Synthesis of (6-[4-^{3}H]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 iodine[2] 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 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 biphenylylisopropoxycarbonyl group. Fragment (**4**) was condensed with (**5**) *via* its azide[4] and the product then coupled to (**6**) using the hydroxybenzotriazole–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 countercurrent distribution. The fully protected tetradecapeptide (**2**) containing 4-iodophenylalanine in place of phenylalanine in position 6 was catalytically tritiated as described for corticotrophin-(1—24)-tetracosapeptide,[3] the product detritylated 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 chromatography[6] having the same elution time as an authentic sample. All intermediates gave satisfactory values on elemental analysis. Tripeptides and larger fragments gave satisfactory amino acid analyses after acid hydrolysis. Amino acid analysis of the radioactive peptide after both acidic and enzymic hydrolysis[7] 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.

H-Ala-Gly-Cys-Lys-Asn-X-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

(**1**)

a; X = Phe(^{3}H)
b; X = Phe

Boc-Ala-Gly-Cys(Trt)-Lys(Boc)-Asn-Phe(I)-Phe-Trp-Lys(Boc)-Thr(Bu^t)-Phe-Thr(Bu^t)-Ser(Bu^t)-Cys(Trt)-OBu^t

(**2**)

Boc-Ala-Gly-Cys(Trt)-Lys(Boc)-NHNH$_2$

(**3**)

Bpoc-Asn-Phe(I)-Phe-NHNH$_2$

(**4**)

H-Trp-Lys(Boc)-Thr(Bu^t)-Phe-OH

(**5**)

H-Thr(Bu^t)-Ser(Bu^t)-Cys(Trt)-OBu^t

(**6**)

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[1] P. Brazeau, W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin, *Science*, 1973, **179**, 77.
[2] J. Epelbaum, P. Brazeau, D. Tsang, J. Brawer, and J. B. Martin, *Brain. Res.*, 1977, **126**, 309.
[3] D. E. Brundish and R. Wade, *J.C.S. Perkin I*, 1973, 2875.
[4] J. Honzl and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2333.
[5] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
[6] C. McMartin and G. E. Purdon, *J. Endocr.*, 1978, **77**, 67.
[7] H. P. J. Bennett, D. F. Elliott, B. E. Evans, P. J. Lowry, and C. McMartin, *Biochem. J.*, 1972, **129**, 695.