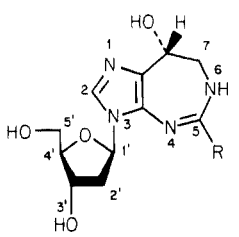
# Adenosine Deaminase Inhibitors. Synthesis and Biological Evaluation of (±)-3,6,7,8-Tetrahydro-3-[(2-hydroxyethoxy)methyl]imidazo[4,5-*d*][1,3]diazepin-8-ol and Some Selected C-5 Homologues of Pentostatin[1]

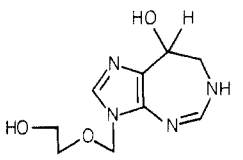
H. D. Hollis Showalter,*,† Sterling R. Putt,† Pal E. Borondy,‡ and Joan L. Shillis§

*Departments of Chemistry, Pharmacokinetics and Drug Metabolism, and Chemotherapy, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105.* *Received November 24, 1982*

The synthesis of several analogues of (8*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (pentostatin, **1a**) is described. Ring closure of 2-amino-1-(5-amino-1*H*-imidazol-4-yl)ethanone dihydrochloride (**3**) with triethyl orthoacetate or triethyl orthopropionate gave the C-5 methyl and ethyl ketoaglycons, 6,7-dihydro-5-methylimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**4b**) and 5-ethyl-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**4c**), respectively. Stannic chloride catalyzed condensation of the pertrimethylsilyl derivatives of **4b** and **4c** with a protected glycosyl halide afforded anomeric mixtures of ketonucleosides 3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β- and -α-D-*erythro*-pentofuranosyl)-6,7-dihydro-5-methylimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**5b** and **6b**) and 3-(2-deoxy-3,5-di-*O*-*p*-toluoyl)-β- and -α-D-*erythro*-pentofuranosyl)-5-ethyl-6,7-dihydroimidazo[4,5-*d*]-[1,3]diazepin-8(3*H*)-one (**5c** and **6c**), respectively. Subsequent separation of the anomers, followed by deprotection and reduction of **5b**, **6b**, and **5c**, afforded the respective 8*R* and 8*S* isomers. Stannic chloride catalyzed condensation of pertrimethylsilyl ketoaglycon **4a** with 2-(chloromethoxy)-1-(*p*-toluoyloxy)ethane to give ketonucleoside 6,7-dihydro-3-[[2-(*p*-toluoyloxy)ethoxy]methyl]imidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**9a**) was followed by deprotection to 6,7-dihydro-3[(2-hydroxyethoxy)methyl]imidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**9b**) and then reduction to the racemic acyclic pentostatin analogue (±)-3,6,7,8-tetrahydro-3-[(2-hydroxyethoxy)methyl]imidazo[4,5-*d*][1,3]diazepin-8-ol (**2**). $K_i$ values for the in vitro adenosine deaminase (EC 3.5.4.4; type I; calf intestinal mucosa) inhibitory activities of **1b**, **1c**, and **2** were determined to be $1.6 \times 10^{-8}$, $1.5 \times 10^{-6}$, and $9.8 \times 10^{-8}$ M, respectively. When compounds **2** and **9b** were tested in combination with vidarabine against herpes simplex virus, type 1, in an HEp-2 plaque reduction assay, only compound **2** was able to potentiate the antiviral activity of vidarabine.

Research efforts into the synthesis and pharmacological evaluation of a number of novel and therapeutically significant inhibitors of adenosine deaminase (adenosine aminohydrolase EC 3.5.4.4) continue unabated.[2] Of these inhibitors, both pentostatin (**1a**)[3,4] and its D-*ribo* analogue





**2**

1a, R = H (pentostatin)
b, R = $CH_3$
c, R = $CH_2CH_3$

coformycin[5] are exceedingly tight-binding inhibitors of this enzyme, showing $K_i = 2.5 \times 10^{-12}$ and $1.0 \times 10^{-11}$ M, respectively, against human erythrocytic adenosine deaminase[6] as shown in studies carried out by Parks and co-workers.[7] Pentostatin, the more potent of the two, has been under extensive preclinical evaluation.[8] It holds considerable promise as a codrug in combination with other therapeutically useful adenosine-type nucleosides for the treatment of both hematologic malignancies and various solid tumors based on human in vitro tissue culture and in vivo xenograft studies.[9] The drug is currently under phase I clinical trials in combination with 9-β-D-arabinofuranosyladenine (vidarabine) against acute myelogenous leukemia.[10]

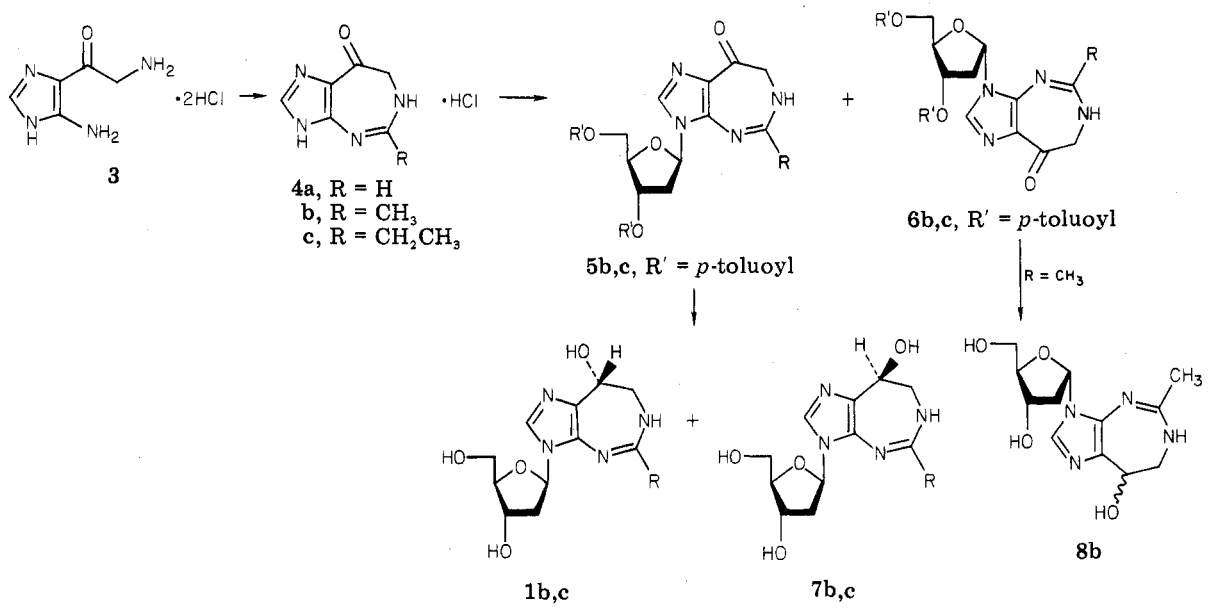
The structure of **1a** incorporates a novel 5-7-membered fused aglyconic moiety, which is believed to mimic the putative tetrahedral transition-state intermediate formed in the course of the enzymatic deamination reaction of adenosine-type nucleosides to their respective inosine counterparts.[11] Recent publications from these laboratories have described various synthetic approaches to pentostatin,[12,13] one of which[12] resulted in a practical route to a multigram preparation of **1a** for expanded drug evaluation. Reported herein are the synthesis and bio-

† Department of Chemistry.
‡ Department of Pharmacokinetics and Drug Metabolism.
§ Department of Chemotherapy.

(1) This paper has been presented. See "Abstracts of Papers"; 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 12–17, 1982; American Chemical Society: Washington, DC, 1982; Abstr MEDI 30.
(2) Glazer, R. I. *Cancer Chemother. Pharmacol.* **1980**, *4*, 227–235. Recent reports include the following: (a) Bastian, G.; Bessodes, M.; Panzica, R. P.; Abushanab, E.; Chen, S.-F.; Stoeckler, J. D.; Parks, R. E., Jr. *J. Med. Chem.* **1981**, *24*, 1383–1385. (b) Woo, P. W. K.; Baker, D. C. *Ibid.* **1982**, *25*, 603–605. (c) Baker, D. C.; Hanvey, J. C.; Kumar, S. D.; Salley, J. J., Jr. *Ibid.* submitted. (d) Baker, D. C.; Hawkins, L. D. *J. Org. Chem.* **1982**, *47*, 2179–2184.
(3) USAN-approved generic name for (8*R*)-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*]-[1,3]diazepin-8-ol. The literature also refers to this as covidarabine (CoV) and 2′-deoxycoformycin (dCoF).
(4) Woo, P. W. K.; Dion, H. W.; Lange, S. M.; Dahl, L. F.; Durham, L. J. *J. Heterocycl. Chem.* **1974**, *11*, 641–643.
(5) Ohno, M.; Yagisawa, N.; Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H. *J. Am. Chem. Soc.* **1974**, *96*, 4327–4328.
(6) Daddona, P. E. *J. Biol. Chem.* **1981**, *256*, 12496–12501.
(7) Agarwal, R. P.; Spector, T.; Parks, R. E., Jr. *Biochem. Pharmacol.* **1977**, *26*, 359–367.
(8) Poster, D. S.; Penta, J. S.; Bruno, S.; Macdonald, J. S. *Cancer Clin. Trials* **1981**, *4*, 209–213.
(9) (a) Cummings, F. J.; Crabtree, G. W.; Spremulli, E.; Rogler-Brown, T.; Parks, R. E., Jr.; Calabresi, R. E. *Proc. Am. Soc. Clin. Oncol.* **1980**, *21*, 332 (abstr C-54). (b) Chu, M. Y.; Dexter, D. L.; Melvin, J. B.; Robinson, B. S.; Parks, R. E., Jr.; Calabresi, P. *Ibid.* **1980**, *21*, 269 (abstr 1080). (c) Spremulli, E. N.; Crabtree, G. W.; Dexter, D. L.; Chu, S. H.; Farineau, D. M.; Ghoda, L. Y.; McGowan, D. L.; Diamond, I.; Parks, R. E., Jr.; Calabresi, P. *Biochem. Pharmacol.* **1982**, *31*, 2415–2421.
(10) Gray, D. P.; Grever, M. R.; Siaw, M. F. E.; Coleman, M. S.; Balcerzak, S. P. *Cancer Treat. Rep.* **1982**, *66*, 253–257.
(11) Wentworth, D. F.; Wolfenden, R. *Biochemistry* **1975**, *14*, 5099–5105.
(12) Chan, E.; Putt, S. R.; Showalter, H. D. H.; Baker, D. C. *J. Org. Chem.* **1982**, *47*, 3457–3464.
(13) Baker, D. C.; Putt, S. R.; Showalter, H. D. H. *J. Heterocycl. Chem.* **1983**, *20*, 629–634.

0022-2623/83/1826-1478$01.50/0 

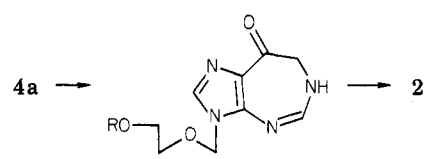
Scheme I




logical evaluation of some selected C-5 alkyl homologues of pentostatin, **1b** and **1c**, as well as acyclic analogue **2** in which the cyclic 2′-deoxy-D-*ribo* moiety is replaced by the (2-hydroxyethoxy)methyl chain. Aglycon substitution with this moiety is gaining increasing attention,[14] as evidenced in the potent antiviral agent acycloguanosine (acyclovir),[15] and is considered to be potentially capable of interfering with the activity of various enzymes for which the natural nucleosides or nucleotides serve as substrates. We were particularly interested in the preparation of **2** based on Schaeffer's earlier report that 9-[(2-hydroxyethoxy)-methyl]adenine is a substrate for adenosine deaminase.[16]

**Chemistry.** The synthesis of target C-5 alkyl homologues **1b** and **1c** is outlined in Scheme I. Ketoaglycons **4b** and **4c** were synthesized in 79 and 89% yields, respectively, by orthoester ring closure of 2-amino-1-(5-amino-1*H*-imidazol-4-yl)ethanone dihydrochloride (**3**) by a process similar to that outlined by us previously for the synthesis of **4a**.[12] Low-temperature condensation of pertrimethylsilyl **4b** with 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride, catalyzed by stannic chloride, under carefully controlled conditions[12,17] gave a 60:40 chromatographically pure mixture of 3-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β- and -α-D-*erythro*-pentofuranosyl)-6,7-dihydro-5-methylimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**5b** and **6b**, respectively) in an 89% yield. The structure of each isomer followed directly from the $^1$H NMR spectrum with resonances and splitting patterns essentially identical with those for the corresponding pentostatin intermediates reported earlier[12] and will not be elaborated upon here. Deprotection of ketonucleoside **5b** via transesterification in methanol–sodium methoxide, followed by aqueous sodium borohydride reduction, provided a 1:1 mixture of 8*R* and 8*S* diastereomeric alcohols **1b** and **7b**, respectively, in 91% yield following preparative HPLC on an octadecylsilyl-derivatized silica gel column operating in the reverse-phase mode.

Scheme II




Deprotection of α-ketonucleoside **6b** via transesterification, followed by borohydride reduction as described above, gave an ca. 45:55 ($^1$H NMR) HPLC-inseparable mixture of 8*R* and 8*S* isomers, **8b**. As these were subsequently shown to be devoid of biological activity, separation of the diastereomers by fractional crystallization was deemed unimportant.

To further assess the effect of added steric bulk and lipophilicity at the C-5 position of pentostatin, we carried out the synthesis of 5-ethyl homologues **1c** and **7c** in an identical manner with the aforementioned procedures. Glycosylation of **4c** afforded a 60:40 β/α mixture of the corresponding ketonucleosides **5c** and **6c** in 77% yield. Deprotection of **5c**, followed by borohydride reduction, provided a 1:1 mixture of the 8*R* and 8*S* diastereomeric alcohols **1c** and **7c**, respectively, in 95% yield following separation by preparative HPLC.

As is often characteristic of this class of compounds, one or both C-8 alcohol diastereomers (e.g., **1b** and **7b**) resisted attempts at fractional crystallization to 100% diastereomeric purity. Hence, for these cases, amorphous solids of 88–99% purity were isolated by precipitation techniques (see Experimental Section).

Unequivocal structural assignments for each pair of 8*R* and 8*S* diastereomeric alcohols (**1b**, **7b** and **1c**, **7c**, re-

(14) Recent reports incorporating this side chain in nucleoside synthesis are the following: (a) Barrio, J. R.; Bryant, J. D.; Keyser, G. E. *J. Med. Chem.* **1980**, *23*, 572–574. (b) Rosowsky, A.; Kim, S.-H.; Wick, M. *Ibid.* **1981**, *24*, 1177–1181. (c) Schroeder, A. C.; Hughes, R. G., Jr.; Bloch, A. *Ibid.* **1981**, *24*, 1078–1083. (d) Bartlett, R. T.; Cook, A. F.; Holman, M. J.; McComas, W. W.; Nowoswait, E. F.; Poonian, M. S.; Baird-Lambert, J. A.; Baldo, B. A.; Marwood, J. F. *Ibid.* **1981**, *24*, 947–954. (e) Kelley, J. L.; Kelsey, J. E., Hall, W. R.; Krochmal, M. P.; Schaeffer, H. J. *Ibid.* **1981**, *24*, 753–756. (f) Abrams, H. M.; Ho, L.; Chu, S. H. *J. Heterocycl. Chem.* **1981**, *18*, 947–951. (g) Parkin, A.; Harnden, M. R. *Ibid.* **1982**, *19*, 33–40. (h) Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547–553.

(15) Schaeffer, H. J.; Beauchamp, L.; Demiranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature (London)* **1978**, *272*, 583–585.

(16) Schaeffer, H. J.; Gurwara, S.; Vince, R.; Bittner, S. *J. Med. Chem.* **1971**, *14*, 367–369.

(17) Showalter, H. D. H.; Putt, S. R. *Tetrahedron Lett.* **1981**, *22*, 3155–3158.

spectively) were difficult to make by $^1$H NMR spectroscopy because of the similarity of resonance and splitting patterns for corresponding positional protons. This is also observed for pentostatin and its 8*S* diastereomer.[12] However, the optical rotation data, relative HPLC mobilities, and the relationship of these values to the inhibition of adenosine deaminase by a single isomer of each C-8 diastereomeric pair (vide infra) are strongly suggestive that the correct assignments have been made.

The synthesis of the acyclic analogue **2** is delineated in Scheme II. Glycosylation of pentostatin ketoaglycon **4a** with 2-(chloromethoxy)-1-(*p*-toluoyloxy)ethane[18] in acetonitrile[17] gave pure ketonucleoside **9a** in 27% yield. Transesterification of **9a** with methanol–sodium methoxide yielded the deprotected ketonucleoside **9b** in 61% yield following purification via preparative reverse-phase HPLC. Reduction of **9b** with sodium borohydride provided pure (±)-3,6,7,8-tetrahydro-3-[(2-hydroxyethoxy)methyl]-imidazo[4,5-*d*][1,3]diazepin-8-ol (**2**) in 45% yield following purification over HP-20 resin.[19]

**Adenosine Deaminase Studies.** Utilizing a modification of a known procedure,[20] we tested compounds **1b,c** **7b,c**, **8b**, and **2** simultaneously with pentostatin (**1a**) in an in vitro assay against calf intestinal mucosal adenosine deaminase (vidarabine as substrate). Only compounds **1b,c** and **2** showed significant activity (17.3, 0.1, and 3.6% as active as pentostatin, respectively); hence, studies were carried out to determine their inhibition constants ($K_i$). When studied under conventional enzyme assay conditions, the non-steady-state nature of the inhibition of adenosine deaminase by pentostatin renders the determination of a meaningful inhibition constant difficult because of its unusually high enzymatic affinity. Theoretical approaches by Cha[21] have provided the basis for the elucidation of the inhibition mechanism of tight-binding inhibitors by determining the manner in which the substrate interferes with the binding of the inhibitor to the enzyme. The procedure involves the determination of the pseudo-first-order rate constants at various concentrations of the substrate and the inhibitor. Whether a compound is a pseudoirreversible, tight-binding, or stoichiometric inhibitor of adenosine deaminase can be determined by employing an Ackermann–Potter analysis[22] as a diagnostic tool. Although this method does not permit direct calculation of the $K_i$ value, the same data may be used by the $I_{50}$ method to estimate the $K_i$ and the molar equivalency of the enzyme. In the present work the Ackermann–Potter plot was constructed by measuring the effect of pentostatin (**1a**) and its homologues **1b** and **1c**, as well as acyclic analogue **2**, on the rate of adenosine deamination. The adenosine deaminase–pentostatin combination clearly demonstrated that pentostatin was a tight-binding inhibitor. On the other hand, **1b,c** and **2** did not produce classical Ackermann–Potter plots. These observations indicated that classical methods of determination of inhibition constants ($K_i$) could be utilized. Using a Michaelis–Menten analysis, we determined $K_i$ values through the use of standard double-reciprocal plots. A classical pattern of competitive inhibition was obtained, and the $K_i$ values for **1b,c** and **2** were estimated to be $1.6 \times 10^{-8}$, $1.5 \times 10^{-6}$, and $9.8 \times 10^{-8}$ M, respectively. For pentostatin, the same data used for the Ackermann–Potter plot were employed to calculate the $K_i$ value by the $I_{50}$ method, and it was found to be $5 \times 10^{-11}$ M.

**Antiviral Studies.** Since pentostatin has proven to be quite effective as a codrug for enhancing the antiviral activity of vidarabine in vitro and in vivo,[23] it was of interest to investigate the ability of some selected target compounds reported herein (**2** and **9b** only) to potentiate the anti herpes simplex virus, type 1, activity of vidarabine, as determined by a plaque-reduction assay in HEp-2 cells. Compounds were tested at 2.5 and 25 μg/mL, alone and in combination with 40, 12.5, 4, 1.25, and 0.4 μg/mL of vidarabine. A 50% or greater reduction in the number of plaques [plaque forming units (pfu)] when compared to virus control cultures was considered to represent significant antiviral activity.

The minimum inhibitory concentration for ≥50% reduction in pfu (≥$MIC_{50}$) against herpes simplex virus was 40 μg/mL for vidarabine alone. However, when combined with either 2.5 or 25 μg/mL of compound **1a** or **2**, only 1.25 μg/mL of vidarabine was required to produce ≥50% reduction in pfu (at a concentration of 2.5 μg/mL, the actual percent plaque reduction was 56% for compound **2** and 100% for compound **1a**). Thus, plaque development was 32-fold more sensitive to combined levels of vidarabine and compound **1a** or **2** than vidarabine alone. In contrast, the addition of similar concentrations of compound **9b** did not potentiate the antiviral activity of vidarabine. No significant reduction in the number of viral plaques was observed with 25 μg/mL of each test compound alone.

## Conclusions

Homologation at the C-5 position of pentostatin with simple alkyl groups results in analogues with reduced binding to calf intestinal mucosal adenosine deaminase. Whether this reduction can be attributed to lipophilicity changes and/or stereoelectronic effects remains to be determined.

Substitution of the 2′-deoxyribosyl sugar moiety of pentostatin with the acyclic (2-hydroxyethoxy)methyl chain gives an analogue that, while displaying a reduced binding to adenosine deaminase, appears to show appreciable potency in potentiating the antiviral effect of vidarabine against herpes simplex virus, type 1.

## Experimental Section

**General.** Melting points were taken on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Digilab FTS-14 instrument. Ultraviolet (UV) spectra were taken on a Cary Model 118C recording spectrophotometer. $^1$H nuclear magnetic resonance ($^1$H NMR) spectra were recorded at 90 MHz on a Varian EM-390 or Bruker WH-90 instrument. Chemical shifts are reported as δ values (parts per million) downfield from internal tetramethylsilane on samples of ~1%, w/v. Optical rotations were taken on a Perkin-Elmer 141 polarimeter. Combustion analyses were performed on a Perkin-Elmer 240 elemental analyzer and are reported within ±0.4% of the theoretical values. Water of crystallization was determined by Karl Fischer titration. $pK_a$ values were determined on a Copenhagen Radiometer TTT60 titrator.

Chromatography was carried out with (a) E. Merck products utilizing silica gel 60 catalog no. 5760 for TLC, catalog no. 7734 for open column chromatography and catalog no. 9385 for flash

(18) Prepared from *p*-toluoylethylene glycol (Robinson, B. *J. Chem. Soc.* **1963**, 2417–2419) by a procedure described for benzoylethylene glycol in ref 14d. The chloro ether is ~90% pure by NMR and is used directly in the glycosylation step.
(19) Available from Gillies International, Inc., La Jolla, CA.
(20) Mitchell, H. K.; McElroy, W. D. *Arch. Biochem.* **1946**, *10*, 343–349.
(21) Cha, S. *Biochem. Pharmacol.* **1976**, *25*, 2695–2702.
(22) Ackermann, W. W.; Potter, V. R. *Proc. Soc. Exp. Biol. Med.* **1949**, *72*, 1–9.
(23) Sloan, B. J.; Kielty, J. K.; Miller, F. A. *Ann. N.Y. Acad. Sci.* **1977**, *284*, 60–80.
(24) Bhat, C. C. *Synth. Proced. Nucleic Acid Chem.* **1968**, *1*, 521–522.

chromatography; (b) Dupont catalog no. 850952-701 for $SiO_2$ analytical HPLC, and (c) Altech catalog no. 600RP for C-18 reverse-phase analytical HPLC. Where specified, preparative separations utilizing $SiO_2$ or C-18 were carried out on a Waters Associates "Prep-500" system. Chromatography solvents were A, (a) 9:1 and (b) 95:5 EtOAc–MeOH; B, 96:4 $CHCl_3$–MeOH; C, 94:6 $CH_2Cl_2$–MeOH; D, (a) 85:15, (b) 4:1, and (c) 9:1 $H_2O$–MeOH, pH 7.5; E, 3:1 $CH_3CN$–0.2 M $NH_4Cl$; F, 9:1 0.005 M $(NH_4)_2HPO_4$–MeOH. All solvents and reagents were "reagent grade" unless otherwise noted.

**6,7-Dihydro-5-methylimidazo[4,5-*d*][1,3]diazepin-8-(3*H*)-one Hydrochloride (4b).** To 2.36 L of absolute EtOH at reflux were added 50.77 g (.238 mol) of 2-amino-1-(5-amino-1*H*-imidazol-4-yl)ethanone dihydrochloride (**3**) and 283 mL of triethyl orthoacetate. The suspension was stirred for 1 h at reflux, during which complete solution resulted. The mixture was ice cooled, and an off-white solid precipitated. The solid was filtered, washed with EtOH, and dried at 25 °C (5 mm) for 24 h to afford 37.78 g (79%) of analytically pure **4b**: mp >300 °C dec; NMR [$(CD_3)_2SO$] $\delta$ 2.5 (s, 3, $CH_3$), 4.15 (s, 2, H-7), 7.98 (s, 1, H-2); IR (KBr) 1687, 1645, 1570, 1392 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) sh 334 nm, 297 ($\epsilon$ 3890), 228 (20 000). Anal. ($C_7H_8N_4O$·HCl) C, H, N, Cl.

**5-Ethyl-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one Hydrochloride (4c)**: 89% yield; mp 260 °C dec; NMR [$(CD_3)_2SO$] $\delta$ 1.23 (t, 3, $J$ = 8 Hz, $CH_3CH_2$), 2.74 (q, 2, J = 8 Hz, $CH_3CH_2$), 4.13 (s, 2, H-7), 7.92 (s, 1, H-2); IR (KBr) 1675, 1560, 1382 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) sh ~330 nm, 296 ($\epsilon$ 4050), 229 (19 900). Anal. ($C_8H_{10}N_4O$·HCl) C, H, N, Cl.

**3-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-$\beta$- and -$\alpha$-D-*erythro*-pentofuranosyl)-6,7-dihydro-5-methylimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (5b and 6b).** To a flame-dried 1-L, three-necked flask equipped with a drying tube, serum stopper, and magnetic stirring bar was added 10.53 g (52.5 mmol) of 6,7-dihydro-5-methylimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one hydrochloride (**4b**), 67.5 mL of bis(trimethylsilyl)trifluoroacetamide (BSTFA; Petrarch or Regis), and 94.5 mL of $CH_3CN$ (Burdick and Jackson spectroquality). The suspension was stirred at room temperature for 30 min, during which complete solution resulted. Solvent and excess BSTFA were evaporated at 25 °C (1 mm), and the resulting syrup was heated at 50–60 °C (1 mm) for 2 h.

After the dark syrupy residue was dissolved in 250 mL of dichloroethane (Burdick and Jackson spectroquality) and 125 mL of $CH_3CN$, the solution was cooled to −25 °C (external). To the stirred solution was added 12.3 mL (0.105 mol) of anhydrous $SnCl_4$ (Ventron or Baker), and the resultant mixture was stirred for 5 min. Crystalline 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride,[24] 19.43 g (0.05 mol), was added in one portion, followed by 250 mL of dichloroethane. After the dark solution was stirred at −20 °C for 1.5 h [after ca. 15 min reaction time, TLC (solvent Aa) revealed complete reaction], the mixture was poured into 600 mL of a stirred solution of saturated aqueous $NaHCO_3$. EtOAc (ca. 1 L) was added, and the mixture was stirred vigorously for 1 h and filtered through Celite, and the layers were separated. The aqueous phase was extracted once with EtOAc, and the combined organic phases were dried ($MgSO_4$) and concentrated to a gold glass. The residue, showing an $\alpha/\beta$ anomeric ratio of 40:60 by HPLC, was dissolved in a minimum volume of EtOAc and flash chromatographed over silica gel (solvent B; 4 psi; 50-mL fractions). Product fractions were assayed by HPLC and then pooled into three lots. Lot 1 contained 6.83 g of >80% $\alpha$ anomer, lot 2 contained 8.60 of 60% $\beta$ anomer, and lot 3 contained 8.83 g of 90% $\beta$ anomer, with the combined lots affording 24.26 g (89% yield). Lots 2 and 3 were combined and dissolved in 200 mL of hot EtOAc. The mixture was cooled to 25 °C, and 240 mL of $Et_2O$ was added. After storage at 3 °C overnight, the crystals were collected by filtration, washed sparingly with cold EtOAc, and dried in vacuo to afford 6.56 g (24%) of >99% $\beta$-anomer (**5b**): mp 177–178.5 °C; $R_f$ 0.41 (solvent Aa); $k'$ (HPLC $SiO_2$, solvent B) = 4.3; $[\alpha]^{23}_D$ −36.2° ($c$ 1, DMF); NMR [$(CD_3)_2SO$] $\delta$ 2.21 (s, 3, C-5 methyl), 2.41, 2.43 (s, 6, aryl $CH_3$'s), 2.60–3.23 (m, 2, H-2′, H-2′a), 3.68 (d, 2, H-7), 4.40–4.90 (m, 3, H-4′, H-5′, 5′a), 5.63–5.95 (m, 1, H-3′), 6.50 (t, 1, H-1′), 7.23–7.60 (m, 4, *p*-toluoyl), 7.80–8.15 (m, 5, H-2 and *p*-toluoyl), 8.52 (br s, 1, NH); IR (KBr) 1720, 1660, 1270, 1100, 750 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 234 nm ($\epsilon$ 49 740), 299 (3420), 346 (4570). Anal. ($C_{28}H_{28}N_4O_6$) C, H, N.

Purification of mixed anomer fractions could be carried out on silica gel by Waters 500 preparative HPLC (solvent C) via recycling to afford a fraction containing 90% of the $\alpha$-anomer (**6b**) as a gum that could not be crystallized: $R_f$ 0.41 (solvent Aa); $k'$ (HPLC $SiO_2$, solvent B) = 3.5; $[\alpha]^{23}_D$ −3.9° ($c$ 1, DMF); NMR [$(CD_3)_2SO$] $\delta$ 2.11 (s, 3, C-5 methyl), 2.36 (s, 6, aryl $CH_3$'s), 2.60–3.30 (m, 2, H-2′, H-2′a), 3.59 (d, 2, H-7), 4.40–4.65 (m, 2, H-5′, H-5′a), 4.82–5.02 (m, 1, H-4′), 5.40–5.69 (m, 1, H-3′), 6.43 (dd, 1, H-1′), 7.17–7.42 (m, 4, *p*-toluoyl), 7.65–8.0 (m, 5, H-2 and *p*-toluoyl), 8.35 (br s, 1, NH); the IR and UV spectra are essentially the same as the $\beta$-anomer. Anal. ($C_{28}H_{28}N_4O_6$) C, H, N.

**3-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-$\beta$- and -$\alpha$-D-*erythro*-pentofuranosyl)-5-ethyl-6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (5c and 6c)**: 77% yield of a 40:60 $\alpha/\beta$ anomeric mixture following rapid column chromatography over silica gel (EtOAc, then solvent Ab). Partial anomer separation was achieved by chromatography over silica gel (gradient elution with $CHCl_3$ to solvent B). $\beta$-Anomer (**5c**): mp 211–213 °C (trituration from EtOAc) $R_f$ 0.46 (solvent Aa); $k'$ (HPLC $SiO_2$, solvent B) = 3.6; $[\alpha]^{23}_D$ −34.8° ($c$ 1,DMF); NMR [$(CD_3)_2SO$] $\delta$ 1.15 (t, 3, $CH_2CH_3$), 2.38 (s, 6, aryl $CH_3$'s), 2.6–3.15 (m, 4, H-2′, H-2′a, $CH_2CH_3$), 3.64 (d, 2, H-7), 4.40–4.75 (m, 3, H-4′, H-5′, H-5′a), 5.60–5.86 (m, 1, H-3′), 6.50 (t, 1, H-1′), 7.22–7.50 (m, 4, *p*-toluoyl), 7.75–8.08 (m, 5, H-2 and *p*-toluoyl), 8.36 (br s, 1, NH); IR (KBr) 1720, 1665, 1272, 1102, 752 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 235 nm ($\epsilon$ 50 060), 347 (4700). Anal. ($C_{29}H_{30}N_4O_6$) C, H, N.

$\alpha$-Anomer (**6c**, glass, 87% isomeric purity by HPLC): $R_f$ 0.46 (solvent Aa); $k'$ (HPLC $SiO_2$, solvent B) = 2.9; $[\alpha]^{23}_D$ −6.6° ($c$ 1, DMF); NMR [$(CD_3)_2SO$] $\delta$ 1.10 (t, 3, $CH_2CH_3$), 2.38 (s, 6, aryl $CH_3$'s), 2.6–3.28 (m, 4, H-2′, H-2′a, $CH_2CH_3$), 3.60 (d, 2, H-7), 4.33–4.70 (m, 2, H-5′, H-5′a), 4.82–5.10 (m, 1, H-4′), 5.48–5.72 (m, 1, H-3′), 6.47 (dd, 1, H-1′), 7.14–7.47 (m, 4, *p*-toluoyl), 7.65–8.03 (m, 5, H-2 and *p*-toluoyl), 8.29 (br s, 1, NH); the IR and UV spectra are essentially the same as the $\beta$-anomer. Anal. ($C_{29}H_{30}N_4O_6$) C, H, N.

**(8*R*)- and (8*S*)-3-(2-Deoxy-$\beta$-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydro-5-methylimidazo[4,5-*d*][1,3]-diazepin-8-ol (1b and 7b).** To a stirred suspension of 4.71 g (9.1 mmol) of ketonucleoside **5b** in 240 mL of absolute MeOH at room temperature was gradually added ~0.45 g (20 mmol) of sodium beads, and the reaction was stirred for 1 h, at which time TLC (solvent Aa) showed complete deprotection of **5b**. Excess dry ice was added, and the solution was evaporated to a solid residue, which was triturated with $Et_2O$. The solid was filtered, washed well with $Et_2O$, and used directly without further purification.

To the above solid dissolved in 150 mL of $H_2O$ at room temperature was added 0.307 g (8.1 mmol) of sodium borohydride. After stirring for 3 h, the solution was adjusted to neutrality with acetic acid and then lyophilized. HPLC revealed an approximate 1:1 mixture of *R* and *S* isomers **1b** and **7b**. Purification was effected over a C-18 reverse-phase column by preparative HPLC (solvent Da). Three major lots containing crude products were collected, and the solvent was lyophilized. Lot 1 contained 670 mg of >97% *S* isomer, lot 2 contained 840 mg of a 55:45 *S*/*R* mixture, and lot 3 contained 980 mg of 97% *R* isomer, with the combined lots affording 2.49 g (91%) of >90% purity products.

Lot 3 was dissolved in a minimum volume of $H_2O$. The aqueous solution was washed with EtOAc and filtered, and the filtrate was then concentrated to an oil, which was diluted with enough MeOH to give solution. Slow addition of this solution to vigorously stirring $Et_2O$ precipitated 870 mg of 97% isomeric purity *R* isomer (**1b**): mp 192–194 °C dec; $R_f$ 0.29 (solvent E); $k'$ (HPLC, C-18; solvent F) = 5.8; p$K_a$ ($H_2O$) = 6.0; $[\alpha]^{23}_D$ +18.0° ($c$ 1, pH 7 buffer); NMR [$(CD_3)_2SO$] $\delta$ 2.00 (s, 3, $CH_3$), 2.0–2.5 (m, 2, H-2′, H-2′a), 2.95–3.25 (m, 2, H-7, H-7a), 3.40–3.62 (m, 2, H-5′, H-5′a), 3.68–3.90 (m, 1, H-4′), 4.20–4.42 (m, 1, H-3′), 4.65–5.35 (m, 4, H-8 and OH's; collapses to t at $\delta$ 4.80 with $D_2O$ wash), 6.22 (dd, 1, H-1′), 7.48 (br s, 1, NH), 7.57 (s, 1, H-2); IR (KBr) 3340, 1630, 1560 $cm^{-1}$; UV $\lambda_{max}$ (pH 7 buffer) 281 nm ($\epsilon$ 9160). Anal. ($C_{12}H_{18}N_4O_4$·0.2$H_2O$) C, H, N.

Crystallization of lot 1 from $H_2O$ afforded 569 mg of the pure *S* isomer (**7b**): mp 230–231 °C; $R_f$ 0.29 (solvent E); $k'$ (HPLC, C-18; solvent F) = 3.6; p$K_a$ ($H_2O$) = 5.9; $[\alpha]^{23}_D$ −84° ($c$ 1, pH 7 buffer); NMR [$(CD_3)_2SO$] $\delta$ 2.00 (s, 3, $CH_3$), 2.0–2.5 (m, 2, H-2′, H-2′a), 2.95–3.25 (m, 2, H-7, H-7a), 3.38–3.66 (m, 2, H-5′, H-5′a), 3.68–3.90 (m, 1, H-4′), 4.20–4.42 (m, 1, H-3′), 4.65–5.10 (m, 3, H-8

and OH's; collapses to t at δ 4.81 with $D_2O$ wash), 5.23 (d, 1, OH), 6.22 (dd, 1, H-1'), 7.49 (br s, 1, NH), 7.58 (s, 1, H-2); the IR and UV spectra are essentially the same as those for **1b**. Anal. ($C_{12}H_{18}N_4O_4$) C, H, N.

Processing of the mother liquors and mixed fractions via recycling on preparative HPLC afforded additional *R* and *S* isomers.

**(8*R*)- and (8*S*)-3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-ethyl-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (1c and 7c)**: 95% yield of an approximate 1:1 mixture of *R* and *S* isomers **1c** and **7c**. Purification was effected over a C-18 reverse-phase column by preparative HPLC (solvent Db).

*R* isomer (**1c**; 88% isomeric purity by HPLC; precipitation by ether from an ethanolic solution): $R_f$ 0.38 (solvent E); *k'* (HPLC, C-18; solvent F) = 12.8; p$K_a$ ($H_2O$) = 6.0; $[\alpha]^{23}_D$ +17.7° (*c* 1, pH 7 buffer); NMR [$(CD_3)_2SO$] δ 1.11 (t, 3, $CH_2CH_3$), 1.95–2.5 (m, 4, H-2', H-2'a, $CH_2CH_3$), 2.95–3.24 (m, 2, H-7, H-7a), 3.38–3.68 (m, 2, H-5', H-5'a), 3.69–3.90 (m, 1, H-4'), 4.18–4.50 (m, 1, H-3'), 4.65–5.40 (m, 4, H-8 and OH's; collapses to t at δ 4.80 with $D_2O$ wash), 6.28 (dd, 1, H-1'), 7.38 (br s, 1, NH), 7.58 (s, 1, H-2); IR (KBr) 3350, 1625, 1550 $cm^{-1}$; UV $\lambda_{max}$ (pH 7 buffer) 281 nm (ε 9310). Anal. ($C_{13}H_{20}N_4O_4 \cdot 0.2H_2O$) C, H, N.

*S* isomer (**7c**; 99% isomeric purity by HPLC): mp 210–213 °C dec (precipitation by ether from a methanolic solution); $R_f$ 0.38 (solvent E); *k'* (HPLC, C-18; solvent F) = 8.7; p$K_a$ ($H_2O$) = 6.1; $[\alpha]^{23}_D$ −85.5° (*c* 1, pH 7 buffer); NMR [$(CD_3)_2SO$] δ 1.12 (t, 3, $CH_2CH_3$), 2.0–2.5 (m, 4, H-2', H-2'a, $CH_2CH_3$), 3.0–3.25 (m, 2, H-7, H-7a), 3.41–3.65 (m, 2, H-5', H-5'a), 3.70–3.95 (m, 1, H-4'), 4.21–4.45 (m, 1, H-3'), 4.70–5.10 (m, 3, H-8 and OH's; collapses to t at δ 4.82 with $D_2O$ wash), 5.24 (d, 1, OH), 6.29 (dd, 1, H-1'), 7.38 (br s, 1, NH), 7.60 (s, 1, H-2); the IR and UV spectra are essentially the same as those for **1c**. Anal. ($C_{13}H_{20}N_4O_4$) C, H, N.

**(8*R*)- and (8*S*)-3-(2-Deoxy-α-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydro-5-methylimidazo[4,5-*d*][1,3]diazepin-8-ols (8b)**: 55% yield of an inseparable mixture of approximate 45:55 mixture, by $^1H$ NMR, of nucleosides following desalting over C-18 preparative HPLC (solvent Da) and precipitation from $Et_2O$; mp >120 °C; $R_f$ 0.27 (solvent E); k' (HPLC, C-18; solvent F) = 5.8; $[\alpha]^{23}_D$ +53.3° (*c* 1.1, pH 7 buffer); UV $\lambda_{max}$ (pH 7 buffer) 280 nm (ε 8550). Anal. ($C_{12}H_{18}N_4O_4 \cdot 0.1H_2O$) C, H, N.

**6,7-Dihydro-3-[[2-(*p*-toluoyloxy)ethoxy]methyl]imidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (9a).** Silylation of 861 mg (3.3 mmol) of 6,7-dihydroimidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one hydrochloride (**4a**), solvated with 1 equiv of $Me_2SO$, with 1.82 mL of bis(trimethylsilyl)trifluoroacetamide and 4 mL of pyridine was carried out as previously described.[12]

The residue was suspended in 27 mL of $CH_3CN$, and the mixture was cooled to −20 °C. To this was added 0.76 mL (6.5 mmol) of anhydrous $SnCl_4$, and the mixture was stirred for 5 min, during which complete solution resulted. Neat, oily 2-(chloromethoxy)-1-(*p*-toluoyloxy)ethane[18] (1.24 g of ~90% purity by $^1H$ NMR, 4.9 mmol) was added all at once. The dark solution was maintained at −10 to −15 °C for 5.25 h and then poured into a solution of saturated aqueous $NaHCO_3$. Further workup as previously described for **5b** gave 308 mg (27%) of ketone **9a** following trituration from hot EtOAc: $Et_2O$; mp 188–191 °C; $R_f$ 0.21 (solvent Aa); NMR [$(CD_3)_2SO$] δ 2.39 (s, 3, $CH_3$), 3.65–4.0 (d overlapping m, 4, H-7 and side-chain $ROCH_2CH_2O$), 4.25–4.50 (m, 2, $ROCH_2CH_2O$), 5.50 (s, 2, anomeric H's), 7.25–7.60 (d overlapping d, 3, *p*-toluoyl H's and H-5), 7.75–8.0 (s, overlapping d, 3, *p*-toluoyl H's and H-2), 8.55 (br s, 1, NH); IR (KBr) 3260, 1712, 1655, 1520, 760 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 350 nm (ε 3830), 232 (32 990). Anal. ($C_{17}H_{18}N_4O_4 \cdot 0.2H_2O$) C, H, N.

**6,7-Dihydro-3-[(2-hydroxyethoxy)methyl]imidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (9b).** A stirred suspension of 110 mg (0.3 mmol) of 6,7-dihydro-3-[[2-(*p*-toluoyloxy)ethoxy]methyl]imidazo[4,5-*d*][1,3]diazepin-8(3*H*)-one (**9a**) in 4 mL of absolute MeOH was treated with sodium beads until TLC (solvent Aa) indicated complete deprotection of **9a**. Dry ice was added to neutralize the solution, and the solvent was evaporated. The solid residue was partitioned between $H_2O$ and EtOAc, and then the aqueous layer was purified over a C-18 reverse phase column by preparative HPLC (solvent Dc) to give a solid, which was boiled in MeOH (ca. 50 mg/50 mL). The solution was filtered hot, and the filtrate was then evaporated to ~5 mL. Addition of $Et_2O$ to the stirred solution, followed by filtration of the precipitate and drying, afforded 45 mg (61%) of **9b**: mp 190 °C dec; $R_f$ 0.44 (solvent E); NMR [$(CD_3)_2SO$] δ 3.52 (s, 4, $HOCH_2CH_2O$), 3.80 (s, 2, H-7), 4.70 (br s, 1, OH), 5.42 (s, 2, anomeric H's), 7.49 (s, 1, H-5), 7.82 (s, 1, H-2), 8.50 (br s, 1, NH); IR (KBr) 3440, 3240, 1665, 1605 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 349 nm (ε 3750), 299 (2940), 251 (5980), 227 (21 680). Anal. ($C_9H_{12}N_4O_3$) C, H, N.

**(±)-3,6,7,8-Tetrahydro-3-[(2-hydroxyethoxy)methyl]imidazo[4,5-*d*][1,3]diazepin-8-ol (2).** The solid residue resulting from reaction of 171 mg (0.5 mmol) of ketone **9b** in 10 mL of absolute MeOH and excess sodium as described above was dissolved in 25 mL of $H_2O$, and the solution was treated with 40 mg (1.1 mmol) of sodium borohydride. After stirring at ~25 °C for 30 min, the solution was adjusted to pH 7 with acetic acid. The aqueous solution was washed with EtOAc and then reduced to 3–5-mL volume. Purification over HP-20 resin, eluting first with $H_2O$ then solvent Dc, gave 84 mg of a solid, which was dissolved in a minimum volume of MeOH. The solution was added to vigorously stirring $Et_2O$, and the precipitate was collected by filtration and then dried to afford 56 mg (45%) of **2**: mp 122–125 °C; $R_f$ 0.24 (solvent E); NMR [$(CD_3)_2SO$] δ 3.21 (d, 2, *J* = 3 Hz, H-7), 3.50 (s, 4, $HOCH_2CH_2O$), 4.86 (t, 1, *J* = 4 Hz, H-8), 5.32 (s, 2, anomeric H's), 7.03 (s, 1, H-5), 7.50 (s, 1, H-2); IR (KBr) 3300, 1630 1360 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 283 nm (ε 8065). Anal. ($C_9H_{14}N_4O_3 \cdot 0.3H_2O$) C, H, N.

**Biological.** For the determination of inhibition constants ($K_i$), adenosine deaminase (EC 3.5.4.4; type I; intestinal mucosa) was obtained from Sigma Chemical Co., St. Louis, Mo. Adenosine deamination was measured by following the decrease of absorbance at 265 nm for the conversion of adenosine to inosine. The assays for the Ackermann–Potter plots were performed at pH 7.5 (phosphate buffer 0.05 M) and ambient temperature with substrate (adenosine at $1 \times 10^{-4}$ M). Inhibitors were incubated with the enzyme (1–8 U/L) for 1 h at room temperature prior to initiation of the enzymatic reaction by the addition of adenosine. Concentrations of the inhibitors were 0.1–0.9 nM for pentostatin, 35–346 nM for **1b**, 1360–10180 nM for **1c**, and 360–4260 nM for **2**.

$K_i$ values for the nontight binding or readily reversible inhibitors were determined by a Michaelis–Menten analysis using standard double-reciprocal and Dixon plots. Adenosine deaminase activity was assayed by the technique of Kalckar.[25] Adenosine concentrations ranged from 3 to 97 μM, and the inhibitor concentrations were 87–346 nM for **1b** 2000–8000 nM for **1c**, and 360–1420 nM for **2**. Spectrophotometric determinations were carried out at room temperature with an Aminco DW-2a spectrophotometer operating in the double-beam mode.

Antiviral studies were conducted by a slight modification of a procedure previously described.[23]

**Acknowledgment.** The authors thank L. M. Werbel for administrative support, F. M. MacKellar and associates for the acquisition of microanalytical and spectral data, and J. Mitulski for enzyme assays. This work was supported in part by the National Cancer Institute of the National Institutes of Health under Contract N01-CM23708.

**Registry No.** **1a**, 53910-25-1; **1b**, 86646-87-9; **1c**, 86688-40-6; (±)-**2**, 86646-88-0; **3**, 69195-92-2; **4a**, 86646-89-1; **4b**, 86668-12-4; **4c**, 86668-13-5; **5b**, 86646-90-4; **5c**, 86646-91-5; **6b**, 86646-92-6; **6c**, 86646-93-7; **7b**, 86646-94-8; **7c**, 86646-95-9; (8*R*)-**8b**, 86646-96-0; (8*S*)-**8b**, 86646-97-1; **9a**, 86646-98-2; **9b**, 86646-99-3; 2-deoxy-3,5-di-*o*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride, 3601-89-6; 2-(chloromethoxy)-1-(*p*-toluoyloxy)ethane, 80585-30-4; adenosine deaminase, 9026-93-1.

(25) Kalckar, H. M. *J. Biol. Chem.* **1947**, *167*, 461–475.