Synthesis of a New Photolabile Support. 4-(2-Chloropropionyl)phenylacetamidomethyl-resin and Its Application in Solid-Phase Peptide Synthesis[1]

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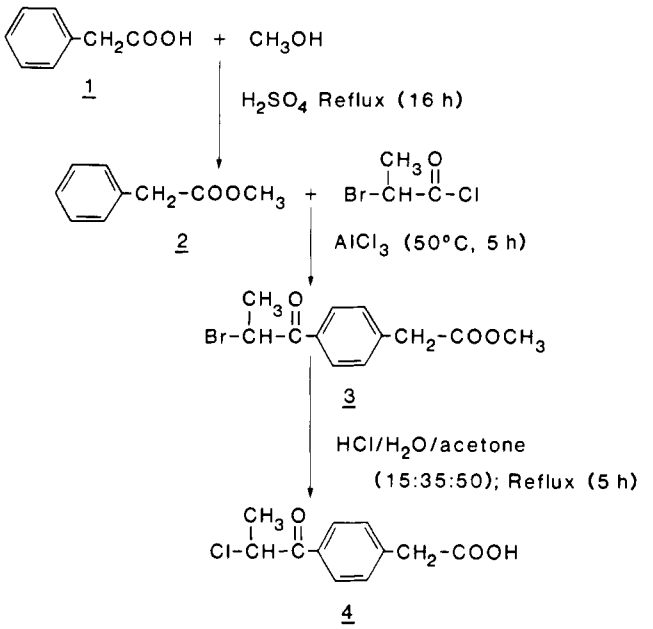
The preparation of a new photolabile resin for solid-phase peptide synthesis is described. [4-(2-Chloropropionyl)phenyl]acetic acid was used as the handle and was obtained in three steps with an 82% overall yield on starting from phenylacetic acid. The incorporation of the handle to the aminomethyl-resin to form 4-(2-chloropropionyl)phenylacetamidomethyl-resin was achieved via its acid chloride or dicyclohexylcarbodiimide mediated coupling. The application of the resin was demonstrated by the stepwise synthesis of two protected peptide segments of thymopoietin II. The peptides were released from the support upon irradiation at a wavelength of 350 nm. The photolytic cleavage yields were in the range of 89–91% for both peptides. The homogeneity of the crude synthetic product, Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)-OH, was examined by high-pressure liquid chromatography after the removal of the blocking groups by catalytic transfer hydrogenation.

The continuing need for the preparation of large biologically active peptides has focused our attention on the chemistry of the photolytically removable polymeric support for the synthesis of protected peptides. Much effort in solid-phase peptide synthesis[2] has recently been directed toward the preparation of peptide segments that can be coupled in solution or on a solid support.[3] This approach, which appears to be the preferred strategy, offers many advantages over the stepwise method in the synthesis of large peptides. It allows for the preparation of analogues without requiring synthesis of the entire sequence. This is especially important when uncertainties exist in the exact structure of the peptide. Furthermore, purification of the segments is possible prior to their coupling, and, therefore, purification of the final product is expected to be easier.

In the preparation of a solid support,[3] the use of a handle which links the peptide to the support has been favored over a direct derivatization of the solid support, since in the latter case the reaction conditions are usually difficult to control. It has also been reported that a higher quality product can be frequently obtained from improved preparations of the solid support.[4,5]

Recently, the syntheses and applications of several photolabile solid supports have been reported. Rich and Gurwara[6] described the use of 4-(bromomethyl)-3-nitrobenzoic acid for the synthesis of protected peptides on polystyrene-based resins. The successful application of the same handle was extended to the liquid-phase method of peptide synthesis on poly(ethylene glycol),[7] multidetachable supports,[5] and polyacrylamide-based resin.[8] Solid phase synthesis of protected peptides up to 16 residues[9] has been achieved by using photolytic cleavage of the α-methylphenacyl ester anchoring linkage.[10] The resin was prepared via a Friedel–Crafts acylation reaction of copolystyrene–divinylbenzene resin with 2-bromopropionyl chloride.[11] A specific substitution level is difficult to obtain by this approach.

Scheme I. Preparation of [4-(2-chloropropionyl)phenyl]acetic Acid



In this paper we describe the synthesis of a new photolabile support, 4-(2-chloropropionyl)phenylacetamidomethyl-resin (**5**) via [4-(2-chloropropionyl)phenyl]acetic acid (**4**)[12] as the handle, for use in solid phase peptide synthesis.

Results and Discussion

(A) Synthesis of the Handle. Several initial attempts to prepare handle **4** via Friedel–Crafts acylation of phe-

(1) Abbreviations used: Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; Bzl, benzyl; ONp, *p*-nitrophenol; DMF, *N,N*-dimethylformamide; DIEA, *N,N*-diisopropylethylamine.

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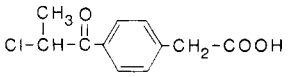

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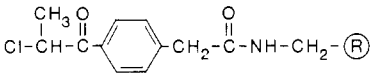

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nylacetic acid by 2-bromopropionyl chloride and aluminum chloride, as described by Drefahl and Fischer,[12] were not completely successful. The product was difficult to isolate and was obtained in low yields (5–10%). Drefahl and Fischer have also described the synthesis of [4-(2-chloropropionyl)phenyl]acetic acid (**4**) from ethyl phenylacetate. Using this method, they obtained the product with an improved yield (25–30%). We have prepared [4-(2-chloropropionyl)phenyl]acetic acid using a similar procedure with the modifications as outlined in Scheme I.

Phenylacetic acid (**1**) was first converted to its methyl ester **2** almost quantitatively. The ester was reacted with 2-bromopropionyl chloride in the presence of aluminum chloride via classical Friedel–Crafts acylation conditions. The acylated ester **3** was obtained by distillation with an 86% overall yield.

Contrary to the report of Drefahl and Fischer, halogen exchange of the bromo ketone was not observed in the acylation. However, when the ester was hydrolyzed by refluxing in an acetone/HCl/H_2O mixture (50:15:35) for 5 h, the product obtained was [4-(2-chloropropionyl)-phenyl]acetic acid (**4**). Identification was based on the elemental analysis and IR, ^1H NMR, and mass spectra. The exchange does not affect the utility of this compound as a handle and is actually advantageous since the chloro compound is less reactive and, therefore, more stable than the bromo compound. Thus, the overall yield of **4** (82%) by the modified procedure was significantly higher than that obtained by Drefahl and Fischer. This may be due to milder hydrolysis conditions and an improved acylation procedure.

(B) Preparation of the 4-(2-Chloropropionyl)-phenylacetamidomethyl-resin (5). The coupling of [4-(2-chloropropionyl)phenyl]acetic acid (**4**) to aminomethyl-resin can be accomplished by several methods that are commonly used in peptide synthesis (Scheme II). The symmetrical anhydride[13] method was less attractive since a quantitative yield could not be achieved even after repetitive coupling as indicated by a strong blue color in the ninhydrin test.[14] A better result could be obtained when the handle was reacted via its acid chloride, which was prepared from the free acid and thionyl chloride. During the coupling, the resin mixture developed a pink color, which disappeared after washing. The resin prepared by this method gave a negative ninhydrin test, indicating the absence of free amino sites. Elemental analysis gave a substitution of 0.65 mmol of chlorine/g of resin. The most convenient and reliable method of attachment was the standard dicyclohexylcarbodiimide-mediated coupling of the free acid with the aminomethyl-resin. A discoloration of the resin was also observed during this coupling procedure, which was removed during the resin washes. By use of this method, a quantitative coupling of the amine was obtained in a single coupling step with a substitution of 0.74 mmol of chlorine/g of resin.

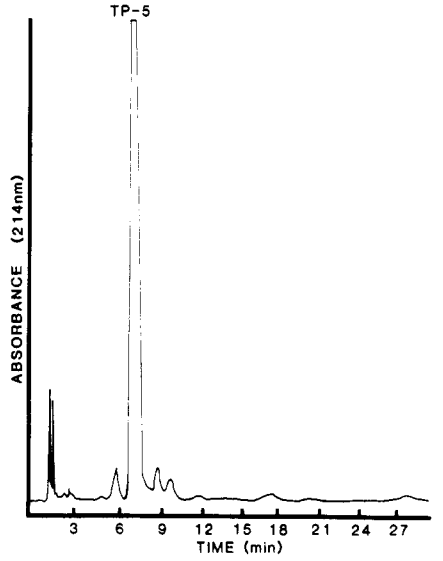

Figure 1. High-pressure liquid chromatography of crude Arg-Lys-Asp-Val-Tyr (TP-5) on a μ-Bondapak C-18 column (3.9 mm × 30 cm; 10% CH_3OH in 0.02 M potassium phosphate as the mobile phase).

(C) Peptide Synthesis. Esterification of the Boc amino acid onto the resin was achieved via the KF method.[15] A substitution level of 0.41 and 0.47 mmol/g of resin was obtained for Boc-Tyr(Bzl)-OH and Boc-Glu(OBzl)-OH, respectively, within 18 h at 50 °C. Following Scheme III, we prepared two protected peptide segments of thymopoietin II,[16] a thymic hormone isolated from bovine thymus, by the solid-phase method. These correspond to segments 32–36, Arg-Lys-Asp-Val-Tyr (**9**, TP-5), and 34–38, Asp-Val-Tyr-Val-Glu. These particular peptides were selected as suitable test peptides both to demonstrate the utility of the resin and because of our ongoing studies on immunomodulating peptides. In addition, they contain most of the blocking groups that are commonly used in peptide synthesis.

The peptides were assembled stepwise by using the standard dicyclohexylcarbodiimide-mediated coupling. The progress of the coupling was monitored by the qualitative ninhydrin test.[14] On the basis of the amount of peptide remaining on the support, the protected peptides were released from the resin by photolysis in 89–91% yield at a wavelength centered at 350 nm. The spectral energy distribution of the lamp is between 320 and 390 nm. The yield of the isolated crude peptides was in the range of 76–78%. In the synthesis of protected segment 32–36 (TP-5), only benzyl-based blocking groups were used for side-chain protection, including the α-amino group of arginine. This strategy enabled us to deprotect the blocked pepide by catalytic transfer hydrogenation[17] without the use of strong acids and to analyze its purity by HPLC.

(D) Purity Analysis of the Pentapeptides. A sample of the unpurified blocked peptide TP-5 (**8**) was deprotected by catalytic transfer hydrogenation with 5% formic acid in methanol and Pd black as the catalyst. The free pentapeptide **9** was then analyzed by HPLC on a μ-Bondapak C-18 reversed-phase column (3.9 mm × 30 cm) with 10%

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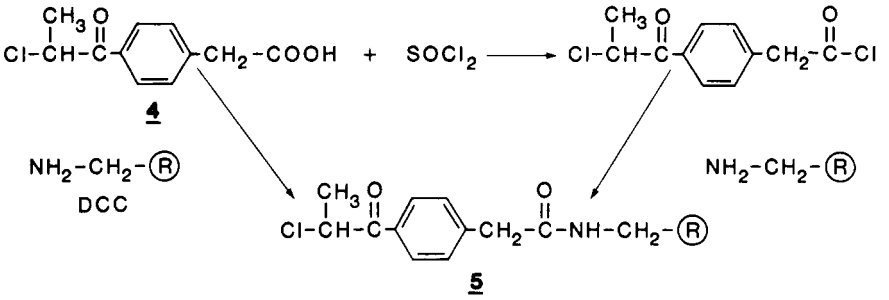
Table I. Amino Acid Analysis of Peptidyl-Resin Hydrolysates

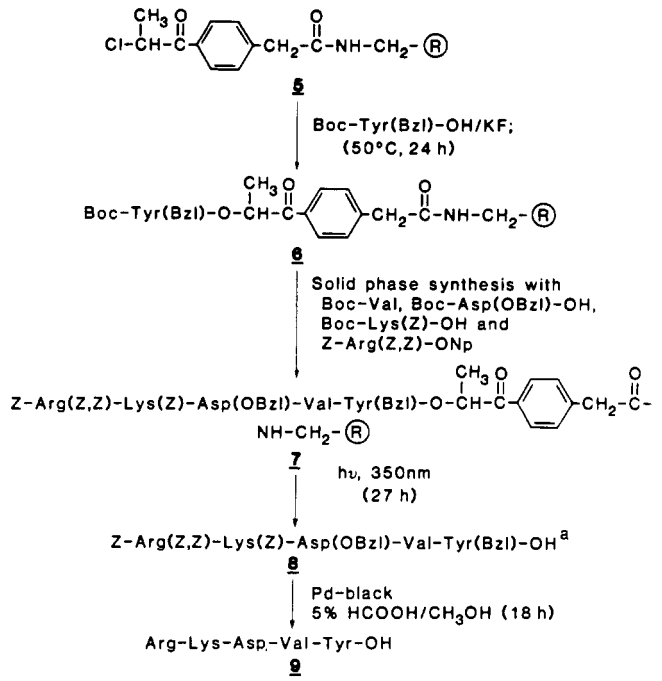
resin	substitution, mmol/g of Ⓡ [a]	amino acid ratios			
		Tyr	Val	Tyr	Glu
Boc-Tyr(Bzl)-Ⓡ	0.410				
Boc-Asp(OBzl)-Val-Tyr(Bzl)-Ⓡ	0.403	1.00	0.94	1.06	
Boc-Glu(OBzl)-Ⓡ	0.470				
Boc-Tyr(Bzl)-Val-Glu(OBzl)-Ⓡ	0.461	0.93	0.95		1.00

[a] Corrected for the peptide weight and expressed as millimoles per gram of resin, based on the first amino acid residue.

Scheme II. Synthesis of 4-(2-Chloropropionyl)phenylacetamidomethyl-resin



Scheme III. Solid-Phase Peptide Synthesis on the New 4-(2-Chloropropionyl)phenylacetamidomethyl-resin [a]



[a] A second protected peptide, Boc-Asp(OBzl)-Val-Tyr(Bzl)-Val-Glu(OBzl)-OH(10) was obtained using the same procedure.

methanol in 0.02 M potassium phosphate (pH 3.0) as the mobile phase.

The pentapeptide eluted with a retention time of 7.65 min and was shown to be 91% pure (Figure 1). UV analysis of free TP-5 in water and a sample prepared by solution synthesis gave identical profiles, indicating that there was no damage or modification of the more light-sensitive tyrosine residue during the photolytic cleavage and hydrogenation (Figure 2). Amino acid analysis of both protected peptides was in good agreement with the theoretical values. Thin-layer chromatography of the peptides showed the desired products to be the major component in several solvent systems. Potential side reactions related to the phenacyl resin, such as intramolecular cyclization at both the first amino acid and the dipeptide stages, would

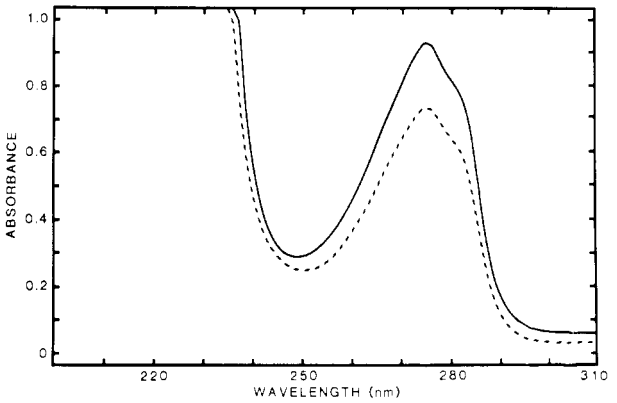
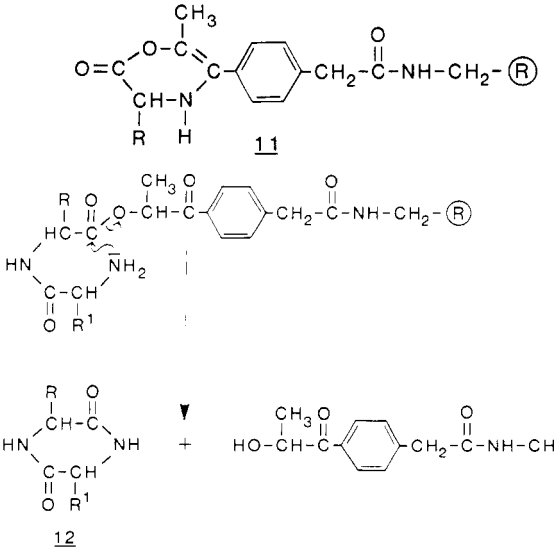


Figure 2. Ultraviolet absorption spectra of Arg-Lys-Asp-Val-Tyr (TP-5) in H_2O: samples from solid phase synthesis (—) and from solution synthesis (- - -).

result in the formation of oxazinone 11 and dioxopiperazine[9] 12, respectively. These reactions occurred at



insignificant levels since no loss of loaded sites from the support was observed as indicated by the amino acid analysis of the peptidyl-resin hydrolyzate at the tripeptide stage (Table I).

Conclusion

These results suggest that 4-(2-chloropropionyl)-phenylacetamidomethyl-resin (**5**) is a suitable resin for peptide synthesis on a solid support. Moreover, handle **4** can be prepared in both large quantity and high purity which allows for a greater flexibility in its application. Therefore, the availability and the improved synthesis of this photolabile handle should also be of interest to the peptide chemist using other polymer-based supports[18-20] and should be useful in the preparation of multidetachable resins[5] as well. In this respect, we have extended the application of this handle to the liquid-phase peptide synthesis using a poly(ethylene glycol)-based support. The results of this work will be reported later. Finally, it is known that phenacyl esters are exceptionally stable to anhydrous acids, including HF and refluxing TFA, while being more susceptible to nucleophiles than the ordinary benzyl esters. Cleavages of peptides from phenacyl resins have been achieved by using NH_3/CH_3OH,[11] N_2H_4,[10,11] thiophenoxide,[11] and KCN/crown ether.[21] Its excellent stability against HF would also allow one to use solid-phase Edman degradation for amino acid sequence analysis of a synthetic peptidyl-resin after removal of the side-chain protecting groups with HF. It has been recognized that the Edman degradation should prove useful in evaluating the progress of solid-phase peptide synthesis.[23,24]

Experimental Section

Aminomethyl-copoly(styrene–1% divinylbenzene) resin (0.7 mmol of NH_2/g of resin) was obtained from Peninsula Laboratories. Amino acid derivatives were obtained from Peninsula Laboratories or Bachem, Inc. Other commerical reagents were as follows: *N,N*-dimethylformamide, 1,1,2,2-tetrachloroethane, 2-bromopropionyl chloride, *N,N*-diisopropylethylamine, thionyl chloride (Aldrich), aluminum chloride, potassium fluoride (Alfa Chemical Co.), trifluoroacetic acid (Halocarbon Co.), phenylacetic acid, dicyclohexylcarbodiimide (Tridom Chemical Co.).

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Nuclear magnetic resonance spectra were recorded with a Varian Model T-60 spectrometer. All photolyses were done in a RPR 100 reactor (Rayonet, The Southern New England Ultraviolet Co., Hamden, CT) equipped with RPR 3500-Å lamps.[25] The air temperature surrounding the sample was maintained at 37 °C by an electric fan. Amino acid analyses were obtained on a Beckman Model 119 CL high-pressure automatic analyzer after hydrolysis of the cleaved peptides with 12 N HCl/acetic acid (1:1) containing a few drops of phenol in sealed tubes at 110 °C for 24 h. For peptidyl-resin, the hydrolysis was done with 12 N HCl/propionic acid (1:1) containing a few drops of phenol at 145 °C for 4 h.

Thin-layer chromatography was performed with precoated silica gel 60 F254 plates (200 μm) obtained from Manufacturing Chemist, Inc. The compound was visualized directly under an ultraviolet light or by spraying with ninhydrin solution and Cl_2/KI–starch solution. The following solvent systems are used: (A) chloroform–acetic acid (97.5:2.5); (B) *n*-butanol–acetic acid–water (3:1:1); (C) *n*-butanol–acetic acid–water (4:1:5, upper phase); (D) ammonium hydroxide–*n*-propanol (37:74). High-pressure column chromatography was performed with a Laboratory Data Control system consisting of a Constametric III solvent delivery system, a Rheodyne 7126 injector, and a Model SM III variable-wavelength UV detector on a μ-Bondapak C-18 column (3.9 mm × 30 cm).

Methyl Phenylacetate (2). Phenylacetic acid (1; 34 g, 0.25 mol) and sulfuric acid (5 mL) were dissolved in methanol (40 g, 1.25 mol). The mixture was heated to reflux overnight. The solvent was evaporated, and the residue was dissolved in ether (200 mL) and then washed with saturated $NaHCO_3$ (3 × 100 mL) and H_2O (2 × 100 mL). The ether phase was dried over sodium sulfate for 4 h and filtered. The filtrate was evaporated to give the product **2** as an oily material: 37 g (99% yield); ^1H NMR ($CDCl_3$) δ 3.45 (2 H, s, CH_2), 3.65 (3 H, s, CH_3), 7.22 (5 H, s, C_6H_5).

Methyl [4-(2-Bromopropionyl)phenyl]acetate (3). 2-Bromopropionyl chloride (50 g, 0.29 mol) was added dropwise into a suspension of aluminum chloride (80 g, 0.6 mol) in tetrachloroethane (200 mL). The mixture was heated to 50 °C for 20 min, and methyl phenylacetate (**2**; 37 g, 0.25 mol) was added at a rate such that the temperature did not exceed 50 °C. The reaction mixture, which turned dark, was stirred for another 4 h at the same temperature and cautiously poured into a beaker with ice (500 g). The solution was acidified with concentrated HCl to pH 1–2 (pH paper) and transferred into a separatory funnel. The aqueous phase was extracted with dichloromethane (2 × 200 mL), and the combined organic extracts were washed with 2% NaOH (3 × 100 mL) and water (2 × 100 mL). The organic phase was dried over sodium sulfate for 4 h and filtered. The solvent was removed by evaporation, and the residue was distilled through a Vigreux column (10 cm) under reduced pressure. The desired product **3** distilled at 145–147 °C (0.2 mmHg) as a light yellowish oil: 60 g (86% yield); ^1H NMR ($CDCl_3$) δ 1.78–1.95 (3 H, d, CH_3), 3.65 (5 H, s, $CH_2CO_2CH_3$) 5.1–5.4 (1 H, m, CH), 7.2–8.0 (4 H, m, C_6H_4); MS, *m/e* 284/286 (M^+, Br isotopes). Anal. Calcd for $C_{12}H_{13}BrO_3$: C, 50.55; H, 4.59; Br, 28.03. Found: C, 50.48; H, 4.71; Br, 27.91.

[4-(2-Chloropropionyl)phenyl]acetic Acid (4).[12] Methyl [4-(2-chloropropionyl)phenyl]acetate (3.19 g, 66.7 mmol) was gently refluxed in a mixture of acetone (50 mL), concentrated HCl (15 mL), and H_2O (35 mL) for 5 h. The progess of the reaction was monitored by thin-layer chromatography (silica gel F60, 200 μm; $CHCl_3$/HOAc, 97.5:2.5) and judged complete by the disappearance of the ester (R_f 0.53). The mixture was filtered, and the acetone was removed with a rotary evaporator. The remaining aqueous phase was extracted with $CHCl_3$ (3 × 50 mL). The acid was then extracted from $CHCl_3$ with saturated $NaHCO_3$ solution (3 × 50 mL). The pH of the sodium bicarbonate solution was brought to 1.5–2.0 with 6 N HCl under ice-bath cooling, and the product was back-extracted with $CHCl_3$ (4 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated to dryness, and the residue was crystallized from CH_2Cl_2/hexane (7:3 v/v) to give a crystalline material **4**: 14 g (94% yield); mp 141–143 °C; R_f 0.23 (solvent system A); MS, *m/e* 226 (M^+); ^1H NMR (Me_2SO-d_6) δ 1.55–1.68 (3 H, d, CH_3), 3.7 (2 H, s, CH_2), 5.55–5.9 (1 H, m, CH), 7.3–8.0 (4 H, m, C_6H_4). Anal. Calcd for $C_{11}H_{11}ClO_3$: C, 58.28; H, 4.88; Cl, 15.64. Found: 3, 57.83; H, 4.88; Cl, 15.75, no detectable bromine.

Preparation of the 4-(2-Chloropropionyl)phenylacetamidomethyl-resin (5). (A) Standard DCC-Coupling Method. Aminomethyl-resin (2.5 g; 1.75 mmol of NH_2) was placed in a reaction vessel on a manual shaker and swelled in dichloromethane (40 mL) overnight. The resin was washed with 5% DIEA/CH_2Cl_2 (40 mL, two times in 5 min) and CH_2Cl_2 (40 mL, five times in 2 min). [4-(2-Chloropropionyl)phenyl]acetic acid (**4**; 0.79 g, 3.5 mmol) and dicyclohexylcarbodiimide (0.72 g, 3.5 mmol) were dissolved in CH_2Cl_2 (20 mL) and added to the resin. The mixture had a yellowish color, which later disappeared. The reaction mixture was shaken overnight. The resin was washed with CH_2Cl_2 (five times in 1 min), 50% HOAc/CH_2Cl_2 (once in 1 min); CH_2Cl_2 (three times in 1 min), CH_3OH (three times in 1 min), and CH_2Cl_2 (five times in 1 min). The product was dried in a desiccator under vacuum to give 2.87 g of resin **5**. A ninhydrin test of the resin was shown to be negative; Cl, 2.61% (0.74 mmol of Cl/g of resin).

(B) Symmetrical Anhydride Method. [4-(2-Chloropropionyl)phenyl]acetic acid (**4**; 1.43 g, 6.3 mmol) and dicyclohexylcarbodiimide (0.65 g, 3.15 mmol) were dissolved in 20 mL

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of CH_2Cl_2 at 0 °C and stirred for 1 h. The mixture was filtered into a reaction vessel containing the prewashed aminomethyl-resin (3 g, 0.7 mmol/g of resin) and shaken for 18 h. The resin was washed with CH_2Cl_2, CH_3OH, and CH_2Cl_2. Ninhydrin testing of a resin sample indicated that the coupling was not completed. A second symmetrical anhydride coupling with [4-(2-chloropropionyl)phenyl]acetic acid (**4**; 1.7 g, 7.5 mmol) and dicyclohexylcarbodiimide (1.55 g, 7.5 mmol) was carried out for 4 h. The ninhydrin test showed no improvement. The resin was washed with CH_2Cl_2, 50% HOAc/CH_2Cl_2, CH_2Cl_2, CH_3OH, and CH_2Cl_2 and dried in a desiccator under vacuum to yield 3.3 g of **5** (Cl, 2.1%; 0.6 mmol of Cl/g of resin). To block the unreacted aminomethyl groups, the resin was further suspended in CH_2Cl_2 (5 mL) and treated with acetic anhydride (5 mL) and pyridine (5 mL) until the ninhydrin test of the resin sample was negative (2 h).

(C) Acid Chloride Method. [4-(2-Chloropropionyl)-phenyl]acetic acid (**4**; 1.6 g, 7 mmol) was dissolved in $CHCl_3$ (5 mL), and thionyl chloride (2 mL, 28 mmol) was slowly added. The mixture was stirred overnight at room temperature. The solvent was evaporated to dryness, and the remaining residue was dissolved in CH_2Cl_2 (10 mL). The acid chloride solution was added into a reaction vessel containing aminomethyl-resin (2.5 g, 1.75 mmol/g of resin), followed by DIEA (0.5 mL). The coupling was complete after 24 h. The resin was washed with CH_2Cl_2 and dried in a desiccator under vacuum to yield 2.8 g of **5**: Cl, 2.36% (0.65 mmol of Cl/g of resin); S, 0.07% (0.022 mmol of S/g of resin).

4-[2-[Boc-Tyr(Bzl)]propionyl]phenylacetamidomethyl-resin (6). 4-(2-Chloropropionyl)phenylacetamidomethyl-resin (**5**, 1.5 g, 1.1 mmol), obtained by method A, was swelled in DMF (20 mL) in a three-necked round-bottomed flask with an overhead stirrer. Potassium fluoride (0.58 g, 10 mmol) and Boc-Tyr(Bzl)-OH (1.85 g, 5 mmol) were slowly added to the resin suspension. The mixture was stirred gently at 50–55 °C for 24 h. The resin was filtered and washed thoroughly with DMF, DMF/H_2O (1:1), H_2O, CH_3OH, and CH_2Cl_2 to yield 1.71 g of **6** (substitution: 0.41 mmol of Tyr/g of resin).

4-[2-[Boc-Glu(OBzl)]propionyl]phenylacetamidomethyl-resin. 4-(2-Chloropropionyl)phenylacetamidomethyl-resin (**5**; 3 g, 2.0 mmol) was suspended in DMF (30 mL). Potassium fluoride (1.16 g, 20 mmol) and Boc-Glu(OBzl)-OH (3.37 g, 10 mmol) were added slowly to the suspension. The mixture was stirred at 50 °C for 24 h. The resin was filtered and washed thoroughly with DMF, DMF/H_2O (1:1), H_2O, CH_3OH, and CH_2Cl_2 to give 3.48 g of resin (substitution: 0.47 mmol of Glu/g of resin).

4-[2-[Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)]-propionyl]phenylacetamidomethyl-resin (7). The peptidyl-resin **7** was assembled stepwise by starting with 4-[2-[Boc-Tyr-(Bzl)]propionyl]phenylacetamidomethyl-resin (**6**; 1.95 g, 0.41 mmol of Tyr/g of resin). One cycle of synthesis consisted of (1) deprotection with 50% TFA in CH_2Cl_2 (once for 2 min, once for 30 min), (2) neutralization with 7% diisopropylethylamine in CH_2Cl_2 (twice for 5 min), and (3) coupling by addition of the Boc amino acid (3 equiv) and dicyclocarbodiimide (3 equiv, 2 h). Steps 2 and 3 were repeated for the second coupling. For the coupling of Z-Arg(Z,Z)-ONp, DMF was used as a solvent. All other couplings and intermediate washes were with dichloromethane. The coupling reaction was monitored by a ninhydrin test. Resin **7** was dried in a desiccator under vacuum to give 1.75 g.

Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)-OH (8). 4-[2-[Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)]propionyl]-phenylacetamidomethyl-resin (**7**, 1 g) was suspended in DMF (35 mL) in a screw-capped glass vial. The suspension was bubbled with argon for 1 h and irradiated at a wavelength of 350 nm for 32 h. The resin was filtered and washed with warm DMF (3 × 30 mL). The filtrate was evaporated to dryness, and the remaining residue was triturated with ether to give a crystalline material, which was collected by filtration and dried in a desiccator under vacuum to yield **8**: 225 mg (78% yield); mp 193–195 °C. Amino acid analysis of the crude peptide: Arg, 0.92; Lys, 0.99; Asp, 1.00; Val, 1.01; Tyr, 0.91. Hydrolysis of the remaining photolyzed peptidyl-resin gave a substitution of 0.045 mmol of Tyr/g of peptidyl-resin (89% cleavage yield).

Arg-Lys-Asp-Val-Tyr (9, TP-5). The crude Z-Arg(Z,Z)-Lys(Z)-Asp(OBzl)-Val-Tyr(Bzl)-OH (50 mg) was deprotected by catalytic transfer hydrogenation with Pd black (50 mg) and 5% formic acid/CH_3OH (25 mL) for 18 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The resulting peptide was analyzed chromatographically as described below.

Analysis of the Pentapeptide TP-5 by HPLC. A sample of the hydrogenated pentapeptide (1 mg) was dissolved in 1 mL of buffer (10% CH_3OH in 0.02 M potassium phosphate solution; pH 3.0). The sample (20 μL) was chromatographed on a μ-Bondapak C-18 column (3.9 mm × 30 cm) with a flow rate of 2 mL/min, using the buffer above as the mobile phase. The peptide was detected at 214 nm with a retention time of 7.65 min and shown to be 91% pure.

4-[2-[Boc-Asp(OBzl)-Val-Tyr(Bzl)-Val-Glu(OBzl)]-propionyl]phenylacetamidomethyl-resin. The protected peptidyl-resin was assembled stepwise by starting with 4-[2-[Boc-Glu(OBzl)]propionyl]phenylacetamidomethyl-resin (3.4 g, 0.47 mmol of Glu/g of resin) and using the same procedure described above. The resin was dried in a desiccator under reduced pressure to yield 3.74 g.

Boc-Asp(OBzl)-Val-Tyr(Bzl)-Val-Glu(OBzl)-OH (10). 4-[2-[Boc-Asp(OBzl)-Val-Tyr(Bzl)-Val-Glu(OBzl)]propionyl]-phenylacetamidomethyl-resin (2 g) was suspended in DMF (4 mL), and the suspension was bubbled with argon for 1 h and photolyzed for 38 h. The resin was filtered and washed with DMF (2 × 35 mL). The filtrate was evaporated to dryness, and the residue was triturated with ether and recrystallized from ethyl acetate/ether to give the peptide: 0.4 g (76%); mp 223–226 °C; R_f 0.85 (system B), 0.78 (system C), 0.61 (system D). Amino acid analysis: Asp, 100; Val, 2.03; Tyr, 0.89, Glu, 1.00. Hydrolysis of the remaining photolyzed peptidyl-resin gave a substitution of 0.043 mmol Glu/g of peptidyl-resin (91% cleavage yield).

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