Comparison of Alternative Deprotection Reagents to Piperidine for the Synthesis of a Poly-Alanine Peptide

INTRODUCTION

In peptide synthesis, piperidine is a common agent for Fmoc removal. However, piperidine is a controlled substance which requires special handling and cannot be used in some countries. Therefore, it would be useful to identify alternative deprotection reagents to piperidine for Fmoc removal.

It is well known that poly-alanine sequences have a high propensity to aggregate after the fifth residue. In this application, $(A)_{10}$K-OH was synthesized using the Tribute's Intellisynth UV-monitoring and Feedback System to compare the efficiency of Fmoc removal by piperidine vs. three alternative bases (pyrrolidine, cyclohexylamine, and *tert*-butylamine) in the last 5 cycles of the synthesis. It was found that pyrrolidine produced a higher purity product with fewer deprotection repeats and shorter deprotection times per cycle than piperidine, proving it to be a highly efficient, viable alternative to piperidine for Fmoc removal.

METHOD

Peptide Synthesis: $(A)_5$K-OH was synthesized on the ***Prelude***® parallel peptide synthesizer (Protein Technologies Inc. Tucson, AZ) at the 50 µmol scale in 4 separate reaction vessels using Fmoc-Lys(Boc)-LL Wang resin (0.35 mmol/g). Deprotection was performed with 20% piperidine in DMF for 2 x 2.5 min, and coupling was performed using 1:1:2 0.1 M AA/0.1 M HCTU/ 0.2 M NMM/DMF (10x excess) for 2 x 5 min. 6 x 30 sec washes were performed after the deprotection and second coupling steps, while a 1 x 30 sec wash was carried out between the first and second couplings. Each reaction vessel was then transferred to a ***Tribute***® peptide synthesizer with the IntelliSynth UV-monitoring and Feedback Control System for the addition of 5 more alanines to make $(A)_{10}$K-OH. The UV-Xtnd function was used to control the deprotection times and repeats to compare the efficiency of the different deprotection reagents. Piperidine, pyrrolidine, cyclohexylamine and *tert*-butylamine were all used at a concentration of 20% in DMF for the deprotection reaction. Coupling was performed using 1:1:2 0.1M AA/0.1M Activator/0.2M NMM in DMF (10x excess) for 2 x 5 min. 5 x 30 sec washes were done after the deprotection and second coupling steps, while a 1 x 30 sec wash was carried out between the first and second couplings. Cleavage was performed by treating the resin with 95:2:2:1 TFA/anisole/water/TIS for 2 hours.

Analysis: Crude peptides were precipitated with anhydrous ether, dried overnight, dissolved in water and analyzed using a Varian ProStar HPLC, equipped with a C18, 300 Å, 5 µm, 250 x 4.6 mm column (Varian Microsorb-MV), over 60 minutes with a flow rate of 1 mL/min and using a gradient of 5-95% B where Buffer A was 0.1% TFA in water and Buffer B was 0.1% TFA in acetonitrile. Detection was at 214 nm. Mass analysis was performed on a Shimadzu LC/MS in the positive ion mode. Separation was performed on a C18, 100 Å, 2.6 µm, 50 x 2.1 mm column (Phenomenex Kinetex), run for 9 minutes with a flow rate of 1 mL/min and using a gradient of 5-50% B, where Buffer A was 0.1% formic acid in water and Buffer B was 0.1% formic acid in acetonitrile.

RESULTS/DISCUSSION

HPLC results and UV data for the last five cycles of $(A)_{10}$K-OH synthesized with each deprotection reagent are shown in Figure 1. Mass analysis data confirmed the identity of the $(A)_{10}$K-OH product peak at an elution time of ~8.2 minutes (m/z = 856), and the Fmoc-$(A)_6$K-OH impurity peak (m/z = 794) at an elution time of ~24.8 minutes (data not shown). Percent purities and total deprotection repeats and times are shown in Table 1. Pyrrolidine produced the highest purity product (46.020%) with the fewest number of deprotection repeats (14 repeats) and shortest total deprotection time (14 minutes) even compared to piperidine (40.846% purity, 19 repeats, 29 minutes). Cyclohexylamine and *tert*-butylamine both performed less efficiently than piperidine with product purities of only 24.432% and 11.877%, respectively, and 35 or more deprotection repeats and total deprotection times of 88 minutes or more. Based on these results,

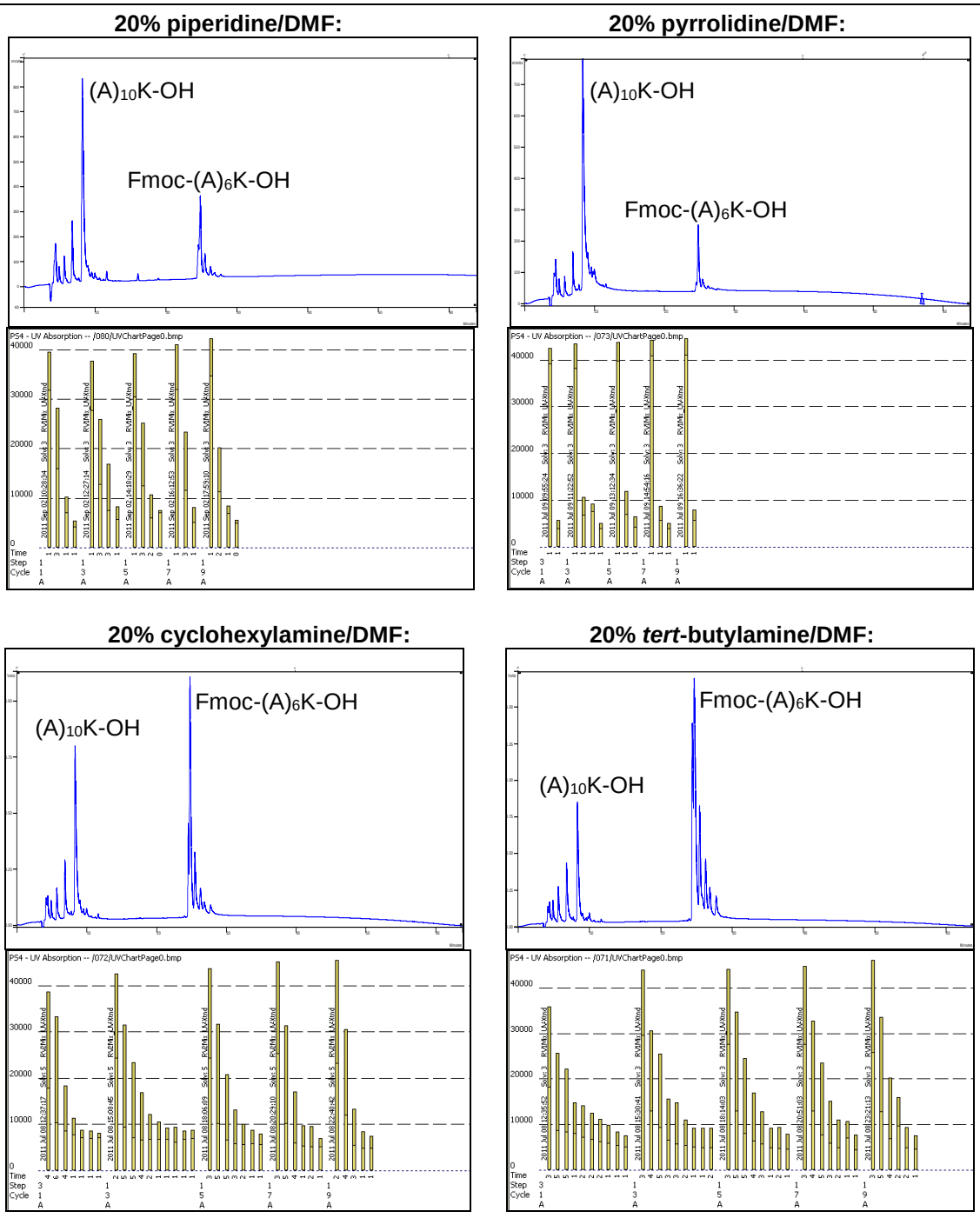

Figure 1: HPLC results and UV data from last five cycles of crude $(A)_{10}K$-OH synthesized on the Prelude® and Tribute® with UV-monitoring. Fmoc-$(A)_6K$-OH impurity peak is also labeled.

Table 1: HPLC percent purities for product $(A)_{10}$K-OH and Fmoc-$(A)_6$K-OH peaks and total deprotection repeats and times for the deprotection reagents used in this study.

Deprotection Reagents	A_{10}K-OH % Purity	Fmoc-A_6K-OH % Purity	Total Dep Reps	Total Dep Time
Piperidine	40.846	13.911	19	29
Pyrrolidine	46.020	9.033	14	14
Cyclohexylamine	24.432	28.522	35	88
tert-Butylamine	11.877	30.586	40	107

it appears that pyrrolidine is the only viable candidate for replacing piperidine during the synthesis of difficult peptides like poly-alanines.

CONCLUSION

20% pyrrolidine produced a higher purity product with fewer repetitions and shorter deprotection times than piperidine. It was the only deprotection reagent in this study proven through UV-monitoring data to be as or even more efficient than piperidine. Based on these results, pyrrolidine may be an effective substitute for piperidine when controlled substances cannot be used.