

# Peptide Bond Isosteres: Ester or (*E*)-Alkene in the Backbone of the Collagen Triple Helix

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Page	Contents
S1	Table of Contents
S2–S4	Experimental Procedures
S5	Figure S1
S6	<sup>1</sup> H NMR spectrum of <b>3</b>
S7	<sup>1</sup> H NMR spectrum of <b>4</b>
S8	<sup>13</sup> C NMR spectrum of <b>4</b>
S9	<sup>1</sup> H NMR spectrum of <b>5</b>
S10	<sup>13</sup> C NMR spectrum of <b>5</b>
S11	<sup>1</sup> H NMR spectrum of <b>6</b>
S12	<sup>13</sup> C NMR spectrum of <b>6</b>

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**General Experimental.** Reagents were obtained from Aldrich Chemical or Fisher Scientific, and used without further purification. Amino acids and their derivatives were obtained from Fisher Scientific, Bachem Bioscience, or Novabiochem. Dichloromethane was distilled over CaH<sub>2</sub>(s) or drawn from a Baker Cycletainer. Thin-layer chromatography was performed by using aluminum-backed plates coated with silica gel containing F<sub>254</sub> phosphor and visualized by UV illumination or staining with I<sub>2</sub>, *p*-anisaldehyde stain, or phosphomolybdic acid stain. Phosphate-buffered saline (PBS) contained (in 1.00 L) KCl (0.20 g), KH<sub>2</sub>PO<sub>4</sub> (0.20 g), NaCl (8.0 g), and Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O (2.16 g) at pH 7.4.

NMR spectra were acquired with a Bruker AC+ 300 spectrometer (<sup>1</sup>H: 300 MHz, <sup>13</sup>C: 75.4 MHz) or Varian UNITY-500 spectrometer (<sup>1</sup>H: 500 MHz, <sup>13</sup>C: 125.7 MHz) at the University of Wisconsin–Madison Chemistry Instrument Center. Compounds with carbamate protecting group (*e.g.*, Boc or Fmoc) exist as mixture of *Z* and *E* isomers that do not interconvert on the NMR time scale. Accordingly, these compounds exhibit two sets of NMR signals.

Mass spectrometry was performed with either a Micromass LCT (electrospray ionization, ESI) mass spectrometer in the University of Wisconsin–Madison Chemistry Instrument Center or with a Perkin–Elmer Voyager (matrix-assisted laser desorption ionization, MALDI) mass spectrometer in the University of Wisconsin–Madison Biophysics Instrumentation Facility.

Circular dichroism (CD) spectroscopy was performed with an Aviv 62A DS spectrometer in the University of Wisconsin–Madison Biophysics Instrumentation Facility.

(ProProGly)<sub>10</sub> was from Peptides International.

***t*-Butyloxycarbonyl-prolylglycine Benzyl Ester (3).** *t*-Butyloxycarbonyl-proline (5.268 g, 24.5 mmol), glycine benzyl ester tosylate salt (9.088 g, 26.9 mmol), dicyclohexylcarbodiimide (5.075 g, 24.5 mmol), and hydroxybenzotriazole hydrate (3.756 g, 24.5 mmol) were dissolved in dry DMF (200 mL) and diisopropylethylamine (12.8 mL, 73.5 mmol) was added. The reaction mixture was stirred overnight, filtered to remove DCU, and concentrated under reduced pressure. The residue was dissolved in EtOAc and washed twice each with aqueous KHCO<sub>3</sub> (5% w/v) and aqueous KHSO<sub>4</sub> (5% w/v) and once with brine. The organic layer was dried over MgSO<sub>4</sub>(s), filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with 1:1 hexane/EtOAc to yield **3** (6.987 g, 79%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$ : 7.39–7.28 (m, 5H), 5.17 (ABq, *J* = 12.3 Hz, 2H), 4.36–3.99 (m, 3H), 3.50–3.30 (m, 2H), 2.36–1.82 (m, 4H), 1.46 (s, 9H).

***N*-(9H-Fluoren-9-ylmethoxycarbonyl)-prolylprolylglycine Benzyl Ester (4).**

BocProGlyOBn (**3**, 4.259 g, 11.8 mmol) was dissolved in dioxane (50 mL) and placed under Ar(g) atmosphere. 4 N HCl in dioxane (29 mL, 116 mmol) was added with a syringe. The reaction mixture was stirred for 1.5 h, and then concentrated to dryness. The residue was dissolved in dry DMF (80 mL), and diisopropylethylamine (4.2 mL, 24.1 mmol) was added, followed by FmocPro pentafluorophenyl ester (5.934 g, 11.8 mmol). Additional DMF (20 mL) was added, then the reaction mixture was stirred overnight and then concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with EtOAc, to yield **4** (5.392 g, 79%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$ : 7.77–7.17 (m, 13H), 5.19–4.94 (m, 2H), 4.73–3.02 (m, 11H), 2.57–1.61 (m, 8H). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>, two rotamers)  $\delta$ : 171.85, 171.75, 171.2, 170.6, 170.1, 170.0, 153.74, 153.67, 144.0, 143.8, 140.7, 140.6, 127.65, 127.57, 127.2, 127.0, 125.1, 124.81, 124.76, 120.1, 120.0, 66.5, 66.1, 59.1, 59.0, 57.8, 57.3, 46.9, 46.6, 46.3, 46.1, 29.5, 28.91, 28.87, 28.6, 24.33, 24.26, 23.7, 22.6.

***N*-(9H-Fluoren-9-ylmethoxycarbonyl)-prolylprolylglycine (5, FmocProProGly).** Pd/C (10% w/w Pd, 0.372 g) was added to solid **4** (3.692 g, 6.35 mmol) under an Ar(g) atmosphere.

MeOH (100 mL) was added and the flask was flushed well with Ar(g). H<sub>2</sub>(g) was added via a balloon, and the reaction mixture was stirred for 4.75 h, filtered through Celite, and concentrated under reduced pressure. The resulting solid was triturated overnight with EtOAc/Et<sub>2</sub>O and then filtered and air-dried to yield **5** (2.7 g, 86%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ: 7.82–7.28 (m, 8H), 4.69–3.35 (m, 11H), 2.29–1.74 (m, 8H). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>, two rotamers): δ: 171.84, 171.76, 170.1, 170.0, 153.8, 153.7, 144.0, 143.8, 143.7, 140.7, 140.6, 127.64, 127.57, 127.1, 127.0, 125.1, 124.81, 124.76, 120.1, 120.0, 66.5, 66.0, 59.2, 59.0, 57.8, 57.3, 46.9, 46.6, 46.3, 46.1, 29.5, 28.91, 28.87, 28.6, 24.33, 24.26, 23.7, 22.6. ESI–HRMS: *m/z* 490.1965 ([M – H]<sup>+</sup>); 490.1978 ([M – H]<sup>+</sup>, calcd).

***N*-(9*H*-Fluoren-9-ylmethoxycarbonyl)-prolylglycolate (**6**, FmocProOGly).**

9-Fluorenylmethoxycarbonylproline (FmocPro, 0.515 g, 1.53 mmol) and hydroxybenzotriazole (0.275 g, 1.80 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (25 mL). Dicyclohexylcarbodiimide (0.325 g, 1.58 mmol) was added, followed by benzyl glycolate (0.25 mL, 1.76 mmol) and diisopropylethylamine (0.55 mL, 3.16 mmol). The reaction mixture was stirred overnight, filtered to remove DCU, and concentrated under reduced pressure. The resulting yellow oil was dissolved in EtOAc, washed twice each with aqueous KHCO<sub>3</sub> (5% w/v) and aqueous KHSO<sub>4</sub> (5% w/v), and then dried over MgSO<sub>4</sub>(s), filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with 1:3 EtOAc/hexanes to yield the benzyl ester (0.281 g, 38%) as a white foam. The benzyl ester (507 mg, 1.04 mmol) was combined with Pd/C (10% w/w Pd, 49 mg) under Ar(g), and to this solution was added MeOH (40 mL). The flask was flushed with Ar(g), and H<sub>2</sub>(g) was introduced via a balloon. The reaction mixture was stirred for 3 h, and then filtered through Celite and concentrated under reduced pressure. The crude product was purified by silica gel chromatography, eluting with MeOH (5–10% v/v) in CH<sub>2</sub>Cl<sub>2</sub> to yield **6** (0.172 g, 42%) as a white solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 9.12 (bs, 1H), 7.75–7.25 (m, 8H), 4.73–4.14 (m, 5H), 3.64–3.41 (m, 2H), 2.27–1.81 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, two rotamers) δ: 171.7, 170.7, 170.6, 155.2, 154.7, 143.9, 143.8, 143.6, 143.5, 141.2, 127.6, 127.0, 125.0, 124.9, 124.8, 124.7, 119.9, 67.7, 67.4, 60.7, 60.5, 58.9, 58.4, 47.0, 46.9, 46.5, 30.7, 29.7, 24.1, 23.1. ESI–HRMS: *m/z* 394.1273 ([M – H]<sup>+</sup>); 394.1291 ([M – H]<sup>+</sup>, calcd).

***N*-(9*H*-Fluoren-9-ylmethoxycarbonyl)-glycylproline (FmocGlyPro).** Glycylproline (1.022 g, 5.94 mmol) was dissolved in aqueous Na<sub>2</sub>CO<sub>3</sub> (10% (w/v), 20 mL), and to this solution was added a solution of Fmoc–Cl (1.696 g, 6.56 mmol) in dioxane (15 mL). Additional dioxane (30 mL) was added, and the reaction mixture was stirred for 3 h. Water (100 mL) was added, and the reaction mixture was washed twice with Et<sub>2</sub>O. The aqueous layer was acidified to pH 2 with 6 N HCl and extracted four times with EtOAc. The combined EtOAc extracts were dried over MgSO<sub>4</sub>(s), filtered, concentrated under reduced pressure, and placed under high vacuum overnight to yield FmocGlyPro (2.255 g, 96%) as a white solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 9.84 (bs, 1H), 7.76–7.26 (m, 8H), 6.19, 5.93 (two broad m, 1H), 4.60–3.41 (m, 8H), 2.31–1.87 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 174.0, 168.5, 156.4, 145.5, 143.8, 141.2, 127.7, 127.0, 125.2, 119.9, 67.6, 67.3, 61.4, 59.3, 47.0, 46.3, 43.3, 28.5, 24.6. ESI–MS: *m/z* 393.1 ([M – H]<sup>+</sup>); 393.2 ([M – H]<sup>+</sup>, calcd).

**Solid-Phase Peptide Synthesis.** Peptides were assembled on solid phase using an Applied Biosystems Model 432A (Synergy) peptide synthesizer using HBTU with HOBt and DIEA as the coupling reagent, or an Applied Biosystems Pioneer instrument using HATU/DIEA as the coupling reagent. Both instruments employ the Fmoc/*t*Bu coupling strategy. It was found that the best resin for collagen mimic peptide synthesis using the Synergy instrument was 2-chlorotrityl

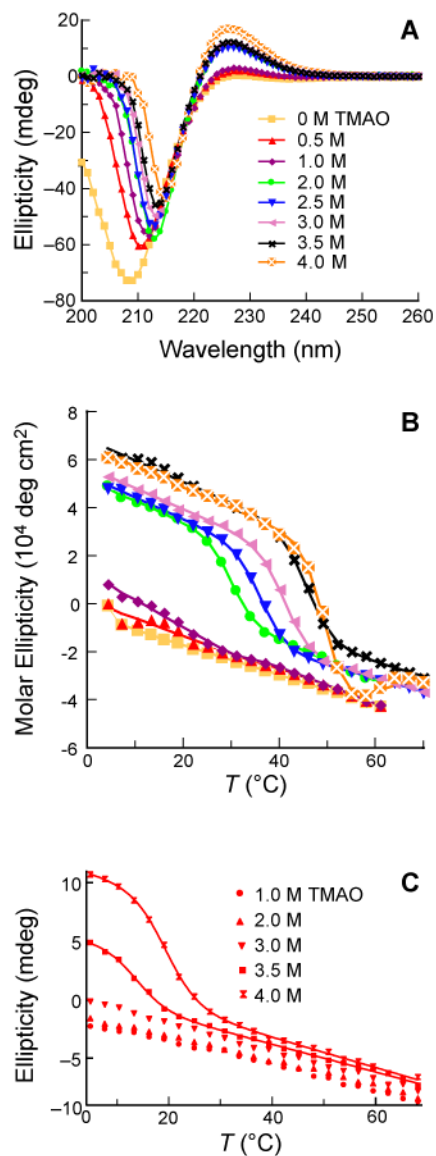
resin, and that the best resin for the Pioneer instrument was polyethyleneglycol-grafted polystyrene (PEG-PS) resin. Syntheses were carried out using 1.5–3.0 equiv of each segment to be coupled. Cleavage of the peptide from the resin was achieved by using 38:1:1 TFA/H<sub>2</sub>O/triisopropyl silane (TIS) for 1.5–3.0 h. Peptides were isolated by precipitation from the cleavage cocktail with *t*-butyl methyl ether, and purified by reverse-phase HPLC on a Varian Dynamax preparative C-18 column with a flow-rate of 4 mL/min and elution with 15% v/v CH<sub>3</sub>CN in water (5 min), 15–50% (30 min), 50–85% (10 min), and 85% (5 min). The purified peptides eluted after 35–40 min, and were analyzed by MALDI-TOF MS (Table S1).

**Table S1.** MALDI-TOF mass spectrometric analysis of depsipeptide **1** and alkenyl peptide **2**.

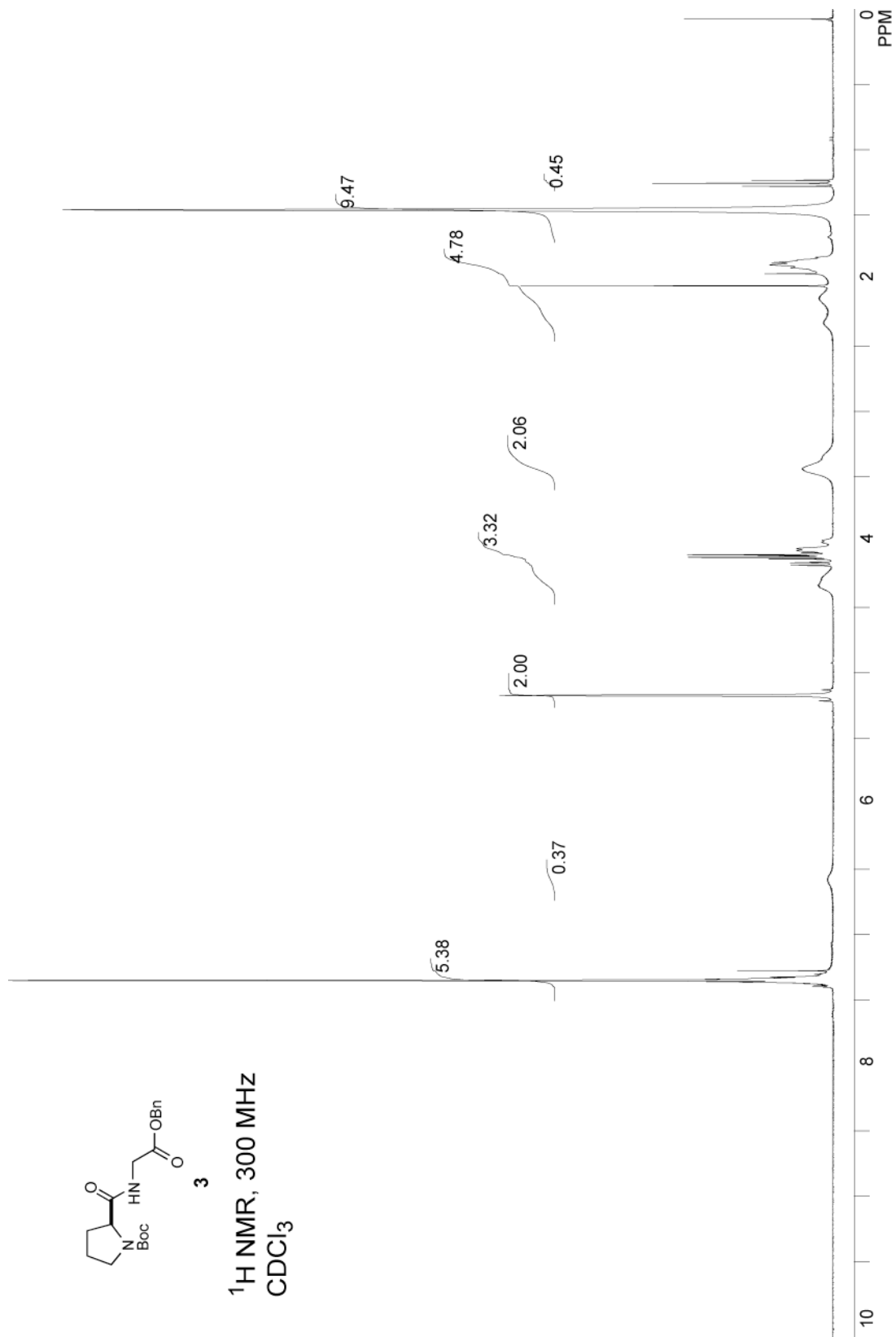
Peptide	MALDI-TOF MS	
	Observed (M + H) <sup>+</sup>	Calculated (M + H) <sup>+</sup>
depsipeptide <b>1</b>	2530.9	2531.3
alkenyl peptide <b>2</b>	2513.8	2513.3

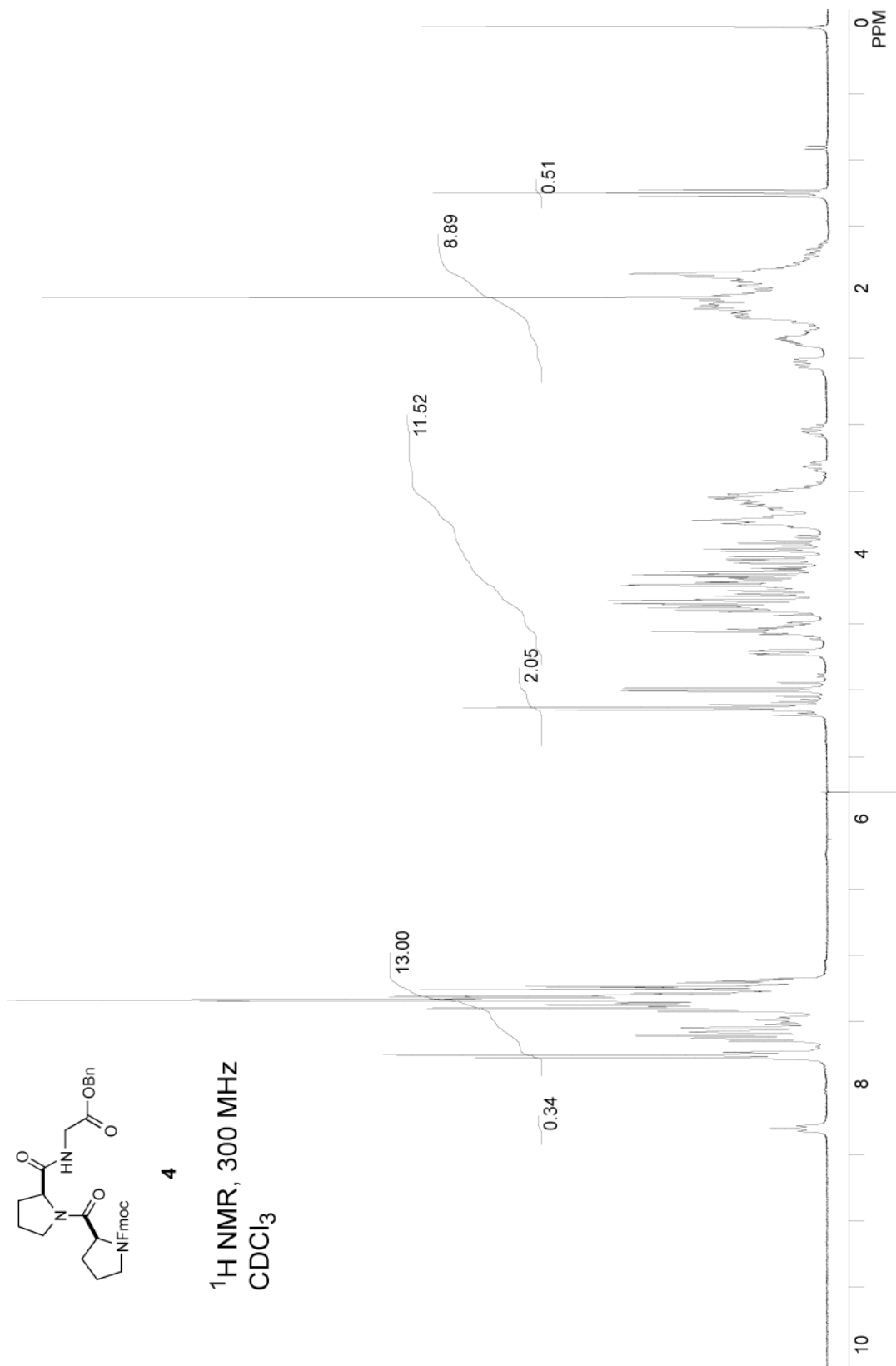
**Circular Dichroism (CD) Spectroscopy of Depsipeptide 1.** Depsipeptide **1** (~0.2 mM) was dissolved in 50 mM AcOH or in PBS containing TMAO (0–4.0 M), and the resulting solutions were incubated at 4 °C for ≥24 h. Solutions were degassed on ice under vacuum for approximately 20 min immediately before placement into pre-cooled 1-mm pathlength quartz cuvettes. Wavelength spectra were recorded at 4 °C from 260–200 nm, sampling every 1 nm and averaging for 3 s at each wavelength (Figure 1S, panel A). Temperature scans were conducted by raising the temperature of the samples in 3-°C increments from 4 °C to 60–80 °C (depending on the *T<sub>m</sub>* value of the triple helices being studied), equilibrating for 5 min at each temperature, and monitoring at 225 nm with a 20-s averaging time for each data point. Wavelength spectra were recorded again at the highest temperature used in the temperature scans, using the same parameters as for the wavelength scan at 4 °C. Values of *T<sub>m</sub>* were determined by fitting the raw CD data (Figure 1S, panel B) using the software package NLREG 4.0 (Phillip Sherrod, 1998).

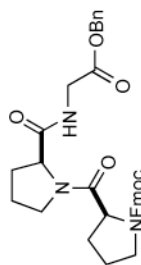
**Circular Dichroism (CD) Spectroscopy of Alkenyl Peptide 2.** Alkenyl peptide **2** was dissolved in 50 mM AcOH (0.36 mM) or in PBS (0.22 mM) containing TMAO (0–4.0 M), and the resulting solutions were incubated at 4 °C for ≥24 h. Solutions were degassed under vacuum for 15 min at 0 °C immediately before placement into pre-cooled 1-mm pathlength quartz cuvettes. Wavelength spectra were recorded at 4 °C from 200–260 nm, sampling every 1 nm and averaging for 3 s at each wavelength (data not shown). Temperature scans were conducted by raising the temperature of the samples in 3-°C increments from 4–73 °C and equilibrating for 10 min at each temperature and recording a spectrum from 210–260 nm, sampling for 3 s every 3 nm. Wavelength spectra were recorded again at 73 °C, using the same parameters as for the wavelength scan at 4 °C. Values of *T<sub>m</sub>* were determined by fitting the raw CD data (Figure 1S, panel C) using the software package NLREG 4.0 (Phillip Sherrod, 1998).



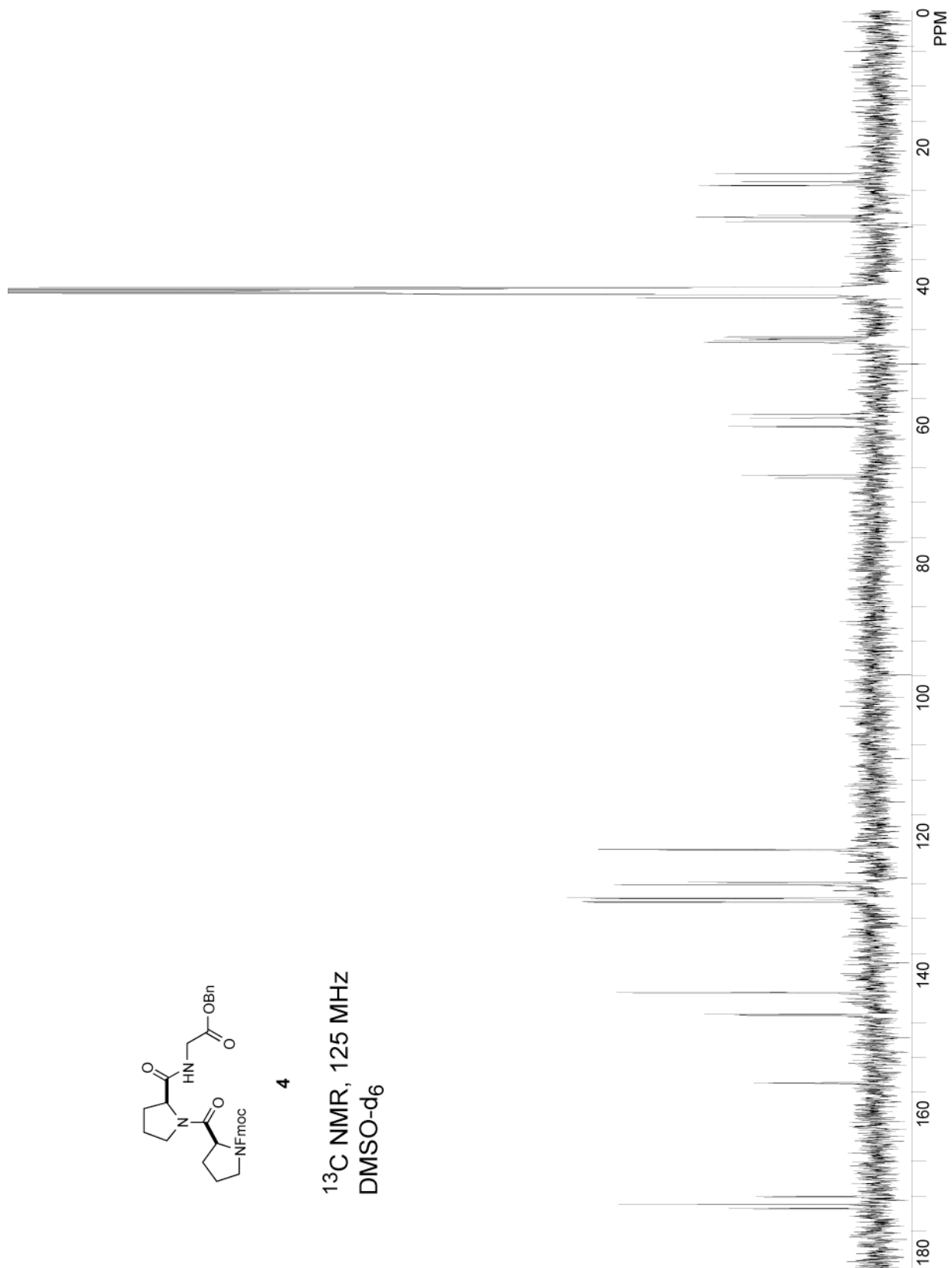
**Figure S1.** (A) Circular dichroism spectra of depsipeptide **1** at 4 °C in PBS containing trimethylamine *N*-oxide (0–4.0 M). (B) Molar ellipticity at 225 nm of depsipeptide **1** at 4–70 °C in PBS containing trimethylamine *N*-oxide (0–4.0 M). (C) Ellipticity at 225 nm of alkenyl peptide **2** at 4–70 °C in PBS containing trimethylamine *N*-oxide (1.0–4.0 M).



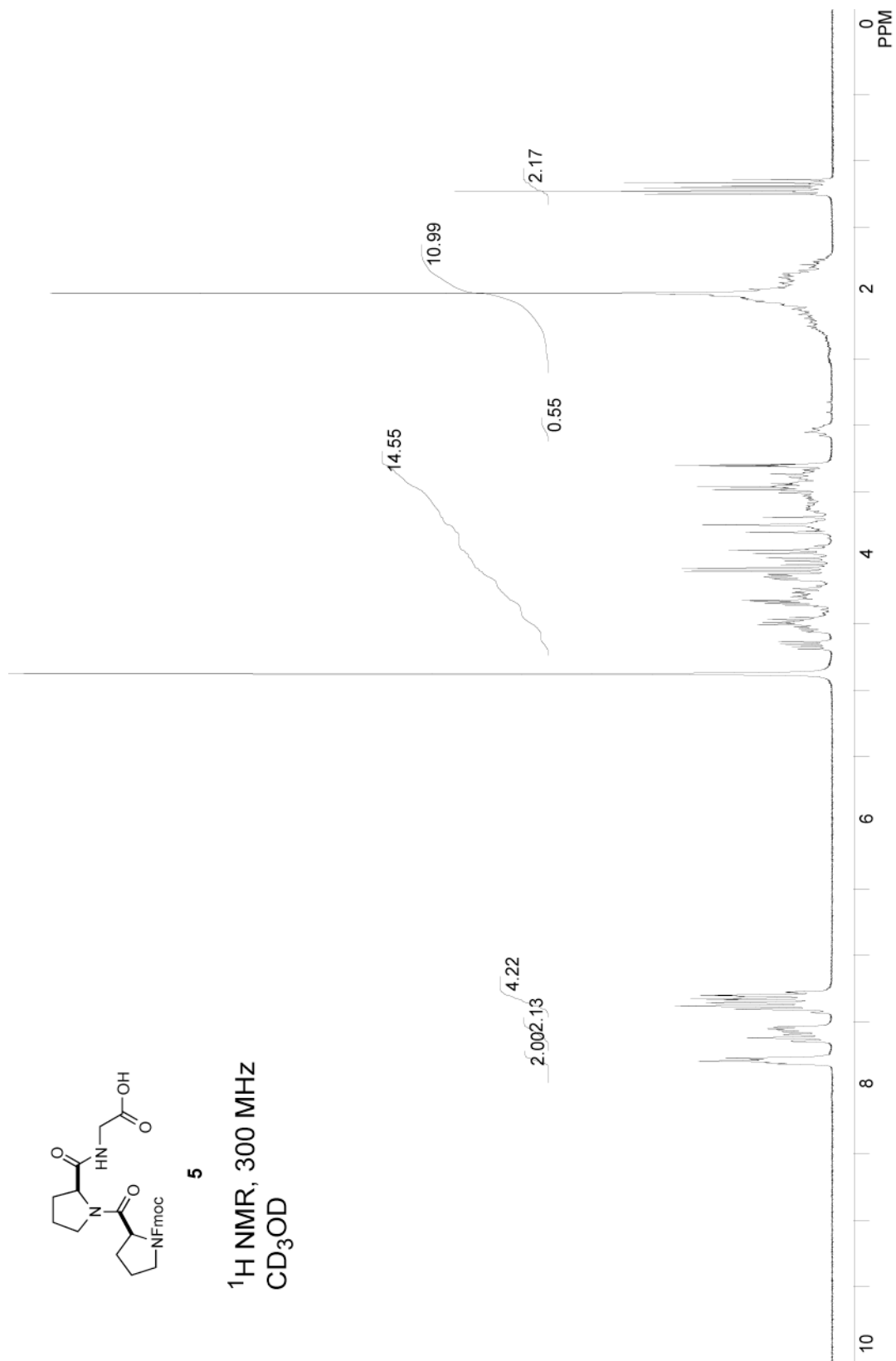


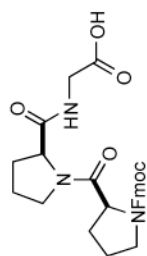
**4**

$^{13}\text{C}$  NMR, 125 MHz  
DMSO- $d_6$







**5**

$^{13}\text{C}$  NMR, 125 MHz  
DMSO- $d_6$

