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# Synthesis and Biological Activity of Somatostatin Analogues modified at the Tryptophan Residue[1]

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Three analogues of somatostatin, in which the tryptophan residue in position 8 was replaced by 3-(1-naphthyl)-L-alanine, 3-(2-naphthyl)-L-alanine and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, were synthesized by the solid-phase method. All of these analogues showed diminished biological activity compared to synthetic somatostatin. The results suggest strongly that the indolyl NH group in the tryptophan residue of somatostatin plays an important role to elicit full hormonal activity and that the function accomplished by the tryptophan residue in somatostatin is different from that in luteinizing hormone-releasing hormone.

**Keywords**——peptide; somatostatin analogue modifying the tryptophan residue; TRH-induced TSH inhibiting activity; structure–activity relationship; somatostatin Nal(1) analogue; somatostatin Nal(2) analogue; somatostatin Tca analogue

After the primary structure of ovine hypothalamic somatostatin was determined to be the tetradecapeptide, H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH,[3] the biological properties against various endocrine glands have been noted by numerous investigators. Structure–activity studies on somatostatin and its analogues have been mainly performed by the Salk Institute group and by Sarantakis, *et al.*, and a large amount of significant information has been obtained. The biological activity of somatostatin is particularly sensitive to replacement of the tryptophan residue at position 8. Thus, whereas the substitution of the tryptophan residue with glycine, alanine, D-alanine and D-tyrosine[4–8] resulted in a marked decrease in activity, the analogue in which replacement was with D-tryptophan was 6—8 times more potent than somatostatin in inhibiting the release of growth hormone, glucagon and insulin.[9]

On the other hand, in contrast to somatostatin, the D-tryptophan analogue of luteinizing hormone-releasing hormone (LH–RH) replaced at position 3 possessed virtually no LH-releas-

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[1] Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature in May 1971: *J. Biol. Chem.*, **247**, 977 (1972). Abbreviations: Nal(1), 3-(1-naphthyl)-L-alanine; Nal(2), 3-(2-naphthyl)-L-alanine; Tca, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; Boc, *tert*-butoxycarbonyl; Bzl, benzyl; Z, benzyloxycarbonyl.

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[3] P. Brazeau, W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin, *Science*, **179**, 77 (1973).

[4] M. Brown, J. Rivier, and W. Vale, *Endocrinology*, **98**, 336 (1976).

[5] W. Vale, P. Brazeau, C. Rivier, M. Brown, B. Boss, J. Rivier, R. Burgus, N. Ling, and R. Guillemin, *Recent Prog. Horm. Res.*, **31**, 365 (1975).

[6] W. Vale, C. Rivier, M. Brown, J. Leppaluoto, N. Ling, M. Monahan, and J. Rivier, *Clin. Endocrinol.* (Oxford), *Suppl.*, **5**, 2615 (1976).

[7] J. Rivier, N. Ling, M. Monahan, C. Rivier, M. Brown, and W. Vale in "Peptides: Chemistry, Structure and Biology," R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, p. 863.

[8] J. Rivier, M. Brown, and W. Vale, *J. Med. Chem.*, **19**, 1010 (1976).

[9] J. Rivier, M. Brown, and W. Vale, *Biochem. Biophys. Res. Commun.*, **65**, 746 (1975).

ing activity.[10] However, we recently found that LH–RH analogues substituted with amino acids having a fused aromatic ring structure in the side-chain at position 3 retained high biological activity.[11] In particular, Nal(1)$^3$–LH–RH was about twice as potent as the original LH–RH, while the potency of Nal(2)$^3$–LH–RH was approximately one-half that of LH–RH.[11,12] These results led us to synthesize somatostatin analogues containing 3-(1-naththyl)-L-alanine and 3-(2-naphthyl)-L-alanine. In addition, in an attempt to obtain more detailed information on the tryptophan residue in somatostatin and to compare the role of somatostatin with that of LH–RH, we have synthesized Nal(1)$^8$-, Nal(2)$^8$- and Tca$^8$-somatostatin and tested their biological activities.

Special amino acids used herein for the substitution, 3-(1-naphthyl)-L-alanine,[11] 3-(2-naphthyl)-L-alanine[11] and 1,2,3,4-tetrahydro-$\beta$-carboline-3-carboxylic acid,[13] were synthesized according to the already described methods. The N$^\alpha$-Boc derivatives of these amino acids were prepared in the usual manner.

The analogues including these amino acids were synthesized by the solid-phase method[14] on a chloromethylated polystyrene. The following side-chain protecting groups were used in the syntheses: Cys, 4-OMe-Bzl; Ser and Thr, Bzl; Lys, 2-Cl-Z. The $\alpha$-amino groups were

Table I. Schedule for Preformed Symmetric Anhydride Coupling

| Step | Operation and reagent[a] | Mixing time (min) |
|---|---|---|
| 1 | $CH_2Cl_2$, 10 ml (3 times) | 1 |
| 2 | 50% TFA/$CH_2Cl_2$, 12 ml (once) | 1 |
| 3 | 50% TFA/$CH_2Cl_2$, 10 ml (once) | 12 |
| 4 | 50% dioxane/$CH_2Cl_2$, 10 ml (3 times) | 1 |
| 5 | $CH_2Cl_2$, 10 ml (3 times) | 1 |
| 6 | 2.5% DIEA/$CH_2Cl_2$, 10 ml (once) | 2.5 |
| 7 | $CH_2Cl_2$, 10 ml (twice) | 1 |
| 8 | Repeat step 6 | |
| 9 | Repeat step 7 | |
| 10 | Repeat step 6 | |
| 11 | $CH_2Cl_2$, 10 ml (4 times) | 1 |
| 12 | Boc-amino acid anhydride (1.5 mmol) in $CH_2Cl_2$ (10 ml) (once) | 120 |
| 13 | DIEA, 0.2 g (once) | 120 |
| 14 | $CH_2Cl_2$, 10 ml (3 times) | 1 |
| 15 | 50% EtOH/$CH_2Cl_2$, 10 ml (3 times) | 1 |

[a] Percentages express v/v ratios.

Table II. Schedule for Active Ester Coupling

| Step | Operation and reagent | Mixing time (min) |
|---|---|---|
| 1—10 | Same as in Table I | |
| 11 | $CH_2Cl_2$, 10 ml (3 times) | 1 |
| 12 | DMF, 10 ml (3 times) | 1 |
| 13 | Boc-Asn-ONp or Boc-Gly-ONp (5.2 mmol) in DMF (10 ml) (once) | 600 |
| 14 | DMF, 10 ml (3 times) | 1 |
| 15 | 50% EtOH/$CH_2Cl_2$, 10 ml (3 times) | 1 |

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10) Y. Hirotsu, D.H. Coy, E.J. Coy, and A.V. Schally, *Biochem. Biophys. Res. Commun.*, **59**, 277 (1974).
11) Y. Yabe, C. Miura, H. Horikoshi, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), **24**, 3149 (1976).
12) K.U. Prasad, R.W. Roeske, F.L. Weitl, J.A. Vilchez-Martinez, and A.V. Schally, *J. Med. Chem.*, **19**, 492 (1976).
13) D.G. Harvey, E.J. Miller, and W. Robson, *J. Chem. Soc.*, **1941**, 153.
14) R.B. Merrifield, *Biochemistry*, 3, 1285 (1964).

protected by the Boc group. Boc-Cys(4-OMe-Bzl) was esterified by the modified Loffet procedure.15) The coupling was carried out by the preformed symmetric anhydride16) of the appropriate Boc-amino acids. The active esters of Boc-Asn and Boc-Gly were used and the coupling was carried out in dimethylformamide (DMF). The schedule used for the syntheses is described in Table I and II.

Cleavage of the peptides from the solid support was performed with anhydrous hydrogen fluoride17) in the presence of anisole. Oxidative cyclization was accomplished in aqueous solution at pH 6.5 with potassium ferricyanide.18) The cyclized peptides were purified by gel filtration on Sephadex G-15 using 50% aqueous acetic acid.19) Final purification was achieved by partition chromatography on Sephadex G-25F20) using a solvent system consisting of *n*-butanol–acetic acid- carbon tetrachloride–water (400: 100: 25: 500).

These purified analogues were characterized by amino acid analyses and their specific optical rotations. All compounds exhibited a single spot on thin-layer chromatography with three different solvent systems (Table III).

TABLE III. Physical Properties and Amino Acid Analyses of Somatostatin Analogues

| Compd. | $[\alpha]_D^{20}$ a) | *Rf* b) | | | Amino acid analyses c) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | deg | II | III | IV | Ala | Gly | Cys | Lys | Asp | Phe | AA d) | Thr | Ser |
| Nal(1)$^8$-somatostatin | −40.9 | 0.58 | 0.17 | 0.46 | 1.00 | 0.97 | 2.07 | 1.95 | 1.01 | 2.88 | 0.97 e) | 1.86 | 0.76 |
| Nal(2)$^8$-somatostatin | −32.4 | 0.57 | 0.17 | 0.46 | 1.00 | 1.00 | 2.08 | 2.02 | 0.97 | 2.74 | 1.18 e) | 1.86 | 0.80 |
| Tca$^8$-somatostatin | −29.8 | 0.52 | 0.16 | 0.43 | 1.00 | 0.99 | 2.07 | 2.00 | 1.04 | 3.02 | 1.00 f) | 1.92 | 0.79 |

*a*) Concentration in 0.1 N AcOH=0.2.
*b*) and *c*) See Experimental Section.
*d*) AA indicates individual amino acids substituted for Trp.
*e*) The elution volume for AA was as follows: Nal(1), 1.33; Nal(2), 0.94 (assuming that the elution volume for Lys is 1.00). CHROMO-BEADS (type C-2, Technicon, 0.6×66 cm) column eluted with 0.35 N citrate buffer pH 5.28 at 50°.
*f*) Tca was identified by Technicon's amino acid analysis assembly using a nine chamber gradient buffer systems. The elution volume based on Lys was 1.26. CHROMO-BEADS (type C-2, Technicon, 0.6×66 cm) column at 70°. Buffer system (Na-citrate buffer) was as follows: (Chamber I) 0.2 N, pH 2.75 (33 ml)+MeOH (2 ml), (II) 0.2 N, pH 2.75 (11 ml)+0.2 N, pH 2.875 (24 ml), (III) 0.2 N, pH 2.875 (35 ml), (IV and V) 0.2 N, pH 3.80 (35 ml), (VI—IX) 1.2 N, pH 6.10 (35 ml).

TABLE IV. Inhibition of TRH Induced TSH Release of Somatostatin Analogues with Modification in Position 8 as compared with Synthetic Somatostatin

| Compd. | % biological activity *in vivo* with 95% confidence limits |
|---|---|
| Synthetic somatostatin | Assumed 100% |
| Nal(1)$^8$-somatostatin | 0.88% (0.35—1.85) |
| Nal(2)$^8$-somatostatin | 1.21% (0.77—1.85) |
| Tca$^8$-somatostatin | 1.48% (0.76—2.78) |

The biological activities of these somatostatin analogues were estimated on the basis of their *in vivo* activities to inhibit thyrotropin releasing hormone (TRH)-induced thyrotropin stimulating hormone (TSH) release in the rat.21) The results are summarized in Table IV.

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15) A. Loffet, *Int. J. Peptide Protein Res.*, **3**, 297 (1971).
16) *a*) H. Hagenmaier and H. Frank, *Z. Phys. Chem.*, **353**, 1973 (1972); *b*) D. Yamashiro and C.H. Li, *Proc. Nat. Acad. Sci. U.S.A.*, **71**, 4945 (1974).
17) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164 (1967).
18) E.B. Hope, V.V.S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962).
19) M. Manning, T.C. Wuu, and J.W.M. Baxter, *J. Chromatogr.*, **38**, 396 (1968).
20) D. Yamashiro, *Nature* (London), **201**, 76 (1964).
21) S. Sawano, Y. Baba, T. Kokubu, and Y. Ishizuka, *Endocrinol. Jpn.*, **21**, 399 (1974).

Contrary to expectation, replacement of the indole ring in the tryptophan residue by the naphthalene ring resulted in a marked diminution in activity. The potency of Nal(1)$^{8}$-somatostatin was somewhat lower than that of Nal(2)$^{8}$-somatostatin unlike the case of Nal(1)$^{3}$- and Nal(2)$^{3}$-LH-RH.[11] On the other hand, Tca$^{8}$-somatostatin, which was expected to have antagonistic action, because we found that Tca$^{3}$-LH-RH was a comparatively potent LH-RH antagonist,[22] showed low but clear agonistic activity. The potency was the highest in the three analogues. The present results suggest strongly that the indolyl NH group in the tryptophan residue of somatostatin is essential for generating the TSH inhibiting activity and that the function achieved by the tryptophan residue in somatostatin is distinct from that in LH-RH.

## Experimental

Melting points were uncorrected. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. Thin-layer chromatography (TLC) was performed on Merck silica gel 60F$_{254}$. *Rf* values refer to the following solvent systems: $Rf^{\mathrm{I}}$, $CHCl_3$–EtOH–AcOH (95: 5: 3, v/v/v); $Rf^{\mathrm{II}}$, $CHCl_3$–MeOH–32% aq. AcOH (60: 45: 20); $Rf^{\mathrm{III}}$, *n*-BuOH–AcOH–$H_2O$ (4: 1: 5, upper phase); $Rf^{\mathrm{IV}}$, *n*-BuOH–AcOH–$H_2O$–pyridine (30: 6: 24: 20). Trifluoroacetic acid (TFA) was distilled before use. N,N′-Dicyclohexylcarbodiimide (DCCD) for solid-phase peptide synthesis was purchased from the Protein Research Foundation (PRF; Minoh-shi, Osaka). Diisopropylethylamine (DIEA) was washed with water, dried over NaOH pellets, and distilled. Dioxane was distilled from sodium and stored at 4°. $CH_2Cl_2$ was refluxed over $P_2O_5$ for 2 hr and then distilled. Chloromethylated polystyrene (2% divinylbenzene, 0.6 mmol of Cl/g, 100—200 mesh) was purchased from PRF. Amino acid analyses of peptides were carried out on samples that had been hydrolyzed with constant boiling HCl containing 4% thioglycolic acid for 24 hr in evacuated, sealed tubes at 110° and also on samples that had been hydrolyzed in the above manner after oxidation by performic acid, and recorded on a Hitachi amino acid analyzer KLA-2 and Technicon's amino acid analysis assembly.

**Boc-Nal(1)** (1)——Nal(1)[11] (1.09 g, 5 mmol) was dissolved in 0.1 N NaOH (75 ml) and dioxane (50 ml). Boc azide (0.86 g, 6 mmol) was added to the solution and the mixture was stirred at room temperature for 39 hr. During the reaction, the pH was kept at 11.0. The reaction mixture was washed with ether and the aqueous layer was acidified with 2 N $KHSO_4$. The precipitated oil was extracted with ether and the organic layer was washed with saturated brine. After evaporation of the solvent, the syrupy residue was redissolved in ether (10 ml). To this solution, *n*-hexane (100 ml) was added and allowed to stand at 4° for 2 days to give colourless needles: yield 1.15 g (72%). mp 94—95°. $[\alpha]_D^{20}$ −55.1° ($c$=0.8, MeOH). $Rf^{\mathrm{I}}$ 0.54. *Anal.* Calcd. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.24; H, 6.59; N, 4.38.

**Boc-Nal(2)** (2)——Prepared from Nal(2)[11] (1.09 g, 5 mmol) and Boc azide (0.86 g, 6 mmol) in the same manner as described in **1**. Recrystallization from *n*-hexane–ether afforded colourless needles: yield 0.95 g (60%). mp 89—91° (lit.[12] 93—95°). $[\alpha]_D^{20}$ 32.9° ($c$=0.88, MeOH). [lit.[12] $[\alpha]_D^{23}$ 42° ($c$=0.5, EtOH)]. $Rf^{\mathrm{I}}$ 0.61. *Anal.* Calcd. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.29; H, 6.75; N, 4.44.

**Boc-Tca** (3)——Tca[13] (2.16 g, 10 mmol) was dissolved in 0.1 N NaOH (150 ml) and dioxane (100 ml), and treated with Boc azide (1.72 g, 12 mmol). The mixture was stirred at room temperature for 12 days while maintaining the pH at 11.0. During the reaction, four portions of Boc azide (1.72 g, 12 mmol) were added. The reaction mixture was washed with ether and the aqueous layer was acidified with 2 N $KHSO_4$ to pH 4.0. The separated oil was extracted with ether and the organic layer was washed with saturated brine. After drying over anhyd. $Na_2SO_4$, the solvent was evaporated to give a colourless solid. Recrystallization from *n*-hexane–ether afforded colourless needles: yield 2.11 g (67%). mp above 270°. $[\alpha]_D^{20}$ 87.0° ($c$=0.96, MeOH). $Rf^{\mathrm{I}}$ 0.89. *Anal.* Calcd. for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.24; H, 6.54; N, 8.65.

**Boc-Cys(4-OMe-Bzl) Polymer** (4)——Boc-Cys(4-OMe–Bzl) (2.74 g, 8 mmol) was dissolved in MeOH (8 ml) and mixed with 10% tetramethylammonium hydroxide in MeOH (7.34 ml). The solution was concentrated *in vacuo* to dryness and placed over $P_2O_5$ for 1.5 hr. Afterwards, the salt was allowed to react with chloromethylated polymer (4.76 g) in DMF (40 ml) for 10.5 hr at 75—80°.[15] The resin was collected on filter with the aid of DMF, and washed successively with DMF, MeOH, water and MeOH. A weight gain to 5.83 g was consistent with a substitution of 0.52 mmol/g obtained by Gisin test.[23]

**Protected Peptide Resins of Nal(1)$^{8}$-, Nal(2)$^{8}$- and Tca$^{8}$-Somatostatin (5, 6 and 7)**——**4** (1 g, 0.52 mmol) was placed in a reaction vessel (2 × 7.5 cm) on a shaker for manual operation and carried through the schedule shown in Tables I and II for incorporating the remaining 13 amino acid residues. $N^{\alpha}$-Boc protection was used throughout with following side-chain protecting groups: Cys, 4-OMe–Bzl; Ser and Thr, Bzl; Lys, 2-Cl-Z.

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[22] Y. Yabe, H. Horikoshi, and Y. Baba, *Endocrinol Jpn.*, in preparation

[23] B.F. Gisin, *Anal. Chim. Acta*, **58**, 248 (1972).

Boc–Asn and Boc–Gly were coupled as their *p*-nitrophenyl esters, as in Table II. The symmetric anhydride of the Boc-amino acid used in all other coupling (Table I) was prepared as follows: Boc-amino acid (3 mmol) in $CH_2Cl_2$ (6 ml) was cooled to 0° and treated with DCCD (0.33 g, 1.65 mmol). After the mixture was stirred for 1 hr at 0°, the precipitate was filtered and washed with $CH_2Cl_2$ (3 ml). The filtrate was added immediately to the resin manually. Boc–Tca (3; 1.3 g, 4.1 mmol) was dissolved in DMF (2 ml) and diluted with $CH_2Cl_2$ (8 ml) before the addition of DCCD (0.47 g, 2.3 mmol). The finished peptide resin was dried under reduced pressure over $P_2O_5$ to yield 1.7—1.9 g. About 20 mg of the resin was removed for analytical purpose during the synthesis.

**Nal(1)$^8$-, Nal(2)$^8$- and Tca$^8$-somatostatin (8, 9 and 10)**——A batch of protected peptide resin (**5**, 1.8 g; **6**, 1.9 g; **7**, 1.7 g) was treated with HF[17] (30 ml) for 1 hr at 0° in the presence of anisole (3 ml). After removal of HF and drying under reduced pressure, the resin was washed with AcOEt (40 ml) and extracted with TFA (25 ml). TFA was evaporated *in vacuo* and the residue was dissolved in 50% aq. acetonitrile (25 ml). The solution was immediately diluted with water (2.2 l), the pH was adjusted to 6.5 with dil. $NH_4OH$, and titrated under stirring with 0.01 M potassium ferricyanide[18] until a permanent yellow colour was observed. After 10 min, AG3-X4A resin (BIO-RAD Laboratories, 200—400 mesh, Cl form, 8 g) was added and stirring was continued for 5 min to remove forrocyanide and excess ferricyanide ions. The resin was filtered off and the filtrate was passed through a 2 × 15.5 cm column of the AG3-X4A resin. The pH of the filtrate was adjusted to 3.0 with AcOH and the solution was lyophilized. Purification was effected by the method of Manning, *et al.*[19] The lyophilisate was dissolved in 50% aq. AcOH (10 ml), applied to the top of column of Sephadex G-15 (Pharmacia Fine Chemicals, column size 3 × 86 cm) which had been preequilibrated with 50% aq. AcOH (500 ml), and eluted with the same solvent. The eluate was collected in 8.4 ml portions (**8** and **9**) or 6.7 ml portions (**10**) and absorbancy at 280 nm (**8** and **10**) or 275 nm (**9**) were measured. The necessary fractions (**8**, No. 22—33; **9**, No. 19—40; **10**, No. 28—45) were combined and concentrated *in vacuo* to yield 0.33 g (**8**), 0.66 g (**9**) or 0.42 g (**10**). The crude peptide (**8**, 140 mg; **9**, 94 mg[24]; **10**, 103 mg) was dissolved in the upper phase (1.5 ml) of *n*-BuOH–AcOH–$CCl_4$–$H_2O$ (400: 100: 25: 500) and the solution was submitted to partition chromatography on Sephadex G-25F[20] (2.5 × 90 cm) with collection of 9.6 ml fractions. The necessary fractions (**8**, No. 70—80; **9**, No. 66—76; **10**, No. 99—114) were combined and concentrated *in vacuo* to give 16.1 mg (**8**), 19.1 mg (**9**) or 23.3 mg (**10**). The product was dissolved in 0.1 N AcOH (1 ml) and the solution was chromatographed on Sephadex G-25F in the same solvent. Lyophilisation of the necessary fractions gave a fluffy powder: 13.0 mg (**8**), 16.1 mg (**9**) and 18.2 mg (**10**).

**Biological Assay**——The *in vivo* biological assay was based on somatostatin or synthesized somatostatin analogues inhibition of TSH release induced by TRH in rats. Male Wister rats, weighing an average of 200 g, were used as assay animals after an overnight fast. TRH at a dose of 500 ng in 0.2 ml physiological saline was injected into the jugular vein of the animals under urethane anesthesia (150 mg/100 g body weight, *i.p.*). Immediately thereafter, somatostatin in 0.5 ml physiological saline was infused into the jugular vein at a rate of 50 ng/min or 500 ng/min for 10 min by a perstaltic pump (Manostat, Model No. SF-0895).[21] Synthesized somatostatin analogues appropriately diluted were infused in the same way. Physiological saline was infused as the control. Blood samples were drawn from the jugular vein before and 10 min after the single injection of TRH. The concentration of serum TSH was measured by double antibody radioimmunoassay employing NIAMDD kits, and expressed in terms of NIAMD-rat TSH-RP-1. The minimum effective dose of somatostatin to suppress the release of TSH stimulated by 500 ng of TRH was 4.0 ng/min in this assay system. The dose-response relationship was established between 4.0 ng/min and 500 ng/min of somatostatin. The details concerning the dose-response curve will appear elsewhere. The relative potency ratios of the somatostatin analogues against somatostatin as the reference were calculated by 3-point analysis.

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24) The crude peptide (0.66 g) was dissolved in the upper phase (10 ml) of *n*-BuOH–AcOH–$CCl_4$–$H_2O$ (400: 100: 25: 500) and the insoluble material was filtered off. The filtrate was concentrated to dryness *in vacuo* to give a solid residue (227 mg). For purification, 94 mg of this crude peptide was used.