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SYNTHESIS OF CYCLIC PEPTIDES BY SOLID PHASE METHODOLOGY

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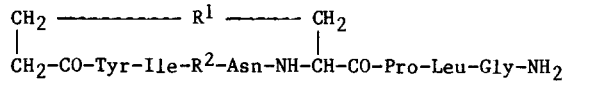
Summary: Four carba analogues of oxytocin and a cyclic analogue of melanocyte stimulating hormone were synthesized using solid phase methodology. Purified compounds were shown to be highly biologically active.

Carba analogues of cyclic hormones which ordinarily contain disulfide bridges (for example oxytocin, vasopressin, vasotocin, somatostatin, calcitonin and insulin) have been prepared to study the importance of disulfide group to biological activity (for references see 1). The general approach for synthesis of these analogues has involved time consuming solution phase techniques. In order to simplify access to this class of compounds we have applied solid phase peptide synthesis methodology to the synthesis of a cyclic MSH and several oxytocin carba analogues.

As model compounds for developing solid phase synthetic methodology we used the highly potent deamino-1-carba-oxytocin (I) and deamino-6-carba-oxytocin (II) since their solution synthesis had been described several years ago[2,3] and we had them in hand as standards. For these syntheses we compared two different approaches.



I, R^1 = CH_2-S, R^2 = Gln III, R^1 = CH_2-S, R^2 = Thr
II, R^1 = S-CH_2, R^2 = Gln IV, R^1 = S-CH_2, R^2 = Thr

In the first one, we utilized a benzhydrylamine resin for solid phase synthesis and the Boc group for α-amino protection. The protected amino acids were coupled in 3 molar excess using the DCC/HOBt protocol until a negative Kaiser's test[4] obtained; for coupling of Boc-Asn and Boc-Gln, p-nitrophenylesters were employed with addition of N-hydroxybenzotriazole (HOBt) as a catalyst. The methyl ester was used for side chain protection of S-γ-carboxypropyl-cysteine. In this approach the carboxyl protection group could not be removed by basic hydrolysis while the peptide was on the resin. Thus the peptide was cleaved from the resin by liquid HF (10% anisole and 5% dithioethane; 45 min at 0°C) with the methyl ester group still intact, and hydrolysis of the octapeptide methyl ester was performed in aqueous solution. The free peptide was transformed to its hydrochloride form and cyclization was performed by a modified active ester method.[5] Deamino-carba-1-oxytocin (I) was purified by gel chromatography (Bio-gel P-4) and high performance liquid chromatography (Vydac C-18); final overall yield, 34%).

The second solid phase synthesis approach utilized Merrifield's resin and the Fmoc α-amino protecting group[6] was used from the fourth to seventh step of the synthesis. After the coupling of Boc-Tyr(Bu^t), all protecting groups were removed by treatment with trifluoroacetic acid and cyclization was performed on the resin with use of dicyclohexylcarbodiimide and N-

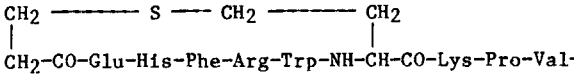
hydroxybenzotriazole. Deamino-carba-6-oxytocin (II) was cleaved from the resin by ammonolysis and purified by HPLC (conditions given above; final overall yield, 12%).

Products of both preparations were compared with authentic samples by TLC in four solvent systems, by HPLC and by ^{1}H NMR (250 MHz) and ^{13}C NMR (90 MHz), and were found to be identical.

For the synthesis of analogue III we used a modification of the first approach. The peptide was cleaved by HF from the resin at the heptapeptide stage. After hydrolysis of the methylester group of the side chain of the modified cysteine residue to give the free peptide (H-Ile-Thr-Asn-Cys(C_3H_6COOH)-Pro-Leu-Gly-NH_2), Boc-Tyr was coupled as its N-hydroxybenzotriazole ester.[8] Cyclization and purification was performed as described for analogue I.

Synthesis of analogue IV (on a benzhydrylamine resin) was accomplished with the free carboxylic group on the side chain of modified homocysteine residue using amino acid symmetrical anhydrides. Boc-Hcy($C_2H_4COOBu^t$)[7] was coupled to the growing peptide chain at the fourth step and all protecting groups were cleaved by trifluoroacetic acid. Symmetrical anhydrides formed immediately prior to the coupling (0.35 eq. of DCC, 30 min 0°C and 1 h room temperature) were used in the next steps. Resin with anchored octapeptide was divided in two parts. One part was treated with trifluoroacetic acid, and the cyclization was performed on the resin with use of DCC and HOBt. The final product was cleaved by liquid HF. Simple gel filtration afforded pure compound IV in 12.6% yield. The other part was first treated with liquid HF and free linear peptide H-Tyr-Ile-Gln-Thr-Asn-Hcy(C_2H_4COOH)-Pro-Leu-Gly-NH_2 was cyclized in solution as described for analogue I. This approach gave a slightly lower yield (9.7%) of a product which was contaminated by the sulfoxide form of IV.* All synthesized analogues were found to be more active than oxytocin in in vitro uterotonic test.

We used the symmetrical anhydride approach, which we have generally found the best, in



V

the synthesis of the cyclic MSH analogue V. After cyclization with DCC/HOBt, the product was cleaved from the resin by liquid HF and purified by ion exchange chromatography and gel filtration; yield 2.4%. Purity was checked by TLC and HPLC, electrophoresis, amino acid analysis, fast atom bombardment mass spectroscopy and elemental analysis. Analogue V was found to be highly active in the frog and lizard skin bioassay systems.[7]

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*The purity of the analogues reported in this paper were determined by TLC in four solvent systems, by HPLC, ^{1}H NMR spectroscopy (250 MHz), and by amino acid and elemental analysis.

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