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New Method for Removing the S-*p*-Methoxybenzyl and S-*t*-Butyl Groups of Cysteine Residues with Mercuric Trifluoroacetate[1,2]

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The S-*p*-methoxybenzyl, S-*t*-butyl and S-1-adamantyl groups of cysteine were smoothly cleaved with mercuric acetate in trifluoroacetic acid or mercuric trifluoroacetate in aqueous acetic acid. The treatment of the formed mercaptides with thiols regenerated cysteine derivatives.

This new method was applied to the synthesis of biologically active polypeptides oxytocin and somatostatin.

Keywords——mercuric trifluoroacetate; S-*p*-methoxybenzyl; S-*t*-butyl; S-1-adamantyl; oxytocin; somatostatin; *p*-nitrobenzyloxycarbonyl

Several methods[4] are available for removing the sulfur-protecting group of cysteine. However, the search for a better method has remained a major pursuit because the unambiguous synthesis of cysteine and cystine-containing peptides requires the use of a variety of removal methods.

Heavy metal salts[5a–d)] like silver and mercury have been widely used to remove S-trityl, S-acetoamidomethyl, S-ethylcarbamoyl and benzylthiomethyl groups. For selective removal, this method is of great value because the reaction conditions are mild and do not affect the other groups protecting amino and/or carboxylic acid.

On the other hand, the S–MBzl group,[6] which is one of the most popular sulfur-protecting groups, is generally removed with strong acids like anhydrous hydrogen fluoride[7] or trifluoromethanesulfonic acid.[8] However, treatment with strong acids makes its selective removal a difficult task. To the best of our knowledge, selective removal of the S–MBzl group using heavy metal salts has not been achieved.

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1) A preliminary communication of this paper by M. Fujino and O. Nishimura appeared in *J. C. S., Chem. Commun.*, **1976**, **998.**

2) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. The abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl; Z(NO_2)=*p*-nitrobenzyloxycarbonyl; Boc=*t*-butoxycarbonyl; MBzl=*p*-methoxybenzyl; Bu^t=*t*-butyl; Bzl=benzyl; Ad=1-adamantyl; HONB and ONB=N-hydroxy-5-norbornene-2,3-dicarboximide and its ester; DMF=dimethylformamide; TFA=trifluoroacetic acid; DCC=dicyclohexylcarbodiimide; DCHA=dicyclohexylamine; Et_3N=triethylamine; *p*-TsOH=*p*-toluene sulfonic acid.

3) Location: *Juso-Honmachi, Yodogawa-ku, Osaka, 532, Japan*; *a*) To whom correspondence should be addressed.

4) See for example: E. Schröder and K. Lübke, "The Peptides," Vol. 1, Academic Press, N.Y., 1965, p. 226; M. Bodanszky, Y. Klausner, and M.A. Ondetti, "Peptide Synthesis," Interscience Pub., N.Y., 1976, p. 60.

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The S-Bu^t group[9] is too stable under practical conditions in peptide synthesis. A few attempts have been made to remove it with $HgCl_2$ by Beyermann, *et al.*[10] and Inoue,[11] but detailed accounts were not presented.

In the course of our investigation on the synthesis of sulfur-containing peptides, we observed that S-MBzl, S-Bu^t and S-Ad groups cleanly split off with an equimolar amount of $(CH_3COO)_2Hg$ in cold TFA within a short period of time.

In the present report, we describe the details of our studies on this new removal method for S-MBzl, S-Bu^t and S-Ad groups of cysteine and its application to peptide synthesis.

An S-protected cysteine derivative [H-Cys(MBzl)-OH,[6] H-Cys(Bu^t)-OH,[9] H-Cys(Ad)-OH or H-Cys(Bzl)-OH[12]] was dissolved in cold TFA containing anisole as a scavenger and allowed to react with an equimolar amount of $(CH_3COO)_2Hg$ for 15 min at 0°. The reaction mixture, after precipitation of the mercuric ion with hydrogen sulfide, was subjected to quantitative amino acid analysis. The results (Table I) show that S-MBzl, S-Bu^t and S-Ad groups were completely cleaved and cysteine was regenerated in quantitative yield, whereas the S-Bzl group was quite stable under these conditions. Other heavy metal ions such as Ag^+, Cu^{2+}, Zn^{2+}, Ni^{2+} and Pb^{2+} (as acetate) were also tested for the same purpose, but none were effective.

TABLE I. Removal of S-Protecting Groups with $(CH_3COO)_2Hg$ in TFA (0°, 15 min) or $(CF_3COO)_2Hg$ in 80% Aqueous Acetic Acid (20°, 60 min)

S-Protected cysteine	Solvent	Regenerated cysteine (%)$^{a)}$	Solvent	Regenerated cysteine (%)$^{a)}$
H-Cys(MBzl)-OH	TFA	100.8	80% aqueous acetic acid	94.0
H-Cys(Bu^t)-OH	TFA	99.8	80% aqueous acetic acid	92.0
H-Cys(Ad)-OH	TFA	102.0	—	—
H-Cys(Bzl)-OH	TFA	0	—	—

a) Amino acid anal.

$$\text{H-Cys(MBzl)-OH} \xrightarrow[\text{in TFA}]{(CH_3COO)_2Hg} \text{H-Cys[Hg(OCOCF}_3\text{)]-OH} \xrightarrow{H_2S} \text{H-Cys-OH (cysteine)}$$

Fig. 1. Reaction Pathway

Furthermore, since the actual mercuric reagent in the reaction mentioned above (Fig. 1) was judged as $(CF_3COO)_2Hg$,[13] this was also tested as a deblocking agent in 80% aqueous acetic acid. H-Cys(MBzl)-OH or H-Cys(Bu^t)-OH was dissolved in 80% aqueous acetic acid and treated with 1.2 equivalents of $(CF_3COO)_2Hg$ for 1 hr at room temperature. The reaction mixture was worked up in the same manner described above. The results (Table I) show that S-MBzl and S-Bu^t groups were removed nearly completely and cysteine was regenerated in almost quantitative yield. These results suggest that the deblocking of S-MBzl and S-Bu^t groups by $(CF_3COO)_2Hg$ in 80% aqueous acetic acid is as effective as that of $(CH_3COO)_2Hg$ in TFA, although the cleavage reaction proceeds at a somewhat slow speed.

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Our next object was to establish the applicability of this method to the actual peptide synthesis. Z-Gly-Cys(MBzl)-Gly-OMe (I) and Z-Gly-Cys(Bu^t)-Gly-OMe (II) were prepared as model peptides by the HONB-activated ester method[14)] starting from H-Gly-OMe in a stepwise manner as shown in Fig. 2. First, compound I was treated under the conditions used in the above-mentioned deblocking procedure to give crystalline Z-Gly-Cys-Gly-OMe (III) in almost quantitative yield. Compound II also gave III, but the yield was somewhat low (Table II). These experimental results indicate that this cleavage method of the S-MBzl group or the S-Bu^t group, especially the S-MBzl group, can be applied to the synthesis of biologically active polypeptides since the reaction is very selective and proceeds rapidly under mild conditions.

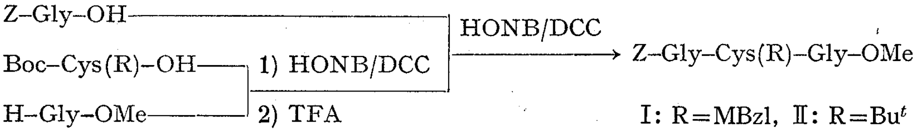

Fig. 2. Synthesis of Z–Gly–Cys(MBzl)–Gly–OMe and Z–Gly–Cys(Bu^t)–Gly–OMe

Table II. Treatment of Z-Gly-Cys(MBzl)-Gly-OMe and Z-Gly-Cys(Bu^t)-Gly-OMe with $(CH_3COO)_2Hg$ in TFA (0°, 15 min) or $(CF_3COO)_2Hg$ in 80% Aqueous Acetic Acid (20°, 60 min)

Starting material	Solvent	Reagent (equiv.)	Yield (%) of Z-Gly-Cys-Gly-OMe
Z-Gly-Cys(MBzl)-Gly-OMe	TFA	1.0	98.1
	80% aqueous acetic acid	1.2	83.9
		2.0	76.5
Z-Gly-Cys(Bu^t)-Gly-OMe	TFA	1.0	71.1
	80% aqueous acetic acid	1.2	Not isolated
		2.0	28.2

Next, as a practical application of this method, oxytocin[15)] was synthesized by the solid phase method[16)] as the first example. Fig. 3 illustrates the synthetic route to oxytocin.

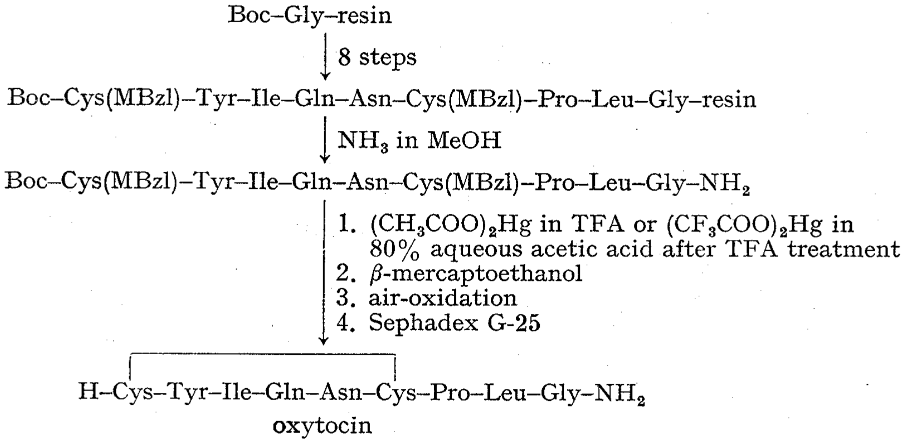

Fig. 3. Synthesis of Oxytocin

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The protected nonapeptide amide Boc-Cys(MBzl)-Tyr-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-NH_2 (IV) was prepared according to the procedure of Hagenmaier and Frank.17) The schedule used for the synthesis is described in the Experimental Section. Compound IV was dissolved in TFA containing anisole and allowed to react with four equivalents of $(CH_3COO)_2$-Hg for 30 min at room temperature. The sulfhydryl groups were liberated from the ensuing mercaptide by treatment with a large amount of β-mercaptoethanol5b) and then air-oxidized (pH 7.3) to form the disulfide bridge. The resulting crude oxytocin was purified by gel filtration on Sephadex G-25 using 50% aqueous acetic acid as the eluting agent. The final product exhibited a single spot on thin-layer chromatography in two solvent systems and the rotation figure was very close to that in literature.18) Alternately, IV was deblocked by TFA giving the nonapeptide amide H-Cys(MBzl)-Tyr-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-NH_2 (V), which was treated with four equimolar amounts of $(CF_3COO)_2Hg$ in 80% aqueous acetic acid for 2 hr at room temperature. The resulting mercaptide was also worked up in the manner stated above to give oxytocin.

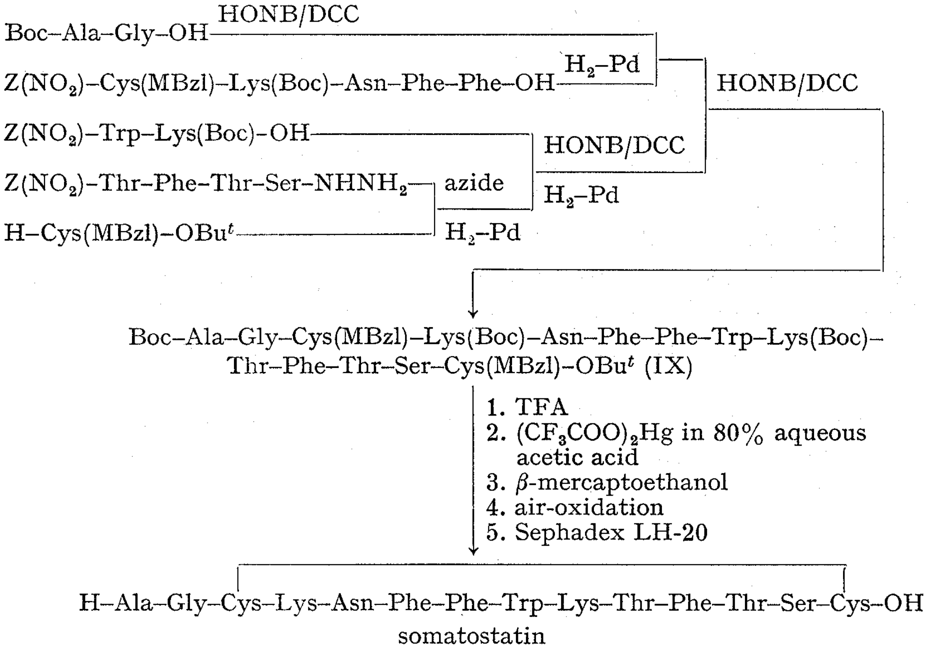

Fig. 4. Synthesis of Somatostatin

As the second example, somatostatin19) was synthesized by the solution method. The synthetic outline is given in Fig. 4. The ε-amino group of Lys and the N-terminal amino group were protected by the Boc group and the C-terminal carboxylic acid was blocked by the Bu^t ester. The $Z(NO_2)$ group,20) removable by catalytic hydrogenation even in the presence of S-MBzl Cys residue, served as a temporal protecting group for the α-amino function of necessary intermediates. The peptide chain was lengthened using the HONB-activated ester method,14) the HONB/DCC method21) and the azide process.

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To prepare the C-terminal heptapeptide ester H-Trp-Lys(Boc)-Thr-Phe-Thr-Ser-Cys-(MBzl)-OBu^t (VI), H-Cys(MBzl)-OBu^t was coupled with Z(NO_2)-Thr-Phe-Thr-Ser-$NHNH_2$ by the azide procedure of Honzl and Rudinger.[22] The resulting protected pentapeptide ester Z(NO_2)-Thr-Phe-Thr-Ser-Cys(MBzl)-OBu^t was submitted to catalytic hydrogenation over Pd-black catalyst in 80% aqueous acetic acid at 50°. Lyophilization and reprecipitation from ethanol–ether or methanol–ether effectively removed the degradation products arising from the Z(NO_2) radical during hydrogenation. The pentapeptide ester H-Thr-Phe-Thr-Ser-Cys-(MBzl)-OBu^t thus obtained was condensed with Z(NO_2)-Trp-Lys(Boc)-OH by the HONB/DCC method[21] giving the protected heptapeptide ester Z(NO_2)-Trp-Lys(Boc)-Thr-Phe-Thr-Ser-Cys(MBzl)-OBu^t (VII). Compound VII was hydrogenated as stated above to yield VI. The N-terminal protected heptapeptide Boc-Ala-Gly-Cys(MBzl)-Lys(Boc)-Asn-Phe-Phe-OH (VIII) was obtained from a coupling reaction between Boc-Ala-Gly-ONB and H-Cys(MBzl)-Lys(Boc)-Asn-Phe-Phe-OH, which was derived from Z(NO_2)-Cys(MBzl)-Lys(Boc)-Asn-Phe-Phe-OH by catalytic hydrogenation. The final coupling reaction between VI and VIII was performed by the HONB/DCC method[21] giving the protected tetradecapeptide ester Boc-Ala-Gly-Cys(MBzl)-Lys(Boc)-Asn-Phe-Phe-Trp-Lys(Boc)-Thr-Phe-Thr-Ser-Cys(MBzl)-OBu^t (IX). Compound IX was treated with TFA to remove the Boc and the Bu^t ester groups. The resulting S-protected tetradecapeptide was allowed to react with four equivalents of $(CF_3COO)_2Hg$ in 80% aqueous acetic acid for 12 hr at room temperature, followed by treatment with β-mercaptoethanol[5b] to yield the tetradecapeptide bearing two sulfhydryl groups. This was then air-oxidized under highly diluted conditions (pH 6.8) at room temperature for 96 hr to establish the intramolecular disulfide bridge. The resulting crude somatostatin was purified by gel filtration on Sephadex LH-20 using 0.1 N acetic acid as the eluting agent. The product thus obtained was found to be homogeneous by thin-layer chromatography in two solvent systems and its optical rotation was very close to those of literature.[23a–f] The acid hydrolysate of this peptide contained constituent amino acids in the ratios predicted by theory.

Thus, the biologically active peptides oxytocin and somatostatin were successfully obtained by this new method. These experimental results demonstrate that this method is promising as a new tool for the synthesis of cysteine and cystine containing peptides and proteins.

Experimental

All melting points were taken by the capillary method and are uncorrected. Rotations were determined with a Perkin-Elmer Model 141 polarimeter. Amino acid analysis was performed on a Hitachi KLB-3B amino acid analyzer. Evaporations were carried out in a rotary evaporator under reduced pressure at a temperature of 40—50°. Catalytic hydrogenation were performed at room temperature for the Z and the Bzl ester groups or at 50° for the Z(NO_2) group with palladium black as catalyst. The purity of the products was tested by thin-layer chromatography on silica gel (Merck's precoated silica gel plate $60F_{254}$) or Avicel (Funakoshi Yakuhin Co. Ltd). Solvent systems used were $CHCl_3$–MeOH–acetic acid (9: 1: 0.5, *Rf* 1), AcOEt–pyridine–acetic acid–H_2O (60: 20: 6: 10, *Rf* 2), *n*-butanol–acetic acid–H_2O (4: 1: 1, *Rf* 3), *n*-butanol–pyridine–acetic acid–H_2O (30: 20: 6: 24, *Rf* 4), AcOEt–*n*-butanol–acetic acid–H_2O (1: 1: 1: 1, *Rf* 5), and $CHCl_3$–MeOH–H_2O (7: 3: 0.5, *Rf* 6).

H-Cys(Ad)-OH (1)——Cysteine (12.1 g, 0.1 mol) and 1-adamantanol (15.2 g, 0.1 mol) were dissolved in 120 ml of TFA. The mixture was stirred at room temperature for 12 hr and then the excess TFA was evaporated off. The oily residue was dissolved in 200 ml of cold H_2O and the solution was adjusted at pH 6.0 with

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conc. NH_4OH. The resulting crystalline materials were collected by filtration and washed with EtOH, ether and then recrystallized from H_2O: yield, 23.0 g (90.0%), mp 227—228° (dec.), $[\alpha]_D^{26}$ $-14.5°$ (c=0.51, acetic acid), *Rf* 2=0.57. *Anal.* Calcd. for $C_{13}H_{22}NO_2S$: C, 60.91; H, 8.65; N, 5.46; S, 12.15. Found: C, 59.51; H, 8.16; N, 5.41; S, 12.33.

H-Cys(Bu^t)-OH (2)——The title compound was prepared in the same manner described in the compound **1**: yield, 30%, mp 244—245°, $[\alpha]_D^{26}$ $-15.4°$ (c=0.57, acetic acid), *Rf* 2=0.51. *Anal.* Calcd. for $C_7H_{15}NO_2S$: C, 45.13; H, 8.65; N, 7.52; S, 17.22. Found: C, 45.11; H, 8.32; N, 7.47; S, 17.77.

Treatment of S-Protected Cysteine Derivative with $(CH_3COO)_2Hg$ in Cold TFA——An S-protected cysteine derivative (1 mmol) was dissolved in cold TFA (10 ml) containing 0.2 ml of anisole. To this solution was added $(CH_3COO)_2Hg$ (319 mg, 1 mmol) and then the reaction mixture was stirred for 15 min at 0°. The excess TFA was removed by evaporation below 25° in 5 min. The resulting residue was dissolved in H_2O and then washed once with ether. Hydrogen sulfide was bubbled through this solution for 15—30 min. The resulting mercuric sulfide was filtered off and the filtrate was concentrated *in vacuo*. The resulting residue was dissolved in H_2O again and then evaporated off. This operation was repeated three times. The resulting crystalline residue was subjected to quantitative amino acid analysis. The results have been shown in Table I. Thin-layer chromatography was performed on silica gel by using the solvent system of *Rf* 2 and 3.

Treatment of S-Protected Cysteine Derivative with CH_3COOAg, $(CH_3COO)_2Cu$, $(CH_3COO)_2Zn$, $(CH_3COO)_2Ni$ or $(CH_3COO)_2Pb$——In place of $(CH_3COO)_2Hg$ in the above experiment, the acetate of Ag^+, Cu^{2+}, Zn^{2+}, Ni^{2+} or Pb^{2+} was used for removing the S–MBzl and S-Bu^t groups. The results have shown that none were effective.

Boc-Cys(MBzl)-Gly-OMe (3)——The title compound was prepared from Boc–Cys(MBzl)–ONB (0.05 mol) and H–Gly–OMe (0.05 mol) in the usual manner: yield, 19.0 g (92.0%), mp 91—92°, $[\alpha]_D^{27}$ $-32.2°$ (c=0.95, DMF), *Rf* 1=0.78. *Anal.* Calcd. for $C_{19}H_{28}N_2O_6S$: C, 55.32; H, 6.84; N, 6.79; S, 7.77. Found: C, 55.58; H, 6.92; N, 6.99; S, 7.65.

Z-Gly-Cys(MBzl)-Gly-OMe (I)——The compound **3** (10.3 g, 0.025 mol) was treated with TFA (70 ml) for 15 min at room temperature and the excess TFA was removed by evaporation. The resulting oily residue was washed well with pet. ether by decantation, dried over NaOH pellets *in vacuo* and then dissolved in 100 ml of DMF. Et_3N (7.2 ml) and Z–Gly–ONB [prepared from Z–Gly–OH (5.2 g, 0.025 mol)] were added and the mixture was stirred at 4° for 48 hr. The solvent was evaporated *in vacuo* and the residue was dissolved in AcOEt. The AcOEt layer was washed with 5% $NaHCO_3$, 0.2 N HCl and H_2O, dried over anhydr. Na_2SO_4 and then evaporated. The residue was treated with ether to give a powder, which was further purified by reprecipitation from AcOEt–ether: yield, 9.0 g (71.4%), mp 90—95°, $[\alpha]_D^{27}$ $-23.1°$ (c=0.55, DMF), *Rf* 1=0.68. *Anal.* Calcd. for $C_{24}H_{29}N_3O_7S$: C, 57.20; H, 5.80; N, 8.34; S, 6.37. Found: C, 57.60; H, 5.81; N, 8.23; S, 6.36.

Boc-Cys(Bu^t)-OH•DCHA Salt (4)——The title compound was prepared according to the method of Nagasawa, *et al.*[24] using the compound **2**: yield, 90%, mp 181—182.5°, $[\alpha]_D^{27}$ $+7.4°$ (c=0.83, MeOH), *Rf* 1=0.61. *Anal.* Calcd. for $C_{24}H_{46}N_2O_4S$: C, 62.93; H, 10.10; N, 6.10; S, 6.99. Found: C, 62.92; H, 10.36; N, 5.95; S, 7.05.

Boc-Cys(Bu^t)-Gly-OMe (5)——The title compound was prepared from Boc-Cys(Bu^t)-ONB (0.02 mol) and H–Gly–OMe (0.02 mol) in the usual manner: yield, 5.9 g (86.9%), mp 73—75°, $[\alpha]_D^{26}$ $-17.9°$ (c=0.56, DMF), *Rf* 1=0.75. *Anal.* Calcd. for $C_{15}H_{28}N_2O_5S$: C, 51.70; H, 8.10; N, 8.04; S, 9.20. Found: C, 51.70; H, 8.12; N, 8.05; S, 9.12.

Z-Gly-Cys(Bu^t)-Gly-OMe (**II**)——The title compound was prepared from the compound **5** in the same manner described in the compound I: yield, 6.1 g (92.5%, liquid), $[\alpha]_D^{26}$ $-11.4°$ (c=0.46, DMF), *Rf* 1=0.67. *Anal.* Calcd. for $C_{20}H_{29}N_3O_6S$: C, 54.65; H, 6.65; N, 9.56; S, 7.30. Found: C, 55.11; H, 6.60; N, 9.48; S, 6.55.

Treatment of the Compound I and II with $(CH_3COO)_2Hg$ in Cold TFA——(a) (1) Z–Gly–Cys[Hg(CF_3-COO)]–Gly–OMe: The compound I (3.02 g, 0.006 mol) was dissolved in cold TFA (45 ml) containing 1.5 ml of anisole. To this solution was added $(CH_3COO)_2Hg$ (1.91 g, 0.006 mol) and then the reaction mixture was stirred for 15 min at 0°. The excess TFA was removed by evaporation below 25° in 5 min. A fine powder formed by addition of ether was collected by filtration and then dried over KOH pellets *in vacuo*: yield, 4.1 g (98.1%), *Anal.* Calcd. for $C_{18}H_{20}F_3HgN_3O_8S$: C, 31.06; H, 2.90; F, 8.19; Hg, 28.82; N, 6.04; S, 4.61. Found: C, 31.02; H, 2.87; F, 8.18; Hg, 28.68; N, 6.18; S, 4.60.

(2) Z–Gly–Cys–Gly–OMe (III): The mercaptide (2.0 g) obtained above was dissolved in DMF (30 ml) and hydrogen sulfide was bubbled through this solution for 90 min. The resulting mercuric sulfide was filtered off through Celite and the filtrate was concentrated *in vacuo*. The crystalline residue was collected by filtration and then recrystallized from MeOH–H_2O: yield, 1.1 g (100%), mp 130—132°, $[\alpha]_D^{26}$ $-5.7°$ (c=0.57, DMF), *Rf* 1=0.66. The nitroprusside test for the sulfhydryl group was strongly positive. *Anal.* Calcd. for $C_{16}H_{21}N_3O_6S$: C, 50.12; H, 5.52; N, 10.96; S, 8.36. Found: C, 49.91; H, 5.18; N, 10.97; S, 8.44; Hg, 0.0071 (atomic absorption spectrophotometry).

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24) T. Nagasawa, K. Kuromizu, K. Narita, and Y. Isowa, *Bull. Chem. Soc. Jpn.*, 46, 1269 (1973).

(b) The compound II was treated in the same manner described in the compound I. The yield of the mercaptide was 84.2% and the compound III was obtained in 84.5%: mp 130—132°, $[\alpha]_D^{26}$ $-5.6°$ ($c=0.61$, DMF), *Rf* 1=0.66. *Anal.* Calcd. for $C_{16}H_{21}N_3O_6S$: C, 50.12; H, 5.52; N, 10.96; S, 8.36. Found: C, 50.13; H, 5.61; N, 10.99; S, 8.27. These results have been summarized in Table II.

Preparation of $(CF_3COO)_2Hg$——Mercuric oxide (red) (21.6 g, 0.1 mol) was dissolved in TFA (200 ml) at room temperature. The excess TFA was removed by evaporation and the resulting crystals were dried over P_2O_5 and NaOH pellets *in vacuo*: yield, 42.7 g (100%). *Anal.* Calcd. for $C_4F_6HgO_4$: C, 11.26; F, 26.72; Hg, 47.02. Found: C, 11.26; H, 26.72; Hg, 46.52.

Treatment of S-Protected Cysteine Derivative with $(CF_3COO)_2Hg$ in 80% Aqueous Acetic Acid——An S-protected cysteine derivative (1 mmol) was dissolved in 80% aqueous acetic acid (20 ml) containing 0.2 ml of anisole. To this solution was added $(CF_3COO)_2Hg$ (512 mg, 1.2 mmol) and the mixture was stirred at room temperature for 1 hr. Hydrogen sulfide, after addition of H_2O (40 ml), was bubbled through this solution for 15 min. The resulting mercuric sulfide was filtered off and the filtrate was subjected to quantitative amino acid analysis. The results have been shown in Table I.

Treatment of the Compound I and II with $(CF_3COO)_2Hg$ in 80% Aqueous Acetic Acid——(a) The compound I (1 mmol) was dissolved in 80% aqueous acetic acid (20 ml). To this solution was added $(CF_3COO)_2Hg$ (512 mg, 1.2 mmol) and the mixture was stirred at room temperature for one hour. The reaction mixture was diluted with 80% aqueous acetic acid (80 ml) and then hydrogen sulfide was bubbled through this solution for 20 min. The resulting mercuric sulfide was filtered off and the filtrate was condensed to dryness. The residue was recrystallized from MeOH–AcOEt: yield, 83.9%, mp 128—130°, $[\alpha]_D^{21}$ $-5.3°$ ($c=0.66$, DMF), *Rf* 1=0.66. *Anal.* Calcd. for $C_{16}H_{21}N_3O_6S$: C, 50.21; H, 5.55; N, 10.96; S, 8.36. Found: C, 50.03; H, 5.27; N, 10.93; S, 8.38; Hg, 0.0058 (atomic absorption spectrophotometry). When two equivalents of $(CF_3COO)_2Hg$ were used, the yield was somewhat low (76.5%). This may be due to adsorption of the peptide on mercuric sulfide.

(b) The compound II (1 mmol) was treated in the same manner as described above. However, the pure compound could not be isolated. Even when two equivalents of $(CF_3COO)_2Hg$ were used, the yield was 28.2%. These results have been summarized in Table II.

Synthesis of Oxytocin

Boc-Cys(MBzl)-Tyr-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-resin——Boc–Gly–resin 8.2 g (Gly content, 2.2 mmol) was placed in a Shimadzu Model APS-800 peptide synthesizer and the introduction of each of the remaining 8 amino acid residues was carried through the schedule shown below. N^{α}–Boc protection was used throughout with S-MBzl group of cysteine. The active ester program was used for the Asn, the Tyr and the Gln residues. (1) CH_2Cl_2 wash (2 min × 4 times), (2) 50% TFA in CH_2Cl_2 (10 min × 2 times), (3) CH_2Cl_2 wash (2 min × 3 times), (4) EtOH wash (2 min × 3 times), (5) $CHCl_3$ wash (2 min × 3 times) (6) 10% Et_3N in $CHCl_3$ (12 min × 1 time), (7) $CHCl_3$ wash (2 min × 3 times), (8) symmetric Boc amino acid anhydride (60 min × 2 times) or Boc amino acid *p*-nitrophenyl ester (6.6 mmol) (12 hr × 2 times), (9) CH_2Cl_2 wash (2 min × 2 times), (10) acetylation of the unreacted amino group by addition of acetic anhydride (10 min × 2 times), (11) CH_2Cl_2 wash (2 min × 3 times). The finished protected peptide resin was washed with acetic acid, DMF and MeOH and then dried over P_2O_5 *in vacuo*: yield, 13.15 g.

Boc-Cys(MBzl)-Tyr-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-NH$_2$ (IV)——The complete resin 12.6 g was suspended in 15.5% ammonia in MeOH (70 ml) and stirred for 46 hr at room temperature. The reaction mixture was filtered and the residue was washed with DMF (20 ml) and the filtrates were concentrated *in vacuo*. The resulting oily product was triturated with ether to give the crude protected nona peptide amide as a powder (2.86 g). This crude product, after treatment with hot EtOH, aqueous EtOH and DMF–EtOH, was purified by column chromatography on silica gel (2.8 × 8.0 cm) using solvent system of $CHCl_3$–MeOH–H_2O–pyridine–acetic acid (1085: 150: 25: 63: 84): yield, 768 mg, $[\alpha]_D^{21}$ $-39.1°$ ($c=0.47$, DMF), *Rf* 6=0.55. *Anal.* Calcd. for $C_{66}H_{98}N_{12}O_{16}S_2 \cdot CH_3COOH \cdot H_2O$: C, 55.52; H, 6.92; N, 11.78; S, 4.49. Found: C, 55.24; H, 7.00; N, 11.92; S, 4.43.

H-$\overline{\text{Cys-Tyr-Ile-Gln-Asn-Cys}}$-Pro-Leu-Gly-NH$_2$ (Oxytocin)——(a) The compound IV (125 mg) was dissolved in TFA (1 ml) containing 0.22 ml of anisole. To this solution was added $(CH_3COO)_2Hg$ (127 mg) and then the reaction mixture was stirred for 30 min at room temperature. Ether was added and the resulting powder was collected by filtration (173 mg) and then dissolved in 70% aqueous acetic acid. To this was added *β*-mercaptoethanol (0.31 ml) and the reaction mixture was stirred for 5 hr at room temperature. The resulting precipitate was filtered off and the filtrate was concentrated to dryness. The residue was then dissolved in a small amount of 50% aqueous acetic acid and the solution was applied to a column of Sephadex G-25 (2.2 × 92.5 cm). The column was then eluted with the same solvent and the desired fractions (149—188 ml) were collected and then lyophilized (85 mg). This was dissolved in 0.1% acetic acid (150 ml) and the pH of the solution was adjusted to 7.3 with 1 N NH_4OH. The solution was oxidized in the air for 4.5 hr at room temperature and then acidified with 1 N acetic acid and lyophilized (76 mg). The residue was dissolved in a small amount of 50% aqueous acetic acid and then the solution was applied to a column of Sephadex G-15 (2.2 × 92.5 cm). The column was developed with the same solvent and the desired fractions (154—188 ml) were pooled and lyophilized: yield, 50 mg, $[\alpha]_D^{22}$ $-29.2°$ ($c=0.47$, 1 N acetic acid), *Rf* 3=0.44, *Rf* 5=0.66. Amino acid ratios in acid hydrolysate (6 N HCl, 110°, 24 hr): Asp, 1.03 (1); Glu, 1.13 (1); Pro, 1.06 (1); Gly, 1.00 (1); 1/2Cys, 1.97 (2); Ile, 1.03 (1); Leu, 1.03 (1); Tyr, 0.90 (1). Average recovery, 83.2%.

(b) The compound IV (500 mg) was treated with TFA (3 ml) for 10 min at room temperature. Ether was added and the resulting powder was collected by filtration. This was purified by a column chromatography on silica gel (2.2 × 5.5 cm) using solvent system of $CHCl_3$–MeOH–acetic acid–H_2O (266: 60: 21: 10): yield, 181 mg, *Rf* 6=0.47. The compound V obtained above was dissolved in 80% aqueous acetic acid (1.5 ml). To this solution was added $(CF_3COO)_2Hg$ (170 mg) and then the reaction mixture was stirred for 2 hr at room temperature. β-Mercaptoethanol (0.31 ml) was added and then the reaction mixture was stirred for 5 hr at room temperature. The resulting precipitate was filtered off and the filtrate was worked up in the same manner described above to yield oxytocin: yield, 27 mg, $[\alpha]_D^{27}$ $-25.9°$ ($c=0.48$, 1 N acetic acid) (lit.18) $-23.1°$), *Rf* 3=0.34, *Rf* 5=0.61. Amino acid ratios in acid hydrolysate (6 N HCl, 110°, 24 hr): Asp, 1.00 (1); Glu, 1.07 (1); Pro, 1.07 (1); Gly, 1.00 (1); 1/2Cys, 1.93 (2); Ile, 1.04 (1), Leu, 1.04 (1); Tyr, 0.89 (1). Average recovery, 79.4%.

Synthesis of Somatostatin

H-Cys(MBzl)-OBu^t·L-tartrate (6)——75 ml of isobutene was bubbled into a solution of H–Cys(MBzl)–OH (4.82 g, 0.02 mol) in a mixture of dioxane (150 ml) and conc. sulfuric acid (4.8 ml) at 0° and then the reaction mixture was left standing at room temperature for 12 hr under the sealed conditions. This was poured into a cold mixture of 1 N NaOH (300 ml) and then the aqueous phase was extracted twice with ether. The combined ether layer was dried over anhydr. Na_2SO_4 and evaporated. The residue was dissolved in 50 ml of ether and to this was added 6.69 N HCl in dioxane (3 ml). The resulting crystals were collected by filtration: yield, 2.4 g. 1.99 g of the hydrochloride was suspended in ether and shaken well with 1 N NaOH (10 ml) until the mixture became clear. The aqueous layer was further extracted twice with ether. The combined ether layer was dried over anhydr. Na_2SO_4 and then evaporated. The residue was dissolved in EtOH with L-tartaric acid (900 mg). The resulting crystals were collected by filtration and recrystallized from EtOH: yield, 2.3 g, mp 100—102°, $[\alpha]_D^{27}$ $-3.2°$ ($c=0.53$, DMF), *Rf* 1=0.66, *Rf* 2=0.90. *Anal.* Calcd. for $C_{15}H_{23}NO_3S \cdot C_4H_6O_6 \cdot 1/2H_2O$: C, 49.99; H, 6.62; N, 3.07; S, 7.02. Found: C, 49.85; H, 6.74; N, 2.98; S, 6.68.

Z-Thr-Ser-OMe (7)——The title compound was prepared from Z–Thr–ONB (0.04 mol) and H–Ser–OMe (0.04 mol) in the usual manner: yield, 10.6 g (74.5%), mp 133—135°, $[\alpha]_D^{23}$ $+10.1°$ ($c=0.86$, DMF), *Rf* 1=0.76. *Anal.* Calcd. for $C_{16}H_{22}N_2O_7$: C, 54.23; H, 6.26; N, 7.91. Found: C, 54.44; H, 6.33; N, 7.84.

Z-Phe-Thr-Ser-OMe (8)——The compound **7** (9.91 g, 0.028 mol) in a mixture of MeOH (100 ml) and 6.69 N HCl in dioxane (6.3 ml) was hydrogenated and the resulting hydrochloride was dissolved in 50 ml of DMF and then neutralized with Et_3N (4.3 ml) at 0°. After filtration, the filtrate was combined with a solution of Z–Phe–ONB [prepared from Z–Phe–OH (8.78 g, 0.028 mol] in dioxane and the mixture was stirred at room temperature for 12 hr. The solvent was evaporated and the residue was dissolved in AcOEt. The AcOEt layer was washed with 1 N HCl, 5% $NaHCO_3$ and H_2O, dried over anhydr. Na_2SO_4 and then evaporated. The resulting crystals were collected by filtration: yield, 11.0 g (80.0%), mp 180—182°, $[\alpha]_D^{23}$ $-3.8°$ ($c=0.67$, DMF), *Rf* 1=0.84. *Anal.* Calcd. for $C_{25}H_{31}N_2O_8 \cdot 1/2H_2O$: C, 58.81; H, 6.12; N, 8.23. Found: C, 58.80; H, 6.25; N, 8.26.

Z(NO$_2$)-Thr-OH·DCHA Salt (9)——The title compound was prepared according to the method of F.H. Carpenter and D.T. Gish20) using $NaHCO_3$ as a base: yield, 50.0%, mp 186—188°, $[\alpha]_D^{23}$ $+14.9°$ ($c=0.77$, DMF), *Rf* 1=0.41. *Anal.* Calcd. for $C_{12}H_{14}N_2O_7 \cdot C_{12}H_{23}N$: C, 60.11; H, 7.78; N, 8.76. Found: C, 60.14; H, 7.81; N, 8.80.

Z(NO$_2$)-Thr-Phe-Thr-Ser-OMe (10)——The compound **8** (11.0 g, 0.022 mol) in MeOH was hydrogenated and the resulting free base was coupled with Z(NO$_2$)-Thr-ONB [prepared from the compound **9** (10.6 g, 0.022 mol)] in tetrahydrofuran. The mixture was stirred for 12 hr at room temperature. The solvent was evaporated and the residue was treated with H_2O. The resulting powder was collected by filtration and recrystallized from MeOH: yield, 12.8 g (90.1%), mp 194—196°, $[\alpha]_D^{23}$ $-0.7°$ ($c=0.72$, DMF), *Rf* 1=0.59, *Rf* 2=0.91. *Anal.* Calcd. for $C_{29}H_{37}N_5O_{12}$: C, 53.78; H, 5.76; N, 10.81. Found: C, 53.80; H, 5.85; N, 10.75.

Z(NO$_2$)-Thr-Phe-Thr-Ser-NHNH$_2$ (11)——To a solution of the compound **10** (12.3 g, 0.019 mol) in DMF (100 ml) was added hydrazine hydrate (9.5 ml) in MeOH (100 ml) and the solution was kept on standing for 48 hr. The crystals formed were collected by filtration and recrystallized from MeOH: yield, 11.4 g (92.7%), mp 229—232° (dec.), $[\alpha]_D^{23}$ $+3.2°$ ($c=0.44$, DMF), *Rf* 1=0.11, *Rf* 2=0.53. *Anal.* Calcd. for $C_{28}H_{37}N_7O_{11} \cdot H_2O$: C, 50.52; H, 5.91; N, 14.81. Found: C, 50.89; H, 5.92; N, 14.90.

Z(NO$_2$)-Thr-Phe-Thr-Ser-Cys(MBzl)-OBu^t (12)——To a solution of the compound **11** (3.24 g, 0.005 mol) in DMF (50 ml), 6.51 N HCl in dioxane (3.1 ml) and then isoamyl nitrite (0.81 ml, 0.006 mol) were added at $-20°$ and the mixture was stirred at $-20°$ for 10 min. After neutralization with Et_3N (2.8 ml), the solution was added to H-Cys(MBzl)-OBu^t [prepared from the compound **6** (2.24 g, 0.005 mol)] in DMF and the mixture was stirred at 4° for 48 hr. The solution was filtered and the filtrate was condensed *in vacuo* to dryness. The residue was treated with H_2O and then recrystallized twice from MeOH: yield, 3.55 g (77.7%), mp 193—195° (dec.), $[\alpha]_D^{25}$ $-18.0°$ ($c=0.56$, DMF), *Rf* 1=0.87. *Anal.* Calcd. for $C_{43}H_{56}N_6O_{14}S$: C, 56.57; H, 6.18; N, 9.21; S, 3.51. Found: C, 56.31; H, 6.08; N, 9.34; S, 3.47.

Z(NO$_2$)-Trp-OH (13)——The title compound was prepared according to the method of F.H. Carpenter and D.T. Gish:20) yield, 88.6%, mp 108—115° (dec.), $[\alpha]_D^{23}$ $-43.5°$ ($c=0.65$, DMF), *Rf* 1=0.80. *Anal.* Calcd. for $C_{19}H_{17}N_3O_4 \cdot 2H_2O$: C, 58.91; H, 5.46; N, 10.85. Found: C, 59.63; H, 5.63; N, 10.74.

Z(NO$_2$)-Trp-Lys(Boc)-OH•DCHA Salt (14)——Z(NO$_2$)-Trp-ONB [prepared from the compound **13** (10.5 g, 0.03 mol)] was added to a solution of H-Lys(Boc)-OH (7.39 g, 0.03 mol) and Et_3N (4.2 ml, 0.03 mol) in DMF and the mixture was stirred for 12 hr at room temperature. The solvent, after neutralization with 1 N HCl (30 ml), was evaporated off and the residue was dissolved in AcOEt. The AcOEt layer was washed with H_2O, dried over anhydr. Na_2SO_4 and then evaporated. The residue was dissolved in acetonitrile and to this was added DCHA (6.0 ml, 0.07 mol). The gelatinous mass formed was collected by filtration and recrystallized from acetonitrile: yield, 12.5 g (52.6%), mp 95—100°, $[\alpha]_D^{25}$ $-20.9°$ ($c=0.56$, DMF), *Rf* 1=0.85. *Anal.* Calcd. for $C_{30}H_{37}N_5O_9 \cdot C_{12}H_{23}N$: C, 63.61; H, 7.63; N, 10.60. Found: C, 63.77; H, 7.67; N, 11.08.

Z(NO$_2$)-Trp-Lys(Boc)-Thr-Phe-Thr-Ser-Cys(MBzl)-OBu^t (VII)——The compound **12** (3.29 g, 0.0036 mol) in 80% aqueous acetic acid (150 ml) was hydrogenated. The catalyst was removed by filtration and the filtrate was lyophilized to give pale yellowish powder. This was treated with EtOH–ether and then MeOH–ether to remove the degradation products from *p*-nitrobenzyl radical: yield, 2.75 g, *Rf* 1=0.06, *Rf* 2=0.43. The powder thus obtained (2.62 g, 0.003 mol) was dissolved in a small amount of DMF and to this was added *p*-TsOH (627 mg, 0.0033 mol) in DMF under ice-cooling. The solvent was evaporated *in vacuo* and the residue was treated with ether, dried over NaOH pellets *in vacuo* and then dissolved in DMF (20 ml). To this solution was added Z(NO$_2$)-Trp-Lys(Boc)-OH [prepared from the compound **14** (2.88 g, 0.0036 mol)], HONB (1.30 g, 0.0072 mol) and DCC (819 mg, 0.004 mol) with N-ethylmorpholine (0.42 ml, 0.0033 mol) at −10°. The mixture was stirred for 3 hr at −10° and then for 48 hr at room temperature. The urea formed was filtered off and the filtrate was condensed *in vacuo*. The residue was treated with H_2O and then recrystallized twice from MeOH: yield, 3.50 g (80.6%), mp 183—185° (dec.), $[\alpha]_D^{25}$ $-26.6°$ ($c=0.67$, DMF), *Rf* 1=0.83, *Rf* 2=0.94. *Anal.* Calcd. for $C_{64}H_{84}N_{10}O_{18}S$: C, 58.52; H, 6.45; N, 10.66; S, 2.44. Found: C, 58.36; H, 6.47; N, 10.49; S, 2.54.

Z-Phe-Phe-OBu^t (15)——The title compound was prepared from Z-Phe-ONB (0.05 mol) and H-Phe-OBu^t (0.05 mol) in the usual manner: yield, 15.4 g (60.0%), mp 107—109°, $[\alpha]_D^{27}$ $-13.5°$ ($c=0.53$, DMF), *Rf* 1=0.97. *Anal.* Calcd. for $C_{30}H_{34}N_2O_5$: C, 71.96; H, 6.82; N, 5.57. Found: C, 71.61; H, 6.64; N, 5.50.

Boc-Asn-Phe-Phe-OBu^t (16)——The compound **15** (13.1 g, 0.026 mol) in MeOH was hydrogenated and the resulting free base was coupled with Boc-Asn-ONB [prepared from Boc-Asn-OH (6.04 g, 0.026 mol)] in DMF. The usual work-up provided a powder, which was recrystallized twice from MeOH: yield, 11.0 g (72.8%), mp 163—166°, $[\alpha]_D^{27}$ $-28.9°$ ($c=0.85$, DMF), *Rf* 1=0.95. *Anal.* Calcd. for $C_{31}H_{42}N_4O_7$: C, 63.90; H, 7.27; N, 9.62. Found: C, 64.12; H, 7.20; N, 9.90.

Z-Lys(Boc)-Asn-Phe-Phe-OH (17)——The compound **16** (10.5 g, 0.018 mol) was treated with TFA for 90 min at room temperature. The excess TFA was removed by evaporation and the residue was treated with ether to form the fine powder, which was collected by filtration, dried over NaOH pellets and then dissolved in DMF (100 ml). To this solution were added Et_3N (5.04 ml, 0.036 mol) and Z-Lys(Boc)-ONB [prepared from Z-Lys(Boc)-OH (8.2 g, 0.0216 mol)] and the mixture was stirred for 12 hr at room temperature. The solvent, after acidification with acetic acid (10 ml), was evaporated *in vacuo* and the residue was triturated with water, collected by filtration and then recrystallized twice from aqueous MeOH and then DMF–AcOEt: yield, 11.1 g (78.1%), mp 194—196° (dec.), $[\alpha]_D^{27}$ $-26.9°$ ($c=0.59$, DMF), *Rf* 1=0.36, *Rf* 2=0.78. *Anal.* Calcd. for $C_{41}H_{52}N_6O_{10}$: C, 62.42; H, 6.64; N, 10.65. Found: C, 62.27; H, 6.66; N, 10.72.

Z(NO$_2$)-Cys(MBzl)-OH (18)——The title compound was prepared according to the method of F.H. Carpenter and D.T. Gish:20) yield, 80%, mp 163—165°, $[\alpha]_D^{23}$ $-51.2°$ ($c=0.61$, DMF), *Rf* 1=0.89. *Anal.* Calcd. for $C_{19}H_{20}N_2O_7S$: C, 54.28; H, 4.79; N, 6.66; S, 7.62. Found: C, 54.18; H, 4.74; N, 6.57; S, 7.56.

Z(NO$_2$)-Cys(MBzl)-Lys(Boc)-Asn-Phe-Phe-OH (19)——The compound **17** (10.3 g, 0.013 mol) in 80% aqueous acetic acid was hydrogenated and the resulting hexapeptide was purified by reprecipitation from hot EtOH: yield, 7.0 g, *Rf* 2=0.19. This (6.55 g, 0.01 mol) was suspended in 100 ml of DMF with Et_3N (1.40 ml, 0.01 mol). To this was added Z(NO$_2$)-Cys(MBzl)-ONB [prepared from the compound **18** (5.05 g, 0.012 mol)] and the mixture was stirred for 12 hr at room temperature. The solvent, after addition of acetic acid (10 ml), was evaporated *in vacuo* and then recrystallized twice from 80% aqueous MeOH: yield, 9.6 g (92.3%), mp 209—210° (dec.), $[\alpha]_D^{27}$ $-24.6°$ ($c=0.74$, DMF), *Rf* 1=0.45, *Rf* 2=0.84. *Anal.* Calcd. for $C_{52}H_{64}N_8O_{14}S$: C, 59.08; H, 6.10; N, 10.60; S, 3.03. Found: C, 59.04; H, 6.02; N, 10.60; S, 3.19.

Boc-Ala-Gly-OBzl (20)——The title compound was prepared from Boc-Ala-ONB (0.023 mol) and H-Gly-OBzl (0.024 mol) in the usual manner: yield, 5.5 g (71.2%), mp 86—87°, $[\alpha]_D^{27}$ $-10.8°$ ($c=0.56$, DMF), *Rf* 1=0.96. *Anal.* Calcd. for $C_{17}H_{24}N_2O_5$: C, 60.70, H, 7.19; N, 8.33. Found: C, 60.82; H, 7.19; N, 8.28.

Boc-Ala-Gly-OH (21)——The compound **20** (5.29 g, 0.015 mol) in MeOH was hydrogenated and the resulting acid was recrystallized from AcOEt: yield, 3.3 g (85.3%), mp 111—113° (dec.), $[\alpha]_D^{27}$ $-6.8°$ ($c=0.56$, DMF), *Rf* 1=0.43, *Rf* 2=0.75. *Anal.* Calcd. for $C_{10}H_{18}N_2O_5 \cdot 1/2H_2O$: C, 47.05; H, 7.50; N, 10.98. Found: C, 46.66; H, 7.65; N, 11.03.

Boc-Ala-Gly-Cys(MBzl)-Lys(Boc)-Asn-Phe-Phe-OH (VIII)——The compound **19** (5.2 g, 0.005 mol) in 80% aqueous acetic acid was hydrogenated. The catalyst was removed by filtration and the filtrate was lyophilized. The lyophilized material was triturated with ether and then reprecipitated from EtOH: yield, 4.05 g, *Rf* 2 = 0.20. This was suspended in 50 ml of DMF with Et_3N (0.70 ml). To this was added a solution of Boc-Ala-Gly-ONB [prepared from the compound **21** (1.48 g, 0.006 mol)] in DMF and the mixture was stirred for 12 hr at room temperature. The solvent, after addition of acetic acid (10 ml), was removed by evaporation *in vacuo*. The residue was triturated with water, collected by filtration and then recrystallized twice from 80% aqueous MeOH: yield, 3.5 g (60.4%), mp 201—203° (dec.), $[\alpha]_D^{25}$ −28.8° (c = 0.69, DMF), *Rf* 1 = 0.15, *Rf* 2 = 0.84. *Anal.* Calcd. for $C_{54}H_{75}N_9O_{14}S$: C, 58.63; H, 6.83; N, 11.40; S, 2.90. Found: C, 58.86; H, 6.83; N, 11.53; S, 2.79.

Boc-Ala-Gly-Cys(MBzl)-Lys(Boc)-Asn-Phe-Phe-Trp-Lys(Boc)-Thr-Phe-Thr-Ser-Cys(MBzl)-OBu^t (IX)——The compound VII (3.29 g, 0.0025 mol) in 80% aqueous acetic acid (150 ml) was hydrogenated. The catalyst was removed by filtration and the filtrate was lyophilized. The lyophilizate was treated twice with EtOH–ether: yield, 2.90 g, *Rf* 1 = 0.06, *Rf* 2 = 0.84. The compound VI obtained above (2.69 g, 0.0023 mol) was dissolved in a small amount of DMF and to this was added *p*-TsOH (437 mg, 0.0023 mol) under ice-cooling. The solvent was evaporated *in vacuo* and the residue was triturated with ether, dried over NaOH pellets *in vacuo* and then dissolved in DMF (20 ml). To this solution was added the compound VIII (2.54 g, 0.0023 mol), HONB (823 mg, 0.0046 mol) and DCC (949 mg, 0.0046 mol) with N-ethylmorpholine (0.29 ml, 0.0023 mol) at −10°. The mixture was stirred for 3 hr at −10° and then for 72 hr at room temperature. The urea formed was filtered off and the filtrate was condensed *in vacuo*. The residue was triturated with H_2O, collected by filtration and then recrystallized twice from MeOH: yield, 3.80 g (75.1%), mp 224—226° (dec.), $[\alpha]_D^{25}$ −18.3° (c = 0.70, DMF), *Rf* 1 = 0.31, *Rf* 2 = 0.90. *Anal.* Calcd. for $C_{109}H_{154}N_{18}O_{26}S_2$: C, 59.62; H, 7.06; N, 11.47; S, 2.92. Found: C, 59.55; H, 6.75; N, 11.32; S, 2.95. Amino acid ratios in acid hydrolysate (6 N HCl, 110°, 24 hr): Lys, 1.86 (2); Asp, 1.14 (1); Thr, 1.74 (2); Ser, 0.85 (1); Gly, 1.00 (1); Ala, 1.06 (1); 1/2 Cys, 0.65 (2); Phe, 3.15 (3) (Trp was destroyed). Average recovery, 84.4%.

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH (Somatostatin)——The compound IX (220 mg, 0.1 mmol) was treated with 90% aqueous TFA (2 ml) under nitrogen gas for 30 min at room temperature and then ether was added. The resulting fine powder was collected by filtration, dried over NaOH pellets *in vacuo*: yield, 221 mg, *Rf* 5 = 0.60. This was then dissolved in 20 ml of 80% aqueous acetic acid. To this was added $(CF_3COO)_2Hg$ (171 mg, 0.4 mmol) and the mixture was stirred for 12 hr at room temperature. To this was added *β*-mercaptoethanol (0.7 ml) and then the mixture was further stirred for 12 hr at room temperature. The resulting precipitates were filtered off and the filtrate was condensed under reduced pressure. The residue was dissolved in a small amount of 50% aqueous acetic acid and applied to a column of Sephadex G-25 (fine, 2.7 × 86.0 cm). The column was developed by the same solvent and the desired fractions (155—205 ml) were pooled and the solvent was evaporated under reduced pressure. The residue was dissolved in 50 ml of water and then treated with Amberlite IRA-410 (acetate form, approximately 10 ml) for 10 min. The resin was filtered off and the filtrate was diluted with 1000 ml of 0.1 N ammonium acetate. The pH of the solution was adjusted to 6.8 with conc. NH_4OH and the solution was kept on standing at room temperature for 96 hr and then lyophilized. The residue was dissolved in a small amount of 0.1 N acetic acid and then applied to a column of Sephadex LH-20 (5.5 × 35.0 cm). The column was then developed with the same solvent and the desired fractions (560—640 ml) were collected and lyophilized: yield, 30.0 mg, $[\alpha]_D^{26}$ −33.6° (c = 0.13, 1% acetic acid), (lit.23a) natural, −32.3°; synthetic, −33.3°; lit.23b) −36°; lit.23c) −34.5°; lit.23d) −29°; lit.23e) −39.5°; lit.23f) −34.8°), *Rf* 4 = 0.60, *Rf* 5 = 0.40. Amino acid ratios in acid hydrolysate (6 N HCl, 110°, 24 hr): Lys, 1.99 (2); Asp, 1.06 (1); Thr, 1.93 (2); Ser, 0.81 (1); Gly, 1.04 (1); Ala, 1.03 (1); 1/2 Cys, 1.75 (2); Phe, 3.07 (3) (Trp was destroyed). Average recovery, 80.0%. Amino acid ratios in an AP–M digest: Lys, 1.95 (2); Trp, 0.84 (1); Thr, 1.84 (2); Ser + Asn, not determined, Gly, 1.00 (1); Ala, 1.05 (1); 1/2 Cys, 1.78 (2); Phe, 3.16 (3). Average recovery, 80.0%.

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