High Throughput Sequential Peptide Synthesis With High Efficiency Solid Phase Peptide Synthesis (*HE*-SPPS)

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Abstract

Peptides play a pivotal role in pharmaceutical and medicinal research as they possess a wide range of biological properties. As such, the increasing demand for peptides requires optimized high throughput synthesis techniques that don't suffer from the typical inefficiencies of SPPS such as long reaction times and large amounts of generated waste [1],[2],[3]. Recently, *HE*-SPPS [4] was introduced which allows for cycle times of only 4 minutes along with 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 under one hour including a rapid 30 minute final cleavage step using microwave energy. Compared to traditional parallel techniques, this new sequential process allows for ultra-fast synthesis times of individual peptides, unique control at each synthesis step, and for purification of each peptide to begin immediately after its synthesis rather than waiting for completion of the entire batch.

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 preps along with 10% w/v piperazine in NMP/EtOH (9/1) as the deblocking solution. 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 crashed out upon addition of ice cold ether and collected.

Liberty Blue HT12

Analysis

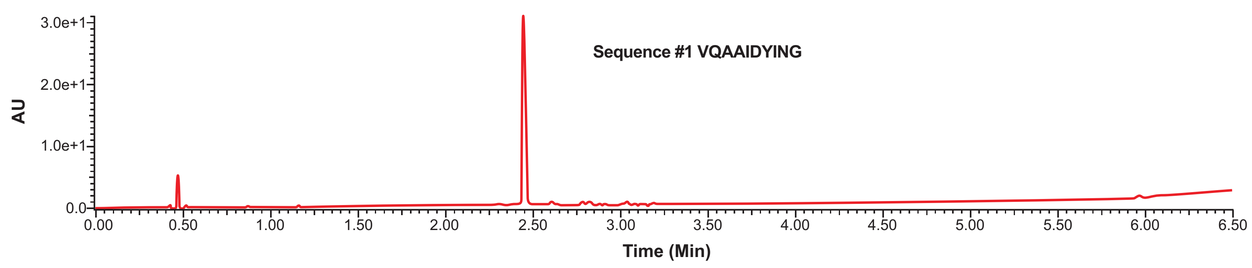
Samples were injected onto a Waters Acquity H-Class UPLC system with PDA detector equipped with an Acquity UPLC BEH C18 column (1.7 mm and d2.1 x 100 mm). The UPLC system was connected to a Waters 3100 Single Quad MS. Peak analysis was achieved on Waters MassLynx software. Separations were performed with a gradient elution of 0.1% TFA in water and acetonitrile.

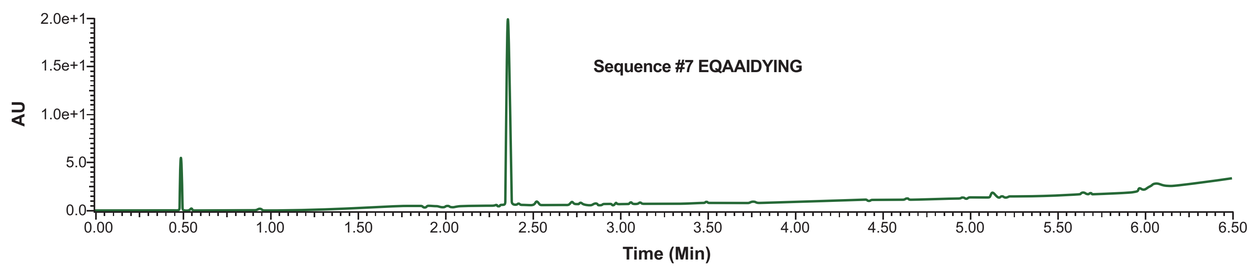
UPLC-MS

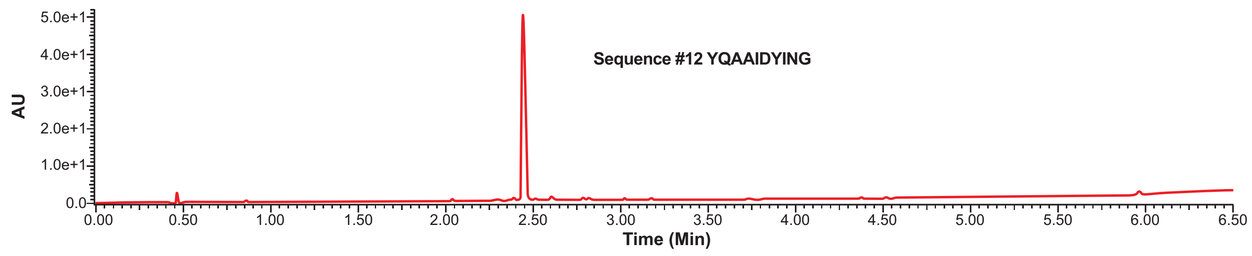
Results

Acyl Carrier Protein ($^{65\text{-}74}$ACP) derivatives, 12 in total, were prepared using the Liberty Blue HT12. The sequential production of the 12 unique peptides was prepared in high purity. UPLC-MS analysis did not reveal any cross-contamination present from any of the 12 peptides run on the peptide instrument.

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%



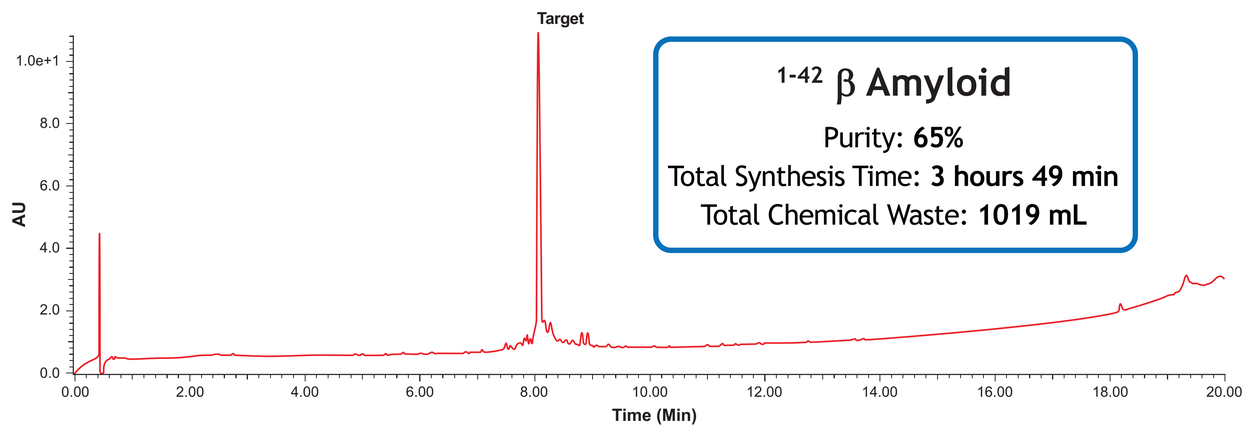




Final parameters for running all 12 peptides on Liberty Blue HT12

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 which avoids a purification bottleneck. Using this same protocol, $^{1\text{-}42}$ β Amyloid (0.1 mmol scale on 0.17 mmol/g PAL PEG PS) was prepared in 65% purity in 3 hr 49 min with only 1019 mL of total waste.



References

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[3] Bray, B. L.; *Nature Reviews*, **2**, 587 (2003)
[4] Collins, J. M., Porter, K. A., Singh, S. K.; Vanier, G. S; *Org. Lett.* **16**, 940 (2014)