Insight into the Polar Reactivity of the Onium Chalcogen Analogues of *S*-Adenosyl-L-methionine†

David F. Iwig and Squire J. Booker*

Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

Received June 23, 2004; Revised Manuscript Received August 18, 2004

ABSTRACT: *S*-Adenosyl-L-methionine (AdoMet) is one of Nature's most diverse metabolites, used not only in a large number of biological reactions but amenable to several different modes of reactivity. The types of transformations in which it is involved include decarboxylation, electrophilic addition to any of the three carbons bonded to the central sulfur atom, proton removal at carbons adjacent to the sulfonium, and reductive cleavage to generate 5′-deoxyadenosyl 5′-radical intermediates. At physiological pH and temperature, AdoMet is subject to three spontaneous degradation pathways, the first of which is racemization of the chiral sulfonium group, which takes place in a pH-independent manner. The two remaining pathways are pH-dependent and include (1) intramolecular attack of the α-carboxylate group onto the γ-carbon, affording L-homoserine lactone (HSL) and 5′-methylthioadenosine (MTA), and (2) deprotonation at C-5′, initiating a cascade that results in formation of adenine and *S*-ribosylmethionine. Herein, we describe pH-dependent stability studies of AdoMet and its selenium and tellurium analogues, *Se*-adenosyl-L-selenomethionine and *Te*-adenosyl-L-telluromethionine (SeAdoMet and TeAdoMet, respectively), at 37 °C and constant ionic strength, which we use as a probe of their relative intrinsic reactivities. We find that with AdoMet intramolecular nucleophilic attack to afford HSL and MTA exhibits a pH−rate profile having two titratable groups with apparent pK_a values of 1.2 ± 0.4 and 8.2 ± 0.05 and displaying first-order rate constants of $<0.7 \times 10^{-6}$ s^{-1} at pH values less than 0.5, $\sim 3 \times 10^{-6}$ s^{-1} at pH values between 2 and 7, and $\sim 15 \times 10^{-6}$ s^{-1} at pH values greater than 9. Degradation via deprotonation at C-5′ follows a pH−rate profile having one titratable group with an apparent pK_a value of ∼11.5. The selenium analogue decays significantly faster via intramolecular nucleophilic attack, also exhibiting a pH−rate profile with two titratable groups with pK_a values of ∼0.86 and 8.0 ± 0.1 with first-order rate constants of $<7 \times 10^{-6}$ s^{-1} at pH values less than 0.9, $\sim 32 \times 10^{-6}$ s^{-1} at pH values between 2 and 7, and $\sim 170 \times 10^{-6}$ s^{-1} at pH values greater than 9. Degradation via deprotonation at C-5′ proceeds with one titratable group displaying an apparent pK_a value of ∼14.1. Unexpectedly, TeAdoMet did not decay at an observable rate via either of these two pathways. Last, enzymatically synthesized AdoMet was found to racemize at rates that were consistent with earlier studies (Hoffman, J. L. (1986) *Biochemistry 25*, 4444−4449); however, SeAdoMet and TeAdoMet did not racemize at detectable rates. In the accompanying paper, we use the information obtained in these model studies to probe the mechanism of cyclopropane fatty acid synthase via use of the onium chalcogens of AdoMet as methyl donors.

S-Adenosyl-L-methionine (AdoMet)[1] is a key metabolite that is ubiquitously distributed throughout all phyla of living systems and may be considered as requisite for life (*1*). It is employed in a multitude of cellular reactions and displays a number of modes of reactivity. For most reactions, the operative functionality of the molecule is a sulfonium group, which is created via a unique condensation reaction between the sulfur of L-methionine and the 5′-carbon of ATP. The second immediate product is tripolyphosphate (PPP_i), which is cleaved further to pyrophosphate (PP_i) and inorganic phosphate (P_i) by a second activity that the enzyme possesses (*2*). The resulting sulfonium group is chiral, having methyl, aminopropylcarboxylate, and 5′-deoxyadenosine substituents bonded to the central sulfur atom, which also carries a lone pair of electrons (Figure 1). The electronic properties of this central, positively charged sulfonium group bestow three distinct reactivities upon the molecule, which are well documented in solution chemistry (*3*, *4*) and which are currently being characterized and established in biological reactions (*5*−*8*).

Historically and pedagogically, AdoMet is known as a cellular alkylating agent. In principle, each of the carbon atoms adjacent to the sulfonium sulfur is activated toward electrophilic addition, resulting in release of one of three different thioether products. Indeed, biological systems exist that exploit the reactivity at each of these positions. The most common alkylation reaction is simple methyl transfer from

† This work was supported by NIH Grant GM-63847 (S.J.B.) and NSF Grant MCB-0133826.

* To whom correspondence should be addressed. Mailing address: 330 South Frear Laboratory, University Park, PA 16802. Phone: 814-865-8793. Fax: 814-863-7024. E-mail: sjb14@psu.edu.

10.1021/bi048693+ CCC: $27.50 © 2004 American Chemical Society
Published on Web 10/02/2004

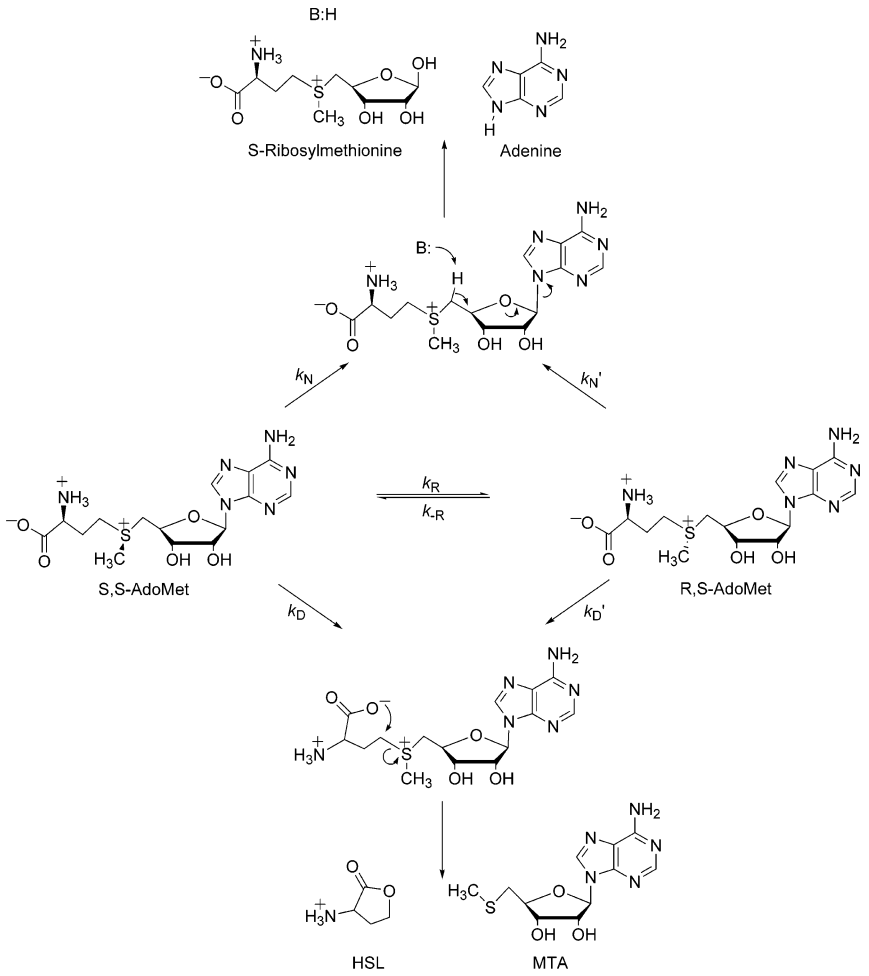

FIGURE 1: Reaction scheme of the three degradation processes associated with (*S*,*S*)-AdoMet: reversible racemization to form (*R*,*S*)-AdoMet through inversion at the sulfonium pole, k_R; deprotonation at the C-5′ and subsequent elimination of the adenine base to form *S*-ribosylmethionine, k_D; and nucleophilic addition by the α-carboxylate onto the γ-carbon of methionine, resulting in MTA and homoserine lactone, k_N.

AdoMet to carbon, nitrogen, oxygen, sulfur, selenium, tellurium, and heavy-metal nucleophiles. These reactions are involved in the modification of DNA and various types of RNA, as well as proteins, carbohydrates, lipids, and an assortment of small molecules (*5*, *9*−*12*). Such simple additions of methyl groups to relevant nucleophiles have broad and far-reaching consequences for the proper functioning of the cell, affecting transcription and translation, tumorigenesis, bioremediation of heavy atoms, and general metabolism (*1*, *12*−*15*). In mammalian cells, biological methylation accounts for ∼80% of the consumption of AdoMet that is produced by the cell (*1*).

Attack at the γ-carbon of AdoMet is also an established reaction; three transformations serve as prototypes. The synthesis of acylhomoserine lactones, small fatty acid-containing compounds that allow Gram-negative bacteria to communicate among each other and regulate a variety of physiological activities, involves acylation of the α-amino group of AdoMet with a fatty acid, followed by intramolecular attack of the α-carboxylate onto the γ-carbon, expelling 5′-methylthioadenosine (MTA) in the process (*16*). Attack at the γ-carbon also takes place in the synthesis of spermine and spermidine after decarboxylation of AdoMet by the pyruvoyl-containing enzyme, AdoMet decarboxylase (*17*, *18*). Last, the formation of aminocyclopropanecarboxylate, the precursor to the plant hormone ethylene, involves deprotonation at the α-carbon and subsequent attack at the γ-carbon by the resulting anion. This reaction is catalyzed by the pyridoxal 5′-phosphate (PLP)-dependent enzyme, aminocyclopropanecarboxylic acid synthase (*19*, *20*). Recently, attack by fluoride ion at the 5′-carbon of AdoMet has been demonstrated to be catalyzed by an enzyme from

[1] Abbreviations: AdoHcys, *S*-adenosyl-L-homocysteine; AdoMet, *S*-adenosyl-L-methionine; BME, 2-mercaptoethanol; CFA, cyclopropane fatty acid; DC-AdoMet, decarboxylated *S*-adenosyl-L-methionine; DC-SeAdoMet, decarboxylated *Se*-adenosyl-L-selenomethionine; DMTA, 5′-dimethylthioadenosine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EPPS, 4-(2-hydroxyethyl)-1-piperazinepropane sulfonic acid; GC, gas chromatography; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); HPLC, high-performance liquid chromatography; HSL, homoserine lactone; IPTG, isopropyl-β-D-thiogalactopyranoside; IS, internal standard; MES, 2-(*N*-morpholino)-ethane sulfonic acid; MS, mass spectroscopy; MtBE, methyl-*tert*-butyl ether; MTA, 5′-methylthioadenosine; Ni−NTA, nickel nitrilotriacetic acid; NMR, nuclear magnetic resonance; PLP, pyridoxal 5′-phosphate; PMSF, phenylmethanesulfonyl fluoride; PPG2000, poly(propylene glycol) 2000-mer; SeAdoHcys, *Se*-adenosyl-L-homoselenocysteine; SeAdoMet, *Se*-adenosyl-L-selenomethionine; SeMTA, 5′-methylselenoadenosine; TeAdoMet, *Te*-adenosyl-L-telluromethionine; TeMTA, 5′-methyltelluroadenosine; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; TMS, trimethylsilane; Tris, tris(hydroxymethyl)aminomethane.

Streptomyces cattleya, producing 5′-fluoro 5′-deoxyadenosine, an intermediate in the biosynthesis of fluoroacetate, a toxin produced by the bacterium, as well as numerous species of plants (*21*).

Two surprising and distinctly different biological reactivities for AdoMet have come to light in recent years. The anticodons of certain tRNAs bear the hypermodified nucleoside queuosine, which contains a cyclopentenediol group appended to a (7-aminomethyl)-7-deazaguanine core structure, termed preQ1 (*8*, *22*). In *Escherichia coli*, the enzyme QueA catalyzes the penultimate step in the biosynthesis of queuosine, which is the transfer of the ribosyl moiety of AdoMet to preQ1 with release of adenine and methionine and concomitant formation of an epoxy-carbocycle. Recent studies are most consistent with removal of a proton from the C-5′ of AdoMet to generate a sulfonium ylide as the key step in the reaction (*8*, *23*).

AdoMet is also a cofactor or cosubstrate in a newly defined class of metalloenzymes called the radical SAM superfamily, wherein it serves as a precursor to a 5′-deoxyadenosyl 5′-radical (5′-dA•) (*6*, *24*−*26*). Bioinformatic studies suggest that this class of enzymes comprises over 500 members, which catalyze a diverse array of reactions, including those involved in DNA biosynthesis and repair, vitamin and cofactor biosynthesis, and modification of proteins and nucleic acids (*27*). In each reaction, the role of the 5′-dA• is to abstract a hydrogen atom from a protein or small-molecule substrate. This event results in either the creation of a protein-derived radical cofactor or the initiation of radical-mediated substrate turnover. The 5′-dA• is generated via a reductive cleavage of AdoMet. The electron is supplied by a $[4Fe-4S]^{+1}$ cluster, a requisite cofactor in each reaction, affording L-methionine as the remaining product.

Under physiological conditions, (*S*,*S*)-AdoMet, the biologically relevant form, degrades to inactive species via three known processes. AdoMet is particularly labile under alkaline conditions, forming adenine and *S*-ribosylmethionine quite readily (*28*). This instability is a direct result of the sulfonium ion of the molecule; it has been demonstrated that *S*-adenosyl-L-homocysteine, which lacks the sulfonium ion, does not degrade under similar conditions, whereas 5′-dimethylthioadenosine (DMTA), also a sulfonium-containing compound, is subject to a similar degradation reaction (*28*). Detailed studies of this reaction demonstrated that it is initiated by deprotonation at C-5′ and proceeds via opening of the ribose ring with elimination of adenine and concomitant formation of a 4′−5′ double bond. Reformation of the ring—via a hemiacetal intermediate—with concomitant loss of the double bond affords *S*-ribosylmethionine (*29*). A competing pathway for AdoMet degradation, which is prominent at even lower pH values, involves an intramolecular attack of the α-carboxylate group onto the γ-carbon of the methionine moiety, resulting in formation of MTA and homoserine lactone (HSL) (*30*). The last process by which the biologically relevant form of AdoMet degrades is racemization of the chiral sulfonium ion, which, in contrast to the aforementioned reactions, is a pH-independent process (*31*−*33*).

Clearly, the widespread use of AdoMet in nature, its importance in the proper functioning of the cell, and the many reactions in which it is involved that serve as potential targets for the design of therapeutics for a variety of illnesses warrant detailed characterizations of it and related molecules. In this study, we use the pathways associated with adenine and MTA formation from AdoMet as model systems to assess the intrinsic polar reactivities of the onium chalcogen analogues of AdoMet, *Se*-adenosyl-L-selenomethionine (SeAdoMet), and *Te*-adenosyl-L-telluromethionine (TeAdoMet) as a function of pH at 37 °C and constant ionic strength. We find that SeAdoMet is a significantly better alkylating agent than AdoMet, while deprotonation at C-5′ occurs more readily with AdoMet than with SeAdoMet under similar experimental conditions. Unexpectedly, TeAdoMet was inert to both processes at 37 °C and pH values that ranged from 0.5 to 12. In the accompanying paper (*40*), we assess the ability of the enzyme cyclopropane fatty acid synthase to use each of the onium chalcogen compounds as a substrate in the reaction and use the information obtained in both studies to formulate a working hypothesis for the mechanism of the enzyme. We propose that these analogues may serve as useful tools for elucidating the mode of reactivity of AdoMet in any given reaction.

MATERIALS AND METHODS

Materials. Yeast extract and tryptone were purchased from Marcor Development Corporation (Carlstadt, NJ). Egg white lysozyme was purchased from EM Science (Gibbstown, NJ). Deoxyribonuclease I (DNase) and ribonuclease A (RNase), both from bovine pancrease, were purchased from ICN Biomedicals Inc. (Aurora, OH). Nickel nitrilotriacetic acid (Ni−NTA) resin was purchased from QIAGEN (Valencia, CA). Sephadex G-25 resin (medium) and Q-Sepharose Fast Flow resin were purchased from Amersham Biosciences (Uppsala, Sweden). CM-52 cation-exchange resin was purchased from Whatman International Ltd (Maidstone, England). Bio-Gel P2 (extra fine) resin and AG 50W-X2 resin were purchased from Bio-Rad Laboratories (Hercules, CA). 1,2-Dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DOPG) in chloroform was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Triethylamine (TEA), (*R*,*S*)-α-amino-γ-butyrolactone hydrobromide, acetic anhydride, methyllithium, tellurium powder (200 mesh), and methyl-*tert*-butyl ether (MtBE) were purchased from Aldrich (Milwaukee, WI). Inorganic pyrophosphatase (Baker's yeast), phenylmethanesulfonyl fluoride (PMSF), L-selenomethionine, L-methionine, and acylase I were purchased from Sigma (St. Louis, MO). All other chemicals and reagents were of reagent grade or better and were purchased from Sigma or Aldrich unless specifically noted.

E. coli strain DM22pK8, which overproduces AdoMet synthetase (*34*), was generously donated by Dr. George Markham of the Fox Chase Cancer Center (Philadelphia, PA). Plasmid pSam18, which encodes human prostate AdoMet decarboxylase, was generously donated by Drs. Margaret A. Phillips and Jeffrey Baldwin of The University of Texas Southwestern Medical Center (Dallas, TX). It was transformed into *E. coli* BL21(DE3), and the protein was expressed and purified as described previously (*35*). The protein was stored in 5 mM Tris-HCl buffer (pH 8.0), containing 25 mM NaCl, 1.25 mM EDTA, 1.25 mM DTT, 1.25 mM putrescine, and 25% glycerol. One unit of enzyme activity is that amount of enzyme that converts 1 μmol of substrate per minute at 37 °C.

General Procedures. Routine UV−visible spectra were recorded on a Cary 300 Bio spectrophotometer in combination with the associated Cary WinUV software package (Varian, Walnut Springs, CA). Sonic disruption of *E. coli* cells was carried out with a 550 sonic dismembrator from Fisher Scientific (Pittsburgh, PA) using a horn containing a $^1/_2$ in. tip. Measurements of pH were carried out with a Beckman 360 pH/Temp/mV meter in combination with a Corning (Corning, NY) combination semi-micro electrode.

High-performance liquid chromatography (HPLC) was carried out on an 1100 system from Agilent (Foster City, CA) with variable wavelength detection or a Beckman (Fullerton, CA) System Gold unit fitted with a 168 diode-array detector. Both HPLC instruments were equipped with autosamplers to facilitate the analysis of multiple samples. Two methods were devised for sample analysis. Method 1 was carried out on the Agilent system using an Agilent Zorbax SB-CN column (4.6 mm × 250 mm, 5 μm) with UV−visible detection at 260 nm and a three-solvent system. Solvent A consisted of 0.4% trifluoroacetic acid (TFA) titrated to pH 1.78 with TEA. Solvent B was methanol, while solvent C was acetonitrile. The column was equilibrated in 95% solvent A/5% solvent B at a flow rate of 1 mL min^{-1}. After injection of the sample, the above initial conditions were maintained for 5 min, upon which simultaneous linear gradients of 5−30% solvent B and 0−50% solvent C were applied from 5 to 15 min. Under these conditions, AdoMet, adenine, *S*-adenosyl-L-homocysteine (AdoHcys), MTA, and tryptophan (internal standard, IS) eluted at 3.2, 4.5, 6.1, 11.2, and 12.6 min, respectively, while SeAdoMet and TeAdoMet eluted at 3.36 and 3.47 min.

Method 2 was carried out on the Beckman System Gold unit using an Agilent Zorbax SB-C8 column (4.6 mm × 250 mm, 5 μm) with UV−vis detection at 260 nm and a two-solvent system. Solvent A contained 100 mM K_2HPO_4 and 1% TFA and was titrated to pH 3.0 with KOH. Solvent B was methanol. The column was equilibrated in 100% solvent A at a flow rate of 1 mL min^{-1}. Upon injection, solvent A was maintained at 100% for 10 min, after which, a gradient of 0−50% solvent B was applied for the following 20 min. Finally, solvent B was increased from 50% to 90% over 5 min, resulting in a total run time of 35 min. Under these conditions, (*S*,*S*)-AdoMet eluted at 11.0 min, and (*R*,*S*)-AdoMet eluted at 12.5 min, while adenine, AdoHcys, and MTA eluted at 19.2, 22.3, and 30.5 min, respectively. The selenium containing compounds, (*S*,*S*)-SeAdoMet, (*R*,*S*)-SeAdoMet, 5′-methylselenoadenosine (SeMTA), and *Se*-adenosyl-L-homoselenocysteine (SeAdoHcys) eluted at 12.3, 13.1, 32.0, and 23.5 min. TeAdoMet eluted at 18.7 min, while the IS eluted at 26.3 min.

All ^{1}H NMR spectra were recorded on a Bruker (Billerica, MA) DRX 400 spectrometer using D_2O as the solvent. Chemical shifts are reported in parts per million (ppm) and are referenced versus contaminating H_2O, which has a chemical shift of ∼4.8 with respect to trimethylsilane (TMS). Electrospray mass spectrometry was carried out in positive ion mode on a Quattro II spectrometer from Micromass MS Technologies (Waters, Milford, MA). Analytical TLC was performed with aluminum-backed silica gel 60 sheets, and column flash chromatography was performed using silica gel grade 62 (60−200 mesh) from EM Science (Gibbstown, NJ). Tetrahydrofuran (THF) was dried by refluxing over $LiAlH_4$ and then distilled and stored over CaH_2 under an argon atmosphere. Methanol, MtBE, and methylene chloride were degassed by bubbling argon through the solvents while stirring for a minimum of 4 h prior to use.

Growth and Preparation of AdoMet Synthetase. AdoMet synthetase was expressed from *E. coli* strain DM22pK8 and prepared by a modification of a previously reported procedure (*2*). In a typical preparation, 200 g of wet cell paste was resuspended in 500 mL of lysis buffer (100 mM Tris-HCl, pH 8.0, 1 mM EDTA, 30 μM PMSF, and 0.1% BME). Lysozyme (0.2 mg mL^{-1} final concentration) and DNase and RNase (each at 20 μg mL^{-1} final concentration) were added, and the mixture was allowed to stir for 30 min at room temperature. The solution was cooled to less than 8 °C by stirring in an ice−water bath for approximately 15 min. The mixture was then subjected to four 60-s bursts of sonic disruption (35% output or 210 W) with intermittent pausing to maintain the temperature at less than 8 °C. All remaining steps were performed at 4 °C. A 5% streptomycin sulfate solution was added to the crude lysate over 10 min to give a final concentration of 1%, and the solution was allowed to stir gently for 15 min before centrifuging at 50 000 × *g* for 60 min at 4 °C. Ammonium sulfate (65% saturation) was added slowly to the supernatant while it was being stirred, and the solution was centrifuged at 15 000 × *g* for 20 min at 4 °C. The pellet was resuspended in 180 mL of buffer A (50 mM Tris-HCl, pH 8.0, 50 mM KCl, 1 mM EDTA, 10% glycerol, and 0.1% BME), concentrated to 30 mL, and passed over a Sephadex G-25 column previously equilibrated in buffer A. Protein-containing fractions were pooled and concentrated by ultrafiltration using an Amicon stirred cell (Millipore, MA) with a YM-10 membrane, mixed with an equal volume of 50% glycerol, and stored at −76 °C after snap freezing in liquid nitrogen.

Synthesis of AdoMet and SeAdoMet. Large-scale AdoMet synthesis was carried out by a modification of known methods (*36*). A typical reaction contained the following in a final volume of 200 mL: 10 mM L-methionine, 15 mM ATP, 100 mM Tris-HCl (pH 8.0), 50 mM KCl, 30 mM $MgCl_2$, 25 U of AdoMet synthetase, 100 U of inorganic pyrophosphatase, and 8% BME. Reactions were carried out at 25 °C for up to 4 h and were monitored by HPLC using method 1. Upon completion, the reaction mixture was titrated to pH 5−6 with HCl, diluted to 2 L with cold 1 mM sodium acetate buffer (pH 5.0), and loaded onto a 400 mL CM-52 column equilibrated in the same buffer. The column was washed with 1 L of the sodium acetate buffer, and AdoMet was eluted with 40 mM H_2SO_4. The pooled fractions were concentrated by rotary evaporation, and excess sulfuric acid was removed by titration of the solution to pH 6.0 with Q-Sepharose Fast Flow resin ($^-$OH form). The solution was further concentrated by rotary evaporation and loaded onto a Bio-Gel P2 (extra fine) gel-filtration column that was equilibrated in water. Fractions were analyzed by UV−vis spectroscopy and HPLC, and those containing AdoMet were pooled and concentrated by rotary evaporation, snap frozen in liquid nitrogen, and stored at −76 °C. Typical yields ranged from 30% to 40%, and the purity of the compound was greater than 98% as judged by HPLC. When synthesized and purified in this fashion and stored at −76 °C, AdoMet displayed no significant degradation for periods of up to 6 months.

SeAdoMet was synthesized in a similar manner using L-selenomethionine in place of L-methionine. However, because of the decreased stability of SeAdoMet, the reaction was allowed to proceed only for 1 h, and a larger amount of AdoMet synthetase (60 U) was used in its synthesis. Yields of SeAdoMet (18%) were lower, primarily because of the significant rate of degradation of the compound to SeMTA during purification. ^{1}H NMR (D_2O, 400 MHz) δ: 2.32 (2H, dd, J = 7.57, 14.46 Hz), 2.84 (3H, s), 3.34−3.42 (1H, m), 3.52−3.60 (1H, m), 3.75 (1H, t, J = 6.43 Hz), 3.82−3.94 (2H, m), 4.51−4.60 (2H, m), 4.91 (1H, t, J = 4.57 Hz), 6.07 (1H, d, J = 4.57 Hz), 8.21 (1H, s), 8.27 (1H, s). ESI-MS: calculated for $C_{15}H_{23}N_6O_5Se$ $[M]^+$, 447.09; found, 447.3.

Synthesis of N-Acetyl-α-amino-γ-butyrolactone. Triethylamine (35 mmol) was added dropwise to a solution of (*R*,*S*)-α-amino-γ-butyrolactone hydrobromide (35 mmol) and acetic anhydride (35 mmol) in 300 mL of cold chloroform under argon while stirring in an ice−water bath. After addition, the solution was removed from the ice−water bath and allowed to stir at ambient temperature. Acylation was complete after 90 min as determined by TLC (CH_2Cl_2/methanol, 1:1, R_f 0.15). The chloroform was removed by rotary evaporation, and the remaining yellow oil was dissolved in hot ethyl acetate. After cooling, the solution was filtered and the ethyl acetate was removed by rotary evaporation. The yield of *N*-acetyl-α-amino-γ-butyrolactone, which was a yellow residue, was 100% as judged by TLC. The NMR spectrum was consistent with the target compound.

Synthesis of (R,S)-N-Acetyltelluromethionine. (*R*,*S*)-*N*-Acetyltelluromethionine was synthesized by modifications of previously published procedures (*37*−*39*). CH_3TeLi was prepared in the dark under argon by freezing tellurium powder (200 mesh, 50 mmol) in THF (150 mL) with liquid nitrogen. The liquid nitrogen bath was removed, and methyllithium (50 mmol in 31.25 mL of diethyl ether) was added dropwise as the THF thawed. After being stirred rapidly for 1 h, the solution turned a pale yellow color. The solution was frozen in liquid N_2, and again the liquid nitrogen bath was removed. As the THF thawed, a solution of *N*-acetyl-α-amino-γ-butyrolactone (35 mmol) and tetramethylethylenediamine (35 mmol) dissolved in 25 mL of THF was added dropwise in the dark, and the mixture turned a reddish orange color. The mixture was stirred for an additional 8 h in the dark, and the formation of (*R*,*S*)-*N*-acetyltelluromethionine was followed by TLC (CH_2Cl_2/methanol, 2:1, R_f 0.2; CH_3CN/water, 4:1, R_f 0.5). The solids were filtered, washed sequentially with degassed MtBE and degassed methylene chloride, dissolved in methanol, and purified over a flash column using CH_2Cl_2/methanol (2:1). The final yield was 69% (24 mmol).

Synthesis of TeAdoMet. (*R*,*S*)-*N*-Acetyltelluromethionine (3.14 g, 11 mmol) was deacetylated with acylase I (314 mg) in 125 mL of an anaerobic solution of 64 mM EPPS buffer (pH 8.0). The reaction was conducted at room temperature inside of a Coy (Grass Lake, MI) anaerobic chamber with gentle stirring. The synthesis was followed by HPLC with UV detection at 235 nm and was complete within 3 h. The reaction was brought to a final concentration of 100 mM Tris-HCl buffer (pH 8.0), 50 mM KCl, 50 mM $MgCl_2$, 24 mM ATP (pH 8.0), and 8% BME in a final volume of 500 mL. AdoMet synthetase (70 U) and inorganic pyrophosphatase (100 U) were then added, and the reaction was allowed to proceed for 30 min. TeAdoMet was purified as described for AdoMet and stored under the same conditions. The purity of the compound was greater than 99% and showed no significant degradation for up to 6 months when stored at −76 °C. UV−visible, NMR, and mass spectra were consistent with the target compound. ^{1}H NMR (D_2O, 400 MHz) δ: 2.21 (3H, s), 2.25−2.32 (2H, m), 3.00−3.10 (1H, m), 3.12−3.21 (1H, m), 3.30−3.40 (1H, m), 3.46 (1H, dd, J = 3.50, 12.54 Hz), 3.71 (1H, dd, J = 5.18, 7.68 Hz), 4.45−4.52 (2H, m), 4.89 (1H, t, J = 4.39 Hz), 6.04 (1H, d, J = 4.01), 8.21 (1H, s), 8.28 (1H, s). ESI-MS: calculated for $C_{15}H_{23}N_6O_5Te$ $[M]^+$, 497; found, 497.

Synthesis of Racemic SeAdoMet. Enzymatically synthesized SeAdoMet was demethylated using *E. coli* cyclopropane fatty acid (CFA) synthase, grown and purified as described in the accompanying paper (*40*). The reaction contained the following in a total volume of 65 mL: 31 mM $KHCO_3$ (pH 7.0), 13 mM DOPG, 9.6 mM (*S*,*S*)-SeAdoMet, and CFA synthase (40 U). The reaction was incubated at 37 °C for 10 h, and the extent of turnover was monitored by HPLC using method 1. Upon completion (57% SeAdoHcys formed), the reaction was quenched with 135 μL of neat formic acid, and the phospholipids and denatured protein were pelleted by centrifugation for 10 min at 10 000 × *g* and 4 °C. The supernatant, which contained SeAdoHcys and SeMTA, was concentrated to 5 mL by rotary evaporation and adjusted to 6% TFA in a final volume of 70 mL. This solution was loaded onto an AG 50W-X2 strong cation exchange column (70 mL), and the column was washed with 200 mL of water followed by 2 L of a solution containing 200 mM formic acid and 100 mM ammonium hydroxide to elute the SeMTA. SeAdoHcys was eluted subsequently with 300 mL of a solution containing 300 mM acetic acid and 275 mM ammonium hydroxide. Solvent was removed by rotary evaporation, and the SeAdoHcys was dissolved in 25 mL of concentrated formic acid.

SeAdoHcys (0.1 mmol in 25 mL in formic acid) was methylated with methyl iodide (7.7 mmol) in the dark under nitrogen according to known methods (*41*). The reaction was followed by HPLC (method 1), and upon completion (26 h), the reaction mixture was extracted twice with diethyl ether, retaining the formic acid layer. The formic acid was removed by rotary evaporation, and the residue was dissolved in 10 mL of water. This process was repeated two additional times. The residue was then dissolved in 500 mL of water and loaded onto a 400 mL CM-52 column equilibrated in 1 mM sodium acetate buffer (pH 5.0). The column was washed with 200 mL of the sodium acetate buffer, and racemic SeAdoMet was eluted with 20 mM H_2SO_4 and further purified as described above for the synthesis and purification of AdoMet. ^{1}H NMR (D_2O, 400 MHz) δ: 2.28−2.37 (2H, m), 2.76 (1.5H, s), 2.80 (1.5H, s), 3.35−3.43 (1H, m), 3.48 (1H, t, J = 7.92 Hz), 3.75−3.79 (1H, m), 3.82−3.94 (2H, m), 4.54−4.61 (2H, m), 4.91−4.96 (1H, m), 6.10 (1H, d, J = 4.25 Hz), 8.28 (1H, s), 8.32 (1H, s).

Decarboxylation of AdoMet, SeAdoMet, or TeAdoMet. Decarboxylation of AdoMet, SeAdoMet, or TeAdoMet was conducted at 37 °C in glass test tubes. Each reaction contained the following in a final volume of 300 μL: 50 mM HEPES (pH 7.5), 1.25 mM dithiothreitol (DTT), 1.9 mM putrescine, 1 mM AdoMet, SeAdoMet, or TeAdoMet, and 0.23 U of AdoMet decarboxylase. All components,

excluding the AdoMet analogue to be decarboxylated, were allowed to preincubate at 37 °C for 4 min. Each reaction was initiated by addition of the AdoMet analogue, and 50 μL aliquots were removed at specified times and quenched by addition of 20 μL of 125 mM H_2SO_4. The precipitated protein was removed by centrifugation, and the supernatant was analyzed by HPLC (method 2). Under these conditions, decarboxylated AdoMet eluted at 17.9 min, decarboxylated SeAdoMet eluted at 18.5 min, and decarboxylated TeAdoMet eluted at 20.3 min.

Decay Studies of AdoMet, SeAdoMet, and TeAdoMet. Decay studies of AdoMet, SeAdoMet, and TeAdoMet were performed at 37 °C and varying pH values, and monitored by HPLC using method 2 for assessment of racemization or method 1 for assessment of adenine or MTA formation. Several three-buffer systems were employed to ensure a constant ionic strength of 100 mM across the pH range evaluated, as well as to minimize buffer effects on the pH profiles (*42*). Buffer system 1 (pH 4.5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0) consisted of 100 mM Tris base, 50 mM MES, and 50 mM acetic acid. Buffer system 2 (pH 0.5, 1.5, and 6.0) consisted of 100 mM Tris base, 50 mM MES, and 50 mM oxalic acid, while buffer system 3 (pH 3.0, 4.5, 6.0, 10.0, 10.5, 11.0, and 12.0) consisted of 100 mM TEA, 50 mM MES, and 50 mM formic acid. Each buffer system was made according to the following procedure. A 10× buffer system was prepared by mixing the three buffer components at concentrations of 1, 0.5, and 0.5 M, respectively. "Working" buffer systems (5×) were then prepared by diluting 50 mL of the 10× buffer system to a final volume of 100 mL while adjusting the pH to the desired value with KOH or HCl.

Prior to initiation of the pH decay studies, calibration curves of each compound expected in the chromatogram (AdoMet, adenine, and MTA) were generated using 1 mM tryptophan as an internal standard. When HPLC method 1 was employed, calibration curves ranged from 2.5 to 750 μM adenine or MTA and 50 μM to 1.5 mM AdoMet. When HPLC method 2 was employed, calibration curves ranged from 10 μM to 1.5 mM for all compounds. Concentrations of calibration standards were determined spectrophotometrically (AdoMet, $\epsilon_{260} = 15\,400$ M^{-1} cm^{-1}; adenine, $\epsilon_{260} = 13\,300$ M^{-1} cm^{-1}; MTA, $\epsilon_{260} = 16\,000$ M^{-1} cm^{-1}) (*43*). The concentrations of SeAdoMet and TeAdoMet were determined from AdoMet calibration curves, while the concentrations of SeMTA and TeMTA were determined from MTA calibration curves.

Each reaction contained the following in a final volume of 500 μL: 100 mM of the necessary buffer system at the desired pH, 1 mM AdoMet, SeAdoMet, or TeAdoMet, and 1 mM tryptophan. All components of the reaction, excluding AdoMet (SeAdoMet, TeAdoMet), were preincubated at 37 °C for 4 min prior to initiation of the reaction by addition of AdoMet. Aliquots (50 μL) were removed at specified times and analyzed immediately by HPLC, or acidified with sulfuric acid (285 mM final concentration) and stored on ice until injection. Control samples demonstrated that breakdown of AdoMet did not occur after quenching as such and storing at 4 °C.

First-order rate constants for racemization (k_R) and formation of MTA (k_N) and adenine (k_D) were obtained by determining the concentrations of each of the nucleoside-containing products as a function of time and fitting the results to the appropriate equation. Racemization was fitted to eq 1, MTA formation was fitted to eq 2, and adenine

$$k_R t = 0.5 \ln\left(\frac{[(S,S)\text{-AdoMet}]_0}{[(S,S)\text{-AdoMet}]_0 - 2[(R,S)\text{-AdoMet}]_t}\right) \quad (1)$$

$$k_N t = \ln\left(\frac{[\text{AdoMet}]_0}{[\text{AdoMet}]_0 - [\text{MTA}]_t}\right) \quad (2)$$

formation was fitted to eq 3; wherein $[\text{AdoMet}]_0$ is the

$$k_D t = \ln\left(\frac{[\text{AdoMet}]_0}{[\text{AdoMet}]_0 - [\text{adenine}]_t}\right) \quad (3)$$

concentration of AdoMet at $t = 0$ and $[\text{AdoMet}]_t$, $[\text{MTA}]_t$, and $[\text{adenine}]_t$ are the concentrations of each of the denoted products at a given time, t (*33*). First-order rate constants obtained for MTA and adenine formation as a function of pH were fitted to eqs 4 and 5, respectively, using the GraFit (Version 5) software package (*44*), which allowed for extraction of relevant pK_a values.

$$y = \frac{\text{Lim}_1 + \text{Lim}_2 10^{\text{pH}-\text{p}K_a}}{10^{\text{pH}-\text{p}K_{a1}} + 1} - \frac{\text{Lim}_2 - \text{Lim}_3 10^{\text{pH}-\text{p}K_{a2}}}{10^{\text{pH}-\text{p}K_{a2}} + 1} \quad (4)$$

$$y = \frac{\text{Lim}_1 + \text{Lim}_2 10^{\text{pH}-\text{p}K_a}}{10^{\text{pH}-\text{pK}_a} + 1} \quad (5)$$

RESULTS

Synthesis and Characterization of AdoMet, SeAdoMet, and TeAdoMet. AdoMet, SeAdoMet, and TeAdoMet were synthesized using AdoMet synthetase in combination with ATP and methionine, selenomethionine, or telluromethionine. Each synthesis was followed by HPLC, and when the reaction reached completion or equilibrium, the respective compound was purified by cation-exchange chromatography and gel filtration. Each compound was ≥95% pure as judged by NMR, mass spectrometry, and HPLC; the major contaminants were MTA in the AdoMet preparation and SeMTA in the SeAdoMet preparation. HPLC method 2 allowed baseline resolution of both (*S*,*S*)-AdoMet and (*R*,*S*)-AdoMet, which displayed retention times of 11.0 and 12.5 min, respectively; MTA displayed a retention time of 30.5 min (Figure 2A). Greater than 98% of the preparation was the biologically relevant (*S*,*S*)-isomer. The final SeAdoMet product was noticeably less pure because the molecule degraded significantly faster than AdoMet during its synthesis and purification. An HPLC chromatogram of the SeAdoMet product is shown in Figure 2B. The retention times for (*S*,*S*)-SeAdoMet and SeMTA (12.3 and 32.0 min, respectively) are slightly longer as compared to AdoMet and MTA, consistent with a decrease in polarity when selenium is substituted for sulfur.

Also apparent in Figure 2B is that there is only one peak present for enzymatically synthesized SeAdoMet. To verify that the peak was not attributable to an inability to separate both isomers of SeAdoMet or an average retention time resulting from rapid racemization of both isomers, SeAdoMet was synthesized chemically by alkylating SeAdoHcys with

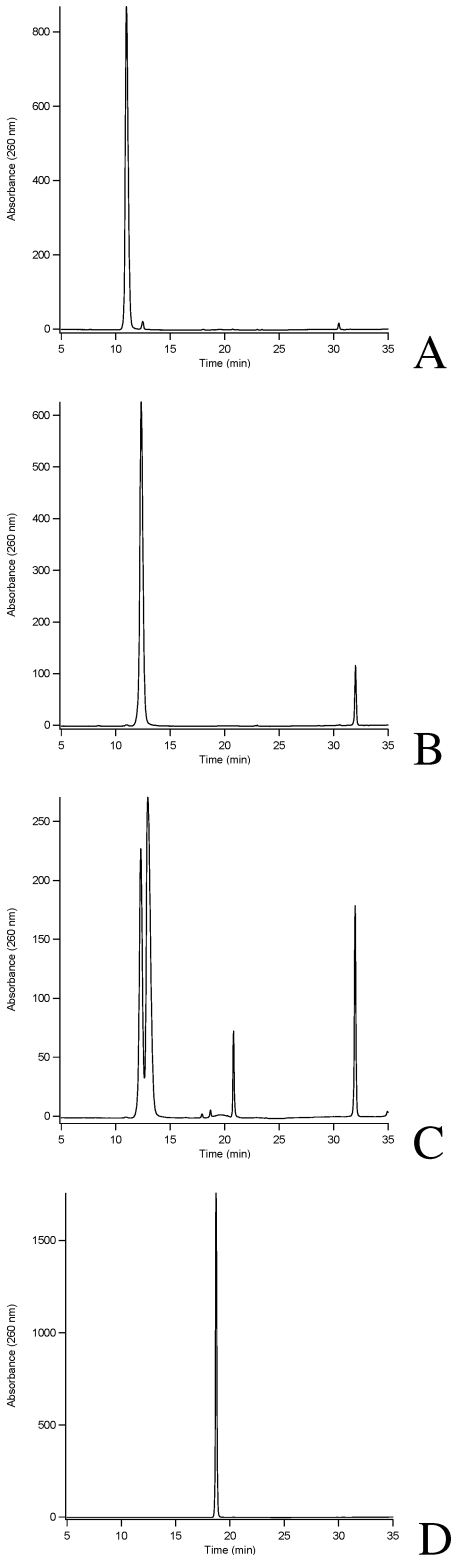

FIGURE 2: HPLC chromatograms of (A) AdoMet, (B) enzymatically synthesized SeAdoMet, (C) chemically synthesized SeAdoMet, and (D) TeAdoMet. HPLC method 2 was used as described in the text. Each chromatogram represents an injection of 25 μL of a 1 mM solution of the compound except in panel D, which was of a 1.5 mM solution of TeAdoMet.

methyl iodide, which takes place with little or no diastereoselectivity. As shown in Figure 2C, the peak that migrates with a retention time of 12.3 min in Figure 2B is now replaced by two peaks. The first peak displays a retention time of 12.3 min, identical to that displayed in Figure 2B, suggesting that it is (*S*,*S*)-SeAdoMet. The second peak displays a retention time of 13.1 min; we assign this peak to (*R*,*S*)-SeAdoMet (vide infra).

To verify the assignments of the (*S*,*S*)- and (*R*,*S*)-stereoisomers of SeAdoMet, the chemically and enzymatically synthesized compounds were treated with AdoMet decarboxylase and reanalyzed by HPLC. In Figure 3A, the HPLC chromatogram of AdoMet before (top trace) and after (bottom trace) a 20 min incubation with AdoMet decarboxylase is shown; the numbers 1, 2, and 3 refer to peaks for (*S*,*S*)-AdoMet, (*R*,*S*)-AdoMet, and decarboxylated AdoMet (DC-AdoMet), respectively. As shown, peak 1 disappears entirely after treatment with AdoMet decarboxylase, and a new peak appears (peak 3), displaying a retention time of 17.9 min. The small peak that migrates with a retention time of 30.5 min corresponds to MTA, which is formed under the conditions of incubation. Although peak 2 is small in intensity, it is noteworthy that it does not appear to be a substrate for the enzyme, consistent with the presumed stereochemical preference for the diastereomers of AdoMet. In Figure 3B, the HPLC chromatogram before (top trace) and after (bottom trace) a 20 min incubation of AdoMet decarboxylase with enzymatically synthesized SeAdoMet (peak 1) is displayed. As shown, SeAdoMet also is a substrate for the enzyme; it is quantitatively converted to decarboxylated SeAdoMet (DC-SeAdoMet) (peak 2; retention time, 18.2 min) within 20 min at 37 °C. The peak that migrates with a retention time of 32 min corresponds to SeMTA. In Figure 3C, the HPLC trace before (top trace) and after (bottom trace) a 10 min incubation of AdoMet decarboxylase with chemically synthesized SeAdoMet (peaks 1 and 2) is displayed. Again, the peak displaying a retention time of 12.3 min disappears, while the peak that migrates with a retention time of 13.1 min remains present. Therefore, the two isomers of SeAdoMet also are separable by HPLC, suggesting that the rate of racemization of (*S*,*S*)-SeAdoMet must be slower than that of (*S*,*S*)-AdoMet, since (*R*,*S*)-SeAdoMet is not observed in enzymatic preparations of SeAdoMet, whereas (*R*,*S*)-AdoMet is observed in enzymatic preparations of AdoMet. Indeed, (*S*,*S*)-AdoMet has been shown to racemize under the experimental conditions described in this study (*33*). Furthermore, extended incubations of enzymatically synthesized SeAdoMet at 37 °C did not result in the formation of (*R*,*S*)-SeAdoMet as judged by HPLC.

D,L-Telluromethionine was synthesized according to known procedures (*37*). As previously established, it was labile in the presence of oxygen, especially in its nonacylated state, and was therefore manipulated under an inert atmosphere (*37*−*39*). By contrast, TeAdoMet did not degrade significantly at room temperature, even in the presence of oxygen. A chromatogram of enzymatically synthesized TeAdoMet (Figure 2D) displays only one peak, having a retention time of 18.7 min, consistent with its reduced polarity as compared to AdoMet and SeAdoMet. Similarly to AdoMet and SeAdoMet, TeAdoMet was a substrate for AdoMet decarboxylase; however, it was decarboxylated at a rate that was 5−6 times slower than that of AdoMet and SeAdoMet under a defined set of conditions (data not shown).

The NMR spectra of TeAdoMet, enzymatically synthesized SeAdoMet, and chemically synthesized SeAdoMet are similar to the published spectrum of AdoMet (*32*) and are

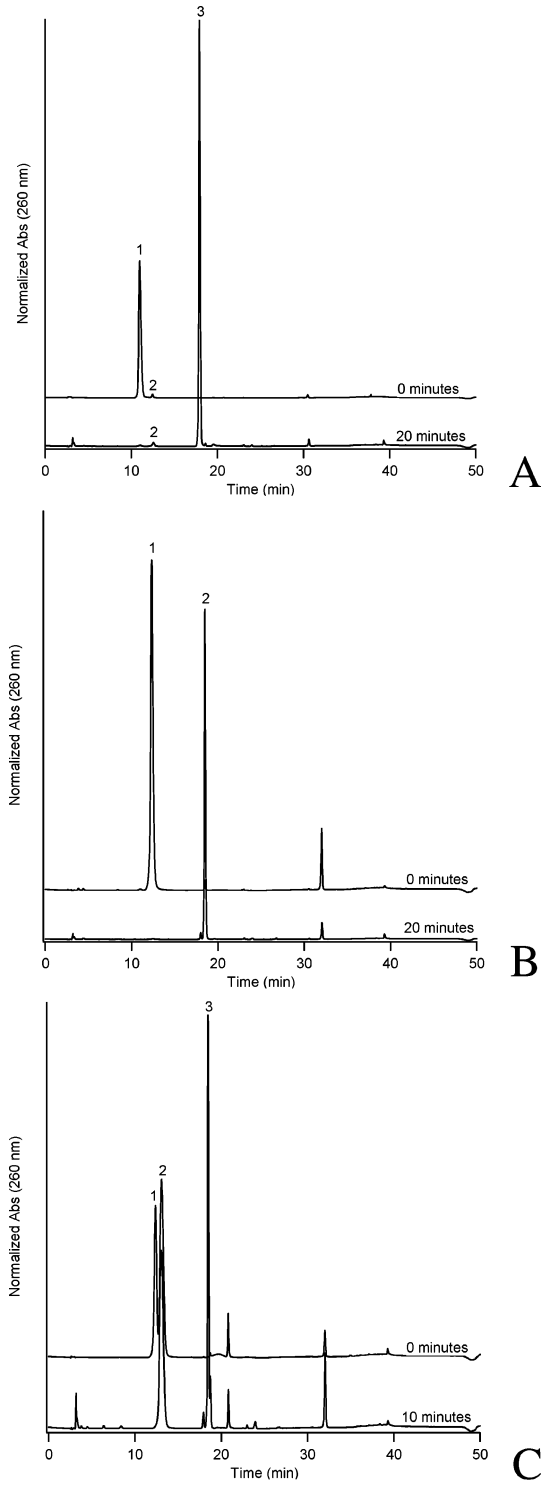

FIGURE 3: Decarboxylation of AdoMet and SeAdoMet using AdoMet decarboxylase. Decarboxylation was performed as described in the text. Chromatograms of (A) AdoMet, (B) enzymatically synthesized SeAdoMet, and (C) chemically synthesized SeAdoMet using HPLC method 2 are shown. Panel A presents 0 and 20 min post-decarboxylation chromatograms showing (1) (*S*,*S*)-AdoMet, (2) (*R*,*S*)-AdoMet, and (3) DC-AdoMet. The intensities of the two chromatograms have been normalized to the same intensities for the (*R*,*S*)-AdoMet peaks. Panel B presents 0 and 20 min post-decarboxylation chromatograms showing (1) (*S*,*S*)-SeAdoMet and (2) DC-SeAdoMet. The intensities of the two chromatograms have been normalized to the same intensities. Panel C presents 0 and 10 min post-decarboxylation chromatograms showing (1) (*S*,*S*)-SeAdoMet, (2) (*R*,*S*)-SeAdoMet, and (3) DC−SeAdoMet. The intensities of the two chromatograms have been normalized to the same intensities for the (*R*,*S*)-SeAdoMet peaks.

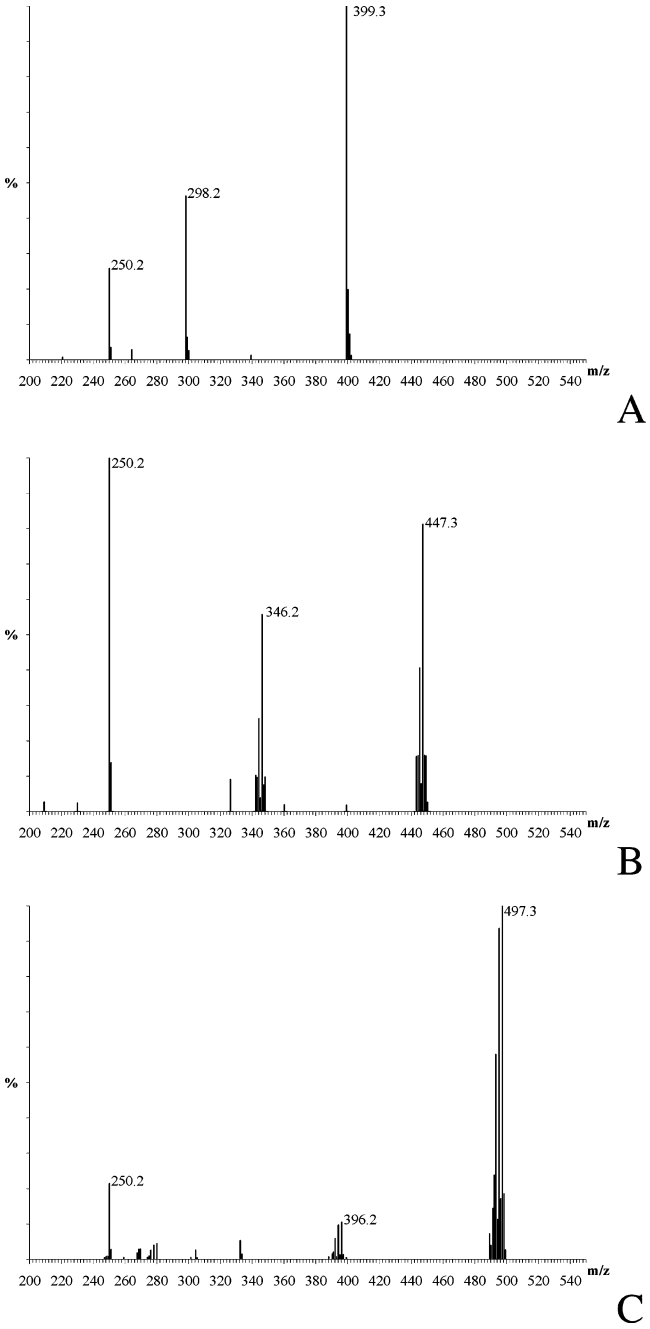

FIGURE 4: Mass spectra of (A) AdoMet, (B) SeAdoMet, and (C) TeAdoMet. The mass spectra were collected as described in the text. The peaks at *m*/*z* values of 399.3 (A), 447.3 (B), and 497.3 (C) represent the parent ions. The peaks at *m*/*z* values of 250.2 represent loss of methionine to yield 5′-deoxyadenosyl fragment, and the peaks at *m*/*z* values of 298.2 (A), 346.2 (B), and 396.2 (C) represent loss of HSL to yield fragments corresponding to MTA, SeMTA, or TeMTA, respectively.

provided in Supporting Information (Figures S1, S2, and S3, respectively), along with a list of relevant assignments. The major differences among the three spectra are found between 1.5 and 3 ppm and are attributed to the differential effects of the respective chalcogen. As shown in Figure 5, increased shielding occurs with an increase in the size of the chalcogen, resulting in upfield shifts of the resonances of the C-5′ protons, the methyl protons, and the γ-protons. The methyl protons display chemical shifts of 3.01 ppm in (*S*,*S*)-AdoMet (*32*), 2.84 ppm in (*S*,*S*)-SeAdoMet (Figure 5A), and 2.21 ppm in (*S*,*S*)-TeAdoMet (Figure 5C), while the methyl

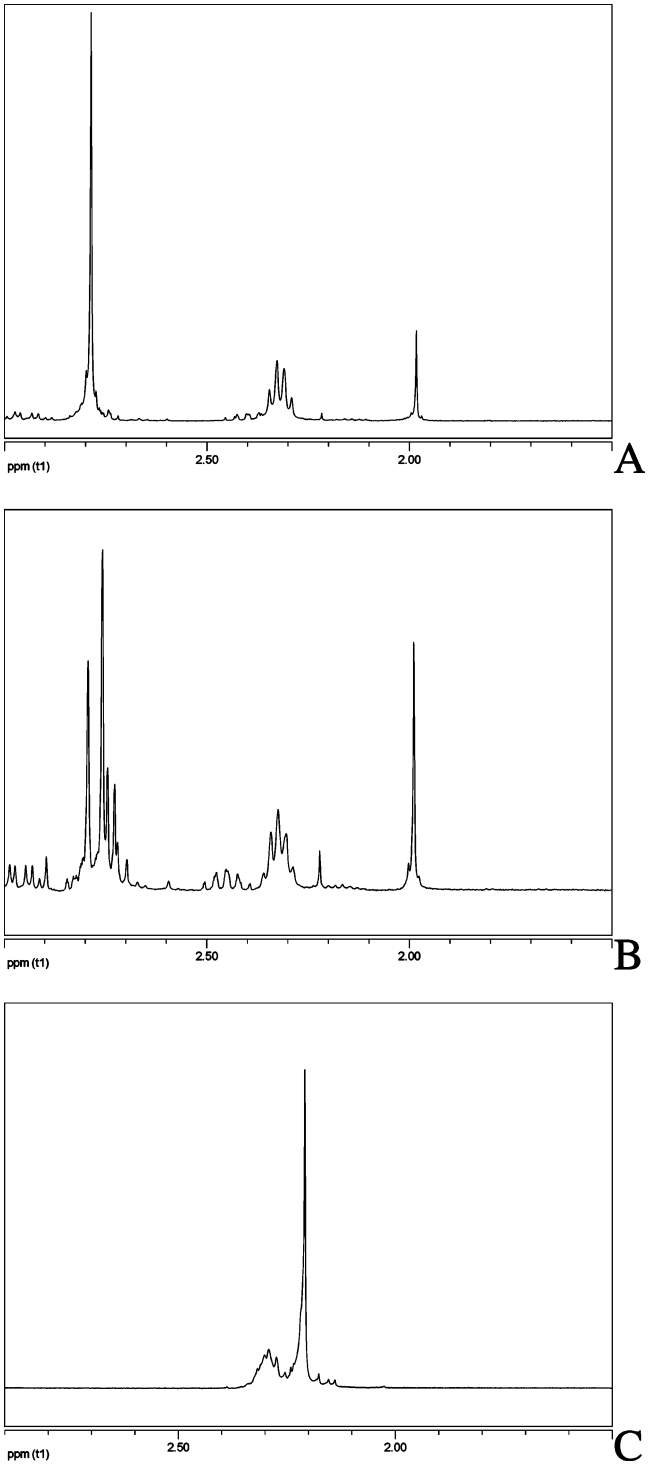

FIGURE 5: Regions from 1.5 to 3.0 ppm of the NMR spectra of (A) enzymatically synthesized SeAdoMet, (B) chemically synthesized SeAdoMet, and (C) TeAdoMet. The NMR spectra were collected in D_2O and are referenced to TMS. The protons on the activated methyl group of enzymatically synthesized SeAdoMet, chemically synthesized SeAdoMet, and enzymatically synthesized TeAdoMet display chemical shifts of 2.84 ppm (A), 2.96 and 2.76 ppm (B), and 2.21 ppm (C), respectively.

protons of (*R*,*S*)-AdoMet and (*R*,*S*)-SeAdoMet display chemical shifts of 2.96 (*32*) and 2.76 ppm (Figure 5B), respectively. The γ-protons display chemical shifts of 3.48 and 3.70 ppm in (*S*,*S*)-AdoMet and (*R*,*S*)-AdoMet (*32*), respectively, while those of TeAdoMet display chemical shifts in the range of 2.25 to 2.32 ppm (Figure 5C). The γ-protons of enzymatically synthesized SeAdoMet display chemical shifts of ~2.32 ppm, while those of chemically synthesized SeAdoMet display chemical shifts between 2.28 and 2.37 ppm (Figure 5B).

The mass spectra of all three compounds are consistent with their expected structures (Figure 4). The fragments at $m/z = 399.3$ (Figure 4A), $m/z = 447.3$ (Figure 4B), and $m/z = 497.3$ (Figure 4C) represent the parent ions, $[M]^+$, of AdoMet, SeAdoMet, and TeAdoMet, respectively. In each spectrum, the fragment at $m/z = 250.2$ corresponds to a 5′-deoxyadenosyl group resulting from fragmentation of the bond between the 5′-carbon and the chalcogen, while the fragments at $m/z = 298.2$ (Figure 4A), $m/z = 346.2$ (Figure 4B), and $m/z = 396.2$ (Figure 4C) represent cleavage of the bond between the γ-carbon of the methionine moiety and the chalcogen, affording $[MTA]^+$, $[SeMTA]^+$, and $[TeMTA]^+$.

Interesting correlations with respect to the relative intensities of each of the fragments and the corresponding onium chalcogen are apparent. For AdoMet (Figure 4A), the parent ion ($m/z = 399.3$) is the base peak (100%), and peaks at m/z values of 250.2 and 298.2 have intensities of 26% and 46%, respectively. For TeAdoMet, which is more stable than AdoMet, the base peak also is the parent ion ($m/z = 497.3$), and the two fragments at $m/z = 250.2$ and $m/z = 396.2$ have intensities of 21% and 11%. The intensity of the fragment at $m/z = 250.2$ is actually an overestimation because there are several isotopes of tellurium with similarly high natural abundances, for example, ^{130}Te (34.08%), ^{128}Te (31.74%), and ^{126}Te (18.84%), that contribute to fragments around $m/z = 396.2$ and $m/z = 497.3$. Those peaks that are around $m/z = 497.3$ are parent ions themselves, representing $[^x\text{TeAdoMet}]^+$, wherein x represents any of the relevant isotopes of tellurium. Renormalization of the intensity at $m/z = 250.2$, accounting for the other major isotopes of tellurium, indicates that the true percentage of the fragment at $m/z = 250.2$ is less than 8%. For SeAdoMet, which is the least stable compound, the base peak is the 5′-deoxyadenosyl fragment at $m/z = 250.2$, while the parent ion at $m/z = 447.3$ and peak at $m/z = 346.2$ have intensities of 81% and 56%, respectively. Renormalization of the percentage at $m/z = 250.2$ to account for the major isotopes of selenium (^{80}Se, 49.61%; ^{78}Se, 23.77%; ^{76}Se, 9.37%; ^{82}Se, 8.73%; ^{77}Se, 7.63%) results in an intensity at $m/z = 250.2$ that is ~60% of the total parent ion species. These spectra suggest that the fragmentation of the parent ion species follows the relative electrophilicities of the molecules, which will be discussed below.

Decay Studies of AdoMet, SeAdoMet, and TeAdoMet. (*S*,*S*)-AdoMet degrades by three independent processes under physiological conditions: the pH-independent racemization about the sulfonium to give the (*R*,*S*)-diastereomer, the pH-dependent intramolecular nucleophilic cyclization to give homoserine lactone and MTA, and the pH-dependent deprotonation at C-5′ with ensuing chemistry that releases adenine and *S*-ribosylmethionine (*33*). (*S*,*S*)-AdoMet, (*S*,*S*)-SeAdoMet, and (*S*,*S*)-TeAdoMet were incubated at 37 °C under conditions of constant ionic strength (100 mM) and varying pH values, and their abilities to form the corresponding (*R*,*S*)-isomer, adenine, MTA, SeMTA, or TeMTA were assessed as a function of time. Racemization was fitted to eq 1, intramolecular nucleophilic addition was fitted to eq

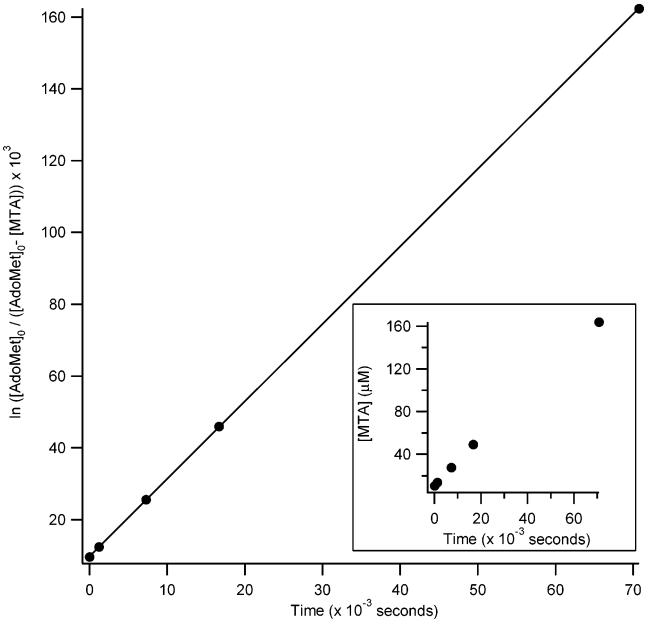

FIGURE 6: Decay study of 1 mM AdoMet at pH 1.5 showing the formation of MTA. The data were fitted to eq 3, and the slope represents the rate constant for nucleophilic addition, k_N. The inset presents the concentration of MTA as a function of time.

2, and deprotonation was fitted to eq 3. The inherent assumption in each of the equations is that the initial concentration of AdoMet ($AdoMet_0$) does not change significantly during the course of the reaction as a result of the other two processes. Therefore, data that were used for fits were obtained for time courses that showed less than 20% degradation of AdoMet. Additionally, it was also assumed that the rate constants for intramolecular nucleophilic attack and deprotonation were equivalent for the (*S*,*S*)- and (*R*,*S*)-isomers of each compound, an assumption that was also made in a previous study (*33*). Racemization was quantified only at low pH values since it is pH-independent (*33*) and because degradation via the other two pathways is significantly faster at higher pH values, which would result in underestimated rates of racemization. Similarly, at high pH values, the first-order rate constant for deprotonation is much higher than that for intramolecular nucleophilic addition, which would result in underestimated rate constants for MTA or SeMTA formation. At high pH values, care was taken to use only the initial linear portion of the plot. In each case, the loss of the onium chalcogen could be accounted for by the sum of the concentrations of adenine and MTA (or SeMTA).

In Figure 6, a representative plot is displayed in which the formation of MTA from AdoMet is graphed according to eq 2. Within the time span of the measurement, the plot is linear and the slope of the line is equal to k_N. Repetition of this experiment at other pH values allowed collection of rate constants, which were plotted as a function of the appropriate pH value to construct a pH−rate profile. In Figure 7, pH−rate profiles for formation of MTA are displayed for AdoMet (Figure 7A), SeAdoMet (Figure 7B), and TeAdoMet (Figure 7C); the corresponding rate constants are listed in Table 1. The results for AdoMet compare favorably to those reported previously (*33*); however, in the present study the pH range was extended from 0.5 to 12.0, and the data were fitted to appropriate equations to extract

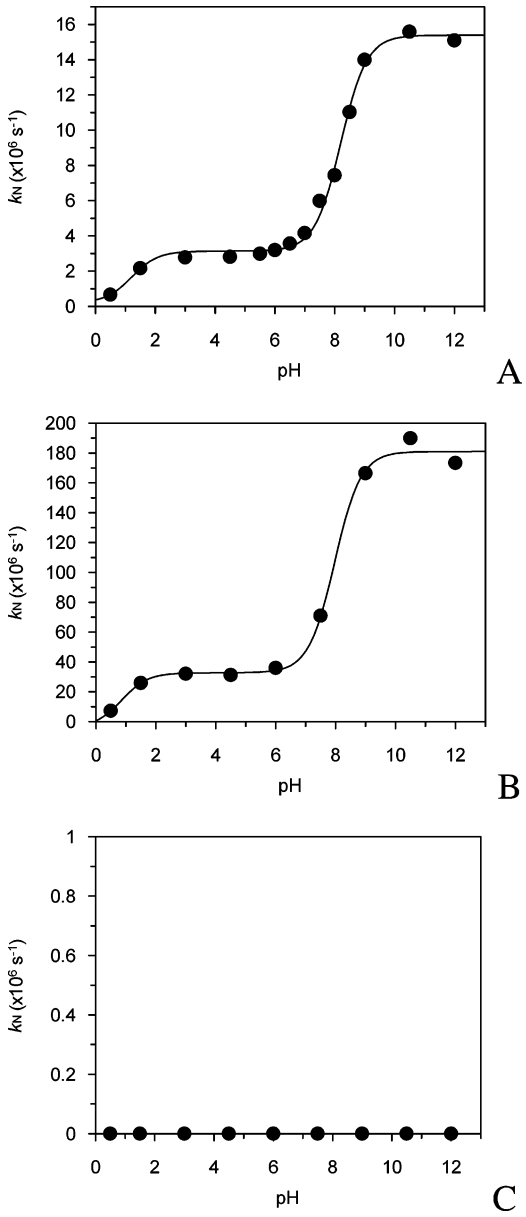

FIGURE 7: pH dependence of the first-order rate constant for intramolecular nucleophilic addition for (A) AdoMet, (B) SeAdoMet, and (C) TeAdoMet. The rates obtained from fits to eq 2 at each pH value were plotted versus pH. The data were fitted to eq 4, yielding pK_a values of 1.2 ± 0.4 and 8.2 ± 0.05 for AdoMet and 0.86 and 8.0 ± 0.1 for SeAdoMet.

apparent pK_a values for functional groups that are involved in the reaction. The formation of MTA from AdoMet was dependent upon two ionizable groups, one with a pK_a value of 1.2 ± 0.4 and one with a pK_a value of 8.2 ± 0.5. We assign the pK_a value of 1.2 to the α-carboxylate group of AdoMet, since the pK_a value for the α-carboxylate of methionine is 2.1 (*45*) and would be expected to be reduced in the presence of the additional positive charge of the sulfonium group. We assign the pK_a value of 8.2 to the α-amino group of AdoMet, since the pK_a value for the α-amino group of methionine is 9.3 (*45*) and would also be expected to be reduced in the presence of the additional positive charge of the sulfonium group. The greater uncertainty in the low pK_a value results from very few data points that are below a pH of 1. The rate constant associated with MTA formation at pH 10.5 (15.6×10^{-6} s^{-1}) is 5-fold greater

Table 1: pH Dependence of First-Order Rate Constants of Nucleophilic Attack (k_N) and Deprotonation (k_D) of AdoMet, SeAdoMet, and TeAdoMet[a]

	AdoMet		SeAdoMet		TeAdoMet	
pH	k_N	k_D	k_N	k_D	k_N	k_D
0.5	0.669	*b*	7.26	*b*	*b*	*b*
1.5	2.16	*b*	25.9	*b*	*b*	*b*
3.0	2.78	*b*	32.1	*b*	*b*	*b*
4.5	2.81	0.0620	31.2	*b*	*b*	*b*
6.0	3.20	0.686	36.0	*b*	*b*	*b*
7.5	5.99	5.48	70.9	*b*	*b*	*b*
9.0	14.0	27.7	166	*b*	*b*	*b*
10.5	15.6	544	190	1.25	*b*	*b*
12.0	15.1	4730	173	10.03	*b*	*b*

[a] All rates are in units of s^{-1} and have been multiplied by 1×10^6. [b] Not detectable.

than that at pH 4.5 (2.81×10^{-6} s^{-1}), suggesting that the positive charge on the α-amino group strongly modulates the nucleophilicity of the α-carboxylate anion.

Very similar results were obtained for the pH-dependent assessment of SeMTA formation from SeAdoMet (Figure 7B). The pH−rate profile showed two ionizable groups, one with a pK_a of ∼0.86 and one with a pK_a of 8.0 ± 0.1, consistent with the α-carboxylate and α-amino groups of SeAdoMet. The apparent first-order rate constant associated with SeMTA formation at pH 10.5 (190×10^{-6} s^{-1}) is 6-fold greater than that at pH 4.5 (31.2×10^{-6} s^{-1}), and both are more than 10-fold greater than the first-order rate constants for MTA formation from AdoMet at the same pH values, suggesting that SeAdoMet is a significantly better alkylating agent under the described experimental conditions. Unexpectedly, TeAdoMet did not degrade to TeMTA to any significant extent under the conditions of the reaction (Figure 7C).

In Figure 8, pH−rate profiles for adenine formation from AdoMet, SeAdoMet, and TeAdoMet are displayed. As previously described (*33*), deprotonation at C-5′ of AdoMet leading to adenine formation does not occur at a significant rate at pH values below 4.5. Formation of adenine from SeAdoMet occurred with a significantly slower first-order rate constant at similar pH values than formation of adenine from AdoMet. Moreover, TeAdoMet did not decay at any significant rate through this pathway at all pH values that were monitored. The data for AdoMet and SeAdoMet degradation were fitted to eq 5, and pK_a values of ∼11.5 and ∼14.1, respectively, were extracted from the fit. It is important to note that these pK_a values are apparent rather than microscopic. It has been suggested that this reaction takes place via a concerted E_2-like elimination reaction, wherein a discrete ylide intermediate is not formed (*29*). However, primary deuterium isotope effects as well as solvent isotope effects suggest that the transition state has significant carbanion character. If a discrete ylide intermediate is formed, it would have to partition to products much faster than it reverts to substrate.

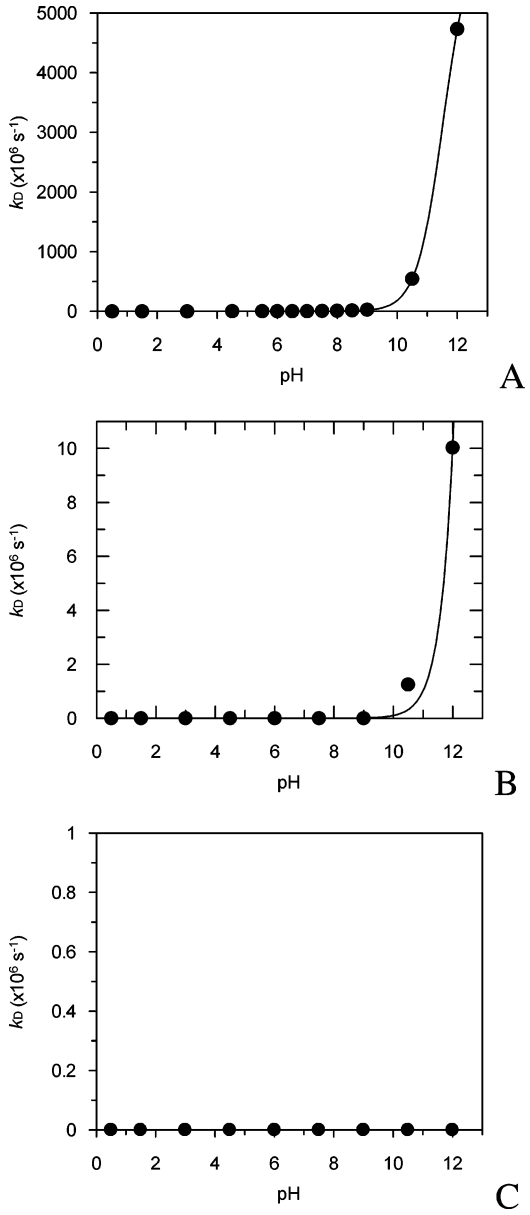

FIGURE 8: pH dependence of the first-order rate constant for deprotonation at C-5′ for (A) AdoMet, (B) SeAdoMet, and (C) TeAdoMet. The rates obtained from fits to eq 3 at each pH value were plotted versus pH. The data were fitted to eq 5, yielding pK_a values of ∼11.5 for AdoMet and ∼14.1 for SeAdoMet.

DISCUSSION

The widespread use and critical importance of AdoMet in nature has led us to synthesize its related onium chalcogens and assess the effect of the central atom on the intrinsic polar reactivity of the respective compound. Early studies by Baddiley et al. demonstrated that the biologically relevant form of AdoMet can degrade by three spontaneous processes (*46*). Two of these processes, intramolecular nucleophilic addition and deprotonation adjacent to the chalcogen, serve as intrinsic model systems for two enzymatic activities in which AdoMet is known to serve as a cosubstrate, alkylation and ylide formation. At pH values less than 2, the predominant mode of decay is via racemization about the central sulfur atom, which bears three carbon-containing substituents, as well as a lone pair of electrons. As the pH is increased, the ionization of the carboxylate group allows for intramolecular nucleophilic addition onto the γ-carbon, forming L-homoserine lactone and releasing MTA. At pH values of 5 or greater, the principle pathway for degradation occurs via deprotonation at C-5′ with several subsequent steps that result in the release of adenine and *S*-ribosylmethionine (*33*).

In this study, we have shown that the selenium analogue of AdoMet is subject to two of these three spontaneous modes of decay (intramolecular nucleophilic addition and deprotonation at C-5′), while the tellurium analogue is inert to all three under the conditions described in Materials and Methods. We reassessed the racemization of (*S*,*S*)-AdoMet and obtained a first-order rate constant of 3.16×10^{-6} s^{-1} at 37 °C, pH 7.5, and constant ionic strength (data not shown), which is similar to the value of 2.1×10^{-6} s^{-1} previously reported for racemization of AdoMet under similar conditions (*33*). This determination was facilitated by our ability to separate cleanly the two isomers of AdoMet by reverse-phase HPLC. Although the (*R*,*S*)-isomer of AdoMet represented less than 5% of the total product after enzymatic synthesis, it was nonetheless clearly observable. In contrast, only one corresponding peak was observable by HPLC upon enzymatic synthesis of either SeAdoMet or TeAdoMet, suggesting that (1) neither SeAdoMet nor TeAdoMet racemize at an appreciable rate under the conditions of incubation, (2) the two isomers of SeAdoMet and TeAdoMet are not separable under the conditions that provide baseline resolution of (*S*,*S*)- and (*R*,*S*)-AdoMet, or (3) the rates of inversion of the two isomers of SeAdoMet and TeAdoMet are very fast, giving rise to one peak with an average retention time for the two isomers. Our finding that chemically synthesized SeAdoMet gave rise to two peaks with retention times that patterned those of the (*S*,*S*)- and (*R*,*S*)-isomers of AdoMet, only one of which was a substrate for AdoMet decarboxylase, clearly ruled out the latter two scenarios, indicating that the compound does not racemize to any significant extent under the conditions of the experiment. Consistent with this finding, the NMR spectrum of enzymatically synthesized SeAdoMet displays one resonance for the protons of the activated methyl group; however, chemically synthesized SeAdoMet displays two resonances for the protons of the activated methyl group.

Our studies with TeAdoMet also are consistent with the premise that this compound does not racemize. Enzymatically synthesized TeAdoMet shows only one major peak when subjected to HPLC under conditions that cleanly separate the two isomers of AdoMet and SeAdoMet. In addition, the NMR spectrum of enzymatically synthesized TeAdoMet shows only one resonance for the protons of the activated methyl group rather than two. Although these results are also consistent with a scenario in which TeAdoMet undergoes rapid racemization, the finding that SeAdoMet does not racemize allows conclusions to be drawn about the rate of racemization of TeAdoMet. Pyramidal inversion barriers are known to increase both with an increase in substituent electronegativity and a decrease in electronegativity of the inverting atom (*47*). Given that tellurium is significantly less electronegative than sulfur and selenium and that the substituents about the respective chalcogen are the same, TeAdoMet would not be expected to racemize at a significant rate at 37 °C. This is consistent with recent ab initio MO calculations of sulfonium, selenonium, and telluronium ylides, wherein the activation energies for inversion at the MP3 level were 21.7, 25.1, and 34.8 kcal mol^{-1}, respectively (*48*).

In a previous study, the stability of AdoMet at five different pH values (1.5, 3.0, 4.5, 6.0, and 7.5) was assessed, and it was found that the largest decrease in the rate constant for MTA formation occurred when the pH fell below the pK_a of the carboxyl group, consistent with the greater nucleophilicity of a carboxylate anion as compared to a carboxylic acid. Unexpectedly, however, we found that the rate constant for MTA formation was governed by two ionizable groups. The first, displaying a pK_a of less than 2, is predicted to be that of the methionine carboxylic acid, as previously noted (*33*). The second, displaying a pK_a of 8.2 ± 0.5, we assign to the α-amino group. From inspection of the rate constants for MTA formation associated with dibasic AdoMet (15.6×10^{-6} s^{-1}) versus monobasic AdoMet (2.81×10^{-6} s^{-1}), we deduce that loss of the positive charge on the α-amino group profoundly affects the stability of the molecule, presumably via electrostatic modulation of the carboxylate nucleophilicity. This finding is reminiscent of one of the early steps in the biosynthesis of biotin, which is the transformation of 7-keto-8-aminopelargonic acid into 7,8-diaminopelargonic acid. This transamination is catalyzed by the pyridoxal 5′-phosphate-dependent enzyme 7,8-diaminopelargonic acid aminotransferase with the source of the amino group stemming from AdoMet. The second immediate product of the reaction is *S*-adenosyl-L-(2-keto-4-methylthio)-butyric acid, in which the α-amino group is replaced by a keto functionality and which has been noted to be extremely labile and often undetected at 37 °C and pH 8.5, the conditions under which turnover is assessed (*49*).

The behavior of SeAdoMet with respect to intramolecular nucleophilic addition was similar to that of AdoMet, except that the rate constants for SeMTA formation from dibasic (190×10^{-6} s^{-1}) and monobasic SeAdoMet (31.2×10^{-6} s^{-1}) were more than 10-fold greater than the corresponding rate constants for AdoMet. This suggests that SeAdoMet is intrinsically a better alkylating agent than AdoMet under the described experimental conditions. We had assumed that TeAdoMet would exhibit an even greater alkylating ability, given the increased lengths of Te−C bonds as compared to Se−C bonds or S−C bonds. Unexpectedly, however, TeAdoMet was inert under the defined experimental conditions. In retrospect, this behavior can be rationalized from the relative electronegativities of C, S, Se, and Te, which are 2.5, 2.4, 2.5, and 2.0 (*50*), respectively. In nucleophilic addition, attack typically occurs at the more electropositive atom with concomitant transfer of the bonding electrons to the more electronegative atom (*51*). The electronegativities of C, S, and Se are approximately the same; however, the electronegativity of Te is significantly less than that of C. Therefore, carbons adjacent to telluronium groups are poorer electrophiles as compared to carbons adjacent to selenonium or sulfonium groups because of the reduced ability of the tellurium atom to induce a positive dipole at the adjacent carbon atom (*51*).

The abilities of AdoMet, SeAdoMet, and TeAdoMet to degrade to adenine and *S*-ribosylmethionine pattern their respective pK_a values at C-5′. A pK_a value of 18.9 has been estimated for the trimethylsulfonium ion in water (*52*). Values for $C_6H_5CH_2S^+Bu_2Br^-$, $C_6H_5CH_2Se^+Bu_2Br^-$, and $C_6H_5CH_2Te^+Bu_2Br^-$ have been determined to be 18.8, 23.5, and 23.8 in dimethyl sulfoxide solution (*53*). Under conditions described in Materials and Methods, TeAdoMet did not degrade to adenine and *S*-ribosyltelluromethionine. By fitting the data obtained for adenine formation for AdoMet and SeAdoMet to eq 5, apparent pK_a values of ~11.5 and

~14.1, respectively, were determined for the C-5′ protons. This reaction is complex, and it is therefore difficult to rationalize these pK_a values at the microscopic level without suitable knowledge of the mechanism of adenine formation. Indeed, in a study of the hydrolysis of DMTA, Borchardt concluded that if a discrete sulfur ylide intermediate is formed, it partitions toward adenine formation much faster than it is reprotonated to give substrate. In fact, the reaction is thought to occur in a concerted fashion (*29*). The important message in the study presented herein is that it is more difficult to generate selenonium or telluronium ylides than sulfonium ylides.

In summary, we have synthesized the AdoMet onium chalcogen analogues, SeAdoMet and TeAdoMet, and found that SeAdoMet is a better alkylating agent than AdoMet but is deprotonated at the carbon adjacent to the chalcogen less efficiently. Moreover, TeAdoMet is resistant to both reactions. In the accompanying manuscript (*40*), we show that SeAdoMet is a better substrate than AdoMet or TeAdoMet in the reaction catalyzed by cyclopropane fatty acid synthase, allowing us to conclude that the reaction most likely does not proceed via ylide intermediates.

ACKNOWLEDGMENT

We thank Dr. George Markham of the Fox Chase Cancer Center (Philadelphia, PA) for the generous donation of *E. coli* strain DM22pK8, which overproduces AdoMet synthetase, as well as his informing us that TeAdoMet can be enzymatically synthesized using AdoMet synthetase. We thank Drs. Margaret A. Phillips and Jeffrey Baldwin of The University of Texas Southwestern Medical Center (Dallas, TX) for the generous donation of plasmid pSam18, which encodes human prostate AdoMet decarboxylase. We thank Dr. Nediljko Budisa for the generous donation of Ac-D,L-Met(Te)-OH.

SUPPORTING INFORMATION AVAILABLE

NMR spectra of AdoMet (Figure S1), SeAdoMet (Figure S2), and TeAdoMet (Figure S3). This information is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Markham, G. D. (2002) in *Encyclopedia of Life Sciences*, Macmillan Publishers Ltd.
2. Markham, G. D., Hafner, E. W., Tabor, C. W., and Tabor, H. (1980) S-Adenosylmethionine synthetase from *Escherichia coli*, *J. Biol. Chem. 255*, 9082−9092.
3. Grimshaw, J. (1981) in *The Chemistry of the Sulphonium Group* (Stirling, C. J. M., and Patai, S., Eds.) John Wiley & Sons Ltd, New York.
4. Knipe, A. C. (1981) in *The Chemistry of the Sulphonium Group* (Stirling, C. J. M., and Patai, S., Eds.) John Wiley & Sons Ltd, New York.
5. Takusagawa, F., Fujioka, M., Spies, A., and Schowen, R. L. (1998) *Comprehensive Biological Catalysis: A Mechanistic Reference*, Vol. 1, Academic Press, San Diego, CA.
6. Frey, P. A., and Booker, S. J. (2001) Radical mechanisms of S-adenosylmethionine-dependent enzymes, *Adv. Protein Chem. 58*, 1−45.
7. Markham, G. D., Norrby, P. O., and Bock, C. W. (2002) S-Adenosylmethionine conformations in solution and in protein complexes: conformational influences of the sulfonium group, *Biochemistry 41*, 7636−7646.
8. Iwata-Reuyl, D. (2003) Biosynthesis of the 7-deazaguanosine hypermodified nucleosides of transfer RNA, *Bioorg. Chem. 31*, 24−43.
9. Cantoni, G. L. (1975) Biological methylation: selected aspects, *Annu. Rev. Biochem. 44*, 435−451.
10. Cheng, X. (1995) Structure and function of DNA methyltransferases, *Annu. Rev. Biophys. Biomol. Struct. 24*, 293−318.
11. Bentley, R., and Chasteen, T. G. (2002) Microbial methylation of metalloids: arsenic, antimony, and bismuth, *Microbiol. Mol. Biol. Rev. 66*, 250−271.
12. Chasteen, T. G., and Bentley, R. (2003) Biomethylation of selenium and tellurium: microoganisms and plants, *Chem. Rev. 103*, 1−25.
13. Choi, S.-W., and Mason, J. B. (2000) Folate and carcinogenesis: an integrated scheme, *J. Nutr. 130*, 129−132.
14. Nau, F. (1976) The methylation of tRNA, *Biochimie 58*, 629−645.
15. Rice, J. C., and Allis, D. C. (2001) Histone methylation versus histone acetylation: new insights into epigenetic regulation, *Curr. Opin. Cell Biol. 13*, 263−273.
16. Miller, M. B., and Bassler, B. L. (2001) Quorum sensing in bacteria, *Annu. Rev. Microbiol. 55*, 165−199.
17. Tabor, C. W., and Tabor, H. (1984) Polyamines, *Annu. Rev. Biochem. 53*, 749−790.
18. Hackert, M. L., and Pegg, A. E. (1997) in *Comprehensive Biochemical Catalysis* (Sinnott, M., Ed.) pp 210−216, Academic Press, New York.
19. Yu, Y. B., Adams, D. O., and Yang, S. F. (1979) 1-Aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis, *Arch. Biochem. Biophys. 198*, 280−286.
20. Ramalingam, K., Lee, K. M., Woodard, R. W., Bleecker, A. B., and Kende, H. (1985) Stereochemical course of the reaction catalyzed by the pyridoxal phosphate-dependent enzyme 1-aminocyclopropane-1-carboxylate synthase, *Proc. Natl. Acad. Sci. U.S.A. 82*, 7820−7824.
21. O'Hagan, D., Schaffrath, C., Cobb, S. L., Hamilton, J. T. G., and Murphy, C. D. (2002) Biosynthesis of an organofluorine molecule, *Nature 416*, 279.
22. Slany, R. K., Bosl, M., and Kersten, H. (1994) Transfer and isomerization of the ribose moiety of AdoMet during the biosynthesis of queuosine tRNAs. A new unique reaction catalyzed by the QueA protein from *Escherichia coli*, *Biochimie 76*, 389−393.
23. Kinzie, S. D., Thern, B., and Iwata-Reuyl, D. (2000) Mechanistic studies of the tRNA-modifying enzyme QueA: a chemical imperative for the use of AdoMet as a "ribosyl" donor, *Org. Lett. 2*, 1307−1310.
24. Fontecave, M., Mulliez, E., and Ollagnier-de Choudens, S. (2001) Adenosylmethionine as a source of 5′-deoxyadenosyl radicals, *Curr. Opin. Chem. Biol. 5*, 506−511.
25. Cheek, J., and Broderick, J. B. (2001) Adenosylmethionine-dependent iron−sulfur enzymes: versatile clusters in a radical new role, *J. Biol. Inorg. Chem. 6*, 209−226.
26. Frey, P. A., and Magnusson, O. T. (2003) S-Adenosylmethionine: a wolf in sheep's clothing, or a rich man's adenosylcobalamin? *Chem. Rev. 103*, 2129−2148.
27. Sofia, H. J., Chen, G., Hetzler, B. G., Reyes-Spindola, J. F., and Miller, N. E. (2001) Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods, *Nucleic Acids Res. 29*, 1097−1106.
28. Parks, L. W., and Schlenk, F. (1958) The stability and hydrolysis of S-adenosylmethionine: isolation of S-ribosylmethionine, *J. Biol. Chem. 230*, 295−305.
29. Borchardt, R. T. (1979) Mechanism of alkaline hydrolysis of S-adenosyl-L-methionine and related sulfonium nucleosides, *J. Am. Chem. Soc. 101*, 458−463.
30. Parks, L. W., and Schlenk, F. (1958) Formation of α-amino-γ-butyrolactone from S-adenosylmethionine, *Arch. Biochem. Biophys. 75*, 291−292.
31. Wu, S.-E., Huskey, W. P., Borchardt, R. T., and Schowen, R. L. (1983) Chiral instability at sulfur of S-adenosylmethionine, *Biochemistry 22*, 2828−2832.
32. 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.
33. Hoffman, J. L. (1986) Chromatographic analysis of the chiral and covalent instability of S-adenosyl-L-methionine, *Biochemistry 25*, 4444−4449.
34. Boyle, S. M., Markham, G. D., Hafner, E. W., Wright, J. M., Tabor, H., and Tabor, C. W. (1984) Expression of the cloned genes encoding the putrescine biosynthetic enzymes and methionine

adenosyltransferase of *Escherichia coli* (*speA*, *speB*, *speC* and *metK*), *Gene 30*, 129−136.
35. Clyne, T., Kinch, L. N., and Phillips, M. A. (2002) Putrescine activation of *Trypanosoma cruzi* S-adenosylmethionine decarboxylase, *Biochemistry 41*, 13207−13216.
36. Park, J., Tai, J., Roessner, C. A., and Scott, A. I. (1996) Enzymatic synthesis of S-adenosyl-L-methionine on the preparative scale, *Bioorg. Med. Chem. 4*, 2179−2185.
37. Karnbrock, W., Weyher, E., Budisa, N., Huber, R., and Moroder, L. (1996) A new efficient synthesis of acetyltelluro- and acetylselenomethionine and their use in the biosynthesis of heavy-atom protein analogues, *J. Am. Chem. Soc. 118*, 913−914.
38. Hope, E. G., Kemmitt, T., and Levason, W. (1988) Synthesis, properties, and multinuclear NMR (^{125}Te {^{1}H}, ^{13}C {^{1}H}, ^{1}H) studies of ditelluroether and polytelluroether ligands, *Organometallics 7*, 78−83.
39. Herrmann, W. A. (1997) in *Sulfur, Selenium, and Tellurium* (Herrmann, W. A., and Zybill, C. E., Eds.) pp 234, Thieme Medical Publishers, Inc., New York.
40. Iwig, D. F., and Booker, S. J. (2004) Isotope and elemental effects indicate a rate-limiting methyl transfer as the initial step in the reaction catalyzed by *Escherichia coli* cyclopropane fatty acid synthase, *Biochemistry 43*, 13510−13524.
41. Gnegy, M. E., and Lotspeich, F. J. (1976) Inhibitors of transfer RNA methyl transferases S-adenosyl sulfonium salts, *J. Med. Chem. 19*, 1191−1195.
42. Ellis, K. J., and Morrison, J. F. (1982) Buffers of constant ionic strength for studying pH dependent processes, in *Methods in Enzymology, Vol. 87. Enzyme Kinetics and Mechanism: Part C: Intermediates, Stereochemistry, and Rate Studies.* (Purich, D. L., Ed.) pp 405−426, Academic Press, Inc.: New York, London.
43. Dawson, R. M. C., Elliott, D. C., Elliott, W. H., and Jones, K. M. (1989) *Data for Biochemical Research*, Oxford University Press, New York.
44. Leatherbarrow, R. J. (2001) *GraFit Version 5*, Erithacus Software Ltd., Horley, U.K.
45. Voet, D., and Voet, J. G. (2004) *Biochemistry*, Vol. 1, 3rd ed., John Wiley & Sons, Ltd., New York.
46. Baddiley, J., Cantoni, G. L., and Jamieson, G. A. (1953) Structural observations on "active methionine", *J. Chem. Soc.*, 2662−2664.
47. Levin, C. C. (1975) A qualitative molecular orbital picture of electronegativity effects on XH_3 inversion barriers, *J. Am. Chem. Soc. 97*, 5649−5655.
48. Shimizu, T., Matsuhisa, A., Kamigata, N., and Ikuta, S. (1995) Activation energies for pyramidal inversion of sulfonium, selenonium, and telluronium ylides based on ab initio MO calculations, *J. Chem. Soc., Perkin Trans. 2*, 1805−1808.
49. Stoner, G. L., and Eisenberg, M. A. (1975) Purification and properties of 7,8-diaminopleargonic acid aminotransferase: an enzyme in the biotin biosynthetic pathway, *J. Biol. Chem. 250*, 4029−4036.
50. Holtzclaw, H. F., Robinson, W. R., and Nebergall, W. H. (1984) *General Chemistry*, 7th ed., D. C. Heath and Company, Lexington, MA.
51. Wade, L. G., Jr. (1995) *Organic Chemistry*, 3rd ed., Prentice Hall, Englewood Cliffs, NJ.
52. Crosby, J., and Stirling, C. J. M. (1970) Elimination and addition reactions. Part XIX. Elimination of phenoxide from β-substituted ethyl phenyl ethers: the nature of activation in 1,2-elimination, *J. Chem. Soc. B*, 671−679.
53. Cheng, J.-P., Liu, B., Zhao, Y., Sun, Y., Zhang, X.-M., and Lu, Y. (1999) Effects of adjacent onium cations and remote substituents on the H−A+ bond equilibrium acidities in dimethyl sulfoxide solution. An extensive ylide thermodynamic stability scale and implication for the importance of resonance effect on ylide stabilities, *J. Org. Chem. 64*, 604−610.

BI048693+