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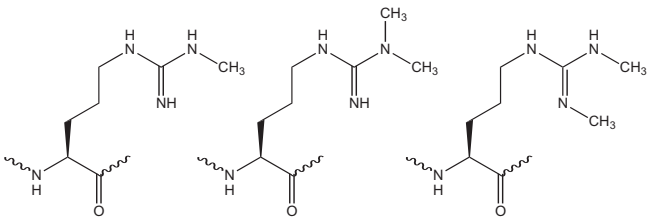
Novabiochem®

innovations 5/06

Synthesis of peptides containing methylated arginine residues



Post-translational methylation of arginine is emerging as an important control mechanism for the regulation of protein expression in eukaryotes [1, 2]. Of the possible combinations of methylated derivatives, mono-methylarginine (Arg(Me)), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) have been found in nature. Methylation is mediated by at least nine different protein arginine-methyl transferases, the most important of which, PRMT1, is responsible for 85% of protein methylation in human cells [3]. PRMTs are classified according to whether they catalyze asymmetric (type-1) or symmetric (type-2) dimethylation [4]. Both classes can catalyze the formation of monomethylarginine. Whilst the exact roles of such modifications are currently poorly understood, they are thought to be involved in RNA processing, transcription regulation, signal transduction, and DNA repair [1, 2, 4]. Until recently, arginine methylation was thought to be irreversible. However, the recent discovery of enzymes capable of converting methylated arginine residues to citrulline have indicated that arginine methylation may play a more important role in cell signaling than previously thought [5].

Research into the role of arginine methylation has until recently been hampered by lack of efficient methods for the synthesis of peptides containing methylated-arginine residues. It is for this reason that Novabiochem® has introduced Fmoc-Arg(Me,Pbf)-OH and Fmoc-ADMA(Pbf)-OH for the incorporation of the most common arginine modifications: Arg(Me) and ADMA. In this innovation, we demonstrate the utility of these derivatives in the synthesis of peptides containing multiple methylated arginine residues.

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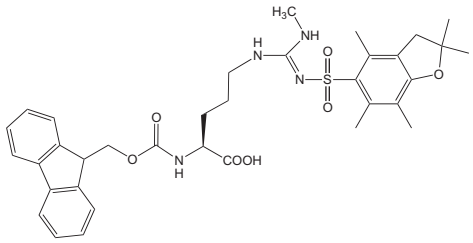
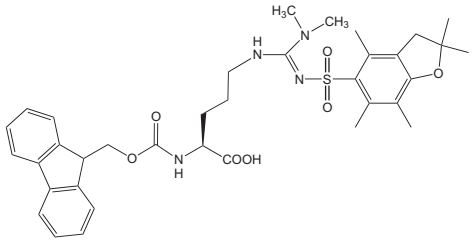
The use of Fmoc-Arg(Me,Pbf)-OH and Fmoc-ADMA(Pbf)-OH

Fmoc-Arg(Me,Pbf)-OH



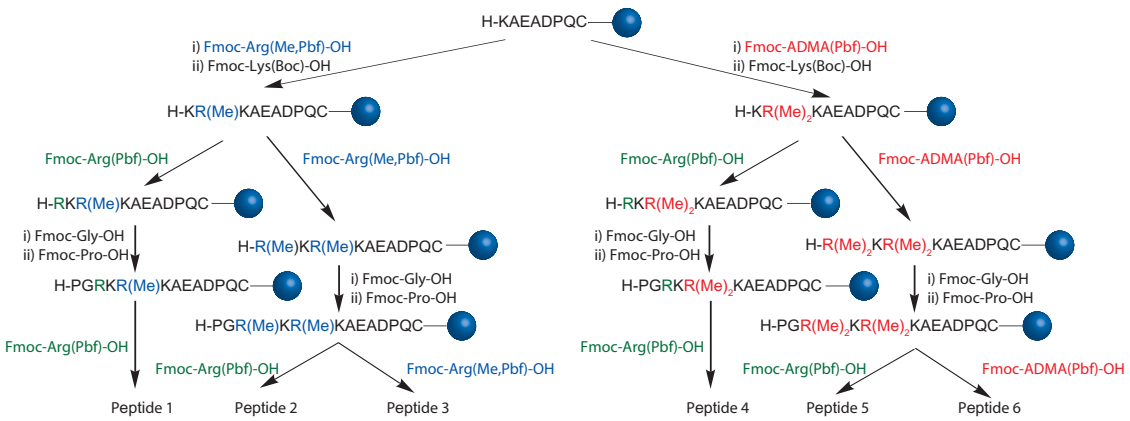
Fmoc-ADMA(Pbf)-OH



Using Fmoc-Arg(Me,Pbf)-OH and Fmoc-ADMA(Pbf)-OH is extremely simple. As they have good solubility in DMF and the reactivity of the guanidino side chain is completely masked by the Pbf group, both can be coupled using standard activation methods such as PyBOP® or TBTU on automated peptide synthesizers. Removal of the Pbf group can be achieved using standard TFA cleavage cocktails in exactly the same manner as the deprotection of Arg(Pbf).

Peptides containing methylated-arginine residues are highly polar, and often elute on RP-HPLC at lower acetonitrile concentrations than the analogous arginine-containing peptides. In order to ensure retention on standard C18 columns, it is advisable to load peptides containing methylated arginines onto the column and initiate elution in buffers containing no acetonitrile.

Fig. 1: Strategy for synthesis of methylated arginine peptides.



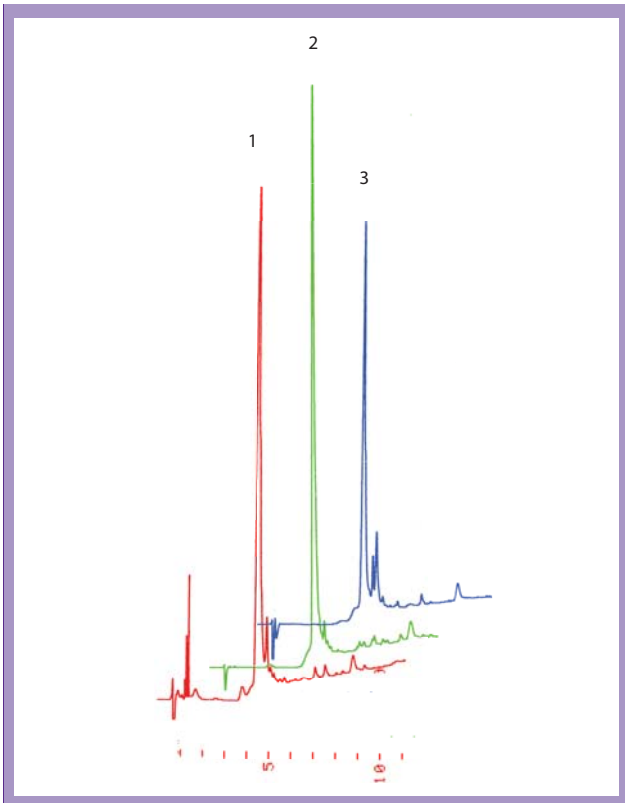
Synthesis of peptides related to methyl-CpG-binding protein

To exemplify the use of Novabiochem®'s derivatives in the synthesis of methylated arginine-containing peptides, the six peptides shown in Table 1 were prepared. The peptides selected are all related to methyl-CpG-binding protein, which is involved with histone deacylase in gene silencing. A C-terminal Cys residue was incorporated in each case to facilitate conjugation to a protein for the purpose of raising antibodies. All syntheses were performed on a NovaSyn Crystal Peptide Synthesizer using NovaSyn® TGR resin. Coupling reactions were carried out for 1 hour using 5-fold excesses of Fmoc-protected amino acids activated with PyBOP®. Fmoc removal was effected by pumping 20% piperidine in DMF through the resin bed until the optical density of the reaction column elute returned to its original value.

The resin was divided during the assembly as shown in Figure 1 to give the six peptides listed in Table 1. All six 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 2). All reduced peptides gave ions of the expected mass on ES-LC (Figure 3).

Table 1: Peptides prepared in this study.

Peptide	Sequence
1	H-Arg-Pro-Gly-Arg-Lys-Arg(Me)-Lys-Ala-Glu-Ala-Asp-Pro-Gln-Cys-NH_2
2	H-Arg-Pro-Gly-Arg(Me)-Lys-Arg(Me)-Lys-Ala-Glu-Ala-Asp-Pro-Gln-Cys-NH_2
3	H-Arg(Me)-Pro-Gly-Arg(Me)-Lys-Arg(Me)-Lys-Ala-Glu-Ala-Asp-Pro-Gln-Cys-NH_2
4	H-Arg-Pro-Gly-Arg-Lys-ADMA-Lys-Ala-Glu-Ala-Asp-Pro-Gln-Cys-NH_2
5	H-Arg-Pro-Gly-ADMA-Lys-ADMA-Lys-Ala-Glu-Ala-Asp-Pro-Gln-Cys-NH_2
6	H-ADMA-Pro-Gly-ADMA-Lys-ADMA-Lys-Ala-Glu-Ala-Asp-Pro-Gln-Cys-NH_2



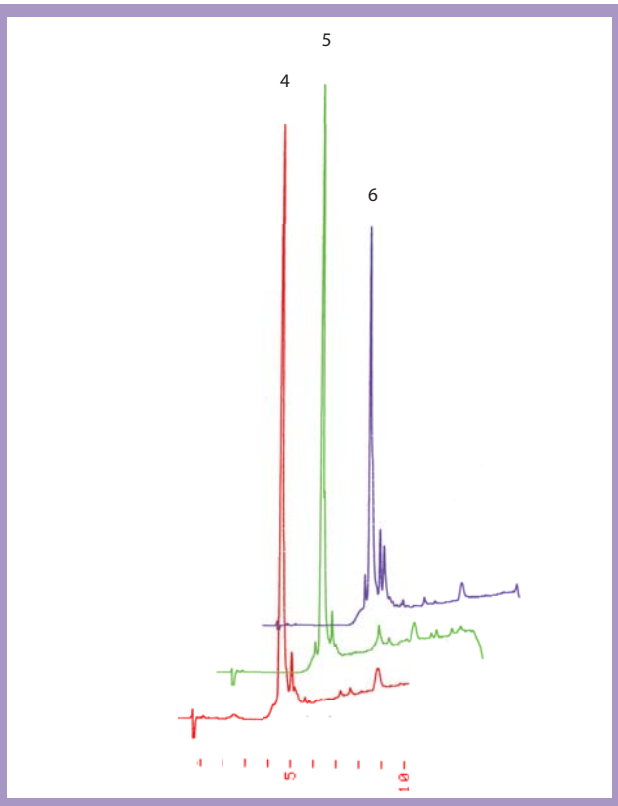
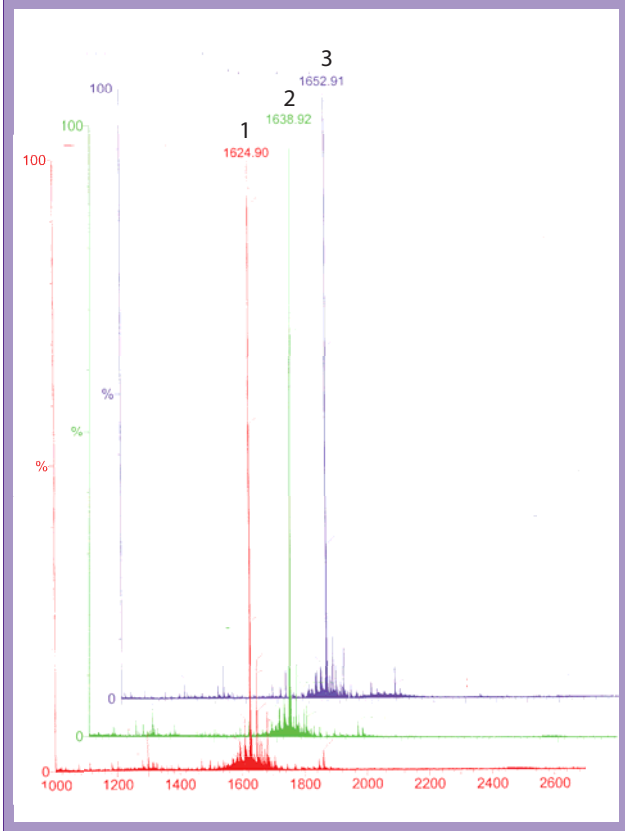


Fig. 2: HPLC profiles of crude peptides 1 - 6. Column: Merck SpeedRod. Buffer A: 0.1 % formic acid in water; buffer B: 90/10/0.1 MeCN/water/formic acid. Gradient 0% B for 2 mins., then 0-50% B in 8 min. Flow rate: 3 ml/min. Detection: 214 nm.



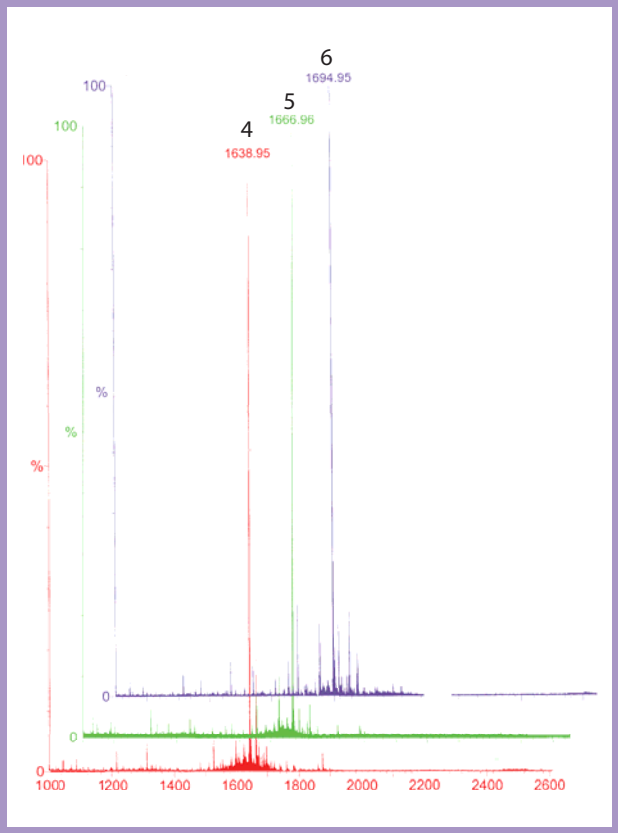


Fig. 3: ES-MS spectra of crude peptides 1-6.

Ordering information

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

Novabiochem®'s methylated lysine derivatives

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

References

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2. M. Bedford & S. Richard (2005) *Mol. Cell.*, **18**, 263.
3. F. Herrmann, et al. (2005) *J. Biol. Chem.*, 280, 38005.
4. J. Wysocka, et al. (2006) *Front. Biosci.*, **11**, 344.
5. R. B. Denman (2005) *BioEssays*, **27**, 242.

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