Minimization and Optimization of Designed β Hairpin Folds

Niels H. Andersen*, Katherine A. Olsen, R. Matthew Fesinmeyer, Xu Tan, F. Michael Hudson, Lisa A. Eidenschink, Shabnam R. Farazi

Department of Chemistry, University of Washington, Seattle WA 98195

Supporting Material

Figure 1S. The backbone CSD comparison of HP5W, (T3V)-HP6, HP6, and Ac-HP6 over the shared portions of the strands -Y(T/V)W-loop-KWTV sequence: Hα (A) and H_N (B) in the two panels. The series presented represents a decreasing hairpin stability. The dramatic differences in Hα CSDs at the Trp and Lys are due to the loop geometry change when NPATG of HP5W is replaced by SNG in the HP6 peptides.

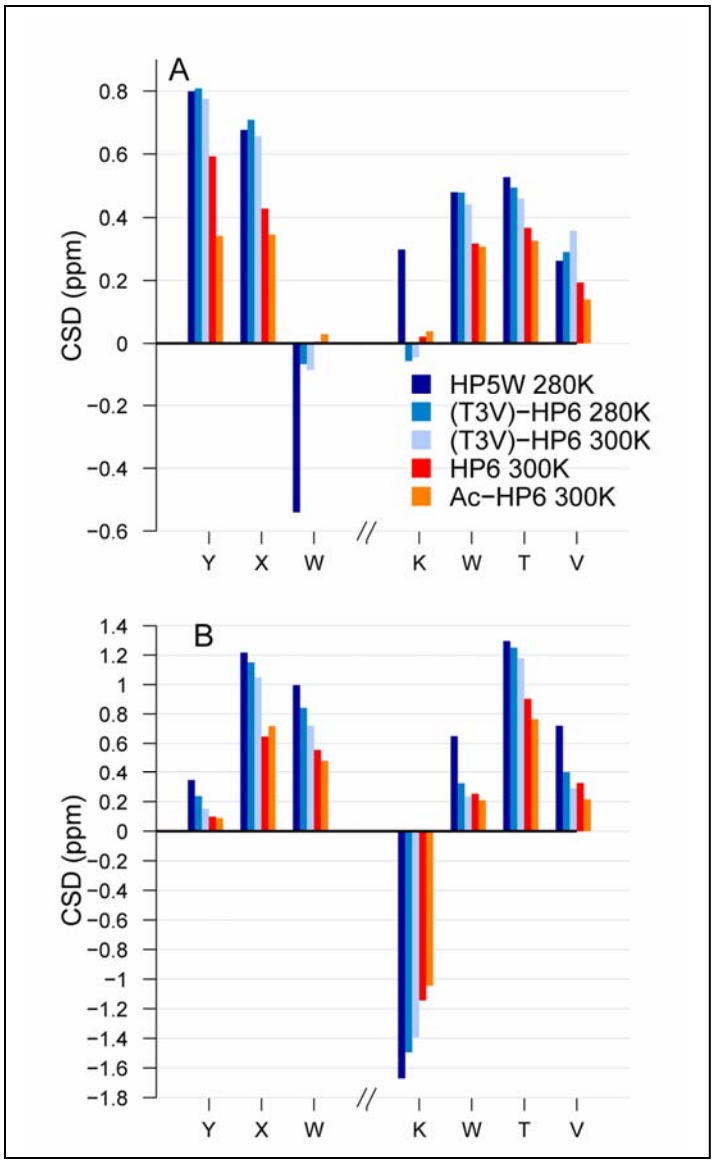
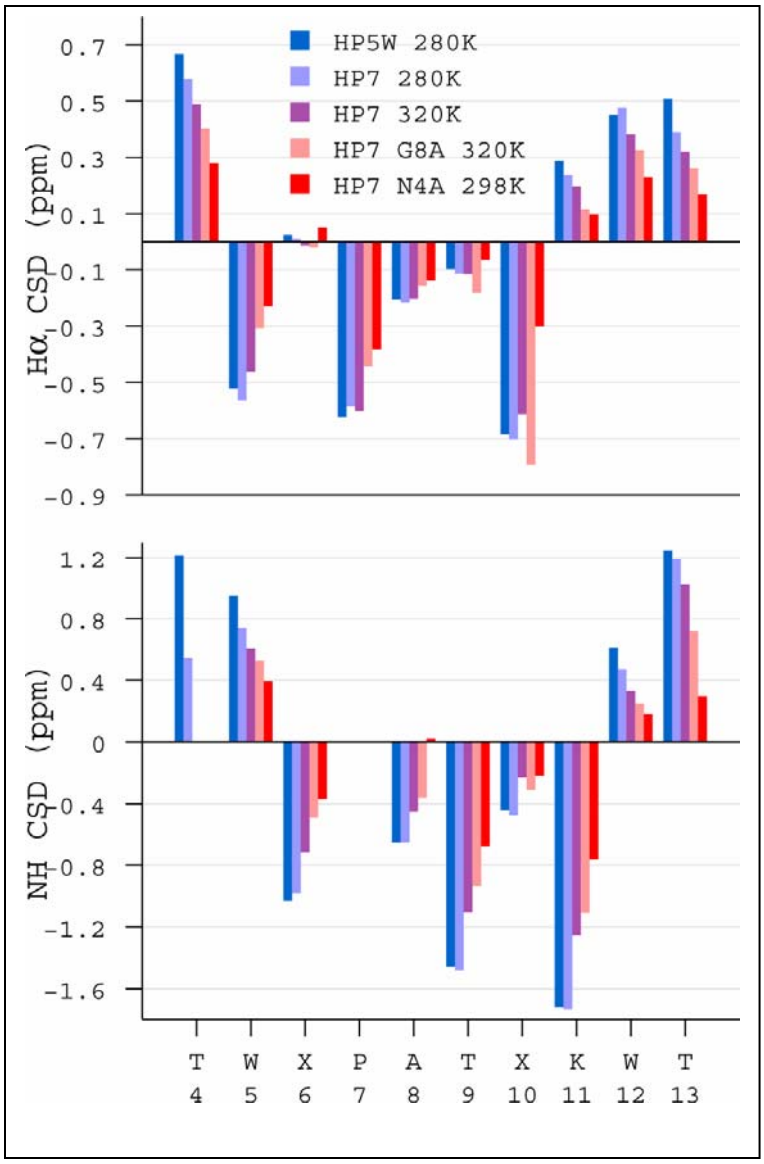


Figure 2S. The backbone CSD comparison of HP5W, HP7, and two HP7 mutants over the shared portions of the -TW(NPATGK)WT- sequence: Hα (A) and H_N (B) in the two panels. At each residue the data is shown in the following order: HP5W at 280K, HP7 at 280 and 320K (illustrating a modest degree of structure melting at the highest temperature), (G8A)-HP7 at 320K (less folded), and (N4A)-HP7 at 298K (much less folded even at this lower temperature). Additional data, including CD melting data, has previously been reported for HP5W.[1] All of the sites with large CSDs, whether positive or negative, show the same order (decreasing from left to right) of fold populations for this series of peptides. The anomaly at Hα of residue 10 is due to the G10A mutation.



NMR Shift Validation of HP5W4 and trpzip4 structure ensembles

We performed a more complete examination of the extent to which the distinctive NMR structure ensembles of trpzip4 *versus* HP5W4 and HP7 predict the chemical shifts observed, with particular attention to the sites along the Trp indole rings that experience ring current shifts, Table 1S. As discussed earlier, while HP5W4 and trpzip4 assume exceedingly similar turn geometries and turn-neighboring Trp sidechain interactions, the packing of the W3 and 14 (HP5W4 numbering used throughout this discussion) sidechains differ. This difference, observed in the NMR structure ensembles, is reinforced in the chemical shifts.

Table **1S**: Chemical Shifts for Trp hydrogens displaying upfield ring current shifts. Hε3 is included for all residues since this shift is particularly diagnostic of the cross-strand 'EtF' interaction geometry. Experimental shifts that correspond to CSDs greater than 0.7ppm are highlighted with the observed shift in bold. Instances in which calculated shifts and the observed values deviate by greater than 0.7 ppm are highlighted with the calculated value italicized.

Site	trpzip4		HP5W4		HP7[4]		δ(ref)[5]
	Obs.[1]	Calc.[2]	Obs.[3]	Calc.	Obs.[3]	Calc.	
Trp 3 Hε3	7.60	7.58±0.07	**6.31**	5.85±0.37	N/A	N/A	7.62
Trp 3 Hz3	7.23	7.21±0.06	6.77	*4.68±0.26*	N/A	N/A	*7.12*
Trp 3 Hz2	7.20	7.23±0.06	7.15	6.97±0.15	N/A	N/A	*7.46*
Trp 3 Hδ1	7.25	7.31±0.05	6.93	6.45±0.20	N/A	N/A	*7.22*
Trp 5 Hε3	**4.97**	5.06±0.05	**5.53**	4.82±0.18	**5.328**	4.84±0.10	7.62
Trp 5 Hz3	**6.27**	6.37±0.03	6.62	6.31±0.11	**6.418**	6.57±0.09	*7.12*
Trp 5 Hz2	7.21	7.22±0.03	7.32	7.16±0.02	7.279	7.24±0.01	*7.46*
Trp 5 Hδ1	6.87	6.84±0.03	**6.47**	6.80±0.02	6.684	6.76±1.03	7.22
Trp 12 Hε3	7.58	7.55±0.06	**6.90**	5.87±0.19	7.380	7.21±0.07	7.62
Trp 12 Hz3	7.21	7.21±0.05	7.10	6.80±0.15	7.161	7.14±0.06	*7.12*
Trp 12 Hδ1	7.17	7.31±0.04	7.42	7.29±0.05	7.557	7.47±0.02	7.22
Trp 14 Hε3	**5.21**	5.23±0.07	**6.74**	*7.60±0.09*	N/A	N/A	7.62
Trp 14 Hz3	6.40	6.48±0.05	6.98	6.96±0.08	N/A	N/A	*7.12*
Trp 14 Hz2	7.25	7.21±0.03	7.23	7.00±0.10	N/A	N/A	*7.46*

[1] The literature data[2] is reported at 288 K. [2] The remarkable match between theoretical and calculated values is presumably related to the Cochran's use of molecular dynamics refinement with AMBER, on which Case's shift-estimation program[3] is based. [3] Observed shifts are at 280 K. [4] Trp 5 and 12 of HP5W4 correspond to Trp 3 and 10 of HP7. [5] Unfolded reference shift values are those observed in this laboratory and are within 0.06 ppm the values reported by several groups[4]. The 7.62 ppm value for Hε3 is also the most downfield observation for unfolded control peptides from the present study.

The W5 sidechain is the only one expected to exhibit identical chemical shift deviations over the three peptides. Throughout, the Hε3, Hz3, Hδ1, and Hz2 positions of W5 display diagnostic upfield deviations (of decreasing magnitude in the order listed). In all three cases, chemical shifts calculated based on the NMR ensemble structures using Shifts 4.1[3] duplicate the pattern and match the observed values to a reasonable degree. HP7 and trpzip4 display an exceedingly good match between the calculated and observed values. In the case of trpzip4, this may be the result of using chemical shifts in the ensemble calculation – the procedure used for HP5W4 and HP7 was purely based on nOe's.

The chemical shifts of the Trp immediately following the turn (W12) are not consistent across the three sequences, reflecting the differing orientations of W3 in trpzip4 and HP5W4 and the lack of the terminal pair of tryptophans in HP7. In trpzip4, the CSDs at W12 are very small. In HP5W4, the largest structuring shift (upfield) at W12 is at Hε3 and, an even larger upfield shift, is predicted by the NMR ensemble. The observed upfield shift at this site is greatly diminished in HP7, indicating that the effect seen in HP5W4 reflects the W3 ring current. The downfield shift at Hδ1 cannot be attributed to W3, since it also appears in HP7.

Given the differences in deviations observed for W12 of HP5W4 and trpzip4, it is not surprising that those observed for W3 and W14 also do not match. One of the largest deviations observed for trpzip4, the 2.37 ppm upfield shift of W14 Hε3, is not replicated in our construct, which moves upfield only 0.84 ppm. This residual upfield shift is not predicted by our structure ensemble. The 1.31 ppm upfield shift of W3 Hε3 in our construct is strikingly different from the near coil value reported for trpzip4 and this shift is predicted by the ensemble. Calculated chemical shift values for HP5W4 do not consistently match the observed values. The largest predicted CSD is at W3 Hz3; in fact, the NMR ensemble would imply that W3 Hε3 should have the largest CSD of all the indole ring CH sites. Hardly any structuring shift is observed at W3 Hz3.

The small standard errors of the HP5W4 shift predictions indicate that the indole ring orientations are well converged in the NMR ensemble. This is probably a case of false convergence in the case of W3 and W14: for example the residual upfield shift at Hε3 of W14 likely implies that the T-orientation of W3 and W14 seen in trpzip4 is also partially populated in HP5W4. Nonetheless, the chemical shifts do serve to confirm the differences in the Trp/Trp interactions between trpzip4 and HP5W4 as seen in the nOe-derived structure ensembles and that only the W5/W12 interaction geometry is preserved throughout this series of peptides.

The $J_{\alpha\beta}$ coupling constants for the Trp residues of HP7 (and HP5W4) provide additional evidence for a defined cross-strand interaction geometry, using HP5W4 numbering: Trp^5 ($J_{\alpha\beta3}$ = 12.1±0.8, $J_{\alpha\beta2}$ 3.5±0.9 Hz) and Trp^{12} ($J_{\alpha\beta3}$ = 2.4±0.5, $J_{\alpha\beta2}$ = 11.8±0.8 Hz). Exact coupling constants were not derived for the more remote Trp pair of HP5W4, but the 1D spectra and TOCSY indicate $J_{\alpha\beta}$ values that are moving toward conformationally-averaged norms.

Table 6. The distance restraints employed in generating the NMR ensemble of HP5W4

Residue1	Proton	Residue 2	Proton	d	d-	d+
1	ha	2	hn	2.44	0.46	0.33
1	hb#	2	hn	3.38	0.74	0.53
1	hb#	14	he3	3.73	0.85	0.86
2	ha	3	hn	2.13	0.23	0.28
2	hb#	3	hn	3.57	0.81	0.82
2	hg#	3	hn	4.49	1.09	1.04
2	hn	15	hn	3.27	0.72	0.52
3	ha	3	hn	3.47	0.78	0.59
3	hb1	3	hn	2.83	0.58	0.41
3	hb2	3	hn	2.67	0.53	0.37
3	hd1	3	hn	4.16	1	1.11
3	ha	4	hn	2.19	0.29	0.29
3	hb1	4	hn	4.45	1.09	1.1
3	hb2	4	hn	5.39	1.39	2.08
3	he3	4	hn	4.88	1.23	1.67
3	hb1	12	hz3	4.45	1.09	1.3
3	he3	13	hn	5.54	1.44	2.51
3	ha	14	he3	4.07	0.97	1.06
3	hb2	14	hh2	4.23	1.02	1.15
3	hb2	14	hz3	4.09	0.98	1.07
3	he3	14	hn	5.31	1.37	2.17
3	hz3	14	he1	4.39	1.07	1.45
3	hz3	14	hn	4.1	0.98	1.07
4	ha	4	hb	3.2	0.35	0.35
4	ha	4	hg2#	3.34	0.66	0.45
4	ha	4	hn	3.13	0.68	0.48
4	hb	4	hn	2.9	0.67	0.37
4	hg2#	4	hn	4.73	1.1	0.93
4	ha	5	hn	2.11	0.21	0.28
4	hb	5	hn	3.74	0.87	0.69
4	hg2#	5	hn	3.92	0.84	0.6
4	hb	13	hn	5.08	1.29	1.68
4	hb	15	hb#	4.79	1.2	1.58
4	hb	15	hg#	4.02	0.94	0.78
4	hg2#	15	hb#	5.57	1.36	1.67
4	hg2#	15	hg#	5.42	1.3	1.25
5	ha	6	hn	2.27	0.37	0.3
5	hb2	6	hn	4.86	1.22	1.44
5	ha	12	he3	4.25	1.03	1.16
5	hb2	12	he1	4.65	1.16	1.45
5	hb2	12	he3	4.98	1.26	1.76
5	hb2	12	hz2	4.1	0.98	1.07
5	hb2	12	hz3	4	0.95	1.01
5	he3	12	hd1	3.9	0.92	1.17
5	he3	12	hn	4.87	1.23	1.66
5	hz3	12	he1	3.88	0.91	1.16
5	hz3	12	hn	4.86	1.22	1.65

5	ha	13	hn	5.1	1.3	1.7
6	ha	7	hd1	2.68	0.53	0.37
6	ha	7	hd2	2.55	0.49	0.35
6	hb1	10	hn	4.66	1.16	1.26
6	hb1	11	hn	3.54	0.81	0.61
6	hn	11	hn	3.82	0.89	0.73
7	ha	5	hd1	4.37	1.07	1.24
7	ha	5	he1	3.27	0.72	0.72
7	ha	5	hz2	3.85	0.9	0.94
7	ha	5	hz3	4.77	1.2	1.56
7	hb1	5	hd1	4.12	0.99	1.08
7	hb1	5	he1	3.46	0.78	0.78
7	hb2	5	he1	5.04	1.28	1.83
7	hd1	5	hd1	3.35	0.74	0.75
7	hg#	5	hd1	3.83	0.9	1.13
7	hg#	5	he1	4.07	0.97	1.25
7	ha	7	hb2	3.01	0.64	0.45
7	hd1	7	hb1	3.57	0.81	0.62
7	hd1	7	hb2	4.37	1.07	1.04
7	hd2	7	hb1	4.19	1.01	0.93
7	hd2	7	hb2	4.12	0.99	0.88
7	ha	8	hn	4.05	0.97	0.84
7	hb1	8	hn	5.14	1.31	1.76
7	hb2	8	hn	3.83	0.9	0.73
7	hd2	8	hn	3.06	0.65	0.46
7	hg#	8	hn	3.65	0.84	0.86
7	ha	10	hn	4.35	1.06	1.03
8	hb#	6	hd21	5.29	1.27	1.26
8	hb#	6	hd22	4.65	1.07	0.88
8	ha	9	hn	3.58	0.82	0.63
8	hb#	9	hn	3.61	0.74	0.52
8	hn	9	hn	2.61	0.51	0.36
9	hg2#	6	hd21	4.49	1.02	0.81
9	hg2#	6	hd22	4.1	0.9	0.66
9	hg2#	8	hn	5.74	1.42	1.62
9	ha	10	hn	4.03	0.96	0.83
9	hg2#	10	hn	5.82	1.44	1.7
9	hb	11	hg#	3.76	0.87	0.9
9	hb	11	hn	4.32	1.05	1.01
10	ha1	5	hh2	4.55	1.12	1.37
10	ha2	5	he1	4.2	1.01	1.13
10	ha2	5	hh2	3.65	0.84	0.85
10	ha2	5	hz2	3.42	0.77	0.77
10	hn	9	hn	2.26	0.36	0.3
10	ha1	11	hn	3.98	0.94	0.81
10	ha2	11	hn	3.46	0.78	0.58
10	hn	11	hn	2.63	0.52	0.36
11	ha	5	hz3	3.64	0.9	0.85
11	ha	12	hd1	3.85	0.9	0.94
11	hb#	12	hn	3.22	0.69	0.38

11	hg#	12	hn	4.25	1.03	1.16
12	hb1	3	he1	5.31	1.36	2.17
12	hb1	3	he3	4.14	1	1.1
12	hb1	3	hz3	5.18	1.32	2
12	hb2	3	he1	5.1	1.3	1.91
12	hb2	3	he3	5.02	1.27	1.81
12	hb2	3	hz2	3.79	0.88	0.92
12	hb1	4	hn	5.41	1.4	2.11
12	he3	4	hn	4.14	1	1.1
12	ha	5	ha	3.01	0.64	0.45
12	ha	5	he3	4.37	1.07	1.24
12	hd1	5	hz3	3.48	0.78	0.99
12	hz3	5	hn	3.97	0.94	1
12	ha	13	hn	2.36	0.43	0.32
12	hb1	13	hn	4.12	0.99	0.88
12	hb2	13	hn	5.22	1.34	1.85
12	he3	13	hn	4.85	1.22	1.63
13	ha	3	hz3	3.32	0.73	0.73
13	hn	4	hn	3	0.72	0.52
13	ha	14	hn	2	0.1	0.35
13	hb	14	hn	2.87	0.59	0.41
13	hg2#	14	hn	4.09	0.9	0.66
14	hb1	1	hb#	4.36	1.05	0.96
14	hb1	1	hd#	4.77	1.18	1.24
14	ha	2	hn	4.94	1.25	1.52
14	he3	2	hn	4.57	1.13	1.39
14	ha	3	he3	4.38	1.07	1.25
14	hb2	3	hz3	4.32	1.05	1.21
14	hz3	3	hn	3.86	0.91	0.95
14	ha	4	hn	4.09	0.98	0.87
14	ha	15	hn	2.38	0.44	0.32
14	hb1	15	hn	4.41	1.08	1.07
14	hb2	15	hn	4.59	1.14	1.21
14	he3	15	hn	4.12	0.99	1.08
15	ha	16	hn	2.22	0.32	0.3
15	hb#	16	hn	3.45	0.78	0.78
15	hg#	16	hn	4.4	1.06	0.98
16	ha	1	hb#	4.69	1.15	1.18
16	ha	2	hn	4.95	1.25	1.54

Table 7. The distance restraints employed in generating the NMR ensemble of HP7

Residue1	Proton	Residue 2	Proton	d	d-	d+
1	hb#	10	he3	2.86	0.49	0.81
1	hb#	10	hb#	3.32	0.61	0.71
1	hd#	10	he3	3.41	0.66	0.97
1	hd#	10	hb#	3.43	0.65	0.74
1	ha	10	he3	3.77	0.78	0.9
1	ha	11	hn	3.85	0.8	0.74
1	ha	1	hb#	2.94	0.51	0.63
1	ha	2	hn	2.16	0.26	0.29
1	hb#	2	hn	3.05	0.55	0.66
2	ha	10	he3	3.24	0.61	0.71
2	hn	11	hn	2.7	0.44	0.38
2	hb	11	hn	3.7	0.75	0.68
3	hz3	10	hd1	2.88	0.49	0.82
3	ha	10	he3	3.06	0.55	0.66
3	ha	10	hn	4.01	0.85	0.82
3	ha	11	hn	3.49	0.69	0.59
3	hb1	3	hn	2.37	0.34	0.32
3	hb1	3	hd1	2.38	0.34	0.52
3	ha	3	he3	3.19	0.59	0.7
3	ha	4	hn	2.13	0.23	0.28
3	hb2	4	hn	3.68	0.75	0.67
3	hb1	4	hn	3.96	0.84	0.8
3	hb1	5	hd1	3.85	0.8	0.74
4	ha	5	hd2	2.23	0.29	0.3
4	ha	5	hd1	2.35	0.33	0.31
4	ha	6	hn	3.44	0.67	0.58
4	hb1	7	hn	3.3	0.63	0.53
4	hb1	8	hn	3.48	0.69	0.59
4	hb1	9	hn	3.08	0.56	0.47
4	hn	9	hn	3.35	0.64	0.54
4	hb2	9	hn	3.73	0.77	0.69
5	hd1	3	hd1	2.71	0.44	0.58
5	hb1	3	he1	2.85	0.49	0.61
5	ha	3	hd1	3.17	0.59	0.69
5	hd2	3	hd1	3.46	0.68	0.78
5	hd1	3	he1	3.66	0.74	0.86
5	hb2	3	hd1	3.89	0.81	0.96
5	hd1	4	hn	4.05	0.86	0.84
5	hd2	4	hn	4.13	0.89	0.89
5	hd2	6	hn	2.7	0.44	0.38
5	hb2	6	hn	3.15	0.58	0.48
5	hd1	6	hn	3.45	0.67	0.58

6	hb#	4	hd22	3.62	0.63	0.52
6	hb#	4	hd21	4.01	0.75	0.63
6	hb#	7	hn	3.35	0.54	0.45
7	hg2#	4	hd22	3.34	0.54	0.45
7	hg2#	4	hd21	3.64	0.63	0.52
7	hn	6	hn	2.4	0.34	0.32
7	hg2#	6	hn	4.73	0.98	0.93
7	hn	8	hn	2.19	0.28	0.29
7	ha	8	hn	3.11	0.57	0.47
7	hb	8	hn	3.6	0.72	0.63
7	hg2#	8	hn	4.24	0.82	0.71
7	hb	9	hn	3.7	0.76	0.68
7	hg2#	9	hn	5.09	1.09	1.13
8	ha2	3	hh2	3.03	0.54	0.65
8	ha1	3	hh2	3.45	0.68	0.78
8	ha2	3	he1	3.47	0.68	0.79
8	ha2	9	hn	2.96	0.52	0.43
8	ha1	9	hn	3.29	0.63	0.53
9	ha	10	hn	2.12	0.22	0.28
9	hb#	10	hn	2.71	0.42	0.37
9	ha	10	hd1	3.43	0.67	0.77
9	ha	10	hb#	4.05	0.85	0.79
9	ha	3	hz3	2.96	0.52	0.64
9	ha	3	hh2	3.85	0.8	0.94
9	hn	8	hn	2.44	0.36	0.33
10	hb1	10	he3	2.6	0.39	0.56
10	ha	11	hn	2.23	0.29	0.3
10	hb#	11	hn	3.05	0.53	0.44
10	hb#	2	hn	3.92	0.8	0.73
10	ha	3	ha	2.6	0.41	0.36
10	hz3	3	hn	2.99	0.53	0.64
10	he3	3	hn	3	0.53	0.64
10	he1	3	hz3	3.17	0.59	0.89
10	hn	3	hz3	3.45	0.68	0.78
10	ha	4	hn	3.03	0.54	0.45
11	hn	10	he3	3.3	0.63	0.73
11	ha	12	hn	2.18	0.27	0.29
11	hb	12	hn	2.51	0.38	0.34
11	hb	2	hn	3.49	0.69	0.59
12	ha	2	hn	3.66	0.74	0.66

Table 8. HP7, pH 6.0 buffer, 290K

Residue	HN	Hα	Hβ, Hβ'	Others
Lys1	Exchange	4.451	2.080, 1.901	γ 1.418;δ 1.258; ε,ε' 2.484,2.391; εNH3 Exchange
Thr2	9.239	4.870	4.043	γ 1.243
Trp3	9.049	4.091	**2.648**, 1.840 $^3J_{\alpha\beta}$: **11.8**, ≤4	ε1 10.002; δ1 6.631; ε3 5.425 ζ3 6.430; η2 6.910; ζ2 7.274
Asn4	7.457	4.920	3.059, 2.373	γNH2 7.19 (δ21), 7.22 (δ22)
Pro5		3.736	2.391, 1.987	γ 2.037; δ,δ' 3.796,3.665
Ala6	7.796	4.138	1.397	
Thr7	6.864	4.262	4.116	γ 0.997
Gly8	7.970	3.734, 3.215		
Lys9	6.621	4.496	1.767, 1.420	γ 1.256; δ,δ' 1.671,1.572; ε,ε' 2.978; εNH3 Exchange
Trp10	8.671	5.139	**3.15**, 3.20 $^3J_{\alpha\beta}$: **2.5**, 12.0	ε1 10.299; δ1 7.564; ε3 7.375; ζ3 7.154; η2 7.181; ζ2 7.286
Thr11	9.402	4.645	4.330	γ 1.222
Glu12	8.452	4.145	2.119, 1.952	γ,γ' 2.317

For the Trp residues the pro-S β proton (Hβ3) is listed first with the shifts and coupling constants shown in bold.

Table 9. (K1R)-HP7, pH 6.0 buffer, 290K

Residue	HN	Hα	Hβ, Hβ'	Others
Arg1	Exchange	4.515	2.065, 1.942	γ 1.641; δ,δ' 2.817,2.737
Thr2	9.264	4.909	4.053	γ 1.256
Trp3	9.103	4.107	**2.629**, 1.805	ε1 9.982; δ1 6.602; ε3 5.419 ζ3 6.431; η2 6.906; ζ2 7.274
Asn4	7.403	4.931	3.072, 2.379	γNH2 7.23
Pro5		3.745	2.403, 1.989	γ 2.057; δ,δ' 3.802,3.689
Ala6	7.799	4.138	1.404	
Thr7	6.846	4.263	4.118	γ 1.001
Gly8	7.965	3.737, 3.215		
Lys9	6.611	4.500	1.777, 1.424	γ 1.257; δ,δ' 1.672,1.579; ε,ε' 2.982; εNH3 Exchange
Trp10	8.668	5.146	~3.16	ε1 10.183; δ1 7.517; ε3 7.358; ζ3 7.102; η2 7.149; ζ2 7.243
Thr11	9.434	4.626	4.329	γ 1.251
Glu12	8.480	4.157	2.145, 1.943	γ,γ' 2.335

Table 10. (G8A)-HP7, pH 6.0 buffer, 290K

Residue	HN	Hα	Hβ, Hβ'	Others
Lys1	Exchange	4.435		γ 1.406; δ 1.301; ε,ε' 2.505,2.401; εNH3 Exchange
Thr2	9.216	4.838	4.047	γ 1.243
Trp3	9.075	4.176	**2.699**, 1.964	ε1 10.100; δ1 6.785; ε3 5.419 ζ3 6.430; η2 6.900; ζ2 7.236
Asn4	7.658	4.914	2.977, 2.385	γNH2 7.26
Pro5		3.839	2.391, 1.987	γ 2.052; δ,δ' 3.807,3.701
Ala6	7.857	4.147	1.402	
Thr7	6.861	4.141	4.114	γ 0.985
Ala8	7.834	3.124	1.356	
Lys9	6.577	4.417	1.690, 1.415	γ 1.295; δ,δ' 1.665,1.562; ε,ε' 2.972; εNH3 Exchange
Trp10	8.630	5.129	~3.20	ε1 10.274; δ1 7.556; ε3 7.401; ζ3 7.154; η2 7.184; ζ2 7.296
Thr11	9.311	4.628	4.316	γ 1.212
Glu12	8.408	4.140	2.119, 1.946	γ,γ' 2.314

Table 11. (N4A)-HP7, pH 6.0 buffer, 298K

Residue	HN	Hα	Hβ, Hβ'	Others
Lys1	Exchange	4.208	1.888	γ 1.497;δ 1.340; ε,ε' 2.667; εNH3 Exchange
Thr2	Exchange	4.557	4.083	γ 1.228
Trp3	8.704	4.411	3.013, 2.664	ε1 10.040; δ1 6.979; ε3 6.746; ζ3 6.837; η2 7.100; ζ2 7.395
Ala4	7.791	4.544	1.165	
Pro5		3.955	2.292, 1.883	γ 1.950; δ,δ' 3.575,3.474
Ala6	8.361	4.216	1.387	
Thr7	7.533	4.312	4.184	γ 1.135
Gly8	8.111	3.841, 3.605		
Lys9	7.439	4.359	1.694, 1.538	γ 1.246; δ,δ' 1.587; ε,ε' 2.923; εNH3 Exchange
Trp10	8.350	4.910	3.23	ε1 10.182; δ1 7.359; ε3 7.528; ζ3 7.142; η2 7.201; ζ2 7.402
Thr11	8.465	4.434	4.199	γ 1.122
Glu12	8.060	4.086	2.053, 1.903	γ,γ' 2.244

Table 12. HP7 (-4), pH 6.0 buffer, 290K

Residue	HN	Hα	Hβ, Hβ'	Others
Trp1	8.325	4.222	2.928, 2.751	ε1 10.082; δ1 6.964; ε3 6.939; ζ3 6.913; η2 7.128; ζ2 7.394
Asn2	7.853	4.852	3.013, 2.451	γNH2 7.39 (δ21), 7.12 (δ22)
Pro3		3.959	2.309, 1.944	γ 1.965; δ,δ' 3.691,3.550
Ala4	8.013	4.234	1.413	
Thr5	7.386	4.306	4.204	γ 1.107
Gly6	8.176	3.867, 3.626		
Lys7	7.541	4.332	1.696, 1.521	γ 1.188; δ,δ' 1.560; ε,ε' 2.904; εNH3 Exchange
Trp8	8.355	4.518	3.225, 3.132	ε1 10.199; δ1 7.318; ε3 7.512; ζ3 7.147; η2 7.229; ζ2 7.433

Table 13. Ac-NPATGK-NH_2, pH 6.0 buffer, 290K

Residue	HN	Hα	Hβ, Hβ'	Others
Asn1	8.458	4.948	2.850, 2.660	γNH2 7.70 (δ21), 7.04 (δ22)
Pro2		4.424	2.310, 1.964	γ 2.024; δ,δ' 3.854,3.732
Ala3	8.480	4.354	1.414	
Thr4	8.072	4.321	4.257	γ 1.229
Gly5	8.435	3.985		
Lys6	8.267	4.301	1.872, 1.750	γ 1.430; δ,δ' 1.680; ε,ε' 2.990; εNH3 Exchange term-COHN2 7.71, 7.19

Table 14. HP6 , pH 6.0 buffer, 290K

Residue	HN	Hα	Hβ, Hβ'	Others
Lys 1	Exchange	4.002	1.912	γ,γ' 1.395; δ,δ' 1.703; ε,ε' 2.992
Tyr 2	8.890	5.152	2.734	δ 6.886; ε 6.676
Thr 3	9.110	4.709	3.995	γ 1.116
Trp 4	8.699	4.631	2.942, 2.213	ε1 9.942; δ1 6.834; ε3 5.994 ζ3 6.639; η2 6.989; ζ2 7.236
Ser 5	8.426	4.352	3.572	
Asn 6	9.018	4.205	2.875, 2.581	γNH2 7.511 (δ21), 6.868(δ22)
Gly 7	7.558	3.885, 3.350		
Lys 8	7.001	4.273	1.767	γ,γ' 1.202; δ,δ' 1.608; ε,ε' 2.944
Trp 9	8.530	5.020	3.025, 2.935	ε1 10.06; δ1 7.28; ε3 7.300 ζ3 7.103; η2 7.179; ζ2 7.28
Thr 10	9.203	4.647	4.066	γ 1.167
Val 11	8.417	4.190	1.906	γ,γ' 0.816
Glu 12	8.300	4.223	2.023, 1.840	γ,γ' 2.290

Table 15. (T3V)-HP6 , pH 6.0 buffer, 290K

Residue	HN	Hα	Hβ, Hβ'	Others
Lys 1	Exchange	4.015	1.931	γ,γ' 1.395; δ,δ' 1.711; ε,ε' 2.976
Tyr 2	8.895	5.278	2.831, 2.724	δ 6.921; ε 6.694
Val 3	9.411	4.575	2.053	γ,γ' 0.893, 0.845
Trp 4	8.883	4.557	**2.865**, 1.987	ε1 9.904; δ1 6.808; ε3 5.660 ζ3 6.545; η2 6.947; ζ2 7.229
Ser 5	8.457	4.344	3.536	
Asn 6	9.036	4.155	2.869, 2.544	γNH2 7.488 (δ21), 6.829 (δ22)
Gly 7	7.398	3.825, 3.261		
Lys 8	6.782	4.209	1.650	γ,γ' 1.186; δ,δ' 1.592; ε,ε' 2.944
Trp 9	8.492	5.149	3.011	ε1 10.06; δ1 7.361; ε3 7.300 ζ3 7.088; η2 7.185; ζ2 7.242
Thr 10	9.429	4.74	4.104	γ 1.181
Val 11	8.367	4.352	1.899	γ,γ' 0.790
Glu 12	8.449	4.154	1.974, 1.78	γ,γ' 2.160

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