Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3229–3236

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Biological Activity of Novel *S*-Adenosyl-L-homocysteine Hydrolase Inhibitors

Jennifer A. Steere and John F. Honek*

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Received 21 January 2003; revised 21 April 2003; accepted 3 May 2003

Abstract—Four potential *S*-adenosyl-L-homocysteine hydrolase inhibitors were prepared and tested against purified recombinant rat liver enzyme. Preliminary studies indicate that three of these compounds, **1**, **2**, and **4**, caused time-dependent inactivation of *S*-adenosyl-L-homocysteine hydrolase but showed a biphasic nature. Compound **3** was found to be a rapid equilibrium inhibitor of this enzyme.

Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) plays an important regulatory role in *S*-adenosyl-L-methionine (AdoMet)-dependent methylation reactions.[1a,b,c,d,e] In AdoMet-dependent transmethylation reactions, the methyl group from AdoMet is transferred to various acceptor molecules such as proteins, nucleic acids, and small molecules by specific methyltransferase enzymes.[2a,b,c] *S*-Adenosyl-L-homocysteine is a product of all AdoMet-dependent transmethylation reactions. In eukaryotes, the only known pathway for the catabolism of AdoHcy is its hydrolysis to homocysteine (Hcy) and adenosine (Ado) by AdoHcy hydrolase (Fig. 1).[3a,b] AdoHcy is a potent competitive inhibitor of all AdoMet-dependent methylation reactions. Inhibition of cellular AdoHcy hydrolase results in an intracellular accumulation of AdoHcy, causing a significant increase in the intracellular AdoHcy/AdoMet ratio and the subsequent inhibition of AdoMet-dependent methylations. Failure to regulate the intracellular levels of AdoHcy can therefore lead to cellular toxicity.

In recent years, AdoHcy hydrolase has become an attractive target for drug design. Inhibitors of this enzyme have been shown to exhibit antiviral,[4a,b,c] antiparasitic,[5a,b,c] anti-arthritic[6] and immunosuppressive effects.[7a,b] The antiviral activity associated with inhibiting AdoHcy hydrolase arises due to the observation that most plant and animal viruses require a methylated cap structure at the 5′-terminus of their mRNA for viral replication.[8] Thus, by inhibiting AdoHcy hydrolase, the virus-encoded methyltransferases that are involved in the formation of this methylated cap structure can be inhibited and viral replication can be slowed. AdoHcy hydrolase inhibitors have been shown to be broad-spectrum antiviral agents interfering with the replication of (−) RNA viruses (e.g., Ebola, Marburg, measles, influenza A and B and rabies), (+) RNA viruses (e.g., reo and rota), and some DNA viruses (e.g., vaccinia and cytomegalovirus).[4a,9a,b,c]

The recent determination of the crystal structure of the rat liver[10a,b] and human[11] AdoHcy hydrolases have greatly contributed to our understanding of this enzyme. Even more recently the crystal structure of rat liver AdoHcy hydrolase with the potent acyclic inhibitor D-eritadenine bound has led to a greater understanding of the interactions between inhibitor and enzyme.[12] In this paper, we describe the biological activity of four nucleosides as potential inhibitors of AdoHcy hydrolase.

The compound, 3′-amino-3′-deoxyadenosine (**1**) (Fig. 2), was first discovered to be a potent antibiotic in 1952;[13a,b] however, this compound, to our knowledge, has never been studied as an inhibitor of AdoHcy hydrolase. The study of this compound should allow for the examination of the interactions an amino group at

*Corresponding author. Tel.: +1-519-888-4567; fax: +1-519-746-0435; e-mail: jhonek@uwaterloo.ca

0968-0896/03/$ - see front matter
doi:10.1016/S0968-0896(03)00301-8

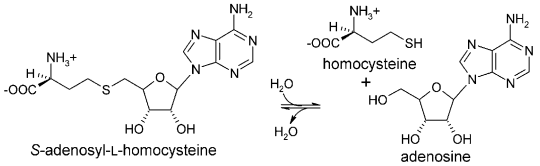


Figure 1. Reaction catalyzed by *S*-adenosyl-L-homocysteine hydrolase.

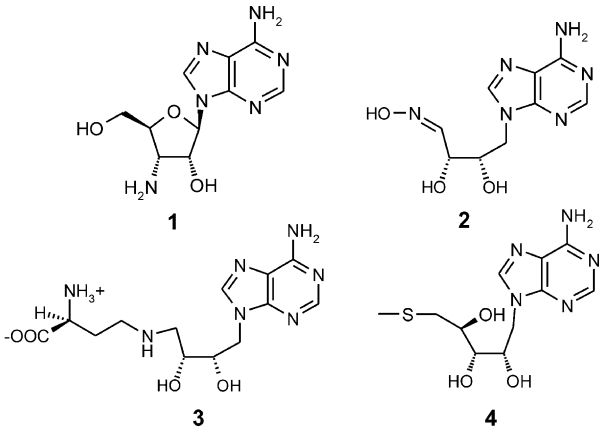


Figure 2. Compounds prepared for this study.

the 3′-position might have with the enzyme and what effect this moiety has on inhibitory activity.

Most of the work previously reported on inhibitors of this enzyme has focused on systematically altering the structure of adenosine and evaluating the derivatives. There have been very few studies on acyclic analogues of adenosine or AdoHcy as inhibitors of AdoHcy hydrolase. A notable exception to this has been the work of Holy et al. which describes a set of ω-carboxyalkyl derivatives and their corresponding MIC_{50} values.[14a] Their work indicated that some of these analogues exhibit rapid irreversible inactivation of SAH hydrolase. In addition, compounds such as (*S*)-9-2,3-dihydroxypropyl)adenine [(*S*)-DHPA], (R,S)-3-adenin-9-yl-2-hydroxypropanoic acid [(*R*,*S*)-AHPA], and D-eritadenine exhibit K_i values of 900 nM,[14b] 40 nM[15] and 3 nM,[16] respectively, against murine L1210 leukemia AdoHcy hydrolase.

We have prepared three acyclonucleosides: 4-(adenin-9-yl)-2,3-dihydroxybutanal oxime (**2**), [4-(adenin-9-yl)-2,3-dihydroxybutyl-L-α-amino]butanoic acid (**3**), and 9-(5′-deoxy-5′-methylthio-D-ribityl)adenine (**4**). Each of these compounds should provide information about the key components of AdoHcy hydrolase interactions with substrates and inhibitors as their cyclic counterparts have been investigated previously (see Kinetic studies).

Results and Discussion

Chemistry

The compound, 3′-amino-3′-deoxyadenosine (**1**) was prepared from adenosine in nine steps utilizing the method by Samano and Robins.[17] We achieved an overall yield of 20% utilizing this method.

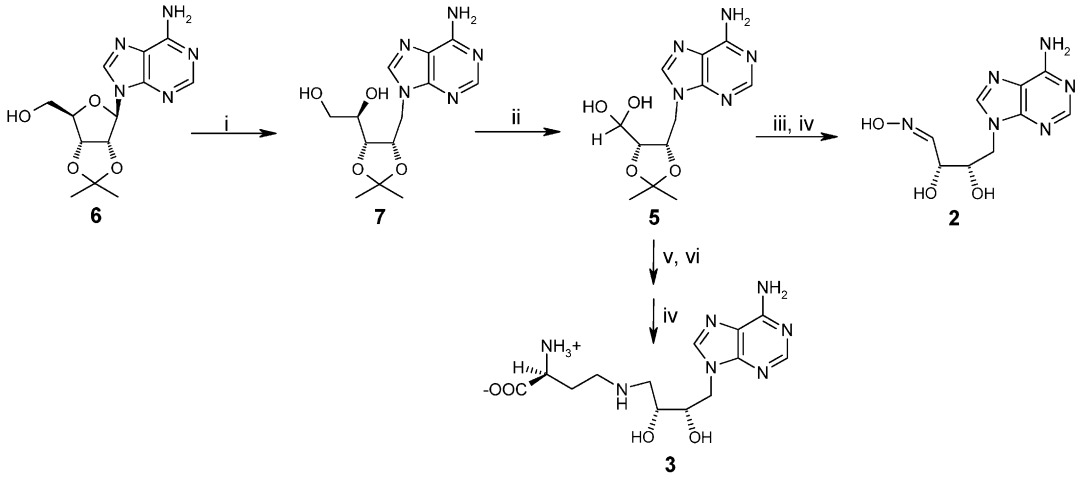
The key intermediate in the preparation of compounds **2** and **3** was 4-(adenin-9-yl)-2,3-*O*-isopropylidenedioxy butanal (**5**) which was obtained by the procedure reported by Hirota et al. (Scheme 1).[18] The first step involves the selective cleavage of the C-1′–O bond of 2′,3′-*O*-isopropylidene adenosine (**6**) with diisobutylaluminum hydride (DIBAL-H) to produce 9-(2,3-*O*-isopropylidene-D-ribityl)adenine (**7**) in 48% yield.[19a,b] Oxidative cleavage of the 2′,3′-diol portion of **7** with sodium periodate gave the key intermediate **5** in quantitative yield as the gem diol.[18] Compound **5** could then be reacted with hydroxylamine hydrochloride and sodium methoxide for 6 h to produce 4-(adenin-9-yl)-2,3-*O*-isopropylidenedioxybutanal oxime **8** in 86% yield as described by Hirota et al.[18] The isopropylidene protecting group was then removed with trifluoroacetic acid (TFA) to give compound **2** as a mixture of E:Z (15:85) isomers in 98% yield. The amine analogue **3** was also prepared from compound **5** by reductive amination with *N*-α-Boc-L-α,γ-diaminobutyric acid, followed by reduction with sodium cyanoborohydride to give [4-(adenin-9-yl)-2,3-*O*-isopropylidenedioxybutanal]-α-*N*-Boc-butanoic acid (**9**) in 36% yield. The protecting groups were then removed with TFA to produce compound **3** in 96% yield.

Compound **4** was prepared by protecting the 2′- and 3′-hydroxyl groups of 5′-deoxy-5′-methylthioadenosine (**10**) using 2,2-dimethoxypropane and *p*-toluenesulfonic acid to give 9-(5′-deoxy-2′,3′-*O*-isopropylidene-5′-methylthio) adenosine (**11**) in 99% yield (Scheme 2).[20] The C-1′–O bond of the now protected derivative **11** was cleaved using DIBAL-H to give 9-(5′-deoxy-2′,3′-*O*-isopropylidene-5′-methylthio-D-ribityl)adenine (**12**) in 17% yield. Compound **12** was then reacted with TFA to remove the protecting group to produce the desired compound **4** in 97% yield.

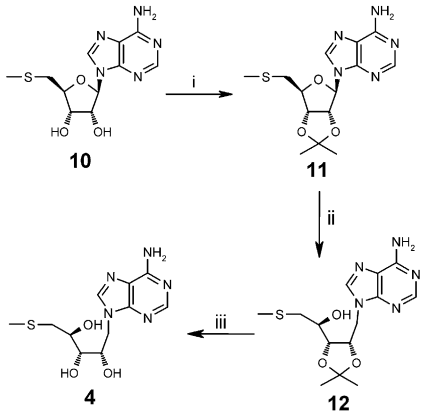
Kinetic studies

The recombinant rat liver AdoHcy hydrolase (MV 1304/pUCSAH) was purified as previously determined by Gomi and coworkers with minor modifications.[21a,b] Enzyme activity was measured in the hydrolytic direction, spectrophotometrically by the method of Mehdi and coworkers.[22] The K_m for *S*-adenosyl-L-homocysteine was determined to be 9.7 μM and the V_{max} was 1.1 μmol/min/mg which compares favourably with previously reported values.[22] The compound, 5′-deoxy-5′-isobutylthioadenosine (SIBA), is a well studied time-dependent inhibitor of AdoHcy hydrolase.[23] When tested, we found it to have a k_{inact} of 0.0488 min^{-1} and a K_i of 105 μM in our system.[21b]

All compounds synthesized were tested as inhibitors against the purified recombinant rat liver AdoHcy hydrolase. Compound **1**, **2**, and **4** were found to inactivate the enzyme in a time-dependent manner. Where possible the kinetic constants for each compound were determined according to the method of Kitz and Wilson.[24] Compound **3** was not found to be a time-dependent inhibitor of this enzyme and was therefore tested as a rapid-equilibrium inhibitor. The kinetic results for all compounds are listed in Table 1.



Scheme 1. The synthesis of compounds **2** and **3**: (i) DIBAL-H (5 equiv), THF (ii) $NaIO_4$, H_2O (iii) $NH_2OH{\cdot}HCl$, NaOMe, MeOH (iv) 8:1 TFA/H_2O (v) *N*-Boc-L-,-diaminobutyric acid, MeOH (vi) $NaCNBH_4$.



Scheme 2. Synthesis of compound **4**: (i) 2,2-dimethoxypropane, TsOH (ii) DIBAL-H (5 equiv), THF (iii) 8/1 TFA/H_2O.

Table 1. Summary of the inhibition studies of AdoHcy hydrolase

Compd	AdoHcy hydrolase inhibition
1	Time-dependent kinetics[a] $K_i = 48$ μM, $k_{inact} = 0.097$ min^{-1}
2	Very rapid biphasic time-dependent kinetics
3	Rapid-equilibrium kinetics[b] $K_i = 66$ uM
4	Very rapid biphasic time-dependent kinetics

[a]The inhibition parameters were calculated from the Kitz and Wilson plot shown in Figure 2.
[b]The inhibition parameters were calculated from the plot of the slope of the lines as a function of [I] shown in Figure 5.

Compound **1** proved to inactivate the rat liver AdoHcy hydrolase in a time-dependent manner, but was biphasic in nature, showing pseudo-first-order kinetics only in the first period of inactivation (Fig. 3). This biphasic process has been observed quite frequently for other AdoHcy hydrolase inhibitors[25a,b] and could be due to a decrease in the concentration of the inhibitor during the experiment or could occur if a generated product was a good inhibitor of the enzyme.[26] In both cases, the earlier time points are more dependable. Further studies will be required in order to determine the cause of this biphasic process; however, it is clear that the presence of an amino function at the

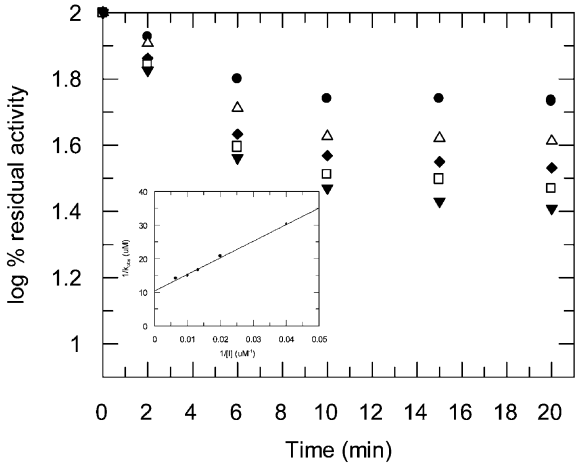


Figure 3. Time-dependent inactivation of AdoHcy hydrolase with compound **1**. The enzyme was preincubated for the indicated times at 37 °C in the presence of 25 μM (●), 50 μM (△), 75 μM (◆), 100 μM (□) or 150 μM (▼) of the compound. At the indicated time points, residual enzyme activity was determined in the hydrolytic direction as stated in the text. Inset: plot of k_{obs}^{-1} versus $[inhibitor]^{-1}$ from which the K_i and k_{inact} values were calculated. The values for compound **1** were determined for time points ≤6 min. Data were the average of triplicate measurements. This was reproducible throughout a number of repeated trials.

3′-position confers the ability of the nucleoside to act as a time-dependent inhibitor of the enzyme.

Compound **2** proved to inactivate the rat liver AdoHcy hydrolase in a time-dependent manner, also with non-pseudo-first-order kinetics (Fig. 4a). There was a very rapid decrease in enzymatic activity from 0 to 2 min followed by a much slower decrease throughout the rest of the experiment. Unfortunately, the initial decrease in enzymatic activity was far too rapid for the extrapolation of data to prepare a Kitz and Wilson plot. Compound **4** also exhibited very similar kinetic results as compound **2** but was not as active (Fig. 4b).

Compound **3** was found not to inhibit the enzyme in a time-dependent manner and was therefore tested as a rapid-equilibrium inhibitor. Preliminary studies have

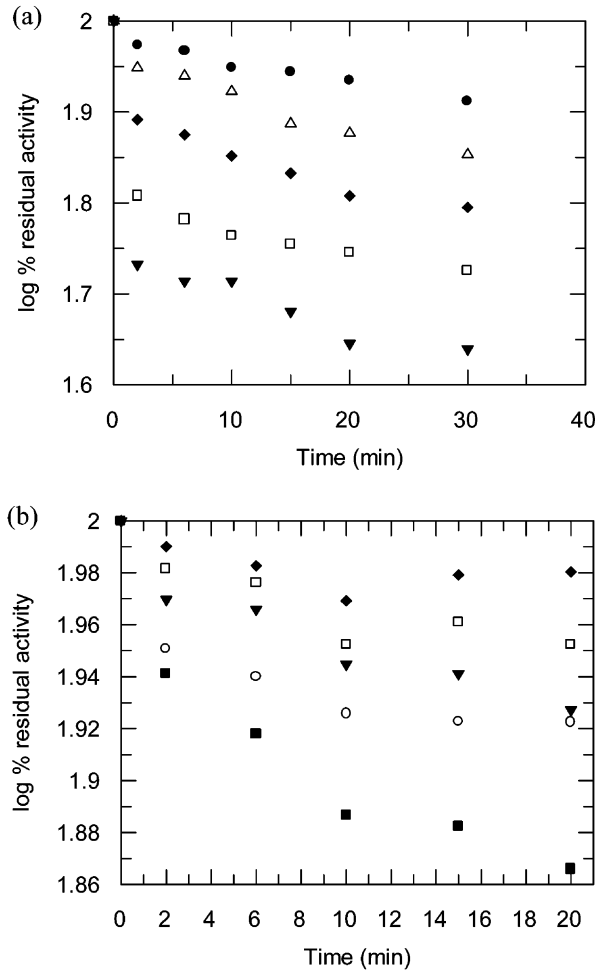


Figure 4. Time-dependent inactivation of AdoHcy hydrolase with (a) compound **2** and (b) compound **4**. The enzyme was preincubated for the indicated times at 37 °C in the presence of 5 μM (●), 10 μM (△), 25 μM (◆), 50 μM (□), 75 μM (▼), 100 μM (○), or 150 μM (■) of the appropriate compound. At the indicated time points, residual enzyme activity was determined in the hydrolytic direction as stated in the text. Data were the average of triplicate measurements. This was reproducible throughout a number of repeated trials.

indicated that this compound is a mixed inhibitor of AdoHcy hydrolase, where both the V_{max} and K_m values are affected by the inhibitor. The K_i, as stated in Table 1, was determined by re-plotting the slope of each line from the Lineweaver–Burk plot as a function of the inhibitor concentration (Fig. 5).[27]

All compounds were tested as possible inhibitors of adenosine deaminase since this protein is present in the assay mixture for AdoHcy hydrolase. None of the compounds showed significant inhibition against adenosine deaminase at the levels measured during this study.

In comparison with previously reported AdoHcy hydrolase inhibitors similar to **1**, **2**, **3**, and **4,** respectively, adenosine weakly inhibits rat liver AdoHcy hydrolase (5% inhibition after 20 min at 20 μM;[28] 35% inhibition after 3 h at 200 μM),[29] adenosine 5′-carboxaldehyde oxime inhibits human placental AdoHcy hydrolase (K_i of 670 nM),[30] N^{γ}-adenosyl-α,γ-diaminobutyric acid inhibits beef liver AdoHcy hydrolase (I_{50} of

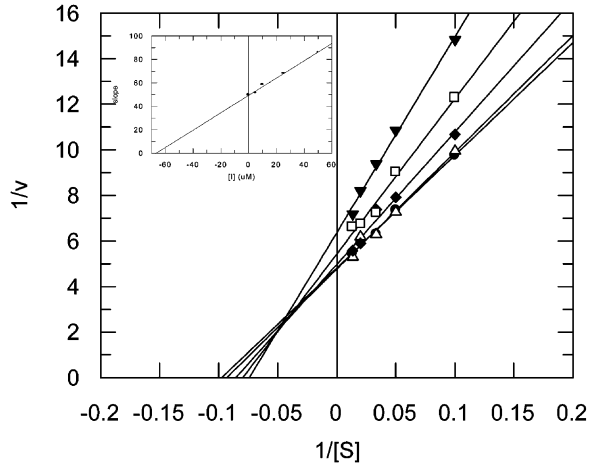


Figure 5. Reversible inactivation of AdoHcy hydrolase with compound **3**. The enzyme was tested in the presence of 0 μM (●), 5 μM (△), 10 μM (◆), 25 μM (□) or 50 μM (▼) of the compound at 37 °C. Inset: Plot of the slope of the lines as a function of [I]. Data were the average of triplicate measurements. This was reproducible throughout a number of repeated trials.

0.14 mM),[23] and 5′-deoxy-5′-methylthioadenosine (MTA) inhibits rat liver AdoHcy hydrolase (K_i of 47 μM).[28] For each of these compounds, except for N^{γ}-adenosyl-α,γ-diaminobutyric acid, the enzymatic activity was measured in the synthetic direction.

Conclusions

Four potential *S*-adenosyl-L-homocysteine hydrolase inhibitors were prepared and tested against purified recombinant rat liver enzyme. One of the more potent compounds tested proved to be compound **1** with K_i and k_{inact} values of 48 μM and 0.097 min^{-1}, respectively. To our knowledge this is the first evaluation of a compound with an amine function at the 3′-position and strongly suggests that this replacement of functionality could lead to more potent and selective inhibitors.

Compounds **2** and **4** showed very similar kinetic results which could be due to a similar mechanism of inactivation. Further detailed biochemical studies would be required to completely elucidate the chemical mechanism of inhibition of AdoHcy hydrolase by these analogues.

Compound **3** is one of the few inhibitors of AdoHcy hydrolase that contains an amino acid moiety. A crystal stucture with this analogue bound to the enzyme should give insight into the binding interactions experienced by the amino acid portion of the natural substrate and may provide additional information about the mechansim of this target enzyme.

Experimental

Thin-layer chromatography was performed on Merck silica gel plates (aluminum backed, 0.2 mm layer of Kieselgel $60F_{254}$). Column chromatography was performed using 70–230 mesh silica gel. Preparative scale

reverse phase separations were performed on a LOBAR apparatus using Merck LiChroprep RP-18 gel. Eluent was delivered through a metering pump (Fluid Metering Inc., USA). Melting points were obtained on a Mel-Temp melting point apparatus and are uncorrected. Fourier transform infrared spectra were recorded on a Perkin–Elmer 1600 FT-IR in chloroform or nujol. Proton (^{1}H) and carbon (^{13}C) magnetic resonance spectra were obtained on Bruker AC-200, AM-250, AC-300 or AC-500 spectrometers. Chemical shifts are reported downfield from TMS ($\delta = 0$) for ^{1}H NMR in $CDCl_3$. For DMSO-d_6 the solvent shift of 2.54 ppm was used. For MeOH-d_4 $\delta_{CD_3OD} = 3.34$ and for D_2O $\delta_{HOD} = 4.67$ ppm was used. For ^{13}C NMR spectra, chemical shifts are reported relative to the central $CDCl_3$ ($\delta = 77.0$), CD_3OD-d_4 ($\delta = 49.5$), or DMSO-d_6 ($\delta = 39.5$). Mass spectra were recorded using FAB or EI on a VG 7070E or a Concept 1S double focussing mass spectrometer.

3′-Amino-3′-deoxyadenosine (1). This compound was prepared as previously described by Samano and Robins[17] in nine steps from adenosine. Mp 259–261 °C (decomp.) (lit. 259–261 °C)[78]; R_f 0.05 (silica gel, 4/1/1 BuOH/H_2O/AcOH); ^{1}H NMR (500 MHz, DMSO) δ 8.36 (s, 1H, H8), 8.12 (s, 1H, H2), 7.28 (s, 2H, NH_2), 5.91 (d, 1H, H1′), 4.36 (dd, 1H, H2′), 3.80 (m, 2H, H4′, H3′), 3.70 (m, 1H, H5′), 3.55 (m, 1H, H5′); ^{13}C NMR (125 MHz, DMSO) δ 156.0 (C6), 152.4 (C2), 148.8 (C4), 139.3 (C8), 119.1 (C5), 89.0 (C1′), 84.9 (C4′), 74.3 (C2′), 60.9 (C5′), 52.2 (C3′); IR ($CHCl_3$) 3339 cm^{-1} (OH); HRMS FAB [m/z 267.11673 ($M + H^+$); {calcd for $C_{10}H_{14}N_6O_3 + H^+$} 267.1127].

9-(2,3-*O*-Isopropylidene-D-ribityl)adenine (7). To a stirred solution of 2′,3′-isopropylidene adenosine (**6**) (1.60 g, 5.234 mmol) in anhydrous THF (100 mL) at 0 °C under argon, was added DIBAL-H (27 mL, 27.23 mmol, 1 M solution in toluene) dropwise under argon. The solution was stirred at room temperature for 24 h, the reaction mixture was quenched at 0 °C with saturated aqueous potassium sodium tartrate (100 mL) and the solution was stirred at room temperature overnight. The mixture was concentrated in vacuo to remove the THF and the aqueous layer was extracted with BuOH (3×150 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (5% MeOH/$CHCl_3$ to 15% MeOH/$CHCl_3$) to give 0.777 g (48%) of a white foam. Mp 154–156 °C (lit. 154–156 °C);[19a] R_f 0.18 (silica gel, 15% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO) δ 8.09 (s, 1H, H8), 8.03 (s, 1H, H2), 7.16 (brs, 2H, NH_2), 5.11 (d, 1H, 4′-OH), 4.62 (t, 1H, 5′-OH), 4.48 (m, 2H, H1′, H2′), 4.16 (dd, 1H, H1′), 4.08 (dd, 1H, H3′), 3.59–3.62 (m, 2H, H4′, H5′), 3.38 (m, 1H, H5′), 1.40 (s, 3H, isopropylidene), 1.15 (s, 3H, isopropylidene); ^{13}C NMR (75 MHz, DMSO) δ 156.5 (C6), 152.8 (C2), 150.1 (C4), 141.9 (C8), 119.3 (C5), 108.9 ($C(CH_3)_2$), 76.4 (C4′), 75.4 (C3′), 69.9 (C2′), 64.4 (C5′), 44.4 (C1′), 28.5 (CH_3), 26.1 (CH_3); IR (nujol) 3331 cm^{-1} (OH); Low Res FAB [m/z 310 ($M + H^+$); {calcd for $C_{13}H_{19}N_5O_4$} 309.3223].

4-(Adenin-9-yl)-2,3-*O*-isopropylidenedioxybutanal (5). To a cold (0 °C) stirring solution of **7** (0.392 g, 1.267 mmol) in 10 mL of H_2O was added $NaIO_4$ (0.407 g, 1.901 mmol) and the mixture was stirred for 2 h. The mixture was quenched with ethylene glycol (0.071 mL, 1.267 mmol) and was further stirred at 0 °C for 1 h. The solvent was evaporated under reduced pressure and the residue was dissolved in anhydrous methanol (25 mL). The precipitate was filtered over a Celite pad and then the filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel (2% MeOH/$CHCl_3$) to give 0.349 g (99%) of the compound as the gem diol structure as a yellow oil. R_f 0.46 (silica gel, 15% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO) δ 8.33 (s, 1H, H8), 8.14 (s, 1H, H2), 7.34 (brs, 2H, NH_2), 6.11 (d, 2H, 4′-OH), 5.33 (dd, 1H, H1′), 5.23 (t, 1H, H4′), 4.95 (dd, 1H, H1′), 4.17–4.23 (m, 1H, H2′), 3.52–3.59 (m, 1H, H3′), 1.54 (s, 3H, isopropylidene), 1.31 (s, 3H, isopropylidene); ^{13}C NMR (75 MHz, MeOH) δ 157.3 (C6), 153.8 (C2), 150.8 (C4), 143.5 (C8), 111.0 (C5), 109.1 ($C(CH_3)_2$), 97.1 (C4′), 79.8 (C3′), 76.5 (C2′), 45.6 (C1′), 28.4 (CH_3), 25.7 (CH_3); IR (neat) 3339 cm^{-1} (OH); Low Res FAB [m/z 300 ($M + Na^+$); {calcd for $C_{12}H_{15}N_5O_3 + Na^+$} 300.261].

4-(Adenin-9-yl)-2,3-*O*-isopropylidenedioxybutanal oxime (8). To a stirred solution of hydroxylamine hydrochloride (0.077 g, 1.107 mmol) in anhydrous MeOH (10 mL) was added NaOMe (0.059 g, 1.107 mmol) at room temperature. The mixture was stirred for 3 h and then the aldehyde **5** (0.229 g, 0.775 mmol) was added and the mixture was stirred at room temperature for 16 h. The mixture was concentrated in vacuo and the residue was purified by silica gel chromatography (2% MeOH/$CHCl_3$ to 15% MeOH/$CHCl_3$) to give 0.195 g (86%, E/Z = 15/85) of a white solid. Mp 234–236 °C (decomp.) (lit. 234–237 °C);[18] R_f 0.32 (silica gel, 15% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO) δ (Z isomer) 11.28 (s, 1H, OH), 8.10 (s, 1H, H8), 8.03 (s, 1H, H2), 7.43 (d, 1H, H4′), 7.21 (brs, 2H, NH_2), 4.66–4.78 (m, 2H, H2′ and H3′), 4.18 (d, 2H, H1′), 1.43 (s, 3H, isopropylidene), 1.22 (s, 3H, isopropylidene); (E isomer) 11.65 (s, 1H, OH), 8.09 (s, 1H, H8), 8.01 (s, 1H, H2), 7.18 (brs, 2H, NH_2), 6.94 (d, 1H, H4′), 5.23 (dd, 1H, H3′), 4.43 (ddd, 1H, H2′), 4.14 (dd, 1H, H1′), 3.96 (dd, 1H, H1′), 1.45 (s, 3H, isopropylidene), 1.26 (s, 3H, isopropylidene); ^{13}C NMR (75 MHz, DMSO) δ 156.5 (C6), 153.0 (C2), 150.0 (C4), 146.0 (C1′), 141.4 (C8), 119.0 (C5), 109.9 ($C(CH_3)_2$), 75.8 (C3′), 75.1 (C2′), 44.1 (C4′), 28.2 (CH_3), 25.5 (CH_3), (the E isomer is difficult to distinguish); IR ($CHCl_3$) 3232 cm^{-1} (OH), 1682 (C=N); Low Res FAB [m/z 293 ($M + H^+$); {calcd for $C_{12}H_{16}N_6O_3$} 292.296].

4-(Adenin-9-yl)-2,3-dihydroxybutanal oxime (2). Compound **8** (0.072 g, 0.246 mmol) was placed in a round bottomed flask and dissolved in 5 mL of 8/1 TFA/H_2O. This was stirred for 1 h. The solvent was evaporated under reduced pressure and redissolved in MeOH and concentrated. This procedure was repeated three times and the residue was dissolved in H_2O and lyophilized to give 0.060 g (98%) of a white powder. Mp 65–67 °C; R_f 0.1 (silica gel, 15% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, DMSO) δ (Z isomer) 11.06 (s, 1H, OH), 8.42 (s, 1H, H8), 8.32 (s, 1H, H2), 7.21 (d, 1H, H4′), 4.33–4.46 (m, 2H, H2′ and H3′), 4.10 (d, 2H, H1′); (the

E isomer is difficult to distinguish); ^{13}C NMR (75 MHz, DMSO) δ 151.5 (C6), 150.5 (C2), 149.4 (C4), 146.3 (C1′), 145.0 (C8), 119.4 (C5), 71.6 (C3′), 63.3 (C2′), 47.3 (C4′); (the *E* isomer is difficult to distinguish); IR ($CHCl_3$) 3257 cm^{-1} (OH), 1674 cm^{-1} (C=N); HRMS FAB [*m*/*z* 253.10811 ($M+H^+$); {calcd for $C_9H_{12}N_6O_3+H^+$} 253.10487].

[4-(Adenin-9-yl)-2,3-*O*-isopropylidenedioxybutanal]-L-α-*N*-*t*-Boc butanoic acid (9). Aldehyde **5** (0.331 g, 1.194 mmol) was placed in a round bottomed flask and dissolved in 20 mL of toluene. This mixture was refluxed with a Dean-Stark trap for 1 h and then concentrated in vacuo. The residue was placed under argon and the *N*-L-α-Boc-α,γ-diaminobutyric acid (0.287 g, 1.313 mmol) was added. Methanol (20 mL) was added and this was stirred at room temperature for 2 h. Sodium cyanoborohydride (0.225 g, 3.582 mmol) was added and the mixture was stirred at room temperature overnight. The reaction was concentrated under reduced pressure and then purified on a reverse phase LOBAR column C-18 (0–10% CH_3CN/H_2O in 2% increments of 100 mL each) to give 0.206 g (36%) of a yellow crystal. Mp 232–234 °C; R_f 0.16 (silica gel, 4/1/1 BuOH/H_2O/AcOH); ^{1}H NMR (300 MHz, MeOH) δ 8.21 (s, 1H, H8), 8.13 (s, 1H, H2), 4.43 (dd, 2H, H1′), 4.11 (dd, 1H, H2′), 3.98 (t, 1H, Hα), 3.82 (dd, 1H, H3′), 2.83 (d, 2H, H4′), 2.71 (dd, 2H, Hγ), 2.01 (m, 1H, Hβ), 1.79 (m, 1H, Hβ), 1.39 (s, 9H, Boc, C(CH_3)$_3$), 1.33 (s, 3H, isopropylidene), 1.21 (s, 3H, isopropylidene); ^{13}C NMR (75 MHz, MeOH) δ 177.9 (acid, C=O), 156.3 (Boc, C=O), 155.9 (C6), 152.6 (C2), 149.6 (C4), 142.3 (C8), 118.2 (C5), 109.6 (*C*(CH_3)$_2$), 78.7 (Boc, *C*(CH_3)$_3$), 77.5 (C3′), 77.1 (C2′), 54.2 (Cα), 50.7 (C4′), 46.2 (Cγ), 44.3 (C1′), 32.6 (Cβ), 27.4 (Boc, C(*C*H_3)$_3$), 26.2 (CH_3), 25.8 (CH_3); IR ($CHCl_3$) 3423 cm^{-1} (OH), 1636 cm^{-1} (C=O); HRMS FAB [*m*/*z* 502.20628 ($M+Na^+$); {calcd for $C_{21}H_{33}N_7O_6+Na^+$} 502.20897].

4-[(Adenin-9-yl)-2,3-dihydroxybutylL–α-amino] butanoic acid (3). Compound **9** (0.029 g, 0.061 mmol) was placed in a round bottomed flask and dissolved in 8/1 TFA/H_2O (5 mL) and stirred overnight at room temperature. The solvent was evaporated under reduced pressure and redissolved in MeOH and concentrated. This procedure was repeated three times and the residue was dissolved in H_2O and lyophilized to give 0.02 g (96%) of a white powder. Mp 158–160 °C; R_f 0.1 (silica gel, 4/1/1 BuOH/H_2O/AcOH); ^{1}H NMR (300 MHz, D_2O) δ 8.31 (s, 1H, H8), 8.27 (s, 1H, H2), 4.28 (dd, 2H, H1′), 3.94 (m, 1H, H2′), 3.76 (m, 2H, H4′), 2.71 (m, 4H, H3′, Hα, Hγ), 2.14 (m, 2H, Hβ); ^{13}C NMR (75 MHz, MeOH) δ 163.0 (C=O), 149.9 (C6), 149.5 (C2), 145.4 (C4), 144.4 (C8), 112.0 (C5), 76.3 (C3′), 69.9 (C2′), 66.9 (Cα), 49.9 (C4′), 47.1 (Cγ), 44.8 (C1′), 26.6 (Cβ); IR ($CHCl_3$) 3301 cm^{-1} (OH), 1676 cm^{-1} (C=O); HRMS FAB [*m*/*z* 340.17447 ($M+H^+$); {calcd for $C_{13}H_{21}N_7O_4+H^+$} 340.17328].

9-(5′-Deoxy-2′,3′-*O*-isopropylidene-5′-thiomethyl) adenosine (11). In a round bottomed flask under argon was placed 5′-deoxy-5′-methylthioadenosine (**10**) (0.1 g, 0.336 mmol), *p*-toluenesulfonic acid monohydrate (0.128 g, 0.673 mmol) and 2,2-dimethyoxypropane (0.332 mL, 2.688 mmol) under argon. This was dissolved in dry acetone (15 mL) and stirred at room temperature for 2 h. The mixture was then neutralized with 10% NH_4OH in H_2O and then evaporated under reduced pressure. The residue was purified by silica gel chromatography (5% MeOH/$CHCl_3$) to give 0.147 g (99%) of a colourless oil. R_f 0.78 (silica gel, 15% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, MeOH) δ 8.24 (s, 1H, H8), 8.19 (s, 1H, H2), 6.15 (d, 1H, H1′), 5.48 (dd, 1H, H2′), 5.02 (dd, 1H, H3′), 4.32 (m, 1H, H4′), 2.72 (m, 2H, H5′), 2.02 (s, 3H, SCH_3), 1.55 (s, 3H, isopropylidene), 1.35 (s, 3H, isopropylidene); ^{13}C NMR (75 MHz, MeOH) δ 156.1 (C6), 152.7 (C2), 148.9 (C4), 140.5 (C8), 119.2 (C5), 114.1 (*C*(CH_3)$_2$), 90.3 (C1′), 86.4 (C4′), 83.8 (C3′), 78.2 (C2′), 35.9 (C5′), 26.1 (CH_3), 24.2 (CH_3), 14.6 (SCH_3); Low Res FAB [*m*/*z* 338 ($M+H^+$); {calcd for $C_{14}H_{19}N_5O_3S$} 337.395].

9-(5′-Deoxy-2′,3′-*O*-isopropylidene-5′-thiomethyl-D-ribityl)adenine (12). To a stirred suspension of **11** (0.048 g, 0.142 mmol) in anhydrous THF (10 mL) at room temperature was added DIBAL-H (0.711 mL, 0.711 mmol) dropwise, under argon. The solution was stirred at room temperature for 24 h and the reaction was quenched with saturated potassium sodium tartrate (10 mL). The solvent was evaporated and the aqueous residue was extracted with *n*-BuOH (30 mL×2). The organic layers were combined, dried (Na_2SO_4) and concentrated in vacuo. This was purified by silica gel chromatography (2% MeOH/$CHCl_3$ to 18% MeOH/$CHCl_3$) to give 0.008 g (17%) of a yellow oil. R_f 0.52 (silica gel, 15% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, MeOH) δ 8.19 (s, 1H, H8), 8.11 (s, 1H, H2), 4.68 (dd, 1H, H4′), 4.58 (m, 1H, H2′), 4.30 (m, 1H, H3′), 4.17 (m, 1H, H1′), 3.93 (m, 1H, H1′), 2.92 (dd, 1H, H5′), 2.60 (dd, 1H, H5′), 2.16 (s, 3H, SCH_3), 1.47 (s, 3H, isopropylidene), 1.26 (s, 3H, isopropylidene); ^{13}C NMR (75 MHz, MeOH) δ 156.2 (C6), 152.3 (C2), 148.8 (C4), 142.0 (C8), 119.6 (C5), 109.2 (*C*(CH_3)$_2$), 78.3 (C3′), 75.6 (C4′), 68.2 (C2′), 44.2 (C1′), 39.4 (C5′), 27.0 (CH_3), 24.3 (CH_3), 15.2 (SCH_3); IR ($CHCl_3$) 3330 cm^{-1} (OH); HRMS FAB [*m*/*z* 340.14297 ($M+H^+$); {calcd for $C_{14}H_{21}N_5O_3S+H^+$} 340.14430].

9-(5′-Deoxy-5′-thiomethyl-D-ribityl)adenine (4). Compound **12** (10 mg, 0.025 mmol) was dissolved in 8/1 TFA/H_2O (0.5 mL) in a round bottom flask was stirred for 1.5 h at room temperature. The solvent was removed in vacuo. Methanol (5 mL) was added to the mixture and subsequently evaporated under reduced pressure. The treatment with methanol was repeated three times. The residue was dissolved in H_2O and lyophilized overnight to give 0.007 g (97%) of a yellow solid. Mp 73–75 °C; R_f 0.15 (silica gel, 15% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, MeOH) δ 8.34 (s, 1H, H8), 8.29 (s, 1H, H2), 4.68 (dd, 1H, H4′), 4.56 (m, 1H, H2′), 4.36 (m, 1H, H3′), 4.06 (m, 1H, H1′), 3.84 (m, 1H, H1′), 2.90 (dd, 1H, H5′), 2.60 (dd, 1H, H5′), 2.11 (s, 3H, SCH_3); ^{13}C NMR (75 MHz, MeOH) δ 156.4 (C6), 151.4 (C2), 148.6 (C4), 145.1 (C8), 119.3 (C5), 74.4 (C3′), 71.4 (C4′), 70.5 (C2′), 44.5 (C1′), 37.5 (C5′), 14.8 (SCH_3); IR ($CHCl_3$) 3342 cm^{-1} (OH); HRMS FAB [*m*/*z* 300.11535 ($M+H^+$); {calcd for $C_{11}H_{17}N_5O_3S+H^+$} 300.15115].

Protein purification. The recombinant rat liver AdoHcy hydrolase was purified from the cell-free extract of *E. coli* transformed with plasmid pUCSAH and grown in the presence of isopropyl β-D-thiogalactopyransoide according to the procedure described by Gomi et al. for the purification, including DEAE Sepharose and Sephacryl S-300 chromatography.[21] About 12 mg of enzyme was obtained from a 1 L culture. The homogeneity of the AdoHcy hydrolase preparation was checked by ESI-MS, the major molecular species being detected at 47,407 Da for each enzyme subunit. The protein concentration was determined by the Lowry method using bovine serum albumin (BSA) as a standard.[31]

Enzyme assay. AdoHcy hydrolase activity was measured spectrophotometrically[22] by following the formation of homocysteine, which was detected by reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to give the chromophoric thiolate ($\varepsilon_{412nm} = 13.6\ mM^{-1}\ cm^{-1}$). The reaction mixture (1 mL total) had the following composition: 100 μM DTNB, 62.5 μM *S*-adenosyl-L-homocysteine, 2 U adenosine deaminase (calf intestine) and AdoHcy hydrolase in 10 mM potassium phosphate buffer, 1 mM EDTA, pH 7.5. The assay buffer contained 10 mM potassium phosphate buffer, 0.1 mM DTNB, 1 mM EDTA, pH 7.5 in which the adenosine deaminase, AdoHcy and AdoHcy hydrolase are added. The resulting mixture was mixed rapidly and the absorbance was measured at 412 nm with respect to time.[22]

Time-dependent inhibition studies. Compounds were evaluated as time-dependent inhibitors of AdoHcy hydrolase by the method of Kitz and Wilson.[21,22] Sufficient quantities of enzyme (2.5 μg) were incubated with various concentrations of each inhibitor in buffer A (10 mM potassium phosphate buffer, 1 mM EDTA, pH 7.5) at 37 °C for the indicated lengths of time. At specific time points, 40 μL of this incubation mixture was withdrawn and added to a cuvette containing 960 μL of the assay buffer (10 mM potassium phosphate buffer, 1 mM EDTA, 0.1 mM DTNB, pH 7.5). The remaining activity was measured in the hydrolytic direction at 37 °C. Each measurement was performed in triplicate.

Rapid-equilibrium inhibition studies. For compound **3**, the K_i value was determined by measuring the enzymatic activity with no inhibitor as well as four additional inhibitor concentrations, at each of four substrate concentrations between 10 and 75 μM. Each point was measured in triplicate. A double reciprocal (Lineweaver–Burk) plot of 1/v as a function of 1/[S] produced a plot indicative of the type of inhibition that was occurring. The K_i value was then determined by replotting the slope of each line from the Lineweaver–Burk plot as a function of the inhibitor concentration.[27]

Inhibition against adenosine deaminase. All compounds were tested as possible inhibitors of calf intestine adenosine deaminase.[32] The reaction mixture (1 mL total) had the following composition: 50 mM potassium phosphate buffer (pH 7.6), 66 μM adenosine, and 100 μM of inhibitor. The adenosine deaminase (0.03 U) was added and the resulting mixture was mixed rapidly and the absorbance was measured at 265 nm, 25 °C.

Acknowledgements

We gratefully acknowledge Drs. R. Aksamit and G. Cantoni for the Escherichia coli MV1304/pUCSAH used in these studies and the Natural Sciences and Engineering Research Council of Canada for financial assistance. Graduate student funding in the form of an OGSST to J.A.S. is also gratefully appreciated. We would also like to thank Tim Jones and Jan Venne for conducting MS and NMR studies, respectively and to A. Litchfield for preliminary synthetic studies.

References and Notes

1. (a) Ueland, P. M. *Pharm. Rev.* **1982**, *34*, 223. (b) Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521. (c) Wolfe, M. S.; Cantoni; G. L.; Chiang, P. K. In *Natural Sulfur Compounds. Novel Biochemical and Structural Aspects*, Cavallini, D., Gaulle, G. E., Zappia, V., Eds.; Plenum: New York, 1980; p. 67 (d) Turner, M. A.; Yang, X.; Yin, D.; Kuczera, K.; Borchardt, R. T.; Howell, P. L. *Cell Biochem. Biophys* **2000**, *33*, 101. (e) Chiang, P. K. *Pharmacol. Ther.* **1998**, *77*, 115.
2. (a) Takusagawa, F.; Fujioka, M.; Spies, A.; Schowen, R. L. In *Comprehensive Biological Catalysis: A Mechanistic Reference*, Sinnott, M., Ed.; Academic: San Diego, 1998; pp 1 (b) Drahovsky, D.; Boehm, T. L. J. *Int. J. Biochem.* **1980**, *12*, 523. (c) In *S-Adenosylmethionine-Dependent Methyltransferases: Structures and Functions*, Cheng, X., Blumenthal, R. M., Eds.; World Scientific: Singapore, 1999.
3. (a) de la Haba, G.; Cantoni, G. L., *J. Biol. Chem.*, **1959**, *234*, 603. (b) Chiang, P. K., In *Methods in Pharmacology*, Paton, D. M., Ed.; Plenum: New York, 1985; Vol 6, p. 127.
4. (a) Bray, M.; Driscoll, J.; Huggins, J. W. *Antiviral Res.* **2000**, *45*, 135. (b) Liu, S.; Wolfe, M. S.; Borchardt, R. T. *Antiviral Res.* **1992**, *19*, 247. (c) De Clercq, E.; Bergstron, D. E.; Holy, A.; Montgomery, J. A. *Antiviral Res.* **1984**, *4*, 119.
5. (a) Bitonti, A. J.; Baumann, J.; Varvi, T.; McCarthy, J. R.; McCann, P. P. *Biochem. Pharmacol.* **1990**, *40*, 601. (b) Whaun, J. M.; Miura, G. A.; Brown, N. D.; Gordon, R. K.; Chiang, P. K. *J. Pharmacol. Exp. Ther.* **1986**, *236*, 277. (c) Nakanishi, M.; Iwata, A.; Yatome, C.; Kitade, Y. *J. Biochem.* **2001**, *129*, 101.
6. Wolos, J. A.; Frondorf, K. A.; Esser, R. E. *J. Immunol.* **1993**, *151*, 526.
7. (a) Saso, Y.; Conner, E. M.; Teegarden, B. R.; Yuan, C. S. *Pharmacology* **2001**, *296*, 106. (b) Wolos, J. A.; Frondorf, K. A.; Davies, G. F.; Jarvi, E. T.; McCarthy, F. R.; Bowlin, T. L. *J. Immunol.* **1993**, *150*, 3264.
8. Banerjee, A. K. *Microbiol. Rev.* **1980**, *44*, 175.
9. (a) Andrei, G.; De Clercq, E. *Antiviral Res.* **1990**, *14*, 287. (b) Villalon, M. D. G.; Gil-Fernandez, C.; De Clercq, E. *Antiviral Res.* **1993**, *20*, 131. (c) Snoeck, R.; Andrei, G.; Neyts, J.; Schols, D.; Cools, M.; Balzarini, J.; De Clercq, E. *Antiviral Res.* **1993**, *21*, 197.
10. (a) Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *Biochemistry* **1999**, *38*, 8323. (b) Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *J. Biol. Chem.* **2000**, *275*, 32147.
11. Turner, M. A.; Yuan, C. S.; Borchardt, R. T.; Hershfield, M. S.; Smith, G. D.; Howell, P. L. *Nature Struct. Biol.* **1998**, *5*, 369.
12. Huang, Y.; Komoto, J.; Takata, Y.; Powell, D. R.; Gomi,

T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. *J. Biol. Chem.* **2002**, *277*, 7477.
13. (a) Suhadolnik, R. J. *Nucleosides as Biological Probes*; Wiley-Interscience: New York, 1979; p. 96. (b) Kutateladze, T.; Beabealashvilt, R.; Azhayev, A.; Krayevsky, A. *FEBS Lett*. **1983**, *153*, 420.
14. (a) Holy, A.; Votruba, I.; De Clercq, E. *Collect. Czech. Chem. Commum* **1985**, *50*, 262. (b) De Clercq, E.; Holy, A. *J. Med. Chem*. **1979**, *22*, 510.
15. De Clercq, E.; Holy, A. *J. Med. Chem*. **1985**, *28*, 282.
16. De Clercq, E.; Bergstron, D. E.; Holy, A.; Montgomery, J. A. *Antiviral Res*. **1984**, *4*, 119.
17. Samano, M. C.; Robins, M. J. *Tetrahedron Lett*. **1989**, *30*, 2329.
18. Hirota, K.; Monguchi, Y.; Sajiki, H.; Sako, M.; Kitade, Y. *J. Chem. Soc., Perkin Trans. 1* **1998**, 941.
19. (a) Hirota, K.; Kitade, Y.; Maki, Y. *Tetrahedron Lett*. **1993**, *34*, 4835. (b) Hirota, K.; Monguchi, Y.; Kitade, Y.; Sajiki, H. *Tetrahedron* **1997**, *53*, 16683.
20. Evans, M. E.; Parrish, F. W.; Long, L., Jr. *Carbohydrate Res*. **1967**, *3*, 453.
21. (a) Gomi, T.; Date, T.; Ogawa, H.; Fujioka, M.; Akasmit, R. R.; Backlund, P. S.; Cantoni, G. L. *J. Biol. Chem*. **1989**, *264*, 1618. (b) Steere, J. A.; Sampson, P. B.; Honek, J. F. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 457.
22. Mehdi, S.; Jarvi, E. T.; Koehl, J. R.; McCarthy, J. R.; Bey, P. *J. Enzym. Inhib* **1990**, *4*, 1.
23. Chiang, P. K.; Richards, H. H.; Cantoni, G. L. *Mol. Pharmacol*. **1977**, *13*, 939.
24. Kitz, R.; Wilson, I. B. *J. Biol. Chem*. **1962**, *237*, 3245.
25. (a) Guillerm, G.; Guillerm, D.; Vandenplas-Witkowski, C.; Rogniaux, H.; Carte, N.; Leize, E.; Van Dorsselaer, A.; De Clercq, E.; Lambert, C. *J. Med. Chem*. **2001**, *44*, 2743. (b) Yuan, C. S.; Wnuk, S. F.; Liu, S.; Robins, M. J.; Borchardt, R. T. *Biochemistry* **1994**, *33*, 12305.
26. Silverman, R. B., In *Methods in Enzymology*, Purich, D. L., Ed.; Academic Press: New York, 1995; Vol. 249, p. 240
27. Copeland, R. A. *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*; Wiley-VCH: New York, 1996; p. 202.
28. Ragione, F. D.; Pegg, A. E. *Biochem. J*. **1983**, *210*, 429.
29. Kajander, E. O.; Raina, A. M. *Biochem. J*. **1981**, *193*, 503.
30. Wnuk, S. F.; Yuan, C. S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J. *J. Med. Chem*. **1997**, *40*, 1608.
31. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem*. **1951**, *193*, 265.
32. Riddles, P. W.; Blakeley, R. L.; Zerner, B. In *Methods in Enzymology*, Purich, D. L., Ed.; Academic Press, Inc: New York, 1983; Vol. 91, p. 49.