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Preparation of the Pure Diastereomeric Forms of *S*-(5'-Deoxy-5'-adenosyl)-1-ammonio-4-methylsulfonio-2-cyclopentene and Their Evaluation as Irreversible Inhibitors of *S*-Adenosylmethionine Decarboxylase from *Escherichia coli*[1]

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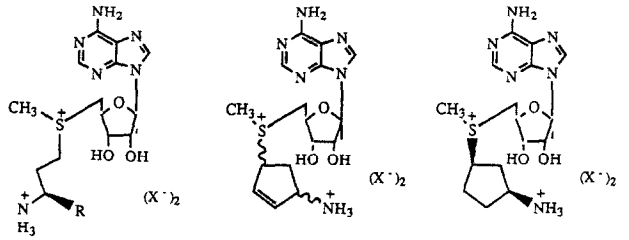
Abstract—The conformationally restricted *S*-adenosylmethionine analogue AdoMac {*S*-(5'-deoxy-5'-adenosyl)-1-ammonio-4-methylsulfonio-2-cyclopentene} has been shown to act as an enzyme activated, irreversible inhibitor of the *Escherichia coli* form of the enzyme *S*-adenosylmethionine decarboxylase. Inactivation of the enzyme is presumably initiated by formation of an imine linkage between the inhibitor and the terminal pyruvate of the enzyme, followed by base-catalyzed elimination of methylthioadenosine and generation of a latent electrophile. Removal of the driving force for the elimination of methylthioadenosine resulted in a reversibly binding inhibitor. Thus, the thioether analogue corresponding to AdoMac, and the corresponding dihydro derivative (H_2-AdoMac), reversibly inhibit the enzyme. AdoMac was resolved into its four pure diastereomeric forms, and each diastereomer was evaluated as an irreversible inhibitor of the enzyme. The K_I values for the individual diastereomers range between 3.83 and 39.6 μM, with the *cis*-1*S*,4*R* diastereomer being the most potent inhibitor. However, the k_{inact} values for the four diastereomers are not significantly different, suggesting that the binding of each diastereomer to the enzyme is configuration-dependent, while the subsequent inactivation likely proceeds through a single intermediate which is formed from each of the four diastereomers. Since each pure diastereomer represents a distinct conformational mimic exhibiting restricted sidechain rotation, the data suggests that these and related analogues may be useful as conformational probes for the catalytic site of AdoMet-DC.

Introduction

The enzyme *S*-adenosylmethionine decarboxylase (AdoMet-DC) is a controlling enzyme in the biosynthesis of cellular polyamines,[2] and as such, represents an important target for pharmacological manipulation of the polyamine biosynthetic pathway.[3] Agents which inhibit AdoMet-DC have the potential to affect a number of diverse biochemical events within the cell, and have been developed as potential antitumor or antiparasitic agents.[3,4] The catalytic subunit of AdoMet-DC from both bacterial and mammalian sources contains a covalently bound pyruvate cofactor at the amino terminus.[5-7] The natural substrate for the enzyme, *S*-adenosylmethionine (AdoMet, **1**, Figure 1), must form an imine linkage with this pyruvate prior to its enzyme-assisted conversion to decarboxylated *S*-adenosylmethionine (dc-AdoMet, **2**, Figure 1), a committed intermediate in the biosynthesis of polyamines. Although the amino acid sequence for the *Escherichia coli* and human forms of the enzyme are known, and specific residues have been identified which may participate in the function of the enzyme,[8-13] no information is available pertaining to the three dimensional structure of the enzyme. In addition, aside from the identification of an N-terminal, pyruvate-containing peptide segment using the AdoMet-DC inhibitor MHZPA,[8] little has been learned about specific amino acid residues which may be present in the catalytic site. Information of this type would prove extremely valuable for the rational design of potent inactivators for AdoMet-DC. To date, a number of potent inhibitors for AdoMet-DC have been synthesized,[13] and some of these agents have been shown to inactivate the enzyme following interaction with the terminal pyruvate moiety.[8,14] To date, the most successful nucleoside-based inhibitor of AdoMet-DC has been 5'-{[(*Z*)-4-amino-2-butenyl]methylamino}-5'-deoxyadenosine (AbeAdo), which serves as an enzyme activated, irreversible inactivator of the enzyme, with a K_I of 0.3 μM and a k_{inact} value of 3.6 min^{-1}.[15] AbeAdo was designed to unmask a latent electrophile in the catalytic site of AdoMet-DC following formation of an imine linkage with the terminal pyruvate cofactor. Presumably, generation of this electrophile would lead to alkylation of specific nucleophilic amino acids, and thus provide data pertaining to the structure of the catalytic site. However, it has recently been demonstrated that AbeAdo inactivates human AdoMet-DC by an unexpected mechanism which leads to transamination of the terminal pyruvate[8] rather than by covalent binding of an electrophile in the active site. The precise mechanism for inactivation of bacterial AdoMet-DC by AbeAdo has not been determined.

It is of interest to note that the Z-isomer of AbeAdo is an extremely potent inhibitor, while the *E*-isomer shows little activity as an inactivator of the *Escherichia coli* form of AdoMet-DC,[15] suggesting that it may be possible to gain information about the catalytic site of the enzyme using specific configurational probes. Our laboratory has recently

reported the synthesis of *S*-(5'-deoxy-5'-adenosyl)-1-ammonio-4-methylsulfonio-2-cyclopentene (AdoMac, **3**, Figure 1), a conformationally restricted analogue of AdoMet, as a mixture of two diastereoisomers having 1*R*,4*R*- and 1*R*,4*S*-configurations at the stereocenters within the cyclopentene ring.[16] In this form, AdoMac is a potent enzyme activated inhibitor of AdoMet-DC isolated from *Escherichia coli,* exhibiting a K_I of 18.3 μM, and a k_{inact} value of 0.133 min^{-1}. Inactivation of the enzyme by AdoMac is also thought to be initiated by formation of an imine linkage between the inhibitor and the terminal pyruvate of the enzyme, followed by base-catalyzed elimination of methylthioadenosine (MTA) and generation of a latent electrophile, as shown in Scheme I. Addition of a nucleophilic amino acid residue to this electrophile by 1,4- or 1,6-addition could then be envisioned within the catalytic site, resulting in irreversible inactivation of the enzyme. We now report the stereospecific synthesis of all four of the diastereomeric forms of AdoMac via a versatile chemoenzymatic pathway, the synthesis of the ring saturated version of 1*R*,4*S*-AdoMac (1*S*,4*R*-H_2-AdoMac, **4**, Figure 1) for use as a control, and the evaluation of each of the above analogues as inhibitors of AdoMet-DC from *Escherichia coli.*



1 R = COOH
2 R = H

3
AdoMac

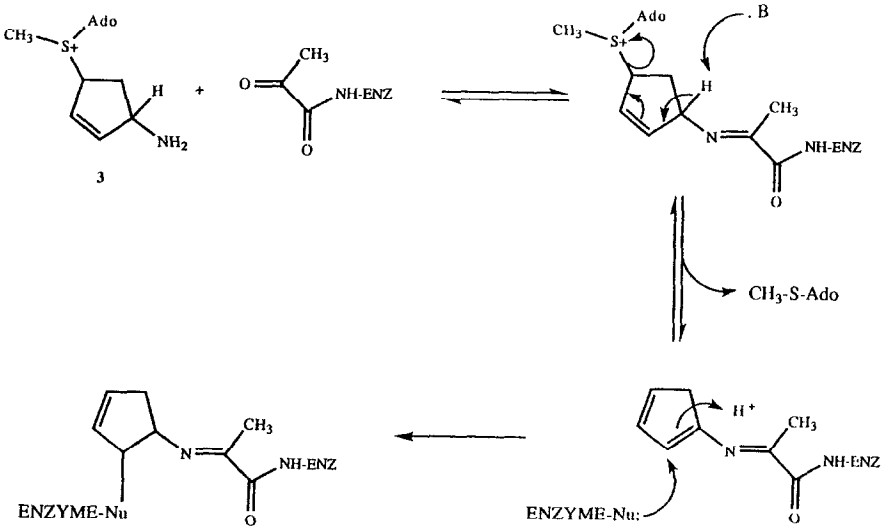
1S,4R-4
H_2-AdoMac

Figure 1. Structures for AdoMet, dc-AdoMet, AdoMac and AdoMac-H_2

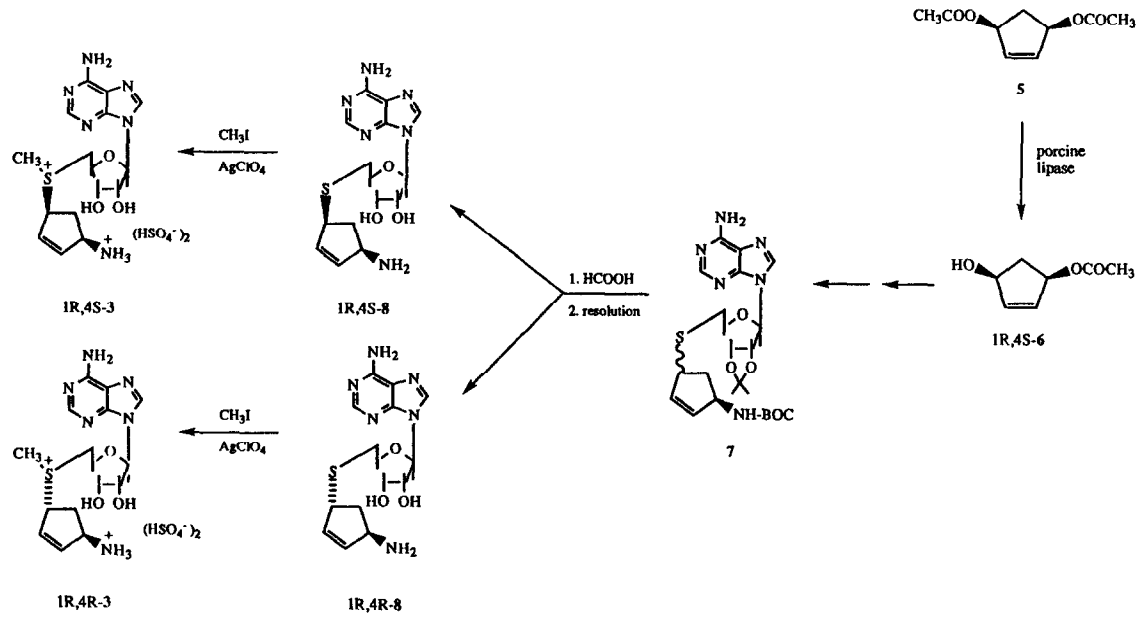
Chemistry

The synthetic route leading to the pure 1*R*,4*R*- and 1*R*,4*S*-diastereomers of AdoMac (**3**) is outlined in Scheme II. The experimental details for the synthesis of **3** as a mixture of diastereomers have previously been described.[16] The *meso*-diacetate **5** was subjected to porcine lipase mediated hydrolysis of the pro-(*S*)-acetyl group[17] to afford 1*R*,4*S*-**6** in 97% e.e. Elaboration of intermediate **6** as previously reported[16] then yielded the fully protected intermediate **7**, which was treated with 88% formic acid to afford thioether **8** as a mixture of the 1*R*,4*R*- and 1*R*,4*S*-diastereomers. At this stage, the mixture of diastereomers was resolved by careful flash chromatography on silica gel (2 x 40 cm column length, $CHCl_3$:methanol:NH_4OH 14:14:1) to afford the 1*R*,4*R*- and 1*R*,4*S*-diastereomers of **8** in pure form. Each pure diastereomer of **8** was then individually methylated using a modification of a literature procedure[18] to provide the desired 1*R*,4*R*- and 1*R*,4*S*-**3**, isolated as the sulfate salts following recrystallization.

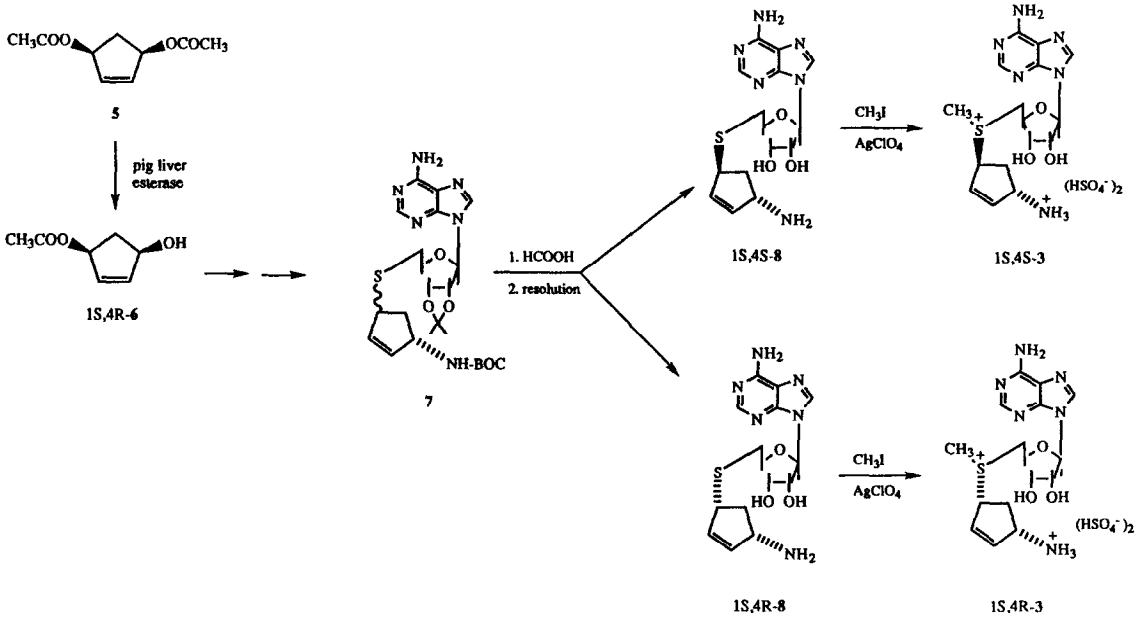
The 1*S*,4*R*- and 1*S*,4*S*-diastereomers of **3** can be accessed in similar fashion using the synthetic pathway shown in Scheme III. Stereospecific hydrolysis of the pro-(*R*)-acetate of **5** using pig liver esterase[19] afforded 1*S*,4*R*-**6** (94% e.e.), which was elaborated as described above to afford **7** as a mixture of the 1*S*,4*R*- and 1*S*,4*S*-diastereomers. The mixture was deprotected and then resolved by careful flash chromatography as described above to yield the pure 1*S*,4*R*- and 1*S*,4*S*-diastereomers of **8**, which were individually methylated to provide the desired 1*S*,4*R*- and 1*S*,4*S*-**3** in diastereomerically pure form, isolated as the sulfate salts following recrystallization. Thus, all four of the possible diastereomers of **3** could be accessed in pure form using a common synthetic scheme.



Scheme I.



Scheme II.



Scheme III.

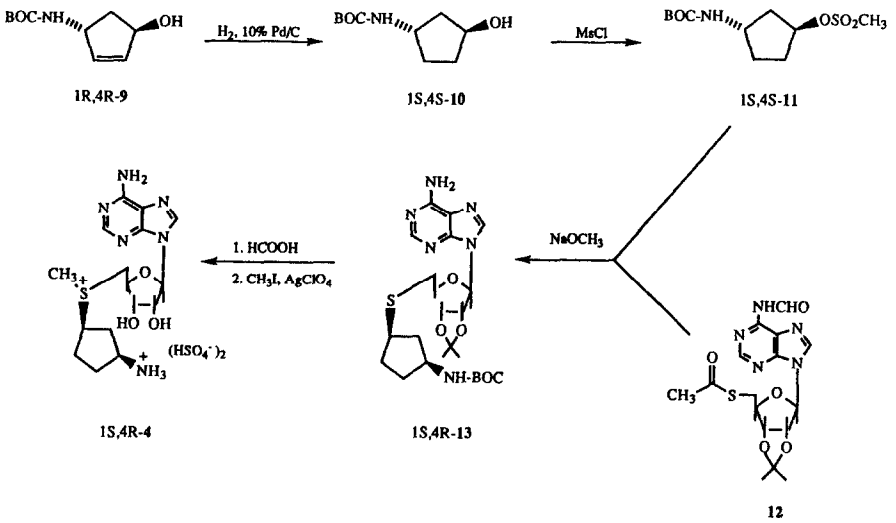
In each case, the diastereomeric excess was determined to be >99% by ^{1}H-NMR spectroscopy, and the absolute stereochemistry of each diastereomer of **3** was assured by comparing the chemical shifts of the methylene protons at C-5 of the cyclopentene ring with the shift of the corresponding protons in known analogues **6** (*cis* orientation) and **9**[16] (*trans* orientation). The results of these studies are summarized in Table 1. For analogues in which the groups at positions 1 and 4 of the cyclopentene ring were in the *cis* configuration, the C-5 proton *cis* to these substituents (H_a) appeared farther downfield than in the corresponding *trans* analogue, and the $\Delta\delta$ value between H_a and H_b was large (between 0.5 and 1.2 ppm). In analogues in which the substituents at positions 1 and 4 of the cyclopentene ring were *trans*, the resonances for H_a were shifted upfield by 0.5–0.7 ppm, and the $\Delta\delta$ value between H_a and H_b was smaller 0.1–0.24 ppm) or undetectable. Thus, the assignment of *cis* and *trans* geometry, as well as the determination of diastereomeric purity, could be accomplished by observing the chemical shift of the C-5 methylene protons. A diastereomeric excess of >99% for each pure diastereomer was further verified by HPLC analysis (Waters C-18 Novapak, 0.8 x 10 cm, 4 μ) according to the reversed-phase ion pairing assay procedure of Wagner.[20]

The synthesis of the saturated analogue H_2-AdoMac, compound **4**, was accomplished starting with derivative *1R,4R*-**9**,[16] as shown in Scheme IV. A portion of *trans*-*1R,4R*-**9** (derived from porcine lipase-mediated hydrolysis of **5**) was hydrogenated (H_2, 10% Pd/C, 50 p.s.i.) to afford *trans*-*1S*, 4*S*-1-hydroxy-4-(*tert*-butyloxycarbonyl) amino-cyclopentane **10**. This derivative was then mesylated[21] to give electrophile **11**, which was coupled to 2',3'-isopropylidene-5'-thioacetyladenosine **12**[22,23] as previously described[16] to afford *cis*-*1S,4R*-{*S*-[5'-deoxy-5'-(2',3'-isopropylideneadenosyl)]}-1-(*tert*-butyloxycarbonyl)amino-4-thiocyclopentane **13** as a single pure diastereomer. Removal of the protecting groups and methylation as described above then afforded the desired *1S,4R*-**4**, H_2-AdoMet, isolated as the sulfate salt following recrystallization. The diastereomeric excess for the *1S,4R*-diastereomer of **4** was found to be >99% by NMR analysis, and by analysis using reversed-phase HPLC,[20] as described above.

Table 1. Chemical shift data for stereochemical characterization of compounds **3**, **6**, **8** and **9**

Compound	H_a (ppm)	H_b (ppm)	$H_a + H_b$	$\Delta\delta$ (ppm)
cis-1R,4S-**6**	2.85	1.65	—	1.2
cis-1S,4R-**6**	2.85	1.65	—	1.2
trans-1R,4R-**9**	2.18	1.94	—	0.24
trans-1S,4S-**9**	2.18	1.94	—	0.24
cis-1R,4S-**8**	2.96	2.37	—	0.59
cis-1S,4R-**8**	2.77	2.32	—	0.45
trans-1R,4R-**8**	2.28	2.04	—	0.24
trans-1S,4S-**8**	2.28	2.18	—	0.10
cis-1R,4S-**3**	2.95	2.40	—	0.55
cis-1S,4R-**3**	2.95	2.39	—	0.56
trans-1R,4R-**3**	—	—	2.55-2.45	—
trans-1S,4S-**3**	—	—	2.60-2.40	—

H_a is defined as the methylene proton at C-5 of the cyclopentene ring which is *cis* to the hydroxyl (compounds **6** and **9**) or amino moiety (compounds **3** and **8**). H_b is the geminal proton at position 5 which is *trans* to these residues. All chemical shifts listed are in ppm referenced to TMS.



Scheme IV.

Results

The irreversible inhibitor AdoMac, **3**, was originally evaluated against the Mg^{2+}-activated form of AdoMet-DC from *Escherichia coli* as a mixture of the *cis*-1*R*,4*S*- and *trans*-1*R*,4*R*-diastereomers. Under these conditions, AdoMac exhibited a K_I of 18.3 μM, and a k_{inact} of 0.133 min^{-1}.[16] In order to assess the configurational dependence of the binding of AdoMac to AdoMet-DC and of the subsequent inactivation process, the pure diastereomers were individually evaluated as inhibitors of the *Escherichia coli* form of AdoMet-DC. The rate of inactivation of AdoMet-DC was followed at four different concentrations of the appropriate diastereomer over a period of 20 min. The resulting inactivation of the enzyme by each diastereomer was both time- and concentration-dependent, as shown in Figure 2. In each case, no inhibition was observed when Mg^{2+} was omitted from the reaction buffer (data not shown). Analysis of the inhibition data according to the method of Kitz and Wilson[24] allowed the derivation of K_I and k_{inact} values for each of the four possible diastereomers of AdoMac, as summarized in Figure 3 and Table 2. The K_I values observed, which range between 3.83 and 39.60 μM, demonstrate that the binding of AdoMac to AdoMet-DC is significantly configuration dependent. However, the k_{inact} values for the four diastereomers are remarkably constant, varying only between 0.064 and 0.099 min^{-1}.

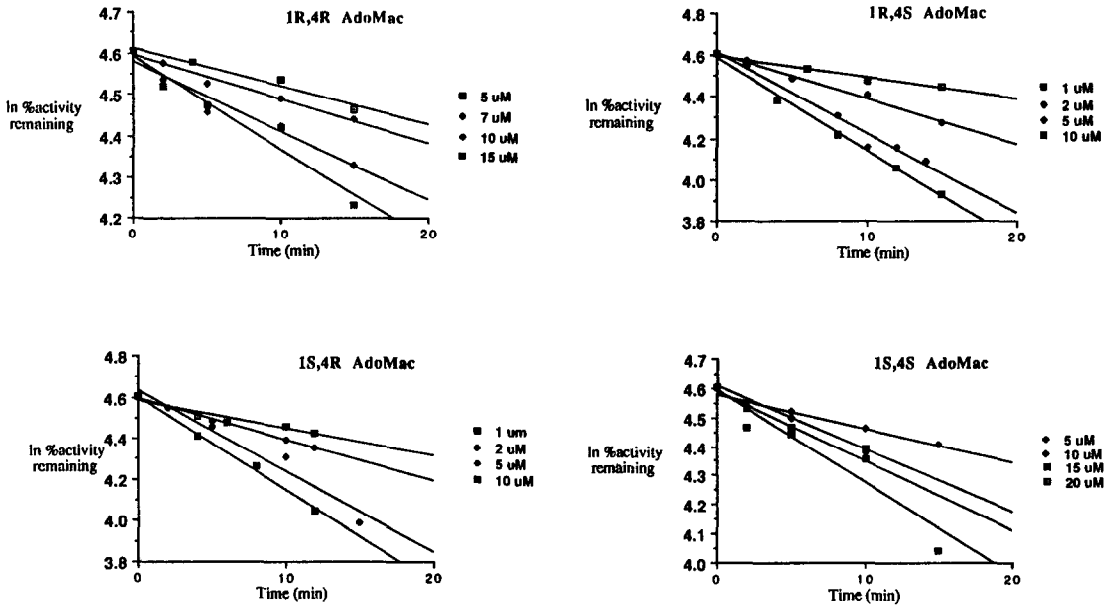


Figure 2. Time inactivation studies for the pure diastereomers of AdoMac. Time-dependent inactivation of AdoMet-DC is monitored as described in the Methods section at a series of four concentrations as shown on each graph. Each data point is the average of two determinations which in each case differ by less than 5%

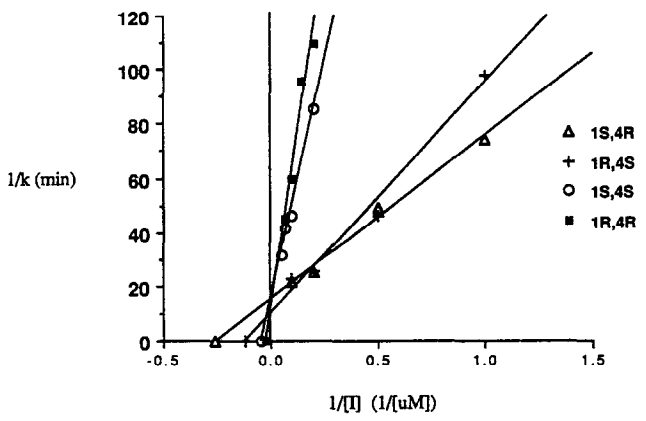


Figure 3. Determination of K_I and k_{inact} values for the individual pure diastereomers of AdoMac. Rate constants derived from the time inactivation studies shown in Figure 2 are replotted according to the method of Kitz and Wilson, and kinetic constants are derived from the equation for the resulting straight line

Table 2. Results of inhibition studies for restricted rotation AdoMet analogs

Compound	K_I (μM)	k_{inact} (min^{-1})	MTA generation	Time dependence
cis-1R,4S + *trans*-1R,4R AdoMac, **3**	18.3	0.133	yes	yes
cis-1R,4S-AdoMac, **3**	8.46	0.099	yes	yes
trans-1R,4R-AdoMac, **3**	39.60	0.079	yes	yes
cis-1S,4R-AdoMac, **3**	3.83	0.064	yes	yes
trans-1S,4S-AdoMac, **3**	23.81	0.068	yes	yes
cis-1R,4S-**14**	293.0	-------	no	no
cis-1S,4R-AdoMac-H_2, **4**	93.0	-------	no	no

As was the case with the diastereomeric mixture of AdoMac, AdoMet-DC was protected from inactivation by the pure diastereomers of AdoMac when the enzyme had been preincubated with the known competitive inhibitor MGBG. In addition, the inhibition produced by each diastereomer was irreversible, as demonstrated by the inability to dialyze away the inhibitor following binding to AdoMet-DC. AdoMet-DC was incubated with 50 μM of the appropriate diastereomer of **3** for 30 min, and then assayed (average of 22% activity remaining). The sample was then dialyzed overnight (62.5 mM Tris-HCl/100 mM $MgSO_4$, pH 7.4, 4 buffer changes) and reassayed. There was no significant change in the activity level of AdoMet-DC following dialysis, suggesting that the inhibition produced by each diastereomer of AdoMac was irreversible. When subjected to the same dialysis conditions in the absence of inhibitor, purified AdoMet-DC retained greater than 95% of its pre-treatment level of activity

HPLC product analysis of the enzymatic reaction mixture for each diastereomer of AdoMac was undertaken to determine whether the expected by-product, MTA, was generated during inactivation of the enzyme. These experiments revealed the time-dependent appearance of a peak which co-eluted with MTA (retention time = 17.08 min), and the corresponding disappearance of the peak co-eluting with AdoMac, suggesting that MTA was generated from AdoMac in the enzymatic reaction as predicted. No other metabolites related to AdoMet could be detected under the assay conditions. In order to rule out the possibility of non-enzymatic generation of MTA from AdoMac under the assay conditions described, control experiments were carried out in the absence of AdoMet-DC, and in the presence of AdoMet-DC which had been inactivated by boiling at 100 °C for 5 min. Under these conditions, no generation of MTA or any other metabolite of **3** was observed during the 20 min assay period.

In theory, the non-methylated precursor to AdoMac, **8**, and the saturated version of AdoMac, H_2-AdoMac (**4**), should also form an imine linkage with the terminal pyruvate of AdoMet-DC. However, neither of these analogues possesses the driving force for elimination of MTA and formation of a latent electrophile within the catalytic site. Thus, it was reasoned that these analogues would, at best, act as competitive inhibitors of the enzyme, and would thereby serve as suitable control compounds with respect to AdoMac. To test this hypothesis, compounds *cis*-1*R*,4*S*-**8** and *cis*-1*S*,4*R*-**4**, each of which possess the same absolute stereochemistry as the potent inhibitor *cis*-1*R*,4*S*-AdoMac, were evaluated for inhibitory activity against AdoMet-DC, as described above. The magnitude of reversible AdoMet-DC inhibition was determined at various concentrations of inhibitor and at two different concentrations of AdoMet (typically, 20.0 and 40.0 μM). The resulting data was analyzed using a Dixon plot to determine the apparent K_I value of each inhibitor. As expected, compounds *cis*-1*R*,4*S*-**8** and *cis*-1*S*,4*R*-**4** act as weak competitive inhibitors of AdoMet-DC, with K_I values of 293.0 and 93.0 μM, respectively, as shown in Figures 4 and 5, and in Table 2. In addition, the inhibition observed in the presence of *cis*-1*R*,4*S*-**8** and *cis*-1*S*,4*R*-**4** was not time-dependent, and no generation of MTA or any other metabolite related to **4** was detected in the enzymatic reaction mixture following exposure to these analogues.

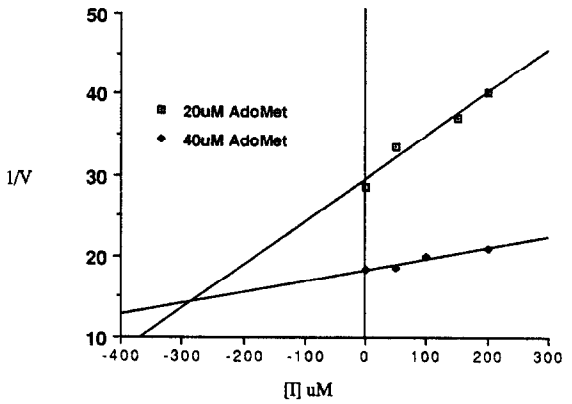


Figure 4. Dixon plot analysis of inhibition of AdoMet-DC by 1*R*,4*S*-**14** in the presence of either 20 or 40 μM concentrations of the substrate, AdoMet

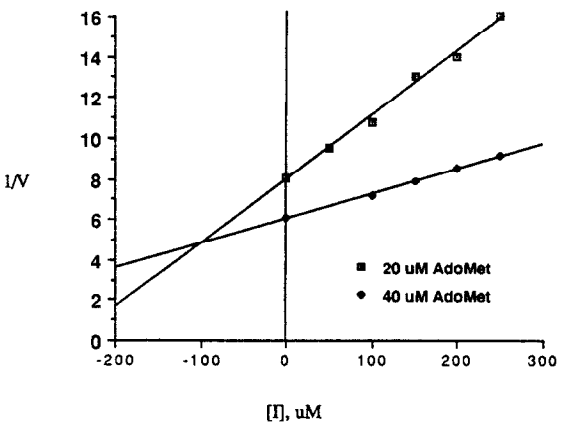


Figure 5. Dixon plot analysis of inhibition of AdoMet-DC by 1*S*,4*R*-AdoMac-H_2, **4**, in the presence of either 20 or 40 μM concentrations of the substrate, AdoMet

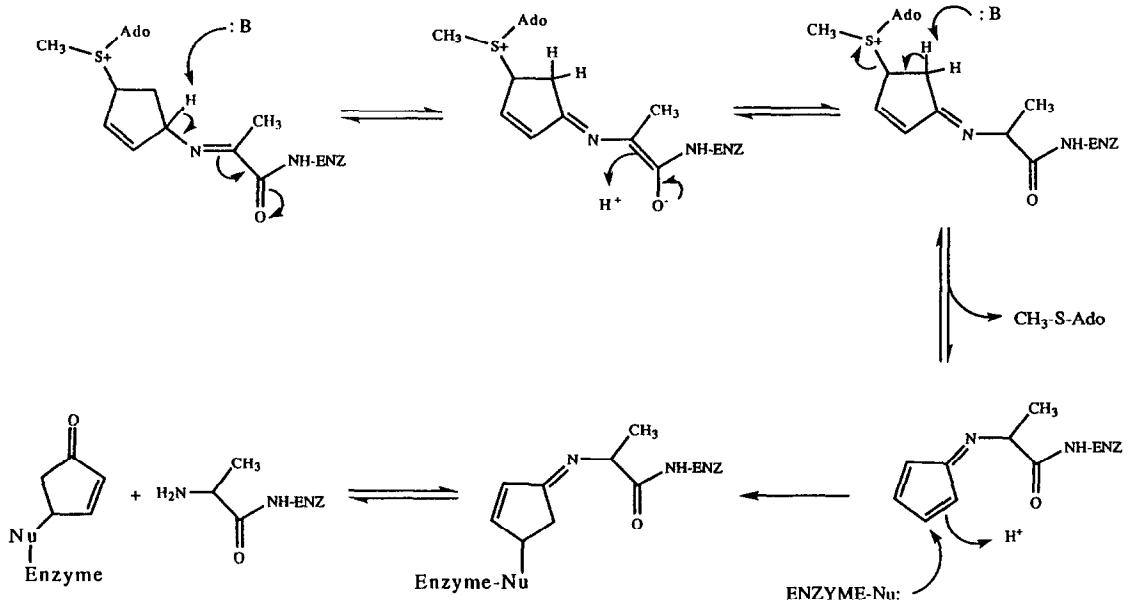
Discussion

Based on the data presented above, it is reasonable to assume that AdoMac inhibits AdoMet-DC from *Escherichia coli* by general base-catalyzed deprotonation of the resulting imino adduct and generation of a latent electrophile, as shown in Scheme I. The observed generation of MTA during inactivation of AdoMet-DC by each diastereomer of AdoMac strongly supports this hypothesis. However, a second mechanism for the generation of MTA can be envisioned. It has recently been demonstrated that AdoMet-DC from *Escherichia coli* undergoes a substrate-dependent transamination/inactivation which involves incorrect protonation of an intermediate enolate following the decarboxylation step.[9] During this process, which occurs once every 6000–7000 turnovers,

the enzyme generates MTA by a β-elimination process which results in the formation of an acrolein-like species. This species has been shown to alkylate cysteine-140 of bacterial AdoMet-DC, which lies in the only region which is conserved between the mammalian and bacterial forms of the enzyme,[10,11] and is probably involved in the catalytic mechanism of AdoMet-DC. Pegg has postulated that inactivation of the human form of AdoMet-DC by the inhibitor AbeAdo may proceed via a similar mechanism,[8] resulting in transamination of the terminal pyruvate. During this process, however, no reactive species was formed within the catalytic site, since no alkylated amino acid residues were detected. In addition, this inactivation may occur without concomitant formation of the by-product 5'-deoxy-5'-methylaminoadenosine, an observation which is consistent with a recent study involving rat liver AdoMet-DC.[25] In the case of AdoMac, inactivation of *Escherichia coli* AdoMet-DC could conceivably proceed by transamination of the pyruvate cofactor, but such a mechanism must also account for the observed production of MTA. Therefore, the mechanism outlined in Scheme V has been postulated. Formation and protonation of the enolate as shown would result in activation of the C-5 methylene protons α to the exocyclic imine functionality. A second base-catalyzed deprotonation could then be envisioned, resulting in β-elimination of MTA by a mechanism similar to substrate-dependent inactivation.[9] However, the resulting enzyme-bound species would be of sufficient reactivity to alkylate a nucleophilic residue prior to hydrolysis of the resulting enamine and diffusion from the catalytic site. Alkylation followed by hydrolysis of the imine linkage would thus result in an enzyme species which has been both transaminated and alkylated. If this proves to be the case, AdoMac may be of value in the identification of specific amino acid residues, other than those adjacent to the terminal pyruvate, which are present in the catalytic site. In order to discriminate between the mechanisms described in Scheme I and Scheme V (or any other potential mechanism of inactivation), and to identify active site amino acid residues, it will be necessary to determine the structure of the adduct formed between AdoMac and AdoMet-DC. These experiments are currently being conducted.

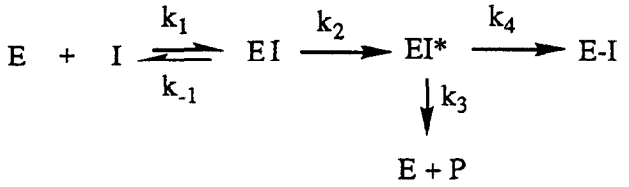
It now appears plausible that AbeAdo may inactivate the rat liver and *Escherichia coli* forms of AdoMet-DC by different mechanisms, since the by-product methylaminoadenosine (MAA) is detected only following inactivation of the bacterial enzyme. In contrast, our preliminary studies show that the generation of MTA following inactivation of AdoMet-DC by AdoMac can be detected using both the bacterial and human forms of the enzyme (unpublished observations). In terms of the inactivation of AdoMet-DC, the implications of this observation are as yet unknown, especially in light of the dramatically different amino acid composition of the *Escherichia coli* and human forms of the enzyme.[10]

Removal of the driving force for the elimination of MTA (as in compounds *cis*-1*R*,4*S*-**8** and *cis*-1*S*,4*R*-**4**) results in compounds which cause a weak competitive inhibition of AdoMet-DC. This data lends support to the mechanisms proposed in Schemes I and V, since elimination of the ability to generate MTA appears to preclude rapid irreversible inactivation. Following formation of the imine linkage, neither compound **8** or compound **4** could undergo a deprotonation/elimination sequence as shown in Scheme I, since the appropriate electron sink is not present. However, compound **4** could potentially inactivate AdoMet-DC by the mechanism shown in Scheme V, although the rate of inactivation would likely be similar to that of substrate-dependent inactivation (1 inactivation every 6000–7000 interactions)[9] and the resulting electrophile would be less reactive than the electrophile formed from **3**.



Scheme V.

The data presented demonstrates that the binding of AdoMac to AdoMet-DC is strongly dependent on configuration. The diastereomers in which the methylsulfonium and amino moieties are *cis* with respect to the cyclopentene ring are roughly 3 to 10-fold more potent than the corresponding *trans* oriented diastereomers. In both the *cis* and *trans* diastereomeric pairs, the isomer in which the amino group is in the *S*-configuration is the more potent isomer. Although the K_I values for the pure diastereomers of AdoMac suggest that association of the inhibitor to the enzyme is configuration dependent, the similarity in the observed k_{inact} values suggests the possibility that the subsequent irreversible inactivation steps may not be influenced by the configuration of the inhibitor. It has been suggested that K_I is reflective of the reversible association of inhibitor to an enzyme, while the k_{inact} value is a complex combination of three rate constants,[26] as shown in the equation below. Once the inhibitor binds to the enzyme (k_1), an



activated species EI* is formed (k_2). This intermediate may then be converted to a false product P, resulting in regeneration of the enzyme (k_3), or the enzyme may be irreversibly inactivated (k_4). Following reversible formation of the imine linkage (k_1), each pure diastereomer of AdoMac appears to inactivate AdoMet-DC at the same rate, suggesting that this inactivation proceeds rapidly, and possibly through a common intermediate or set of intermediates. Thus, the configuration-dependent formation of the Schiff's base between AdoMet-DC and a given diastereomer of AdoMac may result in the observed differences in K_I. A more complete kinetic study of the inactivation of AdoMet-DC by AdoMac is now being conducted to confirm this hypothesis.

Based on the above observations, we feel that the pure diastereomeric forms of AdoMac, as well as those of related analogues, will become useful as conformational probes for the active site of AdoMet-DC. Because of the inherent restricted rotation of the cyclopentenyl "sidechain" portion of the pure diastereomers of AdoMac, each configurational isomer may also be viewed as a distinct conformational mimic. Since these mimics exhibit configuration-dependent K_I values, it should be possible to determine an optimum AdoMet sidechain orientation by developing a series of analogues which vary in both configuration and ring size. Because the precise shape and amino acid composition of the AdoMet-DC active site remains to be fully elucidated, such probes could prove useful in the development of an active site template for the design of additional potent and specific inhibitors for the enzyme. In addition, this approach may be used to discover and exploit the differences in catalytic site shape and composition between the human and bacterial forms of the enzyme. The synthesis and characterization of additional restricted rotation analogues to be used for this purpose are ongoing concerns in our laboratories.

Experimental Section

The compounds *cis*-2-cyclopentene-1,4-diol and *cis*-1,4-diacetoxy-2-cyclopentene **5** were synthesized according to the procedure of Kaneko[27] as modified by Johnson.[28] The *cis*-(+)- 1*R*,4*S*-and *cis*-(–)-1*S*,4*R* enantiomers of compound **6** were synthesized from *cis*-1,4-diacetoxy-2-cyclopentene **5** by modifications of the procedures for enantioselective porcine lipase mediated hydrolysis of the pro-(*S*)-acetate,[17] or enantioselective pig liver esterase mediated hydrolysis of the pro-(*R*)-acetate,[19] respectively. In each case, the desired enantiomer of **6** was determined to be present in 94–97% e.e. by optical rotation, and by NMR analysis of the corresponding Mosher ester.[29] Compounds 1*R*,4*R*-**9** {$[\alpha]^{20}$ +182.1° (c 10.0, $CHCl_3$ + 1% EtOH)} and 1*S*,4*S*-**9** {$[\alpha]^{20}$ –179.2° (c 10.0, $CHCl_3$ + 1% EtOH)} were synthesized from the appropriate enantiomer of **6** as previously described.[16] The previously unreported 1*S*,4*S*-enantiomer of **9** was found to be present in >99% e.e. by comparison of its optical rotation to that of enantiomerically pure 1*R*,4*R*-**9**,[16] and by NMR analysis of the corresponding Mosher ester.[29] *Escherichia coli* (type B, unwashed, enriched, 3/4 log phase) was purchased from Grain Processing Corp., Muscatine, IA. Porcine lipase (Type II, EC 3.1.1.3) and pig liver esterase (EC 3.1.1.1) were purchased from Sigma Chemical Co. (St Louis, MO). Reagents for the determination of protein using the Bradford method[30] were purchased from BioRad Corp., Rockville Center, NY. All other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St Louis, MO) and were used without further purification except as previously described.[16] Spectral and analytical characterization procedures were conducted as previously reported.[16]

1R,4S-cis-*1-Acetoxy-4-hydroxy-2-cyclopentene (6)*

The diacetate **5** (1.53 g, 0.0083 mmol) was suspended in 100 ml of 0.6 M phosphate buffer (pH = 7.5) containing 0.523 g of porcine lipase (Type II, EC 3.1.1.3). The reaction mixture was stirred at room temperature for 55 h, then poured into a 250 mL separation funnel and extracted with three 50 mL portions each of ether and then ethyl acetate. The combined organic layers were washed with 100 mL of saturated NaCl and then dried over $MgSO_4$. Filtration and removal of the solvent *in vacuo* afforded a colorless amorphous solid, which was recrystallized (pentane:ether 1:1) to give 1.02 g (90%) of 1*R*,4*S*-**6** as white needles, m.p. 46–49 °C. The desired 1*R*,4*S*-enantiomer of **6** was determined to be present in 97% e.e. by NMR analysis of the corresponding Mosher ester (see below): {$[\alpha]^{20}_D$ +54.0° (c 10.0, $CHCl_3$ + 1% EtOH}; ^{1}H-NMR ($CDCl_3$) δ 6.12 (m, 1H, H-2), 6.01 (m, 1H, H-3), 5.52 (m, 1H, H-4), 4.75 (m, 1H, H-1), 2.85 (dt, 1H, H-5 *cis* to OH), 2.07 (s, 3H, CH_3), 1.72 (broad s, 1H, OH), 1.65 (dt, 1H, H-5 *trans* to OH); ^{13}C-NMR ($CDCl_3$) δ 170.8 (C=O), 138.5 (C-2), 132.3 (C-3), 77.1 (C-1), 74.5 (C-4), 40.4 (C-5), 21.1 (CH_3); IR cm^{-1} 3353 (O–H), 2950 (aliphatic C–H), 1714 (ester C=O), 1461, 1401 (C=H).

1S,4R-cis-1-Acetoxy-4-hydroxy-2-cyclopentene (6)

Diacetate **5** (1.53 g, 0.0083 mmol) was suspended in 25 mL of 0.1 M phosphate buffer (pH = 7.0) containing 3.7 mg of pig liver esterase. The reaction was allowed to stir at room temperature for 24 h, during which time the pH was maintained at 7.0 by adjusting with 1 N NaOH. The reaction was considered complete when no further pH changes were observed. The reaction mixture was then poured into a 250 mL separation funnel and extracted with three 50 mL portions each of ether and then ethyl acetate. The combined organic layers were washed with 100 mL of saturated NaCl and dried over $MgSO_4$ Filtration and removal of the solvent *in vacuo* afforded a colorless amorphous product, which was recrystallized (pentane:ether 1:1) to give 0.93 g (82%) of *1S,4R*-**6** as white needles, m.p. 47–49 °C. The desired *1S,4R*-enantiomer of **6** was determined to be present in 94% e.e. by NMR analysis of the corresponding Mosher ester (see below): {$[\alpha]^{20}_D$ –52.2° (c 10.0, $CHCl_3$ + 1% EtOH)}; all spectral data (^{1}H-NMR, ^{13}C-NMR, IR) are identical to those reported for *1R,4S-6*, as described above.

Mosher esters of 6 and 9. General procedure

A 0.0007 mol portion of the appropriate alcohol (**6** or **9**) was dissolved in 6 mL of methylene chloride along with 0.187 g (0.0008 mol) of (+)-(α-methoxy-α-trifluoromethyl-α-phenylacetic acid and 0.165 g (0.0008 mol) dicyclohexylcarbodiimide under a nitrogen atmosphere. The reaction was initialized by adding a catalytic amount of 4-dimethylaminopyridine (0.009 g, 0.00007 mol). After the reaction was allowed to stir for 24 h at room temperature, the precipitated dicyclohexylurea was removed by filtration. The filtered solid was washed with 5 mL of hexane, the combined organic layers were removed *in vacuo*, and the residue was purified on silica gel (3:1 hexane:ethyl acetate, R_f = 0.42) to afford the corresponding Mosher ester derivative as white solid.

Mosher ester of 1R,4S-6. ^{1}H-NMR ($CDCl_3$) δ 7.54–7.41 (m, 5H, aromatic H), 6.16 (m, 1H, H-2), 6.08 (m, 1H, H-3), 5.76 (m, 1H, H-4), 5.55 (m, 1H, H-1), 3.55 (s, 3H, OCH_3), 2.92 (dt, 1H, H-5 *cis* to OH), 1.99 (s, 3H, CH_3), 1.89 (dt, 1H, H-5 *trans* to OH); ^{13}C-NMR ($CDCl_3$) δ 170.6 (ester C=O), 166.2 (ester C=O), 136.0 (C-2), 133.4 (C-3), 132.2 (aromatic C-1), 129.7 (aromatic C-2 and C-6), 128.5 (aromatic C-3 and C-5), 127.4 (aromatic C-4), 125.2 (CF_3), 121.4 (CF_3–C), 78.9 (C-1), 76.3 (C-4), 55.4 (OCH_3), 37.0 (C-5), 20.9 (CH_3).

Mosher ester of 1S,4R-6. ^{1}H-NMR ($CDCl_3$) δ 7.54–7.41 (m, 5H, aromatic H), 6.17 (s, 2H, H-2 and H-3), 5.75 (m, 1H, H-4), 5.55 (m, 1H, H-1), 3.55 (s, 3H, OCH_3), 2.87 (dt, 1H, H-5 *cis* to OH), 1.99 (s, 3H, CH_3), 1.89 (dt, 1H, H-5 *trans* to OH); ^{13}C-NMR ($CDCl_3$) δ 170.6 (ester C=O), 166.2 (ester C=O), 136.1 (C-2) 133.4 (C-3), 132.2 (aromatic C-1), 129.7 (aromatic C-2 and C-6), 128.5 (aromatic C-3 and C-5), 127.4 (aromatic C-4), 125.2 (CF_3), 121.4 (CF_3–C), 78.9 (C-1), 76.3 (C-4), 55.4 (OCH_3), 36.8 (C-5), 20.9 (CH_3).

Mosher ester of 1R,4R-9. ^{1}H-NMR ($CDCl_3$) δ 7.60–7.42 (m, 5H, aromatic H), 6.14 (m, 1H, H-2), 6.08 (m, 1H, H-3), 5.98 (m, 1H, H-4), 4.91 (m, 1H, H-1), 4.59 (broad s, 1H, N-H), 3.54 (s, 3H, OCH_3), 2.36 (dt, 1H, H-5 *cis* to OH), 2.01 (dt, 1H, H-5 *trans* to OH), 1.45 (s, 9H, C–CH_3); ^{13}C-NMR ($CDCl_3$) δ 166.4 (ester C=O), 155.3 (carbamate C=O), 140.2 (C-2), 132.2 (C-3), 130.7 (aromatic C-1), 129.6 (aromatic C-2 and C-6), 128.4 (aromatic C-3 and C-5), 127.2 (aromatic C-4), 125.1 (CF_3), 121.3 (CF_3–C), 80.0 (C-1), 77.6 (C-4), 55.6 (CH_3–C), 55.4 (OCH_3), 38.4 (C-5), 28.3 (C–CH_3).

Mosher ester of 1S,4S-9. ^{1}H-NMR ($CDCl_3$) δ 7.52–7.39 (m, 5H, aromatic H), 6.10 (m, 1H, H-2), 5.98 (m, 1H, H-3), 5.95 (m, 1H, H-4), 4.88 (m, 1H, H-1), 4.55 (broad s, 1H, N–H), 3.52 (s, 3H, OCH_3), 2.44 (dt, 1H, H-5 *cis* to OH), 2.05 (dt, 1H, H-5 *trans* to OH), 1.44 (s, 9H, C–CH_3); ^{13}C-NMR ($CDCl_3$) δ 166.4 (ester C=O), 155.3 (carbamate C=O), 140.0 (C-2), 132.1 (C-3), 130.8 (aromatic C-1), 129.6 (aromatic C-2 and C-6), 128.4 (aromatic C-3 and C-5), 127.3 (aromatic C-4), 125.1 (CF_3), 121.3 (CF_3–C), 81.0 (C-1), 77.6 (C-4), 55.6 [$(CH_3)_3$–C], 55.3 (OCH_3), 38.6 (C-5), 28.3 (C–CH_3).

S-(5'-Deoxy-5'-adenosyl)-1-amino-4-thio-2-cyclopentene (8)

Compound **8** was synthesized in 86% yield as a mixture of the *1R,4R* and *1R,4S*-diastereomers, or as a mixture of the *1S,4R* and *1S,4S*-diastereomers, as previously described.[16] The diastereomeric mixtures of **8** described above were separated into their respective pure diastereomers by careful and repeated (typically 3 times) flash chromatography on silica gel (2 x 40 cm column, $CHCl_3$:MeOH:NH_4OH 14:14:1). Fractions containing the desired diastereomeric product were pooled, 50 mL of water was added, and the mixture was concentrated on the rotary evaporator until the pH of the aqueous layer was neutral. The aqueous solution was then lyophyllized to afford diastereomerically pure **8** as a white solid.

cis-*1R,4S-8*. R_f = 0.45; ^{1}H-NMR (CD_3OD) δ 8.30 (s, 1H, H-2), 8.21 (s, 1H, H-8), 6.01 (d, 1H, H-1'), 5.86 (m, 1H, H-2"), 5.67 (m, 1H, H-3"), 4.81 (m, 1H, H-2'), 4.38 (m, 1H, H-3'), 4.25 (m, 1H, H-4'), 3.84 (m, 2H, H-4" and H-1"), 3.05–2.87 (complex m, 3H, H-5' and H-5" *cis* to NH_2), 2.37 (complex m, 1H, H-5" *trans* to NH_2).

trans-*1R,4R-8*. R_f = 0.35; ^{1}H-NMR (CD_3OD) δ 8.30 (s, 1H, H-2), 8.22 (s, 1H, H-8), 6.01 (m, 2H, H-2" and H-3"), 5.82 (m, 1H, H-1'), 4.82 (m, 1H, H-2'), 4.35 (m, 1H, H-3'), 4.19 (m, 2H, H-1" and H-4'), 4.08 (m, 1H, H-4"), 3.08 (m, 2H, H-5'), 2.28 (m, 1H, H-5" *cis* to NH_2), 2.04 (complex m, 1H, H-5" *trans* to NH_2).

cis-*1S,4R-8*. R_f = 0.45; ^{1}H-NMR (CD_3OD) δ 8.28 (s, 1H, H-2), 8.19 (s, 1H, H-8), 5.98 (d, 1H, H-1'), 5.84 (m, 1H, H-2"), 5.71 (m, 1H, H-3"), 4.78 (m, 1H, H-2'), 4.33 (m, 1H, H-3'), 4.16 (m, 1H, H-4'), 3.86 (m, 1H, H-4"), 3.78 (m, 1H, H-1") 3.0 (complex m, 2H, H-5'), 2.77 (m,

1H, H-5" *cis* to NH_2), 2.32 (complex m, 1H, H-5" *trans* to NH_2).

trans-*1S,4S*-**8**. R_f = 0.35; ^{1}H-NMR (CD_3OD) δ 8.50 (s, 1H, H-2), 8.29 (s, 1H, H-8), 6.10 (m, 1H, H-2"), 5.98 (m, 1H, H-1'), 5.81 (m, 1H, H-3"), 4.78 (m, 1H, H-2'), 4.34 (m, 1H, H-3'), 4.19 (m, 1H, H-4'), 4.11 (m, 2H, H-1" and H-4"), 2.98 (m, 2H, H-5'), 2.28 (m, 1H, H-5" *cis* to NH_2), 2.18 (complex m, 1H, H-5" *trans* to NH_2).

S-(5'-Deoxy-5'-adenosyl)-1-ammonio-4-methylsulfonio-2-cyclopentene disulfate, AdoMac (3)

A 0.100 g portion of the appropriate pure diastereomer of **8** (0.00026 mol) was converted to the corresponding pure diastereomer of **3** in 85–96% yield as previously described.[16]

cis-*1R,4S*-*3*. ^{1}H-NMR (CD_3OD) δ 8.30 (s, 1H, H-2), 8.25 (s, 1H, H-8), 6.41 (m, 2H, H-2" and H-3"), 6.05 (d, 1H, H-1'), 5.08 (m, 1H, H-2'), 4.85 (m, 1H, H-3'), 4.58 (m, 1H, H-4'), 4.09–3.85 (complex m, 2H, H-1" and H-4"), 2.95 (complex m, 3H, H-5' and H-5"), 2.79 (s, 3H, CH_3), 2.40 (m, 1H, H-5"); HPLC retention time: 22.03 min.

trans-*1R,4R*-*3*. ^{1}H-NMR (CD_3OD) δ 8.34 (s, 2H, H-2 and H-8), 6.39 (m, 2H, H-2" and H-3"), 6.06 (d, 1H, H-1'), 5.04 (m, 1H, H-2'), 4.58 (m, 2H, H-3' and H-4'), 4.05 (complex m, 1H, H-1"), 3.93 (m, 1H, H-4"), 2.95 (complex m, 2H, H-5'), 2.74 (s, 3H, CH_3), 2.55–2.45 (complex m, 2H, H-5"); HPLC retention time: 13.94 min.

cis-*1S,4R*-*3*. ^{1}H-NMR (CD_3OD) δ 8.45 (s, 2H, H-2 and H-8), 6.10 (d, 1H, H-1'), 5.86 (m, 1H, H-2"), 5.76 (m, 1H, H-3"), 5.08 (m, 1H, H-2'), 4.75 (m, 1H, H-3'), 4.58 (m, 1H, H-4'), 3.91 (complex m, 2H, H-1" and H-4"), 2.95 (complex m, 3H, H-5' and H-5"), 2.90 (s, 3H, CH_3), 2.39 (m, 1H, H-5"); HPLC retention time: 22.93 min.

trans-*1S,4S*-*3*. ^{1}H-NMR (CD_3OD) δ 8.45 (s, 2H, H-2 and H-8), 6.40 (m, 1H, H-2"), 6.29 (m, 1H, H-3"), 6.03 (d, 1H, H-1'), 5.08 (m, 1H, H-2'), 4.78 (m, 1H, H-3'), 4.59 (m, 1H, H-4'), 4.33 (complex m, 1H, H-1"), 4.10 (m, 1H, H-4"), 2.99 (complex m, 2H, H-5'), 2.90 (s, 3H, CH_3), 2.60–2.40 (complex m, 2H, H-5"); HPLC retention time: 18.13 min.

trans-(*1S,4S*)-*1-Hydroxy-4-*(tert-*butyloxycarbonyl)amino-2-cyclopentane (10)*

A 0.220 g portion of *1R,4R*-**9** (0.00055 mol) was dissolved in 10 ml of MeOH and added slowly to a Parr hydrogenation bottle containing 0.160 g 10% Pd/C which was previously wetted with 1 mL of EtOH. The mixture was then hydrogenated at room temperature (50 psi) for 3 days. Filtration (Zetapore 0.45 μ filter) and removal of the solvent then afforded analytically pure *1S,4S*-**10** as white solid (0.212 g, 95.4% yield). ^{1}H-NMR ($CDCl_3$) δ 4.46–4.43 (broad m, 2H, NH and H-4), 4.16 (complex m, 1H, H-1), 2.25 (m, 1H, H-5 *cis* to OH), 2.03 (m, 1H, H-5 *trans* to OH), 1.61 (m, 4H, H-2 and H-3), 1.40 (s, 9H, C–CH_3); ^{13}C-NMR ($CDCl_3$) δ 166.0 (C=O), 77.6 (C-4), 72.3 (C-1), 42.4 (C-5), 33.9 (C-3), 31.4 (C-2), 28.43 (C–CH_3); IR cm^{-1} (KBr) 3342, 3186 (N–H, O–H), 2959 (aliphatic C–H), 1682 (C=O); Anal. ($C_{10}H_{19}NO_3$) C, H, N.

trans-(*1S,4S*)-*1-Methanesulfonyloxy-4-{[*(tert-*butyloxy)-carbonyl]amino}-cyclopentane (11)*

A solution of compound *1S,4S*-**10** (0.101 g, 0.0005 mol) and triethylamine (0.21 mL, 0.0015 mol) in 3.0 mL dichloromethane was cooled to 0 °C under a nitrogen atmosphere, and 0.21 mL (0.0015 mol) of methanesulfonyl chloride was added by dropwise addition. After stirring overnight at room temperature, the reaction was diluted to 10 mL with dichloromethane and washed with two 10 mL portions of 1N HCl, 10 mL of water, and 10 mL of saturated NaCl. The organic layer was then dried over $MgSO_4$. Filtration and removal of solvent afforded the crude product, which was purified on silica gel (hexane: ethyl acetate 6:4) to yield pure *1R,4R*-**11** (0.11 g, 80.1%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 5.14 (complex m, 1H, H-1), 4.49 (broad, 1H, NH), 4.15 (complex m, 1H, H-4), 2.93 (s, 3H, CH_3), 2.32 (m, 1H, H-5 *cis* to OH), 2.13 (m, 2H, H-3), 1.90 (m, 1H, H-5 *trans* to OH), 1.75 (m, 2H, H-3), 1.37 (s, 9H, C–CH_3); ^{13}C-NMR ($CDCl_3$) δ 155.3 (C=O), 82.2 (C-1), 69.1 (C-4), 40.5 (C-5), 38.6 (C–CH_3), 31.7 (C-2), 30.7 (C-3), 28.37 (C–CH_3); IR cm^{-1} (KBr) 3338 (N–H), 2918 (aliphatic C–H), 1675 (C=O), 1450 (S=O); Anal. ($C_{11}H_{21}NO_5S$) C, H, N.

cis-(*1S,4R*)-S-*(2',3'-Isopropylidene-5'-deoxy-5'-adenosyl)-1-{[*(tert-*butyloxy)carbonyl]amino}-4-thiocyclopentane (13)*

A 0.10 g portion (0.000375 mol) of compound *1S,4S*-**11** and 0.221 g (0.000563 mol) of N^6-formyl-2',3'-isopropylidene-5'-thioacetyladenosine **12**[16,22,23] were dissolved in 20 mL of a 1:1 mixture of dry DMF and dry methanol under an argon atmosphere. The solution was frozen and thawed 5 times in succession with liquid nitrogen while allowing a vigorous stream of argon to bubble through the solution, and then 0.061 g (0.00113 mol) of sodium methoxide powder was added through the condenser. The reaction was allowed to stir under argon, protected from light, for three days. The solvent was then removed (rotary evaporator, 0.2 mm Hg), and the residue was partitioned between 50 mL each of water and chloroform. The aqueous layer was washed with two additional 50 mL portions of $CHCl_3$, and the combined organic layers were washed with 50 mL of saturated sodium chloride and dried over anhydrous $MgSO_4$. Filtration, removal of the chloroform and chromatography on silica gel (ethyl acetate:isopropanol 24:1) afforded *1S,4R*-**13** as a pale yellow foam (0.10 g, 55.9% yield). ^{1}H-NMR ($CDCl_3$) δ 8.37 (s, 1H, H-2), 7.96 (s, 1H, H-8), 6.10 (d, 1H, H-1'), 5.62 (br s, 2H, NH_2), 5.56 (m, 1H, H-2'), 5.08 (m, 1H, H-3'), 4.73 (m, 1H, H-1"), 4.41 (m, 1H, H-4"), 4.22 (m, 1H, H–4'), 3.98 (br s, 1H, NH–Boc), 3.16 (m, 2H, H-5'), 2.86–2.73 (complex m, 4H, H-2" and H-3"), 2.37 (complex m, 1H, H-5" *cis* to S), 1.94 (m, 1H,

H-5" *trans* to S), 1.63 (s, 3H, acetonide CH_3), 1.44 (s, 9H, Boc–CH_3), 1.42 (s, 3H, acetonide CH_3). IR cm^{-1} (KBr) 3345 (N–H), 2957 (aliphatic C–H), 1643 (C=O), 1476, 1375 (C=C); Anal. ($C_{23}H_{34}N_6O_5S$) C, H, N.

cis-(1S,4R)-S-(5'-Deoxy-5'-adenosyl)-1-amino-4-methylsulfonio-cyclopentane disulfate (4)

A 0.10 g portion of **1S,4R-13** (0.000198 mol) was converted to the corresponding deprotected analogue, *cis*-(1*S*,4*R*)-*S*-(5'-deoxy-5'-adenosyl)-1-amino-4-thiocyclo-pentane, which was isolated as a white solid (0.070 g, 96.4%) using the method described for the preparation of compound **8**. ^{1}H-NMR (CD_3OD) δ 8.47 (br s, 2H, NH_2), 8.32 (s, 1H, H-2), 8.21 (s, 1H, H-8), 6.01 (d, 1H, H-1'), 4.77 (m, 1H, H-2'), 4.37 (m, 1H, H-1"), 4.26 (m, 1H, H-3'), 3.54 (m, 1H, H-4"), 3.10 (m, 1H, H-4'), 3.04 (m, 1H, H-5'), 2.55 (complex m, 1H, H5"*cis* to S), 2.05 (m, 2H, H-3"), 1.78 (m, 2H, H-2"), 1.52 (m, 1H, H-5" *trans* to S); IR cm^{-1} (KBr) 3306 (N–H), 3201 (O–H), 2917 (aliphatic C–H). This compound was used immediately in the subsequent reaction without further purification.

A 0.020 g portion of the deprotected analogue described above (0.0000545 mol) was methylated using the procedure described for the preparation of **3** to afford **4** (0.018 g, 91.6%) as a white solid. ^{1}H-NMR (CD_3OD) δ 8.48 (s, 1H, H-2), 8.35 (s, 1H, H-8), 6.10 (m, 1H, H-1'), 4.72 (m, 1H, H-2'), 4.44 (m, 1H, H-3'), 3.55–3.16 (m, 3H, H-1", H-4" and H-4'), 2.91 (m, 2H, H-5'), 2.81 (s, 3H, CH_3), 2.30–2.04 (complex m, 2H, H-5"), 1.91–1.72 (complex m, 4H, H-2" and H-3"); IR cm^{-1} (KBr) 3436, 3393 (N–H, O–H), 2905 (aliphatic C–H); HPLC retention time: 20.96 min. Anal. ($C_{16}H_{26}N_6O_3S{\cdot}2H_2SO_4{\cdot}0.5EtOH$) C, H, N.

HPLC Analysis of MTA from enzyme assay mixtures

AdoMet-DC was incubated at an inhibitor concentration of 100 μM in the assay medium described below at 4 °C, with a total volume 0.5 mL. Samples were incubated in duplicate for 2, 6 and 12 h time intervals for each determination. The resulting solutions were filtered through a Centricon-30 device at 5000 x *g*, and 30 μL of the supernatant was analyzed by HPLC (Waters C-18 Novapak, 0.80 x 10 cm, 4 μ) according to the reversed-phase ion pairing assay procedure of Wagner.[20]

Enzyme isolation and assay procedure

AdoMet-DC was isolated from *Escherichia coli* using a modification of the methylglyoxal-bis-guanylhydrazone (MGBG)–Sepharose affinity column procedure of Anton and Kutny[12] as was previously reported.[16] The resulting AdoMet-DC was greater than 90% pure as determined by gel electrophoresis, and the specific activity was typically determined to be 0.80 μmol/min/mg protein at 37 °C. The enzyme was stored in 20 mM potassium phosphate, 0.1 M KCl, 0.5 mM EDTA and 0.5 mM dithiothreitol, pH 7.4 at 4 °C. AdoMet-DC activity was monitored by following the evolution of ^{14}C–CO_2 from *S*-adenosyl-L-[^{14}C–COOH]–methionine using a modification of the procedure of Markham,[31] as previously described.[16] Enzyme activity (V) is expressed as μmol of ^{14}C–CO_2 produced per minute per milligram of protein x 10^{-7}. Each data point presented represents the average of two determinations which in each case differed by less than 5%.

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