SUPPORTING INFORMATION

Heterocyclic Peptide Backbone Modifications in an α-Helical Coiled Coil

W. Seth Horne, Maneesh K. Yadav, C. David Stout, and M. Reza Ghadiri*

Departments of Chemistry, Molecular Biology, and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

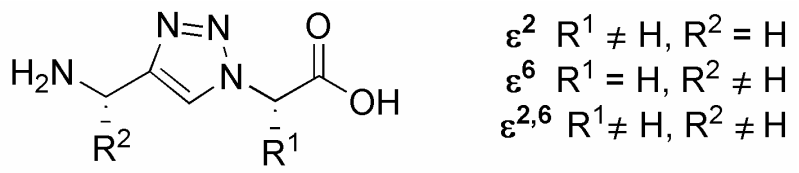


Figure S1. Illustration depicting the nomenclature used for the triazole ε-amino acids.[1] The Greek letter ε refers to the shortest atom connectivity between the amine and acid. The numeric superscripts ε^2, ε^6, and $\varepsilon^{2,6}$ refer to the substitution pattern at the C^2 and C^6 stereogenic carbon atoms.

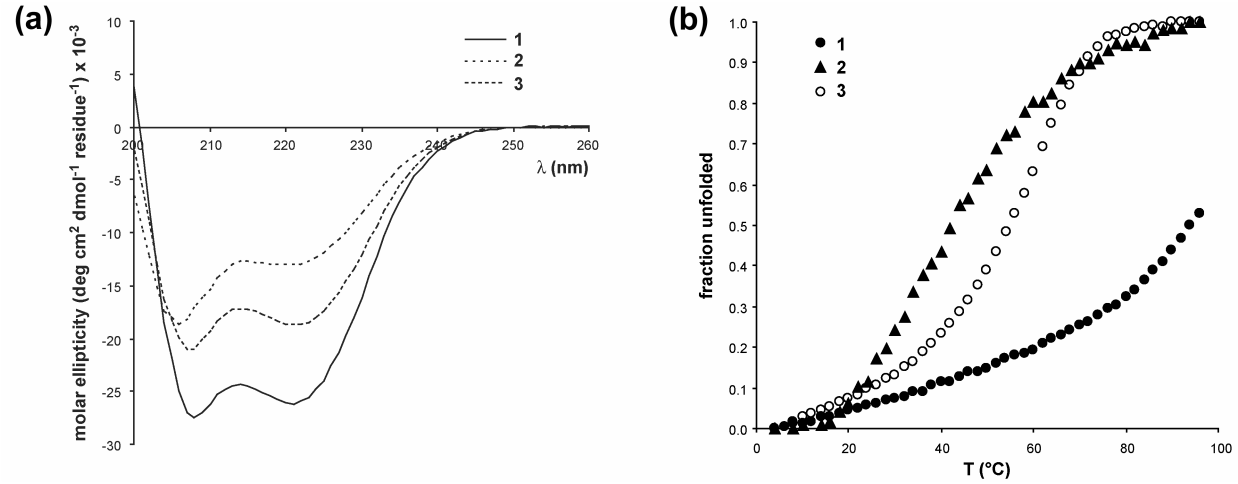


Figure S2. (a) CD spectra of peptides **1**-**3** at 25°C; (b) fraction unfolded for **1**-**3** as a function of temperature based on the intensity of the negative peak at 222 nm. All experiments were carried out with 50 μM peptide solutions in 10 mM MOPS buffer, pH 7.0.

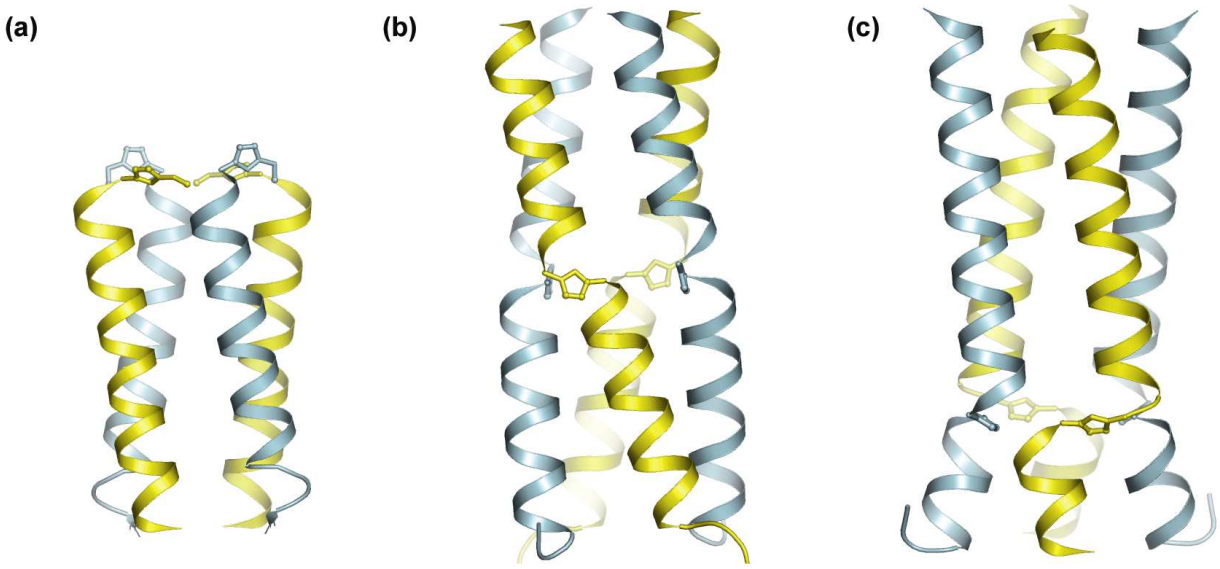


Figure S3. Ribbon representation of the crystal structure of peptides **1** (a), **2** (b), and **3** (c) with atomic positions shown for the triazole residues. Each four-helix bundle superposes a crystallographic 2-fold axis and unique chains in each structure are indicated by different colors. Electron density is not visible for the N-terminal portion of **1**, likely due to disorder in the crystal.

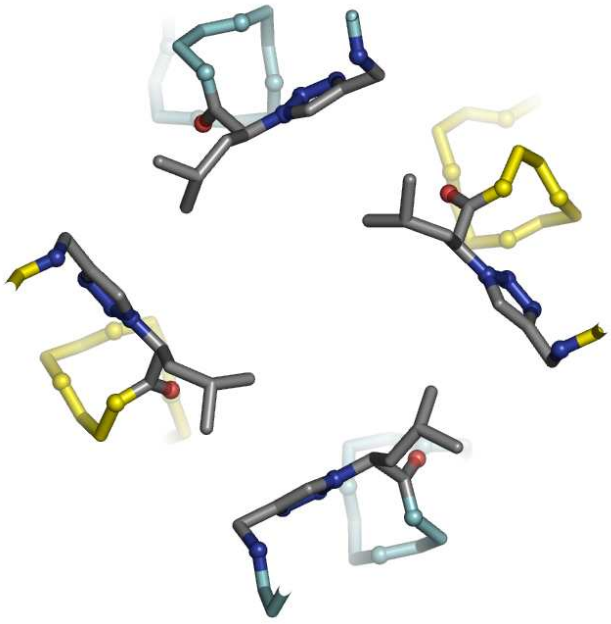


Figure S4. Top down view of the ε residues in one bundle from the crystal structure of **2**.

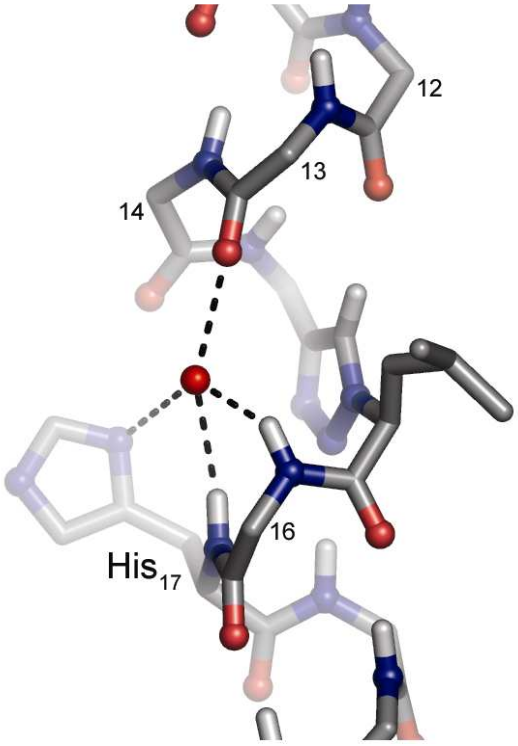


Figure S5. View of the water filled cavity adjacent to the triazole in the crystal structure of **2**.

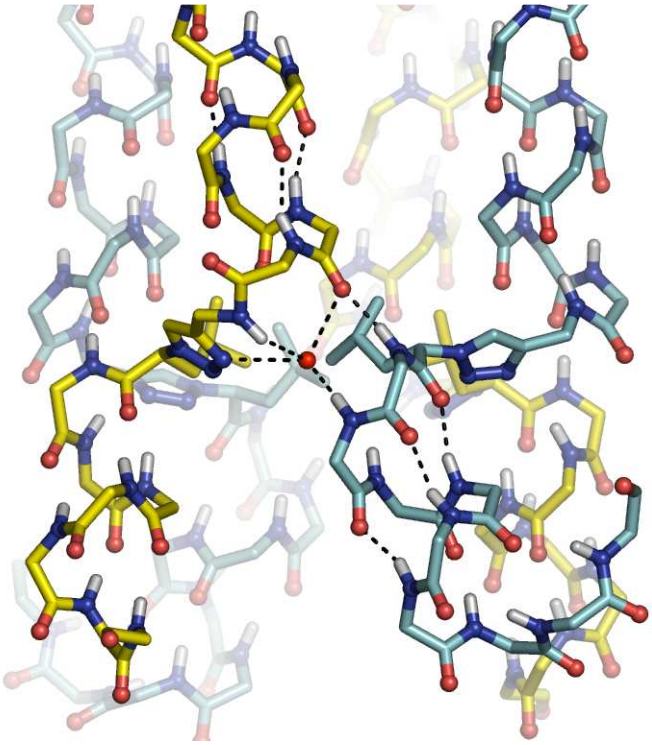
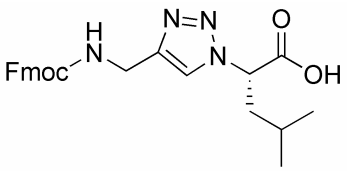


Figure S6. Alternate view of the interhelical crossing in **3**.

Experimental Methods

Materials. Fmoc-Arg(Pbf) Wang Resin, hydroxybenzotriazole (HOBT), and all protected amino acids were obtained from Novabiochem. Solvents and all other chemicals were purchased from Aldrich or Fisher and used as received. HPLC was carried out using gradients between 99:1:0.1 and 10:90:0.1 water / acetonitrile / trifluoroacetic acid.

Fmoc-L-Leu-triazole-ε²-amino acid: Fmoc-L-Leu-triazole-ε²-amino acid was prepared



in 91% yield from L-azido leucine[2] and Fmoc-propargylamine[3] as previously reported for the D-enantiomer.[4] An analytically pure sample was obtained by recrystallization from hot EtOAc/hexanes. $[\alpha]^{22}_{D}$ = +2.5 (c=10 mg/ml in MeOH); ^{1}HNMR (500 MHz, DMSO) δ 8.00 (s, 1H), 7.87 (d, *J* = 8 Hz and s, total 3H), 7.69 (d, *J* = 8 Hz, 2H), 7.40 (t, *J* = 7 Hz, 2H), 7.31 (t, *J* = 7 Hz, 2H), 5.39 (dd, *J* = 5, 11 Hz, 1H), 4.31 (d, *J* = 7 Hz, 2H), 4.27 (*J* = 6 Hz, 2H), 4.22 (t, *J* = 7 Hz, 1H), 2.16 (m, 1H), 1.93 (m, 1H), 1.15 (m, 1H), 0.85 (d, *J* = 7 Hz, 3H), 0.80 (d, *J* = 7 Hz, 3H); ^{13}CNMR 170.6, 156.2, 145.0, 143.9, 140.7, 127.6, 127.0, 125.2, 122.6, 120.1, 65.6, 60.5, 46.7, 36.0, 24.3, 22.5, 20.9; ESI-TOF MS $[M+H]^{+}$ obsd. = 435.2038 (calc. = 435.2027).

Peptide Synthesis: Peptides were prepared on Fmoc-Arg(Pbf) Wang Resin using an Advanced Chemtech 348Ω automated peptide synthesizer with DIC/HOBT mediated coupling reactions. All peptides were purified by RP-HPLC on a C_{18} column. Identity and purity of final products were confirmed by MALDI-MS and analytical HPLC respectively.

Circular Dichroism: CD measurements were carried out on an Aviv Model 62DS Circular Dichroism Spectrometer. Samples consisted of 50 μM peptide in 10 mM MOPS at pH 7.0. Peptide concentrations were determined from UV absorption at 280 nm using an extinction coefficient of 1680 mol^{-1} cm^{-1} L^{-1} based on a contribution of 1280 from tyrosine[5] and 400 from the triazole (estimated from UV measurements of a tripeptide containing the non-natural amino acid). Samples were filtered through a 0.2 μm PSU syringe filter prior to measurement. Spectra were acquired in triplicate, averaged, and background corrected against the buffer alone.

Thermal denaturing experiments were carried out in the same concentration and buffer conditions as above, monitoring $[\theta]_{222nm}$ between 4°C and 96°C in steps of 2°C. Data were analyzed using Mathematica (Wolfram Research). Temperature dependent molar ellipticity was fit to a 4th order polynomial, and T_m calculated by evaluating where the second derivative of the resulting polynomial equaled zero.

Gel Filtration Chromatography: Gel filtration chromatography was performed on a Superdex 75 10/300 column (Amersham Biosciences) eluted at 0.5 ml/min with 50 mM phosphate buffer, pH 7.0, 150 mM NaCl. A mixture containing equal volumes from stock

solutions of bovine erythrocyte carbonic anhydrase (2 mg/ml, MW 29000), horse heart cytochrome C (1 mg/ml, MW 12384), bovine oxidized insulin chain B (2 mg/ml, MW 3495), and cyanocobalamin (1 mg/ml, MW 1355) was injected as a standard. The retention times were fit to the following equation:

$$m = e^{at-b}$$

where *a* and *b* are constants, *m* is the molecular weight of the molecule, and *t* its retention time. This fit gave values for *a* and *b* that were used, in turn, to calculate *m* from values of *t* obtained from injections of peptides **1**-**3** (100 µl of 40 µM stock). This *m*, divided by the molecular weight for each peptide, gave N_{agg} as reported in Table 1.

Crystallization: Peptides were initially screened against 50 buffers from the Crystal Screen Cryo Kit available from Hampton Research. Crystallizations were performed in hanging drops consisting of 1 µl peptide (20 mg / ml in water) and 0.1 µl buffer over a well containing 0.4 ml buffer. Buffers yielding crystals were repeated in varying conditions with 1-2 µl of peptide stock and 0.1-1 µl of buffer. The individual buffers used to obtain crystals for each peptide are listed below.

Crystal Data Collection and Structure Determination: Each crystal was mounted on a cryo-loop directly from the crystallization drop. Data were collected on an Raxis IV image plate detector equipped with Osmic confocal mirrors and Xstream cryo-device (100K) using CuK_α radiation (λ = 1.5418 Å) from a Ru200 X-ray generator operated at 50 kV, 100 mA. Data were processed using MSC Crystal Clear with the exception of space group determination which was carried out using SHELX Xprep.

Structures were solved using the CCP4 software suite.[6] Molecular replacement was carried out using the program Phaser[7] with truncated helices of pLI GCN4 used as the search models.[8] This process eventually confirmed the space group chirality. Restrained refinement was carried out using iterative cycles of Refmac and manual fitting using XtalView.[9] Electron density maps were improved using free atom density modification with the program ARP/wARP.[10] After building in and refining the remaining peptide, the triazole ε^2 residues were docked to unbiased electron density occupying the appropriate region of the backbone. Restraints for bond distances and angles for the triazole portion of the non-natural residue were assembled from a survey of 1,4-disubstituted-1,2,3-triazoles in the Cambridge Structural Database. These restraints were used in remaining cycles of refinement.

Due to disorder in the termini of the chains, we observed somewhat higher R_{free} values compared to R and the resolution. This observation is consistent with structures obtained from a series of side chain substitutions in pLI-GCN4.[11]

Peptide 1: Ac-RMKQIED**X**EEILSKLYHIENELARIKKLLGER-OH
MALDI-TOF $[M+H]^+$ obsd. = 4031.1 (calc. = 4031.2)
Crystallization Buffer: 0.4 M lithium sulfate monohydrate, 12% w/v PEG 8000, 20% v/v anhydrous glycerol

Diffraction Data and Refinement Statistics	
PDB id	1U9G
Resolution (Å)	45.17 – 2.20
Unit Cell (Å, °)	a = b = c = 78.23, $\alpha = \beta = \gamma = 90$
Space Group	$P4_132$
Reflections (total / unique)	25292 / 4526
Avg. Multiplicity	5.6
Completeness (%)	100.0 (98.8)*
R_{merge}	0.031 (0.326)*
Mean $I/\sigma(I)$ unaveraged	11.0 (2.2)*
Asymmetric unit contents:	
peptide chains	2
H_2O	19
SO_4^-	1
R_{factor}	24.9
R_{free}	32.6
Average B factor (Å^2)	46.6
ϕ, ψ outliers	none

* statiscics for outer shell (2.28 – 2.20)

Peptide 2: Ac-RMKQIEDKLEEILS**X**YHIENELARIKKLLGER-OH
MALDI-TOF $[M+H]^+$ obsd. = 4030.8 (calc. = 4031.2)
Crystallization Buffer: 0.085M HEPES – Na, pH7.5, 8.5% v/v isopropanol, 17% w/v PEG4000, 15% v/v anhydrous glycerol

Diffraction Data and Refinement Statistics	
PDB id	1U9F
Resolution (Å)	31.49 – 2.20
Unit Cell (Å, °)	a = b = 67.60, c = 86.70, $\alpha = \beta = \gamma = 90$
Space Group	$P4_32_12$
Reflections (total / unique)	93963 / 10727
Avg. Multiplicity	8.8
Completeness (%)	100 (99.5)*
R_{merge} (%)	0.050 (0.314)*
Mean $I/\sigma(I)$	7.4 (2.3)*
Asymmetric unit contents:	
peptide chains	4
H_2O	79
R_{factor} (%)	25.2
R_{free} (%)	32.0
Average B factor (Å^2)	34.5
ϕ, ψ outliers	none

* statiscics for outer shell (2.28 – 2.20)

Peptide 3: Ac-RMKQIEDKLEEILSKLYHIEN**X**RIKKLLGER-OH

MALDI-TOF $[M+H]^+$ obsd. = 4030.3 (calc. = 4030.3)
Crystallization Buffer: 0.16 M magnesium acetate tetrahydrate, 0.08 M sodium cacodylate, pH 6.5, 16% w/v PEG 8000, 20% v/v anhydrous glycerol

Diffraction Data and Refinement Statistics	
PDB id	1U9H
Resolution (Å)	40.62 – 2.17
Unit Cell (Å, °)	a = b = 45.40, c = 90.92, $\alpha = \beta = \gamma = 90$
Space Group	$P4_322$
Reflections (total / unique)	33578 / 5435
Avg. Multiplicity	6.2
Completeness (%)	98.9 (95.7)*
R_{merge}	0.052 (0.310)*
Mean I/σ(I)	7.2 (2.4)*
Asymmetric unit contents:	
peptide chains	2
H_2O	46
R_{factor} (%)	24.9
R_{free} (%)	28.4
Average B factor (Å^2)	36.1
ϕ, ψ outliers	none

* statiscics for outer shell (2.25 – 2.17)

References:

(1) This nomenclature is adapted from that reported by Seebach for the description of β-amino acids; see, for example: Seebach, D.; Matthews, J. L. *Chem. Comm.* **1997**, *21*, 2015-2022.
(2) Lundquist IV, J. T.; Pelletier, J. C. *Org Lett.* **2001**, *3*, 781-783.
(3) Tong, G.; Lawlor, J. M.; Tregear, G. W.; Haralambidis, J. *J. Org. Chem.* **1993**, *58*, 2223-2231.
(4) Horne, W. S.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *125*, 9372-9376.
(5) Gill, S. C.; Vonhippel. P. H. *Anal. Biochem.* **1989**, *182*, 319-326.
(6) (a) Collaborative Computational Project Number 4. *Acta Crystallogr.* **1994**, *D50*, 760-763. (b) Potterton, E.; Briggs, P.; Turkenburg, M.; Dodson, E. *Acta Crystallogr.* **2003**, *D59*, 1131-1137.
(7) Storoni, L. C.; McCoy, A. J.; Read, R. J. *Acta Crystallogr.* **2004**, *D60*, 432-438.
(8) Harbury, P. B.; Zhang, T.; Kim, P. S.; Alber, T. *Science* **1993**, *262*, 1401-1407. PDB ID: 1GCL.
(9) McRee, D. E. *J. Mol. Graphics* **1992**, *10*, 44-46.
(10) (a) Perrakis, A.; Harkiolaki, M.; Wilson, K. S.; Lamzin, V. S. *Acta Crystallogr.* **2001**, *D57*, 1445-1450. (b) Lamzin, V. S.; Wilson, K. S. *Acta Crystallogr.* **1993**, *D49*, 129-147.
(11) Yadav, M. K.; Redman, J. E.; Gutierrez, J. A.; Zhang, Y.; Stout, C. D.; Ghadiri, M. R. unpublished results.