Peptide Palmitoylation Procedure

Introduction

This procedure details the simple, HE-SPPS coupling of palmitic acid onto the N-terminus of peptides with the Liberty series automated microwave peptide synthesizers at the 0.10 mmol scale. For the Parameter Values for other synthesis scales, contact synthesis.support@cem.com.

This document should be used in conjunction with the Liberty Manual and the Safety Data Sheet (P/N 601400). Read and fully understand all documentation before operating the instrument.

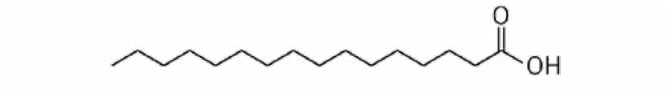


Figure 1. Palmitic Acid

⚠ WARNING
Proper precautions must be taken to avoid contact with reagents or reagent vapors. Protective gear should be worn as outlined in the user's safety program for hazardous materials and the reagent manufacturer's safety data sheet. Refer to these guidelines for proper handling and disposal of the reagents. Dispose of all waste in accordance with all applicable local, state, and federal health and safety.

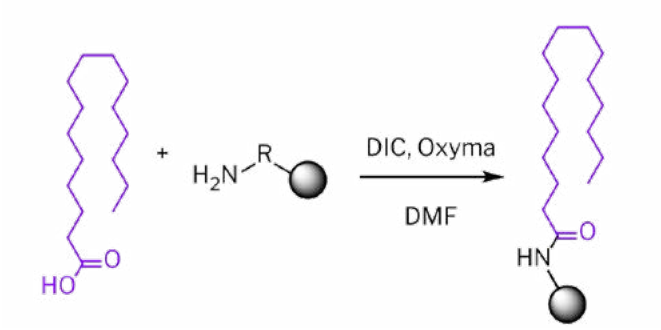


Figure 2. Palmitic Acid to Peptide Coupling

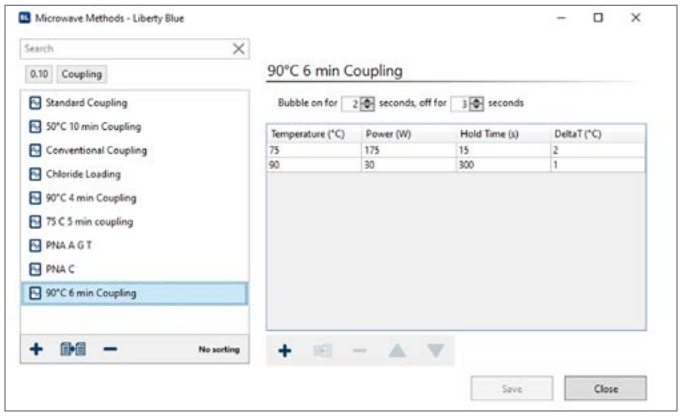
Step 1: Program Microwave Method

Create the "90° C 6 min Coupling" Microwave Method

1. From the "Edit" tab, select "Microwave Methods".
2. Select the following filters: "0.10" synthesis scale and "Coupling".
3. Create a copy of the "Standard Coupling" microwave method by clicking the duplicate button.
4. Rename as "90° C 6 min Coupling".
5. Edit the "90° C 6 min Coupling" microwave method to utilize the following Parameter Values:

Temperature	Power	Hold Time(s)	DeltaT(C)
75	Do Not Adjust	15	2
90	Do Not Adjust	350	1

6. Once all the parameter values are entered select “Save”.



Step 2: Program Cycle with Programmed Microwave Method

Create the “0.10-Double 90° C 6 min Coupling (HS)” Cycle Editor

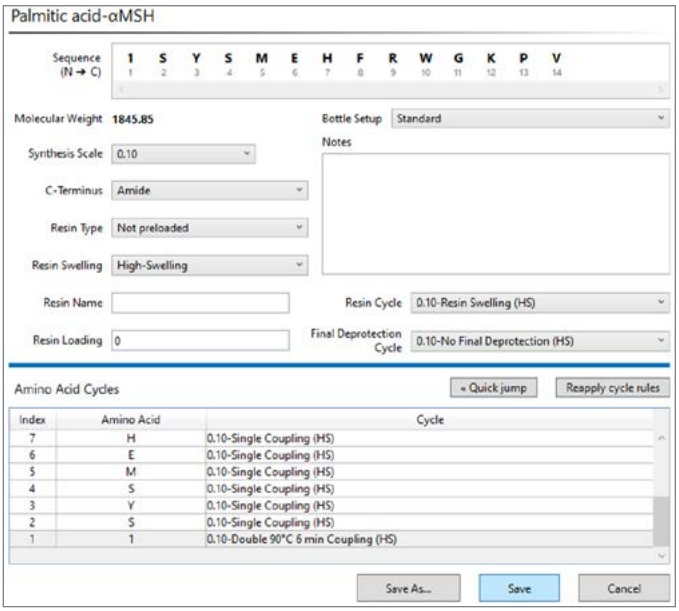
1. From the “Edit” tab, select “Cycles”.
2. Select the following filters: “0.10” synthesis scale, “Amino Acid” and “High-Swelling”.
3. Create a copy of the “0.10-Single Coupling (HS)” by highlighting and clicking the duplicate button. Rename as “0.10-Double 90° C 6 min Coupling (HS)”.
4. Edit the “0.10-Double 90° C 6 min Coupling (HS)” cycle to utilize the following Cycle Steps and Parameter Values:

Step	Operation	0.2 M Amino Acid Parameter Values
1	Deprotection	Microwave Method: Standard Deprotection Deprotection Volume: 4
2	Wash	Volume: 4.0 mL Drain Time: 5.0 mL
3	Wash	Volume: 4.0 mL Drain Time: 5.0 mL
4	Wash	Volume: 4.0 mL Drain Time: 5.0 mL
5	Wash	Volume: 4.0 mL Drain Time: 5.0 mL
5	Coupling	Microwave Method: 90° C 6 min Coupling Amino Acid: (from method) Amino Acid Volume: 2.5 mL Activator Bottle Position: Activator Activator Volume: 1.0 mL Activator Base Bottle Position: Activator Base Activator Base Volume: 0.5 mL
6	Coupling	Microwave Method: 90° C 6 min Coupling Amino Acid: (from method) Amino Acid Volume: 2.5 mL Activator Bottle Position: Activator Activator Volume: 1.0 mL Activator Base Bottle Position: Activator Base Activator Base Volume: 0.5 mL

5. Once all cycle steps and parameters are entered select “Save.”

Step 3: Apply Cycle to a Liberty Method

1. From the “Edit” tab, select “Liberty Methods” to create a new Liberty Method.
2. Add a value (one-letter code) on the N-terminus of the sequence for the position containing the palmitic acid solution.
3. Edit the Liberty Method, utilizing the following method options:
   - C–Terminus: As indicated by resin linker
   - Resin–Type: As appropriate
   - Resin Cycle: As appropriate
   - Final Deprotection Cycle: 0.10-No Final Deprotection
4. Amino Acid Cycles grid, double-click the position for palmitic acid coupling and select the “0.10-90 °C 6 min Double Coupling” cycle from the drop-down menu.
5. Select “Save” and close the Cycle Editor.



6. Load the Liberty Method into the resin indicator position.

7. To ensure sufficient reagent solutions are prepared, select the “Calculators” tab followed by “Usage Calculator.” See “**Step 4: Prepare Reagents**” for important reagent preparation warnings.

Step 4: Prepare Reagents

1. Prepare a 0.2 M solution of palmitic acid in DMF and transfer into a clean, dry centrifuge tube. Load onto desired position as indicated by method.

> ⚠ **WARNING**
> If the palmitic acid solution does not readily dissolve, sonicate for 10 minutes.

2. Weigh desired resin and transfer into clean reaction vessel. Secure the reaction vessel onto the attenuator and place into microwave cavity.

3. Prepare any additional necessary reagents, load onto instrument, and begin the Liberty Method.

Case Study: Automated palmitoylation of α-Melanocyte-stimulating hormone ($CH_3(CH_2)_{14}$CONH-SYSMEHFRWGKPV-NH_2)

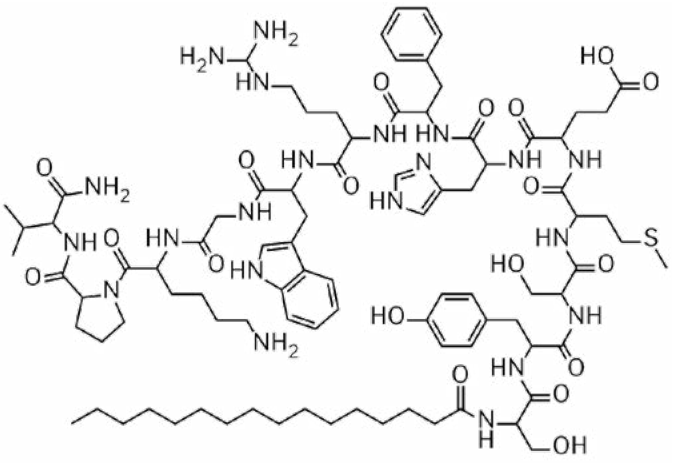


Figure 3. Palmitoylated α-Melanocyte-stimulating Hormone

Peptide Synthesis

A palmitoylated variant of α-Melanocyte-stimulating hormone **$CH_3(CH_2)_{14}$CONH-SYSMEHFRWGKPV-NH_2** (**Figure 3**) was prepared at 0.1 mmol scale using the CEM Liberty Blue automated microwave peptide synthesizer on 0.50 g Rink Amide ProTide LL resin (0.20 meq/g substitution). Deprotection was performed with 20% piperidine in DMF. Coupling reactions were performed with a 5-fold excess of Fmoc-AA-OH and palmitic acid, 1.0 M DIC in DMF, and 1.0 M Oxyma Pure in DMF. Cleavage was performed using the CEM Razor high-throughput peptide cleavage system with 92.5:2.5:2.5:2.5 TFA/H_2O/TIS/DODT. Following cleavage, the peptide was precipitated in Et_2O and lyophilized overnight.

Analysis

The peptide was analyzed on a Thermo Scientific Vanquish UPLC system with PDA detector equipped with an Acquity UPLC BEH C8 column (1.7 µm and 2.1 x 100 mm). The UPLC system was connected to a Thermo Exactive Plus Orbitrap, for structural determination. Peak analysis was achieved on Thermo Chromeleon software. Separations were performed with a gradient elution of 0.1% TFA in (i) H_2O and (ii) MeCN.

Results & Conclusions

Microwave-enhanced SPPS of $CH_3(CH_2)_{14}$CONH-SYSMEHFRWGKPV-NH_2 (mass = 1861) on the Liberty Blue automated microwave peptide synthesizer produced the target peptide in 75% purity (**Figure 4** and **Figure 5**, on page 4). The total synthesis time was 1 hour and 41 minutes, generating 300 mL of total waste.

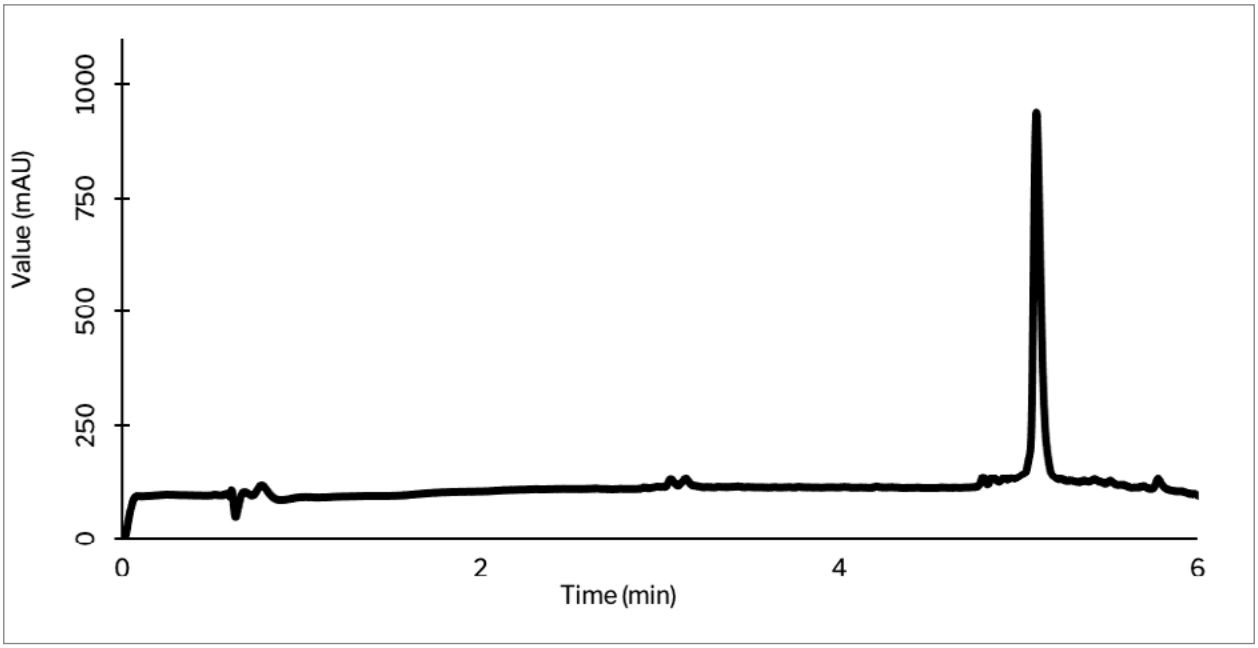


Figure 4. UPLC Chromatogram of $CH_3(CH_2)_{14}CONH\text{-}SYSMEHFRWGKPV\text{-}NH_2$

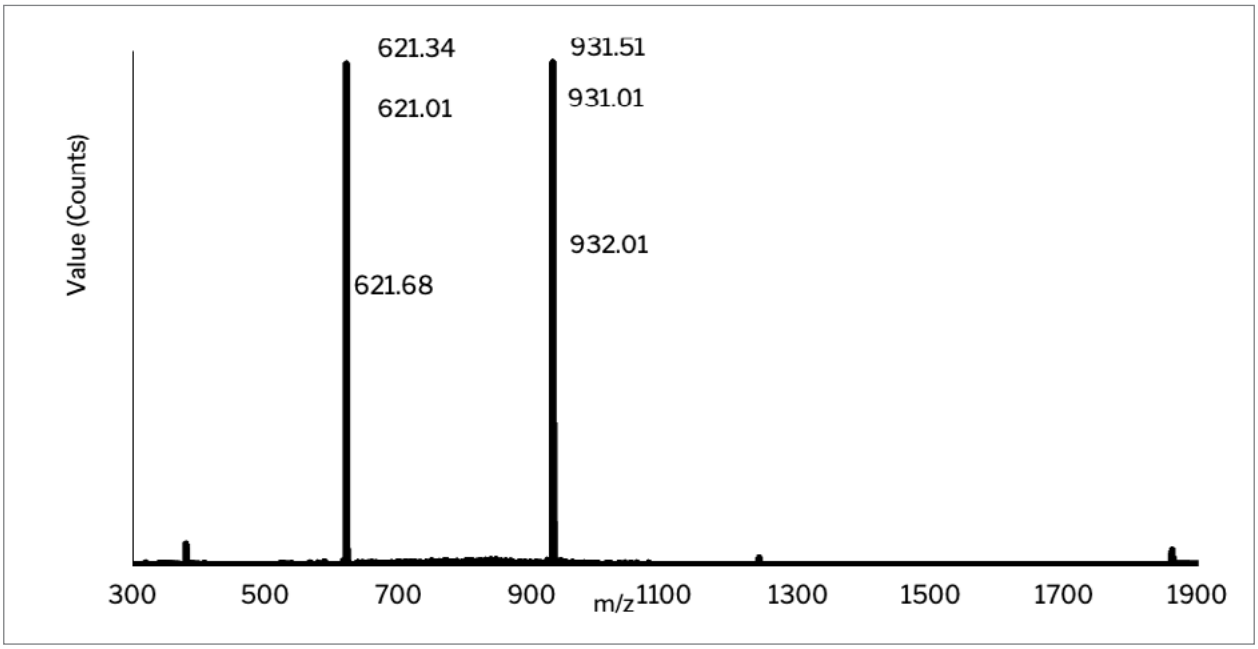


Figure 5. Mass Spectrum of $CH_3(CH_2)_{14}CONH\text{-}SYSMEHFRWGKPV\text{-}NH_2$ at Retention Time 5.13 min

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