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Protein Arginine Allylation and Subsequent Fluorophore Targeting

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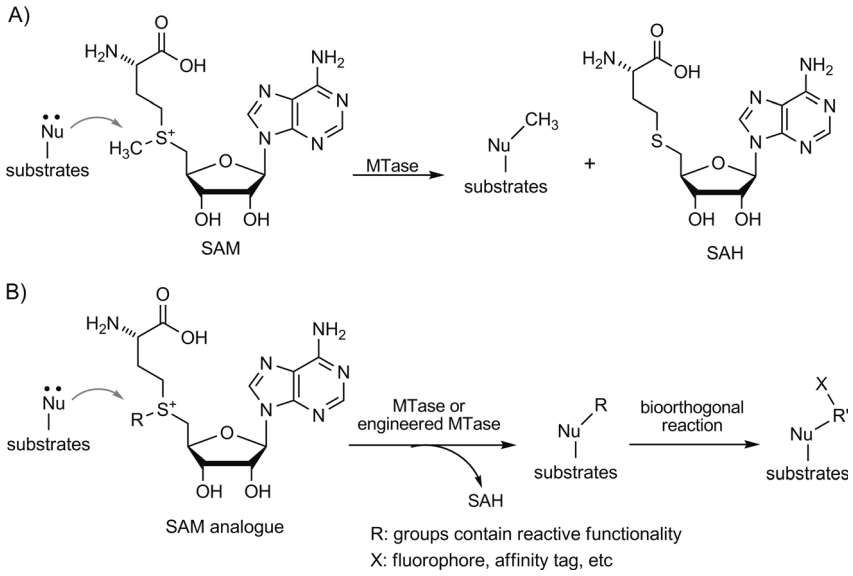
Protein posttranslational modifications, such as phosphorylation, acetylation, and methylation, play important roles in almost all biological processes. Protein methylation by a methyltransferase (MTase) transfers a methyl group from *S*-adenosyl-L-methionine (SAM) to a substrate protein and releases *S*-adenosyl-L-homocysteine (SAH) as a co-product (Scheme 1 A). Methylated proteins are key to several cellular processes, including signal transduction, transcriptional regulation, DNA repair, and RNA processes.[1] In contrast to protein phosphorylation, it remains a challenge to analyze methylated proteins and peptides, largely because the methyl group is small and chemically inert. Although many analytical techniques have been developed, difficulties still exist to achieve a systems-level understanding of the methylproteome, especially methylated proteins in low abundance.[2] To advance this area, SAM analogues bearing reactive functionalities have recently been explored to specifically label the methylation sites by using natural[3] or engineered MTases.[4] Once these reactive functionalities have been incorporated, further derivation can introduce chemical entities to study methylation with readily available analytical and biochemical tools (Scheme 1 B).



Scheme 1. Protein alkylation with MTase. A) MTase-catalyzed transmethylation from SAM, B) Chemical biology strategy for labeling MTase substrates with SAM analogues.

Among the bioorthogonal reactions that have been developed,[5] only copper-catalyzed azide–alkyne cycloaddition has been successfully applied in protein methylation studies, in which a terminal alkyne group was incorporated into SAM analogues to ensure labeling and detection of the protein methylation substrates.[3,4,6] Because natural MTases have limited flexibility for cofactor binding,[4] the structural diversity for functional SAM analogues is limited. It is important to develop new methods to expand the bioorthogonal labeling toolbox for protein methylation studies. Recently, photoinducible tetrazole–alkene cycloaddition ("photo-click chemistry") has been developed as a bioorthogonal reaction for protein labeling in living cells.[7] This reaction features full water compatibility, fast reaction kinetics, and formation of fluorescent pyrazoline cycloadducts. To apply this photo-click chemistry in a protein methylation study, it is possible to use of an allylated SAM analogue (allyl-SAM) as a SAM surrogate for protein MTases. In this work, we established an alternative procedure for the preparation of allyl-SAM and demonstrated protein allylation by using the natural yeast protein arginine MTase Hmt1 and its protein substrate Npl3. We observed mono-allylation, as well as di-allylation, on Npl3 at almost all known methylation sites. The incorporated allyl group enabled subsequent fluorophore targeting upon reaction with tetrazole compounds (Scheme 2).

Although allyl-SAM and a number of other SAM analogues have been prepared previously by a one-step alkylation of SAH with activated electrophiles,[8] pursuing an alternative route remains attractive in order to bypass the expensive starting material, SAH. To prepare allyl-SAM on a larger scale, we established an efficient route starting from readily available adenosine (Scheme 3). The adenosine was first converted to an ace-

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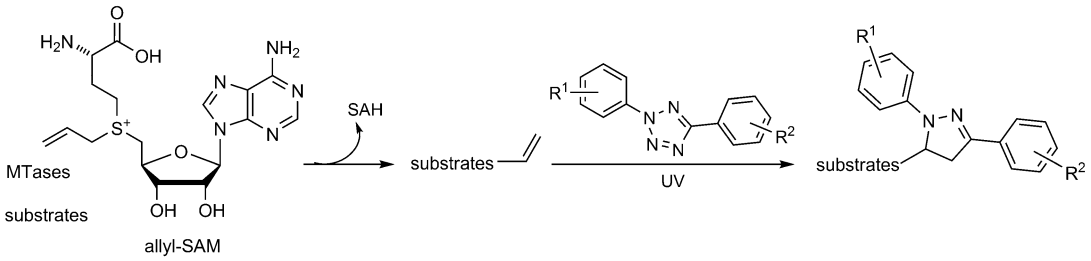
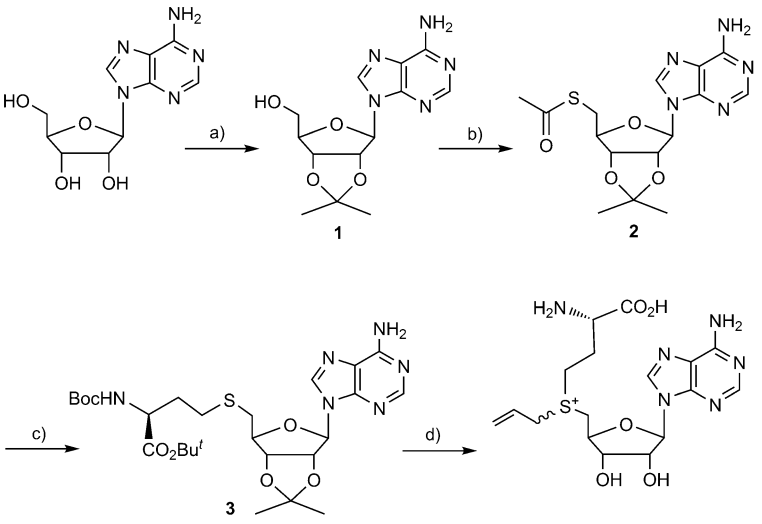


Scheme 2. Strategy for fluorophore-targeting MTase substrates.

tonide-protected nucleotide **1**, followed by the introduction of a sulfur atom with thioacetate under Mitsunobu reaction conditions to give thioester **2**.[9] In parallel, N-Boc-γ-tosyl-L-homoserine-*tert*-butylester was produced from Boc-Asp-*t*Bu according to a literature procedure.[10] In situ cleavage of the acetylthio functionality of thioester **2** with fresh CH_3ONa in MeOH under O_2-free conditions was followed by coupling with N-Boc-γ-tosyl-L-homoserine-*tert*-butylester to give **3**. There was a strong tendency for disulfide formation in the presence of O_2.[9,10] The reaction time and the basicity of solution should also be carefully controlled to avoid a MeOH-involved transesterification product, as it could render the downstream deprotection process problematic. Treatment of **3** with allylbromide in the presence of $AgClO_4$ gave allyl-SAM under acidic conditions. This step involved efficient formation of the carbon–sulfonium bond by alkylation and removal of three different protecting groups. The synthesized SAM analogue, with two diastereomers, was used directly without further purification. It should be noted that compound **3** is a fully protected SAH, which could also be deprotected to form SAH for preparation of SAM analogues according to previously established procedures.[8]

We selected the predominant protein arginine methyltransferase (PRMT) Hmt1 and its representative substrate Npl3, found in the yeast *Saccharomyces cerevisiae*, to demonstrate the usefulness of allyl-SAM. Hmt1 can methylate both histone and non-histone proteins and is responsible for the formation of 89% of the asymmetric dimethylarginine and 66% of the monomethylarginine in yeast.[11] As a type I PRMT, Hmt1 has high homology with the rat PRMT1 and other mammalian PRMTs.[12] Npl3 is a yeast mRNA-binding protein that has up to 17 potential arginine methylation sites.[1a,13] Therefore, it is an interesting pair with which to explore protein allylation and subsequent fluorophore targeting.

We cloned the corresponding genes, enabled heterogeneous production, and purified recombinant Hmt1 and Npl3 from engineered *Escherichia coli* cells. After incubation of Hmt1, Npl3, and allyl-SAM at 30 °C for 20 h, the reaction mixture was mixed with various tetrazole compounds and subjected to 312 nm UV light irradiation. As fluorescent pyrazoline can be generated from the photoinducible bioorthogonal reaction between tetrazole and alkene, the samples were then separated by SDS-PAGE and analyzed by in-gel fluorescence analysis. As shown in Figure 1 A, fluorescent signals were observed for the three samples in the presence of Hmt1, whereas negligible signal was seen in the absence of Hmt1, suggesting that both protein allylation and photoinducible tetrazole–alkene cycloaddition occurred. It was clear that tetrazole **2** showed the strongest fluorescence intensity, which might be due to its high reaction rate or fluorescent quantum yield.[7c] Thus, tetrazole **2** was used in the following experiments.



Scheme 3. Synthesis of allyl-SAM.: a) *p*-TsOH, acetone, room temperature, 95%; b) PPh_3, DEAD, AcSH, THF, −10 °C then 0 °C, 77%; c) *N*-Boc-γ-tosyl-homoserine-*tert*-butylester, CH_3ONa, MeOH, −20 °C then room temperature, 32%; d) allylbromide, $AgClO_4$, 0 °C then room temperature, 55%.

As multiple modification sites are available within Npl3, it was necessary to pursue a more detailed analysis. To determine the effects of allyl-SAM concentration on Npl3 allylation, we performed an assay in the presence of 0 to 800 μM of allyl-SAM, followed by a photo-click reaction with tetrazole **2**. The fluorescence intensity was used to roughly estimate the extent of protein allylation. Figure 2 shows that 400 μM allyl-SAM afforded the strongest fluores-

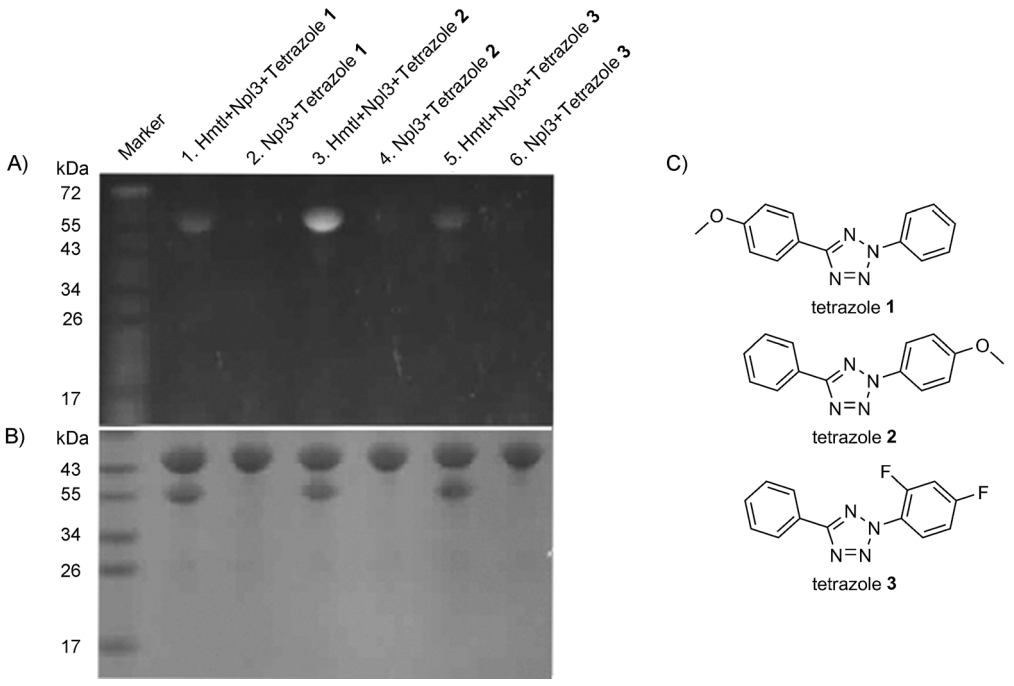


Figure 1. SDS-PAGE analysis of Hmt1-catalyzed allylation of Npl3. A) Fluorescence image, B) Coomassie Blue staining, C) Structures of tetrazole compounds used in the photo-click reaction.

cence intensity. Thus, extensively modified Npl3 samples were chose for additional characterization.

To further confirm that the allyl group of allyl-SAM was incorporated into the arginine residues of Npl3 and investigate the selectivity for potential modification sites, labeled Npl3 samples were digested with trypsin and analyzed by high resolution LC–MS/MS. It has been demonstrated that native Npl3 purified from yeast has 17 methylated arginine residues; dimethylation was observed at 10 sites, whereas both monomethylation and dimethylation were observed for the other sites (Table 1, Figure 3).[1a] Based on mass increments in the MS spectra that are 40.0313 Da and 80.0626 Da higher than the unmodified peptide alone for mono- and diallylation, respectively. Through further MS/MS data analysis, we were able to identify 15 allylated arginine residues. Among these, seven sites that were previously identified as being dimethylated in vivo were found to be diallylated in our studies. To the best of our knowledge, this is the first time that protein arginine dialkylation has been realized enzymatically by using a SAM analogue as the donor of the alkyl group. Among the 17 methylation sites described previously, 13 sites were found allylated in our studies as well; and allylation of the remaining four sites were found in other samples in the presence of different amounts of allyl-SAM. Two arginine residues, Arg370 and Arg391, were found to be allylated here; methylation of these residues has not previously been observed. These results indicated that allyl-SAM can be used as an effective SAM surrogate by Hmt1 for protein allylation, and the selectivity for methyla-

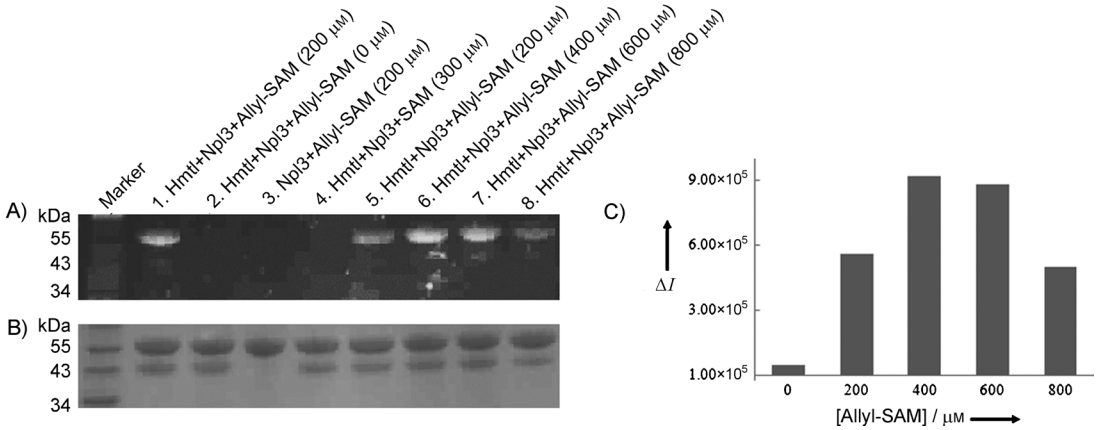


Figure 2. Allyl-SAM concentration dependence of Hmt1-catalyzed allylation of Npl3. A) Fluorescence image, B) Coomassie Blue staining, C) Relative fluorescence intensity (ΔI) of allylated Npl3 samples by using different concentrations of allyl-SAM.

Table 1. Allylation and methylation sites of Npl3 based on LC–MS/MS analysis and literature.

	Modification site[a]	Sample 1[b] Run 1[c]	Run 2	Sample 2 Run 1	Run 2	Allylation state	Methylation state
1	Arg284	–	–	–	M	M	M
2	Arg288[d]	–	–	–	–	–	M
3	Arg290[d]	–	–	–	–	–	D
4	Arg294	M	D	–	D	M/D	D
5	Arg298	M	D	–	D	M/D	D
6	Arg302	D	D	M	M	M/D	D
7	Arg307	M	–	M	M	M	D
8	Arg314	D	M	–	D	M/D	D
9	Arg321	M	M	D	M	M/D	D
10	Arg329	M	M	D	M	M/D	D
11	Arg337[d]	–	–	–	–	–	D
12	Arg344[d]	–	–	–	–	–	M
13	Arg351	–	–	–	D	D	M
14	Arg358	M	M	M	D	M/D	M
15	Arg363	D	M	–	–	M/D	D
16	Arg370	M	D	D	M	M/D	–
17	Arg377	D	D	D	M	M/D	M
18	Arg384	D	M	–	M	M/D	M
19	Arg391	D	D	M	–	M/D	–

[a] Location of alkyl–arginine residues in Npl3 was shown as below. RSNR^{284}GGFR^{288}GR^{290}GGFR^{294}GGFR^{298}GGFR^{302}GGFSR^{307}GGFGGPR^{314}GGFGGPR^{321}-GGYGGYSR^{329}GGYGGYSR^{337}GGYGGSR^{344}GGYDSPR^{351}GGYDSPR^{358}GG YSR^{363}GGYGGPR^{370}NDYGPPR^{377}GSYGGSR^{384} GGYDGPR^{391}GDYGPPRDA. [b] Two sets of parallel samples were evaluated with Hmt1 (10 μM), Npl3 (10 μM), and allyl-SAM (400 μM). [c] All samples were run twice in parallel by LC–MS/MS; –/M/D represent no/monoalkyl/dialkyl modification. [d] Allylation of Arg288, Arg290, Arg337, and Arg344 was not observed in these sets of samples but were detected in the other samples when using different amounts of allyl-SAM.

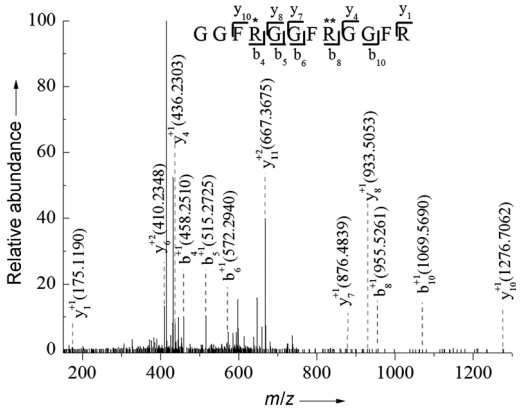


Figure 3. MS/MS spectra of one peptide fragment (GGFRGGFRGGFR) detected by arginine monoallylation (*) and diallylation (**).

tion was largely retained. By measuring the formation of SAH by HPLC, the specific activities of Hmt1 in the presence of allyl-SAM and SAM were determined as 0.46 mol g^{-1} min^{-1} and 11.19 mol g^{-1} min^{-1}, respectively. Thus, the Hmt1–allyl-SAM pair retained about 4% activity of that of the Hmt1–SAM pair in terms of releasing the alkyl group. Hmt1, as a predominant type I PRMT, is capable of catalyzing both monomethylation and dimethylation of protein arginine residues. It is closely related to other members of the PRMT1 family by primary sequence as well as tertiary structure.[14] Previously, allyl-SAM has been documented to efficiently allylate peptide substrates by natural MTases belonging to the distinct SET-domain-containing protein lysine methyltransferases (PKMTs).[15] Thus, allyl-SAM might be compatible with both PRMTs and PKMTs.

Stable, less bulky SAM analogues are essential to study protein methylation behavior with native MTases. Among many known SAM analogues, only the selenium-based analogue SeAdoYn showed excellent compatibility with most wild-type protein MTases for protein alkylation. However, the half-life of SeAdoYn was only about 1.5 h at pH 7.5.[3c,16] Because allyl-SAM is relative stable and the allyl group is relative small, allyl-SAM has a high likelihood of being recognized as a SAM surrogate by wild-type MTases. Furthermore, the presence of an allyl group can be easily detected by a visible fluorescent signal introduced by a photo-click chemical reaction. In terms of protein methylation and DNA/RNA methylation, the application of allyl-SAM provides a terminal alkene functionality that is orthogonal to other functional groups on these macromolecules, offering many benefits for biochemical and biological studies. For example, it can be used to find more protein methylation substrates or to visualize methylated products. The photoinducible bioorthogonal strategy has already been used to study protein modification such as lipidation[17] and to spatiotemporally control imaging of newly synthesized proteins.[7d] More interestingly, allyl-SAM might also be used to tailor natural products by using the corresponding MTase found in the biosynthetic pathway.

In summary, we developed an alternative procedure for the preparation of allyl-SAM and found that arginine residues of the yeast nuclear ribonucleoprotein Npl3 were extensively modified by a Hmt1-catalyzed allylation reaction with allyl-SAM as the allyl group donor. The allylated protein was further labeled by using a photoinducible cycloaddition reaction with tetrazole compounds, leading to formation of protein-attached

fluorescent products. We are now exploring allyl-SAM to study other methylation events in the biological system.

Experimental Section

General: All reagents and chemicals were obtained from commercial suppliers in analytical or higher grade and were used without further purification unless otherwise noted. Compound **2**,[9] *N*-Boc-γ-tosyl-homoserine-*tert*-butylester,[10] and all tetrazole compounds[18] were synthesized as previously described in literature. The silica gel used for flash column chromatography was received from Yantai Yuanbo Silica Gel Co., China. The IRC 84 weak acid resin was purchased from the Chemical Plant of NanKai University, China. TLC plates were visualized by using a combination of UV, iodine, or staining with ninhydrin. Primers *HMT1*-F: GGAATTCCATATGAGCAAGACAGCCGTGAAAGAT, *HMT1*-R: CCGCTCGAGTTAATGCATTAAATAAGAACCTTCGTTTT, *NPL3*-F: GGAATTCCATATGTCTGAAGCTCAAGAAACTCAC, and *NPL3*-R: CCGCTCGAGCCTGGTTGGTGATCTTTCACGT (restriction sites underlined), were synthesized by Takara Bio (Dalian, China).

NMR spectra were recorded at room temperature on a Bruker DRX 400 instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C). Accurate mass measurements of synthesized compounds were performed by using a Q-TOF Micro mass spectrometer (Waters) equipped with a Z-spray ionization source. Analytical HPLC was conducted on a Dionex Controller by using a C_{18} 4.6 mm × 200 mm reverse phase column with UV detection at 260 nm. The handheld UV lamp used in the photo-click reaction was a Spectroline E-series ultraviolet hand lamp (312 nm, 220 V). For in-gel fluorescence imaging, the gel was illuminated from one side by using a handheld 365 nm UV lamp. Fluorescence images and Coomassie Blue-stained protein bands were recorded by gel documentation and analysis systems (InGenius LHR, British). Mass spectrometric analysis of the enzymatic reaction mixtures were carried out by an RP LC–MS/MS system consisting of an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific) with a nanospray source.

Synthesis of 2′,3′-*O*-isopropylidene-*S*-adenosyl-*N*-Boc-L-homocysteine-*tert*-butylester (3): A fresh solution of CH_3ONa (54 mg, 0.84 mmol) in MeOH (3 mL) was added to a solution of compound **2** (141 mg, 0.40 mmol) and *N*-Boc-γ-tosyl-L-homoserine-*tert*-butylester (180 mg, 0.42 mmol) in dry MeOH (5 mL) at −20 °C. The solution was stirred at −20 °C for 30 min and then at room temperature for an additional 15 h. The reaction was quenched by adding aqueous KOAc solution (pH 4.0) and extracting with $CHCl_3$. The organic layers were combined, and the solvents were removed in vacuo. Purification of the crude product by flash chromatography on silia gel with petrol ether/EtOAc (1:5 to 0:1) as the eluent yielded SAH precursor **3** as a white solid (75 mg, 32.3%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.38 (s, 3H), 1.40 (s, 9H), 1.42 (s, 9H), 1.59 (s, 3H), 1.75–2.05 (m, 2H), 2.49–2.57 (m, 2H), 2.71–2.87 (m, 2H), 4.20–4.25 (m, 1H), 4.33–4.37 (m, 1H), 5.02 (dd, J_1 = 3.2, J_2 = 6.4 Hz, 1H), 5.49 (d, J = 6.0 Hz, 1H), 6.07 (d, J = 2.0 Hz, 1H), 7.92 (s, 1H), 8.32 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.9, 29.6, 30.5, 30.9, 35.6, 36.8, 55.8, 82.3, 84.7, 86.3, 86.6, 89.1, 93.4, 117.0, 122.8, 142.6, 151.7, 155.6, 157.9, 158.3, 173.8 ppm.

Synthesis of allyl-SAM: Allyl bromide (2.27 mL, 11.0 mmol) was added dropwise to a stirred solution of compound **3** (80 mg, 0.14 mmol) in formic acid/acetic acid (7 mL, 1:1) at 0 °C; $AgClO_4$ (132 mg, 0.55 mmol) was added subsequently. The solution was further stirred in the dark at room temperature for 24 h. The reaction was quenched by adding water (15 mL) and was washed with diethyl ether (3 × 20 mL). The aqueous portion was lyophilized and then redissolved in KH_2PO_4 buffer (pH 7.0). After adjusting the pH to 4.8–5.2, the crude product was purified over an IRC 84 weak acid resin, eluting with KH_2PO_4 buffer, 0.02 M HCl, 0.04 M HCl and 0.1 M HCl, sequentially. The eluents were concentrated, and the product was collected as a white solid. The concentration of allyl-SAM was determined by UV absorption to be ε_{260} = 15400 L mol^{-1} cm^{-1}. The bioactive epimer was estimated to be 50% of the total diastereomeric mixture and was not further purified (32 mg, 55%): ^{1}H NMR (400 MHz, D_2O): δ = 2.35–2.41(m, 2H, Hβ), 3.55–3.59 (m, 2H, Hγ), 3.90–3.95 (m, 2H, H5′), 4.09–4.14 (m, 1H, Hα), 4.14–4.19 (m, 2H, H1″), 4.51–4.56 (m, 1H, H4′), 4.59–4.64 (m, 1H, H3′), 4.84 (dd, J_1 = 9.4 Hz, J_2 = 3.8 Hz, 1H, H2′), 5.58–5.68 (m, 2H, H3″), 5.77–5.91 (m, 1H, H2″), 6.14 (d, J = 3.6 Hz, 1H, H1′), 8.46 (s, 1H, arom. H), 8.47 ppm (s, 1H, arom. H); ^{13}C NMR (100 MHz, D_2O): δ = 27.6, 27.7, 38.1, 38.4, 43.7, 44.3, 45.4, 45.8, 53.9, 75.3, 75.7, 81.3, 81.6, 92.6, 92.7, 122.0, 125.2, 131.6, 146.3, 147.2, 150.7, 152.6, 173.0 ppm; ESI-MS *m/z* (calcd): allyl-SAM, 425.1590 (424.1602); [5′-(prop-2-enyl)thio-5′-deoxyadenosine+H]$^+$, 324.1158 (324.1130).

Cloning, expression, and purification of Hmt1 and Npl3: The genes encoding *HMT1* (YBR034C) and *NPL3* (YDR432W) were amplified from *S. cerevisiae* BY4741 genome with primer pairs HMT1-F/HMT1-R and NPL3-F/NPL3-R and were cloned into the Nde I-Xho I site of expression plasmids pET15b and pET24b, respectively. The resulting expression vectors pET15b-Hmt1 and pET24b-Npl3 were transformed into *E. coli* BL21 (DE3). The corresponding proteins Hmt1 and Npl3 were overexpressed, purified, and concentrated as described previously.[19] The purified proteins were stored in aliquots at −80 °C in elution buffer (pH 8.0) with 10% glycerol.

Hmt1-catalyzed protein allylation of Npl3 in the presence of Allyl-SAM: Enzymatic reactions were carried out in 20 mM MOPS, 120 mM NaCl, and 2 mM EDTA (pH 7.2) at 30 °C for 20 h. The reaction mixture (200 μL) contained Hmt1 (10 μM), Npl3 (10 μM), and allyl-SAM (400 μM). Control experiments were run in the absence of Hmt1. With the exception of MS analysis, all reaction samples were then subjected to the photo-click reaction as described below.

Fluorophore targeting via a tetrazole–alkene photo-click reaction: The enzymatic reaction mixtures were mixed with tetrazole compounds (300 μM), irradiated with 312 nm UV light for 5 min at room temperature, and additional incubation was carried out at room temperature for 1.5 h without UV irradiation. Teterazole **2** (1 mM) was used to determine the effects of allyl-SAM concentration on Npl3 allylation. The samples were resolved by SDS-PAGE. The protein gels were first subjected to fluorescence analysis and then stained with Coomassie Blue.

HPLC analysis of the specific activity of Hmt1 toward allyl-SAM and SAM: The activity assays were carried out by measuring SAH concentrations in the reaction mixtures over time. Enzymatic reactions were initiated by adding 10 μM Hmt1 to 200 μL assay buffer containing 2 μM Npl3 and 200 μM allyl-SAM at 30 °C. Reaction mixtures were collected every 15 min, quenched by adding an equal volume of chloroform, centrifuged, and filtered by using a 0.22 μm filter unit. The samples were analyzed on a SinoChrom ODS-BP C_{18} column (5 μm, 4.6 mm × 200 mm), eluting as described in the literature.[20] For activity assays in the presence of SAM, only Hmt1 (1.0 μM) was used to ensure similar amounts of SAH in the reaction mixtures. Control experiments were run in the absence of Npl3. Each sample was analyzed three times. Samples from at least five time points were analyzed for each data set to calculate enzymatic activity. The specific activity was calculated as *v*/*c* (*v*: the initial ve-

locity of SAH formation [$\mu mol\,mL^{-1}\,min^{-1}$], c: concentration of Hmt1 [$mg\,mL^{-1}$]).

Methods for mass spectroscopic analysis: The enzymatic reaction mixtures were first digested with trypsin at 37 °C for 16–20 h. After desalting, the digestion products were then analyzed by an RP LC–MS/MS system consisting of an LTQ-Orbitrap mass spectrometer with a nanospray source. A capillary column was first manually pulled to a fine point as a spray tip and then was packed with C_{18} AQ beads (3 μm, 120 Å, Michrom Bioresources). Formic acid (0.1%, *v*/*v*) in water and 0.1% (*v*/*v*) formic acid in CH_3CN were applied as the mobile phase. Gradient elution from 5 to 35% (*v*/*v*) of the 0.1% (*v*/*v*) formic acid in CH_3CN in 30 min was performed to elute each sample. All MS and MS/MS spectra were acquired in the data-dependent mode with the ten most intense ions fragmented by CID.

For identification, the MS/MS spectra in one acquired raw file was converted to a single .mgf file by using MSQuant (http://msquant.alwaysdata.net/msq). These .mgf files were queried against the NPL3 protein database by using Mascot (Matrix Science). FDRs given here are those originating from the internal Mascot decoy database search function. Peptides with FDR < 1% (rank 1 and bold red) and Mascot ion scores > 10 were accepted for identification. The .mgf files were searched by using fully tryptic cleavage and as many as five missed cleavage site; oxidation of methionine (+15.9949 Da), monoallylation of arginine (+40.0313 Da), and diallylation of arginine (+80.0626 Da) were set as variable modifications. The mass tolerances were 10 ppm for parent masses and 0.8 Da for fragment masses.

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Keywords: bioorthogonal reaction · cycloaddition · fluorescence labeling · methyltransferase · protein methylation · *S*-adenosyl-L-methionine

[1] a) A. E. McBride, J. T. Cook, E. A. Stemmler, K. L. Rutledge, K. A. McGrath, J. A. Rubens, *J. Biol. Chem.* **2005**, *280*, 30888 – 30898; b) B. Polevoda, F. Sherman, *Mol. Microbiol.* **2007**, *65*, 590 – 606; c) M. Mohan, H.-M. Herz, A. Shilatifard, *Cell* **2012**, *149*, 498 – 498.e1; d) K. Nimura, K. Ura, Y. Kaneda, *J. Mol. Med.* **2010**, *88*, 1213 – 1220.

[2] M. A. Erce, C. N. I. Pang, G. Hart-Smith, M. R. Wilkins, *Proteomics* **2012**, *12*, 564 – 586.

[3] a) T. Osborne, R. L. W. Roska, S. R. Rajski, P. R. Thompson, *J. Am. Chem. Soc.* **2008**, *130*, 4574 – 4575; b) S. Willnow, M. Martin, B. Luescher, E. Weinhold, *ChemBioChem* **2012**, *13*, 1167 – 1173; c) W. Peters, S. Willnow, M. Duisken, H. Kleine, T. Macherey, K. E. Duncan, D. W. Litchfield, B. Luscher, E. Weinhold, *Angew. Chem.* **2010**, *122*, 5296 – 5299; *Angew. Chem. Int. Ed.* **2010**, *49*, 5170 – 5173.

[4] a) R. Wang, W. H. Zheng, H. Q. Yu, H. T. Deng, M. K. Luo, *J. Am. Chem. Soc.* **2011**, *133*, 7648 – 7651; b) K. Islam, W. Zheng, H. Yu, H. Deng, M. Luo, *Acs Chem. Biol.* **2011**, *6*, 679 – 684.

[5] E. M. Sletten, C. R. Bertozzi, *Angew. Chem.* **2009**, *121*, 7108 – 7133; *Angew. Chem. Int. Ed.* **2009**, *48*, 6974 – 6998.

[6] M. Luo, *ACS Chem. Biol.* **2012**, *7*, 443 – 463.

[7] a) W. Song, Y. Wang, J. Qu, Q. Lin, *J. Am. Chem. Soc.* **2008**, *130*, 9654 – 9655; b) W. Song, Y. Wang, J. Qu, M. M. Madden, Q. Lin, *Angew. Chem.* **2008**, *120*, 2874 – 2877; *Angew. Chem. Int. Ed.* **2008**, *47*, 2832 – 2835; c) Y. Wang, W. Song, W. J. Hu, Q. Lin, *Angew. Chem.* **2009**, *121*, 5434 – 5437; *Angew. Chem. Int. Ed.* **2009**, *48*, 5330 – 5333; d) W. Song, Y. Wang, Z. Yu, C. I. R. Vera, J. Qu, Q. Lin, *Acs Chem. Biol.* **2010**, *5*, 875 – 885; e) Z. Yu, L. Y. Ho, Q. Lin, *J. Am. Chem. Soc.* **2011**, *133*, 11912 – 11915.

[8] C. Dalhoff, G. Lukinavicius, S. Klimasauakas, E. Weinhold, *Nat. Protoc.* **2006**, *1*, 1879 – 1886.

[9] M. Pignot, G. Pljevaljcic, E. Weinhold, *Eur. J. Org. Chem.* **2000**, 549 – 555.

[10] C. Dalhoff, M. Hüben, T. Lenz, P. Poot, E. Nordhoff, H. Köster, E. Weinhold, *ChemBioChem* **2010**, *11*, 256 – 265.

[11] J. K. K. Low, M. R. Wilkins, *FEBS J.* **2012**, *279*, 4423 – 4443.

[12] V. H. Weiss, A. E. McBride, M. A. Soriano, D. J. Filman, P. A. Silver, J. M. Hogle, *Nat. Struct. Biol.* **2000**, *7*, 1165 – 1171.

[13] C. M. Wong, H. M. V. Tang, K. Y. E. Kong, G. W. O. Wong, H. Qiu, D. Y. Jin, A. G. Hinnebusch, *Nucleic Acids Res.* **2010**, *38*, 2217 – 2228.

[14] X. Zhang, X. D. Cheng, *Structure* **2003**, *11*, 509 – 520.

[15] R. Wang, G. Ibánez, K. Islam, W. H. Zheng, G. Blum, C. Sengelaub, M. K. Luo, *Mol. BioSyst.* **2011**, *7*, 2970 – 2981.

[16] I. R. Bothwell, K. Islam, Y. L. Chen, W. H. Zheng, G. Blum, H. T. Deng, M. K. Luo, *J. Am. Chem. Soc.* **2012**, *134*, 14905 – 14912.

[17] W. Song, Z. Yu, M. M. Madden, Q. Lin, *Mol. BioSyst.* **2010**, *6*, 1576 – 1578.

[18] S. Ito, Y. Tanaka, A. Kakehi, K. Kondo, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1920 – 1923.

[19] J. Wang, H. Tan, Z. Zhao, *Protein Expression Purif.* **2007**, *53*, 97 – 103.

[20] C. Birsan, S. C. Litescu, G. L. Radu, *Anal. Lett.* **2010**, *43*, 793 – 803.

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