Technology Offer

Proline-Stabilized SAM Analogs for Sequence-Specific Biomolecule Labeling

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Abstract

This invention introduces a new class of S-adenosyl-L-methionine (SAM) analogs containing a proline moiety. These analogs serve as efficient cofactors for methyltransferase-mediated biomolecule modification, overcoming key limitations of natural SAM such as poor stability and degradation under enzymatic conditions. The invention enables precise, sequence-specific labeling of biomolecules including DNA, RNA, proteins, and small molecules. The improved stability—up to 100-fold over SAM—makes these analogs especially suitable for reactions requiring prolonged incubation or elevated temperatures. Furthermore, these analogs can be functionalized with diverse chemical groups, allowing for direct or biorthogonal labeling strategies. This platform supports advanced applications in diagnostics, therapeutics, and chemical biology, providing a flexible and robust tool for targeted biomolecular modifications.

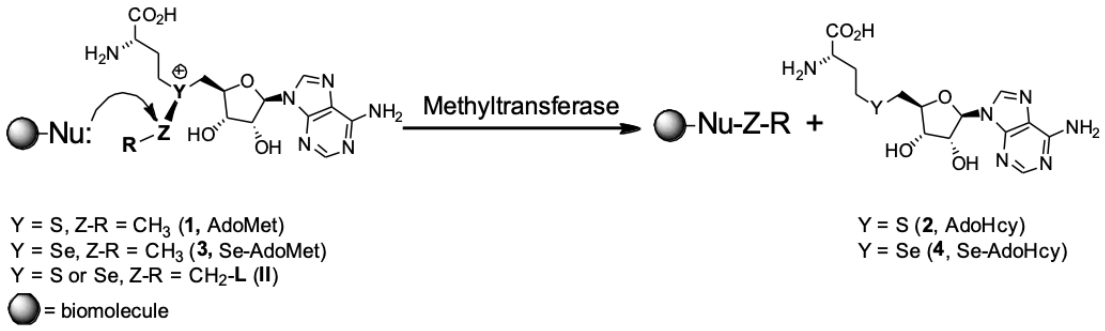

Figure 1: Methyltransferases catalyze reaction between various nucleophiles present in biomolecules and the electrophilic carbon next to the sulfonium center of S-adenosyl-L-methionine (1) or S-adenosyl-L-selenomethionine (3).

Background

S-adenosyl-L-methionine is a ubiquitous biological methyl donor used by methyltransferases to modify DNA, RNA, proteins, and other molecules (compare Figure 1). However, SAM and previously developed analogs suffer from chemical instability, especially under enzymatic reaction or storage conditions. Instability mechanisms include cyclization to homoserine lactone and depurination, which limit their use in applications requiring high precision or durability. Moreover, earlier analogs often lack the chemical handles necessary for downstream conjugation or labeling. These limitations restrict the use of SAM analogs in advanced labeling techniques or in environments with elevated temperatures, such as those used for thermophilic enzymes. A more stable and chemically versatile cofactor is therefore needed.

Technology

The invention discloses a new class of SAM analogs based on a proline core, where the natural homocysteine side chain of SAM is substituted with a proline residue (Figure 2). This structural modification fundamentally improves the chemical stability of the cofactor by preventing intramolecular cyclization, a major degradation pathway of traditional SAM and its analogs. Furthermore, selenium-containing variants of these proline-based analogs exhibit up to 100-fold enhanced stability compared to native SAM, even under conditions of elevated temperature or extended storage. These analogs retain full functionality with methyltransferases, enabling the enzymatic transfer of diverse chemical groups onto target biomolecules.

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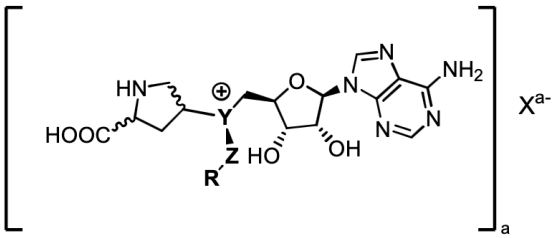

Figure 2: Invented compound. Wherein X^{a-} is an inorganic or organic anion, Y is S, Se or Te, Z is $-CR^1R^2R^3$, $-CR^1R^2-$, $-SiR^1R^2-$, -O-, -S-, -Se-, -Te- or $-NR^3-$ and R is selected from an extensive list of substituents such as aliphatic alkyl groups and cycloalkyl groups.

The cofactors are engineered to carry reactive or label-bearing side chains, such as fluorophores, alkynes, azides, or bioorthogonal groups. This allows their use in two distinct labeling strategies: (1) one-step labeling, in which the cofactor is already carrying the suitable label, which can be directly transferred my a methyltransferase, and (2) two-step labeling, wherein the cofactor is carrying a reactive group which is used to modify the target molecule via a methyltransferase, afterwards the target molecule can be labeled. The system has been successfully demonstrated with DNA methyltransferases like M.TaqI and M.HhaI, enabling sequence- and base-specific labeling of DNA. Additionally, the analogs are compatible with RNA and protein methyltransferases, expanding their utility for site-selective labeling of a broad range of biological targets in vitro and in vivo.

Advantages

- **Enhanced chemical and thermal stability**: Proline-based SAM analogs resist degradation, maintaining functionality in long-term storage and at elevated temperatures.
- **Wide enzyme compatibility**: Functional with a variety of methyltransferases targeting DNA, RNA, proteins, and small molecules.
- **Modular chemical design**: Functional groups such as fluorophores, alkynes, or affinity tags can be integrated for diverse labeling strategies.
- **Precise, sequence-specific modification**: Enables accurate targeting of labeling sites using well-characterized methyltransferases like M.TaqI and M.HhaI.
- **Biocompatibility**: Reactions can be performed in aqueous or mixed environments, making the technology suitable for in vitro and potential in vivo use.

Potential applications

- **Fluorescent labeling of DNA/RNA** for visualization, epigenetic mapping, or nucleic acid tracking in live or fixed samples.
- **Selective protein labeling** to study interactions, structure, or localization in biochemical and cellular assays.
- **Diagnostic assays** employing labeled probes for sequence-specific detection of genetic or infectious targets.
- **Two-step bioconjugation workflows**, enabling site-specific attachment of drugs, nanoparticles, or imaging agents via click chemistry.
- **Thermostable enzymatic labeling systems**, supporting workflows with thermophilic enzymes or harsh processing conditions.

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