Trapping a Folding Intermediate of the α-Helix: Stabilization of the π-Helix

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Supporting Information

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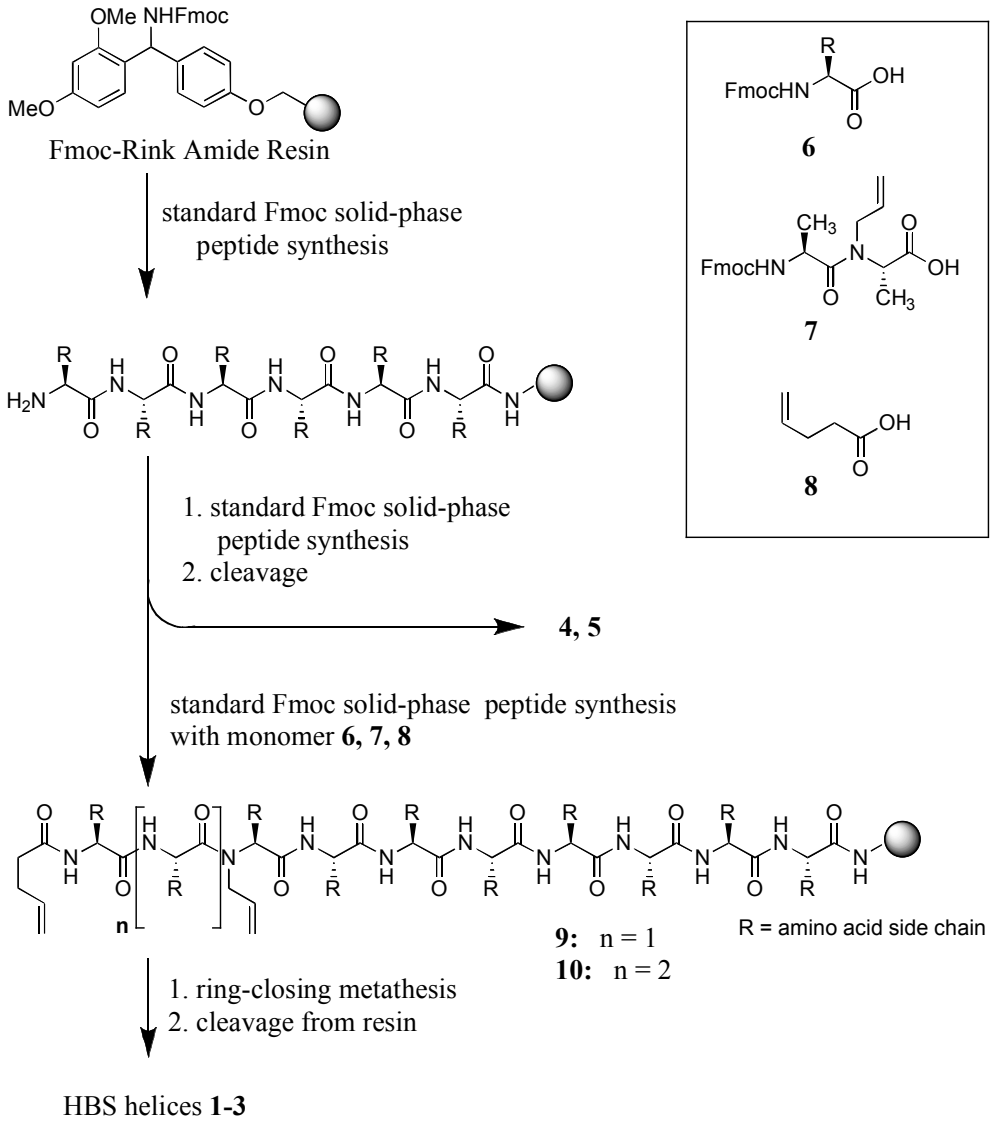
Synthesis and Characterization of Peptides 1-5

The synthesis and characterization of HBS α-helix **1** and the unconstrained peptide **4** have been previously described.[1] HBS π-helices **2** and **3** were prepared and characterized as described.[2]



Scheme 1. Synthesis of HBS helices **1-3** and peptides **4-5**.

1. Wang, D., Chen, K., Kulp, J. L., III & Arora, P. S. (2006) *J. Am. Chem. Soc.* **128,** 9248-9256.

2. Chapman, R. N. & Arora, P. S. (2006) *Org. Lett.* **8,** 5825-5828.

Synthesis and Characterization of HBS Peptides, Side Constrained Peptides and Unconstrained Peptides

Synthesis of Resin Bound Bis-Olefin Peptides 9-10.[3] Resin bound bis-olefin peptides were synthesized by conventional Fmoc solid-phase chemistry on Rink Amide or Knorr resin (loading = 0.4mmole/g) with appropriate substitutions of *N*-allyl dipeptide **7**[4] and 4-pentenoic acid. In each coupling step, the appropriate Fmoc amino acid (5 equiv) was activated with HBTU (4.9 equiv) in 10% DIPEA/NMP solution for 10 min and added to the resin bound free amine. The resulting mixture was shaken for 30–45 min. The coupling efficiency was monitored by the ninhydrin test. After each coupling step, the Fmoc group was removed by treatment with 20% piperidine in NMP (2 x 20 min). The bis-olefin containing resin was thoroughly washed with DMF and DCM respectively, and dried under vacuum overnight. Peptides were stored at 4°C until needed.

General method for RCM in the Microwave.[2] The resin bound bis-olefin (0.1mmole) was placed in a thick wall glass tube (CEM) with 15 mol% of Hoveyda Grubbs II catalyst and sealed with a cap. To this tube was added 900µl of freshly distilled dichlorobenzene, the tube was purged with argon and the resin allowed to swell for 20 min. All reactions were carried out in the CEM discover microwave reactor with a run time of 2 minutes, hold time of 5 minutes and temperature of 200 °C as previously reported.[3] All microwave reactions were conducted in a sealed glass tube; the pressure was monitored and did not exceed 40 psi, while the temperature was monitored by an IR sensor at the base of the reaction vessel. After the indicated time had

3. Dimartino, G., Wang, D., Chapman, R. N. & Arora, P. S. (2005) *Org. Lett.* **7,** 2389-2392.

4. Chapman, R. N., Dimartino, G. & Arora, P. S. (2004) *J. Am. Chem. Soc.* **126,** 12252-12253.

elapsed, the solution was cooled rapidly by compressed air, and the resin washed with DCM (3x), 10% 1,3-bis(diphenylphosphino)propane in DCM (6x) and DCM (3x), then dried for 3 h under vacuum. The peptide was cleaved from the resin with 95% TFA: 2.5% triisopropylsilane : 2.5% water for 90 min, filtered, placed on a rotary evaporator for 5 minutes at 40 ^{o}C, then dissolved in 60% 0.1% aqueous TFA and 40% acetonitrile. The resulting solution was then purified by preparative HPLC.

Synthesis of Unconstrained Peptides. Unconstrained peptides were synthesized by standard Fmoc solid-phase peptide synthesis methodology.

Table 2. Mass spectrometry results. LCMS data was obtained on an Agilent 1100 series LC/MSD (XCT) electrospray trap.

Compound	Calculated Mass $[M+H]^+$	Observed Mass $[M+H]^+$
2	1354.8	1355.5
3	1354.8	1355.3
5	1289.7	1289.5

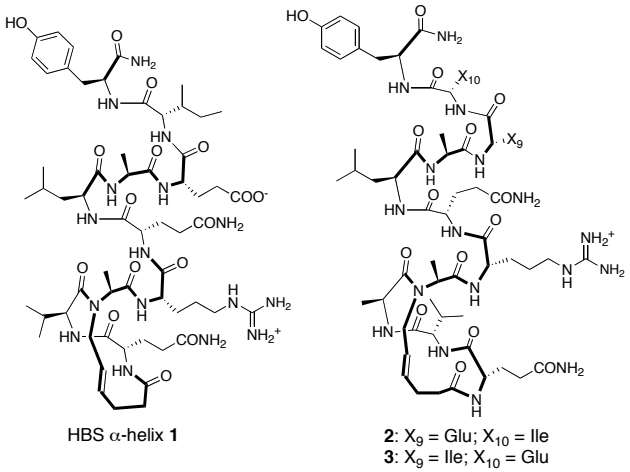


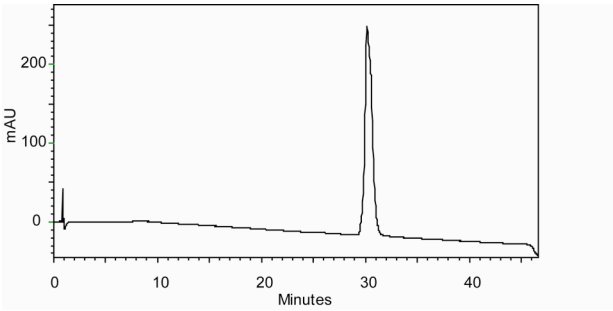
Fig. 6. Chemical structures of HBS helices **1-3**.

4: AcQVARQLAEIY-NH_2

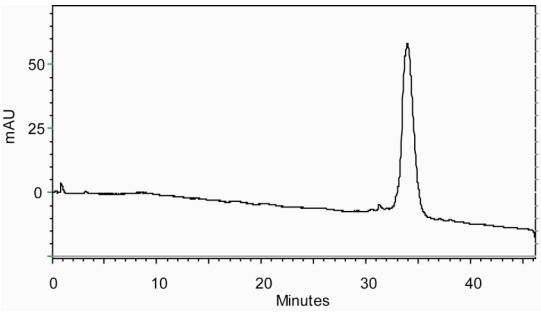
5: AcQVAARQLAIEY-NH_2

Fig. 7. Sequences of peptides **4-5**.

(a) HBS **2**



(b) HBS **3**



(c) Unconstrained peptide **5**

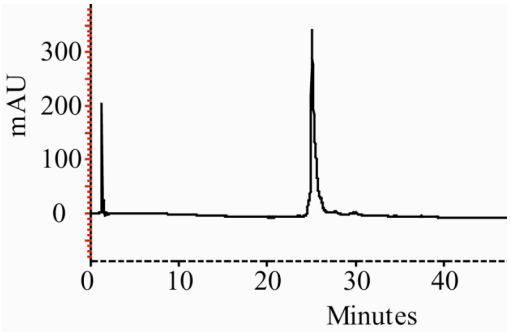


Figure 8. Analytical HPLC traces of peptides **2**, **3** and **7** (222 nm). HPLC conditions: C_{18} reversed-phase column. 0 % B to 40% B in 40 min; A: 0.1% aqueous TFA, B: acetonitrile; flow rate: 1.4 mL/min. HPLC experiments were conducted with 4.6 x 150 mm Waters C_{18} column using a Beckman Coulter HPLC equipped with a System Gold 168 Diode array detector.

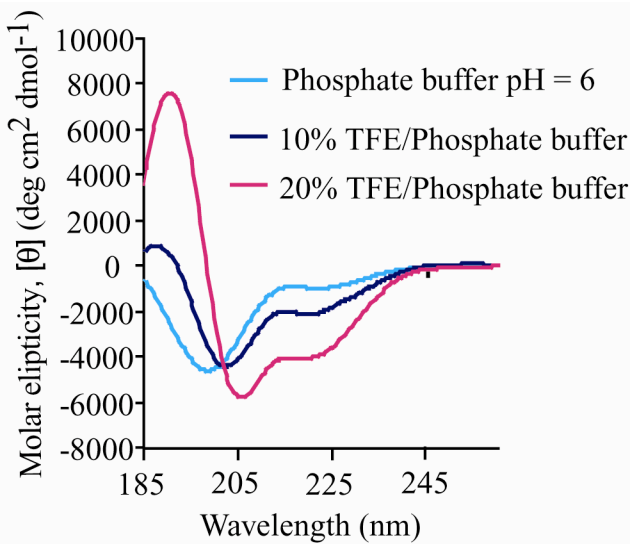
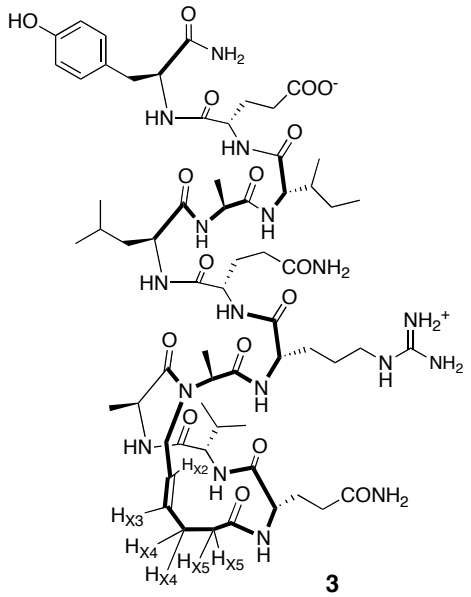


Figure 9. CD spectra of peptide **5** in different solvents at 298 K

2D NMR Studies on HBS π-Helix 3

Table 3. ^{1}H NMR chemical shift assignment of HBS π-helix **3**

residue	NH	H_α	H_β	H_γ	H_δ	other
Q_1	8.31	4.21	2.09	2.46, 2.50		
V_2	7.81	4.1	2.01	0.93		
A_3	8.74	4.29				
A_4	N/A	4.14				
R_5	8.3	4.49	1.9	1.68	3.19	
Q_6	8.55	4.44	2.03, 2.16	2.4		
L_7	8.43	4.38	1.65	1.42	0.95, 0.92	
A_8	8.37	4.33	1.33			
I_9	8.05	4.06	1.82	1.45, 1.47	0.81, 0.88	
E_{10}	8.29	4.31	1.97	2.27, 2.36		
Y_{11}	8.17	4.56	3.17, 2.92			7.19, 7.54
X5						2.46, 2.22
X4						3.68
X3						5.17
X2						5.88
X1						3.67, 4.27



3

Table 4. NOE distances and J_{NH}-$C_{\alpha H}$-derived ϕ angle restraints used for the structure determination of HBS π–helix 3 in TFE-d3/D2O/phosphate buffer (20/5/75).

Atom 1	Atom 2	Category	Correction*
X1	X2	VS	0.6
X1	X3	S	0.6
X1	Q1-HA	VW	1.0
X1	V2-NH	VW	1.0
X1	A3-Hα	M	1.0
X2	X3	S	0.0
X2	X5	VS	0.6
X2	A3-Hβ	M	1.0
X2	A4-Hβ	S	1.0
X3	X4	VS	0.6
X3	X5	M	0.6
X3	Q1-NH	W	0.0
X3	V2-NH	W	0.0
X3	A3-NH	S	0.0
X3	A4-Hβ	W	1.0
X4	X5	VS	0.6
X4	Q1-NH	VS	1.0
X4	V2-NH	M	1.0
X4	A3-NH	M	1.0
X5	Q1-NH	S	1.0
X5	Q1-HA	M	1.0
X5	V2-NH	W	1.0
X5	A3-NH	W	1.0
X5	A3-Hα	VW	1.0
X5	A3-Hβ	W	2.0
X5	A4-Hβ	VS	2.0
X5	R5-NH	S	1.0
Q1-NH	Q1-Hα	S	0.0
Q1-NH	Q1-Hβ	VS	0.6
Q1-Hα	Q1-Hβ	VS	0.6
Q1-NH	V2-NH	S	0.0
Q1-Hα	V2-NH	M	0.0
Q1-Hα	V2-Hγ	M	2.4
Q1-Hβ	V2-NH	S	1.0
Q1-Hβ	V2-Hγ	M	3.4
Q1-NH	Q6-Hα	VW	

Q1-Hα	R5-NH	M	0.0
Q1-Hβ	Q6-Hα	W	1.0
Q1-Hα	Q6-NH	VW	0.0
V2-NH	V2-Hα	S	0.0
V2-NH	V2-Hβ	S	0.0
V2-NH	V2-Hγ	S	1.7
V2-Hα	V2-Hβ	S	0.0
V2-Hα	V2-Hγ	S	1.7
V2-Hβ	V2-Hγ	S	1.7
V2-NH	A3-NH	M	0.0
V2-NH	A3-Hα	VW	0.0
V2-NH	A3-Hβ	VW	1.0
V2-Hα	A3-NH	M	0.0
V2-Hβ	A3-NH	M	0.0
V2-Hβ	A3-Hα	W	0.0
V2-Hβ	A3-Hβ	VW	1.0
V2-Hγ	A3-NH	S	2.4
V2-Hγ	A3-Hβ	W	3.4
V2-Hγ	R5-Hδ	VW	3.4
V2-NH	Q6-Hα	VW	0.0
A3-NH	A3-Hα	S	0.0
A3-NH	A3-Hβ	S	0.6
A3-Hα	A3-Hβ	VS	0.6
A3-NH	A4-Hβ	VW	1.0
A3-NH	L7-Hα	VW	0.0
A3-Hβ	R5-NH	VW	1.0
A3-Hβ	Q6-NH	VW	1.0
A3-Hβ	L7-NH	M	1.0
A4-Hα	A4-Hβ	VS	0.6
A4-Hβ	R5-NH	S	1.0
A4-Hβ	A8-Hα	VW	1.0
R5-NH	R5-Hα	S	0.0
R5-NH	R5-Hβ	VS	0.6
R5-NH	R5-Hγ	M	1.0
R5-NH	R5-Hδ	W	1.0
R5-Hα	R5-Hγ	VS	0.6
R5-Hβ	R5-Hγ	VS	0.6
R5-Hβ	R5-Hδ	VS	0.6
R5-Hβ	R5-NHε	W	2.4
R5-Hγ	R5-Hδ	VS	0.6

R5-Hγ	R5-NHε	M	1.7
R5-NH	Q6-NH	S	0.0
R5-Hα	Q6-NH	M	0.0
R5-Hα	Q6-Hβ	W	1.0
R5-Hβ	Q6-NH	S	1.0
R5-Hγ	Q6-NH	M	1.0
R5-Hδ	Q6-NH	W	1.0
R5-Hα	L7-NH	VW	
R5-Hα	E10-Hβ	VW	1.0
R5-Hβ	L7-NH	VW	1.0
R5-Hβ	I9-Hα	VW	1.0
R5-NH	E10-Hα	VW	0.0
R5-Hγ	E10-NH	VW	1.0
Q6-NH	Q6-Hα	S	0.0
Q6-NH	Q6-Hβ	VS	0.6
Q6-Hα	Q6-Hβ	VS	0.6
Q6-Hβ	Q6-NHδ	S	1.0
Q6-NH	L7-NH	S	0.0
Q6-NH	L7-Hδ	VW	2.4
Q6-Hα	L7-NH	M	0.0
Q6-Hβ	L7-NH	W	1.0
Q6-NHδ	L7-Hβ	W	2.0
Q6-NHδ	L7-Hδ	W	3.4
Q6-NH	A8-Hβ	VW	1.0
Q6-Hβ	A8-NH	VW	1.0
Q6-NH	E10-Hα	VW	0.0
Q6-Hβ	E10-Hα	VW	1.0
L7-NH	L7-Hα	S	0.0
L7-NH	L7-Hβ	VS	0.6
L7-NH	L7-Hγ	S	0.0
L7-NH	L7-Hδ	M	2.0
L7-Hα	L7-Hδ	S	1.7
L7-NH	A8-NH	S	0.0
L7-NH	A8-Hβ	VW	1.0
L7-Hα	A8-NH	M	0.0
L7-Hβ	A8-NH	VS	1.0
L7-Hδ	A8-NH	S	2.4
L7-Hβ	Y11-aro	VS	3.4
A8-NH	A8-Hβ	S	0.6
A8-NH	I9-NH	S	0.0

A8-NH	I9-Hα	VW	0.0
A8-NH	I9-Hδ	VW	1.0
A8-Hβ	I9-NH	S	1.0
A8-Hβ	Y11-aro	VW	3.4
I9-NH	I9-Hα	S	0.0
I9-NH	I9-Hβ	S	0.0
I9-NH	I9-Hγ	S	1.7
I9-NH	I9-Hδ	M	1.0
I9-Hα	I9-Hβ	S	0.0
I9-Hα	I9-Hδ	M	0.6
I9-Hβ	I9-Hδ	M	0.6
I9-NH	E10-NH	S	0.0
I9-NH	E10-Hα	VW	0.0
I9-Hα	E10-NH	S	0.0
I9-Hβ	E10-NH	S	0.0
I9-Hγ	E10-NH	VS	2.4
I9-Hδ	E10-NH	VW	1.0
I9-Hδ	E10-Hα	M	1.0
E10-NH	E10-Hα	S	0.0
E10-NH	E10-Hβ	VS	0.6
E10-NH	E10-Hγ	M	0.6
E10-Hα	E10-Hγ	S	0.6
E10-Hβ	E10-Hγ	S	0.6
E10-NH	Y11-NH	S	0.0
E10-NH	Y11-Hα	VW	0.0
E10-Hα	Y11-NH	M	0.0
E10-Hα	Y11-aro	VW	2.4
E10-Hγ	Y11-NH	M	1.0
E10-Hγ	Y11-aro	VW	3.4
Y11-NH	Y11-Hα	S	0.0
Y11-NH	Y11-Hβ	S	0.6
Y11-NH	Y11-aro	M	2.4
Y11-Hα	Y11-aro	M	2.0

*Pseudoatom corrections were applied to nonstereospecifically assigned protons. Ile methyl and methylene Hγ protons could not be differentiated. The Ile Hγ pseudoatoms were corrected as Leu Hγ pseudoatoms. Wuthrich, K. NMR of Proteins and Nucleic Acids, Wiley, New York.

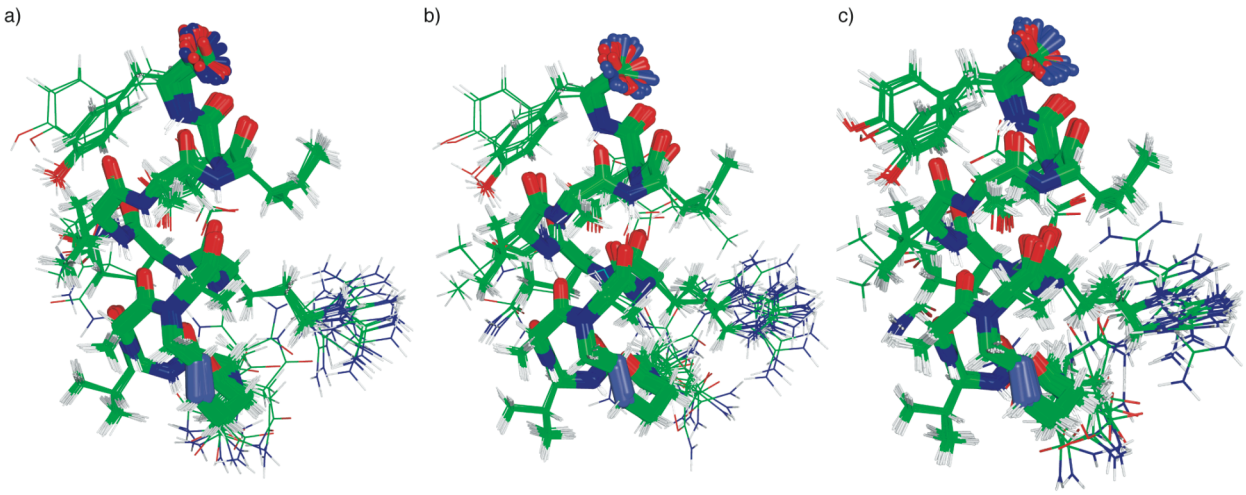


Figure 10. Comparison of the 20 lowest energy conformations resulting from three different starting conformations: (a) π-helix, (b) α-helix and (c) extended strand, respectively.

Table 5. Structural Statistics for 20 final NMR structures.

	20 Lowest Energy Structures	Lowest Energy Structure
Violations (mean & standard deviation)		
Distance constraints (Å)	0.171 ± 0.004	0.166
number of distance constraint violations > 1.0 Å	0	0
j angle restraints (°)	1.793 ± 0.110	1.847
# of angle contraint violations > 5°	0	0
Deviation from idealized geometry		
Bond lengths (Å)	0.00534 ± 0.00008	0.00543
Bond angles (°)	1.715 ± 0.015	1.724
Impropers (°)	2.572 ± 0.093	2.584
R.m.s.d. deviation of each structure to the rest (Å)		
Heavy	1.03 ± 0.20	0.78 ± 0.11
Backbone	0.33 ± 0.15	0.38 ± 0.08
Distance constraints		
Total NOE	147	
Intraresidue	55	
Sequential	74	
Medium and long range	27	
Dihedral angles	10	

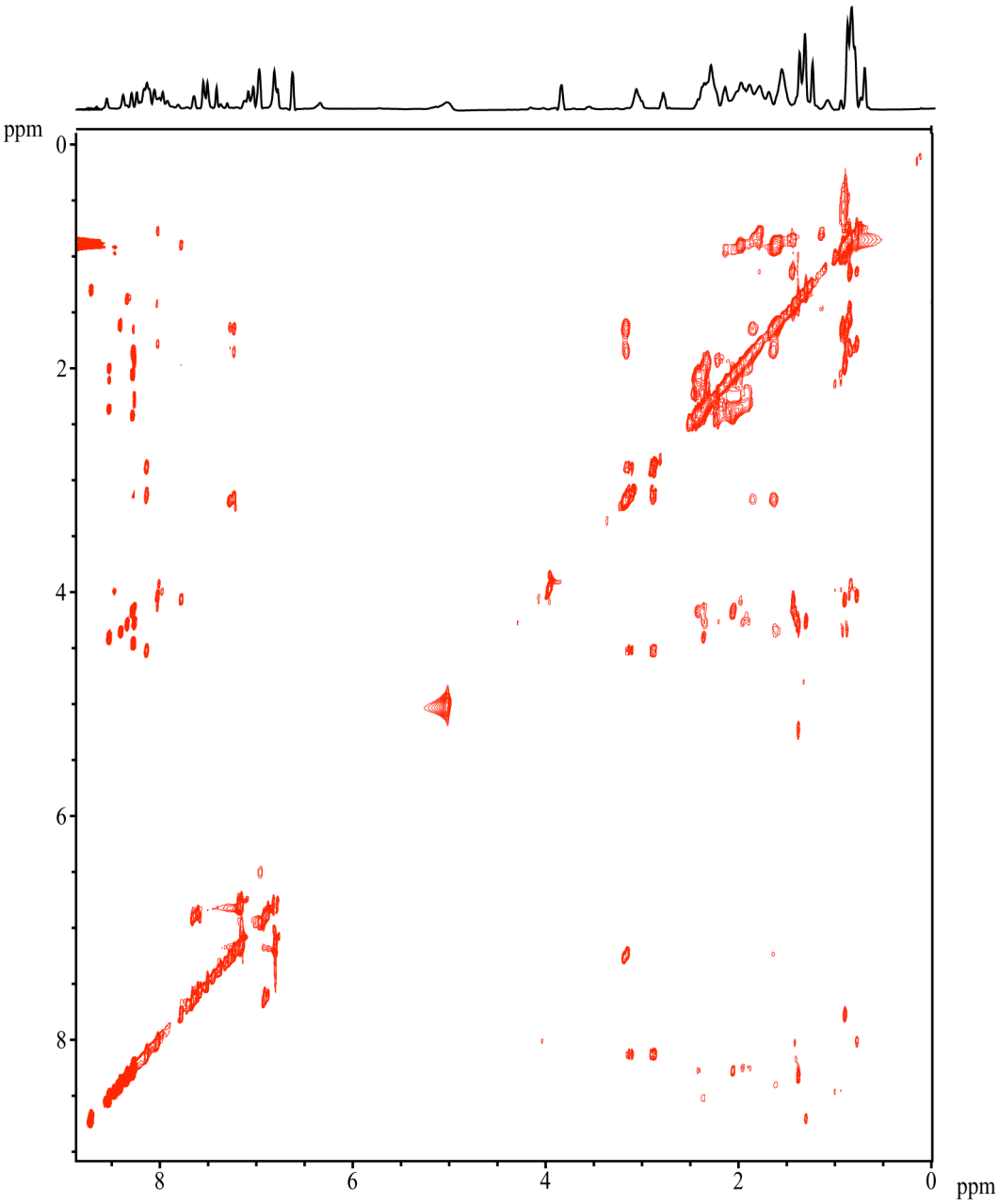


Figure 11. TOCSY spectrum (600 MHz, 268 K) of peptide **3** in TFE-d2/Phosphate buffer (20/80)

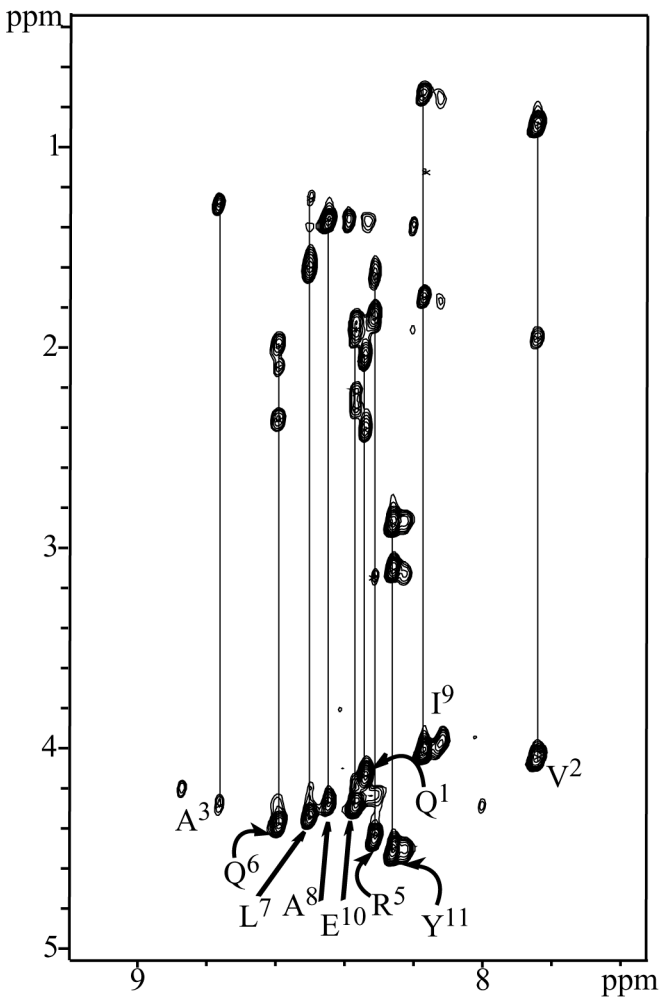


Figure 12. NH-Cα region of the 600 MHz TOCSY spectrum (268 K) of peptide **3** in TFE-d2/phosphate buffer (20/80). Amino acid spin systems are identified by standard one letter codes, their sequence position by solid lines connecting the cross-peak

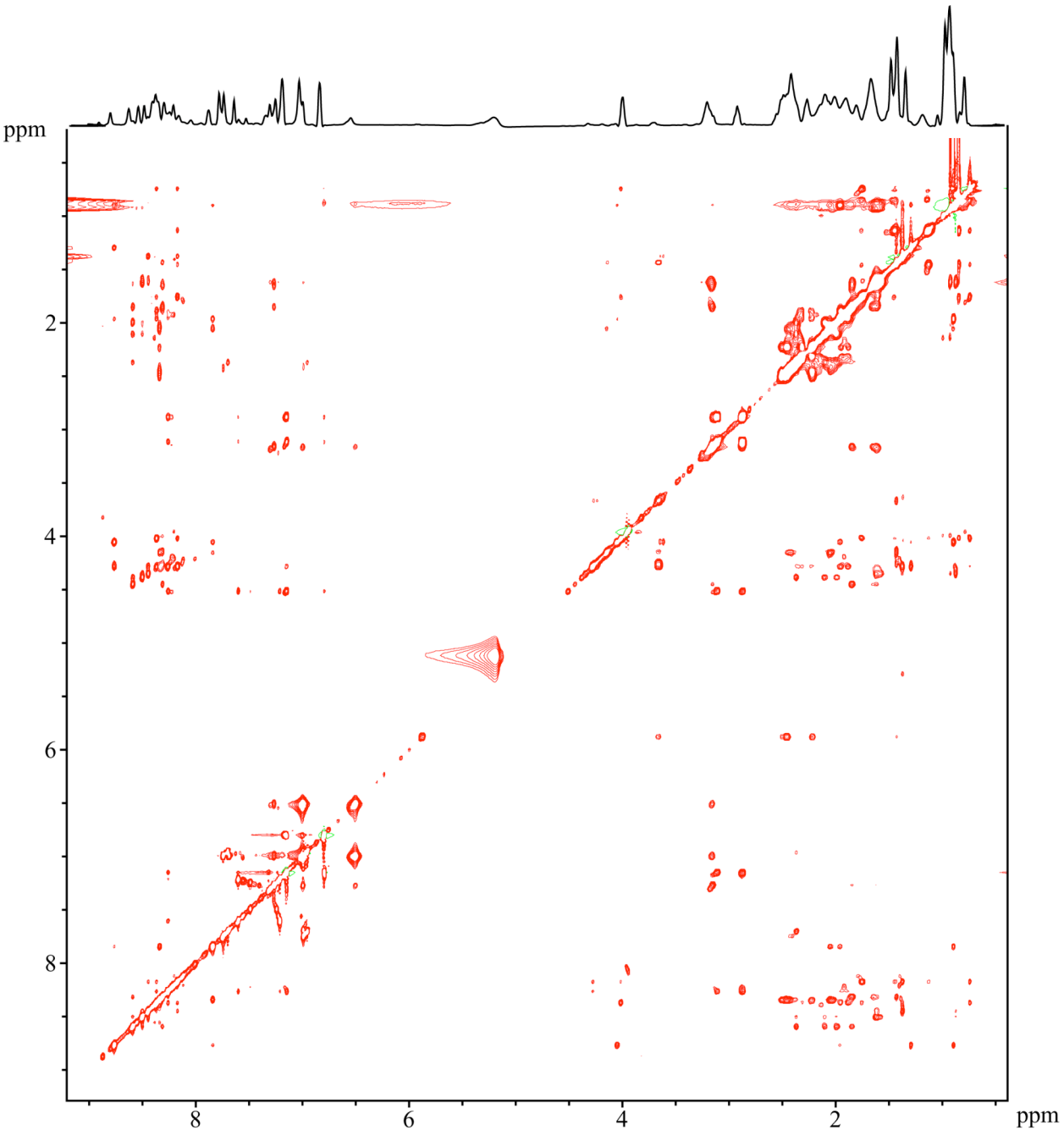


Figure 13. Full NOESY spectrum (600 MHz, 268 K) of peptide **3** in TFE-d2/Phosphate buffer (20/80)

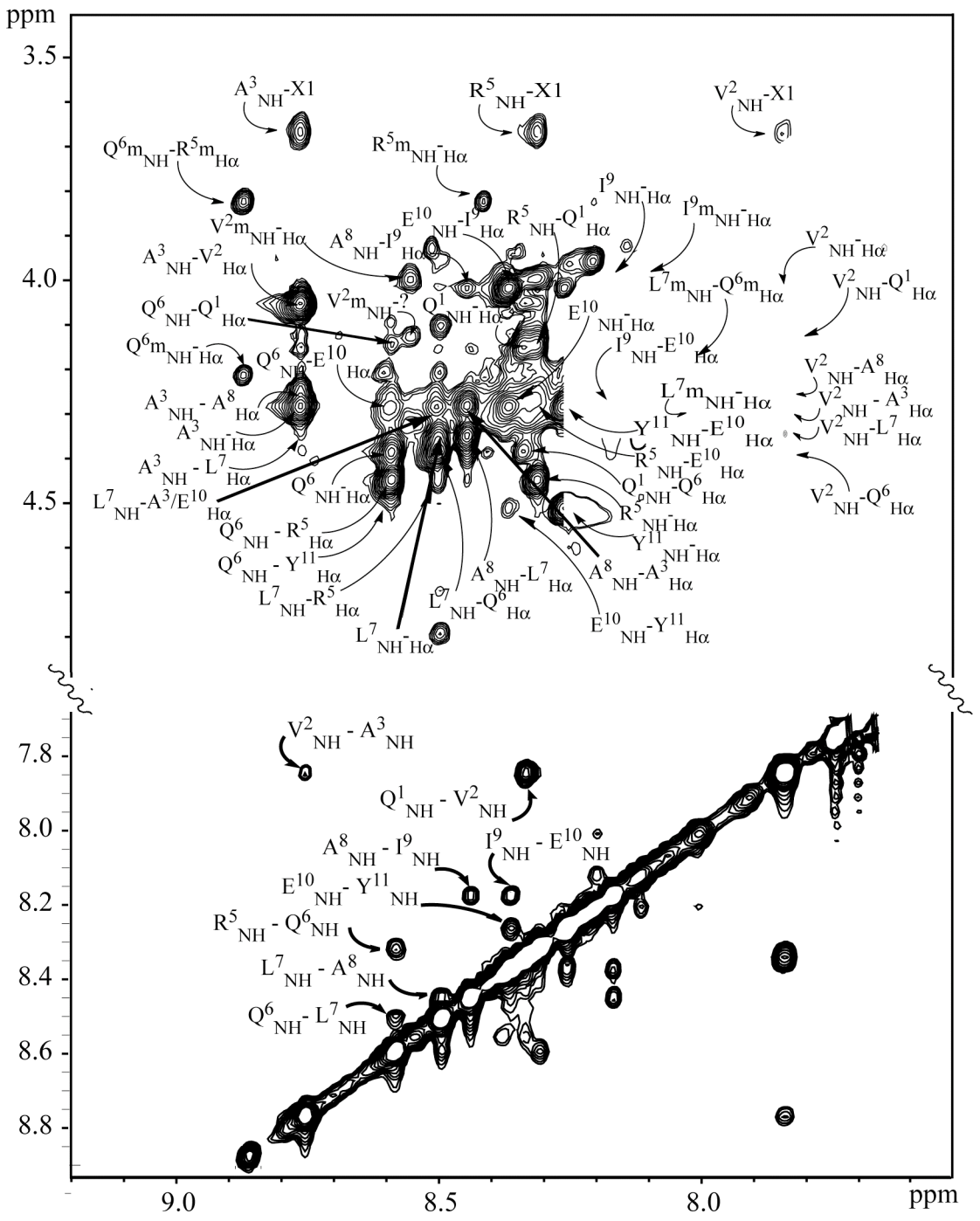


Figure 14. The NH-NH and NH-Cα regions of the 600 MHz NOESY spectrum (268K) of peptide **3** in TFEd2/phosphate buffer (20/80)

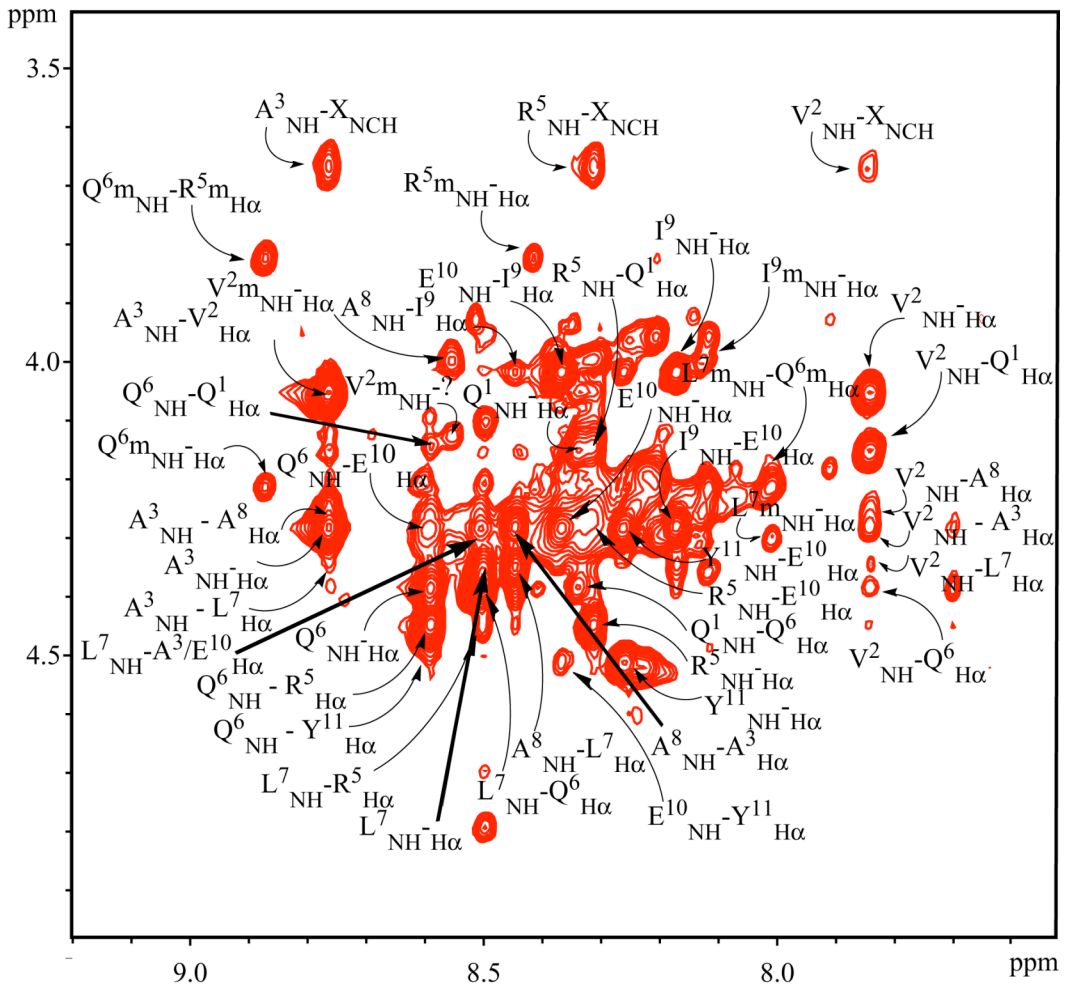


Figure 15. Fingerprint region of NOESY spectra of **3.**

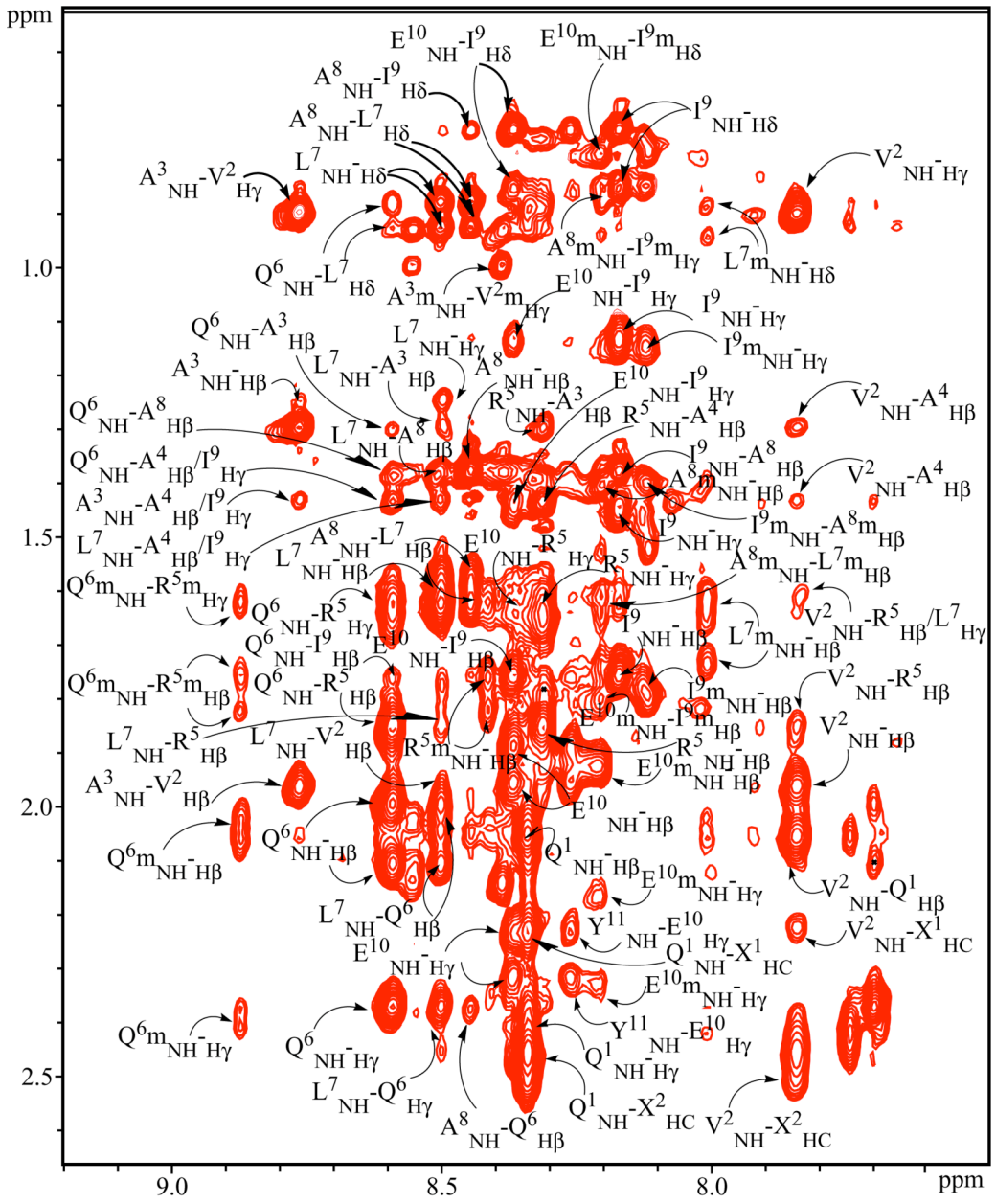


Figure 16. Fingerprint region of NOESY spectra of **3.**

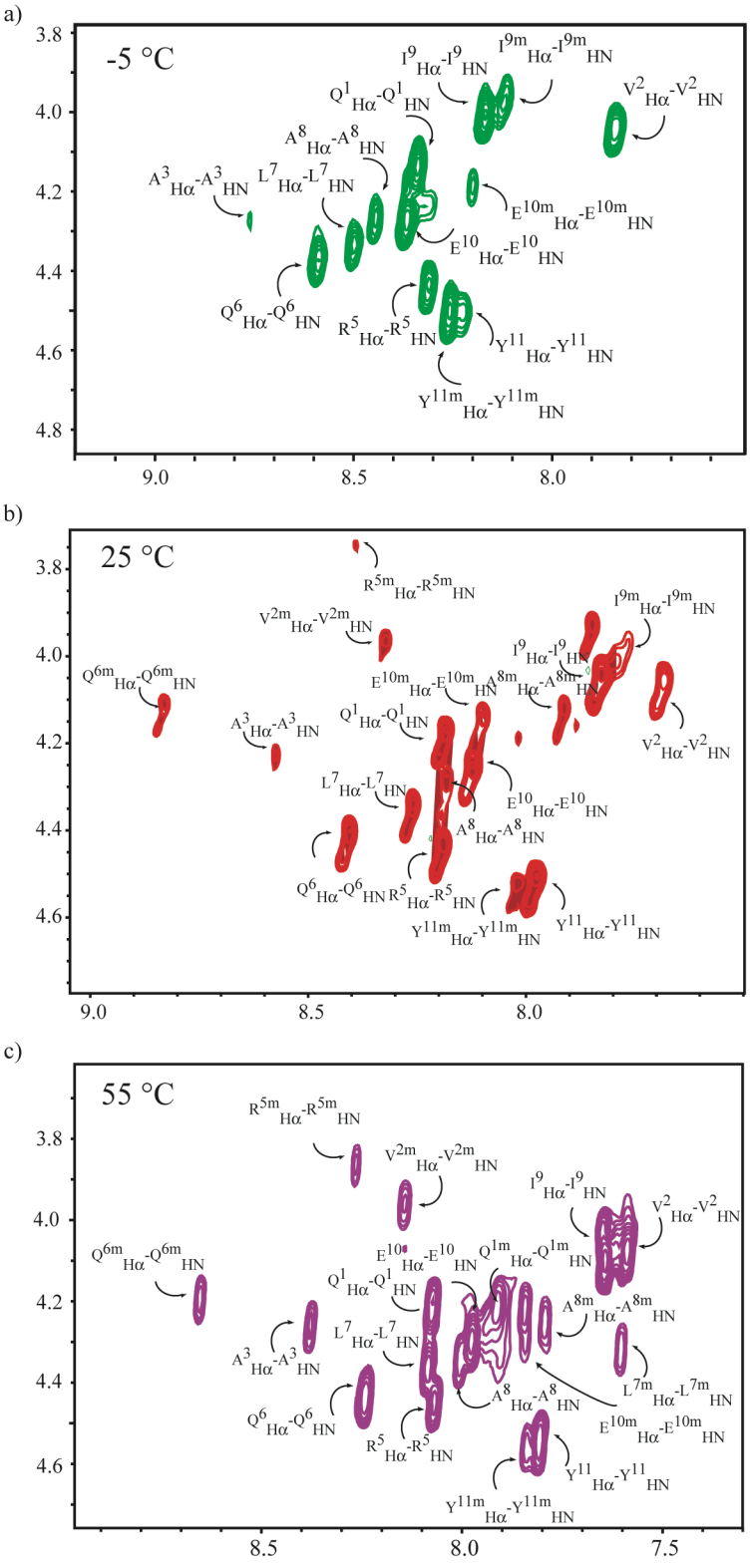


Figure 17. Fingerprint regions of the 600 MHz TOCSY spectra at (a) 268 K, (b) 298 K, and (c) 328 K of peptide **3** in TFEd2/phosphate buffer (20/80). The crosspeaks which appear at higher temperatures are marked "m" (minor conformation).

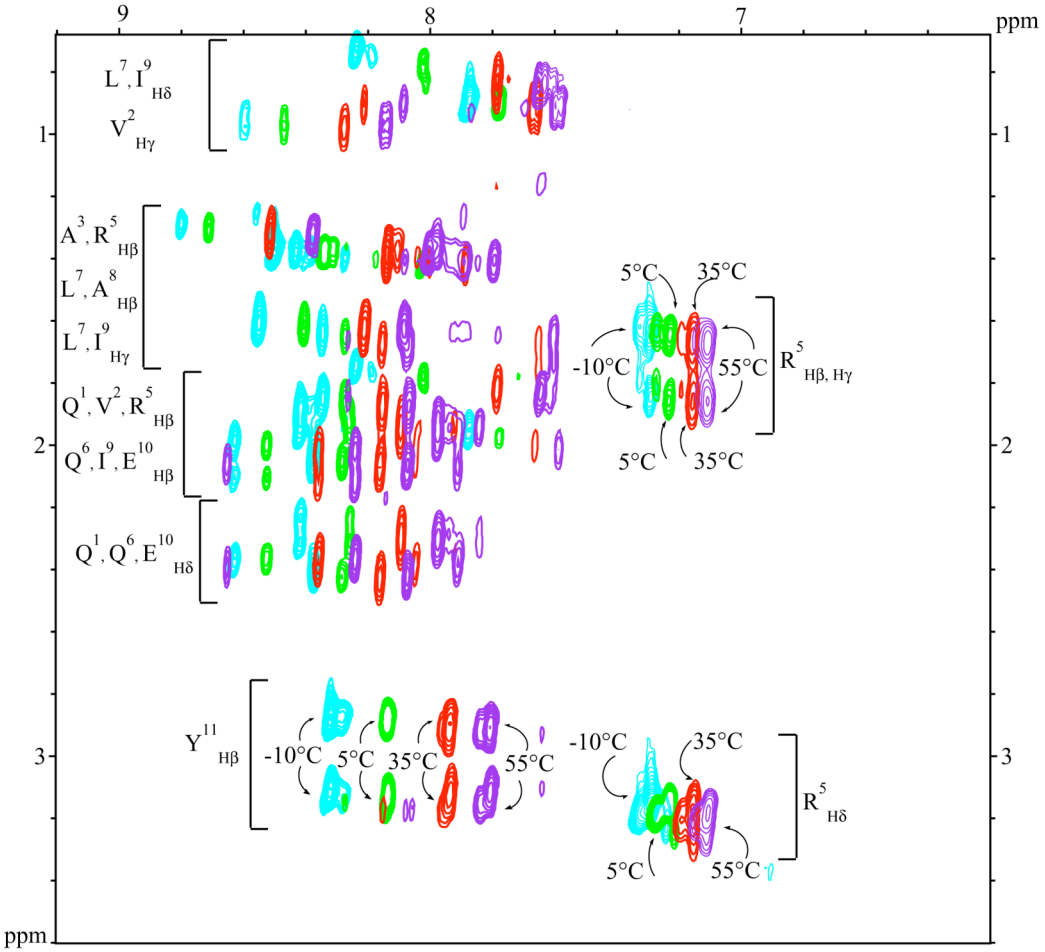


Figure 18. Fingerprint region of the 600 MHz TOCSY spectrum at different temperatures for HBS π-helix **3**.

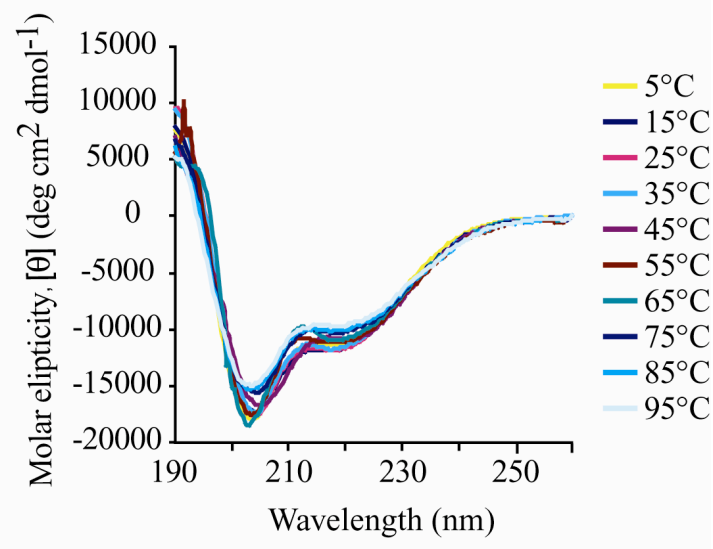
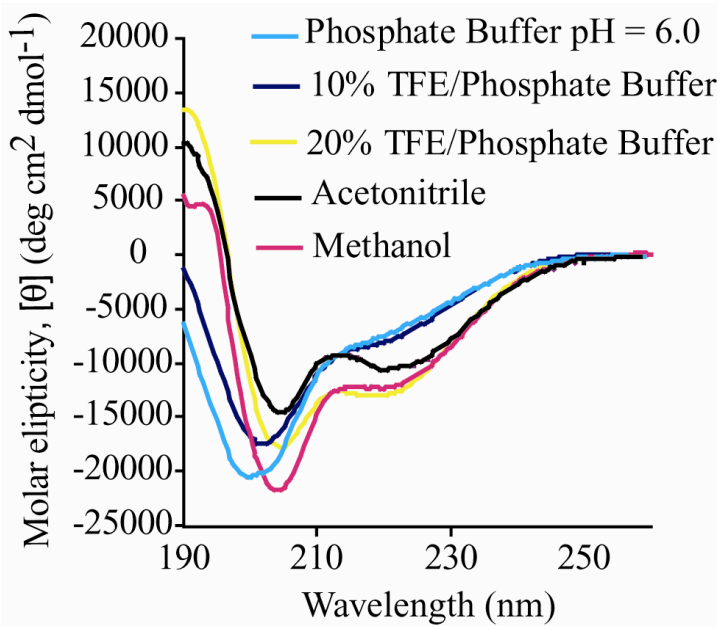


Figure 19. Temperature-dependent CD spectra of HBS peptide **3** in 20% TFE/phosphate buffer.

(a)



(b)

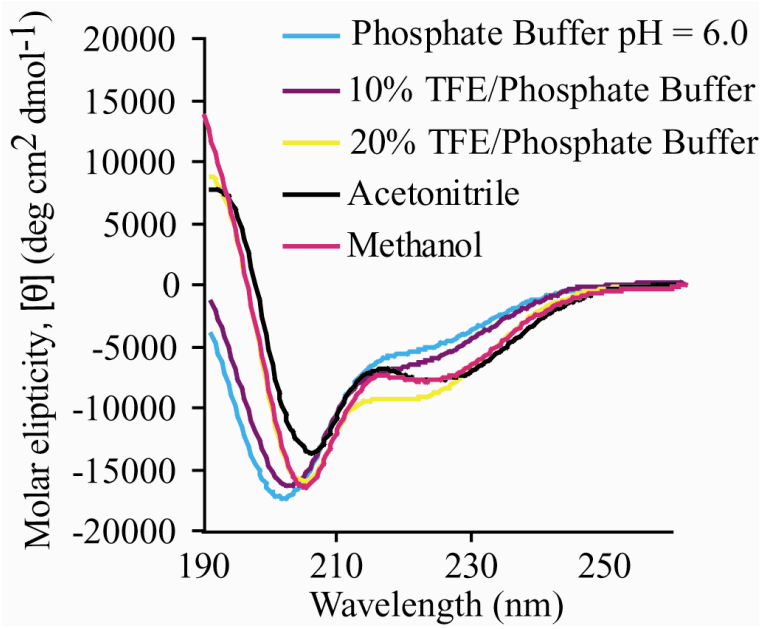


Figure 20. CD spectra of HBS peptides (a) **2** and (b) **3** in various solvents.