

INTRODUCTION

The 65-74 fragment of the acyl carrier protein (⁶⁵⁻⁷⁴ACP) is a known peptide sequence that has been used as a good example of the sequence-dependent problems of solid phase synthesis¹. Its synthesis has become a standard for testing peptide synthesizers as well as the efficacy of different synthesis protocols²⁻⁴. Protein Technologies, Inc. (PTI) considered it important to find out how to improve its quality and speed of synthesis by using **CONVENTIONAL SOLID PHASE CHEMISTRY** and utilizing the unique features of the *Symphony*[®] and *Prelude*[™] peptide synthesizers.

H-VQAAIDYING-OH

Figure 1: Sequence of ⁶⁵⁻⁷⁴ACP

BACKGROUND

⁶⁵⁻⁷⁴ACP was first synthesized using PTI's standard quality control protocol (2 x 2.5 min deprotection, 2 x 10 min coupling, 6 x 0.5 min washes between reaction steps, 2 hour cleavage). The following times were then minimized:

- Deprotection
- Washing
- Coupling
- Cleavage

In addition, more efficient coupling reagents⁵ and a solvent known to reduce internal aggregation⁶ were tested to achieve shorter coupling times.

EXPERIMENTAL

Peptide synthesis (PTI Standard Protocol): The peptides were synthesized on the PTI *Symphony*[®] Peptide Synthesizer at the 25 μmol scale using a 5-fold excess of Fmoc-amino acids relative to the Fmoc-Gly-Wang resin (0.41 mmol/g). Deprotection was performed using 20% piperidine/DMF for 2 x 2.5 min. Coupling was performed using 1:1:4 amino acid/HBTU/NMM in DMF at 2 x 10 minutes. 6 x 0.5 min washes were performed between deprotection and coupling steps, and cleavage was performed with 95:2:2:1 TFA/water/anisole/EDT for 2 hours.

Synthesis Optimization: Optimization was performed by sequentially varying the coupling reagent, deprotection time, coupling time, coupling solvent, wash number, and cleavage time and selecting the conditions for each variable that produced the peptide in the shortest amount of time without a significant loss of purity. The peptide was synthesized using this optimized fast protocol on the *Symphony*[®] and *Prelude*[™].

Peptide Analysis: The crude peptides were analyzed on a Varian Microsorb-MV 300-5 C18 column, 250 x 4.6 mm, over 40 minutes using a gradient of 5-40% aqueous ACN with 0.1% TFA at 1 mL/min. Detection was at 214 nm.

RESULTS

Syntheses were performed in which one variable was optimized per synthesis as shown in **Table 1**. Standard protocol values were replaced with optimized fast protocol values in a cumulative fashion for each successive synthesis. HPLC results from successive syntheses on the *Symphony*[®] are shown in **Figure 2**. HPLC's comparing ⁶⁵⁻⁷⁴ACP synthesized using the standard and fast protocols on the *Symphony*[®] and *Prelude*[™] are shown in **Figure 3**. Synthesis and cleavage times for the standard and fast protocols are shown in **Table 2**.

Table 1: Optimized variables by synthesis. Standard and Fast protocol values are shown.

SYN#	VARIABLE	STANDARD PROTOCOL	FAST PROTOCOL
1	Coupling Reagent	HBTU	HCTU
2	Deprotection Time	2 x 2.5 min	2 x 0.5 min
3	Coupling Time	2 x 10 min	2 x 1 min*
4	Coupling Solvent	DMF	1:1 DMF/ DMSO
5	Wash Number	6 x 0.5 min	2 x 0.5 min
6	Cleavage Time	120 min	30 min

* Valine was coupled for 2 x 5 min

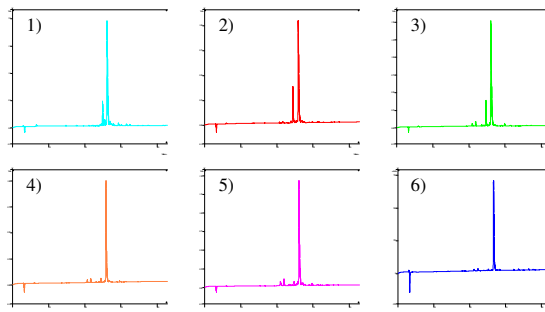


Figure 2: HPLC results of ⁶⁵⁻⁷⁴ACP syntheses on the *Symphony*[®]. One variable was optimized cumulatively per synthesis as follows: 1) Coupling Reagent, 2) Deprotection Time, 3) Coupling Time, 4) Coupling Solvent, 5) Wash Number, 6) Cleavage Time. The optimized result for each synthesis is shown.

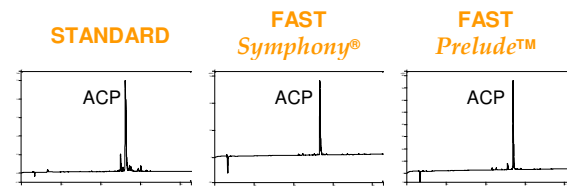


Figure 3: HPLC results of ⁶⁵⁻⁷⁴ACP synthesized using standard and fast protocols on the *Symphony*[®] and *Prelude*[™].

Table 2: Synthesis and cleavage times for standard and fast protocols on the *Symphony*[®] and *Prelude*[™]. Cleavage time includes time for final deprotection, rinsing and drying.

PROTOCOL:	STANDARD	FAST <i>Symphony</i> [®]	FAST <i>Prelude</i> [™]
Synthesis Time:	8.4 hrs	2.9 hrs	1.9 hrs
Cleavage Time:	3.1 hrs	1.1 hrs	1 hr

CONCLUSIONS

- By using HCTU and DMSO in the coupling reaction, shorter synthesis times and higher purities were obtained using **conventional solid phase peptide chemistry** on the *Symphony*[®] and *Prelude*[™].
- Reaction & washing mix times were reduced from 31 to 5 minutes per cycle
- Cleavage reaction times were reduced from 2 hours to 30 minutes

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