Communications to the Editor

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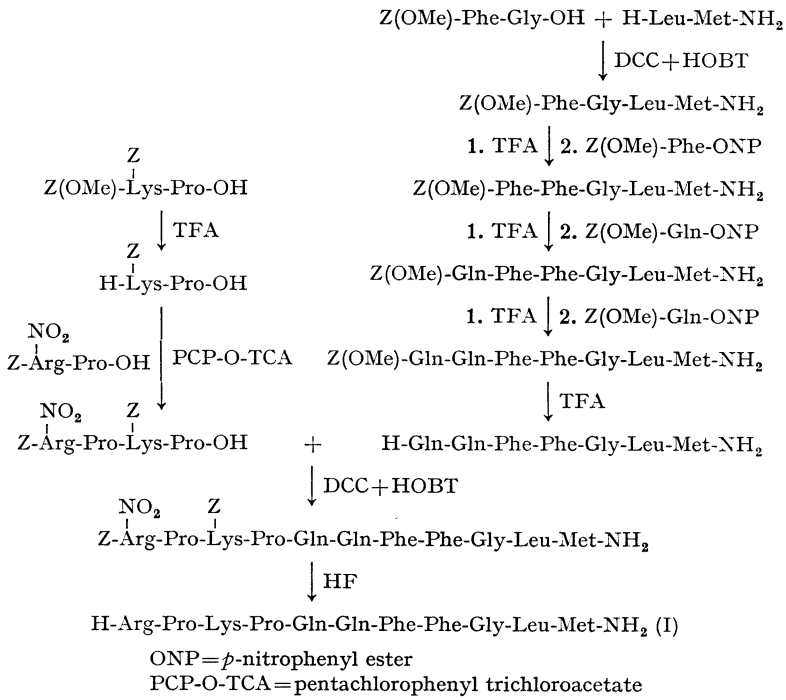
Studies on Peptides. XXXIV.[1,2] Conventional Synthesis of the Undecapeptide Amide corresponding to the Entire Amino Acid Sequence of Bovine Substance P

The amino acid sequence of bovine substance P was established by Chang, *et al.*[3] in 1971 and its solid phase synthesis has been reported by the same group of investigators.[4] We have synthesized the undecapeptide amide (I) corresponding to the entire amino acid sequence of this important hypothalamic principle by the conventional chain elongation procedure as shown in Chart 1.



ONP=*p*-nitrophenyl ester
PCP-O-TCA=pentachlorophenyl trichloroacetate

Chart 1. Synthetic Route to Substance P

The c-terminal tetrapeptide amide, Z(OMe)-Phe-Gly-Leu-Met-NH_2 (*Anal.* Calcd. for $C_{31}H_{43}O_7N_5S$: C, 59.12; H, 6.88; N, 11.12. Found: C, 58.90; H, 6.74; N, 11.25. mp 208—209°, $[\alpha]_D^{22}$ −33.3° in DMF), was obtained by condensation of Z(OMe)-Phe-Gly-OH and H-Leu-

1) Part XXXIII: H. Yajima, H. Watanabe, and M. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 2186 (1971).
2) The peptides and peptide derivatives mentioned in this communication are of the L-configuration. Abbreviations: Z=benzyloxycarbonyl; Z(OMe)=*p*-methoxybenzyloxycarbonyl; DCC=dicyclohexylcarbodiimide; HOBT=N-hydroxybenztriazole; TFA=trifluoroacetic acid; DMF=dimethylformamide; DMSO=dimethylsulfoxide.
3) M.M. Chang, S.E. Leeman, and H.D. Niall, *Nature New Biol.*, **232**, 86 (1971).
4) G.W. Tregear, H.D. Niall, J.T. Potts, Jun., S.E. Leeman, and M.M. Chang, *Nature New Biol.*, **232**, 87 (1971).

Met-NH_2[5] with DCC in the presence of HOBT.[6] The latter reagent suppresses the formation of acylurea, which is a known side reaction of DCC coupling reaction.

Combination of the TFA treatment[7] and the *p*-nitrophenyl ester procedure[8] was applied to the elongation of the tetrapeptide amide in a stepwise manner for the synthesis of the protected heptapeptide amide, Z(OMe)-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (*Anal.* Calcd. for $C_{50}H_{68}O_{12}N_{10}S \cdot H_2O$: C, 57.12; H, 6.71; N, 13.33. Found: C, 56.93; H, 6.60; N, 13.51. mp 257—259°, $[\alpha]_D^{24}$ −17.9° in DMSO). The intermediates, Z(OMe)-Phe-Phe-Gly-Leu-Met-NH_2 and Z(OMe)-Gln-Phe-Phe-Gly-Leu-Met-NH_2, were also obtained in analytically pure form.

For the synthesis of the N-terminal tetrapeptide, Z-Arg(NO_2)-Pro-Lys(Z)-Pro-OH (*Anal.* Calcd. for $C_{38}H_{51}O_{11}N_9$: C, 56.35; H, 6.35; N, 15.56. Found: C, 56.15; H, 6.48; N, 15.58. mp 101—103°, $[\alpha]_D^{29}$ −63.0° in methanol), the pentachlorophenyl trichloroacetate procedure[9] was applied to condense Z-Arg(NO_2)-Pro-OH[10] and H-Lys(Z)-Pro-OH derived from Z(OMe)-Lys(Z)-Pro-OH (oil) by the TFA treatment.

For the final coupling reaction, the protected heptapeptide amide obtained above was treated with TFA in the presence of anisole and the product, after conversion to the corresponding hydrochloride followed by neutralization with triethylamine, was condensed with Z-Arg(NO_2)-Pro-Lys(Z)-Pro-OH by the DCC plus HOBT procedure[6] to give the fully protected undecapeptide amide, Z-Arg(NO_2)-Pro-Lys(Z)-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (*Anal.* Calcd. for $C_{79}H_{109}O_{19}N_{19}S \cdot 2\ H_2O$: C, 55.91; H, 6.71; N, 15.68. Found: C, 55.53; H, 6.67; N, 15.44. mp 224—225°, $[\alpha]_D^{26}$ −37.0° in DMF). This was then treated with HF[11] in the presence of anisole at 0° for 30 minutes to remove all the protecting groups. The deblocked undecapeptide amide was purified by column chromatography on CM-cellulose using the pH 6.9, 0.1M ammonium acetate buffer. The synthetic undecapeptide amide corresponding to the entire amono acid sequence of substance P (I) thus purified was homogeneous on thin layer chromatography and its purity was further assessed by elemental and amino acid analyses (*Anal.* Calcd. for $C_{63}H_{98}O_{13}N_{18}S \cdot 3CH_3COOH \cdot 4H_2O$: C, 51.80; H, 7.43; N, 15.76. Found: C, 51.75; H, 7.25; N, 15.66. $[\alpha]_D^{27}$ −76.0° in 5% acetic acid); *Rf* 0.55 (lit.[12] 0.6) on silica gel (*n*-butanol, pyridine, acetic acid, water: 30: 20: 6: 24 v/v); amino acid ratios in an acid hydrolysate $Arg_{1.03}$ $Pro_{2.07}$ $Lys_{0.83}$ $Glu_{2.08}$ $Phe_{2.30}$ $Gly_{0.90}$ $Leu_{1.00}$ $Met_{0.90}$ (average recovery 96%); complete digestion of the synthetic I by aminopeptidase-M (AP-M) has been unsuccessful because of lack of the prolidase activity of this enzyme.[13]

Biological activity of the synthetic undecapeptide amide (I) was measured by Dr. M. Otsuka of Tokyo Medical and Dental University in comparison with the solid phase synthetic peptide of known activity.[4] When contractility on isolated guinea-pig ileum was examined, our synthetic peptide was active as the standard sample or even more potent. Detail account of these results will be published by Otsuka, *et al.*

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