Synthesis of N-{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-Protected (Fmoc) β -Amino Acids (= Homo- α -Amino Acids) by Direct Homologation

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The successful application of the *Arndt-Eistert* protocol starting from commercially available N-{[(9*H*-fluoren-9-yl)methoxy]carbony]-protected (Fmoc) α -amino acids leading to enantiomerically pure *N*-Fmoc-protected β -amino acids in only two steps and with high yield is reported.

Introduction. – Interest in the synthesis of enantiomerically pure β -amino acids (= homo- α -amino acids) has enormously increased in the last decade due to the pharmacological and biological effects shown by the latter in free form and incorporated in peptides [1]. Only recently, the synthesis and extraordinary properties of a hexapeptide consisting of β -amino acids have been published [2].

With respect to the synthesis of peptides N-{[(9*H*-fluoren-9-yl)methoxy]carbony]}protected (Fmoc) β -amino acids should be of great value since these derivatives could be used in the well-established solid-phase method, eventually also in computer-based peptide synthesis¹). Furthermore, these compounds would also be of great interest in the combinatorial-chemistry strategy [4].

The homologation of commercially available *N*-Fmoc-protected α -amino acids following the *Arndt-Eistert* pathway is a very attractive route, especially since the rearrangement using *N*-[(*tert*-butoxy)carbonyl](Boc)- and *N*-[(benzyloxy)carbonyl](Z)-protected intermediates has been shown to proceed stereospecifically to a high degree in most cases [5]. Unfortunately, the standard conditions used therein do not seem to be ideally suited for preserving the *N*-Fmoc protection, as the crucial step involves the presence of an excess of strongly basic amines. An alternate, less straightforward route of homologation involves reduction, substitution, and hydrolysis and leads to *N*-Fmoc derivatives *via N*-Boc deprotection and subsequent *N*-Fmoc protection of the target compond [4].

Results and Discussion. – We here report the successful application of the *Arndt-Eistert* protocol starting from the commercially available *N*-Fmoc-protected α -amino acids L-alanine (1), D-alanine (2), L-isoleucine (3), and L-proline (4) leading to enantiomerically pure *N*-Fmoc-protected β -amino acids by homologation in only two steps and with high yield²). The amino acids primarily investigated were selected for the following

¹) While this paper was finalized, *Guichard* and *Seebach* published a note confirming the great value of these compounds in the construction of peptides containing nonproteinogenic amino acids [3].

²) Presented at the poster session of the '13. Wissenschaftl. Tagung der Österr. Pharmazeut. Gesellschaft,' Wien, April, 1997 [6].

reasons: N-Fmoc-L- and N-Fmoc-D-alanine (1 and 2, resp.) should provide us with samples of both enantiomers to enable the planned development of an HPLC-based method for the determination of the enantiomeric purity of β -amino acids [7]. N-Fmoc-L-Isoleucine (3) – due to the second stereogenic centre that is not involved in the applied reactions – has the essential prerequisites to investigate the outcome of the rearrangement by NMR spectroscopy. N-Fmoc-L-Proline (4), finally, should allow us to explore the limitations of the method and the applicability to heterocyclic amino acids.



The synthesis of the diazo ketone intermediates was easily performed by applying the mixed-anhydride method: the reaction with ethyl chloroformate (= ethyl carbonochloridate) was carried out at temperatures below -20° avoiding an excess of Et₃N. After the addition of a freshly prepared solution of CH₂N₂ in Et₂O at $< -20^{\circ}$, the reaction mixture was allowed to warm up to 0° before workup. Thus, in all transformations investigated, the diazo ketones resulted in high yield and in enantiomerically pure form (see *Scheme* and *Exper. Part*). The structures of the yellow *N*-Fmoc-amino diazo ketones **5**–**8** were established by the characteristic strong absorption at 2100 cm⁻¹ in the IR spectra and by the outstanding signals in their NMR spectra: the most notable features are the ¹H-resonances near 5 ppm (in CDCl₃) or 6 ppm (in (D₆)DMSO) (H–CN₂), the ¹³C-signal near 52 ppm (CHN₂), and the large ¹J(H,C) coupling constant of *ca.* 200 Hz for *C*HN₂.

Rearrangement to the β -amino-acid derivatives can be initiated either by UV light ($\lambda \ge 254$ nm) or by silver salts, preferably complexed in Et₃N [8]. Orienting small-scale

experiments led us to the decision to apply the Ag-induced reaction³). To minimize the *N*-Fmoc deprotection under the strongly basic conditions, the reaction mixture was actively warmed up from -20° to *ca*. 0° and acidified by addition of 10% aqueous citric acid immediately after the rearrangement was complete. The desired *N*-Fmoc-protected β -amino acids 9–12 could be isolated in high yield after the usual workup procedure (see *Scheme* and *Exper. Part*). The structures of the homologated amino acids were established mainly by NMR spectroscopy, the most striking signals being due to the diastereotopic protons of the new CH₂(α) group (*AB* part of an *ABX* spin system).

The supposed retention of configuration in the β -amino acids was strongly suggested by the NMR spectra of *N*-Fmoc-L- β -homoisoleucine (11) in which any epimerization should be detectable *a priori* due to the presence of two stereogenic centres. Under standard conditions in CDCl₃, 11 shows various broadened signals of very low intensity, but on warming up to 60°, the spins do not exchange fast enough to simplify the spectrum; thus, the presence of the epimeric compound cannot be excluded. However, the ¹H-NMR spectra of 11 recorded in (D₆)DMSO or (D₆)acetone exhibit a single set of data which suggest its enantiomeric purity, at least after recrystallization. Thus, the additional signals found for 11 in CDCl₃ solution may be due to the presence of conformational isomers. To further support this finding, we also converted the Fmoc-Ile-OH derived diazo ketone 7 into the methyl ester of 11 using a similar procedure. Both ¹H- and ¹³C-NMR spectra gave no hints for the formation of stereoisomers during the rearrangement.

The stability of the *N*-Fmoc-protected β -amino acids 9–12 at elevated temperatures in (D₆)DMSO solution is limited, and, therefore, ¹³C-NMR data obtained at this temperature always showed signals of decomposition products (9-methylidene-9*H*-fluorene, deprotected amino acid) beside the expected signals. This problem required a special recording procedure in the case of the most unstable β -amino-acid derivative *N*-Fmoc- β homoproline (12), which exhibited two sets of ¹³C-NMR data in CDCl₃ and in (D₆)DMSO solution at 30° that could not be brought to coalescence at higher temperatures. However, application of the new time-saving pulsed-field-gradient-supported 2D sequences enabled us to prove the exchange of the corresponding C-atoms of 12 and to completely assign the signals also at elevated temperatures (see *Fig.*).

Finally, comparison of the determined specific rotation of the diazo ketones derived from N-Fmoc-L-alanine (1) and N-Fmoc-D-alanine (2) and of the corresponding homologated amino acids confirm that the *Wolff* rearrangement proceeds stereospecifically.

Conclusions and Perspectives. – The *Arndt-Eistert* pathway is a fast and convenient way to synthesize *N*-Fmoc-protected β -amino acids⁴), at least at a laboratory scale⁵). The great and increasing number of commerically available *N*-Fmoc-protected α -amino

³) Small-scale experiments monitored by TLC and NMR spectroscopy showed that the light-induced reaction led to mixtures containing olefinic by-products, presumably dimers of carbene intermediates. Nevertheless, the application of the 'Photo-*Wolff* conditions' should also lead to the desired enantiomerically pure products.

⁴) The methodology was also successfully applied to the orthogonally diprotected N^{α} -Boc- N^{ϵ} -Fmoc-lysine [10].

⁵) For safety reasons, in our laboratory, all reactions involving diazomethane are performed at a scale not greater than 0.2μ [11].





acids makes this route a solid basis for the synthesis of β -amino-acid-based peptides either by the conventional solid-phase synthesis or by the recently developed methodology of *Seebach* and coworkers [12].

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Experimental Part

General. Solvents were either used as purchased from *Fluka* (*puriss. p.a.* or *puriss.* quality) or distilled over appropriate drying agents. Amino-acid derivatives were purchased from *Senn.* TLC: *Macherey-Nagel Polygram** *SIL G/UV*₂₅₄ anal. plates; detection either with UV or by spraying Ce(SO₄)₂ soln. and heating on a hot plate. M.p.: *Reichert* microscope with micro hot stage; uncorrected. Optical rotations: 10-cm, 1-ml cell at 20°; *Perkin-Elmer 141* polarimeter. IR Spectra: in cm⁻¹; *Galaxy 3000 FT* spectrometer, CHCl₃ soln. or KBr. NMR Spectra: chemical shifts δ in ppm and coupling constants *J* in Hz, using the appropriate solvent as internal standard; *Varian Gemini 200* (¹H 200 MHz; ¹³C 50 MHz) or *Bruker AM 300* (¹H 300 MHz; ¹³C 75 MHz) or *Varian Unityplusm 500* (¹H, 500 MHz; 125 MHz) spectrometer; assignments based on DEPT spectra or 2D correlated spectroscopy (COSY, HMQC or HSQC). MS: *Finnigan MAT 95*; Cs gun, 20 kV, $2 \cdot 10^{-6}$ A; 3-nitrobenzyl alcohol (NOBA) matrix. Elemental analyses were performed at the Microanalytical Laboratory of the Institute of Physical Chemistry at the University of Vienna.

N-Fmoc-Protected Amino Diazo Ketones: General Procedure (GP1). A soln. of the N-Fmoc-protected α -amino acid in dry THF is cooled to -25° under Ar, Et₃N (1.1 mol-equiv.) and ethyl chloroformate (1.1 mol-equiv.) are added subsequently with stirring while the temp. is kept below -20° . After 15 min, a freshly prepared CH₂N₂ soln. in dry Et₂O [13][14] is added until the yellow colour of the soln. persists. The mixture is allowed to warm up to 0°, and stirring is continued for 2 to 12 h. Acidification with dil. acetic or citric acid and addition of an equal volume of Et₂O is followed by washing the org. layer with H₂O, sat. NaHCO₃ soln., and brine. Drying (Na₂SO₄) and evaporation yields the crude diazo ketone which is further purified by suspending and stirring for 30 min in pentane and by recrystallization from the appropriate solvent.

(3S)-1-Diazo-3-{{[(9H-fluoren-9-yl)methoxy]carbonyl}amino}butan-2-one (5). From Fmoc-Ala-OH (1; 9.2 g, 29.5 mmol) according to *GP1*: 9.9 g (100%) of essentially pure 5 (by ¹H-NMR). Recrystallization from *t*-BuOMe improved the purity of the material only slightly. Yellow crystals. M.p. 116°. For anal. purposes, the substance was recrystallized twice from *t*-BuOMe. $[z]_{2}^{00} = -50.2$ (c = 1.5, DMF). IR (CHCl₃): 3434w, 3116w, 3011m, 2976m, 2894w, 2113s, 1719s, 1644m, 1504s, 1451m, 1386m, 1362s, 1331w, 1232m, 1147w, 1047m, 1032m, 877w. ¹H-NMR ((D₆)DMSO, 300 MHz, 30°): 7.88 (d, 2 arom. H); 7.70 (br. d, 2 arom. H, NH (overlapping)); 7.41 (t, 2 arom. H); 7.32 (t, 2 arom. H); 5.95 (br. s, H-C(1)); 4.33 (m, CH₂O); 4.21 (m, H-C(9')); 4.03 (m, H-C(3)); 1.19 (d, J = 7.4, Me). ¹³C-NMR (CDCl₃, 75 MHz, 30°): 195.54 (s, CO); 155.66 (s, NHCOO); 143.74, 140.70 (2s), 127.55, 126.97, 125.17, 125.10, 120.02 (5d, 12 arom. C); 65.45 (t, CH₂O); 53.83 (d, C(3)); 52.19 (d, C(1)); 46.69 (d, C(9')); 16.84 (q, C(4)). FAB-MS: 337.1 (23.9, [M + 2]⁺), 336.1 (100, [M + 1]⁺), 335.1 (41.2, M^+). Anal. calc. for C₁₉H₁₇N₃O₃ · 0.15 C₅H₁₂O ('348.59'): C 68.05, H 5.44, N 12.05; found: C 68.53, H 5.28, N 11.91.

(3R)-1-Diazo-3-{{[(9H-fluoren-9-yl)methoxy]carbonyl}amino}butan-2-one (6). From Fmoc-D-Ala-OH · H₂O (2; 10.4 g, 31.6 mmo]) according to *GP1*: 10.6 g (100 %) of essentially pure 6 (by ¹H-NMR). Yellow crystals. M.p. 118–119°. For anal. purposes, the substance was recrystallized from t-BuOMe. $[\alpha]_D^{20} = +50.1$ (c = 0.9, DMF). IR (CHCl₃): 3435w, 3116w, 3014m, 2977m, 2894w, 2113s, 1718s, 1645m, 1504s, 1451m, 1386m, 1362s, 1331w, 1232m, 1216m, 1147w, 1047m, 1031m, 876w. ¹H-NMR (CDCl₃, 300 MHz, 26°): 7.75 (d, 2 arom. H); 7.57 (br. m, 2 arom. H); 7.38 (t, 2 arom. H); 7.30 (t, 2 arom. H); 5.39 (br. d, NH); 5.29 (br. s, H–C(1)); 4.42 (m, CH₂O); 4.42 (br. m, H–C(3)); 4.19 (m, H–C(9')); 1.33 (d, J = 6.8, Me). ¹³C-NMR ((D₆)DMSO, 75 MHz, 30°): 195.66 (s, CO); 155.71 (s, NHCOO); 143.77, 140.75 (2s), 127.61, 127.03, 125.24, 125.17, 120.11 (6d, 12 arom. C); 65.49 (t, CH₂O); 53.88 (d, C(3)); 52.28 (d, C(1)); 4.6.71 (d, C(9')); 16.89 (q, C(4)). FAB-MS: 37.1 (22.3, [M + 2]⁺), 336.1 (100, [M + 1]⁺), 335.1 (35.8, M⁺). Anal. calc. for C₁₉H₁₇N₃O₃ (335.36): C 68.05, H 5.11, N 12.53; found: C 68.25, H 5.09, N 12.27.

(3S,4S)-1-Diazo-3-{{[(9H-fluoren-9-yl)methoxy]carbonyl}amino}-4-methylhexan-2-one (7). From Fmoc-Ile-OH (3; 25.0 g, 70.7 mmol) according to *GP1*. Recrystallization from THF/pentane 1:2 (ν/ν) gave 22.0 g (82%) of 7. Yellow crystals. M.p. 136–137°. For anal. purposes, the substance was recrystallized twice from THF/pentane. [α]₂^D = - 46.6 (c = 1, DMF). IR (KBr): 3302s, 3082m, 3016w, 2965m, 2097s, 1692s, 1634s, 1537m, 1452w,

1389*m*, 1370*m*, 1321*w*, 1260*m*, 1238*w*, 1225*m*, 1028*m*. ¹H-NMR (CDCl₃, 300 MHz, 26°): 7.75 (*d*, 2 arom. H); 7.57 (br. *d*, 2 arom. H); 7.30 (*td*, 2 arom. H); 5.35 (br. *d*, J = 8.4, NH); 5.29 (br. *s*, H–C(1)); 4.42 (*m*, CH₂O); 4.20 (*m*, H–C(9')); 4.13 (*m*, H–C(3)); 1.84 (*m*, H–C(4)); 1.44, 1.11 (2*m*, 2 H–C(5)); 0.93 (*d*, J = 7.1, Me–C(4)); 0.90 (*t*, J = 7.5, Me(6)). ¹³C-NMR (CDCl₃, 75 MHz, 26°): 193.23 (*s*, CO); 156.17 (*s*, MHCOO); 143.77, 141.33 (2*s*), 127.70, 127.07, 125.07, 125.00, 119.97 (5*d*, 12 arom. C); 66.79 (*t*, CH₂O); 62.28 (*d*, C(3)); 54.85 (*d*, C(1)); 47.27 (*d*, C(9')); 37.62 (*d*, C(4)); 24.58 (*t*, C(5)); 15.64 (*q*, C(6)); 11.51 (*q*, (*Me*–C(4)). FAB-MS: 379.1 (24.2, [*M* + 2]⁺), 378.1 (100, [*M* + 1]⁺), 377.1 (41.3, *M*⁺). Anal. calc. for C₂₂H₂₃N₃O₃ · 0.2 C₅H₁₂ ('391.88'): C 70.50, H 6.53, N 10.72; found: C 70.40, H 6.40, N 10.67.

2-Diazo-1-{(2'S)-1-{[(9H-Fluoren-9-yl)methoxy]carbonyl}pyrrolidin-2-yl}ethan-1-one (8). From Fmoc-Pro-OH (4; 9.9 g, 2.9 mmol) according to GP1: 9.55 g (90%) of essentially pure 8 (by ¹H-NMR). Recrystallization from MeOH yielded 8.88 g (84%) of yellow crystals. M.p. 136–138°. [z]_D²⁰ = -69.7 (c = 0.9, DMF). IR (CHCl₃): 3011m, 2985w, 2956w, 2110s, 1697s, 1642s, 1478m, 1451s, 1416s, 1356s, 1325m, 1122m. ¹H-NMR (CDCl₃, 500 MHz, 26°): 7.75 (br. *t*, 2 arom. H); 7.60 (*m*, 1 arom. H); 7.53 (*t*, 1 arom. H); 7.38 (br. *t*, 2 arom. H); 7.30 (*t*, 2 arom. H); 5.28 (br. *s*, H–C(2) (conformer A)); 4.94 (br. *s*, H–C(2) (conformer B)); 4.35 (br. *m*, CH₂O) (conformer B)); 4.23 (*t*, J = 6.7, H–C(") (conformer A)); 4.16 (*t*, J = 5.2, H–C(9") (conformer B)); 3.96 (br. *m*, H–C(2') (conformer A)); 3.47 (*m*, CH₂(5')); 2.15–1.95 (*m*, CH₂(3')); 1.95–1.75 (*m*, CH₂(4')). ¹³C-NMR ((D₆)DMSO, 75 MHz, 30°): 194.66 (*s*, CO); 153.99, 153.73 (2s, NHCOO); 143.72, 143.69, 140.68 (3s), 127.57, 127.04, 126.91, 125.03, 124.94, 120.01 (6d, 12 arom. C); 66.63, 66.49 (2*t*, CH₂O); 63.50, 62.95 (2*d*, C(2')); 52.30 (2*d*, C1); 46.97, 46.37 (2*t*, C(5')); 46.64 (*d*, C(9")); 30.62, 29.46 (2*t*, C(3')); 23.75, 23.73 (2*t*, C(4')). FAB-MS: 363.1 (26.1, [M + 2]⁺), 362.1 (100, [M + 1]⁺), 361.1 (44.9, *M*⁺). Anal. calc. for C₂₁H₁₉N₃O₃ (361.40): C 69.79, H 5.30, N 11.63; found: C 70.08, H 5.24, N 11.49.

N-Fmoc-Protected β -Amino Acids: General Procedure for the Wolff Rearrangement (GP2). A soln. of AgOAc (0.11 mol-equiv.) in Et₃N (3 mol-equiv.) is added to a soln. of the diazo compound in THF containing 10% H₂O (ν/ν) at -15° . The mixture is actively warmed up within 10 min to -5 to 0° where the N₂ evolution starts; the temp. is kept below 5° until the reaction is complete (10 to 30 min, TLC monitoring). The mixture is filtered over *Celite*, the filtrate acidified by addition of 10% aq. citric acid soln. and diluted with an equal volume of AcOEt, and the org. layer washed with brine, dried (Na₂SO₄), and evaporated to yield the crude Fmoc- β -amino acid which can be further purified by recrystallization from the given solvent.

(3S)-3-{{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino}butanoic Acid (= N-{[(9H-Fluoren-9-yl)methoxy]carbonyl}-L-β-homoalanine; Fmoc-β-HAla-OH; **9**). From **5** (1.0 g, 3 mmol) according to GP2: 0.9 g (92%) of essentially pure **9** (by ¹H-NMR). Colourless crystals. M.p. 116–118°. For anal. purposes, the substance was recrystallized from EtOH/H₂O. [α]_D²⁰ = + 5.3 (c = 0.9, DMF). IR (KBr): 3322m, 3065w, 2961m, 1693s, 1536s, 1477m, 1450s, 1412m, 1378m, 1339m, 1261s, 1105s, 1086s, 1057s, 1032m, 977w, 935w, 758m, 737s. ¹H-NMR ((D₆)DMSO, 300 MHz, 30°): 12.13 (br.s, COOH); 7.88 (d, 2 arom. H); 7.67 (br. d, 2 arom. H); 7.40 (br. t, 2 arom. H); 7.21 (d, J = 8.2, NH); 4.28 (m, CH₂O); 4.20 (m, H–C(9)); 3.84 (m, H–C(3)); 2.35 (AB of ABX, J = 15.4, 7.2, 6.6, CH₂(2)); 1.07 (d, J = 6.7, Me). ¹³C-NMR ((D₆)DMSO, 75 MHz, 30°): 172.26 (s, COOH); 155.15 (s, NHCOO); 143.88, 143.81, 140.66 (3s), 127.51, 126.97, 125.07, 120.01 (4d, 12 arom. C); 65.12 (t, CH₂O); 46.70 (d, C(3)); 43.70 (d, C(9')); 40.78 (t, C(2)); 20.39 (q, C(4)). FAB-MS: 327.1 (19.2, [M + 2]⁺), 326.2 (100, [M + 1]⁺), 325.1 (16.0, M⁺). Anal. calc. for C₁₉H₁₉NO₄ (325.37): C 70.14, H 5.89, N 4.30; found: C 69.90, H 6.15, N 4.35.

(3R)-3-{{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino}butanoic Acid (= N-{[(9H-Fluoren-9-yl)methoxy]carbonyl}-D- β -homoalanine; Fmoc-D- β -HAla-OH; 10). From 6 (1.0 g, 2.67 mmol) according to GP2: 0.97 g (100%) of essentially pure 10 (by ¹H-NMR). Colourless crystals. M.p. 115–118°. For anal. purposes, the substance was recrystallized from t-BuOMe. [α]_D² = -4.7 (d = 1.1, DMF). IR (KBr): 3328m, 3066w, 3048w, 3040w, 3018w, 2965w, 2955w, 2949w, 2926w, 2853w, 1689s, 1540m, 1535m, 1451w, 1286w, 1262m, 1059w, 1028w, 737m. ¹H-NMR ((D₆)DMSO, 300 MHz, 26°): 12.17 (br. *s*, COOH); 7.87 (*d*, 2 arom. H); 7.68 (br. *d*, 2 arom. H); 7.40 (br. *t*, 2 arom. H); 7.32 (*tt*, 2 arom. H); 7.26 (*d*, *J* = 8.5, NH); 4.28 (m, CH₂O); 4.20 (m, H–C(9)); 3.85 (m, H–C(3)); 2.35 (AB of ABX, J = 15.4, 7.2, 6.6, CH₂(2)); 1.08 (*d*, J = 7.0, Me). ¹³C-NMR ((D₆)DMSO, 75 MHz, 26°): 172.39 (*s*, COOH); 155.22 (*s*, NHCOO); 143.93, 143.87, 140.73 (3*s*), 127.58, 127.04, 125.13, 120.09 (4d, 12 arom. C); 65.17 (*t*, CH₂O); 46.75 (*d*, C(3)); 43.77 (*d*, C(9')); 4.08 (*t*, C(2)); 20.47 (*q*, C(4)). FAB-MS: 327.1 (30.2, [*M* + 2]⁺), 326.2 (100, [*M* + 1]⁺), 325.1 (16.0, *M*⁺). Anal. calc. for C₁₉H₁₉NO₄ · 0.96 H₂O ('342.66'): C 66.60, H 6.15, N 4.09; found: C 66.55, H 5.58, N 3.98.

(3R,4S)-3-{{[(9H-Fluoren-9-yl)methoxy]carbonyl}amino}-4-methylhexanoic Acid (= N-{[(9H-Fluoren-9-yl)methoxy]carbonyl}-L- β -homoisoleucine; Fmoc- β -HIle-OH; 11). From 7 (2.1 g, 6 mmol) according to GP2: 2.0 g (98%) of essentially pure 11 (by ¹H-NMR). Colourless crystals. M.p. 98–100°. For anal. purposes,

the substance was recrystallized from EtOH/H₂O. $[z]_{2}^{D0} = + 16.6$ (c = 0.8, DMF). IR (KBr): 3324m, 3067w, 2965m, 2932w, 2876w, 1698s, 1543m, 1451m, 1304w, 1262m, 1236m (sh), 1125w, 1042w, 739m. ¹H-NMR ((D₆)DMSO, 500 MHz, 26°): 12.06 (br. s, COOH); 7.87 (d, 2 arom. H); 7.69 (t, 1 arom. H); 7.67 (t, 1 arom. H); 7.32 (t, 2 arom. H); 7.30 (t, 2 arom. H); 7.24 (d, J = 8.7, NH); 4.25 (m, CH₂O); 4.20 (m, H–C(9)); 3.78 (m, H–C(3)); 2.31 (*AB* of *ABX*, J = 15.4, 9.4, 4.4, CH₂(2)); 1.47 (m, H–C(4)); 1.34 (m, 1 H–C(5)); 1.05 (m, 1 H–C(5)); 0.83 (t, J = 7.3, Me(6)); 0.79 (d, J = 6.9, Me–C(4)). ¹³C-NMR ((D₆)DMSO, 75 MHz, 30°): 172.88 (s, COOH); 155.47 (s, NHCOO); 143.95, 143.73, 140.65 (3s), 127.50, 126.92, 125.14, 119.99 (4d, 12 arom. C); 65.10 (t, CH₂O); 51.86 (d, C(3)); 46.76 (d, C(9')); 38.32 (d, C(4)); 35.76 (t, C(2)); 24.91 (t, C(5)); 14.74 (q, Me–C(4)); 11.42 (q, C(6)). FAB-MS: 369.2 (25.6, $[M + 2]^+$), 368.2 (100, $[M + 1]^+$), 367.2 (7.6, M^+). Anal. calc. for C₂₂H₂₅NO₄ (367.44): C 71.91, H 6.86, N 3.81; found: C 71.97, H 7.09, N 3.90.

Methyl (3R,4S)-3-{{[(9H-Fluoren-9-yl)methoxy]carbonyl}annio}-4-methylhexanoate (= N-{[(9H-Fluoren-9-yl)methoxy]carbonyl}-L- β -homoisoleucine Methyl Ester; Fmoc- β -HIle-OMe). From 7 (3.0 g, 8 mmol) according to GP2 with THF containing 40% of MeOH as solvent: 2.9 g (95%) of essentially pure Fmoc- β -HIle-OMe (by ¹H-NMR). Colourless crystals. M.p. 120°. For anal. purposes, the substance was recrystallized from THF/Et₂O. [x]₂²⁰ = 19.1 (c = 0.9, DMF). IR (KBr): 3318s, 3085w, 3019w, 2962s, 2932m, 2888w, 2875m, 2857w, 1745s, 1693s, 1546s, 1464m, 1450s, 1417w, 1378w, 1345m, 1321s, 1309s, 1236s, 1218m, 1191m, 1176m, 1121s, 1080w, 1045s, 760m, 738s. ¹H-NMR (CDCl₃, 300 MHz): 7.74 (d, 2 arom. H); 7.58 (d, 2 arom. H); 7.38 (t, 2 arom. H); 7.29 (td, 2 arom. H); 5.16 (d, J = 9.0, NH); 4.38 (d, J = 7, CH₂O); 4.21 (t, J = 7, H–C(9)); 3.86 (m, H–C(5)); 1.10 (m, 1H–C(5)); 0.89 (t, J = 7.4, Me(6)); 0.87 (d, J = 6.5, Me–C(4)). ¹³C-NMR (CDCl₃, 75 MHz): 172.36 (s, COOMe); 155.90 (s, NHCOO); 144.01, 143.92, 141.31 (3s), 127.62, 126.99, 125.05, 119.92 (d, 2 arom C); 66.55 (t, CH₂O); 52.47 (d, C(3)); 51.75 (q, MeO); 47.30 (d, C(9)); 38.08 (d, C(4)); 36.25 (t, C(2)); 25.48 (t, C(5)); 15.27 (q, Me-C(4)); 11.36 (q, C(6)). FAB-MS: 383.1 (25.5, [M + 2]⁺), 382.1 (100, [M + 1]⁺), 381.1 (3.6, M^+). Anal. cale. for C₂₃H₂₇NO₄ (381.47): C 72.42, H 7.13, N 3.67; found: C 72.20, H 7.29, N 3.69.

(2S)-1-{[(9H-Fluoren-9-yl)methoxy]carbonyl}pyrrolidine-2-acetic Acid (Fmoc-β-HPro-OH, **12**). From **8** (1.0 g, 2.67 mmol) according to *GP2*: 0.9 g (93%) of essentially pure **12** (by ¹H-NMR). Recrystallization from MeOH yielded 0.8 g (83%) of colourless crystals. M.p. 191–192°. $|x|_{D}^{20} = -33.6$ (c = 0.9, DMF). IR (CHCl₃): 3068m, 3027m, 3008m, 2982m, 2955m, 1699s, 1451s, 1421s, 1355m, 1335m, 1121m. ¹H-NMR ((D₆)DMSO, 500 MHz, 26°): 12.19 (br. s, COOH); 7.87 (br. m, 2 arom. H); 7.64 (d, 2 arom. H); 7.40 (br. m, 2 arom. H); 7.32 (br. m, 2 arom. H); 4.45–4.20 (br. m, CH₂O, H–C(9')); 3.97 (m, H–C(2) (conformer A)); 3.89 (m, H–C(2) (conformer B)); 3.23 (m, CH₂(5)); 2.45 (*AB* of *ABX*, CH₂COOH (conformer A)); 2.20 (*AB* of *ABX*, CH₂COOH (conformer B)); 1.95 (br. m, 1 H of CH₂(3)); 1.83 (m, 1 H of CH₂(4)); 1.74 (m, 1 H of CH₂(4)); 1.63 (m, 1 H of CH₂(3)). ¹³C-NMR ((D₆)DMSO, 75 MHz, 26°): 172.47, 172.29 (2s, COOH); 153.67 (s, NHCOO); 143.88, 140.77 (2s), 127.60, 127.06, 125.05, 124.88, 120.09 (5d, 12 arom. C); 66.15 (t, CH₂O); 54.18, 53.48 (2d, C(2)); 46.74 (d, C(9')); 46.30, 45.86 (2t, C(5)); 38.34, 37.58 (2t, CH₂COOH); 30.67, 29.90 (t, C(3)); 22.92, 22.02 (2t, C(4)). FAB-MS: 353.2 (23.4, [M + 2]⁺), 352.2 (100, [M + 1]⁺), 351.2 (5.9, M⁺). Anal. calc. for C₂₁H₂₁NO₄ (351.40): C 71.78, H 6.02, N 3.99; found: C 72.04, H 6.29, N 4.04.

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