Supplementary Information

CH-π Interaction Between Cross-Strand Amino Acid Pair Stabilizes β-hairpins

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Materials and Methods:

General Information

All the Fmoc and orthogonally protected amino acids, 1-hydroxybenzotriazole (HOBt) and Rink Amide AM resin were purchased from GL Biochem, Shanghai, China. *N,N'*-Dimethylformamide (DMF), *N,N'*-Diisopropylcarbodiimide (DIC), *N, N*-diisopropylethylamine (DIPEA), Trifluoroacetic acid (TFA), Triisopropylsilane (TIPS), Glacial acetic acid, Calcium hydride, Piperidine and Iodine were purchased from Sigma-Aldrich. All the above reagents were used as commercially supplied. Solvents for RP-HPLC were purchased as HPLC grade and used without further purification. Dichloromethane was dried with Calcium hydride. All the other solvents were used as commercially supplied.

All the reactions were performed in oven-dried glass apparatus. Reactions on solid support were carried out in plastic syringes (10 ml) fitted with a frit column plate.

High-resolution mass spectra were recorded on a BrukerDaltonics ESI Q TOF- (Maxix Impact) with Nano LC (Proxeon easy nLC) mass spectrometer. ESI mass spectra were recorded in positive ion mode on a HCT ultra ETD II ion trap spectrometer (PTM Discovery System, BrukerDaltonics, Germany). MALDI mass spectra were recorded on UltrafleXtreme TOF/TOF (BrukerDaltonics, Germany) and the data were processed and analysed using the Flex Analysis 3.1 software.

Nuclear magnetic resonance (NMR) spectra were recorded either on a 700 MHz BrukerAvance spectrometer (Bruker, Karlsruhe, Germany), or a 500 MHz Agilent NMR spectrometer.

Analytical RP-HPLC was performed on a Shimadzu UFLC system equipped with Prominence Diode Array (PDA) UV Detector at 210 nm, 254 nm and 270 nm using an analytical column (Phenomenex C18, 250 mm x 4.6 mm I.D., 5 μm) at a flow rate of 1 mL/min. Purifications were performed using a semi-preparative column (Phenomenex C18, 250 mm x 10 mm I.D., 5 μm) at a flow rate of 4 mL/min.

Peptide Synthesis

Peptides **1** to **10**, peptides designed for double mutant cycle, their respective cyclic and unfolded controls were synthesized on Rink Amide AM resin (0.7 mmolg-1) on 200 mg scale (0.16 mmol) using standard Fmoc-based strategy. The resin was swollen in DMF and deprotected with 20% piperidine in DMF (5 min x 1, 15 min x 1) followed by thorough washing with DMF (3 times). The C-teminal amino acid, Fmoc-Gln(Trt)-OH (2.5 eq.) was loaded onto the resin by using standard coupling reagents (2.5 equiv HOBt, 2.5 equiv DIC) in DMF for 2 hours at room temperature. The entire peptide was assembled with this same protocol as well. In case of the cyclic (fully folded) controls of the respective peptides, amino acids were assembled on Rink Amide AM resin in a similar fashion to its linear counterparts with exception of the incorporation of Fmoc-Cys(Acm)-OH at both the N- and C-termini. The peptides were cyclized by oxidation using 4 equiv. of iodine in DMF for 2 hours.

N-terminal Acetylation of the Peptide

After the final Fmoc deprotection of all the peptides and their respective unfolded and fully folded controls, the N-terminal was acetylated with acetic anhydride (2.5 equiv) and DIPEA (2.5 equiv) for 5 mins twice in DCM at room temperature. The resin was then washed thoroughly with DMF (5 times) followed by DCM (2 times).

Global Deprotection and Cleavage from the Resin

All the peptides and their controls were cleaved off from the resin and globally deprotected by using the cleavage cocktail TFA: TIPS: H_2O (95: 2.5: 2.5) for 30 minutes at room temperature. The cleaved peptide solution was then precipitated in chilled diethyl ether, centrifuged twice and dissolved in water for purification by RP-HPLC.

Purification by RP-HPLC

A suitably adjusted 20-minute gradient of 15% B to 45% B was used for purification of compounds **1** to **10**, peptides of double mutant cycles and their respective unfolded and cyclic controls, where solvent A was 0.1% TFA in H_2O and B was 0.1% TFA in acetonitrile.

NMR Acquisition

For acquiring NMR all the peptides were dissolved in 100 mM sodium acetate buffer (pH 3.8) H_2O: D_2O **(**9:1). In all the compounds, 0.1% TMSP was used as an internal standard (δ = 0 ppm). Standard Bruker pulse sequences *zgesgp* for ^1H, *mlevesgpph* (60 ms mixing time) for TOCSY and *roesyesgpph* (100 ms mixing time) for ROESY were used to acquire the NMR data. Two-dimensional data were obtained using 2048 data points in the direct dimension and 512 data points in the indirect dimension.

The NMR spectra were acquired using peptide concentrations of 1-2 mM at room temperature (298K).

All NMR data were processed using iNMR (www.inmr.net), and the 2D NMR data were analyzed with SPARKY.[1] The chemical shift tables were generated from TOCSY and ^1H spectra. The sequential assignments and inter- and intra-residue NOEs were determined through ROESY. The NOEs were then integrated and the integration values were converted to distances using the formula $D= A*(B/X)^{1/6}$, where X is the integrated peak volume, B is the peak volume of Gly7-HA, A is the distance between the two HA protons of Gly7.

Determination of folding free energy (ΔG_f) of the peptides

The percentage of β-sheet population and ΔG_f were calculated from the H^α chemical shifts for each peptide at the reporter residues (Val3, Val5, Lys8 and Ile10). These reporter residues are located at the hydrogen-bonded position which allows accurate determination of β--sheet folded fraction. Fraction folded at each residue were calculated by Equation 1;

$$\textbf{Percentage of Fraction Folded} = [(\delta_{obs}-\delta_U) / (\delta_F-\delta_U)]*100$$

Where, δ_{obs} is the H^α chemical shift of the peptide of interest, δ_U is the H_α chemical shift of its unfolded control in which the D-amino acid is replaced with the L-amino acid and δ_F is the H^α chemical shift of its fully folded control obtained by cyclizing the peptide through disulfide bond formation. The error in NMR shift assignment was considered to be 0.01ppm and the final values were calculated using error propagation method.[2]

The equilibrium constant was calculated using Equation 2:

$$\mathbf{K} = \textit{(Fraction Folded) / (1- Fraction Folded)}$$

Finally, the ΔG_f was calculated using Equation 3:

$$\Delta G_f = -RTlnK$$

The ΔG_f was calculated at each reporter positions and the average of the four values have been reported.

Table S1: Characterization of compounds through RP-HPLC and Mass Spectrometry.

Peptides	Sequence	RP-HPLC	Mass spectrometry	
		Retention time (mins)	Calculated $[M+H]^+$ (Da)	Observed $[M+H]^+$ (Da)
1	Ac-R**Y**VEV-pG-KKI**L**Q-NH_2	13.9	1470.7840	1470.8061
2	Ac-R**F**VEV-pG-KKI**L**Q-NH_2	14.6	1454.7850	1455.5200
3	Ac-R**W**VEV-pG-KKI**L**Q-NH_2	14.3	1493.8220	1493.9939
4	Ac-R**H**VEV-pG-KKI**L**Q-NH_2	13.3	1444.7540	1445.1933
5	Ac-R**W**VEV-pG-KKI**Nva**Q-NH_2	14.5	1479.7950	1479.8090
6	Ac-R**W**VEV-pG-KKI**Nle**Q-NH_2	14.8	1493.8220	1493.9181
7	Ac-R**W**VEV-pG-KKI**M**Q-NH_2	17.5	1551.8550	1552.4176
8	Ac-R**W**VEV-pG-KKI**I**Q-NH_2	17.2	1493.8220	1494.5462
9	Ac-R**W**VEV-pG-KKI**V**Q-NH_2	14.5	1479.7950	1479.4407
10	Ac-R**W**VEV-pG-KKI**T**Q-NH_2	16.1	1481.7670	1481.8062
B	Ac-R**W**VEV-pG-KKI**A**Q-NH_2	14.2	1451.7450	1452.4146
C	Ac-R**A**VEV-pG-KKI**L**Q-NH_2	15.1	1378.6870	1378.8104
D	Ac-R**A**VEV-pG-KKI**A**Q-NH_2	12.3	1336.6060	1337.6654
F	Ac-R**A**VEV-PG-KKI**Nva**Q-NH_2	13.5	1364.6603	1364.7944
1 cont	Ac-R**Y**VEV-PG-KKI**L**Q-NH_2	13.8	1470.7840	1471.0103
2 cont	Ac-R**F**VEV-PG-KKI**L**Q-NH_2	14.7	1454.7850	1454.8937
3 cont	Ac-R**W**VEV-PG-KKI**L**Q-NH_2	14.3	1493.8220	1493.8507
4 cont	Ac-R**H**VEV-PG-KKI**L**Q-NH_2	13.1	1444.7540	1445.5408
5 cont	Ac-R**W**VEV-PG-KKI**Nva**Q-NH_2	14.5	1479.7950	1479.8093
6 cont	Ac-R**W**VEV-PG-KKI**Nle**Q-NH_2	15.0	1493.8220	1493.9889
7 cont	Ac-R**W**VEV-PG-KKI**M**Q-NH_2	17.4	1551.8550	1551.9909
8 cont	Ac-R**W**VEV-PG-KKI**I**Q-NH_2	17.2	1493.8220	1494.9804
9 cont	Ac-R**W**VEV-PG-KKI**V**Q-NH_2	14.5	1479.7950	1481.2131
10 cont	Ac-R**W**VEV-PG-KKI**T**Q-NH_2	16.1	1481.7670	1481.8111
B cont	Ac-R**W**VEV-PG-KKI**A**Q-NH_2	14.2	1451.7450	1453.0167
C cont	Ac-R**A**VEV-PG-KKI**L**Q-NH_2	13.6	1378.6870	1378.7919
D cont	Ac-R**A**VEV-PG-KKI**A**Q-NH_2	12.3	1336.6060	1336.9959
F cont	Ac-R**A**VEV-PG-KKI**Nva**Q-NH_2	13.5	1364.6603	1364.7702
1 cyc	**S-S** Ac-C**RY**VEV-pG-KKI**L**QC-NH_2	15.6	1675.0460	1676.0980
2 cyc	**S-S** Ac-C**RF**VEV-pG-KKI**L**QC-NH_2	15.9	1659.0470	1659.8080
3 cyc	**S-S** Ac-C**RW**VEV-pG-KKI**L**QC-NH_2	16.0	1698.0840	1699.8928
4 cyc	**S-S** Ac-C**RH**VEV-pG-KKI**L**QC-NH_2	15.0	1649.0120	1648.9058
5 cyc	**S-S** Ac-C**RW**VEV-pG-KKI**Nva**QC-NH_2	16.5	1684.0570	1685.3365
6 cyc	**S-S** Ac-C**RW**VEV-pG-KKI**Nle**QC-NH_2	17.1	1698.0840	1698.8921
7 cyc	**S-S** Ac-C**RW**VEV-pG-KKI**M**QC-NH_2	18.2	1716.1170	1716.1908
8 cyc	**S-S** Ac-C**RW**VEV-pG-KKI**I**QC-NH_2	18.3	1698.0840	1698.7207
9 cyc	**S-S** Ac-C**RW**VEV-pG-KKI**V**QC-NH_2	16.5	1684.0570	1683.6229
10 cyc	**S-S** Ac-C**RW**VEV-pG-KKI**T**QC-NH_2	16.7	1686.0290	1686.0620
B cyc	**S-S** Ac-C**RW**VEV-pG-KKI**A**QC-NH_2	15.9	1656.0030	1657.2315
C cyc	**S-S** Ac-C**RA**VEV-pG-KKI**L**QC-NH_2	15.1	1582.9490	1583.1732
D cyc	**S-S** Ac-C**RA**VEV-pG-KKI**A**QC-NH_2	12.8	1540.8680	1541.3212
F cyc	**S-S** Ac-C**RA**VEV-pG-KKI**Nva**QC-NH_2	13.3	1568.9220	1568.8993

Characterization of compounds through NMR spectroscopy.

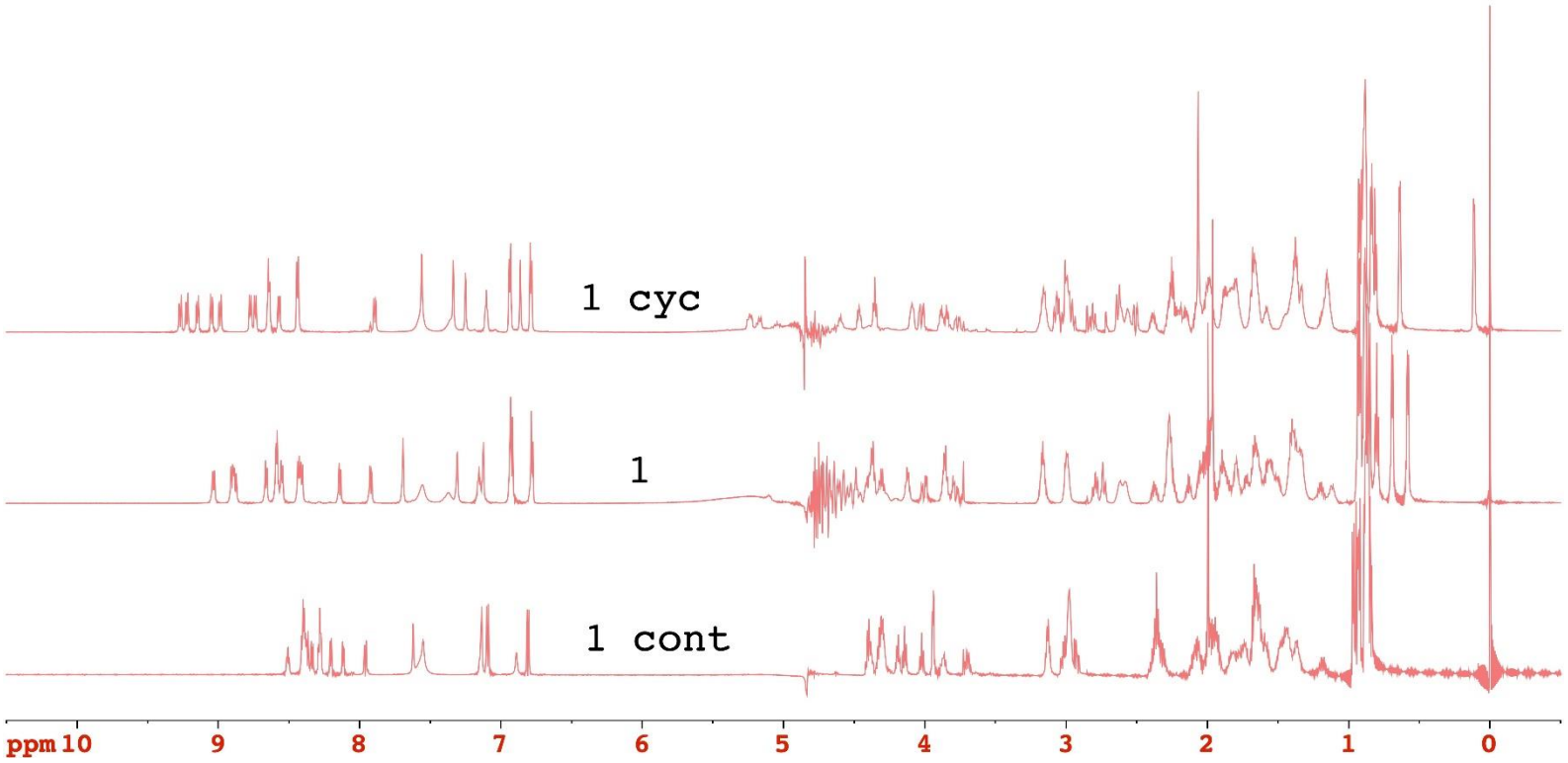


Figure S1: ^{1}H NMR overlay of **1 cyclic**, **1** and **1 random coil control** (top to bottom), acquired at 298K, in 100mM sodium acetate buffer : D_2O (9:1), pH 3.8.

Table S2: Chemical shifts of peptide 1 cyc, 1 and 1 cont (random coil).

(only HN and HA chemical shifts were provided for the cyclic and random coil controls for all the peptides)

Residues		Peptides		
		1 cyc	**1**	**1 cont**
N-term Cys	HN	8.44	-	-
	HA	5.22	-	-
Arg1	HN	8.74	8.14	8.19
	HA	4.60	4.36	4.18
	HB		1.68	
	HG		1.54	
	HD		3.17	
	NAc		1.96	
Tyr2	HN	8.78	8.40	8.27
	HA	5.17	5.10	4.62
	HB		2.76	
Val3	HN	9.27	8.87	7.95
	HA	4.50	4.41	4.02
	HB		2.01	
	HG		0.88	
Glu4	HN	8.57	8.54	8.38
	HA	5.05	5.00	4.30
	HB		1.95	
	HG		2.26	
Val5	HN	9.05	8.89	8.33
	HA	4.64	4.61	4.41
	HB		1.98	
	HG		0.93	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.37	4.36	4.11
	HB		2.06/2.12	
	HG		1.98	
	HD		3.85	
Gly7	HN	8.65	8.57	8.50
	HA	3.77/4.02	3.77/4.01	3.95
Lys8	HN	7.89	7.92	8.38
	HA	4.61	4.56	4.31
	HB		1.79	
	HG		1.39	
	HD		1.68	
	HE		2.99	
Lys9	HN	8.44	8.43	8.11
	HA	4.86	4.65	4.33
	HB		1.58	
	HG		1.14	
	HD		1.33	
	HE		2.98	
Ile10	HN	9.22	9.02	8.28
	HA	4.59	4.48	4.13
	HB		1.89	
	HG		1.39	
	HD		0.87	
Leu11	HN	8.65	8.58	8.40
	HA	4.09	4.11	4.38
	HB		1.40/1.58	
	HG		1.17	
	HD		0.58/0.66	
Gln12	HN	9.14	8.65	8.36
	HA	4.62	4.30	4.30
	HB		2.03	
	HG		2.27	
C-term Cys	HN	8.98	-	-
	HA	5.09	-	-

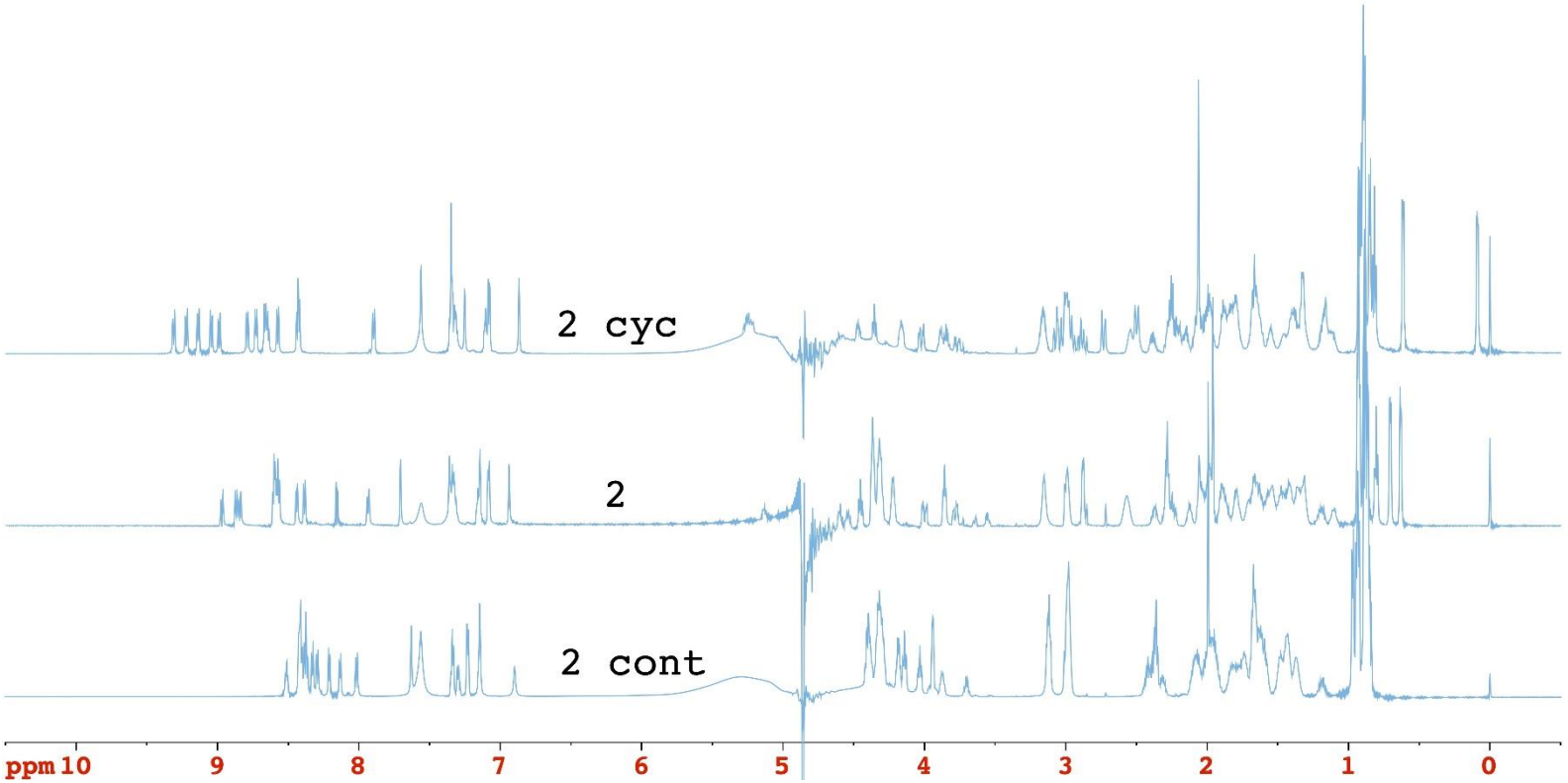


Figure S2: ^{1}H NMR overlay of **2 cyclic**, **2** and **2 random coil control** (top to bottom), acquired at 298K, in 100mM sodium acetate buffer : D_2O (9:1), pH 3.8.

Table S3: Chemical shifts of peptide 2 cyc, 2 and 2 cont (random coil).

Residues		Peptides		
		2 cyc	**2**	**2 cont**
N-term Cys	HN	8.43	-	-
	HA	5.22	-	-
Arg1	HN	8.72	8.15	8.21
	HA	4.57	4.32	4.17
	HB		1.69	
	HG		1.52	
	HD		3.16	
	NAc		1.95	
Phe2	HN	8.79	8.38	8.33
	HA	5.25	5.13	4.67
	HB		2.87	
Val3	HN	9.30	8.84	8.00
	HA	4.45	4.36	4.02
	HB		2.01	
	HG		0.90	
Glu4	HN	8.57	8.56	8.38
	HA	5.03	4.98	4.30
	HB		1.91/2.00	
	HG		2.29	
Val5	HN	9.04	8.86	8.38
	HA	4.60	4.58	4.39
	HB		1.99	
	HG		0.94	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.36	4.36	4.10
	HB		2.13/2.37	
	HG		1.99	
	HD		3.85	
Gly7	HN	8.65	8.58	8.51
	HA	3.77/4.02	3.78/3.99	3.93
Lys8	HN	7.89	7.93	8.41
	HA	4.60	4.54	4.33
	HB		1.80	
	HG		1.39	
	HD		1.68	
	HE		2.99	
Lys9	HN	8.42	8.43	8.13
	HA	4.81	4.61	4.30
	HB		1.58	
	HG		1.11	
	HD		1.36	
	HE		2.99	
Ile10	HN	9.22	8.96	8.30
	HA	4.60	4.46	4.13
	HB		1.89	
	HG		1.20/1.40	
	HD		0.88	
Leu11	HN	8.66	8.59	8.43
	HA	4.16	4.23	4.35
	HB		1.47/1.59	
	HG		1.14	
	HD		0.63/0.70	
Gln12	HN	9.13	8.61	8.37
	HA	4.61	4.31	4.30
	HB		1.88/2.05	
	HG		2.28	
C-term Cys	HN	8.99	-	-
	HA	5.05	-	-

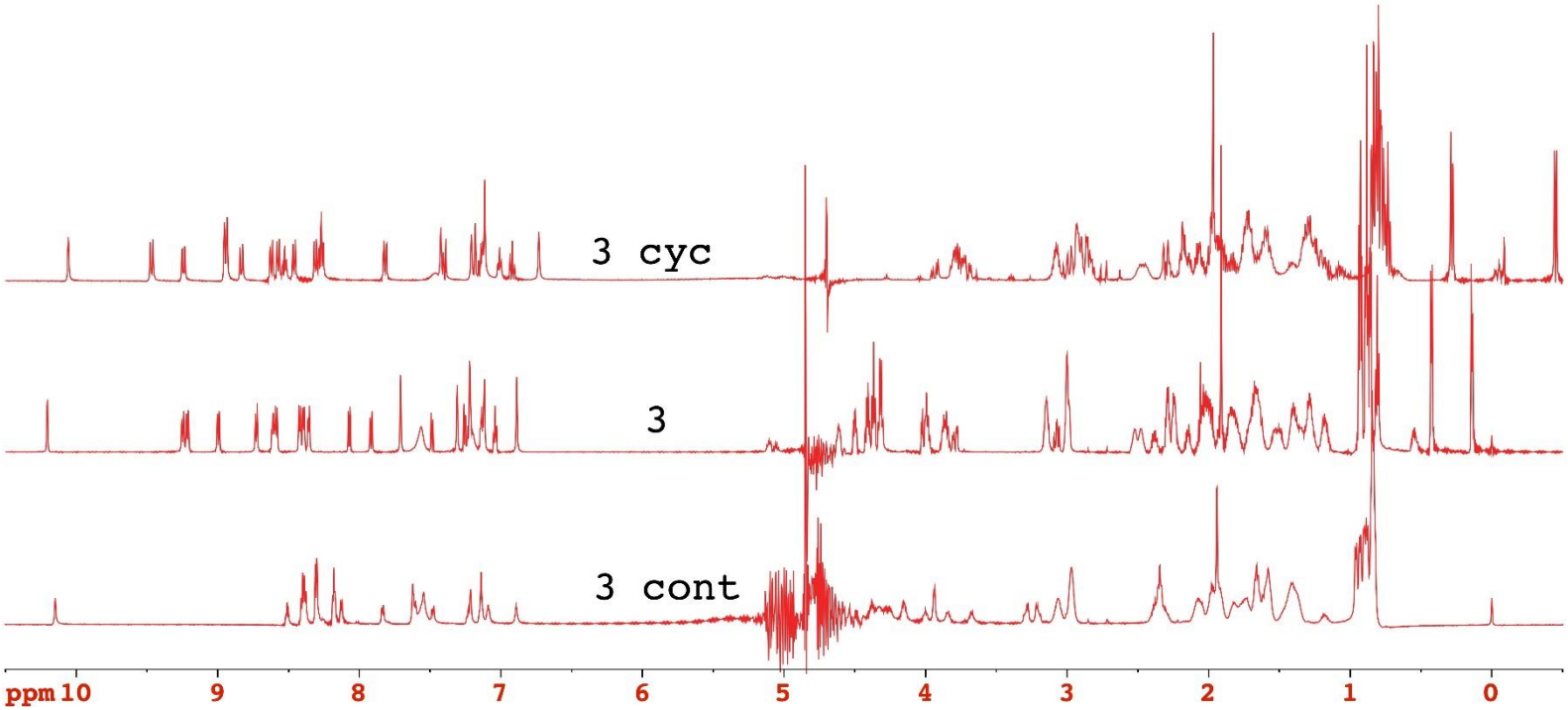


Figure S3: ^{1}H NMR overlay of **3 cyclic**, **3** and **3 random coil control** (top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8

Table S4: Chemical shifts of peptide 3 cyc, 3 and 3 cont (random coil).

Residues		Peptides		
		3 cyc	**3**	**3 cont**
N-term Cys	HN	8.40	-	-
	HA	5.23	-	-
Arg1	HN	8.73	8.04	8.17
	HA	4.60	4.41	4.16
	HB		1.66	
	HG		1.52	
	HD		3.14	
	NAc		1.91	
Trp2	HN	8.71	8.35	8.18
	HA	5.10	5.11	4.73
	HB		3.02	
Val3	HN	9.57	9.25	7.83
	HA	4.55	4.50	4.00
	HB		2.04	
	HG		0.87	
Glu4	HN	8.58	8.58	8.30
	HA	5.09	5.06	4.38
	HB		1.89/2.01	
	HG		2.29	
Val5	HN	9.06	8.96	8.30
	HA	4.63	4.63	4.39
	HB		1.98	
	HG		0.92	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.37	4.37	4.09
	HB		2.11/2.37	
	HG		2.00	
	HD		3.85	
Gly7	HN	8.66	8.60	8.51
	HA	3.79/4.03	3.80/4.02	3.94
Lys8	HN	7.91	7.90	8.40
	HA	4.64	4.62	4.32
	HB		1.81	
	HG		1.40	
	HD		1.67	
	HE		3.00	
Lys9	HN	8.44	8.42	8.12
	HA	5.04	4.86	4.32
	HB		1.65	
	HG		1.18	
	HD		1.31	
	HE		3.00	
Ile10	HN	9.34	9.21	8.30
	HA	4.69	4.61	4.14
	HB		1.85	
	HG		1.17/1.40	
	HD		0.86	
Leu11	HN	8.39	8.35	8.40
	HA	3.88	4.01	4.37
	HB		0.93/1.30	
	HG		0.56	
	HD		0.14/0.42	
Gln12	HN	9.06	8.72	8.38
	HA	4.60	4.32	4.29
	HB		1.83/2.01	
	HG		2.23	
C-term Cys	HN	8.96	-	-
	HA	5.05	-	-

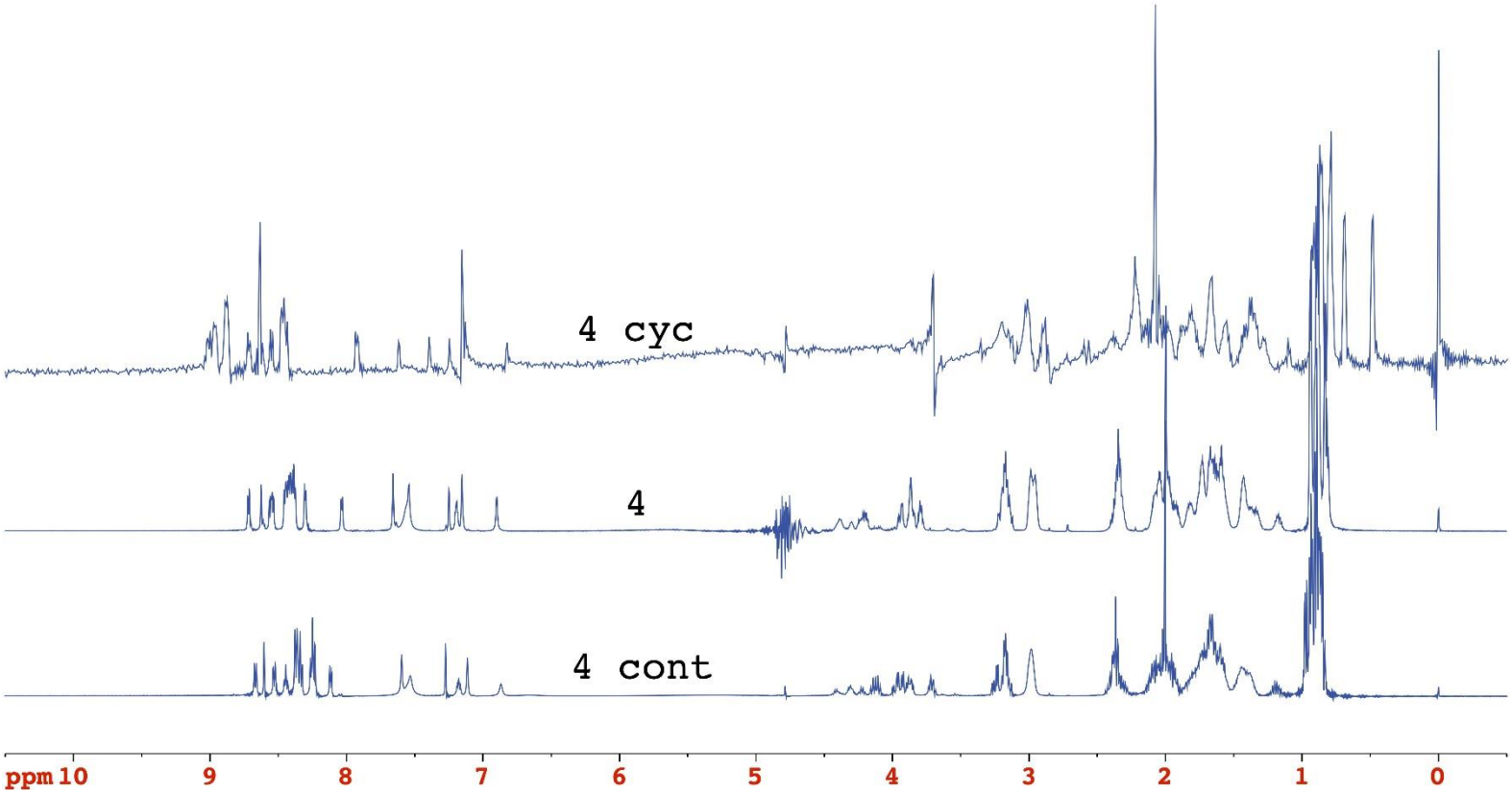


Figure S4: ^{1}H NMR overlay of **4 cyclic**, **4** and **4 random coil control** (top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8

Table S5: Chemical shifts of peptide 4 cyc, 4 and 4 cont (random coil).

Residues		Peptides		
		4 cyc	**4**	**4 cont**
N-term Cys	HN	8.42	-	-
	HA	5.09	-	-
Arg1	HN	8.54	8.30	8.25
	HA	4.49	4.24	4.23
	HB		1.71	
	HG		1.58	
	HD		3.18	
	NAc		1.99	
His2	HN	8.70	8.72	8.65
	HA	5.09	4.86	4.74
	HB		3.19	
Val3	HN	9.00	8.40	8.23
	HA	4.61	4.20	4.14
	HB		2.03	
	HG		0.86	
Glu4	HN	8.54	8.54	8.32
	HA	5.11	4.61	4.28
	HB		1.96	
	HG		2.36	
Val5	HN	8.86	8.56	8.34
	HA	4.59	4.51	4.44
	HB		2.03	
	HG		0.94	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.35	4.41	4.21
	HB		2.04/2.35	
	HG		1.98	
	HD		3.84	
Gly7	HN	8.60	8.40	8.43
	HA	3.77/4.00	3.91	3.93
Lys8	HN	7.92	8.03	8.36
	HA	4.58	4.38	4.32
	HB		1.82	
	HG		1.41	
	HD		1.70	
	HE		3.01	
Lys9	HN	8.54	8.44	8.10
	HA	5.10	4.39	4.29
	HB		1.60/1.74	
	HG		1.21	
	HD		1.32	
	HE		3.00	
Ile10	HN	8.95	8.38	8.23
	HA	4.58	4.21	4.10
	HB		1.85	
	HG		1.16/1.42	
	HD		0.89	
Leu11	HN	8.60	8.45	8.38
	HA	4.35	4.40	4.37
	HB		1.59	
	HG		1.21	
	HD		0.87	
Gln12	HN	8.96	8.42	8.51
	HA	4.59	4.30	4.39
	HB		2.09	
	HG		2.35	
C-term Cys	HN	8.99	-	-
	HA	5.03	-	-

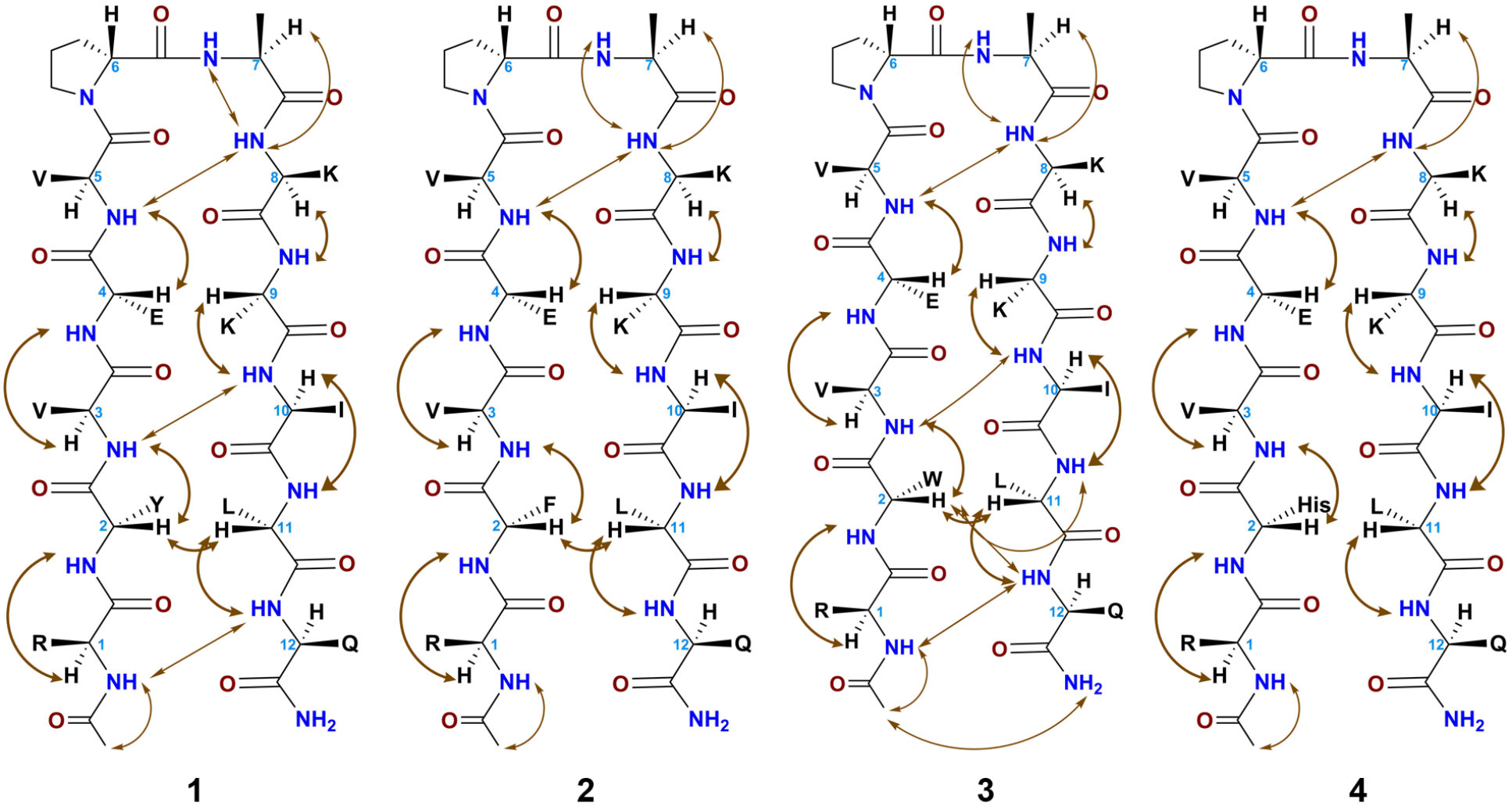


Figure S5: The characteristic backbone β-hairpin NOEs observed in **1-4**, where the thick arrows denote strong (1.8–2.2 Å), the thin arrows denote medium to weak NOEs (> 2.2 Å). The distances were resolved as mentioned in the previous section, taking the distance between the two HA protons of Gly7 as 1.77 Å.

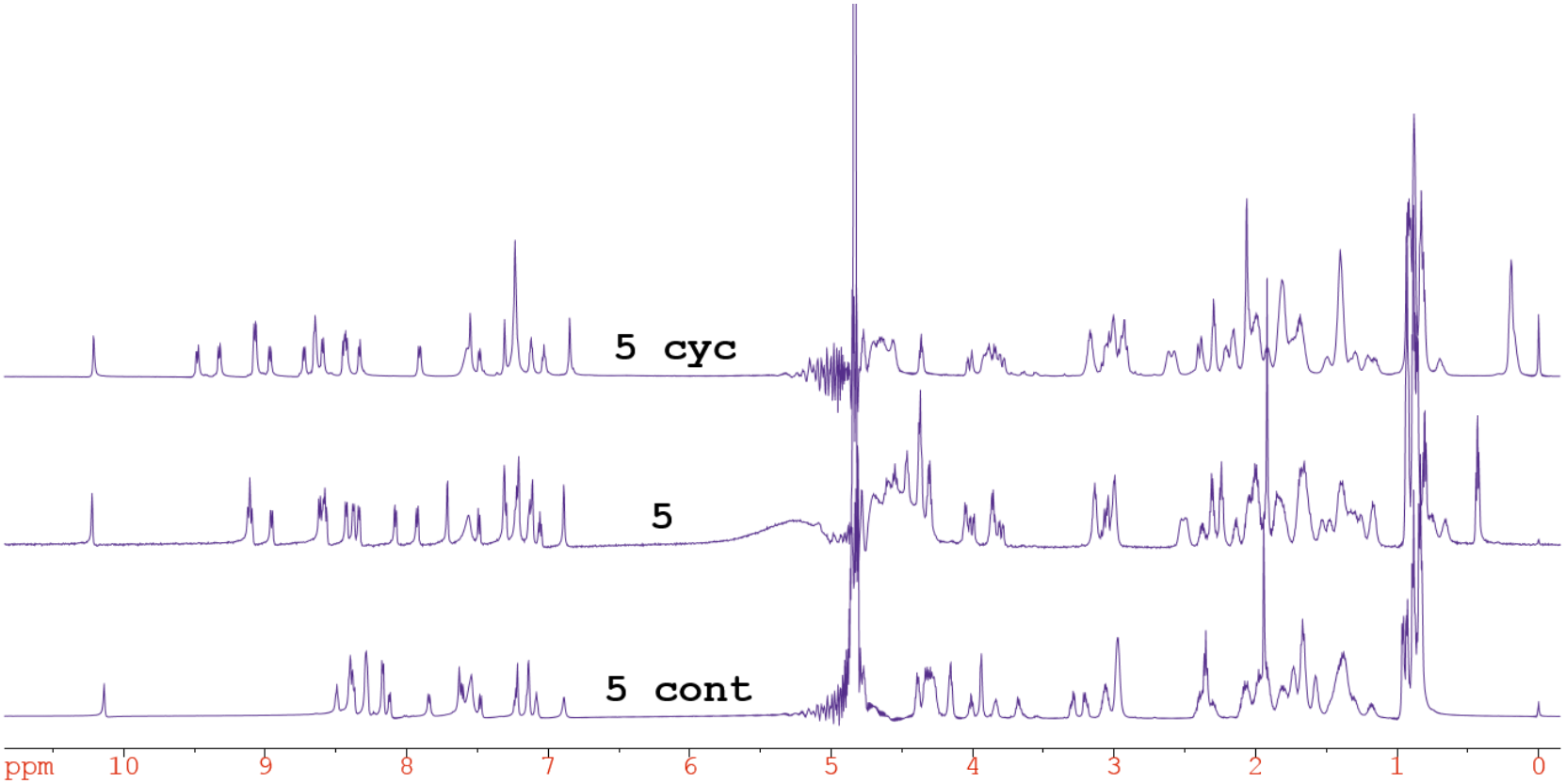


Figure S6: ^{1}H NMR overlay of **5 cyclic**, **5** and **5 random coil** control (from top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8.

Table S6: Chemical shifts of peptide 5 cyc, 5 and 5 cont (random coil).

Residues		Peptides		
		5 cyc	**5**	**5 cont**
N-term Cys	HN	8.42	-	-
	HA	5.13	-	-
Arg1	HN	8.64	8.07	8.16
	HA	4.63	4.37	4.15
	HB		1.66	
	HG		1.54	
	HD		3.14	
	NAc		1.92	
Trp2	HN	8.72	8.33	8.16
	HA	5.14	5.08	4.73
	HB		3.04	
Val3	HN	9.48	9.08	7.83
	HA	4.56	4.47	4.00
	HB		2.06	
	HG		0.88	
Glu4	HN	8.59	8.56	8.28
	HA	5.07	5.02	4.28
	HB		1.94/2.03	
	HG		2.31	
Val5	HN	9.07	8.95	8.27
	HA	4.60	4.59	4.39
	HB		2.00	
	HG		0.95	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.36	4.38	3.99
	HB		2.00/2.37	
	HG		1.90	
	HD		3.84	
Gly7	HN	8.64	8.58	8.49
	HA	3.79/4.02	3.80/4.01	3.94
Lys8	HN	7.90	7.92	8.39
	HA	4.64	4.58	4.31
	HB		1.79	
	HG		1.39	
	HD		1.65	
	HE		2.99	
Lys9	HN	8.44	8.42	8.11
	HA	5.00	4.80	4.32
	HB		1.70	
	HG		1.14	
	HD		1.38	
	HE		3.01	
Ile10	HN	9.32	9.11	8.28
	HA	4.66	4.56	4.14
	HB		1.85	
	HG		1.18	
	HD		0.89	
Nva11	HN	8.33	8.37	8.36
	HA	3.92	4.04	4.29
	HB		0.85/1.39	
	HG		0.65/0.74	
	HD		0.43	
Gln12	HN	9.06	8.60	8.38
	HA	4.55	4.30	4.29
	HB		1.86/2.03	
	HG		2.26	
C-term Cys	HN	8.96	-	-
	HA	5.02	-	-

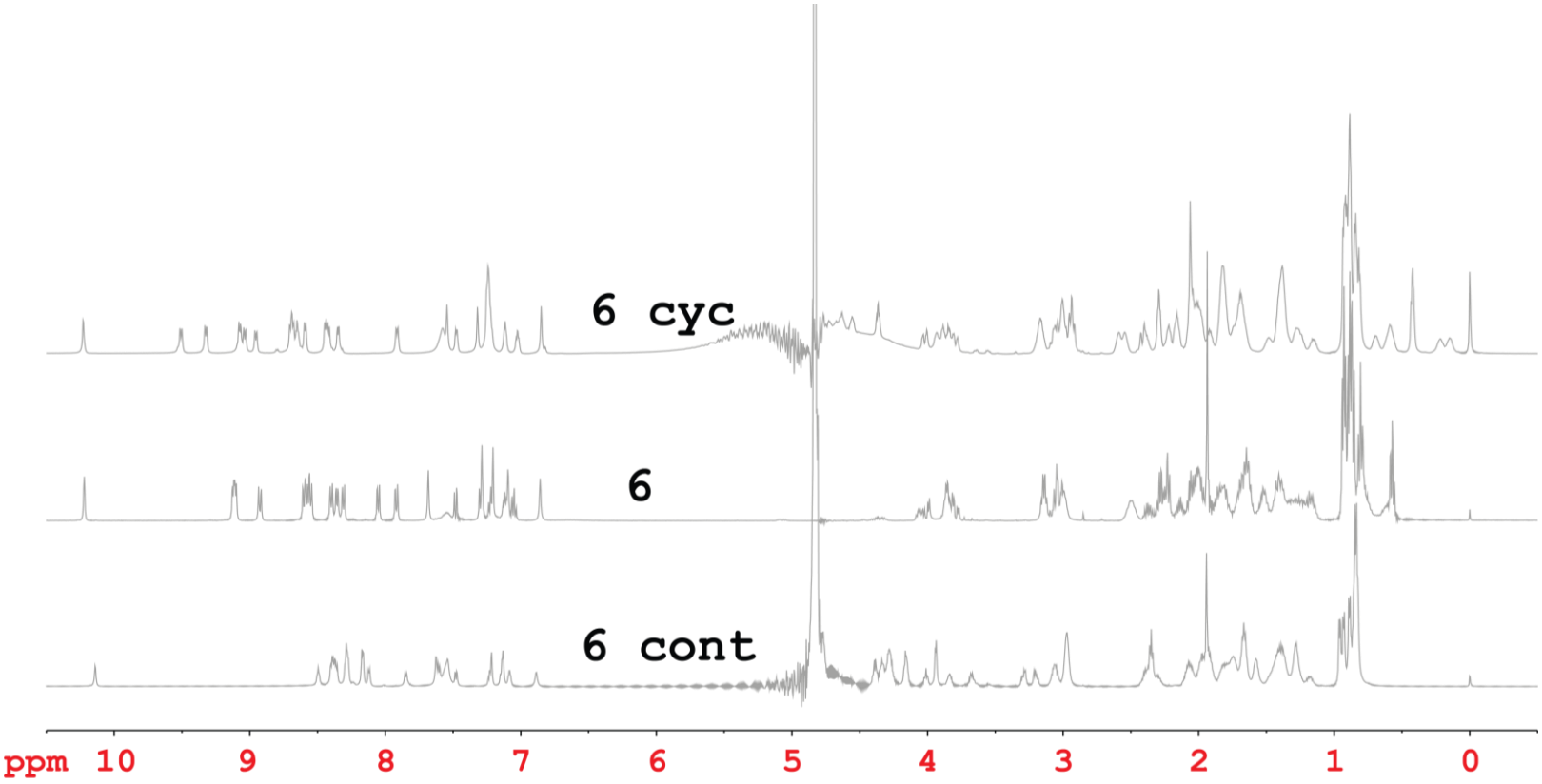


Figure S7: ^{1}H NMR overlay of **6 cyclic**, **6** and **6 random coil** control (from top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8.

Table S7: Chemical shifts of peptide 6 cyc, 6 and 6 cont (random coil).

Residues		Peptides		
		6 cyc	**6**	**6 cont**
N-term Cys	HN	8.68	-	-
	HA	5.14	-	-
Arg1	HN	8.69	8.04	8.16
	HA	4.63	4.40	4.16
	HB		1.71	
	HG		1.52	
	HD		3.15	
	NAc		1.93	
Trp2	HN	8.41	8.30	8.16
	HA	5.20	5.07	4.73
	HB		3.05	
Val3	HN	9.55	9.11	7.84
	HA	4.55	4.52	4.00
	HB		2.07	
	HG		0.91	
Glu4	HN	8.58	8.55	8.29
	HA	5.07	5.05	4.26
	HB		1.98/2.04	
	HG		2.28	
Val5	HN	9.07	8.92	8.27
	HA	4.64	4.61	4.39
	HB		2.01	
	HG		0.93	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.38	4.37	4.16
	HB		2.01/2.37	
	HG		1.91	
	HD		3.84	
Gly7	HN	8.64	8.57	8.49
	HA	3.80/4.02	3.80/4.01	3.93
Lys8	HN	7.91	7.91	8.11
	HA	4.65	4.58	4.32
	HB		1.85	
	HG		1.43	
	HD		1.71	
	HE		2.99	
Lys9	HN	8.43	8.39	8.39
	HA	5.02	4.83	4.33
	HB		1.64	
	HG		1.16	
	HD		1.37	
	HE		3.00	
Ile10	HN	9.32	9.11	8.27
	HA	4.68	4.51	4.15
	HB		1.89	
	HG		1.40	
	HD		0.90	
Nle11	HN	8.34	8.36	8.35
	HA	3.94	4.06	4.28
	HB		1.45	
	HG		0.80	
	HD		0.89	
	HE		0.58	
Gln12	HN	9.03	8.61	8.39
	HA	4.57	4.32	4.29
	HB		1.85/2.01	
	HG		2.24	
C-term Cys	HN	8.95	-	-
	HA	5.04	-	-

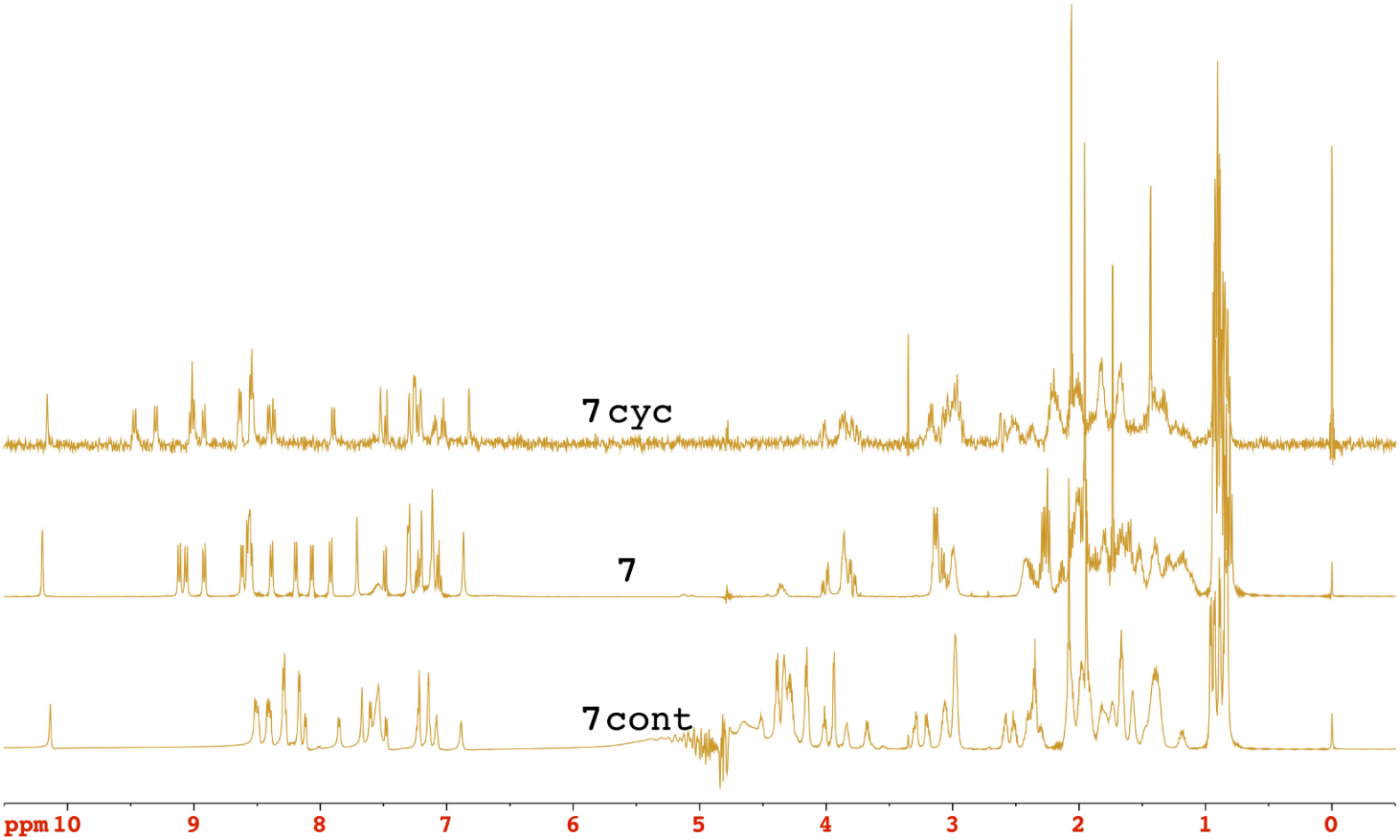


Figure S8: ^{1}H NMR overlay of **7 cyclic**, **7** and **7 random coil control** (top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8

Table S8: Chemical shifts of peptide 7 cyc,7 and7 cont (random coil).

Residues		Peptides		
		7 cyc	**7**	**7 cont**
N-term Cys	HN	8.37	-	-
	HA	5.16	-	-
Arg1	HN	8.64	8.07	8.16
	HA	4.61	4.38	4.17
	HB		1.68	
	HG		1.54	
	HD		3.14	
	NAc		1.94	
Trp2	HN	8.55	8.20	8.16
	HA	5.10	5.12	4.74
	HB		3.09	
Val3	HN	9.47	9.06	7.85
	HA	4.54	4.47	4.01
	HB		2.05	
	HG		0.88	
Glu4	HN	8.55	8.57	8.27
	HA	5.05	5.08	4.25
	HB		1.94/2.04	
	HG		2.27	
Val5	HN	9.00	8.92	8.27
	HA	4.63	4.61	4.39
	HB		1.99	
	HG		0.94	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.35	4.36	4.16
	HB		2.10/2.37	
	HG		1.89	
	HD		3.86	
Gly7	HN	8.64	8.56	8.49
	HA	3.79/4.03	3.80/4.00	3.94
Lys8	HN	7.90	7.92	8.39
	HA	4.64	4.57	4.33
	HB		1.79	
	HG		1.41	
	HD		1.67	
	HE		2.99	
Lys9	HN	8.40	8.39	8.11
	HA	5.02	4.80	4.33
	HB		1.72	
	HG		1.29	
	HD		1.39	
	HE		2.99	
Ile10	HN	9.31	9.11	8.28
	HA	4.71	4.55	4.14
	HB		1.91	
	HG		1.40	
	HD		0.81	
Met11	HN	8.55	8.55	8.51
	HA	4.20	4.37	4.51
	HB		1.87	
	HG		1.76	
	HD		1.33	
Gln12	HN	9.03	8.62	8.42
	HA	4.57	4.33	4.30
	HB		1.86/2.05	
	HG		2.25	
C-term Cys	HN	8.92	-	-
	HA	5.04	-	-

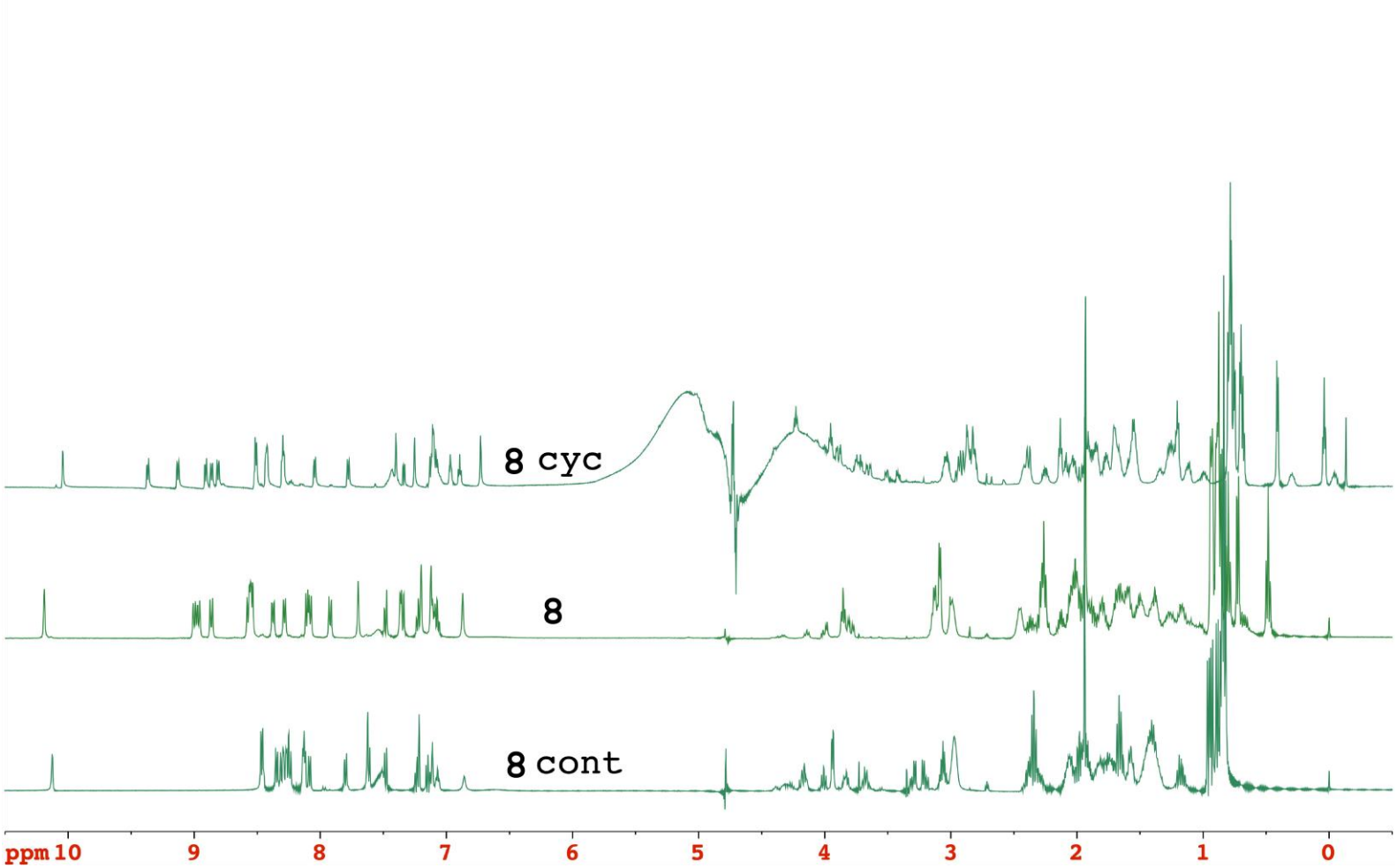


Figure S9: ^{1}H NMR overlay of **8 cyclic**, **8** and **8 random coil** control (from top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8.

Table S9: Chemical shifts of peptide 8 cyc, 8 and 8 cont (random coil).

Residues		Peptides		
		8 cyc	**8**	**8 cont**
N-term Cys	HN	8.43	-	-
	HA	5.13	-	-
Arg1	HN	8.64	8.09	8.12
	HA	4.64	4.31	4.15
	HB		1.67	
	HG		1.52	
	HD		3.17	
	NAc		1.93	
Trp2	HN	8.55	8.06	8.11
	HA	5.10	5.07	4.72
	HB		3.07	
Val3	HN	9.49	8.95	7.79
	HA	4.52	4.40	3.99
	HB		2.03	
	HG		0.88	
Glu4	HN	8.55	8.52	8.25
	HA	5.09	5.01	4.26
	HB		1.91/2.02	
	HG		2.26	
Val5	HN	9.03	8.85	8.23
	HA	4.63	4.58	4.38
	HB		1.98	
	HG		0.93	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.36	4.37	4.11
	HB		2.36	
	HG		1.81	
	HD		3.85	
Gly7	HN	8.64	8.53	8.45
	HA	3.79/4.02	3.79/4.00	3.93
Lys8	HN	7.90	7.91	8.34
	HA	4.64	4.55	4.31
	HB		1.77	
	HG		1.38	
	HD		1.66	
	HE		3.00	
Lys9	HN	8.41	8.36	8.07
	HA	5.06	4.73	4.31
	HB		1.73	
	HG		1.16	
	HD		1.37	
	HE		2.99	
Ile10	HN	9.26	8.98	8.26
	HA	4.82	4.56	4.16
	HB		1.89	
	HG		1.17	
	HD		0.88	
Ile11	HN	8.17	8.26	8.37
	HA	4.08	4.13	4.17
	HB		1.46	
	HG		0.72/1.02	
	HD		0.48	
Gln12	HN	8.94	8.56	8.45
	HA	4.55	4.31	4.28
	HB		1.87/2.02	
	HG		2.24	
C-term Cys	HN	8.99	-	-
	HA	5.03	-	-

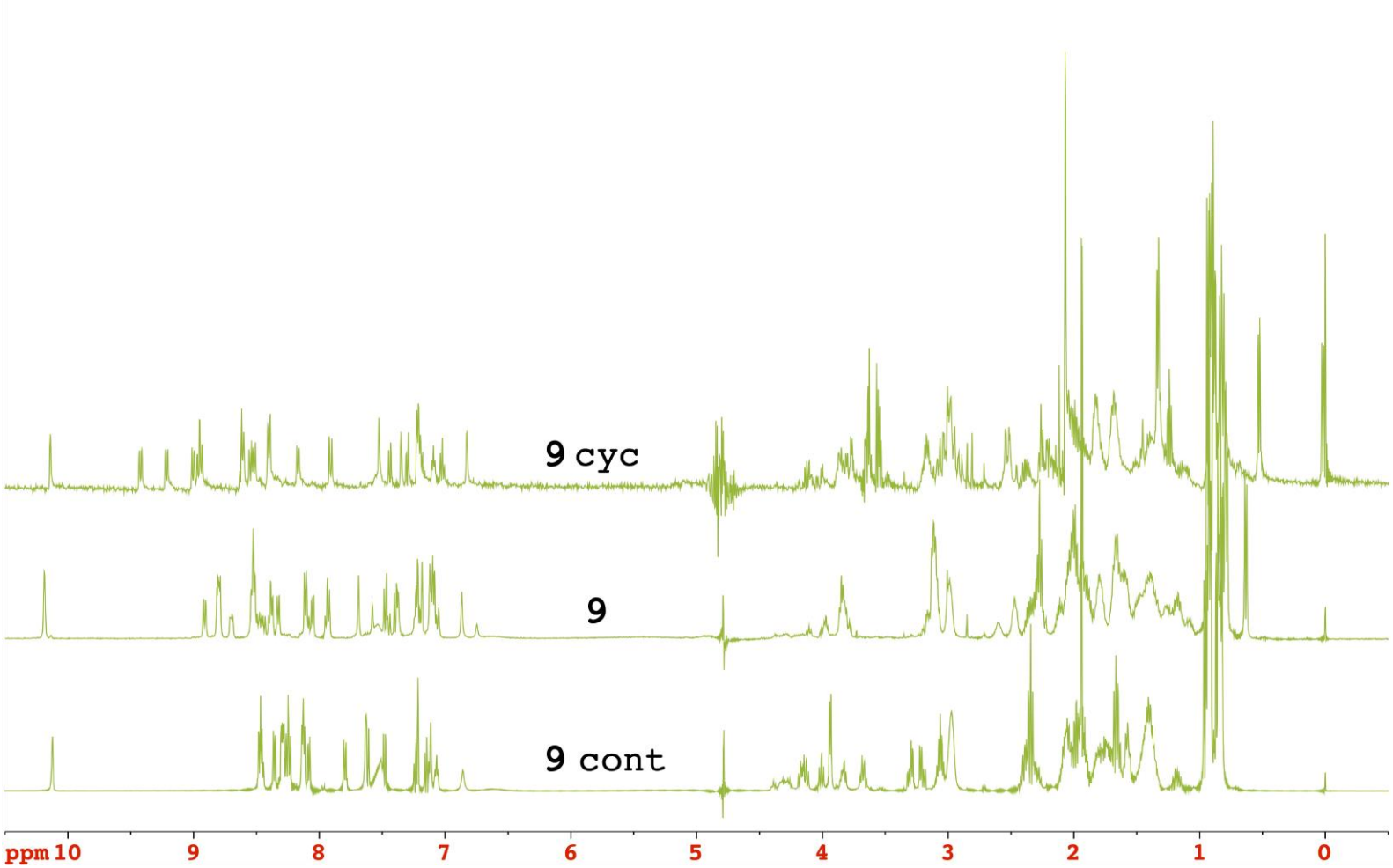


Figure S10: ^{1}H NMR overlay of **9 cyclic**, **9** and **9 random coil control** (top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8

Table S10: Chemical shifts of peptide 9 cyc, 9 and 9 cont (random coil).

Residues		Peptides		
		9 cyc	**9**	**9 cont**
N-term Cys	HN	8.40	-	-
	HA	5.11	-	-
Arg1	HN	8.61	8.09	8.12
	HA	4.65	4.25	4.14
	HB		1.71	
	HG		1.57	
	HD		3.16	
	NAc		1.93	
Trp2	HN	8.51	8.04	8.11
	HA	5.17	5.06	4.73
	HB		3.11	
Val3	HN	9.42	8.78	7.79
	HA	4.53	4.36	3.98
	HB		1.98	
	HG		0.91	
Glu4	HN	8.54	8.52	8.24
	HA	5.10	4.96	4.25
	HB		1.92/2.03	
	HG		2.31	
Val5	HN	9.00	8.68	8.22
	HA	4.63	4.55	4.39
	HB		2.00	
	HG		0.87	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.36	4.37	4.07
	HB		2.11/2.38	
	HG		1.84	
	HD		3.87	
Gly7	HN	8.62	8.50	8.44
	HA	3.80/4.02	3.80/3.98	3.92
Lys8	HN	7.91	7.92	8.35
	HA	4.63	4.50	4.31
	HB		1.78	
	HG		1.40	
	HD		1.68	
	HE		2.98	
Lys9	HN	8.40	8.37	8.07
	HA	5.00	4.64	4.31
	HB		1.77	
	HG		1.28	
	HD		1.42	
	HE		3.00	
Ile10	HN	9.21	8.90	8.28
	HA	4.74	4.47	4.15
	HB		1.90	
	HG		1.18	
	HD		0.89	
Val11	HN	8.16	8.31	8.29
	HA	4.11	4.09	4.13
	HB		1.77	
	HG		0.63/0.78	
	HD		-	
Gln12	HN	8.95	8.51	8.46
	HA	4.60	4.31	4.28
	HB		1.87/2.03	
	HG		2.28	
C-term Cys	HN	8.96	-	-
	HA	5.06	-	-

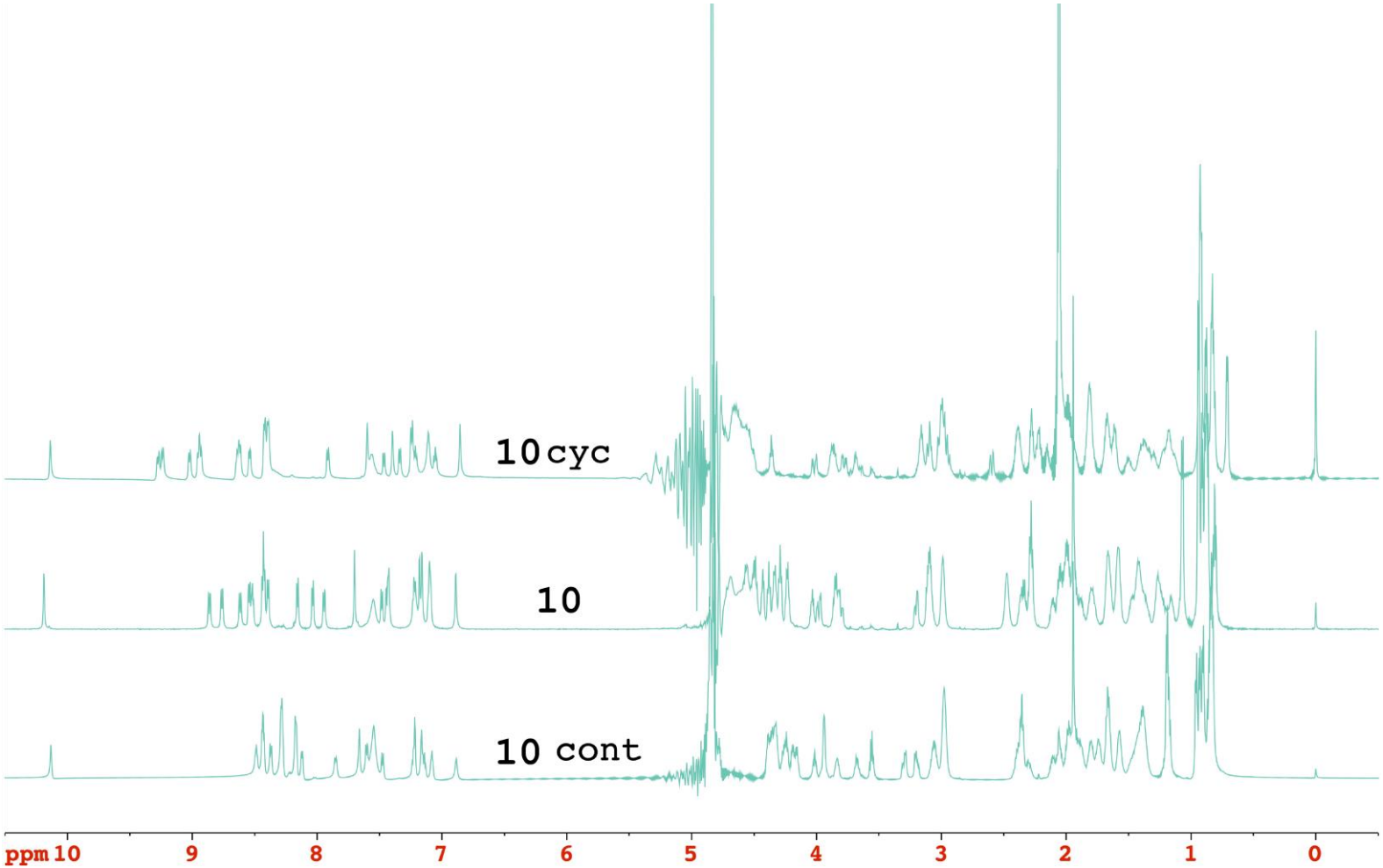


Figure S11: ^{1}H NMR overlay of **10 cyclic**, **10** and **10 random coil control** (top to bottom), acquired at 298K, in 100mM sodium acetate buffer: D_2O (9:1), pH 3.8

Table S11: Chemical shifts of peptide 10 cyc, 10 and 10 cont (random coil).

Residues		Peptides		
		10 cyc	**10**	**10 cont**
N-term Cys	HN	8.41	-	-
	HA	5.02	-	-
Arg1	HN	8.53	8.15	8.16
	HA	4.56	4.23	4.15
	HB		1.71	
	HG		1.58	
	HD		3.18	
	NAc		1.94	
Trp2	HN	8.38	8.02	8.16
	HA	5.29	5.04	4.73
	HB		3.20	
Val3	HN	9.26	8.75	7.83
	HA	4.50	4.42	4.01
	HB		2.01	
	HG		0.94	
Glu4	HN	8.60	8.53	8.28
	HA	5.13	4.91	4.28
	HB		1.91/2.01	
	HG		2.31	
Val5	HN	9.01	8.61	8.26
	HA	4.63	4.29	4.38
	HB		2.02	
	HG		0.88	
D-Pro6 / L-Pro6	HN	-	-	-
	HA	4.35	4.36	4.01
	HB		2.37	
	HG		1.92	
	HD		3.86	
Gly7	HN	8.63	8.51	8.47
	HA	3.78/4.01	3.80/3.97	3.92
Lys8	HN	7.90	7.93	8.42
	HA	4.61	4.50	4.32
	HB		1.77	
	HG		1.40	
	HD		1.69	
	HE		2.99	
Lys9	HN	8.41	8.41	8.11
	HA	4.89	4.55	4.33
	HB		1.74	
	HG		1.29	
	HD		1.40	
	HE		2.99	
Ile10	HN	9.22	8.85	836
	HA	4.78	4.48	4.24
	HB		1.94	
	HG		1.21	
	HD		0.91	
Thr11	HN	8.38	8.37	8.23
	HA	4.53	4.37	4.19
	HB		4.04	
	HG		1.09	
	HD		-	
Gln12	HN	8.92	8.43	8.42
	HA	4.60	4.33	4.32
	HB		1.90/2.04	
	HG		2.27	
C-term Cys	HN	8.94	-	-
	HA	4.99	-	-

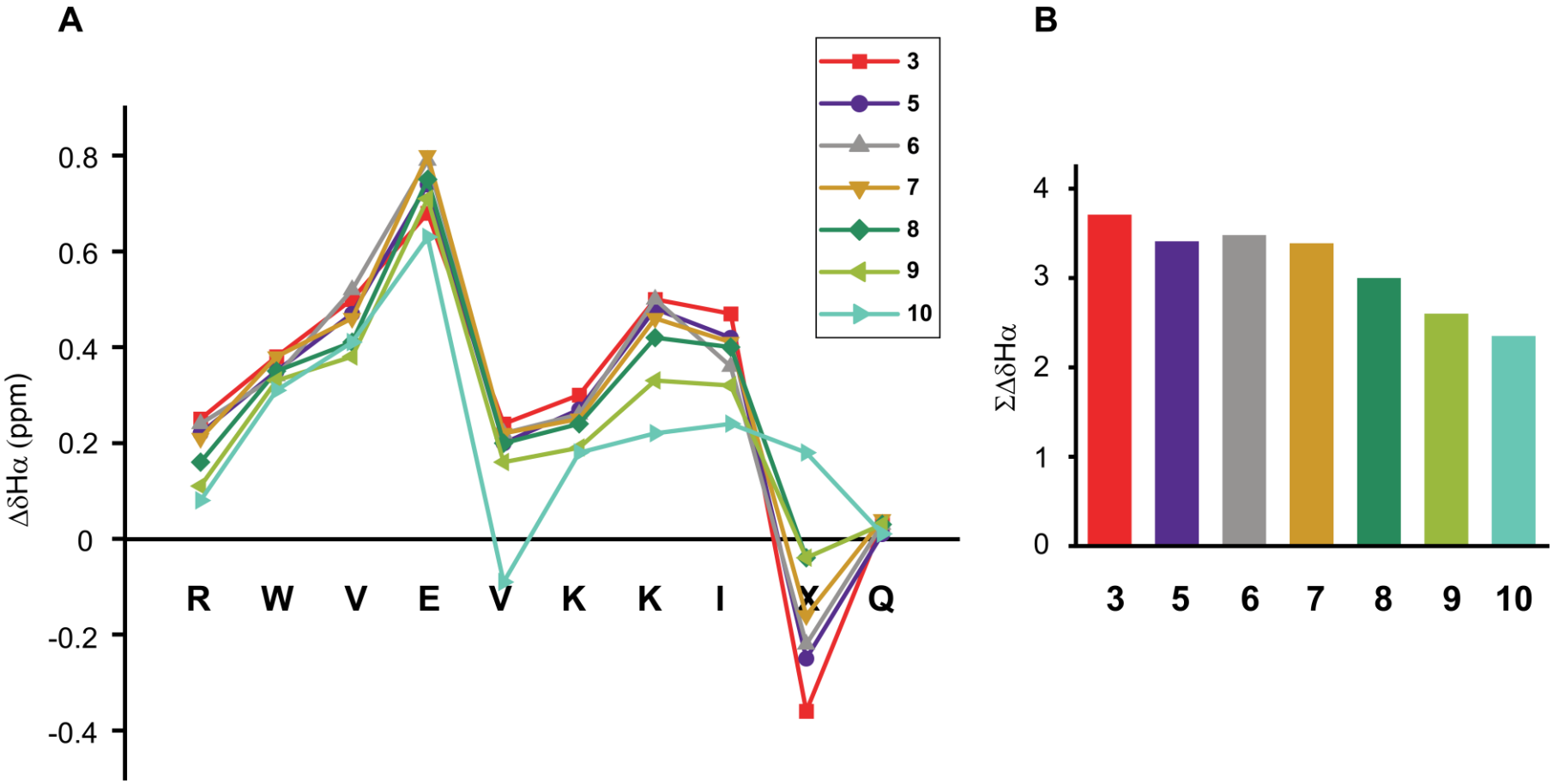


Figure S12: Chemical shift deviation (A) and summation of the chemical shifts (ΣCSD) of peptides **3**, **5-10** (B).

Relative upfield shifts of the side chains of 11th residue in 3, 5-10:

Peptide 3: Ac-RWVEV-pG-KKILQ-NH2

Peptides	HN	HA	HB	HG	HD
3	8.35	4.01	0.93/1.30	0.56	0.14/0.42
3 cont	8.40	4.37	1.36/1.43	1.60	0.84/0.87

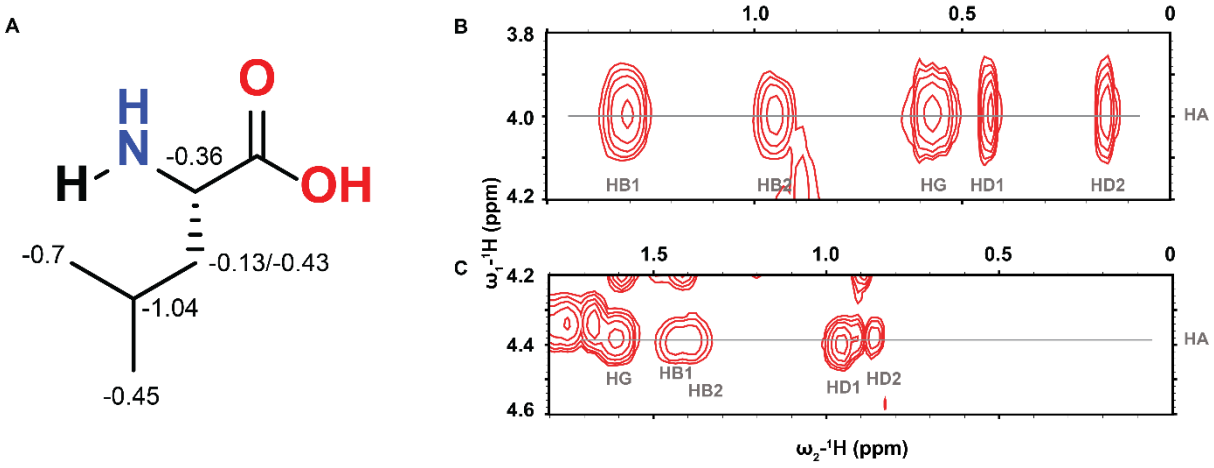


Figure S13: (A) Relative upfield shift of Leu11 side-chain protons w.r.t its unfolded control. TOCSY slices of **3**(B) and **3 cont**(C).

Peptide 5: Ac-RWVEV-pG-KKINvaQ-NH2

Peptides	HN	HA	HB	HG	HD
5	8.37	4.04	0.85/1.39	0.65/0.74	0.43
5 cont	8.36	4.29	1.42/1.67	1.36	0.88

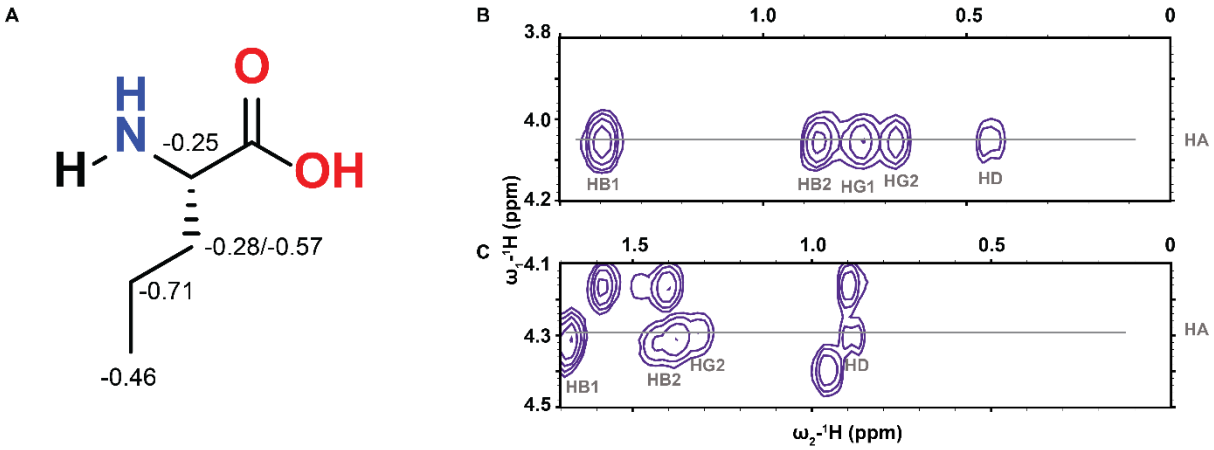


Figure S14: (A) Relative upfield shift of Nva11 side-chain protons w.r.t its unfolded control. TOCSY slices of **5**(B) and **5 cont**(C).

Peptide 6: Ac-RWVEV-pG-KKINleQ-NH$_2$

Peptides	HN	HA	HB	HG	HD	HE
6	8.37	4.06	1.45	0.80	0.89	0.58
6 cont	8.35	4.28	1.79	1.75	1.67	1.29

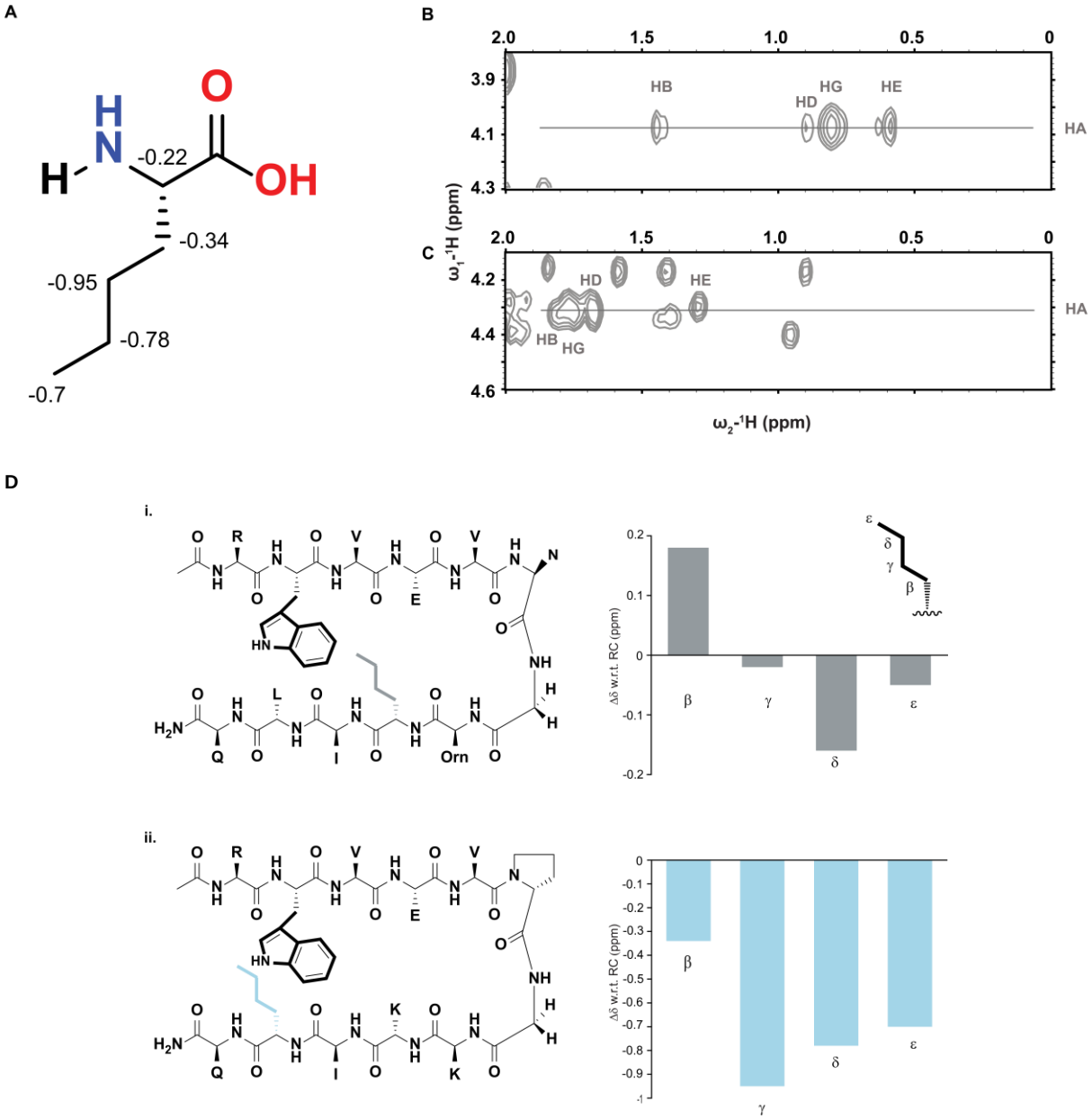


Figure S15: (A) Relative upfield shift of Nle11 side-chain protons w.r.t its unfolded control. TOCSY slices of **6**(B) and **6 cont**(C). (D) Comparison of the relative upfield shifts when Nle is placed at the diagonal(i) or at the cross-strand position(ii) w.r.t Trp2. The Δδ values for D(i) has been adopted from the work of Waters et al.[3]

Peptide 7: Ac-RWVEV-pG-KKIMQ-NH$_2$

Peptides	HN	HA	HB	HG	HD
6	8.55	4.37	1.87	1.76	1.33
6 cont	8.51	4.51	2.16	2.48	1.60

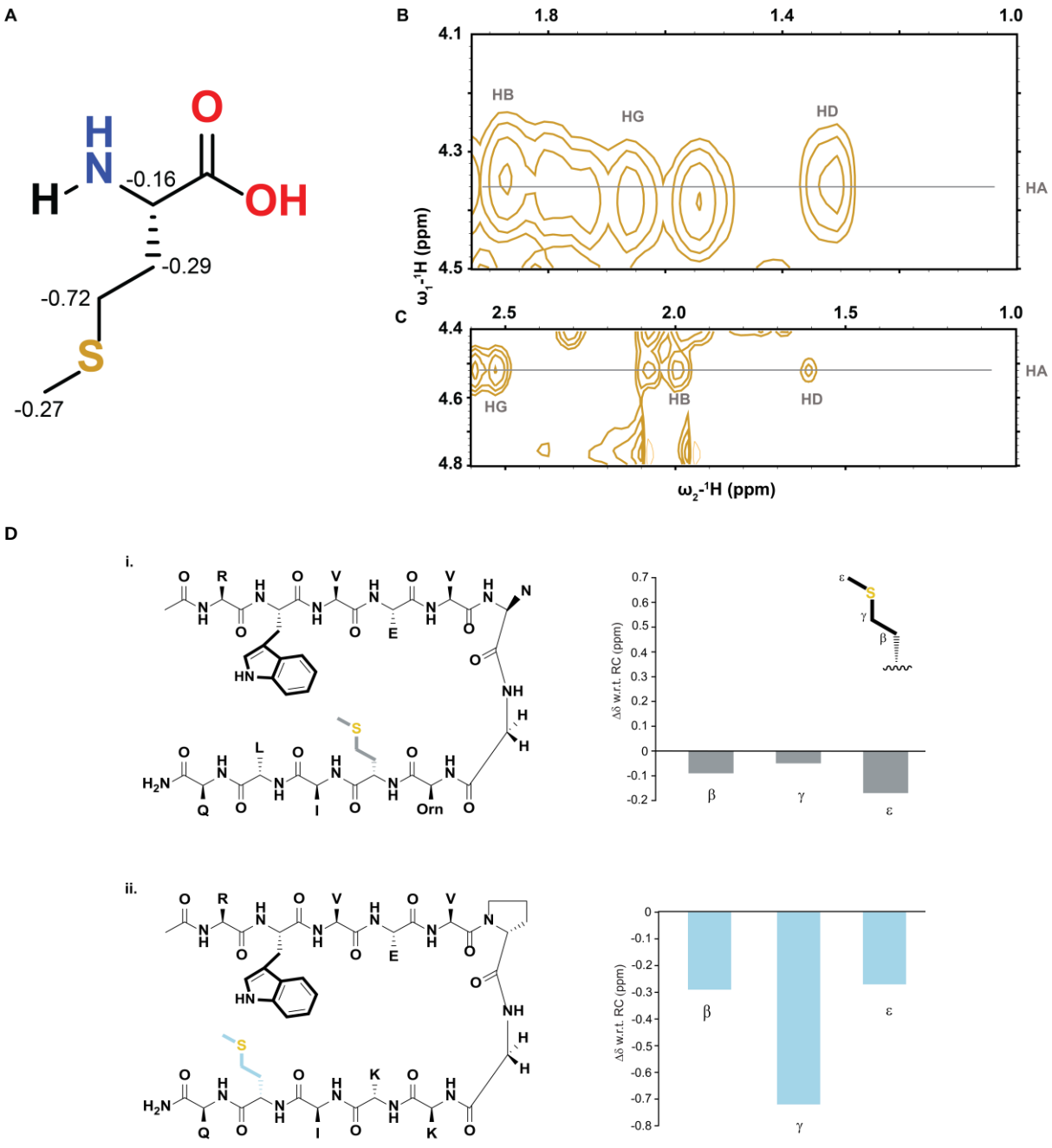


Figure S16: (A) Relative upfield shift of Met11 side-chain protons w.r.t its unfolded control. TOCSY slices of **7**(B) and **7 cont**(C). (D) Comparison of the relative upfield shifts when Met is placed at the diagonal(i) or at the cross-strand position(ii) w.r.t Trp2. The Δδ values for D(i) has been adopted from the work of Waters et al.[3]

Peptide 8: Ac-RWVEV-pG-KKIIQ-NH_2

Peptides	HN	HA	HB	HG	HD
7	8.26	4.13	1.46	0.72/1.02	0.48
7 cont	8.37	4.17	1.81	0.88/1.19	0.84

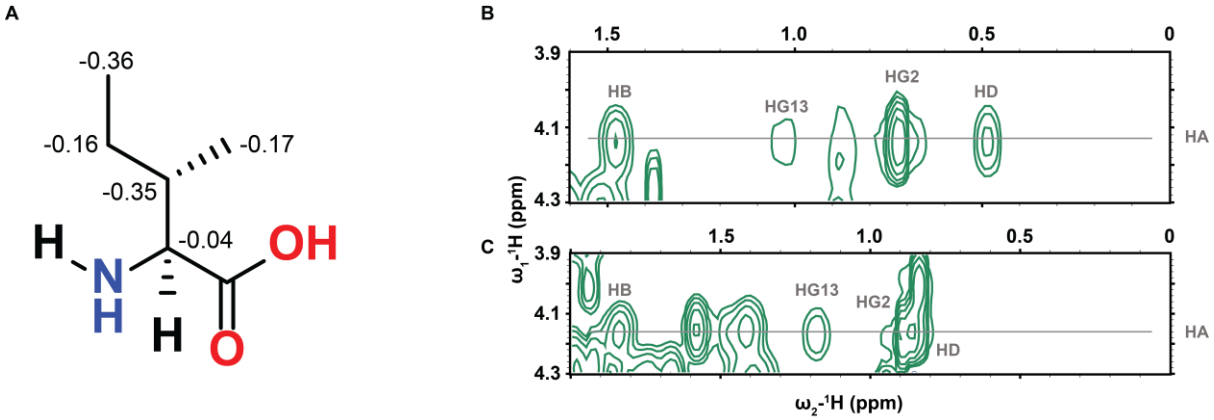


Figure S17: (A) Relative upfield shift of Ile11 side-chain protons w.r.t its unfolded control. TOCSY slices of **8**(B) and **8 cont**(C).

Peptide 9: Ac-RWVEV-pG-KKIVQ-NH_2

Peptides	HN	HA	HB	HG
8	8.31	4.09	1.77	0.63/0.78
8 cont	8.29	4.13	1.91	0.91/0.98

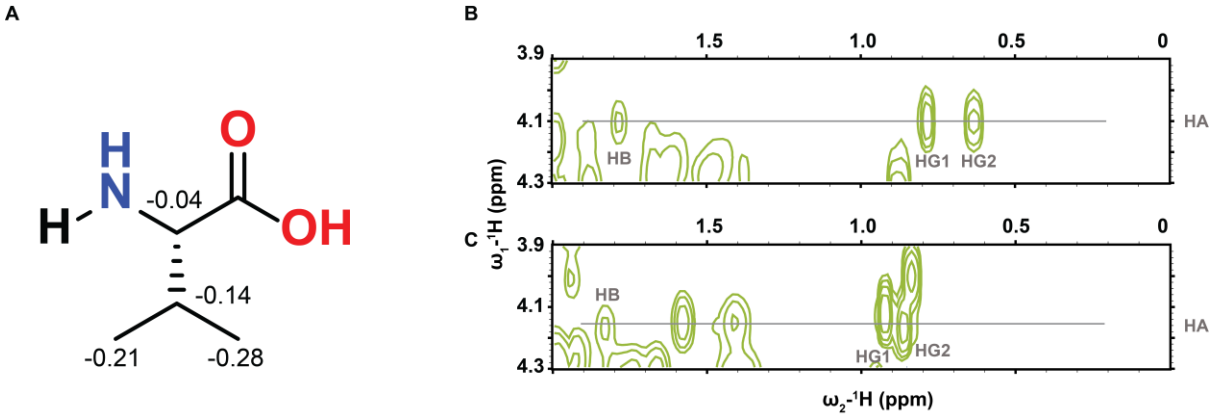


Figure S18: (A) Relative upfield shift of Val11 side-chain protons w.r.t its unfolded control. TOCSY slices of **9**(B) and **9 cont**(C).

Peptide 10: Ac-RWVEV-pG-KKITQ-NH$_2$

Peptides	HN	HA	HB	HG
9	8.37	4.37	4.04	1.09
9 cont	8.23	4.19	4.22	1.24

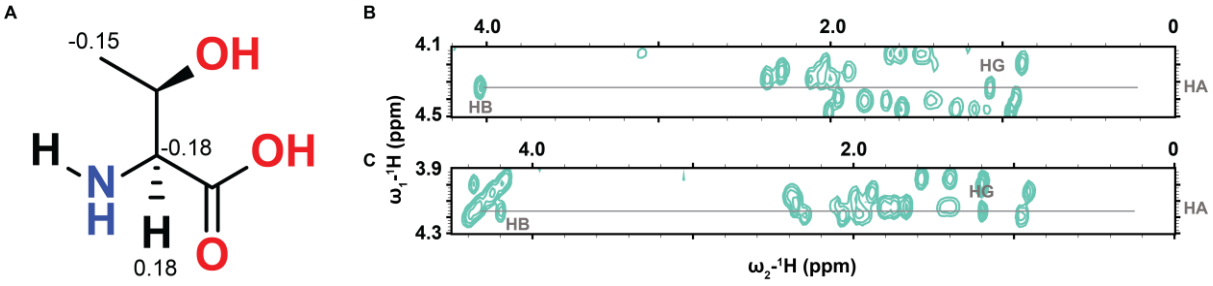
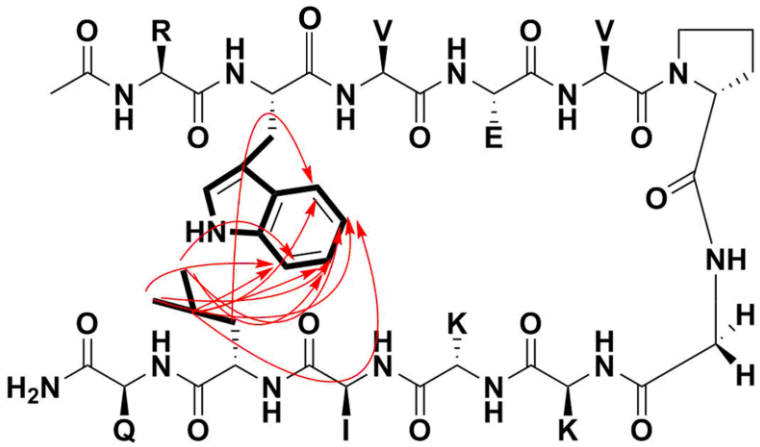


Figure S19: (A) Relative upfield shift of Thr11 side-chain protons w.r.t its unfolded control. TOCSY slices of **10**(B) and **10 cont**(C).

Observed NOEs between Trp2 and different aliphatic residues in 3, 5-10:

Peptide 3: Ac-RWVEV-pG-KKILQ-NH$_2$

A



B

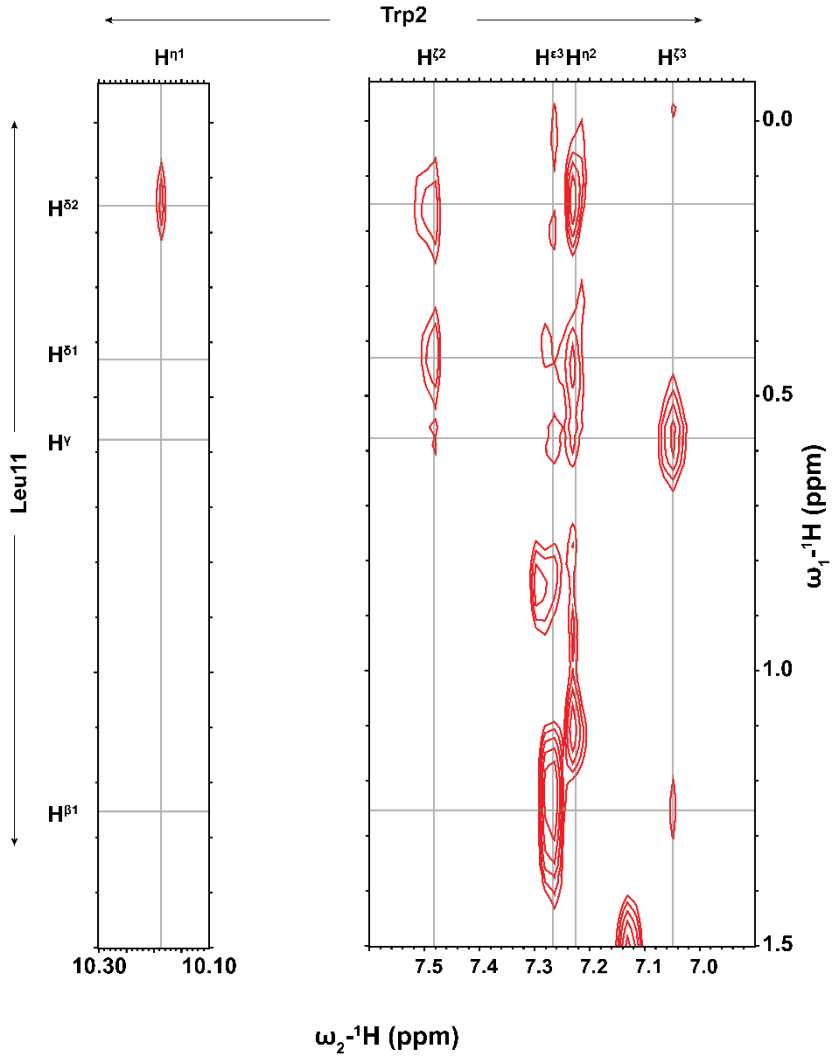
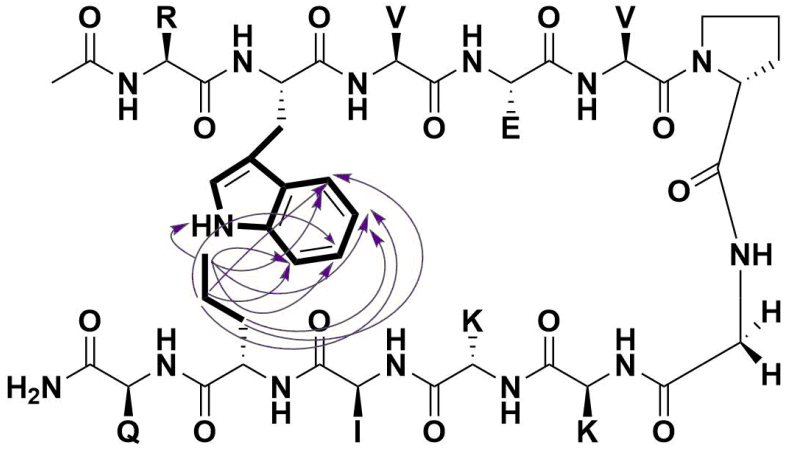


Figure S20: Observed NOEs between the indole aromatic ring of Trp2 and isopropyl side chain of Leu11.

Peptide 5: Ac-RWVEV-pG-KKINvaQ-NH$_2$

A



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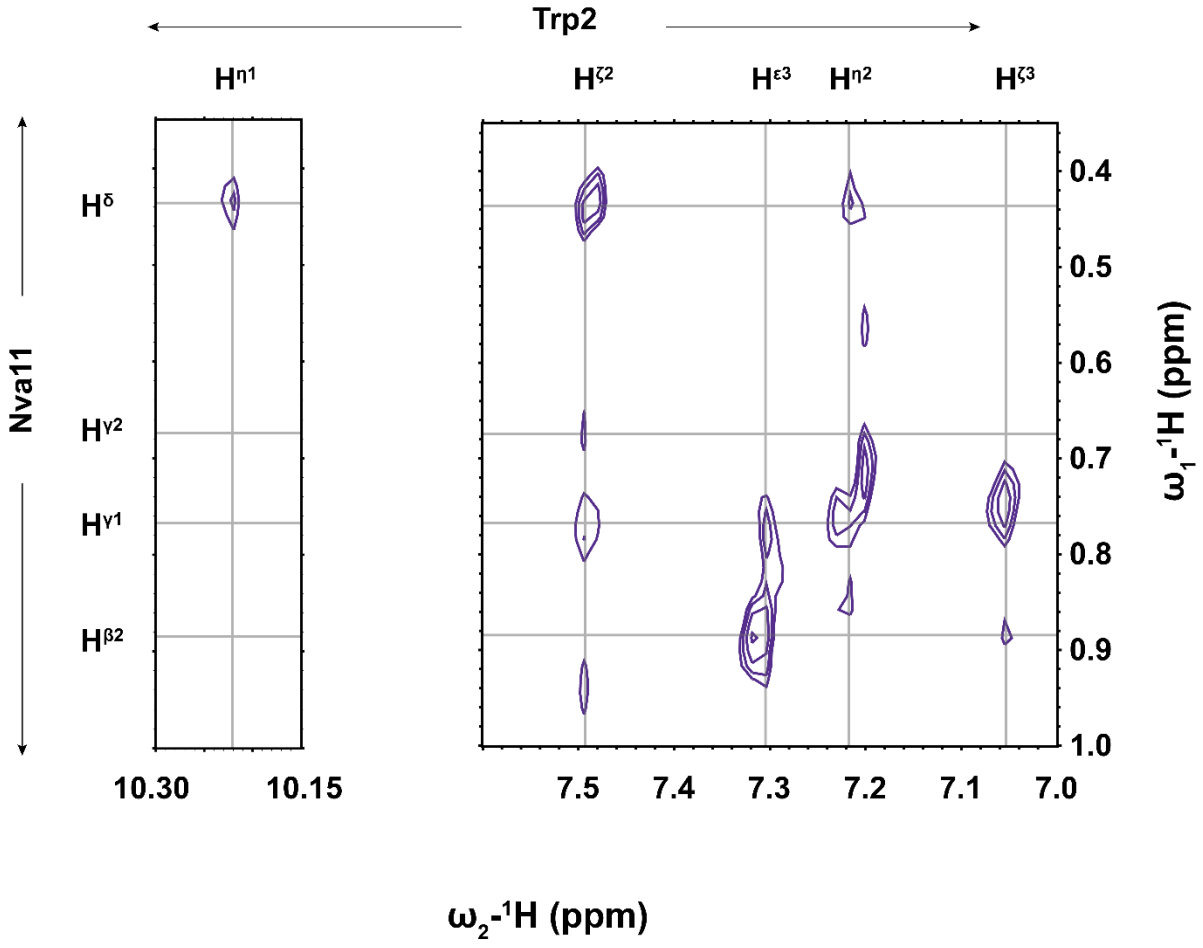
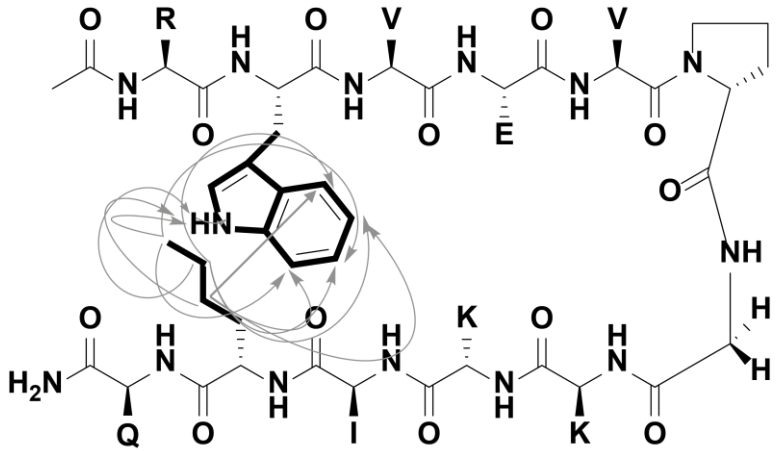


Figure S21: Observed NOEs between the indole side chain of Trp2 and Nva11.

Peptide 6: Ac-RWVEV-pG-KKINleQ-NH$_2$

A



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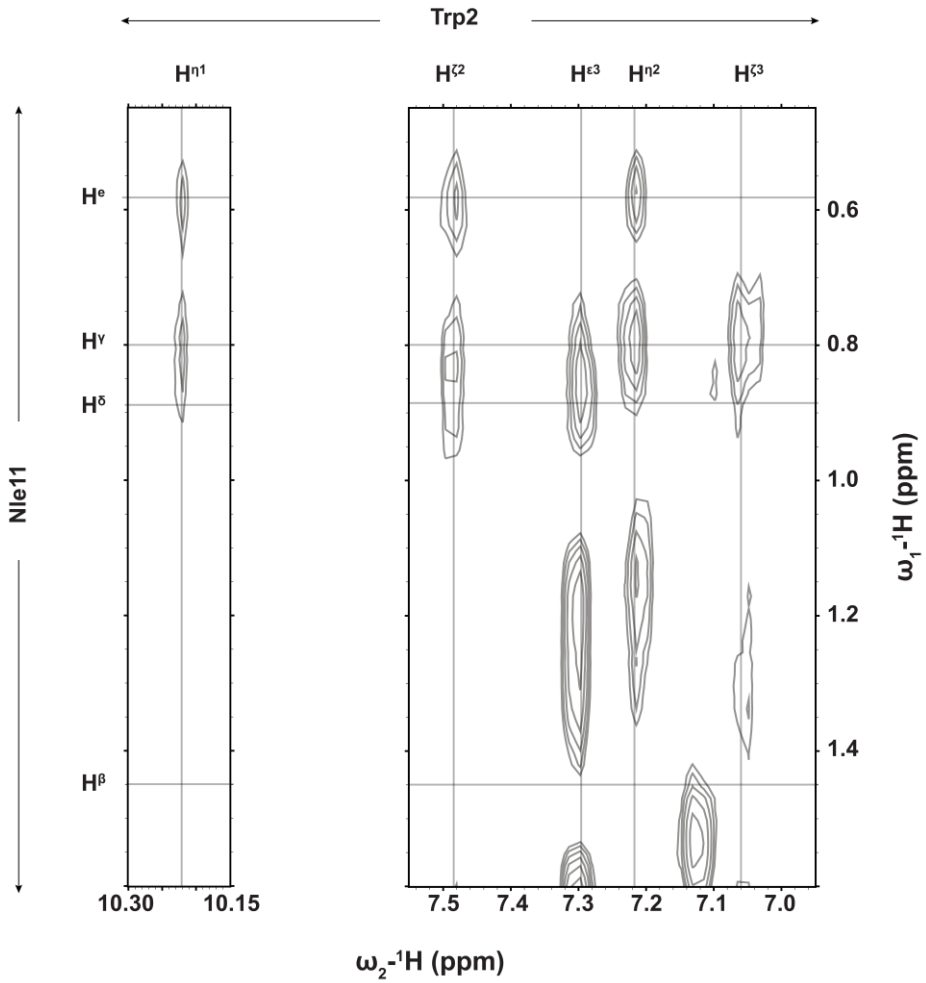
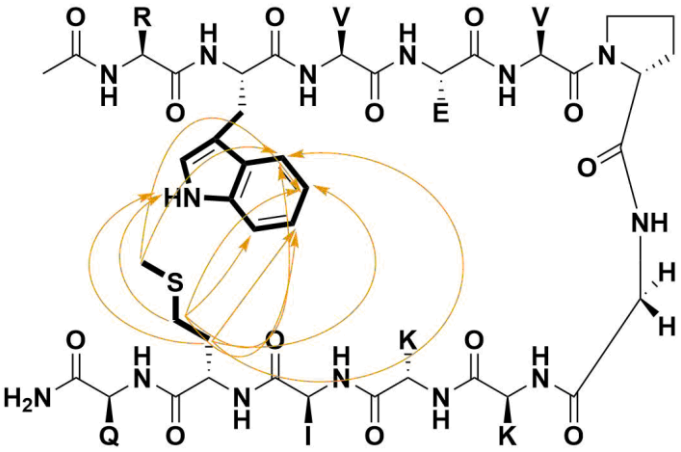


Figure S22: Observed NOEs between the indole side chain of Trp2 and Nle11.

Peptide 7: Ac-RWVEV-pG-KKIMQ-NH$_2$

A



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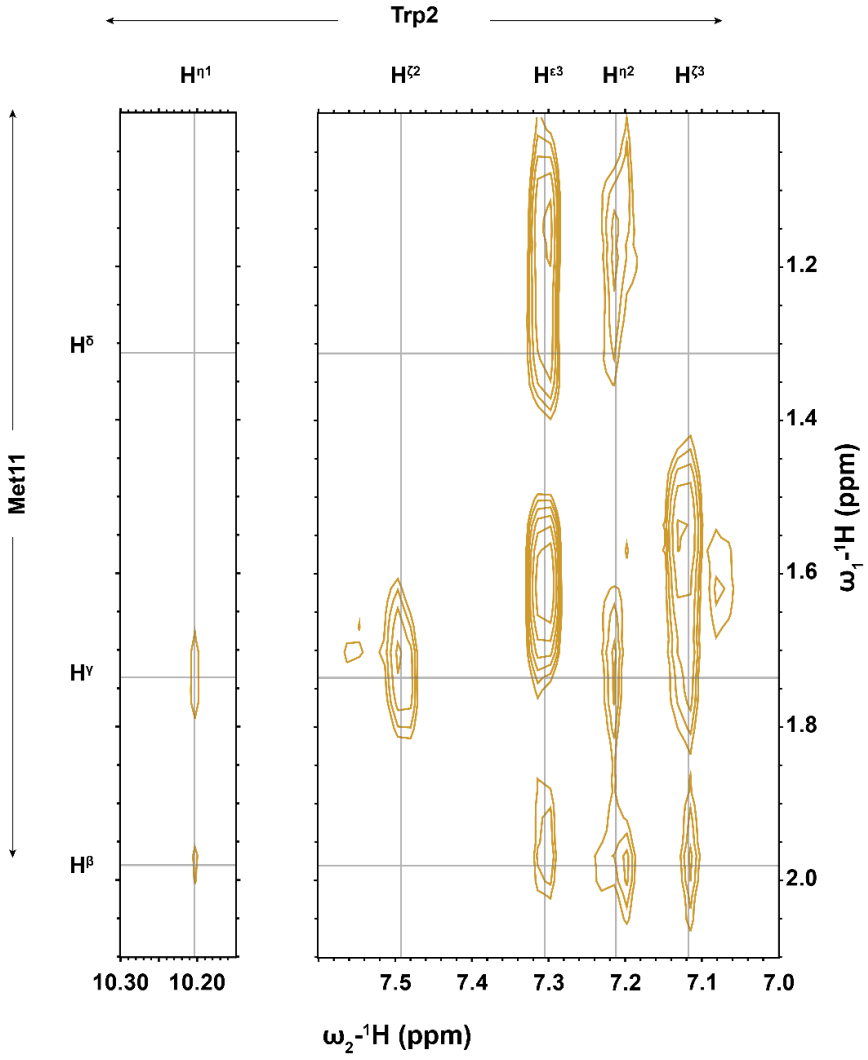
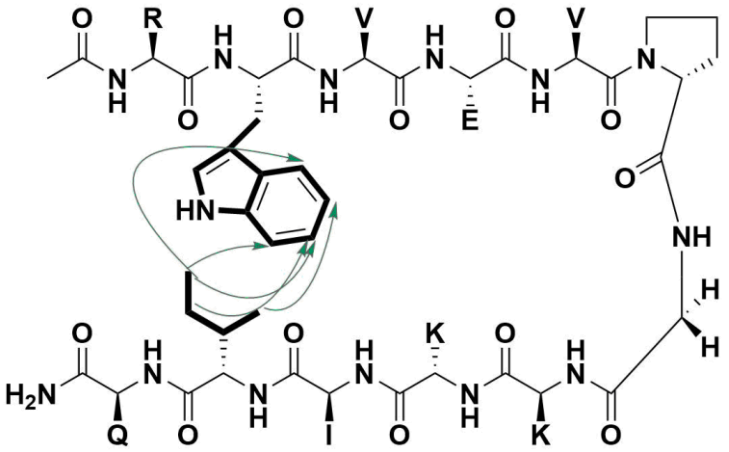


Figure S23: Observed NOEs between the indole side chain of Trp2 and Met11.

Peptide 8: Ac-RWVEV-pG-KKIIQ-NH$_2$

A



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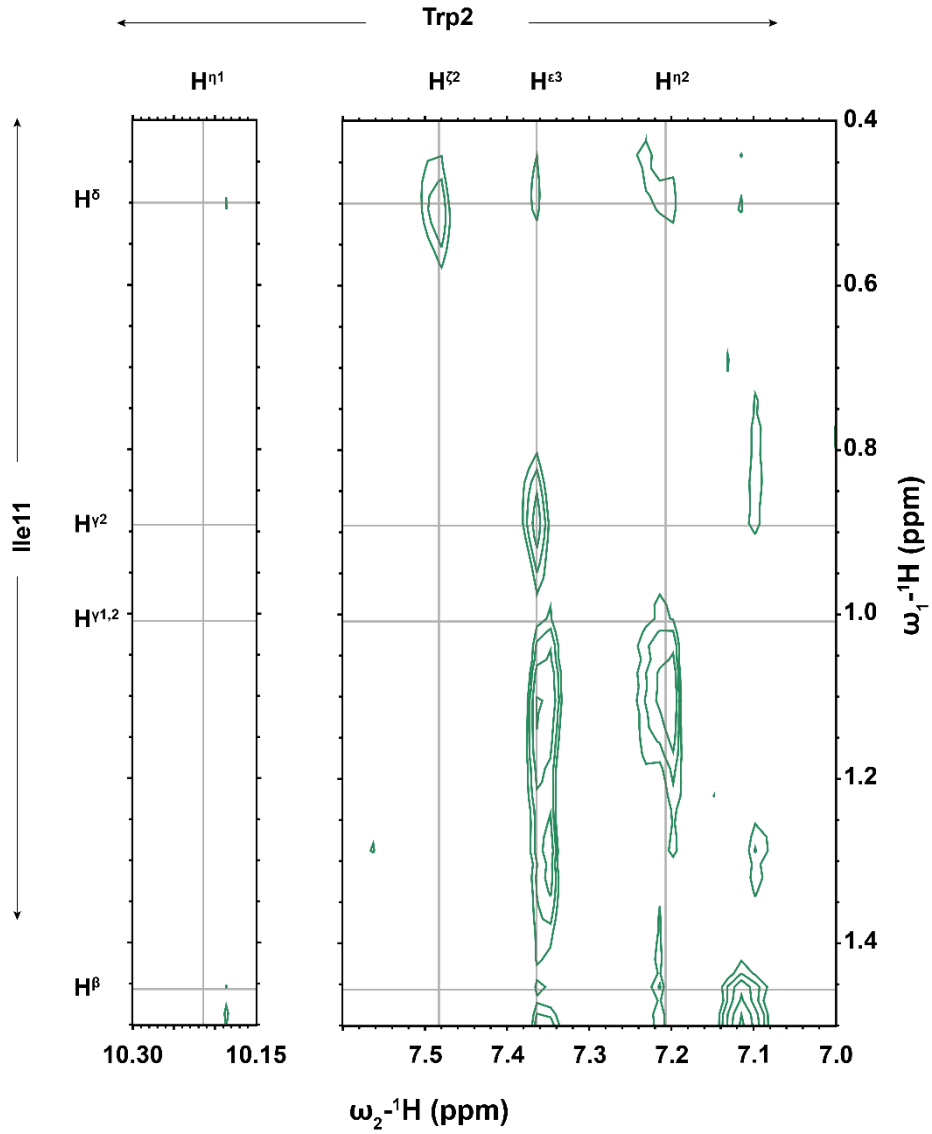
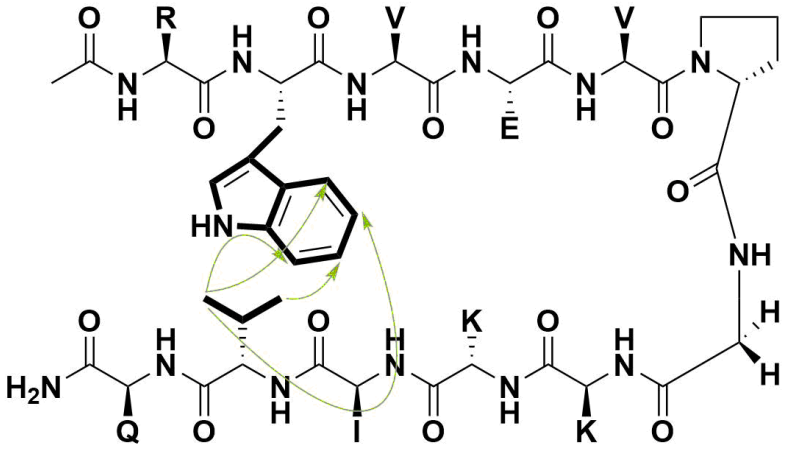


Figure S24: Observed NOEs between the indole side chain of Trp2 and Ile11.

Peptide 9: Ac-RWVEV-pG-KKIVQ-NH$_2$

A



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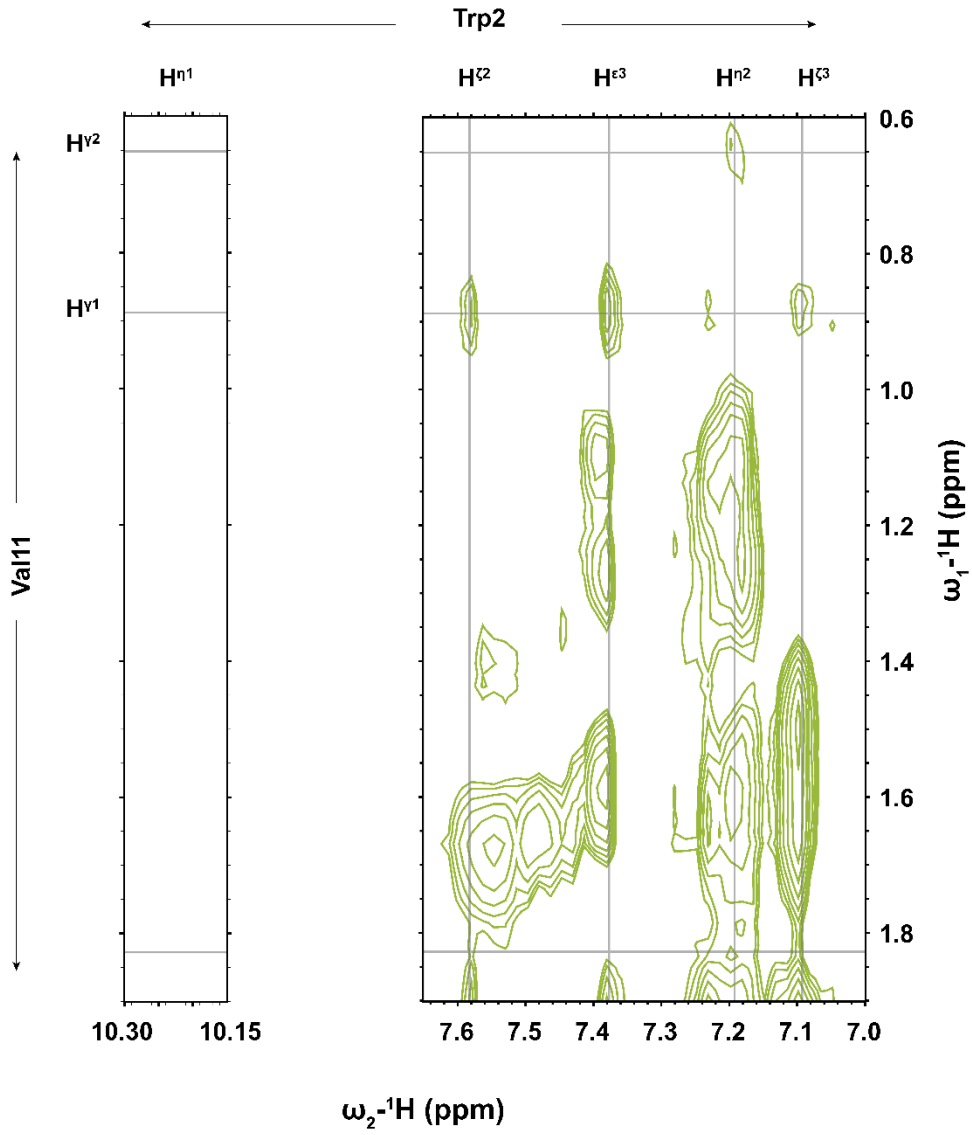
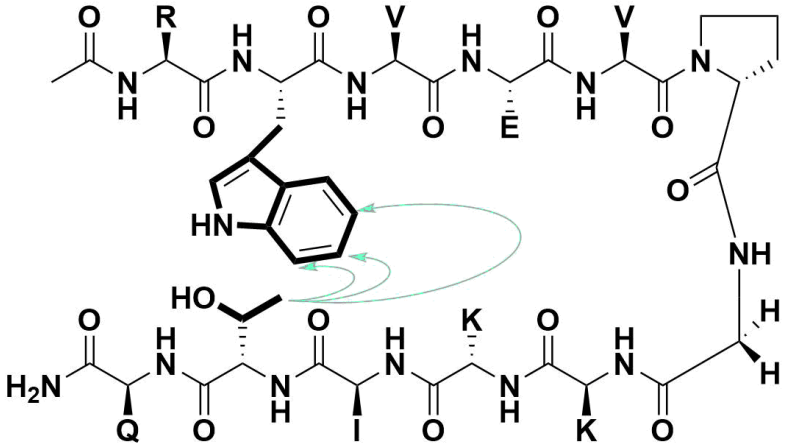


Figure S25: Observed NOEs between the indole side chain of Trp2 and Val11.

Peptide 10: Ac-RWVEV-pG-KKITQ-NH$_2$

A



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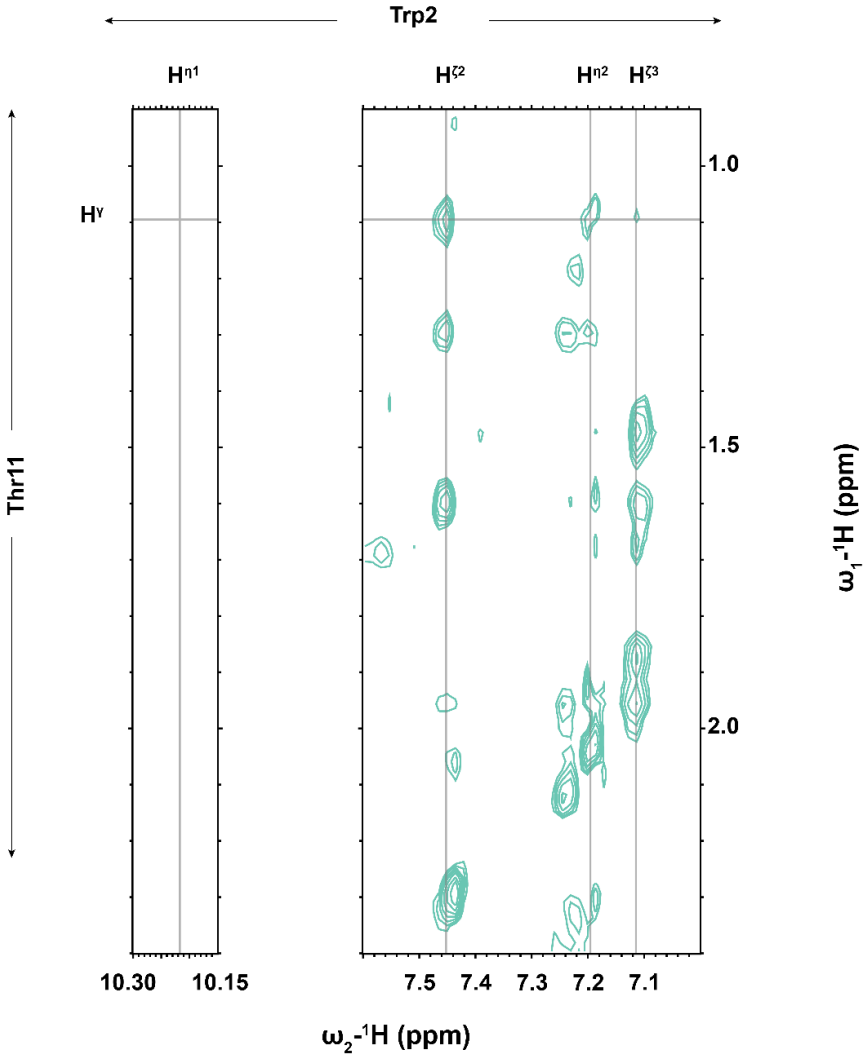


Figure S26: Observed NOEs between the indole side chain of Trp2 and Thr11.

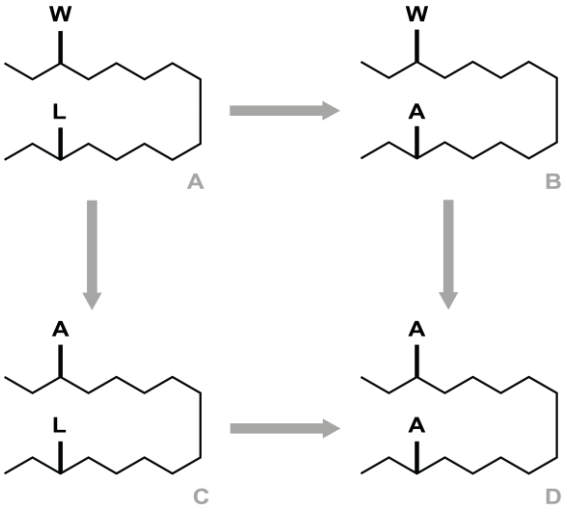
Table S12: Chemical shifts of the peptides of double mutant cycles, their cyclic and random coil (unfolded) control.

	A*	B	C	D	E*	F
Xaa2	Trp	Trp	Ala	Ala	Trp	Ala
Yaa11	Leu	Ala	Leu	Ala	Nva	Nva

*The chemical shifts of A, E and their controls are given in Table S4 and S6 under Peptide **3** and **5.**

Residues		**Compounds**											
		B cyc	**B**	**B_cont**	**C cyc**	**C**	**C cont**	**D cyc**	**D**	**D cont**	**F cyc**	**F**	**F cont**
N-term Cys	HN	8.47	-	-	8.46	-	-	8.46	-	-	8.47	-	-
	HA	5.09	-	-	5.10	-	-	5.10	-	-	5.09	-	-
Arg1	HN	8.37	8.03	8.16	8.44	8.26	8.25	8.46	8.26	8.26	8.65	8.27	8.27
	HA	4.53	4.24	4.14	4.73	4.29	4.27	4.71	4.28	4.27	4.55	4.29	4.27
Xaa2	HN	8.60	8.09	8.15	8.64	8.41	8.39	8.55	8.39	8.42	8.59	8.40	8.38
	HA	5.21	5.06	4.71	4.98	4.52	4.32	4.58	4.42	4.33	4.94	4.40	4.30
Val3	HN	9.31	8.78	7.78	9.05	8.36	8.15	8.56	8.24	8.15	8.84	8.30	8.16
	HA	4.54	4.33	3.98	4.47	4.20	4.08	4.35	4.14	4.07	4.40	4.17	4.07
Glu4	HN	8.59	8.50	8.28	8.51	8.41	8.35	8.55	8.38	8.37	8.53	8.42	8.41
	HA	5.08	4.96	4.24	4.99	4.71	4.30	4.46	4.66	4.28	4.97	8.38	4.31
Val5	HN	9.02	8.78	8.22	8.89	8.63	8.36	8.90	8.55	8.35	8.97	8.60	8.38
	HA	4.62	4.56	4.24	4.61	4.52	4.42	4.59	4.50	4.42	4.60	4.52	4.42
D-Pro6/L-	HN	-	-	-	-	-	-	-	-	-	-	-	-
Pro6	HA	4.35	4.31	4.28	4.32	4.30	4.26	4.35	4.25	4.27	4.36	4.36	4.29
Gly7	HN	8.64	8.49	8.49	8.62	8.44	8.49	8.57	8.41	8.49	8.64	8.45	8.51
	HA	3.78/4.02	3.79/3.96	3.93	3.77/4.01	3.82/3.97	3.93	3.79/4.00	3.81/3.95	3.93	3.77/4.02	3.82/3.96	3.94
Lys8	HN	7.89	7.91	8.42	7.88	7.96	8.12	7.87	7.96	8.12	7.88	7.98	8.13
	HA	4.61	4.50	4.32	4.60	4.45	4.30	4.57	4.42	4.30	4.60	4.44	4.30
Lys9	HN	8.41	8.36	8.11	8.68	8.41	8.25	8.67	8.45	8.42	8.44	8.44	8.40
	HA	4.91	4.60	4.32	4.55	4.43	4.27	4.53	4.40	4.34	4.69	4.42	4.31
Ile10	HN	9.29	8.90	8.25	9.06	8.49	8.27	8.87	8.34	8.26	9.02	8.44	8.30
	HA	4.64	4.42	4.13	4.62	4.23	4.13	4.41	4.19	4.13	4.46	4.21	4.14
Yaa11	HN	8.48	8.54	8.44	8.42	8.45	8.42	8.64	8.47	8.45	8.49	8.45	8.37
	HA	4.10	4.24	4.30	4.70	4.46	4.36	4.55	4.35	4.33	4.67	4.43	4.37
Gln12	HN	8.94	8.23	8.35	8.68	8.45	8.45	8.73	8.38	8.44	8.98	8.39	8.47
	HA	4.50	4.26	4.24	4.55	4.33	4.40	4.53	4.29	4.40	4.63	4.29	4.39
C-term Cys	HN	8.99	-	-	8.98	-	-	8.94	-	-	8.94	-	-
	HA	5.03	-	-	5.04	-	-	5.04	-	-	5.01	-	-

Double mutant cycle analysis to calculate pairwise interaction energy between Trp-Leu pair in Peptide 3:



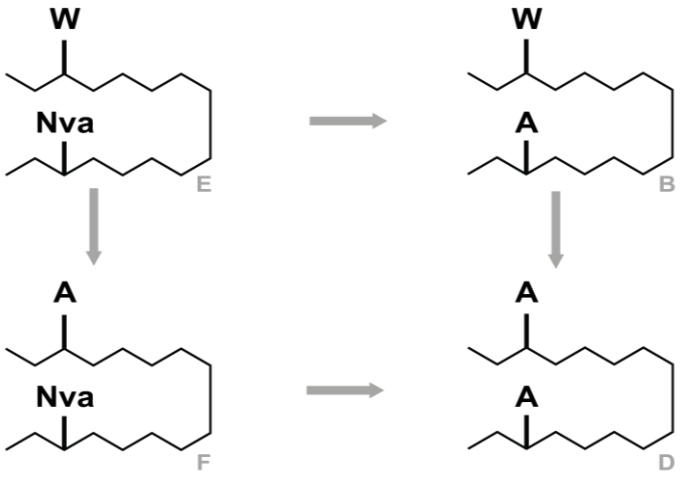
$$\Delta\Delta G_{dmut} = (\Delta G_A + \Delta G_D) - (\Delta G_B + \Delta G_C)$$

Figure S27: Schematic representation of the double mutant cycle which varies W and L individually and jointly. The pairwise interaction between W and L is calculated by the subtracting the stabilities of "B" and "C" from that of "A" and "D".

Table S13: Folding free energies of the peptides involved in double mutant cycle analysis.

	Sequence	ΔG_f (kcal/mol)	$\Delta\Delta G_{dmut}$ (kcal/mol)
A	Ac-^{1}R**W**VEV--pG--KKI**L**Q^{12}-NH_2	-1.68 ± 0.8	-1.14
B	Ac-^{1}R**W**VEV--pG--KKI**A**Q^{12}-NH_2	-0.44 ± 0.2	
C	Ac-^{1}R**A**VEV--pG--KKI**L**Q^{12}-NH_2	0.30 ± 0.2	
D	Ac-^{1}R**A**VEV--pG--KKI**A**Q^{12}-NH_2	0.40 ± 0.2	

Double mutant cycle analysis to calculate pairwise interaction energy between Trp-Nva pair in Peptide 5:



$$\Delta\Delta G_{dmut} = (\Delta G_E + \Delta G_D) - (\Delta G_B + \Delta G_F)$$

Figure S28: Schematic representation of the double mutant cycle which varies W and Nva individually and jointly. The pairwise interaction between W and Nva is calculated as discussed previously.

Table S14: Folding free energies of the peptides involved in double mutant cycle analysis.

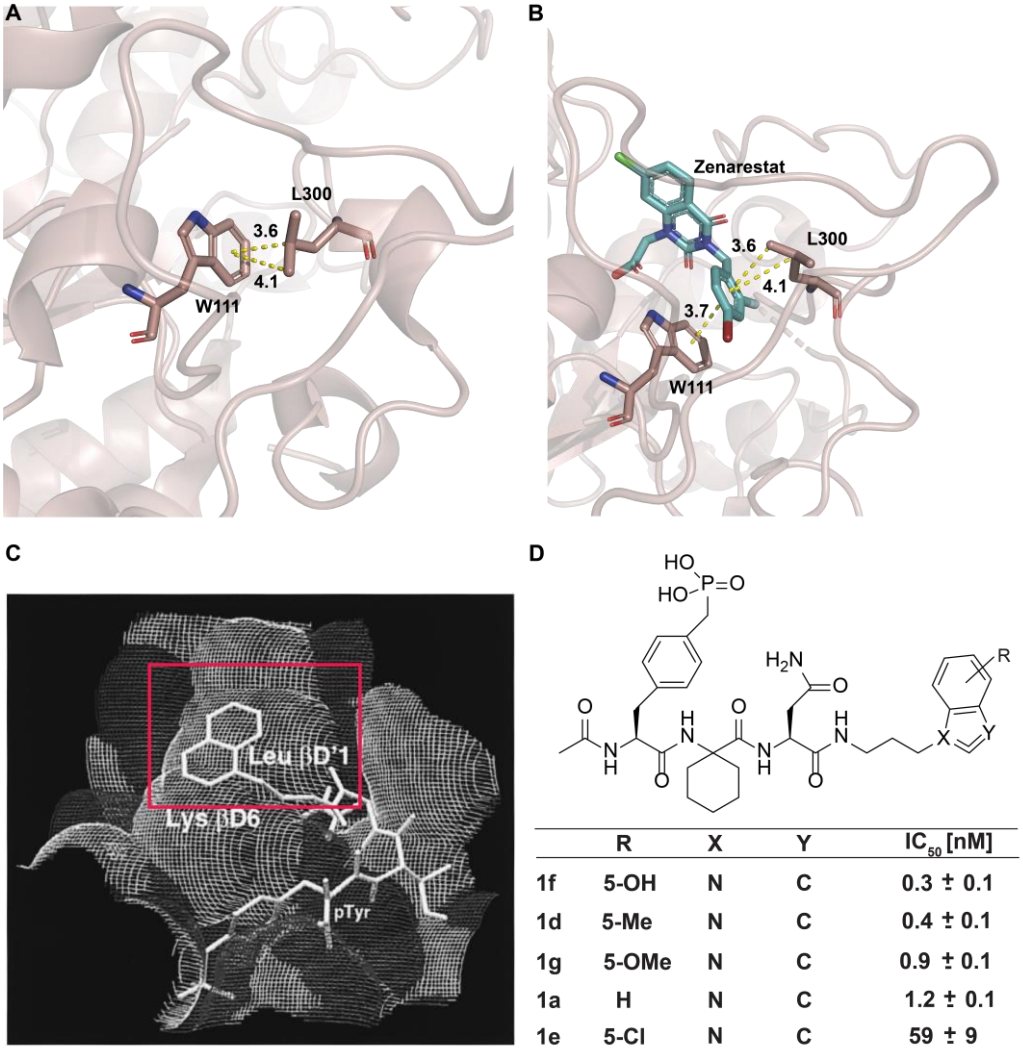
	Sequence	ΔG_f (kcal/mol)	$\Delta\Delta G_{dmut}$ (kcal/mol)
E	Ac-^{1}R**W**VEV--pG--KKI**Nva**Q^{12}-NH_2	-1.12 ± 0.3	-0.58
B	Ac-^{1}R**W**VEV--pG--KKI**A**Q^{12}-NH_2	-0.44 ± 0.2	
F	Ac-^{1}R**A**VEV--pG--KKI**Nva**Q^{12}-NH_2	0.30 ± 0.2	
D	Ac-^{1}R**A**VEV--pG--KKI**A**Q^{12}-NH_2	0.40 ± 0.2	

Table S15: Fraction folded and folding free energies of all the peptides.

Peptides	Fraction folded (%)				% folded population	ΔG_f (kcal/mol)
	Val3	Val5	Lys8	Ile10		
1	81	87	83	78	82 ± 2	-0.90 ± 0.1
2	79	90	78	70	79 ± 4	-0.83 ± 0.2
3 (A)	91	100	94	85	93 ± 3	-1.68 ± 0.8
4	40	20	37	43	35 ± 5	0.39 ± 0.1
5 (E)	84	95	82	81	85 ± 3	-1.12 ± 0.3
6	95	88	79	68	83 ± 6	-1.02 ± 0.3
7	87	92	77	72	82 ± 4	-0.95 ± 0.2
8	77	80	73	61	73 ± 4	-0.59 ± 0.1
9	69	67	59	54	62 ± 3	-0.31 ± 0.1
10	84	NA	62	44	63 ± 11	-0.37 ± 0.3
B	63	84	62	57	66 ± 6	-0.44 ± 0.2
C	31	53	50	20	38 ± 5	0.30 ± 0.2
D	25	47	44	21	34 ± 6	0.40 ± 0.2
F	30	56	47	22	39 ± 8	0.30 ± 0.2

Potential of Trp-Leu pairing in protein-protein interactions and ligand designing:

To demonstrate whether this weak interaction could be harnessed to design inhibitors of enzymes and protein-protein interaction, we came across the study by Kinoshita et al., where they report the CH-π interaction between Leu300 and Trp111 at the active site of the human aldose reductase holoenzyme (Fig S29 A).[4,5] However, the binding of a potent inhibitor, zenarestat, disrupts the native CH-π interaction and the phenyl ring of zenarestat engages in a CH-π interaction with Leu300 and π-π interaction with Trp111 (Fig S29 B). In another study, Schoepfer et al. designed highly potent inhibitors of Grb2-SH2 domain by exploiting the CH-π interaction between indolyl moiety of the peptidic inhibitor and alkyl groups of LeuβD'1 and LysβD6 in the protein.[6] By enriching the electron density of the indolyl ring through substitution with electron donating groups, they amplified the CH-π interaction, which directly correlated with enhanced affinity of the inhibitor.[7]



	R	X	Y	IC_{50} [nM]
1f	5-OH	N	C	0.3 ± 0.1
1d	5-Me	N	C	0.4 ± 0.1
1g	5-OMe	N	C	0.9 ± 0.1
1a	H	N	C	1.2 ± 0.1
1e	5-Cl	N	C	59 ± 9

Figure S29: (A) CH-π interaction between Trp111 and Leu300 in Aldose Reductase (1ads).[5] (B) Co-crystal structure of Human Aldose Reductase with Zenarestat, displaying a CH-π-π interaction between the enzyme and its inhibitor (1iei).[4] (C) CH-π interaction between indolyl moiety of the peptidic inhibitor and alkyl groups of Leu βD'1 in Grb2-SH2 domain.[6] (D) Modulating CH-π interaction, by enriching the electron density of the indolyl ring through substitution with electron withdrawing/donating groups which directly correlated with their IC_{50}.[7]

1. T. D. Goddard, D. G. Kneller, SPARKY 3, University of California, San Francisco.
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