Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Comparative analysis of cross strand aromatic-Phe interactions in designed peptide β-hairpins

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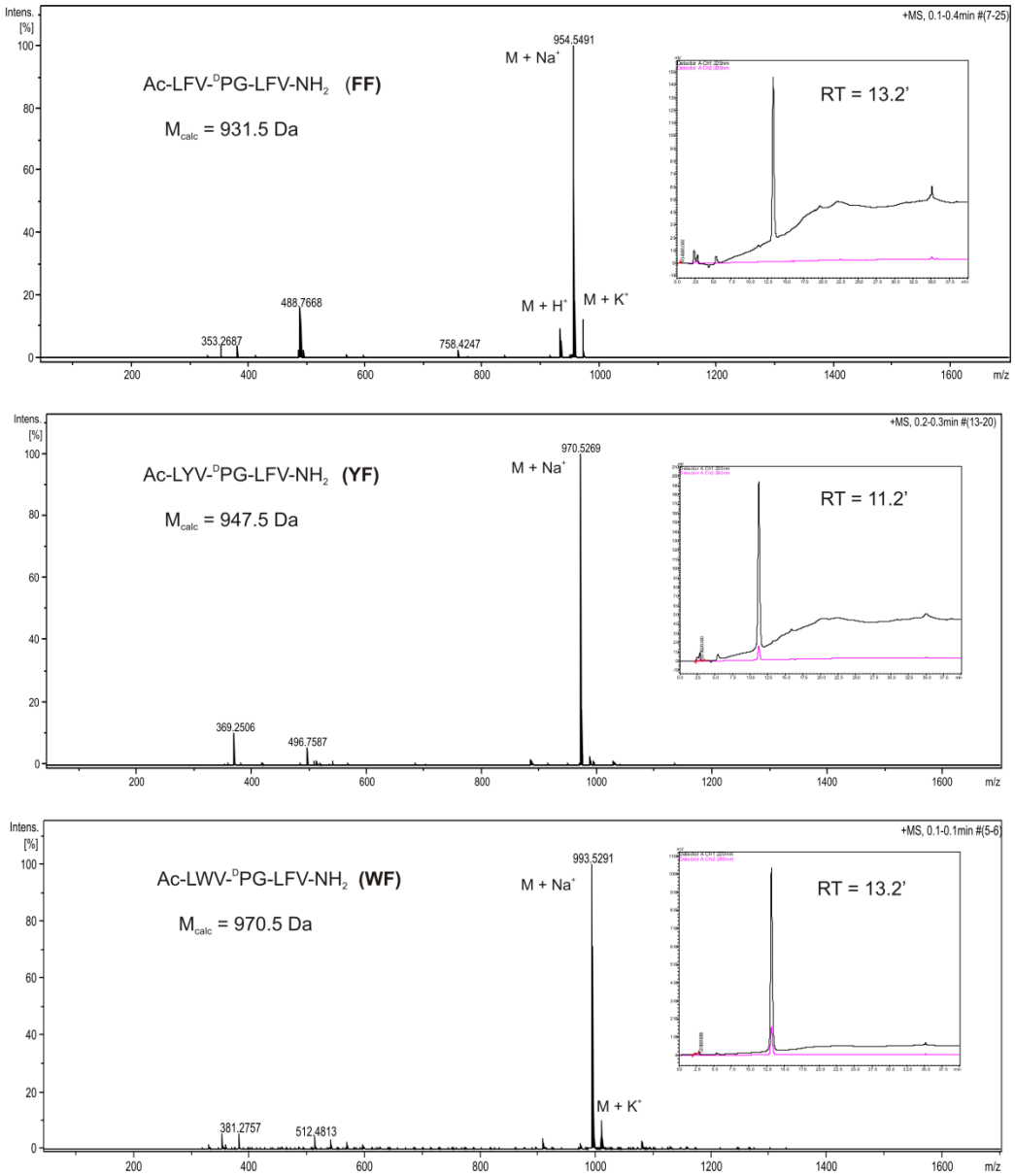
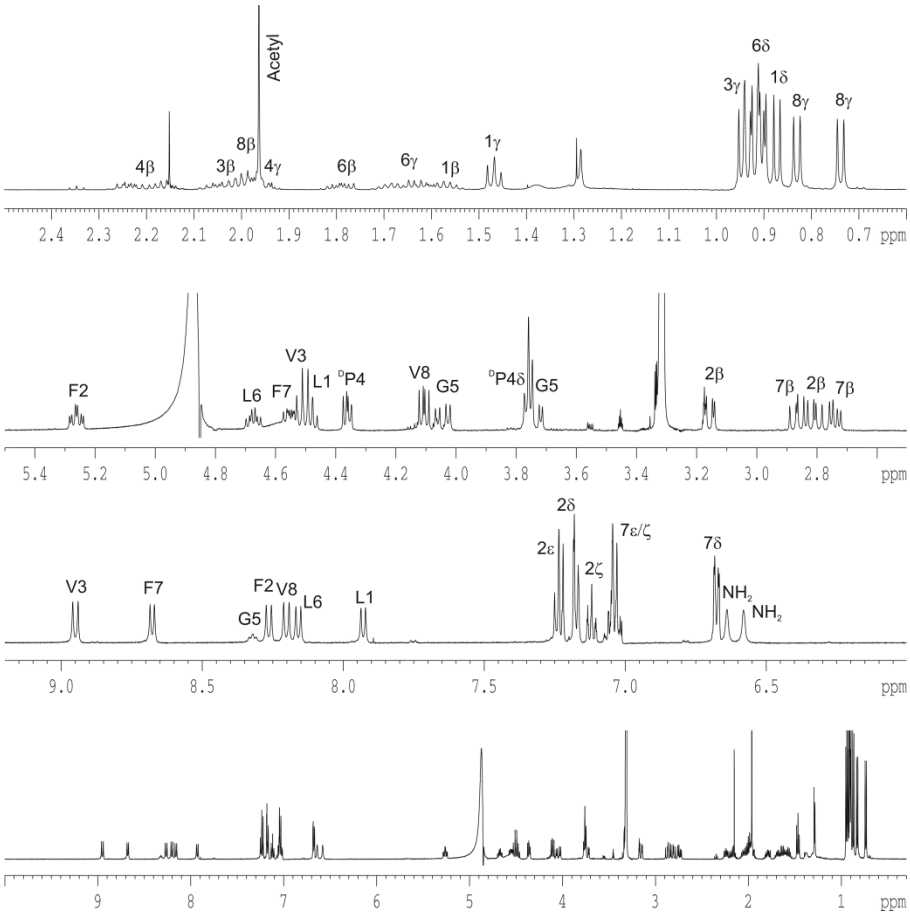
Supplementary Experimental Section



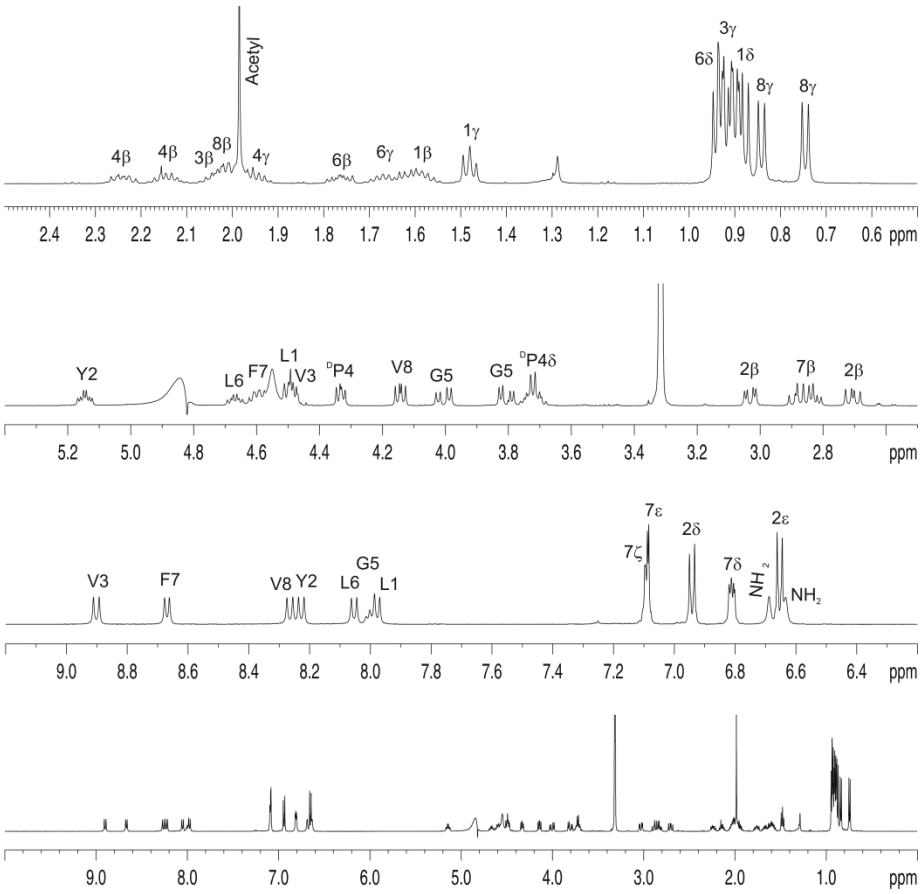
Mass spectra and HPLC profiles: Mass spectra were recorded on a micrOTOF-Q II mass spectrometer (Bruker Daltonik GmbH). Calculated molecular weights and observed peptide adducts are annotated. Analytical HPLC profiles of purified peptides were obtained on a Shimadzu UFLC system on a C_{18} reverse phase column (5µm particle size) using methanol-water gradients. The HPLC profile is provided as an inset and peak retention times (RT) of the desired peptide (in minutes) are also indicated. A water-methanol gradient of 70-100% methanol in 30 minutes and 100-100% methanol in next 10 minutes was used in each case.

Supplementary Figures



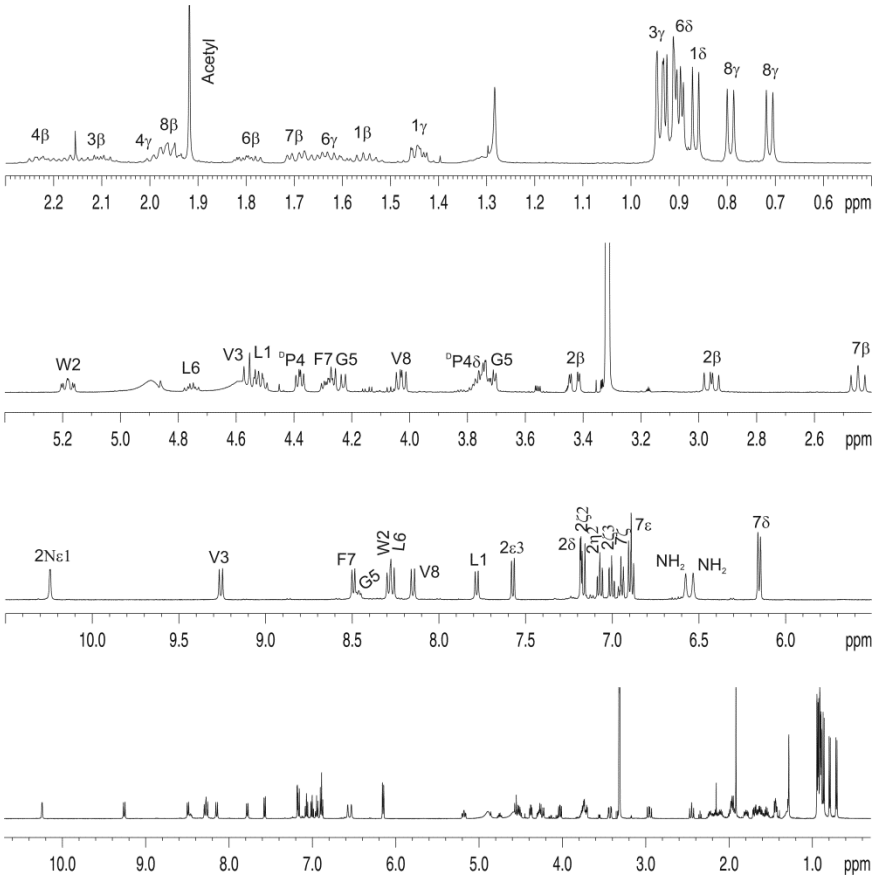
Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	Others	$^{3}J_{NH\text{-}C^{\alpha}H}$	dδ/dT
	ppm	ppm	ppm	ppm	ppm	Hz	ppb/K
Leu1	7.93	4.48	1.48	1.47	$C^{\delta}H$: 0.89	8.34	-3.7
Phe2	8.12	5.25	3.15 2.79	-	$C^{\delta}H$: 7.17 $C^{\varepsilon}H$: 7.23 $C^{\zeta}H$: 7.11	9.18	-8.0
Val3	8.94	4.50	2.04	0.925	-	9.45	-4.8
^{D}Pro4	-	4.35	2.22	1.99	$C^{\delta}H$: 3.75	-	-
Gly5	8.31	4.04 3.73	-	-	-	-	-10.2
Leu6	8.15	4.67	1.78	1.63	$C^{\delta}H$: 0.92	8.84	-2.2
Phe7	8.66	4.54	2.85 2.74	-	$C^{\delta}H$: 6.67 $C^{\varepsilon}H$: 7.03 $C^{\zeta}H$: 7.04	7.79	-9.6
Val8	8.20	4.10	1.98	0.82 0.73	-	9.59	-3.6

Figure S1a: ^{1}H 1D spectrum (top) and NMR parameters (table) of peptide **FF** in CD_3OH at 303K.



Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	Others	$^{3}J_{NH\text{-}C^{\alpha}H}$	dδ/dT
	ppm	ppm	ppm	ppm	ppm	Hz	ppb/K
Leu1	7.99	4.49	1.58	1.48	$C^{\delta}H$: 0.87	8.17	-3.2
Tyr2	8.25	5.14	3.03 2.70	-	$C^{\delta}H$: 6.93 $C^{\varepsilon}H$: 6.65	9.19	-7.3
Val3	8.92	4.48	2.03	0.90	-	9.58	-4.3
^{D}Pro4	-	4.32	2.24 2.14	1.95	$C^{\delta}H$: 3.72	-	-
Gly5	8.00	4.00 3.81	-	-	-	-	-11.7
Leu6	8.06	4.67	1.76	1.61	$C^{\delta}H$: 0.92	8.90	-3.8
Phe7	8.69	4.60	2.85	-	$C^{\delta}H$: 6.79 $C^{\varepsilon}H$: 7.07 $C^{\zeta}H$: 7.08	7.88	-9.4
Val8	8.29	4.14	2.01	0.84 0.74	-	9.63	-1.6

Figure S1b: ^{1}H 1D spectrum (top) and NMR parameters (table) of peptide **YF** in CD_3OH at 303K.



Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	Others	$^{3}J_{NH\text{-}C^{\alpha}H}$	dδ/dT
	ppm	Ppm	ppm	ppm	ppm	Hz	ppb/K
Leu1	7.78	4.51	1.55	1.44	$C^{\delta 1}H$: 0.89	8.51	-3.4
Trp2	8.28	5.18	3.43 2.95	-	$C^{\delta 1}H$: 7.18 $N^{\varepsilon 1}H$: 10.24 $C^{\varepsilon 3}H$: 7.57 $C^{\zeta 2}H$: 7.17 $C^{\zeta 3}H$: 7.00 $C^{\eta 2}H$: 7.07	9.21	-7.9
Val3	9.25	4.55	2.09	0.92	-	9.71	-6.3
DPro4	-	4.38	2.21	1.97	$C^{\delta 1}H$: 3.75	-	-
Gly5	8.45	4.24 3.72	-	-	-	-	-11.0
Leu6	8.26	4.75	1.79	1.63	$C^{\delta 1}H$: 0.91	9.50	-3.0
Phe7	8.48	4.28	2.45 1.70	-	$C^{\delta}H$: 6.15 $C^{\varepsilon}H$: 6.89 $C^{\zeta}H$: 6.95	8.24	-8.5
Val8	8.15	4.02	1.94	0.79 0.71	-	9.75	-1.7

Figure S1c: ^{1}H 1D spectrum (top) and NMR parameters (table) of peptide **WF** in CD_3OH at 303K.

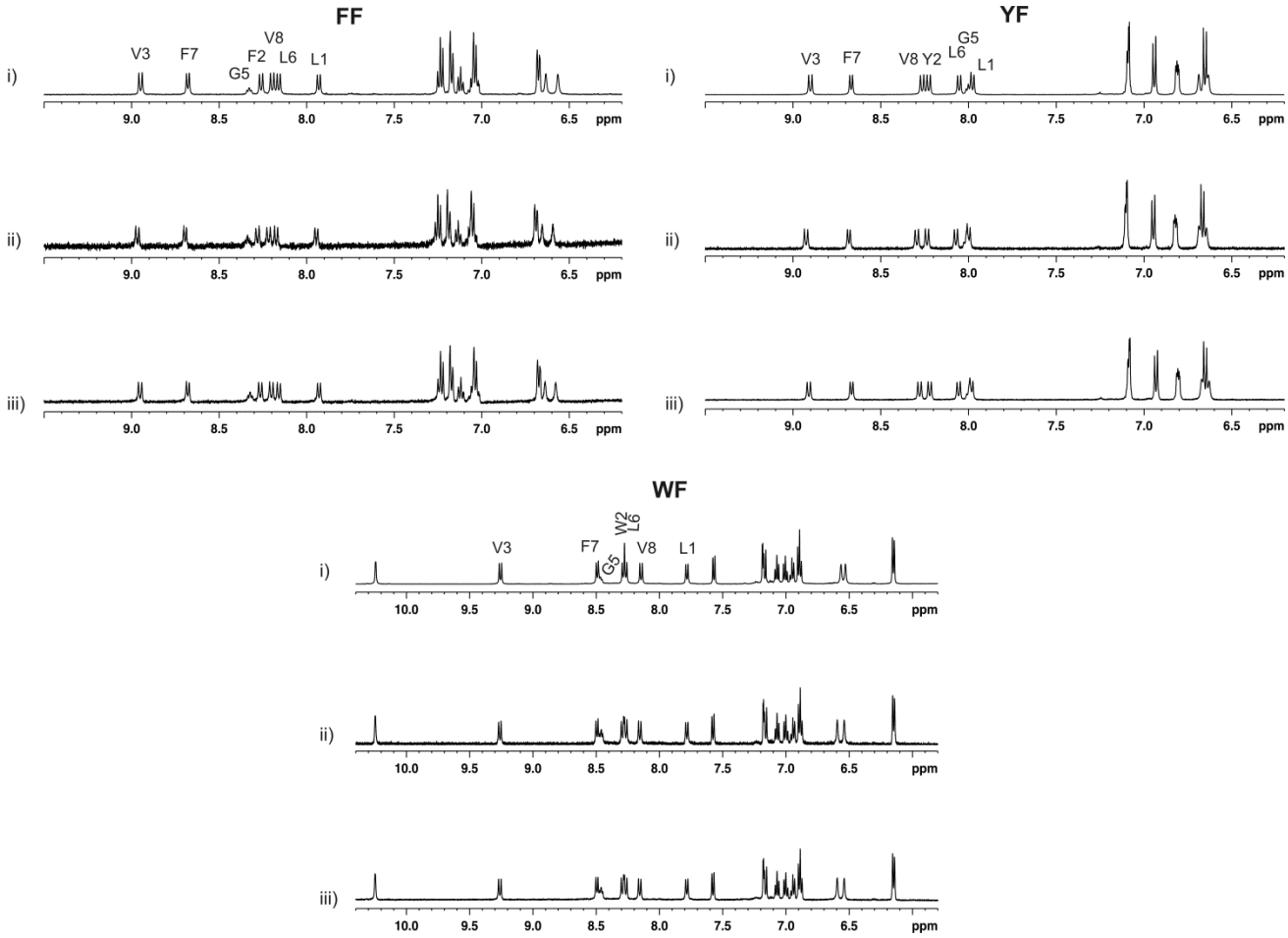


Figure S2: Comparison of the amide region of the ^{1}H 1D spectra of the three peptides in CD_3OH at 303K to check for peptide aggregation. The top spectrum (i) was recorded with 16 scans and a peptide concentration of ~2-3mM. Spectra (ii) and (iii) were recorded after 10-fold dilution of (i), using 16 scans (ii) or 96 scans (iii). Resonance assignments are shown only for the top spectrum in each peptide. Complete assignment for (i) is available in Figure S1.

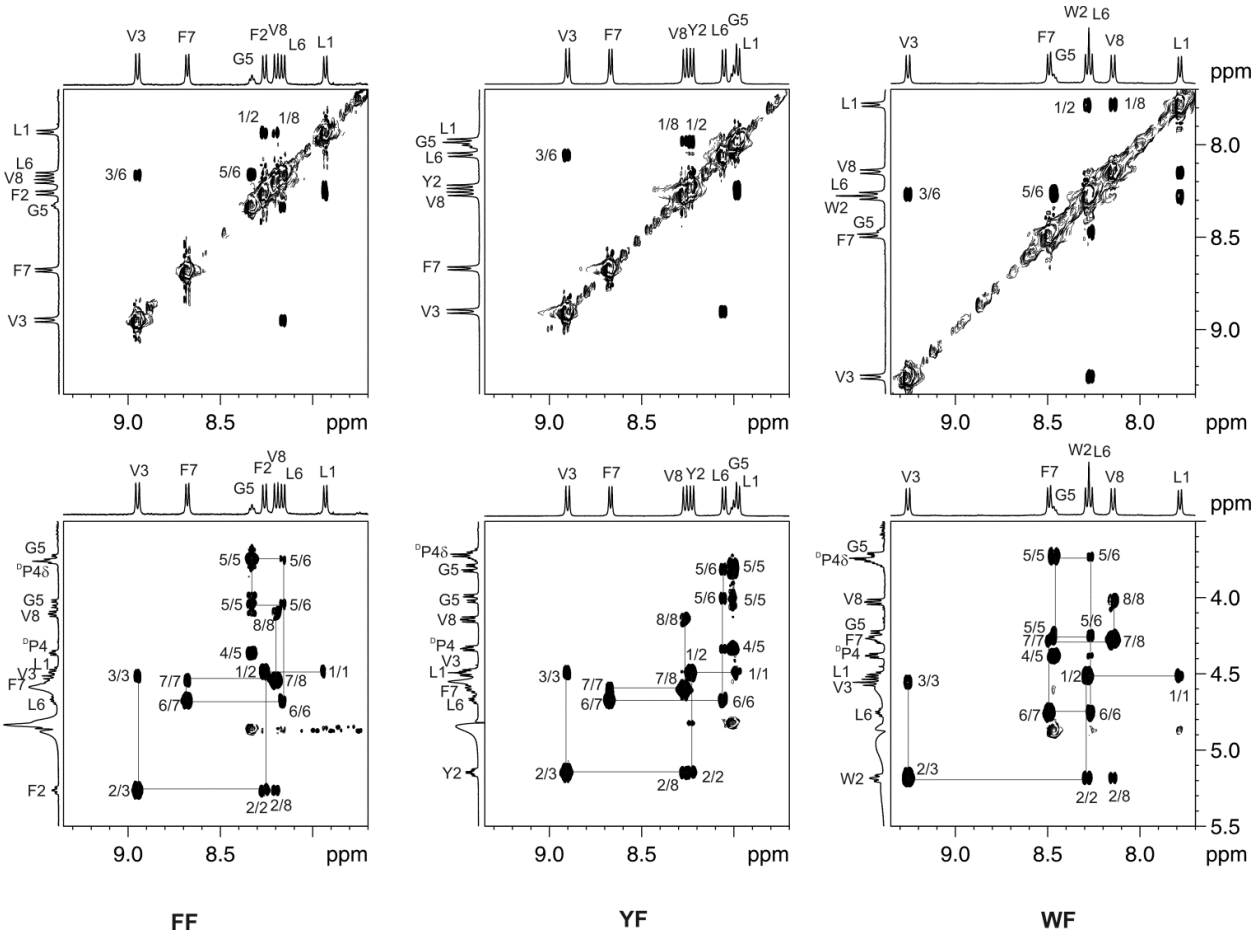


Figure S3: Partial expansions of homonuclear ^{1}H-^{1}H NMR ROESY spectra of **FF**, **YF** and **WF**, obtained in CD_3OH, highlighting diagnostic β-hairpin NH-NH (top) and NH-C$^{\alpha}$H (bottom) NOEs. Note that the NH-NH region is also shown in Fig. 2 of the main text, but is included here for completion.

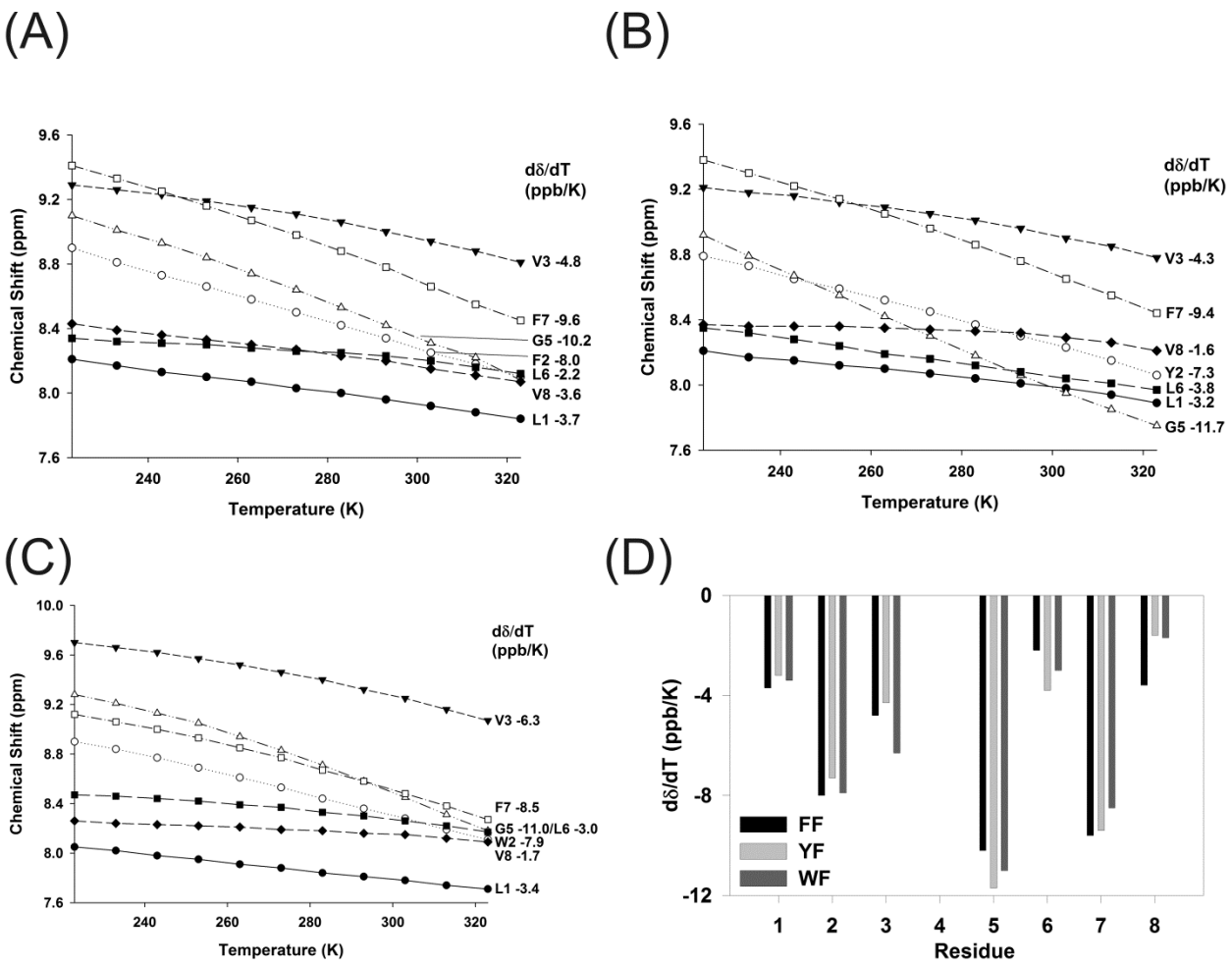


Figure S4: Temperature dependence of exposed versus hydrogen bonded amides, probed by monitoring ^{1}H chemical shifts with temperature, from 223K to 323K at intervals of 10K, for (A) **FF**, (B) **YF** and (C) **WF**. Residues involved in internal hydrogen bonds in the folded hairpin conformation are indicated as filled symbols and those in the non-hydrogen bonding position are represented as open symbols. The change in chemical shift for each residue across the three peptides is compared in (D).

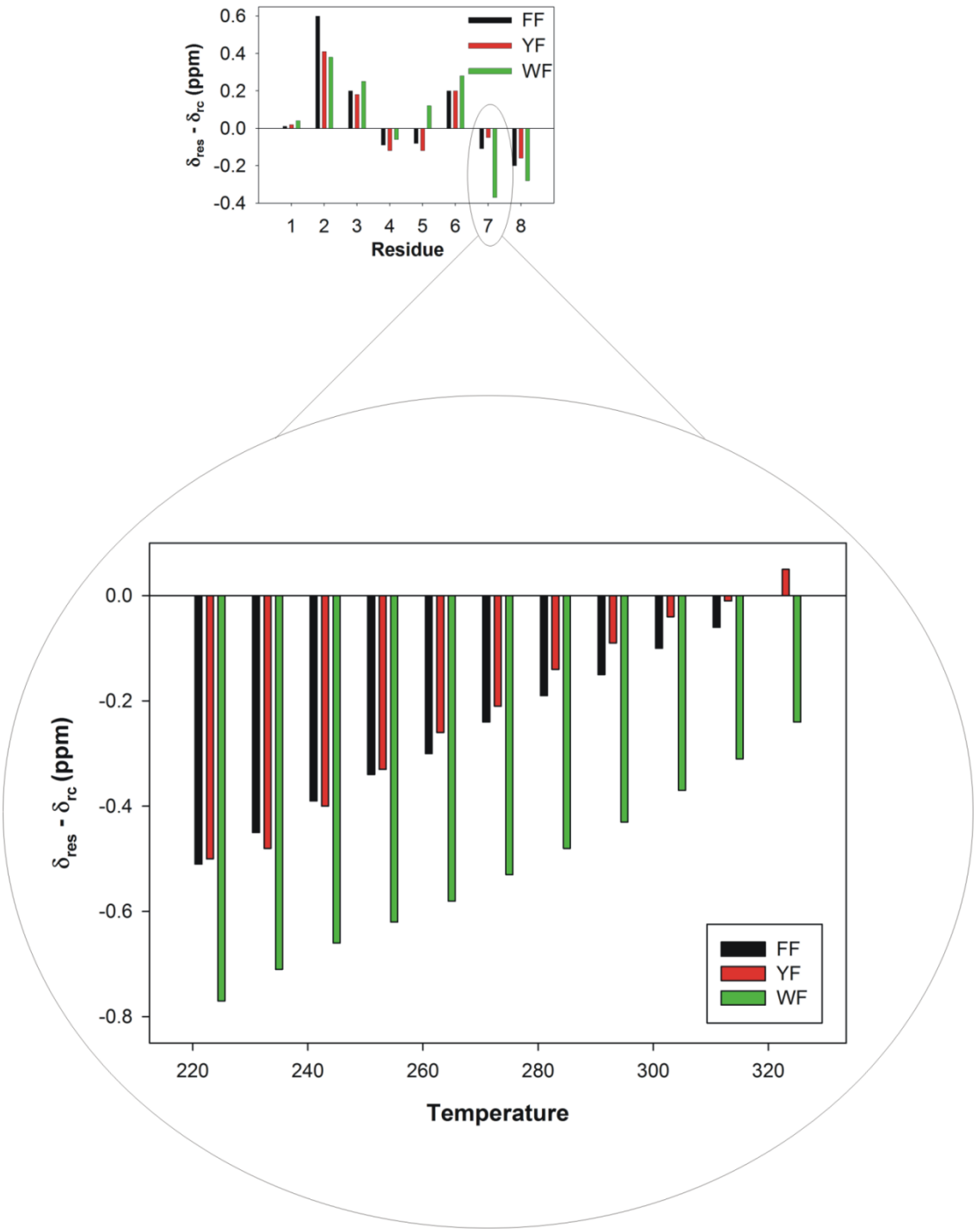


Figure S5: Chemical Shift Indexing (CSI) highlighting the variation of Phe7 $C^{\alpha}H$ chemical shift against random coil values, with temperature. Anomalous CSI values (in turn suggesting a stronger aromatic influence on the chemical shift) are in the order **WF>FF>YF**

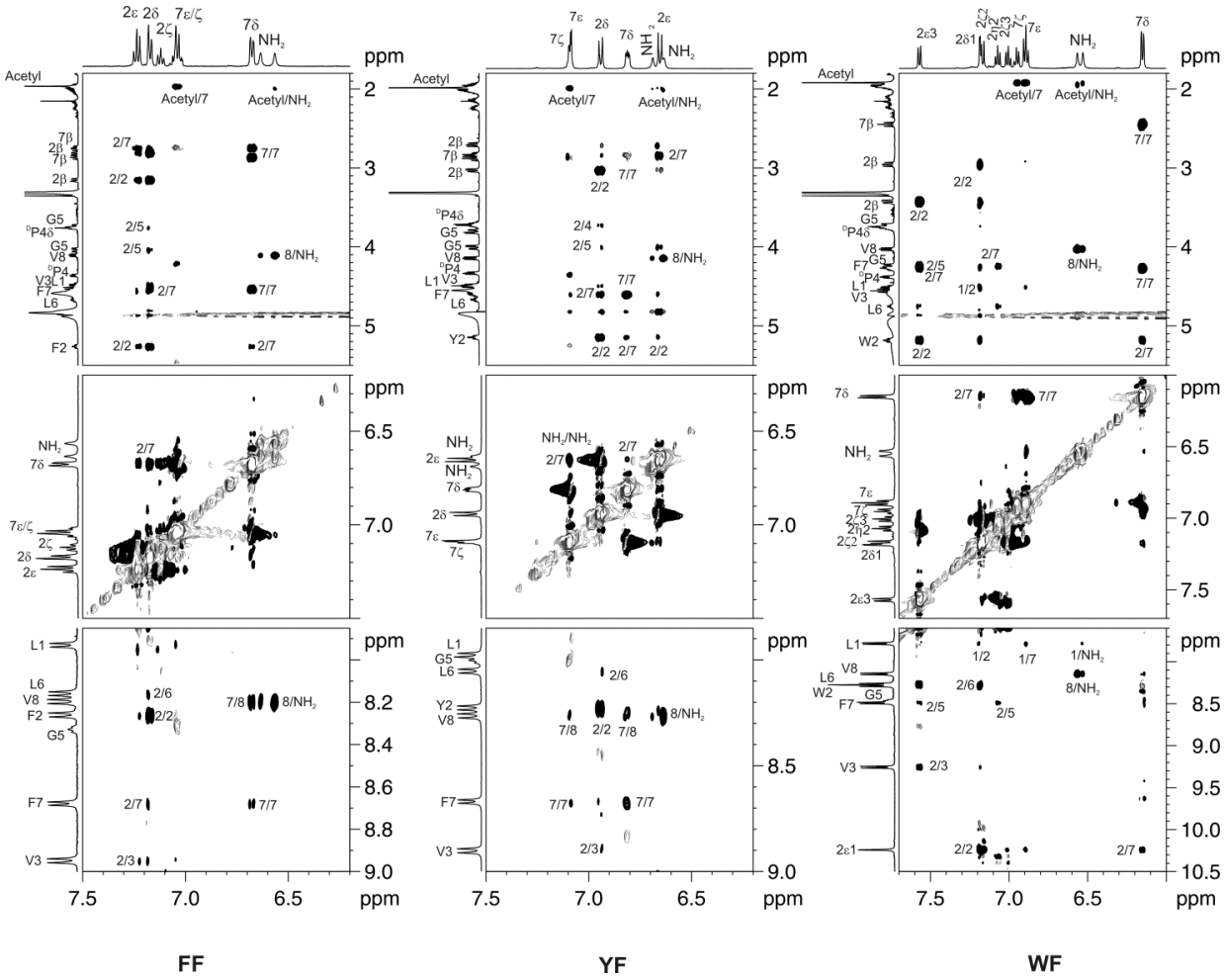


Figure S6: Partial expansions of the ROESY spectra in CD_3OH, highlighting key NOEs that establish ring orientation in the three peptides. NOEs observed from the aromatic ring protons to the $C^{\alpha/\beta/\gamma}H$ region (top), aromatic resonances (middle) and amide resonances (bottom) are indicated

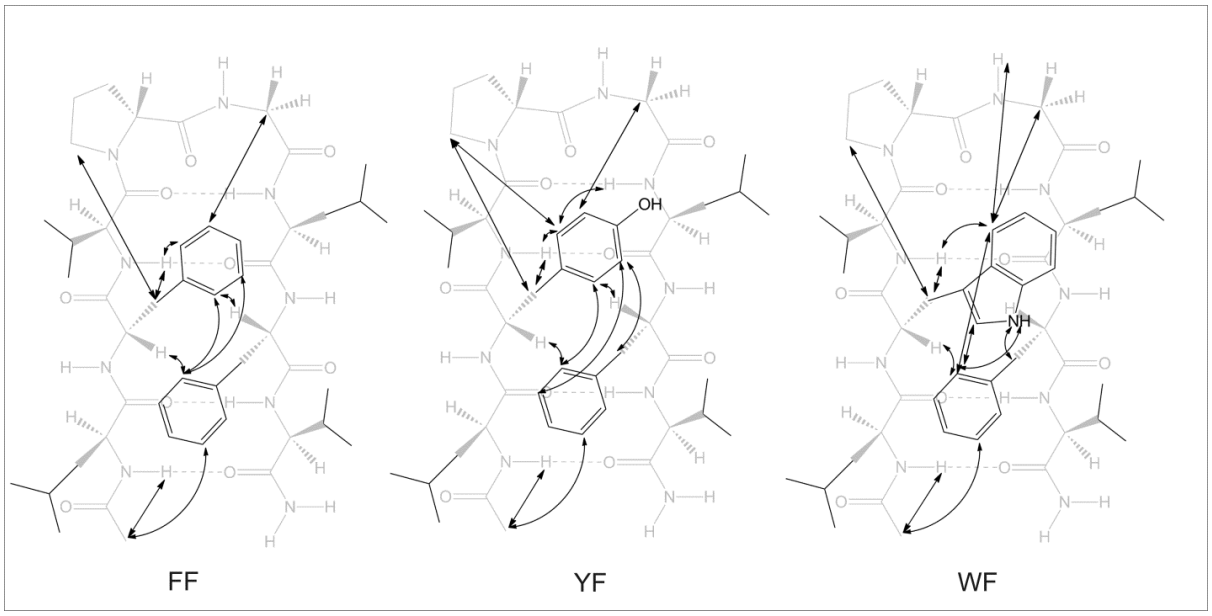


Figure S7: Schematic representation of key cross-strand ring proton NOEs observed in the ROESY spectrum of the three peptides.

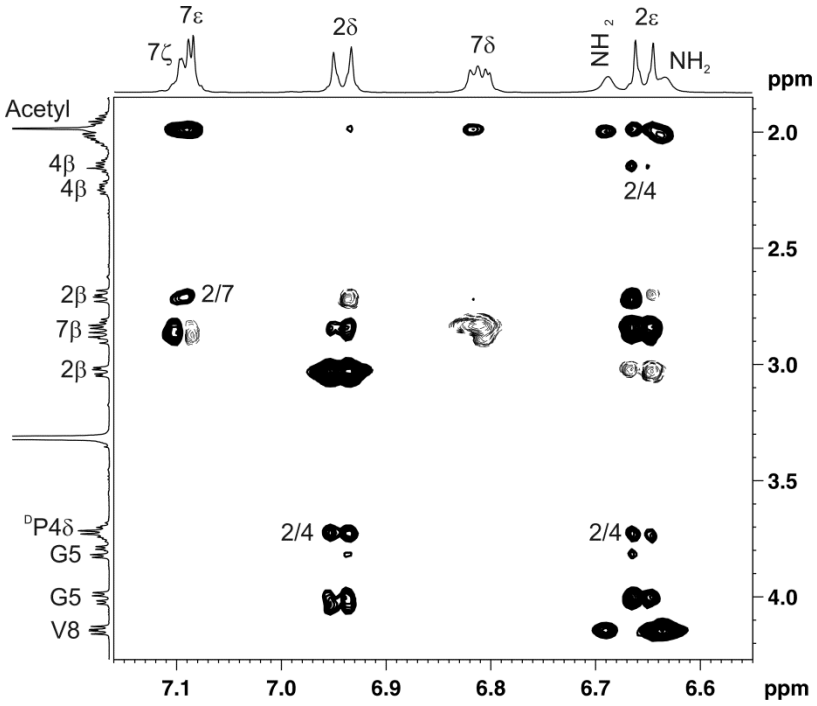


Figure S8: Partial expansion of the ROESY spectrum of peptide **YF**, highlighting the corresponding diagnostic NOEs for conformer II,[1] which confirms the presence of two alternate ring orientations in this peptide with sufficient occupancy.

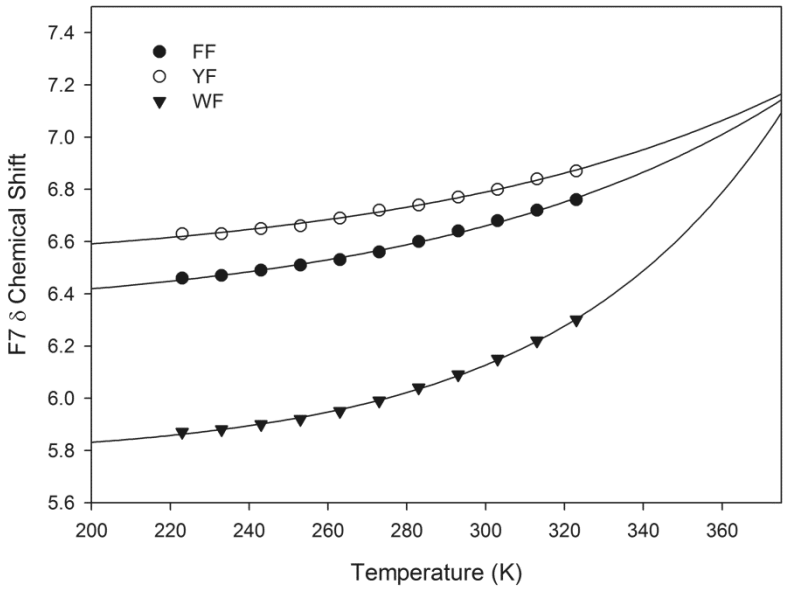


Figure S9: Data from the observed Phe7 $C^{\delta}H$ proton chemical shifts in CD_3OH at the respective temperatures (shown as symbols) were fit to an exponential function to predict the $C^{\delta}H$ shift at higher (to ~375K) and lower (200K) temperatures. The rate of change in chemical shift for this resonance is dramatic in **WF**, while it is nearly linear for **FF** and **YF**, over the temperature range examined. This anomalous upfield shift in **WF** is likely to arise from strong indole-benzene interactions in the peptide upon adopting a β-hairpin conformation.

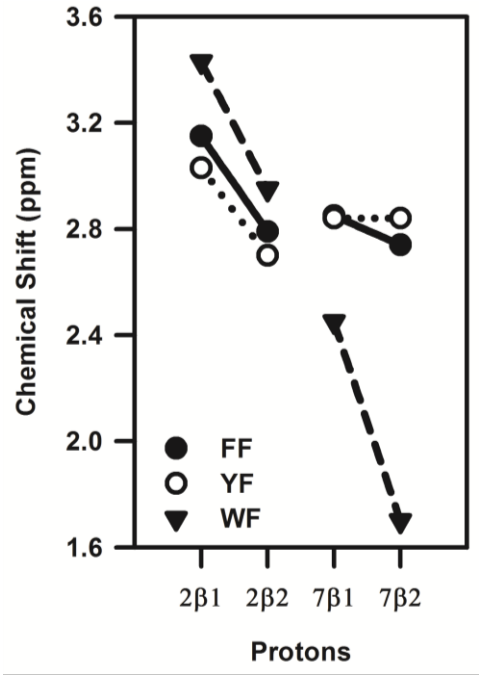


Figure S10: Influence of ring currents on ^{1}H NMR chemical shift of geminal C$^{\beta}$H protons of F/Y/W2 and F7. An ~0.4ppm upfield shift is seen for the geminal 7C$^{\beta}$H in **FF** and **YF**, when compared with 2C$^{\beta}$H in **FF**, despite variation in the chemical nature of residues at position 2, while in **WF**, an additional 0.4-1.2ppm change is obtained.

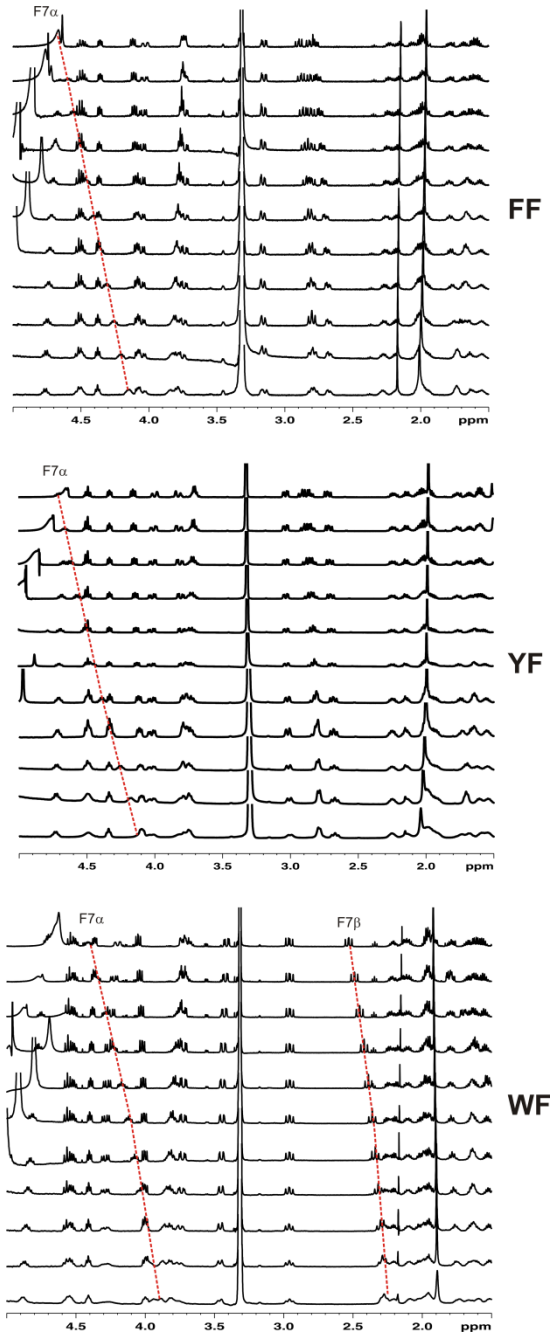


Figure S11: Temperature dependence of Phe7 $C^{\alpha}H$ proton resonance under the influence of aromatic interactions. Anomalous upfield chemical shift can be observed in all three peptides, which is a direct evidence for the resonance being under the shielding zone of the N-terminal ring. The dδ/dT values for each peptide are: -5.1ppb/K (**FF**); -5.5ppb/K (**YF**); -5.3ppb/K (**WF**). The corresponding upfield shifted Phe7 $C^{\beta}H$ resonance is in line with this observation.

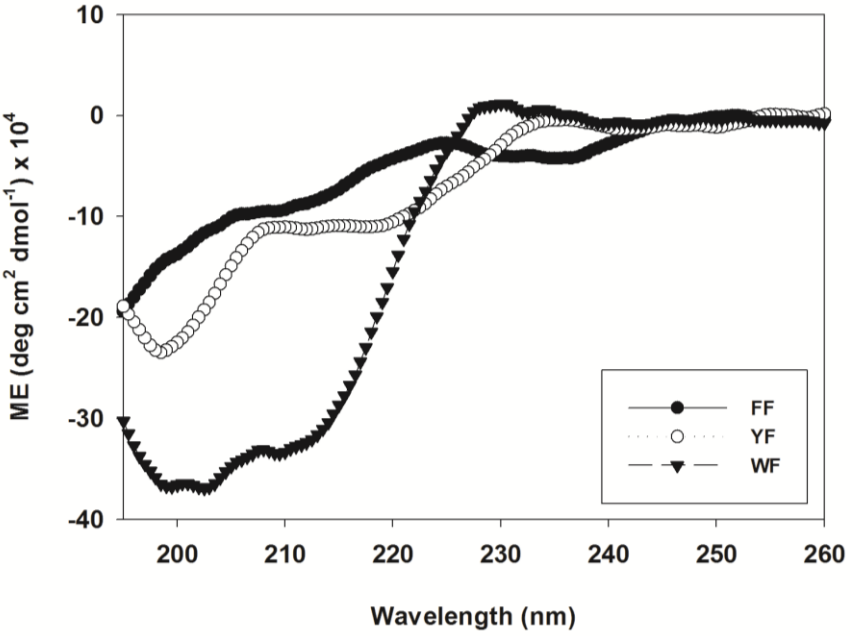


Figure S12: Comparison of the far-UV electronic circular dichroism (ECD) spectra of the three peptides in methanol. The far-UV CD arising from the backbone n→π* and π→π* transitions of the peptide unit are often masked by contributions from aromatic interactions. We observe anomalous spectra in all three cases, due to significant contribution of aromatic residues to the far-UV CD and the characteristic exciton couplet at 195nm and 215nm for a β-sheet structure is not obtained. In **FF**, we observe two negative bands at ~195nm and ~210nm arising from Phe-Phe interactions. Similarly, the spectrum for **YF** displays a shallow trough centred at ~220nm and a second negative maximum at ~198nm, and resembles the spectrum observed for Tyr-Tyr interactions, suggesting that the contributions of Phe to far-UV CD is surpassed by the influence of Tyr. The anomalous contribution becomes even stronger in the case of **WF**, with a minor positive band at 228nm and negative maxima at ~200nm and ~210nm.

Supplementary Tables

Table S1: Summary of experimental constraints used in the NMR structure calculation and observed violations in the calculated structures.

Experimental Constraints	No. of Constraints		
	FF	YF	WF
Intraresidue NOEs	38	37	40
Sequential NOEs	13	13	15
Long range NOEs	14	20	25
Hydrogen bonds	4	4	4
Angle constraints	12	12	12
Violations observed	0	0	0

Table S2: Average backbone torsion angles calculated for 35 best structures for the three peptides.

FF: Ac-LFV-^{D}PG-LFV-NH_2		
Residue	Phi[a]	Psi[a]
Leu1	-	75.5 +/- 5.0
Phe 2	-126.1 +/- 6.2	137.3 +/- 7.5
Val 3	-136.0 + 4.5	73.4 +/- 2.6
^{D}Pro 4	69.7 +/- 0.03	-113.3 +/- 1.6
Gly 5	-90.1 +/- 0.1	15.7 +/- 0.4
Leu 6	-133.1 +/- 2.9	156.7 +/- 7.8
Phe 7	-130.2 +/- 5.0	141.5 +/- 6.0
Val 8	-129.5 +/- 6.0	-

YF: Ac-LYV-^{D}PG-LFV-NH_2		
Residue	Phi[a]	Psi[a]
Leu1	-	81.3 +/- 2.4
Tyr 2	-130.3 +/- 8.6	143.1 +/- 7.9
Val 3	-136.0 + 5.7	73.2 +/- 2.5
^{D}Pro 4	69.7 +/- 0.03	-113.0 +/- 2.2
Gly 5	-90.5 +/- 0.5	16.4 +/- 4.9
Leu 6	-128.5 +/- 6.1	168.5 +/- 11.9
Phe 7	-129.6 +/- 8.0	147.3 +/- 2.7
Val 8	-129.1 +/- 7.6	-

WF: Ac-LWV-^{D}PG-LFV-NH_2		
Residue	Phi[a]	Psi[a]
Leu1	-	84.8 +/- 0.1
Trp 2	-99.1 +/- 0.1	125.8 +/- 0.9
Val 3	-126.1 + 0.6	75.3 +/- 0.9
^{D}Pro 4	69.7 +/- 0.04	-111.8 +/- 0.9
Gly 5	-91.4 +/- 0.1	15.7 +/- 2.0
Leu 6	-128.0 +/- 2.1	142.3 +/- 0.0
Phe 7	-119 +/- 0.2	144.2 +/- 0.3
Val 8	-140.2 +/- 0.2	-

[a]Values in degree

Supplementary References

1. R. Sonti, R. Rai, S. Ragothama and P. Balaram, *J. Phys. Chem. B*, 2012, **116**, 14207-14215.