BIG SAVINGS Through Speed and Throughput

Protein Technologies, Inc. - PTI Chemistry Team

The cost of peptide production by solid phase peptide synthesis is affected by many factors, including the choice of chemistry and the choice of instrumentation.

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 always the case. When the chemistry is optimized, high purity peptide can be obtained at amazing speeds using conventional Fmoc chemistry.

To illustrate this, a difficult peptide (the 65-74 fragment of the acyl carrier protein) and a long peptide (chain A of the human proinsulin C-peptide[1]) were synthesized at the 25 μmol scale on a Protein Technologies, Inc. Symphony® peptide synthesizer under optimized conditions[2] (**Figures 1 & 2**). Deprotections were completed in 3 minutes or less, and couplings were completed in 4 minutes or less (**Table 1**), resulting in a total peptide production time of 4 hours for the 10-mer, and under 14 hours for the 31-mer.

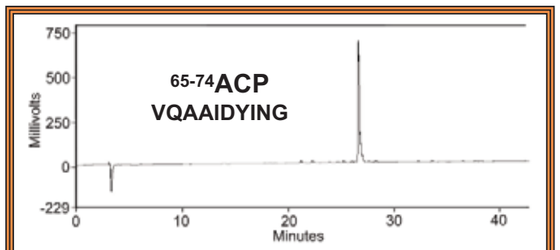

Figure 1: HPLC of Crude 65-74ACP.

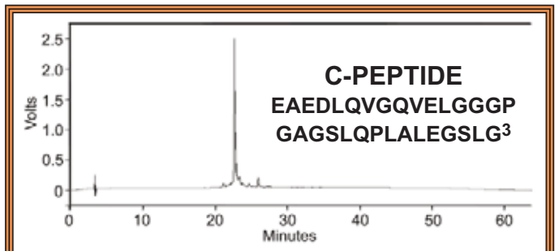

Figure 2: HPLC of Crude C-peptide.

Table 1: Reaction, synthesis and cleavage times for 65-74ACP and C-peptide. Cleavage time includes time for final deprotection, rinsing and drying.

Peptide:	65-74ACP	C-peptide
Deprotection Time:	2 x 30 sec	2 x 1.5 min
Coupling Time:	2 x 1 min	2 x 2 min
Synthesis Time:	2.9 h	12.8 h
Cleavage Time:	1.1 h	0.9 h

When developing an optimized protocol, the ability to test different reaction conditions (resin, activator, solvent, deprotectant) in parallel increases throughput, which saves time and money. The Symphony® peptide synthesizer from Protein Technologies, Inc. comes with 12 independent reaction vessels that are capable of running under different conditions simultaneously, giving you the power of 12 peptide synthesizers in one.

Protein Technologies, Inc. has been a global leader in automated peptide synthesis instrumentation since 1990 and is ready to supply you with the synthesizers and support you need to reach your peptide synthesis goals. Innovators of the venerable PS3™, Symphony®, Prelude™ and Sonata® suite of synthesizers, PTI's greatest commitment is to its loyal and growing customer base. For the best in performance, reliability, and throughput, along with unparalleled field support, choose PTI synthesizers and reagents for your lab.

Peptide Synthesis and Analysis. Peptides were synthesized at the 25 μmol scale on Fmoc-Gly-Wang resin (0.41 mmol/g). Deprotection was accomplished with 20% piperidine in DMF, and coupling was performed with 1:1:4 amino acid/HCTU/NMM in DMSO and 0.9/1/1 amino acid/PyBOP/DIPEA in DMF for 65-74ACP and C-peptide, respectively. 65-74ACP was cleaved with 95:2:2:1 TFA/anisole/water/EDT for 30 minutes, while C-peptide was cleaved with 94:1:2.5:2.5 TFA/TIS/water/EDT for 18 minutes. Peptides were analyzed on a Varian ProStar HPLC using a Varian Microsorb-MV 300-5 C18 column, 250 x 4.6 mm, over 40 minutes using a gradient of 5-40% aqueous acetonitrile with 0.1% TFA or over 60 minutes using a 5-95% gradient at a 1 mL/min flow rate. Detection was at 214 nm.

About the Protein Technologies, Inc. Chemistry team: The Protein Technologies, Inc. chemistry team has more than 30 years of experience in peptide chemistry and is eager to serve PTI instrument users by providing technical support and developing new applications on PTI instruments.

Questions? Contact a member of the PTI Chemistry Team at
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References:
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. Slieker, K.L. Sundell, R.D. Dimarchi. *J. Biol. Chem.* **267**, 419 (1992).
2. G. Fuentes, C. Hood, K. Page, H. Patel, J.H. Park, M. Menakuru, Fast Conventional Synthesis of 65-74ACP on the *Symphony*® and *Prelude*™, European Peptide Symposium, (2006). http://www.peptideinstruments.com/images/29EPS_P559.pdf
3. C-terminal Q was replaced with a G.