Disulfide bond-forming reaction using a dimethyl sulfoxide/aqueous HCl system and its application to regioselective two disulfide bond formation

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Received 29 April, accepted for publication 14 August 1994

Disulfide bond formation in S-acetamidomethyl (Acm) cysteine-containing peptides by successive treatments with silver trifluoromethanesulfonate (AgOTf) and dimethyl sulfoxide (DMSO)/aqueous HCl is described. An S-Acm cysteine was found to be quantitatively converted into cystine by deprotection of the Acm group with AgOTf followed by DMSO/aqueous HCl treatment. Under these reaction conditions, no significant side reactions were observed with oxidation-sensitive amino acids such as Met, Tyr and Trp. Oxytocin and a Trp-containing peptide, urotensin II, were prepared by this method. Furthermore, regioselective two disulfide bond formation was found to be feasible by the combination of air oxidation and the AgOTf-DMSO/HCl system. This strategy has been successfully applied to the syntheses of tachyplesin I and endothelin I, which have two disulfide bonds and a Trp residue in the molecule.

Key words: dimethyl sulfoxide; endothelin I; HCl; regioselective disulfide bond formation; S-acetamidomethyl cysteine; silver trifluoromethanesulfonate; tachyplesin I

For the synthesis of cystine-containing peptides, the disulfide bond-forming reaction is one of the most important steps. As a conventional method, air oxidation or K3Fe(CN)6 oxidation has been commonly used (1). In the case of the syntheses of multi-disulfide-containing peptides, it is desirable that each disulfide bond be formed regioselectively in order to suppress the formation of isomers. However, this regioselective synthesis cannot be attained by air oxidation or K3Fe(CN)6 oxidation. The differential deprotection of several S-protecting groups of the cysteinyl residues and the following stepwise disulfide formation must be carried out regioselectively. We have developed several disulfide-bond-forming reactions. Thallium(III) trifluoroacetate (2), DMSO (3) or sulfoxide-silylating reagent (4) was found to convert some S-protected cysteines and cysteine to cystine in TFA. Iodine oxidation is also conventionally used for the conversion of S-Acm cysteine residues [Cys(Acm)] (5) to a cystinyl residue (6). The regioselective syntheses using these reactions were also reported (7-10). However, one potential limitation to the use of these reactions for peptide synthesis is the modification of Trp. Thus, our research interest has been focused on the development of a facile and side-reaction-free method for disulfide bond formation of Trp, Cys-containing peptides.

We have preliminarily reported that S-Acm cysteine can be converted to cystine by deprotection of the Acm group with AgOTf (11) followed by DMSO/aqueous HCl (1 N) treatment (12) (Fig. 1). The DMSO/HCl system conducts two reactions: the conversion of H-Cys(Ag)-OH to cysteine besides precipitation of AgCl by the action of aqueous HCl (13), and the oxidation of regenerating cysteine to cystine by the action of aqueous HCl.
of DMSO in aqueous media (14, 15). In the present paper we report the experimental details of the syntheses of cystine-containing peptides using the AgOTf-DMSO/HCl system, and also describe its application to regioselective two disulfide bond formation.

RESULTS AND DISCUSSION

In a preliminary study, the rate of cystine generation with 10% DMSO/1 N HCl or 50% DMSO/1 N HCl was examined using an amino acid analyzer. First, an S-Acm group of H-Cys(Acm)-OH was deprotected with AgOTf in TFA-anisole. During treatment of the resulting H-Cys(Ag)-OH with 10% DMSO/1 N HCl or 50% DMSO/1 N HCl, the generated cystine was quantified using an amino acid analyzer (Table 1). Nearly quantitative generation of cystine from H-Cys(Ag)-OH was accomplished with 10% DMSO/1 N HCl or 50% DMSO/1 N HCl treatment at room temperature within 24 h. In view of its greater reactivity, 50% DMSO/1 N HCl was preferred to 10% DMSO/1 N HCl. Next, the stabilities of the oxidation-sensitive amino acids were examined under this reaction condition. Met, Tyr, and Trp were treated with 50% DMSO/1 N HCl at room temperature. After 20 h, each amino acid was quantitatively recovered without detection of other peaks on amino acid analysis. Increasing the acidity of the reaction may be partially responsible for inducing the sulfioxide-mediated side reaction on Trp, occurring in the DMSO or sulfioxide-silylating reagent/TFA treatment (16). We previously reported that during AgOTf treatment, Met, Tyr, and Trp remain intact (11).

In order to estimate the usefulness of 50% DMSO/1 N HCl for disulfide bond formation during the syntheses of cystine-containing peptides, the syntheses of oxytocin (17) and urotensin II (18) (Fig. 2) were attempted. Oxytocin is a simple model for the synthesis of a cystine-containing peptide. Urotensin II contains a Trp residue in the molecule. Each purified Cys(Acm)-peptide, synthesized by Fmoc-based solid-phase techniques, was treated with AgOTf/TFA-anisole followed by 50% DMSO/1 N HCl oxidation to yield a solution containing the corresponding cystine peptide. Analytical HPLC of the reaction mixture showed a single main peak having the same retention time as that of each authentic sample (Fig. 3).

We next examined the feasibility of the regioselective two disulfide bond formation by the combination of the air-oxidation method and DMSO/HCl system. For the synthesis of a two disulfide-containing peptide, two different groups, MBzl (19) and Acm, were employed for the protection of the sulfhydryl groups of the cysteinyl residues. We devised the synthetic scheme with the following description. At first, MBzl groups are removed from Cys(MBzl) residues by 1 M TMSBr-thioanisole/TFA (20) or 1 M TMSOTf-thioanisole/TFA (21) treatment, and subsequently the first disulfide bond is formed by air oxidation between the regenerating cysteinyl residues. Next, Cys(Acm) residues are converted into the cystinyl residue by the AgOTf-DMSO/HCl method to construct the second disulfide bond.

As a model of regioselective synthesis, tachyplesin I (22), which has two disulfide bonds and a Trp residue in the molecule, was selected. The peptidyl resin of tachyplesin I was synthesized by Fmoc-based solid-phase techniques. For the protection of the four sulfhydryl groups of the cysteinyl residues, MBzl and Acm groups were employed with suitable pairing; MBzl groups for Cys7 and Cys12, and Acm groups for Cys3 and Cys16 (Fig. 4). Treatment of the constructed peptidyl resin with 1 M TMSBr-thioanisole/TFA resulted in the cleavage of the peptide from the resin with the simultaneous removal of all protecting groups except for the S-Acm groups. The resulting dihydropeptide, [Cys(SH)7,12, Cys(Acm)3-16]-tachyplesin I, was air-oxidized to form the first disulfide bridge. After HPLC purification, monocyclic product was subjected to treatment with AgOTf-DMSO/HCl to construct the second disulfide bridge. HPLC examination of the crude peptide showed the presence of a major component possessing the same retention time as that of an authentic sample (23) (Fig. 5a). Ion spray mass spectrometry analysis of the HPLC-purified peptide showed it to be the monomeric form. Disulfide-pairings proved to be identical with those of tachyplesin I by the amino acid analysis of peptide fragments derived from the tryptic digest of the synthetic peptide (23).
As a comparative study, conventional iodine oxidation was applied to the formation of the second disulfide bridge. Purified monocyclic \([\text{Cys(Acm)}]\)-tachyplesin I was treated with \(I_2\) (9). The progress of the reaction was monitored by HPLC of the reaction mixture. After a 1 h treatment, the complete disappearance of the starting material and the formation of tachyplesin I were recognized (Fig. 5b); however, many components due to the \(I_2\)-mediated side reaction were observed.

Further comparative examination on the above two oxidation methods for the synthesis of endothelin I (24) was attempted. Endothelin I has two disulfide bonds besides the oxidation-sensitive Met and Trp residues in the molecule. Air-oxidation of fully reduced tetrahydroendothelin I was reported to afford two major products corresponding to native endothelin I (type I) and to its disulfide isomer (type II) in a ratio of 3:1, respectively (9) (Fig. 6). Based on these facts, we realized that en-
dothelin I was a synthetic target suitable for examining the degree of disulfide exchange in utilizing the regioselective disulfide bond-forming reactions. The peptidyl resin of endothelin I was synthesized by Z(OMe)-based solid-phase techniques (25) starting from the H-Trp(Mts)-OCH₂-PAM resin (Fig. 7). For the S-protection of four cysteiny1 residues, MBzl groups for Cys³ and Cys¹¹ and Acm groups for Cys¹ and Cys¹₅ were employed. The fully protected peptidyl resin was treated with 1 M TMSOTf-thioanisole/TFA to cleave the peptide from the resin with the simultaneous removal of all protecting groups except for O of Met(O) and for the S-Acm groups. Next, the peptide was treated with 1 M TMSBr-thioanisole/TFA to reduce Met(O) to Met. The resulting dihydropeptide, [Cys(SH)³,¹¹, Cys(Acm)³,¹₅]-endothelin I, was subjected to the two-step disulfide bond formation using air oxidation and AgOTf-DMSO/HCl or iodine oxidation, as described in the synthesis of tachyplesin I. The resulting crude peptide from the above two methods was analyzed using HPLC (Fig. 8). Treatment with AgOTf-DMSO/HCl gave the endothelin I (type I) and type II isomers in a ratio of 8:1 with little other by-products. Compared to air oxidation, the AgOTf-DMSO/HCl method significantly suppressed the formation of the type II isomer. The use of iodine oxidation gave no type II isomer; however, many by-products were observed. In terms of minor side reactions and higher yield (AgOTf-DMSO/HCl method: 62%, I₂ oxidation: 30%), our method was superior to iodine oxidation.

FIGURE 6
Primary structure and three possible disulfide isomers of endothelin I.

H-Cys-Ser-Cys-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp-OH

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**FIGURE 7**
Synthetic scheme for endothelin I.
In this study, the synthetic usefulness of the AgOTf-DMSO/HCl system for disulfide bond formation in Cys(Acm)-containing peptides was demonstrated. This procedure caused no side reactions to the oxidation-sensitive amino acids such as Met, Tyr and Trp. Furthermore, the solvent systems (TFA and DMSO/HCl) circumvent the solubility problems of hydrophobic or basic peptides. The syntheses of tachyplesin I and endothelin I were accomplished utilizing the regioselective disulfide bond forming method composed of air-oxidation and the AgOTf-DMSO/HCl system. On the other hand, the conventional successive air and iodine oxidation method led to less satisfactory results, which may be attributable to I₂-induced side reactions to the oxidation sensitive amino acids. The present regioselective method provides useful tactics for the synthesis of two disulfide-containing peptides, particularly when Trp residues are present.

**EXPERIMENTAL PROCEDURES**

Amino acid analysis was conducted using a Hitachi 835 instrument. HPLC was performed on a Waters model 600M or a Waters LC module I equipped with a waters 741 data module. The solvents for HPLC were H₂O and CH₃CN, both containing 0.1% (v/v) TFA. For analytical HPLC, μBondasphere 5 μC18-100 Å (3.9 x 150 mm) was eluted with a linear gradient of CH₃CN (gradient I, 10-40%, 30 min; gradient II, 25-40%, 45 min) at a flow rate of 1 mL/min. Preparative HPLC was performed on Cosmosil 5C18 (10 x 250 mm) at a flow rate of 4 mL/min or Cosmosil 5C18-AR (20 x 250 mm) at a flow rate of 8 mL/min. The eluate was monitored by UV absorption measurements at 220 nm. FAB mass spectra were recorded using a VG Analytical ZAB-SE instrument. Ion-spray mass spectra were obtained with a Sciex API IIIE biomolecular mass analyzer. Fmoc-Val-p-benzoylbenzyl alcohol (Alko) resin and Boc-Trp(Mts)-OCH₃-PAM resin were purchased from Watanabe Chemical Industries, Ltd. 5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid resin (PAL™-resin) was purchased from Millipore.

The rate measurement of cystine generation using 10% DMSO/1 N HCl or 50% DMSO/1 N HCl. Treatment of HCl-H-Cys(Acm)-OH (4.6 mg, 20 pmol) and H-Gly-OH (internal standard, 10 pmol) with AgOTf (26 mg, 5 equiv.) in TFA (500 μL)-anisole (4.8 μL, 2 equiv.) was performed at 4 °C for 1 h. Dry ether (2 mL) was added to the reaction mixture and the resulting powder, H-Cys(Ag)-OH, was collected by centrifugation. After being washed three times with dry ether (6 mL), H-Cys(Ag)-OH was treated with 10% DMSO/1 N HCl or 50% DMSO/1 N HCl (2 mL each) at room temperature. At specific intervals (1, 3, 7, 24 h), an aliquot (500 μL each) was sampled and diluted with H₂O (10 mL). The amount of cystine in the diluted solution (50 μL) was quantified using an amino acid analyzer (Table 1).

Examination of the stabilities of oxidation-sensitive amino acids. H-Met-OH, H-Tyr-OH, H-Trp-OH, and H-Gly-OH (internal standard) (50 pmol each) were treated with 10% DMSO/1 N HCl or 50% DMSO/1 N HCl (2 mL each) at room temperature. At specific intervals (1, 3, 7, 24 h), an aliquot (500 μL each) was sampled and diluted with H₂O (10 mL). The amount of each amino acid in the solution (50 μL) was quantified.
using an amino acid analyzer. The recovery of each amino acid was: Met 92%, Tyr 99%, Trp 102%.

**Oxytocin.** [Cys(Acm)]<sup>6-11</sup>-oxytocin (3) (0.79 μmol) was treated with AgOTf (8 mg, 40 eq) in TFA (200 μL) in the presence of anisole (3 μL) at 4°C for 1.5 h. Dry ether (1 mL) was added to the reaction mixture to afford powder. After being washed three times with dry ether (3 mL), the product was treated with 50% DMSO/1 N HCl (1.3 mL) at room temperature for 7 h. After removal of the AgCl precipitate by filtration, the filtrate was diluted with H₂O (10 mL). The analytical HPLC (gradient I) pattern of the crude peptide is shown in Fig. 3a. The crude peptide was purified by HPLC (gradient of CH₃CN: 20–25%, 30 min); yield 0.57 μmol (72% based on [Cys(Acm)]<sup>6-11</sup>-oxytocin, calculated from the HPLC peak area of oxytocin using standard peptide solutions of known concentrations), and a retention time on analytical HPLC (gradient I) of 26.0 min. Amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Asp 1.11 (1), Thr 1.00 (1), Gly 1.10 (1), Ala 2.08 (2), cystine N.D. (1), Val 1.00 (1), Tyr 1.05 (1), Phe 1.01 (1), Lys 1.25 (1). FAB-MS m/z: 1383.7 (M + Na)<sup>+</sup> [calcd. 1383.5 for (M + Na)<sup>+</sup> of C₆₂H₆₆N₁₂O₁₂S₂].

**/[Cys(Acm)]<sup>6-11</sup>-urotensin II.** The protected urotensin II resin was manually constructed using the Fmoc-based solid-phase method on Fmoc-Val-Alko resin (0.52 mmol/g, 0.1 mmol scale). The Fmoc amino acid derivative (2.5 equiv.) was successively condensed using DIPEA (5 equiv.) for 1 h in combination with treatment using 2% DBU/DMF (7 min) to remove the Fmoc group. The following side-chain protected Fmoc amino acids were used: Cys(Acm), Tyr(Bu<sup>′</sup>), Lys(Boc), Asp(Obu<sup>′</sup>) and Thr(Bu<sup>′</sup>). The protected urotensin II resin (120 mg, 32 μmol) was treated with TFA—m-cresol—EDT-thioanisole—H₂O (80:5:5:5.5: v/v, 10 mL) at room temperature for 1.5 h (3). After removal of the resin by filtration, the filtrate was concentrated in vacuo. Dry ether was then added to precipitate the product. The product was purified by HPLC (gradient of CH₃CN: 26–30%, 30 min) to afford a white powder: yield 44 mg (78% based on Fmoc-Val-Alko resin), and a retention time on analytical HPLC (gradient I) of 26.0 min. Amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Asp 1.13 (1), Thr 1.03 (1), Gly 1.13 (1), Ala 2.02 (2), Cys(Acm) N.D. (2), Val 1.00 (1), Tyr 0.98 (1), Phe 1.06 (1), Lys 1.13 (1). FAB-MS m/z: 1505.8 (M + H)<sup>+</sup> [calcd. 1505.7 for (M + H)<sup>+</sup> of C₆₈H₆₆N₁₂O₁₂S₂].

**Urotensin II.** [Cys(Acm)]<sup>6-11</sup>-urotensin II (2.6 μmol) was treated with AgOTf—DMSO/1 N HCl as described in the synthesis of oxytocin. The analytical HPLC (gradient I) pattern of the reaction mixture is shown in Fig. 3b. The crude peptide was purified by HPLC (gradient of CH₃CN: 29–32%, 30 min); yield 2.1 μmol (80% based on [Cys(Acm)]<sup>6-11</sup>-urotensin II), and a retention time on analytical HPLC (gradient I) of 29.5 min (same as the authentic sample, which was prepared by treatment of [Cys(Acm)]<sup>6-11</sup>-urotensin II with the iodine oxidation). Amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Asp 1.11 (1), Thr 1.00 (1), Gly 1.10 (1), Ala 2.08 (2), cystine N.D. (1), Val 1.00 (1), Tyr 1.05 (1), Phe 1.01 (1), Lys 1.25 (1). FAB-MS m/z: 1383.7 (M + Na)<sup>+</sup> [calcd. 1383.5 for (M + Na)<sup>+</sup> of C₆₂H₆₆N₁₂O₁₂S₂].

**/[Cys(Acm)]<sup>2-16</sup>-tachyplesin I.** The protected tachyplesin I resin was constructed using the Fmoc-based solid-phase method on PAL<sup>™</sup>-resin (0.15 mmol/g, 0.1 mmol scale) using a MilliGen/Biosearch model 9050 automatic peptide synthesizer. The Fmoc amino acid derivative (5 equiv.) in DMF (2 mL) was successively condensed by means of TBTU (27)–HOBt (5 equiv.) each in the presence of DIPEA (5 equiv.) for 1 h. After removal of the resin by filtration, the filtrate was concentrated in vacuo. Dry ether was added to precipitate the product. The product was dissolved in 50% AcOH (5 mL). The total volume of solution was brought to 1 L with H₂O and its pH was adjusted to 7.5 with concentrated NH₄OH. After 2 days, the pH was adjusted to 5 with AcOH, followed by the addition of Diaion HP-20 resin (ca. 10 g). After the mixture was stirred for 1 h, the resin was collected by filtration. The peptide was eluted from the resin with 80% CH₃CN in 1 N AcOH (100 mL). The solvent was removed by evaporation and lyophilization. The crude peptide was purified by HPLC (gradient of CH₃CN: 21–24%, 30 min) to give a white powder: yield 11 mg (16% based on the PAL<sup>™</sup>-resin), and a retention time on analytical HPLC (gradient I) of 24.0 min. Amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Gly 1.00 (1), Cys(Acm) N.D. (2), cystine N.D. (1), Val 0.91 (1), Ile 0.81 (1), Tyr 1.80 (2), Phe 0.92 (1), Lys 0.91 (1), Trp 1.00 (1), Tyr 1.05 (1), Phe 1.01 (1), Lys 0.91 (1), Trp N.D. (1), Arg 4.57 (5). Ion-spray mass (reconstructed) m/z: 2407.0 (calcd. 2406.2 for C₁₀₆H₁₆₈N₃₂O₃₂S₂).
0.17 μmol (79%) based on [Cys(Acm)]
-tachyplesin I, and a retention time on analytical HPLC (gradient I) of 23.6 min [same as the authentic sample (23)]. Amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Gly 1.00 (l), cystine N.D. (2), Val 0.96 (l), Ile 0.90 (1), Tyr 1.96 (2), Phe 0.93 (1), Lys 0.95 (1), Trp N.D. (1), Arg 0.71 (5). Ion-spray mass (reconstructed) m/z: 2262.9 (calcd. 2262.1 for C_{99}H_{151}N_{37}O_{19}S_{4}). The positions of the disulfide linkages of the product were determined according to the reported procedure (23).

Synthesis of tachyplesin I using the iodine oxidation. [Cys(Acm)]
-tachyplesin I (0.11 μmol) was dissolved in MeOH-H_{2}O = 8.2 (200 μL) in the presence of 15 equiv. of HCl. MeOH solution (20 μL) containing I_{2} (15 equiv.) was added at room temperature (9). At intervals (5, 15, 30, 60 and 120 min) part of the reaction mixture (40 μL each) was sampled and the reaction was stopped by adding saturated ascrobic acid solution (50 μL). The progress of the reaction was monitored by analytical HPLC. The HPLC (gradient I) elution pattern of the sample for a 1 h treatment is shown in Fig. 5b. Yield: 58%.

[Cys(Acm)]
-endothelin I. The protected endothelin I resin was constructed manually using Z(Ome)-based solid-phase method on a Boc-Trp(Mts)-OCH_{2}-PAM resin (0.40 mmol/g, 0.2 mmol scale). The Z(Ome) amino acid derivative (2.5 equiv.) was condensed successively using DIPCDI (2.5 equiv.) in the presence of HOBT (2.5 equiv.) in combination with treatment by 0.1 M MSA, 20% m-cresol/CH_{2}Cl_{2} to remove Z(Ome) group, according to the reported schedule (25). The following side-chain protected Z(Ome) amino acids were used: Asp(OBzl), His(Bom), Tyr(Cl-Bzl), Glu(OBzl), Lys(Z), Met(O) and Ser(Bzl). For the S-protection of the four Cys residues, two kinds of protecting groups were employed; MBzl groups for Cys’ and Cys”, and Acm groups for Cys’ and Cyslj. The protected endothelin I resin (50 mg, 16 pmol) was treated with 1 M TMSOTf-thioanisole-I TFA (5 mL) at 4°C for 2 h. After the removal of the resin by filtration, dry ether was added to precipitate the product. The product was dissolved in a 0.1 M AcOH containing 8 M urea (200 mL), and the pH of the solution was adjusted to 7.5 with concentrated NH_{4}OH. After 1 day, the product was isolated by adsorption on Diaion HP-20 resin (ca. 5 g) as mentioned in the synthesis of [Cys(Acm)]
-tachyplesin I, and purified by HPLC (gradient of CH_{3}CN: 30-35%, 60 min); yield 2.4 mg (6%, based on Boc-Trp(Mts)-OCH_{2}-PAM Resin), with a retention time on analytical HPLC (gradient II) of 29.5 min. Amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Asp 1.67 (2), Ser 2.02 (3), Glu 0.96 (1), cystine N.D. (2), Val 0.96 (1), Met 0.98 (1), Ile 1.55 (2), Leu 2.00 (2), Tyr 0.99 (1), Phe 1.15 (1), Lys 1.07 (1), His 0.96 (1), Trp N.D. (1). FAB-MS m/z: 2636.8 (M + H)^{+} [calcd. 2635.1 for (M + H)^{+} of C_{112}H_{171}N_{27}O_{34}S_{5}].

Synthesis of endothelin I using AgOTf-DMSO/1 N HCl method. [Cys(Acm)]
-endothelin I (0.41 μmol) was treated with AgOTf-DMSO/1 N HCl as previously mentioned. The HPLC (gradient II) elution pattern of the crude peptide is shown in Fig. 8a. Two peaks, with retention times identical to those of endothelin I and type II isomer, were observed in a ratio of 8:1 (HPLC peak area). Each product was purified by HPLC (CH_{3}CN 30%, isocratic); [endothelin I] yield: 62% (based on [Cys(Acm)]
-endothelin I), retention time on analytical HPLC (gradient II): 28.2 min, amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Asp 1.59 (2), Ser 2.05 (3), Glu 0.95 (1), cystine N.D. (2), Val 0.91 (1), Met 0.92 (1), Ile 1.53 (2), Leu 2.00 (2), Tyr N.D. (1), Phe 1.36 (1), Lys 1.22 (1), His 1.06 (1), Trp (1), FAB-MS m/z: 2492.8 (M + H)^{+} [calcd. 2491.0 for (M + H)^{+} of C_{109}H_{150}N_{25}O_{32}S_{5}], [type II isomer] yield: 7.5%, retention time on an analytical HPLC (gradient II): 27.4 min, amino acid ratios after 6 N HCl hydrolysis (values in parentheses are theoretical): Asp 1.33 (2), Ser 1.68 (3), Glu 0.91 (1), cystine N.D. (2), Val 0.95 (1), Met 0.98 (1), Ile 1.62 (2), Leu 2.00 (2), Tyr N.D. (1), Phe 1.26 (1), Lys 0.94 (1), His 1.05 (1), Trp N.D. (1). FAB-MS m/z: 2492.8 (M + H)^{+} [calcd. 2491.0 for (M + H)^{+} of C_{109}H_{150}N_{25}O_{32}S_{5}].

Synthesis of endothelin I using the iodine oxidation. [Cys(Acm)]
-endothelin I (0.53 μmol) was treated with I_{2} in MeOH-H_{2}O = 8:2 containing HCl. The progress of the reaction was monitored by an analytical HPLC. The HPLC (gradient II) elution pattern of the crude peptide with a 30 min treatment time is shown in Fig. 8b. Yield: 30%.

ACKNOWLEDGMENTS

We are grateful to Drs. Nobuharu Shigematsu and Hirokazu Tanaka, Fujisawa Pharmaceutical Co., Ltd., for measurement of the FAB-mass spectra.

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Disulfide bond formation