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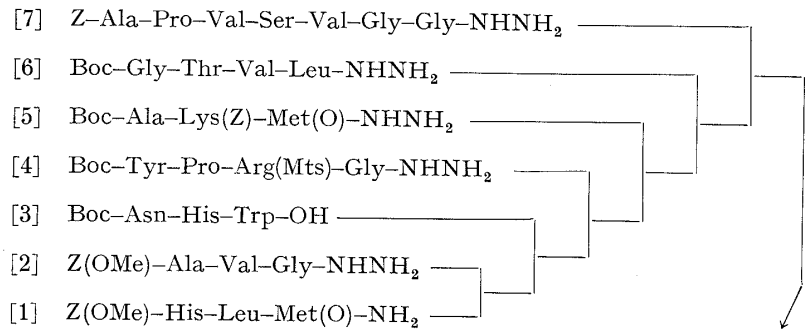
Synthesis of a Heptacosapeptide Amide corresponding to the Entire Amino Acid Sequence of Gastric Gastrin Releasing Peptide[1]

The heptacosapeptide amide corresponding to the entire amino acid sequence of gastric gastrin releasing peptide was synthesized using a new carboxyl-activating reagent, thiazoline-2-thione, and a new deprotecting system, 1 M trifluoromethanesulfonic acid (TFMSA)-thioanisole in TFA.

Keywords——synthesis of gastric gastrin releasing peptide; 1 M solution of trifluoromethanesulfonic acid-thioanisole (1: 1 equiv.) in TFA as a deprotecting system; N^G-mesitylene-2-sulfonylarginine; carboxyl-activating reagent, thiazoline-2-thione; side reactions at the Trp residue; ethanedithiol as a cation scavenger; reduction of Met(O) under acid treatment with thioanisole; m-cresol as a scavenger to suppress the O-sulfonylation at the Tyr residue

Recently, McDonald *et al.*[2] assigned tentatively the complete amino acid sequence of a bombesin-like heptacosapeptide amide, with potent gastrin releasing activity, which was isolated from porcine non-antral gastric and intestinal tissue.



H–Ala–Pro–Val–Ser–Val–Gly–Gly–Gly–Thr–Val–Leu–Ala–Lys–Met–Tyr–
Pro–Arg–Gly–Asn–His–Trp–Ala–Val–Gly–His–Leu–Met–NH_2

Fig. 1. Synthetic Route for the Gastric Gastrin Releasing Peptide

We wish to report preliminarily the synthesis of the heptacosapeptide amide corresponding to the proposed amino acid sequence of this new gastrointestinal peptide (Fig. 1). A new deprotecting procedure, trifluoromethanesulfonic acid (TFMSA)–thioanisole,[3,4] was employed in the final step of the synthesis. Thus, amino acid derivatives bearing protecting groups removable by combination of these two reagents were employed; *i. e.*, Lys (Z) and Arg (Mts).[5]

1) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Mts=mesitylene-2-sulfonyl, DCC=dicyclohexylcarbodiimide, HOBT=N-hydroxybenzotriazole, DMF=dimethylformamide, DMSO=dimethylsulfoxide, EDT=ethanedithiol, TFA=trifluoroacetic acid, MSA=methanesulfonic acid.

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Each Met residue was protected as the corresponding sulfoxide[6] in order to prevent partial oxidation during the synthesis.

For construction of the peptide backbone, seven peptide fragments were selected. The C-terminal tripeptide amide [1] was synthesized in the sulfoxide form alternative to our partial synthesis[7] of bombesin,[8] which shares the common C-terminal heptapeptide sequence with gastrin releasing peptide. These fragments with different protecting groups and bombesin itself were alternatively synthesized by other authors.[9] The fragments, [1] and [3], were prepared by the conventional amide forming reactions and the rests of the fragments, ([2], [4]—[7]) were synthesized in some part using a new carboxyl-activating reagent, thiazoline-2-thione.[10] They were assembled by the Honzl and Rudinger's azide procedure,[11] except for fragment [2], Boc–Asn–His–Trp–OH, since the azide procedure is not ideal for Trp-containing peptides. This fragment was thus introduced by DCC in the presence of HOBT[12] in order to suppress possible racemization. After incorporation of the Trp residue into the chain, the Boc group was adopted as the N^{α}-protecting group for fragments [3]—[6], since much less side reactions at the Trp residue was involved during the N^{α}-deprotection with TFA,[13] compared with the TFA deprotection of Z(OMe).[14] In addition, anisole containing EDT[15] was applied as a cation scavenger to minimize the side reactions at the Trp residue during the TFA treatment.

The protected heptacosapeptide amide, Z–Ala–Pro–Val–Ser–Val–Gly–Gly–Gly–Thr–Val–Leu–Ala–Lys(Z)–Met(O)–Tyr–Pro–Arg(Mts)–Gly–Asn–His–Trp–Ala–Val–Gly–His–Leu–Met-(O)–NH_2 (mp 236—239°, $[\alpha]_D^{23}$ −50.8° in DMSO, *Rf* 0.53 in *n*-BuOH–AcOH–AcOEt–H_2O= 1:1:1:1, amino acid ratios in 4 N MSA hydrolysate: Asp 1.08, Thr 0.96, Ser 1.01, Pro 1.63, Gly 5.17, Ala 3.11, Val 4.22, Met+Met(O) 1.83, Leu 2.00, Tyr 1.03, Trp 0.71, Lys 1.01, His 2.08, Arg 1.01 (recovery of Leu 78%). *Anal.* Calcd for $C_{151}H_{220}N_{38}O_{39}S_3 \cdot 2H_2O$: C, 54.56; H, 6.79; N, 16.01. Found: C, 54.52; H, 6.77; N, 15.73), was purified by gel-filtration on Sephacryl S-200 with DMF–5% H_2O as eluent[16] and subjected to deprotection with 1 M TFMSA-thioanisole in TFA in an ice-bath for 60 min. *m*-Cresol was used as an additional scavenger to suppress a side reaction at the Tyr residue, *i. e.*, O-sulfonylation.[5] Tlc examination revealed that the most of the Met(O) residue was reduced back to Met under this acid treatment with thioanisole. In order to ensure the complete reduction, the deprotected peptide, after brief treatment with

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dil. NH_4OH at pH 10, was incubated, at pH 6.0, with dithiothreitol at 60° for 12 hr. The reduced peptide was then purified by gel-filtration on Sephadex G-25 and ionexchange chromatography on CM-cellulose with a gradient elution with pH 7.9, 0.1 M NH_4HCO_3 buffer to yield a homogeneous product. ($[\alpha]_D^{23}$ −89.5° in 1% AcOH, *Rf* 0.48 in *n*-BuOH–AcOH–Pyr–H_2O=4: 1: 1: 2, 0.61 in *n*-BuOH–AcOH–Pyr–H_2O=30: 6: 20: 24, Amino acid ratios in 4 N MSA hydrolysate: Asp 1.01, Thr 0.96, Ser 0.91, Pro 1.81, Gly 4.98, Ala 3.00, Val 4.09, Met 1.85, Leu 2.00, Tyr 1.08, Trp 0.88, Lys 1.05, His 2.08, Arg 1.06 (recovery of Leu 89%). *Anal.* Calcd for $C_{126}H_{198}N_{38}O_{41}S_2 \cdot 3CH_3COOH \cdot 7H_2O$: C, 50.95; H, 6.80; N, 17.11. Found: C, 51.03; H, 6.78; N, 17.35).

It was found that by an intravenous administration to dog, our synthetic peptide exhibited remarkable gastrin releasing activity. Details of biological assays will be published in a separated paper.

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