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# ADENOSINE DIALDEHYDE ANALOGS I: REGIOSELECTIVE SYNTHESIS OF ADENOSINE MONOALDEHYDES

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**Abstract:** Selective borohydride reduction of adenosine dialdehyde (**1**) gave the known adenosine 2'-monoaldehyde (**2**). Reaction of **1** with N,N'-diphenylethylenediamine, followed by reduction and deblocking gave adenosine 3'-monoaldehyde (**5**), a new compound. Unlike **2**, compound **5** inhibited *S*-AdoHcy hydrolase and showed antiviral activity in vitro. Copyright © 1996 Elsevier Science Ltd

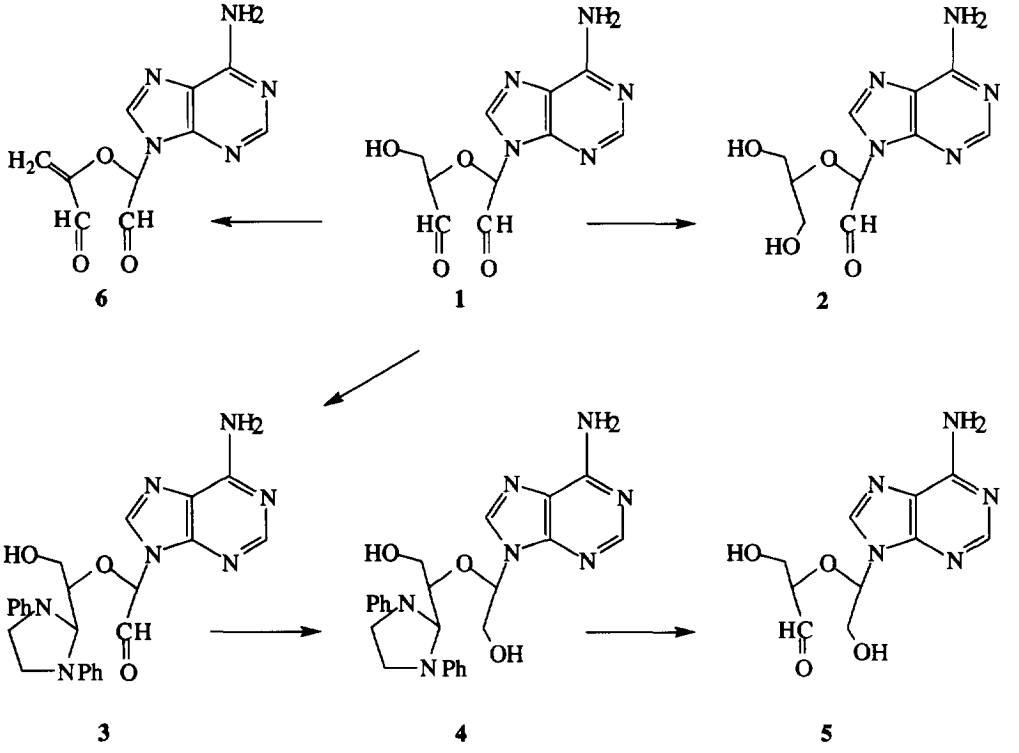
## Introduction

Periodate-oxidized ribonucleosides have shown a broad spectrum of pharmacological activities. Inosine dialdehyde demonstrated positive responses against human cancer in phase I clinical trials[1] and was the second most active of 49 antitumor drugs tested against *Trypanosoma rhodesiense*, the causative agent of African sleeping sickness, in mice.[2] Adenosine dialdehyde (**1**) cured 4 of 5 mice of *T. rhodesiense* at 3.3 mg/kg (s.c.) and the single fatality at this dose had a 350% increase in survival times over untreated controls. In contrast, inosine dialdehyde was inactive against the parasite below 53 mg/kg, but was much less toxic than **1** at higher dose[3]. Compound **1** was also active against L1210 leukemia in mice[4] as well as vaccinia virus[5,6] and vesicular stomatitis virus (VSV)[7] in vitro. Part of the toxicity of nucleoside dialdehydes might be caused by their ability to crosslink proteins.[8] In efforts to reduce the murine toxicity of adenosine dialdehyde, we regioselectively reduced one of the dialdehyde groups to afford nucleoside monoaldehydes incapable of crosslinking reactions. Khym and Cohn[9] previously reported regioselective synthesis of nucleoside 2'-monoaldehydes. We report herein what we believe is the first isolation of a nucleoside 3'-monoaldehyde.

**Synthesis and Characterization**

The synthesis of the adenosine monoaldehydes is shown in Scheme I. Compound **1** was prepared as described by Dvonch et al.[4] Selective borohydride reduction of **1** gave adenosine 2'-monoaldehyde (**2**)[9] which

**Scheme I**



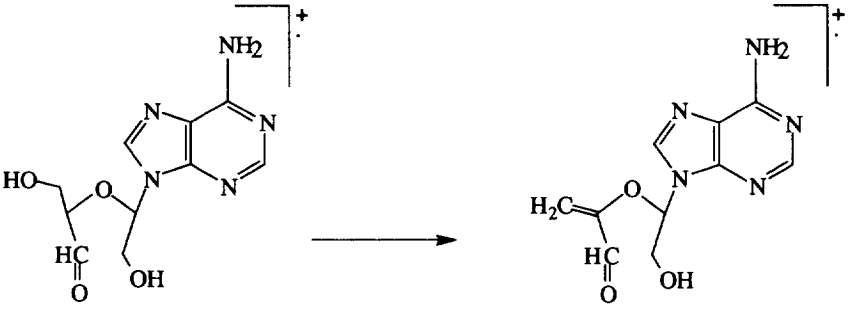
was obtained in a 44% yield by preparative HPLC.[10] Adenosine 3'-monoaldehyde was regioselectively obtained by employing a modification[11] of the method that Nemec and Rhoades[12] applied to 6-azauridine dialdehyde. Initially, **1** was treated with N,N'-diphenylethylenediamine to give, after flash chromatography (silica gel column, 0 – 5% vol. MeOH in $CHCl_3$ gradient), a 42% yield of imidazolidine (**3**). Subsequently, **3** was reacted with aqueous sodium borohydride in $CHCl_3$–MeOH (3:20) at -5 °C to 0 °C; the reaction was monitored by silica gel TLC in 9:1 $CHCl_3$–MeOH. After completion, the pH was adjusted to 7.3 with 2 *N* acetic acid, and the reaction mixture concentrated in vacuo to a small volume. Extraction with $CH_2Cl_2$ gave, after drying ($MgSO_4$), solvent evaporation, and crystallization from ethyl acetate, a 72% yield of reduced imidazolidine (**4**).[13] We obtained adenosine 3'-monoaldehyde (**5**) by treating a stirred solution of **4** (1.17 g, 2.53 mmole) in $CH_2Cl_2$ (500 ml) and MeOH (50 ml) at 0 °C with a solution of *p*-toluenesulfonic acid monohydrate in acetone (5 ml). After 1.5 hours, the precipitate was collected by filtration and dissolved in $H_2O$-MeOH (1:1, 300 ml), stirred with 20 g of Bio-Rad AG 1-X8 anion exchange resin (acetate form) for a few minutes and then filtered. The filtrate was kept at -20 °C overnight, and the resulting crystals were

removed by filtration. The filtrate was extracted twice with $CH_2Cl_2$. The aqueous layer was concentrated in vacuo at 35 °C to a small volume and lyophilized twice to give 496 mg of white powder; a 71% total yield. The product, adenosine 3'-monoaldehyde (**5**), was found to be at least 96% pure by analytical HPLC ($C_{18}$ column, mobile phase: 5 % $CH_3CN$ in $H_2O$).[14] Compound **6**, 4',5'-dehydroadenosine dialdehyde, was prepared by modification of the method of Grant and Lerner.[15] Compound **1** was heated under reflux in $H_2O$ under $N_2$ for 1 hour to give **6**, which was isolated in 17% yield after repeated preparative HPLC.[10]

The $^1H$ and $^{13}C$ NMR spectra of nucleoside dialdehydes are known to be extremely complex. Haworth et al.[16] found that the solution structure of uridine dialdehyde consisted of several isomers in dynamic equilibrium resulting in a 400 MHz $^1H$ NMR spectrum that was too complicated to analyze. They were, however, able to analyze two uridine dialdehyde derivatives and found the presence of three diastereoisomeric cyclic acetals. The 200 and 400 MHz $^1H$ spectra that we observed with compounds **2** and **5**,[17] although difficult to fully analyze, indicated that the nucleoside monoaldehydes also exist in solution mainly as cyclic acetals.

The facile tendency of compound **1** to form the conjugated 4',5'-dehydroadenosine dialdehyde (**6**) upon heating was pertinent to our ability to distinguish between the monoaldehyde isomers **2** and **5**. When **2** was heated for 1 hour in refluxing pH 7 phosphate buffer, it was found to be stable by analytical HPLC. Under the same conditions, the 3'-monoaldehyde (**5**) decomposed at approximately half the rate of the dialdehyde, **1**. We ascribe the thermal instability of **5** to its ability to dehydrate to the conjugated aldehyde (**7**), see Scheme II.

**Scheme II**



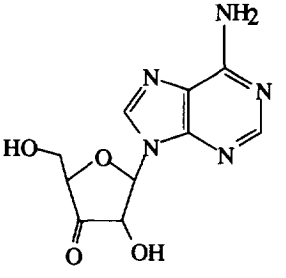

**5** **7**

Indeed, the mass spectrum (DCI, $NH_3$) of **5** showed a peak at *m/z* 250 ($MH^+ - H_2O$) corresponding to **7**, as the most prominent in the spectrum, with only a minor peak at *m/z* 268 ($MH^+$). The relative intensities were

10:1. In contrast, **2** displayed a peak at *m/z* 268 ($MH^+$) as the major ion, with only a minor (20:1) peak at *m/z* 250.

**Biological Activity**

The antiviral activity of adenosine dialdehyde (**1**) has been correlated with its potent competitive inhibition of the enzyme *S*-adenosylhomocysteine (SAH) hydrolase.[18] A number of inhibitors of this enzyme have shown antiviral activity.[19, 20] Hoffman[21] has attributed the potent inhibition of SAH hydrolase by **1** ($K_i$ = 3.3 nM) to its structural resemblance to the 3'-ketoadenosine transition state intermediate (**8**) formed




**8**

at the active site in the mechanism proposed by Palmer and Abeles.[22] In our assay,[23] adenosine 3'-monoaldehyde (**5**), which like **1** resembles **8**, gave 89% inhibition of SAH hydrolase at a concentration of 10 μM; whereas adenosine 2'-monoaldehyde (**2**), which is not a transition state analog, gave only 4% inhibition. Both **5** and **6** showed competitive inhibition with $K_i$ values of 1.9 and 0.5 μM, respectively. The antiviral $IC_{50}$ values (with minimal toxic concentrations in parentheses) of compounds **1**, **5**, and **6** were, respectively, 0.41 (100), 0.41 (119) and 11.5 (100) μg/ml against vaccinia virus in Vero cells and 3.1 (201), 1.9 (198) and 8.9 (100) μg/ml against VSV in L929 cells, whereas compound **2** was inactive and nontoxic against both viruses at concentrations as high as 320 μg/ml.[24]

Conversion of antiviral and antitumor nucleosides to monoaldehydes might allow them to form Schiff bases with lysine residues on monoclonal antibodies or with poly(*L*-lysine) for carrier-mediated drug delivery. LeMaitre et al.[25] found inhibition of VSV by an antisense oligonucleotide poly(*L*-lysine) conjugate. Also, retrograde axonal transport of the antiherpes drug 5'-amino-5-iodo-2',5'-dideoxyuridine[26] linked to the periodate-oxidized carbohydrate moiety of horseradish peroxidase by Schiff base formation has been demonstrated.[27] We believe that further investigation of nucleoside monoaldehydes as potential medicinal agents is warranted.

**Acknowledgment**

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**References and Notes**

1. Kaufman, J.; Mittle, A. *Cancer Chemother. Rep. Pt. 1* **1975**, *59*, 1007.
2. Kinnamon, K. E.; Steck, E. A.; Rune, D. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 157.
3. Neenan, J. P.; Schram, K. H. *Abstracts of Papers*, 185th National Meeting of the American Chemical Society, Seattle, WA, American Chemical Society: Washington, D. C., **1983**; MEDI-17.
4. Dvonch, W.; Fletcher, W.; Gregory, E-M.; Healy, H.; Warren, G. H.; Alburn, H. E. *Cancer Res.* **1966**, *26*, 2386.
5. Keller, B. T.; Borchardt, R. T. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1983**, *42*, 2139.
6. Keller, B. T.; Borchardt, R. T. *Molec. Pharmac.* **1987**, *31*, 485.
7. Neenan, J. P.; Opitz, S. M.; Borges, K. M.; Grinnell, J. D.; Canonico, P. G.; Ussery, M. A. *Abstracts of Papers*, 192nd National Meeting of the American Chemical Society, Anaheim, CA, American Chemical Society: Washington, D. C., **1986**; MEDI-87.
8. Cysyk, R. L.; Adamson, R. H. *Cancer Treatment Reports* **1976**, *60*, 563.
9. Kyhm, J. X.; Cohn, W. E. *J. Amer. Chem. Soc.* **1960**, *82*, 6380.
10. Preparative HPLC was performed on a Waters 500A system (50 x 30 cm, $C_{18}$ column). Mobile phases: 1.5, 3% $CH_3CN$ in $H_2O$ (compounds **2** and **6**, respectively).
11. To a stirred solution of **1** (3.18 g, 12 mmol) in absolute MeOH (200 ml) was added a solution of N,N'-diphenylethylenediamine (2.55 g, 12 mmol) in $CHCl_3$ (10 ml). After stirring 2 days at room temperature, under $N_2$, solvents were removed in vacuo. The residue was redissolved in $CHCl_3$ (75 ml) and MeOH (10 ml), and stirred an additional 18 days at room temperature. Solvents were removed in vacuo and **3** was isolated by flash chromatography (see text). An analytical sample was obtained by recrystallization from THF/ether (6:1). Elemental analysis of compound **3** ($C_{24}H_{25}O_3N_7 \cdot C_4H_8O$): anal. calcd; C, 63.26; H, 6.26; N, 18.44 and found; C, 63.19; H, 6.28; N, 18.24. $^1H$ NMR of compound **3**: ($Me_2SO$-$d_6$/$D_2O$) δ 8.22-8.04 (m, 2 H; H-2 and H-8); 7.25-6.48 (m, 10 H; phenyl rings); 5.97-5.79 (m, 1 H; H-1'); 5.41-5.11 (m, 2 H; H-2' and H3'); 4.31-3.31 (m, 11 H; H-4', 2 H-5', -$NCH_2CH_2N$-, 2 -$CH_2O$ of THF); and 1.78-1.72 (m, 4 H; - $CH_2CH_2$- of THF).
12. Nemec, J.; Rhoades, J. M. *Nucleosides and Nucleotides* **1983**, *2*, 99.
13. An analytical sample of **4** (m.p. 157-159 °C) was obtained by semipreparative HPLC (7.8 x 30 cm, $C_{18}$ column; mobile phase: gradient from 40 to 70% $CH_3CN$ in $H_2O$). Elemental analysis of compound **4** ($C_{24}H_{27}O_3N_7 \cdot 0.2H_2O$): anal. calcd; C, 61.96; H, 5.94; N, 21.08 and found; C, 61.98; H, 6.00; N, 21.02.

$^1$H NMR of compound **4**: ($CDCl_3$) δ 8.32 (s, 1 H; H-8); 7.99 (s, 1 H; H-2); 7.26-6.75 (m, 10 H; phenyl rings); 6.02-5.98 (t, $J$ = 3.5, 3.8, 1 H; H-1'); 5.71 (s, 2 H; $NH_2$); 5.45-5.41 (d, $J$ = 7.1, 1 H; H3'); 4.76 (s, broad, 1 H; 2'-OH); 3.97-3.50 (m, 10 H; 5'-OH, -$NCH_2CH_2N$-, H-4', $CH_2$-5', and $CH_2$-2').

14. Elemental analysis of compound **2** ($C_{10}H_{13}O_4N_5$•1.2 $H_2O$): anal. calcd; C, 41.58; H, 5.37; N, 24.24 and found; C, 41.59; H, 5.27; N, 24.26. Elemental analysis of compound **5** ($C_{10}H_{13}O_4N_5$•0.6 $H_2O$): anal. calcd; C, 43.20; H, 5.15; N, 24.19 and found; C, 43.59; H, 5.16; N, 24.64. The IR spectra of **2** and **5** showed a broad band at 1100 $cm^{-1}$, as reported for other periodate oxidized nucleosides.[28]
15. Grant, A. J.; Lerner, L. M. *J. Med. Chem.* **1980**, *23*, 795.
16. Howarth, O.; Jones, A. S.; Walker, R. T.; Wyatt, P. G. *J. Chem. Soc., Perkins Trans. II* **1984**, 261.
17. The following resonances are tentatively assigned for compounds **2** and **5**. Compound **2**: $^1$H NMR ($D_2O$) δ 8.77-8.11 (m, 2 H; H-2 and H-8); 6.11-5.31 (m, 2 H; H-1' and H-2'); 4.39-3.57 (m, 5 H; 2 H-3', H-4' and 2 H-5'). Compound **5**: $^1$H NMR ($D_2O$) δ 8.37-8.16 (m, 2 H; H-2 and H-8); 6.14-5.99 (m, 1 H; H-1'); 5.17-4.70 (m, 5 H; 2 H-2', H-4' and 2 H-5').
18. Keller, B. T.; Borchardt, R. T. *Molec. Pharmac.* **1987**, *31*, 485.
19. De Clercq, E.; Cools, M. *Biochem. Biophys. Res. Commun.* **1985**, *129*, 306.
20. De Clercq, E. *Biochem. Pharmac.* **1987**, *36*, 2567.
21. Hoffman, J. L. *Transmethylation*; Usdin, E.; Borchardt, R. T.; Creveling, C. R., Eds.; Elsevier: New York, 1979; pp 181-186.
22. Palmer, J. L.; Abeles, R. H. *J. Biol. Chem.* **1976**, *251*, 5817.
23. SAH hydrolase activity was measured in the direction of *S*-AdoHcy synthesis: each assay mixture contained 0.3 μg of enzyme;[19] 120 mM Na phosphate, pH 7.6; 1 mM homocysteine; 2 mM dithiothreitol; 1 mM EDTA; 0.3 - 3.0 μM [8-$^{14}$C] adenosine, 54.3 mCi/mmol (New England Nuclear); and inhibitor in a final volume of 0.2 mL. The reaction was started by addition of enzyme followed by incubation at 37 °C for 2.5 min. The reaction was terminated by heating in boiling water for 2 min., followed by centrifugation. After addition of 5 μl each of 5 mM solutions of adenosine, inosine, and product AdoHcy as markers, aliquots (5 x 5 μl each) were spotted on aluminum-supported silica gel 60 F254 TLC sheets (E. Merck). Chromatograms were developed in 1-butanol : acetic acid : water (12:3:5) for 100 min. Spots containing radioactive product were visualized under UV light, cut out, and counted after addition of Scintisol fluor. Each reaction was run in duplicate. Kinetic parameters were determined by Lineweaver-Burk plots.
24. Antiviral data on compounds **1**, **2**, **5** and **6** were obtained from the U. S. Army Medical Research Institute of Infectious Diseases, Frederick, MD, USA.
25. LeMaitre, M.; Bayard, B.; Lebleu, B. *Proc. Natl. Acad. Sci., U.S.A.* **1987**, *84*, 648.
26. Cheng, Y-C.; Neenan, J. P.; Goz, B.; Ward, D. C.; Prusoff, W. H. *J. Virol.* **1975**, *15*, 1284.
27. Hasehke, R. H.; Ordronneau, J. M.; Bunt, A. H. *J. Neurochem.* **1980**, *35*, 1431.
28. Hansske, F.; Cramer, F. *Carbohydr. Res.* **1977**, *54*, 75.

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