Microwave Synthesis of Peptide Thioesters

Introduction

Although great advances have been made in the synthesis of peptides, synthesis of long peptides and whole proteins at reasonable purity remains difficult.\(^1\) To minimize these problems, the sequence can be broken up into a series of shorter peptides that are synthesized separately and then coupled together to form the large target peptide/protein.\(^1\)

Native chemical ligation (NCL) is a method of assembly of large peptides and proteins from smaller fragments.\(^2\) When ligating two fragments, the N-terminal fragment must contain a C-terminal thioester. Generation of C-terminal thioesters has historically proven difficult with Fmoc chemistry,\(^1,2\) but recent advances in resins have streamlined the process. Here, we demonstrate the resin loading, synthesis, and cleavage of a peptide thioester.

Materials and Methods

Reagents

All Fmoc amino acids were obtained from Peptides International (Louisville, KY). \(N-[(1H-1,2,3-Benzotriazol-1-yl oxy)(dimethylamino)methylene]-N-methyl- methanaminium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole monohydrate (HOBt), (benzotriazol-1-yl oxy)tripyrrloidinophosphonium hexafluorophosphate (PyBOP)\) were obtained from Anaspec (Fremont, CA). 4-Sulfambytyryl NovaSyn TG resin was obtained from EMD Chemicals (San Diego, CA). Diisopropylethylamine (DIEA), iodoacetonitrile (ICH\(_2\)CN), piperidine, piperazine, tetrahydrofuran (THF), trifluoroacetic acid (TFA), triisopropylsilane (TIS), benzyl hydrosulfide (BnSH), and 3,6-dioxa-1,8-octanedithiol (DODT) were obtained from Sigma Aldrich (St. Louis, MO). Dichloromethane (DCM) and \(N,N\)-dimethylformamide (DMF) were obtained from Pharmaco-AAPER (Brookfield, CT). \(N\)-Methylpyrrolidone (NMP), anhydrous diethyl ether, acetic acid, HPLC grade water and acetonitrile were obtained from VWR (West Chester, PA).

Peptide Synthesis:

Loading of Sulfamylbutyryl Resin

Loading of the resin was performed using the CEM Liberty automated peptide synthesizer. Fmoc-AA-OH (Fmoc-Gly-OH for ACP, Fmoc-Tyr(tBu)-OH for MCoTI-13, 0.2 M in DMF, 1.25 mL), PyBOP (0.2 M in DMF, 1.25 mL), and DIEA (1 M in NMP, 0.5 mL) were added to 4-sulfamylbutyryl NovaSyn TG resin (0.25 meq/g loading, 0.2 g) and heated in the Discover (2x30 min, 75 °C). The resin was then washed with DMF (3x5 mL).

Synthesis of \(^{65-74}\)ACP Thioester

![Figure 1. \(^{65-74}\)ACP thioester structure](image)

The peptide was prepared using the CEM Liberty automated microwave peptide synthesizer. Deprotection with 20% piperidine in DMF was performed in two stages with an initial...
deprotection of 30 s followed by 3 min at 75 °C. Coupling reactions were performed with a 5 fold excess of Fmoc-AA-OH with 1:1:2 AA/HBTU/DIEA for 5 min at 75 °C. Cleavage was performed in three steps. First, the resin was treated with ICH2CN and DIEA in NMP (24 hr, RT). Next, the resin was treated with BnSH in THF (24 hr, RT). Finally, the peptide thioester was cleaved from the resin using 92.5:2.5:2.5:2.5 TFA/H2O/TIS/DODT (4 hr, RT). Following cleavage the peptide was precipitated and washed in diethyl ether.

MCoTI-I Peptide Thioester

![MCoTI-I Peptide Thioester](image)

Figure 2. MCoTI-I thioester structure.

The peptide was prepared using the CEM Liberty automated microwave peptide synthesizer. Deprotection with 5% piperazine with 0.1 M HOBt in DMF was performed in two stages with an initial deprotection of 30 s followed by 3 min at 75 °C. Coupling reactions were performed with a 5 fold excess of Fmoc-AA-OH with 1:1:2 AA/HBTU/DIEA for 5 min at 75 °C (50 °C for Cys). Cleavage was performed in three steps. First, the resin was treated with ICH2CN and DIEA in NMP (24 hr, RT). Next, the resin was treated with BnSH in THF (24 hr, RT). Finally, the peptide thioester was cleaved from the resin using 92.5:2.5:2.5:2.5 TFA/H2O/TIS/DODT (4 hr, RT). Following cleavage the peptide was precipitated and washed in diethyl ether.

**Peptide Analysis**

The peptides were analyzed on an XBridge C18 column (9×50 mm) at 214 nm with a gradient of 5 - 70% MeCN with 0.1% formic acid, 0 - 20 min, with the column heated to 40 °C. Mass analysis was performed using an LCQ Advantage ion trap mass spectrometer with electrospray ionization (Thermo Electron).

**Results**

For the 65-74ACP peptide, the first residue (Fmoc-Gly-OH) was loaded on the 4-sulfamylbutyryl NovaSyn TG resin as described above and the peptide synthesized at 64% crude purity (Figure 3). Following lyophilization, the peptide was weighed and a crude yield of 78% was obtained.

![LC chromatogram for ACP-thioester synthesized on sulfambutyryl resin.](image)
For MCoTI-I peptide, the first residue (Fmoc-Tyr(tBu)-OH) was loaded on the 4-sulfamylbutyryl NovaSyn TG resin as described above and the peptide thioester synthesized at 90% crude purity (Figure 4). For this peptide, crude yield was not determined.

Figure 4. LC chromatogram for MCoTI-I thioester synthesized on sulfambutyrryl resin.

Conclusion

The synthesis of peptide thioesters can be successfully accomplished using Fmoc chemistry using microwave energy. These peptide thioesters can be obtained with good purity and at a reasonable yield.

References

2. Dittmann M; Sadek M; Seidel R; Engelhard M; Native chemical ligation in dimethylformamide can be performed chemoselectively without racemization. J. Pept. Sci. 2012, 18, 312-316.