Journal of Peptide Science
J. Peptide Sci. **9**: 312–319 (2003)
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.458

JOURNAL OF PEPTIDE SCIENCE

Efficient Protocols for the Synthesis of Enantiopure γ-Amino Acids with Proteinogenic Side Chains

VASSILIOS LOUKAS, CATERINA NOULA and GEORGE KOKOTOS*

Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece

Received 26 August 2002
Accepted 13 December 2002

Abstract: The synthesis of enantiopure γ-substituted γ-amino acids with proteinogenic side chains, starting from the corresponding natural α-amino acids, was studied. *N*-Protected amino aldehydes containing various protective groups were prepared from the corresponding amino alcohols by oxidation with NaOCl in the presence of AcNH-TEMPO and directly reacted with methyl, benzyl and *tert*-butyl phosphoranylidene acetate to produce α,β-unsaturated γ-amino esters. Simultaneous hydrogenation of the double bond and removal of either the benzyl or benzyloxycarbonyl group led to *N*- or *C*-protected γ-amino acids in high yield. The enantiomeric purity was studied by ^{1}H NMR analysis of Mosher amides and chiral HPLC analysis.

Keywords: γ-amino acids; amino alcohols; amino aldehydes; enantiopurity; Wittig reaction

INTRODUCTION

Unnatural amino acids play an important role in the design and synthesis of pharmacologically relevant molecules, analogues of bioactive peptide and peptide mimetics [1–6]. New bioorganic methodologies, such as cell-free synthesis including *in vitro* suppression, have enabled the incorporation of unnatural amino acids into the framework of proteins [7]. Of special interest is that oligopeptides containing unnatural amino acids are able to form well-defined secondary structures. Recent investigations by Seebach [8,9], Gellman [10,11] and Hanessian [12] have shown that short chain peptides comprising β-amino acids can adopt helix, sheet or reverse turn conformations in solution or solid state as evidenced by NMR, CD, x-ray or modelling studies. Furthermore, γ-peptides with four or six amino acid residues, derived from doubly homologated L-alanine, L-valine and L-leucine, form stable helical secondary structure in solution [13,14]. The surprising difference between the natural α-, and the analogous β- and γ-peptides is that the helix stability increases upon homologation of the residues [14]. It should be emphasized that, as most recently demonstrated [15], β- and γ-peptides are completely stable to common proteases, without inhibiting their normal activity. This is of considerable importance as it suggests that β- and γ-peptides may be suitable for pharmaceutical applications.

There are two general routes for the synthesis of γ-amino acids: modification of glutamic acid and homologation of α-amino acids. The multistep derivatization of the α-carboxy function of glutamic acid using alkyl cuprates [16] has a major limitation, i.e. the incompatibility of cuprates with many functionalities, while the use of β-amido zinc derivatives obtained from glutamic acid has been demonstrated only for the synthesis of homophenylalanine derivatives [17]. Boc-protected

*Correspondence to: Dr George Kokotos, Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece;
e-mail: gkokotos@cc.uoa.gr
Contract/grant sponsor: Greek Government and European Commission.
Contract/grant sponsor: University of Athens.

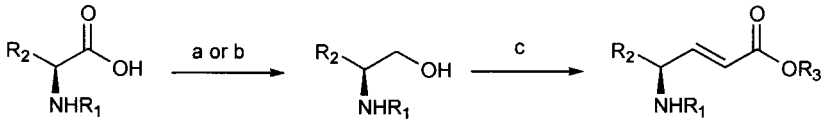
γ-amino acids have been prepared by a double Arndt-Eistert homologation of α-amino acids [14] or an olefinative homologation of α-amino aldehydes [14,18]. Another method involves the reduction of the keto functionality of α-amino-acyl Meldum's acid, followed by thermal decarboxylative ring closure to a 5-substituted pyrrolidinone, and basic hydrolysis to the γ-amino acid [19]. The aim of this work was to develop efficient protocols for the synthesis of γ-substituted γ-amino acids with proteinogenic side chains starting from α-amino acids.

RESULTS AND DISCUSSION

The approach studied here for the synthesis of γ-amino acids was based on a Wittig–type olefination reaction of *N*-protected α-amino aldehydes, leading to chain homologation. Such aldehydes may be prepared either by reduction of a carboxy derivative of amino acids or by oxidation of 2-amino alcohols [20]. Both of the published methods for the homologation of α-amino aldehydes to γ-amino acids are based on reduction of either amino acid methyl esters by DIBAL [18] or Weinreb amides [14]. It was decided to prepare α-amino aldehydes by oxidation of 2-amino alcohols, using NaOCl in the presence of a catalytic amount of a TEMPO derivative, a method that appears to be superior to the reductive methods in terms of preservation of the enantiomeric purity [21]. In addition, the use of a variety of *N*-protective groups of amino acids and *C*-protective groups of the phosphoranylidene acetate was investigated.

N-Protected amino acids **1a–h** were converted into alcohols **2a–h** by reduction with $NaBH_4$ of either their corresponding mixed anhydrides [22] or their corresponding acyl fluorides [23] (Scheme 1), as previously described by the authors. The widely used *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (Z) and 9-fluorenylmethoxycarbonyl (Fmoc) groups were used for protection of the α-amino group. The ε-amino group of lysine was protected by Boc or Z, while the γ-carboxy group of glutamic acid was protected as the methyl ester. Alcohols **2a–h** were oxidized to the corresponding aldehydes by NaOCl in the presence of a catalytic amount of 4-acetamido-2,2,6,6-tetramethylpiperidine-1-yloxy free radical (AcNH-TEMPO) [24,25] and the aldehydes were directly used for the Wittig–type reaction without any purification. Treatment of the aldehydes with the stabilized ylides methyl, benzyl or *tert*-butyl (triphenylphosphoranylidene)acetate in THF at 50 °C led to α,β-unsaturated esters **3a–h**. The geometry of the double bond was *E*, as indicated by ^{1}H NMR analysis.

As depicted in Scheme 2, catalytic hydrogenation of the double bond with simultaneous removal of the benzyl group of **3d,e** led to Boc-protected γ-amino

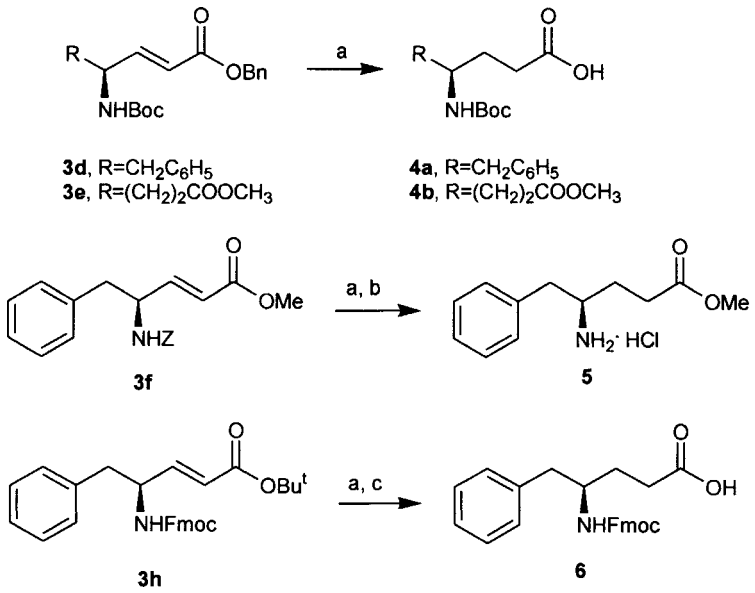


1a-h **2a-h** **3a-h**

1, 2, 3	R_1	R_2	R_3
a	Boc	$CH_2C_6H_5$	Me
b	Boc	$(CH_2)_4NHBoc$	Me
c	Boc	$(CH_2)_4NHZ$	Me
d	Boc	$CH_2C_6H_5$	Bn
e	Boc	$(CH_2)_2COOCH_3$	Bn
f	Z	$CH_2C_6H_5$	Me
g	Fmoc	$CH_2C_6H_5$	Me
h	Fmoc	$CH_2C_6H_5$	Bu^t

[a]Reagents and conditions: (a) (i) $ClCO_2Et$, *N*-methylmorpholine, THF, –10 °C, (ii) $NABH_4$, MeOH, 0 °C to rt; (b) (i) $C_3N_3F_3$, pyridine, CH_2Cl_2, –15 °C, (ii) $NaBH_4$, MeOH, rt; (c) (i) NaOCl, AcNH-TEMPO, $NaHCO_3$, NaBr, H_2O/EtOAc/$PhCH_3$, –5 °C, (ii) $Ph_3P{=}CHCOOR_3$, THF, 50 °C.

Scheme 1 Synthesis of *N*-Protected α,β-Unsaturated γ-Amino Acid Esters **3a-h**[a].



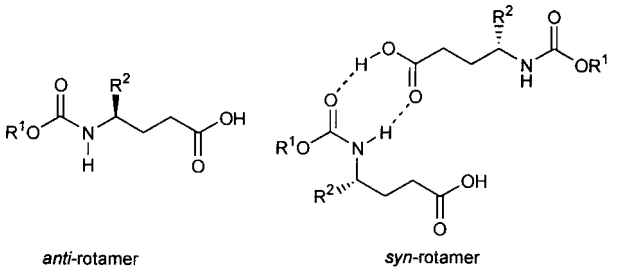
[a]Reagents and conditions: (a) H_2, 10% Pd/C; (b) HCl/THF, rt; (c) TFA/CH_2Cl_2, rt.

Scheme 2 Synthesis of *N*- and *C*-Protected γ-Amino Acids.

acids **4a,b**, while simultaneous removal of the Z group of **3f** produced the γ-amino acid methyl ester **5**. Fmoc protected γ-amino acid **6** was prepared by catalytic hydrogenation of **3h** followed by treatment with TFA. It should be noted that the simultaneous hydrogenation of the double bond and the removal of either benzyl group or benzyloxycarbonyl group led to *N*-protected or *C*-protected γ-amino acids in high yields, suitable for peptide synthesis.

It should be noticed that in the ^{1}H-NMR ($CDCl_3$) spectra of *N*-protected γ-substituted γ-amino acids **4a,b** and **6**, two signals correspond to the carbamate NH proton, attributed to the existence of rotamers. As has been proposed for α-amino acid carbamate derivatives [26], the high field peak corresponds to the *anti*-rotamer and the low field peak corresponds to the *syn*-rotamer (Scheme 3). The *syn*-rotamer is possibly stabilized by formation of intermolecular H-bond complexes with another carboxylic acid moiety.

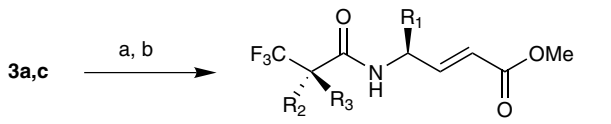
The key step for the synthesis of γ-substituted γ-amino acids is the preparation of *N*-protected amino aldehydes and the subsequent Wittig olefination. Thus, the enantiomeric purity of the final products depends on the conditions used for both the preparation of α-amino aldehydes, which present a high tendency for racemization, and the Wittig reaction. To confirm if any racemization had occurred to the stereogenic centre, compounds **3a** and **3c** were deprotected and coupled almost



Scheme 3 *Anti*- and *syn*- Rotamers of γ-Amino Acids[a].

quantitatively with (S)-(−)- and (*R*)-(+)-α-methoxy-α-trifluoromethyl phenylacetic acid (MTPA) [27] using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) as a condensing agent in the presence of 1-hydroxybenzotriazole (HOBt) (Scheme 4). The ^{1}H-NMR spectra of the pairs of MTP amides were compared. An enantiomeric excess of >95% was indicated by the absence of any diastereomeric proton signal in the spectrum of each MTP amide, since the protons of the double bond and the methoxy protons are well resolved. The most characteristic ^{1}H-NMR chemical shift in the spectra of α,β-unsaturated γ-amino esters (**12a–d**) is that corresponding to the α proton; a chemical shift difference of $\Delta\delta = 0.11–0.20$ ppm was observed. Furthermore, the ^{19}F-NMR spectra of MTP amides showed only one signal.

HPLC analysis of Fmoc-protected γ-amino acid **6**, using a ChiraDex® column, confirmed that the



12a, R_1=$CH_2C_6H_5$, R_2=MeO, R_3=Ph
12b, R_1=$CH_2C_6H_5$, R_2=Ph, R_3=MeO
12c, R_1=$(CH_2)_4$NHZ, R_2=MeO, R_3=Ph
12d, R_1=$(CH_2)_4$NHZ, R_2=Ph, R_3=MeO

[a]Reagents and conditions: (a) 4 N HCl/THF, rt; (b) (*S*)-(–)- or (*R*)-(+)-MTPA, EDC, HOBt, Et_3N, CH_2Cl_2, 0 °C to rt.

Scheme 4 Mosher Amides Prepared for Determination of Enantiopurity[a].

enantiomeric purity was at least 95%. Thus, the production of α-amino aldehydes by NaOCl/AcNH-TEMPO oxidation, in combination with the reaction with stabilized ylides, leads to products of high enantiomeric purity.

CONCLUSION

Efficient protocols for the synthesis of enantiopure γ-substituted γ-amino acids with proteinogenic side chains are proposed in the present study. The proposed method is compatible with *N*-protective groups widely applied in peptide chemistry (*tert*-butoxycarbonyl, benzyloxycarbonyl, 9-fluorenylmethoxycarbonyl) and leads to products of high enantiomeric purity.

EXPERIMENTAL SECTION

Melting points were determined on a melting point apparatus and are uncorrected. Specific rotations were measured at 25 °C on a polarimeter using a 10 cm cell. NMR spectra were recorded on a 200 MHz spectrometer. All amino acid derivatives were of L-configuration and were purchased from Fluka Chemical Co. TLC plates (silica gel 60 F_{254}) and silica gel 60 (70–230 or 230–400 mesh) for column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in ethanol stain. THF, toluene and Et_2O were dried by standard procedures and stored over molecular sieves or Na. All other solvents and chemicals were of reagent grade and used without further purification. The ylides $XOOCCH=PPh_3$ (X = Me, *t*-Bu, Bn) were prepared [28] by treatment of the corresponding triphenylphosphonium bromide with NaOH in water at 0 °C for 15 min and used in the Wittig reactions without purification.

General Procedure for the Preparation of *N*-Protected α,β-Unsaturated γ-Amino Acid Esters (Compounds 3a–h)

To a solution of *N*-protected 2-amino alcohol **2a–h** (5.00 mmol) in a mixture of toluene/EtOAc 11 (30 ml) a solution of NaBr (0.54 g, 5.25 mmol) in water (2.5 ml) was added followed by AcNH-TEMPO (11 mg, 0.05 mmol). To the resulting biphasic system, which was cooled to −5 °C, an aqueous solution of 0.35 M NaOCl (15.7 ml, 5.5 mmol) containing $NaHCO_3$ (1.26 g, 15 mmol) was added dropwise under vigorous stirring, at −5 °C over a period of 1 h. After the mixture had been stirred for a further 15 min at 0 °C, EtOAc (30 ml) and water (10 ml) were added. The aqueous layer was separated and washed with AcOEt (10 ml). The combined organic layers were washed with 5% aqueous citric acid (30 ml) containing KI (0.18 g), 10% aqueous $Na_2S_2O_3$ (30 ml) and brine, and dried (Na_2SO_4). The solvents were evaporated under reduced pressure and the crude aldehyde obtained was immediately used for the next step.

To a solution of the above *N*-protected α-amino aldehyde in dry THF (50 ml) $XOOCCH=PPh_3$ (X = Me, *t*-Bu, Bn) (5.50 mmol) was added and the solution heated at 50 °C for 1–2 h. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl (45 ml) and extracted with Et_2O (3 × 10 ml). The combined organic phases were washed with brine and dried (Na_2SO_4). The solvent was removed and the residue was purified by column chromatography using a mixture of EtOAcpetroleum ether 37 as eluent.

Methyl (2E,4S)-4-((tert-butoxycarbonyl)amino)-5-phenylpent-2-enoate (3a). Yield 82%; white solid; mp 73°–74 °C; $[\alpha]^{25}D + 4.5$ (*c* 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 9H, $C(CH_3)_3$), 2.89 (m, 2H, CH_2), 3.70 (s, 3H, OCH_3), 4.60 (m, 2H, NH, CH), 5.87 (d, 1H, J = 15.4 Hz, CH=C*H*COO), 6.90 (dd, 1H, J = 15.4, 4.8 Hz, C*H*=CHCOO), 7.15–7.35 (m, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.3, 40.7, 51.6, 52.3, 79.8, 120.6, 126.9, 128.5, 128.7, 129.2, 129.5, 136.3, 147.7, 154.9, 166.5. Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.68; H, 7.72; N, 4.51.

Methyl (2E,4S)-4,8-(bis(tert-butoxycarbonyl)amino)okt-2-enoate (3b). Yield 62%; white solid; mp 68°–69 °C; $[\alpha]_D^{25} - 10.0$ (*c* 1.0, $CHCl_3$); ^1H NMR

(200 MHz, $CDCl_3$) δ 1.20–1.58 (m, 24H, $CH_2(CH_2)_3$ CH, $C(CH_3)_3$), 3.05 (m, 2H, $NHCH_2CH_2$), 3.68 (s, 3H, OCH_3), 4.22 (m, 1H, CH), 4.69 (br, 1H, NH), 4.82 (d, 1H, $J = 8.2$ Hz, NH), 5.87 (d, 1H, $J = 15.6$ Hz, CH=C*H*CO), 6.80 (dd, 1H, $J = 15.6$, 5.3 Hz, C*H*=CHCO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.6, 28.3, 29.6, 33.9, 39.8, 51.4, 78.9, 79.5, 120.1, 148.7, 155.2, 156.0, 166.7. Anal. Calcd for $C_{19}H_{34}N_2O_6$: C, 59.05; H, 8.87; N, 7.25. Found: C, 59.38; H, 9.09; N, 7.25.

Methyl (2E,4S)-8-{[(benzyloxy)carbonyl]amino}-4-[(tert-butoxycarbonyl)amino]okt-2-enoate (3c). Yield 61%; white oil; $[\alpha]_D^{25} - 7.2$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25–1.65 (m, 13H, $CH_2(CH_2)_2$, $C(CH_3)_3$), 1.91 (m, 2H, CH_2), 3.18 (m, 2H, $NHCH_2$), 3.71 (s, 3H, OCH_3), 4.21 (m, 1H, CH), 4.75 (br, 1H, NH), 4.95 (br, 1H, NH), 5.08 (s, 2H, $CH_2C_6H_5$), 5.89 (d, 1H, $J = 15.6$ Hz, CH=C*H*CO), 6.82 (dd, 1H, $J = 15.6$, 5.4 Hz, C*H*=CHCO), 7.25–7.40 (m, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.6, 28.3, 29.5, 33.9, 40.4, 51.6, 66.5, 79.7, 120.2, 128.2, 136.4, 148.8, 155.2, 156.5, 166.7. Anal. Calcd for $C_{22}H_{32}N_2O_6$: C, 62.84; H, 7.67; N, 6.66. Found: C, 62.98; H, 7.83; N, 6.52.

Benzyl (2E,4S)-4-[(tert-butoxycarbonyl)amino]-5-phenylpent-2-enoate (3d). Yield 73%; white solid; mp 88°–90 °C; $[\alpha]_D^{25} + 1.8$ (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (s, 9H, $C(CH_3)_3$), 2.88 (d, 2H, $J = 6.4$ Hz, $C_6H_5CH_2$), 4.42–4.71 (m, 2H, NH, CH), 5.17 (s, 2H, OCH_2), 5.91 (d, 1H, $J =$ 15.6 Hz, CH=C*H*CO), 6.96 (dd, 1H, $J = 15.6$, 4.8 Hz, C*H*=CHCO), 7.10–7.38 (m, 10H, $2 \times C_6H_5$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.3, 40.7, 52.2, 66.3, 79.9, 120.7, 127.0, 128.4, 129.5, 135.8, 136.3, 148.1, 154.9, 165.9; FAB MS *m/z* (%) 404 ($M^+ + Na$, 22), 326 (5), 282 (11), 190 (7), 174 (14), 91 (100), 57 (49). Anal. Calcd for $C_{23}H_{27}NO_4$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.38; H, 7.21; N, 3.64.

1-Benzyl 7-methyl (2E,4S)-4-[(tert-butoxycarbonyl)amino]hept-2-enedioate (3e). Yield 48%; oil; $[\alpha]_D^{25} - 8.0$ (*c* 1.3, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.44 (s, 9H, $C(CH_3)_3$), 1.82 (m, 2H, CH_2CH), 2.41 (t, 2H, $J = 7.2$ Hz, CH_2CO), 3.68 (s, 3H, CH_3O), 4.35 (m, 1H, CH), 4.61 (m, 1H, NH), 5.18 (s, 2H, OCH_2), 5.97 (d, 1H, $J = 15.8$ Hz, CH=C*H*CO), 6.88 (dd, 1H, $J = 15.8$, 5.4 Hz, C*H*=CHCO), 7.32–7.43 (m, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.3, 29.3, 30.4, 51.1, 51.8, 66.4, 80.0, 120.9, 126.9, 128.3, 128.6, 135.8, 148.1, 155.0, 165.9, 173.4; FAB MS *m/z* (%) 755 ($2M^+ + 1$, 5), 400 ($M^+ + Na$, 32), 378 ($M^+ + 1$, 34), 322 (100), 278 (95), 246 (8), 217 (15), 170 (54) 138 (25), 91 (86), 57 (97). Anal. Calcd for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.89; H, 7.35; N, 3.53.

Methyl (2E,4S)-4-{[(benzyloxy)carbonyl]amino}-5-phenylpent-2-enoate (3f). Yield 67%; white solid; mp 79°–80 °C; $[\alpha]_D^{25} + 4.1$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.89 (d, 2H, $J = 6.6$ Hz, $CH_2C_6H_5$), 3.71 (s, 3H, OCH_3), 4.68 (m, 1H, CH), 5.01 (d, 1H, $J = 7.8$ Hz, NH), 5.06 (s, 2H, CH_2OCO), 5.88 (d, 1H, $J = 15.8$Hz, CH=C*H*CO), 6.92 (dd, 1H, $J = 15.8$, 5.2 Hz, C*H*=CHCO), 7.10–7.40 (m, 10H, $2 \times C_6H_5$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 40.4, 51.6, 52.7, 66.8, 120.8, 127.0, 128.3, 128.6, 129.1, 129.4, 136.0, 147.4, 155.5, 166.4. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.91; H, 6.31; N, 4.11.

Methyl (2E,4S)-4-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-5-phenylpent-2-enoate (3g). Yield 69%; white solid; mp 113°–115 °C; $[\alpha]_D^{25} - 9.1$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.92 (m, 2H, $CH_2C_6H_5$), 3.74 (s, 3H, OCH_3), 4.17 (t, 1H, $J = 6.6$Hz, $CHCH_2OCO$), 4.41 (m, 2H, $CHCH_2OCO$), 4.70 (m, 1H, CH), 5.01 (d, 1H, $J = 8.0$Hz, NH), 5.88 (d, 1H, $J = 15.4$Hz, CH=C*H*CO), 6.95 (dd, 1H, $J = 15.4$, 4.8 Hz, C*H*=CHCO), 7.10–7.45 (m, 9H, C_6H_5, 3,4,7,8-H Fmoc), 7.48–7.60 (m, 2H, 2,9-H Fmoc), 7.78 (d, 2H, $J = 7.2$Hz, 5,6-H Fmoc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 40.5, 47.2, 51.5, 52.8, 66.6, 119.7, 119.9, 120.8, 124.6, 126.9, 127.4, 127.6, 128.6, 129.0, 129.3, 135.9, 141.1, 143.5, 147.2, 155.3, 166.2. Anal. Calcd for $C_{27}H_{25}NO_4$: C, 75.86; H, 5.89; N, 3.28. Found: C, 75.68; H, 5.75; N, 3.42.

tert-Butyl (2E,4S)-4-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-5-phenylpent-2-enoate (3h). Yield 65%; white solid; mp 138°–140 °C; $[\alpha]_D^{25} - 14.5$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48 (s, 9H, $C(CH_3)_3$), 2.85 (m, 2H, $CH_2C_6H_5$), 4.18 (t, 1H, $J = 6.6$Hz, $CHCH_2OCO$), 4.39 (m, 2H, $CHCH_2OCO$), 4.60–4.78 (m, 2H, NH, CH), 5.80 (d, 1H, $J =$ 15.6Hz, CH=C*H*CO), 6.82 (dd, 1H, $J = 15.6$, 4.6 Hz, C*H*=CHCO), 7.12–7.55 (m, 11H, C_6H_5, 2,3,4,7,8,9-H Fmoc), 7.78 (d, 2H, $J = 7.2$Hz, 5,6-H Fmoc); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.1, 40.7, 47.2, 52.6, 66.7, 80.6, 119.8, 120.1, 123.0, 125.1, 127.2, 127.6, 127.8, 128.8, 129.5, 136.2, 141.3, 143.7, 145.7, 155.4, 165.3; FAB MS *m/z* (%) 492 ($M^+ + Na$, 100), 436 (91), 400 (5), 241 (11), 57 (32). Anal. Calcd for $C_{30}H_{31}NO_4$: C, 76.73; H, 6.65; N, 2.98. Found: C, 76.54; H, 6.63; N, 2.94.

General Procedure for the Preparation of Boc-Protected γ-Amino Acids (Compounds 4a,b)

To a solution of **3d,e** (2.00 mmol) in MeOH (20.0 ml), 10% Pd/C (20 mg) was added. The reaction mixture was stirred overnight under H_2 at rt. The catalyst was removed by filtration through a pad of celite and the organic solvent evaporated under reduced pressure. The product was purified by recrystallization from Et_2O/petroleum ether.

(4R)-4-((tert-Butoxycarbonyl)amino)-5-phenylpentanoic acid (4a). Yield 87%; white crystalline solid; mp 132°–134 °C (lit.[12] 114°–114.5 °C); $[\alpha]_D^{25}$ – 2.5 (*c* 1.0, EtOH) (lit.[12] $[\alpha]_D^{25}$ – 0.7 (*c* 0.87, EtOH)); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 9H, $C(CH_3)_3$), 1.78 (m, 2H, CH_2CH_2CO), 2.35 (m, 2H, CH_2CH_2CO), 2.75 (m, 2H, $C_6H_5CH_2$), 3.73 (m, 1H, CH), 4.44 (d, 3/5H, J = 9.0Hz, NH), 6.07 (d, 2/5H, J = 9.0Hz, NH), 7.16–7.33 (m, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.3, 29.4, 31.0, 41.7, 51.2, 79.5, 126.4, 128.4, 129.3, 137.6, 155.7, 178.3; FAB MS *m/z* (%) 316 (M^+ + Na, 4), 294 (M^+ + 1, 32), 238 (66), 194 (27), 176 (31), 102 (31), 57 (100).

(4R)-7-Methoxy-4-((tert-butoxycarbonyl)amino)-7-oxoheptanoic acid (4b). Yield 66%; white solid; mp 78°–79 °C; $[\alpha]_D^{25}$ – 5.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9H, $C(CH_3)_3$), 1.64–1.90 (m, 4H, CH_2CHCH_2), 2.35–2.42 (m, 4H, 2 × CH_2CO), 3.60 (m, 1H, CH), 3.67 (s, 3H, OCH_3), 4.45 (d, 2/3H, J = 9.4Hz, NH), 5.89 (d, 1/3H, J = 9.4Hz, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.3, 30.7, 50.0, 51.7, 79.5, 155.9, 173.9, 178.1; FAB MS *m/z* (%) 312 (M^+ + Na, 23), 290 (M^+ + 1, 62), 234 (97), 190 (100), 172 (23), 158 (18), 140 (36), 116 (30), 102 (16), 71 (4), 57 (93), 41 (44). Anal. Calcd for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.84. Found: C, 53.78; H, 7.95; N, 4.99.

Methyl (4R)-4-amino-5-phenylpentanoate hydrochloride (5). To a solution of **3f** (339 mg, 1.00 mmol) in MeOH (10.0 ml), 10% Pd/C (30 mg) was added. The reaction mixture was stirred overnight under H_2. The catalyst was removed by filtration through a pad of celite and the organic solvent evaporated under reduced pressure. The residue was treated with 4 N HCl/THF for a few minutes. The excess of acid and the solvent were evaporated under reduced pressure, the residue taken up and concentrated twice from THF and finally treated with dry Et_2O; yield 92%; white solid; mp 97°–99 °C; $[\alpha]_D^{25}$ + 9.1 (*c* 0.87, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.04 (m, 2H, CH_2CH_2CO), 2.57 (m, 2H, CH_2CO), 2.94 (dd, 1H, J = 13.3, 8.9 Hz, $CHHC_6H_5$), 3.29 (dd, 1H, J = 13.3, 5.0 Hz, $CHHC_6H_5$), 3.52–3.64 (m, 4H, CH, OCH_3), 7.20–7.35 (m, 5H, C_6H_5), 8.35 (br, 2H, NH_2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.9, 30.0, 39.2, 51.9, 53.1, 127.4, 129.2, 129.5, 135.5, 173.0; FAB MS *m/z* (%) 208 (M^+ + 1, 100), 159 (12), 117 (50), 91 (10). Anal. Calcd for $C_{12}H_{17}NO_2 \cdot HCl$: C, 59.13; H, 7.44; N, 5.75. Found: C, 59.21; H, 7.46; N, 5.76.

(4R)-4-{((9H-Fluoren-9-ylmethoxy)carbonyl)amino}-5-phenylpentanoic acid (6). To a solution of **3h** (1.13 g, 2.40 mmol) in a mixture of $MeOHCH_2Cl_2$ 21 (45 ml), 10% Pd/C (120 mg) was added. The reaction mixture was stirred overnight under H_2. The catalyst was removed by filtration through a pad of celite and the organic solvents evaporated under reduced pressure. The product was purified by column chromatography using a mixture of EtOAcpetroleum ether (28) as eluent.

To a solution of the above Fmoc-protected γ-amino *tert*-butylester (472 mg, 1 mmol) in CH_2Cl_2 (3 ml) TFA (3 ml) was added and the mixture stirred at rt for 20 min. CH_2Cl_2 was added (10 ml), the excess of acid and the solvent were evaporated under reduced pressure, and the residue taken up and concentrated twice from CH_2Cl_2. The residue was finally treated with petroleum ether and filtered; yield 58%; white solid; mp 149°–151 °C; $[\alpha]_D^{25}$ – 20.0 (*c* 1.0, MeOH); ^{1}H NMR (200 MHz, d_6-DMSO) δ 1.62 (m, 2H, $CHCH_2CH_2$), 2.22 (m, 2H, CH_2COOH), 2.68 (d, 2H, J = 6.8 Hz, $CH_2C_6H_5$), 3.59 (m, 1H, CH), 4.08–4.28 (m, 3H, $CHCH_2OCO$), 6.74 (m, 1/3H, NH), 6.96 (m, 2/3H, NH), 7.15–7.45 (m, 9H, C_6H_5, 3,4,7,8-H Fmoc), 7.64 (d, 2H, J = 7.2Hz, 2,9-H Fmoc), 7.89 (d, 2H, J = 7.2Hz, 5,6-H Fmoc); ^{13}C NMR (50 MHz, CD_3OD) δ 31.0, 31.7, 42.5, 53.6, 67.4, 120.9, 126.2, 127.3, 128.1, 128.7, 129.3, 130.4, 139.9, 142.6, 145.2, 145.4, 158.5, 177.2; FAB MS *m/z* (%) 438 (M^+ + Na, 100). Anal. Calcd for $C_{26}H_{25}NO_4 \cdot 1/4H_2O$: C, 74.35; H, 6.12; N, 3.34. Found: C, 74.48; H, 6.09; N, 3.32.

General Procedure for the Preparation of Mosher Amides (Compounds 12a–d)

Compounds **3a** and **3c** (0.20 mmol) were treated with 4 N HCl in MeOH (2.5 ml) for 30 min at rt. The excess of acid and the solvent were evaporated under reduced pressure, the residue taken up and concentrated twice from MeOH and finally treated with dry Et_2O and filtered. The product was dissolved in CH_2Cl_2 (2.0 ml) under stirring at 0 °C,

and Et_3N (0.061 ml, 0.44 mmol), (*S*)-(−)- or (*R*)-(+)-MTPA (56 mg, 0.24 mmol), EDC (46 mg, 0.24 mmol) and HOBt (25 mg, 0.24 mmol) were subsequently added. The reaction mixture was stirred at 0 °C for 1 h and overnight at rt. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc (5 ml), washed with 10% citric acid solution, brine, 5% $NaHCO_3$ and brine, and dried ($MgSO_4$). The solvent was evaporated and the residue was dissolved in $CDCl_3$ and subjected to NMR analysis.

Characteristic ^{1}H NMR Chemical Shifts (in ppm)

(S)-Mosher amide of methyl (2E,4S)-4-amino-5-phenylpent-2-enoate (12a). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.24 (q, 3H, $J = 1.6$ Hz), 5.95 (dd, 1H, $J = 1.8$, 15.9 Hz), 6.95 (d, 1H, $J = 8.7$ Hz), 6.98 (dd, 1H, $J = 5.1$, 15.9 Hz); ^{19}F NMR (188 MHz, $CDCl_3$, reference with external TFA) δ 8.88 (s).

(R)-Mosher amide of methyl (2E,4S)-4-amino-5-phenylpent-2-enoate (12b). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.19 (q, 3H, $J = 1.6$ Hz), 5.75 (dd, 1H, $J = 1.8$, 15.9 Hz), 6.72 (d, 1H, $J = 8.7$ Hz), 6.88 (dd, 1H, $J = 5.5$, 15.9 Hz); ^{19}F NMR (188 MHz, $CDCl_3$, reference with external TFA) δ 9.06 (s).

(S)-Mosher amide of methyl (2E,4S)-4-amino-8-{[(benzyloxy)carbonyl]amino}oct-2-enoate (12c). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.41 (q, 3H, $J = 1.6$ Hz), 5.93 (dd, 1H, $J = 1.5$, 15.9 Hz); ^{19}F NMR (188 MHz, $CDCl_3$, reference with external TFA) δ 9.19 (s).

(R)-Mosher amide of methyl (2E,4S)-4-amino-8-{[(benzyloxy)carbonyl]amino}oct-2-enoate (12d). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.38 (q, 3H, $J = 1.6$ Hz), 5.82 (dd, 1H, $J = 1.5$, 15.9 Hz); ^{19}F NMR (188 MHz, $CDCl_3$, reference with external TFA) δ 9.04 (s).

Acknowledgement

V.L. thanks the Greek Government and the European Commission for a fellowship (E.P.E.A.E.K.). This work was supported in part by the University of Athens (Special Account for Research Grants).

REFERENCES

1. Williams RH. *Synthesis of Optically Active α-Amino Acids*. Pergamon Press: New York, 1989.
2. Giannis A, Kolter T. Peptide mimetics for receptor ligands: discovery, development, and medicinal perspectives. *Angew. Chem., Int. Ed. Engl.* 1993; **32**: 1244–1267.
3. Duthaler RO. Recent developments in the stereoselective synthesis of α-amino acids. *Tetrahedron* 1994; **50**: 1539–1650.
4. Sardina FJ, Rapoport H. Enantiospecific synthesis of heterocycles from α-amino acids. *Chem. Rev.* 1996; **96**: 1825–1872.
5. Kokotos G, Martin V, Constantinou-Kokotou V, Gibbons WA. Synthesis of medicinally useful lipidic α-amino acids, 2-amino alcohols and diamines. *Amino Acids* 1996; **11**: 329–343.
6. Hanessian S, McNughton-Smith G, Lombart HG, Lubell WD. Design and synthesis of conformationally constrained amino acids as versatile scaffolds and peptide mimetics. *Tetrahedron* 1997; **53**: 12 789–12 854.
7. Goody RS, Alexandrov K, Engelhard M. Combining chemical and biological techniques to produce modified proteins. *ChemBioChem* 2002; **3**: 399–403.
8. Etezady-Esfarjani T, Hilty C, Wuthrich K, Rueping M, Schreiber J, Seebach D. NMR-structural investigations of a β^3-dodecapeptide with proteinogenic side chains in methanol and in aqueous solutions. *Helv. Chim. Acta* 2002; **85**: 1197–1209.
9. Rueping M, Schreiber JV, Lelais G, Jaun B, Seebach D. Mixed β^2/β^3-hexapeptides and β^2/β^3-nonapeptides folding to (*P*)-helices with alternative twelve- and ten-membered hydrogen-bonded rings. *Helv. Chim. Acta* 2002; **85**: 2577–2593.
10. Cheng RP, Gellman SH, DeGrado WF. β-Peptides: From structure to function. *Chem. Rev.* 2001; **101**: 3219–3232.
11. Porter EA, Wang XF, Schmitt MA, Gellman SH. Synthesis and 12-helical secondary structure of β-peptides containing (2*R*, 3*R*)-aminoproline. *Org. Lett.* 2002; **4**: 3317–3319.
12. Hanessian S, Yang H. Design of a novel secondary structure scaffolding device: Induction of a reverse turn in tetrapeptides by incorporating a β-amino acid and stereocontrolled free radical α-substitution reactions in peptide motifs. *Tetrahedron Lett.* 1997; **38**: 3155–3158.
13. Hanessian S, Luo X, Schaum R, Michnick S. Design of secondary structures in unnatural peptides: Stable helical γ-tetra-, hexa-, and octapeptides and consequences of α-substitution. *J. Am. Chem. Soc.* 1998; **120**: 8569–8570.
14. Hintermann T, Gademann K, Jaun B, Seebach D. γ-Peptides forming more stable secondary structures than α-peptides: Synthesis and helical NMR-solution structure of the γ-hexapeptide analog of H-(Val-Ala-Leu)$_2$-OH. *Helv. Chim. Acta* 1998; **81**: 983–1002.
15. Frackenpohl J, Arvidsson PI, Schreiber JV, Seebach D. The outstanding biological stability of β- and γ-peptides toward proteolytic enzymes: An *in vitro*

investigation with fifteen peptidases. *ChemBioChem* 2001; **2**: 445–455.
16. El Marini A, Roumenstat ML, Viallefont P, Razafindramboa D, Bonato M, Follet M. Synthesis of enantiomerically pure β- and γ-amino acids from aspartic and glutamic acid derivatives. *Synthesis* 1992; 1104–1108.
17. Dexter CS, Jackson RFW, Elliott J. Synthesis of enantiomerically pure β- and γ-amino acid derivatives using funtionalized organozinc reagents. *J. Org. Chem.* 1999; **64**: 7579–7585.
18. Hanessian S, Schaum R. 1,3-Asymmetric induction in enolate alkylation reactions of *N*-protected γ-amino acid derivatives. *Tetrahedron Lett.* 1997; **38**: 163–166.
19. Smrcina M, Majer P, Majerová E, Guerassina TA, Eissenstat MA. Facile stereoselective synthesis of γ-substituted γ-amino acids from the corresponding α-amino acids. *Tetrahedron* 1997; **53**: 12 867–12 874.
20. Jurczak J, Golebiowski A. Optically active *N*-protected α-amino aldehydes in organic synthesis. *Chem. Rev.* 1989; **89**: 149–164.
21. Jurczak J, Kobrzycka E, Gruza H, Prokopowicz P. Effective and mild method for preparation of optically active α-amino aldehydes via TEMPO oxidation. *Tetrahedron* 1998; **54**: 6051–6064.
22. Kokotos G. A convenient one-pot conversion of *N*-protected amino acids and peptides into alcohols. *Synthesis* 1990; 299–301.
23. Kokotos G, Noula C. Selective one-pot conversion of carboxylic acids into alcohols. *J. Org. Chem.* 1996; **61**: 6994–6996.
24. Leanna MR, Sowin TJ, Morton HE. Synthesis of α-amino and α-alkoxy aldehydes via oxoammonium oxidation. *Tetrahedron Lett.* 1992; **33**: 5029–5032.
25. Ma Z, Bobbitt JM. Organic oxoammonium salts. A new convenient method for the oxidation of alcohols to aldehydes and ketones. *J. Org. Chem.* 1991; **56**: 6110–6114.
26. Marcovici-Mizrahi D, Gottlieb HE, Marks V, Nudelman A. On the stabilization of the *syn*-rotamer of amino acid carbamate derivatives by hydrogen bonding. *J. Org. Chem.* 1996; **61**: 8402–8406.
27. Dale JA, Dull DL, Mosher H. α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. *J. Org. Chem.* 1969; **34**: 2543–2549.
28. Hands AR, Mercer AJ. Reaction of sodium hydride with ω-hydroxyalkyl-phosphonium, -arsonium and -ammonium salts. *J. Chem. Soc.* 1968; 1331–1337.

