ture with stirring, and the mixture was filtered. The filtrate was evaporated to dryness and the residue was extracted with 1.2 l of $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated to one third of its initial volume. To the resulting solution was added 0.5 l of ether and the precipitate of 280 g (86%) of **4**, mp 135—136°, was collected. *Anal.* Calcd. for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.24; H, 5.62; N, 4.42. NMR (Me_2SO-d_6) δ: 5.58 [1H, s, CH(OMe)$_2$].

5-Formyl-8-hydroxycarbostyril (5)——To a solution of 280 g (0.86 mol) of **4** in 10 l of EtOH was added 10 g of 5% palladium carbon, and reduction was carried out in a 50 l hydrogenator under 5 atomospheres of hydrogen gas at 50°. After 1 hr the catalyst was removed and the filtrate was evaporated to dryness. To the residue was added 9 l of MeOH and 2 l of 0.5 N hydrochloric acid, and the resulting solution was stirred for 1 hr at room temperature and cooled. The precipitate was collected and washed with water and MeOH to give 150 g (92%) of **5** as pale straw colored crystals, mp 315—317° (dec.). *Anal.* Calcd. for $C_{10}H_7NO_3$: C, 63.49; H, 3.73; N, 7.40. Found: C, 63.50; H, 3.42; N, 7.77. NMR (Me_2SO-d_6) δ: 10.05 (1H, s, CHO), 9.04 and 6.74 [1H, d, J=9.6 Hz, C_4–H and C_3–H], and 7.69 and 7.16 [1H, d, J=8.4 Hz, CH (Ar)].

Oxidation of Procaterol (1) with *m*-Chloroperoxybenzoic Acid——To a solution of 25 g (0.081 mol) of 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril monohydrate [(**1**), mp 149—151° (dec.)] in 150 ml of DMF was added 25 g (0.145 mol) of *m*-chloroperoxybenzoic acid (Aldrich Chemical Company, Inc.) in small portions with stirring at room temperature. After 1 hr the reaction mixture was poured into 1 l of ice-water. The resulting precipitate was collected, washed with EtOH and recrystallized with DMF to give 5.5 g (36%) of **5**.

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The β-*p*-Nitrobenzyl Ester to Minimize Side Reaction during Treatment of Aspartyl Peptides with Methanesulfonic Acid[1,2]

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When Boc-Asp(OBzl)-Ser(Bzl)-OBzl was deblocked with methanesulfonic acid–anisole to obtain free dipeptide, a few degree of α to β shift was observed. Boc-Asp-(OBzl)-Thr(Bzl)-OBzl had similar property. When β-carboxyl group was protected with *p*-nitrobenzyl group stable to methanesulfonic acid–anisole, no detectable α to β shift was observed. Boc-Asp(ONb)-Ser(Bzl)-Asp(OBzl)-Pro-Arg(MBS)-ONb was treated with methanesulfonic acid-anisole, followed by catalytic hydrogenation or treatment with zinc powder in acetic acid for the cleavage of *p*-nitrobenzyl group to give Asp-Ser-Asp-Pro-Arg.

Keywords——Boc-Asp(ONb)-Ser(Bzl)-OBzl; Boc-Asp(ONb)-Thr(Bzl)-OBzl; Z-Asp-(OBzl)-His-OBzl; Boc-Asp(OBzl)-Gly-OBzl; Z-Asp(OBzl)-Val-OBzl

The methanesulfonic acid (MsOH)–anisole reagent has been found to cleave efficiently a number of protecting groups currently employed in peptide chemistry without significant side reactions.[4]

We have found that α to β shift of aspartyl peptides occurs during the treatment of β-benzyl aspartyl peptides with this reagent and this side reaction can be minimized when *p*-nitrobenzyl ester (ONb) is used as a protecting group of β-carboxyl group of the aspartic residue.

1) A part of this work was presented at the 15th Symposium on Peptide Chemistry, Osaka, 1977.
2) Abbreviations used are those recommended by IUPAC–IUB Commission of Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: DMF=dimethylformamide, AP-M=amino-peptidase M.
3) Location: *Komatsushima, Sendai 983, Japan.*
4) H. Yajima, Y. Kiso, N. Fujii and H. Irie, *Chem. Pharm. Bull.* (Tokyo), **23**, 1164 (1975).

Firstly, protected α- and β-aspartyl dipeptides, shown in Table I, were synthesized in a usual manner and all of the protecting groups were removed by catalytic hydrogenation or trifluoroacetic acid (TFA) to give the corresponding dipeptides. Deblocked α- and β-aspartyl dipeptides thus obtained were used for authentic samples as analyzed the product in the reaction mixture of the protected α-aspartyl dipeptides and MsOH–anisole. Protected α-aspartyl dipeptides shown in Table I, except XI and XII, were also used as substrate toward the action of MsOH–anisole. *Rf* values of paper chromatography and retention times on a long column in 4 hours standard amino acid analysis of the authentic dipeptides are shown in Table II.

Secondly, the products in the reaction mixture of the five kinds of the protected α-aspartyl peptides and MsOH–anisole were isolated and analyzed according to the procedure shown in Chart 1. From these investigations, it was found that III and IX among five kinds of

Table I. Protected Aspartyl Dipeptides

Boc-Asp(OBzl)-Gly-OBzl[a] (I)
Gly-OBzl[b]
Z-Asp-OBzl (II)
Boc-Asp(OBzl)-Ser(Bzl)-OBzl[c] (III)
Ser(Bzl)-OBzl[c]
Boc-Asp-ONb (IV)
Z-Asp(OBzl)-His-OBzl[d] (V)
His-OBzl[d]
Z-Asp-OBzl (VI)
Z-Asp(OBzl)-Val-OBzl (VII)
Val-OBzl
Z-Asp-OBzl (VIII)
Z-Asp(OBzl)-Thr(Bzl)-OBzl (IX)
Thr(Bzl)-OBzl
Z-Asp-OBzl (X)
Boc-Asp(ONb)-Ser(Bzl)-OBzl (XI)
Boc-Asp(ONb)-Thr(Bzl)-OBzl (XII)

a) Y. Ogata, K. Igano, K. Inoue and S. Sakakibara, "Proceeding of the 12th Symposium on Peptide Chemistry," ed. by H. Yajima, Protein Research Foundation, Minoh, Osaka, 1975, p. 35.
b) G. Losse, H. Jeschkeit and D. Knoft, *Chem. Ber.*, **97**, 1789 (1964).
c) See ref. 5.
d) See ref. 7.

Table II. *Rf* Values of Paper Chromatography and Retention Times on a Long Column in 4 hr Standard Amino Acid Analysis

		Asp-Gly		Asp-Ser		Asp-His		Asp-Val		Asp-Thr	
		α	β	α	β	α	β	α	β	α	β
Rf value[a]	A	0.27	0.19	0.22	0.14	0.17[b]	0.12[b]	0.58	0.43	0.27	0.27
	B	0.42	0.33	0.40	0.27	0.35	0.26	0.70	0.59	0.49	0.48
Retention time (min)		102	102	87[c]	35[c]			115	115	77	35

a) A: BuOH–AcOH–H_2O (3:1:1), Toyo Roshi No. 51. B: BuOH–AcOH–H_2O (2:1:1), Toyo Roshi No. 50.
b) See ref. 7.
c) See ref. 5.

protected aspartyl dipeptide
↓ 1. MsOH containing anisole, at room temperature for 30 min
2. addition of dry ether, centrifugation and washing with dry ether
3. neutralization with 1N NH_4OH
4. treatment with 1% NH_4HCO_3, at 40° for 24 hr
5. lyophilization
↓
aspartyl dipeptide
↓ paper or ion exchange column chromatography
↓
α- and β-aspartyl dipeptide

Chart 1. Analysis of Aspartyl Peptide Treated with MsOH–anisole

the protected peptides examined were derived to β-aspartyl peptides in a few percent yield. Namely, the contents of Asp-Ser[5] and $\overset{\text{Asp}}{\ulcorner}$—Ser[5] were 97.6% and 2.4% respectively, and the contents of Asp-Thr and $\overset{\text{Asp}}{\ulcorner}$—Thr were 96% and 4% respectively. When III was treated with MsOH–anisole as described above, minor amount (1.2%) of aspartic acid and serine was detected besides β-aspartyl peptide as by-product when analyzed the reaction mixture with amino acid analyzer. IX gave also similar results, the other protected α-aspartyl dipeptides shown in Table I gave no component amino acids, this may be explained that ester bond of the N to O shifted product[6] is hydrolyzed in part. On the contrary of the fact that Asp(OBzl)-His derivative had tendency to give $\overset{\text{Asp}}{\ulcorner}$—His[7] during treatment with anhydrous hydrogen fluoride,[8] V did not give $\overset{\text{Asp}}{\ulcorner}$—His during treatment with MsOH–anisole. The other two kinds of the protected aspartyl dipeptides were also derived to the corresponding α-aspartyl peptide without any detectable formation of β-aspartyl peptide. From the reaction mixture of III with MsOH–anisole, two isomers were separated preparatively with the use of Dowex 1×2 (acetate form) column chromatography. Asp-Ser and $\overset{\text{Asp}}{\ulcorner}$—Ser thus obtained were assessed to be identical with the authentic sample in physical and chemical properties. Similarly, Asp-Thr and $\overset{\text{Asp}}{\ulcorner}$—Thr were separated and assessed to be identical with the authentic sample.

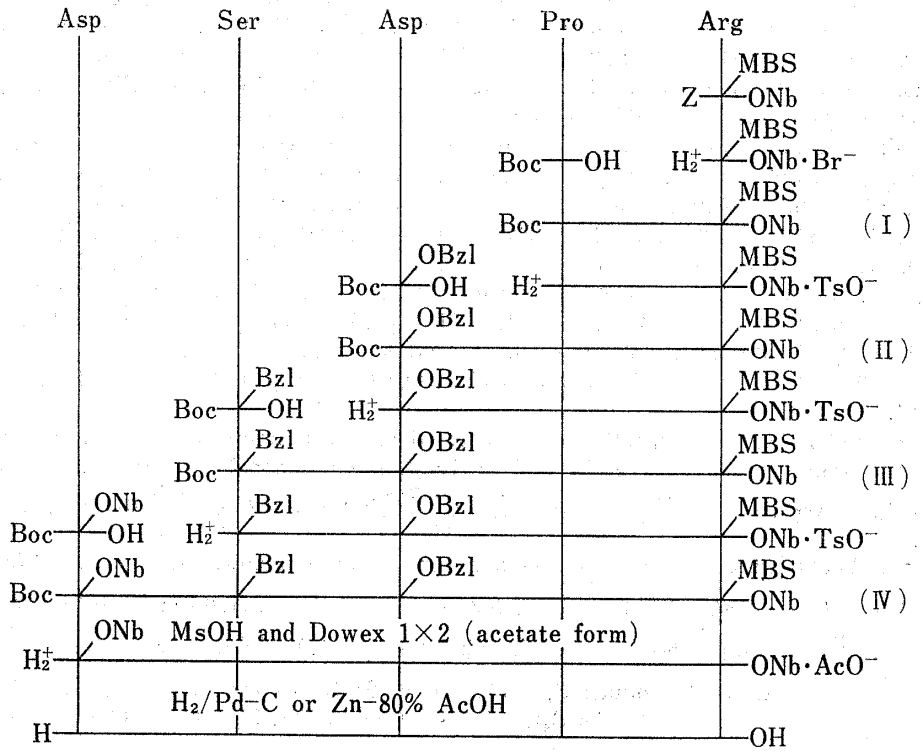

Chart 2. Synthetic Route for the Pentapeptide

5) K. Suzuki, K. Nitta and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 3025 (1976).
6) M. Fujino, M. Wakimasu, S. Shinagawa, C. Kitada and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **26**, 539 (1978).
7) M. Hirata, K. Noda and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **45**, 1790 (1972).
8) T. Baba, H. Sugiyama and S. Seto, *Chem. Pharm. Bull.* (Tokyo), **23**, 1164 (1975).

XI[5] was treated with MsOH–anisole and the resulting product was hydrogenated over 10% palladium-carbon to remove the ONb group. The desired Asp-Ser was detected in the reaction mixture without any detectable $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{Ser}$ and the component amino acids as analyzed with amino acid analyzer. Similarly, XII, which was prepared from Boc-Asp(ONb)[5] and Thr(Bzl)-OBzl derived from the hemioxalate,[9] did not give $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{Thr}$ and the component amino acids. However, minor rate of the N to O acyl migration in the reaction mixture of the two kinds of the protected dipeptide with MsOH–anisole was observed as analyzed by paper electrophoresis in a similar manner described by Fujino, *et al.*[6]

On the basis of these findings during the above investigations, immunogloblin E fragment, Asp-Ser-Asp-Pro-Arg, was synthesized to examine the utility of Asp(ONb). Fully protected pentapeptide, Boc-Asp(ONb)-Ser(Bzl)-Asp(OBzl)-Pro-Arg(MBS)-ONb, was synthesized in a virtually similar manner described in a previous paper.[5] In this investigation, *p*-methoxybenzenesulfonyl (MBS) group labile to MsOH was applied for the protection of guanidium group of the arginine residue.[10] Boc group in the intermediates was removed by the treatment with 2 M *p*-toluenesulfonic acid (TsOH) in dioxane. The synthetic route is shown in Chart 2. In the final step, ONb group was cleaved with zinc powder in 80% acetic acid at 0°[11] or catalytic hydrogenation. Asp-Ser-Asp-Pro–Arg thus obtained was identical with the authentic peptide.[5]

Experimental

All melting points are uncorrected. Unless otherwise mentioned, Boc group of the protected peptide was deblocked with 2 M TsOH in dioxane, and the paper chromatography was performed on Toyo Roshi No. 51 with the following solvent systems: *Rf*(A), BuOH–AcOH–H_2O (4:1:5. upper layer);[12] *Rf*(B), BuOH–AcOH–pyridine–H_2O (15:3:10:12).[13] Amino acid analysis was carried out on a Hitachi Model KLA-3B amino acid analyzer according to the directions given by Moore, *et al.*[14]

Protected α- and β-Aspartyl Dipeptides——Protected aspartyl dipeptides shown in Table I were synthesized in a virtually similar manner described in a previous paper.[5] Analytical data of new compounds were described below. VII; oil, $[\alpha]_D^{25}$ −28.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{28}H_{36}N_2O_7$: C, 65.61; H, 7.08; N, 5.47. Found: C, 65.69; H, 7.06; N, 5.57. VIII; mp 127—131°, $[\alpha]_D^{17}$ −9.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{31}H_{34}N_2O_7$: C, 68.11; H, 6.27; N, 5.13. Found: C, 67.92; H, 6.47; N, 5.30. IX; mp 72—84°, $[\alpha]_D^{17}$ −10.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{37}H_{38}N_2O_8$: C, 69.58; H, 6.00; N, 4.39. Found: C, 69.40; H, 5.99; N, 4.56. X; mp 69—74°, $[\alpha]_D^{17}$ −6.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{37}H_{38}N_2O_8$: C, 69.58; H, 6.00; N, 4.39. Found: C, 69.86; H, 6.07; N, 4.51. XI; mp 104—105°, $[\alpha]_D^{23}$ −22.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{33}H_{37}N_3O_9$: C, 63.96; H, 6.02; N, 6.78. Found: C, 63.77; H, 5.96; N, 6.88. XII; mp 93—94°, $[\alpha]_D^{23}$ −15.0° (c=1.0, DMF). *Anal.* Calcd. for $C_{34}H_{39}N_3O_9$: C, 64.44; H, 6.20; N, 6.63. Found: C, 64.21; H, 6.04; N, 6.53.

Authentic α- and β-Aspartyl Dipeptides——Protected aspartyl dipeptides, except XI and XII, were deblocked and the products were purified in a virtually similar manner described in a previous paper[5] to give free dipeptides, Asp-Gly,[8] $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{Gly}$,[8] Asp-Ser,[5] $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{Ser}$,[5] Asp-His,[7] $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{His}$,[7] Asp-Val, $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{Val}$, Asp-Thr and $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{Thr}$. Analytical data of new compounds were described below. Asp-Val; mp 175—189° (dec.), $[\alpha]_D^{17}$ +8.0° (c=1.0, H_2O). *Anal.* Calcd. for $C_9H_{16}N_2O_5 \cdot 2H_2O$: C, 40.29; H, 6.01; N, 10.44. Found: C, 40.60; H, 5.98; N, 10.40. $\overset{\text{Asp}}{\lceil}\!\!-\!\!\text{Val}$; mp 145—158° (dec.), $[\alpha]_D^{17}$ −9.0° (c=1.0, H_2O). *Anal.* Calcd. for $C_9H_{16}N_2O_5 \cdot 2H_2O$: C, 40.29: H, 6.01; N, 10.44, Found: C, 39.99; H, 6.20; N, 10.32. Asp-Thr; mp 144—160° (dec.), $[\alpha]_D^{14}$ +4.0° (c=1.0, H_2O). *Anal.* Calcd. for $C_8H_{14}N_2O_6 \cdot H_2O$: C, 38.09; H, 6.39; N, 11.11. Found: C, 38.22;

9) T. Mizoguchi, G. Levin, D.W. Woolley and J.M. Stewart, *J. Org. Chem.*, 33, 903 (1968).
10) O. Nishimura and M. Fujino, *Chem. Pharm. Bull.* (Tokyo), **24**, 1568 (1976).
11) K. Suzuki, N. Endo, K. Nitta and Y. Sasaki, "Peptide Chemistry 1976," ed. by T. Nakajima, Protein Research Foundation, Minoh, Osaka, 1977, p. 45.
12) S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).
13) S.G. Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953).
14) S. Moore, D.H. Spackman and W.H. Stein, *Anal. Chem.*, **30**, 1185 (1958).

H, 6.55; N, 10.95. $\overset{\text{Asp}}{\text{|}}\!\!-\!\!-\text{Thr}$; mp 140—160° (dec.), $[\alpha]_D^{14}$ 0° (c=1, H_2O). *Anal.* Calcd. for $C_8H_{14}N_2O_6 \cdot H_2O$: C, 38.09; H, 6.39; N, 11.11. Found: C, 38.15; H, 6.25; N, 10.88.

Preparation and Analysis of Aspartyl Dipeptide——The protected α-aspartyl peptides, except XI and XII, were treated as shown in Chart 1. Namely, treatment of the protected aspartyl dipeptides, I, V and VII, (0.5 mmol) with MsOH (100 eq) containing anisole (5 eq) was performed at room temperature for 30 min. The reaction mixture was diluted with dry ether. The resulting product was collected by centrifugation and precipitated from MeOH and dry ether, and followed by neutrallization with 1 N NH_4OH under cooling. One % NH_4HCO_3 (2 ml) was added to the solution and the solution was allowed to stand at 40° for 24 hr. After lyophilization of the reaction mixture, the product was applied on paper chromatography or amino acid analyzer. The results are shown in Table II.

Preparation of α- and β-Aspartyl Dipeptides from the Reaction Mixture of III and IX with MsOH–anisole——III (200 mg) was treated as described above. The product was applied to a column (1.8×16.5 cm) of Dowex 1×2 (acetate form) and eluted with a linear gradient of 0.2 M AcOH (300 ml) in the mixing chamber to 2 M AcOH (300 ml) in the reservoir. Asp-Ser and $\overset{\text{Asp}}{\text{|}}\!\!-\!\!-\text{Ser}$ were eluted in 160 to 220 ml and 310 to 380 ml respectively. Similarly, IX (200 mg) was treated and the product was worked up in a virtually similar manner described above. Asp-Thr and $\overset{\text{Asp}}{\text{|}}\!\!-\!\!-\text{Thr}$ were eluted in 190 to 230 ml and 320 to 380 ml respectively.

Treatment of XI and XII with MsOH–anisole——XI (50 mg) was dissolved in MsOH (0.15 ml) containing anisole (0.1 ml) and allowed to stand at room temperature for 30 min. The reaction mixture was worked up as described above. The resulting product (27 mg) in MeOH–H_2O (1: 1) (15 ml) was hydrogenated over 10% Pd–C for 6 hr. After the removal of the catalyst, the solvent was evaporated to dryness. The product in H_2O (4 ml) was applied to a column (1.8×15 cm) of Dowex 1×2 (acetate form) and eluted with H_2O (150 ml) and 2 M AcOH (150 ml). Fractions positive to ninhydrin reagent were pooled, evaporated and lyophilized to give 16 mg (90% yield); mp 121—130°; $[\alpha]_D^{14}$ +21.5° (c=1.0, H_2O), (lit.[5] mp 119—129°; $[\alpha]_D^{16}$ +23.9° (c=0.6, H_2O)). *Anal.* Calcd. for $C_9H_{12}N_2O_6 \cdot H_2O$: C, 34.89; H, 5.76; N, 11.59. Found: C, 35.11; H, 5.79; N, 11.63.

XII (25 mg) was treated with MsOH (0.08 ml) containing anisole (0.05 ml) and followed by hydrogenation in the same manner described above to give 9 mg (98% yield); mp 142—158° (dec.); $[\alpha]_D^{14}$ +5.0° (c=1.0, H_2O). *Anal.* Calcd. for $C_8H_{14}N_2O_6 \cdot H_2O$: C, 38.09; H, 6.39; N, 11.11. Found: C, 38.21; H, 6.34; N, 10.98.

Boc-Pro-Arg(MBS)-ONb (I)——Z-Arg(MBS)-ONb[10] (500 mg) was treated with 4.6 N HBr in AcOH (1.5 ml) containing anisole (0.15 ml) in the usual manner. The title compound was prepared from the product thus obtained and Boc-Pro (175 mg) as described for the preparation of the protected aspartyl dipeptide; yield 530 mg (96%); mp 70—86°; $[\alpha]_D^{30}$ −18.7° (c=1.1, DMF); *Rf*(A) 0.80, *Rf*(B) 0.90, single spot positive to ninhydrin reagent. *Anal.* Calcd for $C_{30}H_{40}N_6O_{10}S$: C, 53.24; H, 5.96; N, 12.42. Found: C, 53.37; H, 6.04; N, 12.59.

Boc-Asp(OBzl)-Pro-Arg(MBS)-ONb (II)——Boc group in I (490 mg) was deblocked with 2 M TsOH in dioxane[15] and the resulting product was coupled with Boc-Asp(OBzl) (223 mg) in a similar manner described above; yield 428 mg (68%); mp 77—89; $[\alpha]_D^{30}$ −24.0° (c=1.0, DMF); *Rf*(A) 0.76, *Rf*(B) 0.86, single spot positive to ninhydrin reagent. *Anal.* Calcd. for $C_{41}H_{51}N_7O_{13}S$: C, 55.83; H, 5.83; N, 11.12. Found: C, 56.03; H, 6.04; N, 10.97.

Boc-Ser(Bzl)-Asp(OBzl)-Pro-Arg(MBS)-ONb (III)——This compound was prepared from II (441 mg) and Boc-Ser(Bzl) (148 mg) in the same manner described above; yield 430 mg (81%); mp 76—84°; $[\alpha]_D^{30}$ −35.4° (c=1.1, DMF); *Rf*(A) 0.79, *Rf*(B) 0.92, single spot positive to ninhydrin reagent. *Anal.* Calcd. for $C_{51}H_{62}N_8O_{15}S$: C, 57.83; H, 5.94; N, 10.58. Found: C, 57.72; H, 6.01: N, 10.34.

Boc-Asp(ONb)-Ser(Bzl)-Asp(OBzl)-Pro-Arg(MBS)-ONb (IV)——This compound was prepared from III (460 mg) and Boc-Asp(ONb)[5] (160 mg) in the same manner described above; yield 560 mg (98%); mp 82—87°; $[\alpha]_D^{30}$ −26.0° (c=1.0, DMF); *Rf*(A) 0.85, *Rf*(B) 0.89, single spot positive to ninhydrin reagent. *Anal.* Calcd. for $C_{62}H_{72}N_{10}O_{26}S$: C, 56.87; H, 5.54; N, 10.70. Found: C, 57.06; H, 5.53; N, 10.54.

Asp-Ser-Asp-Pro-Arg[5]——IV (200 mg) was dissolved in MsOH (4.1 ml, 100 eq) containing anisole (0.32 ml, 5 eq) and allowed to stand at room temperature for 1 hr. The oily product which was formed by addition of dry ether to the reaction mixture was collected by centrifugation and washed with dry ether. The resulting product was dissolved in H_2O (8 ml), washed with EtOAc and treated with Dowex 1×2 (acetate form, 15 g) for 1 hr. The resin was removed by filtration and the filtrate was condensed in vacuum and lyophilized over KOH pellets; weight 156 mg.

(A): An aliquot (70 mg) of the crude partially protected peptide thus obtained in MeOH–H_2O (1: 1) (15 ml) was hydrogenated over 10% Pd–C for 14 hr. The hydrogenated product was worked up in the same manner described in a previous paper;[5] yield 21 mg (52%); mp 180—193° (dec.); $[\alpha]_D^{21}$ −85.0° (c=1.0, H_2O), (lit.[5] mp 185—200° (dec.); $[\alpha]_D^{10}$ −87.7° (c=0.5, H_2O)); *Rf*(A) 0.04, *Rf*(B) 0.10, single spot positive to ninhydrin and Sakaguchi reagents; amino acid ratios in the acid hydrolysate: Arg 0.86, Asp 2.03, Ser 1.04, Pro

15) K. Suzuki, N. Endo, K. Nitta and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **26**, 2198 (1978).

1.08 (average recovery 92%); amino acid ratios in AP-M digest:[5] Arg 0.93, Asp 1.03, Ser 1.05 (average recovery 90%).

(B): An aliquot (40 mg) in 80% AcOH (2 ml) was treated with zinc powder (65 mg, 20 eq) in three portions at 5 min intervals under cooling and the mixture was stirred at 0° for 1.5 hr. The unchanged zinc powder was filtered off and washed with 80% AcOH (1 ml×4) on filter. After lyophilization of the filtrate, the resulting product was dissolved in H_2O (15 ml), extracted with EtOAc and treated with dithizone-$CHCl_3$ solution to remove zinc ion. The aqueous solution was condensed in vacuum and lyophilized. The product thus obtained was purified by column chromatography as described above; yield 15 mg (65%); mp 188—192° (dec.); $[\alpha]_D^{21}$ −88.0° (c=1.0, H_2O), (lit.[5] mp 185—200° (dec.); $[\alpha]_D^{19}$ −87.7° (c=0.5, H_2O)); *Rf*(A) 0.05, *Rf*(B) 0.11, single spot positive to ninhydrin and Sakaguchi reagents; amino acid ratios in the acid hydrolysate: Arg 0.98, Asp 1.98, Ser 1.06, Pro 1.07 (average recovery 88%); amino acid ratios in AP-M digest:[5] Arg 1.16, Asp 0.86, Ser 1.00 (average recovery 96%).