

Synthesis of (6-[4-³H]Phenylalanine)-somatostatin of High Specific Radioactivity

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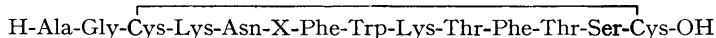
Summary Somatostatin labelled with tritium in the 6-phenylalanine residue (**1a**) has been synthesised *via* catalytic deiodination of the fully protected intermediate (**2**).

SOMATOSTATIN (**1b**) the hypothalamic tetradecapeptide with growth hormone release-inhibiting properties has been the subject of much interest since its recognition.¹ For the purposes of radioimmunoassay, analogues containing

tyrosine residues have been used as vehicles for introducing radioactive iodine² but no synthesis of the parent hormone with the high radioactivity necessary for examining its handling *in vivo* has been described.

We here report the synthesis of tritium-labelled somatostatin (**1a**) using the catalytic deiodination procedure employed by us previously for corticotrophin analogues.³ The problem of catalyst poisoning by the disulphide bridge of the target compound was solved by introducing the radioisotope into the fully protected open chain tetradecapeptide (**2**) and cyclising at the next synthetic step. Fragments (**3**)—(**6**) were constructed by stepwise addition of suitably

(**2**) containing 4-iodophenylalanine in place of phenylalanine in position **6** was catalytically tritiated as described for corticotrophin-(1—24)-tetracosapeptide,³ the product de-tritylated and cyclised with iodine in methanol. Removal of all protecting groups was effected with 90% trifluoroacetic acid. The free peptide was purified by chromatography on carboxymethylcellulose using a linear gradient (0—0.5 M) of pH 5.0 trimethylammonium acetate and appeared homogeneous by high pressure liquid chromatography⁶ having the same elution time as an authentic sample. All intermediates gave satisfactory values on elemental analysis. Tripeptides and larger fragments gave satis-



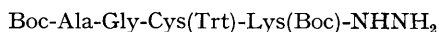
(1)

a; X = Phe(³H)

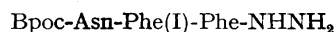
b; X = Phe



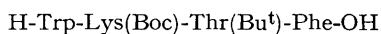
(2)



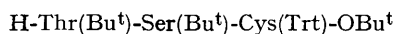
(3)



(4)



(5)



(6)

protected intermediates. Permanent side chain protection was by *t*-butyl or *t*-butoxycarbonyl group, and amino protection was by benzyloxycarbonyl group or, when iodine or sulphur was present, by biphenylisopropoxycarbonyl group. Fragment (**4**) was condensed with (**5**) *via* its azide⁴ and the product then coupled to (**6**) using the hydroxy-benzotriazole-dicyclohexylcarbodi-imide procedure.⁵ After *N*-deprotection the resulting decapeptide was coupled with the azide derived from (**3**). Intermediates were purified by crystallisation in most cases or by counter-current distribution. The fully protected tetradecapeptide

factory amino acid analyses after acid hydrolysis. Amino acid analysis of the radioactive peptide after both acidic and enzymic hydrolysis⁷ confirmed that the product contained no detectable quantity of *D*-amino acid residues. Radioactivity was associated exclusively with phenylalanine in these hydrolysates. The product had a specific activity of 15.5 Ci mmol⁻¹ which is adequate for a range of biochemical studies.

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