Supporting Information

Reciprocity of Steric and Stereoelectronic Effects in the Collagen Triple Helix

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Experimental Section

General. Commercial chemicals were of reagent grade or better, and were used without further purification. Anhydrous THF, DMF, and CH_2Cl_2 were obtained from CYCLE-TAINER® solvent delivery systems (J. T. Baker, Phillipsburg, NJ). Other anhydrous solvents were obtained in septum-sealed bottles. In all reactions involving anhydrous solvents, glassware was either oven- or flame-dried. $NaHCO_3$ and brine (NaCl) refer to saturated aqueous solutions unless specified otherwise. Flash chromatography was performed with columns of silica gel 60, 230–400 mesh (Silicycle, Québec City, Canada). Semi-preparative HPLC was performed with a Zorbax C-8 reversed-phase column. Analytical HPLC was performed with an Agilent C-8 reversed-phase column using linear gradients of solvent A (H_2O with 0.1% v/v TFA) and solvent B (CH_3CN with 0.1% v/v TFA).

The term "concentrated under reduced pressure" refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining the water-bath temperature below 40 °C. Residual solvent was removed from samples at high vacuum (<0.1 torr). The term "high vacuum" refers to vacuum achieved by a mechanical belt-drive oil pump.

NMR spectra were acquired with a Bruker DMX-400 Avance spectrometer (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) at the National Magnetic Resonance Facility at Madison (NMRFAM). NMR spectra were obtained at ambient temperatures on samples dissolved in $CDCl_3$ unless indicated otherwise. Coupling constants *J* are provided in Hertz. Compounds with a carbamate protecting group (*e.g.*, Boc or Fmoc) exist as mixtures of *Z* and *E* isomers that do not interconvert on the NMR time scale at ambient temperatures. Accordingly, these compounds exhibit two sets of NMR signals, except when spectra are obtained at higher temperature (as indicated).

Mass spectrometry was performed with either a Micromass LCT (electrospray ionization, ESI) in the Mass Spectrometry Facility in the Department of Chemistry or an Applied Biosystems Voyager DE-Pro (matrix-assisted laser desorption/ionization, MALDI) mass spectrometer in the University of Wisconsin Biophysics Instrumentation Facility.

N-*tert*-Butyloxycarbonyl–(2*S*,4*R*)-4-methylproline (**1**) and (*S*)-2-*tert*-butyldimethylsilyloxymethyl-*N*-*tert*-butyloxycarbonyl–4-methylenepyrrolidine (**7**) were synthesized by the method of Del Valle and Goodman.[S1] *N*-*tert*-Butyloxycarbonyl–(2*S*)-prolyl–glycine benzyl ester **(4)** was synthesized by the method of Jenkins et al.[S2] Synthetic routes to *N*-(9-fluorenylmethoxycarbonyl)–(2*S*,4*R*)-4-methylprolyl–(2*S*)-prolyl–glycine **6** and *N*-(2-$^{13}CH_3$-acetyl)–(2*S*,4*R*)-4-methylproline methyl ester **(2)**, *N*-(9-fluorenylmethoxycarbonyl)–(2*S*)-prolyl–(2*S*,4*S*)-4-methylprolyl–glycine **(13)** and *N*-(2-$^{13}CH_3$-acetyl)–(2*S*,4*S*)-4-methylproline methyl ester (**10**), and *N*-(9-fluorenylmethoxycarbonyl)–(2*S*,4*R*)-4-methylprolyl–(2*S*,4*S*)-4-methylprolyl–glycine (**15**) are summarized in Schemes S1, S2, and S3, respectively.

***N*-(2-$^{13}CH_3$-Acetyl)–(2*S*,4*R*)-4-methylproline methyl ester (2).** Following the method of Nudelman et al,[S3] compound **1** (80 mg, 0.35 mmol) was dissolved in anhydrous MeOH (11 mL), and the resulting solution was cooled to 0 °C. Acetyl chloride (12.1 g, 150 mmol) was added dropwise and the reaction mixture was allowed to warm slowly to room temperature and stirred for 6 h. The resulting solution was concentrated under reduced pressure and the residue dissolved in anhydrous CH_2Cl_2 (15 mL). *N*,*N*-4-Dimethylaminopyridine (385 mg, 3.2 mmol) was added, followed by the dropwise addition of $H_3{}^{13}CC(O)Cl$ (250 mg, 3.0 mmol) and the reaction mixture was stirred for 24 h. MeOH (5 mL) was added to quench the reaction. The resulting solution was concentrated under reduced pressure, and the residue was dissolved in 10% w/v aqueous citric acid, extracted with CH_2Cl_2 (2 × 40 mL), dried over anhydrous $MgSO_4$(s), and concentrated

under reduced pressure. The crude product was purified by flash chromatography (50% v/v EtOAc in hexane to elute byproducts followed by 6% v/v MeOH in EtOAc) to afford **2** (50 mg, 0.27 mmol, 77%) as a colorless oil. ^{1}H NMR δ:1.08 and 1.07 (2 d, J = 5.8, 3H), 1.76–1.86 (m, 1H), 2.08 (d, $J_{C–H}$ = 128, 3H), 2.06–2.09 (m, 1H), 2.52 (m, 1H), 3.05 (t, J = 9.2, 1H), 3.71–3.83 (m, 4H), 4.41 and 4.54 (2 dd, J = 2.4, 9.0, 1H); ^{13}C NMR δ: 17.5, 17.9, 22.1, 22.3, 32.7, 37.2, 52.4, 53.3, 54.9, 58.7, 60.7, 169.3, 169.8, 173.0; HRMS–ESI (m/z): $[M + Na]^+$ calcd for $C_8{}^{13}CH_{15}NO_3Na$, 209.0983; found, 209.0987.

***N*-(9-Fluorenylmethoxycarbonyl)–(2*S*,4*R*)-4-methylproline (3).** Compound **1** (0.88 g, 3.8 mmol) was dissolved in 4 N HCl in dioxane (20 mL) under Ar(g) and stirred for 1.5 h. The resulting solution was concentrated under reduced pressure and the residue dissolved in dioxane and concentrated under reduced pressure again. The resulting free amine was dissolved in 10% w/v aqueous $NaHCO_3$ (55 mL), and a solution of Fmoc–OSu (1.41 g, 4.2 mmol) in dioxane (18 mL) was added. Additional dioxane (40 mL) was added, and the resulting white suspension stirred for 27 h. The dioxane was removed under reduced pressure and the aqueous solution was diluted with water (100 mL) and washed with ether (4 × 60 mL). The aqueous layer was acidified to pH 1.5 with 2 M HCl, extracted with EtOAc (4 × 160 mL), dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure to afford **3** (1.11 g, 3.2 mmol, 87%) as a white solid. ^{1}H NMR δ: 1.04 and 1.08 (d, J = 6.5, 3H), 1.73–1.94 (m, 1H), 2.11–2.20 and 2.31–2.50 (m, 2H), 2.97–3.05 (m, 1H), 3.68–3.79 (m, 1H), 4.11–4.52 (m, 4H), 7.24–7.45 (m, 4H), 7.51–7.63 (m, 2H), 7.68–7.81 (m, 2H); ^{13}C NMR δ: 17.3, 31.2, 32.4, 36.7, 38.7, 47.1, 47.3, 53.6, 53.8, 58.9, 59.8, 66.0, 67.5, 68.2, 120.1, 120.2, 125.0, 125.2, 125.2, 127.2, 127.2, 127.8, 127.9, 141.5, 143.8, 143.9, 156.4, 175.3, 177.8; ESI–MS (m/z): $[M – H]^–$ calcd for $C_{21}H_{20}NO_4Na$, 350.1; found, 350.6.

***N*-9-Fluorenylmethoxycarbonyl–(2*S*,4*R*)-4-methylprolyl–(2*S*)-prolyl–glycine benzyl ester (5).** Compound **4** (1.34 g, 3.8 mmol) was dissolved in 4 N HCl in dioxane (27 mL) under Ar(g) and stirred for 1.4 h. The resulting solution was concentrated under reduced pressure and the residue was dissolved in anhydrous CH_2Cl_2 (30 mL) and cooled to 0 °C. Compound **3** (430 mg, 1.3 mmol) was added to the solution, followed by PyBroP (606 mg, 1.3 mmol) and DIEA (1.26 g, 9.8 mmol). The reaction mixture was allowed to warm slowly to room temperature, stirred for 36 h, and then washed with 10% w/v aqueous citric acid (100 mL), $NaHCO_3$ (100 mL), water (100 mL), and brine (100 mL), dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient: 100% hexane to 100% EtOAc) to afford **5** (442 mg, 0.7 mmol, 57%) as a white solid containing an unidentified impurity which was removed after the succeeding step. HRMS–ESI (m/z): $[M + Na]^+$ calcd for $C_{35}H_{37}N_3O_6Na$, 618.2580; found, 618.2594.

***N*-9-Fluorenylmethoxycarbonyl–(2*S*,4*R*)-4-methylprolyl–(2*S*)-prolyl–glycine (6).** MeOH (50 mL) was added carefully to a mixture of compound **5** (420 mg, 0.7 mmol) and Pd/C (10% w/w, 90 mg, 0.1 mmol) under Ar(g), and the resulting black suspension was stirred under H_2(g) for 5 h. Careful monitoring by TLC was necessary to prevent hydrogenolysis of the Fmoc group. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc to elute byproducts, then 25% v/v MeOH in CH_2Cl_2 with 0.1% formic acid). The fractions containing **6** were concentrated under reduced pressure, and the formic acid was removed by dissolving the residue in 10% v/v MeOH in toluene and concentrating under reduced pressure to afford **6** (300 mg, 0.6 mmol, 84%) as a white solid. The purity of **6** was determined to be >90% by analytical HPLC (gradient: 15% B to 85% B over 50 min). A small sample was purified further by semi-

preparative HPLC for use in NMR experiments. ^{1}H NMR (spectrum obtained at 343 K in DMSO-d_6) δ: 1.06–0.93 (m, 3H), 1.7–2.47 (m, 7H), 2.81–3.75 (m, 6H), 4.11–4.58 (m, 5H), 7.26–7.46 (m, 4H), 7.50–7.92 (m, 5H); ^{13}C NMR (DMSO-d_6) δ: 17.4, 17.5, 24.3, 24.3, 28.9, 29.0, 30.0, 31.3, 36.3, 37.2, 46.1, 46.5, 46.6, 46.9, 53.1, 53.7, 57.6, 58.0, 59.1, 59.2, 66.0, 66.5, 120.0, 120.1, 124.8, 124.9, 125.1, 127.1, 127.2, 127.6, 127.7, 140.7, 143.9, 144.0, 153.6, 153.8, 158.1, 158.4, 158.8, 169.9, 170.0, 171.2, 171.8, 172.0; HRMS–ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{28}H_{31}N_3O_6Na$, 528.2111; found, 528.2108.

***N*-*tert*-Butyloxycarbonyl–(2*S*,4*S*)-2-hydroxymethyl–4-methylpyrrolidine (8).** Following the method of Del Valle and Goodman,[S1] a 0.1 M solution of compound **7** (9.78 g, 29.8 mmol) in MeOH was prepared. Raney-nickel (~1.00 g) that had been washed repeatedly with MeOH was added to the solution, and the flask was flushed repeatedly with H_2(g). The solution was stirred under H_2(g) for 30 h and then filtered through a Celite pad. (**Caution:** Do not allow the filter cake to dry during filtration as Raney-nickel can rapidly ignite.) The resulting solution was concentrated under reduced pressure and then dissolved in 0.5 M TBAF in THF (120 mL). After stirring for 15 h, the solution was concentrated under reduced pressure. The synthetic procedure was expected to yield both **8** and its trans diastereomer in a 3:1 ratio.[S1] The major diastereomer **8** was purified by flash chromatography (7% v/v EtOAc in hexane). Compound **8** was obtained (3.82 g, 17.7 mmol, 59% (2 steps)) as a colorless oil. The ratio of **8** to its 4*R* diastereomer was determined to be >30:1 by gas chromatography with a Supelco β-Dex-250 chiral column (17 m) and N_2 as the carrier gas at a column temperature of 110 °C. ^{1}H NMR δ: 1.02 (d, *J* 6.1, 3H), 1.08 (m, 1H), 1.47 (s, 9H), 2.04–2.21 (m, 2H), 2.77 (t, *J* = 10.1, 1H), 3.53–3.73 (m, 3H), 3.82–3.98 (m, 1H), 5.33 (d, *J* = 8.4, 1H); ^{13}C NMR δ: 17.0, 28.5, 28.7, 37.5, 54.5, 55.0, 61.6, 67.9, 80.4, 157.1; ESI–MS (*m*/*z*): $[M + Na]^+$ calcd for $C_{11}H_{21}NO_3Na$, 238.1; found, 238.3.

***N*-*tert*-Butyloxycarbonyl–(2*S*,4*S*)-4-methylproline (9).** Following the method of Del Valle and Goodman,[S1] three solutions were prepared prior to the oxidation. The first solution consisted of $NaClO_2$ (1.33 g, 14.7 mmol) in water (7.4 mL). The second solution consisted of bleach (436 μL) in water (7.4 mL). The third solution consisted of compound **8** (1.60 g, 7.4 mmol) dissolved in 100 mL of 3:2 CH_3CN:NaH_2PO_4 buffer (pH 6.6, 0.67 M). The solution containing **8** was heated to 45 °C, and TEMPO (193 mg, 0.7 mmol) was added. The two oxidant solutions were added simultaneously in 618 μL portions over 1 h, and the resulting solution was stirred at 40 °C for 18 h. After cooling to room temperature, the reaction was quenched by dropwise addition of saturated aqueous Na_2SO_3 until the solution became colorless. The acetonitrile was removed under reduced pressure, and the resulting aqueous solution basified to pH 10 with 1 M NaOH. The basic solution was washed with ether (5 × 125 mL) and then acidified to pH 2 with 2 M HCl. The acidic solution was extracted with ether (4 × 200 mL), and the organic layer was dried over anhydrous $MgSO_4$(s) and concentrated under reduced pressure to afford **9** (1.60 g, 7.0 mmol, 94%) as a white solid. ^{1}H NMR δ: 1.09 (d, *J* = 6.0, 3H), 1.44 and 1.50 (s, 9H), 1.58–1.70 and 1.88–2.00 (m, 1H), 2.21–2.31 (m, 1H), 2.31–2.52 (m, 1H), 2.89–3.04 (m, 1H), 3.67–3.82 (m, 1H), 4.20–4.38 (2m, 1H); ^{13}C NMR δ: 16.9, 17.2, 28.2, 28.3, 32.7, 36.4, 38.8, 53.3, 54.1, 59.4, 59.5, 80.4, 81.6, 159.4, 162.1, 174.9, 179.6; ESI–MS (*m*/*z*): $[M - H]^-$ calcd for $C_{11}H_{18}NO_4$, 228.1; found, 228.4.

***N*-(2-^{13}CH$_3$-Acetyl)–(2*S*,4*S*)-4-methylproline methyl ester (10).** Following the method of Nudelman et al,[S3] compound **9** (100 mg, 0.44 mmol) was dissolved in anhydrous MeOH (10 mL), and the resulting solution was cooled to 0 °C. Acetyl chloride (11.80 g, 150 mmol) was added dropwise and the reaction mixture was allowed to warm slowly to room temperature and stirred for 10 h. The resulting solution was concentrated under reduced pressure and the residue

dissolved in anhydrous CH_2Cl_2 (15 mL). *N*,*N*-4-Dimethylaminopyridine (450 mg, 3.7 mmol) was added, followed by the dropwise addition of $H_3{}^{13}CC(O)Cl$ (99 mg, 1.2 mmol). The reaction mixture was stirred for 9 h. Additional unlabeled acetyl chloride was added to ensure complete reaction, followed by MeOH (10 mL) to quench the reaction. The resulting solution was concentrated under reduced pressure, and the residue was dissolved in 10% w/v aqueous citric acid, extracted with CH_2Cl_2 (2 × 40 mL), dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure. The crude product was purified by flash chromatography (50% v/v EtOAc in hexane to elute byproducts followed by 6% v/v MeOH in EtOAc) to afford **10** (40 mg, 0.21 mmol, 52%) as a yellow oil. ^{1}H NMR δ: 1.06 and 1.10 (2 d, J = 6.4, 3H), 1.56 (q, J = 10.5, 1H), 2.09 (d, $J_{C–H}$ = 128, 3H), 2.28–2.46 (m, 2H), 3.18 (t, J = 9.8, 1H), 3.69 (m, 1H), 3.74 and 3.78 (2 s, 3H), 4.36 (t, J = 8.4, 1H); ^{13}C NMR δ: 17.0, 21.8, 22.4, 33.9, 37.6, 52.3, 55.1, 59.3, 168.9, 169.4, 173.1, 173.2; HRMS–ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_8{}^{13}CH_{15}NO_3Na$, 209.0983; found, 209.0980.

***N*-*tert*-Butyloxycarbonyl–(2*S*,4*S*)-4-methylprolyl–glycine benzyl ester (11).** Compound **9** (1.6 g, 7.0 mmol), glycine benzyl ester tosylate (3.07 g, 9.1 mmol), and PyBOP (3.64 g, 7.0 mmol) were dissolved in anhydrous CH_2Cl_2 (80 mL). DIEA (2.26 g, 17.5 mmol) was added, and the resulting solution was stirred for 27 h under Ar(g). The reaction mixture was washed with 10% w/v aqueous citric acid (3 × 50 mL), $NaHCO_3$ (3 × 50 mL), water (50 mL), and brine (50 mL), dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure. The crude oil was purified by flash chromatography (1:1 EtOAc:hexane) to afford **11** (2.13 g, 5.9 mmol, 84%) as a colorless, sticky liquid. ^{1}H NMR δ: 1.03 and 1.04 (d, J = 3.2, 3H), 1.44 (bs, 9H), 1.55–2.50 (m, 4H), 2.90 (t, J = 9.8, 1H), 3.65–3.94 (m, 1H), 4.01–4.34 (m, 3H), 5.18 (s, 2H), 7.36 (bs, 5H); HRMS–ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{20}H_{28}N_2O_5Na$, 399.1896; found, 399.1897.

***N*-9-Fluorenylmethoxycarbonyl–(2*S*)-prolyl–(2*S*,4*S*)-4-methylprolyl–glycine benzyl ester (12).** Compound **11** (1.18 g, 3.3 mmol) was dissolved in 4 N HCl in dioxane (30 mL) under Ar(g) and stirred for 2.5 h. The resulting solution was concentrated under reduced pressure and the residue dissolved in anhydrous DMF (50 mL). DIEA (1.60 g, 12.2 mmol) was added, followed by Fmoc–Pro–OPfp (3.52 g, 7.0 mmol), and additional anhydrous DMF (20 mL). The solution was stirred for 48 h and then concentrated by rotary evaporation under high vacuum. Flash chromatography (gradient: 25% v/v EtOAc in hexane to 95% v/v EtOAc in hexane) afforded **12** (800 mg, 1.3 mmol, 40%) as a white solid. ^{1}H NMR δ: 1.04 and 1.07 (d, J = 6.5, 3H), 1.76–2.60 (m, 8H), 3.44–3.75 (m, 2H), 3.91–4.61 (m, 8H), 5.27 (s, 2H), 7.04–7.79 (m, 13H); HRMS–ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{35}H_{37}N_3O_6Na$, 618.2580; found, 618.2558.

***N*-9-Fluorenylmethoxycarbonyl–(2*S*)-prolyl–(2*S*,4*S*)-4-methylprolyl–glycine (13)**. MeOH (130 mL) was added carefully to a mixture of compound **12** (800 mg, 1.3 mmol) and Pd/C (10% w/w, 160 mg, 0.2 mmol) under Ar(g), and the resulting black suspension was stirred under H_2(g) for 2 h. Careful monitoring by TLC was necessary to prevent hydrogenolysis of the Fmoc group. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc to elute byproducts, then 12% v/v MeOH in CH_2Cl_2 containing 0.1% v/v formic acid). The fractions containing **13** were concentrated under reduced pressure and the formic acid was removed by dissolving the residue in 10% v/v MeOH in toluene and concentrating under reduced pressure to afford **13** (500 mg, 1.0 mmol, 73%) as a white solid. The purity of **13** was determined to be >90% by analytical HPLC (gradient: 15% B to 85% B over 50 min). ^{1}H NMR (spectrum obtained at 343 K in DMSO-d_6) δ: 1.02 (d, J = 6.5, 3H), 1.37–1.49 (m, 1H). 1.70–2.00 (m, 3H), 2.06–2.27 (m, 2H), 2.87–3.46 (m, 6H), 3.74–4.02 (m, 3H), 4.11–4.57 (m, 5H), 7.25–7.90 (m, 8H); ^{13}C NMR

(DMSO-d_6) δ: 16.8, 22.6, 23.7, 28.5, 29.3, 33.3, 33.4, 37.0, 37.1, 40.7, 46.2, 46.6, 46.8, 46.9, 53.6, 53.8, 57.6, 58.0, 59.8, 59.9, 66.3, 66.5, 120.1, 120.2, 125.0, 125.1, 125.1, 125.3, 127.1, 127.1, 127.2, 127.3, 127.7, 140.7, 140.7, 143.8, 143.9, 144.0, 153.8, 153.8, 169.5, 169.6, 171.2, 171.7, 171.7; HRMS–ESI (*m*/*z*): $[M - H]^-$ calcd for $C_{28}H_{30}N_3O_6$, 504.2135; found, 504.2121.

***N*-9-Fluorenylmethoxycarbonyl–(2*S*,4*R*)-4-methylprolyl–(2*S*,4*S*)-4-methylprolyl–glycine benzyl ester (14).** Compound **11** (980 mg, 2.7 mmol) was dissolved in 4 N HCl in dioxane (30 mL) under Ar(g) and stirred for 1.7 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in anhydrous CH_2Cl_2 (80 mL) and cooled to 0 °C. Compound **3** (430 mg, 1.3 mmol) was added to the solution, followed by PyBroP (653 mg, 1.4 mmol) and DIEA (1.00 g, 7.8 mmol). The resulting solution was allowed to warm slowly to room temperature and then stirred for 40 h. The reaction mixture was diluted with CH_2Cl_2 (125 mL), washed with 10% w/v aqueous citric acid (100 mL), $NaHCO_3$ (100 mL), water (100 mL), and brine (100 mL), dried over anhydrous $MgSO_4$(s), and concentrated under reduced pressure. Flash chromatography (gradient: 35% v/v EtOAc in hexane to 90% v/v EtOAc in hexane) afforded **14** (520 mg, 0.9 mmol, 66%) as a white solid. ^{1}H NMR δ: 0.96–1.30 (m, 6H), 1.63–3.13 (m, 9H), 3.69–4.66 (m, 7H), 5.15 (m, 2H), 7.14–7.79 (m, 13H); HRMS–ESI (*m*/*z*): $[M + Na]^+$ calcd for $C_{36}H_{39}N_3O_6Na$, 632.2737; found, 632.2712.

***N*-9-Fluorenylmethoxycarbonyl–(2*S*,4*R*)-4-methylprolyl–(2*S*,4*S*)-4-methylprolyl–glycine (15).** MeOH (60 mL) was added carefully to a mixture of compound **14** (520 mg, 0.9 mmol) and Pd/C (10% w/w, 160 mg, 0.2 mmol) under Ar(g), and the resulting black suspension was stirred under a hydrogen atmosphere for 2.5 h. Careful monitoring by TLC was necessary to prevent hydrogenolysis of the Fmoc group. The suspension was filtered through a pad of Celite and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc to elute byproducts, then 12% v/v MeOH in CH_2Cl_2 with 0.1% formic acid). The fractions containing **15** were concentrated under reduced pressure, and the formic acid was removed by dissolving the residue in 10% v/v MeOH in toluene and concentrating under reduced pressure to afford **15** (315 mg, 0.6 mmol, 66%) as a white solid. The purity of **15** was determined to be >90% by analytical HPLC (gradient 15% B to 85% B over 50 min). ^{1}H NMR (spectrum obtained at 343 K in DMSO-d_6) δ: 0.9–1.05 (m, 6H), 1.27 (m, 1H), 1.42 (m, 1H), 1.72–1.82 (m, 1H), 2.01–2.35 (m, 4H), 2.84–3.00 (m, 3H), 3.48–3.66 (m, 3H), 3.70–3.80 (m, 1H), 4.23–4.59 (m, 4H), 7.29–7.45 (m, 4H), 7.52–7.77 (m, 2H), 7.84–7.91 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 14.0, 16.8, 17.3, 17.5, 17.6, 18.2, 22.1, 30.0, 30.5, 31.0, 31.2, 31.2, 33.3, 33.4, 33.8. 35.3. 36.1. 37.0. 46.6, 47.0, 51.3, 51.6, 53.0, 53.6, 53.8, 57.9, 58.1, 58.4, 59.8, 59.9, 60.0, 66.2, 66.5, 109.6, 120.1, 120.2, 121.4, 124.9, 125.1, 125.1, 127.1, 127.1, 127.2, 127.3, 127.7, 127.7, 128.9, 140.7, 140.7, 143.8, 152.4, 153.8, 169.4, 169.5, 171.3, 171.5, 171.6; HRMS–ESI (*m*/*z*): $[M - H]^-$ calcd for $C_{29}H_{33}N_3O_6$, 518.2291; found, 518.2307.

Measurement of $K_{trans/cis}$ Values of (2) and (10). Each compound (5–10 mg) was dissolved in D_2O with enough CD_3OD added to solubilize the compound (less than 20% of total volume). The ^{13}C NMR spectra were recorded using an inverse gated decoupling pulse program with a relaxation delay of 100 s and a pulse width of 10 μs. The spectral baselines were corrected and peaks corresponding to the labeled carbon were integrated with the software package NUTS.[S4] Values of $K_{trans/cis}$ were determined by the relative areas of the trans and cis peaks for the labeled carbons.

Attachment of Fmoc–mep–Pro–GlyOH (6) to 2-Chlorotrityl Resin. Under Ar(g), 33 mg (0.053 mmol) of 2-chlorotrityl resin (loading: 1.6 mmol/g) was swelled in anhydrous CH_2Cl_2 (0.7 mL) for 5 min. A solution of compound **6** (25 mg, 0.050 mmol) and DIEA (26 mg,

0.20 mmol) in anhydrous CH_2Cl_2 (0.7 mL) was added by syringe. Additional anhydrous CH_2Cl_2 (0.5 mL) was used to ensure complete transfer of **6**. After 2 h, anhydrous MeOH (0.2 mL) was added to cap any remaining active sites on the resin. The resin-bound peptide was isolated by gravity filtration, washed with several portions of anhydrous CH_2Cl_2 (~25 mL), and dried under high vacuum. The mass of the resin-bound peptide was 57 mg. Loading was measured by ultraviolet spectroscopy[S5] to be 0.69 mmol/g.

Attachment of Fmoc–Pro–Mep–GlyOH (13) and Fmoc–mep–Mep–GlyOH (15) to 2-Chlorotrityl Resin. Fmoc-tripeptides **13** and **15** were loaded onto 2-chlorotrityl resin in similar fashion to that described for **6**. Loadings were measured by ultraviolet spectroscopy[S5] to be 0.56 mmol/g for **13** and 0.60 mmol/g for **15**.

Synthesis of (mep–Pro–Gly)$_7$, (Pro–Mep–Gly)$_7$, and (mep–Mep–Gly)$_7$. The three 21-mer peptides were synthesized by segment condensation of their corresponding Fmoc-tripeptides (**6**, **13**, and **15**) on solid phase using an Applied Biosystems Synergy 432A Peptide Synthesizer at the University of Wisonsin–Madison Biotechnology Center. The first trimer was loaded onto the resin as described above. Fmoc-deprotection was achieved by treatment with 20% (v/v) piperidine in DMF. The trimers (3 equivalents) were converted to active esters by treatment with HBTU, DIEA, and HOBt. Extended couplings (120–200 min) were employed at room temperature.

Peptides were cleaved from the resin in 95:3:2 TFA:triisopropylsilane:H_2O (1.5 mL), precipitated from *t*-butylmethylether at 0 °C, and isolated by centrifugation. Semi-preparative HPLC was used to purify the peptides (mep–Pro–Gly)$_7$ (gradient: 10% B to 40% B over 50 min), (Pro–Mep–Gly)$_7$ (gradient: 15% B to 50% B over 50 min), and (mep–Mep–Gly)$_7$ (gradient: 15% B to 60% B over 60 min). All three peptides were >90% pure by analytical HPLC and MALDI–TOF mass spectrometry (*m*/*z*) $[M + H]^+$ calcd for $C_{91}H_{136}N_{21}O_{22}$ 1876.2, found 1875.6 for (mep–Pro–Gly)$_7$, 1875.4 for (Pro–Mep–Gly)$_7$; calcd for $C_{98}H_{150}N_{21}O_{22}$ 1974.4, found 1973.7 for (mep–Mep–Gly)$_7$.

Circular Dichroism Spectroscopy of (mep–Pro–Gly)$_7$, (Pro–Mep–Gly)$_7$, and (mep–Mep–Gly)$_7$. Peptides were dried under vacuum for at least 24 h before being weighed and dissolved to 0.2 mM in 50 mM acetic acid (pH 2.9). The solutions were incubated at ≤4 °C for ≥24 h before CD spectra were acquired using an Aviv 202SF spectrometer at the University of Wisconsin Biophysics Instrumentation Facility. Spectra were measured with a 1-nm band-pass in cuvettes with a 0.1-cm pathlength. The signal was averaged for 3 s during wavelength scans and either 5 or 15 s during denaturation experiments. During denaturation experiments, CD spectra were acquired at intervals of 1 °C for (mep–Pro–Gly)$_7$ and 3 °C for (Pro–Mep–Gly)$_7$ and (mep–Mep–Gly)$_7$. At each temperature, solutions were equilibrated for a minimum of 5 min before data acquisition. Values of T_m were determined by fitting molar ellipticity at 225 nm (for (Pro–Mep–Gly)$_7$ and (mep–Mep–Gly)$_7$) or 227 nm (for (mep–Pro–Gly)$_7$) to a two-state model.[S6] T_m values were determined in triplicate.

Circular Dichroism Spectroscopy of (mep–Pro–Gly)$_7$ in Solutions Containing Trimethylamine-*N*-Oxide. (mep–Pro–Gly)$_7$ was dried under vacuum for 24 h before being weighed and dissolved to 0.2 mM in solutions of 50 mM acetic acid containing 1.5, 2.0, 2.5, or 3.0 M trimethylamine-*N*-oxide (TMAO), respectively. (Solution pH was corrected to pH = 2.9 by addition of concentrated HCl.) Solutions were incubated at ≤4 °C for ≥24 h before CD spectra were recorded using the methods described in the previous section. Figures S1A and S1B show the CD spectra and the thermal melts for each solution. The CD spectra show the characteristic maximum at ~227 nm seen for all triple helices, and cooperative transitions were observed

during all three thermal melts. Figure S1C is a plot of T_m values for a (mep–Pro–Gly)$_7$ triple helix versus TMAO concentration. Linear regression and extrapolation to 0 M TMAO predicts a T_m value of 17.7 °C for a (mep–Pro–Gly)$_7$ triple helix, which is similar to the T_m value of 13 °C determined by direct measurement (Figure 1B and Table 1).

Sedimentation Equilibrium Experiments on (mep–Pro–Gly)$_7$, (Pro–Mep–Gly)$_7$, and (mep–Mep–Gly)$_7$. Sedimentation equilibrium experiments were performed with a Beckman XL-A Analytical Ultracentrifuge at the University of Wisconsin Biophysics Instrumentation Facility. Samples were diluted to approximately 0.1 mM in 50 mM potassium phosphate buffer (pH 3) and equilibrated at ≤4 °C for ≥24 h. Equilibrium data were collected at multiple speeds at both 4 and 37 °C. Gradients were monitored at 230 nm. Solvent densities of 1.00494 and 0.99800 g/mL at 4 and 37 °C, respectively, were measured by an Anton Paar DMA5000 density meter. Partial specific volumes ($\bar{\nu}$) for (mep–Pro–Gly)$_7$, (Pro–Mep–Gly)$_7$ and (mep–Mep–Gly)$_7$ were calculated based on amino acid content and corrected for the monomer molecular weights determined by sedimentation equilibrium experiments at 37 °C. A $\bar{\nu}$ value of 0.781 cm^3/g was used for (mep–Pro–Gly)$_7$ and (Pro–Mep–Gly)$_7$ and a $\bar{\nu}$ value of 0.770 cm^3/g was used for (mep–Mep–Gly)$_7$. Data were analyzed with programs written for IgorPro (Wavemetrics) by Dr. Darrell R. McCaslin (University of Wisconsin Biophysics Instrumentation Facility).

A log plot of absorbance versus the square of the distance from the center of rotation is shown in Figure S2. The slope at any point is proportional to the weight-averaged molecular weight, provided that the extinction coefficients per unit mass of assembled and monomeric peptides are equivalent. Curvature in such plots demonstrates the presence of multiple species.

Sedimentation equilibrium results at 37 °C are consistent with a single monomeric species for (mep–Pro–Gly)$_7$, (Pro–Mep–Gly)$_7$, and (mep–Mep–Gly)$_7$, as shown in Figure S2. At 4 °C, the dramatic change in gradient for (Pro–Mep–Gly)$_7$ and (mep–Mep–Gly)$_7$ is consistent with the assembly of these species into a triple helix. The fit shown at 4 °C for these two peptides (Figure S2) is based on a mixture of monomer and trimer. The data at 4 °C for (mep–Pro–Gly)$_7$ indicates some assembly for this peptide at low temperature, but to a much lesser extent than is observed for the other two peptides. The fit shown in Figure S2 for (mep–Pro–Gly)$_7$ at 4 °C is for a mixture of monomer and trimer.

Computations. The conformational preferences of 4-methylprolines were examined by hybrid density functional theory as implemented in Gaussian 98.[S7] *N*-Acetyl-4-methylproline methyl esters were used as model compounds in this study. Geometry optimizations and frequency calculations at the B3LYP/6-31+G* level of theory were performed on both the endo and exo conformers of Ac–mep–OMe and Ac–Mep–OMe, which were held in the trans (ω = 180°) conformation. Frequency calculations of the optimized structures yielded no imaginary frequencies, indicating a true stationary point on the potential energy surface. Single-point energy calculations at the B3LYP/6-311+G(2d,p) level of theory were performed on the optimized structures. The resulting self-consistent field (SCF) energies were corrected by the zero-point vibrational energy (ZPVE) determined in the frequency calculations, and are listed in Table S1.

References

(S1) Del Valle, J. R.; Goodman, M. *J. Org. Chem*. **2003,** *68,* 3923–3931.
(S2) Jenkins, C. L.; Vasbinder, M. M.; Miller, S. J.; Raines, R. T. *Org. Lett.* **2005,** *7*, 2619–2622.

(S3) Nudelman, A.; Bechor, Y.; Falb, E.; Fischer, B.; Wexler, B. A.; Nudelman, A. *Synth. Commun*. **1998,** *28,* 471–474.

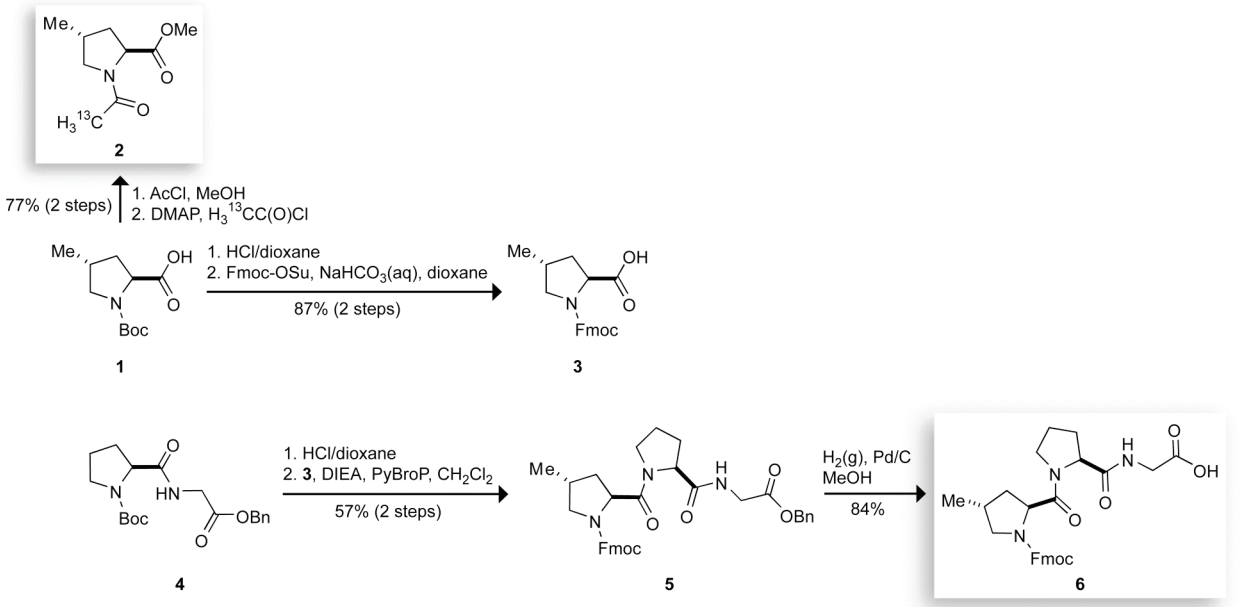
(S4) NUTS–NMR Utility Transform Software, Acorn NMR, Inc., 7670 Las Positas Road, Livermore, CA 94551.

(S5) Applied Biosystems *Determination of the Amino Acid Substitution Level via an Fmoc Assay;* Technical Note 123485 Rev 2; Documents on Demand–Applied Biosystems Web Page, http://docs.appliedbiosystems.com/search.taf (November 30, 2005).

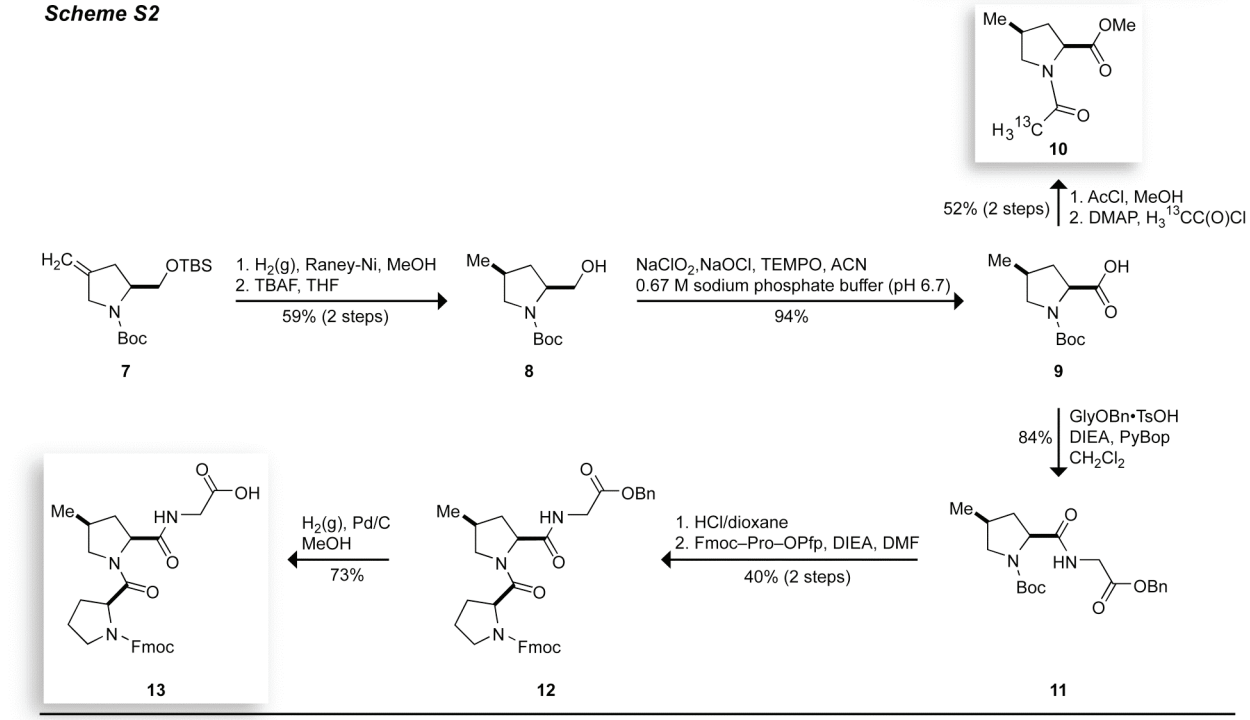
(S6) Becktel, W. J.; Schellman, J. A. *Biopolymers* **1987,** *26,* 1859–1877.

(S7) Gaussian 98, Revision A.9, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, and J. A. Pople, Gaussian, Inc., Pittsburgh PA, 1998.

Scheme S1



Scheme S2



Scheme S3

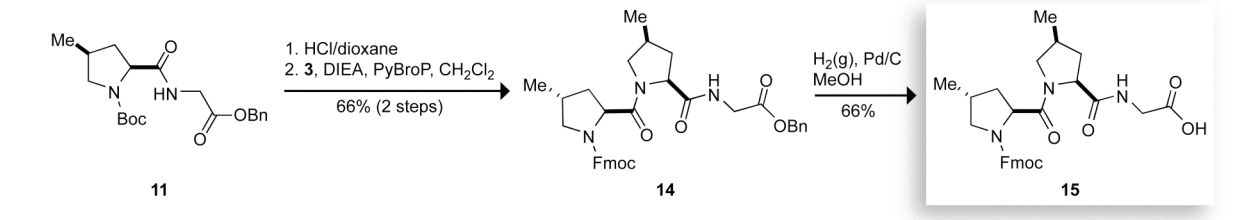


Table S1. SCF energies (atomic units; au) of Ac–Yaa–OMe calculated with B3LYP at 6-311+G(2d,p).

Conformer	Energy	ZPVE	Energy (ZPVE-corrected)
mep endo	–632.642027277	0.238638	–632.4033893
mep exo	–632.640078157	0.238851	–632.4012272
Mep endo	–632.639069142	0.238857	–632.4002121
Mep exo	–632.641497308	0.238639	–632.4028583

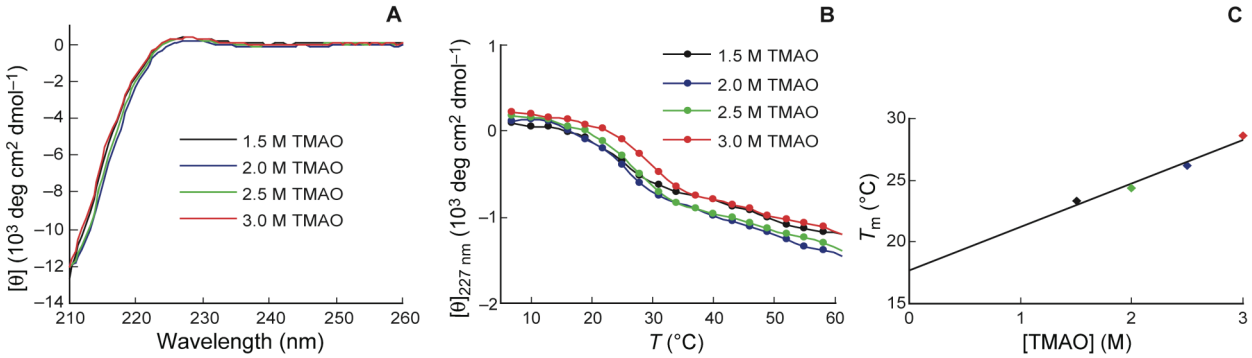


Figure S1. (A) Circular dichroism spectra of $(mep–Pro–Gly)_7$ in the presence of TMAO (1.5, 2.0, 2.5, or 3.0 M) at 4 °C. The maxima at ~225 nm are indicative of a collagen triple helix. (B) Thermal denaturation experiments with $(mep–Pro–Gly)_7$ in the presence of TMAO (1.5, 2.0, 2.5, or 3.0 M). Cooperative transitions are apparent in all four solutions. (C) Plot of T_m values for $(mep–Pro–Gly)_7$ versus TMAO concentration. Linear regression and extrapolation to 0 M TMAO gives T_m = 17.7 °C.

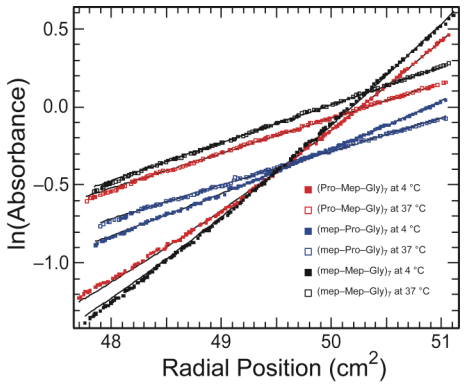


Figure S2. Sedimentation equilibrium data for $(Pro–Mep–Gly)_7$ (red squares), $(mep–Pro–Gly)_7$ (blue squares) and $(mep–Mep–Gly)_7$ (black squares) at a rotor speed of 50,000 rpm. Equilibrium data were collected at 4 °C (filled squares) and 37 °C (open squares). Gradients were monitored at 230 nm. Best fits shown are for solutions containing both trimer and some monomer at 4 °C, and for solutions containing only monomer at 37 °C.