NMR analysis revealed a 3:1 mixture of diastereomeric products: ^{1}H NMR major diastereomer 1.51 (d, 3 H, J = 6.8 Hz), 3.44 (q, 3 H, J = 1.6 Hz), 5.42 (m, 1 H), 7.1-7.6 (m, 10 H), minor diastereomer 1.54 (d, 3 H, J = 6.8 Hz), 3.47 (q, 3 H, J = 1.6 Hz), 5.39 (m, 1 H), 7.1-7.6 (m, 10 H); ^{13}C NMR 20.72, 49.29, 55.11, 123.02, 127.02, 172.16, 127.64, 127.78, 128.42, 128.55, 128.98, 129.46, 133.48, 141.11, 121.90, 125.85; IR (neat) 3329, 2926, 1686, 1508, 1450, 1269, 1165, 1106, 1025, 755 cm^{-1}; HRMS exact mass calcd for $C_{18}H_{17}NO_2BrF_3$ 415.0399, found 415.0346.

MTPA Amide of α-Phenethylamine 29. n-BuLi (1.0 mL of a 1.6 M solution in hexane) was added to a flame-dried flask at -100 °C containing the above aryl bromide **28** (20 mg, 0.048 mmol) in anhydrous THF (4 mL). After the mixture was stirred for 30 min, water was added with vigorous stirring. After this solution was stirred to room temperature, ether (5 mL) was added, and the resulting solution was washed with saturated aqueous NH_4Cl. After drying ($MgSO_4$), solvent removal gave the product as a colorless oil. ^{1}H NMR showed a 3:1 mixture of diastereoisomers, from which the major component was identical to the corresponding MTPA amide of authentic (S)-α-phenethylamine.

MTPA Amide of o-Methyl-α-phenethylamine 30. Partially resolved aryl bromide **28** was dissolved in dry HMPA (1.0 mL) under argon. $Pd(PPh_3)_4$ (4 mg, 0.003 mmol) and tetramethyltin (79.2 mg, 0.44 mmol) were added, and the mixture was heated at 70 °C for 38 h. After cooling to room temperature, the reaction mixture was poured into saturated aqueous NH_4Cl. After extraction three times with Et_2O and drying ($MgSO_4$), the solvent was removed at reduced pressure, and the residue was rechromatographed on silica gel (8-12% ether in pentane). The product (**30**) was a 3:1 mixture of diastereoisomers (^{1}H NMR spectroscopy) whose components were identical to the corresponding MTPA amides of racemic o-methyl-α-phenethylamine: ^{1}H NMR major diastereomer 1.53 (d, 3 H, J = 6.9 Hz), 2.34 (s, 3 H), 3.43 (q, 3 H, J = 1.5 Hz), 5.33 (dq, 1 H, J = 1.5, 6.9 Hz), 6.96 (m, 1 H), 7.1-7.6 (m, 9 H), minor diastereomer 1.50 (d, 3 H, J = 6.9 Hz), 2.40 (s, 3 H), 3.36 (q, 3 H, J = 1.5 Hz), 5.36 (dq, 1 H, J = 1.5, 6.9 Hz), 6.96 (m, 1 H), 7.1-7.6 (m, 9 H); ^{13}C NMR 18.99, 20.64, 20.96, 45.54, 45.61, 121.72, 126.25, 126.30, 127.45, 127.55, 127.67, 128.45, 128.60, 129.42, 130.76, 130.85, 136.00, 140.60; IR (neat) 3415, 3335, 2965, 1686, 1502, 1452, 1266, 1167, 1100, 988, 758, 715 cm^{-1}; HRMS exact mass calcd for $C_{19}H_{20}NO_2F_3$ 351.1446, found 351.1460.

Supplementary Material Available: Crystallographic unit cell parameters and atomic coordinates for the four crystal structures described in the text (5 pages). Ordering information is given on any current masthead page.

Disulfide Bond Formation Using the Silyl Chloride-Sulfoxide System for the Synthesis of a Cystine Peptide

Kenichi Akaji, Tadashi Tatsumi, Makoto Yoshida, Tooru Kimura, Yoichi Fujiwara, and Yoshiaki Kiso*

Contribution from the Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan. *Received November 18, 1991*

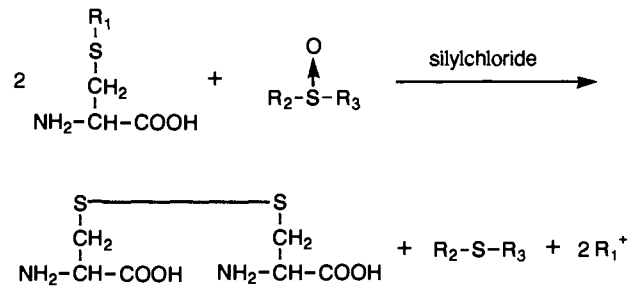
Abstract: An efficient method for disulfide bond formation in peptides by the silyl chloride-sulfoxide system is described. Methyltrichlorosilane in trifluoroacetic acid, in the presence of diphenyl sulfoxide, is found to cleave various S-protecting groups of cysteine to form cystine directly within 10-30 min. No side reactions were observed with nucleophilic amino acids such as Met, His, or Tyr, except for Trp, under the reaction conditions of the silyl chloride-sulfoxide treatment. A chlorination of the indole moiety of unprotected Trp, rather than sulfur-sulfur bond formation, is a dominant reaction when the peptide containing unprotected Trp is treated with the chlorosilane-sulfoxide. However, the disulfide bond can be formed efficiently with no modification at the indole ring by treatment of the peptide having a formyl-protected Trp residue with the silyl chloride-sulfoxide system. The formyl group is removed by a brief treatment at basic pH without affecting the disulfide bond formed by the silyl chloride-sulfoxide treatment. This new disulfide bond forming reaction in trifluoroacetic acid is successfully applied to the syntheses of oxytocin, human brain natriuretic peptide, and somatostatin without any solubility problem.

Introduction

For the synthesis of a cystine-containing peptide such as calcitonin, insulin, or a variety of growth factors, a disulfide bond forming reaction is a key step in both solution- and solid-phase syntheses. In general, air oxidation or iodine oxidation has been employed for this reaction. However, several problems associated with the formation of a disulfide bond using these conventional methods have been overlooked.[1]

Air oxidation is one of the mildest methods to construct a disulfide bond but usually requires a long reaction time (several hours to several days).[2] The reaction also requires a high dilution of reduced peptide to prevent the formation of different conformers or polymers.[3] In addition, hydrophobic or basic peptides tend to aggregate and precipitate out of the solution in spite of these mild reaction conditions since the reaction has to be conducted in aqueous medium at slightly basic pH.[4] In contrast, the disulfide bond can be formed within a relatively short time (several minutes to several hours) by iodine oxidation of the peptide having S-Acm[5] or Trt cysteine.[6] The reaction can be performed in both aqueous and organic medium at acidic pH. However, the iodine oxidation needs particularly controlled conditions since several nucleophilic amino acids such as Met, Tyr, His, and Trp are susceptible to

Scheme I



(1) (a) For earlier review, see: Hiskey, R. G. *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; Vol. 3, pp 137-167. (b) Tam, J. P.; Wu, C.-R.; Liu, W.; Zhang, J.-W. *J. Am. Chem. Soc.* **1991**, *113*, 6657-6662.

(2) Yajima, H.; Fujii, N. *J. Am. Chem. Soc.* **1981**, *103*, 5867-5871.

(3) Akaji, K.; Fujii, N.; Yajima, H.; Hayashi, K.; Mizuta, K.; Aono, M.; Moriga, M. *Chem. Pharm. Bull.* **1985**, *33*, 184-201.

(4) Lin, Y.-Z.; Ke, X.-H.; Tam, J. P. *Biochemistry* **1991**, *30*, 3310-3314.

(5) Kamber, B. *Helv. Chim. Acta* **1971**, *54*, 927-930.

(6) Kamber, B.; Rittel, W. *Helv. Chim. Acta* **1968**, *51*, 2061-2064.

0002-7863/92/1514-4137$03.00/0

Table I. Formation of Cystine by the Treatment of Boc-Cys(Acm)-OH with a Silyl Compound in the Presence of PhS(O)Ph

silyl chloride	temp, °C	10 min Cys(Acm), %	10 min cystine, %	30 min Cys(Acm), %	30 min cystine, %
$SiCl_4$	4	7	93	0	100
CH_3SiCl_3	4	15	79	0	93
$(CH_3)_3SiCl$	4	100	0	100	2
	25	98	4	94	6
$(CH_3)_4Si$	25	82	0	77	0

Table II. Formation of Cystine by the Treatment of Boc-Cys(Acm)-OH with CH_3SiCl_3 in the Presence of Sulfoxide at 4 °C

sulfoxide	10 min Cys(Acm), %	10 min cystine, %	30 min Cys(Acm), %	30 min cystine, %
PhS(O)Ph	15	79	0	93
$CH_3S(O)CH_3$	20	70	0	89
Z(OMe)-Met(O)-OH	ND[a]	90	ND[a]	88

[a] Not determined. Cys(Acm) has the same elution time as Met(O) by amino acid analysis.

iodine and often overoxidized.[7]

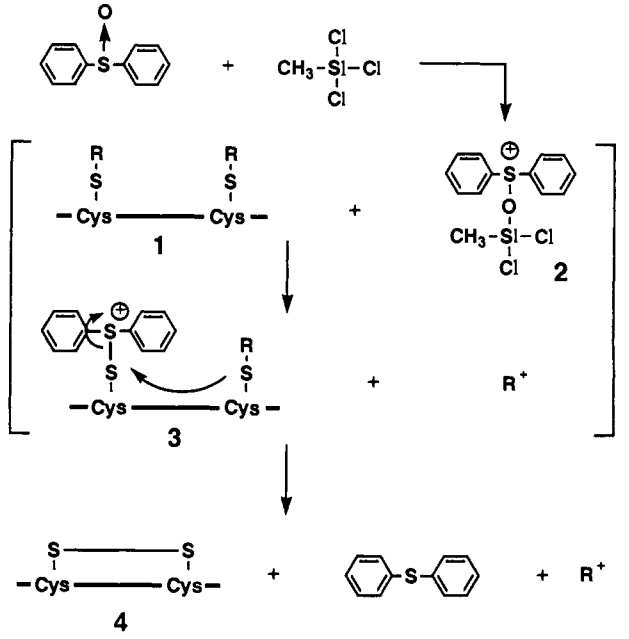
In a preliminary report,[8] we have demonstrated that methyltrichlorosilane, in the presence of diphenyl sulfoxide, can cleave various S-protecting groups of cysteine to form cystine directly (Scheme I). The reaction is rapid and completed within 10–30 min in TFA. Most of the nucleophilic amino acids can be recovered unchanged after treatment with chlorosilane. In this paper, we describe the scope of disulfide bond formation using the silyl chloride–sulfoxide system and present experimental details of the syntheses of cystine-containing peptides using this reagent system.

Results and Discussion

Disulfide Bond Formation Using the Silyl Chloride–Sulfoxide System. First, the effects of silyl chloride, sulfoxide, and the S-protecting group of cysteine on the rate of cystine formation by the silyl chloride–sulfoxide system were examined by using an amino acid analyzer. To do this, Boc-Cys(Acm)-OH[9] in TFA, in the presence of diphenyl sulfoxide, was treated with four different silyl chloride compounds, i.e., $SiCl_4$, CH_3SiCl_3, $(CH_3)_3SiCl$, and $(CH_3)_4Si$, and the recoveries of cystine and parent S-Acm cysteine were examined quantitatively by an amino acid analyzer (Table I). Formation of cystine from Cys(Acm) was completed within 30 min at 4 °C by using tetrachlorosilane or methyltrichlorosilane. Between these two silyl chloride compounds, there is no significant difference regarding the reaction rate. However, trimethylsilyl chloride converted Cys(Acm) to cystine in only 6% yield even at 25 °C after 30 min and tetramethylsilane showed no cystine formation. Since the handling and workup of methyltrichlorosilane are easier than those of tetrachlorosilane, we employed methyltrichlorosilane as a suitable silyl chloride for further experiment.

The influence of the sulfoxide compound on the reaction rate was similarly examined. Boc-Cys(Acm)-OH in TFA was treated with methyltrichlorosilane in the presence of three different sulfoxides (diphenyl sulfoxide, dimethyl sulfoxide, and Z-(OMe)-Met(O)-OH), respectively, and the progress of the reaction was monitored by using an amino acid analyzer (Table II). We observed no significant difference between the three sulfoxides examined regarding the reaction rate. S-Acm cysteine was converted to cystine within 30 min at 4 °C by treatment with

Scheme II



methyltrichlorosilane and each sulfoxide. However, small peaks of unknown products were detected on an amino acid analysis when dimethyl sulfoxide was employed. In addition, diphenyl sulfoxide, which accumulates during the progress of sulfur–sulfur bond formation as discussed below, is known to act as a soft base and have deprotection accelerating ability in TFA.[10] Thus, diphenyl sulfoxide was selected as a suitable sulfoxide compound.

We then examined the behavior under the methyltrichlorosilane–diphenyl sulfoxide treatment of the following three different kinds of S-protecting groups: amidomethyl type (Acm,[9] Tacm,[11] and Bam[12]), benzyl type (MeOBzl,[13] MeBzl,[14] and NO_2Bzl[15]), and alkyl thioether type (*t*-Bu[16] and *i*-Pr[17]). As summarized in Table III, all three protecting groups of the amidomethyl type were cleaved to form cystine as a sole product within 30 min at 4 °C. Among benzyl-type protecting groups, MeOBzl was cleaved quantitatively to form cystine within 10 min at 4 °C, but MeBzl was cleaved incompletely, 26% remaining as parent compound after the 30 min treatment, and no cleavage of NO_2Bzl was observed even after a 60-min treatment. Similarly, *t*-Bu was cleaved quantitatively within 10 min, whereas more acid stable *i*-Pr was kept intact even after a 60-min treatment with the silyl chloride–sulfoxide. Thus, the acid stability of the S-protecting group appears to influence the rate of cystine formation by the silyl chloride–sulfoxide. Among the S-protecting groups of benzyl and alkyl thioether types, only the protecting groups stable to HF[18] were quantitatively recovered unchanged. The other less acid stable S-protecting groups were cleaved to form cystine in more than 80% yield.

Next, the susceptibility of nucleophilic amino acids to the silyl chloride–sulfoxide was examined. In the presence of Met, His, and Tyr, Boc-Cys(Acm)-OH in TFA was treated with methyl-

(7) Kamber, B.; Hartmann, A.; Eisler, K.; Riniker, B.; Rink, H.; Sieber, P.; Rittel, W. *Helv. Chim. Acta* **1980**, *63*, 899–915.
(8) Akaji, K.; Tatsumi, T.; Yoshida, M.; Kimura, T.; Fujiwara, Y.; Kiso, Y. *J. Chem. Soc., Chem. Commun.* **1991**, 167–168.
(9) Veber, D. F.; Milkowski, J. D.; Varga, S. L.; Denkewalter, R. G.; Hirschmann, R. *J. Am. Chem. Soc.* **1972**, *94*, 5456–5461.
(10) Fujii, N.; Otaka, A.; Funakoshi, S.; Bessho, K.; Watanabe, T.; Akaji, K.; Yajima, H. *Chem. Pharm. Bull.* **1987**, *35*, 2339–2347.
(11) Kiso, Y.; Yoshida, M.; Fujiwara, Y.; Kimura, T.; Shimokura, M.; Akaji, K. *Chem. Pharm. Bull.* **1990**, *38*, 673–675.
(12) Chakravarty, P. K.; Olsen, P. K. *J. Org. Chem.* **1978**, *43*, 1270–1271.
(13) Akabori, S.; Sakakibara, S.; Shimonishi, Y.; Nobuhara, Y. *Bull. Chem. Soc. Jpn.* **1964**, *37*, 433–434.
(14) Erickson, B. W.; Merrifield, R. B. *J. Am. Chem. Soc.* **1973**, *95*, 3750–3756.
(15) Bachi, M. D.; Ross-Petersen, K. J. *J. Org. Chem.* **1972**, *37*, 3550–3551.
(16) Callahan, F. M.; Anderson, G. W.; Paul, R.; Zimmerman, J. E. *J. Am. Chem. Soc.* **1963**, *85*, 201–207.
(17) Gawron, O.; Lieb, J. A. *J. Am. Chem. Soc.* **1952**, *74*, 834.
(18) Sakakibara, S.; Shimonishi, Y.; Kishida, Y.; Okada, M.; Sugihara, H. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2164–2167.

Table III. Cleavage of Various S-Protecting Groups of Cysteine and Formation of Cystine by CH_3SiCl_3–PhS(O)Ph at 4 °C[a]

	10 min		30 min		60 min	
	starting compd, %	cystine, %	starting compd, %	cystine, %	starting compd, %	cystine, %
Cys(Acm)	15	79	0	93		
Cys(Tacm)	9	82	0	88		
Cys(Bam)	4	98	0	100		
Cys(*t*-Bu)	0	99				
Cys(*i*-Pr)	94	0	93	0	100	0
Cys(MeOBzl)	0	95				
Cys(MeBzl)	51	49	26	76	16	83
Cys(NO_2Bzl)	97	0	96	0	95	0

[a] Abbreviations: Acm = acetamidomethyl, Tacm = trimethylacetamidomethyl, Bam = benzamidomethyl, MeOBzl = 4-methoxybenzyl, MeBzl = 4-methylbenzyl, NO_2Bzl = 4-nitrobenzyl.

trichlorosilane–diphenyl sulfoxide at 25 °C and the recovery of each amino acid was quantified by an amino acid analysis. Each nucleophilic amino acid, as well as cystine, was recovered quantitatively even after a 60-min treatment at 25 °C. No side peak such as Met(O) was observed on an amino acid analysis. Quantitative recovery of Trp(CHO)[19] under the same reaction conditions was also confirmed similarly. However, the recovery of unmasked Trp was fairly low, which is discussed below in detail.

Possible Mechanism. A proposed pathway for the synthesis of a cystine peptide using the silyl chloride–sulfoxide system is given in Scheme II. The first interaction should be the formation of sulfonium cation **2** derived from diphenyl sulfoxide and oxygenophilic silyl compound. The formation of a sulfonium ion of this type was known and utilized for the reduction of sulfoxides using the trimethylsilyl chloride/thiol system.[20] Subsequent electrophilic attack of **2** on the sulfur atom of S-protected cysteine **1** will lead to the formation of intermediate **3**. The nature of the silyl chloride employed should be one of the main factors that influence the electrophilicity of **2**. The assumed intermediate sulfenyl compound **3** may then function as the electrophile and react with another S-protected cysteine to provide disulfide **4** and diphenyl sulfide. The presence of diphenyl sulfide has been confirmed by mass spectrometry of the reaction mixture as described in the previous paper.[8] This final step would be analogous to the reaction of a sulfenyl iodide with an S-protected cysteine in iodine oxidation studied by Kamber et al.[7] In addition, the mechanism suggests that the ability of S-protecting group to form a stabilized moiety upon cleavage of the bond between the sulfur atom and itself, like the iminium ion from S-Acm group,[7,9] may influence the rate of disulfide bond formation by the silyl chloride–sulfoxide system. We consider the proposed mechanism can provide an explanation for our observation that the reaction rate of cystine formation largely depends on the nature of the silyl chloride and the S-protecting group.

Synthesis of Oxytocin. From the model studies described above, it appears feasible to apply the silyl chloride–sulfoxide system to peptides carrying no further protecting groups other than those of cysteine thiol groups. To examine the feasibility of this method, the simple nonapeptide amide oxytocin (**5**) was synthesized by

H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

5

the combination of the silyl chloride–sulfoxide system and three different S-protecting groups (Acm, Tacm, and *t*-Bu). [Cys(Acm)[1,6]]- or [Cys(Tacm)[1,6]]-oxytocin was prepared by Fmoc-based solid-phase synthesis[21] followed by deprotection with 1 M HBF_4–thioanisole.[22] Conventional solution-phase synthesis and TFA deprotection were employed for the preparation of [Cys(*t*-Bu)[1,6]]-oxytocin. Each purified S-protected oxytocin in TFA was treated with methyltrichlorosilane–diphenyl sulfoxide at 25 °C and the reaction mixture was examined by analytical HPLC (Figure 1). Each reaction was completed within 10 min and no significant intermolecular disulfide bond formation was observed on any chromatogram. The product was isolated by fast protein liquid chromatography (FPLC). The purified oxytocin possessed the same elution time on the basis of analytical HPLC as that of an authentic sample purchased from Peptide Institute Inc., Osaka.

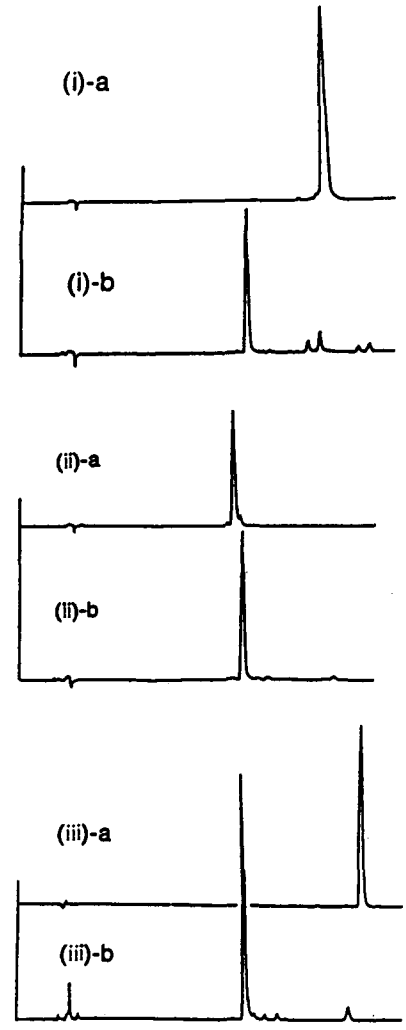


Figure 1. HPLC of the reaction mixture after a 10-min treatment of S-protected oxytocin with CH_3SiCl_3–PhS(O)Ph at 25 °C. Eluate was monitored by UV measurement at 230 nm. (i, a) [Cys(Tacm)[1,6]]-Oxytocin; (i, b) reaction mixture after the treatment of (i, a); (ii, a) [Cys(Acm)[1,6]]-oxytocin; (ii, b) reaction mixture after the treatment of (ii, a); (iii, a) [Cys(*t*-Bu)[1,6]]-oxytocin; (iii, b) reaction mixture after the treatment of (iii, a).

Synthesis of Human Brain Natriuretic Peptide (hBNP). hBNP consists of 32 amino acid residues, including two Met and a His

(19) Previero, A.; Antonia, M.; Previero, C.; Cavadore, J. C. *Biochim. Biophys. Acta* **1967**, *147*, 453–461.
(20) Numata, T.; Togo, H.; Oae, S. *Chem. Lett.* **1979**, 329–332.
(21) For review, see: Atherton, E.; Sheppard, R. C. *The Peptides*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: New York, 1987; Vol. 9, pp 1–38.
(22) Akaji, K.; Yoshida, M.; Tatsumi, T.; Kimura, T.; Fujiwara, Y.; Kiso, Y. *J. Chem. Soc., Chem. Commun.* **1990**, 288–290.

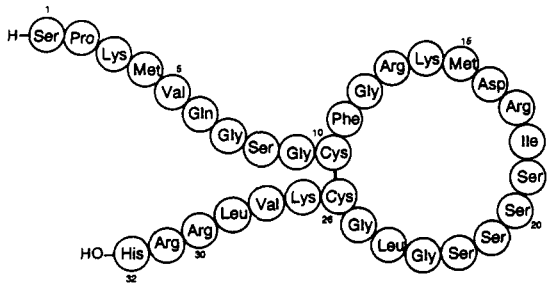


Figure 2. Amino acid sequence and disulfide pairing of human brain natriuretic peptide (hBNP).

Table IV. Recoveries of Trp, Cystine, and Cys(Acm) Derivative after Treatment of Boc-Cys(Acm)-OH with CH_3SiCl_3–PhS(O)Ph at 25 °C in the Absence or Presence of Scavenger

	scavenger					
	none		anisole		3-methylindole	
	30 min	60 min	30 min	60 min	30 min	60 min
Trp	0.47	0.43	0.36	0.27	0.52	0.68
cystine	0.24	0.47	0.37	0.81	0	0
Cys(Acm)	0.74	0.29	0.67	0.27	0.95	0.95
Gly[a]	1.00	1.00	1.00	1.00	1.00	1.00

[a] Internal standard.

residues, and contains one disulfide bond in the molecule[23] (Figure 2). The synthesis of hBNP appears to be a suitable test for the silyl chloride–sulfoxide method in the practical synthesis of a cystine-containing peptide, since we have observed the formation of a large amount of the Met(O) derivative during the synthesis of structurally homologous porcine BNP using an iodine-oxidation method.[24] [Cys(Tacm)^{10,26}]- or [Cys(Acm)^{10,26}]-hBNP was prepared by the combination of Fmoc-based solid-phase peptide synthesis and HBF_4 deprotection followed by FPLC purification as described above in oxytocin synthesis. The purified S-protected hBNP in TFA was treated with methyltrichlorosilane–diphenyl sulfoxide at 25 °C for 10 min to form the disulfide bond (Figure 3). The crude product gave single main peak on HPLC, showing no significant modification occurred on the peptide chain. After purification by FPLC, the homogeneous peptide had physicochemical properties identical with those of an authentic sample prepared by air oxidation.[25]

Behavior of Unmasked Trp. As shown in Table IV, Trp is an amino acid susceptible to the silyl chloride–sulfoxide treatment. The recovery of Trp was 43% after a 60-min treatment of Boc-Cys(Acm)-OH and unmasked Trp with methyltrichlorosilane–diphenyl sulfoxide at 25 °C. On this treatment, 29% of starting Cys(Acm) derivative was recovered unchanged. In an attempt to improve the recovery of Trp by the addition of a cation scavenger, anisole or 3-methylindole, to the reaction mixture, no significant improvement of the Trp recovery was observed, although 3-methylindole has been known to be one of the best cation scavenger to prevent the alkylation of the indole ring of Trp.[26] In addition, no cystine formation was observed even after a 60-min treatment with the chlorosilane–sulfoxide in the presence of 3-methylindole. From these model studies, the presence of an indole ring in the reaction mixture seemed to lower significantly the reaction rate of sulfur–sulfur bond formation by the silyl chloride–sulfoxide system.

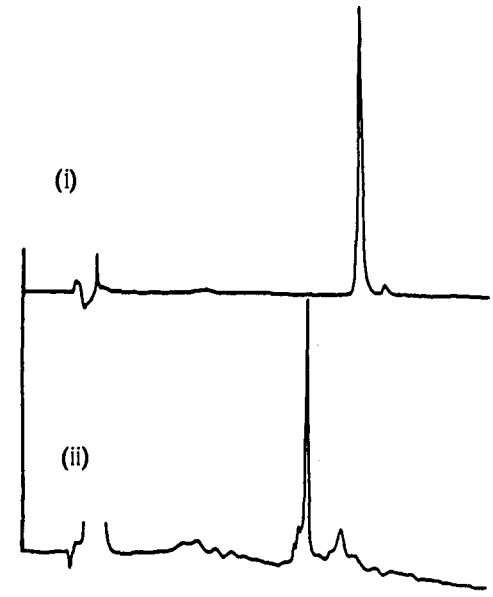


Figure 3. HPLC of the reaction mixture after a 10-min treatment of S-protected hBNP with CH_3SiCl_3–PhS(O)Ph at 25 °C. Eluate was monitored by UV measurement at 230 nm. (i) [Cys(Tacm)^{10,26}]-hBNP; (ii) reaction mixture after the treatment of (i).

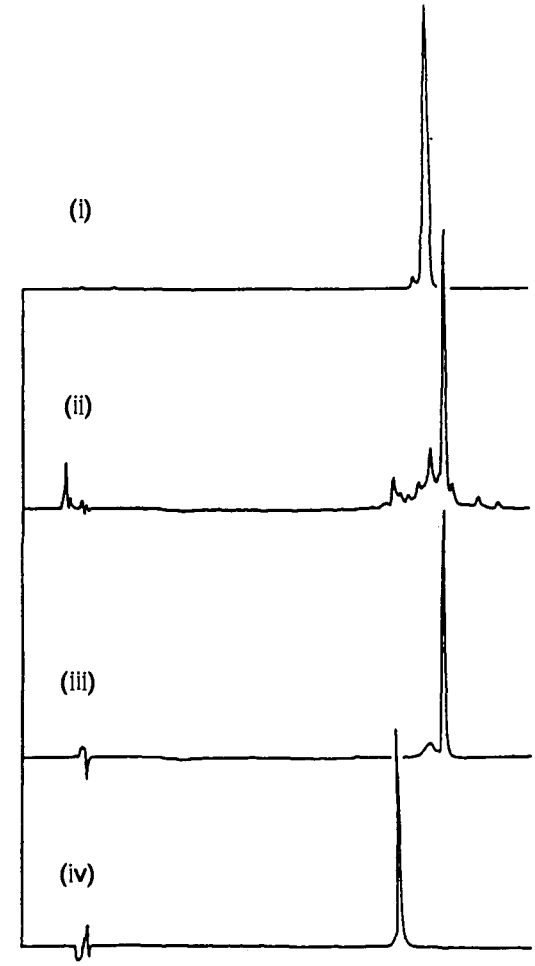
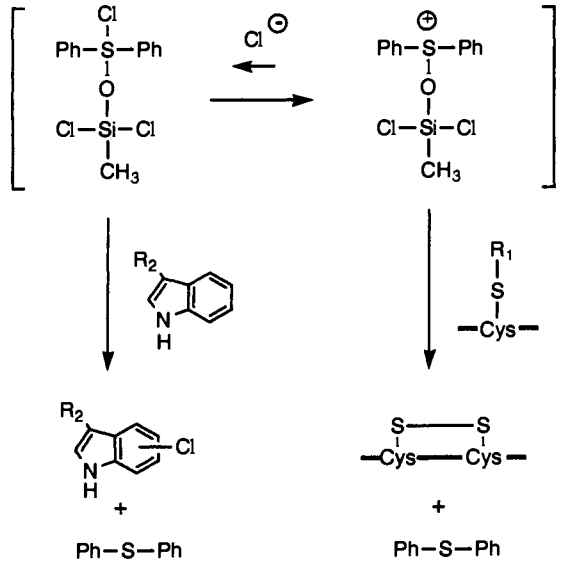


Figure 4. HPLC of the reaction mixture and the product after a 60-min treatment of S-protected somatostatin with CH_3SiCl_3–PhS(O)Ph at 4 °C. Eluate was monitored by UV measurement at 280 nm. (i) [Cys(Tacm)^{3,14}]-Somatostatin; (ii) reaction mixture after the treatment of (i); (iii) purified product of (ii); (iv) an authentic somatostatin.

In order to examine the effect of the unmasked Trp residue for the practical synthesis of a cystine peptide by the silyl chloride–

(23) Sudoh, T.; Maekawa, K.; Kojima, M.; Minamino, N.; Kangawa, K.; Matsuo, H. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 1427–1434.
(24) Yoshida, M.; Tatsumi, T.; Fujiwara, Y.; Iinuma, S.; Kimura, T.; Akaji, K.; Kiso, Y. *Chem. Pharm. Bull.* **1990**, *38*, 1551–1557.
(25) Yoshida, M.; Akaji, K.; Tatsumi, T.; Fujiwara, Y.; Kimura, T.; Kiso, Y. *Peptide Chemistry 1989*; Yanaihara, N., Ed.; Protein Research Foundation: Osaka, 1990; pp 33–38.
(26) (a) Löw, M.; Kisfaludy, L.; Sohar, P. *Z. Physiol. Chem.* **1978**, *359*, 1643–1651. (b) Wünsch, E.; Fontana, A.; Drees, F. *Z. Naturforsch.* **1967**, *22b*, 607–609.

Scheme III



sulfoxide system, we undertook the solid-phase synthesis of somatostatin (**6**). Somatostatin, consisting of 14 amino acid residues

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

6

and containing one disulfide bridge and a Trp residue, has been used as a suitable model peptide to evaluate the methods of disulfide bond formation in the presence of an indole ring.[27] [Cys(Tacm)^{3,14}]-somatostatin was prepared by using the unmasked Trp derivative by the same method as reported before[24] and was purified by FPLC. The S-protected somatostatin was then treated with methyltrichlorosilane–diphenyl sulfoxide at 4 °C and the reaction was monitored by HPLC (Figure 4). After a 60-min treatment, the starting S-protected somatostatin disappeared and a single major peak appeared. The product having a single peak on HPLC was obtained with 26% yield after FPLC purification of the reaction mixture.

However, the retention time of the purified product was different from that of an authentic sample purchased from Peptide Institute Inc. No cystine or Trp was recovered after LAP digestion of the product, whereas Cys(Tacm), instead of cystine, was recovered. By the sequencing of the product, Trp was not detected although all other amino acids, except for Cys, were detected in the order predicted from the sequence. These findings suggest that a single modification at the Trp residue occurred during the treatment of S-protected somatostatin having unprotected Trp with the chlorosilane–sulfoxide system. The purified product gave the mass value $[M + H]^+$ of 1899.9 in the FAB mass spectrum. The value is 34 mass value higher than that expected for [Cys(Tacm)^{3,14}]-somatostatin, which strongly suggests that the replacement of the H atom of the indole ring with a Cl atom occurred during the treatment. In addition, the theoretical isotopic mass distribution expected for the Cl replaced S-protected somatostatin agreed very well with the observed spectrum. The same results were obtained when the [Cys(Acm)^{3,14}]-somatostatin, instead of the corresponding Cys(Tacm) derivative, was treated with the silyl chloride–sulfoxide.

Thus, it has been shown that a chlorination of the indole moiety of unprotected Trp, rather than the disulfide bond formation, is a dominant reaction when the peptide containing an unmasked Trp residue is treated with the silyl chloride–sulfoxide system. We consider that the indole ring may work as an excellent scavenger to push the reaction to simple reduction of the sulfoxide as in the case of reductive acidolysis[28] (Scheme III).

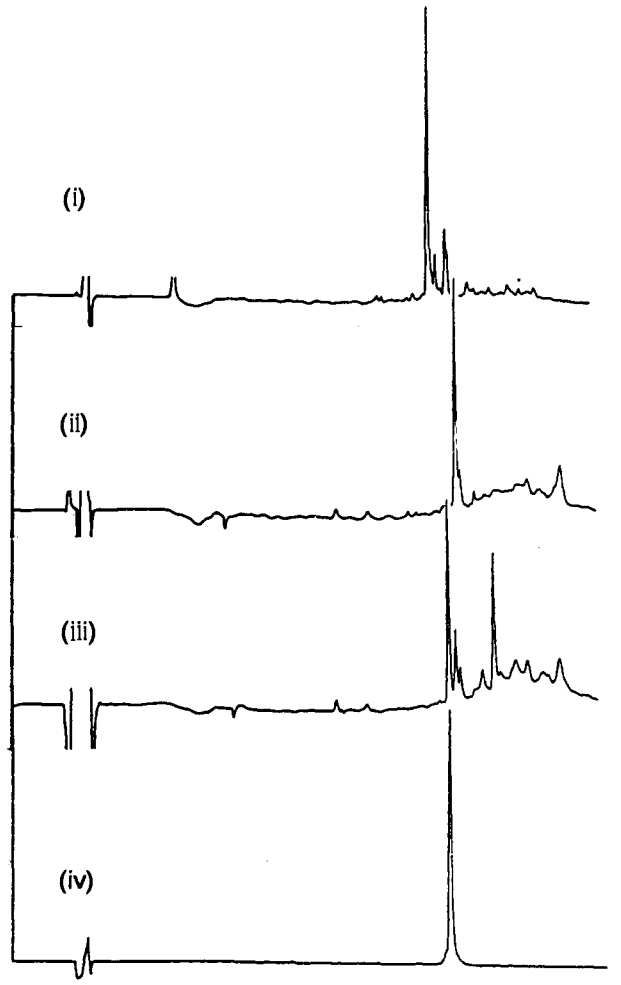


Figure 5. HPLC of the reaction mixture and the product for the synthesis of somatostatin. Eluate was monitored by UV measurement at 280 nm. (i) Crude [Cys(Tacm)^{3,14}, Trp(CHO)^{8}]-somatostatin; (ii) reaction mixture after a 15-min treatment of (i) with CH_3SiCl_3–PhS(O)Ph at 25 °C; (iii) reaction mixture after a 2-min treatment of (ii) at pH 11; (iv) coelution of the purified product and an authentic somatostatin.

Synthesis of Somatostatin. In order to avoid the modification at the Trp residue described above, we have adopted Trp(CHO) for the synthesis of somatostatin using the silyl chloride–sulfoxide system. The N^{in}-CHO group of Trp is known to be stable to acid but removable by a short treatment with NaOH without affecting the disulfide bond.[29] [Cys(Acm)^{3,14},Trp(CHO)^{8}]-Somatostatin was prepared by the conventional Boc-based solid-phase method followed by high HF cleavage.[30] The existence of the CHO group in the product was confirmed by FAB mass spectrometry since the elution time of the product was not distinguishable from that of unformylated [Cys(Acm)^{3,14}]-somatostatin on HPLC.

To construct the disulfide bond, the N^{in}-CHO, S-protected somatostatin in TFA was treated with methyltrichlorosilane–diphenyl sulfoxide at 25 °C. After a 15-min treatment, the starting compound disappeared and the product having a single major peak on HPLC was obtained. The product was then treated with aqueous NaOH for 2 min to give the crude somatostatin. The whole sequence of the reaction can be monitored on HPLC (Figure 5). After purification by FPLC, the homogeneous peptide having the same elution time on HPLC with that of an authentic sample

(27) (a) Sieber, P.; Kamber, B.; Riniker, B.; Rittel, W. *Helv. Chim. Acta* **1980**, *63*, 2358–2363. (b) McCurdy, S. N. *Peptide Res.* **1989**, *2*, 147–152.

(28) Kiso, Y.; Kimura, T.; Yoshida, M.; Shimokura, M.; Akaji, K.; Mimoto, T. *J. Chem. Soc., Chem. Commun.* **1989**, 1511–1513.

(29) Kumagaye, S.; Kuroda, H.; Nakajima, K.; Watanabe, T.; Kimura, T.; Masaki, T.; Sakakibara, S. *Int. J. Peptide Protein Res.* **1988**, *32*, 519–526.

(30) (a) For review on Boc-based solid-phase method, see: Barany, G.; Merrifield, R. B. *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1980; Vol. 2, pp 1–284. (b) For review on HF cleavage, see: Tam, J. P.; Merrifield, R. B. *The Peptides*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: New York, 1987; Vol. 9, pp 185–248.

(Peptide Institute) was obtained. The integrity of the purified product was further determined by FAB mass spectrometry. The observed mass value and the isotopic distribution agreed well with the expected value of $[M + H]^+$ and the theoretical isotopic mass distribution, respectively.

Conclusion

It is shown in this work that a disulfide bond can be formed directly from an S-protected cysteine derivative by treatment with the silyl chloride–sulfoxide system within 10–30 min. The reaction is much faster than conventional air oxidation. During the silyl chloride–sulfoxide treatment, nucleophilic amino acids, except for Trp, can be kept unchanged and no oxidation at Met, Tyr, or His residues is observed. A Trp-containing cystine peptide can be synthesized by employing the Trp(CHO) derivative. The N^{in}-CHO group of Trp is removed by brief treatment with NaOH after the formation of a disulfide bond by using the silyl chloride–sulfoxide system. The reaction of the silyl chloride–sulfoxide system is conducted in TFA, which remarkably alleviates the insolubility problem of the hydrophobic peptide, since TFA dissolves most of the peptide freely. The present alternative disulfide bond forming reaction will be useful for developing a regioselective method to form multiple disulfide bonds by combination with methods hitherto employed for the synthesis of a cystine peptide.

Experimental Section

General. Melting points are uncorrected. Amino acid analysis was conducted with a Hitachi L8500 amino acid analyzer utilizing ion-exchange chromatography, which eluted citrate buffer, and postcolumn ninhydrin detection. Optical rotation was determined with a Union PM-101 polarimeter. TLC was effected with silica gel (Kieselgel 60 F_{254}, Merck) on a precoated aluminum sheet. R_f values refer to the following solvent systems: R_{f_1} $CHCl_3$–CH_3OH–H_2O (8:3:1), R_{f_2} *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2). The TLC spot was detected by visualization with ninhydrin. Analytical HPLC characterization was carried out on a YMC AM302 column (4.6 × 150 mm), which was eluted with a linear gradient of CH_3CN (10–60%, 30 min) in 0.1% aqueous TFA at a flow rate of 0.7 mL/min. FPLC (Pharmacia) was carried out with a YMC ODS-AQ300 column (1.5 × 50 cm) eluted with a linear gradient of 60% CH_3CN/0.1% aqueous TFA (0–100%, 400 min) in 0.1% aqueous TFA at the flow rate of 3.0 mL/min. The eluate was monitored by measuring the UV absorption at 230 and 254 nm. The solvent of desired fractions was removed by lyophilization. Sequence analysis was carried out by using an Applied Biosystems 470A gas-phase protein sequencer equipped with a 120A PTH analyzer. FAB mass spectra were obtained on a JEOL JMS-SX102 spectrometer equipped with the data processor JMA-DA6000 or on a JEOL JMS-D300 instrument. Cys(Tacm), Cys(Bam), Cys(*i*-Pr), and Cys(NO_2Bzl) derivatives were prepared according to the published procedures. Boc-Cys(Acm)-OH, Boc-Cys(MeOBzl)-OH, Boc-Cys(MeBzl)-OH, Boc-Cys(*t*-Bu)-OH, and Boc-Trp(CHO)-OH were purchased from Peptide Institute Inc. (Osaka, Japan). Fmoc amino acid derivatives, 4-(benzyloxy)benzyl alcohol resin (Wang resin), and 4-methylbenzhydrylamine (MBHA) resin were purchased from Nova Biochem (Switzerland). Boc-Cys(Acm) linked to Merrifield resin and Fmoc-His(Bum) linked to Wang resin were purchased from Watanabe Chemical Ind. (Hiroshima, Japan). Leucine-aminopeptidase (LAP, lot No. 117F-8085) was purchased from Sigma.

General Protocol for the Determination of Cystine Formation (Tables I–IV). A mixture of an S-protected cysteine derivative (20 μmol) and Gly (internal standard, ca. 20 μmol) in TFA (500 μL) was treated with silyl chloride (20 equiv) in the presence of sulfoxide (5 equiv). The reaction temperature and the reaction time are shown in each table. Periodically, an aliquot was taken from the reaction mixture. The sample was then diluted with 0.02 N HCl and the solution was analyzed on an amino acid analyzer. The amounts of cystine formed and remaining parent S-protected cysteine derivative are presented in each Table. To examine the susceptibility of nucleophilic amino acids to the silyl chloride–sulfoxide, a mixture of amino acids (Met, His, Tyr, ca. 20 μmol each) and Boc-Trp(CHO)-OH (16 μmol) was added to the reaction mixture as well as Gly. The reaction was conducted at 25 °C and the recovery of each amino acid or Trp(CHO) was also determined similarly by an amino acid analysis. In the experiment of Table IV, Boc-Trp-OH (ca. 20 μmol) or Boc-Trp-OH plus additional scavenger (anisole or 3-methylindole, 5 equiv) was also added to the reaction mixture. The reaction was conducted at 25 °C and the recovery of Trp was determined similarly. The retention times of S-protected cysteine derivatives and side-chain protected amino acid derivatives from an amino acid analysis are as follows: Cys(Acm) 11.94 min, Cys(Tacm) 12.50 min, Cys(Bam) 57.38 min (same as Leu), Cys(*t*-Bu) 43.01 min, Cys(*i*-Pr) 41.22 min, Cys(MeOBzl) 82.50 min (same as Trp), Cys(MeBzl) 85.04 min, Cys(NO_2Bzl) 82.42 min (same as Trp), Met(O) 11.70 min (same as Cys(Acm)), Trp(CHO) 88.00 min (same as Arg). Quantitative recovery of cystine (49.81 min) was somewhat difficult to obtain by amino acid analysis presumably due to the low solubility of cystine in the analysis buffers.

S-Protected Oxytocin. [Cys(Tacm)^{1,6}]- and [Cys(Acm)^{1,6}]-Oxytocin. Both S-Tacm and Acm oxytocin resins were prepared manually by the solid-phase method with MBHA resin (300 mg each, amine content 0.237 mmol). Fmoc amino acid or the S-protected derivative was condensed by the use of DIPCDI (2.5 equiv) in the presence of HOBt (2.5 equiv) according to the published schedule.[31] Each S-protected oxytocin resin (100 mg) was then treated with 1 M HBF_4–thioanisole in TFA (6.5 mL) in the presence of *m*-cresole (0.2 mL) and EDT (0.5 mL) in an ice bath for 90 min. Dry ether was added to precipitate the product. The product was extracted with 6 M guanidine (5 mL) and the resin was removed by filtration. The filtrate was purified by FPLC to afford a white powder. [Cys(Tacm)^{1,6}]-Oxytocin: yield 15.5 mg (31%), R_{f_2} 0.63, retention time on an analytical HPLC 19.85 min. Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.98 (ND); Glu × 1, 1.02 (ND); Pro × 1, 1.01 (0.38); Gly × 1, 1.00 (1.00); Ile × 1, 0.96 (1.09); Leu × 1, 0.99 (1.03); Tyr × 1, 0.97 (1.02); Cys(Tacm) × 2, ND (1.40). [Cys(Acm)^{1,6}]-Oxytocin: yield 15.5 mg (32%), R_{f_2} 0.41, retention time on an analytical HPLC 13.97 min. Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.96 (ND); Glu × 1, 1.00 (ND); Pro × 1, 0.96 (1.09); Gly × 1, 1.00 (1.00); Ile × 1, 0.98 (1.00); Leu × 1, 0.98 (1.01); Tyr × 1, 0.97 (1.00); Cys(Acm) × 2, ND (1.99).

[Cys(*t*-Bu)^{1,6}]-Oxytocin. Starting from H-Pro-Leu-Gly-NH_2,[32] [N^{α}-Boc, Cys(*t*-Bu)^{1,6}]-oxytocin was prepared in solution by using essentially the same stepwise method as described in the literature.[33] Cys(*t*-Bu), Asn, Gln, and Ile were condensed by the active ester method and Tyr was introduced by the azide method: mp 227–229 °C, $[\alpha]_D^{28}$ −42.1° (*c* 0.5, DMF), R_{f_1} 0.61. Anal. Calcd for $C_{56}H_{92}N_{12}O_{14}S_2 \cdot H_2O$: C, 54.26; H, 7.64; N, 13.56. Found: C, 53.98; H, 7.79; N, 13.48. The above protected nonapeptide amide (50 mg) was then treated with TFA–anisole (1 mL–0.44 mL) in an ice bath for 60 min. Dry ether was added to the reaction mixture and the product was extracted with 1 N AcOH (5 mL). The aqueous solution was applied to FPLC to give a purified title compound: yield 42 mg (91%), R_{f_2} 0.78, retention time on an analytical HPLC 23.18 min. Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses), Asp × 1, 0.98 (ND); Glu × 1, 0.97 (ND); Pro × 1, 1.01 (1.05); Gly × 1, 1.00 (0.93); Ile × 1, 0.98 (0.99); Leu × 1, 0.99 (1.00); Tyr × 1, 0.98 (0.96); Cys(*t*-Bu) × 2, ND (2.14).

Oxytocin. [Cys(Tacm)^{1,6}]-Oxytocin (9.8 mg) dissolved in TFA (2.7 mL) was treated with CH_3SiCl_3 (93 μL, 100 equiv) in the presence of PhS(O)Ph (16 mg, 10 equiv) and anisole (86 μL, 100 equiv) at 25 °C. After being stirred for 10 min, dry ether (50 mL) and 1 N AcOH (10 mL) were added to the reaction mixture. The analytical HPLC pattern of the product in the aqueous phase is shown in Figure 1, (i). The product in the aqueous phase was purified by FPLC to give a product having a sharp single peak on an analytical HPLC: yield 5.5 mg (69%), $[\alpha]_D^{30}$ −22.0° (*c* 0.3, H_2O) (lit.[34] −26.2° in H_2O), R_{f_2} 0.5; retention time on an analytical HPLC, 15.26 min (same as the authentic sample purchased from Peptide Institute). Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.98 (ND); Glu × 1, 1.00 (ND); Pro × 1, 0.95 (0.61); Gly × 1, 1.00 (1.00); Cys × 1, 0.76 (0.52); Ile × 1, 0.97 (1.05); Leu × 1, 0.99 (1.08); Tyr × 1, 0.92 (1.09). Purified oxytocin, 6.6 mg (56%) and 8.6 mg (64%), was also obtained from [Cys(Acm)^{1,6}]-oxytocin (13.4 mg in 3.5 mL of TFA) and [Cys(*t*-Bu)^{1,6}]-oxytocin (15.0 mg in 4.5 mL of TFA), respectively, by the same treatment with CH_3SiCl_3–PhS(O)Ph as described above. The analytical HPLC pattern of each crude product is shown in Figure 1, (ii) or (iii).

Synthesis of Human Brain Natriuretic Peptide (hBNP). S-Protected hBNP. Protected hBNP resin was prepared by the solid-phase method starting from Fmoc-His(Bum) linked to a Wang resin (175 mg, 0.1 mmol). The following Fmoc amino acids and derivatives were used to construct the peptide chain on the resin: Gly, Val, Met, Ile, Leu, Phe,

(31) Akaji, K.; Fujii, N.; Tokunaga, F.; Miyata, T.; Iwanaga, S.; Yajima, H. *Chem. Pharm. Bull.* **1989**, *37*, 2661–2664.
(32) Ressler, C.; du Vigneaud, V. *J. Am. Chem. Soc.* **1954**, *76*, 3107–3109.
(33) (a) Vandesande, F. *Bull. Soc. Chim. Belg.* **1969**, *78*, 395–406. (b) Sakakibara, S.; Nobuhara, Y.; Shimonishi, Y.; Kiyoi, R. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 120–123.
(34) du Vigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C. W.; Katsoyannis, P. G.; Gordon, S. *J. Am. Chem. Soc.* **1953**, *75*, 4879–4880.

Pro, Gln, Asp(O-t-Bu), Ser(t-Bu), Lys(Boc), Arg(Mtr), and Cys(Tacm) or Cys(Acm). The construction of the peptide chain was carried out manually as described above in oxytocin synthesis. Fully protected [Cys(Tacm)^{10,26}]-hBNP resin (150 mg) thus obtained was treated with 1 M HBF_4–thioanisole in TFA (8 mL) in the presence of *m*-cresol (0.2 mL) and EDT (0.5 mL) in an ice bath for 90 min. The crude product was extracted with 6 M guanidine (5 mL). The solution was applied to a column of Sephadex G-15 (3.2 × 42 cm), which was eluted with 1 N AcOH. The eluate was monitored by measuring the UV absorption at 254 nm. The solvent of the fractions corresponding to the main peak was removed by lyophilization to give a powder (77 mg). The product was further purified by FPLC to afford a white fluffy powder. Fully protected [Cys(Acm)^{10,26}]-hBNP resin (200 mg) was deprotected with 1 M HBF_4–thioanisole and the product was purified similarly. [Cys(Tacm)^{10,26}]-hBNP: yield 17 mg (28%), retention time on an analytical HPLC 15.7 min. Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.93 (0.95); Ser × 6, 5.05 (5.42); Glu × 1, 0.96 (ND); Pro × 1, 0.97 (0.85); Gly × 5, 4.93 (4.73); Val × 2, 1.84 (1.89); Met × 2, 1.79 (1.96); Ile × 1, 0.96 (0.99); Leu × 2, 2.00 (2.00); Phe × 1, 0.98 (0.96); Lys × 3, 2.94 (2.76); His × 1, 0.98 (0.92); Arg × 4, 3.86 (3.63); Cys(Tacm) × 2, ND (1.99). [Cys(Acm)^{10,26}]-hBNP: yield 15 mg (15%), retention time on an analytical HPLC 13.56 min. Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 1.05 (0.90); Ser × 6, 5.10 (5.28); Glu × 1, 0.93 (ND); Pro × 1, 0.92 (0.78); Gly × 5, 4.86 (4.60); Val × 2, 1.83 (1.84); Met × 2, 1.79 (1.91); Ile × 1, 1.00 (1.01); Leu × 2, 2.00 (2.00); Phe × 1, 0.97 (0.94); Lys × 3, 2.76 (2.70); His × 1, 1.00 (0.95); Arg × 4, 3.80 (3.60); Cys(Acm) × 2, ND (1.83).

hBNP. [Cys(Tacm)^{10,26}]-hBNP (8.4 mg) in TFA (8.4 mL) was treated with CH_3SiCl_3 (27 μL, 100 equiv) in the presence of PhS(O)Ph (4.6 mg, 10 equiv) and anisole (25 μL, 100 equiv) at 25 °C for 10 min. Dry ether (50 mL) and 1 N AcOH (10 mL) were added to the reaction mixture. The analytical HPLC pattern of the product in the aqueous phase is shown in Figure 3. The crude product was purified by FPLC to give a sample having a sharp single peak on an analytical HPLC: yield 3.9 mg (50%), $[\alpha]_D^{30}$ −57.1° (*c* 0.2, 1 N, AcOH), retention time on an analytical HPLC 13.20 min (same as the authentic sample prepared by air oxidation[25]). Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 1.03 (0.90); Ser × 6, 4.66 (5.46); Glu × 1, 0.92 (ND); Pro × 1, 0.97 (0.87); Gly × 5, 4.88 (4.81); Val × 2, 1.93 (1.95); Cys × 1, 0.40 (0.97); Met × 2, 1.87 (2.01); Ile × 1, 1.00 (1.07); Leu × 2, 2.00 (2.00); Phe × 1, 1.03 (1.01); Lys × 3, 2.85 (2.90); His × 1, 0.95 (0.96); Arg × 4, 3.85 (3.88). Purified hBNP, 4.2 mg (44%), was also obtained from [Cys(Acm)^{10,26}]-hBNP (10 mg in 10 mL of TFA) by the 10-min treatment with CH_3SiCl_3 (100 equiv)–PhS(O)Ph (10 equiv) in the presence of anisole (100 equiv).

Treatment of the S-Protected Somatostatin Derivative Having an Unmasked Trp Residue with the Silyl Chloride–Sulfoxide. The crude [Cys(Tacm)^{3,14}]-somatostatin was prepared by Fmoc-based solid-phase synthesis followed by deprotection with HBF_4 according to the published procedure[24] and was purified by FPLC to afford the product having a single main peak on an analytical HPLC. The purified [Cys(Tacm)^{3,14}]-somatostatin (10 mg) in TFA (3.3 mL) was treated with CH_3SiCl_3 (65 μL, 100 equiv) in the presence of PhS(O)Ph (11.2 mg, 10 equiv) and anisole (60 μL, 100 equiv) in an ice bath for 60 min. The crude product was purified by FPLC. The analytical HPLC patterns of crude and purified samples are shown in Figure 4. [Cys(Acm)^{3,14}]-Somatostatin (7.5 mg) was similarly prepared and treated with CH_3SiCl_3–PhS(O)Ph. The product was purified as mentioned above. Product obtained from [Cys(Tacm)^{3,14}]-somatostatin: yield 2.6 mg (26%), R_{f_2} 0.30, retention time on an analytical HPLC 22.76 min. Amino acid ratios after 4 M MSA hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.97 (ND); Thr × 2, 1.58 (2.12); Ser × 1, 0.46 (0.99); Gly × 1, 1.00 (1.00); Ala × 1, 1.00 (0.97); Phe × 3, 2.69 (2.95); Lys × 2, 1.85 (1.94); Cys(Tacm) × 2, ND (1.50). No Trp or Cys was detected. The predicted sequence for somatostatin, except for Cys and Trp, was confirmed by sequencing analysis. An unknown peak was detected at the position of Trp by the sequencing. FAB-MS (obtained on a JEOL JMS-SX102), *m/z* 1899.9 for $[M + H]^+$ and 1900.9 for a base peak in the molecular ion region (calcd 1899.869 for $[M + H]^+$ of $C_{88}H_{128}N_{20}O_{21}S_2Cl$ and 1900.872 for the base peak). Product obtained from [Cys(Acm)^{3,14}]-somatostatin: yield 2.2 mg (29%), R_{f_2} 0.20, retention time on an analytical HPLC 20.11 min. Amino acid ratios after 4 M MSA hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.92 (ND); Thr × 2, 1.71 (1.84); Ser × 1, 0.55 (0.86); Gly × 1, 1.00 (1.00); Ala × 1, 0.96 (0.93); Phe × 3, 2.52 (2.77); Lys × 2, 1.85 (1.90); Cys(Acm) × 2, ND (1.99). No Trp or Cys was detected. The predicted sequence for somatostatin, except for Cys and Trp, was confirmed by sequencing analysis. An unknown peak was detected at the position of Trp by the sequencing. FAB-MS (obtained on a JEOL JMS-SX102), *m/z* 1815.7 for $[M + H]^+$ (calcd 1815.775 for $[M + H]^+$ of $C_{82}H_{115}N_{20}O_{21}S_2Cl$).

Synthesis of Somatostatin. [Cys(Acm)^{3,14}, Trp(CHO)^8]-Somatostatin. A fully protected somatostatin resin was prepared by Boc-based solid-phase synthesis starting from Boc-Cys(Acm) linked to Merrifield resin (250 mg, 0.11 mmol). The following Boc amino acids and derivatives were used: Phe, Asn, Gly, Ala, Ser(Bzl), Thr(Bzl), Lys(ClZ), Trp-(CHO), and Cys(Acm). The construction of the peptide chain was conducted by the use of DIPCDI (2.5 equiv) in the presence of HOBt (2.5 equiv). The resulting protected somatostatin resin (240 mg) was treated with HF (6 mL) in the presence of *m*-cresol (0.14 mL) at 4 °C for 60 min. HF was removed by evaporation at 0 °C. After the residue was washed with dry ether, the product was extracted with 6 M guanidine (5 mL). The solution was filtered and the filtrate was applied to a column of Sephadex G-15 (3.2 × 50 cm), which was eluted with 4 N AcOH. The eluate was monitored by measuring the UV absorption at 280 nm. The solvent of the desired fractions was removed by lyophilization to give a white powder: yield 120 mg (61%), retention time on an analytical HPLC 19.28 min. Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.96 (ND); Thr × 2, 1.66 (2.12); Ser × 1, 0.43 (0.96); Gly × 1, 1.00 (1.00); Ala × 1, 0.99 (1.07); Phe × 3, 2.73 (2.91); Lys × 2, 1.89 (2.00); Trp × 1, ND (0.73); Cys(Acm) × 2, ND (2.13). FAB-MS (obtained on a JEOL JMS-D300), *m/z* 1811 for $[M + H]^+$ (calcd 1811.05 for $C_{83}H_{116}N_{20}O_{22}S_2$).

Somatostatin. [Cys(Acm)^{3,14}, Trp(CHO)^8]-somatostatin (17 mg) in TFA (8.5 mL) was treated with CH_3SiCl_3 (110 μL, 100 equiv) in the presence of PhS(O)Ph (19 mg, 10 equiv) and anisole (102 μL, 100 equiv) at 25 °C for 15 min. Dry ether (50 mL) and 4 N AcOH (10 mL) were added to the reaction mixture. The pH of the aqueous phase was adjusted to 11 with 4 N NaOH and the solution was stirred in an ice bath for 2 min. The pH of the solution was adjusted to 6 with 1 N HCl. The progress of each reaction was monitored on an analytical HPLC (Figure 5). Diaion HP-20 (ca. 10 g) was added to the solution and the mixture was stirred for 60 min. The resin was collected by filtration and washed twice with H_2O (200 mL). The peptide was eluted from the resin with 60% CH_3CN in 1 N AcOH. After the removal of CH_3CN by evaporation at room temperature, the remaining solvent was removed by lyophilization to give a powder. The product was further purified by FPLC to give a product showing a sharp single peak by analytical HPLC: yield 1.5 mg (10%), R_{f_2} 0.23, retention time on an analytical HPLC 20.29 min (same as the authentic sample purchased from Peptide Institute). Amino acid ratios after 6 N HCl hydrolysis and LAP digestion (numbers in parentheses): Asp × 1, 0.99 (ND); Thr × 2, 1.71 (2.06); Ser × 1, 0.84 (1.05); Gly × 1, 1.00 (1.00); Ala × 1, 0.98 (0.98); Cys × 1, 0.35 (0.89); Phe × 3, 2.74 (2.81); Lys × 2, 1.82 (1.80); Trp × 1, 0.41 (0.90). FAB-MS (obtained on a JEOL JMS-SX102), *m/z* 1637.7 for $[M + H]^+$ (calcd 1637.724 for $C_{76}H_{104}N_{18}O_{19}S_2$).

Acknowledgment. This investigation was supported in part by a Grant-in-Aid (No. 03771701) from the Ministry of Education, Science, and Culture, Japan.