Thermodynamic and Structural Impact of α,α-Dialkylated Residue Incorporation in a β-Hairpin Peptide

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SUPPORTING INFORMATION

Contents

General Information. HCTU, Fmoc-protected α-amino acids, and Fmoc-protected α,α-dialkylated α-amino acids were purchased from Chem-Impex. NovaPEG Rink Amide Resin was purchased from NovaBioChem. Solvents and all other reagents were purchased from Fisher Scientific and used as received without further purification.

Peptide Synthesis. Peptides were synthesized using Fmoc solid-phase synthesis methods with a Discover Bio Manual Peptide Synthesizer on NovaPEG Rink Amide resin. Couplings were completed with a 75 second ramp to 75°C with a total run time of 5 minutes using Fmoc-protected amino acid (4 equiv relative to resin), HCTU (4 equiv), and DIEA (6 equiv) in *N*-methyl-2-pyrrolidone. Deprotections were completed with a 75 second ramp to 85°C with a total run time of 3 minutes using an excess of 20% 4-methylpiperidine in DMF. After each coupling or deprotection cycle, the resin was washed three times with DMF. Residue Asn^6 was double coupled in each peptide.

For peptides **1b**-**5b**, residue Phe^{12}, directly following the α,α-dialkylated α-amino acid at position 13, was double coupled. After this double coupling, the peptide was capped by acetylation with a solution of 8:2:1 DMF:DIEA:Ac_2O. In syntheses where capping was not employed, multiple deletion products were seen resulting from incomplete coupling of the residue following the bulky α,α-dialkylated α-residue.

Prior to cleavage, the resin was washed three times each with DMF, dichloromethane, and methanol then dried under vacuum. Peptides were cleaved from resin using a solution of TFA/H_2O/TIS (95%/2.5%/2.5%) for 3 hours. After precipitating in cold diethyl ether, the solutions were centrifuged and the pelleted solids were dissolved in 10% acetonitrile in water containing 0.1% TFA and sonicated 30-45 minutes. Peptides were purified by RP-HPLC using a Phenomenex Luna C18 column with gradients between 0.1% TFA in H_2O and 0.1% TFA in acetonitrile then lyophilized. Peptide identity and purity were determined by mass spectrometry (Voyager DE Pro MALDI-TOF, Table S1) and analytical RP-HPLC, respectively (Figures S2 and S3).

NMR Sample Preparation and Data Collection. NMR samples were prepared by dissolving 2-3 mg peptide in 750-850 μL de-gassed buffer solution (50 mM phosphate, 9:1 H_2O/D_2O, uncorrected pH 6.3) to make ~2 mM solutions. 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS, 50 mM in water) was added to a final concentration of ~0.2 mM DSS in the sample. The NMR tube headspace was purged with a stream of nitrogen prior to capping.

NMR spectra of peptides were recorded on a Bruker Avance-700 spectrometer. Chemical shifts are reported relative to DSS (0 ppm). TOCSY, NOESY, and COSY pulse programs used excitation-sculpted gradient-pulse solvent suppression. All experiments were obtained using 2048 data points in the direction dimension and 512 data points in the indirect dimension. TOCSY were acquired with a mixing time of 80 ms and NOESY were acquired with a mixing time of 200 ms. Previous work has demonstrated that these experimental parameters are appropriate for analysis of peptides with similar sizes.[S1-3]

NMR Data Analysis and Structure Determination. The Sparky software package (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco) was used to analyze 2D NMR data. Backbone chemical shift assignments were generated for peptides **5a** and **5b** and were analyzed for qualitative NOE's indicative of folding (Figure 3). Peptides **5a** and **5b** were fully assigned and inter-residue NOE's were tabulated. NOE integration values were converted to distance restraints using the formula $I = cr^{-6}$ where I is intensity, c is a constant (determined using resolved diastereotopic CH_2 groups) and r is distance. [S4] The distances were then sorted and classified as strong (≤2.7 Å), medium (≤3.5 Å), weak (≤4.5 Å), or very weak (≤5.5 Å) to generate distance restraints (Tables S5 and S6).

The Crystallography and NMR system (CNS) software package was used to generate 3D structures by simulated annealing. Patches were written containing geometric restraints for diethylglycine. Distance restraints tabulated from NOE data were used in 10 simulated annealing runs using default parameters for protein NMR (Figure S4). No structures included any NOE distance-restraint violations (>0.5 Å) and the minimum energy average of these 10 structures was inspected to identify H-bonding contacts. These contacts were then included in an additional restraint file and the annealing process repeated to generate an ensemble of 10 low energy structures (Figure S5) and a minimized average structure (Figure 4) for peptides **5a** and **5b**.

References

(S1) Lengyel, G. A.; Frank, R. C.; Horne, W. S. *J. Am. Chem. Soc.* **2011**, *133*, 4246.
(S2) Lengyel, G. A.; Horne, W. S. *J. Am. Chem. Soc.* **2012**, *134*, 15906.
(S3) Lengyel, G. A.; Eddinger, G. A.; Horne, W. S. *Organic Letters* **2013**, *15*, 944.
(S4) Wüthrich, K. *NMR in structural biology : a collection of papers by Kurt Wüthrich.*; World Scientific: River Edge, 1995.

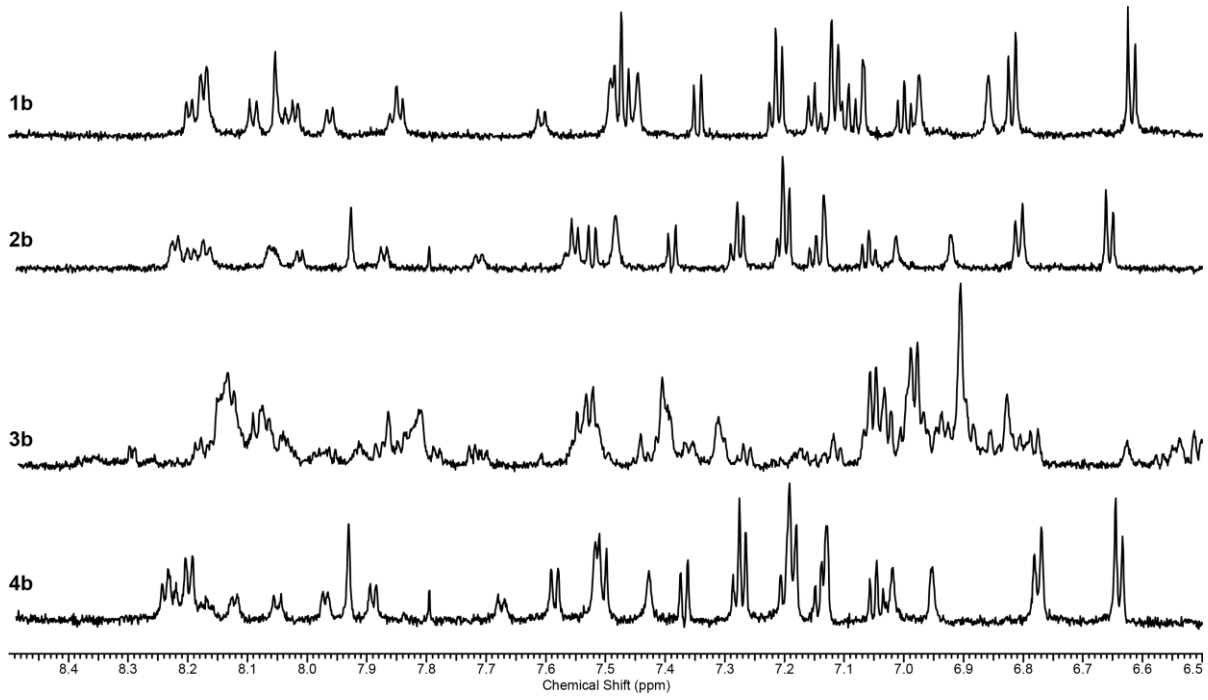

Figure S1. Amide and aromatic region from the ^{1}H-NMR spectra of peptides **1b**-**4b** at 298 K. Sharp resonances support the absence of aggregation.

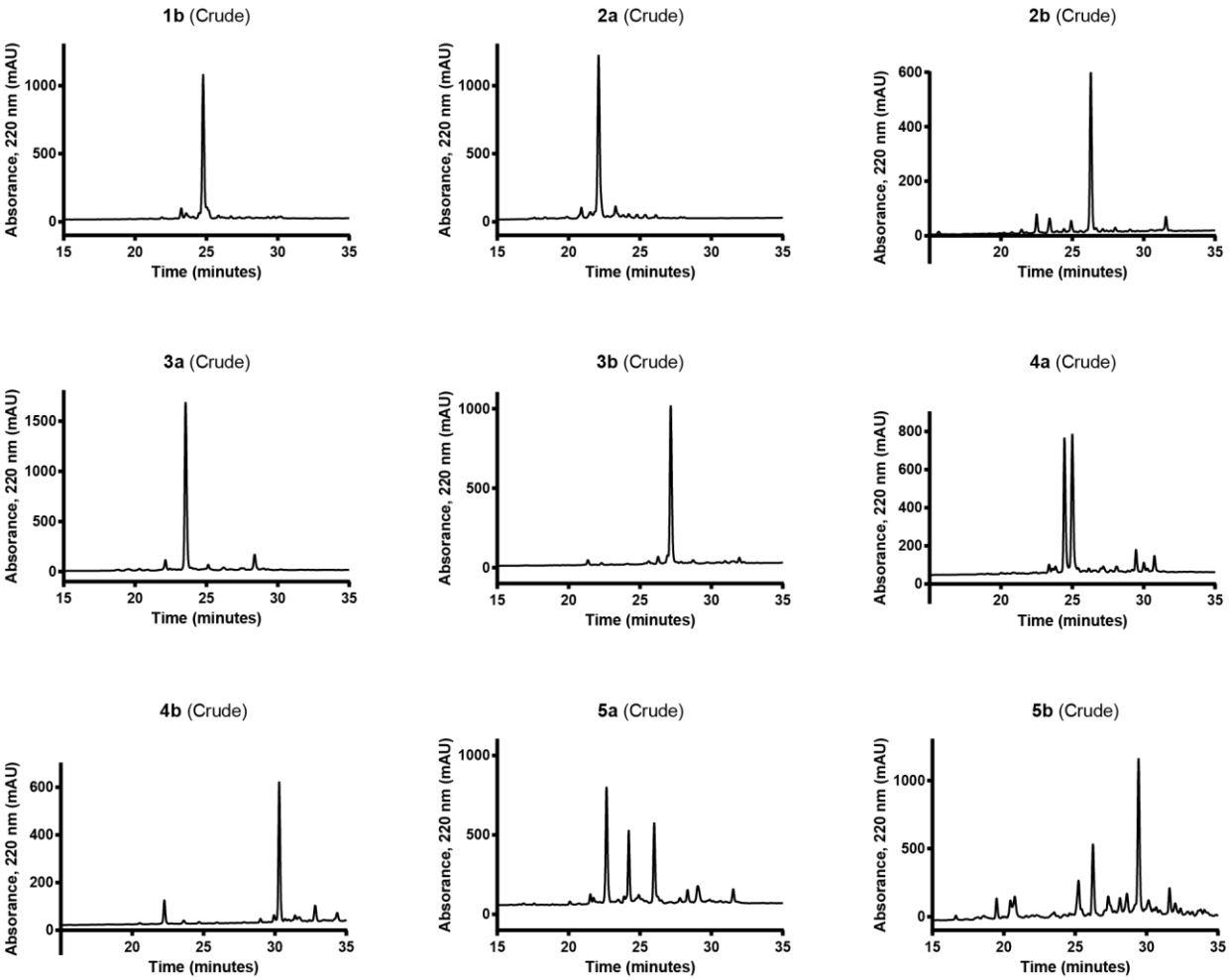

Figure S2. Crude analytical HPLC traces for peptides **2a**-**5a** and **1b**-**5b**.

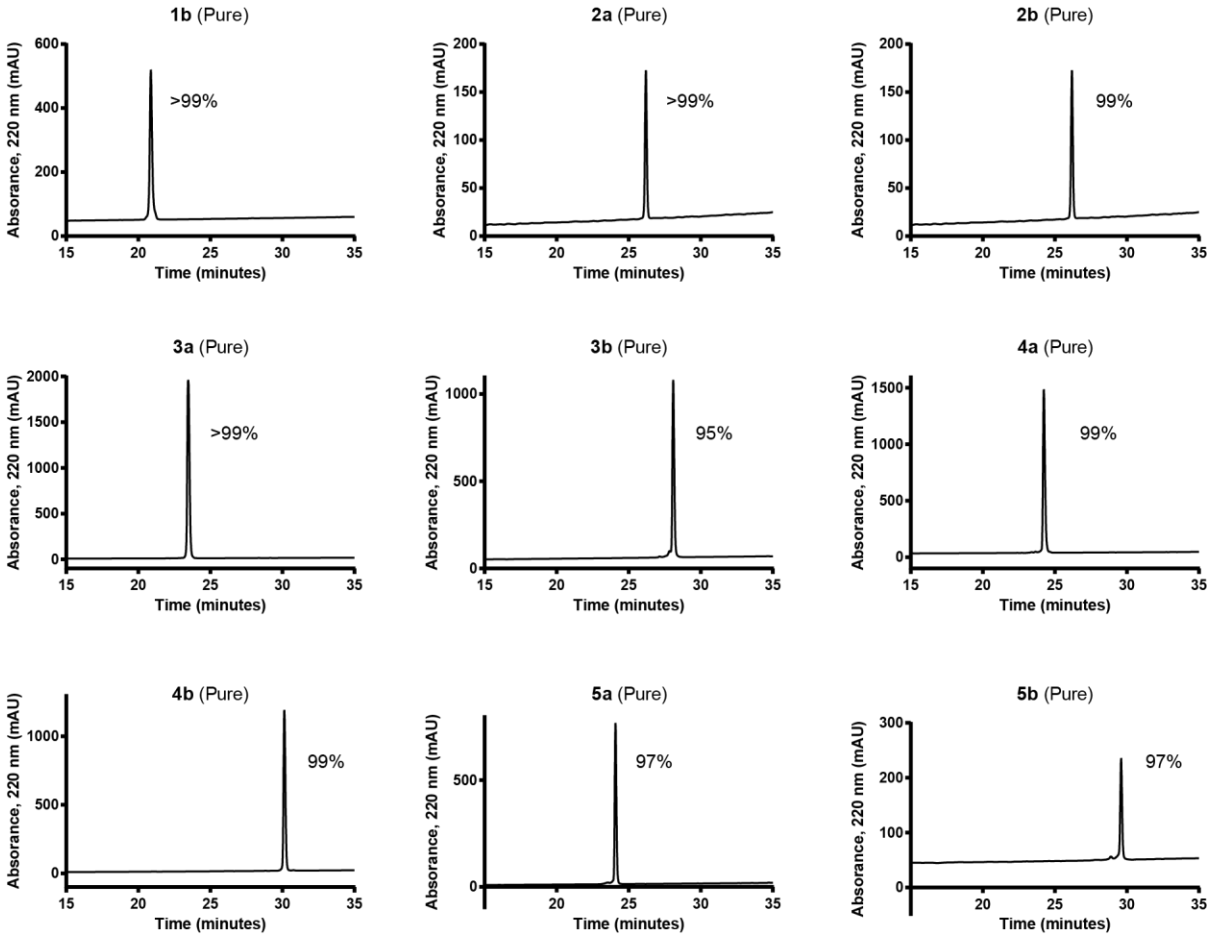

Figure S3. Pure analytical HPLC traces for peptides **2a**-**5a** and **1b**-**5b**. Purity measured by integration is included next to each peak.

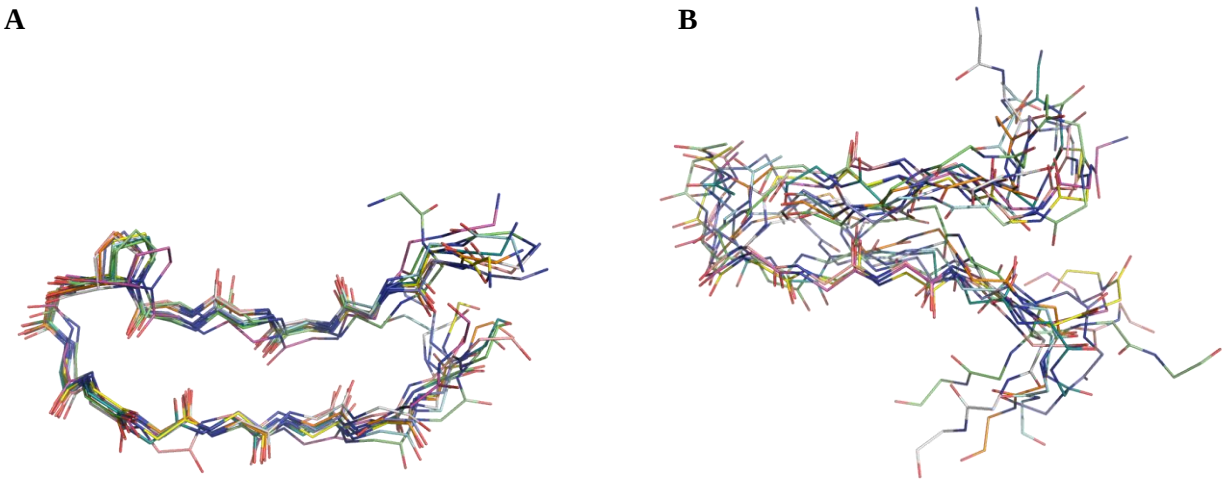

Figure S4. (**A**) Ensemble of lowest 10 energy structures of peptide **5a** from simulated annealing with NOE restraints alone. Side chains removed for clarity. (**B**) Ensemble of 10 lowest energy structures of peptide **5b** from simulated annealing with NOE restraints alone. Side chains removed for clarity.

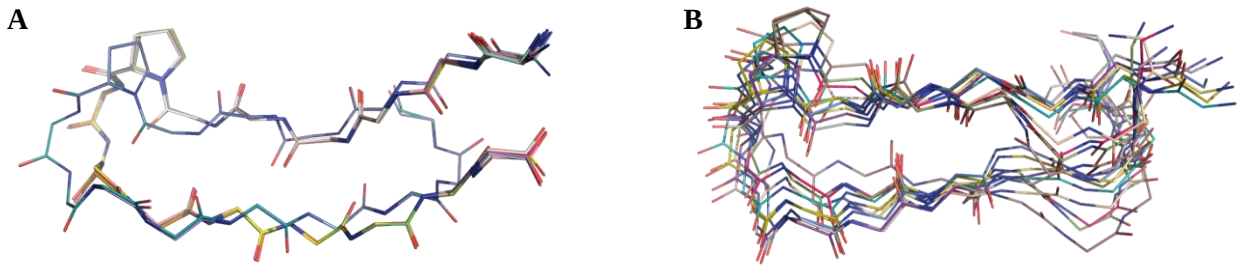

Figure S5. (**A**) Ensemble of the 10 lowest energy structures of peptide **5a** from simulated annealing with NOE and hydrogen-bond restraints. Side chains removed for clarity. (**B**) Ensemble of the 11 lowest energy structures of peptide **5b** from simulated annealing with NOE and hydrogen-bond restraints. Side chains removed for clarity.

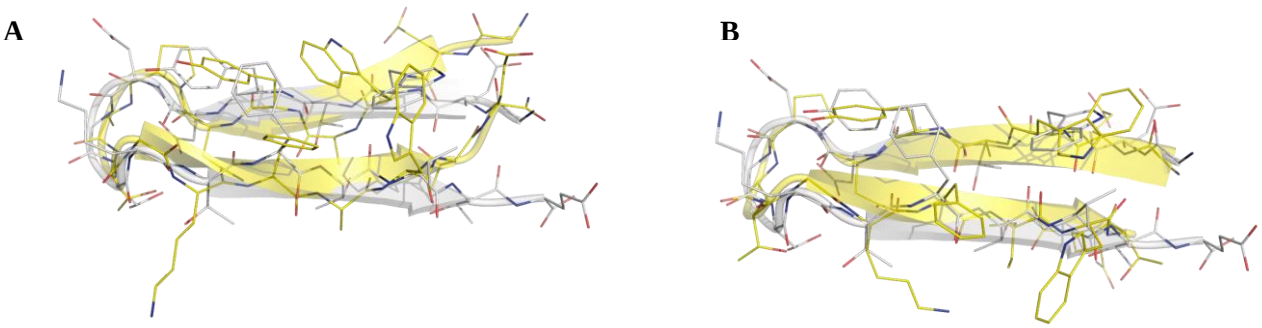

Figure S6. (**A**) Overlay of average structure of peptide **5a** (yellow) and C-terminal hairpin of protein GB1 (white). (**B**) Overlay of average structure of peptide **5b** (yellow) and C-terminal hairpin of protein GB1 (white).

Table S1. MALDI-MS Data for Peptides **2a**-**5a** and **1b**-**5b**.

Peptide	Calculated *m/z* $(M+H)^+$	Observed *m/z* $(M+H)^+$
1b	1753.9	1753.6
2a	1753.9	1753.8
2b	1781.8	1781.9
3a	1767.9	1768.0
3b	1809.9	1809.8
4a	1781.9	1782.0
4b	1837.9	1837.6
5a	1826.9	1827.3
5b	1868.9	1869.3

Table S2. Gly_{10} Chemical Shift Assignments for Peptides **1a**-**4a** and **1b**-**4b**.

Peptide	Gly_{10} Chemical Shifts (ppm)			Temperature (K)
	N_H	$H_{\alpha 1}$	$H_{\alpha 2}$	
2a	8.377	3.801	4.002	278
3a	8.377	3.808	4.016	278
4a	8.372	3.805	4.014	278
1b	8.281	3.925	3.951	298
2b	8.250	3.863	3.943	298
3b	8.396	3.869	3.947	298
4b	8.243	3.858	3.951	298

Table S3. Backbone Chemical Shift Assignments for Peptide **5a**.

Residue	Atom	Chemical Shift (ppm)
G1	$H_{\alpha1}$	3.388
G1	$H_{\alpha2}$	3.684
E2	H	7.816
E2	H_{α}	4.457
W3	H	8.751
W3	H_{α}	4.861
A4	H	8.875
A4	H_{α}	4.765
Y5	H	8.777
Y5	H_{α}	3.646
N6	H	7.719
N6	H_{α}	4.958
P7	H_{α}	3.978
A8	H	7.841
A8	H_{α}	4.217
T9	H	7.063
T9	H_{α}	4.398
G10	H	8.32
G10	$H_{\alpha1}$	3.721
G10	$H_{\alpha2}$	4.031
K11	H	7.344
K11	H_{α}	4.705
F12	H	8.646
F12	H_{α}	4.815
A13	H	8.736
A13	H_{α}	4.688
W14	H	8.663
W14	H_{α}	4.625
T15	H	8.362
T15	H_{α}	4.398
E16	H	8.339
E16	H_{α}	4.083

Table S4. Backbone Chemical Shift Assignments for Peptide **5b**.

Residue	Atom	Chemical Shift (ppm)
G1	$H_{\alpha1}$	*
G1	$H_{\alpha2}$	*
E2	H	*
E2	H_{α}	4.382
W3	H	8.369
W3	H_{α}	4.665
A4	H	8.274
A4	H_{α}	4.357
Y5	H	8.006
Y5	H_{α}	4.238
N6	H	8.035
N6	H_{α}	4.834
P7	H_{α}	4.135
A8	H	7.989
A8	H_{α}	4.24
T9	H	7.41
T9	H_{α}	4.296
G10	H	8.24
G10	$H_{\alpha1}$	3.748
G10	$H_{\alpha2}$	3.899
K11	H	7.535
K11	H_{α}	4.509
F12	H	8.217
F12	H_{α}	4.328
X13	H	8.164
W14	H	7.489
W14	H_{α}	4.808
T15	H	8.03
T15	H_{α}	4.284
E16	H	8.037
E16	H_{α}	*

*Not included due to ambiguous assignment.

Table S5. NOE Distance Restraints for Peptide **5a**.

Residue		Proton	Residue		Proton	Distance
3	W	HA	4	A	H	2.70
4	A	HA	5	Y	H	2.70
5	Y	HA	6	N	H	2.70
6	N	H	5	Y	HA	2.70
6	N	HA	7	P	QD	2.70
9	T	H	8	A	H	2.70
12	F	HA	5	Y	HA	2.70
12	F	HA	13	A	H	2.70
13	A	HA	14	W	H	2.70
14	W	HA	15	T	H	2.70
1	G	HA2	2	E	H	3.50
2	E	HA	3	W	H	3.50
2	E	QG	3	W	H	3.50
3	W	H	2	E	HA	3.50
3	W	HA	14	W	HE3	3.50
3	W	HB2	12	F	QD	3.50
3	W	HE3	12	F	HB1	3.50
4	A	H	3	W	HB2	3.50
5	Y	H	4	A	QXB	3.50
5	Y	HA	12	F	QD	3.50
5	Y	HA	13	A	H	3.50
5	Y	HB2	3	W	HD1	3.50
5	Y	HB2	3	W	HH2	3.50
5	Y	QD	7	P	HA	3.50
6	N	HA	8	A	H	3.50
7	P	HA	5	Y	QD	3.50
7	P	HA	8	A	H	3.50
7	P	QD	8	A	H	3.50
8	A	H	7	P	HA	3.50
8	A	H	7	P	QD	3.50
8	A	HA	9	T	H	3.50
8	A	QXB	9	T	H	3.50
9	T	H	8	A	QXB	3.50
9	T	HB	11	K	H	3.50
10	G	H	7	P	HA	3.50
10	G	HA2	11	K	H	3.50
11	K	H	10	G	HA1	3.50

Residue		Proton	Residue		Proton	Distance
11	K	HB2	12	F	H	3.50
11	K	HG2	12	F	H	3.50
12	F	H	11	K	HG2	3.50
12	F	HA	6	N	H	3.50
12	F	HB1	3	W	HE3	3.50
12	F	HB1	13	A	H	3.50
14	W	HA	3	W	HE3	3.50
14	W	HE3	15	T	H	3.50
15	T	H	4	A	H	3.50
15	T	H	14	W	HE3	3.50
15	T	H	16	E	HA	3.50
2	E	QB	3	W	H	4.50
3	W	H	2	E	QB	4.50
3	W	HB2	4	A	H	4.50
3	W	HE3	4	A	H	4.50
4	A	H	3	W	HE3	4.50
5	Y	QD	6	N	H	4.50
5	Y	QD	7	P	QG	4.50
5	Y	QE	6	N	H	4.50
5	Y	QE	7	P	HA	4.50
5	Y	QE	7	P	QD	4.50
5	Y	QE	7	P	QG	4.50
6	N	H	5	Y	H	4.50
6	N	H	5	Y	QE	4.50
6	N	HA	7	P	QG	4.50
7	P	HA	5	Y	QE	4.50
7	P	QD	5	Y	QD	4.50
7	P	QD	5	Y	QE	4.50
7	P	QD	6	N	H	4.50
7	P	QG	5	Y	QD	4.50
7	P	QG	5	Y	QE	4.50
8	A	H	10	G	H	4.50
8	A	QXB	3	W	HH2	4.50
10	G	HA1	11	K	H	4.50
12	F	HB2	13	A	H	4.50
12	F	QE	5	Y	QE	4.50
14	W	HA	4	A	H	4.50
14	W	HB2	15	T	H	4.50
8	A	H	7	P	QG	5.50

Residue		Proton	Residue		Proton	Distance
9	T	QXGT	8	A	H	5.50
9	T	QXGT	8	A	HA	5.50
9	T	QXGT	10	G	H	5.50

Table S6. NOE Distance Restraints for Peptide **5a**.

Residue		Proton	Residue		Proton	Distance
3	W	HA	4	A	H	2.70
3	W	HA	13	X	H	2.70
5	Y	HA	6	N	H	2.70
6	N	HA	7	P	HD1	2.70
6	N	HA	7	P	HD2	2.70
13	X	HB2B	14	W	H	3.50
14	W	H	13	X	HB2B	3.50
2	E	HA	3	W	H	3.50
2	E	HB2	3	W	H	3.50
3	W	H	2	E	HA	3.50
3	W	HA	12	F	QB	3.50
4	A	H	3	W	H	3.50
4	A	HA	5	Y	H	3.50
5	Y	H	4	A	HA	3.50
7	P	HA	5	Y	QE	3.50
7	P	HA	8	A	H	3.50
7	P	HD2	8	A	H	3.50
7	P	QG	5	Y	QD	3.50
8	A	H	7	P	HA	3.50
9	T	H	11	K	H	3.50
10	G	H	9	T	H	3.50
11	K	H	6	N	HB1	3.50
11	K	H	6	N	HB2	3.50
11	K	HA	12	F	H	3.50
11	K	QD	12	F	H	3.50
13	X	H	4	A	H	3.50
13	X	H	12	F	QB	3.50
13	X	HB1A	14	W	H	3.50
13	X	HB2A	14	W	H	3.50
14	W	H	13	X	HB1A	3.50
14	W	H	13	X	HB2A	3.50
14	W	H	13	X	QQGA	3.50
14	W	H	15	T	H	3.50
14	W	HA	3	W	HE1	3.50
3	W	HB1	4	A	H	4.50
3	W	HB2	4	A	H	4.50
4	A	QXB	5	Y	H	4.50

Residue		Proton	Residue		Proton	Distance
5	Y	H	4	A	QXB	4.50
5	Y	HA	13	X	H	4.50
5	Y	QB	6	N	H	4.50
5	Y	QE	6	N	H	4.50
5	Y	QE	7	P	HA	4.50
6	N	HB1	11	K	H	4.50
6	N	HB2	9	T	H	4.50
7	P	HA	5	Y	QD	4.50
7	P	HA	10	G	H	4.50
7	P	HD1	8	A	H	4.50
7	P	HD2	6	N	H	4.50
7	P	QG	5	Y	QE	4.50
7	P	QG	8	A	H	4.50
8	A	H	9	T	H	4.50
8	A	QXB	9	T	H	4.50
9	T	H	8	A	H	4.50
9	T	H	10	G	H	4.50
9	T	HA	10	G	H	4.50
9	T	HB	10	G	H	4.50
10	G	H	9	T	HA	4.50
10	G	H	11	K	H	4.50
10	G	HA1	11	K	H	4.50
10	G	HA2	11	K	H	4.50
11	K	H	8	A	H	4.50
11	K	H	10	G	H	4.50
11	K	H	10	G	HA1	4.50
11	K	H	10	G	HA2	4.50
11	K	H	12	F	H	4.50
11	K	QB	12	F	H	4.50
11	K	QB	12	F	HA	4.50
11	K	QD	12	F	HA	4.50
12	F	HA	11	K	QB	4.50
12	F	HA	11	K	QD	4.50
12	F	QB	5	Y	QE	4.50
13	X	QQGB	14	W	H	4.50
14	W	H	13	X	H	4.50
14	W	QB	15	T	H	4.50
15	T	H	14	W	QB	4.50
4	A	QXB	13	X	H	5.50

Residue		Proton	Residue		Proton	Distance
5	Y	QD	6	N	H	5.50
6	N	HB2	11	K	H	5.50
7	P	HD1	6	N	H	5.50
7	P	QB	5	Y	QD	5.50
7	P	QB	5	Y	QE	5.50
12	F	QB	5	Y	QD	5.50
13	X	QQGA	12	F	QB	5.50
14	W	H	13	X	HB1B	5.50