Nucleic Acid Related Compounds. 80. Synthesis of 5'-S-(Alkyl and aryl)-5'-fluoro-5'-thioadenosines with Xenon Difluoride or (Diethylamido)sulfur Trifluoride, Hydrolysis in Aqueous Buffer, and Inhibition of S-Adenosyl-L-homocysteine Hydrolase by Derived "Adenosine 5'-Aldehyde" Species[1a]

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Treatment of 5'-S-(alkyl and aryl)-5'-thioadenosine derivatives **2** with XeF_2, or the corresponding sulfoxides **3** with DAST/$SbCl_3$, gave diastereomeric 5'-fluoro compounds which were deprotected to give the 5'-S-(alkyl and aryl)-5'-fluoro-5'-thioadenosine analogues **5**. Stereochemistry was established by X-ray crystallography, and ^{19}F NMR chemical shifts were definitive for configurationally-related 5'-fluoro diastereomers. Sulfoxidation and thermolysis afforded the fluoromethylene analogues with retained relative configuration. The nucleoside 5'-α-fluoro thioethers **5** underwent spontaneous hydrolysis in aqueous buffer to give derived "adenosine 5'-aldehyde" species which caused potent time-dependent inactivation of S-adenosyl-L-homocysteine hydrolase.

Introduction

Numerous biological transmethylation reactions utilize S-adenosyl-L-methionine (AdoMet, SAM) as the methyl donor and release S-adenosyl-L-homocysteine (AdoHcy, SAH) as the nucleoside byproduct.[2a] Since AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes,[2b] AdoHcy hydrolase (EC 3.3.1.1) functions as an essential catabolic enzyme to effect the hydrolytic cleavage of AdoHcy to adenosine and L-homocysteine.[2] Therefore, AdoHcy hydrolase is an attractive target for mechanism-based chemotherapeutic agents.[3,4] Enzymatic decarboxylation of AdoMet gives the (aminopropyl)-(methyl)sulfonium compound that serves as the aminopropyl donor for biosynthesis of the polyamines spermidine and spermine, with release of 5'-S-methyl-5'-thioadenosine (MTA) as the nucleosidic byproduct.[2a,5]

The mechanism of action of AdoHcy hydrolase was established by Palmer and Abeles[6] and has been studied in greater detail by Borchardt,[7] Parry,[8] McCarthy,[9] and Porter[10] and their co-workers. The "carbocyclic" nucleoside antibiotics neplanocin A[11] and aristeromycin[12] are potent inactivators of AdoHcy hydrolase and inhibition of the enzyme has been correlated with antiviral activity.[3,4,13] Synthetic analogues lacking the 4'-hydroxymethyl group have been designed to effect the desired "cofactor depletion" inactivation[4] of AdoHcy hydrolase but preclude toxic effects resulting from phosphorylation of O5' and further anabolic processing. Other recently noted mechanism-based inhibitors of AdoHcy hydrolase include the 4',5'-didehydro-5'-fluoroadenosines[14] and 5'-chloro analogues,[14b,15] 2'-deoxy-2'-methyleneadenosine,[16] a 4'-acetylenic homoadenosine analogue,[17] and "adenosine 5'-aldehyde"[18a] and its oximes.[18b]

Several years ago we designed new types of fluorine-containing nucleoside analogues[19] that could be anticipated to function as inhibitors of AdoHcy hydrolase based on the Palmer-Abeles mechanism (Figure 1). Analogous 5'-fluoro-5'-thionucleoside derivatives were also prepared by McCarthy and converted into diastereomeric 4',5'-dide-

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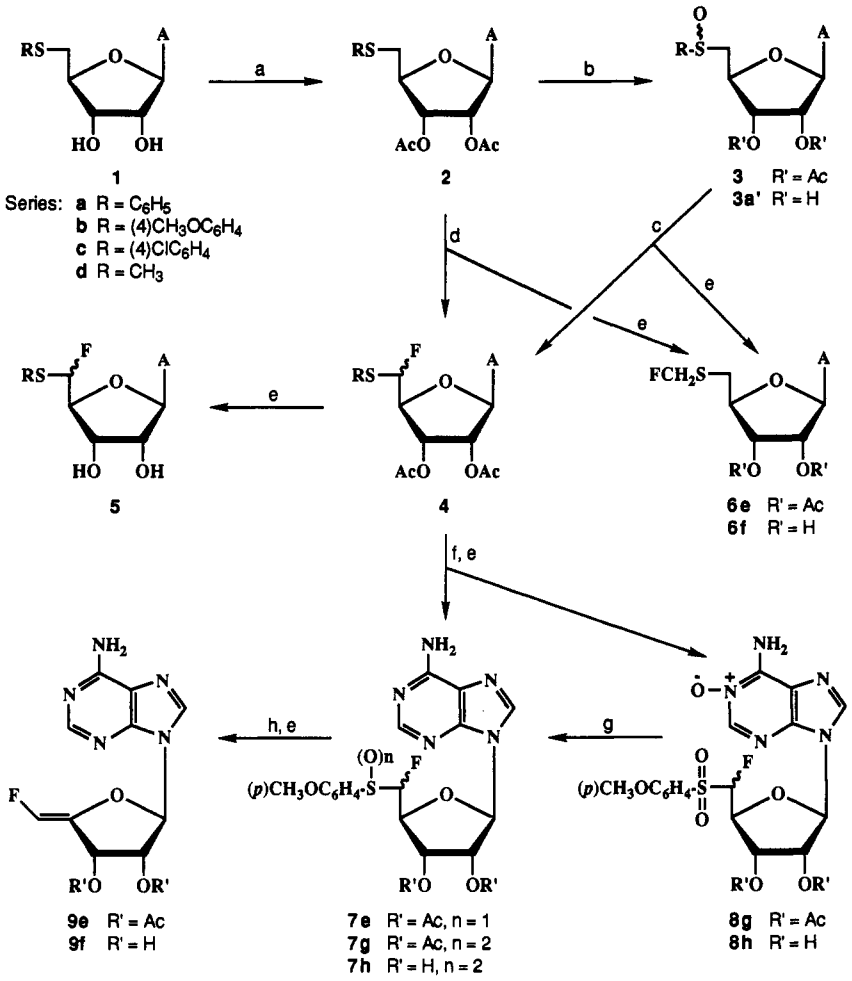
Scheme 1[a]



[a] (a) Ac_2O/pyridine; (b) MCPBA/CH_2Cl_2/−40 °C; (c) DAST/$SbCl_3$/CH_2Cl_2; (d) XeF_2/CH_2Cl_2/−25 °C to ambient; (e) NH_3/MeOH; (f) MCPBA/CH_2Cl_2; (g) Si_2Cl_6/CH_2Cl_2; (h) EtN(*i*-Pr)$_2$/diglyme/145 °C.

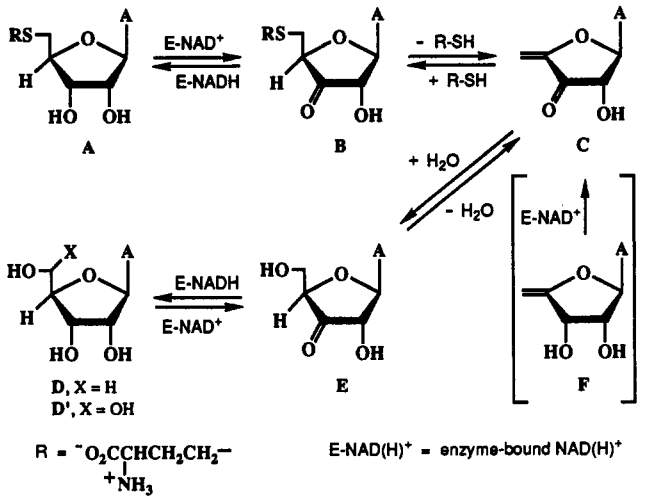


Figure 1. Proposed mechanism for *S*-adenosyl-L-homocysteine hydrolase.

hydro-5′-deoxy-5′-fluoroadenosines.[14] As predicted, the latter fluoromethylene compounds were potent inhibitors of AdoHcy hydrolase[14,15b] and had significant biological activity. Parallel syntheses of 5′-fluoro analogues of MTA and demonstration of the competitive inhibition of methylthioadenosine phosphorylase (MTAPase) have been reported.[20] Fluorinations at C5′ (and C2′) of uridine 5′-(and 2′)-thioethers with xenon difluoride, or the corresponding sulfoxides with (diethylamido)sulfur trifluoride (DAST) and antimony(III) chloride catalysis, gave stable 5′-*S*-aryl-5′-fluoro-5′-thiouridines[21a] and 2′-[alkyl(or aryl)-sulfonyl]-2′-deoxy-2′(*S*)-fluorouridines (and cytidines).[21b]

We now report conversions of adenosine into 5′-*S*-alkyl-(and aryl)-5′-fluoro-5′-thioadenosines and their time-dependent inactivation of AdoHcy hydrolase. The unfluorinated thioether precursors did not undergo alternative substrate oxidation by AdoHcy hydrolase.[15b] Thus, the subsequent elimination and addition pathways (Figure 1) are presumably precluded for their α-fluoro analogues also. However, these α-fluoro thioethers undergo hydrolysis in aqueous buffers to give "adenosine 5′-aldehyde" species that are potent inhibitor(s).[18] Spontaneous hydrolysis of selected adenosine 5′-fluoro-5′-thioethers in aqueous buffer and time-dependent inactivation kinetics with AdoHcy hydrolase are described.

Synthetic Results and Discussion

We developed safe and efficient conversions of adenosine into 5′-*S*-alkyl(and aryl)-5′-thioadenosines (1, Scheme 1)

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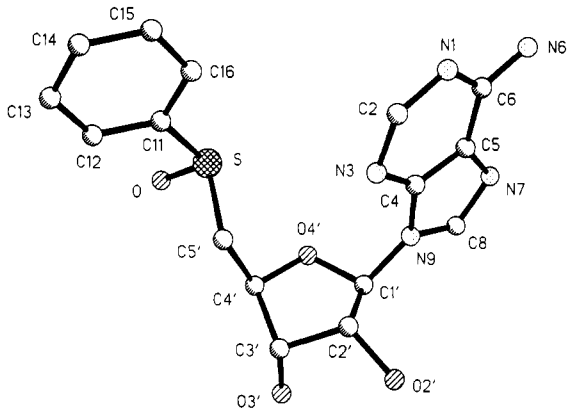

Figure 2. Computer drawing of the crystal structure of 5′-deoxy-5′-[phenylsulfinyl (R_S)]adenosine [**3a′** (R_S)]. Hydrogen atoms were omitted for clarity.

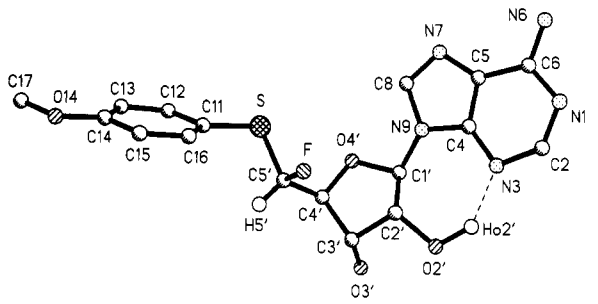

Figure 3. Computer drawing of the crystal structure of 5′(*S*)-fluoro-5′-*S*-(4-methoxyphenyl)-5′-thioadenosine [**5b** (5′*S*)]. Hydrogen atoms were omitted for clarity.

via 5′-chloro-5′-deoxyadenosine[22a] since cyclonucleoside byproducts were noted[22b] when dialkyl disulfides were employed in Hata's disulfide/phosphine procedure.[22c] Acetylation of **1** followed by selective oxidation [1 equiv of 3-chloroperoxybenzoic acid (MCPBA)/CH_2Cl_2/−40 °C] gave the protected sulfoxides **3** [*R*/*S* at sulfur, $(R/S)_S$ ~1:1] quantitatively (~80% overall from adenosine). Treatment of **3a** with DAST/ZnI_2/CH_2Cl_2[23] gave the 2′,3′-di-*O*-acetyl-5′-fluoro-5′-*S*-phenyl-5′-thioadenosine (**4a**) diastereomers as minor products plus major quantities of the deoxygenated starting material **2a**. Deoxygenation of sulfoxides to sulfides with NaI/BF_3·Et_2O had been reported.[24] Therefore, alternative Lewis acid catalysts were examined to circumvent the deoxygenative[25] side reaction. Antimony(III) chloride[19] and SbF_5 exerted potent catalysis of the conversion of sulfoxide **3a** to fluoro nucleoside **4a**. This method has proven to be general[19,21,26] and $SbCl_3$ is convenient to use.

Treatment of **3a** with DAST (2 equiv)/$SbCl_3$ (0.1 equiv)/CH_2Cl_2 at ambient temperature for 10 h and silica flash chromatography gave the fluoro diastereomers **4a** (5′*R*/*S*, 39:61; 69%) with ^{19}F NMR δ −155.62 (dd, $^2J_{F-5'}$ = 53.0 Hz, $^3J_{F-4'}$ = 10.3 Hz, 0.39, F5′*R*) and −159.61 (dd, $^2J_{F-5'}$ = 53.0 Hz, $^3J_{F-4'}$ = 18.4 Hz, 0.61, F5′*S*) (upfield from CCl_3F). The ^{1}H NMR spectrum had two sets of downfield-shifted doublets of doublets at δ 6.82 ($^2J_{5'-F}$ = 53.0 Hz, $^3J_{5'-4'}$ = 5.5 Hz, 0.39, H5′*R*) and 6.75 ($^2J_{5'-F}$ = 53.0 Hz, $^3J_{5'-4'}$ = 5.0 Hz, 0.61, H5′*S*). It is noteworthy that treatment of the **3a** (R_S) diastereomer [see Figure 2 for the X-ray crystal structure of its unprotected precursor **3a′** (R_S)] under identical conditions resulted in formation of the same isomeric mixture of **4a**. Thus, the stereochemistry of this deoxygenative fluorination process is not related to the configuration of the sulfoxide substrate.

The 4-methoxyphenyl thioether **3b** gave improved fluorination rates and yields as noted.[23] Catalysis with ZnI_2 also gave good fluorination yields of **4b** (5′*R*/*S*, 35:65; 68%), but reactions were less clean and proceeded less rapidly. Treatment of **3b** with DAST/$SbCl_3$ afforded **4b** (5′*R*/*S*, 36:64; 74%). Deacetylation (NH_3/MeOH) and fractional crystallization (MeOH) gave **5b** (5′*S*) (33%, 24% from **3b**) whose 5′*S* configuration was established by X-ray crystallography (Figure 3). Characteristic NMR spectral differences between diastereomer pairs include: (i) ^{19}F chemical shifts (5′*R* downfield from 5′*S*); (ii) ^{19}F and ^{1}H coupling constants (5′*S* has a larger $^3J_{F-H4'}$ than 5′*R*); (iii) ^{13}C coupling constants (5′*R* has a larger $^2J_{C4'-F}$ than 5′*S*); (iv) ^{13}C and ^{1}H chemical shifts for C4′ and H4′ (5′*S* is downfield from 5′*R*).

The catalytic efficiency of $SbCl_3$[26a] was demonstrated with the deactivated 4-chlorophenyl thioether **3c**. Standard treatment (DAST/$SbCl_3$/CH_2Cl_2/ambient temperature) gave the **4c** diastereomers plus deoxygenated starting material **2c** (~1:1, 61%). However, slightly more vigorous treatment of **3c** with DAST (5 equiv)/$SbCl_3$ (0.15 equiv)/CH_2Cl_2 with initial heating at 30 °C for 1 h followed by stirring (ambient) for 12 h afforded **4c** (5′*R*/*S*, 37:63; 81%) plus **2c** (~15%). Selective oxidation of **2c** in the crude mixture [MCPBA, (0.2 equiv)/CH_2Cl_2/−40 °C/5 min] produced the polar unfluorinated sulfoxide **3c** which was readily separated from **4c**. Deacetylation of **4c** gave **5c**.

Direct fluorination of **2** with XeF_2[27] gave **4** with an inverted ratio of diastereomers and avoided the oxidation step[1a] via **3**. Thus, treatment of **2b** with XeF_2 (1.13 equiv)/CH_2Cl_2 under Ar at −25 °C to ambient for 1.5 h and flash chromatography gave **4b** (5′*R*/*S*, 62:38; 69%). Thioethers **2a** and **2c** under identical conditions gave **4a** and **4c** (5′*R*/*S*, ~3:1) plus unchanged **2a** and **2c** and the unfluorinated sulfoxides **3a** and **3c**, respectively.

Treatment of the methyl sulfoxide **3d** with DAST/$SbCl_3$ gave the 5′-*S*-(fluoromethyl)-5′-thio (**6e**) and 5′-fluoro-5′-*S*-methyl-5′-thio (**4d**) regioisomers (58% combined after purification). The ^{19}F NMR triplet for **6e** at δ −184.63 ($^2J_{F-CH_2}$ = 52 Hz, 0.42, FCH_2S) and two doublets of doublets for **4d** at δ −163.62 ($^2J_{F-5'}$ = 54 Hz, $^3J_{F-4'}$ = 12 Hz, 0.21, F5′*R*), −165.08 ($J_{F-5'}$ = 54 Hz, $J_{F-4'}$ = 17 Hz, 0.37, F5′*S*) were diagnostic for the regio- (**4d**/**6e**, 63:37) and stereochemical [**4d** (5′*R*/*S*, 21:42)] composition. It is noteworthy that treatment of **2d** with XeF_2 gave a mixture (59% combined) of **4d**/**6e** (39:61) [**4d** (5′*R*/*S*, 50:50)]. The formation of **4d** was unexpected based on the previously observed regioselective formation of α-fluoromethyl sul-

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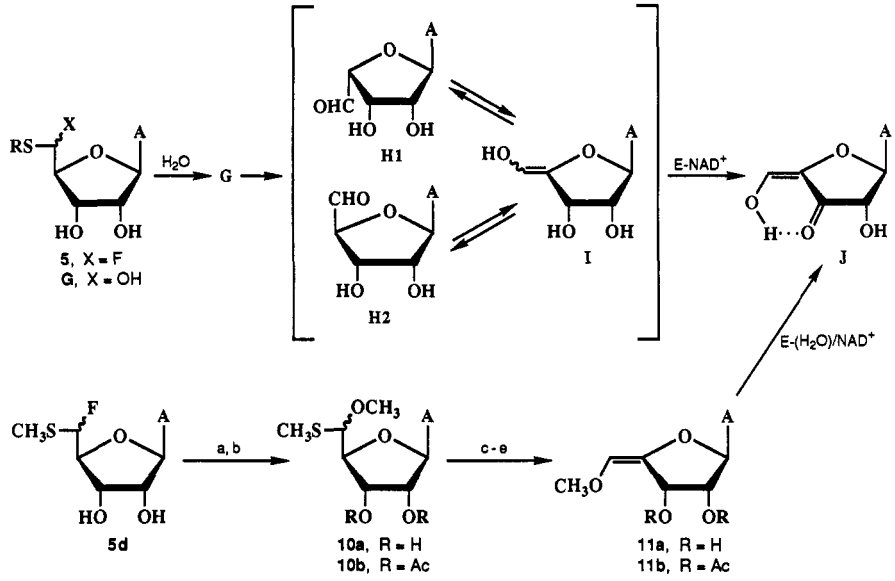
Scheme 2[a]



[a] (a) $CH_3OH/CHCl_3$/silica gel; (b) Ac_2O/pyridine; (c) MCPBA/CH_2Cl_2; (d) 135 °C/EtN(*i*-Pr)$_2$/diglyme; (e) NH_3/MeOH.

fides from either methyl sulfoxides[23] or methyl sulfides.[27c,d] However, fluorination of MTA sulfoxide with DAST gave similar regioisomeric mixtures.[20] In contrast, fluorinations of uridine methyl 2′-sulfides or sulfoxides with XeF_2 or DAST/$SbCl_3$ gave the C2′-fluorinated products.[21b] Deacetylation of **4d**/**6e** (39:61) and preparative TLC gave 5′-*S*-(fluoromethyl)-5′-thioadenosine (**6f**, 44%). The 5′-fluoro-5′-methylthio diastereomers **5d** were unstable and decomposed readily on silica gel columns and TLC plates.[19] Solvolytic replacement of fluoride by methoxide with MeOH/$CHCl_3$ solvent systems (vide infra) was evidenced by loss of ^{19}F NMR signals and appearance of ^1H singlets (OCH_3). However, **5d**[19] could be isolated by anion-exchange column chromatography[20a] or by careful TLC on deacidified plates.

Treatment of α-fluoro sulfoxides with DAST[23] or α-fluoro thioethers with XeF_2[27a] was reported to give α,α-difluoro thioethers. However, our nucleoside analogues did not undergo this second conversion. Selective oxidation of **4b** afforded **7e** (four diastereomers, ^{19}F NMR) plus minor quantities of the overoxidized sulfones **7g**. Treatment of the **7e** mixture with DAST/$SbCl_3$ resulted in deoxygenation to give **4b** with the same diastereomer ratio as before oxidation. Treatment of **4b** with XeF_2 gave sulfoxides **7e** plus recovered **4b**. Acetylation of **5b** (5′*S*) and oxidation gave **7e** [5′*S*, (*R*/*S*)$_S$ 23:77] in harmony with preferential anti-attack of the oxidant relative to the C–F bond.[28] Oxidations of **4b** (5′*R*/*S*, 36:64 and 62:38) afforded different ratios of the fluoro sulfoxide diastereomers **7e**.

Thermolysis of **7e** [5′*S*, (*R*/*S*)$_S$, 23:77] in ethyldiisopropylamine/diglyme at ~145 °C for 48 h gave 5′(*Z*)-fluoromethylene compound **9e** (71%) and recovered **7e** (~15%). Deacetylation of **9e** gave 4′,5′-didehydro-5′-deoxy-5′(*Z*)-fluoroadenosine (**9f**, 88%). It is noteworthy that this approach provides the more active AdoHcy hydrolase inhibitory[14,15b] 5′(*Z*)-isomer **9f** without recourse to column separation of the *E* and *Z* isomers.

More vigorous oxidation (MCPBA, 2.65 equiv) of **4b** (5′*R*/*S*, 36:64) gave the diastereomeric 5′-fluoro sulfones **7g** and their 1-*N*-oxides **8g** which were N-deoxygenated with hexachlorodisilane[29] to give **7g** in high yield. Deacetylation of **7g** gave **7h** (5′*R*/*S*, 40:60), and **8h** (5′*S*) was obtained from **8g** after deprotection and fractional crystallization.

Spontaneous Hydrolysis of the 5′-α-Fluoro Thioethers 5a–c. The 5′-α-fluoro thioethers **5** were unstable in aqueous solutions, whereas solutions of **5b** in Me_2SO-d_6 were stable for days at ambient temperature and months at ~4 °C. The methylthio diasteromers **5d** were especially unstable, and solvolysis to give 5′-*O*-methyl-5′-(methylthio) acetal diastereomers also occurred readily on silica plates or columns with methanol-containing solvents (vide infra). In order to investigate probable conversions of **5** to derived adenosine 5′-aldehyde species in the aqueous buffer solutions used for AdoHcy hydrolysis inhibition assays, hydrolysis kinetics were studied. In neutral phosphate buffer, the 5′-α-fluoro thioethers **5** were shown to hydrolyze spontaneously to adenosine 5′-aldehyde **H2** and its 4′-epimer **H1** (Scheme 2). To analyze the kinetics of these reactions, HPLC systems (e.g., Figure 4) were developed to separate the 5′-α-fluoro thioethers (e.g., **5b**) from possible intermediates (e.g., **G**) and products (e.g., **H1** and **H2**, Scheme 2). As shown in Figure 5, the concentration of 5′-α-fluoro thioether **5b** decreased rapidly at 37 °C with simultaneous appearance and then disappearance of an intermediate (probably **G**, Scheme 2) and the appearance of adenosine 5′-aldehyde **H2** and its 4′-epimer **H1**. The structures of **H2** and **H1** were confirmed by coinjection with authentic synthetic samples.[18a] The experimental data shown in Figure 5 were fitted to a consecutive reaction scheme ($\mathbf{5b} \xrightarrow{k_1} \mathbf{G} \xrightarrow{k_2} \mathbf{H}$), providing estimates of k_1 (0.10 min^{-1}) and k_2 (0.15 min^{-1}). The 5′-α-fluoro thioethers **5a**, **5c**, and **5d** also hydrolyzed to form mixtures of adenosine 5′-aldehyde **H2** and its 4′-epimer **H1**. Qualitatively, the rate of disappearance of **5d** was much faster than the rate of hydrolysis of **5b** (TLC, ambient temperature; disappearance rates: **5d** ≫ **5b** ≥ **5a** > **5c**). However, the rate of degradation of **5d** was not determined since a rapidly

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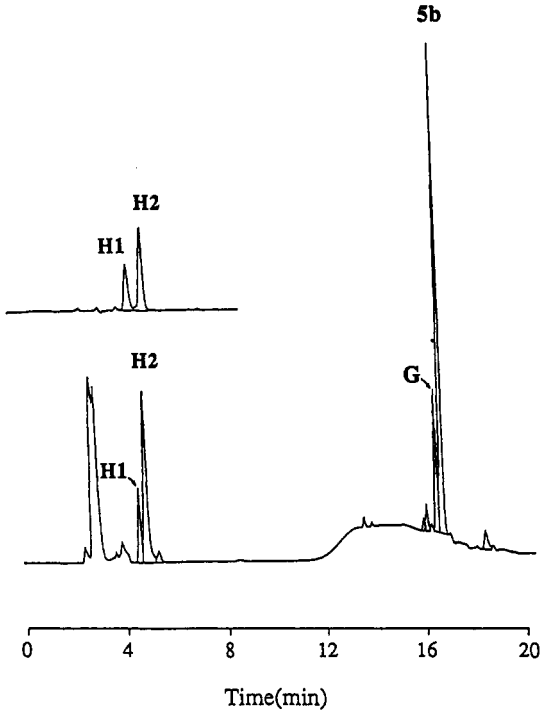

Figure 4. HPLC chromatogram of the hydrolysis products generated upon incubation of **5b** in 50 mM potassium phosphate buffer containing 1 mM EDTA, pH 7.2, at 37 °C for 5 min. An aliquot of the reaction mixture was analyzed by reversed-phase HPLC as described in the Experimental Section. **H1** and **H2**: the diastereomeric adenosine 5′-aldehydes that are formed. Inset: HPLC of standard samples of adenosine 5′-aldehyde **H2** and its 4′-epimer **H1**.

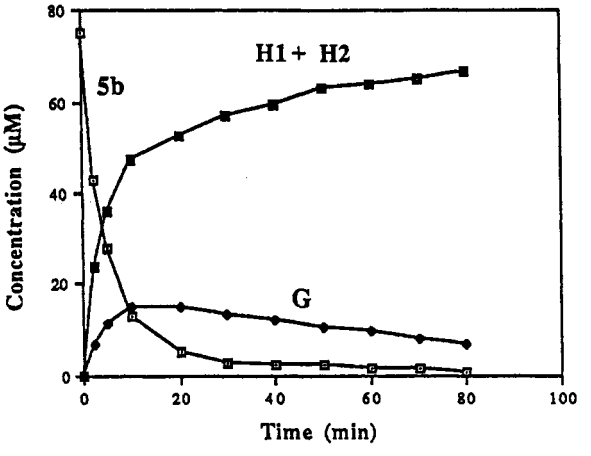

Figure 5. Time course of degradation of 5′-α-fluoro thioether **5b** in phosphate buffer, pH 7.2. Reaction conditions and analytical methods are described in the Experimental Section.

formed intermediate was not separated from **5d** by HPLC. No intermediates were *detected* by HPLC upon decomposition of **5a** or **5c** at 37 °C. The kinetics for formation of **H1** and **H2** from **5a** and **5c** were fitted to a single first-order rate equation (**5** → **H**, $k \approx 0.01$ min^{-1}) for the hydrolytic decomposition of these 5′-α-fluoro thioethers at 37 °C.

The 4-chloro- (**5c**), protio- (**5a**), and methoxy- (**5b**) phenyl thioethers have substituents with decreasing electronic demand for comparison with the labile aliphatic compound **5d**. Since **5d** undergoes solvolysis to methoxy methylthio acetal **10a**, it is reasonable to assume that hydrolysis of the 5′-α-fluoro thioethers **5** involves loss of fluoride to give a carbocationic species that undergoes nucleophilic attack by water and loss of a proton to give an intermediate thiohemiacetal (**G**, Scheme 2). The rates of generation of the initial cationic species and subsequent loss of the elements of methanethiol and benzenethiols from the thiohemiacetals derived from **5d** and **5a–c** to give the aldehydes (**H2** and **H1**, in tautomeric equilibrium with hydroxy enol ethers **I**) should be affected by cationic stabilization by the alkyl relative to the aryl substituents, respectively, as observed.

Time-Dependent Inhibition of AdoHcy Hydrolase. Since 5′-thioether **1b** was not an alternative substrate for AdoHcy hydrolase,[15b] it is unlikely that fluorinated analogue **5b** would undergo enzymatic oxidation (Figure 1). However, **5b**, as well as **5a**, **5c**, and **5d**, were shown to produce potent inhibition of bovine liver AdoHcy hydrolase. All of the 5′-α-fluoro thioethers produced time- and concentration-dependent inactivation of the enzyme. From the kinetics of the enzyme inactivation, K_i and k_{inact} values were determined. K_i and k_{inact} values for the 5′-α-fluoro thioethers **5b** and **5c** and the vinyl fluoride **9f** are presented in Table 3. It should be noted that the values for **5b** and **5c** are apparent K_i and k_{inact} values because, as discussed above, these 5′-α-fluoro thioethers rapidly decompose in aqueous solution to form mixtures of reaction intermediates (e.g., **G**, Scheme 2) and the "adenosine aldehydes" (e.g., **H2**, **H1**, and **I**, Scheme 2). It is noteworthy that the apparent K_i value observed for **5b** (K_i = 300 nM) is substantially greater than that observed for **5c** (K_i = 21.4 nM). This might reflect the fact that hydrolysis of **5b** proceeds via formation of intermediate **G**, which is unlikely to be an inhibitor of AdoHcy hydrolase but might be stabilized by interaction with the enzyme. In contrast, degradation of **5c** might result in higher immediate concentrations of the active AdoHcy hydrolase-inhibitory "adenosine aldehydes." It also is interesting to note that **5c** and the vinyl fluoride **9f** have similar K_i but quite different k_{inact} values. The k_{inact} value for **5c** is similar in magnitude to that observed with adenosine 5′-aldehyde, which would be consistent with the spontaneous hydrolysis of **5c** to this potent inhibitor of AdoHcy hydrolase.

Based on these mechanistic considerations,[15b] "adenosine 5′-aldehydes" (**H2** and **H1**, Scheme 2) were prepared from two independent precursors and their potent time-dependent inhibition of AdoHcy hydrolase was demonstrated.[18a] It was found that a recombinant rat liver AdoHcy hydrolase apoenzyme catalyzed removal of fluoride from the 4′-fluoromethylene analogue **9f** and its 5′*E* diastereomer to give **H2** and **H1**.[7b] Thus, these fluorovinyl compounds[14] function as prodrugs[7b,18a] of the active 5′-aldehyde-derived type I (cofactor-depletion)[4] mechanism-based inhibitor(s).[7b,15b,18a] The present 5′-α-fluoro thioethers **5** (which are two-stage precursors, oxidation and thermolysis, of the fluorovinyl analogues) appear to function as "spontaneous solvolysis prodrugs" of the same inhibitor(s).

The hydroxy enol ethers (**I**, Scheme 2), aldehyde **H2**, or especially the aldehyde-hydrate (**D′**, Figure 1) of **H2** represent plausible inhibitor candidates. Enzyme-mediated oxidation of the "adenosine 5′-aldehydes" results in reduction of NAD^+ to NADH with accompanying type I (cofactor-depletion)[4] mechanism-based inhibition of AdoHcy hydrolase.[7b,18] Aldehyde-hydrate **D′** (of **H2**) closely resembles the natural substrate, adenosine (**D**, Figure 1), and elimination of water in the second enzyme-

Table 1. ^{1}H NMR Spectral Data[a,b]

compound	H1′[c] ($J_{1'-2'}$)	H2′ ($J_{2'-3'}$)	H3′ ($J_{3'-4'}$)	H4′ ($J_{4'-5',5''}$)	H5′,5″[d] ($J_{5'-5''}$)	H2[e]	H8[e]	NH_2[f]	aromatic (J_{A-B})	others
1a	5.93 (6.0)	4.95[g] (5.0)	4.22[g] (4.0)	4.04[g] (5.0, 7.0)	3.48,[h] 3.36[h] (14.0)	8.18	8.38	7.32	7.20–7.40[i]	5.58[c] (6.0,[j] OH2′), 5.43[c] (5.0,[j] OH3′)
1b	5.91 (6.0)	4.84[g] (5.0)	4.19[g] (3.5)	3.98[g] (6.0, 7.0)	3.29,[h] 3.17[h] (13.0)	8.18	8.38	7.34	6.90,[c] 7.37[c] (8.5)	5.55[c] (6.0,[j] OH2′), 5.39[c] (4.5,[j] OH3′), 3.78[e] (CH_3O)
1c[k]	5.88 (5.8)	4.81[g] (5.2)	4.18[g] (3.5)	3.96[g] (5.7, 7.2)	3.43,[h] 3.29[h] (13.9)	8.15	8.35	7.25	7.27–7.49[i]	5.53[c] (6.1,[j] OH2′), 5.37[c] (4.9,[j] OH3′)
1d	5.93 (6.0)	4.80[g] (5.0)	4.19[g] (4.0)	4.08[g] (5.7, 6.5)	2.94,[h] 2.83[h] (14.0)	8.16	8.37	7.33		5.55[c] (6.0,[j] OH2′), 5.38[c] (4.7,[j] OH3′), 2.10[e] (CH_3S)
2a	6.21 (6.0)	6.16[h] (5.0)	5.63[h] (4.0)	4.26[g] (5.5, 7.5)	3.58,[h] 3.46[h] (14.0)	8.19	8.36	7.42	7.20–7.40[i]	2.12,[e] 2.02[e] (Ac's)
2b	6.19 (6.0)	6.16[h] (5.0)	5.61[h] (4.0)	4.18[g] (6.0, 7.0)	3.43,[h] 3.32[h] (13.5)	8.19	8.38	7.42	6.88,[c] 7.35[h]	2.11,[e] 2.00[e] (Ac's), 3.74[e] (CH_3O)
2c[k]	6.18 (5.9)	6.14[h] (6.0)	5.61[h] (4.2)	4.24[g] (4.9, 6.9)	3.56,[h] 3.46[h] (13.5)	8.17	8.35	8.35	7.32–7.48[i]	2.09,[e] 2.00[e] (Ac's)
2d	6.21 (6.0)	6.13[h] (5.5)	5.59[h] (4.5)	4.32[g] (6.0, 7.0)	3.03,[h] 2.93[h] (14.0)	8.20	8.42	7.40		2.14,[e] 2.01[e] (Ac's), 2.06[e] (CH_3S)
3a′ (R_S)	5.98 (6.0)	4.90[g] (5.0)	4.21[g] (3.0)	4.35[g] (10.5, 2.5)	3.54,[h] 3.08[h] (13.0)	8.15	8.40	7.35	7.51–7.70[i]	5.50[c] (4.5,[j] OH3′), 5.58[c] (5.7,[j] OH2′)
3a (R_S)	6.30 (6.0)	6.20[h] (5.1)	5.58[h] (3.6)	4.57[g] (10.7, 3.0)	3.68,[h] 3.24[h] (13.0)	8.17	8.44	7.42	7.54–7.68[i]	2.00,[e] 2.11[e] (Ac's)
3a (S_S)[k,l]	6.15 (6.0)	6.20[h] (5.1)	5.72[h] (3.0)	4.37[g] (7.0, 5.0)	3.60–3.78[i]	8.20	8.30	7.60	7.44–7.62[i]	2.04,[e] 2.16[e] (Ac's)
3b (R_S)	6.31 (6.0)	6.24[h] (5.5)	5.58[h] (4.0)	4.56[g] (11.0, 3.0)	3.62,[h] 3.26[h] (13.0)	8.20	8.44	7.43	7.55,[c] 7.14[c] (8.5)	2.12,[e] 1.99[e] (Ac's), 3.82 (CH_3O)
3b (S_S)	6.13 (6.0)	6.21[h] (5.0)	5.65[h] (3.5)	4.30[g] (7.0, 5.0)	3.59,[h] 3.53[h] (13.0)	8.18	8.33	7.41	7.59,[c] 7.00[c] (8.5)	2.12,[e] 2.02[e] (Ac's), 3.78 (CH_3O)
3c (R_S)[k]	6.30 (6.0)	6.18[h] (5.2)	5.57[h] (3.9)	4.56[g] (10.4, 3.3)	3.69,[h] 3.27[h] (13.0)	8.20	8.45	7.42	7.70[f]	2.02,[e] 2.14[e] (Ac's)
3c (S_S)[k]	6.10 (6.3)	6.22[h] (5.3)	5.65[h] (3.4)	4.40[g] (6.1)	3.64[c]	8.19	8.27	7.43	7.46,[c] 7.58[c] (8.5)	1.99,[e] 2.14[e] (Ac's)
3d [$(R/S)_S$][k]	6.28, 6.25 (5.0)	6.10[h] (6.0)	5.70[h] (4.0)	4.60–4.69,[i] 4.52–4.59[i]	3.21–3.52[i]	8.20	8.40	7.42		2.04,[e] 2.05,[e] 2.12,[e] 2.14[e] (Ac's), 2.56,[e] 2.60[e] (CH_3S)
4a (5′R)[m]	6.55 (5.5)	6.42[h] (5.6)	6.00[h] (4.0)	4.60[g] (5.5,[n] 10.2[o])	6.82[h] (53.0[p])	8.44	8.74	7.73	7.70–7.86[i]	1.97,[e] 2.09[e] (Ac's)
4a (5′S)[m]	6.55 (5.5)	6.25[h] (5.2)	6.08[h] (5.0)	4.65[g] (5.0,[n] 18.2[o])	6.75[h] (53.0[p])	8.50	8.72	7.73	7.70–7.86[i]	1.98,[e] 2.08[e] (Ac's)
4b (5′R)[m]	6.30 (5.5)	6.18[h] (5.5)	5.78[h] (4.0)	4.38[g] (6.0,[n] 10.0[o])	6.36[h] (53.0[p])	8.14	8.40	7.44	6.98,[c] 7.42[c] (8.8)	2.02,[e] 2.13[e] (Ac's), 3.78[e] (CH_3O)
4b (5′S)[m]	6.30 (5.0)	6.04[h] (6.0)	5.88[h] (5.0)	4.44[g] (5.5,[n] 17.0[o])	6.30[h] (53.0[p])	8.16	8.37	7.44	7.02,[c] 7.47[c] (8.8)	2.04,[e] 2.14[e] (Ac's), 3.79[e] (CH_3O)
4c (5′R)[m]	6.29 (5.5)	6.16[h] (5.5)	5.77[h] (4.0)	4.48[g] (5.5,[n] 10.4[o])	6.57[h] (52.5[p])	8.10	8.37	7.43	7.42–7.53[i]	2.12,[e] 2.01[e] (Ac's)
4c (5′S)[m]	6.29 (5.5)	6.01[h] (5.7)	5.84[h] (5.4)	4.52[g] (4.9,[n] 18.3[o])	6.49[h] (53.0[p])	8.15	8.35	7.43	7.42–7.53[i]	2.12,[e] 2.03[e] (Ac's)
5a (5′R)[m]	5.95 (6.0)	4.84[g] (5.0)	4.36[g] (3.2)	4.12[g] (5.4,[n] 12.0[o])	6.38[h] (53.5[p])	8.08	8.32	7.30	7.32–7.54[i]	5.63[c] (5.0,[j] OH3′), 5.65[c] (5.0,[j] OH2′)
5a (5′S)[m]	6.00 (5.8)	4.72[g] (5.5)	4.42[g] (3.5)	4.19[g] (5.0,[n] 21.0[o])	6.40[h] (53.5[p])	8.16	8.30	7.34	7.32–7.54[i]	5.60[c] (5.0,[j] OH3′), 5.67[c] (5.5,[j] OH2′)
5b (5′R)[q]	5.95 (6.5)	4.85[g] (5.0)	4.33[g] (3.0)	4.03[g] (6.0,[n] 11.3[o])	6.16[h] (53.5[p])	8.13	8.33	7.32	6.95,[c] 7.40[c] (9.0)	5.61[c] (5.6,[j] OH3′), 5.63[c] (6.5,[j] OH2′), 3.76[e] (CH_3O)
5b (5′S)	5.98 (6.0)	4.72[g] (5.0)	4.41[g] (3.5)	4.12[g] (5.5,[n] 18.8[o])	6.15[h] (53.5[p])	8.15	8.31	7.34	7.00,[c] 7.57[c] (9.0)	5.58[c] (5.0,[j] OH3′), 5.65[c] (6.0,[j] OH2′), 3.78[e] (CH_3O)
5c (5′R)[m]	5.97 (6.2)	4.86[g] (5.5)	4.39[g] (3.5)	4.17[g] (5.3,[n] 12.2[o])	6.43[h] (53.0[p])	8.09	8.34	7.42	7.43–7.56[i]	5.58–5.71[i] (OH)
5c (5′S)[m]	6.01 (5.8)	4.71[g] (5.9)	4.42[g] (3.5)	4.21[g] (5.2,[n] 21.0[o])	6.42[h] (53.6[p])	8.14	8.31	7.42	7.43–7.56[i]	5.58–5.71[i] (OH)
4d[r]	6.26, 6.28 (6.0, 5.5)	6.08,[h] 5.97[h] (5.5, 6.0)	5.72–5.78[i]	4.41–4.50[i] (12.0,[o] 17.0[o])	6.17,[h] 6.20[h] (54.0[p])	8.18	8.31, 8.33	7.41		2.02,[e] 2.04,[e] 2.11,[e] 2.13[e] (Ac's), 2.23,[e] 2.25[e] ($^4J_{CH_3-F}$ = 1.5 Hz, CH_3S)
6e[r]	6.20 (6.0)	6.12[h] (5.5)	5.58[h] (4.5)	4.30–4.36[i]	3.17–3.28[i]	8.17	8.38	7.39		2.00,[e] 2.12[e] (Ac's), 5.65[c] (^2J = 52.0 Hz, FCH_2S)
6f	5.88 (6.0)	4.76[g] (5.0)	4.15[g] (4.0)	4.06[g] (5.5, 7.0)	3.12,[g] 3.06[g] (13.5, 2.0[o])	8.16	8.34	7.30		5.36[c] (5.0,[j] OH3′), 5.53[c] (6.0,[j] OH2′), 5.64[h] (^2J = 53.0 Hz, ^4J = 2.0 Hz, CH_2F)
7e (5′S,S_S)[k,t]	6.36 (4.4)	6.03[h] (6.0)	5.93[h] (5.9)	4.72[g] (1.8,[n] 27.9[o])	5.56[h] (46.2[p])	8.35	8.18	7.42	7.17,[c] 7.72[c] (9.0)	2.08,[e] 2.13[e] (Ac's)
7g (5′R)[m]	6.27 (6.0)	6.11[h] (5.5)	5.82[g,u] (2.8)	4.68[g] (6.0,[n] 13.4[o])	6.15[h] (44.5[p])	8.08	8.25	7.40	7.02,[c] 7.73[c] (9.0)	1.99,[e] 2.14[e] (Ac's), 3.82[e] (CH_3O)
7g (5′S)[m]	6.22 (6.0)	6.01[h] (5.5)	5.88[h] (4.1)	4.72[g] (3.0,[n] 25.8[o])	6.27[h] (44.5[p])	8.12	8.34	7.38	7.02,[c] 7.75[c] (9.0)	2.04,[e] 2.11[e] (Ac's), 3.78[e] (CH_3O)
8g (5′R)[m]	6.27 (6.0)	6.15[h] (5.5)	5.80[h] (2.8)	4.69[g] (6.0,[n] 13.0[o])	6.18[h] (44.5[p])	8.53	8.32	*v*	6.98,[c] 7.74[c] (9.0)	4.98,[e] 2.20[e] (Ac's), 3.82[e] (CH_3O)
8g (5′S)[m]	6.19 (6.0)	5.96[h] (6.0)	5.78[h] (4.0)	4.77[g] (2.8,[n] 27.0[o])	6.14[h] (44.5[p])	8.51	8.22	*v*	6.93,[c] 7.70[c] (9.0)	2.03,[e] 2.14[e] (Ac's), 3.78[e] (CH_3O)
7h (5′R)[m]	5.92 (6.5)	4.91[g] (6.0)	4.33[g] (4.0)	4.30[g] (6.0,[n] 15.0[o])	6.06[h] (45.0[p])	8.10	8.22	7.32	7.02,[c] 7.72[c] (9.0)	5.76[c] (5.5,[j] OH2′), 5.65[c] (6.5,[j] OH3′), 3.85[e] (CH_3O)
7h (5′S)[m]	5.91 (6.0)	4.69[g] (5.5)	4.47[g] (4.0)	4.40[g] (3.0,[n] 26.3[o])	6.04[h] (45.0[p])	8.02	8.12	7.30	7.04,[c] 7.78[c] (9.0)	5.75[c] (5.0,[j] OH2′), 5.71[c] (6.0,[j] OH3′), 3.82[e] (CH_3O)
8h (5′S)	5.84 (6.0)	4.68[g] (5.5)	4.42[g] (3.4)	4.47[g] (2.8,[n] 28.5[o])	6.02[h] (45.0[p])	8.60	8.12	*w*	6.95,[c] 7.72[c] (9.0)	5.79[c] (5.0,[j] OH2′), 5.77[c] (6.0,[j] OH3′), 3.78[e] (CH_3O)
9e (Z)[k,w]	6.48 (5.2)	6.14[h] (7.1)	6.03[c]		6.59[c] (73.7[p])	8.38	7.98	5.95		2.06,[e] 2.14[e] (Ac's)
9f (Z)[k]	6.24 (7.1)	5.04[g] (4.8)	4.62[h]		6.69[c] (76.0[p])	8.42	8.18	7.40		5.82[c] (6.6,[j] OH2′), 5.67 (4.6,[j] OH3′)
10a[x]	5.92 (6.5)	4.74[g] (3.0)	4.19,[g] 4.26[g] (4.2, 5.0)	4.00,[h] 4.07[h] (6.0,[n] 6.5[n])	4.65,[c] 4.72[c]	8.14, 8.15	8.30, 8.32	7.32		5.49[c] (5.0,[j] OH3′), 5.57[c] (6.0,[j] OH2′), 1.98[e], 2.04[e] (CH_3S), 3.35,[e] 3.41[e] (CH_3O)

[a] Chemical shifts (δ) at 400 MHz unless otherwise noted. [b] "Apparent" first-order coupling constants (in parentheses). [c] Doublet. [d] Upfield peak assigned to H5″ (pro-R). [e] Singlet. [f] Broad singlet. [g] Doublet of doublets of doublets. [h] Doublet of doublets. [i] Multiplet. [j] ($^3J_{HO-CH}$). [k] 200 MHz. [l] Subtraction of signals for **3a** (R_S) from a spectrum of **3a** [$(R/S)_S$]. [m] Signals for the 5′ (R and S) diastereomers were assigned from a spectrum of the mixture by correlation with ^{19}F NMR intensities. [n] ($^3J_{4'-5'}$). [o] ($^3J_{4'-F}$). [p] ($^2J_{5'-F}$). [q] Subtraction of peaks for **5b** (5′S) from a spectrum of **5b** (5′R/S). [r] Signals were assigned by correlation with ^{19}F NMR intensities from a spectrum of the mixture of regio/stereo isomers. [s] ($^4J_{FC-S-CH}$). [t] Shifts for the minor **7e** (5′S, R_S) isomer were the same except δ 6.27 (d, $J_{1'-2'}$ = 5.6 Hz, H1′). [u] ($^4J_{3'-F}$ = 1.5 Hz). [v] No signal observed for NH_2. [w] $CDCl_3$ solution. [x] Mixture of diastereomers (~4:1), major diastereomer peaks are quoted first.

Table 2. ^{13}C NMR Spectral Data[a,b]

compd	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′	aromatic C1″	aromatic C2″	aromatic C3″	aromatic C4″	XCH_3
1a[c]	153.70	150.11	119.67	156.44	141.14	88.44	73.65[d]	73.26[d]	83.92	36.16	136.32	130.11	129.35	127.14	
1b[c]	152.62	149.47	119.20	156.06	139.94	87.33	72.57[d]	72.52[d]	82.96	37.41	125.50	132.99	114.71	158.46	55.71
1c	153.03	149.81	119.47	156.49	140.27	87.65	72.81[d]	72.70[d]	82.89	35.22	135.27	130.12	129.18	130.71	
1d[c]	153.03	149.83	119.39	156.42	140.18	87.50	72.74	72.74	83.85	36.07					15.52
5a[e]	153.37, 153.29	149.91	119.31	156.25	140.46	87.87	73.37, 72.65	71.21, 71.07	84.33,[f] 85.42[h]	101.33,[g] 100.39[g]	132.24, 131.97	131.91, 131.74	130.05, 130.65	128.79	
5b (5′*R*)[i]	153.14	149.90	119.29	156.40	140.00	87.19	72.89	71.10	83.62[j]	101.32[k]	121.07	135.44	115.15	160.25	55.43
5b (5′*S*)	153.14	149.83	119.29	156.50	139.64	87.48	73.04	71.10	85.00[l]	100.85[m]	121.70	135.81	115.25	160.25	55.43
5c[e]	153.17, 153.07	149.87	119.29	156.36, 156.43	140.08, 139.55	87.50, 87.45	72.58, 72.98	70.88, 70.72	83.75,[n] 84.92[o]	100.62,[g] 99.87[p]	130.98, 131.27	133.28, 133.49	129.64	130.15	
6f[q]	152.94	149.60	119.24	156.08	140.25	87.73	72.67[d]	72.55[d]	84.07	34.53					
7h[e]	153.00, 152.95	149.72	119.25	156.32	140.05, 139.11	87.08, 86.79	72.45, 71.97	70.77	81.11,[r] 80.31[t]	100.38,[s] 99.34[u]	127.00, 126.73	131.97, 131.87	114.63	164.50	55.87
8h (5′*S*)	143.49	142.15	118.50	148.47	141.91	87.15	72.30	70.81	80.58[v]	100.63[w]	127.03	132.03	114.34	164.39	55.84
9f (*Z*)	153.25	149.77	119.21	156.11	140.61	87.74	71.75	67.34[x]	142.42[y]	131.18[z]					

[a] Chemical shifts (δ) at 75.5 MHz. [b] Proton-decoupled singlets unless otherwise noted. [c] Literature[22a] values. [d] Assignments might be reversed. [e] Spectrum of the 5′(*R*/*S*) mixture. [f] (d, $^2J_{4'\text{-}F}$ = 22.6 Hz). [g] (d, $^1J_{5'\text{-}F}$ = 219.2 Hz). [h] (d, $^2J_{4'\text{-}F}$ = 20.5 Hz). [i] Signals for **5b** (5′*S*) were subtracted from a spectrum of **5b** (5′*R*/*S*). [j] (d, $^2J_{4'\text{-}F}$ = 24.6 Hz). [k] (d, $^1J_{5'\text{-}F}$ = 220.3 Hz). [l] (d, $^2J_{4'\text{-}F}$ = 20.4 Hz). [m] (d, $^1J_{5'\text{-}F}$ = 223.8 Hz). [n] (d, $^2J_{4'\text{-}F}$ = 25.2 Hz). [o] (d, $^2J_{4'\text{-}F}$ = 19.7 Hz). [p] (d, $^1J_{5'\text{-}F}$ = 222.5 Hz). [q] Peak also at δ 84.92 (d, $^1J_{C\text{-}F}$ = 279.0 Hz, FCH_2S). [r] (d, $^2J_{4'\text{-}F}$ = 23.9 Hz). [s] (d, $^1J_{5'\text{-}F}$ = 220.1 Hz). [t] (d, $^2J_{4'\text{-}F}$ = 17.0 Hz). [u] (d, $^1J_{5'\text{-}F}$ = 218.2 Hz). [v] (d, $^2J_{4'\text{-}F}$ = 17.1 Hz). [w] (d, $^1J_{5'\text{-}F}$ = 221.3 Hz). [x] (d, $^3J_{3'\text{-}F}$ = 4.5 Hz). [y] (d, $^2J_{4'\text{-}F}$ = 2.6 Hz). [z] (d, $^1J_{5'\text{-}F}$ = 245.6 Hz).

Table 3. Kinetic Constants for Inhibition of Bovine Liver AdoHcy Hydrolase by 5b, 5c, and 9f, and Human Recombinant Placental AdoHcy Hydrolase by 11a

inhibitor	K_i (μM)	apparent k_{inact} (min^{-1})	k_{inact}/K_i ($M^{-1}\ min^{-1}$) × 10^6
5b (5′*R*/*S* = 2.7)	~0.3	~4	13.3
5c (5′*R*/*S* = 1.6)	0.0214	0.24	11.2
9f (*Z*-isomer)	0.022	0.042	1.91
11a	1.59	0.13	0.083

[a] Assays were performed at 37 °C in 50 mM potassium phosphate buffer, 1 mM EDTA, pH 7.2, in the synthetic direction as described in the Experimental Section.

mediated step would produce the same 3′-oxidized (keto) form of the hydroxy enol ethers (**J**, Scheme 2) that would result from 3′-oxidation of hydroxy enol ethers **I** (Scheme 2). The latter oxidation step also is plausible since 4′-methylene compound **F** (Figure 1) is oxidized by AdoHcy hydrolase, and its 5′-fluoro analogue (**9f**, Scheme 1) is a potent prodrug inhibitor which has been shown to undergo both hydrolysis (of the vinyl fluoride) and oxidation (at C3′) by the two functional activities of this enzyme.[7b]

In order to probe this possibility further, a 5′-*O*-methyl derivative of hydroxy enol ether **I** was prepared (Scheme 2). Solvolysis of **5d** by its slow passage through a column of silica gel in chloroform/methanol gave the 5′-*O*-methyl-5′-(methylthio)adenosines (**10a**). This mixture was acetylated (**10b**) and oxidized to sulfoxides, and the latter were thermolyzed to give the methoxyvinyl ethers (**11b**). Deacetylation of **11b** and careful chromatographic purification of the sensitive vinyl diether nucleoside(s) gave a single 5′-*O*-methyl-4′,5′-didehydroadenosine (**11a**) diastereomer. The 9-[5(*E*)-*O*-methyl-β-D-*erythro*-pent-4-enofuranosyl]adenine configuration of **11a** is *tentatively* assigned. Its chemical shifts and coupling constants are similar to those of 5′(*E*)-halovinyl analogues in the adenosine series[14,15a] but NOSY and NOE ^1H NMR experiments were inconclusive. Significant NOE enhancements and NOSY crosspeaks were observed between the 5′ (vinyl) and methoxy protons of **11a**, but ≤2% NOE enhancements and no NOSY crosspeaks between the 5′ and 3′ protons were detected. This is consistent with a 5′(*E*) configuration with the methoxy group extended away from the furanose ring but remains *tentative* in the absence of both (*E* and *Z*) isomers. It is noteworthy that the vinyl diether **11a** was stable in buffer solutions used for the enzyme assays, and no decomposition to "adenosine 5′-aldehydes" was observed by HPLC. However, it functioned as a moderately potent time-dependent inactivator of AdoHcy hydrolase (Table 3). Incubation of **11a** with AdoHcy hydrolase generated HPLC peaks with the same retention times found for "3′-oxidized adenosine 5′-aldehydes" obtained by enzyme processing of other adenosine 5′-aldehyde precursors.[7b,18] Thus, AdoHcy hydrolase-mediated hydrolysis/oxidation of the vinyl diether **11a** occurred with accompanying time-dependent inactivation of the enzyme. This provides further support for the ability of AdoHcy hydrolase to oxidize and/or hydrate hydroxy enol ether intermediate(s) such as **I** (Scheme 2).

Our original choice of *O*-acetyl protection[19] allowed isolation and evaluation of **5**. The 2′,3′-*O*-isopropylidene protection strategy[14] was successful for the preparation of **9f** only because the 5′-fluoro substituent provides dramatic stabilization of the otherwise extremely acid-labile vinyl ether function[30] during hydrolysis of the acetal protecting group. Acidic deprotection conditions are precluded with the 5′-α-fluoro thioethers **5**.

Structure Determinations. The configurations and conformations of **3a′** (R_S) and **5b** (5′*S*) were established by X-ray crystallography (see Figures 2 and 3). Structure determination summaries for these compounds with tables containing position and thermal parameters, bond lengths and angles, torsion angles, least-squares planes and dihedral angles, and hydrogen-bond data are available.[31] The structure of **3a′** (R_S) was refined anisotropically, but because of the limited number of observed data for **5b** (5′*S*), its structure was refined isotropically (except the F and S atoms which were refined anisotropically). Positions of all hydrogen atoms that appeared to be involved in H-bonds were located in the respective difference maps for both molecules. Positions for the remaining hydrogen atoms were calculated.

(30) McCarthy, J. R., Jr.; Robins, R. K.; Robins, M. J. *J. Am. Chem. Soc.* **1968**, *90*, 4993.

(31) X-Ray data, analyses, and experimental details are available from the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

Both compounds are adenosine derivatives with similar groups at C5′, but major conformational differences exist in the base–sugar portions of the two molecules (see Figures 2 and 3). The dihedral angle between the least-squares planes of the base and sugar is 91.8° in **3a′** (R_S) and 153.6° in **5b** (5′*S*). The pseudorotation angle for **3a′** (R_S) is 178° corresponding to a $^2T_3$ furanose conformation, and 163° in **5b** (5′*S*) corresponding to a ^2E furanose pucker. These ring orientations were confirmed using the least-squares plane definition for sugar conformations.[32] The glycosyl torsion angle (O4′–C1′–N9–C8) for **3a′** (R_S) is −107° in the syn range whereas that for **5b** (5′*S*) is −20° in the anti range. The C3′–C4′–C5′–S torsion angles for **3a′** (R_S) and **5b** (5′*S*) are both 170° in the "t" (gt) range.

A rationalizing factor for the conformational differences is the intramolecular H-bond between O2′ and N3 in **5b** (5′*S*) (Figure 3). All four hydrogen atoms bonded to N and O atoms in each of the two molecules are involved in H-bonds. However, these interactions are intermolecular except for the intramolecular O2′–HO2′···N3 H-bond in **5b** (5′*S*). An intermolecular H-bond in **3a′** (R_S) involves an O2′–HO2′ interaction with an oxygen atom of a symmetry-related molecule as acceptor (Table 7S in the supplementary material). Forces including the intramolecular O2′–HO2′···N3 H-bond result in crystallization of **5b** (5′*S*) as an elongated molecule with the plane of the sugar nearly parallel to that of the base. In contrast, **3a′** (R_S), which has no intramolecular sugar–base H-bonds, crystallized in a U-shaped conformation with the least-squares planes of the sugar and base almost perpendicular.

Experimental Section

Uncorrected melting points were determined on a microstage block. UV spectra were determined with solutions in MeOH. NMR spectra were determined with solutions in Me_2SO-d_6 unless otherwise noted with Me_4Si as internal [^1H (400 MHz); ^{13}C (100 MHz)] and CCl_3F as external [^{19}F (376.5 MHz)] standards. ^{19}F shifts upfield from CCl_3F have negative values. High-resolution EI mass spectra were determined at 70 eV and low-resolution spectra at 20 eV. Aldrich DAST was used as recieved. Aldrich 85% MCPBA gave equivalent yields before or after extraction to remove 3-chlorobenzoic acid. XeF_2 was obtained from PCR, Inc. Reagent grade chemicals were used and solvents were distilled before use. Pyridine was dried by reflux over and distillation from CaH_2. Silica TLC was performed with the upper phase of EtOAc/PrOH/H_2O (4:1:2) unless otherwise noted with visualization under 254-nm light. Sulfur-containing compounds were detected by spraying TLC plates with a solution of $PdCl_2$ (0.4 g) in concentrated hydrochloric acid/H_2O (1:9, 100 mL). Merck kieselgel 60 (230–400 mesh) was used for column chromatography. Solvents were flash evaporated at <25 °C under water aspirator or mechanical oil pump vacuum. Solid products were dried in vacuo over P_4O_{10} at elevated temperatures.

5′-*S*-(4-Chlorophenyl)-5′-thioadenosine (1c). Treatment of 5′-chloro-5′-deoxyadenosine[22a] (4.28 g, 15 mmol) with 4-chlorobenzenethiolate [generated from 4-chlorobenzenethiol (2.28 g, 15.75 mmol) and sodium hydride (792 mg of 50% NaH/mineral oil, 16.5 mmol)] in DMF as described[22a] gave **1c** [5.02 g, 85% (from MeOH/H_2O, 3:1)]: mp 89–92 °C (softening), 164–166 °C; UV max 259 nm (ε 19 500), min 230 nm (ε 5700); MS *m/z* 393 (19, M^+, ^{35}Cl), 395 [8, M^+, ^{37}Cl (and ^{35}Cl, ^{34}S)]. Anal. Calcd for $C_{16}H_{16}ClN_5O_3S$ (393.85): C, 48.79; H, 4.09; N, 17.78. Found: C, 48.81; H, 4.08; N, 17.90.

2′,3′-Di-*O*-acetyl-5′-*S*-phenyl-5′-thioadenosine (2a). Procedure A. A stirred suspension of 5′-*S*-phenyl-5′-thioadenosine[22a] (**1a**, 1.44 g, 4 mmol) in Ac_2O (1.04 mL, 1.12 g, 11 mmol) was cooled in an ice–H_2O bath and pyridine (6 mL) was added. Stirring was continued for 7 h at 0 °C (TLC indicated complete reaction), MeOH (20 mL) was added, and stirring was continued for 30 min. The solution was evaporated and the residue was partitioned (2% HOAc/H_2O//$CHCl_3$). The organic phase was washed (H_2O, $NaHCO_3$/H_2O, brine, and H_2O), dried (Na_2SO_4), and evaporated to give **2a** (1.74 g, 98%) as a TLC-homogeneous solid foam of sufficient purity for use in subsequent reactions: MS *m/z* 443.1265 (5.9, M^+ [$C_{20}H_{21}N_5O_5S$] = 443.1263).

2′,3′-Di-*O*-acetyl-5′-*S*-(4-methoxyphenyl)-5′-thioadenosine (2b). Acetylation of 5′-*S*-(4-methoxyphenyl)-5′-thioadenosine[22a] (**1b**, 1.56 g, 4 mmol) by procedure A gave **2b** (1.87 g, 99%): MS *m/z* 473.1377 (2.6, M^+ [$C_{21}H_{23}N_5O_6S$] = 473.1369).

2′,3′-Di-*O*-acetyl-5′-*S*-(4-chlorophenyl)-5′-thioadenosine (2c). Acetylation of 5′-*S*-(4-chlorophenyl)-5′-thioadenosine (**1c**, 4.16 g, 10.56 mmol) by procedure A gave **2c** (4.89 g, 97%): MS *m/z* 477 (82, M^+, ^{35}Cl), 479 [36, M^+, ^{37}Cl (and ^{35}Cl, ^{34}S)].

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-(phenylsulfinyl)adenosine (3a). Procedure B. MCPBA (621 mg as 85% reagent, 3.06 mmol) in CH_2Cl_2 (30 mL) was added dropwise to a cold (−40 °C) stirred solution of **2a** (1.33 g, 3 mmol) in CH_2Cl_2 (30 mL) and TLC indicated complete reaction. The solution was poured into ice-cold saturated $NaHCO_3$/H_2O (70 mL) and extracted ($CHCl_3$, 2 × 50 mL). The combined organic phase was washed (brine and H_2O), dried (Na_2SO_4), and concentrated to give **3a** [$(R/S)_S$, 42:58; 1.34 g, 97%] as an amorphous glass: MS *m/z* 459.1216 (12, M^+ [$C_{20}H_{21}N_5O_6S$] = 459.1213), 136.0623 (96, BH_2).

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-[(4-methoxyphenyl)-sulfinyl]adenosine (3b). Treatment of **2b** (1.89 g, 4 mmol) by procedure B gave **3b** [$(R/S)_S$, 46:54; 1.91 g, 98%]: MS *m/z* 489.1324 (9.2, M^+ [$C_{21}H_{23}N_5O_7S$] = 489.1318), 155.0173 (76, ArSO), 136.0620 (61, BH_2). Silica chromatography (2% MeOH/$CHCl_3$) afforded partial separation of the diastereomers with **3b** (R_S) eluted first.

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-[(4-chlorophenyl)sulfinyl]adenosine (3c). Treatment of **2c** (1.91 g, 4 mmol) by procedure B gave **3c** [$(R/S)_S$, 46:54; 1.96 g, 99%]: MS *m/z* 493 (90, M^+, ^{35}Cl), 495 [38, M^+, ^{37}Cl (and ^{35}Cl, ^{34}S)]. Silica chromatography (3% MeOH/EtOAc) afforded partial separation of the diastereomers with **3c** (R_S) eluted first.

2′,3′-Di-*O*-acetyl-5′-fluoro-5′-*S*-phenyl-5′-thioadenosine (4a). Procedure C. DAST (0.528 mL, 0.645 g, 4 mmol) was added by syringe to a stirred solution of **3a** (918 mg, 2 mmol) and $SbCl_3$ (45 mg, 0.2 mmol) in CH_2Cl_2 (20 mL) under N_2 at ambient temperature. TLC indicated complete reaction after 10 h. Excess DAST was destroyed by addition of ice-cold saturated $NaHCO_3$/H_2O and stirring for 30 min. The organic layer was separated and the H_2O layer extracted ($CHCl_3$). The combined organic phase was washed ($NaHCO_3$/H_2O, brine, and H_2O), dried (Na_2SO_4), and concentrated, and the residue was chromatographed (1.5% MeOH/$CHCl_3$) to give amorphous **4a** (5′*R*/

(32) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.

S, 39:61; 636 mg, 69%): ^{19}F NMR δ −155.62 (dd, $^{2}J_{F-5'}$ = 53 Hz, $^{3}J_{F-4'}$ = 10.3 Hz, 0.39, F5′*R*), −159.61 (dd, $^{2}J_{F-5'}$ = 53 Hz, $^{3}J_{F-4'}$ = 18.4 Hz, 0.61, F5′*S*); MS *m/z* 461.1173 (9.5, M$^+$ [$C_{20}H_{20}FN_5O_5S$] = 461.1169). This material failed to crystallize.

2′,3′-Di-*O*-acetyl-5′-fluoro-5′-*S*-(4-methoxyphenyl)-5′-thioadenosine (4b). Treatment of **3b** (978 mg, 2 mmol) by procedure C (8 h) gave **4b** (5′*R*/*S*, 36:64; 719 mg, 74%): ^{19}F NMR δ −156.47 (dd, $^{2}J_{F-5'}$ = 53 Hz, $^{3}J_{F-4'}$ = 10 Hz, 0.36, F5′*R*), −159.68 (dd, $^{2}J_{F-5'}$ = 53 Hz, $^{3}J_{F-4'}$ = 17 Hz, 0.64, F5′*S*): MS *m/z* 491.1264 (8.4, M$^+$ [$C_{21}H_{22}FN_5O_6S$] = 491.1274).

Procedure D. A solution of **2b** (473 mg, 1 mmol) in CH_2Cl_2 (5 mL) was injected into a cold (−25 °C) stirred suspension of XeF_2 (174 mg, 1.03 mmol) in CH_2Cl_2 (2 mL) under Ar in a round-bottom flask fitted with a rubber septum. Xenon was evolved immediately and gas evolution continued (reversal of the syringe plunger) as the sample warmed to ambient temperature. After 1 h the reaction mixture was cooled to −25 °C and solid XeF_2 (17 mg, 0.1 mmol) was added quickly. The mixture was stirred for 30 min at ambient temperature and $CHCl_3$ (20 mL) and saturated $NaHCO_3/H_2O$ were added. The organic layer was washed ($NaHCO_3/H_2O$, brine, and H_2O), dried (Na_2SO_4), concentrated, and the residue was chromatographed (1.5% MeOH/$CHCl_3$) to give **4b** (5′*R*/*S*, 62:38; 339 mg, 69%; R_f same as **2b**). This product had spectroscopic data identical to that obtained by procedure C except for the diastereomer ratio.

Treatment of **2a** (155 mg, 0.35 mmol) and **2c** (167 mg, 0.35 mmol) by procedure D with XeF_2 (62 mg, 0.37 mmol) in CH_2Cl_2 (5 mL) or CH_3CN (5 mL) followed by XeF_2 (12 mg, 0.07 mmol) gave **4a** (5′*R*/*S*, 75:25)/**2a** (~3:1, 82 mg) and **4c** (5′*R*/*S*, 75:25)/**2c** (~2:1, 91 mg), respectively. The sulfoxides **3a** [(*R*/*S*)$_S$, ~1:1.2; 51 mg, 32%] and **3c** [(*R*/*S*)$_S$, ~1:1.2; 45 mg, 27%] also were eluted from the respective columns.

2′,3′-Di-*O*-acetyl-5′-fluoro-5′-*S*-(4-chlorophenyl)-5′-thioadenosine (4c). Treatment of **3c** (300 mg, 0.61 mmol) by procedure C (the reaction mixture was heated at 30 °C during the first hour) with DAST (0.403 mL, 491 mg, 3.05 mmol) and $SbCl_3$ (21 mg, 0.092 mmol) for 12 h gave **4c** (5′*R*/*S*, 37:63; 245 mg, 81%) plus **2c** (~15%, ^{1}H NMR). Treatment of this mixture by procedure B (MCPBA, 20 mg as 85% reagent, 0.1 mmol) and chromatography (2% MeOH/EtOAc) gave **4c** (5′*R*/*S*, 37:63; 178 mg, 59%): ^{19}F NMR δ −155.62 (dd, $^{2}J_{F-5'}$ = 52.5 Hz, $^{3}J_{F-4'}$ = 10.7 Hz, 0.37, F5′*R*), −160.02 (dd, $^{2}J_{F-5'}$ = 53.5 Hz, $^{3}J_{F-4'}$ = 18.3 Hz, 0.63, F5′*S*): MS *m/z* 495 (12, M$^+$, ^{35}Cl), 497 [5, M$^+$, ^{37}Cl (and ^{35}Cl, ^{34}S)]. Recovered **3c** [(*R*/*S*)$_S$, ~1:1; 29 mg, 10%] was eluted in later fractions.

5′-Deoxy-5′-[phenylsulfinyl (*R*$_S$)]adenosine [3a′ (*R*$_S$)]. MCPBA (313 mg of 85% reagent, 1.55 mmol) in CH_2Cl_2 (40 mL) was added slowly to a stirred solution of **1a** (538 mg, 1.5 mmol) in EtOH/CH_2Cl_2 (1:1, 100 mL) at −20 °C. After 30 min the mixture was evaporated and the residue suspended ($CHCl_3$) and washed (minimum volume of $NaHCO_3/H_2O$ to remove 3-chlorobenzoic acid). The insoluble product was recrystallized (hot EtOH/H_2O, 1:5) to give the title compound (270 mg, 48%): mp 196–197 °C; UV max 257 nm (ε 22 000), min 226 nm (ε 7200); MS *m/z* 375.0991 (3, M$^+$ [$C_{16}H_{17}N_5O_4S$] = 375.1001). Anal. Calcd for $C_{16}H_{17}N_5O_4S$ (375.4): C, 51.19; H, 4.56; N, 18.66; S, 8.54. Found: C, 51.27; H, 4.48; N, 18.67; S, 8.61. The R_S configuration was established by X-ray crystallography (Figure 2).

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-[phenylsulfinyl (*R*$_S$)]-adenosine [3a (*R*$_S$)]. Treatment of **3a′** (*R*$_S$) (94 mg, 0.25 mmol) by procedure A (Ac_2O, 66 μL, 71 mg, 0.7 mmol; pyridine, 0.75 mL) gave **3a** (*R*$_S$) (111 mg, 97%) as a solid foam: MS *m/z* 459.1220 (16, M$^+$[$C_{20}H_{21}N_5O_6S$] = 459.1213).

Treatment of 3a (*R*$_S$) by Procedure C (DAST/$SbCl_3$) gave the same diastereomer ratio of **4a** (5′*R*/*S*, 39:61; 72%) (^{19}F NMR) as obtained from the **3a** [(*R*/*S*)$_S$, ~1:1] mixture.

5′-Fluoro-5′-*S*-phenyl-5′-thioadenosine (5a). Procedure E. A solution of **4a** (5′*R*/*S*, 39:61, from procedure C; 184 mg, 0.4 mmol) in MeOH (15 mL) was stirred with saturated NH_3/MeOH (15 mL) at ambient temperature for 2 h and evaporated. The white solid was recrystallized (2 × MeOH) to give **5a** (5′*R*/*S*, 77:23; 49 mg, 33%): mp 110–113 °C; UV max 257 nm (ε 17 000), min 227 nm (ε 5900); ^{19}F NMR δ −154.36 (dd, $^{2}J_{F-5'}$ = 53.5 Hz, $^{3}J_{F-4'}$ = 12 Hz, 0.77, F5′*R*), −160.25 (dd, $^{2}J_{F-5'}$ = 53.5 Hz, $^{3}J_{F-4'}$ = 21 Hz, 0.23, F5′*S*); MS (FAB) *m/z* 378 (41, MH$^+$). Anal. Calcd for $C_{16}H_{16}FN_5O_3S$ (377.4): C, 50.92; H, 4.27; N, 18.56; S, 8.50. Found: C, 51.01; H, 4.23; N, 18.56; S, 8.39. The residue from evaporated mother liquors was crystallized (MeOH) to give **5a** (5′*R*/*S*, 32:68; 69 mg, 46%): mp 131–132 °C (79% total).

5′(*S*)-Fluoro-5′-*S*-(4-methoxyphenyl)-5′-thioadenosine [5b (5′*S*)]. A solution of **4b** (5′*R*/*S*, 36:64, from procedure C; 1.30 g, 2.64 mmol) in MeOH (30 mL) was treated by procedure E to give **5b** (5′*S*) (350 mg, 33%) (Figure 3): mp 170–172 °C dec; UV max 249 nm (ε 19 700), min 225 nm (ε 8400); ^{19}F NMR δ −160.24 (dd, $^{2}J_{F-5'}$ = 53.5 Hz, $^{3}J_{F-4'}$ = 18.8 Hz); MS *m/z* 407 (4, M$^+$). Anal. Calcd for $C_{17}H_{18}FN_5O_4S$ (407.4): C, 50.12; H, 4.45; N, 17.19; S, 7.87. Found: C, 50.09; H, 4.69; N, 16.87; S, 8.09. The second crop of crystals (MeOH) was **5b** (5′*R*/*S*, 76:24; 548 mg, 51%). Pure **5b** (5′*R*): ^{19}F NMR δ −155.05 (dd $^{2}J_{F-5'}$ = 53.5 Hz, $^{3}J_{F-4'}$ = 11.3 Hz) was not obtained.

5′-Fluoro-5′-*S*-(4-chlorophenyl)-5′-thioadenosine (5c). Method A. Treatment of **4c** (5′*R*/*S*, 37:63, from procedure C; 150 mg, 0.30 mmol) by procedure E gave **5c** (5′*R*/*S*, 61:39; 49 mg, 40%): mp 112–116 °C; UV max 253 nm (ε 21 400), min 231 nm (ε 8900); ^{19}F NMR δ −154.78 (dd, $^{3}J_{F-5'}$ = 53.3 Hz, $^{3}J_{F-4'}$ = 12.2 Hz, 0.61, F5′*R*), −160.20 (dd, $^{2}J_{F-5'}$ = 53.4 Hz, $^{2}J_{F-4'}$ = 21.2 Hz, 0.39, F5′*S*); MS *m/z* 411 (2, M$^+$, ^{35}Cl). Anal. Calcd for $C_{16}H_{15}ClFN_5O_3S$ (411.8): C, 46.66; H, 3.67; N, 17.01. Found: C, 46.74; H, 3.87; N, 16.78. The residue from evaporated mother liquors was crystallized (MeOH) to give **5c** (5′*R*/*S*, 23:77; 47 mg, 38%) (78% total).

Method B. Treatment of crude **4c** (5′*R*/*S*, 37:63, from procedure C; 150 mg, ~0.3 mmol; contaminated ~15% with **2c**) by procedure E gave an off-white solid that was chromatographed (EtOAc followed by the usual TLC solvent) and crystallized (MeOH) to give **5c** (5′*R*/*S*, 64:36; 52 mg, 42%) and a second crop (5′*R*/*S*, 37:63; 18 mg, 15%) (57% total).

2′,3′-Di-*O*-acetyl-5′-*S*-methyl-5′-thioadenosine (2d). Treatment of **1d**[22a] (1.19 g, 4 mmol) by procedure A gave **2d** (1.49 g, 98%) as a solid foam: MS *m/z* 381.1105 (9.8, M$^+$ [$C_{15}H_{19}N_5O_5S$] = 381.1107), 136.0623 (100, BH_2).

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-(methylsulfinyl)adenosine (3d). Treatment of **2d** (1.14 g, 3 mmol) in CH_2Cl_2 (40 mL) at −50 °C by procedure B with additional stirring

(30 min) gave complete reaction (TLC). The combined aqueous phase was back-extracted (2 × $CHCl_3$) and the combined organic phase was washed with a small amount of brine, dried (Na_2SO_4), and evaporated to give **3d** [(*R*/*S*)$_S$, ~1:1; 1.03 g, 86%]: MS *m*/*z* 382.0818 (98, M^+ [$C_{15}H_{19}N_5O_6S$] − CH_3 = 382.0821); CI-MS (NH_3) *m*/*z* 398 (100, MH^+).

2′,3′-Di-*O*-acetyl-5′-fluoro-5′-*S*-methyl-5′-thioadenosine (4d) and 2′,3′-Di-*O*-acetyl-5′-*S*-(fluoromethyl)-5′-thioadenosine (6e). Method A. Treatment of **3d** (794 mg, 2 mmol) by procedure C [DAST (0.49 mL, 645 mg, 4 mmol), $SbCl_3$ (45 mg, 0.2 mmol), CH_2Cl_2 (25 mL), under Ar] and flash chromatography (Merck silica No. 9385; 1.5% MeOH/$CHCl_3$) gave **4d** (5′*R*/*S*, 21:42)/**6e** (63:37; 460 mg, 58%): ^{19}F NMR δ −163.62 (dd, $^2J_{F-5'}$ = 54 Hz, $^3J_{F-4'}$ = 12 Hz, 0.21, F5′*R*), −165.08 (dd, $^2J_{F-5'}$ = 54 Hz, $^3J_{F-4'}$ = 17 Hz, 0.42, F5′*S*), −184.63 (t, $^2J_{F-H}$ = 52 Hz, 0.37, FCH_2S); MS *m*/*z* 399.1009 (9, M^+ [$C_{15}H_{18}FN_5O_5S$] = 399.1013), 384.0779 (28, M − CH_3), 366.0879 (22, M − FCH_2). Different ratios of products were obtained under different reaction and workup conditions.

Method B. Treatment of **2d** (1.14 g, 3 mmol) in CH_2Cl_2 (10 mL) by procedure D [XeF_2 (543 mg, 3.21 mmol) in CH_2Cl_2 (4 mL) and then XeF_2 (66 mg, 0.39 mmol)] and purification as in method A gave **4d** (5′*R*/*S*, ~50:50)/**6e** (~39:61; 706 mg, 59%).

5′-*S*-(Fluoromethyl)-5′-thioadenosine (6f) and 5′-*O*-Methyl-5′-(methylthio)adenosine (10a). Treatment of **4d**/**6e** (method B; 200 mg, 0.5 mmol) by procedure E gave a white solid which was dissolved (EtOH) and subjected to preparative TLC (developed 2 × MeOH/$CHCl_3$, 1:6). The lower band was extracted (MeOH/$CHCl_3$) and the extract was evaporated. The residue was dissolved (EtOH, 3 mL) and Et_2O was added to precipitate **6f** (70 mg, 44%; 74% based on the ratio of **6e**): mp 132–134 °C dec; UV max 262 nm (ε 12 700), min 229 nm (ε 1800); ^{19}F NMR δ −184.01 (t, $^2J_{F-H}$ = 53 Hz, FCH_2S); MS *m*/*z* 282 (4, M − FCH_2), 135 (100, BH^+). Anal. Calcd for $C_{11}H_{14}FN_5O_3S$·0.5 C_2H_5OH (338.4): C, 42.60; H, 5.06; N, 20.70. Found: C, 42.39; H, 4.98; N, 20.61. (EtOH was confirmed by ^1H NMR.)

Extraction of the higher PTLC band (when deacetylation was allowed to proceed for 4 h) gave the 5′-*O*-methyl-5′-(methylthio)adenosine diastereomers (**10a**, ~4:1; 35 mg, 22%; 55% based on the ratio of **4d**): MS *m*/*z* 327.0985 (1, M^+ [$C_{12}H_{17}N_5O_4S$] = 327.1001), 312.0763 (76, M − CH_3).

2′,3′-Di-*O*-acetyl-5′-deoxy-5′-fluoro-5′-[(4-methoxyphenyl)sulfonyl]adenosine (7g) and 2′,3′-Di-*O*-acetyl-5′-deoxy-5′-fluoro-5′-[(4-methoxyphenyl)sulfonyl]adenosine 1-*N*-Oxide (8g). MCPBA (319 mg of 85% reagent, 1.58 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirred solution of **4b** (5′*R*/*S*, 36:64, from procedure C; 480 mg, 0.98 mmol) in CH_2Cl_2 (20 mL) at −50 °C. After 10 min, MCPBA (206 mg of 85% reagent, 1.02 mmol) in CH_2Cl_2 (10 mL) was added, the temperature was allowed to rise to 4 °C, and stirring was continued at ~4 °C for 16 h and at ambient temperature for 6 h. Saturated $NaHCO_3/H_2O$ (20 mL) was added, stirring was continued for 10 min, and the layers were separated. The aqueous layer was extracted (2 × $CHCl_3$) and the combined organic phase was washed (H_2O and brine), dried ($MgSO_4$), and evaporated. The solid foam was chromatographed (2% MeOH/$CHCl_3$) to give colorless foams of **7g** (5′*R*/*S*, 38:62; 200 mg, 39%): UV max 248 nm (ε 23 500), min 222 nm (ε 4500); ^{19}F NMR δ −187.97 (dd, $^2J_{F-5'}$ = 44.5 Hz, $^3J_{F-4'}$ = 13.4 Hz, 0.38, F5′*R*), −193.21 (dd, $^2J_{F-5'}$ = 44.5 Hz, $^3J_{F-4'}$ = 25.8 Hz, 0.62, F5′*S*); MS *m*/*z* 523 (24, M^+); and **8g** (5′*R*/*S*, 38:62; 270 mg, 51%): UV max 236, 261 (sh), 304 nm (ε 40 900, 12 600, 2100), min 218, 285 nm (ε 12 500, 1900); ^{19}F NMR δ −187.69 (dd, $^2J_{F-5'}$ = 44.5 Hz, $^3J_{F-4'}$ = 13.0 Hz, 0.38, F5′*R*), −193.52 (dd, $^2J_{F-5'}$ = 44.5 Hz, $^3J_{F-4'}$ = 27.0 Hz, 0.62, F5′*S*); MS *m*/*z* 523 (20, M − 16).

Deoxygenation of 8g to 7g. Si_2Cl_6 (53 mg as 96% reagent, 0.19 mmol) was added to a solution of **8g** (5′*R*/*S*, 38:62; 100 mg, 0.18 mmol) in CH_2Cl_2 (5 mL) and stirring was continued at ambient temperature for 2.5 h. Evaporation followed by chromatography of the residue (10% MeOH/$CHCl_3$) gave **7g** (5′*R*/*S*, 38:62; 75 mg, 77%) as a colorless solid foam.

5′-Deoxy-5′-fluoro-5′-[(4-methoxyphenyl)sulfonyl]-adenosine (7h). Treatment of **7g** (5′*R*/*S*, 38:62; 300 mg, 0.57 mmol) by procedure E gave a white solid which was recrystallized (MeOH/H_2O, 9:1) to give **7h** (5′*R*/*S*, 40:60; 200 mg, 80%): mp 172–185 °C; UV max 247 nm (ε 24 300), min 224 nm (ε 6600); ^{19}F NMR δ −186.38 (dd, $^2J_{F-5'}$ = 45 Hz, $^3J_{F-4'}$ = 15 Hz, 0.40, F5′*R*), −192.03 (dd, $^2J_{F-5'}$ = 45 Hz, $^3J_{F-4'}$ = 26.3 Hz, 0.60, F5′*S*); MS *m*/*z* 439 (0.5, M^+). Anal. Calcd from $C_{17}H_{18}FN_5O_6S$ (439.4): C, 46.47; H, 4.13; N, 15.94; S, 7.30. Found: C, 46.06; H, 4.03; N, 15.75; S, 7.52.

5′-Deoxy-5′(*S*)-fluoro-5′-[(4-methoxyphenyl)sulfonyl]adenosine 1-*N*-Oxide [8h (5′*S*)]. Treatment of **8g** (5′*R*/*S*, 38:62; 400 mg, 0.74 mmol) by procedure E gave a solid which was recrystallized (MeOH/H_2O, 4:1) to give **8h** (5′*S*) (125 mg, 36%): mp 264–265 °C; UV max 235, 260 (sh), 300 nm (ε 45 200, 11 800, 2300), min 215, 288 nm (ε 12 200, 2000); ^{19}F NMR δ −192.85 (dd, $^2J_{F-5'}$ = 45 Hz, $^3J_{F-4'}$ = 28.5 Hz, 1, F5′*S*); MS *m*/*z* 455 (10, M^+). Anal. Calcd for $C_{17}H_{18}FN_5O_7S$ (455.4): C, 44.83; H, 3.98; N, 15.38; S, 7.08. Found: C, 44.48; H, 3.93; N, 15.16; S, 7.18.

2′,3′-Di-*O*-acetyl-5′(*S*)-fluoro-5′-[(4-methoxyphenyl)-sulfinyl (*R*/*S*)$_S$]adenosine [7e (5′*S*,(*R*/*S*)$_S$)]. MCPBA (43 mg as 85% reagent, 0.21 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a solution of **4b** (5′*S*) [95 mg, 0.19 mmol; obtained from **5b** (5′*S*) by procedure A] in CH_2Cl_2 (5 mL) at −40 °C. Stirring was continued at −20 to 0 °C for 2 h, MCPBA (4 mg as 85% reagent, 0.02 mmol) was added at −10 °C, and stirring for 45 min completed the oxidation. The solution was poured into $NaHCO_3/H_2O$ and the organic layer was washed ($NaHCO_3/H_2O$ and brine), dried ($MgSO_4$), and evaporated to give **7e** [5′*S*, (*R*/*S*)$_S$, 23:77; 94 mg, 98%] as a white foam: ^{19}F NMR δ −195.16 (dd, $^2J_{F-5'}$ = 46.5 Hz, $^3J_{F-4'}$ = 27.5 Hz, 0.77, F5′*S*, S_S), −198.23 (dd, $^2J_{F-5'}$ = 46.5 Hz, $^3J_{F-4'}$ = 21.0 Hz, 0.23, F5′*S*, R_S); MS *m*/*z* 507.1239 (1, M^+ [$C_{21}H_{22}FN_5O_7S$] = 507.1224), 352.1070 (71, M − ArSO).

Analogous oxidation of **4b** (5′*R*/*S*, 36:64, from procedure C; 245 mg, 0.50 mmol) gave **7e** [5′*R*/*S*, (*R*/*S*)$_S$; 248 mg, 98%]: ^{19}F NMR δ −187.10 (dd, $^2J_{F-5'}$ = 47.0 Hz, $^3J_{F-4'}$ = 10.5 Hz, 0.28, F5′*R*, R_S), −195.16 (dd, $^2J_{F-5'}$ = 46.5 Hz, $^3J_{F-4'}$ = 27.5 Hz, 0.45, F5′*S*, S_S), −198.23 (dd, $^2J_{F-5'}$ = 46.5 Hz, $^3J_{F-4'}$ = 21.0 Hz, 0.15, F5′*S*, R_S), −199.65 (dd, $^2J_{F-5'}$ = 47.0 Hz, $^3J_{F-4'}$ = 7.5 Hz, 0.07, F5′*R*, S_S). Oxidation of **4b** (5′*R*/*S*, 62:38, from procedure D; 49 mg, 0.10 mmol) gave **7e** (48 mg, 97%): ^{19}F NMR δ −187.10 (0.44), −195.16 (0.32), −198.23 (0.09), −199.65 (0.10). These mixtures also contained ~5% of the **7g** diastereomers.

9-[2,3-Di-*O*-acetyl-5-deoxy-5(*Z*)-fluoro-β-D-*erythro*-pent-4-enofuranosyl]adenine (9e). A solution of **7e** [5′*S*, (*R*/*S*)$_S$] (88 mg, 0.17 mmol) and EtN(*i*-Pr)$_2$ (110 mg, 0.15 mL, 0.85 mmol) in diglyme (3 mL) was purged with N_2 for

30 min and heated at 145 ± 2 °C (oil bath temperature). After 24 h EtN(*i*-Pr)$_2$ (88 mg, 0.12 mL, 0.58 mmol) was added and heating was continued for 24 h. Volatiles were evaporated and the residue was chromatographed (3% MeOH/EtOAc) to give **9e** (43 mg, 71%) as a slightly yellow foam: MS *m/z* 351 (22, M$^+$) plus recovered **7e** (13 mg, 15%).

9-[5-Deoxy-5(*Z*)-fluoro-β-D-*erythro*-pent-4-enofuranosyl]adenine (9f). Treatment of **9e** (40 mg, 0.11 mmol) by procedure E and crystallization of the residue (EtOAc/hexanes) gave **9f** (26 mg, 88%): mp 216–219 °C (lit.[14b] mp 218–221 °C); ^{19}F NMR δ −166.76 (d, $^2J_{F-5'}$ = 76.0 Hz); MS *m/z* 267 (81, M$^+$).

9-[5(*E*)-*O*-Methyl-β-D-*erythro*-pent-4-enofuranosyl]adenine (11a). Fluorination (2 h/ambient temperature) of **2d** (1.52 g, 4 mmol) with DAST (1.16 mL, 1.42 g, 8.8 mmol) and $SbCl_3$ (91 mg, 0.4 mmol) in CH_2Cl_2 (20 mL) as described in ref 1a, supplementary material, gave **4d** (5′*R*/*S*, 1:1.2)/**6e** (~3.5:1; 1.80 g) as a solid yellow foam after workup[1a] (or by procedure C). (Further chromatography[1a] of this material gave a colorless mixture of **4d**/**6e** with different ratios of regio- and diastereoisomers.) Deacetylation of this **4d**/**6e** mixture (1.80 g) by procedure E gave a yellow residue which was passed slowly (~10 h) through a silica column (2 → 8% MeOH/$CHCl_3$) to give 5′-*O*-methyl-5′-(methylthio)adenosine (**10a**; diastereomers, ~4:1; 500 mg, 38% from **2d**) as a white solid foam. Acetylation of this material (500 mg, 1.53 mmol) by procedure A gave 2′,3′-di-*O*-acetyl-5′-*O*-methyl-5′-(methylthio)adenosine (**10b**; diastereomers, ~4:1; 615 mg, 98%): ^{1}H NMR ($CDCl_3$, major diastereomer) δ 2.11, 2.15 (s, s; 3, 3; Ac's), 2.16 (s, 3, CH_3S), 3.54 (s, 3, CH_3O), 4.44 (dd, $J_{4'-5'}$ = 3.7 Hz, $J_{4'-3'}$ = 2.2 Hz, 1, H4′), 4.57 (d, 1, H5′), 5.71 (dd, $J_{3'-2'}$ = 5.3 Hz, 1, H3′), 5.90 (dd, $J_{2'-1'}$ = 7.1 Hz, 1, H2′), 6.31 (d, 1, H1′), 8.12 (s, 1, H2), 8.37 (s, 1, H8); MS (CI) *m/z* 412 (100, MH$^+$). Oxidation [MCPBA (1.05 equiv)/−60 → −40 °C/15 → 30 min] of crude **10b** (615 mg, 1.5 mmol) by procedure B [back-extraction (3 × $CHCl_3$) as described for **3d** is crucial] gave four diastereomers of 2′,3′-di-*O*-acetyl-5′-*O*-methyl-5′-(methylsulfinyl)adenosine (544 mg, 85%): MS (CI) *m/z* 428 (30, MH$^+$), 364 (100, M − CH_3SO). Over-oxidized sulfone byproducts also were formed (5–10%). Crude **10b** (544 mg, 1.27 mmol) in diglyme (20 mL) containing EtN(*i*-Pr)$_2$ (1.32 mL, 0.985 g, 7.62 mmol) was purged (Ar) for 30 min and heated at 135 °C (±2 °C) for 3.5 h. The solution was evaporated and the residue was chromatographed on silica (EtOAc → 4% MeOH/EtOAc) to give 9-[2′,3′-di-*O*-acetyl-5′(*E*)-*O*-methyl-β-D-*erythro*-pent-4-enofuranosyl]adenine (**11b**; 175 mg, 38%) as a yellow foam (purity, 80–90%): ^{1}H NMR ($CDCl_3$) δ 1.98, 2.16 (s, s; 3, 3; Ac's), 3.56 (s, 3, OCH_3), 6.06 (dd, $J_{2'-3'}$ = 5.4 Hz, $J_{2'-1'}$ = 7.3 Hz, 1, H2′), 6.26 (d, $J_{5'-3'}$ = 1.0 Hz, 1, H5′), 6.30 (dd, 1, H3′), 6.34 (d, 1, H1′), 7.99 (s, 1 , H2), 8.36 (s, 1, H8); MS (CI) *m/z* 364 (100, MH$^+$). The sulfone byproduct was stable to these thermolysis conditions and was eluted after the methoxyvinyl compound **11b**. Deacetylation of crude **11b** by procedure E gave a yellow unstable solid which was purified by RP-HPLC (preparative C_{18} column, two injections; 10% CH_3CN/H_2O for 20 min followed by a gradient of 10 → 35% CH_3CN/H_2O for 40 min at 3 mL/min; t_R 38 min). Pooled fractions were evaporated in vacuo at <25 °C and the solid was dried in vacuo (P_4O_{10}, ambient temperature) for 8 h to give **10a** (57 mg, 13% from **10a**; 5% overall yield from **2d**) as a white powder: mp 192–198 °C dec; UV max 259 nm (ε 14 300), min (ε 7500); ^{1}H NMR (DMSO-d_6) δ 3.50 (s, 3, OCH_3), 4.68 (dd, $J_{3'-2'}$ = 5.1 Hz, $J_{3'-OH3'}$ = 3.5 Hz, 1, H3′), 4.97 (ddd, $J_{2'-1'}$ = 7.9 Hz, $J_{2'-OH2'}$ = 6.9 Hz, 1, H2′), 5.42 (d, 1, OH3′), 5.58 (d, 1, OH2′), 6.06 (d, 1, H1′), 6.12 (s, 1, H5′), 8.17 (s, 1, H2), 8.44 (s, 1, H8); ^{13}C NMR (DMSO-d_6) δ 60.28 (CH_3O), 65.92 (C3′), 72.15 (C2′), 86.18 (C1′), 119.23 (C5), 130.10 (C5′), 140.38 (C8), 144.58 (C4′), 150.04 (C4), 153.16 (C2), 156.06 (C6); MS (CI) *m/z* 280 (100, MH$^+$). Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot 0.5H_2O$ (288.3): C, 45.83; H, 4.90; N, 24.30. Found: C, 45.94; H, 4.89; N, 24.20. Yields in this sequence were not readily repeatable and average values are quoted. Repetition of the RP-HPLC purification was sometimes required to give homogeneous analytically pure material.

Determination of Kinetic Constants for Inhibition of AdoHcy Hydrolase. To determine the kinetic constants (K_i and k_{inact}) of enzyme inactivation, various concentrations of the potential inhibitors were preincubated with purified bovine liver[33] or recombinant human placental[34] AdoHcy hydrolase (20 nM) for various times at 37 °C in 0.5 mL of 50 mM potassium phosphate buffer, pH 7.2, containing 1 mM EDTA. Residual enzyme activity was determined in the synthetic direction by adding 10 μL of 10 mM adenosine and 40 μL of 68.7 mM homocysteine to the mixture and continuing the incubation for an additional 5 min. The reaction was terminated by adding 25 μL of 5 N perchloric acid, and the formed AdoHcy was analyzed by HPLC on a C-18 reversed-phase column (Econosphere, Alltech, 250 × 4.6 mm). The elution was carried out in two sequential linear gradients: 6–15% A over 0–9 min, 15–50% A over 9–15 min, where mobile phase A was acetonitrile and mobile phase B was 50 mM sodium phosphate buffer (pH 3.2) containing 10 mM heptanesulfonate. Quantitation of AdoHcy was monitored by UV at 258 nm. The pseudo-first-order rate constants (K_{app}) were obtained from plots of the log % of remaining activity vs preincubation time at each concentration of the inhibitor. K_i and k_{inact} values were obtained from plots of $1/K_{app}$ vs 1/[inhibitor] using the equation

$$1/K_{app} = 1/k_{inact} + K_i/k_{inact}[I]$$

Kinetics of Spontaneous Hydrolysis of 5′-α-Fluoro Thioethers. Stock solutions of 2 mM of the 5′-α-fluoro thioethers **5** were prepared in Me_2SO-d_6 and stored at 4 °C. For stability studies, the stock solutions were diluted 20-fold with 50 mM potassium phosphate buffer, pH 7.2, containing 1 mM EDTA, and incubated at 37 °C. At various time intervals, aliquots of the reaction mixture were withdrawn and frozen instantly in an acetone/dry ice bath. The samples were then subjected to HPLC analysis using a C-18 reversed-phase column (Econosphere, Alltech, 250 × 4.6 mm). The elution gradient consisted of the following sequential steps: 6% A over 0–5 min, 6–80% A over 5–15 min, 80% A over 15–17 min, 6% A over 17–27 min, where mobile phase A is acetonitrile and mobile phase B is 50 mM sodium phosphate buffer, pH 3.2, containing 10 mM heptanesulfonate.

Data were analyzed using equations of $A = A_0e^{-k_1t}$ and $B = k_1A_0/(k_2 - k_1)(e^{-k_1t} - e^{-k_2t})$ with a XYMATH computer program, where *A* is the concentration of **5** at reaction

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time t, A_0 is the initial concentration of **5**, B is the concentration of the formed intermediate (**G**) at reaction time t, k_1 and k_2 are the rate constants as shown in the text.

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Supplementary Material Available: ^{1}H NMR spectra of compounds **2a**, **2b**, **2c**, **3a** $(R/S)_S$, **3b** $(R/S)_S$, **3c** $(R/S)_S$, **4a** (5′R/S), **4b** (5′R/S), **4c** (5′R/S), **3a** (R_S), **2d**, **3d** $(R/S)_S$, **4d** (5′R/S) + **6e**, **7g** (5′R/S), **8g** (5′R/S), **7e** [5′S,$(R/S)_S$], **9e**(Z), and **10a** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.