# Protection of Tryptophan in Peptide Synthesis. The Use of Crown Ethers

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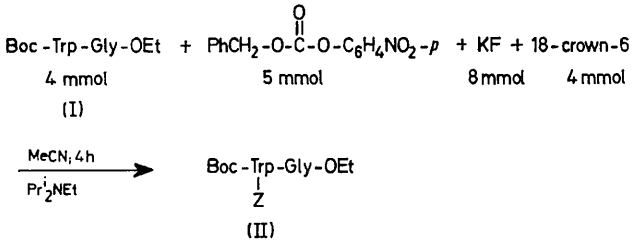
*Summary* Protection of the indole ring of tryptophan by the benzyloxycarbonyl group is achieved by acylation with *p*-nitrophenyl benzyl carbonate in the presence of unsolvated fluoride anion, generated by crown ethers.

PROTECTION of the indole ring in tryptophan-containing peptides is often required especially when acidic conditions are needed for the removal of $N^{\alpha}$ protecting groups. This



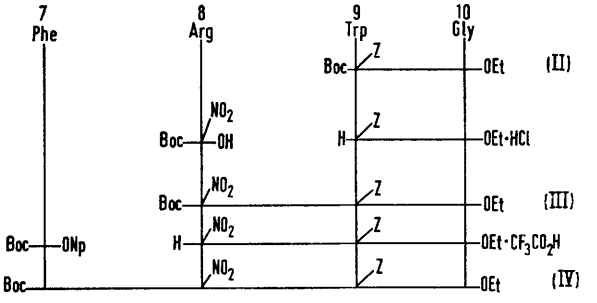

SCHEME 1.

goal may be achieved in two ways: either by the addition of scavengers or by attachment of a protecting group to the indole nitrogen. The formyl group has been employed successfully.[1,2] In order to increase the scope of the methods employed for protecting the indole ring, the applicability of the benzyloxycarbonyl group was investigated. The key feature of our method is the use of $F^-$, which presumably acts as the base that abstracts the proton from the indole nitrogen and thus enables its rapid acylation. Solubilization of KF[3] is brought about by complex formation between 18-crown-6[3] or dicyclohexyl-18-crown-6 and the potassium cation. This leaves the fluoride anion unsolvated[4] and its basicity is increased. A typical acylation is shown in Scheme 1.

TABLE

| | M.p./°C | $[\alpha]_D^{24}$ [a] | Yield/%[e] |
|---|---|---|---|
| (I) | 112—113 | −18 | 98[b,c] |
| (II) | 95—96 | −2 | 55[d,f,g] |
| (III) | 144—145 | −11 | 74[b,d] |
| (IV) | 140—141 | −11 | 97[d] |

[a] $c = 1$, in dimethylformamide. [b] Prepared by the dicyclohexylcarbodi-imide–1-hydroxybenzotriazole method (W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788). [c] Positive Ehrlich test. [d] Negative Ehrlich test. [e] Purity was shown by t.l.c., elemental analysis, and n.m.r. spectroscopy. [e] Purified by column chromatography (neutral $Al_2O_3$; silica gel; ethyl acetate–methylene chloride, 4:1). [g] The low yield can be explained by side reactions (Y. S. Klausner and M. Chorev, unpublished work).




SCHEME 2.

The benzyloxycarbonyl group can be removed by catalytic hydrogenation, hydrazine, or liquid HF. It is stable to 4M HCl in ethyl acetate, 0·12N HCl in formic acid,[1] and short exposure (5 min) to trifluoroacetic acid. A fully protected tetrapeptide, which constitutes positions 7—10 in ACTH, has been synthesized (Scheme 2). Yields and physical characteristics are given in the Table. In order to test whether racemisation has occurred during the acylation step, (II) was converted into (I) by catalytic hydrogenation and the product was digested with α-chymotrypsin; complete cleavage was observed.

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[2] D. Yamashiro and C. H. Li, *J. Org. Chem.*, 1973, **38**, 2594.
[3] C. J. Pedersen, *J. Amer. Chem. Soc.*, 1967, **89**, 7017.
[4] C. L. Liotta and H. P. Harris, *J. Amer. Chem. Soc.*, 1974, **96**, 2250.