

Trimethylacetamidomethyl (Tacm) Group, a New Protecting Group for the Thiol Function of Cysteine¹⁾

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S-Trimethylacetamidomethyl-L-cysteine [Cys(Tacm)] was easily prepared from *N*-hydroxymethyltrimethylacetamide and L-cysteine in trifluoroacetic acid. The *S*-Tacm group is stable to HF, but cleavable with mercury(II) acetate in trifluoroacetic acid or iodine in aqueous acetic acid. Cys(Tacm) is less susceptible to sulfoxide formation than three related groups, *i.e.*, acetamidomethyl (Acm), benzamidomethyl (Bam) and (2-oxo-1-pyrrolidinyl)methyl (Pym).

Keywords cysteine *S*-protecting group; *S*-trimethylacetamidomethyl-L-cysteine; peptide synthesis; iodine-oxidation; mercury ion deprotection; *S*-protected cysteine sulfoxide

Protection of the thiol function of cysteine is important in peptide synthesis, and many thiol protecting groups have been studied.²⁾ Among them, the widely used *S*-acetamidomethyl (Acm) group³⁾ has the advantage of being stable to HF but removable with mercury(II) ion or iodine.⁴⁾ However, the preparation of Cys(Acm) is rather troublesome because thiazolidine-2-carboxylic acid is formed as a by-product,³⁾ and the activation of Boc-Cys(Acm)-OH using the DCC-HOBt method is known to be accompanied by a side reaction.⁵⁾ These complications were not observed when *S*-benzamidomethyl (Bam)⁶⁾ and *S*-(2-oxo-1-pyrrolidinyl)methyl (Pym)⁷⁾ groups were used, but these groups were not sufficiently stable to HF or alkaline conditions, as described later. Thus, we have introduced a new derivative, *S*-trimethylacetamidomethyl-L-cysteine [Cys(Tacm)], which can be easily prepared. This new *S*-Tacm group is stable to acidic conditions and is removable with iodine, and the Cys(Tacm) derivative was applied to the synthesis of porcine brain natriuretic peptide. The results have been outlined in preliminary reports.^{8,9)} In the present paper, we wish to describe details of the preparation and properties of Cys(Tacm) and related compounds.

As shown in Fig. 1, the Cys(Tacm) derivatives were prepared without any serious side reaction according to the procedure described for Fmoc-Cys(Acm)-OH.¹⁰⁾ *N*-Hydroxymethyltrimethylacetamide (1) was obtained almost quantitatively from trimethylacetamide and formaldehyde-H₂O (7:13) in the presence of KOH. Subsequently, the Tacm group was incorporated into L-cysteine by treatment with 10% excess of *N*-hydroxymethyltrimethylacetamide

(1) in trifluoroacetic acid (TFA) at room temperature for 1 h. Without further purification, H-Cys(Tacm)-OH (2) thus obtained was reacted with di-*tert*-butyl dicarbonate. Boc-Cys(Tacm)-OH was isolated and characterized as its CHA salt. *N*^z-Z(OMe) and Fmoc derivatives were also prepared by the reaction with Z(OMe)-N₃ or Fmoc-Cl, respectively. The Cys(Pym) derivatives were also prepared and characterized similarly.

The chemical properties of the *S*-Tacm group under various conditions used in practical peptide synthesis were examined by thin-layer chromatography (TLC). As shown in Table I, the *S*-Tacm group was stable under acidic conditions (TFA, 0.5 M MSA,¹¹⁾ 1 M TFMSA,¹²⁾ or HF¹³⁾), basic conditions (NH₂NH₂ or 1 N NaOH) and Zn/AcOH treatment, but partial cleavage was observed in the case of 25% HBr/AcOH¹⁴⁾ treatment, as with the *S*-Acm group.³⁾ Next, removal of the *S*-Tacm group was examined using

TABLE I. Stability of the *S*-Tacm Group under Various Conditions

Reagents	Temp. (°C)	Time (h)	Stability
TFA	r.t.	24	Stable
0.5 M MSA/CH ₂ Cl ₂ :dioxane (9:1)	r.t.	24	Stable
1 M TFMSA-thioanisole/TFA	0	2	Stable
HF- <i>m</i> -cresol	0	1	Stable
Zn-90% AcOH	r.t.	3	Stable
25% HBr/AcOH	r.t.	2	Unstable
NH ₂ NH ₂ /MeOH	r.t.	24	Stable
1 N NaOH-MeOH	0	1	Stable

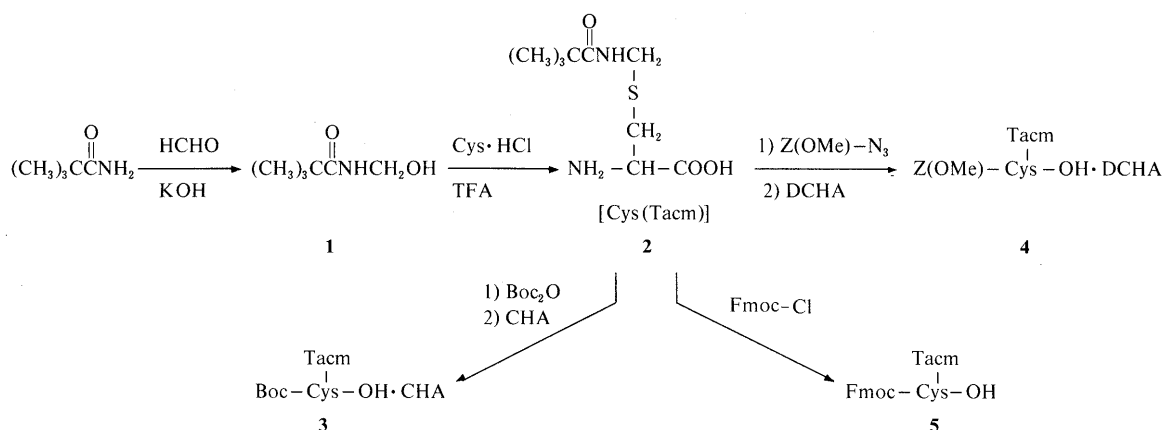


Fig. 1. Preparation of *N*^z-Protected Cys(Tacm) Derivatives

Hg(OAc)₂ or I₂. Boc-Cys(Tacm)-OH was quantitatively converted to cysteine (98%) by treatment with Hg(OAc)₂ in TFA, followed by DTT treatment. The Tacm group of H-Cys(Tacm)-OH was also oxidatively cleaved by I₂ in 90% AcOH to form cystine with 83%¹⁵⁾ conversion yield. When Boc-Cys(Tacm)-OH was treated with I₂ in 90% AcOH, bis Boc-cystine¹⁶⁾ was produced as a sole product on TLC.

It has been shown that partial loss of the *S*-Acm group occurs during HF cleavage of resin-bound Cys(Acm)-containing peptides.¹⁷⁾ In addition, it was reported briefly that the Bam group was not adequately stable to the action of alkalis.⁷⁾ These findings prompted us to compare the stability of the Tacm group to HF and alkalis with those of Acm and the related Bam and Pym groups in detail. Each Boc-Cys(R)-OH (R = Tacm, Acm, Bam or Pym) was treated with HF in the presence of *m*-cresol at 0 °C or room temperature, and the recovery of H-Cys(R)-OH was examined on an amino acid analyzer. As summarized in Table II, Cys(Tacm) and Cys(Acm) derivatives were recovered nearly quantitatively (98.1% and 92.0%, respectively), but partial loss of Bam and Pym groups was detected in the treatment at 0 °C. At elevated temperature, the recovery of each derivative decreased as expected. Among them, the *S*-Tacm derivative gave the best recovery (86.8%). Next, the recovery of each *S*-protected cysteine derivative after 0.05 M NaOH or NH₂NH₂ treatment under the conditions listed in Table II was examined similarly. *S*-Tacm, Acm and Pym derivatives were recovered quantitatively (95.2–97.1%) after these treatments. Partial cleavage of the *S*-Bam group was detected (56.4% and 38.0% recovery), as described in the literature.⁷⁾

Once the sulfoxide of Cys(Acm) was formed by oxidation, Hg(OAc)₂ or I₂ failed to cleave the Acm group

TABLE II. Recovery of *S*-Protected Cys-Derivatives after HF or Alkaline Treatment^{a)}

Cys deriv.	HF		0.05 M NaOH	NH ₂ NH ₂ (10eq)
	0 °C, 1 h	r.t., 1 h	0 °C, 1 h	25 °C, 24 h
H-Cys(Acm)-OH	92.0	85.1	95.8	95.2
H-Cys(Bam)-OH	50.8	26.5	56.4	38.0
H-Cys(Pym)-OH	84.2	72.3	97.1	96.3
H-Cys(Tacm)-OH	98.1	86.8	96.7	95.7

a) Val was used as an internal standard.

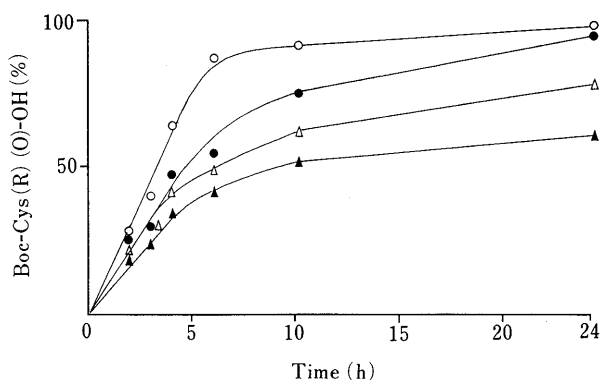


Fig. 2. Oxidation of Boc-Cys(R)-OH by NaBO₃ (1.2 eq) in AcOEt-H₂O (1:1) at 25 °C

—○—, R = Acm; —●—, R = Bam; —△—, R = Pym; —▲—, R = Tacm.

from the sulfoxide.¹⁸⁾ In addition, *S*-Acm-Cys sulfoxide [Cys(Acm)(O)] could not be reduced back to Cys(Acm) by usual thiol treatment. Thus, we examined the susceptibility of Cys(Tacm) and the related *S*-Acm, Bam and Pym derivatives to oxidation with NaBO₃ by using a TLC-scanner. As shown in Fig. 2, 70–100% of Boc-Cys(R)-OH (R = Acm, Bam and Pym) was oxidized to the corresponding sulfoxide by NaBO₃ after 24 h, whereas 55% of Boc-Cys(Tacm)-OH was oxidized to its sulfoxide under the same conditions. The results indicated that Cys(Tacm) is less susceptible to air-oxidation than Cys(Acm), Cys(Bam), or Cys(Pym), presumably because of the steric hindrance of the bulky side chain, and the use of Cys(Tacm) seems to be advantageous for the syntheses of relatively large peptides.

In conclusion, the *S*-Tacm group was judged to fulfill several criteria for practical peptide synthesis in respect of stability to HF or alkalis, susceptibility to Hg(OAc)₂ or I₂ and resistance to air-oxidation.

Experimental

TLC was performed on silica gel (Kieselgel 60F₂₅₄, Merck). *R_f* values refer to the following solvent systems: *R_{f1}*, CHCl₃-MeOH-H₂O (8:3:1, lower phase); *R_{f2}*, CHCl₃-MeOH-AcOH (9:1:0.5). The melting points are uncorrected. Optical rotations were determined with a Union PM-101 polarimeter. Mass spectra were obtained on a JEOL JMS-DX 300 spectrometer and data processor (JEOL JMA-DA 5000). Proton nuclear magnetic resonance (¹H-NMR) spectra were obtained on a JEOL JMX-GX 270 (270 MHz) instrument. Amino acid analysis was performed on a Hitachi L-8500 amino acid analyzer.

***N*-Hydroxymethyltrimethylacetamide (1)** Trimethylacetamide (5.0 g, 49.4 mmol) and KOH (0.30 g, 5.0 mmol) were dissolved in a solution of formaldehyde-water (7:13) (3.0 ml, 34.9 mmol) at 80 °C and then stirred at room temperature overnight. The solution was acidified with 6 N HCl and evaporated *in vacuo*. The residue was extracted with acetone (50 ml). The acetone extracts were dried over Na₂SO₄ and evaporated to dryness. The product, a colorless oil, solidified on standing. *M*⁺ = 131, ¹H-NMR (CDCl₃) δ: 1.21 (s, 9H, *tert*-Bu), 4.74 (d, *J* = 6.6 Hz, 2H, CH₂), 6.83 (br, 1H, NH).

H-Cys(Tacm)-OH (2) A mixture of L-cysteine hydrochloride monohydrate (3.35 g, 19.1 mmol), *N*-hydroxymethyltrimethylacetamide (1) (2.76 g, 21.0 mmol) and TFA (15 ml) was stirred at room temperature for 1 h. The TFA was removed *in vacuo*, the residue was dissolved in EtOH (20 ml) and evaporation was repeated. The product was precipitated with ether: yield 4.10 g (94%), *R_{f1}* 0.26. The analytical sample was prepared by recrystallization of 0.5 g of the crystalline product from a minimum volume of water; mp 188 °C (dec.), [*α*]_D²⁰ −20.8° (*c* = 0.5, H₂O). *Anal.* Calcd for C₉H₁₈N₂O₃S: C, 46.13; H, 7.74; N, 11.96. Found: C, 45.98; H, 7.57; N, 11.94.

Boc-Cys(Tacm)-OH·CHA (3) A solution of H-Cys(Tacm)-OH (2) (4.00 g, 17.1 mmol) in 10% aqueous Na₂CO₃ (30 ml)-dioxane (30 ml) was cooled in an ice-bath and di-*tert*-butyl dicarbonate (5.22 g, 23.9 mmol) was added. The mixture was stirred at room temperature overnight. The solution was concentrated *in vacuo* and acidified with 5% citric acid. The aqueous phase was extracted with AcOEt (50 ml) twice. The combined organic phase was washed with NaCl-H₂O, dried over Na₂SO₄ and concentrated. The residue was dissolved in ether (50 ml) and CHA (1.69 ml) was added. The mixture was concentrated and the residue was recrystallized from MeOH with ether; yield 6.94 g (94%), *R_{f1}* 0.48, mp 142–144 °C, [*α*]_D²⁰ −14.7° (*c* = 0.7, MeOH). *Anal.* Calcd for C₁₄H₂₆N₂O₅·C₆H₁₃N: C, 55.40; H, 9.07; N, 9.69. Found: C, 55.63; H, 9.30; N, 9.63.

Z(OMe)-Cys(Tacm)-OH·DCHA (4) A solution of H-Cys(Tacm)-OH (2) (1.87 g, 5.37 mmol) and Et₃N (2.39 ml, 17.2 mmol) in H₂O (25 ml)-THF (25 ml) was cooled in an ice-bath and 4-methoxybenzyloxycarbonyl azide (1.34 g, 6.45 mmol) was added. The mixture was stirred at room temperature overnight. The solution was washed with ether (twice 75 ml each) and concentrated *in vacuo*. The remaining aqueous solution was acidified with 5% citric acid. The oily product was extracted with AcOEt (50 ml) twice. The organic phase was washed with NaCl-H₂O, dried over Na₂SO₄ and concentrated. The residue was converted to the DCHA salt as described above and the salt recrystallized from MeOH with ether; yield

2.38 g (77%), R_f 0.81, mp 82–84 °C, $[\alpha]_D^{25} - 17.1^\circ$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{18}H_{26}N_2O_5S \cdot C_{12}H_{23}N$: C, 62.14; H, 8.52; N, 7.25. Found: C, 62.43; H, 8.89; N, 7.08.

Fmoc-Cys(Tacm)-OH (5) A solution of H-Cys(Tacm)-OH (2) (1.0 g, 4.27 mmol) in 10% aqueous Na_2CO_3 (20 ml)-dioxane (20 ml) was cooled in an ice-bath and Fmoc-Cl (1.32 g, 5.12 mmol) was added. The mixture was stirred at room temperature overnight. The solution was concentrated *in vacuo* and acidified with 5% citric acid. The aqueous phase was extracted with AcOEt (25 ml) twice. The combined organic phase was washed with $NaCl-H_2O$, dried over Na_2SO_4 and concentrated. The product was further purified by column chromatography on silica using $CHCl_3$ -MeOH (10:0.5) as an eluant and was precipitated with *n*-hexane; yield 1.24 g (64%), mp 62–65 °C, $[\alpha]_D^{25} - 40.8^\circ$ ($c=0.5$, MeOH), R_f 0.42. *Anal.* Calcd for $C_{24}H_{28}N_2O_5S$: C, 63.14; H, 6.18; N, 6.14. Found: C, 62.87; H, 6.18; N, 5.91.

H-Cys(Pym)-OH·HCl (6) A mixture of L-cysteine hydrochloride monohydrate (3.46 g, 19.8 mmol), *N*-hydroxy- α -pyrrolidone¹⁹⁾ (2.74 g, 23.8 mmol) and TFA (10 ml) was stirred at room temperature for 1 h. The TFA was removed *in vacuo*, the residue was dissolved in 1 *N* HCl and evaporation was repeated. The crude hydrochloride was recrystallized from 2-propanol with ether; yield 3.10 g (92%), R_f 0.19, mp 178–180 °C [lit.⁷⁾ mp 174–175 °C], $[\alpha]_D^{17} - 14.9^\circ$ ($c=0.6$, H_2O) [lit.⁷⁾ $[\alpha]_D^{20} + 4.7^\circ$ ($c=1.0$, H_2O)]. *Anal.* Calcd for $C_8H_{13}N_2O_3 \cdot HCl$: C, 37.72; H, 5.94; N, 11.00. Found: C, 37.86; H, 5.87; N, 10.84.

Boc-Cys(Pym)-OH·DCHA (7) Compound 7 was prepared by treatment of H-Cys(Pym)-OH·HCl (6) (3.0 g, 11.8 mmol) in 10% Na_2CO_3 (50 ml)-dioxane (50 ml) with di-*tert*-butyl dicarbonate (3.60 g, 16.5 mmol) as described for the preparation of 3; yield 5.10 g (87%), R_f 0.55, mp 107–109 °C, $[\alpha]_D^{17} - 8.0^\circ$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{13}H_{22}N_2O_5S \cdot C_{12}H_{23}N$: C, 60.09; H, 9.08; N, 8.41. Found: C, 59.97; H, 9.06; N, 8.28.

Z(OMe)-Cys(Pym)-OH·DCHA (8) Compound 8 was prepared by treatment of H-Cys(Pym)-OH·HCl (6) (1.19 g, 4.68 mmol) and Et_3N (2.08 ml, 15.0 mmol) in H_2O (25 ml)-THF (25 ml) with 4-methoxybenzylloxycarbonyl azide (1.16 g, 5.60 mmol) as described for the preparation of 4; yield 1.89 g (72%), R_f 0.83, mp 131–133 °C, $[\alpha]_D^{15} - 13.1^\circ$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{17}H_{22}N_2O_6S \cdot C_{12}H_{23}N$: C, 61.78; H, 8.05; N, 7.45. Found: C, 61.74; H, 7.95; N, 7.24.

Stability Studies of the S-Tacm Group Samples of H-Cys(Tacm)-OH (0.04 mmol) were treated under the conditions shown in Table I. Each reaction was monitored by TLC (stained with ninhydrin). The results are summarized in Table I.

Deprotection of Boc-Cys(Tacm)-OH with $Hg(OAc)_2$ In the presence of anisole (10 eq), Boc-Cys(Tacm)-OH (prepared from 0.05 mmol of the CHA salt) was treated with $Hg(OAc)_2$ (1 eq) in TFA (2.0 ml) in an ice-bath for 30 min. The excess TFA was removed by evaporation at room temperature, then dry ether was added. The residue was dissolved in H_2O (3.0 ml) and the solution, after being adjusted to pH 8 with 5% NH_4OH , was incubated with DTT (10 eq) at 37 °C for 6 h. The resulting precipitate was filtered off. The filtrate was concentrated and the residue was subjected to an amino acid analyzer; recovery of cysteine was 98.0%. No spot corresponding to the starting material was detected on TLC.

Deprotection of H-Cys(Tacm)-OH with Iodine H-Cys(Tacm)-OH (0.04 mmol) in 90% AcOH was treated with 20% iodine/EtOH (10 eq) at 25 °C and cystine-formation was determined with an amino acid analyzer; recoveries of cystine were 61% (15 min), 74% (30 min) and 83% (60 min).

Deprotection of Boc-Cys(Tacm)-OH with Iodine Boc-Cys(Tacm)-OH (0.05 mmol) in 90% AcOH (3.0 ml) was also treated with 20% iodine/EtOH (10 eq) at 25 °C for 60 min. The spot corresponding to the starting material (R_f 0.48) disappeared on TLC, and only the spot corresponding to bis Boc-cystine (R_f 0.22)¹⁶⁾ was detected.

Treatment of Boc-Cys(Tacm)-OH with HF In the presence of *m*-cresol (0.1 ml), Boc-Cys(Tacm)-OH (prepared from 0.04 mmol of the CHA salt) was treated with HF (ca. 2.0 ml) at 0 °C (or room temperature) for 1 h, then excess HF was removed *in vacuo*. The residue was subjected to an amino acid analyzer; recovery of Cys(Tacm) was 98.1% (or 86.8%). No spot corresponding to cysteine was detected on TLC after the treatment at 0 °C. Boc-Cys(R)-OH (R = Ac, Bam and Pym) were also treated under the same conditions. The results are summarized in Table II.

Treatment of H-Cys(Tacm)-OH with 0.05 *M* NaOH in Aqueous MeOH H-Cys(Tacm)-OH (0.04 mmol) was treated with 0.05 *M* NaOH in aqueous MeOH (1.0 ml) at 0 °C for 30 min, then MeOH was removed *in vacuo*. The residue was subjected to an amino acid analyzer; no peak corresponding to cysteine or cystine was detected. H-Cys(R)-OH (R = Ac, Bam and Pym) were also treated under the same condition. The

results are summarized in Table II.

Treatment of H-Cys(Tacm)-OH with NH_2NH_2 in Aqueous MeOH H-Cys(Tacm)-OH (0.04 mmol) was treated with $NH_2NH_2 \cdot H_2O$ (10 eq) in aqueous MeOH (1.0 ml) at 25 °C for 24 h, and then the treated material was analyzed on an amino acid analyzer as stated above. H-Cys(R)-OH (R = Ac, Bam and Pym) were also treated under the same conditions. The results are summarized in Table II.

Boc-Cys(Tacm)(O)-OH (9) A solution of Boc-Cys(Tacm)-OH [prepared from 1.0 g (2.31 mmol) of the CHA salt] in AcOEt- H_2O (1:1, 30 ml) was stirred in the presence of $NaBO_3 \cdot 4H_2O$ (1.2 eq) at 25 °C for 24 h; the reaction was monitored by TLC. The solution was acidified with 5% citric acid. The separated AcOEt layer was washed with $NaCl-H_2O$, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica using $CHCl_3$ -MeOH (10:0.5) as an eluant to give an amorphous powder; yield 0.46 g (42%), R_f 0.35, $[\alpha]_D^{17} - 37.1^\circ$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{14}H_{26}N_2O_6S \cdot 1/2 H_2O$: C, 46.78; H, 7.29; N, 7.79. Found: C, 46.70; H, 7.05; N, 7.67.

For comparison, Boc-Cys(R)-OH (R = Ac, Bam, and Pym) (0.04 mmol) in AcOEt- H_2O (1:1, 4.0 ml) were oxidized with $NaBO_3 \cdot 4H_2O$ (1.2 eq). The progress of oxidation was monitored with a Shimadzu dual-wavelength TLC-scanner and the results are shown in Fig. 2.

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References and Notes

- 1) The following abbreviations are used: Cys = L-cysteine, Z(OMe) = 4-methoxybenzylloxycarbonyl, Boc = *tert*-butoxycarbonyl, Fmoc = 9-fluorenylmethylloxycarbonyl, Ac = acetamidomethyl, Bam = benzamidomethyl, Pym = (2-oxo-1-pyrrolidinyl)methyl, TFA = trifluoroacetic acid, MSA = methanesulfonic acid, TFMSA = trifluoromethanesulfonic acid, THF = tetrahydrofuran, DCHA = dicyclohexylamine, CHA = cyclohexylamine, DCC = dicyclohexylcarbodiimide, HOBT = 1-hydroxybenzotriazole, DTT = dithiothreitol.
- 2) R. G. Hiskey, "The Peptides," Vol. 3, ed. by E. Gross and J. Meienhofer, Academic Press, New York, 1981, p. 37.
- 3) D. F. Veber, J. D. Milkowski, R. G. Denkwalter, and R. Hirschmann, *Tetrahedron Lett.*, **1968**, 3057; D. F. Veber, J. D. Milkowski, S. L. Varga, and R. Hirschmann, *J. Am. Chem. Soc.*, **94**, 5456 (1972).
- 4) B. Kamber, A. Hartmann, K. Eisler, B. Riniker, H. Rink, P. Sieber, and W. Rittel, *Helv. Chim. Acta*, **63**, 899 (1980); P. Sieber and W. Rittel, *ibid.*, **63**, 2358 (1980).
- 5) B. J. Williams and G. T. Young, "Peptides: Structure and Biological Function, Proc. 6th Am. Peptide Symp.," ed. by E. Gross and J. Meienhofer, Pierce Chem. Co., Rockford, Illinois, 1979, p. 321.
- 6) P. K. Chakravarty and R. K. Olsen, *J. Org. Chem.*, **43**, 1270 (1978).
- 7) O. S. Papusuevich, G. I. Aukone, S. Ya. Miksta, and U. O. Kalei, *J. Gen. Chem., USSR*, **52**, 404 (1984).
- 8) Y. Kiso, M. Yoshida, T. Kimura, Y. Fujiwara, and M. Shimokura, *Tetrahedron Lett.*, **30**, 1979 (1989).
- 9) Y. Kiso, M. Yoshida, T. Kimura, Y. Fujiwara, and M. Shimokura, in "Peptide Chemistry 1988," ed. by M. Ueki, Protein Res. Found., Osaka, 1989, p. 201.
- 10) F. Albelicio, A. Grandas, A. Porta, E. Pedrosa, and E. Giralt, *Synthesis*, **1987**, 271.
- 11) Y. Kiso, T. Kimura, Y. Fujiwara, M. Shimokura, and A. Nishitani, *Chem. Pharm. Bull.*, **36**, 5024 (1988).
- 12) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, **1974**, 107; Y. Kiso, M. Satomi, K. Ukawa, and T. Akita, *ibid.*, **1980**, 1063.
- 13) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Jpn.*, **38**, 1412 (1965).
- 14) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952); *ibid.*, **19**, 62 (1954).
- 15) Amounts of cystine calculated by the amino acid analysis. In this case, quantitative data were not obtained owing to poor solubility of cystine in amino acid analyzer buffers.
- 16) I. Photaki, *J. Am. Chem. Soc.*, **88**, 2292 (1966).
- 17) S. F. Brady, W. J. Pareveda, and R. F. Nutt, "Peptides: Chemistry and Biology, Proc. 10th Am. Peptide Symp.," ed. by G. R. Marshall, ESCOM, Sci. Publisher B. V., Leiden, Netherlands, 1988, p. 192.
- 18) H. Yajima, K. Akaji, S. Funakoshi, N. Fujii, and H. Irie, *Chem. Pharm. Bull.*, **28**, 1942 (1980).
- 19) A. Einhorn, *Justus Liebigs Ann. Chem.*, **343**, 207 (1905).