Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Antimicrobially Active Cycloundecapeptides Related to Gramicidin S Having A Novel Turn Structure with *cis* D-Phe-Pro Amido Bond

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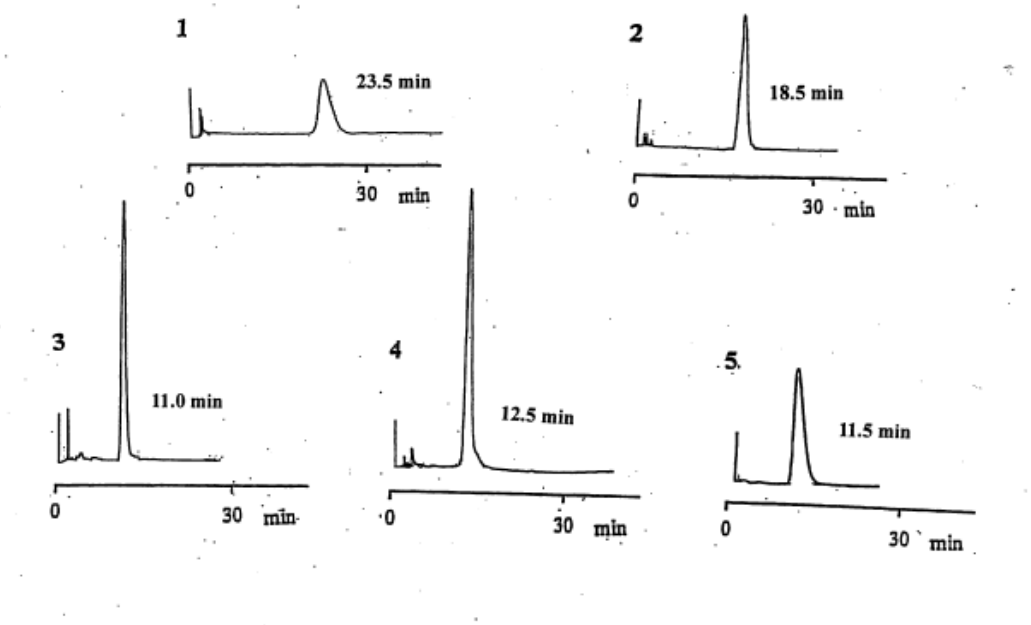
HPLC analyses of 1-5

HPLC analyses of **1-5** were achieved using analytical reverse phase HPLC system (800 Series, JASCO LTD., Tokyo, Japan), equipped with an 880 intelligent HPLC pump, an 875-UV intelligent UV/Vis detector, an 860-CD column oven, and a TSK-Gel C18 column (4.6 x 150 mm, 10 mm particle size, Tosoh Co., Tokyo, Japan). Chromatographies were carried out by a flow rate of 1 ml/min at 30 ºC and monitored at 220 nm. Elution solvents used methanol-0.1% TFAaq (70:30) for **1**, methanol-0.1 % TFAaq (65:35) for **2** and methanol-0.1 % TFAaq (55:45) for **3**-**5**. HPLC profiles of **1-5** were shown in next figures.



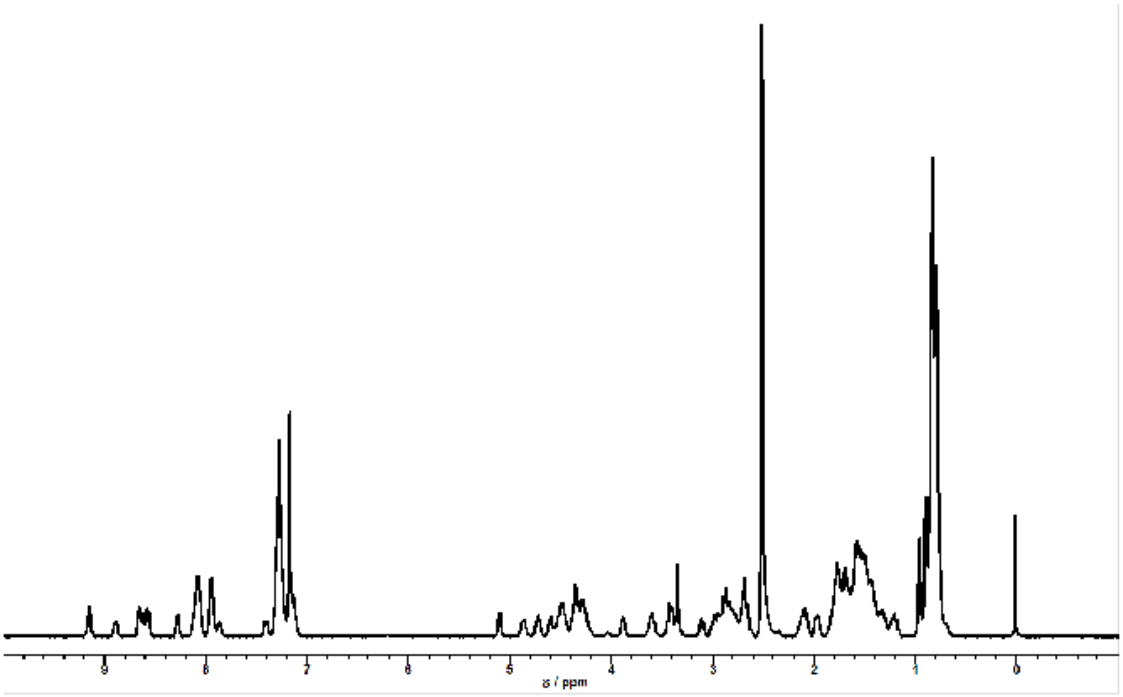


Figure 2. ^{1}H NMR spectrum of **1** in DMSO-d_6 at 30 °C.

Table 1. All ^{1}H chemical shifts (δ in ppm) of **1**.

		$^{\alpha}$NH	$^{\alpha}$H	$^{\beta}$H	$^{\gamma}$H	$^{\delta}$H	$^{\delta}$NH	others
δ (ppm)	Val[1]	7.28	4.48	2.09	0.82			
	Orn[2]	8.59	4.88	1.57	1.59	2.68	8.09	
	Leu[3]	8.56	4.53	1.55	1.57	0.81		
	Leu[4]	8.03	3.88	1.51 1.35	1.77	0.89		
	D-Phe[5]	8.67	4.28	3.12 2.67				Ar 7.34-7.1
	Pro[6]		5.11	2.12	1.73	3.50 3.34		
	Val[7]	7.40	4.32	1.77	0.82			
	Orn[8]	8.87	4.72	1.66 1.82	1.55 1.73	2.76 2.93	7.95	
	Leu[9]	8.28	4.59	1.21 1.44	1.48	0.82		
	D-Phe[10]	9.11	4.38	3.00 2.87				Ar 7.34-7.1
	Pro[11]		4.34	1.99 1.51	1.62	3.58 2.52		

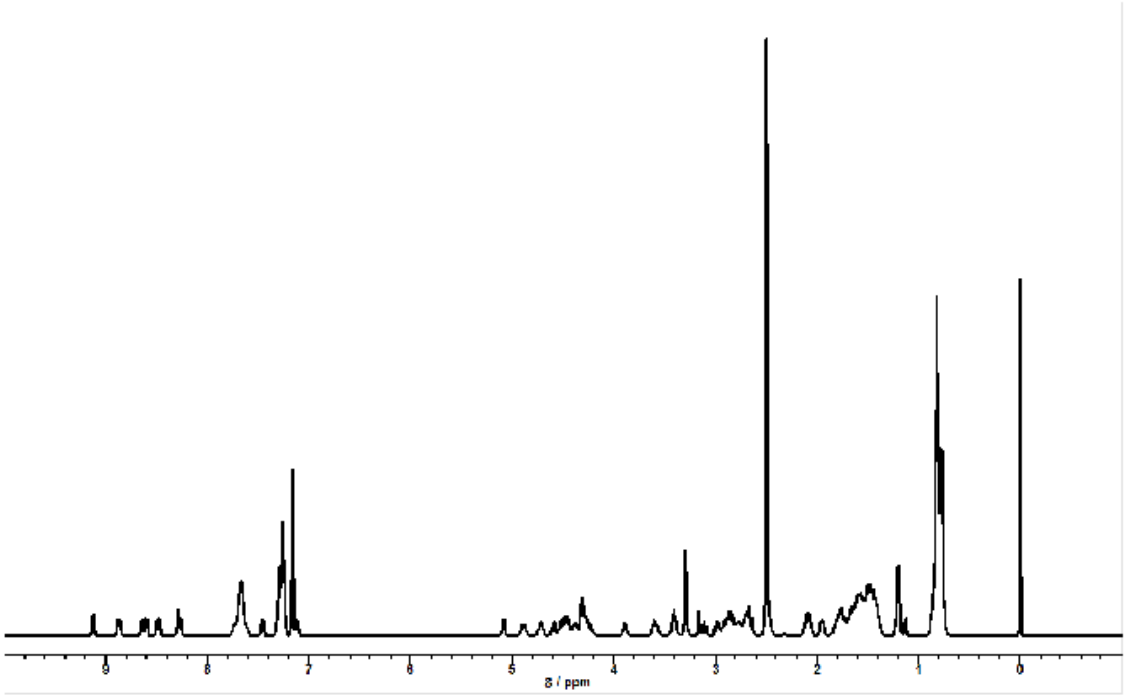


Figure 3. ^{1}H NMR spectrum of **2** in DMSO-d_6 at 30 °C.

Table 2. All ^{1}H chemical shifts (δ in ppm) of **2**.

		$^{\alpha}$NH	$^{\alpha}$H	$^{\beta}$H	$^{\gamma}$H	$^{\delta}$H	$^{\delta}$NH	others
δ (ppm)	Val^{1}	7.24	4.47	2.10	0.81			
	Orn^{2}	8.66	4.88	1.60 1.48	1.45	2.71	7.67	
	Leu^{3}	8.49	4.53	1.55 1.48	1.55	0.78		
	Ala^{4}	8.31	3.88	1.22				
	D-Phe^{5}	8.60	4.27	3.12 2.68				Ar 7.1-7.32
	Pro^{6}		5.09	2.10	1.77	3.42		
	Val^{7}	7.45	4.29	1.77	0.81			
	Orn^{8}	8.87	4.70	1.88 1.77	1.60	2.71	7.67	
	Leu^{9}	8.27	4.59	1.45 1.22	1.39	0.84		
	D-Phe^{10}	9.13	4.38	2.98 2.86				Ar 7.1-7.32
	Pro^{11}		4.30	1.97 1.48	1.60	3.62 2.46		

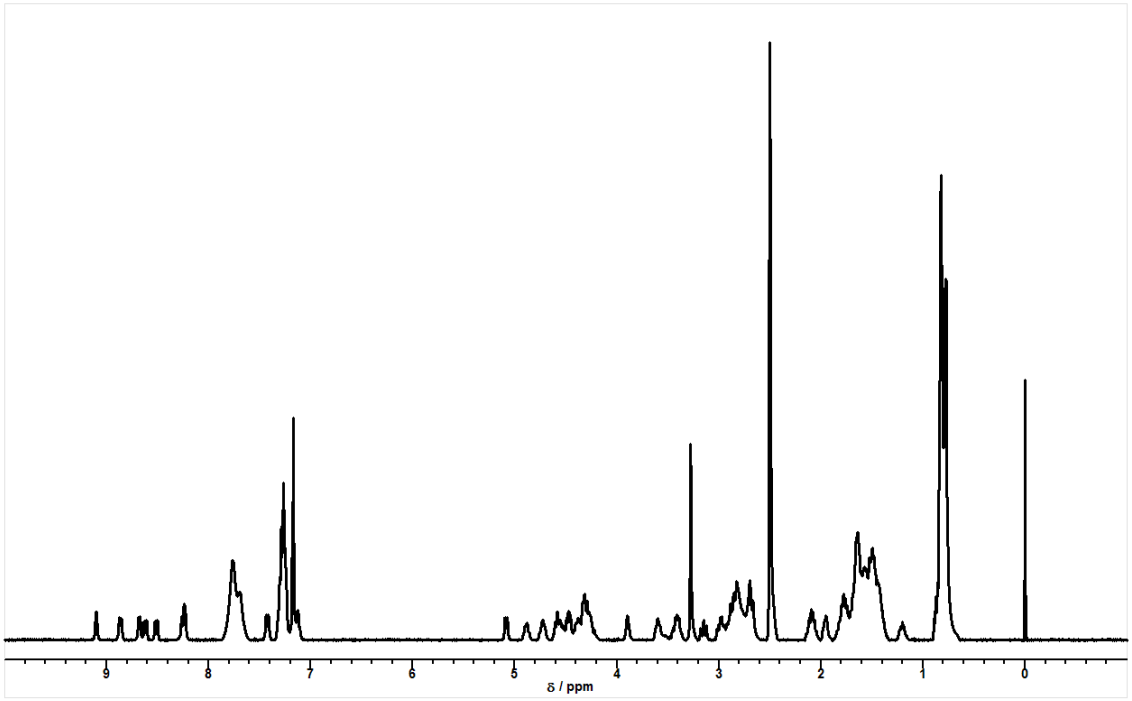


Figure 4. ^{1}H NMR spectrum of **3** in DMSO-d_6 at 30 °C.

Table 3. All ^{1}H chemical shifts (δ in ppm) of **3**.

		$^{\alpha}$NH	$^{\alpha}$H	$^{\beta}$H	$^{\gamma}$H	$^{\delta}$H	$^{\delta}$NH	others
δ (ppm)	Val^{1}	7.25	4.47	2.08	0.81			
	Orn^{2}	8.62	4.88	1.61 1.54	1.48	2.71	7.74	
	Leu^{3}	8.53	4.54	1.51	1.55	0.81		
	Orn^{4}	8.22	3.88	1.64	1.57	2.84	7.66	
	D-Phe^{5}	8.68	4.28	3.15 2.68				Ar 7.09-7.32
	Pro^{6}		5.10	2.11	1.86 1.71	3.41		
	Val^{7}	7.43	4.31	1.77	0.81			
	Orn^{8}	8.87	4.72	1.80 1.73	1.61	2.78	7.78	
	Leu^{9}	8.25	4.56	1.44	1.50	0.81		
	D-Phe^{10}	9.10	4.38	3.00 2.89				Ar 7.09-7.32
	Pro^{11}		4.32	1.93 1.50	1.50	3.59 2.49		

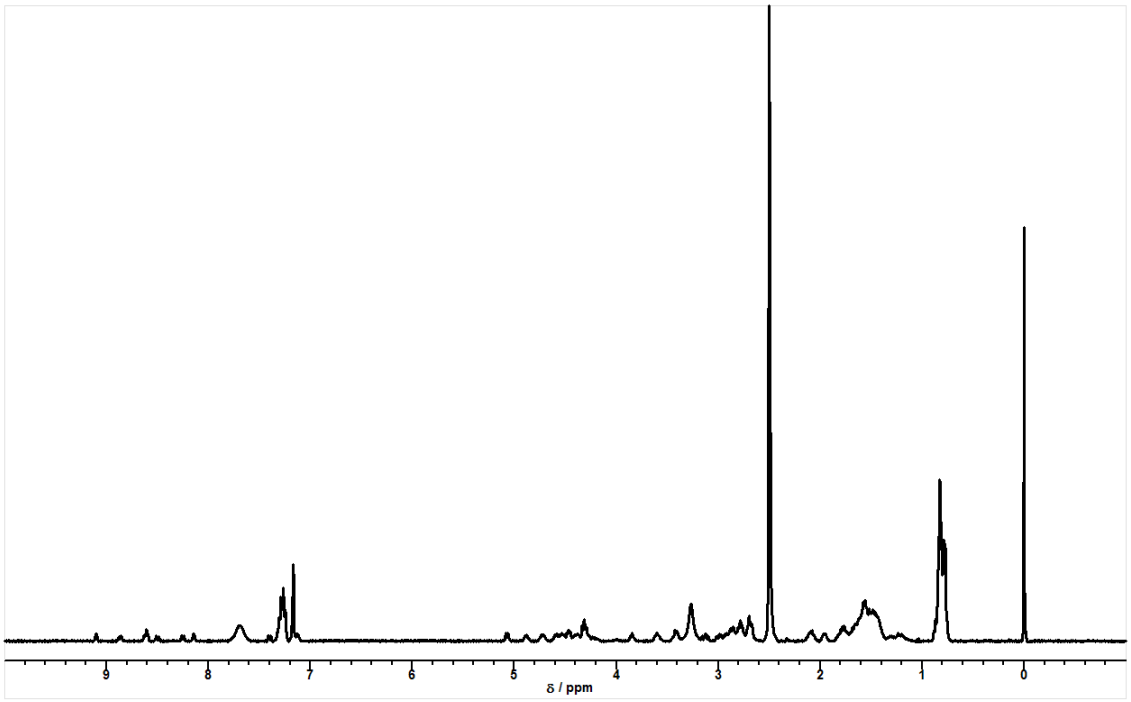


Figure 5. ^{1}H NMR spectrum of **4** in DMSO-d_6 at 30 °C.

Table 4. All ^{1}H chemical shifts (δ in ppm) of **4**.

		$^{\alpha}$NH	$^{\alpha}$H	$^{\beta}$H	$^{\gamma}$H	$^{\delta}$H	$^{\delta}$NH	others
δ (ppm)	Val^{1}	7.24	4.47	2.09	0.80			
	Orn^{2}	8.62	4.88	1.59 1.48	1.45	2.70	7.68	
	Leu^{3}	8.51	4.53	1.55 1.48	1.52	0.80		
	Lys^{4}	8.13	3.85	1.56	1.41	1.30		$^{\varepsilon}$H 2.78 $^{\varepsilon}$NH 7.68
	D-Phe^{5}	8.60	4.29	3.14 2.68				Ar 7.34 - 7.10
	Pro^{6}		5.06	2.09	1.69	3.41		
	Val^{7}	7.39	4.32	1.77	0.80			
	Orn^{8}	8.86	4.73	1.82 1.77	1.59	2.79	7.69	
	Leu^{9}	8.24	4.59	1.41 1.22	1.48	0.80		
	D-Phe^{10}	9.09	4.38	2.97 2.86				Ar 7.34 - 7.10
	Pro^{11}		4.30	1.96 1.50	1.50	3.60 2.50		

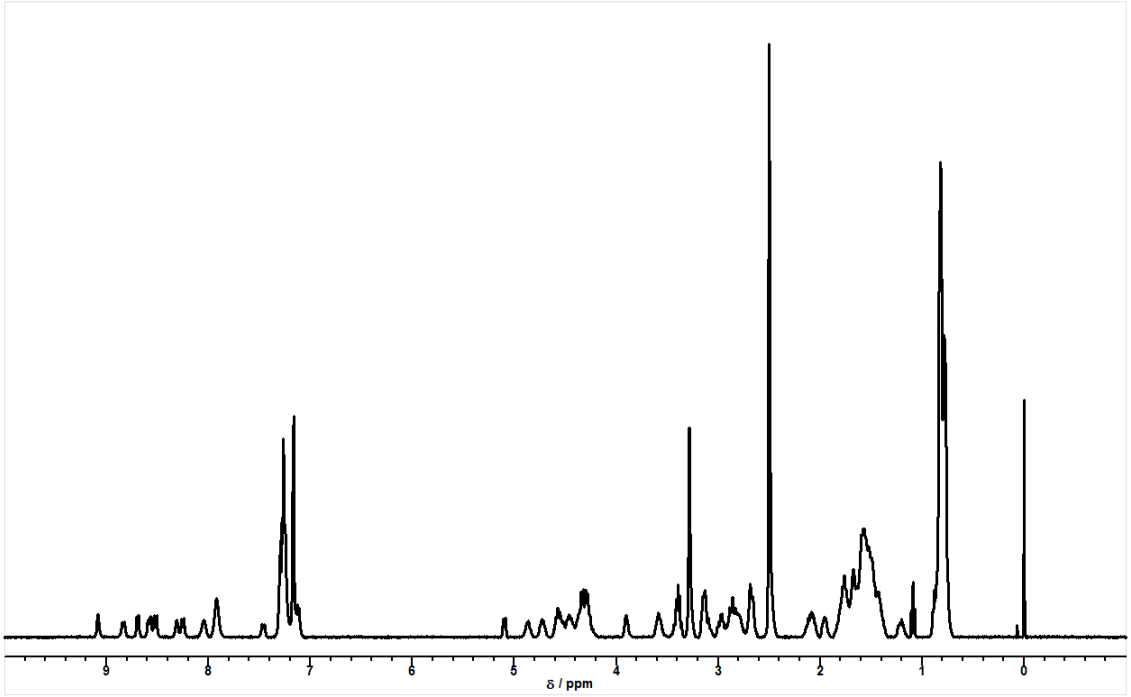


Figure 6. ^{1}H NMR spectrum of **5** in DMSO-d_6 at 30 °C.

Table 5. All ^{1}H chemical shifts (δ in ppm) of **5**.

		$^{\alpha}$NH	$^{\alpha}$H	$^{\beta}$H	$^{\gamma}$H	$^{\delta}$H	$^{\delta}$NH	others
δ (ppm)	Val^{1}	7.27	4.47	2.10	0.81			
	Orn^{2}	8.59	4.88	1.57	1.48	2.71	8.04	
	Leu^{3}	8.51	4.56	1.51	1.57	0.81		
	Arg^{4}	8.29	3.90	1.63	1.68 1.66	2.88 2.82	7.95	NH 7.9-7.95
	D-Phe^{5}	8.69	4.29	3.12 2.68				Ar 7.1-7.32
	Pro^{6}		5.11	2.13	1.77	3.41		
	Val^{7}	7.46	4.29	1.77	0.84			
	Orn^{8}	8.84	4.73	1.80	1.63	3.15	7.90	
	Leu^{9}	8.25	4.59	1.51	1.57	0.81		
	D-Phe^{10}	9.08	4.34	3.02 2.97				Ar 7.1-7.32
	Pro^{11}		4.32	1.98 1.48	1.49	3.59 2.51		

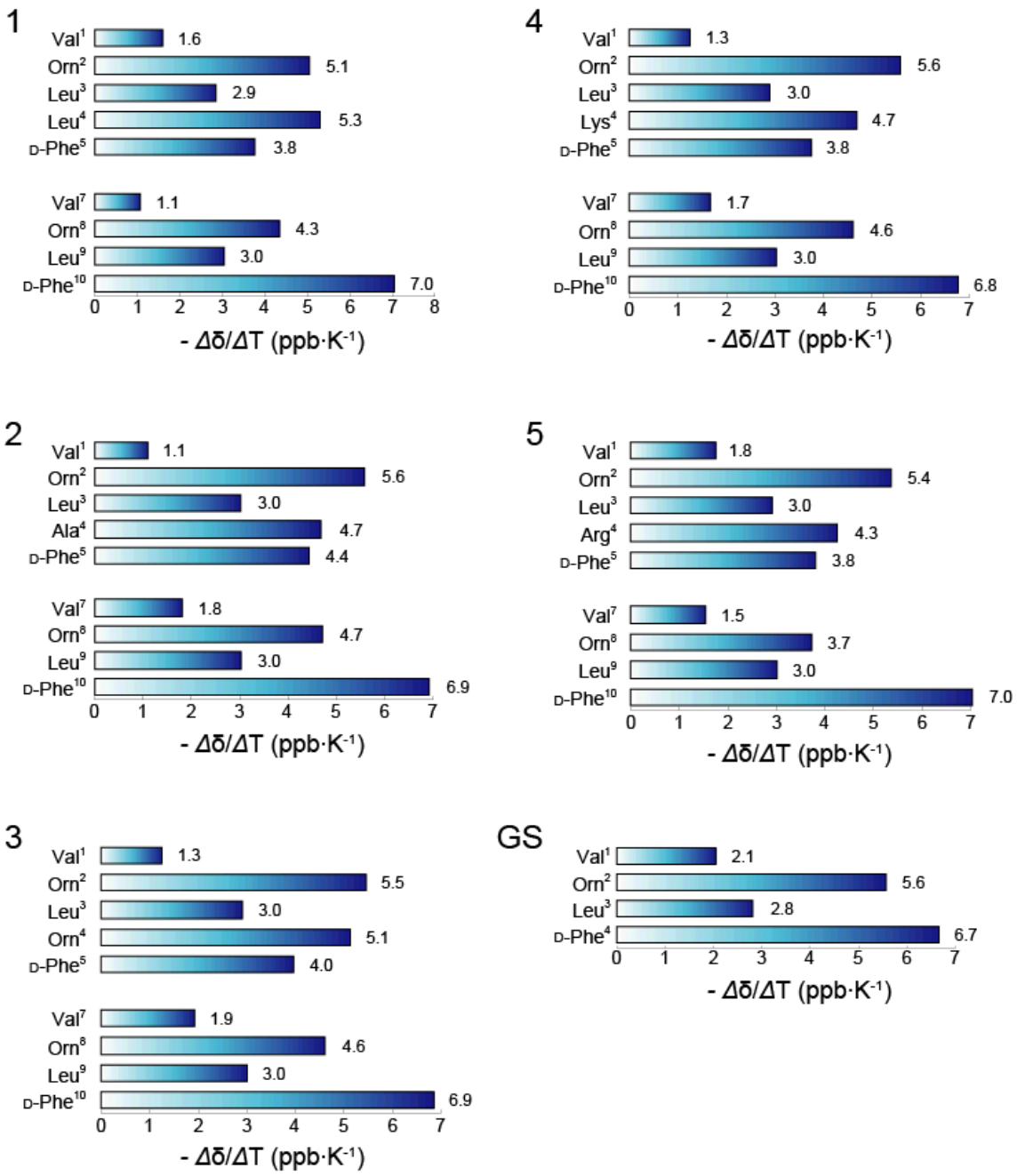


Figure 7. Temperature dependences of amide protons in **1**-**5** and **GS**.

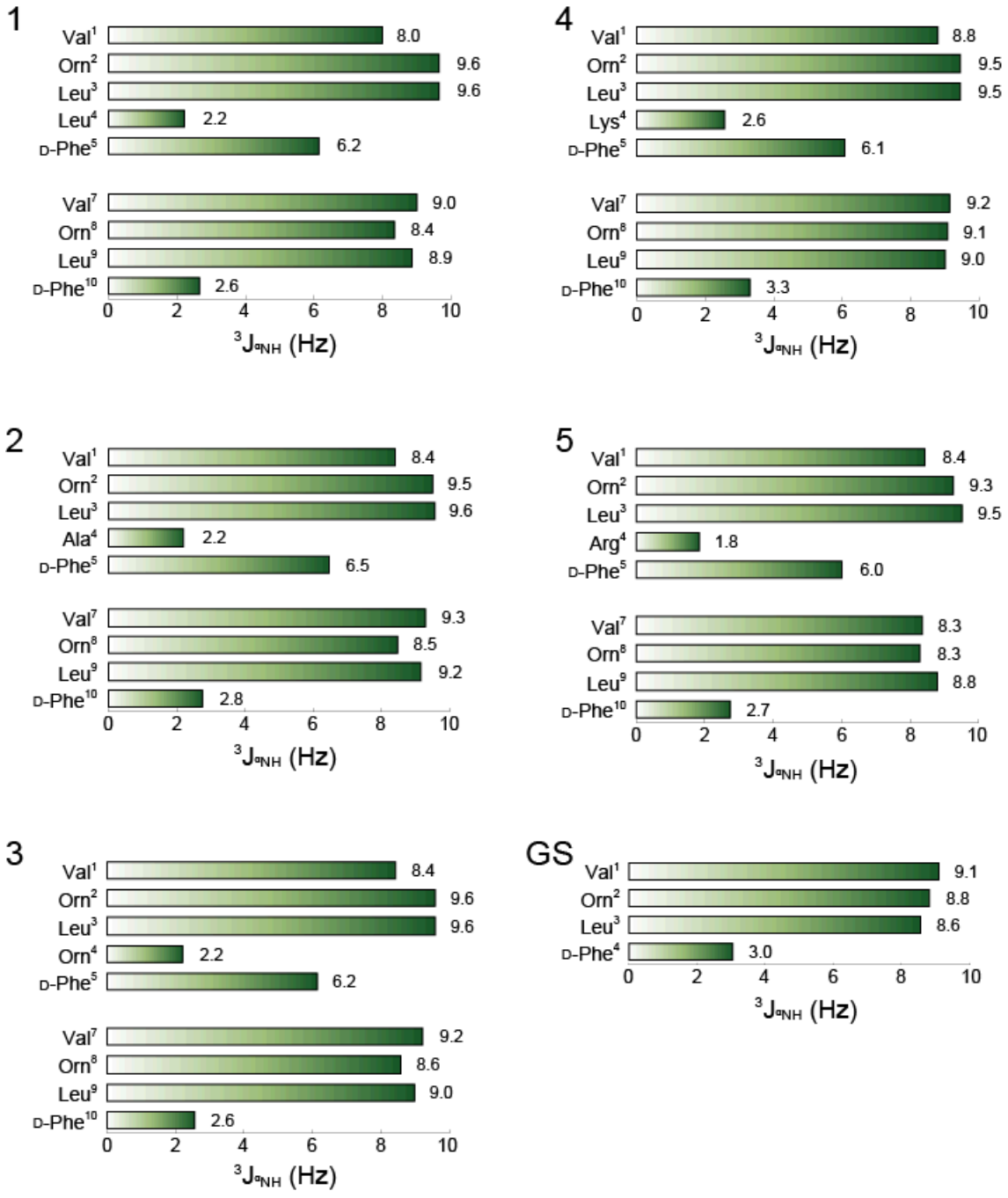


Figure 8. $J_{NH\text{-}}{}^{\alpha}{}_{CH}$ values of amide protons in **1-5** and **GS**.

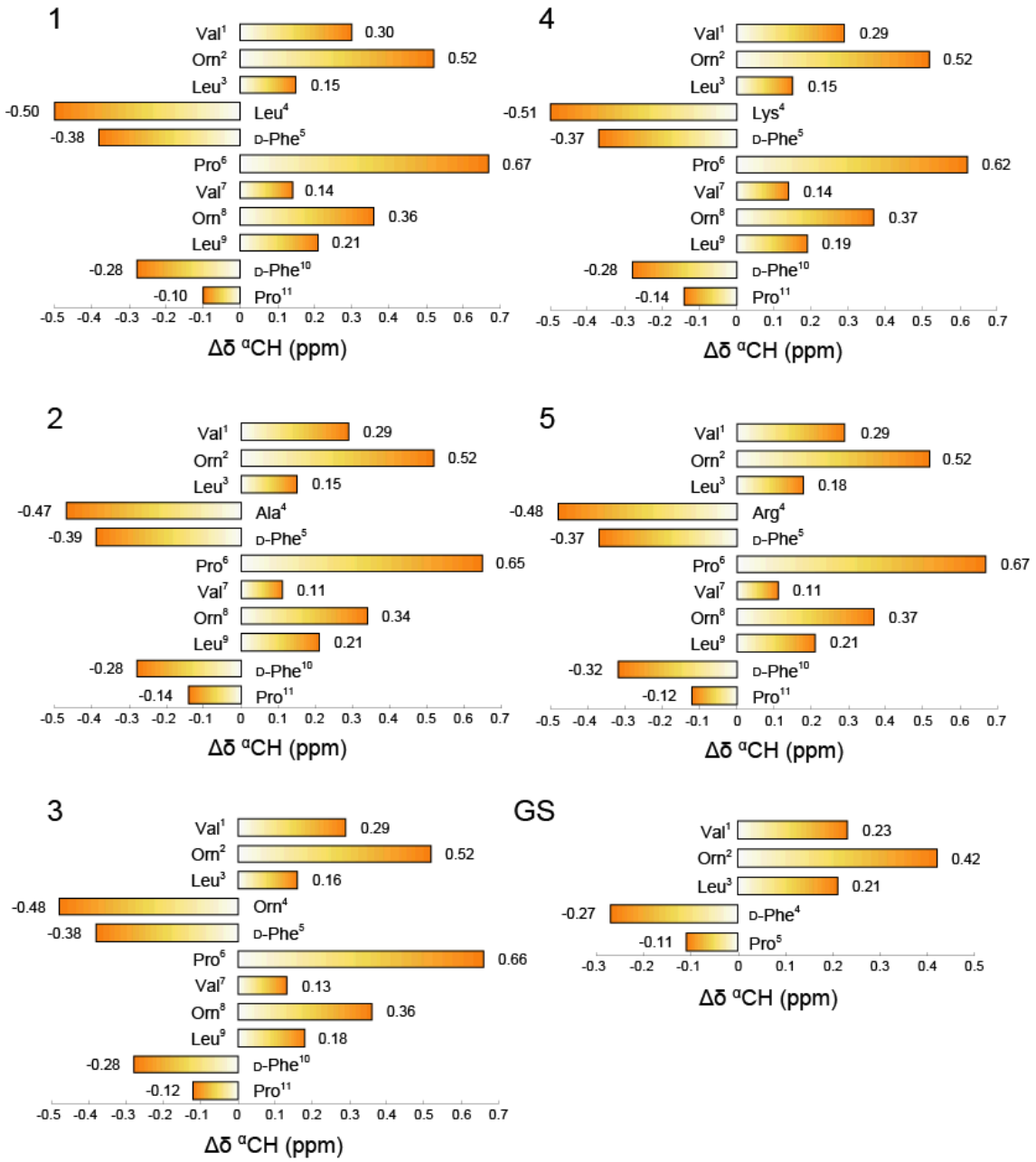


Figure 9. Chemical shift perturbations of α protons in **1**-**5** and **GS**.

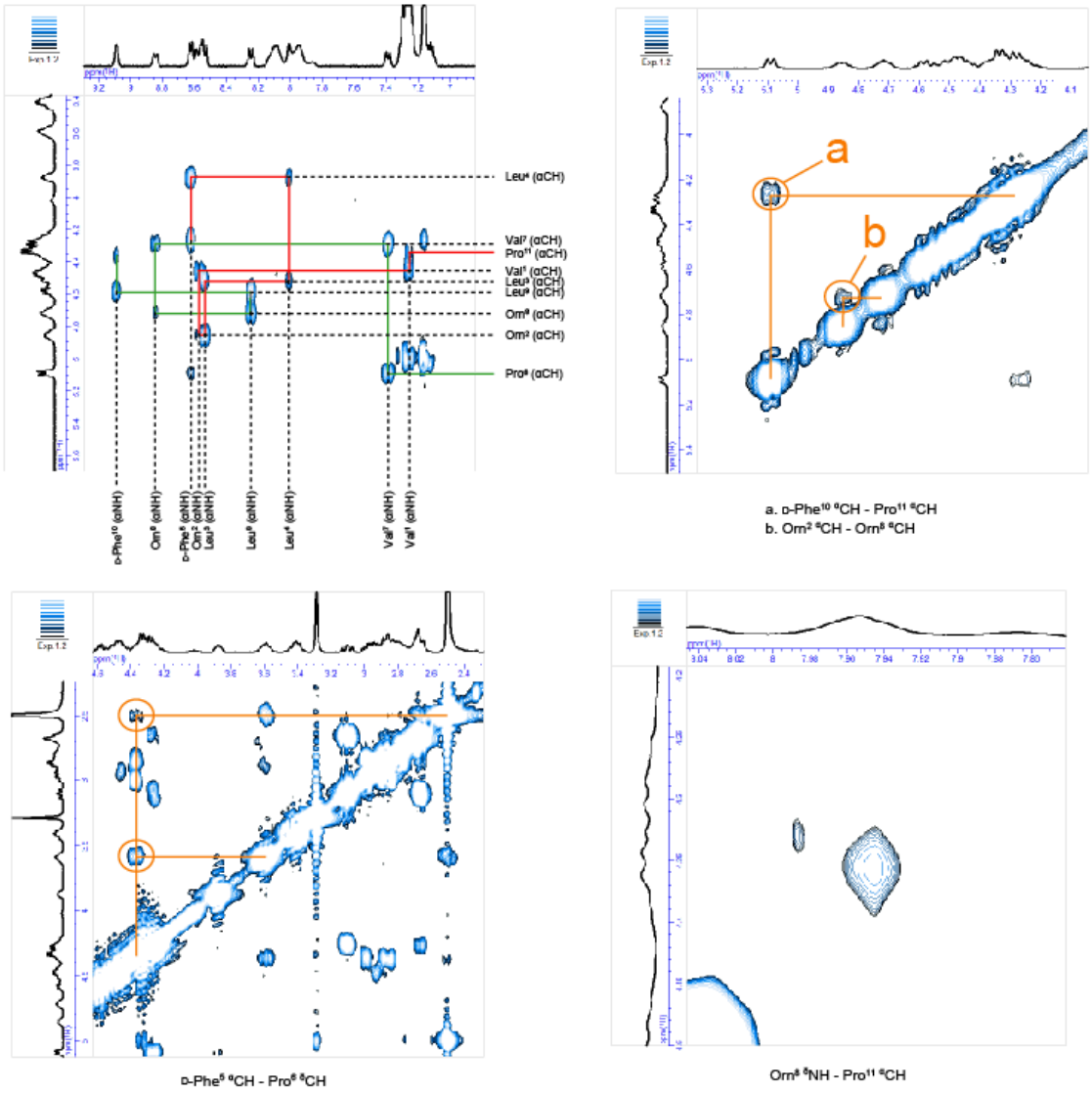


Figure 10. ROESY spectra of **1**.

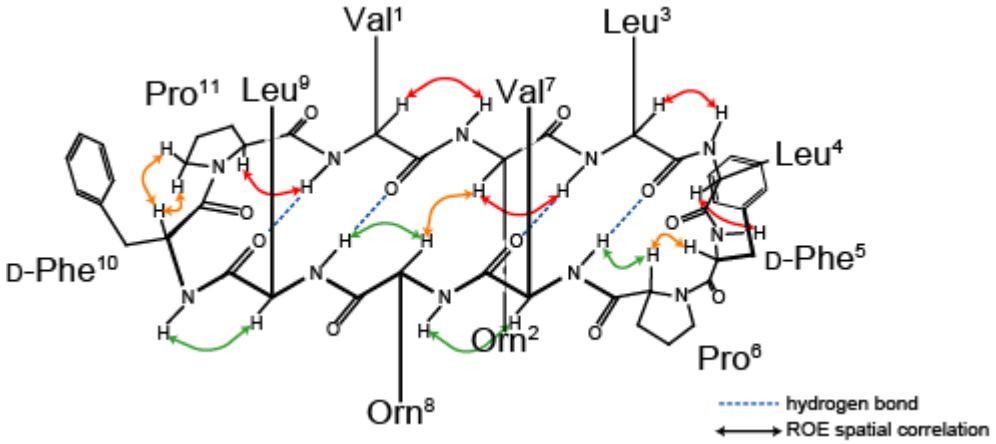


Figure 11. Proposed secondary structure and spatial ROE crosspeaks of **1**.

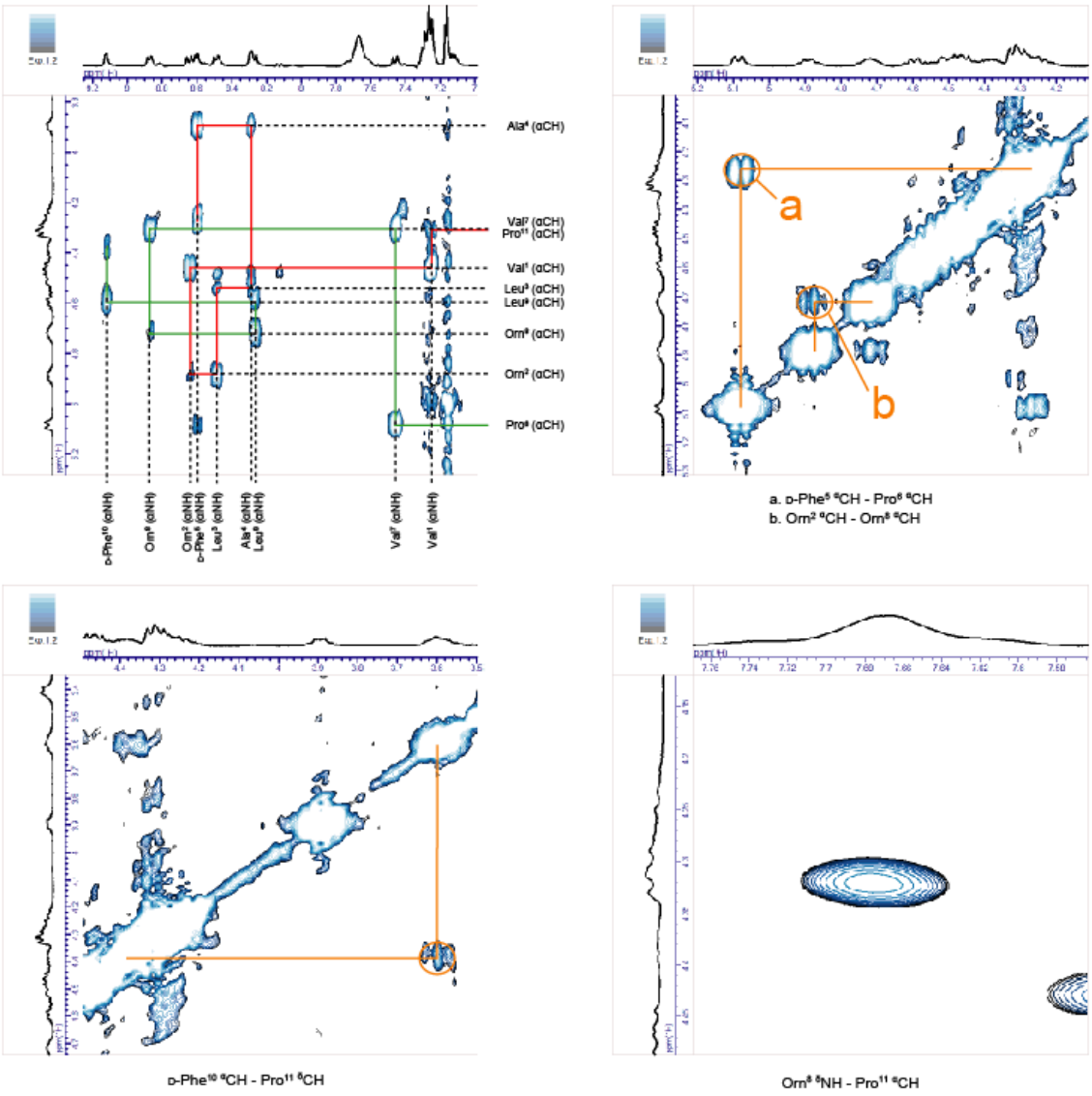


Figure 12. ROESY spectra of **2**.

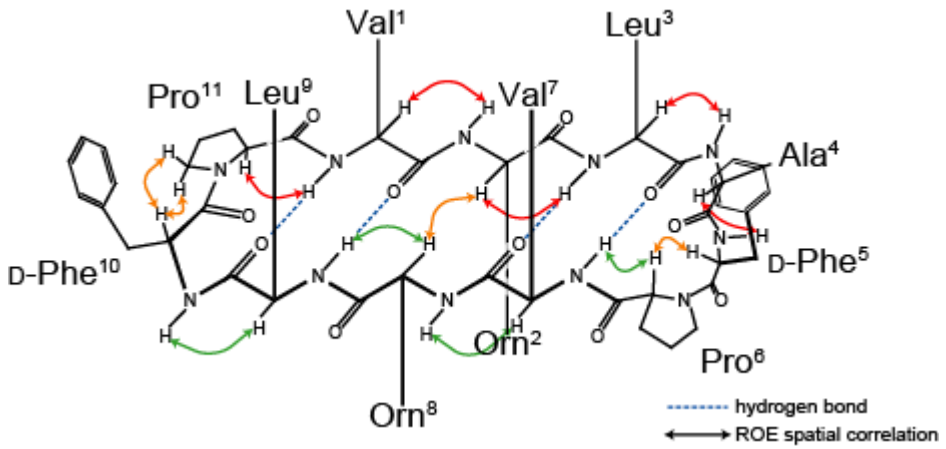


Figure 13. Proposed secondary structure and spatial ROE crosspeaks of **2**.

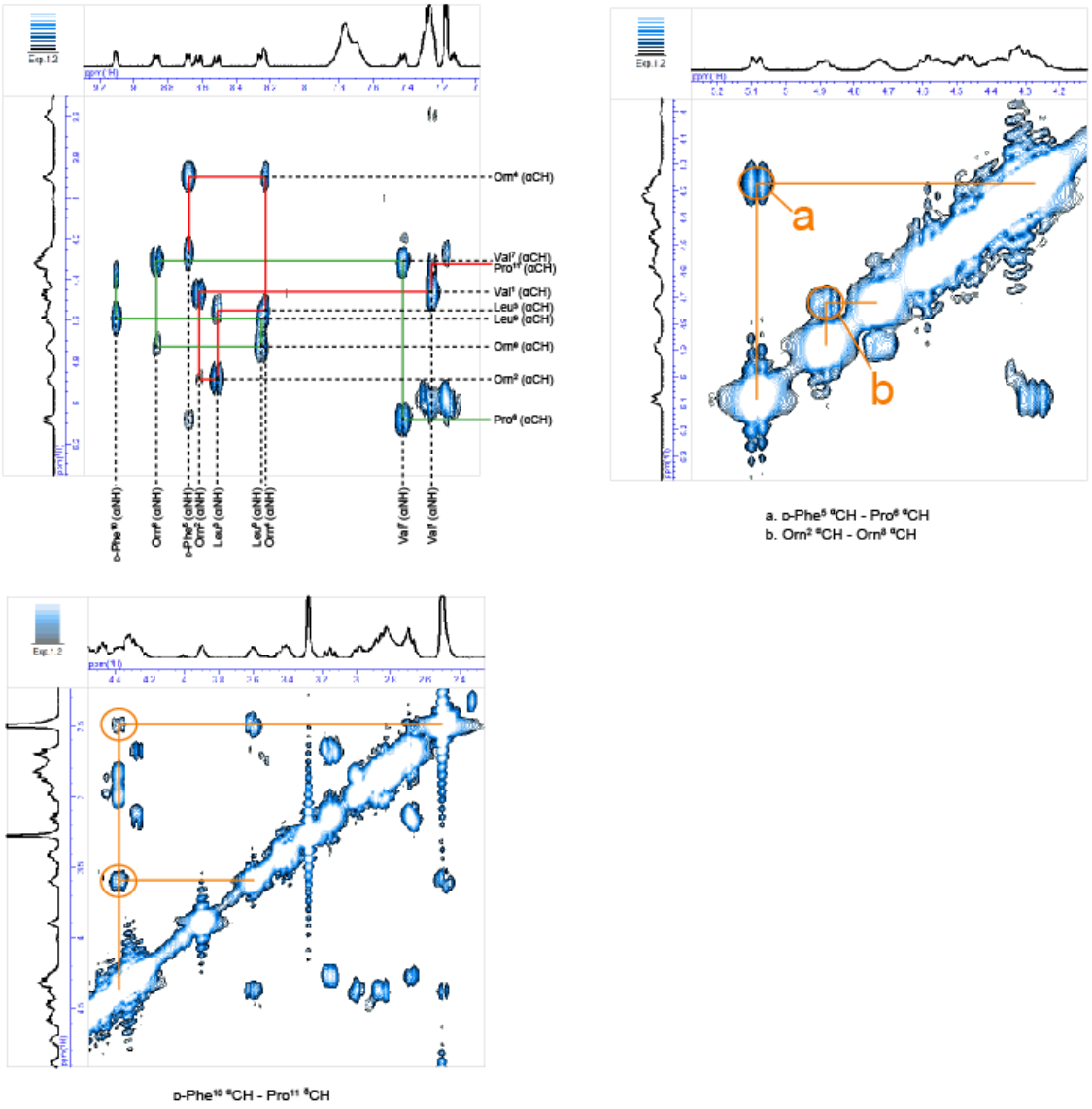


Figure 14. ROESY spectra of **3**

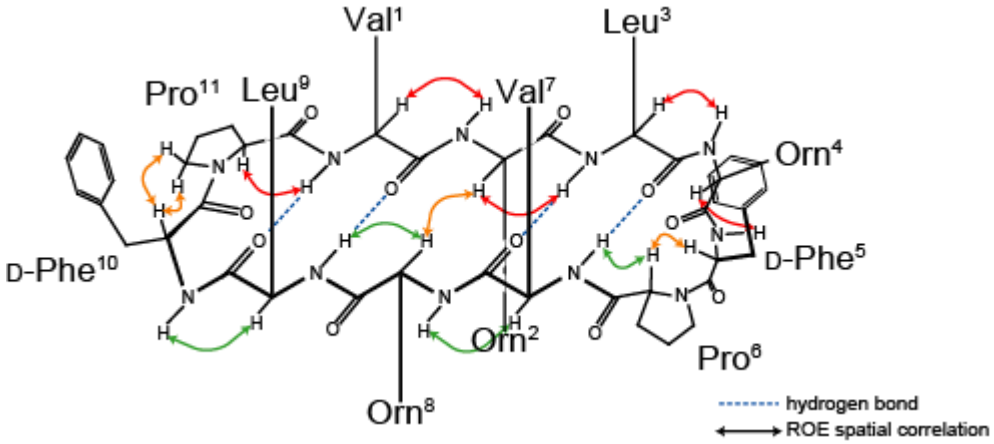


Figure 15. Proposed secondary structure and spatial ROE crosspeaks of **3**.

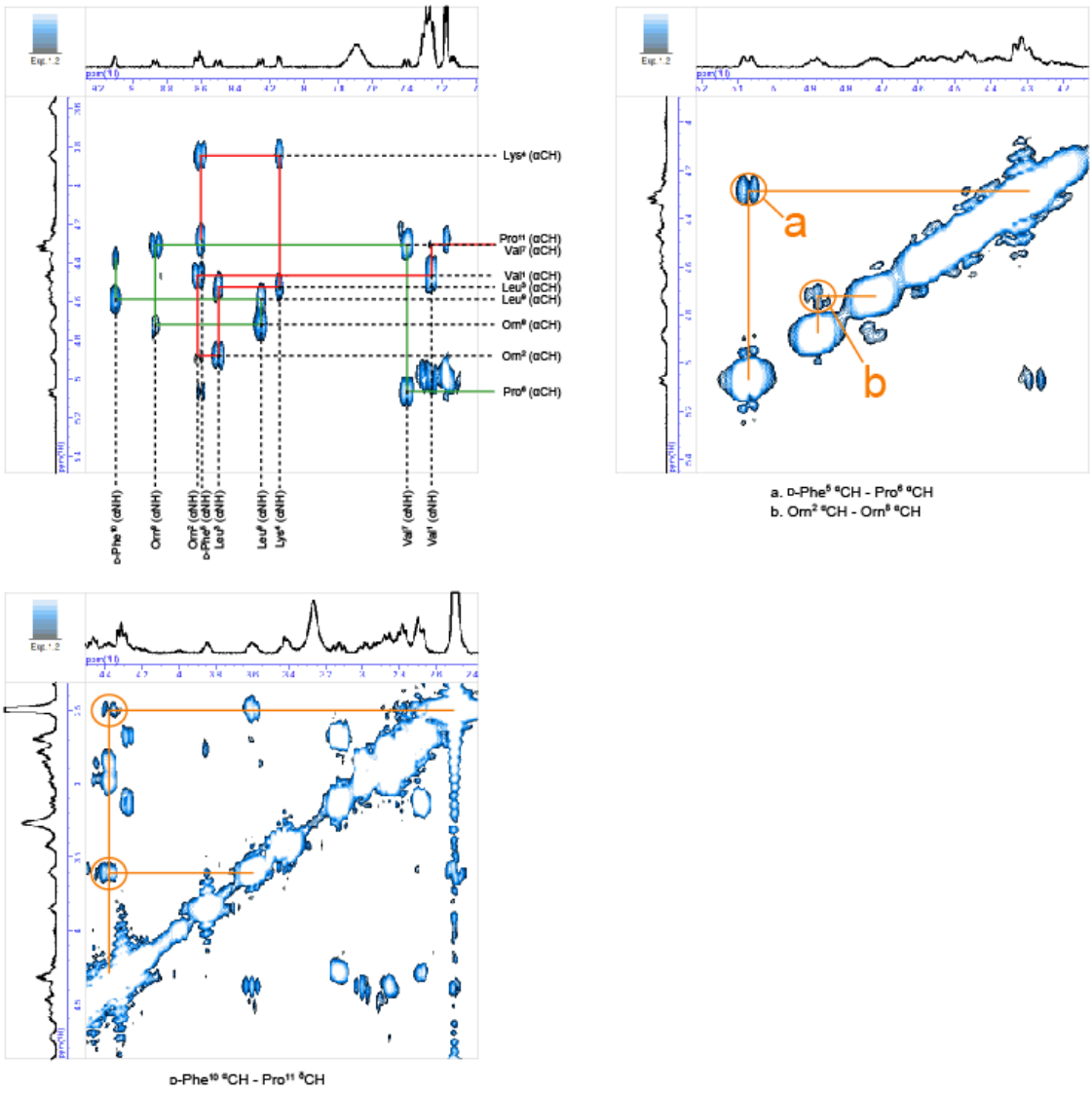


Figure 16. ROESY spectra of **4**

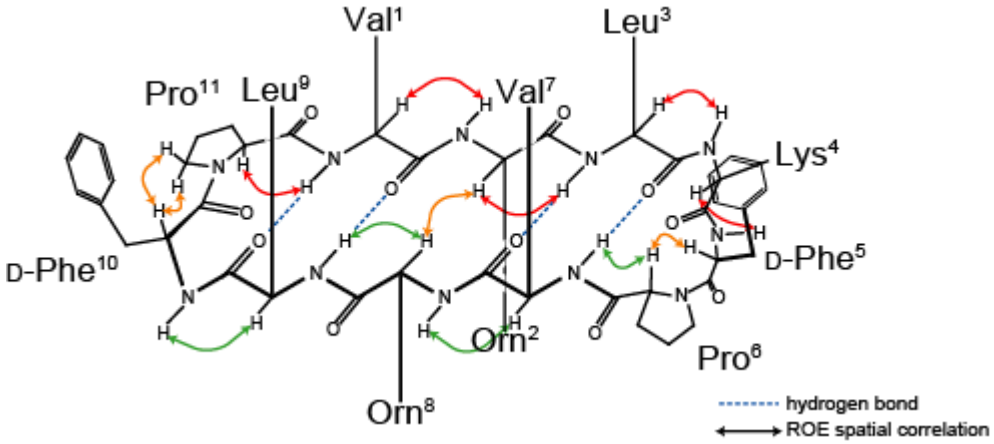


Figure 17. Proposed secondary structure and spatial ROE crosspeaks of **4**.

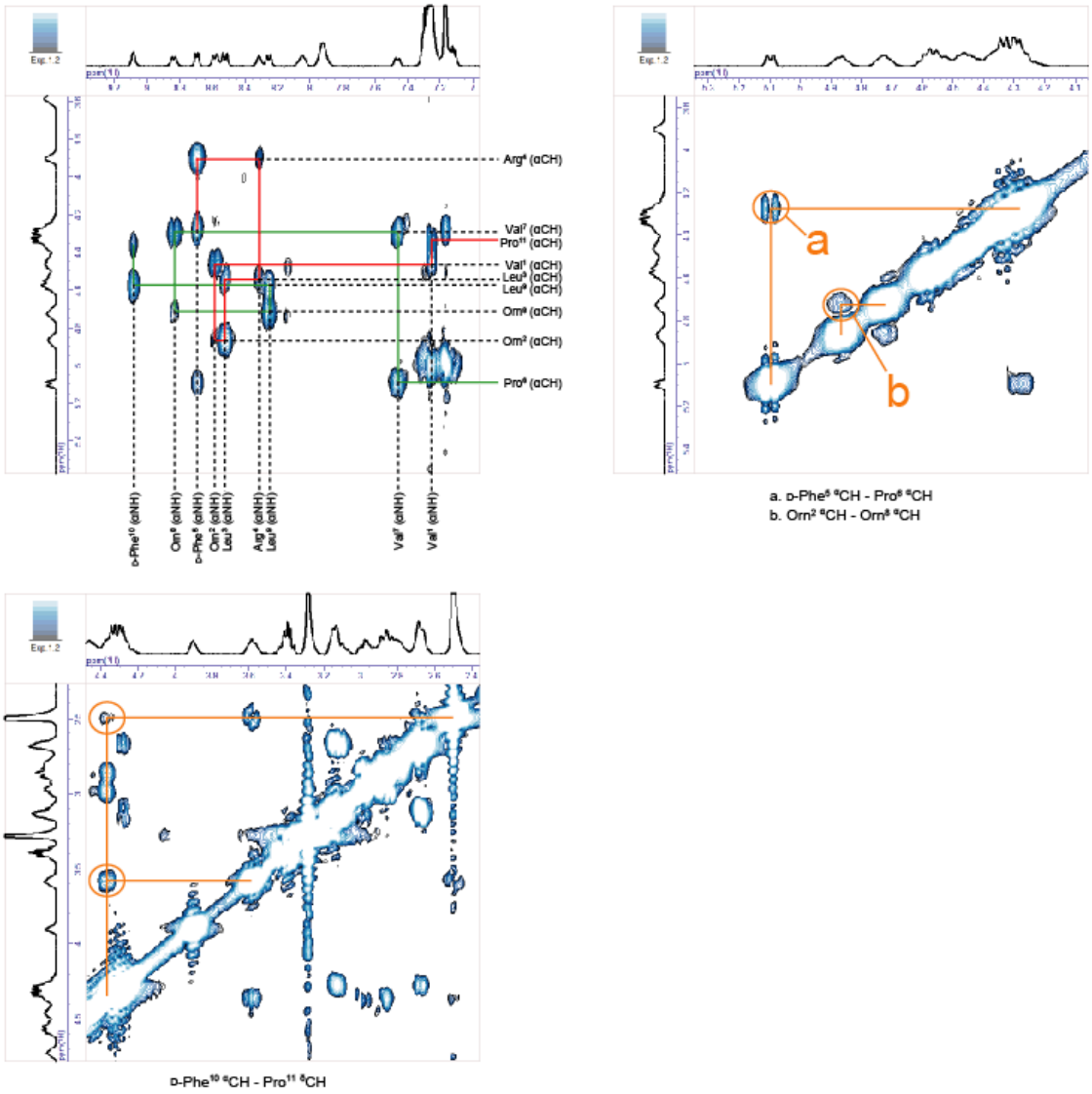


Figure 18. ROESY spectra of **5**

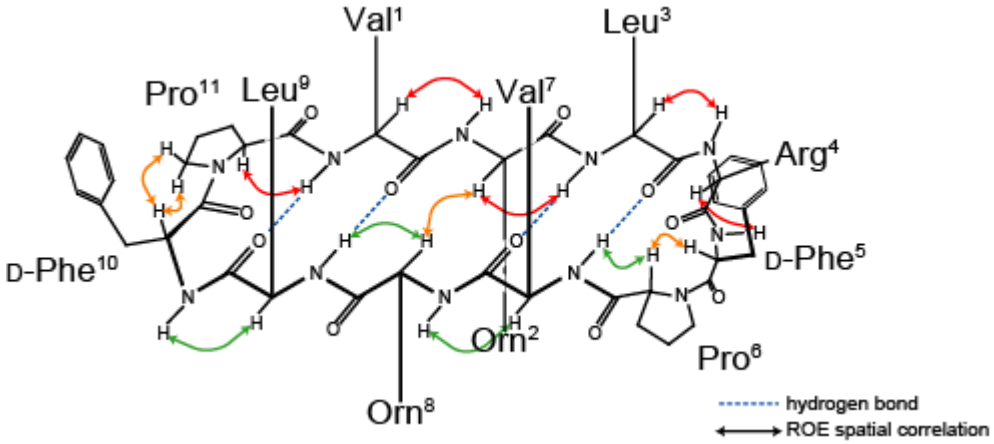


Figure 19. Proposed secondary structure and spatial ROE crosspeaks of **5**.

Table 6. Hemolytic activities of GS and **1**-**5** (% hemolysis).

	Peptide concentration (μM)					
Peptide	1	10	20	30	40	50
GS	0.49±0.57	10.27±0.59	27.04±1.16	58.64±0.89	84.06±0.67	94.80±0.75
1	2.27±0.12	15.20±0.61	44.42±0.63	80.02±0.40	99.88±0.31	100.00±1.22
2	1.95±0.32	6.68±0.09	8.97±0.44	12.30±0.79	17.97±0.53	23.29±0.52
3	1.78±0.68	4.49±0.62	6.04±0.30	6.95±0.46	7.85±0.76	8.32±0.03
4	2.15±0.19	4.26±0.65	5.73±0.78	6.44±0.55	7.60±0.93	8.20±0.21
5	1.88±0.40	5.20±0.49	8.47±0.45	11.12±0.76	12.91±0.55	13.86±0.82

	Peptide concentration (μM)		
Peptide	60	80	100
GS	100.00±0.53	100.00±0.56	100.00±0.75
1	100.00±1.17	100.00±0.67	100.00±0.57
2	26.69±0.36	38.36±0.51	53.69±0.41
3	9.19±0.41	9.97±0.38	11.65±0.78
4	9.46±0.13	10.67±0.15	11.85±0.74
5	15.32±0.49	17.68±0.55	19.98±0.79

The experiments were carried out three times for each peptide.