Microwave Cleavage for Rapid Reaction Monitoring

INTRODUCTION
In the development of antibodies directed against specific peptide sequences, T-helper epitopes are often tagged onto the peptide sequence to aid in the stimulation of the immune response. One such sequence is the universal Pan HLA DR-binding epitope (PADRE). Synthesis of the PADRE peptide has proven to be difficult. The Accent Microwave Peptide Cleavage System allows for rapid microcleavage during peptide synthesis for analysis, providing quick determination of coupling efficiency.

MATERIALS AND METHODS
Reagents
Fmoc-Cha-OH (N-α-Fmoc-β-cyclohexyl-L-alanine), Fmoc-α-Ala-OH, and Rink Amide MBHA resin were obtained from Novabiochem. All other Fmoc amino acids, O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) and N-hydroxybenzotriazole (HOBt) were obtained from CEM Corporation. Diisopropylethylamine (DIEA), piperidine, trifluoroacetic acid (TFA), triisopropylsilane (TIS), and 3,6-dioxa-1,8-octanedithiol (DODT) were obtained from Sigma Aldrich. Dichloromethane (DCM), N,N-dimethylformamide (DMF), anhydrous diethyl ether, acetic acid, HPLC grade water and acetonitrile were obtained from VWR.

Peptide Synthesis:
(D-Ala)-K-(Cha)-VAAWTLKA-(D-Ala)-NH₂
The peptide was prepared using the CEM Liberty automated microwave peptide synthesizer on 0.1403 g of Rink Amide MBHA resin (0.72 meq/g substitution). Deprotection (20% piperidine with 0.1 M HOBt in DMF) was performed for 3 min with 25 W at a maximum temperature of 75 °C. Coupling reactions were performed with 5 fold excess Fmoc-AA-OH with 1:0.9:2 AA/HBTU/DIEA for 5 min with 15 W at a maximum temperature of 75 °C. Following the coupling of Lys³, the synthesis was paused and a small amount of resin (~50 μg) was removed from the reaction vessel for microcleavage using the CEM Accent Microwave Peptide Cleavage System. Cleavage was performed using 1.5 mL of 92.5:2.5:2.5:2.5 TFA/H₂O/TIS/DODT for 5 min with 25 W at 38 °C. Following cleavage the peptide was precipitated and washed with diethyl ether. Based on the microcleavage results, the synthesis was restarted with a double-coupling of Lys³. Cleavage of the finished peptide was performed using 92.5:2.5:2.5:2.5 TFA/H₂O/TIS/DODT for 30 min with 25 W at 38 °C. Following cleavage the peptide was precipitated and washed with diethyl ether.

Peptide Analysis
The microcleavage product was analyzed on a Waters Atlantis C₁₈ column (2.1 x150 mm) at 214 nm with a gradient of 5 – 70% MeCN (0.1% formic acid), 0 – 10 min. The finished peptide was analyzed on a Waters Atlantis C₁₈ column (2.1 x150 mm) at 214 nm with a gradient of 5 – 70% MeCN (0.1% formic acid), 0 – 20 min. Mass analysis was
performed using an LCQ Advantage ion trap mass spectrometer with electrospray ionization (Thermo Electron).

RESULTS
The total time required for microcleavage and analysis of the peptide was 28 minutes. The analysis showed only 82% coupling of Lys\(^2\). The finished peptide, following a second coupling of Lys\(^2\), gave complete coupling of Lys\(^2\) with a final crude purity of 93%. After lyophilization, 0.125 g of peptide were obtained, a 96% yield.

CONCLUSION
The Accent microwave cleavage system allows for the rapid evaluation of difficult couplings. By analyzing the peptide during the synthesis, potentially problematic peptides can be synthesized at greater purity.

REFERENCES

Figure 1. Microcleavage of PADRE after Lys\(^2\).

Figure 2. Final Analysis of PADRE.