Chem. Pharm. Bull. 35(7)2804-2809(1987)

## Studies on $\alpha_2$ -Plasmin Inhibitor Fragment T-11. II.<sup>1,2)</sup> Synthesis of the Entire Amino Acid Sequence of $\alpha_2$ -Plasmin Inhibitor Fragment T-11

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(Received October 14, 1986)

The hexacosapeptide corresponding to the entire amino acid sequence of human  $\alpha_2$ -plasmin inhibitor fragment T-11 was synthesized by a conventional solution method. Three newly synthesized fragments were combined successively with the protected C-terminal hexadecapeptide ester previously obtained by using the dicyclohexylcarbodiimide-*N*-hydroxybenzotriazole and azide procedures to afford the protected hexacosapeptide ester. The trifluoromethanesulfonic acidthioanisole-trifluoroacetic acid procedure was employed to remove all protecting groups of the protected peptide ester at the final stage. The dissociation constant for the interaction between the synthetic  $\alpha_2$ -plasmin inhibitor fragment T-11 and plasmin was equal to that of the native T-11 from human  $\alpha_2$ -plasmin inhibitor.

**Keywords**— $\alpha_2$ -plasmin inhibitor;  $\alpha_2$ -plasmin inhibitor fragment synthesis; peptide synthesis; thioanisole-mediated TFMSA-TFA deprotection; dissociation constnat

As described in the preceding paper,<sup>1)</sup> the human  $\alpha_2$ -plasmin inhibitor fragment named T-11 was found to show high binding activity to plasmin.<sup>3)</sup> To clarify the structure-activity relationship of this important plasma protein inhibitor fragment, we undertook the synthesis of this fragment and its intermediates by a conventional solution method. In this paper, we describe the synthesis of the hexacosapeptide that covers the entire amino acid sequence of T-11 by further chain elongation with the three newly synthesized fragments of the intermediate, hexadecapeptide ester, whose synthesis has already been reported,<sup>1)</sup> followed by the thioanisole–mediated deprotection of all protecting groups with TFMSA–TFA<sup>4a,b)</sup> and purification steps.

The strategy employed here for the synthesis was essentially the same as that described in the preceding paper.<sup>1)</sup> Amino acid derivatives having side chain-protecting groups removable by the above deprotecting reagents at the final step were employed, *i.e.*, Asp(OBzl), Lys(Z) and Glu(OBzl). The Met residue was protected as its sulfoxide as previously described.<sup>1)</sup>

The first fragment, Boc–Asp(OBzl)–Leu–Lys(Z)–OH (I-11), was prepared stepwise starting with the condensation of H–Lys(Z)–OH and Boc–Leu–OH by means of the pentachlorophenyl ester procedure.<sup>5)</sup> Next, the *p*-nitrophenyl ester procedure<sup>6)</sup> was used for the combination of Boc–Asp(OBzl)–OH with a TFA–treated sample of the above dipeptide, Boc–Leu–Lys(Z)–OH.

The fragment, Boc-Leu-Phe-Gly-Pro-NHNH<sub>2</sub> (I-12), was prepared from the dipeptides Boc-Gly-Pro-OBzl and Boc-Leu-Phe-NHNH<sub>2</sub>. The DCC method<sup>7</sup> was useful for condensation of Boc-Gly-OH and H-Pro-OBzl to give the above dipeptide. Boc-Leu-OH and H-Phe-OMe were combined by the DCC-HOBT method<sup>8</sup> and the resulting dipeptide ester was converted to the corresponding hydrazide with hydrazine hydrate. A TFA-treated sample of Boc-Gly-Pro-OBzl was condensed with Boc-Leu-Phe-NHNH<sub>2</sub>, *via* the azide,<sup>9</sup> and the

resulting tetrapeptide ester, Boc-Leu-Phe-Gly-Pro-OBzl, was then converted to the corresponding hydrazide in the usual manner.

The N-terminal fragment of T-11, Boc–Gly–Asp(OBzl)–Lys(Z)–OH (I-13), was also prepared in a stepwise manner starting with H–Lys(Z)–OH, which was successively condensed with Boc–Asp(OBzl)–OH and Boc–Gly–OH by the *p*-nitrophenyl ester procedure.

Subsequent fragment condensation was carried out as follows. The fragment Boc–Asp(OBzl)–Leu–Lys(Z)–OH (I-11) was condensed with a TFA–treated sample of the protected hexadecapeptide ester  $(I-10)^{11}$  by the DCC–HOBT method to give the nona-decapeptide ester (I-14). Then, Boc–Leu–Phe–Gly–Pro–NHNH<sub>2</sub> (I-12) was coupled with a TFA–treated sample of I-14 by the azide procedure. The DCC–HOBT method was used for the final condensation of Boc–Gly–Asp(OBzl)–Lys(Z)–OH (I-13) and a TFA–treated sample of I-15 as indicated in Fig. 1.

After completion of the coupling reactions had been confirmed by the ninhydrin test, each product was purified by batchwise washing with 5% citric acid and 5% NaHCO<sub>3</sub>, followed by precipitation.

The purity of these intermediates was checked by TLC, elemental analysis and amino acid analysis. The amino acid ratios of the acid hydrolysates of these intermediates are shown in Table I.

The deprotection procedure and the subsequent purification methods are shown in Fig. 2. The protected hexacosapeptide ester (I-16) thus obtained was treated with 1 M TFMSA in



- 6. DEAE-cellulose (gradient elution with NH<sub>4</sub>HCO<sub>3</sub>)
- purified T-11
  - Fig. 2. Deprotection of (I-16) and Purification of T-11

	I-14 <sup><i>a</i>)</sup>	I-15 <sup>a)</sup>	I-16 <sup><i>a</i>)</sup>	T-11 <sup>a)</sup>	T-11 <sup>b)</sup>
Asp	2.28 (2)	1.85 (2)	3.01 (3)	3.01 (3)	2.84 (3)
Ser	0.82(1)	0.68 (1)	0.73 (1)	0.85(1)	0.92 (1)
Glu	3.16 (3)	2.72 (3)	2.98 (3)	3.01 (3)	2.04 (2)
Gln					1.02 (1)
Pro	4.24 (4)	5.17 (5)	4.55 (5)	5.21 (5)	5.31 (5)
Gly	1.01 (1)	1.95 (2)	3.16 (3)	3.01 (3)	3.33 (3)
Val	1.13 (1)	1.12 (1)	1.23 (1)	1.02 (1)	1.05 (1)
Met	0.56 (1)	0.58 (1)	0.84 (1)	0.79(1)	0.92 (1)
Leu	2.18 (2)	3.04 (3)	3.40 (3)	3.11 (3)	3.09 (3)
Tyr	0.86 (1)	0.69 (1)	0.77 (1)	0.92 (1)	1.09 (1)
Phe	1.00 (1)	2.00 (2)	2.00 (2)	2.00 (2)	2.00 (2)
Lys	2.02 (2)	1.97 (2)	3.13 (3)	2.85 (3)	2.98 (3)
Rec. (%)	92	90	89	91	81

 TABLE I.
 Amino Acid Ratios in 6 N HCl Hydrolysates and Aminopeptidase M

 Digests of Synthetic T-11 and Its Intermediates

a) A 6 N HCl hydrolysate. b) Aminopeptidase M digest.

TFA in the presence of thioanisole<sup>4a)</sup> and *m*-cresol<sup>4b)</sup> in an ice-bath for 1 h to remove all protecting groups employed. After precipitation of the peptide with ether, the deprotected peptide was converted to the corresponding acetate by treatment with Amberlite IRA-410 (acetate form). The product was next treated with diluted ammonia in order to reverse the possible  $N \rightarrow O$  shift at the Ser residue.<sup>10)</sup> To ensure the complete reduction of the Met(O) residue, the deprotected peptide was incubated with dithiothreitol,<sup>11)</sup> and then subjected to gel-filtration on Sephadex G-25. The main peak portions were collected and lyophilized. The lyophilized product was further purified by ion-exchange chromatography on DEAE-cellulose. In elution with a gradient up to 0.20 m NH<sub>4</sub>HCO<sub>3</sub>, minor peaks were detected before the main peak (Fig. 3). The main product thus obtained exhibited a single peak on HPLC (Fig. 4) and a single spot on TLC in two different solvent systems. Its purity was further confirmed by elemental analysis and amino acid analysis after acid hydrolysis and enzymatic digestion.

The dissociation constant for the interaction between our synthetic peptide and plasmin measured by the method of Wiman *et al.*<sup>12)</sup> was determined to be 4—7  $\mu$ M, which was the same as that reported for the native T-11 (5.5  $\mu$ M).<sup>3)</sup>

## Experimental

Melting points are uncorrected. Optical rotations were determined with a JASCO DIP 140 digital polarimeter. Acid hydrolysis was performed in 6 N HCl at 110 °C for 24 h in evacuated tubes. The amino acid compositions of acid hydrolysates were determined with a Hitachi amino acid analyzer 835 and are not corrected for amino acid destruction. TLC was performed on silica-gel plates (Kiesel gel 60 F<sub>254</sub>, Merck) and *Rf* values refer to the following solvent systems:  $Rf^1$  CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (8:3:1),  $Rf^2$  *n*-butanol-pyridine-acetic acid-H<sub>2</sub>O (4:1:1:2),  $Rf^3$  *n*-butanol-pyridine-H<sub>2</sub>O (1:1:1).

Analytical HPLC was conducted on a JASCO TWINCLE apparatus equipped with a Cosmosil  $5C_{18}$ -P ( $5\mu$ , Nakarai Chem. Co.) column ( $4.6 \times 150$  mm) by linear gradient elution with acetonitrile (10% to 50%, 15 min) in 0.05% TFA at a flow rate of 1.0 ml/min with monitoring at 230 nm. Aminopeptidase M (Lot. 012583-1) was purchased from Pierce Chemical Co., Ltd.

**Boc-Leu-Lys(Z)-OH**—Boc-Leu-OPcp (3.88 g) was added to a suspension of H-Lys(Z)-OH (2.59 g) and  $Et_3N$  (2.47 ml) in DMSO-DMF (10 ml-15 ml) and the mixture was stirred at room temperature for 48 h. The solvent was removed by evaporation and the residue was dissolved in 5% NaHCO<sub>3</sub>. This solution was washed with AcOEt, then acidified with citric acid and the precipitate was extracted with AcOEt. The AcOEt layer was washed with 5%

citric acid and H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub> and then evaporated. The resulting oily residue was applied to a silica-gel column(65 g,  $2.5 \times 24$  cm) and eluted with CHCl<sub>3</sub>. The purified dipeptide was treated with IPE and recrystallized from AcOEt and IPE; yield 2.80 g (72%), mp 72—74 °C,  $[\alpha]_{25}^{D}$  -11.9 ° (*c*=1.1, MeOH), *Rf*<sup>1</sup> 0.51. *Anal*. Calcd for C<sub>25</sub>H<sub>39</sub>N<sub>3</sub>O<sub>7</sub> · 0.5H<sub>2</sub>O: C, 59.74; H, 8.02; N, 8.36. Found: C, 60.18; H, 7.82; N, 8.33.

**Boc-Asp(OBzI)-Leu-Lys(Z)-OH (I-11)**—Boc-Leu-Lys(Z)-OH (2.47 g) was treated with TFA (10 ml) in the usual manner and the resulting oily product was dissolved in THF (20 ml) and neutralized with Et<sub>3</sub>N. To this solution, Boc-Asp(OBzI)-ONp (2.29 g) and Et<sub>3</sub>N (1.43 ml) were added, and the mixture was stirred at room temperature overnight. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in AcOEt and the AcOEt layer was washed with 5% citric acid and H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The resulting residue was crystallized from *n*-hexane and recrystallized from AcOEt and IPE; yield 2.56 g (71%), mp 98—100 °C,  $[\alpha]_{D}^{24} - 25.0^{\circ}$  (*c*=1.1, MeOH), *Rf*<sup>1</sup> 0.78. *Anal*. Calcd for C<sub>36</sub>H<sub>50</sub>N<sub>4</sub>O<sub>10</sub>: C, 61.87; H, 7.21; N, 8.02. Found: C, 61.68; H, 7.18; N, 8.09.

**Boc–Gly–Pro–OBzl**—DCC (3.21 g) was added to a mixture of Boc–Gly–OH (2.51 g) and H–Pro–OBzl (prepared from 3.80 g of the hydrochloride with 2.18 ml of  $Et_3N$ ) in THF–CHCl<sub>3</sub> (10 ml–5 ml), and the solution was stirred at room temperature for 18 h, then filtered. The filtrate was evaporated, and the residue was dissolved in AcOEt. This solution was washed with 5% citric acid, 5% NaHCO<sub>3</sub> and H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to give an oily residue; yield 4.98 g (96%),  $Rf^{-1}$  0.67.

**Boc–Leu–Phe–OMe**—DCC (4.49 g) and HOBT  $\cdot$  H<sub>2</sub>O (3.06 g) were added to a solution of Boc–Leu–OH  $\cdot$  H<sub>2</sub>O (5.17 g) in dioxane–THF (10 ml–20 ml). After stirring of the mixture for 15 min, H–Phe–OMe (prepared from 4.31 g of the hydrochloride with 2.79 ml of Et<sub>3</sub>N) in CHCl<sub>3</sub>–dioxane (20 ml–10 ml) was added, and the whole was stirred at room temperature for 18 h, then filtered. The filtrate was evaporated, and the residue was extracted with AcOEt. The AcOEt layer was washed with 5% citric acid, 5% NaHCO<sub>3</sub> and H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub> and then evaporated. The resulting residue was triturated with IPE and *n*-hexane and recrystallized from ether and IPE–*n*-hexane; yield 7.61 g (97%), mp 86–87 °C (Lit.<sup>13)</sup> 78–79 °C), [ $\alpha$ ]<sub>25</sub><sup>25</sup> – 20.7 ° (c = 1.2, MeOH) (Lit.<sup>13)</sup> [ $\alpha$ ]<sub>25</sub><sup>25</sup> – 27.6 ° (c = 1.0, MeOH), *Rf*<sup>1</sup> 0.83. *Anal.* Calcd for C<sub>21</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub>: C, 64.24; H, 8.22; N, 7.14. Found: C, 64.19; H, 8.25; N, 7.08.

**Boc–Leu–Phe–NHNH**<sub>2</sub>—Hydrazine hydrate (80%, 11.70 ml) was added to a solution of Boc–Leu–Phe–OMe (7.55 g) in MeOH (50 ml), and the gelatinous mass that formed after standing overnight was collected by filtration and recrystallized from MeOH and IPE; yield 6.95 g (92%), mp 165–166 °C,  $[\alpha]_D^{25} - 36.0^\circ$  (c = 1.0, MeOH),  $Rf^1$  0.80. *Anal.* Calcd for C<sub>20</sub>H<sub>32</sub>N<sub>4</sub>O<sub>4</sub>: C, 61.20; H, 8.22; N, 14.28. Found: C, 61.06; H, 8.08; N, 14.17.

**Boc–Leu–Phe–Gly–Pro–OBzl**—Boc–Gly–Pro–OBzl (2.62 g) was treated with TFA (5 ml) in the usual manner, and the excess TFA was removed by evaporation. The residue was decanted with IPE and *n*-hexane, and the residue thus obtained was dissolved in DMF (10 ml) and neutralized with Et<sub>3</sub>N. This solution was combined with the azide (prepared from 2.35 g of Boc–Leu–Phe–NHNH<sub>2</sub> with 3.72 ml of 3.87 N HCl–DMF, 0.97 ml of isoamyl nitrite and 2.83 ml of Et<sub>3</sub>N) in DMF (10 ml). The mixture was stirred at 4 °C for 48 h, then evaporated *in vacuo*, and the residue was extracted with AcOEt. The extract was washed with 5% citric acid, 5% NaHCO<sub>3</sub> and H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue was triturated with IPE and *n*-hexane, followed by recrystallization from ether and IPE*n*-hexane; yield 3.40 g (91%), mp 78–80 °C,  $[\alpha]_{25}^{25}$  – 84.4 ° (*c*=1.0, MeOH), *Rf*<sup>1</sup> 0.90. Anal. Calcd for C<sub>34</sub>H<sub>46</sub>N<sub>4</sub>O<sub>7</sub> · 0.5H<sub>2</sub>O: C, 64.64; H, 7.50; N, 8.87. Found: C, 64.82; H, 7.30; N, 8.85.

**Boc–Leu–Phe–Gly–Pro–NHNH**<sub>2</sub> (I-12) — Hydrazine hydrate (80%, 3.84 ml) was added to a solution of the above protected tetrapeptide ester (3.94 g) dissolved in MeOH (40 ml). The mixture was left to stand at room temperature for 18 h, then the solvent was removed by evaporation. The residue was dissolved in AcOEt, and the organic phase was washed with NaCl–H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub> and then evaporated. The residue was triturated with IPE, and the mass obtained was recrystallized from MeOH and IPE; yield 3.04 g (88%), mp 113–115 °C,  $[\alpha]_{25}^{25}$  – 80.5 ° (*c* = 1.1, MeOH), *Rf*<sup>1</sup> 0.48. *Anal*. Calcd for C<sub>27</sub>H<sub>42</sub>N<sub>6</sub>O<sub>6</sub> · 0.5H<sub>2</sub>O: C, 58.36; H, 7.80; N, 15.13. Found: C, 58.36; H, 7.80; N, 15.19.

**Boc-Asp(OBzl)-Lys(Z)-OH**—Boc-Asp(OBzl)-ONp (8.89 g) was added to a suspension of H-Lys(Z)-OH (6.92 g) in DMF (70 ml) containing  $Et_3N$  (3.63 ml) and *N*-methylmorpholine (2.20 ml), and the mixture was stirred at room temperature for 48 h. Work-up as described for Boc-Leu-Lys(Z)-OH afforded an oily product; yield 10.23 g (87%),  $Rf^1$  0.54.

**Boc-Gly-Asp(OBzl)-Lys(Z)-OH (I-13)**—Boc-Asp(OBzl)-Lys(Z)-OH (5.65 g) was treated with TFA (15 ml) in an ice-bath for 45 min, then the excess TFA was removed by evaporation. IPE was added to the residue. The resulting powder was dried over KOH pellets *in vacuo* for 1 h and then dissolved in THF (20 ml) followed by neutralization with Et<sub>3</sub>N. To this solution, Boc-Gly-ONp (3.27 g) and Et<sub>3</sub>N (1.33 ml) were added, and the mixture was stirred at room temperature for 48 h. After usual work-up as described above, the residue was triturated with ether and the gelatinous mass obtained was precipitated from AcOEt-MeOH with ether-IPE; yield 3.32 g (54%), mp 81–83 °C, [ $\alpha$ ]<sub>25</sub><sup>25</sup> – 5.6 ° (c=1.0, MeOH),  $Rf^{1}$  0.43. Anal. Calcd for C<sub>32</sub>H<sub>42</sub>N<sub>4</sub>O<sub>10</sub>: C, 59.80; H, 6.59; N, 8.72. Found: C, 59.95; H, 6.62; N, 9.01.

Boc-Asp(OBzl)-Leu-Lys(Z)-Leu-Val-Pro-Pro-Met(O)-Glu(OBzl)-Glu(OBzl)-Asp(OBzl)-Tyr-Pro-Gln-Phe-Gly-Ser-Pro-Lys(Z)-OBzl (I-14) — The protected hexadecapeptide ester (I-10) (2.07 g) was treated with TFA (10 ml) in the usual manner, and the resulting dried powder was dissolved in DMF (10 ml) and neutralized with  $Et_3N$ . DCC (0.28 g) and HOBT  $\cdot$  H<sub>2</sub>O (0.13 g) were added to a solution of Boc–Asp(OBzl)–Leu–Lys(Z)–OH (I-11) (0.83 g) in DMF (5 ml). The above N<sup>*x*</sup>-deprotected peptide solution was added to this mixture. The reaction mixture was stirred at room temperature for 18 h, then DCurea was filtered off and the filtrate was concentrated under reduced pressure. The residue was treated with 5% citric acid and ether, and the resulting powder was collected and washed batchwise with 5% citric acid, 5% NaHCO<sub>3</sub> and H<sub>2</sub>O, followed by precipitation from MeOH with AcOEt; yield 2.35 g (92%), mp 139–141 °C, [x]<sub>2</sub><sup>25</sup> – 58.9 ° (c = 1.1, DMF), Rf<sup>1</sup> 0.64. Anal. Calcd for C<sub>156</sub>H<sub>202</sub>N<sub>22</sub>O<sub>38</sub>S · 3H<sub>2</sub>O: C, 60.84; H, 6.81; N, 10.01. Found: C, 60.78; H, 6.72; N, 10.02.

**Boc-Leu-Phe-Gly-Pro-Asp(OBzl)-Leu-Lys(Z)-Leu-Val-Pro-Pro-Met(O)-Glu(OBzl)-Glu(OBzl)-Asp-(OBzl)-Tyr-Pro-Gln-Phe-Gly-Ser-Pro-Lys(Z)-OBzl (1-15)** The above nonadecapeptide ester (1-14) (1.70 g) was treated with TFA (10 ml) as stated above and the excess TFA was removed by evaporation. The residue was treated with ether, and the resulting powder was dried over KOH pellets *in vacuo* and then dissolved in DMF (5 ml). After neutralization with Et<sub>3</sub>N, the azide (prepared from 0.46 g of Boc-Leu-Phe-Gly-Pro-NHNH<sub>2</sub> with 0.65 ml of 3.87 N HCl-DMF, 0.17 ml of isoamyl nitrite and 0.43 ml of Et<sub>3</sub>N) in DMF (5 ml) was combined with the above  $N^{\alpha}$ -deprotected peptide solution and the mixture was stirred at 4 °C for 48 h. After work-up as described above, the product was precipitated from MeOH with ether; yield 1.86 g (96%), mp 133–136 °C,  $[\alpha]_{D}^{25}$  -62.7 ° (*c*=1.1, DMF), *Rf*<sup>-1</sup> 0.66. *Anal.* Calcd for C<sub>178</sub>H<sub>232</sub>N<sub>26</sub>O<sub>42</sub>S·4H<sub>2</sub>O: C, 60.87; H, 6.89; N, 10.37. Found: C, 60.64; H, 6.69; N, 10.44.

**Boc-Gly-Asp(OBzl)-Lys(Z)-Leu-Phe-Gly-Pro-Asp(OBzl)-Leu-Lys(Z)-Leu-Val-Pro-Pro-Met(O)-Glu-(OBzl)-Glu(OBzl)-Asp(OBzl)-Tyr-Pro-Gln-Phe-Gly-Ser-Pro-Lys(Z)-OBzl (1-16)**—The above compound (I-15) (1.40 g) was treated with TFA (10 ml) as stated above, and ether was added. The resulting powder was dissolved in DMF (7 ml) and neutralized with Et<sub>3</sub>N. This solution was added to a mixture of Boc-Gly-Asp(OBzl)-Lys(Z)-OH (0.39 g), DCC (0.15 g) and HOBT  $\cdot$  H<sub>2</sub>O (0.10 g) in DMF (3 ml) and the mixture was stirred at room temperature for 18 h, then filtered. The filtrate was evaporated, and the residue was treated with ether and H<sub>2</sub>O. The resulting powder was washed batchwise as stated above and precipitated from MeOH-THF with ether; yield 1.41 g (88%), mp 198—202 °C,  $[\alpha]_{25}^{25}$  - 58.6 ° (c = 1.0, DMF),  $Rf^1$  0.64. Anal. Calcd for C<sub>205</sub>H<sub>264</sub>N<sub>30</sub>O<sub>49</sub>S · 4H<sub>2</sub>O: C, 60.99; H, 6.79; N, 10.41. Found: C, 60.77; H, 6.63; N, 10.40.

H-Gly-Asp-Lys-Leu-Phe-Gly-Pro-Asp-Leu-Lys-Leu-Val-Pro-Met-Glu-Glu-Asp-Tyr-Pro-Gln-Phe-Gly-Ser-Pro-Lys-OH (T-11) The above protected hexacosapeptide ester (1-16) (200 mg) was treated with 1 M TFMSA-thioanisole in TFA (3.97 ml) in the presence of *m*-cresol (0.53 ml) in an ice-bath for 1 h, then the solvent was removed by evaporation and dry ether was added to the residue. The resulting powder was collected by filtration, and dried over KOH pellets *in vacuo* for 30 min. This treatment was repeated twice to ensure complete deprotection. The deprotected peptide thus obtained was dissolved in H<sub>2</sub>O (4 ml), and treated with Amberlite IRA-410 (acetate form) for 30 min with stirring in an ice-bath. The mixture was filtered, and the pH of the filtrate was adjusted to 8.0 with 5% NH<sub>4</sub>OH and after 30 min to 4.0 with AcOH in an ice-bath. The solution was lyophilized to give a hygroscopic powder, which was incubated with dithiothreitol (200 mg) in H<sub>2</sub>O (5 ml) in an atmosphere of nitrogen at 37 °C for 19 h. The solution was applied to a Sephadex G-25 column (3.3 × 133 cm) and eluted with 1 N AcOH at a flow rate of 71.2 ml/h. The UV absorption at 275 nm was determined in each fraction (10.4 ml). The fractions corresponding to the front main peak (tube Nos. 53—66) were collected and the solvent was removed by lyophilization to give a white powder; yield 114 mg (78%).



Fig. 3. Purification of Crude Synthetic T-11 by Ion-Exchange Chromatography on DEAE-Cellulose



The crude product was dissolved in  $H_2O$  (3 ml), and the solution was applied to a column of DEAE-cellulose (1.5 × 34.5 cm), which was first washed with the starting buffer 0.01 M NH<sub>4</sub>HCO<sub>3</sub> (pH 8.1) and then eluted with a linear gradient formed from the starting buffer (500 ml) and 0.20 M NH<sub>4</sub>HCO<sub>3</sub> (500 ml) at a flow rate of 41 ml/h. The UV absorption at 275 nm in each fraction (10.2 ml) was determined (Fig. 3). The fractions corresponding to the main peak (tube Nos. 59–70) were combined and the solvent and ammonium salt were removed by lyophilization. After the addition of 1 N AcOH to the residue, the solvent was removed by lyophilization to give a fluffy powder; yield 52 mg (36%),  $[\alpha]_D^{25} - 76.0^\circ$  (c = 0.2, 0.1 N AcOH),  $Rf^3 0.12, Rf^4 0.70$ . The synthetic peptide exhibited a single peak at a retention time of 15.4 min on HPLC with a Cosmosil 5C<sub>18</sub>-P column (4.6 × 150 mm) (Fig. 4). Amino acid ratios in a 6 N HCl hydrolysate and an aminopeptidase M digest of the synthetic peptide are shown in Table I. *Anal.* Calcd for C<sub>134</sub>H<sub>202</sub>N<sub>30</sub>O<sub>40</sub>S · 4AcOH · 15.5H<sub>2</sub>O: C, 49.80; H, 7.33; N, 12.27. Found: C, 49.97; H, 7.52; N, 12.53.

Acknowledgement We are grateful to the staff of the Analytical Section of this Institute for elemental and amino acid analyses. We also thank Mr. Sugiyama, Laboratory of Biochemistry of this Institute, for the determination of the dissociation constants of the synthetic peptide.

## **References and Notes**

- 1) Part I: T. Sasaki and S. Isoda, Chem. Pharm. Bull., 35, 2797 (1987).
- 2) Amino acid and peptide derivatives mentioned in this paper are of the L-configuration. The following abbreviations were used: Z = benzyloxycarbonyl, Z(OMe)=p-methoxybenzyloxycarbonyl, Bzl = benzyl, Boc = tert-butoxycarbonyl, Np=p-nitrophenyl, Pcp=pentachlorophenyl, DCC=dicyclohexylcarbodiimide, DCurea=dicyclohexylurea, DEAE=diethylaminoethyl, DMSO=dimethylsulfoxide, AcOEt=ethyl acetate, DMF=dimethylformamide, HPLC=high performance liquid chromatography, HOBT=N-hydroxybenzotriazole, IPE=isopropyl ether, MeOH=methanol, THF=tetrahydrofuran, TLC=thin-layer chromatography, Et<sub>3</sub>N=triethylamine, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid, UV=ultraviolet.
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