Alternative to Piperidine in Fmoc Solid-Phase Synthesis

John Hachmann and Michal Lebl*

Illumina, Inc., 9885 Towne Centre Drive, San Diego, California 92130

Received September 21, 2005

Piperidine is the most common reagent in solid phase and combinatorial syntheses utilizing the Fmoc protecting group.[1] Piperidine is a controlled substance and according to United States Code, Title 21, Chapter 13, Subchapter I, Part C−Registration of Manufacturers, Distributors, and Dispensers of Controlled Substances, its distribution is strictly monitored. Piperidine has to be stored in locked cabinets with restricted access, and its use has to be reported (see http://www.lacofd.org/HHMD/Documents/RS.Reg.8.04.doc). Therefore, the use of piperidine is quite a nuisance.

Recent publication has argued for the use of a lower concentration of piperidine to mitigate the incovenience associated with its use.[2] We have looked into the possibility of replacing piperidine with reagents which would not be controlled substances, have the same kinetics of Fmoc group removal, potentially better physical properties, and which would be in the same price category. One may speculate that wide use of piperidine was caused by economical considerations, and this would be true in comparison to other candidates with higher boiling point (lower odor), such as 4-piperidinemethanol, 4-piperidinopiperidine, and 4-phenylpiperidine, which are by orders of magnitude more expensive than piperidine.

We decided to test several piperidine derivatives and 4-methylpiperazine as a replacement for piperidine in solid-phase peptide synthesis. Kinetics of Fmoc group release from Fmoc-Ile attached to chlorotrityl resin was followed by UV absorbance of the resultant dibenzofulvene adduct. As shown in Table 1, only 3- and 4-methylpiperidine had identical efficiency in Fmoc group removal, as compared to piperidine, and surprisingly enough, 4-methylpiperidine costs approximately the same as piperidine.

We then synthesized four model sequences (Table 2), both manually in a plastic syringe equipped with a frit and automatically in our tilted plate centrifugation-based peptide synthesizer,[3] using piperidine and 4-methylpiperidine. In the second case, we have prepared 24 copies of each sequence in a 96-well microtiterplate. All individual syntheses were evaluated by RP-HPLC, and no significant differences were found between the use of piperidine and 4-methylpiperidine.

On the basis of these results, we decided to switch completely to the use of 4-methylpiperidine, and in the course of the last two years, we have synthesized thousands of peptide sequences with lengths from 5 to 24 amino acids. We have not observed any difficulties which we can ascribe to the use of this deprotection reagent. Most importantly, we did not have to fill in any paperwork.

Table 1. Half-Life of Fmoc Group Removal[a]

reagent (25% in dimethylformamide)	$T_{1/2}$ (min)
piperidine	2.0
2-methylpiperidine	3.0
3-methylpiperidine	1.9
4-methylpiperidine	2.0
4-methylpiperazine	7.8

[a] Fmoc-Ile attached to chlorotrityl-resin (1 mg) was placed in a glass cuvette and reagent (1 ml) was added. Cuvette was shaken intermittently and UV absorbance (301 nm) of the solution was read at 2-min intervals.

Table 2. Sequences Used for Test Syntheses Using Piperidine and 4-Methylpiperidine

		purity (HPLC, %)	
peptide[a]	difficulty[b]	piperidine	4-methyl-piperidine
$(Enkephalin)_2$ (YGGFLYGGFL)	1.00, 1.00	92.3	92.5
LHRH (EHWSYGWLPG)	0.96, 0.89	91.7	91.2
ACP (65−74) (VQAAIDYING)	1.40, 1.20	83.6	84.0
β-amyloid (25−34) (GSNKGAIIGL)	1.35, 1.18	93.6	93.5

[a] Identity of synthesized peptide was confirmed by mass spectroscopy. [b] Most difficult coupling (first value) and average value for difficulty of couplings (second value) (from "Peptide Companion", www.promptscientific.com/peptidecompanion); scale: <0.8 easy, 0.8−1.2 normal, >1.2 difficult.

References and Notes

(1) Podlech, J.; Gurrath, M.; Muller, G. 9-Fluorenylmethoxycarbonyl Group. In *Synthesis of Peptides and Peptidomimetics*; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Thieme: Stuttgart, 2004.

(2) Zinieris, N.; Leondiadis, L.; Ferdefigos, N. *J. Comb. Chem.* **2005**, *7*, 4−6.

(3) Lebl, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1305−1310.

* To whom correspondence should be addressed. E-mail: mlebl@illumina.com.

CC050123L

10.1021/cc050123l CCC: $33.50
Published on Web 12/21/2005