Synthesis of 4-(Fmoc-aminoacyloxymethyl)phenoxyacetic Acids for Use in Solid-Phase Peptide Synthesis

Nikos Zinieris,^a Stella Kokinaki,^a Leondios Leondiadis,^b Nikolas Ferderigos*^a

^a Laboratory of Organic Chemistry, Chemistry Department, University of Athens, 157 71 Athens, Greece

^b Mass Spectrometry and Dioxine Analysis Lab, IRRP, National Centre for Scientific Research 'Demokritos', 153 10 Athens, Greece Fax +30(210)7274761; E-mail: nferder@chem.uoa.gr

Received 7 April 2006; revised 20 April 2006

Abstract: The synthesis of 4-(Fmoc-aminoacyloxymethyl)phenoxyacetic acids was achieved in high yield by the reaction of Fmoc-amino acids with (4-iodomethylphenoxy)acetic acid 2-oxo-2-phenylethyl ester. The removal of the temporary protecting group, phenacyl ester, was effectively achieved by reductive cleavage with magnesium turnings.

Key words: solid-phase synthesis, magnesium, peptides

The solid-phase methodology proposed by Merrifield¹ and fine-tuned over time is presently based on a Boc/Bn or Fmoc/*t*-Bu strategy using polystyrene (PS) or PEG-PS solid supports.²

The anchoring of the first amino acid residue to the solid support is one of the most critical steps in solid-phase peptide synthesis. The process by which the C-terminal N^{α}-protected amino acid is attached to the resin and the nature of the anchoring linkage ultimately contributes to the yield and purity of the final product. Sites on the resin not initially blocked by this process can be potentially acylated in subsequent steps, leading to the generation of related C-terminally truncated by-products.

The Fmoc/t-Bu strategy has gained considerable popularity over the last decade due to the use of milder reagents and the high yields usually obtained. However, the formation of the ester bond, required when attaching the first amino acid to the alkoxybenzyl resin in the Fmoc/t-Bu process, is considerably more difficult than that of the corresponding amide bond used in the Boc/Bn strategy for the attachment of N^{α} -Boc-aminoacyloxymethyl acetic acid to aminomethyl resins (Scheme1).

In the Boc/Bn approach, a controlled loading is performed and no by-products are produced. In contrast, the *p*-alkoxybenzyl ester anchoring linkage introduced by Wang et al.^{3,4} and its subsequent evolution by Sheppard et al.^{5,6} for the Fmoc/*t*-Bu strategy, requires harsher conditions which may lead to low substitution or enantiomerization,⁷ especially for cysteine and histidine, and formation of dipeptide by-products.⁸ To ensure satisfactory results when using this methodology, it is important that the following precautions are taken: a) all reagents and glassware must be dried thoroughly before use, since the presence of moisture can severely affect loading efficiencies; b) reactions should be conducted using the minimum volumes of solvent to ensure maximum reagent concentration; c) after each reaction, the loading efficiency should be checked and, if necessary, the reaction should be repeated with fresh reagents; and d) a strong acetylating reagent must be used in order to cap unreacted resinbound hydroxyl groups to prevent the growth of deletion sequences at these sites.



Scheme 1 A, Boc/Bn strategy; B Fmoc/*t*-Bu strategy; A.A., amino acid.

An alternative method for the attachment of the C-terminal N^{α} -Fmoc-amino acid to the *p*-alkoxybenzyl-type resin utilizes novel active esters of Na-Fmoc-aminoacyl-4oxymethylphenoxy acetic acids9 which directly acylate amino functionalized polymers. These methods suffer from a number of disadvantages, namely: a) from our experience and the experience of others,¹⁰ the reaction of an active ester derivative with a protected-amino acid gives rise to the formation of a multiplicity of by-products and b) active esters are not the method of choice for activation of a carboxyl function in order to achieve high loaded resins. The need for an improved methodology led us to employ the new approach described here. This new approach utilizes a phenacyl group (PAC), which has proved to be an important reagent for protecting carboxyl functions during orthogonal synthesis.¹¹After insertion of the desired Fmoc-amino acid, the PAC group is removed utiliz-

SYNTHESIS 2006, No. 16, pp 2789–2793 Advanced online publication: 12.07.2006 DOI: 10.1055/s-2006-942485; Art ID: Z07506SS © Georg Thieme Verlag Stuttgart · New York



Scheme 2 *Reagents and conditions*: i. Phenacyl bromide, Et₃N; ii. NaI, TMSCI, CH₃CN; iii. Fmoc A.A.OH, DIPEA; iv. Mg turnings, AcOH, DMF–MeOH.

ing our recently developed methodology,¹² which tolerates the rest of the functional groups present.

Specifically, 4-[(hydroxymethyl)phenoxy]acetic acid (1) was protected with a PAC group under standard conditions (Scheme 2).¹³

The hydroxyl group was converted to the corresponding iodide using the chlorotrimethylsilane/sodium iodide method¹⁴ and reacted with Fmoc-amino acids (Table 1) in the presence of DIPEA to form the ester bond. Iodide derivatives/DIPEA were preferred for the formation of the ester bond, since no by-products were observed; in contrast, when we used the corresponding bromide derivatives/potassium fluoride^{15,16} or the cesium carbonate method,¹⁷ a low yield of the desired product was obtained. All isolated yields were greater than 80%; the products were of high purity (> 95%) (Table 1).

Finally, to test the ability of the synthesized derivatives **5** to bind to the aminomethyl resins,¹⁹ 4-[Fmoc-(γ -*tert*-bu-tyl)glutamyloxymethyl]phenoxyacetic acid (**5c**) activated by PyBOP,²⁰ was reacted with aminomethyl PS-DVB resin (1 mmol NH₂/g). The yield was quantitative, as confirmed by the Kaiser ninhydrine test and estimation of the Fmoc group.²¹

In conclusion, we have developed a practical, controlled, and versatile methodology for the anchoring of N^{α} -Fmocamino acids as *p*-alkoxybenzyl esters in solid-phase peptide synthesis.

All reagents and solvents were obtained commercially (Aldrich, LabScan) and used without further purification. PE with a bp range 40–60 °C was used. Fmoc-amino acids were provided by CBL, Patras, Greece. The reactions were monitored by TLC on Merck silica gel plates (60F, 254) using UV light as a visualizing agent. ¹H NMR and ¹³C NMR spectra were obtained on a Mercury Varian Spectrometer (200 MHz). Chemical shifts are reported relative to residual undeuterated solvent as the internal reference. Optical rotations were performed on a Perkin Elmer 343 Polarimeter. Elemental analyses were performed on a Perkin Elmer Elemental Analyzer 2400. For ESI mass spectral analysis a test solution in 50% aq MeOH was infused into an electrospray interface mass spectrometer (MSQ Surveyor, Finnigan) at a flow rate of 1 mL/min. UV measurements were obtained on a UV-VIS spectrophotometer Varian

Downloaded by: Collections and Technical Services Department. Copyrighted material.

	Amino acid	Esters 4			Acids 5		
		Yield (%)	Mp (°C)	$[\alpha]_D^{20,a}$	Yield (%)	Mp (°C)	$[\alpha]_D^{20,a}$
a	Asn	95	133–136	+6.2	90	189–192	-10.7
b	Arg	80	oil	-	60	126–132	-27.4
c	Glu	94	84–85	+1.9	90	78-82	+0.4
d	Gly	88	101	_	87	172–175	-
e	Lys	98	105–110	-3.5	94	102–104	-16.7
f	Met	95	85-87	+12	87	oil	_
g	Phe	97	123–126	-4.1	94	105–107	-1.21
h	Ser	92	oil	_	90	119–122	+2.3
k	Trp	91	100-104	-31.5	87	131–134	-20.8
I	Tyr	93	92–94	-4.2	93	105–107	-4.57

 Table 1
 Fmoc-amino Acid Derivatives 4 and 5

^a c = 1, DMF.

Cary 50. Mps were determined on a Büchi 535 apparatus and are uncorrected. Column chromatography purifications were performed using Merck 70–230 mesh silica gel 60.

(4-Hydroxymethylphenoxy)acetic Acid 2-Oxo-2-phenylethyl Ester (2)

To a cooled (0 °C) solution of 4-[(hydroxymethyl)phenoxy]acetic acid (1.82 g, 10 mmol) and phenacyl bromide (2.0 g, 10 mmol) in EtOAc (20 mL), Et₃N (1.1 g, 11 mmol) was added and the reaction mixture was left overnight at r.t. The reaction mixture was diluted with Et₂O (20 mL), and the ethereal layer was washed successively with H₂O (10 mL), 5% NaHCO₃ (10 mL), and brine (10 mL). The organic phase was dried over anhyd Na₂SO₄ and the solvents were evaporated in vacuo. The residue was recrystallized from EtOAc– PE; yield: 2.7g (90%); mp 95–97 °C.

¹H NMR (200 MHz, CDCl₃): δ = 4.63 (s, 2 H), 4.85 (s, 2 H), 5.47 (s, 2 H), 6.98 (d, *J* = 8.6 Hz, 2 H), 7.32 (d, *J* = 8.6 Hz, 2 H), 7.46–7.63 (m, 3 H), 7.91 (d, *J* = 7.1 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl_3): δ = 191.73, 168.89, 157.42, 134.69, 134.34, 134.06, 129.16, 128.84, 128.00, 114.93, 66.71, 65.33, 64.87.

Anal. Calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.67; H, 5.25.

(4-Iodomethylphenoxy)acetic Acid 2-Oxo-2-phenylethyl Ester (3)

To a solution of 1 (2.5 g, 8.3 mmol) and NaI (1.25 g, 8.3 mmol) in MeCN (10 mL) covered with aluminum foil was slowly added TMSCl (0.9 g, 8.3 mmol) with continuous stirring. After 2 h the reaction was complete (monitored by TLC), the reaction mixture was taken up in Et₂O (100 mL) and washed successively with H₂O (10 mL), a 10% solution of Na₂S₂O₃ (25 mL), brine (20 mL), dried over Na₂SO₄, and concentrated in vacuo to dryness. The residue was purified by recrystallization from EtOAc–PE (1:4); yield: 3.0 g (90%); mp 116–117 °C.

¹H NMR (200 MHz, CDCl₃): δ = 4.45 (s, 1 H), 4.83 (s, 1 H), 5.46 (s, 2 H), 6.91 (d, *J* = 8.8 Hz, 2 H), 7.34 (d, *J* = 8.8 Hz, 2 H), 7.45–7.63 (m, 3 H), 7.91 (d, *J* = 7.0 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 191.46, 168.61, 157.55, 134.35, 134.10, 132.78, 130.40, 129.19, 128.00, 115.29, 66.75, 65.35, 6.27.

Anal. Calcd for $C_{17}H_{15}IO_4$: C, 49.78; H, 3.69. Found: C, 49.82; H, 3.52.

4-(Fmoc-asparaginyloxymethyl)phenoxyacetic Acid Phenacyl Ester (4a); Typical Procedure

To a stirred and cooled (0 °C) solution of Fmoc-asparagine (2.6 g, 6 mmol) and DIPEA (0.61 g 5.5 mmol) under an Ar atmosphere was added **3** (2.0 g, 10 mmol), the resulting solution was left overnight at 0 °C. The mixture was taken up in EtOAc (70 mL) and washed successively with H_2O (10 mL), a 5% solution of NaHCO₃ (25 mL), a 10% solution of Na₂S₂O₃ (25 mL), brine (10 mL), and dried over Na₂SO₄. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (CHCl₃– MeOH, 9:1).

¹H NMR (200 MHz, DMSO- d_6): $\delta = 2.52-2.64$ (m, 2 H), 4.14–4.34 (m, 4 H), 4.34–4.50 (m, 1 H), 4.91 (s, 2 H), 5.03 (s, 2 H), 5.60 (s, 2 H), 6.91 (d, J = 8.7 Hz, 2 H), 7.20–7.74 (m, 11 H), 7.86 (d, J = 7.1 Hz, 2 H), 7.95 (d, J = 7.1 Hz, 2 H).

¹³C NMR (75 MHz, DMSO-*d*₆): δ = 193.03, 172.28, 171.45, 169.04, 158.02, 156.52, 144.44, 141.37, 134.75, 134.35, 130.18, 129.61, 129.38, 128.51, 128.31, 127.77, 125.89, 120.79, 115.09, 67.66, 66.45, 65.12, 51.39, 47.24, 37.31.

Anal. Calcd for $C_{36}H_{32}N_2O_8$: C, 69.67; H, 5.20; N, 4.51. Found: C, 69.42; H, 5.45; N, 4.70.

4-[Fmoc-(N^G -Pbf)arginyloxymethyl]phenoxyacetic Acid Phenacyl Ester (4b)

¹H NMR (200 MHz, CDCl₃): δ = 1.39 (s, 6 H), 1.52–1.89 (m, 4 H), 2.04 (s, 3 H), 2.48 (s, 3 H), 2.56 (s, 3 H), 2.86 (s, 2 H), 2.94–3.19 (m, 2 H), 4.01–4.18 (m, 1 H), 4.22–4.39 (m, 3 H), 4.76 (s, 2 H), 4.98 (d, *J* = 11.7 Hz, 1 H), 5.07 (d, *J* = 12.2 Hz, 1 H), 5.40 (s, 2 H), 5.80 (d, *J* = 8.0 Hz, 1 H), 5.90 (br, 1 H), 6.20 (br, 2 H), 6.88 (d, *J* = 8.4 Hz, 2 H), 7.21–7.59 (m, 11 H), 7.71 (d, *J* = 7.4 Hz, 2 H), 7.85 (d, *J* = 8.4 Hz, 2 H).

 13 C NMR (75 MHz, CDCl₃): δ = 191.62, 172.31, 169.00, 158.91, 158.01, 156.49, 156.42, 144.08, 143.91, 141.44, 138.52, 134.42, 133.94, 133.20, 132.44, 130.53, 129.19, 128.86, 128.00, 127.34, 125.39, 124.89, 120.19, 117.72, 114.93, 86.60, 67.30, 67.05, 66.87, 66.80, 65.11, 53.93, 47.28, 43.36, 40.84, 29.88, 28.77, 25.41, 19.56, 18.22, 12.72.

Anal. Calcd for $C_{51}H_{54}N_4O_{11}S$: C, 65.79; H, 5.85; N, 6.02. Found: C, 65.53; H, 5.73; N, 5.80.

4-[Fmoc-(γ-*tert*-butyl)glutamyloxymethyl]phenoxyacetic Acid Phenacyl Ester (4c)

¹H NMR (200 MHz, CDCl₃): δ = 1.44 (s, 9 H), 1.81–2.11 (m, 2 H), 2.21–2.39 (m, 2 H), 4.08–4.19 (m, 1 H), 4.26–4.50 (m, 3 H), 4.75 (s, 2 H), 5.09 (s, 2 H), 5.38 (s, 2 H), 5.73 (d, *J* = 8.1 Hz, 1 H), 6.92 (d, *J* = 8.6 Hz, 2 H), 7.23–7.58 (m, 11 H), 7.72 (d, *J* = 7.4 Hz, 2 H), 7.84 (d, *J* = 8.5 Hz, 2 H).

 13 C NMR (CDCl₃): δ = 191.39, 172.19, 172.12, 168.52, 158.17, 156.25, 144.21, 143.96, 141.49, 134.22, 134.11, 130.36, 129.10, 128.77, 127.99, 127.94, 127.32, 125.40, 125.35, 120.20, 115.03, 80.91, 67.24, 67.12, 66.68, 65.18, 53.86, 47.36, 31.64, 28.32, 27.61.

Anal. Calcd for C₄₁H₄₁NO₁₀: C, 69.58; H, 5.84; N, 1.98. Found: C, 69.32; H, 5.82; N, 1.67.

4-(Fmoc-glycyloxymethyl)phenoxyacetic Acid Phenacyl Ester (4d)

¹H NMR (200 MHz, DMSO- d_6): δ = 3.84 (d, J = 5.9 Hz, 1 H), 4.18– 4.34 (m, 3 H), 4.95 (s, 2 H), 5.08, (s, 2 H), 5.62 (s, 2 H), 6.97 (d, J = 8.6 Hz, 2 H), 7.27 –7.79 (m, 11 H), 7.86 (d, J = 7.0 Hz, 2 H), 7.97 (d, J = 7.1 Hz, 2 H).

¹³C NMR (75 MHz, DMSO-*d*₆): δ = 192.95, 170.83, 169.04, 158.15, 157.25, 144.47, 141.43, 134.71, 134.33, 130.59, 129.57, 129.32, 128.50, 128.31, 127.74, 125.86, 120.76, 115.14, 67.63, 66.50, 66.35, 65.12, 47.28, 42.89.

Anal. Calcd for $C_{34}H_{29}NO_8$: C, 70.46; H, 5.04; N, 2.42. Found: C, 69.82; H, 5.22; N, 2.27.

$\label{eq:linear} \begin{array}{l} \mbox{4-[Fmoc-(ϵ-Boc)-lysyloxymethyl]phenoxyacetic Acid Phenacyl Ester (4e)} \end{array}$

¹H NMR (200 MHz, CDCl₃): δ = 1.42 (s, 9 H), 1.57–2.12 (m, 6 H), 2.93–3.14 (m, 2 H), 4,12–4.22 (m, 1 H), 4.29–4.45 (m, 3 H), 4.60 (br, 1 H), 4.80 (s, 2 H), 5.06 (d, *J* = 11.8 Hz, 1 H), 5.15 (d, *J* = 11.8 Hz, 1 H), 5.44 (s, 2 H), 6.95 (d, *J* = 8.5 Hz, 2 H), 7.25–7.50 (m, 9 H), 7.58 (d, *J* = 7.3 Hz, 2 H), 7.75 (d, *J* = 7.2 Hz, 2 H), 7.88 (d, *J* = 8.6 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl₃): δ = 191.46, 172.54, 168.64, 158.16, 156.29, 156.21, 144.17, 143.96, 141.50, 134.33, 134.08, 130.48, 129.16, 128.83, 127.98, 127.93, 127.30, 125.35, 120.19, 115.02, 67.23, 67.03, 66.72, 65.24, 54.05, 47.37, 40.24, 32.28, 29.80, 28.65, 22.52.

Anal. Calcd for $C_{43}H_{46}N_2O_{10}$: C, 68.78; H, 6.18; N, 3.73. Found: C, 68.66; H, 6.35; N, 3.57.

4-(Fmoc-methionyloxymethyl)phenoxyacetic Acid Phenacyl Ester (4f)

¹H NMR (200 MHz, CDCl₃): δ = 2.01 (s, 3 H), 1.98–2.22 (m, 2 H), 2.30–2.53 (m, 2 H), 4.06–4.60 (m, 4 H), 4.77 (s, 2 H), 5.03–5.17 (m,

Synthesis 2006, No. 16, 2789-2793 © Thieme Stuttgart · New York

2 H), 5.40 (s, 2 H), 5.68 (d, J = 7.9 Hz, 1 H), 6.94 (d, J = 8.5 Hz, 2 H), 7.20–7.60 (m, 11 H), 7.74 (d, J = 7.3 Hz, 2 H), 7.86 (d, J = 8.4 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl₃): δ = 191.46, 172.11, 168.57, 158.20, 156.17, 144.15, 143.95, 141.51, 134.28, 134.08, 130.46, 129.14, 128.74, 127.33, 125.33, 120.23, 115.06, 67.19, 66.73, 65.21, 53.50, 47.38, 31.98, 30.06, 15.64.

Anal. Calcd for $C_{37}H_{35}NO_8S$: C, 67.98; H, 5.40; N, 2.14. Found: C, 68.25; H, 5.57; N, 2.43.

4-(Fmoc-phenylalanyloxymethyl)phenoxyacetic Acid Phenacyl Ester (4g)

¹H NMR (200 MHz, CDCl₃): δ = 3.10 (d, *J* = 5.7 Hz, 2 H), 4.16–4.25 (m, 1 H), 4.29–4.47 (m, 2 H), 4.64–4.74 (m, 1 H), 4.85 (s, 2 H), 5.05 (d, *J* = 11.8 Hz, 1 H), 5.14 (d, *J* = 11.9 Hz, 1 H), 5.27 (d, *J* = 8.3 Hz, 1 H), 5.47 (s, 2 H), 6.97 (d, *J* = 8.6 Hz, 2 H), 7.21–7.63 (m, 16 H), 7.77 (d, *J* = 7.2 Hz, 2 H), 7.90 (d, *J* = 8.6 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 191.44, 171.56, 168.62, 158.23, 155.75, 144.13, 143.97, 141.53, 135.80, 134.34, 134.10, 130.75, 129.60, 129.18, 128.81, 127.99, 127.95, 127.30, 125.36, 125.32, 120.21, 115.02, 67.18, 66.72, 65.28, 55.02, 47.38, 38.38.

Anal. Calcd for $C_{41}H_{35}NO_8$: C, 73.53; H, 5.27; N, 2.09. Found: C, 73.25; H, 5.26; N, 2.37.

4-[Fmoc-(*tert*-butyl)serinyloxymethyl]phenoxyacetic Acid Phenacyl Ester (4h)

¹H NMR (200 MHz, $CDCl_3$): $\delta = 1.11$ (s, 9 H), 3.60 (dd, J = 8.9, 2.8 Hz, 1 H), 3.84 (dd, J = 8.9, 2.3 Hz, 1 H), 4.19–4.56 (m, 4 H), 4.78 (s, 2 H), 5.09 (d, J = 11.9 Hz, 1 H), 5.20 (d, J = 12.1 Hz, 1 H), 5.41 (s, 2 H), 5.79 (d, J = 8.9 Hz, 1 H), 6.96 (d, J = 8.5 Hz, 2 H), 7.26–7.64 (m, 11 H), 7.75 (d, J = 7.4 Hz, 2 H), 7.86 (d, J = 7.2 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl₃): δ = 191.47, 170.83, 168.62, 158.08, 156.39, 144.26, 144.03, 141.49, 134.33, 134.04, 130.37, 129.16, 129.05, 127.99, 127.35, 125.49, 125.43, 120.24, 114.96, 73.67, 67.40, 66.99, 66.74, 65.22, 62.33, 54.98, 47.35, 27.52.

Anal. Calcd for $C_{39}H_{39}NO_9$: C, 70.36; H, 5.90; N, 2.10. Found: C, 70.27; H, 6.12; N, 2.35.

4-(Fmoc-tryptophyloxymethyl])phenoxyacetic Acid Phenacyl Ester (4k)

¹H NMR (200 MHz, CDCl₃): δ = 3.19–3.29 (m, 2 H), 4.13–4.26 (m, 1 H), 4.30–4.45 (m, 2 H), 4.72–4.81 (m, 1 H), 4.85–4.97 (m, 3 H), 5.20 (d, *J* = 11.6 Hz, 1 H), 5.37 (d, *J* = 8.5 Hz, 1 H), 5.51 (s, 2 H), 6.94 (d, *J* = 8.6 Hz, 2 H), 7.02–7.65 (m, 16 H), 7.76 (d, *J* = 7.1 Hz, 2 H), 7.91 (d, *J* = 8.6 Hz, 2 H), 8.31 (br, 1 H).

 13 C NMR (75 MHz, CDCl₃): δ = 191.52, 180.12, 171.91, 169.30, 157.98, 155.97, 144.19, 144.01, 141.49, 136.21, 134.55, 133.92, 131.42, 129.28, 128.03, 127.89, 127.28, 125.44, 123.59, 121.97, 120.16, 119.69, 118.69, 114.63, 111.45, 109.01, 67.28, 66.90, 64.76, 55.06, 47.37, 28.29.

Anal. Calcd for $C_{43}H_{36}N_2O_8{:}$ C, 72.87; H, 5.12; N, 3.95. Found: C, 73.05; H, 5.06; N, 3.78.

4-[Fmoc-(*tert*-butyl)tyrosyloxymethyl]phenoxyacetic Acid Phenacyl Ester (4l)

¹H NMR (200 MHz, CDCl₃): δ = 1.31 (s, 9 H), 2.95–3.15 (m, 2 H), 4.09–4.20 (m, 1 H), 4.28–4.45 (m, 2 H), 4.62–4.72 (m, 1 H), 4.81 (s, 2 H) 5.04 (d, *J* = 11.8 Hz, 1 H), 5.12 (d, *J* = 12.0 Hz, 1 H), 5.42 (s, 2 H), 5.48 (d, *J* = 8.3 Hz, 1 H), 6.83–7.00 (m, 5 H), 7.25–7.61 (m, 12 H), 7.75 (d, *J* = 7.2 Hz, 2 H), 7.86 (d, *J* = 8.6 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 191.48, 171.70, 168.64, 158.19, 155.83, 154.64, 144.14, 143.99, 141.51, 134.34, 134.03, 130.71, 130.08, 129.16, 128.63, 128.00, 127.34, 125.38, 124.40, 120.25, 115.00, 67.14, 66.77, 65.20, 55.18, 47.36, 37.71, 29.08.

Anal. Calcd for $C_{45}H_{43}N_2O_9$: C, 72.86; H, 5.84; N, 1.89. Found: C, 73.15; H 5.75; N, 2.07.

4-(Fmoc-asparaginyloxymethyl)phenoxyacetic Acid (5a); Typical Procedure

To a solution of 4-(Fmoc-asparaginyloxymethyl)phenoxy acetic acid phenacyl ester (**4a**; 1.27 g, 2 mmol) in MeOH–DMF (8:2, 20 mL), AcOH (24 mmol, 1.5 mL) and Mg turnings (0.32 g, 14 mmol) were added. After stirring for 120 min at r.t. the reaction mixture was filtered. The filtrate was concentrated in vacuo and the residue was diluted with a 5% solution of NaHCO₃ (20 mL) and Et₂O– EtOAc (1:1, 20 mL), and the organic layer was extracted with a 5% solution of NaHCO₃ (2 × 20 mL). The aqueous layer was acidified to pH 2–3 with a sat. solution of KHSO₄ and extracted with EtOAc (2 × 20 mL). The organic layer was washed with brine (2 × 20 mL), dried over Na₂SO₄, and filtered. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (CHCl₃–MeOH, 9:1).

¹H NMR (200 MHz, CDCl₃): δ = 2.48–2.60 (m, 2 H), 4.18–4.29 (m, 3 H), 4.37–4.49 (m, 1 H), 4.61 (s, 2 H), 5.01 (s, 2 H), 6.83 (d, *J* = 8.6 Hz, 2 H), 6.94 (br, 2 H), 7.19–7.43 (m, 5 H), 7.68–7.73 (m, 3 H), 7.87 (d, *J* = 7.4 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 172.27, 171.45, 170.80, 158.26, 156.51, 144.44, 141.37, 130.17, 128.99, 128.32, 127.77, 125.89, 120.79, 114.95, 66.50, 65.14, 51.41, 47.27, 37.31.

MS (ESI): $m/z = 517.2 [M - H]^{-}$.

4-[Fmoc-(*N*^{*G*}**-Pbf)-arginyloxymethyl]phenoxyacetic Acid (5b)** ¹H NMR (200 MHz, CDCl₃): δ = 1.37 (s, 6 H), 1.42–1.75 (m, 4 H), 2.01 (s, 3 H), 2.43 (s, 3 H), 2.50 (s, 3 H), 2.83 (s, 2 H), 4.02–4.17 (m, 1 H), 4.18–4.35 (m, 3 H), 4.48 (s, 2 H), 4.82 (br, 1 H), 5.85 (br, 1 H), 6.33 (br, 2 H), 6.76 (d, *J* = 6.0 Hz, 2 H), 7.14–7.36 (m, 6 H),

7.51 (d, *J* = 6.9 Hz, 2 H), 7.69 (d, *J* = 7.2 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 172.21, 170.10, 159.13, 158.10, 156.46, 156.39, 143.98, 143.80, 141.43, 138.72, 132.64, 130.79, 128.69, 127.94, 127.30, 125.32, 125.00, 120.18, 117.86, 114.79, 86.73, 67.31, 53.68, 47.16, 43.24, 28.73, 28.56, 19.47, 18.08, 12.69.

MS (ESI): $m/z = 811.5 [M - H]^{-}$.

4-[Fmoc-(γ*-tert*-butyl)glutamyloxymethyl]phenoxyacetic Acid (5c)

¹H NMR (200 MHz, CDCl₃): δ = 1.38 (s, 9 H), 1.67–2.10 (m, 2 H), 2.12–2.38 (m, 2 H), 4.03–4.19 (m, 1 H), 4.23–4.54 (m, 5 H), 4.97 (s, 2 H), 5.70 (d, *J* = 7.7 Hz, 1 H), 6.76 (d, *J* = 7.0 Hz, 2 H), 7.12–7.38 (m, 6 H), 7.53 (d, *J* = 7.0 Hz, 2 H), 7.70 (d, *J* = 7.3 Hz, 2 H).

 13 C NMR (75 MHz, CDCl₃): δ = 172.39, 172.19, 157.83, 156.34, 144.11, 143.84, 143.45, 141.42, 130.25, 127.90, 127.58, 127.48, 127.27, 127.11, 125.31, 120.17, 81.11, 67.31, 67.08, 53.77, 47.25, 31.62, 30.75, 28.25, 27.50, 25.27, 25.04.

MS (ESI): $m/z = 588.3 [M - H]^{-}$.

4-(Fmoc-glycyloxymethyl)phenoxyacetic Acid (5d)

¹H NMR (200 MHz, DMSO- d_6): δ = 3.75 (d, *J* = 6.1 Hz, 2 H), 4.19–4.29 (m, 3 H), 4.60 (br, 1 H), 4.62 (s, 2 H), 5.01 (s, 2 H), 6.84 (d, *J* = 7.1 Hz, 2 H), 7.23–7.77 (m, 8 H), 7.85 (d, *J* = 7.3 Hz, 2 H).

¹³C NMR (75 MHz, DMSO-*d*₆): δ = 170.76, 158.33, 157.16, 144.44, 141.38, 130.55, 128.92, 128.29, 127.73, 125.83, 120.77, 114.98, 66.42, 66.34, 65.09, 47.23, 42.86.

MS (ESI): $m/z = 460.2 [M - H]^{-}$.

4-[Fmoc-(ε-Boc)-lysyloxymethyl]phenoxyacetic Acid (5e)

¹H NMR (200 MHz, CDCl₃): δ = 1.39 (s, 9 H), 0.81–1.86 (m, 6 H), 2.84–3.11 (m, 2 H), 4.06–4.18 (m, 1 H), 4.32–4.76 (m, 5 H), 4.95 (d, *J* = 11.6 Hz, 1 H), 5.07 (d, *J* = 11.7 Hz, 1 H), 5.67 (d, *J* = 7.0

Hz, 1 H), 6.80 (d, *J* = 7.3 Hz, 2 H), 7.15–7.39 (m, 6 H), 7.55 (d, *J* = 7.3 Hz, 2 H), 7.72 (d, *J* = 7.4 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl₃): δ = 172.60, 158.21, 156.52, 156.33, 144.11, 143.91, 141.47, 130.43, 127.93, 127.28, 125.33, 120.20, 115.27, 115.22, 79.69, 67.22, 66.92, 53.99, 47.29, 40.32, 32.17, 29.69, 28.61, 22.44.

MS (ESI): $m/z = 631.4 [M - H]^{-}$.

4-(Fmoc-methionyloxymethyl)phenoxyacetic Acid (5f)

¹H NMR (200 MHz, CDCl₃): $\delta = 2.00$ (s, 3 H), 1.85–2.23 (m, 2 H), 2.33–2.52 (m, 2 H), 4.07–4.43 (m, 4 H), 4.51 (s, 2 H), 5.06 (s, 2 H), 5.53 (br, 1 H), 6.83 (d, J = 7.6 Hz, 2 H), 7.16–7.46 (m, 6 H), 7.56 (d, J = 7.5 Hz, 2 H), 7.74 (d, J = 7.3 Hz, 2 H).

¹³C NMR (75 MHz, CDCl₃): δ = 174.18, 172.23, 157.90, 156.24, 144.03, 143.80, 141.45, 130.39, 128.73, 127.95, 127.28, 125.27, 120.21,115.17, 67.24, 53.37, 47.26, 31.90, 29.99, 15.62.

MS (ESI): $m/z = 534.3 [M - H]^{-}$.

4-(Fmoc-phenylalanyloxymethyl)phenoxyacetic Acid (5g)

¹H NMR (200 MHz, CDCl₃): δ = 2.96–3.10 (m, 2 H), 4.18–4.55 (m, 6 H), 4.97 (s, 2 H), 5.41 (d, *J* = 7.8 Hz, 1 H), 6.80 (d, *J* = 7.5 Hz, 2 H), 6.96–7.39 (m, 11 H), 7.50 (d, *J* = 6.3 Hz, 2 H), 7.72 (d, *J* = 7.3 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl₃): δ = 171.73, 163.73, 158.13, 155.91, 144.05, 143.90, 141.48, 135.87, 130.60, 129.57, 128.79, 128.54, 127.95, 127.30, 125.37, 125.32, 120.21, 115.18, 67.20, 67.11, 55.05, 47.27, 47.13, 38.26, 37.12, 32.00.

MS (ESI): $m/z = 550.3 [M - H]^{-}$.

4-[Fmoc-(*tert*-butyl)serinyloxymethyl]phenoxyacetic Acid (5h) ¹H NMR (200 MHz, CDCl₃): $\delta = 1.10$ (s, 9 H), 3.59 (dd, J = 9.0, 3.0 Hz, 1 H), 3.83 (dd, J = 9.1, 2.7 Hz, 1 H), 4.19–4.29 (m, 1 H), 4.34–4.43 (m, 2 H), 4.50–4.55 (m, 1 H), 4.60 (s, 2 H), 5.08 (d, J = 12.0 Hz, 1 H), 5.18 (d, J = 12.1 Hz, 1 H), 5.82 (d, J = 9.0 Hz, 1 H), 6.87 (d, J = 8.6 Hz, 2 H), 7.21–7.49 (m, 6 H), 7.61 (d, J = 7.3 Hz, 2 H), 7.76 (d, J = 7.2 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl₃): δ = 172.88, 170.94, 157.87, 156.58, 144.18, 143.94, 141.49, 130.42, 129.06, 127.96, 127.62, 127.34, 127.16, 125.45, 125.39, 125.14, 120.22, 114.84, 73.80, 67.55, 67.04, 65.06, 62.29, 54.94, 47.29, 27.48, 25.18, 25.04.

MS (ESI): $m/z = 546.2 [M - H]^{-}$.

4-(Fmoc-tryptophyloxymethyl]) (5k)

¹H NMR (200 MHz, CDCl₃): δ = 2.94–3.27 (m, 2 H), 4.01–4.18 (m, 1 H), 4.195–4.558 (m, 3 H), 4.601–5.037 (m, 3 H), 5.53 (d, *J* = 7.7 Hz, 1 H), 6.45 (s, 1 H), 6.66 (d, *J* = 6.3 Hz, 2 H), 6.81–7.49 (m, 12 H), 7.71 (d, *J* = 7.4 Hz, 2 H), 8.44 (s, 1 H).

¹³C NMR (75 MHz, CDCl₃): δ = 172.18, 157.78, 156.28, 144.07, 143.86, 141.45, 136.33, 130.71, 130.60, 127.95, 127.81, 127.31, 125.39, 123.52, 122.21, 120.20, 119.80, 118.66, 114.95, 111.66, 109.24, 67.39, 66.87, 55.11, 47.23.

MS (ESI): $m/z = 589.5 [M - H]^{-}$.

4-[Fmoc-(O-tert-butyl)-tyrosyloxymethyl] (5l)

¹H NMR (200 MHz, CDCl₃): $\delta = 1.27$ (s, 9 H), 2.83–3.14 (m, 2 H), 3.98–4.15 (m, 1 H), 4.21–4.47 (m, 4 H), 4.53–4.69 (m, 1 H), 4.95 (s, 2 H), 5.48 (d, J = 6.7 Hz, 1 H), 6.70–6.90 (m, 5 H), 7.10–7.39 (m, 8 H), 7.51 (d, J = 6.5 Hz, 2 H), 7.72 (d, J = 7.2 Hz, 2 H).

 ^{13}C NMR (75 MHz, CDCl₃): δ = 171.75, 158.01, 155.92, 154.53, 144.07, 143.92, 141.48, 130.71, 130.43, 130.00, 127.93, 127.62, 127.47, 127.37, 127.28, 127.15, 127.12, 125.33, 124.35, 120.19, 115.36, 78.74, 67.23, 67.03, 55.09, 47.30, 37.68, 29.02, 25.27, 24.98.

MS (ESI): $m/z = 622.4 [M - H]^{-}$.

4-[Fmoc-(γ-tert-butyl)glutamyloxymethyl]phenoxyacetate Resin

Aminomethyl PS-DVB resin (100 mg, 1 mmol NH₂/g) was placed into a syringe for solid-phase peptide synthesis and left to swell for 30 min. Derivative **5c** (240 mg, 0.4 mmol), HOBt (64 mg, 0.4 mmol), and PyBOP (0.4 mmol) were dissolved into DMF (1 mL) and after addition of DIPEA (104 mg, 0.8 mmol) the solution was transferred into the reaction syringe. The resin was gently agitated for 4 h. Then the reaction mixture was washed with DMF (6 × 2 mL), CH₂Cl₂ (6 × 2 mL), Et₂O (2 × 2 mL), and dried; yield: 152 mg (97%); the Kaiser ninhydrine test was negative; Fmoc estimation 0.66 mmol/g.

Acknowledgment

This work was supported by funds from 'Special Account for Research Grants' of the National and Capodistrian University of Athens.

References

- (1) Merrifield, R. B. J. Am. Chem. Soc. 1963, 85, 2145.
- (2) (a) Chan, W. C.; White, P. D. *Fmoc Solid Phase Peptide Synthesis*; OUP: Oxford, **2000**. (b) Fields, G. B. *Methods in Enzymology*, Vol. 289; Academic Press: New York, **1997**.
- (3) Wang, S.-S. J. Am. Chem. Soc. 1973, 95, 1328.
- (4) Wang, S.-S.; Tam, J. P.; Wang, B. S. H.; Merrifield, R. B. Int. J. Pept. Protein Res. 1981, 18, 459.
- (5) Atherton, E.; Logan, C. J.; Shepard, R. C. J. Chem. Soc., Perkin Trans. 1 1981, 538.
- (6) Shepard, R. C.; Williams, B. J. Int. J. Pept. Protein Res. 1982, 20, 451.
- (7) Atherton, E.; Benoiton, N. L.; Brown, E.; Sheppard, R. C.; Williams, B. J. J. Chem. Soc., Chem. Commun. 1981, 336.

Downloaded by: Collections and Technical Services Department. Copyrighted material

- (8) Atherton, E.; Logan, C. J.; Sheppard, R. C. J. Chem. Soc., *Perkin Trans. 1* **1981**, 538.
- (9) Bernatowicz, M. S.; Kearney, T.; Neves, R. S.; Köster, H. *Tetrahedron Lett.* **1989**, *30*, 4341.
- Mitchell, A. R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. J. Org. Chem. 1978, 43, 2845.
- (11) (a) Greene, T. W.; Wuts, P. G. M. Protective Groups in Organic Synthesis, 3rd ed.; J. Wiley & Sons: New York, 1999, 393–394. (b) Greene, T. W.; Wuts, P. G. M. Protective Groups in Organic Synthesis, 3rd ed.; J. Wiley & Sons: New York, 1999, 728.
- (12) Kokinaki, S.; Leondiadis, L.; Ferderigos, N. *Org. Lett.* **2005**, 7, 1723.
- (13) Stelakatos, G. C.; Zervas, L. J. Chem. Soc. C 1966, 1191.
- (14) Olah, G. A.; Narang, S. C.; Balaram Gupta, B. G.; Malhotra, R. J. Org. Chem. 1979, 44, 1247.
- (15) Tam, J. P.; Kent, S. B. H.; Wong, T. W.; Merrifield, R. B. Synthesis 1980, 955.
- (16) Clark, J. H.; Miller, M. Tetrahedron Lett. 1977, 599.
- (17) Wang, S.-S.; Gisin, B. F.; Winter, D. P.; Makofske, R.;
 Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. J. Org. Chem. 1977, 42, 1286.
- (18) Hendrickson, J. B.; Kandall, C. Tetrahedron Lett. 1970, 343.
- (19) Zikos, C. C.; Ferderigos, N. *Tetrahedron Lett.* **1995**, *36*, 3741.
- (20) Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.
- (21) Chang, C. D.; Waki, M.; Ahmad, M.; Meienhofer, J.; Lundell, E. O.; Haug, J. D. *Int. J. Pept. Protein Res.* **1980**, *15*, 59.