

A Highly Stable Short α -Helix Constrained by a Main Chain Hydrogen Bond Surrogate

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Supporting Information

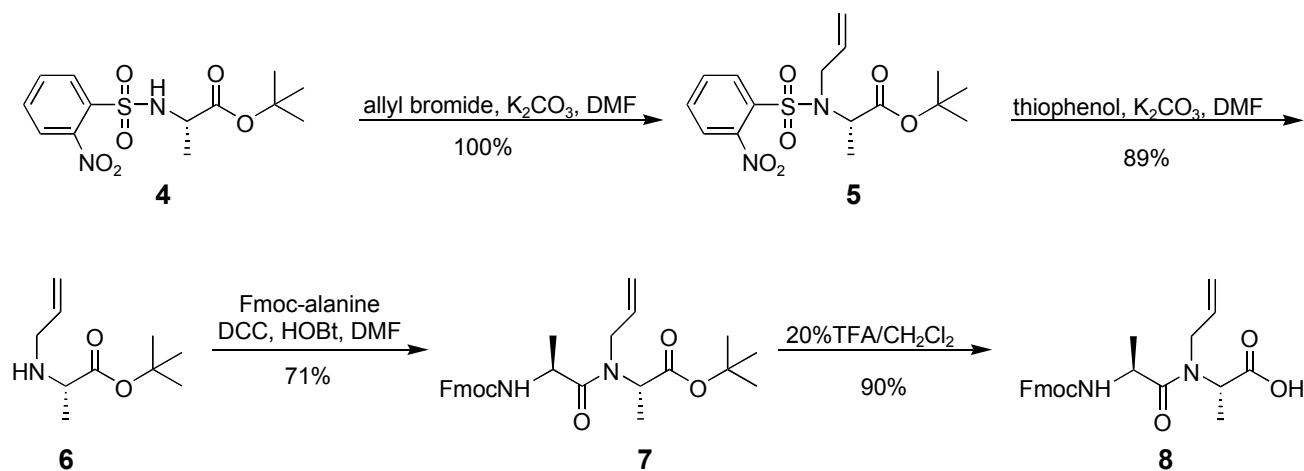
General. Commercial-grade reagents and solvents were used without further purification except as indicated. CH_2Cl_2 and DMF were dried prior to use by percolation through anhydrous Al_2O_3 as described by Grubbs and coworkers.¹ All reactions were stirred magnetically; moisture-sensitive reactions were performed under nitrogen in flame-dried glassware. Thin-layer chromatography (TLC), usually using either ethyl acetate/hexane or methanol/ CH_2Cl_2 as the solvent system, was used to monitor reactions. Visualization was accomplished by either ultraviolet light or by immersing the plate in a 1% aqueous solution of potassium permanganate and heating. Flash chromatography with silica gel was performed following the conditions described by Still and coworkers.² Solvents were removed by rotary evaporation under reduced pressure; where appropriate, the residue was further dried using a vacuum pump. Reverse-phase HPLC experiments were conducted with 4.6 x 150 mm (analytical scale) or 21.4

¹ Pangborn, A. B.; Giardello, M. A.; Grubbs, R. A.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.

² Burfield, D. R. and Smithers, R. H. *J. Org. Chem.* **1978**, *43*, 3966-3968.

x 150 mm (preparative scale) Waters C18 reverse phase columns using a Beckman Coulter HPLC equipped with a System Gold 168 Diode array detector. The typical flow rates for analytical and preparative HPLC were 1 mL/min and 8 mL/min, respectively. In all cases, 0.1% aqueous TFA and acetonitrile buffers were used. Proton NMR spectra were obtained on a Bruker AV-400 (400 MHz), Bruker AV-500 (500 MHz), or Varian-200 (200 MHz) spectrometer. Carbon NMR spectra were obtained on a Bruker (100.5 MHz) spectrometer. Proton chemical shifts are reported as δ values relative to tetramethylsilane (0.00 ppm) or to the particular solvent used in the experiment (CDCl_3 : 7.26 ppm or D_2O : 4.80 ppm). Carbon chemical shifts are reported as δ values relative to the particular solvent used in the experiment (CDCl_3 : 77.0 ppm). Data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, br = broad), coupling constant, and integration. Infrared (IR) spectra were obtained with a Thermo Nicolet Avatar 360 FTIR. High-resolution mass spectra (HRMS) were obtained by fast atom bombardment (FAB) of samples in *m*-nitrobenzyl alcohol with Cs^+ ions. FAB experiments were performed by MSU-NIH Mass Spectrometry Facility, East Lansing, MI. LCMS data was obtained on an Agilent 1100 series LC/MSD (XCT) electrospray trap.

Scheme S1. Synthesis of Dipeptide 8



***o*-NBS-N(Allyl)-Ala-OtBu (5).** Allyl bromide (0.650 mL, 7.60 mmol) was added to a solution of *o*-NBS-Ala-OtBu³ **4** (1.36 g, 4.12 mmol) and K₂CO₃ (1.15 g, 8.34 mmol) in dry DMF (20 mL). The mixture was stirred overnight, and then poured into water (20 mL). The aqueous layer was extracted with ether (3 x 30 mL) and the combined organic layers were dried (MgSO₄), filtered, and concentrated *in vacuo* to afford 1.53 g of **5** (100%) as a pale yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 8.10-8.05 (m, 1H), 7.69-7.66 (m, 2H), 7.62-7.59 (m, 1H), 5.85 (ddt, *J* = 17.2, 10.2, 6.74 Hz, 1H), 5.20 (dd, *J* = 10.2, 1.3 Hz, 1H), 5.08 (dd, *J* = 17.2 Hz, *J* = 1.2, 1H), 4.69 (q, *J* = 7.3 Hz, 1H), 4.18 (dd, AB pattern, *J* = 16.8, 6.2 Hz, 1H), 3.84 (dd, AB pattern, *J* = 16.9, 6.2 Hz, 1H), 1.48 (d, *J* = 7.3 Hz, 3H), 1.35 (s, 9H); ¹³C NMR (100 MHz) δ 170.26, 148.08, 135.11, 133.99, 133.37, 131.60, 131.27, 124.06, 117.86, 82.07, 56.64, 48.52, 27.80, 16.86; IR (film) 1733 cm⁻¹; HRMS (FAB) *m/z* for C₁₆H₂₃N₂O₆S [M+H]⁺, calcd 371.1229, found 371.1277.

HN(Allyl)-Ala-OtBu (6). Thiophenol (0.135 mL, 1.32 mmol) was added to a solution of *o*-NBS-N(Allyl)-Ala-OtBu, **5**, (0.400 g, 1.09 mmol) and K₂CO₃ (0.450 g, 3.25 mmol) in DMF (3 mL). After 2 h, the reaction mixture was poured into water (3 mL). The aqueous layer was extracted with ether (3 x 30 mL), and the combined organic layers were extracted with 1 M HCl (3 x 25 mL). The pH of the combined aqueous layers was adjusted with saturated aqueous NaHCO₃ to pH 7.5. The aqueous layer was then extracted with ether (3 x 30 mL), dried (MgSO₄), filtered, and concentrated to yield 0.18 g of **6** (89%) as a yellow oil: ¹H NMR (400 MHz, CDCl₃) δ 5.88 (ddt, *J* = 17.1, 10.2, 6.1 Hz, 1H), 5.19 (dd, *J* = 17.1, 1.5 Hz, 1H), 5.09 (dd, *J* = 10.2, 1.5 Hz, 1H), 3.29-3.11 (m, 3H), 1.47 (s, 9H), 1.26 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (100 MHz) δ 175.06, 136.41, 116.11, 80.77, 56.47, 50.47, 28.44, 19.07; IR (film) 3299, 1723 cm⁻¹; HRMS (FAB) *m/z* for C₁₀H₂₀NO₂ [M+H]⁺, calcd 186.1448, found 186.1494.

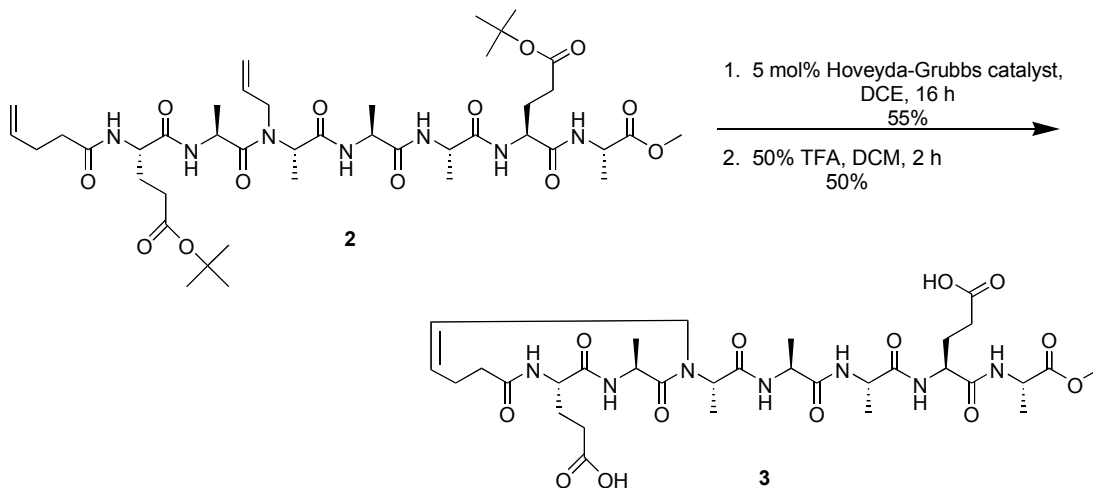
³ Turner, J. J.; Wilschut, N.; Overkleeft, H. S.; Klaffke, W.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1999**, *40*, 7039-7042.

Fmoc-Ala-N(Allyl)-Ala-OtBu (7). *N,N*-Dicyclohexylcarbodiimide (0.250 g, 1.19 mmol) was added to a solution containing Fmoc-Ala-OH (0.370 g, 1.19 mmol) and 1-hydroxybenzotriazole (0.160 g, 1.19 mmol) in DMF (10 mL). The mixture was stirred for 10 min and then **6** (0.202 g, 1.09 mmol) was added. After 18 h, the reaction mixture was filtered, poured into 10 mL water, and extracted with ether (3 x 30 mL). The organic layer was dried (MgSO₄), filtered, and concentrated. The crude product was purified by flash chromatography (5:1 hexane-ethyl acetate) to afford 0.37 g of **7** (71 %) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 5.90 (ddt, *J* = 16.9, 10.1, 8.1 Hz, 1H), 5.73 (d, *J* = 8.1 Hz, 1H) 5.23-5.31 (m, 2H), 4.72-4.79 (m, 1H), 4.63 (quintet, *J* = 7.1 Hz, 1H), 4.31-4.44 (m, 2H), 4.21 (t, *J* = 7.1 Hz, 1H), 4.05 (dd, AB pattern, *J* = 17.2, *J* = 4.4, 1H), 3.87 (dd, AB pattern *J* = 17.5, 5.0 Hz, 1H), 1.44 (s, 9H), 1.39 (d, *J* = 3.0 Hz, 3H), 1.37 (d, *J* = 2.3 Hz, 3H); ¹³C NMR (100 MHz) δ 173.15, 170.58, 155.44, 143.96, 141.29, 133.86, 127.68, 127.05, 125.18, 119.96, 117.63, 81.59, 66.92, 54.33, 48.32, 47.41, 47.17, 27.99, 19.41, 14.62; IR (film) 3299, 1730 cm⁻¹; HRMS (FAB) *m/z* for C₂₈H₃₅N₂O₅ [M+H]⁺ calcd 479.2501, found 479.2546.

Fmoc-Ala-N(Allyl)-Ala-OH (8). Fmoc-Ala-N(Allyl)-Ala-OtBu **7** (0.270 g, 0.650 mmol) was dissolved in a solution of trifluoroacetic acid (2 mL) and CH₂Cl₂ (10 mL). The mixture was stirred at room temperature and the reaction progress monitored by TLC. After 2 h, the mixture was concentrated and the residue was redissolved in 10 mL of ethyl acetate and washed with water (2 x 10 mL). The organic layer was extracted with saturated aqueous NaHCO₃ (3 x 10 mL), and the combined aqueous layers were washed with EtOAc (2 x 10 mL). The aqueous fraction was then acidified to pH 2 with concentrated aqueous HCl and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated to afford 0.250 g (90%) of **8** as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.48-7.59 (m, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.4, 2H), 5.97-5.84 (m, 1H), 5.83 (d, *J* = 8.3 Hz, 1H), 5.26-5.33 (m, 2H), 5.32-5.21 (m, 2H), 4.63-4.72 (m, 1H), 4.66-4.58 (m, 1H), 4.35 (d, *J* = 7.3 Hz, 2H), 4.18-4.22 (m, 1H), 4.08 (dd, AB pattern, *J* = 11.8,

5.4 Hz, 1 H), 4.97 (dd, AB pattern, $J = 12.8, 4.7$ Hz, 1 H), 1.48 (d, $J = 7.3$ Hz, 3H), 1.39 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz) δ 175.61, 173.94, 155.66, 143.93, 143.81, 141.28, 133.27, 127.69, 127.05, 125.22, 119.9, 118.93, 67.04, 54.06, 48.97, 47.41, 47.11, 27.59, 19.05, 15.87, 14.37; IR (film) 3400, 3297, 1719 cm^{-1} ; HRMS (FAB) m/z for $\text{C}_{24}\text{H}_{27}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$ calcd 423.1874, found 423.1920.

Scheme S2. Synthesis of constrained peptide 3.



Constrained Peptide (3). The modified octapeptide **2** was prepared by conventional Fmoc solid phase chemistry. The pre-loaded Fmoc-Ala Wang resin was purchased from NovaBiochem. The Fmoc group was removed from Fmoc-Ala (0.3mmol, 441.18mg) by treatment with 20% piperidine in NMP (2 x 20 min), followed by washings with DMF (3x), DCM (3x), MeOH (3x) and ether (3x). The resulting amine was treated with activated Fmoc-Glu(OtBu)-OH (0.750 mmol, 0.319 g), HBTU (0.675 mmol, 0.256 g) and 5% DIEA/NMP for 45 mins, followed by washings with DMF (3x), DCM (3x), MeOH (3x) and ether (3x). This procedure was repeated for the introduction of the remaining amino acids, dipeptide **8**, and pentenoic acid residues. Treatment of the resin with TEA:MeOH (1:10) at 50 °C for 16 h afforded crude **2**. Column chromatography (gradient:1% MeOH/DCM to 4% MeOH/DCM) afforded 171 mg (64.8%) of **2** as a white solid. ESIMS for $\text{C}_{42}\text{H}_{70}\text{N}_7\text{O}_{13}$ $[\text{M} + \text{H}]^+$ calcd 880.5, found 880.4.

To a solution of bis olefin **2** (60.0 mg, 68.0 μmol) in 2 mL of anhydrous dichloroethane at 50 $^{\circ}\text{C}$ was added a solution of Hoveyda-Grubbs catalyst (2.14 mg, 3.40 μmol) in 0.270 mL anhydrous dichloroethane. After 16 h, the reaction mixture was concentrated under vacuum and purified by HPLC to afford 31.8 mg (55%) of the *t*-butyl ester protected constrained peptide as a white solid. ESIMS for $\text{C}_{40}\text{H}_{66}\text{N}_7\text{O}_{13}$ $[\text{M} + \text{H}]^+$ calcd 852.5, found 852.5.

The protected peptide (20.0 mg, 23.5 μmol) was dissolved in TFA (2.5 mL) and CH_2Cl_2 (2.5 mL), and the reaction progress was monitored by TLC. After 3 h, the reaction mixture was concentrated and purified by HPLC (Figure S1) to yield the cyclized deprotected peptide **3** as a white solid in 8.7 mg (50.2%). ESIMS for $\text{C}_{32}\text{H}_{50}\text{N}_7\text{O}_{13}$ $[\text{M} + \text{H}]^+$ calcd 740.3, found 740.2.

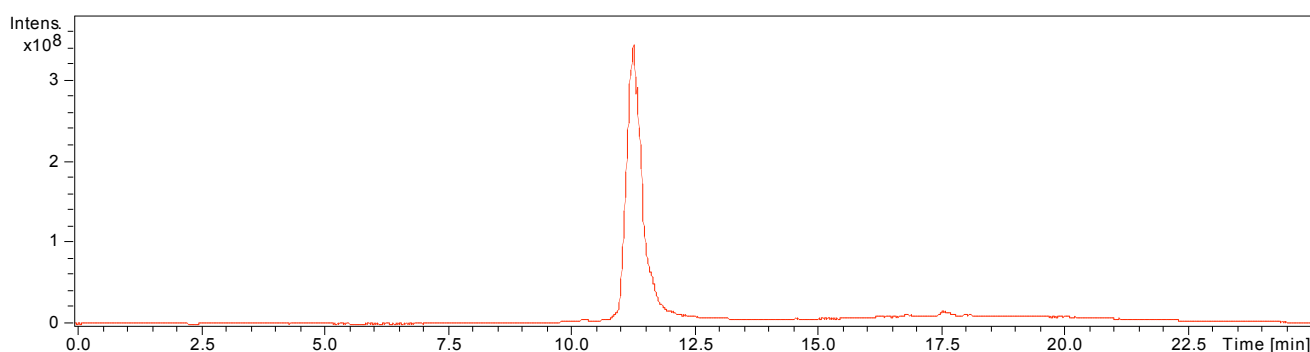
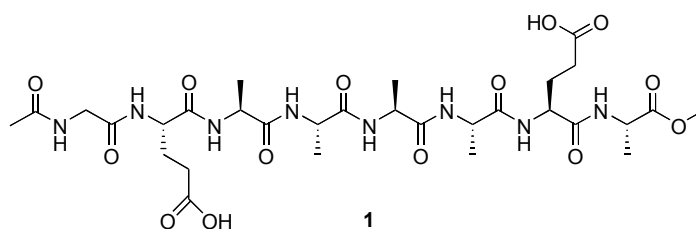


Figure S1. Reverse-phase HPLC plot of purified constrained helix **3**. HPLC conditions: C_{18} reverse phase column. 20-100% B in 15 mins; A: 0.1% aqueous TFA, B: acetonitrile; flow rate: 0.2 mL/min.



Control peptide (1). The control peptide **1** (AcGEAAAAEA-OMe) was prepared by conventional Fmoc solid phase chemistry on Wang resin and was cleaved as a methyl ester with methanol and triethylamine as described above. ESIMS for $\text{C}_{30}\text{H}_{49}\text{N}_8\text{O}_{14}$ $[\text{M} + \text{H}]^+$ calcd 745.3, found 745.7.

Circular Dichroism Studies. CD spectra were recorded on an AVIV 202SF CD spectrometer equipped with a temperature controller using 1mm path length cells. The scan speed was set to 5 nm/min and spectra were averaged over 5 scans. Spectral baselines were obtained under analogous conditions as that for the samples. All spectra are baseline subtracted, converted to a uniform scale of molar ellipticity, and replotted. The helix content of each peptide was determined from the mean residue CD at 222 nm, $[\theta]_{222}$ (deg cm² dmol⁻¹) corrected for the number of amide bonds.⁴ Percent helicity was calculated from ratio $[\theta]_{222}/[\theta]_{\max}$, where $[\theta]_{\max} = -43000^* \times [1-(2.57/n)]$ (= 27213 for $n = 7$; *this value is obtained from reference 4a). CD measurements were performed at a peptide concentration of 50 μ M in 30 mM phosphate buffer (pH 7.0) at 25 °C unless indicated otherwise. The mean residue ellipticity of the constrained peptide remains constant between the range of 25 μ M to 100 μ M indicating that aggregation is not influencing CD signal in this concentration range. The peptide solutions of desired concentrations were prepared by appropriate dilutions of 3-5 mg of the lyophilized peptide. The concentration of the peptide solution was further confirmed with the BCA protein assay kit (Pierce); control peptide **1** was used to obtain standard curve for concentration measurements with this kit. Figure S2 shows the CD spectra of **1** in phosphate buffer and in increasing concentrations of TFE; the unconstrained peptide does not show measurable helical character. Figure S3 shows the CD spectra of **3** at various temperatures between 5 °C and 95 °C. The intensity of the 222 nm band remains essentially constant while that of the 202 nm band changes slightly. Varied temperature CDs were allowed to equilibrate at the given temperature for 30 minutes before each run.

⁴ (a) Chin, D. H.; Woody, R. W.; Rohl, C. A.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15416-15421. (b) Chen, Y. H.; Yang, J. T.; Martinez, H. M. *Biochemistry* **1972**, *11*, 4120-4131.

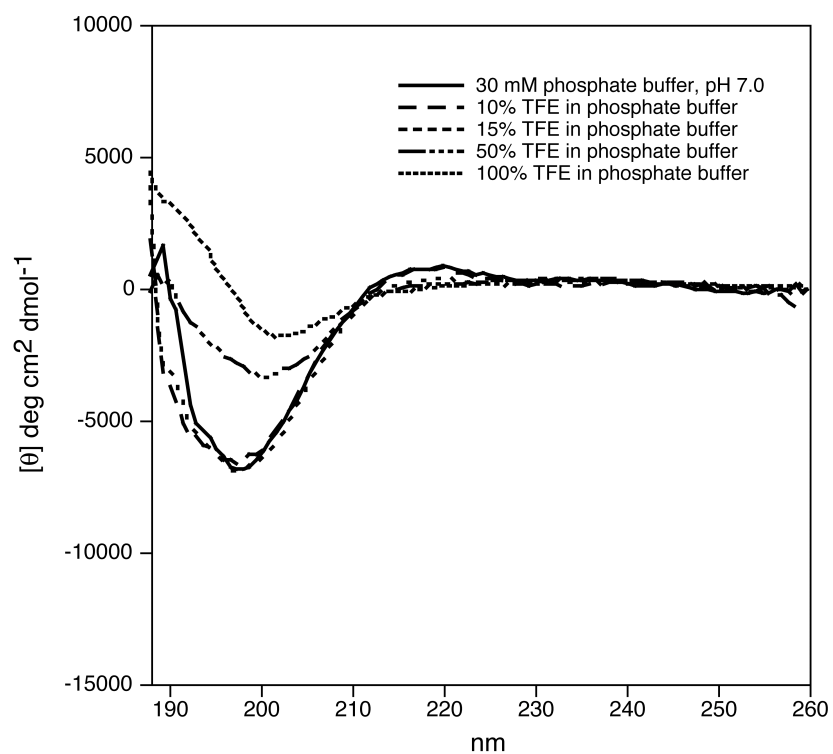


Figure S2. CD spectra of control peptide **1** in increasing concentrations of TFE.

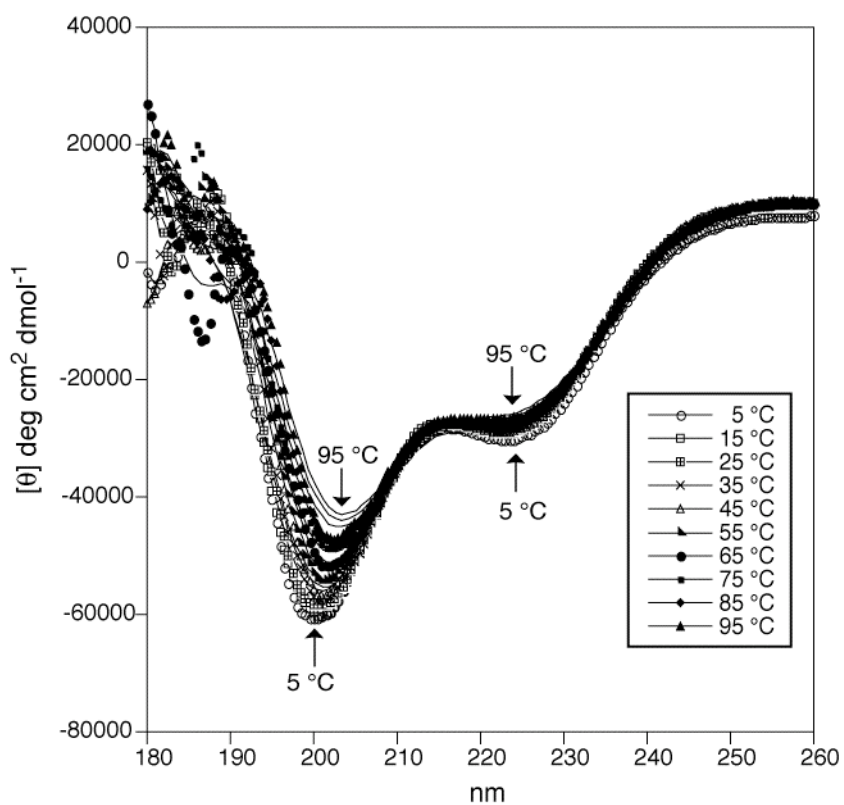


Figure S3. Effect of temperature on the helicity of **3**.

NMR experiments

¹H NMR sample preparation. The ROESY and TOCSY experiments were performed at 40 °C in slightly acidic phosphate buffer (pH 6.2) which was determined to be the optimum pH for the ROESY experiments. We used this unusually high temperature because aggregation (as detected by ¹H NMR) of this very hydrophobic peptide was observed at lower temperatures for samples at NMR concentration (1 mM) in the acidic buffer. It is a further indication of the stability of this helix that strong ROESY crosspeaks can be observed at 40 °C. All experiments were performed at a peptide concentration of 1 mM. The proton resonances for the constrained peptide were numbered according to amino acid residues X₁E₂A₃A₄^{*}A₅A₆E₇A₈, where X refers to the pentenoic acid residue and A^{*} to the *N*-allylalanine residue. These resonances were assigned by means of TOCSY and ROESY experiments.

¹H NMR TOCSY Experiments on 3. TOCSY studies on the constrained helix **3** were performed on Bruker Avance 500 spectrometer. TOCSY spectra were recorded at 313 K with a mixing time of 70 ms. An 8.1 μsec 90° pulse was employed to collect 2k points in *f2* domain and 256 points in *f1* domain. The data were processed using Bruker XWINNMR software on a HP Workstation X1100. A 90° sine-squared window function was applied in both directions. The data were zero-filled to give a final matrix of 1k by 1k real points. The TOCSY spectrum is shown in Figure S4.

¹H NMR pfg-ROESY Experiments on 3. The pfg-ROESY studies on **3** were performed on Bruker Avance 500 spectrometer. The ROESY spectra were recorded at 313 K with a mixing time of 200 ms using the pfg-ROESY pulse sequence. A ROESY continuous wave spin-lock of 1.5 KHz was used to collect 2k points in the *f2* domain and 256 points in the *f1* domain. The data were processed using Bruker XWINNMR software on an HP Workstation X1100. A 90° sine-squared window function was applied in both directions. The data were zero-filled once in *f1* domain to give a final matrix of 1k by 1k real points. The important ROESY cross-peaks are shown in Figures S5 and S6.

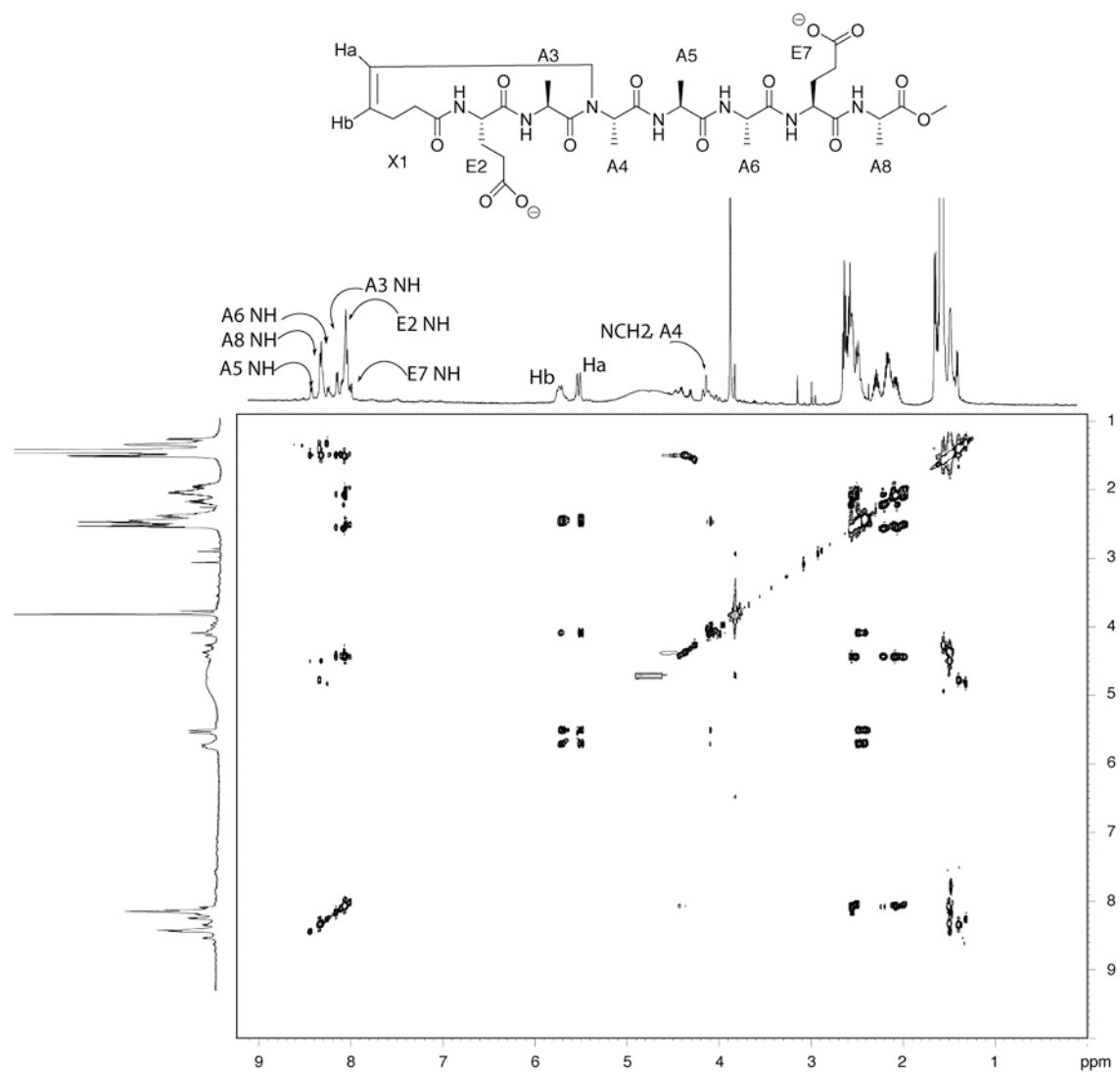


Figure S4. TOCSY spectrum of **3**. The region between 3.80 and 5.00 ppm has suppressed peaks in the 1D ^1H NMR due to the water peak suppression-related effects.

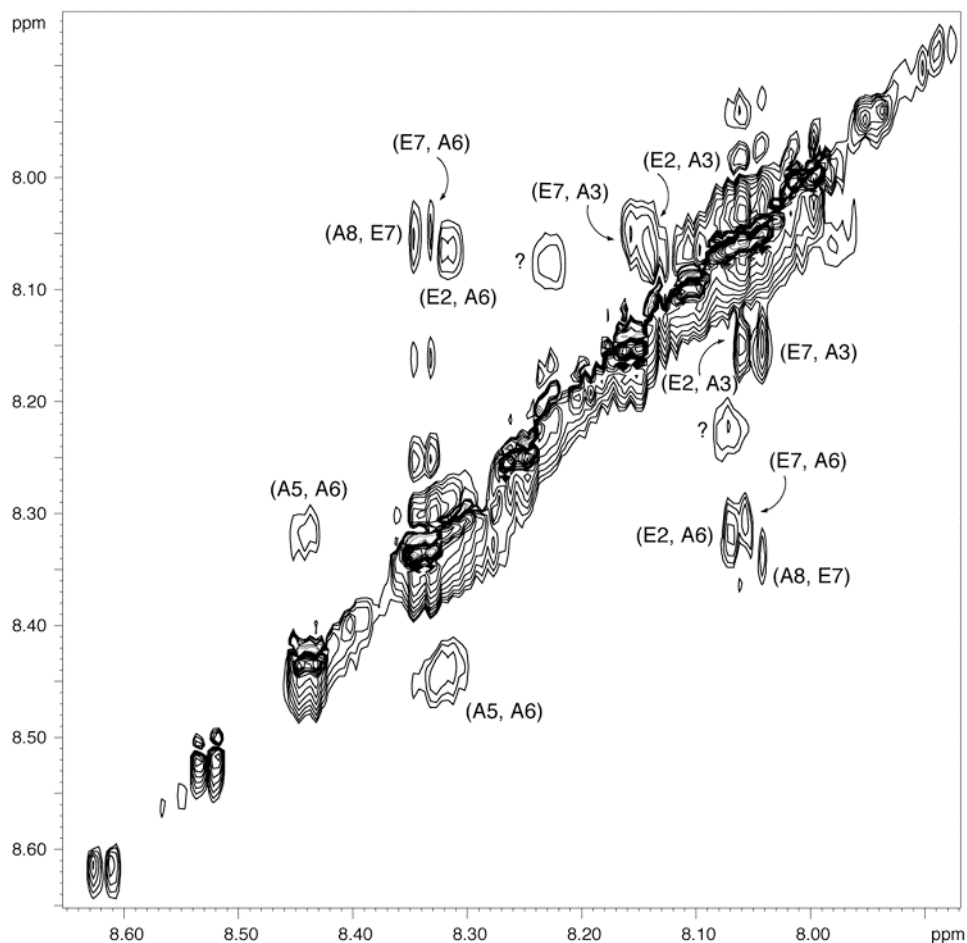


Figure S5. Amide NH-NH region from the ROESY spectrum of **3**.

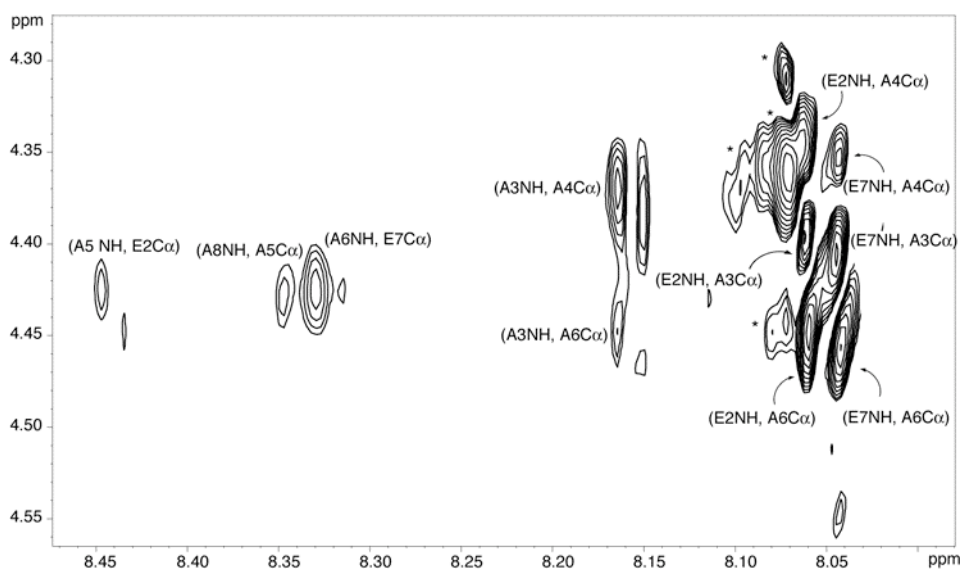


Figure S6. Amide NH-C α region from the ROESY spectrum of **3**. * indicates NOE crosspeaks (at 8.07 ppm) that result from peptide aggregation over time.