Concise, Stereoselective Route to the Four Diastereoisomers of 4-Methylproline

Annabel C. Murphy, Maya I. Mitova, John W. Blunt, and Murray H. G. Munro*

Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

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The full stereochemical characterization of 4-methylproline, a rare amino acid found in a number of peptidic secondary metabolites, has often been hindered by long reaction sequences or low stereoselectivity in the synthesis leading to reference samples. The preparation of the four diastereoisomers of 4-methylproline by a concise and stereoselective route is presented and features a six-step route with late-stage stereodivergence, good stereoselectivity for both *cis*- and *trans*-series (75% and 88% de, respectively), and good overall yields (cumulative yields of 30–40%). Additional data on the Marfey's derivatives of the stereoisomers are also presented.

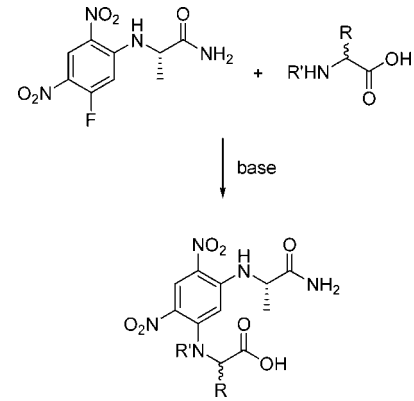
The rare amino acid 4-methylproline is an unusual structural feature of the pteratides, a series of cytotoxic, cyclic depsipeptides isolated from a Malaysian Basidiomycete identified as a *Pterula* species.[1] 4-Methylproline has also been characterized from other natural sources, being first isolated from young apples,[2–4] and then found in bacteria,[5,6] cyanobacteria,[7–9] the sponge *Theonella* sp.,[10] and terrestrial fungal sources.[11–14] Labeling studies by Leusch et al. showed that this amino acid was leucine-derived in the cyanobacterial secondary metabolite nostopeptolide A1.[15] The biosynthetic route was examined via the cloning and characterization of two crucial nonribosomal peptide synthetases (NRPSs) in the pathway. However, the stereochemistry of 4-methylproline residues in secondary metabolites has often remained unassigned or been assigned by comparison to the known physical data of the amino acid.[16] When isolating trace secondary metabolites, insufficient material often precludes such an approach. Access to reference samples of the four diastereoisomers of 4-methylproline and the physical data for key derivatives, such as derivatives with Marfey's chiral reagent (Scheme 1),[17,18] allows unambiguous assignment of the natural residue by chromatographic methods. To assign the absolute stereochemistry of the pteratide series, this approach was adopted and the synthesis of the four diastereoisomers of 4-methylproline undertaken.

Literature reports for a unified, stereoselective approach to all four diastereoisomers are few. Heindl et al. reported a stereospecific synthesis of protected 4-methylproline derivatives,[19,20] and Del Valle and Goodman have published a stereoselective route to 4-methylproline.[21] The method of Heindl et al. proved difficult due to low yields and irreproducible results during a key cuprate alkylation reaction, so the method of Del Valle and Goodman was examined. This route relied on stereodivergent hydrogenations of a 4-exomethyleneproline. To provide a more succinct synthesis and to improve the stereoselectivity for the *cis*-series (3:1 reported), an ester was used at the α-position rather than a protected alcohol, as a means to increase the steric bulk crucial for facial selectivity during heterogeneous hydrogenation. Since esters are reported to be capable of directing Crabtree's catalyzed hydrogenations almost as efficiently as alcohols, a significant reduction in stereoselectivity in the *trans*-series (>40:1 reported) was not anticipated.[22] Furthermore, this change would remove the need for redox manipulation at the α-carbon, thus abbreviating the synthesis. We report here an improved, six-step stereoselective synthesis of the 4-methylproline diastereoisomers.

Results and Discussion

The route (Scheme 2) employed the key exomethylene intermediates **2** and *ent*-**2** and used *trans*-4-hydroxy-L-proline and *cis*-4-hydroxy-D-proline, the least expensive of the four diastereoisomers of 4-hydroxyproline (all four available commercially), as starting materials. Esterification and CBz amine protection proceeded cleanly. Several of the CBz-protected amino acid derivatives described below were observed as inseparable mixtures of *syn*- and *anti*-rotamers by NMR spectroscopy, which is readily explained by the tendency of secondary amino acids to promote the less-favored *syn*-conformation on attached amide or carbamate bonds. Oxidation using classic chromium conditions occurred in good yield to give **1**/*ent*-**1**. Olefination was next undertaken on **1**/*ent*-**1**, initially employing the Wittig conditions utilized by Chirgadze et al.[23] However, the exocyclic methylene products obtained displayed no optical activity, suggesting that epimerization of the α-center had occurred under the basic Wittig conditions. This was confirmed when, after divergent hydrogenation and deprotection of both enantiomers, HPLC analysis of Marfey's derivatives of each of the 4-methylprolines showed the same two major peaks present in equal abundance for each series (see Figure S2; Supporting Information). The milder Petasis reagent for olefination of **1**/*ent*-**1**[24,25] was therefore employed, and modest isolated yields were obtained (42–56%).

Scheme 1. General Procedure for Marfey's Derivatization of Amino Acids



Heterogeneous hydrogenation of **2**/*ent*-**2** using a Pd/C catalyst proceeded cleanly, with concomitant cleavage of the CBz group to give **3**/*ent*-**3**. Hydrogenation using Crabtree's homogeneous catalyst was slow, taking around 5 days to complete. It was found that the initially formed product of the reaction was an isomerization product where the exocyclic methylene double bond had migrated to an endocyclic position to give the enamines **4**/*ent*-**4**. This product could be isolated in near quantitative yield after 16 h. HRESIMS established that the mass of the pseudomolecular ion of **4** was isobaric with the starting material, **2**, suggestive of an isomerization product. ^{1}H NMR data (1:1 mixture of rotamers around the

* To whom correspondence should be addressed. E-mail: murray.munro@canterbury.ac.nz. Tel: +64 3364 2434. Fax: +64 3364 2110.

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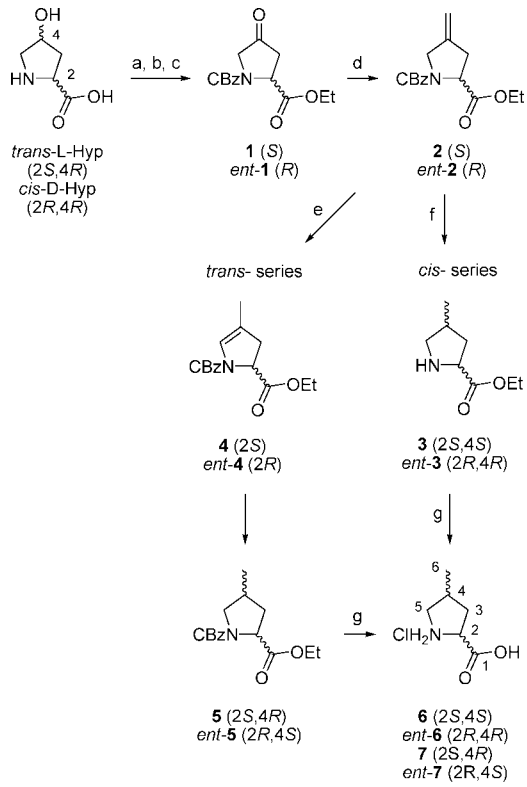
Scheme 2[a]



[a] Reagents and conditions: (a) $SOCl_2$, EtOH, reflux, 4 h; (b) CBzCl, TEA, MeOH, rt, 16 h; (c) CrO_3, pyridine, CH_2Cl_2, rt, 16 h (**1**: 80%, *ent*-**1**: 67%); (d) dimethyltitanocene, toluene, 90 °C, 3 h (**2**: 56%, *ent*-**2**: 42%); (e) Crabtree's catalyst, $CHCl_3$, H_2, rt, 5 d (**5**: 84%, *ent*-**5**: 80%), (f) Pd/C, CH_2Cl_2, H_2, rt, 16 h (**3**: 67%, *ent*-**3**: 100%); (g) $HCl_{(aq)}$ 6 M, 70 °C, 6 h (**6**: 100%, *ent*-**6**: 87%, **7**: 88%, *ent*-**7**: 80%).

carbamate observed) established the presence of broadened singlet olefinic protons (δ 6.40 and 6.32) and broadened singlet methyl groups (δ 1.70 and 1.68). COSY NMR spectroscopy confirmed the proposed structure, with correlations between the H-2 (δ 4.70 and 4.64) and H-3 (δ 2.99 and 2.53) protons, as well as an allylic coupling between the H-5 proton and H-6 protons. Alkene migration under Crabtree's hydrogenation conditions has been reported by a number of groups.[26–28] Interestingly, these groups reported that no hydrogenation of the isomerized alkene could be achieved; however with extended reaction times **4**/*ent*-**4** were cleanly hydrogenated to the protected 4-methylprolines **5**/*ent*-**5**. The intermediacy of **4**/*ent*-**4** was confirmed by purification of **4** and placing it under identical hydrogenation conditions, whereupon its consumption and the appearance of **5** could be monitored by proton NMR spectroscopy over the course of several days. Deprotection of **3** and **5** and their enantiomers was achieved under acid hydrolysis conditions to give the 4-methylproline diastereoisomers as hydrochloride salts. Analysis of the ^{1}H NMR data showed that a good degree of stereoselectivity had been achieved by both hydrogenation routes (see Figure S1; Supporting Information). NMR data for both diastereoisomeric pairs were in good accordance with literature values, and 1D NOESY NMR experiments confirmed the assigned stereochemistry.[29]

Marfey's derivatization of each of the diastereoisomers of 4-methylproline and HPLC analysis (see Figure S3; Supporting Information) allowed the optical purity of the final products to be determined. The *cis*-series showed a diastereomeric ratio of around 7:1, an improvement on the method of Del Valle and Goodman, who obtained a ratio of 3:1. The *trans*-series showed a diastereomeric ratio of 15:1, which is a moderately significant reduction compared to that reported by Del Valle and Goodman of >40:1. This is likely due to less efficient binding of the ester to the catalyst compared to an alcohol, leading to a reduced ability to direct the hydrogenation. With the directing effect diminished, competing, nondirected hydrogenations took place, leading to a lowering of the observed stereoselectivity. However, the excess obtained is still more than sufficient for the purposes of natural product stereochemical assignments.

This route to the 4-methylprolines represents an improvement on the current literature procedures and offers a succinct route (six steps) to all four diastereoisomers of 4-methylproline from inexpensive starting materials, with late-stage stereodivergence. High yields were obtained in all but one step, with maximum cumulative yields of 46% for the *cis*-series and 34% for the *trans*-series. More consistent stereoselectivities were also obtained than the route of Del Valle and Goodman, with both series obtained in moderately high stereochemical excess (75% de for *cis* and 88% de for *trans*).

Experimental Section

General Experimental Procedures. Optical rotation data were recorded on a Perkin-Elmer 341 polarimeter. IR measurements were taken on either a Shimadzu FTIR-8201PC spectrophotometer or a Perkin-Elmer Spectrum One FTIR spectrophotometer. NMR spectra were recorded on either a 500 or 75 MHz instrument and were referenced using tetramethylsilane (TMS) for spectra recorded in $CDCl_3$ and *tert*-butyl alcohol (δ_C 30.3 ppm) and residual solvent peaks for those measured in D_2O (δ_H 4.70 ppm). Samples were analyzed on a micromass LCT mass spectrometer equipped with an electrospray ionization (ESI) probe. Analytical high-pressure liquid chromatography (HPLC) was carried out on a Dionex HPLC instrument. Thin-layer chromatography (TLC) was done on 250 μm fluorescent plates and visualized using UV and/or PMA spray reagent (10% w/v phosphomolybdic acid in ethanol). Normal-phase flash column chromatography was performed using silica gel (230–400 mesh, 60 Å pore size) and commercial grade solvents. MeOH was distilled under nitrogen over magnesium and stored over 4 Å sieves. Toluene was distilled under nitrogen over calcium hydride. Reagents obtained from commercial suppliers were used without further purification.

***N*-CBz-*trans*-4-Hydroxy-L-proline Ethyl Ester.** Preparation of *trans*-4-hydroxy-L-proline ethyl ester was performed according to the literature (*trans*-4-hydroxy-L-proline ethyl ester hydrochloride: white, amorphous solid, 96%).[18] *trans*-4-Hydroxy-L-proline ethyl ester hydrochloride (1 g, 5.11 mmol) was dissolved in anhydrous MeOH (20 mL) and cooled to 0 °C. Triethylamine (2.15 mL, 15.4 mmol, 3.0 equiv) was added followed by the dropwise addition of benzylchloroformate (950 μL, 6.75 mmol, 1.3 equiv). The reaction was warmed to room temperature and stirred for 16 h. The solvent was removed *in vacuo*, EtOAc (30 mL) was added, and then the mixture was washed with citric $acid_{(aq)}$ (5% w/v, 3 × 30 mL) and H_2O (2 × 20 mL). The organic phase was dried over $MgSO_4$ and filtered and the solvent removed *in vacuo*. The crude product was purified on silica using a stepwise gradient of 5−50% EtOAc in petroleum ether to give *N*-CBz-*trans*-4-hydroxy-L-proline ethyl ester (1.46 g, 4.98 mmol, 98%) as a colorless oil (an inseparable ~1:1 mixture of *syn*- and *anti*-rotamers around the carbamate was obtained): R_f 0.27 (silica, 1:1 petroleum ether−EtOAc, visualization UV/PMA); $[\alpha]^{20}_D$ –55 (*c* 1.00, $CHCl_3$); IR (thin film) ν_{max} 1743, 1707, 1421, 1358, 1196 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40–7.29 (5H, m, CBz), 5.16–5.03 (2H, m, CBz), 5.05 (1H, d, 12.7 Hz, O*H*), 4.51–4.46 (2H, m, H-4 and H-2), 4.21 (1H, q, 7.2 Hz, Et), 4.02 (1H, m, Et), 3.69–3.54 (2H, m, H-5), 2.31 (1H, m, H-3a), 2.10 (1H, m, H-3b), 1.27 (3/2H, t, 7.1 Hz, Et), 1.11 (3/2H, t, 7.1 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1 (C, ester C═O), 172.9 (C, ester C═O), 155.4 (C, CBz C═O), 155.0 (C, CBz C═O), 136.9 (C, CBz), 136.7 (C, CBz), 128.9 (CH, CBz), 128.8 (CH, CBz), 128.4 (CH, CBz), 128.3 (CH, CBz), 128.2 (CH, CBz), 70.6 (CH, C-4), 69.9 (CH, C-4), 67.7 (CH_2, CBz), 67.6 (CH_2, CBz), 61.7 (CH_2, Et), 61.6 (CH_2, Et), 58.4 (CH, C-2), 58.2 (CH, C-2), 55.6 (CH_2, C-5), 55.0 (CH_2, C-5), 39.6 (CH_2, C-3), 38.9 (CH_2, C-3), 14.5 (CH_3, Et), 14.4 (CH_3, Et); HRESIMS *m*/*z* 294.1344 $[M + H]^+$ 1.0 ppm (294.1341 calcd for $C_{15}H_{20}NO_5$).

***N*-CBz-*cis*-4-Hydroxy-D-proline Ethyl Ester.** *cis*-4-Hydroxy-D-proline ethyl ester hydrochloride[18] (white, amorphous solid, 77%) was protected as above to give *N*-CBz-*cis*-4-hydroxy-D-proline ethyl ester as a colorless oil in approximately quantitative yield (an inseparable

~1:1 mixture of *syn*- and *anti*-rotamers around the carbamate was obtained): R_f 0.24 (silica, 1:1 petroleum ether−EtOAc, visualization UV/PMA); $[\alpha]^{20}_D$ +20 (*c* 0.10, $CHCl_3$); IR (thin film) ν_{max} 1748, 1705, 1418, 1351, 1201 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.30 (5H, m, CBz), 5.14 (2H, m, CBz), 4.43 (1H, d, 10.0 Hz, H-2), 4.38 (1H, m, H-4), 4.26 (1H, q, 7.0 Hz, Et), 4.08 (1H, m, Et), 3.78 (1/2H, d, 11.7 Hz, H-5a), 3.73 (1/2H, d, 11.7 Hz, H-5a), 3.64 (1/2H, dd, 12.2 Hz, 4.6 Hz, H-5b), 3.60 (1/2H, dd, 11.8 Hz, 4.5 Hz, H-5b), 2.34 (1H, m, H-3a), 2.12 (1H, m, H-3b), 1.31 (3/2H, t, 7.3 Hz, Et), 1.15 (3/2H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.7 (C, ester C═O), 174.5 (C, ester C═O), 154.9 (C, CBz C═O), 154.2 (C, CBz C═O), 136.3 (C, CBz), 136.2 (C, CBz), 128.5 (CH, CBz), 128.4 (CH, CBz), 128.1 (CH, CBz), 127.9 (CH, CBz), 127.8 (CH, CBz), 71.3 (CH, C-4), 70.3 (CH, C-4), 67.3 (CH_2, CBz), 62.0 (CH_2, Et), 61.9 (CH_2, Et), 58.3 (CH, C-2), 57.9 (CH, C-2), 56.2 (CH_2, C-5), 55.9 (CH_2, C-5), 38.6 (CH_2, C-3), 37.7 (CH_2, C-3), 13.9 (CH_3, Et); HRESIMS *m*/*z* 294.1329 $[M + H]^+$ 4.1 ppm (294.1341 calcd for $C_{15}H_{20}NO_5$).

***N*-CBz-4-Keto-L-proline Ethyl Ester, 1.** Chromium trioxide (6.30 g, 63.0 mmol, 13 equiv) was dissolved in a mixture of pyridine (10.2 mL) and CH_2Cl_2 (50 mL) and stirred at room temperature for 15 min. After this time *N*-CBz-*trans*-4-hydroxy-L-proline ethyl ester (1.4 g, 4.77 mmol) dissolved in CH_2Cl_2 (20 mL) was added to the mixture. The reaction was stirred at room temperature for 16 h, and then filtered through a silica pad using 50% EtOAc in petroleum ether (200 mL). After removal of the solvents *in vacuo*, the product (1.18 g, 4.05 mmol, 85%) was obtained as a colorless oil (an inseparable ~1:1 mixture of *syn*- and *anti*-rotamers around the carbamate was obtained): R_f 0.43 (silica, 2:1 petroleum ether−EtOAc, visualization UV/PMA); $[\alpha]^{20}_D$ –4 (*c* 1.00, $CHCl_3$); IR (thin film) ν_{max} 1767, 1743, 1713, 1416, 1192, 1158 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.32 (5H, m, CBz), 5.20 (2H, m, CBz), 4.85 (1/2H, d, 10.8 Hz, H-2), 4.81 (1/2H, d, 10.8 Hz, H-2) 4.22 (1H, q, 7.2 Hz, Et), 4.09 (1H, m, Et), 3.96 (2H, m, H-5), 2.95 (1H, m, H-3a), 2.60 (1H, dd, 18.8 Hz, 2.6 Hz, H-3b), 1.27 (3/2H, t, 7.2 Hz, Et), 1.16 (3/2H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.8 (C, C-4), 207.1 (C, C-4), 171.3 (C, ester C═O), 154.9 (C, CBz C═O), 154.1 (C, CBz C═O), 135.9 (C, CBz), 129.0 (CH, CBz), 128.5 (CH, CBz), 128.3 (CH, CBz), 128.0 (CH, CBz), 67.7 (CH_2, CBz), 61.8 (CH_2, Et), 56.0 (CH, C-2), 52.6 (CH_2, C-5), 52.4 (CH_2, C-5), 41.1 (CH_2, C-3), 40.6 (CH_2, C-3), 14.0 (CH_3, Et); HRESIMS *m*/*z* 292.1191 $[M + H]^+$ 2.1 ppm (292.1185 calcd for $C_{15}H_{18}NO_5$).

***N*-CBz-4-Keto-D-proline Ethyl Ester, *ent*-1.** As described above using *N*-CBz-*cis*-4-hydroxy-D-proline ethyl ester: colorless oil obtained in 87% yield; $[\alpha]^{20}_D$ +4 (*c* 1.00, $CHCl_3$).

***N*-CBz-4-Exomethylene-L-proline Ethyl Ester, 2.** To *N*-CBz-4-keto-L-proline ethyl ester (**1**, 100 mg, 0.34 mmol) dissolved in anhydrous toluene (1 mL) was added a toluene solution of dimethyltitanocene[24] (~0.88 mmol in ~ 1 mL, 2.6 equiv), and the reaction was heated to 90 °C for 3 h. After cooling to room temperature the mixture was added dropwise to stirred petroleum ether (50 mL), whereupon a yellow precipitate formed and was stirred for a further 30 min. The precipitate was removed by filtration through a bed of Celite, and the solvents were removed *in vacuo*. The crude residue was purified by chromatography on silica using a stepwise gradient of 2–10% diethyl ether in petroleum ether to give the product (54 mg, 0.19 mmol, 56%) as a colorless oil (an inseparable ~1:1 mixture of *syn*- and *anti*-rotamers around the carbamate was obtained): R_f 0.57 (silica, 2:1 petroleum ether−EtOAc, visualization UV/PMA); $[\alpha]^{20}_D$ –16 (*c* 1.00, $CHCl_3$); IR (thin film) ν_{max} 1747, 1714, 1417, 1360, 1196, 896 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.29 (5H, m, CBz), 5.14 (2H, m, H-6), 5.02 (2H, m, CBz), 4.55 (1/2H, dd, 9.6 Hz, 2.9 Hz, H-2), 4.50 (1/2H, dd, 9.6 Hz, 2.9 Hz, H-2), 4.17 (3H, m, H-4, Et), 4.07 (1H, m, Et), 2.98 (1H, m, H-3a), 2.64 (1H, br d, 16.1 Hz, H-3b), 1.26 (3/2H, t, 7.2 Hz, Et), 1.14 (3/2 H, t, 7.2 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.1 (C, ester C═O), 172.0 (C, ester C═O), 154.9 (C, CBz C═O), 154.3 (C, CBz C═O), 142.9 (C, C-4), 142.0 (C, C-4), 136.5 (C, CBz), 136.4 (C, CBz), 128.8 (CH, CBz), 128.6 (CH, CBz), 128.5 (CH, CBz), 128.4 (CH, CBz), 128.01 (CH, CBz), 127.97 (CH, CBz), 127.9 (CH, CBz), 127.8 (CH, CBz), 108.3 (CH_2, C-6), 108.2 (CH_2, C-6), 67.13 (CH_2, CBz), 67.07 (CH_2, CBz), 61.3 (CH_2, Et), 61.2 (CH_2, Et), 59.0 (CH, C-2), 58.9 (CH, C-2), 51.0 (CH_2, C-5), 50.5 (CH_2, C-5), 36.8 (CH_2, C-3), 36.0 (CH_2, C-3), 14.1 (CH_3, Et), 14.0 (CH_3, Et); HRESIMS *m*/*z* 290.1404 $[M + H]^+$ 4.1 ppm (290.1392 calcd for $C_{16}H_{20}NO_4$).

***N*-CBz-4-Exomethylene-D-proline Ethyl Ester, *ent*-2.** As described above, using *N*-CBz-4-keto-D-proline ethyl ester, *ent*-**1**: colorless oil obtained in 42% yield; $[\alpha]^{20}_D$ +15 (*c* 1.00, $CHCl_3$).

***cis*-4-Methyl-L-proline Ethyl Ester, 3.** *N*-CBz-4-Exomethylene-L-proline ethyl ester (**2**, 15 mg, 0.052 mmol) was dissolved in CH_2Cl_2 (1 mL) and 10% Pd/C (1.5 mg) added. The reaction was placed under an atmosphere of $H_{2(g)}$ by means of a balloon and stirred at room temperature for 16 h. The catalyst was removed by filtration through a pad of Celite using CH_2Cl_2 (15 mL) and the solvent removed *in vacuo* to give the product (5.5 mg, 0.035 mmol, 67%) as a colorless oil: $[\alpha]^{20}_D$ –36 (*c* 0.31, $CHCl_3$) (*cis*:*trans*, ~7:1); IR (thin film) ν_{max} 3418, 1743, 1263, 1230 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.47 (1H, t, 8.8 Hz, H-2), 4.30 (2H, q, 7.1 Hz, Et), 3.68 (1H, m, H-5a), 2.98 (1H, br t, 10.5 Hz, H-5b), 2.63–2.56 (2H, m, H-3a and H-4), 1.66 (1H, m, H-3b), 1.32 (3H, t, 7.1 Hz, Et), 1.13 (3H, d, 6.4 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1 (C, ester C═O), 62.9 (CH_2, Et), 59.0 (CH, C-2), 52.3 (CH_2, C-5), 36.8 (CH_2, C-3), 33.2 (CH, C-4), 16.4 (CH_3, C-6), 14.0 (CH_3, Et); HRESIMS *m*/*z* 158.1183 $[M + H]^+$ 1.3 ppm (158.1181 calcd for $C_8H_{16}NO_2$).

***cis*-4-Methyl-D-proline Ethyl Ester, *ent*-3.** As described above, using *N*-CBz-4-exomethylene-D-proline ethyl ester, *ent*-**2**: colorless oil obtained in quantitative yield; $[\alpha]^{20}_D$ +39 (*c* 0.30, $CHCl_3$) (*cis*:*trans*, ~7:1).

***N*-CBz-*trans*-4-Methyl-L-proline Ethyl Ester, 5.** *N*-CBz-4-Exomethylene-L-proline ethyl ester (**2**, 30 mg, 0.104 mmol) was dissolved in $CHCl_3$ (1 mL), and Ir(cod)pyr(PCy_3)]PF_6 (Crabtree's catalyst, 3 mg, 0.004 mmol, 4 mol %) was added. The reaction was placed under an atmosphere of $H_{2(g)}$ by means of a balloon and stirred at room temperature for 5 days. The crude product was purified on silica using a stepwise gradient of 2% to 6% diethyl ether in petroleum ether to give the product (25 mg, 0.087 mmol, 84%) as a colorless oil, plus isomerization product **4** (3 mg, 0.010 mmol, 10%) as a colorless oil. Inseparable ~1:1 mixtures of *syn*- and *anti*-rotamers around the carbamate were obtained for both products. **4**: R_f 0.29 (silica, 4:1 petroleum ether−EtOAc, visualization UV/PMA); IR (thin film) ν_{max} 1751, 1709, 1427, 1203, 1183 cm^{-1}; $[\alpha]^{20}_D$ –87 (*c* 0.30, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.30 (5H, m, CBz), 6.40 (1/2H, br s, H-5), 6.32 (1/2H, br s, H-5), 5.15 (2H, m, CBz), 4.70 (1/2H, dd, 12.0 Hz, 5.0 Hz, H-2), 4.64 (1/2H, dd, 12.0 Hz, 5.0 Hz, H-2), 4.23 (1H, q, 7.0 Hz, Et), 4.08 (1H, q, 7.1 Hz, Et), 2.99 (1H, m, H-3a), 2.53 (1H, m, H-3b), 1.70 (3/2H, br s, H-6), 1.68 (3/2H, br s, H-6), 1.28 (3/2H, t, 7.1 Hz, Et), 1.15 (3/2H, t, 7.1 Hz, Et); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 128.5 (CH, CBz), 128.0 (CH, CBz), 127.9 (CH, CBz), 124.3 (CH, C-5), 123.7 (CH, C-5), 67.3 (CH_2, CBz), 67.1 (CH_2, CBz), 61.4 (CH_2, Et), 58.7 (CH, C-2), 58.6 (CH, C-2), 39.6 (CH_2, C-3), 14.1 (CH_3, Et), 13.2 (CH_3, C-6), quaternary carbons not observed; HRESIMS *m*/*z* 290.1404 $[M + H]^+$ 4.1 ppm (290.1392 calcd for $C_{16}H_{20}NO_4$). **5**: R_f 0.24 (silica, 4:1 petroleum ether−EtOAc, visualization UV/PMA); $[\alpha]^{20}_D$ –43 (*c* 1.00, $CHCl_3$) (*cis*:*trans*, ~1:15); IR (thin film) ν_{max} 1747, 1710, 1417, 1357, 1194 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.28 (5H, m, CBz), 5.18–5.04 (2H, m, CBz), 4.41 (1/2H, dd, 9.0 Hz, 2.6 Hz, H-2), 4.36 (1/2H, dd, 9.0 Hz, 2.6 Hz, H-2), 4.19 (1H, q, 7.4 Hz, Et), 4.05 (1H, m, Et), 3.78 (1H, m, H-5a), 3.05 (1/2H, dd, 10.1 Hz, 8.5 Hz, H-5b), 2.99 (1/2H, dd, 10.1 Hz, 8.8 Hz, H-5b), 2.41 (1H, m, H-4), 2.09 (1H, m, H-3a), 1.84 (1H, m, H-3b), 1.26 (3/2H, t, 7.2 Hz, Et), 1.14 (3/2H, t, 7.2 Hz, Et), 1.05 (3/2H, d, 6.7 Hz, H-6), 1.03 (3/2H, d, 6.7 Hz, H-6); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.8 (C, ester C═O), 172.6 (C, ester C═O), 154.2 (C, CBz C═O), 136.7 (C, CBz), 128.4 (CH, CBz), 128.3 (CH, CBz), 127.91 (CH, CBz), 127.85 (CH, CBz), 127.8 (CH, CBz), 127.7 (CH, CBz), 66.94 (CH_2, CBz), 66.88 (CH_2, CBz), 61.1 (CH_2, Et), 61.0 (CH_2, Et), 59.5 (CH, C-2), 59.1 (CH, C-2), 53.6 (CH_2, C-5), 53.3 (CH_2, C-5), 38.5 (CH_2, C-3), 37.6 (CH_2, C-3), 32.0 (CH, C-4), 31.1 (CH, C-4), 17.33 (CH_3, C-6), 17.29 (CH_3, C-6), 14.14 (CH_3, Et), 14.05 (CH_3, Et); HRESIMS *m*/*z* 292.1545 $[M + H]^+$ 1.4 ppm (292.1549 calcd for $C_{16}H_{22}NO_4$).

Note. If this reaction was allowed to proceed for 16 h and then purified, no hydrogenated product **5** was isolated, and an approximately quantitative yield of **4** was recovered. If purified **4** was reacted under the same conditions as above, its conversion to **5** could be observed by proton NMR spectroscopy over the course of several days.

***N*-CBz-*trans*-4-Methyl-D-proline Ethyl Ester, *ent*-5.** As described above, using *N*-CBz-4-exomethylene-D-proline ethyl ester, *ent*-**2**: colorless oil obtained in 80% yield; $[\alpha]^{20}_D$ +41 (*c* 0.31, $CHCl_3$) (*cis*:*trans*, ~1:15).

***cis*-4-Methyl-L-proline Hydrochloride, 6.** *cis*-4-Methyl-L-proline ethyl ester (**3**, 3.3 mg, 0.021 mmol) was dissolved in 6 M $HCl_{(aq)}$ (300 μL), sealed in a thick-walled glass tube, and heated to 70 °C for 6 h. After cooling to room temperature, the reaction was extracted with

EtOAc (2 × 300 μL) and the $HCl_{(aq)}$ removed by evaporation under a stream of $N_{2(g)}$ to give the product (2.8 mg, 0.021 mmol, quantitative) as an off-white solid: $[\alpha]^{20}_D$ –41 (*c* 0.07, H_2O) (*cis*:*trans*, ~7:1); IR (diffuse reflectance) ν_{max} 3100–2900, 1738, 1455, 1405, 1213 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 4.30 (1H, dd, 8.2 Hz, 9.8 Hz, H-2), 3.43 (1H, dd, 11.4 Hz, 7.4 Hz, H-5a), 2.87 (1H, br t, 10.6 Hz, H-5b), 2.52 (1H, dt, 13.1 Hz, 7.6 Hz, H-3a), 2.38 (1H, m, H-4), 1.64 (1H, dt, 13.1 Hz, 9.8 Hz, H-3b), 0.99 (3H, d, 6.7 Hz, H-6); ^{13}C NMR (D_2O, 75 MHz) δ 173.0 (C, C═O), 60.7 (CH, C-2), 52.7 (CH_2, C-5), 36.9 (CH_2, C-3), 33.7 (CH, C-4), 16.4 (CH_3, C-6); HRESIMS *m*/*z* 130.0862 $[M + H]^+$ 4.6 ppm (130.0868 calcd for $C_6H_{12}NO_2$).

***cis*-4-Methyl-D-proline Hydrochloride, *ent*-6.** As described above, using *cis*-4-methyl-D-proline ethyl ester, *ent*-**3**: amorphous, white solid obtained in 87% yield; $[\alpha]^{20}_D$ +31 (*c* 0.12, H_2O) (*cis*:*trans*, ~7:1) (lit.[16] free amine +85.2, *c* 1.68, H_2O; +47.9, *c* 1.28, 3 N HCl).

***trans*-4-Methyl-L-proline Hydrochloride, 7.** As described above using *trans*-4-methyl-L-proline ethyl ester, **5**: amorphous, white solid obtained in 88% yield; $[\alpha]^{20}_D$ –33 (*c* 0.21, H_2O) (*cis*:*trans*, ~1:15) (lit.[16] free amine –56.6, *c* 1.03, H_2O; –23.9, *c* 1.54, 3 N HCl); IR (diffuse reflectance) ν_{max} 1736, 1456, 1358, 1265, 1211 cm^{-1}; ^1H NMR (D_2O, 500 MHz) δ 4.38 (1H, dd, 9.6 Hz, 5.1 Hz, H-2), 3.49 (1H, dd, 11.6 Hz, 7.2 Hz, H-5a), 2.83 (1H, dd, 11.5 Hz, 8.6 Hz, H-5b), 2.38–2.25 (2H, m, H-3a and H-4), 1.94 (1H, dt, 13.2 Hz, 9.0 Hz, H-3b), 0.99 (3H, d, 6.6 Hz, H-6); ^{13}C NMR (D_2O, 75 MHz) δ 172.8 (C, C═O), 59.9 (CH, C-2), 52.9 (CH_2, C-5), 36.4 (CH_2, C-3), 32.3 (CH, C-4), 16.6 (CH_3, C-6); HRESIMS *m*/*z* 130.0862 $[M + H]^+$ 4.6 ppm (130.0868 calcd for $C_6H_{12}NO_2$).

***trans*-4-Methyl-D-proline Hydrochloride, *ent*-7.** As described above, using *trans*-4-methyl-D-proline ethyl ester, *ent*-**5**: amorphous, white solid obtained in 80% yield; $[\alpha]^{20}_D$ +31 (*c* 0.04, H_2O) (*cis*:*trans*, ~1:15).

Marfey's Derivatization[17] and HPLC Analysis. A 1% (w/v) solution (100 μL) of FDAA (Marfey's reagent, N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide) in acetone was added to an aliquot (50 μL) of a 50 mM solution of each amino acid. After addition of $NaHCO_3$ solution (1 M; 20 μL), the mixture was incubated (1 h at 40 °C). The reaction was stopped by addition of HCl (2 M; 10 μL), the solvents were evaporated to dryness, and the residue was redissolved in MeOH−H_2O (1:1; 1 mL). An aliquot of each of these solutions (10 μL) was analyzed by HPLC (Prodigy C18, 250 × 4.6 mm, 5 μm; solvents: A: H_2O + 0.05% TFA, B: MeOH; 0 min 45% B, 30 min 65% B; 25 °C; 1 mL·min^{-1}; detection at 330 nm). Retention times for the derivatized amino acids under the reported conditions were as follows: **6**, 16.62 min; *ent*-**6**, 18.48 min; **7**, 18.26 min; *ent*-**7**, 19.25 min.[1]

^1H NMR data (CD_3OD, 500 MHz, capillary probe) for the Marfey's derivatives of each diastereoisomer.

***trans*-4-Methyl-L-proline derivative:** δ 8.69 (1H, s), 5.79 (1H, s), 4.50 (1H, t, 6.8 Hz), 4.18 (1H, q, 6.9 Hz), 3.57 (1H, dd, 10.3 Hz, 6.6 Hz), 2.95 (1H, dd, 10.1 Hz, 5.0 Hz), 2.55 (1H, m), 2.24 (1H, m), 2.12 (1H, m), 1.54 (3H, d, 6.8 Hz), 1.04 (3H, d, 6.9 Hz).

***trans*-4-Methyl-D-proline derivative:** δ 8.66 (1H, s), 5.79 (1H, s), 4.37 (1H, m), 4.18 (1H, m), 3.54 (1H, dd, 10.5 Hz, 6.8 Hz), 2.94 (1H, dd, 10.4 Hz, 5.5 Hz), 2.55 (1H,m), 2.21 (1H, m), 2.06 (1H, m), 1.57 (3H, d, 6.8 Hz), 1.03 (3H, d, 6.8 Hz).

***cis*-4-Methyl-L-proline derivative:** δ 8.71 (1H, s), 5.85 (1H, s), 4.26–4.17 (2H, m), 3.33 (1H, m), 2.96 (1H, m), 2.51 (1H, m), 2.18 (1H, m), 1.71 (1H, q, 11.1 Hz), 1.50 (3H, d, 6.8 Hz), 1.10 (3H, d, 6.5 Hz).

***cis*-4-Methyl-D-proline derivative:** δ 8.73 (1H, s), 5.84 (1H, s), 4.54 (1H, m), 4.17 (1H, q, 6.9 Hz), 3.28 (1H, m), 3.18 (1H, m), 2.61 (1H, m), 2.30 (1H, m), 1.68 (1H, m), 1.60 (3H, d, 7.0 Hz), 1.13 (3H, d, 6.4 Hz).

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Supporting Information Available: ^1H NMR spectra of 4-methylproline hydrochloride residues and HPLC chromatograms of Marfey's derivatives of all 4-methylproline stereoisomers. This material is available free of charge via the Internet at http://pubs.acs.org.

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