

Fast Synthesis Study – Optimized Fmoc Chemistry Used to Rapidly Synthesize 32-mer C-Peptide in 8.7 Hours

INTRODUCTION

Very fast synthesis times are achievable using optimized Fmoc conditions. Traditional peptide chemistry typically resorts to using extended reaction times to maintain synthesis yields, often resulting in deprotection times of 20-30 minutes and coupling times of an hour or more. It is accepted that speed is gained at the expense of purity. However, this is not the case. In fact, when the chemistry is optimized, high purity peptide can be obtained at amazing speeds using conventional Fmoc chemistry. To illustrate this, chain A, human proinsulin C-peptide¹ was synthesized with 2 x 1.5 minute deprotection times, 2 x 2 minute coupling times, and an 18 minute cleavage time.

Sequence²: H-EAEDLQVGQVELGGGPGAGSLQ
PLALEGSLG-OH

METHOD

Peptide Synthesis: Peptides were synthesized at the 25 µmol scale on a *Symphony*[®] peptide synthesizer and at the 20 µmol scale on a *Prelude*[™] peptide synthesizer using Fmoc-Gly-Wang resin (0.41 mmol/g). Deprotection was performed with 20% piperidine in DMF for 2 x 1.5 minutes. Coupling was performed with 0.1 M amino acid with a ratio of 0.9/1/1 amino acid/PyBOP/DIPEA in DMF for 2 x 2 minutes. Cleavage was performed with 94:1:2.5:2.5 TFA/TIS/water/EDT for 18 minutes.

Analysis: Peptides were analyzed on a Varian Microsorb C-18 column (4.5 x 250 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 60 minutes. Detection was at 214 nm.

RESULTS

The results are shown in Figures 1 and 2.

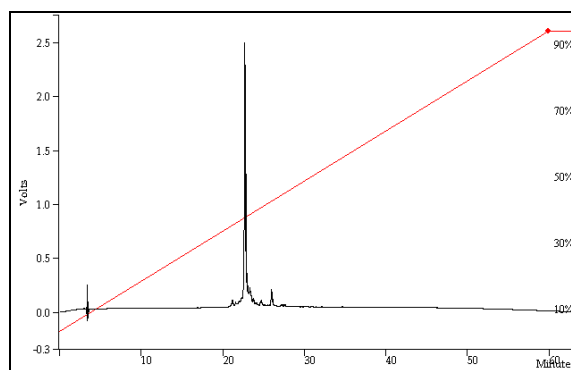


Figure 1: HPLC results for C-peptide on *Symphony*[®].

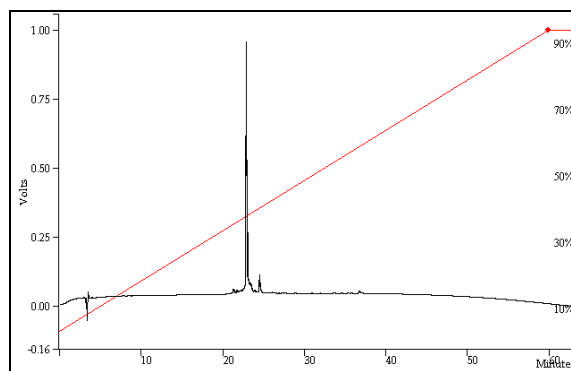


Figure 2: HPLC results for C-peptide on *Prelude*[™].

CONCLUSION

Conventional Fmoc-chemistry is capable of extremely fast reaction times when optimized. The 32-mer C-peptide was successfully synthesized in 15.3 hours on the *Symphony*[®] and in 8.7 hours on the *Prelude*[™].

¹ W.F. Heath, R.M. Belagaje, G.S. Brooke, R.E. Chankce, J.A. Hoffmann, H.B. Long, S.G. Reams, C. Roundtree, W.N. Shaw, L.J. Sliker, K.L. Sundell, R.D. Dimarchi. *J. Biol. Chem.* **267**, 419 (1992).

² C-terminal Q was replaced with a G