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ORIGINAL ARTICLE

A convenient preparation of N^{ε}-methyl-L-lysine derivatives and its application to the synthesis of histone tail peptides

Hongfang Chi · Md. Shahidul Islam · Tienabe K. Nsiama · Tamaki Kato · Norikazu Nishino

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Abstract A convenient route is established for the preparation of N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine and N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine as building blocks to be used for the synthesis of methylated peptides. This methodology is based on the use of malonate derivatives and dibromobutane to produce key intermediates, L-2-amino-6-bromohexanoic acid derivatives, which could be modified to the required group at the ε-position. Fmoc-protection is accessible, so these compounds can be used in solution as well as in solid-phase peptide synthesis. Also the peptides containing these methylated lysines have been proved to resist the action of trypsin and lysyl endopeptidase. Thus, this new method could be considered as an improvement of the synthesis of N^{ε}-methyl-L-lysine derivatives.

Keywords L-2-Amino-6-bromohexanoic acid · N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine · N^{α}-Fmoc-N^{ε}- dimethyl-L-lysine

Abbreviations

AcOH	Acetic acid
AMC	7-Amino-4-methylcoumarin
Boc	*tert*-Butyloxycarbonyl
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
Fmoc	9-Fluorenylmethyloxycarbonyl
Fmoc-OSu	*N*-(9-Fluorenylmethoxycarbonyloxy)succinimide
HBTU	*O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HOBt·H_2O	1-Hydroxybenzotriazole hydrate
HPLC	High-performance liquid chromatography
HRMS (FAB)	High-resolution fast atom bombardment mass spectrometry
LCMS	Liquid chromatography mass spectrometry
MCA	4-Methylcoumaryl-7-amide
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography

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H. Chi · Md. S. Islam · T. K. Nsiama · T. Kato (✉) · N. Nishino
Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Wakamatsu, Kitakyushu 808-0196, Japan
e-mail: tmkato@life.kyutech.ac.jp

Introduction

The facile and selective synthesis of N^{ε}-methyl-L-lysine derivatives is important because of their biological and medicinal application. Generally, lysine methylation, which can be found in mono-, di-, or trimethylation state in vivo, is one of the important post-translational modifications on the N-terminal peptide chain of the histone proteins (Fig. 1).

It has been studied that the transition between mono-, di-, and trimethylation may control transcriptional regulation, RNA maturation, DNA repair and genomic imprinting (Grunstein 1998; Martin and Zhang 2005; Mai and Altucci 2009; Gavin and Sharma 2010). For the elucidation of the specific biological function of different state of lysine methylation, the preparation of site-specifically methylated

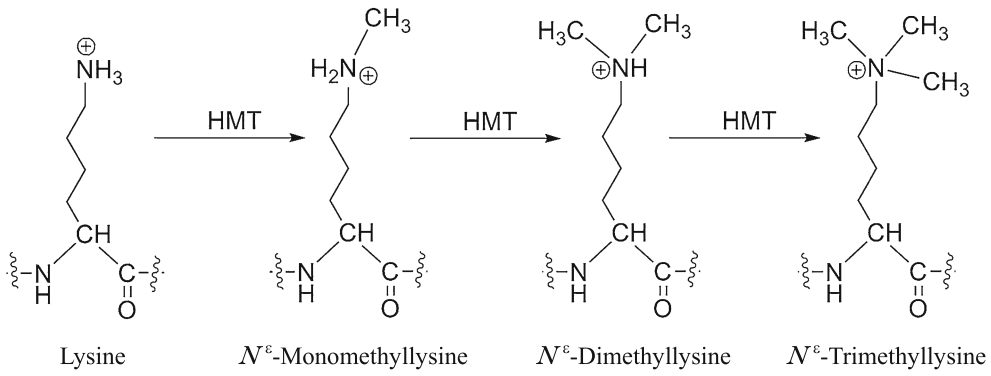
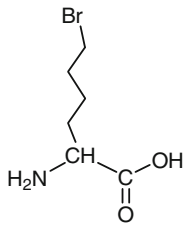


Fig. 1 N^{ε}-Methylation of lysine by histone methyltransferases (HMT)

model to mimic histone called for the synthesis of N^{ε}-methyl-L-lysine derivatives urgently. In recent years, the developments of synthesis provided some methods to prepare N^{α}-methylated amino acids and their derivatives. However, these methods are poor in the preparation of methylated lysine at ε-position, and achievement of selectivity for mono-, di-, and trimethylated lysine (Aurelio et al. 2004; White and Konopelski 2005). But some impressive progresses in the synthetic strategy of N^{ε}-methyl-L-lysine were also succeeded. Benoiton obtained N^{ε}-methyl-L-lysine by the formaldehyde–formic acid methylation of N^{α}-carbobenzoxy-N^{ε}-benzyl-L-lysine (Benoiton 1964). The direct methylation of N^{α}-Fmoc-lysine was also developed by reductive methylation with formaldehyde and sodium cyanoborohydride and quaternization with excess amount of methyl iodide (Huang et al. 2006, 2007). The earlier methods are costly and no large scale synthesis is shown. Herein, we describe a different methodology from the past to prepare N^{ε}-methyl-L-lysine derivatives. In this strategy, protected 2-amino-6-bromohexanoic acid (Ab6, Fig. 2) has been employed. Our group has reported before the use of derivatives of 2-amino-*n*-bromo-alkanoic acid (Abn) as intermediates of functional amino acids (Watanabe et al. 2004a, b, 2005). By reacting mono- and dimethylamine to protected Ab6, useful intermediates are available for N^{ε}-methyl-lysine derivatives such as N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine and N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine. This method does not require expensive reagents to allow the preparation of designed methylated lysine in multi-grams. Thus, the convenient and economical preparation of useful N^{ε}-methyl-L-lysine derivatives has been achieved in large scale. Not only the L-form but also the D-form of N^{ε}-methyl-lysine derivatives could be recovered if necessary. Since the methylation of histone H3 protein is often observed at 4- and 9-positions, H3 (1–12) peptides containing N^{ε}-monomethyl-lysine has been synthesized by standard solid-phase method in Fmoc-chemistry. In addition, the fluorescent peptide (H3, 1–4)-MCAs have also been synthesized to examine the susceptibility to trypsin and lysyl endopeptidase. As a result, we observed that these peptides are hardly hydrolyzed at mono- and dimethylated lysines.

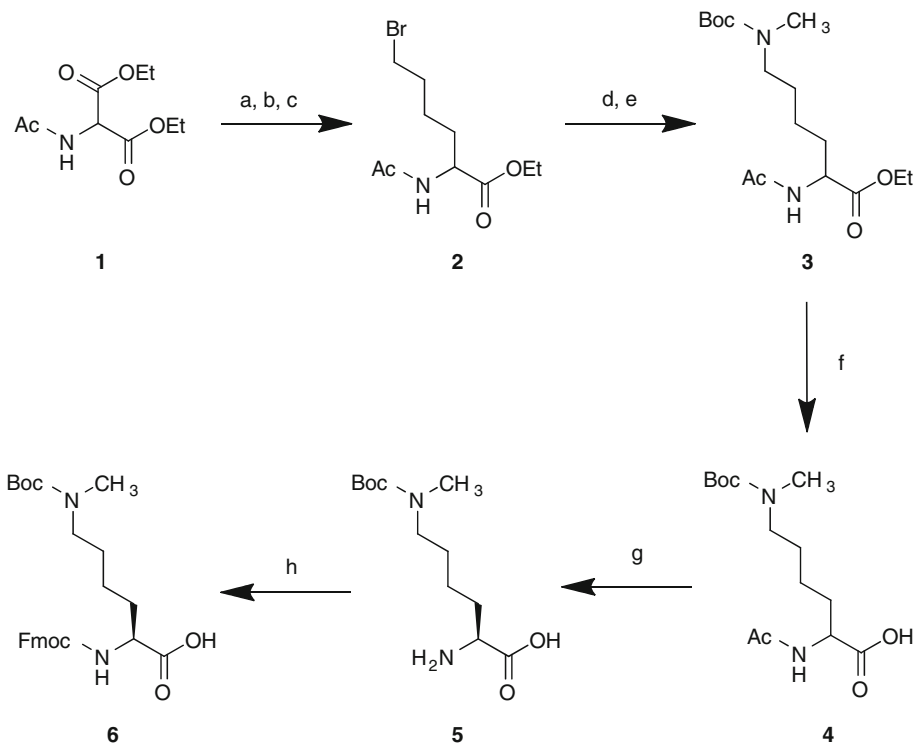
Fig. 2 Structure of 2-amino-6-bromohexanoic acid (Ab6)



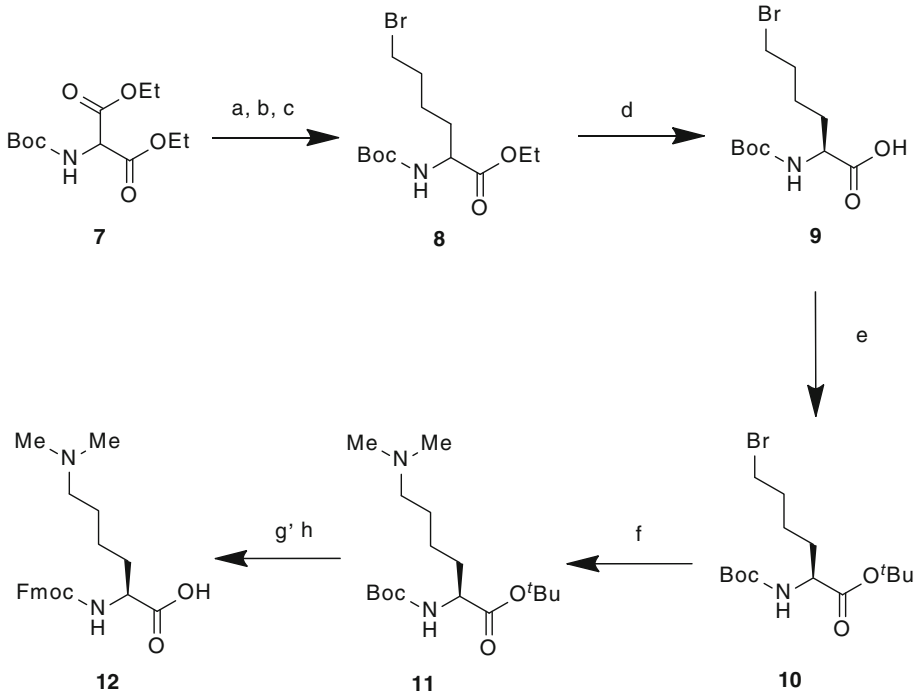
Synthetic routes to N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine and N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine

Since free Ab6 readily cyclizes to afford L-pipecolic acid (Watanabe et al. 2005), we reacted methylamine to Ac-DL-Ab6-OEt to prepare N^{α}-Ac-N^{ε}-methyl-DL-lysine ethyl ester at the first step (Scheme 1). Subsequently, the side chain was protected with Boc-group to N^{α}-Ac-N^{ε}-(Boc, methyl)-DL-lysine ethyl ester (**3**). The racemic mixture of N^{α}-Fmoc-N^{ε}-(Boc, methyl)-DL-lysine (**4**) was subjected to the action of L-aminoacylase to obtain optically pure N^{ε}-(Boc, methyl)-L-lysine (**5**), whose α-amino group was finally protected with Fmoc-group to give a useful derivative (**6**) for peptide synthesis.

On the other hand, since diethyl Boc-aminomalonate is also commercially available, we prepared optically pure Boc-L-Ab6-OH at first by the aid of subtilisin (Watanabe et al. 2005) then fully protected it as *tert*-butyl ester (Scheme 2, **10**). Substitution reaction of **10** with dimethylamine gave **11**, whose two *tert*-butyl groups were helpful to extract **11** into ethyl acetate and in purification. Finally, protecting groups of **11** were removed and reacted with Fmoc-OSu to give **12**. When we tried to synthesis of **12** by the same procedure as described for **6**, the water soluble property of N^{α}-Ac-N^{ε}-dimethyl-DL-lysine reduced the yield drastically. In addition, the isolation of the desired optically pure product required tedious ion exchange procedure.



Scheme 1 Synthesis of N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine (**6**). Reagents and conditions: (*a*) (i) ethanol, sodium ethoxide, reflux, 30 min; (ii) 1, 4-dibromobutane, reflux, 6 h; (*b*) NaOH aq, 0 °C, 4 h; (*c*) toluene, reflux, 4 h; (*d*) KI, methylamine/methanol, 30 min; (*e*) dioxane, Boc_2O, DIEA; (*f*) NaOH aq., 2 h; (*g*) L-aminoacylase, $CoCl_2{\cdot}6H_2O$, pH 7–8, 38 °C; (h) Na_2CO_3 aq., dioxane, Fmoc-OSu, pH 10



Scheme 2 Synthesis of N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine (**12**). Reagents and conditions: (*a*) (i) ethanol, sodium ethoxide, reflux, 30 min; (ii) 1,4-dibromobutane, reflux, 6 h; (*b*) NaOH aq., 0 °C, 4 h; (*c*) toluene, reflux, 6 h; (*d*) subtilisin, DMF/H_2O (3/1, v/v), pH 7–8, 38 °C, 3 h; (*e*) *tert*-butyl alcohol, Boc_2O, DMAP; (*f*) DMF, KI, dimethylamine hydrochloride; (*g*) TFA; (*h*) Na_2CO_3 aq., dioxane, Fmoc-OSu, pH 10

Materials and methods

Unless otherwise noted, all solvents and reagents were reagent grade obtained from WAKO, Japan. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh) eluting with solvents as indicated. All compounds were routinely checked by thin layer chromatography (TLC) and/or high-performance liquid chromatography (HPLC). TLC was performed on aluminum-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}) with spots visualized by UV light or charring. Analytical HPLC was performed on a Hitachi L-7100 instrument equipped with a

chromolith performance RP-18e column (4.6 × 100 mm, Merck). The mobile phases used were A: H_2O with 0.1 % TFA, B: CH_3CN with 0.1 % TFA using a solvent gradient of A–B over 15 min with a flow rate of 2 mL/min, with detection at 220 nm. HRMS (FAB) measurements were performed on a JEOL JMS-SX 102A instrument.

Preparation of N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine

*Ac-DL-Ab6-OEt (**2** in Scheme 1)*

Metallic sodium (2.23 g, 95 mmol) was dissolved in dehydrated ethanol (100 mL) and diethyl acetamidomalonate **1** (21.7 g, 100 mmol) was added to the solution. The mixture was refluxed for 30 min for the complete dissolution and then 1,4-dibromobutane (64.2 g, 300 mmol) was added. After 3 h reflux, the reaction mixture was cooled on an ice bath and hydrolysis was carried out by the addition of 1 M NaOH (50 mL). After selective hydrolysis, ethanol was evaporated and the unreacted 1,4-dibromobutane was removed by extraction with diethyl ether under alkaline condition. Then the desired half ester was extracted with ethyl acetate (pH about 3 with solid citric acid) and washed with brine, dried over $MgSO_4$ and evaporated to obtain white solid of monoester monoacid (19.6 g, 61 mmol). The solution of the half ester in toluene (100 mL) was refluxed for 5 h. The removal of solvent gave an oil, which was purified by silica gel column chromatography using hexane/ethyl acetate (2/1, v/v) to give an oily **2** (15.5 g, 55 mmol, 55 %). LCMS *m/z* found: 280, calcd. for $[M + H]^+$ $C_{10}H_{19}BrNO_3$: 280.

*N^{α}-Ac-N^{ε}-(Boc, methyl)-DL-lysine ethyl ester (**3**)*

To a solution of **2** (14.1 g, 50 mmol) in DMF (50 mL), KI (8.3 g, 50 mmol) was dissolved and then added methylamine in methanol (9 M, 55 mL, 500 mmol) and the mixture was allowed to stand for 30 min at room temperature. Solvents and excess methylamine were removed by evaporation to give a syrup, which was dissolved in dioxane (100 mL). To this solution, DIEA (13.1 mL, 75 mmol) and di-*tert*-butyl dicarbonate (Boc_2O) (13.1 g, 60 mmol) were added at room temperature. After overnight reaction the desired compound **3** was isolated and purified with a silica gel column using hexane/ethyl acetate (1/4, v/v). Colorless oil was obtained as **3** (15.8 g, 48 mmol, 96 %). LCMS *m/z* found: 331.2, calcd. for $[M + H]^+$ $C_{16}H_{31}N_2O_5$: 331.2.

*N^{α}-Ac-N^{ε}-(Boc, methyl)-DL-lysine (**4**)*

The ethyl ester **3** (15.8 g, 48 mmol) was dissolved in ethanol (50 mL) and flashed once to remove ethyl acetate from the oily starting material. To the solution of the ester in ethanol (100 mL), 2 M NaOH (36 mL) was added. After 2 h, reaction mixture was acidified with AcOH (5.72 mL, 100 mmol) and concentrated to give an oil. The acid was extracted into ethyl acetate at pH 3–4 using solid citric acid. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to get compound **4** (14.5 g, 48 mmol, 100 %). LCMS *m/z* found: 303.1, calcd. for $[M + H]^+$ $C_{14}H_{27}N_2O_5$: 303.1.

*N^{ε}-(Boc, methyl)-L-lysine (**5**)*

The racemic acid **4** (14.5 g, 48 mmol) was dissolved in water (240 mL) and pH of the solution was adjusted to 7 by the addition of 1 M NaOH at 38 °C. To this neutral solution, $CoCl_2 \times 6H_2O$ (57 mg) and *Aspergillus genus* L-aminoacylase (1.20 g, TCI) were added and incubated overnight. The pH of the solution was maintained neutral by the addition of 1 M NaOH. The reaction mixture was acidified with dilute hydrochloric acid and unreacted N^{α}-Ac-N^{ε}-(Boc, methyl)-D-lysine was extracted into ethyl acetate.

*N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine (**6**)*

To the aqueous solution containing **5**, 10 % Na_2CO_3 (15.3 g, 144 mmol) and dioxane (200 mL) were added and allowed to react with Fmoc-OSu at pH around 10 at room temperature overnight. After evaporation of dioxane, the reaction mixture was shaken with ether (100 mL) and acidified with 1 M hydrochloric acid to pH 2–3. The desired product **6** was extracted into ethyl acetate and purified with a silica gel column using chloroform/methanol (49/1, v/v). The yield of **6** was 9.3 g (19.2 mmol, 80 %). HRMS (FAB) *m/z* found: 483. 2530, calcd. for $[M + H]^+$ $C_{27}H_{35}N_2O_6$; 483.2495.

Preparation of N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine

*Boc-DL-Ab6-OEt (**8**)*

Compound **8** was prepared by the same procedure as described for compound **2** except that diethyl Boc-aminomalonate **7** (21.1 g, 100 mmol) was employed as starting material. Compound **8** could be obtained in 40–60 % yield. LCMS *m/z* found: 338.1, calcd. for $[M + H]^+$ $C_{13}H_{25}BrNO_4$: 338.1.

*Boc-L-Ab6-OH (**9**)*

Compound **8** (4.39 g, 13 mmol) was suspended on a mixed solvent of DMF (39 mL) and water (13 mL) (3/1, v/v) at 38 °C using a mechanical stirrer. The pH was adjusted at 7–8 by adding 1 M ammonia. Then subtilisin Carlsberg

from *Bacillus licheniformis* (13 mg, 1 mg enzyme per mmol of substrate, SIGMA) was added. The reaction was completed within 3 h and then the mixture was concentrated. The unreacted Boc-D-Ab6-OEt was extracted with diethyl ether under alkaline condition. The desired compound **4** was extracted with ethyl acetate at pH 3–4 by acidification with solid citric acid. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to dryness. The yield of **9** was 1.95 g (6.30 mmol, 48 %). LCMS *m/z* found: 310, calcd. for $[M + H]^+$ $C_{11}H_{21}BrNO_4$: 310.

*Boc-L-Ab6-O^{t}Bu (**10**)*

Compound **9** (1.12 g, 3.62 mmol) was dissolved in 10 mL of *t*-butyl alcohol. To this solution, Boc_2O (1.11 g, 5.10 mmol) and DMAP (0.14 g, 1.10 mmol) were added. After 1 h reaction at room temperature, the solution was concentrated and the residues were passed through a silica gel column using ethyl acetate/hexane (1/6, v/v). Colorless oil was obtained as **10** (1.1 g, 3.0 mmol, 66 %). LCMS *m/z* found: 366.1, calcd. for $[M + H]^+$ $C_{15}H_{29}BrNO_4$: 366.1.

*Boc-L-Lys(Me)$_2$-O^{t}Bu (**11**)*

Compound **10** (1.1 g, 3.0 mmol) was dissolved in 5 mL of DMF. To this solution, KI (0.48 g, 2.40 mmol), dimethylamine hydrochloride $[(CH_3)_2NH\times HCl]$ (0.5 g, 6 mmol) and triethylamine (0.3 mL, 6 mmol) were added and stirred overnight. After completion of the reaction, DMF was evaporated and the residue was dissolved in ethyl acetate. The solution was washed with saturated sodium carbonate and then dried over anhydrous Na_2CO_3, filtered and evaporated to get the oily **11** (0.8 g, 2.4 mmol, 80 %). LCMS *m/z* found: 331.2, calcd. for $[M + H]^+$ $C_{17}H_{35}N_2O_4$: 331.2.

*N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine (**12**)*

Compound **11** (0.8 g, 2.4 mmol) was dissolved in TFA (2.4 mL) at 0 °C and kept for 3 h at room temperature. After evaporation of TFA, the residue was crystallized with the mixture of ether/petroleum ether (1/4) as TFA salt. It was then dissolved in the 10 % Na_2CO_3 (1.21 g, 11.4 mmol) and dioxane (14 mL) and allowed to react with Fmoc-OSu at pH around 10 with stirring at room temperature overnight. After evaporation of dioxane, the reaction mixture was washed with ether (10 mL) and acidified with 1 M hydrochloric acid to adjust pH about 3. The purification with a silica gel column using chloroform/methanol (19/1, v/v) as eluent, gave compound **12** (0.75 g, 1.15 mmol, 60 %). HRMS (FAB) *m/z* found: 397.2049, calcd. for $[M + H]^+$ $C_{23}H_{28}N_2O_4$: 397.2127.

Table 1 Mass spectrum data and HPLC purities for all synthetic peptides

Histone tail peptides	MS $[M + H]^+$	Purity (%)
ARTK(Me)QTARK(Me)STG (H3 1–12)	1,332.8 (calcd. 1,332.8)	98
Ac-ARTK(Me)-MCA (H3 1–4)	688.4 (calcd. 688.4)	100
Ac-ARTK(Me)$_2$-MCA (H3 1–4)	702.4 (calcd. 702.4)	100

Peptides synthesis

Automated solid-phase peptide synthesis

As an example of the use of N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine in the automated solid-phase peptide synthesis, we synthesized H3 (1–12) peptide with monomethylation at 4- and 9-positions. The protected peptide fragments were assembled on Wang resin (substitution 0.7 mmol/g) by peptide synthesizer (Applied Biosystems 433A) using Fmoc/piperidine strategy in FastMoc Chemistry at 0.25 mmol scale. HBTU and $HOBt\cdot H_2O$ were used as activating agents. The cleavage and removal of protecting groups were carried out by the treatment with TFA containing phenol (5 %), thioanisole (5 %), ethanedithiol (2.5 %) and water (5 %). The peptides were precipitated by *tert*-butyl methyl ether. The methylated peptide ARTK(Me)QTARK(Me)STG was purified by Sephadex G-25 with 10 % AcOH and HPLC, analyzed by LCMS (Table 1). HPLC profiles and LCMS data are provided in Supplementary data.

Fluorescent peptide-MCAs

To a chilled solution of N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine (1.9 g, 4 mmol) in DCM (10 mL), DCC (0.42 g, 2 mmol) was added. After 1 h on ice bath, AMC (0.36 g, 2 mmol) was added to the mixture. The suspension was stirred at room temperature overnight, then the product was filtered and washed with methanol. N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine-MCA was obtained as crystalline solid (2.3 g, 90 %). N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine-MCA was synthesized as described in the same manner above. Yield, 2.0 g, 92 %.

N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine-MCA (160 mg, 0.25 mmol) was dissolved in 20 % piperidine/DMF (2 mL) at room temperature. After 30 min, the reagent and solvent were removed by evaporation. The residues were extracted into ethyl acetate, the solution being washed with sat. Na_2CO_3 and dried over anhydrous Na_2CO_3. The filtrate was concentrated to yield N^{ε}-(Boc, methyl)-L-lysine-MCA as free amine (104 mg, 0.25 mmol, 100 %). N^{α}-Fmoc-N^{ε}-

dimethyl-L-lysine-MCA was treated with 20 % piperidine in DMF as described above. 82 mg (0.25 mmol, 100 %) of free amine N^{ε}-dimethyl-L-lysine-MCA was obtained.

The protected peptide Ac-AR(Pmc)T(^{t}Bu)-OH was assembled on Barlos resin (substitution 0.6 mmol/g) by manual solid-phase synthesis using Fmoc/piperidine strategy. HBTU and HOBt·H_2O were used for condensation. After capping the N-terminal with acetic anhydride, the protected peptide was cleaved from the resin in DCM containing TFE and AcOH for 2 h. The protected peptide (177 mg, 0.25 mmol) was condensed with N^{ε}-(Boc, methyl)-L-lysine-MCA (104 mg, 0.25 mmol) and N^{ε}-dimethyl-L-lysine-MCA (82 mg, 0.25 mmol) by the aid of HBTU/HOBt method, respectively. The protected peptide-MCAs were treated with deprotection cocktail by the same procedure as described in automated synthesis. The HPLC purified peptide-MCAs (Table 1) were analyzed by HRMS (FAB). HPLC profiles and HRMS (FAB) data are provided in Supplementary data.

Results and discussion

In the present synthetic routes, the key intermediates are Ab6 derivatives, which are conveniently prepared from diethyl acetamido- or Boc-aminomalonate and dibromobutane by a few steps. Since the N^{ε}-modification with Boc-group is provided enough hydrophobicity for the extraction of the intermediate such as N^{α}-Ac-N^{ε}-(Boc, methyl)-DL-lysine (**4**), acetamide malonate was better choice. However, dimethyl modification afforded hydrophilic property to the intermediate, therefore, resulted in difficulty in isolation. To clean this serious problem, we employed diethyl Boc-aminomalonate (**7**). In both routes (Schemes 1, 2), the L-amino acid derivatives were obtained in high optical purity by enzymatic resolution with L-aminoacylase and subtilisin, respectively, for well-established in high optical purity.

For the monomethylation, the synthesis started with commercially available diethyl acetamidomalonate **1**, the reaction of **2** with methyl amine in DMF in the presence of KI was followed by Boc-protection without further purification. After saponification of **3**, racemic **4** was subjected to the resolution by *Aspergillus genus* L-aminoacylase (TCI). The N^{α}-Ac-N^{ε}-(Boc, methyl)-D-lysine could be recovered by extraction into ethyl acetate. The free amino acid N^{ε}-(Boc, methyl)-L-lysine in the aqueous layer was reacted with Fmoc-OSu in the presence of Na_2CO_3. The desired compound N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine was obtained in good yield.

By using dimethylamine as methylation agent, N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine has been produced starting with diethyl Boc-aminomalonate **7**. The key intermediate in Scheme 2 is compound **11**, which has two *tert*-butyl groups and increased in hydrophobicity. Actually this compound was easily extracted into ethyl acetate for isolation. Since Boc-group cannot be cleaved by L-aminoacylase, the enzymatic resolution was carried out by the enantio-specific hydrolysis of ester bond by subtilisin.

Two peptide-MCAs corresponding to the H3 (1–4) sequence were synthesized. Upon the action of trypsin and lysyl endopeptidase, neither Ac-ARTK(Me)-MCA nor Ac-ARTK(Me)$_2$-MCA was hydrolyzed. This fact suggests the drastic change in susceptibility by methylation toward these enzymes.

Conclusion

In summary, the convenient and economical routes have been developed for the synthesis of N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine and N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine to be used as building blocks for the synthesis of methylated peptides. N^{ε}-Methyllysine residues have been efficiently incorporated to the H3 (1–12) peptide by automated solid-phase peptide synthesis. N^{α}-Fmoc-N^{ε}-(Boc, methyl)-L-lysine and N^{α}-Fmoc-N^{ε}-dimethyl-L-lysine are condensed with 7-amino-4-methylcoumarin to prepare fluorescent peptide-MCAs, which are used to examine the susceptibilities to trypsin and lysyl endopeptidase. They showed no activity to mono- and dimethylated lysine peptides.

Conflict of interest The authors declare that they have no conflict of interest.

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