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AdoMet analog synthesis and utilization: Current state of the art

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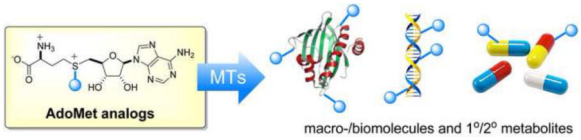
Abstract

S-Adenosyl-L-methionine (AdoMet) is an essential enzyme cosubstrate in fundamental biology with an expanding range of biocatalytic and therapeutic applications. In recent years, technologies enabling the synthesis and utilization of novel functional AdoMet surrogates have rapidly advanced. Developments highlighted within this brief review include improved syntheses of AdoMet analogs, unique *S*-adenosyl-L-methionine isosteres with enhanced stability, and corresponding applications in epigenetics, proteomics and natural product/small molecule diversification ('alkylrandomization').

Graphical abstract



Keywords

Methyltransferase; methione adenosyltransferase; *S*-adenosylmethionine; SAM; halogenase

Introduction

Methyltransferase (MT)-catalyzed *S*-adenosyl-L-methionine (AdoMet, SAM, or SAMe)-dependent methylation is a key enzymatic reaction that enables the functional modulation of a vast array of biomolecules ranging from small metabolites to macromolecules (Fig. 1a; Fig. 2a) [1–5]. Consistent with this, alterations in methylation are associated with a wide range of human pathologies and variability in drug response [2–4]. Despite great advances in methylation-dependent bioinformatics and disease-associated biomarkers, the study of intracellular MT spatial/temporal resolution, specificity and/or function remains a challenge [3,4]. Within this context, the early proof of concept studies revealing synthetic non-native

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AdoMet analogs to function as efficient cosubstrates for DNA [6] or natural product (NP) [7] MTs inspired a range of subsequent conceptually similar strategies to study NP [8–11], protein [12–18], and nucleic acid [19–23] methylation. Subsequent development of permissive enzyme-based strategies for the synthesis of differentially *S*-alkylated AdoMet analogs has further simplified access to these unique cosubstrates [11,14,19,24] and also facilitated emerging cell-based applications [14]. Within this context, this brief review attempts to highlight recent advances in the generation and application of differentially *S/Se*-alkylated AdoMet analogs and what are perceived to be key remaining challenges in further advancing the impact of these unique reagents. While a wide array of AdoMet adenosyl and/or L-methionine (L-Met) chain modified analogs have been pursued within the context of inhibitor design, it is important to note that these fall outside the scope of this review [25–27].

Chemical synthesis of AdoMet analogs

Differentially *S/Se*-alkylated AdoMet surrogates have been constructed via both chemical and enzyme-catalyzed synthesis, the former of which is briefly summarized within this section with an emphasis on analogs demonstrated as functional cosubstrates for downstream AdoMet-utilizing enzymes. Alkylation of *S*-adenosyl-L-homocysteine (AdoHcy) with alkyl halides in HCOOH/AcOH in the presence or absence of Lewis acid ($AgClO_4$ or AgOTf) as the predominate synthetic strategy of choice, has enabled the synthesis of >20 chemically diverse *S/Se*-alkylated AdoMets (Fig. 2b). Table 1 highlights functionally active analogs synthesized to date, where subtle variations from the conventional synthetic strategy are noted. While synthetic strategies opened the door to the interrogation of methyltransferases [6,7,21,28,29], typical synthetic yields range from 3% to 90% of (*S/R*)-sulfonium diastereomeric mixtures where residual starting materials (AdoHcy, a potent product inhibitor of AdoMet-utilizing enzymes) are commonly detrimental to the target enzymes to be studied [30,31]. Thus, purification via reverse-phase chromatography [32–34] or cation-exchange HPLC [35] is typically required, the nature of which often restricts practical scale. AdoMet chemical lability can also be disadvantageous to lengthy synthetic manipulations and/or purification schemes where intramolecular cyclization, depurination and sulfonium epimerization contribute to AdoMet $t_{1/2}$ (Fig. 1b) [19,20,35,36].

Chemoenzymatic synthesis of AdoMet analogs

The complement to conventional AdoMet cosubstrate synthesis is enzyme-catalyzed production. Two distinct enzymes have been employed (methionine adenosyltransferases and halogenases, Table 1), a main advantage of which is the potential to directly couple AdoMet analog production to downstream utilization reactions and thereby circumvent the fundamental AdoMet stability issues and/or the need for tedious purification procedures noted in previous section.

Methionine adenosyltransferases (MATs, EC 2.5.1.6)

MATs (also known as *S*-adenosylmethionine synthetase/synthase, SAMS) catalyze the formation of AdoMet from adenosine triphosphate (ATP) and (L-Met) as a predominate strategy for AdoMet production in nature (Fig. 2c). Within this context, Singh *et al.* surveyed

the capabilities of a representative set of wild-type bacterial, archaeal and mammalian MATs with 44 structurally diverse differentially *S/Se*-alkylated L-Met analogs. This cumulative effort highlighted human MAT II catalytic alpha subunit (hMAT2A) and the archael thermophilic *Methanocaldococcus jannaschii* MAT (mMAT) as notably permissive [11]. Using the same suite of putative substrates, Wang and Singh *et al*. reported similar promiscuity for the archael *Sulfolobus solfataricus* MAT (sMAT) and, notably, the corresponding first structural elucidation for a thermostable MAT (sMAT, PDB ID 4HPV) and corresponding non-native ligand-bound complex (*S*-adenosylethionine, AdoEth; PDB ID 4L2Z) [24]. The Luo group also reported the successful hMAT2A-catalyzed synthesis of two differentially *S*-alkylated AdoMet analogs carrying bulky chemoselective handles and the design of key hMAT2A mutants to improve activity toward targeted non-native L-Met analogs [14]. In a similar fashion, a wild-type MAT from *Bacillus subtilis* was recently reported to accept four of 11 differentially *S*-alkylated methionine analogs tested along with key mutants that displayed improved proficiency, permissivity and an apparent reduction in product (AdoMet) inhibition [37]. In addition to L-Met analogs bearing alternative *S*-alkyl groups, six different carboxyl- and/or amino-modified L-Met analogs were also recently assessed for their viability as alternative cosubstrates of pathogenic bacterial MATs [38]. Cumulatively, well over 50 L-Met analogs have been assessed as putative substrates for a wide array of wild-type and mutant MATs within the last 5 years toward enabling non-native AdoMet production and, in many cases, subsequent utilization in coupled systems [8,11,14,24,37–39]. In addition, tetrazole-based surrogates Ado^{t}Met and ^{7dz}Ado^{t}Met (Fig. 2d) recently generated via hMAT2A-catalyzed synthesis were demonstrated to serve as functional cosubstrates for the prototypical class I MT DnrK involved in daunorubicin biosynthesis [40]. This latter study notably highlighted a dramatic improvement in the corresponding ^{7dz}Ado^{t}Met isostere stability where structure elucidation of DnrK ligand-bound structures also revealed Ado^{t}Met to occupy the AdoMet site with a slight shift toward the DnrK-bound acceptor coinciding with a slight improvement in k_{cat}.

Halogenases (SalL, EC 2.5.1.94; FDAS, EC 2.5.1.63)

The innovative application of two wild-type microbial halogenases (5′-chloro-5′-deoxyadenosine synthase or adenosyl-chloride synthase, SalL; 5′-fluoro-5′-deoxyadenosine synthase or adenosyl-fluoride synthase, FDAS) have recently been reported for differentially *S*-alkylated AdoMet production. SalL and FDAS catalyze the reversible formation of L-Met and 5′-chloro or 5’-fluoro-5′-deoxyadenosine (ClDA or FDA, respectively) from AdoMet and chloride or fluoride, respectively, where the equilibrium typically favors the reactants (Fig. 2e) [41–45]. In this pioneering work [19], SalL and FDAS were found to catalyze the production of six differentially *S*-alkylated AdoMet analogs from their respective L-Met analogs and commercially available ClDA or FDA. Structure-based rational design of SalL (PDB ID 2Q6I and 2Q6L) and FDAS (PDB ID 1RQR) mutants also led to catalytic improvements with targeted non-native substrates [19]. In addition, SalL-catalyzed AdoMet analog production has been successfully coupled to the model MTs arginine methyltransferase 1 (PRMT1), DNA MT HhaI and the natural product MT MtfA [19,46]. Reminiscent of the ^{7dz}Ado^{t}Met isosteres described in the prior section, SalL-catalyzed synthesis of the thieno[3,4-*d*]pyrimidine-based thAdoMet was also recently reported [47]. While thAdoMet stability was not assessed, thAdoMet served as a functional cosubstrate for

the model DNA MT M.*Taq*I. Like MATs, halogenases importantly enable coupling to downstream AdoMet-utilizing processes in vitro. Whether ClDA/FDA uptake (compared to readily available cellular ATP for MAT) impacts cell-based applications remains to be determined.

AdoMet analog applications

The pioneering applications of non-native AdoMet cosubstrates in MT-catalyzed reactions to afford non-native alkylation of DNA [6] and the indolocarbazole rebeccamycin [7] reported in 2006 by Weinhold group and the Rajski/Thorson collaborative team, respectively, served as the key proof of concept for an array of subsequent innovative advances and applications (Table 1). This section briefly summarizes recent representative examples in the context of modifying nucleic acids, proteins and complex natural products.

Nucleic acids

DNA/RNA methylation plays a key role in epigenetic regulation of gene expression where the vast temporal and spatial complexity presents a notable technological challenge to molecular and mechanistic study, further complicated by the high structural conservation among nucleic acid MTs (NAMTs) [48,49]. While cytosine methylation is a highly conserved modification across many species and among the best understood nucleic acid modifications, many other nucleic acid methylation events also contribute to epigenetic regulation [49]. AdoMet analogs present a valuable new tool to study these essential processes via MT-catalyzed installation of isotopic or chemoselective handles as a framework for epigenetic mapping [6,20–23,50–52]. This concept has been further extended to track RNA modification [53–55]

Proteins

Protein methylation is a key post-translational protein function modulator as exemplified by the role of histone and transcription factor methylation in cellular differentiation and proliferation [56–58]. Here again, the structural conservation among protein MTs (PMTs), vast array of protein targets, and corresponding temporal and spatial occurrence present significant experimental barriers [3]. As with the nucleic acid strategies highlighted in the previous section, AdoMet analogs also enable selective installation of novel chemoselective handles to track and identify methylation events catalyzed by PMTs [12–18,57–66], the proof of concept of which was first demonstrated by Weinhold and coworkers using the wild-type PMT Dim-5 and AdoMet analog **18** (Table 1) [60]. Interestingly, while **18** is also a validated substrate of other wild-type PMTs and NAMTs [50,53], Luo and collaborators more recently reported the need for engineered PMTs to accommodate this AdoMet analog in the pursuit of putative bioorthogonal reagents to study PMT-catalyzed methylation events *in vitro* and living cells [14,17,18,59,63,65,67]. Isotopic tags have also been installed using corresponding PMTs and suitably-labeled AdoMet analogs (Table 1, entries **1 – 3**) [68–73].

Natural products

Natural product (NP) methylation is a highly prevalent biosynthetic reaction where natural product methyltransferase (NPMT)-catalyzed regio/stereospecific *O-, N-, S-* and/or *C-*

methylation of the fundamental NP core can contribute to bioactivity modulation [5,74]. Within this context, AdoMet analogs in conjunction with both NPMT domains of large multi-functional modular enzyme complexes and standalone late-stage tailoring NPMTs have enabled NP 'alkylrandomization' (*i.e.*, differential alkylation, the terminology reminiscent of NP 'glycorandomization' [75,76]) to afford novel coumarins [9], fungal polyketides [10], indolocarbazoles [7,11], macrolides [8], nonribosomal peptides [46] and related small molecules [9,40,77]. Importantly, these technologies present a clear complement to conventional synthesis to extend NP structure-activity relationships (SAR) via selective NPMT-catalyzed installation of non-native alkyl groups, protecting groups and/or uniquely functionalized handles for subsequent downstream chemoselective diversification where *C*-MTs also offer new avenues to potentially access synthetically difficult C-C bond-forming operations [9].

Conclusions

As exemplified by the platform development and innovative applications summarized within this brief review, *S*-alkylated AdoMet analogs serve as useful chemical biology tools, where practical access has paved the way for a rapidly expanding array of opportunities in fundamental discovery and targeted synthesis. A perceived area for considerable growth in this regard are cell-based applications, the key for which will be the development of universal bioorthogonal AdoMet surrogate/catalyst pairings with high catalytic turnover and exquisite selectivity. 'Bump-and-hole' technologies [78], such as those pioneered by Shokat and colleagues [79], serve as the basis for similar AdoMet adenine-modified strategies to achieve MT bioorthogonality as exemplified by the early work of Schultz and Gray [80] and a more recent example by the Zhou group [81]. Alternatively, Luo and collaborators have pursued putative bioorthogonality via targeting specific AdoMet *S*-alkyl modifications [17,67]. This growing precedent suggests a vibrant future for cell-based, and possibly even whole animal, applications where the fundamental key to achieving true bioorthogonality will depend on the development of AdoMet surrogate/catalyst pairings that display suitable selectivity for the targeted process/reaction over native biochemical processes/enzymes [82].

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Highlights

- AdoMet is one of the most essential cosubstrates in nature.
- Practical access to AdoMet analogs enables new tools, technologies, leads and discoveries.
- Both synthetic and chemoenzymatic strategies for AdoMet production have been advanced.
- Chemoenzymatic strategies set the stage for cell-based or whole animal applications.
- Bioorthogonal catalyst/AdoMet pairings are anticipated to have a dramatic impact.

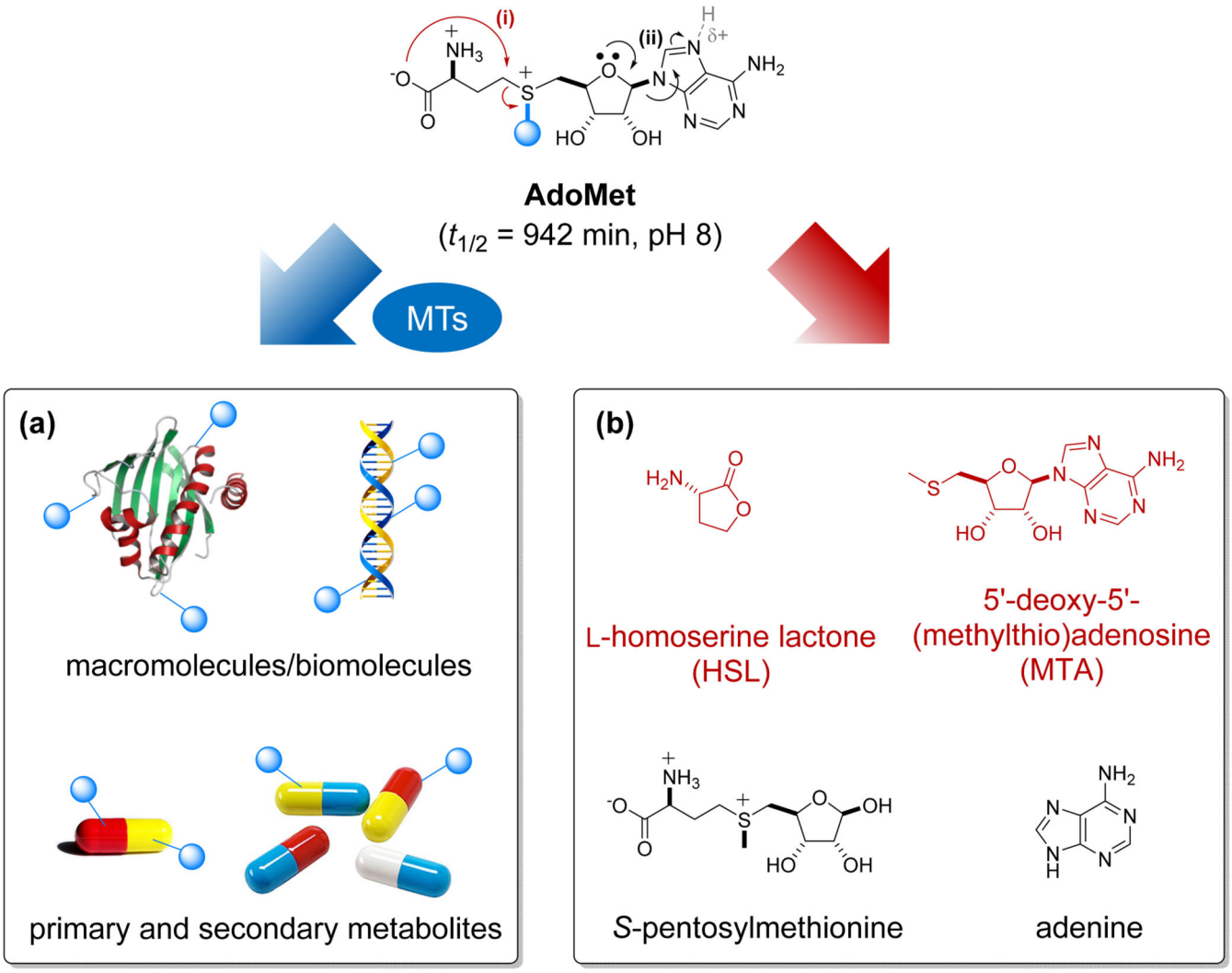


Figure 1. Representative AdoMet utilization and degradation pathways

(a) AdoMet serves as a critical alkyl donor in most MT-catalyzed reactions within the context of modifying nucleic acids, proteins and small molecule-based metabolites (blue sphere signifies methyl in native systems). **(b)** AdoMet chemically degrades via intramolecular cyclization **(pathway i)** and depurination **(pathway ii)**.

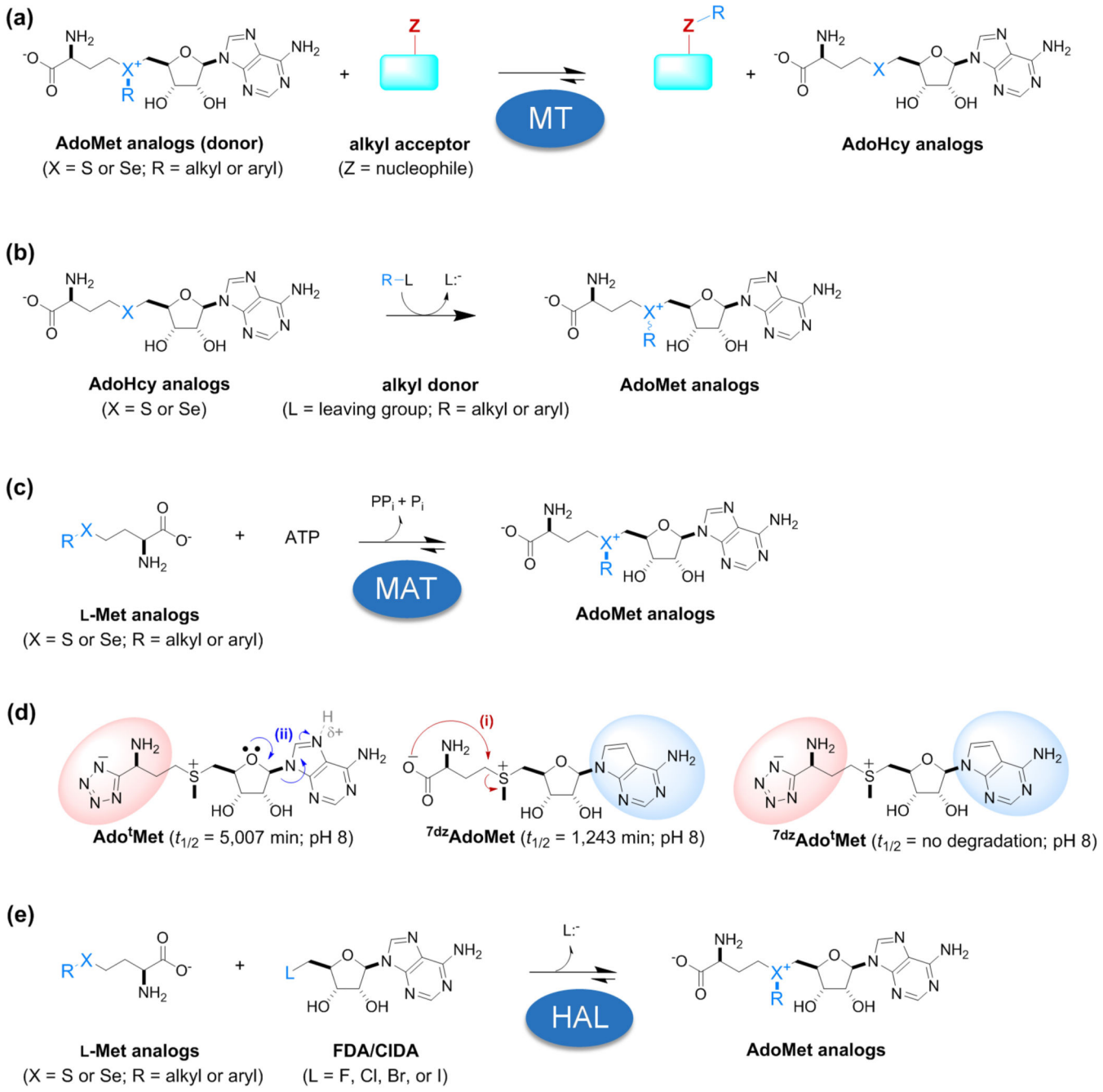


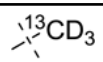
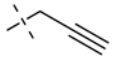
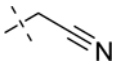
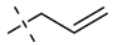
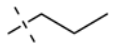
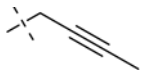
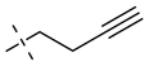
Figure 2. Key reactions and reagents

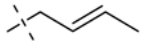
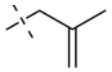
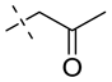
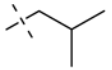
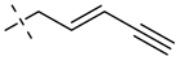
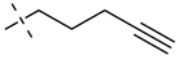
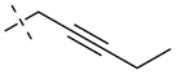
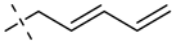
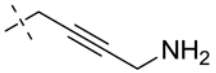
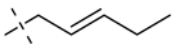
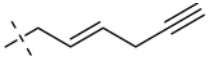
(a) General MT-catalyzed reaction scheme (AdoHcy, *S*-adenosyl-L-homocysteine; also known as SAH). MTs can catalyze *C*-, *O*-, *N*- or *S*-methylation. **(b)** Typical synthetic strategy for AdoMet analog chemical synthesis where common leaving groups include halides, triflates, mesylates and tosylates. **(c)** General methionine adenosyl transferase (MAT; also known as *S*-adenosylmethionine synthetase/synthase, SAMS)-catalyzed reaction scheme. **(d)** Stabilized functional AdoMet surrogates afforded via MAT-catalyzed turnover of (*S*)-3-(methylthio)-1-(1*H*-tetrazol-5-yl)propan-1-amine (tetrazole-L-methionine, L-^{t}Met)

and ATP or L-^{t}Met and 7-deaza-ATP (^{7dz}ATP) to give Ado^{t}Met and ^{7dz}Ado^{t}Met, respectively. **(e)** General halogenase-catalyzed reaction scheme (HAL: adenosyl-chloride synthase, SalL, or adenosyl-fluoride synthase, FDAS).

Table 1

Summary of AdoMet Analog Syntheses and Applications.

Entry	Thio/Seleno-Alkyl Substitution	Heteroatom	Method for Analog Synthesis			Analog Application		
			Chemical	*MAT*	*Halogenase*	*NA*	*P*	*SM*
1		S		[37,68]			[68]	
2		S		[69–72]			[69–72]	
3		S				[53]	[73]	
4		S		[83]				
5		S, Se	[6]	[8,11,24,37,83]	[19]	[6]	[19]	[8,11]
6		S, Se	[9,12,13,33,54,63]	[11,24]		[52]	[12,13,33]	[9–11]
7		S, Se		[11,24]				[11]
8		S, Se	[6,9,32,59,62,64]	[8,11,24,83]	[19]	[6,32]	[17–19,59,62,64–66]	[8,9,11]
9		S, Se	[6,59]	[11,24,37,84]	[19]	[6]		[84]
10		S, Se		[11,24]				
11		S	[6,9,20,32,54]			[6,20,32,54]	[18]	[9]
12		S, Se	[63]	[11,24]			[63]	

Entry	Thio/Seleno-Alkyl Substitution	Heteroatom	Method for Analog Synthesis			Analog Application		
			Chemical	*MAT*	*Halogenase*	*NA*	*P*	*SM*
13		S	[9]	[11,24]			[17]	[9]
14		S, Se		[11,24]				
15		S	[77]					[77]
16		S, Se		[11,24,37,84]	[19]			
17		S		[11,24]				
18		S, Se	[13,50,59,60,62,63]	[14]		[50,53]	[13,14,17,18,59,60,63,65]	
19		S, Se	[59,62,63]				[63]	
20		S	[20]			[20]		
21		S		[11,24]				
22		S	[20]			[20]		
23		S	[17]				[17]	
24		S, Se	[59,63]	[14]			[14,17,59,63,65]	

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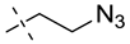
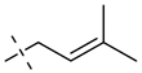
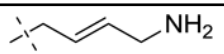
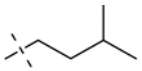
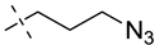
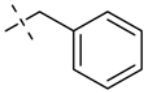
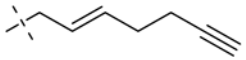
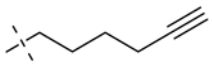
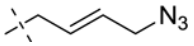
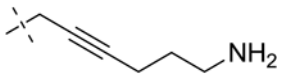
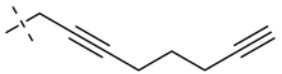
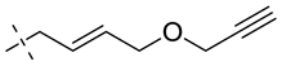
Entry	Thio/Seleno-Alkyl Substitution	Heteroatom	Method for Analog Synthesis			Analog Application		
			Chemical	*MAT*	*Halogenase*	*NA*	*P*	*SM*
25		S		[11,24]				
26		S, Se		[11,24]				
27		Se		[11,24]				
28		S		[11,24]				
29		S		[11,24]				
30		S	[9]		[19]		[19]	[9]
31		S	[17]				[17]	
32		S, Se	[59]				[59]	
33		S, Se	[15,16]	[11,24]			[15,16]	
34		S	[20]			[20]		
35		S	[20]			[20]		
36		S	[62]				[18,62]	

Entry	Thio/Seleno-Alkyl Substitution	Heteroatom	Method for Analog Synthesis			Analog Application		
			Chemical	*MAT*	*Halogenase*	*NA*	*P*	*SM*
37		S	[20]			[20]		
38		S	[20,22]			[22]		
39		S	[20,52,54]			[20,52]		