Application of *S*-1-Adamantylcysteine to the Synthesis of a 37-Residue Peptide Amide corresponding to Human Calcitonin Gene-related Peptide (hCGRP)

Nobutaka Fujii,[a] Haruaki Yajima,*[a] Akira Otaka,[a] Susumu Funakoshi,[a] Motoyoshi Nomizu,[a] Kenichi Akaji,[a] Itsuo Yamamoto,[b] Kanji Torizuka,[b] Kouki Kitagawa,[c] Tadashi Akita,[c] Kenshi Ando,[c] Tatsuhiko Kawamoto,[c] Yasutsugu Shimonishi,[d] and Toshifumi Takao[d]

[a] *Faculty of Pharmaceutical Sciences and* [b] *School of Medicine, Kyoto University, Kyoto 606, Japan*
[c] *Faculty of Pharmaceutical Sciences, Tokushima University, Tokushima 743, Japan*
[d] *Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan*

An *S*-1-adamantyl (Ad) group on cysteine was found to be cleaved smoothly by 1 M trifluoromethanesulphonic acid–thioanisole in trifluoroacetic acid or thallic trifluoroacetate; the usefulness of Cys(Ad) in practical peptide synthesis was demonstrated by synthesizing human calcitonin gene-related peptide, a 37-residue peptide amide with one disulphide bridge using a conventional solution method.

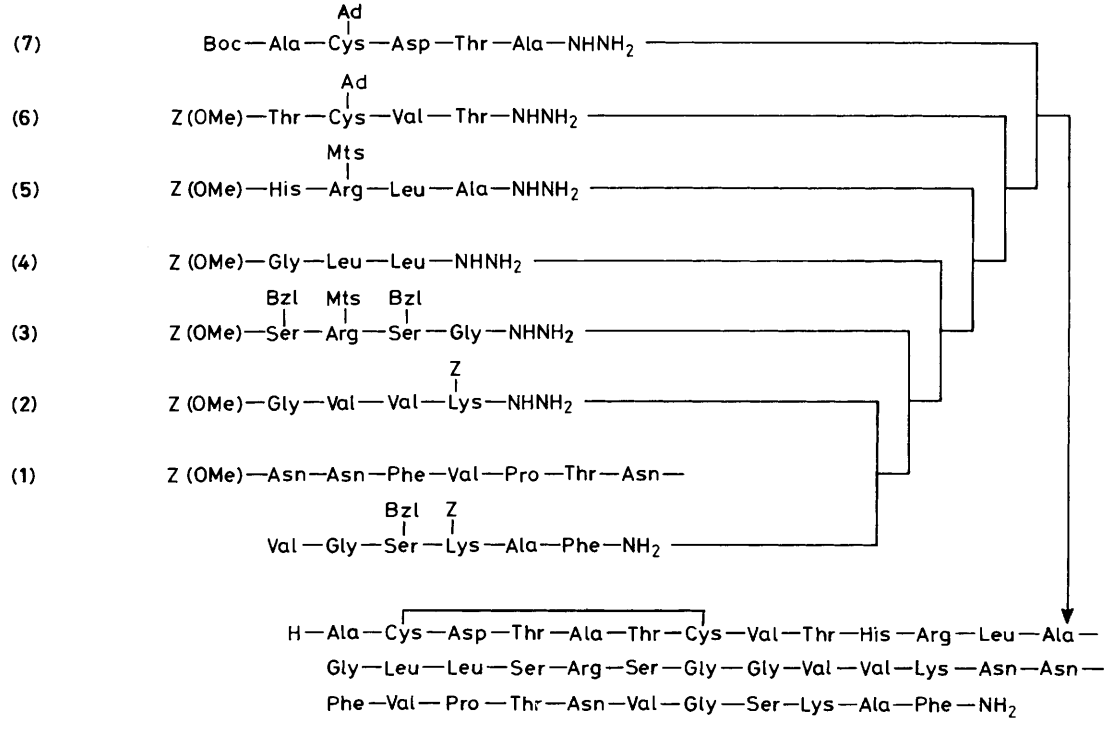
Human calcitonin gene-related peptide, hCGRP, has been isolated from human medullary thyroid carcinoma by Morris *et al.*[1] This 37-residue peptide amide with one disulphide bridge was synthesized using a conventional solution method using Cys(Ad) [Ad = *S*-1-adamantyl].[2]

The *S*-Ad group is cleaved by $(CH_3CO_2)_2Hg$ in trifluoroacetic acid (TFA), but this reaction has never been used in peptide synthesis. In comparison with the *S*-*p*-methoxybenzyl (*S*-MBzl) group,[3] the *S*-Ad group is more stable to N^{α}-deprotection by TFA and resistant to sulphoxide formation.[4] This group is cleaved smoothly by 1 M trifluoromethanesulphonic acid (TFMSA)–thioanisole in TFA[5] or alternatively by $(CF_3CO_2)_3Tl$ in an ice bath within 1 h.

For the synthesis of hCGRP, besides Cys(Ad), amino acid derivatives bearing protecting groups removable by 1 M TFMSA–thioanisole in TFA were employed, *i.e.*, Lys(Z), Ser(Bzl), Asp(OBzl), and Arg(Mts).[6] The TFA-labile Z(OMe) and Boc groups gave temporary N^{α}-protection of various acyl components (Scheme 1) [Z(OMe) = *p*-methoxybenzyloxycarbonyl, Z = benzyloxycarbonyl, Boc = t-butoxycarbonyl, Bzl = benzyl, Mts = mesitylene-2-sulphonyl].

Seven peptide fragments were selected as building blocks to construct the entire amino acid sequence of hCGRP using Honzl and Rudinger's azide procedure.[7] The peptide hydrazides were synthesized by known amide forming reactions. The *C*-terminal 13-residue peptide amide (**1**) was prepared by azide condensation of Z(OMe)-Phe-Val-Pro-Thr-$NHNH_2$ and H-Asn-Val-Gly-Ser(Bzl)-Lys(Z)-Ala-Phe-NH_2 followed by successive *p*-nitrophenyl ester condensations[8] of two Asn residues (positions 25 and 26). The Bzl protecting group of the Asp residue (position 3) was deliberately removed by hydrogenolysis, before condensation of the Cys(Ad) residue, at the stage of fragment synthesis in order to avoid base-catalysed ring closure between the Asp(OBzl)-Thr linkage.[9]

The necessary fragments thus obtained were then assembled according to Scheme 1. Using excess of acyl components (1.5—5.0 equiv.), each coupling reaction was performed in a mixture of dimethylformamide (DMF) and dimethylsulphoxide (DMSO) or in hexamethylphosphoramide. The azide condensation of fragment (**6**) had to be performed at a lower temperature (−15 °C) than usual (−4 °C), otherwise acid hydrolysis of the product gave a low recovery of Thr, the



Scheme 1

C-terminal amino acid of fragment (**6**), presumably owing to partial Curtius rearrangement.[10] Protected intermediates, with two exceptions were purified by precipitation from DMF or DMSO with appropriate solvents, such as MeOH or AcOEt. The protected hCGRP and the protected 32-residue peptide (position 6—37) were purified by gel-filtration on Sephadex LH-60 using DMSO–DMF (3:7) as eluant. Throughout this synthesis, Phe was selected as a diagnostic amino acid in acid hydrolysis. By comparing the quantity of Phe recovered with the amount of newly incorporated amino acid after each condensation, incorporation of each fragment was ascertained. Homogeneity of each intermediate was also confirmed by elemental analysis and t.l.c. That no sulphoxide was formed on Cys(Ad) was confirmed by hydrolysis of the protected hCGRP with 6 M HCl in the presence of phenol. No *S*-*p*-hydroxyphenylcysteine was detected.[4]

For deprotection, the protected hCGRP was first treated with 1 M TFMSA–thioanisole in TFA in the presence of *m*-cresol (0 °C, 4 h). The deprotected peptide was reduced with mercaptoethanol in 6 M guanidine–HCl in Tris-HCl buffer (pH 8.0) and, after gel-filtration on Sephadex G-25, was oxidized in air to form a disulphide bridge. A diluted solution (peptide concentration 74.5 mg dm^{-3}) in 0.08 M $AcONH_4$ (pH 7.5) kept standing at 25 °C for 2 days, was used to monitor the progress of air oxidation by Ellman's test.[11] The crude product was purified by gel-filtration on Sephadex G-50, followed by ion-exchange chromatography on CM-Biogel A using gradient elution with 0.2 M $AcONH_4$ (pH 6.8). The final purification was achieved by reverse-phase h.p.l.c. on Nucleosil $5C_{18}$ column using gradient elution with acetonitrile (30 to 35% for 1 h) in 0.2% TFA. Purity of the product (yield 17%) was confirmed by acid hydrolysis, h.p.l.c., and disc isoelectrophoresis (Pharmalite pH 3—10) and its mass was determined by fast atom bombardment mass spectrometry ($M + H^+$ 3789).

Alternatively, the protected hCGRP was treated with TFA in the presence of thioanisole and *m*-cresol (25 °C, 24 h) to remove the protecting groups, Boc, Z, Bzl, and Mts. This thioanisole mediated deprotection[12] was repeated to ensure complete deprotection. The *S*-Ad group was removed by treatment with $(CF_3CO_2)_3Tl$ in TFA (0 °C, 1 h). The desired product was obtained in 8% yield. That no thallium contamination had occurred in the synthetic peptide was confirmed by atomic absorption analysis.

Synthetic hCGRP (2×10^{-7} M) increased the level of cAMP in the monolayered culture cells from the kidney and brain of new born rats, and it (1×10^{-7} M) suppressed bone ^{45}Ca-release stimulated by a synthetic sample of human parathyroid hormone 1–34 purchased from Toyo Jozo Co.

The authors are grateful to Dr. Mistunobu Kitamura, Environmental Preservation Center, Kyoto University, for atomic absorption analysis of the synthetic peptide.

Received, 2nd January 1985; Com. 018

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