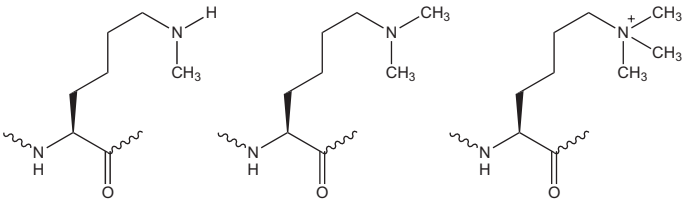
Novabiochem®

innovations 2/07

Synthesis of peptides containing methylated lysine residues

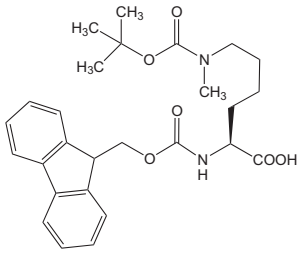


Post-translational methylation of histone lysine residues is emerging as an important control mechanism for the regulation of protein expression, X chromosome activation, genomic imprinting and DNA repair [1, 2]. All possible combinations of methylated lysine residues (Lys(Me), Lys(Me)$_2$ and Lys(Me)$_3$) have been found in nature. Histones H3 and H4 appear to be the primary sites for modification, where multiple lysine residues are methylated by at least a dozen histone methyl transferases (HMT). Whilst the exact roles of such modifications are currently poorly understood, methylation of H3 lysines-36 and -79 appears to be associated with transcription activation, whereas methylation of lysines-4, -9, -27 and lysine-20 on H3 and H4, respectively, is thought to lead to transcription repression [1]. The recent discovery of enzymes capable of demethylating lysine residues would indicate that lysine methylation is a dynamic process, akin to phosphorylation or acetylation. These demethylases are generally highly selective regarding the location and methylation state of the substrate lysine residue. LSD1, for instance, demethylates mono- and dimethyllysine-4 and -9 on H3, whereas JMJD2B demethylates trimethyllysine-9 on H3 [2].

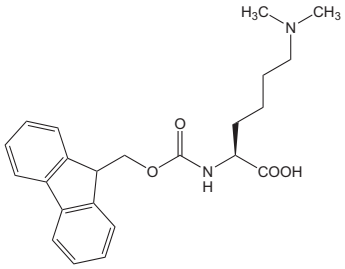
A prerequisite to research into the role of lysine methylation is ready access to peptides containing methylated lysine residues. It is for this reason that the Novabiochem® brand has introduced Fmoc-Lys(Me,Boc)-OH, Fmoc-Lys(Me)$_2$-OH and Fmoc-Lys(Me)$_3$-OH for the incorporation of methylated lysine residues by Fmoc SPPS. In this innovation, we demonstrate the utility of these derivatives in the synthesis of peptides related to histone 2B (Figure 1).

The use of Fmoc-Lys(Me,Boc)-OH, Fmoc-Lys(Me)$_2$-OH, and Fmoc-Lys(Me)$_3$-OH

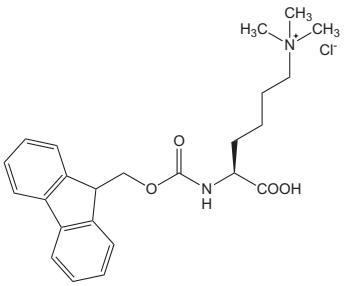
Fmoc-Lys(Me,Boc)-OH



Fmoc-Lys(Me)$_2$-OH



Fmoc-Lys(Me)$_3$-OH



Fmoc-Lys(Me,Boc)-OH, Fmoc-Lys(Me)$_2$-OH, and Fmoc-Lys(Me)$_3$-OH are extremely simple to use, as they have good solubility in DMF and can be coupled using standard activation methods such as PyBOP® or TBTU on automated peptide synthesizers. In the case of Fmoc-Lys(Me)$_2$-OH, some users have observed low levels of double incorporation of residues immediately following the lysine. This presumably results from loss of Fmoc-groups promoted by the basic side chain of this amino acid. Using acidic coupling mixtures may help ameliorate this side reaction.

Synthesis of histone 2B related peptides

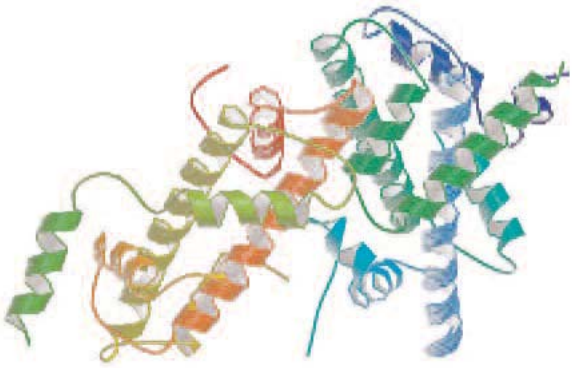


Fig. 1: Histone octamer. Green: H2A; blue: H2B; red: H3; light green H4. For clarity only one of each histone is shown [3].

To exemplify the use of Novabiochem derivatives in the synthesis of methylated lysine-containing peptides, the five histone 2B related peptides shown in Table 1 were prepared. A C-terminal Cys residue was incorporated in each case to facilitate conjugation to a protein for the purpose of raising antibodies.

The syntheses of peptides 1-4 (Table 1) were performed on a NovaSyn Crystal Peptide Synthesizer using NovaSyn® TGR resin. Coupling reactions were carried out for 1 hour using standard PyBOP® protocols and 5-fold excesses of Fmoc-protected amino acids. Fmoc removal was effected by pumping 20% piperidine in DMF through the resin bed until the optical density of the reaction column eluent returned to its original value. Peptide 5 was prepared using an ABi 433A peptide synthesizer on Rink amide MBHA resin. All couplings were performed using FastMoc protocols with 10-fold excesses of Fmoc-amino acids for 30 minutes. Fmoc removal was carried out by three treatments with 20% piperidine in DMF for 3 minutes.

All five peptidyl resins were cleaved by treatment with TFA/TIS/water (95:2.5:2.5) for 4 hours and the crude peptides were isolated by ether precipitation. HPLC analyses were performed on dimeric peptides, which were obtained by dissolving small samples of each peptide in water and allowing to fully oxidize (Figure 4). All reduced peptides gave ions of the expected mass by mass spectrometry. Some representative mass spectra are shown in Figures 2 and 3.

Table 1: Peptides prepared in this study.

Peptide	Sequence
1	H-Asn-Ala-Ala-Arg-Asp-Asn-Lys-Lys(Me)-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2
2	H-Asn-Ala-Ala-Arg-Asp-Asn-Lys(Me)-Lys(Me)-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2
3	H-Asn-Ala-Ala-Arg-Asp-Asn-Lys-$Lys(Me)_2$-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2
4	H-Asn-Ala-Ala-Arg-Asp-Asn-$Lys(Me)_2$-$Lys(Me)_2$-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2
5	H-Asn-Ala-Ala-Arg-Asp-Asn-Lys-$Lys(Me)_3$-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2

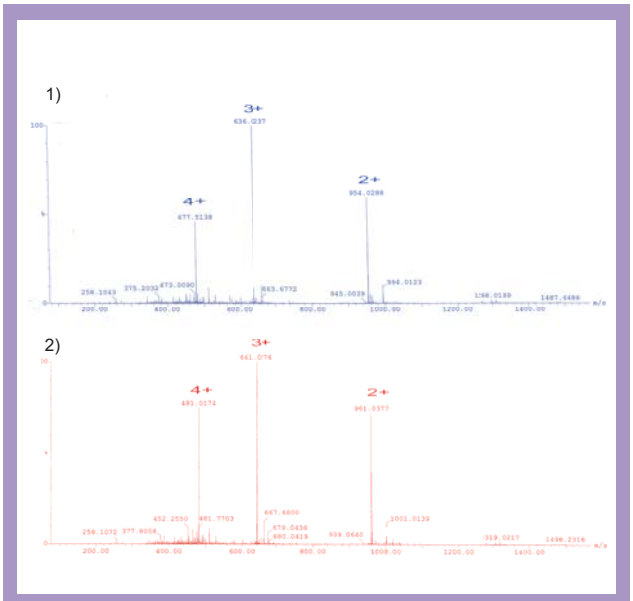


Fig. 2: ES-MS spectra of 1) peptide 1, H-Asn-Ala-Ala-Arg-Asp-Asn-Lys-Lys(Me)-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2 and 2) peptide 3, H-Asn-Ala-Ala-Arg-Asp-Asn-Lys-$Lys(Me)_2$-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2.

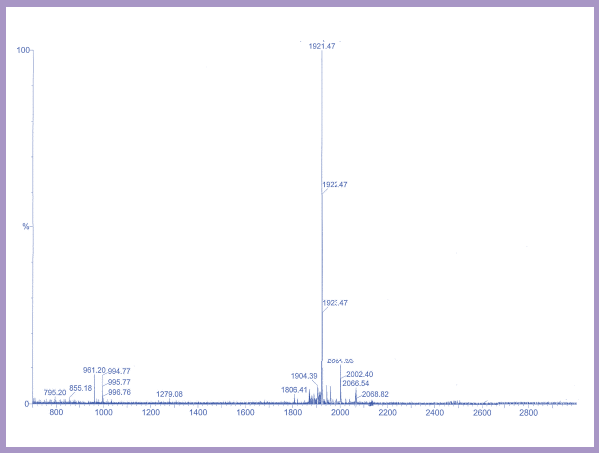


Fig. 3: MALDI-TOF spectrum of peptide 2, H-Asn-Ala-Ala-Arg-Asp-Asn-Lys(Me)-Lys(Me)-Thr-Arg-Ile-Ile-Pro-Arg-His-Cys-NH_2.

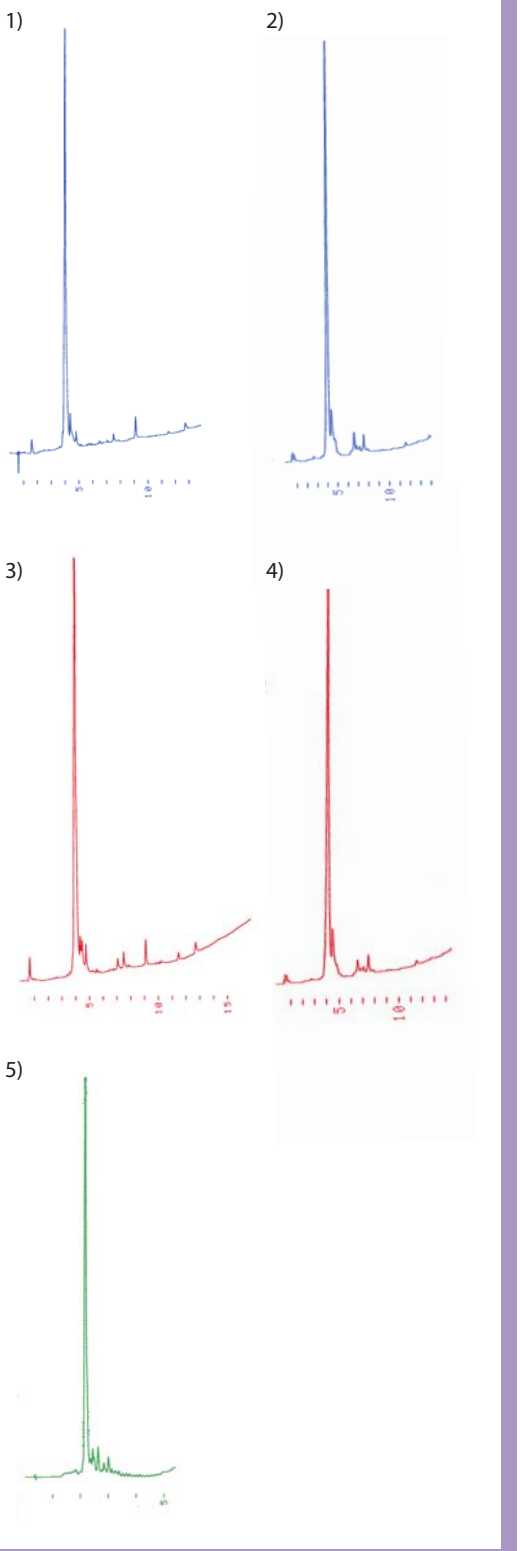


Fig. 4: HPLC profiles of crude peptides 1 - 5. Column: Merck SpeedRod. Buffer A: 0.1 % TFA in water; buffer B: 90/10/0.1 MeCN/water/TFA. Peptides 1-4: Gradient 0% B for 0.2 min, then 0-100% B in 18 min; flow rate: 3 ml/min. Detection: 214 nm. Peptide 5: Gradient 0% B for 0.2 min, then 0-100% B in 7 min; flow rate: 5 ml/min.

Ordering information

04-12-1263	Fmoc-Lys(Me,Boc)-OH	500 mg 1 g
04-12-1269	Fmoc-Lys(Me)$_2$-OH · HCl	1 g 5 g
04-12-1270	Fmoc-Lys(Me)$_3$-OH chloride	500 mg 1 g

Novabiochem methylated arginine derivatives

04-12-1261	Fmoc-Arg(Me,Pbf)-OH	1 g
04-12-1264	Fmoc-ADMA(Pbf)-OH	1 g 5 g

References

1. W. K. Paik, et al. (2007) Trends Biochem. Sci., doi: 10.1016/j.tibs.2007.01.006.
2. X. Tian & J. Fang (2007) Acta Biochim. Biophys. Sinica, **39**, 81.
3. G. Arents, et al. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 10148. (PDB ID: 1HIO).

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