Tetrahedron Letters,Vol.28,No.52,pp 6617-6620,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

REMOVAL OF 9-FLUORENYLMETHYLOXYCARBONYL (Fmoc) GROUP WITH TETRABUTYLAMMONIUM FLUORIDE

Masaaki Ueki* and Masahide Amemiya

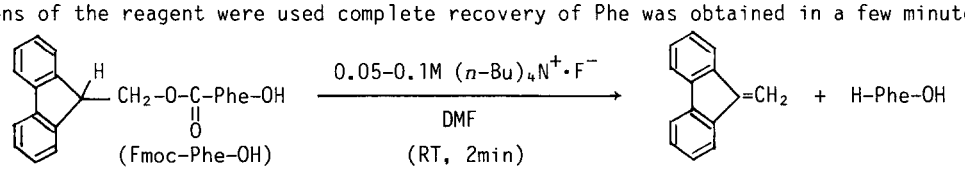
Department of Applied Chemistry, Faculty of Science, Science University of Tokyo, Kagurazaka, Shinjuku-ku, Tokyo 162, Japan

Summary: Tetrabutylammonium fluoride in N, N-dimethylformamide (DMF) is an effective alternative to the piperidine reagent for the removal of 9-fluorenylmethyloxycarbonyl (Fmoc) group in the solid phase peptide synthesis.

Since the introduction by R. B. Merrifield[1] a set of acid-labile protecting groups have been used in the solid phase peptide synthesis.[2] But, the problem of some side reactions during the acidolytic deprotection has not so far been fully overcome. From this reason the base-labile 9-fluorenylmethyloxycarbonyl (Fmoc) group[3] has recently attracted special interest as a temporary amino protecting group. However, there are some problems also in the Fmoc strategy. Especially, the loss of a peptide chain from the resin support at the dipeptide level by the diketopiperadine formation[4] is one of the important problems. Since the piperidine which has been exclusively used for the removal of the Fmoc group[5,6] is at the same time a good catalyst for this side reaction,[4] development of a better reagent is needed.

Unsolvated fluoride ion has been used as a base in organic synthesis.[7] 2-(trimethylsilyl)ethyl ester group[8] for the carboxyl protection and 2-(trimethylsilyl)ethoxycarbonyl[9] and 2-(trimethylsilyl)ethanesulfonyl[10] groups for the amine protection are known as protecting groups removable with the fluoride ion. Recently a new handle for anchoring a protected amino acid to a solid support which is cleaved by attack of fluoride ion has also been reported.[11] Fluoride ion is also useful for removal of organophosphorus protecting groups for thiol[12,13] and phenolic hydroxyl[14] groups. In this communication we would like to report the first application of tetrabutylammonium fluoride to the cleavage of the base-labile Fmoc group.

In the first trial Fmoc-L-phenylalanine was deprotected with 0.05-2.0M tetrabutylammonium fluoride (TBAF) trihydrate in DMF and an amount of phenylalanine liberated was determined on an amino acid analyzer. Contrary to the usual fluoride ion promoted reactions in which the presence of water should carefully be avoided, removal of the Fmoc group with TBAF proceeded smoothly in presence of water of crystallization of TBAF. As shown in Fig. 1 when 0.05-0.1M solutions of the reagent were used complete recovery of Phe was obtained in a few minutes. De-



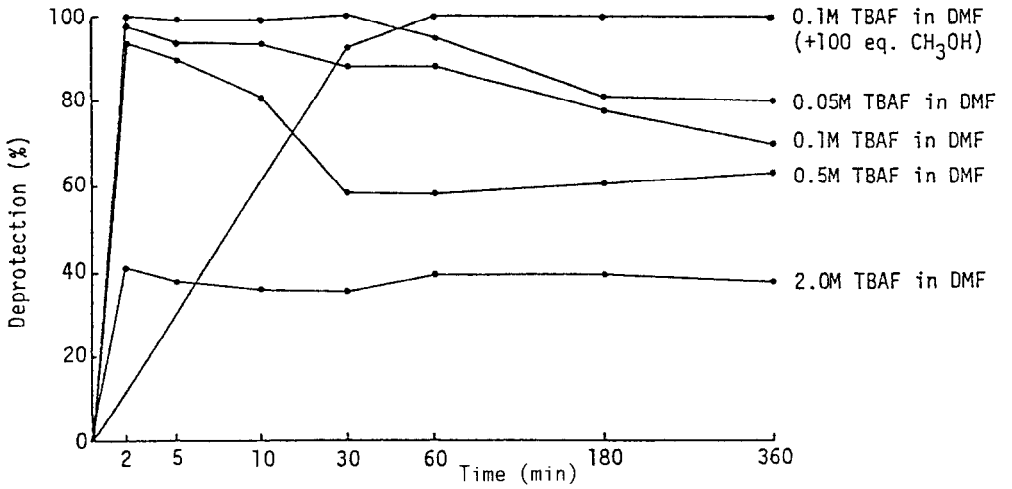


Fig. 1. Effects of Concentration of TBAF and an Additive on Removal of Fmoc Group

crease in the recovery after longer periods would probably be attributed to the secondary reaction of the free amino group with dibenzofulvene, a cleavage product from the Fmoc group. Such lowering of the recovery of the amino acid could be avoided by the addition of a large excess of methanol (100 eq.) to the reagent.

The new removal conditions of the Fmoc group were tested through solid phase synthesis of a model peptide, L-Leu-L-Ala-Gly-L-Val. Starting from Fmoc-L-Val-$OCH_2C_6H_4OCH_2$-resin a peptide chain was elongated according to the standard protocol[15] except for the deprotection step in which piperidine was replaced by TBAF. The Fmoc group was removed by treating twice with 0.1M TBAF in DMF (2 min). Couplings of Fmoc-amino acids were carried out using preformed symmetric

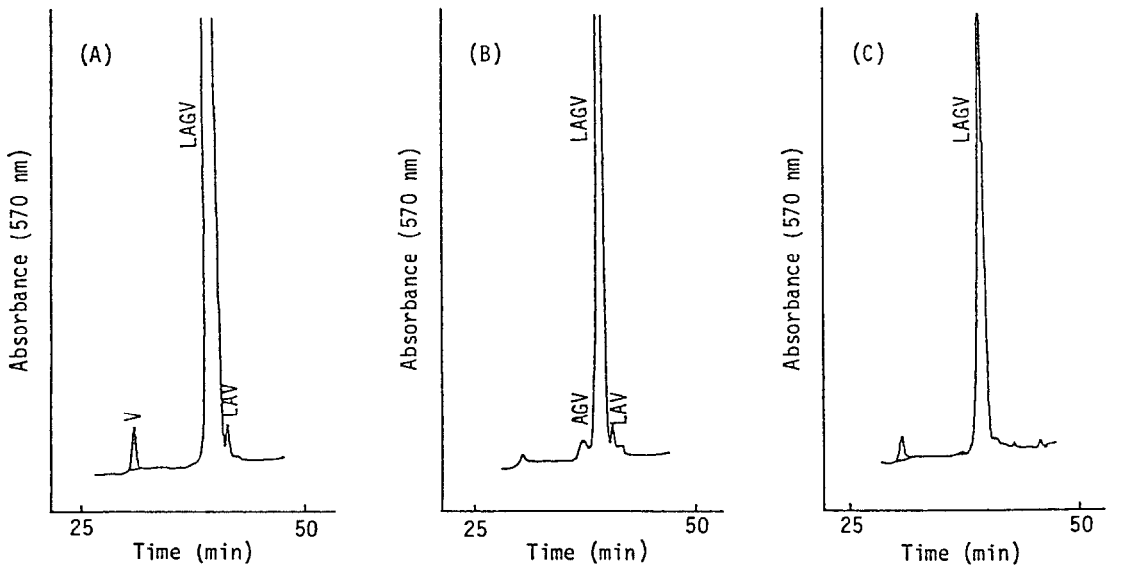


Fig. 2. Ion-exchange Chromatograms of LAGV Synthesized Using Various Removal Conditions; (A)double deprotection with 0.1M TBAF in DMF for 2 min, (B)single deprotection with 0.1M TBAF in DMF for 2 min, and methanol was added before filtration, (C)deprotection with 0.02M TBAF in DMF for 20 min in the Continuous Flow System

anhydrides. Amino acid ratios of an acid hydrolyzate of the protected tetrapeptide resin were Leu, 1.01; Ala, 1.03; Gly, 1.00; Val, 1.08. A portion of the resin was treated with 90% aqueous trifluoroacetic acid to afford Fmoc-L-Leu-L-Ala-Gly-L-Val-OH, mp 197-200°C(dec), $[\alpha]_D^{20}$ -25.5°(c1, EtOH); lit.[16] mp 198-200°C(dec), $[\alpha]_D^{20}$ -25.7°(c1, EtOH), in 73% yield.

From the rest of the protected peptide resin the Fmoc group was removed and then the free peptide was cleaved from the resin support. An ion-exchange chromatogram of the crude tetrapeptide (Fig. 2a) did not show the presence of any deficient peptides except for Val(1.5%) and Leu-Ala-Val(0.9%) arising from incomplete cleavage of the Fmoc group on the C-terminal Val.

Next, the loss of a peptide chain during the removal of Fmoc group with TBAF was studied. Since D-Val-L-Pro sequence is known to be very prone to cyclize[17] Fmoc-D-Val-L-Pro-$OCH_2C_6H_4O$-CH_2CO-L-Leu-NH-resin containing Leu as an internal reference[18] was used for this study. When the resin was deprotected by treating with 0.1M TBAF in DMF (10 eq.) for 2 min and washed in the usual manner 75% of the peptide chain was liberated from the resin. When the reaction time for deprotection was extended to 4 min 92% of the chain was lost. But, when the TBAF solution was diluted by methanol before filtration, loss of the peptide chain was appreciably suppressed. However, the amounts of deficient peptides, Leu-Ala-Val (1.4%) and Ala-Gly-Val(2.5%), are greater (Fig. 2b) than those in the former synthesis. Therefore, these revised conditions are recommended only for the deprotection step in the dipeptide level. Dichloromethane and water were not effective for this purpose.

Table 1. Loss of the Peptide Chain from Fmoc-D-Val-L-Pro-resin during Deprotection

Reagent	Quenching*	Time(min)	Peptide Chains Retained(%)
0.1M TBAF in DMF		2	25
0.1M TBAF in DMF		4	8
0.1M TBAF in DMF	CH_2Cl_2	2	24
0.1M TBAF in DMF	H_2O	2	24
0.1M TBAF in DMF	CH_3OH	2	59
0.1M TBAF in DMF	CH_3OH	3	49
50% piperidine in DMF		5	4

*)The cleaving solution was diluted with the solvent indicated before filtration.

As the most effective application of the new removal reaction, its use in peptide synthesis by continuous flow system[19,20] was investigated, since dibenzofulvene, the reactive cleavage product from the Fmoc group, would not be accumulated in this system. The synthesis was performed on a Pepsyn KA resin, which was specially designed for use in solid phase synthesis by the continuous flow system.[19,20] Deprotection and, at the same time, washing were performed by one-way flow of 0.02M TBAF in DMF for 20 min. Couplings with Fmoc-amino acid symmetric anhydrides were carried out, in this case, as in the usual synthesis using a swelling gel resin without recirculating. As expected, deficient peptides except for those arising from incomplete deprotection in the C-terminal valine were not detected in an ion-exchange chromatogram of the LAGV obtained (Fig. 2c).

As limitation of the new removal method using the TBAF in DMF, instability of benzyl ester group[8,21] and aspartyl peptide bonds[8] toward this reagent has already been noted. Solution of this problem and applications of the new removal conditions to the synthesis of biologically active peptides are now in progress.

References

1. R. B. Merrifield, J. Am. Chem. Soc., 85, 2149(1963).
2. G. Barany and R. B. Merrifield, "Solid-Phase Peptide Synthesis," in "The Peptides," ed by E. Gross and J. Meienhofer, Academic Press, New York (1979), Vol. 2, Chap. 1, pp. 100-118.
3. L. A. Carpino and G. Y. Han, J. Am. Chem. Soc., 92, 5748(1970).
4. E. Pedroso, A. Grandas, X. d. l. Heras, R. Eritja, and E. Giralt, Tetrahedron Lett., 27, 743(1986).
5. C.-D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E. O. Lundell, and J. D. Hang, Int. J. Pept. Prot. Res., 15, 59(1980).
6. E. Atherton, C. J. Logan, and R. C. Sheppard, J. Chem. Soc., Perkin Trans I, 538(1981).
7. J. H. Clark, Chem. Rev., 80, 429(1980).
8. P. Sieber, Helv. Chim. Acta, 60, 2711(1977).
9. L. A. Carpino and J.-H. Tsao, J. Chem. Soc., Chem. Commun., 358(1978).
10. S. M. Weinreb, D. M. Demko, and T. A. Lessen, Tetrahedron Lett., 27, 2099(1986).
11. D. G. Mullen and G. Barany, Tetrahedron Lett., 28, 491(1987).
12. L. Horner, R. Gehring, and H. Lindel, Phosphorus and Sulfur, 11, 349(1981).
13. M. Ueki and K. Shinozaki, Bull. Chem. Soc. Jpn., 57, 2156(1984).
14. M. Ueki, Y. Sano, I. Sori, K. Shinozaki, S. Ikeda, and H. Oyamada, Tetrahedron Lett., 35, 4181(1986).
15. J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," 2nd ed, Pierce Chemical Co., Rockford (1984), pp.82.
16. J. Meienhofer, M. Waki, E. P. Heimer, T. J. Lmbros, R. C. Makofske, and C.-D. Chang, Int. J. Pept. Prot. Res., 13, 35(1979).
17. B. F. Gisin and R. B. Merrifield, J. Am. Chem. Soc., 94, 3102(1972).
18. G. Barany and R. B. Merrifield, J. Am. Chem. Soc., 107, 4936(1985).
19. T. Lucas, M. B. Prystowsky, and B. W. Erickson, Proc. Nat. Acad. Sci., US, 78, 2791(1981).
20. E. Atherton, E. Brown, and R. C. Sheppard, J. Chem. Soc., Chem. Commun., 1151(1981).
21. G. Barany, and F. Albericio, J. Am. Chem. Soc., 107, 4936(1985).

(Received in Japan 21 July 1987)