Electronic Supplementary Material (ESI) for Chemical Communications

Promoting Peptide α-Helix Formation with Dynamic Covalent Oxime Side-Chain Cross-Links

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SUPPORTING INFORMATION

Contents

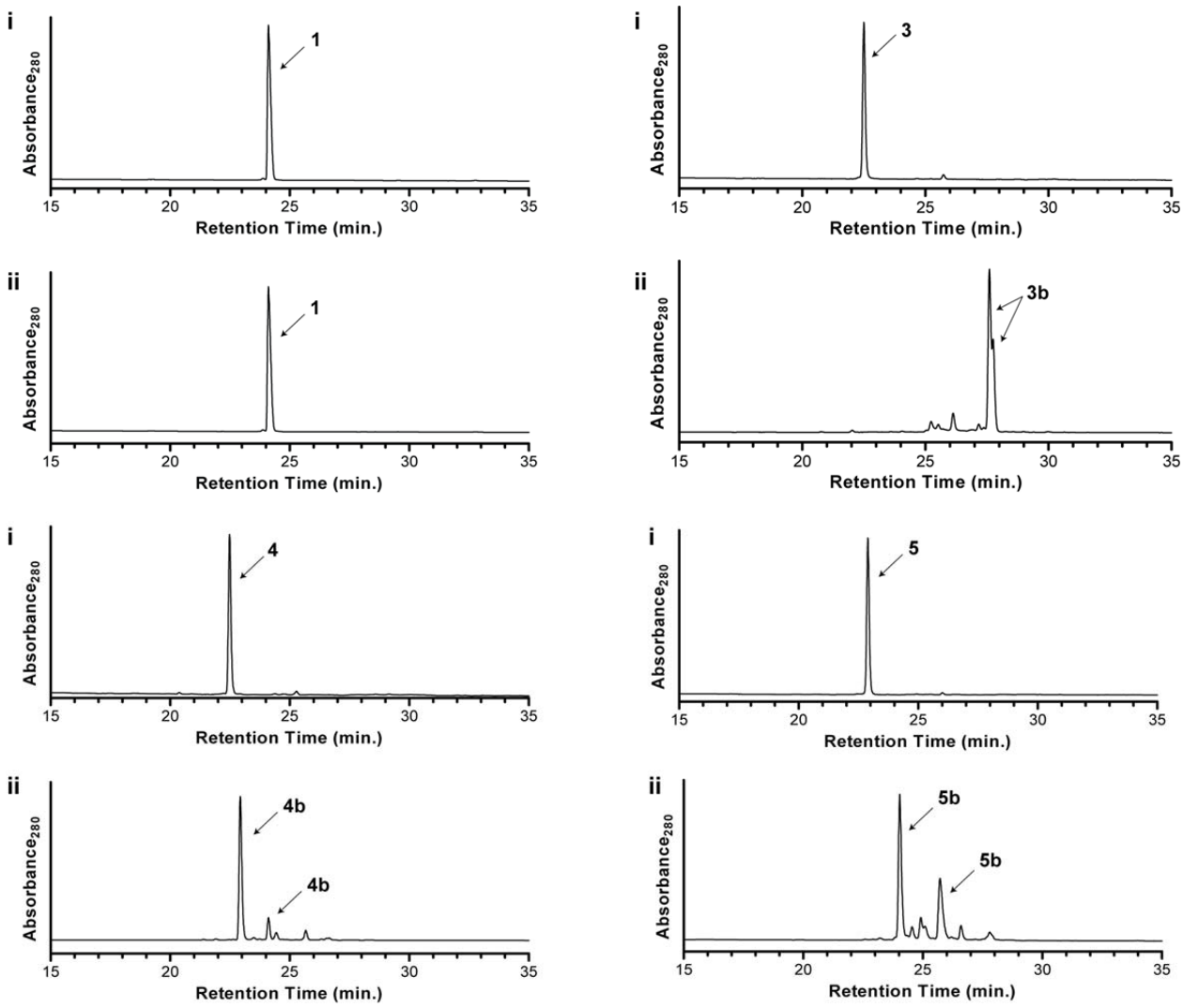


Fig. S1. HPLC analyses of peptides **1**, **3**, **4** and **5**. The purified peptide was injected prior to addition of $NaIO_4$ (i) and 10 minutes after addition of $NaIO_4$ (ii) in 100 mM phosphate buffer pH 7.

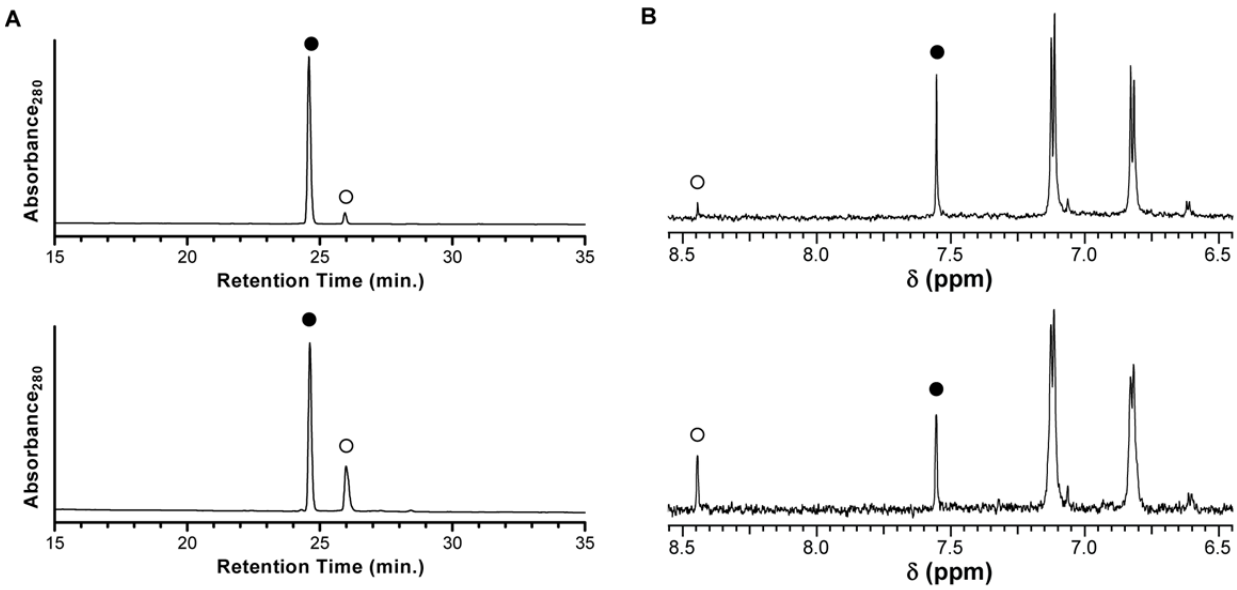


Fig. S2. Assignment of absolute stereochemistry of **2b** oxime isomers. Preparative HPLC of a reaction mixture of **2b** yields two fractions: one composed of ~95% major **2b** isomer and one composed of a mixture of major and minor isomer at a ratio similar to that in the parent reaction mixture. (A) Representative analytical HPLC traces showing the typical composition of fractions obtained after isomer separation. (B) ^{1}HNMR analysis in D_2O shows two singlets in the range expected for the oxime C−H resonance. The isomers are assigned as *E* (open circles) and *Z* (closed circles) based on the relative chemical shifts of the two signals.[S1]

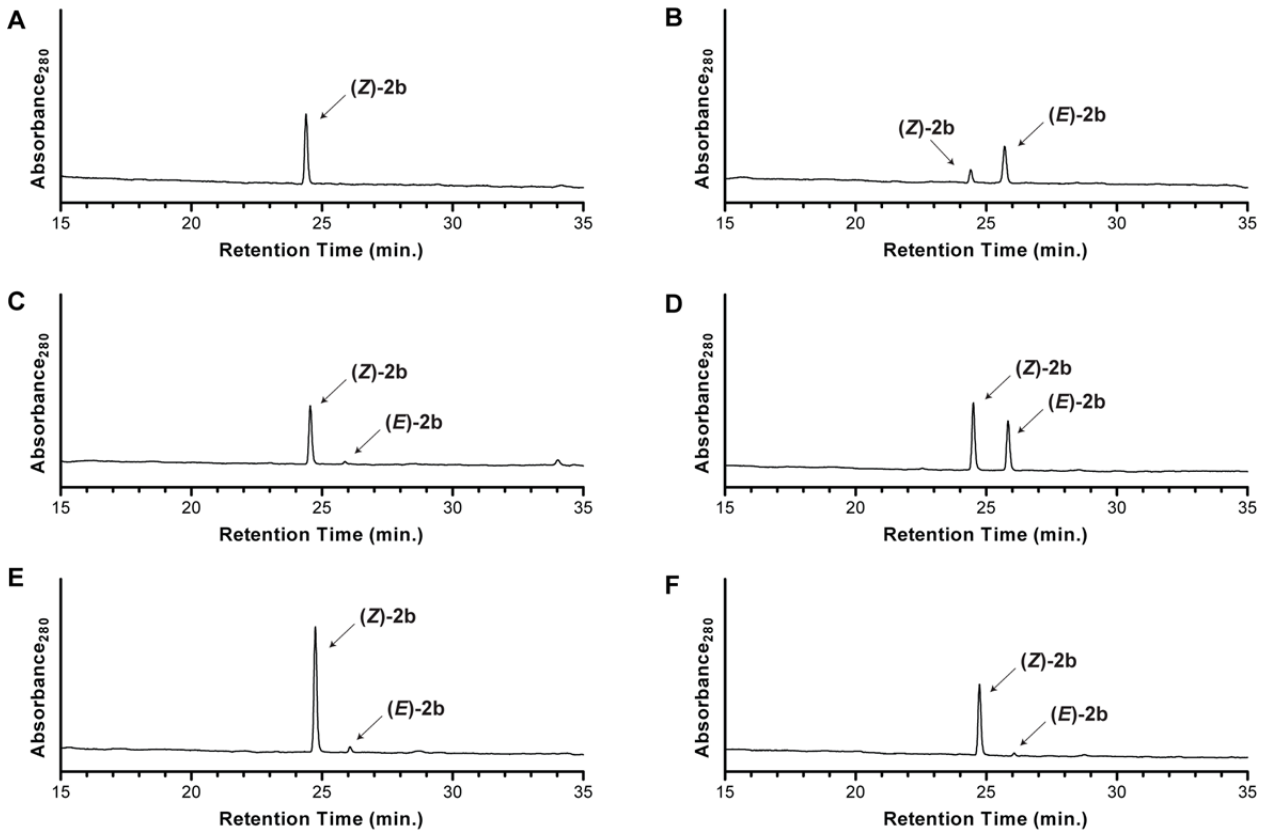


Fig. S3. HPLC analysis of the isomerization between **(*E*)-2b** and **(*Z*)-2b**. (A, B) Major (A) and minor (B) oxime isomer peaks in the oxidation/cyclization reaction of peptide **2** were isolated by analytical HPLC, frozen, and injected immediately after thawing. (C, D) Lyophilized samples enriched in **(*Z*)-2b** (C) and **(*E*)-2b** (D) were each redissolved in 100 mM phosphate buffer pH 7 and analyzed after 24 hours. Partial interconversion is seen, but the two solutions do not reach the same ratio of **(*Z*)-2b** / **(*E*)-2b**. (E, F) Lyophilized fractions enriched in **(*Z*)-2b** (E) and **(*E*)-2b** (F) were each redissolved in 0.1% TFA in H_2O and analyzed after 10 minutes. Rapid interconversion to the same **(*Z*)-2b** / **(*E*)-2b** ratio is observed.

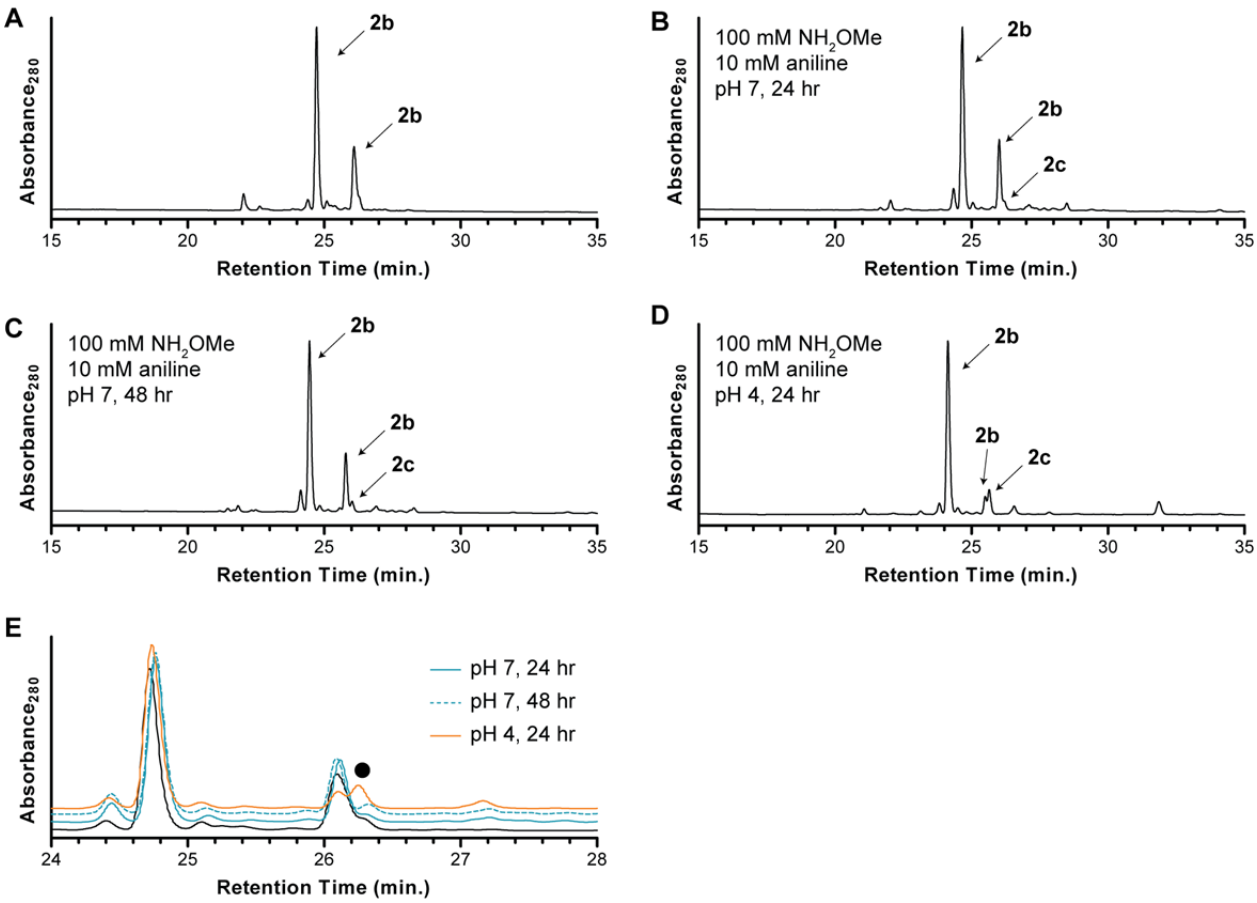


Fig. S4. HPLC analysis of the exchange reaction between cyclic oxime **2b** and NH_2OMe. (A) Oxidation and cyclization of peptide **2** was carried out as described in the text and split into two portions. (B,C) In one experiment, NH_2OMe (1000 equiv.) and aniline (100 equiv.), each buffered to pH 7, were added, and the reaction analyzed after 24 hours (B) and 48 hours (C). (D) In a parallel experiment, NH_2OMe (1000 equiv.) and aniline (100 equiv.) were added, the pH lowered to 4, and the reaction monitored after 24 hours. (E) An overlay of HPLC chromatograms from A-D illustrate the change over time in the peak assigned as **2c** (closed circle) based on MS of the isolated fraction.

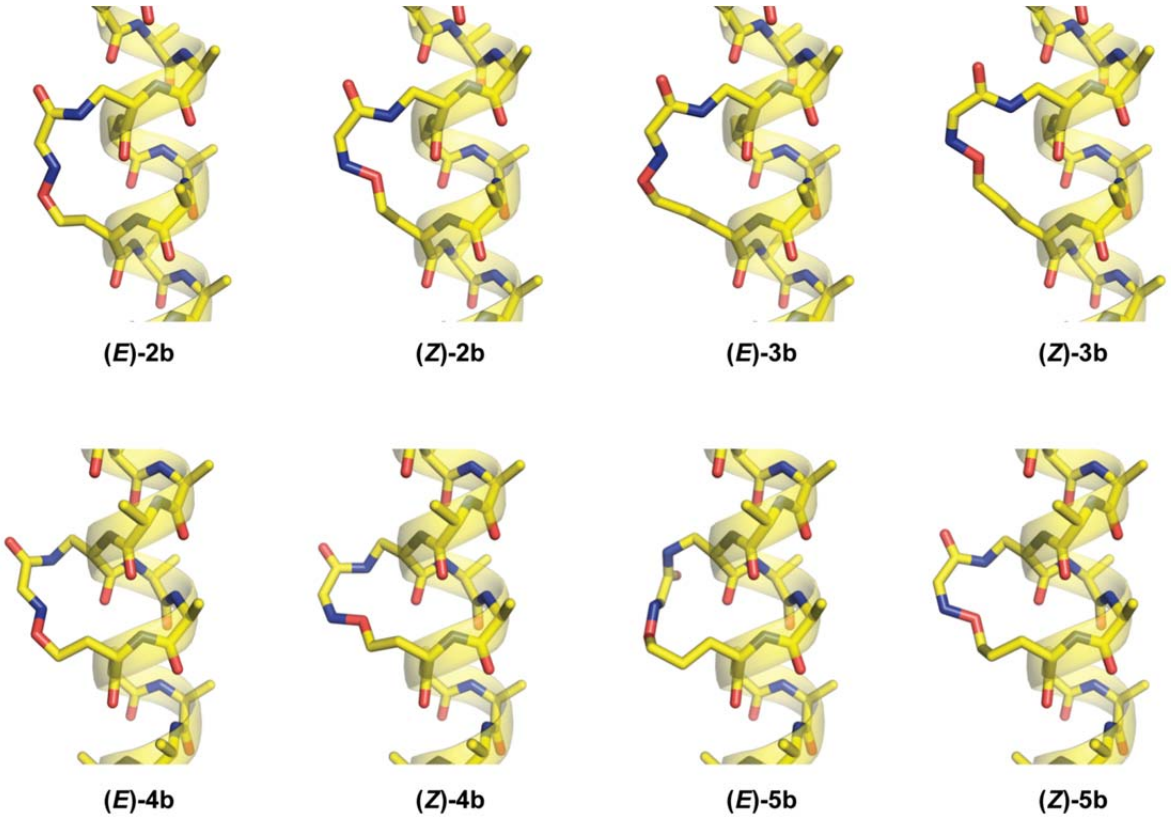


Fig. S5. Molecular models showing possible side-chain conformations of oxime bridges in peptides **2b**-**5b** in the context of an idealized α-helix. Backbone atom coordinates were fixed during minimization. Side chain amides of the **U** residues in **4b** and **5b**, regardless of oxime stereochemistry, showed significant deviation from planarity; similar deviations were not observed in **2b** or **3b**. This result supports the observation that the macrocycles in **4b** and **5b** are not compatible with an ideal α-helical fold.

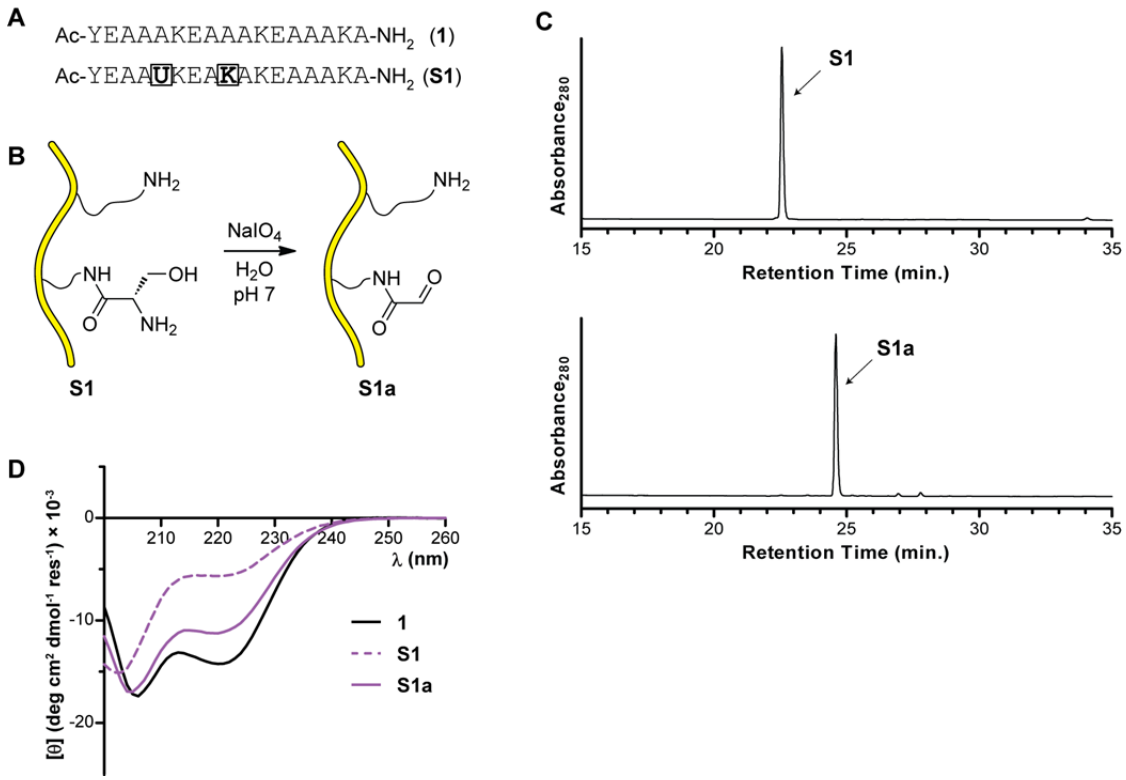


Fig. S6. (A) Sequences of control peptides analyzed by HPLC and circular dichroism. (B) Conversion of the **U** residue in **S1** to a glyoxyl aldehyde in **S1a**. (C) HPLC analysis of peptide **S1** prior to addition of $NaIO_4$ (top) and 10 min after addition of $NaIO_4$ (bottom) in 100 mM phosphate buffer pH 7. (D) Circular dichroism spectra of 100 μM solutions of control peptide **1**, peptide **S1** prior to oxidation, and after treatment with 200 μM $NaIO_4$.

Table S1. Biophysical data for peptides **1** and **2b**-**5b**.[a]

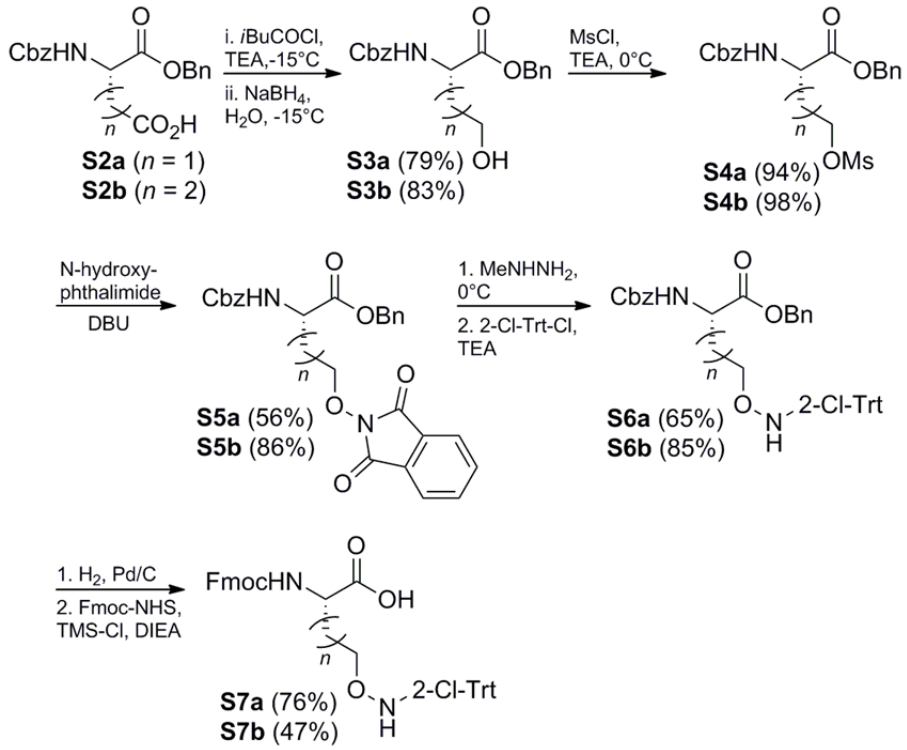
Peptide	$[\theta]_{222}$ at 20 °C (deg cm^2 dmol^{-1} res^{-1})	Fraction helix at 20 °C (%)	T_m (°C)
1	−14,000	41	3
2b	−17,100	52	16
3b	−18,000	53	21
4b	−4,600	0	–
5b	−9,000	23	−3

[a] All measurements were made on 100 μM concentration peptide solutions in 100 mM phosphate, pH 7. Details for determination of fraction helix and T_m are given in the experimental.

Table S2. MALDI data for peptides.

Peptide	$[M+H]^+$ (*m*/*z*)	
	Calculated	**Observed**
1	1704.9	1704.6
2	1851.9	1851.5
3	1865.9	1865.8
4	1851.9	1851.8
5	1865.9	1866.1
S1	1863.9	1864.0
2b	1802.9	1802.5
2c	1849.9	1850.1
3b	1816.9	1816.8
4b	1802.9	1802.3
5b	1816.9	1817.1
S1b	1854.9[a]	1854.6[a]

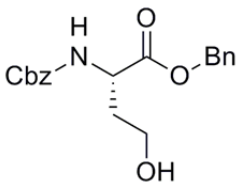
[a] Calculated and observed for $[M+Na]^+$



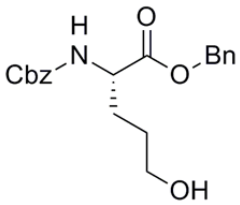
Scheme S1. Synthesis of protected amino acid monomers **S7a** and **S7b**.

General Information. Optical rotations were measured on a Perkin-Elmer 241 digital polarimiter with a sodium lamp at ambient temperature. NMR spectra were recorded on a Bruker Avance-400 spectrometer. Cbz-Asp-OBn and Cbz-Glu-OBn, 2-chlorotrityl chloride, Boc-Ser(tBu)-OH, and Fmoc-Dap-OH were purchased from Chem-Impex. 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU), (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), NovaPEG Rink Amide Resin, Fmoc-OSu, and Fmoc-protected α-amino acids were purchased from Novabiochem and used as received. Solvents and all other reagents were purchased from Aldrich, Baker, EMD, Fisher, or TCI and used as received. Flash column chromatography was performed using Silicycle SiliaFlash P60 (230-400 mesh) silica gel.

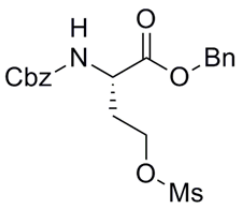
Synthesis of Monomers



S3a was synthesized by modification of a known route.[S2] Cbz-Asp-OBn (2.0g, 5.59 mmol) (**S2a**) was dissolved in 15 mL anhydrous THF and cooled to -15 °C under nitrogen. Triethylamine (858 µL, 6.16 mmol) and then isobutyl chloroformate (798 µL, 6.16 mmol) were added. Sodium borohydride (634 mg, 16.76 mmol) in 5.0 mL of water was then added. The reaction was stirred approximately 10 minutes, until vigorous bubbling stopped, and the reaction vessel poured into 100 mL water. The solution was extracted three times with 50 mL ethyl acetate. The organics were combined, washed with 50 mL brine, dried over anhydrous sodium sulfate and concentrated. The resulting pale yellow oil was purified by silica gel chromatography (25% → 50% ethyl acetate in hexanes) to yield product **8a** as a white solid (1.51 g, 4.42 mmol, 79% yield). $[\alpha]_D = +0.6°$ (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.36 (10H, m), 5.67 (1H, d, *J* = 7.4 Hz), 5.16 (4H, m), 4.60 (1H, m), 3.68 (2H, m), 2.76 (1H, br s) 2.19 (1H, m), 1.71 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.47, 156.88, 136.12, 135.23, 128.74, 128.67, 128.38, 128.25, 67.48, 67.38, 58.43, 51.45, 35.65; HRMS *m/z* calculated for $C_{19}H_{21}NO_5Na$ $[M+Na]^+$: 366.1317; found: 366.1322

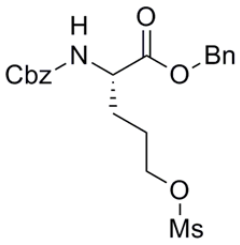


S2b (2.0g, 5.38 mmol) was converted to **S3b** by the same route as **S3a**. The product was isolated as a white solid (1.61 g, 4.49 mmol, 83%). $[\alpha]_D = -1.9°$ (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.35 (10H, m), 5.48 (1H, d, *J* = 8.0 Hz), 5.17 (2H, m), 5.10 (2H, s) 4.47 (1H, m), 3.62 (2H, t, *J* = 5.7 Hz), 1.95 (1H, m), 1.78 (1H, m), 1.56 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.40, 156.17, 136.30, 135.36, 128.71, 128.61, 128.51, 128.40, 128.26, 128.18, 67.27, 67.10, 61.99, 53.77, 29.29, 28.18; HRMS *m/z* calculated for $C_{20}H_{23}NO_5Na$ $[M+Na]^+$: 380.1474; found: 380.1477.

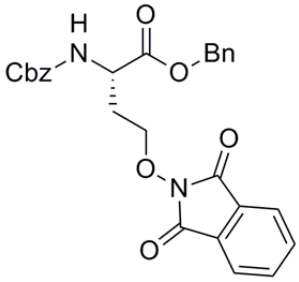


S4a was synthesized by modification of a known route.[S3] Compound **S3a** (1.41 g, 4.11 mmol) was dissolved in 10 mL anhydrous dichloromethane and cooled to 0 °C under nitrogen. Triethylamine (858 µL, 6.16 mmol) is added and the solution was stirred 15 minutes. Methanesulfonyl chloride (383 µL, 4.93 mmol) was added and reaction was stirred for one hour at 0 °C. The reaction was then diluted with 100 mL dichloromethane, and washed twice with 40 mL brine. The organics were dried over anhydrous sodium sulfate and concentrated. The resulting yellow oil (1.62 g, 3.85 mmol, 94%) was used directly in the next

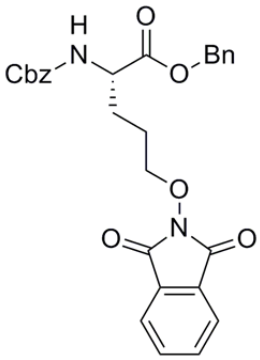
step without further purification. $[\alpha]_D$ = +3.8° (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.38 (10H, m), 5.61 (1H, d, *J* = 7.5 Hz), 5.20 (2H, s), 5.13 (2H, s), 4.57 (1H, m), 4.29 (2H, m), 2.92 (3H, s), 2.38 (1H, m), 2.19 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.34, 155.99, 136.11, 135.01, 128.76, 128.64, 128.50, 128.35, 128.19, 67.74, 67.25, 65.75, 50.97, 45.85, 37.13, 31.79.



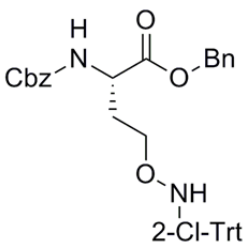
S3b (1.55 g, 4.33 mmol) was converted to **S4b** by the same route as **S4a**. The product was isolated as a yellow oil (1.85 g, 4.24 mmol, 98%). $[\alpha]_D$ = +0.6° (*c* = 1, $CHCl_3$). Spectra agreed with previously reported data.[S3]



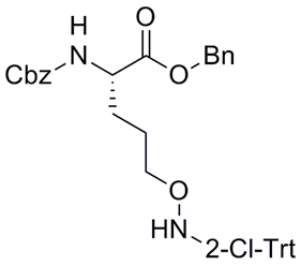
S5a was synthesized by modification of a known route.[S3] N-hydroxypthalimide (1.26 g, 7.72 mmol) was dissolved in 7 mL anhydrous DMF and cooled to 0 °C on an ice bath under nitrogen. DBU (1.15 mL, 7.72 mmol) was added and solution stirred for 20 minutes at 0 °C. The mesylate intermediate **S4a** (1.62 g, 3.85 mmol) was added in 10 mL anhydrous DMF. The reaction was allowed to warm to room temperature and stirred under nitrogen for 2 days. The reaction was then diluted into 250 mL ethyl acetate, washed twice with 75 mL water, once with 75 mL saturated aqueous sodium carbonate, and once with 75 mL brine. The resulting organic layer was dried over sodium sulfate and concentrated. The resulting oil was purified by silica gel chromatography (20% → 25% ethyl acetate in hexanes) to yield product as a white solid (1.05 g, 2.16 mmol, 56% yield). $[\alpha]_D$ = +1.0° (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.79 (4H, m), 7.29 (10H, m), 6.22 (1H, d, *J* = 8.3 Hz), 5.19 (4H, m), 4.68 (1H, m), 4.28 (2H, m), 2.35 (2H, q, *J* = 5.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.49, 163.55, 156.31, 135.41, 134.72, 128.93, 128.63, 128.58, 128.55, 128.44, 128.14, 128.09, 74.94, 67.43, 67.03, 51.68, 30.30; HRMS *m/z* calculated for $C_{27}H_{24}N_2O_7Na$ $[M+Na]^+$: 511.1481; found 511.1484.



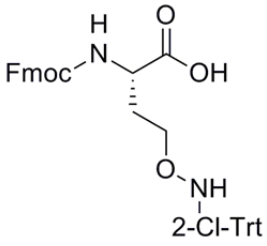
S4b (1.85 g, 4.24 mmol) was converted to **S5b** by the same route as **S5a**. The product was isolated as a white solid (1.83 g, 3.65 mmol, 86%). $[\alpha]_D$ = −6.8° (*c* = 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 7.78 (4H, m), 7.34 (10H, m), 5.51 (1H, d, *J* = 8.2 Hz), 5.20 (2H, s), 5.10 (2H, s), 4.49 (1H, m), 4.18 (2H, m), 2.16 (1H, m), 2.01 (1H, m), 1.80 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 172.13, 163.76, 156.12, 136.39, 135.40, 134.64, 129.02, 128.74, 128.62, 128.56, 128.40, 128.24, 128.16, 123.68, 77.58, 67.38, 67.08, 53.81, 28.96, 24.44; HRMS *m/z* calculated for $C_{27}H_{24}N_2O_7Na$ $[M+Na]^+$: 525.1638; found 525.1613.



S6a was synthesized by modification of a known route.[S3] **S5a** (871 mg, 1.78 mmol) was dissolved in 8 mL anhydrous methylene chloride and cooled to 0 °C under nitrogen. Methyl hydrazine (141 µL, 2.67 mmol) was added and the reaction was stirred for one hour at 0 °C. The reaction was then filtered through celite, washed with methylene chloride, and concentrated. The resulting oil was used directly in the next step. The intermediate (1.78 mmol) was dissolved in 8 mL anhydrous methylene chloride under nitrogen. Diisopropylethylamine (620 µL, 3.56 mmol) was added, followed by 2-chlorotrityl chloride (836 mg, 2.67 mmol). The reaction was stirred at room temperature for two hours. After this time, the reaction was diluted with 100 mL ethyl acetate and washed with 40 mL water, and 40 mL brine. The resulting organic layer was dried over anhydrous sodium sulfate and concentrated. Column chromatography (15% → 25% ethyl acetate in hexanes) yielded a viscous, light yellow oil (733 mg, 1.15 mmol, 65% yield). $[\alpha]_D$ = −10.0° (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 (24H, m), 5.28 (1H, d, *J* = 7.7 Hz), 5.09 (2H, s), 5.03 (2H, s), 4.70 (1H, s), 4.37 (1H, m), 3.81 (2H, m), 1.98 (2H, q, *J* = 5.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.72, 156.04, 143.11, 142.94, 140.37, 136.46, 135.56, 133.86, 132.76, 131.64, 128.81, 128.75, 128.70, 128.59, 128.49, 128.37, 128.32, 128.24, 128.00, 127.97, 127.18, 127.15, 126.49, 74.90, 70.46, 67.13, 66.98, 52.68, 30.51; HRMS *m/z* calculated for $C_{38}H_{35}ClN_2O_5Na$ $[M+Na]^+$: 657.2132; found: 657.2145.

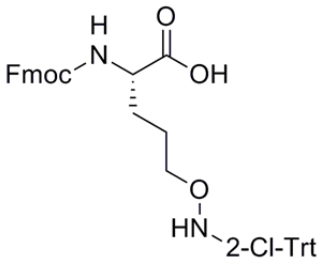


S5b (647 mg, 1.28 mmol) was converted to **S6b** as described for **S6a**. The product was isolated as pale yellow oil (709 mg, 1.09 mmol, 85%). $[\alpha]_D$ = +1.4° (*c* = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29 (24H, m), 5.19 (1H, d, *J* = 6.2 Hz), 5.12 (2H, s), 5.09 (2H, s), 4.30 (1H, m), 3.20 (2H, t, *J* = 5.4 Hz), 1.69 (1H, m), 1.48 (3H, m); ^{13}C NMR (100 MHz, DMSO) δ = 172.18, 156.12, 142.09, 141.05, 138.86, 135.92, 133.36, 131.75, 131.04, 128.81, 128.38, 128.32, 128.00, 127.81, 127.73, 127.39, 126.65, 126.49, 73.89, 71.87, 65.84, 65.47, 59.73, 53.76, 27.25, 24.50, 20.73, 14.06; HRMS *m/z* calculated for $C_{39}H_{37}ClN_2O_5Na$ $[M+Na]^+$: 671.2288; found: 671.2299.

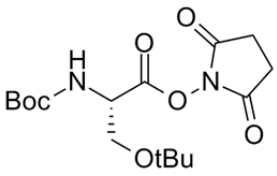


S7a was synthesized by modification of a known route.[S3] **S6a** (643 mg, 1.01 mmol) was dissolved in 8 mL anhydrous methanol. The reaction vessel was flushed with nitrogen and Pd/C, 20 wt% (50 mg, 8% w/w) was added. The vessel was fitted with a hydrogen-filled balloon and stirred until TLC showed complete loss of starting material (2 hours). After this time, the reaction was filtered through celite, washed with methanol, and concentrated. The resulting white solid was dissolved in 8 mL anhydrous methylene chloride and placed under nitrogen atmosphere. Diisopropylethylamine (703 µL, 4.04 mmol) was added, followed by addition of trimethylsilyl chloride (256 µL, 2.02 mmol). The reaction was stirred for 15 minutes. Fmoc-OSu (408 mg, 1.21 mmol) was then added and the reaction stirred overnight under nitrogen. Following this time, the reaction was diluted with 100 mL ethyl acetate, washed with 25 mL saturated aqueous ammonium chloride solution, and 25 mL saturated aqueous sodium chloride solution. The resulting organic layer was dried over sodium sulfate and concentrated. Flash column chromatography (33% → 100% ethyl acetate in hexanes) yielded a white solid (489 mg 0.773

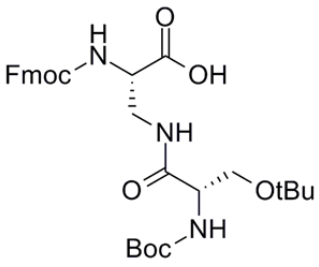
mmol, 76%). $[\alpha]_D = -2.7°$ (c = 1, $CHCl_3$). ^1H NMR (400 MHz, DMSO) δ = 12.31 (1H, s), 7.90 (1H, d, J = 7.5 Hz), 7.73 (1H, m), 7.58 (1H, s), 7.52 (2H, m), 7.42 (2H, m), 7.29 (17H, m), 5.03 (1H, s), 4.25 (2H, m), 3.90 (1H, m), 3.61 (2H, m), 1.84 (1H, m), 1.70 (1H, m); ^{13}C NMR (100 MHz, DMSO) δ = 173.76, 156.01, 143.83, 142.07, 140.97, 140.72, 136.97, 133.36, 131.79, 131.09, 128.86, 128.33, 127.77, 127.47, 127.45, 127.07, 126.74, 126.59, 125.30, 120.11, 79.18, 73.94, 69.47, 65.61, 65.40, 51.02, 46.63, 29.69; HRMS *m/z* calculated for $C_{38}H_{33}ClN_2O_5Na$ $[M+Na]^+$: 655.1976; found: 655.1963.



S6b (640 mg, 0.990 mmol) was converted to **S7b** as described for **S7a**. The product was isolated as a white solid (302 mg, 0.466 mmol, 47%). $[\alpha]_D = +9.5°$ (c = 1, $CHCl_3$). ^1H NMR (400 MHz, DMSO) δ = 12.16 (1H, s), 7.90 (1H, d, J = 7.5 Hz), 7.72 (1H, d, J = 7.5 Hz), 7.54 (3H, m), 7.42 (2H, t, J = 7.4 Hz), 7.30 (17H, m), 5.03 (1H, s), 4.27 (2H, m), 3.85 (1H, m), 3.53 (2H, m), 1.53 (1H, m), 1.42 (3H, m); ^{13}C NMR (100 MHz, DMSO) δ = 173.84, 156.07, 143.84, 143.77, 142.16, 141.09, 140.69, 137.00, 133.39, 131.08, 128.84, 128.31, 127.62, 127.43, 126.04, 126.69, 126.54, 125.27, 120.08, 114.15, 79.18, 73.92, 72.13, 65.56, 65.35, 53.64, 46.65, 27.41, 24.72; HRMS *m/z* calculated for $C_{39}H_{35}ClN_2O_5Na$ $[M+Na]^+$: 669.2132; found: 669.2129.



Boc-Ser(*t*Bu)-NHS was prepared by modification of a known route.[S4] Boc-Ser(*t*Bu)-OH (500 mg, 1.91 mmol) was dissolved in 7.5 mL water and 7.5 mL dioxane. N-hydroxysuccinimide (242 mg, 2.10 mmol) was added and the reaction was cooled to 0 °C under nitrogen. Dicyclohexylcarbodiimide (433 mg, 2.10 mmol) was added. The reaction was allowed to warm to room temperature and stirred for 3 hours. Following this time, the reaction was filtered through celite, washed with ethyl acetate and concentrated. The resulting oil was dissolved in 50 mL ethyl acetate and washed twice with 15 mL saturated aqueous sodium bicarbonate solution, twice with 15 mL water, and twice with 15 mL brine. The organics were then dried over anhydrous sodium sulfate and concentrated to yield a white solid (606 mg, 1.69 mmol, 88%). ^1H NMR (400 MHz, $CDCl_3$) δ = 5.40 (1H, d, J = 9.0 Hz), 4.75 (1H, m), 3.89 (1H, m), 3.64 (1H, m), 2.80 (4H, s), 1.43 (9H, s), 1.18 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.59, 166.96, 155.13, 80.43, 73.94, 61.81, 53.07, 28.34, 27.22, 25.64; HRMS *m/z* calculated for $C_{16}H_{26}N_2O_7Na$ $[M+Na]^+$: 381.1638; found: 381.1645.



S8 was prepared by modification of a known route.[S4] Fmoc-diaminopropionic acid (Fmoc-Dap-OH) (545 mg, 1.69 mmol) was suspended in 5 mL anhydrous methylene chloride under nitrogen. N-methyl-N-(trimethylsilyl) trifluoroacetamide (713 µL, 3.85 mmol) was added and the reaction was refluxed overnight. After this time, Boc-Ser(tBu)-NHS (606 mg, 1.69 mmol) was added and the reaction was stirred for 3 days. The reaction was diluted with 50 mL ethyl acetate and washed twice with 20 mL brine. Column chromatography (0% → 5% methanol in methylene chloride) yielded a white solid (394 mg, 0.69 mmol, 41% yield). $[\alpha]_D = +11.2°$ (c = 1, $CHCl_3$). ^1H NMR (400 MHz, DMSO) δ = 12.84 (1H, s), 8.33 (1H, s), 7.95 (1H, m), 7.90 (2H, d, J = 7.53), 7.70 (2H, d, J = 7.53), 7.49 (1H, d, J = 8.0), 7.43 (2H, t,

J = 7.28), 7.35 (2H, m), 6.57 (1H, d, *J* = 8.3), 4.32 (1H, m), 4.24 (2H, m), 4.09 (1H, m), 4.03 (1H, m), 3.62 (1H, m), 3.46 (1H, m), 3.40 (1H, m), 3.26 (1H, m), 1.38 (9H, s), 1.09 (9H, s); ^{13}C NMR (100 MHz, DMSO) δ = 171.98, 156.01, 155.16, 143.80, 143.75, 140.74, 127.66, 127.12, 125.24, 120.15, 79.20, 78.18, 72.78, 65.78, 61.95, 55.05, 53.99, 46.61, 28.17, 26.17, 25.25; HRMS *m/z* calculated for $C_{30}H_{39}N_3O_8Na$ $[M+Na]^+$: 592.2635; found: 592.2648.

Peptide Synthesis and Purification. Peptides **1-5** were synthesized by standard microwave-assisted Fmoc solid-phase synthesis techniques on NovaPEG Rink Amide resin. Amino acids **S7a** and **S7b** were coupled to resin with PyBOP, due to the observation of guanidinium-capped peptide impurities at the site of inclusion of these monomers in initial peptide synthesis attempts using HCTU. Cleavage of the peptides from resin was achieved by treatment with 94% trifluoroacetic acid, 2.5% ethanedithiol, 2.5% water, and 1% triisopropylsilane for 2 to 3 hours. Following cleavage from resin, the peptides were precipitated from the concentrated TFA solution by addition of diethyl ether (~20 mL). Following centrifugation, the ether was decanted and the peptide pellet suspended in 0.1% TFA in water for purification. Peptides were purified by HPLC on a Phenomenex Luna (Phenomenex cat. #00G-4253-P0-AX) C_{18} preparative column using gradients between 0.1% TFA in water and 0.1% TFA in acetonitrile. HPLC fractions containing the desired peptide product were pooled, frozen immediately and lyophilized. Formation of an unidentified contaminant was observed when an aminooxy peptide was allowed to remain in acetonitrile solution for extended periods,[S5] necessitating immediate freezing of desired fractions after elution. Identity of the purified peptides was confirmed by mass spectroscopy using a Voyager DE Pro MALDI-TOF instrument. A table of calculated and observed *m/z* values is included (Table S2). Peptide purity was determined by analytical HPLC on a Phenomenex Luna C_{18} (Phenomenex cat. #00G-4252-E0) column (Fig. S1).

Peptide Oxidation and HPLC Analysis. Peptide stock solutions of **1-5** and **S1** were each prepared in 100 mM aqueous sodium phosphate buffer, pH 7. Peptide concentrations were determined on Tyr absorbance at 276 nm (ε = 1450 M^{-1} cm^{-1}).[S6] Oxidation reactions were performed by addition of aqueous sodium periodate to a final concentration of 100 μM peptide and 200 μM sodium periodate in 100 mM sodium phosphate buffer at pH 7. Aliquots were removed from the reaction solution, diluted by 10% with 0.1% TFA, and immediately analyzed by HPLC on Phenomenex Luna C_{18} column (cat. #00G-4252-E0). Complete consumption of the starting peptide was observed within 10 minutes with the formation of two new peaks comprising 85-90% of observed peptide. The identity and ratio of the new peaks did not change significantly over the course of two days in aqueous buffered solution. The major peaks by HPLC were isolated by collection of the eluent from analytical HPLC and identified by MALDI-TOF MS on a Voyager DE Pro instrument (Table S2).

Assignment of Absolute Stereochemistry of 2b Isomers. An oxidation reaction of **2** was carried out as described above on large scale (2 mL, 100 μM peptide, 200 μM $NaIO_4$). After 15 minutes, the oxidized peptide was diluted by 10% with 0.1% TFA solution and injected onto preparative-scale HPLC. Two fractions, corresponding to the peak for each **2b** oxime isomer, were collected, frozen immediately, and lyophilized. It is difficult to obtain a pure sample of the minor isomer on scale; it partially equilibrates to the major isomer in the time it takes to freeze fractions collected from preparative HPLC. Nevertheless, using the above method, we were able to routinely obtain (1) a sample composed of ~95% major **2b** isomer and (2) a sample with a

ratio of major/minor isomer similar to that in the parent reaction mixture but free of other impurities. The lyophilized powder from each fraction was taken up into D_2O containing 100 mM phosphate buffer at pH 7 (uncorrected) with 20 μM DSS as an internal standard. The sample was allowed to stand for 1 hour to fully exchange amide protons with deuterium. Following this time, the samples were analyzed by ^{1}HNMR at 700 MHz using excitation sculpting to suppress the residual H_2O signal. Two singlets were observed in the region expected for the oxime C–H (Fig. S2); isomers are assigned as *E* and *Z* based on the relative chemical shift of the oxime C–H resonance.[S1]

Oxime Isomer Exchange Experiments. An oxidation reaction of peptide **2** was carried out as described above. Peaks corresponding to the E- and Z- oxime isomers were collected from the analytical HPLC eluent (total volume ~300 μL). Approximately one third of the eluent was frozen until completion of the previous HPLC run, then thawed and reinjected immediately to confirm composition of the isolated material. Partial conversion of **(*E*)-2b** to **(*Z*)-2b** was observed even in the short time between thawing and injection. The remaining frozen eluent of each peak was lyophilized. Following lyophilization, samples of each isomer were dissolved in either (1) 200 μL of 100 mM sodium phosphate buffer at pH 7.0 or (2) 200 μL of 0.1 % TFA in water. In 0.1% TFA / water, it was observed that **(*Z*)-2b** and **(*E*)-2b** rapidly interconvert and reach the same product ratio within minutes. At neutral pH, there is some interconversion, but the rate of isomerization is slower, and the reaction does not reach equilibrium at up to 24 h.

Oxime Exchange Experiments. Stock solutions were prepared of $NH_2OMe{\cdot}HCl$ at 1 M concentration in 100 mM phosphate at either pH 7 or pH 1. An aniline stock solution was prepared at 0.1 M in 100 mM phosphate at pH 7. An oxidation/cyclization reaction of peptide **2** was performed by addition of sodium periodate to a final concentration of 125 μM peptide and 250 μM sodium periodate in 100 mM phosphate buffer at pH 7. Following oxidation, the reaction was split into two aliquots. Each aliquot was diluted with a combination of NH_2OMe stock, aniline stock, and 100 mM phosphate to give the following final compositions: (1) 100 μM peptide, 100 mM NH_2OMe (from the pH 7 stock), 10 mM aniline, 100 mM phosphate, pH 7; (2) 100 μM peptide, 100 mM NH_2OMe (from the pH 1 stock), 10 mM aniline, 100 mM phosphate, pH 4. The reactions were monitored by collection of HPLC eluent and MALDI-TOF MS (Fig. S3, Table S2) as described above.

Circular Dichroism Spectroscopy. Measurements were made on an Olis DSM17 Circular Dichroism Spectrometer using quartz cuvettes with a 0.2 cm path length. 100 μM concentration peptide solutions were prepared in 100 mM phosphate buffer at pH 7.0, with or without 200 μM sodium periodate. CD scans were carried out from 260 to 200 nm with 5 second averaging times, 1 nm step size, and 2 nm bandwidth at 20 °C. Spectra were corrected for a buffer blank and baseline molar ellipticity at 260 nm. Variable temperature CD data were obtained by monitoring molar ellipticity at 222 nm from 2-95 °C at 3 °C intervals with a dead band of 0.5 °C, 2 minute equilibration time between data points and 5 second averaging times. Scan data were smoothed by the Savistsky-Golay method as implemented in GraphPad Prism. The % helicity for each peptide was calculated as previously described.[S7] Fraction helical content (f_{helix}) was calculated from eq. 1

$$f_{helix} = ([\theta]_{222} - [\theta]_{coil})/([\theta]_{helix} - [\theta]_{coil}) \qquad [1]$$

where $[\theta]_{222}$ is the observed molar ellipticity at 222 nm, $[\theta]_{helix}$ is the molar ellipticity at 222 nm for a fully folded helix and $[\theta]_{coil}$ is the molar ellipticity at 222 nm for a random coil. Values of $[\theta]_{coil}$ for each peptide were determined from high-temperature baselines in thermal unfolding experiments. The value of $[\theta]_{helix}$ was calculated to be −29,800 deg cm^2 dmol^{-1} res^{-1} according to eq. 2

$$[\theta]_{helix} = (-44000 + 250T)(1 - k/n) \qquad [2]$$

where T is the temperature in °C, n the number of residues in the peptide, and k a constant.[S7] We used a value of $k = 4.0$ in the calculations based precedent suggesting this value is appropriate for helices longer than ~11 residues.[S7] Temperature-dependent CD data were fit to simple two-state unfolding model to obtain apparent melting temperatures (T_m). The fully folded baseline used in the fit was determined from eq. 2.

Molecular Modeling. Computations of energy minimized coordinates were carried out using the Scigress software package (Fujitsu). Coordinates for an Ala_{17} peptide in an idealized α-helix fold were built. Backbone atoms were locked, and oxime side chains corresponding to peptides **2b**-**5b** were constructed (*E* and *Z*-oxime isomers for each peptide). The eight starting models were subjected to MM3 molecular dynamics (100 ps at 5000 K) to generate a randomized set of side chain conformers. For each molecular dynamics run, five conformers were selected at random and minimized. The lowest energy model for each peptide/isomer combination that maintained the starting oxime stereochemistry is shown in Fig. S5. This protocol is not meant to represent an exhaustive search for a global energy minimum. Instead, it is intended to provide a plausible side chain conformation for each oxime macrocycle size in the context of an idealized α-helix. The significant distortions from idealized geometry observed in the oxime bridges of peptides **4b** and **5b** suggest incompatibility of the macrocycles based on $i \rightarrow i+3$ residue spacing with an α-helical fold.

Supporting Information References

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