Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 457–460

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of an α-Aminophosphonate Nucleoside as an Inhibitor of *S*-Adenosyl-L-Homocysteine Hydrolase

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Received 26 September 2001; accepted 16 November 2001

Abstract—A phosphonic acid analogue of *S*-adenosyl-L-homocysteine was prepared by a novel method and the epimeric mixture separated. Preliminary studies indicate that each epimer causes time-dependent inactivation of *S*-adenosyl-L-homocysteine hydrolase, however each presented distinct kinetic characteristics. © 2002 Elsevier Science Ltd. All rights reserved.

S-Adenosyl-L-methionine (AdoMet) serves as a methyl donor for a variety of transmethylation reactions.[1a,b] In these reactions, the methyl group from AdoMet is transferred to various acceptor molecules such as proteins, nucleic acids, phospholipids, and small molecules by specific methyltransferase enzymes.[2a,b] AdoMet-dependent methyltransferase reactions are indirectly controlled by the intracellular levels of S-adenosyl-L-homocysteine (AdoHcy), as AdoHcy is a potent feedback inhibitor of AdoMet-dependent methyltransferases.[3a,b] In eukaryotes, the intracellular levels of AdoHcy and the ratio of AdoHcy/AdoMet are regulated by the hydrolysis of AdoHcy to adenosine (Ado) and homocysteine (Hcy) by the enzyme S-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1) (Fig. 1).[4a,b] Inhibition of AdoHcy hydrolase leads to a rapid accumulation of intracellular AdoHcy and the consequent inhibition of AdoMet-dependent transmethylation reactions. Failure to regulate the intracellular levels of AdoHcy leads to cellular toxicity.

In recent years, AdoHcy hydrolase has become an attractive target for drug design since its inhibitors have been shown to exhibit antiviral,[5a,b,c] antiparasitic,[6] antiarthritic,[7] and immunosuppressive effects.[8a,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.[9] 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. The recent determination of the crystal structure of the rat liver[10] and human[11] AdoHcy hydrolases have further contributed to our understanding of this enzyme.

There have been very few studies on inhibitors of AdoHcy hydrolase with variations in the amino acid portion of AdoHcy.[12] We wish to investigate further the importance of the amino acid portion of AdoHcy in regards to its inhibitory activity as well as providing information about the key components of AdoHcy hydrolase interactions with substrates and inhibitors. In this regard, we were interested in the bioisosteric replacement of the carboxylate by a phosphonate moiety.

The phosphonic acid moiety has long been established as a bioisostere of carboxylic acids[13] and organophosphorus analogues have been shown to be inhibitors of numerous metabolic processes.[14a,b,c] The phosphonic and phosphinic acid analogues of AdoHcy and AdoMet have previously been prepared by Khomutov and coworkers.[15] The compound, 5′-deoxy-5′-chloroadenosine

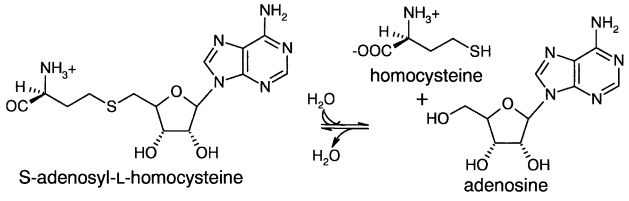

Figure 1. The reaction catalyzed by *S*-adenosyl-L-homocysteine hydrolase.

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PII: S0960-894X(01)00789-2

was alkylated with γ-mercapto-α-aminopropyl phosphinic or phosphonic acid in the presence of liquid ammonia to afford the desired analogue in unspecified yield. Preliminary studies pertaining to the biological evaluation of these compounds were reported on epimeric material. Both the phosphinic and phosphonic acid analogues of AdoMet and AdoHcy were shown to inhibit AdoMet decarboxylase. With respect to RNA methyltransferase, the AdoMet derivatives appeared to act as substrates, whereas the AdoHcy analogues acted as inhibitors of the enzyme.

We have successfully synthesized the α-aminophosphonate AdoHcy analogue by a novel method and have separated the epimeric mixture into each individual epimer (A and B). The epimeric mixture and epimers A and B were evaluated against recombinant rat liver AdoHcy hydrolase.

Chemistry

Preparation of the phosphonate nucleoside is outlined in Scheme 1. Diphenylmethylamine was reacted with formalin to give 2,4,6-tris(benzhydryl)hexahydro-1,3,5-triazine **1** in 99% yield upon recrystallization.[16] Addition of diethylphosphite to **1** gave diethyl *N*-(diphenylmethyl)methylphosphonate **2** in 96% yield. Compound **2** was oxidized with DDQ in benzene in the presence of crushed 4 Å molecular sieves to give the imine **3** in 99% yield.[17] Next, compound **3** was alkylated with excess 1,2-dibromoethane to give **4** in 38% yield. The 5′-thio nucleoside analogue **5** was prepared as previously described.[18] The thiolate anion of **5** was generated from methanolic potassium hydroxide and compound **4** was added to perform the alkylation to give fully protected AdoHcy analogue **6** in 63% yield. As was expected, ^{1}H NMR analyses of the phosphonate adduct **6** revealed that the compound was generated as a 1:1 mixture of epimers. Full deprotection was achieved with trimethylsilyl iodide (TMSI)[19a,b] to generate the desired compound **7** in 75% yield. The AdoHcy analogue **7** was purified on DOWEX 50W-X8 (elution: H_2O and then 1 N NH_4OH) and reverse-phase HPLC served to separate the 9′-epimers.[20]

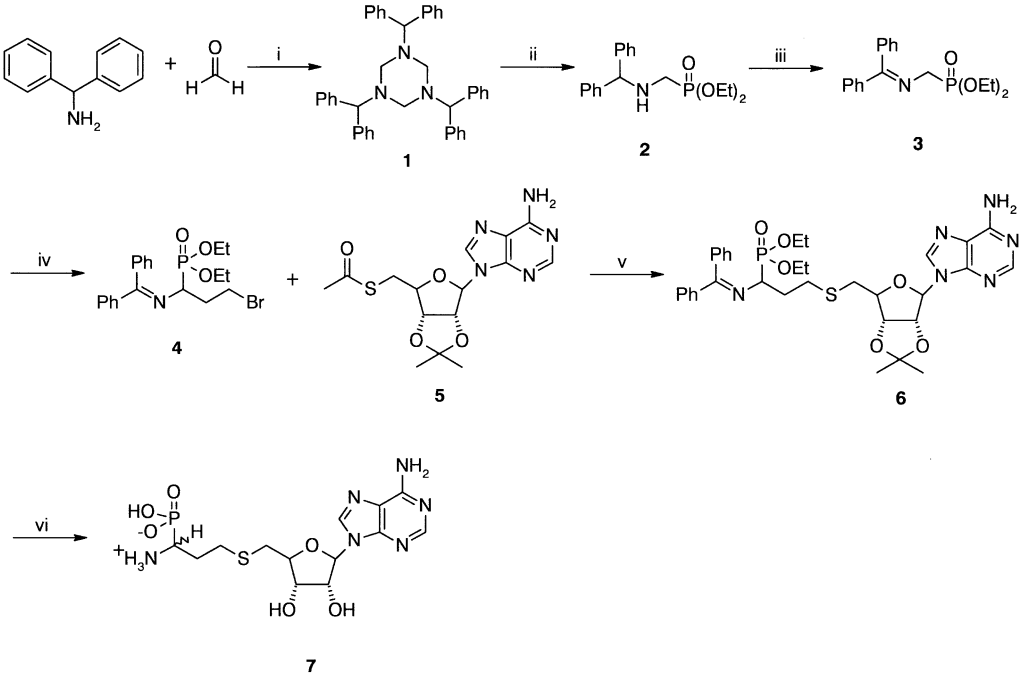
Kinetic Studies

The recombinant rat liver AdoHcy hydrolase (MV 1304/pUCSAH) was purified as previously determined by Gomi and coworkers with minor modifications.[21] Enzyme activity was measured in the hydrolytic direction, through the use of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), as described previously.[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 104.7 μM in our system.

Table 1. Inhibition constants for epimers A and B with AdoHcy hydrolase[a]

Compd	K_i (μM)	k_{inact} (min^{-1})
A	345±3	0.0049
B	1094±49	0.0145

[a]The inhibition parameters were calculated from the Kitz and Wilson plots shown in Figure 2.



Scheme 1. (i) Benzene reflux, Dean-Stark, 99%, (ii) diethylphosphite, 96%; (iii) DDQ, 4 Å sieves, benzene, 99%, (iv) *n*-BuLi, THF, −78 °C, $BrCH_2CH_2Br$ (7 equiv) 38%; (v) MeOH, KOH, 63%; (vi) TMSI, H_2O, 75%.

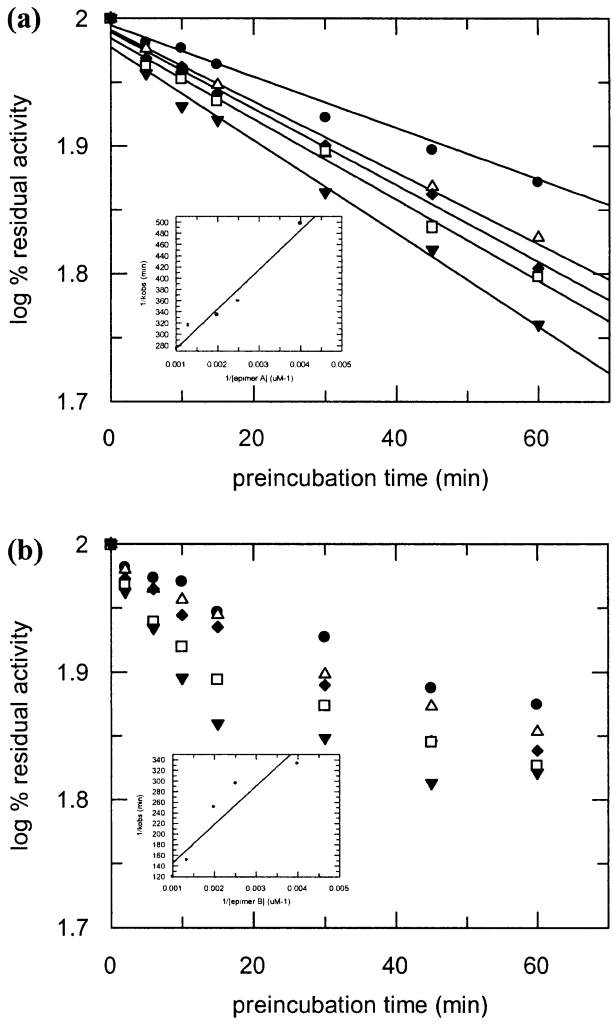


Figure 2. Time-dependent inactivation of AdoHcy hydrolase with (a) epimer A and (b) epimer B. The enzyme was preincubated for the indicated times at 37 °C in the presence of 250 μM (●), 400 μM (△), 500 μM (◆), 750 μM (□) or 1000 μM (▼) of the appropriate epimer. At the indicated time points, residual 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 epimer B were determined for time points $\leq$15 min. Data were the average of triplicate measurements. This was reproducible throughout a number of repeated trials.

The inactivation of AdoHcy hydrolase by the epimeric mixture of the phosphonate nucleoside appeared to occur by a complex process that was investigated further by studying each individual epimer (A and B). Each epimer was tested in a time-dependent manner. Kinetic constants for epimers A and B were determined according to the method of Kitz and Wilson[24] and are listed in Table 1.

Epimer A proved to inactivate the rat liver AdoHcy hydrolase in a time-dependent and pseudo-first-order process, as shown in Figure 2. The K_i and k_{inact} values are listed in Table 1. Epimer B also proved to be time-dependent, but biphasic in nature, showing pseudo-first-order kinetics only in the first period of inactivation (Fig. 2). This biphasic process has been observed quite frequently by 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 need to be performed in order to determine the cause of this biphasic process. However, it was determined that both epimers were not substrates for AdoHcy hydrolase as there was no turnover of either epimer at a concentration of 2.5 mM when incubated for 2 h with the enzyme. The K_i observed for epimer B was much larger than that for epimer A, which may be explained if epimer B contained the unnatural D-configuration on the amino acid side chain. Attempts to obtain the X-ray structure of one of the epimers is ongoing in our laboratory.

Both epimer A and B appear to inactive the enzyme irreversibly since the lost enzymatic activity could not be restored after dialysis (24 h) against 10 mM potassium phosphate buffer, pH 7.2.

This is the first evaluation of a phosphonate nucleoside as a probe for the enzyme AdoHcy hydrolase. It is expected that the phosphonate group will have substantial dianionic character throughout these experiments, as the pH of the assay buffer is 7.2. Hence, the effect of pH on the extent and type of inhibition by these analogues as well as the structure determination of one of the epimers are the focus of ongoing research.

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 scholarship to J.A.S. and OGS to P.B.S. is also gratefully appreciated. We would also like to thank Tim Jones and Jan Venne for conducting MS and NMR studies, respectively.

References and Notes

1. (a) Chiang, P. K.; Gordon, R. K.; Tal, J.; Zeng, G. C.; Doctor, B. P.; Pardhasaradhi, K.; McCann, P. P. *FASEB J.* **1996**, *10*, 471. (b) Yuan, C. S.; Liu, S.; Wnuk, S. F.; Robins, M. J.; Borchardt, R. T. *Adv. Antiviral Drug Des.* **1996**, *2*, 41.
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; p 1. (b) Drahovsky, D.; Boehm, T. L. J. *Int. J. Biochem.* **1980**, *12*, 523.
3. (a) Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521. (b) 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.
4. (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.
5. (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.

6. Bitonti, A. J.; Baumann, J.; Varvi, T.; McCarthy, J. R.; McCann, P. P. *Biochem. Pharmacol.* **1990**, *40*, 601.
7. Wolos, J. A.; Frondorf, K. A.; Esser, R. E. *J. Immunol.* **1993**, *151*, 526.
8. (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.
9. Banerjee, A. K. *Microbiol. Rev.* **1980**, *44*, 175.
10. Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *Biochemistry* **1999**, *38*, 8323.
11. Turner, M. A.; Yuan, C. S.; Borchardt, R. T.; Hershfield, M. S.; Smith, G. D.; Howell, P. L. *Nat. Struct. Biol.* **1998**, *5*, 369.
12. Chiang, P. K.; Richards, H. H.; Cantoni, G. L. *Mol. Pharmacol.* **1977**, *13*, 939.
13. Burger, A. *Prog. Drug Res.* **1991**, *37*, 287.
14. (a) Lowther, W. T.; Zhang, Y.; Sampson, P. B.; Honek, J. F.; Matthews, B. W. *Biochemistry* **1999**, *38*, 14810. (b) Lejczak, B.; Kafarski, P.; Zygmunt, J. *Biochemistry* **1989**, *28*, 3549. (c) Atherton, F. R.; Hassall, C. H.; Lambert, R. W. *J. Med. Chem.* **1986**, *29*, 29.
15. Syrku, V. I.; Zavalova, L. L.; Khomutov, R. M. *Bioorg. Khim.* **1986**, *12*, 839.
16. Smolin, E. M.; Rapoport, L. In *The Chemistry of Heterocyclic Compounds; s-Triazenes and Derivatives*; Weissberger, A., Ed.; Interscience: New York, 1959; p 473.
17. Sampson, P. B.; Honek, J. F. *Org. Lett.* **1999**, *1*, 1395.
18. Baddiley, J.; Jamieson, G. A. *J. Chem. Soc.* **1955**, 1085.
19. (a) Rudisill, D. E.; Whitten, J. P. *Synthesis* **1994**, *851*. (b) TMSI (6 equiv) from a new ampoule must be utilized. Old TMSI that contained red colouration was ineffective.
20. Epimer A: HPLC (C18: 25 mm × 10 cm) retention time 18 min at flow rate 3 mL/min, (30% CH_3CN/H_2O); ^1H NMR (500 MHz, DMSO) δ 8.38 (s, 1H, H-2), 8.18 (s, 1H, H-8), 5.90 (d, 1H, $J=5.6$ Hz, H-1′), 4.70–472 (m, 1H, H-2′), 4.18–4.21 (m, 1H, H-3′), 4.07–4.11 (m, 1H, H-4′), 2.94–3.10 (m, 2H, H_2-5′), 2.65–2.85 (m, 3H, H-9′, H_2-7′), 2.05–2.10 (m, 1H, H-8′), 1.82–1.92 (m, 1H, H-8′); ^{13}C NMR (125 MHz, DMSO) δ 155.6 (C-6), 152.7 (C-2), 149.3 (C-4), 139.8 (C-8), 118.7 (C-5), 87.0 (C-1′), 83.5 (C-2′), 72.7 (C-3′), 72.1 (C-4′), 34.5 (C-5′), 29.7 (C-7′), 28.6 (C-8′), (C-9′ obscured by DMSO); ^{31}P NMR (80.0 MHz, DMSO) δ 14.1; high-resolution FAB *m/z* [421.09080 (M + 1); (calcd for $C_{13}H_{22}SPN_6O_6$) 421.12586]. Epimer B: HPLC (C18: 25 mm × 10 cm); retention time 22 min at flow rate 3 mL/min, (30% CH_3CN/H_2O); ^1H NMR (500 MHz, DMSO) δ 8.38 (s, 1H, H-2), 8.08 (s, 1H, H-8), 5.86 (d, 1H, $J=5.5$ Hz, H-1′), 4.63–4.68 (m, 1H, H-2′), 4.11–4.18 (m, 1H, H-3′), 3.99–4.03 (m, 1H, H-4′), 2.86–2.92 (m, 2H, H_2-5′), 2.65–2.85 (m, 3H, H-9′, H_2-7′), 1.92–2.04 (m, 1H, H-8′), 1.76–1.82 (m, 1H, H-8′); ^{13}C NMR (125 MHz, DMSO) δ 153.4 (C-6), 153.2 (C-2), 152.6 (C-4), 140.3 (C-8), 116.3 (C-5), 87.8 (C-1′), 84.9 (C-2′), 73.7 (C-3′), 73.2 (C-4′), 34.2 (C-5′), 31.2 (C-7′), 29.7 (C-8′), (C-9′ obscured by DMSO); ^{31}P NMR (80.0 MHz, DMSO) δ 13.2; high-resolution FAB *m/z* [421.08658 (M + 1); (calcd for $C_{13}H_{22}SPN_6O_6$) 421.12586].
21. Gomi, T.; Date, T.; Ogawa, H.; Fujioka, M.; Akasmit, R. R.; Backlund, P. S.; Cantoni, G. L. *J. Biol. Chem.* **1989**, *264*, 1618.
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-Witkowki, 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: New York, 1995; Vol. 249, p 240.