High-Throughput Automated Microwave-Enhanced Peptide Synthesis

Summary

The ability to incorporate high-throughput automation for sequential microwave-assisted SPPS provides significant advantages for peptide production. Batch sizes up to 24 peptides can be synthesized in higher purity, with less waste, and extremely rapidly. Compared to parallel approaches, individual peptides can advantageously be moved to analysis and purification without waiting for the entire batch to be completed. The use of automated sequential microwave SPPS was demonstrated on a Liberty PRIME™ 2.0 HT24 with the rapid production of 20 distinct 16mer neo-antigen peptides.

Introduction

Modern peptide research is fast paced and often involves screening libraries of peptides to discover and develop functional peptides. Automated microwave-assisted SPPS has greatly reduced the time required to synthesize peptides, but synthesizing one peptide at a time requires user input between each sequence, if libraries need to be prepared. CEM's microwave peptide synthesizers (Liberty™ 2.0, Liberty Blue™ 2.0, and Liberty PRIME™ 2.0) can be paired with an HT transfer system, to allow the user to queue and automatically synthesize up to 24 peptides consecutively. Batches of individual resins are pre-loaded onto the HT for each peptide in the queue, and then automatically transferred to the Liberty synthesizer for peptide synthesis.

Automatic resin transfer removes the need for user input in between each queued peptide, maximizing productivity during the work-day and allows multiple peptides to run overnight. HT systems are designed to transfer 4, 12, or 24 batches of resin per queue, with ideal usage, based on the cycle times of the synthesizer. The fastest synthesizer, Liberty PRIME 2.0, can be equipped with HT4, HT12, or HT24, depending on the needs of the researcher. Liberty Blue 2.0 can be equipped with HT4, or HT12, while the base model Liberty 2.0 can be equipped with an HT4. Furthermore, the Liberty PRIME 2.0 synthesizer features extra reagent and bottle capacity for running large batches with the HT24 setup, without refilling, along with the optional ability to be used in cGMP settings for peptide production.

To demonstrate, a published set of 20 neoantigen peptides[1, 2] with diverse sequences and an average length of 16 residues was synthesized with the Liberty PRIME 2.0 using an HT24. The total synthesis time for the set of peptides was 24 h 14 min. The peptides were synthesized on 0.1 mmol scale using CarboMAX™ and yielded crude peptides with purities that ranged from 47% – 90%, and an average purity of 69% (**Table 1** on page 2 and **Figure 1** on page 3).[3] The total usage of DMF was 6056 mL and the total amount of waste generated was 6979 mL (**Table 2** on page 2). These neo-antigens were further purified by elevated temperature chromatography to purities suitable for biological studies.[4]

Materials and Methods

Reagents

The following Fmoc amino acids were obtained from CEM Corporation (Matthews, NC) and contain the indicated side chain protecting groups: Ala, Asn(Trt), Arg(Pbf), Asp(OMpe), Cys(Trt), Gln(Trt), Glu(OtBu), Gly, His(Boc), Ile, Leu, Lys(Boc), Pro, Met, Ser(tBu), Thr(tBu), Trp(Boc), Tyr(tBu), and Val. Rink Amide ProTideTM LL resin (0.20 meq/g substitution) was also obtained from CEM Corporation. N,N'-Diisopropylcarbodiimide (DIC), pyrrolidine, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), and triisopropylsilane (TIS) were obtained from Sigma-Aldrich (St. Louis, MO). Dichloromethane (DCM), N,N-Dimethylformamide (DMF), anhydrous diethyl ether (Et_2O), and acetic acid were obtained from VWR (West Chester, PA). LC-MS grade water (H_2O) and LC-MS grade acetonitrile (MeCN) were obtained from Fisher Scientific (Waltham, MA).

Peptide Synthesis

All peptides were synthesized on a 0.1 mmol scale using the CEM Liberty PRIME 2.0 automated microwave peptide synthesizer on Rink Amide ProTide resin LL. Deprotection was performed with pyrrolidine in DMF. Coupling reactions were performed with 5-equivalents of Fmoc-AA with DIC and Oxyma Pure in DMF (CarboMAX).2 Cleavage was performed at 38 °C using TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated with Et_2O and lyophilized overnight.

Peptide Analysis

The peptides were analyzed on a ThermoFisher UPLC system with Q Exactive plus MS equipped with an Acquity UPLC BEH C8 column (1.7 mm x 100 mm). Peak analysis was achieved on Chromeleon software. Separations were performed with a gradient elution of 0.05% TFA in (i) H_2O and (ii) MeCN.

Results

Table 1. Peptide sequences and respective crude purities achieved from consecutive synthesis on a Liberty PRIME 2.0 using an HT24.

Peptide Sequence	Crude Purity (%)
GWVKPIIIGHHAYGDQYRAT	73
TLYEQEIEV	49
HGSRKNITDMVEGAKKANG	73
SLLNQPKAV	79
EDPYLFELPVLKYLDMGTT	76
ALAVLSNYDA	84
TMEDKIYDQQVTKQCLCF	47
YSYPETPLYMQTASTSYYE	47
KVGYTERQRWDFLSEASIM	61
RLRMREHMMKNVDTNQD	65
VYEKNGYIYF	90
ALAVLCNYDA	73
ALVPPSKRKMWVVSPAEKA	78
ISTPTPTIVHPGSLPLHLG	75
IVQENNTPGTYLLSVSARD	74
RFHMKVSVYLLAPLREALS	75
ENLKQNDISAEFTYQTKDA	82
YMMPVNSEV	70
TNDVKTLADLNGVIEEEFT	59
SAWLFRMWYIFDHNYLKPL	48

Table 2. Combined synthesis time, DMF, and waste for the 20 peptides.

Total Synthesis Time	24 h 14 min
Total DMF Used	6056 mL
Total Waste	6979 mL

Conclusions

Automated sequential microwave SPPS using the HT resin transfer modules provides a powerful setup for synthesis of large batches of peptides. This was demonstrated through the high purity total batch synthesis of 20 different neo-antigen peptides in only one day. Individual peptides were completed in around 1 hour in high purity and with extremely low waste generated. All peptides in the batch were able to be quickly purified in high yield using elevated temperature preparative HPLC with the Prodigy system.[4] The available HT modules (HT4, HT12, HT24) provide a powerful upgrade to the Liberty 2.0, Liberty Blue 2.0, and Liberty PRIME 2.0 microwave peptide synthesizers.

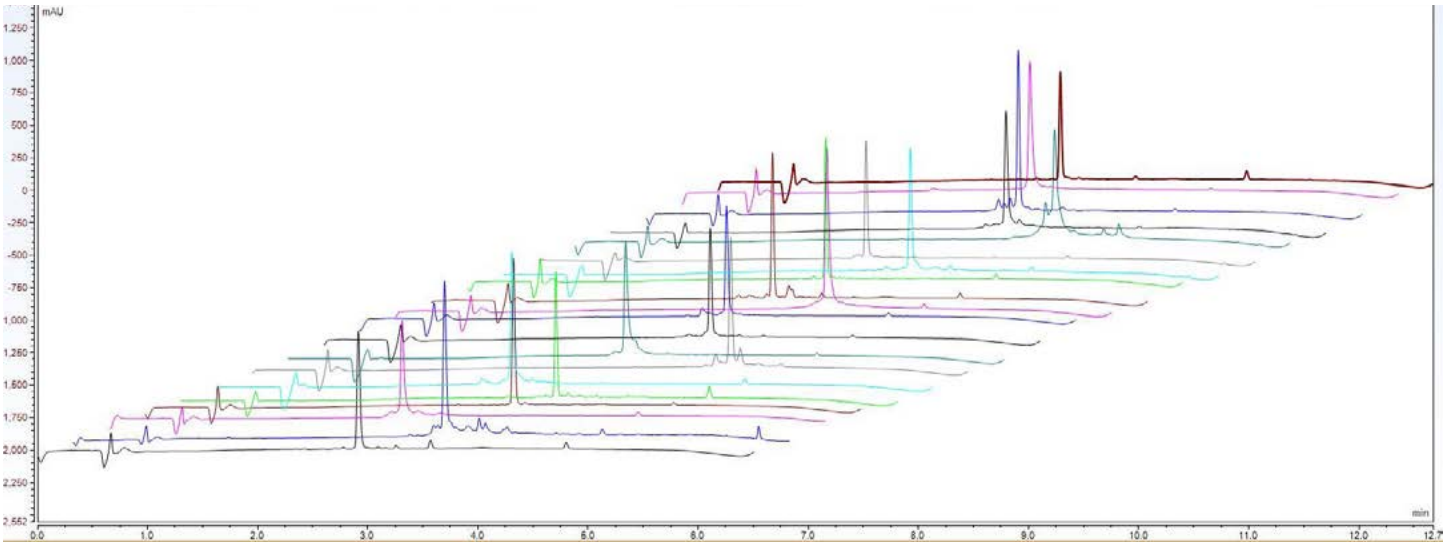

Figure 1. Overlaid chromatograms from UPLC analysis of the crude peptides.

References

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