Technical Support
Information Bulletin 1208

Fmoc-Lys(Boc,Me)-OH, Fmoc-Lys(Me_2)-OH HCl, Fmoc-Lys(Me_3)-OH Cl

Methylation of lysine on the epsilon nitrogen is a regulatory post-translational modification common in histones. Mono-, di- and tri-methylated lysine residues may be found in the N-terminal tail of the four histone proteins. p53 and several other non-histone proteins are also methylated post-translationally.

To study the effects, mechanisms and enzymes involved in lysine methylation, synthesis of peptides containing methylated lysine residues is required. Fmoc-Lys(Boc,Me)-OH, Fmoc-Lys(Me_2)-OH HCl and Fmoc-Lys(Me_3)$^+$-OH Cl^- are available for synthesizing peptides containing, respectively, monomethylated, dimethylated or trimethylated lysine. Fmoc-Lys(Boc,Me)-OH can be utilized in peptide synthesis with standard coupling procedures. The Boc protecting group is removed during the trifluoroacetic acid (TFA) cleavage of the peptide from the resin.

Fmoc-Lys(Me_3)$^+$-OH Cl^- can be coupled utilizing standard peptide coupling procedures, but the coupling may be difficult with a second coupling required. Overall quality of the crude peptide may be reduced, especially if aspartimide formation is likely.[1]

The Lys(Me_2) sidechain is basic and can prematurely remove Fmoc groups over extended periods. Fmoc-Lys(Me2)-OH and all subsequent residues should be with diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt). Under these slightly acidic conditions, the sidechain is protonated and unable to cause Fmoc deprotection.

<u>DIC/HOBt Coupling for Fmoc-Lys(Me_2)-OH and Following Residues</u>

1. Remove the N-terminal Fmoc group according to standard Fmoc deprotection procedures.
2. Wash the resin 3 to 5 times with fresh solvent.
3. In a separate flask, dissolve 2.5 equivalents (based on the resin substitution) of the amino acid and 3.5 equivalents (based on the resin substitution) of HOBt in dimethylformamide (DMF) or N-methylpyrrole (NMP) (approximately 1 mL per mmol of Fmoc-amino acid).
4. Add 2.5 equivalents (based on the resin substitution) of DIC and stir the mixture under inert gas for 3 minutes.
5. Transfer the solution of activated amino acid to the reaction flask containing the N-deprotected resin.
6. Agitate the mixture at room temperature for 30 minutes to 1 hour. Remove and rinse a few resin beads, and test the completeness of the coupling with a Kaiser test.
7. When the coupling is complete, filter the resin and wash it 3 to 5 times with fresh solvent.
8. Repeat this procedure for each remaining amino acid in the sequence.

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[1] S.B. Rothbart, K. Krajewski, B.D. Stahl, S.M. Fuchs, *Methods in Enzymology*, **2012**, *512*, 107-135.