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Effects of Two Synthetic Serum Thymic Factor Analogues and Four Thymic Factor Fragments on the Low E-Rosette Forming Cells in Patients with Chronic Renal Failure[1)]

Takashi Abiko* and Hiroshi Sekino

Kidney Center, Sendai Insurance Hospital, Tsutsumimachi 3-16-1, Sendai, 980, Japan

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Two new analogues of serum thymic factor (STF), [Dab^3]- and [Asn^5, Gln^9]-STF and four thymic factor fragments, H-Lys-Ser-Gln-OH, H-Lys-Ser-Gln-Gly-OH, H-Lys-Ser-Gln-Gly-Gly-OH and H-Lys-Ser-Gln-Gly-Gly-Ser-OH, were synthesized by the solution method, and were tested to determine their effects on the low E-rosette forming cells in patients with chronic renal failure. The analogue [Dab^3]-STF and three fragments, H-Lys-Ser-Gln-Gly-OH, H-Lys-Ser-Gln-Gly-Gly-OH and H-Lys-Ser-Gln-Gly-Gly-Ser-OH, increased E-rosette forming capacity when incubated *in vitro* with patient's blood, but [Asn^5, Gln^9]-STF and the tripeptide, H-Lys-Ser-Gln-OH, had no effect.

Keywords——serum thymic factor; E-rosette forming cells; chronic renal failure; HOBT-DCC procedure; Lys-Ser-Gln-Gly

One of the active thymus hormones, termed serum thymic factor (STF), is a nonapeptide (H-Pyr-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH) with a molecular weight of 847.[2)] This factor has been reported to exhibit various immunological activities, including induction of T-cell markers on T-cell precursors.[3)]

In order to evaluate the structure-activity relationship in the STF molecule, many analogues and shorter-chain fragments have been synthesized.[4-7)] These results indicate that the pentapeptide moiety (Lys-Ser-Gln-Gly-Gly) is the minimum essential structure for the expression of immunological activity.[7)]

On the other hand, it is well known that cell-mediated immunity is impaired in chronic renal failure.[8-10)] Peripheral blood lymphopenia is well recognized in uremic subjects,[11)] with a reduction in the total numbers of both T- and B-cells.

Studies gave also shown that serum from patients with renal failure depresses the number of E-rosette forming cells.[12)] In previous reports[13,14)] from this laboratory, we described the syntheses of a series of STF analogues and fragments and reported some of their immunological properties in the uremic state. In these reports,[13,14)] we concluded that the lysine residue and the Lys-Ser-Gln-Gly-Gly-Ser-Asn moiety of STF probably play an important role in the activity on E-rosette forming cells in the uremic state.

We describe here the syntheses of two further STF analogues, [Dab^3]- and [Asn^5, Gln^9]-STF and four fragments, H-Lys-Ser-Gln-OH, H-Lys-Ser-Gln-Gly-OH, H-Lys-Ser-Gln-Gly-Gly-OH and H-Lys-Ser-Gln-Gly-Gly-Ser-OH. Further, we have compared the *in vitro* effects of these peptides on low E-rosette forming cells from patients with chronic renal failure.

In the present synthesis, as illustrated in Figs. 1, 2 and 3, amino acid derivatives bearing protecting groups, *i.e.*, Asn-ONb, Lys(Z), Dab(Z), Z-Pyr, Gln-ONb, Gly-ONb, Ser-ONb and Gly-OBzl, which could be removed by catalytic hydrogenation were used. Hydroxy groups of serine residues were not protected. These protecting groups survive mostly intact during careful TFA treatment for the removal of the Boc group, employed as a temporary α-amino protecting group.

As shown in Fig. 1, two peptide subunits, Boc-Gln-Gly-Gly-Ser-Asn-ONb (V) and Z-Pyr-Ala-Dab(Z)-Ser-$NHNH_2$ (IX) served as building blocks for the construction of [Dab^3]-STF. First, the C-terminal pentapeptide, Boc-Gln-Gly-Gly-Ser-Asn-ONb (V), was synthe-

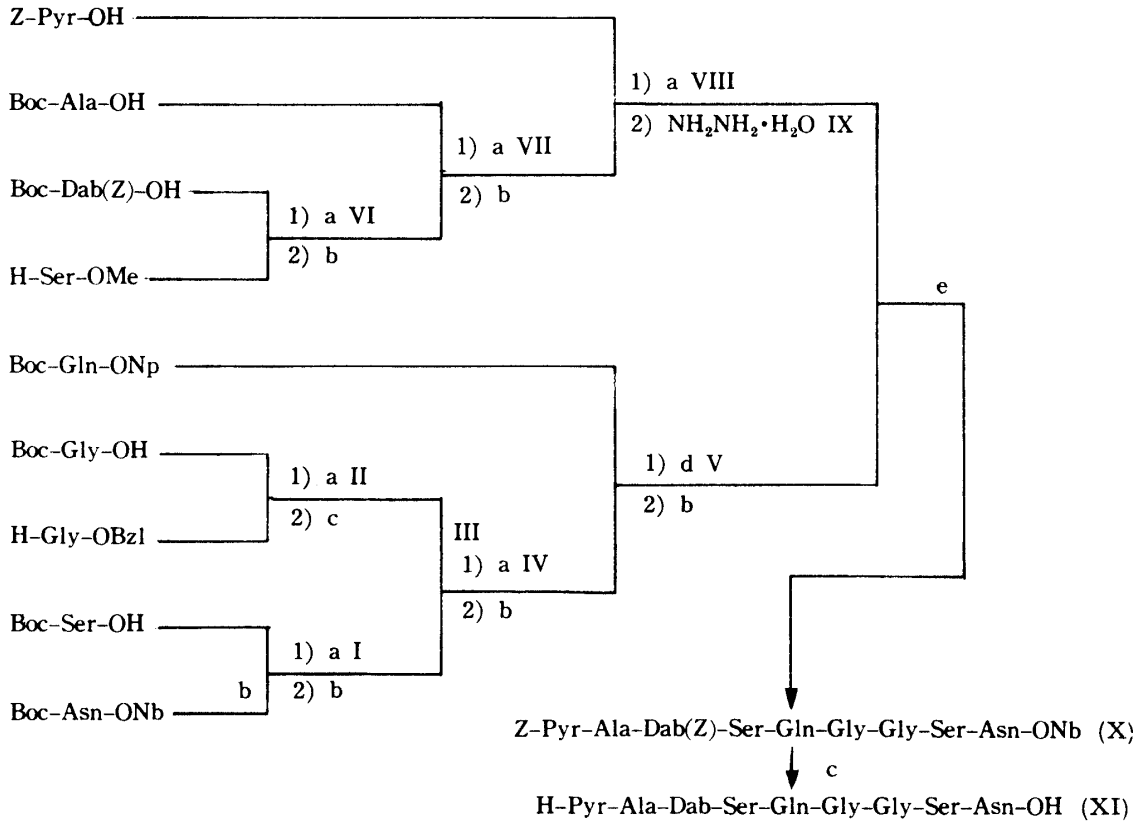

Fig. 1. Synthetic Scheme for [Dab^3]-STF

a, HOBT-WSCI; b, TFA-anisole; c, H_2/Pd-charcoal; d, active ester; e, azide.

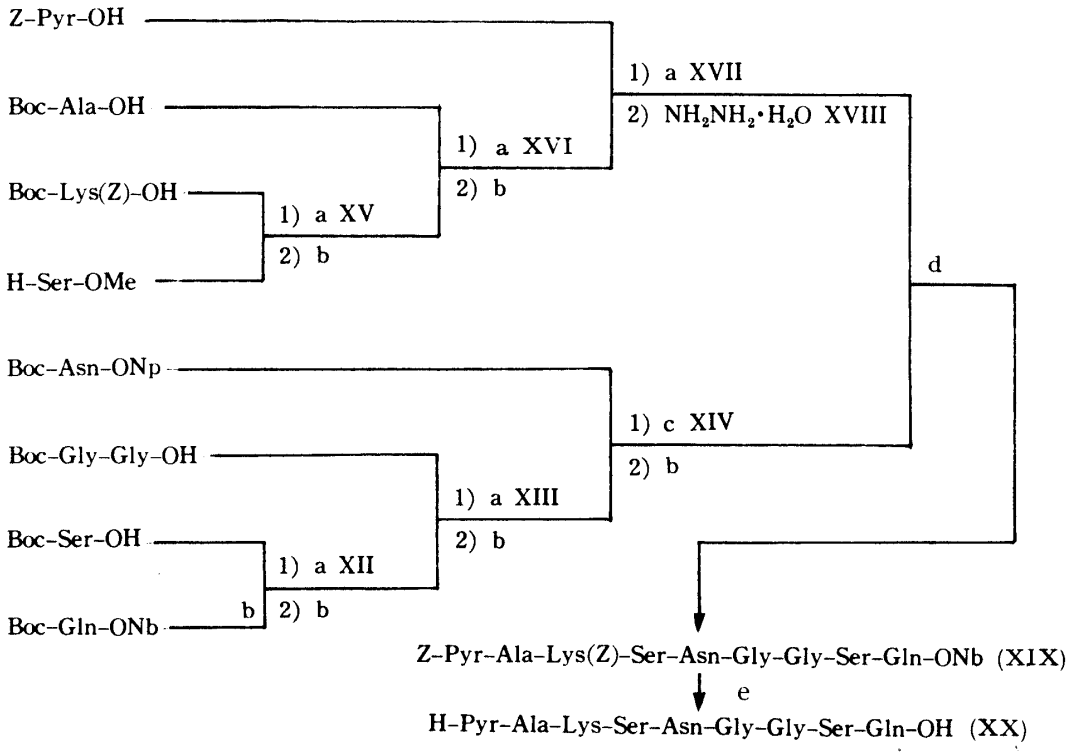

Fig. 2. Synthetic Scheme for [Asn^5, Gln^9]-STF

a, HOBT-WSCI; b, TFA-anisole; c, active ester; d, azide; e, H_2/Pd-charcoal.

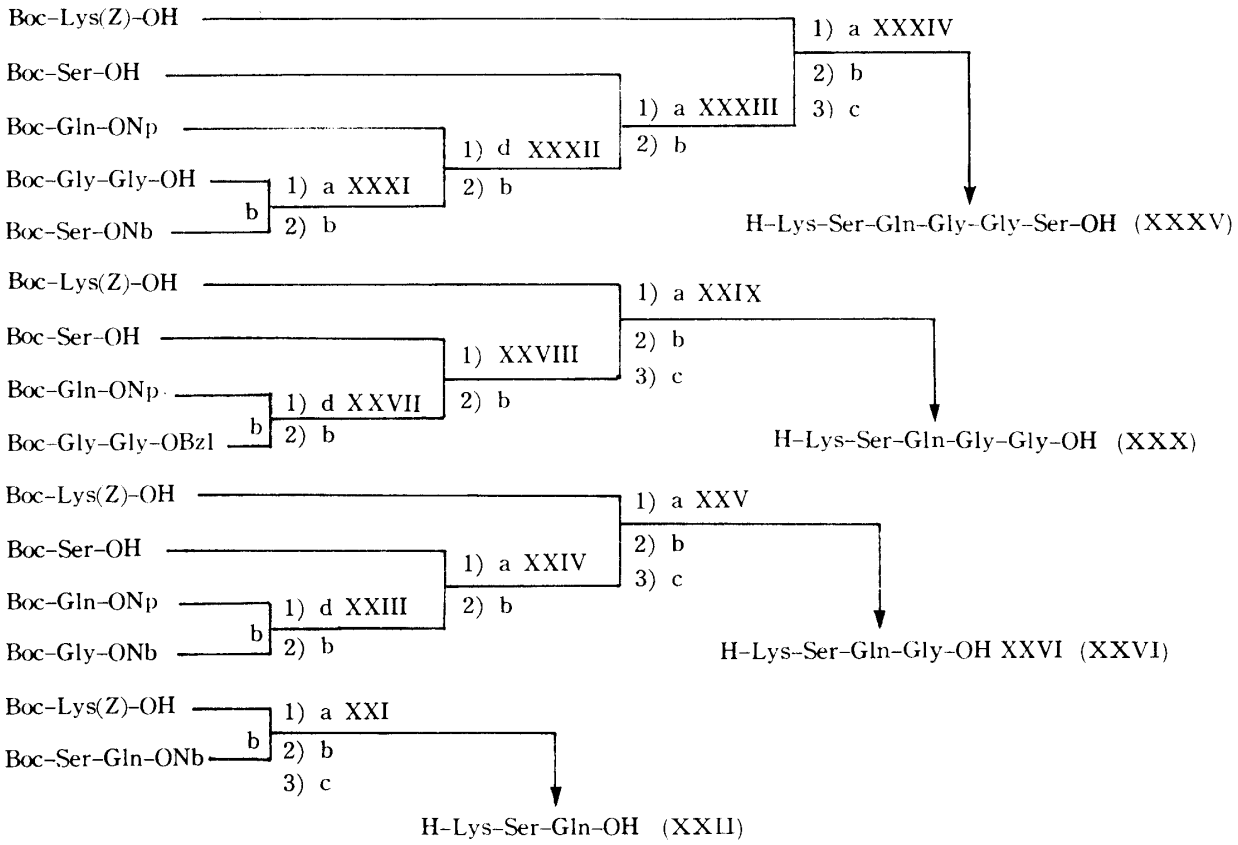

Fig. 3. Synthetic Scheme for the STF Fragments

a, HOBT-WSCI; b, TFA-anisole; c. H_2/Pd-charcoal; d. active ester.

sized by stepwise and fragment condensation methods. The protected tetrapeptide ester, Boc–Gly–Gly–Ser–Asn–ONb (IV), was prepared by the HOBT–DCC procedure[15] starting from Boc–Asn–ONb. After TFA–anisole treatment of IV, the resulting tetrapeptide ester was condensed with Boc–Gln–ONp to give the protected pentapeptide, Boc–Gln–Gly–Gly–Ser–Asn–ONb (V). Next, in order to prepare the protected tetrapeptide hydrazide, Z–Pyr–Ala–Dab(Z)–Ser–$NHNH_2$ (IX), the tetrapeptide ester, Z–Pyr–Ala–Dab(Z)–Ser–OMe (VIII), was synthesized stepwise by the HOBT–DCC procedure[15] starting from H–Ser–OMe·HCl. The Boc groups of intermediates were removed by treatment with TFA–anisole prior to the next coupling reaction. This peptide ester was therefore converted to the corresponding hydrazide, Z–Pyr–Ala–Dab(Z)–Ser–$NHNH_2$ (IX), which permitted a check on its homogeneity by elemental analysis. After TFA–anisole treatment of V, the resulting pentapeptide amide was coupled with IX by the azide procedure[16] to give the protected nonapeptide ester, Z–Pyr–Ala–Dab(Z)–Ser–Gln–Gly–Gly–Ser–Asn–ONb (X). X was further purified by Sephadex LH-20 column chromatography using water-saturated BuOH as an eluent. The protected nonapeptide (X) thus obtained was found to be homogeneous by paper chromatography in two different solvent systems. The elemental analysis and the amino acid analysis of X gave values in good agreement with the theoretical values. X was hydrogenated over 10% Pd–charcoal in aqueous AcOH for 20 h. The hydrogenated product was purified by column chromatography on Sephadex G-15. The nonapeptide (XI) thus obtained was found to be homogeneous on paper chromatography using two different solvent systems. The amino acid analysis of XI also gave values in good agreement with the theoretical values.

[Asn^5, Gln^9]-STF (XX) was prepared as described for the preparation of XI (Fig. 2). The nonapeptide (XX) was found to be homogeneous on paper chromatography using two different

solvent systems. Ratios of amino acids in the acid hydrolysate of XX agreed well with the theoretical values.

As shown in Fig. 3, each STF fragment was obtained by condensation from the C-terminus to the N-terminus by the HOBT–DCC procedure[15] and the *p*-nitrophenyl ester procedure, after TFA–anisole treatmnet. Following the final condensation, the Boc group was removed by TFA–anisole treatment and then the other protecting groups were removed by catalytic hydrogenation and the product was purified by column chromatography on Sephadex G-10. The four STF fragments thus obtained were found to be homogeneous by paper chromatography in two different solvent systems. The amino acid analyses of these four STF fragments gave values in good agreement with the theoretical values.

The *in vitro* effects of the two STF analogues and four fragments on low E-rosette forming cells of patients with chronic renal failure are shown in Tables I and II.

Table I. Effect of the Two STF Analogues on Low E-Rosette Forming Cells from Patients with Chronic Renal Failure

Peptide	Dose (molar concentration)	E-Rosette forming cells[d] (%)
——[a]		72.6±7
——[b]		41.2±7
STF[b,c]	1.25×10^{-4}	65.3±6
	2.48×10^{-4}	70.8±7
[Dab^3]-STF[b,c]	1.20×10^{-4}	51.2±6
	2.41×10^{-4}	56.8±7
[Asn^5, Gln^9]-STF[b,c]	1.25×10^{-4}	39.4±6
	1.48×10^{-4}	38.2±7

a) Normal venous blood.
b) Patient's venous blood.
c) Incubation was carried out for 30 min at 37°C.
d) Each value represents the mean ± S.D. of triplicate measurements.

Table II. Effect of the Four Synthetic STF Fragments on Low E-Rosette Forming Cells from Patients with Chronic Renal Failure

Peptide	Dose (molar concentration)	E-Rosette forming cells[d] (%)
——[a]		74.1±7
——[b]		42.6±6
STF[b,c]	1.25×10^{-4}	63.8±7
	2.48×10^{-4}	68.3±6
H–Lys–Ser–Gln–Gly–Gly–Ser–OH[b,c]	1.77×10^{-4}	58.2±7
H–Lys–Ser–Gln–Gly–Gly–OH[b,c]	4.12×10^{-4}	57.4±6
H–Lys–Ser–Gln–Gly–OH[b,c]	6.14×10^{-4}	56.8±5
H–Lys–Ser–Gln–OH[b,c]	9.49×10^{-4}	43.1±7
	1.19×10^{-3}	40.5±7[e]

a) Normal venous blood.
b) Patient's venous blood.
c) Incubation was carried out for 30 min at 37°C.
d) Each value represents the mean ± S.D. of triplicate measurements.
e) Inactive by 1.19×10^{-3} M.

Incubation of blood from patients in the presence of various amounts of synthetic peptides from 1.25×10^{-4} to 1.19×10^{-3} M mostly resulted in recovery of E-rosette formation (Tables I and II). The activities of [Dab^3]-STF, H–Lys–Ser–Gln–Gly–Gly–Ser–OH, H–Lys–Ser–Gln–Gly–Gly–OH and H–Lys–Ser–Gln–Gly–OH were lower than that of STF. However, [Asn^5,

Gln[9]]-STF (1.48×10^{-4} M) and H–Lys–Ser–Gln–OH (1.19×10^{-3} M) had no effect on the low E-rosette forming cells in patients. These results strongly indicate that the tetrapeptide moiety, Lys–Ser–Gln–Gly, is the minimum essential structure for the expression of restoration activity on low E-rosette forming cells in cases of chronic renal failure.

Experimental

Melting points are uncorrected. Optical rotations were measured with an Atago Polax machine (cell length: 10 cm). Amino acid compositions of acid hydrolysates were determined with a JEOL JLC-8AH amino acid analyzer (one-column system). Evaporation of solvents was carried out with a rotary evaporator under reduced pressure at 35 to 45°C. Boc groups of the protected peptides were removed by TFA–anisole treatment. The resulting amino components were chromatographed on filter paper, Toyo Roshi No. 51, at room temperature. Rf^a values refer to the Partridge system[17] and Rf^b values refer to BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24).[18] For paper chromatography, α-Z groups of protected peptides were not deblocked.

Venous blood was obtained from five patients suffering from chronic renal failure. Venous blood samples from healthy donors (21—31 years old) were used as a control. Standard STF was purchased from the Protein Research Foundation, Osaka, Japan.

Boc-Ser-Asn-ONb (I)——Boc-Asn-ONb (1.8 g) was dissolved in TFA (6 ml)–anisole (1.2 ml) and the solution was allowed to stand at room temperature for 20 min. The TFA was evaporated off and the residue was treated with dry ether, and collected by filtration. The powder obtained was dried over KOH pellets *in vacuo* and then dissolved in DMF (10 ml) and the solution was neutralized with NMM (0.6 ml). To this ice-chilled solution, Boc-Ser-OH (1.1 g), HOBT (744 mg) and WSCI (1.1 g) were successively added. After being stirred overnight at 4°C, the mixture was extracted with EtOAc, and the extract was washed successively with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O, dried over $MgSO_4$ and then concentrated *in vacuo*. The residue was reprecipitated from EtOAc and *n*-hexane; yield 1.9 g (86%), mp 73—75°C, $[\alpha]_D^{26}$ −36.4° (c=1.0, DMF), Rf^a 0.65, Rf^b 0.76, single ninhydrin-positive spot. *Anal.* Calcd for $C_{19}H_{26}N_4O_9$: C, 50.22; H, 5.77; N, 12.33. Found: C, 50.38; H, 6.05; N, 12.15.

Boc-Gly-Gly-OBzl (II)——HOBT (495 mg) and WSCI (725 mg) were added to a solution of Boc–Gly–OH (642 mg) and H–Gly–OBzl Tos (1.1 g) in tetrahydrofuran (10 ml) containing NMM (to keep the solution slightly alkaline) with stirring at 0°C. The solution was stirred for 16 h at 4°C. Then, the mixture was extracted with EtOAc and the extract was washed successively with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was reprecipitated from EtOAc and petroleum ether; yield 1 g (oily material) (91%), Rf^a 0.64, Rf^b 0.68, single ninhydrin-positive spot. *Anal.* Calcd for $C_{16}H_{22}N_2O_5$: C, 59.62; H, 6.88; N, 8.69. Found: C, 59.91; H, 7.20; N, 8.46.

Boc-Gly-Gly-OH (III)——A solution of II (900 mg) in MeOH (15 ml) and 1 N AcOH (5 ml) was hydrogenated in the presence of 10% Pd–charcoal in the usual manner for 6 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* and then dried over KOH pellets *in vacuo*. The dried product was recrystallized from EtOAc and *n*-hexane; yield 600 mg (93%), mp 61—63°C, Rf^a 0.18, Rf^b 0.27, single ninhydrin-positive spot. *Anal.* Calcd for $C_9H_{16}N_2O_5$: C, 46.55; H, 6.95; N, 12.02. Found: C, 46.21; H, 7.04; N, 11.75.

Boc-Gly-Gly-Ser-Asn-ONb (IV)——This compound was prepared from I (901 mg), III (511 mg), HOBT (297 mg) and WSCI (436 mg) essentially as described for the preparation of I; yield 879 mg (73%), mp 104—107°C, $[\alpha]_D^{26}$ −5.1° (c=1.0, DMF), Rf^a 0.56, Rf^b 0.75, single ninhydrin-positive spot. *Anal.* Calcd for $C_{23}H_{32}N_6O_{11}\cdot 2H_2O$: C, 45.69; H, 6.00; N, 13.90. Found: C, 45.78; H, 6.29; N, 13.69.

Boc-Gln-Gly-Gly-Ser-Asn-ONb (V)——IV (569 mg) was treated with TFA (3 ml)–anisole (0.6 ml) as described above and the resulting powder was dissolved in DMF (5 ml) together with NMM (0.11 ml). Boc–Gln–ONp (470 mg) was added, and the whole was stirred at room temperature for 18 h. The reaction mixture was then diluted with 1 N NH_4OH (1 ml) with stirring to saponify the unchanged *p*-nitrophenyl ester. After 1 h, the mixture was extracted with EtOAc and the extract was washed successively with 1 N NH_4OH, H_2O, 1 N citric acid and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from EtOAc and *n*-hexane; yield 454 mg (65%), mp 135—139°C, $[\alpha]_D^{26}$ −11.3° (c=1.0, DMF), Rf^a 0.64, Rf^b 0.81, single ninhydrin-positive spot. *Anal.* Calcd for $C_{28}H_{40}N_8O_{13}\cdot H_2O$: C, 47.06; H, 5.92; N, 15.68. Found: C, 47.21; H, 6.34; N, 15.37.

Boc-Dab(Z)-Ser-OMe (VI)——This compound was prepared from H–Ser–OMe·HCl (778 mg), Boc–Dab–(Z)–OH (2 g), HOBT (744 mg) and WSCI (978 mg) essentially as described for the preparation of II; yield 1.7 g (74%), mp 89—93°C, $[\alpha]_D^{26}$ −30.4° (c=1.0, DMF), Rf^a 0.68, Rf^b 0.79, single ninhydrin-positive spot. *Anal.* Calcd for $C_{21}H_{31}N_3O_8\cdot H_2O$: C, 53.50; H, 7.06; N, 8.91. Found: C, 53.29; H, 7.34; N, 8.56.

Boc-Ala-Dab(Z)-Ser-OMe (VII)——This compound was prepared essentially in the same manner as described for the preparation of I by using VI (468 mg), Boc–Ala–OH (208 mg), HOBT (149 mg) and WSCI (217 mg). The product was reprecipitated from EtOAc and petroleum ether; yield 439 mg (84%), mp

114—119°C, $[\alpha]_D^{25}$ −26.7° (c=1.0, DMF), Rf^a 0.74, Rf^b 0.84, single ninhydrin-positive spot. *Anal.* Calcd for $C_{24}H_{36}N_4O_9$: C, 54.95; H, 6.92; N, 10.68. Found: C, 55.21; H, 7.10; N, 10.60.

Z-Pyr-Ala-Dab(Z)-Ser-OMe (VIII)——VII (269 mg) was treated with TFA (3 ml)–anisole (0.6 ml) as described above and the resulting powder was dissolved in DMF (3 ml) together with NMM (0.06 ml) at 0°C. Z-Pyr-OH (145 mg), HOBT (75 mg) and WSCI (109 mg) were added to the solution. After being stirred overnight at 4°C, the mixture was extracted with EtOAc and the extract was washed successively with 1 N $NaHCO_3$, H_2O, 1 N HCl and H_2O, dried over $MgSO_4$ and then concentrated *in vacuo*. The residue was reprecipitated from EtOAc and ether; yield 228 mg (68%), mp 121—125°C, $[\alpha]_D^{26}$ −18.3° (c=1.0, DMF). *Anal.* Calcd for $C_{32}H_{39}N_5O_{11}$: C, 57.39; H, 5.87; N, 10.46. Found: C, 57.26; H, 6.18; N, 10.25.

Z-Pyr-Ala-Dab(Z)-Ser-NHNH$_2$ (IX)——VIII (167 mg) was dissolved in DMF (1 ml)–MeOH (1 ml), then hydrazine hydrate (0.15 ml) was added and the solution was kept standing at room temperature for 48 h. The precipitate thereby formed was collected on filter paper and washed with MeOH. This product was recrystallized from MeOH; yield 167 mg (94%), mp 189—194°C, $[\alpha]_D^{26}$ −10.9° (c=1.0, DMF). *Anal.* Calcd for $C_{31}H_{36}N_7O_{13}$: C, 52.10; H, 5.08; N, 13.72. Found: C, 52.33; H, 5.40; N, 13.84.

Z-Pyr-Ala-Dab(Z)-Ser-Gln-Gly-Gly-Ser-Asn-ONb (X)——V (116 mg) was treated with TFA (2 ml)–anisole (0.4 ml) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* and dissolved in DMF (2 ml) containing NMM (0.02 ml). The azide[16] (prepared from 143 mg of IX with 0.14 ml of 6 N HCl in dioxane and 0.1 ml of isoamylnitrite at −60°C) in DMF (1 ml)–DMSO (1 ml) and NMM (0.1 ml) were added to the above ice-chilled solution and the mixture was stirred for 48 h at 4°C then poured into ice-chilled 1 N $NaHCO_3$ with stirring. Next, 50% NH_4OAc was added dropwise with stirring to form a precipitate. The precipitate was collected and washed successively with 1 N $NaHCO_3$, H_2O, 1 N HCl and H_2O. The product was precipitated from MeOH and ether. The dried powder was next purified by gel-filtration on Sephadex LH-20 (2.8×98 cm), eluted with water-saturated BuOH. Individual fractions (5 ml each) were collected and the absorption at 230 nm was determined. The fractions corresponding to the main peak (tube Nos. 37—44) were combined and the solvent was evaporated off. The residue was treated with ether to give a precipitate; yield 119 mg (58%), mp 193—198°C, $[\alpha]_D^{26}$ −36.8° (c=1.0, DMF), Rf^a 0.71, Rf^b 0.84, single chlorine–tolidine-positive spot. *Anal.* Calcd for $C_{54}H_{67}N_{13}O_{21}$: C, 52.55; H, 5.47; N, 14.75. Found: C, 52.45; H, 5.26; N, 14.89. Amino acid ratios in the acid hydrolysate: Gly 2.18, Ala 1.00, Ser 1.72, Dab 0.91, Glu 1.70, Asp 1.03 (recovery of Ala 82%).

H-Pyr-Ala-Dab-Ser-Gln-Gly-Gly-Ser-Asn-OH (XI)——X (80 mg) was hydrogenated in 50% AcOH (12 ml) over 10% Pd–charcoal for 20 h. The catalyst was removed with the aid of celite. The solution was evaporated to dryness and the residue was dried over KOH pellets *in vacuo*. The crude peptide thus obtained was dissolved in 1% AcOH (2 ml), applied to a column of Sephadex G-15 (2.8×92 cm), and eluted with the same solvent. Fractions corresponding to the front main peak (tube Nos. 55—62) were combined and lyophilized; yield 24 mg (44%), mp 184—190°C, $[\alpha]_D^{26}$ −51.5° (c=1.0, H_2O), Rf^a 0.06, Rf^b 0.12, single chlorine–tolidine-positive spot. Amino acid ratios in the acid hydrolysate: Dab 0.83, Gly 2.09, Ala 1.00, Ser 1.71, Glu 1.85, Asp 0.80 (recovery of Ala 84%).

Boc-Ser-Gln-ONb (XII)——This compound was prepared from Boc–Gln–ONb (1.1 g), Boc–Ser–OH (645 mg), HOBT (425 mg) and WSCI (603 mg) essentially as described for the preparation of I. The product was recrystallized from EtOAc and ether; yield 1.3 g (93%), mp 82—84°C, $[\alpha]_D^{26}$ −35.4° (c=1.0, DMF), Rf^a 0.69, Rf^b 0.83, single ninhydrin-positive spot. *Anal.* Calcd for $C_{20}H_{28}N_4O_9$: C, 51.28; H, 6.03; N, 11.96. Found: C, 51.29; H, 5.82; N, 11.67.

Boc-Gly-Gly-Ser-Gln-ONb (XIII)——This compound was prepared from XII (1.2 g), III (639 mg), HOBT (372 mg) and WSCI (543 mg) essentially as described for the preparation of I; yield 1.3 g (87%), mp 91—94°C, $[\alpha]_D^{26}$ −5.2° (c=1.0, DMF), Rf^a 0.34, Rf^b 0.59, single ninhydrin-positive spot. *Anal.* Calcd for $C_{24}H_{34}N_6O_{11}$: C, 49.48; H, 5.88; N, 14.43. Found: C, 49.25; H, 5.18; N, 14.16.

Boc-Asn-Gly-Gly-Ser-Gln-ONb (XIV)——This compound was prepared from XIII (596 mg) and Boc–Asn–ONp (400 mg) essentially as described for the preparation of V; yield 454 mg (65%), mp 135—139°C, $[\alpha]_D^{26}$ −11.3° (c=1.0, DMF), Rf^a 0.64, Rf^b 0.81, single ninhydrin-positive spot. *Anal.* Calcd for $C_{28}H_{40}N_8O_{13}\cdot H_2O$: C, 47.06; H, 5.92; N, 15.68. Found: C, 47.21; H, 6.34; N, 15.37.

Boc-Lys(Z)-Ser-OMe (XV)——This compound was prepared from H–Ser–OMe·HCl (1.6 g), Boc–Lys(Z)–OH DCHA (5.6 g), HOBT (1.6 g) and WSCI (2.1 g) essentially as described for the preparation of II; yield 4.7 g (94%), mp 72—76°C, $[\alpha]_D^{26}$ −40.9° (c=1.0, DMF), Rf^a 0.63, Rf^b 0.79, single ninhydrin-positive spot. *Anal.* Calcd for $C_{23}H_{35}N_3O_8\cdot 2H_2O$: C, 53.37; H, 7.60; N, 8.12. Found: C, 53.25; H, 7.48; N, 7.86.

Boc-Ala-Lys(Z)-Ser-OMe (XVI)——This compound was prepared from XV (2.4 g), Boc–Ala–OH (1 g), HOBT (744 mg) and WSCI (1.1 g) essentially as described for the preparation of I: yield 2.1 g (75%), mp 103—111°C, $[\alpha]_D^{26}$ −33.8° (c=1.0, DMF), Rf^a 0.80, Rf^b 0.82, single ninhydrin-positive spot. *Anal.* Calcd for $C_{26}H_{40}N_4O_9$: C, 56.51; H, 7.30; N, 10.14. Found: C, 56.61; H, 7.03; N, 9.90.

Z-Pyr-Ala-Lys(Z)-Ser-OMe (XVII)——This compound was prepared from XVII (553 mg), Z–Pyr–OH (289 mg), HOBT (149 mg) and WSCI (218 mg) essentially as described for the preparation of VIII. The product was recrystallized from MeOH and ether, and then recrystallized from EtOAc; yield 516 mg (74%), mp 171—179°C, $[\alpha]_D^{28}$ −10.1° (c=1.0, DMF). *Anal.* Calcd for $C_{34}H_{43}N_5O_{11}$: C, 58.53; H, 6.22; N, 10.04. Found: C, 58.25; H, 5.87; N, 10.07.

Z-Pyr-Ala-Lys(Z)-Ser-NHNH$_2$ (XVIII)——This compound was prepared from XVII (349 mg) and hydrazine hydrate (0.3 ml) essentially as described for the preparation of IX; yield 350 mg (94%), mp 193—197°C, $[\alpha]_D^{26}$ −21.6° (c=1.0, DMF). *Anal.* Calcd for $C_{33}H_{40}N_7O_{13}$: C, 49.73; H, 5.43; N, 13.20. Found: C, 49.72; H, 5.65; N, 12.86.

Z-Pyr-Ala-Lys(Z)-Ser-Asn-Gly-Gly-Ser-Gln-ONb (XIX)——XIV (116 mg) was treated with TFA (2 ml)–anisole (0.4 ml) as described above. The resulting pentapeptide ester trifluoroacetate was dissolved in DMF (2 ml) containing NMM (0.02 ml). The azide (prepared from 186 mg of XVIII with 0.14 ml of 6 N HCl in dioxane and 0.1 ml of isoamylnitrite at −60°C) in DMF (1 ml)–DMSO (1 ml) and NMM (0.09 ml) were added to the above ice-chilled solution and the mixture was stirred for 48 h at 4°C. After that, the mixture was poured into ice-chilled 1 N $NaHCO_3$ with stirring. The precipitate thus formed was washed successively with 1 N $NaHCO_3$, H_2O, 1 N HCl and H_2O. The product was further purified by column chromatography on Sephadex LH-20 (2.8×98 cm), equilibrated and eluted with water-saturated BuOH. The desired fractions (5 ml each, tube Nos. 37—44) were collected and the solvent was removed by evaporation. Ether was added to the residue to give a precipitate; yield 123 mg (59%), mp 184—189°C, $[\alpha]_D^{26}$ −16.9° (c=1.0, DMF), Rf^a 0.76, Rf^b 0.91, single chlorine–tolidine-positive spot. *Anal.* Calcd for $C_{56}H_{71}N_{13}O_{21}$: C, 53.29; H, 5.67; N, 14.43. Found: C, 53.23; H, 5.33; N, 14.08. Amino acid ratios in the acid hydrolysate: Gly 2.20, Ala 1.00, Ser 1.69, Lys 0.92, Glu 1.72, Asp 1.01 (recovery of Ala 80%).

H-Pyr-Ala-Lys-Ser-Asn-Gly-Gly-Ser-Gln-OH (XX)——XIX (80 mg) was hydrogenated in 50% AcOH (12 ml) over 10% Pd–cahrcoal for 24 h. The catalyst was removed with the aid of celite. The solution was evaporated to dryness and the residue was dried over KOH pellets *in vacuo*. The solution of deprotected nonapeptide in 1% AcOH (2 ml) was added to a Sephadex G-15 column (2.8×90 cm) and eluted with 1% AcOH. Fractions of 4 ml each were collected at a flow rate of 4 ml per 18 min with an automatic fraction collector, and the absorbancy of each fraction was determined at 230 nm. The eluate in tube Nos. 52 to 57 containing the nonapeptide was pooled and lyophilized; yield 28 mg (52%), mp 192—199°C, $[\alpha]_D^{26}$ −48.6° (c=1.0, H_2O), Rf^a 0.05, Rf^b 0.11, single chlorine–tolidine-positive spot. Amino acid ratios in the acid hydrolysate: Lys 0.92, Gly 2.16, Ala 1.00, Ser 1.70, Glu 1.72, Asp 0.84 (recovery of Ala 81%).

Boc-Lys(Z)-Ser-Gln-ONb (XXI)——This compound was prepared from XII (234 mg), Boc–Lys(Z)–OH DCHA (309 mg), HOBT (75 mg) and WSCI (109 mg) essentially as described for the preparation of I. The product was recrystallized from MeOH and ether; yield 278 mg (76%), mp 78—83°C, $[\alpha]_D^{26}$ −25.1° (c=1.0, DMF), Rf^a 0.88, Rf^b 0.93, single ninhydrin-positive spot. *Anal.* Calcd for $C_{34}H_{46}N_6O_{12}$: C, 55.88; H, 6.35; N, 11.50. Found: C, 55.87; H, 6.29; N, 11.11.

H-Lys-Ser-Gln-OH (XXII)——XXI (100 mg) was treated with TFA (2 ml)–anisole (0.4 ml) as described above. The de-Boc peptide ester was hydrogenated in 50% AcOH (12 ml) over 10% Pd–charcoal for 20 h. The catalyst was removed with the aid of celite. The filtrate was evaporated to dryness and the residue was dried over KOH pellets *in vacuo*. The hydrogenated product thus obtained was dissolved in 1% AcOH (2 ml) and applied to a column of Sephadex G-10 (2.8×90 cm), eluting with the same solvent. Individual fractions (4 ml each) were collected and the absorbancy at 230 nm was determined. Fractions corresponding to the main peak were combined and the solvent was removed by lyophilization; yield 42 mg (73%), mp 197—208°C, $[\alpha]_D^{26}$ −9.1° (c=1.0, H_2O), Rf^a 0.07, Rf^b 0.16, single ninhydrin-positive spot. Amino acid ratios in the acid hydrolysate: Lys 1.00, Ser 0.87, Glu 1.09 (recovery of Lys 78%).

Boc-Gln-Gly-ONb (XXIII)——This compound was prepared from Boc–Gly–ONb (310 mg) and Boc–Gln–ONp (470 mg) essentially as described for the preparation of V. The product was recrystallized from EtOAc; yield 389 mg (85%), mp 134—135°C, $[\alpha]_D^{26}$ −4.7° (c=1.0, DMF), Rf^a 0.59, Rf^b 0.67, single ninhydrin-positive spot. *Anal.* Calcd for $C_{19}H_{26}N_4O_8 \cdot H_2O$: C, 50.00; H, 6.18; N, 12.27. Found: C, 50.21; H, 6.37; N, 11.90.

Boc-Ser-Gln-Gly-ONb (XXIV)——This compound was prepared from XIII (219 mg), Boc–Ser–OH (113 mg), HOBT (75 mg) and WSCI (109 mg) essentially as described for the preparation of I; yield 218 mg (83%), mp 67—68°C, $[\alpha]_D^{26}$ −50.3° (c=1.0, DMF), Rf^a 0.59, Rf^b 0.74, single ninhydrin-positive spot. *Anal.* Calcd for $C_{22}H_{31}N_5O_{10}$: C, 50.28; H, 5.95; N, 13.33. Found: C, 50.09; H, 6.32; N, 12.85.

Boc-Lys(Z)-Ser-Gln-Gly-ONb (XXV)——This compound was prepared from XXIV (175 mg), Boc–Lys(Z)–OH DCHA (206 mg), HOBT (50 mg) and WSCI (73 mg) essentially as described for the preparation of I. The product was recrystallized from MeOH and ether; yield 187 mg (71%), mp 149—151°C, $[\alpha]_D^{26}$ −18.2° (c=1.0, DMF), Rf^a 0.75, Rf^b 0.82, single ninhydrin-positive spot. *Anal.* Calcd for $C_{36}H_{49}N_7O_{13}$: C, 54.89; H, 6.27; N, 12.45. Found: C, 55.16; H, 6.41; N, 12.68.

H-Lys-Ser-Gln-Gly-OH (XXVI)——This compound was prepared from XXV (100 mg) essentially as described for the preparation of XXII. The fractions corresponding to the main peak (tube Nos. 46—51) were combined and evaporated to dryness *in vacuo*, and then the residue was lyophilized; yield 41 mg (67%), mp 176—183°C, $[\alpha]_D^{26}$ −40.3° (c=1.0, H_2O), Rf^a 0.03, Rf^b 0.10, single ninhydrin-positive spot. Amino acid ratios in the acid hydrolysate: Gly 1.00, Ser 0.74, Glu 0.86, Lys 0.89 (recovery of Gly 84%).

Boc-Gln-Gly-Gly-OBzl (XXVII)——This compound was prepared from II (322 mg) and Boc–Gln–ONp (460 mg) essentially as described for the preparation of V. The product was recrystallized from EtOAc and ether; yield 421 mg (94%), mp 138—139°C, $[\alpha]_D^{26}$ −5.9° (c=1.0, DMF), Rf^a 0.81, Rf^b 0.74, single ninhydrin-positive spot. *Anal.* Calcd for $C_{21}H_{30}N_4O_7$: C, 55.99; H, 6.71; N, 12.44. Found: C, 56.25; H, 6.38; N, 12.87.

Boc-Ser-Gln-Gly-Gly-OBzl (XXVIII)——This compound was prepared from XXVII (375 mg), Boc-Ser-OH (188 mg), HOBT (124 mg) and WSCI (182 mg) essentially as described for the preparation of I; yield 339 mg (76%), mp 76—78°C, $[\alpha]_D^{27}$ −12.4° ($c=1.0$, DMF), Rf^a 0.64, Rf^b 0.74, single ninhydrin-positive spot. *Anal.* Calcd for $C_{24}H_{35}N_5O_9$: C, 53.62; H, 6.56; N, 13.03. Found: C, 53.91; H, 6.20; N, 13.14.

Boc-Lys(Z)-Ser-Gln-Gly-Gly-OBzl (XXIX)——This compound was prepared from XXVIII (269 mg), Boc-Lys(Z)-OH DCHA (309 mg), HOBT (75 mg) and WSCI (109 mg) essentially as described for the preparation of I. The product was recrystallized from MeOH and ether; yield 216 mg (54%), mp 94—97°C, $[\alpha]_D^{26}$ −34.7° ($c=1.0$, DMF), Rf^a 0.79, Rf^b 0.82, single ninhydrin-positive spot. *Anal.* Calcd for $C_{38}H_{53}N_7O_{12}$: C, 57.06; H, 6.68; N, 12.26. Found: C, 56.82; H, 6.83; N, 12.49.

H-Lys-Ser-Gln-Gly-Gly-OH (XXX)——This compound was prepared from XXIX (100 mg) essentially as described for the preparation of XXII. The fractions corresponding to the main peak (tube Nos. 43—49) were collected and evaporated to dryness and the residue was lyophilized; yield 39 mg (57%), mp 201—207°C, $[\alpha]_D^{24}$ −21.5° ($c=1.0$, H_2O), Rf^a 0.03, Rf^b 0.12, single ninhydrin-positive spot. Amino acid ratios in the acid hydrolysate: Lys 1.00, Ser 0.90, Glu 1.06, Gly 2.19 (recovery of Lys 79%).

Boc-Gly-Gly-Ser-ONb (XXXI)——This compound was prepared from Boc-Ser-ONb (681 mg), III (512 mg), HOBT (297 mg) and WSCI (435 mg) essentially as described for the preparation of I; yield 604 mg (65%), mp 74—76°C, $[\alpha]_D^{26}$ −15.4° ($c=1.0$, DMF), Rf^a 0.72, Rf^b 0.81, single ninhydrin-positive spot. *Anal.* Calcd for $C_{20}H_{28}N_4O_9$: C, 51.28; H, 6.03; N, 11.96. Found: C, 51.62; H, 5.92; N, 11.66.

Boc-Gln-Gly-Gly-Ser-ONb (XXXII)——This compound was prepared from XXXI (454 mg) and Boc-Gln-ONp (470 mg) essentially as described for the preparation of V. The product was reprecipitated from MeOH and ether; yield 406 mg (68%), mp 76—80°C, $[\alpha]_D^{26}$ −41.3° ($c=1.0$, DMF), Rf^a 0.69, Rf^b 0.80, single ninhydrin-positive spot. *Anal.* Calcd for $C_{25}H_{36}N_6O_{11}\cdot 3H_2O$: C, 46.15; H, 6.51; N, 12.92. Found: C, 46.10; H, 6.79; N, 12.70.

Boc-Ser-Gln-Gly-Gly-Ser-ONb (XXXIII)——This compound was prepared from XXXII (298 mg), Boc-Ser-OH (123 mg), HOBT (75 mg) and WSCI (109 mg) essentially as described for the preparation of I; yield 251 mg (73%), mp 83—86°C, $[\alpha]_D^{26}$ −10.4° ($c=1.0$, DMF), Rf^a 0.71, Rf^b 0.84, single ninhydrin-positive spot. *Anal.* Calcd for $C_{28}H_{41}N_7O_{13}\cdot 2H_2O$: C, 46.73; H, 6.30; N, 13.62. Found: C, 46.66; H, 6.54; N, 13.47.

Boc-Lys(Z)-Ser-Gln-Gly-Gly-Ser-ONb (XXXIV)——This compound was prepared from XXXIII (214 mg), Boc-Lys(Z)-OH DCHA (194 mg), HOBT (46 mg) and WSCI (68 mg) essentially as described for the preparation of I. The product was reprecipitated from EtOAc and ether; yield 202 mg (68%), mp 101—108 °C, $[\alpha]_D^{26}$ −29.8° ($c=1.0$, DMF), Rf^a 0.74, Rf^b 0.78, single ninhydrin-positive spot. *Anal.* Calcd for $C_{42}H_{59}N_9O_{16}$: C, 53.33; H, 6.29; N, 13.33. Found: C, 53.51; H, 6.46; N, 12.89.

H-Lys-Ser-Gln-Gly-Gly-Ser-OH (XXXV)——This compound was prepared from XXXIV (100 mg) essentially as described for the preparation of XXII. The fractions corresponding to the main peak (tube Nos. 39—44) were combined and evaporated to dryness *in vacuo*, and then the residue was lyophilized; yield 33 mg (50%), mp 216—223°C, $[\alpha]_D^{25}$ −33.9° ($c=1.0$, H_2O), Rf^a 0.05, Rf^b 0.10, single ninhydrin-positive spot. Amino acid ratios in the acid hydrolysate: Lys 1.00, Gly 2.14, Ser 1.70, Glu 1.03 (recovery of Lys 80%).

E-Rosette Formation——A patient's blood was incubated with a synthetic peptide for 30 min at 37°C and then lymphocytes were isolated in a Hypaque-Ficoll gradient[19] for the testing of E-rosette formation. Isolated lymphocytes were adjusted to 5×10^5 cells/ml with PBS. Contamination by monocytes and polymorphonuclear cells amounted to less than 7%.[20] Sheep erythrocytes (Kyokutō Pharmaceutical Co.) were washed with PBS, and a suspension (8×10^6/ml) was prepared. The lymphocytes were washed with GVB^{2+} and centrifuged for 10 min at 1500 rpm, then suspended in FCS (Dainippon Pharmaceutical Co.) (1 ml). The suspension was mixed with the suspension of sheep erythrocytes (0.5 ml) and incubated for 12 h at 4°C. The mixture was then centrifuged for 5 min at 900 rpm. Triplicate wet-cell preparations were checked by phase contrast microscopy. For each preparation, 200 lymphocytes were counted, and the proportion binding more than three erythrocytes was determined.

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References and Notes

1) The amino acid residues except glycine are of the L-configuration. The abbreviations used to denote amino acid derivatives and peptides are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: Z, benzyloxycarbonyl; OBzl, benzyl ester; ONb, *p*-nitrophenyl ester; OMe, methyl ester; Boc, *tert*-butoxycarbonyl; DMF, dimethylformamide; TFA, trifluoroacetic acid; WSCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DCC, dicyclohexylcarbodiimide; HOBT, *N*-hydroxybenzotriazole; Tos, *p*-toluenesulfonic acid; NMM, *N*-methylmorpholine; EtOAc, ethyl acetate; AcOH, acetic acid; FCS, fetal calf serum; E-rosette, a rosette with sheep erythrocytes; GVB^{2+}, gelatin veronal buffer; PBS, phosphate-buffered saline; DMSO, di-

methylsulfoxide; Dab, L-2,4-diamino-*n*-butyric acid; DCHA, dicyclohexylamine.

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