Stabilization of the Collagen Triple Helix by O-Methylation of Hydroxyproline Residues

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General Experimental. Chemicals were from Aldrich or Acros (reagent grade or better), and were used without further purification. Amino acids were from Novabiochem, with the exception of Boc-Hyp-OH, which was from Chem-Impex International (Wood Dale, IL). (ProHypGly)₁₀·10H₂O was from Peptides International (Louisville, KY). Anhydrous DMF and CH₂Cl₂ were obtained from a CYCLE-TAINER[®] solvent delivery system from J. T. Baker (Phillipsburg, NJ). All other solvents were from Fisher Scientific (Pittsburgh, PA). Flash chromatography was performed with columns of silica gel 60, 230–400 mesh (Silicycle, Québec City, QC, Canada). Semi-preparative HPLC was performed with a Zorbax C8 reversed-phase column and analytical HPLC was performed with an Agilent C8 reversed-phase column. Linear gradients of solvent A (H₂O with 0.1% v/v TFA) and solvent B (CH₃CN with 0.1% v/v TFA) were used for HPLC analysis and purification.

The removal of solvents and other volatile materials "under reduced pressure" refers to the use of a rotary evaporator at water-aspirator pressure (<20 torr) and a water bath of <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 recorded on either a Bruker DMX-400 Avance spectrometer or a Bruker DMX-500 Avance spectrometer at the National Magnetic Resonance Facility at Madison (NMRFAM). Some compounds with a carbamate protecting group exist as mixtures of rotomers that do not interconvert on the NMR time scale at ambient temperatures and therefore exhibit two sets of NMR signals (as indicated).

Mass spectrometry was performed with either a Micromass LCT (electrospray ionization, ESI) in the Mass Spectrometry Facility in the University of Wisconsin 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.

Fmoc-Pro-Mop-Gly-OH was synthesized in 6 steps (overall yield: 30%) by the route shown in Scheme S-1.

Scheme S-1



N-tert-Butoxycarbonyl-(2S,4R)-4-methoxyproline (Boc-Mop-OH). This preparation is similar to that reported by Krapcho et al. for Cbz-Mop-OH.^{S1} To a solution of N-tert-Butoxycarbonyl-(2S,4R)-4-hydroxyproline (Boc-Hyp-OH, 2.00 g, 8.65 mmol) and CH₃I (1.90 mL, 30.3 mmol) in acetone (50 mL) was added Ag₂O (6.50 g, 28.1 mmol). The vessel was purged with Ar(g) and sealed with a septum, and the resulting suspension was stirred at room temperature for 24 h. The mixture was filtered and evaporated to a residue under reduced pressure. The residue was dried under high vacuum for 1 h, and then resubmitted to the reaction conditions a second time. After filtering and evaporating, the residue was dissolved in H₂O (60 mL), a solution of NaOH (0.36 g, 9.08 mmol) in 1 mL H₂O was added, and the solution was stirred at room temperature for 24 h (to hydrolyze any methyl ester that might have formed during alkylation). The aqueous mixture was acidified to pH 1 by the addition of 3 N HCl(aq) and extracted with CH₂Cl₂ (3×60 mL). The combined organic layers were dried over MgSO₄(s) and evaporated under reduced pressure, and the product was then isolated by flash chromatography (100:0-95:5 CH₂Cl₂/MeOH) affording Boc-Mop-OH (0.97 g, 46%) as a tinted residue. ¹H NMR (500 MHz, CDCl₃, ~3:2 ratio of 2 rotamers): δ 4.44 (t, J = 7.5 Hz, 0.6 H), 4.34 (t, J = 7.9 Hz, 0.4 H), 4.01-3.95 (m, 1 H), 3.70-3.65 (m, 0.4 H), 3.60-3.52 (m, 1 H), 3.49 (dd, J= 11.7, 4.8 Hz, 0.6 H), 3.33 (overlapping s, 3 H), 2.46–2.36 (m, 1 H), 2.32–2.24 (m, 0.6 H), 2.17-2.07 (m, 0.4 H), 1.49 (s, 5.4 H), 1.43 (s, 3.6 H); ¹³C NMR (125 MHz, CDCl₃, ~3:2 ratio of 2 rotamers): δ 178.9, 176.0, 156.1, 154.0, 81.7, 80.9, 78.4, 78.2, 57.9 (2 signals), 56.9 (2 signals), 51.8, 51.0, 36.4, 34.3, 28.5, 28.4; HRMS (ESI): m/z calcd for C₁₁H₁₉NO₅Na ([M+Na]⁺) 268.1161. found 268.1151.

N-tert-Butoxycarbonyl-(2S,4R)-4-methoxyprolylglycine Benzyl Ester (Boc-Mop-Gly-**OBn**). A solution of Boc-Mop-OH (0.42 g, 1.71 mmol), H-Gly-OBn TsOH (0.64 g, 1.88 mmol), DCC (0.35 g, 1.71 mmol), HOBt·H₂O (0.26 g, 1.71 mmol) and DIEA (0.89 mL, 5.13 mmol) in DMF (30 mL) was stirred at room temperature under Ar(g) for 20 h. The mixture was filtered to remove insoluble DCU and concentrated by rotary evaporation under high vacuum. The product was isolated by flash chromatography (two columns were needed; the first was run in 8:2 EtOAc/hexanes and the second in 7:3 EtOAc/hexanes) affording Boc-Mop-Gly-OBn (0.55 g, 82%) as a clear oil. ¹H NMR (500 MHz, CDCl₃, ~3:2 ratio of 2 rotamers): δ 7.41–7.32 (m, 5 H), 6.53 (app bs, 0.4 H), 5.22–5.15 (m, 2 H), 4.44 (dd, J = 7.9, 5.6 Hz, 0.6 H); 4.31 (t, J = 7.8 Hz, 0.4 H), 4.16–3.97 (m, 2.6 H), 3.93 (app bs, 0.4 H), 3.81–3.72 (m, 0.6 H), 3.53–3.43 (m, 1.4 H), 3.32 and 3.31 (overlapping s, 3 H), 2.49–2.42 (m, 0.6 H), 2.41–2.33 (m, 0.4 H), 2.16–2.05 (m, 1 H), 1.89 (bs, 0.6 H), 1.47 (s, 5 H), 1.42 (s, 4 H); ¹³C NMR (125 MHz, CDCl₃, ~3:2 ratio of 2 rotamers): δ 173.0, 172.2, 169.8, 169.6, 156.0, 154.8, 135.3, 135.1, 128.8 (2 signals), 128.7, 128.6, 128.5, 81.1, 81.0, 78.7, 78.2, 67.5, 67.3, 60.0, 58.7, 57.0, 56.9, 51.7, 51.5, 41.6, 41.2, 36.8, 33.7, 28.5, 28.4; HRMS (ESI): m/z calcd for $C_{20}H_{28}N_2O_6Na$ ([M+Na]⁺) 415.1845, found 415.1852.

N-9-Fluorenylmethoxycarbonyl-(2*S*)-prolyl-(2*S*,4*R*)-4-methoxyprolylglycine Benzyl Ester (Fmoc-Pro-Mop-Gly-OBn). Boc-Mop-Gly-OBn (0.54 g, 1.38 mmol) was dissolved in 14 mL of 4N HCl in dioxane (56 mmol) and the solution was stirred at room temperature under Ar(g) for 2.5 h. The mixture was evaporated under reduced pressure, the remaining white solid (H-Mop-Gly-OBn·HCl) was azeotroped twice with toluene and dried under high vacuum for 2 h. The solid was dissolved in DMF (20 mL), FmocPro pentafluorophenyl ester (0.69 g, 1.38 mmol) and DIEA (0.48 mL, 2.75 mmol) were added, and the mixture was stirred at room temperature under Ar(g) for 20 h. The mixture was concentrated by rotary evaporation under high vacuum,

and the product was isolated by flash chromatography (9:1 EtOAc/hexanes) affording Fmoc-Pro-Mop-Gly-OBn (0.78 g, 93% over 2 steps) as a clear residue. ¹H NMR (400 MHz, CDCl₃, mixture of 3 or more rotomers, integrations are approximate): δ 8.28 (t, J = 6.1 Hz, 0.3 H), 7.78–7.72 (m, 2 H), 7.65–7.51 (m, 2 H), 7.45–7.28 (m, 8 H), 7.27–7.23 (m, 1 H + CHCl₃), 7.21–7.18 (m, 1 H), 5.13 (app dd, J = 22.4, 12.3 Hz, 1.3 H), 5.00 (app dd, J = 25.5, 12.3 Hz, 0.7 H), 4.76 (dd, J = 4.4, 4.0 Hz, 0.4 H),), 4.63 (dd, J = 4.4, 4.0 Hz, 0.2 H), 4.55–4.30 (m, 3.2 H), 4.28–4.07 (m, 2.6 H), 4.07–3.86 (m, 2.2 H), 3.76–3.46 (m, 4 H), 3.39 (dd, J = 5.3, 5.0 Hz, 0.2 H), 3.34 (s, 1.3 H), 3.29 (s, 1 H), 3.21 (s, 0.6 H), 2.55–2.45 (m, 1 H), 2.44–2.36 (m, 0.4 H), 2.25–1.80 (m, 6 H); ¹³C NMR (100 MHz, CDCl₃, mixture of 3 or more rotomers): δ 172.5 (2 signals), 172.1, 171.9, 171.5, 171.3, 169.6, 169.5, 169.3, 162.7, 155.5, 155.1, 154.6, 144.6, 144.3, 144.2, 144.0, 143.8, 141.5 (multiple signals), 135.4, 128.8, 128.7, 127.8 (multiple signals), 127.2, 125.4, 125.2, 125.1, 125.0, 120.1 (2 signals), 79.1, 79.0, 67.9, 67.6, 67.3, 67.2, 67.1, 59.6, 58.8, 58.6, 58.1, 58.0, 57.2, 57.1, 51.5, 51.0, 47.6, 47.4, 47.2 (2 signals), 46.9, 41.6, 41.5, 37.3, 32.5, 32.2, 30.5, 29.4 (2 signals), 24.8, 24.5, 23.3; HRMS (ESI): m/z calcd for C₃₅H₃₇N₃O₇Na ([M+Na]⁺) 634.2529, found 634.2517.

N-9-Fluorenvlmethoxycarbonyl-(2S)-prolyl-(2S,4R)-4-methoxyprolylglycine (Fmoc-Pro-Mop-Gly-OH). A suspension of Fmoc-Pro-Mop-Gly-OBn (0.74 g, 1.21 mmol) and Pd/C (0.07 g) in MeOH (25 mL) was stirred under an atmosphere of $H_2(g)$ for 2 h. The mixture was filtered through a pad of Celite[®] and evaporated leaving a white solid. The product was isolated by flash chromatography (9:1 CH₂Cl₂/MeOH-EtOH) affording Fmoc-Pro-Mop-Gly-OH (0.54 g, 86%) as a white solid. ¹H NMR (500 MHz, DMSO- d_6 , ~1:1 ratio of 2 rotamers): δ 7.91–7.82 (m, 2 H), 7.65 (t, J = 7.4 Hz, 1 H), 7.58 (d, J = 7.4 Hz, 0.5 H), 7.55 (d, J = 7.5 Hz, 0.5 H), 7.44–7.28 (m, 5 H), 4.60 (dd, J = 8.6, 3.1 Hz, 0.5 H), 4.52 (dd, J = 8.6, 2.7 Hz, 0.5 H), 4.45–4.40 (m, 0.5 H), 4.34 (app t, J = 7.4 Hz, 0.5 H), 4.27–4.10 (m, 3 H), 4.04–3.97 (m, 1 H), 3.76–3.70 (m, 0.5 H), 3.67– 3.57 (m, 1.5 H), 3.42–3.29 (signals under H₂O peak), 3.22 (s, 1.5 H), 3.11 (s, 1.5 H), 2.32–2.29 (m, 0.5 H), 2.28–2.18 (m, 0.5 H), 2.17–2.06 (m, 1.5 H), 2.02–1.73 (m, 4.5 H); ¹³C NMR (125 MHz, DMSO-*d*₆, ~1:1 ratio of 2 rotamers): δ 171.7, 171.0, 170.3, 170.2, 153.8, 143.9 (3 signals), 143.8, 140.7, 140.6, 127.7 (2 signals), 127.2, 127.1, 125.3, 125.1, 120.1, 120.0 (2 signals), 78.6, 78.4, 66.8, 66.5, 58.3 (2 signals), 57.9, 57.6, 56.1, 56.0, 55.9, 51.3, 51.2, 47.0, 46.7, 46.6, 46.3, 42.0, 34.3, 29.6, 28.6, 23.7, 22.6; HRMS (ESI): m/z calcd for $C_{28}H_{31}N_3O_7Na$ ([M+Na]⁺) 544.2060, found 544.2039.

N-(2-¹³CH₃-Acetyl)-(2*S*,4*R*)-4-methoxyproline methyl ester (2-¹³CH₃-Ac-Mop-OMe). Following the method of Nudelman et al., ^{S2} a solution of Boc-Mop-OH (100 mg, 0.41 mmol) in anhydrous MeOH (12.5 mL) was cooled to 0 °C. Acetyl chloride (12.5 mL) was added dropwise over 5 min., the mixture was allowed to warm to room temperature and stirred under Ar(g) for 7 h. The solvent was evaporated under reduced pressure, and the resulting residue (H-Mop-OMe·HCl) was dried under high vacuum for 1 h. The residue was dissolved in CH₂Cl₂ (20 mL), *N*,*N*-Dimethylaminopyridine (500 mg, 3.84 mmol) was added, followed by dropwise addition of H₃¹³CC(O)Cl (273 mL, 3.84 mmol) and the mixture was stirred at room temperature under Ar(g) for 24 h. MeOH (5 mL) was added to quench the reaction. The mixture was concentrated under reduced pressure, the remaining residue was dissolved in CH₂Cl₂ (40 mL) and washed with 10% w/v aqueous citric acid. The aqueous layer was back-extracted with CH₂Cl₂ (3x 20 mL), and the combined organic layers were dried over MgSO₄(s) and evaporated under reduced pressure. The product was isolated by flash chromatography (1:1 EtOAc/hexanes to elute byproducts followed by 94:6 EtOAc/MeOH) affording 2-¹³CH₃-Ac-Mop-OMe (71 mg, 86%) as a white solid. ¹H NMR (500 MHz, CDCl₃, ~4:1 ratio of *trans:cis* amide bond rotomers): δ 4.50

(app t, J = 7.8 Hz, 1 H), 4.11–4.07 (m, 0.8 H), 4.02–3.98 (m, 0.2 H), 3.96–3.91 (m, 0.2 H), 3.82– 3.78 (m, 0.8 H), 3.79 (s, 0.6 H), 3.75 (s, 2.4 H), 3.58–3.51 (m, 1 H), 3.35 (s, 2.4 H), 3.32 (s, 0.6 H), 2.52–2.45 (m, 0.2 H), 2.40–2.32 (m, 0.8 H), 2.27–2.19 (m, 0.2 H), 2.11–2.03 (m, 0.8 H), 2.10 (d, $J_{C-H} = 128$ Hz, 2.4 H), 1.97 (d, $J_{C-H} = 128$ Hz, 0.6 H); ¹³C NMR (125 MHz, CDCl₃, ~4:1 ratio of *trans:cis* amide bond rotomers): δ 173.0, 172.8, 170.2 (d, J = 51 Hz), 169.6 (d, J = 52 Hz), 79.0, 77.4, 58.8, 57.4, 56.9, 52.9, 52.5, 50.8, 37.3, 34.5, 22.5 and 21.9 (labeled ¹³C); HRMS (ESI): m/z calcd for C₈¹³CH₁₅NO₄Na ([M+Na]⁺) 225.0932, found 225.0925.

Attachment of Fmoc-Pro-Mop-Gly-OH onto 2-chlorotrityl chloride resin. Under Ar(g), 69 mg (0.110 mmol) of 2-chlorotrityl chloride resin (loading 1.6 mmol/g) was swollen in dry CH₂Cl₂ (0.4 mL). A solution of Fmoc-Pro-Mop-Gly-OH (40 mg, 0.077 mmol) in dry CH₂Cl₂ (1.0 mL) was added followed by DIEA (58 μ L, 0.330 mmol), and the resin suspension was agitated gently for 2.5 h. Anhydrous CH₃OH (0.2 mL) was added to the mixture and the suspension was agitated for an additional 15 min (to cap any remaining active sites on the resin). The resin-bound peptide was isolated by gravity filtration, washed with dry CH₂Cl₂ (20 x 1 mL), and dried over KOH under high vacuum for 18 h. Loading was measured by UV spectroscopy using the reported protocol^{S3} to be 0.57 mmol/g.

Peptide Synthesis. (Pro-Mop-Gly)₁₀ was synthesized with free N- and C-termini by segment condensation of Fmoc-Pro-Mop-Gly-OH on solid phase using an Applied Biosystems Synergy 432A Peptide Synthesizer at the University of Wisconsin–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 Fmoc-tripeptides (3 equiv) were converted to active esters by treatment with HBTU, DIEA, and HOBt. Couplings were allowed to proceed for 45–60 min at room temperature. The peptide was cleaved from the resin in 38:1:1 TFA/H₂O/triisopropylsilane (1 mL), precipitated from *tert*-butylmethylether at 0 °C, and isolated by centrifugation. Semi-preparative HPLC was used to purify (Pro-Mop-Gly)₁₀ (gradient: 10% B to 30% B over 60 min). The peptide was >90% pure by analytical HPLC and characterized by MALDI–TOF mass spectrometry: $(m/z) [M + H]^+$ calcd for (Pro-Mop-Gly)₁₀ 2830.4, found 2831.3.

Circular Dichroism (CD) Spectroscopy. CD spectra were recorded with an Aviv 202SF circular dichroism spectrometer. Spectra were recorded on peptide solutions (100 μ M in 50 mM HOAc (pH 2.9)) that had been incubated at ≤ 4 °C for ≥ 48 h in 1-nm increments with a 3-s averaging time, 1-nm bandpass, and 0.1-cm pathlength. Samples for thermal stability experiments were generated by incubating peptide solutions (200 μ M) at 4 °C for ≥ 48 h. The solutions were then heated from 4 °C to 97 °C (for samples in 50 mM HOAc (aq) (pH 2.9)) or to 106 °C (for samples in 2:1 ethylene glycol/50 mM HOAc(aq)) at 3-°C increments with a 5-min equilibration at each step. The ellipticity at 225 nm was monitored with a 5-s averaging time, 1-nm bandpass, and 0.1-cm pathlength. Values of $T_{\rm m}$ were determined by fitting the data to a two-state model.⁸⁴

Differential Scanning Calorimetry (DSC). DSC measurements were conducted on a VP-DSC instrument (MicroCal, LLC, Northampton, MA). For each peptide, an instrument baseline was established by filling both the sample and the reference cell with degassed 50 mM HOAc(aq) (pH 2.9) and scanning from 5–98 °C at 15 °C/h until at least 3 consecutive overlaying scans were observed. The last of these scans was used as the baseline for each subsequent peptide scan.

Peptide solutions (~0.5 mg/mL in 50 mM HOAc(aq)), incubated at 4 °C for \geq 48 h, were degassed and loaded into the sample cell (without removing the reference solution) during the

Supporting Information

cool down from the final baseline scan (at ~15 °C). Samples were scanned from 5–98 °C at 15 °C/h; the first scan of each sample was used in the analysis. Subsequent scans of the same sample showed a decreased melting enthalpy indicative of incomplete recovery of triple helix (60–80%); because complete helical folding of these peptides requires a few hours at low temperature, and the sample cells were cooled at ~15 °C/min, incomplete folding is expected during the rapid cool down cycle. Nevertheless, a second run with a fresh sample (incubated at 4 °C for \geq 48 h) gave a trace that overlaid with the initial scan for both peptides.

After DSC measurements, peptide concentrations were determined by quantitative amino acid analysis (Scientific Research Consortium, Inc., St. Paul, MN). Peptide concentrations of 231 μ M for (ProHypGly)₁₀ and 129 μ M for (ProMopGly)₁₀ were calculated from the average of Gly and Pro content.

Data processing was done using the MicroCal software in the Origin 7 program (OriginLab Corp., Northampton, MA). For each sample, the appropriate reference scan was subtracted from the sample scan and the data were normalized to the monomer concentrations (determined above). A progress baseline was then subtracted from the data, giving the traces shown in Figure 1C of the manuscript.

Values of ΔH (per mole of monomer) were obtained by direct integration of the DSC exotherms. Using ΔH and the exotherm maxima as the $T_{\rm m}$ (64.3 °C for (ProHypGly)₁₀ and 73.8 °C for (ProMopGly)₁₀), $T\Delta S$ (at $T_{\rm m}$) was calculated with the equation: $T_{\rm m} = \Delta H/(\Delta S + R \cdot \ln(0.75c^2))$, where *c* is the concentration of monomeric peptide.^{S5} Values of ΔG were then calculated with the equation: $\Delta G = \Delta H - T\Delta S$. Table 1 in the manuscript lists only data for (ProMopGly)₁₀, comparing these data to those reported by Kobayashi and coworkers^{S6} for (ProHypGly)₁₀ and (ProFlpGly)₁₀. The data of Kobayashi and coworkers were normalized to $T^{\circ} = 71.9 \ ^{\circ}$ C (which is the T° for (ProProGly)₁₀, *i.e.* where $\Delta G = 0$). Because our thermodynamic data for (ProMopGly)₁₀ were determined at nearly the same temperature (73.8 °C), comparisons of the three peptides are meaningful.

The values for $(ProHypGly)_{10}$, however, were determined at 64.3 °C, and such comparisons become unsound with larger differences in temperature. For $(ProHypGly)_{10}$, the thermodynamic parameters at 64.3 °C were $\Delta H = -29.5$ kcal/mol, $T\Delta S = -27.3$ kcal/mol, and $\Delta G = -2.2$ kcal/mol. Note that ΔG agrees with that reported by Kobayashi and coworkers (-2.0 kcal/mol).

Measurement of the Amide Bond *trans/cis* Equilibrium Constant ($K_{t/c}$) in [¹³CH₃]Ac-Mop-OMe. [¹³CH₃]Ac-Mop-OMe (10 mg) was dissolved in 94:6 D₂O/CH₃OD (850 µL). The ¹³C NMR spectrum was recorded using an inverse gated decoupling pulse program with a relaxation delay of 60 s and a pulse width of 10 µs. A total of 32 transients were collected. The spectral baseline was corrected and peaks corresponding to the labeled carbon were integrated with the software package NUTS.⁸⁷ Values of $K_{t/c}$ were determined by the relative areas of the *trans* and *cis* peaks for the labeled carbon.

Crystallization of Ac-Mop-OMe. $[^{13}CH_3]$ Ac-Mop-OMe (10 mg) was dissolved in 1:1 CH₂Cl₂/hexanes (1 mL) and the solution was allowed to stand at room temperature in a loosely-capped vial. Slow evaporation afforded crystals suitable for X-ray analysis after ~72 h (all solvent had evaporated).

Crystallographic Data Collection. A colorless crystal with approximate dimensions $0.49 \times 0.41 \times 0.23 \text{ mm}^3$ was selected under oil under ambient conditions and attached to the tip of a nylon loop. The crystal was mounted in a stream of cold N₂(g) at 100(2) K and centered in the X-ray beam by using a video camera.

The crystal evaluation and data collection were performed on a Bruker CCD-1000 diffractometer with Mo K_{α} ($\lambda = 0.71073$ Å) radiation and the diffractometer-to-crystal distance of 4.9 cm.

The initial cell constants were obtained from three series of scans at different starting angles. Each series consisted of 20 frames collected at intervals of 0.3° in a 6° range about ω with the exposure time of 10 s per frame. A total of 69 reflections were obtained. The reflections were indexed successfully by an automated indexing routine built in the SMART program. The final cell constants were calculated from a set of 4805 strong reflections from the actual data collection.

Data were collected by using the full-sphere data collection routine. The reciprocal space was surveyed to the extent of a full sphere to a resolution of 0.80 Å. A total of 6155 data were harvested by collecting three sets of frames with 0.3° scans in ω and ϕ with an exposure time 10 s per frame. These highly redundant datasets were corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements.^{S8}

Crystallographic Structure Solution and Refinement. The systematic absences in the diffraction data were consistent for the space groups $P2_1$ and $P2_1/m$. The *E*-statistics strongly suggested the non-centrosymmetric space group $P2_1$ that yielded chemically reasonable and computationally stable results of refinement.^{S8}

A successful solution by the direct methods provided most non-hydrogen atoms from the *E*-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were found in the difference map and refined independently. The absolute configuration was assigned from the known synthetic procedure.

The final least-squares refinement of 187 parameters against 2089 data resulted in residuals *R* (based on F^2 for $I \ge 2\sigma$) and *wR* (based on F^2 for all data) of 0.0273 and 0.0706, respectively. The final difference Fourier map was featureless.

The molecular diagram is drawn with 50% probability ellipsoids.

References

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Identification code Empirical formula Formula weight Temperature Wavelength Crystal system Space group Unit cell dimensions	raines07 $C_9H_{15}NO_4$ 201.22 100(2) K 0.71073 Å Monoclinic P2 ₁ a = 5.8595(7) Å b = 13.4363(16) Å c = 6.5774(8) Å	$\alpha = 90^{\circ}$ $\beta = 98.802(2)^{\circ}$ $\nu = 90^{\circ}$
Volume Z Density (calculated) Absorption coefficient	$\begin{array}{c} c = 0.3774(8) \text{ A} \\ 511.74(11) \text{ Å}^{3} \\ 2 \\ 1.306 \text{ Mg/m}^{3} \\ 0.103 \text{ mm}^{-1} \end{array}$	γ = 90
<i>F</i> (000) Crystal size Theta range for data collection Index ranges Reflections collected	216 $0.49 \times 0.41 \times 0.23 \text{ mm}^3$ $3.03 \text{ to } 26.41^\circ$ $-7 \le h \le 7, -16 \le k \le 16, -6155$	$-8 \le l \le 8$
Independent reflections Completeness to theta = 26.41° Absorption correction Max. and min. transmission Performment method	2089 $[R_{int} = 0.0187]$ 99.9% Multi-scan with SADABS 0.9768 and 0.9515 Full matrix least squares of	$h E^2$
Data / restraints / parameters Goodness-of-fit on F^2 Final <i>R</i> indices $[I > 2\sigma(I)]$ <i>R</i> indices (all data) Absolute structure parameter Largest diff peak and hole	$R_{1} = 0.0273, wR_{2} = 0.0700$ $R_{1} = 0.0279, wR_{2} = 0.0700$ Assigned from synthesis 0.213 and -0.144 e Å ⁻³)
Largest diff. peak and hole	0.213 and -0.144 e.A ⁻³	

Table S-1. Crystal Data and Structure Refinement for Ac-Mop-OMe

	x	У	Z	$U_{ m eq}^{\ \ a}$
O(1)	10655(2)	6883(1)	939(2)	23(1)
O(2)	8162(2)	5980(1)	-3279(1)	25(1)
O(3)	11278(1)	4985(1)	-2570(1)	18(1)
O(4)	6862(2)	3977(1)	3304(1)	23(1)
N(1)	7697(2)	5826(1)	968(2)	17(1)
C(1)	8741(2)	6708(1)	1419(2)	18(1)
C(2)	7454(2)	7452(1)	2526(2)	22(1)
C(3)	8812(2)	5058(1)	-90(2)	16(1)
C(4)	9347(2)	5422(1)	-2146(2)	17(1)
C(5)	11860(3)	5185(1)	-4590(2)	24(1)
C(6)	7016(2)	4215(1)	-386(2)	19(1)
C(7)	5668(2)	4353(1)	1412(2)	20(1)
C(8)	6832(3)	2915(1)	3420(3)	32(1)
C(9)	5545(2)	5481(1)	1635(2)	19(1)

Table S-2. Atomic Coordinates (×10⁴) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for Ac-Mop-OMe

 $\overline{{}^{a}U_{eq}}$ is defined as $\frac{1}{3}$ of the trace of the orthogonalized U^{ij} tensor.

O(1)-C(1)	1.2333(15)
O(2)-C(4)	1.2002(16)
O(3)-C(4)	1.3415(15)
O(3)-C(5)	1.4465(15)
O(4)-C(7)	1.4237(16)
O(4)-C(8)	1.4283(17)
N(1)-C(1)	1.3460(16)
N(1)-C(3)	1.4540(15)
N(1)-C(9)	1.4721(15)
C(1)-C(2)	1.5054(17)
C(2)-H(2A)	0.961(19)
C(2)-H(2B)	0.968(18)
C(2)-H(2C)	0.94(2)
C(3)-C(4)	1.5154(16)
C(3)-C(6)	1.5388(17)
C(3)-H(3)	0.958(14)
C(5)-H(5A)	0.93(3)
C(5)-H(5B)	0.966(19)
C(5)-H(5C)	0.952(17)
C(6)-C(7)	1.5310(17)
C(6)-H(6A)	0.961(18)
C(6)-H(6B)	0.947(17)
C(7)-C(9)	1.5256(17)
C(7)-H(7)	0.968(15)
C(8)-H(8A)	0.98(2)
C(8)-H(8B)	0.94(2)
C(8)-H(8C)	0.97(2)
C(9)-H(9A)	1.007(17)
C(9)-H(9B)	0.935(17)
	0000(11)
C(4)-O(3)-C(5)	115.76(10)
C(7)-O(4)-C(8)	113.15(11)
C(1)-N(1)-C(3)	120.53(9)
C(1)-N(1)-C(9)	126.33(10)
C(3)-N(1)-C(9)	112.81(10)
O(1)-C(1)-N(1)	120.75(11)
O(1)-C(1)-C(2)	122.68(12)
N(1)-C(1)-C(2)	116.57(10)
C(1)-C(2)-H(2A)	108.6(11)
C(1)-C(2)-H(2B)	110.2(11)
H(2A)-C(2)-H(2B)	107.8(15)
C(1)-C(2)-H(2C)	110.0(11)
H(2A)-C(2)-H(2C)	111.4(16)
H(2B)-C(2)-H(2C)	108.8(15)
N(1)-C(3)-C(4)	111.56(10)
N(1)-C(3)-C(6)	103.56(9)
C(4)-C(3)-C(6)	110.83(10)
N(1)-C(3)-H(3)	110.9(8)
C(4)-C(3)-H(3)	108.0(8)
C(6)-C(3)-H(3)	112.1(9)
O(2)-C(4)-O(3)	124.79(12)

Table S-3. Bond Lengths [Å] and Angles [°] for Ac-Mop-OMe

125.23(11)
100.00.00
109.89(10)
110.8(14)
107.8(10)
108.4(17)
108.6(10)
110.9(18)
110.3(15)
103.67(10)
111.2(10)
111.5(10)
107.2(10)
109.7(10)
113.0(13)
107.05(11)
112.93(10)
103.45(10)
106.7(9)
113.7(9)
113.1(9)
111.9(13)
112.7(13)
104.6(17)
108.0(12)
107.0(17)
112.6(17)
103.22(10)
111.7(10)
110.2(10)
111.5(10)
108.9(10)
111.0(14)

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	22(1)	19(1)	30(1)	0(1)	9(1)	-4(1)
O(2)	30(1)	26(1)	20(1)	4(1)	4(1)	9(1)
O(3)	19(1)	21(1)	16(1)	2(1)	6(1)	2(1)
O(4)	26(1)	19(1)	24(1)	3(1)	6(1)	1(1)
N(1)	15(1)	17(1)	18(1)	-1(1)	5(1)	1(1)
C(1)	20(1)	16(1)	16(1)	3(1)	2(1)	1(1)
C(2)	28(1)	17(1)	23(1)	0(1)	7(1)	2(1)
C(3)	15(1)	16(1)	18(1)	0(1)	2(1)	1(1)
C(4)	18(1)	16(1)	18(1)	-2(1)	2(1)	-1(1)
C(5)	27(1)	29(1)	18(1)	3(1)	9(1)	2(1)
C(6)	18(1)	16(1)	21(1)	-2(1)	2(1)	-2(1)
C(7)	15(1)	19(1)	25(1)	1(1)	5(1)	0(1)
C(8)	43(1)	21(1)	35(1)	6(1)	12(1)	3(1)
C(9)	16(1)	19(1)	25(1)	-1(1)	7(1)	0(1)

Table S-4. Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for Ac-Mop-OMe^a

^aThe anisotropic displacement factor exponent takes the form: $-2p^2[h^2 a^{*2}U^{11} + ... + 2h k a^{*} b^{*} U^{12}]$.

	x	У	Z	$U_{ m eq}$
H(2A)	8130(30)	8097(14)	2420(30)	27(4)
H(2B)	7600(30)	7286(14)	3970(30)	30(4)
H(2C)	5880(40)	7451(14)	1960(30)	27(4)
H(3)	10230(20)	4851(11)	720(20)	9(3)
H(5A)	12190(40)	5858(19)	-4730(30)	48(6)
H(5B)	13230(30)	4803(14)	-4730(30)	27(4)
H(5C)	10600(30)	4983(15)	-5600(30)	25(4)
H(6A)	7740(30)	3572(14)	-340(20)	22(4)
H(6B)	5970(30)	4322(13)	-1610(30)	18(4)
H(7)	4170(30)	4032(11)	1190(20)	13(3)
H(8A)	7810(40)	2608(17)	2510(30)	44(5)
H(8B)	5360(40)	2648(15)	2990(30)	36(5)
H(8C)	7470(30)	2724(15)	4820(30)	33(5)
H(9A)	4140(30)	5749(13)	730(30)	23(4)
H(9B)	5510(30)	5637(12)	3020(30)	19(4)

Table S-5. Hydrogen Coordinates (×10⁴) and Isotropic Displacement Parameters ($Å^2 \times 10^3$) for Ac-Mop-OMe

C(3)-N(1)-C(1)-O(1)	0.20(17)
C(9)-N(1)-C(1)-O(1)	173.14(11)
C(3)-N(1)-C(1)-C(2)	-179.69(10)
C(9)-N(1)-C(1)-C(2)	-6.75(17)
C(1)-N(1)-C(3)-C(4)	-58.09(14)
C(9)-N(1)-C(3)-C(4)	128.08(11)
C(1)-N(1)-C(3)-C(6)	-177.34(10)
C(9)-N(1)-C(3)-C(6)	8.82(13)
C(5)-O(3)-C(4)-O(2)	-2.82(19)
C(5)-O(3)-C(4)-C(3)	173.71(10)
N(1)-C(3)-C(4)-O(2)	-35.67(16)
C(6)-C(3)-C(4)-O(2)	79.17(15)
N(1)-C(3)-C(4)-O(3)	147.82(10)
C(6)-C(3)-C(4)-O(3)	-97.34(11)
N(1)-C(3)-C(6)-C(7)	-27.98(12)
C(4)-C(3)-C(6)-C(7)	-147.73(10)
C(8)-O(4)-C(7)-C(9)	171.94(11)
C(8)-O(4)-C(7)-C(6)	-74.87(14)
C(3)-C(6)-C(7)-O(4)	-78.66(12)
C(3)-C(6)-C(7)-C(9)	36.72(12)
C(1)-N(1)-C(9)-C(7)	-159.42(12)
C(3)-N(1)-C(9)-C(7)	13.99(13)
O(4)-C(7)-C(9)-N(1)	88.55(11)
C(6)-C(7)-C(9)-N(1)	-30.94(12)

Table S-6. Torsion angles [°] for Ac-Mop-OMe



















