Studies on $\alpha_2$-Plasmin Inhibitor Fragment T-11. I. Synthesis of the Protected Hexadecapeptide Ester Corresponding to Positions 11 through 26 of $\alpha_2$-Plasmin Inhibitor Fragment T-11

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The protected hexadecapeptide ester corresponding to positions 11 through 26 of human $\alpha_2$-plasmin inhibitor fragment T-11, which consists of 26 amino acids and binds to the plasmin(ogen) lysine-binding site(s), was synthesized by assembling five peptide fragments by the azide method or the dicyclohexylcarbodiimide-N-hydroxybenzotriazole method.

Keywords—$\alpha_2$-plasmin inhibitor; $\alpha_2$-plasmin inhibitor fragment synthesis; protected hexadecapeptide ester; peptide synthesis; fragment condensation

$\alpha_2$-Plasmin inhibitor is physiologically the most important inhibitor of plasmin in blood plasma. This inhibitor is a single-chain glycoprotein with a molecular weight of 67000. It contains about 500 amino acid residues and 12% carbohydrates. This protein inhibits plasmin very quickly, and its inhibitory reaction involves two steps: the first step is reversible binding to the lysine-binding site(s) of plasmin, and the second is irreversible binding to the active site of plasmin. The high reaction rate of this inhibition is due to the first step of interaction between $\alpha_2$-plasmin inhibitor and plasmin.

In the previous paper, we reported that the human $\alpha_2$-plasmin inhibitor fragment named T-11 showed high binding activity to the lysine binding site(s) of plasmin with a dissociation constant of 5.5 $\mu$M. This indicated that T-11 contains the binding site of $\alpha_2$-plasmin inhibitor to the lysine-binding site(s) of plasmin. The peptide was shown to have 26 amino acid residues, and its amino acid sequence determined by the Edman method is illustrated in Fig. 1.

Only 300 $\mu$g of this peptide was obtained from 10 mg of human $\alpha_2$-plasmin inhibitor. It was necessary to obtain a larger amount of this fragment for further studies on the interaction between $\alpha_2$-plasmin inhibitor and plasmin. Thus, we wished to obtain the peptide in quantity by chemical synthesis.

We have synthesized the hexacosapeptide corresponding to the entire amino acid sequence of T-11 and made a preliminary examination of the physiological properties of this synthetic peptide and some of its intermediates. In a series of studies described in this and the following papers, we intend to give a detailed account of our synthetic studies on the inhibitor...
fragment, which may cast some light on the structure–activity relationship of this important plasma protein inhibitor fragment.

This paper describes the synthesis of the protected C-terminal hexadecapeptide (positions 11—26 in T-11) as a starting material for the synthesis of T-11. A conventional solution method was used for the synthesis of T-11 and its fragments in order to obtain sufficient amounts of these peptides and to ensure their purity. As α-amino protecting groups, the TFA-labile Boc group was chosen for temporary protection, and the α-amino group of the Ser derivative was protected with the Z(OMe) group. TFMSA–thioanisole–TFA was used as the final deprotecting reagent. Amino acid derivatives with side-chain protecting groups, i.e., Asp(OBzl), Glu(OBzl) and Lys(Z), easily removable by the above mixed reagent and capable of surviving under the restricted TFA treatment conditions were employed. In order to prevent partial oxidation of the Met residue during the various steps involved, the Met residue was protected as Met(O) and deprotected with dithiothreitol at the final stage.

The scheme employed in the synthesis of the hexadecapeptide ester is illustrated in Fig. 2. We used the azide method and the DCC–HOBT method as fragment condensing techniques. As building blocks for the construction of the C-terminal portion of T-11, five relatively small peptide fragments were synthesized, namely, Z(OMe)–Ser–Pro–Lys(Z)–OBzl (I-1) (positions 24—26), Boc–Gln–Phe–Gly–NHNH₂ (I-2) (positions 21—23), Boc–Asp(OBzl)–Tyr–Pro–OH (I-3) (positions 18—20), Boc–Met(O)–Glu(OBzl)–OH (I-4) (positions 15, 16), and Boc–Leu–Val–Pro–Pro–NHNH₂ (I-5) (positions 11—14).

The protected C-terminal tripeptide ester (I-1) was synthesized from H–Lys(Z)–OBzl by stepwise chain elongation with Boc–Pro–OH and Z(OMe)–Ser–NHNH₂ by the DCC and azide procedures, respectively. The reason for the usage of Z(OMe)–Ser–NHNH₂ instead of Boc–Ser–NHNH₂ was that the resulting tripeptide is easily crystallized, whereas the Boc derivative is not.

For the synthesis of (I-2), Boc–Phe–Gly–OMe was prepared by the DCC–HOBT procedure. The resulting dipeptide ester, after treatment with TFA, was condensed with Boc–Gln–OH by the p-nitrophenyl ester procedure. Boc–Gln–Phe–Gly–OMe was then converted to the corresponding hydrazide.

The azide condensation of Boc–Tyr–NHNH₂ and H–Pro–OH afforded Boc–Tyr–Pro–OH, which was treated with TFA and condensed with Boc–Asp(OBzl)–OH by the p-nitrophenyl ester procedure in the presence of N-methylmorpholine to give the protected tripep-
The oily compound Boc-Met-Glu(OBzl)-OH, which was prepared by condensation of Boc-Met-OH and H—Glu(OBzl)-OH by the trichlorophenyl ester procedure, was oxidized with sodium periodate to give the easily crystallized compound (I-4).

The protected tetrapeptide hydrazide (I-5) was synthesized as illustrated in Fig. 3. The azide procedure was used to condense the TFA-treated sample of Boc-Pro-Pro-OBzl (obtained by the DCC-HOBT technique) and Boc—Leu-Val-NHNH₂, which was prepared from Boc—Leu-Val-OMe by hydrazine hydrate treatment. The resulting tetrapeptide ester, Boc—Leu-Val-Pro-Pro—NHNH₂ (I-5), was converted to the corresponding hydrazide (I-5) in the usual manner.

As shown in Fig. 2, two fragments, Boc—Gln—Phe—Gly—NHNH₂ (I-2) and Boc—Leu—Val—Pro—Pro—NHNH₂ (I-5), were assembled by the azide procedure to avoid racemization. The other two fragments, Boc—Met(O)—Glu(OBzl)—OH (I-4) and Boc—Asp(OBzl)—Tyr—Pro—OH (I-3), were condensed by the DCC—HOBT method to minimize racemization. Only one amino acid residue, Boc—Glu(OBzl)—OH (position 17 in T-11), was introduced by the p-nitrophenyl ester procedure. All of the protected peptide esters, (I-7) to (I-10), except for (I-6), obtained in the chain elongation steps were readily soluble in ethyl acetate. Therefore, purification of (I-7) to (I-10) was performed by washing of the ethyl acetate extract with diluted citric acid and sodium bicarbonate solution, followed by precipitation. The less soluble

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**Table 1. Amino Acid Ratios in 6 N HCl Hydrolysates of the Intermediates of Synthetic T-11**

<table>
<thead>
<tr>
<th></th>
<th>I-6</th>
<th>I-7</th>
<th>I-8</th>
<th>I-9</th>
<th>I-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>1.03 (1)</td>
<td>1.00 (1)</td>
<td>1.07 (1)</td>
<td>1.09 (1)</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>0.81 (1)</td>
<td>0.87 (1)</td>
<td>0.87 (1)</td>
<td>0.81 (1)</td>
<td>0.77 (1)</td>
</tr>
<tr>
<td>Glu</td>
<td>0.96 (1)</td>
<td>0.96 (1)</td>
<td>1.90 (2)</td>
<td>3.44 (3)</td>
<td>3.30 (3)</td>
</tr>
<tr>
<td>Pro</td>
<td>0.89 (1)</td>
<td>1.86 (2)</td>
<td>1.91 (2)</td>
<td>2.03 (2)</td>
<td>4.22 (4)</td>
</tr>
<tr>
<td>Gly</td>
<td>0.96 (1)</td>
<td>1.20 (1)</td>
<td>0.95 (1)</td>
<td>1.21 (1)</td>
<td>1.29 (1)</td>
</tr>
<tr>
<td>Val</td>
<td></td>
<td></td>
<td></td>
<td>1.24 (1)</td>
<td></td>
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<tr>
<td>Met+Met(O)</td>
<td></td>
<td></td>
<td></td>
<td>1.01 (1)</td>
<td>0.95 (1)</td>
</tr>
<tr>
<td>Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.35 (1)</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.86 (1)</td>
<td>0.97 (1)</td>
<td>0.77 (1)</td>
<td>0.71 (1)</td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>1.00 (1)</td>
<td>1.00 (1)</td>
<td>1.00 (1)</td>
<td>1.00 (1)</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>0.89 (1)</td>
<td>0.95 (1)</td>
<td>1.06 (1)</td>
<td>0.89 (1)</td>
<td>0.92 (1)</td>
</tr>
<tr>
<td>Rec. (%)</td>
<td>89</td>
<td>91</td>
<td>86</td>
<td>91</td>
<td>89</td>
</tr>
</tbody>
</table>
compound (I-6) was washed batchwise.

The purity of these synthetic protected peptides was assessed by thin-layer chromatography, elemental analysis and amino acid analysis (Table I) of 6 N HCl hydrolysates. The well characterized protected hexadecapeptide thus synthesized served as an amino component for the total synthesis of T-11, which will be described in the following paper.

**Experimental**

All melting points are uncorrected. Thin-layer chromatography was performed on silica gel plates (Kiesel gel 60F254, Merck) and Rf values refer to the following solvent systems: Rf1 CHCl3-MeOH-H2O (8:3:1), Rf2 CHCl3-MeOH-AcOH (9:1:0.5). Optical rotations were determined with a JASCO DIP 140 digital polarimeter. Acid hydrolysates were performed in 6 N HCl at 110 °C in evacuated tubes for 24 h. The amino acid compositions of acid hydrolysates were determined with a Hitachi amino acid analyzer 835 and are not corrected for amino acid destruction.

**Boc–Pro–Lys(Z)–OBzl** — DCC (11.24 g) was added to a solution of Boc–Pro–OH (10.81 g) and H–Lys(Z)–OBzl (prepared from 27.13 g of the tosylate with 6.93 ml of Et3N) in DMF (90 ml) and the mixture was stirred at room temperature overnight. After filtration, the filtrate was concentrated in vacuo and the residue was dissolved in ether. The ether phase was washed with 5% citric acid, 5% NaHCO3 and H2O, and dried over Na2SO4. Evaporation of the solvent gave an oily residue; yield 24.20 g (85%), Rf1 0.87.

**Z(OMe)–Ser–Pro–Lys(Z)–OBzl (I-1)** — Boc–Pro–Lys(Z)–OBzl (8.45 g) was treated with TFA (15 ml) with iced-cooling for 45 min. The excess TFA was removed by evaporation in vacuo and the residue was washed twice with n-hexane by decantation. This oily residue was then dissolved in DMF (20 ml) and neutralized with Et3N. The azide (prepared from 4.64 g of Z(OMe)–Ser–NHNHz with 12.71 ml of 3.87 N HCl-DMF, 3.31 ml of isoamyl nitride and 8.89 ml of Et3N) in DMF (15 ml) was added to the N-deprotected peptide solution and the mixture was stirred at 4 °C for 48 h. The solvent was removed by evaporation and the residue was dissolved in AcOEt. The AcOEt phase was washed with 5% citric acid, 5% NaHCO3 and H2O, and then evaporated. The residue was triturated with IPE and a white powder was obtained after recrystallization from MeOH and ether; yield 5.78 g (54%), mp 78–80 °C, [z]20D −49.4 ° (c = 1.1, MeOH), Rf1 0.58. Anal. Calcld for C38H46N4O10-0.5H2O: C, 62.71; H, 5.16; N, 14.22. Found: C, 62.81; H, 5.13; N, 14.26.

**Boc–Phe–Gly–OMe** — DCC (6.13 g) and HOBT·H2O (4.59 g) were combined with a solution of Boc–Phe–OH (7.96 g) in THF (50 ml) and H–Gly–OMe (prepared from 4.24 g of the hydrochloride with 4.57 ml of Et3N) in CHCl3 (50 ml). The solution was stirred at room temperature overnight. After filtration, the solvent was removed by evaporation and the residue was dissolved in AcOEt. The organic phase was washed with 5% citric acid, 5% NaHCO3 and H2O, and dried over Na2SO4. After evaporation of the AcOEt, the residue was triturated with n-hexane. White crystals were obtained after recrystallization from ether and n-hexane; yield 9.54 g (95%), mp 69–71 °C, [z]20D −4.2 ° (c = 1.0, MeOH), Rf1 0.86. Anal. Calcld for C17H24N2O10: C, 56.88; H, 6.94; N, 12.07. Found: C, 56.69; H, 7.07; N, 12.14.

**Boc–Gln–Phe–Gly–OMe** — Boc–Phe–Gly–OMe (6.12 g) was treated with TFA (15 ml) at 0 °C for 45 min. The excess TFA was removed by evaporation in vacuo and the residue was washed with n-hexane twice, dissolved with DMF (30 ml), and neutralized with Et3N. Boc–Gln–ONp (6.69 g) and Et3N (2.52 ml) were combined with the above solution and the mixture was stirred at room temperature overnight, then evaporated. The residue was triturated with ether and 5% citric acid, 5% NaHCO3 and H2O, followed by recrystallization from MeOH–THF and IPE; yield 6.83 g (81%), mp 164–165 °C, [z]20D −30.4 ° (c = 1.0, MeOH), Rf1 0.66. Anal. Calcld for C38H46N4O11·0.5H2O: C, 62.71; H, 6.94; N, 12.06. Found: C, 62.69; H, 6.77; N, 12.14.

**Boc–Gln–Phe–Gly–NHNHz (I-2)** — Hydrazine hydrate (80%, 8.93 ml) was added to a solution of Boc–Gln–Phe–Gly–OMe (6.83 g) in MeOH–dioxane (15 ml–15 ml), and the mixture was left to stand overnight at room temperature. The resulting crystalline mass was collected by filtration and recrystallized from MeOH–dioxane and ether; yield 4.89 g (72%), mp 174–176 °C, [z]20D −39.0 ° (c = 1.0, DMF), Rf1 0.41. Anal. Calcld for C21H23N2O5·0.5H2O: C, 53.26; H, 6.83; N, 17.59. Found: C, 53.21; H, 6.58; N, 17.59.

**Boc–Tyr–Pro–OH** — A solution of proline (4.14 g) in H2O–DMSO (5 ml–45 ml) containing Et3N (9.15 ml) was added to the azide (prepared from 8.86 g of Boc–Tyr–NHNHz with 18.6 ml of 3.87 N HCl-DMF, 4.85 ml of isoamyl nitrite and 9.98 ml of Et3N) in DMF (10 ml). The mixture was stirred at 4 °C for 48 h, then evaporated, and the residue was dissolved in 5% NaHCO3. This solution was washed with AcOEt, then the aqueous phase was acidified with citric acid and the resulting precipitate was extracted with AcOEt. The AcOEt phase was washed with H2O, dried over Na2SO4 and then evaporated. IPE was added to the residue and the resulting powder was recrystallized from MeOH and IPE; yield 7.63 g (67%), mp 114–116 °C, [z]20D −26.6 ° (c = 1.0, MeOH), Rf1 0.40. Anal. Calcld for C19H32N4O10·0.25H2O·C, 59.95; H, 7.24; N, 7.32. Found: C, 59.70; H, 7.07; N, 7.07.

**Boc–Asp(OBzl)–Tyr–Pro–OH (I-3)** — The protected dipeptide (6.50 g) mentioned above was treated with TFA (20 ml) in an ice-bath for 1 h, then dry IPE and n-hexane were added to the solution. The resulting powder was dried
over KOH pellets in vacuo for 1 h and then dissolved in DMF (50 ml) and neutralized with Et3N. To this solution, Boc–Asp(OBzI)–ONp (7.64 g) and N-methylmorpholine (1.89 ml) were added and the mixture was stirred at room temperature overnight. The solvent was removed by evaporation under reduced pressure and the residue was dissolved in 5% Na2CO3. This solution was washed with AcOEt, then acidified with citric acid and the precipitate was washed with AcOEt. The organic extract was washed with 5% citric acid and H2O, dried over Na2SO4 and then evaporated. The residue was triturated with IPE and the resulting powder was recrystallized from MeOH–AcOEt and IPE; yield 8.56 g (85%), mp 93–96 °C, [α]23; −43.2° (c = 1.0, MeOH), Rf 0.65. Anal. Calcd for C35H37N3O13: C, 61.74; H, 6.39; N, 7.20. Found: C, 61.48; H, 6.43; N, 6.98.

**Boc–Met(O)–Glu(OBzI)–OH (I-4)**—H-Glu(OBzI)-OH (5.69 g) in DMF–DMSO (20 ml–20 ml) and Et3N (3.33 ml) were added to a solution of Boc–Met–OTcp (8.58 g) in THF (40 ml) containing N-methylmorpholine (2.20 ml), and the mixture was stirred at room temperature for 18 h. Evaporation of the solvent gave a residue which was dissolved in 5% Na2CO3. The aqueous phase was washed with AcOEt and acidified with citric acid, and the precipitate was washed with AcOEt. The organic phase was washed with H2O, dried over Na2SO4 and then evaporated. The resulting oily residue was dissolved in MeOH (75 ml) followed by the addition of sodium periodate (4.49 g) in H2O (90 ml). The mixture was stirred at room temperature for 2 h, and the precipitated white mass was filtered off. The filtrate was evaporated and the residue was added to the extract. After being washed with H2O and dried over Na2SO4, the extract was evaporated and IPE was added to the residue. The resulting white mass was recrystallized from AcOEt–MeOH and IPE; yield 8.15 g (84%), mp 93–95 °C, [α]23; −5.2° (c = 1.0, MeOH), Rf 0.41. Anal. Calcd for C27H32N4O17: C, 55.79; H, 9.36; N, 16.01. Found: C, 55.74; H, 9.27; N, 16.01.

**Boc–Val–OH (I-5)**—Boc–Leu–Val–OMe (3.57 g) and HOBT·H2O (2.43 g) were added to a solution of Boc–Leu–OH·H2O (4.10 g) in DMF (10 ml). The mixture was stirred for 15 min, then H-Val–OMe (prepared from 2.91 g of the hydrochloride with 2.42 ml of Et3N) in CHCl3 (10 ml) was added and the whole was stirred at room temperature for 18 h, and filtered. The filtrate was evaporated and the residue was extracted with AcOEt. After washing in the usual manner, the organic phase was washed over Na2SO4 and the oily residue was obtained by evaporation; yield 12.0 g (99%), Rf 0.81.

**Boc–Leu–Val–OMe (I-6)**—DCC (6.13 g) and HOBT·H2O (1.0 g) were added to a solution of Boc–Pro–OH (6.49 g) in DMF (30 ml) and the mixture was stirred for 15 min. Then H–Pro–OBzl (prepared from 8.70 g of the hydrochloride with 4.99 ml of Et3N) in CHCl3 (25 ml) was added and the white was stirred at room temperature for 18 h, and filtered. The filtrate was evaporated and the residue was extracted with AcOEt. After washing in the usual manner, the organic phase was washed over Na2SO4 and the oily residue was obtained by evaporation; yield 3.36 g (80%), mp 118–121 °C, [α]i; −155.8° (c = 1.0, MeOH), Rf 0.64. Anal. Calcd for C26H46N6O6·H2O: C, 59.09; H, 9.25; N, 8.16.

**Boc–Leu–Val–NNNH2 (I-7)**—Hydrazine hydrate (80%, 7.39 ml) was added to a solution of Boc–Leu–Val–OMe (4.19 g) in MeOH (15 ml), and the mixture was stirred overnight. The solvent was removed by evaporation in vacuo, and the residue was triturated with H2O. The resulting mass was reprecipitated from MeOH and H2O; yield 3.07 g (74%), mp 143–145 °C, [α]23; −52.4° (c = 1.0, MeOH), Rf 0.61. Anal. Calcd for C16H32N4O4: C, 55.79; H, 9.36; N, 16.01. Found: C, 55.74; H, 9.27; N, 16.01.

**Boc–Leu–Val–Pro–Pro–NHNH2 (I-8)**—Boc–Pro–Pro–OBzl (3.78 g) was treated with TFA (15 ml) in the usual manner, and dry ether and n-hexane were added and decanted off twice. The resulting residue was dissolved in DMF (10 ml) and neutralized with Et3N. To this ice-chilled solution, the azide (prepared from 4.48 g of Boc–Gln–Phe–Gly–Ser–Pro–Lys(Z)-OBzl (I-5) Boc–Pro–Pro–OBzl (6.13 g) and HOBT·H2O (1.0 g) were added to a solution of Boc–Pro–OH (6.49 g) in DMF (30 ml) and the mixture was stirred for 15 min. Then H–Pro–OBzl (prepared from 8.70 g of the hydrochloride with 4.99 ml of Et3N) in CHCl3 (25 ml) was added and the whole was stirred at room temperature for 18 h, and filtered. The filtrate was evaporated and the residue was extracted with AcOEt. After washing in the usual manner, the organic phase was washed over Na2SO4 and the oily residue was obtained by evaporation; yield 3.07 g (80%), mp 143–145 °C, [α]23; −52.4° (c = 1.0, MeOH), Rf 0.61. Anal. Calcd for C22H32N2O8S: C, 54.53; H, 6.66; N, 5.78. Found: C, 54.56; H, 6.58; N, 5.73.

**Boc–Gln–Phe–Gly–Ser–Pro–Lys(Z)–OBzl (I-9)**—Z(OMe)–Ser–Pro–Lys(Z)–OBzl (5.78 g) was treated with TFA (20 ml)–anisole (2.0 ml) in an ice-bath for 1 h and then the excess TFA was removed by evaporation. IPE and n-hexane were added to the residue. The resulting powder was dissolved in KOH in vacuo for 1 h, then dissolved in DMF (10 ml) and neutralized with Et3N. DCC (0.90 g) and HOBT·H2O (0.62 g) were added to a solution of Boc–Asp(OBzI)–Lys–Pro–OH (2.14 g) in DMF (10 ml),
and the reaction mixture was stirred for 15 min. Then the solution containing the N°-deprotected hexapeptide ester was added and the whole was stirred at room temperature for 18 h. After work-up as described for Boc–Phe–Gly–OMe, the product was precipitated from AcOEt with ether; yield 3.24 g (96%), mp 115–118 °C, [c] 5  52.9 (c 1.1, DMF), R f  0.67. Anal. Calcd for C 78 H 106 N 12 O 22.2H 2 O: C, 61.18; H, 6.49; N, 9.84. Found: C, 61.14; H, 6.33; N, 9.77.

Boc–Glu(OBzl)–Asp(OBzl)–Tyr–Pro–Gln–Phe–Gly–Ser–Pro–Lys(Z)–OBzl (I–9)—The above protected nonapeptide ester (I–7) (3.24 g) was treated with TFA (15 ml) in the usual manner, and the N°-deprotected peptide, isolated as stated above, was dissolved in DMF (15 ml) and neutralized with Et 3 N. To this i-collchilled solution, Boc–Glu(OBzl)–ONp (1.53 g) and N-methylmorpholine (0.25 ml) were added, and the mixture was stirred at room temperature for 18 h. After usual work-up as stated above, the residue was treated with ether and the resulting powder was collected by filtration. The product was further purified by silica-gel column chromatography (SiO 2 150 g, 4 × 21.5 cm) using MeOH–CHCl 3 –acetic acid (85 : 10 : 5) as an eluent. The desired product was finally precipitated from AcOEt with ether; yield 1.70 g (46%), mp 105–118 °C, [c] 5  52.9 (c 1.1, DMF), R f  0.67. Anal. Calcd for C 39 H 46 N 12 O 22 : C, 66.67; H, 6.49; N, 9.83. Found: C, 66.45; H, 6.35; N, 9.68.

Boc–Met(O)–Glu(OBzl)–Glu(OBzl)–Asp(OBzl)–Tyr–Pro–Gln–Phe–Gly–Ser–Pro–Lys(Z)–OBzl (I–10)—The above protected decapetide ester (I–9) (1.67 g) was treated with TFA (5 ml) in the usual manner and dry ether was added to the residue. The resulting powder was collected by filtration and dried over KOH pellets in vacuo for 1 h, then dissolved in DMF (7 ml), and neutralized with Et 3 N. To the N°-deprotected peptide solution, Boc–Met(O)–Glu(OBzl)–OH (0.63 g) in THF (3 ml), DCC (0.32 g) and HOBT·H 2 O (0.15 g) were added, and the mixture was stirred at room temperature for 18 h. DCurea was removed by filtration and the filtrate was evaporated in vacuo. The residue was extracted with AcOEt, and the organic phase was washed with 5% citric acid, 5% NaHCO 3 and H 2 O, then dried over Na 2 SO 4 . Evaporation and trituration with IPE gave a white powder, which was precipitated from MeOH with ether; yield 1.97 g (97%), mp 105–118 °C, [c] 5  52.9 (c 1.1, DMF), R f  0.67. Anal. Calcd for C 87 H 106 N 12 O 22.2H 2 O: C, 61.18; H, 6.49; N, 9.84. Found: C, 61.14; H, 6.33; N, 9.77.

Boc–Leu–Val–Pro–Pro–Met(O)–Glu(OBzl)–Glu(OBzl)–Asp(OBzl)–Tyr–Pro–Gln–Phe–Gly–Ser–Pro–Lys(Z)–OBzl (I–11) —The above protected nonapeptide ester (I–10) (1.86 g) was treated with TFA (6 ml) in the usual manner, and the deprotected peptide, isolated as stated above, was dissolved in DMF (3 ml) and neutralized with Et 3 N. To this ice-chilled solution, the azide (prepared from 0.79 g of Boc–Leu–Val–Pro–Pro–NHNH 2 with 0.99 ml of Et 3 N) was added and the whole was stirred at room temperature for 18 h. After usual work-up as stated above, the residue was treated with ether and the resulting powder was collected by filtration. The product was further purified by silica-gel column chromatography (SiO 2 150 g, 4 × 21.5 cm) using MeOH–CHCl 3 –acetic acid (85 : 10 : 5) as an eluent. The desired product was finally precipitated from AcOEt with ether; yield 1.47 g (93%), mp 123–126 °C, [c] 5  63.1 (c 1.1, DMF), R f  0.30. Anal. Calcd for C 39 H 44 N 12 O 22 : C, 66.67; H, 6.49; N, 9.83. Found: C, 66.45; H, 6.33; N, 9.68.

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References and Notes

1) Amino acid and peptide derivatives mentioned in this paper are of the L-configuration. The following abbreviations were used: Z = benzoyloxycarbonyl, Z(Ome) = p-methoxybenzoyloxycarbonyl, Bzl = benzyl, Boc = tert-butoxycarbonyl, Np = p-nitrophenyl, Tcp = 2,4,5-trichlorophenyl, DCC = dicyclohexylcarbodiimide, DCurea = dicyclohexylurea, DMF = dimethylformamide, DMSO = dimethylsulfoxide, AcOEt = ethyl acetate, HOBT = N-hydroxybenzotriazol, IPE = isopropyl ether, MeOH = methanol, THF = tetrahydrofuran, Et 3 N = triethylamine, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid.


