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SAM/SAH Analogs as Versatile Tools for SAM-Dependent Methyltransferases

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Abstract

S-Adenosyl-*L*-methionine (SAM) is a sulfonium molecule with a structural hybrid of methionine and adenosine. As the second largest cofactor in the human body, its major function is to serve as methyl donor for SAM-dependent methyltransferases (MTases). The resultant transmethylation of biomolecules constitutes a significant biochemical mechanism in epigenetic regulation, cellular signaling, and metabolite degradation. Recently, numerous SAM analogs have been developed as synthetic cofactors to transfer the activated groups on MTase substrates for downstream ligation and identification. Meanwhile, new compounds built upon or derived from the SAM scaffold have been designed and tested as selective inhibitors for important MTase targets. Here, we summarized the recent development and application of SAM analogs as chemical biology tools for MTases.

Graphical Abstract

1. INTRODUCTION

S-Adenosyl-*L*-methionine (AdoMet or SAM) is a sulfonium compound with a structural hybrid of methionine and adenosine. The asymmetric substitution of sulfonium ion presents a chiral center, and both (*R*)- and (*S*)-epimers are optically stable and can be separated. In living cells, the natural SAM ((*S*)-epimer) is biosynthesized from *L*-methionine and ATP by methionine adenosyltransferase (MAT; step one in Figure 1a).

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Notes

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As the second most ubiquitous cofactor, one major function of SAM is to serve as a methyl donor to the N-, C-, O- or S-nucleophiles under the catalysis of methyltransferases (MTases).^{1–5} Various substrates (DNA, RNA, protein, carbohydrate, and small molecule metabolite) can be adopted, and their methylation plays a crucial role in epigenetic regulation, protein function modulation, synthesis and degradation of metabolites and signaling molecules, etc. Therefore, this transmethylation reaction is of wide biological significance. Generally, the reaction is carried out through an S_N2-type mechanism with a nucleophilic attack to the methyl group adjacent to the sulfonium center. Besides the methylated product, it generates *S*-adenosyl-*L*-homocysteine (AdoHcy, SAH) as the byproduct (step two in Figure 1a). Early studies showed that the reaction is very stereospecific. Only the natural (*S*)-epimer is preferentially utilized by most of the enzymes.^{6,7} The (*R*)-epimer which shares similar binding pocket on MTases, however, fails in the methyl transfer step due to unfavorable geometry of the sulfonium center.

SAM-dependent MTases are a large and diversified class of enzymes with a cofactor (SAM) binding site and a substrate binding site.⁸ Based on structural characteristics, MTases can be divided into five classes (I–V).⁹ For simplicity, we discuss MTases in this review based on their substrates. The DNA methyltransferases catalyze the transfer of methyl groups to N4 and C5 positions of cytosine or the N6 position of adenine bases in DNA (Figure 1b),¹⁰ and the C5-cytosine methyltransferases include DNMT1 and DNMT3. DNMT1 is a maintenance DNA methyltransferase, mainly responsible for methylating the newly synthesized DNA strands, while DNMT3 (mainly DNMT3A and 3B) is in charge of *de novo* DNA methylation. In humans, DNA methylation occurs at C5 of cytosine at CpG dinucleotide, and the DNA methylation is usually associated with gene repression. Protein methyltransferases (PMTs), also referred to as histone methyltransferases (HMTs), catalyze the methylation of lysine or arginine residues on the histone or nonhistone substrates. They can be simply divided into protein lysine methyltransferase (PKMT), protein arginine methyltransferase (PRMT), and other PMTs. The PKMT is responsible for the generation of mono-, di-, and trimethylated lysine residues, while the PRMT is responsible for the generation of monomethyl arginine (MMA), symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA; Figure 1b). The PKMT is a large class of enzymes with more than 50 members and can be divided into eight groups (KMT1–KMT8). The PRMT can be distinguished into three subtypes based on the methylation products: type I (PRMT1–4, 6, 8) for ADMA and MMA generation, type II (PRMT5 and PRMT9) for SDMA and MMA generation, and type III (PRMT7) for MMA generation only. Other PMTs include those methyltransferases which target C-terminal, N-terminal, and peptidyl side chains other than lysine and arginine residues. Particularly, the discovery of protein N-terminal methyltransferases (NTMTs) has received much attention lately.^{11–14} Besides DNA and protein MTases, many MTases exist for the methylation of small molecules. For instance, the catechol *O*-methyltransferase (COMT), which catalyzes the methyl transfer to the catecholamines for neurotransmitter degradation, is implicated in numerous neurological disorders such as Parkinson's disease (PD).^{15–17}

SAM and SAH have long been recognized as of great value in exploring the functions of MTases. In the past, many SAM/SAH analogs have been synthesized as MTase inhibitors. However, due to the restriction of protein expression and purification techniques, many of

these studies are quite limited. In recent years, SAM/SAH analogs have found more diversified applications in the emerging fields of novel MTases such as DNMTs and PMTs. Herein, we summarize the recent applications of SAM/SAH analogs as chemical biology tools for studying MTases, with particular relevance to epigenetic regulation. We will first discuss their development as MTase inhibitors and then their contributions as synthetic cofactors and others to study enzyme substrate profiles and MTase functions. In the end, we will discuss limitations and potentials. There exist several other excellent reviews to which the readers may be directed for further details on chemical biology of methyltransferase, ^{3,9,18–25} protein methyltransferase probes, ^{26–30} and DNA methyltransferase probes. ^{31–35}

2. DEVELOPMENT OF SAH ANALOGS AS MTASE INHIBITORS

DNMT and PMT are two important families of epigenetic enzymes responsible for regulation of DNA and histone functions, respectively. It is well-known that the aberrant DNA/histone methylation accounts for a plethora of diseases, especially cancer, ³⁶ and DNMT and PMT are validated targets for cancer therapy with several drugs approved or in clinical trials. Therefore, development of MTase inhibitors has garnered great attention. They not only serve as potential therapeutics but also are useful chemical probes for MTase biology study. SAM/SAH analogs have long been recognized and studied as small molecule inhibitors, with much work on structural modifications on the amino acid chain, sugar, and base portions by Borchardt, Wu, and others. ^{37–46} Below, we discuss the recent development of SAM/SAH-based MTase inhibitors.

2.1. Pan Inhibitors

Pan-MTase inhibitors such as SAH, sinefungin, and methylthioadenosine (MTA) are SAH mimics (Figure 2) and generally lack specificities among different MTases.

2.1.1. SAH—As a product of SAM-dependent transmethylation reaction, SAH has been known to be a non-selective feedback inhibitor for many MTases. ⁴⁷ It can be degraded into adenosine and homocysteine by SAH hydrolase. Therefore, in cellular studies, SAH hydrolase inhibitors such as adenosine dialdehyde are used as SAM-dependent MTase inhibitors by blocking the degradation of SAH.

2.1.2. Sinefungin—Sinefungin is a natural nucleoside analog of SAH with the capability to inhibit a variety of SAM-dependent MTases. Especially, virus-encoded mRNA cap methyltransferase is more sensitive to sinefungin than other MTases; therefore, sinefungin may be developed as a useful antiviral agent. ^{48,49} Besides this, it could also serve as a starting point for structural modification. In 2012, the Luo group developed a series of sinefungin analogs based on careful analysis of the transition state of protein lysine methylation. ⁵⁰ They discovered that *n*-propyl sinefungin **3** was a selective inhibitor for SETD2 ($IC_{50} = 0.8 \pm 0.02 \mu M$) because the flexible binding pocket of SETD2 is capable of accommodating larger groups such as propyl. Recently, compound **4** with a cyclohexyl substitution of the amino acid side chain was reported to be a EHMT1 and EHMT2 inhibitor ($IC_{50} = 1.5$ and $1.6 \mu M$, respectively) with excellent selectivity over PRMT1 and SET7/9. ⁵¹

2.1.3. Methylthioadenosine—Methylthioadenosine (MTA) is a natural sulfur-containing nucleoside widely present in different organisms.⁵² It is reported as a pan-inhibitor of MTases,⁵³ possibly by directly inhibiting activity of MTases or by blocking the activity of SAH hydrolase. It is cell permeable, and therefore, it is one of the most popular pan-inhibitors of MTases.

2.2. Specific MTase Inhibitors

2.2.1. Allele-Specific Inhibitors—The paucity of specific inhibitors for MTases poses a great challenge for enzyme function studies and substrate identification. One chemical genetic approach named “bump-and-hole” may provide a new way around this.⁵⁴ In this approach, both the wild-type enzyme and its natural cofactor are manipulated to construct a “pair”: the enzyme is engineered to generate a “hole,” while the cofactor is modified with large substituent to come up with a “bump” to fit into the “hole.” The modified cofactor should not pair with the wild-type enzyme; neither should the engineered enzyme and natural cofactor. In 2001, the first application of this strategy in Rmt1 (a yeast PRMT1) was reported.⁵⁵ A single E117G mutation in the cofactor-binding pocket of the enzyme was made, and a series of 6-substituted SAH/SAM analogs were screened. It is found that N⁶-benzyl SAM **6** was an effective and selective cofactor for the mutant enzyme. Meanwhile, the N⁶-naphthylmethyl SAH derivative **8** is an allele-specific inhibitor for the mutant enzyme with a K_i of 5.0 μ M and >20-fold selectivity over the wild-type enzyme (Figure 3a). By monitoring the gene and mRNA expressions after applying probe **8** or **9** (Figure 3b), the authors were able to identify a possible connection between PRMT activity and kinase activity. Later, the Zhou lab also identified a vSET mutant and its matched 2',3'-dibenzyl SAM (compound **7** in Figure 3a) cofactor pair.⁵⁶

The “bump and hole” strategy possesses great potential for substrate labeling and enzyme functional study. However, in some cases, the modified enzyme-cofactor pairs showed significantly reduced enzymatic activities (K_{cat}/K_m).⁵⁵ Besides this, the limited stability and bioavailability of the synthetic inhibitors or cofactors also limit their further applications.

2.2.2. Bisubstrate Inhibitors—Usually, SAM/SAH-based inhibitors show inadequate selectivity due to the high homology of SAM binding domains of different MTases. In this respect, bisubstrate analogs have the potential to afford increased potency and selectivity by combining the free binding energies of the cofactor and substrate interactions without suffering entropic loss (Figure 4a).⁵⁷ Ideal bisubstrate MTase inhibitors should have an effective linker between the two fragments that mimic the transition state of the methyl transfer reaction (Figure 4b), with one fragment of the inhibitor targeting the SAM-binding site and the other targeting the substrate-binding site. Such an optimal linker is essential for the synergistic effect of the two components, which dictates the affinity and selectivity of the bisubstrate inhibitor.

2.2.2.1. COMT Inhibitors: COMT inhibitors are important for alleviating Parkinson's disease (PD) as they can decrease the deactivation of L-dopa in the periphery, and two 3-nitrocatechol containing COMT inhibitors (tolcapone and entacapone) have been introduced to the market. In 2000, the first bisubstrate inhibitor of COMT was reported by the

Diederich group.^{58,59} Compound **10** ($IC_{50} = 2 \mu M$ for COMT) was designed via a computational method based on the crystal structure of the COMT complex with Mg^{2+} , SAM, and 3,5-dinitrocatechol (Figure 5a). Kinetic analysis showed that this compound is a competitive inhibitor for SAM and a noncompetitive inhibitor for catechol, suggesting that it may go through a “two-step” binding by occupying the SAM binding site first. Later modifications on the linker part based on the molecular modeling led to compound **11** with dramatically increased potency ($IC_{50} = 9 nM$).⁶⁰ The X-ray cocrystal structure of this compound with COMT confirmed its bisubstrate binding mode. Since the nitrocatechol structure may cause hepatotoxicity, the nitro group was later replaced with a series of substituted phenyl moieties, and the resulted compound **12** could still maintain potent COMT inhibition ($IC_{50} = 21 nM$).⁶¹ A further modification on the ribose part and adenine part also yielded compounds with high potency as exemplified by 3'-deoxyl compound **13** and 6-substituted compound **14** with IC_{50} 's of 40 nM and 9 nM, respectively.^{62–64}

2.2.2.2. PRMT and PKMT Inhibitors: Protein arginine methyltransferase 1 (PRMT1) is the most prevalent PRMT in cells and accounts for over 80% of arginine methylation. In 2010, the first synthetic bisubstrate inhibitors of PRMT1 were reported.⁶⁵ They are 5'-N SAM analogs with the methyl group extended to accommodate the guanidinium group of arginine residue (Figure 5b). Different carbon linkers between guanidinium and the 5'-N moiety ($n = 1–3$) were used. Compound **15** shows moderate inhibition for both PRMT1 and CARM1 (for PRMT1, $IC_{50} = 6.2 \mu M$; for CARM1, $IC_{50} = 13.3 \mu M$), and its analog **16** with one methylene extension improved the potency and selectivity (for PRMT1, $IC_{50} = 2.9 \mu M$; for CARM1, > 50% remaining enzyme activity at 100 μM).⁶⁶

In 2015, another type of PRMT bisubstrate (compounds **17–20**) was reported, in which the amino acid side chain of compounds **15** and **16** was omitted and the guanidinium group was directly attached to the 5' position of adenosine via C/N/S linkers.⁶⁷ It is found that potency and selectivity of inhibitors respond dramatically to small changes in the chemical structure. For example, compound **17** showed excellent inhibition for CARM1 ($IC_{50} = 0.12 \mu M$) with ca. 100-fold selectivity against PRMT1 and PRMT6, while compound **18** with one methylene extension generally lost selectivity (for PRMT1, $IC_{50} = 1.30 \mu M$; for CARM1, $IC_{50} = 0.56 \mu M$; for PRMT6, $IC_{50} = 0.72 \mu M$). Compound **19** with an S-linker is a selective inhibitor for G9a ($IC_{50} = 3.18 \mu M$) while compound **20** with an N-linker is selective for PRMT6 ($IC_{50} = 3.2 \mu M$). Another structure manipulation of the guanidinium moiety of compound **19** yielded the urea compound **DS-437 (21)**. An inhibition profiling of this compound over 27 PMTs showed that it is devoid of G9a activity. Instead, it is a dual PRMT5-PRMT7 inhibitor, with IC_{50} 's of $5.9 \pm 1.4 \mu M$ and $6 \pm 0.5 \mu M$ for the PRMT5-MEP50 complex and PRMT7, respectively.⁶⁸ In addition, the application of this bisubstrate inhibitor design in PKMT is also reported.⁶⁹ The typical compound is shown as compound **22** with a terminal substituted amine group. It displayed a remaining activity of ca. 10% for SET7/9 at 100 μM .

2.2.2.3. NTMT Inhibitor: Protein N-terminal methyltransferase 1 (NTMT1) is one type of enzyme for methylating the N-terminal amine of protein. Specifically, it recognizes a canonical motif of X-P-K (X = A, P or S). Recently, the Huang group reported the first

NTMT1 inhibitor using a bisubstrate inhibitor strategy. The compound **NAM-TZ-SPKRIA** (**23**, Figure 5b) was a conjugate of 5'-N SAM and hexapeptide (SPKRIA) via a triazole moiety.⁷⁰ It had an IC₅₀ value of $0.81 \pm 0.13 \mu\text{M}$ for NTMT1 and over 60-fold selectivity over other major PMTs. A kinetic study showed that it exhibited a competitive inhibition pattern for both substrates, confirming its bisubstrate inhibition pattern.

Generally, a SAH-based bisubstrate inhibitor is a promising strategy to generate potent and selective compounds. It can be seen that the X-ray structure based design is quite successful in the development of COMT bisubstrate inhibitors.⁵⁹ However, for PMT bisubstrate inhibitors, the inhibition profiles in response to structure change seem to be difficult to predict, probably due to dedicated structural difference of binding domains in different MTases subtypes. Therefore, vigorous examination of the crystal structure of the enzyme complex is desired. Meanwhile, docking studies also provide a good way to aid inhibitor design and bioactivity rationalization. Right now, only CARM1 crystal structure in PMT has been successfully used for docking studies.^{66,67} For instance, Ward *et al.* performed docking studies of compounds **15** and **16** with CRAM1 and PRMT1.⁶⁶ They found that the guanidine group of compound **15** extends into the substrate channel and interacts with the “double-E loop” (E258 and E267) of CRAM1. For compound **16**, the longer chain pushed the guanidine group away from E258 leading to dramatically reduced CRAM1 inhibition. However, PRMT1 inhibition of this compound could be maintained because the guanidine group could also interact with a unique E47 in the substrate channel of PRMT1. Besides the above-discussed methyltransferase inhibitors, the concept of bisubstrate inhibitor design can also be extended to other methyltransferase members. For example, Vaubourgeix *et al.* found that S-adenosyl-N-decylaminoethyl, in which the amino acid side chain of SAH was replaced by a lipid chain, is a potent inhibitor of *Mycobacterium tuberculosis* mycolic acid methyltransferases.⁷¹

2.2.3. Inhibitors Containing Amended SAM Scaffolds

2.2.3.1. DNMT Inhibitors: DNMTs are validated anticancer targets with two inhibitors (Decitabine and Azacitidine) approved. However, as mechanism-based inhibitors, they need to be incorporated into the DNA to exert their biological activities. Moreover, they are not DNMT selective and also suffer from stability problems.^{34,35} Therefore, developing new DNMT inhibitors is of significant value. Most DNMT inhibitors are nucleoside (mainly cytidine) analogs. Recently MethylGene Inc. conducted structural modifications on the amino acid chain and adenine part of SAH.^{72,73} They found that the sterically restricted proline mimic (as in compound **24** in Figure 6) can serve as a substituent for a free amino acid chain with similar activity, while the six-membered and the decarboxylate counterparts were less active. On the adenine part, they conducted multiple substitutions on the 2-position of adenine and replaced adenine with other heterocycles. It is found that the 6-amine of SAH is crucial for the inhibition, and the 7-deaza SAH (compound **25**) could maintain the activity of SAH. The same trend for the 7-deaza analog was also observed for DOT1L inhibitors. In addition, DNA adenine methyltransferase is a potential antimicrobial target since this type of MTases does not exist in eukaryotes. Different modifications on the 5'- and N-6 positions have been conducted, and micromolar potency on the inhibition of bacterial MTase could be reached.^{74–76}

2.2.3.2. PMT Inhibitors: DOT1L is a unique PKMT in that it does not possess a SET domain, and it is the only enzyme to catalyze the methylation of H3K79, which is located in the globular domain of the histone octamer core. DOT1L is an important target for acute leukemia with mix lineage leukemia (MLL) translocations.^{77–79} As a first potent and selective DOT1L inhibitor, compound EPZ004777 (**26**, Figure 7) was developed by Epizyme Inc. in 2011.⁸⁰ This compound is a SAM-competitive inhibitor with an IC_{50} of 0.4 ± 0.1 nM and showed excellent selectivity over a panel of other MTases. In cellular studies, EPZ004777 could selectively inhibit the H3K79 methylation and block the MLL fusion target (*HOXA9* and *MEIS1*) expression. Besides, it also showed antiproliferative effects in MLL-rearranged cell lines with a delay (6–8 days), but had no effect on the non-MLL-rearranged cell line. The antitumor effect of this compound was further confirmed in the mouse xenograft model of MLL. However, due to the poor pharmacokinetic properties, mini-pumps had to be used for its delivery in animal studies. Later modifications based on EPZ004777 yielded compound EPZ-5676.^{81,82} Compared with EPZ004777, it displayed improved DOT1L inhibition (K_i value of 0.08 ± 0.03 nM vs 0.3 ± 0.02 nM) and residence time (>24 h vs 1 h) and was more potent in cellular studies. In September 2012, compound EPZ-5676 was advanced for phase 1 clinical study. Currently, it is under clinical study for the treatment of adult MLL-r and pediatric MLL-r.⁸³

The crystal structure of EPZ004777 in complex with DOT1L by Structural Genomics Consortium (SGC) revealed that this compound occupied the SAM-binding site. The ability of DOT1L to accommodate the bulky *t*-butylphenyl moiety is due to the conformational rearrangement of Tyr312 and Thr139 at substrate-binding and activation loops.⁸⁴ This conformational remodeling mechanism also agrees with the previous crystal structure of the other EPZ004777 analog with DOT1L.⁸⁵ And based on the crystal structure, a 7-bromo analog SGC0946 was designed to explore a hydrophobic cleft in this position. Compound SGC0946 showed increased potency ($K_D = 0.06 \pm 0.01$ nM) as compared with EPZ004777 ($K_D = 0.25 \pm 0.02$ nM). It also demonstrated increased residence time and permeability leading to greater efficacy in cellular studies. It should be noted that 7- position is an important site for DOT1L binding because 7-bromo-deaza-SAH was also reported to be a potent and selective DOT1L inhibitor ($IC_{50} = 77$ nM).⁸⁶

In the meantime, the Song group also reported an extensive structure modification on the 5'- and 6-N positions of SAH.^{87,88} Among 55 different carbamate, amide, and urea analogs, they found that compound **28** and its 6-methyl analog **29** are highly potent and selective DOT1L inhibitors with IC_{50} values of 0.46 nM and 0.76 nM (Figure 7), respectively. In two MLL-rearranged leukemia cell lines, these two compounds could block the cell growth slowly at 10 μ M and 30 μ M levels with EC_{50} 's of 4.4–11.0 μ M under a 20 day treatment. The Song group further reported a cyclopentane-containing compound **30** with improved metabolic stability in human liver and plasma microsomes.⁸⁹

Besides serving as a promising treatment for MLL, this series of compounds also opened opportunities for exploring the DOT1L inhibition in other diseases or other applications.^{90–92} For example, a fluorescent probe based on compound EPZ004777 was designed and can be used for high-throughput screening.⁹³ By using compound EPZ004777, it is also shown that DOT1L inhibition could enhance the reprogramming of somatic cells.⁹⁴

3. DEVELOPMENT OF SAM ANALOGS AS SYNTHETIC COFACTORS

Besides the use as MTase inhibitors, SAM analogs and derivatives may serve as synthetic cofactors for substrate labeling. It is well-known that PMTs also possess the ability to methylate other noncanonical protein targets. For understanding the functional activities of MTases and the methylations thereof, it is important to elucidate their substrates profiles. However, one impediment for substrates identification and assay development comes from the small size and inherent inert chemical reactivity of methyl group. Traditionally, radioactive SAM, methyl-specific antibody, and mass spectrometry are generally exploited as “methylation reporters”. Recently, it is shown that the modified SAM analogs could be effective synthetic cofactors (i.e., cofactor surrogates) to label substrates by selectively introducing activated groups on substrates for later derivatization. Since the sulfonium center is the key moiety for transmethylation, most of the current modifications on synthetic cofactors are centered on this moiety. These SAM analogs can be mainly divided into two groups: aziridinoadenosines and double-activated SAM analogs. In both cases, SAM analogs serve as bioorthogonal probes, leading to “Sequence-specific Methyltransferase-Induced Labeling (SMILing)” and “methyltransferase-directed Transfer of Activated Groups (mTAG),” respectively.^{95,96} Usually, C8- and N6- positions of the adenine part are major sites for introducing reporter or affinity groups because MTases are more tolerable with these modifications. In addition, due to the instability of some double-activated synthetic cofactors, the selenium-based SAM analogs have also been explored.

3.1. Aziridinoadenosines

Aziridines are highly active electrophiles featured by a three-membered ring containing one nitrogen. When the 5'-sulfonium substitution of SAM is replaced by an aziridine group, the resulted aziridinoadenosine could be attacked by nucleophiles in an S_N2 mechanism leading to ring opening. Different from the SAM-mediated transmethylation reaction, which yields a methylated product, the aziridinoadenosine-mediated reaction yields a conjugate of the cofactor and substrate. It is hypothesized that this alkylation could be carried out in a highly selective way at the recognition sites of SAM and substrates under the catalysis of MTases. Early exploration of this “MTase-driven alkylation” was done by the Weinhold group in 1998.⁹⁷ In the presence of compound **32** (Table 1) and a stoichiometric amount of M *TaqI* (a type of N₆-adenine DNA methyltransferase), a short oligodeoxynucleotide duplex could be coupled with the synthetic cofactor as evidenced by the RP-HPLC/ESI-MS, and no nonenzymatic reaction was observed. Based on this compound, a fluorescent cofactor was developed by attaching a fluorophore at the 8-position.⁹⁸ Compound **33**, which is an 8-azide derivative of compound **32**, was later developed by the Rajske group in 2005. The DNA adduct of this probe was shown to be capable of coupling with biotin or fluorophore via Staudinger ligation.^{99,100}

Shortly after, compound **34** with a propargyl substitution on 5'-N was proposed to improve the reactivity and stability.¹⁰¹ These types of compounds show great advantage in that they can be generated *in situ* from N-mustard analogs by intramolecular cyclization between the beta-halide and amine. The transferred propargyl group also enables further bioorthogonal ligation. Later, structural modification on this probe showed that analogs **35** and **36**, in

which the amino acid side chain of SAM was maintained, displayed more enhanced DNA alkylation by DNA MTase M. *EcoRI*, probably because the optimal interaction between MTase and SAM was retained.^{102,103} The versatility of this probe was also demonstrated by rebeccamycin MTase (RebM) to generate different small molecule derivatives.¹⁰⁴

In 2008, the Thompson group applied probe **35** for PRMT1.¹⁰⁵ The authors showed that PRMT1 could direct the transfer of synthetic cofactor fragment to the H4–21 peptide substrate region-specifically at Arg-3 in an enzyme-dependent manner. Further investigation on the resulted conjugated bisubstrates showed that it had an IC₅₀ value of lower than 18.5 μM with a 4.4-fold selectivity for PMRT1 over CARM1. This might provide a new way to generate PRMT1 inhibitors *in situ*. Recently, azide and alkyne derivatives of this compound on 6- and 8- positions were exploited for the same enzyme.¹⁰⁶ It is found that PRMT1 can only accommodate small substitutions such as azide on the 8- position (exemplified by compound **37**). For 6- modification, it can accommodate more extended chains (exemplified by compound **38**).

Another interesting application of this type of compound comes from the Song lab as DOT1L inhibitors.¹⁰⁷ They found that extending the amino acid side chain with one more methylene group could significantly increase the DOT1L inhibition. The IC₅₀ of compound **39** decreased to 0.038 μM as compared with 15.7 μM of compound **35**. The enhanced potency may be because ethylene extension can more replicate the SAM binding, since the C–N bonds in the aziridine SAM analogs are shorter than the C–S bonds in SAM (~1.47 Å compared with ~1.82 Å). This compound also showed moderate inhibition toward CARM1, PRMT1, and G9a with IC₅₀'s of 1.1, 2.7, and 1.8 μM , respectively. In addition, by examining the crystal structures of DOT1L-SAM, CARM-SAH, and G9a-SAH, the authors found that DOT1L possesses a unique hydrophobic pocket with only one single hydrogen bond with 6-NH₂. Therefore, with an extra methyl or benzyl group at the 6-NH₂ position (compound **40**), the selectivity for DOT1L (IC₅₀ = 0.12 μM) can be greatly enhanced with IC₅₀ >100 μM for the three other enzymes.

Using the aziridinoadenosines to covalently attach the activated group to MTase substrates offers a convenient way for substrate labeling. However, this method also suffers from some pitfalls. First, because of the “product inhibition” nature of this type compound,¹⁰⁵ stoichiometric amounts of methyltransferase are needed. Complicated enzyme kinetics can also be expected, and IC₅₀ measurement requires careful tuning of the enzyme and SAM concentrations.¹⁰⁷ Second, the aziridinoadenosines or their N-mustard precursors are highly reactive, which could give rise to stability concerns and potential nonspecific and nonenzymatic alkylations. For example, it was reported that successful PRMT1-directed alkylation using probes **37** and **38** may require low-dithiothreitol (DTT) or a DTT-deficient buffer.¹⁰⁶ In addition, some probes also have limited membrane permeability. Because of these reasons, the aziridinoadenosines are seldom applied to cellular studies. One example is that the precursor of compound **39** was reported to have no inhibition in MLL-rearranged leukemia cells.⁸⁷ In 2009, Thomas *et al.* reported a photocaged aziridine precursor **41** with 6-nitroveratryl group (Table 1) as a photocleavable moiety.¹⁰⁸ This compound is stable under normal conditions and could quickly transform to aziridine after 2 min UV irradiation. It may provide an alternative way to improve the stability of aziridinoadenosines.

3.2. Double-Activated SAM Analogs

Usually, if the methyl group adjacent to the sulfonium center of SAM is replaced by an ethyl or propyl group, the enzyme catalysis rate for transferring the extended group is greatly decreased due to steric effects. For the allele-specific cofactors, the authors are referred to the allele-specific inhibitor part of this review. In 2006, the Weinhold group reported a new type of SAM analog in which the methyl group is replaced by activated groups such as allyl or propargyl. For such compounds as **42** and **43** (Table 2), the reaction rate could be maintained because the steric hindrance for the S_N2 -like transition state could be partially compensated by the conjugative stability offered by the β -double bond or triple bond.^{109,110} This type of double-activated SAM analog could be synthesized through nucleophilic substitution of the sulfur center of SAH with alkyl bromide or alkyl triflate under acidic conditions, in which the nucleophilic amino group of SAH is in the protonated state.¹¹⁰ Using this method, a mixture of (*S*)- and (*R*)-epimers are obtained. Since only the (*S*)-epimer is active for transmethylation reaction, the separation is desired and could possibly be reached by HPLC.

This transalkylation reaction results in functionalization of MTase substrates with a transferrable tag for further ligation. One advantage over the aziridine counterpart is that its resultant complex does not inhibit MTases; therefore a stoichiometric amount of enzyme is not needed. In a related version, another SAM analog with terminal amine for the labeling of DNA methyltransferase substrates was reported. The extended propargylic chain in compound **44** could be efficiently transferred on DNA substrates and then conjugated with the NHS-ester of an amine-reactive probe.⁹⁶

The terminal alkynyl-containing analog is of special value because it can serve as an alkyne end for azide–alkyne cycloaddition. However, compound **43** is unstable in basic solution with a half-life below several minutes due to the hydrolysis of terminal alkynyl group.^{111–113} This instability restricts its application for MTases which require longer incubation time and also may complicate the biological results. For example, the diastereomer mixture of **43** was reported to display specificity for SETDB1 (a type of histone H3 lysine 9 methyltransferase) over other methyltransferases such as SETDB1, SET7, SMYD2, PRMT1, and CARM1,¹¹⁴ and it is speculated that the selectivity may be due to its reduced efficacy as a result of instability of compound **43**.¹⁸ Therefore, another SAM analog attached with a pent-2-en-4-ynyl side chain (AdoEnYn, **45**) was reported to circumvent this stability issue.¹¹⁵ Using this probe, it was demonstrated that the transfer of a pent-2-en-4-ynyl side chain to histone H3 could be reached using Dim-5 (a histone H3 lysine methyltransferase) or a MLL MTase complex at a pH of 9. The resultant H3 modified with terminal alkyne could further couple with azide-PEG-biotin by CuAAC Click chemistry. Later, this probe was also used for site-specific RNA labeling by Trm1.¹¹⁶

Besides the allyl or propargyl SAM analogs, the ketone analog was also reported by the Zhou group in 2010 (**Keto-AdoMet**, **46**).¹¹⁷ Using the human thiopurine *S*-methyltransferase (TPMT), the authors demonstrated that it shared a similar substrate profile with SAM. The high reactivity of ketone toward hydroxylamine or hydrazide makes it easy for derivatization. For example, the substrate could be labeled with Alexa Fluor 647

hydroxyamine, leading to a strong signal at 650 nm, and the background can be minimized due to the absence of ketone in protein or DNA.

For the application of these probes to cellular studies, it is desirable that the probe is selective for the target enzyme, excluding the interference from other endogenous MTases. However, this specificity requirement is hard to meet. Likely, the transferrable group of the probe is either “too small and non-specific” or “too big and inactive.” To tackle this problem, the Luo group developed a high throughput assay to screen the synthetic cofactors against mutant PMTs¹¹⁸ and developed a strategy called “bioorthogonal profiling of protein methylation (BPPM)” by incorporating the “bump and hole” strategy. For a comprehensive review of BPPM, the readers are recommended to Luo’s review article.¹¹⁹ In this strategy (Figure 8),^{120,121} the PMTs are engineered to harbor the bulky SAM synthetic cofactors. It brings about dual benefits: on the one hand, more synthetic cofactors could be adopted by PMTs since the enzymes’ binding pockets are enlarged; and on the other hand, the effect of endogenous PMTs can be minimized. It should be noted that the mutations were located in the SAM-binding site and remote from substrate-binding site; therefore, substrate methylomes are not supposed to be affected. This strategy is mainly applied to cell lysates due to the limited membrane permeability of SAM and its analogs (“*in-vitro*” part of Figure 8). Later, the application in living cells was realized using biosynthesized SAM analogs by engineered methionine adenosyltransferase (MAT; “*in-vivo*” part of Figure 8).

Using the BPPM strategy, the Luo group found that the **Pob-SAM (47)**/PRMT1 Y39FM48G pair possesses enough activity with a K_{cat}/K_m value 20-fold lower than the native SAM/PRMT1 pair.¹¹¹ In addition, the mutant enzyme has no activity on SAM, making this pair desirable for substrate labeling in the presence of closely related enzymes and SAM. Then, two other pairs were also discovered: compound **Hey-SAM (48)**/G9a Y1154A¹²² and **Ab-SAM (49)** for EuHMT1/2 mutants.¹²³ Using **Ab-SAM**, the authors were able to identify several EuHMT1/2 nonhistone substrates and revealed their different substrates patterns.^{123,124} Using a similar strategy, the authors also identified the PRMT3 substrates.¹²⁵ Besides these, using *in vivo* BPPM, the Luo group successfully profiled the chromatin-modifying activities of G9a and GLP1 in living cells.¹²⁶ The BPPM strategy was also applied to the DNMT for sequence-specific labeling of DNA.⁹⁵

3.3. Selenium- and Tellurium-based SAM Analogs

As discussed above, the stability of some SAM-based synthetic cofactors could significantly limit their further applications. Generally, there are three degradation pathways for SAM, leading to epimerized SAM, methylthioadenosine (MTA), and adenine, respectively, with the increasing of pH.^{2,127–129} For synthetic cofactors, the degradation could be faster because their side chains could also go through degradation. For example, the Klimašauskas group has examined the stability of a series of alkynyl-containing SAM cofactors and found that they possessed half-lives between 0.05 and 5 h at pH 7.4 as compared with a half-life of 17 h for SAM.¹¹³

Previous research found that the electrophilicity of SAM and its onium congeners followed the series SeAM > SAM > TeAM, and the acidity of 5'-C may follow the series SAM > SeAM > TeAM.¹³⁰ Based on this reactivity difference, selenium-based synthetic cofactors

were proposed (Figure 9).^{112,131,132} It is found that at pH 7.5, **AdoYn (43)** could rapidly decay to a hydrated intermediate (possibly **Keto-AdoMet** by hydration of the propargyl side chain) with a half-life of about 5 min, then gradually convert to an MTA analog. In regard to **SeAdoYn (52)**, no hydrated intermediate was observed, and it gradually degraded to the propargyl-Se-MTA derivative with a half-life of 1.5 h.¹¹² This also agrees with the Luo group's finding.¹³¹ In addition to the enhanced stability, the **SeAdoYn** is also expected to be more reactive for enzymatic transfer because of the increased activation of the selenonium center as compared to the sulfonium counterpart. The advantage of this probe was demonstrated in the efficient labeling of a series of PKMT substrates.^{112,131,133}

4. DEVELOPMENT OF SAM ANALOGS AS CAPTURE COMPOUNDS AND FLUORESCENT COFACTORS

Besides serving as MTase inhibitors and synthetic cofactors, SAM/SAH analogs can also be used as capture compounds (CC) for a capture compound mass spectroscopy (CCMS) study.¹³⁴ Usually, a CC is comprised of three moieties (Figure 10a): (1) one selectivity moiety responsible for interacting with the target proteins, (2) one photoactive moiety responsible for covalent cross-linking of the bond proteins, and (3) one sorting moiety for isolating the captured proteins for further analysis. The procedure is initiated by the interaction between the selectivity moiety of the probe and its binding proteins, then followed by irreversible fixation of the captured proteins upon UV irradiation, and ended by isolation/characterization of the probe–protein complex. Since SAH is more stable and has a similar K_d with SAM, the Weinhold group constructed the first CC for SAM-dependent MTases based on SAH (Figure 10a).^{135–137} In these compounds, C8- and N6- positions of adenine were conjugated with photo-cross-linking groups (azide or diazine) and biotin (exemplified by N6-modified compounds **53** and **54** in Figure 10a). These compounds were demonstrated to be effective to capture a series of SAM-binding proteins from *E. coli* lysate. It should be noted that two SAH-binding proteins were also isolated in this case.

Fluorescent SAM analogs are often used to measure SAM binding. For example, four SAM analogs (compounds **56–59**) with modifications on adenine moieties were enzymatically prepared (Figure 10b).¹³⁸ They possess maximum excitation/emission wavelengths at 278/365, 297/344, 305/372, and 280/360 nM, respectively. By applying **DAPSM (59)** and measuring fluorescence intensity, the authors were able to determine the binding constant of **DAPSAM** and SAM-III riboswitch RNA ($K_d = 0.38 \mu\text{M}$). Additionally, CC can also be fluorescently labeled. Compound **55** which is a fluorescein conjugate was used to measure binding affinity via fluorescence anisotropy.¹³⁹

5. LIMITATION AND PERSPECTIVE

Biological methylation is a powerful mechanism to regulate cellular processes. The methyl donor SAM, also referred to as “mother nature’s methyl iodide,” is the second largest cofactor after ATP in the human body. In the past, a plethora of SAM analogs were designed as either MTase inhibitors or bioorthogonal probes or other kinds of probes. They hold great promise as new cancer therapeutics and are significantly accelerating the biological studies of MTases. For example, a DOT1L inhibitor has entered clinical study for MLL treatment,

and double-activated SAM analogs are valuable probes to identify MTase substrates. Nevertheless, there is still much to explore for their biological and biomedical applications. Stable, selective, cell permeable, and fully characterized probes are still in high demand.

Both SAM and SAH have poor membrane permeability. Besides this, SAM suffers from pH-dependent lability, while SAH could be easily degraded by SAH hydrolase. These two limitations (membrane permeability and stability) pose challenges for further application of SAM and SAH analogs. For SAM analogs, their major application is to serve as synthetic cofactors for cell lysate. For living cell profiling, *in vivo* synthesis of SAM analogs using mutant MAT enzymes has to be adopted due to the poor cellular permeability. For SAH analogs, they mainly serve as inhibitors and capture compounds for MTases. However, it is unknown whether these SAH analogs could be SAH hydrolase substrates or inhibitors. Since SAH is a feedback inhibitor of MTases, it is expected that the cellular concentrations and ratios SAM and SAH will have impacts on the bioactivity of MTases.

For SAM/SAH binding, structurally different binding pockets of MTases are involved. It can be seen that highly selective synthetic cofactors or inhibitors can be obtained by carefully tuning their structures. It is expected that systematic examination and computational studies of these binding pockets would greatly accelerate the probe development and also provide rationale for the observed selectivity. Meanwhile, a full characterization of SAM/SAH analogs over a broad spectrum of MTases or even other related enzymes is needed. In this respect, development of robust and high throughput screening methods is still of great value to substitute current methods which are time- and resource-demanding.

Currently, the application of the SAM/SAH analogs is largely limited to cellular models, and the synthetic cofactors are mainly applied on cell lysate contexts. With the advancement of new SAM/SAH probes, we would wish to see more studies on animal treatments and living cells or even organ profiling in future. From probe design to synthesis, bioactivity evaluation, cellular study, animal testing, or even human trial, it requires a collective effort of structural biology, medicinal chemistry, assay development, biochemistry, pharmacology, and many other scientific branches. It is no doubt that application of new techniques for the MTase study will greatly accelerate the progress of this field. Meanwhile, a large number of other SAM-dependent enzymes have been uncovered, such as the “radical SAM” superfamily. This provides opportunities to test the existing probes and also calls for new SAM-based probes specially designed to fit with these new enzymes.

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References

1. Fontecave M, Atta M, Mulliez E. S-adenosylmethionine: nothing goes to waste. Trends Biochem Sci. 2004; 29:243–249. [PubMed: 15130560]

2. Dalhoff, C., Weinhold, E. Modified Nucleosides: in Biochemistry, Biotechnology and Medicine. Wiley-VCH Verlag GmbH & Co. KGaA; 2008. S-Adenosyl-L-Methionine and Related Compounds; p. 223-247.
3. Struck AW, Thompson ML, Wong LS, Micklefield J. S-adenosyl-methionine-dependent methyltransferases: highly versatile enzymes in biocatalysis, biosynthesis and other biotechnological applications. *ChemBioChem*. 2012; 13:2642–2655. [PubMed: 23180741]
4. Frey PA, Magnusson OT. S-Adenosylmethionine: a wolf in sheep's clothing, or a rich man's adenosylcobalamin? *Chem Rev*. 2003; 103:2129–2148. [PubMed: 12797826]
5. Loenen WA. S-adenosylmethionine: jack of all trades and master of everything? *Biochem Soc Trans*. 2006; 34:330–333. [PubMed: 16545107]
6. Borchardt RT, Wu YS. Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 5 Role of the asymmetric sulfonium pole in the enzymatic binding of S-adenosyl-L-methionine. *J Med Chem*. 1976; 19:1099–1103. [PubMed: 978674]
7. de la Haba G, Jamieson GA, Mudd SH, Richards HH. S-Adenosylmethionine: The Relation of Configuration at the Sulfonium Center to Enzymatic Reactivity1. *J Am Chem Soc*. 1959; 81:3975–3980.
8. Martin JL, McMillan FM. SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold. *Curr Opin Struct Biol*. 2002; 12:783–793. [PubMed: 12504684]
9. Schubert HL, Blumenthal RM, Cheng X. Many paths to methyltransfer: a chronicle of convergence. *Trends Biochem Sci*. 2003; 28:329–335. [PubMed: 12826405]
10. Jeltsch A. Beyond Watson and Crick: DNA methylation and molecular enzymology of DNA methyltransferases. *ChemBioChem*. 2002; 3:274–293. [PubMed: 11933228]
11. 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]
12. 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]
13. Petkowski JJ, Bonsignore LA, Tooley JG, Wilkey DW, Merchant ML, Macara IG, Schaner Tooley CE. NRMT2 is an N-terminal monomethylase that primes for its homologue NRMT1. *Biochem J*. 2013; 456:453–462. [PubMed: 24090352]
14. 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]
15. Bonifacio MJ, Palma PN, Almeida L, Soares-da-Silva P. Catechol-O-methyltransferase and its inhibitors in Parkinson's disease. *CNS Drug Rev*. 2007; 13:352–379. [PubMed: 17894650]
16. Espinoza S, Manago F, Leo D, Sotnikova TD, Gainetdinov RR. Role of catechol-O-methyltransferase (COMT)-dependent processes in Parkinson's disease and L-DOPA treatment. *CNS Neurol Disord: Drug Targets*. 2012; 11:251–263. [PubMed: 22483291]
17. Marsala SZ, Gioulis M, Ceravolo R, Tinazzi M. A systematic review of catechol-O-methyltransferase inhibitors: efficacy and safety in clinical practice. *Clin Neuropharmacol*. 2012; 35:185–190. [PubMed: 22805229]
18. Luo M. Current chemical biology approaches to interrogate protein methyltransferases. *ACS Chem Biol*. 2012; 7:443–463. [PubMed: 22220966]
19. Carlson SM, Gozani O. Emerging technologies to map the protein methylome. *J Mol Biol*. 2014; 426:3350–3362. [PubMed: 24805349]
20. Liu Y, Liu K, Qin S, Xu C, Min J. Epigenetic targets and drug discovery: part 1: histone methylation. *Pharmacol Ther*. 2014; 143:275–294. [PubMed: 24704322]
21. Klimasauskas S, Weinhold E. A new tool for biotechnology: AdoMet-dependent methyltransferases. *Trends Bio-technol*. 2007; 25:99–104.
22. Copeland RA, Solomon ME, Richon VM. Protein methyltransferases as a target class for drug discovery. *Nat Rev Drug Discovery*. 2009; 8:724–732. [PubMed: 19721445]
23. Kouzarides T. SnapShot: Histone-modifying enzymes. *Cell*. 2007; 131:822. [PubMed: 18022374]
24. Li KK, Luo C, Wang D, Jiang H, Zheng YG. Chemical and biochemical approaches in the study of histone methylation and demethylation. *Med Res Rev*. 2012; 32:815–867. [PubMed: 22777714]

25. Fuhrmann J, Clancy KW, Thompson PR. Chemical biology of protein arginine modifications in epigenetic regulation. *Chem Rev.* 2015; 115:5413–5461. [PubMed: 25970731]
26. Cole PA. Chemical probes for histone-modifying enzymes. *Nat Chem Biol.* 2008; 4:590–597. [PubMed: 18800048]
27. Kaniskan HU, Jin J. Chemical probes of histone lysine methyltransferases. *ACS Chem Biol.* 2015; 10:40–50. [PubMed: 25423077]
28. Kaniskan HU, Konze KD, Jin J. Selective inhibitors of protein methyltransferases. *J Med Chem.* 2015; 58:1596–1629. [PubMed: 25406853]
29. Zheng YG, Wu J, Chen Z, Goodman M. Chemical regulation of epigenetic modifications: opportunities for new cancer therapy. *Med Res Rev.* 2008; 28:645–687. [PubMed: 18271058]
30. Bissinger EM, Heinke R, Sippl W, Jung M. Targeting epigenetic modifiers: Inhibitors of histone methyltransferases. *MedChemComm.* 2010; 1:114–124.
31. Lyko F, Brown R. DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. *J Natl Cancer Inst.* 2005; 97:1498–1506. [PubMed: 16234563]
32. Gnyszka A, Jastrzebski Z, Flis S. DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer Res.* 2013; 33:2989–2996. [PubMed: 23898051]
33. Foulks JM, Parnell KM, Nix RN, Chau S, Swierczek K, Saunders M, Wright K, Hendrickson TF, Ho KK, McCullar MV, Kanner SB. Epigenetic drug discovery: targeting DNA methyltransferases. *J Biomol Screening.* 2012; 17:2–17.
34. Martinet N, Michel BY, Bertrand P, Benhida R. Small molecules DNA methyltransferases inhibitors. *MedChemComm.* 2012; 3:263–273.
35. Erdmann A, Halby L, Fahy J, Arimondo PB. Targeting DNA methylation with small molecules: what's next? *J Med Chem.* 2015; 58:2569–2583. [PubMed: 25406944]
36. Campbell RM, Tummino PJ. Cancer epigenetics drug discovery and development: the challenge of hitting the mark. *J Clin Invest.* 2014; 124:64–69. [PubMed: 24382391]
37. Coward JK, Slisz EP. Analogs of S-adenosylhomocysteine as potential inhibitors of biological transmethylation. Specificity of the S-adenosylhomocysteine binding site. *J Med Chem.* 1973; 16:460–463. [PubMed: 4718456]
38. Chang CD, Coward JK. Analogues of S-adenosylhomocysteine as potential inhibitors of biological trans-methylation. Synthesis of analogues with modifications at the 5'-thioether linkage. *J Med Chem.* 1976; 19:684–691. [PubMed: 1271409]
39. Crooks PA, Tribe MJ, Pinney RJ. Inhibition of bacterial DNA cytosine-5-methyltransferase by S-adenosyl-L-homo-cysteine and some related compounds. *J Pharm Pharmacol.* 1984; 36:85–89. [PubMed: 6143811]
40. Hildesheim J, Hildesheim R, Lederer E. New syntheses of S-adenosyl homocysteine analogues, potential methyltransferase inhibitors. *Biochimie.* 1972; 54:431–437. [PubMed: 4646815]
41. Borchardt RT, Huber JA, Wu YS. Potential inhibitor of S-adenosylmethionine-dependent methyltransferases. 2 Modification of the base portion of S-adenosylhomocysteine. *J Med Chem.* 1974; 17:868–873. [PubMed: 4845380]
42. Borchardt RT, Wu YS. Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 1 Modification of the amino acid portion of S-adenosylhomocysteine. *J Med Chem.* 1974; 17:862–868. [PubMed: 4845379]
43. Borchardt RT, Wu YS. Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 3 Modifications of the sugar portion of S-adenosylhomocysteine. *J Med Chem.* 1975; 18:300–304. [PubMed: 1133821]
44. Borchardt RT, Shiong Y, Huber JA, Wycpalek AF. Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 6 Structural modifications of S-adenosylmethionine. *J Med Chem.* 1976; 19:1104–1110. [PubMed: 978675]
45. Balzarini J, De Clercq E, Serafinowski P, Dorland E, Harrap KR. Synthesis and antiviral activity of some new S-adenosyl-L-homocysteine derivatives. *J Med Chem.* 1992; 35:4576–4583. [PubMed: 1335077]
46. Backstrom R, Honkanen E, Pippuri A, Kairisalo P, Pystynen J, Heinola K, Nissinen E, Linden IB, Mannisto PT, Kaakkola S, et al. Synthesis of some novel potent and selective catechol O-methyltransferase inhibitors. *J Med Chem.* 1989; 32:841–846. [PubMed: 2704029]

47. Richon VM, Johnston D, Sneeringer CJ, Jin L, Majer CR, Elliston K, Jerva LF, Scott MP, Copeland RA. Chemogenetic analysis of human protein methyltransferases. *Chem Biol Drug Des.* 2011; 78:199–210. [PubMed: 21564555]
48. Zheng S, Hausmann S, Liu Q, Ghosh A, Schwer B, Lima CD, Shuman S. Mutational analysis of *Encephalitozoon cuniculi* mRNA cap (guanine-N7) methyltransferase, structure of the enzyme bound to sinefungin, and evidence that cap methyltransferase is the target of sinefungin's antifungal activity. *J Biol Chem.* 2006; 281:35904–35913. [PubMed: 16971388]
49. Pugh CS, Borchardt RT, Stone HO. Sinefungin, a potent inhibitor of virion mRNA(guanine-7)-methyl-transferase, mRNA(nucleoside-2'-)-methyltransferase, and viral multiplication. *J Biol Chem.* 1978; 253:4075–4077. [PubMed: 659406]
50. Zheng W, Ibanez G, Wu H, Blum G, Zeng H, Dong A, Li F, Hajian T, Allali-Hassani A, Amaya MF, Siarheyeva A, Yu W, Brown PJ, Schapira M, Vedadi M, Min J, Luo M. Sinefungin derivatives as inhibitors and structure probes of protein lysine methyltransferase SETD2. *J Am Chem Soc.* 2012; 134:18004–18014. [PubMed: 23043551]
51. Devkota K, Lohse B, Liu Q, Wang MW, Staerk D, Berthelsen J, Clausen RP. Analogues of the Natural Product Sinefungin as Inhibitors of EHMT1 and EHMT2. *ACS Med Chem Lett.* 2014; 5:293–297. [PubMed: 24900829]
52. Avila MA, Garcia-Trevijano ER, Lu SC, Corrales FJ, Mato JM. Methylthioadenosine. *Int J Biochem Cell Biol.* 2004; 36:2125–2130. [PubMed: 15313459]
53. Williams-Ashman HG, Seidenfeld J, Galletti P. Trends in the biochemical pharmacology of 5'-deoxy-5'-methylthioadenosine. *Biochem Pharmacol.* 1982; 31:277–288. [PubMed: 6803807]
54. Islam K. Allele-specific chemical genetics: concept, strategies, and applications. *ACS Chem Biol.* 2015; 10:343–363. [PubMed: 25436868]
55. Lin Q, Jiang F, Schultz PG, Gray NS. Design of allele-specific protein methyltransferase inhibitors. *J Am Chem Soc.* 2001; 123:11608–11613. [PubMed: 11716715]
56. Li J, Wei H, Zhou MM. Structure-guided design of a methyl donor cofactor that controls a viral histone H3 lysine 27 methyltransferase activity. *J Med Chem.* 2011; 54:7734–7738. [PubMed: 21958314]
57. Huang R, Martinez-Ferrando I, Cole PA. Enhanced interrogation: emerging strategies for cell signaling inhibition. *Nat Struct Mol Biol.* 2010; 17:646–649. [PubMed: 20520657]
58. Masjost B, Ballmer P, Borroni E, Zurcher G, Winkler FK, Jakob-Roetne R, Diederich F. Structure-based design, synthesis, and in vitro evaluation of bisubstrate inhibitors for catechol O-methyltransferase (COMT). *Chem - Eur J.* 2000; 6:971–982. [PubMed: 10785817]
59. Ma Z, Liu H, Wu B. Structure-based drug design of catechol-O-methyltransferase inhibitors for CNS disorders. *Br J Clin Pharmacol.* 2014; 77:410–420. [PubMed: 23713800]
60. Lerner C, Masjost B, Ruf A, Gramlich V, Jakob-Roetne R, Zurcher G, Borroni E, Diederich F. Bisubstrate inhibitors for the enzyme catechol-O-methyltransferase (COMT): influence of inhibitor preorganisation and linker length between the two substrate moieties on binding affinity. *Org Biomol Chem.* 2003; 1:42–49. [PubMed: 12929389]
61. Paulini R, Lerner C, Jakob-Roetne R, Zurcher G, Borroni E, Diederich F. Bisubstrate inhibitors of the enzyme catechol O-methyltransferase (COMT): efficient inhibition despite the lack of a nitro group. *ChemBioChem.* 2004; 5:1270–1274. [PubMed: 15368579]
62. Ellermann M, Jakob-Roetne R, Lerner C, Borroni E, Schlatter D, Roth D, Ehler A, Rudolph MG, Diederich F. Molecular recognition at the active site of catechol-o-methyltransferase: energetically favorable replacement of a water molecule imported by a bisubstrate inhibitor. *Angew Chem, Int Ed.* 2009; 48:9092–9096.
63. Ellermann M, Paulini R, Jakob-Roetne R, Lerner C, Borroni E, Roth D, Ehler A, Schweizer WB, Schlatter D, Rudolph MG, Diederich F. Molecular recognition at the active site of catechol-O-methyltransferase (COMT): adenine replacements in bisubstrate inhibitors. *Chem - Eur J.* 2011; 17:6369–6381. [PubMed: 21538606]
64. Paulini R, Trindler C, Lerner C, Brandli L, Schweizer WB, Jakob-Roetne R, Zurcher G, Borroni E, Diederich F. Bisubstrate inhibitors of catechol O-methyltransferase (COMT): the crucial role of the ribose structural unit for inhibitor binding affinity. *ChemMedChem.* 2006; 1:340–357. [PubMed: 16892369]

65. Dowden J, Hong W, Parry RV, Pike RA, Ward SG. Toward the development of potent and selective bisubstrate inhibitors of protein arginine methyltransferases. *Bioorg Med Chem Lett*. 2010; 20:2103–2105. [PubMed: 20219369]
66. Dowden J, Pike RA, Parry RV, Hong W, Muhsen UA, Ward SG. Small molecule inhibitors that discriminate between protein arginine N-methyltransferases PRMT1 and CARM1. *Org Biomol Chem*. 2011; 9:7814–7821. [PubMed: 21952734]
67. van Haren M, van Ufford LQ, Moret EE, Martin NI. Synthesis and evaluation of protein arginine N-methyltransferase inhibitors designed to simultaneously occupy both substrate binding sites. *Org Biomol Chem*. 2015; 13:549–560. [PubMed: 25380215]
68. Smil D, Eram MS, Li F, Kennedy S, Szewczyk MM, Brown PJ, Barsyte-Lovejoy D, Arrowsmith CH, Vedadi M, Schapira M. Discovery of a Dual PRMT5-PRMT7 Inhibitor. *ACS Med Chem Lett*. 2015; 6:408–412. [PubMed: 25893041]
69. 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]
70. 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]
71. Vaubourgeix J, Bardou F, Boissier F, Julien S, Constant P, Ploux O, Daffe M, Quemard A, Mourey L. S-adenosyl-N-decyl-aminoethyl, a potent bisubstrate inhibitor of mycobacterium tuberculosis mycolic acid methyltransferases. *J Biol Chem*. 2009; 284:19321–19330. [PubMed: 19439410]
72. Isakovic L, Saavedra OM, Llewellyn DB, Claridge S, Zhan L, Bernstein N, Vaisburg A, Elowe N, Petschner AJ, Rahil J, Beaulieu N, Gauthier F, MacLeod AR, Delorme D, Besterman JM, Wahhab A. Constrained (1-)-S-adenosyl-L-homocysteine (SAH) analogues as DNA methyltransferase inhibitors. *Bioorg Med Chem Lett*. 2009; 19:2742–2746. [PubMed: 19364644]
73. Saavedra OM, Isakovic L, Llewellyn DB, Zhan L, Bernstein N, Claridge S, Raeppl F, Vaisburg A, Elowe N, Petschner AJ, Rahil J, Beaulieu N, MacLeod AR, Delorme D, Besterman JM, Wahhab A. SAR around (1)-S-adenosyl-L-homocysteine, an inhibitor of human DNA methyltransferase (DNMT) enzymes. *Bioorg Med Chem Lett*. 2009; 19:2747–2751. [PubMed: 19362833]
74. Kumar R, Srivastava R, Singh RK, Surolia A, Rao DN. Activation and inhibition of DNA methyltransferases by S-adenosyl-L-homocysteine analogues. *Bioorg Med Chem*. 2008; 16:2276–2285. [PubMed: 18083524]
75. Hobley G, McKelvie JC, Harmer JE, Howe J, Oyston PC, Roach PL. Development of rationally designed DNA N6 adenine methyltransferase inhibitors. *Bioorg Med Chem Lett*. 2012; 22:3079–3082. [PubMed: 22483584]
76. Wahnon DC, Shier VK, Benkovic SJ. Mechanism-based inhibition of an essential bacterial adenine DNA methyltransferase: rationally designed antibiotics. *J Am Chem Soc*. 2001; 123:976–977. [PubMed: 11456633]
77. Chen CW, Armstrong SA. Targeting DOT1L and HOX gene expression in MLL-rearranged leukemia and beyond. *Exp Hematol*. 2015; 43:673–684. [PubMed: 26118503]
78. Napper AD, Watson VG. Targeted drug discovery for pediatric leukemia. *Front Oncol*. 2013; 3:170. [PubMed: 23847761]
79. Anglin JL, Song Y. A medicinal chemistry perspective for targeting histone H3 lysine-79 methyltransferase DOT1L. *J Med Chem*. 2013; 56:8972–8983. [PubMed: 23879463]
80. Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, Johnston LD, Scott MP, Smith JJ, Xiao Y, Jin L, Kuntz KW, Chesworth R, Moyer MP, Bernt KM, Tseng JC, Kung AL, Armstrong SA, Copeland RA, Richon VM, Pollock RM. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell*. 2011; 20:53–65. [PubMed: 21741596]
81. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, Allain CJ, Klaus CR, Raimondi A, Scott MP, Waters NJ, Chesworth R, Moyer MP, Copeland RA, Richon VM, Pollock RM. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood*. 2013; 122:1017–1025. [PubMed: 23801631]

82. Basavapathruni A, Olhava EJ, Daigle SR, Therkelsen CA, Jin L, Boriack-Sjodin PA, Allain CJ, Klaus CR, Raimondi A, Scott MP, Dovletoglou A, Richon VM, Pollock RM, Copeland RA, Moyer MP, Chesworth R, Pearson PG, Waters NJ. Nonclinical pharmacokinetics and metabolism of EPZ-5676, a novel DOT1L histone methyltransferase inhibitor. *Biopharm Drug Dispos.* 2014; 35:237–252. [PubMed: 24415392]
83. DOT1L Inhibitor – EPZ-5676 for Acute Leukemias with Genetic Alterations of MLL. <http://www.epizyme.com/programs/dot1l-inhibitor/>
84. Yu W, Chory EJ, Wernimont AK, Tempel W, Scopton A, Federation A, Marineau JJ, Qi J, Barsyte-Lovejoy D, Yi J, Marcellus R, Jacob RE, Engen JR, Griffin C, Aman A, Wienholds E, Li F, Pineda J, Estiu G, Shatseva T, Hajian T, Al-Awar R, Dick JE, Vedadi M, Brown PJ, Arrowsmith CH, Bradner JE, Schapira M. Catalytic site remodelling of the DOT1L methyltransferase by selective inhibitors. *Nat Commun.* 2012; 3:1288. [PubMed: 23250418]
85. Basavapathruni A, Jin L, Daigle SR, Majer CR, Therkelsen CA, Wigle TJ, Kuntz KW, Chesworth R, Pollock RM, Scott MP, Moyer MP, Richon VM, Copeland RA, Olhava EJ. Conformational adaptation drives potent, selective and durable inhibition of the human protein methyltransferase DOT1L. *Chem Biol Drug Des.* 2012; 80:971–980. [PubMed: 22978415]
86. Yu W, Smil D, Li F, Tempel W, Fedorov O, Nguyen KT, Bolshan Y, Al-Awar R, Knapp S, Arrowsmith CH, Vedadi M, Brown PJ, Schapira M. Bromodeaza-SAH: a potent and selective DOT1L inhibitor. *Bioorg Med Chem.* 2013; 21:1787–1794. [PubMed: 23433670]
87. Anglin JL, Deng L, Yao Y, Cai G, Liu Z, Jiang H, Cheng G, Chen P, Dong S, Song Y. Synthesis and structure-activity relationship investigation of adenosine-containing inhibitors of histone methyltransferase DOT1L. *J Med Chem.* 2012; 55:8066–8074. [PubMed: 22924785]
88. Liu W, Deng L, Song Y, Redell M. DOT1L inhibition sensitizes MLL-rearranged AML to chemotherapy. *PLoS One.* 2014; 9:e98270. [PubMed: 24858818]
89. Deng L, Zhang L, Yao Y, Wang C, Redell MS, Dong S, Song Y. Synthesis, Activity and Metabolic Stability of Non-Ribose Containing Inhibitors of Histone Methyltransferase DOT1L. *MedChemComm.* 2013; 4:822–826. [PubMed: 23795283]
90. Gibbons GS, Owens SR, Fearon ER, Nikolovska-Coleska Z. Regulation of Wnt signaling target gene expression by the histone methyltransferase DOT1L. *ACS Chem Biol.* 2015; 10:109–114. [PubMed: 25361163]
91. Sarkaria SM, Christopher MJ, Klco JM, Ley TJ. Primary acute myeloid leukemia cells with IDH1 or IDH2 mutations respond to a DOT1L inhibitor in vitro. *Leukemia.* 2014; 28:2403–2406. [PubMed: 25092143]
92. Zhang L, Deng L, Chen F, Yao Y, Wu B, Wei L, Mo Q, Song Y. Inhibition of histone H3K79 methylation selectively inhibits proliferation, self-renewal and metastatic potential of breast cancer. *Oncotarget.* 2014; 5:10665–10677. [PubMed: 25359765]
93. Yi JS, Federation AJ, Qi J, Dhe-Paganon S, Hadler M, Xu X, St Pierre R, Varca AC, Wu L, Marineau JJ, Smith WB, Souza A, Chory EJ, Armstrong SA, Bradner JE. Structure-guided DOT1L probe optimization by label-free ligand displacement. *ACS Chem Biol.* 2015; 10:667–674. [PubMed: 25397901]
94. Onder TT, Kara N, Cherry A, Sinha AU, Zhu N, Bernt KM, Cahan P, Marcarci BO, Unternaehrer J, Gupta PB, Lander ES, Armstrong SA, Daley GQ. Chromatin-modifying enzymes as modulators of reprogramming. *Nature.* 2012; 483:598–602. [PubMed: 22388813]
95. Lukinavicius G, Lapinaite A, Urbanaviciute G, Gerasimaite R, Klimasauskas S. Engineering the DNA cytosine-5 methyltransferase reaction for sequence-specific labeling of DNA. *Nucleic Acids Res.* 2012; 40:11594–11602. [PubMed: 23042683]
96. Lukinavicius G, Lapiene V, Stasevskij Z, Dalhoff C, Weinhold E, Klimasauskas S. Targeted labeling of DNA by methyltransferase-directed transfer of activated groups (mTAG). *J Am Chem Soc.* 2007; 129:2758–2759. [PubMed: 17309265]
97. Pignot M, Siethoff C, Linscheid M, Weinhold E. Coupling of a Nucleoside with DNA by a Methyltransferase. *Angew Chem, Int Ed.* 1998; 37:2888–2891.
98. Pljevaljcic G, Pignot M, Weinhold E. Design of a new fluorescent cofactor for DNA methyltransferases and sequence-specific labeling of DNA. *J Am Chem Soc.* 2003; 125:3486–3492. [PubMed: 12643710]

99. Comstock LR, Rajski SR. Conversion of DNA methyltransferases into azidonucleosidyl transferases via synthetic cofactors. *Nucleic Acids Res.* 2005; 33:1644–1652. [PubMed: 15778434]
100. Comstock LR, Rajski SR. Methyltransferase-directed DNA strand scission. *J Am Chem Soc.* 2005; 127:14136–14137. [PubMed: 16218579]
101. Weller RL, Rajski SR. DNA methyltransferase-moderated click chemistry. *Org Lett.* 2005; 7:2141–2144. [PubMed: 15901154]
102. Weller RL, Rajski SR. Design, synthesis, and preliminary biological evaluation of a DNA methyltransferase-directed alkylating agent. *ChemBioChem.* 2006; 7:243–245. [PubMed: 16365907]
103. Du Y, Hendrick CE, Frye KS, Comstock LR. Fluorescent DNA labeling by N-mustard analogues of S-adenosyl-L-methionine. *ChemBioChem.* 2012; 13:2225–2233. [PubMed: 22961989]
104. Zhang C, Weller RL, Thorson JS, Rajski SR. Natural product diversification using a non-natural cofactor analogue of S-adenosyl-L-methionine. *J Am Chem Soc.* 2006; 128:2760–2761. [PubMed: 16506729]
105. Osborne T, Roska RL, Rajski SR, Thompson PR. In situ generation of a bisubstrate analogue for protein arginine methyltransferase 1. *J Am Chem Soc.* 2008; 130:4574–4575. [PubMed: 18338885]
106. Hymbaugh Bergman SJ, Comstock LR. N-mustard analogs of S-adenosyl-L-methionine as biochemical probes of protein arginine methylation. *Bioorg Med Chem.* 2015; 23:5050–5055. [PubMed: 26037613]
107. Yao Y, Chen P, Diao J, Cheng G, Deng L, Anglin JL, Prasad BV, Song Y. Selective inhibitors of histone methyltransferase DOT1L: design, synthesis, and crystallographic studies. *J Am Chem Soc.* 2011; 133:16746–16749. [PubMed: 21936531]
108. Townsend AP, Roth S, Williams HE, Stylianou E, Thomas NR. New S-adenosyl-L-methionine analogues: synthesis and reactivity studies. *Org Lett.* 2009; 11:2976–2979. [PubMed: 19552444]
109. Dalhoff C, Lukinavicius G, Klimasauskas S, Weinhold E. Direct transfer of extended groups from synthetic cofactors by DNA methyltransferases. *Nat Chem Biol.* 2006; 2:31–32. [PubMed: 16408089]
110. Dalhoff C, Lukinavicius G, Klimasauskas S, Weinhold E. Synthesis of S-adenosyl-L-methionine analogs and their use for sequence-specific transalkylation of DNA by methyltransferases. *Nat Protoc.* 2006; 1:1879–1886. [PubMed: 17487172]
111. Wang R, Zheng W, Yu H, Deng H, Luo M. Labeling substrates of protein arginine methyltransferase with engineered enzymes and matched S-adenosyl-L-methionine analogues. *J Am Chem Soc.* 2011; 133:7648–7651. [PubMed: 21539310]
112. Willnow S, Martin M, Luscher B, Weinhold E. A selenium-based click AdoMet analogue for versatile substrate labeling with wild-type protein methyltransferases. *ChemBioChem.* 2012; 13:1167–1173. [PubMed: 22549896]
113. Lukinavicius G, Tomkuvienė M, Masevicius V, Klimasauskas S. Enhanced chemical stability of adomet analogues for improved methyltransferase-directed labeling of DNA. *ACS Chem Biol.* 2013; 8:1134–1139. [PubMed: 23557731]
114. Binda O, Boyce M, Rush JS, Palaniappan KK, Bertozzi CR, Gozani O. A chemical method for labeling lysine methyltransferase substrates. *ChemBioChem.* 2011; 12:330–334. [PubMed: 21243721]
115. Peters W, Willnow S, Duisken M, Kleine H, Macherey T, Duncan KE, Litchfield DW, Luscher B, Weinhold E. Enzymatic site-specific functionalization of protein methyltransferase substrates with alkynes for click labeling. *Angew Chem, Int Ed.* 2010; 49:5170–5173.
116. Motorin Y, Burhenne J, Teimer R, Koynov K, Willnow S, Weinhold E, Helm M. Expanding the chemical scope of RNA:methyltransferases to site-specific alkylation of RNA for click labeling. *Nucleic Acids Res.* 2011; 39:1943–1952. [PubMed: 21037259]
117. Lee BW, Sun HG, Zang T, Kim BJ, Alfaro JF, Zhou ZS. Enzyme-catalyzed transfer of a ketone group from an S-adenosylmethionine analogue: a tool for the functional analysis of methyltransferases. *J Am Chem Soc.* 2010; 132:3642–3643. [PubMed: 20196537]

118. Wang R, Ibanez G, Islam K, Zheng W, Blum G, Sengelaub C, Luo M. Formulating a fluorogenic assay to evaluate S-adenosyl-L-methionine analogues as protein methyltransferase cofactors. *Mol BioSyst.* 2011; 7:2970–2981. [PubMed: 21866297]
119. Wang R, Luo M. A journey toward Bioorthogonal Profiling of Protein Methylation inside living cells. *Curr Opin Chem Biol.* 2013; 17:729–737. [PubMed: 24035694]
120. Blum G, Bothwell IR, Islam K, Luo M. Profiling protein methylation with cofactor analog containing terminal alkyne functionality. *Curr Protoc Chem Biol.* 2013; 5:67–88. [PubMed: 23788324]
121. Blum G, Islam K, Luo M. Bioorthogonal profiling of protein methylation (BPPM) using an azido analog of S-adenosyl-L-methionine. *Curr Protoc Chem Biol.* 2013; 5:45–66. [PubMed: 23667794]
122. Islam K, Zheng W, Yu H, Deng H, Luo M. Expanding cofactor repertoire of protein lysine methyltransferase for substrate labeling. *ACS Chem Biol.* 2011; 6:679–684. [PubMed: 21495674]
123. Islam K, Bothwell I, Chen Y, Sengelaub C, Wang R, Deng H, Luo M. Bioorthogonal profiling of protein methylation using azido derivative of S-adenosyl-L-methionine. *J Am Chem Soc.* 2012; 134:5909–5915. [PubMed: 22404544]
124. Islam K, Chen Y, Wu H, Bothwell IR, Blum GJ, Zeng H, Dong A, Zheng W, Min J, Deng H, Luo M. Defining efficient enzyme-cofactor pairs for bioorthogonal profiling of protein methylation. *Proc Natl Acad Sci U S A.* 2013; 110:16778–16783. [PubMed: 24082136]
125. Guo H, Wang R, Zheng W, Chen Y, Blum G, Deng H, Luo M. Profiling substrates of protein arginine N-methyltransferase 3 with S-adenosyl-L-methionine analogues. *ACS Chem Biol.* 2014; 9:476–484. [PubMed: 24320160]
126. Wang R, Islam K, Liu Y, Zheng W, Tang H, Lailier N, Blum G, Deng H, Luo M. Profiling genome-wide chromatin methylation with engineered posttranslation apparatus within living cells. *J Am Chem Soc.* 2013; 135:1048–1056. [PubMed: 23244065]
127. Hoffman JL. Chromatographic analysis of the chiral and covalent instability of S-adenosyl-L-methionine. *Biochemistry.* 1986; 25:4444–4449. [PubMed: 3530324]
128. Iwig DF, Booker SJ. Insight into the polar reactivity of the onium chalcogen analogues of S-adenosyl-L-methionine. *Biochemistry.* 2004; 43:13496–13509. [PubMed: 15491157]
129. Parks LW, Schlenk F. The stability and hydrolysis of S-adenosylmethionine; isolation of S-ribosylmethionine. *J Biol Chem.* 1958; 230:295–305. [PubMed: 13502398]
130. Iwig DF, Grippe AT, McIntyre TA, Booker SJ. Isotope and elemental effects indicate a rate-limiting methyl transfer as the initial step in the reaction catalyzed by Escherichia coli cyclopropane fatty acid synthase. *Biochemistry.* 2004; 43:13510–13524. [PubMed: 15491158]
131. Bothwell IR, Islam K, Chen Y, Zheng W, Blum G, Deng H, Luo M. Se-Adenosyl-L-selenomethionine Cofactor Analogue as a Reporter of Protein Methylation. *J Am Chem Soc.* 2012; 134:14905–14912. [PubMed: 22917021]
132. Bothwell IR, Luo M. Large-scale, protection-free synthesis of Se-adenosyl-L-selenomethionine analogues and their application as cofactor surrogates of methyltransferases. *Org Lett.* 2014; 16:3056–3059. [PubMed: 24852128]
133. Shimazu T, Barjau J, Sohtome Y, Sodeoka M, Shinkai Y. Selenium-based S-adenosylmethionine analog reveals the mammalian seven-beta-strand methyltransferase METTL10 to be an EF1A1 lysine methyltransferase. *PLoS One.* 2014; 9:e105394. [PubMed: 25144183]
134. Koster H, Little DP, Luan P, Muller R, Siddiqi SM, Marappan S, Yip P. Capture compound mass spectrometry: a technology for the investigation of small molecule protein interactions. *Assay Drug Dev Technol.* 2007; 5:381–390. [PubMed: 17638538]
135. Lenz T, Poot P, Weinhold E, Dreger M. Profiling of methyltransferases and other S-Adenosyl-L-homocysteine-binding proteins by Capture Compound mass spectrometry. *Methods Mol Biol.* 2012; 803:97–125. [PubMed: 22065221]
136. Lenz T, Poot P, Grabner O, Glinski M, Weinhold E, Dreger M, Koster H. Profiling of methyltransferases and other S-adenosyl-L-homocysteine-binding Proteins by Capture Compound Mass Spectrometry (CCMS). *J Visualized Exp.* 2010:2264.

137. Dalhoff C, Huben M, Lenz T, Poot P, Nordhoff E, Koster H, Weinhold E. Synthesis of S-adenosyl-L-homocysteine capture compounds for selective photoinduced isolation of methyltransferases. *ChemBioChem*. 2010; 11:256–265. [PubMed: 20049756]
138. Ottink OM, Nelissen FH, Derks Y, Wijmenga SS, Heus HA. Enzymatic stereospecific preparation of fluorescent S-adenosyl-L-methionine analogs. *Anal Biochem*. 2010; 396:280–283. [PubMed: 19748477]
139. Brown LJ, Baranowski M, Wang Y, Schrey AK, Lenz T, Taverna SD, Cole PA, Sefkow M. Using S-adenosyl-L-homocysteine capture compounds to characterize S-adenosyl-L-methionine and S-adenosyl-L-homocysteine binding proteins. *Anal Biochem*. 2014; 467:14–21. [PubMed: 25172130]

Glossary

Biological methylation, the biochemical process of adding methyl group(s) to a biological substrate (e.g., nucleic acids, proteins, carbohydrates, and small molecule metabolites) which is catalyzed by different members of methyltransferases—SAM is the most prevalent methyl donor for biological methylation; epigenetics, the regulation of transcription and other DNA-templated processes by molecular events that cause changes in the chromatin state without directly altering the genetic DNA sequence—most commonly studied epigenetic mechanisms are DNA methylation and histone modifications; Pan-MTase inhibitors, inhibitors which lack specificities for a particular methyltransferase but instead target multiple methyltransferases indiscriminately; bioorthogonal probes, Non-native, nonperturbing chemical handles that can be modified in living systems through highly selective reactions with exogenously delivered reporter molecules; allele-specific chemical probes, a protein of interest is genetically engineered such that only the mutated allele, instead of the wild-type allele, shows a sensitive response to a particularly designed chemical probe—the allele-specific probe can be either an inhibitor or a substrate; bisubstrate inhibitors, a type of inhibitors in which two substrates are covalently coupled together in order to gain high affinity by the product of the two association constants of those substrates—the design of bisubstrate inhibitors generally requires a ternary complex mechanism of the enzymatic reaction; capture compounds (CCs), compounds that are designed for rapid and qualitative investigation of small molecule–protein interactions—CCs contain trifunctional motifs in one structure: a reversibly interacting selectivity group, a covalent bond-forming reactivity group, and a sorting/pulldown affinity tag

Figure 1.

(a) Overview of SAM-dependent MTase-catalyzed transmethylation reactions. (b) The products of DNA and histone methylation.

Figure 2.
Structures of pan-MTase inhibitors.

Figure 3.
(a) Structures of allele-specific cofactors and inhibitors. (b) Application of allele-specific inhibitors.

Figure 4.

(a) Schematic illustration of bisubstrate inhibitor concept. (b) Hypothesized PRMT bisubstrate transition state.

Figure 5.

(a) Representative COMT bisubstrate inhibitors. (b) Representative PMT bisubstrate inhibitors.

Figure 6.
Representative DNMT inhibitors.

Figure 7.
Representative DOT1L inhibitors.

Figure 8.
In-vitro and *in vivo* Bioorthogonal Profiling of Protein Methylation (BPPM).

SAM: X = S

SeAM (50): X = Se

TeAM (51): X = Te

SeAdoYn, ProSeAM (52)

Figure 9.
Representative selenium- and tellurium-based SAM analogs.

Figure 10.

(a) Representative SAH-based capture compounds (CCs). (b) Representative fluorescent SAM analogs.

Table 1

Structures of Representative Aziridinoadenosines As Synthetic Cofactors

Precursor for compounds 34-40
41: R₁ = 6-nitroveratryl, R₂ = R₃ = H

Compound	R ₁	R ₂	R ₃
32	H	H	H
33	H	N ₃	H
34	-CH ₂ C≡CH	H	H
35		H	H
36		-NHCH ₂ C≡CH	H
37		N ₃	H
38		H	-(CH ₂) ₄ C≡CH
39		H	H
40		H	CH ₃

Table 2

Structures of Representative Double-Activated SAM Analogs As Synthetic Cofactors

Compounds	R
Allyl-SAM (42)	
AdoYn, Propargyl SAM (43)	
44	
AdoEnYn, EnYn-SAM (45)	
Keto-AdoMet (46)	
Pob-SAM (47)	
Hey-SAM (48)	
Ab-SAM (49)	