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Studies on Peptides. CLI.[1,2] Syntheses of Cystine-Peptides by Oxidation of S-Protected Cysteine-Peptides with Thallium(III) Trifluoroacetate

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Thallium(III) trifluoroacetate, a mild oxidant with a soft-acid character, was found to cleave various S-protecting groups of cysteine in trifluoroacetic acid, with spontaneous formation of cystine. Except for unmasked Trp and Met, other amino acids, including His and Tyr, remained intact in the presence of this oxidant. The usefulness of this oxidant for intramolecular disulfide bond-forming reactions was demonstrated by direct conversion of three model S-protected cysteine-peptides into cystine-peptides, *i.e.*, oxytocin, urotensin II and human calcitonin gene-related peptide.

Keywords——thallium trifluoroacetate; soft-acid metal; intramolecular disulfide bond-forming reaction; cystine-peptide synthesis; S-*p*-methoxybenzylcysteine; S-1-adamantylcysteine; S-acetamidomethylcysteine; oxytocin; urotensin II; calcitonin gene-related peptide

Recently, we completed the synthesis of a 37-residue peptide[3] corresponding to the entire amino acid sequence of human calcitonin gene-related peptide (hCGRP),[4] for which a new cysteine derivative, Cys(Ad),[5] was employed. In the final step of the synthesis, the S–Ad group was cleaved by treatment with 1 M TFMSA/TFA[6] in the presence of thioanisole,[7] together with other protecting groups employed, then as usual, the deprotected peptide, after reduction with 2-mercaptoethanol, was submitted to air-oxidation to establish the disulfide bond in a highly diluted solution.

In this synthesis, we found that the S–Ad group could be cleaved by treatment with thallium (III) trifluoroacetate [$(CF_3COO)_3Tl$],[5b] a soft-acid metal which is known to have an appreciable affinity for the sulfur atom.[8] Thus, the S–Ad group was cleaved from protected hCGRP by treatment with $(CF_3COO)_3Tl$, after removing the rest of the protecting groups with TFA in the presence of thioanisole.[7b] The product thus obtained by two steps of deprotection was reduced with 2-mercaptoethanol, then submitted to air-oxidation as described above.

Hg(II) salts are known to have an ability to cleave various S-protecting groups of cysteine.[9] The resulting mercaptides have to be treated with thiols to regenerate cysteine. As in the case of Hg(II) treatment, we applied thiol treatment as described above, expecting the complete removal of the Tl salt. Later, it was found that, in contrast to Hg(II) treatment, the product formed by treatment of Z(OMe)–Cys(Ad)–OH with $(CF_3COO)_3Tl$ in TFA is cystine, not cysteine. The results indicated that $(CF_3COO)_3Tl$ acts first as a soft acid to cleave the S–Ad group, then as a mild oxidant[10] to form the disulfide bond as shown in Fig. 1. This finding suggested that S–Ad-cysteine-peptides can be directly converted to the cystine-peptides, eliminating both thiol treatment and the laborious and time-consuming air-oxidation reaction which is usually accompanied with undesired polymer formation. Before

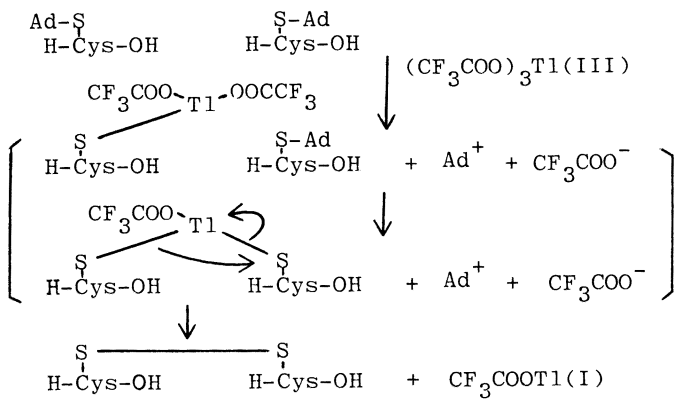


Fig. 1. Formation of Cystine by Treatment of Cys(Ad) with $(CF_3COO)_3Tl$

TABLE I. Oxidative Cleavage of Various S-Protecting Groups of Cysteine by Treatment with $(CF_3COO)_3Tl$ (0 °C, 60 min)

Derivative	Cystine formed (%)	Cysteine regenerated after reduction (%)
H–Cys(MBzl)–OH	86.7	98.5
Boc–Cys(Bu^t)–OH	80.5	96.9
Z(OMe)–Cys(Ad)–OH	83.0	89.3
Boc–Cys(Acm)–OH	81.0	95.3
H–Cys(Tri)–OH	80.2	93.6
Z(OMe)–Cys(Dbs)–OH	81.1	87.0
Boc–Cys(4-Me-Bzl)–OH	74.5[a]	89.7
H–Cys(Bzl)–OH	0[a]	0

a) A by-product, presumably the sulfoxide, was detected.

re-examination of our previous hCGRP synthesis, the responses of other S-protected cysteines to the action of this Tl salt were investigated.

In the literature, the oxidative character of iodine has been applied to convert directly Cys(Tri)-peptides and Cys(Acm)-peptides into cystine-peptides.[11] In this reaction, solvent effects have to be taken into consideration to minimize iodination at several amino acid residues, such as Tyr, His, Met and Trp. We preferred TFA as a solvent to other organic solvents, since TFA is the best solvent for free peptides, as well as protected peptides. Each Cys-derivative dissolved in TFA was treated with $(CF_3COO)_3Tl$ (1 eq) in an ice-bath for 60 min, then part of the solution was subjected to amino acid analysis. Anisole (*ca.* 2 eq) was used to trap alkyl cations. Cys(Bzl)[12] resisted the action of this reagent, but other protecting groups, including the Ad group, so far examined here (MBzl,[13] Bu^t,[14] Acm,[15] Tri,[16] and Dbs[17]) were cleaved to produce cystine as a sole product as shown in Table I. Cys(4-Me-Bzl)[18] generated cystine, but a small amount of a by-product (presumably the sulfoxide) was detected. When each cleaved sample was incubated with ethanedithiol, cysteine was regenerated quantitatively. The above results suggested that the $(CF_3COO)_3Tl$ oxidation procedure can be applied to convert not only Cys(Ad)-peptides, but also other S-protected cysteine-peptides to cystine-peptides, as well as into cysteine-peptides, if necessary.

Prior to applying this newly found disulfide-bond-forming reaction to practical peptide syntheses, the side effects of this oxidant on other functional amino acids were examined. We noticed that cystine was inert in TFA in the presence of $(CF_3COO)_3Tl$, but a small amount of

cysteic acid formed when cystine was exposed to $(CF_3COO)_3Tl$ in an aqueous solution. Thus, in practical peptide synthesis, precipitation of treated peptides with ether is recommended to remove the Tl salt, before further purification, since the Tl salt is ether-soluble. Unmasked Trp suffered modification to produce several unidentified products (recovery of Trp, 38%), but Trp(Mts)[19] remained intact after a 60 min treatment at 0 °C. Met was partially oxidized to the corresponding sulfoxide[20] (34%), but not to the sulfone. It seems worthwhile to note that His and Tyr survived unchanged after this $(CF_3COO)_3Tl$ treatment. From these model experiments, we reached the conclusion that two amino acids, Trp and Met, must be protected during the $(CF_3COO)_3Tl$ treatment.

Next, the stability of the disulfide bond of cystine under the conditions required for removal of N^{in}-protecting groups from Trp and reduction of Met(O) was examined. Recently we found that the N^{in}-Mts group of Trp could be cleaved by treatment with 1 M TMSOTf/TFA[21] in the presence of a soft base,[22] such as thioanisole[7] or diphenylsulfide[23] (PhSPh), more readily than with 1 M TFMSA/TFA,[6] together with other Bzl-type protecting groups and the N^G-Mts group from Arg.[24] Thus, as a model experiment, cystine was treated with 1 M TMSOTf/TFA or 1 M TFMSA/TFA in an ice-bath for 60 min and the effect of an added soft base was examined by measuring the recovery of cystine with an amino acid analyzer. As shown in Table II, dimethylsulfide (MeSMe) or PhSPh gave a much better result than thioanisole. Of these, MeSMe[25] (recommended for the removal of Bzl-protecting groups by TFMSA/TFA) was found not to be effective enough to assist the cleaving reaction of Trp(Mts), as well as Arg(Mts), in either 1 M TMSOTf/TFA or 1 M TFMSA/TFA treatment, as shown in Table III. Thus, the conclusion we reached here is that Trp-containing cystine-peptides can be obtained by $(CF_3COO)_3Tl$ treatment of Trp(Mts)-peptides, followed by 1 M TMSOTf/TFA treatment in the presence of PhSPh, rather than MeSMe, to remove the Mts group and other protecting groups. PhSPh is not freely soluble in TFA. Thus, a concentration

Table II. Stability of Cystine under Treatment with 1 M TMSOTf/TFA or 1 M TFMSA/TFA in the Presence of Sulfur Compounds

Reagent (0 °C, 60 min)	Cystine recovered (%)	
	1 M TFMSA/TFA	1 M TMSOTf/TFA
None	93.8	83.8
Thioanisole[a]	64.2	64.7
MeSMe[a]	92.2	86.9
PhSPh[b]	80.4	85.8

a) To a concentration of 1 M. *b*) To a concentration of 0.5 M.

Table III. Effects of Sulfur Compounds on Deprotection of Trp(Mts), Arg(Mts) and Asp(OBzl)

Additive	Deprotecting reagent (0 °C, 60 min)	Amino acid regenerated (%) from		
		Trp(Mts)	Arg(Mts)	Asp(OBzl)
PhSPh[a]	1 M TMSOTf/TFA	100.0	97.0	100.0
	1 M TFMSA/TFA	76.3	81.3	97.2
MeSMe[b]	1 M TMSOTf/TFA	34.0	22.1	98.3
	1 M TFMSA/TFA	16.7	20.1	81.4

a) To a concentration of 0.5 M. *b*) To a concentration of 1 M.

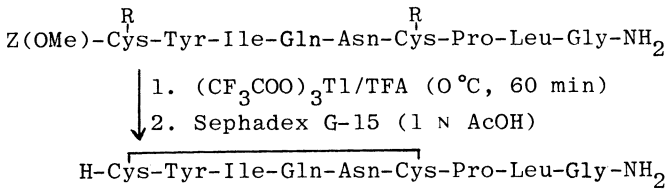
of 0.5 M PhSPh in TFA was judged to be suitable for practical use.

Next, selective deoxygenation of Met(O) in the presence of cystine was examined. It is a difficult problem to reduce Met(O) without affecting the disulfide bond, since hitherto known sulfhydryl-type reducing reagents of Met(O)[26] do not fulfill our present demand. Thioanisole[27] or MeSMe[25] in TFMSA/TFA was found to reduce Met(O) partially during TFMSA/TFA treatment, but PhSPh which we selected above as a soft base was found not to exert any noticeable reducing effect on Met(O) during TMSOTf/TFA treatment, or TFMSA/TFA treatment. Apart from sulfur compound, we were interested in examining the reducing properties of ammonium iodide.[28] A mixture of Z(OMe)-Met(O)-OH and cystine in TFA was treated with ammonium iodide in an ice-bath for 60 min, then subjected to amino acid analysis. This reagent gave tolerably high recoveries of Met and cystine as shown in Table IV. When Trp was added to this mixture, its recovery was *ca.* 76%. Thus, for the synthesis of Met- and Trp-containing cystine-peptides, further investigation seems to be required to find more suitable conditions.

After these model experiments, the usefulness of $(CF_3COO)_3Tl$ for intramolecular disulfide bond-forming reactions was examined by preparing two model peptides, oxytocin[29] and urotensin II.[30] In addition, hCGRP was re-synthesized by the present method. Each product was compared with the respective authentic samples by high-performance liquid chromatography (HPLC).

TABLE IV. Reduction of Met(O) in the Presence of Cystine and Tryptophan

Reagent (20 eq) (0 °C, 60 min)	Amino acid recovered (%)		
	Met	Cys	Trp
NH_4I	89.7	90.2	75.8



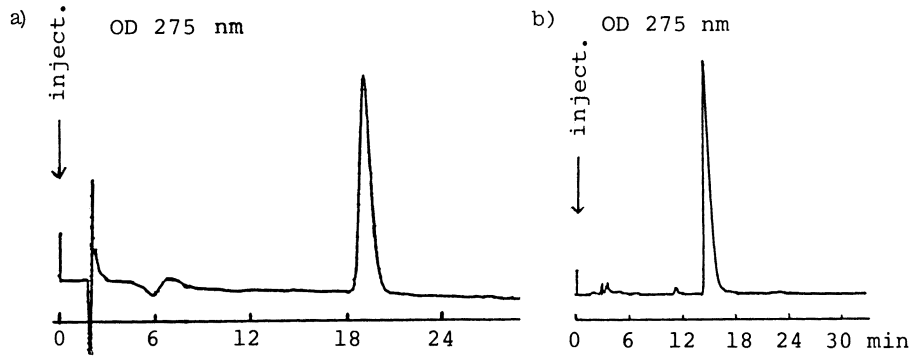


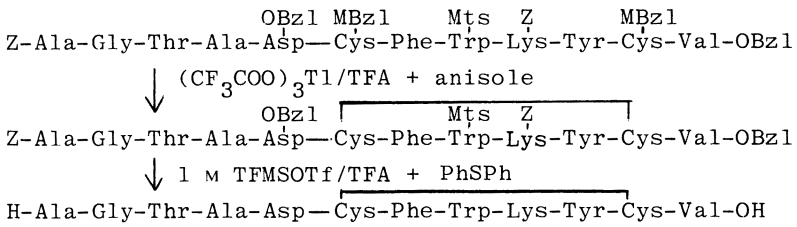
Fig. 2. HPLC of Crude Oxytocin Prepared by $(CF_3COO)_3Tl$ Oxidation

a) From the R = MBzl derivative. b) From the R = Acm derivative.

Oxytocin was prepared alternatively with Cys(MBzl) or Cys(Acm). First, when Z(OMe)–Cys(MBzl)–Tyr–Ile–Gln–Asn–Cys(MBzl)–Pro–Leu–Gly–NH_2 in TFA was treated with $(CF_3COO)_3Tl$ in an ice-bath for 60 min, oxytocin was directly obtained as a main product. The crude sample obtained after gel-filtration was examined by HPLC in comparison with an authentic sample of oxytocin. As shown in Fig. 2a, a product possessing a retention time identical with that of the authentic sample was obtained directly, without the laborious air-oxidation reaction. A similar result was obtained when the Cys(Acm) derivative, Z(OMe)–Cys(Acm)–Tyr–Ile–Gln–Asn–Cys(Acm)–Pro–Leu–Gly–NH_2, in TFA was treated with $(CF_3COO)_3Tl$, as can be seen from the HPLC elution pattern of its gel-filtered product (Fig. 2b).

Next, as an example of a Trp-containing peptide, a dodecapeptide corresponding to the entire amino acid sequence of urotensin II, a caudal neurosecretory hormone of the toleost fish, was prepared. In our previous synthesis,[31] Trp was not protected. Thus, protected urotensin II was re-synthesized using Trp(Mts). Z–Ala–Gly–Thr–Ala–Asp(OBzl)–Cys(MBzl)–Phe–Trp(Mts)–Lys(Z)–Tyr–Cys(MBzl)–Val–OBzl thus obtained was treated with $(CF_3COO)_3Tl$ in TFA as described above, then with 1 M TMSOTf/TFA in the presence of PhSPh in an ice-bath for 120 min, a longer time than in the model experiments, to ensure complete deprotection. The crude product obtained after gel-filtration was examined by HPLC. As shown in Fig. 3, a product possessing a retention time identical with that of an authentic sample of synthetic urotensin II emerged from the column as a main component. The desired product was isolated by preparative HPLC in 34% yield, while the yield of the authentic sample prepared by the usual air-oxidation procedure was 16%.

hCGRP was re-synthesized by using the present method. Protected hCGRP was first treated with $(CF_3COO)_3Tl$ in TFA to form the disulfide bond and then with 1 M TMSOTf/TFA in the presence of PhSPh as stated above to remove other protecting groups (Mts from Arg, Bzl from Ser and Z from Lys). The product was purified by gel-filtration, followed by ion-exchange chromatography on CM-Trisacryl. The elution pattern of the CM-



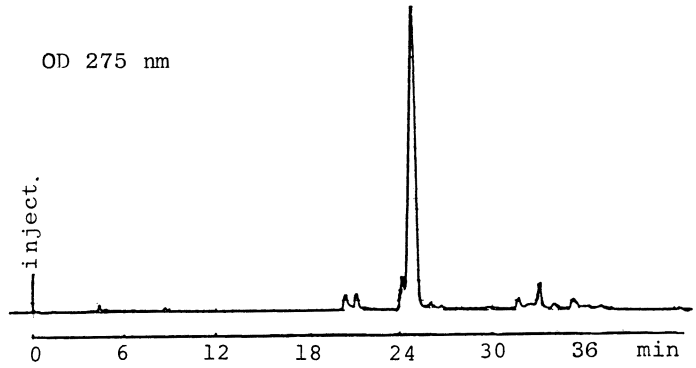


Fig. 3. HPLC of Crude Synthetic Urotensin II Prepared by $(CF_3COO)_3Tl$ Oxidation, Followed by TMSOTf/TFA Deprotection

Boc-Ala-Cys(Ad)-Asp-Thr-Ala-Thr-Cys(Ad)-Val-Thr-His-Arg(Mts)-Leu-Ala-Gly-Leu-Leu-Ser(Bzl)-Arg(Mts)-Ser(Bzl)-Gly-Gly-Val-Val-Lys(Z)-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser(Bzl)-Lys(Z)-Ala-Phe-NH_2

1. $(CF_3COO)_3Tl$/TFA + anisole
2. TMSOTf/TFA + PhSPh + *m*-cresol

H-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2

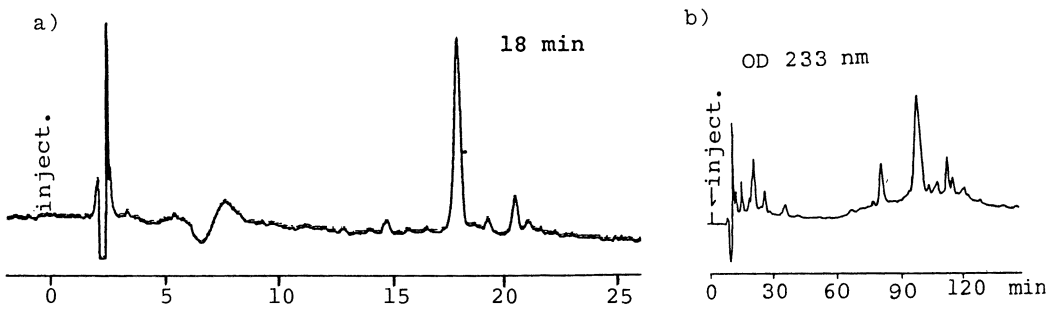


Fig. 4. HPLC of CM-Purified hCGRP

a) Sample obtained by $(CF_3COO)_3Tl$ oxidation method [on Nucleosil $5C_{18}$ (0.4 × 15 cm), flow rate 1 ml/min].

b) Sample obtained by air-oxidation method[3] [on Nucleosil $5C_{18}$ (1 × 25 cm), flow rate 2 ml/min].

purified product is shown in Fig. 4a, in comparison with that of the CM-purified product obtained by the air-oxidation procedure (Fig. 4b).[3] It can be judged that the product obtained by the present method possessed much higher purity than the previous one. After HPLC purification, the product was obtained in a somewhat better yield (11%) than before (8%).

No thallium contamination was detected in the synthetic peptides by X-ray energy spectroscopy. As described earlier, one of the attractive features on this direct oxidative disulfide bond-forming reaction is that the reaction can be performed in TFA without any solubility problem. A new route has thus been opened to synthesize peptides containing one disulfide bond without laborious and time-consuming air-oxidation.We intend to discuss some remaining problems involved in the syntheses of Met-containing cystine-peptides in a separate paper.

Experimental

Amino acid analysis and HPLC were conducted with a Hitachi 835-02 analyzer and a Waters 204 compact model, respectively. Rotation and ultraviolet absorption (UV) were determined with a Union PM-101 polarimeter and a Hitachi 100-20 spectromer, respectively. Thin layer chromatography (TLC) was conducted on silica gel (Kieselgel G, Merck) and *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 $CHCl_3$–MeOH (10:5), and Rf_3 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2). Fast atom bombardment mass spectra (FAB-MS) were obtained on a ZAB SE instrument (VG Analytical Co., England).

Model Experiments

$(CF_3COO)_3Tl$ Treatment of S-Protected Cysteine Derivatives——A mixture of an S-protected cysteine derivative (57 μmol each) and Gly (internal standard) in TFA (140 μl) was treated with $(CF_3COO)_3Tl$ (1 eq) in the presence of anisole (*ca.* 2 eq) in an ice-bath for 60 min. An aliquot, after being diluted with H_2O containing Met (5 eq, to quench the reagent), was analyzed on an amino acid analyzer. The results of cystine formation are shown in Table I. Presumably due to low solubility of cystine in the analysis buffers, quantitative recovery of cystine was difficult to obtain in amino acid analysis.

Reduction of $(CF_3COO)_3Tl$-Treated Samples——An aliquot of each treated sample was adjusted to pH 7.5 with 5% NH_4OH, then incubated with ethanedithiol (10 eq) at 40 C for 5 h and submitted to amino acid analysis. The results of cysteine regeneration are listed in Table I.

Oxidation of Cystine to Cysteic Acid by $(CF_3COO)_3Tl$ in an Aqueous Solution——A mixture of cystine and $(CF_3COO)_3Tl$ (1 eq) in TFA was kept in an ice-bath for 60 min. No cysteic acid was detected. The solution was diluted with H_2O (5 times), then kept at room temperature for 24 h and subjected to amino acid analysis. Formation of cysteic acid was 11%.

Stability of Amino Acids in TFA to the Action of $(CF_3COO)_3Tl$——A mixture of Met, Tyr, His, and Trp (0.2 mmol each) in TFA (5.0 ml) was treated with $(CF_3COO)_3Tl$ (1 eq) in an ice-bath for 30 min and an aliquot was subjected to amino acid analysis. Recoveries of amino acids were: Met 66.0%, Met(O) 34.0%, Tyr 97.9% (no other peak was detected); His 96.8% (no other peak was detected), Trp 38.4%.

Stability of Cystine during 1 M TFMSA/TFA or 1 M TMSOTf/TFA Treatment in the Presence of Thioether Compounds——A mixture of cystine (45 μmol) and Gly (55 μmol, internal standard) was treated with 1 M TFMSA/TFA (0.45 ml) or 1 M TMSOTf/TFA (0.45 ml) in the presence of a thioether compound, thioanisole (to a concentration of 1 M) or MeSMe (to a concentration of 1 M) or PhSPh (to a concentration of 0.5 M), in an ice-bath for 60 min. Recoveries of cystine determined by an amino acid analyzer are listed in Table II.

Effects of Sulfur Compounds on the Deprotection of Trp(Mts), Arg(Mts) and Asp(OBzl)——A mixture of three amino acid derivatives (50 μmol each), Trp(Mts), Arg(Mts) and Asp(OBzl), was treated with 1 M TMSOTf/TFA (0.5 ml) or 1 M TFMSA/TFA (0.5 ml) in the presence of PhSPh (to a concentration of 0.5 M) or MeSMe (to a concentration of 1 M) in an ice-bath for 60 min. Recoveries of amino acids determined with an analyzer are listed in Table III.

Deoxygenation of Met(O) in the Presence of Cystine——A mixture of Z(OMe)-Met(O)-OH (35.3 mmol), cystine (27.4 mmol), H-Trp-OH (41.6 mmol), Gly (internal standard) and PhSPh (20 eq) in TFA (1 ml) was treated with NH_4I (20 eq, for Met(O)) in an ice-bath for 60 min. Recoveries of Met, Cystine and Trp are listed in Table IV.

Synthesis of Oxytocin

From Cys(MBzl)-Derivative——Z(OMe)-Cys(MBzl)-Tyr-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-NH_2 was prepared by the azide condensation of Z(OMe)-Cys(MBzl)-Tyr-$NHNH_2$ with a TFA-treated sample of Z(OMe)-Ile-Gln-Asn-Cys(MBzl)-Pro-Leu-Gly-NH_2, prepared in a stepwise manner by means of known amide-forming reactions; mp 250—251 C, $[\alpha]_D^{20}$ −37.6° (c=0.6, DMSO), Rf_1 0.50. *Anal.* Calcd for $C_{68}H_{92}N_{12}O_{17}S_2 \cdot 3.5H_2O$: C, 55.30; H, 6.76; N, 11.38. Found: C, 55.23; H, 6.51; N, 11.17.

The above protected nonapeptide amide (37 mg) dissolved in TFA (1 ml) was treated with $(CF_3COO)_3Tl$ (15.5 mg, 1.1 eq) in the presence of anisole (0.1 ml) in an ice-bath for 60 min, then *n*-hexane was added to precipitate the product. *n*-Hexane was removed by decantation and the residue was treated with dry ether. The resulting powder was washed thoroughly with ether and dissolved in 1 N AcOH. The solution was applied to a column of Sephadex G-15 (1.6 × 103 cm), which was eluted with the same solvent. The desired fractions (4 ml each, tube Nos. 22—30, monitored by UV absorption measurement at 275 nm) were combined and the solvent was removed by lyophilization to give a powder; yield 12 mg (45%). The HPLC pattern of the product obtained at this stage is shown in Fig. 2a. A mixture of the synthetic product and an authentic sample of oxytocin (10 μg each) emerged from a Nucleosil $5C_{18}$ (0.4 × 15 cm) column as a single peak (retention time 19 min), on isocratic elution with 16% MeCN in 0.1% TFA. For characterization, the gel-filtered sample was purified by HPLC on a TSK-GEL 410 KG column (2.45 × 30 cm) with isocratic elution using 23% MeCN in 0.1% TFA aq.; yield 7.2 mg (27%), $[\alpha]_D^{20}$ −25.2° (c=0.2, H_2O) (lit.[29] −26.2° in H_2O). Amino acid ratios in a 6 N HCl hydrolysate: Cystine 0.71, Tyr 0.91, Ile 1.01, Glu 1.00, Asp 1.02, Pro 0.96, Leu 1.00, Gly 1.00 (recovery of Gly, 79%).

From Cys(Acm)-Derivative——Boc-Cys(Acm)-Ile-Gln-Asn-Cys(Acm)-Pro-Leu-Gly-NH_2 was similarly synthesized. mp 228—230 C, $[\alpha]_D^{19}$ −30.5° (c=0.5, DMSO), Rf_1 0.21. *Anal.* Calcd for $C_{54}H_{86}N_{14}O_{16}S_2 \cdot 0.5H_2O$: C, 51.45; H, 6.96; N, 15.56. Found: C, 51.40; H, 7.00; N, 15.27.

The protected nonapeptide obtained above (50 mg) in TFA (5.0 ml) was treated with $(CF_3COO)_3Tl$ (25.8 mg, 1.1 eq) in the presence of anisole (50 μl) and the product precipitated with ether was purified by gel-filtration on Sephadex G-15 (yield 15.1 mg, 39%), followed by HPLC as described above; yield 8.3 mg (22%). A mixture of the synthetic peptide and an authentic sample of oxytocin (10 μg each) emerged from a Vydac $5C_{18}$ (0.46 × 25 cm) column as a single peak (retention time, 14 min) on isocratic elution with 16% MeCN.

Synthesis of Urotensin II

Boc-Trp(Mts)-Lys(Z)-Tyr-Cys(MBzl)-Val-OBzl——Boc-Trp(Mts)-OSu was allowed to react with a TFA-treated sample of Z(OMe)-Lys(Z)-Tyr-Cys(MBzl)-Val-OBzl[31] in DMF in the presence of NMM until the solution became negative to the ninhydrin test. The solvent was removed by evaporation and the residue was treated with 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O (washing procedure) and precipitated from DMF with isopropyl alcohol; yield 66%, mp 129—130 C, $[\alpha]_D^{20}$ −27.1° (c=0.6, DMF), Rf_1 0.83. *Anal.* Calcd for $C_{71}H_{85}N_7O_{14}S_2 \cdot H_2O$: C, 63.51; H, 6.53; N, 7.30. Found: C, 63.48; H, 6.49; N, 7.46.

Boc-Cys(MBzl)-Phe-Trp(Mts)-Lys(Z)-Tyr-Cys(MBzl)-Val-OBzl——This protected heptapeptide ester was prepared by the azide condensation of Boc-Cys(MBzl)-Phe-$NHNH_2$ with a TFA-treated sample of the above

pentapeptide ester and purified by means of the above washing procedure, followed by precipitation from MeOH; yield 78%, mp 192—193 °C, $[\alpha]_D^{20}$ −26.0° (c=0.5, DMF), Rf_1 0.81. *Anal.* Calcd for $C_{91}H_{107}N_9O_{17}S_3 \cdot H_2O$: C, 63.79; H, 6.41; N, 7.36. Found: C, 63.79; H, 6.33; N, 7.38.

Z(OMe)–Asp(OBzl)–Cys(MBzl)–Phe–Trp(Mts)–Lys(Z)–Tyr–Cys(MBzl)–Val–OBzl——This protected octapeptide ester was prepared by reaction of Z(OMe)–Asp(OBzl)–OSu and a TFA-treated sample of the above protected heptapeptide ester and purified by means of the washing procedure, followed by precipitation from DMF with MeOH; yield 65%, mp 212—213 °C, $[\alpha]_D^{20}$ −59.6° (c=0.2, DMF), Rf_2 0.79. *Anal.* Calcd for $C_{106}H_{118}N_{10}O_{21}S_3 \cdot H_2O$: C, 64.22; H, 6.10; N, 7.07. Found: C, 64.18; H, 6.06; N, 7.21.

Z–Ala–Gly–Thr–Ala–Asp(OBzl)–Cys(MBzl)–Phe–Trp(Mts)–Lys(Z)–Tyr–Cys(MBzl)–Val–OBzl——Protected urotensin II was prepared by the azide condensation of Z–Ala–Gly–Thr–Ala–$NHNH_2$ with a TFA-treated sample of the above protected octapeptide ester and purified by means of the washing procedure followed by precipitation from DMF with MeOH; yield 77%, mp 274—276 °C, $[\alpha]_D^{20}$ −40.8° (c=0.3, DMF), Rf_1 0.69, Amino acid ratios in a 6 N HCl hydrolysate: Asp 0.92, Thr 0.99, Gly 1.03, Ala 1.83, Val 0.94, Tyr 1.17, Phe 1.00, Lys 1.05 (recovery of Phe, 85%). *Anal.* Calcd for $C_{117}H_{136}N_{14}O_{25}S_3 \cdot 2H_2O$: C, 61.89; H, 6.22; N, 8.64. Found: C, 61.81; H, 6.00; N, 8.61.

Deprotection for the Synthesis of Urotensin II——Protected urotensin II (50 mg) in TFA (5.0 ml) was treated with $(CF_3COO)_3Tl$ (14.6 mg, 1.2 eq) in the presence of anisole (50 μl) in an ice-bath for 60 min, then TFA was removed by evaporation *in vacuo* and dry ether was added. The resulting powder was collected by centrifugation, then treated with 1 M TMSOTf/TFA (4 ml) in the presence of PhSPh (0.79 ml) and *m*-cresol (0.25 ml) in an ice-bath for 120 min. Finally isopropyl ether was added. The resulting powder was collected by centrifugation, washed with isopropyl ether and dissolved in H_2O (5 ml). The pH of the ice-chilled solution was adjusted to 7.5 with 5% NH_4OH and after 10 min, to 3 with AcOH. The solution was applied to a column of Sephadex G-25 (2.0 × 137 cm), which was eluted with 1 N AcOH. The desired fractions (6.7 ml each, tube Nos. 60—67, monitored by UV absorption measurement at 275 nm) were combined and the solvent was removed by lyophilization. The HPLC elution pattern of this crude product was shown in Fig. 3. The product was next purified by HPLC on a Cosmosil $5C_{18}$ column with a gradient of MeCN (23 to 35%, for 20 min) in 0.1% TFA. The desired eluate (retention time, 25 min) was collected and the solvent was removed by lyophilization to give a fluffy white powder; yield 10.4 mg (34%). $[\alpha]_D^{20}$ −59.0° (c=0.2, 1% AcOH), (lit.[31] −60.4° in 1% AcOH). FAB-MS *m/z*: 1361 $(M+H)^+$. Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.02, Thr 0.92, Gly 1.01, Ala 1.96, Cys 0.86, Val 1.00, Tyr 1.00, Phe 1.02, Lys 1.05 (recovery of Val, 78%). A mixture of the samples obtained by the present method and the previous method emerged from a Nucleosil $5C_{18}$ column as a single peak (retention time, 25 min), when eluted with the same gradient employed above.

Re-synthesis of hCGRP

Protected hCGRP (75 mg) in TFA (7.5 ml) was treated with $(CF_3COO)_3Tl$ (9.7 mg, 1.2 eq) in the presence of anisole (75 μl) in an ice-bath for 60 min, then TFA was removed by evaporation *in vacuo* and dry ether was added. The resulting powder was collected by centrifugation, and treated with 1 M TMSOTf/TFA (5.0 ml) in the presence of PhSPh (1.2 ml) and *m*-cresol (0.37 ml) in an ice-bath for 2 h, then dry ether was added. The resulting powder was collected by centrifugation and dissolved in H_2O (5 ml). The pH of the ice-chilled solution was adjusted to 7.5 with 5% NH_4OH and after 10 min, to 3.0 with AcOH. The solution was gel-filtered on Sephadex G-25 using 1 N AcOH as an eluant. The desired fractions (6.7 ml each, tube Nos. 43—55, monitored by means of the Folin–Lowry test at 750 nm) were combined and the solvent was removed by lyophilization; yield 41 mg (73%). The product was next purified by ion-exchange chromatography on a CM-Trisacryl column (2 × 5 cm) with a gradient of NaCl (0 to 0.2 M) in pH 6.5, 0.01 M $AcONH_4$ buffer. The desired fractions (5.8 ml each, tube Nos. 53—62, monitored by means of the Folin–Lowry test at 750 nm) were combined and the solvent was removed by lyophilization. The residue was desalted by gel-filtration on Sephadex G-15 using 1 N AcOH as an eluant as stated above. Lyophilization of the desired fractions gave a fluffy powder; yield 12 mg (30%). This CM-purified product was further purified by HPLC on a Nucleosil $5C_{18}$ column with a gradient of MeCN (25—40%, 30 min) in 0.1% TFA aq. at a flow rate of 1.0 ml/min. Its HPLC pattern is shown in Fig. 4. After a further run of HPLC, the desired eluate (retention time, 18 min) was taken and the solvent was removed by lyophilization to give a white fluffy powder; yield 3.0 mg (52%), overall yield was 11%. $[\alpha]_D^{20}$ −81.1° (c=0.1, 0.5 N AcOH) (lit.[3] −84.4° in 0.5 N AcOH), FAB-MS *m/z*: 3789 $(M+H)^+$. Amino acid ratios in a 6 N HCl hydrolysate (numbers in parentheses are theoretical): Asp. 4.21 (4), Thr 3.75 (4), Ser 3.01 (3), Pro 0.97 (1), Gly 4.09 (4), Ala 4.08 (4), Cys 0.69 (1), Val 4.29 (5), Leu 3.14 (3), Phe 2.00 (2), Lys 2.05 (2), His 0.96 (1), Arg 2.09 (2), (recovery of Phe was 80%, low recovery of Val was due to incomplete hydrolysis of the Val–Val linkage).

The product obtained here was identical in terms of *Rf* values on TLC (Rf_3 0.39) and retention time (18 min) on HPLC with the product obtained by the previous air-oxidation procedure under the conditions stated above.

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References and Notes

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2) Amino acids and peptide derivatives mentioned in this paper are of the L-configuration. The following abbreviations are used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, MBzl = *p*-methoxybenzyl, Bu^t = *tert*-butyl, Ad = 1-adamantyl, Acm = acetamidomethyl, Tri = triphenylmethyl, Dbs = dibenzosuberyl, Bzl = benzyl, Mts = mesitylenesulfonyl, TFMSA = trifluoromethanesulfonic acid, TMSOTf = trimethylsilyl trifluoromethanesulfonate, TFA = trifluoroacetic acid, DMF = dimethylformamide, DMSO = dimethylsulfoxide, NMM = *N*-methylmorpholine.
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