

Alternative and Chemoselective Deprotection of the α -Amino and Carboxy Functions of *N*-Fmoc-Amino Acid and *N*-Fmoc-Dipeptide Methyl Esters by Modulation of the Molar Ratio in the AlCl_3 /*N,N*-Dimethylaniline Reagent System

Maria Luisa Di Gioia,^[a] Antonella Leggio,^[a] Adolfo Le Pera,^[a] Angelo Liguori,^{*,[a]} Francesca Perri,^[a] and Carlo Siciliano^[a]

Keywords: Aluminium trichloride / Amino acids / Deprotection / Dipeptides / *N,N*-Dimethylaniline

The amino and carboxy functions in *N*-Fmoc- α -amino acid and *N*-Fmoc-peptide methyl esters can be alternatively and chemoselectively deprotected by treatment with the reagent system AlCl_3 /*N,N*-dimethylaniline (DMA). The chemoselec-

tivity of the process is controlled by modulating the relative molar ratio of the Lewis acid and DMA.

(© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2004)

Introduction

The use of orthogonal protecting groups to mask the α -amino, side chain and terminal carboxy functions is of great importance for successful peptide synthesis, both in solution and under solid phase conditions.

The protection of the terminal carboxy moiety is a very important step in solution peptide synthesis. From previous experience, in many applications it would be extremely convenient to mask the carboxy group as a methyl ester^[1,2] when the 9-fluorenylmethyloxycarbonyl group is used to protect the terminal α -amino function.

The availability of simple and straightforward procedures that would allow the alternative unblocking of the carboxy or α -amino functions with high chemoselectivity represents a challenging field in the synthesis of peptides, especially when peptide chains are synthesized by a building-block approach.^[3,4] Alternative deprotection of amino and carboxy functions could also be useful in multi-step total synthesis.

Results and Discussion

The selective cleavage of an ester function without removing the Fmoc protecting group on the amino moiety^[5–21] is an important strategic element in peptide synthesis. In a previous paper,^[22] we reported a facile procedure for the chemoselective unblocking of the carboxy group in *N*-Fmoc-protected α -amino acid and peptide

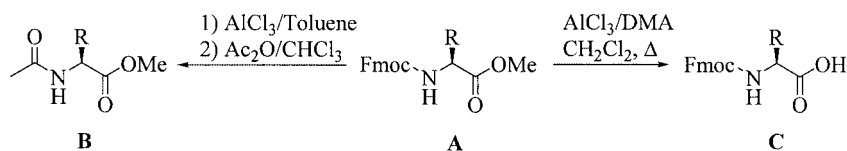
methyl esters using the reagent system AlCl_3 /*N,N*-dimethylaniline (DMA). We also reported^[23,24] that the reagent system AlCl_3 /toluene easily removes the *N*-9-fluorenylmethyloxycarbonyl protecting group from the amino function of α -amino acid and short peptide methyl esters without demolition of the methyl ester moiety and with retention of the configuration of the carbon atoms of the peptide systems.

The analogy between the two reagent systems containing the same Lewis acid species, AlCl_3 , which remove either the ester function (AlCl_3 /DMA) or the urethane group (AlCl_3 /toluene), cannot provide a clear explanation for the diverse behaviour observed for the two reagent systems considered (Scheme 1).

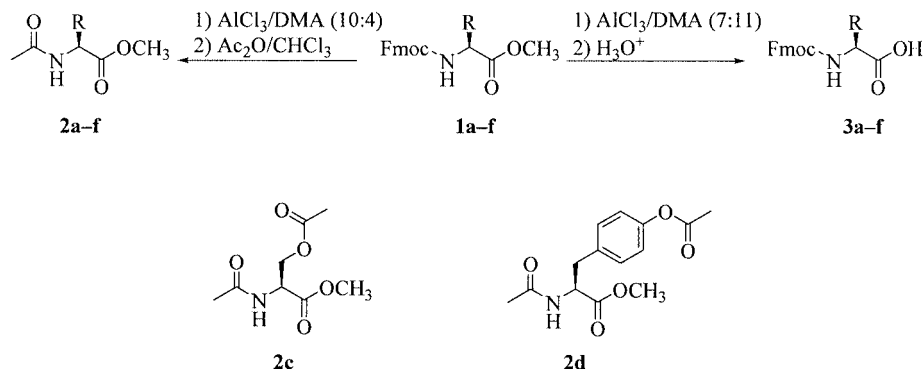
We have carried out some experiments in order to establish the optimal molar ratio of the constituents in the reagent system AlCl_3 /DMA for removal of the Fmoc group from the α -nitrogen atom. Fast chemoselective removal of the Fmoc protecting group was obtained with a 10:4 molar ratio of AlCl_3 and DMA (Scheme 2, Table 1).

The compatibility of the reagent system AlCl_3 /DMA with aromatic moieties on the side chains of *N*-Fmoc-amino acid methyl esters, was checked by treating *N*-Fmoc-phenylalanine methyl ester (**1b**) and *N*-Fmoc-tyrosine methyl ester (**1d**) under the conditions typically used for the chemoselective removal of the Fmoc group (molar ratio AlCl_3 /DMA, 10:4). The *N*-deprotected methyl esters obtained were converted, by treatment with acetic anhydride, into the corresponding *N*-acetyl derivatives **2b** and **2d** (Scheme 2) in 77% and 94% yields, respectively. Successively, the same substrates **1b** and **1d** were treated with AlCl_3 /DMA in a 7:11 molar ratio to unblock the carboxy function. Under these conditions, the reagent system AlCl_3 /DMA operates by removing the methyl group from the ester function of **1b** and

^[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi della Calabria, Via P. Bucci Cubo 15/C, 87036 Arcavacata di Rende (CS), Italy
Fax: (internat.) + 39-0984-4928-55
E-mail: A.Liguori@unical.it



Scheme 1



Scheme 2

Table 1. Yields of compounds **2a–f** and **3a–f**

	R	Yield (%)	
		2	3
a	CH(CH ₃)CH ₂ CH ₃	83	84
b	CH ₂ Ph	77	84
c	CH ₂ OH	93	83
d	CH ₂ C ₆ H ₄ OH	94	78
e	CH ₂ CH ₂ SCH ₃	98	98
f	CH ₂ SCH ₂ NHCOCH ₃	67	83

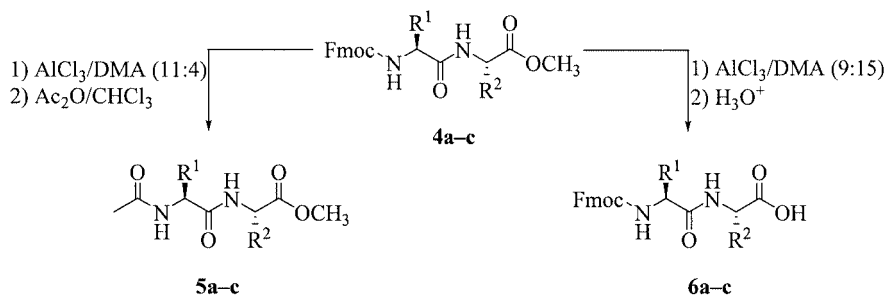
1d without demolition of the urethane moiety present on the α -nitrogen atom. In this case, the treatment afforded *N*-Fmoc-phenylalanine (**3b**) and *N*-Fmoc-tyrosine (**3d**) in 84% and 78% yields, respectively. In all the reactions performed with the substrates **1b** and **1d**, either the removal of the Fmoc protecting group or, alternatively, the demethylation of the ester function, were chemoselective as determined by chromatographic analysis of the respective reaction mixtures. Furthermore, the experimental data confirmed that the reagent system AlCl₃/DMA does not promote the formation of side products arising from possible electrophilic attacks of the methyl and 9-fluorenylmethyl cations on the aromatic rings of the side chains present in the starting protected substrates.

In order to establish the capabilities of the reagent system AlCl₃/DMA, other *N*-Fmoc- α -amino acid methyl esters, characterized by a nucleophilic site on the side chains, were subjected to both the reaction conditions described above. This new set of experiments allowed the evaluation of the possible competition between the reactions of the nucleophilic sites and DMA with the 9-fluorenylmethyl or, alternatively, the methyl cations generated during the removal of the Fmoc protecting group and the demethylation of the carboxymethyl function, respectively.

Substrates **1c** and **1e** were treated under the appropriate experimental conditions, which regulate each of the two different reactive pathways, to allow the specific unblocking of the amino and the carboxy functions. The results obtained are summarized in Table 1. Upon treatment with the reagent system AlCl₃/DMA in the 7:11 molar ratio, the two substrates generated the corresponding *N*-Fmoc-amino acids **3c** and **3e** in 83% and 98% yields, respectively. When a 10:4 molar ratio of the Lewis acid and DMA was applied instead, the corresponding methyl esters were recovered and isolated as the respective *N*-acetyl derivatives **2c** and **2e** in 93% and 98% yields, respectively, after treatment with Ac₂O. Mass spectrometric and ¹H NMR analysis of the crude materials resulting from these reactions revealed that alkylation of the nucleophilic functionalities on the side chains of the starting substrates does not occur under the experimental conditions used. The *N*-Fmoc-*S*-(acetamidomethyl)cysteine methyl ester (**1f**) was also subjected to both experimental conditions. Compounds **2f** and **3f**, obtained by removing the urethanic or the methyl ester function, respectively, preserve the acetamidomethyl protecting group on the side chain.

The chemoselectivity of the removal of the Fmoc protecting group or, alternatively, the demethylation of the ester function was also studied in simple peptide systems. Dipeptides **4a–c** (Scheme 3) were treated with the reagent system AlCl₃/DMA in the molar ratio 11:4. This new composition of the reagent system used for deprotection was justified by the higher number of functionalities present in the dipeptides that are capable of coordinating to the electrophilic reagent.

The intermediate dipeptides, which bear a free terminal amino function, were quenched by acetylation with Ac₂O. The *N*-acetylated dipeptidyl derivatives **5a–c** were recovered in yields ranging from 71% to 74% (Table 2). The

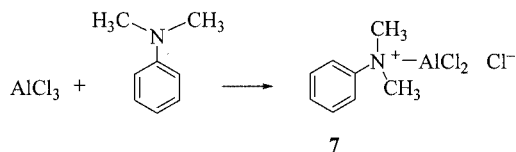


Scheme 3

Table 2. Yields of compounds **5a–c** and **6a–c**

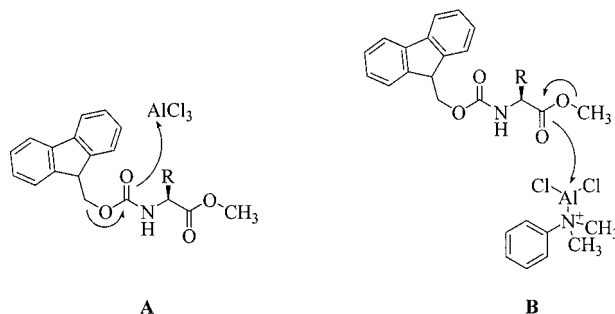
	R ¹	R ²	Yield (%)	
			5	6
a	CH ₂ CH(CH ₃) ₂	CH ₂ Ph	71	78
b	CH(CH ₃) ₂	CH ₂ CH(CH ₃) ₂	73	85
c	CH ₂ Ph	CH(CH ₃) ₂	74	75

same substrates **4a–c**, upon treatment with the reagent system AlCl₃/DMA in a molar ratio of 9:15, gave the *N*-Fmoc protected dipeptides **6a–c** (Scheme 3) in 75–85% yields (Table 2). Two different reaction pathways generate the peptide systems; the carboxymethyl moiety is maintained whilst the urethane protecting group is removed or, alternatively, the protection on the α -nitrogen atom is retained and the methyl from the ester function is lost. This particular behaviour could be of great importance when the availability of differently protected dipeptide precursors is required for the preparation of more complex peptides through a building-block synthetic strategy. The different compositions of the reagent system AlCl₃/DMA can satisfactorily explain both the reaction pathways observed for the alternative and chemoselective removal of the protecting groups in *N*-Fmoc- α -amino acid and *N*-Fmoc-peptide methyl esters. The species responsible for the unblocking are different in the two cases studied. The 1:1 AlCl₃/DMA adduct **7** (Scheme 4) plays a key role in the deprotection of the substrates analysed in this study.



Scheme 4

The formation of the equimolecular adduct of the Lewis acid and DMA could explain the dissimilarities observed when different molar ratios of AlCl₃ and DMA are used to compose the reagent system. Both the adduct **7** and AlCl₃ are the real species present in the reaction mixtures when the reagent system has an excess of AlCl₃. The free Lewis acid coordinates to the most basic site, the carbonyl oxygen



Scheme 5

atom of the urethane protecting group (**A**, Scheme 5). This moiety, upon reaction with AlCl₃, generates the transient 9-fluorenylmethyl cation, which is quenched by the adaptable adduct **7**, inducing the demolition of the masking group placed on the α -nitrogen atom. The adduct between DMA and the 9-fluorenylmethyl cation was identified by GC-MS analysis of the crude material recovered after work up of the reaction mixtures.

When the reagent system is prepared with an excess of DMA, the reactive Lewis acid is **7** (**B**, Scheme 5), which is more sterically hindered and cannot coordinate to the urethane carbonyl group and thus prefers to attack the ester function. The consequent demethylation proceeds via formation of the methyl cation, which in turn is quenched by DMA present in good excess with respect to the Lewis acid. In this case the adduct produced from the reaction between DMA and the methyl cation was also identified by GC-MS analysis. Alternatively it can be supposed that the DMA demethylates, by a S_N2 mechanism, the ester function activated by the AlCl₃/DMA adduct because of the absence in the crude reaction materials of methylated products in the side chains.

Conclusion

The chemoselective removal of the Fmoc protecting group from the α -amino function or, alternatively, demethylation of the ester moiety in *N*-Fmoc- α -amino acid and peptide methyl esters can be performed by modulating the molar ratio of the two components of the reagent system.

Changes in the molar ratio of AlCl_3 and DMA correspond to changes in the reactive species responsible for the removal of the target protecting group.

Experimental Section

General Remarks: All reagents were purchased from Aldrich Co. and Senn Chemical. All solvents were purified and dried by standard procedures and distilled prior to use. Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. NMR characterization of all the compounds was performed with a Bruker Avance 300 spectrometer. ^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75.5 MHz, respectively, with CDCl_3 or $[\text{D}_6]\text{DMSO}$ as solvent. GC-MS analyses were carried out using a 30-m HP-35MS capillary column with a 0.25 mm internal diameter and a 0.25 μm film thickness. The mass detector was operated in the electron impact ionization mode (EI-MS) at an electron energy of 70 eV. Mass spectra were recorded with a Vacuum Generator ZAB-2F spectrometer, with 3-nitrobenzyl alcohol as the matrix, by fast atom bombardment (FAB^+ MS) with a neutral Xenon beam operating at 8 keV and a total current of 10 μA . Reaction mixtures were monitored by TLC with silica gel 60-F254 precoated glass plates purchased from Merck. Short column flash chromatography (SCFC) was performed on Kieselgel 60 H without gypsum. All reactions were carried out under an inert atmosphere (N_2).

Removal of the Fmoc Group of *N*-Fmoc- α -Amino Acid Methyl Esters 1a–f. **General Procedure:** AlCl_3 (10 mmol) and *N,N*-dimethylaniline (4 mmol) were added to a suspension of the appropriate *N*-Fmoc- α -amino acid methyl ester 1a–f (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was stirred under reflux for 4 h until TLC analysis of the reaction mixture (chloroform/methanol, 90:10, v/v) showed complete conversion of the precursor. Methanol was then added dropwise until effervescence stopped, and the solvents were evaporated to dryness. The chromatographic purification of the crude product (chloroform/methanol, 95:5, v/v) afforded the corresponding α -amino acid methyl ester, which was successively dissolved in an aqueous 5% Na_2CO_3 solution (5 mL) and treated with acetic anhydride (3 mL) in dichloromethane (10 mL). After 2 h at room temp., the organic layer was separated and the aqueous layer was extracted with dichloromethane (3×3 mL). The combined organic extracts were washed once with brine, dried with Na_2SO_4 and then evaporated to dryness to give the *N*-acetyl- α -amino esters 2a–f in 67–98% overall yields. The compound characterization data for 2a–f fitted those observed for authentic samples of *N*-acetyl- α -protected amino esters.

Removal of the Methyl Ester Group of *N*-Fmoc- α -Amino Acid Methyl Esters 1a–f:^[22] A suspension of the appropriate *N*-Fmoc- α -amino acid methyl ester 1a–f (1 mmol) in dry dichloromethane was treated with the reagent system AlCl_3 (7 mmol)/*N,N*-dimethylaniline (11 mmol). Chromatographic purification of the crude reaction products enabled a simple removal of the excesses of the reagents used to afford the respective *N*-Fmoc-amino acids 3a–f in high yields. The compound characterization data for 3a–f fitted those observed for authentic samples of *N*-Fmoc-protected amino acids.

Synthesis of Dipeptides 4a–c: A solution of the appropriate *N*-Fmoc- α -amino acid chloride (0.9 mmol) in ethanol-free chloroform (10 mL) was added dropwise to a magnetically stirred solution of the commercially available α -amino ester hydrochlorides (1 mmol) dissolved in an aqueous 5% Na_2CO_3 solution (5 mL). The resulting

mixture was stirred at room temp. for 2 h. The organic layer was separated and the aqueous layer was extracted with chloroform (2×5 mL). The combined organic extracts were washed with aqueous 1 M HCl (2×3 mL), washed once with brine, dried with Na_2SO_4 and then evaporated to dryness to give the dipeptides 4a–c in 86–92% overall yields.

The compound characterization data for 4b fitted those observed for an authentic sample of *N*-Fmoc-valyl-leucine methyl ester.^[22]

Compound 4a: White solid (1.45 g, 86% yield), m.p. 137–140 $^\circ\text{C}$. IR (KBr): $\tilde{\nu} = 3305, 3090, 2965, 1742, 1692, 1641, 1532, 1241, 760, 741$ cm^{-1} . ^1H NMR (CDCl_3): $\delta = 0.88\text{--}0.98$ [m, 6 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 1.45–1.70 [m, 3 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 2.98 (dd, $J_1 = 5.8, J_2 = 13.9$ Hz, 1 H, CH_2Ph), 3.13 (dd, $J_1 = 5.9, J_2 = 13.9$ Hz, 1 H, CH_2Ph), 3.72 (s, 3 H, OCH_3), 4.20 (m, 1 H, Fmoc-CH), 4.30–4.51 (m, 3 H, CHNH), 4.85 (m, 1 H, CHCH_2Ph), 5.21 (d, $J = 8.4$ Hz, 1 H, OCONH), 6.51 (d, $J = 7.4$ Hz, 1 H, CHCONH), 7.01–7.28 (m, 5 H, Ar-H), 7.31–7.83 (m, 8 H, Ar-H) ppm. ^{13}C NMR: $\delta = 15.3, 22.0, 22.9, 24.6, 37.8, 41.4, 47.1, 53.2, 53.4, 67.1, 120.0, 125.0, 125.1, 127.1, 127.2, 127.7, 128.6, 129.3, 136.6, 141.3, 143.8, 171.7, 171.8$ ppm. FAB^+ MS: m/z (%) = 515 (12) $[\text{M} + \text{H}]^+$, 179 (100), 178 (78), 165 (39). $\text{C}_{31}\text{H}_{34}\text{N}_2\text{O}_5$ (514.61): calcd. C 72.35, H 6.66, N 5.44, O 15.55; found C 72.45, H 6.63, N 5.40.

Compound 4c: White solid (1.62 g, 91% yield), m.p. 167–169 $^\circ\text{C}$. IR (KBr): $\tilde{\nu} = 3300, 3078, 2964, 1744, 1701, 1654, 1542, 1261, 758, 739$ cm^{-1} . ^1H NMR (CDCl_3): $\delta = 0.81$ [d, $J = 6.8$ Hz, 3 H, $\text{CH}(\text{CH}_3)_2$], 0.87 [d, $J = 6.9$ Hz, 3 H, $\text{CH}(\text{CH}_3)_2$], 2.10 [m, 1 H, $\text{CH}(\text{CH}_3)_2$], 3.16–3.25 (m, 2 H, CH_2Ph), 3.71 (s, 3 H, OCH_3), 4.20 (m, 1 H, CHCH_2Ph), 4.30–4.55 (m, 4 H, Fmoc-CH_2 , $\text{CHCH}(\text{CH}_3)_2$, Fmoc-CH), 5.53 (d, $J = 7.1$ Hz, 1 H, OCONH), 6.41 (d, $J = 7.5$ Hz, 1 H, CHCONH), 7.15–7.88 (m, 13 H, Ar-H) ppm. ^{13}C NMR: $\delta = 17.6, 30.5, 37.5, 47.1, 51.9, 55.1, 55.7, 67.4, 126.0, 126.8, 127.8, 128.2, 128.4, 128.8, 129.0, 139.5, 141.0, 143.6, 156.0, 171.6, 171.8$ ppm. FAB^+ MS: m/z (%) = 501 (100) $[\text{M} + \text{H}]^+$, 279 (61), 179 (62), 178 (38), 165 (25). $\text{C}_{30}\text{H}_{32}\text{N}_2\text{O}_5$ (500.59): calcd. C 71.98, H 6.44, N 5.60, O 15.98; found C 72.09, H 6.40, N 5.57.

Removal of the Fmoc Group of Dipeptides 4a–c. General Procedure: AlCl_3 (11 mmol) and *N,N*-dimethylaniline (4 mmol) were added to a suspension of the appropriate dipeptide 4a–c (1 mmol) in dry dichloromethane (10 mL). The resulting mixture was stirred under reflux for 4 h, until TLC analysis (chloroform/methanol, 90:10, v/v) showed complete conversion of the precursor. Methanol was then added dropwise until effervescence stopped and the solvents were evaporated to dryness. The chromatographic purification of the crude product (chloroform/methanol, 95:5, v/v) afforded the corresponding dipeptides which were successively dissolved in an aqueous 5% solution of Na_2CO_3 (5 mL) and treated with acetic anhydride (3 mL) in chloroform (10 mL). After 2 h at room temp., the organic layer was separated and the aqueous layer was extracted with dichloromethane (3×3 mL). The combined organic extracts were washed once with brine, dried with Na_2SO_4 and then evaporated to dryness to give the dipeptides 5a–c in 71–74% overall yields.

Compound 5a: Oil (0.37 g, 71% yield). IR (neat): $\tilde{\nu} = 3275, 3048, 2968, 1749, 1657, 1642, 1251$ cm^{-1} . ^1H NMR (CDCl_3): $\delta = 0.90\text{--}0.95$ [m, 6 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 1.53–1.70 [m, 3 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 1.99 (s, 3 H, CH_3CONH), 3.09 (dd, $J_1 = 6.5, J_2 = 13.7$ Hz, 1 H, CH_2Ph), 3.18 (dd, $J_1 = 5.5, J_2 = 13.7$ Hz, 1 H, CH_2Ph), 3.74 (s, 3 H, OCH_3), 4.45 [m, 1 H, $\text{CHCH}_2\text{CH}(\text{CH}_3)_2$], 4.87 (m, 1 H, CHCH_2Ph), 5.82 (d, $J = 8.2$ Hz, 1 H, OCONH), 6.51 (d, $J = 7.5$ Hz, 1 H, CHCONH), 7.09–7.30

(m, 5 H, Ar-*H*) ppm. ^{13}C NMR: δ = 22.1, 22.6, 22.9, 37.0, 41.2, 51.1, 51.9, 53.2, 126.0, 127.8, 128.7, 139.5, 170.7, 171.6, 171.8 ppm. EI-MS: m/z (%) = 334 (2) [M^+], 162 (40), 128 (74), 86 (100), 44 (17), 43 (14). $\text{C}_{18}\text{H}_{26}\text{N}_2\text{O}_4$ (334.41): calcd. C 64.65, H 7.84, N 8.38, O 19.14; found C 64.75, H 7.81, N 8.34.

Compound 5b: Yellow solid (0.40 g, 73% yield), m.p. 112–114 °C. IR (KBr): $\tilde{\nu}$ = 3278, 2963, 1744, 1672, 1643, 1264 cm^{-1} . ^1H NMR (CDCl_3): δ = 0.91 [d, J = 2.8 Hz, 3 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 0.93 [d, J = 2.9 Hz, 3 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 0.96 [d, J = 6.6 Hz, 3 H, $\text{CH}(\text{CH}_3)_2$], 0.99 [d, J = 6.5 Hz, 3 H, $\text{CH}(\text{CH}_3)_2$], 1.50–1.70 [m, 3 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 2.01 (s, 3 H, CH_3CONH), 2.07 [m, 1 H, $\text{CH}(\text{CH}_3)_2$], 3.73 (s, 3 H, OCH_3), 4.40 [dd, J_1 = 7.2, J_2 = 8.9 Hz, 1 H, $\text{CHCH}(\text{CH}_3)_2$], 4.55 [m, 1 H, $\text{CHCH}_2\text{CH}(\text{CH}_3)_2$], 6.55 (d, J = 8.9 Hz, 1 H, OCONH), 6.85 (d, J = 7.7 Hz, 1 H, CHCONH) ppm. ^{13}C NMR: δ = 18.3, 18.9, 21.8, 22.7, 23.2, 24.7, 31.5, 40.9, 50.9, 52.2, 58.2, 170.1, 171.5, 173.1 ppm. EI-MS: m/z (%) = 286 (1) [M^+], 230 (5), 144 (85), 100 (14), 86 (38), 72 (100), 55 (11), 43 (17). $\text{C}_{14}\text{H}_{26}\text{N}_2\text{O}_4$ (286.37): calcd. C 58.72, H 9.15, N 9.78, O 22.35; found C 58.82, H 9.11, N 9.74.

Compound 5c: Oil (0.53 g, 74% yield). IR (neat): $\tilde{\nu}$ = 3287, 3055, 2959, 1746, 1658, 1635, 1263 cm^{-1} . ^1H NMR (CDCl_3): δ = 0.82 [d, J = 6.8 Hz, 3 H, $\text{CH}(\text{CH}_3)_2$], 0.90 [d, J = 6.8 Hz, 3 H, $\text{CH}(\text{CH}_3)_2$], 2.01 (s, 3 H, CH_3CONH), 2.08 [m, 1 H, $\text{CH}(\text{CH}_3)_2$], 3.00–3.10 (m, 2 H, CH_2Ph), 3.70 (s, 3 H, OCH_3), 4.45 [dd, J_1 = 5.0, J_2 = 8.1 Hz, 1 H, $\text{CHCH}(\text{CH}_3)_2$], 4.70 (m, 1 H, CHCH_2Ph), 6.23 (d, J = 7.5 Hz, 1 H, OCONH), 6.32 (d, J = 8.1 Hz, 1 H, CHCONH), 7.20–7.38 (m, 5 H) ppm. ^{13}C NMR: δ = 17.7, 18.7, 29.7, 31.1, 38.2, 40.7, 52.2, 54.5, 57.4, 112.7, 116.7, 127.0, 128.7, 170.2, 171.2, 172.8 ppm. EI-MS: m/z (%) = 320 (4) [M^+], 162 (78), 114 (31), 43 (100). $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_4$ (320.38): calcd. C 63.73, H 7.55, N 8.74, O 19.98; found C 63.84, H 7.52, N 8.70.

Removal of the Methyl Ester Group of Dipeptides 4a–c:^[22] Removal of the methyl ester group of the dipeptides **4a–c** (1 mmol) was carried out with the reagent system AlCl_3 (9 mmol)/*N,N*-dimethylaniline (15 mmol). Chromatographic purification of the crude reaction products afforded the respective *N*-Fmoc-dipeptides **6a–c** in high yields. The compound characterization data for **6b** fitted those observed for an authentic sample of *N*-Fmoc-valyl-leucine.^[22]

Compound 6a: White solid (0.25 g, 78% yield), m.p. 167–170 °C. IR (KBr): $\tilde{\nu}$ = 3390, 2962, 1707, 1670, 1646, 1554, 1246, 761, 740 cm^{-1} . ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 0.73–0.89 [m, 6 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 1.41–1.70 [m, 3 H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$], 2.92–3.02 (m, 2 H, CH_2Ph), 3.98–4.38 (m, 4 H, Fmoc- CH_2 , Fmoc- CH , CHCH_2Ph), 4.72 [m, 1 H, $\text{CHCH}_2\text{CH}(\text{CH}_3)_2$], 7.08–8.29 (m, 15 H, OCONH , CHCONH , Ar-*H*) ppm. FAB⁺ MS: m/z (%) = 501 (17) [$\text{M} + \text{H}$]⁺, 179 (100), 178 (62), 165 (40). $\text{C}_{30}\text{H}_{32}\text{N}_2\text{O}_5$ (500.59): calcd. C 71.98, H 6.44, N 5.60, O 15.98; found C 72.09, H 6.40, N 5.57.

Compound 6c: White solid (0.31 g, 75% yield), m.p. 183–186 °C. IR (KBr): $\tilde{\nu}$ = 3408, 2961, 1692, 1656, 1254, 759, 740 cm^{-1} . ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 0.78–0.94 [m, 6 H, $\text{CH}(\text{CH}_3)_2$], 2.05 [m, 1 H, $\text{CH}(\text{CH}_3)_2$], 2.70–3.00 (m, 2 H, CH_2Ph), 4.03–4.31 [m, 4 H, Fmoc- CH_2 , Fmoc- CH , $\text{CHCH}(\text{CH}_3)_2$], 4.37 (m, 1 H, CHCH_2Ph), 7.13–7.90 (m, 15 H, OCONH , CHCONH , Ar-*H*) ppm. FAB⁺ MS: m/z (%) = 487 (13) [$\text{M} + \text{H}$]⁺, 179 (100), 178 (64), 165 (29). $\text{C}_{29}\text{H}_{30}\text{N}_2\text{O}_5$ (486.56): calcd. C 71.59, H 6.21, N 5.76, O 16.44; found C 71.71, H 6.17, N 5.73.

- [1] For a comprehensive review, see: E. Haslam, *Tetrahedron* **1980**, *36*, 2409–2434.
- [2] R. W. Roeske, *The Peptides*, Academic Press, New York, **1981**, vol. 3, p. 102.
- [3] J. Jones, *The Chemical Synthesis of Peptides*, Clarendon Press, Oxford, **1991**, p. 103.
- [4] M. Bodanzski, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin, **1984**, 2nd ed., p. 221.
- [5] S. Liu, C. Dockendorff, S. D. Taylor, *Org. Lett.* **2001**, *3*, 1571–1574.
- [6] B. Ye, T. R. Burke, *Tetrahedron Lett.* **1995**, *36*, 4733–4736.
- [7] R. Pascal, R. Sola, *Tetrahedron Lett.* **1998**, *39*, 5031–5034.
- [8] M. G. Organ, J. Xu, B. N'Zemba, *Tetrahedron Lett.* **2002**, *43*, 8177–8180.
- [9] R. W. Jackson, *Tetrahedron Lett.* **2001**, *42*, 5163–5165.
- [10] M. J. O'Donnell, F. Delgado, *Tetrahedron* **2001**, *57*, 6641–6650.
- [11] Y.-Q. Wu, D. C. Limburg, D. E. Wilkinson, M. J. Vaal, G. S. Hamilton, *Tetrahedron Lett.* **2000**, *41*, 2847–2849.
- [12] G. M. Makara, G. R. Marshall, *Tetrahedron Lett.* **1997**, *38*, 5069–5072.
- [13] C. W. West, M. A. Estiarte, D. H. Rich, *Org. Lett.* **2001**, *3*, 1205–1208.
- [14] D. Filippov, E. K. Yeheskiely, G. A. van der Marel, G. I. Tesser, J. H. van Boom, *Tetrahedron Lett.* **1998**, *39*, 3597–3600.
- [15] K. J. Hale, J. Cai, V. M. Delisser, *Tetrahedron Lett.* **1996**, *37*, 9345–9348.
- [16] W.-C. Chen, M. D. Vera, M. M. Joullie, *Tetrahedron Lett.* **1997**, *38*, 4025–4028.
- [17] M. Nishizawa, H. Yamamoto, K. Seo, H. Imagawa, T. Sugihara, *Org. Lett.* **2002**, *4*, 1947–1949.
- [18] Y. Luo, M. A. Blaskovich, G. A. Lajoie, *J. Org. Chem.* **1999**, *64*, 6106–6111.
- [19] P. Gomez-Martinez, A. M. Kimbonguila, F. Guibe, *Tetrahedron* **1999**, *55*, 6945–6960.
- [20] J. M. Sanderson, P. Singh, C. W. G. Fishwick, J. B. C. Findlay, *J. Chem. Soc., Perkin Trans. 1* **2000**, 3227–3231.
- [21] J. M. Caba, I. M. Rodriguez, I. Manzanares, E. Giralt, F. Albericio, *J. Org. Chem.* **2001**, *66*, 7568–7574.
- [22] M. L. Di Gioia, A. Leggio, A. Le Pera, C. Siciliano, G. Sindona, A. Liguori, *J. Pept. Res.* **2004**, *63*, 383–387.
- [23] A. Leggio, A. Liguori, A. Napoli, C. Siciliano, G. Sindona, *Eur. J. Org. Chem.* **2000**, 573–575.
- [24] M. L. Di Gioia, A. Leggio, A. Liguori, A. Napoli, C. Siciliano, G. Sindona, *J. Org. Chem.* **2001**, *66*, 7002–7007.

Received May 7, 2004