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Convenient Route to Enantiopure Fmoc-Protected Morpholine-3-carboxylic Acid

Filippo Sladojevich, Andrea Trabocchi, and Antonio Guarna*

Dipartimento di Chimica Organica "Ugo Schiff" and Laboratorio di Progettazione, Sintesi e Studio di Eterocicli Biologicamente Attivi (HeteroBioLab), Università degli Studi di Firenze, Polo Scientifico e Tecnologico, Via della Lastruccia 13, I-50019 Sesto Fiorentino, Firenze, Italy

antonio.guarna@unifi.it

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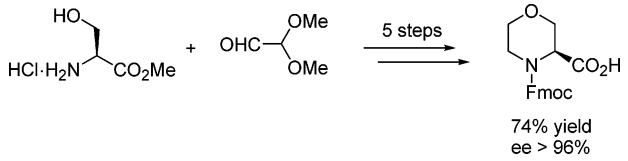

Enantiopure Fmoc-protected morpholine-3-carboxylic acid was synthesized from dimethoxyacetaldehyde and serine methyl ester through a short and practical synthetic route. The preparation consisted of a five-step process based on reductive amination, intramolecular acetalization, and concomitant elimination of the anomeric methoxy substituent, followed by hydrogenation of the double bond and final acidic ester hydrolysis. The optical purity of both enantiomers of the title amino acid was demonstrated by HPLC analysis of the corresponding amide derivatives obtained from coupling with chiral (*S*)-(−)-1-phenylethylamine. Moreover, the synthesis of a model tripeptide showed full compatibility of the title Fmoc-amino acid with solid-phase peptide synthesis, thus allowing the application of Fmoc-morpholine-3-carboxylic acid in peptidomimetic chemistry on the solid phase.

Over the years the synthesis and applications of cyclic amino acids has attracted considerable attention from synthetic and medicinal chemists, especially in the area of peptidomimetics.[1] The incorporation of cyclic secondary amino acids[2] has profound effects on the conformation of peptides, due to the inability of the nitrogen atom to act as a hydrogen bond donor unless it is located at the *N*-terminal position of the molecule, and to the conformational strain imparted by the cyclic structure. Also, *cis/trans* isomerism of the tertiary amide bond formed by cyclic amino acids is responsible for the modulation of the conformational preferences. Cyclic secondary amino acids have been applied in several biological issues, and their incorporation into bioactive peptides has been reported over the years.[3,4] In particular, morpholine-3-carboxylic acid has been used to synthesize several bioactive molecules, such as TACE,[5] MMP and TNF inhibitors,[6] and a potent orally active VLA-4 antagonist.[7] Also, it has been included in the core structure of tricyclic benzodiazepines,[8] 6-methylidene penems as β-lactamase inhibitors,[9] β-carbolines as IKK-2 inhibitors,[10] 8,6-fused bicyclic peptidomimetic compounds as interleukin-1β converting enzyme inhibitors,[11] and in the structure of benzoxazepines as stimulators of AMPA receptor.[12] The first syntheses of morpholine-3-carboxylic acid were reported in 1981 by Anteunis et al. as a racemate,[13] and by Brown et al. describing the preparation of the cyclic amino acid as the *N*-benzyl, ethyl ester derivative from serine in 6% overall yield.[14] In the same years, Kogami and Ogawa reported the most convenient synthesis of morpholine-3-carboxylic acid,[15] which employed chiral benzyl *N*-Cbz-2-aziridine-carboxylate, derived from serine, to give the title amino acid after three steps in 66% overall yield. Recently, Dave and Sasaki reported the synthesis of enantiomerically pure Boc-morpholine-3-carboxylic acid from the corresponding alcohol derivative in 81% yield,[16] which in turn was obtained from a protected serinol derivative in six steps and in 50% overall yield. In addition, an enzymatic synthesis of cyclic amino acids, including morpholine-3-carboxylic acid, has been proposed recently.[17]

* Author to whom correspondence should be addressed. Fax: +39-055-457-3569. Phone: +39-055-457-3481.

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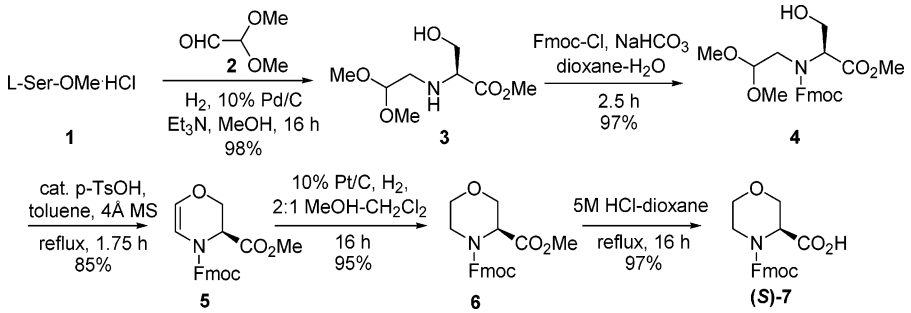
SCHEME 1

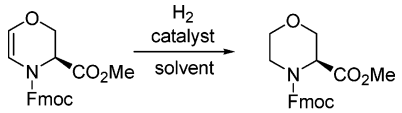


Given the importance of this amino acid in medicinal and organic chemistry, we envisioned morpholine-3-carboxylic acid coming from the condensation of serine methyl ester with dimethoxyacetaldehyde to give the enantiomerically pure title amino acid *N*-protected with the Fmoc group, which is of special interest for solid-phase peptide and organic synthesis. Reductive amination of dimethoxyacetaldehyde with L-serine methyl ester hydrochloride was carried out under a hydrogen atmosphere in the presence of catalytic amounts of 5% Pd/C, giving compound **3** in 50% yield. Optimization of the reaction conditions consisted of the addition of 1 molar equiv of triethylamine, thus furnishing clean adduct **3** in 98% yield after overnight stirring at room temperature (Scheme 1).

Subsequent amine protection as Fmoc-urethane was achieved by using Fmoc-Cl in water−dioxane as the solvent system, and in the presence of $NaHCO_3$ as base, giving **4** in 97% yield. Acid-catalyzed cyclization by acetalization and *in situ* elimination of the methoxy group was achieved in one pot, by refluxing compound **4** in toluene in the presence of catalytic quantities of *p*-toluenesulfonic acid, thus giving **5** as a single stereoisomer in 85% yield. When the reaction was carried out without using molecular sieves, we observed a decrease of the yield to 67%, and the reaction time was prolonged to 4 h to achieve complete conversion of the starting material. Hydrogenation of the double bond at C-5 and C-6 carbon atoms of **5** was initially attempted by treatment with H_2 and catalytic 10% Pd/C, leading to partial deprotection of the amine function (Table 1). The extent of such deprotection was found to be dependent on the choice of the solvent, as when 2:1 MeOH−CH_2Cl_2 was used as the solvent mixture the amount of deprotected amino ester increased, compared to MeOH (Table 1, entries 1 and 2). The use of Raney-Ni as catalyst was unsuccessful, as only starting material was recovered from the reaction mixture.

Finally, 10% Pt/C catalyst gave clean reduction of the double bond and allowed preservation of the amine protecting group. The reaction proceeded in 91% with MeOH as solvent, and upon application of 2:1 MeOH−CH_2Cl_2 as the solvent system, the yield was further optimized to 95% (Table 1, entry 5). Hydrolysis of the ester function to give the title Fmoc-protected amino acid was carried out in an acidic medium to preserve the base-labile Fmoc group. Optimization of the reaction conditions required several attempts to achieve high yield. Hydrolysis of **6** in 2:1 acetonitrile−4 M HCl with overnight stirring at room temperature resulted in poor conversion, giving **7** in 15% yield. Replacement of acetonitrile with dioxane, without altering the other reaction conditions, produced **7** in 29% yield. A marked improvement was obtained by raising the reaction temperature. In fact, the hydrolysis was carried out by refluxing the Fmoc-amino ester **6** in a 0.07 M 1:1 dioxane−4 M HCl solution for 2.5 h, giving **7** in 69% yield. Further optimization gave **7** in 97% yield by overnight refluxing a 0.2 M solution of **6** in a 1:1 mixture of 5 M HCl−dioxane. Consequently, compound (*S*)-**7** was obtained in five steps and an overall yield of 74%. Also, the synthesis of the corresponding enantiomeric (*R*)-Fmoc-morpholine-3-carboxylic acid [(*R*)-**7**] was carried out starting from D-serine methyl ester, giving the title amino acid in 73% overall yield.

TABLE 1. Optimization of Reaction Conditions for the Double Bond Hydrogenation of 5



entry	catalyst	solvent	yield, %
1	10% Pd/C	MeOH	67[a]
2	10% Pd/C	2:1 MeOH−CH_2Cl_2	25[a]
3	Raney Ni	MeOH	*b*
4	10% Pt/C	MeOH	91
5	10% Pt/C	2:1 MeOH−CH_2Cl_2	95

[a] Partial deprotection of the Fmoc group was observed [b] Only starting material was recovered

Enantiomeric purity of (*R*)-**7** and (*S*)-**7** was determined by preparing and analyzing the corresponding diastereomeric amides obtained by reaction with a chiral amine, followed by HPLC analysis,[18] according to methods reported for similar compounds.[19] Thus, coupling between (*S*)-(−)-1-phenylethylamine (98% pure) and (*S*)-**7** or (*R*)-**7** was carried out with HBTU/HOBt and DIPEA in CH_2Cl_2 at room temperature for 3 h, giving the corresponding diastereomeric amides in quantitative yields. Since the 1:1 mixture of the two diastereoisomers could not be separated by reverse-phase HPLC due to similar retention times, we found it more convenient to deprotect a 1 mg/mL analytical sample of each of the two diastereomeric amides in acetonitrile with a drop of piperidine. The two deprotected samples showed different retention times, and the enantiomeric purity of the final product was measured by HPLC, giving a de >96%, without any detectable traces of the parent isomer in both cases (see the Supporting Information).

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(18) NMR analysis of the diastereomeric amides derived from (*S*)-**7** and (*R*)-**7** for assessing the enantiomeric purity of the Fmoc-amino acids was complicated by the presence of rotamers at the urethane bond.

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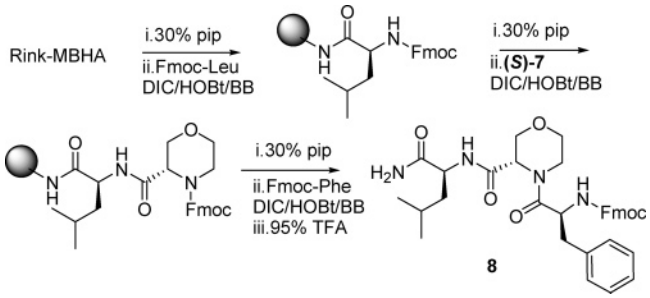
SCHEME 2



Also, Fmoc-3-morpholine carboxylic acid was applied in the solid-phase synthesis of a model tripeptide with use of Rink-HMBA resin and DIC/HOBt as the activating mixture. The internal colorimetric Bromophenol Blue test, as reported by Krchnák et al.,[20] was used to monitor the coupling efficiency on both amino and carboxylic functions of morpholine-3-carboxylic acid. Thus, Fmoc-Phe-morpholine-3*S*-CO-Leu-NH_2 **8** was synthesized. The coupling reactions proved to proceed slower on both functions compared to those on acyclic α-amino acids. The crude tripeptide was obtained in 90% yield after cleavage from the resin, and in 86% purity as determined by HPLC, thus showing full compatibility with solid-phase peptide chemistry.

In summary, we have shown a convenient synthetic strategy for the preparation of enantiomerically pure *N*-Fmoc-morpholine-3-carboxylic acid starting from cheap commercially available serine methyl ester hydrochloride and dimethoxyacetaldehyde, resulting in the preparation of both enantiomers on a gram scale. Determination of enantiomeric purity by HPLC revealed the proposed synthetic strategy proceeded with high stereoconservation, without any traces of racemization. Finally, the synthesis of a model tripeptide by solid-phase techniques proved the title amino acid to be compatible with the solid-phase peptide synthesis, although showing lower reactivity compared to that of proteinogenic acyclic α-amino acids, thus demonstrating the application of the title amino acid in solid-phase protocols for medicinal chemistry.

Experimental Section

(2*S*)-2-(2,2-Dimethoxyethylamino)-3-hydroxypropionic Acid Methyl Ester (3). L-Serine methyl ester hydrochloride (1.00 g, 6.47 mmol) was dissolved in MeOH (20 mL), then triethylamine (902 μL, 6.47 mmol), a 60% aqueous solution of dimethoxyacetaldehyde (1.11 g, 6.47 mmol), and 10% Pd/C (90 mg) were successively added, and the resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. Next, the suspension was filtered on Celite and the organic solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2−MeOH 12:1, R_f 0.43) to yield **3** as a colorless oil (1.31 g, 98%). $[\alpha]^{24}_D$ −28.5 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.44 (t, J = 4.5 Hz, 1 H), 3.77 (dd, J = 11.2, 4.5 Hz, 1 H), 3.74 (s, 3 H), 3.59 (dd, J = 12.5, 8.0 Hz, 1 H), 3.40 (t, J = 4.5 Hz, 1 H), 3.36 (s, 6 H), 2.84 (dd, J = 12.5, 4.5 Hz, 1 H), 2.65 (dd, J = 12.5, 4.5 Hz, 1 H), 2.39 (br, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.1 (s), 103.5 (d), 62.7 (d), 62.5 (t), 53.9 (q), 53.1 (q), 52.0 (q), 49.1 (t). MS *m*/*z* 207 (M^+, 26), 149 (13), 133 (18). Anal. Calcd for $C_8H_{17}NO_5$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.40; H, 8.31; N, 6.69.

(2*S*)-2-[(2,2-Dimethoxyethyl)(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-hydroxypropionic Acid Methyl Ester (4). To a solution of **3** (1.14 g, 5.5 mmol) in 2:1 water−dioxane (15 mL) was added $NaHCO_3$ (0.92 g, 11.0 mmol), and the mixture was cooled to 0 °C with an ice bath, then a solution of Fmoc-Cl (1.42 g, 5.5 mmol) in dioxane (15 mL) was added dropwise over 15 min. The ice bath was removed, and the reaction mixture was left stirring for 2.5 h. Successively, the mixture was partitioned between EtOAc (40 mL) and water (20 mL), and the organic phase was washed with 1 M HCl and brine and dried over Na_2SO_4. The organic solvents were then removed under reduced pressure, and the crude product was purified by flash column chromatography (EtOAc−hexanes 3:2, R_f 0.53), thus giving pure **4** as a colorless oil (2.29 g, 97%). $[\alpha]^{22}_D$ −31.6 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) 3:2 mixture of rotamers δ 7.76 (d, J = 7.2 Hz, 2 H), 7.60 (d, J = 6.0 Hz, 1 H), 7.55−7.53 (m, 1 H), 7.42−7.39 (m, 2 H), 7.35−7.29 (m, 2 H), 4.76−4.69 (m, 2 H), 4.61−4.47 (m, 2 H), 4.24−4.21 (m, 1 H), 3.97−3.94 (m, 1 H), 3.86−3.80 (m, 1 H), 3.69 and 3.61 (s, 3 H), 3.69−3.59 (m, 1 H), 3.49 and 3.43 (2s, 2.4 H), 3.16 and 3.11 (2s, 3.6 H), 3.21−3.11 (m, 0.4 H), 2.97 (dd, J = 15.2, 7.2 Hz, 0.6 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.0 (s), 156.6 (s), 143.4 (s, 2 C), 141.2 (s, 2 C), 127.6 (d, 2 C), 127.1 and 126.9 (d, 2 C), 124.6 and 124.5 (d, 2 C), 119.9 (d, 2 C), 103.3 and 103.0 (d), 67.7 and 66.6 (t), 62.9 and 62.1 (d), 60.7 and 60.2 (t), 55.6 (q), 54.7 (q), 52.2 (q), 49.1 and 48.9 (t), 47.3 and 47.0 (d). MS *m*/*z* 367 [0.2, $M^+ - (OCH_3)_2$], 324 (0.2). Anal. Calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.30; H, 6.39; N, 3.21.

(3*S*)-2,3-Dihydro[1,4]oxazine-3,4-dicarboxylic Acid 4-(9*H*-Fluoren-9-ylmethyl) Ester 3-Methyl Ester (5). A solution of compound **4** (1.1 g, 2.56 mmol) in toluene (25 mL) containing a catalytic amount of *p*-toluenesulfonic acid monohydrate (49 mg, 0.26 mmol) was placed in a single-necked round-bottomed flask equipped with a reflux condenser and dropping funnel containing approximately 13 g of 4 Å molecular sieves. The mixture was refluxed for 1.75 h. Then it was cooled to room temperature and filtered through a thin layer of $NaHCO_3$. Toluene was removed under reduced pressure, and the crude product was purified by flash column chromatography (hexanes−EtOAc 3:1, R_f 0.55) to yield compound **5** as a white foam (795 mg, 85%). $[\alpha]^{23}_D$ +6.2 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) 3:2 mixture of rotamers δ 7.77 (t, J = 8.0 Hz, 1 H), 7.61 (t, J = 8.0 Hz, 1 H), 7.50 (m, 1 H), 7.41 and 7.32 (m, 2 H), 6.42 (d, J = 5.2 Hz, 0.4 H), 6.36 (d, J = 5.2 Hz, 0.6 H), 6.02 (d, J = 5.2 Hz, 0.4 H), 5.98 (d, J = 5.2 Hz, 0.6 H), 4.97 (s, 0.4 H), 4.68 (d, J = 11.2 Hz, 0.4 H), 4.60−4.40 (m, 3.2 H), 4.32 (t, J = 7.2 Hz, 0.6 H), 4.23 (t, J = 7.2 Hz, 0.4 H), 3.99 (dd, J = 11.2, 3.2 Hz, 0.6 H), 3.87 (dd, J = 11.2, 3.2 Hz, 0.4 H), 3.86 (s, 1.8 H), 3.71 (s, 1.2 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 168.2 (s), 151.9 and 151.2 (s), 143.4 and 143.2 (s, 2 C), 141.0 (s, 2 C), 129.7 and 128.8 (d), 127.6 (d, 2 C), 126.9 (d, 2 C), 124.9 (d), 124.8 (d), 124.8 and 124.5 (d), 119.9 (d, 2 C), 105.9 and 105.3 (d), 68.3 and 67.8 (t), 65.4 and 64.9 (t), 54.5 and 53.9 (d), 52.8 (q), 47.0 and 46.9 (d). MS *m*/*z* 365 (M^+, 7), 306 (0.4, $M^+ - CO_2$-Me), 179 (100). Anal. Calcd for $C_{21}H_{19}NO_5$: C, 69.03; H, 5.24; N, 3.83. Found: C, 69.16; H, 5.31; N, 3.80.

(3*S*)-Morpholine-3,4-dicarboxylic Acid 4-(9*H*-Fluoren-9-ylmethyl) Ester 3-Methyl Ester (6). Compound **5** (1.30 g, 3.3 mmol) was dissolved in a 2:1 mixture of MeOH−CH_2Cl_2 (30 mL), and 10% Pt/C (166 mg) was added. The suspension was hydrogenated overnight at room temperature, and then filtered over Celite. The organic solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (hexanes−EtOAc 2:1, R_f 0.48) to yield pure **6** as a white foam (1.15 g, 95%). $[\alpha]^{24}_D$ −51.5 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers δ 7.78−7.75 (t, J = 6.9 Hz, 2 H), 7.60 (t, J = 6.5 Hz, 1 H), 7.50 (t, J = 7.1 Hz, 1 H), 7.43−7.38 (m, 2 H), 7.35−7.28 (m, 2 H), 4.65 (m, 0.5 H), 4.56−4.46 (m, 1.5 H), 4.44−4.37 (m, 1 H), 4.33−4.28 (m, 1.5 H), 4.24−4.20 (m, 0.5 H), 3.91−3.84 (m, 1.5 H), 3.78 and 3.73 (s, 3 H), 3.66 (dd, J = 12.0, 2.4 Hz, 1.5 H), 3.58 (dd, J = 12.0, 2.4 Hz, 0.5 H), 3.50−3.40 (m, 1.5 H), 3.22 (td, J = 12.0, 4.0 Hz, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 169.9 (s), 143.7 and 143.5 (s, 2 C), 141.1 (s, 2 C), 127.5 (d, 2 C), 126.9 (d, 2 C), 124.8 (d), 124.6 and 124.5 (d), 119.8 (d, 2 C),

67.8 and 67.5 (t), 67.5 and 67.2 (t), 66.5 and 66.1 (t), 54.6 and 54.3 (d), 52.5 (q), 47.0 (d), 41.5 and 41.0 (t). MS *m*/*z* 367 (M^+, 0.6), 278 (0.8), 178 (9), 57 (100). Anal. Calcd for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.70; H, 5.77; N, 3.82.

(3*S*)-Morpholine-3,4-dicarboxylic Acid 4-(9*H*-Fluoren-9-ylmethyl) Ester [(*S*)-7]. Ester **6** (1.70 g, 4.6 mmol) was dissolved in dioxane (12 mL) and 5 M HCl (12 mL) was added. The reaction was refluxed for 18 h and then diluted with 5% Na_2CO_3 (120 mL). The resulting solution was washed with diethyl ether and then the aqueous layer was acidified to pH 1 with concentrated HCl and the organic phase was extracted with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to yield compound (*S*)-**7** as a white solid (1.57 g, 97%). Mp 128−130 °C. $[\alpha]^{24}_D$ −56.9 (*c* 1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) mixture of rotamers δ 8.44 (br, 1 H), 7.69−7.62 (m, 2 H), 7.52−7.50 (m, 1 H), 7.44−7.39 (m, 1 H), 7.33−7.17 (m, 4 H), 4.62 (s, 0.5 H), 4.51−4.41 (m, 1.5 H), 4.38−4.32 (m, 1 H), 4.25−4.11 (m, 2 H), 3.83−3.81 (m, 1 H), 3.71 (d, J = 13.0 Hz, 0.5H), 3.65−3.50 (m, 1 H), 3.45 (dd, J = 11.8, 3.8 Hz, 0.5 H), 3.42−3.31 (m, 1.5 H), 3.22−3.16 (td, J = 12.5, 3.4 Hz, 0.5 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.7 and 173.5 (s), 156.1 and 155.5 (s), 143.5 and 143.3 (s, 2 C), 141.0 (s, 2 C), 127.5 (d, 2 C), 126.8 (d, 2 C), 124.7 (d), 124.5 and 124.4 (d), 119.7 (d, 2 C), 67.9 and 67.4 (t), 67.4 and 67.0 (t), 66.4 and 66.0 (t), 54.5 and 54.1 (d), 47.0 (d), 41.5 and 41.0 (t). ESI-MS *m*/*z* 354.09 (M^+ + H, 8), 376.18 (M^+ + Na, 100), 392.18 (M^+ + K, 46). Anal. Calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 67.90; H, 5.43; N, 3.98.

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Supporting Information Available: Experimental data for (*R*)-**3**−(*R*)-**7** and **8**, and experimental procedure for assessing the enantiomeric purity of (*S*)-**7** and (*R*)-**7**; HPLC chromatograms of Fmoc-deprotected diastereomeric amides of (*S*)-**7** and (*R*)-**7**; copies of the ^{1}H and ^{13}C NMR spectra of compounds **3**−(*S*)-**7**; HPLC chromatograms and ESI-MS of tripeptide **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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