Synthesis of Chirally Pure 2,5-Disubstituted Diketopiperazines Derived from Trisubstituted Phenylalanines

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Some new chirally pure 2,5-substituted diketopiperazines were synthesized starting from 2-methoxybenzyl alcohol. This multistep synthesis proceeds through the enzymatic synthesis of chirally pure amino acids, protection and dipeptide coupling, cyclization of dipeptide ester formates, and nitration of the resulting diketopiperazines.

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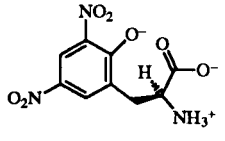
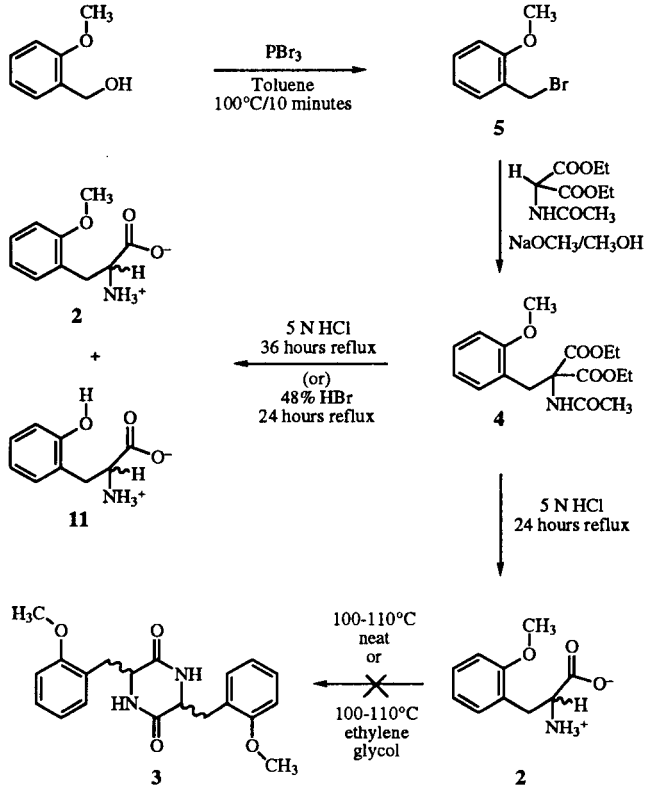
Amino acids, being polar molecules, do not diffuse into the brain efficiently. The endogenous amino acids which are required in brain tissue and cannot be synthesized therein compete for the same few amino acid carrier systems present in the blood-brain barrier [1], and exogenous amino acids must compete for these same carrier systems. We are attempting to develop some new general strategies to deliver amino acids to the brain, using the triply ionized compound L-2-hydroxy-3,5-dinitrophenylalanine (**1**) [2] as a prototypical amino acid. Compound **1** is a selective antagonist at the α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid subtype of glutamate receptors. Such antagonists have therapeutic potential in the treatment of stroke [3], epilepsy [4], and even drug addiction [5]. We are in the process of synthesizing lipophilic precursors of **1** that could freely diffuse into the brain. Further, this work begins an empirical search for bioprecursors that are converted back to amino acids selectively in certain regions of the brain. In this paper we describe the synthesis of diketopiperazines derived from 2-methoxy-3,5-dinitrophenylalanine.



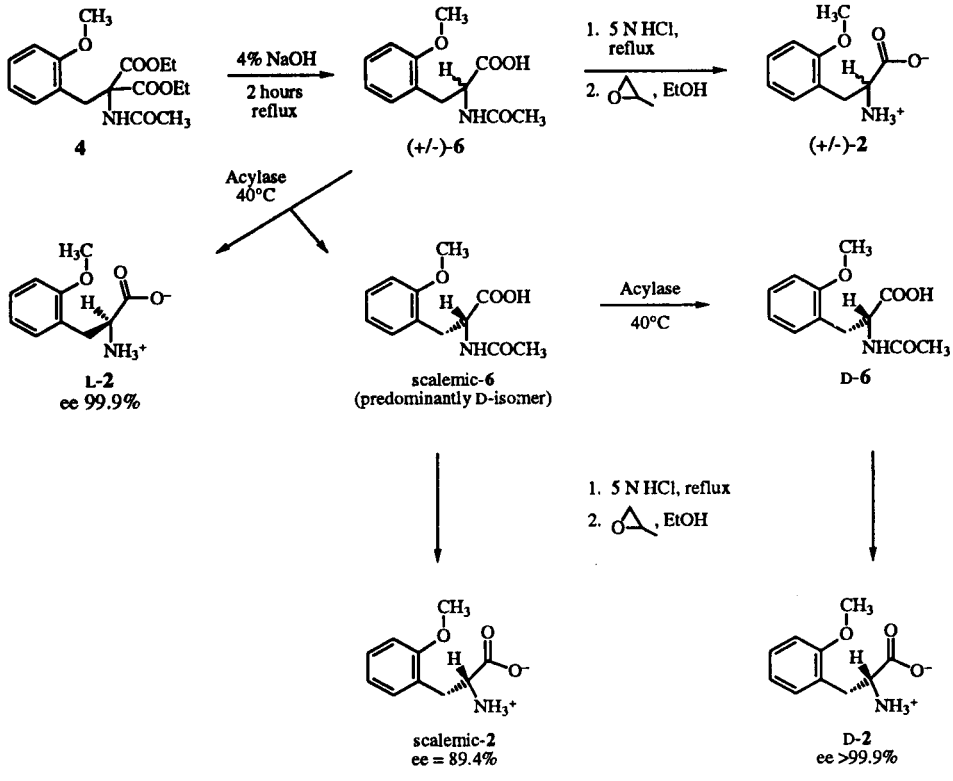
Methods for diketopiperazine synthesis have been recently reviewed by Rajappa and Natekar [6]; only a few general methods have been explored. Attempted cyclization of racemic 2-methoxyphenylalanine (**2**), either by dry-heating or heating in ethylene glycol [7], did not give the diastereomeric diketopiperazines **3** (Scheme 1). The racemic amino acid **2** was synthesized by acid hydrolysis of the acetamidomalonic acid diethyl ester derivative **4**, following the method used by Baudy *et al.* [8] for the synthesis of some quinoxalinepropanoic acids. Compound **4** was obtained by the reaction of acetamidomalonic acid diethyl ester sodium salt (formed *in situ*) with 2-methoxybenzyl bromide (**5**).

L-2-Methoxyphenylalanine (L-**2**) was synthesized by enzymatic hydrolysis [9] (Scheme 2) of the L enantiomer in racemic *N*-acetyl-2-methoxyphenylalanine ((±)-**6**), using *Aspergillus* acylase (Sigma). The unreacted *N*-acetylamino acid was found to be scalemic. Adding more acylase in this reaction after the first 24 hours or 48 hours of the reaction did not improve the enantiomeric purity of the D isomer, which suggests product inhibition of the acylase by L-**2**. Addition of cobalt chloride [9] did not improve chiral purity or degree of completion. In a separate experiment, the scalemic *N*-acetylamino acid resulting from a single treatment with acylase was subjected to enzymatic hydrolysis once again, and subsequent acid-hydrolysis of the remaining *N*-acetylamino acid gave chirally pure D-**2**. The chiral purity of each amino acid was determined by

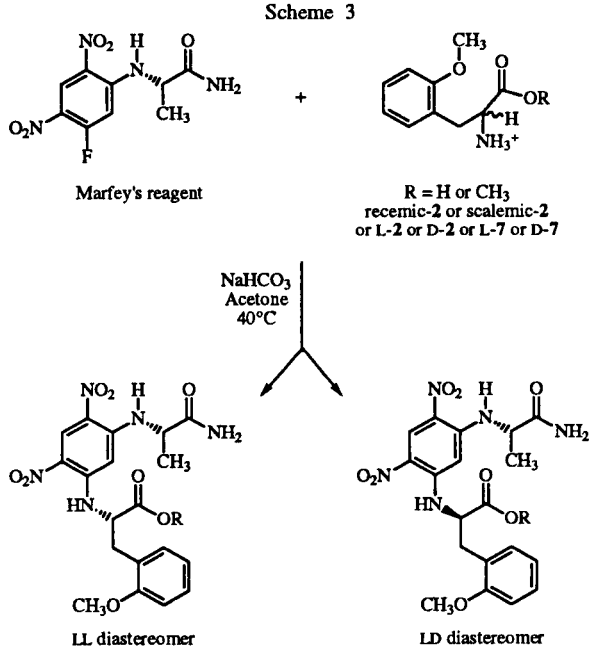
Scheme 1



Scheme 2



Scheme 3



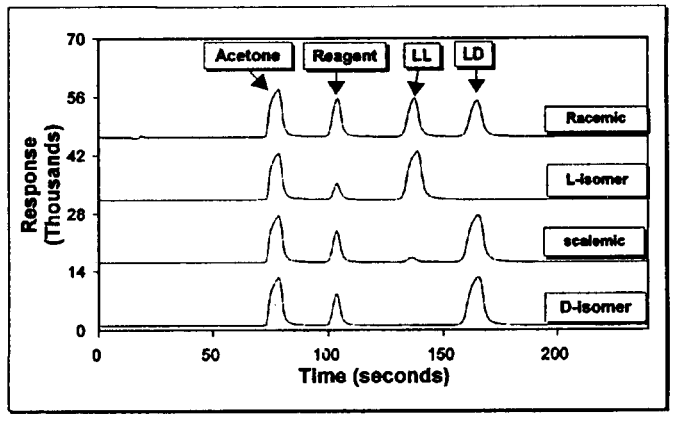
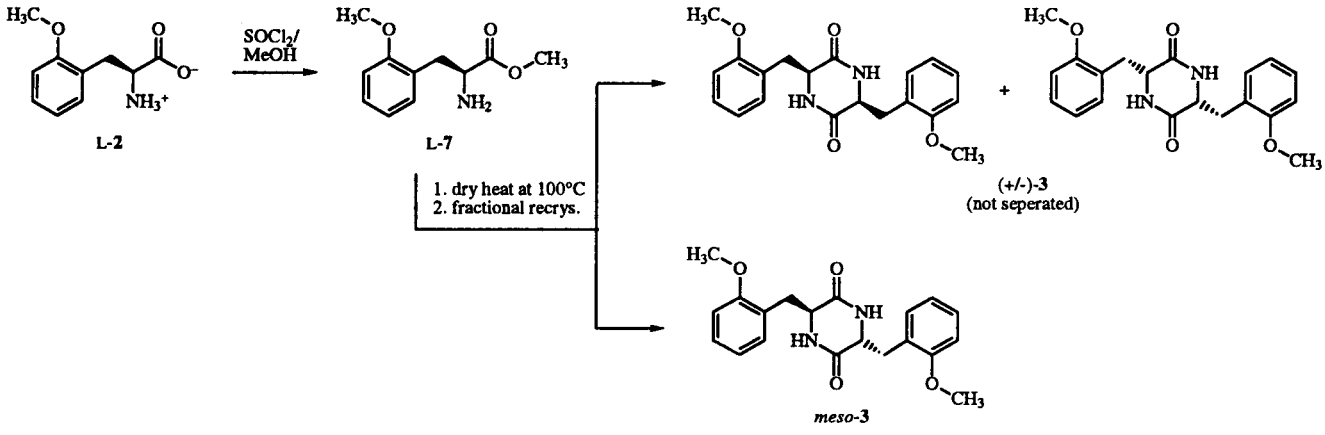


Figure 1. Hplc chromatogram for the chiral purity of the enantiomers of 2-methoxyphenylalanine. Conditions: 50% acetonitrile/0.1% CF_3COOH/H_2O, 1.5 ml/minute; column: 5 µm Spherisorb C18, 4.6 mm ID x 25 cm L; detection: uv at 254 nm.

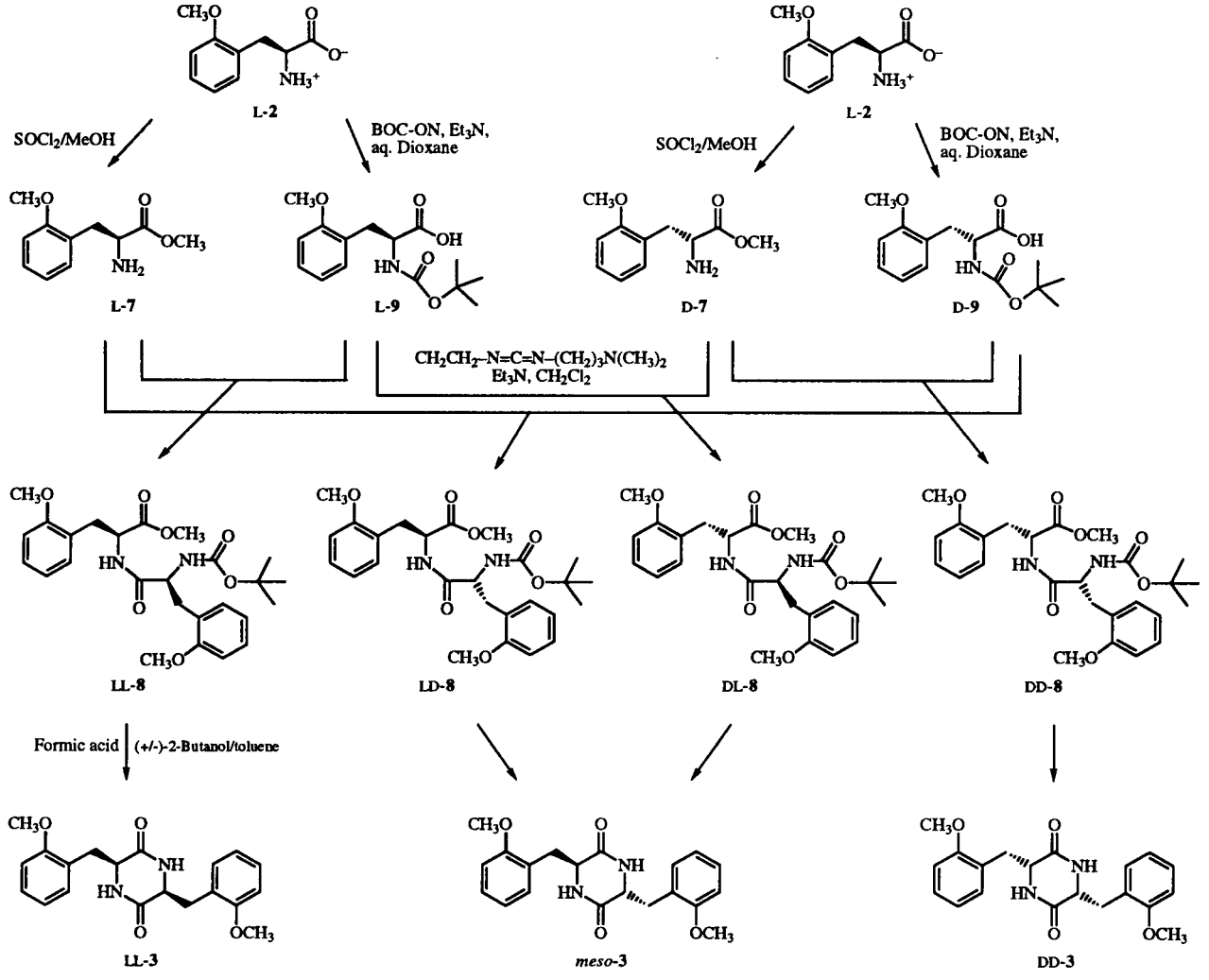
derivatization (Scheme 3) using Marfey's reagent [10] and quantitation using reversed-phase hplc. The enantiomeric excess of L-2 and D-2 was >99.9% and that of scalemic-2 was about 90% (Figure 1).

L-2-Methoxyphenylalanine methyl ester (L-7) was prepared from L-2 (Scheme 4) by the method of Brenner and Huber [11], and the chiral purity was characterized in the same manner as for the amino acid, reacting the ester with Marfey's reagent and directly chromatographing (Scheme 3). The enantiomeric excess of L-7 was >99.99% and that of D-7 was 98.6%. Cyclic dimerization of L-7 produced an approximately equal mixture of diketopiperazine diastereomers 3 (Scheme 4). Compound (±)-3 was separated from the *meso*-3 diastereomer by fractional crystallization from ethanol, and each of the two diastereomers

Scheme 4



Scheme 5



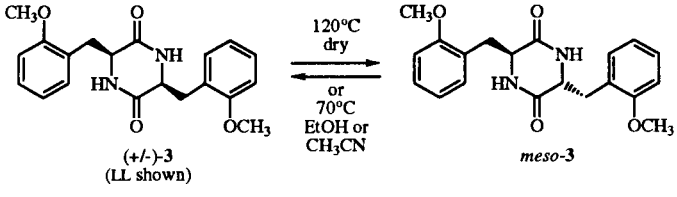
was characterized by ^{1}H- and ^{13}C-nmr, and by mass spectral analysis. The correct stereochemical assignments were based on the subsequent chiral syntheses (see below).

Chirally pure diketopiperazines **3** were obtained (Scheme 5) following the procedure of Nitecki *et al.* [12]. The linear dipeptides **8** were obtained by reacting one of

the D- or L-amino acid esters **7** with either of the D- or L-*N*-BOC-protected amino acids **9**, using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride as the coupling agent. The coupling efficiencies were considerably greater than indicated by the given yields, the problem being incomplete crystallization. The butoxycarbonyl group was removed from each dipeptide using formic acid, and the resulting dipeptide ester formate was cyclized *in situ* by azeatropically removing formic acid in boiling (±)-2-butanol/toluene. Following this procedure all three possible diketopiperazines, namely LL-**3**, DD-**3**, and *meso*-**3**, were synthesized; *meso*-**3** was obtained from both DL-**8** and LD-**8**, thus verifying the correct structural assignments of (±)-**3** and *meso*-**3** obtained from L-**7**.

The chiral purity of each isomer was established initially by tlc, and then more rigorously by hplc methods; in each case the opposite diastereomer was not observed. The compounds (±)-**3** and *meso*-**3** were interconvertable at about 120° (Scheme 6), or at a considerably lower temperature in ethanol or acetonitrile solutions. This in part explains why

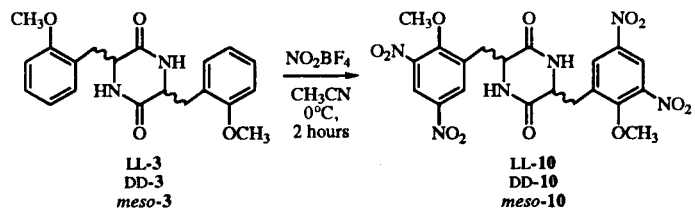
Scheme 6



simply heating the methyl esters did not produce chirally pure diketopiperazines; however, it is noteworthy that significant interconversion did not occur in refluxing 2-butanol/toluene, perhaps due to the low dielectric constant (Nitecki *et al.* [12] did not explain their selection of this solvent combination). All the isomeric diketopiperazines were characterized by hplc, nmr, ir, mass spectral analysis, and optical rotation, as well as by elemental analysis. The nmr spectra were surprisingly similar for the *cis* and *trans* isomers. Stereochemical considerations in diketopiperazine rings leading to such similarities were recently studied by Yamazaki *et al.* [13] in some detail.

The diketopiperazines were then nitrated using nitronium tetrafluoroborate in acetonitrile (Scheme 7). Each resulting tetranitrated derivative was characterized using hplc, nmr,

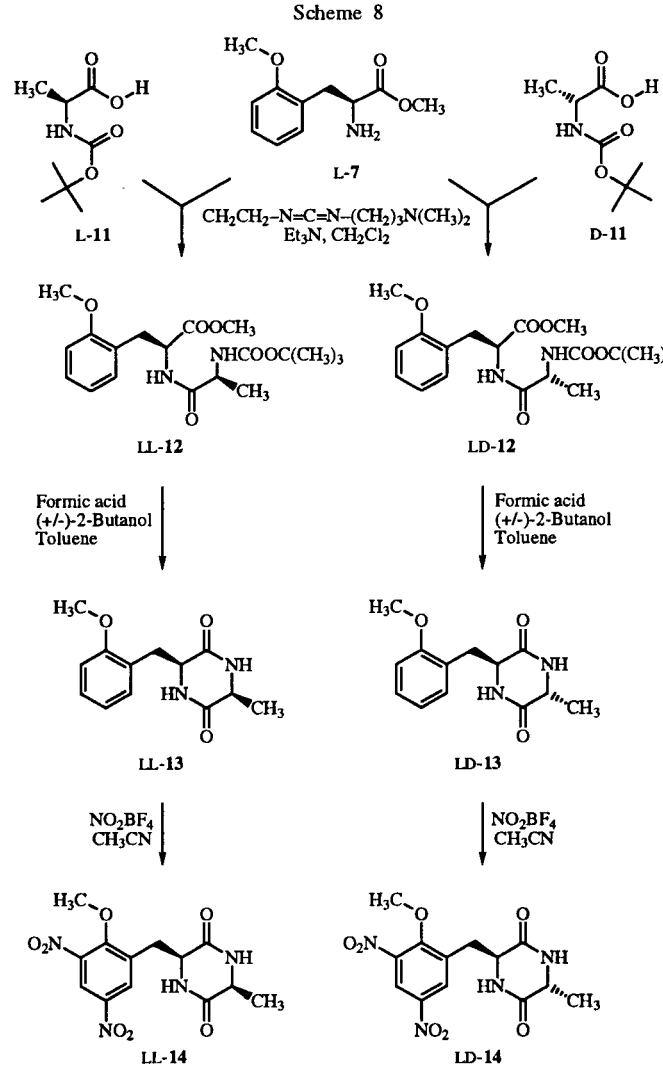
Scheme 7



mass spectrometry, optical rotation, and elemental analysis. We are so far unable to find in the literature an instance of nitration in the presence of the diketopiperazine moiety.

The diketopiperazines shown in Scheme 8 were synthesized and characterized in the same manner as described above for the dimers, except the chirally pure *N*-*t*-butoxycarbonylalanines replaced the *N*-*t*-butoxycarbonylphenylalanines in the dipeptide coupling. Diasteromers LL-**13** and LD-**13** as well as diasteromers LL-**14** and LD-**14** could be distinguished by significant differences in both the ^{1}H- and ^{13}C-nmr spectra. Diastereomeric purities were again analyzed by hplc.

Scheme 8



Experimental

Melting points were determined on a Thomas-Hoover melting point apparatus and are reported uncorrected. The ir spectra were recorded as potassium bromide pellets with a Nicolet Impact 400D FT spectrophotometer. The nmr spectra were recorded on a JEOL

FX 90Q spectrophotometer. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane or, in the case of deuterium oxide solutions, 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt. Elemental analyses were obtained either from Galbraith Laboratories, Inc., Knoxville, TN or Atlantic Microlab, Inc., Norcross, GA. The hplc analyses were carried out on an ISCO binary gradient chromatograph equipped with two model 2350 pumps and a model UA-5 fixed-wavelength uv/visible detector equipped with a 254 nm filter set (ISCO, Inc., Lincoln, NE). Chromatograms were obtained on an ISCO 5 μm Spherisorb C18 column, 4.6 mm ID x 25 cm L. Samples were injected with a Valco Model C6W injection valve (VICI, Inc.). The chromatograms were analyzed using ChemResearch® software (ISCO) and Quattro Pro® for Windows, Version 5.0 (Borland International, Inc., Scotts Valley, CA). Mobile phases were isocratic or gradient combinations of the following solutions: Solvent A, 20% acetonitrile/0.1% trifluoroacetic acid/water; Solvent B, 50% acetonitrile/0.1% trifluoroacetic acid/water; Solvent C, 75% acetonitrile/0.1% trifluoroacetic acid/water; Solvent D, 10% acetonitrile; Solvent E, 40% acetonitrile, Solvent F, 98% acetonitrile, Solvent G, 99% acetonitrile, Solvent H, 100% acetonitrile. The tlc was carried out on 250-μm hard-layer silica HLF UNIPLATES/organic binder (Analtech, Inc., Wilmington, DE), with one of the following eluents: Eluent A, diisopropylether:chloroform:glacial acetic acid (6:3:1); Eluent B, hexane:ethyl acetate (3:1).

2-Methoxybenzyl Bromide (**5**).

The procedure was essentially that of Kelley *et al.* [14], with minor but noteworthy modifications. Phosphorus tribromide (10.2 g, 38 mmoles) was added to a well-stirred solution of 2-methoxybenzyl alcohol (15.0 g, 108 mmoles) and toluene (125 ml) at 40°. The temperature of the solution was raised to 100° in about 15 minutes and maintained at 100° for 10 minutes, and then the reaction was allowed to cool to ambient room temperature. The liquid was decanted into a separatory funnel and washed with water (2 x 250 ml) and brine (2 x 250 ml). The combined aqueous washes were extracted with ether (375 ml). The organic fractions were combined and washed with water (2 x 250 ml) and then with brine (250 ml). The washed organic phase was spin-evaporated to a golden-yellow oily liquid, which was then vacuum-distilled to provide 17.4 g (80%) of **5** as a colorless liquid, bp (0.004 mm of mercury) 70-72°; the ^{1}H nmr spectrum is in accord with the literature [14].

Diethyl 1-Acetamido-2-(2'-methoxyphenyl)-1,1-ethanedicarboxylate (**4**).

Diethyl acetamidomalonate (11.9 g, 55 mmoles) was added to a stirred solution of ethanolic sodium ethoxide, freshly prepared by dissolving sodium spheres (1.3 g, 56 mmoles) in anhydrous ethanol (60 ml). After dissolving the malonate, 2-methoxybenzyl bromide (**5**, 10.3 g, 51 mmoles) was added and the reaction mixture was stirred under nitrogen for 3 hours, monitoring the reaction by tlc (Eluent B) for the disappearance of **5** (R_f = 0.81), after which the solution was spin-evaporated to dryness. The residue was partitioned between ethyl acetate (100 ml) and water (20 ml). The ethyl acetate layer was washed with brine (50 ml), dried over anhydrous sodium sulfate, filtered, and spin-evaporated to dryness. The residue was recrystallized from ethyl acetate to give 16.0 g (92%) of **4** as a white crystalline powder, mp 87.5-88.5°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.81 (s,1H, NH, deuterium oxide-exchangeable), 6.83-7.61 (m, 4H, phenyl), 4.14 (q, 4H, J = 7.1 Hz, CH_2CH_3), 3.69 (s, 3H, OCH_3), 3.47 (s, 2H, benzyl), 1.87 (s, 3H, $COCH_3$), 1.18 (t, 6H, J = 7.1 Hz, CH_2CH_3); ^{13}C nmr: δ 169.4 (COO), 167.6 (CONH), 158.0 (C2'), 131.8 (C6'), 128.5 (C4'), 123.5 (C1'), 120.2 (C5'), 110.7 (C3'), {66.2, 61.6 (OCH_2, CNH)}, 55.4 (OCH_3), 32.1 (CH_2), 22.3 ($COCH_3$), 14.0 (CH_2CH_3); ir (potassium bromide): 3288 (amide NH), 3006 (Ar C-H stretch), {2979, 2945, 2838 (C-H)}, {1756,1729 (C=O)}, 1642, 1609 (N-H), 1521 (C=C), 1374 (C-N), {1287, 1125, 1024 (C-O)} cm^{-1}.

Anal. Calcd. for $C_{17}H_{23}NO_6 \cdot 1/4H_2O$: C, 59.73; H, 6.93; N, 4.10.Found: C, 60.03; H, 6.90; N, 4.15.

(±)-2-Methoxyphenylalanine ((±)-**2**).

The substituted malonate **4** (20.2 g, 60 mmoles) was refluxed with 5 *N* hydrochloric acid (100 ml), monitoring the reaction by hplc (Solvent A, 1.5 ml/minute, 1 drop of the reaction mixture was diluted with 9 drops of water and 10 μl was injected into the hplc; t_R = 3.0 minutes for **2**). After 24 hours (if the reflux was continued for a further 6 hours, there was significant formation of 2-hydroxyphenylalanine (**11**), t_R = 2.1 minutes), the reaction mixture was spin-evaporated to dryness and the residue was dissolved in ethanol (50 ml). Propylene oxide (10 ml) was added and the mixture was stirred for 3 hours. The white precipitate of the zwitterionic amino acid was collected and dried under vacuum to yield 10.0 g (86%) of **2**, mp 240-242°; ^{1}H nmr (Deuterium oxide, 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt): δ 6.91-7.48 (m, 4H, phenyl), 4.01 (m, 1H, CH), 3.87 (s, 3H, OCH_3), 3.36 (dd, 1H, J = 13.5, 4.9 Hz, CH_aH_b), 3.02 (dd, 1H, J = 13.4, 8.1 Hz, CH_aH_b); ^{13}C nmr: δ 176.5 (CO), 160.2 (C2), 134.0 (C6), 132.0 (C4), 126.5 (C1), 123.8 (C5), 114.5 (C3), 58.0 (overlapped CH, OCH_3), 33.8 (CH_2).

(±)-*N*-Acetyl-2-methoxyphenylalanine ((+)-**6**).

The aryl-substituted malonate **4** (16.9 g, 50 mmoles) was refluxed with 1 *N* sodium hydroxide (100 ml) for 6 hours, monitoring the reaction by tlc (1 drop of the reaction mixture was added to two drops of 5 *N* hydrochloric acid; this aqueous sample was extracted with ten drops of ethyl acetate, and the ethyl acetate extract was spotted on the tlc plate, which was eluted with hexane:ethyl acetate 1:1) for the disappearance of the starting material (R_f = 0.46). The reaction mixture was washed with ethyl acetate (2 x 200 ml), and the aqueous phase was then acidified to *p*H 2 with concentrated hydrochloric acid and extracted with ethyl acetate (3 x 200 ml). The ethyl acetate extract was washed with brine (50 ml) and concentrated by spin-evaporation to approximately 10 ml. This concentrate was stored in a freezer at approximately -5-(-2)° for about 12 hours, and the resulting crystals were collected by suction-filtration and dried in a vacuum desiccator to give 11.1 g (94%) of (±)-**6**, mp 166-167°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.12 (d, 1H, J = 8.4 Hz, NH, deuterium oxide-exchangeable), 6.76-7.17 (m, 4H, phenyl), 4.46 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 3.13 (dd, 1H, J = 13.6, 5.4 Hz, CH_aCH_b), 2.74 (dd, 1 H, J = 13.7, 9.5 Hz, CH_aCH_b), 1.77 (s, 3H, $COCH_3$); ^{13}C nmr: δ 173.5 (COO), 169.2 (NHCO), 157.4 (C2), 130.6 (C6), 128.0 (C4), 125.6 (C1), 120.1 (C5), 110.7 (C3), {55.4, 51.9 (CH, OCH_3)}, 31.9 (CH_2), 22.4 ($COCH_3$); ir (potassium bromide): 3500-2475 (OH), 3295 (amide NH), 3087 (Ar C-H), {2972, 2945, 2845 (C-H)}, 1703 (C=O), 1609 (amide C=O), 1562 (C=C), 1387 (C-N), 1246 (C-O) cm^{-1}.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.64; H, 6.49; N, 5.88.

L-2-Methoxyphenylalanine (L-**2**).

(±)-*N*-Acetyl-2-methoxyphenylalanine ((±)-**6**, 5.9 g, 25 mmoles) was suspended in distilled water (60 ml) and sufficient 2 *N* potassium hydroxide was added to adjust the final *p*H to 7.5-8.0, resulting in a clear solution. The volume was adjusted to 100 ml with distilled water, and 1 ml was set aside as the standard for hplc analysis. *Aspergillus* acylase I (Sigma, 221 mg) was added, and the reaction mixture was stirred and maintained at 40 ± 2°. The reaction was monitored by hplc (Solvent A, 1.5 ml/minute; 1 drop of the reaction mixture was diluted with 9 drops of water and 10 µl was injected into the hplc; for comparision one drop of the standard solution was diluted with 19 drops of water and injected into the hplc; t_R = 3 minutes for L-**2**). After 72 hours, when the chromatographic peak height due to the *N*-acetylamino acid **6** (t_R = 5.8 minutes) from the standard and sample preparations were nearly equal and the height ratio of the peaks due to **6** and **2** in the sample preparation remained constant, the solution was acidified to *p*H 2 with 5 *N* hydrochloric acid and extracted with ethyl acetate (4 x 200 ml). This extract was found to contain only the *N*-acetylamino acid by hplc. The combined aqueous phase, found to contain only the amino acid by hplc, was spin-evaporated to dryness, the residue was dissolved in ethyl alcohol (25 ml), and the resulting solution was filtered. The filtrate was treated with propylene oxide (3 ml) and stirred for 3 hours at room temperature; the resulting white precipitate was collected by suction-filtration and dried to give 1.6 g (63%) of L-**2** [15], mp 237-239°; $[\alpha]_D^{20}$ = -52.85° (Water, c = 1); ^{1}H nmr and ^{13}C nmr spectra were essentially identical with those reported for (±)-**2**.

Anal. Calcd. for $C_{10}H_{13}NO_3 \cdot 1/2H_2O$: C, 58.81; H, 6.91; N, 6.85. Found: C, 58.50; H, 6.99; N, 6.82.

D-*N*-Acetyl-2-methoxyphenylalanine (D-**6**).

The ethyl acetate extract set aside after the extraction of L-**2** in the above procedure was concentrated by spin-evaporation and stored in a refrigerator (2-5°) overnight. The crystals formed were collected by filtration and dried in a vacuum desiccator to give 2.9 g of **6**. When acid-hydrolyzed to the amino acid **2** (see procedure below), the enantiomeric excess was about 90%. This scalemic *N*-acetyl-2-methoxyphenylalanine (scalemic-**6**) was again treated with acylase (100 mg), this time providing 2.5 g (85%) of chirally pure D-**6**, mp 166-167.5°; $[\alpha]_D^{20}$ = +5.98° (ethyl alcohol, c = 1); ^{1}H nmr and ^{13}C nmr spectra were essentially identical with those reported for (±)-**6**.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found C, 60.76; H, 6.46; N, 5.94.

D-2-Methoxyphenylalanine (D-**2**).

D-*N*-Acetyl-2-methoxyphenylalanine (D-**6**, 2.7 g, 10 mmoles) was refluxed with 5 *N* hydrochloric acid (50 ml), monitoring the reaction by hplc (Solvent A, 1.5 ml/minute, 1 drop of the sample was diluted with 19 drops of water; t_R = 3 minutes for D-**2** and t_R = 5.8 minutes for D-**6**). After about 5 hours all the starting material had reacted (when the reaction was scaled up four-fold, the reaction went to completion in 2.5 hours), and the solution was then spin-evaporated to dryness. The residue was dissolved in ethyl alcohol (25 ml). Propylene oxide (10 ml) was added and the mixture was stirred for 2 hours. The precipitate that formed was filtered and dried to provide 1.4 g (72%) of D-**2** [15], mp 238-240°; $[\alpha]_D^{20}$ = +52.27° (Water, c = 1); ^{1}H nmr and ^{13}C nmr were identical with those reported for (±)- **2**.

Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.17. Found: C, 61.43; H, 6.93; N, 6.99.

Chiral Purity of 2-Methoxyphenylalanines L-**2** and D-**2**.

The method is a modification of the procedure of Szókán *et al.* [10]. Marfey's reagent solution (0.20 ml, 7.4 µmoles), prepared by dissolving 10 mg of Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide in 1.0 ml of acetone, was added to 0.1 ml (0.5 µmole) of a solution of 19.52 mg of 2-methoxyphenylalanine (racemic, or scalemic, or L, or D) in 2.0 ml of water. After the addition of 0.04 ml of 1 *M* sodium bicarbonate solution, the mixture was heated at 40° for 1 hour. After cooling, 0.02 ml of 2 *M* hydrochloric acid solution was added. The samples (10 µl) were injected into the hplc (Solvent B, 1.5 ml/min). The retention time for the diastereomer obtained from L- **2** was 2.1 minutes and that for the diastereomer obtained from D-**2** was 2.8 minutes. The enantiomeric excess for L-**2** was >99.99%, that for D-**2** was 99.99%, and that for scalemic-**2** was 90.4%.

L-2-Methoxyphenylalanine Methyl Ester (L-**7**).

Methanol (4.6 ml, 120 mmoles) contained in a 3-necked flask under a positive pressure of dry nitrogen was cooled to -5° with a saltwater/ice-bath; thionyl chloride (1.23 ml, 16.8 mmoles) was then added. To this solution L-**2** (2.9 g, 15 mmoles) was slowly added, during which time the temperature was maintained at -5°. Thereafter the suspension was slowly warmed to 40° and further stirred at 40° for 3 hours; during this time, the suspension slowly became a solution. Methanol was distilled off by spin-evaporation, and the residue was dried under vacuum at room-temperature for 1 hour. The residue was dissolved in a minimum amount of water (about 10 ml), diethyl ether (50 ml) was added, and the *p*H was adjusted to 10 with concentrated ammonium hydroxide. The aqueous layer was then extracted with additional ether (5 x 100 ml). The combined ether extracts were washed with brine (50 ml) and spin-evaporated to give 2.4 g (77%) of L-**7** as an oily liquid [16]; $[\alpha]_D^{20}$ = +29.07° (methylene chloride, c = 24); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 6.79-7.21 (m, 4H, phenyl), 3.76 (s, 3H, $ArOCH_3$), 3.57 (m, 1 H, CH), 3.54 (s, 3H, $COOCH_3$), 2.79 (dd, 2H, J = 7.0, 2.2 Hz, CH_2); ^{13}C nmr: δ 176.0 (COO), 157.7 (C2), 131.3 (C6), 128.2 (C4), 126.1 (C1), 120.3 (C5), 110.8 (C3), {55.5, 54.6 (CH, OCH_3)}, 51.5 ($COOCH_3$), 36.1 (CH_2).

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.05; H, 7.18; N, 6.73.

D-2-Methoxyphenylalanine Methyl Ester (D-**7**).

By using D-**2** instead of L-**2** in the above procedure for L-**7**, 2.4 g (77%) of D-**7** was obtained as an oily liquid [16]; $[\alpha]_D^{20}$ = -29.10° (methylene chloride, c = 24); ^{1}H nmr and ^{13}C nmr were identical to those reported for L-**7**.

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 62.92; H, 7.41; N, 6.72.

Chiral Purity of 2-Methoxyphenylalanine Methyl Esters L-**7** and D-**7**.

The method is similar to that used for determining the chiral purity of the amino acids L-**2** and D-**2**. Marfey's reagent solution (0.40 ml, 7.4 µmoles), prepared by dissolving 5 mg of Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide in 1.0 ml of acetone, was added to 0.1 ml (0.5 µmole) of a solution of 100 mg of 2-methoxyphenylalanine methyl ester (L or D) in 10 ml of 1:1 acetone/water. After the addition of 0.04 ml of 1 *M* sodium bicarbonate solution, the mixture was heated over a water bath at 40° for 1 hour and allowed to cool. Hydrochloric acid solution (2 *M*, 0.02 ml) was added. The samples (10 µl) were injected into the hplc

(Solvent B, 1.5 ml/min). The retention time for the diastereomer obtained from L-**7** was 5.5 minutes and that for the diastereomer obtained from D-**7** was 5.9 minutes. The enantiomeric excess for L-**7** was >99.99% and that for D-**7** was 98.6%.

3,6-bis(2-Methoxybenzyl)piperazine-2,5-diones (±)-**3** and *meso*-**3**.

2-Methoxyphenylalanine methyl ester (3.1 g, 15 mmoles) was heated neat in an oil bath at 100-110° for 24 hours, monitoring the reaction by tlc (Eluent A, R_f = 0.28 for (±)-**3**; R_f = 0.56 for *meso*-**3**). The residue was dissolved in ethanol (10 ml) and two compounds were obtained by fractional crystallization. The (±)-**3** crystallized first (0.2 g, 8% after repeated recrystallizations), and *meso*-**3** crystallized second (0.3 g, 12% after repeated recrystallizations). The stereochemistry was established by unambiguous synthesis of the diketopiperazines LL-**3**, DD-**3**, and *meso*-**3** (see below); mp of (±)-**3** 190-192°; mp of *meso*-**3**: 186-188°. Elemental analyses for (±)-**3** and *meso*-**3** were not obtained at this point, but were obtained for the compounds synthesized through the linear dipeptide route given below. The spectral characteristics of (±)-**3** were identical with those of LL-**3** and DD-**3**; and those of *meso*-**3** were identical with those of *meso*-**3** obtained from linear dipeptides LD-**8** or DL-**8** (see below).

Interconversion of (±)-**3** and *meso*-**3**.

About 5 mg each of (±)-**3** and *meso*-**3** were heated to 120° for 5 minutes in two separate capillary tubes sealed at one end. The compounds were then dissolved with ethyl alcohol (5 ml). The solutions were spotted on a tlc plate, with the unheated samples as reference (Eluent A, R_f = 0.28 for (±)-**3** and R_f = 0.56 for *meso*-**3**). It was evident that (±)-**3** was partially converted to *meso*-**3** and *meso*-**3** was found to be partially converted to (±)-**3**. In multiple experiments, the rate and extent of conversion were clearly dependent on the temperature and duration of heating, but detailed kinetic investigations were not undertaken.

L-*N*-*tert*-Butoxycarbonyl-2-methoxyphenylalanine (L-**9**).

A solution of L-**2** (3.9 g, 20 mmoles) and triethylamine (4.2 ml, 30 mmoles) in 50% aqueous dioxane (24 ml) was prepared, and 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile (5.4 g, 22 mmoles) was added in one portion. The solution was stirred at room temperature for 5 hours, monitoring the reaction by hplc (Solvent B, flow rate 1.2 ml/minute; 1 drop of the reaction mixture was diluted with 19 drops of water and 20 drops of acetonitrile, and 10 µl was injected into the chromatograph; t_R = 2.4 minutes for compound L-**2** and t_R = 4.7 minutes for compound L-**9**). When the starting material was no longer in evidence, the solution was diluted with water (30 ml) and washed with ethyl acetate (4 x 100 ml). The ethyl acetate washings were combined, extracted with water (100 ml), and then discarded. The aqueous phases were combined, acidified with citric acid (10 g) and 1.2 *N* hydrochloric acid (40 ml), and extracted with ethyl acetate (4 x 100 ml). The combined ethyl acetate extracts were washed with brine (50 ml), dried over anhydrous sodium sulfate, and spin-evaporated to a volume of about 5 ml. Hexane (5 ml) was added, and crystallization was allowed to proceed in a freezer at -5-(-2)° for about 4 hours. The crystals were filtered and dried to obtain 4.5 g (77%) of L-**9**, mp 153-154°; $[\alpha]_D^{20}$ = -7.94° (ethanol, c = 1); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 6.76-7.21 (m, 4H, phenyl), 4.10-4.30 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 3.10 (dd, 1H, J = 13.5, 4.5 Hz, CH_aH_b), 2.78 (dd, 1H, J = 13.5, 7.5 Hz, CH_aH_b), 1.31 (s, 9H, $C(CH_3)_3$); ^{13}C nmr: δ 174.0 (COO), 157.5 (NHCO), 155.6 (C2), 131.5 (C6), 128.7 (C4), 125.8 (C1), 120.2 (C5), 110.7 (C3), 78.1 ($C(CH_3)_3$), {55.3, 53.4 (CH, OCH_3)}, 32.0 (CH_2), 28.3 ($C(CH_3)_3$); ir (potassium bromide): 3500-2500 (OH), 3349 (NH), {2972, 2932, 2838 (C-H)}, {1743,1662 (C=O)}, 1501 (C=C), 1347 (C-N), 1253 (C-O),1119 (C-O-C), 1025 cm^{-1}.

Anal. Calcd. for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.04; H, 7.18; N, 4.79.

D-*N*-*tert*-Butoxycarbonyl-2-methoxyphenylalanine (D-**9**).

By using D-**2** instead of L-**2** in the above procedure for L-**9**, 4.7 g (79%) of D-**9** was obtained, mp 151-152°; $[\alpha]_D^{20}$ = +7.98° (ethanol, c = 1); ^{1}H nmr, ^{13}C nmr, and ir were identical to those reported for L-**9**.

Anal. Calcd. for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 60.75; H, 7.20; N, 4.73.

N-tert-Butoxycarbonyl-L-2-methoxyphenylalanyl-L-2-methoxyphenylalanine Methyl Ester (protected linear dipeptide LL-**8**).

To a solution of *N*-BOC-L-2-methoxyphenyl alanine L-**9** (0.59 g, 2.0 mmoles) in methylene chloride (10 ml) at 0° was added L-2-methoxyphenylalanine methyl ester L-**7** (0.42 g, 2.0 mmoles) and triethylamine (0.28 ml, 2.0 mmoles). *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.38 g, 2.0 mmoles) was added, and the solution was stored in a freezer at -5-(-2)°. The reaction was monitored by hplc (Solvent B, flow rate 2.0 ml/minute; samples were prepared by evaporating 1 drop of the reaction mixture to dryness on a steam bath and dissolving the residue in 10 drops of acetonitrile). After 72 hours, when there was no further product formation, the reaction mixture was diluted to a volume of 25 ml with methylene chloride, washed with 0.5 *N* sodium hydroxide solution (25 ml), water (25 ml), 1 *N* hydrochloric acid (25 ml), water (25 ml), and finally with brine (25 ml), and dried over anhydrous sodium sulfate. The solution was spin-evaporated to dryness, the residue was taken up in ethyl acetate (20 ml), and this solution was filtered, concentrated to about 5 ml, and stored in a freezer at -5-(-2)° overnight. The crystals formed were collected by filtration and dried to yield 0.23 g (24%) of LL-**8**, mp 132-133°; $[\alpha]_D^{20}$ = +4.02 (methylene chloride, c = 0.5); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.96 (d, 1H, J = 7.82 Hz, amide NH), 6.63-7.29 (m, 8H, phenyl), 4.31-4.60 (m, 1H, CH), 3.98-4.30 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.54 ($COOCH_3$), 2.90-3.19 (m, 2H, CH_2), 2.62-2.89 (m, 2H, CH_2), 1.25 (s, 9H, $C(CH_3)_3$); ^{13}C nmr: 171.9 (COO), 171.8 (NHCOO), 157.3 (NHCO), 154.9 (C2', C2"), {130.8, 128.2 (C6', C6")}, {128.2, 127.6 (C4', C4")}, {125.7, 124.6 (C1', C1")}, 119.9 (C5', C5"), {110.5, 110.4 (C3', C3")}, 78.1 (NHCOO*C*), 55.3 (both OCH_3), 54.1 ($COOCH_3$), 52.1 (CHNHBOC), 51.6 ($CHCOOCH_3$), {32.2, 31.8, (both CH_2)}, 28.0 ($C(CH_3)_3$); ir (potassium bromide): {3349, 3315 (NH)}, {3093, 3053, 3033, 3012 (Ar C-H)}, {2972, 2946, 2838 (C-H)}, {1750, 1696, 1663 (C=O)}, {1609, 1589 (NH)}, {1548, 1521 (C=C)}, {1394, 1374 (C-N)}, {1300, 1246 (C-O)}, 1186 (C-O-C), 1024 (C-O) cm^{-1}.

Anal. Calcd. for $C_{26}H_{34}N_2O_7$: C, 64.18; H, 7.04; N, 5.76. Found: C, 64.01; H, 7.21, N, 6.02.

N-*tert*-Butoxycarbonyl-D-2-methoxyphenylalanyl-D-2-methoxyphenylalanine Methyl Ester (protected linear dipeptide DD-**8**).

By using D-**9** and D-**7** in the place of L-**9** and L-**7** in the above procedure for LL-**9**, 0.16 g (16%) of DD-**8** was obtained, mp 132-134°; $[\alpha]_D^{20}$ = -4.08 (methylene chloride, c = 0.5); ^{1}H nmr, ^{13}C nmr, and ir were identical to those reported for LL-**8**.

Anal. Calcd. for $C_{26}H_{34}N_2O_7 \cdot 1/4H_2O$: C, 63.59; H, 7.08; N, 5.70. Found: C, 63.47; H, 7.13; N, 5.59.

N-tert-Butoxycarbonyl-L-2-methoxyphenylalanyl-D-2-methoxyphenylalanine methyl ester (protected linear dipeptide LD-**8**).

By using D-**9** and L-**7** in the place of L-**9** and L-**7** in the above procedure for LL-**9**, 0.20 g (20%) of LD-**8** was obtained as a waxy semisolid; ^{1}H nmr: δ 8.06 (d, 1H, J = 7.6 Hz, amide NH), 6.65-7.20 (m, 8H, phenyl), 4.32-4.63 (m, 1H, CH), 4.02-4.31 (m, 1H, CH), 3.78 (s, 3H, OCH_3) unresolved from 3.79 (s, 3H, OCH_3), 3.59 (s, 3H, $COOCH_3$), 2.72-3.20 (m, 4H, both CH_2 signals), 1.27 (s, 9H, $C(CH_3)_3$); ms (FAB): m/z 487.1 (calcd. 487.6 (M+1)), 387.1.

N-tert-Butoxycarbonyl-L-2-methoxyphenylalanyl-D-2-methoxyphenylalanine Methyl Ester (protected linear dipeptide DL-**8**).

By using L-**9** and D-**7** in the place of L-**9** and L-**7** in the above procedure for LL-**9**, 0.19 g (19%) of DL-**8** was obtained as a waxy semi-solid; ^{1}H nmr and ms were identical to those reported for LD-**8**.

3*S*,6*S*-bis(2-Methoxybenzyl)piperazine-2,5-dione (LL-**3**).

The linear dipeptide LL-**8** (400 mg, 0.8 mmole) was dissolved in 97% formic acid (40 ml), and the solution was kept at ambient temperature for 2 hours. After removal of most of the formic acid by spin-evaporation (<30°), the residue containing the crude dipeptide ester formate was dissolved in (±)-2-butanol (40 ml) and toluene (10 ml). The solution was boiled (about 120°) for 1 hour, and the solvent level was meanwhile maintained by the addition of fresh butanol, monitoring the reaction by tlc (Eluent A; R_f = 0.28 for LL-**3**, R_f = 0.69 for LL-**8**). When the starting material was no longer in evidence, the solution was spin-evaporated to dryness. The residue was dissolved in ethyl acetate (50 ml), concentrated by spin-evaporation, and placed in a refrigerator overnight. The resulting white crystals were collected and dried at room-temperature to yield 0.18 g (63%) of LL-**3**, mp 194-195°; $[\alpha]_D^{20}$ = -255.3° (CH_2Cl_2, c = 0.5); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.73 (d, 2H, J = 2.93 Hz, both NH signals), 6.88-7.33 (m, 8H, phenyl), 3.83-4.05 (m, 2H, both CH signals), 3.76 (s, 6H, both OCH_3 signals), 2.97 (dd, 2H, J = 13.5, 5.2 Hz, both CH_aH_b signals), 2.39 (dd, 2H, J = 13.5, 7.5 Hz, both CH_aH_b signals); ^{13}C nmr: δ 167.0 (both CONH), 157.8 (C2', C2"), 131.6 (C6', C6"), 128.2 (C4', C4"), 124.8 (C1', C1"), 120.3 (C5', C5"), 111.0 (C3', C3"), {55.5, 54.6 (both CH, both OCH_3)}, 35.1 (both CH_2); ms (EI): m/z 354 (calcd. 354.2), 121.1 (base), 91; ir (potassium bromide): 3194 (N-H), 3046 (Ar C-H), {2959, 2899, 2839 (C-H)}, 1669 (C=O), {1602, 1582 (N-H)}, {1501, 1461 (C=C)}, 1246 (C-O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{22}N_2O_4 \cdot 1/8H_2O$: C, 67.35; H, 6.29; N, 7.85. Found: C, 67.05; H, 6.33; N, 7.79.

3*R*,6*R*-bis(2-Methoxybenzyl)piperazine-2,5-dione (DD-**3**).

By using the linear peptide DD-**8** instead of LL-**8** in the above procedure for LL-**3**, 0.16 g (56%) of DD-**3** was obtained, mp 193-195°; $[\alpha]_D^{20}$ = +253.8° (methylene chloride, c = 0.5); ^{1}H nmr, ^{13}C nmr, and ir were identical to those reported for LL-**3**.

Anal. Calcd. for $C_{20}H_{22}N_2O_4 \cdot 1/8H_2O$: C, 67.35; H, 6.29; N, 7.85. Found: C, 67.41; H, 6.50; N, 7.68.

3*S*,6*R*-bis(2-Methoxybenzyl)piperazine-2,5-dione (or 3*R*,6*S*-bis(2-Methoxybenzyl)piperazine-2,5-dione, *meso*-**3**).

The compound *meso*-**3** was obtained by using either the linear peptide LD-**8** or DL-**8** instead of LL-**8** in the above procedure for LL-**3**. From LD-**8**, 0.11 g (39%) of *meso*-**3** was obtained, mp 185-187°. From DL-**8**, 0.10 g (35%) of *meso*-**3** was obtained, mp 186-187°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 7.51 (d, 2H, both NH signals), 6.75-7.31 (m, 8H, phenyl), 3.74 (m, 6H, both OCH_3 signals), 3.65-3.80 (m, 2H, both CH signals), 3.1 (dd, 2H, J = 13.5, 5.0 Hz, both CH_aH_b signals), 2.78 (dd, 2H, J = 13.5, 7.5 Hz, both CH_aH_b signals); ^{13}C nmr: δ 167.6 (both CONH), 157.4 (C2', C2"), 130.8 (C6, C6"), 127.9 (C4', C4"), 124.6 (C1', C"), 120.0 (C5', C5"), 111.7 (C3', C3"), {55.2, 54.0 (both CH, both OCH_3)}, 32.2 (both CH_2); ms (EI): same as LL-**3**; ir (potassium bromide): {3288, 3214 (N-H)}, 3006 (Ar C-H), {2939, 2905, 2838 (C-H)}, {1690, 1649 (C=O)}, {1602, 1582 (N-H)}, {1495, 1461 (C=C)}, 1313 (CN), 1253 (C-O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{22}N_2O_4 \cdot 1/8H_2O$: C, 67.35; H, 6.29; N, 7.85. Found: C, 67.15; H, 6.63; N, 7.66.

Diastereomeric Purity of LL-**3**, DD-**3**, and *meso*-**3**.

The diastereomeric purities of LL-**3**, DD-**3**, and *meso*-**3** were characterized by hplc (Solvent H, 0.75 ml/minute, 5 mg of the sample was dissolved in 1 ml of ethyl alcohol and 10 µl was injected into the hplc; t_R = 5.0 minutes for LL-**3** and DD-**3**, t_R = 3.6 minutes for *meso*-**3**); LL-**3** and DD-**3** were free from *meso*-**3** (de >99.99%), and *meso*-**3** did not contain the *cis* diastereomer (de >99.99%).

3*S*,6*S*-bis(2-Methoxy-3,5-dinitrobenzyl)piperazine-2,5-dione (LL-**10**).

A suspension of the diketopiperazine LL-**3** (0.35 g, 1.0 mmole) in reagent-grade acetonitrile (4 ml) was cooled in an ice bath to 0°, and nitronium tetrafluoroborate (95%, 0.70 g, 5.0 mmoles) was added in small portions over 20 minutes with continued stirring under an argon atmosphere, monitoring the reaction by hplc (flow rate 1.2 ml/minute, eluting 2 minutes with Solvent A, then a linear gradient to Solvent B over 5 minutes, and finally eluting with solvent B for 4 minutes). When no further starting material (t_R = 6.0 minutes) was in evidence and only one product (t_R = 6.6 minutes) was greatly predominant, the reaction mixture was poured onto ice (50 g) and the resulting mixture was stored in a refrigerator overnight. The precipitated product was filtered off and dried in a vaccum dessicator at room-temperature to give 0.21 g (37%) of LL-**10**, mp 210-212°; $[\alpha]_D^{20}$ = -81.1 (acetone, c = 0.5); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.71 (d, 2H, J = 2.7 Hz, phenyl H4' and H4"), 8.45 (d, 2H, J = 2.7 Hz, phenyl H6' and phenyl H6"), 8.28 (m, 2H, both NH signals, deuterium oxide-exchangeable), 4.05-4.30 (m, 2H, both CH signals), 3.93 (s, 6H, both OCH_3 signals), 3.08-3.65 (m, 4H, both CH_2 signals); ^{13}C nmr: δ 166.6 (both NHCO signals), 156.5 (C2', C2"), 142.8 (C5', C5"), 141.9 (C3', C3"), 135.2 (C1', C1"), 130.5 (C6', C6"), 120.2 (C4', C4"), 63.0 (both OCH_3 signals), 54.2 (both CH signals), 34.9 (both CH_2 signals); ms (EI): m/z 535.4 (calcd. 535.4; (M+1)), 136.1 (base); ir (potassium bromide): {3355, 3208 (N-H)}, 3093 (Ar C-H), {2945, 2872 (C-H)}, 1676 (C=O), 1608 (N-H), 1535 (NO_2), 1481 (C-N), 1347 (NO_2), 1273 (C-O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{18}N_6O_{12} \cdot 1.5H_2O$: C, 42.79; H, 3.77; N, 14.79; Found: C, 42.77; H, 3.48; N, 14.97.

3*R*,6*R*-bis(2-Methoxy-3,5-dinitrobenzyl)piperazine-2,5-dione (DD-**10**).

The diketopiperazine DD-**3** was nitrated following the same procedure as above, to yield 0.26 g (46%) of DD-**10**, mp 211-213°; $[\alpha]_D^{20}$ = +81.7 (acetone, c = 0.5); ^{1}H nmr, ^{13}C nmr, ms, and ir were the same as those of LL-**10**.

Anal. Calcd. for $C_{20}H_{18}N_6O_{12} \cdot H_2O$: C, 43.49; H, 3.65; N, 15.21; Found for DD-**10**: C, 43.77; H, 3.59; N, 15.19.

3*S*,6*R*-bis(2-Methoxy-3,5-dinitrobenzyl)piperazine-2,5-dione (or 3*R*,6*S*-bis(2-Methoxy-3,5-dinitrobenzyl)piperazine-2,5-dione, *meso*-**10**).

The diketopiperazine *meso*-**3** was nitrated following the same procedure as given for LL-**10** above, to yield 0.16 g (29%) of *meso*-**10**, mp 218-222°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.68 (d, 2H, J = 2.9 Hz, phenyl H4' and H4"), 8.47 (d, 2H, J = 2.9 Hz, phenyl H6' and H6"), 8.25 (m, 2H, both NH signals, deuterium oxide-exchangeable), 4.10-4.33 (m, 2H, both CH signals), 3.92 (s, 6H, both OCH_3 signals), 3.15-3.65 (m, 4H, both CH_2 signals); ^{13}C nmr: δ 167.2 (both NHCO signals), 156.9 (C2', C2"), 143.0 (C5', C5"), 142.2 (C3', C3"), 135.4 (C1', C1"),131.0 (C6', C6"), 120.0 (C4', C4"), 63.1 (both OCH_3 signals), 53.8 (both CH signals), 33.5 (both CH_2 signals); ms (EI) for *meso*-**10** was essentially identical to that of LL-**10** and DD- **10**; ir (potassium bromide): 3228 (N-H), 3087 (Ar C-H), {2993, 2966, 2939, 2878 (C-H)}, {1690, 1656 (C=O)}, 1608 (N-H), 1548 (NO_2), 1481 (C-N), 1340 (NO_2), 1219 (C-O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{18}N_6O_{12}$•1.5H_2O: C, 42.79; H, 3.77; N, 14.79; Found: C, 42.97; H, 3.68; N, 14.61.

Diastereomeric Purity of LL-**10**, DD-**10** and *meso*-**10**.

The diastereomeric purities of LL-**10**, DD-**10**, and *meso*-**10** were characterized by hplc (Solvent E, 1.2 ml/minute, 1 mg of the sample was dissolved in 1 ml of acetonitrile and 10 µl was injected into the hplc; t_R = 9.9 minutes for LL-**10** and DD-**10**, t_R = 11.2 minutes for *meso*-**10**). The diastereomeric excess for LL-**10** was 97.0%, that for DD-**10** was 96.5% and that for *meso*-**10** was >99.99%.

3*S*-(2-Methoxybenzyl)-6*S*-methylpiperazine-2,5-dione (LL-**13**).

To a solution of L-*N*-*tert*-butoxycarbonylalanine (L-**11**, 0.76 g, 4.0 mmoles) in methylene chloride (20 ml) at 0° was added L-2-methoxyphenylalanine methyl ester (L-**7**, 0.84 g, 4.0 mmoles) and triethylamine (0.56 ml, 4.0 mmoles). *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.77 g, 4.0 mmoles) was added, and the solution was stored in a freezer at -5-(-2)°. After 72 hours, the reaction mixture was diluted to a volume of 25 ml with methylene chloride, washed with 0.5 *N* sodium hydroxide solution (25 ml), water (25 ml), 1 *N* hydrochloric acid (25 ml), water (25 ml), and finally with brine (25 ml), and dried over anhydrous sodium sulfate. The solution was spin-evaporated to dryness, and the residue was taken up in ethyl acetate (20 ml). This solution was filtered and evaporated to a semi solid mass which was dissolved in 97% formic acid (20 ml). The solution was kept at ambient temperature for 2 hours. After removal of most of the formic acid by spin-evaporation (<30°), the residue containing the crude dipeptide ester formate was dissolved in (±)-2-butanol (40 ml) and toluene (10 ml). The solution was boiled (about 120°) for 1 hour, and the solvent level was meanwhile maintained by the addition of fresh butanol. Thereafter, the solution was spin-evaporated to dryness, and the residue was dissolved in ethanol (50 ml). This solution was concentrated by spin-evaporation and placed in a refrigerator overnight. The resulting white crystals were collected and dried at room-temperature to yield 0.50 g (46%) of LL-**13**, mp 229.5-230.5°; $[\alpha]_D^{20}$ = -57.8° (methylene chloride, c = 0.5); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.05, (bs, 1H, NH), 7.67 (bs, 1H, NH), 6.86-7.33 (m, 4H, phenyl), 3.98-4.05 (m, 1 H, CH), 3.75 (s, 4H, both OCH_3 and CH signals), 3.14 (dd, 1H, J = 13.5, 5.1 Hz, CH_aH_b), 2.85 (dd, 1H, J = 13.5, 4.9 Hz, CH_aH_b), 0.86 (d, 3H, J = 7.1 Hz, CH_3); ^{13}C nmr: δ 168.3 (CO), 167.0 (CO), 158.0 (C2'), 131.5 (C6'), 128.3 (C4'), 124.8 (C1'), 120.3 (C5'), 111.1 (C3'), {55.6, 55.0 (CH, OCH_3)}, 50.1 (CH), 33.6 (CH_2), 19.8 ($CHCH_3$); ir (potassium bromide): 3180, (N-H), 3106 (Ar C-H), {2985, 2871 (C-H)}, {1676, 1642 (C=O)}, {1600, 1585 (N-H)}, {1494, 1461 (C=C)}, 1239 (C-O) cm^{-1}.

Anal. Calcd. for $C_{13}H_{16}N_2O_3$•1/2CH_3CH_2OH: C, 62.41; H, 6.79; N, 10.78. Found: C, 62.33; H, 6.57; N, 10.89. The nmr spectra also indicated the hemiethanolate.

3*S*-(2-Methoxybenzyl)-6*R*-methylpiperazine-2,5-dione (LD-**13**).

By using D-*N*-*tert*-butoxycarbonylalanine (D-**11**) instead of L-**11** in the above procedure for the synthesis of LL-**13**, 0.52 g (48%) of LD-**13** was obtained, mp 193-196°; $[\alpha]_D^{20}$ = -6.02° (methylene chloride, c = 0.5); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.06 (bs, 1H, NH), 7.83 (bs, 1H, NH), 6.86-7.33 (m, 4H, phenyl), 3.98-4.05 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 3.25-3.50 (q, 1H, CH), 2.95-3.10 (m, 2H, CH_2), 1.13 (d, 3H, J = 6.8 Hz, CH_3); ^{13}C nmr: δ 168.9 (CO), 168.4 (CO), 157.9 (C2'), 131.6 (C6'), 128.5 (C4'), 124.6 (C1'), 120.3 (C5'), 111.0 (C3'), 55.5, (unresolved CH, OCH_3), 48.8 (CH), 33.8 (CH_2), 17.8 ($CHCH_3$); ir (potassium bromide): 3200, (N-H), 3093 (Ar C-H), {2985, 2905 (C-H)}, {1689, 1671 (C=O)}, {1602, 1582 (N-H)}, {1494, 1461 (C=C)}, 1240 (C-O) cm^{-1}.

Anal. Calcd. for $C_{13}H_{16}N_2O_3$•1/2CH_3CH_2OH: C, 62.41; H, 6.79; N, 10.78. Found: C, 62.63; H, 6.60; N, 10.89. The nmr spectra also indicated the hemiethanolate.

Diastereomeric Purity of LL-**13** and LD-**13**.

The diastereomeric purities of LL-**13** and LD-**13** were characterized by hplc (Solvent F, 0.75 ml/minute, 1 mg of the sample was dissolved in 2 ml of ethyl alcohol and 10 µl was injected into the hplc; t_R = 5.1 minutes for LL-**13** and t_R = 3.6 minutes for LD-**13**). The diastereomeric excess for LL-**13** was 99.9% and that of LD-**13** was 94.1%

3*S*-(2-Methoxy-3,5-dinitrobenzyl)-6*S*-methylpiperazine-2,5-dione (LL-**14**).

A suspension of the diketopiperazine LL-**13** (0.25 g, 1.0 mmole) in reagent-grade acetonitrile (4 ml) was cooled in an ice bath to 0°, and nitronium tetrafluoroborate (95%, 0.35 g, 2.5 mmoles) was added in small portions over 20 minutes with continued stirring and cooling under an argon atmosphere. The reaction mixture was stirred 30 minutes further at room-temperature and then poured onto ice (50 g); the resulting mixture was stored in a refrigerator overnight. The precipitated product was filtered off and dried in a vacuum dessicator at room-temperature to give 0.11 g (32.5%) of LL-**14**, mp 254-256°; $[\alpha]_D^{20}$ = -60.4° (acetonitrile, c = 0.5); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.69 (d, 1H, J = 2.9 Hz, phenyl H4'), 8.46 (d, 1H, J = 2.9 Hz, phenyl H6'), 8.29 (bs, 1H, NH), 8.06 (bs, 1H, NH), 4.15-4.30 (m, 1H, CH), 3.92 (s, 3H, OCH_3), 3.80 (m, 1H, CH), 3.22 (m, 2H, CH_2), 1.17 (d, 3H, J = 7.08 Hz, CH_3); ^{13}C nmr: δ 168.7 (CO), 166.6 (CO), 156.5 (C2'), 142.9 (C5'), 141.9 (C3'), 135.3 (C1'), 130.2 (C6'), 119.9 (C4'), 62.9 (OCH_3), 54.1 (CH), 50.5 (CH), 33.6 (CH_2), 19.7 (CH_3); ir (potassium bromide): 3207 (N-H), 3080 (Ar C-H), {2979, 2945 (C-H)}, {1682, 1645 (C=O)}, 1602 (N-H), 1541 (NO_2), 1468 (C-N), 1347 (NO_2), 1289 (C-O) cm^{-1}.

Anal. Calcd. for $C_{13}H_{14}N_4O_7$: C, 46.16; H, 4.17; N, 16.56; O, 33.11. Found: C, 46.38; H, 4.34; N, 16.74; O, 33.27.

3*S*-(2-Methoxy-3,5-dinitrobenzyl)-6*R*-methylpiperazine-2,5-dione (LD-**14**).

By using LD-**13** instead of LL-**13** in the above procedure, 0.09 g (26.6%) of LD-**14** was obtained, mp 217-220°; $[\alpha]_D^{20}$ = +10.2°

(acetonitrile, c = 0.5); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.70 (d, 1H, J = 2.93 Hz, phenyl H4'), 8.45 (d, 1H, J = 2.69 Hz, phenyl H6'), 8.27 (bs, 1H, NH), 8.06 (bd, 1H, NH), 3.85-4.15 (m, 2H, CH), 3.93 (s, 3H, OCH_3), 3.22 (m, 2H, CH_2), 1.24 (d, 3H, J = 6.83 Hz, CH_3); ir (potassium bromide): 3221 (N-H), 3080 (Ar C-H), {2979, 2945 (C-H)}, {1682, 1645 (C=O)}, 1602 (N-H), 1541 (NO_2), 1468 (C-N), 1347 (NO_2), 1289 (C-O) cm^{-1}.

Anal. Calcd. for $C_{13}H_{14}N_4O_7$: C, 46.16; H, 4.17; N, 16.56. Found: C, 46.26; H, 4.31; N, 16.68.

Diastereomeric Purity of LL-**14** and LD-**14**.

The diastereomeric purities of LL-**14** and LD-**14** were characterized by hplc (Solvent G, 0.96 ml/minute, 1 mg of the sample was dissolved in 2 ml of acetonitrile and 10 µl was injected into the hplc; t_R = 3.4 minutes for LL-**14** and t_R = 2.9 minutes for LD-**14**). Isomer LL-**14** was free from LD-**14** (de >99.99%), and LD-**14** did not contain LL-**14** (de >99.99%).

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[15] There was racemization observed after 30 days when this compound was stored at room temperature; hence it was stored in a freezer below -5°, whereupon no racemization was observed for at least 3 months.

[16] When this compound was stored at room temperature or in a freezer for 30 days, racemization was observed. If stored as a solution in methylene chloride, no racemization was observed for at least 3 months.