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Synthesis and Immunological Effects of Thymosin α_1 and Its Fragments on Inhibitory Factor in Minimal Change Nephrotic Syndrome[1]

TAKASHI ABIKO, IKUKO ONODERA, and HIROSHI SEKINO

Kidney Center, Sendai Insurance Hospital[2]

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The octacosapeptide corresponding to the entire amino acid sequence of thymosin α_1 was synthesized using the catalytic hydrogenation procedure at the final deprotection step. The depressed E-rosette forming capacity of lymphocytes caused by a serum factor from a patient in the active stage of MCNS was significantly restored when the lymphocytes were incubated with thymosin α_1. The relative potency of the decapeptide fragment (positions 19—28) was 1.04 and that of the pentadecapeptide fragment (positions 14—28) was 9.83 based on synthetic thymosin α_1 (100.00) as a standard.

Keywords——thymosin α_1; minimal change nephrotic syndrome; E-rosette forming cells; HONB–DCC method; HOBT–DCC method

The complete amino acid sequence of thymosin α_1 from calf thymus was determined by Goldstein *et al.*[3,4] This peptide is a heat-stable, highly acidic molecule composed of 28 amino acid residues.[5] On the other hand, an extract of thymus, thymosin, induces an increase in the percentage of T-cell rosettes when incubated *in vitro* with sheep erythrocytes and lymphocytes from patients with primary immunodeficiency disease or viral illness.[6]

Recently, Tomizawa *et al.*[7] reported that MCNS may be a disorder of T-lymphocytes function. They also suggested the presence of a humoral E-rosette formation inhibitory factor in patients with active MCNS and showed that extract of calf thymus, thymosin, induces some recovery of E-rosette formation.[7] Then we reported that the C-terminal decapeptide (positions 19—28), induces some recovery of E-rosette formation in the presence of inhibitory factor from a patient with nephrotic syndrome,[8] and later the C-terminal pentadecapeptide was prepared to study its immunological activity.[9] The present report describes the synthesis of the entire amino acid sequence of thymosin α_1. Further, the relative responses of the E-rosette formation inhibiting activity of active MCNS serum to the decapeptide fragment (positions 19—28)[8] and the pentadecapeptide fragment (positions 14—28)[9] were compared

1) Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: DMF, dimethylformamide; MCNS, minimal change nephrotic syndrome; EDTA, ethylenediamine tetraacetic acid; E-rosette, a rosette with sheep erythrocytes; GVB^{2+}, gelatin–veronal buffer; WSCI, water-soluble carbodiimide; TFA, trifluoroacetic acid; HOBT, N-hydroxybenzotriazole; DCC, dicyclohexylcarbodiimide; HONB, N-hydroxy-5-norbornene-2,3-dicarboximide; PBS, phosphate-buffered saline; Tos, *p*-toluenesulfonate; THF, tetrahydrofuran.

2) Location: *Tsutsumimachi 3-16-1, Sendai, 980, Japan.*

3) A.L. Goldstein, A. Guha, M.M. Zatz, M.A. Hardy, and A. White, *Proc. Natl. Acad. Sci. USA*, **69**, 1800 (1972).

4) A.L. Goldstein, T.L.K. Low, M. McAdoo, J. McClure, G.B. Thurman, J. Rossio, C. Lai, D. Chang, S. Wang, C. Harvey, A.H. Ramel, and J. Meinhofer, *Proc. Natl. Acad. Sci. USA*, **74**, 725 (1977).

5) T.L.K. Low, G.B. Thurman, M. McAdoo, J. McClure, J.L. Rossio, P.H. Naylor, and A.L. Goldstein, *J. Biol. Chem.*, **254**, 981 (1979).

6) J.J. Costanzi, R.G. Gagliano, F. Delaney, N. Harris, G.B. Thurman, H. Sakai, A.L. Goldstein, D. Loukas, G.B. Cohen, and P.D. Thomson, *Cancer*, **40**, 14 (1977).

7) S. Tomizawa, S. Suzuki, M. Oguri, and T. Kuroume, Abstracts of Papers, 20th Annual Meeting of the Nephrological Society of Japan, Nagoya, December, 1977, p. 157.

8) T. Abiko, I. Onodera, and H. Sekino, *Chem. Pharm. Bull.*, **27**, 3171 (1979).

9) T. Abiko, H. Sekino, and H. Higuchi, *Chem. Pharm. Bull.*, **28**, 3411 (1980).

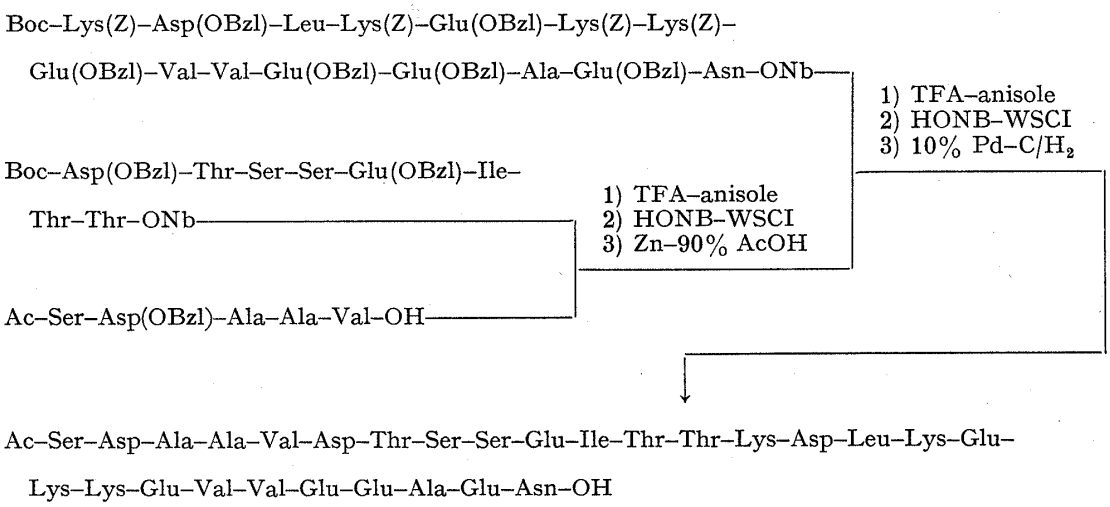

Fig. 1. Synthetic Route for Thymosin α_1

with that to thymosin α_1 by taking synthetic thymosin α_1 as a standard. The synthesis of the protected C-terminal pentadecapeptide, Boc–Lys(Z)–Asp(OBzl)–Leu–Lys(Z)–Glu(OBzl)–Lys-(Z)–Lys(Z)–Glu(OBzl)–Val–Val–Glu(OBzl)–Glu(OBzl)–Ala–Glu(OBzl)–Asn–ONb, was described in the preceding paper.[9] The synthetic scheme for thymosin α_1 is illustrated in Fig. 1. This route is different from those employed for the synthesis of thymosin α_1 by other workers.[10] In these examples, the DCC condensation of acylamino acids and suitable peptide fragments was performed in the presence of a racemization suppressor, such as HOBT[11] or HONB.[12] The synthetic scheme for the protected fragment, Boc–Asp(OBzl)–Thr–Ser–Ser–Glu(OBzl)–Ile–Thr–Thr–ONb, is illustrated in Fig. 2. Boc–Thr–ONb was treated with TFA–anisole to remove the Boc group and the product was coupled with Boc–Thr–OH by the HOBT–DCC method[11] to give Boc–Thr–Thr–ONb (I). The Boc group of the dipeptide I was similarly removed and the free base was condensed with Boc–Ile–OH by the HOBT–DCC method to give Boc–Ile–Thr–Thr–ONb (II). The Boc group of the tripeptide II was again similarly

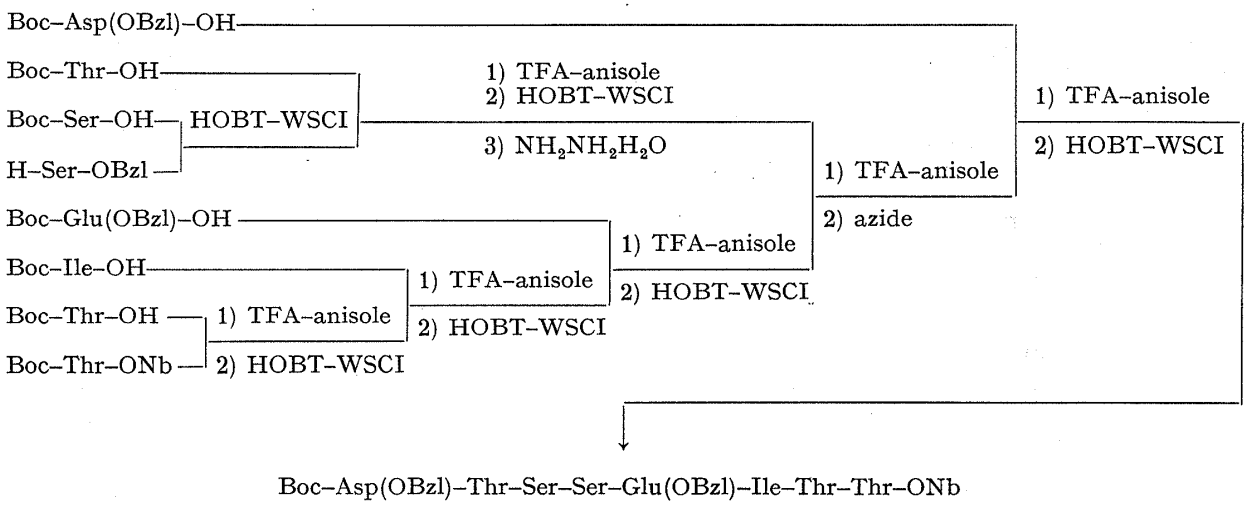

Fig. 2. Synthetic Route for the Protected Octapeptide (Positions 6—13)

10) S.S. Wang, R. Makofske, A. Bach, and R.B. Merrifield, *Int. J. Peptide Protein Res.*, **15**, 1 (1980).
11) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
12) M. Fujino, S. Kobayashi, M. Obayashi, J. Fukuda, S. Shinagawa, and O. Nishimura, *Chem. Pharm. Bull.*, **22**, 1857 (1974).

removed and the free base was condensed with Boc–Glu(OBzl)–OH by the HOBT–DCC method to afford Boc–Glu(OBzl)–Ile–Thr–Thr–ONb (III). H–Ser–OBzl Tos was condensed with Boc–Ser–OH by the HOBT–DCC method to give Boc–Ser–Ser–OBzl (IV). As before, the Boc group of the dipeptide IV was removed and the free base was condensed with Boc–Thr–OH by the HOBT–DCC method to afford Boc–Thr–Ser–Ser–OBzl (V). The tripeptide V was treated with hydrazine hydrate to give Boc–Thr–Ser–Ser–$NHNH_2$ (VI). After removal of the Boc group of III, the resulting tetrapeptide ester was condensed with the azide prepared from VI according to Rudinger's procedure[13] to give Boc–Thr–Ser–Ser–Glu(OBzl)–Ile–Thr–Thr-ONb (VII). The Boc group of the heptapeptide VII was again similarly removed and the free base was condensed with Boc–Asp(OBzl)–OH by the HOBT–DCC method to afford Boc–Asp(OBzl)–Thr–Ser–Ser–Glu(OBzl)–Ile–Thr–Thr–ONb (VIII). The synthetic scheme for the acylpeptide fragment, Ac–Ser–Asp(OBzl)–Ala–Ala–Val–OH, is illustrated in Fig. 3. Z–

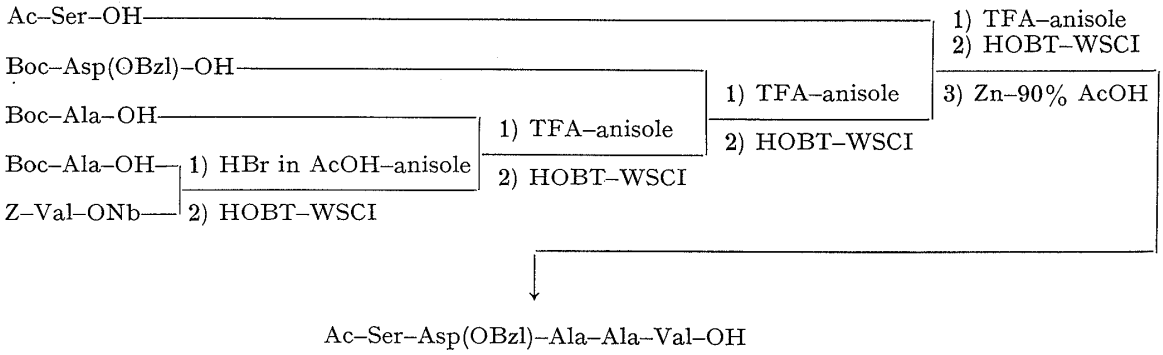

Fig. 3. Synthetic Route for the Protected Pentapeptide (Positions 1—5)

Val–ONb was treated with 25% HBr in AcOH–anisole to remove the Z group and the product was coupled with Boc–Ala–OH by the HOBT–DCC method to give Boc–Ala–Val–ONb (IX). The Boc group of the dipeptide IX was similarly removed and the free base was condensed with Boc–Ala–OH by the HOBT–DCC method to afford Boc–Ala–Ala–Val–ONb (X). The Boc group of the tripeptide X was similarly removed and the free base was condensed with Boc–Asp(OBzl)–OH by the HOBT–DCC method to give Boc–Asp(OBzl)–Ala–Ala–Val–ONb (XI). This, after treatment with TFA–anisole, was condensed with Ac–Ser–OH[14] by the HOBT–DCC method to give Ac–Ser–Asp(OBzl)–Ala–Ala–Val–ONb (XIII), from which the *p*-nitrobenzyl ester group was removed by the treatment with Zn in 90% AcOH.[15] The last traces of metal contamination were removed by treatment with 1% EDTA. The Boc group of the octapeptide VIII was removed and the corresponding free base was condensed with the N-terminal moiety, Ac–Ser–Asp(OBzl)–Ala–Ala–Val–OH (XIII), by the HONB–DCC method[12] to yield Ac–Ser–Asp(OBzl)–Ala–Ala–Val–Asp(OBzl)–Thr–Ser–Ser–Glu(OBzl)–Ile–Thr–Thr–ONb (XIV), from which the *p*-nitrobenzyl ester group was removed by treatment with Zn in 90% AcOH. After removal of the Boc group of Boc–Lys(Z)–Asp(OBzl)–Leu–Lys(Z)–Glu(OBzl)–Lys(Z)–Lys(Z)–Glu(OBzl)–Val–Val–Glu(OBzl)–Glu(OBzl)–Ala–Glu(OBzl)–Asn–ONb,[9] the resulting pentadecapeptide ester was condensed with the N-terminal moiety, Ac–Ser–Asp(OBzl)–Ala–Ala–Val–Asp(OBzl)–Thr–Ser–Ser–Glu(OBzl)–Ile–Thr–Thr–OH (XV), by the HONB–DCC method to yield Ac–Ser–Asp(OBzl)–Ala–Ala–Val–Asp(OBzl)–Thr–Ser–Ser–Glu(OBzl)–Ile–Thr–Lys(Z)–Asp(OBzl)–Leu–Lys(Z)–Glu(OBzl)Lys(Z)–Lys(Z)–Glu(OBzl)–Val–Val–Glu(OBzl)–Glu(OBzl)–Ala–Glu(OBzl)–Asn–ONb (XVI). N–Methyl-2-pyrrolidone had

13) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).
14) Z. Bohak and E. Katchalski, *Biochemistry*, **2**, 228 (1963).
15) K. Suzuki, N. Endo, K. Nitta, and Y. Sasaki, Proceedings of the 14th Symposium on Peptide Chemistry, Hiroshima, 1976, p. 45.

to used as a solvent, because of the poor solubility of the amino component during this coupling reaction. Finally, the protected octacosapeptide ester was hydrogenated over 10% Pd–C to remove the protecting groups, The deblocked product was purified by gel filtration on a Sephadex G-25 column followed by partition column chromatography on Sephadex G-25 according to Yamashiro.[16] A solvent system consisting of BuOH–AcOH–H_2O (4: 1: 5) was used to elute the desired compound. The absorbancy (230 nm) due to the peptide bond was used as a guide for this chromatographic purification. The octacosapeptide (XVII) thus obtained was found to be homogeneous by paper chromatography with two different solvent systems. The amino acid compositions in the acid hydrolysate of XVII agreed well with the theoretical values. Incubation of lymphocytes with serum from a patient with active MCNS decreased the proportions of T-cell rosettes from 77% to 45%. After incubation of cell suspensions with various amounts of the synthetic thymosin α_1 and its two fragments[9] from 1 μg/ml to 100 μg/ml, recovery of T-cell rosette formation was observed (Table I). The potency of the synthetic thymosin α_1 was more than 10 times the potency of the synthetic pentadecapeptide (positions 14—28) (Table II).[9] The synthetic decapeptide (positions 19—28)[8] showed 1/100 of the activity of the synthetic thymosin α_1.

Table I. Effects of the Synthetic Thymosin α_1 and Its Two Fragments on the Action of Inhibiting Factor of Serum from a Patient with Active MCNS

Peptides	Dose (μg/ml)	E-Rosette formation (%)
—[a,b]		76±4
—[b,c]		77±5
—[c,d]		45±4
Thymosin α_1[c,d]	1	58±5
	10	67±4
	100	74±5
Pentadecapeptide[c,d] (positions 14—28)	1	46±3
	10	56±4
	100	69±4
Decapeptide[c,d] (positions 19—28)	1	47±4
	10	46±4
	100	59±5

a) Normal lymphocytes.
b) Control serum: incubation was carried out for 30 min at a concentration of 20%.
c) Patient's lymphocytes.
d) Patient's serum: incubation was carried out for 30 min at a concentration of 20%.

Table II. Relative Potencies of Thymosin α_1 and Its Two Fragments on E-Rosette Formation Inhibiting Factor of Serum from a Patient with Active MCNS

Peptides	Relative potency (molar basis)
Thymosin α_1	100.00
Pentadecapeptide (positions 14—28)	9.83
Decapeptide (positions 19—28)	1.04

Experimental

All melting points are uncorrected. Rotations were determined with an Atago Polax machine. The amino acid analysis was performed with a JEOL JLC-8AH amino acid analyzer. Unless otherwise men-

16) D. Yamashiro, *Nature* (London), **201**, 76 (1964).

tioned, Z groups of the protected amino acids and peptides were deblocked with HBr in AcOH, and Boc groups with TFA. The resulting amino components were chromatographed on filter paper, Toyo Roshi No. 51, at room temperature. Rf^1 values refer to the Partridge system[17] and Rf^2 values refer to BuOH–pyridine–AcOH–H_2O (30 : 20 : 6 : 24).[18] Concentration procedure were carried out in a rotary evaporator under reduced pressure at a temperature of 35—40°. The blood samples for this study were obtained from one nephrotic child and three healthy persons. The nephrotic child case was in the active stage, exhibiting proteinurea and biochemical abnormality in the blood. The blood was centrifuged and the separated serum was kept at −20° until use.

Boc-Thr-Thr-ONb (I)——Boc–Thr–ONb (3.5 g) was treated with TFA (7 ml) in the presence of anisole (0.7 ml) at room temperature for 30 min, then excess TFA was removed by evaporation. The residue was washed with *n*-hexane and then dried over KOH pellets *in vacuo*. The product was dissolved in THF (15 ml) and the solution was neutralized with N-methylmorpholine (1.5 ml). To this ice-chilled solution, a solution of Boc–Thr–OH (2.4 g) in THF (10 ml), HOBT (1.5 g) and WSCI (1.8 g) were added and the whole was stirred at 0° for 16 hr. The solution was concentrated *in vacuo* and then extracted with EtOAc. The EtOAc layer was washed successively with 1 N citric acid, H_2O, 1 N $NaHCO_3$ and H_2O, dried over $MgSO_4$ and then concentrated *in vacuo*. The residue was recrystallized from hot EtOAc: 2.8 g (62%), mp 136°, $[\alpha]_D^{30}$ −23.5° (c=1.0, DMF), Rf^1 0.86, Rf^2 0.92, single ninhydrin–positive spot. *Anal.* Calcd for $C_{20}H_{29}N_3O_9$: C, 52.74; H, 6.42; N, 9.23. Found: C, 52.45; H, 6.29; N, 9.04.

Boc-Ile-Thr-Thr-ONb (II)——I (2.3 g) was treated with TFA (5 ml)-anisole (0.5 ml) and the deprotected peptide isolated as stated above was dissolved in DMF (20 ml). To this ice-chilled solution, N-methylmorpholine (0.8 ml), Boc–Ile–OH (1.3 g), HOBT (0.543 g) and WSCI (0.754 g) were successively added. After stirring at 0° for 16 hr, the mixture was extracted with EtOAc and then washed successively with 1 N citric acid, H_2O, 1 N $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*, than *n*-hexane was added to the residue: 1.6 g (52%), mp 95—98°, $[\alpha]_D^{30}$ −18.9° (c=1.0, DMF), Rf^1 0.80, Rf^2 0.91, single ninhydrin–positive spot. *Anal.* Calcd for $C_{26}H_{40}N_4O_{10}$: C, 54.92; H, 7.09; N, 9.85. Found: C, 54.69; H, 7.41; N, 9.65.

Boc-Glu(OBzl)-Ile-Thr-Thr-ONb (III)——II (608 mg) was treated with TFA (2 ml)–anisole (0.4 ml) as described above. The resulting tripeptide ester was condensed with Boc–Glu(OBzl)–OH (372 mg), in the presence of HOBT (149 mg) and WSCI (171 mg) essentially as described for the preparation of II. After standing for 16 hr, the mixture was poured into cold 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 citric acid and H_2O. The product was reprecipitated from DMF and 1 N citric acid: 456 mg (58%), mp 85—96°, $[\alpha]_D^{30}$ −36.5° (c=1.0, DMF), Rf^1 0.92, Rf^2 0.95, single ninhydrin–positive spot. *Anal.* Calcd for $C_{38}H_{53}N_5O_{13}$: C, 57.93; H, 6.78; N, 8.89. Found: C, 57.89; H, 7.01; N, 8.87.

Boc-Ser-Ser-OBzl (IV)——This compound was prepared from H–Ser–OBzl Tos (3.7 g), HOBT (1.5 g), Boc–Ser–OH (2.2 g) and WSCI (1.8 g) essentially as described for the preparation of II: 2.9 g (74%), mp 63—64°, $[\alpha]_D^{30}$ −15.6° (c=1.0, DMF), Rf^1 0.61, Rf^2 0.78, single ninhydrin–positive spot. *Anal.* Calcd for $C_{18}H_{26}N_2O_7H_2O$: C, 54.00; H, 7.05; N, 7.00. Found: C, 53.72; H, 7.42; N, 6.93.

Boc-Thr-Ser-Ser-OBzl (V)——This compound was prepared from IV (1300 mg), HOBT (495 mg), Boc–Thr–OH (804 mg) and WSCI (570 mg) essentially as described for the preparation of II. The product was reprecipitated from MeOH and ether: 841 mg (53%), mp 146—152°, $[\alpha]_D^{30}$ −18.7° (c=1.0, DMF), Rf^1 0.61, Rf^2 0.83, single ninhydrin-positive spot. *Anal.* Calcd for $C_{22}H_{33}N_3O_9$: C, 54.65; H, 6.88; N, 8.69. Found: C, 54.26; H, 6.49; N, 9.01.

Boc-Thr-Ser-Ser-NHNH$_2$ (VI)——V (242 mg) was dissolved in MeOH (3 ml). Hydrazine hydrate (0.2 ml) was added and the solution was left to stand at room temperature. The gelatinous mass formed on standing overnight was collected by filtration and recrystallized from MeOH: 154 mg (81%), mp 184—185°, $[\alpha]_D^{30}$ −10.4° (c=1.0, DMF). *Anal.* Calcd for $C_{15}H_{29}N_5O_8H_2O$: C, 42.35; H, 7.35; N, 16.46. Found: C, 41.96; H, 7.51; N, 16.38.

Boc-Thr-Ser-Ser-Glu(OBzl)-Ile-Thr-Thr-ONb (VII)——III (394 mg) was treated with TFA (3 ml)–anisole (0.5 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 (3 ml) containing N-methylmorpholine (0.08 ml). The azide[13] (prepared from 209 mg of Boc–Thr–Ser–Ser–NHNH$_2$) in DMF (3 ml) and N-methylmorpholine (0.08 ml) were added to the above ice-chilled solution and the mixture was stirred at 4° for 48 hr. The mixture was poured into 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 citric acid and H_2O. The product was recrystallized from hot EtOAc: 291 mg (55%), mp 126—131°, $[\alpha]_D^{32}$ −5.8° (c=1.0, DMF), Rf^1 0.82, Rf^2 0.95, single ninhydrin–positive spot. *Anal.* Calcd for $C_{48}H_{70}N_8O_{19}$: C, 54.23; H, 6.64; N, 10.54. Found: C, 53.89; H, 6.93; N, 10.42.

Boc-Asp(OBzl)-Thr-Ser-Ser-Glu(OBzl)-Ile-Thr-Thr-ONb (VIII)——This compound was prepared from VII (133 mg), Boc–Asp(OBzl)–OH (44 mg), HOBT (19 mg) and WSCI (21 mg) essentially as described for

17) S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).
18) S.G. Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953).

the preparation of III. The product was recrystallized from EtOAc: 103 mg (65%), mp 108—111°, $[\alpha]_D^{32}$ −61.0° (c=1.0, DMF), Rf^1 0.86, Rf^2 0.91, single ninhydrin-positive spot. *Anal.* Calcd for $C_{59}H_{81}N_9O_{22}$: C, 55.87; H, 6.44; N, 9.94. Found: C, 56.01; H, 6.41; N, 10.12.

Boc-Ala-Val-ONb (IX)——Z-Val-ONb (1300 mg) was dissolved in AcOH (5 ml), anisole (0.5 ml) and 25% HBr in AcOH (5 ml). After 40 min at room temperature, the reaction mixture was shaken vigorously with dry ether. The precipitate was washed with dry ether and dried over KOH pellets *in vacuo*. The resulting amino acid ester was condensed with Boc-Ala-OH (693 mg), HOBT (500 mg) and WSCI (570 mg) essentially as described for the preparation of II: 1003 mg (71%), mp 55—57°, $[\alpha]_D^{30}$ −19.1° (c=1.0, DMF), Rf^1 0.85, Rf^2 0.86, single ninhydrin-positive spot. *Anal.* Calcd for $C_{20}H_{29}N_3O_7$: C, 56.72; H, 6.90; N, 9.92. Found: 56.36; H, 7.23; N, 9.81.

Boc-Ala-Ala-Val-ONb (X)——This compound was prepared from IX (1000 mg), Boc-Ala-OH (492 mg), HOBT (351 mg) and WSCI (404 mg) essentially as described for the preparation of II: 834 mg (76%), mp 53—56°, $[\alpha]_D^{30}$ −24.0° (c=1.0, DMF), Rf^1 0.83, Rf^2 0.85, single ninhydrin-positive spot. *Anal.* Calcd for $C_{23}H_{34}N_4O_8$: C, 55.86; H, 6.93; N, 11.33. Found: C, 55.42; H, 7.24; N, 10.98.

Boc-Asp(OBzl)-Ala-Ala-Val-ONb (XI)——This compound was prepared from X (618 mg), Boc-Asp-(OBzl)-OH (444 mg), HOBT (186 mg) and WSCI (213 mg) essentially as described for the preparation of I. The product was recrystallized from EtOAc: 701 mg (80%), mp 120°, $[\alpha]_D^{32}$ −35.7° (c=1.0, DMF), Rf^1 0.83, Rf^2 0.91, single ninhydrin-positive spot. *Anal.* Calcd for $C_{34}H_{45}N_5O_{11}$: C, 58.36; H, 6.48; N, 10.01. Found: C, 57.97; H, 6.66; N, 9.92.

Ac-Ser-Asp(OBzl)-Ala-Ala-Val-ONb (XII)——This compound was prepared from XI (583 mg), Ac-Ser-OH (220 mg), HOBT (124 mg) and WSCI (142 mg) essentially as described for the Preparation of III. The product was reprecipitated from DMF and 1 N citric acid: 601 mg (88%), mp 149—153°, $[\alpha]_D^{30}$ −41.1° (c=1.0, DMF), *Anal.* Calcd for $C_{34}H_{44}N_6O_{12}$: C, 56.03; H, 6.09; N, 11.53. Found: C, 55.72; H, 6.35; N, 11.48.

Ac-Ser-Asp(OBzl)-Ala-Ala-Val-OH (XIII)——Zn dust (100 mg) was added to a solution of XII (273 mg) in 90% AcOH (5 ml) and the mixture was stirred at 0° for 30 min and then at room temperature for 90 min. The solution was filtered, the filtrate was concentrated *in vacuo* and the residue was treated with ice-chilled 1% EDTA (40 ml). The gelatinous mass formed on standing in the refrigerator overnight was collected by filtration, washed with 1 N citric acid and H_2O and then crystallized three times from hot MeOH–H_2O (2: 1): 131 mg (58%), mp 195—199°, $[\alpha]_D^{30}$ −29.4° (c=1.0, DMF), *Anal.* Calcd for $C_{27}H_{39}N_5O_{10}$: C, 54.63; H, 6.62; N, 11.80. Found: C, 54.41; H, 6.91; N, 12.09.

Ac-Ser-Asp(OBzl)-Ala-Ala-Val-Asp(OBzl)-Thr-Ser-Ser-Glu(OBzl)-Ile-Thr-Thr-ONb (XIV)——This compound was prepared from VIII (85 mg), XIV (69 mg), HONB (14 mg) and WSCI (13 mg) essentially as described for the preparation of III. The product was recrystallized from hot MeOH: 103 mg (88%), mp 208—221°, $[\alpha]_D^{30}$ −48.1° (c=1.0, DMF). *Anal.* Calcd for $C_{81}H_{110}N_{14}O_{29}$: C, 55.79; H, 6.36; N, 11.25. Found: C, 56.12; H, 6.85; N, 10.94.

Ac-Ser-Asp(OBzl)-Ala-Ala-Val-Asp(OBzl)-Thr-Ser-Ser-Glu(OBzl)-Ile-Thr-Thr-OH (XV)——XIV (87 mg) was dissolved in DMF–90% AcOH (1 ml—3 ml) and then treated with Zn dust (17 mg) essentially as described for the preparation of XIII. The product was extracted with *n*-BuOH and washed successively with 1 N citric acid and H_2O. The *n*-BuOH layer was concentrated *in vacuo* and then the residue was dissolved in DMF (2 ml). The solution was poured into 1 N citric acid with stirring and the precipitate thereby formed was washed successively with 1 N citric acid and H_2O: 34 mg (43%), mp 164—172°, $[\alpha]_D^{30}$ −56.0° (c=1.0, DMF). *Anal.* Calcd for $C_{74}H_{105}N_{13}O_{27}H_2O$: C, 63.36; H, 7.69; N, 12.99. Found: C, 62.98; H, 8.01; N, 13.18.

Ac-Ser-Asp(OBzl)-Ala-Ala-Val-Asp(OBzl)-Thr-Ser-Ser-Glu(OBzl)-Ile-Thr-Thr-Lys(Z)-Asp(OBzl)-Leu-Lys(Z)-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-ONb (XVI)——The Boc-Lys(Z)-Asp(OBzl)-Leu-Lys(Z)-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu-(OBzl)-Ala-Glu(OBzl)-Asn-ONb[9] (52 mg) was treated with TFA (1 ml)–anisole (0.1 ml) as described above. To an ice-chilled solution of this product in N-methyl-2-pyrrolidone (2 ml), XV (30 mg), HONB (4 mg) and WSCI (4 mg) were added, followed by N-methylmorpholine to keep the solution slightly alkaline. After 48 hr at −10°, the reaction mixture was poured into 1 N $NaHCO_3$ with stirring. The precipitate thus formed was washed successively with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O. The dried product was recrystallized from hot EtOH: 69 mg (90%), mp 186—193°, $[\alpha]_D^{30}$ −36.0° (c=0.3, DMF). *Anal.* Calcd for $C_{231}H_{298}N_{34}O_{65}$: C, 60.43; H, 6.54; N, 10.37. Found: C, 60.09; H, 6.98; N, 10.48.

Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH (XVII)——The protected octacosapeptide XVI (50 mg) was hydrogenated in AcOH (10 ml)–H_2O (5 ml) over 10% Pd-C for 30 hr. The catalyst was removed with aid of cellite. The filtrate was evaporated to dryness, and the residue was dissolved in 1% AcOH (2 ml) and the solution was applied to a column of Sephadex G-25 (2.6×80 cm). Individual fractions (10 ml each) eluted with 1% AcOH were collected and the absorbancy at 230 nm was determined. The fractions corresponding to the front peak (tube No. 20—25) were combined and concentrated *in vacuo*. Analysis by paper chromatography revealed the presence of two chlorine–*o*-tolidine–positive spots with Rf^1 0.03 (major), 0.18 (minor) and Rf^2 0.10 (major), 0.31 (minor). The product was dissolved in a small amount of the upper phase of a solvent system consisting of *n*-BuOH–AcOH–H_2O (4: 1: 5). The solution was applied to a column of Sephadex G-25 (2.6×73 cm) previously equilibrated with the same upper phase and individual fractions (8 ml each)

were collected. The absorbancy at 230 nm was determined. The fractions corresponding to the main peak (tube No. 59—65) were combined. The solvent was removed by evaporation and the residue was lyophilized from 3% AcOH to give a fluffy powder: 17 mg (51%), mp 173—184°, $[\alpha]_D^{24}$ −91.0° ($c=0.3$, 2 N AcOH), Rf^1 0.03, Rf^2 0.10, single chlorine–*o*-tolidine–positive spot. Amino acid compositions in an acid hydrolysate: Lys 3.79, Ala 2.86, Val 3.14, Thr 2.79, Leu 1.02, Ile 0.93, Ser 2.73, Glu 5.67, Asp 3.78 (average recovery 81%).

E-Rosette Formation——Peripheral blood was obtained from a patient with active MCNS. Lymphocytes were isolated in a Hypaque–Ficoll gradient[19] for T cell rosette formation. Isolated lymphocytes were adjusted to 5×10^5 cells/ml with PBS. Contamination by monocytes and polymorphonuclear cells amounted to less than 5%.[20] Sheep erythrocytes were washed with PBS, and a suspension (1×10^6/ml) was prepared. Lymphocytes were suspended in GVB^{2+} (0.8 ml) and incubated for 30 min at 37° with the patient's serum (0.2 ml) and the thymosin α_1. Next, they were washed with GVB^{2+} and centrifuged for 10 min at 1500 rpm, then suspended in GVB^{2+} (1.0 ml). The suspension was mixed with the suspension of sheep erythrocytes (0.5 ml) and incubated for 18 hr at 4°. 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. Monocytes or polymorphonuclear cells forming rosettes were excluded.

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