Enhanced Chemical Stability of AdoMet Analogues for Improved Methyltransferase-Directed Labeling of DNA

ARTICLE *in* ACS CHEMICAL BIOLOGY · APRIL 2013

Impact Factor: 5.33 · DOI: 10.1021/cb300669x · Source: PubMed

4 AUTHORS, INCLUDING:

Grazvydas Lukinavicius
École Polytechnique Fédérale de Lausanne
23 PUBLICATIONS **545** CITATIONS

SEE PROFILE

Saulius Klimasauskas
Institute of Biotechnology Vilnius University
78 PUBLICATIONS **3,580** CITATIONS

SEE PROFILE

ACS chemical biology

Letters

pubs.acs.org/acschemicalbiology

Enhanced Chemical Stability of AdoMet Analogues for Improved Methyltransferase-Directed Labeling of DNA

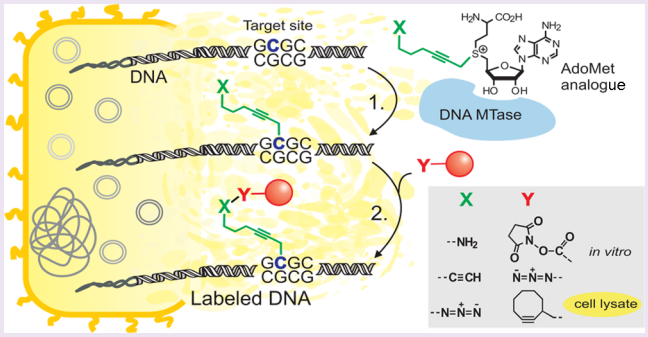
Gražvydas Lukinavičius,[†,§] Miglė Tomkuvienė,[†] Viktoras Masevičius,[†,‡] and Saulius Klimašauskas*[,†]

[†]Institute of Biotechnology, Vilnius University, LT-02241 Vilnius, Lithuania

[‡]Faculty of Chemistry, Vilnius University, LT-03225 Vilnius, Lithuania

Supporting Information

ABSTRACT: Methyltransferases catalyze specific transfers of methyl groups from the ubiquitous cofactor *S*-adenosyl-L-methionine (AdoMet) to various nucleophilic positions in biopolymers like DNA, RNA, and proteins. We had previously described synthesis and application of AdoMet analogues carrying sulfonium-bound 4-substituted but-2-ynyl side chains for transfer by methyltransferases. Although useful in certain applications, these cofactor analogues exhibited short lifetimes in physiological buffers. Examination of the reaction kinetics and products showed that their fast inactivation followed a different pathway than observed for AdoMet and rather involved a pH-dependent addition of a water molecule to the side chain. This side reaction was eradicated by synthesis of a series of cofactor analogues in which the separation between an electronegative group and the triple bond was increased from one to three carbon units. The designed hex-2-ynyl moiety-based cofactor analogues with terminal amino, azide, or alkyne groups showed a markedly improved enzymatic transalkylation activity and proved well suitable for methyltransferase-directed sequence-specific labeling of DNA *in vitro* and in bacterial cell lysates.



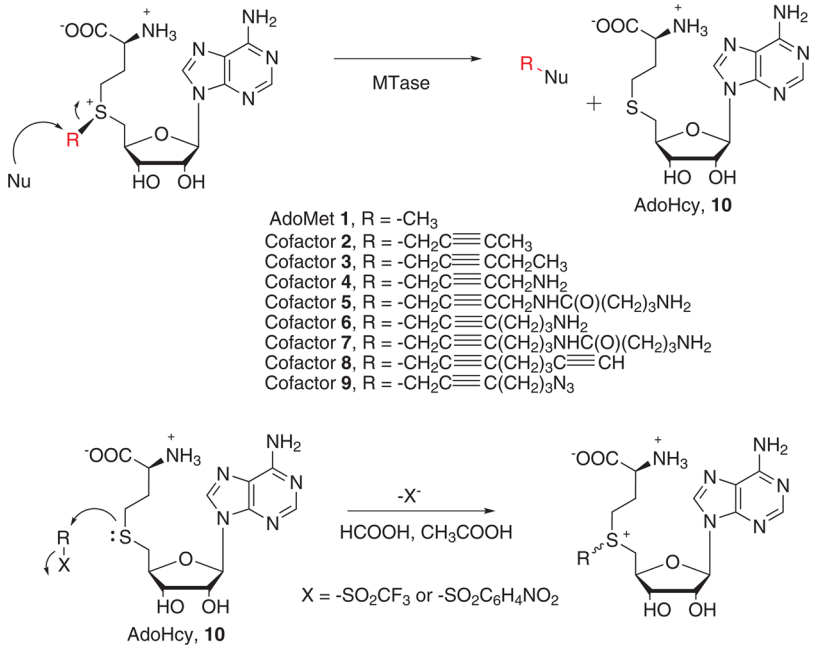
Methyltransferases (MTases) naturally catalyze the transfer of methyl group from the cofactor *S*-adenosyl-L-methionine (AdoMet, cofactor **1**) to a variety of biomolecules; the reaction typically occurs via an S_N2 transfer of an activated sulfonium-bound methyl group leading to a methylated product and demethylated cofactor *S*-adenosyl-L-homocysteine (AdoHcy). To expand the practical utility of this highly specific enzymatic reaction, two major classes of synthetic AdoMet analogues have been developed.[1] One strategy exploits aziridine[2] or *N*-mustard[3] mimics of the sulfonium center whereby a whole cofactor molecule is transferred as the anchoring unit for attaching desired reporters to the target biomolecule (named sequence-specific methyltransferase-induced labeling, SMILing). Subsequently, cofactors with activated sulfonium-bound side-chains have been produced, which permit direct transfer of these extended side chains alone (named methyltransferase-directed transfer of activated groups, mTAG).[4,5] The required activation of the side chain was achieved by incorporating a double or a triple bond next to the transferrable carbon atom (β position to the sulfonium center). On the basis of our experience with bacterial DNA MTases and short model compounds (cofactors **2** and **3**, Scheme 1),[4] we had designed a series of triple bond containing side chains with a terminal functional group. To this end, we had synthesized and investigated properties of AdoMet analogues with 4-aminobut-2-yn-1-yl or 4-(4-amino butan-amido)-but-2-yn-1-yl side chains (cofactors **4** and **5**).[5] Despite suitability for certain applications, further studies revealed shortcomings of these cofactors associated with a rapid loss of activity under physiological conditions.

In the present work, we have investigated the kinetics and decay pathways of a series of AdoMet analogues with extended propargylic side chains. We find that the but-2-ynyl cofactors containing an electronegative group (amino or amido) at position 4 of the side chain undergo a pH-dependent addition of a water molecule to the side chain. These observations suggested that close proximity of electron withdrawing groups makes the side chain highly susceptible to the addition of nucleophiles such as hydroxyl anion or water. This problem was resolved by synthesis of a series of cofactor analogues in which the separation between the electronegative group and the triple bond is increased from one to three carbon units. The hex-2-ynyl moiety containing cofactor analogues with terminal amino, azide, or alkyne groups showed a markedly improved enzymatic transalkylation activity and proved highly useful for two-step sequence-specific *in vitro* and *ex vivo* labeling of DNA.

The general structure and synthetic strategy for AdoMet analogues containing an activating triple C−C bond is shown in Scheme 1. The first in the series of functional cofactors were those in which a single carbon unit stands between a polar (amino or amido, respectively) group and the triple bond (cofactors **4** and **5**).[5] As mentioned above, these compounds

Received: December 5, 2012
Accepted: April 4, 2013
Published: April 4, 2013

Scheme 1. Methyltransferase-Directed Labeling of DNA Using AdoMet Analogues with Extended Propargylic Transferrable Groups (Upper) and Their Chemical Synthesis from AdoHcy (Lower)



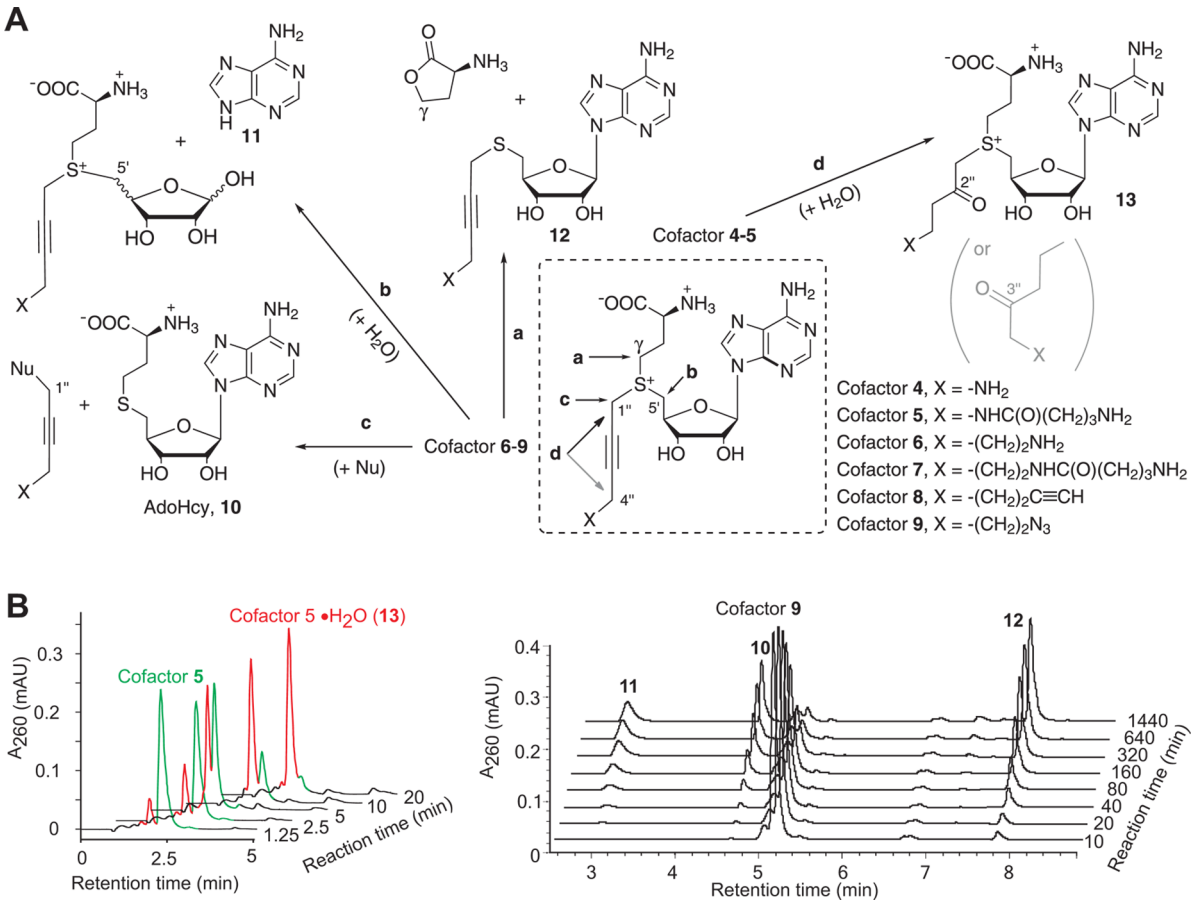


Figure 1. Chemical decomposition of AdoMet analogues in aqueous buffers. (A) Observed routes (a–d) of hydrolytic decomposition of extended AdoMet analogues carrying propargyllic side chains. (B) Time-course HPLC chromatograms of decomposition products obtained with cofactors **5** (left) and **9** (right) in M.*Hha*I buffer (pH 7.4) at 37 °C.

turned out to be problematic in terms of their chemical stability under enzymatic reaction conditions. In particular, cofactor **4** showed lifetimes in the order of minutes, which manifested in poor reproducibility of enzymatic assays (Supplementary

dx.doi.org/10.1021/cb300669x | *ACS Chem. Biol.* 2013, 8, 1134–1139

Figure S5). The natural cofactor AdoMet itself is a high energy compound and spontaneously decomposes in water *via* two pathways (Figure 1a, routes a and b).[6,7] We therefore systematically evaluated the stability of our propargylic AdoMet analogues in standard MTase reaction buffers (pH 7.4). LC/MS analysis of reaction products showed that the model AdoMet analogues **2** and **3** essentially followed the same decomposition routes, however slightly more rapidly than the natural cofactor (Supplementary Figures S1 and S2 and Table 1). Unexpectedly, incubation of the but-2-ynyl cofactors **4** and **5** in aqueous buffers revealed that they both are rapidly converted to more polar (Figure 1, route d) compounds with a molecular mass of +18 amu (Figure 1b). High resolution MS/MS analysis of the reaction product obtained from cofactor **5** clearly indicated that a water molecule is added to the side chain of the cofactor, and the reaction is strongly pH dependent (Supplementary Figures S3 and S4). Since such products were not observed with cofactors **2** or **3** under similar conditions, we concluded that the presence of an electronegative group (protonated amine or amide, respectively) in proximity to the sulfonium-activated triple bond may facilitate the addition of nucleophiles (see discussion on mechanism below). The hydration of the more stable cofactor **5** was ∼150-fold faster than decomposition of AdoMet (Table 1), and the resulting hydration product was found to be inactive in enzymatic transalkylation reactions (see below). Altogether, these observations prompted us to conclude that the but-2-yn-1-yl moiety is not a promising unit for building AdoMet analogues with polar functional groups.

Table 1. Cofactor Lifetimes ($\tau 1/2$) and Proton Chemical Shifts (δ) in M.*Hha*I Reaction Buffer (pH 7.4) at 37 °C

cofactor	transferrable group, R	$\tau_{1/2}$ (h)	δ (4″-CH_2) (ppm)
1	methyl	17 ± 0.4	none
2	but-2-ynyl	3.2 ± 0.2	1.7−1.9
3	pent-2-ynyl	4.6 ± 0.2	n.r.
4	4-aminobut-2-ynyl	<0.05	3.7−3.9
5	4-(4-aminobutanamido)but-2-ynyl	0.11 ± 0.01	4.1
6	6-aminohex-2-ynyl	1.9 ± 0.1	2.0−2.3
7	6-(4-aminobutanamido)hex-2-ynyl	2.5 ± 0.1	2.2−2.4
8	oct-2,7-diynyl	3.0 ± 0.1	2.0−2.3
9	6-azidohex-2-ynyl	3.1 ± 0.2	2.0−2.1

To circumvent this problem, we prepared and explored similar cofactor analogues in which the separation between the electronegative group and the triple bond was increased from one to three carbon units. Synthesis of 6-substituted hex-2-yn-1-yl side chains was generally based on 5-chloropent-1-yne, which was converted to 6-chlorohex-2-yn-1-ol as described previously for the butynyl system.[5] Further transformations involved replacing the 6-chloro substituent with *N*-Boc-amino, 4-*N*-Boc-aminobutanamido, or azide groups (see Supporting Information for details). Synthesis of a side chain carrying a terminal alkyne group was achieved *via* hydroxymethylation of hept-1,6-diyne to oct-2,7-diyn-1-ol. Subsequently, all obtained end-substituted alkynols were converted to corresponding *O*-nosylates, which were used for *S*-selective charging of the side chains onto AdoHcy under acidic conditions.[4] The *N*-Boc protecting group was removed by mild acid treatment, as appropriate. The cofactors were isolated by a combination of ion-exclusion flash chromatography and reversed-phase HPLC as diastereomeric mixtures or as enriched sulfonium epimers and characterized by NMR and high-resolution mass spectrometry (Supporting Information).

The novel AdoMet analogues were examined for their stability in the MTase reaction buffer. We found that the lifetimes of the hexynyl cofactors were much improved as compared to those of cofactors 4 and 5 and ranged between 1.9 to 3.5 h under the same conditions (Supplementary Figure S1 and Table 1). No hydration products were detectable under these conditions, and the decay pathways were similar to those reported for AdoMet (Figure 1b, right). In addition, cofactors showed the formation of detectable amounts of AdoHcy and corresponding hexynols or hexynthioethanols indicating that the transferable 1″-methylene carbon can be attacked by nucleophiles (water and 2-mercaptoethanol) present in reaction buffers (Figure 1a, route c).

The sulfonium center in AdoMet induces a partial positive charge on all three adjacent carbon atoms (transferrable methyl group, ribose 5′-carbon, and methionine γ-carbon), which manifests itself by a nearly 1 ppm high field shift of proton chemical shifts as compared with those in methionine and AdoHcy (to 3.0, 3.9, and 3.5 ppm, respectively).[7,8] Under physiological conditions, both adjacent methylene groups are susceptible to attack leading to slow decomposition to inactive species. One pathway involves an intramolecular attack of the α-carboxylate group onto the γ-carbon of the methionine moiety, resulting in methylthioadenosine and homoserine lactone. In parallel, deprotonation at 5′-C leads to formation of adenine and *S*-ribosylmethionine.[6] Notably, no significant nucleophilic attack on the methyl group of AdoMet by water is detectable under physiological conditions, but in the case of extended AdoMet analogues, the presence of the activating triple bond seems to make the transferable carbon (C1″) more electron deficient (proton chemical shifts of 4.2−4.4 ppm, see Supplementary Table S1) and thus more reactive toward nucleophiles. Moreover, the close proximity of an electron withdrawing group in the butynyl cofactors **4** and **5** significantly increases the electron deficiency on C4″ and even further on C1″ (see Table 1 and Supplementary Table S1). Taking into account that deprotonation of α-C−H bonds next to the positively charged sulfur atom is well-known to be much easier than that next to the positively charged nitrogen atom,[9,10] one would assume that an electron deficient but-2-yn-1-yl system could readily rearrange into a but-1,2-dien-1-yl (allenic) system with subsequent fast addition of water to give a buten-2-oxo-1-yl product (a but-2,3-dien-1-yl intermediate and then buten-2-oxo-1-yl product are less likely). A similar mechanism has also been proposed for the fast inactivation of AdoMet analogues carrying unsubstituted sulfonium-bound prop-2-yn-1-yl side chains.[11,12] Although the hydrated prop-2-oxo-1-yl cofactor proved active in transfer reactions toward strong sulfur nucleophiles,[13] the reactivity of such ketone derivatives appears insufficient with the majority of DNA and protein MTases.

The novel cofactors were then examined using engineered versions[14] of the DNA cytosine-5 MTases. Enzymatic transalkylations were monitored using a DNA protection assay, which exploits resistance of MTase-modified target sites to cleavage with cognate restriction endonucleases. Our analyses showed that the newly designed AdoMet analogues showed a markedly improved enzymatic transalkylation activity as compared to cofactors 4 and 5 (Supplementary Figure S5 and Table S2). Composition analysis of the modified DNA

dx.doi.org/10.1021/cb300669x | *ACS Chem. Biol.* 2013, 8, 1134−1139

substrates confirmed enzymatic transfer of all side chains from all hexynyl cofactors (Supplementary Figure S6).

During the past years, a number of mTAG cofactors with short side chains were synthesized and studied for derivatization and labeling of DNA, RNA, and protein targets.[11,12,15−17] These cofactors were typically intended for work with wild-type MTases. This strategy permits interrogation of a wide spectrum of cellular enzymes and even entire methylomes in cells or cell extracts. However, our selection of a larger hex-2-ynyl moiety as the basic transfer unit permits a better accessibility and enhanced reactivity of the attached terminal group, which is beneficial for efficient conjugation of the derivatized molecule with other bulky compounds or large biomolecules. In the case of DNA modification, bulky moieties attached on longer linear side chains are generally better tolerated by DNA polymerases as compared to shorter linkers[18] permitting efficient amplification and analysis of subsequently labeled DNA by PCR and sequencing techniques. However, an increased steric bulk of the AdoMet analogues makes them poor substrates for wild-type MTases.[14] This is a useful feature for numerous other *in vivo* and *ex vivo* studies in which selective transalkylation activity with a designated tailor-engineered MTase is sought in the context of a vast variety of endogenous AdoMet-dependent MTases. However, this would be especially useful if an mTAG labeling reaction could be performed in the presence of the natural AdoMet cofactor. To this end, we have recently reported successful transfer of aliphatic primary amino groups from cofactor **7** in direct competition with AdoMet using an engineered version of the *Hha*I MTase,[14] and this appears to hold for cofactors **8** and **9** as well (Supplementary Figure S7).

Since bioorthogonal conjugation reactions offer added benefits, we therefore examined if similar two-step labeling of DNA is possible in crude bacterial cell extract with cofactor **9**. First, using a cleavage assay combining methylation sensitive endonucleases R.Hin6I and McrBC we show that the mTAG reaction with the engineered version of *Hha*I (eM.HhaI) is strongly in favor of transferring the extended groups, resulting in most of the *Hha*I sites carrying azide modification (Supplementary Figure S8). As a final proof of principle, we performed mTAG transalkylation and subsequent copper-free click labeling of DNA in a crude bacterial extract. For this, *E. coli* cells carrying a multicopy recombinant plasmid were gently lysed and treated with eM.*Hha*I and cofactor **9**. In the second step, incubation was continued in the presence of a commercial cyclooctyne-based fluorogenic reagent (Figure 2A). Purified plasmid DNA was then digested with suitable restriction endonucleases and analyzed by agarose gel electrophoresis to reveal three clearly resolved DNA fragments. The observed fluorescence intensity distribution was fully consistent with the number of the GCGC target sites in the plasmid-derived fragments demonstrating a sequence-specific labeling of the cellular DNA (Figure 2B). The experimental procedure is simple and specific even in crude cell lysates attesting its suitability for routine laboratory use.

In conclusion, this work presents the first detailed study of the chemical stability of AdoMet analogues used for selective labeling of biomolecular targets. Altogether our results indicate that the newly engineered series of AdoMet analogues based on the hex-2-ynyl moiety provide a robust platform for a variety of applications involving two-step sequence-specific labeling of DNA. Combination of different MTases and cofactor chemistries potentially permits a multicolor sequence-selective labeling of DNA. Useful *in vitro* applications of mTAG labeling

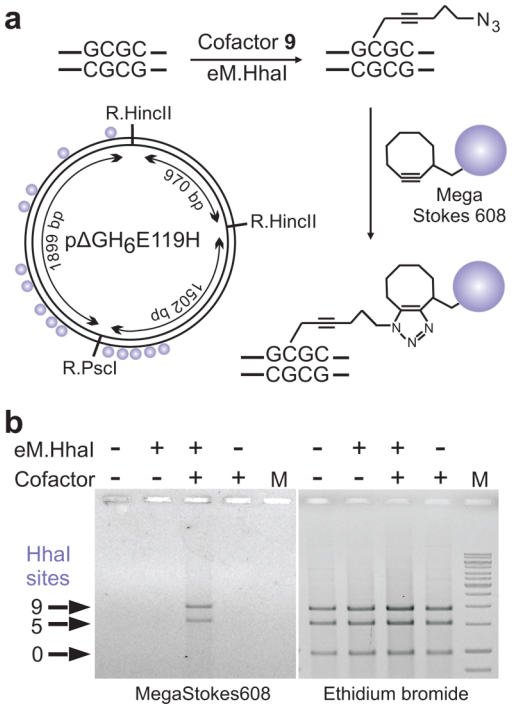


Figure 2. Sequence-specific two-step mTAG labeling of plasmid DNA using copper-free click reaction in crude cell extract. (a) *Escherichia coli* ER2267 cells carrying the pΔGH$_6$E119H plasmid were harvested, and crude lysate was treated with eM.*Hha*I (engineered *Hha*I MTase) and cofactor **9** for 3 h at 37 °C. Alkyne MegaStokes 608 dye was added, and incubation continued for another 3 h, followed by plasmid DNA isolation by column purification. (b) Image of agarose gel electrophoresis of the labeled plasmid fragmented with R.HincII and R.PscI endonucleases. Number of *Hha*I sites is indicated on the left of the fragment-pointing arrows. Control samples lacked M.*Hha*I or cofactor as indicated. M, DNA size marker.

using amino cofactors **6** and **7** have recently been demonstrated for single molecule fluorescence-based genotyping of large natural DNA substrates[19] and for covalent tagging and enrichment of epigenomic target sites (E. Kriukienė, V. Labrie, T. Khare, S. C. Wang, A. Petronis, S. Klimašauskas, et al., unpublished data). Furthermore, deposition of alkyne and azide groups onto DNA in combination with bioortogonal click labeling paves the way to specific *in vivo* labeling of these biologically important macromolecules.

METHODS

Materials. The pΔGH$_6$E119H plasmid encoding a catalytically inactive E119H M.*Hha*I mutant was prepared from pΔ324GH$_6$[20] by site specific mutagenesis following previously described procedures.[21] *S*-adenosyl-L-methionine (**1**) and *S*-adenosyl-L-homocysteine (**10**) were purchased from Sigma-Aldrich.

Chemical Synthesis and Purification of AdoMet Analogues. Chemical synthesis and purification of AdoMet analogues were performed as previously described by direct chemoselective *S*-alkylation of *S*-adenosyl-L-homocysteine with corresponding trifluoromethylsulfonates (cofactors **2** and **3**)[4,21] or 4-nitrobenzenesulfonates (cofactors **4**−**9**).[5] The cofactor analogues were obtained either as diasteromeric mixtures of *R*,*S*- and *S*,*S*-isomers or were chromatographically enriched in the enzymatically active *S*,*S*-isomer (see Supporting Information for details).

HPLC-MS Analysis of Cofactor Decomposition Kinetics and Products. The stability of the synthesized AdoMet analogues was evaluated in mTAG reaction buffer (50 mM Tris-HCl, 10 mM NaCl, 0.5 mM EDTA, 2 mM 2-mercaptoethanol, 0.2 mg mL^{-1} BSA, pH 7.4)

dx.doi.org/10.1021/cb300669x | *ACS Chem. Biol.* 2013, 8, 1134−1139

at 37 °C at a starting concentration of 300 μM. Twenty microliter samples were taken into two volumes of HPLC buffer A (20 mM HCOONH4, pH 3.5) and flash frozen in liquid nitrogen. Ten microliter aliquots were loaded onto a HPLC column (Discovery HS C18 75 × 2.1 mm). Samples were eluted with a linear gradient of solvents A (20 mM $HCOONH_4$, pH 3.5) and B (80% methanol solution in water) at a flow of 0.3 mL min^{-1} and detected by a diode array UV absorbance detector followed by an in-line electrospray ionization single quadruple mass spectrometer (Hewlett-Packard 1100 series system) using postcolumn equal coflow 99% methanol and 1% CF_3COOH. Mass spectra were acquired in 50−800 m/z range in positive ion mode. Ionization capillary voltage was 5000 V, fragmentor voltage 100−120 V, drying gas temperature 300−350 °C, and flow rate 10−12 L min^{-1}.

High-resolution mass spectra of modification products were acquired on an Agilent Q-TOF 6250 mass spectrometer equipped with a Dual-ESI source. HR-MS/MS analysis of cofactor **5** and its hydration product was performed with an Agilent 6520 Q-TOF spectrometer, precursor ions (555.2344 and 537.2238) selected with a window of ±4 m/z, using collision energy of 20.0 V, and nitrogen as the collision gas.

HPLC-MS Analysis of DNA Transalkylation Products. mTAG transalkylation reactions were performed with eM.*Hha*I (variant Q82A/Y254S/N304A, 0.5 μM), oligonucleotide duplex (pATTGCGC, 33 μM), and cofactors **1**−**4**, **6**−**8** or **9** (300 μM) in reaction buffer at 37 °C for 4 h and then heated at 80 °C for 20 min. The modified duplexes were desalted by ethanol precipitation. Nuclease PI buffer (10 mM Tris-HCl, 10 mM magnesium chloride, and 1 mM zinc acetate, pH 7.5) containing Nuclease PI (0.05 au) and shrimp alkaline phosphatase (0.1 au) was added, and the samples were incubated at 37 °C for 4 h. Hydrolyzate was analyzed on an integrated HPLC/ESI-MS system as described above.

mTAG Derivatization of DNA in the Presence of Competing AdoMet *in Vitro*. eM.*Hha*I (0.125 μM) and pΔGH_6E119H plasmid DNA (0.25 μM total GCGC sites) were combined with cofactor **8** or **9** and AdoMet (total cofactor concentration 50 μM) at specified ratios. Reactions were incubated at 37 °C for 1 h. Completeness of DNA modification was monitored by incubating reaction aliquots with restriction endonuclease R.Hin6I (2−5 au per 1 μg of DNA) in accordance to manufacturer recommendations (Fermentas).

DNA Labeling *via* Cu(I)-Assisted Click Chemistry *in Vitro* (Cofactor 8). An aliquot of a mTAG modification reaction (5 μL) was diluted with an equal volume of DMSO and 1.5 μL of freshly prepared Cu-TBTA solution was added followed by 1 μL of 2.5 mM Alexa647 azide in DMSO. The reaction mixture was incubated 45 min at 37 °C, and DNA purified by ethanol precipitation. Cu-TBTA solutions were prepared by combining 0.03 M CuBr (Sigma-Aldrich) in DMSO with 100 mM tris-(benzyltriazolylmethyl)amine (TBTA) (Sigma-Aldrich) in DMSO/*tert*-butanol.

For analysis of site-specific labeling, modified DNA was digested with R.HindII and R.PscI restriction enzymes for 5 h at 37 °C to generate three fragments: 1900 bp with 9 *Hha*I targets, 1500 bp with 5 *Hha*I targets, and 970 bp with no *Hha*I targets. Fragmented DNA was resolved on a 1% agarose gel and stained with EtBr. Gels were scanned on a Fuji FLA-5100 gel imagining system using 635 (for Alexa647) and 473 nm (for EtBr) lasers.

DNA Labeling *via* Cu-Free Click Chemistry *in Vitro* (Cofactor 9). Cofactor **9** mTAG modification reactions were directly treated with Alkyne MegaStokes608 for Cu-free click-chemistry (Sigma-Aldrich). MegaStokes608 dye (Sigma-Aldrich) was added to 100 μM, and the mixtures were incubated at 37 °C for 1 h. Afterward, the DNA was column-purified (Zymo Research IIIC columns). Finally, it was fragmented and agarose-gel resolved as described above (DNA labeling *via* Cu-assisted click chemistry *in vitro*). For MegaStokes608, fluorescence gels were scanned using a 473 nm laser and then stained with EtBr (0.1 mg mL^{-1}) and scanned again to visualize bulk DNA.

DNA Modification in Crude Cell Lysates (Cofactor 9). *E.coli* ER2267 cells were transformed with the pΔGH_6E119H plasmid. After overnight growth in LB medium, the cells were spun down and resuspended in TE buffer (100 mM Tris·HCl, pH = 8.0, and 100 mM EDTA). Proteinase inhibitor PMSF to 2.5 mM, RNase A/T1 mix, and lysozyme to 0.25 mg mL^{-1} were added, and the suspension was incubated 1 h at 37 °C. Cell debris and unlysed cells were spun down, supernatant was combined with eM.*Hha*I (2 μM) and cofactor **9** or **1** (50 μM) in TE buffer and incubated for 3 h at 37 °C. PMSF was replenished every hour. Finally, plasmids were purified using a GeneJET Plasmid Miniprep kit (Fermentas).

For fluorescent labeling of the azide derivatized DNA (Figure 2), an equal volume of DMSO and MegaStokes608 alkyne dye to 200 μM were added and incubated for 3 h at 37 °C. Modified DNA was column-purified (Zymo Research IIIC columns) and digested with R.HincII and R.PscI as described above (DNA labeling *via* click chemistry *in vitro*).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures, spectral data and complete characterization of extended cofactors. Supplementary Figures S1−S8 and Supplementary Tables S1−S2. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(S.K.) E- mail: saulius.klimasauskas@bti.vu.lt.

Present Address

§Institute of Chemical Sciences and Engineering, Ecole Polytechnique Federal de Lausanne, 1015 Lausanne, Switzerland.

Notes

The authors declare the following competing financial interest(s): G.L and S.K. are inventors on related pattents.

■ ACKNOWLEDGMENTS

We are grateful to V. Lapienė, Z. Liutkevičiūtė, M. Nainytė,and R. Gerasimaitė for technical assistance and advice, and G. Sasnauskas for a gift of the McrBC enzyme. This work was supported by funding from the National Institutes of Health [grant HG004535] and the European Social Fund under the Global Grant measure [grant VP1-3.1-ŠMM-07-K-01-105].

■ REFERENCES

(1) Klimašauskas, S., and Weinhold, E. (2007) A new tool for biotechnology: AdoMet-dependent methyltransferases. *Trends Biotechnol. 25*, 99−104.

(2) Pljevaljcic, G., Schmidt, F., and Weinhold, E. (2004) Sequence-specific methyltransferase-induced labeling of DNA (SMILing DNA). *ChemBioChem 5*, 265−269.

(3) Comstock, L. R., and Rajski, S. R. (2005) Conversion of DNA methyltransferases into azidonucleosidyl transferases via synthetic cofactors. *Nucleic Acids Res. 33*, 1644−1652.

(4) Dalhoff, C., Lukinavičius, G., Klimašauskas, S., and Weinhold, E. (2006) Direct transfer of extended groups from synthetic cofactors by DNA methyltransferases. *Nat. Chem. Biol. 2*, 31−32.

(5) Lukinavičius, G., Lapienė, V., Staševskij, Z., Dalhoff, C., Weinhold, E., and Klimašauskas, S. (2007) Targeted labeling of DNA by methyltransferase-directed transfer of activated groups (mTAG). *J. Am. Chem. Soc. 129*, 2758−2759.

(6) Hoffman, J. L. (1986) Chromatographic analysis of the chiral and covalent instability of *S*-adenosyl-L-methionine. *Biochemistry 25*, 4444−4449.

(7) Klimašauskas, S., and Lukinavičius, G. (2008) Chemistry of AdoMet-dependent methyltransferases. In *Wiley Encyclopedia of Chemical Biology* (Begley, T. P., Ed.), pp 1−10, John Wiley & Sons, Inc., New York.

(8) Stolowitz, M. L., and Minch, M. J. (1981) *S*-Adenosyl-L-methionine and *S*-adenosyl-L-homocysteine, an NMR study. *J. Am. Chem. Soc. 103*, 6015−6019.
(9) Johnson, A. W., and Amel, R. T. (1968) The chemistry of ylids. XVI. The acidifying effects of 'onium groups. *Can. J. Chem. 46*, 461−463.
(10) Cheng, J.-P., Liu, B., and Zhang, X.-M. (1998) Radical substituent effects of alpha-sulfonium groups. *J. Org. Chem. 63*, 7574−7575.
(11) Willnow, S., Martin, M., Lüscher, B., and Weinhold, E. (2012) A selenium-based click adomet analogue for versatile substrate labeling with wild-type protein methyltransferases. *ChemBioChem 13*, 1167−1173.
(12) Bothwell, I. R., Islam, K., Chen, Y., Zheng, W., Blum, G., Deng, H., and Luo, M. (2012) Se-adenosyl-L-selenomethionine cofactor analogue as a reporter of protein methylation. *J. Am. Chem. Soc. 134*, 14905−14912.
(13) Lee, B. W. K., Sun, H. G., Zang, T., Kim, B. J., Alfaro, J. F., and Zhou, Z. S. (2010) Enzyme-catalyzed transfer of a ketone group from an *S*-adenosylmethionine analogue: a tool for the functional analysis of methyltransferases. *J. Am. Chem. Soc. 132*, 3642−3643.
(14) Lukinavičius, G., Lapinaitė, A., Urbanavičiūtė, G., Gerasimaitė, R., and Klimašauskas, S. (2012) Engineering the DNA cytosine-5 methyltransferase reaction for sequence-specific labeling of DNA. *Nucleic Acids Res. 40*, 11594−11602.
(15) Peters, W., Willnow, S., Duisken, M., Kleine, H., Macherey, T., Duncan, K. E., Litchfield, D. W., Lüscher, B., and Weinhold, E. (2010) Enzymatic site-specific functionalization of protein methyltransferase substrates with alkynes for click labeling. *Angew. Chem., Int. Ed. 49*, 5170−5173.
(16) Islam, K., Zheng, W., Yu, H., Deng, H., and Luo, M. (2011) Expanding cofactor repertoire of protein lysine methyltransferase for substrate labeling. *ACS Chem. Biol. 6*, 679−684.
(17) Tomkuvienė, M., Clouet-d'Orval, B., Černiauskas, I., Weinhold, E., and Klimašauskas, S. (2012) Programmable sequence-specific click-labeling of RNA using archaeal box C/D RNP methyltransferases. *Nucleic Acids Res. 40*, 6765−6773.
(18) Weisbrod, S. H., and Marx, A. (2008) Novel strategies for the site-specific covalent labelling of nucleic acids. *Chem. Commun.*, 5675−5685.
(19) Neely, R. K., Dedecker, P., Hotta, J.-I., Urbanavičiūtė, G., Klimašauskas, S., and Hofkens, J. (2010) DNA fluorocode: A single molecule, optical map of DNA with nanometre resolution. *Chem. Sci. 1*, 453−460.
(20) Daujotytė, D., Vilkaitis, G., Manelytė, L., Skalicky, J., Szyperski, T., and Klimašauskas, S. (2003) Solubility engineering of the HhaI methyltransferase. *Protein Eng. 16*, 295−301.
(21) Gerasimaitė, R., Vilkaitis, G., and Klimašauskas, S. (2009) A directed evolution design of a GCG-specific DNA hemimethylase. *Nucleic Acids Res. 37*, 7332−7341.