# Automated N-terminal Acetylation

## Introduction

Synthetic peptides make promising drug candidates because their structural diversity and similarity to endogenous peptides and proteins grants them specificity and selectivity. Unfortunately, serum proteases will rapidly break down peptides in the body, limiting their efficacy as drugs. A number of modifications have been developed to extend serum half-life, with N-terminal acetylation being one of the simplest. Vogel et al[1] demonstrated that N-terminal acetylation of a small antimicrobial peptide (Lfc) extends the half-life in human serum from 0.5 hours to 1.5 hours. Synthesis of N-terminally acetylated peptides can be easily automated using the Liberty Blue.

## Materials and Methods

### Reagents

Amino acids, Oxyma Pure, and Rink Amide ProTide™ LL resin were obtained from CEM Corporation (Matthews, NC). Dichloromethane (DCM), N,N-dimethyl-formamide (DMF), anhydrous diethyl ether, acetic acid, HPLC grade water and acetonitrile were obtained from VWR (West Chester, PA).


### Peptide Synthesis: RRWQWR-$NH_2$ (Lfc2) and $CH_3CO$-RRWQWR-$NH_2$ (Lfc4)

The model peptide was prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on Rink Amide ProTide resin (0.61 meq/g substitution). Deprotection with 20% piperidine in DMF was performed in a single step of 1 min at 90 °C. Coupling reactions were performed with a 5 fold excess of Fmoc-AA-OH with 1:1:1 AA/DIC/Oxyma Pure for 2 min at 90 °C.

Following final deprotection, N-terminal acetylation of Lfc4 with 10% acetic anhydride in DMF was performed in a single step of 2 min at 65 °C. Because of the highly polar nature of acetic anhydride, a modified microwave method was used to minimize temperature overshoot as shown in Table 1. A Wash Thru Manifold is recommended after the acetylation microwave step to remove any residual acetic anhydride, and a minimum of 3-4 washes (2-4 mL each) is recommended following initial deprotection and the Wash Thru Manifold.

| Stage | Temperature (°C) | Power (Watts) | Hold Time (sec) | Delta T |
|---|---|---|---|---|
| 1 | 65 | 40 | 30 | 5 |
| 2 | 65 | 0 | 30 | 5 |
| 3 | 65 | 40 | 30 | 5 |
| 4 | 65 | 0 | 30 | 5 |

*Table 1. N-terminal acetylation microwave method*

Cleavage was performed using 92.5:2.5:2.5:2.5 TFA/H2O/TIS/DODT for 30 min at 42 °C. Following cleavage the peptide was precipitated and washed twice in diethyl ether.

## Results

Lfc2, the peptide with the free N-terminus, was synthesized at **78% crude purity** (Figure 1). Lfc4, the peptide with the N-terminus acetylated, was synthesized at **72% crude purity** (Figure 2). No unacetylated peptide was detected in the analysis of Lfc4.

 

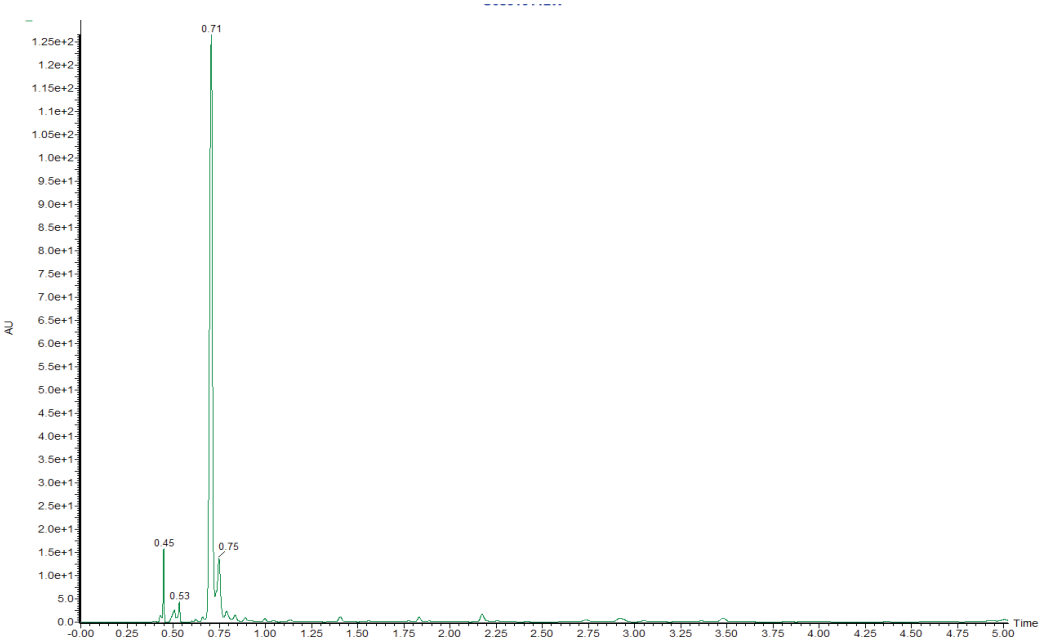


*Fig 1. UPLC Chromatogram of RRWQWR-$NH_2$ (Lfc2)*

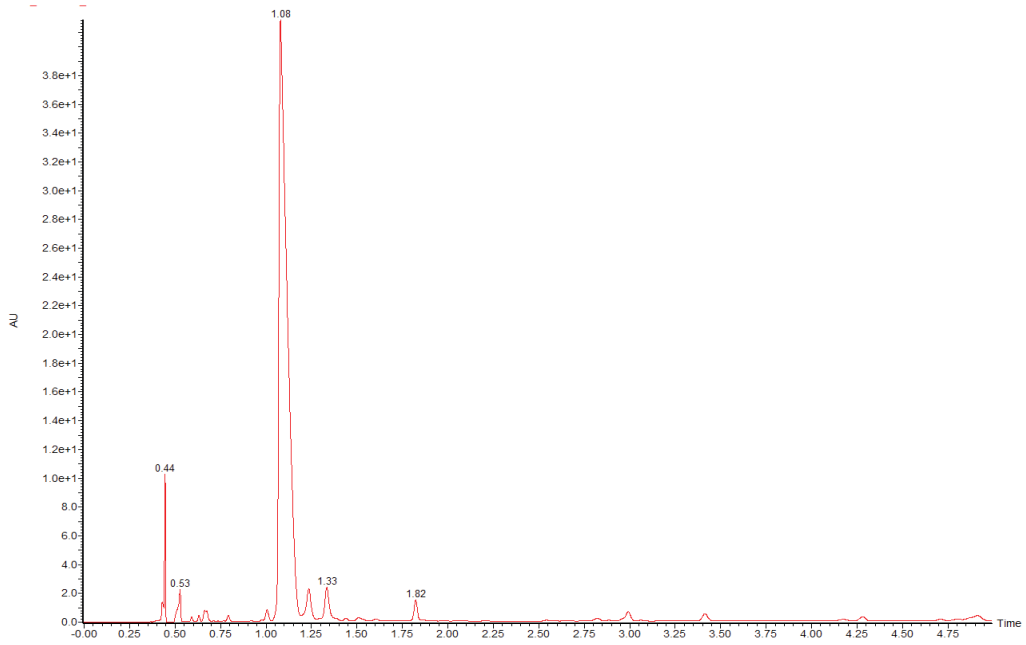


*Fig 2. UPLC Chromatogram of $CH_3CO$-RRWQWR-$NH_2$ (Lfc4)*

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[1] *Nguyen, L., Chau, J., Perry, N., de Boer, L., Zaat, S., Vogel, H. PLoS ONE. 2010, 5(9), e12684.*