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Facile synthesis of N^{ε}-(benzyl, methyl)-lysine as a building block for site-specifically lysine monomethylated peptides

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Abstract—Facile, mild and efficient one-pot preparation of N^{α}-Fmoc-N^{ε}-(benzyl, methyl)-lysine, a building block for monomethylated peptide synthesis, was described. This building block was proved to be efficient for the synthesis of site-specifically monomethylated peptide. Benzyl group, which was incorporated by reductive benzylation and removed via catalytic hydrogenolysis, served as an excellent protecting group.

Lysine methylation, one of the post-translational modifications affecting histones,[1–4] can be found in mono-, di-, or trimethylated state in vivo (Fig. 1).[5,6] Regulation of the transition between mono-, di-, and trimethylation of lysine residues may control dynamic processes such as transcription and DNA repair.[7] For instance, dimethylation or trimethylation of histone H3 lysine 4 has been correlated with transcriptionally active genes, whereas monomethylation of H3 lysine 4 has been implicated in silent genes. This dualistic nature of the H3 lysine 4 methyl mark has far remained unresolved.[8,9] In order to elucidate the specific biological function of different state of lysine methylation, site-specifically methylated peptides and full-length histones are necessary to be synthesized. As a result, N^{ε} methylated lysine as a building block for site-specifically methylated peptide synthesis is required.

The development of synthetic routes to N^{α}-Fmoc protecting N^{ε} methylated lysine and subsequent peptide synthesis based on Fmoc chemistry strategy has been the focus of our research group. Herein a method for the preparation of N^{α}-Fmoc-N^{ε}-(benzyl, methyl)-lysine (**1**), a building block for the synthesis of peptides which

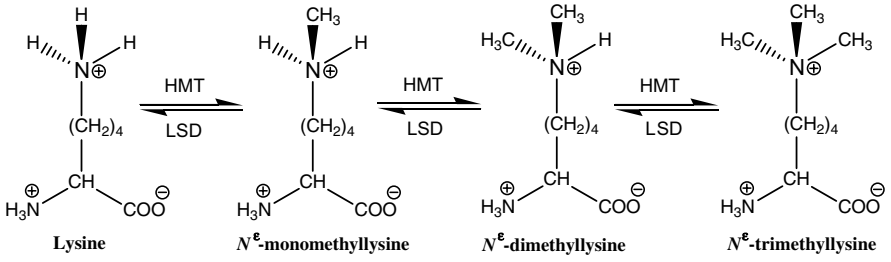

Figure 1. Chemical structures of lysine and its methylated derivatives. The action of histone methyltransferases (HMTs) and lysine-specific demethylase (LSD) is indicated.

Keywords: Lysine; Methylation; Benzylation; Monomethylated peptide.
Abbreviations: Fmoc, 9-fluorenylmethoxycarbonyl; Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; Trt, Trityl; TFA, trifluoroacetic acid; ESI-MS, electrospray ionization-mass spectroscopy; RP-HPLC, reversed-phase high-performance liquid chromatography.
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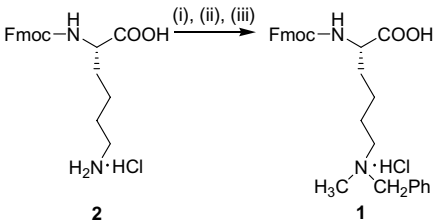
contain monomethylated lysine, is described. Compound **1** is obtained, without resorting to carboxyl group protection, via a consecutive sequence of reductive benzylation and reductive methylation using N^{α}-Fmoc-lysine (**2**) as the starting material. Both reductive alkylations are mild and efficient in the same flask without isolation. Finally, monomethylation of lysine side chain is specifically achieved by selective removal of the benzyl group after peptide chain assembly using N^{α}-Fmoc-N^{ε}-(benzyl, methyl)-lysine as a building block. Benzyl group serves as a great protecting group, because it can be conveniently incorporated and removed. Moreover, an important advantage of benzyl over other protecting groups for lysine side chain such as Boc, Z and Trt is that another methyl group can be further introduced by reductive methylation. Reductive methylation is possible for the benzylated lysine because it is a secondary amine, which has the potential of being transformed further to a tertiary amine.

Reductive alkylation of amino groups using aldehyde and hydride reagents provides a direct path from primary or secondary amino groups to tertiary amino groups.[10–13] As shown in Scheme 1, the benzyl group was introduced by treatment of **2** in ethanol with benzaldehyde in the presence of 3 Å molecular sieves for 1 h, followed by overnight reduction with sodium cyanoborohydride. The thus-formed benzylated lysine, N^{α}-Fmoc-N^{ε}-benzyl-lysine, was then treated in situ with 37% (w/w) formaldehyde solution. Another equivalent of sodium cyanoborohydride was added and allowed to react for additional 30 min to produce the desired compound **1**. The reaction mixture was acidified with 0.2 M hydrochloric acid and filtrated to remove the by-product solid. Compound **1** was finally obtained as a white solid by purification with silica gel chromatography and characterized with NMR and ESI-MS. ESI-MS 473.6 $[M+H]^+$, mp 90–92 °C, $[\alpha]_D^{20}$ $+5.0 \pm 0.2°$ ($c = 0.005$ in methanol). N^{α}-Fmoc-N^{ε}-benzyl-lysine was also isolated and characterized. ESI-MS 459.3 $[M+H]^+$, mp 185–187 °C, $[\alpha]_D^{20}$ $-2.0 \pm 0.1°$ ($c = 0.011$ in methanol). The total yield was 85% for the one-pot reaction that involved reductive benzylation and reductive methylation. The isolated yield was 89% for the reductive benzylation and 95% for the reductive methylation.

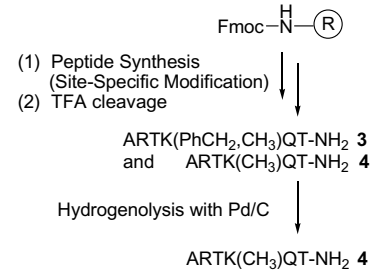
Note that only one benzyl group was attached to the amino group as expected, and no dibenzylated derivative was observed by reductive benzylation with appropriate amount of benzaldehyde. The steric hindrance of benzaldehyde precluded dibenzylation, however, methylation to the benzylated secondary amine was still available. The actual reaction process was that N^{α}-Fmoc-lysine reacted with benzaldehyde in the presence of 3 Å molecular sieves to first form a Schiff base and was then reduced by sodium cyanoborohydride. Among the hydride reagents, sodium cyanoborohydride has been widely employed for this kind of transformation. Sodium cyanoborohydride is used instead of sodium borohydride because the former is a much weaker reducing agent that does not reduce aldehydes or ketones at neutral pH, but readily reduces Schiff bases.[11] Moreover, reductive benzylation using sodium cyanoborohydride is a mild reaction condition and Fmoc group is just tolerable to such condition. Benzyl group can also be introduced by the reaction of benzyl bromide with amine in the presence of a mild base. However, dibenzyl derivatives might be produced from primary amines in this case.

Additionally, the sequence of reductive benzylation and reductive methylation was crucial to the success of this method. If reductive methylation was performed first, the product was dimethylated lysine (ESI-MS 397.5 $[M+H]^+$) but not monomethylated lysine. Consequently, the benzyl group cannot be further introduced to provide the desired product **1**. Therefore, reductive benzylation must proceed first, followed by reductive methylation.

Subsequent peptide synthesis was carried out as illustrated in Scheme 2. In order to synthesize a fragment [1–6] of histone H3 *N*-terminal tail (Ala-Arg-Thr-Lys(CH_3)-Gln-Thr) containing a monomethylated lysine, compound **1** was used as a building block in the following peptide synthesis. Compound **1** was first incorporated into the peptide chain on rink-amide resin with the standard Fmoc-based chemistry. When the peptide chain assembly on resin was completed, a peptide (Ala-Arg-Thr-Lys($PhCH_2$, CH_3)-Gln-Thr-NH_2) (**3**) (ESI-MS 807.6 $[M+H]^+$), in which the lysine residue was both methylated and benzylated, was obtained predominantly upon TFA-based cleavage from resin. Surprisingly, a lysine monomethylated peptide (Ala-Arg-Thr-Lys(CH_3)-Gln-Thr-NH_2) (**4**) (ESI-MS 717.5 $[M+H]^+$) was also observed from the ESI-MS spectrum of crude peptide, which indicated that benzyl groups on



Scheme 1. Reagents and conditions: (i) PhCHO, 3 Å molecular sieve, $NaBH_3CN$, ethanol; (ii) HCHO, $NaBH_3CN$, ethanol; (iii) HCl.



Scheme 2. Scheme for the synthesis of site-specifically monomethylated peptide.

some of peptide **3** were already cleaved by cleavage solution. Fortunately, peptide **4** was the desired peptide. Therefore, the peptide mixture was subject to further debenzylation without separation. Debenzylation of the peptide mixture via catalytic hydrogenolysis on Pd/C gave the desired monomethylated peptide **4** as the only product. The synthetic building block was efficiently coupled into peptide chain according to Kaiser Test[14] result. Furthermore, RP-HPLC analysis and ESI-MS spectra of the synthetic peptide indicated high purity (95%) and homogeneity of the crude peptide and thus confirmed the reliability and efficiency of the method.

In conclusion, N^{α}-Fmoc-N^{ε}-(benzyl, methyl)-lysine was prepared by a consecutive process of reductive benzylation and methylation of N^{α}-Fmoc-lysine in a one-pot reaction and then successfully incorporated into a fragment [1–6] of histone H3 *N*-terminal tail. TFA-based cleavage, followed by debenzylation via catalytic hydrogenolysis, gave the corresponding site-specifically monomethylated peptide. The procedure was facile, mild and efficient, and benzyl group was proved to be an excellent protecting group. It is believed that the developed method can be extensively applied to the selective monomethylation of N^{α} of amino acids and many other primary amines.

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Supplementary data

Supplementary data contain experimental procedures, ESI-MS, ^{1}H and ^{13}C NMR data and spectra of all synthetic compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.05.190.

References and notes

1. Murray, K. *Biochemistry* **1964**, *3*, 10–15.
2. Kouzarides, T. *Curr. Opin. Genet. Dev.* **2002**, *12*, 198–209.
3. Lachner, M.; Jenuwein, T. *Curr. Opin. Cell Biol.* **2002**, *14*, 286–298.
4. Sims, R. J., III; Nishioka, K.; Reinberg, D. *Trends Genet.* **2003**, *19*, 629–639.
5. Bannister, A. J.; Schneider, R.; Kouzarides, T. *Cell* **2002**, *109*, 801–806.
6. Kubicek, S.; Jenuwein, T. *Cell* **2004**, *119*, 903–906.
7. Shahbazian, M. D.; Zhang, K. L.; Grunstein, M. *Mol. Cell* **2005**, *19*, 271–277.
8. Santos-Rosa, H.; Schneider, R.; Bannister, A. J.; Sherriff, J.; Bernstein, B. E.; Tolga Emre, N. C.; Schreiber, S. L.; Mellor, J.; Kouzarides, T. *Nature* **2002**, *419*, 407–411.
9. van Dijk, K.; Marley, K. E.; Jeong, B. R.; Xu, J. P.; Hesson, J.; Cerny, R. L.; Waterborg, J. H.; Cerutti, H. *Plant Cell* **2005**, *17*, 2439–2453.
10. Borch, R. F.; Hassid, A. I. *J. Org. Chem.* **1972**, *37*, 1673–1674.
11. Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897–2904.
12. Barney, C. L.; Huber, E. V.; McCarthy, J. R. *Tetrahedron Lett.* **1990**, *31*, 5547–5550.
13. White, K. N.; Konopelski, J. P. *Org. Lett.* **2005**, *7*, 4111–4112.
14. Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147–157.