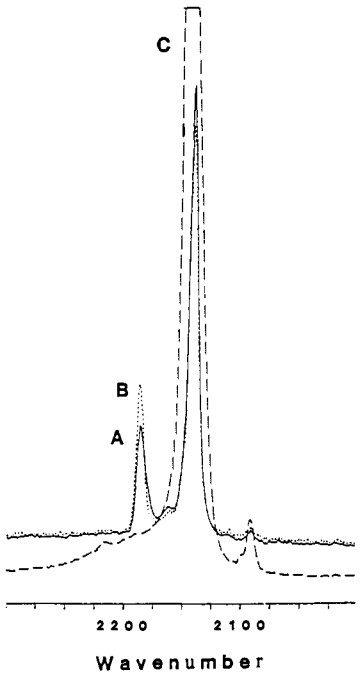

Figure 1. Infrared spectrum of trimethylaluminum and carbon monoxide. A: TMA + CO in argon. B: After warming matrix to allow migration. C: CO without TMA.

report a monomer:dimer ratio of 7:1 with no heating of the gas prior to deposition and an increase to >200:1 when the gas is heated to 350 °C.

Attempts to observe the complex at room temperature using an IBM 95 FTIR instrument with 0.25-cm^{-1} resolution revealed no observable band at 2185 cm^{-1}. Ab initio calculations indicate that the oscillator strengths for complexed CO and free CO are nearly identical. With the sensitivity of this instrument at pressures of 0.1 atm for CO and 0.01 atm for $AlMe_3$, we would detect the complex if the equilibrium constant for complex formation were 1 or greater. Assuming $\Delta S \approx -40$ cal K^{-1} mol^{-1} for formation of the complex gives, as an upper limit for the exothermicity of complex formation, $\Delta H = -12$ kcal mol^{-1}. We did not observe any ultraviolet absorption above 200 nm in experiments carried out on a similar apparatus using a sapphire window.

In order to gain further insight into the nature of the aluminum–carbon monoxide bonding, MNDO and ab initio calculations were carried out with different substituents bonded to aluminum. Using the MOPAC4 program, several features of the reaction system were calculated: (1) the enthalpy change associated with formation of the complex; (2) the structure of the complex; (3) the vibrational frequencies and intensities; and (4) charge distribution.

These computational results indicate that as carbon monoxide approaches the aluminum atom in TMA from a distance of 4.0 Å, the energy decreases with a barrier less than 1 kcal mol^{-1} to a minimum at a distance of 1.86 Å with an overall decrease in energy of 28 kcal mol^{-1}. The angle from CH_3 to Al to CO is 104°, as would be expected from theory when the aluminum atom accepts the lone pair from CO and rehybridizes toward sp^3. The most intense vibration was calculated to be the CO stretch at 2430 cm^{-1}. By comparison, the stretch in free CO was calculated to be 2382 cm^{-1}. The formation of the complex results in an increase in wavenumber of the CO stretch of 48 cm^{-1}.

The ab initio calculations of AlH_3–CO at the 6-31G* level gave similar results. The calculated binding energy of CO to trimethylaluminum is 7 kcal mol^{-1}, which is substantially less than the value calculated by MNDO. The calculated Al–CO bond length is 2.37 Å. The calculated CO stretching wavenumber is 2506 cm^{-1} compared to a calculated value of 2438 cm^{-1} for free CO: an increase of 68 cm^{-1}.

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The increase of the CO stretching frequency upon complex formation with TMA is an indication of a difference in the bonding compared with that of CO bonded to Al atoms or surfaces. In the latter cases, P_π–π^* bonding is significant and contributes to a weakening of the CO bond.[7] In the case of the complex with TMA, the interaction is primarily that of the lone pair on carbon, the 5σ orbital of CO, occupying the vacant orbital on aluminum. Removal of electron density from this orbital on CO, which is weakly antibonding, results in an increase in the CO bond strength. This effect is noted in both the ab initio and MNDO calculations.

A similar increase in CO stretching frequency is found in BH_3·CO, which has a CO stretch at 2165 cm^{-1} in the gas phase.[8] Thus, both BH_3 and $Al(CH_3)_3$ complexes have little contribution to bonding from P_π–π^* interaction.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for the support of this research. We also acknowledge support from the NSF, the Pew Memorial Trust, the Dreyfus Foundation, and the Duke Endowment.

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5′-{[(*Z*)-4-Amino-2-butenyl]methylamino}-5′-deoxyadenosine: A Potent Enzyme-Activated Irreversible Inhibitor of *S*-Adenosyl-L-methionine Decarboxylase from *Escherichia coli*

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The polyamine biosynthetic pathway is an important target for the design of chemotherapeutic agents.[1] The actual rate-limiting step in the formation of spermidine and spermine is catalyzed by *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50, AdoMetDC), a pyruvoyl-containing enzyme.[2] A number of reversible and irreversible potent AdoMetDC inhibitors have been reported.[3] However, they were neither sufficiently potent, nor sufficiently stable, nor sufficiently selective to permit investigation of AdoMetDC importance in cellular physiology in vivo.[3] An enzyme-activated irreversible inhibitor may fulfill these requirements.

The mechanism of *Escherichia coli* AdoMetDC inactivation by AdoMet was recently demonstrated.[4] It involves the transamination of the nascent reaction product to the pyruvoyl group, followed by the elimination of methylthioadenosine and the generation of the Michael acceptor 2-propenal, which could alkylate a nucleophilic residue in the active site. Furthermore, it has been recognized that analogues of decarboxylated AdoMet with a nitrogen atom in place of the sulfur produced also some time-dependent inhibition of mammalian AdoMetDC.[5]

On the basis of these data, we have designed 5′-{[(*Z*)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine (**1**) as a potential enzyme-activated irreversible inhibitor of AdoMetDC.

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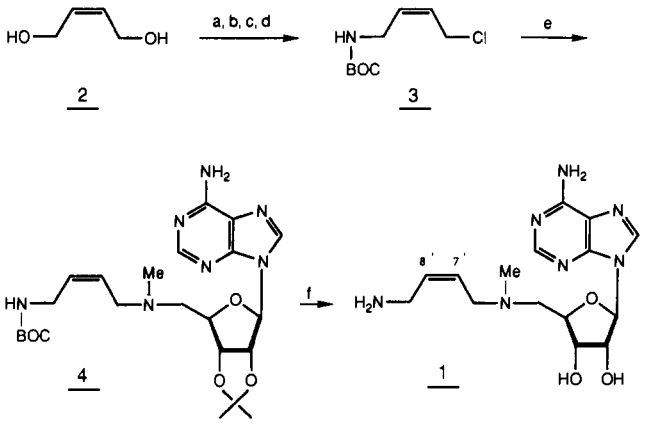
Scheme I[a]



[a] (a) Dihydropyran, CH_2Cl_2, 0 °C, 2 days. (b) Phthalimide, PPh_3, diethyl azodicarboxylate, THF, room temperature, 12 h. (c) (1) NH_2NH_2, EtOH, Δ, 12 h; (2) 1 N HCl, Δ, 2 h; (3) $(BOC)_2O$, NEt_3, CH_2Cl_2, Δ, 12 h. (d) MsCl, NEt_3, CH_2Cl_2, room temperature, 12 h. (e) 5′-(Methylamino)-5′-deoxy-2′,3′-isopropylideneadenosine, K_2CO_3, CH_3CN, Δ, 12 h. (f) (1) 1 N H_2SO_4, room temperature, 2 days; (2) EtOH.

Here, we report the synthesis of **1** and we show that the compound is an extremely potent and effective inhibitor of AdoMetDC purified from *E. coli*.[6]

The synthetic access to **1** is depicted in Scheme I. 5′-N-Alkylation of 5′-(methylamino)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine[8] with (*Z*)-*N*-(*tert*-butoxycarbonyl)-4-chloro-2-butenamine (**3**) in acetonitrile and a stoichiometric amount of potassium carbonate gives the adduct 5′-{[(*Z*)-4-[(*tert*-butoxycarbonyl)amino]-2-butenyl]methylamino}-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**4**) in 55% yield. The acid-labile protecting groups in **4** were removed simultaneously in a one-pot reaction by treatment with 1 N H_2SO_4, to afford **1** in 60% yield after recrystallization in ethanol.[9] The chloride **3** was readily synthesized in four steps from (*Z*)-2-butene-1,4-diol (**2**) in 15% overall yield.[10]

Incubation of *E. coli* AdoMetDC with **1** resulted in a time-dependent loss of enzyme activity, which followed pseudo-first-order kinetics. A Kitz and Wilson replot of the data[11] indicated that saturation was attained. The K_i value for **1** was 0.3 ± 0.1 μM, and the k_{inact} value was 3.6 ± 0.9 min^{-1}. MGBG, a competitive inhibitor, protected the enzyme from inactivation. Furthermore, the inactivation was not detectable when Mg^{2+}, which is required for enzyme activity,[4] was omitted. These results demonstrate that the inactivation takes place in the enzyme active site. The presence of dithiothreitol (1 mM) in the preincubation medium ruled out the possibillity that the species responsible for inactivation was released from the enzyme active site.[12] Incubation with 0.2 μM **1** for 14 min at 37 °C resulted in complete

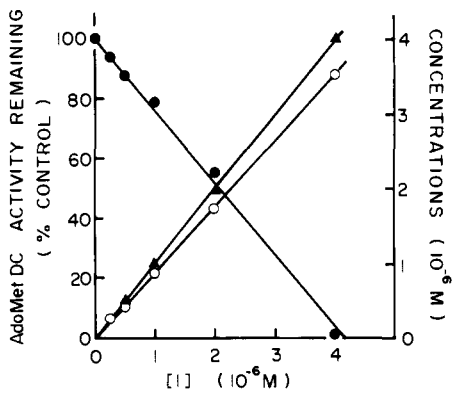
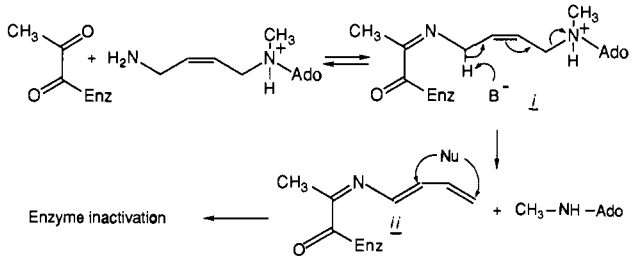


Figure 1. AdoMetDC inactivation (●), consumption of **1** (▲), and formation of 5′-(methylamino)-5′-deoxyadenosine (○) as a function of the initial concentrations of **1**. AdoMetDC (final concentration: 4 μM) was incubated at 37 °C with various concentrations of **1** between 0 and 4 μM. After a 10-min incubation, two aliquots were withdrawn in parallel: (1) 5-μL aliquots, which were diluted in 295 μL of 50 mM HEPES buffer (pH 7.4), 50 μL of this mixture serving to measure enzyme activity remaining;[7] (2) 25-μL aliquots, to which was added 5 μL of 0.05 N $HClO_4$ to halt the reaction. Reversed-phase HPLC analysis of **1** and 5′-(methylamino)-5′-deoxyadenosine was performed after injection of 20 μL of these mixtures.[13]

Scheme II



inactivation of the enzyme. Prolonged dialysis of this inactivated enzyme for 24 h at 4 °C did not produce any recovery of enzyme activity, suggesting the formation of a covalent linkage of the inhibitor to the enzyme active site.

In a separate experiment, we studied the decrease of enzyme activity as a function of the inhibitor concentration. Results shown in Figure 1 demonstrate that the stoichiometry of enzyme inactivation and consumption of **1** is close to one. We identified by HPLC the nucleoside product of the inactivation as being 5′-(methylamino)-5′-deoxyadenosine.[13] This product was formed with a quasi-stoichiometry (Figure 1). Scheme II represents the mechanism we propose to explain these results.

Recognition of the protonated form of the 5′-tertiary amine of **1** should be favored by AdoMetDC, which recognizes a sulfonium on the natural substrate. Formation of the Schiff base i between the pyruvoyl prosthetic group of the enzyme and **1** would be followed by abstraction of an α proton of **1**. This should facilitate the elimination of 5′-(methylamino)-5′-deoxyadenosine and result in the formation of the conjugated imine ii, which could undergo addition of a nucleophilic residue of the enzyme. Finally, it is worth noting that we found the trans isomer[14] of **1** as well as various substituted derivatives of 5′-[(3-aminopropyl)-methylamino]-5′-deoxyadenosine[15] to be at least 1000-fold less

(6) The enzyme was purified essentially as described in ref 7 from *E. coli* MRE 600 cell paste purchased from the Public Health Laboratory Service, Porton Product Ltd., Salisbury, Wilts, U.K. The specific activity of the purified AdoMetDC was 1.4 units/mg (micromoles/minute per milligram) at 37 °C.

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(9) **1**: ^1H NMR (0.1 N NaOD, 360 MHz, TSP as reference, *J* in Hz) δ (ppm) 2.37 (s, 3 H, CH_3N), 2.92 (d, 2 H, $H_{5'}$, $J(H_{5'}H_{4'})$ = 5.70), 3.30 (d, 2 H, $H_{6'}$, $J(H_{6'}H_{7'})$ = 6.70), 3.65 (m, 2 H, $H_{9'}$, $J(H_{9'A}H_{9'B})$ = 14.20, $J(H_{9'A}H_{8'})$ = $J(H_{9'B}H_{8'})$ = 6.85), 4.22 (dd, 1 H, $H_{3'}$, $J(H_{3'}H_{2'})$ = 5.30, $J(H_{3'}H_{4'})$ = 5.45), 4.31 (dt, 1 H, $H_{4'}$, $J(H_{4'}H_{3'})$ = 5.45, $J(H_{4'}H_{5'})$ = 5.70), 4.68 (dd, 1 H, $H_{2'}$, $J(H_{2'}H_{1'})$ = 4.45, $J(H_{2'}H_{3'})$ = 5.30), 5.73 (dt, 1 H, $H_{8'}$, $J(H_{8'}H_{7'})$ = 11.20, $J(H_{8'}H_{9'})$ = 6.85), 5.81 (dt, 1 H, $H_{7'}$, $J(H_{7'}H_{8'})$ = 11.20, $J(H_{7'}H_{6'})$ = 6.70), 5.96 (d, 1 H, $H_{1'}$, $J(H_{1'}H_{2'})$ = 4.45), 8.01 (s, 1 H, H_2), 8.16 (s, 1 H, H_8); MS (FAB, Xe) *m/e* 350 MH^+. Elemental anal. found for $C_{15}H_{23}N_7O_3 \cdot 1.5H_2SO_4 \cdot 1H_2O$: C, 35.02; H, 5.49; N, 19.06. Calcd: C, 34.60; H, 5.55; N, 18.83.

(10) NMR spectra, IR spectra, CHN analysis, or high-resolution MS agreed with the proposed structure of all the intermediates.

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(13) 5′-(Methylamino)-5′-deoxyadenosine and **1** were determined according to the HPLC method previously developed for decarboxylated AdoMet analogue determination (Wagner, J.; Hirth, Y.; Claverie, N.; Danzin, C. *Anal. Biochem.* **1986**, *154*, 604–617). Authentic 5′-(methylamino)-5′-deoxyadenosine was readily obtained by treatment of **1** with 1 N H_2SO_4 overnight and precipitation by ethanol.

(14) The *E* isomer of **1** was synthesized through a sequence similar to that described in Scheme I, starting from (*E*)-1,4-dichlorobutene. The ^1H NMR spectrum of the *E* isomer differed mainly from the spectrum of **1** in the chemical shifts of the CH_3N signal (δ = 2.42 ppm) and the vinylic protons $H_{7'}$ and $H_{8'}$ (m, δ = 5.94 ppm). On the basis of the integration of the CH_3N signals, each isomer contained less than 1% of the other one.

active than **1** as time-dependent inhibitors of *E. coli* AdoMetDC, showing the importance of the steric factors in the inactivation process.

In conclusion, inactivation of *E. coli* AdoMetDC by **1** represents the first example of potent enzyme-activated irreversible inhibition of a pyruvoyl enzyme. Inactivation studies on AdoMetDC prepared from rat tissues will be described elsewhere.

Acknowledgment. We thank Annie Clauss for technical assistance in the synthesis of the described compounds.

Registry No. 1, 123642-27-3; **2**, 6117-80-2; **3**, 123642-28-4; **4**, 123642-29-5; 5'-(methylamino)-5'-deoxy-2',3'-*O*-isopropylideneadenosine, 34245-49-3; adenosylmethionine decarboxylase, 9036-20-8.

(15) These compounds were 5'-[(3-amino-4-pentenyl)methylamino]-5'-deoxyadenosine; 5'-[(3-amino-4-pentynyl)methylamino]-5'-deoxyadenosine; 5'-[(3-amino-4,5-hexadienyl)methylamino]-5'-deoxyadenosine; 5'-[(3-amino-4-fluorobutyl)methylamino]-5'-deoxyadenosine. Detailed data regarding these compounds will be published elsewhere.

Synthesis and X-ray Crystal Structure of the Zirconocene Complex of a Cyclopentyne and Its Use To Prepare Bicyclic Cyclopentenones

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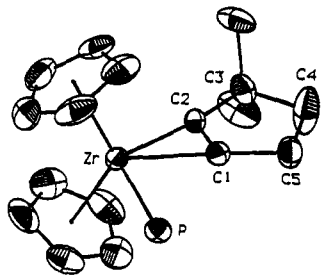
Cyclohexyne is the smallest cyclic alkyne to be prepared in a form stabilized by complexation to a transition metal.[2-4] We now describe the preparation, characterization by X-ray crystallography, and a preliminary study of the reactivity of the zirconocene complex of 5,5-dimethylcyclopentyne stabilized as its trimethylphosphine adduct.

We recently reported the preparation of the zirconocene complex of cyclohexyne **2**.[2] Compound **2** was formed via loss of methane from **1** with $\tau_{1/2}$ = 40 min at 20 °C (Scheme I). Studies of the reactions of **2** indicated that this compound experienced little angle distortion, the strain being relieved by the π-backbonding from the electron-rich zirconium center. The ease of formation of **2** and its relatively unstrained structure suggested that zirconocene complexes of even smaller cyclic alkynes ought to be accessible. Thus, we were surprised when (1-cyclopentenyl)methylzirconocene (**3**) did not lose methane to form the corresponding cyclopentyne complex **4**, even upon prolonged heating at elevated temperatures (120 °C). Subsequent work indicated that having sufficient overlap of the vinyl C-H bond with the Zr-centered LUMO was the key to inducing methane loss.[5,6] In order to probe whether a derivative of **3** could be prepared which possessed the necessary interaction of the C-H bond with the LUMO, we synthesized (5,5-dimethylcyclopentenyl)methylzirconocene (**5**). Thermolysis of **5**, at 90 °C ($\tau_{1/2}$ = 4 h), in the presence of excess trimethylphosphine provides a 48% isolated yield of complex **6**, which has been characterized by ^{1}H, ^{13}C, and ^{31}P NMR, X-ray crystallography, IR, and combustion analysis. The X-ray crystal structure of **6** is shown in Figure 1. It is interesting to note that the presence of the geminal dimethyl groups at C-3 does not cause a perceptible lengthening, in the solid state, of the C2-Zr bond relative to the analogous bond in **2**.[2] We believe, however, that the presence of these methyl groups in **5** effects the necessary overlap by causing the movement of C3 away from the Cp_2Zr fragment, with a concomitant decrease in the distance between C1 and the Cp_2Zr unit as shown in Scheme I.[7]



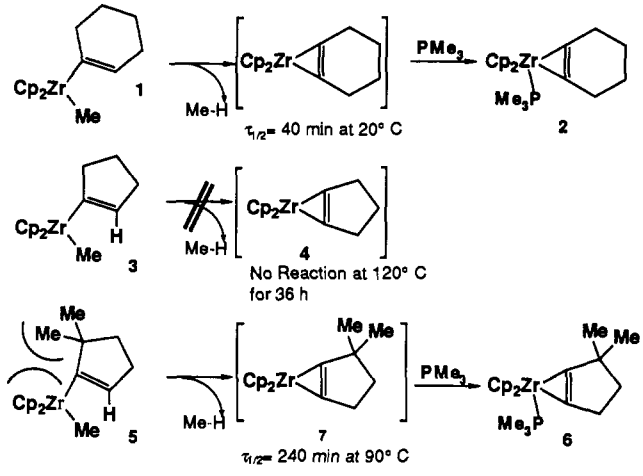
Selected Bond Lengths and Angles

Zr-C1	2.201(4)Å
Zr-C2	2.169(4)
C1-C2	1.295(6)
C1-C5	1.528(6)
C2-C3	1.515(6)
Zr-P	2.698(2)
C1-Zr-C2	34.5(2)°
C1-C2-C3	114.4(4)
C2-C1-C5	111.1(5)

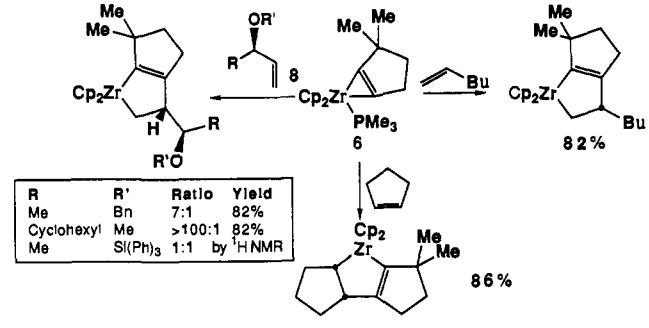
Carbons removed from Phosphorus for clarity

Figure 1.

Scheme I



Scheme II



R	R'	Ratio	Yield
Me	Bn	7:1	82%
Cyclohexyl	Me	>100:1	82%
Me	Si(Ph)3	1:1	by ^{1}H NMR

Complex **6** and its ligand-free version **7**, which can be generated and used in situ, manifest a number of important and synthetically useful differences in reactivity as compared to **2**. In particular, **2** fails to react with olefins at room temperature. The corresponding ligand-free complex of **2**, generated in situ, reacts at room temperature with 1-hexene to give a ca. 1:1 mixture of regioi-

(1) Camille & Henry Dreyfus Teacher-Scholar Awardee, 1989-1994; American Cancer Society Junior Faculty Research Awardee, 1987-1989; Eli Lilly Grantee, 1988-1990; Alfred P. Sloan Research Fellow, 1988-1990; Union Carbide Innovation Recognition Awardee, 1989.

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