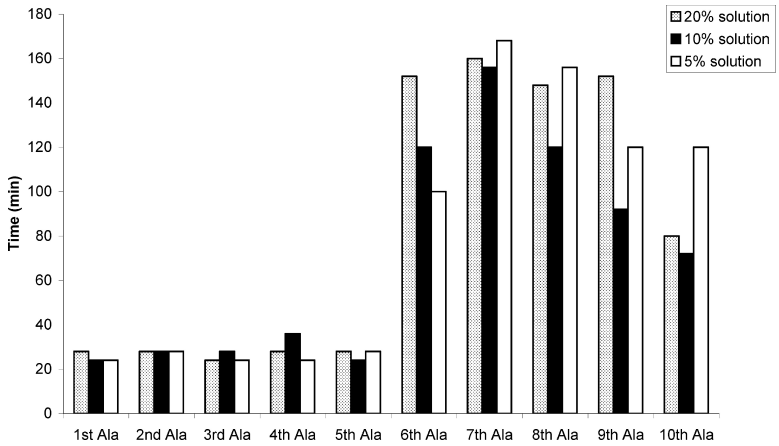
JOURNAL OF combinatorial CHEMISTRY

Report

*N*α-Fmoc Removal from Resin-Bound Amino Acids by 5% Piperidine Solution

Nikos Zinieris, Leondios Leondiadis, and Nikolas Ferderigos

J. Comb. Chem., **2005**, 7 (1), 4-6• DOI: 10.1021/cc049872d • Publication Date (Web): 20 November 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Combinatorial Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

*N*α-Fmoc Removal from Resin-Bound Amino Acids by 5% Piperidine Solution

Nikos Zinieris,[†] Leondios Leondiadis,*[,‡] and Nikolas Ferderigos*[,†]

Laboratory of Organic Chemistry, Chemistry Department, University of Athens, 157 71 Greece, and Mass Spectrometry and Dioxin Analysis Lab, IRRP, National Centre for Scientific Research "Demokritos", Athens, 153 10 Greece

Received July 30, 2004

In solid-phase peptide synthesis (SPPS), introduced by B. R. Merrifield[1] in 1963, the growing peptide chain is assembled by alternating acylation and deprotection reactions in a stepwise manner. It is very important that the repetitive steps proceed rapidly, in high yields, and with minimal side reactions to prevent formation of byproducts.

Two methods are applied for the elongation of the peptide chain on the solid support. The original Boc/Bz approach developed by Merrifield utilizes a regime of graduated acidolysis to achieve selectivity in the removal of temporary and permanent protection. The Fmoc/*t*-Bu method introduced later by Atherton[2] and Meienhofer[3] is based on an orthogonal protecting group strategy, using the base-labile Fmoc group[4] for protection of the α-amino group and acid labile side-chain protecting groups and resin-linkage agents. Fmoc-based solid phase peptide synthesis is now firmly established alongside the Merrifield technique.

One of the major steps in SPPS is the Nα-deprotection cycle. The deprotection reagent is required in large quantities in peptide chemistry and can be a significant expense, especially for high-volume labs and core facilities. In Fmoc/*t*-Bu SPPS,[5] a 20% v/v solution of piperidine in DMF is required in common usage conditions. This was established by two studies of the lability of Fmoc derivatives to various bases. In the first, the rates of cleavage of Fmoc valine in a piperidine/DMF solution were determined by quantitative amino acid analysis.[6] In the second, the rate of disappearance of Fmoc alanine *tert*-butyl ester in dichloromethane was followed qualitatively by TLC.[7] In both cases, base strength and steric factors were found to be important. The cleavage reaction was evidently much faster in DMF than in a less polar medium, such as dichloromethane. It was found that the approximate half-life of Fmoc cleavage by 20% piperidine was 6 s.

Experience earned during the synthesis of various peptides by Fmoc SPPS revealed that in the case of sequences which have become aggregated, the standard treatment with 20% piperidine in DMF may not always be effective. In these cases, it is advisable to increase the time required for deprotection or to use a stronger base, such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[8,9]

Taking in consideration that piperidine is not only an expensive reagent but also a controlled substance according to the 92/109/EC recommendation, we decided to test the efficacy of more dilute solutions of piperidine in *N*α-Fmoc removal from amino acids bonded to resin in SPPS. This were based on the observation that the time needed for deprotection in the SPPS was much longer than the time measured using amino acids in solution. Obviously, the diffusion of the reagents through the solid phase support plays an important role in the overall time needed.

Therefore, we measured the half-lives needed for Fmoc-removal when Fmoc-L-Lys (Boc), Fmoc-L-Val, Fmoc-L-Pro, or Fmoc-Gln was bound on a chlorotrityl resin.[10] The capacity of the synthesized resins was measured after treatment by 20% piperidine in DMF by UV absorbance of the resultant piperidine-dibenzofulvene adducts (301 nm, $\epsilon = 7800\ cm^{-1}\ M^{-1}$)[11] and found to be 0.20, 0.19, 0.20, and 0.21 mmol g^{-1}, respectively.

For the half-life measurement, deprotection solution (1 mL) was added to the resin sample (20 mg) and after 10 s was filtered out, and the UV absorption at 301 nm was measured. The procedure was repeated until the UV absorption of the filtrate dropped to 0.05 AU. The percent of the Fmoc removal was expressed as a percentage of the absorption measured when deprotection solution was added to the same amount of resin sample for 20 min.[12] Then the calculated percent of the Fmoc removal was plotted against time. The time needed for 50% Fmoc removal corresponds to the half-life of the deprotection step. The half-lives measured and the time needed for 99.99% deprotection are shown in Table 1.

One can see that the time needed for deprotection depended on the amino acid bound to the resin, with the Lys derivative having the shortest time and the Val derivative having the longest. One can also notice that the longer the time needed for deprotection, the less differences observed between the 20, 10, or 5% piperidine solutions. This could mean that when longer time is needed for deprotection, the rate of Fmoc removal depends on the diffusion of piperidine inside the beads rather than on its concentration.[13]

To examine the possibility of using piperidine solutions more dilute than 20%, for cost-effectiveness and environmental protection reasons, we applied 20, 10, or 5% piperidine solutions in the synthesis of H−Ala10-Lys−OH, which is a known sterically hindered "difficult" sequence.[14]

The synthesis was carried out on a chlorotrityl resin[10] (100 mg, 0.20 mmol g^{-1}). Piperidine/DMF 20, 10, and 5% solution washes (4-min intervals) were used for the removal of the Fmoc-protected group until the UV absorption at 301 nm dropped to 0.05 AU. We measured the time needed for

* To whom correspondence should be addressed. (Leondiadis) Phone: +30(210) 6503610. Fax: +30(210) 6536873. E-mail: leondi@rrp.demokritos.gr. (Ferderigos) Phone: +30 210 7274475. E-mail: nferder@chem.uoa.gr.

[†] University of Athens.

[‡] National Centre for Scientific Research "Demokritos".

10.1021/cc049872d CCC: $30.25
Published on Web 11/20/2004

Table 1. Half Lives Measured and the Time Needed for 99.99% Deprotection for Fmoc-Gln, Fmoc-Val, Fmoc-Val, and Fmoc-Lys-Boc Resin-Bound Amino Acids

	20% piperidine solution in DMF		10% piperidine solution in DMF		5% piperidine solution in DMF	
resin-bound amino acid	$t_{1/2}$ (s)	time needed for 99.99% deprotection (min)[a]	$t_{1/2}$ (s)	time needed for 99.99% deprotection (min)[a]	$t_{1/2}$ (s)	time needed for 99.99% deprotection (min)[a]
Fmoc-Gln	20.5	4.5	23.2	5.1	53.1	11.8
Fmoc-Val	41.5	9.2	44.6	9.9	47.1	10.4
Fmoc-Pro	25.4	5.6	27	5.9	32	7.1
Fmoc-Lys (Boc)	10.3	2.3	22.3	4.9	27.4	6.1

[a] Time needed for 99.99% deprotection $= -\ln 0.0001\ t_{1/2}/0.693$

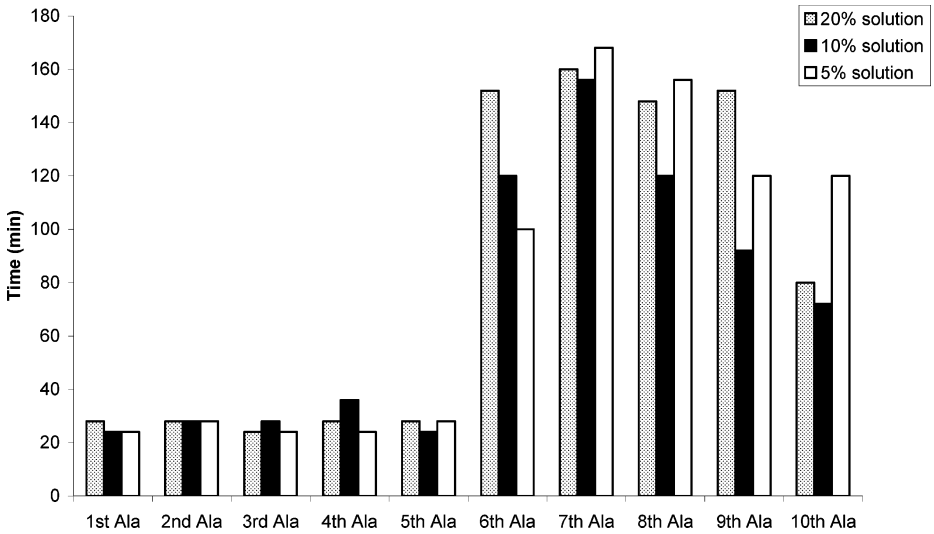


Figure 1. Time needed for complete deprotection before every amino acid coupling, using 20, 10 and 5% piperidine/DMF solution washes.

complete deprotection before every amino acid coupling. The results are shown in Figure 1.

During the peptide synthesis, two different behaviors were observed. No difficulty was observed before Ala6. From Ala6 to Ala10, the deprotection time needed was much longer. As one can see in Figure 1, all three different piperidine solutions tested needed about the same deprotection time, without any obvious differences. On the other hand, when a more dilute piperidine solution was used (2%, data not shown), Fmoc removal was accomplished in a much longer time. In all syntheses, the final product ($[M + H]^+$ at 857.9 amu) was obtained with 60% overall yield.

Finally, we tested the use of 5% piperidine solution in the synthesis of acyl carrier protein (65−74)[15] and Leu-enkephaline.[16] The syntheses were carried out on 100 mg of chlorotrityl resin,[10] 0.26 and 0.25 mmol g^{-1}, respectively. Fmoc-removal washes were repeated in 4-min intervals until the UV absorption at 301 nm dropped to 0.05 AU. The HPLC profiles, ESI-MS spectra, and overall yields were the same as those obtained when 20% piperidine was used in a parallel synthesis. Acyl carrier protein synthesis was accomplished with 75% overall yield ($[M + H]^+$ at 1063.8 amu) and Leu-enkephaline synthesis with 95% overall yield ($[M + H]^+$ at 556.4 amu).

In conclusion, 5% piperidine solution in DMF can be used instead of the usual 20% solution in SPPS for Fmoc removal from the resin-bound peptide. This can provide improved convenience and lower costs to peptide labs because piperidine is not only an expensive reagent but is also a controlled substance according to the 92/109/EC recommendation. Of course, for difficult sequences, Fmoc removal completion must always be checked before moving to the next coupling.

Acknowledgment. This work was supported by funds from the Special Account for Research Grants of the National and Kapodistrian University of Athens.

References and Notes

(1) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.
(2) Atherton, E. H.; Harkins, D.; Logan, C. J.; Williams, B. J. *J. Chem. Soc., Chem. Commun.* **1978**, 537−539.
(3) Chang, C. D.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1978**, *11*, 246−249.
(4) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404−3409.
(5) Chang, C. D.; White P. D. *Fmoc Solid-Phase Peptide Synthesis. A Practical Approach*; Oxford University Press: New York, 2000, p 52.
(6) Atherton, E.; Logan, C. J.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans.* **1981**, 538−546.
(7) Chang, C. D.; Waki, M.; Ahmad, M.; Meienhofer, J.; Lundell, E. O.; Haug, J. D. *Int. J. Pept. Protein Res.* **1980**, *15*, 59−66.
(8) Wade, J. D.; Bedford, J.; Sheppard, R. C.; Tregear, G. W. *Pept. Res.* **1991**, *4*, 194−199.
(9) Tickler, A. K.; Barrow, C. J.; Wade, J. D. *J. Pept. Sci.* **2001**, *7*, 488−494.
(10) Zikos, C. C.; Ferderigos, N. G. *Tetrahedron Lett.* **1994**, *35*, 1767−1768.

(11) Jensen, J. K.; Alsina, J.; Songster, F. M.; Vagner, J.; Fernando Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452.
(12) Chang, C. D.; White P. D. *Fmoc Solid-Phase Peptide Synthesis. A Practical Approach*; Oxford University Press: New York, 2000, p 63.
(13) Fields, G. B.; Fields, C. *J. Am. Chem. Soc.* **1991**, *113*, 4202−4207.
(14) Larsen, B. D.; Holm, A. *Int. J. Pept. Protein Res.* **1994**, *43*, 1−9.
(15) Hancock, W. S.; Prescott, D. J.; Vagelos, P. R.; Marshall, G. R. *J. Org. Chem.* **1973**, *38*, 774−781.
(16) Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature* **1975**, *258*, 577−580.

CC049872D