[Chem. Pharm. Bull. 24(7)1568—1575(1976)]

UDC 547.466.1.04 : 547.541.04

p-Methoxybenzenesulfonyl as a Protecting Group of Guanidino Function in Peptide Synthesis[1]

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(Received November 11, 1975)

The *p*-methoxybenzenesulfonyl (MBS) group was used to protect the ω-guanidino function in arginine and was found to be more easily and completely removed by treatment with methanesulfonic acid and borontristrifluoroacetate (BTFA) than the *p*-toluenesulfonyl group.

In order to evaluate the usefulness of this new protecting group in peptide synthesis, bradykinin and tuftsin were prepared by using the MBS group.

The tosyl (*p*-toluenesulfonyl)-protecting group[3] has been widely used to protect the ω-guanidino group in arginine. It is generally removed by sodium–liquid ammonia reduction[3] or by the action of hydrogen fluoride.[4] However, these removal conditions seem to be somewhat drastic for the complicated biologically active peptides, as some side reactions[5] are known to occur during these treatments.

Although Yajima, *et al.*[6] and Pless and Bauer[7] recently reported some new methods for removing the protecting groups, which are currently used in peptide synthesis, by means of various sulfonic acids, such as methanesulfonic acid and trifluoromethanesulfonic acid, and borontristrifluoroacetate (BTFA), respectively, the tosyl group is hard to remove with these reagents under the practical conditions.

In order to solve this problem, we tried to use the *p*-methoxybenzenesulfonyl (MBS) group for the ω-guanidino in the arginine residue and found that the MBS group was more easily cleaved by the treatment with methanesulfonic acid or BTFA than the tosyl group.

Z-Arg(MBS)-OH was prepared from Z-Arg-OH[8] and *p*-methoxybenzenesulfonyl chloride[9] using a procedure similar to that described in the preparation of Z-Arg(Tos)-OH,[3] and the resulting Z-Arg(MBS)-OH was crystallized from acetonitrile as dicyclohexylamine salt (I).

1) The amino acids, peptides and their derivatives (except glycine) in this paper are of the L-configuration. Their abbreviated designations are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967).

Other abbreviations used are: MBS=*p*-methoxybenzenesulfonyl; BTFA=borontristrifluoroacetate; HONB=N-hydroxy-5-norbornene-2,3-dicarboximide; ONB=N-hydroxy-5-norbornene-2,3-dicarboximide ester; DCC=N,N′-dicyclohexylcarbodiimide; ONBzl=*p*-nitrobenzylester; OTCP=2,4,5-trichlorophenylester; DCHA=dicyclohexylamine.

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Hydrogenation of Z-Arg(MBS)-OH over Pd black afforded H-Arg(MBS)-OH (II), which was easily crystallized from water.

For comparison of removal properties of H–Arg(MBS)–OH and H–Arg(Tos)–OH,[3] both compounds were treated with methanesulfonic acid or BTFA.

As shown in Fig. 1, the MBS group was quantitatively removed by methanesulfonic acid and 8 equivalents of BTFA, while the tosyl group was only partially removed under identical conditions. These results indicate that the MBS group has many more advantageous features than the tosyl group as a protecting group of the ω-guanidino function in arginine.[10]

To confirm the usefulness of the MBS group in peptide synthesis, bradykinin and tuftsin were synthesized. The synthesis of bradykinin is outlined in Fig. 2. The N^{α}-amino group

	Reagent				
	(a) CH_3SO_3H	(b) CH_3SO_3H-CF_3COOH (1:1)	(c) BTFA 6 eq	(c) BTFA 8 eq	(c) BTFA 10 eq
H-Arg(Tos)-OH	24.1%	8.2%	1.3%	3.3%	11.5%
H-Arg(MBS)-OH	99.1%	92.4%	72.1%	84.5%	100%

Fig. 1. Removal of MBS group by CH_3SO_3H and BTFA

H-Arg(MBS)-OH and H-Arg(Tos)-OH were treated with (a) methanesulfonic acid or (b) methanesulfonic acid–trifluoroacetic acid (1:1) in the presence of anisole at room temperature (21°) for 35—40 min and also (c) 6, 8, and 10 equivalents of BTFA in trifluoroacetic acid at 0° for 60 min. The reaction products were subjected to quantitative amino acid analysis to estimate the content of regenerated arginine (%).

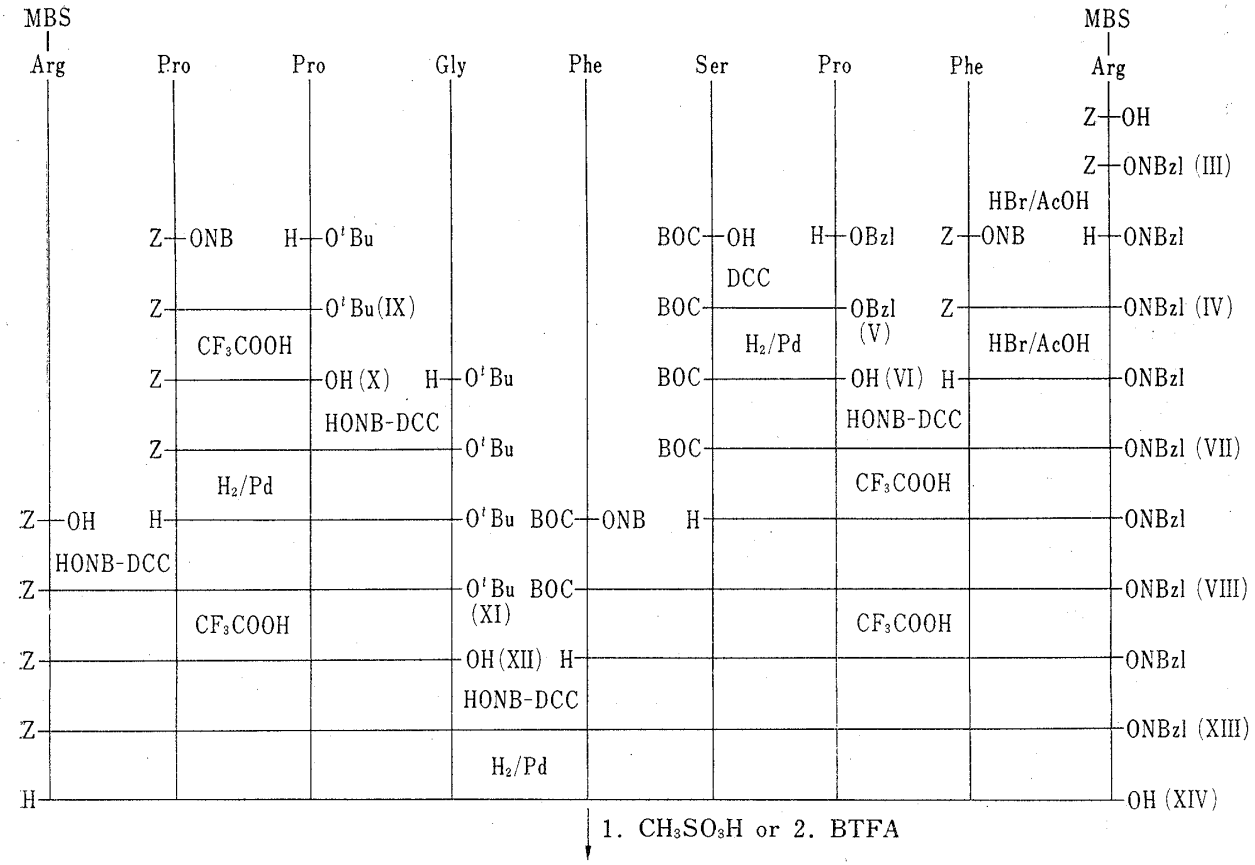

Fig. 2. Synthesis of Bradykinin

10) The MBS group is also completely cleaved by hydrogen fluoride.

and C-terminal carboxylic acid were protected by benzyloxycarbonyl (Z) or *t*-butyloxycarbonyl group (BOC) and *p*-nitrobenzylester (ONBzl), respectively. HONB active ester[11] and HONB–DCC method[11] were extensively used to extend the peptide chain. Z–Arg(MBS)–OH, derived from I, was esterified with *p*-nitrobenzyl bromide in the presence of triethylamine to give Z–Arg(MBS)–ONBzl (III) in analytically pure state. H–Arg(MBS)–ONBzl, which was derived from III by treatment with HBr in acetic acid, was acylated with Z–Phe–ONB to afford Z–Phe–Arg(MBS)–ONBzl (IV) in crystalline form.

For the synthesis of BOC–Ser–Pro–OH (VI), BOC–Ser–OH and H–Pro–OBzl were coupled with DCC in acetonitrile to give BOC–Ser–Pro–OBzl (V) in crystalline form. V was converted into VI by hydrogenation over Pd black as catalyst. VI thus obtained and H–Phe–Arg(MBS)–ONBzl, which was derived from IV by treatment with HBr in acetic acid, were condensed with DCC in the presence of HONB[11] to yield BOC–Ser–Pro–Phe–Arg(MBS)–ONBzl (VII), which was deblocked by treatment with trifluoroacetic acid and then acylated with BOC–Phe–ONB[11] to give BOC–Phe–Ser–Pro–Phe–Arg(MBS)–ONBzl (VIII) in crystalline form.

To prepare Z–Arg(MBS)–Pro–Pro–Gly–O^{t}Bu (XI), H–Pro–O^{t}Bu was allowed to react with Z–Pro–ONB[11] to afford Z–Pro–Pro–O^{t}Bu (IX) in crystalline form. Acid solvolysis of IX with trifluoroacetic acid afforded the corresponding acid, Z–Pro–Pro–OH (X),[12] in good yield. X and H–Gly–O^{t}Bu were coupled with DCC in the presence of HONB[11] to afford Z–Pro–Pro–Gly–O^{t}Bu as an oil. The oil was hydrogenated over Pd black and the resulting H–Pro–Pro–Gly–O^{t}Bu was condensed with Z–Arg(MBS)–OH using the HONB/DCC method[11] to give XI.

Z–Arg(MBS)–Pro–Pro–Gly–OH (XII) and H–Phe–Ser–Pro–Phe–Arg–(MBS)–ONBzl, which were derived from VIII and XI, respectively, by treatment with trifluoroacetic acid, were coupled with DCC plus HONB[11] to yield the fully protected bradykinin, Z–Arg–(MBS)–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg(MBS)–ONBzl (XIII), in analytically pure form. Hydrogenation of XIII over Pd black as catalyst gave H–Arg(MBS)–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg–(MBS)–OH (XIV), which was treated with methanesulfonic acid in the presence of anisole as a scavenger at room temperature (21°) for 40 min or 50 equivalents of BTFA in trifluoroacetic acid at 0° for 60 min to give crude bradykinin.

The resulting methanesulfonate or trifluoroacetate of the products was exchanged for the corresponding acetate by Amberlite IRA-400 or 410 (acetate form) and purified by chromatography on carboxymethylcellulose using gradient elution with ammonium acetate buffer, on Amberlite XAD-2 using gradient elution with aqueous ethanol and on Bio-Gel P-6 using 1N acetic acid as an eluting agent.

Both final products thus obtained behaved exactly like the authentic bradykinin[13] when compared by thin-layer chromatography, paper chromatography, paper electrophoresis and bioassay[14] (guinea pig ileum contraction), and the amino acid and elemental analyses values also agreed well with theoretical values.

In these syntheses of bradykinin, treatment of BTFA seemed to be more difficult to handle due to its instability than that of methanesulfonic acid. Therefore, methanesulfonic acid was judged as more practical for removal of the MBS group.

Tuftsin[15,16] was synthesized as shown in Fig. 3. The N$^{\alpha}$-amino group was protected by the Z or BOC group and the N$^{\varepsilon}$-amino group by the Z group. The C-terminal carboxylic acid

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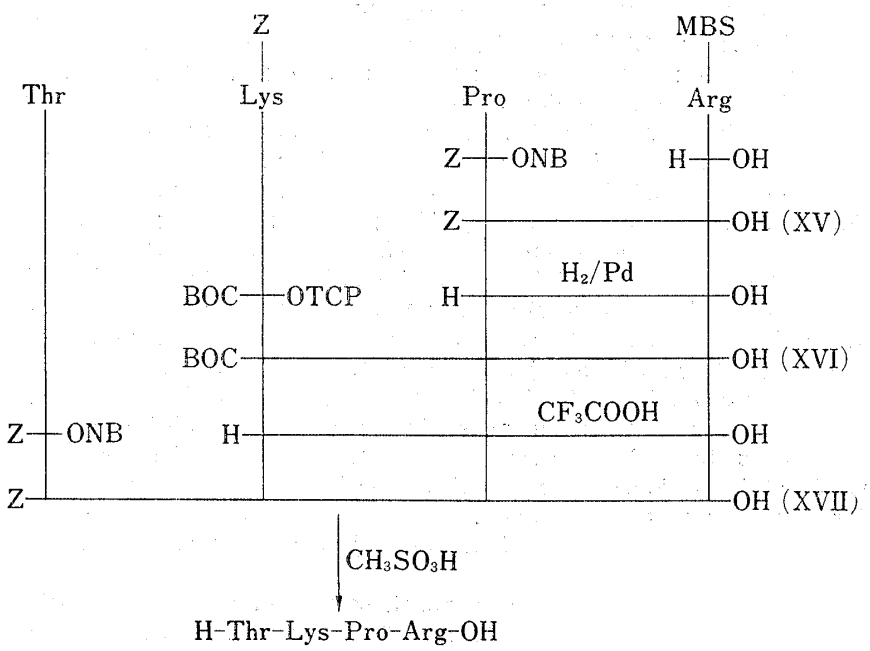

Fig. 3. Synthesis of Tuftsin

was blocked by salt formation. The peptide chain was lengthened stepwise using HONB active ester and 2,4,5-trichlorophenylester and the final protected tuftsin, Z-Thr-Lys(Z)-Pro-Arg(MBS)-OH (XVII), was deblocked using methanesulfonic acid.

The resulting methanesulfonate was exchanged for the corresponding acetate by Amberlite IRA-410 (acetate form) and purified by chromatography on carboxymethylcellulose using gradient elution with ammonium acetate and on Sephadex LH-20 using 0.1N acetic acid as an eluting agent.

The tetrapeptide thus obtained exhibited apparently a single spot on thin-layer chromatography and its optical rotation was the same as the literature value.[16] Amino acid and elemental analyses values also agreed well with theoretical values.[17]

The experimental results presented here indicate that the MBS group is promising as a protector of the ω-guanidino function in arginine during peptide synthesis. Further applications of this method to the synthesis of complicated biologically active peptides are now in progress in these laboratories.

Experimental

All melting points were taken by the capillary method and are uncorrected. The purity of the products was tested by thin-layer chromatography on silica gel or Avicel. Solvent systems used were: $CHCl_3$–MeOH–AcOH (9: 1: 0.5, *Rf* 1), AcOEt–pyridine–AcOH–H_2O (60: 20: 6: 10, *Rf* 2), *n*-BuOH–AcOH–H_2O (4: 1: 1, *Rf* 3), *n*-BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24, *Rf* 4), AcOEt–*n*-BuOH–AcOH–H_2O (1: 1: 1: 1, *Rf* 5).

Z-Arg(MBS)-OH·DCHA Salt (I)——Z-Arg-OH[8] (55.5 g, 0.18 mole) was dissolved in a mixture of 180 ml of 4 N sodium hydroxide and 1300 ml of acetone at room temperature, cooled to 0° and stirred vigorously. To this solution, *p*-methoxybenzenesulfonyl chloride[9] (74.4 g, 0.36 mole) dissolved in 300 ml of acetone was added dropwise at 0° during a period of 30 min. After being stirred for 2 hr at 0° and then for 2 hr at room temperature, the reaction mixture was acidified with citric acid and then evaporated under reduced pressure at 40°. The resulting oily residue was dissolved in AcOEt. The AcOEt layer was washed twice with water, and then extracted three times with 400 ml of 5% sodium bicarbonate solution. This extract was acidified with citric acid and the resulting oily residue was extracted with AcOEt. The AcOEt layer was washed twice with water and dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* to give an oil: yield 86.0 g.

The oily residue was dissolved in 600 ml of AcOEt and to this solution, dicyclohexylamine (36.0 ml, 0.18 mole) was added. The mixture was allowed to stand in a refrigerator overnight. The resulting crystal-

17) The biological activity of this synthetic tuftsin will be reported elsewhere.

line material was collected by filtration and then recrystallized twice from acetonitrile: yield, 77.0 (61.1%); mp 110.0—112.0°; $[\alpha]_D^{23}$ +5.1° (c=1.35 in MeOH); Rf 1=0.18, Rf 2=0.69. *Anal.* Calcd. for $C_{33}H_{49}O_7N_5S \cdot CH_3CN$: C, 59.98; H, 7.48; N, 11.99; S, 4.58. Found: C, 59.97; H, 7.74; N, 11.72; S, 4.59.

H-Arg(MBS)-OH (II)——The crude oily Z-Arg(MBS)-OH (2.0 g) was dissolved in 20 ml of MeOH and hydrogenated over Pd catalyst (0.2 g) for 3 hr. Pd catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. The resulting residue was recrystallized from boiling water: yield, 900 mg; mp 144.0—146.0° (decomp.); $[\alpha]_D^{23}$ −6.1° (c=0.71 in MeOH); Rf 2=0.08, Rf 3=0.25. *Anal.* Calcd. for $C_{13}H_{20}O_5N_4S \cdot 1/2H_2O$: C, 44.18; H, 5.99; N, 15.85; S, 9.07. Found: C, 44.05; H, 5.80; N, 15.75; S, 8.99.

Removal of MBS Group by Methanesulfonic Acid——(a) Compound II (34.4 mg, 0.1 mmole) was treated with 0.5 ml of methanesulfonic acid containing 0.025 ml of anisole at 21° for 40 min. To this reaction mixture, 10 ml of ether was added and the resulting oily precipitate was washed three times with ether by decantation and then subjected to quantitative amino acid analysis. The result is shown in Fig. 1 (a).

(b) Compound II (34.4 mg, 0.1 mmole) was previously dissolved in 0.25 ml of trifluoroacetic acid and then treated with 0.25 ml of methanesulfonic acid containing 0.025 ml of anisole at 21° for 35 min. This reaction mixture was worked up in the manner described above. The result is shown in Fig. 1 (b).

Removal of MBS Group by BTFA——Compound II (34.4 mg, 0.1 mmole) was dissolved in 0.5 ml of trifluoroacetic acid and to this solution, 6, 8, or 10 equivalents of BTFA[7] in trifluoroacetic acid was added under ice-cooling. After being stirred at 0° for 60 min, the reaction mixture was diluted with 10 ml of ice-cooled water and then lyophilized. The resulting residue was subjected to quantitative amino acid analysis. The result is shown in Fig. 1 (c).

Z-Arg(MBS)-ONBzl (III)——In a separatory funnel, compound I (7.0 g, 0.01 mole) was suspended in 200 ml of AcOEt and to this, 60 ml of ice-cooled 0.2 N H_2SO_4 was added. The mixture was shaken vigorously until I dissolved completely. The organic layer was washed twice with water and dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* to give an oily residue. This oily residue was dissolved in 30 ml of DMF and to this, triethylamine (1,68 ml, 0.012 mole) and *p*-nitrobenzyl bromide (2.5 g, 0.012 mole) were added under ice-cooling. The reaction mixture was stirred for 2 hr at 80° and poured into 300 ml of water. The resulting oily residue was extracted with AcOEt. The AcOEt layer was washed with 1 N HCl and 5% sodium bicarbonate and dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo*, then the resulting oily residue was triturated with pet-ether: yield, 6.2 g (100%); mp 90.0—110.0° (decomp.); $[\alpha]_D^{21}$ −3.4° (c=0.59 in DMF); Rf 1=0.60. *Anal.* Calcd. for $C_{28}H_{31}O_9N_5S$: C, 54.80; H, 5.09; N, 11.41; S, 5.28. Found: C, 55.49; H, 5.15; N, 11.15; S, 4.36.

Z-Phe-Arg(MBS)-ONBzl (IV)——Compound III (6.1 g, 0.01 mole) was dissolved in 35 ml of 25% HBr in acetic acid. After 30 min, ether was added to this solution and the resulting precipitate was collected by filtration and washed with ether then dried over NaOH pellets *in vacuo* for 12 hr. The dried powder was dissolved in 30 ml of DMF. This solution was cooled to 0°, then triethylamine (2.8 ml, 0.02 mole) was added. The resulting salt was filtered off. To the filtrate, Z-Phe-ONB[11] (4.6 g, 0.01 mole) was added and the reaction mixture was stirred for 12 hr at room temperature. The reaction mixture was diluted with 300 ml of water and the resulting oily product was extracted with AcOEt. The AcOEt layer was washed with 1 N HCl and 5% $NaHCO_3$ then dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* to give an oil, which was crystallized by addition of pet-ether. Recrystallization from EtOH gave 4.5 g (59.2%) of IV: mp 130.0—132.0°; $[\alpha]_D^{21}$ −6.8° (c=0.66 in DMF); Rf 1=0.62. *Anal.* Calcd. for $C_{32}H_{40}O_{11}N_6S$: C, 58.41; H, 5.30; N, 11.05; S, 4.22. Found: C, 58.52; H, 5.19; N, 10.98; S, 4.36.

BOC-Ser-Pro-OBzl (V)——H-Pro-OBzl·HCl (8.5 g, 0.035 mole) was dissolved in 100 ml of acetonitrile. To this solution, triethylamine (4.9 ml, 0.035 mole) and BOC-Ser-OH (6.2 g, 0.03 mole) were added under ice cooling. Next, DCC (6.5 g, 0.0325 mole) was added and the reaction mixture was stirred for 48 hr at 4°. The insoluble materials formed were filtered off and the filtrate was evaporated *in vacuo*. The residue was dissolved in 200 ml of AcOEt and washed with 1 N HCl and 5% $NaHCO_3$. The solvent was evaporated *in vacuo*. The resulting oily product was crystallized by addition of hexane and recrystallized twice from EtOH: yield, 8.6 g (72.0%); mp 65.0—67.0°; $[\alpha]_D^{21}$ −55.0° (c=0.57 in DMF); Rf 1=0.83. *Anal.* Calcd. for $C_{20}H_{28}O_6N_2 \cdot 1/2H_2O$: C, 59.84; H, 7.28; N, 6.98. Found: C, 59.95; H, 7.38; N, 6.59.

BOC-Ser-Pro-OH (VI)——Compound V (2.0 g, 0.005 mole) was dissolved in 50 ml of MeOH and hydrogenated over Pd black (0.2 g) for 3 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. The resulting residue was crystallized by adding hexane and recrystallized from AcOEt–pet-ether: yield, 1.5 g (100%); mp 147.0—148.0° (decomp.); $[\alpha]_D^{22}$ −52.6° (c=0.56 in DMF); Rf 1=0.20. *Anal.* Calcd. for $C_{13}H_{22}O_6N_2$: C, 51.64; H, 7.34; N, 9.27. Found: C, 51.42; H, 7.30; N, 9.27.

BOC-Ser-Pro-Phe-Arg(MBS)-ONBzl (VII)——Compound IV (3.8 g, 0.005 mole) was dissolved in 20 ml of 25% HBr in acetic acid at room temperature. After 40 min, ether was added to this solution. The resulting precipitate was collected by filtration and dried over NaOH pellets *in vacuo* for 12 hr. The dried powder was dissolved in 20 ml of DMF and to this solution, triethylamine (1.4 ml, 0.001 mole) was added under ice-cooling. The resulting salt was filtered off and to the filtrate, VI (1.5 g, 0.005 mole) and HONB[11] (900 mg, 0.005 mole) were added. The mixture was cooled to 0°, then DCC (1.03 g, 0.005 mole) was added. The reaction mixture was stirred for 48 hr at 4° and then for 24 hr at room temperature. The solvent was evaporated *in vacuo* to dryness and the residue was dissolved in 100 ml of AcOEt. The AcOEt layer was

washed with 0.2 N HCl and 5% $NaHCO_3$, then dried over anhyd. Na_2SO_4. AcOEt was evaporated *in vacuo* and the resulting oily product crystallized upon adding pet-ether: yield, 3.8 g (83.4%); mp 155.0—160.0° (decomp.); $[\alpha]_D^{21}$ −26.0° (c=0.52 in DMF); *Rf* 1=0.61. *Anal.* Calcd. for $C_{42}H_{54}O_{13}N_8S \cdot 1/2H_2O$: C, 54.83; H, 6.02; N, 12.18; S, 3.49. Found: C, 54.81; H, 6.00; N, 11.87; S, 3.53.

BOC-Phe-Ser-Pro-Phe-Arg(MBS)-ONBzl (VIII)——Compound VII (3.64 g, 0.004 mole) was dissolved in 30 ml of trifluoroacetic acid at room temperature. After 30 min, the excess of trifluoroacetic acid was evaporated off. The residue was triturated with ether, collected by filtration and then dried over NaOH pellets *in vacuo* for 12 hr. The dried powder was dissolved in 20 ml of DMF and to this solution, triethylamine (0.56 ml, 0.004 mole) and BOC-Phe-ONB[11] (1.72 g, 0.004 mole) were added. The reaction mixture was stirred for 6 hr and the solvent was evaporated *in vacuo* to dryness. The resulting residue was dissolved in 100 ml of AcOEt. The AcOEt layer was washed with 0.2 N HCl and 5% $NaHCO_3$ and dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* to give a crystalline residue, which was recrystallized from AcOEt: yield, 4.0 g (94.6%); mp 165.0—170.0°; $[\alpha]_D^{21}$ −22.2° (c=0.59 in DMF); *Rf* 1=0.65. *Anal.* Calcd. for $C_{51}H_{63}O_{14}N_8S \cdot H_2O$: C, 56.92; H, 6.09; N, 11.71; S, 2.92. Found: C, 56.58; H, 5.76; N, 11.66; S, 3.17.

Z-Pro-Pro-O^{t}Bu (IX)——Z-Pro-O^{t}Bu (24.4 g, 0.08 mole) was dissolved in 250 ml of methanol and hydrogenated over Pd black (3.0 g) for 5 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in 100 ml of DMF and to this solution, Z-Pro-ONB[11] (32.8 g, 0.08 mole) was added. After being stirred at 80° for 8 hr, the reaction mixture was allowed to stand for 12 hr at room temperature and then was poured into 1000 ml of water. The resulting oily product was extracted with AcOEt. The AcOEt layer was washed with 0.2 N HCl and 5% $NaHCO_3$, then dried over anhyd. Na_2SO_4. The solvent was removed *in vacuo* and the resulting residue was crystallized by addition of pet-ether: yield, 24.8 g (60.0%); mp 84.0—85.0°; $[\alpha]_D^{21}$ −79.2° (c=0.52 in DMF), *Rf* 1=0.85. *Anal.* Calcd. for $C_{22}H_{30}O_5N_2$: C, 65.65; H, 7.51; N, 6.96. Found: C, 65.87; H, 7.54; N, 7.04.

Z-Pro-Pro-OH (X)——Compound IX (4.1 g. 0.0106 mole) was dissolved in 25 ml of trifluoroacetic acid at room temperature. After 30 min, the excess of trifluoroacetic acid was evaporated off and the resulting residue was dissolved in 100 ml of AcOEt. The AcOEt layer was washed with water, then dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was crystallized by adding pet-ether. Recrystallization from AcOEt gave 2.2 g (60.0%) of X: mp 189.0—190.0° (lit.[12] mp 187.0—190.0°); $[\alpha]_D^{21}$ −79.8° (c=0.56 in DMF), *Rf* 1=0.40. *Anal.* Calcd. for $C_{15}H_{22}O_5N_2$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.32; H, 6.51; N, 8.01.

Z-Arg(MBS)-Pro-Pro-Gly-O^{t}Bu (XI)——Compound X (4.45 g, 0.0129 mole) and HONB[11] (2.31 g, 0.0129 mole) were dissolved in 50 ml of dioxane, then DCC (2.6 g, 0.0129 mole) was added under ice-cooling After 4 hr, the urea formed was filtered off and the filtrate was combined with H-Gly-O^{t}Bu (3.28 g, 0.025 mole). The reaction mixture was stirred for 48 hr at room temperature, then the solvent was removed *in vacuo* to dryness. The resulting oil was dissolved in 100 ml of AcOEt. The AcOEt layer was washed with 0.2 N HCl and 5% $NaHCO_3$, then dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* and the resulting oil was washed three times with pet-ether by decantation: yield, 4.9 g (82.2%), *Rf* 1=0.80. This oily product, Z-Pro-Pro-Gly-O^{t}Bu (4.9 g, 0.0106 mole), was dissolved in 70 ml of MeOH and hydrogenated over Pd black (0.5 g) for 24 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. The resulting residue, Z-Arg(MBS)-OH [prepared from I (7.42 g, 0.0106 mole)] and HONB[11] (2.0 g, 0.0111 mole) were dissolved in a mixture of 10 ml of tetrahydrofuran and 90 ml of dioxane. To this solution, DCC (2.4 g, 0.0117 mole) was added under ice-cooling. The reaction mixture was stirred for 48 hr at 4° and then the urea formed was filtered off and the filtrate was evaporated *in vacuo* to dryness. The resulting oily product was dissolved in 100 ml of AcOEt. The AcOEt layer was washed with 0.2 N HCl and 5% $NaHCO_3$, then dried over anhyd. Na_2SO_4. The solvent was removed *in vacuo* and the residue was triturated with ether. This product was purified by reprecipitation from AcOEt-ether: yield, 5.5 g (66.2%); mp 120.0—130.0° (decomp.); $[\alpha]_D^{21}$ −47.7° (c=0.53 in DMF), *Rf* 1=0.70. *Anal.* Calcd. for $C_{37}H_{51}O_{10}N_7S \cdot 1/2H_2O$: C, 55.90; H, 6.59; N, 12.33; S, 4.03. Found: C, 55.89; H, 6.62; N, 12.31; S, 4.05.

Z-Arg(MBS)-Pro-Pro-Gly-OH (XII)——Compound XI (4.72 g, 0.006 mole) was dissolved in 30 ml of trifluoroacetic acid and the solution was allowed to stand for 30 min at room temperature. The excess of trifluoroacetic acid was removed *in vacuo* and the residue was dissolved in 150 ml of AcOEt. The AcOEt layer was washed three times with water and dried over anhyd. Na_2SO_4. AcOEt was evaporated *in vacuo* and the resulting oily residue was triturated with ether. The product was purified by reprecipitation from hot AcOEt: yield, 3.5 g (85.4%); mp 80.0—90.0° (decomp.); $[\alpha]_D^{21}$ −46.4° (c=0.56 in DMF), *Rf* 1=0.44. *Anal.* Calcd. for $C_{33}H_{43}O_{10}N_7S \cdot H_2O$: C, 53.00; H, 6.06; N, 13.11; S, 4.29. Found: C, 53.13; H, 6.03; N, 12.60; S, 4.26.

Z-Arg(MBS)-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg(MBS)-ONBzl (XIII)——Compound VIII (1.06 g, 0.001 mole) was dissolved in 12 ml of cold trifluoroacetic acid and the solution was allowed to stand for 30 min at room temperature. To this solution, 0.17 ml of 5.85 N HCl in dioxane was added, then the excess of trifluoroacetic acid was removed *in vacuo*. The residue was triturated with ether and dried over NaOH pellets *in vacuo* for 12 hr. The dried powder was dissolved in 20 ml of DMF, then 1.28 ml of 10% N-ethylmorpholine in DMF was added under ice-cooling, followed by XII (730 mg, 0.001 mole) and HONB[11] (270 mg, 0.0015 mole). To this mixture, DCC (247 mg, 0.0012 mole) was added and the reaction mixture was stirred for 72

hr at 4°, then for 24 hr at room temperature. The urea formed was filtered off and the filtrate was evaporated *in vacuo* to dryness. The residue was triturated with ether. The product was purified by reprecipitation from EtOH: yield, 1.4 g (84.0%), mp 120.0—125.0° (decomp.); $[\alpha]_D^{22}$ −37.1° (c=0.56 in DMF), *Rf* 1=0.60. *Anal.* Calcd. for $C_{79}H_{96}O_{21}N_{16}S \cdot H_2O$: C, 55.62; H, 5.91; N, 13.14; S, 3.76. Found: C, 55.62; H, 5.87; N, 12.97; S, 3.87. Amino acid *Anal.* (6 N HCl. 110°, 24 hr): Arg, 1.94 (2); Ser, 0.96 (1); Pro, 3.08 (3); Gly, 0.96 (1); Phe, 1.98 (2). Average recovery 98.0%.

H-Arg(MBS)-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg(MBS)-OH (XIV)——Compound XIII (4.0 g, 0.0024 mole) was dissolved in 100 ml of 80% aqueous acetic acid and hydrogenated over Pd black (0.5 g) for 8 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. The resulting oily residue was triturated with acetone. The product was collected by filtration and washed with hot acetone: yield, 3.4 g (100%), mp 170.0—180.0° (decomp.); $[\alpha]_D^{24}$ −51.3° (c=0.50 in acetic acid), *Rf* 2=0.06, *Rf* 3=0.21. *Anal.* Calcd. for $C_{64}H_{85}O_{17}N_{15}S_2 \cdot H_2O$: C, 54.19; H, 6.18; N, 14.81; S, 4.52. Found: C, 54.16; H, 6.35; N, 14.76; S, 4.58. Amino acid *Anal.* (6 N HCl, 110°, 24 hr): Arg, 2.07 (2); Ser, 0.93 (1); Pro, 2.90 (3); Gly, 1.00 (1); Phe, 1.95 (2). Average recovery 100%.

Preparation of H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH (bradykinin) with Methanesulfonic Acid——Compound XIV (280 mg, 0.2 mmole) was treated with 3.0 ml of methanesulfonic acid containing 0.12 ml of anisole at 21° for 40 min. To this reaction mixture, 100 ml of ether was added, then the resulting oily precipitate was washed with ether by decantation. The residue was dissolved in 10 ml of water, then passed through a column of Amberlite IRA-410 (acetate form; 2.5×10 cm). The eluate (100 ml) was then subjected to chromatography on carboxymethylcellulose column (2.5×12 cm). The column was washed with 100 ml of 0.01 M ammonium acetate (pH 6.9), then eluted by linear gradient method using ammonium acetate (0.01 M/0.3 M=500 ml/500 ml). The desired fractions (200—270 ml) were collected and then lyophilized: 178 mg. This material was purified by rechromatography on a column of carboxymethylcellulose in the same manner, then passed through a column of Bio-Gel P-6 (5×35 cm) using 1 N acetic acid as eluting agent. The desired fractions were collected and lyophilized: yield, 170 mg; $[\alpha]_D^{24}$ −80.8° (c=0.49 in H_2O), *Rf* 3=0.06, *Rf* 4=0.72 (Avicel), *Rf* 5=0.11. *Anal.* Calcd. for $C_{50}H_{73}O_{11}N_{15} \cdot 3CH_3COOH \cdot 4H_2O$: C, 51.25; H, 7.14; N, 16.01. Found: C, 51.02; H, 6.62; N, 16.94. Amino acid *Anal.* (6 N HCl, 110°, 24 hr): Arg, 2.03 (2); Ser, 0.91 (1); Pro, 3.00 (3); Gly, 1.03 (1); Phe, 1.98 (2). Average recovery 88.3%. Paper electrophoresis: −4.2 cm (pH 1.9, 500 V, 60 hr).

Preparation of H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH (bradykinin) with BTFA——Compound XIV (280 mg, 0.2 mmole) was treated with 50 equivalents of BTFA in 30 ml of trifluoroacetic acid at 0° for 1 hr. The excess of trifluoroacetic acid was evaporated *in vacuo*, then the residue was dissolved in 100 ml of water. The insoluble material was filtered off and the filtrate was lyophilized. The lyophilized material was dissolved in 20 ml of water, then passed through a column of Amberlite IRA-400 (acetate form; 2.5 cm×10.0 cm). Next the eluate (100 ml) was subjected to chromatography on a carboxymethylcellulose column (3.0 cm×10.0 cm). The column was washed with 400 ml of 0.01 M ammonium acetate, then eluted by exponential gradient method using ammonium acetate (0.01 M/0.3 M=700 ml/700 ml). The desired fractions (200—400 ml) were collected, then lyophilized: 190 mg. This product was dissolved in 10 ml of 0.01 M ammonium acetate, then applied to a column of Amberlite XAD-2 (2.5 cm×10.0 cm) and washed with 100 ml of 0.01 mole ammonium acetate. The desired material was eluted by exponential gradient method (0.01 M ammonium acetate/50% aqueous EtOH containing 0.5% acetic acid=500 ml/500 ml) and lyophilized. The lyophilized material was dissolved in 5 ml of 1 N acetic acid and passed through a column of Bio-Gel P-6 (5.0 cm×35.0 cm) using 1 N acetic acid as an eluant. The desired fractions were collected and lyophilized; yield, 153 mg; $[\alpha]_D^{21}$ −80.9° (c=0.43 in H_2O), *Rf* 3–0.06, *Rf* 4=0.72 (Avicel), *Rf* 5=0.11. *Anal.* Calcd. for $C_{59}H_{73}O_{11}N_{15} \cdot 3CH_3COOH \cdot 4H_2O$: C, 51.25; H, 7.14; N, 16.01. Found: C, 51.02; H, 7.14; N, 16.47. Amino acid *Anal.* (6 N HCl, 110°, 24 hr): Arg, 2.02 (2); Ser, 0.96 (1); Pro, 2.93 (3); Gly, 1.02 (1); Phe, 1.98 (2). Average recivery 87.0%.

Z-Pro-Arg(MBS)-OH (XV)——Compound II (2.76 g, 8 mmoles) was dissolved in 15 ml of DMF and to this solution were added triethylamine (1.12 ml, 8 mmoles) and Z-Pro-ONB[11] (3.28 g, 8 mmoles). The reaction mixture was stirred for 6 hr at room temperature, then 1.5 ml of glacial acetic acid was added. After evaporation of the solvent, the residue was dissolved in AcOEt. AcOEt layer was washed three times with water, then evaporated. The residue was triturated with ether and reprecipitated from chloroform–ether: yield, 3.9 g (85%); mp 80—85.0° (decomp.); $[\alpha]_D^{24}$ −16.3° (c=0.58 in DMF); *Rf* 1=0.20. *Anal.* Calcd. for $C_{26}H_{33}O_8N_5S \cdot 1/2H_2O$: C, 53.41; H, 5.86; N, 12.00; S, 5.44. Found: C, 53.37; H, 5.78; N, 11.66; S, 5.20.

BOC-Lys(Z)-Pro-Arg(MBS)-OH (XVI)——Compound XV (3.46 g, 6 mmoles) was dissolved in 100 ml of a mixture of MeOH–water–acetic acid (6 : 3 : 1) and hydrogenated over Pd black (0.3 g) for 8 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. The residue was dissolved in 10 ml of DMF together with triethylamine (0.84 ml, 6 mmoles) and BOC-Lys(Z)-OTCP (3.36 g, 6 mmoles). The reaction mixture was stirred for 12 hr at room temperature, then 2 ml of glacial acetic acid was added. After evaporation of the solvent, the residue was dissolved in AcOEt. The AcOEt layer was washed three times with water, then evaporated. The residue was triturated with ether and reprecipitated from chloroform–ether: yield, 3.4 g (70.9%); mp 103—109.0° (decomp.); $[\alpha]_D^{24}$ −19.4° (c=0.70 in DMF); *Rf* 1=0.25. *Anal.* Calcd. for $C_{37}H_{53}O_{11}N_7S \cdot 1/2H_2O$: C, 54.67; H, 6.69; N, 12.06; S, 3.94. Found: C, 54.72; H, 6.70; N, 11.78; S, 3.80.

Z-Thr-Lys(Z)-Pro-Arg(MBS)-OH (XVII)——Compound XVI (1.85 g, 2.3 mmoles) was treated with trifluoroacetic acid (10 ml) for 20 min at 10°. After evaporation of the acid, the residue was triturated with ether, collected by filtration, then dried over NaOH pellets. The dried powder was dissolved in 10 ml of DMF together with triethylamine (0.64 ml, 2.3 mmoles) and Z-Thr-ONB[11] (952 mg, 2.3 mmoles). The reaction mixture was stirred for 12 hr at room temperature, then 2 ml of glacial acetic acid was added. After evaporation of the solvent, the residue was triturated with ether, then reprecipitated from AcOEt–ether: yield, 2.1 g (95.5%); mp 90.0—95.0° (decomp.); $[\alpha]_D^{24}$ −15.3° (c=0.62 in DMF); *Rf* 1=0.15. *Anal.* Calcd. for $C_{44}H_{58}O_{14}N_8S$: C, 56.28; H, 6.23; N, 11.93; S, 3.42. Found: C, 55.99; H, 6.22; N, 11.56; S, 3.36.

Preparation of H-Thr-Lys-Pro-Arg-OH (tuftsin) with Methanesulfonic Acid——Compound XVII (1.88 g, 2 mmoles) was treated with methanesulfonic acid (17 ml) in the presence of anisole (1.0 ml) at 21° for 45 min, then ether was added. The resulting oily precipitate was washed with ether by decantation. This was then dissolved in water (10 ml) and passed through a column of Amberlite IRA-410 (acetate form; 2.5×10.0 cm). The effluent (200 ml) was pooled, then applied to a column of carboxymethylcellulose (2.5×15.0 cm), which was washed with water (500 ml), then eluted by linear gradient method using ammonium acetate buffer (water/0.2 M=500 ml/500 ml). The desired fractions (located by Ninhydrin and Sakaguchi tests; 250—310 ml) were pooled and lyophilized. The lyophilized material was dissolved in a small amount of 0.1 N acetic acid, then passed through a column of Sephadex LH-20 (3.0×130.0 cm) using 0.1 N acetic acid as an eluting agent. The desired fractions were pooled and lyophilized to constant weight: yield, 840 mg; $[\alpha]_D^{23}$ −60.2° (c=0.55 in 5% acetic acid) [lit.[16] $[\alpha]_D^{22}$ −60.8° (c=0.60 in 5% acetic acid)]; *Rf* 3=0.04, *Rf* 4=0.20, *Rf* 5=0.14. Amino acid *Anal.* (6 N HCl, 110°, 24 hr): Lys, 1.01 (1); Arg, 1.00 (1); Thr, 0.99 (1); Pro, 1.06 (1). Average recovery 100%. *Anal.* Calcd. for $C_{21}H_{40}O_6N_8 \cdot 2CH_3COOH \cdot 1.5H_2O$: C, 46.35; H, 7.93; N, 17.30. Found: C, 46.06; H, 7.89; N, 17.28.

Acknowledgement We wish to express our deep appreciation to Prof. H. Yajima of Kyoto University for his helpful advice. We are also grateful to Drs. E. Ohmura and K. Morita of this division for their encouragement throughout this work.