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Azadideoxyadenosine: Synthesis, enzymology, and anti-HIV activity

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Abstract—Synthesis of an azanucleoside, a new analogue of dideoxyadenosine, is described. This compound is only slowly deaminated by mammalian adenosine deaminase and it is a substrate for adenosine kinase. It exhibits in vitro anti-HIV activity.

There is continuing interest in the synthesis of new nucleoside analogues with potential antiviral activity. Synthesis and anti-HIV activities of new dideoxynucleosides, of both the D- and L-related families, in which the furanose oxygen is transposed to a new endocyclic position within the cyclopentane ring have been the subject of a number of studies.[1,2] The family of nucleoside analogues referred to as carbocyclic nucleosides has been the subject of intense investigation, both in synthesis and antiviral studies.[3–7] For example, the enantiomerically pure carbocyclic analogue of 2′-deoxyadenosine (Fig. 1) has been synthesized and showed activity against DNA viruses.[8,9]

Conformational studies on the pyrrolidine and 4-hydroxyproline rings have indicated substantial similarity to the cyclopentyl and tetrahydrofuran ring structures.[10] While a few pyrrolidine nucleosides have been synthesized,[11,12] antiviral or other biological studies of these compounds are largely lacking. Also, some interesting azanucleosides, such as the title compound (Fig. 1), have not been synthesized or examined for antiviral activity. This communication describes the synthesis, enzymology and in vitro anti-HIV studies of 3′-aza-2′,4′-dideoxyadenosine, a new carbocyclic nucleoside analogue.

The starting compound for the synthesis (Scheme 1) was *trans*-4-hydroxy-L-proline (**1**). Epimerization of *trans*-4-

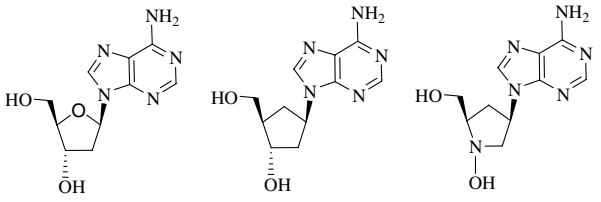
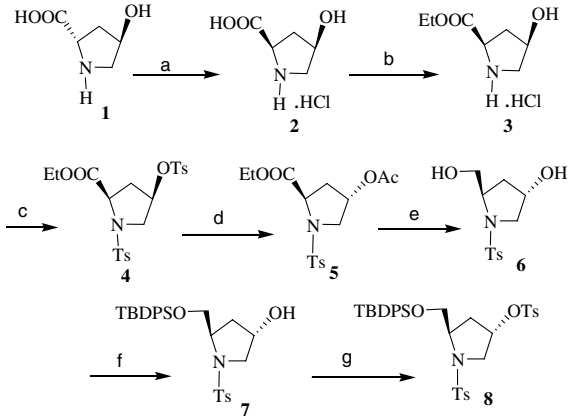


Figure 1. Structures of three related adenine nucleosides (L to R) 2′-deoxyadenosine, carba-2′-deoxyadenosine, and 3′-azadide oxyadenosine.



Scheme 1. Reagents and conditions: (a) Ac_2O, AcOH, reflux, 5.5 h, 2 N HCl, reflux, 3 h, 65%; (b) absolute EtOH, HCl (gas), reflux, 5 h, 67%; (c) Et_3N, Py, TsCl, −5 °C, 1 h, refrigerated 12 h, 5 h at rt, 68%; (d) $(Et)_4N^{+-}OAc$, toluene, reflux 12 h, 75%; (e) $LiBH_4$, THF, 0 °C 30 min, rt 12 h, 76%; (f) *t*-TBDPSCl, imidazole, DMAP, dry CH_2Cl_2, at 0 °C then 18 h at rt, 47%; (g) *p*-TsCl, CH_2Cl_2, DMAP, 48 h, rt, 99%.

Keywords: Azanucleoside; Synthesis; Adenosine deaminase; Kinase phosphorylation; Anti-HIV activity.
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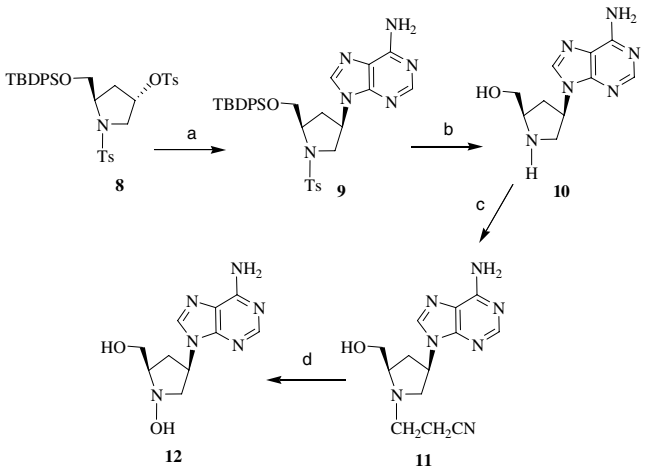

Scheme 2. Reagents and conditions: (a) adenine, K_2CO_3, 18-crown-6, DMF, 80 °C, 12 h, 59%; (b) HBr, HOAc, phenol, 90 °C, 16 h, 88%; (c) acrylonitrile, 0 °C, 1 h, to rt, 4 h, 58%; (d) MeOH–$CHCl_3$ (1:1), *m*-CPBA, −78 °C, K_2CO_3, 3 h, rt, 16 h, 90%.

hydroxy-L-proline under the conditions described[13] gave the *cis*- or *allo*-isomer **2**. The product crystallized from aqueous HCl as its hydrochloride salt, which was free from the *trans*-compound. The hydrochloride salt **2** was esterified with ethanol to **3**, and the amine and hydroxyl functionalities were then blocked with tosyl groups to give **4**.

Displacement (S_N2) of the tosyloxy group of **4** with acetate ions gave the inverted acetate **5**, which was converted to **6** with lithium borohydride. Selective protection of the primary hydroxyl group with TBDPS (to give **7**), followed by tosylation of the secondary hydroxyl functionality, gave key intermediate **8**. For the main coupling step to the aza nucleoside (Scheme 2), intermediate **8** was treated with adenine in the presence of 18-crown-6 and K_2CO_3 in DMF,[14,15] which produced nucleoside **9**. The structure of **9** was confirmed by NMR data and NOE experiments. Reductive cleavage of the *N*-tosyl protecting group of **9** was carried out with HBr in acetic acid in the presence of phenol. Under these reaction conditions, the silyl protecting group was also removed to produce the azanucleoside **10**. Cyanoethylation of **10** with acrylonitrile gave the cyanoethyl derivative **11**, which was oxidized with 77% *m*-chloroperbenzoic acid in MeOH/$CHCl_3$ (1:1) to give the desired hydroxylamine product **12**. Purification of the crude product by crystallization from ethanol gave the pure target compound. Its structure was confirmed by ^1H and ^{13}C NMR, UV, and HRMS data.[16] Compound **12** was optically active and dextrorotatory.

One key question pertaining to compound **12** was its stability toward deamination by mammalian adenosine deaminase (ADA). Kinetic parameters were obtained for **12** and compared to adenosine as the positive standard. Compound **12** was a poor substrate for ADA (Fig. 2 and Table 1). It is not an inhibitor of this enzyme. Phosphorylation studies using mammalian adenosine kinase (AK) showed that **12** was a substrate for AK and was slowly converted to its monophosphate, the formation of which could be analyzed by HPLC analysis.

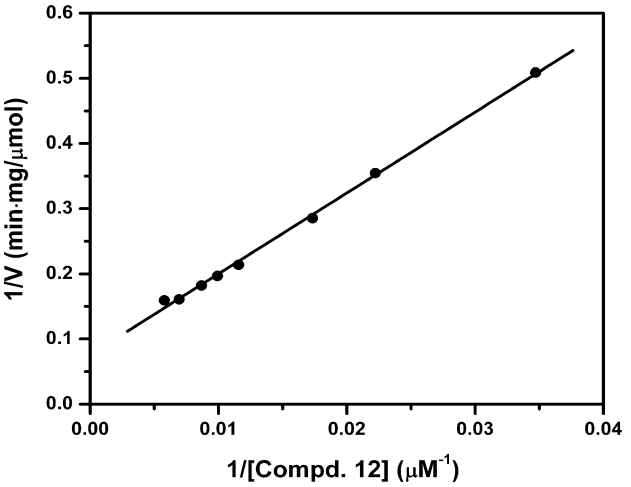

Figure 2. Lineweaver–Burk plot of the deamination of compound **12**.

Table 1. Kinetic parameters for substrate activity for **12** compared to adenosine toward bovine adenosine deaminase

Substrate	λ^a (nm)	K_m (μM)	V_{max} μmole min^{-1} mg $protein^{-1}$	V_{max}/K_m relative (%)
Adenosine	265	32 ± 2.7	246 ± 9.1	100
Compound **12**	265	131 ± 15.4	12 ± 0.8	1.2

[a] Deamination carried out in phosphate buffer (pH 7) and monitored at 265 nm, the wavelength of maximum difference between substrate and product.

Compound **12** was tested in a PBMC-based, microtiter anti-HIV assay against the clinical isolate, HIV-1_{TEKI} (NSI phenotype) and HIV-1_{NL4-3} (SI phenotype). All antiviral determinations were performed in triplicate with serial 1/2 log10 dilution of the test materials (six to nine concentrations total).[17] The overall performance of both assays was validated by the MOI-sensitive positive control compound, AZT, exhibiting the expected level of antiviral activity [IC_{50} = 1.98 nM (HIV-1_{TEKI}) and 1.75 nM (HIV-1_{NL4-3}), CC_{50} >1000 nM, PBMC]. Compound **12** was found to have anti-HIV activity [IC_{50} = 3.52 μM (HIV-1_{TEKI}) and 3.11 μM (HIV-1_{NL4-3})]. The CC_{50} value in PBMC was 46.6 μM.

In summary, an azadideoxyadenosine analogue of 2′-deoxyadenosine was synthesized, and its structure and stereochemistry were confirmed by optical rotation, UV, ^1H and ^{13}C NMR spectroscopy, and HRMS data. This compound exhibits resistance toward deamination by ADA. It possesses in vitro anti-HIV activity with IC_{50s} in the low micromolar range. As the compound is phosphorylated by AK, it seems likely that the anti-HIV activity could be attributed to the inhibition of HIV reverse transcriptase by its 5′-triphosphate.

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References and notes

1. Nair, V.; Jahnke, T. S. *Antimicrob. Agents Chemother.* **1995**, *39*, 1017.
2. Nair, V. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Chu, C. K., Ed.; Elsevier: Amsterdam, 2002.
3. Marquez, V. E.; Lim, M. I. *Med. Res. Rev.* **1986**, *6*, 1.
4. Montgomery, J. A. *Antiviral. Res.* **1989**, *12*, 113.
5. White, E. L.; Parker, W. B.; Macy, L. J.; Shaddix, S. C.; McCaleb, G.; Secrist, J. A., III; Vince, R.; Shannon, W. M. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 393.
6. Vince, R.; Hua, M. J. *J. Med. Chem.* **1990**, *33*, 17.
7. Melroy, J.; Nair, V. *Curr. Pharm. Des.* **2005**, *11*, 3847.
8. Beres, J.; Sagi, Gy.; Baitz-Gacs, E.; Tomoskozi, I.; Otvos, L. *Tetrahedron* **1988**, *44*, 6207.
9. Beres, J.; Sagi, Gy.; Tomoskozi, I.; Gruber, L.; Baitz-Gacs, E.; Otvos, L.; De Clercq, E. *J. Med. Chem.* **1990**, *33*, 1353.
10. DeTar, D. F.; Luthra, N. P. *J. Am. Chem. Soc.* **1977**, *99*, 1232.
11. Ng, K. E.; Orgel, L. E. *J. Med. Chem.* **1989**, *32*, 1754.
12. Peterson, M. L.; Vince, R. *J. Med. Chem.* **1991**, *34*, 2787.
13. Baker, G. L.; Fritschel, S. J.; Stille, J. R.; Stille, J. K. *J. Org. Chem.* **1981**, *46*, 2954.
14. Nair, V.; Nuesca, Z. M. *J. Am. Chem. Soc.* **1992**, *114*, 7951.
15. Guenther, S.; Balzarini, J.; De Clercq, E.; Nair, V. *J. Med. Chem.* **2002**, *45*, 5426.
16. *cis*-4-(6-Amino-9*H*-purin-9-yl)-D-prolinol (**12**). Acrylonitrile (0.25 ml, 1.58 mmol) was added dropwise over 15 min to a cooled (ice-salt bath) solution of **10** (0.37 g, 1.58 mmol) in H_2O (10 ml). Stirring was continued for 1 h and for an additional 4 h with the bath removed. The reaction mixture was evaporated to dryness and the residue crystallized from methanol to give *N*-(cyanoethyl)-*cis*-4-(6-amino-9*H*-purin-9-yl)-D-prolinol (**11**) (0.26 g, 0.91 mmol, 58%). ^{1}H NMR (500 MHz, CD_3OD + $CDCl_3$): δ 1.99 (m, 1H), 2.70 (m, 4H), 2.83 (m, 1H), 2.91 (m, 1H), 3.43 (d, 1H), 3.67 (m, 2H), 5.17 (m, 1H), 8.28 (s, 1H), 8.59 (s, 1H). A solution of **11** (0.076 g, 0.27 mmol) in methanol and chloroform (1:1, 10 ml) was cooled to −78 °C, and *m*-chloroperbenzoic acid (0.068 g, 0.4 mmol) and K_2CO_3 (0.55 g, 0.4 mmol) were added. After stirring for 3 h at −78 °C, the reaction mixture was warmed to room temperature and stirred for an additional 16 h. The resulting suspension was filtered through a $MgSO_4$ pad and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 10:1) and then crystallized from ethanol to give pure *cis*-4-(6-amino-9*H*-purin-9-yl)prolinol (**12**) as a white solid (0.06 g, 0.24 mmol, 90%): mp 201–202 °C. ^{1}H NMR (500 MHz, CD_3OD): δ 2.00 (m, 1H), 2.76 (m, 1H), 3.05 (br s, 1H), 3.44, 3.78, 3.83–3.86 (m, 3H), 5.26 (br s, 1H), 8.23 (s, 1H), 8.50 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 32.5, 48.2, 61.0, 63.4, 68.8, 118.4, 140.2, 149.0, 152.2, 155.9, HRMS-FAB calcd for $C_{10}H_{15}N_6O_2$ $(M+H)^+$: 251.1256, found: 251.1252. $[\alpha]_D^{24}$ +30.5 (*c* 2 in MeOH); UV λ_{max} 261 nm (ε 15,700).
17. Nair, V.; Chi, G.; Ptak, R.; Neamati, N. *J. Med. Chem.* **2006**, *49*, 445.