Synthesis of Galanin, a New Gastrointestinal Polypeptide

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A new gastrointestinal polypeptide, galanin, which consists of 29 amino acids, was synthesized using the trifluoromethanesulphonic acid deprotecting procedure: two new amino acid derivatives, Trp(Mts) [Mts = N^{in}-mesitylene-2-sulphonyl] and Asp(OChp) [Chp = β-cycloheptyl], were employed for this synthesis to suppress possible side reactions, *i.e.*, indole-alkylation and succinimide formation respectively; the synthetic peptide exhibited contractile activity in isolated rat ileum and hyperglycemic activity in dogs.

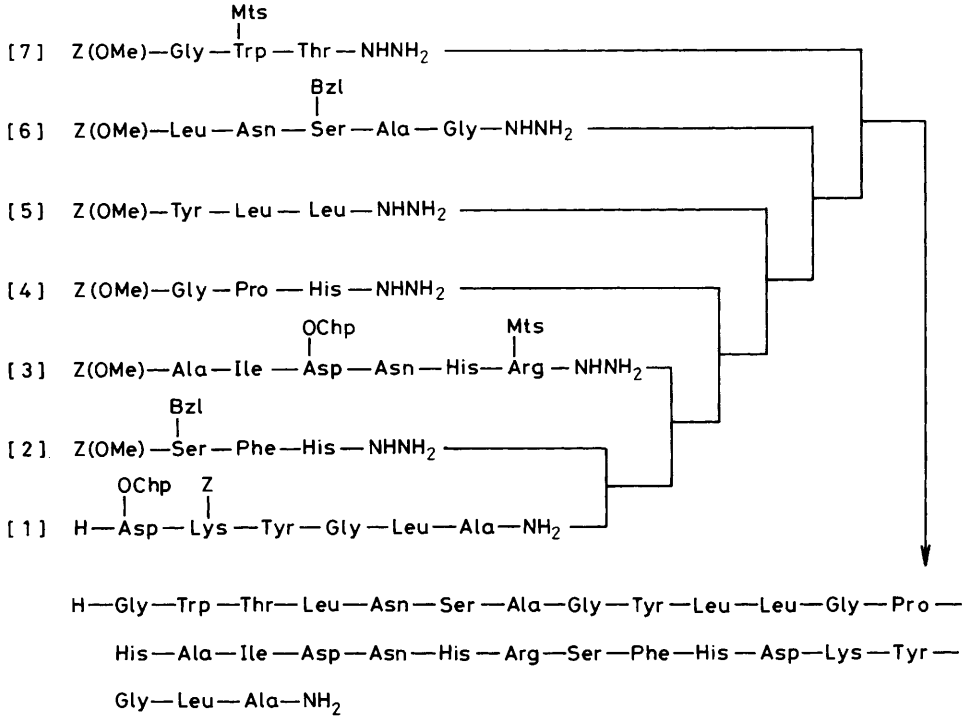
Galanin is a 29 amino acid peptide which was isolated from porcine intestine by Tatemoto *et al.*[1] in 1983. We have synthesized this novel peptide by the conventional solution method using the trifluoromethanesulphonic acid (TFMSA) deprotecting procedure.[2]

Besides Arg(Mts),[3] Lys(Z), and Ser(Bzl), new Trp and Asp derivatives, Trp(Mts)[4] and Asp(OChp),[5] were employed for the present synthesis (Mts = mesitylene-2-sulphonyl, Z = benzyloxycarbonyl, Bzl = benzyl, Chp = cycloheptyl). The former N^{in}-protecting group was found to suppress indole-alkylation[6] during the N^{α}-deprotection by trifluoroacetic acid (TFA). The latter derivative was prepared according to the procedures of Blake[7] and Tam *et al.*[8] and its β-carboxy protecting group was found to suppress base-catalysed succinimide formation effectively.[9] Both protecting groups are stable to TFA, but are cleaved smoothly by 1 M TFMSA–thioanisole in TFA[2] within 60 min at room temperature.

The TFA-labile Z(OMe) group[10] was employed for N^{α}-protection and seven peptide fragments were selected as building blocks to construct the peptide backbone, Scheme 1. They were synthesized by the known amide-forming reactions; fragment [3] containing Asp(OChp) was prepared starting with Troc-$NHNH_2$ (Troc = β,β,β-trichloroethyloxycarbonyl),[11] the protecting group of which is known to be cleaved by Zn[12] or Cd[13] in acetic acid.

Seven fragments thus prepared were assembled successively by Honzl and Rudinger's azide procedure[14] to minimize possible racemization. This method is still the best method to introduce the unprotected His residue with negligible racemization.[15] Each product was purified by precipitation from *N,N'*-dimethylformamide (DMF) with methanol or ethyl acetate and the protected galanin by gel-filtration on Sephadex LH-60 using DMF as an eluant. The purity of these derivatives was confirmed by t.l.c., elemental analysis, and amino acid analysis after acid hydrolysis. In the last instance, Leu was selected as a diagnostic amino acid. By comparison of the recovery of Leu with those of newly incorporated amino acids, satisfactory condensation of each fragment was ascertained.

For deprotection, the protected galanin was treated with 1 M TFMSA–thioanisole in TFA in the presence of additional



Scheme 1

scavengers, *m*-cresol[16] and ethanedithiol,[17] in an ice-bath for 2 h. The deprotected peptide, after brief treatment with dil. ammonia at pH 8,[18] was purified by gel-filtration on Sephadex G-25 with 0.5 M acetic acid as an eluant, followed by h.p.l.c. on Nucleosil 5C18 with an isocratic elution using MeCN–0.2% TFA (35 : 65) as an eluant to yield a homogeneous product (yield 33%). The purified product exhibited a single peak on h.p.l.c. with an identical retention time to that of natural galanin. Its homogeneity was further assessed by disc isoelectrofocusing (pH 3—10), acid hydrolysis, and aminopeptidase digestion.

Galanin is reported to cause sustained hyperglycemia and have an ability to contract smooth muscle preparations.[1] When administered intravenously to dogs, our synthetic peptide (2 μg/kg) exhibited remarkable hyperglycemic activity. Its contractile activity on isolated rat ileum was 5 times more active than that of synthetic substance P.[19]

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