CHEMISTRY LETTERS, pp. 721-724, 1993.

One-pot Deprotection and Coupling of Peptides by the Fmoc Strategy

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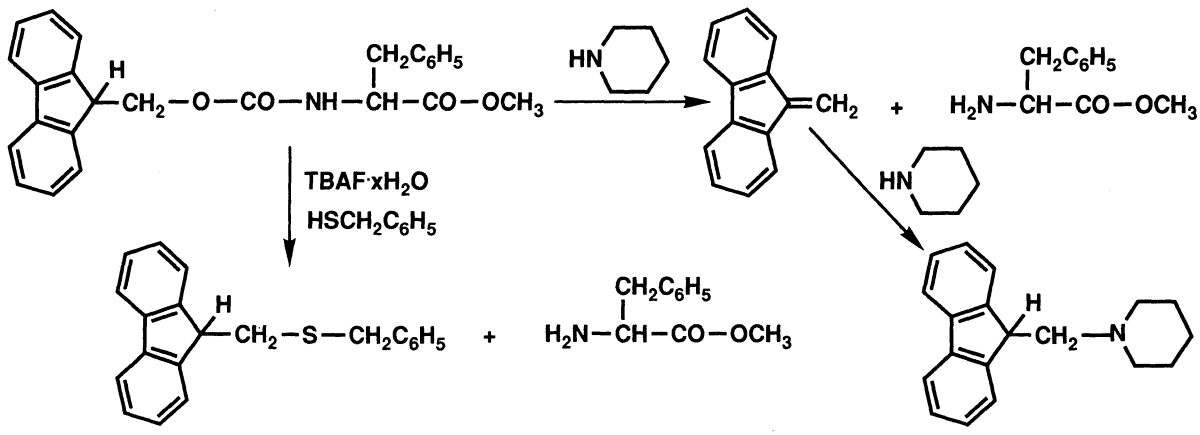
9-Fluorenylmethyloxycarbonyl (Fmoc) group can be quickly removed with tetrabutylammonium fluoride hydrate (TBAF•xH_2O) in the presence of a large excess of phenylmethanethiol or 1-octanethiol. Subsequent oxidation of the thiol with bis(1-methyl-1*H*-tetrazol-5-yl) disulfide simultaneously inactivated the TBAF and enabled one-pot peptide bond elongation by the Fmoc strategy.

Fmoc strategy that does not use strong acids for deprotection has been the preferred method in solid phase peptide synthesis.[1] However, its use in solution phase synthesis is restricted since an excess secondary amine used for deprotection must be removed before coupling.[2] Recently we reported that tetrabutylammonium fluoride hydrate (TBAF•xH_2O) could be used for removal of the Fmoc group.[3] Although the reaction is rapid and simple possible subsequent reactions of liberated amino components with dibenzofulvene, the cleavage product of the Fmoc group,[4] was a new problem. To overcome this problem use of an additive which adds to dibenzofulvene like piperidine should be considered.

On the other hand, in the study of the cleavage reaction of phenacyl esters with TBAF•xH_2O, we found that the addition of a large excess of phenylmethanethiol or 1-octanethiol not only accelerated the reaction but also reduced the necessary amounts of TBAF•xH_2O.[5] Since thiols are expected to scavenge dibenzofulvene we applied the TBAF-thiol system to the cleavage of Fmoc group in this study.[6]

When Fmoc-phenylalanine methyl ester, Fmoc-Phe-OMe, was treated with TBAF•xH_2O (2 equiv.) in presence of phenylmethanethiol (10 equiv.) in tetrahydrofuran (THF) or *N,N*-dimethylformamide (DMF), 9-benzylthiomethylfluorene[7] was obtained in quantitative yield. This indicates that the dibenzofulvene is completely trapped by the thiol. Then, the optimal conditions for cleavage were studied using 1-octanethiol, which is preferable



to phenylmethanethiol because of less repugnant odor. The results were summarized in Table 1.

Cleavage of the Fmoc group from Fmoc-Phe-OMe with 2 equiv. of TBAF•xH_2O alone took 30 min (entry 1). The cleavage time could be reduced dramatically by addition of 1-octanethiol (entry 2). Reduction of the reaction time could also be achieved when ultrasound was applied (entry 3). However, in the absence of the thiol the reaction mixture took yellow color and many byproducts probably arising from polymerization of dibenzofulvene were observed on TLC. In this case the addition product of H-Phe-OMe with dibenzofulvene[4] could not be isolated. For scavenging dibenzofulvene completely 10 equiv. of the thiol was necessary (entries 2, 4, and 5). When the amount of the thiol was reduced to 5 equiv. the reaction proceeded still rapidly (entry 6), while the trapping of dibenzofulvene was incomplete. The amount of the TBAF•xH_2O could be reduced to 1.5 equiv. without substantial decrease in reaction rate (entry 7). The solvent THF (entry 8) as well as DMF could be used, while in dichloromethane no reaction occurred.

Table 1. Cleavage of Fmoc Group of Fmoc-Phe-OMe with TBAF•xH_2O-*n*-$C_8H_{17}SH$ System

Entry	TBAF•xH_2O	*n*-$C_8H_{17}SH$	Solvent	Concn/M[a]	Ultrasound	Time/min[b]
1	2.0 equiv.	0	DMF	0.001	--	30
2	2.0 equiv.	10 equiv.	DMF	0.001	--	3
3	2.0 equiv.	0	DMF	0.001	+	2
4	2.0 equiv.	10 equiv.	DMF	0.001	+	2
5	2.0 equiv.	10 equiv.	DMF	0.01	+	1
6	2.0 equiv.	5 equiv.	DMF	0.01	+	1
7	1.5 equiv.	10 equiv.	DMF	0.01	+	2
8	2.0 equiv.	10 equiv.	THF	0.01	+	1

a)Concentration of Fmoc-Phe-OMe. b)Time required for complete disappearance of Fmoc-Phe-OMe on TLC.

Table 2 shows recovery of phenylalanine when Fmoc-Phe-OH was treated with TBAF•xH_2O (2 equiv.) in the presence of various amounts of 1-octanethiol for 30 min with ultrasound for the initial 5 min. The recovery increased as the amount of the thiol increased.

Table 2. Amino Acid Recovery in Deprotection of Fmoc-Phe-OH with TBAF•xH_2O-*n*-$C_8H_{17}SH$[a]

Amount of *n*-$C_8H_{17}SH$/equiv.	0	2	5	8	10
Recovery of Phe/%	92.6	98.9	98.9	99.9	100

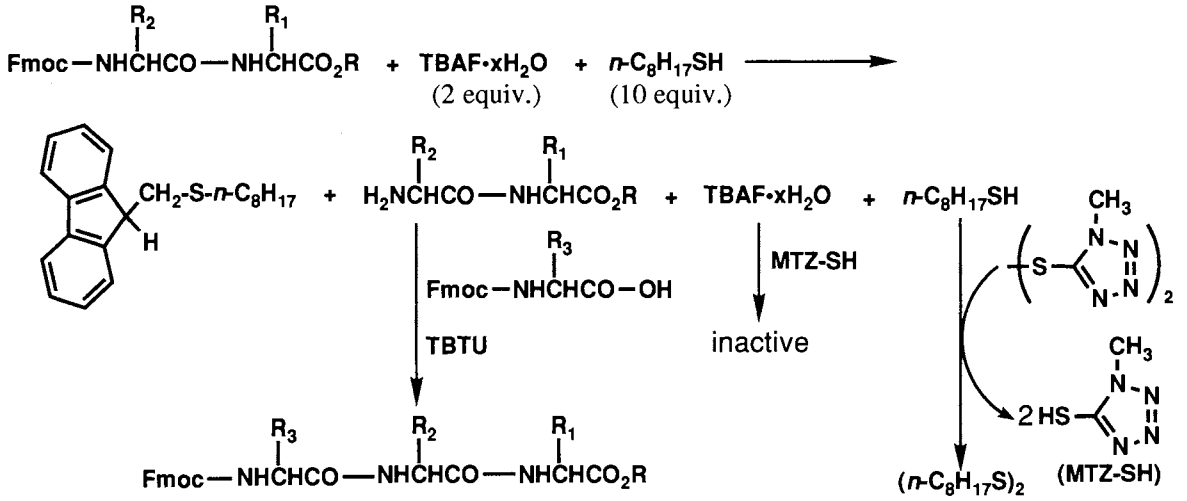
a)Recovery of amino acid was determined by an amino acid analyzer.

From the above results it was concluded that the optimal conditions for quick cleavage of Fmoc group are to apply 2 equiv. of TBAF•xH_2O in the presence of 10 equiv. of 1-octanethiol. Under these conditions Fmoc groups of Fmoc-Ala-Phe-OtBu, Fmoc-Leu-Phe-OBzl, Fmoc-Phe-Leu-OtBu, Fmoc-Val-Glu(OBzl)$_2$, Fmoc-D-Val-Pro-OBzl, Fmoc-Gly-Phe-Leu-OtBu and Fmoc-Gly-Gly-Phe-Leu-OtBu could also be removed completely within 1 min. However, the TBAF•xH_2O-thiol system could not have been applied to a solid phase synthesis by the continuous flow system.[8] In this case, oxidation of the thiol occurred to result in deactivation of the fluoride

ion. Fluoride ion is known to catalyze the air oxidation of thiols,[9] but the reason of the deactivation of the fluoride ion has not so far been specified.

Then we tried to use the new cleavage system to a one-pot synthesis of peptides, for which complete inactivation of the deprotecting reagent before coupling was necessary. Thiols having strong nucleophilicity comparable to amines should also be removed. Their easily oxidizable character was used for this purpose.

Oxidation of thiols by the thiol-disulfide exchange reaction is mild enough for use in peptide synthesis. Among several disulfides developed for this purpose bis(1-methyl-1*H*-tetrazol-5-yl) disulfide[10] was selected because the oxidation of 1-octanethiol with this disulfide completed within 3 min. In addition 1-methyl-1*H*-tetrazol 5-thiol (MTZ-SH) produced completely deactivated TBAF•xH_2O. Based upon these findings we planned the one-pot deprotection and coupling of peptides by the Fmoc strategy as sketched in the following scheme.



Scheme 1. One-pot deprotection and coupling of peptides by the Fmoc strategy.

In a typical experiment 1-octanethiol (346 μl, 2 mmol) followed by TBAF•xH_2O (126.2 mg, 0.4 mmol) was added to a solution of Fmoc-Phe-Leu-OtBu (111.3 mg, 0.2 mmol) in DMF (1 ml) under an argon atmosphere. The mixture was stirred with ultrasound for 1 min. To this was added bis(1-methyl-1*H*-tetrazol-5-yl) disulfide (276.3 mg, 1.2 mmol) and stirring was continued for 3 min. Then, diisopropylethylamine (125.4 μl, 0.72 mmol), Fmoc-Gly-OH (71.4 mg, 0.24 mmol) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[11] (77.1 mg, 0.24 mmol) were added in this order. The mixture was stirred for 10 min. To this 5% aq. $NaHCO_3$, ether and a small amount of petroleum ether were added and precipitates were collected by filtration (method A). In case of oil the product was extracted with ethyl acetate and isolated by column chromatography on silica gel using gradient elution with $CHCl_3$ to $CHCl_3$-CH_3OH (30:1) (method B).

Table 3 summarizes the results of several one-pot syntheses of Fmoc-tripeptide esters. Except for the case starting from Fmoc-D-Val-Pro-OBzl, which has strong tendency to the diketopiperadine formation,[12] the desired tripeptides were obtained in greater than 90% yields. Pentafluorophenyl active ester[13] as well as TBTU could be used for the coupling. In all examples the total reaction time was less than 20 min.

In conclusion a one-pot method for peptide bond elongation by the Fmoc strategy was established. However, development of a carboxyl protecting group that makes ease of the isolation of the products is required to achieve literal rapid solution phase synthesis.

Table 3. One-pot Deprotection and Coupling of Fmoc-dipeptides

Entry	Dipeptide	Acylation		Isolation	Yield/%[c]
		Reagent (Amount/equiv.)	Time/min[a]	method[b]	
1	Fmoc-Phe-Leu-OtBu	Fmoc-Gly-OPfp (1.2)	10	B	quant.
2	Fmoc-Phe-Leu-OtBu	Fmoc-Gly-OH (1.2) / TBTU (1.2)	10	B	quant.
3	Fmoc-Phe-Leu-OtBu	Fmoc-Gly-OH (1.2) / TBTU (1.2)	10	A	91
4	Fmoc-D-Val-Pro-OBzl	Fmoc-Gly-OH (1.2) / TBTU (1.2)	15	B	10
5	Fmoc-Val-Glu(OBzl)$_2$	Fmoc-Phe-OH (1.2) / TBTU (1.2)	15	B	95
6	Fmoc-Val-Glu(OBzl)$_2$	Fmoc-Phe-OH (1.05) / TBTU (1.2)	15	A	92
7	Fmoc-Leu-Phe-OBzl	Fmoc-Met-OH (1.2) / TBTU (1.2)	15	B	70
8	Fmoc-Ala-Phe-OtBu	Fmoc-Gly-OH (1.2) / TBTU (1.2)	15	B	96
9	Fmoc-Leu-Phe-OBzl	Fmoc-Val-OH (1.2) / TBTU (1.2)	15	B	92

a)Time required for complete disappearance of the amino component on TLC. b)See text. c)Isolated yield.

This work was financially supported by Grant-in-Aid for Scientific Research No. 03215106 from the Ministry of Education, Science and Culture. We thank Dr. Catherine Gehrig de Chavez for reading the manuscript.

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(Received January 25, 1993)