

**COMBINED X-RAY AND NMR ANALYSIS OF THE STABILITY OF THE CYCLOTIDE
CYSTINE KNOT FOLD THAT UNDERPINS ITS INSECTICIDAL ACTIVITY AND
POTENTIAL USE AS DRUG SCAFFOLD**

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Running head: cyclotide structure and stability

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Supplemental Data

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Table S1. Hydrogen Bond Interactions with Water Molecules in varv F

residue			WAT ^a	distance (Å)	temperature factor	residue			WAT ^a	distance (Å)	temperature factor
CYS	1	N	49	2.81	23.13	TYR	11	OH	59	2.75	53.20
CYS	1	O	39	3.17	36.31	THR	12	O	38	2.79	25.76
CYS	1	N	43	3.42	36.33	THR	12	O γ 1	39	2.67	36.31
GLY	2	N	49	2.89	23.13	ALA	13	N	41	3.11	30.25
GLY	2	N	43	2.74	36.33	GLY	14	O	34	2.72	22.98
GLY	2	O	69	2.63	39.47	GLY	14	N	55	2.97	42.83
GLU	3	N	49	3.46	23.13	CYS	15	O	38	3.35	25.76
GLU	3	O ϵ 1	35	2.95	28.42	CYS	15	O	71	2.91	44.86
THR	4	O	32	3.32	18.64	CYS	15	O	65	2.67	51.00
THR	4	O γ 1	32	2.72	18.64	SER	16	N	34	3.35	22.98
THR	4	O	35	2.98	28.42	SER	16	O γ	34	2.84	22.98
THR	4	O γ 1	54	2.60	39.52	SER	16	O	61	3.39	34.96
THR	6	N	32	3.08	18.64	CYS	17	N	30	3.30	5.44
THR	6	O γ 1	32	2.83	18.64	CYS	17	O	47	2.70	34.20
LEU	7	O	37	2.64	29.94	SER	18	O γ	61	2.74	34.96
LEU	7	N	52	3.14	32.44	SER	18	O γ	62	2.69	44.46
GLY	8	O	51	2.75	39.76	TRP	19	O	42	2.65	27.88
GLY	8	O	48	3.45	41.24	TRP	19	O	68	3.40	35.93
GLY	8	O	74	3.14	43.54	CYS	22	O	49	2.70	23.13
THR	9	O	35	3.24	28.42	THR	23	O	31	3.41	16.83
THR	9	O γ 1	37	2.66	29.94	THR	23	O γ 1	31	3.02	16.83
THR	9	O	52	3.26	32.44	THR	23	O	34	3.22	22.98
THR	9	O γ 1	45	3.42	38.35	THR	23	O γ 1	61	3.09	34.96
THR	9	O γ 1	48	2.84	41.24	THR	23	O γ 1	62	3.33	44.46
CYS	10	N	30	3.29	5.44	ASN	25	N	46	2.90	36.67
CYS	10	O	38	3.07	25.76	ASN	25	O δ 1	46	3.49	36.67
CYS	10	O	44	2.81	29.39	GLY	26	N	31	3.13	16.83
CYS	10	O	71	3.05	44.86	GLY	26	O	33	2.81	33.46
TYR	11	OH	40	3.14	28.64	PRO	28	O	43	2.90	36.33
TYR	11	O	53	2.78	44.43	ILE	29	O	50	3.13	35.76

^aWater molecules

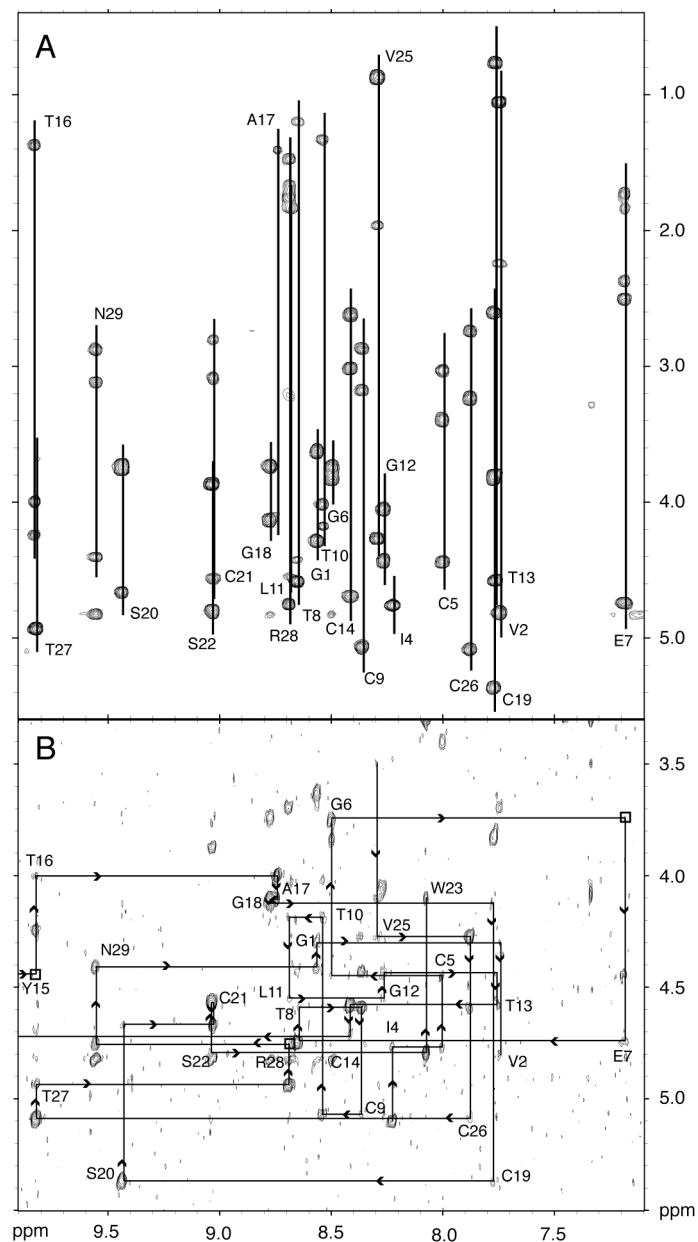


Fig. S1. TOCSY and NOESY spectra of varv F. Fingerprint regions of a 80 ms TOCSY spectrum (A) and a 200 ms NOESY spectrum in 90% H₂O and 10% D₂O at 298K. Spin systems are shown in the TOCSY spectrum and the sequential connectivities in the NOESY spectrum. The one-letter code for amino acids as well as the residue number is used for the sequence assignments. Tyr-15 has a NH chemical shift of 11.61 ppm and is not included in the diagram to improve visibility of the other peaks. Some peaks that are only visible at lower threshold levels are indicated by boxes.

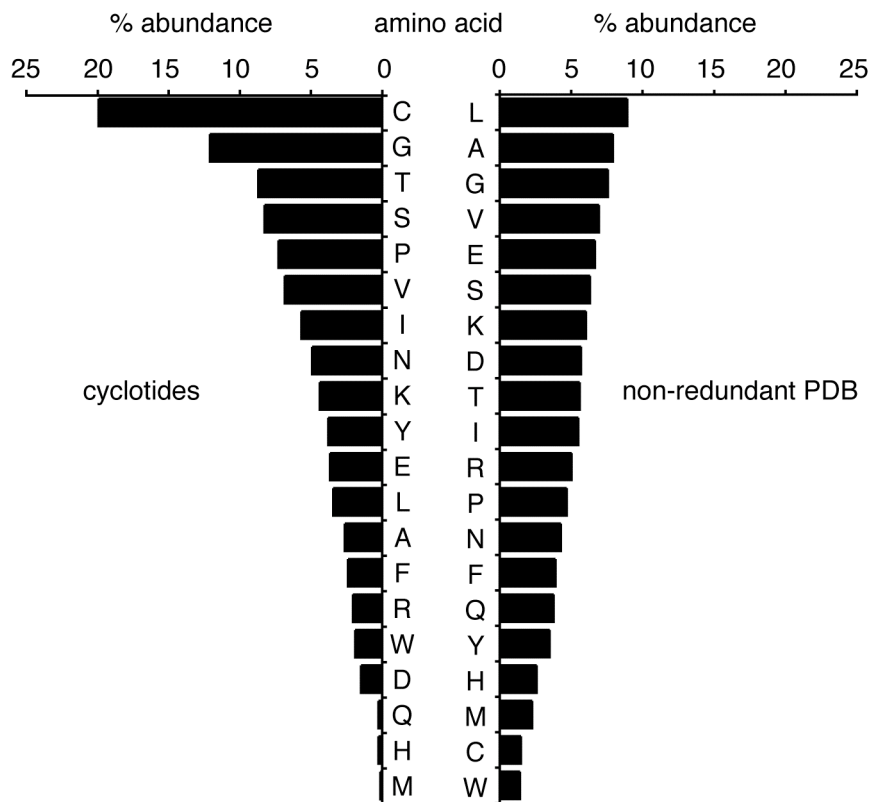


Fig. S2. Amino acid composition of cyclotide sequences and sequences from the PDB. Amino acid compositions were calculated for cyclotides (extracted from CyBase; <http://research1t.imb.uq.edu.au/cybase>) and are shown on the left of the figure. Amino acid compositions for a non-redundant compilation of the PDB (from PISCES; <http://dunbrack.fccc.edu/Guoli/PISCES.php>) are shown on the right of the figure.