Supporting Information

Relationship Between Salt-Bridge Identity and 14-Helix Stability of β^3-peptides in Aqueous Buffer

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I. General Information. Fmoc-protected α-amino acids, PYBOP®, HBTu, HOBt, and Wang resin were purchased from Novabiochem (San Diego, CA). Dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), N-methylmorpholine (NMM), trifluoroacetic acid (TFA), and piperidine were purchased from American Bioanalytical (Natick, MA). CD_3OH (99.5% d_3) was obtained from Cambridge Isotopes (Andover, MA). All other reagents were purchased from Sigma-Aldrich. Mass spectra were acquired with Applied Biosystems Voyager-DE-Pro MALDI-TOF mass spectrometer (Foster City, CA). Reverse-phase HPLC was performed using a Varian ProStar HPLC system using Vydac analytical (C4, 300 Å, 5 μM, 4.6 mm x 250 mm) or semi-preparative column (C_8, 300 Å, 10 μM, 10 mm x 250 mm), or Waters SymmetryPrep semi-preparative column (C_8, 100 Å, 7 μM, 7.8 mm x 300 mm) columns, using water/acetonitrile gradients containing 0.1% TFA. Separations were performed as indicated with flow rates of 1

mL/min (analytical), and 4 mL/min or 5 mL/min (semi-preparative). β^3-peptides and Fmoc-β^3-(L)-amino acids were detected spectrophotometrically at 214 nm and 280 nm using the Varian Prostar PDA UV/VIS detector. β-peptides were synthesized using a Symphony/Multiplex peptide synthesizer (Protein Technologies, Tuscon, AZ). Circular dichroism experiments were performed on an AVIV Model 202 or Model 212 spectrometer (Proterion Corporation, Piscataway, NJ). NMR spectroscopy was performed on an 800 MHz Varian INOVA NMR spectrometer (Varian, Palo Alto, CA).

II. β-peptide preparation

A. β-amino acid synthesis

Fmoc-protected β ^{3}-L-amino acids were prepared following methods described by Seebach.[1,2-4] β-peptides were synthesized on a 25 μmole scale using standard Fmoc chemistry and Wang resin loaded with β^3-homotyrosine(OtBu) (**2, 2KE, 2OD, 2KD, 2DabD** and **2DabE**) or β^3-homoaspartate(OtBu) (**β53-1D**), as described.[5] **β53-1D, 2DabD** and **2DabE** were synthesized manually using a glass peptide synthesis apparatus with fritted glass at the top and bottom and a sidearm for addition of reagents (Ace Glass, Vineland, NJ).

Analytical data for Fmoc-β^3-HDab (Fmoc-L-β-(γ-Boc)-2,4-homodiaminobutyric acid): White powder (0.166 g, 20%), $[\alpha]_D$ = -4.2 (c=1, $CHCl_3$), IR ($CHCl_3$): 3683.7*w*, 3623.6*w*, 3019.8*m*, 2926.4*w*, 2400.3*w*, 1710.6*w*, 1514.0*w*, 1478.2*w*, 1425.5*w*, 1215.5*s*, 1045.5*w*, 929.0*w*, 795.0*s*, 669.4*s*. ^{1}H-NMR (400 MHz, CD_3COCD_3) 1.59 (tBu, 9H, s), 1.87-1.98 (CH_2, 2H, d), 2.77-2.80 (CH_2, 2H, t), 3.18-3.21 (CH_2, 1H, t), 3.46 (CH_2, 1H, s), 4.25-4.26 (CH, 1H, d), 4.41-4.44 (CH, 1H, t), 4.49-4.58 (CH_2, 2H, m), 6.14 (NH, 1H, s), 6.68-6.70 (NH, 1H, d), 7.50-8.06 (CH, 8H, m). ^{13}C-NMR (400 MHz, CD_3COCD_3, rotamers expressed in italics) *29.05*, 29.68-30.83 (CH_3, 3C, m), 36.18 (CH_2, 1C, s), 38.35 (CH_2, 1C, s), 40.23 (CH_2, 1C, s), 47.36 (CH, 1C, s), 48.52 (CH, 1C, s), 67.21 (CH_2,

1C, s), 78.93 (C, 1C, s), 121.19 (CH, 1C, s), 126.54 (CH, 1C, s), 128.34 (CH, 1C, s), 128.89 (CH, 1C, s), 142.51 (C, 1C, s), 145.56 (C, 1C, s), 173.00 (CO, C, s), 206.39-206.98 (CO, C, s), 210.37 (CO, C, s). ESI-MS: 477.3 (M + Na) (expected mass 454.5).

C. β-peptide synthesis (automated method): β-peptides **2**, **2KE**, **2OD**, **2KD** and β**53-1D** were synthesized using automated solid phase methods as described previously.[6] One cycle of peptide elongation consisted of the following steps. The loaded resin was first washed with N-methyl-2-pyrrolidone (NMP) (1 x 20 min, 3 x 3 min for initial resin swell, 3 x 30 sec between all other elongation steps) and the terminal Fmoc protecting group removed with 20% v/v piperidine/DMF (1 x 2 min, 2 x 8 min). Note that following the addition of the sixth residue an additional deprotection step, 2% v/v piperidine, 2% v/v 1,8-diazobiocyclo[5.4.0]-undec-7-ene (DBU) (1 x 8 min) is included, as reported previously.[7,8] The deprotected resin was then washed with NMP (6 x 30 sec) and treated for 30 min with a cocktail containing 3 equivalents of the appropriate β^3-amino acid, 3 eq of PyBOP® or 2.5 eq of HBTu, 3 eq HOBt, and 8 eq diisopropylethylamine (DIEA). The coupled resin was then washed once with NMP (1 x 30 sec), unreacted amino groups acetylated upon treatment with 6% v/v acetic anhydride and 6% v/v N-methyl morpholine (NMM) in NMP (1 x 8 min) and the capped resin washed with NMP (6 x 30 sec). These steps were repeated until the β-peptide sequence was complete. Once the final Fmoc protecting group was removed, the resin was washed with NMP (8 x 30 sec) and methylene chloride (8 x 30 sec) dried 20 min under N_2, and then treated for 90 min with a cleavage cocktail composed of 2% v/v water and 2% v/v tri-isopropylsilane (TIPS) in trifluoroacetic acid (TFA). The cleaved resin was washed once with the cleavage cocktail (1 x 30 sec) and the cleaved β-peptide was collected,

concentrated by rotary evaporation, washed with acetonitrile, and reconstituted in 1:1 H_2O/CH_3CN. The final product was lyophilized to dryness.

D. **β-peptide synthesis (manual method)**: The procedure for manual peptide synthesis follows closely the procedure described for automated peptide synthesis, with the following differences: **(1)** Removal of Fmoc protecting groups during the first six cycles was accomplished using 5 mL of 20% v/v piperidine in DMF (1 x 1 min, 2 x 8 min). Removal of the Fmoc protecting groups during the remaining cycles was achieved by 5 mL of 20% v/v piperidine in DMF (1 x 1 min, 2 x 8 min) followed by 5 mL of 2% v/v piperidine, 2% v/v DBU in NMP (1 x 8 min). **(2)** After the deprotection and NMP washing steps, three equivalents of the appropriate β^3-amino acid were reconstituted by a cocktail of 2.5 eq HBTu, 3 eq HOBt, and 8 eq diisopropyethylamine (DIEA) in a glass vial followed by immediate addition to the resin and a 30 min coupling step. (**3**) Unreacted amino groups were treated with Ac_2O:DIEA:NMP (1:1:6, total 5.33 mL; 1 x 8 min). Once the final Fmoc protecting group was removed, the resin was washed with NMP (9 x 4mL), dichloromethane (9 x 4 mL), and methanol (9 x 4 mL) and allowed to dry overnight under N_2. Cleavage of the β-peptide from the Wang resin occurred by shaking the resin 2h at RT in the presence of 2% v/v water, 2% v/v TIPS in TFA (10 mL). The free β-peptide was then blown through the RV frit by N_2 and collected, washed with the cleavage cocktail, rotary evaporated, washed with CH_3CN, and reconstituted in H2O/CH3CN (1:1). The final product was lyophilized to dryness.

E. **β-peptide purification and analysis**: The success of each β−peptide synthesis was assessed initially by HPLC and MALDI-TOF analysis of the crude reaction mixture. β-peptides were then purified to homogeneity by reverse-phase HPLC. The identities and purities of purified β-peptides were assessed by analytical HPLC

and mass spectrometry (Table S-1). Following purification, β-peptides were lyophilized, kept at -20 ^{0}C, and reconstituted in PBC buffer (1 mM each phosphoric, boric, and citric acids, adjusted to pH 7.0 with NaOH) immediately prior to use.

F. Mass spectrometry data for β-peptides used in this study.

β ^{3}-peptide	*[M + H]* (calc)	*[M + H]* (obs)
2	1333.4	1332.4
2KE	1361.4	1358.7
2OD	1305.4	1304.0
2KD	1333.4	1331.9
β53-1D	1361.7	1360.6
2DabD	1277.4	1277.1
2DabE	1305.4	1306.3

III. Circular Dichroism Spectroscopy: CD spectra were acquired using AVIV Model 215 or 202 spectrometers at room temperature in PBC buffer using a 2mm pathlength CD cell. Spectra represent the average of three scans (100 ms time constant, 2 nm bandwidth) and were background-corrected and smoothed over 3 data points. Each β-peptide was analyzed at a concentration of 80 μM, however for **2**, **2KE** and **2OD**, CD experiments were repeated at a concentration of 40 μM, showing no change in minimum mean residue ellipticity (MRE). For the salt titration study CD spectra were obtained at 25 ^{0}C using a 1 cm pathlength CD cell. Spectra represent one scan taken over 2 seconds with a 30 min stir averaging time.

IV. NMR Spectroscopy of β53-1D

A. Sample preparation and data acquisition

β**53-1D** (~4.4 mg) was dissolved in CD_3OH (~430 μL) for NMR analysis. All data were acquired at 10 ^{0}C on an 800 MHz Varian Inova NMR spectrometer (Varian, Palo Alto, CA) with a 5 mm triple resonance (HCN) probe equipped with triple axis (XYZ) pulsed magnetic field gradients. All pulse sequences were part of the Varian Biopack user library. Homonuclear two-dimensional (2D) NMR spectra were acquired with spectral widths of 8000 Hz in both dimensions, a 3 s recycle delay between successive scans, and acquisition times of 0.256 and

0.032 s along F2 and F1, respectively. For the z-filtered[9] 2D TOCSY[10] NMR experiment, isotropic mixing was applied for 100 ms using an ~11 kHz DIPSI-2[11] subsequence. Similarly, spin-locking during the 2D ROESY[12] NMR experiment was achieved using a 5 kHz continuous radiofrequency field applied during the 300 ms ROE mixing period. The solvent resonance was suppressed in both experiments using WET[13,14] subsequences containing 5 ms selective sinc pulses. Acquisition required a total of 15 and 52 hours due to signal averaging of 32 and 128 scans for the TOCSY and ROESY experiments, respectively. Assignments were aided by the collection of natural abundance ^{1}H, ^{13}C-HSQC NMR spectra with a 2 second recycle delay between scans, and acquisition times of 0.205 and 0.022 seconds for the F2 and F1 dimensions, respectively. Total acquisition time required for each HSQC (one for aliphatic-region carbons, one for aromatic-region carbons) was 20.3 hours due to signal averaging of 64 scans. All spectra were processed using NMPpipe[15] and analyzed using the Sparky[16] software package. Unambiguously-identified ROEs observed between sequentially non-adjacent residues are shown as solid arrows in the following diagram. There were additional potential ROEs corresponding to 14-helical structure that may have been present but were obscured by resonance overlap.

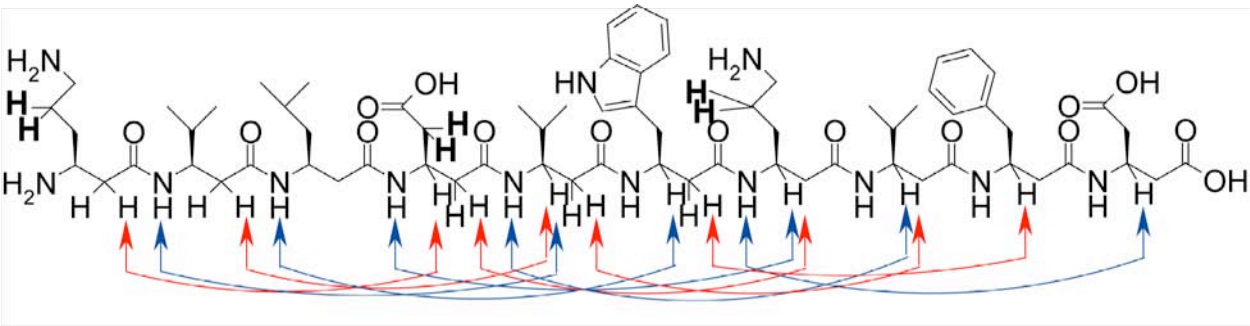

Figure. S1. Unambiguous $C_N(i)\rightarrow C_\beta(i+3)$ ROEs (red) and $C_\alpha(i)\rightarrow C_\beta(i+3)$ (blue) observed for β**53-1D** in CD_3OH.

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