Supporting Information

Selective modification of tryptophan in polypeptides via C-N coupling with azoles using *in-situ* generated iodine-based oxidants in aqueous media

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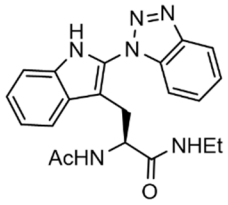
Supporting data



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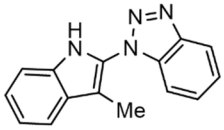


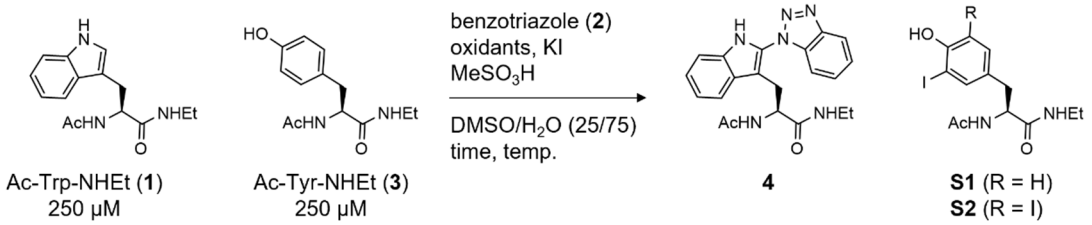
compound **I**

^{1}H NMR (δ, ppm)	
4 (500 MHz, $CDCl_3$)	**I** (400 MHz, $CDCl_3$)
7.22-7.25 (m, 1H)	7.22−7.26 (m, 1H)
7.31-7.34 (m, 1H)	7.34 (td, *J* = 8.0, 1.2 Hz, 1H)
7.41 (d, *J* = 8.2 Hz, 1H),	7.42−7.47 (m, 2H)
7.47 (ddd, J = 2.5, 5.4, 8.1 Hz, 1H)	7.48−7.51 (m, 1H)
7.57-7.58 (m, 2H),	7.54−7.58 (m, 1H)
7.79 (d, *J* = 8.0 Hz, 1H),	7.68 (d, *J* = 8.0 Hz, 1H)
8.11 (d, *J* = 8.4 Hz, 1H),	8.14 (d, *J* = 8.0 Hz, 1H)
9.06 (s, 1H).	8.49 (s, 1H)

^{13}C NMR (δ, ppm)	
4 (126 MHz, $CDCl_3$)	**I** (100 MHz, $CDCl_3$)
107.4	106.8
110.3	110.3
111.8	111.3
120.2	119.7
120.6	120.3
121.3	120.4
124.5	123.9
125.4	124.6
127.0	125.8
127.1	127.9
129.5	128.7
133.8	133.9
134.4	134.3
145.6	145.4

Fig. S1 Structural confirmation of **4** by comparison of ^{1}H and ^{13}C NMR spectra of aromatic region (^{1}H NMR: δ 7.00-10.00, ^{13}C NMR: δ 100.0-150.0) between **4** and 2-(benzotriazol-1-yl)-3-methylindole (**I**).[1]

Table S1 Optimization of reaction conditions for C–N coupling of tryptophan **1** with benzotriazole (**2**)



Ac-Trp-NHEt (**1**) 250 μM | Ac-Tyr-NHEt (**3**) 250 μM | **4** | **S1** (R = H), **S2** (R = I)

entry	oxidant	[oxidant] (mM)	[KI] (mM)	[CH_3SO_3H] (mM)	[**2**] (mM)	time (min)	temp.	conversion (%)[a]		yield (%)[b]		
								1	**3**	**4**	**S1**	**S2**
1	I_2	1	0	0	25	30	r.t.	78	33	15	20	6
2	NIS	1	0	0	25	30	r.t.	88	>95	21	7	>95
3	HIO_3	1	0	0	25	30	r.t.	8	<5	<5	<5	<5
4	HIO_3	1	0.5	0	25	30	r.t.	36	9	9	7	<5
5	KIO_3	1	0.5	1	25	30	r.t.	38	10	9	7	<5
6	KIO_3	1	0.5	10	25	30	r.t.	>95	31	21	21	9
7	KIO_3	1	0.5	20	25	10	r.t.	>95	8	16	8	<5
8	KIO_3	1	0.5	20	50	10	r.t.	>95	13	25	8	<5
9	KIO_3	1	0.5	20	100	10	r.t.	>95	15	38	8	<5
10	KIO_3	1	0.5	20	100	30	0 ℃	85	<5	47	<5	<5
11	KIO_3	0.5	0.25	20	100	60	0 ℃	86	5	49	<5	<5
12	KIO_3	1	0.25	20	100	60	0 ℃	>95	<5	61	<5	<5
13	I_2	1	0	20	100	60	0 ℃	73	6	47	9	<5
14	NIS	1	0	20	100	60	0 ℃	>95	13	40	<5	<5
15	KIO_3	1	0	20	100	60	0 ℃	53	<5	20	<5	<5

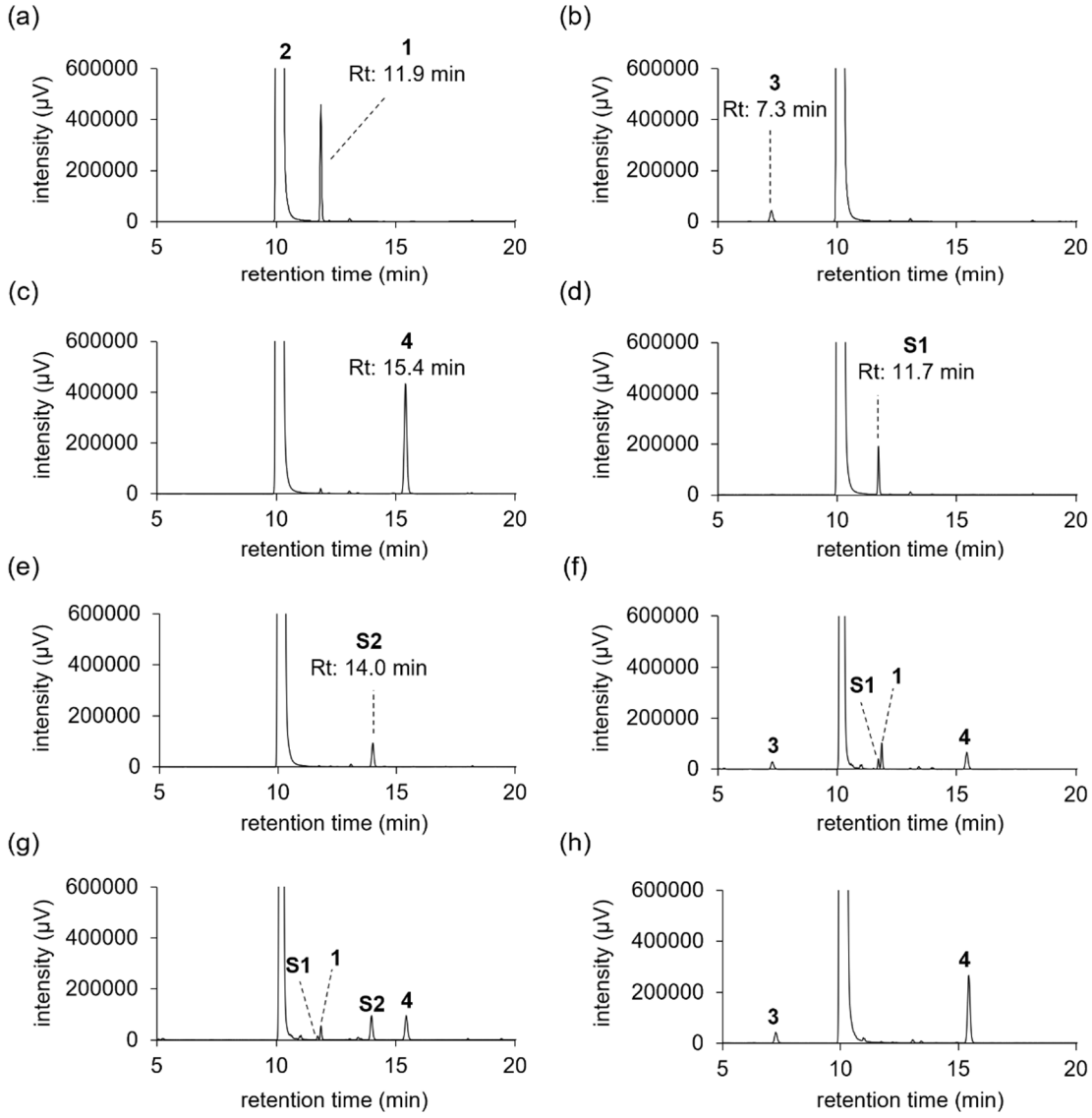
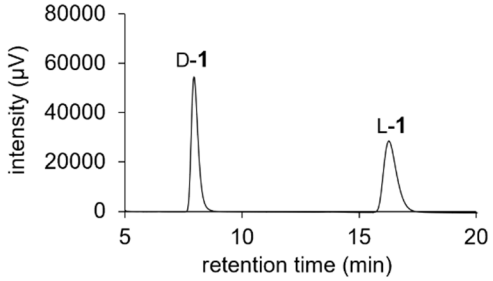


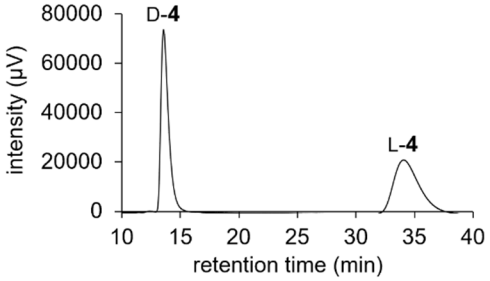
Fig. S2 RP-HPLC chromatograms of the authentic samples of (a) **1**, (b) **3**, (c) **4**, (d) **S1**, and (e) **S2** in the presence of **2** and the reaction mixtures of (f) entry 1, (g) entry 2, and (h) entry 12 in Table S1.

(a)



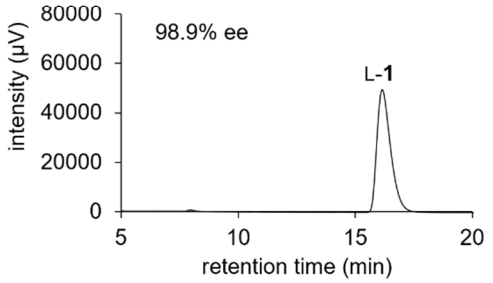
Peak#	Retention time (min)	Area
1	7.94	1148636
2	16.259	1150957

(b)



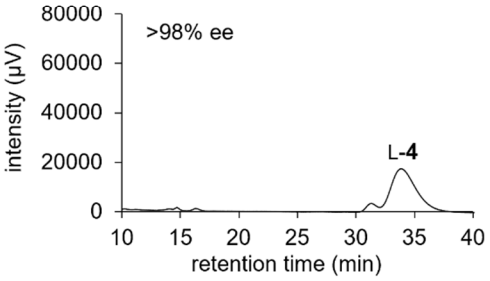
Peak#	Retention time (min)	Area
1	13.576	3424957
2	34.056	3407249

(c)



Peak#	Retention time (min)	Area
1	7.974	11621
2	16.158	2029240

(d)



Peak#	Retention time (min)	Area
1	14.098	29057
2	14.729	36627
3	16.343	38982
4	31.319	222027
5	33.88	2694488

D-isomer was not detected.

Fig. S3 HPLC chromatograms and peak tables for (a) racemic **1**, (b) racemic **4**, (c) L-**1**, and (d) the crude product of the C–N coupling of **1** and **2**. Peaks No.1–4 in (d) were judged not to be the D-**4** according to the UV spectral data collected with the PDA detector.

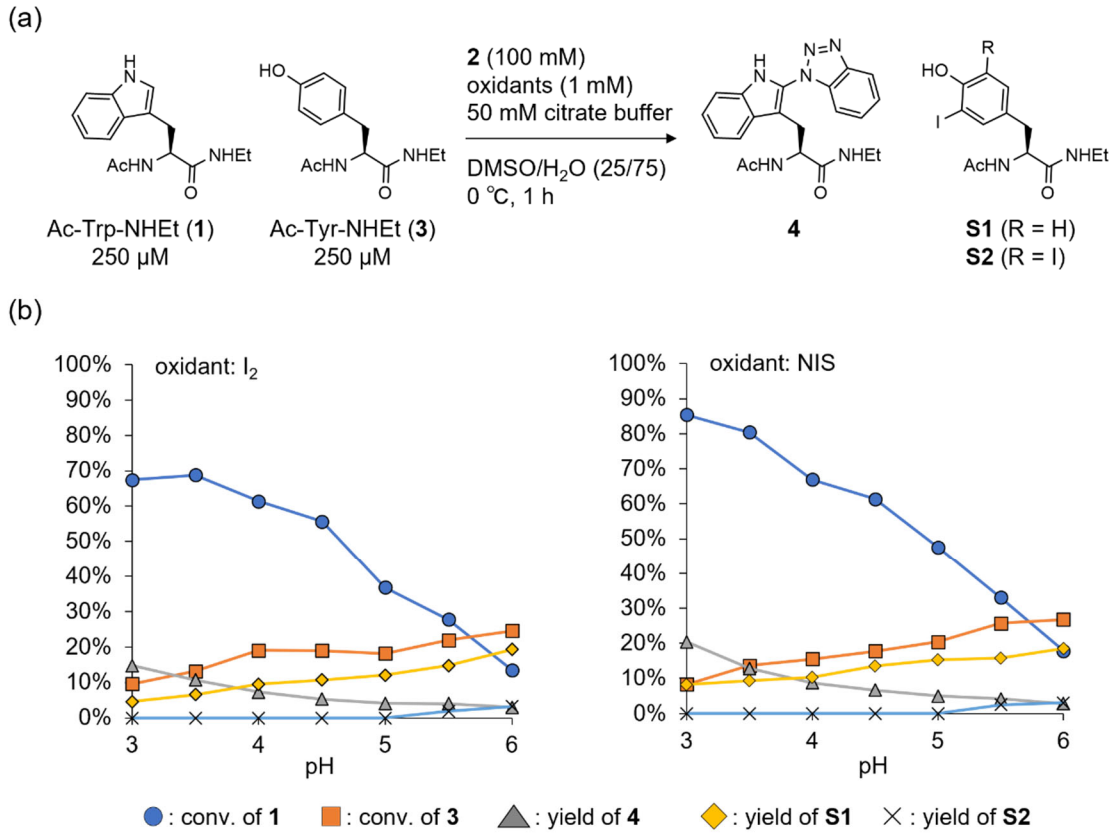


Fig. S4 (a) C–N coupling of tryptophan **1** and **2** with I_2 or NIS as an oxidant at various pH in the presence of tyrosine **3**. (b) pH dependency of the conversions of **1** and **3** and the yields of **4**, **S1**, and **S2**.

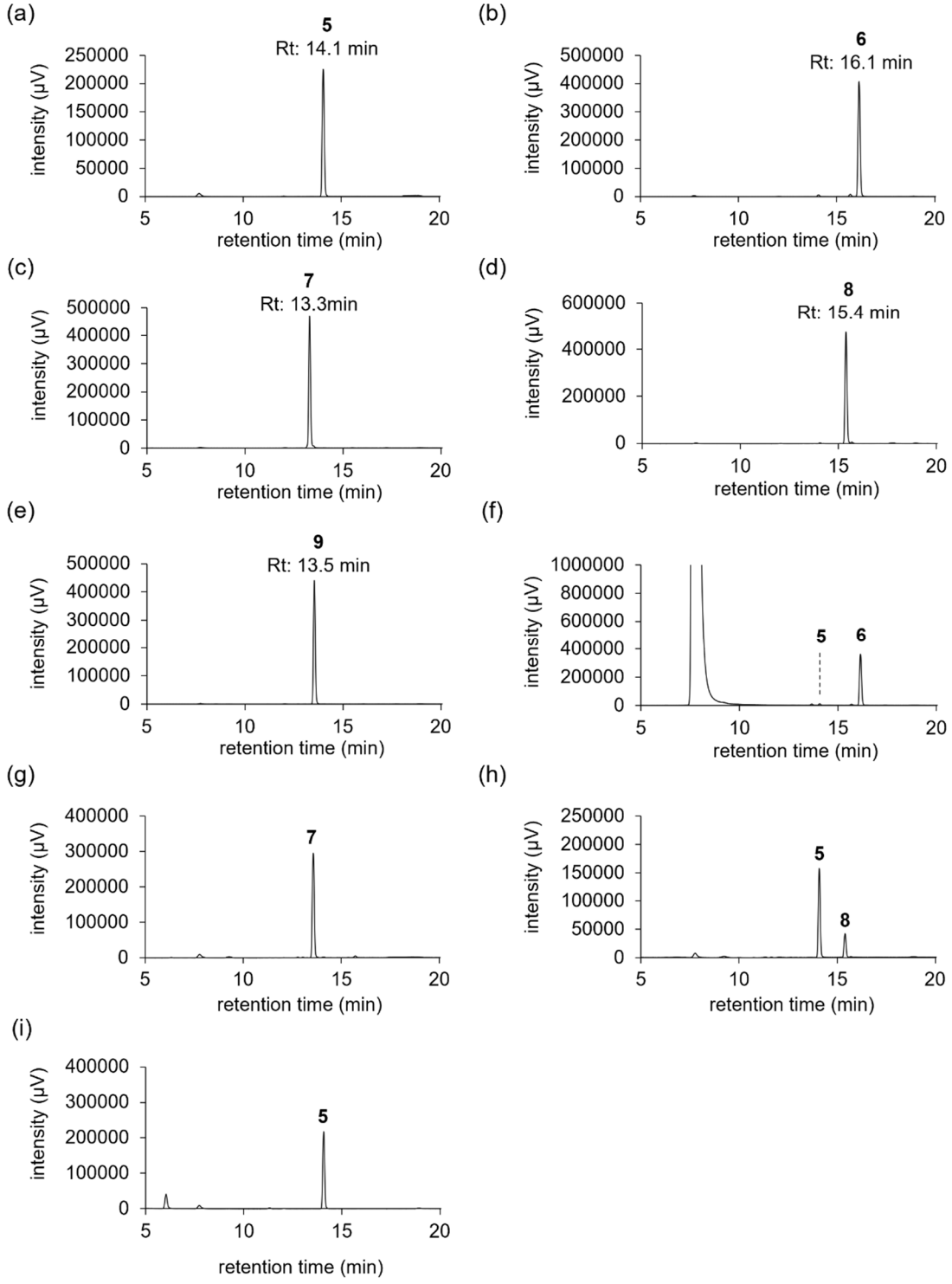


Fig. S5 RP-HPLC chromatograms of the authentic samples of (a) **5**, (b) **6**, (c) **7**, (d) **8**, and (e) **9** and the reaction mixtures with (f) benzotriazole, (g) triazole, (h) pyrazole, and (i) benzimidazole.

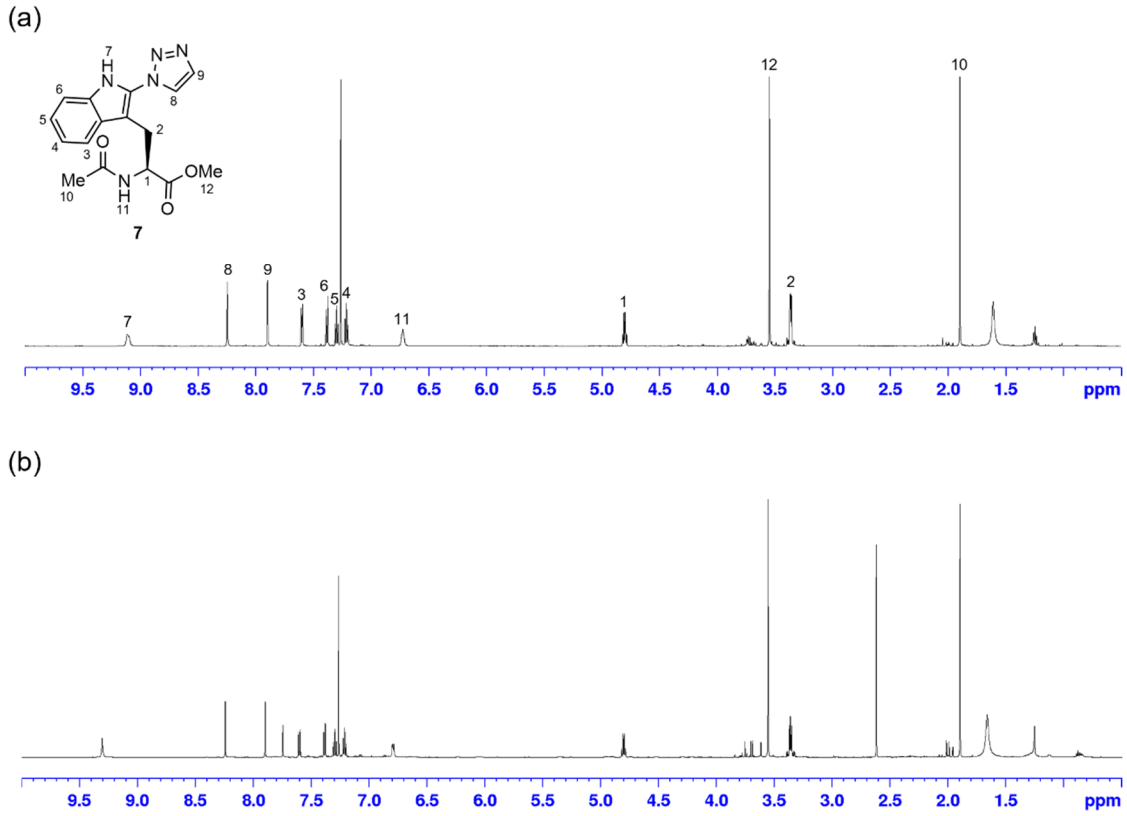


Fig. S6 Confirmation of the C–N coupling of 1,2,3-triazole at the N1 nitrogen by ^{1}H NMR in $CDCl_3$. Numbers on each signal correspond to numbers on the structures. (a) The authentic sample of **7** and (b) the crude product of the C–N coupling of **5** and 1,2,3-triazole.

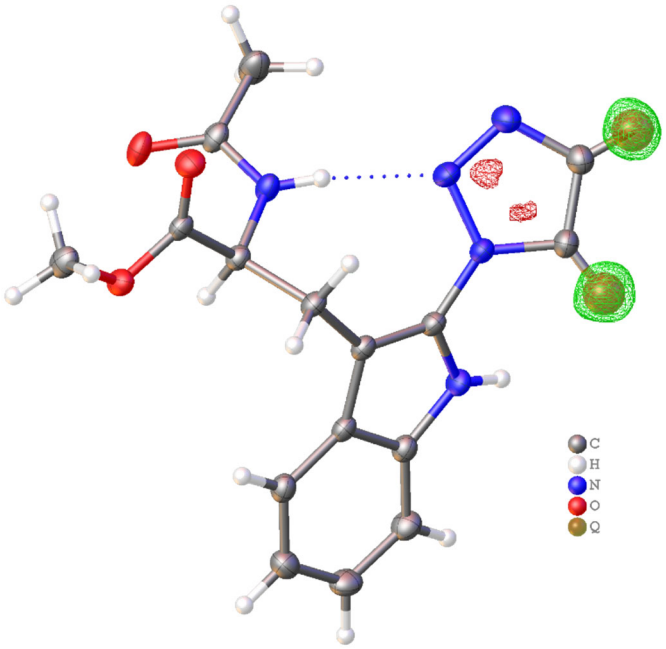


Fig. S7 The crystal structure of *N*-acetyl-2-(*1H*-1,2,3-triazol-1-yl)-L-tryptophan methyl ester (**7**). The positive peaks (green wire mesh) in the difference electron density map (0.2 eÅ^{-3} level) suggested the presence of the hydrogen atoms, which indicated the attachment of the triazole ring at the N1 position. The dotted line indicated the hydrogen bonding.

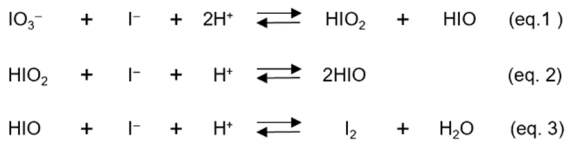


Fig. S8 Equilibrium of iodine species in an aqueous condition.

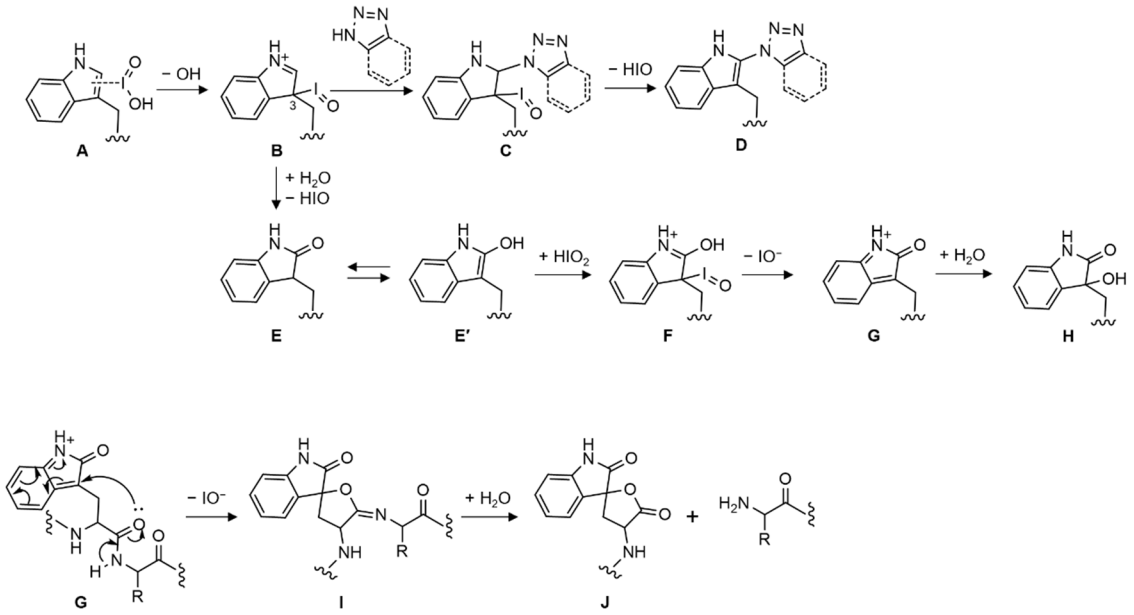


Fig. S9 Possible reaction mechanisms of the C–N coupling reaction and the side reactions.

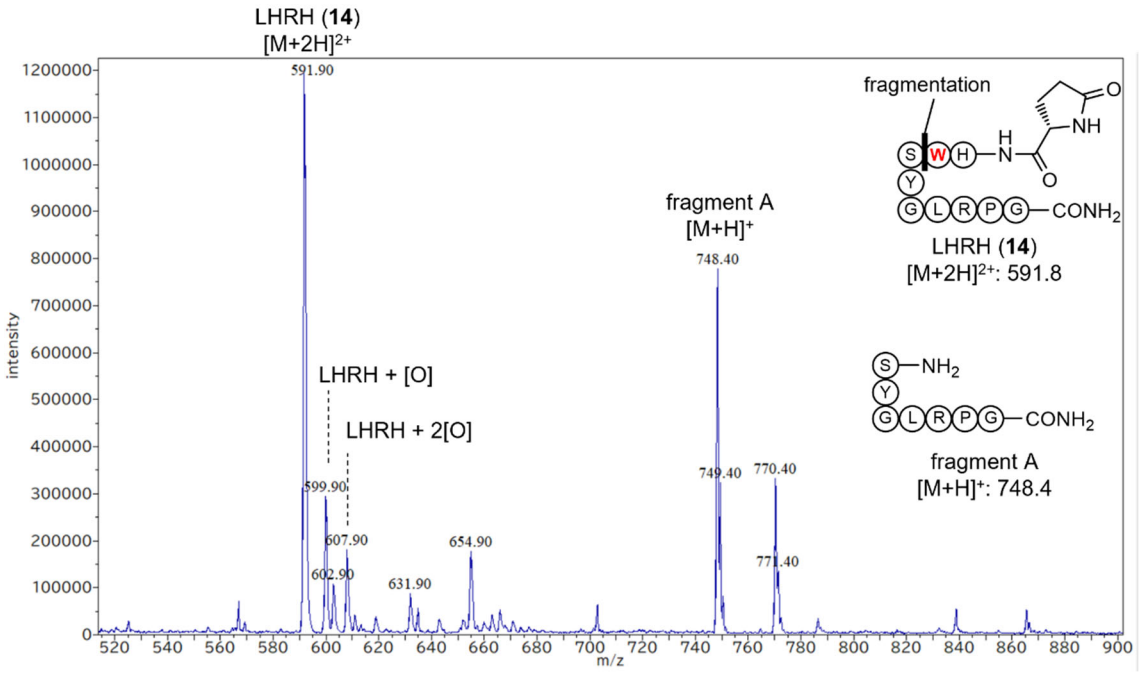
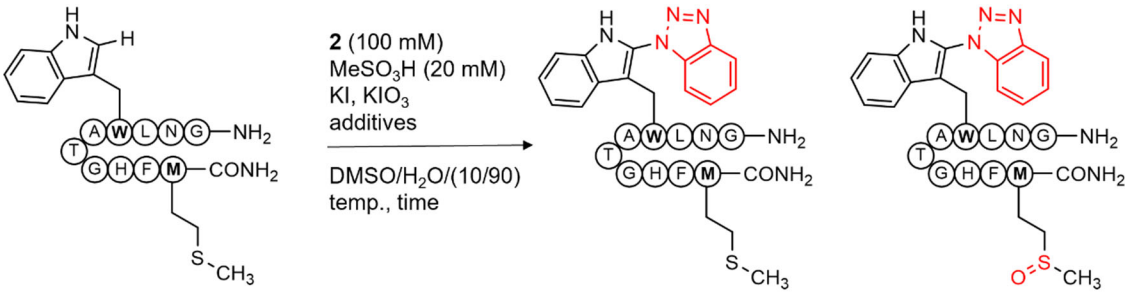


Fig. S10 Fragmentation of LHRH (**14**) under the reaction conditions in the absence of **2**. ESI-MS spectrum of the reaction mixture.

Additional experimental data for tryptophan modification of polypeptides via C–N coupling with benzotriazole 2

Table S2 Exploration of additives for suppression of undesirable methionine oxidation



neuromedin B (**10**)
[M+H]$^+$: 1132.5

19
[M+H]$^+$: 1249.5

19$_{ox}$
[M+H]$^+$: 1265.5

entry	[KI] (mM)	[KIO_3] (mM)	additives	[additives] (mM)	temp.	time (min)	area (**19**) /area (**10**+**19$_{ox}$**)
1	0.5	2	–	0	r.t.	30	0.17
2	0.5	2	–	0	0 ℃	30	3.55
3	0.5	2	thiourea	0.25	0 ℃	30	3.22
4	0.5	2	thiourea	2	0 ℃	30	1.75
5	0.5	2	HCO_2H	5	0 ℃	30	3.82
6	0.5	2	HCO_2H	20	0 ℃	30	4.35
7	0.25	1	HCO_2H	20	0 ℃	60	5.85

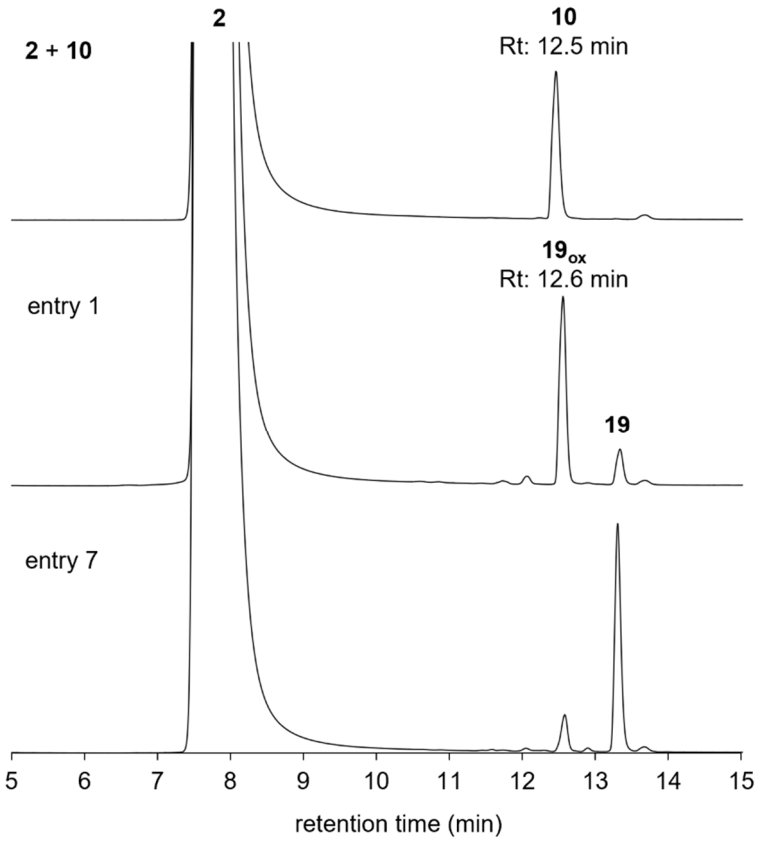
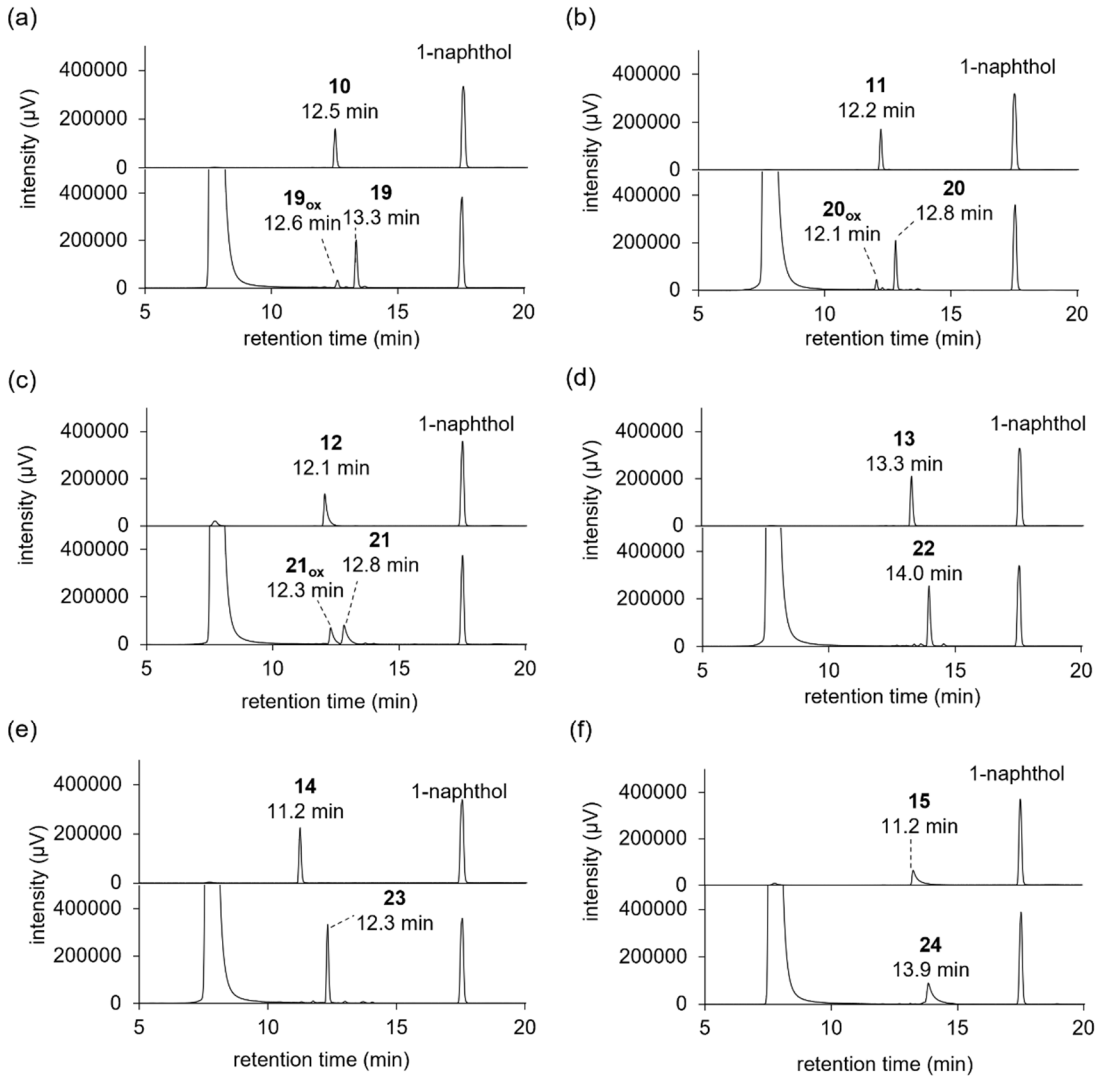


Fig. S11 RP-HPLC chromatograms for Table S2.


(a)
1-naphthol
10
12.5 min
19ox
12.6 min
19
13.3 min
(b)
1-naphthol
11
12.2 min
20ox
12.1 min
20
12.8 min
(c)
1-naphthol
12
12.1 min
21ox
12.3 min
21
12.8 min
(d)
1-naphthol
13
13.3 min
22
14.0 min
(e)
1-naphthol
14
11.2 min
23
12.3 min
(f)
1-naphthol
15
11.2 min
24
13.9 min
intensity (μV)
400000
200000
0
5
10
15
20
retention time (min)

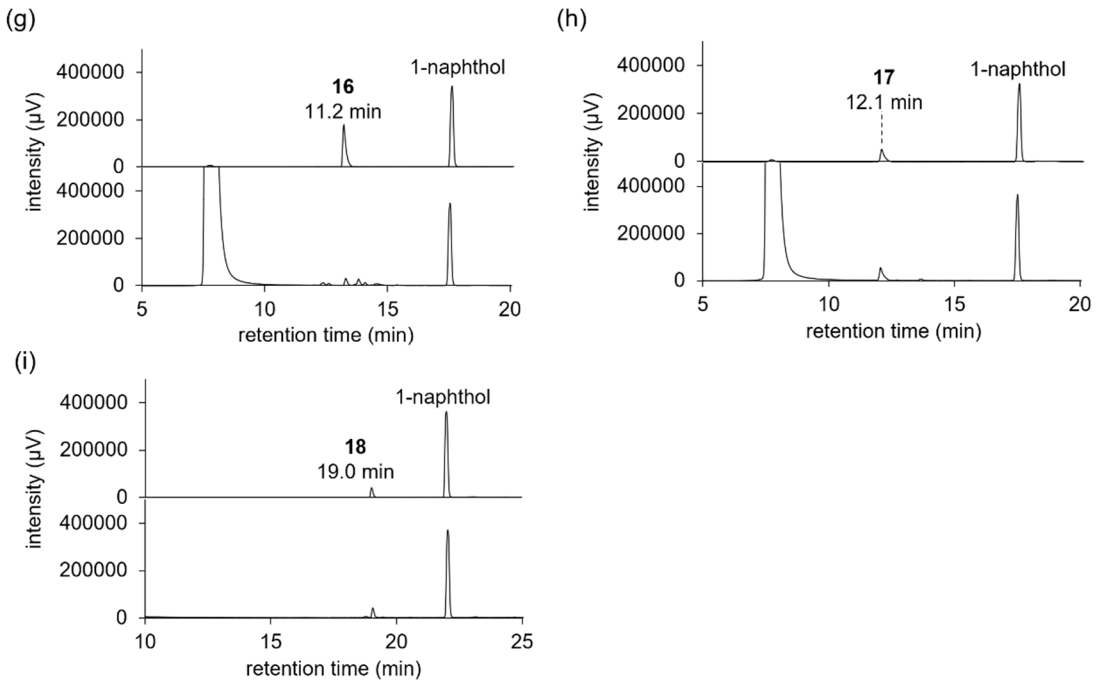
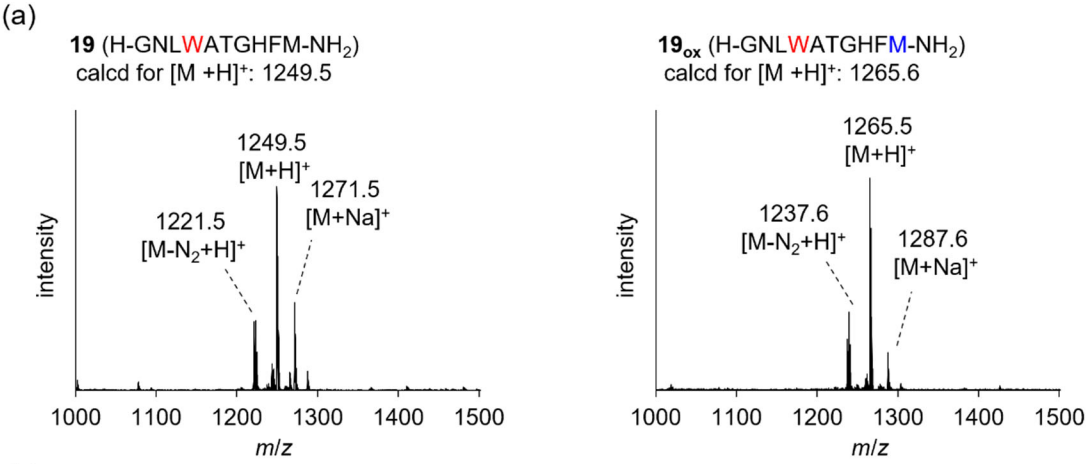
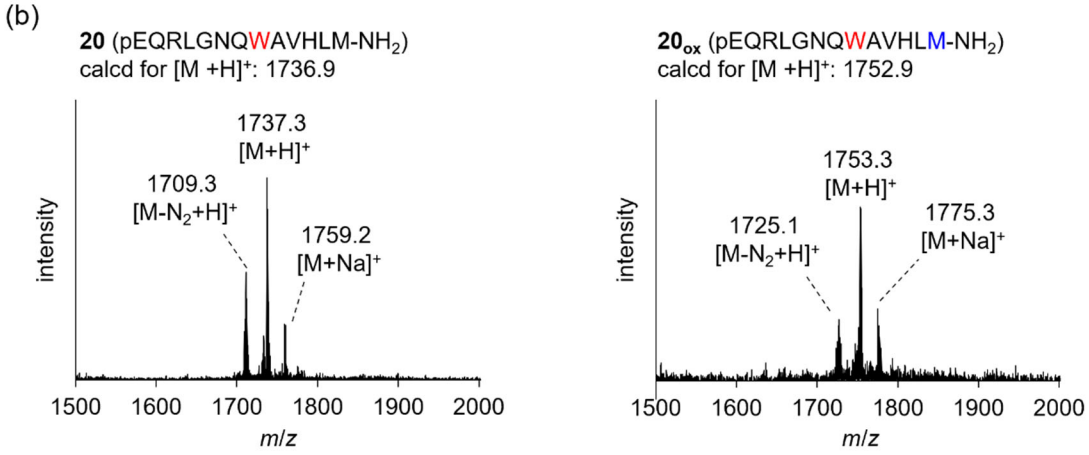


Fig. S12 Tryptophan modification of polypeptides via C–N coupling with **2**. RP-HPLC chromatograms of the parent peptides (top) and the reaction mixtures (bottom): (a) neuromedin B (**10**) (H-GNLWATGHFM-NH_2), (b) bombesin (**11**) (pEQRLGNQWAVHLM-NH_2), (c) α-MSH (**12**), (Ac-SYSMEHFRWGKPV-NH_2), (d) kisspeptin-10 (**13**) (H-YNWNSFGLRF-NH_2), (e) LHRH (**14**) (pEHWSYGLRPG-NH_2), (f) somatostatin (**15**) [H-AGCKNFFWKTFTSC-OH (C^3-C^{13})], (g) synthetic peptide **16** (H-WRVYI-OH), (h) synthetic peptide **17** (H-YRVYI-OH), and (i) endokinin D (**18**) (H-GAYQLEHTFQGLL-NH_2).

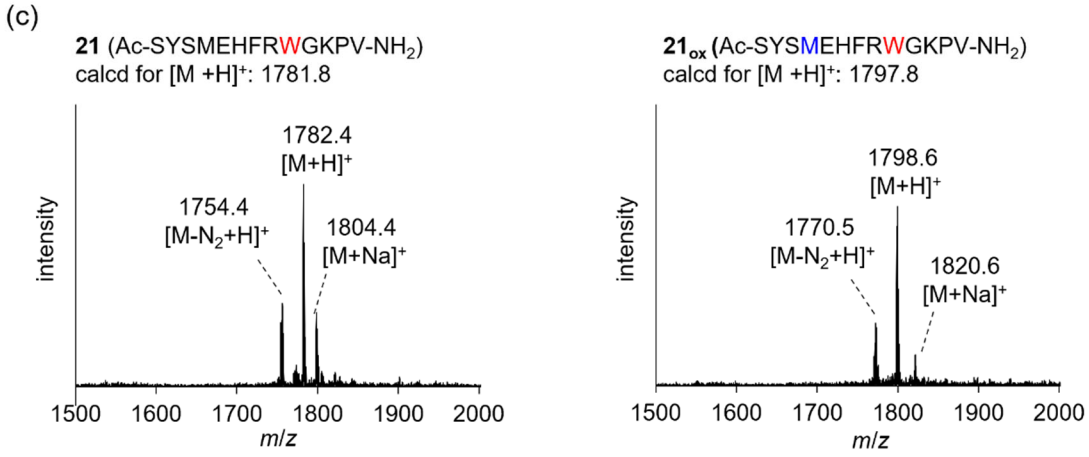
(a)


19 (H-GNLWATGHFM-NH2)
calcd for [M +H]+: 1249.5
1249.5
[M+H]+
1271.5
[M+Na]+
1221.5
[M-N2+H]+
intensity
1000 1100 1200 1300 1400 1500
m/z
19ox (H-GNLWATGHFM-NH2)
calcd for [M +H]+: 1265.6
1265.5
[M+H]+
1237.6
[M-N2+H]+
1287.6
[M+Na]+
intensity
1000 1100 1200 1300 1400 1500
m/z

(b)


20 (pEQRLGNQWAVHLM-NH2)
calcd for [M +H]+: 1736.9
1737.3
[M+H]+
1709.3
[M-N2+H]+
1759.2
[M+Na]+
intensity
1500 1600 1700 1800 1900 2000
m/z
20ox (pEQRLGNQWAVHLM-NH2)
calcd for [M +H]+: 1752.9
1753.3
[M+H]+
1725.1
[M-N2+H]+
1775.3
[M+Na]+
intensity
1500 1600 1700 1800 1900 2000
m/z

(c)


21 (Ac-SYSMEHFRWGKPV-NH2)
calcd for [M +H]+: 1781.8
1782.4
[M+H]+
1754.4
[M-N2+H]+
1804.4
[M+Na]+
intensity
1500 1600 1700 1800 1900 2000
m/z
21ox (Ac-SYSMEHFRWGKPV-NH2)
calcd for [M +H]+: 1797.8
1798.6
[M+H]+
1770.5
[M-N2+H]+
1820.6
[M+Na]+
intensity
1500 1600 1700 1800 1900 2000
m/z

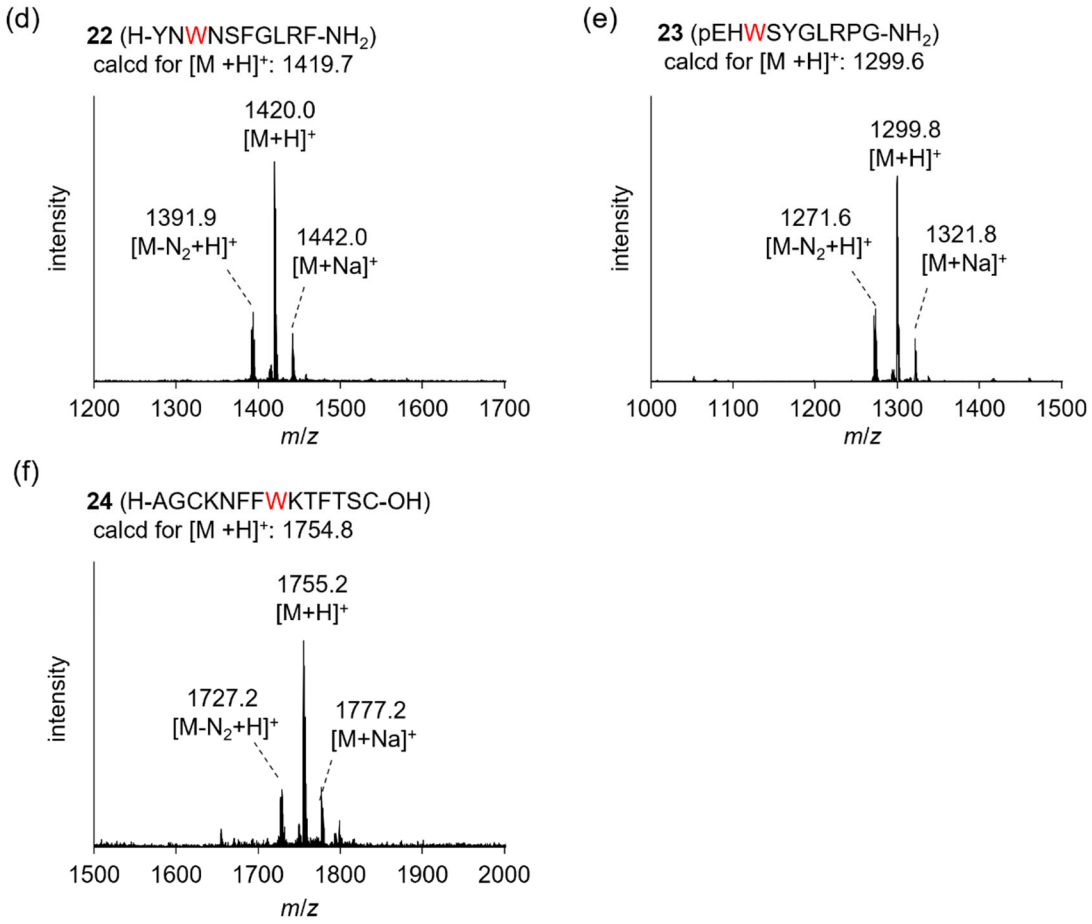
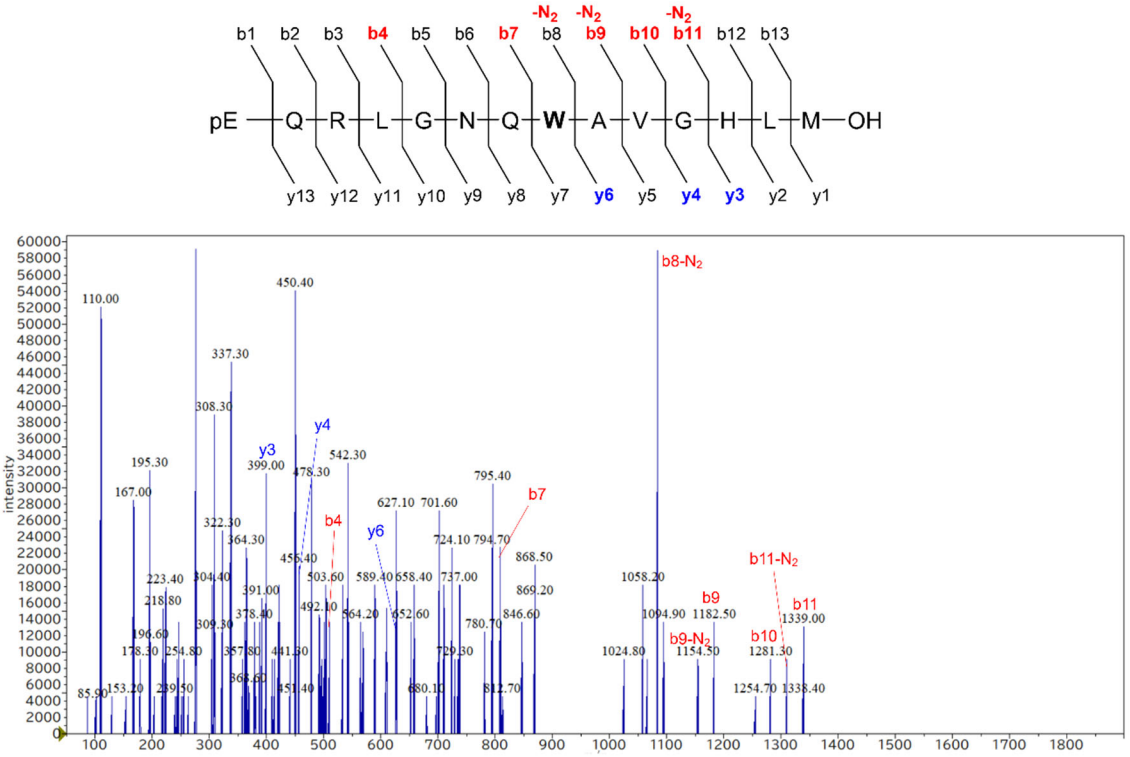


Fig. S13 MALDI-TOF MS spectra of modified peptides **19**—**24** after RP-HPLC purification. An additional $[M-28+H]^+$ peak derived from N_2 loss from the benzotriazole was observed.[2]

Precursor ion: 869.0 (m/z), Collision energy: -35.0 V

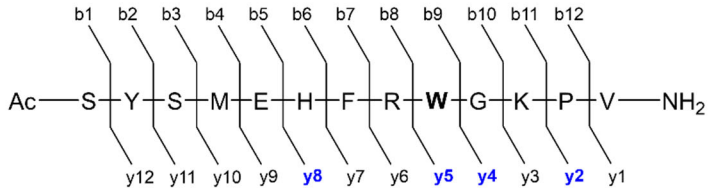


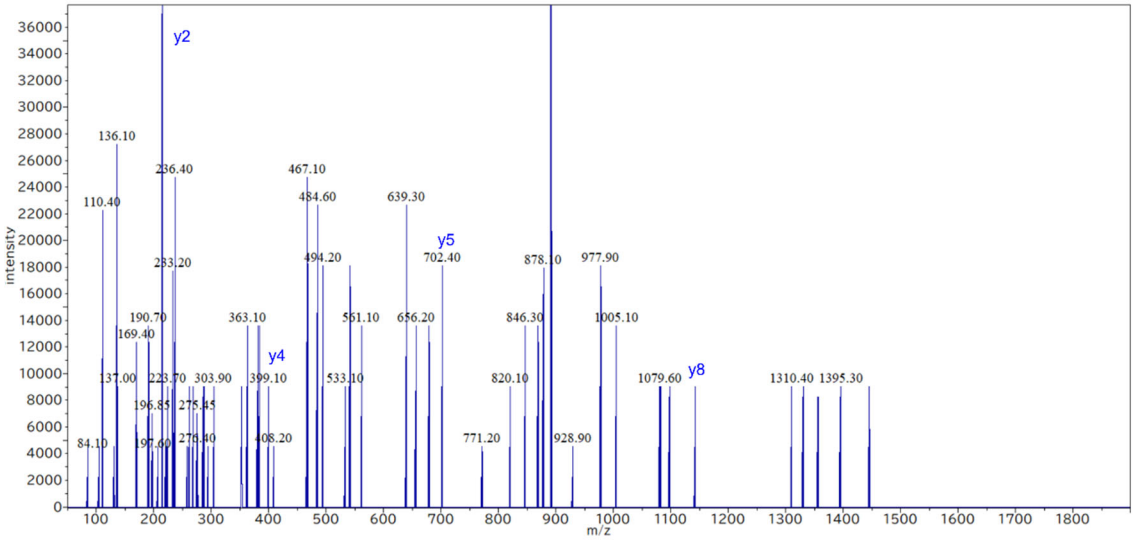
Simulated fragment list

b-N_2		b			y		y-N_2	
				pE				
		b2	240.1	Q	y13	1625.8	y13-N_2	1597.8
		b3	396.2	R	y12	1497.8	y12-N_2	1469.8
		b4	**509.3**	L	y11	1341.7	y11-N_2	1313.7
		b5	566.3	G	y10	1228.6	y10-N_2	1200.6
		b6	680.3	N	y9	1171.6	y9-N_2	1143.6
		b7	**808.4**	Q	y8	1057.5	y8-N_2	1029.5
b8-N_2	**1083.5**	b8	1111.5	**W**	y7	929.5	y7-N_2	901.5
b9-N_2	**1154.5**	b9	**1182.6**	A	y6	**626.3**		
b10-N_2	1253.6	b10	**1281.6**	V	y5	555.3		
b11-N_2	**1310.6**	b11	**1338.6**	G	y4	**456.2**		
b12-N_2	1447.7	b12	1475.7	H	y3	**399.2**		
b13-N_2	1560.8	b13	1588.8	L	y2	262.2		
				M-NH_2	y1	149.1		

Fig. S14 LC-MS/MS analysis data of modified bombesin **20**. Denitrogenated b ions (b-N_2) and y ions (y-N_2) were also observed. Detected fragment ions were highlighted in red (b ions) or blue (y ions).

Precursor ion: 891.8 (m/z), Collision energy: -35.0 V



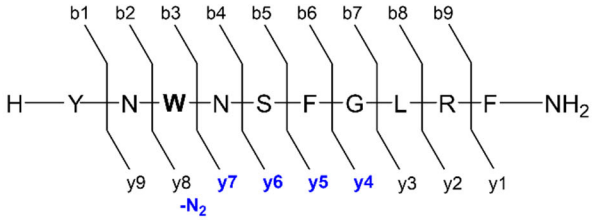


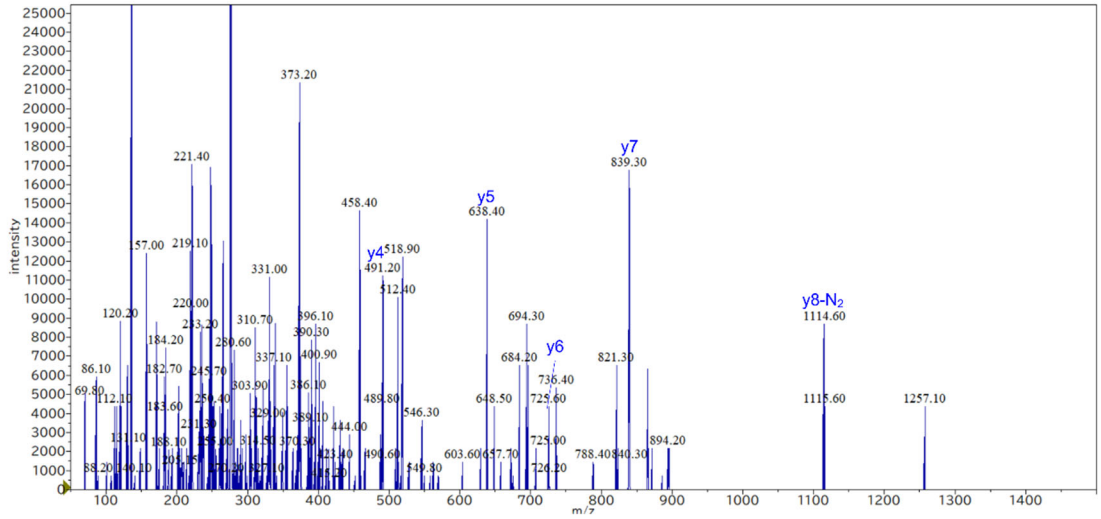
Simulated fragment list

b-N_2		b			y		y-N_2	
				Ac-S				
		b2	293.1	Y	y12	1652.8	y12-N_2	1624.8
		b3	380.1	S	y11	1489.7	y11-N_2	1461.7
		b4	511.2	M	y10	1402.7	y10-N_2	1374.7
		b5	640.2	E	y9	1271.7	y9-N_2	1243.6
		b6	777.3	H	y8	**1142.6**	y8-N_2	1114.6
		b7	924.2	F	y7	1005.6	y7-N_2	977.5
		b8	1080.5	R	y6	858.5	y6-N_2	830.5
b9-N_2	1355.6	b9	1383.6	**W**	y5	**702.4**	y5-N_2	674.4
b10-N_2	1412.6	b10	1440.6	G	y4	**399.3**		
b11-N_2	1540.7	b11	1568.7	K	y3	342.3		
b12-N_2	1637.7	b12	1665.7	P	y2	**214.2**		
				V-NH_2	y1	117.1		

Fig. S15 LC-MS/MS analysis data of modified α-MSH **21**. Denitrogenated b ions (b-N_2) and y ions (y-N_2) were also observed. Detected fragment ions were highlighted in red (b ions) or blue (y ions).

Precursor ion: 710.6 (m/z), Collision energy: -35.0 V



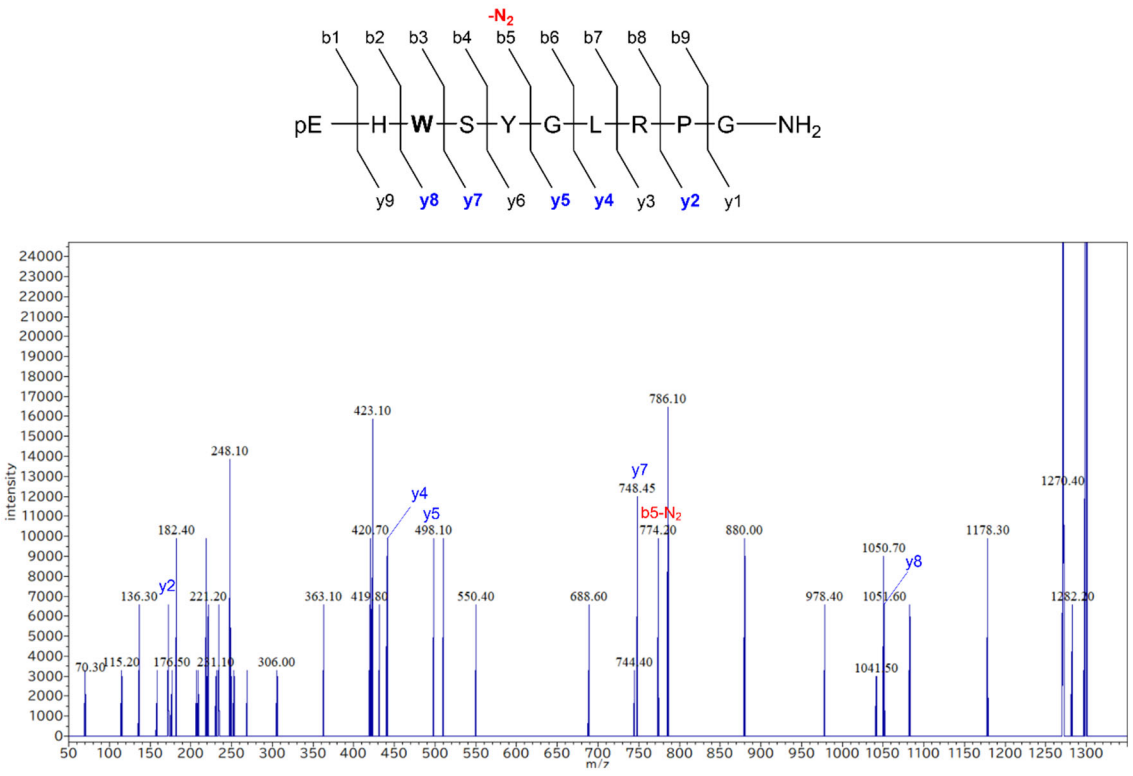


Simulated fragment list

b-N_2		b			y		y-N_2	
				Y				
		b2	278.1	N	y9	1256.6	y9-N_2	1228.6
b3-N_2	553.2	b3	581.2	**W**	y8	1142.6	y8-N_2	**1114.6**
b4-N_2	667.3	b4	695.3	N	y7	**839.5**		
b5-N_2	754.3	b5	782.3	S	y6	**725.4**		
b6-N_2	901.4	b6	929.4	F	y5	**638.4**		
b7-N_2	958.4	b7	986.4	G	y4	**491.3**		
b8-N_2	1071.5	b8	1099.5	L	y3	434.3		
b9-N_2	1227.6	b9	1255.6	R	y2	321.2		
				F-NH_2	y1	165.1		

Fig. S16 LC-MS/MS analysis data of modified kisspeptin-10 **22**. Denitrogenated b ions (b-N_2) and y ions (y-N_2) were also observed. Detected fragment ions were highlighted in red (b ions) or blue (y ions).

Precursor ion: 1299.5 (m/z), Collision energy: -35.0 V

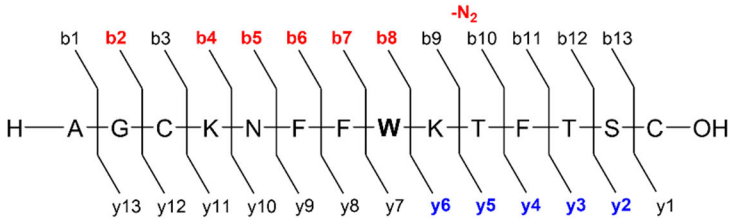


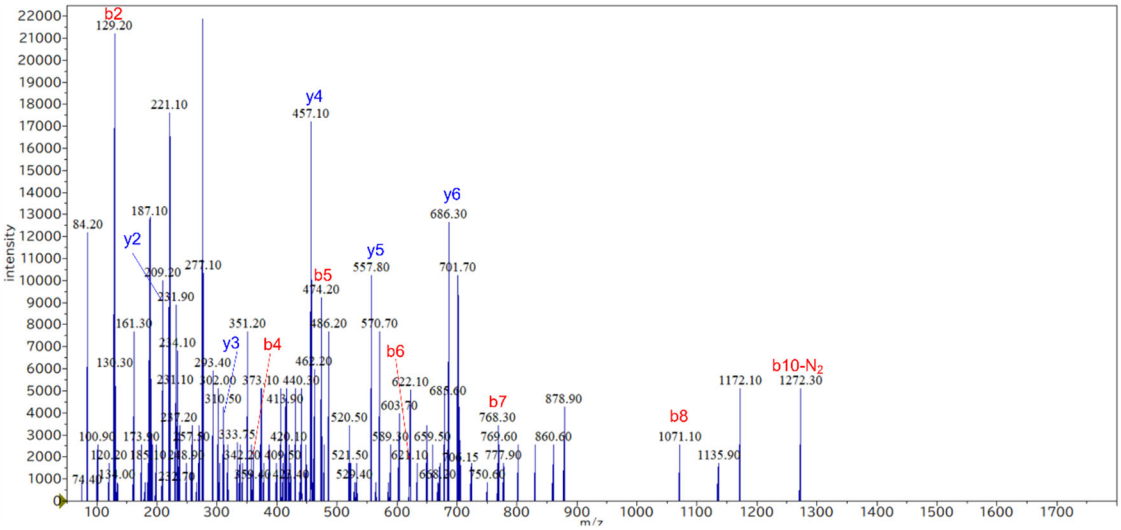
Simulated fragment list

b-N_2		b			y		y-N_2	
				pE				
		b2	249.1	H	y9	1188.6	y9-N_2	1160.5
b3-N_2	524.2	b3	552.2	**W**	y8	**1051.5**	y8-N_2	1023.5
b4-N_2	611.2	b4	639.2	S	y7	**748.4**		
b5-N_2	**774.3**	b5	802.3	Y	y6	661.4		
b6-N_2	831.3	b6	859.3	G	y5	**498.3**		
b7-N_2	944.4	b7	972.4	L	y4	**441.3**		
b8-N_2	1100.5	b8	1128.5	R	y3	328.2		
b9-N_2	1197.6	b9	1225.6	P	y2	**172.1**		
				G-NH_2	y1	75.1		

Fig. S17 LC-MS/MS analysis data of modified LHRH **23**. Denitrogenated b ions (b-N_2) and y ions (y-N_2) were also observed. Detected fragment ions were highlighted in red (b ions) or blue (y ions).

Precursor ion: 878.9 (m/z), Collision energy: -35.0 V





Simulated fragment list

b-N_2		b			y		y-N_2	
				A				
		b2	**129.1**	G	y13	1685.7	y13-N_2	1657.7
		b3	232.1	C	y12	1628.7	y12-N_2	1600.7
		b4	**360.2**	K	y11	1525.7	y11-N_2	1497.7
		b5	**474.2**	N	y10	1397.6	y10-N_2	1369.6
		b6	**621.3**	F	y9	1283.6	y9-N_2	1255.6
		b7	**768.3**	F	y8	1136.5	y8-N_2	1108.5
b8-N_2	1043.5	b8	**1071.5**	**W**	y7	989.4	y7-N_2	961.4
b9-N_2	1171.6	b9	1199.6	K	y6	**686.3**		
b10-N_2	**1272.6**	b10	1300.6	T	y5	**558.2**		
b11-N_2	1419.7	b11	1447.7	F	y4	**457.2**		
b12-N_2	1520.7	b12	1548.7	T	y3	**310.1**		
b13-N_2	1607.7	b13	1635.8	S	y2	**209.1**		
				C	y1	122.0		

Fig. S18 LC-MS/MS analysis data of modified somatostatin **24**. Denitrogenated b ions (b-N_2) and y ions (y-N_2) were also observed. Detected fragment ions were highlighted in red (b ions) or blue (y ions).

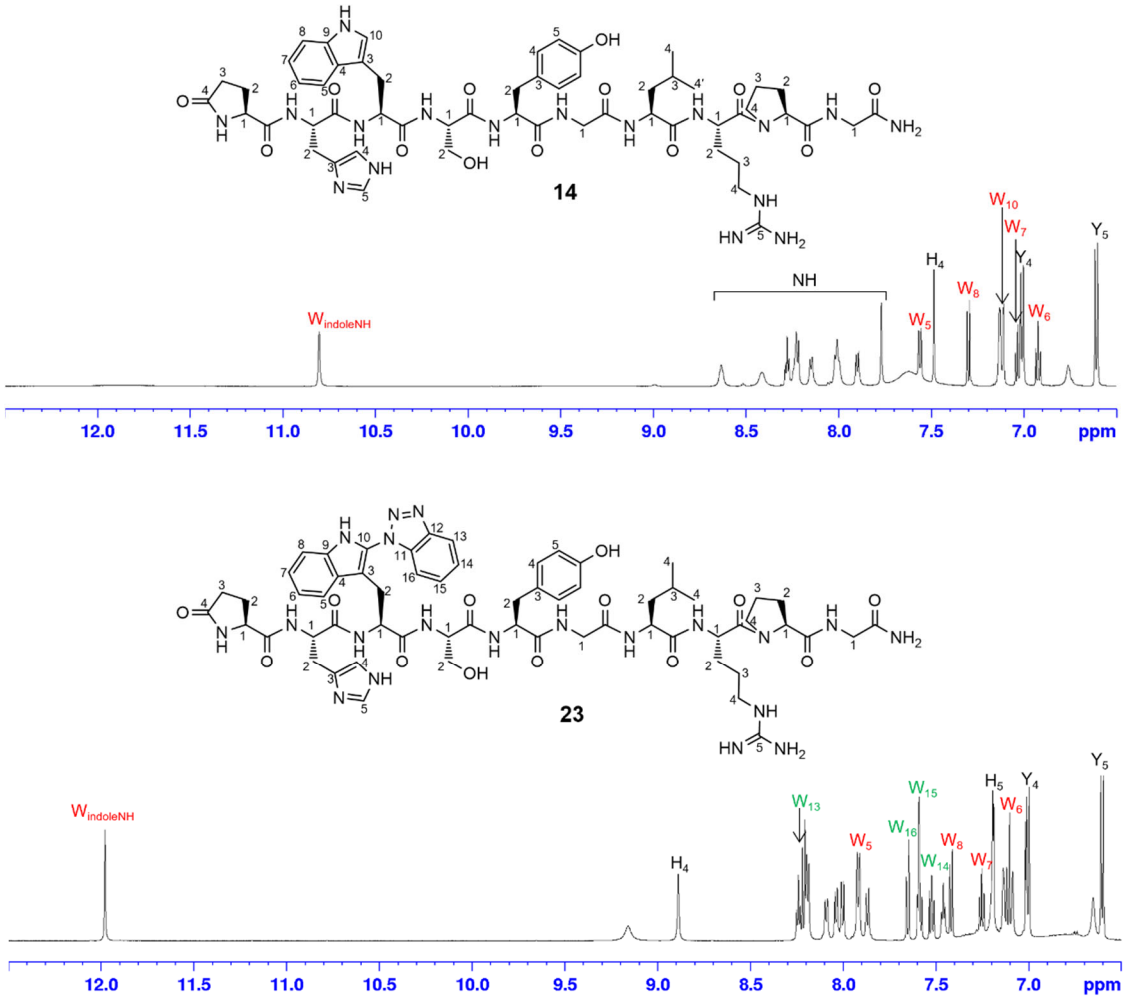


Fig. S19 Aromatic regions of ^{1}H NMR spectra of **14** and **23**. The red and green letters indicated the signals of the indole and the benzotriazole, respectively. Numbers on each signal corresponded to numbers on the structures.

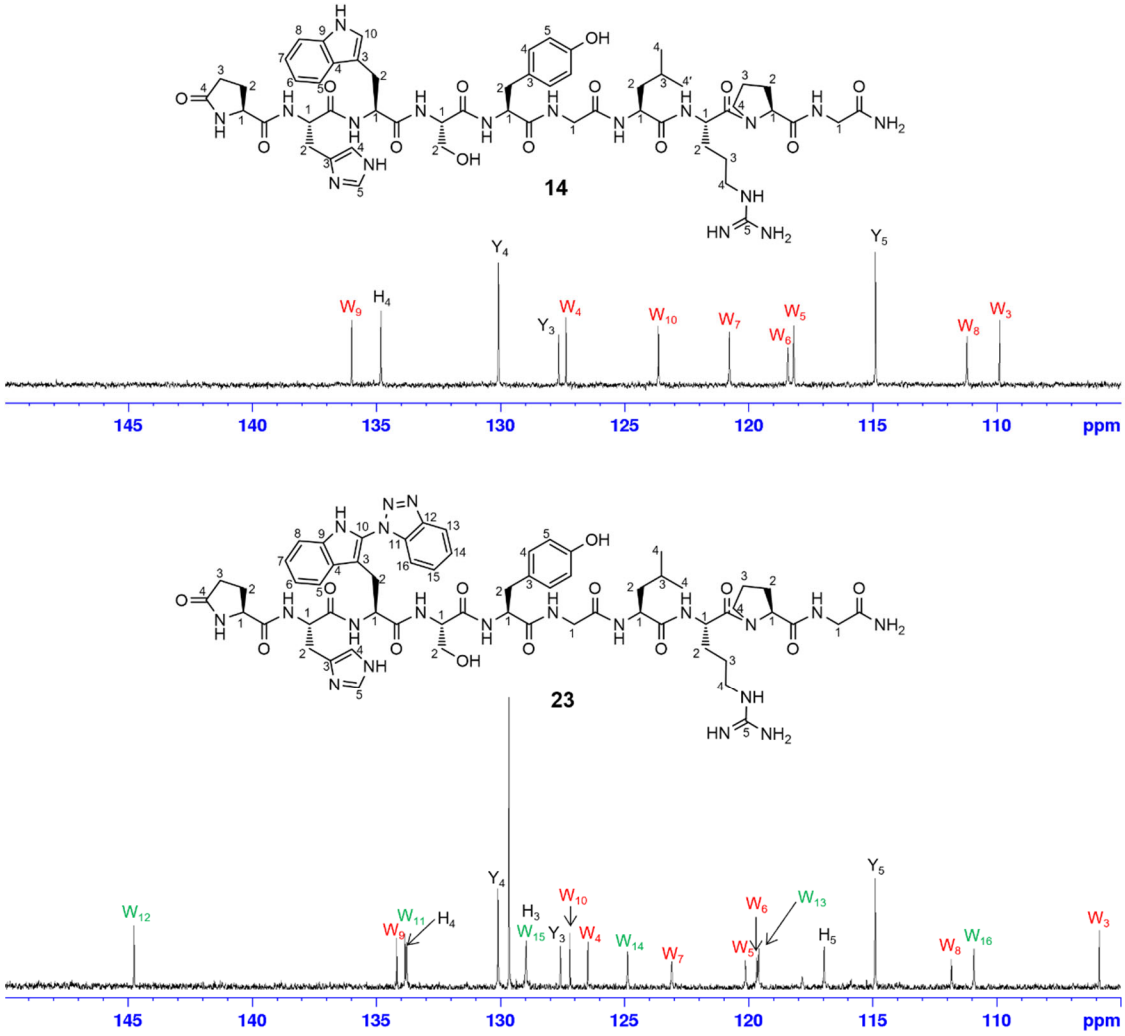


Fig. S20 Aromatic region of ^{13}C NMR spectra of **14** and **23**. The red and green letters indicated the signals of the indole and the benzotriazole, respectively. Numbers on each signal corresponded to numbers on the structures.

Table S3 ^{1}H NMR assignment for LHRH (**14**) and the modified LHRH **23**. N.D.: not determined.

		LHRH (**14**)		**23**	
		^{1}H	^{13}C	^{1}H	^{13}C
pE1	NH	7.77	–	7.60-7.57	–
	1	3.97-3.95	55.5	3.96-3.94	55.4
	2	2.17-2.12, 1.76-1.73	25.2	2.14-2.06, 1.66-1.61	24.9
	3	2.10-2.00	29.1	2.05-1.98	N.D.
	4	–	178.0	–	177.4
H2	NH	8.02-8.01	–	8.00 (d, J = 8.2 Hz)	–
	1	4.43-4.38	52.9	4.46-4.42	51.4
	2	2.89-2.86, 2.78-2.75	N.D.	2.95-2.92, 2.82-2.78	26.9
	3	–	N.D.	–	129.0
	4	7.48 (d, J = 0.6 Hz)	134.8	8.89	133.8
	5	N.D.	N.D.	7.19	116.9
W3	NH	8.15 (d, J = 7.3 Hz)	–	8.04 (d, J = 7.6 Hz)	–
	1	4.56-4.53	53.5	4.70-4.66	53.1
	2	3.17-3.14, 2.97-2.95	27.4	3.22-3.19, 2.90-2.86	26.3
	3	–	109.9	–	105.8
	4	–	127.4	–	126.5
	5	7.56 (d, J = 8.0 Hz)	118.2	7.92 (d, J = 8.0 Hz)	120.1
	6	.92 (dd, J = 8.0, 7.1 Hz)	118.4	7.10 (dd, J = 8.0, 7.3 Hz)	119.7
	7	7.03 (dd, J = 8.1, 7.1 Hz)	120.8	7.25 (dd, J = 8.2, 7.3 Hz)	123.1
	8	7.30 (d, J = 8.1 Hz)	111.2	7.42 (d, J = 8.2 Hz)	111.8
	9	–	136.0	–	134.2
	10	7.11	123.6	–	127.2
	11	–	–	–	133.9
	12	–	–	–	144.8
	13	–	–	8.21 (d, J = 8.4 Hz)	119.6
	14	–	–	7.52 (dd, J = 7.2 Hz)	124.9
	15	–	–	7.60-7.57	129.0
	16	–	–	7.65 (d, J = 8.3 Hz)	110.9
	indole NH	10.81	–	11.98	–
S4	NH	8.41	–	8.09 (d, J = 7.5 Hz)	–
	1	4.31-4.28	55.4	4.22-4.19	54.8
	2	3.59-3.57, 3.50-3.48	61.5	3.56-3.51	61.7
Y5	NH	8.02-8.01	–	7.92 (d, J = 8.0 Hz)	–
	1	4.41-4.38	54.6	4.41-4.38	54.5
	2	2.96-2.93, 2.75-2.72	36.4	2.92-2.90, 2.72-2.68	36.5
	3	–	127.7	–	127.6
	4	7.01 (d, J = 8.5 Hz)	130.1	7.01 (d, J = 8.5 Hz)	130.1
	5	6.61 (d, J = 8.5 Hz)	114.9	6.61 (d, J = 8.5 Hz)	114.9
	6	–	155.8	–	155.8
G6	NH	8.28	–	8.24 (t, J = 5.9 Hz)	–
	1	3.59-3.57	41.9	3.65-3.61, 3.59-3.55	41.8
L7	NH	7.90 (d, J = 8.1 Hz)	–	7.87 (d, J = 8.2 Hz)	–
	1	4.38-4.33	50.7	4.35-4.31	50.7
	2	1.47-1.39	40.9	1.41-1.38	41.1
	3	1.60-1.57	24.1	1.58-1.53	24.1
	4	0.86 (d, J = 6.6 Hz) 0.83 (d, J = 6.6 Hz)	23.1, 21.5	0.85 (d, J = 6.5 Hz) 0.82 (d, J = 6.5 Hz)	23.1, 21.5
R8	NH	8.23-8.21	–	8.20-8.18	–
	1	4.56-4.53	50.1	4.46-4.42	50.1
	2	1.72-1.68, 1.60-1.54	27.9	1.71-1.68, 1.58-1.53	28.1
	3	1.54-1.47	24.4	1.53-1.49	24.5
	4	3.02-2.99	40.4	3.12-3.04	40.6
	5	–	157.3	–	156.6
	quanidineNH	8.62	–	7.47-7.45	–
P9	1	4.26-4.23	59.9	4.27-4.25	59.8
	2	2.10-1.98, 1.85-1.82	29.0	2.05-1.98	N.D.
	3	1.97-1.92, 1.85-1.82	24.6	1.94-1.90, 1.83-1.79	24.6
	4	3.56-3.53	46.9	3.70-3.66, 3.56-3.51	47.0
G10	NH	8.24-8.22	–	8.20-8.18	–
	1	3.75-3.66	42.0	3.70-3.66	42.0

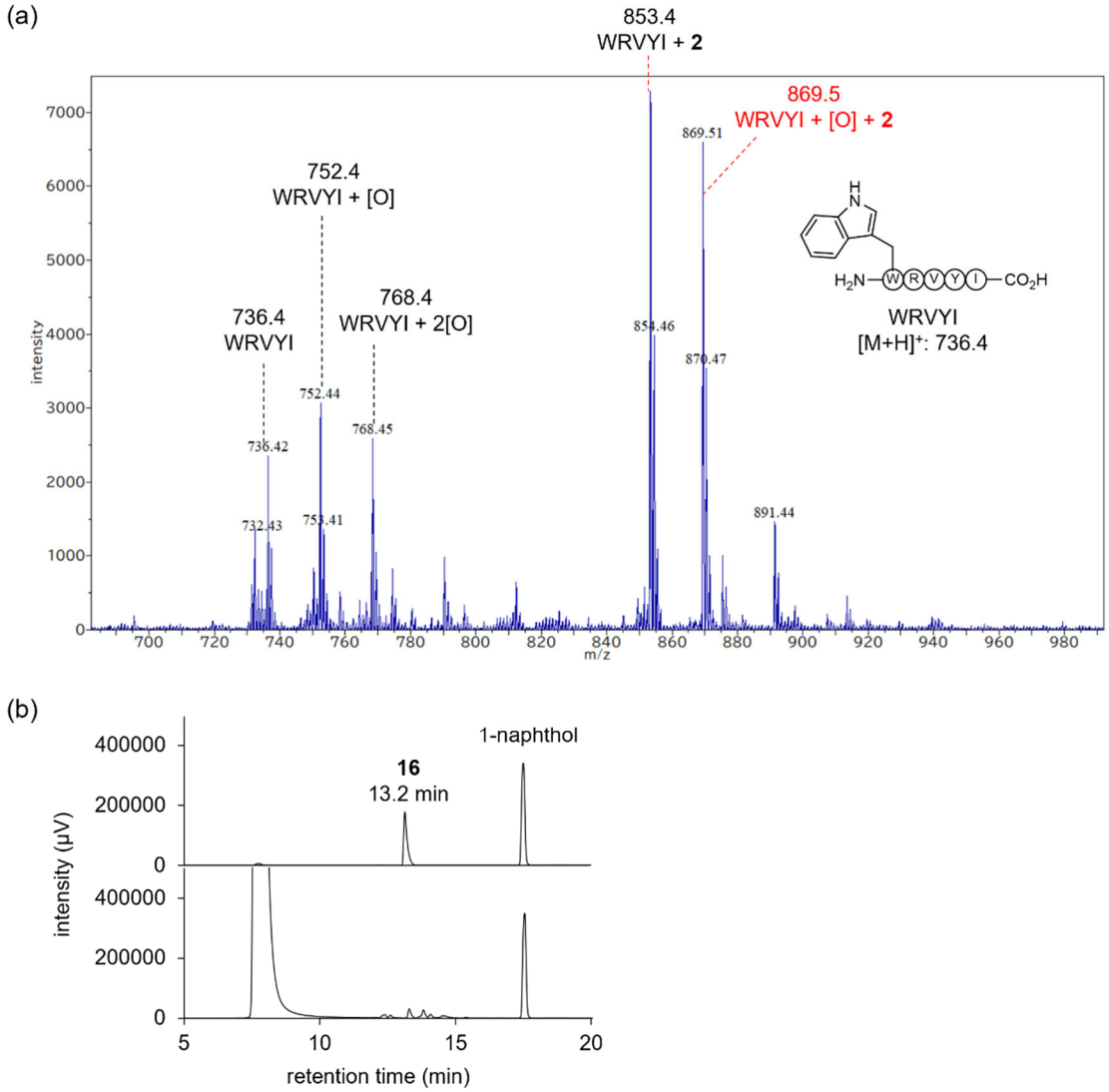


Fig. S21 Tryptophan modification of WRVYI via the C–N coupling with **2**. (a) MALDI-TOF MS spectrum of the crude reaction mixture. (b) RP-HPLC chromatograms of **16** (top) and the reaction mixture (bottom).

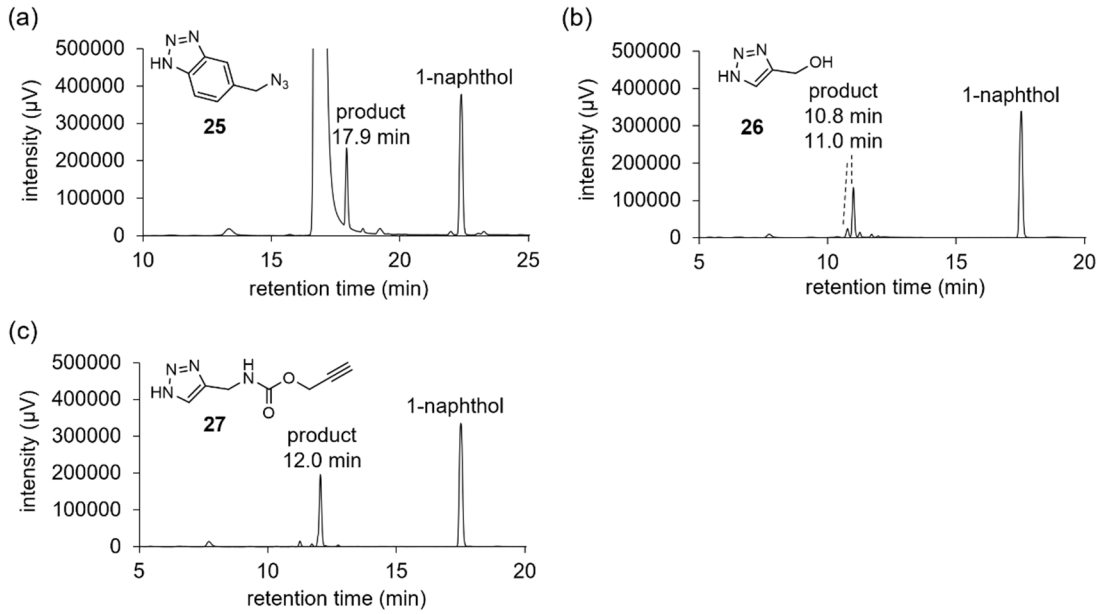


Fig. S22 Tryptophan modification of LHRH (**14**) via the C–N coupling with **25**—**27**. RP-HPLC chromatograms of the crude reaction mixtures with (a) **25**, (b) **26**, and (c) **27.**

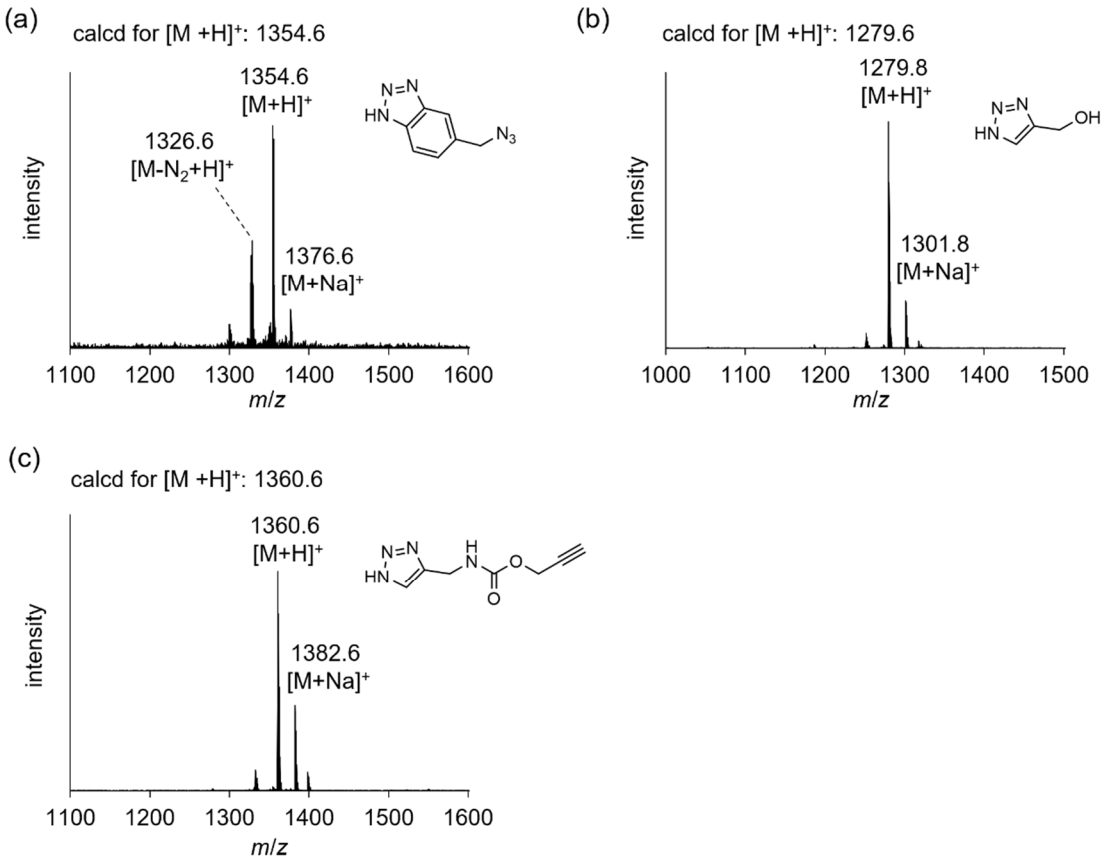


Fig. S23 MALDI-TOF MS spectra of modified peptides with **25**—**27** after RP-HPLC purification. An additional $[M-28+H]^+$ peak derived from N_2 loss from the benzotriazole was observed.[2]

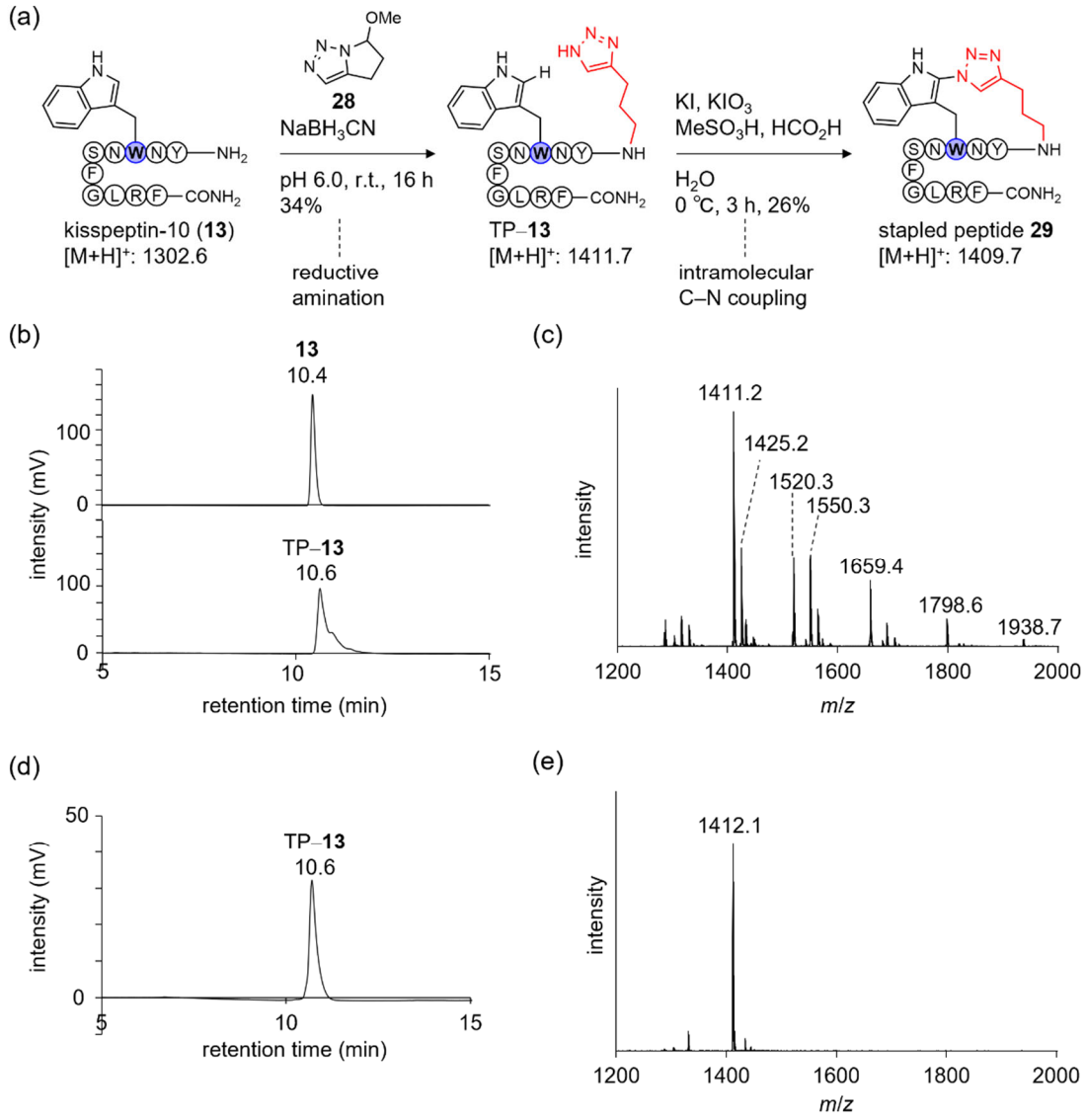


Fig. S24 (a) The two-step reaction scheme for stapling of kisspeptin-10 (**13**). (b) Analytical RP-HPLC chromatograms of kisspeptin-10 (**13**) (top) and the reaction mixture of reductive amination of **13** with **28** and $NaBH_3CN$ (bottom). (c) MALDI-TOF MS spectrum of the crude reaction mixture of the reductive amination. (d) RP-HPLC chromatogram and (e) MALDI-TOF MS spectrum of purified TP–**13**.

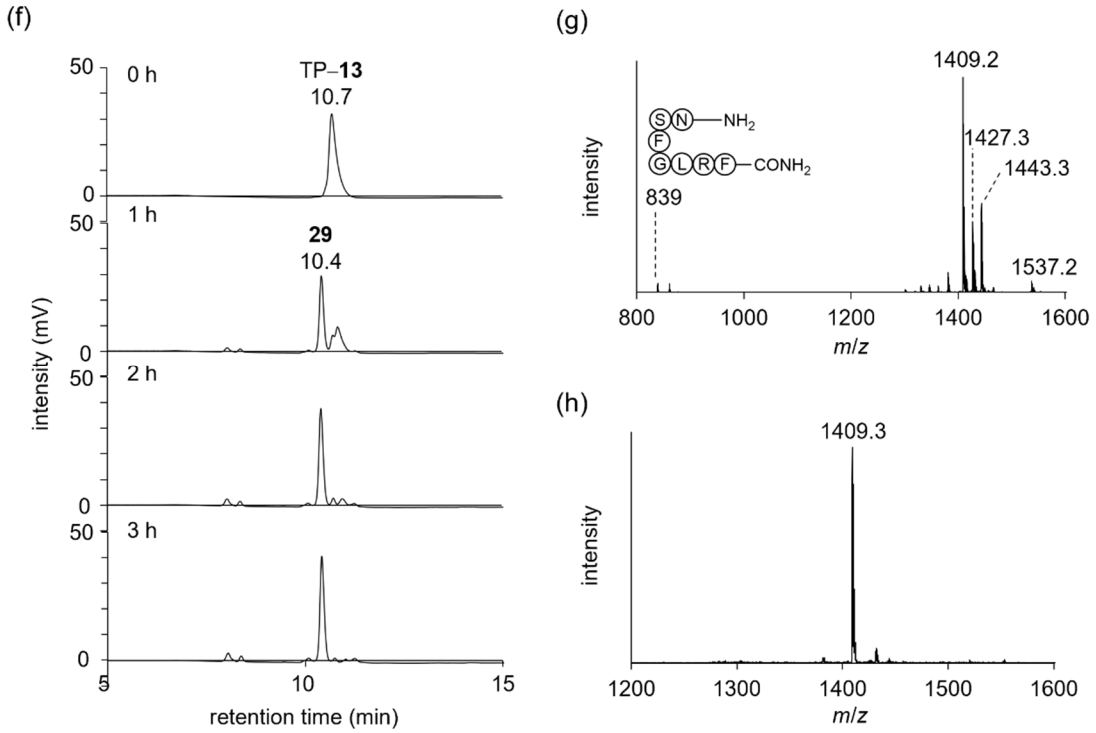


Fig. S24 (continued) (f) RP-HPLC chromatograms of the crude reaction mixture of intramolecular C-N coupling after incubation for a given time period. (g) MALDI-TOF MS spectrum of the crude reaction mixture of intramolecular C-N coupling after incubation for 3 h. A peak at m/z 839 was assigned to the peptide fragment which was formed through cleavage at C-terminal side of tryptophan.[3] Peaks at m/z 1427 (ΔM = +16 amu) and 1443 (ΔM = +32 amu) were assumed to be oxidized TP–**13** (m/z 1411). (h) MALDI-TOF MS spectrum of purified **29**.

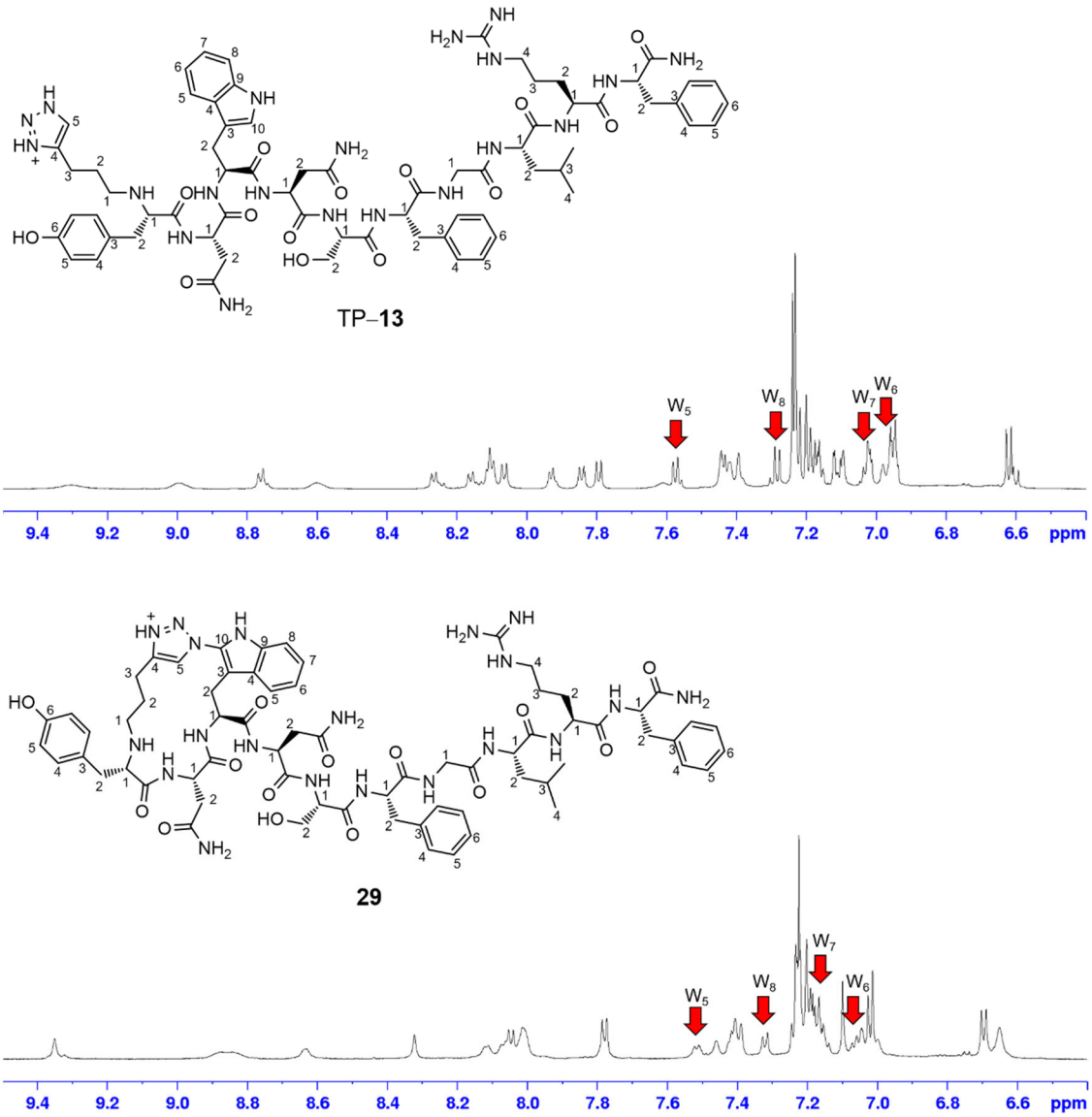


Fig. S25 Aromatic region of ^{1}H NMR spectra of TP–**13** and **29**. The red arrows indicates the signals of the indole rings. Numbers on each signal correspond to numbers on the structures.

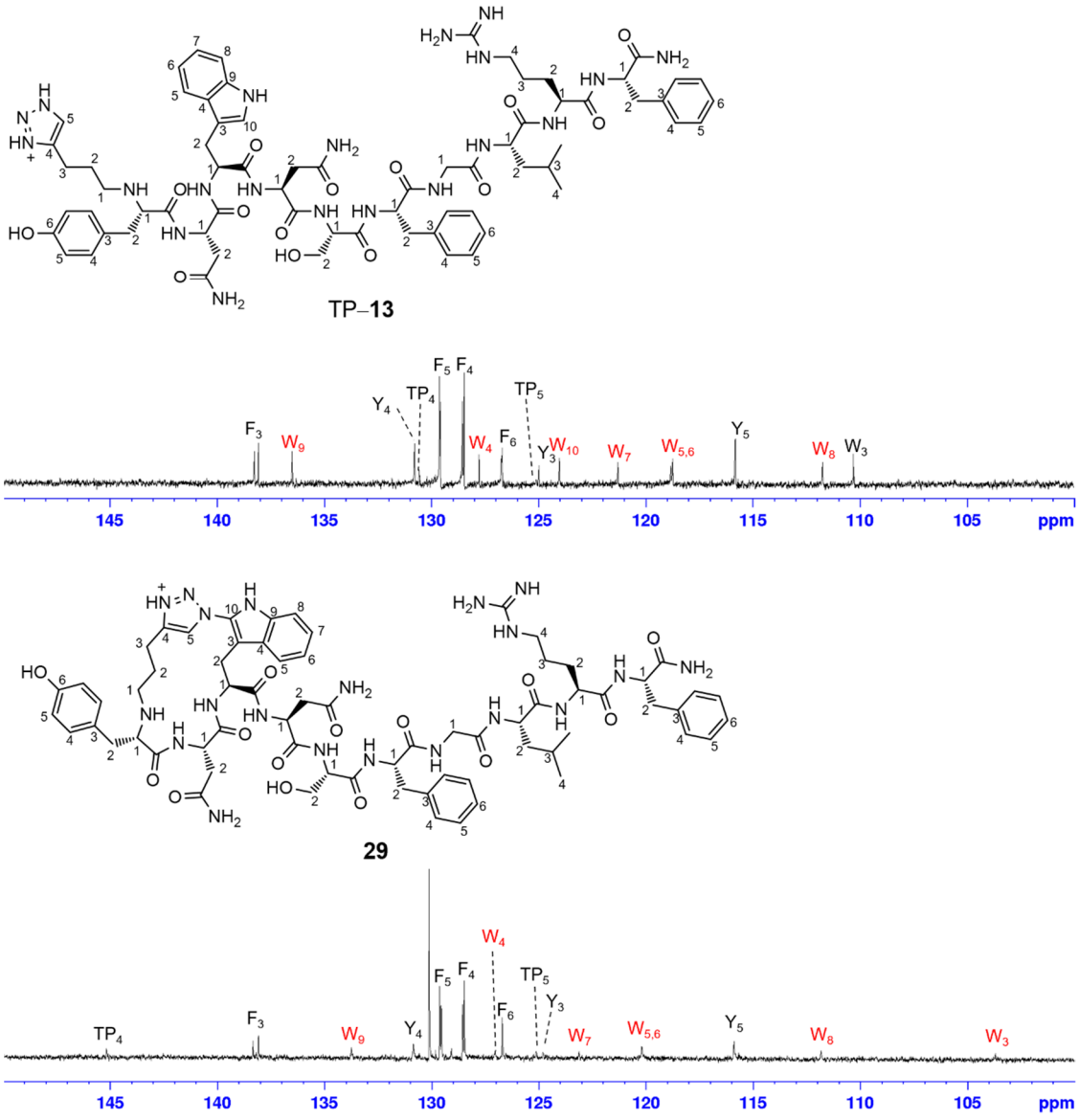


Fig. S26 Aromatic region of ^{13}C NMR spectra of TP–**13** and **29**. The signals of the indole rings in each spectrum were highlighted in red. Numbers on each signal correspond to numbers on the structures.

Table S4 ^{1}H NMR assignment for kisspeptin-10 (**13**), TP–**13**, stapled peptide **29**. N.D.: not determined.

		kisspeptin-10 (**13**)		TP-**13**		stapled peptide **29**	
		^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
TP	1			2.83-2.72	45.7	3.03-2.92	45.3
	2			1.91-1.79	25.5	2.08-2.03	24.9
	3			2.65-2.61	22.0	2.96-2.81	22.3
	4			–	130.6	–	145.2
	5			N.D.	125.1	8.32	125.1
Y1	NH		–	8.99, 8.60	–	8.63	–
	1	3.29-3.26	56.7	3.97-3.93	60.8	3.88	62.7
	2	2.84-2.79, 2.41-2.37	N.D.	2.93-2.79	35.8	3.00-2.91	35.7
	3	–	129.0	–	124.0	–	124.8
	4	6.97 (d, J = 8.5 Hz)	130.6	6.95 (d, J = 8.5 Hz)	130.8	7.02 (d, J = 8.2 Hz)	130.9
	5	6.66 (d, J = 8.5 Hz)	115.5	6.62 (d, J = 8.5 Hz)	115.8	6.70 (d, J = 8.2 Hz)	115.9
	6	–	156.2	–	157.0	–	157.1
N2	NH	8.32	–	8.75	–	8.62	–
	1	4.62-4.58	50.3	4.71-4.65	50.2	4.18	51.6
	2	2.62-2.58, 2.52-2.48	37.6	2.62-2.57, 2.44-2.38	37.6	2.70-2.67, 2.52-2.46	36.3
W3	NH	8.11	–	8.176	–	8.00	–
	1	4.48-4.45	54.2	4.52-4.48	54.2	4.44-4.39	54.3
	2	3.17-3.14, 2.98-2.94	27.7	3.15-3.12, 2.98-2.93	28.0	3.05-3.01, 2.97-2.94	26.7
	3	–	110.3	–	110.3	–	103.7
	4	–	127.8	–	127.8	–	127.1
	5	7.54 (d, J = 7.9 Hz)	118.8	7.57 (d, J = 7.7 Hz)	118.8	7.52 (d, J = 7.8 Hz)	120.3
	6	6.94-6.91	118.7	6.97-6.93	118.7	7.09-7.05	120.3
	7	7.03-7.01	121.2	7.04-7.01	121.3	7.17-7.14	123.1
	8	7.28 (d, J = 8.1 Hz)	111.7	7.28 (d, J = 8.0 Hz)	111.7	7.32 (d, J = 8.0 Hz)	111.9
	9	–	136.5	–	136.5	–	133.8
	10	7.15 (d, J = 2.0 Hz)	124.2	7.12 (d, J = 2.2 Hz)	124.0	–	N.D.
	indole NH	10.79	–	10.75	–	11.95	–
N4	NH	8.19-8.16	–	8.26	–	8.12	–
	1	4.54-4.52	49.7	4.71-4.65	50.2	4.60-4.55	50.1
	2	2.49-2.45	37.8	2.65-2.57, 2.47-2.38	37.9	2.56-2.52, 2.34-2.31	38.1
S5	NH	7.99	–	7.93	–	7.94	–
	1	4.19-4,17	56.3	4.22-4.17	56.1	3.96-3.91	56.3
	2	3.58-3.56, 3.52-3.49	61.8	N.D.	61.8	3.54-3.50, 3.48-3.43	61.6
F6	NH	8.19-8.16	–	8.1	-	8.07	-
	1	4.44-4.39	54.2	4.44-4.40	54.0	4.44-4.40	54.0
	2	3.01-2.97, 2.84-2.79	38.0	3.01-2.96, 2.83-2.79	38.0	3.07-3.01, 2.89-2.85	37.1
	3	–	138.4 or 138.2	–	138.3 or 138.1	-	138.3 or 138.1
	4	7.24-7.22	128.6 or 128.5	7.24-7.20	128.6 or 128.5	7.26-7.15	128.6 or 128.5
	5	7.24-7.20	129.6	7.24-7.17	129.6	7.26-7.15	129.6
	6	7.17-7.14	126.7	7.19-7.15	126.7	7.26-7.15	126.7
G7	NH	8.19-8.16	–	8.11	–	8.01	–
	1	3.77-3.74, 3.70-3.66	42.7	3.72-3.70	42.6	3.67	42.6
L8	NH	7.91	–	7.84	–	7.78	–
	1	4.32-4.28	51.6	4.32-4.28	51.5	4.30-4.24	51.5
	2	1.47-1.39	41.0	1.47-1.36	41.2	1.45-1.35	41.1
	3	1.61-1.56	24.5	1.61-1.55	24.5	1.61-1.51	24.5
	4	0.87 (d, J = 6.7 Hz) 0.82 (d, J = 6.5 Hz)	23.6 22.0	0.86 (d, J = 6.6 Hz) 0.82 (d, J = 6.5 Hz)	23.6 22.0	0.86 (d, J = 6.6 Hz) 0.82 (d, J = 6.5 Hz)	23.6 22.0
R9	NH	8.19-8.16	–	8.06	-	7.41	–
	1	4.20-4.16	52.9	4.22-4.17	52.8	4.19	52.8
	2	1.63-1.58	28.9	1.65-1.60, 1.52-1.47	29.3	1.66-1.47	29.4
	3	1.42-1.34	25.2	1.45-1.35	25.4	1.45-1.35	25.4
	4	3.01-2.97	40.5	3.07-3.01	40.9	3.07-3.03	40.9
F10	NH	7.88	–	7.79	–	7.71	–
	1	4.44-4.39	55.1	4.45-4.41	55.1	4.40-4.35	55.2
	2	3.10-3.06, 2.91-2.87	37.3	3.08-3.04, 2.92-2.87	37.4	3.03-2.99, 2.85-2.79	38.1
	3	–	138.4 or 138.2	-	138.3 or 138.1	-	138.3 or 138.1
	4	7.24-7.22	128.6 or 128.5	7.24-7.20	128.5 or 128.6	7.26-7.15	128.5 or 128.6
	5	7.24-7.20	129.6	7.24-7.17	129.6	7.26-7.15	129.6
	6	7.17-7.14	126.7	7.19-7.15	126.7	7.26-7.15	126.7

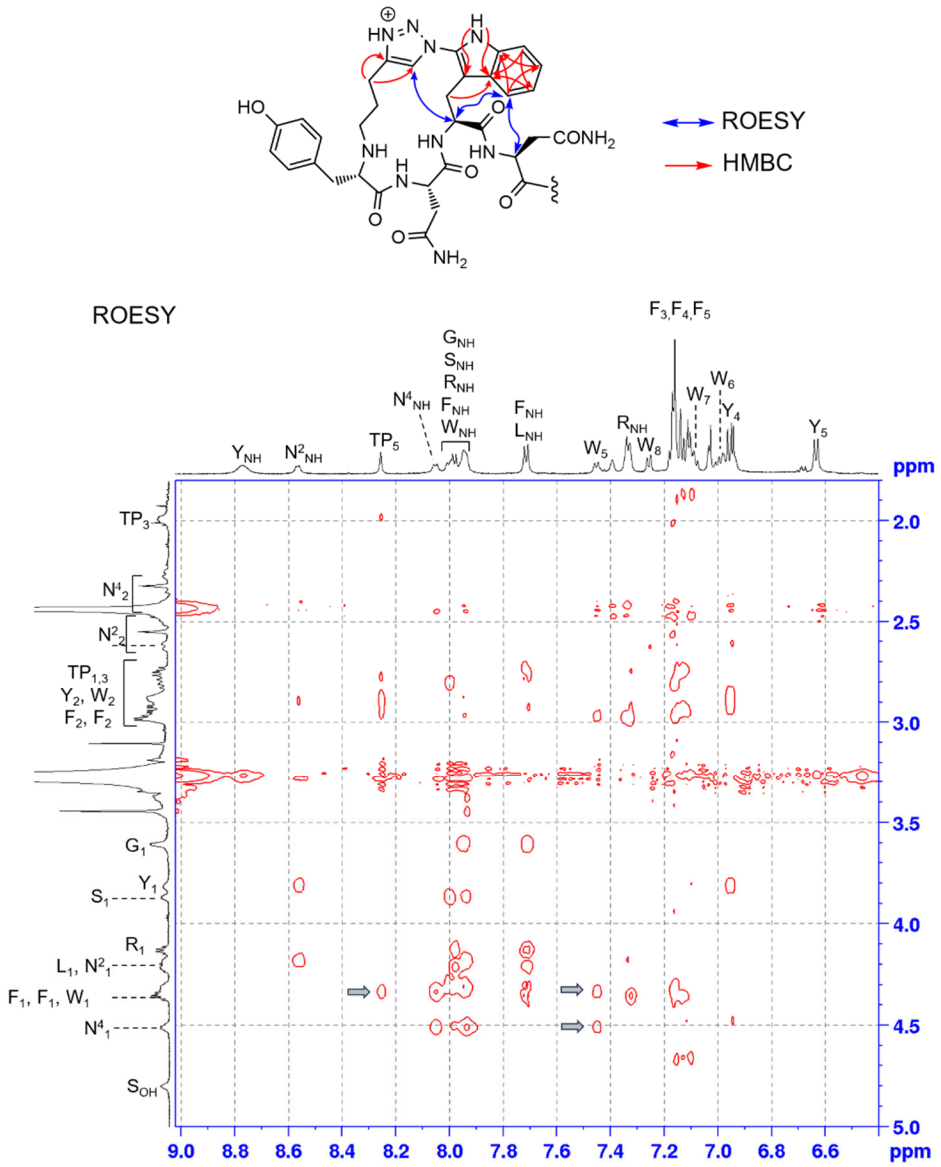
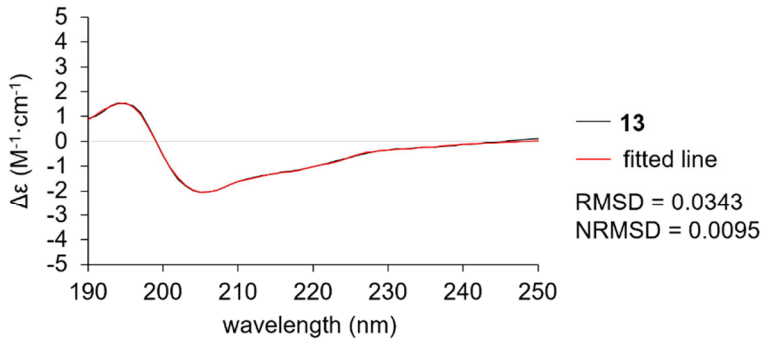


Fig. S27 ROESY experiment data. Gray arrows indicated the correlation suggesting C–N bond formation.

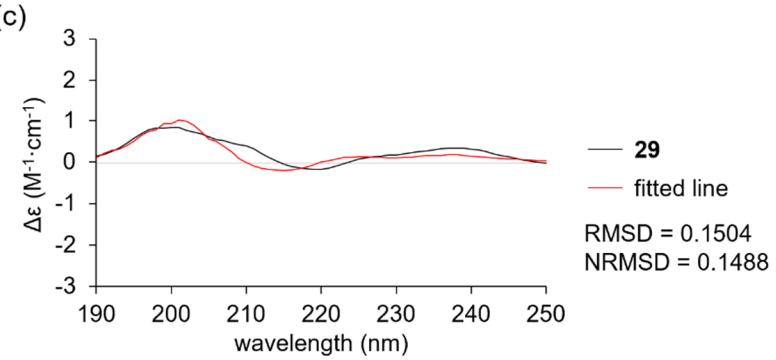
(a)



(b)

Secondary structure	Content (%)
Helix	6.8
Antiparallel	25.8
Parallel	0
Turn	13.5
Others	53.8

(c)



(d)

Secondary structure	Content (%)
Helix	0
Antiparallel	50.5
Parallel	0
Turn	12.9
Others	36.5

Fig. S28 The results of secondary structure analyses by a BeStSel program. (a) (c) Fitting results for **13** and **29**. (b) (d) The tables of sceondary structure contents for **13** and **29**.

Experimental information

1. General information

All chemical reagents and solvents, which were purchased were used without further purification. Neuromedin B (4152-v), bombesin (4086-v), somatostatin (4023-v), luteinizing hormone-releasing hormone (human) (4013-v), α-melanocyte-stimulating hormone (4057-v), and endokinin D (4412-v) were purchased from peptide institute. Kisspeptin-10 (human) was purchased from peptide institute (4389-v) and Selleck chemicals (E2037). WRVYI and YRVYI were synthesized by Fmoc-based solid-phase peptide synthesis. The molar extinction coefficient at 280 nm of each peptide was computed by Expasy ProtParam tool.

Silica gel column chromatography was performed using Wakosil® C-300 (spherical and neutral, 40-64 μm, 233-01677, Fujifilm Wako pure chemical Co.), and the Isolera One flash purification system (Biotage). Preparative TLC was performed with PLC silica gel 60 F254, 0.5 mm (Merck). IR spectra were measured on a Jeol FT-IR SPX60 spectrometer. ^{1}H NMR spectra were measured at 500 MHz on Bruker AVANCE 500 spectrometers or 600 MHz on Bruker AVANCE 600 spectrometers. ^{13}C NMR spectra were measured at 126 MHz on a Bruker AVANCE 500 spectrometers or 151 MHz on a Bruker AVANCE 600 spectrometers. $CDCl_3$, DMSO-d_6, and methanol-d_4 were used as a solvent and the residual solvent peaks were used as an internal standard (^{1}H NMR: $CDCl_3$ 7.26 ppm, methanol-d_4 3.31 ppm, DMSO-d_6 2.50 ppm; ^{13}C NMR: $CDCl_3$ 77.16 ppm, methanol-d_4 49.00 ppm, DMSO-d_6, 39.52 ppm). High resolution mass spectra (HRMS) were recorded on JEOL JMS-T100LP AccuTOF. Reverse-phase high-performance liquid chromatography (RP-HPLC) was performed with CBM-20A, SPD-10A VP, LC-20AD, and CTO-10AS VP (Shimadzu) using CAPCELL PAK C18 MGII (4.6 mm×250 mm, Shiseido), Discovery® BIO Wide Pore C18 (4.6 mm×250 mm, Sigma), or COSMOSIL 5C18-MS-II (10 x 250 mm, Nacalai tesque). The flow rates were 1.0 mL/min for analytical scale and 4.0 mL/min for preparative scale. In every RP-HPLC analysis, a gradient of 0.1% trifluoroacetic acid (TFA) in H_2O (mobile phase A) and 0.1% TFA in acetonitrile (mobile phase B) was employed. Compounds were detected by UV detector at 280 nm. Enantiomeric excesses of compounds were analyzed by HPLC using CBM-20A, SPD-20A, SPD-M20A, LC-20AB, CTO-10AS VP (Shimadzu) and CHIRALPAK® ID-3 (4.6 mm×250 mm, DAICEL) with hexane and 2-propanol as mobile phases. Compounds were detected by UV detector at 254 nm. MALDI-TOF MS analysis was performed on MALDI-8020 (Shimadzu). α-Cyano-4-hydroxycinnamic acid (CHCA) (20 mg/mL solution in 50:50:0.1 H_2O/MeCN/TFA) or 3,5-dimethoxy-4-hydroxycinnamic acid (SA) (20 mg/mL solution in 50:50:0.1 H_2O/MeCN/TFA) was used as a matrix in MALDI-TOF MS analysis. LC-MS and LC-MS/MS analyses were conducted on CBM-20A, SPD-20A, LC-20AD, CTO-20A, SIL-20AC HT, and LCMS-8050 (Shimadzu) with CAPCELL PAK C18 MGII (4.6 mm×250 mm, Shiseido). A gradient of 0.1% formic acid (FA) in H_2O (mobile phase A) and 0.1% FA in acetonitrile (mobile

phase B) was employed. UV-vis spectra were recorded on V-770 UV-VIS-NIR spectrometer (JASCO). CD spectra were recorded on J-1100 (JASCO).

2. Methods for HPLC analyses for analytical scale and preparative scale

Method A:
gradient: 10% B for 3 min, 10–30% B over 1 min, 30% B for 5 min, 30–95% B over 10 min, 95% B for 5 min. column: Discovery® BIO Wide Pore C18 (4.6 mm×250 mm, Sigma), flow rate: 1.0 mL/min temperature: 30 °C.

Method B:
gradient: 20% B for 3 min, 20–95% B over 16 min, 95% B for 5 min, column: CAPCELL PAK C18 MGII (4.6 mm×250 mm, Shiseido), flow rate: 1.0 mL/min, temperature: 30 °C.

Method C (for endokinin D):
gradient: 20% B for 10 min, 20–95% B over 10 min, 95% B for 5 min, column: CAPCELL PAK C18 MGII (4.6 mm×250 mm, Shiseido), flow rate: 1.0 mL/min, temperature: 30 °C.

Method D (for LHRH modified with **25**):
gradient: 20% B for 8 min, 20–95% B over 16 min, 95% B for 5 min, column: CAPCELL PAK C18 MGII (4.6 mm×250 mm, Shiseido), flow rate: 1.0 mL/min, temperature: 30 °C.

Method E (for peptide stapling):
gradient: 20% B for 3 min, 20–95% B over 16 min, 95% B for 5 min, column: CAPCELL PAK C18 MGII (4.6 mm×250 mm, Shiseido), flow rate: 1.0 mL/min, temperature: 30 °C.

Method F (for purification of TP–**13** and stapled peptide **23**):
gradient: 20% B for 8 min, 20–95% B over 16 min, 95% B for 5 min, column: COSMOSIL 5C18-MS-II (10 x 250 mm), flow rate: 4.0 mL/min, temperature: r.t..

Method G (for the analysis of enantiomeric excesses of **1** and **4**)
Isocratic elution: 25% 2-propanol, column: CHIRALPAK ID-3 (4.6 mm×250 mm, DAICEL), flow rate: 1.0 mL/min, temperature: 25 °C.

3. Methods for LC-MS and LC-MS/MS

Method A:
gradient: 20% B for 3 min, 20–95% B over 16 min, 95% B over 5 min, column: CAPCELL

PAK C18 MGII (4.6 mm×250 mm, Shiseido), flow rate: 1.0 mL/min.

Method B:
gradient: 20% B for 10 min, 20–95% B over 25 min, 95% B for 10 min, column: CAPCELL PAK C18 MGII (4.6 mm×250 mm, Shiseido), flow rate: 0.2 mL/min.

4. Experimental procedures

4.1. C–N coupling of Ac-Trp-NHEt (1) and benzotriazole (2) in the presence of Ac-Tyr-NHEt (3)

To a mixture of Ac-Trp-NHEt (**1**) [2 μL, 5 mM in DMSO (final conc.: 0.25 mM)], Ac-Tyr-NHEt (**3**) [2 μL, 5 mM in DMSO (final conc.: 0.25 mM)], benzotriazole (**2**) [4 μL, 250, 500 or 1000 mM in DMSO (final conc.: 25, 50 or 100 mM)], $MeSO_3H$ [4 μL, 10 or 100 mM in H_2O (final conc.: 1 or 10 mM), or 8 μL, 100 mM in H_2O (final conc. 20 mM], and KI [2 μL, 5 mM in H_2O (final conc.: 0.25 mM), or 4 μL, 5 mM in H_2O (final conc.: 0.5 mM)], DMSO and H_2O (required volume to adjust the total volume of the reaction mixture and the DMSO/H_2O ratio to 40 μL and 25/75), was added I_2, NIS [2 μL, 20 mM in DMSO (final conc.: 1 mM)] or KIO_3 [4 μL, 5 mM or 10 mM in H_2O (final conc.: 0.5 or 1 mM)]. The mixture was incubated at given temperature for a given time period. The mixture was quenched with Na_2SO_3 (10 μL, 500 mM in H_2O) and diluted with H_2O (30 μL). The resulting solution (40 μL) was analyzed by RP-HPLC using method A.
Retention times: **1**: 11.9 min; **3**: 7.3 min; **4**: 15.4 min; **S1**: 11.7 min; **S2**: 14.0 min.

Yields of **4**, **S1** and **S2** were determined by comparison of peak area with that of separately prepared authentic samples (see section 6). Conversion of the substrates were defined as (100% – residual rate).

4.2. Determination of the degree of racemization of 4 during the C–N coupling of Ac-Trp-NHEt (1) and benzotriazole (2)

To a mixture of Ac-Trp-NHEt (**1**) [4 μL, 5 mM in DMSO (final conc.: 0.25 mM)], benzotriazole (**2**) [8 μL, 1000 mM in DMSO (final conc.: 100 mM)], $MeSO_3H$ [16 μL, 100 mM in H_2O (final conc. 20 mM], and KI [4 μL, 5 mM in H_2O (final conc.: 0.25 mM)], DMSO (8 μL) and H_2O (32 μL), was added KIO_3 [8 μL, 10 mM in H_2O (final conc.: 1 mM)]. The mixture was incubated in an ice bath for 1 h. The mixture was quenched with Na_2SO_3 (20 μL, 500 mM in H_2O) and diluted with H_2O (160 μL). The organic materials were extracted with AcOEt (200 μL and 100 μL×2). The combined organic layers were evaporated and dissolved in 2-propanol (50 μL). The resulting solution (20 μL) was analyzed by HPLC using method G.
Retention times: L-**1**: 16.2 min, D-**1**: 7.9 min, L-**4**: 34.1 min, D-**4**: 13.6 min.
The enantiomeric excesses of L-**1** and **4** were calculated by comparison of the peak areas of L-

and D-isomers.

4.3. C–N coupling of Ac-Trp-NHEt (1) and benzotriazole (2) in the presence of Ac-Tyr-NHEt (3) with I_2 or NIS under buffered condition

To an ice-cold mixture of Ac-Trp-NHEt (**1**) [2 μL, 5 mM in DMSO (final conc.: 0.25 mM)], Ac-Tyr-NHEt (**3**) [2 μL, 5 mM in DMSO (final conc.: 0.25 mM)], benzotriazole (**2**) [4 μL, 1 M in DMSO (final conc.: 100 mM)], H_2O (10 μL) and citrate buffer (20 μL, 100 mM in H_2O, a given pH) was added I_2 or NIS [2 μL, 20 mM in DMSO (final conc.: 1 mM)]. The reaction mixture was incubated in an ice bath for 1 h. Work-up and RP-HPLC analysis were performed in the same manner as section 4.1.

4.4. C–N coupling of Ac-Trp-OCH_3 (5) and azoles with KI and KIO_3

To an ice-cold mixture of Ac-Trp-OMe (**5**) [2 μL, 5 mM in DMSO (final conc.: 0.25 mM)], azoles [4 μL, 1 M in DMSO or H_2O (final conc.: 100 mM)], $MeSO_3H$ [8 μL, 100 mM in H_2O (final conc.: 20 mM)], KI [2 μL, 5 mM in H_2O (final conc.: 0.25 mM), or 4 μL, 5 mM in H_2O (final conc.: 0.5 mM)], DMSO and H_2O (required volume to adjust the total volume of the reaction mixture and the DMSO/H_2O ratio to 40 μL and 25/75), was added KIO_3 [4 μL, 10 or 20 mM in H_2O (final conc.: 1 or 2 mM)]. The reaction mixture was incubated in an ice bath for 1 h and was quenched with Na_2SO_3 (10 μL, 500 mM in H_2O) and diluted with H_2O (30 μL). The resulting solution (40 μL) was analyzed by RP-HPLC using method B.

Retention times: **5**: 14.1 min; **6**: 16.1 min; **7**: 13.3 min; **8**: 15.4 min; **9**: 13.5 min.

Yields of **6**, **7**, and **8** were determined by comparison of the peak areas with those of separately prepared authentic samples (see section 6). Conversion of the substrates were defined as (100% – residual rate).

4.5. Structural confirmation of 7 by ^{1}H NMR

To an ice-cold mixture of Ac-Trp-OMe (**5**) (3.0 mg, 0.012 mmol), 1,2,3-triazole (232 μL, 4.00 mmol), $MeSO_3H$ (52 μL, 0.80 mmol), and KI (3.5 mg, 0.021 mmol) in DMSO (2 mL) and H_2O (38 mL), was added KIO_3 (17 mg, 0.079 mmol). The reaction mixture was incubated in an ice bath for 1 h and was quenched with Na_2SO_3 (630 mg). The organic materials were extracted with $CHCl_3$ (10 mL×3). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated. The resulting residue (4 mg) was analyzed by ^{1}H NMR in $CDCl_3$.

4.6. X-ray crystallographic analysis of *N*-acetyl-2-(*1H*-1,2,3-triazol-1-yl)-L-tryptophan methyl ester (7)

The analysis of **7** was performed on a on a Rigaku Synergy-R/DWTI APEX II instrument with a

Hypix-6000HE detector (Cu-*K*α, λ = 1.514184 Å, T = 123.15K). The structures were solved by Dual space methods (SHELXT-2018) and refined by full-matrix least squares calculations on F^2 (SHELXL-2018) using the SHELX-TL program package. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were created with ideal geometry and refined using a riding model.

Crystallographic data have been deposited with Cambridge Crystallographic Data Centre: Deposition number CCDC-2298364. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk). The single crystal of **7** was prepared by a vapor diffusion method. *N*-Acetyl-2-(*1H*-1,2,3-triazol-1-yl)-L-tryptophan methyl ester (**7**) was dissolved in AcOEt. The solution in a micro vial was put into a vial filled with hexane and was stayed overnight. Crystal data of **7**: $C_{16}H_{17}N_5O_3$, colorless, 0.72 × 0.52 × 0.15 mm^3, monoclinic, space group $P2_1$ (No. 4), a = 8.78319(6), b = 10.61767(7), c = 9.51916(6) Å, α = 90°, β = 114.9580(8)°, γ = 90°, V = 804.830(11) $Å^3$, ρ_{calcd} = 1.351 g/cm^3 , Z = 2, 3159 unique reflections (R_{int} = 0.0278, R_{sigma} = 0.0139) out of 3170 with $I > 2\sigma(I)$ reflections measured, R_1 = 0.0231, and wR_2 =0.0640 [I > 2σ(I)], R_1 = 0.0240, and wR_2 = 0.0674 for all data, GOF = 1.051, and flack parameter = 0.001(6). When the structure was solved as the N2-coupling adduct, the R factor increased from 2.66 % to 3.89 %, supporting the structure assignment. Furthermore, the crystal structure of *N*-acetyl-2-(*1H*-1,2,3-triazol-1-yl)-L-tryptophan methyl ester with difference electron density map (0.2 e$Å^{-3}$ level) confirmed the synthesis of N1 coupling adduct (Fig.S7).

4.7. General procedure for modification of peptides through C–N coupling with benzotriazole (2)

To an ice-cold mixture of peptides [2 μL, 2.5 mM in H_2O (final conc.: 0.25 mM)], benzotriazole [2 μL, 1 M in DMSO (final conc.: 100 mM)], $MeSO_3H$ [4 μL, 100 mM in H_2O (final conc.: 20 mM)], HCO_2H [2 μL, 200 mM in H_2O (final conc.: 20 mM)], KI [1 μL, 5 mM in H_2O (final conc.: 0.25 mM), or 2 μL, 5 mM in H_2O (final conc.: 0.5 mM)], and H_2O (6 or 7 μL, required volume to adjust the total volume of the reaction mixture to 20 μL), was added KIO_3 [2 μL, 10 or 20 mM in H_2O (final conc.: 1 or 2 mM)]. The reaction mixture was incubated in an ice bath for 1 h, quenched with Na_2SO_3 (5 μL, 500 mM in H_2O), and diluted with H_2O (3 μL). Only for somatostatin, sodium ascorbate (8 μL, 100 mM in H_2O) was used instead of Na_2SO_3 to prevent from reduction of the disulfide bond. To the mixture was added 1-naphthol (2 μL, 10 mM in DMSO). The resulting solution (20 μL) was analyzed by RP-HPLC using method B except for endokinin D (method C). After separation by RP-HPLC, each product was identified by MALDI-TOF MS and LC-MS/MS analyses. For LC-MS/MS analysis of modified somatostatin, reaction mixture was quenched with

Na_2SO_3 (5 μL, 500 mM in H_2O) and was incubated overnight at room temperature before the analysis (method A).

4.8. Modification of LHRH through C–N coupling with azoles

benzotriazole **25**:

To a cold mixture of LHRH [2 μL, 2.5 mM in H_2O (final conc.: 0.25 mM)], **25** [2 μL, 1 M in DMSO (final conc.: 100 mM)], $MeSO_3H$ [4 μL, 100 mM in H_2O (final conc.: 20 mM)], HCO_2H [2 μL, 200 mM in H_2O (final conc.: 20 mM)], KI [2 μL, 5 mM in H_2O (final conc.: 0.5 mM)], DMSO (3 μL) and H_2O (3 μL), was added KIO_3 (2 μL, 20 mM in H_2O). The mixture was incubated at 10 ℃ in a cryostat (UC reactor, Techno Sigma) for 1 h. Work-up and RP-HPLC and MALDI-TOF MS analysis were performed in a similar manner to section 4.7. using RP-HPLC method D instead of method B.

1,2,3-triazole derivatives **26** and **27**:

To an ice-cold mixture of LHRH [2 μL, 2.5 mM in H_2O (final conc.: 0.25 mM)], triazoles [8 μL, 250 mM in H_2O (final conc.: 100 mM)], $MeSO_3H$ [4 μL, 100 mM in H_2O (final conc.: 20 mM)], HCO_2H [2 μL, 200 mM in H_2O (final conc.: 20 mM)] and KI [2 μL, 5 mM in H_2O (final conc.: 0.5 mM)], was added KIO_3 (2 μL, 20 mM in H_2O). The mixture was incubated in an ice bath for 2 h. Work-up and RP-HPLC and MALDI-TOF MS analyses were performed in the same manner as section 4.7.

4.9. Analytical-scale preparation of stapled peptide 29 via the intramolecular C–N coupling for analytical scale

Kisspeptin-10 (**13**) (200 μL, 2.3 mM in H_2O) was incubated with **28** (100 μL, 50 mM in DMSO) and $NaBH_3CN$ (100 μL, 125 mM in 20 mM citrate buffer at pH 6.0) in citrate buffer (2100 μL, 20 mM, pH 6.0) for 16 h at rt. The reaction was quenched with TFA (100 μL). The resulting solution was purified by preparative RP-HPLC (Method F). The fraction at 10.4 min was collected and lyophilized to obtain TP–**13** as a colorless powder. The powder was dissolved in H_2O (100 μL) and analyzed by analytical RP-HPLC and MALDI-TOF MS. The concetration of TP–**13** was estimated to be 0.78 mM based on UV absorbace at 280 nm. The yield of this step was 34%.

To a cold mixture of TP–**13** (200 μL, 0.78 mM in H_2O), $MeSO_3H$ (400 μL, 100 mM in H_2O), HCO_2H (200 μL, 200 mM in H_2O), KI (200 μL, 5 mM in H_2O), and H_2O (800 μL), was added KIO_3 (200 μL, 20 mM in H_2O). The mixture was incubated in an ice bath for 3 h. The reaction was quenched with sodium ascorbate (200 μL, 100 mM in H_2O). The resulting solution was purified by preparative RP-HPLC (Method F). The fraction at 13.4 min was collected and lyophilized to obtain **29** as a colorless powder. The powder was dissolved in a 1:1 mixture of H_2O and DMSO (200 μL) and

analyzed by analytical RP-HPLC and MALDI-TOF MS. The concetration of **29** was estimated to be 0.20 mM based on UV absorbace at 280 nm. The yield of this step was 26%.

4.10. Scale-up preparation of stapled peptide 29 for NMR experiments

Kisspeptin-10 (**13**) (5.2 mg) was incubated with **28** (0.8 mL, 50 mM in DMSO) and $NaBH_3CN$ (0.8 mL, 125 mM in 20 mM citrate buffer at pH 6.0) in citrate buffer (18.4 mL, 20 mM, pH 6.0) for 16 h at rt. The reaction was quenched with TFA (0.8 mL). The resulting solution was purified by preparative RP-HPLC (Method F). The fraction at 10.4 min was collected and lyophilized to obtain TP–**13** as a colorless powder (5.0 mg). The powder was dissolved in DMSO-d_6 (0.7 mL) and analyzed by NMR experiments.

To a cold mixture of a solution of TP–**13** in DMSO (0.7 mL), $MeSO_3H$ (3.2 mL, 100 mM in H_2O), HCO_2H (1.6 mL, 200 mM in H_2O), KI (1.6 mL, 5 mM in H_2O), and H_2O (6.4 mL), was added KIO_3 (1.6 mL, 20 mM in H_2O). The mixture was incubated in an ice bath for 3 h. The reaction was quenched with sodium ascorbate (1.6 mL, 100 mM in H_2O). The resulting solution was purified by preparative RP-HPLC (Method F). The fraction at 13.4 min was collected and lyophilized to obtain **23** as a colorless powder (2.4 mg). The powder was dissolved in DMSO-d_6 (0.7 mL) and analyzed by NMR experiments.

4.11. CD spectroscopy of peptides

Kisspeptin-10 (**13**) (15 μL, 2.3 mM in H_2O) was dissolved in trifluoroethanol (TFE) (285 μL). A solution of **29** in DMSO-d_6 (50 μL, 1.2 mM) was evaporated, and the resulting solids were dissoleved in H_2O (15 μL) and TFE (285 μL). CD spectra of **13** and **29** were recorded at room temperature using 0.1 cm cuvette.

5. Method for quantification of modified peptides

Yields of modified peptides were estimated by using peak areas on RP-HPLC chromatograms and molar extinction coefficients of modified peptides. A molar extinction coefficient of an original peptide (sub.) at 280 nm (ε_{280}) can be calculated as eq. 1.[4]

ε_{280}(sub.) = 5500×(number of Trp) + 1490×(number of Tyr) + 125×(number of disulfide bond) (eq. 1)

Considering molar extinction coefficients of Ac-Trp-OMe (**5**), Ac-2-(benzotriazol-1-yl)-Trp-OMe (**6**), and Ac-2-(triazol-1-yl)-Trp-OMe (**7**) at 280 nm (Table S5), molar extinction coefficients of modified Trp residues in peptides were calculated to be 10010 for a benzotriazole adduct and 8855 for a triazole adduct, respectively. Thus, molar extinction coefficients of

peptides modified with benzotriazole and triazole can be described as eq. 2 and eq. 3.
For a benzotriazole-modified peptide
ε_{280}(benzotriazole) = 10010×(Trp) + 1490×(Tyr) + 125×(disulfide bond) (eq. 2),
and for a triazole-modified peptide
ε_{280}(triazole) = 8855×(Trp) + 1490×(Tyr) + 125×(disulfide bond) (eq. 3)

Table S5 Molar extinction coefficients of **5**, **6**, and **7** at 280 nm

compound	ε_{280} ($M^{-1}cm^{-1}$)	ε_{280}(**6** or **7**) / ε_{280}(**5**)
5	5.82×10^3	-
6	1.06×10^4	1.82
7	9.36×10^3	1.61

When the Trp modification proceeds quantitatively, the peak area (S_{max}) of the modified peptide can be calculated by using the initial peak area of the original peptide (S_0).
$S_{max} = \varepsilon_{280}$(benzotriazole or triazole)/ε_{280}(sub.) ×S_0

The yield of the modified peptide was calculated from the observed peak area (S) on RP-HPLC chromatograms of a reaction mixture.
yields of the modified peptide = S/S_{max}×100

Error of the amount of injection was corrected using 1-naphthol as an internal standard.

6. Preparation of amino acid derivatives as authentic samples for RP-HPLC analyses

N-Acetyl-L-tryptophan ethylamide (1)

Ac-Trp-OEt (841 mg, 3.06 mmol) was dissolved in 70% aqueous ethylamine solution (6 mL). The mixture was stirred for 4 d at room temperature. The reaction mixture was diluted with water (40 mL). The organic materials were extracted with EtOAc (40 mL×3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (40 mL) and brine (40 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was washed with water and dried in *vacuo* to afford **1** (640 mg, 76% yield) as colorless solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.94 (dd, J = 7.2, 7.2 Hz, 3H), 1.78 (s, 3H), 2.87 (dd, J = 8.6, 14.5 Hz, 1H), 3.01-3.07 (m, 3H), 4.44 (ddd, J = 5.7, 8.4, 8.4 Hz, 1H), 6.95-6.98 (m, 1H), 7.03-7.06 (m, 1H), 7.10 (d, J = 2.1 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 7.9 Hz, 1H), 7.91 (dd, J =5.4, 5.5 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 10.77 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 14.6, 22.6, 28.0, 33.4, 53.4, 110.3, 111.2, 118.1, 118.5, 120.8, 123.4, 127.3, 136.0, 168.9, 171.2. IR (neat, cm^{-1}): 3402, 3294, 3102, 2974, 1631, 1539, 1454, 1374, 1287, 1247, 1151, 1092, 1011, 946,

892, 813, 739, 716, 650, 602, 553, 506. HRMS (ESI): calcd for $C_{15}H_{19}N_3NaO_2$ $[M+Na]^+$ 296.1375, found 296.1372.

_N_-Acetyl-L-tyrosine ethylamide (2)

N-Acetyl tyrosine ethyl ester monohydrate (137 mg, 0.509 mmol) was dissolved in 70% aqueous ethylamine solution (1 mL). The mixture was stirred for 4 d at room temperature. The reaction mixture was acidified with aqueous hydrochloric acid solution (4 M, 10 mL) and saturated with NaCl. The organic materials were extracted with EtOAc (20 mL×2 and 10 mL). The combined organic layers were dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was washed with diethyl ether and dried in air to afford **2** (93 mg, 73% yield) as colorless solids. ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.92 (dd, *J* = 7.2, 7.2 Hz, 3H), 1.76 (s, 3H), 2.60 (dd, *J* = 9.1, 13.7 Hz, 1H), 2.79 (dd, *J* = 5.5, 13.7 Hz, 1H), 2.99-3.07 (m, 2H), 4.31 (ddd, *J* = 5.5, 8.7, 8.9 Hz, 1H), 6.62 (d, *J* = 8.5 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 7.88 (dd, *J* = 5.1, 5.3 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 9.14 (d, *J* = 1.1 Hz, 1H). These values were in good agreement with those reported in the literature.[5]

_N_-Acetyl-2-(1H-benzo[d][1,2,3]triazol-1-yl)-L-tryptophan ethylamide (4)

To a mixture of Ac-Trp-NHEt (**1**) (55 mg, 0.20 mmol) and benzotriazole (60 mg, 0.50 mmol) in MeOH (800 μL) was added *N*-iodosuccinimide (90 mg, 0.40 mmol). The mixture was stirred in an ice bath for 1 h. The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ solution (2 mL) and diluted with water (5 mL). The organic materials were extracted with EtOAc (30 mL and 20 mL). The combined organic layers were washed with 2 M aqueous Na_2CO_3 solution (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with Isolera One flash purification system (hexane/ EtOAc, 75–100%) to afford **4** (39 mg, 49% yield) as colorless solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (dd, *J* = 7.3, 7.3 Hz, 3H), 1.90 (s, 3H), 3.04-3.12 (m, 3H), 3.17 (dd, *J* = 5.4, 14.8 Hz, 1H), 4.58-4.62 (m, 1H), 6.27 (dd, *J* = 5.3, 5.4 Hz, 1H), 7.18 (d, *J* = 6.6 Hz, 1H), 7.22-7.25 (m, 1H), 7.31-7.34 (m, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.47 (ddd, *J* = 2.5, 5.4, 8.1 Hz, 1H), 7.57-7.58 (m, 2H), 7.79 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 9.06 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 14.6, 23.1, 26.0, 34.5, 53.6, 107.4, 110.3, 111.8, 120.2, 120.6, 121.3, 124.5, 125.4, 127.0, 127.1, 129.5, 133.8, 134.4, 145.6, 170.9, 171.2. IR (neat, cm^{-1}): 3199, 1739, 1658, 1630, 1548, 1530, 1501, 1469, 1440, 1371, 1342, 1280, 1211, 1048, 1008, 742. HRMS (ESI): calcd for $C_{21}H_{22}N_6NaO_2$ $[M+Na]^+$ 413.1701, found 413.1701.

_N_-Acetyl-3-iodo-L-tyrosine ethylamide (S1)

To a solution of Ac-Tyr-NHEt (**3**) (44 mg, 0.20 mmol) and *p*-toluenesulfonic acid monohydrate (37 mg, 0.19 mmol) in MeCN (4 mL), was added *N*-iodosuccinimide (45 mg 0.10 mmol). The mixture was stirred at room temperature for 3 h. To the reaction mixture was added Na_2SO_3 (38 mg) and concentrated in *vacuo*. The resulting residue was purified with Isolera One flash purification system (EtOAc/ acetone, 0–50%) to afford **S1** (48 mg, 64% yield) as colorless solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.96 (dd, J = 7.2, 7.2 Hz, 3H), 1.76 (s, 3H), 2.57 (dd, J = 9.2, 13.6 Hz, 1H), 2.77 (dd, J = 5.4, 13.6 Hz, 1H), 2.97-3.10 (m, 2H), 4.31 (ddd, J = 5.4, 8.7, 8.9 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 7.02 (dd, J = 2.1, 8.2 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.93 (dd, J = 5.5, 5.6 Hz, 1H), 8.05 (d, J = 8.5 Hz, 1H), 10.07 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 14.6, 22.5, 33.3, 36.6, 54.3, 84.1, 114.5, 130.3, 130.6, 139.1, 155.0, 168.9, 170.7. IR (neat, cm^{-1}): 3248, 3068, 2976, 1643, 1536, 1502, 1415, 1367, 1274, 1225, 1146, 1037, 960, 892, 824, 787, 741, 708, 663, 649, 609, 582, 533. HRMS (ESI): calcd for $C_{13}H_{17}IN_2NaO_3$ $[M+Na]^+$ 399.0182, found 399.0175.

***N*-Acetyl-3,5-diiodo-L-tyrosine ethylamide (S2)**

To a solution of Ac-Tyr-NHEt (**3**) (45 mg, 0.20 mmol) and $Na_2HPO_4 \cdot 12H_2O$ (210 mg, 0.609 mmol) in MeOH (1 mL) and H_2O (1 mL) was added I_2 (126 mg 0.512 mmol). The mixture was stirred overnight at room temperature. The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ solution (8 mL) and acidified with 4 M aqueous HCl solution (2 mL) to pH ~4. The organic materials were extracted with EtOAc (40 mL×2). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with flush silica gel column chromatography (CH_2Cl_2/MeOH, 19:1 to 9:1) to afford **S2** (66 mg, 65% yield) as colorless solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.97 (dd, J = 7.2, 7.2 Hz, 3H), 1.77 (s, 3H), 2.56 (dd, J = 9.3, 13.6 Hz, 1H), 2.76 (dd, J = 5.3, 13.6 Hz, 1H), 2.98-3.10 (m, 2H), 4.31 (ddd, J = 5.3, 8.8, 9.0 Hz, 1H), 7.59 (s, 2H), 7.97 (dd, J = 5.5, 5.5 Hz, 1H), 7.88 (dd, J = 5.1, 5.3 Hz, 1H), 8.05 (d, J = 8.6 Hz, 1H), 9.34 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ14.7, 22.4, 33.3, 36.0, 54.0, 84.8, 134.2, 139.6, 153.6, 168.9, 170.4. IR (neat, cm^{-1}): 3440, 3266, 3062, 2929, 1644, 1532, 1454, 1401, 1371, 1309, 1275, 1257, 1233, 1185, 1146, 1037, 950, 903, 869, 789, 727, 706, 692, 596. HRMS (ESI): calcd for $C_{13}H_{16}I_2N_2NaO_3$ $[M+Na]^+$ 524.9148, found 524.9143.

***N*-Acetyl-L-tryptophan methyl ester (5)**

To a mixture of L-tryptophan methyl ester hydrochloride (2.53 g, 9.93 mmol) in THF (50 mL), were added Ac_2O (1.1 mL, 12 mmol) and triethylamine (2.8 mL, 20 mmol) in an ice bath. The mixture was stirred overnight at room temperature. The reaction mixture was diluted with H_2O (50 mL). The organic materials were extracted with EtOAc (50 mL×2). The combined organic

layers were washed with 2 M aqueous HCl solution (40 mL), 2 M aqueous $NaHCO_3$ solution (40 mL) and brine (40 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was washed with diethyl ether to afford **5** (2.07 g, 80% yield) as colorless solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.96 (s, 3H), 3.28-3.37 (m, 2H), 3.70 (s, 3H), 4.96 (ddd, *J* = 5.3, 5.3, 7.9 Hz, 1H), 5.97 (d, *J* = 7.3 Hz, 1H), 6.98 (d, *J* = 2.4 Hz, 1H), 7.11-7.14 (m, 1H), 7.18-7.22 (m, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 8.10 (s, 1H). These values were in good agreement with those reported in the literature.[6]

***N*-Acetyl-2-(1H-benzo[d][1,2,3]triazol-1-yl)-L-tryptophan methyl ester (6)**

To a mixture of Ac-Trp-OMe (**5**) (52 mg, 0.20 mmol), I_2 (12 mg, 0.047 mmol) and benzotriazole (60 mg, 0.50 mmol) in a test tube were added MeOH (400 μL) and H_2O (400 μL). The mixture was stirred for 5 min at room temperature. Then, 70% aqueous TBHP solution (56 μL, 0.40 mmol) was added to the mixture and the mixture was stirred for 24 h at 40 ℃ in ChemiStation™ (EYELA). The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ solution (2 mL) and diluted with H_2O (5 mL). The organic materials were extracted with EtOAc (30 mL and 20 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with Isolera One flash purification system (hexane/EtOAc, 50–100%) to afford **6** (58 mg, 74% yield) as colorless solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.86 (s, 3H), 3.22 (dd, *J* = 6.9, 14.8 Hz, 1H), 3.32 (dd, *J* = 4.9, 14.8 Hz, 1H), 3.51 (s, 3H), 4.80 (ddd, *J* = 5.0, 6.9, 6.9 Hz, 1H), 6.62 (d, *J* = 7.2 Hz, 1H), 7.26-7.30 (m, 1H), 7.36-7.39 (m, 1H), 7.45 (d, *J* = 7.1 Hz, 1H), 7.49-7.54 (m, 2H), 7.60-7.63 (m, 1H),7.74 (d, *J* = 7.9 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 8.47 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 23.0, 26.1, 52.6, 52.7, 106.6, 110.1, 111.8, 119.9, 120.6, 121.3, 124.6, 125.3, 127.1, 127.2, 129.4, 133.8, 134.4, 145.6, 170.3, 172.0. IR (neat, cm^{-1}): 3199, 1739, 1658, 1530, 1501, 1469, 1440, 1371, 1342, 1280, 1211, 1048, 1008, 742, 649, 635, 625. HRMS (ESI): calcd for $C_{20}H_{19}N_5NaO_3$ $[M+Na]^+$ 400.1386, found 400.1382.

***N*-Acetyl-2-(1*H*-1,2,3-triazol-1-yl)-L-tryptophan methyl ester (7)**

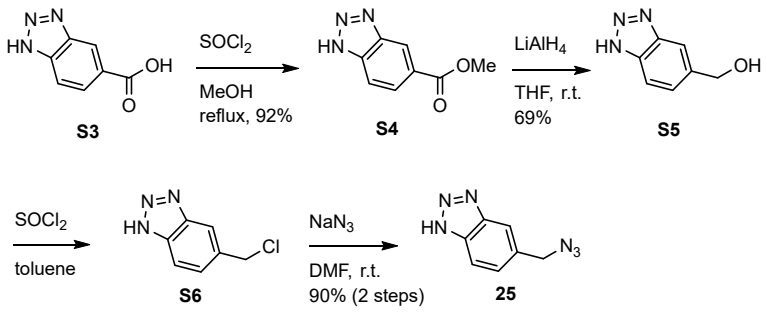
To a mixture of Ac-Trp-OMe (**1**) (52 mg, 0.20 mmol), I_2 (12 mg, 0.047 mmol) and 1,2,3-triazole (30 μL, 0.52 mmol) in a test tube were added MeOH (400 μL) and H_2O (400 μL). The mixture was stirred for 5 min at room temperature. Then, 70% aqueous TBHP solution (56 μL, 0.40 mmol) was added to the mixture and the mixture was stirred for 24 h at 40 ℃ in ChemiStation™ (EYELA). The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ solution (2 mL) and diluted with H_2O (5 mL). The organic materials were extracted with EtOAc (30 mL and 20 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with Isolera

One flash purification system (hexane/ EtOAc, 50–100%) to afford 7 (55 mg, 84% yield) as colorless solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.89 (s, 3H), 3.31-3.36 (m 2H), 3.55 (s, 3H), 4.80 (ddd, *J* = 6.5, 6.6, 6.7 Hz, 1H), 6.81 (d, *J* = 6.9 Hz, 1H), 7.19-7.22 (m, 1H), 7.27-7.30 (m, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 1.1 Hz, 1H), 8.23 (d, *J* = 1.1 Hz, 1H), 9.23 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 23.1 26.5 52.7 53.0 102.4 111.7 119.5 121.2 124.3, 124.4 127.4 128.6 133.6 134.6 170.5 172.2. IR (neat, cm^{-1}): 3211, 3136, 3080, 1742, 1655, 1568, 1507, 1462, 1429, 1367, 1343, 1278, 1235, 1215, 1194, 1165, 1125, 1041, 1012, 791, 762, 716, 649, 546. HRMS (ESI): calcd for $C_{16}H_{17}N_5NaO_3$ $[M+Na]^+$ 350.1229, found 350.1228.

***N*-Acetyl-2-(pyrazol-1-yl)-L-tryptophan methyl ester (8)**

To a mixture of Ac-Trp-OMe (**1**) (52 mg, 0.20 mmol), I_2 (12 mg, 0.047 mmol) and pyrazole (35 mg, 0.51 mmol) in a test tube were added MeOH (400 μL) and H_2O (400 μL). The mixture was stirred for 5 min at room temperature. Then, 70% aqueous TBHP solution (56 μL, 0.40 mmol) was added to the mixture and the mixture was stirred for 48 h at 40 °C in ChemiStation^{TM} (EYELA). The reaction was quenched by addition of saturated aqueous $Na_2S_2O_3$ solution (2 mL) and diluted with H_2O (5 mL). The precipitates were collected by filtration and washed thoroughly with H_2O to afford **8** (43 mg, 65% yield) as colorless solids. ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.76 (s, 3H), 3.15 (dd, *J* = 7.8, 14.3 Hz, 1H), 3.27 (dd, *J* = 6.9, 14.3 Hz, 1H), 3.44 (s, 3H), 4.45 (ddd, *J* = 7.1, 7.1, 7.4 Hz, 1H), 6.62 (dd, *J* = 2.0, 2.2 Hz, 1H), 7.06-7.09 (m, 1H), 7.14-7.17 (m, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 1.6 Hz, 1H), 8.23 (d, *J* = 2.4 Hz, 1H), 8.49 (d, *J* = 6.9 Hz, 1H), 11.74 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 22.2, 25.6, 51.7, 53.0, 99.4, 107.3, 111.4, 118.4, 119.6, 122.0, 127.4, 130.6, 132.4, 133.0, 141.1, 169.2, 172.2. IR (neat, cm^{-1}): 3220, 3052, 1733, 1651, 1574, 1525, 1467, 1439, 1416, 1366, 1335, 1282, 1225, 1211, 1172, 1129, 1055, 1011, 978, 953, 926, 875, 747, 732, 639, 603. HRMS (ESI): calcd for $C_{17}H_{18}N_4NaO_3$ $[M+Na]^+$ 349.1276, found 349.1277.

7. Preparation of azole derivatives



Methyl 1*H*-benzo[d][1,2,3]triazole-5-carboxylate (S4)

To a suspension of carboxylic acid **S3** (816 mg, 5.00 mmol) in MeOH (40 mL) was added thionyl chloride (430 μL, 5.9 mmol) dropwise in an ice bath. The mixture was stirred for 5 min. The temperature of the mixture was raised to reflux and maintained overnight under reflux. The reaction mixture was cooled to room temperature and concentrated in *vacuo*. The residue was diluted with a mixture of EtOH and EtOAc (10 mL and 60 mL) and washed with brine (40 mL). The remained organic materials in the aqueous layer were extracted with a mixture of EtOH and EtOAc (10 mL and 40 mL). The combined organic layers were dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo* to afford **S4** (813 mg, 92% yield) as brown solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.00 (s, 3H), 7.87 (br s, 1H), 8.19, (d, J = 8.7 Hz), 8.73 (s, 1H). These values were in good agreement with those reported in the literature.[7]

5-Hydroxymethyl-1*H*-benzo[d][1,2,3]triazole (S5)

To a suspension of $LiAlH_4$ (228 mg, 6.01 mmol) in anhydrous THF (20 mL) under argon atmosphere was added ester **S4** (708 mg, 4.00 mmol) in five portions over 1 h. The mixture was stirred overnight at room temperature. The mixture was quenched with EtOAc (200 μL) and stirred for 1 h. The resulting mixture was poured into ice water (50 mL), acidified with 2 M aqueous HCl solution to pH ~3, and saturated with NaCl. The organic materials were extracted with EtOAc (50 mL) and THF (30 mL×2). The combined organic layers were concentrated in *vacuo*. The resulting residue was purified with Isolera One flash purification system ($CHCl_3$/MeOH, 19:1 to 10:1) to afford **S5** (411 mg, 69% yield) as orange solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.66 (s, 2H), 5.36 (s, 1H), 7.37 (s, 1H), 7.77 (br s, 1H), 7.86 (br s, 1H), 15.59, (s, 1H). These values were in good agreement with those reported in the literature.[7]

5-(Azidomethyl)-1*H*-benzo[d][1,2,3]triazole (25)

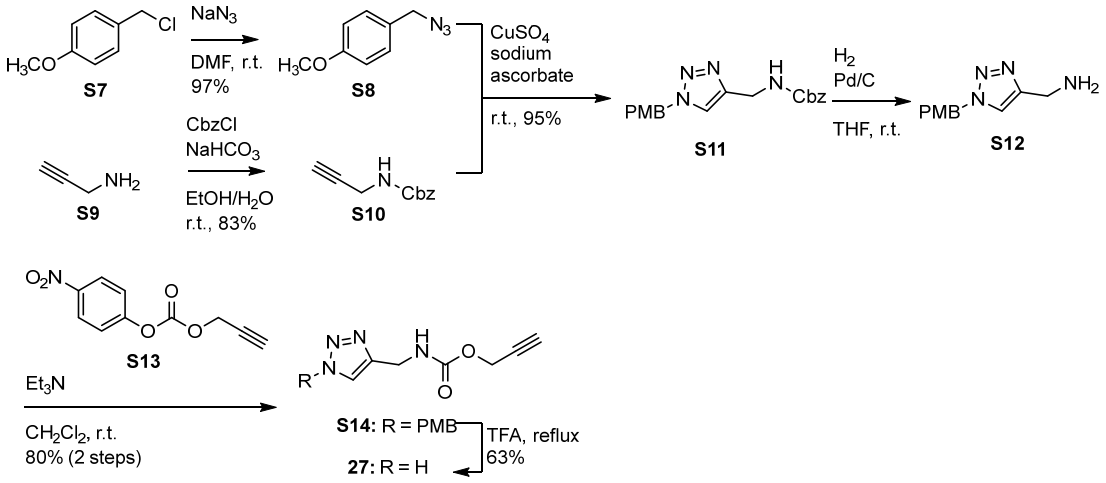
To a suspension of alcohol **S5** (296 mg, 2.0 mmol) in toluene (3.0 mL) was added thionyl chloride (2.0 mL) dropwise in an ice bath. The mixture was stirred for 5 min at the same temperature. The temperature of the mixture was raised to room temperature and the mixure was stirred for 3 h. The resulting precipitates were collected by filtration, washed with *n*-hexane (40 mL), and dried in *vacuo*. The resulting solids (372 mg) were directly used for the next step.

To a solution of the above solids in DMF (4 mL) was added NaN_3 (391 mg, 6.01 mmol). The mixture was stirred for 3 days at room temperature. The reaction mixture was diluted with H_2O (10 mL) and the oraganic materials were extracted with EtOAc (30 mL, 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with Isolera One flash purification

system (hexane/ EtOAc, 40–50%) to afford **25** (313 mg, 90% yield from **S5**) as colorless solids. ^{1}H NMR (600 MHz, 5% TFA-*d* in CD_3OD): δ 4.56 (s, 2H), 7.48 (dd, *J* = 1.3, 8.6 Hz, 1H), 7.87 (s, 1H), 7.90 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (151 MHz, 5% TFA-*d* in CD_3OD): δ 55.4, 115.0, 116.2, 127.9, 136.0, 139.8, 140.1. IR (neat, cm^{-1}): 2980, 2773, 2084, 1523, 1473, 1409, 1381, 1347, 1304, 1267, 1236, 1209, 1194, 1150, 1018, 998, 961, 892, 861, 827, 808, 772, 760, 706. HRMS (ESI): calcd for $C_7H_5N_6$ $[M-H]^-$ 173.0576, found 173.0571.

5-Hydroxymethyl-1*H*-1,2,3-triazole (26)

To a solution of CuI (39 mg, 0.20 mmol) in anhydrous DMF (4 mL) and MeOH (1 mL) under argon atmosphere were added propargyl alcohol (120 μL, 2.1 mmol) and $TMSN_3$ (390 μL, 3.0 mmol). The mixture was stirred at 80 °C for 21 h. Then, the reaction mixture was cooled to room temperature and concentrated in *vacuo*. The residue was suspended with EtOAc (30 mL) and the precipitates were removed by filtration through celite pad. The filtrate was concentrated in *vacuo* and the residue was purified by flush silica gel column chromatography (EtOAc) to afford **26** (151 mg, 74% yield) as colorless amorphous solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.90 (s, 1H), 4.86 (s, 2H), 7.72 (s, 1H). These values were in good agreement with those reported in the literature.[8]



1-(Azidomethyl)-4-methoxybenzene (S8)

To a solution of 4-methoxybenzyl chloride (**S7**) (827 mg, 5.27 mmol) in DMF (10 mL) was added sodium azide (406 mg, 6.25 mmol). The mixture was stirred at room temperature for 24 h. The mixture was diluted with Et_2O (40 mL), washed with H_2O (40 mL×2) and brine (40 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo* to afford **S8** (831 mg, 97% yield) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.82 (s, 3H), 6.90-6.92 (m, 2H), 7.24-7.26 (m, 2H). These values were in good agreement with those reported in the literature.[9]

Benzyl prop-2-yn-1-ylcarbamate (S10)

To a solution of amine **S9** (320 μL, 5.0 mmol) and $NaHCO_3$ (839 mg, 9.99 mmol) in THF was added benzyl chloroformate (850 μL, 6.0 mmol). The mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with H_2O (10 mL) and the organic materials were extracted with Et_2O (30 mL×3). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with Isolera One flash purification system (hexane/EtOAc, 10–20%) to afford **S10** (787 mg, 83% yield) as colorless solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.24 (t, *J* =2.5 Hz, 1H), 4.00 (br d, *J* = 3.1 Hz, 2H) 4.92 (br s, 1H), 5.13(s, 2H), 7.31-7.38 (m, 5H). These values were in good agreement with those reported in the literature.[10]

1-(4-Methoxybenzyl)-4-({[(benzyloxy)carbonyl]amino}methyl)-1*H*-1,2,3-triazol (S11)

To a solution of azide **S8** (655 mg, 10.1 mmol) and carbamate **S10** (755 mg, 3.99 mmol) in *t*-BuOH (10 mL) and H_2O (10 mL) were added $CuSO_4 \cdot 5H_2O$ (51 mg, 0.21 mmol) and sodium ascorbate (79 mg, 0.40 mmol). The reaction mixture was stirred at room temperature for 13 h. The reaction mixture was diluted with EtOAc (100 mL) and filtered through celite pad. The organic layer was separated, washed with 100 mM aqueous EDTA·4Na solution (40 mL) and brine (40 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo* to afford **S11** (1.34 g, 95%) as colorless solids. ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.94 (s, 3H), 4.41 (d, *J* = 6.0 Hz, 2H), 5.07 (s, 2H), 5.40 (s, 2H), 5.46 (s, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 8.5 Hz, 2H), 7.29-7.33 (m, 5H), 7.40 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 36.6, 53.8, 55.4, 66.9, 114.6, 121.7, 126.6, 128.1, 128.2, 128.6, 129.8, 136.5, 145.4, 156.4, 160.0. IR (neat, cm^{-1}): 3382, 3323, 3107, 3062, 3007, 2952, 2837, 1687, 1612, 1587, 1512, 1456, 1426, 1377, 1325, 1303, 1240, 1174, 1144, 1127, 1106, 1056, 1033, 975, 920, 843, 826, 742, 709. HRMS (ESI): calcd for $C_{19}H_{20}N_4NaO_3$ $[M+Na]^+$ 375.1433, found 375.1430.

4-Nitrophenyl prop-2-yn-1-yl carbonate (S13)

To a solution of 4-nitrophenyl chloroformate (1.01 g, 5.01 mmol) in anhydrous THF (20 mL) under argon atmosphere were added trimethylamine (830 μL, 6.0 mmol) and propargyl alcohol (295 μL, 5.0 mmol) in an ice bath. The mixture was stirred for 1 h. The mixture was diluted with EtOAc (20 mL), washed with sat. NH_4Cl aq. soln. (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with Isolera One flash purification system (hexane/EtOAc, 10:1 to 1:1) to afford **S13** (445 mg, 40% yield) as colorless solids. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.62 (t, *J* = 2.5 Hz, 1H), 4.88 (d, *J* = 2.5 Hz, 2H), 7.39-7.42 (m, 2H), 8.28-8.30 (m, 2H). These values were in good agreement with

those reported in the literature.[11]

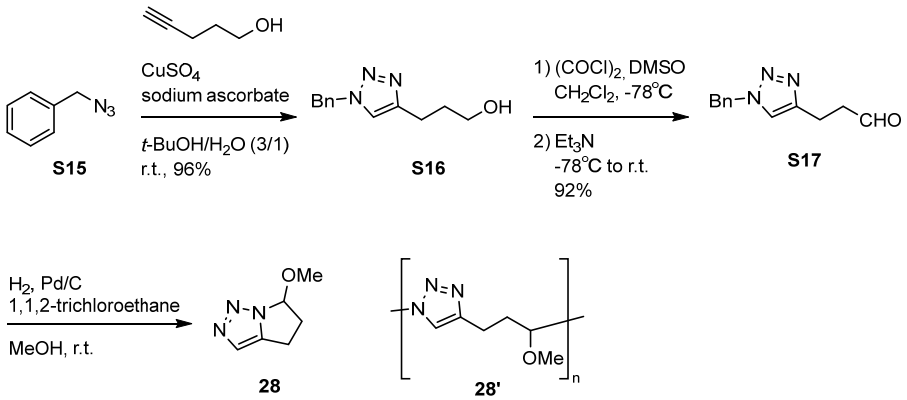
1-(4-Methoxybenzyl)-4-({[(prop-2-yn-1-yloxy)carbonyl]amino}methyl)-1*H*-1,2,3-triazol (S14)

To a solution of carbamate **S11** (353 mg, 1.00 mmol) in THF (10 mL) was added 10% Pd/C (47 mg). The mixture stirred overnight at room temperature under H_2 atmosphere. The reaction mixture was filtered through a celite pad and concentrated in *vacuo*. The resulting solid (224 mg) was directly used for the next step.

To a solution of the residue and trimethylamine (210 μL, 1.5 mmol) in CH_2Cl_2 (10 mL) was added carbonate **S13** (206 mg, 0.931 mmol). The mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with saturated aqueous Na_2CO_3 solution (20 mL) and the organic materials were extracted with CH_2Cl_2 (40 mL×3). The combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with flush silica gel column chromatography (CH_2Cl_2 /MeOH, 19:1) to afford **S14** (241 mg, 80% yield from **S11**) as colorless solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.47 (t, J = 2.4 Hz), 3.73 (s, 3H), 4.21 (d, J = 5.9 Hz, 2H), 4.61 (d, J = 2.4 Hz, 2H), 5.47 (s, 2H) 6.92 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 8.7 Hz, 2H), 7.83 (t, J = 5.7 Hz, 1H), 7.90 (s, 1H). These values were in good agreement with those reported in the literature.[12]

4-({[(Prop-2-yn-1-yloxy)carbonyl]amino}methyl)-1*H*-1,2,3-triazol (27)

To carbamate **S14** (132 mg, 0.440 mmol) was added TFA (1.6 mL). The mixture was stirred for 2 days under reflux. The reaction mixture was cooled to room temperature and concentrated in *vacuo*. The residue was dissolved in EtOAc (30 mL) and the desired product was extracted with 2 M aqueous NaOH solution (10 mL and 5 mL). The combined aqueous layers were acidified to pH ~3 with 2 M aqueous HCl solution and the organic materials were extracted with EtOAc (20 mL×3). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified with silica gel column chromatography (hexane/ EtOAc, 1:2) to afford **27** (50 mg, 63% yield) as colorless solids. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.48 (t, J = 2.2 Hz, 1H, major and minor tautomers), 4.26 (s, 2H, major and minor), 4.63 (d, J = 2.2 Hz, 2H major and minor), 7.50 (s, 1H, minor), 7.59 (s, 1H, major), 7.86 (s, 1H, major and minor), 14.69 (s, 1H, major), 15.08 (s, 1H, minor). ^{13}C NMR (126 MHz, DMSO-d_6, major tautomer): δ 35.8, 51.7, 77.2, 79.3, 132.1, 145.2, 155.4. IR (neat, cm^{-1}): 3296, 3163, 3112, 3078, 3006, 2360, 1692, 1555, 1443, 1396, 1355, 1308, 1276, 1220, 1147, 1126, 1050, 1025, 1004, 992, 961, 885, 849, 781, 764, 664, 636. HRMS (ESI): calcd for $C_7H_8N_4NaO_2$ $[M+Na]^+$ 203.0545, found 203.0542.



3-(1-Benzyl-1H-1,2,3-triazol-4-yl)propan-1-ol (S17)

To a solution of benzyl bromide (594 μL, 5.00 mmol) in acetone (80 mL) and H_2O (20 mL) was added sodium azide (487 mg, 7.50 mmol). The mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with H_2O (40 mL) and the organic materials were extracted by CH_2Cl_2 (50, 20, 20 mL). The combined organic layers were dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo* to afford **S15** (658 mg) as a colorless oil.

To a solution of **S15** in *t*-BuOH (15 mL) and H_2O (5 mL) were added 4-pentyn-1-ol (456 μL, 4.93 mmol), $Cu(OAc)_2 \cdot H_2O$ (101 mg, 0.503 mmol), and sodium ascorbate (197 mg, 0.996 mmol). The reaction mixture was stirred at room temperature for 20 h under Ar atomosphere and concentrated in *vacuo*. The residue was suspended with H_2O (10 mL) and the organic materials were extracted with $CHCl_3$ (20 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered through cotton plug, and concentrated in *vacuo*. The resulting residue was purified using Isolera One system ($CHCl_3$/MeOH, 2—18%) to afford **S16** (1032 mg, 95% yield from **S15**) as colorless solids. ^{1}H NMR (600 MHz, $CDCl_3$/TMS): δ 1.92 (tt, J = 6.0, 7.3 Hz, 2H), 2.81 (t, J = 7.3 Hz, 2H), 3.70 (t, J = 6.0 Hz, 2H), 5.49 (s, 2H), 7.22 (s, 1H), 7.25–7.27 (m, 2H), 7.35–7.38 (m, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 22.1, 31.9, 54.1, 61.8, 120.9, 128.0, 128.7, 129.1, 134.8, 148.0. HRMS (ESI): calcd for $C_{12}H_{15}N_3NaO$ $[M+Na]^+$ 240.1113, found 240.1121.

3-(1-Benzyl-1H-1,2,3-triazol-4-yl)propanal (S17)

To a solution of oxalyl chloride (685 μL, 7.99 mmol) in CH_2Cl_2 (6 mL) in three-necked flask fitted with an Ar ballon and a thermometer at −70 °C in a dry ice–acetone bath was added dropwise a solution of DMSO (850 μL, 12.0 mmol) in CH_2Cl_2 (6 mL). The mixture was stirred for 5 min at the same temperature. To the mixture was added dropwise a solution of **S16** (874 mg, 4.02 mmol) in CH_2Cl_2 (6 mL) over 10 min. The resulting mixture was stirred for 1 h at the same temperature. To the mixture was added Et_3N (3.35 mL, 24.0 mmol). A dry ice–acetone bath was

removed, and the mixture was stirred for 30 min. The mixture was quenched with sat. NH_4Cl aq. solution (40 mL). The organic materials were extracted with $CHCl_3$ (30 mL x 2). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified by using Isolera One system ($CHCl_3$/MeOH, 2—18%) to afford **S17** (793 mg, 92% yield) as orange oil. ^{1}H NMR (600 MHz, $CDCl_3$/TMS): δ 2.91 (dt, J = 0.9, 7.0 Hz, 2H), 3.01 (t, J = 7.0 Hz, 2H), 5.48 (s, 2H), 7.25–7.26 (m, 3H), 7.36–7.37 (m, 3H), 9.82 (t, J = 0.9 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 201.2, 146.6, 134.8, 129.1, 128.7, 128.0, 121.2, 54.1, 43.0, 18.2. HRMS (ESI): calcd for $C_{12}H_{13}N_3NaO$ $[M+Na]^+$ 238.0956, found 238.0958.

6-Methoxy-5,6-dihydro-4H-pyrrolo[1,2-c][1,2,3]triazole (28)

To a solution of **S17** (97 mg, 0.45 mmol) in MeOH (5 mL) were added 10% Pd/C (29 mg) and 1,1,2-trichloroethane (44.5 μL, 0.497 mmol). The suspension was stirred for 48 h under 1 atom of hydrogen at rt. The mixture was filtered through a celite pad and the filtrate was concentrated in *vacuo* to afford colorless oil (69 mg). Low-resolution MS data indicated that the product was a mixture of **28** and **28′** (Fig. S29). The resulting oil was dissolved in DMSO and was directly used for stapling of **13** without further purification.

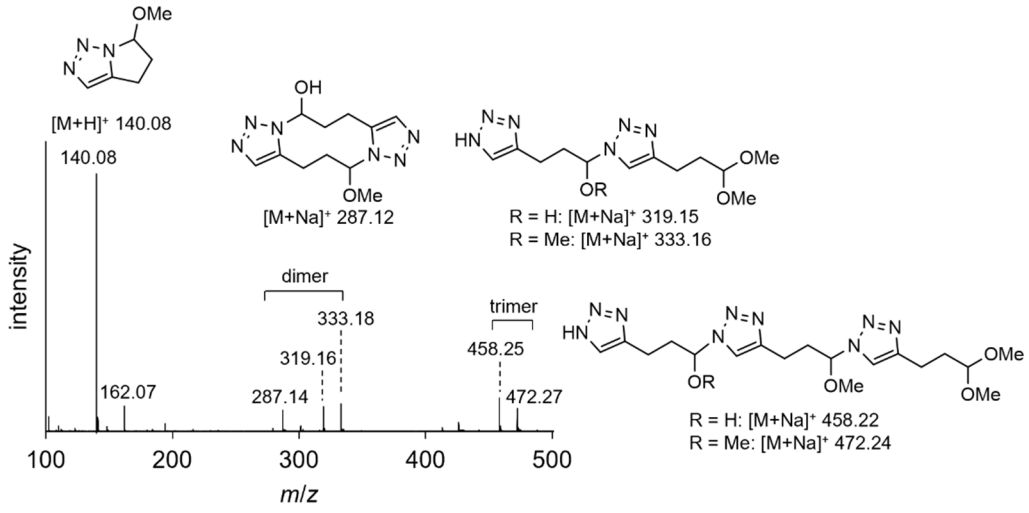
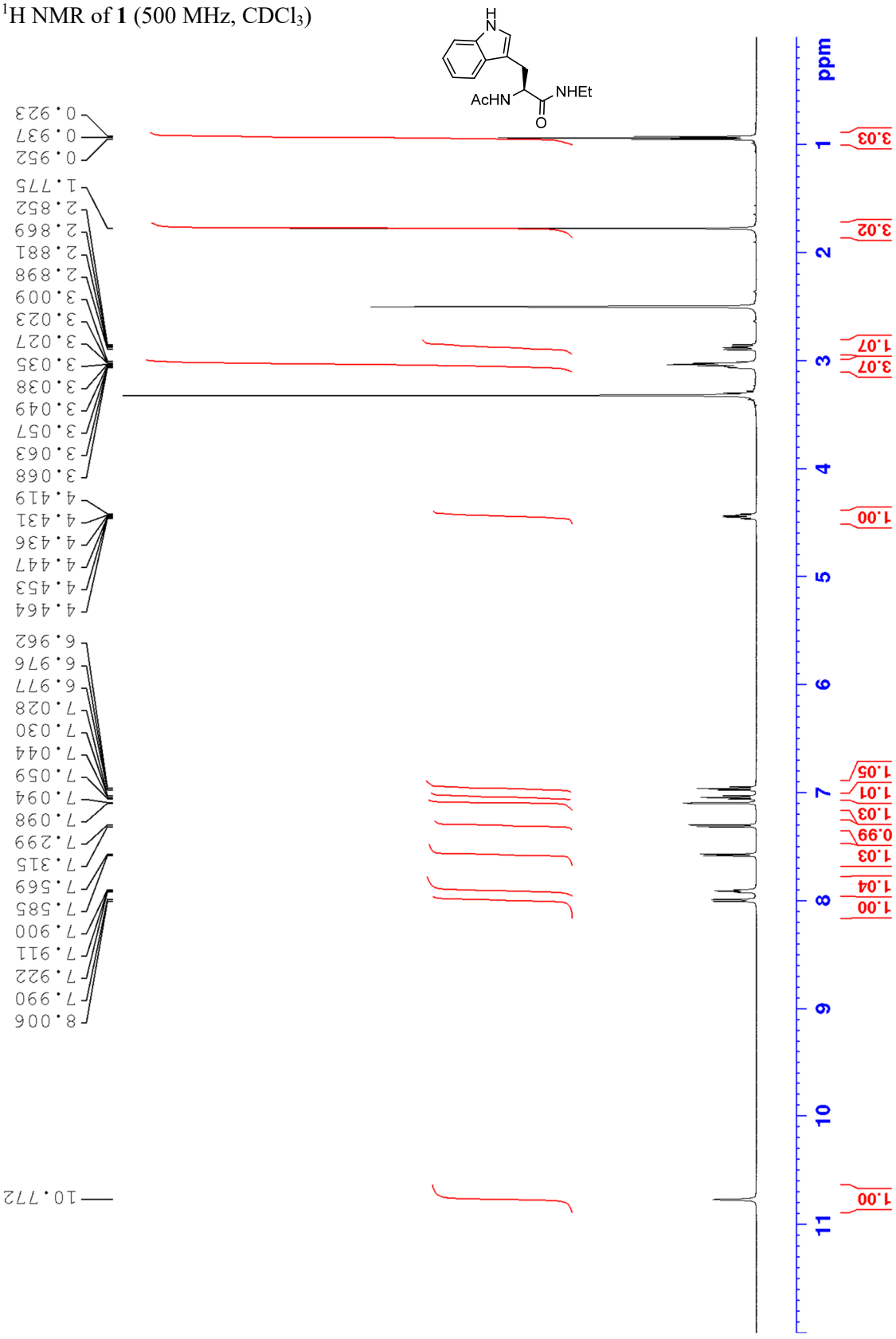


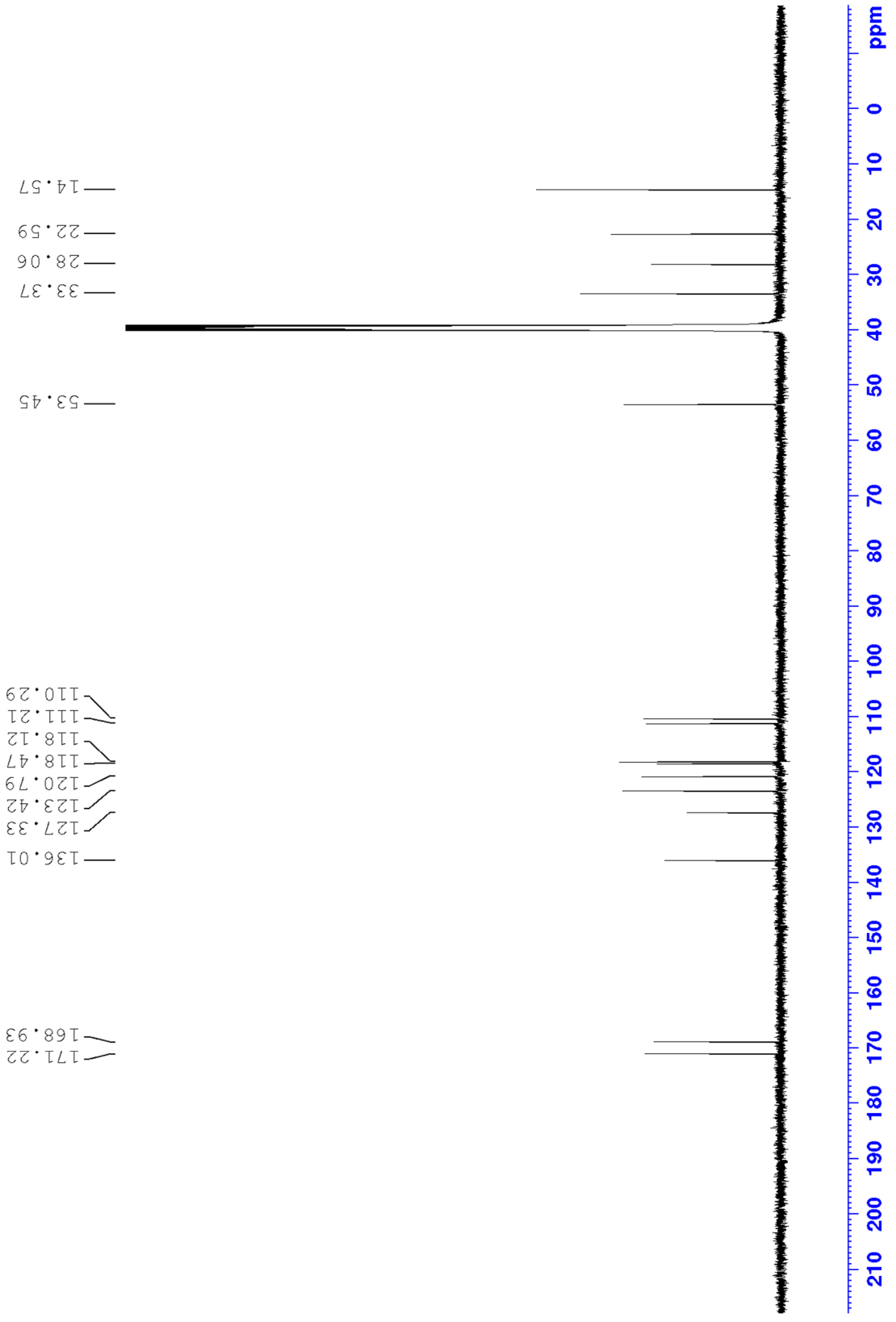
Fig. S29 Low-resolution MS data of **28.**

^{1}H and ^{13}C NMR spectra of synthesized compounds

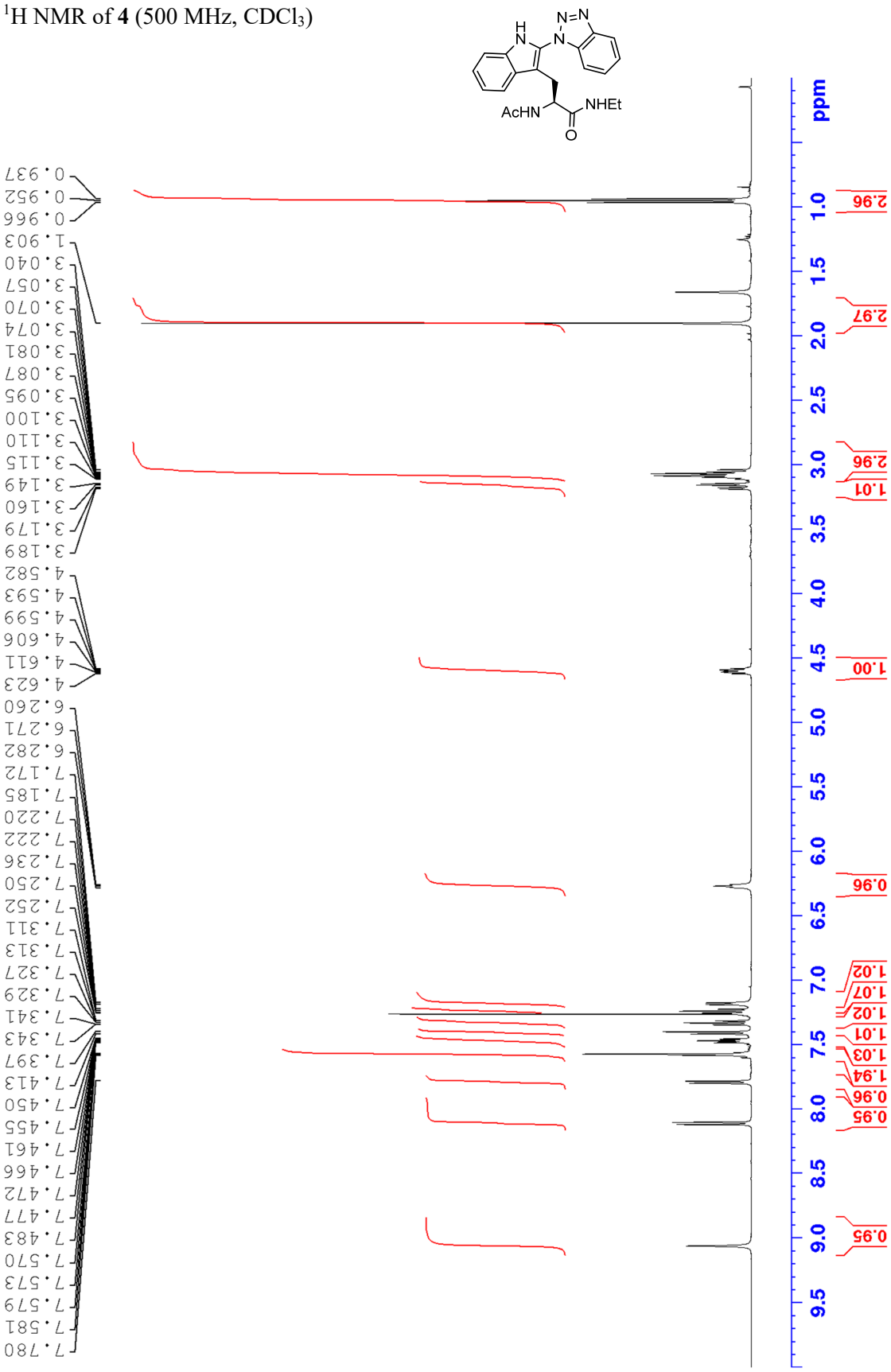
^{1}H NMR of **1** (500 MHz, $CDCl_3$)



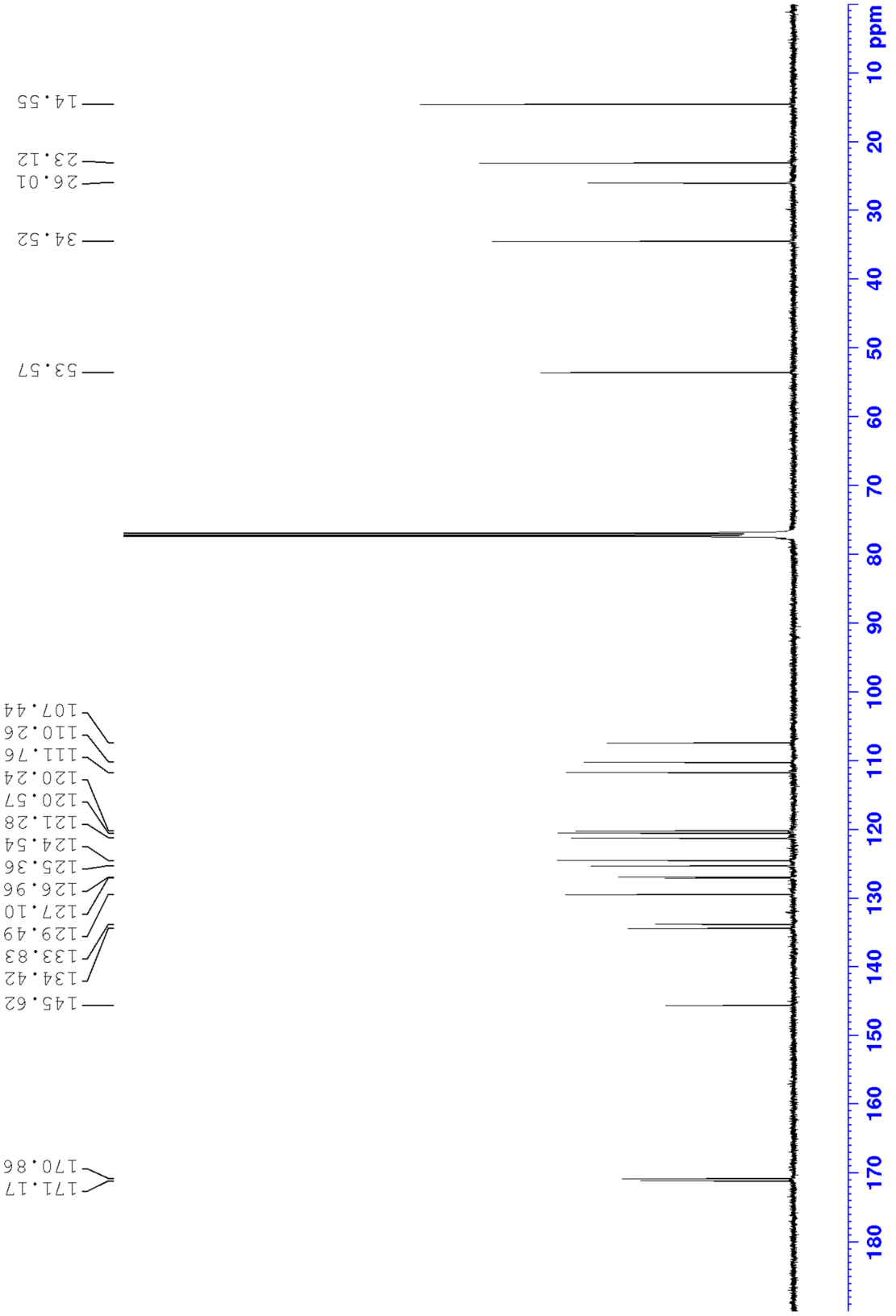
^{13}C NMR of **1** (126 MHz, $CDCl_3$)



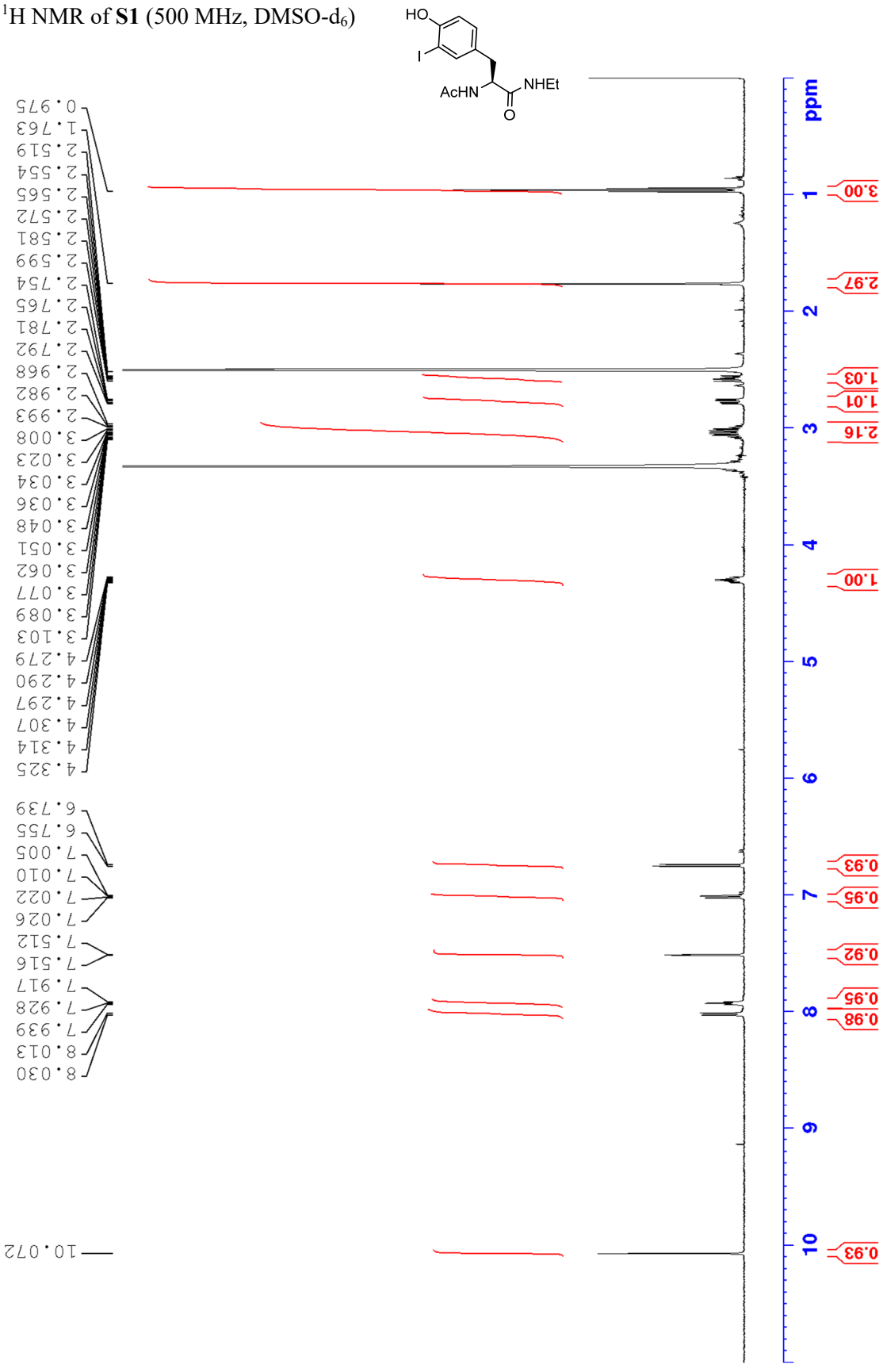
^{1}H NMR of **4** (500 MHz, $CDCl_3$)



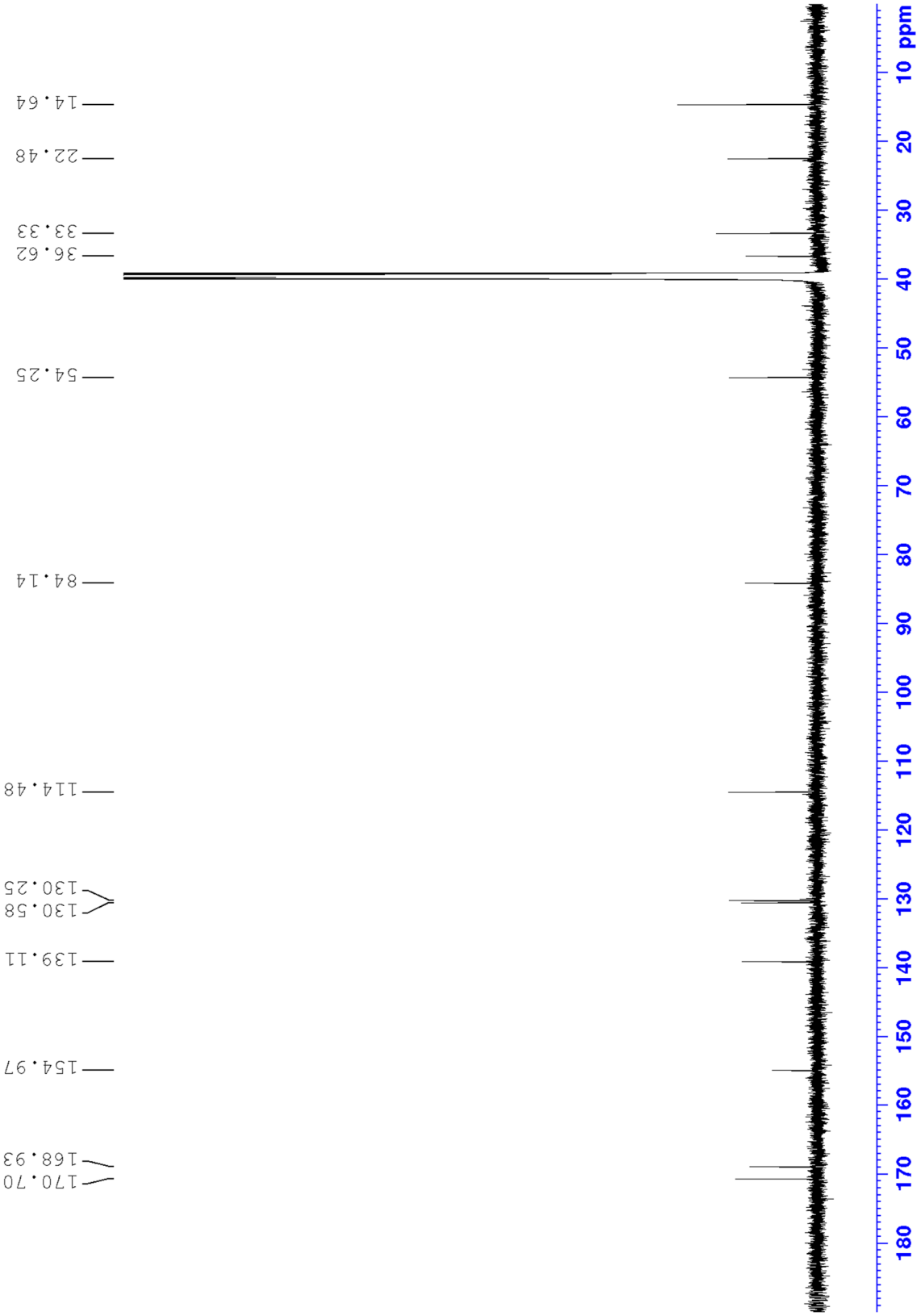
^{13}C NMR of 4 (126 MHz, $CDCl_3$)



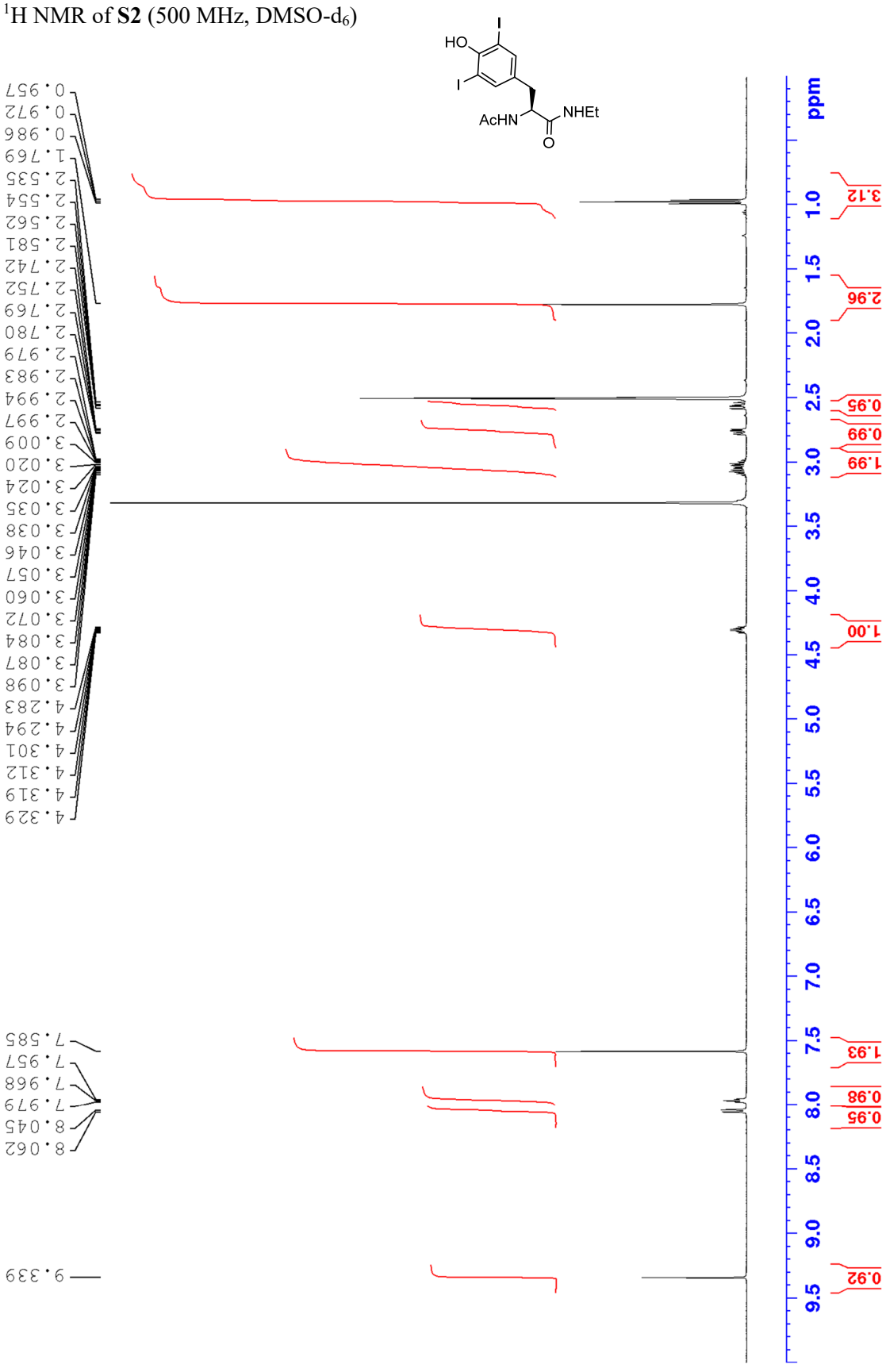
^{1}H NMR of **S1** (500 MHz, DMSO-d$_6$)



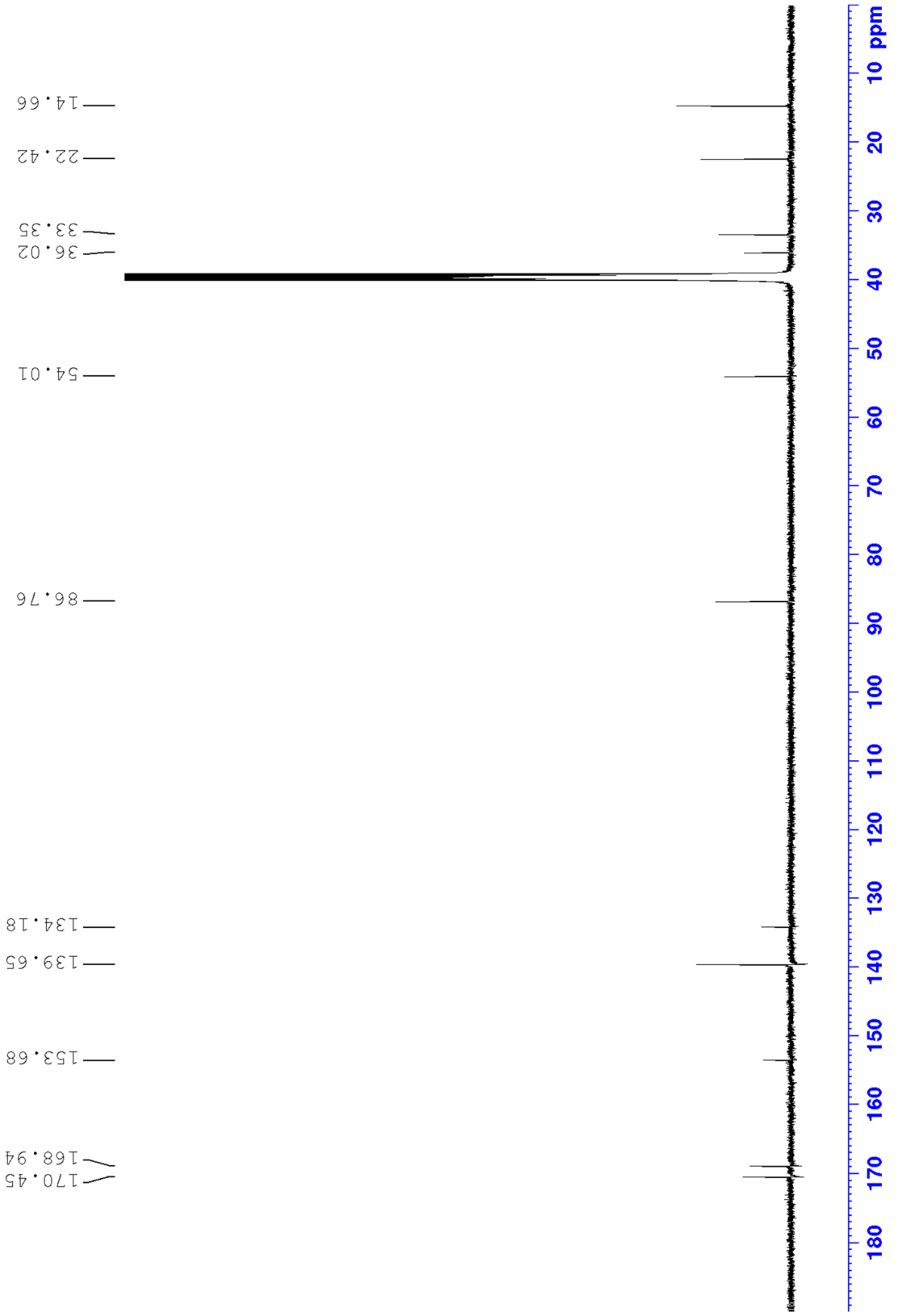
^{13}C NMR of **S1** (126 MHz, DMSO-d_6)



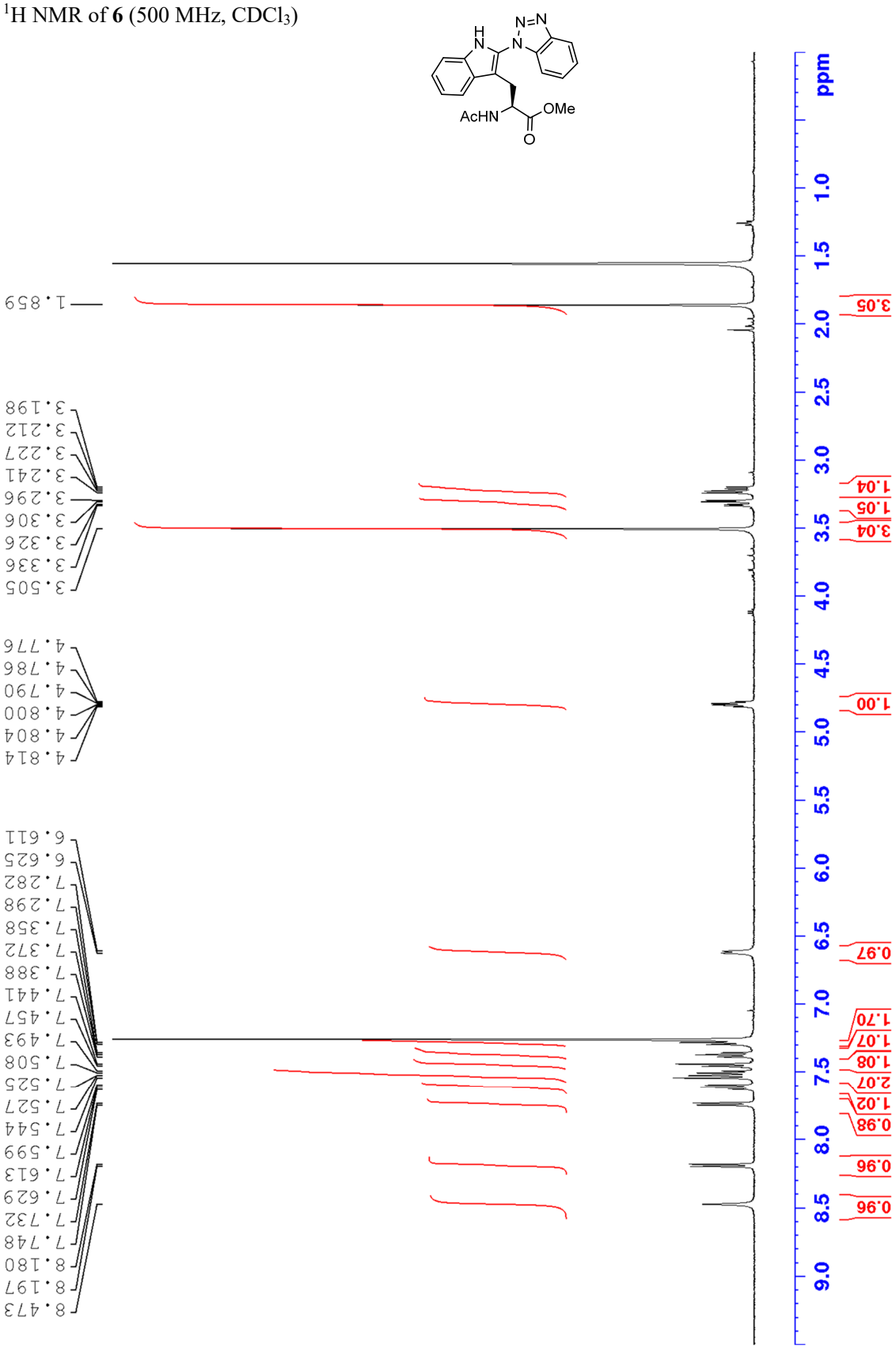
^{1}H NMR of **S2** (500 MHz, DMSO-d_6)



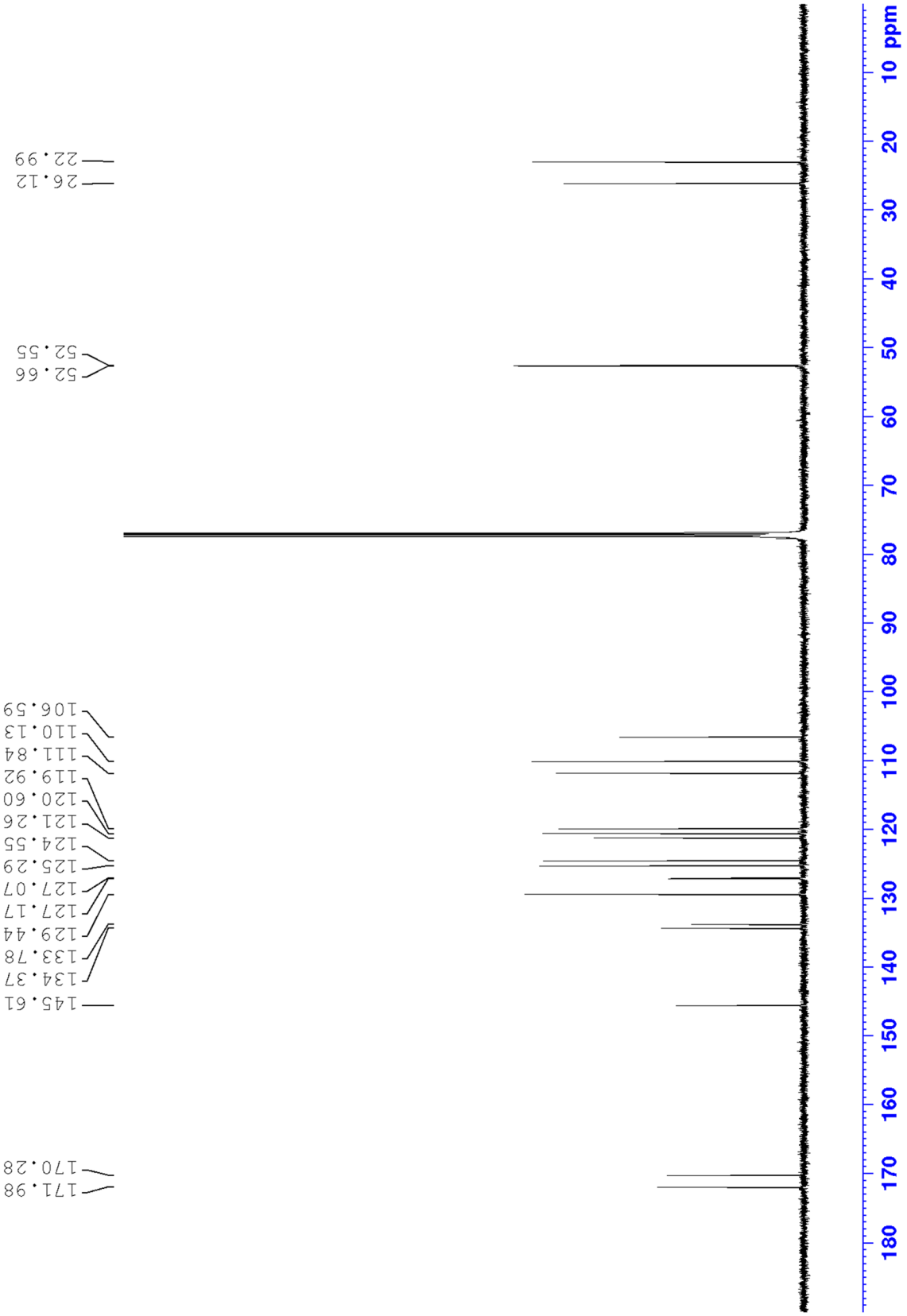
^{13}C NMR of **S2** (126 MHz, DMSO-d_6)



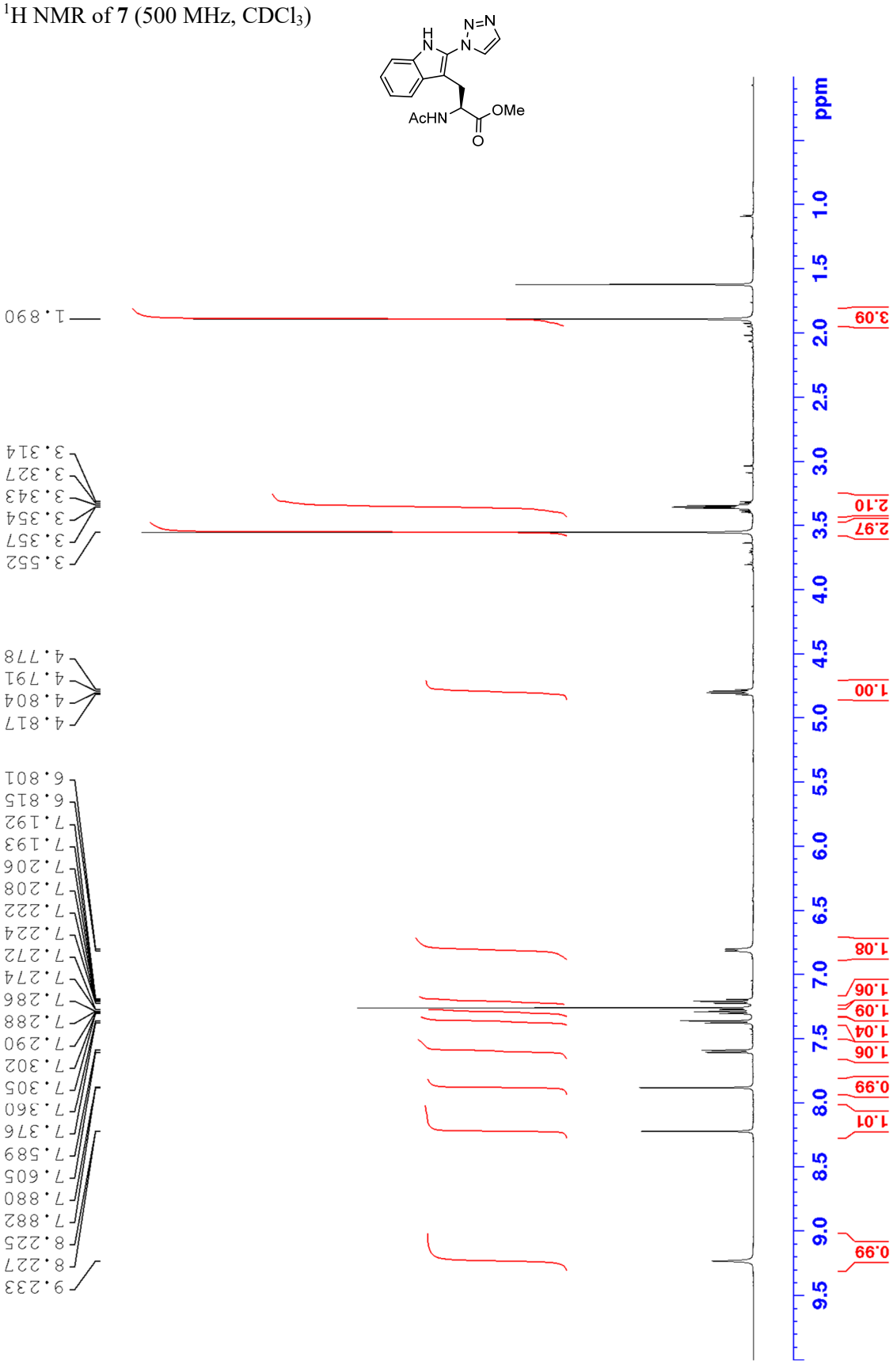
^{1}H NMR of **6** (500 MHz, $CDCl_3$)



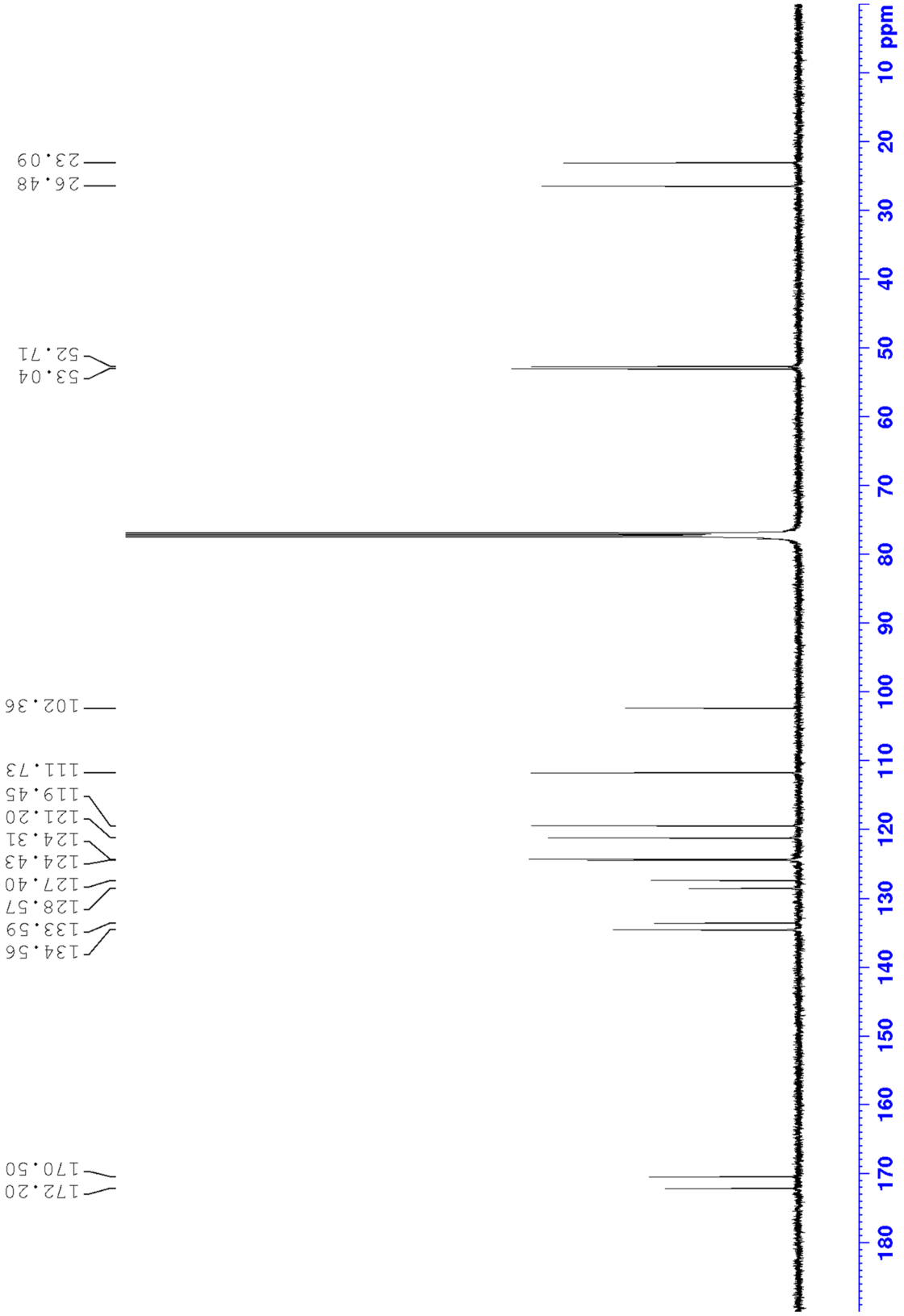
^{13}C NMR of **6** (126 MHz, $CDCl_3$)



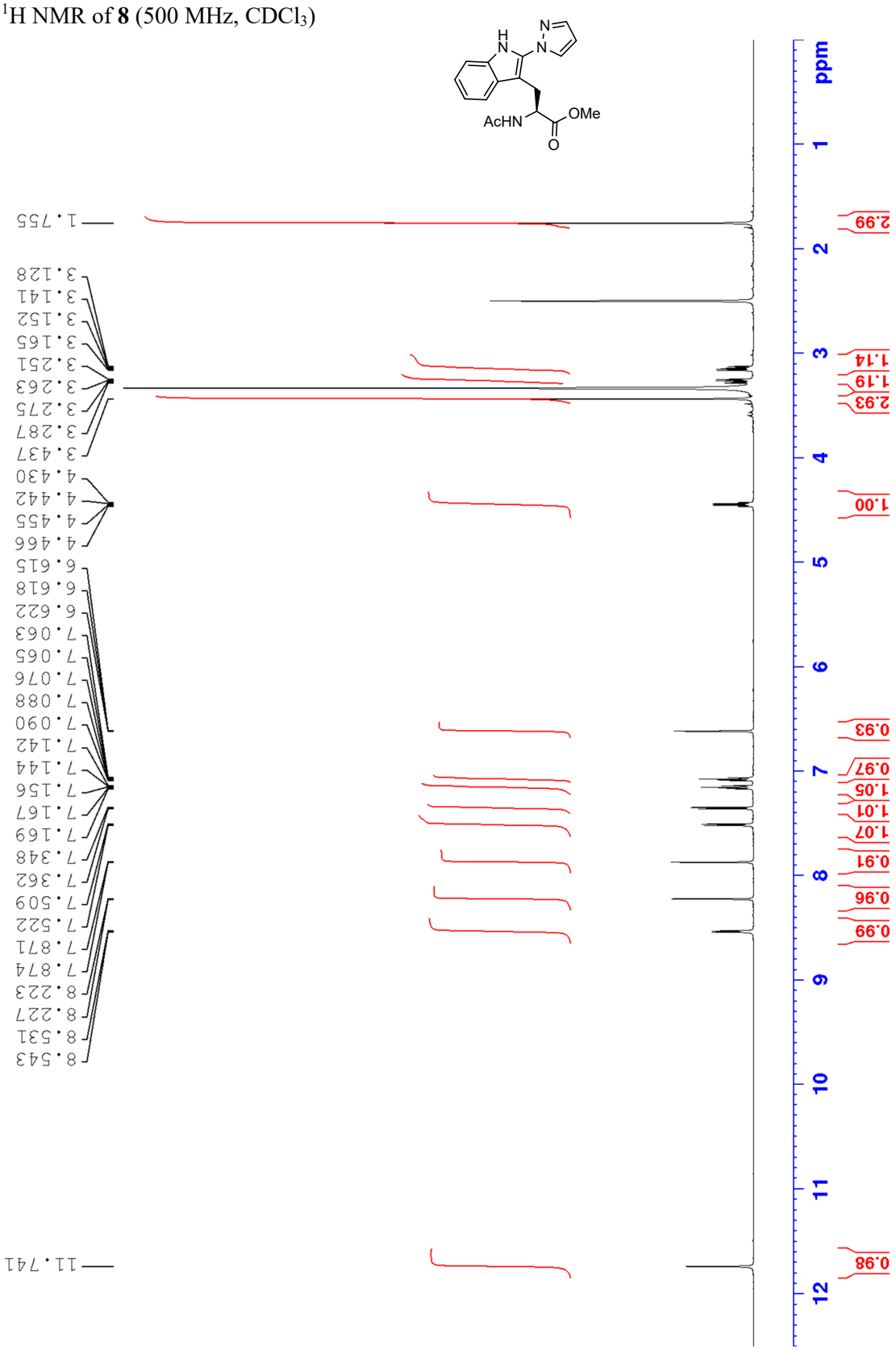
^{1}H NMR of **7** (500 MHz, $CDCl_3$)



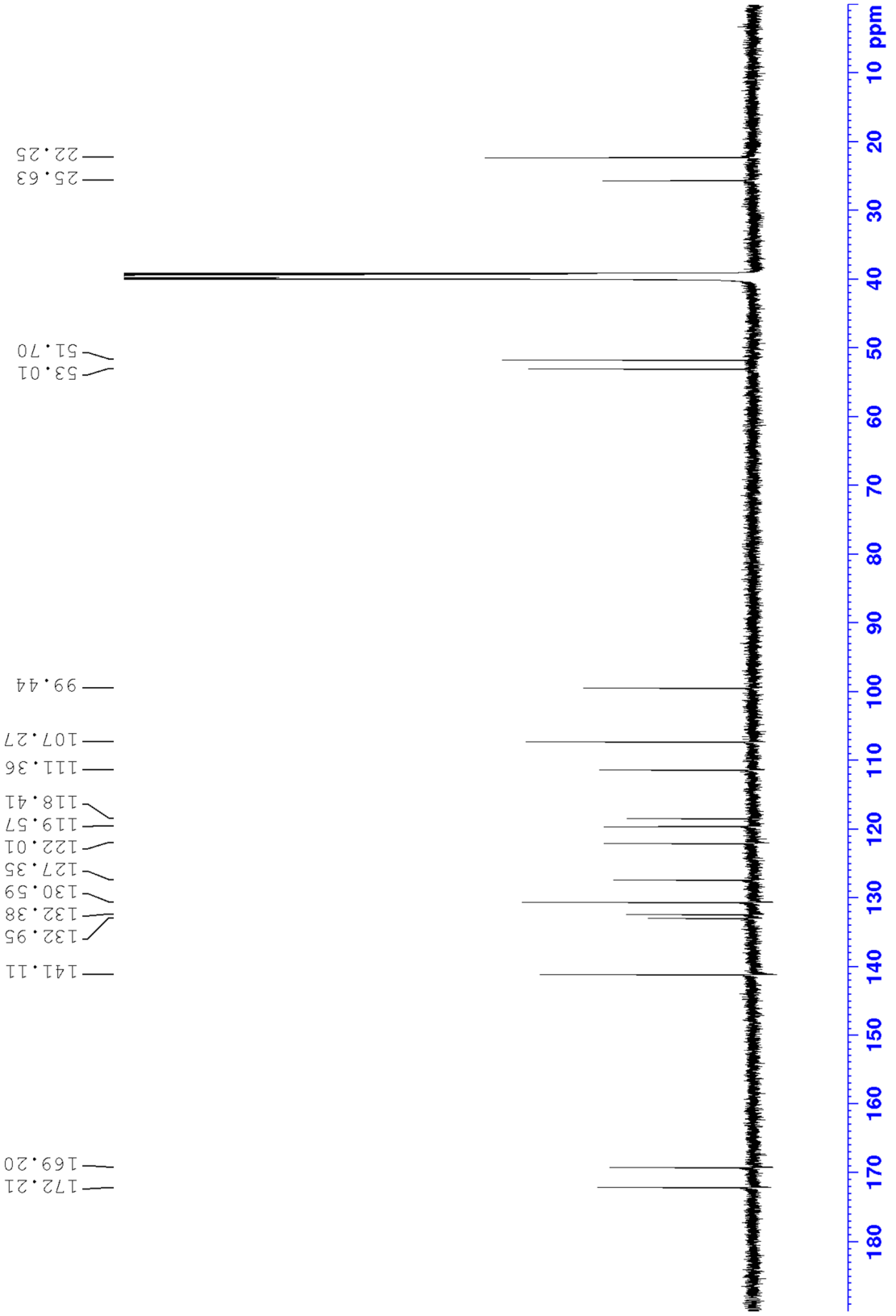
^{13}C NMR of **7** (126 MHz, $CDCl_3$)



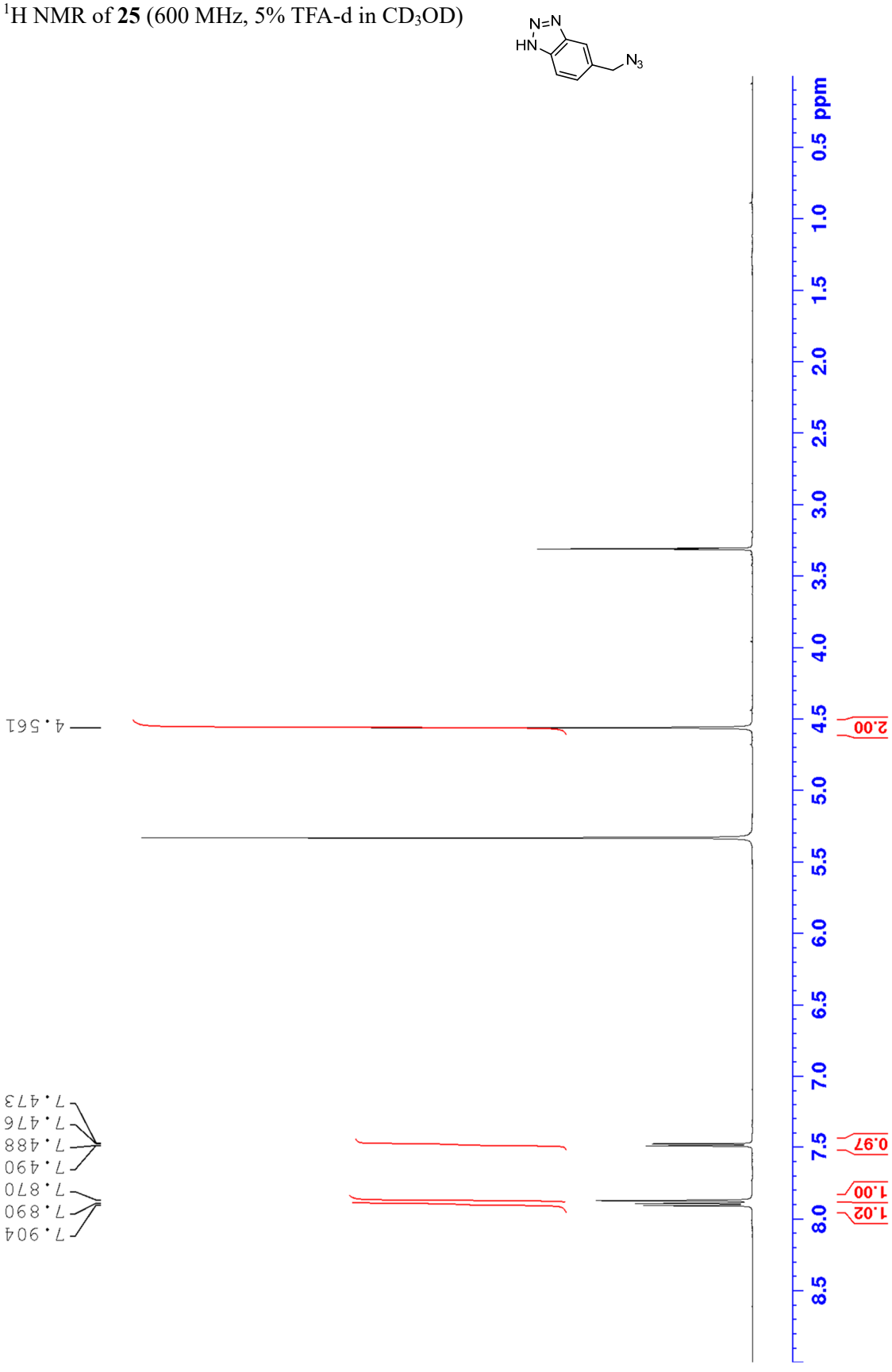
^{1}H NMR of **8** (500 MHz, $CDCl_3$)



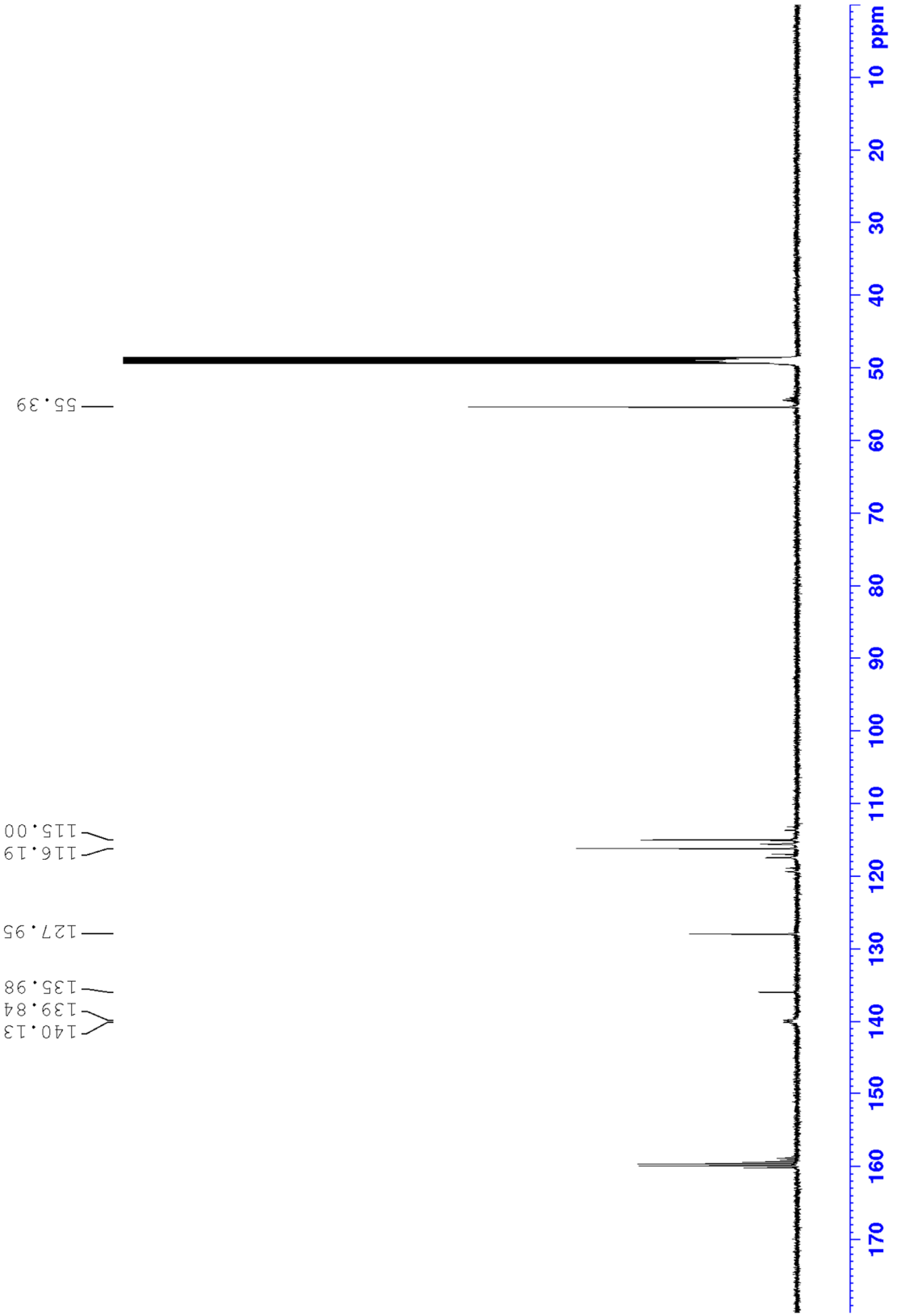
^{13}C NMR of **8** (126 MHz, $CDCl_3$)



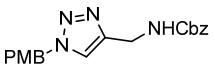
^{1}H NMR of **25** (600 MHz, 5% TFA-d in CD_3OD)

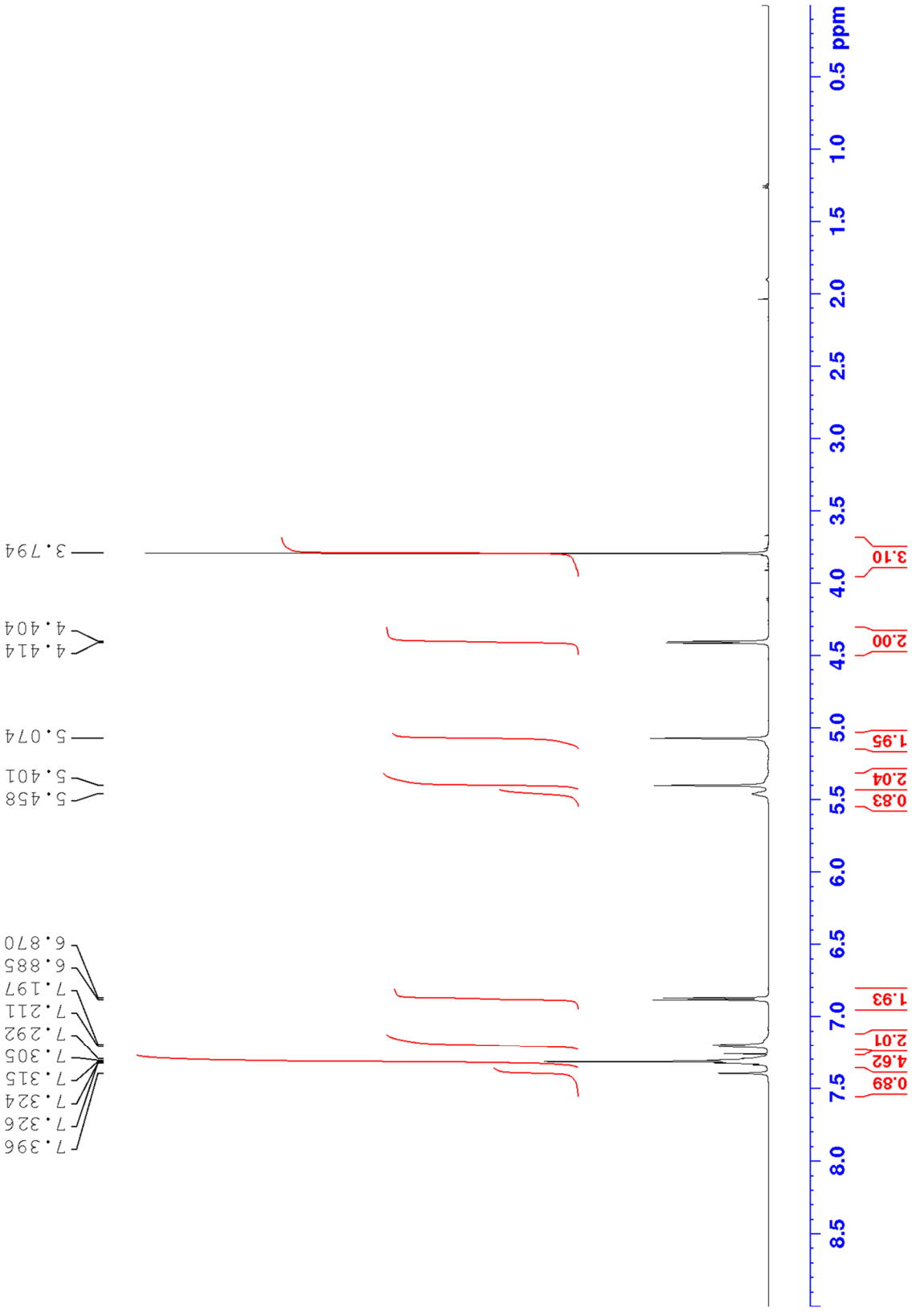


^{13}C NMR of **25** (151 MHz, 5% TFA-d in CD_3OD)

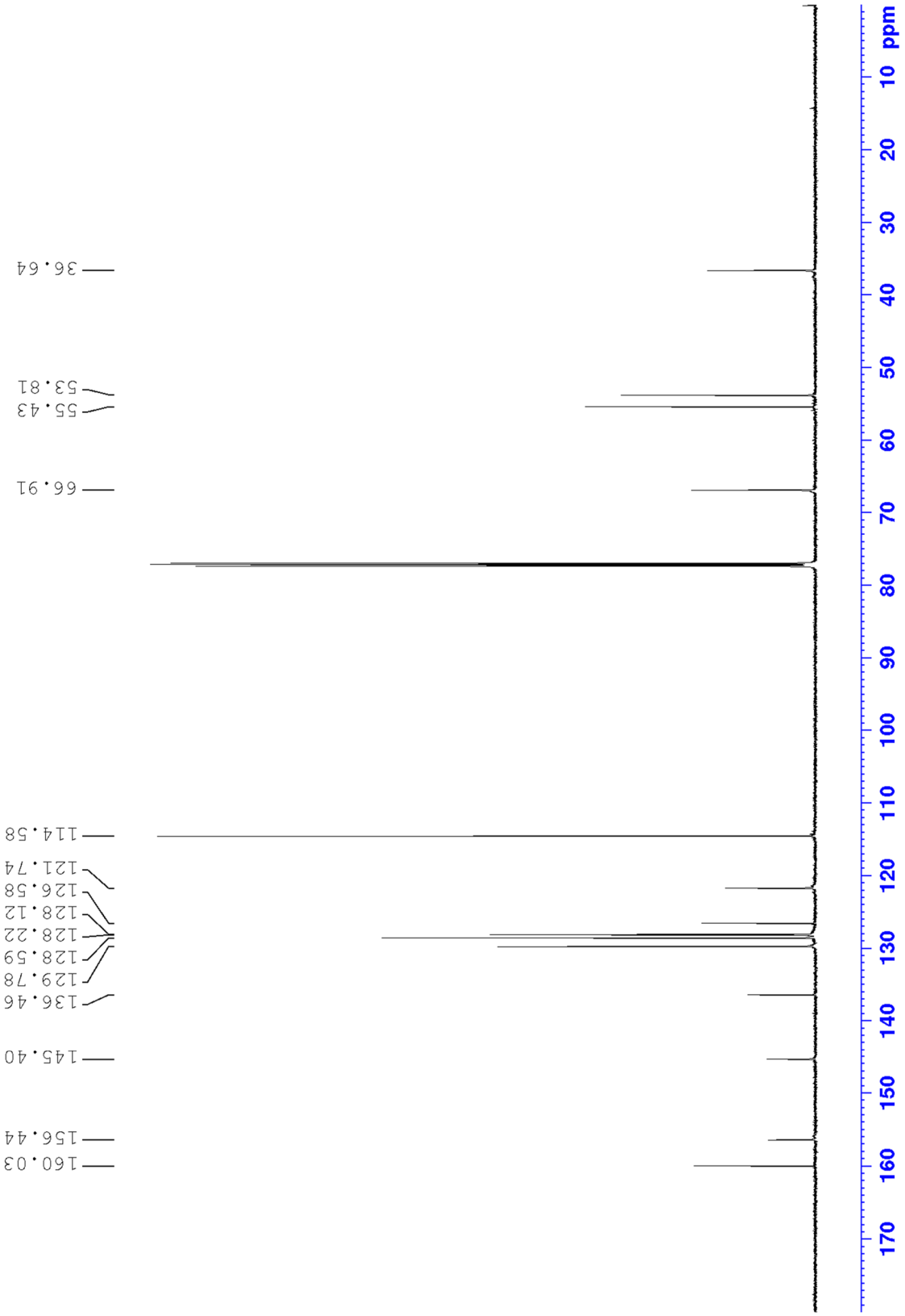


^{1}H NMR of **S11** (600 MHz, $CDCl_3$)

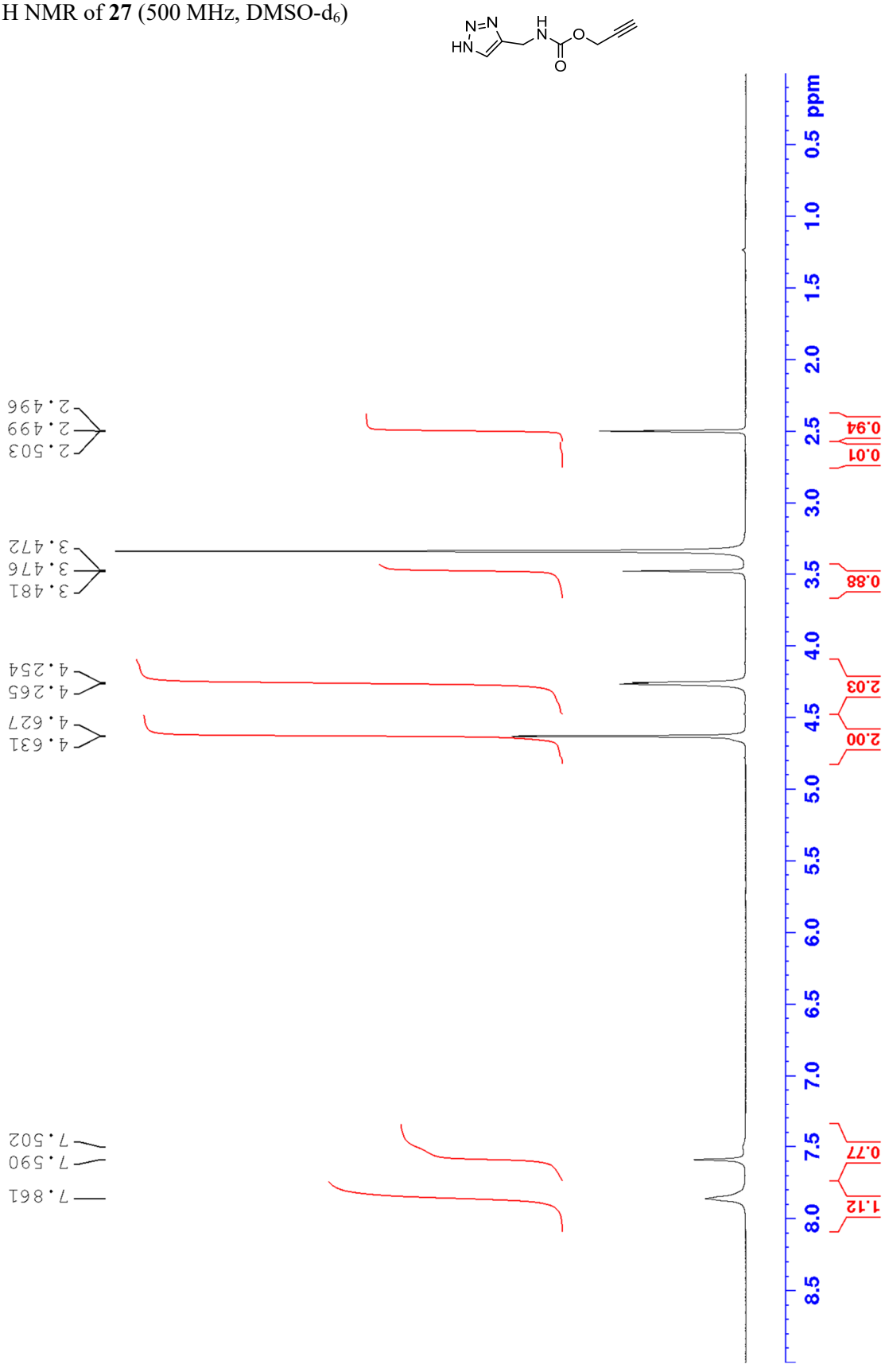




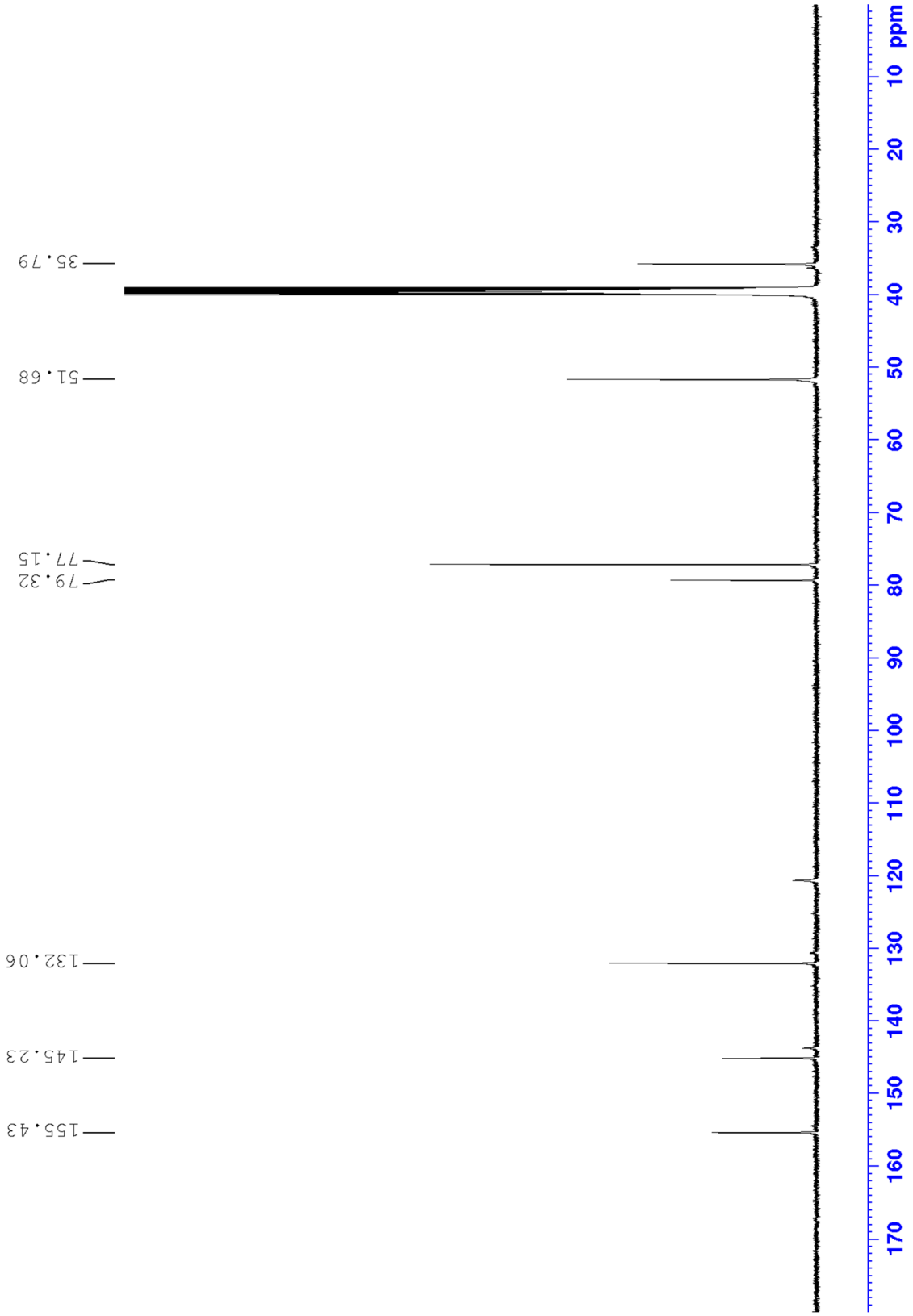
^{13}C NMR of **S11** (151 MHz, $CDCl_3$)



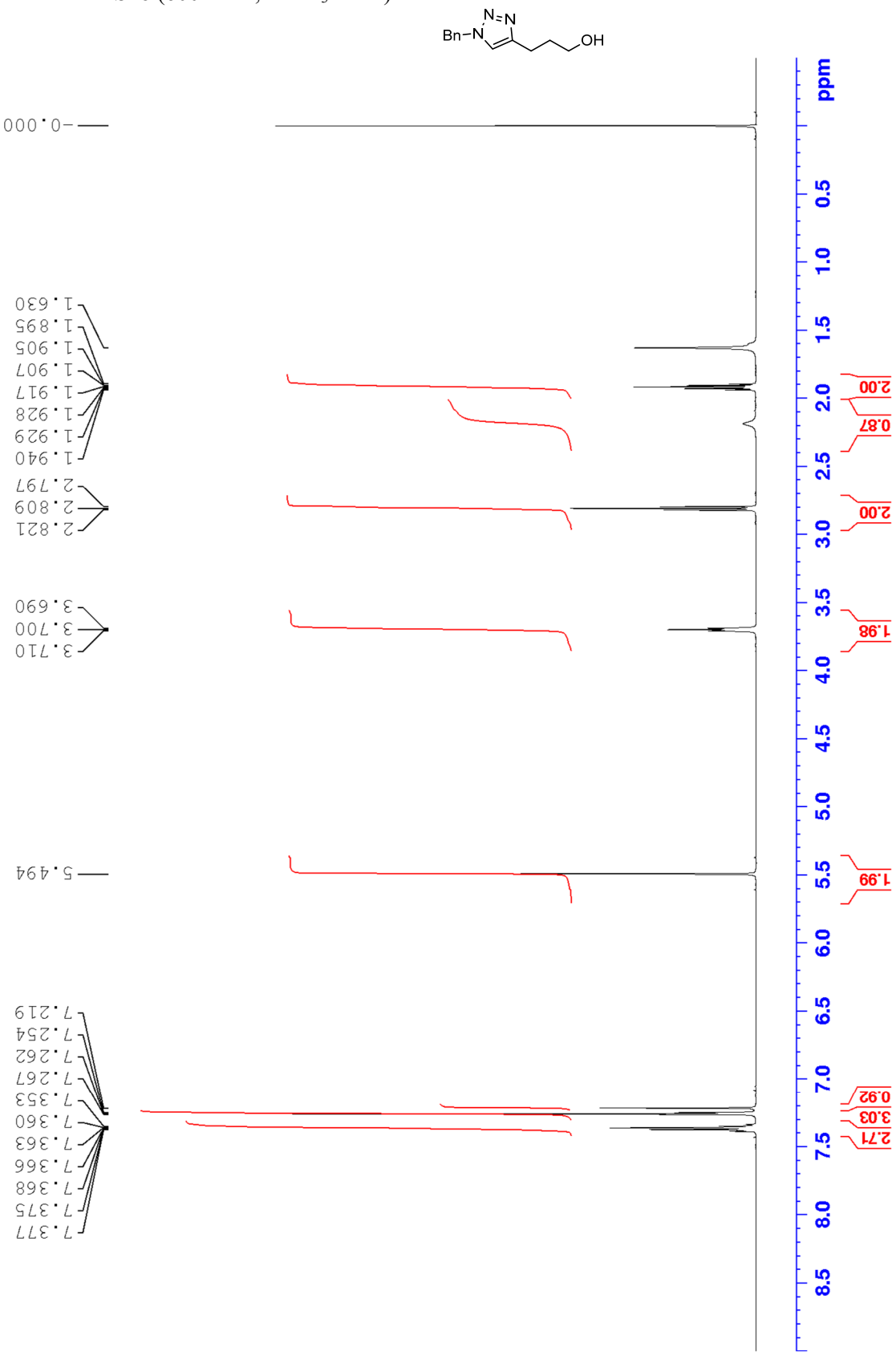
^{1}H NMR of **27** (500 MHz, DMSO-d_6)



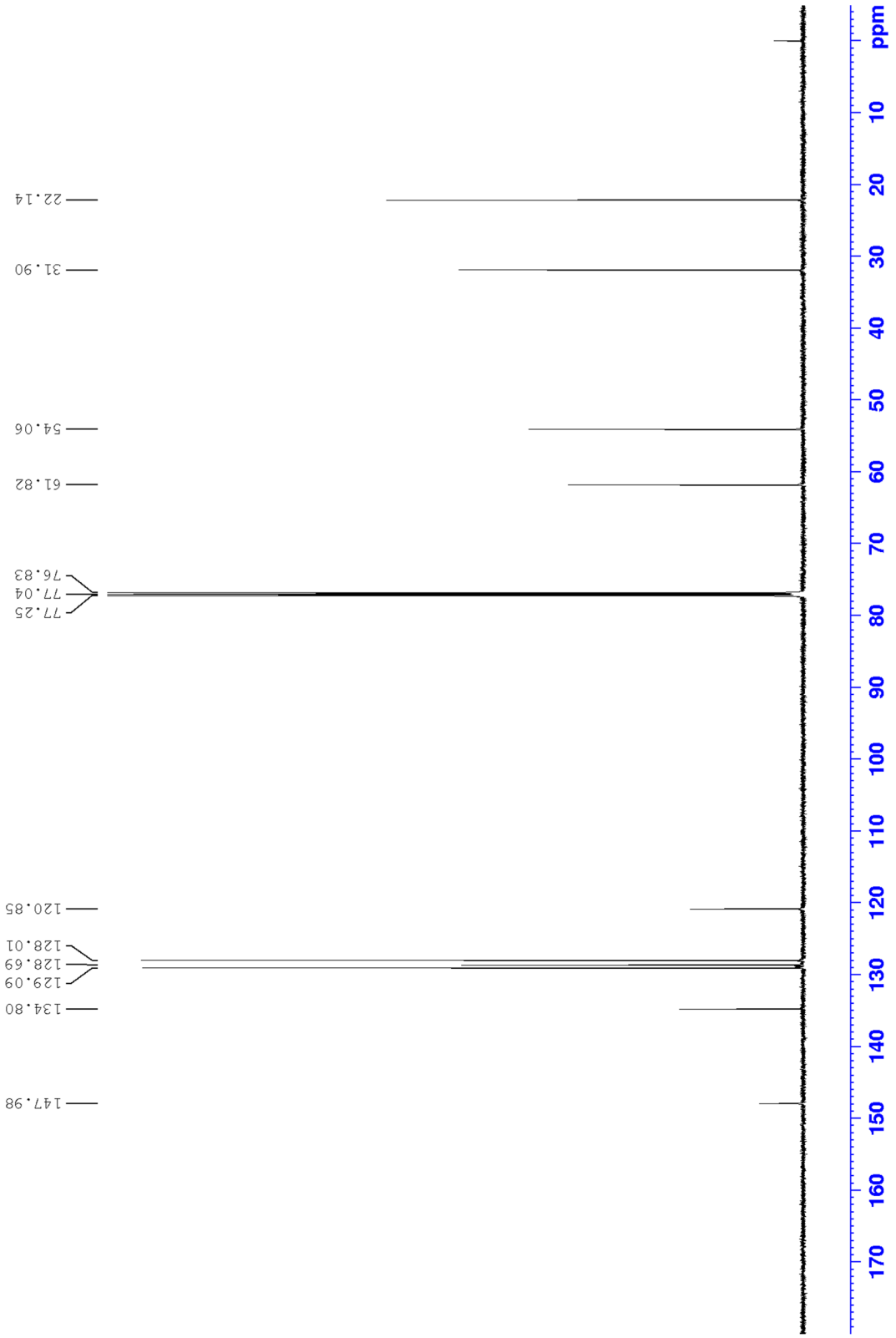
^{13}C NMR of **27** (126 MHz, DMSO-d_6)



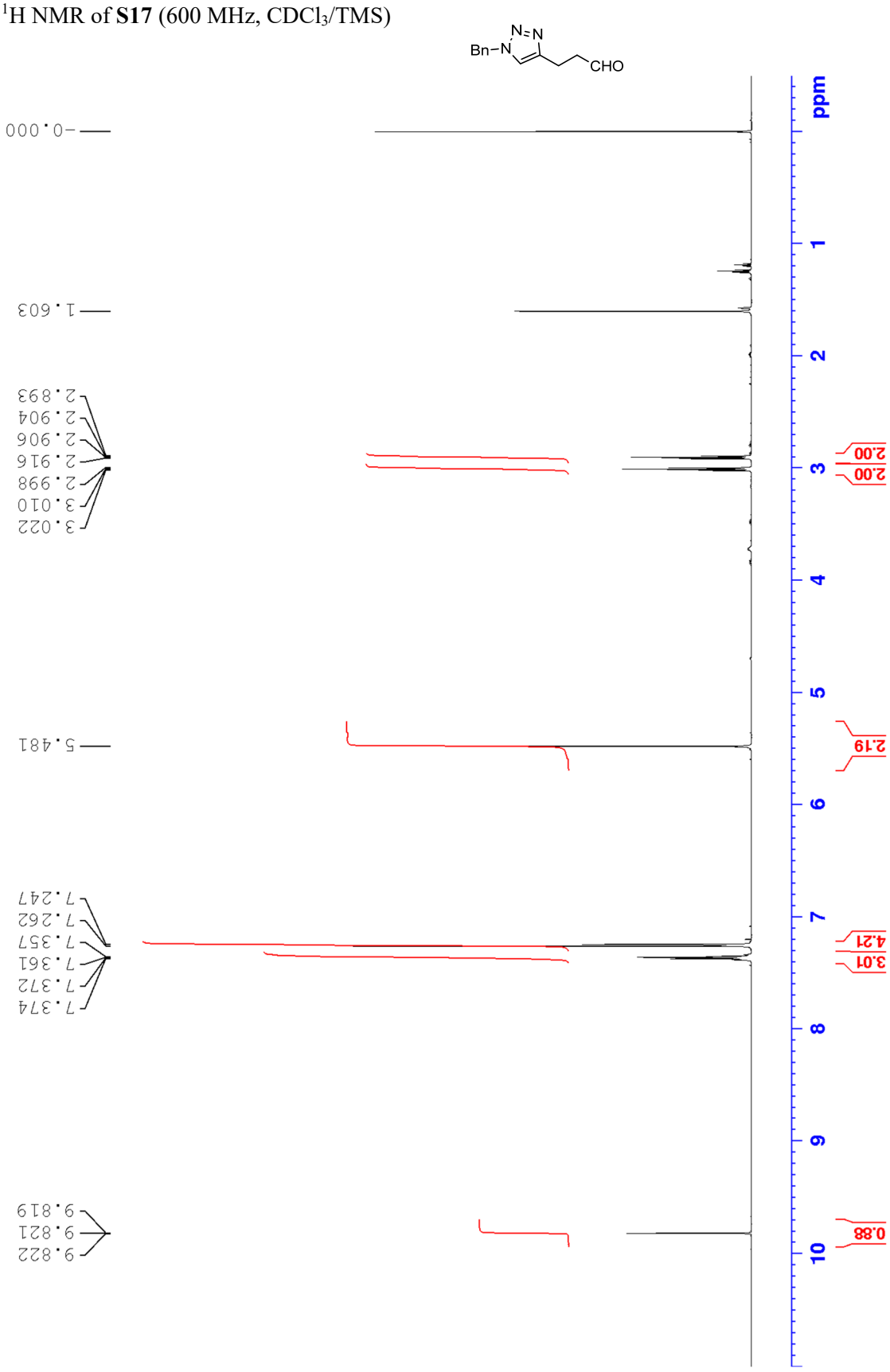
^{1}H NMR of **S16** (600 MHz, $CDCl_3$/TMS)



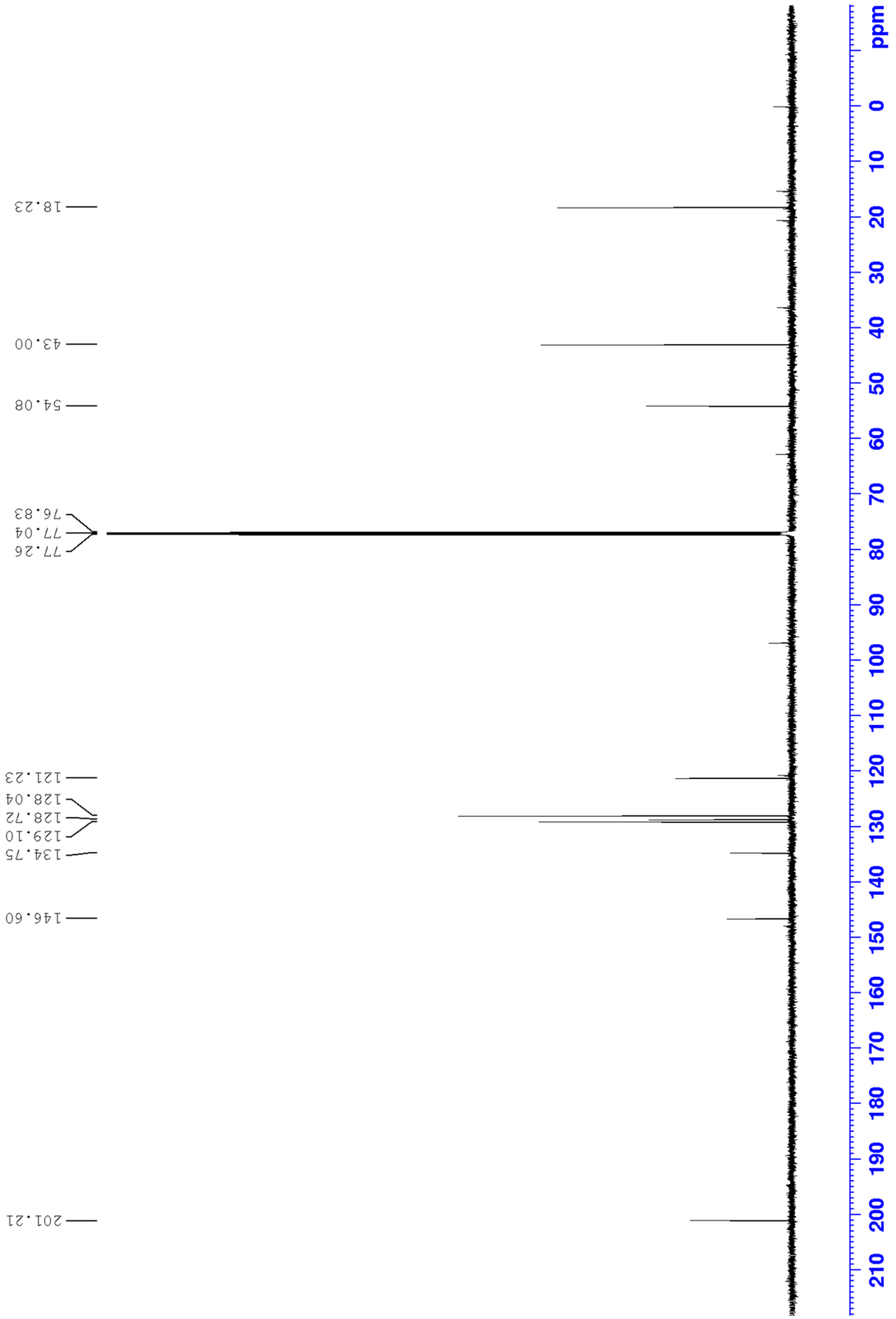
^{13}C NMR of **S16** (151 MHz, $CDCl_3$)



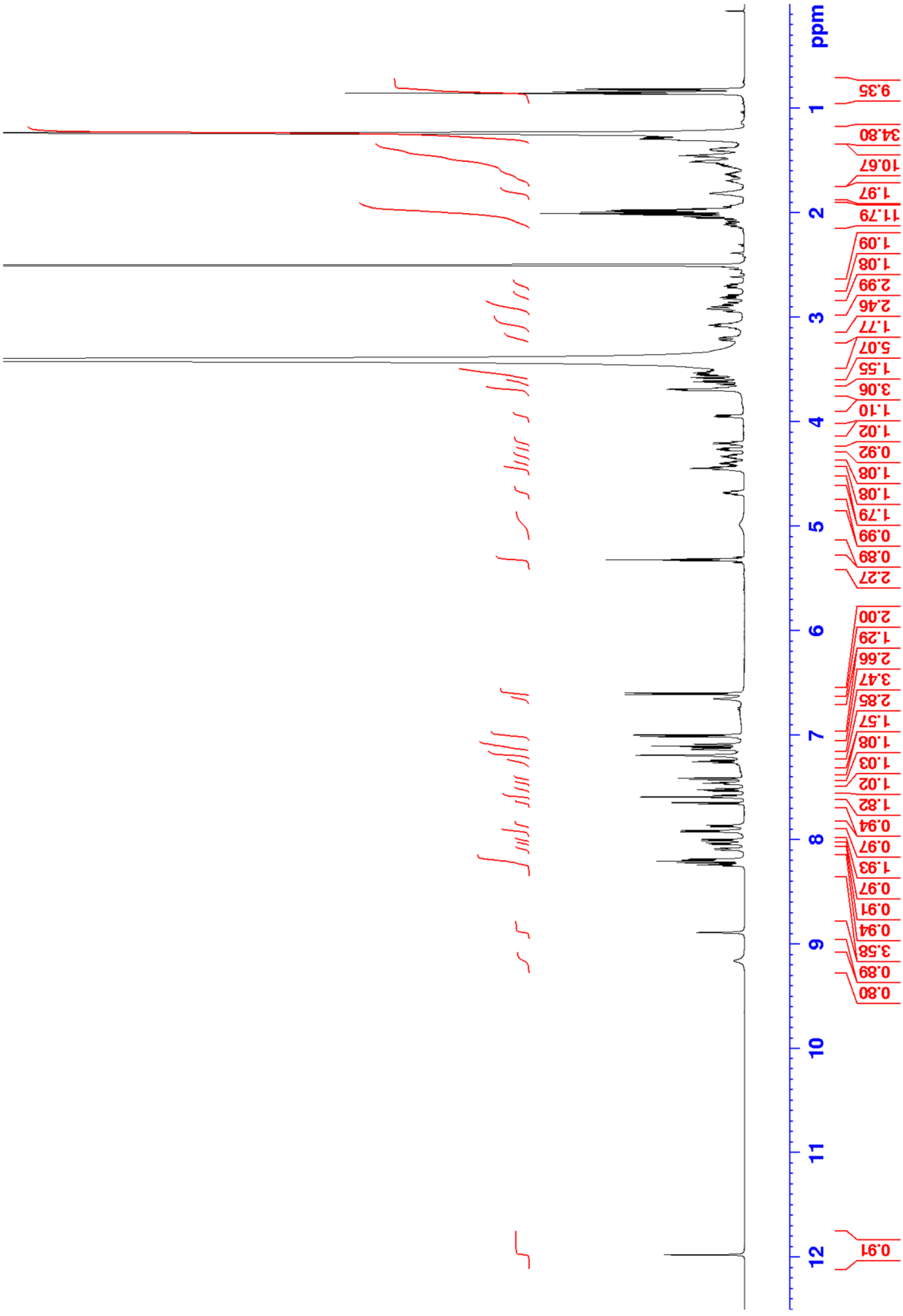
^{1}H NMR of **S17** (600 MHz, $CDCl_3$/TMS)



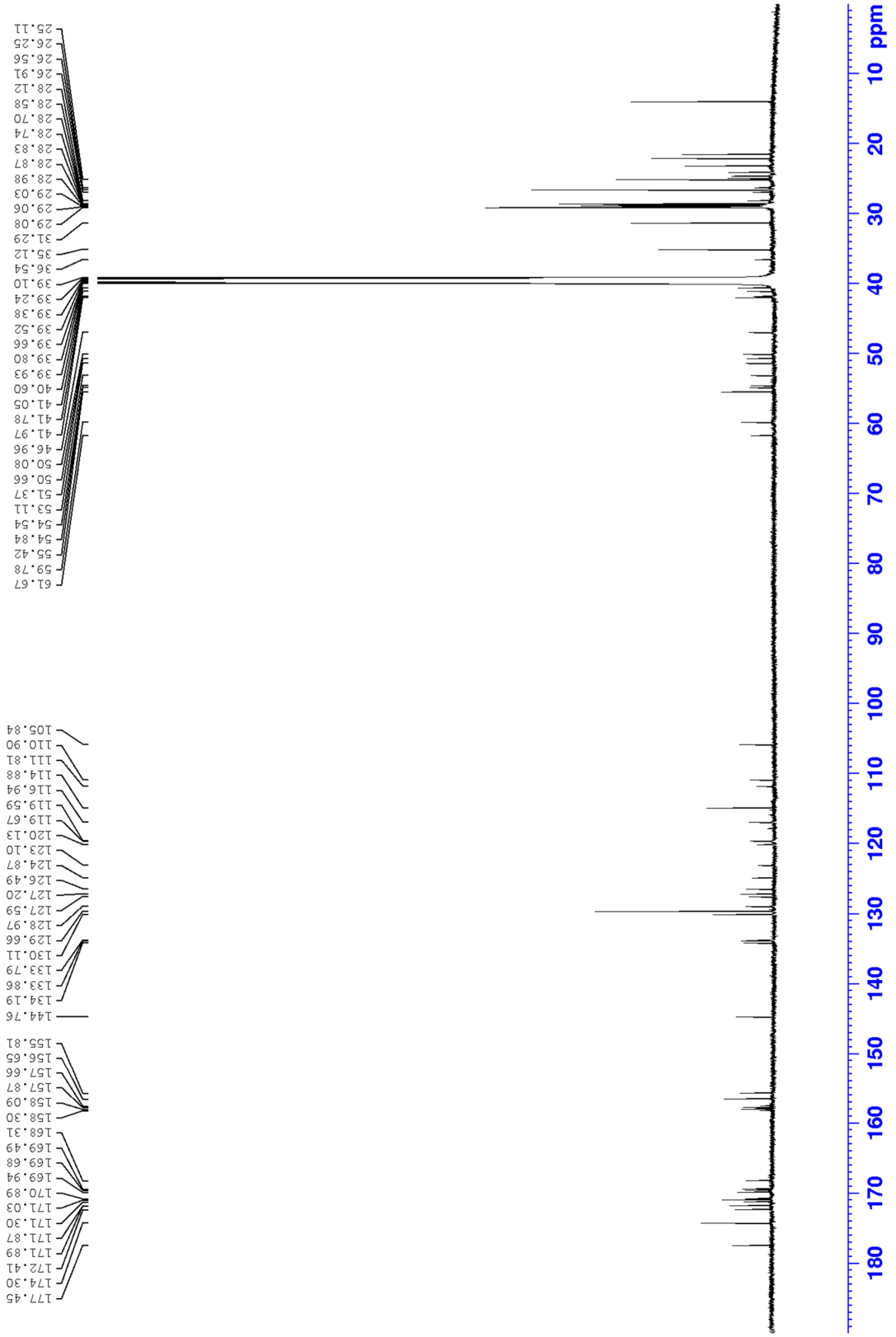
^{13}C NMR of **S17** (151 MHz, $CDCl_3$)



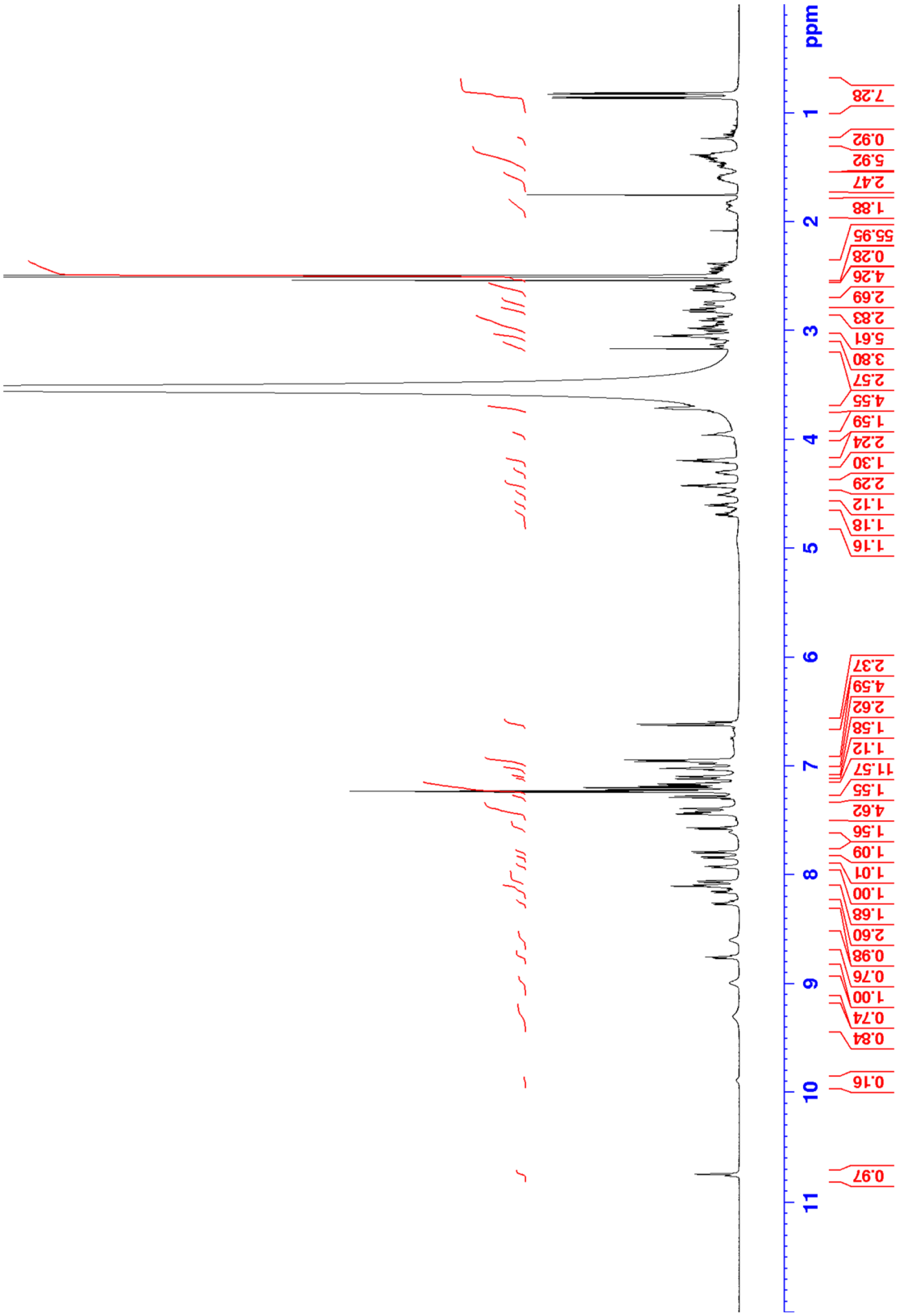
^{1}H NMR of **23** (600 MHz, DMSO-d_6)



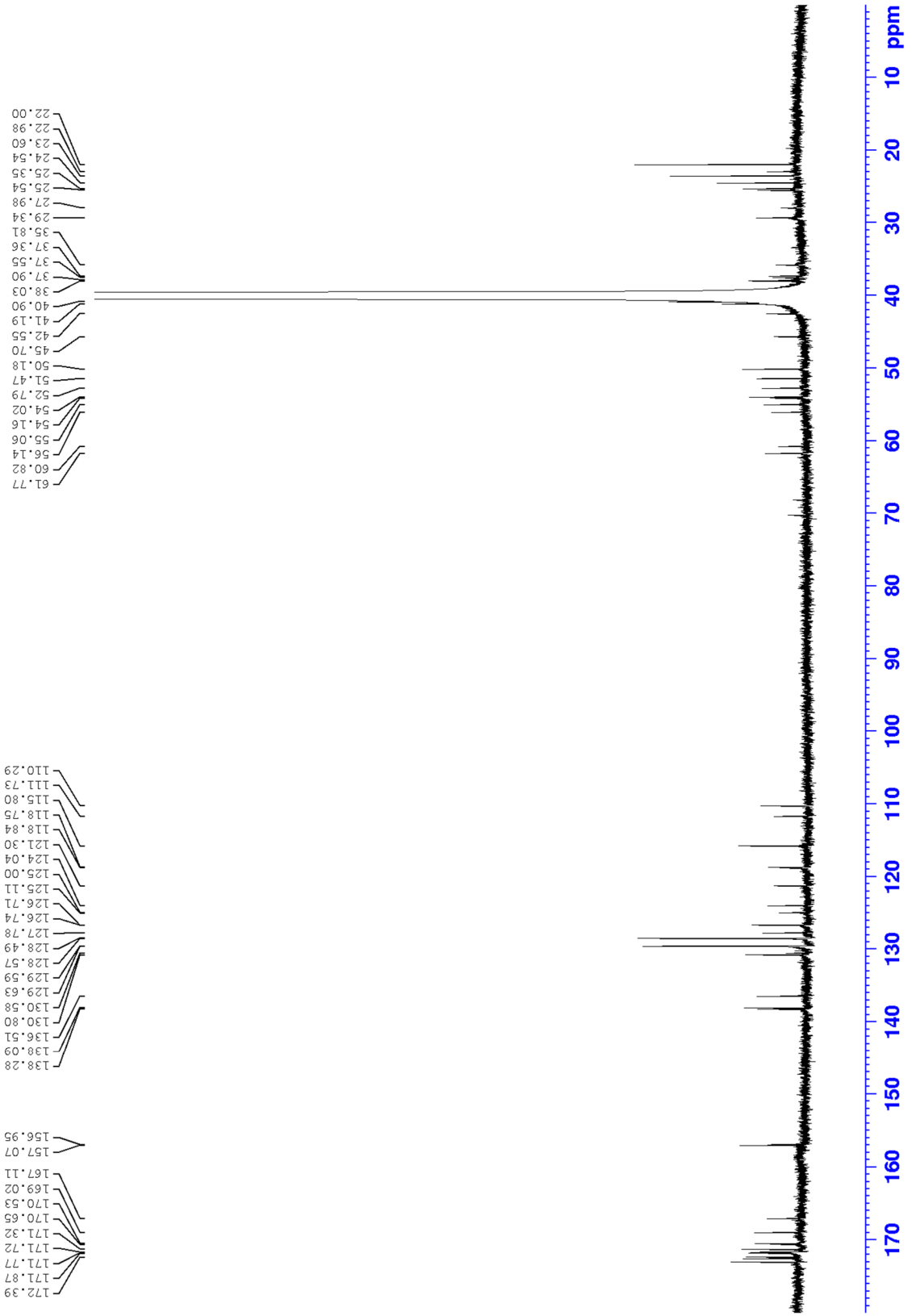
^{13}C NMR of **23** (151 MHz, DMSO-d_6)



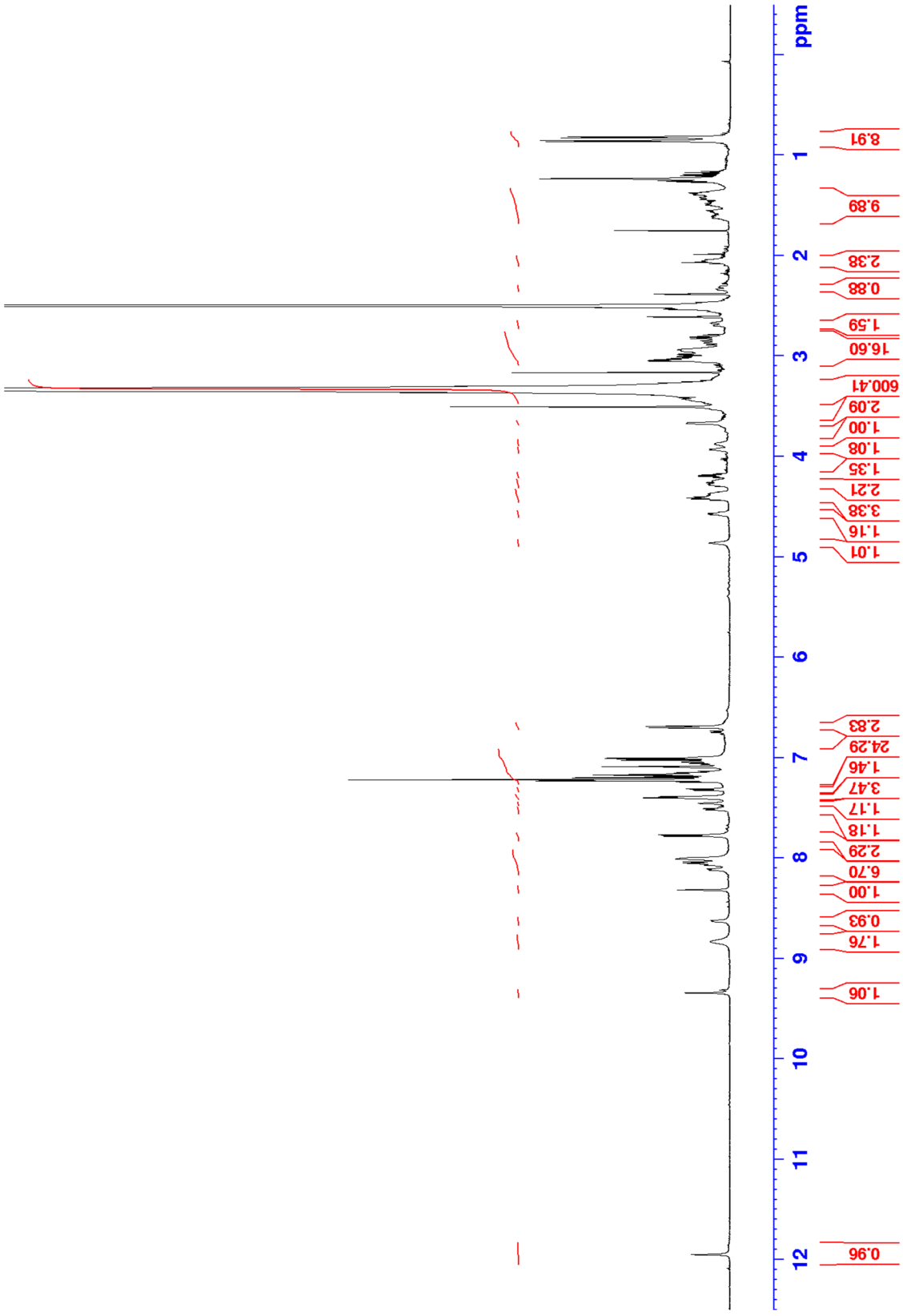
^{1}H NMR of **TP–13** (600 MHz, DMSO-d_6)



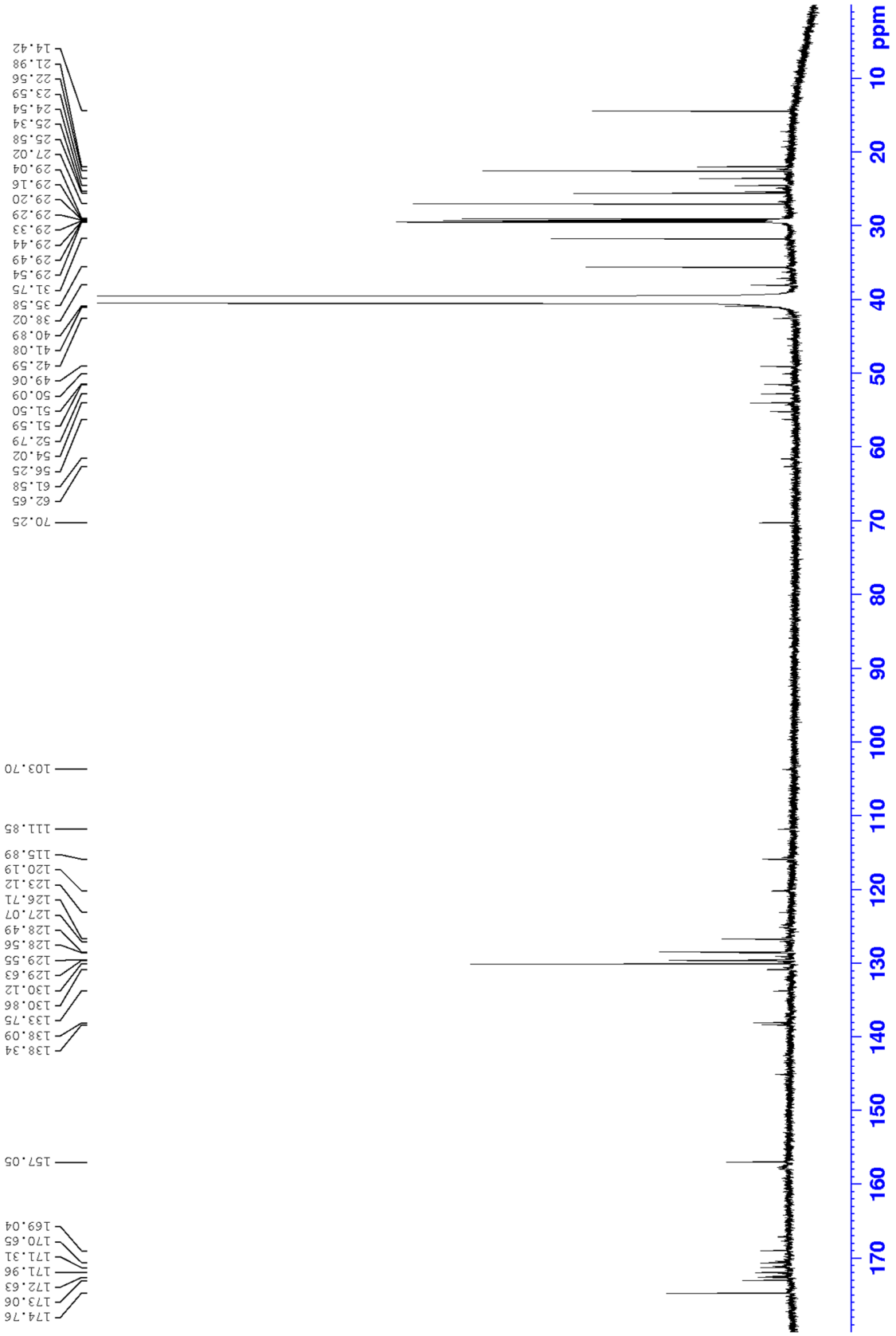
^{13}C NMR of **TP–13** (151 MHz, DMSO-d_6)



^{1}H NMR of **29** (600 MHz, DMSO-d_6)



^{13}C NMR of **29** (151 MHz, DMSO-d_6)



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