

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

INACTIVATION OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE WITH FLUORINATED ANALOGS OF 2'- AND 3'-DEOXY-5'-METHYLTHIOADENOSINE

D. Guillerm^a; G. Guillerm^a; C. Witkowski-Vandenplas^a

^a Université de Reims, Reims, Cédex 2, France

Online publication date: 31 March 2001

To cite this Article Guillerm, D. , Guillerm, G. and Witkowski-Vandenplas, C.(2001) 'INACTIVATION OF S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE WITH FLUORINATED ANALOGS OF 2'- AND 3'-DEOXY-5'-METHYLTHIOADENOSINE', *Nucleosides, Nucleotides and Nucleic Acids*, 20: 4, 689 – 693

To link to this Article: DOI: 10.1081/NCN-100002352

URL: <http://dx.doi.org/10.1081/NCN-100002352>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

INACTIVATION OF *S*-ADENOSYL-L-HOMOCYSTEINE HYDROLASE WITH FLUORINATED ANALOGS OF 2'- AND 3'-DEOXY-5'-METHYLTHIOADENOSINE

D. Guillermin, G. Guillermin,* and C. Witkowski-Vandenplas

Laboratoire des Réactions Sélectives et Applications - UMR 6519,
Université de Reims, BP 1039, 51687 Reims Cédex 2, France

ABSTRACT

Fluorinated analogs of 2'- and 3'-deoxy-5'-methylthioadenosine **1–4** caused irreversible inactivation of AdoHcy hydrolase. Based on the ESI-Mass spectra analysis of the inactivated enzyme with the fluorinated analog **1** a mechanism of inactivation is proposed.

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase has emerged as a target for molecular design of antiviral agents because of its important role in regulating *S*-adenosyl-L-methionine-dependent methylation reactions (1).

We have recently found that 5'-deoxy-5'-difluoromethylthio-adenosine (DFMTA) and 5'-deoxy-5'-trifluoromethylthioadenosine (TFMTA) were potent mechanism-based inactivators of AdoHcy hydrolase (2). In order to clarify the mechanism and the binding mode of this new series of inhibitors, we prepared their 2'- and 3'-deoxy analogs **1–4** (Fig. 1), for assays on AdoHcy hydrolase activity.

3'-deoxy-5'-deoxy-5'-*S*-difluoromethyl-5'-thioadenosine **1** and 2'-deoxy-5'-deoxy-5'-*S*-difluoromethyl-5'-thioadenosine **2**, 3'-deoxy-5'-deoxy-5'-*S*-trifluoromethyl-5'-thioadenosine **3** and 2'-deoxy-5'-deoxy-5'-*S*-trifluoromethyl-5'-thioadenosine **4** have been synthesised in two steps (3) from their corresponding fluorinated analogs DFMTA and TFMTA, already prepared by us (4). **1** and **2**,

*Corresponding author.

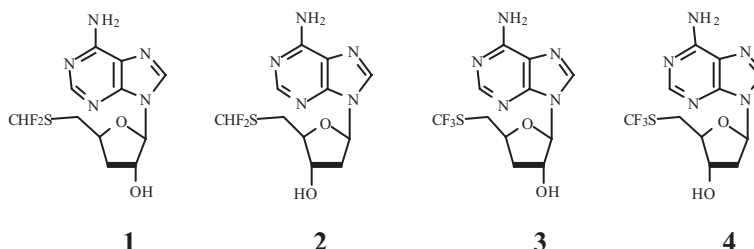
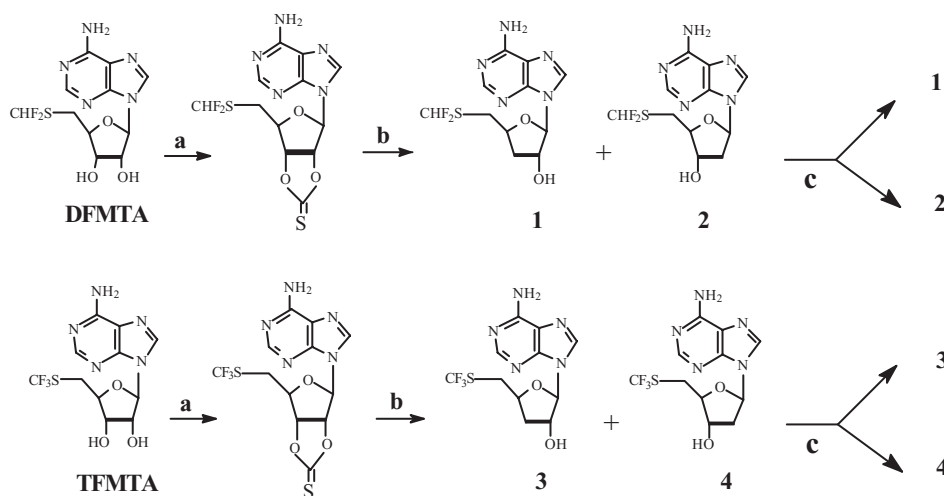


Figure 1.

3 and **4** were easily separated as described in Scheme 1 and fully identified from their ^1H NMR Spectra (5).

1, **2** and **3**, **4** were tested on the activity of recombinant human placental AdoHcy hydrolase purified to homogeneity as previously reported (6). AdoHcy hydrolase activity was assayed in the direction of AdoHcy synthesis using (8- ^{14}C)-Adenosine (7). **1–4** derivatives caused time-dependent and irreversible inactivation of the enzyme. The Kitz and Wilson method (8) was used for kinetic constants determination. (Table 1).

These results indicated that **1**, **2**, **3** and **4** are weaker inhibitors than the corresponding parent DMFTA and TFMTA (2), showing the importance of 2'- and 3'-hydroxyl groups for proper binding with AdoHcy hydrolase as previously reported (9). Upon complete inactivation of AdoHcy hydrolase by fluorinated nucleosides **1–4**, the reaction products were analysed by HPLC/MS. No traces of dehydronucleosides **5** or **6** (Scheme 2), used as authentics, were detected.



Scheme 1. a) 1,1'-thiocarbonyldiimidazole, DMF, 60% b) nBu_3SnH , AIBN, toluene (reflux) 45% c) Flash-column chromatography (Merck Kieselgel 60/230-400 mesh): AcOEt/MeOH 9/1.



Table 1.

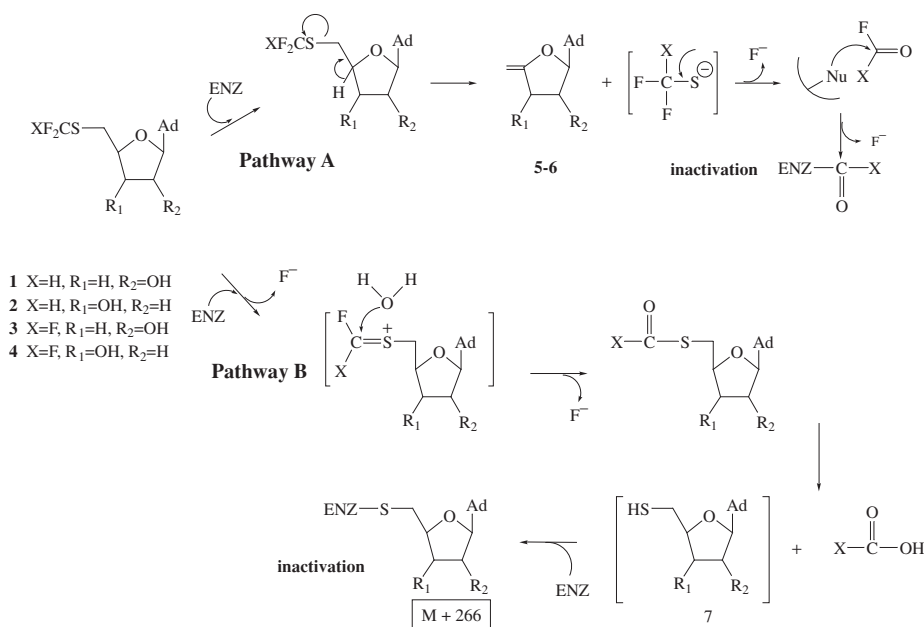
Compounds	K _i (μM)	K _{inact} (min ⁻¹)
3'-deoxy-DFMTA 1	95	1.8 10 ⁻²
2'-deoxy-DFMTA 2	55	1 10 ⁻²
3'-deoxy-TFMTA 3	400	2.5 10 ⁻²
2'-deoxy-DFMTA 4	190	1.4 10 ⁻²

NAD⁺/NADH content was not modified after incubation of AdoHcy hydrolase with inactivators **2** and **4** (data not shown).

The mechanism of inactivation was further investigated using the difluoromethyl derivatives **1** and **2**. Inactivation of AdoHcy hydrolase with **1** and **2** was accompanied by release of fluorine ion (measured by ¹⁹F NMR spectroscopy). When an 8 fold excess of **1** or **2** per mole of enzyme subunit was incubated with purified AdoHcy hydrolase, unreacted **1** and **2** were still present after complete inactivation of the enzyme with the release of # 1.8 mole of fluorine ion per mole of inactivated enzyme.

The mechanism of inactivation by inhibitors **1–4** might involve two catalytic pathways (Scheme 2).

In pathway A, a β-elimination step (without prior oxidation at C-3') of difluoromethyl or trifluoromethylthiolates ions might produce in the enzyme cavity highly reactive acylating agents such as thioformylfluoride or carbonothionic difluoride



Scheme 2.



(XFC=S, X=H, F) which could irreversibly acylate nucleophilic residues at the active site.

A second hypothetical mode of inactivation of AdoHcy hydrolase via the formation of thiol-nucleoside intermediate **7**, generated from inhibitors **1–4** by the “hydrolytic activity” of the enzyme, was also considered (pathway B).

Further mechanistic studies were investigated with compound **1** including Electrospray Ionization Mass Spectra (ESI-MS) analysis of the inactivated enzyme. When AdoHcy hydrolase was inactivated by **1** (50%) the inactivated enzyme subunit was detected at 47900 ± 5 Da showing that the inhibition of AdoHcy hydrolase was accompanied by a covalent linkage of 270 ± 5 Da (the native enzyme used in this experiment was detected at 47630 ± 5 Da). This result argues in favor of the second hypothetical mode of inactivation of AdoHcy hydrolase (pathway B). Formation of a disulfide bond with a thiol-nucleoside intermediate like **7** and a cysteine residue present (10) in the active site of the enzyme, may be proposed to explain the irreversible covalent inactivation process with the fluorinated nucleosides **1–4**.

Additional studies are in progress using nano ESI-MSⁿ techniques to elucidate the exact localisation of the covalent linkage induced on AdoHcy with DFMTA, TFMTA and their 2'- and 3'-deoxy analogs.

ACKNOWLEDGMENTS

The authors express their thanks to Pr. Michael S. Hershfield and Dr. Francisco Arredondo (Duke University School of Medicine) for providing sample of *E. Coli* transformed with a plasmid encoding for human placental Ado Hcy hydrolase, Dr. Helene Rogniaux and Dr. Alain Van Dorselaer (University Louis Pasteur, France) for carrying out the mass spectra analysis in this study.

REFERENCES

1. Liu, S.; Wolfe, M.S.; Borchardt, R.T. *Antiviral Research*, **1992**, *19*, 247–265.
2. Muzard, M.; Vandenplas, C.; Guillerm, D.; Guillerm, G. *J. Enzyme Inhibition*, **1998**, *13*, 443–456.
3. Barton, D.R.; Mc Combie, H.S.W. *J. C. S. Perkin I*, **1975**, 1574–1585.
4. Gatel, M.; Muzard, M.; Guillerm, D.; Guillerm, G. *Eur. J. Med. Chem.*, **1996**, *31*, 37–41.
- 5-1. mp 190–192°C- HRMS (DCI/NH₃) 318,088 (MH)⁺, calc. 317,259- [α]_D²⁰ -21° (c 1,6 × 10⁻²; CHCl₃/MeOH: 4/1)- ¹H NMR, CDCl₃, ϵ CD₃OD δ (ppm), J (Hz): 2.02 (ddd, 1H, H-3' β , J 13.35, 5.72, 9.91); 2.23 (ddd, 1H, H-3' α , J 13.35, 2.29, 5.34); 3.21 (d, 2H, H-5', J 5.34); 4.56 (m, 1H, H-2'); 4.79 (m, 1H, H-4'); 5.94 (d, 1H, H-1', J 1.53); 6.90 (t, 1H, SCHF₂, J 55); 8.07 and 8.28 (2 s, 2H, H-2 and H-8). ¹³C NMR, CDCl₃, ϵ CD₃OD, δ (ppm), J (Hz): 30.8 (C-5'); 36.4 (C-3'); 76.2 (C-4'); 80.0 (C-2'); 93.2 (C-1'); 119.8 (C-6); 120.3 (d, SCHF₂, J 275); 138.1 and 151.8 (C-2 and C-8);

- 148.2 and 155.2 (C-4 and C-5). ^{19}F NMR, CDCl_3 ε CD_3OD , δ (ppm), J (Hz) -93.0 (2 dd, 2F, J 240, 55).
2. mp $62-64^\circ\text{C}$ - HRMS (DCI/ NH_3) 318,030 (MH) $^+$, calc. 317,259- $[\alpha]_{\text{D}}^{20} +11^\circ$ (c 3.1×10^{-2} ; $\text{CHCl}_3/\text{MeOH}$: 4/1)- ^1H NMR, CDCl_3 , ε CD_3OD δ (ppm), J (Hz): 2.48 (ddd, 1H, H-2' β , J 13,74, 6,49, 4,2); 2.80 (ddd, 1H, H-2' α , J 13.74, 6,86, 6,49); 3.13 and 3.22 (dd, 2H, H-5'a, H-5'b, J 13,73, 5,72) ; 4.17 (ddd, 1H, H-4', J 5.72, 5.72, 3,81); 4.48 (m, 1H, H-3'); 6.36 (dd, 1H, H-1', J 6.49, 6,86); 6.83 (t, 1H, SCHF_2 , J 56,5); 7,99 and 8,26 (2 s, 2H, H-2 and H-8). ^{13}C NMR, CDCl_3 , ε CD_3OD , δ (ppm), J (Hz): 29.5 (C-5'); 39.5 (C-2'); 72.9 (C-4'); 84.4 (C-3'); 85.8 (C-1'); 119.5 (C-6); 120.3 (dd, SCHF_2 , J 275); 139.0 and 152,4 (C-2 and C-8); 149.0 and 155.3 (C-4 and C-5). ^{19}F NMR CDCl_3 , ε CD_3OD , δ (ppm), J (Hz) -93.0 (2 dd, 2F, J 244,3, 56,5).
 3. mp $194-196^\circ\text{C}$ - HRMS (DCI/ NH_3) 336,108 (MH) $^+$, calc. 335,250- $[\alpha]_{\text{D}}^{20} -17^\circ$ (c 1.5×10^{-2} ; $\text{CHCl}_3/\text{MeOH}$: 4/1)- ^1H NMR, CDCl_3 , ε CD_3OD δ (ppm), J (Hz): 2.02 (ddd, 1H, H-3' β , J 13,36, 5,72, 9,91); 2.24 (ddd, 1H, H-3' α , J 13.36, 1,91, 5,34); 3.25 (dd, 1H, H-5'a, J 14,11, 5,72); 3.31 (dd, 1H, H-5'b, J 14.11, 5,34); 4.58 (m, 1H, H-2'); 4.76 (m, 1H, H-4'); 5.91 (d, 1H, H-1', J 1,53); 7.98 and 8.24 (2 s, 2H, H-2 and H-8). ^{13}C NMR CDCl_3 , ε CD_3OD , δ (ppm), J (Hz): 33.4 (C-5'); 36.4 (C-3'); 75.9 (C-4'); 79.0 (C-2'); 93.0 (C-1'); 119.8 (C-6); 130.6 (ddd, SCF_3 , J 305); 137.8 and 152.3 (C-2 and C-8); 148.1 et 155.6 (C-4 and C-5). ^{19}F NMR CDCl_3 , ε CD_3OD , δ (ppm): -41.5 (s, SCF_3).
 4. mp $48-50^\circ\text{C}$ - HRMS (DCI/ NH_3) 336,072 (MH) $^+$, calc. 335,250- $[\alpha]_{\text{D}}^{20} +5.8^\circ$ (c 1.5×10^{-2} ; $\text{CHCl}_3/\text{MeOH}$: 4/1)- ^1H NMR CDCl_3 , ε CD_3OD δ (ppm), J (Hz): 2; 40 (ddd, 1H, H-2' β , J 13,74, 6,48, 3,81); 2.84 (ddd, 1H, H-2' α , J 13.74, 6,87, 6,48); 3.18 (dd, 1H, H-5'a, J 13,73, 6,48); 3,28 (dd, 1H, H-5'b, J 13.73, 5,34); 4.11 (ddd, 1H, H-4', J 6.48, 5.34, 3,82); 4.43 (m, 1H, H-3'); 6.26 (dd, 1H, H-1', J 6.48, 6,87); 7.91 and 8.13 (2 s, 2H, H-2 and H-8). RMN, ^{13}C , CDCl_3 ε CD_3OD , δ (ppm), J (Hz): 31.9 (C-5'); 38.9 (C-2'); 72.9 (C-4'); 84.7 (C-3'); 84.9 (C-1'); 119.5 (C-6); 130.6 (ddd, SCF_3 , J 305); 139.2 and 152.3 (C-2 and C-8); 148.8 and 155.3 (C-4 and C-5). ^{19}F NMR CDCl_3 , ε CD_3OD , δ (ppm): $-41,5$ (s, SCF_3).
 6. Yuan, C.-S.; Yeh, J.; Liu, S.; Borchardt, R. *J. Biol. chem.* **1993**, 268, 17030-17037.
 7. Della Ragione, F.; Pegg, A.E. *Biochem. J.* **1983**, 210, 429-434.
 8. Kitz, K.; Wilson, I.B. *J. Biol. chem.* **1962**, 237, 3245-3249.
 9. Robins, M.J.; Neschadimenko, V.; Ro, B.-O.; Yuan, C.-S.; Borchardt, R.J.; Wnuk, S.F.; *J. Org. Chem.*, **1998**, 63, 1205-1211.
 10. Turner, M.A.; Yuan, C.-S.; Borchardt, R.T. Hershfield, M.S.; Smith, G.D.; Howell, P.L. *Nature Structural Biology*, **1998**, 5, 369-376.



Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the [U.S. Copyright Office](#) for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on [Fair Use in the Classroom](#).

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our [Website User Agreement](#) for more details.

[Order now!](#)

Reprints of this article can also be ordered at

<http://www.dekker.com/servlet/product/DOI/101081NCN100002352>