Are L-Adenosine and Its Derivatives Substrates for *S*-Adenosyl-L-homocysteine Hydrolase?

Mengmeng Wang,[§] Jinsong Zhang,[‡] Daniela Andrei,[†] Krzysztof Kuczera,[§] Ronald T. Borchardt,[‡] and Stanislaw F. Wnuk*,[†]

Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, and Departments of Pharmaceutical Chemistry and Molecular Biosciences, The University of Kansas, Lawrence, Kansas 66047

Received November 24, 2004

Moffatt oxidation of 2′,3′-*O*-isopropylidene-L-adenosine and treatment of the resulting crude 5′-aldehyde with hydroxylamine followed by deprotection gave L-adenosine 5′-carboxaldehyde oximes, whose enantiomers are known to be potent inhibitors of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase. The L-adenosine and its 5′-aldehyde oxime derivatives were found to be inactive as inhibitors of AdoHcy hydrolase. Docking calculations showed that binding of L-adenosine to AdoHcy hydrolase is weaker (higher energy) and less specific (larger number of clusters) compared to D-Ado.

Introduction

The mammalian *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) effects hydrolytic cleavage of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy).[1] The cellular levels of AdoHcy and Hcy are critical because AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes[1,2] and Hcy appears to be a risk factor for coronary artery diseases.[3]

A number of inhibitors, which function as substrates for the "hydrolytic activity" of AdoHcy hydrolase and utilize unusual spatial leniency within the enzyme occupied by C4′ constituents have been developed.[4] Palmer and Abeles found that 4′,5′-didehydro-5′-deoxyadenosine **A** is an alternative substrate of AdoHcy hydrolase[5] (Figure 1). On the basis of this finding, McCarthy et al. designed the 5′-fluoro exomethylene analogue **B**[6a] that led to the discovery that adenosine 5′-carboxaldehyde and its 4′-epimer are equally potent inhibitors.[6b,c] The halo- and dihalo(homovinyl)adenosine analogues **C** and **D,** having isolated double bonds between C5′ and C6′, have been good substrates for the "hydrolytic activity" of the enzymes.[7] The 4′-(halo)-acetylenic[8a] and other 5′-modified adenosine analogues[8b,c] as well as fluoroneplanocin[9a] (but not L-fluoroneplanocin[9b]) are also good inhibitors. The X-ray crystal structures of AdoHcy hydrolase revealed an unusual dual role for a catalytic water molecule at the active site.[10] Recent computational, kinetic, and crystallographic studies provided insight into the conformational changes of the enzyme required for substrate binding and catalysis.[11]

Porter showed that 9-(α-L-lyxofuranosyl)adenine **E**, which contained a 5′-hydroxyl group in a stereochemically incorrect position (the H4′ is on the opposite side of the ribose ring relative to adenosine), rapidly reduced AdoHcy hydrolase, thus suggesting that there was considerable steric tolerance in the vicinity of the binding site for the C4′ of adenosine.[12] However, the lack of epimerization of **E** to adenosine after oxidation (i.e., the enzyme does not catalyze dehydration of 3′-keto-**E**) was consistent with stereospecific removal of the 4′-hydrogen during the dehydration reaction.[13]

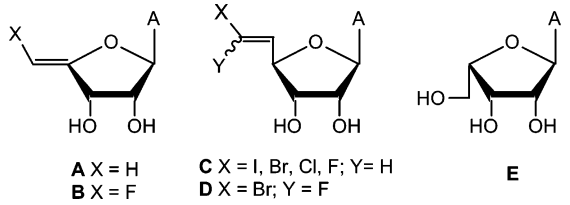
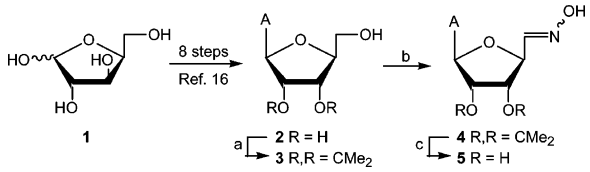


Figure 1. Selected inhibitors of AdoHcy hydrolase.

Scheme 1[a]



[a] Reagents: (a) $CH(OEt)_3$, Me_2CO, *p*-$CH_3C_6H_4SO_3H$; (b) (i) DMSO, DCC, Cl_2CHCO_2H, (ii) NH_2OH, pyridine; (c) TFA, H_2O.

Potent antiviral activity of the unnatural L-nucleosides against HIV and HBV viruses has been demonstrated in the past decade.[14] Also, very recently the halohomovinyl and acetylenic derivatives of L-adenosine were prepared but showed only modest cytostatic activities and were found to be inactive or moderately active as AdoHcy hydrolase inhibitors.[15] In view of these findings, herein, we report enzymatic studies on the interaction of L-adenosine and its 5′-oxime derivatives with AdoHcy hydrolase and computational studies of substrate specificity of the enzyme toward L-adenosine.

Chemistry

The L-adenosine **2** was prepared from L-xylose **1** in eight steps following the Moyround and Strazewski protocol (Scheme 1).[16] Moffatt oxidation of 2′,3′-*O*-isopropylidene-L-adenosine **3** gave crude 5′-aldehyde that was treated with hydroxylamine hydrochloride in pyridine to give the protected oximes **4** (*E*/*Z*, ∼6:1, 80%).

* To whom correspondence should be addressed. Phone: 305-348-6195. Fax: 305-348-3772. E-mail: wnuk@fiu.edu
§ Department of Molecular Biosciences, The University of Kansas.
‡ Department of Pharmaceutical Chemistry, The University of Kansas.
† Florida International University.

10.1021/jm0490484 CCC: $30.25 © 2005 American Chemical Society
Published on Web 04/21/2005

Table 1. Summary for AutoDock Results for D-Ado and L-Ado Docking to the Closed Form of AdoHcy Hydrolase

	total no. of clusters[a]	av energy of top cluster[b] (kcal/mol)	no. of solutions in top cluster
D-Ado	16	-18.9 ± 0.3[c]	21
L-Ado (**2**)	24	-16.3 ± 0.3[c]	26

[a] Clustering of a total of 128 runs. [b] Average binding energy of the first cluster for L-Ado and second cluster for D-Ado. The first cluster for D-Ado was rejected as described in text. [c] Standard deviation over solutions within cluster.

Acid-catalyzed removal of isopropylidene group gave **5** (*E*/*Z*, ~6:1, 89%). Oximes **5** have spectroscopic properties identical to those of their known enantiomer, adenosine 5′-carboxaldehyde oximes.[17a]

Inactivation of *S*-Adenosyl-L-homocysteine Hydrolase

L-Adenosine **2** and its 5′-oxime derivatives **5** were evaluated for their ability to inhibit the activity of recombinant human placental AdoHcy hydrolase by incubating the enzyme with the compounds at 200 μM for 20 min at 37 °C. The AdoHcy hydrolase activity was determined by assaying the enzyme's ability to catalyze the conversion of Ado and Hcy to AdoHcy. Under these conditions, **2** and **5** were inactive as inhibitors of the AdoHcy hydrolase (data not shown). In contrast, adenosine-5′-carboxaldehyde oximes under these conditions produce 72% inhibition of the enzyme. Adenosine-5′-carboxaldehyde oxime is known to be a potent inhibitor of AdoHcy hydrolase with K_i and k_{inact} values of 0.67 μM and 0.16 min^{-1}, respectively,[17a] and is a substrate for the enzyme hydrolytic activity.[17b]

Computational Results

AutoDock simulations enable the prediction of preferred protein−ligand binding modes, interaction strengths, and binding specificity from calculations of binding energies and ligand spatial distributions. For each protein−ligand pair, 128 LGA (Lamarckian genetic algorithm) docking runs were performed, with each run producing one possible binding mode or solution. The 128 solutions were first sorted in terms of the binding mode, i.e., the position and orientation of the ligand relative to the protein target. The solutions having rms (root mean square) deviations in ligand atomic positions of less than 0.5 Å were grouped into a cluster. The total number of generated low-energy clusters measures the specificity of binding.[18] A small number of clusters indicates that the ligand has only a few possible binding modes and interacts with a specific site (or sites) on the target protein. On the other hand, a large number of clusters implies existence of a wide range of binding modes and lack of specific ligand−target interactions. The second step in sorting solutions involves identification of the solution of lowest binding energy within each cluster and ranking the different clusters according to this energy value. The solution with the lowest energy in the top-ranked (i.e., lowest-energy) cluster and all solutions with energies higher by up to 5.0 kcal/mol were considered as possible binding modes for ligand and target.

Docking of D-Ado and L-Ado to the Closed Structure. A summary of the AutoDock results is presented in Table 1; the docked structures of the first and second clusters are shown in Figure 2A. The docking of D-Ado to the closed form of AdoHcy hydrolase produced 16 clusters out of 128 runs. There were 77 solutions in the first cluster with an average docking energy of −19.2 kcal/mol. The binding site defined by the first cluster is in the cleft between the NAD binding domain and the substrate-binding domain, very close to the NAD cofactor. Since it is impossible for the D-Ado to stay at that site in the presence of the whole protein, this binding mode is an artifact due to removal of the NAD binding domain and the C-terminal domain and will not be considered further. Our best model for a possible D-Ado/protein complex is thus described by the second cluster. This cluster has 21 solutions with average energy of −18.9 kcal/mol. This binding mode has a low energy, and the ligand orientation is quite similar to that of 3′-keto of DHCeA [9-(2,3-dihydroxycyclopent-4-en-1-yl)-adenine] in the crystal structure 1A7A.[10a] The only difference is a tilt of the ribose ring by about 30° in the D-Ado-docked model, compared with the cyclopentene ring of 3′-keto-DHCeA in the crystal structure (Figure 2B). This is quite consistent with previous docking results.[11a]

For L-Ado, AutoDock produced 24 clusters out of 128 runs (Table 1). The first cluster consisted of 26 solutions, with an average docking energy of −16.3 kcal/mol. The positions of the first and second clusters are about 2 Å away from the substrate in the 1A7A crystal structure (Figure 2C), indicating that L-Ado does not fit into the inhibitor/substrate binding site. Comparison of the AutoDock results suggests that L-Ado should be a poor substrate of AdoHcy hydrolase compared to D-Ado. The binding of L-Ado to the protein is weaker (higher energy) and less specific (larger number of clusters) compared to D-Ado. The binding energy difference $\Delta E = -18.9 - (-16.3) = -2.6$ kcal/mol corresponds to a change in the binding constant by a factor of $\exp[-\Delta E/(RT)] = 76$ at room temperature. The microscopic reason for these effects appears to be a lack of fit between L-Ado and the inhibitor/substrate binding site. Lack of the structural fit was recently observed by computer overlaid structures of D and L enantiomers of 6′(*E*)-(bromohomovinyl)adenosine (e.g., **C**, X = Br, Y = H).[15]

The docking results are approximate. The scoring is based on an empirical energy function, solvation effects treated with a highly simplified model, and only ligand flexibility taken into account, with the protein structure kept fixed.[18] The binding energy difference calculated here, −2.6 kcal/mol, is only slightly greater in magnitude than the estimated standard error of the method, 2 kcal/mol. Thus, the AutoDock results should be only considered as qualitative. The calculated binding energy results are in qualitative agreement with the observed inhibitory effects. Additionally, the simulations suggest that L-Ado has a lower specificity and worse fit into the known active site than D-Ado.

Summary and Conclusions

On the basis of the findings that unnatural L-nucleoside analogues possess potent antiviral activity against HIV and HBV viruses and the fact that the potency of AdoHcy inhibitors (derived from D-nucleosides) has been correlated with their antiviral activity, we examined the possibility of whether L-adenosine and its derivatives

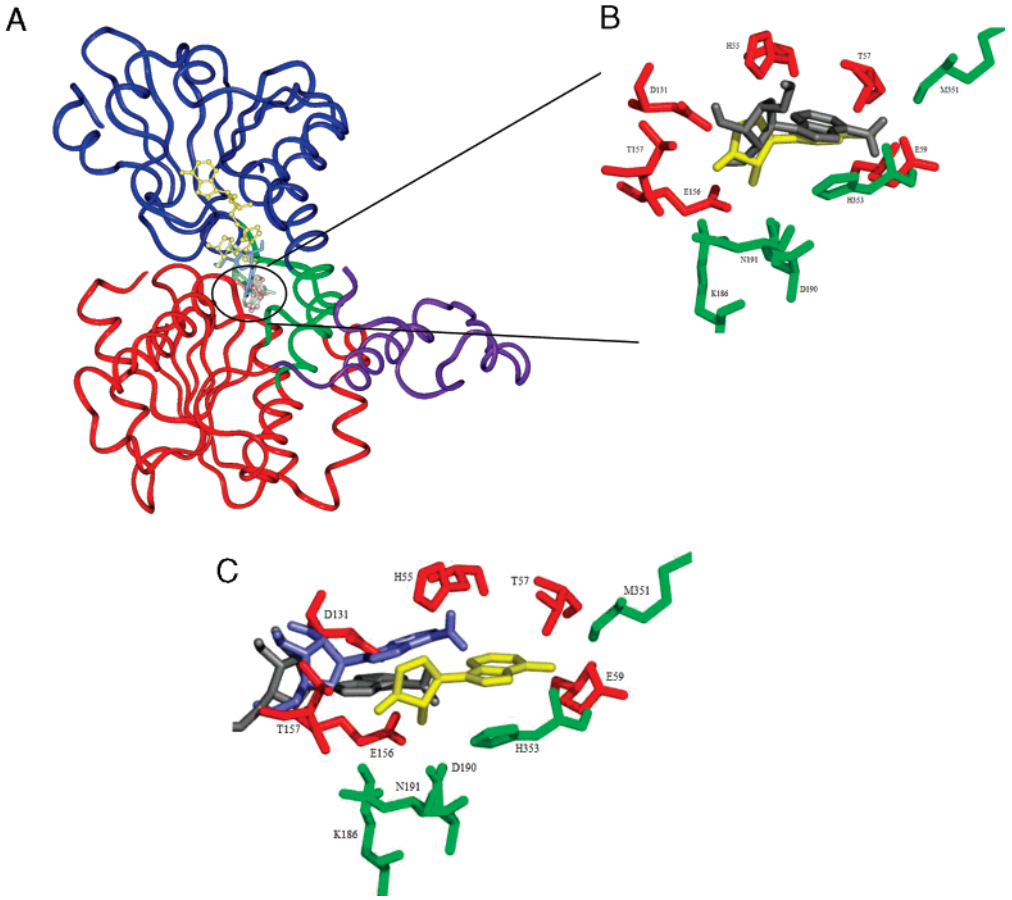


Figure 2. Docking results from Autodock. (A) The lowest-energy structures of docked D-Ado from the first two clusters are overlaid on the full closed form of AdoHcy hydrolase (PDB code 1A7A). Shown are the substrate (catalytic) binding domain (SBD, red), cofactor binding domain (CBD, blue) with cofactor in yellow, the hinges between the SBD and CBD (green), and the smaller C-terminal domain (magenta), which extends into the neighboring subunit (not shown). Cofactor NADH (yellow) and inhibitor 3′-keto-DHCeA (pink) are in ball-and-stick form. The structure from the first cluster (light-blue) is located between the two large domains and overlaps the NADH. The structure from the second cluster (light-green) overlaps the 3′-keto-DHCeA inhibitor. (B) The orientation of the docked D-Ado from the second cluster (gray) is quite similar to that of the 3′-keto-DHCeA inhibitor in the 1A7A crystal structure (yellow) except for a 30° tilt of the ribose ring. (C) The positions of docked L-Ado from the first two clusters (blue and gray) in the binding site of the closed form of AdoHcy hydrolase are both about 2 Å away from the bound inhibitor 3′-keto-DHCeA in the 1A7A crystal structure (yellow). The residues involved in the binding of the adenine and ribose moieties of the inhibitor are shown colored as in (A). The pictures are generated with InsightII (Accelrys Inc., 2000).

can act as (un)likely substrates and/or inhibitors of the enzyme. The L-adenosine **2** and its 5′-oxime **5** (the enantiomer of which is a potent inhibitor[17]) were found to be inactive as inhibitors of AdoHcy hydrolase. This corroborates a recent report[15] that the halohomovinyl and acetylenic derivatives of L-adenosine were found to be much weaker inhibitors than the corresponding analogues derived from adenosine.[7a] Docking calculations showed that binding of L-Ado is not as specific as that of D-Ado for human AdoHcy hydrolase and that the binding energy of the D-Ado/enzyme complex is lower than that of the L-Ado/enzyme complex. These results might explain why L-Ado and its analogues are inactive as inhibitors of AdoHcy hydrolase and therefore not good candidates for drug design targeting AdoHcy hydrolase and/or transmethylation enzymes. The results also indicate that AdoHcy hydrolase has much more careful substrate specificity than some virus enzymes from HIV and HBV, where L-analogues show very potent activity.

Experimental Section

Computational Procedures. The AutoDock program (version 3.0.5) was used for docking calculations.[18c] The graphical program SYBYL (version 16.91, Tripos, Inc.) was used for model building of the ligand structures to generate missing hydrogen atoms and assign partial charges for protein targets used in AutoDock runs and for energy minimization of initial ligand structures. Structural figures were generated using MolScript[19] and Raster3D.[20] Preparation of protein and ligand coordinates and binding simulation using AutoDock are described in Supporting Information. The three-dimensional structure of the closed form of AdoHcy hydrolase complex with the inhibitor (3′-keto-DHCeA) has been described,[10a] and model docking calculations were reported.[11a]

Chemical Synthesis and Enzymatic Studies. Details on the synthetic and enzymatic experimental procedures were described in our recent publications.[8a,b]

L-2′,3′-*O*-Isopropylideneadenosine (3). L-Adenosine[16] (**2**, 200 mg, 0.75 mmol) was suspended in dried acetone (6 mL) containing *p*-toluenesulfonic acid monohydrate (470 mg, 2.5 mmol). Triethyl orthoformate (1.5 mL, 1.34 mg, 9.0 mmol) was then added over a period of 10−20 min at ambient temperature with vigorous mechanical stirring until a clear solution was obtained. After 18 h, water (4.5 mL) and concentrated ammonium hydroxide (0.1 mL) were added (to adjust the pH to ∼7−8). Volatiles were evaporated and the residue was column-chromatographed (EtOAc → 5% MeOH/EtOAc) to give **3** (206 mg, 90%) with spectroscopic data identical to the data from the commercial sample of 2′,3′-*O*-isopropylideneadenosine.

L-2′,3′-*O*-Isopropylideneadenosine-5′-carboxaldehyde Oximes (4). A solution of **3** (100 mg, 0.33 mmol) and

N,*N*′-dicyclohexylcarbodiimide (DCC, 235 mg, 1.13 mmol) in dried DMSO (1.5 mL) was stirred under argon at ambient temperature. The Cl_2CHCO_2H (0.013 mL, 21 mg, 0.16 mmol) was then added, and stirring was continued for 90 min. Pyridine (0.5 mL) and $NH_2OH{\cdot}HCl$ (226 mg, 3.25 mmol) were added to the solution of the crude L-adenosine-5′-carboxaldehyde, and stirring was continued at ambient temperature overnight. Volatiles were evaporated, $CHCl_3$ was added, and the precipitated dicyclohexylurea (DCU) was filtered. The mother liquor was partitioned (1% $AcOH/H_2O//CHCl_3$), and the aqueous layer was extracted (4 × $CHCl_3$). The combined organic phase was washed ($NaHCO_3/H_2O$, brine), dried (Na_2SO_4), concentrated, and column chromatographed (2% → 4% MeOH/$CHCl_3$) to give **4** (*E*/*Z*, ∼ 6:1, 83 mg, 80%), MS (APCI) *m*/*z* 321 (100, MH^+), and other spectroscopic data as described for the D-enantiomer.[17a]

L-Adenosine-5′-carboxaldehyde Oximes (5). A solution of **4** (83 mg, 0.08 mmol) in CF_3CO_2H/H_2O (9:1, 5 mL) was stirred at 0 °C for 45 min under argon. Volatiles were evaporated, and the residue was coevaporated (3 × toluene) and then column chromatographed (EtOAc → 8% MeOH/EtOAc) to give **5** (*E*/*Z*, ∼ 6:1, 20 mg, 89%) as an amorphous white solid: MS (APCI) *m*/*z* 281 (100, MH^+); UV (MeOH) λ_{max} = 260 nm (ϵ = 14 100), λ_{min} = 228 nm (ϵ = 4000); ^{1}H NMR (MeOH-d_4) for **5** (*E*), δ 4.50 (t, J = 4.7, 1, H3′), 4.58 (dd, J = 6.9, 4.4 Hz, 1, H4′), 4.83 (t, J = 5.0 Hz, 1, H2′), 6.06 (d, J = 5.0 Hz, 1, H1′), 7.64 (d, J = 6.9 Hz, 1, H5′), 8.24 (s, 1, H2), 8.32 (s, 1, H8); ^{1}H NMR (MeOH-d_4) for **5** (*Z*), δ 4.38 (dd, J = 4.6, 1.5 Hz, 1, H3′), 5.08 (dd, J = 7.5, 4.8 Hz, 1, H2′), 5.22 (dd, J = 5.2, 1.6 Hz, 1, H4′), 6.03 (d, J = 7.5 Hz, 1, H1′), 7.28 (d, J = 5.2 Hz, 1, H5′), 8.24 (s, 1, H2), 8.32 (s, 1, H8). Anal. ($C_{10}H_{12}N_6O_4$) C, H, N.

Acknowledgment. This work was supported by NIH/NIGMS (Grant S06GM08205 to S.F.W.) and the U.S. Public Health Service (Grant GM-29332 to R.T.B.) awards.

Supporting Information Available: Experimental details for inactivation of AdoHcy hydrolase and computational procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Yuan, C.-S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. Design and Synthesis of *S*-Adenosylhomocysteine Hydrolase Inhibitors as Broad-Spectrum Antiviral Agents. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed; JAI Press: Greenwich, CT, 1996; Vol. 2, pp 41−88. (b) Turner, M. A.; Yang, X.; Yin, D.; Kuczera, K.; Borchardt, R. T.; Howell, P. L. Structure and function of *S*-adenosylhomocysteine hydrolase. *Cell Biochem. Biophys.* **2000**, *33*, 101−125.

(2) Yuan, C.-S.; Saso, Y.; Lazarides, E.; Borchardt, R. T.; Robins, M. J. Recent advances in *S*-adenosyl-L-homocysteine hydrolase inhibitors and their potential clinical applications. *Expert Opin. Ther. Pat.* **1999**, *9*, 1197−1206.

(3) (a) Schneyder, G.; Roffi, M.; Pin, R.; Flammer, Y.; Lange, H.; Eberli, F. R.; Meier, B.; Turi, Z. G.; Hess, O. M. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N. Engl. J. Med.* **2001**, *345*, 1593−1600. (b) Langheinrich, A. C.; Braun-Dullaeus, R. C.; Walker, G.; Jeide, I.; Schiling, R.; Tammoscheit, K.; Dreyer, T.; Fink, L.; Bohle, R. M.; Haberbosch, W. Effects of 3-deazaadenosines on homocysteine and atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis* **2003**, *171*, 181−192.

(4) Wnuk, S. F. Targeting "hydrolytic" activity of the *S*-adenosyl-L-homocysteine hydrolase. *Mini-Rev. Med. Chem.* **2001**, *1*, 307−316.

(5) Palmer, J. L.; Abeles, R. H. The mechanism of action *S*-adenosylhomocysteine hydrolase. *J. Biol. Chem.* **1979**, *254*, 1217−1226.

(6) (a) McCarthy, J. R.; Jarvi, E. T.; Matthews, D. P.; Edwards, M. L.; Prakash, N. J.; Bowlin, T. L.; Mehdi, S.; Sunkara, P. S.; Bey, P. 4′,5′-Unsaturated 5′-fluoroadenosine nucleosides: Potent mechanism-based inhibitors of *S*-adenosyl-L-homocysteine hydrolase. *J. Am. Chem. Soc.* **1989**, *111*, 1127−1128. (b) Yuan, C.-S.; Yeh, J.; Liu, S.; Borchardt, R. T. Mechanism of inactivation of *S*-adenosyl-L-homocysteine hydrolase by fluorine-containing adenosine analogs. *J. Biol. Chem.* **1993**, *268*, 17030−17037. (c) Liu, S.; Wnuk, S. F.; Yuan, C.; Robins, M. J.; Borchardt, R. T. Adenosine 5′-carboxaldehyde: A potent inhibitor of *S*-adenosyl-L-homocysteine hydrolase. *J. Med. Chem.* **1993**, *36*, 883−887.

(7) (a) Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J. Nucleic acid related compounds. 84. Synthesis of 6′(*E* and *Z*)-halohomovinyl derivatives of adenosine, inactivation of *S*-adenosyl-L-homocysteine hydrolase, and correlation of anticancer and antiviral potencies with enzyme inhibition. *J. Med. Chem.* **1994**, *37*, 3579−3587. (b) Yuan, C.-S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. A Novel mechanism-based inhibitor (6′-bromo-5′, 6′-didehydro-6′-deoxy-6′-fluorohomoadenosine) that covalently modifies human placental *S*-adenosylhomocysteine hydrolase. *J. Biol. Chem.* **1998**, *273,* 18191−18197.

(8) (a) Yang, X.; Yin, D.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. Mechanism of Inactivation of human *S*-adenosyl-L-homocysteine hydrolase by 5′,5′,6′,6′-tetrahydro-6′-deoxy-6′-halohomoadenosines. *Biochemistry* **2000**, *39*, 15234−15241. (b) Wnuk, S. F.; Lewandowska, E.; Sacasa, P. R.; Crain, L. N.; Zhang, J.; Borchardt, R. T.; De Clercq, E. Stereoselective synthesis of sugar-modified enyne analogues of adenosine and uridine. Interaction with *S*-adenosyl-L-homocysteine hydrolase, and antiviral and cytotoxic effects. *J. Med. Chem.* **2004**, *47*, 5251−5257. (c) Guillerm, G.; Muzard, M.; Glapski, C.; Pilard, S. Inactivation of human *S*-adenosylhomocysteine hydrolase by covalent labeling of cysteine 195 with thionucleoside derivatives. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5803−5807.

(9) (a) Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. Design, synthesis, and biological evaluation of fluoroneplanocin A as the novel mechanism-based inhibitor of *S*-adenosylhomocysteine hydrolase. *J. Med. Chem.* **2003**, *46*, 201−203. (b) Yun, M.; Moon, H. R.; Lee, K. M.; Park, H. l.; Kim, H. P.; Jeong, L. S. A short synthesis of L-ribose and its utility to L-fluoro-neplanocin A. *Abstracts of Papers, Carbohydrate Division*, 229th National Meeting of the American Chemical Society, San Diego, CA, March 13−17, 2005; American Chemical Society: Washington, DC, 2005; CARB-042.

(10) (a) Turner, M. A.; Yuan, C.-S.; Borchardt, R. T.; Hershfield, M. S.; Smith, G. D.; Howell, P. L. Structure determination of selenomethionyl *S*-adenosylhomocysteine hydrolase using data at a single wavelength. *Nat. Struct. Biol.* **1998**, *5*, 369−376. (b) Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. Crystal structure of *S*-adenosylhomocysteine hydrolase from rat liver. *Biochemistry* **1999**, *38*, 8323−8333.

(11) (a) Hu, Y.; Yang, X.; Yin, D.; Mahadevan, J.; Kuczera, K.; Schowen, R. L.; Borchardt, R. T. Computational characterization of substrate binding and catalysis in *S*-adenosylhomocysteine hydrolase. *Biochemistry* **2001**, *40*, 15143−15152. (b) Yang, X.; Hu, Y.; Yin, D. H.; Turner, M. A.; Wang, M.; Borchardt, R. T.; Howell, P.; Kuczera, K.; Schowen, R. L. Catalytic strategy for *S*-adenosylhomocysteine hydrolase: transition-state stabilization and the avoidance of abortive reactions. *Biochemistry* **2003**, *42*, 1900−1909.

(12) Porter, D. J. T. *S*-Adenosylhomocysteine hydrolase: Stereochemistry and kinetics of hydrogen transfer. *J. Biol. Chem.* **1993**, *268*, 66−73.

(13) Parry, R.; Askonas, L. J. Studies of enzyme stereochemistry. Elucidation of the stereochemistry of the reaction catalyzed by *S*-adenosylhomocysteine hydrolase. *J. Am. Chem. Soc.* **1985**, *107*, 1417−1418.

(14) (a) Gumina, G.; Chong, Y.; Choo, H.; Song, G.-Y.; Chu, C. K. L-nucleosides: antiviral activity and molecular mechanism. *Curr. Top. Med. Chem.* **2002**, *2*, 1065−1086. (b) Sommadossi, J.-P. Antiviral β-L-Nucleosides Specific for Hepatitis B Virus Infection. In *Recent Advances in Nucleosides*; Chu, C. K., Ed; Elsevier Science: Amsterdam, 2002; pp 417−432. (c) Chong, Y.; Stuyver, L.; Otto, M. J.; Schinazi, R. F.; Chu, C. K. Mechanism of antiviral activities of 3′-substituted L-nucleosides against 3TC-resistant HBV polymerase: A molecular modeling approach. *Antiviral Chem. Chemother.* **2003**, *14*, 309−319. (d) Zhu, W.; Chong, Y.; Choo, H.; Mathews, J.; Schinazi, R. F.; Chu, C. K. Synthesis, structure−activity relationships, and mechanism of drug resistance of D- and L-β-3′-fluoro-2′,3′-unsaturated-4′-thionucleosides as anti-HIV agents. *J. Med. Chem.* **2004**, *47*, 1631−1640.

(15) Wang, J.-F.; Yang, X.-D.; Zhang, L.-R.; Yang, Z.-J.; Zhang, L.-H. Synthesis and biological activities of 5′-ethylenic and acetylenic modified L-nucleosides and isonucleotides. *Tetrahedron* **2004**, *38*, 8535−8546.

(16) Moyround, E.; Strazewski, P. L-Ribonucleosides from L-xylose. *Tetrahedron* **1999**, *55*, 1277−1284.

(17) (a) Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq E.; Robins, M. J. Anticancer and antiviral effects and inactivation of *S*-adenosyl-L-homocysteine hydrolase with 5′-carboxaldehydes and oximes synthesized from adenosine and

sugar-modified analogues. *J. Med. Chem.* **1997**, *40*, 1608−1618. (b) Huang, H.; Yuan, C.-S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. The mechanism of inactivation of human placental *S*-adenosylhomocysteine hydrolase by (*E*)-4′,5′-didehydro-5′-methoxyadenosine (DMAO) and adenosine 5′-carboxaldehyde oxime (ACAO). *Arch. Biochem. Biophys.* **1997**, *343*, 109−117.

(18) (a) Goodsell, D. M.; Olson, A. J. Automated docking of substrates to proteins by simulated annealing. *Proteins* **1990**, *8*, 195−202. (b) Morris, G. M.; Goodsell, D. S.; Huey, R.; Olson, A. J. Distributed automated docking of flexible ligands to proteins: Parallel applications of AutoDock 2.4. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 293−304. (c) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J. Comput. Chem.* **1998**, *19,* 1639−1662.

(19) Kraulis, P. J. "MOLSCRIPT": A program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **1991**, *24*, 946−950.

(20) Merritt, E. A.; Murphy M. E. P. Raster3D version 2.0. A program for photorealistic molecular graphics. *Acta Crystallogr.* **1994**, *D50*, 869−873.

JM0490484