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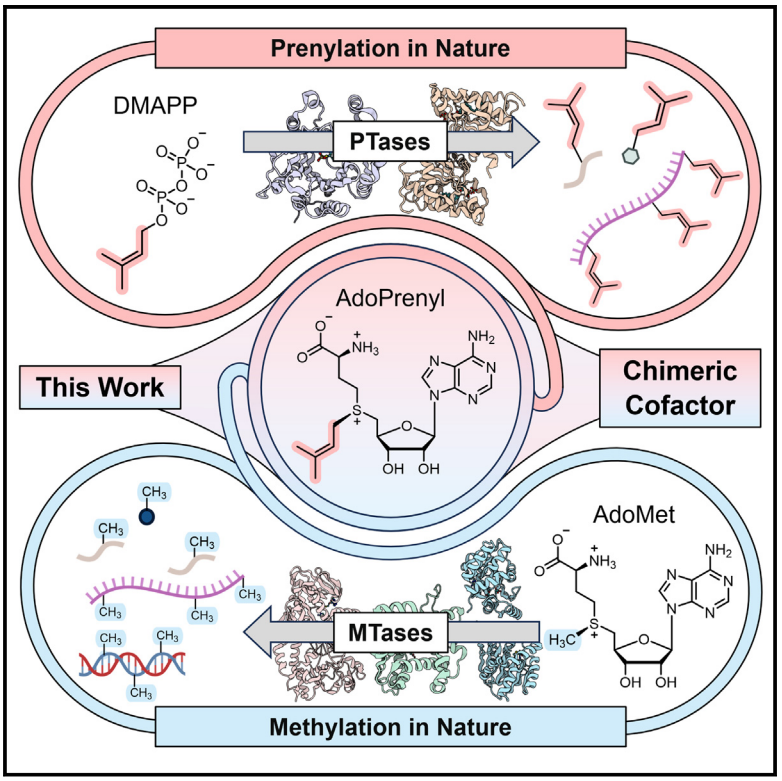
# Chimeric cofactors enable methyltransferase-catalyzed prenylation

## Graphical abstract




## Highlights

- Chimeric cofactors make methyltransferases act as prenyltransferases
- The tested methyltransferases favor prenylation over natural methylation
- This strategy can transfer prenyl, geranyl, farnesyl, and clickable groups

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## In brief

In nature, many methyltransferases ($C_1$) use *S*-adenosyl-L-methionine (AdoMet or SAM) as a cofactor for methyl transfer, whereas prenyltransferases ($C_5$) use dimethylallyl diphosphate (DMAPP). We engineered a chimeric cofactor, termed AdoPrenyl, that enables methyltransferase-catalyzed prenylation. AdoPrenyl is highly reactive and is preferred over AdoMet in direct competition by the methyltransferases tested. The concept extends to the transfer of geranyl ($C_{10}$), farnesyl ($C_{15}$), and a clickable prenyl analog. This work unlocks hundreds of methyltransferases for the generation of pseudo-natural products and new-to-nature bioconjugates.

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# Chimeric cofactors enable methyltransferase-catalyzed prenylation

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**THE BIGGER PICTURE** Enzymes can perform alkylation reactions of their target substrate with high selectivity under mild conditions. Suitable transferases can be found in different enzyme classes and rely on different cofactor scaffolds depending on whether they transfer one-carbon or non-methyl groups. *S*-Adenosyl-L-methionine (AdoMet or SAM) is characteristic of methyltransferases ($C_1$), whereas prenyltransferases ($C_5$) rely on dimethylallyl diphosphate (DMAPP). We engineered a chimeric AdoMet/DMAPP cofactor and found that it is efficiently converted by various methyltransferases acting on different targets at C, N, and O atoms. The chimeric cofactor is highly reactive, and prenylation is preferred over the methylation in direct competition with the natural AdoMet.

Our work unlocks hundreds of methyltransferases as biocatalysts for regioselective prenylation and will prove useful in making pseudo-natural products and new-to-nature bioconjugates. We also show that the concept can be extended to $C_{10}$ and $C_{15}$ units as well as clickable groups. The finding that the AdoMet scaffold can be used for efficient prenyl transfer by wild-type methyltransferases shows that there is no inherent chemical or enzymatic reason that $C_1$ and $C_5$ transfer is catalyzed by different enzyme classes. This relaxes the paradigm of one-carbon- versus non-methyl-group-transferring enzyme classes for biotransformations and therefore opens new doors in biocatalytic alkylation.

## SUMMARY

Enzymatic alkylation is known for its selectivity and specificity. Transferases are found in enzymes that transfer one-carbon groups and in those that transfer non-methyl groups. Both classes catalyze the attack of a nucleophilic substrate but use different cofactors. *S*-Adenosyl-L-methionine (AdoMet or SAM) is characteristic of methyltransferases (MTases), whereas prenyltransferases (PTases) rely on dimethylallyl diphosphate (DMAPP). It is unclear whether this preference originates from inherent chemical or enzymatic requirements. We find that DNA, RNA, and small-molecule MTases acting on C, N, and O atoms function as PTases when offered a chimeric AdoMet-DMAPP cofactor (AdoPrenyl). This cofactor is highly reactive, necessitating its enzymatic *in situ* formation and leading to preferential MTase-catalyzed prenylation. The DNA-MTase M.TaqI efficiently transfers geranyl ($C_{10}$) and farnesyl ($C_{15}$) moieties as well. Our work shows that the AdoMet scaffold can function as an efficient prenyl donor. Because there are hundreds of MTases, this route to regio- and sequence-selective prenylation is versatile for forming pseudo-natural products and new-to-nature bioconjugates.

## INTRODUCTION

Alkylation is an important strategy for modulating the chemical and biological properties of small molecules, lipids, nucleic acids, and proteins. Methylation or alkylation can enhance the potency of active pharmaceutical ingredients by several orders of magnitude.[1] Strategies for selective late-stage methylation or alkylation are therefore highly sought after. However, chemical alkylation from alkyl halides—although simple and cheap—lacks selectivity on complex molecules.

Alkylating enzymes show regio-, stereo-, and chemoselectivity. Transferases for alkylation can be found in enzyme class

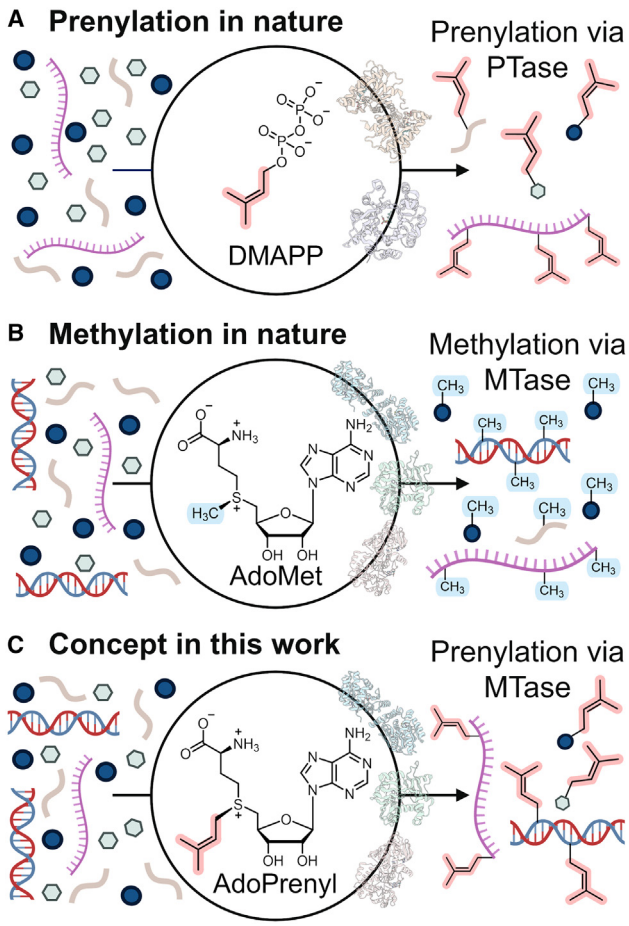


**Figure 1. Concept of natural and new chimeric cofactors for enzyme-catalyzed alkylation**

(A) Prenyltransferases (PTases) use dimethylallyl diphosphate (DMAPP) to transfer the prenyl group to diverse natural products and tRNA.

(B) Methyltransferases (MTases) catalyze the methyl transfer from *S*-adenosyl-L-methionine (AdoMet or SAM) to small molecules, proteins, RNA, or DNA.

(C) The chimeric cofactor AdoPrenyl merges parts from DMAPP and AdoMet. Various MTases use it to prenylate small molecules or nucleic acids in a regio- or sequence-specific manner.

(EC) 2.1 for one-carbon-group transfer[2,3] and in EC 2.5 for non-methyl-group transfer.[4] They share the attack of a nucleophilic substrate on a cofactor-derived electrophile but use different cofactor scaffolds. Prenyltransferases (PTases) rely on dimethylallyl diphosphate (DMAPP) as cofactor for the transfer of $C_5$ units (Figure 1A). The reaction between DMAPP and its isomer, isopentenyl diphosphate (IPP), generates longer-chain linear isoprenes geranyl diphosphate (GPP or $C_{10}$) and farnesyl diphosphate (FPP or $C_{15}$) and is also catalyzed by PTases. Prenylation is a ubiquitous modification in nature; it occurs in natural products[5,6] (e.g., flavonoids, isoflavonoids, and chalconoids), cytokinin precursors, tRNA,[7,8] cyclic peptides,[9] and proteins and is biologically and medically important. Cofactor analogs of DMAPP have been synthesized and converted with PTases.[10–15]

Conversely, methyltransferases (MTases) utilize *S*-adenosyl-L-methionine (SAM or AdoMet) as the cofactor for $C_1$ transfer (Figure 1B).[16,17] Various AdoMet analogs have been used for alkyl randomization,[18–20] labeling,[21–23] and photocaging.[24,25] AdoMet analogs bearing allylic moieties can enhance the MTase-catalyzed non-methyl transfer by stabilizing the transition state.[26,27]

The current understanding is that nature uses two distinct classes of enzyme-cofactor pairs to transfer methyl and $C_5$ units, each of which relies on different cofactor scaffolds. It is unclear whether this is necessitated by the underlying alkylation chemistry. MTases are abundant and act on a broad variety of substrates that are not targeted by PTases; however, the transfer of prenyl, geranyl, or even farnesyl residues with MTases has not been described. We reason that a cofactor chimera of DMAPP and AdoMet could turn MTases into prenyl-transferring enzymes (Figure 1C). Such an approach could drastically expand the scope of prenylation and potentially even lead to the geranylation or farnesylation of MTase substrates. It would also answer the question of whether there is an inherent chemical reason for the distinct ECs for $C_1$ and $C_5$ transfer.

## RESULTS

On the basis of these considerations, we designed the chimeric cofactor AdoPrenyl, which features a dimethylallyl (or prenyl) group at the sulfonium center of the AdoMet scaffold (Figure 1C). Our attempts to synthesize AdoPrenyl from *S*-adenosyl-L-homocysteine (AdoHcy) and prenyl bromide, in analogy to the synthesis of other AdoMet analogs, indicated the formation of AdoPrenyl; however, the isolation failed (Figure S1).

AdoMet and analogs can also be synthesized enzymatically by various approaches,[28–30] including the methionine adenosyltransferase (MAT)-catalyzed reaction of methionine (**1a**) (or analogs) with ATP. We switched to enzymatic *in situ* generation and synthesized prenyl-homocysteine (**1b**, $C_5$; Figures 2 and S2–S6), a methionine analog bearing a prenyl moiety. We converted **1b** with ATP by using a MAT variant from *Methanocaldococcus jannaschii* (PC-MjMAT) engineered to accept sterically demanding methionine analogs[24,31] (Figure 2A). At elevated temperature (65°C), we observed the unexpected formation of AdoHcy (**4**) from **1b** and ATP (Figure 2C).

As a negative control, we omitted methionine and found only the degradation of ATP to ADP (Figure S7). The control reaction with **1a** at 65°C led to the expected AdoMet degradation product methylthioadenosine (MTA, **3a**) (Figure 2B), which forms via intramolecular cyclization and releases homoserine lactone (HSL) (Figure 2A).[17,32,33] Analysis was performed by liquid chromatography-mass spectrometry (LC-MS) (Figures S8–S17).

These results indicate that AdoPrenyl (**2b**) can be generated chemically and enzymatically but is short lived and potentially highly reactive. The prenyl group in **2b** leads to a different degradation pathway than AdoMet and the reported AdoMet analogs.

Next, we wanted to test whether the *in-situ*-formed AdoPrenyl can serve as a cofactor in MTase-catalyzed prenylation reactions and chose *O*-, *C*-, and *N*-MTases belonging to small-molecule, DNA, and RNA MTases to perform cascade reactions (Figure 3A) starting from methionine (**1a**) or prenylhomocysteine (**1b**).

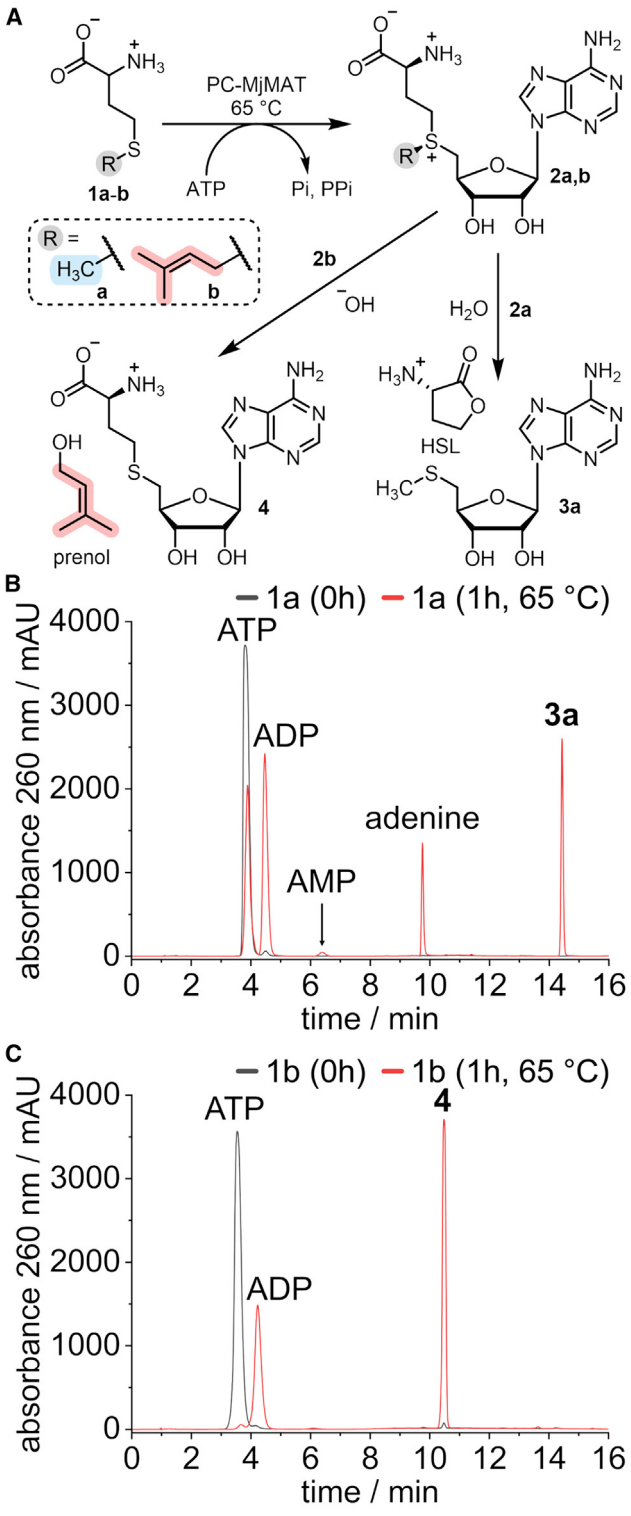


**Figure 2. *In situ* generation and decomposition of AdoMet and AdoPrenyl**

(A) Methionine (**1a**) and prenyl-D/L-homocysteine (**1b**) are converted to AdoMet (**2a**) and AdoPrenyl (**2b**) by PC-MjMAT in the presence of ATP. AdoMet (**2a**) follows the expected decomposition pathway, yielding methylthioadenosine (**3a**) at 65°C. AdoPrenyl **2b** decomposes to *S*-adenosyl-L-homocysteine (**4**) and prenol.

(B and C) HPLC analysis of the enzymatic reactions starting from **1a** (B) or **1b** (C) with ATP catalyzed by PC-MjMAT. Conditions: 5 mM **1a** and **1b**, 5 mM ATP, and 100 μM PC-MjMAT incubated at 65°C for 1 h. Reaction buffer (1×): 50 mM HEPES, 10 mM $MgCl_2$, 5 mM KCl (pH = 7.4).

RnCOMT$^{M40A/Y200L}$, a variant of the catechol-*O*-MTase from *Rattus norvegicus*, is reported to methylate dihydroxybenzaldehyde (**5**) with high regioselectively.[37,38] When we exposed it to *in-situ*-formed AdoPrenyl (**2b**), we observed efficient formation of the prenylated product (**5b**) (75% conversion in 1 h) with a ratio of prenylvanillin (**5b$^{meta}$**) to prenylisovanillin (**5b$^{para}$**) of 92:8 (Figures 3B and 3C). The other regioisomer did not accumulate over time, confirming that the observed prenylation was catalyzed by MTase. The correct annotation of the regioisomers was ensured by comparison to a standard of **5b$^{para}$** synthesized for that purpose (Figures S18 and S19).

C–C bond formation is highly desired in enzymatic reactions. The MTase NovO is known to alkylate the C8 position of various 7-hydroxycoumarin scaffolds.[20,34,39] In the cascade reaction with *in-situ*-formed AdoPrenyl (**2b**), the substrate 4,5,7-trihydroxy-3-phenylcoumarin (**6**) was readily converted to **6b**. The conversion reached 85% after 1 h, and no other products were observed by high-performance liquid chromatography (HPLC) analysis (Figures 3D and 3E).

As the *N*-MTase, we used Ecm1, the mRNA cap guanine-N7 MTase from *Encephalitozoon cuniculi*.[35,40] With *in-situ*-formed AdoPrenyl, we again observed efficient prenylation of the substrate, i.e., the dinucleotide GpppA (**7**) (Figures 3F and 3G). Conversion to product **7b** reached 98% after 30 min. We observed a small amount of intermediates **2b** and **4** in this case (formed at 30 min but consumed after 60 min) given that the detection at 260 nm is most sensitive for these compounds. The adenine peak originated from the MTAN-catalyzed degradation of **4**. Cascade products **7a** and **7b** showed no degradation under assay conditions for 24 h, whereas prenolether **5b** was labile when strong acids were added (Figures S20 and S21). Complete HPLC traces for the MTase-catalyzed prenylations and methylations are shown in Figures S22 and S23, and cascade products were verified by liquid chromatography-quadrupole mass spectrometry (LC-Q-MS) (Figures S24–S29). For all reactions, we performed negative controls without MTase to rule out uncatalyzed product formation (Figure S30)

When we compared the prenylation reactions (**5b–7b**) with the respective methylation control reactions (**5a–7a**) performed under identical conditions, we noticed that prenylation proceeded faster, suggesting that AdoPrenyl—although being an AdoMet/DMAPP chimera and non-natural co-substrate—was more efficiently converted by MTases than the natural AdoMet itself.

This prompted us to set up a direct competition assay starting from equimolar concentrations of **1a** and **1b** and perform the MAT/MTase cascade reactions. Analysis of the product distribution (prenyl/methyl) should be indicative of the co-substrate preference (Figure 4A).

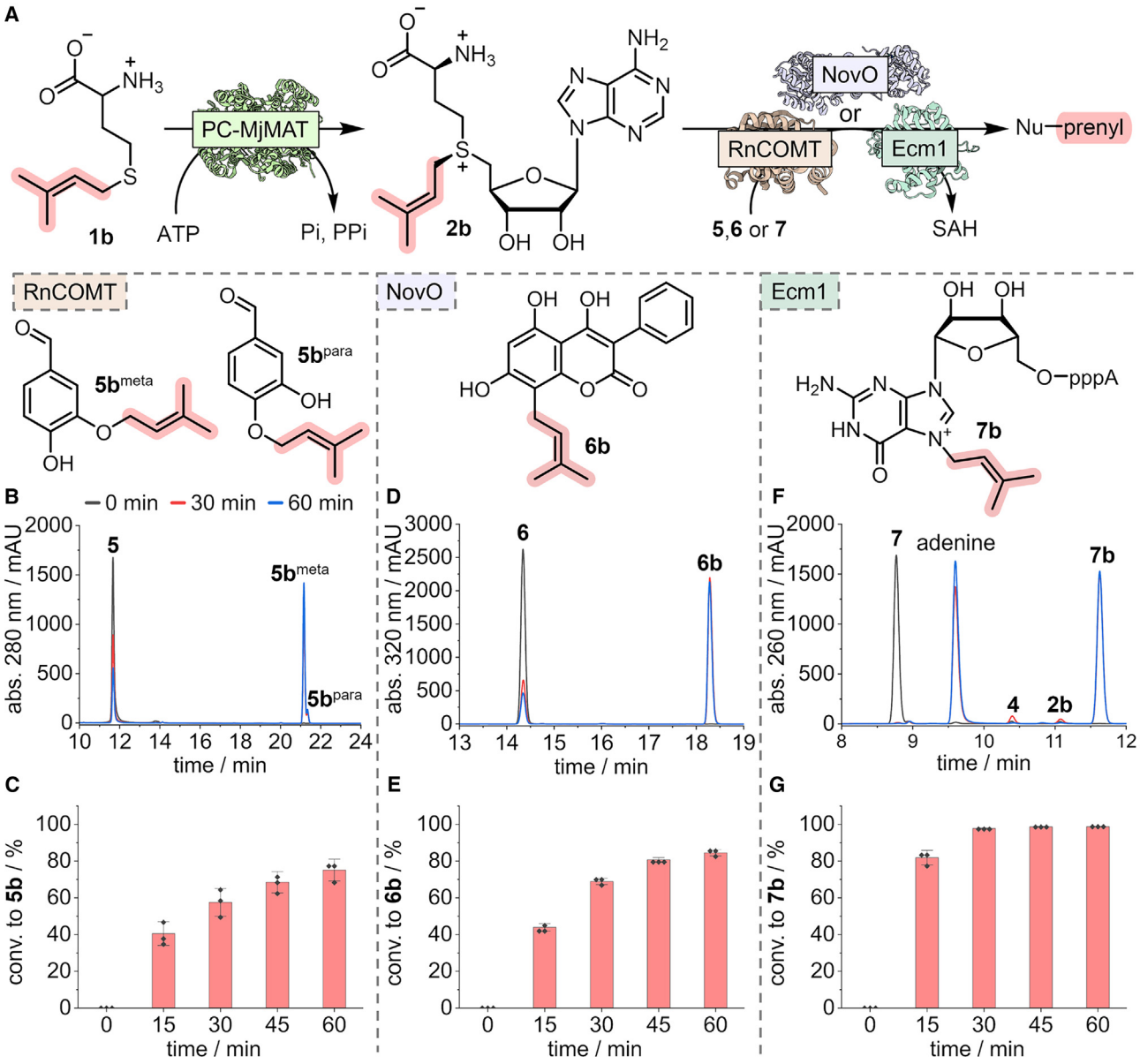


**Figure 3. MTase-catalyzed prenyl transfer from AdoPrenyl formed *in situ***

(A) Reaction scheme. Methionine analog **1b** is converted into AdoPrenyl by the MAT/MTase cascade to give prenylated products **5b**, **6b**, and **7b** (Nu-prenyl). PC-MjMAT (PDB: 7P84),[31] NovO (PDB: 5MGZ),[34] Ecm1 (PDB: 1Z3C),[35] and RnCOMT (PDB: 1H1D).[36]

(B and C) Representative HPLC analysis (B) and time course (C) of the PC-MjMAT/RnCOMT$^{M40AY200L}$ cascade reactions.

(D and E) Representative HPLC analysis (D) and time course (E) of the PC-MjMAT/NovO cascade reactions.

(F and G) Representative HPLC analysis (F) and time course (G) of the PC-MjMAT/Ecm1 cascade reactions.

Conditions: 2.5 mM (for RnCOMT$^{M40AY200L}$) or 5 mM (**1b**), 5 mM ATP, 100 μM PC-MjMAT, 50 μM MTase, 1 mM **5** or **6** or 500 μM **7**, and 10 μM MTAN at 37°C. Reaction buffer (1×): 50 mM HEPES, 10 mM $MgCl_2$, 5 mM KCl (pH = 7.4). Bar plots show average and standard deviation of triplicates.

These competition experiments yielded higher amounts of prenylation than methylation product for all three tested MTases (Figure S31). Specifically, the PC-MjMAT/RnCOMT$^{M40A/Y200L}$ cascade yielded 6% **5a** versus 49% **5b** after 1 h, corresponding to an 8-fold preference for prenylation (Figure 4B). The PC-MjMAT/NovO cascade yielded 12% **6a** versus 80% **6b** after 1 h (Figure 4C), corresponding to a ~7-fold preference for prenylation. The PC-MjMAT/Ecm1 cascade resulted in 5% **7a** versus 95% **7b** after 1 h, corresponding to a remarkable 19-fold preference for prenylation (Figure 4D).

Starting from identical conditions, all three cascade reactions led to more prenylation than methylation product, suggesting that the chimeric AdoPrenyl is a better co-substrate than AdoMet to promiscuous MTases. The observed and maintained

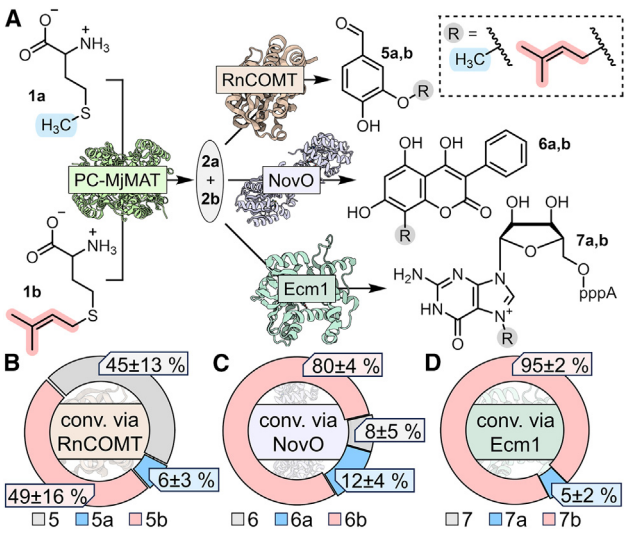


**Figure 4. Competition assays using 1a and 1b in the same MAT/MTase cascade reactions**

(A) Reaction scheme. PC-MjMAT (PDB: 7P84),[31] NovO (PDB: 5MGZ),[34] Ecm1 (PDB: 1Z3C),[35] and RnCOMT (PDB: 1H1D).[36]

(B–D) Products of PC-MjMAT/RnCOMT (B), PC-MjMAT/NovO (C), and PC-MjMAT/Ecm1 (D) competition cascade reactions ($n$ = 3). Conversions are given as the average and standard deviations of triplicates.

Conditions: 2.5 mM (for RnCOMT) or 5 mM (**1a** and **1b**), 5 mM ATP, 100 μM PC-MjMAT, 50 μM MTase, 1 mM **5** or **6** or 500 μM **7**, and 10 μM MTAN at 37°C. Reaction buffer (1×): 50 mM HEPES, 10 mM $MgCl_2$, 5 mM KCl (pH = 7.4).

regioselectivity in the MTase-based prenylation reactions, together with the rapid decay of AdoPrenyl in aqueous solution, further excludes that the prenylation preference is the result of the nonenzymatic background reaction.

An alternative partial explanation for our observation could be the preferential formation of AdoPrenyl versus AdoMet by PC-MjMAT. However, looking at the compounds resulting from **2a** versus **2b**, we observed only a slight preference of 1.5-fold for prenyl moiety (Figures 2B and 2C).

The fact that three different MTases preferred prenylation strongly supports the hypothesis that AdoPrenyl is a better substrate from a chemical perspective. This conclusion is strengthened by the effects of stabilization of hyperconjugation for different alkyl halides. The isoprenyl residue results in an allyl cation that is tertiary at one end, which is drastically stabilized with respect to simple tertiary or simply allylic cations.[41] Given that the generally accepted mechanism for alkyl transfer from AdoMet analogs is $S_N2$, this stabilization can be expected to be reflected in the transfer reactions. Of course, for enzymatic reactions, the chemical effects can be perturbed by steric constraints of the enzymes. We had deliberately picked MTases known to be promiscuous to mitigate such steric constraints.

We wondered whether we could also target a natural prenyl acceptor by the chimeric AdoMet/DMAPP cofactor. The $N^6$ isoprenylation of adenine ($i^6A$) has been found in cytokinins[15] and tRNA. $N^6$-isoprenyladenosine[7] is discussed for its antiproliferative activity in inhibiting protein prenylation.[42,43] Adenine is also frequently methylated at the $N^6$ position, a modification found

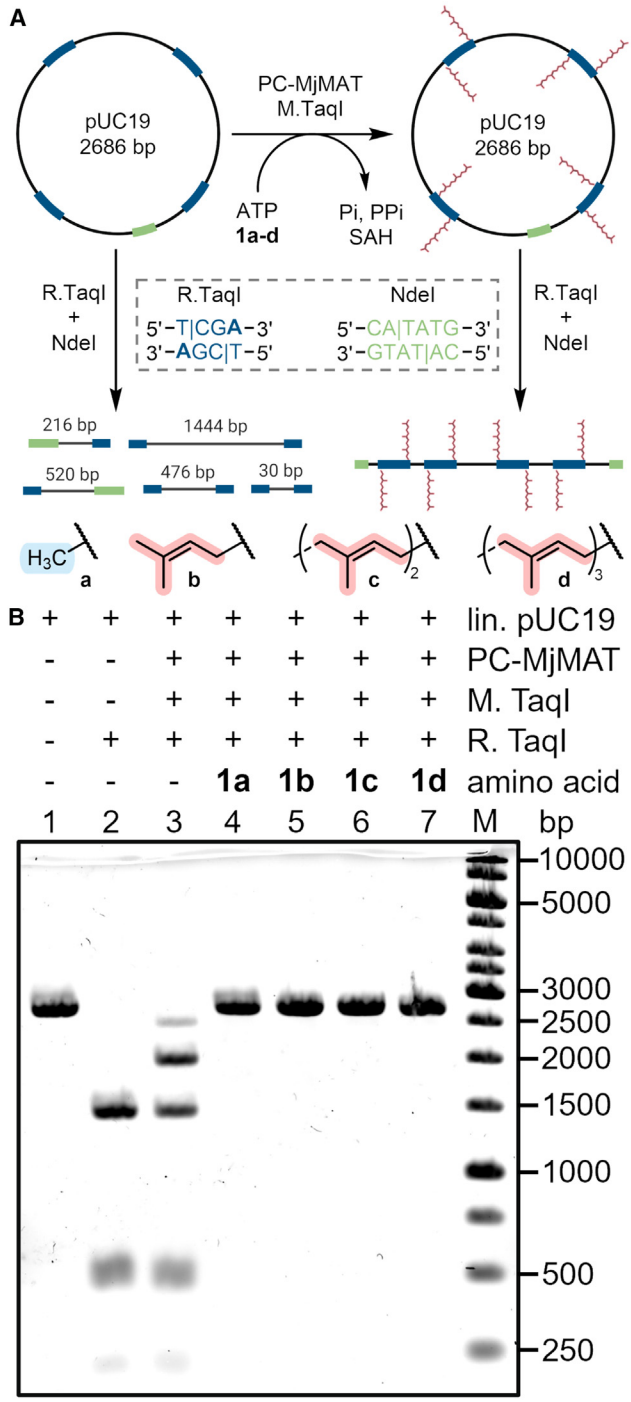


**Figure 5. Prenylation, geranylation, and farnesylation of plasmid DNA**

(A) Reaction scheme of the PC-MjMAT/M.TaqI cascade protecting the pUC19 plasmid from restriction by R.TaqI.

(B) Gel analysis of pUC19 plasmid treated with indicated PC-MjMAT/M.TaqI cascade reactions. Conditions: 5 mM **1a–1d**, 600 ng pUC19, 15 mM ATP, 80 μM PC-MjMAT, 1.5 U M.TaqI, and 10 μM MTAN at 37°C for 5 h; 1% agarose gel.

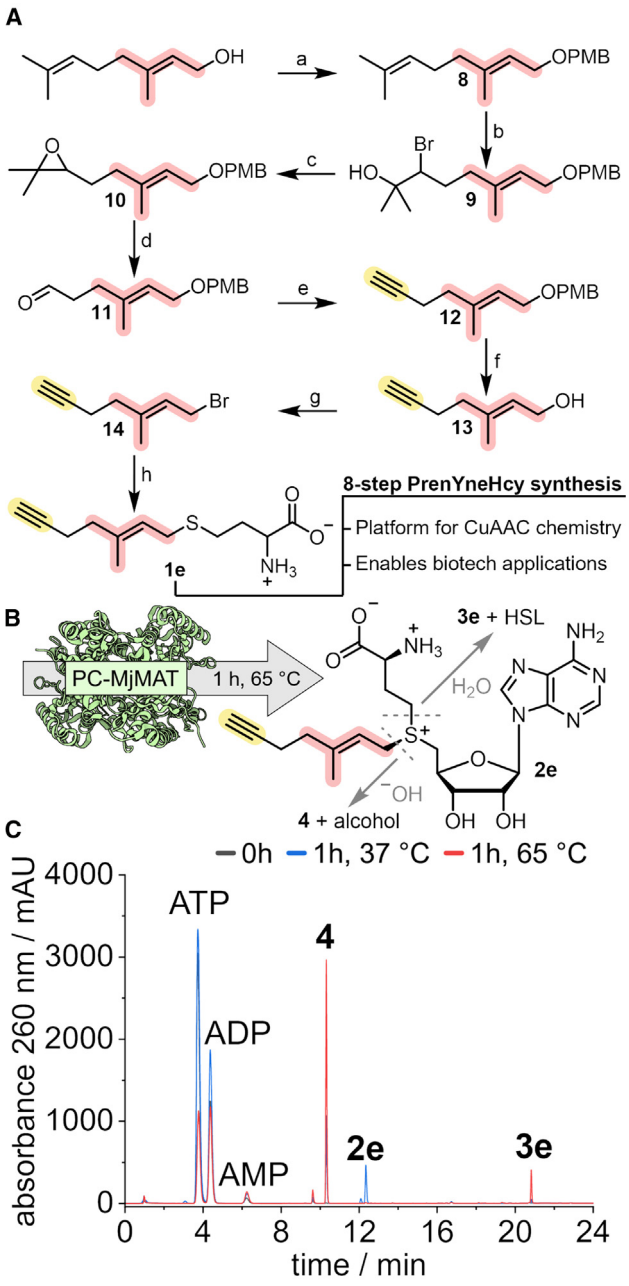


**Figure 6. Design and synthesis of clickable prenyl methionine and AdoMet analogs**

(A) Synthesis of PrenYneHcy (**1e**). The prenyl part (red) for efficient transfer is extended with a terminal alkyne (yellow) for click chemistry and biotechnological applications. (a) NaH, dry DMF, PMBCl, room temperature (RT), 14 h; (b) NBS, THF/$H_2O$ (5:1), RT, 1 h; (c) $K_2CO_3$, anhydrous MeOH, 0°C, 1 h; (d) $NaIO_4$, $HIO_4$, THF/$H_2O$ (10:1), RT, 1 h; (e) $K_2CO_3$, anhydrous MeOH, Ohira-Bestmann reagent, RT, 14 h; (f) DDQ, DCM/$H_2O$ (10:1), 0°C, 2.5 h; (g) $CBr_4$, $PPh_3$, 2,6-lutidine, dry DCM, 0°C, 30 min; (h) NaOH (aq.), D,L-homocysteine thiolactone, dioxane, RT, 4 h.

(B) Enzymatic generation and decomposition of AdoPrenYne (**2e**) from PrenYneHcy (**1e**) and ATP using PC-MjMAT. Degradation pathways to *S*-adenosyl-L-homocysteine (**4**) and the alkyl-thioadenosine (**3e**) are shown.

(C) HPLC analysis of enzymatic reactions starting from **1e** with ATP catalyzed by PC-MjMAT. At 37°C, AdoPrenYne (**2e**) is observed. At 65°C, decomposition products **4** (major) and **3e** (minor) are observed. Conditions: 5 mM **1e**, 5 mM ATP, 100 μM PC-MjMAT. Incubation at 37°C or 65°C for 1 h. Reaction buffer: 50 mM HEPES, 10 mM $MgCl_2$, 5 mM KCl (pH = 7.4). Abbreviations: PMBCl, *para*-methoxybenzylchloride; NBS, *N*-bromosuccinimide.

in DNA and RNA.[26,44,45] The DNA-MTase M.TaqI can selectively methylate the $N^6$ position of adenine in the sequence 5′-TCGA-3′ to protect the DNA from cleavage by the restriction enzyme R.TaqI (5′-T|CGA-3′). We performed the cascade reaction by starting from **1b** with PC-MjMAT/M.TaqI on DNA. Specifically, we used the pUC19 plasmid (2,686 bp) with four recognition sites on each DNA strand (Figure 5A).

We observed that, similar to the positive control starting from **1a** (Figure 5B, lane 4), DNA was protected from restriction when **1b** was used in the MAT/MTase cascade (Figure 5B, lane 5), whereas unprotected DNA (without cascade or with cascade without amino acid) was restricted (Figure 5B, lanes 2 and 3). As a control, pUC19 linearized by NdeI is shown in lane 1. These results show that a natural methylation site can also be prenylated by the chimeric AdoPrenyl in combination with a suitable MTase.

To see whether the concept of chimeric AdoMet/DMAPP cofactors can be further extended to longer-chain isoprene moieties, we synthesized the methionine analogs geranyl-D/L-homocysteine (**1c**, $C_{10}$) and farnesyl-D/L -homocysteine (**1d**, $C_{15}$) and tested their conversion with ATP by PC-MjMAT. At 65°C, we observed the formation of AdoHcy (**4**), suggesting the formation and degradation of the respective AdoMet analogs, albeit with less efficiency than for **1b** (Figures S7–S15). These data indicate that chimeric AdoMet/DMAPP cofactors with higher isoprene moieties are formed and that the efficiency declines with longer chains.

We then tested **1c** and **1d** in cascade reactions to modify DNA. To our delight, methionine analogs **1c** and **1d** were also accepted by the PC-MjMAT/M.TaqI cascade and showed complete protection of the DNA (Figure 5B, lanes 6 and 7). These results show that **1a**–**1d** can be converted to **2a**–**2d**, which are accepted by M.TaqI to methylate, prenylate, geranylate, and farnesylate DNA, and that complete modification of the plasmid can be achieved.

We wondered whether the finding that the prenyl unit is a preferred moiety for transfer by MTases could be translated into a biotechnological application. AdoMet analogs facilitate the site-specific transfer of functional groups and are therefore excellent tools for labeling and isolating biomolecules.[44–48] In particular, the transfer of clickable groups has proven useful for downstream applications. However, a main caveat of the approach is that the transfer efficiency is reduced when AdoMet analogs are used and is commonly low in the presence of AdoMet. The latter is present in samples of cellular origin and is often co-purified with MTase enzymes. We therefore wondered whether we could engineer superior clickable AdoMet analogs that benefit from the preferred transfer observed in the prenylation reaction.

To this end, we designed the "clickable prenyl" PrenYneHcy (**1e**), a prenyl-based methionine analog extended with a terminal alkyne for click chemistry (Figure 6A). We reasoned that such a residue could still be privileged in enzymatic transfer because it would proceed via the allylic cation, which is tertiary at one end.

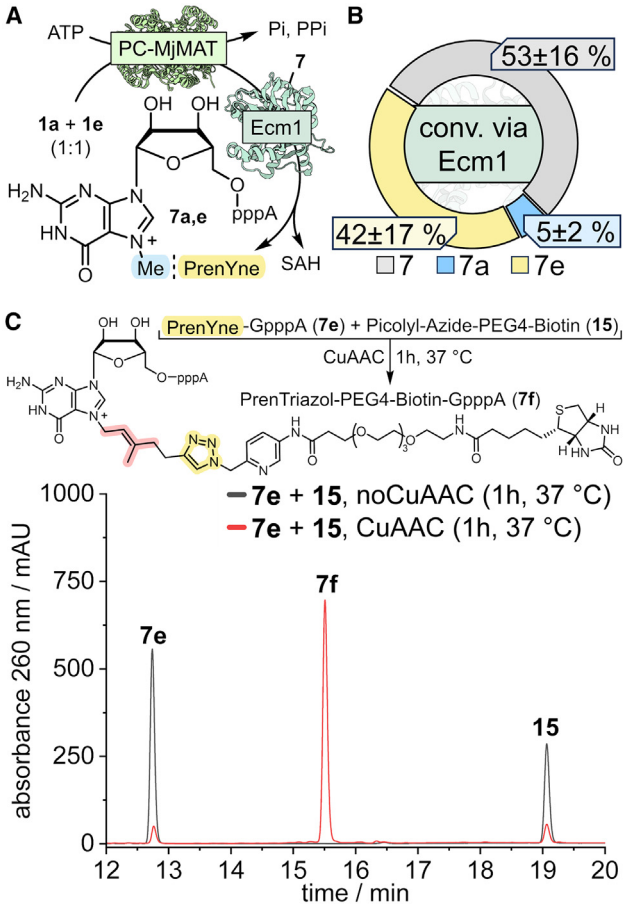


**Figure 7. Labeling of the mRNA 5′ cap with a clickable prenyl and subsequent CuAAC**

(A) PC-MjMAT converts ATP and PrenYneHcy (**1e**) to AdoPrenYne, which can be utilized by Ecm1.

(B) Competition assays using **1a** and **1e** in the same PC-MjMAT/Ecm1 cascade reaction. Conversions are reported as standard deviations of triplicates.

(C) Purified **7e** is clicked to biotin-azide to form the biotinylated mRNA 5′ cap (**7f**).

We synthesized **1e** in eight steps starting from geraniol and characterized the products by NMR and MS (Figures S32–S40). We then tested the enzymatic formation of the respective AdoMet analog, i.e., AdoPrenYne (**2e**), as well as the degradation pathways (Figures 6A and 6B). In the enzymatic reaction at 37°C, we observed the formation of **2e**. At 65°C, we observed only degradation products. AdoHcy (**4**) was the main degradation product, but small amounts of the respective alkyl-thioadenosine (**3e**) were also observed. These data suggest that **2e** primarily retains the degradation and transfer properties of AdoPrenyl (Figure 2) despite the addition of a clickable group.

We then used **1e** to perform a PC-MjMAT/Ecm1 cascade reaction. We confirmed the efficient formation of the expected modified mRNA 5′ cap dinucleotide **7e** from **1e** (Figure S41A). To test whether the clickable prenyl retains superior transfer properties, we performed competition experiments with the natural AdoMet (Figures 7A, 7B, and S41B). To this end, we set up cascades starting from equimolar amounts of **1a** and **1e** in the same reaction and found an >8-fold preference for transfer of the clickable prenyl over methylation (42% **7e** versus 5% **7a**) formed in 1 h (Figures 7B and S41B).

We then used the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) to conjugate **7e** with biotin-azide (**15**), forming the biotinylated 5′ cap **7f** in 94% conversion (Figures 7C and S42–S44). This strategy thus enables efficient click labeling in the presence of AdoMet.

## DISCUSSION

Our work shows that promiscuous MTases can efficiently use the AdoMet scaffold to transfer prenyl moieties. We demonstrated the chemo-enzymatic formation of novel chimeric cofactors AdoPrenyl, AdoGeranyl, and AdoFarnesyl and tested their compatibility with different MTases. These chimeric cofactors quickly decompose via a different route than most AdoMet analogs and form AdoHcy instead of the MTA analog. The rapid decomposition prevents its isolation and necessitates *in situ* formation and use in cascade reactions. We synthesized methionine analogs with isoprene moieties of various lengths ($C_5$, $C_{10}$, and $C_{15}$), and these analogs are converted by an engineered MAT enzyme (PC-MjMAT). The prenylation ($C_5$) cascades are broadly applicable, exemplified by *C*-, *N*-, and *O*-MTases for small molecules, DNA, and RNA. We show that prenylation even outcompetes natural methylation in direct competition cascades. Additionally, we demonstrate that PrenYneHcy can be converted into AdoPrenYne and utilized by an RNA MTase and can be applied to labeling the mRNA 5′ cap dinucleotide with biotin via a clickable handle and subsequent CuAAC reaction. The privileged character of the prenyl core structure in the transfer is maintained. This finding has practical implications for efficient labeling in the presence of AdoMet both *in vitro* and in cellular environments.

Our results demonstrate that cofactor engineering readily turns many representatives of the abundant class of MTases (EC 2.1) into efficient prenyl-transferring enzymes, which in nature are found in the group of PTases (EC 2.5) and rely on the respective DMAPP cofactor scaffold. Hence, there is no inherent chemical or enzymatic reason that $C_1$ transfer and $C_5$ transfer are catalyzed by different ECs. It is tempting to speculate that the AdoPrenyl cofactor was too unstable to become a useful cofactor in the complex cellular system. However, for bioconversions, the high reactivity and efficient conversion by various MTases are advantageous. These findings significantly broaden the scope of prenylation by including MTase substrates and give access to MTase-assisted regio- and sequence-selective prenylation of small molecules to form pseudo-natural products and new-to-nature isoprene-DNA conjugates.

## METHODS

Detailed methods can be found in the supplemental information.

## RESOURCE AVAILABILITY

### Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Andrea Rentmeister (a.rentmeister@lmu.de).

### Materials availability

All reagents generated in this study will be made available on request, but we may require a payment and/or a completed materials transfer agreement if there is potential for commercial application.

### Data and code availability

All data are available from the Open Data LMU repository under a CC-BY-NC-ND 4.0 license at https://doi.org/10.5282/ubm/data.571.

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## AUTHOR CONTRIBUTIONS

N.V.C. and A.R. conceived the study. N.V.C., A.H., Y.L.P., P.G., and M.E. performed the experiments. N.V.C., A.H., M.E., P.G., and A.R. analyzed the data. A.H. visualized the data. N.V.C. and A.R. wrote the initial draft. A.R. supervised the project and acquired the funding. All authors approved the final version.

## DECLARATION OF INTERESTS

A.R. is a member of the *Chem* advisory board.

## SUPPLEMENTAL INFORMATION

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## REFERENCES

1. Schönherr, H., and Cernak, T. (2013). Profound methyl effects in drug discovery and a call for new C-H methylation reactions. Angew. Chem. Int. Ed. *52*, 12256–12267. https://doi.org/10.1002/anie.201303207.
2. Deen, J., Vranken, C., Leen, V., Neely, R.K., Janssen, K.P.F., and Hofkens, J. (2017). Methyltransferase-directed labeling of biomolecules and its applications. Angew. Chem. Int. Ed. *56*, 5182–5200. https://doi.org/10.1002/anie.201608625.
3. Wessjohann, L.A., Keim, J., Weigel, B., and Dippe, M. (2013). Alkylating enzymes. Curr. Opin. Chem. Biol. *17*, 229–235. https://doi.org/10.1016/j.cbpa.2013.02.016.
4. de Bruijn, W.J.C., Levisson, M., Beekwilder, J., van Berkel, W.J.H., and Vincken, J.P. (2020). Plant aromatic prenyltransferases: tools for microbial cell factories. Trends Biotechnol. *38*, 917–934. https://doi.org/10.1016/j.tibtech.2020.02.006.
5. Tao, Z., Liu, J., Jiang, Y., Gong, L., and Yang, B. (2017). Synthesis of prenylated flavonols and their potents as estrogen receptor modulator. Sci. Rep. *7*, 12445. https://doi.org/10.1038/s41598-017-12640-9.
6. Kuzuyama, T., Noel, J.P., and Richard, S.B. (2005). Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products. Nature *435*, 983–987. https://doi.org/10.1038/nature03668.
7. Lamichhane, T.N., Mattijssen, S., and Maraia, R.J. (2013). Human cells have a limited set of tRNA anticodon loop substrates of the tRNA isopentenyltransferase TRIT1 tumor suppressor. Mol. Cell. Biol. *33*, 4900–4908. https://doi.org/10.1128/MCB.01041-13.
8. Schweizer, U., Bohleber, S., and Fradejas-Villar, N. (2017). The modified base isopentenyladenosine and its derivatives in tRNA. RNA Biol. *14*, 1197–1208. https://doi.org/10.1080/15476286.2017.1294309.
9. Zhang, Y., Hamada, K., Satake, M., Sengoku, T., Goto, Y., and Suga, H. (2023). Switching prenyl donor specificities of cyanobactin prenyltransferases. J. Am. Chem. Soc. *145*, 23893–23898. https://doi.org/10.1021/jacs.3c07373.
10. Elshahawi, S.I., Cao, H., Shaaban, K.A., Ponomareva, L.V., Subramanian, T., Farman, M.L., Spielmann, H.P., Phillips, G.N., Jr., Thorson, J.S., and Singh, S. (2017). Structure and specificity of a permissive bacterial C-prenyltransferase. Nat. Chem. Biol. *13*, 366–368. https://doi.org/10.1038/nchembio.2285.
11. Bandari, C., Scull, E.M., Masterson, J.M., Tran, R.H.Q., Foster, S.B., Nicholas, K.M., and Singh, S. (2017). Determination of alkyl-donor promiscuity of tyrosine-*O*-prenyltransferase SirD from *Leptosphaeria maculans*. Chembiochem *18*, 2323–2327. https://doi.org/10.1002/cbic.201700469.
12. Johnson, B.P., Scull, E.M., Dimas, D.A., Bavineni, T., Bandari, C., Batchev, A.L., Gardner, E.D., Nimmo, S.L., and Singh, S. (2020). Acceptor substrate determines donor specificity of an aromatic prenyltransferase: expanding the biocatalytic potential of NphB. Appl. Microbiol. Biotechnol. *104*, 4383–4395. https://doi.org/10.1007/s00253-020-10529-8.
13. Colombano, A., Dalponte, L., Dall'Angelo, S., Clemente, C., Idress, M., Ghazal, A., and Houssen, W.E. (2023). Chemoenzymatic late-stage modifications enable downstream click-mediated fluorescent tagging of peptides. Angew. Chem. Int. Ed. *62*, e202215979. https://doi.org/10.1002/anie.202215979.
14. Johnson, L.A., Dunbabin, A., Benton, J.C.R., Mart, R.J., and Allemann, R.K. (2020). Modular chemoenzymatic synthesis of terpenes and their analogues. Angew. Chem. Int. Ed. *59*, 8486–8490. https://doi.org/10.1002/anie.202001744.
15. Couillaud, J., Duquesne, K., and Iacazio, G. (2022). Extension of the terpene chemical space: the very first biosynthetic steps. Chembiochem *23*, e202100642. https://doi.org/10.1002/cbic.202100642.
16. Fontecave, M., Atta, M., and Mulliez, E. (2004). S-adenosylmethionine: nothing goes to waste. Trends Biochem. Sci. *29*, 243–249. https://doi.org/10.1016/j.tibs.2004.03.007.
17. Hoffman, J.L. (1986). Chromatographic analysis of the chiral and covalent instability of *S*-adenosyl-L-methionine. Biochemistry *25*, 4444–4449. https://doi.org/10.1021/bi00363a041.
18. Singh, S., Zhang, J., Huber, T.D., Sunkara, M., Hurley, K., Goff, R.D., Wang, G., Zhang, W., Liu, C., Rohr, J., et al. (2014). Facile chemoenzymatic strategies for the synthesis and utilization of *S*-adenosyl-L-methionine analogues. Angew. Chem. Int. Ed. *53*, 3965–3969. https://doi.org/10.1002/anie.201308272.
19. Law, B.J.C., Struck, A.W., Bennett, M.R., Wilkinson, B., and Micklefield, J. (2015). Site-specific bioalkylation of rapamycin by the RapM 16-O-methyltransferase. Chem. Sci. *6*, 2885–2892. https://doi.org/10.1039/c5sc00164a.
20. Stecher, H., Tengg, M., Ueberbacher, B.J., Remler, P., Schwab, H., Griengl, H., and Gruber-Khadjawi, M. (2009). Biocatalytic Friedel-Crafts alkylation using non-natural cofactors. Angew. Chem. Int. Ed. *48*, 9546–9548. https://doi.org/10.1002/anie.200905095.
21. Wang, R., Islam, K., Liu, Y., Zheng, W., Tang, H., Lailler, N., Blum, G., Deng, H., and Luo, M. (2013). Profiling genome-wide chromatin methylation with engineered posttranslation apparatus within living cells. J. Am. Chem. Soc. *135*, 1048–1056. https://doi.org/10.1021/ja309412s.

22. Islam, K., Bothwell, I., Chen, Y., Sengelaub, C., Wang, R., Deng, H., and Luo, M. (2012). Bioorthogonal profiling of protein methylation using azido derivative of *S*-adenosyl-L-methionine. J. Am. Chem. Soc. *134*, 5909–5915. https://doi.org/10.1021/ja2118333.

23. Lukinavicius, G., Lapiene, V., Stasevskij, Z., Dalhoff, C., Weinhold, E., and Klimasauskas, S. (2007). Targeted labeling of DNA by methyltransferase-directed transfer of activated groups (mTAG). J. Am. Chem. Soc. *129*, 2758–2759. https://doi.org/10.1021/ja0691876.

24. Michailidou, F., Klöcker, N., Cornelissen, N.V., Singh, R.K., Peters, A., Ovcharenko, A., Kümmel, D., and Rentmeister, A. (2021). Engineered SAM synthetases for enzymatic generation of AdoMet Analogs with photocaging groups and reversible DNA modification in cascade reactions. Angew. Chem. Int. Ed. *60*, 480–485. https://doi.org/10.1002/anie.202012623.

25. Anhäuser, L., Muttach, F., and Rentmeister, A. (2018). Reversible modification of DNA by methyltransferase-catalyzed transfer and light-triggered removal of photo-caging groups. Chem. Commun. *54*, 449–451. https://doi.org/10.1039/c7cc08300a.

26. Dalhoff, C., Lukinavičius, G., Klimasăuskas, S., and Weinhold, E. (2006). Direct transfer of extended groups from synthetic cofactors by DNA methyltransferases. Nat. Chem. Biol. *2*, 31–32. https://doi.org/10.1038/nchembio754.

27. Hoffmann, A., Schülke, K.H., Hammer, S.C., Rentmeister, A., and Cornelissen, N.V. (2023). Comparative *S*-adenosyl-L-methionine analogue generation for selective biocatalytic Friedel-Crafts alkylation. Chem. Commun. *59*, 5463–5466. https://doi.org/10.1039/d3cc01036h.

28. Schülke, K.H., Ospina, F., Hörnschemeyer, K., Gergel, S., and Hammer, S.C. (2022). Substrate profiling of anion methyltransferases for promiscuous synthesis of s-adenosylmethionine analogs from haloalkanes. Chembiochem *23*, e202100632. https://doi.org/10.1002/cbic.202100632.

29. Sadler, J.C., Humphreys, L.D., Snajdrova, R., and Burley, G.A. (2017). A tandem enzymatic $sp^2$-C-methylation process: coupling in situ *S*-adenosyl-L-methionine formation with methyl transfer. Chembiochem *18*, 992–995. https://doi.org/10.1002/cbic.201700115.

30. Liao, C., and Seebeck, F.P. (2020). Asymmetric beta-methylation of L- and D-alpha-amino acids by a self-contained enzyme cascade. Angew. Chem. Int. Ed. *59*, 7184–7187. https://doi.org/10.1002/anie.201916025.

31. Peters, A., Herrmann, E., Cornelissen, N.V., Klöcker, N., Kümmel, D., and Rentmeister, A. (2022). Visible-light removable photocaging groups accepted by MjMAT variant: structural basis and compatibility with DNA and RNA methyltransferases. Chembiochem *23*, e202100437. https://doi.org/10.1002/cbic.202100437.

32. Huber, T.D., Wang, F., Singh, S., Johnson, B.R., Zhang, J., Sunkara, M., Van Lanen, S.G., Morris, A.J., Phillips, G.N., Jr., and Thorson, J.S. (2016). Functional AdoMet isosteres resistant to classical adomet degradation pathways. ACS Chem. Biol. *11*, 2484–2491. https://doi.org/10.1021/acschembio.6b00348.

33. Cornelissen, N.V., Mineikaitė, R., Erguven, M., Muthmann, N., Peters, A., Bartels, A., and Rentmeister, A. (2023). Post-synthetic benzylation of the mRNA 5′ cap via enzymatic cascade reactions. Chem. Sci. *14*, 10962–10970. https://doi.org/10.1039/d3sc03822j.

34. Sadler, J.C., Chung, C.H., Mosley, J.E., Burley, G.A., and Humphreys, L.D. (2017). Structural and functional basis of C-methylation of coumarin scaffolds by NovO. ACS Chem. Biol. *12*, 374–379. https://doi.org/10.1021/acschembio.6b01053.

35. Hausmann, S., Zheng, S., Fabrega, C., Schneller, S.W., Lima, C.D., and Shuman, S. (2005). Encephalitozoon cuniculi mRNA cap (guanine N-7) methyltransferase: methyl acceptor specificity, inhibition by *S*-adenosylmethionine analogs, and structure-guided mutational analysis. J. Biol. Chem. *280*, 20404–20412. https://doi.org/10.1074/jbc.M501073200.

36. Bonifácio, M.J., Archer, M., Rodrigues, M.L., Matias, P.M., Learmonth, D.A., Carrondo, M.A., and Soares-Da-Silva, P. (2002). Kinetics and crystal structure of catechol-*O*-methyltransferase complex with co-substrate and a novel inhibitor with potential therapeutic application. Mol. Pharmacol. *62*, 795–805. https://doi.org/10.1124/mol.62.4.795.

37. Law, B.J.C., Bennett, M.R., Thompson, M.L., Levy, C., Shepherd, S.A., Leys, D., and Micklefield, J. (2016). Effects of active-site modification and quaternary structure on the regioselectivity of catechol-*O*-methyltransferase. Angew. Chem. Int. Ed. *55*, 2683–2687. https://doi.org/10.1002/anie.201508287.

38. Herbert, A.J., Shepherd, S.A., Cronin, V.A., Bennett, M.R., Sung, R., and Micklefield, J. (2020). Engineering orthogonal methyltransferases to create alternative bioalkylation pathways. Angew. Chem. Int. Ed. *59*, 14950–14956. https://doi.org/10.1002/anie.202004963.

39. McKean, I.J.W., Sadler, J.C., Cuetos, A., Frese, A., Humphreys, L.D., Grogan, G., Hoskisson, P.A., and Burley, G.A. (2019). *S*-Adenosyl methionine cofactor modifications enhance the biocatalytic repertoire of small molecule C-alkylation. Angew. Chem. Int. Ed. *58*, 17583–17588. https://doi.org/10.1002/anie.201908681.

40. Hausmann, S., Vivarès, C.P., and Shuman, S. (2002). Characterization of the mRNA capping apparatus of the microsporidian parasite Encephalitozoon cuniculi. J. Biol. Chem. *277*, 96–103. https://doi.org/10.1074/jbc.M109649200.

41. Streitwieser, A. (1956). Solvolytic displacement reactions at saturated carbon atoms. Chem. Rev. *56*, 571–752. https://doi.org/10.1021/cr50010a001.

42. Laezza, C., Notarnicola, M., Caruso, M.G., Messa, C., Macchia, M., Bertini, S., Minutolo, F., Portella, G., Fiorentino, L., Stingo, S., and Bifulco, M. (2006). N6-isopentenyladenosine arrests tumor cell proliferation by inhibiting farnesyl diphosphate synthase and protein prenylation. FASEB J. *20*, 412–418. https://doi.org/10.1096/fj.05-4044lsf.

43. Gelb, M.H., Brunsveld, L., Hrycyna, C.A., Michaelis, S., Tamanoi, F., Van Voorhis, W.C., and Waldmann, H. (2006). Therapeutic intervention based on protein prenylation and associated modifications. Nat. Chem. Biol. *2*, 518–528. https://doi.org/10.1038/nchembio818.

44. Hartstock, K., Kueck, N.A., Špaček, P., Ovcharenko, A., Hüwel, S., Cornelissen, N.V., Bollu, A., Dieterich, C., and Rentmeister, A. (2023). MePMe-seq: antibody-free simultaneous $m^6A$ and $m^5C$ mapping in mRNA by metabolic propargyl labeling and sequencing. Nat. Commun. *14*, 7154. https://doi.org/10.1038/s41467-023-42832-z.

45. Hartstock, K., Nilges, B.S., Ovcharenko, A., Cornelissen, N.V., Püllen, N., Lawrence-Dörner, A.M., Leidel, S.A., and Rentmeister, A. (2018). Enzymatic or in vivo installation of propargyl groups in combination with click chemistry for the enrichment and detection of methyltransferase target sites in RNA. Angew. Chem. Int. Ed. *57*, 6342–6346. https://doi.org/10.1002/anie.201800188.

46. Muttach, F., and Rentmeister, A. (2016). A biocatalytic cascade for versatile one-pot modification of mRNA starting from methionine analogues. Angew. Chem. Int. Ed. *55*, 1917–1920. https://doi.org/10.1002/anie.201507577.

47. 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. https://doi.org/10.1021/ja304782r.

48. Gasiulė, L., Stankevičius, V., Kvederavičiu Tė, K., Rimšelis, J.M., Klimkevičius, V., Petraitytė, G., Rukšėnaitė, A., Masevičius, V., and Klimašauskas, S. (2024). Engineered methionine adenosyltransferase cascades for metabolic labeling of individual DNA methylomes in live cells. J. Am. Chem. Soc. *146*, 18722–18729. https://doi.org/10.1021/jacs.4c06529.