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# THE DEBLOCKING OF t-BUTYLOXYCARBONYL-PEPTIDES WITH FORMIC ACID

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The removal of the t-butyloxycarbonyl (t-boc) groups with trifluoroacetic acid or hydrogen chloride in a neutral medium are widely used methods in peptide chemistry[1-6]; however, our attempts to deblock t-boc-L-threonyl-ε-cbz-lysine benzyl ester with these reagents resulted in low yields, due to the formation of several side products. The complex reaction mixtures are due to a partial removal of the carbobenzoxy (cbz) protecting group when anhydrous or 90% trifluoroacetic acid is used and the formation of substantial amounts of the methyl ester when methanolic hydrogen chloride is the reagent. In addition, hydrolysis of the benzyl ester is observed whenever hydrogen chloride in an inert medium is used, due to the hygroscopic nature of the reagent.

We now report that t-boc-peptides are readily deblocked by treatment with formic acid (98%) at room temperature for 1-3 hours. Under these conditions, the commonly used carbobenzoxy, formyl, and the O-benzyl ether protecting groups remain intact and both alkyl and benzyl ester functions are completely stable. The other acid labile blocking groups such as the N-trityl, N-o-nitrophenylsulphenyl and the O-t-butyl ether and ester functions are also smoothly cleaved with this reagent. Among the advantages of the formic acid procedure are the commercial availability and good solvent properties of the reagent, the retention of steric homogeneity of the peptides and greatly improved product yields whenever acid sensitive amino acids, such as tryptophane or threonine, are present in the peptide.

In a typical experiment t-boc-L-threonyl-ε-cbz-lysine benzyl ester (2.7 g; m.p. 82-83°, $[\alpha]_D^{25}$ - 22.7) was dissolved in formic acid (98%, 80 ml) and the solution kept at room temperature for 2-1/2 hours. The reagent was then removed in vacuo (bath temperature <30°) and the residue

triturated with anhydrous ether (20 ml). The crude solid was then recrystallized from ethyl acetate to give 2.26 g (93% yield) of the formate salt of L-threonyl-ε- cbz-lysine benzyl ester (m.p. 93-94°; $[\alpha]_D^{25}$ - 15.4°).

TABLE

Physical Constants of Formic Acid Salts of Peptide Esters[a]

| Peptide[b][c] | Melting Point °C | Rotation $[\alpha]_D^{25}$ Degrees | Molecular Formula | Analysis Calcd % C | H | N | Found % C | H | N |
|---|---|---|---|---|---|---|---|---|---|
| H-L-val-D-ala-OBZ | 117-119 | +56.7 | $C_{16}H_{24}N_2O_5$ | 59.24 | 7.46 | 8.64 | 59.24 | 7.44 | 8.87 |
| H-L-val-L-ala-OMe[d] | dec.[e] | -16.6 | $C_{10}H_{20}N_2O_5$ | 48.37 | 8.12 | 11.28 | 48.50 | 8.17 | 11.14 |
| H-L-ala-L-ala-OMe[d] | 120-128 | -35.9 | $C_8H_{16}N_2O_5$ | 43.63 | 7.32 | 12.72 | 43.77 | 7.32 | 12.75 |
| H-L-leu-L-try-OMe | 134-136 | +4.0 (methanol) | $C_{19}H_{27}N_3O_5$ | 60.46 | 7.21 | 11.13 | 60.69 | 7.27 | 11.50 |
| H-L-threo-ε-cbz-L-Lys-OBZ | 93-94 | -15.4 | $C_{26}H_{35}N_3O_8$ | 60.33 | 6.82 | 8.12 | 60.51 | 6.87 | 8.32 |
| H-L-leu-L-threo-ε-cbz-L-lys-OBZ | 139-141 | -17.9 | $C_{32}H_{46}O_9N_4$ | 60.93 | 7.35 | 8.88 | 60.81 | 7.37 | 8.89 |

(a) Melting points are uncorrected and the rotations are for 1% ethanol solutions unless otherwise specified. Analyses were performed by the Microanalytical Service, Chemistry Department, Stanford University.

(b) The abbreviations used are as described by Young[7].

(c) The purity of the reaction mixtures and of the final products were established by t.l.c. on silica G plates using the solvent system, chloroform-methanol-acetic acid (70:30:5). The chromatograms were developed with ninhydrin and by the chlorination technique[8].

(d) The steric purity of the compound was established by g.l.c. of the trifluoroacetyl derivative[9].

(e) Diketopiperazine formation.

The formate salts of peptide esters (Table) are non-hygroscopic, crystalline solids, which have been kept by us over several months without any noticeable deterioration in the chemical purity of the samples. We have observed, however, that the action of heat on the formates of dipeptide methyl esters results in a facile conversion to the corresponding diketopiperazines. We will report later on this cyclisation reaction which offers a convenient route to sterically pure diketopiperazines.

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REFERENCES

1. L. A. Carpino, J. Amer. Chem. Soc., 79, 4427 (1957).

2. F. C. McKay and N. F. Albertson, Ibid., 79, 4686 (1957).

3. R. Schwyzer, P. Sieber and H. Kappeler, Helv. Chim. Acta., 42, 2622 (1959).

4. G. W. Anderson and A. C. McGregor, J. Amer. Chem. Soc., 79, 6180 (1957).

5. R. B. Merrifield, Endeavour, 24, 3 (1965).

6. M. Bodansky, M. A. Ondetti, S. D. Levine, V. L. Narayanan, M. Saltza, J. T. Sheehan, N. J. Williams and E. F. Sabo, Chem. Ind., 1757 (1966).

7. G. T. Young, Peptides: Proceedings of the 5th Symposium, Oxford, England. Pergamon Press, Oxford, 261 (1962).

8. D. E. Nitecki and J. W. Goodman, Biochem., 5, 665 (1966).

9. F. Weygand, A. Prox, L. Schmidhammer and W. Konig, Angew, Chem. Internat. Edit., 2, 183 (1963).