Fast, Sequential High Throughput Peptide Synthesis

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Introduction

Peptides are an interesting class of compounds that have proven to be useful in materials science, as therapeutics, and as tools in medicinal research. Typically, such peptides are prepared via Solid Phase Peptide Synthesis (SPPS). Increasing demand of these highly valuable compounds however,requires an optimized high throughput technique that doesn't suffer from the typical inefficiencies such as long reaction times, low purities, and large amounts of generated waste [1],[2],[3].

Recently, *HE*-SPPS [4] was introduced to reduce these inefficiencies. *HE*-SPPS allows for cycle times of only 4 minutes as well as a 90% reduction in total waste. This new process utilizes microwave energy, thereby allowing synthesis of high purity peptides in less time and with less waste. The *HE*-SPPS process was applied to a series of 12 different peptides using a Liberty Blue™ microwave peptide synthesizer incorporating a 12 channel high throughput option (HT12). The total synthesis for each peptide was completed in less than one hour including a rapid 30 minute final cleavage step using microwave energy. Compared to traditional parallel techniques, *HE*-SPPS represents a great alternative for high throughput sequential synthesis and allows for:

Benefits of Sequential SPPS over Parallel SPPS

- 4 min cycle times (15 cycles per hr), resulting in finished peptides in 1-2 hrs
- 90% reduction in total waste
- Unique control at each synthesis step
- Purification of each peptide to begin immediately
- High purity from μw heating during both coupling and deprotection
- Instrumentation is much less complex

Methods

All peptides were synthesized sequentially using a Liberty Blue™ HT12 under *HE*-SPPS conditions at 0.1 mmol scale using 5-fold excess of reagents [0.2 M amino acid solution (in DMF) with 0.5 M DIC (in DMF) and 1.0 M Oxyma Pure (in DMF)]. Rink amide MBHA PS (0.38 mmol/g) was used in all preparations, except in the case of $^{1\text{-}42}$β-Amyloid and Ubiquitin (76-mer) in which PAL PEG PS was used. Deprotections were carried out with 10% w/v piperazine in NMP/EtOH (9/1). The peptide resin was washed three times with DCM immediately after synthesis. The peptides were cleaved with TFA/TIS/H_2O/DODT (92.5/2.5/2.5/2.5); 30 min at 38 °C using an Accent™ microwave cleavage system. The resin was filtered and the peptide precipitated upon addition of ice cold ether. The peptide was collected and analyzed.

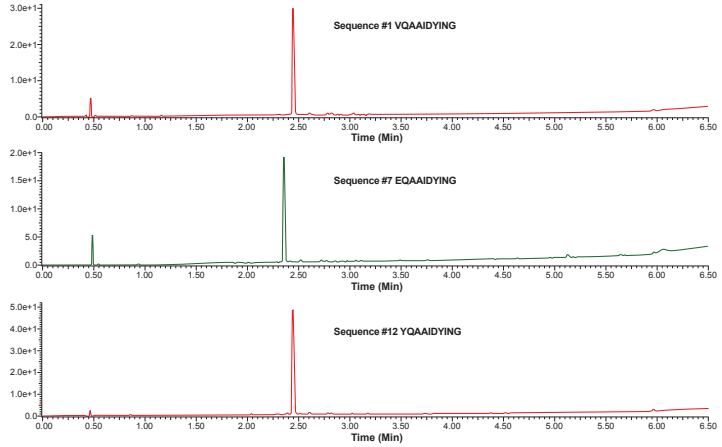
Liberty Blue HT12

Results and Discussion

To meet the high peptide demand, parallel, room temperature SPPS is often utilized. Several reactions are performed simultaneously to offset the extremely slow room temperature couplings/deprotections conditions. However, in this setup, difficult sequences and/or long sequences suffer from poor purities, use of large excess of reagents, and large amounts of solvent/waste generated. In addition, results can take days, thereby complicating the analysis.

To test these theories, Acyl Carrier Protein ($^{65\text{-}74}$ACP) derivatives, 12 in total, were prepared using the Liberty Blue HT12. All 12 peptides were produced sequentially in high purity. UPLC-MS analysis did not show any evidence of cross-contamination from peptide to peptide. The entire library was synthesized in less than 14 hours.

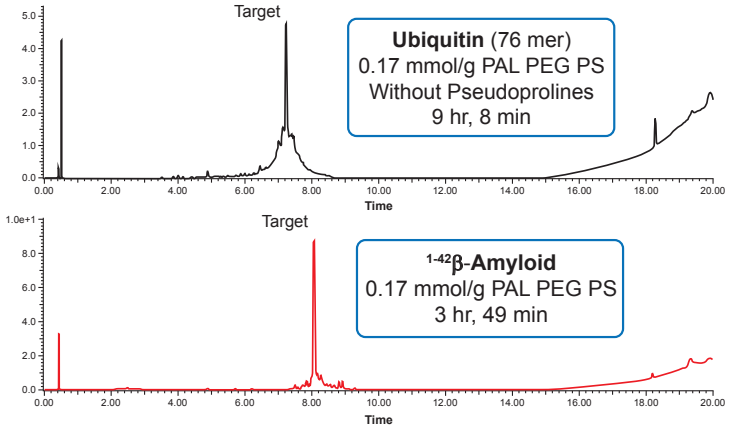
Sequence #	Peptide Sequence	Peptide MW	UPLC Crude Purity
1	VQAAIDYING	1062	95%
2	AQAAIDYING	1034	96%
3	IQAAIDYING	1076	96%
4	GQAAIDYING	1020	88%
5	DQAAIDYING	1078	96%
6	QQAAIDYING	1091	81%
7	EQAAIDYING	1092	98%
8	NQAAIDYING	1077	98%
9	MQAAIDIYING	1094	87%
10	SQAAIDYING	1050	94%
11	TQAAIDYING	1064	94%
12	YQAAIDYING	1126	98%



Final parameters for running all 12 peptides on Liberty Blue HT12

Total Synthesis Time	Total Wash Solvent Usage (DMF)	Total Chemical Waste
13 hr 28 min	1.16 L	2.04 L

The *HE*-SPPS method provides extremely high purity synthesis in a high throughput sequential format thereby avoiding a purification bottleneck. Using this same protocol, difficult and long sequences such as $^{1\text{-}42}$β-Amyloid [5] and Ubiquitin (76mer) [6] were prepared in 3 hr 49 min and 9 hr 8 min respectively.



References

[1] Lax, R. The Future of Peptide Development in the Pharmaceutical Industry. *PharManufacturing: The International Peptide Review* **2010**, 10-15.
[2] Chan, W., White, P. *Fmoc Solid Phase Synthesis - A Practical Approach*; Oxford University Press: New York, 2000.
[3] Bray, B. L. Large-scale manufacture of peptide therapeutics by chemical synthesis. *Nat. Rev. Drug Discov.* **2003**, *2*, 587-593.
[4] Collins, J. M.; Porter, K. A.; Singh, S. K.; Vanier, G. S. High-Efficiency Solid Phase Peptide Synthesis (*HE*-SPPS). *Org. Lett.* **2014**, *16*, 940 – 943.
[5] All amino acid cycles used standard *HE*-SPPS conditions: single 1 min/90 °C deprotection step, a single 2 min/90 °C coupling. Arginine: 2 x 90 °C 2 min coupling.
[6] All amino acid cycles used a single 1 min/90 °C deprotection step, a single 2 min/90 °C coupling for the first 30 amino acid residues, and a double 2 min/90°C coupling for the rest of the sequence. Pseudoprolines were not used.