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Histone Methyltransferase

Selective Reagent for Detection of *N*-ε-Monomethylation of a Peptide Lysine Residue through S_NAr Reaction

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Abstract: Methylations of specific lysine residues of histone proteins are catalyzed by histone methyltransferases (HMTs) and play key roles in the epigenetic control of gene expression. Several methods to detect *N*-ε-methylation of the lysine residue have been established in order to evaluate the activity of HMTs, to develop inhibitors, and to identify substrates. However, they mostly employ specific antibodies or enzymes such as peptidases, and their reliability and reproducibility often depend on the quality of the protein reagents and the reaction conditions. Here, we describe a convenient method to detect *N*-ε-monomethylation of the lysine residue through a simple chemical reaction. We focused on nucleophilic aromatic substitution reaction (S_NAr reaction) between an aromatic electrophile and a primary or monomethylated amino group. Screening of various electrophiles indicated that 4-fluoro-2-nitroacetophenone (**1g**) has high selectivity for the *N*-ε-monomethylated amino group of lysine. Furthermore, the reaction products of **1g** with lysine and *N*-ε-monomethylated lysine, **5g** and **6g**, respectively, show different absorption spectra, that is, the absorbance at 350 nm of **6g** is 13 times larger than that of **5g**. We show that these characteristic properties of **1g** can be utilized for the selective detection of the methylation state of lysine residues in HMT substrate peptides, and for an assay of HMT activity.

Introduction

Reversible methylations of specific lysine residues of histone proteins are mediated by "writer" histone lysine methyltransferases (HMTs) and "eraser" demethylases such as Lys-specific demethylases (LSDs), and serve to regulate gene transcription.[1–4] However, the substrates of HMTs are not limited to histone proteins.[5] For example, G9a methylates not only H3K9 and H3K27, but also tumor suppressor p53.[6] Moreover, Set7/9 methylates p53[7] and estrogen receptor (ER) α,[8] one of the nuclear hormone receptors. Hence, methylation-modifying enzymes could play roles in various diseases involving these substrate proteins (such as breast cancer in the case of ER).[9,10] Development of selective inhibitors for each HMT has been attempted[11–14] in order to elucidate in detail the physiological functions of HMTs and also for therapeutic purposes. Effective assay systems for HMT activity are required to assist the discovery and development of inhibitors, and several methods have been established.[15] For example, an enzymatic reaction using *S*-adenosylmethionine (SAM) with an *S*-[^{3}H]-methyl group is one approach,[16] and antibody-based methods such as the AlphaLISA system have also been applied.[17,18] However, the use of radioisotope-labelled compounds or antibodies is relatively expensive and inconvenient. Other methods that involve coupling to other enzymes, such as *S*-adenosyl-homocysteine (SAH) hydrolase,[19] SAH nucleosidase[20,21] and peptidase,[22] have been reported, though their reliability and reproducibility often depend on the quality of the protein reagents and the reaction conditions. False positives owing to inhibition of the coexisting coupled enzyme are also a concern.

Therefore, there is still need for development of a simple, inexpensive method for assaying HMT activity. We considered that it would be feasible to utilize a chemical reaction to distinguish the functional groups of substrate and product, as has been reported for other purposes. For example, in the case of serine kinase, β elimination of phosphorylated serine under basic conditions affords dehydroalanine, which can react with a thiolate compound for selective sensing.[23–25] For an epigenetic modification other than methylation, that is acetylation of the lysine residue, the difference between amino and acetamido groups in the substrate tolerance of peptidase[26] or in chemical reactivity[27] has been utilized. The difference in chemical properties between the primary amino group of the lysine residue and the methylated amino group may be small compared to those in the cases of phosphorylation and acetylation. Nevertheless, in this work, we found a selective reaction that can distinguish the methylation state of a peptide lysine residue, and we show that it can be used to construct an assay system for HMT activity.

Results and Discussion

Several chemical reagents that label amino groups have been reported. For example, trinitrobenzenesulfonic acid (TNBS) re-

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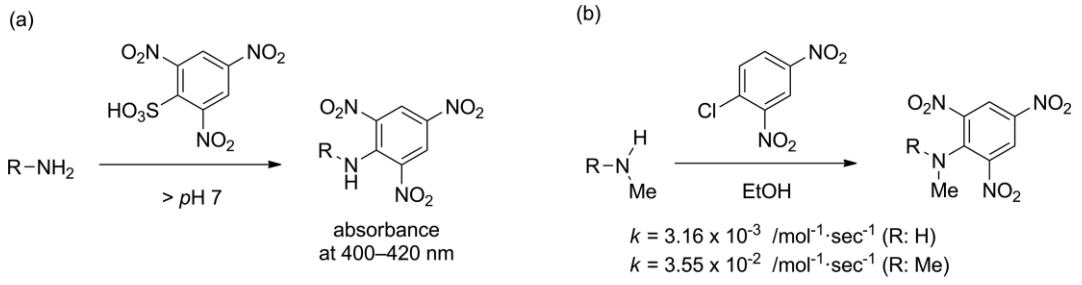


Figure 1. S_NAr reactions and reactivities of amines with aromatic electrophiles, (a) TNBS and (b) 1-chloro-2,4-dinitrobenzene.

acts with an amino group through nucleophilic aromatic substitution (S_NAr), yielding a product with strong absorbance at around 400–420 nm (Figure 1a).[28,29] This reagent reacts selectively with primary amino groups such as the N-terminal amino group or ε-amino group of lysine residues in peptides, while the secondary amino group of the proline residue is not labeled.[30,31] Interestingly, another electrophile that undergoes S_NAr reaction with amino groups, 1-chloro-2,4-dinitrobenzene, is reported to react with secondary amino groups 10-fold faster than with primary amino groups (Figure 1b).[32]

However, there are few reports on the selectivity in S_NAr reactions between primary and secondary amino groups,[32–34] and the effects of substituents and leaving groups of electrophiles have not been well studied. Therefore, we set out to find a suitable electrophile for distinguishing primary and *N*-ε-monomethylated lysines in order to construct an assay system for the activity of HMTs, especially monomethyltransferases such as Set7/9. First, the reactivities of various aromatic electrophiles **1a**–**g** and **2a** containing fluoride or sulfonate as the leaving group were examined (Figure 2).

For the evaluation of **1a**–**g** and **2a** as candidates to distinguish the methylation state of the lysine residue, we prepared lysine and *N*-ε-monomethylated lysine with a Cbz-protected α-amino group, **3** and **4**, as well as their reaction products **5** and **6** (see the Supporting Information, Scheme S1). The S_NAr reaction of each electrophile with **3** or **4** was monitored by recording the ^{1}H-NMR spectra in a mixture of [D_3]acetonitrile and deuterated water in the presence of K_2CO_3, and the initial rate with **3** (v_1) or **4** (v_2) was determined (Table 1).

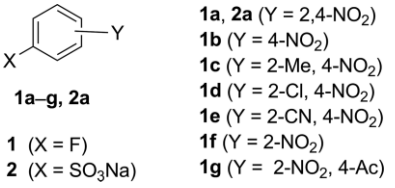
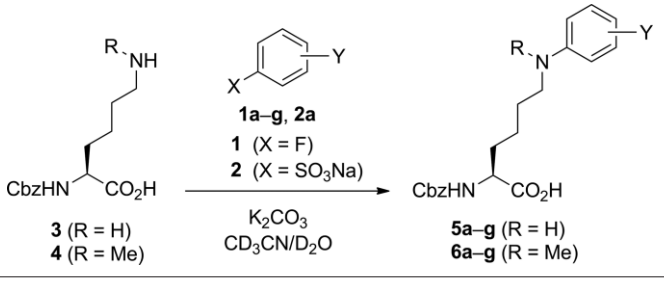


Figure 2. Structures of aromatic electrophiles **1a**–**g** and **2a**.

The electrophile **2a** with a sulfonate group as the leaving group reacted with lysine **3** faster than with *N*-ε-monomethyllysine **4**. In contrast, the electrophiles **1a**–**g** with a fluorine group as the leaving group reacted preferentially with **4**, except for **1c**, which reacted almost equally with **3** and **4**. The reaction rates and ratios (v_2/v_1) appear to be influenced by the steric effect of substituent groups and the *N*-methyl group, as well as the nucleophilicity of the amino group. Thus, the bulky leaving group of **2a**, sulfonate, suppresses the reaction with the *N*-monomethylamino group compared to that with the primary amino group. On the other hand, compounds **1** with the smaller fluoride group as the leaving group would show less

Table 1. Initial reaction rates of S_NAr reaction of lysine moieties **3** and **4** with various electrophiles.



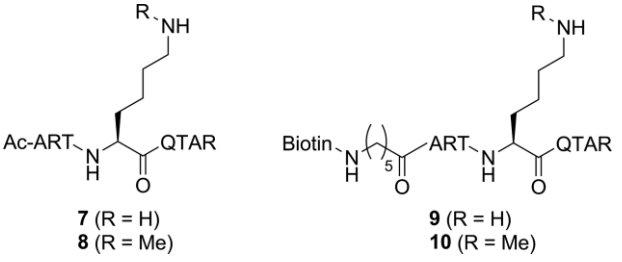
Electrophile	Substituent (Y)	Temp. [°C]	Reaction rate with **3** (v_1)[a]	Reaction rate with **4** (v_2)[a]	$V_2/V1$
1a	2,4-$(NO_2)_2$	20	5.0×10^{0}	3.8×10^{1}	7.5
1b	4-NO_2	60	2.0×10^{-3}	1.8×10^{-2}	9.0
1c	2-CH_3, 4-NO_2	60	3.7×10^{-3}	2.8×10^{-3}	0.77
1d	2-Cl, 4-NO_2	20	2.2×10^{-2}	4.4×10^{-2}	2.0
1e	2-CN, 4-NO_2	20	1.3×10^{-1}	1.5×10^{0}	12
1f	2-NO_2	60	1.2×10^{-2}	4.1×10^{-2}	3.4
1g	2-NO_2, 4-Ac	20	1.9×10^{-1}	3.2×10^{0}	17
2a	2,4-$(NO_2)_2$	60	9.8×10^{-2}	1.3×10^{-3}	0.013

[a] v_1 and v_2 indicate the initial rates [mM min^{-1}] of the S_NAr reaction of each electrophile with **3** or **4**, respectively.

steric hindrance, so that the higher nucleophilicity of the *N*-monomethylamino group would dominate the reactivity, resulting in larger values of the ratio (v_2/v_1). In compounds with one electron-withdrawing group, **1b** (4-NO_2), **1c** (2-CH_3, 4-NO_2), and **1f** (2-NO_2), the reaction rates with **3** or **4** were slow even at 60 °C, though **1b** showed moderate selectivity with a ratio (v_2/v_1) of 9.0. Compounds with two electron-withdrawing groups reacted faster, and among them, **1a** [2,4-$(NO_2)_2$], **1e** (2-CN, 4-NO_2), and **1g** (2-NO_2, 4-Ac) showed relatively high selectivity for *N*-ε-monomethyllysine **4**, with ratios (v_2/v_1) of 7.5, 12, and 17, respectively. However, **1d** (2-Cl, 4-NO_2) showed lower selectivity with a ratio (v_2/v_1) of 2.0.

Three electrophiles, **1a**, **1e**, and **1g**, exhibited relatively high selectivity for *N*-ε-monomethyllysine **4** with a sufficiently fast reaction rate. If the reaction products possessed specific absorption, like TNBS, this could be utilized for selective detection. Accordingly, we measured the absorption spectra of the S_NAr reaction products with **3** or **4** (Figure 3). In every pair examined, a bathochromic shift of the absorption maximum wavelength of the product of *N*-ε-monomethyllysine, **6**, was observed, compared with that of the corresponding nonmethylated product **5**. Focusing on the difference in absorbance at specific wavelengths, the difference at 350 nm between **5g** and **6g** was especially large, that is, the absorbance was 13-fold larger for **6g**. Thus, on the basis of the 17-fold faster reaction rate and 13-fold larger absorption at 350 nm of the reaction product for *N*-ε-monomethyllysine, **1g** was considered as a suitable electrophile for our purpose.

Next, we examined whether **1g** could be applied to monitor the methylation state of the lysine residue of a substrate peptide. For this study, histone 3 peptide (residues 1–8) **7**, whose N-terminal amino group is capped by an acetyl group, was prepared, together with its monomethylated derivative, **8** (Figure 4). Compound **1g** was added to solutions containing these peptides in various ratios, and the absorption spectrum of each solution was measured after 2 h to follow the S_NAr reaction (Figure 5). As expected, the absorbance around 300–370 nm increased with increasing ratio of monomethylated peptide **8**. The absorbance at 350 nm, where unreacted **1g** showed negligible absorbance (Figure S1), showed a good linear relationship



7 (R = H)
8 (R = Me)

9 (R = H)
10 (R = Me)

Figure 4. Structures of substrate peptides **7** and **8**, and their biotin-conjugated derivatives **9** and **10**.

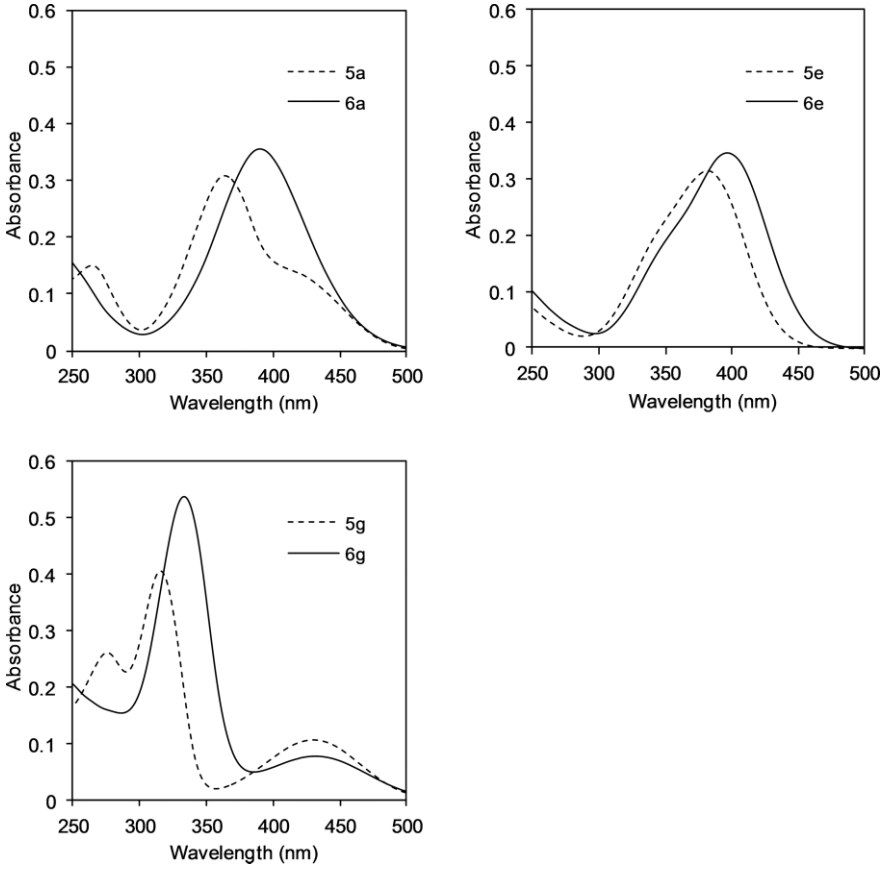


Figure 3. Absorption spectra of the products of the S_NAr reaction in carbonate buffer (pH 10).

with the content ratio of **8** (R^2 = 0.997), indicating that **1g** is able to monitor the methylation state of histone peptide (Figure 6).

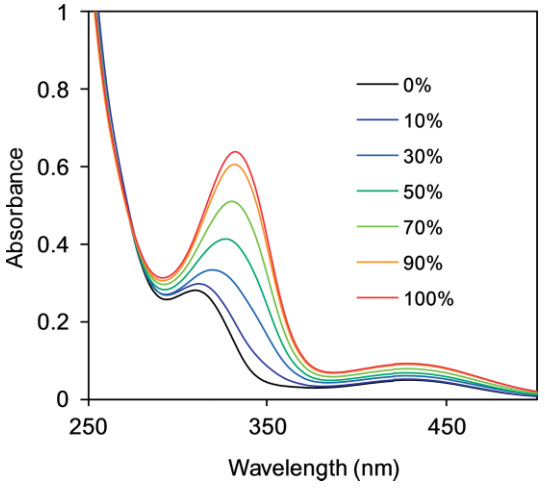


Figure 5. Absorption spectra of solutions of **7** and **8** at various ratios after S_NAr reaction with **1g** in 0.1 M HCl aq. Total concentration of **7** and **8** was 150 μM. Percentages indicate the ratio of monomethylated peptide **8**.

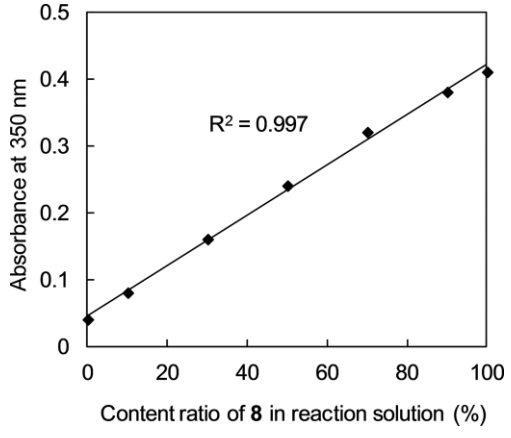


Figure 6. Relationship between the absorbance at 350 nm of solutions of **7** and **8** at various ratios after S_NAr reaction with **1g**, and content ratio of monomethylated peptide **8**.

Finally, we aimed to construct an assay system for HMT activity. Set7/9 was selected as the target, because it is reported to monomethylate the 4th lysine residue of histone 3 (H3K4). First, we prepared the substrate peptide, H3 peptide (1–8 residues), with a biotin moiety at the N terminus (**9**) and its monomethylated peptide **10** (Figure 4). As in the case of *N*-acetyl peptides (**7** and **8**), we confirmed that **1g** could monitor the methylation state of the biotinated peptide (data not shown). In an enzymatic assay, buffer components such as tris(hydroxymethyl)-aminomethane (Tris) and dithiothreitol (DTT) might interfere with the S_NAr reaction; hence, dialysis and lyophilization processes were introduced before the reaction. As shown in Figure 7, the absorbance induced by the enzymatic reaction of Set7/9 ("enzymatic reaction") increased to the level of the positive control ("100 % conversion"), which contained monomethylated peptide **10** instead of substrate peptide **9**. On the other hand, thermal denaturation of Set7/9 ("thermal denaturation") blocked the absorbance increase, and the absorbance was similar to that of the negative control without coenzyme, AdoMet ("no SAM"). These data suggested that **1g** is available for monitoring the enzymatic activity of Set7/9 without the need for any radioisotope, antibody, or other enzyme. Although the change of absorption induced by Set7/9 is still relatively small, partially due to the rather high background derived from the presence of Set7/9 and other coexistences even under the optimized conditions, we regard this result as a proof of principle for a new assay system for HMT activity. We consider that this strategy could be modified to obtain greater sensitivity, for example, by adapting it to use another output, such as fluorescence change. Work along this line is in progress.

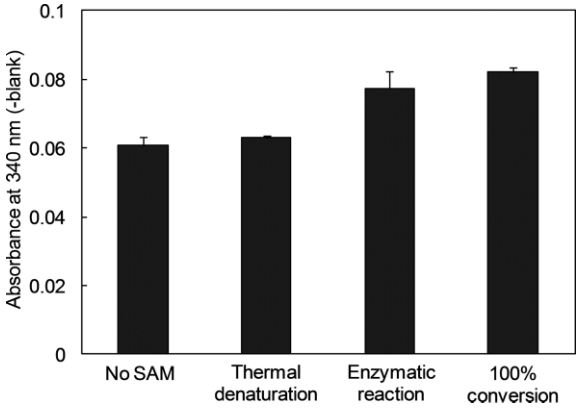


Figure 7. Absorption change induced by Set7/9 after S_NAr reaction of substrate peptide with **1g**. Enzymatic reaction was performed with 10 μM peptide, 20 μM SAM, and 2.5 μM Set7/9, in HMT reaction buffer. After dialysis and lyophilization, the peptide was concentrated to 150 μM and the S_NAr reaction was performed by addition of 2 mM **1g**. After 2 h, the reaction solution was diluted with 0.1 M HCl, and the absorbance at 340 nm was measured. No SAM: without coenzyme *S*-adenosylmethionine; thermal denaturation: Set7/9 was inactivated by boiling; enzyme reaction: containing all components required for the reaction; 100 % conversion: monomethylated peptide **10** was added instead of substrate peptide **9**.

Conclusions

We have developed a simple and convenient method, based on selective recognition of *N*-ε-monomethyllysine through S_NAr reaction, for monitoring the methylation state of the ε-amino group of peptide lysine residues. For proof of principle, the method was applied to assay the activity of Set7/9 as a model HMT. This system should not only be useful for the development of new HMT inhibitors, but also for the identification of HMT substrates and methylation sites.

Experimental Section

General: All reagents were purchased from Sigma–Aldrich, Tokyo Kasei, Wako Chemicals, Kanto Chemicals, and Nacalai tesque and used without further purification. NMR spectra were recorded with Bruker AVANCE 400 and Bruker AVANCE 500 spectrometers. Chemical shifts for NMR are reported as parts of per million (ppm) relative to chloroform (δ = 7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C NMR). Mass spectra were recorded with a Bruker Daltonics micro-TOF-2focus spectrometer in the positive ion mode. UV spectra were

recorded with a JASCO V-550. For the assay of HMT activity, OD 340 was recorded with a Beckman Coulter DTX 880 Multimode Detector. Compound **4** was synthesized according to the reported method.[35]

General Procedure for Synthesis of the Products of the S_NAr Reaction (Scheme 1)**:** Electrophile **1** (0.30 mmol) was added to a suspension of **3** or **4** (0.20 mmol) and K_2CO_3 (0.50 mmol) in a mixture of H_2O (5 mL) and dioxane (1 mL). The mixture was stirred in the dark for a suitable time (1 h to 24 h) at room temperature. After the reaction was finished, the solution was diluted with saturated $NaHCO_3$ aq. and the mixture was washed with CH_2Cl_2. The aqueous layer was acidified with saturated citric acid solution, and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and the solvents evaporated. The precipitate was collected, and dried in vacuo to afford the corresponding compound **5** or **6**.

***N*-α-Carbobenzoxy-*N*-ε-(2,4-dinitrophenyl)-L-lysine (5a):** Yield: 69 %, yellow oil. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 8.99 (d, J = 2.4 Hz, 1 H), 8.80 (s, 1 H), 8.29 (ddd, J = 9.6, 2.8, 0.8 Hz, 1 H), 7.38–7.29 (m, 6 H), 6.55 (d, J = 8.4 Hz, 1 H), 5.08 (s, 2 H), 4.29–4.23 (m, 1 H), 3.62 (q, J = 6.8 Hz, 2 H), 1.95–1.59 (m, 6 H) ppm. ^{13}C NMR (125 MHz, [D_6]acetone): δ = 174.5, 157.6, 150.0, 138.5, 136.9, 131.5, 131.3, 129.8, 129.3, 129.1, 125.2, 116.1, 67.5, 55.0, 44.2, 32.2, 29.1, 24.0 ppm. HRMS (ESI): calcd. for $C_{20}H_{21}N_4O_8$ [M – H]$^-$ 445.1365; found 445.1366.

***N*-α-Carbobenzoxy-*N*-ε-(2-cyano-4-nitrophenyl)-L-lysine (5e):** Yield: 78 %, yellow oil. ^{1}H NMR (400 MHz, [D_3]acetonitrile): δ = 8.35 (d, J = 2.4 Hz, 1 H), 8.19 (ddd, J = 9.6, 2.8, 0.8 Hz, 1 H), 7.41–7.29 (m, 5 H), 6.81 (d, J = 9.6 Hz, 1 H), 6.02 (s, 1 H), 5.93 (d, J = 7.6 Hz, 1 H), 5.07 (s, 2 H), 4.16–4.11 (m, 1 H), 3.32 (q, J = 6.8 Hz, 2 H), 1.89–1.39 (m, 6 H) ppm. ^{13}C NMR (125 MHz, [D_6]acetone): δ = 174.3, 157.6, 155.6, 138.5, 137.9, 131.7, 131.1, 129.8, 129.3, 129.1, 117.1, 111.8, 96.1, 67.5, 55.0, 44.1, 32.2, 29.0, 24.0 ppm. HRMS (ESI): calcd. for $C_{21}H_{21}N_4O_6$ [M – H]$^-$ 425.1467; found 425.1461.

***N*-α-Carbobenzoxy-*N*-ε-(4-acetyl-2-nitrophenyl)-L-lysine (5g):** Yield: 96 %, yellow oil. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 8.70 (d, J = 1.6 Hz, 1 H), 8.37 (s, 1 H), 8.01 (dd, J = 7.2, 1.6, Hz, 1 H), 7.38–7.30 (m, 5 H), 7.02 (d, J = 7.2 Hz, 1 H), 5.93 (d, J = 6.4 Hz, 1 H), 5.06 (s, 2 H), 4.17–4.12 (m, 1 H), 3.41 (q, J = 5.6 Hz, 2 H), 2.50 (s, 3 H), 1.90–1.46 (m, 6 H) ppm. ^{13}C NMR (125 MHz, [D_6]acetone): δ = 196.2, 174.4, 157.6, 149.2, 138.5, 136.2, 132.3, 129.8, 129.5, 129.3, 129.1, 125.9, 115.6, 67.5, 55.0, 43.9, 32.2, 29.2, 26.7, 24.1 ppm. HRMS (ESI): calcd. for $C_{22}H_{25}N_3O_7Na$ [M + Na]$^+$ 466.1585; found 466.1583.

***N*-α-Carbobenzoxy-*N*-ε-(2,4-dinitrophenyl)-*N*-ε-methyl-L-lysine (6a):** Yield: quant., yellow oil. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 8.59 (d, J = 2.8 Hz, 1 H), 8.21 (dd, J = 9.6, 2.8 Hz, 1 H), 7.39–7.29 (m, 6 H), 6.52 (d, J = 8.4 Hz, 1 H), 5.08 (s, 2 H), 4.27–4.22 (m, 1 H), 3.54 (t, J = 7.6 Hz, 2 H), 1.94–1.48 (m, 6 H) ppm. ^{13}C NMR (125 MHz, [D_6]acetone): δ = 174.3, 157.6, 150.3, 138.5, 137.3, 137.2, 129.8, 129.3, 129.1, 128.8, 125.3, 119.3, 67.5, 54.9, 54.7, 41.4, 32.2, 27.3, 23.7 ppm. HRMS (ESI): calcd. for $C_{21}H_{23}N_4O_8$ [M – H]$^-$ 459.1521; found 459.1527.

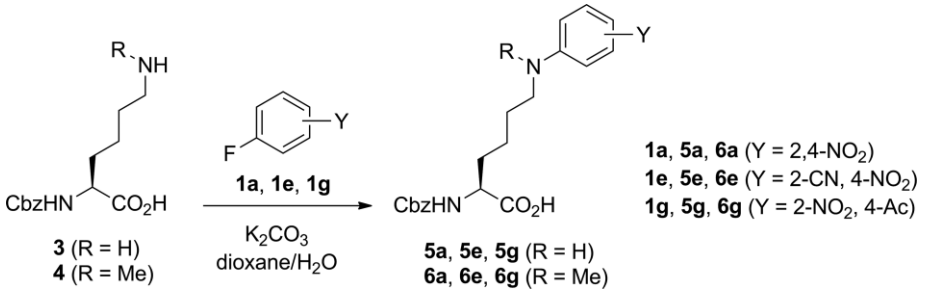
***N*-α-Carbobenzoxy-*N*-ε-(2-cyano-4-nitrophenyl)-*N*-ε-methyl-L-lysine (6e):** Yield: 70 %, yellow oil. ^{1}H NMR (400 MHz, [D_3]acetonitrile): δ = 8.36 (d, J = 3.2 Hz, 1 H), 8.11 (dd, J = 9.6, 2.8 Hz, 1 H), 7.39–7.30 (m, 5 H), 6.91 (d, J = 9.6 Hz, 1 H), 5.92 (d, J = 7.2 Hz, 1 H), 5.07 (s, 2 H), 4.17–4.12 (m, 1 H), 3.61–3.57 (m, 2 H), 3.21 (s, 3 H), 1.89–1.33 (m, 6 H) ppm. ^{13}C NMR (125 MHz, [D_6]acetone): δ = 174.8, 157.6, 156.7, 138.6, 138.1, 134.3, 129.8, 129.7, 129.3, 129.1, 120.0, 116.8, 95.8, 67.4, 55.2, 41.4, 32.4, 28.0, 23.7 ppm. HRMS (ESI): calcd. for $C_{22}H_{23}N_4O_6$ [M – H]$^-$ 439.1623; found 439.1622.

***N*-α-Carbobenzoxy-*N*-ε-(4-acetyl-2-nitrophenyl)-*N*-ε-methyl-L-lysine (6g):** Yield: 14 %, yellow oil. ^{1}H NMR (400 MHz, [D_6]acetone): δ = 8.26 (d, J = 2.0 Hz, 1 H), 7.93 (dd, J = 7.2, 1.6, Hz, 1 H), 7.37–7.32 (m, 5 H), 7.14 (d, J = 7.2 Hz, 1 H), 5.90 (d, J = 6.0 Hz, 1 H), 5.06 (s, 2 H), 4.13–4.09 (m, 1 H), 3.32 (t, J = 6.0 Hz, 2 H), 2.82 (s, 3 H), 2.48 (s, 3 H), 1.84–1.59 (m, 6 H) ppm. ^{13}C NMR (125 MHz, [D_6]-acetone): δ = 196.2, 174.3, 157.6, 149.3, 133.5, 129.8, 129.3, 129.2, 129.1, 127.0, 119.2, 67.5, 55.0, 54.5, 40.1, 32.2, 27.4, 26.9, 23.8 ppm. HRMS (ESI): calcd. for $C_{23}H_{27}N_3O_7Na$ [M + Na]$^+$ 480.1741; found 480.1738.

Measurement of the Initial Rate of the S_NAr Reaction: A solution of electrophile (96 mM) in [D_3]acetonitrile (500 μL) was added to a solution of lysine **3** or *N*-methyl lysine **4** (26.6 mM) in D_2O (300 μL) containing K_2CO_3 (80 mM), and the reaction mixture was stirred at the appropriate temperature. At the desired time point, the reaction was quenched with deuterium chloride solution (2 M) in D_2O, and the ^{1}H NMR spectrum was measured. Product concentrations were calculated from the peak integrals and plotted against reaction time. The initial rate was calculated from the slope.

Estimation of the Amount of *N*-Methyllysine-containing Peptide 8 Using 1g: A solution of **1g** in acetonitrile (33 μL) was added to mixtures of peptide **7** (100–0 %) and **8** (0–100 %) at different ratios in water (20 μL) containing K_2CO_3 [final concentrations: substrate peptide (10 mM), K_2CO_3 (30 mM), and **1g** (30 mM)]. Each mixture was incubated at 20 °C for 120 min, and then the reaction was quenched by 1/200 dilution with HCl aq. (0.1 M) solution. The absorption spectra were measured.

Determination of HMT Activity Using 1g: Human Set7/9 (residues 111–366) with an artificial N-terminal Gly-Ser-Ser-Gly-Ser-Ser-Gly sequence was synthesized and purified as described previously[36] for use in the enzymatic assay. A mixture of peptide **9** (10 μM), Set7/9 (2.5 μM), and SAM (20 μM) in the reaction buffer [Tris (50 mM), $MgCl_2$ (5 mM), DTT (4 mM), pH 8.8] was incubated at 35 °C for 24 h. The mixture was dialyzed against distilled water for 6 h with use of a PlusOne Mini Dialysis Kit (1 kDa MWCO, GE Healthcare) and lyophilized for 12 h. The obtained colorless powder was dissolved in K_2CO_3



Scheme 1. Synthesis of the S_NAr reaction products (**5** and **6**).

aq. (240 mM) solution. Aliquots (10 μL) of the solution were applied to a 96-well plate, and an acetonitrile solution of **1g** (20 μL) was added to each well [final concentration: peptide (75 μM), K_2CO_3 (80 mM), and **1g** (2 mM)]. The plate was incubated at room temperature for 90 min, and then HCl aq. (0.1 M, 170 μL) was added to each well. The absorbance of each well at 340 nm was measured with a plate reader.

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[1] S. Rea, F. Eisenhaber, N. O'Carroll, B. D. Strahl, Z. W. Sun, M. Schmid, S. Opravil, K. Mechtler, C. P. Ponting, C. D. Allis, T. Jenuwein, *Nature* **2000**, *406*, 593–599.
[2] Y. Zhang, D. Reinberg, *Genes Dev.* **2001**, *15*, 2343–2360.
[3] M. Lachner, T. Jenuwein, *Curr. Opin. Cell Biol.* **2002**, *14*, 286–298.
[4] J. C. Black, J. R. Whetstine, *Mol. Cell* **2012**, *48*, 491–507.
[5] X. Zhang, H. Wen, X. Shi, *Acta Biochim. Biophys. Sin.* **2012**, *44*, 14–27.
[6] J. Huang, S. Dorsey, L. Chuikov, L. Perez-Burgos, X. Zhang, T. Jenuwein, D. Reinberg, S. L. Berger, *J. Biol. Chem.* **2010**, *285*, 9636–9641.
[7] S. Chuikov, J. K. Kurash, J. R. Wilson, B. Xiao, N. Justin, G. S. Ivanov, K. McKinney, P. Tempst, C. Prives, S. J. Gamblin, N. A. Barlev, D. Reinberg, *Nature* **2004**, *432*, 353–360.
[8] K. Subramanian, D. Jia, K.-V. Priya, D. R. Powell, R. E. Collins, D. Sharma, J. Peng, X. Cheng, P. M. Vertino, *Mol. Cell* **2008**, *30*, 336–347.
[9] R. A. Copeland, M. E. Solomon, V. M. Richon, *Nat. Rev. Drug Discovery* **2009**, *8*, 724–732.
[10] R. A. Varier, H. T. M. Timmers, *Biochim. Biophys. Acta* **2011**, *1815*, 75–89.
[11] C. Zagni, U. Chiacchio, A. Rescifina, *Curr. Med. Chem.* **2013**, *20*, 167–185.
[12] T. Fujiwara, K. Ohira, K. Urushibara, A. Ito, M. Yoshida, M. Kanai, A. Tanatani, H. Kagechika, T. Hirano, *Bioorg. Med. Chem.* **2016**, *24*, 4318–4323.
[13] D. Li, F. Barsyte-Lovejoy, M. J. Oudhoff, J. H. Tatlock, A. Dong, H. Zeng, H. Wu, S. A. Freeman, M. Schapira, G. A. Senisterra, E. Kuznetsova, R. Marcellus, A. Allali-Hassani, S. Kennedy, J. P. Lambert, A. L. Couzens, A. Aman, A. C. Gingras, R. Al-Awar, P. V. Fish, B. S. Gerstenberger, L. Roberts, C. L. Benn, R. L. Grimley, M. J. Braam, F. M. Rossi, M. Sudol, P. J. Brown, M. E. Bunnage, D. R. Owen, C. Zaph, M. Vedadi, C. H. Arrowsmith, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 12853–12858.
[14] S. Mori, K. Iwase, N. Iwanami, Y. Tanaka, H. Kagechika, T. Hirano, *Bioorg. Med. Chem.* **2010**, *18*, 8158–8166.
[15] M. Luo, *ACS Chem. Biol.* **2012**, *7*, 443–463.
[16] D. Greiner, T. Bonaldi, R. Eskeland, E. Roemer, A. Imhof, *Nat. Chem. Biol.* **2005**, *1*, 143–145.
[17] A. M. Quinn, A. Allali-Hassani, M. Vedadi, A. Simeonov, *Mol. BioSyst.* **2010**, *6*, 782–788.
[18] N. Gauthier, M. Caron, L. Pedro, M. Arcand, J. Blouin, A. Labonte, C. Normand, V. Paquet, A. Rodenbrock, M. Roy, N. Rouleau, L. Beaudet, J. Padros, R. Rodriguez-Suarez, *J. Biomol. Screening* **2012**, *17*, 49–58.
[19] E. Collazo, J. F. Couture, S. Bulfer, R. C. Trievel, *Anal. Biochem.* **2005**, *342*, 86–92.
[20] K. M. Dorgan, W. L. Wooderchak, D. P. Wynn, E. L. Karschner, J. F. Alfaro, Y. Q. Cui, Z. S. Zhou, J. M. Hevel, *Anal. Biochem.* **2006**, *350*, 249–255.
[21] G. Ibanez, J. L. McBean, Y. M. Astudillo, M. Luo, *Anal. Biochem.* **2010**, *401*, 203–210.
[22] H. Chi, Y. Takemoto, T. K. Nsiama, T. Kato, N. Nishino, A. Ito, M. Yoshida, *Bioorg. Med. Chem.* **2014**, *22*, 1268–1275.
[23] J. Howard, Veeranna, C. P. Harish, *Biochemistry* **1998**, *37*, 16211–16224.
[24] Z. A. Knight, B. Schilling, R. H. Row, D. M. Kenski, B. W. Gibson, K. M. Shokat, *Nat. Biochem.* **2003**, *21*, 1047–1054.
[25] S. Akita, N. Umezawa, T. Higuchi, *Org. Lett.* **2005**, *7*, 5565–5568.
[26] D. Wegener, F. Wirsching, D. Riester, A. Schwienhorst, *Chem. Biol.* **2003**, *10*, 61–68.
[27] R. Baba, Y. Hori, S. Mizukami, K. Kikuchi, *J. Am. Chem. Soc.* **2012**, *134*, 14310–14313.
[28] K. Satake, T. Okuyama, M. Ohashi, T. Shinoda, *J. Biochem.* **1960**, *47*, 654–660.
[29] A. F. S. A. Habeeb, *Anal. Biochem.* **1966**, *14*, 328–336.
[30] H. H. Brown, *Clin. Chem.* **1968**, *14*, 967–978.
[31] J. B. Bateson, *J. Inst. Brew.* **1970**, *76*, 150–152.
[32] L. O. Brady, R. F. Cropper, *J. Chem. Soc.* **1950**, 507–516.
[33] C. D. Ritchie, M. Sawada, *J. Am. Chem. Soc.* **1977**, *99*, 3754–3761.
[34] I.-H. Um, L.-R. Im, J.-S. Kang, S. S. Bursey, J. M. Dust, *J. Org. Chem.* **2012**, *77*, 9738–9746.
[35] D. C. Tully, A. K. Chatterjee, A. Vidal, H. M. J. Petrassi, Z. Wang, B. Bursulaya, G. A. Spraggon, WO2007137080, **2007**.
[36] H. Niwa, N. Handa, Y. Tomabechi, K. Honda, M. Toyama, N. Ohsawa, M. Shirouzu, H. Kagechika, T. Hirano, T. Umehara, S. Yokoyama, *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2013**, *69*, 595–602.

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