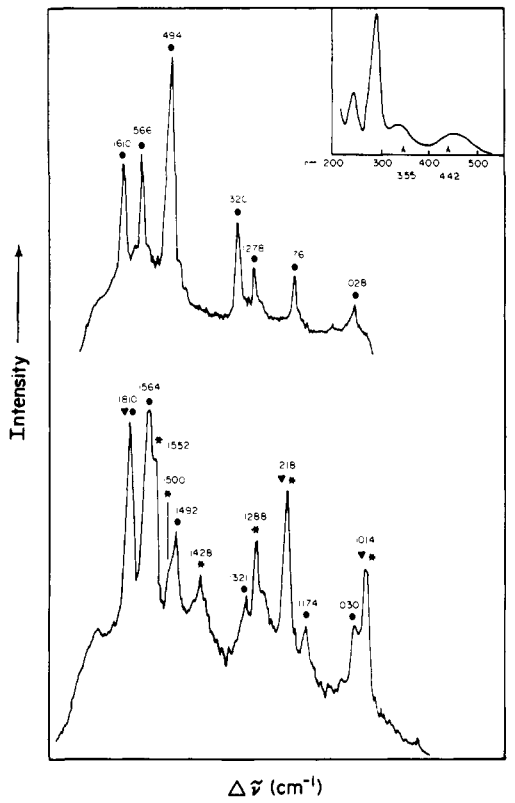

Figure 2. Resonance Raman spectra of deoxygenated 10^{-4} M aqueous solutions of $Ru^{II}(bpy)_2(py)_2(BF_4)_2$. Top frame: under CW excitation at 441.6 nm. Bottom frame: under pulsed excitation at 354.7 nm. Wavenumber shifts are given above the peaks. Key to band assignments: (●) bpy, (*) $bpy^{-\cdot}$, (▼) py. (Inset: Absorption spectrum in the 250–600-nm region.)

is observed at this excitation wavelength, which is resonant with metal-to-bpy CT absorption. The bottom frame of Figure 2 displays the RR spectrum of **3** upon excitation with ~5-mJ pulses at 355 nm. In comparison to the top frame, the more intense, higher energy illumination produces many additional peaks and gives rise to strong variations in relative peak intensities. Although **3** is being excited in resonance with Ru-to-py CT absorption, rapid internal energy relaxation apparently leaves a significant proportion of the complex in its lowest MLCT excited state, such that RR scattering characteristic of bpy* is observed. However, the spectrum differs from that of **1** excited under similar conditions[1,2,4,5] in that ground-state bpy scattering predominates for **3**. (For example, the excited-state peak at 1552 cm^{-1} is much stronger than the ground-state mode at 1564 cm^{-1} in the RR spectrum of **1** excited by 355-nm laser pulses.) Moreover, the intensity pattern is altered by the underlying presence of ground-state py scattering.[7] The origins of the peaks are denoted directly in the figure.

The wavennumber shifts and relative intensities of the Raman peaks observed for **3** under 355-nm excitation suggest that the localized exctation model applies as well to this mixed ligand system; i.e., the species responsible for the excited state scattering can be represented as $Ru^{III}(bpy^{-\cdot})(bpy)(py)_2^{2+}$ (**3***). Because the excitation is resonant primarily to metal-to-py CT absorption and alternative decay channels exist (e.g., photochemistry, internal conversion), the relative population of **3*** suffers in comparison to the corresponding state in the tris bpy complex. The Raman probe photons in a given laser pulse encounter a larger concentration of ground-state molecules in **3** than in **1**, giving rise to a relatively large contribution to the Raman scattering from $Ru^{II}(bpy)_2(py)_2^{2+}$. Within the limitations set by the time scale of these experiments and the requirement that the pump radiation used to excite the sample molecules be in resonance with an absorption of the electronically excited species so prepared, our results indicate that the localized excitation model previously advanced for $Ru(bpy)_3^{2+}$ and its analogues applies also to transition-metal complexes having multiple ligands with different low-lying π^* levels.

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Registry No. $Ru^{II}(bpy)_2(acpy)_2(BF_4)_2$, 94570-84-0; $Ru^{II}(bpy)_2(py)_2(BF_4)_2$, 94596-79-9.

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Studies of Enzyme Stereochemistry. Elucidation of the Stereochemistry of the Reaction Catalyzed by *S*-Adenosylhomocysteine Hydrolase

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S-Adenosylhomocysteine (SAH, **1**) (Scheme I) is a product of biological transmethylation reactions that utilize *S*-adenosylmethionine as methyl donor. SAH acts as a potent inhibitor of most methyltransferases thus far examined and this finding has led to proposals that SAH plays a regulatory role in vivo.[1] The only known mechanism for the catabolism of SAH in eucaryotic cells is via its reversible hydrolysis to adenosine (**2**) and homocysteine (**3**) catalyzed by the enzyme *S*-adenosylhomocysteine hydrolase (Scheme I). This enzyme was first isolated from rat liver[2] and subsequently found to occur in a variety of eucaryotes[3] and procaryotes.[4] The inhibitory effects of SAH and the fact that adenosine is cytotoxic to individuals lacking adenosine deaminase have made SAH hydrolase an attractive target for pharmacological studies.[5] The enzyme from beef liver has been crystallized,[3d] and elegant mechanistic studies have been carried out.[3e] These studies established that the enzyme contains bound NAD and that the cleavage of SAH to homocysteine and adenosine is accomplished by oxidation of the 3′-hydroxyl group of SAH followed by β-elimination of homocysteine to yield 3′-keto-4′,5′-dehydro-5′-deoxyadenosine. The latter substance then undergoes a Michael-type addition of water to produce 3′-ketoadenosine, which is finally reduced to adenosine.[3e] The importance of *S*-adenosylhomocysteine hydrolase in mammalian systems and its novel mechanistic features prompted us to carry out a stereochemical analysis whose results are summarized here.

The analysis was carried out in two stages. The first stage began with the synthesis of (5′*S*)- and (5′*R*)-(5′-$^2H_1$)-*S*-adenosyl-

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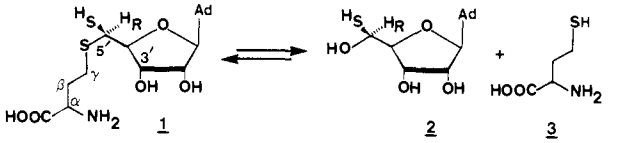
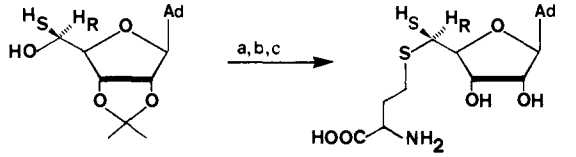
Scheme I



Scheme II

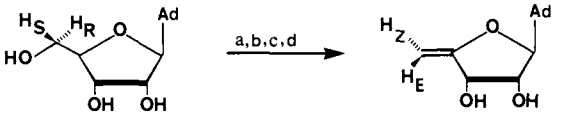


4 H_R=D, H_S=H 6 H_R=H, H_S=D

5 H_R=H, H_S=D 7 H_R=D, H_S=H

[a] TsCl, C_5H_5N. [b] Na, NH_3, L-Homocystine. [c] H^+

Scheme III



8 H_R=D, H_S=H 10 H_Z=D, H_E=H

9 H_R=H, H_S=D 11 H_Z=H, H_E=D

[a] $SOCl_2$, HMPA. [b] $PhSe^-$. [c] H_2O_2. [d] Δ

homocysteine (**6** and **7**) from (5′*R*)- and (5′*S*)-(5′-$^2H_1$)-*O*-2′,3′-isopropylideneadenosine (**4** and **5**)[6] via literature methods (Scheme II).[7] The 270-mHz proton NMR spectra of **6** and **7** are shown in Figure 1. It is clear from these spectra that **6** and **7** are diastereotopically deuterated at C-5′. The configuration assigned to **6** and **7** at C-5′ is based upon the expectation that displacement at C-5′ will proceed with inversion of configuration.[8]

Samples of chirally deuterated SAH having been prepared, (5′*R*)-(5′-$^2H_1$)- and (5′*S*)-(5′$^2H_1$)-adenosine[6] were incubated with purified *S*-adenosylhomocysteine hydrolase isolated from beef liver.[3d] The two samples of chirally deuterated SAH produced by the enzyme were isolated, and their chirality was determined by comparison of their NMR spectra with those of the reference compounds. The results of the comparison indicated that the conversion of adenosine to SAH by *S*-adenosylhomocysteine hydrolase occurs with overall *retention* of configuration at C-5′.

A complete stereochemical analysis of the reaction catalyzed by SAH hydrolase requires elucidation of the stereochemistry of one of the elimination or the addition steps. The key to the elucidation is provided by the observation[3e] that 4′,5′-dehydro-5′-deoxyadenosine serves as a substrate for the enzyme. Elucidation of the stereochemistry of the addition reaction involving homocysteine was accomplished by using (*Z*)- and (*E*)-(5′-$^2H_1$)-4′,5′-dehydroadenosine (**10** and **11**) as substrates. These

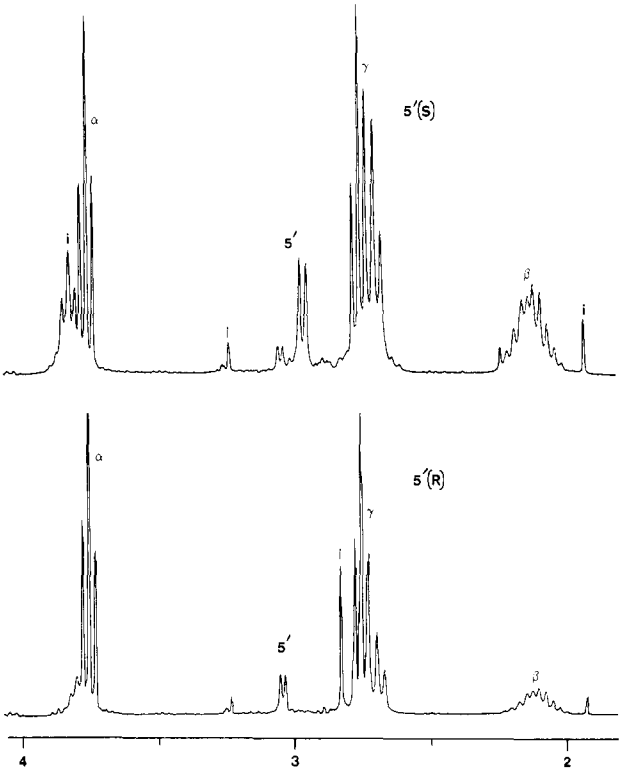


Figure 1. ^1H NMR spectra (270 MHz) (D_2O) of (5′-$^2H_1$)-*S*-adenosylhomocysteine (i = impurity).

specifically labeled forms of 4′,5′-dehydroadenosine were synthesized from (5′*R*)- and (5′*S*)-(5′-$^2H_1$)-adenosine (**8** and **9**)[6] via (5′*S*)- and (5′*R*)-(5′-$^2H_1$)-5′-chloroadenosine,[11] using the chemistry of Zylber et al.[12] (Scheme III). The stereochemistry assigned to **10** and **11** is based upon the fact that H_Z exhibits a larger coupling constant to the 3′-hydrogen than H_E,[6] and it is consistent with inversion in the phenyl selenide displacement step and syn elimination[13] of the selenoxide.

Incubation of **10** and **11** with *S*-adenosylhomocysteine hydrolase and L-homocysteine yielded two samples of chirally deuterated SAH. NMR analysis of these two samples showed that **10** yields (5′*R*)-(5′-$^2H_1$)-SAH while **11** yields the 5′*S* derivative. The addition of homocysteine to 3-keto-4′,5′-dehydro-5′-deoxyadenosine therefore takes place with syn geometry. Since the overall reaction catalyzed by SAH hydrolase proceeds with retention of configuration at C-5′, it follows that the elimination step catalyzed by the enzyme must also exhibit syn geometry. These results suggest a single base mechanism and provide an interesting parallel with the β-elimination–addition reactions of α-amino acids catalyzed by pyridoxal phosphate since the latter also proceed with syn geometry at each step.[14]

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Supplementary Material Available: ^1H NMR spectra (270 mHz) of SAH and of labeled SAH derived from **4**, **5**, **10**, and **11** (5 pages). Ordering information is given on any current masthead page.

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