CHEMISTRY
A EUROPEAN JOURNAL

Supporting Information

Effects of Chain Length and N-Methylation on a Cation-p Interaction in a b-Hairpin Peptide

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Contents:

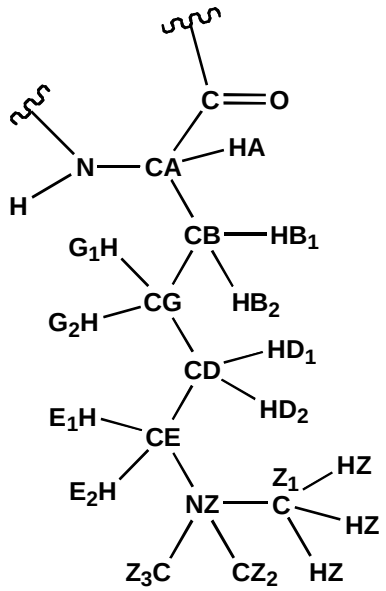
Peptide Synthesis

The synthesis of all peptides was performed on an Applied Biosystems Pioneer peptide synthesizer using Applied Biosystems PEG-PAL amide resin. Peptides were synthesized on a 0.1 or 0.07 mmole scale. All amino acids with functionality were protected during synthesis as follows: Arg(Pbf), Asn(trt), Lys(Boc), Orn(Boc), Gln(trt), and Glu(tBu). Coupling reagents were HBTU/HOBt. The N-terminus was acylated for all peptides with a solution of 5% acetic anhydride and 6% 2,6-lutidine in DMF. Cleavage conditions removed all side chain protection with a cocktail of 90% TFA/5% Triisopropylsilane/5% H_2O. Peptides were purified by RP-HPLC on a C18 column. Peptides were purified with a gradient of A and B (A: 95% H_2O/5% ACN with 0.1% TFA, B: 95% ACN/5% H_2O with 0.1% TFA). Once purified, peptides were lyophilized to powder and characterized by MALDI mass spectroscopy and NMR.

Trimethylated lysine, ornithine, and diaminobutyric acid containing peptides were synthesized following the procedure of Kretsinger and Schneider.[1] Peptides were synthesized using Fmoc-Lys(Me)$_2$-OH purchased from either Anaspec or Bachem, Fmoc-Orn(Me)2-OH, or Fmoc-Dab(Me)2-OH prepared as described. The dimethylated amino acid-containing peptides (0.100 mmol scale) were reacted prior to cleavage from the resin with MTBD (18 μL, 0.125 mmol) and methyl iodide (62 μL, 1 mmol) in DMF (5 mL) for 5 hours with bubbling N_2 in a peptide synthesis flask stoppered with a vented septum. After washing the resin with DMF (3X), CH_2Cl_2 (3X), and drying, the peptide was cleaved with a cocktail of 90% TFA/5% Triisopropylsilane/5% H_2O for 3 hours.

Additional Details for Molecular Dynamics Simulations in Amber.

Atomic charges of trimethylated lysine for use in Amber ff99 were parameterized using Merz-Kollman charge calculated with Gaussian 03 [b3lyp/cc-pvDZ SCF=Tight Test Pop=MK iop(6/33=2,6/41=10,6/42=10) Nosymm] and processed using Antechamber. Charges were averaged for equivalent groups (i.e., methyls on ammonium headgroup) and are shown below:



Atom Name	Charge
N	-0.3479
H	0.2747
C	0.7341
O	-0.5894
CA	-0.2400
HA	0.1426
CB	-0.0094
HB1,HB2	0.0362
CG	0.1501
HG1,HG2	-0.0280
CD	0.0698
HD1,HD2	0.0275
CE	-0.3796
HE1,HE2	0.1785
NZ	0.1814
CZ_{1-3}	-0.3099
HZ_{1-9}	0.1683

Figure S1. Atomic charges calculated for use with trimethylated lysine in Amber (ff99).

Pre-production runs included minimization of the solvent (TIP3P water) and counterions, minimization of the entire system, and heating of the system from 0 to 300K over 20 ps.The 2 ns MD simulation was run under constant temperature (300K; Langevin temperature) and pressure (1 atm), using the SHAKE algorithm and a timestep of 2 fs. Plots of Energy, Temperature, and Pressure from the simulation are shown below.

Figure S2. (a) Energy plots from the 2 ns simulation (Kinetic Energy: Red; Potential Energy: Black; Total Energy: Green) (b) Temperature during the simulation. (c) Pressure fluctuation during the simulation.

Quantification of Folding

To determine the chemical shifts of the fully folded state, 14-residue disulfide-linked analogs of peptides **WKL**, **WKMeL**, **WKMe2L**, **WKMe3L**, **WKT**, **WKMeT**, **WOrnL**, and **WDabL** were synthesized with a sequence of Ac-CRWVEVNGO**X**ILQC-NH, where X represents the designated cationic residue. The disulfide bond between Cys1 and Cys14 constrains the peptide to a β-hairpin. To determine the unfolded chemical shifts, 7-mers were synthesized with sequences Ac-RWVEVNG-NH2, Ac-NGO**X**ILQ-NH2, and Ac-NGO**X**ITQ-NH2, where X represents the designated cationic residue. The chemical shifts for residues in the strand and one turn residue were obtained from each 7-mer peptide. The fraction folded was determined from equation 1.

$$\text{Fraction Folded (f)} = [\delta_{obs} - \delta_0]/[\delta_{100} - \delta_0] \quad \text{(eqn 1)}$$

$$\Delta G = =-0.001987*298*\ln(f/(1-f)) \quad \text{(eqn 2)}$$

Characterization of Structure

Methods used to indicate the formation of β-hairpin structure in peptide **WK*L**, **WK*T**, **WOrn*L**, and **WDab*L** include the analysis of Hα shifting relative to random coil, backbone amide shifts relative to random coil, and the identification of cross strand NOEs. The β-hairpin should have backbone hydrogen bonded amides between cross-strand residue pairs Arg-Gln,

Val-Ile, and Val-Orn in all hairpin peptides. The presence of these hydrogen bonds is readily demonstrated by downfield shifting of the amide hydrogens in these positions relative to random coil. As seen below, the selected peptides exhibit significant downfield shifting at key positions along the strand. As expected for β-hairpins, the termini are frayed and show little or no amide shifting. The Asn amide shows significant downfield shifting as expected for a Type I' turn.

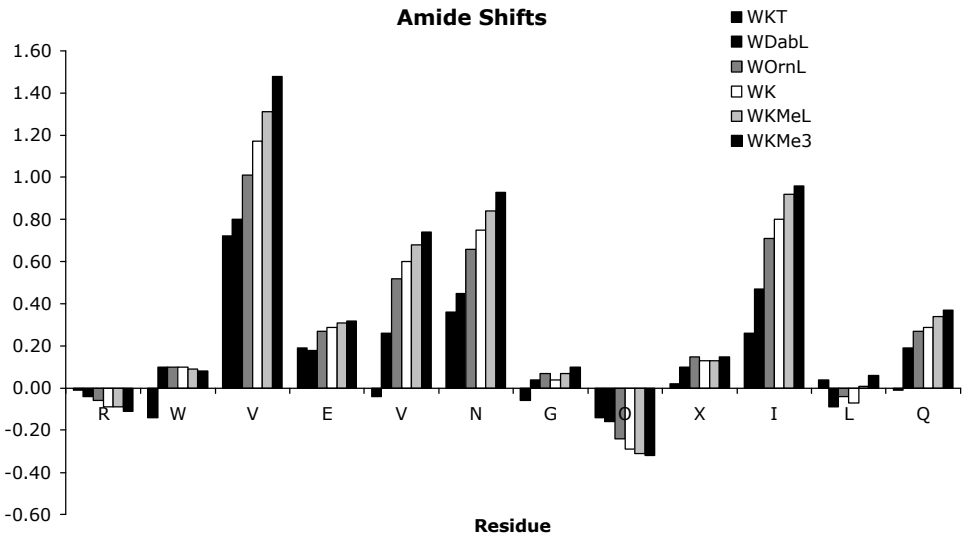


Figure S3. Backbone amide chemical shifts for selected peptides.

Furthermore, analysis of the H_α fraction folded plot for selected peptides **WKL**, **WKMeL**, **WKMe3L**, **WOrnL**, **WDabL**, and **WKT**, as calculated by equation **1,** shows cooperative folding along the entire strand, with the exception of fraying at the termini.

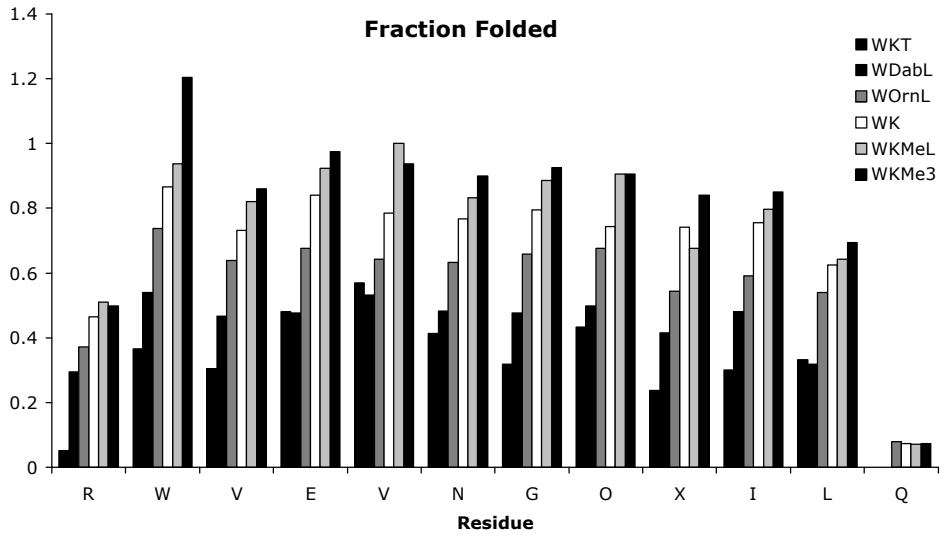


Figure S4. Fraction folded values for selected peptides.

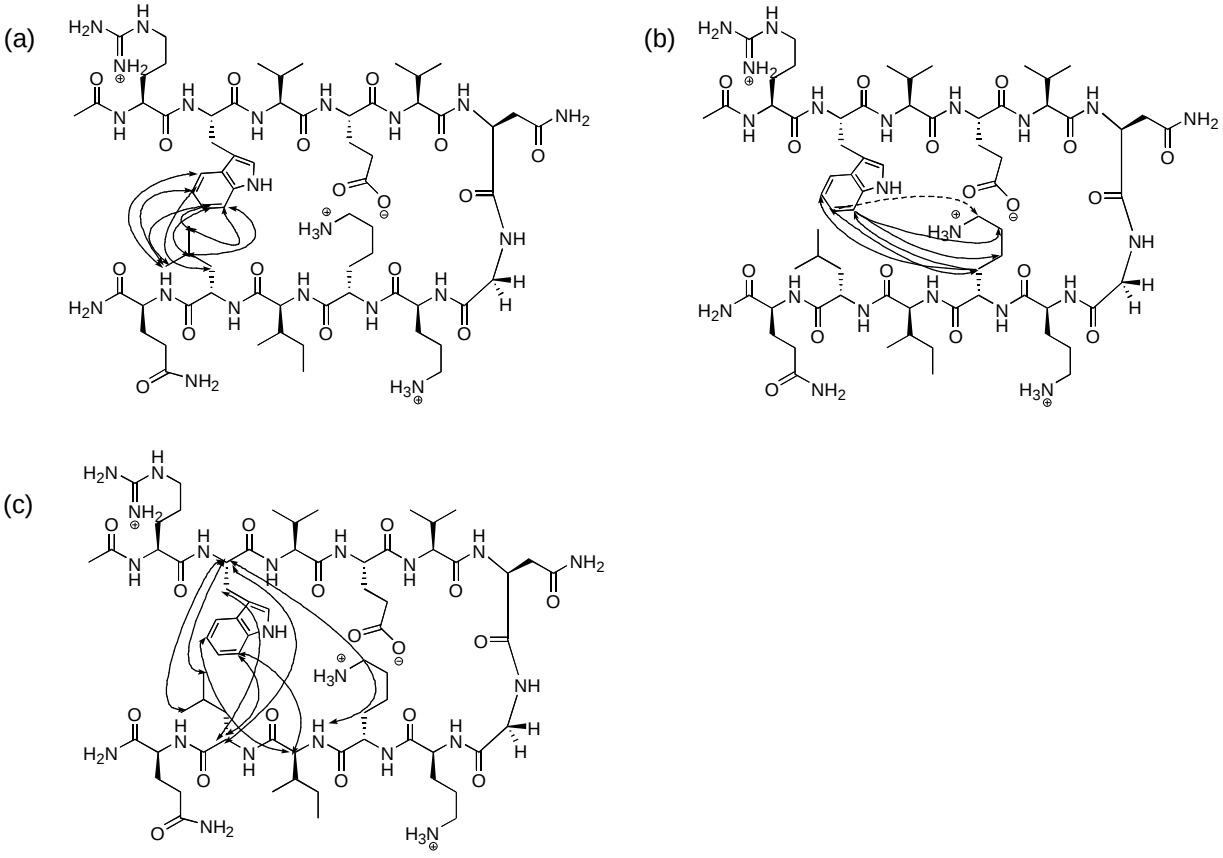


Figure S5. WKL Cross-strand and Diagonal NOEs.

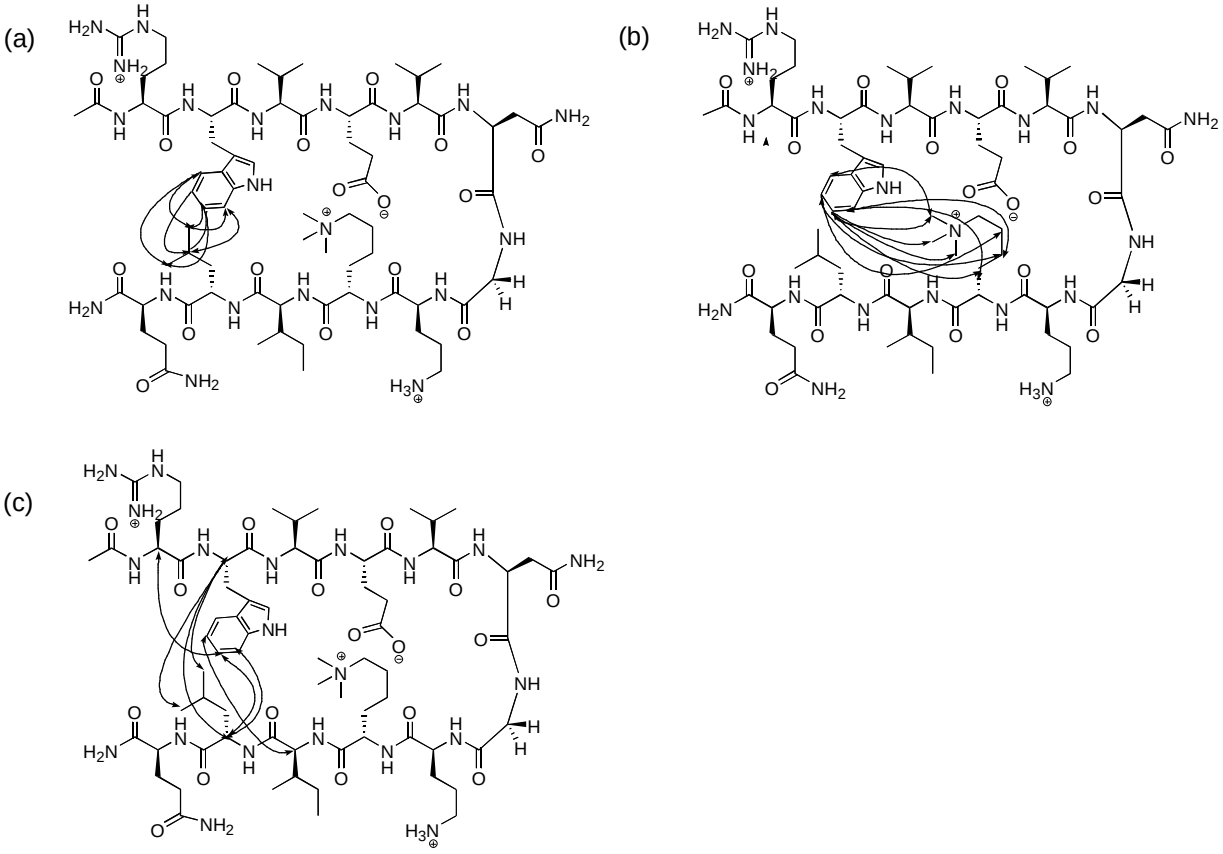


Figure S6. WKMe3L Cross-strand and Diagonal NOEs.

(a)

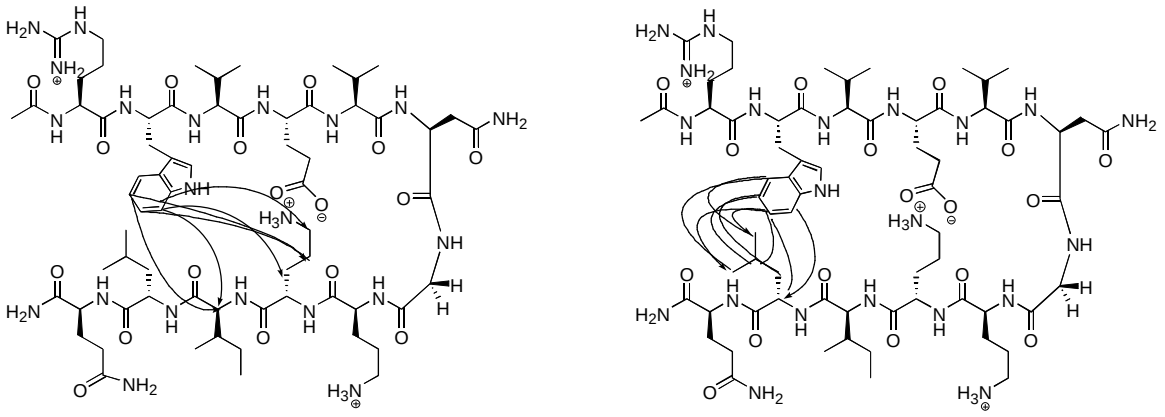
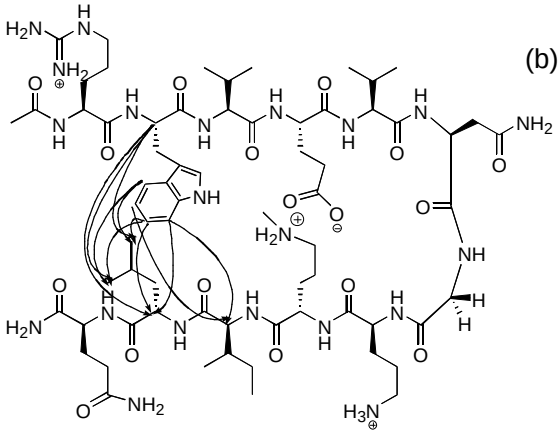


Figure S7. WOrnL Cross-strand and Diagonal NOEs.

(a)



(b)

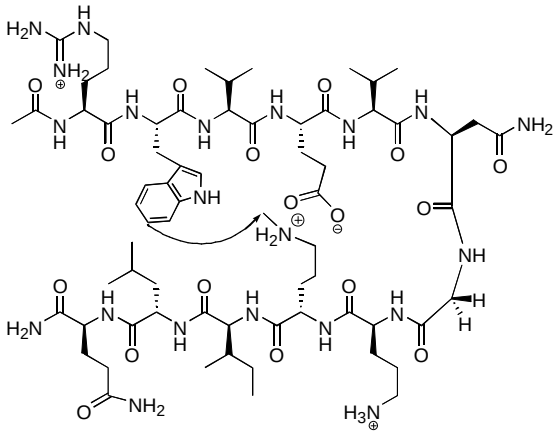


Figure S8. WOrnMeL Cross-strand and Diagonal NOEs.

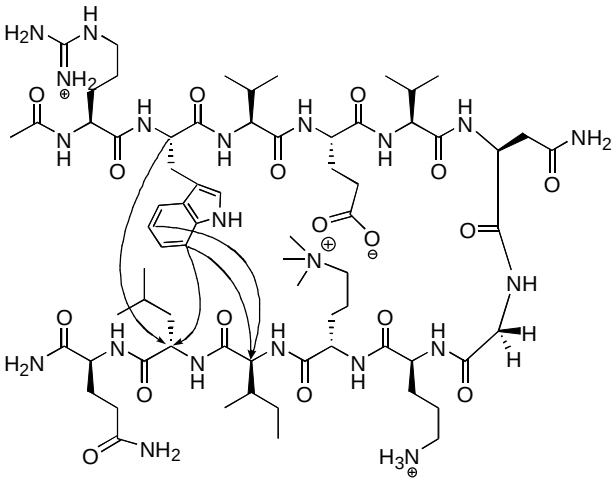


Figure S9. WOrnMe3L Cross-strand NOEs.

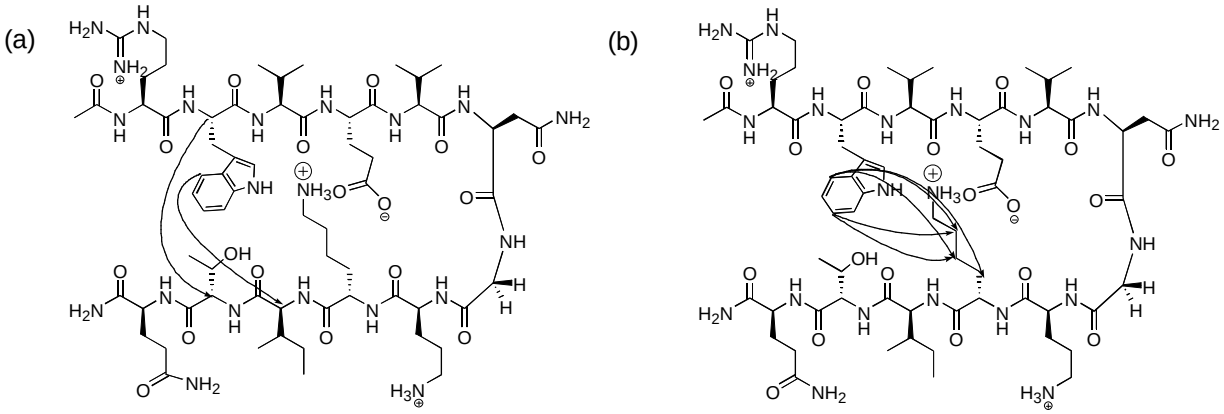


Figure S10. WKT Cross-strand and Diagonal NOEs.

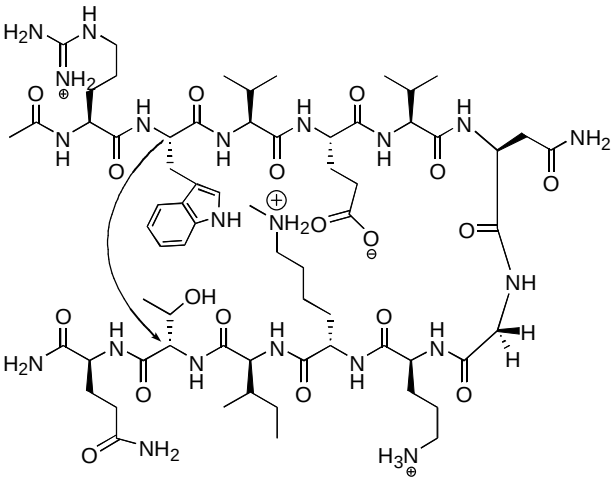


Figure S11. WKMeT Cross-strand NOEs.

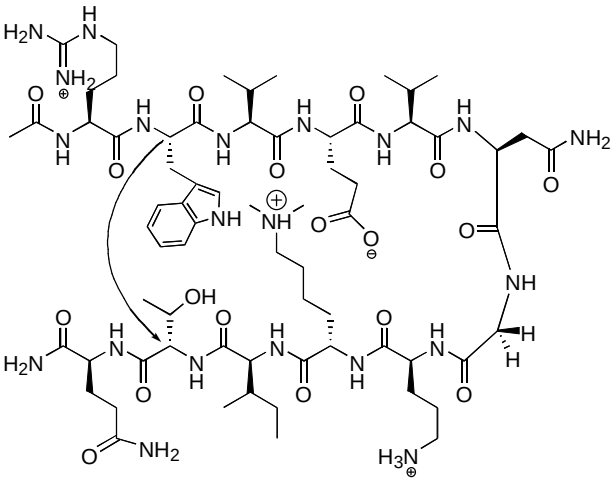


Figure S12. WKMe2T Cross-strand NOEs.

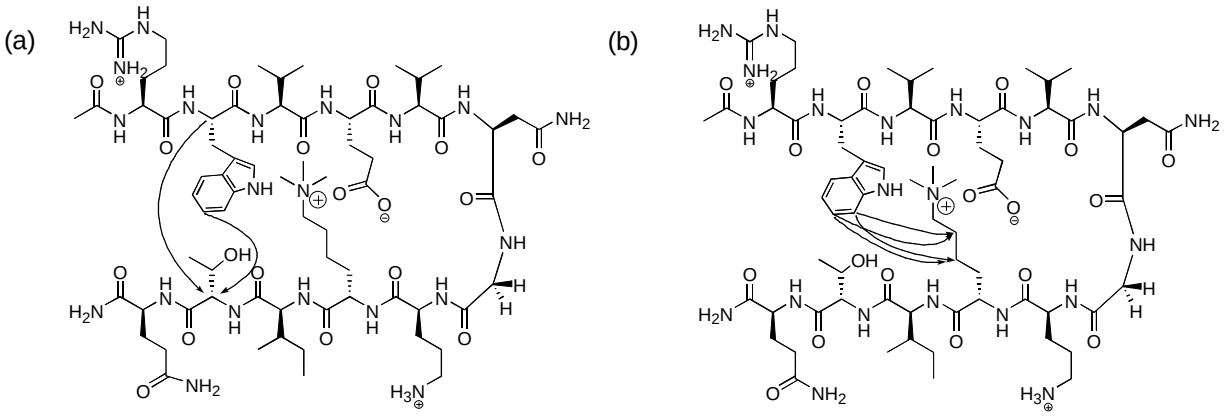


Figure S13. WKMe3T Cross-strand and Diagonal NOEs.

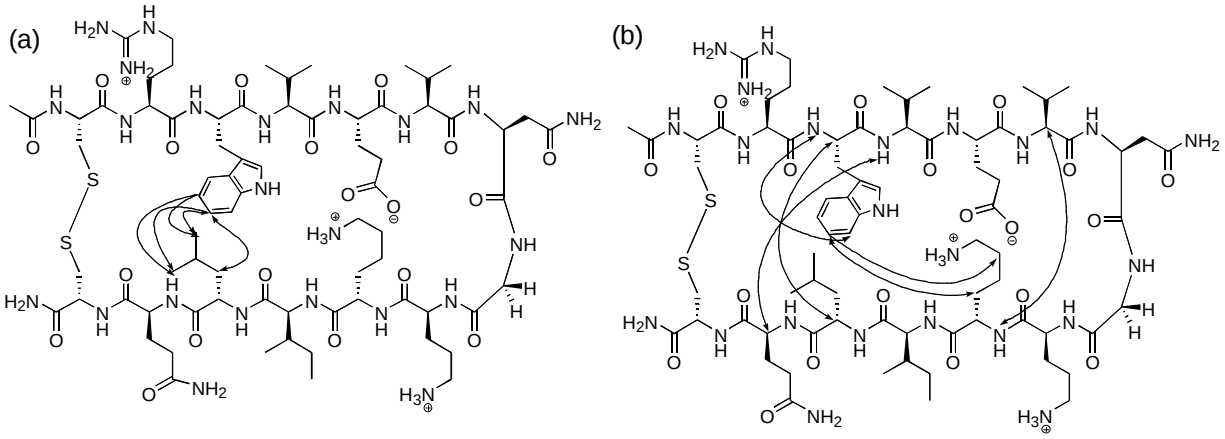


Figure S14. WKLcyc Cross-strand and Diagonal NOEs.

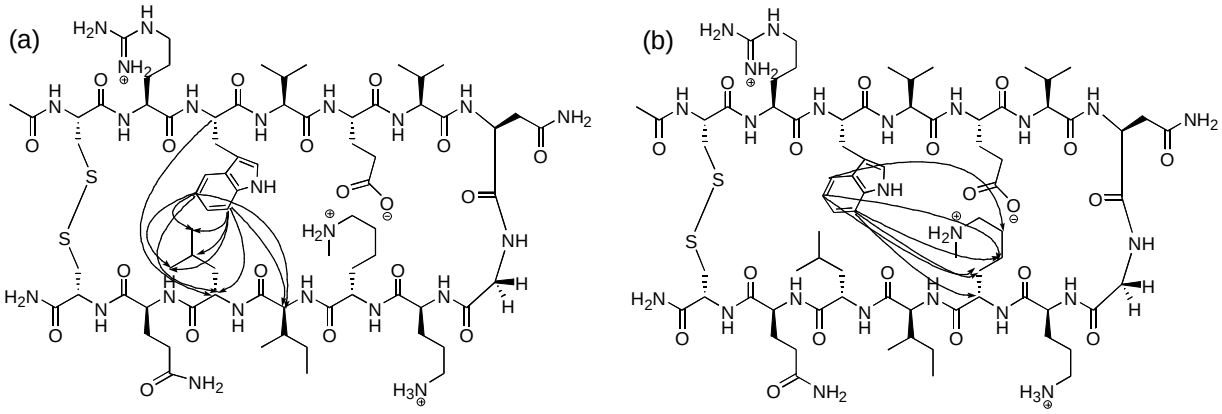


Figure S15. WKMeLcyc Cross-strand and Diagonal NOEs.

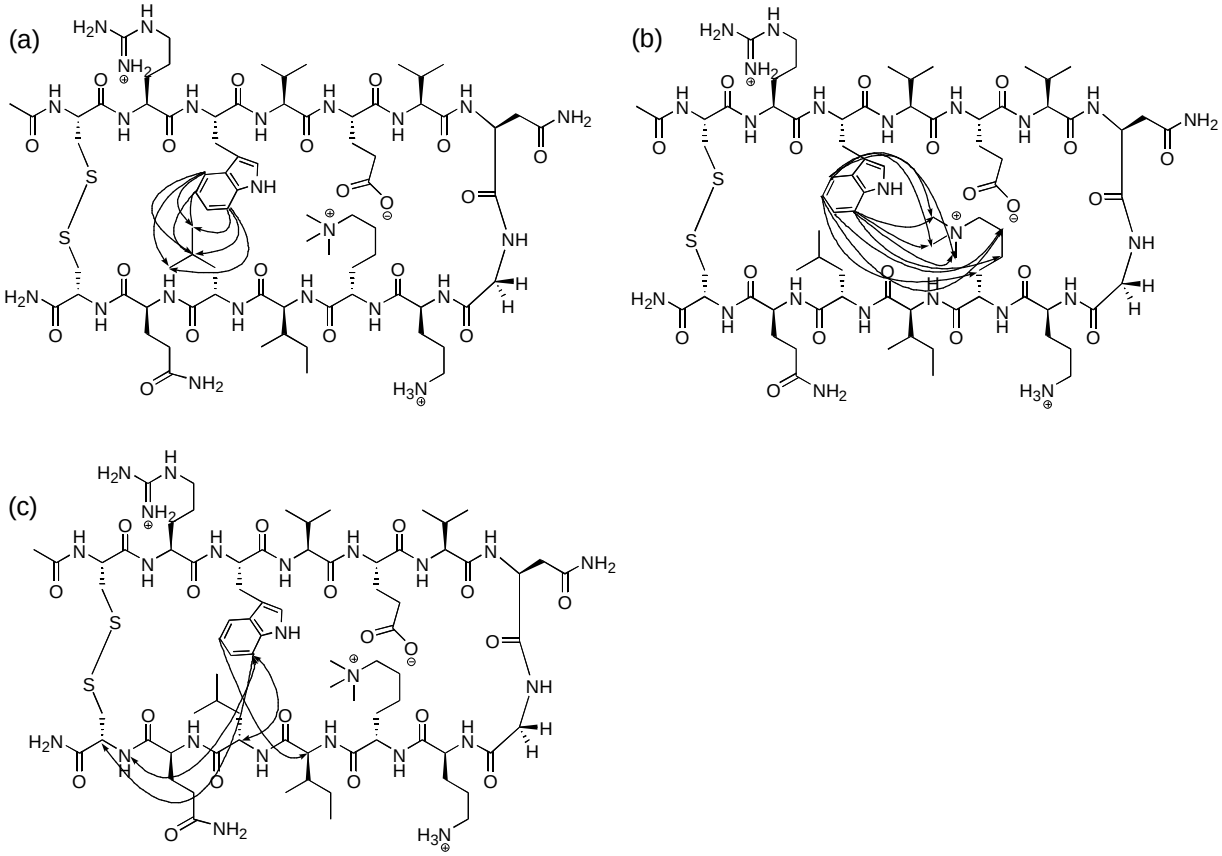


Figure S16. WKMe3Lcyc Cross-strand and Diagonal NOEs**.**

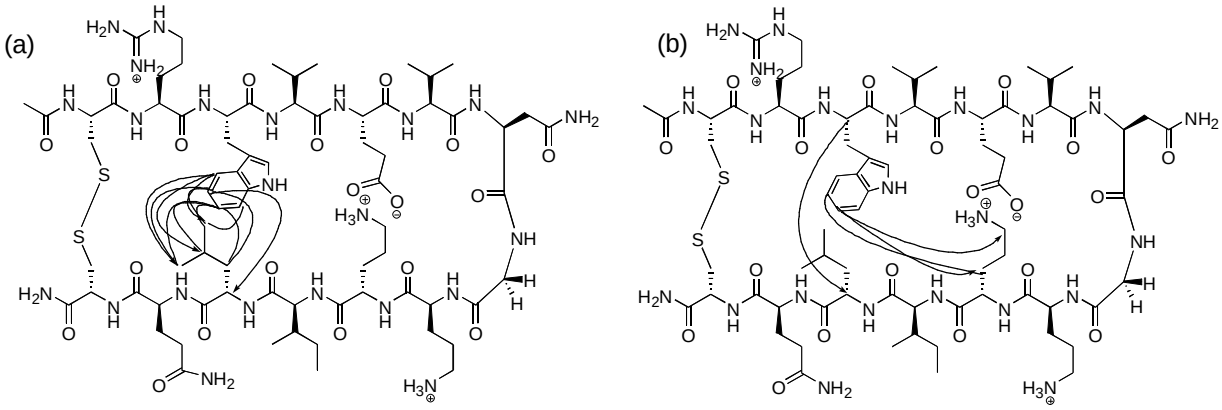


Figure S17. WOrnLcyc Cross-strand NOEs.

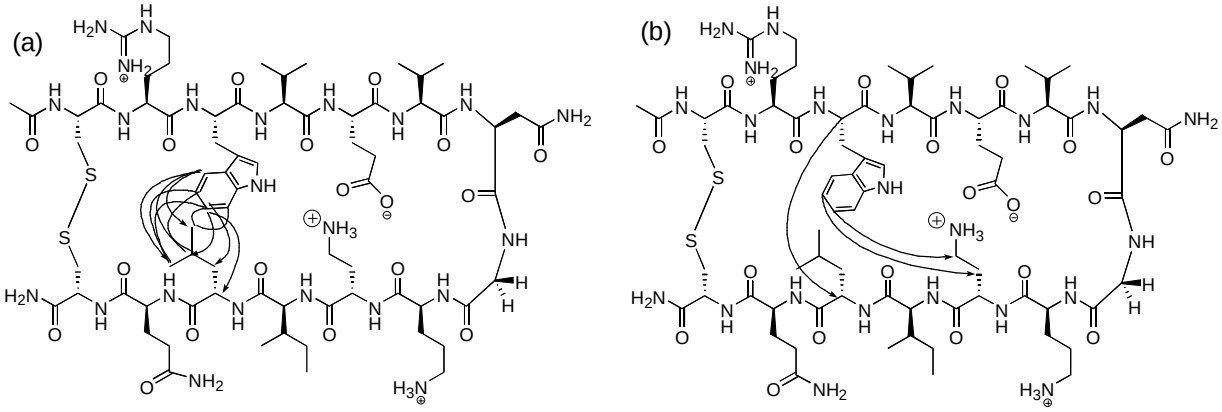


Figure S18. WDabLcyc Cross-strand NOEs.

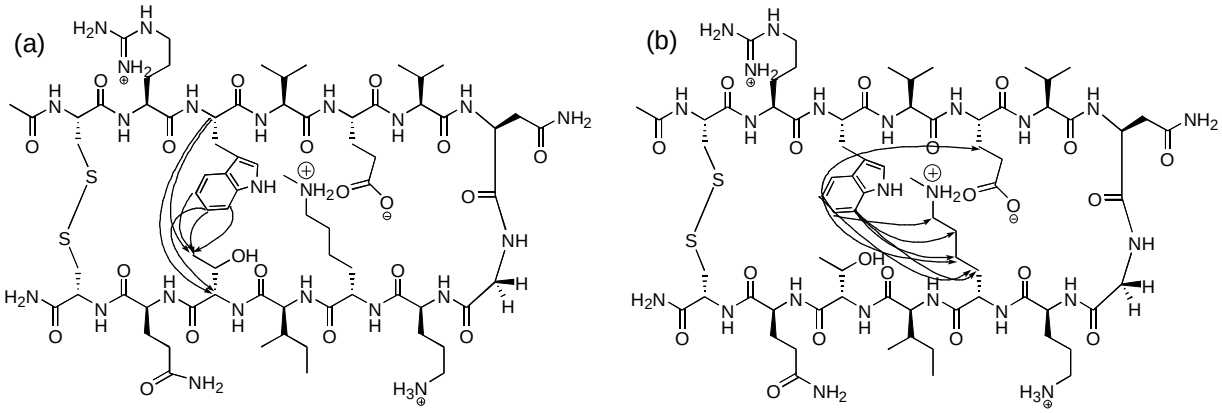


Figure S19. WKMeTcyc Cross-strand and Diagonal NOEs.

Thermodynamic Analysis

Peptides were analyzed assuming a two-state system. The equilibrium constant was determined from the fraction folded (f) by K = f/(1-f). The free energy was then calculated from ? G° = -RTlnK. In order to determine the thermodynamic parameters, ? H°, ? S°, and ? Cp°, the temperature dependence of the Gly chemical shift difference was fit to the following equation:[2]

$$\text{Fraction folded} = [\exp(x/RT)]/[1 + \exp(x/RT)]$$

$$x = [T(?\,S°298 + ?\,Cp° \ln(T/298)) - (?\,H°298 + ?\,Cp°(T - 298))]$$

Table S1. Temperature Dependence of the Fraction Folded from Glycine Chemical Shift Data for the WKL series peptides.

WKL		WKMeL		WKMe2L		WKMe3L	
Temp (K)	fraction folded	Temp (K)	fraction folded	Temp (K)	fraction folded	Temp (K)	fraction folded
276	0.806	276	0.849	276	0.883	276	0.894
281	0.806	281	0.856	281	0.886	281	0.901
285	0.804	285	0.855	285	0.894	285	0.908
289	0.801	289	0.859	289	0.893	290	0.912
294	0.793	294	0.855	294	0.896	294	0.918

298	0.783	298	0.853	298	0.894	299	0.919
302	0.773	302	0.844	302	0.892	303	0.919
307	0.754	307	0.834	307	0.886	308	0.915
311	0.734	311	0.820	311	0.878	312	0.910
316	0.709	316	0.798	316	0.865	317	0.903
320	0.680	320	0.776	320	0.849	321	0.892
324	0.650	324	0.751	324	0.830	325	0.878
329	0.615	329	0.718	329	0.803	330	0.859
						334	0.832
						339	0.804
						343	0.773
						348	0.737

Table S2. Temperature Dependence of the Fraction Folded from Glycine Chemical Shift Data for the **WOrnL** and **WDabL** peptides.

WOrnL		WDabL	
Temp (K)	fraction folded	Temp (K)	fraction folded
275	0.722	275	0.557
280	0.717	280	0.547
284	0.711	284	0.533
289	0.701	289	0.514
293	0.688	293	0.492
298	0.671	298	0.468
303	0.649	303	0.441
307	0.622	307	0.410
312	0.594	312	0.380
316	0.564	316	0.346
321	0.530	321	0.315
325	0.493	325	0.279
330	0.454	330	0.244

Table S3. Temperature Dependence of the Fraction Folded from Glycine Chemical Shift Data for **WK*T** series peptides.

	WKT	WKMeT	WKMe2T	WKMe3T
Temp (K)	fraction folded	fraction folded	fraction folded	fraction folded
276	0.453	0.583	0.706	0.784
280	0.449	0.573	0.701	0.780
285	0.438	0.564	0.692	0.778
289	0.426	0.549	0.680	0.769
294	0.414	0.531	0.664	0.760
298	0.398	0.514	0.647	0.744
303	0.379	0.491	0.624	0.729
307	0.358	0.467	0.602	0.707
312	0.337	0.441	0.571	0.684
316	0.315	0.420	0.542	0.656
321	0.292	0.388	0.510	0.626
326	0.270	0.359	0.477	0.595
330	0.245	0.331	0.445	0.559

Table S4. Parameters Derived from Fitting of Thermal Denaturation Data for Peptides **WK*T** at 298 K(Errors shown in parenthesis).

	WKT	WKMeT	WKMe2T	WKMe3T
ΔS (cal/mol*K)	-10.2 (0.04)	-10.4(0.1)	-10.1(0.1)	-8.3 (0.1)
ΔC_p(cal/mol*K)	-99 (2)	-98 (3)	-116 (3)	-134 (3)
ΔH (kcal/mol)	-2.8 (0.01)	-3.1(0.02)	-3.4(0.03)	-3.11 (0.03)

Tables S5-S45. NMR Assignments of protons in peptides at 298K

Table S5. Peptide **WKL**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.38	1.69	1.51, 1.65	3.14		8.05	6.62,7.09
W	5.09	3.10	7.23,7.49	7.07,7.23	7.23	8.29	10.17
V	4.43	2.03	0.87			8.92	
E	4.92	2.02,1.91	2.28			8.49	
V	4.21	1.95	0.91			8.82	
N	4.47	3.05,2.76				9.35	
G	4.08,3.73					8.59	
O	4.61	1.86	1.75	3.04		7.84	7.64
K	4.77	1.69	1.24	1.35	2.56	8.46	7.28
I	4.57	1.88	0.89,1.42,1.19	0.89		9.05	
L	4.08	1.36,1.06	0.77	0.3,0.51		8.30	
Q	4.32	2.04,1.88	2.27			8.62	

Table S6. Peptide **WKMeL**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.39	1.72	1.53	3.14		8.05	7.11,6.64
W	5.14	3.08				8.28	10.19
V	4.48	2.03	0.87			9.06	
E	5.01	1.97	2.28			8.51	
V	4.23	1.93	0.91			8.90	
N	4.45	3.06,2.75				9.44	
G	4.09,3.70					8.63	
O	4.64	1.83	1.75	3.03		7.82	7.62
K(Me)	4.73	1.67	1.28	1.28	2.33	8.46	7.66

					CH3=2.21		
I	4.58	1.88	0.87,1.43,1.18	0.87		9.16	
L	4.11	1.36,1.06	0.87	0.34		8.37	
Q	4.32	1.93	2.23			8.66	

Table S7. Peptide **WKMe2L**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)$_2$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.38	1.73	1.59	3.08		8.05	7.10
W	5.18	3.08				8.27	10.21
V	4.48	2.02	0.86			9.16	
E	5.05	1.96	2.27			8.54	
V	4.22	1.92	0.90			8.91	
N	4.42	3.00				9.51	
G	4.08,3.68					8.64	
O	4.62	1.78	1.78	3.00		7.81	7.66
K(Me)$_2$	4.75	1.67	1.25	1.25	2.27 **(CH3)$_2$**=2.32, 2.29	8.47	8.40
I	4.62	1.80	0.88,1.43,1.18	0.88		9.22	
L	4.11	1.37	0.89	0.36		8.42	
Q	4.31	1.96	2.27			8.68	

Table S8. Peptide **WKMe3L**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)$_3^{\oplus}$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.39	1.72	1.63, 1.52	3.14		8.03	7.11,6.64
W	5.23	3.12,2.96	7.05,7.23,7.30,7.5			8.27	10.22
V	4.51	2.05	0.87			9.23	
E	5.09	1.96	2.30			8.52	
V	4.25	1.92	0.91			8.96	
N	4.43	3.10,2.76				9.53	
G	4.095					8.65	
	3.675						
O	4.65	1.83	1.76	3.04		7.79	7.64
K(Me)$_3$	4.8	1.7	1.18	1.32	2.37	8.48	**(CH$_3$)$_3$**=2.49
I	4.65	1.83	1.42,1.20,0.89	0.89		9.26	
L	4.14	1.39	0.92,0.86	0.44,0.17		8.43	
Q	4.33	2.01,1,86	2.26			8.70	

Table S9. Peptide **WOrnL**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Orn-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.34	1.65	1.51	3.13		8.08	6.63,7.12
W	5.01	3.12	7.22,7.49	7.06,7.22	7.36	8.29	10.17
V	4.39	2.02	0.86			8.76	
E	4.83	1.97	2.24			8.47	
V	4.19	1.96	0.91			8.74	

N	4.51	3.02,2.75				9.26	
G	4.06,3.77					8.59	
O	4.58	1.89	1.7	3.03		7.91	7.66
O	4.73	1.72	1.60	2.67		8.55	7.66
I	4.47	1.84	0.88,1.41,1.18	0.88		8.96	
L	4.12	1.39	1.11,0.86	0.55,0.41		8.33	
Q	4.33	2.04	2.27			8.59	

Table S10. Peptide **WOrnMeL**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Orn(Me)-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.32	1.70	1.50	3.12		8.07	6.63,7.11
W	5.04	3.13				8.29	10.20
V	4.38	2.03	0.91			8.77	
E	4.82	1.97	2.23			8.50	
V	4.18	1.94	0.91			8.72	
N	4.48	3.03,2.75				9.29	
G	4.03,3.72					8.60	
O	4.56	1.82	1.72	3.03		7.90	7.65

O(Me)	4.74	1.72	1.60	2.61	**CH_3**=2.43	8.56	8.16
I	4.50	1.84	0.87,1.42	0.87		8.97	
L	4.10	1.39	1.15,0.90	0.54,0.38, 0.90		8.36	
Q	4.28	1.98	2.33			8.60	

Table S11. Peptide **WOrnMe2L**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Orn$(Me)_2$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.33	1.61	1.52	3.12		8.07	6.61,7.10
W	5.06	3.10				8.25	10.21
V	4.40	2.02	0.86			8.82	
E	4.88	1.94	2.23			8.51	
V	4.20	1.92	0.89			8.73	
N	4.47	3.04,2.75				9.32	
G	4.06,3.74					8.59	
O	4.58	1.84	1.72	3.03		7.86	7.64
$O(Me)_2$	4.77	1.68	1.57	2.61	**$(CH_3)_2$**=2.54,2.50	8.57	8.16
I	4.51	1.86	0.87,1.43,1.18	0.87		9.00	
L	4.14	1.4	1.14,0.92	0.56, 0.38		8.38	
Q	4.32	2.05,1.87	2.28			8.59	

Table S12. Peptide **WOrnMe3L**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Orn(Me)$_3$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.33	1.64	1.52	3.12			
W	5.07	3.10					
V	4.39	2.03	0.87				
E	4.92	1.96	2.24				
V	4.20	1.94	0.91				
N	4.47	3.01,2.76					
G	4.07,3.73						
O	4.60	1.83	1.75	3.02			
O(Me)$_3$	4.81	1.69	1.60	2.71	**(CH$_3$)$_3$**=2.70		
I	4.51	1.85	0.89,1.43,1.2	0.89			
L	4.15	1.61	1.17,1.39,0.92	0.57,0.4			
Q	4.31	1.97	2.30				

Table S13. Peptide **WDabL**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Dab-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.31	1.66	1.48	3.12		8.10	7.11,6.62
W	4.90	3.16	7.23,7.49	7.07,7.23	7.43	8.29	10.17
V	4.31	2.02	0.85			8.55	
E	4.72	2.00	2.29			8.38	
V	4.18	1.98	0.92			8.48	

N	4.56	2.97,2.76			9.05		
G	4.02,3.81				8.54		
O	4.52	1.83	1.76	3.02	8.01	7.66	
Dab	4.72	2.09	3.03		8.64	7.66	
I	4.4	1.85	0.87,1.42,1.17	0.87	8.76		
L	4.14	1.39	1.20,0.97	0.61,0.49	8.33		
Q	4.31	2.05,1.9	2.30		8.54		

Table S14. Peptide **WDabMeL**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Dab(Me)-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.33	1.64	1.52	3.13		8.09	7.11, 6.63
W	4.97	3.14	7.62,7.39,7.23,7.23,7.06			8.30	10.16
V	4.35	2.03	0.86			8.68	
E	4.82	1.98	2.29			8.42	
V	4.20	1.96	0.92			8.63	
N	4.53	3.02,				9.17	

		2.76				
G	4.05, 3.80				8.57	
O	4.56	1.81	1.81	3.02	7.98	7.65
Dab(Me)	4.76	2.09	3.02	**CH3**=2.40	8.68	8.58
I	4.46	1.86	1.43,1.16,0.87	0.87	8.89	
L	4.14	1.40	1.17,0.92	0.50	8.36	
Q	4.32	1.92	2.30		8.57	

Table S15. Peptide **WDabMe2L**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Dab(Me)$_2$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.33	1.63	1.51	3.12			
W	5.02	3.11					
V	4.38	2.04	0.85				
E	4.89	1.97	2.27				
V	4.20	1.95	0.90				
N	4.48	3.04,2.69					
G	4.047, 3.750						
O	4.55	1.77	1.77	3.02			
Dab(Me)$_2$	4.77	2.06	3.07	**(CH3)$_2$**=2.457, 2.542			
I	4.48	1.87	0.87,1.38,1.17	0.87			
L	4.13	1.38	1.13,0.90	0.45			
Q	4.30	1.96	2.27				

Table S16. Peptide **WDabMe3L**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Dab(Me)$_3$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.33	1.66	1.54	3.12			
W	5.07	3.13					
V	4.41	2.06	0.87				
E	4.96	1.98	2.30				
V	4.22	1.92	0.91				
N	4.48	3.06,2.73					
G	4.062,3.742						
O	4.59	1.79	1.79	3.03			
Dab(Me)$_3$	4.79	2.11	3.24	**(CH3)$_3$**= 2.715			
I	4.51	1.88,1.51	0.88, 1.26	0.88			
L	4.16	1.40	0.95	0.46			
Q	4.31	1.93	2.27				

Table S17. Peptide **WKT**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.20	1.58	1.50	3.08		8.13	7.07
W	4.92	3.25,3.19				8.05	10.14
V	4.18	2.00	0.87			8.18	

E	4.66	1.98	2.37			8.39	
V	4.17	2.01	0.92			8.47	
N	4.58	2.76,2.96				8.96	
G	4.00,3.86					8.49	
O	4.49	1.81	1.75	3.02		7.98	7.64
K	4.45	1.69	1.25	1.37	2.68	8.39	7.35
I	4.40	1.92	0.91	0.91		8.60	
T	4.42	4.11	1.15			8.30	
Q	4.34	1.95	2.33			8.39	

Table S18. Peptide **WKMeT**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.21	1.61	1.45	3.08			
W	4.99	3.20				8.00	
V	4.23	1.99	0.86			8.38	
E	4.76	1.97	2.35				
V	4.17	1.98	0.92			8.60	
N	4.54	2.76,2.97					

G	4.02,3.80					8.55	
O	4.51	1.77	1.77	3.01			
K(Me)	4.47	1.65	1.17	1.33	2.50 **CH3**=2.39		
I	4.44	1.94	0.91	0.91		8.77	
T	4.44	4.10	1.12				
Q	4.34	2.09,1.91	2.30				

Table S19. Peptide **WKMe2T**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)$_2$-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.23	1.62	1.46	3.09			
W	5.06	3.14				7.97	
V	4.27	2.01	0.87			8.53	
E	4.87	1.97	2.33				
V	4.19	1.96	0.92			8.69	
N	4.51	2.75,3.00					
G	4.04,3.76						
O	4.54	1.79	1.73	3.02			
K(Me)$_2$	4.51	1.65	1.11	1.29	2.44 **(CH3)$_2$**=2.46, 2.44		
I	4.50	1.95	0.91	0.91		8.86	
T	4.38	4.19	1.18				
Q	4.33	1.95	2.30				

Table S20. Peptide **WKMe3T**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)$_3$-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.25	1.62	1.48	3.09			
W	5.12	3.14				7.95	
V	4.31	2.02	0.86			8.68	
E	4.96	1.97	2.33			8.55	
V	4.22	1.94	0.92			8.77	
N	4.49	2.75,3.04					
G	4.05,3.73					8.62	
O	4.57	1.81	1.71	3.02			
K(Me)$_3$	4.57	1.67	1.11	1.28	2.52 **(CH3)$_2$**=2.61		
I	4.55	1.99	0.91	0.91		8.97	
T	4.49	4.09	1.11				
Q	4.36	1.88	2.28				

Table S21. Peptide **WKLcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys-Ile-Leu-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.04	2.95				8.91	
R	4.61	1.80	1.66,1.51	3.18		8.71	7.11,6.67

W	5.15	3.09,2.94	7.00,7.23,7.28,7.49			8.64	
V	4.58	2.05	0.87			9.54	
E	5.04	2.00,1.93	2.27			8.54	
V	4.24	1.92	0.89			9.09	
N	4.40	3.10,2.75				9.6	
G	4.115, 3.675					8.68	
O	4.70	1.85	1.71	3.05		7.76	7.66
K	4.93	1.72	1.29	1.38	2.53	8.52	7.21
I	4.71	1.85	1.43,1.16,0.87	0.87		9.40	
L	3.90	1.29,0.76	0.37	0.07, -0.35		8.32	
Q	4.57	2.07,1.82	2.24,2.16			9.04	
C	5.22	3.00,2.40				8.36	

Table S22. Peptide **WKMe2Lcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)$_2$-Ile-Leu-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.04	2.92					
R	4.60	1.86	1.70	3.16			
W	5.12	3.03					
V	4.49	1.96	0.89				
E	5.11	1.93	2.28				

V	4.25	1.94	0.91				
N	4.40	3.04,2.75					
G	4.11,3.66						
O	4.75	1.79	1.79	3.00			
K(Me)$_2$	4.90	1.72	1.31	1.31	2.25	**(CH$_3$)$_2$=** 2.27, 2.23	
I	4.83	1.88	0.89	0.89			
L	4.01	1.34	0.81,0.41,0.31	-0.36			
Q	4.59	1.93	2.17				
C	5.22	3.05					

Table S23. Peptide **WKMeLcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)-Ile-Leu-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.04	2.95					
R	4.59	1.73	1.50	3.16		8.72	
W	5.17	3.09,2.97				8.61	
V	4.58	2.07	0.87			9.53	
E	5.07	1.93	2.19			8.51	
V	4.23	1.91	0.89			9.05	
N	4.40	3.10,2.75					
G	4.115, 3.668						
O	4.67	1.81	1.77	3.04		7.76	7.27

K(Me)	4.92	1.71	1.33	1.33	2.36, 2.24	**(CH_3)=**2.14
I	4.72	1.86	1.41,1.17	0.87	9.40	
L	3.96	1.33	0.79,0.35	0.19, -0.38	8.38	
Q	4.58	1.87	2.16		9.04	
C	5.21	2.97,2.41				

Table S24. Peptide **WKMe3Lcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)$_3^{\oplus}$-Ile-Leu-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.04	2.92,3.10				8.92	
R	4.6	1.80	1.68,1.48	3.16		8.71	6.64,7.10
W	5.14	2.96,3.11	7.02,7.25,7.28	7.50		8.64	10.17
V	4.59	2.08	0.87			9.57	
E	5.11	1.93	2.29			8.50	
V	4.26	1.91	0.90			9.05	
N	4.40	3.09,2.75				9.61	
G	4.105, 3.660					8.70	
O	4.68	1.84	1.74	3.03		7.76	7.65
K(Me)$_3$	4.89	1.77	1.22	1.35	2.36	8.48	**(CH_3)$_3$**=2.46,2.44
I	4.74	1.87	1.17,1.42,0.88	0.88		9.42	
L	4.03	1.35,0.79	0.32	-0.39		8.46	
Q	4.58	2.08,1.82	2.20			9.06	
C	5.21	2.96,3.11				8.59	

Table S25. Peptide **WOrnLcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Orn-Ile-Leu-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.04	2.91					
R	4.61	1.79	1.65,1.50	3.16		8.73	
W	5.12	3.09	7.00,7.23,7.27,7.49	6.99		8.67	
V	4.60	2.07	0.86			9.51	
E	5.09	1.97	2.17				
V	4.24	1.90	0.90			9.04	
N	4.40	3.09,2.75					
G	4.12,3.67						
O	4.69	1.83	1.83	3.04			
Orn	5.04	1.78	1.67	2.64			
I	4.69	1.83	1.39,1.16,0.87	0.87		9.40	
L	3.89	1.29	0.74,0.37	0.059, -0.33		8.35	
Q	4.56	2.05,1.82	2.18				
C	5.22	2.99,2.40					

Table S26. Peptide **WDabLcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Dab-Ile-Leu-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.04	2.94					
R	4.62	1.80	1.67,1.50	3.17		8.72	
W	5.07	3.05	7.00,7.23,7.27,7.49	6.96		8.68	

V	4.64	2.09	0.86		9.53	
E	5.19	1.97	2.21			
V	4.25	1.89	0.89		8.98	
N	4.41	3.11,2.74				
G	4.11,3.67					
O	4.69	1.88	1.75	3.04	7.82	
Dab	5.07	2.11	3.05			
I	4.68	1.83	1.37,1.15,0.84	0.84	9.35	
L	3.84	1.24	0.71,0.35	-0.09, -0.34	8.28	
Q	4.54	2.09,1.82	2.15		9.01	
C	5.22	2.99,2.39				

Table S27. Peptide **WKTcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys-Ile-Thr-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.00	2.95,2.62					
R	4.56	1.82	1.50	3.16			
W	5.30	3.08				8.33	10.10
V	4.51	2.03	0.85			9.19	
E	5.06	1.97	2.25			8.59	
V	4.24	1.90	0.90			9.02	
N	4.41	3.10,2.76					

G	4.11,3.67						
O	4.66			3.03		7.77	7.66
K	4.80	1.62	1.17	1.25	2.36		
I	4.77	1.99	0.92	0.92		9.25	
T	4.54	3.71	0.73			8.33	
Q	4.57	2.02,1.83	2.21			8.91	
C	4.98	3.08,2.96					

Table S28. Peptide **WKMeTcyc**: Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-Lys(Me)-Ile-Thr-Gln-Cys-NH2

	α	β	γ	δ	ε	Amide	Amine
C	5.03	2.96,2.63					
R	4.55	1.82	1.51	3.16			
W	5.36	3.08					
V	4.51	2.03	0.85				
E	5.11	1.98	2.28				
V	4.25	1.90	0.91				
N	4.41	3.10,2.76					
G	4.10,3.67						
O	4.66	1.82	1.73	3.03			
K	4.80	1.64	1.19	1.21	2.13	**CH3=** 2.09	
I	4.80	2.00	0.92	0.92			
T	4.59	3.74	0.78				
Q	4.60	2.03,1.84	2.22				
C	4.98	3.09,2.97					

Table S29. Peptide KL7: Ac-Asn-Gly-Orn-Lys-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.67	2.82				8.44	
G	3.94					8.55	
O	4.35	1.88,1.78	1.7	3.01		8.13	7.64
K	4.31	1.77	1.42	1.69	2.99	8.33	7.56
I	4.14	1.85	1.48,1.20,0.89	0.89		8.25	
L	4.38	1.65	1.61	0.93,0.88		8.37	
Q	4.3	2.12,1.99	2.37			8.33	

Table S30. Peptide **KMeL7**: Ac-Asn-Gly-Orn-Lys(Me)-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.68	2.81				8.43	
G	3.94					8.56	
O	4.35	1.78	1.68	3.01		8.13	7.61
K(Me)	4.30	1.75	1.40	1.68	3.02	8.33	8.05
					CH3=2.701		
I	4.13	1.85	1.49,1.23,0.89	0.89		8.24	
L	4.38	1.63	1.63	0.86		8.36	
Q	4.3	2.10,1.96	2.37			8.32	

Table S31. Peptide **KMe2L7**: Ac-Asn-Gly-Orn-Lys(Me)$_2$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.7	2.81				8.54	
G	3.96					8.58	
O	4.36	1.75	1.75	3.02		8.12	7.60

$K(Me)_2$	4.30	1.95,1.79	1.40	1.69	3.12 $(CH3)_2$=2.87	8.34	8.83
I	4.14	1.84	0.89	0.89		8.28	
L	4.39	1.66	1.66	0.90		8.39	
Q	4.3	2.01	2.33			8.35	

Table S32. Peptide **KMe3L7**: Ac-Asn-Gly-Orn-Lys$(Me)_3^{\oplus}$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.71	2.81				8.42	
G	3.94					8.55	
O	4.36	1.84	1.69	3.02		8.11	7.64
$K(Me)_3$	4.33	1.84,1.73	1.43	1.66	3.3	8.33	$(CH_3)_3$=3.1
I	4.14	1.84	1.49,1.19,0.89	0.89		8.30	
L	4.39	1.7	1.61	0.92		8.37	
Q	4.31	2.04	2.36			8.33	

Table S33. Peptide **W7**: Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-NH2

	α	β	γ	δ	ε	Amide	Amine
R	4.18	1.60	1.48	3.08		8.14	6.63,7.12
W	4.70	3.27				8.19	10.11
V	4.02	1.96	0.85			7.75	
E	4.29	1.99	2.43			8.20	
V	4.10	2.07	0.94			8.22	
N	4.70	2.82				8.60	
G	3.91					8.38	

Table S34. Peptide **OrnL7**: Ac-Asn-Gly-Orn-Orn-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.69	2.80				8.41	
G	3.95					8.52	

O	4.35	1.85	1.72	3.02		8.15	7.6
O	4.36	1.84	1.70	3.02		8.40	7.6
I	4.15	1.85	1.35,1.14,0.90	0.90		8.25	
L	4.39	1.71	1.71	0.90		8.37	
Q	4.31	2.04	2.35			8.32	

Table S35. Peptide **OrnMeL7**: Ac-Asn-Gly-Orn-Orn(Me)-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.69	2.80				8.45	
G	3.93					8.55	
O	4.35	1.84	1.76	3.04		8.16	7.6
O(Me)	4.35	1.84	1.76	3.00	CH3=2.71	8.45	8.14
I	4.13	1.83	1.48,1.13,0.89	0.89		8.31	
L	4.38	1.64	1.59,0.93,0.86	0.86		8.39	
Q	4.29	2.09,1.97	2.34			8.36	

Table S36. Peptide **OrnMe2L7**: Ac-Asn-Gly-Orn-Orn$(Me)_2$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.69	2.82				8.45	
G	3.94					8.56	
O	4.34	1.77	1.65	3.01		8.16	7.63
$O(Me)_2$	4.38	1.77	1.65	3.17	$(CH3)_2$=2.88,2.87	8.44	8.16
I	4.14	1.84	1.48,1.1,0.90	0.90		8.33	
L	4.38	1.63	1.63	0.90		8.40	
Q	4.31	2.10,1.98	2.36			8.36	

Table S37. Peptide **OrnMe3L7**: Ac-Asn-Gly-Orn-Orn(Me)$_3$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.67	2.80					
G	3.93						
O	4.33	1.79	1.65	3.01			
O(Me)$_3$	4.38	1.81	1.69	3.34	(CH3)$_3$=3.11		
I	4.13	1.84	1.48,1.1,0.88	0.88			
L	4.37	1.69	1.69	0.90			
Q	4.30	2.09,1.97	2.36				

Table S38. Peptide **DabL7**: Ac-Asn-Gly-Orn-Dab-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.69	2.81				8.41	
G	3.95					8.5	
O	4.35	1.85	1.69	3.01		8.17	7.6
Dab	4.47	2.12	3.06			8.54	7.6
I	4.14	1.81	1.37,1.1,0.90	0.90		8.29	
L	4.28	1.66	1.66	0.98,0.84		8.42	
Q	4.31	2.12,1.99	2.38			8.35	

Table S39. Peptide **DabMeL7**: Ac-Asn-Gly-Orn-Dab(Me)-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.68	2.81					
G	3.95						
O	4.35	1.88	1.70	3.01			
Dab(Me)	4.47	2.11	3.09		CH3=2.73		
I	4.14	1.85	1.47,1.17,0.90	0.90			
L	4.39	1.63	0.91	0.91			
Q	4.31	1.99	2.37				

Table S40. Peptide **DabMe2L7**: Ac-Asn-Gly-Orn-Dab(Me)$_2$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.68	2.83					
G	3.95						
O	4.34	1.82	1.73	3.02			
Dab(Me)$_2$	4.48	2.20	3.20		(CH3)$_2$=2.901		
I	4.13	1.85	1.45,0.91	0.91			
L	4.40	1.65	0.92	0.92			
Q	4.305	2.03	2.35				

Table S41. Peptide **DabMe3L7**: Ac-Asn-Gly-Orn-Dab(Me)$_3$-Ile-Leu-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.68	2.84					
G	3.94						
O	4.32	1.77	1.77	3.01			
Dab(Me)$_3$	4.51	2.246	3.395		(CH3)$_3$=3.142		
I	4.14	1.74	0.91	0.91			
L	4.39	1.644	1.644, 0.926	0.926			
Q	4.29	2.00	2.36				

Table S42. Peptide **KT7**: Ac-Asn-Gly-Orn-Lys-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.69	2.81				8.44	
G	3.94					8.55	
O	4.36	1.84	1.72	3.01		8.12	7.60

K	4.34	1.87	1.43	1.70	3.01	8.37	7.53
I	4.24	1.89	1.50,1.21,0.93	0.90		8.34	
T	4.36	4.19	1.20			8.26	
Q	4.34	2.14,2.00	2.37			8.40	

Table S43. Peptide **KMeT7**: Ac-Asn-Gly-Orn-Lys(Me)-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.69	2.80					
G	3.94					8.55	
O	4.37	1.76	1.76	3.01		8.11	
K(Me)	4.34	1.75	1.42	1.70	3.01		
					CH3=2.70		
I	4.24	1.89	1.49,1.22,0.91	0.91		8.33	
T	4.37	4.19	1.20			8.26	
Q	4.34	2.00	2.37			8.39	

Table S44. Peptide **KMe2T7**: Ac-Asn-Gly-Orn-Lys$(Me)_2$-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.70	2.81				8.43	
G	3.94					8.55	
O	4.37	1.76	1.76	3.01		8.12	
$K(Me)_2$	4.33	1.77	1.41	1.70	3.10		
					CH3=2.87		
I	4.24	1.89	0.90	0.90		8.35	
T	4.37	4.19	1.20			8.25	
Q	4.33	2.00	2.37			8.40	

Table S45. Peptide **KMe3T7**: Ac-Asn-Gly-Orn-Lys$(Me)_3$-Ile-Thr-Gln-NH2

	α	β	γ	δ	ε	Amide	Amine
N	4.67	2.80				8.42	
G	3.94					8.55	
O	4.37	1.78	1.78	3.01			
$K(Me)_3$	4.36	1.79	1.40	1.79	3.31		
					CH3=3.10		
I	4.24	1.89	1.50,1.18,0.91	0.91		8.36	
T	4.37	4.19	1.19			8.24	
Q	4.33	2.09,2.00	2.37			8.39	

[1] Kretsinger, J. and Schneider, J. P. *J. Am. Chem. Soc*. **2003**, *125*, 7907-7913.
[2] Maynard, A.J. Sharman, G. J. and Searle, M. S. *J. Am. Chem. Soc*. **1998**, *120*, 1996-2007.