SUPPORTING INFORMATION

Solution Structure of a Sponge-Derived Cystine Knot Peptide and Its Notable Stability

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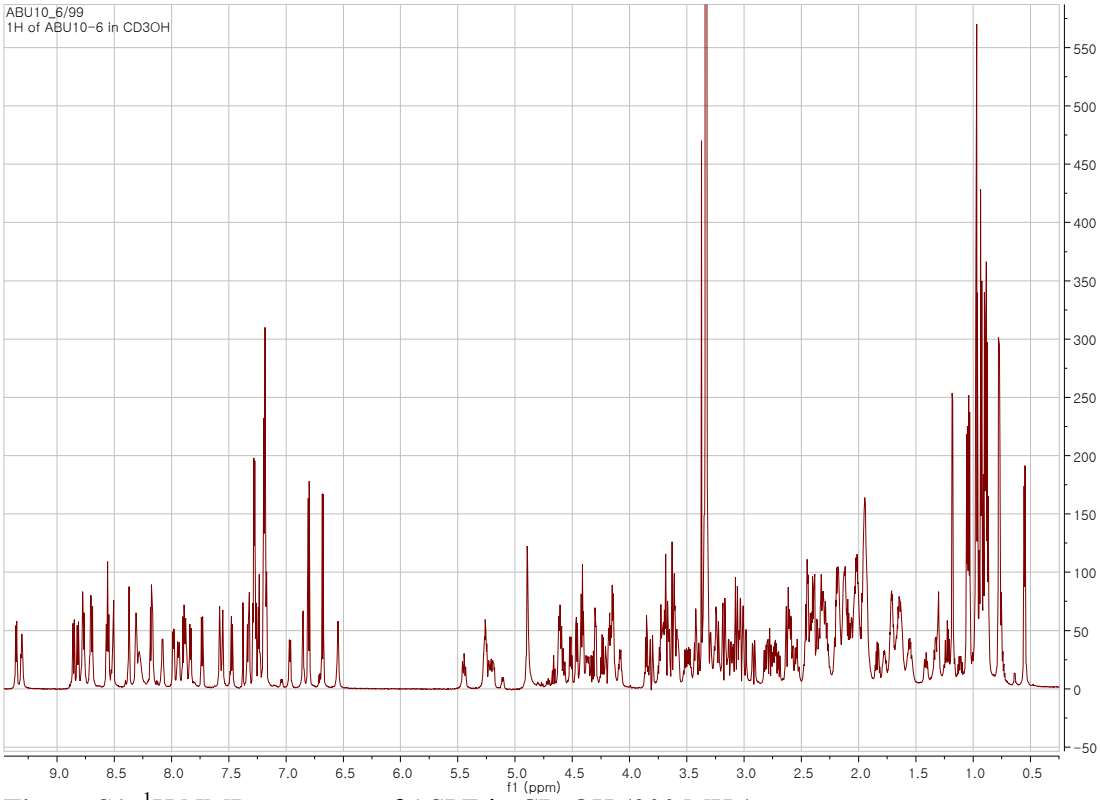

Figure S1. ^{1}H NMR spectrum of ASPE in CD_3OH (900 MHz).

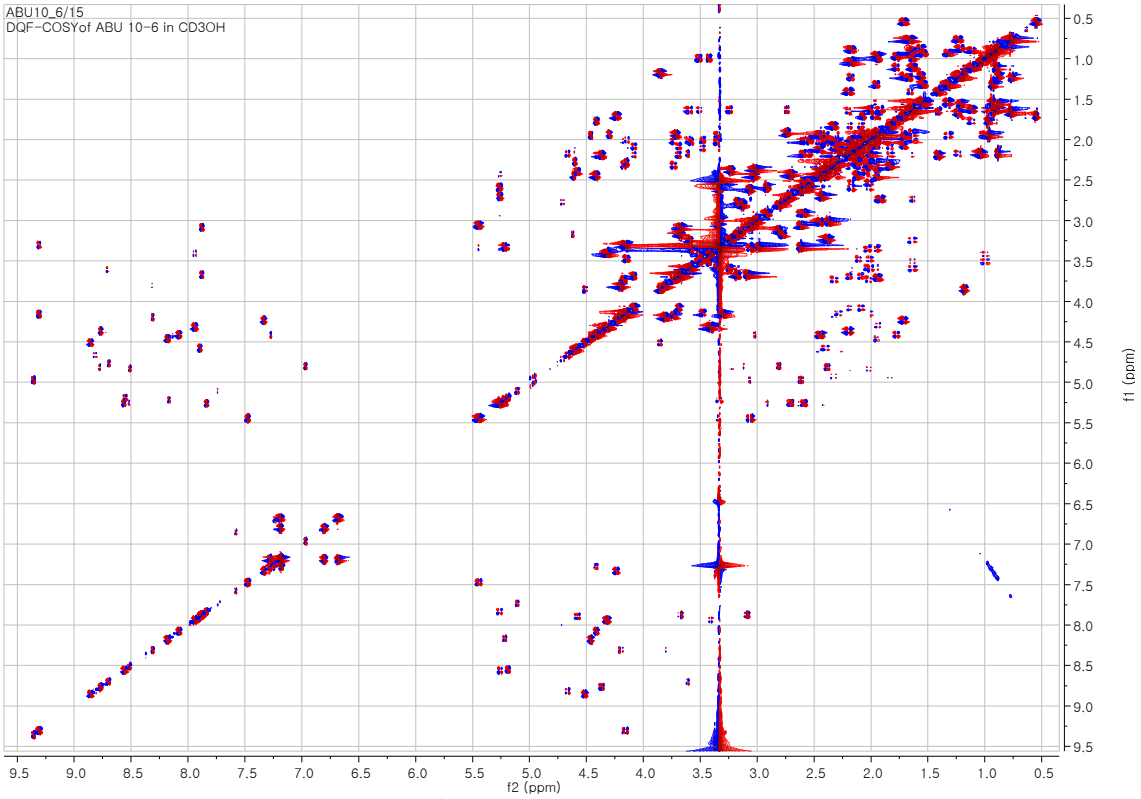

Figure S2. DQF-COSY spectrum of ASPE in CD_3OH (900 MHz)

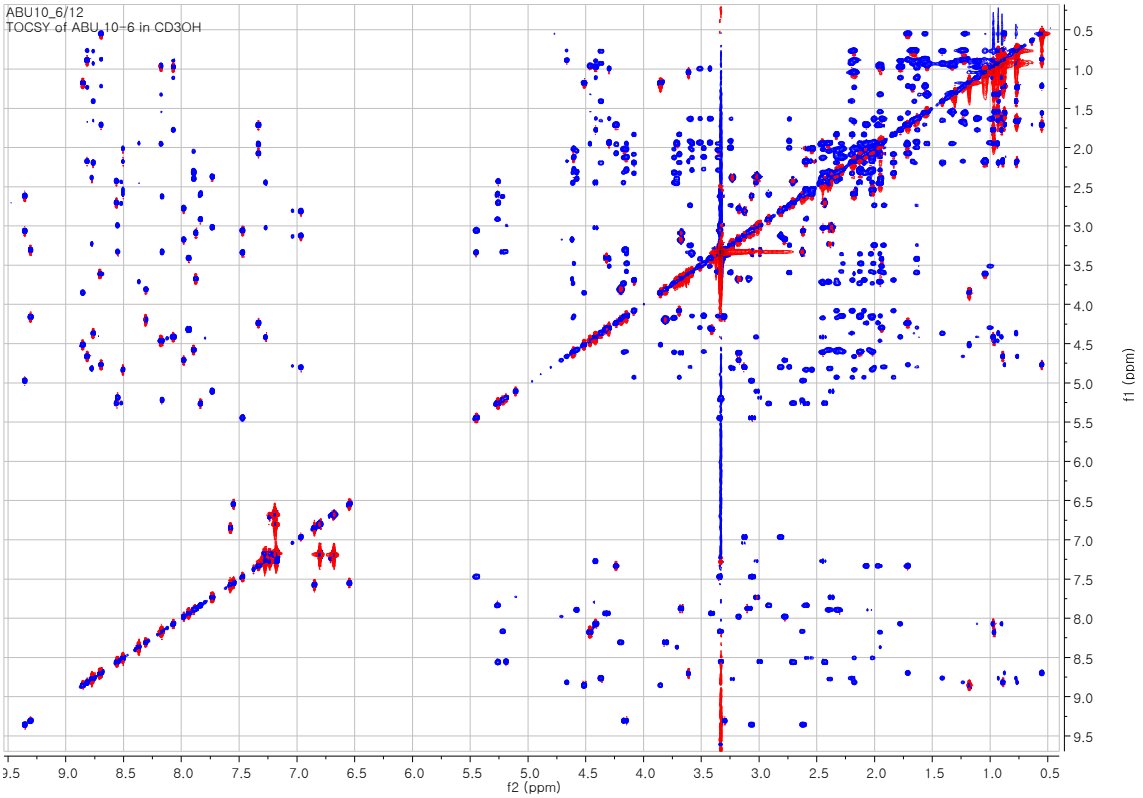

Figure S3. TOCSY spectrum of ASPE in CD_3OH (900 MHz, mixing time = 80 ms).

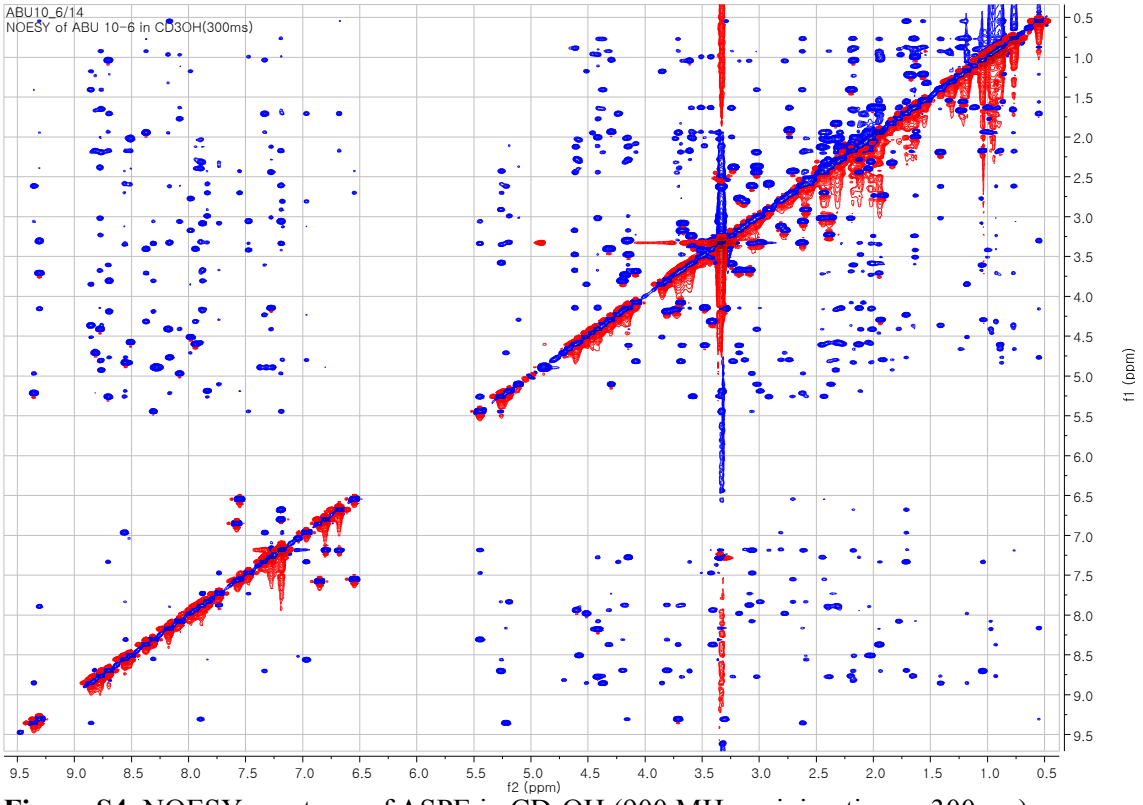

Figure S4. NOESY spectrum of ASPE in CD_3OH (900 MHz, mixing time = 300 ms).

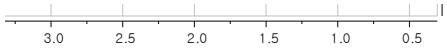

Figure S5. HSQC spectrum of ASPE in CD_3OH (900 MHz).

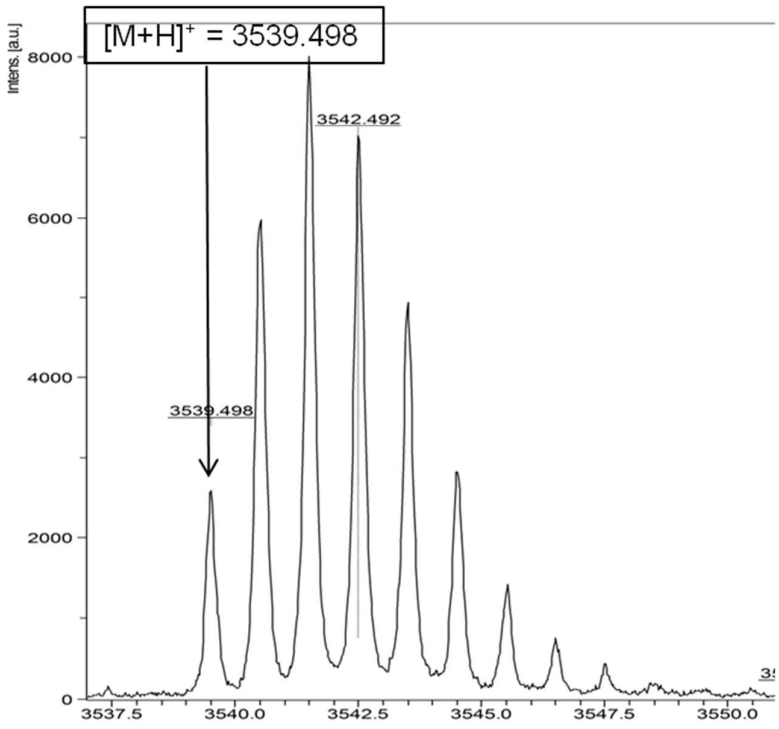

Figure S6. MALDI-TOF MS spectrum of ASPE.

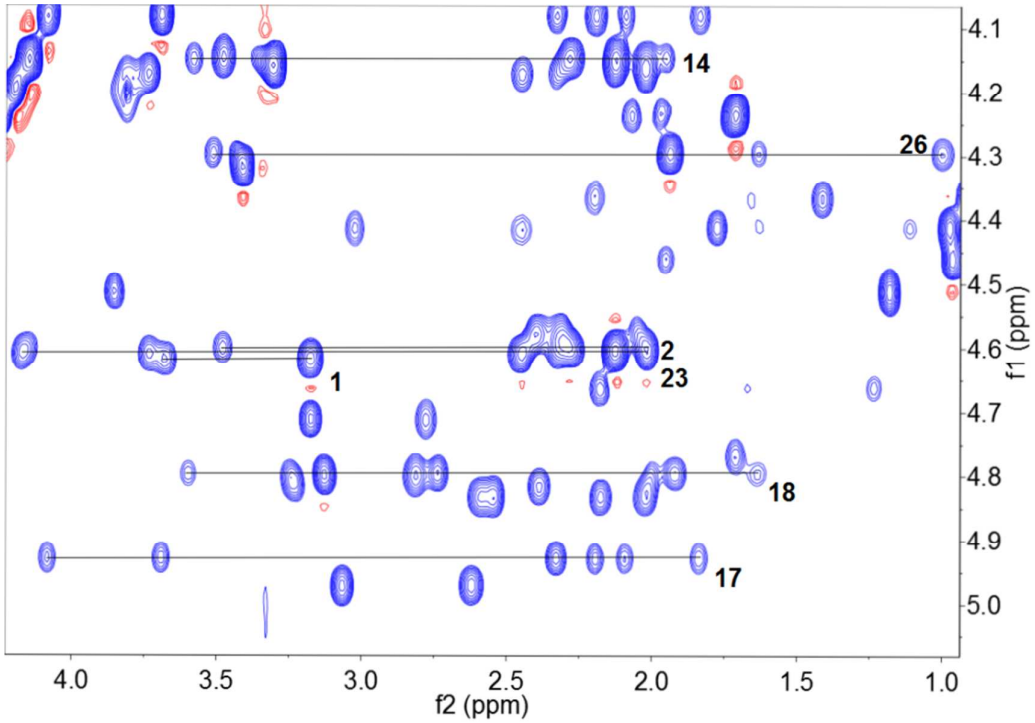

Figure S7. The $C^{\alpha}H$-$C^{\alpha}H$ region of TOCSY spectrum (in CD_3OH, mixing time = 80 ms, 900 MHz) of ASPE showing the intra-residue scalar connectivities of six Pro and *N*-terminal residues.

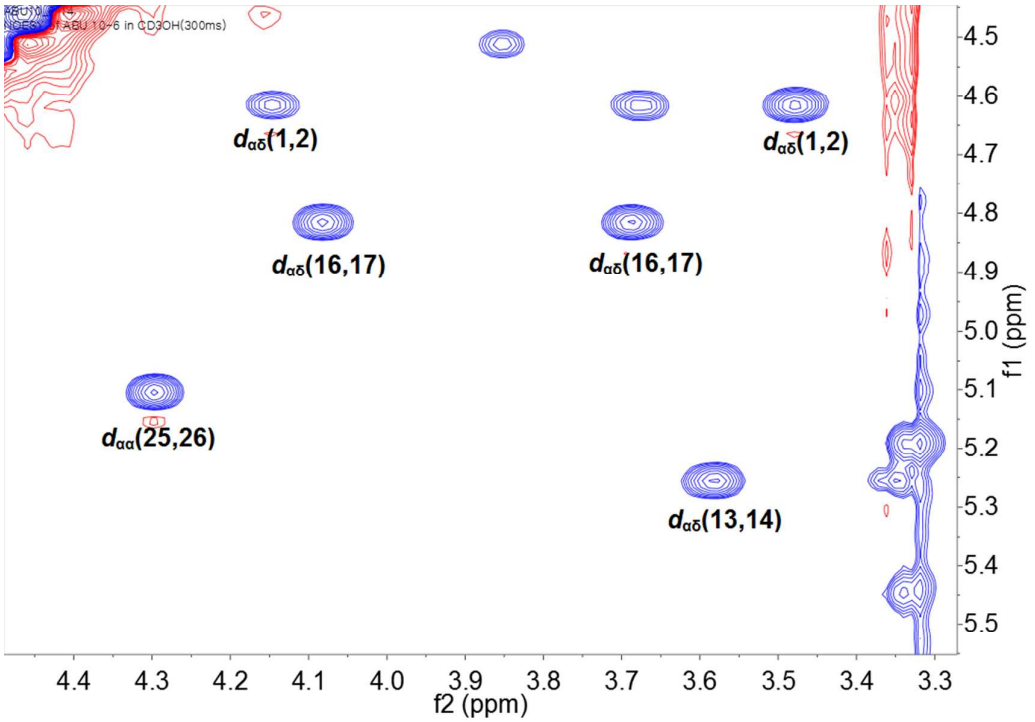

Figure S8. The $C^{\alpha}H$-$C^{\alpha}H$ region of NOESY spectrum (in CD_3OH, mixing time = 300 ms, 900 MHz) of ASPE showing the $d_{\alpha\alpha(i-1,i)}$ and $d_{\alpha\delta(i-1,i)}$ connectivities of Pro residues.

Figure S9. Picture of the marine sponge *Asteropus* sp.

Table S1. Analysis of the possible disulfide bonding patterns in ASPE.

Disulfide pattern	Mean distance (Å)[a]
1-8	8.9 ± 1.6
1-15	8.1 ± 0.3
1-16	**2.8 ± 2.0**
1-21	11.0 ± 1.0
1-30	8.6 ± 0.1
8-15	3.0 ± 0.8
8-16	10.1 ± 0.8
8-21	**3.3 ± 1.5**
8-30	3.5 ± 1.2
15-16	8.4 ± 0.2
15-21	5.4 ± 0.4
15-30	**1.8 ± 0.4**
16-21	12.4 ± 0.5
16-30	8.8 ± 0.3
21-30	6.1 ± 0.1

[a] Mean S-S distances (Å) calculated from the 20 lowest energy structures without disulfide bond restraints

Table S2. ^{1}H NMR Chemical Shifts of ASPE (in CD_3OH, 900 MHz).

No.	Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	Others
1	Cys		4.61	3.67, 3.17	
2	Pro		4.60	2.28	$C^{\gamma}H_2$ 2.12, 2.01; $C^{\delta}H_2$ 4.15, 3.48
3	Gly	7.94	4.32, 3.41		
4	Glu	8.37	3.71	1.95	$C^{\gamma}H_2$ 2.46, 2.40
5	Gly	9.31	4.16, 3.30		
6	Glu	7.89	4.58	2.40, 2.04	$C^{\gamma}H_2$ 2.31
7	Gln	8.51	4.83	2.18, 2.02	$C^{\gamma}H_2$ 2.60, 2.54; $N^{\varepsilon}H_2$ 7.57, 6.85
8	Cys	8.55	5.19	3.33, 2.99	
9	Asp	7.84	5.26	2.91, 2.59	
10	Val	8.71	3.61	2.18	$C^{\gamma}H_3$ 1.05, 1.04
11	Glu	7.33	4.24	1.71	$C^{\gamma}H_2$ 2.07, 1.96
12	Phe	6.97	4.80	3.13, 2.81	$C^{\delta}H_2$ 7.18; $C^{\varepsilon}H_2$ 7.28; $C^{\zeta}H$ 7.23
13	Asn	8.56	5.26	2.70, 2.43	$N^{\delta}H_2$ 7.55, 6.55
14	Pro		4.15	2.29, 2.13	$C^{\gamma}H_2$ 2.03, 1.95; $C^{\delta}H_2$ 3.58, 3.35
15	Cys	7.27	4.42	3.02, 2.45	
16	Cys	8.78	4.82	3.23, 2.39	
17	Pro		4.93	2.33, 1.83	$C^{\gamma}H_2$ 2.19, 2.09; $C^{\delta}H_2$ 4.08, 3.69
18	Pro		4.80	2.74, 1.92	$C^{\gamma}H_2$ 2.03, 1.63; $C^{\delta}H_2$ 3.59, 3.24
19	Leu	8.77	4.37	2.20, 1.41	$C^{\gamma}H$ 1.65; $C^{\delta}H_3$ 0.93, 0.77
20	Thr	8.86	4.51	3.85	$C^{\gamma}H_3$ 1.18
21	Cys	7.99	4.71	3.18, 2.78	
22	Ile	8.82	4.66	2.17	$C^{\gamma}H_2$ 1.67, 1.23; $C^{\gamma}H_3$ 0.89; $C^{\delta}H_3$ 0.77
23	Pro		4.61	2.45, 2.13	$C^{\gamma}H_2$ 2.32, 2.02; $C^{\delta}H_2$ 4.17, 3.73
24	Gly	7.88	3.67, 3.09		
25	Asp	7.73	5.11	3.02, 2.37	
26	Pro		4.30	1.94	$C^{\gamma}H_2$ 1.63, 1.00; $C^{\delta}H_2$ 3.51, 3.42
27	Tyr	7.47	5.45	3.34, 3.06	$C^{\delta}H_2$ 7.18; $C^{\varepsilon}H_2$ 6.80
28	Gly	8.31	4.20, 3.81		
29	Ile	8.70	4.77	1.71	$C^{\gamma}H_2$ 1.56, 1.22; $C^{\gamma}H_3$ 0.55; $C^{\delta}H_3$ 0.88
30	Cys	8.17	5.22	3.33, 2.62	
31	Tyr	9.36	4.97	3.06, 2.62	$C^{\delta}H_2$ 7.19; $C^{\varepsilon}H_2$ 6.68
32	Ile	8.07	4.41	1.77	$C^{\gamma}H_2$ 1.64, 1.11; $C^{\gamma}H_3$ 0.97; $C^{\delta}H_3$ 0.90
33	Ile	8.18	4.46	1.95	$C^{\gamma}H_2$ 1.54, 1.32; $C^{\gamma}H_3$ 0.97; $C^{\delta}H_3$ 0.93

Table S3. ^{13}C NMR Chemical Shifts of ASPE Assigned on the Basis of HSQC Spectroscopy (in CD_3OH, 900 MHz).

No.	Residue	C^{α}	C^{β}	C^{γ}	Others
1	Cys	49.3	38.6		
2	Pro	60.0	30.2	25.4	C^{δ} 48.0
3	Gly	39.4			
4	Glu	54.6	24.1	29.4	
5	Gly	42.8			
6	Glu	51.8	28.0	31.2	
7	Gln	54.0	27.6	31.9	
8	Cys	50.8	48.5		
9	Asp	49.4	37.9		
10	Val	63.2	29.3	19.4, 17.1	
11	Glu	54.2	26.5	29.9	
12	Phe	55.8	39.2		C^{δ} 129.0, C^{ε} 128.3 C^{ζ} 126.9
13	Asn	48.9	36.2		
14	Pro	60.0	29.4	24.2	C^{δ} 47.3
15	Cys	53.2	40.1		
16	Cys	49.6	35.2		
17	Pro	58.9	28.1	25.1	C^{δ} 47.0
18	Pro	61.0	28.7	21.5	C^{δ} 45.7
19	Leu	53.8	39.0	26.3	C^{δ} 23.2, 22.3
20	Thr	58.3	68.3	18.4	
21	Cys	52.3	37.2		
22	Ile	54.7	38.2	24.2, 13.4	C^{δ} 9.4
23	Pro	61.8	31.4	22.7	C^{δ} 47.3
24	Gly	44.5			
25	Asp	48.1	35.9		
26	Pro	60.9	31.7	20.5	C^{δ} 47.6
27	Tyr	54.7	37.9		C^{δ} 129.6, C^{ε} 115.5
28	Gly	44.3			
29	Ile	56.3	38.6	25.1, 14.8	C^{δ} 9.3
30	Cys	52.8	39.0		
31	Tyr	54.8	40.6		C^{δ} 130.7, C^{ε} 114.8
32	Ile	58.1	36.9	25.7, 14.2	C^{δ} 10.5
33	Ile	56.9	36.9	24.9, 14.9	C^{δ} 10.6