



Solid Phase Synthesis of a Fluorescent Peptide: Comparison of Fmoc-Lys(5-FAM)-Resin and Fmoc-Lys[5-FAM(Trt)]-Resin

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Introduction

Many FRET (Fluorescent Resonance Energy Transfer) peptides, as well as non-FRET peptides, requiring C-terminal fluorescent labels can be accomplished using either solution phase or solid phase synthesis. In solid phase synthesis, the use of the fluorophore, 5-carboxyfluorescein (5-FAM) generates stable amide bonds. In order to help facilitate the synthesis of C-terminal fluorescent peptides, we have prepared and performed comparison studies using two kinds of resins, Fmoc-Lys(5-FAM)-Rink Amide resin (I) and Fmoc-Lys[5-FAM(trt)]-Rink Amide resin (II), the latter contains a phenolic hydroxyl group protected with a trityl group.¹ The reason for introducing the protecting group was to prevent the phenolic hydroxyl groups from reacting with the activated amino acids during peptide synthesis. EREQTVDSLVRPRTGRKKRRQRRRK(5-FAM)-NH₂, a fluorescent peptide was synthesized using these 2 resins. Syntheses were carried out under the same standard conditions and the peptides obtained showed no significant difference in purity. The results of these studies showed that resin (I) is adequate for synthesis of C-terminal fluorescent labeled peptide.

Results and Discussion

Preparation of Resins (I) and (II)

Fmoc-Rink amide resin was deprotected with 20% piperidine in DMF and reacted with Fmoc-Lys(Mtt)-OH [Resin:Fmoc-Lys(Mtt)-OH:HBTU:HOBt:DIEA, 1:3:3:3:6]. After 1h, the resin was thoroughly washed and the Mtt group deprotected with TFA/TIS/DCM (1:2:97). Resin was washed with DCM (twice), MeOH (twice), DCM (twice), 1% DIEA in DMF (twice) and DMF (twice) successively. Fmoc-Lys-Rink amide resin was reacted with 5-FAM [Resin: 5-FAM:DIC:HOBt, 1:2:2:2] in DMF. After the reaction is completed, washed and dried, resin (I) was obtained. Resin (I) was treated twice with Trt-Cl [resin:Trt-Cl:DIEA, 1:9:9] in DCM for 16 h, then washed and dried to obtain resin (II).

Synthesis of EREQTVDSLVRPRTGRKKRRQRRRK(5-FAM)-NH₂

Synthesis was performed on a SYMPHONY/Multiplex peptide synthesizer (Protein Technologies, Tucson, AZ). Peptide was synthesized using solid phase peptide synthesis employing Fmoc chemistry using resin (I) or (II). Side chain protecting groups for the amino acids are: t-butyl for Asp, Glu, Ser, Thr, Tyr; trityl for Asn, Cys, Gln, His; Pbf for Arg and Boc for Lys. Couplings were performed with sixfold excess of activated amino acids. Fmoc-amino acids were activated using Fmoc-amino acid: HBTU:HOBt:DIEA, 1:1:1:2, with NMP as the coupling medium. Fmoc protecting groups were removed using 20% piperidine/NMP.

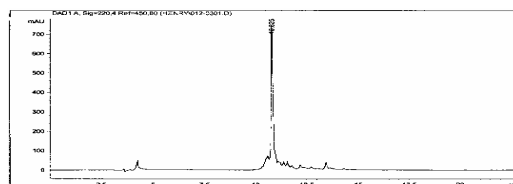


Figure 1. HPLC profile of crude EREQTVDSLVRPRTGRKKRRQRRRK(5-FAM)-NH₂ using resin (II). Analysis: A-0.1% TFA/H₂O, B-0.09% TFA/MeCN; 1mL/min, 5-65% B for 20 min.

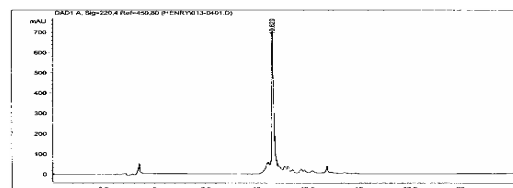


Figure 2. HPLC profile of crude EREQTVDSLVRPRTGRKKRRQRRRK(5-FAM)-NH₂ using resin (I). Analysis: A-0.1% TFA/H₂O, B-0.09% TFA/MeCN; 1mL/min, 5-65% B for 20 min.

Upon completion of the chain assembly, the peptide-resin was cleaved with TFA:thioanisole:water:phenol:EDT for 4h. Crude peptides were checked by RP-HPLC (Figure 1-2) and MALDI TOF MS (Figure 3, Theoretical [M+H]⁺ 3938.5).

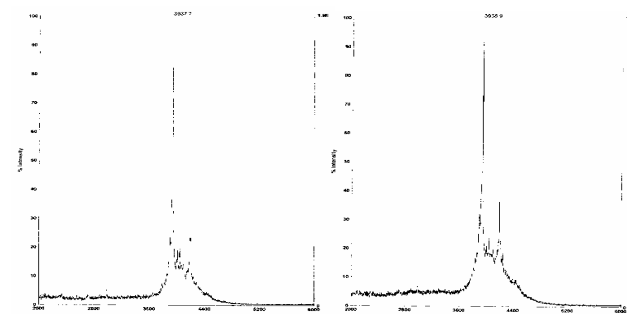


Figure 3. MALDI-TOF-MS of the crude peptide: (A) Resin (II); (B) Resin (I).

Conclusion

From the HPLC profiles, the crude peptides obtained with resin (I) and resin (II) showed no significant difference in purity (Fig. 1 and 2). The crude peptides also showed similar MS data having the correct major MS peak. From these results, the protecting phenolic hydroxyl group with a trityl group in resin (II) does not seem to have any apparent advantages; and the unprotected resin (I) is adequate for synthesis of C-terminal fluorescent peptide under normal coupling condition.

Reference

1. Fischer, R. et al. *Bioconjugate Chem.* **2003**, *14*, 653-660.