4',5'-Unsaturated 5'-Fluoroadenosine Nucleosides: Potent Mechanism-Based Inhibitors of S-Adenosyl-L-homocysteine Hydrolase

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The inhibition of S-adenosyl-L-homocysteine (SAH) hydrolase (EC 3.3.1.1) is an attractive target for developing antiviral agents in general and antiretroviral agents in particular.[1] The enzyme catalyzes the hydrolytic cleavage of SAH (**1**) to adenosine (**6**) and L-homocysteine (Scheme I). It regulates biological methylation reactions indirectly by controlling intracellular levels of SAH, which is a potent inhibitor of S-adenosylmethione-(SAM)-dependent transmethylation reactions.[1b,c,2] For proper binding to ribosomes the m-RNA of many viruses requires methyl group 5'-capping, which is catalyzed by SAM-dependent transmethylases.[1e,3]

Palmer and Abeles[4] established that SAH hydrolase contains bound NAD. During the conversion of SAH to adenosine NAD is reduced to NADH, while the 3'-hydroxy of the substrate is oxidized leading to a 3'-keto-5'-methylene intermediate (**2a**). It was of interest to us that 4',5'-didehydro-5'-deoxyadenosine (**4**)[5] is a substrate for SAH hydrolase and is converted to both adenosine (**6**) and SAH (**1**) via **2a** (Scheme I).[4] This led us to design the corresponding vinyl fluorides **10** and **11** as potential mechanism-based inhibitors of SAH hydrolase. Enzymatic turnover of **10** and **11** (depicted for **10** in Scheme I) would give a β-fluoro-α,β-unsaturated ketone (**2b**) which is susceptible to addition of an enzyme nucleophile followed by elimination of fluoride in a Michael-type reaction to provide **3**. Natural products or analogues thereof have recently been reported to be time-dependent inhibitors of this enzyme.[1e,e,f,6] However, these compounds apparently inactivate SAH hydrolase by reduction of the tightly bound cofactor NAD to form a stable E·NADH complex or by virtue of a tight binding complex of enzyme and product. (For a discussion, see ref 1f and 7.) We now wish to report the synthesis of **10** and **11** and their inhibitory activity toward purified SAH hydrolase from mouse liver and rat liver.

Two synthetic routes to **10** and **11** are outlined in Scheme II, and full synthetic procedures are available in the Supplementary Material section. Key steps in the first route include the conversion of N^6,N^6-bis-benzoyl-2',3'-O-isopropylideneadenosine-5'-aldehyde[8] to **7** with 3 equiv of diethylaminosulfur trifluoride (DAST) in 18% yield and dehydrofluorination of **7** to a 2:1 mixture of geometric isomers **8** with KO-*t*-Bu in DMSO. The geometric isomers **9** were separated by flash chromatography. Alternatively, isopropylideneadenosine was treated with *p*-anisylidenedisulfide under the conditions of Nakagawa[9] to provide the thioether **12** in 98% yield. Either diastereomeric sulfoxide **13** or the mixture was transformed to the same diastereomeric mixture of fluorosulfoxides **15** (54%) by our recently reported method.[10] The vinyl fluorides **10** and **11** were separated on a Dekker column[11] (MeOH). NOE experiments established the configuration of each isomer.

Partially purified SAH hydrolase was prepared from mouse liver, and the enzyme activity was assayed by following the formation of tritiated SAH as previously described.[12] Pseudo-first-order loss of SAH hydrolase activity was observed on incubation with varying concentrations of **10** (see Figure 1, Supplementary Material for plot of log percent of enzyme activity remaining vs time). The kinetic parameters k_{inact} and K_I (Table I) were estimated from a plot of $t_{1/2}$ vs $[I]^{-1}$.[13] Adenosine-protected SAH hydrolase from inhibition by **10**, and neither extensive dialysis, gel filtration, nor addition of NAD reversed the inhibition. When **10** was mixed with purified enzyme from rat liver, the formation of NADH (stoichiometric with the amount of enzyme present) was observed spectrophotometrically. This is consistent with the oxidation of the 3'-hydroxy group of **10** to a 3'-keto group. Incubation of rat liver SAH hydrolase with tritiated **10**[14] followed by gel filtration yielded labeled protein (0.8 mol of inhibitor per mol of enzyme subunit). These facts suggest that **10** and **11** are mechanism-based substrates for SAH hydrolase.

The proposed scheme of inactivation of SAH hydrolase by **10** by the formation of a covalent bond to the enzyme is further supported by the release of 1 mol of fluoride anion per mol of enzyme inactivated as demonstrated by ^{19}F NMR (δ -118.8 ppm vs $CFCl_3$, singlet) when a 7.7-fold excess of **10** was incubated with 12 μM purified SAH hydrolase from rat liver for 10 min. Unreacted **10** was shown to still be present by ^{19}F NMR (δ -166.76 vs $CFCl_3$, doublet, J = 75.2 Hz) in a ratio of 5.7 of **10** to 1 of fluoride anion. This result also shows that the partition ratio is close to zero, i.e., that every turnover leads to an inactivation event since the integrals for vinyl fluoride vs fluoride anion in the ^{19}F NMR would be predicted to be 6.7:1 for a partition ratio of zero. It is interesting to note that the higher reactivity (k_{inact}/K_I) of **10** vs **11** is similar to kinetic results reported for the attack of nucleophiles on (*E*)-β-halo-α,β-unsaturated ketones vs the (*Z*) geometric isomer.[15]

Antiretroviral activity was determined against Moloney leukemia virus (MoLV) in the XC plaque assay as previously described.[16] Compounds were added 2 h prior to the addition of virus, and both **10** and **11** were effective inhibitors of replication in vitro, with IC_{50}'s of 50 and 250 ng/mL, respectively. It should be noted that increased potency of **10** over **11** correlates with a higher k_{inact}/K_I for **10** (Table I).

In summary, a novel class of mechanism-based inhibitors of SAH hydrolase have been designed based on the enzymatic pathway for the conversion of SAH to adenosine With the recent assignment of the sequence of SAH hydrolyase from rat liver[17]

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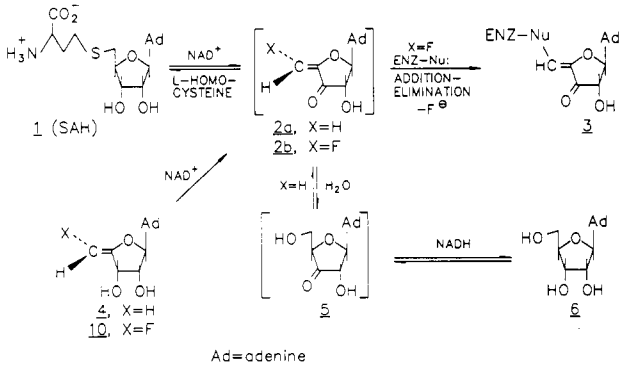
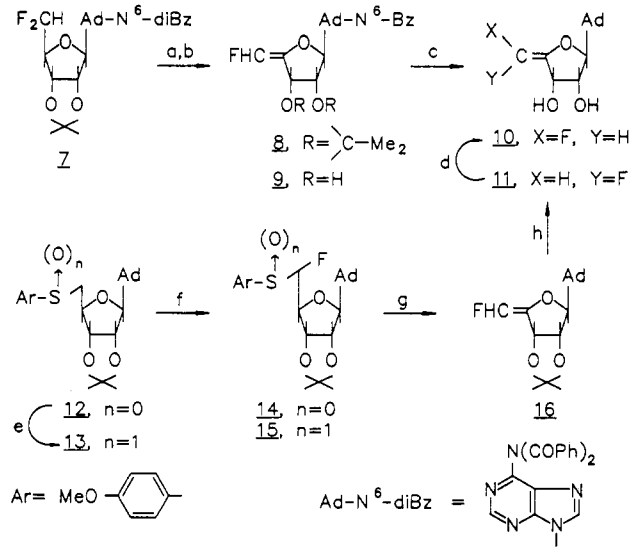
Scheme I. Mechanism of Action of SAH Hydrolase and the Incorporation of **4** and **10** into the Enzymatic Pathway



Scheme II[a]



[a] Reagents: (a) KO-*t*-Bu, DMSO (59%); (b) 80% TFA, flash chromatography (*E*, 20%) (*Z*, 42%); (c) NH_3, EtOH (**10**, 61%) (**11**, 68%); (d) *hν*, sunlamp, acetone, MeOH (60%), (e) MCPBA (86%); (f) DAST; MCPBA (54%); (g) 140 °C, diglyme (77%); (h) 75% TFA, Dekker column (**10**, 48%) (**11**, 19%).

Table I. Kinetic Constants for SAH-Hydrolase Inhibitors

compound	K_I (μM)	k_{inact} (min^{-1})	k_{inact}/K_I (M^{-1} min^{-1})
10	0.55	0.277	504 000
11	1.04	0.23	221 000

and the availability of crystalline enzyme,[18] it may be possible to define the active site of the enzyme with these inhibitors.

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Supplementary Material Available: Figure 1, which shows time- and concentration-dependent inhibition of SAH hydrolase by **10**, and experimental procedures and spectral data (NMR, IR, MS) for compounds **7**-**16** (12 pages). Ordering information is given on any current masthead page.

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Shape-Selective Access to Zeolite Supercages. Arene Charge-Transfer Complexes with Viologens as Visible Probes

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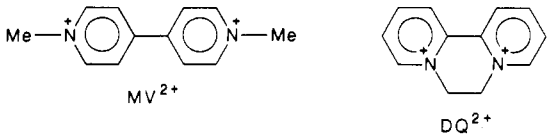
Restricted yet ready access to the supercage represents an important facet of shape selectivity in zeolite catalysis.[1] However there is extant no general method to readily assess the structural constraints for the passage of hydrocarbon substrates in the liquid-phase through the zeolite framework. Our recent studies of intermolecular interactions[2] suggested that the stepwise assemblage of charge-transfer (CT) complexes directly within the zeolite cavity offers a viable approach to this problem. As a test case, we now report the shape-selective formation of various aromatic CT complexes in zeolites with the bipyridinium acceptors methylviologen MV^{2+} and diquat DQ^{2+} shown below.[3]



The cations MV^{2+} and DQ^{2+} are readily incorporated into zeolite-Y by ion exchange of Na^+ at an 80% level of supercage occupancy.[4] Mere exposure of these acceptor-doped colorless powders (400 mesh) to dilute solutions of anthracene, dimethylnaphthalene, and durene in dichloromethane leads immediately to distinctively colored purple, orange, and yellow zeolites, respectively, while the supernatant solutions remain colorless.[5] The diffuse reflectance spectra of the colored zeolites show the presence of new bands that bear striking resemblance to the charge-transfer spectra of the corresponding arenes with MV^{2+} and DQ^{2+} in solution (Table I). Importantly, the absorption maxima (λ_{CT}) vary uniformly with the ionization potentials of different arene donors,[8] and the linear correlation in Figure 1 confirms their charge-transfer character according to Mulliken.[9] Furthermore, the subtle difference between the MV^{2+} and DQ^{2+} acceptors is clearly reflected in the displacement of the solid-state CT bands by an amount $\Delta h\nu_{CT}$ corresponding to the difference in their reduction potentials of $\Delta E^0_{red} = 70$ mV.[10]

The successful isolation of the bright orange, single crystal of the MV^{2+} complex with dimethoxynaphthalene allows X-ray crystallography (Figure 2)[11] to establish the relevant face-to-face

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