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High-Throughput Process Optimization and Difficult Peptide Synthesis on the Symphony® X

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Introduction

The Symphony X® multiple peptide synthesizer offers the greatest number of independent protocols of any instrument on the market, making it an ideal platform for high-throughput process development. Furthermore, the addition of rapid IR heating provides a powerful tool for completing even the most difficult peptide sequences.

A range of conditions, including variations in the resin, deprotection and coupling reagents, activation, and reaction time was screened simultaneously to optimize the synthesis of the 31-mer C-Peptide (Figure 1). Temperature may also be varied on the Symphony X® through the use of IR heating, as applied to the synthesis of the challenging peptide thymosin α1 (Figure 2).

EAEDLQVGQVELGGGPGAGSLQPLALEGSLG

Fig. 1. Sequence of C-Peptide.

Ac-SDAAVDTSSEITTKDLKEKKEVVEEAEN

Fig. 2. Sequence of Thymosin α1.

Results and Discussion

The power of the Symphony X™ for process optimization has been demonstrated through the synthesis of C-Peptide using multiple reaction conditions. The target 31-mer peptide was obtained with crude purities ranging from 80 to 92%. Among the variations in the 22 conditions screened, reaction time, coupling reagent, and the choice of resin all had an effect on the product purity. For example, under otherwise equivalent conditions, slightly higher purities were noted for the synthesis on Tentagel S RAM than with standard Rink MBHA polystyrene resin (Figure 3).

Illustrating the advantage of rapid heating to 85°C during deprotection and coupling, thymosin α1 was prepared with 80% purity (Figure 4). This is the highest purity that has been reported for the synthesis of this peptide. Impressively, this result was produced using just one minute deprotections and two minutes coupling times.

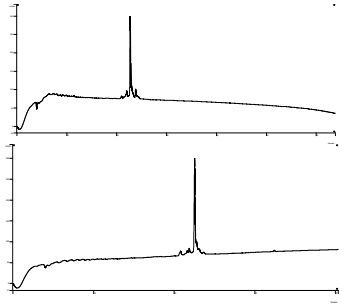

Fig. 3. HPLC traces for C-Peptide synthesized using Rink MBHA PS (left, 82% purity) and Tentagel S RAM resin (right, 88% purity).

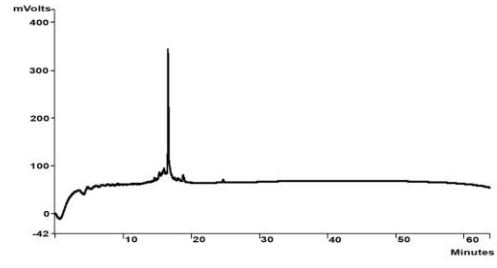

Fig. 4. HPLC trace of thymosin α1 synthesized in 80% purity using IR heating.