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SOLID PHASE SYNTHESIS OF TYROSINE-CONTAINING HISTONE FRAGMENTS

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Abstract—The solid phase synthesis of Ac-Val-Val-Tyr-Ala-Leu-OH (**1**), Ac-Glu-Ala-Tyr-Leu-Val-OH (**2**) and Ac-Leu-Tyr-Gly-Phe-Gly-Gly-OH (**3**), segments 86–90 of histone H4, 97–101 of histone H3 and 97–102 of histone H4, respectively, is described using chloromethylresin and chloromethyl-Pab-resin as solid supports. In the synthesis of peptides **2** and **3** using 2, 6-diclorobenzyl ether as tyrosine side chain protection, 7% and 8% of the 3-dichlorobenzyltyrosine-rearranged peptide could be isolated by Bio-Gel P-2 or diethylaminoethylcellulose chromatography. Alternative use of cyclohexyl ether as tyrosine protection has been explored with satisfactory results.

Eucharistic chromatin is constituted by linear arrays of repetitive units called nucleosomes.[1,2] The nucleosome has been defined as a nucleoprotein complex made up of a superhelical DNA filament of about 140 base pairs wound around a protein octamer formed by two copies of each of the histones H2A, H2B, H3 and H4.[3] Although considerable advances have made in recent years in the understanding of nucleosome morphology, most details about its internal architecture still remain to be ellucidated. A simplified inroad to this latter problem has been the study of the interactions between histone peptide models and DNA. In this context, the chemical synthesis of significant histone fragments is undoubtedly of great interest.[4–7]

In this paper we wish to describe the synthesis of the peptides Ac-Val-Val-Tyr-Ala-Leu-OH (**1**), Ac-Glu-Ala-Tyr-Leu-Val-OH (**2**) and Ac-Leu-Tyr-Gly-Phe-Gly-Gly-OH (**3**), which correspond to the segments 86–90 of histone H4, 97–101 of histone H3 and 97–102 of histone H4, respectively. These peptide sequences have been synthesized with the aim of deepening our present knowledge of the role played by tyrosine residues in histone-DNA interactions in the nucleosome. Gabbay et al[8,9] and Mayer et al.[10] have approached this problem by studying the interactions of DNA with small aromatic peptides, generally not natural histone sequences, and have reached divergent conclusions concerning the possibility of intercalation of the aromatic ring of tyrosine between DNA base pairs. Peptides **1**–**3** have been synthesized by solid phase procedures.[11] Attention has also been directed to some related methodological aspects such as tyrosine side chain protection and the use of the recently developed Pab-resin[12] of improved acid stability.

RESULTS AND DISCUSSION

Ac-Val-Val-Tyr-Ala-Leu-OH **1** was synthesized on a chloromethyl-polystyrene resin. Boc-L-Leu-OCH_2-resin was prepared by the cesium salt procedure.[13] Boc-L-Ala-OH, Boc-L-Tyr(Dcbzl)-OH and Boc-L-Val-OH were subsequently assembled onto the desired sequence by stepwise coupling using the protocol described in the experimental section. The extension of the coupling was systematically monitored after each synthetic cycle by the ninhydrin test.[14] The amine content of the polymer was also checked at several stages of the synthesis by the picric acid method.[15] After HF treatment, crude **1** was obtained, which was found to be not totally homogeneus by TLC (chloroform–methanol–acetic acid 14:4:1). Attempts to purify peptide **1** either by adsorption chromatography on silica gel using the above solvent system or by gel filtration on Sephadex G-10 were not successful. Finally, **1** was readily purified by anion exchange chromatography on diethylaminoethylcellulose. The main peak yielded pure Ac-Val-Val-Tyr-Ala-Leu-OH in a 51% combined synthesis-purification yield. Amino acid analysis after 24 hr hydrolysis showed a low valine content: $Ala_{1.00}Val_{1.24}Leu_{1.05}Tyr_{0.95}$ due to incomplete cleavage of the Val-Val bond. However, amino acid composition after 72 hr hydrolysis was in good agreement with the expected structure: $Ala_{1.00}Val_{1.93}Leu_{1.03}Tyr_{0.99}$. Peptide **1** was shown to be homogeneus by both TLC and HPLC and was further characterized by ^1H NMR spectroscopy as well as mass spectrometry of its permethylated derivative.[16]

Ac-Glu-Ala-Tyr-Leu-Val-OH **2** was synthesized on a chloromethyl-Pab-resin. Boc-L-Val-OCH_2-Pab-resin was prepared by the cesium salt method. Boc-L-Leu-OH, Boc-L-Tyr(Dcbzl)-OH, Boc-L-Ala-OH and Boc-L-Glu(Bzl)-OH were incorporated following the same protocol used for peptide **1**. After HF treatment (67% yield) the crude peptide was purified by gel filtration on Bio-Gel P-2. All peptide material eluted as a single peak ($Glu_{1.00}Ala_{1.02}Val_{1.02}Leu_{0.93}Tyr_{0.81}$), the remaining peaks being non-peptide impurities derived from the HF reaction. Next, pep-

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tide **2** was chromatographed on diethylaminoethylcellulose ($Glu_{1.00}Ala_{1.03}Val_{1.00}Leu_{1.00}Tyr_{0.87}$). The global purification yield was 60%. Peptide **2** was found to be homogeneus by both TLC and HPLC and was additionally characterized by ^1H NMR spectroscopy.

Amino acid analysis of the slowest running fraction (7%) showed no tyrosine content: $Glu_{1.05}Ala_{1.06}Val_{0.90}Leu_{0.99}$. However, this fraction exhibited an UV absorption spectrum characteristic of tyrosine derivatives and gave a positive Pauly reaction in TLC. It was characterized as Ac–Glu–Ala–Tyr(3-Dcbzl)–Leu–Val–OH, arising from rearrangement during HF treatment of the 2, 6-dichlorobenzyl protection group of tyrosine,[17] ^1H NMR spectrum of this product was nearly identical with that of peptide **2** except in the aromatic region, where a complex group of signals appeared instead of the double doublet pattern typical of tyrosine (Table 1). Mass spectrum of the permethylated derivative of this fraction showed a peak at *m/e* 634 corresponding to Ac–Me_2Glu–MeAla–Me_2Tyr(3-Dcbzl)$^+$ which supported the above assignment.

Ac–Leu–Tyr–Gly–Phe–Gly–Gly–OH **3** was first synthesized on a chloromethylated polystyrene support. Boc–Gly–OCH_2-resin was prepared by the cesium salt method. After coupling of Boc–Gly–OH, Boc–L–Phe–OH, Boc–L–Tyr(Dcbzl)–OH and Boc–L–Leu–OH, HF treatment of the polymer afforded crude **3** in a 79% yield. Purification started with a Bio–Gel P-2 filtration, which allowed two fractions to be separated. The major component, ($Gly_{3.11}Leu_{0.95}Phe_{1.03}Tyr_{0.92}$, 61% yield), contained the desired product and was further purified by anion exchange chromatography on diethylaminoethylcellulose to give pure Ac–Leu–Tyr–Gly–Phe–Gly–Gly–OH ($Gly_{2.91}Leu_{1.00}Phe_{1.04}Tyr_{0.94}$) in a 77% yield. Peptide **3** was homogeneus by both HPLC and TLC and was also characterized by ^1H NMR spectroscopy.

The minor component (8%) contained practically no tyrosine ($Gly_{3.00}Leu_{0.96}Phe_{1.00}Tyr_{0.13}$) and exhibited a similar behavior than the rearranged product formed in the HF removal of peptide **2** (similar UV and positive Pauly reaction in TLC). It was likewise characterized as Ac–Leu–Tyr(3-Dcbzl)–Gly–Phe–Gly–Gly–OH.

As can be seen, significant amounts of tyrosine-rearranged peptides have been detected in the synthesis of both **2** and **3** using 2, 6-dichlorobenzyl ether as tyrosine side chain protection. In an attempt to decrease the extent of this undesirable side reaction, we have explored in a second synthesis of **3** the use of cyclohexyl ether protection for tyrosine, recently introduced by Engelhard and Merrifield.[18] In this case the synthesis was carried out on a chloromethyl–Pab–resin, which had been shown to be rather advantageous in the synthesis of peptide **2** described above. Previous utilization of Pab–resin includes the synthesis of the 39–43 fragment of the histone H4[12] and of somatostatin-28 and catfish somatostatin.[19] Boc–L–Tyr(cHex)–OH was synthesized following an improved procedure, based on that of Engelhard and Merrifield,[18] which is described in the experimental section. The remaining amino acids were incorporated as in the previous synthesis of **3**. HF treatment yielded crude **3** in a 75% cleavage yield. Purification by Bio–Gel P-2 gave three fractions. The main component was further purified by anion exchange chromatography on diethylaminoethylcellulose and characterized as pure **3** ($Gly_{3.03}Leu_{1.00}Tyr_{0.88}Phe_{0.99}$; global purification yield 65%). In contrast with the previous synthesis using 2, 6-dichlorobenzyl ether protection for tyrosine, in this case no rearranged 3-cyclohexyltyrosine-containing peptide could be found on careful HPLC scrutiny.

It can be seen from the above results that the extent of the 2, 6-dichlorobenzyl group rearrangement under the standard cleavage conditions used throughout this work (HF–anisole 9:1) is certainly dependent on the peptide sequence. Thus, no rearranged peptides were detected neither in the synthesis of Ac–Val–Val–Tyr–Ala–Leu–OH **1** nor in that of Ac–His–Arg–Tyr–Arg–Pro–OH.[12] In contrast, tyrosine-rearranged peptides were found in the syntheses of both **2** and **3** in 7% and 8% amounts relative to the main product, respectively. These byproduct formation levels, still higher than the 5% reported by Erickson and Merrifield[17] for the free amino acid, seem to indicate that serious purification problems could be expected

Table 1. ^1H NMR spectra of the aromatic region of the tyrosine rearrangement products of peptides **2** and **3**

Proton	Chemical shift (δ) A[+]	B
CH_2 (Dcbzl)	4.09 (s)	4.09 (s)
C_2-H (Tyr)	6.25 (s)	6.20 (s)
C_5-H (Tyr)	6.71 (d)	6.68 (d)
C_6-H (Tyr)	6.88 (d)	6.87 (d)
$C_{4'}$-H (Dcbzl)	7.36 (d)	7.29 (d)
$C_{3',5'}$-H (Dcbzl)	7.50 (d)	7.48 (d)

[+]A and B denote the rearrangement products of peptides **2** and **3**, respectively.

to arise with peptides containing several tyrosine residues synthesized by this particular protection strategy. On the other hand, our results seem to confirm that cyclohexyl ether protection can be an advantageous alternative in those cases. Finally, Pab-resin has again been found to be a very convenient solid support for peptide synthesis.

EXPERIMENTAL

Dichloromethane was dried over anhydrous potassium carbonate and distilled over it immediately before use. Dimethylformamide was dried over 4 Å molecular sieves and freed of amines by nitrogen bubbling until a negative 1-fluoro-2, 4-dinitrobenzene test was observed. Hydrolysates for amino acid analyses from peptide or peptide-resin samples were prepared by treatment with 6N hydrochloric acid for 24 hr or with 12N hydrochloric acid/glacial acetic acid (1:1) for 48 hr in vacuum-degassed sealed tubes at 110° with norleucine added as an internal standard. Amino acid analyses were obtained in a Beckman 120 autoanalyzer. TLC was performed on precoated silica gel plates (Merck, 0.2 mm). Analytical HPLC was carried out in a Waters ALC/GPC 205 U system. Preparative HPLC was done in a Waters Prep LC/500 instrument. ^{1}H NMR spectra were recorded at 200 MHz in a Varian XL-200 spectrometer, operating in the Fourier transform mode. Permethylated peptide derivatives were prepared as described by Thomas[20] and subsequently analyzed in an AEI MS 902S spectrometer at 70 eV. Copoly (styrene-1%-divinylbenzene) beads (Bio-Beads SX1) and its chloromethylated derivative were purchased from Bio-Rad Laboratories. Chloromethyl-Pab-resin was prepared in our laboratory as previously described.[12] Boc-L-Ala-OH, Boc-Gly-OH, Boc-L-Glu(Bzl)-OH, Boc-L-Leu-OH.H_2O, Boc-L-Phe-OH and Boc-L-Val-OH were from Fluka and its purity was checked by TLC. Boc-L-Tyr(Dcbzl)-OH was prepared in our laboratory as described by Yamashiro and Li.[21]

Boc-(0-cyclohexyl)-L-tyrosine dicyclohexylammonium salt[18]

L-Tyrosine (30 g, 0.17 mole) was quantitatively converted into its methyl ester hydrochloride by three successive treatments with methanolic 1.5 M HCl (300 mL each) for 2 hr at room temperature. After each treatment the solvent was removed and the residue was triturated with anhydrous benzene and evaporated to dryness. The resulting ester (15 g, 0.06 mole) was suspended in 200 mL of CH_2Cl_2 and 20 g of trifluoroacetic anhydride in 50 mL of CH_2Cl_2 were slowly added with vigorous stirring. After 2 hr at room temperature, the resulting yellowish solution was evaporated to dryness yielding 18.9 g (100%) of pure Tfa-L-Tyr-OMe, which was used without further purification. Tfa-L-Tyr-OMe (20 g, 0.07 mole), cyclohexene (50 mL, previously distilled from sodium) and BF_3-etherate (2 mL, freshly distilled from sodium hydride) were dissolved in 100 mL of CH_2Cl_2 and refluxed under nitrogen with vigorous magnetic stirring for 24 hr. After 12 hr, additional amounts of cyclohexene (25 mL) and BF_3-etherate (1 mL) were added. The progress of the reaction was monitored by TLC as described. When the reaction was complete (usually after 24 hr), the phenolic components of the mixture were removed by extraction with 2 N NaOH and the resulting organic phase was washed with 1 N HCl and water, dried and evaporated to dryness. The residue was filtered through a 3 × 40 cm silica gel column eluted with chloroform. 10 g of this purified crude were submitted to preparative HPLC using two Waters PrepPack silica gel cartridges (in series) eluted with CH_2Cl_2 at 200 mL/min. Fractions corresponding to the major component (2.4–3.0 L.) were pooled and freed of solvent, affording 7.5 g (59% yield) of white, crystalline Tfa-L-Tyr(cHex)-OMe. Analysis: Calculated for $C_{18}H_{22}F_3O_4N$: C, 57.9%; H, 5.90%; N, 3.75%. Found: C, 57.3%; H, 5.82%; N, 4.04%. $[\alpha]_D^{20} = +98.2°$ (c 1, chloroform); +7.4° (c 1, methanol). Hydrolysis of Tfa-L-Tyr (cHex)-OMe and conversion of the resulting H-L-Tyr (cHex)-OH into Boc-L-Tyr(cHex)-OH were carried out as described by Engelhard and Merrifield (1978). For H-L-Tyr(cHex)-OH, $[\alpha]_D^{20} = -9.6°$ (c 1, glacial acetic acid), and for Boc-L-Tyr(cHex)-OH.DCHA, $[\alpha]_D^{20} = +29.5°$ (c 1, methanol). The optical purity of both compounds (> 99.5%) was checked by their conversion into H-Leu-Tyr-OH and ion exchange chromatographic analysis of the potential diastereomers.[22]

Ac-Val-Val-Tyr-Ala-Leu-OH **1**

Cesium Boc-L-leucinate (1.0 g, 2.8 mmole) was reacted with 2.5 g of chloromethyl-polystyrene (0.89 mmole Cl/g) in DMF at 50° for 18 hr (Gisin, 1973). 2.5 g of the resulting Boc-L-Leu-OCH_2-resin (0.48 mmole/g) were then placed in the reaction vessel of a custom-made synthesizer[23] and submitted to the following synthetic protocol for the incorporation of each amino acid residue: (1) 1 × 2 min 30% TFA-CH_2Cl_2; (2) 1 × 30 min 30% TFA-CH_2Cl_2; (3) 4 × 2 min CH_2Cl_2; (4) 1 × 2 min 5% DIEA-CH_2Cl_2; (5) 1 × 30 min 5% DIEA-CH_2Cl_2; (6) 4 × 2 min CH_2Cl_2; (7) shake with 2.5 equivalent of Boc-amino acid for 10 min; (8) without filtering, add 2.5 equivalent of DCC and shake for 120 min (eventually overnight); (9) 4 × 2 min CH_2Cl_2; (10) 4 × 2 min DMF; (11) 4 × 2 min CH_2Cl_2; (12) repeat from step (4). Final acetylation was performed according to the same program introducing 5 equiv of glacial AcOH and DCC in steps (7) and (8), respectively. The resulting Ac-Val-Val-Tyr(Dcbzl)-Ala-Leu-OCH_2-resin was then treated with 60 mL of anhydrous HF and 6 mL of anisole at 0° for 1 hr to give crude **1** (800 mg). Anion exchange chromatography was performed on a 20 × 2 cm diethylaminoethylcellulose (Whatman DE 52) column loaded with 80 mg crude and eluted with a linear gradient of ammonium carbonate (0.6–10 mS) at pH 8.5 (30 mL/hr). Peptide **1** was characterized as follows: TLC: single spot, R_f 0.60; chloroform–methanol–acetic acid (14:4:1). hplc: single peak, 11.6 min; Waters μ-Bondapack C_{18} column; elution with a 30–60% acetonitrile-0.05 M triethylamine phospate buffer (pH 3.0) gradient for 10 min; flow: 2 mL/min. ^{1}H NMR(d_6-DMSO): δ 0.73–0.79 (m, $^{\gamma}CH_3$(Val); 0.83–0.86 (dd), $^{\delta}CH_3$(Leu); 1.17 (d), $^{\beta}CH_3$(Ala); 1.32–1.70 (m), $^{\gamma}$CH(Leu) and $^{\beta}CH_2$(Leu); 1.80–1.90 (m). $^{\beta}CH_2$(Val); 1.84 (s), CH_3(Ac); 2.64–2.97 (m), $^{\beta}CH_2$(Tyr); 3.95–4.35 (m), $^{\alpha}$CH(Leu, Val); 4.22(m), $^{\alpha}$CH(Ala); 4.45(m), $^{\alpha}$CH(Tyr); 6.59 (d), $C_{3,5}$-H(Tyr); 6.99 (d), $C_{2,6}$-H(Tyr); 7.60–8.09 (5 × d), NH(Ala, Val, Leu, Tyr). MS: *m/e* 703 (Ac-MeVal-MeVal-Me_2Tyr-MeAla-MeLeu-OMe^+); 545 (Ac-MeVal-MeVal-Me_2Tyr-MeAla$^+$); 460 (Ac-MeVal-MeVal-Me_2Tyr$^+$); 269 (Ac-MeVal-MeVal$^+$); 156 (Ac-MeVal$^+$). $[\alpha]_D^{20} = -85°$ (c 0.25, 0.25 M acetic acid).

Ac-Glu-Ala-Tyr-Leu-Val-OH **2**

Cesium Boc-L-valinate (0.88 g, 2.5 mmole) was reacted with 3.2 g of chloromethyl-Pab-resin (0.63 mmole Cl/g) in DMF at 50° for 18 hr to give Boc-L-Val-OCH_2-Pab-resin with a substitution of 0.57 mmole/g. Peptide synthesis was then performed upon 2.0 g of this polymer following the same protocol described for peptide **1**. 2.5 g of the resulting Ac-Glu(bzl)-Ala-Tyr(Dcbzl)-Leu-Val-OCH_2-Pab-resin were treated with 60 mL of anhydrous HF and 6 mL of anisole at 0° for 1 hr yielding 1.0 mmole of crude **2**. Bio-Gel P-2 chromatography was performed on a 100 × 2 cm column loaded with 42 mg of crude and eluted with 0.1 M ammonium acetate, pH 8.5, at 35 mL/hr. Anion-exchange chromatography was performed on a 20 × 2 cm diethylaminoethylcellulose (Whatman DE 52) column loaded with 82 mg of peptide and eluted with a linear gradient of ammonium acetate (2–25 mS) at pH 8.5 (35 mL/hr). Peptide **2** was characterized as follows: TLC: single spot, R_f 0.65, isopropanol-formic acid–water (44:5:1) single spot, R_f 0.83, chloroform–methanol–ammonium hydroxide (15:9:2). HPCL: single peak, 3.4 min, Merck Lic-

hrosorb RP-18 column; elution with 22% acetonitrile in 0.01 M ammonium acetate (pH 4.0); flow rate: 2 mL/min ^{1}H NMR (d_6-DMSO): δ 0.83–0.89 (m), $^{\gamma}CH_3$(Val) and $^{\delta}CH_3$(Leu); 1.14 (d), $^{\beta}CH_3$(Ala); 1.48 (m), $^{\beta}CH_2$(Leu) and $^{\gamma}CH_2$(Leu); 1.8–2.0 (m), $^{\beta}CH$(Val) and $^{\beta}CH_2$(Glu); 1.84 (s), CH_3(Ac); 2.24 (t), $^{\gamma}CH_2$(Glu); 2.80–2.90 (m), $^{\beta}CH_2$(Tyr); 3.96 (dd), $^{\alpha}CH$(Val); 4.1–4.5 (m), $^{\alpha}CH$(Glu, Ala, Leu, Tyr); 6.61 (d), $C_{3,5}$–H(Tyr); 6.99 (d), $C_{2,6}$–H(Tyr); 7.60–8.14 (5 × d), NH(Glu, Ala, Val, Leu, Tyr). $[\alpha]_D^{20} = -76.5°$ (c 0.4, 0.1 M AcOH).

The tyrosine C-benzylated peptide, Ac–Glu–Ala–Tyr(3-Dcbzl)–Leu–Val–OH, was characterized as follows: TLC: single spot, R_f 0.67, isopropanol-formic acid–water (44:5:1) ^{1}H NMR: see Table 1. MS: *m/e* 634 (Ac–Me_2Glu–MeAla–Me_2Tyr(3-Dcbzl)$^+$), 285 (Ac–Me_2Glu–MeAla$^+$), 200 (Ac–Me_2Glu$^+$).

Ac–Leu–Tyr–Gly–Phe–Gly–Gly–OH **3**

Synthesis on a chloromethyl-resin. Cesium Boc-glycinate (1.1 g, 3.4 mmole) was reacted with 3.2 g of chloromethyl-polystyrene (0.89 mmole Cl/g) in DMF at 50° for 18 hr to give Boc–Gly–OCH_2–resin (0.61 mmole/g) Peptide synthesis was performed upon 2.0 g of this polymer as described above. After HF treatment of 2.8 g of peptide–OCH_2–resin (40 mL HF; 4 mL anisole; 1 hr, 0°), 620 mg of crude **3** were obtained. Bio–Gel P-2 chromatography was performed on a 100 × 2 cm column loaded with 220 mg of crude and eluted with 0.1 M ammonium acetate, pH 10.0, at 36 mL/hr. Anion-exchange chromatography was performed on a 20 × 2 cm diethylaminoethylcellulose (Whatman DE 52) column loaded with 190 g of peptide and eluted with a linear gradient of ammonium acetate (2–30 mS, pH 8.5), at 38 mL/hr. Peptide **3** was characterized as follows: TLC: single spot, R_f 0.19, chloroform–methanol–acetic acid (14:4:1), single spot, R_f 0.56 *n*-butanol acetic acid–water (4:2:1). HPLC: single peak, 8.2 min; Waters μ-Bondapack C_{18} column; elution with a 20–80% acetonitrile-0.05 triethylamine phosphate buffer (pH 3.0) gradient for 25 min; flow rate: 2 mL/min. ^{1}H NMR (d_6-DMSO): δ 0.71–0.80 (dd), $^{\delta}CH_3$(Leu); 1.2–1.4 (m), $^{\gamma}CH$ and $^{\beta}CH_2$(Leu); 1.79 (s), CH_3(Ac); 2.76–2.96 (m), $^{\beta}CH_2$(Tyr); 2.76–3.12 (m), $^{\beta}CH_2$(Phe); 3.40–4.02 (m), $^{\alpha}CH_2$(Gly); 4.14–4.18 (m), $^{\alpha}CH$(Leu); 4.33 (m), $^{\alpha}CH$(Phe, Tyr); 6.59 (dd), $C_{3,5}$–H(Tyr); 7.00 (dd), $C_{2,6}$–H(Tyr); 7.24 (s), Ar–H(Phe); 8.32 (d), NH (Phe); 8.48, 8.57 (2 × s), NH(Gly); 8.90 (d), NH(Leu); 9.14 (d), NH(Tyr).

The rearrangement product, Ac–Leu–Tyr(3-Dcbzl)–Gly–Phe–Gly–Gly–OH, was characterized as follows: TLC: single spot, R_f 0.62, *n*-butanol–acetic acid–water (4:2:1) single spot, R_f 0.70, isopropanol–formic acid–water (44:5:1) ^{1}H NMR: See Table 1. MS: *m/e* 519 (Ac–MeLeu–Me_2Tyr (Dcbzl)$^+$). $[\alpha]_D^{20} = -22°$ (c 0.4, 0.1 M acetic acid).

Synthesis on a chloromethyl-Pab-resin. Cesium Boc-glycinate (0.33 g, 1.05 mmole) was reacted with 1.28 g of chloromethyl–Pab–resin (0.68 mmole Cl/g) in DMF at 50°C for 18 hr to give Boc–Gly–OCH_2–Pab–resin with a substitution of 0.56 mmole/g. Peptide synthesis was performed upon 1.61 g of this polymer as described above except for the use of the cyclohexyl-protected tyrosine derivative. After HF treatment of 1.33 g of peptide–OCH_2–Pab–resin (30 mL HF, 3 mL anisole; 1 hr, 0°), crude **3** (230 mg) was obtained. Bio–Gel P-2 chromatography was carried out on a 100 × 2 cm column loaded with 115 mg of crude and eluted with 0.1 M ammonium acetate, pH 8.5, at 27 mL/hr. Anion exchange chromatography was performed on a 20 × 2 cm diethylaminoethylcellulose column loaded with 78 mg of peptide and eluted with a linear gradient of ammonium acetate (5–25 mS, pH 8.5) at 40 mL/hr. Peptide **3** thus pruified was characterized as above.

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