S-(5′-Deoxy-5′-adenosyl)-1-aminoxy-4-(methylsulfonio)-2-cyclopentene (AdoMao): An Irreversible Inhibitor of S-Adenosylmethionine Decarboxylase with Potent *in Vitro* Antitrypanosomal Activity

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The *S*-adenosylmethionine (AdoMet) analogue *S*-(5′-deoxy-5′-adenosyl)-1-aminoxy-4-(methylsulfonio)-2-cyclopentene (AdoMao) was synthesized in two of its four possible diastereomeric forms using a facile chemoenzymatic route. The *trans*-1*R*,4*R*- and *trans*-1*S*,4*S*-diastereomers of AdoMao, as well as the corresponding diastereomers of the unmethylated precursor molecule nor-AdoMao, were then evaluated as inhibitors of *S*-adenosylmethionine decarboxylase (AdoMet-DC) from both bacterial and human sources. All four of the analogues acted as time-dependent, irreversible inhibitors of AdoMet-DC from *Escherichia coli*, exhibiting remarkably constant K_i values ranging between 20.6 and 23.7 μM. These analogues also inhibited the human form of AdoMet-DC, although this form of the enzyme was able to discriminate between AdoMao (K_i values of 21.2 μM for the *trans*-1*R*,4*R* form and 19.6 μM for the *trans*-1*S*,4*S* form) and nor AdoMao (K_i values of 95.2 μM for the *trans*-1*R*,4*R* form and 30.9 μM for the *trans*-1*S*,4*S* form). The *trans* diastereomers of AdoMao and nor-AdoMao were next evaluated for their ability to inhibit trypanosomal growth *in vitro* against cultured *Trypanosoma brucei brucei* bloodforms. All four of these analogues were effective growth inhibitors, with IC_{50} values ranging between 0.9 and 10.1 μM. The two most effective analogues, *trans*-1*S*,4*S*-AdoMao (IC_{50} 0.9 μM) and *trans*-1*S*,4*S*-AdoMao (IC_{50} 3.0 μM) were also effective against two clinical isolates of the pathogenic organism *Trypanosoma brucei rhodesiense*, KETRI 243 and KETRI 269. The most promising analogue in all respects was *trans*-1*S*,4*S*-AdoMao, which was subsequently found to have minimal effects on cell growth, AdoMet-DC activity, and intracellular polyamine levels in the sensitive human promyelocytic leukemia cell line HL60. Thus, the *S*-adenosylmethionine analogue *trans*-1*S*,4*S*-AdoMao acts as an effective inhibitor of AdoMet-DC and appears to serve as a parasite-specific trypanocidal agent *in vitro*.

Introduction

The enzyme *S*-adenosylmethionine decarboxylase (AdoMet-DC, EC 4.1.1.50) catalyzes the decarboxylation of *S*-adenosylmethionine (AdoMet, **1**, Figure 1) to produce decarboxylated S-adenosylmethionine (dc-AdoMet, **2**, Figure 1), a required intermediate for the biosynthesis of cellular polyamines. AdoMet-DC plays a regulatory role in the biosynthesis of polyamines in a variety of organisms and as such has become a target for the development of novel chemotherapeutic agents.[1] The catalytic subunit of all of the known forms of AdoMet-DC contains a covalently bound pyruvate, which acts as a cofactor by forming an imine linkage with the substrate prior to enzymatic decarboxylation.[2-4] On the basis of this mechanistic feature, a number of inhibitors of AdoMet-DC have been synthesized as potential antitumor and/or antiparasitic agents,[5-12] but to date, none of these compounds has been developed for clinical use.

It has recently been demonstrated that trypanosomes such as *Trypanosoma brucei brucei* do not synthesize purine derivatives but rather import adenosine and its analogues using a specific, energy-dependent adenosine transport system.[13] Our group[14] as well as others[15] have proposed that analogues of AdoMet can act as substrates for this transporter, which is not present in mammalian cells, raising the possibility that AdoMet-DC inhibitors which are transported could act as parasite-specific toxins. Along these lines, we recently described the synthesis and biological evaluation of AdoMac (**3**, Figure 1), a potent, enzyme activated irreversible inhibitor of AdoMet-DC.[10] AdoMac is a conformationally restricted analogue of dc-AdoMet and inactivates both the human and *Escherichia coli* forms of AdoMet-DC in a configuration-dependent manner.[11] Despite being a potent inactivator of the enzyme (K_i = 18 μM as a mixture of the 1*R*,4*R*- and 1*R*,4*S*-diastereomers), AdoMac has little growth inhibitory effect on the L1210 and HT29 mammalian cell lines.[14] However, this analogue inhibits growth of cultured *Trypanosoma brucei brucei* blood forms with an IC_{50} value of 5.2 μM.[14] In addition, parasite-specific toxicity has been observed following treatment with a number of AdoMet analogues synthesized in our laboratories.[14]

As an extension of our studies involving AdoMac, the aminoxy cogener AdoMao (**4**, Figure 1) was designed and synthesized. It was reasoned that the aminoxy amine-surrogate functional group would show enhanced nucleophilicity at the terminal pyruvate of AdoMet-DC, thus making AdoMao a more effective inhibitor. In addition, AdoMao would be expected to form a stable oxime linkage with the enzyme, rather than the more easily hydrolyzable imine formed by AdoMac. It is likely that AdoMet-DC would be irreversibly inactivated by AdoMao subsequent to this transformation. Following formation of the oxime linkage between AdoMao and

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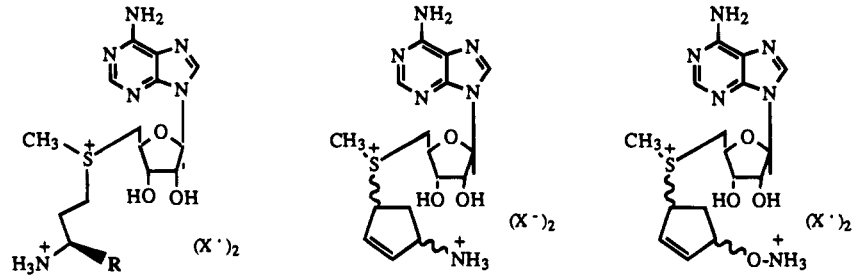


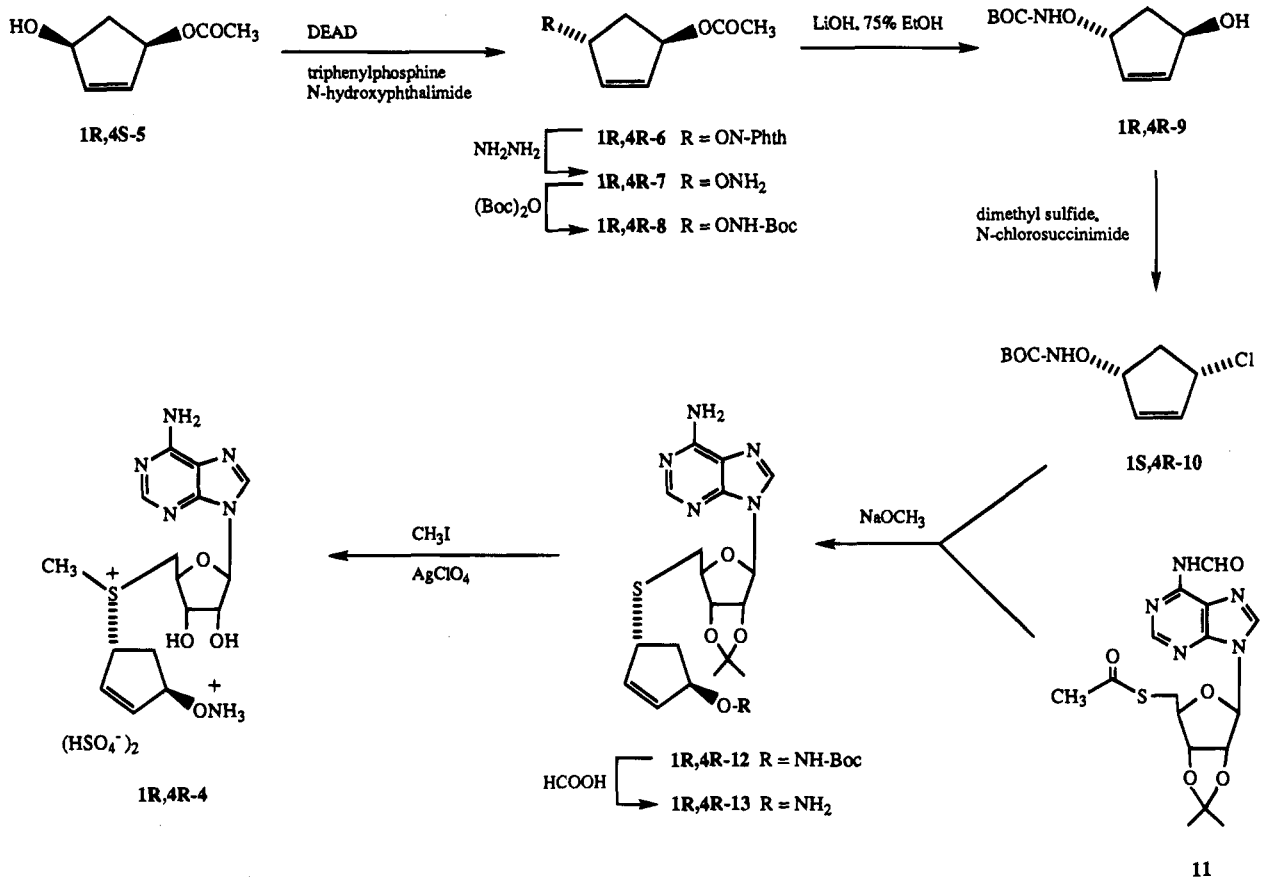
1 R = COOH
2 R = H

3

4

Figure 1. Structures of AdoMet (1), dc-AdoMet (2), AdoMac (3), and AdoMao (4).

Scheme 1



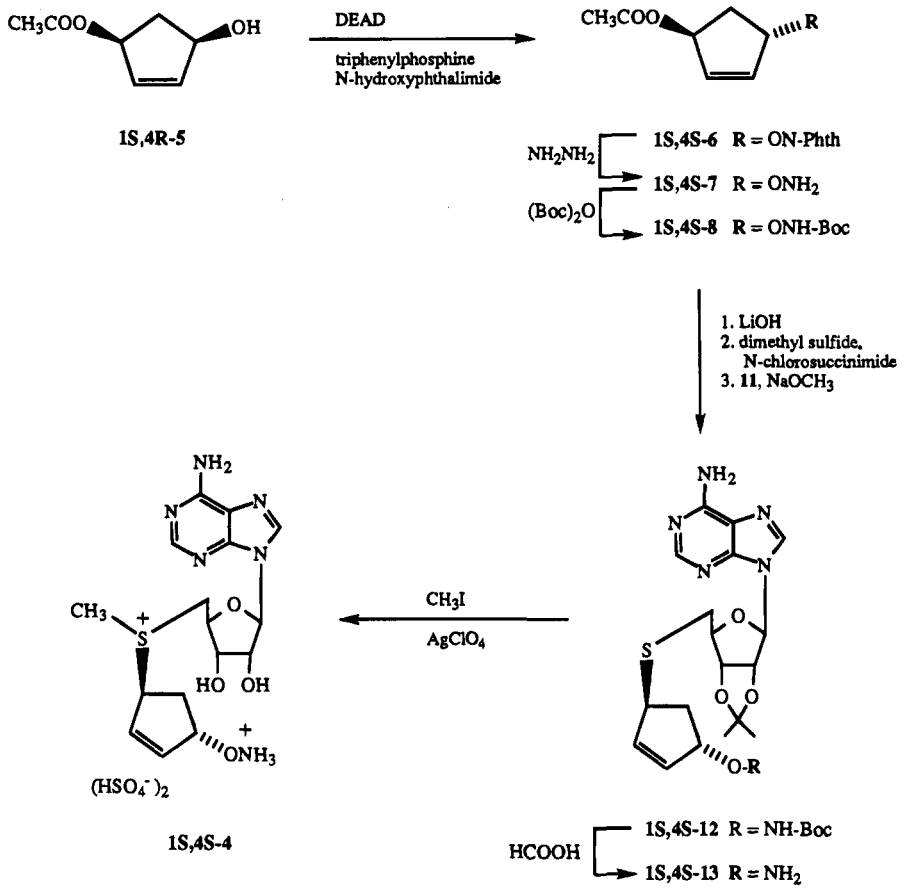
the terminal pyruvate of AdoMet-DC, it is conceivable that there are additional steps involved in the inactivation process. However, the data presented in this paper does not allow speculation as to what these steps may be. More detailed kinetic studies are required in order to elucidate the precise mechanism for inactivation of AdoMet-DC by AdoMao. Nonetheless, based on the observations and hypotheses described above, it was postulated that AdoMao could act as a trypanosome-specific growth inhibitor by virtue of being selectively imported by the parasitic adenosine transport system and then by irreversibly inactivating trypanosomal AdoMet-DC. We now report the synthesis of AdoMao in two of its pure diastereomeric forms and the results of the associated enzymatic characterization and biological evaluation studies.

Chemistry

The synthetic route leading to the proposed AdoMet-DC inhibitor *trans*-1*R*,4*R*-**4** is outlined in Scheme 1. The starting alcohol, (+)-*cis*-(1*R*,4*S*)-1-acetoxy-4-hydroxy-2-cyclopentene **5**, was synthesized from *cis*-1,4-diacetoxy-2-cyclopentene[16,17] in 97% ee by enzymatic hydrolysis of the *pro-S* acetate using porcine lipase, as previously described.[10,11,18] *N*-Hydroxyphthalimide was then added at the free hydroxyl position under Mitsunobu conditions[7] to afford the protected aminoxyester *trans*-1*R*,4*R*-**6**. The phthalimide protecting group was removed (NH_2NH_2) to produce *trans*-1*R*,4*R*-**7**, which was then reprotected as the corresponding *N*-Boc[19] derivative to yield *trans*-1*R*,4*R*-**8**. Subsequently, the acetate at position 1 was cleaved to afford the protected aminoxy-alcohol *trans*-1*R*,4*R*-**9**. The desired *trans*-1*R*,4*R*-enantiomer of intermediate **9** was found to be present in >97% ee, as determined by NMR analysis of the corresponding Mosher ester,[20] demonstrating that no racemization had occurred during the preceding steps.

During the synthesis of the known inhibitor AdoMac (**3**), chlorination of the allylic alcohol *trans*-(1*R*,4*R*)-1-hydroxy-4-{[(*tert*-butyloxy)carbonyl]amino}-2-cyclopen-

Scheme 2



tene resulted in the formation of the corresponding chloride in a 64:36 ratio of *cis* to *trans* isomers, as determined by NMR analysis.[10,11] The *cis* and *trans* diastereomeric forms for 1,4-substituted 2-cyclopentene derivatives can be readily discriminated by the ^{1}H-NMR resonances of the diastereotopic protons at C-5.[11] The intermediate *cis*-1*S*,4*R*-1-chloro-4-{[(*tert*-butyloxy)carbonyl]amino}-2-cyclopentene shows resonances for the C-5 methylene protons which are well separated (2.93 and 1.97 ppm), while the C-5 protons in the corresponding *trans* derivative are more proximal and are shifted slightly upfield (2.64 and 2.05 ppm).[11] Synthesis of this chloride by the method of Meyers[21] (LiCl, methanesulfonyl chloride) and by the method of Corey[22] (dimethyl sulfide, *N*-chlorosuccinimide) resulted in similar mixtures of the *cis* and *trans* diastereomers. This unanticipated racemization ultimately led to the ability to separate the pure diastereomeric forms of AdoMac, since substitution at the racemized cyclopentenyl chloride led to the formation of a mixture of diastereomeric thionucleosides which could be resolved by flash chromatography.[11] When this chemistry was extended to the chlorination of the protected allylic aminoxy alcohol *trans*-1*R*,4*R*-**9**, using the method of Corey[22] (dimethyl sulfide, *N*-chlorosuccinimide), the desired product, allylic chloride **10**, was isolated only as the *cis*-1*S*,4*R*-isomer, consistent with 100% inversion of configuration at the allylic center. The ^{1}H-NMR spectrum for *cis*-1*S*,4*R*-**10** showed characteristic C-5 resonances at 2.88 and 2.23, while no resonances appeared that could be attributed to *trans*-C-5 methylene protons.

Formation of the fully protected thionucleoside *trans*-1*R*,4*R*-**12** was accomplished by treatment of N^6-formyl-5′-deoxy-5′-thioacetyl-2′,3′-*O*-isopropylideneadenosine (**11**) with sodium methoxide in the presence of *cis*-1*R*,4*S*-**10**, as previously described.[10,23] The *N*-Boc and acetonide protecting groups were then removed simultaneously (88% HCOOH) to afford the aminoxy thionucleoside **13** as the *trans*-1*R*,4*R*-diastereomer. Compound **13** was then methylated according to a modification of the method of Samejima[10,24] to afford the desired target molecule *trans*-1*R*,4*R*-**4**. This analogue was found to be present in 99% diastereomeric excess by ^{1}H-NMR and by HPLC analysis (Waters C-18 Novapak, 0.8 × 10 cm, 4 μm) using the reversed-phase ion pairing assay described by Wagner.[25]

The *trans*-1*S*,4*S*-diastereomer of the proposed AdoMetDC inhibitor **4** was also synthesized, using an analogous route which is outlined in Scheme 2. The starting alcohol, *cis*-1*S*,4*R*-**5**, was synthesized from *cis*-1,4-diacetoxy-2-cyclopentene[16,17] in 97% ee by enzymatic hydrolysis of the *pro-R* acetate using pig liver esterase, as previously described.[10,11,26] This alcohol was then elaborated to the *cis*-1*R*,4*S*-enantiomer of allylic chloride **10**, as described in Scheme 1, which was appended to thioacetyladenosine **11**[10,23] to afford the 1*S*,4*S*-diastereomer of **12**. Hydrolysis of the *N*-Boc and acetonide protecting groups, followed by methylation as described above,[10,24] then produced the desired analogue *trans*-1*S*,4*S*-**4**. This analogue was found to be present in 99% diastereomeric excess by ^{1}H-NMR and by HPLC analysis (Waters C-18 Novapak, 0.8 × 10 cm, 4 μm) using the reversed-phase ion pairing assay described by Wagner.[25]

Biological Evaluation

The *trans*-1*R*,4*R*- and *trans*-1*S*,4*S*-diastereomers of **4**, as well as the corresponding isomers of the unmethy-

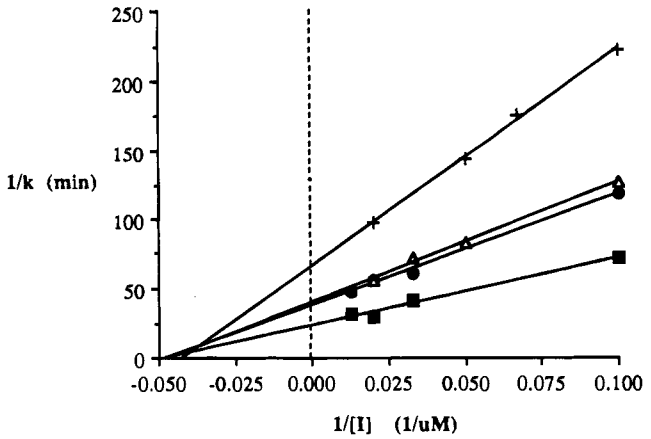


Figure 2. Kitz–Wilson plot for the inhibition of AdoMet-DC from *Escherichia coli* by the pure diastereomeric forms of **13** and **4**: (■) *trans*-1*R*,4*R*-**13**, (●) trans-1*S*,4*S*-**13**, (△) *trans*-1*R*,4*R*-**4**, (+) *trans*-1*S*,4*S*-**4**.

Table 1. Kinetic Constants for the Inhibition of AdoMet-DC by the Pure Diastereomeric Forms of Nor-AdoMao (**13**) and AdoMao (**4**)

	E. coli AdoMet-DC		human AdoMet-DC	
compound	K_i (μM)	k_{inact} (min^{-1})	K_i (μM)	k_{inact} (min^{-1})
trans-1*R*,4*R*-**13**	20.6	0.043	95.2	0.298
trans-1*S*,4*S*-**13**	21.2	0.027	30.9	0.149
trans-1*R*,4*R*-**4**	21.5	0.025	21.2	0.079
trans-1*S*,4*S*-**4**	23.7	0.015	19.6	0.115

lated precursor **13**, were evaluated as inhibitors of AdoMet-DC from both human and bacterial sources. Against the *E. coli* form of the enzyme, each analogue acted as an effective time-dependent, irreversible inactivator. For each analogue, time-dependent loss of enzyme activity was monitored at four concentrations of the inhibitor, and a value for the pseudo-first-order rate constant of inactivation k_{obs} was determined graphically (data not shown). The resulting k_{obs} values were then replotted according to the method of Kitz and Wilson,[27] as shown in Figure 2, and the inhibitor constants K_i and k_{inact} were then determined by linear regression. The resulting values are reported in Table 1. Interestingly, the K_i and k_{inact} values were remarkably constant for the four analogues, ranging between 20.6 and 23.7 μM and between 0.043 and 0.015 min^{-1}, respectively. The enzyme was protected from inactivation by both AdoMao and nor-AdoMao when it was precincubated with the known competitive inhibitor methylglyoxal–bisguanylhydrazone (MGBG). In addition, the observed inhibition was shown to be irreversible, since it was not possible to regenerate enzyme activity following extensive dialysis of the enzyme–inhibitor complex.

As was the case with the bacterial form of the enzyme, the pure diastereomeric forms of **13** and **4** acted as time-dependent, irreversible inhibitors of human AdoMet-DC. Kinetic constants were again derived by the method of Kitz and Wilson,[27] as described above (see Figure 3 and Table 1). In contrast to the bacterial enzyme, the human form of AdoMet-DC was able to readily discriminate between the four AdoMet analogues. The methylsulfonium analogues *trans*-1*R*,4*R*-**4** and *trans*-1*S*,4*S*-**4** (K_i values of 21.2 and 19.6 μM, respectively) were significantly more potent than the corresponding thioethers **13**, as outlined in Table 1. As before, protection from inactivation was observed for each analogue following preincubation with MGBG, and irreversibility

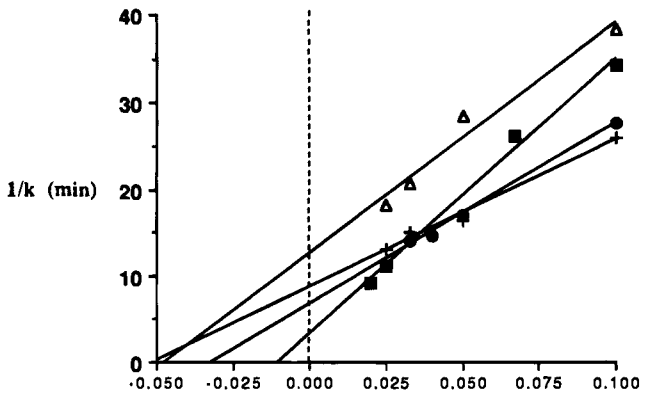


Figure 3. Kitz–Wilson plot for the inhibition of human AdoMet-DC by the pure diastereomeric forms of **13** and **4**: (■) *trans*-1*R*,4*R*-**13**, (■) *trans*-1*S*,4*S*-**13**, (△) *trans*-1*R*,4*R*-**4**, (+) *trans*-1*S*,4*S*-**4**.

Table 2. Inhibition of Trypanosomal Growth by the Pure Diastereomeric Forms of Nor-AdoMao (**13**) and AdoMao (**4**)

	IC_{50} (μM)		Ketri 269 %
compound	*T. brucei brucei*	Ketri 243	inhn (100 μM)
trans-1*R*,4*R*-**13**	10.1		
trans-1*S*,4*S*-**13**	3.0	82	21
trans-1*R*,4*R*-**4**	10.0		
trans-1*S*,4*S*-**4**	0.90	10	49

was assured by demonstrating the inability to remove the inhibitor by dialysis.

Since the four AdoMet analogues described above acted as effective inhibitors of bacterial and human AdoMet-DC, each was evaluated for its ability to inhibit growth in bloodforms of the parasitic organism *T. brucei brucei* in an *in vitro* assay system. Each analogue was found to be an effective growth inhibitor; however, in this case, the *trans*-1*S*,4*S*-diastereomers of **13** (IC_{50} = 3.0 μM) and **4** (IC_{50} = 0.9 μM) were both significantly more potent than the corresponding *trans*-1*R*,4*R*-isomers of **13** and **4**, as outlined in Table 2. The two most potent analogues, *trans*-1*S*,4*S*-**13** and *trans*-1*S*,4*S*-**4**, were also evaluated against two clinical isolates of the pathogenic parasite *Trypanosoma brucei rhodesiense* (KETRI 243 and KETRI 269) and were found to be reasonably effective trypanocides in each case, as shown in Table 2.

In order to assess the specificity of AdoMao for trypanosomal cells, the most potent trypanocidal AdoMet analogue, *trans*-1*S*,4*S*-**4**, was evaluated for its ability to inhibit growth in the sensitive HL60 human promyelocytic leukemia cell line. Cells were exposed to the appropriate concentration of the inhibitor for 96 h and were then disrupted by sonication. AdoMet-DC activity was then monitored by the method of Anton and Kutny,[28] as previously described,[10] and intracellular polyamines were measured by HPLC using the method of Danzin.[25] Each data point presented in Table 3 is the average of two determinations which in each case differed by less than 5%. During the course of the experiment, cell growth was minimally affected by *trans*-1*S*,4*S*-**4**, and no significant changes in AdoMet-DC activity were observed under the assay conditions, as shown in Table 3. In addition, there were no detectable changes in the levels of intracellular putrescine, spermidine, and spermine, suggesting that the

Table 3. Effects of *trans*-1*S*,4*S*-4 on AdoMet-DC and Polyamine Levels in Cultured HL60 Cells

μM inhibitor	AdoMet-DC, pmol/h^{-1} mg^{-1} of protein	putrescine, nmol/mg of protein	spermidine, nmol/mg of protein	spermine, nmol/mg of protein
none	315.3	0.92	10.1	34.0
0.1	355.3	0.71	7.9	30.4
1.0	346.6	0.94	10.1	38.1
10.0	293.0	0.71	8.4	31.6
50.0	448.8	0.79	11.2	28.0
100.0	488.9	0.94	16.1	27.0

analogue was unable to penetrate into HL60 cells in sufficient quantities to inhibit or induce AdoMet-DC.

Discussion

We have previously described the irreversible, enzyme-activated AdoMet-DC inhibitor AdoMac (**3**),[10] which appears to inactivate the enzyme by rearranging to a reactive intermediate following formation of an imine linkage with the terminal pyruvate of AdoMet-DC. This inhibition appears to be configuration dependent, since the four pure diastereomeric forms of AdoMac inhibit the *E. coli* form of the enzyme with K_i values ranging from 3.8 to 39.6 μM.[11] Because the side chain portion of AdoMac is conformationally restricted, each pure diastereomeric form of the analogue represents a distinct conformational mimic. Thus, it has been postulated that the pure diastereomers of AdoMac could be used as active site conformational probes for the various isozymic forms of AdoMet-DC. Interestingly, the unmethylated form of AdoMac (nor-AdoMac) acts as a reversible inhibitor of bacterial AdoMet-DC, presumably because it does not possess the driving force for the elimination of methylthioadenosine (MTA) following formation of the requisite imine linkage. As an extension of these studies, we have recently shown that the inhibition of human AdoMet-DC by AdoMac is also configuration dependent and that nor-AdoMac acts as a reversible inhibitor for this form of the enzyme as well.[29] However, the human enzyme preferentially binds to a distinctly different isomer of AdoMac, suggesting that there are significant differences in the active site topology of the human and bacterial forms of the enzyme. In contrast to these observations, the unmethylated precursor to AdoMao, **13**, and AdoMao, **4**, both irreversibly inactivate AdoMet-DC from human and bacterial sources (see Table 1). In the case of AdoMet-DC from *E. coli*, this inactivation does not appear to be configuration dependent. Since both **13** and **4** must form a stable oxime with the terminal pyruvate of AdoMet-DC, the enzyme is most likely inactivated prior to the elimination of MTA, and thus this additional driving force may not be necessary for inactivation to proceed. Experiments are now underway to determine whether MTA is released following inactivation of either form of AdoMet-DC by **4**. In contrast to the results of kinetic studies involving bacterial AdoMet-DC, inactivation of the human enzyme by **13** appears to depend on configuration, since there are distinct differences in activity between the *trans*-1*R*,4*R*- and *trans*-1*S*,4*S*-diastereomers of **13** (Table 1). Interestingly, both diastereomers of **4** inactivate human AdoMet-DC with equal potency and with K_i values similar to those derived from the bacterial AdoMet-DC inactivation studies. These experiments further demonstrate that the human and bacterial forms of AdoMet-DC have distinctly different binding sites and support the contention that it may be possible to design isozyme-specific inhibitors of AdoMet-DC.

The analogues described above, and in particular *trans*-1*S*,4*S*-**4**, appear to act as promising antitrypanosomal agents *in vitro*. Interestingly, the observed growth inhibition is also configuration-dependent, since the *trans*-1*S*,4*S*-forms of both **13** and **4** are the more potent trypanocides. This may indicate that the trypanosomal adenosine transporter and/or the trypanosomal form of AdoMet-DC prefer substrates in the *S*-configuration and suggests that conformational probes such as **13** and **4** may be of use to study the spatial requirements of these proteins. The excellent trypanocidal potency of *trans*-1*S*,4*S*-**4** makes this analogue a candidate for *in vivo* antiparasitic studies, and such experiments are now underway. Perhaps the most remarkable feature of this analogue is its ability to act as a parasite-specific toxin. This observation is most likely the result of preferential uptake by the trypsonomal adenosine transporter,[13] combined with the fact that mammalian cells, which normally conduct the *de novo* biosynthesis of purines, lack such a transporter. For this reason, the synthesis of additional AdoMet analogues for evaluation as potential specific antiparasitic agents is an ongoing concern in our laboratories.

Experimental Section

cis-2-Cyclopentene-1,4-diol and *cis*-1,4-diacetoxy-2-cyclopentene were synthesized according to the procedure of Kaneko[16] as modified by Johnson.[17] Compounds 1*R*,4*S*-**5** and 1*S*,4*R*-**5** were synthesized from *cis*-1,4-diacetoxy-2-cyclopentene by enzyme-mediated hydrolysis of the *pro*-*S*[18] or *pro*-*R*[26] acetate as previously described.[11] 5′-Thioacetyl-2′,3′-isopropylideneadenosine **11** was synthesized from 2′,3′-isopropylideneadenosine in two steps as previously described.[23,30] 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). All other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. and were used without further purification except as noted below. Pyridine was dried by passing it through an aluminum oxide column and then stored over KOH. Triethylamine was distilled from potassium hydroxide and stored in a nitrogen atmosphere. Methanol was distilled from magnesium and iodine under a nitrogen atmosphere and stored over molecular sieves. Methylene chloride was distilled from phosphorus pentoxide and chloroform was distilled from calcium sulfate. Tetrahydrofuran was purified by distillation from sodium and benzophenone. Dimethyl formamide was dried by distillation from anhydrous calcium sulfate and was stored under nitrogen. Preparative scale chromatographic procedures were carried out using E. Merck silica gel 60, 230–440 mesh. Thin layer chromatography was conducted on Merck precoated silica gel 60 F-254. All ^{1}H- and ^{13}C-NMR spectra were recorded on a General Electric QE-300 spectrometer, and all chemical shifts are reported as δ values referenced to TMS. Infrared spectra were recorded on a Nicolet 5DXB FT-IR spectrophotometer and are referenced to polystyrene. Ultraviolet spectra were recorded on a Beckman DU-8B spectrophotometer. In all cases, ^{1}H-NMR, ^{13}C-NMR, and IR spectra were consistent with assigned structures. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN, and are within 0.4% of calculated values.

For biological studies, *S*-adenosyl-L-[^{14}C-COOH]methionine was purchased from Amersham Life Science Division, Arling-

ton Heights, IL, and *E. coli* (¾ log phase) were purchased from Grain Processing Corp., Muscatine, IA. Reagents for the determination of protein were purchased from BioRad Corp., Rockville Center, NY. Centrifugation procedures were carried out on a Beckman L7 ultracentrifuge, and radioactivity was measured using an LKB 1209 Rackbeta liquid scintillation counter. Sonic disruption of *E. coli* was carried out using a Beckman 4710 series ultrasonic homogenizer.

(1*R*,4*R*)-*trans*-1-Acetoxy-4-(*N*-phthalimidooxy)-2-cyclopentene (6). A 0.300 g portion of 1*R*,4*S*-**5** (2.112 mmol) was dissolved in 3 mL of dry THF under a nitrogen atmosphere along with 0.666 g (2.535 mmol) of triphenylphosphine and 0.414 g (2.535 mmol) of *N*-hydroxyphthalimide. Diethyl azodicarboxylate (0.441 g, 2.535 mmol) dissolved in 3 mL of THF was added slowly *via* syringe, and the reaction mixture was allowed to stir at room temperature overnight under nitrogen. The solvent was then removed *in vacuo* to obtain a yellow residue which was purified on silica gel (hexane:ethyl acetate, 6:4) to afford 0.541 g (89% yield) of 1*R*,4*R*-**6** as a white solid: ^{1}H-NMR ($CDCl_3$) δ 7.85 (m, 2H, aromatic H-3 and H-6), 7.78 (m, 2H, aromatic H-4 and H-5), 6.25 (m, 2H, H-2 and H-3), 5.85 (m, 1H, H-1), 5.53 (m, 1H, H-4), 2.67 (m, 1H, H-5 *cis* to OH), 2.23 (m, 1H, H-5 *trans* to OH), 2.02 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 170.7 (ester C=O), 164.2 (phthalimide C=O), 137.6 (C-2), 134.5 (aromatic C-1 and C-2), 134.2 (aromatic C-3 and C-6), 128.9 (C-3), 123.6 (aromatic C-4 and C-5), 91.9 (C-4), 78.3 (C-1), 36.5 (C-5), 21.2 (CH_3); IR (cm^{-1}, KBr) 2944 (aliphatic C−H), 1728 (phthalimido C=O), 1709 (ester C=O), 1470, 1383 (C=C). Anal. ($C_{15}H_{13}NO_5$) C, H, N.

(1*S*,4*S*)-*trans*-1-Acetoxy-4-(*N*-phthalimidooxy)-2-cyclopentene (6). A 0.100 g portion of compound 1*S*,4*R*-**5** (0.704 mmol) was converted to compound 1*S*,4*S*-**6** in 94% yield exactly as described for the preparation of the enantiomeric compound, 1*R*,4*R*-**6**. All spectral data (^{1}H-NMR, ^{13}C-NMR, and IR) were identical to those reported for 1*R*,4*R*-**6**.

(1*R*,4*R*)-*trans*-1-Acetoxy-4-aminoxy-2-cyclopentene (7). Compound 1*R*,4*R*-**6** (0.270 g, 0.940 mmol) was dissolved in 10.8 mL of 0.2 M methanolic hydrazine and allowed to reflux for 4 h. After cooling, a white precipitate was filtered off, and the solvent was removed *in vacuo*. The resulting solid was then dissolved in chloroform and refiltered. The organic layer was concentrated *in vacuo* to afford relatively pure 1*R*,4*R*-**7** (as estimated by NMR) as a yellow oil (0.141 g, 95%) which was immediately used in the subsequent step without further purification: ^{1}H-NMR ($CDCl_3$) δ 6.15 (m, 1H, H-2), 6.10 (m, 1H, H-3), 5.78 (m, 1H, H-1), 5.28 (broad, 2H, ONH_2), 4.93 (m, 1H, H-4), 2.25 (m, 1H, H-5 *cis* to OH), 2.04 (s, 3H, CH_3), 1.83 (m, 1H, H-5 *trans* to OH); ^{13}C-NMR ($CDCl_3$) δ 170.9 (C=O), 139.4 (C-2), 128.8 (C-3), 87.3 (C-4), 79.0 (C-1), 36.2 (C-5), 21.1 (CH_3); IR (cm^{-1}, KBr) 3340 (N−H), 1713 (ester C=O), 1460, 1371 (C=C).

(1*S*,4*S*)-*trans*-1-Acetoxy-4-aminoxy-2-cyclopentene (7). A 0.150 g portion (0.522 mmol) of 1*S*,4*S*-**6** was converted to 1*S*,4*S*-**7** in 99% yield exactly as described for the preparation of the enantiomeric compound, 1*R*,4*R*-**7**. All spectral data (^{1}H-NMR, ^{13}C-NMR, and IR) were identical to those reported for 1*R*,4*R*-**7**.

(1*R*,4*R*)-*trans*-1-Acetoxy-4-{*N*-[(*tert*-butyloxy)carbonyl]aminoxy}-2-cyclopentene (8). To a solution of $NaHCO_3$ (0.107 g, 1.274 mmol) and NaCl (0.186 g, 3.185 mmol) in 1.15 mL of H_2O was added 0.200 g (1.274 mmol) of compound 1*R*,4*R*-**7** in 1.15 mL of $CHCl_3$, and the mixture was cooled to 0 °C in an ice bath. A 0.278 g (1.274 mmol) portion of di-*tert*-butyl dicarbonate was added slowly, and the two-phase mixture was allowed to warm to room temperature. The reaction mixture was refluxed for 3 h and then allowed to cool, and the chloroform was removed *in vacuo*. The residual aqueous layer was diluted to 20 mL and then washed with three 20 mL portions of EtOAc. The combined organic phases were washed with 25 mL each of 5% $NaSO_4$, water, and saturated NaCl, and then dried over anhydrous $MgSO_4$. Filtration and removal of the solvent afforded a clear, colorless oil which was chromatographed on silica gel (hexane/ethyl acetate, 6:4) to give 0.239 g (89%) of 1*R*,4*R*-**8** as a white solid: ^{1}H-NMR ($CDCl_3$) δ 6.17 (m, 2H, H-2 and H-3), 5.80 (m, 1H, H-1), 5.14 (m, 1H, H-4), 2.43 (m, 1H, H-5 *cis* to OH), 2.05 (m, 1H, H-5 *trans* to OH), 2.03 (s, 3H, CH_3), 1.48 (s, 9H, C-CH_3); ^{13}C-NMR ($CDCl_3$) δ 170.8 (ester C=O), 157.1 (carbamate C=O), 136.2 (C-2), 128.8 (C-3), 88.9 (C-4), 78.6 (C-1), 68.1 (tert-C), 35.8 (C-5), 28.5 (C-CH_3), 27.4 (ester CH_3); IR (cm^{-1}, KBr) 3367 (N−H), 2924 (aliphatic C−H), 1728 (ester C=O), 1679 (carbamate C=O), 1461, 1377 (C=C). Anal. ($C_{12}H_{19}NO_5$) C, H, N.

(1*S*,4*S*)-*trans*-1-Acetoxy-4-{*N*-[(*tert*-butyloxy)carbonyl]-aminoxy}-2-cyclopentene (8). A 0.082 g (0.522 mmol) portion of 1*S*,4*S*-**7** was converted to 1*S*,4*S*-**8** in 81% yield exactly as described for the preparation of the enantiomeric compound, 1*R*,4*R*-**8**. All spectral data (^{1}H-NMR, ^{13}C-NMR, and IR) were identical to those reported for 1*R*,4*R*-**8**.

(1*R*,4*R*)-*trans*-1-Hydroxy-4-{*N*-[(tert-butyloxy)carbonyl]-aminoxy}-2-cyclopentene (9). A 0.250 g (0.972 mmol) portion of compound 1*R*,4*R*-**8** was dissolved in 19.5 mL of 75% ethanol, and 0.061 g (1.458 mmol) of $LiOH{\cdot}H_2O$ was then added with stirring. The reaction mixture was allowed to stir at room temperature overnight and was then diluted with 25 mL of water and extracted with three 25 mL portions of ethyl acetate. The combined organic layers were washed with 25 mL each of 5% $NaSO_4$, water, and saturated NaCl and then dried over anhydrous $MgSO_4$. Filtration and removal of the solvent afforded a yellow oil which was chromatographed on silica gel (hexane:ethyl acetate, 6:4) to give 0.162 g (78%) of 1*R*,4*R*-**9** as a white solid. Analysis of the product by the Mosher procedure[20] indicated the desired 1*R*,4*R*-enantiomer was present in >97% ee: ^{1}H NMR ($CDCl_3$) δ 6.15 (m, 1H, H-2), 6.06 (m, 1H, H-3), 5.15 (m, 1H, H-1), 5.04 (m, 1H, H-4), 2.38 (m, 1H, H-5 *cis* to OH), 2.10 (broad, 1H, OH), 1.95 (m, 1H, H-5 *trans* to OH), 1.48 (s, 9H, C-CH_3); ^{13}C-NMR ($CDCl_3$) δ 157.2 (carbamate C=O), 140.6 (C-2), 132.5 (C-3), 89.5 (C-4), 81.6 (C-1), 76.0 (*tert*-C), 39.3 (C-5), 28.2 (C-CH_3); IR (cm^{-1}, KBr) 3339, 3186 (N−H, O−H), 2959 (aliphatic C−H), 1698 (carbamate C=O), 1461, 1377 (C=C). Anal. ($C_{10}H_{17}NO_4$) C, H, N.

(1*S*,4*S*)-*trans*-1-Hydroxy-4-{*N*-[(*tert*-butyloxy)-carbonyl]aminoxy}-2-cyclopentene (9). A 0.109 g (0.424 mmol) portion of compound 1*S*,4*S*-**8** was converted to 1*S*,4*S*-**9** in 53% yield exactly as described for the preparation of the enantiomeric compound, 1*R*,4*R*-**9**. Analysis of the product by the Mosher procedure[20] indicated the desired 1*R*,4*R*-enantiomer was present in >97% ee. All spectral data (^{1}H-NMR, ^{13}C-NMR, and IR) were identical to those reported for 1*R*,4*R*-**9**.

(1*S*,4*R*)-*cis*-1-Chloro-4-{*N*-[(*tert*-butyloxy)carbonyl]aminoxy}-2-cyclopentene (10). A 0.047 g portion of dimethyl sulfide (0.756 mmol) was added in dropwise fashion to a cold (0 °C) solution containing 0.093 g (0.699 mmol) of *N*-chlorosuccinimide in 3.18 mL of CH_2Cl_2 under a nitrogen atmosphere. The reaction mixture was then cooled to −20 °C, and 0.136 g (0.635 mmol) of compound 1*R*,4*R*-**9** in 0.32 mL of CH_2Cl_2 was added. The solution was warmed to 0 °C, allowed to stir for 3 h, and then poured into 2 mL of ice-cold brine. The aqueous phase was extracted with three 4 mL portions of ethyl ether, and the combined organic layers were washed with 4 mL of cold brine and dried over anhydrous $MgSO_4$. The solvent was then removed *in vacuo*, and the residue was purified on silica gel (hexane:ethyl acetate, 6:4) to afford 0.059 g (40%) of compound 1*S*,4*R*-**10** as a clear, colorless oil: ^{1}H-NMR ($CDCl_3$) δ 6.12 (m, 2H, H-2 and H-3), 4.97 (m, 1H, H-1), 4.81 (m, 1H, H-4), 2.88 (m, 1H, H-5 *cis* to Cl), 2.23 (m, 1H, H-5 *trans* to Cl), 1.48 (s, 9H, C-CH_3); ^{13}C-NMR ($CDCl_3$) δ 157.1 (carbamate C=O), 137.8 (C-2), 132.7 (C-3), 88.3 (C-4), 81.7 (C-1), 60.4 (*tert*-C), 39.5 (C-5), 28.2 (C-CH_3). Although reasonable stable for short periods at low temperature, compound **10** proved to be too unstable to obtain a suitable combustion analysis and was immediately used in the subsequent step.

(1*R*,4*S*)-*cis*-1-Chloro-4-{*N*-[(*tert*-butyloxy)carbonyl]aminoxy}-2-cyclopentene (10). A 0.270 g portion of compound 1*S*,4*S*-**9** (1.255 mmol) was converted to 1*R*,4*S*-**10** in 48% yield exactly as described for the preparation of the enantiomeric compound, 1*S*,4*R*-**10**. All spectral data (^{1}H-NMR and ^{13}C-NMR) were identical to those reported for 1*S*,4*R*-**10**.

(1*R*,4*R*)-*trans*-S-(2′,3′-O-Isopropylidene-5′-deoxy-5′-adenosyl)-1-{*N*-[(*tert*-butyloxy)carbonyl]aminoxy}-4-mercapto-2-cyclopentene (12). A 0.059 g portion of 1*S*,4*R*-**10** (0.254 mmol) and 0.150 g (0.381 mmol) of 2′,3′-*O*-isopropy-

lidene-5′-deoxy-5′-thioacetyladenosine (11) were dissolved in 6.8 mL of a 1:1 mixture of dry DMF and dry methanol under an argon atmosphere. In order to remove all traces of dissolved oxygen, the solution was frozen and thawed five times in succession with liquid nitrogen while a vigorous stream of argon was bubbled through the solution. A 0.062 g (1.143 mmol) portion of sodium methoxide powder was then quickly added, and the reaction mixture was allowed to stir under argon, protected from light, for 3 days. The solvent was then removed *in vacuo* (0.5 mmHg), and the residual brown gum was partitioned between 20 mL each of water and chloroform. The aqueous layer was washed with two additional 20 mL portions of chloroform, and the combined organic layers were washed with 20 mL of saturated NaCl and dried over anhydrous $MgSO_4$. Filtration, removal of the solvent *in vacuo*, and chromatography on silica gel (ethyl acetate: 2-propanol, 24:1) afforded 1*R*,4*R*-**12** (0.104 g, 79%) as a pale yellow foam: ^{1}H-NMR ($CDCl_3$) δ 8.34 (s, 1H, H-2), 7.93 (s, 1H, H-8), 6.08 (m, 1H, H-1′), 6.01 (m, 1H, H-2″), 5.94 (m, 1H, H-3″), 5.53 (m, 1H, H-2′), 5.05 (m, 1H, H-3′), 5.00 (m, 1H, H-1″), 4.39 (m, 1H, H-4′), 3.99 (m, 1H, H-4″), 2.74 (m, 1H, H-5′), 2.40 (m, 1H, H-5″ *cis* to adenosyl), 2.04 (m, 1H, H-5″ *trans* to adensoyl), 1.61 (s, 3H, acetonide CH_3), 1.47 (s, 9H, C-CH_3) 1.40 (s, 3H, acetonide CH_3). Anal. ($C_{23}H_{32}N_6O_6S$) C, H, N.

(1*S*,4*S*)-*trans*-S-(2′,3′-*O*-Isopropylidene-5′-deoxy-5′-adenosyl)-1-{*N*-[(*tert*-butyloxy)carbonyl]aminoxy}-4-mercapto-2-cyclopentene (12). A 0.141 g portion of compound 1*R*,4*S*-**10** (0.606 mmol) was converted to 1*S*,4*S*-**12** in 72% yield exactly as described for the preparation of the diastereomeric compound, 1*R*,4*R*-**12**. All spectral data (^{1}H-NMR) were identical to those reported for 1*R*,4*R*-**12**.

(1*R*,4*R*)-*trans*-S-(5′-Deoxy-5′-adenosyl)-1-aminoxy-4-mercapto-2-cyclopentene (13). A 0.046 g portion of compound 1*R*,4*R*-**12** (0.089 mmol) was dissolved in 1.5 mL of 88% aqueous formic acid and allowed to stir at room temperature for 2 days. The reaction mixture was then poured into 20 mL of water, and the aqueous layer was washed with three 15 mL portions of diethyl ether and lyophyllized to afford a fluffy, off-white solid. This solid was chromatographed on silica gel ($CHCl_3$:MeOH:NH_4OH, 7:7:1). The fractions containing the desired product were pooled and diluted with 30 mL of water, and the resulting solution was rotary evaporated until the pH was neutral. The aqueous solution was then lyophyllized to afford 1*R*,4*R*-**13** as a white, fluffy solid (0.025 g, 72% yield): ^{1}H-NMR (CD_3OD) δ 8.31 (s, 1H, H-2), 8.21 (s, 1H, H-8), 6.00 (m, 1H, H-1′), 5.86 (m, 1H, H-2″), 5.83 (m, 1H, H-3″), 4.78 (m, 1H, H-3′), 4.32 (m, 1H, H-4′), 4.20 (m, 1H, H-1″), 3.78 (m, 1H, H-4″), 2.71 (m, 1H, H-5′), 2.12 (m, 1H, H-5″ *cis* to adenosyl group), 1.60 (m, 1H, H-5″ *trans* to adenosyl group). Anal. ($C_{15}H_{20}N_6O_4S$) C, H, N.

(1*R*,4*R*)-*trans*-S-(5′-Deoxy-5′-adenosyl)-1-aminoxy-4-mercapto-2-cyclopentene (13). A 0.250 g portion of compound 1*S*,4*S*-**12** (0.482 mmol) was converted to 1*S*,4*S*-**13** in 99% yield exactly as described for the preparation of the diastereomeric compound, 1*R*,4*R*-**13**. All spectral data (^{1}H-NMR) were identical to those reported for 1*R*,4*R*-**13**.

(1*R*,4*R*)-*trans*-S-(5′-Deoxy-5′-adenosyl)-1-aminoxy-4-(methylsulfonio)-2-cyclopentene Disulfate (4). A 0.015 g portion of compound 1*R*,4*R*-**13** (0.0396 mmol) was dissolved in 0.78 mL of a 1:1 mixture of HCOOH and CH_3COOH, and to this mixture were added 0.1 mL of iodomethane (1.596 mmol) and 0.016 g of silver perchlorate (0.079 mmol) in 0.165 mL of 1:1 HCOOH:CH_3COOH. The reaction mixture was allowed to stir at room temperature, protected from light, overnight. A yellow precipitate was removed by filtration, and the filtered solution was diluted with 25 mL of water and washed with three 20 mL portions of diethyl ether. The aqueous layer was then lyophyllized to give crude yellow 1*R*,4*R*-**4**, which was purified by chromatography on silica gel (water:acetic acid:butanol, 1:1:1). The fractions containing the product were pooled, diluted with 30 mL of water, and washed with three 20 mL portions of ethyl ether. The water layer was then lyophyllized, and the residue was applied to an anion-exchange column (Dowex 1X8-200, 100-200 mesh) which had been previously converted to the sulfate form using 1 N H_2SO_4. Column fractions containing the product were then pooled and lyophyllized to afford pure 1*R*,4*R*-**4** as a fluffy white, hygroscopic solid (0.014 g, 90%): ^{1}H-NMR (CD_3OD) δ 8.26 (m, 2H, H-2 and H-8), 6.50 (m, 1H, H-1′), 6.39 (m, 1H, H-2″), 6.26 (m, 1H, H-3″), 5.88 (m, 1H, H-2′), 5.79 (m, 1H, H-3′), 4.55 (m, 1H, H-4′), 4.02 (m, 1H, H-1″), 3.89 (m, 1H, H-4″), 2.89 (complex m, 1H, H-5′), 2.75 (s, 3H, CH_3), 2.22 (m, 1H, H-5″ *cis* toadenosyl), 1.48 (m, 1H, H-5″ *trans* to adenosyl). Anal. ($C_{16}H_{26}N_6O_{12}S_3$) C, H, N.

(1*R*,4*R*)-*trans*-S-(5′-deoxy-5′-adenosyl)-1-aminoxy-4-(methylsulfonio)-2-cyclopentene Disulfate (4). A 0.150 g portion of compound 1*S*,4*S*-**13** (0.0396 mmol) was converted to 1*S*,4*S*-**4** in 81% yield exactly as described for the preparation of the diastereomeric compound, 1*R*,4*R*-**4**. All spectral data (^{1}H-NMR) were identical to those reported for 1*R*,4*R*-**4**.

Enzyme Isolation and Assay Procedure. AdoMet-DC was isolated from *E. coli* using a modification of the methylglyoxal–bisguanylhydrazone (MGBG)-Sepharose affinity column procedure of Anton and Kutny[28] as previously described.[10] 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^{-1} mg^{-1} protein at 37 °C. The bacterial 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. Protein concentrations were measured by the method of Bradford[31] using bovine serum albumin as a standard. For the *E. coli* form of the enzyme, 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,[32] as previously described.[10]

The human form of AdoMet-DC was a generous gift from Dr. Anthony E. Pegg of the Penn State University Hershey Medical Center, Hershey, PA. For studies involving the human form of AdoMet-DC (specific activity 0.3 μmol min^{-1} mg^{-1} of protein) enzyme activity was determined using a modification of the procedure described for the bacterial enzyme assay.[10] The enzyme was incubated with the appropriate concentration of inhibitor in incubation buffer (10 mM Tris, 2.5 mM DTT, 0.1 mM EDTA, 50 μM putrescine, pH 7.5), and at various time points, aliquots were withdrawn and used to initiate the assay reaction. Each reaction mixture contained 50 μg of AdoMet-DC, 20 μL of 1 mM *S*-adenosyl-L-[^{14}C-COOH]methionine (0.9 mCi/mmol, 20 μM final concentration) in reaction buffer (0.1 M sodium phosphate, 1 mM putrescine, 2.5 mM DTT, pH 7.5), with a final volume of 1 mL. All reactions were carried out at 37 °C in a tightly closed scintillation vial, and radiolabeled CO_2 was trapped on a filter disk in the vial cap soaked with 40 μL of 1.0 M hyamine hydroxide. Assay reactions were terminated after 20 min by addition of 1 N $HClO_4$. After an additional 20 min at 37 °C, the filter disk was placed in a scintillation vial with 10 mL of scintillation cocktail and counted (counting efficiency 95% or greater). In all cases, enzyme activity (V) is expressed as micromoles of [^{14}C]CO_2 produced per minute per milligram of protein $\times$ 10^{-7}. Each data point reported represents the average of two determinations which in each case differed by less than 5%.

Kinetic Analysis of *E. coli* and Human AdoMet-DC Inhibition. Time-dependent decreases in enzyme activity were monitored for at least four concentrations of each inhibitor (typically, 5-50 μM) over a period of 25 min. The rate of irreversible inhibition was monitored by withdrawing aliquots for assay at four or five time intervals following partial inactivation in the presence of the appropriate concentration of inhibitor, and in the absence of AdoMet, as described above. In each case, the time-dependent decrease in activity (expressed as % ln A) was linear, and a pseudo-first-order rate constant was derived from each line. Replotting of the rate constants (k_{obs}) using the method of Kitz and Wilson[27] then allowed the K_i and k_{inact} values to be determined graphically.

Determination of Trypanosomal Growth Inhibition. Antitrypanosomal activity was determined on blood forms of *T. brucei brucei* grown *in vitro* using a [^{3}H]hypoxanthine incorporation assay or by direct cell count. Continuous cultures of bloodforms were initiated in a feeder layer-free system by inoculating wells of a 24-well culture dish (Falcon 3047) containing 1 mL of modified Iscove's medium[33] with 10^5

trypanosomes from mouse blood. Plates were incubated in 3% CO_2 in air at 37 °C. One half of the volume of medium was replaced daily, and trypanosomes achieved peak densities of 5×10^6 mL^{-1} in 4 days. Inhibitor sensitivity tests were done by dissolving the agent in sterile medium and replacing one half of the volume daily with medium containing double strength inhibitor. Cell counts were made daily, and IC_{50} values were calculated after 48 h exposure. Alternatively, trypanosomes were incubated in modified Iscove's medium for 24 h with varying concentrations of inhibitor, washed, and then incubated for an additional 24 h with medium containing 1.0 μCi of [^{3}H]hypoxanthine. The cells (5×10^6 per well) were then harvested by filtration, and the filters were scintillation counted. Each data point reported represents the average of two determinations which in each case differed by less than 5%.

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