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Conformationally Constrained Amino Acids. Synthesis and Optical Resolution of 3-Substituted Proline Derivatives

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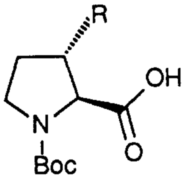
The literature route to racemic cis- and trans-3-substituted prolines starting from acetamidomalonate and α,β-unsaturated aldehydes has been refined and applied to the synthesis of 3-phenyl- and 3-*n*-propylproline. A key improvement in the sequence was the acid-catalyzed silane reduction of the initial hydroxylactam Michael adduct **2**, which allowed subsequent transformations to proceed cleanly and in high yield. Both the *N*- and 3-substitutents were found to have an effect on selectivity in the saponification of trans-3-substituted proline esters in the presence of the corresponding cis esters. The trans isomers in each series were resolved via the diastereomeric (*S*)-α-methylbenzylamides, and the absolute configurations of the resulting pure optical isomers were assigned.

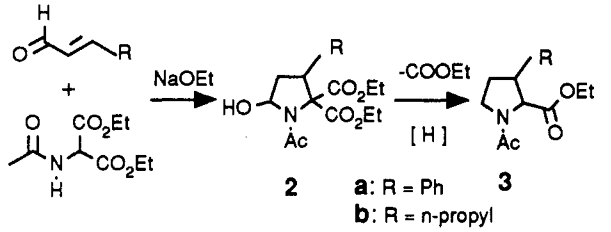
Proline is a well-known means of inducing conformational constraints into peptides.[1] Because of motional restrictions inherent to the pyrrolidine ring, the presence of a proline residue greatly reduces the available conformational space of a peptide and gives rise to conformers separated by relatively high interconversion barriers.[2] Thus, information may be obtained about the bioactive conformation of a peptide, and the biological potency may be increased by incorporation of a proline residue. However, when substitution of an amino acid residue with proline leads to a reduction in biological activity, the question arises whether the loss is due to conformational or steric considerations or to loss of a favorable interaction associated with the side chain of the original amino acid residue. In this vein, we have examined the incorporation of 3-substituted proline derivatives into peptides, where the 3-substituent corresponds to the substituent on the β-carbon of standard amino acids. For example, 3-substituted prolines **1a–c** are conformationally constrained analogues of phenylalanine, norleucine, and aspartate, respectively.



1a, R = Ph
1b, R = $CH_2CH_2CH_3$
1c, R = CO_2R'

In this paper, we describe our work on the synthesis and enantiomeric resolution of derivatives of 3-phenyl- and 3-*n*-propylproline. A stereospecific synthesis of *N*-Boc-*trans*-3-*n*-propyl-L-proline, **1b**, from 4-hydroxy-L-proline was recently developed in our laboratory,[3] but the route was not applicable to the synthesis of **1a** and was not amenable to large-scale work. We were attracted to the schemes outlined by Cox et al.,[4] Mauger et al.,[5] and Sarges et al.[6] for the synthesis of racemic 3-methyl- and 3-phenylproline, which entail condensation of an (acylamino)malonate to the appropriate α,β-unsaturated aldehyde, followed by a sequence of reactions that effect overall 5-deoxygenation and 2-dealkoxycarbonylation (Scheme I). Here we report observations made during our work on the synthesis of 3-phenylproline according to the literature method[6] and describe some refinements in the published procedure. In addition, further modifications were required during the application of these procedures to the synthesis of 3-*n*-propylproline. Finally, we report the optical resolution of the trans isomer of each analogue via the diastereomeric α-methylbenzylamides and the assignment of absolute stereochemistry to the pure optical isomers.

Scheme I



Results and Discussion

The literature reports suggest several procedural variations for effecting conversion of the initial Michael adduct **2** to a mixture of cis- and trans-3-substituted prolines **3** (Scheme I). The major options essentially reduce to whether the reduction in oxidation state at C-5 precedes or follows the dealkoxycarbonylation at C-2. In the reported synthesis of 3-phenylproline,[6] C-5 reduction followed by a standard malonate-type dealkoxycarbonylation sequence gave the cis isomer as the major product, whereas significant quantities of the trans isomer were obtained only when dealkoxycarbonylation preceded C-5 reduction or when the cis ester was saponified under forcing conditions.

During our efforts at replicating these procedures, it became evident that some refinements in the protocol should afford a more attractive method for preparing either or both isomers of the target structure. In particular, deoxygenation at C-5 early in the sequence is clearly preferable in terms of yield and ease of handling, since

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[1] (a) Momany, F. A; Chuman, H. *Methods Enzymol.* **1986**, *124*, 3. (b) Marshall, G. R. In *Chemical Recognition in Biological Systems*; Creighton, A. M., Turner, S., Eds.; The Chemical Society: London, 1982; p 279. (c) Arison, B. H.; Hirschmann, R.; Veber, D. F. *Bioorg. Chem.* **1978**, *7*, 447.

[2] (a) Hollósi, M.; Radics, L.; Wieland, T. *Int. J. Peptide Protein Res.* **1977**, *10*, 286. (b) Delaney, N. G.; Madison, V. *Int. J. Peptide Protein Res.* **1982**, *19*, 543, and references therein.

[3] Holladay, M. W.; May, C. S.; Arnold, W. A., unpublished results. **1b**: mp 88–90 °C; $[\alpha]_D^{25} = -40.6°$ (*c* 1, $CHCl_3$). Optical purity of this product was assessed as >98% based on HPLC analysis of derived α-methylbenzylamides.

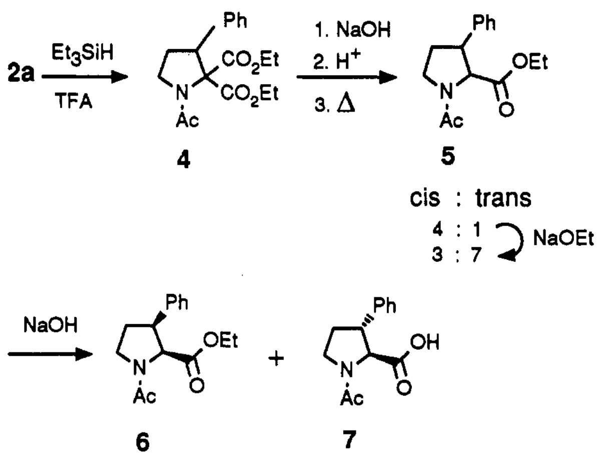
[4] Cox, D. A.; Johnson, A. W.; Mauger, A. B. *J. Chem. Soc.* **1964**, 5024.

[5] Mauger, A. B.; Irreverre, F.; Witkop, B. *J. Am. Chem. Soc.* **1966**, *88*, 2019.

[6] Sarges, R.; Tretter, J. R. *J. Org. Chem.* **1974**, *39*, 1710.

0022-3263/90/1955-0270$02.50/0

Scheme II

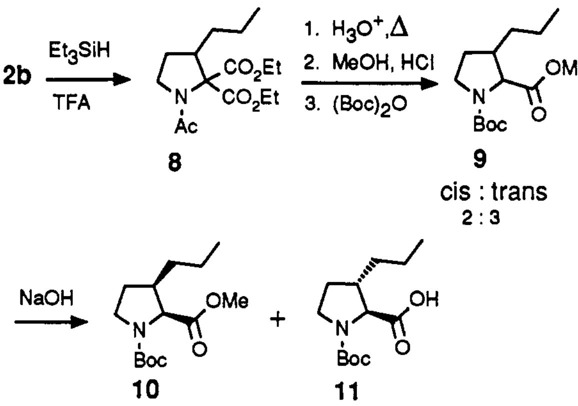


there is significantly greater tendency for the occurrence of side reactions, including retro-Michael reversion of 2 to its educts, while C-5 is still at the aldehyde oxidation state. Moreover, C-5 deoxygenation is readily effected in a single step in high yield using an acid-catalyzed silane reduction procedure.[7] Although the approach involving early C-5 reduction does ultimately lead to a predominance of cis isomer as reported, predominant trans isomer may be readily obtained, if desired, by epimerization of the cis ester under anhydrous conditions.[8]

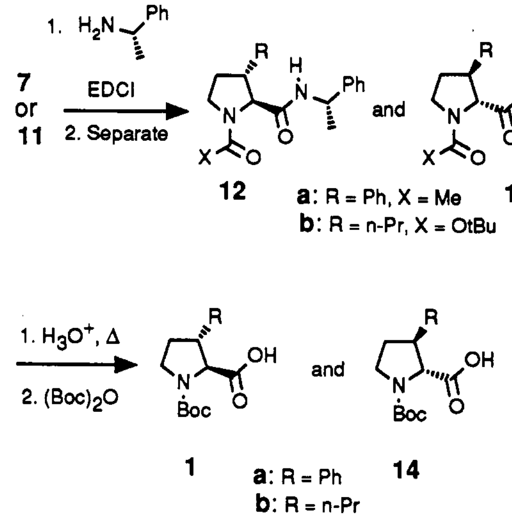
In accord with the above discussion, the sequence described by Scheme II was found to proceed in high yields and in a straightforward fashion. Treatment of **2a** with Et_3SiH/CF_3CO_2H in chloroform[7] provided the pyrrolidine diester **4** (94%), which after monosaponification and decarboxylation was converted to a 1:4 mixture of trans:cis esters **5** in 89% yield. Selective saponification cleanly removed the minor trans isomer as the acid, as reported for the corresponding 4,5-dehydro series,[6] whereas epimerization with NaOEt/EtOH provided a 7:3 equilibrium mixture of trans:cis esters, which were similarly separated by selective saponification to racemic **6** and **7**.

Unexpectedly, an additional modification was required in the 3-*n*-propyl series. In contrast to the 3-phenyl series, mild saponification of a mixture of *cis*- and *trans*-*N*-acetyl-3-*n*-propylproline ethyl esters, **3b**, resulted in partial saponification of the cis ester to afford a 30:70 ratio of cis:trans acids. In contrast, we had found in earlier work[3] on the synthesis of 3-*n*-propyl-L-proline by a different route that the selective saponification of the corresponding mixture of *N*-Boc methyl esters provided a clean separation of the cis and trans isomers. Thus, steric bulk both in the *N*-acyl group and in the 3-substituent apparently contributes to reducing the susceptibility of a cis-3-substituted-proline ester to saponification. To efficiently incorporate the required change of *N*-acyl group into the synthetic route, the modified sequence described in Scheme III was developed. After 5-deoxygenation of **2b** with Et_3SiH/CF_3COOH, the resulting diester **8** was subjected to aqueous acidic conditions at elevated temperature to effect hydrolysis and decarboxylation in a single step, to afford a 2:3 mixture of *cis*- and *trans*-3-*n*-propylproline hydrochlorides. Esterification with methanolic HCl followed by protection with a Boc group provided the corresponding mixture of racemic *trans*- and *cis*-3-*n*-propylproline methyl esters **9** in 80% overall yield from **2b**, which were then separated by selective saponification to racemic **10** and **11**.

Scheme III



Scheme IV



Among several methods examined for optical resolution of racemic *trans*-3-*n*-propylproline, conversion to the α-methylbenzylamides **12b**/**13b** followed by recrystallization or chromatographic separation provided the best results (Scheme IV). In the 3-phenyl series, **12a** and **13a** were readily separable by chromatography, but thus far all attempts to crystallize either diastereomer have been unsuccessful. Acid hydrolysis of the pure diastereomeric amides provided the optically pure free amino acids, which after N-protection to **1** and **14** were ready for incorporation into peptides using standard methods.

The absolute stereochemistry of the 3-*n*-propyl series was assigned by correlation to the product synthesized from 4-hydroxy-L-proline[3] and confirmed by single-crystal X-ray analysis of **12b** (Figure 1).

The absolute configuration of the 3-phenyl series was determined by N-deprotection followed by decarboxylation[9] of **1a** and **14a** to the corresponding enantiomers of 3-phenylpyrrolidine, for which absolute stereochemistry has been assigned.[10] Both isomers of 3-phenylpyrrolidine

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[7] Auerbach, J.; Zamore, M.; Weinreb, S. M. *J. Org. Chem.* **1976**, *41*, 725.

[8] When we repeated the procedure reported in ref 6 for conversion of *N*-acetyl-2,2-bis(ethoxycarbonyl)-3-phenylpyrrolidine to *trans*-*N*-acetyl-3-phenylproline (NaOH, dioxane/H_2O, 80 °C), the crude product contained a 2:1 mixture of trans:cis acids; one recrystallization from EtOAc/hexane afforded a 6:1 trans:cis mixture in the precipitate fraction, whereas the mother liquors contained a 1:4 trans:cis mixture.

[9] Hashimoto, M.; Eda, Y.; Osanai, Y.; Iwai, T.; Aoki, S. *Chem. Lett.* **1986**, *893*.

[10] (a) Bettoni, G.; Cellucci, C.; Tortorella, V. *J. Heterocycl. Chem.* **1976**, *13*, 1053. (b) Tseng, C. C.; Terashima, S.; Yamada, S.-I. *Chem. Pharm. Bull.* **1977**, *25*, 166.

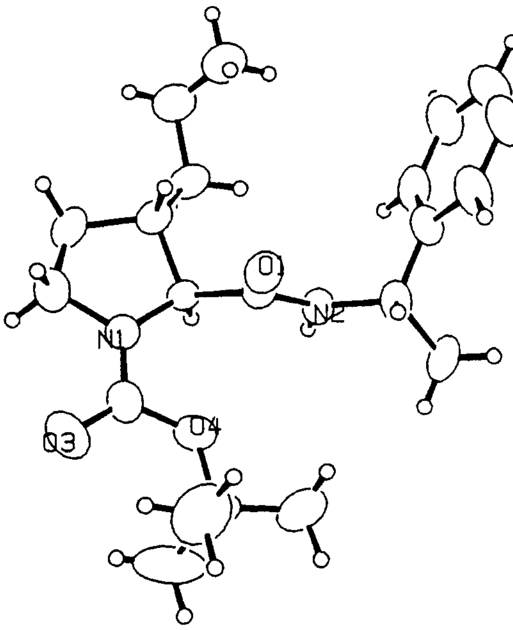

Figure 1. ORTEP drawing of **12b**.

were converted to Mosher[13] amide derivatives, which were examined by ^{19}F NMR and determined to have enantiomeric excesses of 86% and 96% for the *R* and *S* isomers, respectively. The low values observed for the optical rotations of the 3-phenylpyrrolidines are not consistent with this analysis and thus presumably were influenced by the presence of optically active impurities. Accordingly, it was desirable to substantiate the stereochemical assignment by appropriate comparisons with an authentic sample of optically active 3-phenylpyrrolidine. The preparation of (*S*)-3-phenylpyrrolidine from (*S*)-2-phenylsuccinic acid was carried out by a modification of the literature method.[10a] The Mosher amide from this product corresponded by ^{19}F NMR to the product obtained from **14a**, which confirms that **14a** has the D-trans stereochemistry.

In summary, literature methods for the preparation of 3-substituted proline derivatives have been refined to provide convenient procedures for the preparation of these analogues.[11] The modifications include the use of Et_3SiH/TFA for the reduction of **2** and the use of a Boc group on **9** for enhanced selectivity during selective saponification of the trans ester in the presence of the cis isomer. The racemic trans isomers of 3-phenyl- and 3-*n*-propylproline were resolved, and the absolute configurations of optically pure derivatives were assigned. The overall sequence was easily carried out on a 200-g scale, which makes these analogues readily available for peptide studies. Application of this method to other 3-substituted prolines is currently underway.[12]

Experimental Section

Proton magnetic resonance spectra were obtained on a Nicolet QE-300 (300 MHz) or a General Electric GN-300 (300 MHz) instrument. Chemical shifts are reported as δ values (ppm) relative to Me_4Si as an internal standard unless otherwise indicated. Spin-spin decoupling and NOE difference spectra were obtained on a General Electric GN-500 (500 MHz) instrument. Mass spectra were obtained with Hewlett-Packard HP5965 (CI) and Kratos MS50 (FAB, HRMS) spectometers. Infrared spectra were recorded on a Perkin-Elmer 283 spectrophotomer. Elemental analyses and the above determinations were performed by the Analytical Research Department, Abbott Laboratories.

Thin-layer chromatography (TLC) was carried out using E. Merck precoated silica gel F-254 plates (thickness 0.25 mm). Preparative thin-layer chromatography (PTLC) was carried out using Analtech 20 × 20 cm precoated silica gel GF plates (thickness 1.00 mm). Flash column chromatography was performed on Merck Silica Gel 60, 200–400 mesh.

Melting points are uncorrected and were determined on either a Thomas-Hoover or a Büchi 510 melting point apparatus. Optical rotation data were obtained on Perkin-Elmer Model 241 polarimeter. Anhydrous solvents were purchased from Aldrich (Milwaukee, WI), and reactions requiring anhydrous solvents were performed under a nitrogen atmosphere.

Abbreviations used are as follows: HOBt, 1-hydroxybenzotriazole; EDCI, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride.

Diethyl 1-Acetyl-3-phenylpyrrolidine-2,2-dicarboxylate (4). To a solution of **2a**[4] (4.35 g, 12.4 mmol) and triethylsilane (3.0 mL, 18.6 mmol) in 25 mL of $CHCl_3$ was added trifluoroacetic acid (9.5 mL, 124 mmol) dropwise with stirring over 10 min. After stirring for 2.5 h at room temperature, the solution was concentrated in vacuo, and then an EtOAc solution of the residue was washed with aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated to 4.3 g of oily residue, which was sufficiently pure for direct use. A 444-mg sample was distilled (Kugelrohr: 206–214 °C bath temperature, 0.7 mmHg) to afford 400 mg (94%) of pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 7.5 Hz, 3 H), 1.30 (t, J = 7.5 Hz, 3 H), 2.15 (s, 3 H), 2.25 (quintet of doublets, J_1 = 1.8 Hz, J_2 = 6 Hz, 1 H), 2.60 (m, 1 H), 3.75 (m, 2 H), 3.90 (m, 3 H), 4.30 (q, J_1 = 7.5 Hz, 2 H), 7.20 (m, 2 H), 7.30 (m, 3 H); HRMS calcd for $C_{18}H_{23}NO_5$ *m/e* 333.1576, found *m/e* 333.1569.

***N*-Acetyl-3-phenylproline Ethyl Ester (5, Mixture of Cis and Trans Isomers).** Crude diester 4 (1.8 g, 5.4 mmol) was suspended in 50 mL of 0.5 N NaOH and stirred at room temperature for 21 h. The resultant solution was extracted once with EtOAc and then acidified to pH 2 with 3 N HCl. The precipitate was extracted into $CHCl_3$, and the aqueous phase twice extracted with $CHCl_3$. The combined $CHCl_3$ fractions were concentrated to 1.58 g of solid residue, which was heated for 1 h at 75 °C in 20 mL of toluene. Evaporation of the solvent afforded 1.2 g of **5** (cis:trans = 4:1): MS (CI), *m/e* 262 ((M + H)$^+$); partial ^{1}H NMR ($CDCl_3$) cis isomer (two conformers) δ 0.73 (t, J = 7.5 Hz, 2.2 H), 0.82 (t, J = 7.5 Hz, 0.8 H), 4.56 (d, J = 8.4 Hz, 0.2 H), 4.62 (d, J = 9 Hz, 0.8 H), trans isomer two conformers) δ 1.20 (t, J = 7.5 Hz, 2.1 H), 1.28 (t, J = 7.5 Hz, 0.8 H), 4.39 (d, J = 4.5 Hz, 0.25 H), 4.52 (d, J = 6 Hz, 0.75 H). Anal. Calcd for $C_{15}H_{19}HO_3 \cdot 0.1H_2O$: C, 68.47; H, 7.35; N, 5.32. Found: C, 68.42; H, 7.35; N, 5.29.

***N*-Acetyl-*trans*-3-phenylproline (7).** A total of 950 mg (3.6 mmol) of **5** was heated at reflux for 2 h with 22 mL of 1 M NaOEt in EtOH, which contained 430 μL of ethyl trifluoroacetate as an H_2O scavenger. After cooling, water (15 mL) was added, the solution was stirred for 2.5 h at ambient temperature, and then the acidic and neutral components were separated by standard extractive techniques to afford 664 mg (78%) of **7** and 200 mg (21%) of cis-ester **6**. Recrystallization of 135 mg of crude **7** from MeOH/EtOAc afforded 112 mg of analytically pure material: mp 182–184 °C (lit.[4] mp 180–181 °C); MS (CI), *m/e* 251 ((M + NH_4)$^+$), 234 ((M + H)$^+$); ^{1}H NMR (Me_2SO-d_6, two conformers) δ 1.89 (s, 0.7 H), 2.0–2.1 (cmplx, 3.1 H, includes 2.02 (s, 2.3 H)), 2.18 (m, 0.2 H), 2.30 (m, 1 H), 3.38 (m, 1 H, partially obscured by H_2O), 3.60 (m, 1 H), 3.73 (m, 1 H), 4.15 (d, J = 6 Hz, 0.8 H), 4.98 (d, J = 6 Hz, 0.2 H). Anal. Calcd for $C_{13}H_{15}NO_3 \cdot 0.1MeOH$: C, 66.54; H, 6.56; N, 5.92. Found: C, 66.42; H, 6.36; N, 5.85. Partial ^{1}H NMR spectrum for **6** ($CDCl_3$) δ 0.64 (t, J = 7.5 Hz, 2.25 H), 0.83 (t, J = 7.5 Hz, 0.75 H), 2.0 (s, 0.75 H), 2.12 (s, 2.25 H), 4.56 (d, J = 8 Hz, 0.25 H), 4.73 (d, J = 9 Hz, 0.75 H).

Preparation and Separation of 12a and 13a. A solution of **7** (917 mg, 3.92 mmol), HOBt·H_2O (659 mg, 4.31 mmol), and (*S*)-(−)-α-methylbenzylamine (555 μL, 521 mg, 4.31 mmol) in 10

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(11) After a substantial amount of this work had been completed, publication of the preparation of 3-substituted prolines from a glycine-nickel complex and α,β-unsaturated aldehydes became known: Belokon', Y. N.; Bulychev, A. G.; Pavlov, V. A.; Fedorova, E. B.; Tsyryapkin, V. A.; Bakhmutov, V. A.; Belikov, V. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2075.

(12) In a preliminary study, treatment of the methyl ester of **1a** with RuO_4 gave methyl *N*-[(*tert*-butyloxy)carbonyl]-*trans*-3-carboxypyroglutamate in good yield.

(13) Dale, J. A.; Dull, D. A.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

mL of DMF was cooled to 0° C and then EDCI (823 mg, 4.31 mmol) was added. The mixture was allowed to warm to room temperature while stirring for 3 h and then was diluted with EtOAc and 1 M $KHSO_4$. The layers were separated, and the organic phase was washed successively with H_2O, saturated aqueous $NaHCO_3$, and brine, then dried (Na_2SO_4), and concentrated. The residue was chromatographed over 125 g of silica gel (1.5% HOAc/EtOAc) to provide 562 mg (42%) of **12a** (97:3 mixture with **13a** by ^{1}H NMR) and 655 mg (50%) of **13a** (>98% diastereomeric purity by ^{1}H NMR). **12a**: R_f = 0.24 (2.5% HOAc/EtOAc); $[\alpha]^{24}{}_{D}$ = +13.8° (*c* 0.88, MeOH); MS (CI) *m/e* 337 ($(M + H)^+$), 188; ^{1}H NMR ($CDCl_3$) δ 1.45 (d, *J* = 7 Hz, 3 H), 2.1 (m, 1 H), 2.19 (s, 3 H), 2.5 (m, 1 H), 3.67 (t, *J* = 7.5 Hz, 2 H), 3.82 (m, 1 H), 4.51 (d, *J* = 4.5 Hz, 1 H), 5.05 (m, 1 H), 7.0 (br d, *J* = 7.5 Hz, 1 H), 7.1–7.35 (cmplx, 10 H). **13a:** R_f = 0.40 (2.5% HOAc/EtOAc); $[\alpha]^{24}{}_{D}$ = −147.8° (*c* 0.68, MeOH); MS (CI) *m/e* 337 ($(M + H)^+$), 188; ^{1}H NMR ($CDCl_3$) δ 1.42 (d, *J* = 6 Hz, 2.5 H), 1.47 (d, *J* = 7.5 Hz, 0.5 H), 2.05 (m, 1 H), 2.08 (s, 0.5 H), 2.15 (s, 2.5 H), 2.5 (m, 1 H), 3.6 (m, 2 H), 3.93 (m, 1 H), 4.25 (d, *J* = 4.5 Hz, 0.2 H), 4.61 (d, *J* = 4.5 H, 0.8 H), 5.05 (m, 0.8 H), 5.20 (m, 0.2 H), 7.15–7.38 (complex, 11 H).

***N*-[(*tert*-Butyloxy)carbonyl]-*trans*-3-phenyl-L-proline (1a).** Amide **12a** (528 mg, 1.57 mmol, 97:3 mixture with **13a**) was dissolved in 4 mL of HOAc and 12 mL of 8 N HCl and heated at reflux for 18 h. The mixture was concentrated to dryness, the residue was taken up in H_2O and extracted once with an equal volume of EtOAc, and the aqueous phase was again concentrated to dryness. The residue was dissolved in 8 mL of 1:1 H_2O/dioxane and treated with NEt_3 (484 μL, 353 mg, 3.50 mmol) and di-*tert*-butyl dicarbonate (753 mg, 3.45 mmol). After stirring for 4 h, additional NEt_3 was added to ca. pH 9, then stirring was continued for an additional 18 h. Extractive isolation of the acidic component afforded 313 mg of a tan foam. Chromatography over silica gel (1:1 EtOAc/hexane, containing 1% HOAc) afforded 260 mg (57%) of **1a**: mp 158–160 °C; $[\alpha]^{24}{}_{D}$ = +33.7° (*c* 1.0, $CHCl_3$); MS (CI), *m/e* 309 ($(M + NH_4)^+$), 292 ($(M + H)^+$), 253, 236, 192, 146; ^{1}H NMR ($CDCl_3$) (two conformers) δ 1.43 (s, 4 H), 1.52 (s, 5 H), 2.03 (m, 1 H), 2.33 (m, 1 H), 3.50 (m, 1 H), 3.6–3.8 (complex, 1.45 H), 3.87 (m, 0.55 H), 4.30 (d, *J* = 6.3 Hz, 0.45 H), 4.45 (d, *J* = 4.8 Hz, 0.55 H), 7.2–7.4 (complex, 5 H). Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.95; H, 7.27; N, 4.81. Found: C, 65.81; H, 7.27; N, 4.73.

***N*-[(*tert*-Butyloxy)carbonyl]-*trans*-3-phenyl-D-proline (14a)** was prepared similarly from **13a** (453 mg, 1.34 mmol, >98% diastereomeric purity), except in this case, purification of 320 mg of crude product was effected by recrystallization from EtOAc/hexane to afford 182 mg (47%) of **14a**: mp 162–163 °C; $[\alpha]^{24}{}_{D}$ = −35.9° (*c* 1.0, $CHCl_3$). Found: C, 66.10; H, 7.31; N, 4.78. NMR and MS data were identical with those for **1a**. A second crop of 56 mg (14%), mp 159–160 °C, was obtained from the mother liquors.

(*S*)-(+)-3-Phenylpyrrolidine. a. The Boc group was removed from acid **14a** (91 mg, 0.31 mmol) by treatment with 1:1 CH_2Cl_2/CF_3CO_2H for 1 h at room temperature. The volatile components were evaporated under reduced pressure, and then the residue was dissolved in 1 mL of H_2O and applied to a column (2-mL bed volume) of Dowex-50 (H^+ form). The column was washed with H_2O, and then the amino acid was eluted with 2 N NH_4OH. Combined ninhydrin-positive fractions were lyophilized to give 63 mg of the free amino acid in zwitterionic form: ^{1}H NMR (D_2O) δ 2.22 (m, 1 H), 2.50 (m, 1 H), 3.51 (m, 1 H), 3.61 (m, 1 H), 4.12 (d, *J* = 9 Hz, 1 H), 7.45 (m, 5 H). A 59-mg sample of this product was heated with stirring under N_2 in a 160 °C oil bath in the presence of 1 mL of cyclohexanol and 5 μL of 2-cyclohexen-1-one until the solid had dissolved (ca. 15 min.). After the solution had cooled, it was treated with 5 mL each of H_2O and Et_2O, the aqueous phase was made acidic with 3 N HCl, and then the layers were separated. The aqueous phase was washed with three additional portions of Et_2O and then was made basic by addition of solid K_2CO_3. The resulting solution was continuosuly extracted with Et_2O, and then the organic phase was dried over $MgSO_4$ and evaporated to 49 mg of a pale yellow residue, which was distilled by using a microsublimation apparatus. After collection of a forerun fraction at 60 °C (bath temperature) and 4.2 mmHg, a second fraction (8.9 mg) was collected at 105 °C (bath temperature) and 4.2 mmHg; MS (CI), *m/e* 148 ($(M + H)^+$); ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 1 H), 2.31 (m, 1 H), 2.89 (br m, NH), 2.98 (t, *J* = 10 Hz, 1 H), 3.21 (m, 1 H), 3.35 (m, 2 H), 3.50 (dd, *J* = 7.5, 10 Hz, 1 H), 7.28 (m, 5 H); $[\alpha]^{24}{}_{D}$ = +16.0° (*c* 0.04, MeOH); $[\alpha]^{24}{}_{D}$ = +13.8° (*c* 0.48, EtOH), lit. for *R* isomer $[\alpha]_D$ = −24° (*c* 4.7%, MeOH),[10a] for the *S* isomer $[\alpha]^{20}{}_{D}$ = +22.7° (*c* 2.36, EtOH).[10b] The (α-methoxy-α-(trifluoromethyl)phenyl)acetyl (MTPA) amide was prepared by condensation with (−)-MPTA chloride in CCl_4/pyridine;[13] MS (CI), *m/e* 364 ($(M + H)^+$); ^{19}F NMR (Me_2SO-d_6, 145 °C) (ppm, downfield relative to the center peak of the trifluoroethanol triplet) δ 5.82 (s), 6.18 (s) in a ratio of 45:1.

b. (*S*)-*N*-Benzyl-3-phenylpyrrolidine was prepared from (*S*)-(+)-2-phenylsuccinic acid by a procedure similar to that described.[10a] Debenzylation was carried out by catalytic hydrogenolysis over 20% PdC in MeOH (4 atm, room temperature). After removal of the catalyst by filtration, the solvent was evaporated to provide the crude product, which was confirmed by comparison of the ^{1}H NMR spectrum with that from (a). ^{19}F NMR of the product from (−)-MPTA chloride (same conditions as above) δ 5.82 (s).

(*R*)-(−)-3-Phenylpyrrolidine was obtained from **1a** in a manner analogous to that described for the preparation of the *S* isomer from **14a**; ^{1}H NMR and MS data were identical with those for the *S* isomer; $[\alpha]^{23}{}_{D}$ = −12.4° (*c* 0.9, MeOH); $[\alpha]^{24}{}_{D}$ = −14.6° (*c* 0.6, EtOH); ^{19}F NMR of the MTPA amide from (−)-MTPA chloride (same conditions as above) δ 5.82 (s), and 6.12 (s) in a ratio of 1:13.

Diethyl 1-Acetyl-5-hydroxy-3-*n*-propylpyrrolidine-2,2-dicarboxylate (2b). Sodium (3.48 g, 0.15 mol) was dissolved in a stirred solution of diethyl acetamidomalonate (201.6 g, 0.93 mol) in anhydrous ethanol (1200 mL) at room temperature under nitrogen. The reaction mixture was cooled to 0 °C, and *trans*-2-hexenal (100.0 g, 1.02 mol) was then added dropwise. The resulting mixture was allowed to warm to room temperature. After stirring for 3 h at 23 °C, the reaction was quenched with 24 mL of acetic acid. The solution was concentrated in vacuo, and the resulting residue was taken up in EtOAc and washed successively with saturated aqueous $NaHCO_3$ (2×) and brine, then dried over $MgSO_4$, and concentrated. The residue was crystallized from EtOAc/hexane to give 271.4 g (93%) of **2b** as fine needles: mp 105–106 °C; TLC R_f = 0.43 (9:1 $CHCl_3$/MeOH); IR ($CDCl_3$) 3460, 1750, 1665 cm^{-1}; MS(CI), *m/e* 316 ($(M + H)^+$), 298 ($(MH - H_2O)^+$); ^{1}H NMR (CD_3OD) δ 5.64 (d, *J* = 5.2 Hz, 1 H), 4.26–4.10 (m, 4 H), 2.83 (m, 1 H), 2.21 (s, 3 H), 2.08 (dd, *J* = 12.8, 6.4 Hz, 1 H), 1.94 (dd, *J* = 12.8, 5.2 Hz, 1 H), 1.90–1.78 (m, 1 H), 1.47–1.11 (m, 9 H), 0.94 (t, *J* = 7.2 Hz, 3 H). Anal. Calcd for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44. Found: C, 57.28; H, 8.08; N, 4.42.

Diethyl 1-Acetyl-3-*n*-propylpyrrolidine-2,2-dicarboxylate (8). To a solution of **2b** (271.0 g, 0.86 mol) and triethylsilane (206 mL, 1.29 mol) in CH_2Cl_2 (3 L) was added trifluoroacetic acid (663 mL, 8.6 mol) dropwise with stirring while controlling the internal temperature at 25–30 °C by means of an ice bath. After stirring for 3 h at 23 °C, the solution was concentrated in vacuo, and the residue was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution until all the TFA was neutralized. The organic phase was dried (Na_2SO_4) and concentrated to give 350 g of oil. The oily product containing silicon impurities was used directly in the subsequent step. An analytically pure sample was obtained as an oil after flash chromatography (EtOAc/hexane, 1:2, then 1:1): TLC, R_f = 0.55 (9:1 $CHCl_3$/MeOH); IR ($CDCl_3$) 1745, 1655 cm^{-1}; MS(CI), *m/e* 300 ($(M + H)^+$); ^{1}H NMR ($CDCl_3$) δ 4.28–4.17 (m, 4 H), 3.76 ("dt", *J* = 9.6, 1.1 Hz, 1 H), 3.57 (ddd, *J* = 10.7, 9.6, 6.2 Hz, 1 H), 2.51–2.4 (m, 1 H), 2.16–2.01 (m, 4 H), 1.85–1.70 (m, 2 H), 1.42–1.15 (m, 9 H), 0.93–0.89 (t, *J* = 7.0 Hz, 3 H). Anal. Calcd for $C_{15}H_{25}NO_5$: C, 60.18; H, 8.42; N, 4.68. Found: C, 59.90; H, 8.34; N, 4.65.

3-*n*-Propylproline Hydrochloride (Cis:Trans Mixture). The crude ester 8 (350 g) was suspended in 6 N HCl (2 L) and acetic acid (500 mL) and heated at reflux for 17 h. The reaction mixture was extracted with EtOAc (2×), and the aqueous phase was concentrated on a rotary evaporator. The residue was then triturated with ether to crystallize the product. The solid was collected by filtration, washed with ether, and dried in a vacuum oven to give 152.3 g of the hydrochloride salt. An analytically pure sample was obtained by recrystallization from acetone/ether; mp 131–133 °C; TLC R_f = 0.26 (10:4:1 $CHCl_3$/MeOH/NH_4OH);

IR (KBr) 3420, 1735 cm^{-1}; MS (CI), *m/e* 158 (free base M + H$^+$); ^{1}H NMR (D_2O) major isomer δ 3.93 (d, *J* = 7.4 Hz, 1 H), 3.58–3.29 (m, 2 H), 2.50–2.43 (m, 1 H), 2.32–2.16 (m, 1 H), 1.90–1.68 (m, 2 H), 1.50–1.18 (m, 3 H), 0.91 (t, *J* = 7.2 Hz, 3 H), minor isomer δ 4.32 (d, *J* = 8.1 Hz, 1 H), 3.58–3.29 (m, 2 H), 2.66–2.60 (m, 1 H), 2.32–2.16 (m, 1 H), 1.90–1.68 (m, 2 H), 1.50–1.18 (m, 3 H), 0.89 (t, *J* = 6.8 Hz, 3 H). Anal. Calcd for $C_8H_{16}NO_2Cl$: C, 49.61; H, 8.33; N, 7.23. Found: C, 49.35; H, 8.17; N, 7.18.

***N*-[(*tert*-Butyloxy)carbonyl]-3-*n*-propylproline Methyl Ester (9).** The hydrochloride salt (152.3 g) from the above reaction was dissolved in MeOH (1.5 L), and the solution was charged with HCl gas until it was saturated. After stirring overnight, the reaction mixture was concentrated to give an oil. This was taken up in 1 N HCl and extracted with EtOAc (2×). The aqueous layer was carefully made basic with K_2CO_3 and extracted with $CHCl_3$ exhaustively. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated to give 122 g of oil: TLC R_f = 0.70 (90:10:0.1 $CHCl_3$/MeOH/NH_4OH); MS (CI), *m/e* 272 ((M + H)$^+$); partial ^{1}H NMR ($CDCl_3$) major isomer δ 3.74 (s, 3 H), 3.36 (d, *J* = 6.3 Hz, 1 H), minor isomer δ 3.83 (d, *J* = 8.1 Hz, 1 H), 3.72 (s, 3 H).

The oil (122 g) was taken up in MeOH (1 L), and then $NaHCO_3$ (180 g) and di-*tert*-butyl dicarbonate (171.4 g, 0.79 mol) were added slowly. After stirring overnight at 23 °C, the mixture was filtered, and the filtrate concentrated. The residue was triturated with EtOAc, filtered again, and concentrated to give 186.1 g of oil as mixture of cis and trans isomers: TLC R_f = 0.31 (1:6 EtOAc/hexane); IR ($CDCl_3$) 1700, 1745 cm^{-1}; MS (CI), *m/e* 272 ((M + H)$^+$); partial ^{1}H NMR ($CDCl_3$) cis isomer (two conformers) δ 4.32 (d, *J* = 8.1 H, 0.4 H), 4.23 (d, *J* = 8.5 Hz, 0.6 H), 3.72 (s, 1.2 H), 3.71 (s, 1.8 H), 0.91 (t, *J* = 7.0 Hz, 3 H), trans isomer (two conformers) δ 3.94 (d, *J* = 5.5 Hz, 0.4 H), 3.81 (d, *J* = 6.2 Hz, 0.6 H), 3.74 (s, 1.2 H), 3.73 (s, 1.8 H), 3.68–3.56 (m, 1 H), 3.50–3.42 (m, 1 H), 2.25–2.13 (m, 1 H), 2.03–1.92 (m, 1 H), 1.78–1.06 (m, 14 H), 0.92 (t, *J* = 7.0 Hz, 3 H). Anal. Calcd for $C_{14}H_{25}NO_4$: C, 61.97; H, 9.29; N, 5.16. Found: C, 61.93; H, 9.30; N, 5.11.

***N*-[(*tert*-Butyloxy)carbonyl]-*trans*-3-*n*-propylproline (11) and *N*-[(*tert*-Butyloxy)carbonyl]-*cis*-3-*n*-propylproline Methyl Ester (10).** To a solution of 9 (186.0 g, 0.685 mol) in MeOH (685 mL) was added 1 N NaOH (685 mL) at 23 °C. After stirring for 20 h, the solution was concentrated to remove MeOH and then extracted with EtOAc (3×). The extracts were dried ($MgSO_4$), filtered, and concentrated to give 71.4 g (38%) of cis ester **10**: TLC R_f = 0.54 (1:4 EtOAc/hexane); IR ($CDCl_3$) 1745, 1700 cm^{-1}; HRMS (CI), *m/e* 272.1869 ((M + H)$^+$, for $C_{14}H_{26}NO_4$, calcd 272.1862); ^{1}H NMR ($CDCl_3$) cis isomer (two conformers) δ 4.32 (d, *J* = 8.1 Hz, 0.4 H), 4.23 (d, *J* = 8.5 Hz, 0.6 H), 3.72 (s, 1.2 H), 3.71 (s, 1.8 H), 3.68–3.56 (m, 1 H), 3.34–3.25 (m, 1 H), 2.41–2.25 (m, 1 H), 2.12–1.90 (m, 1 H), 1.78–1.64 (m, 1 H), 1.45–1.23 (m, 12 H), 1.20–1.07 (m, 1 H), 0.91 (t, *J* = 7.0 Hz, 3 H). The aqueous phase was acidified with solid citric acid and extracted 2× with EtOAc. The combined extract was washed with H_2O and brine, dried ($MgSO_4$), filtered, and concentrated to give 94.2 g (53%) of trans-acid **11**: TLC R_f = 0.45 (9:1 $CHCl_3$/MeOH); IR ($CDCl_3$) 3050, 1720, 1690 cm^{-1}; MS (CI), *m/e* 258 ((M + H)$^+$); ^{1}H NMR ($CDCl_3$) (two conformers) δ 3.98 (br d, *J* = 4.4 Hz, 0.6 H), 3.84 (br d, *J* = 6.2 Hz, 0.4 H), 3.64–3.34 (m, 2 H), 2.50 (m, 0.6 H), 2.30 (m, 0.4 H), 2.12–1.97 (m, 1 H), 1.69–1.28 (m, 14 H), 0.93 (m, 3 H). Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.66; H, 8.91; N, 5.44.

Preparation and Separation of 12b and 13b. To a solution of **11** (94.2 g, 0.37 mol) and HOBt·H_2O (54.4 g, 0.40 mol) in CH_2Cl_2 (1 L) was added EDCI (76.9 g, 0.40 mol) under nitrogen at 0 °C. After stirring overnight at 23 °C, the reaction mixture was diluted with EtOAc, washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in a minimum amount of ether, and **12b** preferentially crystallized out upon standing (34.0 g, 9:1 **12b**/**13b**). The mother liquor was concentrated, and the resulting residue was chromatographed on silica gel using 35% EtOAc/hexane as eluant to provide fractions containing 46.5 g of **13b** (35%, contains <5% **12b**), 6.9 g of a ca. 1:1 mixture, and 16.3 g of 9:1 **12b**/**13b**. The mixed fraction (6.9 g) and the fractions enriched in **12b** (total 50.3 g) were combined and recrystallized three times from ether/hexane to give a total of 40.2 g (30%) of **12b** (>99% pure). Alternatively, the mixture could be separated by preparative HPLC with about 80% recovery. **12b**: mp 112–114 °C (Et_2O/hexane); TLC, R_f = 0.39 (EtOAc:hexane = 1:2); $[\alpha]^{23}_D$ = −56.3° (*c* 0.75, MeOH); MS (CI), *m/e* 361 ((M + H)$^+$, base), 305, 261, 112; ^{1}H NMR (Me_2SO-d_6, 138 °C) δ 7.65–7.15 (m, 5 H), 4.99 ("q", *J* = 7.1 Hz, 1 H), 3.77 (d, *J* = 5.1 Hz, 1 H), 3.55–3.30 (m, 2 H), 2.14 (m, 1 H), 1.97 (m, 1 H), 1.55–1.25 (m, 5 H), 1.43 (d, *J* = 7.1 Hz, 3 H), 1.40 (s, 9 H), 0.88 (br t, *J* = 7.0 Hz, 3 H). Anal. Calcd for $C_{21}H_{32}N_2O_3$: C, 69.97; H, 8.95; N, 7.77. Found: C, 69.99; H, 9.05; N, 7.73.

13b: mp 104–105 °C (Et_2O/hexane); TLC R_f = 0.45 (EtOAc:hexane = 1:2); $[\alpha]^{25}_D$ = −65.8° (*c* 0.76, MeOH); MS (CI) *m/e* 361 ((M + H)$^+$, base), 305, 261, 112; ^{1}H NMR (Me_2SO-d_6, 138 °C) δ 7.60–7.17 (m, 5 H), 4.98 ("q", *J* = 7.1 Hz, 1 H), 3.79 (d, *J* = 4.9 Hz, 1 H), 3.51–3.33 (m, 2 H), 2.18 (m, 1 H), 2.00 (m, 1 H), 1.58–1.45 (m, 2 H), 1.43 (d, *J* = 7.1 Hz, 3 H), 1.42–1.28 (m, 3 H), 1.36 (s, 9 H), 0.91 (br t, *J* = 7.0 Hz, 3 H). Anal. Calcd for $C_{21}H_{32}N_2O_3$: C, 69.97; H, 8.95; N, 7.77. Found: C, 70.27; H, 9.25; N, 7.73.

X-ray Crystallographic Absolute Stereostructure Determination of 12b. Colorless single crystals suitable for the collection of X-ray diffraction data were obtained by recrystallization from a solution of 8:1:1 acetonitrile:acetone:water by vapor diffusion against 9:1 water:acetone. A crystal (dimensions 0.25 × 0.02 × 0.90 mm) was selected for data collection and mounted on a Rigaku AFC5 automated four-circle diffractometer. The crystal was found to be orthorhombic, and unit cell parameters and the orientation matrix were obtained. Data collection was carried out using the ω profile mode with real-time Lehmann–Larsen profile analysis: formula $C_{21}H_{32}N_2O_3$; formula weight, 360.50; space group $P2_12_12_1$; a = 12.685 (4) Å; b = 17.094 (4) Å; c = 9.829 (2) Å; V = 2125.2 (8) Å^3; Z = 4; diffractometer, Rigaku AFC5; radiation, Cu Kα; filter, Ni; scan type, ω profile with real-time Lehmann–Larsen profile analysis; scan speed, 1°/min; scan range, 1.5 °; 2θ scan limits, 5–90°; 3 per 150 reflections; indices (−3,1,0), (−3,−1,0), 3,−1,1); crystal stability, standard reflections increased 1.2% during data collection; total reflections scanned, 1033; unique reflections $[I > 3\sigma(I)]$ = 788; R, $(\sum|K|F_o| - |F_c||)/(\sum K|F_o|)$ = 0.047; R_ω ($\omega = 1/\sigma^2$) 0.058. The data were corrected for absorption, Lorentz, and polarization factors. The structure was solved by SHELXS, a direct-methods program. The structure was refined through the least-squares procedure with the complete matrix of normal equations. Non-hydrogen atoms were refined anisotropically. The hydrogen atom position on N2 was found by fourier difference all other hydrogen atom positions were calculated. The largest and the smallest peaks in the final difference map were +0.174 and −0.133 e/Å^3. (List of positional and anisotropic thermal parameters of non-hydrogen atoms, positional and thermal parameters of hydrogen atoms, bond distances, and bond angles are available as supplementary material (see paragraph at end of paper)).

***N*-[(*tert*-Butyloxy)carbonyl]-*trans*-3-*n*-propyl-L-proline (1b).** A solution of **12b** (40.2 g, 0.11 mol, >95% diastereomerically pure) in 8 N HCl (870 mL) and glacial acetic acid (220 mL) was heated at reflux overnight. The solution was concentrated on a rotary evaporator, and the residue taken into H_2O and extracted with ether. The aqueous phase was concentrated and azeotroped 3× with toluene to give 43.0 g of a mixture of *trans*-3-*n*-propyl-L-proline hydrochloride and α-methylbenzylamine hydrochloride. The salts were taken up in dioxane/H_2O (1:1, 400 mL) and then treated carefully with *N,N*-diisopropylethylamine (35.5 g, 0.275 mol) and di-*tert*-butyl dicarbonate (60.0 g, 0.275 mol) sequentially at 0 °C. After stirring overnight at 23 °C, the mixture was diluted with EtOAc, and the two layers were separated. The organic layer was extracted with 0.5 N NaOH (2×). The combined aqueous layers were extracted with EtOAc once, and the separated organic layer was back-extracted with 0.5 N NaOH. The combined aqueous fractions were cooled to 0–5 °C and acidified to pH 1.0 with cold 4 N HCL and extracted immediately with EtOAc (2×). The combined EtOAc extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was dried under reduced pressure over P_2O_5 to give 26.1 g (92%) of **1b** as a white solid: mp 88–89 °C (hexane); TLC, R_f = 0.15 (9:1 $CHCl_3$/MeOH); $[\alpha]^{24}_D$ = −38.3° (*c* 1.0, $CHCl_3$); $[\alpha]^{25}_D$ = −42.5° (*c* 0.095, $CHCl_3$); MS (CI) *m/e* 258 ((M + H)$^+$), 219 (base), 202, 158; ^{1}H NMR (360 mHz, Me_2SO-d_6, 100 °C) δ 3.67 (d, *J* = 4.0 Hz, 1 H), 3.40 (m, 1 H), 3.30 (m, 1 H), 2.20 (m, 1 H), 1.98 (m, 1

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H), 1.55–1.20 (m, 15 H), 0.90 (t, J = 7.0 Hz, 3 H). Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.85; H, 8.97; N, 5.44.

***N*-[(*tert*-Butyloxy)carbonyl]-*trans*-3-*n*-propyl-D-proline (14b).** Amide **13b** (>99% diastereomeric purity) was converted similarly to **14b**: mp 90–92 °C (hexane); $[\alpha]^{24}_D$ = +43.2° (c 1.0, $CHCl_3$).

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Registry No. **1a**, 123724-21-0; **1b**, 123724-22-1; **2a**, 3005-63-8; **2b**, 123724-23-2; **4**, 123724-24-3; *cis*-**5**, 123807-01-2; *trans*-**5**, 123807-02-3; **7**, 123807-03-4; **8**, 123724-25-4; *cis*-**9**, 123724-26-5; *trans*-**9**, 123724-27-6; **11**, 123877-36-1; **12a**, 123724-28-7; **12b**, 123724-29-8; **13a**, 123724-30-1; **13b**, 123724-31-2; **14a**, 123724-32-3; **14b**, 123724-33-4; (−)-MTPA-Cl, 39637-99-5; (*S*)-(−)-PhCH(Me)NH_2, 2627-86-3; (*S*)-(+)-HOOCCH(Ph)CH_2COOH, 4036-30-0; AcNHCH$(COOEt)_2$, 1068-90-2; (*E*)-$H_3CCHCHCH_2CH_2CH_3$, 6728-26-3; (*S*)-(−)-PhCH(Me)NH_2·HCl, 17279-30-0; D-*trans*-3-phenylproline, 118758-50-2; (*S*)-(+)-3-phenylpyrrolidine, 62624-46-8; (*S*)-(*R**,*R**)-*N*-[[α-methoxy-α-(trifluoromethyl)phenyl]acetyl]-3-phenylpyrrolidine, 123724-34-5; (*S*)-*N*-benzyl-3-phenylpyrrolidine, 59349-74-5; (*R*)-3-phenylpyrrolidine, 61586-46-7; (±)-*cis*-3-*n*-propylproline hydrochloride, 123807-04-5; (±)-*trans*-3-*n*-propylproline hydrochloride, 123807-05-6; (±)-*cis*-3-*n*-propylproline methyl ester hydrochloride, 123724-35-6; (±)-*trans*-3-*n*-propylproline methyl ester hydrochloride, 123724-36-7.

Supplementary Material Available: List of positional and anisotropic thermal parameters of non-hydrogen atoms, positional and thermal parameters of hydrogen atoms, and bond distances and bond angles for compound **12b** (5 pages). Ordering information can be found on any current masthead page.

Urocanic Acid Photobiology. Identification and Characterization of the Major Photoadducts Formed between Urocanic Acid and Thymidine[1]

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Two products (adducts I and II), formed under conditions known to photochemically bind urocanic acid (UA) covalently to DNA, have been isolated from the photolysis of UA and thymidine. The gross structural features of the adducts were determined by using photolytic cleavage, mass spectrometry, UV absorption spectroscopy, ^1H and ^{13}C NMR, and ^1H–^{13}C correlation spectroscopy (COLOC). The regiochemistries were assigned on the basis of the pH dependence of hydrogen chemical shifts. The relative stereochemistries were determined by using nuclear Overhauser effects and spin–lattice relaxation times. All of these data support the assignment of the adducts as diastereomerically related cyclobutane structures resulting from 2 + 2 cycloaddition of the UA acrylic acid and thymidine double bonds.

Urocanic acid (2-propenoic acid, 3-(1*H*-imidazol-4-yl)-, UA, Figure 1) is a major component of the stratum corneum and recently has been a subject of intense photobiological interest.[2] Early studies hypothesized the biological role of UA as both a natural sunscreen and a photoprotecting agent against UV damage to DNA.[2] It is a major absorber of UV light in the skin and has been found to undergo efficient E/Z isomerization as its primary, unimolecular photochemical reaction.[2] UA is also quite reactive with singlet oxygen[2] and has been shown to photolytically generate superoxide.[3] However, more recent work has emphasized the potential deleterious effects of UA when exposed to UV light and has focused on the direct photochemical interaction of UA and biologically important components. Examples are the UA-sensitized photoinactivation of bacteriophage G4 single-stranded DNA[4] and the photochemical incorporation of UA into bovine serum albumin.[5] It has been determined that UA photochemically binds to native calf thymus DNA when these are irradiated at $\lambda > 270$ nm, with some evidence that one of the bases responsible for the photochemical incorporation is thymidine.[6] In addition, two independent studies have found that irradiated samples of (*E*)-UA can cause immune suppression in mice (there is good evidence that the causative agent is (*Z*)-UA)[7] and may increase the animals' susceptibility to cancer.[8] The results of these studies suggest that UA photochemistry may be important to the phenomenon of photocarcinogenesis. The present

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