

Synthesis of ⁶⁵⁻⁷⁴ACP and ABRF Using 4-Methylpiperidine as Deprotectant

INTRODUCTION

In peptide synthesis and peptide library synthesis, piperidine is a common reagent for Fmoc removal. However, piperidine is a controlled substance, which requires special handling. Hackmann and Lebl published a paper entitled "Alternative to Piperidine in Fmoc Solid-Phase Synthesis¹," in which piperidine was substituted with 4-methylpiperidine and produced a similar result to piperidine. In this application note, 4-methylpiperidine and piperidine are compared in the synthesis of two test peptides used for *Prelude*TM quality control: ⁶⁵⁻⁷⁴ACP (H-VQAAIDYING-OH) and ABRF (H-VKKRCSMWIIPDDEA-OH). Both bases are found to produce similar results.

METHOD

Peptide Synthesis: Peptides were synthesized on a *Prelude*TM peptide synthesizer at the 20 μmol scale. ⁶⁵⁻⁷⁴ACP was synthesized with Fmoc-Gly-Wang resin (0.41 mmol/g) while ABRF was synthesized on Fmoc-Ala-Wang resin (0.40 mmol/g). Deprotection was performed with either 20% piperidine in DMF or 20% 4-methylpiperidine in DMF for 2 x 2.5 minutes. Coupling was performed with 1:1:4 amino acid/HCTU/NMM in DMF for 2 x 10 minutes. Cleavage was performed with 95:2:2:1 TFA/water/anisole/EDT for 2 hours.

Analysis: Peptide were analyzed on a Varian Microsorb C-18 column (4.5 x 50 mm) on a Varian Pro-Star HPLC using an aqueous acetonitrile, 0.1% TFA buffer system with an increasing gradient of 5-95% acetonitrile over 7 minutes. Detection was at 214 nm.

RESULTS/DISCUSSION

The results for each peptide are shown in Figures 1 & 2.

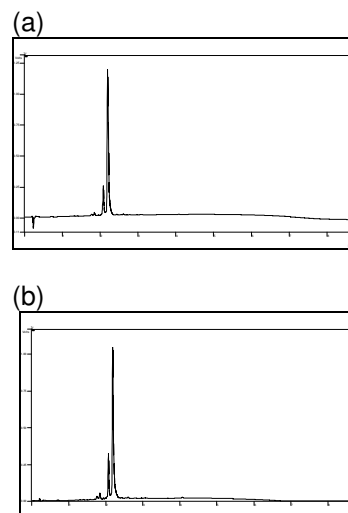


Figure 1: ⁶⁵⁻⁷⁴ACP synthesized with (a) piperidine; (b) 4-methylpiperidine.

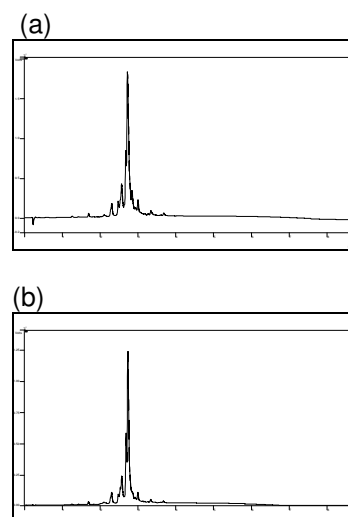


Figure 2: ABRF synthesized with (a) piperidine, (b) 4-methylpiperidine.

¹ J. Hachmann and M. Lebl, *J. Comb. Chem.*, **8**, 149 (2006).

CONCLUSION

There is no significant difference between peptides synthesized with piperidine and 4-methylpiperidine.