# *Escherichia coli* Mediated Biosynthesis and in Vitro Anti-HIV Activity of Lipophilic 6-Halo-2',3'-dideoxypurine Nucleosides

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A series of 6-substituted 2',3'-dideoxypurine ribofuranosides (ddP) was enzymatically synthesized with live *E. coli* in an effort to enhance the lipophilicity of this class of anti-human immunodeficiency virus (HIV) compounds and thereby facilitate drug delivery into the central nervous system. All 6-halo-substituted ddPs were substantially more lipophilic, as defined by their octanol–water partition coefficient (*P*), than their nonhalogenated congeners 2',3'-dideoxyinosine (ddI) or 2',3'-dideoxyguanosine (ddG). For this class of compounds, log *P*'s ranged from +0.5 to −1.2 in the following order: 6-iodo, 2-amino-6-iodo > 6-bromo, 2-amino-6-bromo > 6-chloro, 2-amino-6-chloro > 6-fluoro, 2-amino-6-fluoro ≫ ddG > ddI. These compounds were evaluated in vitro for ability to suppress the infectivity, replication, and cytopathic effect of HIV. 2-Amino-6-fluoro-, 2-amino-6-chloro-, and 6-fluoro-ddP exhibited a potent activity against HIV comparable to that of ddI or ddG and completely blocked the infectivity of HIV without affecting the growth of target cells. The comparative order of in vitro anti-HIV activity was 2-amino-6-fluoro, 2-amino-6-chloro, 6-fluoro > 2-amino-6-bromo > 2-amino-6-iodo, 6-chloro > 6-bromo > 6-iodo. These compounds also exhibited potent in vitro activity against HIV-2 and 3'-azido-3'-deoxythymidine-resistant HIV-1 variants. All 2-amino-6-halo-ddPs and 6-halo-ddPs were substrates for adenosine deaminase (ADA) and were converted to ddG or ddI, respectively. In the presence of the potent ADA inhibitor 2'-deoxycoformycin, 6-halo-substituted ddPs failed to exert an in vitro antiretroviral effect. These dideoxypurine nucleoside analogues represent a new class of lipophilic prodrugs of ddG and ddI that possess the potential for more effective therapy of HIV-induced neurologic disorders.

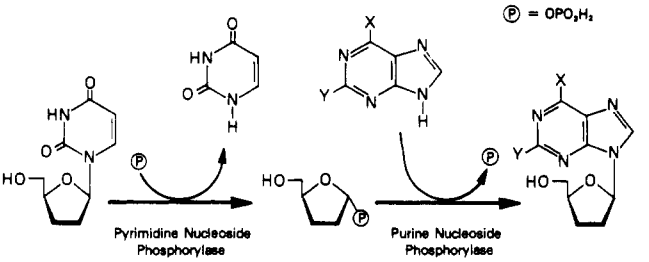
## Introduction

In the past 6 years, a number of potentially useful approaches for the therapy of human immunodeficiency virus (HIV) infection have emerged.[1-3] One such approach is the use of the broad family of 2',3'-dideoxynucleosides (ddN) as therapeutic agents against HIV infection.[4,5] It should be noted that in the past 25 years a variety of ddN analogues have been synthesized and studied by pioneering researchers[6-9] (see refs 1-3 for review). 3'-Azido-3'-deoxythymidine (AZT or zidovudine), one of the ddN analogues, was identified in 1985 as a potent antiretroviral agent against HIV in vitro.[5] AZT has now been formally proven to reduce the morbidity and mortality of patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC).[10-12] Two other pyrimidine analogues of the ddN family, 2',3'-dideoxycytidine (ddC)[13,14] and 2',3'-didehydro-2',3'-dideoxythymidine (D4T),[15] have demonstrated activity against HIV-1 in some patients with AIDS and ARC in Phase I clinical trials. A purine ddN member, 2',3'-dideoxyinosine (ddI or didanosine), has also recently been shown to be active against HIV-1 in patients with AIDS and advanced ARC in short-term Phase I clinical trials.[16-19]

HIV not only causes severe forms of immunodeficiency but also often causes a variety of neurological disorders.[20,21] HIV in the central nervous system (CNS) may replicate more actively than in other tissues and, indeed, the CNS may serve as a principal reservoir of the virus in the whole body.[22-24] Thus, the capability of antiretroviral agents to penetrate into the CNS may constitute an important feature of therapeutics against HIV infection. However, the lipophilicity of purine 2',3'-dideoxynucleosides, especially ddI, is generally low and perhaps, in part, this limits the penetration of such 2',3'-dideoxypurine nucleoside analogues into the CNS.

In the present work, we synthesized a variety of 2- and 6-substituted 2',3'-dideoxypurine ribofuranoside (ddP) analogues by an enzymatic method employing *E. coli* JA-300 and identified several 6-halo-substituted ddP ana-

**Scheme I**




| | X | Y |
|---|---|---|
| 1 | F | $NH_2$ |
| 2 | Cl | $NH_2$ |
| 3 | Br | $NH_2$ |
| 4 | I | $NH_2$ |
| 5 | F | H |
| 6 | Cl | H |

| | X | Y |
|---|---|---|
| 7 | Br | H |
| 8 | I | H |
| 11 | Cl | Cl |
| 12 | H | $NH_2$ |
| 13* | OH | OH |
| 14 | H | H |

* Nucleoside rather than base was used as the purine precursor.

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(1) Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990**, *249*, 1533.
(2) Mitsuya, H.; Broder, S. *Nature* **1987**, *325*, 773.
(3) Yarchoan, R.; Mitsuya, H.; Meyer, C. E.; Broder, S. *N. Engl. J. Med.* **1989**, *321*, 726.
(4) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911.
(5) Mitsuya, H.; Weinhold, K. J.; Furman, F. A.; St. Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096.
(6) Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076.
(7) Robins, M. J.; Robins, R. K. *J. Am. Chem. Soc.* **1964**, *86*, 3585.
(8) Horwitz, J. P.; Chua, J.; Noel, M.; Donatti, J. T. *J. Org. Chem.* **1967**, *32*, 817.
(9) Lin, T. S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109.
(10) Yarchoan, R.; Klecker, R.; Weinhold, K. J.; Markham, P. D.; Lyerly, H. K.; Durack, D. T.; Gelmann, E.; Lehrman, S. N.; Blum, R. M.; Barry, D. W.; Shearer, G. M.; Fischl, M. A.; Mitsuya, H.; Gallo, R. C.; Collins, J. M.; Bolognesi, D. P.; Myers, C. E.; Broder, S. *Lancet* **1986**, *i*, 575.
(11) Fischl, M. A.; Richman, D. D.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Schooley, R. T.; Jackson, G. G.; Durack, D. T.; King, D.; the AZT Collaborative Group *N. Engl. J. Med.* **1987**, *317*, 185.

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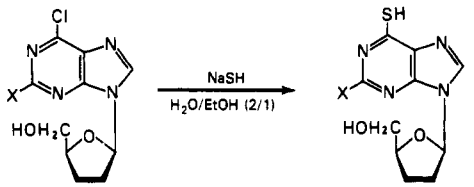
**Table I.** In Vitro Antiviral Activity of 6-Halogen-Containing Dideoxypurine Ribofuranosides

| compound | $CD_{50}$[c] | HIV-1[a] $ED_{50}$[d] | HIV-1[a] $CD_{50}/ED_{50}$ | HIV-2[b] $ED_{50}$ | HIV-2[b] $CD_{50}/ED_{50}$ |
|---|---|---|---|---|---|
| (1) 2-amino-6-fluoro-ddP | >300[e] | 2.4 | >125 | 14.5 | >20.7 |
| (2) 2-amino-6-chloro-ddP | >300[e] | 5.5 | >55 | 12.5 | >24 |
| (3) 2-amino-6-bromo-ddP | >300[e] | 6.9 | >43 | ND[f] | ND |
| (4) 2-amino-6-iodo-ddP | 210 | 7.9 | 27 | ND | ND |
| (5) 6-fluoro-ddP | 190 | 2.8 | 68 | 16.0 | 11.9 |
| (6) 6-chloro-ddP | 164 | 6.9 | 24 | 42.0 | 3.9 |
| (7) 6-bromo-ddP | 96 | 7.7 | 13 | ND | ND |
| (8) 6-iodo-ddP | 86 | 46 | 2 | ND | ND |
| (9) 2-amino-6-mercapto-ddP | 9 | >200 | <0.1 | ND | ND |
| (10) 6-mercapto-ddP | 150 | >200 | <0.8 | ND | ND |
| (11) 2,6-dichloro-ddP | 36 | >200 | <0.2 | ND | ND |
| (12) 2-amino-ddP | >200 | >200 | | ND | ND |
| (13) 2',3'-dideoxyxanthosine | >200 | >200 | | ND | ND |
| (14) 2',3'-dideoxynebularine | >200 | >200 | | ND | ND |
| (15) 2',3'-dideoxyinosine | >900 | 5.6 | >161 | ND | ND |
| (16) 2',3'-dideoxyguanosine | >900 | 3.5 | >257 | ND | ND |
| (17) 3'-azido-3'-deoxythymidine (AZT)[g] | >20 | 5.0 | 4 | ND | ND |

[a] The target ATH8 cells were exposed to a 4200 50% tissue culture infectious dose ($TCID_{50}$) of HIV-$1_{IIIB}$ per cell. [b] The target ATH8 cells were exposed to a 7700 $TCID_{50}$ dose of HIV-$2_{LAV}$ per cell. [c] The $CD_{50}$ values represent the drug concentrations ($\mu$M) required to suppress the growth of target ATH8 cells by 50% in vitro. [d] The $ED_{50}$ values represent the drug concentrations ($\mu$M) required to inhibit the cytopathic effect of HIV by 50% in vitro. [e] Because of the inherent toxicity of the cosolvent used, DMSO, concentrations higher than 300 and 200 $\mu$M were not tested for 1–3 and 12–14, respectively. [f] ND, not determined. [g] 3'-Azido-3'-deoxythymidine (AZT) is listed as a reference compound.

logues which have an enhanced lipophilicity and a potent antiretroviral activity against various strains of HIV in vitro. A number of chemical methods for synthesizing a variety of ddN analogues have been developed.[6-9] However, the requirement for a multiple-step synthesis using expensive reagents in such chemical methods has often prevented adaptation to a large-scale production of ddN analogues. In addition, many chemical methods can inherently have a serious disadvantage such as formation of unnecessary byproducts, i.e., 2'- or 3'-deoxynucleosides and the $\alpha$-anomer of 2',3'-dideoxynucleosides. Pioneering studies using bacterial enzyme systems for the synthesis of nucleoside analogues through transribosylation,[30,31] transdeoxyribosylation,[32,33] transarabinosylation,[34,35] and transdideoxyribosylation[36] have been reported. However, transdideoxyribosylation with transfer of the dideoxy-

**Scheme II**




(12) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Nusinoff-Lehrman, S.; the AZT Collaborative Working Group *N. Engl. J. Med.* **1987**, *317*, 192.
(13) Yarchoan, R.; Perno, C.-F.; Thomas, R. V.; Klecker, R. W.; Allain, J. P.; Wills, J.; McAtee, N.; Fischl, M. A.; Dubinski, R.; McNeely, M. C.; Mitsuya, H.; Pluda, J. M.; Lawley, T. J.; Leuther, M.; Safai, B.; Collins, J. M.; Myers, C. E.; Broder, S. *Lancet* **1988**, *i*, 76.
(14) Merigan, T. C.; Skowron, G.; Bozzette, S. A.; Richman, D.; Uttamchandani, R.; Fischl, M.; Schooley, R.; Hirsch, M.; Soo, W.; Pettinelli, C.; Schaumburg, H.; the ddC Study Group of the AIDS Clinical Trials Group *Ann. Int. Med.* **1989**, *110*, 189.
(15) Browne, M.; Curt, G.; Brown University Phase I Working Group, unpublished data.
(16) Yarchoan, R.; Mitsuya, H.; Thomas, R. V.; Pluda, J. M.; Hartman, N. R.; Perno, C.-F.; Marczyk, K. S.; Allin, J.-P.; Johns, D. G.; Broder, S. *Science* **1989**, *245*, 412.
(17) Lambert, J. S.; Seidlin, M.; Reichman, R. C.; Plank, C. S.; Laverty, M.; Morse, G. D.; Knupp, C.; McLaren, C.; Pettinelli, C.; Valentine, F. T.; Dolin, R. *N. Engl. J. Med.* **1990**, *322*, 1333.
(18) Cooley, T. P.; Kunches, L. M.; Saunders, C. A.; Ritter, J. K.; Perkins, C. J.; McLaren, C.; McCaffrey, R. P.; Liebman, H. A. *N. Engl. J. Med.* **1990**, *322*, 1340.
(19) Yarchoan, R.; Mitsuya, H.; Pluda, J. M.; Marczyk, K. S.; Thomas, R. V.; Hartman, N. R.; Browers, P.; Perno, C.-F.; Allain, J.-P.; Johns, D. G.; Broder, S. *Rev. Infect. Dis.* **1990**, *12*, S522.
(20) Snider, W. D.; Simpson, D. M.; Nielsen, S.; Gold, J. W. M.; Metroka, C. E.; Posner, J. B. *Ann. Neurol.* **1983**, *14*, 403.
(21) Koralnik, I. J.; Beaumanoir, A.; Häusler, R.; Kohler, A.; Safran, A. B.; Delacoux, R.; Vibert, D.; Mayer, E.; Burkhard, P.; Nahory, A.; Magistris, M.; Sanches, J.; Myers, P.; Paccolat, F.; Quoëx, F.; Gabriel, V.; Perrin, L.; Mermillod, B.; Gauthier, G.; Waldvogel, F. A.; Hirshel, B. *N. Engl. J. Med.* **1990**, *323*, 864.
(22) Gartner, S.; Markovits, P.; Markovits, D. M.; Kaplan, M. H.; Gallo, R. C.; Popovic, M. *Science* **1986**, *233*, 215.
(23) Koenig, S.; Gendelman, H. E.; Orenstein, J. M.; Dal Canto, M. C.; Pezeshpour, G. H.; Yungbluth, M.; Janotta, F.; Aksamit, A.; Martin, M. A.; Fauci, A. S. *Science* **1986**, *233*, 1089.
(24) Watkins, B. A.; Dorn, H. H.; Kelly, W. B.; Armstrong, R. C.; Potts, B. J.; Michaels, F.; Kufta, C. V.; Dubois-Dalcq, M. *Science* **1990**, *249*, 549.
(25) Reference deleted in press.
(26) Reference deleted in press.
(27) Reference deleted in press.
(28) Reference deleted in press.
(29) Chu, C. K.; Ullas, G. V.; Jeong, L. S.; Ahn, S. K.; Doboszewski, B.; Lin, Z. K.; Beach, J. W.; Schinazi, R. F. *J. Med. Chem.* **1990**, *33*, 1553.
(30) Kalckar, H. M. *J. Biol. Chem.* **1947**, *167*, 477.
(31) Imada, A.; Igarashi, S. *J. Bacteriol.* **1967**, *94*, 1551.
(32) Ott, J. L.; Werkmann, C. H. *Arch. Biochem. Biophys.* **1954**, *48*, 483.
(33) Ott, J. L.; Werkmann, C. H. *Arch. Biochem. Biophys.* **1957**, *69*, 264.
(34) Yokozeki, K.; Yamanaka, S.; Utagawa, T.; Takinami, K.; Hirose, Y.; Tanaka, A.; Sonomoto, K.; Fukui, S. *Appl. Microbiol. Biotechnol.* **1982**, *14*, 225–231.
(35) Utagawa, T.; Morisawa, H.; Yoshinaga, F.; Yamazaki, A.; Mitsugi, K.; Hirose, Y. *Agric. Biol. Chem.* **1985**, *49*, 1053.
(36) (a) Shiragami, H.; Irie, Y.; Shirae, H.; Yokozeki, K.; Yasuda, N. *J. Med. Chem.* **1988**, *53*, 5170–5173. (b) Carson, D. A.; Wasson, D. B. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 829–834. (c) Shirae, H.; Kobayashi, K.; Shiragami, H.; Irie, Y.; Yasuda, N.; Yokozeki, K. *Appl. Environ. Microbiol.* **1989**, *55*, 419–424.

ribosyl group to modified bases, in particular 6-halopurines, has not been reported. Our observations described here should provide additional structure–activity relationships useful in developing therapeutic purine nucleosides.

## Chemistry

2′,3′-Dideoxypurine nucleosides (1–8, 11–14) were synthesized through a bacterial transglycosylation reaction mediated by nucleoside phosphorylases derived from intact *E. coli* JA-300 cells[37] (Scheme I). In addition two dideoxypurine nucleoside analogues (9, 10) were chemically synthesized (Scheme II) from enzymatically synthesized 6-halo-2′,3′-dideoxynucleoside precursors. In preliminary studies, eight bacterial strains (*E. coli* JA-300, IAM-1239, IAM-1264, IAM-12119, and JC-411; *Klebsiella pneumoniae* IFO-3321; *Erwinia hebicola* IFO-12686; and *Enterobactor aerogenes* IFO-13534) were tested for their capacity to produce 2′,3′-dideoxyadenosine (ddA) through *N*-ribosyl transfer reaction between 2′,3′-dideoxyuridine (ddU) and adenine, and JA-300 was chosen as the best source of the nucleoside phosphorylases. ddU was used as a common starting material in the *E. coli* mediated biosynthesis of ddP analogues described in this report, since the synthesis of ddU is readily adapted to a large-scale production and the cost of uridine as a raw material is relatively low.

## In Vitro Antiviral Activity against HIV-1

All four 2-amino-6-halo-ddPs (1–4) exerted a potent antiretroviral activity against HIV-$1_{IIIB}$ in vitro in the HIV cytopathic effect inhibition assay with respective $ED_{50}$ values of 2.4, 5.5, 6.9, and 7.9 μM (Table I). Three of the 6-halo-ddPs (5–7) also showed a significant antiviral activity against HIV-$1_{IIIB}$ with respective $ED_{50}$ values of 2.8, 6.9, and 7.7 μM, although these three compounds were substantially more suppressive of cell growth than the reference compounds ddI (15) and ddG (16). Among the ddPs tested, compounds substituted with a fluorine, chlorine, or bromine atom at the 6-position were generally less toxic to cell growth as compared to those substituted with an iodine. Two 6-mercapto-ddPs (9, 10), 2,6-dichloro-ddP (11), 2-amino-ddP (12), 2′,3′-dideoxyxanthosine (13), and 2′,3′-dideoxynebularine (14) were not active against HIV under the conditions used.

Compounds 1, 2, 5, and 6 were further tested for their in vitro antiviral activity against HIV-$2_{LAV2}$, another virus strain which can cause a clinical syndrome resembling AIDS,[38] and a pair of AZT-sensitive and AZT-resistant HIV-1 strains, which were isolated from one patient before and after the AZT therapy, respectively.[39] As in the assays for activity against HIV-$1_{IIIB}$, 1, 2, 5, and 6 were found to be potent inhibitors of HIV-$2_{LAV2}$ in vitro (Table I). Three compounds, 1, 2, and 5, were also tested against AZT-sensitive and -resistant HIV-1 strains (HIV-$1_{pre018}$ and HIV-$1_{post018}$, respectively) and found to be active against both HIV-1 strains at concentrations of 20 and 50 μM (Figure 1).

## Partition Coefficients

Antiretroviral drugs with a high degree of lipophilicity may theoretically possess an enhanced ability to penetrate the blood–brain barrier and may efficiently inhibit infectivity and replication of HIV-1 in the CNS. We, therefore, determined an *n*-octanol–water partition coefficient (*P*) for each drug by a micro shake-flask prodecure.[40] All eight 6-halo-substituted ddPs (1–8) had substantially higher octanol–water partition coefficients than the reference purine dideoxynucleosides ddI and ddG (15, 16, respectively) (Table II). Six of the 6-halo-substituted ddPs (2–4, 6–8) had higher log *P* values than AZT, which has been proven to penetrate relatively well into the cerebrospinal fluid in patients with HIV-infection.[41,42]

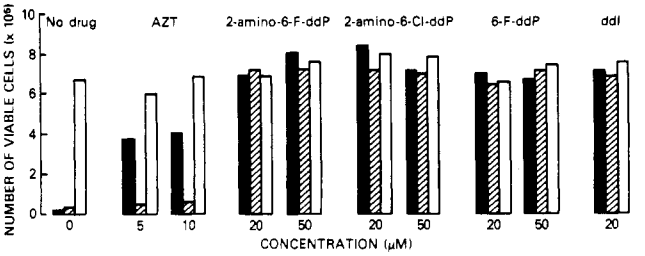



**Figure 1.** Activity of 1, 2, and 5 against an AZT-resistant HIV variant in vitro. Susceptible $CD4^+$ ATH8 cells (200 000) were exposed to an AZT-sensitive HIV isolate (HIV-$1_{pre018}$; closed column) or AZT-resistant HIV isolate (HIV-$1_{post018}$; hatched column), which were derived from a patient before therapy and after more than 6 months of AZT therapy, respectively (both kind gifts from Dr. D. D. Richman),[39] at a 1000 $TCID_{50}$ viral dose. The ATH8 cells were cultured in the continuous presence of 1, 2, and 5 (20 and 50 μM). Control cells were similarly treated with compound but not exposed to the virus (open column). The number of viable ATH8 cells were counted on day 7 of culture by using the trypan blue dye exclusion method. Each drug was tested for antiviral activity more than three times with a wide range of concentrations. Data shown represent the most representative results. The activity of ddI (20 μM) against both HIV-1 strains is shown as a reference.

**Table II.** Partition Coefficients (*P*) of 6-Halo-Substituted 2′,3′-Dideoxypurine Nucleoside Analogues[a]

| compound | *P* | *P* ratio[b] |
|---|---|---|
| 1 (2-amino-6-fluoro-ddP) | 0.89 ± 0.01 | 11.0 |
| 2 (2-amino-6-chloro-ddP) | 1.62 ± 0.04 | 20.0 |
| 3 (2-amino-6-bromo-ddP) | 2.17 ± 0.04 | 26.8 |
| 4 (2-amino-6-iodo-ddP) | 3.33 ± 0.06 | 41.1 |
| 16 (ddG) | 0.08 ± 0.01 | |
| 5 (6-fluoro-ddP) | 0.99 ± 0.01 | 17.4 |
| 6 (6-chloro-ddP) | 1.72 ± 0.03 | 30.1 |
| 7 (6-bromo-ddP) | 2.25 ± 0.03 | 39.5 |
| 8 (6-iodo-ddP) | 3.35 ± 0.09 | 58.6 |
| 15 (ddI) | 0.05 ± 0.01 | |
| 17 (AZT) | 1.12 ± 0.02 | |

[a] Measured for 1-octanol and 0.01 M potassium phosphate buffer (pH 7.0) as described in the Experimental Section. [b] *P* ratio = (*P* value of the 6-halo-substituted ddP)/(*P* value of the corresponding ddI or ddG).

## Adenosine Deaminase Activation

Since several 2-amino-6-halopurine and 6-halopurine ribofuranosides are known to be hydrolyzed by adenosine deaminase (ADA),[43,44] it was likely that 6-halo-substituted

(37) Tschumper, G.; Carbon, J. *Gene* **1980**, *10*, 157.
(38) Barré-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, T.; Charmaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vésinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868.
(39) Larder, B. A.; Darby, G.; Richman, D. D. *Science* **1989**, *243*, 1731.
(40) Ford, H., Jr.; Merski, C. L.; Kelley, J. A. *Abstracts of Papers*; 41st Pittsburgh Conference, New York, NY; The Pittsburgh Conference: Pittsburgh, PA; Abstract 1174, 1990.
(41) Yarchoan, R.; Berg, G.; Brouwers, P.; Fischl, M. A.; Spitzer, A. R.; Wichman, A.; Grafman, J.; Thomas, R. V.; Safai, B.; Brunetti, A.; Perno, C. F.; Schmidt, P. J.; Larson, S. M.; Myers, C. E.; Broder, S. *Lancet* **1987**, *i*, 132.
(42) Pizzo, P. A.; Eddy, J.; Balis, F. M.; Murphy, R. F.; Moss, H.; Wolters, P.; Brouwers, P.; Jarosinski, P.; Rubin, M.; Broder, S.; Yarchoan, R.; Brunetti, A.; Maha, M.; Nusinoff-Lehrman, S.; Poplack, D. G. *N. Engl. J. Med.* **1988**, *319*, 889.
(43) Chassy, B. M.; Suhadolnick, R. J. *J. Biol. Chem.* **1967**, *242*, 3655.

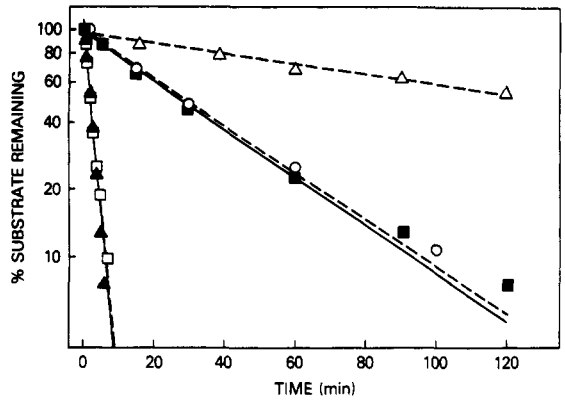



**Figure 2.** Enzymatic hydrolysis of 2-amino-6-halo-ddPs by adenosine deaminase. ADA was incubated with equimolar solutions of 1 (open square), 2 (closed square), 3 (open circle), 4 (open triangle), or ddA (closed triangle) as described in the Experimental Section. The decrease in substrate concentration was determined at the indicated time points by HPLC analysis. Data shown were generated in consecutive individual determinations using an identical enzyme preparation. Similar results were obtained in a duplicate analysis.

ddPs would also be substrates for this enzyme. This was indeed the case as can be seen in Figure 2, which depicts the ADA enzyme kinetics for four 2-amino-6-halo-ddPs (1–4) in the presence of an excess of isolated ADA. In the case of 1–4, ddG was formed at a rate which corresponded to substrate disappearance, while ddI was produced (data not shown) upon enzymatic hydrolysis of 5–8. 2-Amino-6-fluoro-ddP (1) showed essentially the same rate as did ddA (Figure 2). When the kinetic experiments were performed in RPMI-1640 culture media containing 15% fetal calf serum, the 2-amino-6-halo-ddPs were still hydrolyzed to ddG, but at a rate that was ca. 60 times slower than the rate in the presence of an excess of isolated enzyme. For example, 1 had a ca. 2 h half-life in the culture media, as did ddA.

We then asked if 1, 2, and 5 could have antiviral activity against HIV-1 in the presence of 2'-deoxycoformycin (2'-dCF), the potent inhibitor of ADA. We found that all these compounds lost their antiviral activity in the presence of 2'-dCF and essentially all the target ATH8 cells were destroyed by the virus (Figure 3). Taking data shown in Figure 2 and Table I into consideration, it appears that the better substrates for ADA possess better anti-HIV activity. This would further support the interpretation that 6-halo-substituted ddP analogues exert their antiviral activity against HIV upon conversion to ddG or ddI.

## Discussion

One of the devastating features of HIV infection is in neurological abnormalities.[20,21] A recent controlled study of neurologic changes in men with asymptomatic HIV infection has revealed that abnormalities detected by electrophysiologic tests including electroencephalography are far more common in asymptomatic carriers of HIV than in controls ($P < 0.00005$) and tend to progress over time.[21] While the pathogenesis of the HIV-caused neurological abnormalities is as yet incompletely understood, such a disorder may be directly linked to structural and functional changes due to infection and/or replication of HIV in the CNS, particularly in cells of a monocyte/macrophage lineage.[22-24] In this regard, HIV-associated neurological disorders in both adults and children with

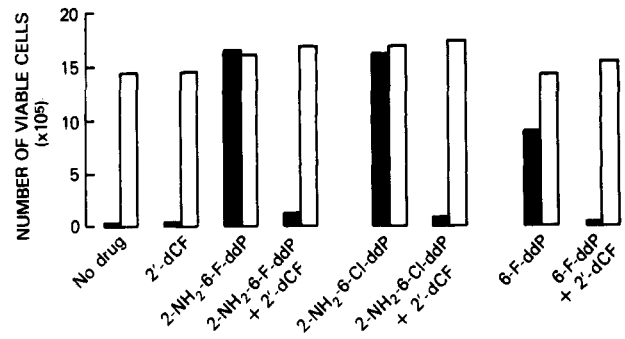



**Figure 3.** Effects of 2'-deoxycoformycin (dCF) on in vitro antiretroviral activity of 6-halo-substituted ddPs. ATH8 cells were preincubated with or without 5 μM dCF, exposed to HIV-$1_{IIIB}$ (closed column), and cultured with or without 50 μM of 1, 2, or 5 in the continuous presence of 5 μM dCF. On day 6, the total viable cells were counted as described in the legend to Figure 1. Control ATH8 cells (open column) were treated similarly but not exposed to the virus.

AIDS or ARC have been substantially improved during therapy with AZT.[41,42] These relatively prompt improvements of neurological abnormalities in some patients following therapy with AZT[41,42] may represent the effect of antiretroviral activity of the drug in the CNS. Thus, lipophilicity of dideoxynucleosides could constitute an important feature of antiretroviral therapies against HIV infection, while it should be stressed that the lipophilicity of a given drug may not necessarily determine its CNS-penetration potential, and improved CNS penetration may not necessarily improve the therapeutic index of the drug.

The 6-halo versions of ddPs are of interest in view of structure/activity relationships, since the substitution with a halogen atom confers substantial lipophilicity on ddP analogues with a retention of antiretroviral activity. In this regard, 6-halo-ddP analogues appear to exert antiviral activity only upon conversion to ddG or ddI. This is intriguing in view that there is still no reliable algorithm for predicting which congeners will exert more antiretroviral activity against HIV or less toxicity to target cells. For example, replacement of the aromatic oxygen of ddI by a hydrogen, generating 2',3'-dideoxypurine ribofuranoside, or 2',3'-dideoxynebularine (14), negates the potent antiretroviral activity of ddI (Table I). The same replacement in ddG, generating 2-amino-2',3'-dideoxypurine ribofuranoside (12), also abolishes the antiretroviral activity of ddG (Table I). We also find that two 6-mercapto analogues (9, 10) exert no antiretroviral activity in vitro (Table I). The lack of antiretroviral activity for 9 and 10 may be because these analogues are not converted to ddG or ddI. Indeed, 2-amino-6-mercapto-ddP is not a substrate for ADA and is not converted to ddG at all in the presence of an excess of isolated ADA.[45]

The enzymatic method we have exploited for the synthesis of 6-halo-substituted ddPs has several advantages. (1) Its reaction steps are simple and less labor-intensive than conventional chemical methods. (2) Product purification is straightforward. (3) Reactive functional groups (e.g. iodo) can be handled easily. (4) Minimal byproducts are formed such as 2'- or 3'-deoxynucleosides or the α-anomer of 2',3'-dideoxynucleosides.

Most ddP analogues are synthesized with yields in a range of 20–40% in the current enzymatic system. However, compounds 11 and 13 are obtained in substantially lower yields (9.7% and 5%, respectively). This is in part because the starting materials, 2,6-dichloropurine and

(44) Baer, H. P.; Drummond, G. I.; Gillis, G. *Arch. Biochem. Biophys.* **1967**, *123*, 172.

(45) Ford, H., Jr.; Kelley, J. A.; Mitsuya, H., unpublished data.

xanthosine (xanthine was not used since it was even more insoluble), respectively, are used in emulsion since they are much less soluble in the buffer than other starting materials. It is worth stressing, however, that unused starting materials can be recovered simply by centrifugation and can be used for the next reaction.

Although only free cells were employed for the trans-dideoxyribosylation in the current work, the present enzymatic method has already been adapted for large-scale production of the 6-halo-substituted nucleoside analogues described here by immobilizing JA-300 cells with strontium arginate gel in a bioreactor.[46] Use of immobilized *E. coli* offers advantages: (1) enzymes can be used repeatedly; (2) continual nucleoside synthesis is possible for at least 1 month; (3) the purity of the products is higher with less labor, in part because of the ease in separating cells and cell-derived products; and (4) enzymatic reactions can be carried out in buffers containing organic solvents such as dimethyl sulfoxide (DMSO). It should be noted, however, that long term use tends to limit the enzymatic utility to about 1 month.

In summary, some of the 2-amino-6-halo-ddPs and 6-halo-ddPs represent a new class of lipophilic prodrugs for ddG and ddI, respectively. In particular, six of these compounds (**1–6**) possess a virtually equivalent antiretroviral activity to that of ddG and ddI and appear to be active against a wide range of HIV strains. Our current observations may also offer a new strategy to develop lipophilic purine nucleoside derivatives for other clinical applications.

## Experimental Section

Melting points were determined on a Yanaco melting point apparatus and were not corrected. $^1H$ and $^{13}C$ NMR spectra were recorded on a JEOL FX60Q instrument. Proton chemical shifts are expressed as $\delta$ values with reference to $Me_4Si$. $^{13}C$ chemical shifts are expressed as $\delta$ values with reference to 2,2-dimethyl-2-silapentane-5-sulfonate. UV spectra were recorded with a Hitachi instrument Model 150-20 spectrophotometer. Positive ion fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS-AX505H double-focusing mass spectrometer. The *E. coli* mediated biosynthesis as well as chemical synthesis of ddP analogues were monitored by high-performance liquid chromatography (HPLC). Thin-layer chromatography with E. Merck 60F 254 precoated silica gel plates was also carried out for monitoring. The chemical synthesis and in vitro anti-HIV activity of compounds **6**, **10**, and **14** have been published elsewhere.[29] ddA, ddI, and ddG were provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, while 3′-azido-3′-deoxythymidine (AZT) was purchased from Sigma Chemical Co. 2-Amino-6-chloropurine and 2-amino-6-iodopurine were purchased from Sigma Chemical Co. 2-Amino-6-fluoropurine, 2-amino-6-bromopurine, and 2′,3′-dideoxyuridine (ddU) were synthesized by published methods.[47-49]

Under the conditions of the *E. coli* mediated biosynthesis described below, the activity of ADA inherent to *E. coli* was substantially diminished at 50 °C. For example, when adenine and ddU were incubated in a reaction mixture containing JA-300 cells in pH 6.5, 50 mM phosphate buffer for 3 h at 50 °C, the yield of ddA was 71%, while 3% ddA was found to be converted to ddI. Following immobilization of JA-300 cells using strontium arginate gel, the activity of ADA was further diminished.[46] Under the same conditions described above but with immobilized cells, the yield of ddA was 50.1%, while only 0.1% ddI was recovered.

**HPLC Analysis of Dideoxypurine Nucleoside Analogues.** The synthesis of various 2′,3′-dideoxynucleoside analogues was monitored with a Waters Associates Model 411 Liquid Chromatograph. A TSK-ODS-80 TM column (4.6 mm diameter, 150 mm long) protected by a TSK guard gel ODS-80TM was eluted with 3% $CH_3CN$ in pH 7.0 potassium phosphate buffer at a flow rate of 1.0 mL/min. The products were detected by UV absorbance at 254 nm and the chromatogram was recorded and integrated with a Shimadzu CR-6A Chromatopac data processor.

**Antiviral Test Procedures.** The HIV cytopathic effect inhibition assay was performed as previously described.[4,5] Briefly, target $CD4^+$ ATH8 cells ($2 \times 10^5$) were exposed to a $4.2 \times 10^3$ 50% tissue culture infectious dose ($TCID_{50}$) of HIV-$1_{IIIB}$ per cell (1000 viral particles per cell), a $1 \times 10^3$ $TCID_{50}$ dose of HIV-$1_{pre018}$ (100 virus particles per cell) or HIV-$1_{post018}$ (160 virus particles per cell),[39] or a $7.7 \times 10^3$ $TCID_{50}$ dose of HIV-$2_{LAV2}$ per cell (10 viral particles per cell) for 1 h and resuspended in 2 mL of fresh complete medium (RPMI 1640 supplemented with 4 mM L-glutamine, 15% undialyzed and heat-inactivated fetal calf serum, 50 units/mL of penicillin, and 50 $\mu$g/mL of streptomycin) containing 15% (v/v) interleukin-2 (IL-2, lectin-depleted; Advanced Biotechnologies Inc., Silver Spring, MD) and 50 units/mL of recombinant IL-2 (Amgen, Thousand Oaks, CA) in the presence or absence of various concentrations of drugs. The drugs were added only once throughout the experiment. The ATH8 cells were then incubated at 37 °C at 5% $CO_2$ containing humidified air. Control cells were treated similarly but were not exposed to the virus. On day 7, viable cells were counted in a hemocytometer under the microscope by the trypan blue dye exclusion method. Each drug was tested for antiviral activity more than three times with a wide range of concentrations. Variability in cell number determination is ±10% of the value shown.[4]

**Octanol–Water Partition Coefficients.** Octanol–water partition coefficients were determined by a micro-flask method, as previously described.[40] Briefly, octanol-saturated, pH 7.0, 0.01 M potassium phosphate buffer containing 10 $\mu$g of a test compound (1 mL) was mixed with 1.0 mL of buffer-saturated 1-octanol. Partitioning was performed with a 2-mL Lidex Mixxor apparatus. The phases were separated and centrifuged, and the relative concentration of sample in each phase was determined by HPLC analysis. Reported partition coefficients are the means of at least three independent determinations.

**Enzymatic Hydrolysis.** Relative rates of hydrolysis of ddPs by ADA and characterization of their products were determined as previously described.[50] Briefly, 0.1 unit of ADA (10 $\mu$L) was added to a 400 $\mu$M solution of a given compound in 0.01 M Tris buffer (pH 7.5) and incubated at 37 °C. A 20-$\mu$L aliquot was then withdrawn and quenched by mixing with 1.0 mL of 2 $\mu$M 2′-deoxycoformycin. The diluted sample was ultrafiltered to remove enzyme. The decrease in substrate concentration and the formation of product was followed over time by HPLC analysis of 100-$\mu$L aliquots from each sample. A 4.6 × 250 mm 5-mm Ultrasphere-ODS column, protected by a Waters guard column packed with 37–50 $\mu$m Vydac 201SC, was eluted isocratically with 10–20% $CH_3CN$ in 0.01 M pH 7.0 phosphate buffer at a flow rate of 1.0 mL/min. 2′,3′-Dideoxynucleoside analogues were detected at the appropriate wavelength of maximum absorption with a Gilson 116 variable-wavelength detector. Peak identity was determined from coincidence of retention times with standards and by comparison of on-the-fly UV spectra obtained with a Perkin-Elmer LC-235 diode-array detector. Peak areas and peak heights were measured simultaneously on a Spectra-Physics SP4200 computing integrator. Concentration vs time data were then fit to either first-order exponential decay or appearance equations using Graph Pad, a personal computer based curve-fitting program.

**2-Amino-6-fluoro-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (1).** 2-Amino-6-fluoropurine (0.457 g, 3.0 mmol), ddU (0.637 g, 3.0 mmol), and free JA-300 cells (13.2 g) which served as a source of nucleoside phosphorylases were mixed and incubated for 3 h in 0.05 M potassium phosphate buffer (pH 6.5) at 50 °C. At the end of the reaction, the mixture was centrifuged at 8000 rpm for 20 min at 10 °C. The supernatant

(46) Murakami, K.; et al. unpublished data.
(47) Kiburis, J.; Lister, J. H. *J. Chem. Soc. C* **1971**, 3942.
(48) Beaman, G. A.; Gerster, J. F.; Robins, R. K. *J. Org. Chem.* **1962**, *27*, 986.
(49) Furukawa, Y.; Yoshioka, Y.; Imai, K.; Honjo, M. *Chem. Pharm. Bull.* **1970**, *18*, 554.
(50) Marquez, V. E.; Tseng, C. K. H.; Mitsuya, H.; Aoki, S.; Kelley, J. A.; Ford, H.; Roth, J. S.; Broder, S.; Driscoll, J. S. *J. Med. Chem.* **1990**, *33*, 978.

was collected, chromatographed on a DIAION HP-20 column containing 60 mL of HP-20 resin (Mitsubishi Kasei Co.), and eluted successively with water, 10% methanol, and 50% methanol. The 50% methanol fraction was evaporated to give 1 (164 mg, 21.7%): mp 138–140 °C; TLC $R_f$ ($CHCl_3$/methanol, 9/1) 0.58; $\lambda_{max}$ (0.01 N NaOH) 245 ($\epsilon$ 7970), 284 nm ($\epsilon$ 6530); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.74–2.70 (m, 4 H, H-2' and -3'), 3.43–3.79 (m, 2 H, H-5'), 3.79–4.35 (m, 1 H, H-4'), 4.96 (t, 1 H, OH), 6.14 (t, 1 H, H-1), 6.93 (bs, 2 H, $NH_2$), 8.36 (s, 1 H, H-8); FAB-HRMS (high resolution) ($m/z$) calcd for $C_{10}H_{13}O_2N_5F$ ($MH^+$) 254.1053, measured 254.1057. Anal. ($C_{10}H_{12}O_2N_5F \cdot 0.25H_2O$) C, H, N, F.

**2-Amino-6-chloro-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (2).** 2-Amino-6-chloropurine (3.39 g, 20.0 mmol), ddU (4.24 g, 20.0 mmol), and JA-300 cells (85 g) were incubated for 3 h in 0.05 M potassium phosphate buffer (pH 6.5) at 50 °C, followed by centrifugation. The supernatant was chromatographed on a DIAION HP-20 column and eluted with water, 20% methanol, and 50% methanol. The 50% methanol fraction was evaporated and **2** was produced as white crystals (970 mg, 3.6 mmol, 18%) by recrystallization from water: mp ($H_2O$) 138–140 °C; TLC $R_f$ ($CHCl_3$/methanol, 9/1) 0.58; $\lambda_{max}$ (0.001 N NaOH) 222 ($\epsilon$ 27 310), 248 ($\epsilon$ 8800), 307 nm ($\epsilon$ 9460); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.74–2.67 (m, 4 H, H-2' and H-3'), 3.44–3.73 (m, 2 H, H-5'), 3.86–4.34 (m, 1 H, H-4'), 4.97 (t, 1 H, OH), 6.13 (t, 1 H, H-1'), 6.95 (bs, 2 H, $NH_2$), 8.39 (s, 1 H, H-8); FAB-HRMS ($m/z$) calcd for $C_{10}H_{13}O_2N_5Cl$ ($MH^+$) 270.0758, found 270.0718. Anal. ($C_{10}H_{12}O_2N_5Cl \cdot 0.25H_2O$) C, H, N, Cl.

**2-Amino-6-bromo-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (3).** 2-Amino-6-bromopurine (3.98 g, 18.6 mmol), ddU (3.95 g, 18.6 mmol), and JA-300 cells (93 g) were incubated for 3 h in 0.05 M potassium phosphate buffer (pH 6.5) at 50 °C, followed by centrifugation. The supernatant was chromatographed on a DIAION HP-20 column and eluted with water, 20% methanol, and 50% methanol. The 50% methanol fraction was treated with activated charcoal and evaporated to give partially purified **3**. Pale yellow crystals (1.25 g, 3.98 mmol, 21.3%) were produced after recrystallization from water: mp 137–142 °C; TLC $R_f$ ($CHCl_3$/methanol, 9/1) 0.62; $\lambda_{max}$ (0.001 N NaOH) 221 ($\epsilon$ 29 740), 249 ($\epsilon$ 9940), 319 nm ($\epsilon$ 10 330); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.73–2.67 (m, 4 H, H-2' and H-3'), 3.43–3.79 (m, 2 H, H-5'), 3.81–4.34 (m, 1 H, H-4'), 4.95 (t, 1 H, OH), 6.11 (t, 1 H, H-1'), 6.94 (bs, 2 H, $NH_2$), 8.38 (s, 1 H, H-8); FAB-HRMS ($m/z$) calcd for $C_{10}H_{13}O_2N_5Br$ ($MH^+$) 314.0253, found 314.0193. Anal. ($C_{10}H_{12}O_2N_5Br$) C, H, N, Br.

**2-Amino-6-iodo-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (4).** 2-Amino-6-iodopurine (4.43 g, 17.0 mmol), ddU (3.61 g, 17.0 mmol), and JA-300 cells (76 g) were incubated for 3 h in 0.05 M potassium phosphate buffer (pH 6.5) at 50 °C, followed by centrifugation. The supernatant was then chromatographed on a DIAION HP-20 column and eluted with water, 20% methanol, and 50% methanol. The 50% methanol fraction was evaporated to give **4**. White crystals (2.0 g, 5.5 mmol, 32.3%) were produced after two recrystallizations from water: mp 143–146 °C; TLC $R_f$ ($CHCl_3$/methanol, 9/1) 0.65; $\lambda_{max}$ (0.001 N NaOH) 223 ($\epsilon$ 28 110), 249 ($\epsilon$ 13 860), 312 nm ($\epsilon$ 10 950); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.72–2.63 (m, 4 H, H-2' and H-3'), 3.38–3.72 (m, 2 H, H-5'), 3.79–4.30 (m, 1 H, H-4'), 4.94 (t, 1 H, OH), 6.01 (t, 1 H, H-1'), 6.84 (bs, 2 H, $NH_2$), 8.33 (s, 1 H, H-8); FAB-HRMS ($m/z$) calcd for $C_{10}H_{13}O_2N_5I$ ($MH^+$) 362.0114, found 362.0065. Anal. ($C_{10}H_{12}O_2N_5I$) C, H, N, I.

**6-Fluoro-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (5).** 6-Fluoropurine (0.456 g, 3.3 mmol) and ddU (0.70 g, 3.3 mmol) were added to 165 mL of pH 6.5, 0.05 M potassium phosphate buffer. To the mixture was added 16 g of live *E. coli* JA-300 cells and the combined reaction mixture was incubated for 4 h at 50 °C, followed by centrifugation. The supernatant was collected and chromatographed on a DIAION HP-20 column and eluted with water, 20% methanol, and 100% methanol. The fraction of 100% methanol was concentrated, purified by silica gel column chromatography (ethyl acetate), and the eluent treated with activated charcoal to give **5** (0.18 g, 0.76 mmol, 23%): mp ($CH_3COOC_2H_5$) 109–112 °C; TLC $R_f$ ($CHCl_3$/methanol, 95/5) 0.40; $\lambda_{max}$ ($H_2O$) 250 nm ($\epsilon$ 6720); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.80–2.76 (m, 4 H, H-2' and H-3'), 3.44–3.76 (m, 2 H, H-5'), 3.99–4.40 (m, 1 H, H-4'), 4.99 (t, 1 H, OH), 6.41 (t, 1 H, H-1'), 8.71 (s, 1 H, H-8), 8.92 (s, 1 H, H-2); FAB-HRMS ($m/z$) calcd for $C_{10}H_{12}O_2N_4F$ ($MH^+$) 239.0944, found 239.0987. Anal. ($C_{10}H_{11}O_2N_4F$) C, H, N.

**6-Chloro-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (6).** 6-Chloropurine (1.78 g, 11.5 mmol) and ddU (2.44 g, 11.5 mmol) were added to 230 mL of pH 6.5, 0.05 M potassium phosphate buffer. To the mixture, 50 g of pelleted JA-300 cells were added and incubated for 2 h at 50 °C. Following removal of the cells, the supernatant was chromatographed on a DIAION HP-20 column and eluted with water, 10% methanol, and 50% methanol. The 50% methanol fraction was treated with the activated charcoal and concentrated by evaporation to give crude **6**. Recrystalization from ethyl acetate gave 0.955 g (3.7 mmol, 32.6%) of white crystals: mp ($CH_3COOC_2H_5$) 104–105 °C; TLC $R_f$ ($CHCl_3$/methanol, 95/5) 0.45; $\lambda_{max}$ ($H_2O$) 265 nm ($\epsilon$ 9810); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.82–2.76 (m, 4 H, H-2' and H-3'), 3.41–3.78 (m, 2 H, H-5'), 3.91–4.40 (m, 1 H, H-4'), 5.00 (t, 1 H, OH), 6.39 (t, 1 H, H-1'), 8.79 (s, 1 H, H-8), 8.94 (s, 1 H, H-2); FAB-MS ($m/z$) 255 ($MH^+$). Anal. ($C_{10}H_{11}O_2N_4Cl \cdot 0.5H_2O$) C, H, N, Cl.

**6-Bromo-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (7).** 6-Bromopurine (1.79 g, 9.0 mmol), ddU (1.91 g, 9.0 mmol), and JA-300 cells (40 g) were mixed and incubated at 50 °C for 3 h in pH 6.5, 0.05 M potassium phosphate buffer, followed by centrifugation. The supernatant was chromatographed on a DIAION HP-20 column and eluted with water, 20% methanol, and 40% methanol. The fraction of 40% methanol was treated with activated charcoal and concentrated by evaporation to give **7**. Pure **7** was produced by recrystallization from $CH_3COOC_2H_5$ to give white crystals (0.572 g, 1.9 mmol, 21.2%): mp ($CH_3COOC_2H_5$) 106–108 °C; TLC $R_f$ ($CHCl_3$/methanol, 95/5) 0.46; $\lambda_{max}$ ($H_2O$) 267 nm ($\epsilon$ 9920); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.80–2.76 (m, 4 H, H-2' and H-3'), 3.42–3.75 (m, 2 H, H-5'), 3.90–4.40 (m, 1 H, H-4'), 4.98 (t, 1 H, OH), 6.38 (t, 1 H, H-1'), 8.73 (s, 1 H, H-8), 8.93 (s, 1 H, H-2); FAB-HRMS ($m/z$) calcd for $C_{10}H_{12}O_2N_4Br$ ($MH^+$) 299.0144, found 299.0108. Anal. ($C_{10}H_{11}O_2N_4Br \cdot 0.5H_2O$) C, H, N.

**6-Iodo-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (8).** 6-Iodopurine (1.5 g, 6.1 mmol), ddU (1.3 g, 6.1 mmol), and JA-300 cells (28.5 g) were mixed and incubated for 3 h in pH 6.5, 0.05 M potassium phosphate buffer at 50 °C. Following centrifugation, the supernatant was collected and chromatographed on a DIAION HP-20 column and eluted with water, 30% methanol, 60% methanol, and 80% methanol. The 60% and 80% methanol fractions were mixed and evaporated to give the crude title compound. The compound was recrystallized from ethanol to yield 0.458 g (1.3 mmol) of white needlelike crystals of **8** (21.6%): mp (ethanol) 108–111 °C; TLC $R_f$ ($CHCl_3$/methanol, 95/5) 0.46; $\lambda_{max}$ ($H_2O$) 276 nm ($\epsilon$ 11 650); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.73–2.74 (m, 4 H, H-2' and H-3'), 3.43–3.75 (m, 2 H, H-5'), 3.92–4.38 (m, 1 H, H-4'), 4.98 (t, 1 H, OH), 6.35 (t, 1 H, H-1'), 8.63 (s, 1 H, H-8), 8.89 (s, 1 H, H-2); FAB-MS ($m/z$) 347 ($MH^+$). Anal. ($C_{10}H_{11}O_2N_4I$) C, H, N, I.

**2-Amino-6-mercapto-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (9).** A mixture of **2** (0.27 g, 1 mmol) and sodium hydrosulfide (1.0 g, 17.8 mmol) in 13.2 mL of distilled water/ethanol (2/1) was refluxed for 2 h. The reaction mixture was cooled to room temperature and further cooled to 0 °C in an ice/water bath. The solution was neutralized to pH 8 with 10% aqueous acetic acid and then stirred for several hours in the ice/water bath. After the pH of the solution had stabilized between 7 and 8, the solvent was removed in vacuo (20 mmHg, 50 °C). The residue was purified by silica gel column chromatography using methanol/chloroform (1/9) as eluent to yield the title compound as a white crystalline solid (0.23 g, 86.8%) after recrystalization from methanol/chloroform (1/4): mp 201–203 °C; TLC $R_f$ ($CHCl_3$/methanol, 9/1) 0.18; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.73–2.72 (m, 4 H, H-2' and H-3'), 3.36–3.74 (m, 2 H, H-5'), 3.82–4.43 (m, 1 H, H-4'), 4.97 (t, 1 H, OH), 6.02 (t, 1 H, H-1'), 6.81 (bs, 2 H, $NH_2$), 8.14 (s, 1 H, H-8), 11.96 (bs, 1 H, SH); FAB-HRMS ($m/z$) calcd for $C_{10}H_{14}O_2N_5S$ ($MH^+$) 268.3197, found 268.3173. Anal. ($C_{10}H_{13}O_2N_5S$) C, H, N, S.

**6-Mercapto-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (10).** A mixture of **6** (0.25 g, 1 mmol) and sodium hydrosulfide (1.0 g, 17.8 mmol) in 13.2 mL of distilled water/ethanol (2/1) was refluxed for 2 h. The reaction mixture was cooled to room temperature and further cooled to 0 °C in an ice/water bath. The solution was neutralized to pH 8 with 10% aqueous acetic acid and then stirred for several hours at 0 °C.

After the pH of the solution had stabilized between 7 and 8, the solvent was removed in vacuo (20 mmHg, 50 °C). The residue was purified by silica gel column chromatography using methanol/chloroform (1/9) as eluent to yield **10** as a white crystalline solid (0.20 g, 81%) after recrystalization from methanol/chloroform (1/4): mp 187–189 °C; TLC $R_f$ ($CHCl_3$/methanol, 9/1) 0.35; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.77–2.66 (m, 4 H, H-2′ and H-3′), 3.35–3.69 (m, 2 H, H-5′), 3.90–4.48 (m, 1 H, H-4′), 4.99 (bs, 1 H, OH), 6.24 (t, 1 H, H-1′), 8.21 (s, 1 H, H-8), 8.53 (s, 1 H, H-2), 13.7 (bs, 1 H, SH); FAB-HRMS ($m/z$) calcd for $C_{10}H_{13}O_2N_4S$ ($MH^+$) 253.3050, found 253.3032. Anal. ($C_{10}H_{12}O_2N_4S$) C, H, N, S.

**2,6-Dichloro-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (11).** 2,6-Dichloropurine (0.376 g, 2 mmol), ddU (0.424 g, 2 mmol), and JA-300 cells (9 g) were mixed and incubated for 3 h in pH 6.5, 0.05 M potassium phosphate buffer at 50 °C. At the end of the reaction the mixture was centrifuged at 8000 rpm for 20 min at 10 °C. The supernatant was chromatographed on a DIAION HP-20 column and eluted with water, 20% methanol, 40% methanol, and 80% methanol. The 80% methanol fraction was evaporated to yield **11** as a white crystalline solid (0.056 g, 9.7%) following recrystalization from methanol: mp 141–143 °C; TLC $R_f$ ($CHCl_3$/methanol, 95/5) 0.43; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.80–2.75 (m, 4 H, H-2′ and H-3′), 3.44–3.77 (m, 2 H, H-5′), 3.93–4.40 (m, 1 H, H-4′), 4.98 (t, 1 H, OH), 6.33 (t, 1 H, H-1′), 8.96 (s, 1 H, H-8); FAB-HRMS ($m/z$) calcd for $C_{10}H_{11}O_2N_4Cl_2$ ($MH^+$) 289.1602, measured 289.1584. Anal. ($C_{10}H_{10}O_2N_4Cl_2$) C, H, N, Cl.

**2-Amino-9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (12).** 2-Aminopurine (0.946 g, 7 mmol) and ddU (1.48 g, 7 mmol) were added to 140 mL of pH 6.5, 50 mM potassium phosphate buffer. To the mixture were added pelleted JA-300 cells (30 g), and the mixture was incubated for 2 h at 50 °C. The cells were removed by centrifugation at 8000 rpm for 20 min at 10 °C. The supernatant was chromatographed on a DIAION HP-20 column and eluted with water and 20% and 40% methanol. The fractions of 20% and 40% methanol were concentrated and purified by silica gel column chromatography ($CHCl_3$/methanol) to give **12** (0.727 g, 44%): mp 168–169 °C; TLC $R_f$ ($CHCl_3$/MeOH, 9/1) 0.5; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.77–2.78 (m, 4 H, H-2′, and H-3′), 3.42–3.69 (m, 2 H, H-5′), 3.78–4.29 (m, 1 H, H-4′), 4.92 (t, 1 H, OH), 6.13 (t, 1 H, H-1′), 6.51 (bs, 2 H, $NH_2$), 8.30 (s, 1 H, H-6), 8.57 (s, 1 H, H-8); FAB-MS ($m/z$) 236 ($MH^+$).

**2′,3′-Dideoxyxanthosine, 9-(2,3-Dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-xanthosine (13).** Xanthosine (4.26 g, 15 mmol; in emulsion) and ddU (3.18 g, 15 mmol) were suspended in 750 mL of pH 7.0, 0.05 M potassium phosphate buffer. The live *E. coli* JA-300 cells (150 g) were added and the reaction mixture was incubated for 4 h at 50 °C. The incubation mixture was then centrifuged at 8000 rpm for 20 min at 10 °C. The supernatant was chromatographed on a DIAION HP-20 column and eluted with water and 30% methanol. The fraction of 30% methanol was concentrated and purified by HPLC using TOSOH SC-8070 System. The purification process was monitored by a TOSOH UV-8070 P&P detector at 254 nm. A TSK gel ODS-120T column was eluted with 2% $CH_3CN$ in pH 7.0 potassium phosphate buffer at a flow rate of 30 mL/min. The products were detected at a wavelength of 254 nm. The fraction corresponding to **13** was concentrated and desalted by DIAION HP-20 column chromatography. **13** was eluted with 50% methanol and crystallized from water–ethanol (0.20 g, 5.3%): mp >378 °C dec; $\lambda_{max}$ (0.1 N NaOH) 290 nm ($\epsilon$ 8320); $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.52–2.60 (m, 4 H, H-2′ and H-3′), 3.46–3.80 (m, 2 H, H-5′), 3.81–4.37 (m, 1 H, H-4′), 5.04 (t, 1 H, OH), 6.13–6.44 (dd, 1 H, H-1′), 8.32 (s, 1 H, H-8), 10.9 (bs, 1 H, NH), 11.5 (bs, 1 H, NH); $^{13}C$ NMR ($D_2O$ + NaOD) $\delta$ 27.6 (C-3′), 35.2 (C-2′), 65.8 (C-5′), 85.2 (C-4′), 89.7 (C-1′), 109.5 (C-5), 143.5 (C-8), 161.7 (C-4), 163.6 (C-2), 164.5 (C-6); FAB-HRMS ($m/z$) calcd for $C_{10}H_{13}O_4N_4$ ($MH^+$) 253.0937, found 253.0900. Anal. ($C_{10}H_{12}O_4N_4 \cdot 0.5H_2O$) C, H, N.

**2′,3′-Dideoxynebularine, 9-(2,3-Dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purine (14).** Purine (0.84 g, 7 mmol), ddU (1.49 g, 7 mmol), and JA300 cells (32 g) were mixed and incubated for 4 h in pH 6.5, 0.05 M potassium phosphate buffer at 50 °C, followed by centrifugation. The supernatant was chromatographed on a DIAION HP-20 column and eluted with water and 100% methanol. The methanol fraction was evaporated and purified by silica gel column chromatography using methanol/chloroform (1/9) as eluent to yield the title compound as a white crystalline solid (0.66 g, 43%) after recrystallization from methanol: mp 157–159 °C; TLC $R_f$ ($CHCl_3$/methanol, 9/1) 0.60; $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.80–2.80 (m, 4 H, H-2′ and H-3′), 3.38–3.77 (m, 2 H, H-5′), 3.82–4.39 (m, 1 H, H-4′), 4.97 (t, 1 H, OH), 6.39 (t, 1 H, H-1′), 8.84 (s, 1 H, H-6), 8.94 (s, 1 H, H-8), 9.17 (s, 1 H, H-2): FAB-MS ($m/z$) 221 ($MH^+$). Anal. ($C_{10}H_{12}O_2N_4$) C, H, N.

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# Synthesis and in Vitro Evaluation of 2,3-Dimethoxy-5-(fluoroalkyl)-Substituted Benzamides: High-Affinity Ligands for CNS Dopamine $D_2$ Receptors[1]

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A number of 2,3-dimethoxy-5-(fluoroalkyl)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamides (with or without a 6-hydroxy group) were synthesized and evaluated as dopamine $D_2$ receptor ligands. The parent acids were synthesized via the Claisen rearrangement of the appropriate *O*-allyl ethers, which were derived from *o*-vanillic acid or 2,3-dimethoxysalicylic acid. A decrease in reactivity was found to be characteristic of pentasubstituted benzoates, and difficulties were encountered with the introduction of fluorine onto the ethyl side chains. The (fluoroethyl)- and (fluoropropyl)salicylamides were 5 times more potent than the corresponding benzamides in inhibiting [$^3H$]spiperone binding to the $D_2$ receptor. These (fluoroalkyl)salicylamides are of potential value for in vivo positron emission tomography (PET) studies upon the basis of their relatively selective, high potency binding affinity for the $D_2$ receptor.

During the past decade the binding properties of a variety of benzamides and salicylamides containing *N*-[(1-alkyl-2-pyrrolidinyl)methyl] side chains have been reported.[2-5] Many of these amides are selective and potent

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(2) Jenner, P.; Marsden, C. D. *Neuropharmacology* **1981**, *20*, 1285.

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