Facile Removal of a Peptide Chain from a Solid Phase Resin Support by Hydrolysis Using Tetrabutylammonium Fluoride Trihydrate in *N,N*-Dimethylformamide

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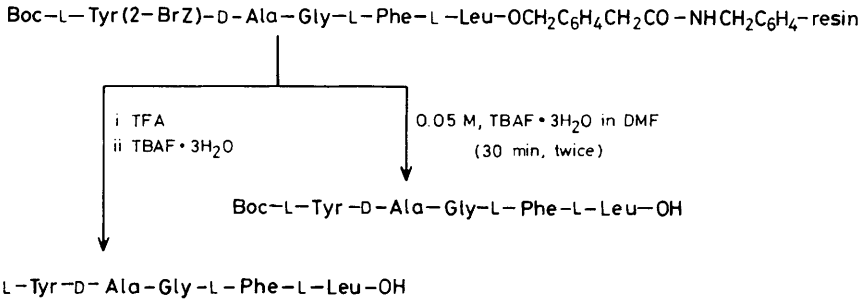
The ester hydrolysis reaction, using tetrabutylammonium fluoride trihydrate [0.05 M TBAF·$3H_2O$ in *N,N*-dimethylformamide (DMF), 25 °C, 30 min, twice], has been successfully applied to the removal of a peptide chain from a solid phase resin support.

The lability of some ester groups during deprotection of the 2-(trimethylsilyl)ethyl ester group with tetrabutylammonium fluoride (TBAF) under anhydrous conditions has been reported by Sieber.[1] The ester cleavage reaction by fluoride-induced fragmentation has been further studied to develop a new linker to a resin support in solid phase synthesis;[2,3] however, the possibility of the direct ester bond cleavage promoted by fluoride ion has not so far attracted special attention. During our study related to the protection of cysteine thiol[4] and tyrosine hydroxy[5] groups, it was found that some ester groups were rapidly hydrolysed by TBAF trihydrate in *N,N*-dimethylformamide (DMF), and that practical applications would be possible.

First, the stability of various esters of benzoic acid was examined using TBAF·$3H_2O$ (0.05 M, 5 equiv.) in DMF at 25 °C. Percentage cleavage after 6 h reaction for the various esters was as follows: methyl, 49%; cyclohexyl, 28%; benzyl, 77%; *p*-methoxybenzyl, 53%; and cinnamyl, 83%. The unsubstituted benzyl ester was cleaved fairly rapidly, but 6% of the ester remained unreacted after 24 h. Phenacyl ester was cleaved completely within 10 min and 9-fluorenylmethyl ester was cleaved instantaneously. *p*-Nitrobenzyl ester was cleaved very rapidly, but the reaction mixture became deep blue in colour as a result of a complex reaction. t-Butyl ester was almost completely stable under these conditions (cleavage: 0.7% for 24 h and 7.5% for 96 h). From these results, TBAF·$3H_2O$ would be expected to act as a good cleavage reagent for some ester groups.

The mechanism of this ester cleavage reaction has not been studied in detail, but the substituent effects in the reaction of benzyl esters and the high recovery (85% after 24 h) of benzyl alcohol from benzyl benzoate suggest that the cleavage occurs



by direct attack of water supported by fluoride ion at the ester carbonyl group.

In solid phase peptide synthesis using the t-butoxycarbonyl (Boc) group as a temporary amine protecting group, resins with a benzyl or *p*-(carbamoylmethyl)benzyl (Pam)[6] ester bond to the first protected amino acids have generally been used.[7] The presence of an electron-withdrawing substituent at the *para* position in the Pam ester resin made the ester bond more acid stable on the one side and more base labile on the other. As a result, lability of the Pam ester bond towards $TBAF{\cdot}3H_2O$ would be expected, and an attempt was made to apply the fluoride ion catalysed ester hydrolysis, to effect removal of a peptide chain from a solid phase resin support.

A fully protected peptide with the sequence [D-Ala,[2] L-Leu[5]]enkephalin was assembled on a Beckman Model 990 instrument using symmetric anhyrides for couplings.

A part of the peptide resin thus obtained was treated with trifluoroacetic acid (TFA) (25%) in dichloromethane, and the peptide chain was cleaved by treatment with $TBAF{\cdot}3H_2O$ in DMF (0.05 M, 5 equiv.) for 30 min at room temperature. After purification by gel filtration on LH-20 and preparative t.l.c. pure [D-Ala,[2] L-Leu[5]]enkephalin (DALE) {m.p. 160.0—163.5 °C(decomp.), $[\alpha]_D^{27}$ +21° (*c* 0.1, DMF)}, was obtained in 48 and 6% yields from the first and second treatments with $TBAF{\cdot}3H_2O$, respectively. Under these conditions the 2-bromobenzyloxycarbonyl (2-BrZ) group on the tyrosine side-chain hydroxy group was not removed completely. The 2-BrZ group was removed from a minor product by catalytic hydrogenation to give an additional 7% yield of DALE, a total yield of 61%.

Literature racemization of phenylalanine in the reaction of benzyloxycarbonyl-L-phenylalanine methyl ester with TBAF (refluxing in tetrahydrofuran for 14 h) gave an oxazolone, which was rapidly hydrolysed.[8] The crude DALE obtained above was analysed by reversed phase h.p.l.c. to check the possibility of racemization during cleavage from the resin support. Since the reaction conditions were milder than those of the reported reaction, no trace of [D-Ala,[2] D-Leu[5]]enkephalin could be detected in the crude product.

Direct treatment of the other part of the protected peptide resin with $TBAF{\cdot}3H_2O$ (0.05 M) in DMF twice (each for 30 min), gave Boc–L-Tyr–D-Ala–Gly–L-Phe–L-Leu–OH {m.p. 146.5—152.0 °C, $[\alpha]_D^{25}$ −14.4° (*c* 1, DMF)}, in an overall yield of 46%.

These results show that tetrabutylammonium fluoride trihydrate is a useful reagent, not only for the general hydrolysis of esters, but also as a reagent for cleavage of peptide chains from the solid phase resin supports affording free peptides or protected peptide segments.

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References

1 P. Sieber, *Helv. Chim. Acta*, 1977, **60**, 2711.
2 D. G. Mullen and G. Barany, *Tetrahedron Lett.*, 1987, **28**, 491.
3 R. Ramage, C. A. Barron, S. Bielecki, and D. W. Thomas, *Tetrahedron Lett.*, 1987, **28**, 4105.
4 M. Ueki and K. Shinozaki, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2156.
5 M. Ueki, Y. Sano, I. Sori, K. Shinozaki, S. Ikeda, and H. Oyamada, *Tetrahedron Lett.*, 1986, **27**, 4181.
6 A. R. Mitchell, E. W. Erickson, M. n. Ryabtsev, R. S. Hodges, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1976, **98**, 7357.
7 G. Barany and R. B. Merrifield, 'Solid Phase Peptide Synthesis,' in 'The Peptides,' ed. E. Gross and J. Meienhofer, Academic Press, New York, 1979, vol. 2, p. 100.
8 J. Pless, *J. Org. Chem.*, 1974, **39**, 2644.