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Discovery of Bisubstrate Inhibitors for Protein N-Terminal Methyltransferase 1

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Abstract

Protein N-terminal methyltransferase 1 (NTMT1) plays an important role in regulating mitosis and DNA repair. Here, we describe the discovery of a potent NTMT1 bisubstrate inhibitor **4** ($IC_{50} = 35 \pm 2$ nM) that exhibits greater than 100-fold selectivity against a panel of methyltransferases. We also report the first crystal structure of NTMT1 in complex with an inhibitor, which revealed that **4** occupies substrate and cofactor binding sites of NTMT1.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](https://doi.org/10.1021/acs.jmedchem.9b00206) at DOI: 10.1021/acs.jmedchem.9b00206.

General procedures for the synthesis of intermediates **8** and **13**; NMR and MS spectra of **8**, **10**, **11**, and **13**; NMR, MALDI-MS, and LC-MS spectra of **14**; MALDI-MS and LC-MS spectra of **4**, **5**, and **6**; biochemical assay; Figure S1, IC_{50} determination of **14**; Figure S2, MALDI-MS results of MALDI-MS methylation inhibition assay for **4**; Figure S3, IC_{50} curve of **4** for MALDI-MS methylation inhibition assay; Figure S4, selectivity of **4**; cocrystallization and structure determination; Table S1, crystallography data and refinement statistics (PDB code 6DTN) ([PDF](#))

Molecular formula strings ([CSV](#))

Accession Codes

The coordinates for the structure of human NTMT1 in complex with **4** have been deposited under PDB code 6DTN. Authors will release the atomic coordinates and experimental data upon article publication.

The authors declare no competing financial interest.

INTRODUCTION

Protein post-translational modifications not only increase proteomic diversity but also play an important role in the regulation of gene expression and cell function. Therefore, dysregulation of such modifications is involved in many human diseases including inflammation, cancer, and neurodegenerative and metabolic diseases.^{1–3}

Protein N-terminal methyltransferase 1 (NTMT1/NRMT1) catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to protein α -N-terminal amines.^{4,5} It recognizes a specific motif X-P-K/R (X represents any amino acid other than D/E).^{6,7} Protein α -N-terminal methylation was observed on ribosome proteins, histone H2B, cytochrome c-557, and myosin light chain proteins over 4 decades ago.^{8–10} Recently, regulator of chromatin condensation 1 (RCC1), the tumor suppressor retinoblastoma 1 (RB1), oncoprotein SET, centromere protein A/B (CENP-A/B), damaged DNA-binding protein 2 (DDB2), and poly(ADP-ribose) polymerase 3 (PARP3) have been reported to undergo N-terminal methylation.^{4,11–17} The function of α -N-terminal methylation is traditionally inferred to regulate protein stability and protein–protein interaction. Recently, its role in protein–DNA interaction has been uncovered in chromosome segregation, mitosis, and DNA damage repair.^{11–19} For example, N-terminal methylation of RCC1 strengthens its interaction with chromatin during mitosis.²⁰ Additionally, N-terminal methylation of DDB2 promotes its recruitment to DNA damage site and facilitates nucleotide excision repair.¹⁴ Besides the aforementioned, NTMT1 is overexpressed in various cancer patient tissues including malignant melanoma and colorectal and brain cancer compared with normal tissue according to Protein Atlas. Knockdown of NTMT1 leads to hypersensitivity of breast cancer cell lines MCL-7 and LCC9 to etoposide and γ irradiation treatment.¹⁸ NTMT1 knockout mice exhibit developmental defects and impaired DNA repair.¹⁹

Such critical cellular processes and dysfunction in which NTMT1 is implicated impose an urgent need for potent and selective NTMT1 inhibitors as chemical probes to delineate the roles of NTMT1 under physiological and pathological conditions. So far, two NTMT1 inhibitors **1** ($IC_{50} = 0.81 \pm 0.13 \mu M$) and **2** ($IC_{50} = 0.94 \pm 0.16 \mu M$) (Figure 1) have been reported.^{21,22} They displayed 30- to 60-fold selectivity over protein lysine methyltransferase G9a and arginine methyltransferase 1 (PRMT1).^{21,22} Both inhibitors were designed to mimic the ternary complex as guided by a random sequential Bi–Bi mechanism, for which either peptide substrates or SAM cofactor could bind to NTMT1 first followed by binding the other substrate to form a ternary complex intermediate.²³ Although both compounds proved the principle of this mechanism-based design strategy, their potency and selectivity were modest. Furthermore, no structural information has been reported on how these inhibitors interact with NTMT1.

Recently, cocrystal structures of NTMT1 in complex with both S-adenosyl homocysteine (SAH) and peptide substrates have been disclosed.^{6,7} Among all tested peptide substrates, PPKRIA has the tightest binding affinity to NTMT1.⁶ Motivated by the structural information, we chose to covalently link the Pro-containing peptide substrate with a SAM analogue (**3**) in an attempt to obtain more potent NTMT1 bisubstrate inhibitors (Figure 2). However, previous synthetic routes to prepare known inhibitors **1** and **2** are not feasible to

prepare such bisubstrate analogues with a Pro at the first position of the peptide substrate portion.^{21,22,24} Here, we describe a new synthesis route to prepare a new series of bisubstrate inhibitors **4–6** (Scheme 1). The top inhibitor **4** ($IC_{50} = 158 \pm 20$ nM and $K_i = 39 \pm 9.5$ nM) is highly potent and selective in a fluorescence-based assay. Furthermore, **4** displays an IC_{50} of 35 ± 2 nM in a MALDI-MS based assay. Importantly, we have obtained the cocrystal structure of the inhibitor **4** in complex with NTMT1, which is the first complex structure of NTMT1 with its inhibitor to our knowledge. This complex structure clearly illustrates that our designed bisubstrate inhibitor binds to both SAM and peptide substrate binding sites of NTMT1. These data provide a framework toward the development of cell-potent inhibitors for NTMT1 to decipher its physiological role and pharmacological potential.

RESULTS AND DISCUSSION

Design.

There are two adjacent binding pockets that are occupied by SAH and the substrate peptide in the crystal structure of the NTMT1–PPKRIA–SAH ternary complex (PDB code 5E1M). The distance between the SAH sulfur atom and the α -nitrogen atom of the first Pro residue is ~ 5 Å. Therefore, we hypothesized that using a three-carbon atom linker (total linear distance of ~ 5 Å) to covalently link a SAM analogue **3** with a peptide substrate moiety to mimic the transition state would provide potent and selective bisubstrate analogues (Figure 2). The propylene linker for proposed compounds **4–6** is also supported by inhibitor **2**. We decided to incorporate Pro at the first position in this design because peptide substrate starting with Pro shows the highest binding affinity among all tested peptide substrates.^{6,7} We chose three different peptides PPKRIA, PPRRRS, and PPKR to generate **4–6** as bisubstrate inhibitors of NTMT1 (Figure 2) in order to explore how C-terminal sequence and length affect the activity. The PPKRIA peptide is derived from the N-terminus of mouse RCC1 protein. The PPRRRS is a mutant peptide derived from N-terminus of human CENP-A, where Pro replaces Gly at the first position.

Synthesis.

Compound **9** was synthesized as previously described.²¹ Then **9** was treated with p-NBS-Cl to protect amine group, followed by reacting with **8** to yield **10**.²⁵ Removal of NBS group provided **11**, which was subjected to hydrolysis and subsequent reductive amination with aldehyde **12** to provide **13**.^{25–27} Then **13** was coupled with peptides on resin and followed by cleavage to produce the bisubstrate analogues **4–6**. Meanwhile, direct deprotection of **13** offered **14**.²⁸

Biochemical Characterization.

SAH hydrolase (SAHH)-coupled fluorescence assay was employed to evaluate the inhibitory activities of all synthesized compounds by monitoring the production of SAH.^{23,29} SAM and peptide substrate GPKRIA were at their K_m values.

Compound **4** ($IC_{50} = 158 \pm 20$ nM, $K_i = 39 \pm 9.5$ nM) showed top inhibition against NTMT1 among all synthesized compounds (Figure 3A). When the PPKRIA peptide portion

of **4** was replaced by PPRRRS to yield **5** ($IC_{50} = 485 \pm 74$ nM, $K_i = 121 \pm 18$ nM) (Figure 3B), the inhibitory activity decreased about 3-fold. A similar result was observed when peptide PPKRIA was shortened to PPKR to offer **6** ($IC_{50} = 414 \pm 60$ nM, $K_i = 103 \pm 15$ nM) (Figure 3C). Since **14** only contains Pro instead of X-P-K/R peptide motif for NTMT1, we expected **14** to be less potent than **4–6**. Indeed, **14** did not show any significant inhibition against NTMT1 at 10 μ M (Figure S1).

MALDI-MS Methylation Inhibition Assay.

To validate the inhibition effect on NTMT1, we performed an orthogonal MALDI-MS methylation assay to directly evaluate the inhibitory activity effects of **4** on α -N-amine methylation progression over 20 min (Figures 4 and S2).³⁰ Even in the presence of 50 nM **4**, trimethylation of substrate peptide GPKRIA was substantially reduced to less than 10% (Figure S2). Neither di- nor trimethylation of GPKRIA was detected at 100 nM **4** (Figure 4A). Fitting these data yielded an IC_{50} of 35 ± 2 nM for **4** in this MALDI-MS based assay (Figure S3).

Selectivity Studies.

To evaluate the selectivity of **4**, we investigated its inhibitory activity over a panel of methyltransferases including two representative members from protein lysine methyltransferase PKMT (G9a and SETD7) and protein arginine methyltransferase PRMT (PRMT1 and *Tb*PRMT7), respectively. We also include nicotinamide *N*-methyltransferase (NNMT) that shares a SAM cofactor binding pocket. In addition, SAHH is included in the selectivity study because it has a SAH binding site and is used in the coupled fluorescence assay. As shown in Table 1 and Figure S4, **4** barely displayed any inhibition against all the enzymes at 3.3 μ M. At 33 μ M, **4** showed less than 20% inhibition on G9a, SETD7, and NNMT and less than 40% inhibition on PRMT1. At 100 μ M, **4** exhibited less than 30% inhibition on G9a, SETD7, and NNMT. The selectivity of **4** for NTMT1 is over 600-fold over G9a, SETD7, and NNMT and 200-fold over PRMT1, manifesting the high selectivity of the bisubstrate analogues. For *Tb*PRMT7 and SAHH, compound **4** showed less than 40% inhibition at 10 μ M. According to the estimated IC_{50} values for both enzymes, the selectivity of **4** is more than 100-fold over *Tb*PRMT7 and SAHH. The interaction of **4** with SAHH also explains the difference of IC_{50} values obtained from SAHH-coupled fluorescence and MALDI-MS based assay.

Inhibition Mechanism Studies.

To determine the inhibition mechanism of **4**, we performed kinetic analysis of **4** to determine the inhibition mechanism using the SAHH-coupled fluorescence-based assay (Figure 5).²³ Compound **4** showed an unambiguous pattern of competitive inhibition for the peptide substrate and SAM, as demonstrated by an ascending, linear dependence of the IC_{50} values on the peptide substrate or SAM concentration. This result indicated that **4** is a bisubstrate inhibitor that occupies cofactor and peptide substrate binding sites. In addition, this is consistent with its random sequential Bi–Bi mechanism, where peptide substrate or SAM cofactor can bind to NTMT1 first and followed by binding the other to form a ternary complex.²³

Cocrystal Structure of Compound **4** in Complex with NTMT1.

To elucidate the molecular interactions between the NTMT1 and **4**, we determined the first X-ray cocrystal structure of NTMT1 in complex with its inhibitor (PDB code 6DTN) (Figure 6A,B). Compound **4** was found to bind to the cofactor and substrate binding sites of NTMT1. Super-imposition of our NTMT1-**4** structure with the published NTMT1–PPKRIA–SAH ternary complex (PDB code 5E1M) gave an RMSD value of 0.35 Å (across all residues of chain A).⁶ The propylene linker (C3) mediates **4** binding at both sites simultaneously, which corroborated our design strategy and inhibition mechanism study. Specifically, the SAM analogue moiety (NAM) of **4** in the binary complex binds nearly identically with SAH. The inhibitor–protein interaction retains the same manner as previously observed with SAH-protein in the ternary complex of substrate peptide/SAH (Figure 6B–D).⁶ For example, the carboxyl group of NAM portion forms a salt bridge interaction with the side chain of Arg74 and the amino group forms two H-bonds with Gly69 and Gln135 (Figure 6A,D). Meanwhile, the adenine moiety of **4** forms two H-bonds with the backbone amide group of Leu119 and the side chain of Gln120. Hydroxyl groups of the ribose also form two H-bonds with side chains of Asp91 and Thr93. Meanwhile, the peptide portion of **4** also binds very similarly as the peptide substrate PPKRIA. The carbonyl oxygen of the first residue Pro interacts with the side chain of Asn168 through hydrogen bonding. The second Pro occupies a hydrophobic pocket that is formed by Leu31, Ile37, and Ile214. In addition, the ϵ -amine of the third Lys forms electronic interactions with carboxylate groups of Asp177 and Asp180.⁶ Last, direct H-bonds exist between the carbonyl oxygen of the fourth Arg and Try215, the amide group of the fifth residue and Glu213, and the amide group of the sixth residue with the side chain of Try215.

CONCLUSIONS

In summary, we designed and synthesized a new series of potent and selective bisubstrate inhibitors **4–6** of NTMT1.^{6,7,23} The top inhibitor, **4**, showed an IC₅₀ of 158 ± 20 nM in SAHH-coupled fluorescence assay. We confirmed its potent inhibition through an orthogonal MS-based assay, which displayed an IC₅₀ of 35 ± 2 nM. Compound **4** exhibited more than 100-fold selectivity for NTMT1 over other methyltransferases and SAHH. Kinetic analysis revealed **4** was a competitive inhibitor for SAM and peptide substrate. Furthermore, the cocrystal structure of NTMT1 in complex with **4** clearly showed that the bisubstrate inhibitor occupied cofactor SAM and substrate binding site, which is consistent with the inhibition mechanism. Despite its high potency and selectivity, **4** has poor cell permeability that restricts it from cell-based studies (data were not shown). However, these valuable results, especially the first cocrystal structure of inhibitors with NTMT1, provide the framework for future development of cell-potent inhibitors to decipher the physiological roles of NTMT1 and to validate its pharmacological potential.

EXPERIMENTAL SECTION

Chemistry General Procedures.

The reagents and solvents were purchased from commercial sources (Fisher) and used directly. Final compounds were purified on preparative high-pressure liquid chromatography

(RP-HPLC) Agilent 1260 series system. Systems were run with 0–20% methanol/water gradient with 0.1% TFA. NMR spectra were acquired on a Bruker AV500 instrument (500 MHz for ^1H NMR, 126 MHz for ^{13}C NMR). Matrix-assisted laser desorption ionization mass spectra (MALDI-MS) data were acquired in positive-ion mode using a Sciex 4800 MALDI TOF/TOF MS. The peptides (PKR, PKRIA, and PRRRS) were synthesized on a CEM Liberty Blue automated microwave peptide synthesizer with the manufacturer's standard coupling cycles at 0.1 mmol scale. The purity of final compounds was confirmed by Waters LC–MS system. Systems were run with 0–5% or 0–30% methanol/water gradient with 0.1% TFA. All the target compounds showed a purity of >95%.

NAM-C3-PPKRIA (4).

To a suspension of PKRIA on resin (0.1 mmol, 1.0 equiv) in DMF (3 mL) were added **13** (144 mg, 0.2 mmol, 2 equiv), DIC (31 μL , 0.2 mmol, 2 equiv), and HOBt (27 mg, 0.2 mmol, 2 equiv). The mixture was shaken overnight at rt. After filtration, the resin was subsequently washed with DMF (3 mL $\times$ 3), MeOH (3 mL $\times$ 3), and CH_2Cl_2 (3 mL $\times$ 3). The peptide conjugates were mixed with a cleavage mixture (10 mL) containing TFA/2,2'-(ethylenedioxy)diethanethiol/triisopropylsilane (TIPS)/water (94:2.5:1:2.5 v/v), and the suspension was shaken at rt for 4–5 h. The solvent of the filtrate was removed by nitrogen gas flow, and the residue was washed with 10 vol of cold anhydrous ether. After centrifugation, the supernatant was discarded. The residue was purified by reverse phase HPLC using an Agilent 1260 series system with 0.1% TFA in water (A) and MeOH (B) as the mobile phase. MALDI-MS (positive) m/z : calcd for $\text{C}_{48}\text{H}_{83}\text{N}_{18}\text{O}_{11}$ $[\text{M} + \text{H}]^+$ m/z 1087.6489, found m/z 1087.7162. LC–MS purity: >95%.

NAM-C3-PPRRRS (5).

Compound **5** was prepared according to the procedure for **4** and purified by reverse phase HPLC. MALDI-MS (positive) m/z : calcd for $\text{C}_{48}\text{H}_{83}\text{N}_{23}\text{O}_{12}$ $[\text{M} + \text{H}]^+$ m/z 1174.6670, found m/z 1174.9718. LC–MS purity: >95%.

NAM-C3-PPKR (6).

Compound **6** was prepared according to the procedure for **4** and purified by reverse phase HPLC. MALDI-MS (positive) m/z : calcd for $\text{C}_{39}\text{H}_{67}\text{N}_{16}\text{O}_9$ $[\text{M} + \text{H}]^+$ m/z 903.5277, found m/z 903.6199. LC–MS purity: >95%.

(3-(((S)-3-Amino-3-carboxypropyl)(((2R,3S,4R,5S)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-methyl)amino)propyl)-l-proline (14).

To a suspension of compound **13** (12 mg) in ddH_2O (0.3 mL) in an ice bath was added 4 N HCl in dioxane (0.6 mL). The mixture was stirred for 6 h at rt, and the volatiles were removed in vacuo. The residue was purified by cation-exchange chromatography (0.1 M NH_4HCO_3 aq solution). After lyophilization, 4 mg of white solid (46%) was obtained. ^1H NMR (500 MHz, D_2O) δ 8.15 (s, 1H), 8.09 (s, 1H), 5.91–5.85 (m, 1H), 4.17–4.07 (m, 2H), 3.69–3.62 (m, 1H), 3.62–3.55 (m, 1H), 3.47–3.38 (m, 1H), 3.07–2.88 (m, 2H), 2.88–2.65 (m, 4H), 2.65–2.42 (m, 4H), 2.26–2.14 (m, 1H), 1.94–1.72 (m, 4H), 1.72–1.58 (m, 3H).

MALDI-MS (positive) m/z : calcd for $C_{22}H_{35}N_8O_7$ $[M + H]^+$ m/z 523.2629, found m/z 523.4034. LC-MS purity: >95%.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

NTMT1	protein N-terminal methyltransferase 1
SAM	<i>S</i> -5'-adenosyl-L-methionine
SAH	<i>S</i> -5'-adenosyl-L-homocysteine
SAHH	SAH hydrolase
PKMT	protein lysine methyltransferase
PRMT	protein arginine methyltransferase
NNMT	nicotinamide <i>N</i> -methyltransferase
RCC1	regulator of chromatin condensation 1
RB1	tumor suppressor retinoblastoma 1
CENP-A/B	centromere protein A/B
DDB2	damaged DNA-binding protein 2
PARP3	poly(ADP-ribose) polymerase 3
MALDI-MS	matrix-assisted laser desorption ionization mass spectra
TFA	trifluoroacetic acid

REFERENCES

- (1). Scaramuzzino C; Casci I; Parodi S; Lievens PMJ; Polanco MJ; Milioto C; Chivet M; Monaghan J; Mishra A; Badders N; Aggarwal T; Grunseich C; Sambataro F; Basso M; Fackelmayer FO; Taylor JP; Pandey UB; Pennuto M Protein Arginine Methyltransferase 6 Enhances Polyglutamine-Expanded Androgen Receptor Function and Toxicity in Spinal and Bulbar Muscular Atrophy. *Neuron* 2015, 85, 88–100. [PubMed: 25569348]
- (2). Tsai W; Niessen S; Goebel N; Yates JR; Guccione E; Montminy M PRMT5 Modulates the Metabolic Response to Fasting Signals. *Proc. Natl. Acad. Sci. U. S. A* 2013, 110, 8870–8875. [PubMed: 23671120]

- (3). Cho M; Park J; Choi H; Park M; Won H; Park Y; Lee CH; Oh S; Song Y; Kim HS; Oh YH; Lee JY; Kong G DOT1L Cooperates with the C-Myc-P300 Complex to Epigenetically Derepress CDH1 Transcription Factors in Breast Cancer Progression. *Nat. Commun* 2015, 6, 7821. [PubMed: 26199140]
- (4). Schaner Tooley CE; Petkowski JJ; Muratore-Schroeder TL; Balsbaugh JL; Shabanowitz J; Sabat M; Minor W; Hunt DF; Macara IG NRMT Is an Alpha-N-Methyltransferase That Methylates RCC1 and Retinoblastoma Protein. *Nature* 2010, 466, 1125–1128. [PubMed: 20668449]
- (5). Webb KJ; Lipson RS; Al-Hadid Q; Whitelegge JP; Clarke SG Identification of Protein N-Terminal Methyltransferases in Yeast and Humans. *Biochemistry* 2010, 49, 5225–5235. [PubMed: 20481588]
- (6). Dong C; Mao Y; Tempel W; Qin S; Li L; Loppnau P; Huang R; Min J Structural Basis for Substrate Recognition by the Human N-Terminal Methyltransferase 1. *Genes Dev.* 2015, 29, 2343–2348. [PubMed: 26543161]
- (7). Wu R; Yue Y; Zheng X; Li H Molecular Basis for Histone N-Terminal Methylation by NRMT1. *Genes Dev.* 2015, 29, 2337–2342. [PubMed: 26543159]
- (8). Henry GD; Dalgarno DC; Marcus G; Scott M; Levine BA; Trayer IP The Occurrence of α -N-Trimethylalanine as the N-Terminal Amino Acid of Some Myosin Light Chains. *FEBS Lett.* 1982, 144, 11–15. [PubMed: 7106295]
- (9). Chen R; Brosius J; Wittmann-Liebold B; Schäfer W Occurrence of Methylated Amino Acids as N-Termini of Proteins from Escherichia Coli Ribosomes. *J. Mol. Biol* 1977, 111, 173–181. [PubMed: 323502]
- (10). Nomoto M; Kyogoku Y; Iwai K N-trimethylalanine, a novel blocked N-terminal residue of Tetrahymena Histone H2B. *J. Biochem* 1982, 92, 1675–1678. [PubMed: 6818230]
- (11). Sathyan KM; Fachinetti D; Foltz DR α -Amino Trimethylation of CENP-A by NRMT Is Required for Full Recruitment of the Centromere. *Nat. Commun* 2017, 8, 14678. [PubMed: 28266506]
- (12). Hao Y; Macara IG Regulation of Chromatin Binding by a Conformational Switch in the Tail of the Ran Exchange Factor RCC1. *J. Cell Biol* 2008, 182, 827–836. [PubMed: 18762580]
- (13). Dai X; Otake K; You C; Cai Q; Wang Z; Masumoto H; Wang Y Identification of Novel Alpha-N-Methylation of CENP-B That Regulates Its Binding to the Centromeric DNA. *J. Proteome Res* 2013, 12, 4167–4175. [PubMed: 23978223]
- (14). Cai Q; Fu L; Wang Z; Gan N; Dai X; Wang Y α -N-Methylation of Damaged DNA-Binding Protein 2 (DDB2) and Its Function in Nucleotide Excision Repair. *J. Biol. Chem* 2014, 289, 16046–16056. [PubMed: 24753253]
- (15). Dai X; Rulten SL; You C; Caldecott KW; Wang Y Identification and Functional Characterizations of N-Terminal Alpha-N-Methylation and Phosphorylation of Serine 461 in Human PARP3. *J. Proteome Res* 2015, 14, 2575–2582. [PubMed: 25886813]
- (16). Hitakomate E; Hood FE; Sanderson HS; Clarke PR The Methylated N-Terminal Tail of RCC1 Is Required for Stabilisation of Its Interaction with Chromatin by Ran in Live Cells. *BMC Cell Biol.* 2010, 11, 43. [PubMed: 20565941]
- (17). Huang R Chemical Biology of Protein N-terminal Methyltransferases. *ChemBioChem* 10.1002/cbic.201800615. Published Online: November 26, 2018.
- (18). Bonsignore LA; Butler JS; Klinge CM; Schaner Tooley CE Loss of the N-Terminal Methyltransferase NRMT1 Increases Sensitivity to DNA Damage and Promotes Mammary Oncogenesis. *Oncotarget* 2015, 6, 12248–12263. [PubMed: 25909287]
- (19). Bonsignore LA; Tooley JG; Van Hoose PM; Wang E; Cheng A; Cole MP; Schaner Tooley CE NRMT1 Knockout Mice Exhibit Phenotypes Associated with Impaired DNA Repair and Premature Aging. *Mech. Ageing Dev* 2015, 146–148, 42–52.
- (20). Chen T; Muratore TL; Schaner-Tooley CE; Shabanowitz J; Hunt DF; Macara IG N-Terminal Alpha-Methylation of RCC1 Is Necessary for Stable Chromatin Association and Normal Mitosis. *Nat. Cell Biol* 2007, 9, 596–603. [PubMed: 17435751]
- (21). Zhang G; Richardson SL; Mao Y; Huang R Design, Synthesis, and Kinetic Analysis of Potent Protein N-Terminal Methyltransferase 1 Inhibitors. *Org. Biomol. Chem* 2015, 13, 4149–4154. [PubMed: 25712161]

- (22). Zhang G; Huang R Facile Synthesis of SAM-Peptide Conjugates through Alkyl Linkers Targeting Protein N-Terminal Methyltransferase 1. *RSC Adv.* 2016, 6, 6768–6771. [PubMed: 27588169]
- (23). Richardson SL; Mao Y; Zhang G; Hanjra P; Peterson DL; Huang R Kinetic Mechanism of Protein N-Terminal Methyltransferase 1. *J. Biol. Chem* 2015, 290, 11601–11610. [PubMed: 25771539]
- (24). Mori S; Iwase K; Iwanami N; Tanaka Y; Kagechika H; Hirano T Development of Novel Bisubstrate-Type Inhibitors of Histone Methyltransferase SET7/9. *Bioorg. Med. Chem* 2010, 18, 8158–8166. [PubMed: 21036620]
- (25). Fukuyama T; Jow CK; Cheung M 2- and 4-Nitro-benzenesulfonamides: Exceptionally Versatile Means for Preparation of Secondary Amines and Protection of Amines. *Tetrahedron Lett.* 1995, 36, 6373–6374.
- (26). Karoyan P; Chassaing G New Strategy for the Synthesis of 3-Substituted Prolines. *Tetrahedron Lett.* 1997, 38, 85–88.
- (27). Van Haren MJ; Taig R; Kuppens J; Sastre Toraño J; Moret EE; Parsons RB; Sartini D; Emanuelli M; Martin NI Inhibitors of Nicotinamide N-Methyltransferase Designed to Mimic the Methylation Reaction Transition State. *Org. Biomol. Chem* 2017, 15, 6656–6667. [PubMed: 28758655]
- (28). Babault N; Allali-Hassani A; Li F; Fan J; Yue A; Ju K; Liu F; Vedadi M; Liu J; Jin J Discovery of Bisubstrate Inhibitors of Nicotinamide N-Methyltransferase (NNMT). *J. Med. Chem* 2018, 61, 1541–1551. [PubMed: 29320176]
- (29). Collazo E; Couture JF; Bulfer S; Trievel RC A Coupled Fluorescent Assay for Histone Methyltransferases. *Anal. Biochem* 2005, 342, 86–92. [PubMed: 15958184]
- (30). Richardson SL; Hanjra P; Zhang G; Mackie BD; Peterson DL; Huang R A Direct, Ratiometric, and Quantitative MALDI-MS Assay for Protein Methyltransferases and Acetyltransferases. *Anal. Biochem* 2015, 478, 59–64. [PubMed: 25778392]

Figure 1.
Chemical structures of NTMT1 inhibitors.

Figure 2.
Design of the bisubstrate inhibitors for NTMT1.

Figure 3.
IC₅₀ determination for **4–6** against NTMT1 ($n = 3$).

Figure 4. MALDI-MS methylation inhibition assay of **4**: (A) MALDIMS results of MALDI-MS methylation inhibition assay for **4**; (B) quantification of methylation progression of GPRIA by NTMT1 with **4** at 20 min ($n = 3$).

Figure 5. Inhibition mechanism studies of **4**: (A) IC₅₀ curves of **4** at varying concentrations of SAM with fixed concentration of GPKRIA; (B) linear regression plot of IC₅₀ values with corresponding concentrations of SAM; (C) IC₅₀ curves of **4** at varying concentrations of GPKRIA with fixed concentration of SAM; (D) linear regression plot of IC₅₀ values with corresponding concentrations of GPKRIA.

Figure 6.

X-ray crystal structure. (A) X-ray crystal structure of **4** (green) in complex with NTMT1 (gray) in a binary complex (PDB code 6DTN). Analysis of the interactions with NTMT1 (gray ribbon) is depicted here with hydrogen bonds shown as yellow dashes and interacting residues from NTMT1 shown in gray stick. (B) Comparison of **4** to substrate bound complex. The similarity of the binding mode of compound **4** (left, green stick) within NTMT1 to those observed previously with SAH and PPKRIA (right, gray stick, PDB code 5E1M) is illustrated here with a side-by-side comparison. (C) Compound **4** (green stick) bound to NTMT1 (gray cartoon) with the $F_o - F_c$ omit electron density map contoured at 3.0σ depicted as a transparent green isomesh. (D) Compound **4** interaction diagram (Schrödinger Maestro) with NTMT1.

Scheme 1. Synthetic Route^a

^aReagents and conditions: (a) p-NBS-Cl, K₂CO₃, DMF; (b) **8**, Cs₂CO₃, DMF, 80 °C, 56% in two steps; (c) Cs₂CO₃, mercaptoethanol, rt, 52%; (d) LiOH, MeOH, H₂O, quantitative yield; (e) NaBH₃CN, MeOH, 75%; (f) 4 N HCl in dioxane, H₂O, 0 °C to rt, 46%; (g) (i) peptide on resin, DIC, HOBT, DMF; (ii) TFA:DODT:H₂O:TIPS; (h) 1-bromo-3-chloropropane, K₂CO₃, KI, acetone, reflux, 80%.

Table 1.

Selectivity Evaluation of Compound 4

	enzyme activity (%) ^a				
	concentration of 4				
	3.3 μ M	10 μ M	33 μ M	100 μ M	IC ₅₀ (μ M)
NTMT1	14	14			0.158 $\pm$ 0.02
G9a	106	82	81	78	>100
PRMT1	97	92	64	39	>33
SETD7	94	99	97	83	>100
7bPRMT7	89	73	40	21	>10
NNMT	98	94	85	74	>100
SAHH	78	61	43	25	>10

^aThe values of enzyme activity for NTMT1 are mean values of triplicate experiments ($n = 3$). The values of enzyme activity for other enzymes are mean values of duplicate experiments ($n = 2$).