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Synthesis of an Immunologically Active Analog of Thymic Humoral Factor-γ 2 with Enhanced Enzymatic Stability

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Abstract—Acetyl-thymic humoral factor-γ 2 chloromethyl ketone [Ac–Leu–Glu–Asp–Gly–Pro–Lys–Phe–Leu–CH_2Cl], an analog of thymic humoral factor-γ 2, was synthesized and studied for its immunological effects on the impaired blastogenic response of T-lymphocytes isolated from uremic patients. Synthetic thymic humoral factor-γ 2 and the synthetic acetyl-thymic humoral factor-γ 2 chloromethyl ketone both restored the impaired blastogenic response of T-lymphocytes of uremic patients. However, the synthetic thymic humoral factor-γ 2 is susceptible to proteolytic digestion. On the other hand, the synthetic acetyl-thymic humoral factor-γ 2 chloromethyl ketone retained activity and was shown to exhibit a high degree of stability when incubated in human serum, These results indicate that N-terminal acetylation and the introduction of a chloromethyl ketone residue into the C-terminal residue of thymic humoral factor-γ 2 increase resistance to proteolytic degradation by exopeptidases without loss of immunological activity.

Introduction

Thymic humoral factor-γ 2 (THF-γ 2), an octapeptide essential for immune regulation, was isolated from calf thymus by Burnstein *et al.*[2] THF-γ 2 has the amino acid sequence, H–Leu–Glu–Asp–Gly–ProLys–Phe–Leu–OH, with no homology to the sequence of other thymic hormones. THF-γ 2 was found to be one of the factors that modulated steps in the maturation of T-lymphocytes.[2]

Uremic patients suffer from an abnormal cellular immune function. This includes frequent infections[3] and a high incidence of neoplasms.[4] In experimental chronic uremia in the rat, the depressed cellular immunity has been well documented.

In a previous paper,[5] we reported syntheses and immunological effects of THF-γ 2 and its analogues and showed that the synthetic THF-γ 2 and also some analogues could have restoring effects on the impaired cell-mediated immunological functions. However, a major problem in the development of THF-γ 2 as a therapeutic agent is the relatively short duration of action of the naturally occurring peptide; the *in vivo* plasma half-life of this peptide, which is not unlike other peptide hormones.

This report deals with the synthesis of Ac-THF-γ 2-CH_2Cl, which was expected to be a more enzymatic stable analogue of THF-γ 2. The N- and C-terminal amino acids of THF-γ 2 were modified with an acetyl group and a chloromethyl ketone group to increase resistance to proteolytic degradation by exopeptidases. The desired Ac-THF-γ 2-CH_2Cl was synthesized by the conventional solution method according to the scheme shown in Figure 1.

As shown, the TFA-labile Boc group, for N^{α}-protection, and amino acid derivatives bearing protecting groups removable by catalytic hydrogenation, were employed, i.e. Glu(OBzl), Asp(OBzl) and Lys(Z). The substituted hydrazine, Troc-$NHNH_2$,[6] was employed for the preparation of the fragment, Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly–NHNH-Troc, containing the Glu(OBzl) and Asp(OBzl) residues. This Troc group is known to be cleaved by Zn^7 in AcOH without affecting other functional groups. Boc–Leu–CH_2Cl was obtained by the mixed anhydride coupling of Boc–Leu–OH and diazomethane, followed by the addition of HCl/doixance.[8] Throughout the syntheses of intermediates and fragments, the purity of every intermediate was checked by TLC, elemental analysis and amino acid analysis. The analytical results were within ± 0.4 % of theoretical values in all cases.

Starting from Boc–Leu–CH_2Cl, fragment **IV**, Boc–Pro–Lys(Z)–Phe–Leu–CH_2Cl, was prepared by stepwise elongation using the Su active ester procedure.[9] Fragment **VII**, Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly–NHNH-Troc, was also prepared by stepwise elongation using the Su active ester procedure[9] except for introduction of the Ac–Leu residue, for which the HOBT–WSCI procedure[10] was employed.

Fragment **VII** was treated with Zn^7 in AcOH and DMF to remove the Troc group, and the last trace of zinc acetate was removed by treatment with EDTA to give Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly–$NHNH_2$ **VIII** in analytically pure form. The hydrazine test on the thin-layer chromatograms and elemental analytical data were consistent with homogeneity of the desired product. The resulting hydrazide **VIII** was condensed with the protected C-teminal tetrapeptide chloromethyl ketone **IV** by the azide

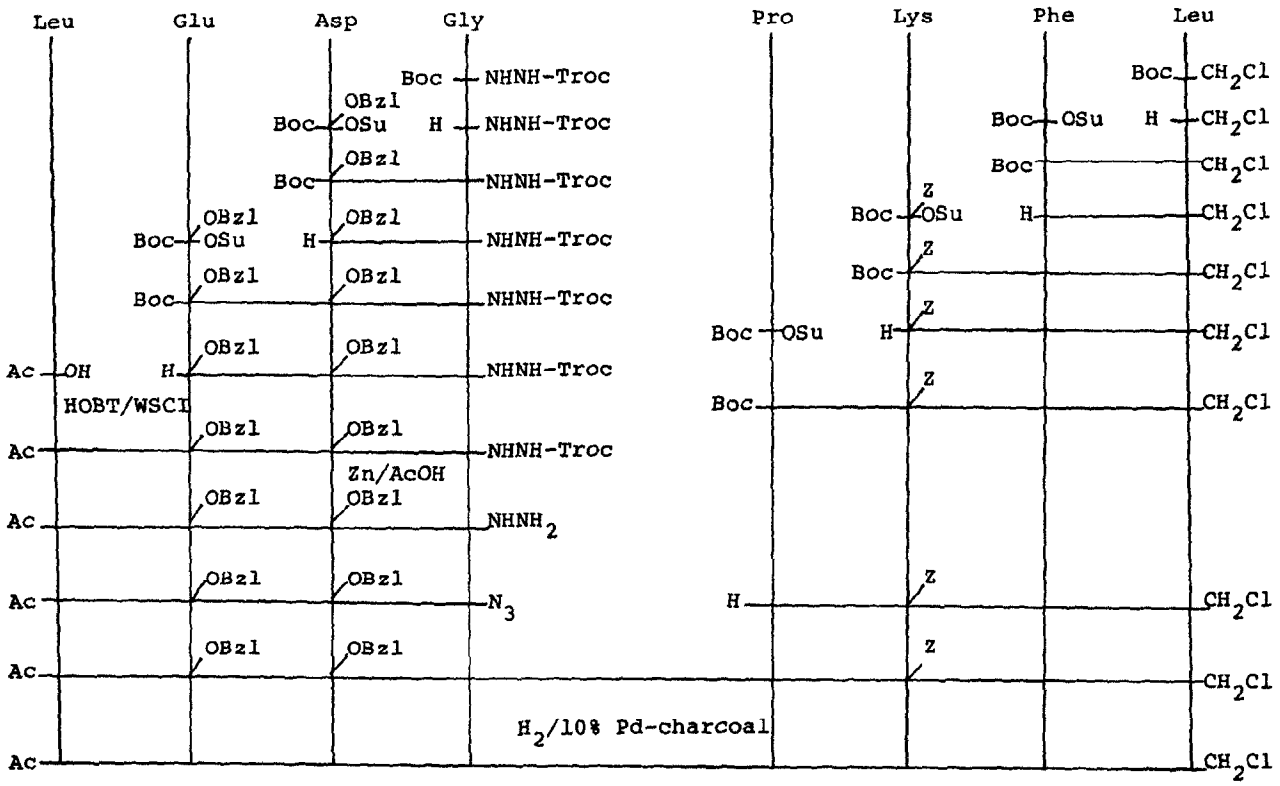
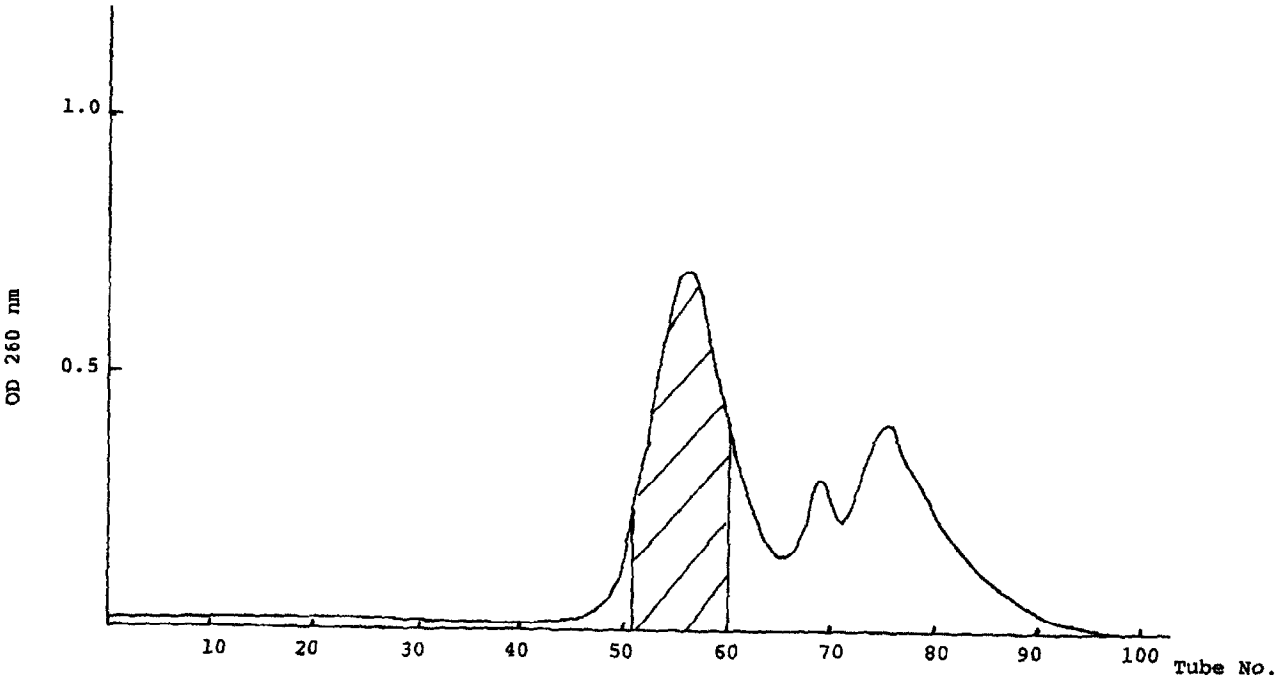


Figure 1. Synthetic route to Ac-THF-γ2-CH_2Cl



solvent: 2 % AcOH
column: 3.0 × 97 cm

Figure 2. Purification of synthetic Ac-THF-g2-CH2Cl by gel-filtration chromatography on a*Sephadex G-15 column*

procedure[11] to yield Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly–Pro–Lys(Z)–Phe–Leu–CH_2Cl **IX**. The homogeneity of the protected octapeptide **IX** was also assessed by elemental analysis, TLC and amino acid analysis of the acid hydrolysate.

In the final step of the synthesis, the protected octapeptide chloromethyl ketone was hydrogenated over 10 % Pd-charcoal in AcOH–H_2O–MeOH (5:1:4) for 24 h. The hydrogenated product was purified by gel-filtration on Sephadex G-15, followed by preparative TLC. Desalting on

a Sephadex G-15 column gave a fluffy powder, which exhibited a single chlorine-tolidine-positive spot on TLC. Its purity was further confirmed by amino acid analysis after acid hydrolysis. The peptide also exhibited a single peak on HPLC (Figure 3).

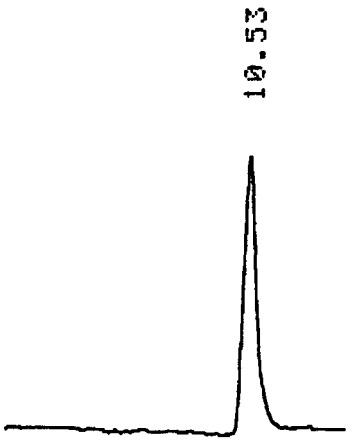


Figure 3. HPLC of Synthetic Ac-THF-γ2-CH_2Cl

The molecular weight of the synthetic peptide was ascertained by FAB-MS spectrometry. The immunological effect of the synthetic Ac–THF-γ 2-CH_2Cl and THF-γ 2[5] was examined by means of the JIMRO (Japan Immunoresearch Laboratory Co., Ltd) fluorometric blast-formation test according to Itoh and Kawai.[12] Responses of T-lymphocytes to mitogenic stimulation were significantly lower in uremic patients than those of normal persons. The *in vitro* effect of these two synthetic peptides after treatment of human serum on the impaired PHA response of T-lymphocytes from the uremic patients is shown in Table 1.

Results and Discussion

Peptide hormones have several shortcomings. Among them, rapid degradation by proteolytic enzymes precludes oral dosage and the maintenance of significant blood levels. Slow infusion protocols are necessary to achieve an adequate immunological effect in many cases. The practical difficulties of slow infusion could be overcome by design of a THF-γ 2 analogue with enhanced stability to enzymatic degradation.

Low and Goldstein reported[13] that N-terminal acetylation of thymosin β_4 increases resistance to proteolytic degradation by exopeptidases. It is known that peptidyl chloromethyl ketone derivatives have inhibitory activity against endopeptidase (EC 3.4.22.19).[14] These results prompted us to synthesize Ac-THF-γ 2-CH_2Cl.

Comparison of the stimulation index (SI) values of the blastogenic transformation of T-lymphocytes into lymphoblasts with mitotic activity upon PHA stimulation shows that restorative effect of both peptides, Ac-THF-γ 2-CH_2Cl and THF-γ 2, was approximately equal in potency without prior incubation in human serum. The synthetic Ac-THF-γ 2-CH_2Cl, however, still demostrated a restorative effect in the uremic patients investigated after treatment of human serum, although the restorative effect after incubation in human serum was a little weaker than that of the same peptide without incubation. In contrast, *in*

Table 1. Effect of the synthetic Ac–THF–γ2–CH_2Cl and THF–γ2 on the impaired PHA-stimulation of T-lymphocytes of uremic patients after treatment of human serum

Peptide	Dose (μg/ml)	SI[a,b]
—[c]	—	281.6 ± 49.6
—[d]	—	112.4 ± 48.3[g]
Ac-THF-γ2-CH_2Cl[d,e]	1.0	220.8 ± 50.2[h]
THF-γ2[d,e]	1.0	226.1 ± 47.9[h]
Ac-THF-γ2-CH_2Cl[d,f]	1.0	198.5 ± 50.2[h]
THF-γ2[d,f]	1.0	110.9 ± 48.7[h]

[a]Each value represent the mean ± S.D. of triplicate measurements.
[b]SI (stimulation index) was calculated according to the following formula: $SI = \frac{I_2 - I_o}{I_1 - I_o} \times 100$,
I_2 = mean fluorescence intensity of PHA-activated lymphocytes, I_1 = fluorescence intensity of PHA-nonactivated lymphocytes and I_o = fluorescence intensity of ethidium bromide.
[c]Normal venous lymphocytes.
[d]Patient's venous lymphocytes.
[e]Incubation was carried out at 37 °C in a humidified atmosphere of 5 % CO_2 in air for 12 h using each synthetic peptide without treatment of human serum.
[f]Each synthetic peptide was incubated in human serum at 37 °C for 30 min and then lymphocytes were incubated with one of the human serum treated peptides at 37 °C for 12 h.
[g]$p<0.05$, when compared to the normal persons using Student's t test.
[h]$p<0.01$, when compared to the uremic patients using Student's t test.

vitro addition of the synthetic THF-γ 2 after treatment of human serum had no effect on the mitotic activity induced by PHA stimulation under the same conditions.

These results seem to suggest that N-terminal acetylation and the introduction of chloromethyl ketone into the C-terminal residue of THF-γ 2 increase resistance to inactivation by enzyme degradation. In the case of normal subjects, *in vitro* addition of these peptides had no effect on the mitotic activity induced by PHA stimulation under the same conditions (data not shown).

Experimental

General experimental procedures used in this paper are essentially the same as described in the previous papers.[15,16] Melting points are uncorrected. Rotations were measured on an Atago Polax machine (cell length: 10 cm). The amino acid compositions of the hydrolysates were determined with a Hitachi type 835-50 amino acid analyzer. HPLC was conducted with a Shimadzu LC-3A apparatus coupled to a Cosmosil 5C18 column (4.6 × 100 mm). FAB-MS spectra were obtained on an Auto Spec Q instrument (UQ Analytical Co., England) mass spectrometer equipped with an OPUS data processor. Solutions were concentrated in a rotary evaporator under reduced pressure at a temperature of 30–45 °C. Boc groups of the protected peptides were removed by TFA–anisole treatment. The resulting amino components were chromatographed on silica gel plates (Kieselgel G, Merck) and R_f^1 values refer to the following solvent system: $CHCl_3$–MeOH–H_2O (90:8:2). The final product corresponding to Ac-THF-γ 2-CH_2Cl was chromatographed on a cellulose plate (Merck). R_f^2 value refers to BuOH–AcOH–H_2O (4:1:5, upper layer). Patient selection involved two uremic patients who were suffering from recurrent infectious diseases; examination of the cellular immunocompetence of these patients revealed a significant decrease in blast-formation by PHA. ^{3}H-Thymidine incorporation values of these patients were 11089 and 10423 cpm respectively (normal values: 42009–42694 cpm). Venous blood was obtained from these patients for the fluorometric blast-formation test. Venous blood samples from three healthy donors were used as a control. The fluorescence excitation spectrum was measured with an Oyo-Bunko ulog-fluospec 11 A fluorometer. Kits for the fluorometric blast-formation test were purchased from Japan Immunoresearch Laboratories Co., Ltd, Japan.

Boc–Leu–CH_2Cl (I)

Diazomethane [prepared from nitrosomethylurea (6.1 g) in ether (50 mL)] was added to a mixed anhydride [prepared from Boc–Leu-OH (6.9 g), Et_3N (4.2 mL) and ethylchloroformate (2.8 mL) as usual] in tetrahydrofuran (100 mL) at –15 °C and the reaction mixture was stirred at 4 °C for 12 h. Then 6 N HCl/dioxane (9 mL) was added to the reaction mixture at 0 °C and the resultant solution was stirred at 0 °C for 3 h. After neutralization of the solution with Et_3N and removal of the solvent, the residue was extracted with EtOAc. The extract was washed with 5 % citric acid, H_2O, 5 % $NaHCO_3$ and H_2O, dried over $MgSO_4$ and evaporated *in vacuo*. *n*-Hexane was added to the residue to afford the product, which was recrystallized from EtOH. Yield 5,3 g (67 %), mp 58–61 °C, $[\alpha]_D^{21}$ –37.4 ° (c = 1.0, DMF), R_f^1 0.85, single ninhydrin-positive spot. Anal. calcd for $C_{12}H_{22}ClNO_3$: C, 54.46; H, 8.41; N, 5.31. Found: C, 54.58; H, 8.69; N, 5.07.

Boc–Phe–Leu–CH_2Cl (II)

Compound **I** (3.8 g) was treated with TFA–anisole (30 mL–6 mL) in an ice-bath for 40 min, and TFA was then removed by evaporation. The residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 2 h, and then dissolved in DMF (15 mL) containinq NMM (1.8 mL). To this solution, Boc–Phe-OSu (5.2 g) was added, and the mixture was stirred at room temperature for 7 h. The product was extracted with EtOAc and the extract was washed successively with 5 % citric acid, H_2O, 5 % $NaHCO_3$ and H_2O, dried over $MgSO_4$ and then concentrated *in vacuo*. The residue was precipitated from EtOAc with petroleum ether and then recrystallized from EtOH. Yield 4.0 g (65 %), mp 141–151 °C, $[\alpha]_D^{21}$ –54.3 ° (c = 1.0, DMF), R_f^1 0.82, single ninhydrin-positive spot. Anal. calcd for $C_{21}H_{31}ClN_2O_4\ H_2O$: C, 58.80; H, 7.76; N, 6.53. Found: C, 59.16; H, 7.84; N, 6.33.

Boc–Lys(Z)–Phe–Leu–CH_2Cl (III)

This compound was prepared essentially in the same manner as described for the preparation of **II** using **II** (1.9 g) and Boc–Lys(Z)-OSu (2.6 g). The product was reprecipitated from EtOAc with *n*-hexane. Yield 2.6 g (77 %), mp 119–125 °C, $[\alpha]_D^{21}$ –28.9 ° (c = 1.0, DMF), R_f^1 0.77, single ninhydrin-positive spot. Anal. calcd for $C_{35}H_{49}ClN_4O_5\ 2H_2O$: C, 62.07; H, 7.89; N, 8.27. Found: C, 61.83; H, 8.09; N, 8.43.

Boc–Pro–Lys(Z)–Phe–Leu–CH_2Cl (IV)

This compound was prepared essentially in the same manner as described for the preparation of **II** using **III** (1.4 g) and Boc–Pro-OSu (687 mg). The product was reprecipitated from EtOAc with ether. Yield 1.1 g (73 %), mp 127–134 °C, $[\alpha]_D^{21}$ –18.3 ° (c = 1.0, DMF), R_f^1 0.80, single ninhydrin-positive spot. Anal. calcd for $C_{40}H_{56}ClN_5O_6$: C, 65.07; H, 7.65; N, 9.49. Found: C, 65.39; H, 7.92; N, 9.50.

Boc–Asp(OBzl)–Gly–NHNH-Troc (V)

This compound was prepared essentially in the same manner as described for the preparation of **II** using Boc–Gly–NHNH-Troc[17] (2.4 g) and Boc–Asp(OBzl)-OSu (3.1 g). The product was reprecipitated from EtOAc with *n*-hexane. Yield 2.8 g (72 %), mp 90–96 °C, $[\alpha]_D^{21}$ –9.2 ° (c = 1.0, DMF), R_f^1 0.64, single ninhydrin-positive spot. Anal. calcd for $C_{21}H_{27}Cl_3N_3O_8\ 2H_2O$: C, 42.62; H, 5.28; N, 7.10. Found: C, 42.86; H, 5.51; N, 7.39.

Boc–Glu(OBzl)–Asp(OBzl)–Gly–NHNH-Troc (VI)

This compound was prepared from **V** (2 g) and Boc–Glu(OBzl)-OSu (1.6 g) essentially as described for the

preparation of **II**. The product was reprecipitated from EtOAc with ether. Yield 1.9 g (68 %), mp 114–121 °C, $[\alpha]_D^{21}$ −13.6 ° (c = 1.0, DMF), R_f^1 0.75, single ninhydrin-positive spot. Anal. calcd for $C_{33}H_{40}Cl_3N_4O_{11}$ $3H_2O$: C, 47.81; H, 5.59; N, 6.76. Found: C, 47.54; H, 5.92; N, 6.39.

Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly–NHNH-Troc (VII)

Compound **VI** (1.8 g) was treated with TFA–anisole (18 mL–3.2 mL) in an ice-bath for 40 min, and TFA was removed by evaporation. The residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 2 h, and then dissolved in DMF (15 mL) containing NMM (0.25 mL). To this ice-chilled solution, Ac–Leu-OH (381 mg), HOBT (359 mg) and WSCI (422 mg) were successively added. After having been stirred at 0 °C for 8 h, the mixture was extracted with EtOAc, and the extract was washed successively with 1 N HCl, H_2O, 5 % $NaHCO_3$ and H_2O, dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was reprecipitated from MeOH with ether. Yield 1.4 g (78 %), mp 130–137 °C, $[\alpha]_D^{21}$ −14.2° (c = 1.0, DMF), R_f^1 0.77, chlorine-tolidine-positive spot. Anal. calcd for $C_{36}H_{45}Cl_3N_5O_{11}$ H_2O: C, 50.98; H, 5.59; N 8.26. Found: C, 50.69; H, 5.76; N, 8.07.

Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly–$NHNH_2$ (VIII)

Compound **VII** (1.1 g) in a mixture of AcOH (6 mL) and DMF (3 mL) was treated with Zn dust (408 mg) at 4 °C for 12 h. The solution was filtered, and the residue was treated with 3 % EDTA and then with $NaHCO_3$ to adjust the pH to neutral. The resulting gelatinous mass was washed with H_2O and reprecipitated from DMF with H_2O. The dried product was reprecipitated from MeOH with ether. Yield 812 mg (90 %), mp 163–173 °C, $[\alpha]_D^{21}$ −29.4 ° (c = 1.0, DMF), R_f^1 0.59, single hydrazine-test-positive spot. Anal. calcd for $C_{33}H_{44}N_5O_9$ $3H_2O$: C, 56.33; H, 5.77; N, 10.02. Found: C, 56.45; H, 6.10; N, 9.79.

Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly–Pro–Lys(Z)–Phe–Leu–CH_2Cl (IX)

Compound **IV** (492 mg) was treated with TFA–anisole (5 mL–1 mL) as usual. The resulting powder was dissolved in DMF (4 mL) containing NMM (0.08 mL). The azide [prepared from 699 mg of Ac–Leu–Glu(OBzl)–Asp(OBzl)–Gly-$NHNH_2$ **VIII** (1.5 eq)] in DMF (4 mL) and NMM (0.34 mL) were added to the above ice-chilled solution. The mixture, after being stirred at −10 °C for 32 h, was neutralized with a few drops of AcOH and concentrated. The residue was treated with 5 % citric acid and the precipitate thereby formed was washed with 5 % citric acid and H_2O. The resulting powder was purified by gel-filtration on a Sephadex LH-20 column (2.8 × 102 cm) with DMF containing 3 % H_2O. The UV absorption at 260 nm was determined in each fraction (5 mL) and the desired fractions containing a substance of R_f^1 0.76 were combined. The solvent was removed by evaporation and the residue was treated with ether to afford a powder. The product was reprecipitated from hot EtOAc. Yield 536 mg (62 %), mp 146–153 °C, $[\alpha]_D^{21}$ −37.4 ° (c = 1.0, DMF), R_f^1 0.76, single chlorine-tolidine-positive spot. Anal. calcd for $C_{68}H_{88}ClN_9O_{15}$: C, 62.49; H, 6.79; N, 9.65. Found: C, 62.31; H, 6.98; N, 9.49. Amino acid ratios in a 6 N HCl hydrolysate: Leu 2.03, Phe 0.97, Pro 0.91, Gly 1.00, Glu 0.92, Asp 0.95, Lys 1.02 (recovery of Gly 85 %).

Ac–Leu–Glu–Asp–Gly–Pro–Lys–Phe–Leu–CH_2Cl (X)

The fully protected octapeptide **IX** (261 mg) was hydrogenated in a mixture of AcOH–H_2O–MeOH (5:1:4, 15 mL) for 24 h over 10 % Pd-charcoal (100 mg). The catalyst was removed by the aid of cellite. The solvent was evaporated to dryness *in vacuo* and the residue was dried over KOH pellets *in vacuo*. The powder was dissolved in 2 % AcOH (2 mL), applied to a column of Sephadex G-15 (3.0 × 97 cm) and eluted with 2 % AcOH. Individual fractions (5 mL each) were collected and absorbancy at 260 nm was determined. The first peak (tube Nos 51–60) was collected and the solvent was removed by lyophilization. Analysis by TLC revealed the presence of two chlorine-tolidine-positive spots with R_f^2 0.12 (main) and 0.46 (minor). The crude product was dissolved in a small amount of water and subjected to preparative TLC (cellulose plate, 20 × 40 cm) using BuOH–AcOH–H_2O (4:1:5, upper layer) as a developing solvent. The zone corresponding to R_f^2 0.12 was separated and extracted with 2 % AcOH. The extracts were concentrated to a small volume, applied to a Sephadex G-15 column (3.0 × 97 cm) and eluted with 2 % AcOH as described above and the solvent was removed by lyophilization. Yield 64 mg (32 %), $[\alpha]_D^{21}$ −72.9 ° (c = 1.0, 2 % AcOH), R_f^2 0.12, single chlorine-tolidine-positive spot. Amino acid ratios in a 6 N HCl hydrolysate: Leu 2.02, Phe 0.93, Pro 0.90, Gly 1.00, Glu 0.95, Asp 0.97, Lys 1.01 (recovery of Gly 86 %). FAB-MS m/z: 993 $(M + H)^+$. The synthetic peptide exhibited a single peak on HPLC using a Cosmosil 5C18 column (4.6 × 100 mm) at a retention time of 10.53 min when eluted with a gradient of acetonitrile (32 to 45 % in 15 min) in 0.1 % TFA at a flow rate of 1.0 mL per min.

Stability studies of the two synthetic peptides

Treatment with human serum. The synthetic peptides were treated with human serum to evaluate the preservation of their immunological activity. Blood was collected from normal healthy volunteers in serum separation tubes (Beckton–Dickson SST vacutainer brand tubes No. 6572) and allowed to clot at room temperature. The tubes were then centrifuged at 2000 *g* for 15 min, and the serum was immediately transferred to sterile conical centrifuge tubes (Corning No. 25310) and placed on ice. Peptide solutions (1.0 mg/mL) in 0.01 M sodium phosphate buffer (pH 7.4) were prepared. To 900 μL of serum in 1.5 mL polypropyrene centrifuge tubes equilibrated at 37 °C was added 50 μg of peptide solution. The mixture was incubated at 37 °C for 30 min and then the pH of the solution was adjusted to acidic area with dil. AcOH and lyophilized.

Fluorometric blast-formation test. A 3-mL aliquot of venous blood from uremic patients was drawn into a syringe containing 25 U/mL of heparin and then mixed

with 3 mL of PBS. Lymphocytes were isolated in Hypaque–Ficoll gradient.[18] Isolated lymphocytes were adjusted to 1.0×10^6/mL with PBS. The lymphocytes were cultured in 0.5 mL of RPMI 1640 (Gibco) with FCS (Dainippon Pharmaceutical Co.) in microplates. Cultures of each combination were incubated at 37 °C in the presence of the peptide in a humidified atmosphere of 5 % CO_2 in air for 12 h and PHA (0.125 %, 0.5 mL) was added to each well. Incubation was continued under the same conditions for 60 h. T-lymphocytes in each well were transferred to a test tube and centrifuged for 10 min at 240 *g*, then the supernatant was removed. A 2-mL aliquot of 0.125 % SDS was added to the residue and stirred for 20 min at room temperature; lymphocytes were completely destroyed by this procedure. Ethidium bromide solution was added to the above solution and the mixture was stirred for 15 min at room temperature. The fluorescence excitation spectrum was measured accordinq to Itoh and Kawai.[12]

References and Notes

1. Abbreviations used: TFA, trifluoroacetic acid; Z, benzyloxycarbonyl; OBzl, benzyl ester; Ac, acetyl; CH_2Cl, chloromethyl; Troc, β,β,β-trichloroethyloxycarbonyl; NMM, *N*-methylmorpholine; Et_3N, triethylamine; DMF, dimethylformamide; OSu, *N*-hydroxysuccinimide ester; HOBT, 1-hydroxybenzotriazole; Boc, *tert*-butoxycarbonyl; WSCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EDTA, ethylenediaminetetraacetic acid; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; PHA, phytohemagglutinin; AcOH, acetic acid; EtOAc, ethyl acetate; MeOH, methanol; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline; RPMI, Rosewell Park Memorial Institute; FCS, fetal calf serum; FAB-MS, fast atom bombardment mass spectrometry.

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