H, ArH), 4.59 (m, 1 H, CH), 4.16 (q, 2 H, CH_2), 1.18 (t, 3 H, CH_3), 1.16 (d, 6 H, 2 × CH_3).

4-Isopropoxybenzyl Alcohol (73). Ethyl 4-isopropoxybenzoate (25.6 g, 123 mmol) was added dropwise over 30 min to a refluxing solution of Vitride (70% solution in toluene) (42 mL, 150 mmol) and toluene (100 mL). After 1 h additional Vitride (25 mL) and toluene (50 mL) were added. After 4 h additional Vitride (50 mL) was added, and the solution was refluxed for 2 h. The reaction was cooled to ambient temperature, added dropwise to 6 N hydrochloric acid (600 mL), and stirred for 16 h. Toluene (200 mL) was added with stirring, and the layers were separated. The aqueous phase was washed with toluene (150 mL), and the combined toluene extracts were washed with water (2 × 450 mL). The organic layer was filtered and concentrated to an oil under reduced pressure. The oil was dissolved in ethanol and reevaporated. The residual oil was dissolved in ethyl acetate and added to 50 g of silica gel 60. The volatiles were removed by spin evaporation in vacuo, and the residual solids were added to a column (7 cm × 18 cm) of silica gel 60 wetted with hexane. The column was eluted with ethyl acetate–hexane (1:30), and the appropriate fractions were combined and spin evaporated in vacuo to give 8.45 g (41%) of 4-isopropoxybenzyl alcohol:[23] NMR (DMSO-d_6) δ 6.94 (AB q, 4 H, ArH), 4.45 (m, 1 H, CH), 4.27 (s, 2 H, CH_2), 3.14 (s, 1 H, OH), 1.09 (d, 6 H, CH_3).

Acknowledgment. We thank Mr. C. J. Bradley and Mr. A. Emmerson for skilled technical assistance in determining the antiviral activities. Dr. B. S. Hurlbert and has staff provided some of the NMR. We acknowledge the assistance of Ms. T. Cozart, S. Paris, and D. Tabon in preparation of the manuscript and thank Mr. Allen Jones for proofreading the manuscript.

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Nucleic Acid Related Compounds. 57. Synthesis, X-ray Crystal Structure, Lipophilic Partition Properties, and Antiretroviral Activities of Anomeric 3′-Azido-2′,3′-dideoxy-2,6-diaminopurine Ribosides[1]

Morris J. Robins,*[,†] Steven G. Wood,[†] N. Kent Dalley,[2,†] Piet Herdewijn,[‡] Jan Balzarini,[‡] and Erik De Clercq[‡]

Department of Chemistry, Brigham Young University, Provo, Utah 84602, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. *Received January 9, 1989*

Trimethylsilyl triflate-catalyzed transfer glycosylation of 2,6-diacetamidopurine (**2**) with 3′-azido-3′-deoxythymidine (AZT, **1**) as donor followed by deprotection gave 2,6-diamino-9-(3-azido-2,3-dideoxy-α- and -β-D-*erythro*-pentofuranosyl)purines (**3** and **4**) in low yields. Selective 2′-O-tosylation of 2,6-diamino-9-(β-D-ribofuranosyl)purine (2,6-diaminopurine riboside, DAPR, **5**) followed by our lithium triethylborohydride promoted 1,2-hydride rearrangement gave 2,6-diamino-9-(2-deoxy-β-D-*threo*-pentofuranosyl)purine (**7**). Tritylation of **7** followed by mesylation at O3′, deprotection, and displacement of the 3′-mesylate with azide provided a stereodefined synthesis of 2,6-diamino-9-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)purine (AzddDAPR, **4**). X-ray crystallographic analysis of **4** showed two orientations of the azido group, but consistent conformational features in the remainder of the molecule. In contrast, two independent conformations have been found for AZT. The azido function confers enhanced lipophilicity, which could be expected to contribute significantly to nonselective transport across membranes. A large difference in the octanol/water partition coefficients of the α (**3**) and β (**4**) anomers was found. The β anomer (**4**) exerts potent inhibition of HIV-induced cytopathogenicity in human MT-4 cells (ED_{50}: 0.3 μM). This concentration is an order of magnitude lower than that required for ddDAPR, AzddAdo, and AzddGuo. Potent inhibition of Moloney sarcoma virus induced transformation of murine C3H cells by AzddDAPR (**4**) was also observed. The α anomer (**3**) had no observed antiviral activity.

The discovery that 3′-azido-3′-deoxythymidine (AZT, **1**) exerts potent inhibitory activity against the human immunodeficiency virus (HIV), considered to be the causitive agent for acquired immunodeficiency syndrome (AIDS), has stimulated extensive efforts in nucleoside chemistry and biomedical applications.[3] A number of pyrimidine[4,5a] and purine[5] nucleoside analogues with the 2′,3′-dideoxypentofuranosyl skeleton have been prepared and tested. This includes the parent 2′,3′-unsaturated-2′,3′-dideoxy- and 2′,3′-dideoxynucleosides as well as 2′- or 3′-substituted-2′,3′-dideoxynucleoside derivatives. Attachment of an electronegative substituent at C3′,[5a,b] or especially at C2′,[5b,c] enhances the stability of such acid-sensitive purine nucleosides at lower pH values. However, it also markedly reduces the antiviral selectivity relative to that of the parent dideoxynucleoside in most cases, except for substitution of the *pro-S* hydrogen at C3′ by azide.[5a,b]

We have reported potent anti-HIV activity and enzymatic deamination of 2,6-diamino-9-(2,3-dideoxy-β-D-

† Brigham Young University.
‡ Katholieke Universiteit Leuven.

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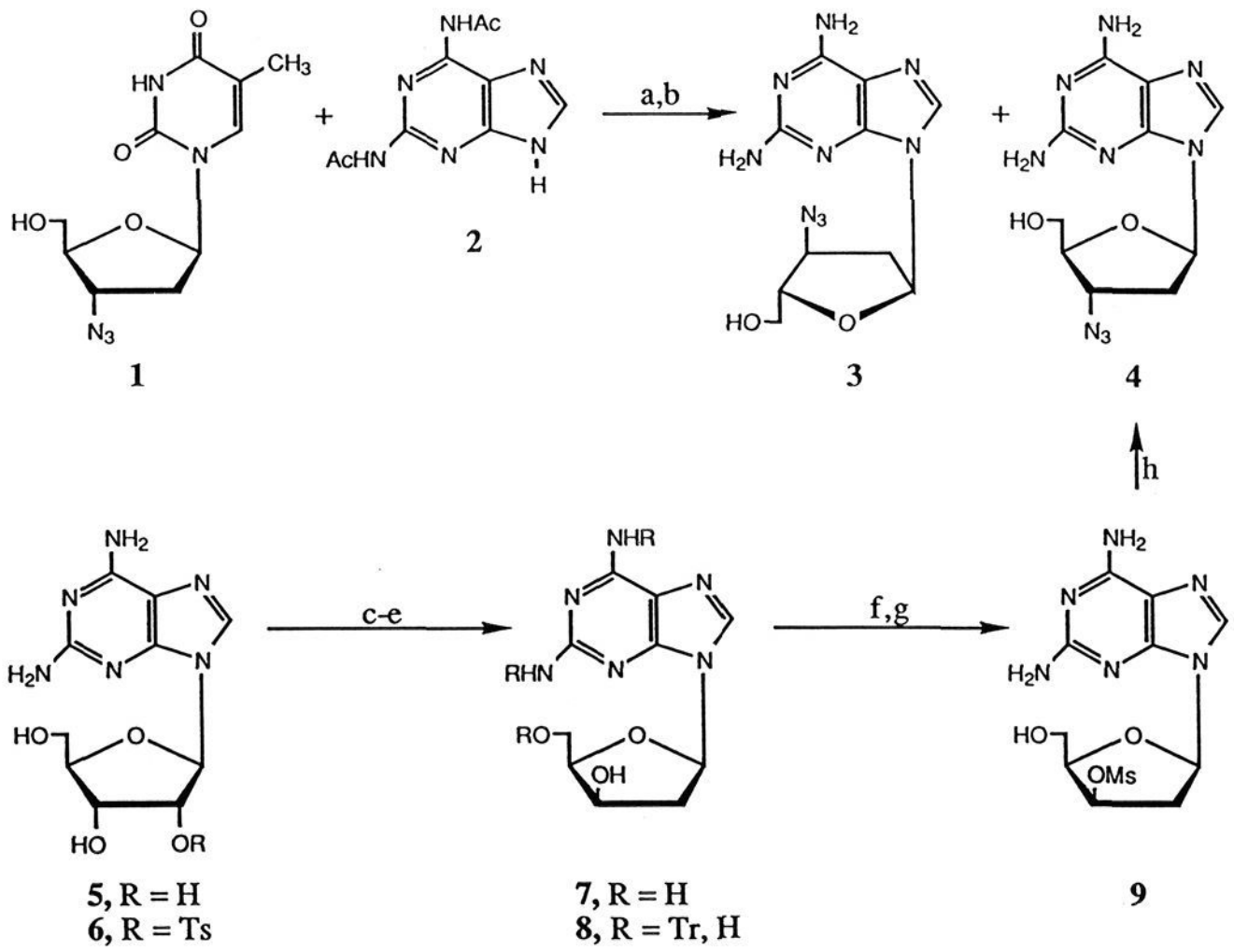
Scheme I[a]



1 2 3 4

5, R = H
6, R = Ts

7, R = H
8, R = Tr, H

9

[a] (a) (i) Bis(trimethylsilyl)acetamide/MeCN/Δ; (ii) trimethylsilyl triflate/Δ. (b) NH_3/MeOH/H_2O/Δ. (c) (i) Bu_2SnO/MeOH/Δ; (ii) TsCl/Et_3N. (d) $LiEt_3BH$/THF/DMSO. (e) TrCl/Pyridine. (f) MsCl/Pyridine. (g) HOAc/H_2O (8:2)/80 °C. (h) LiN_3/DMF/100 °C.

glycero-pentofuranosyl)purine (2′,3′-dideoxy-2,6-diaminopurine riboside, ddDAPR)[6] and biological studies on 3′-azido and 3′-fluoro derivatives of 2,6-diamino-9-(β-D-ribofuranosyl)purine (2,6-diaminopurine riboside, DAPR) and related purine nucleoside analogues.[7] We now describe chemical syntheses, crystal structural features, and lipophilic partitioning. Antiretroviral activities of 2,6-diamino-9-(3-azido-2,3-dideoxy-α- and -β-D-*erythro*-pentofuranosyl)purine (α- and β-AzddDAPR, **3** and **4**) are compared with related reference compounds.

Chemistry

The trimethylsilyl triflate catalyzed transfer glycosylation procedure reported by Imazawa and Eckstein for the preparation of AzddGuo[8] was employed for the first synthesis of AzddDAPR, with 3′-azido-3′-deoxythymidine (AzdThd, AZT, **1**) as the glycosyl donor and 2,6-diacetamidopurine[9] (**2**) as acceptor. Thus, a mixture of **1** and **2** in acetonitrile was treated with bis(trimethylsilyl)acetamide to give a clear solution. Trimethylsilyl triflate was then added, and the solution was heated at reflux. Attempts were made to increase the low yields realized with this procedure, but <25% of the deprotected mixture of α- (**3**) and β-AzddDAPR (**4**) was obtained. The glycosyl configurations of **3** and **4** were indicated by the empirical anomeric proton NMR line shape criterion[10] with the expected "apparent triplet" observed for the H1′ peak of the β (**4**) and a doublet of doublets for the α (**3**) anomer. X-ray crystallographic analysis of **4** verified the β configuration.

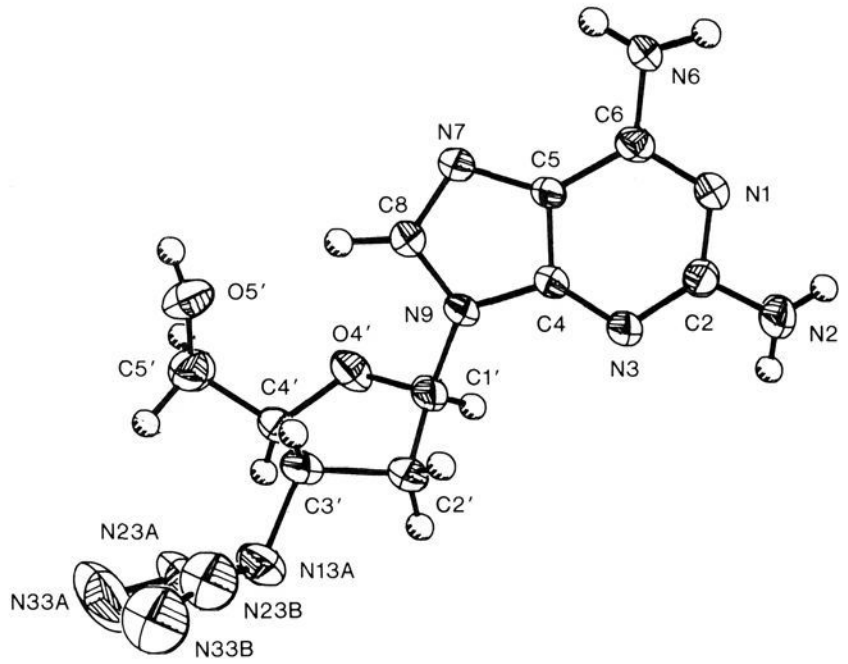


Figure 1.

A more effective route utilized treatment of 2,6-diamino-9-(β-D-ribofuranosyl)purine (DAPR, **5**) with di-

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Table I. Antiretrovirus Activity of α-AzddDAPR and β-AzddDAPR

compd	HIV-induced cytopathogenicity in human MT-4 cells			MSV-induced transformation of murine C3H cells	
	ED_{50},[a] μM	CD_{50},[b] μM	sel index[c]	ED_{50},[d] μM	TD,[e] μM
α-AzddDAPR	>50	36 ± 7.5		>200	>200
β-AzddDAPR	0.3[f]	44[f]	147	1.56 ± 0.17	>200
ddDAPR	3.5[f]	404[f]	115	19[f]	>200[f]
AzddGuo	2.8[f]	165[f]	59	6.2[f]	>400[f]
AzddAdo	5[g]	10[g]	2	69[g]	>200[g]

[a] 50% effective dose, or dose required to inhibit HIV-induced cytopathogenicity in MT-4 cells by 50%. [b] 50% cytotoxic dose, or dose required to reduce MT-4 cell viability by 50%. [c] Selectivity index (CD_{50}/ED_{50}). [d] Dose required to inhibit MSV-induced C3H cell transformation by 50%. [e] Toxic dose, or dose required to cause an alteration of normal cell morphology upon microscopic examination. [f] See ref 7. [g] See ref 6c.

butyltin oxide and *p*-toluenesulfonyl chloride in methanol[11] to give 2′-*O*-tosyl-DAPR (**6**). Our 1,2-hydride shift rearrangement[12] of **6** with lithium triethylborohydride gave 2,6-diamino-9-(2-deoxy-β-D-*threo*-pentofuranosyl)purine (**7**) in 94% yield. Tritylation of **7** in the usual manner gave a mixture of *N*,5′-*O*-ditrityl and *N*,*N*,5′-*O*-tritrityl derivatives, each in 47% yield. The tritrityl compound was treated with methanesulfonyl chloride in pyridine, and the crude product obtained after workup was deprotected[13] with 80% aqueous acetic acid at 80 °C to give 2,6-diamino-9-(2-deoxy-3-*O*-methanesulfonyl-β-D-*threo*-pentofuranosyl)purine (**9**) in 57% yield for the two steps. Treatment of **9** with lithium azide in dimethylformamide at 100 °C gave the desired AzddDAPR (**4**) in 85% yield.

X-ray Crystal Structure of AzddDAPR (4)

A composite computer-generated drawing of **4** with both orientations of the disordered azido group is shown in Figure 1. The positions of two fractional sets of nitrogen atoms (N23 and N33) were resolved by using difference maps, but with lower precision than for the base and sugar atoms. The other fractional nitrogen atoms (N13) of the azide group could not be resolved. There is a 70% ("A" atoms) to 30% ("B" atoms) distribution between the two populated sites with N–N–N angles of 170.0 (5)° in the more populated (A) and 165 (1)° in the less (B). It is of interest to compare the orientations of the azido group of **4** with those in AZT (**1**) and related structures with the same pentofuranosyl moiety. At least six X-ray analyses of the crystal structure of **1** have been reported.[14] The unit cell of AZT contains two crystallographically independent molecules. The azido group of AZT is directed away from the sugar ring in both molecules with C2′–C3′–N–N torsion angles of 177.5 (3)° and 176.2 (3)°.[14f] The disordered azido group in **4** also is directed away from the sugar ring, but to lesser extents with C2′–C3′–N–N torsion angles of 158.9 (3)° for the "A" and 124.1 (6)° for the "B" sites. Four different molecular conformations were observed for 1-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-ethyluridine (3′-azido-2′,3′-dideoxy-5-ethyluridine).[14f] One of these molecules (labeled CS-85D[14f]) had an azido group disordered similarly to that found by us with N–N–N angles of 174 (1)° and 168 (1)° [4 has 170.0 (5)° and 165 (1)°]. However, the C2′–C3′–N–N torsion angles for CS–85D[14f] were −26 (1°) and 6 (1)° in contrast to the analogous angles of 158.9 (3°) and 124.1 (6°) in **4**. These angles are closer to their CS–85A molecule[14f] in which only one orientation of the azide group was found with a C2′–C3′–N–N torsion angle of 150.0 (4)°.

The base moiety of **4** is planar as expected with the largest deviation from the ring least-squares plane at N3 (0.0175 Å). The sugar ring has a pseudorotation angle of 13.2° ($^3T_2$ conformation range).[15] The C4–N9–C1′–O4′ torsion angle is −141.3 (2)° (anti range). There is an HC8–O5′ intramolecular interaction (HC8–O5′ interatomic distance: 2.38 Å) between the base and the sugar in **4** analogous to that involving HC6–O5′ in AZT. This constrains the C3′–C4′–C5′–O5′ torsion angle [51.6 (3)°] in **4** to the g^+ range. There are five hydrogen atoms bonded to nitrogen or oxygen atoms in **4**. However, only the three hydrogens bonded to O5′ and N6 are involved in strong hydrogen bonding (with the intermolecular binding interactions: HO5′–N3, HN61–N1, HN62–N7). The hydrogen atoms of N2 do not participiate in significant hydrogen bonding as defined by criteria that H···A interatomic distances should be significantly shorter than the sum of the van der Waals radii of H and A. When A is O or N the sum of the radii are 2.6 or 2.7 Å, respectively. However, there are weak interactions with the H···A distances less than 0.2 Å of the limiting values (see supplementary material, Table III). While both the H···A and D···A interatomic distances are rather long to be considered hydrogen bonds, the fact that angles at the hydrogen atoms are within 30° of 180° suggests that they do have significance in affecting the crystal structure. The weaker interactions involving N2 and its hydrogens are consistent with a large equivalent isotropic thermal parameter of N2 relative to that of N6.

Biological Results and Discussion

The α (**3**) and β (**4**) anomers of AzddDAPR were evaluated for antiretroviral activity in comparison with the parent ddDAPR and the "natural" purine analogues 3′-azido-2′,3′-dideoxyadenosine (AzddAdo) and 3′-azido-2′,3′-dideoxyguanosine (AzddGuo). As seen in Table I, the α anomer (**3**) was devoid of any anti-HIV activity at subtoxic concentrations (ED_{50} = >50 μM) and also failed to inhibit MSV-induced transformation of C3H cells at 200 μM. In contrast, AzddDAPR (**4**) exerted very potent inhibition of HIV-induced cytopathogenicity in MT-4 cells

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Table II. Partition Coefficients for Purine 2′-Deoxy-, 2′,3′-Dideoxy-2′,3′-didehydro-, 2′,3′-Dideoxy-, and 3′-Azido-2′,3′-dideoxynucleoside Analogues

compd	partition coeff P
dAdo	0.283 ± 0.06
ddeAdo	0.440 ± 0.03
ddAdo	0.605 ± 0.12
AzddAdo	2.249 ± 0.16
dDAPR	0.300 ± 0.09
ddeDAPR	0.333 ± 0.05
ddDAPR	0.344 ± 0.05
α-AzddDAPR (3)	1.004 ± 0.13
β-AzddDAPR (4)	1.725 ± 0.12
dGuo	0.050 ± 0.02
ddeGuo	0.061 ± 0.01
ddGuo	0.098 ± 0.05
AzddGuo	0.472 ± 0.05

(ED_{50} = 0.3 μM) with toxicity at 2 orders of magnitude higher concentration (CD_{50} = 44 μM).[7] AzddDAPR (4) was active against HIV at an order of magnitude lower concentration than the parent ddDAPR and also the AzddAdo and AzddGuo analogues. It was also superior to ddDAPR, AzddAdo, and AzddGuo in inhibiting MSV-induced transformation of C3H cells (see Table I). It is clear that AzddDAPR (4) has the highest selectivity index (CD_{50}/ED_{50} ratio) of the 3′-azido-2′,3′-dideoxy purine nucleoside analogues presently examined. However, the ratios are not significantly different for AzddDAPR and ddDAPR.

It has recently been shown that AzdThd (AZT, 1) permeates cell membranes independently of the nucleoside transport carrier, and this is likely related to its enhanced lipophilic character.[16] We have previously speculated that the greater lipid solubility of AzddDAPR (4) may result in its more rapid entry into cells and thereby account for its more pronounced anti-HIV activity relative to that of its parent ddDAPR.[7] Although cellular uptake studies with radiolabeled AzddDAPR will be required to support this hypothesis, we have determined that the azido function uniformly confers enhanced lipophilicity upon all of the nucleosides that we have examined.

Partitioning of a number of nucleosides between 1-octanol and an aqueous potassium phosphate buffer has been measured to evaluate relative lipid solubilities. Table II contains partition coefficient data for the 2′-deoxy-β-D-*erythro*-pentofuranosyl (2′-deoxyriboside), 2′,3′-dideoxy-β-D-*glycero*-pent-2′-enofuranosyl (2′,3′-dideoxy-2′,3′-didehydroriboside), 2′,3′-dideoxy-β-D-*glycero*-pentofuranosyl (2′,3′-dideoxyriboside), and 3′-azido-2′,3′-dideoxy-β-D-*erythro*-pentofuranosyl (3′-azido-2′,3′-dideoxyriboside) 9-nucleosides of adenine, 2,6-diaminopurine, and guanine. It is seen that minor chemical changes in the sugar moiety significantly influence the relative lipid solubilities. Parallel effects on the lipid partitioning were observed with a given change in the sugar irrespective of the structure of the purine (or pyrimidine, data not shown) base. Lipid solubilities of the 2′,3′-dideoxy-2′,3′-didehydro compounds were uniformly greater than those of the corresponding 2′-deoxynucleosides, and the 2′,3′-dideoxy derivatives were in turn more lipid soluble than their 2′,3′-dideoxy-2′,3′-didehydro precursors. A dramatic increase in lipophilicity was observed with all 3′-azido-2′,3′-dideoxynucleosides relative to the corresponding parent 2′,3′-dideoxy compounds. The adenine and 2,6-diaminopurine products were especially lipophilic (AzddAdo, P = 2.249; AzddDAPR, P = 1.725). The increase in lipophilicity of AzddDAPR relative to ddDAPR (P = 1.725 vs. 0.344) is slightly greater than that for AzdThd relative to dThd (P = 0.964 vs. 0.233). It is remarkable that the relative lipid solubilities of the α (3) and β (4) diastereomers are so different. Inversion of the anomeric configuration of AzddDAPR (4, P = 1.725) resulted in a reduction of the lipophilicity of 3 (P = 1.004) to a value comparable with that of the pyrimidine β analogue AzdThd (1, P = 0.964).

It is clear that the pronounced antiretroviral activity of AzddDAPR justifies its further evaluation as a putative new therapeutic agent. The demonstration of its activity as an efficient substrate of adenosine deaminase suggests its role as a prodrug of AzddGuo.[7] However, its relative effectiveness as an inhibitor of HIV- and MSV-induced transformation in the presence of the potent adenosine deaminase inhibitor 2′-deoxycoformycin suggests that it may also function as an antiviral agent per se, in contrast to results with its parent ddDAPR.[7] Additional studies with this promising new agent are in progress.

Experimental Section

General experimental considerations and equipment have been described.[5b,17] Column chromatography was performed on Merck silica gel. Acetonitrile was refluxed for several hours with and distilled from P_4O_{10}. Pyridine was refluxed overnight with and distilled from potassium hydroxide. Methanol was refluxed overnight with and distilled from magnesium/iodine. Dimethylformamide and dimethyl sulfoxide were purified by distillation in vacuo.

2,6-Diamino-9-(3-azido-2,3-dideoxy-α- and -β-D-*erythro*-pentofuranosyl)purine (3 and 4). Method A. To a magnetically stirred suspension of AzdThd (1, 134 mg, 0.5 mmol) and 2,6-diacetamidopurine[9] (2, 210 mg, 0.92 mmol) in 4 mL of dried acetonitrile was added bis(trimethylsilyl) acetamide (1 mL, ~4 mmol). The mixture was heated to reflux, and heating was continued for 15 min after a clear solution was obtained. The solution was cooled, and trimethylsilyl trifluoromethanesulfonate (120 μL, ~0.9 mmol) was added. This solution was heated at reflux for 2.5 h, cooled to ambient temperature, and 4 mL of methanolic ammonia (presaturated at 0 °C) was added. Stirring was continued for 20 min at ambient temperature, precipitated solids were filtered, and the filtrate was evaporated. This sequence was repeated four times at the noted 0.5-mmol scale, and the combined residues were dissolved in MeOH (7 mL). An equal volume of concentrated NH_3/H_2O was added, and the solution was heated for 18 h at 80 °C in a small Teflon-lined pressure vessel. The cooled solution was evaporated, and the residue was dissolved in H_2O and applied to a column (2 × 10 cm) of Dowex 1 × 2 (OH^-) resin packed in H_2O. Elution with H_2O gave a reasonably good separation of the anomers. Fractions containing only the faster migrating 4 were combined and evaporated, and the residue was recrystallized from H_2O to give 15 mg (3%) of pure 4 as colorless prisms: mp 239–241 °C dec; ^1H NMR δ 6.15 ("t", J = 6.5 Hz, H1′), 7.93 (s, H8); MS m/z 291.1195 ($M^+[C_{10}H_{13}N_9O_2]$ = 291.1192). See method B below for further data.

Fractions containing primarily 3 were combined and evaporated to give 15 mg (3%) of product. This material was dissolved in MeOH, adsorbed on 0.5 mL of silica gel by evaporation, and applied to a small column (0.3 × 10 cm) of silica gel packed in CH_2Cl_2. The column was eluted with successive 5-mL volumes of CH_2Cl_2/MeOH containing 0–25% MeOH in 5% increments. Fractions containing pure 3 were combined, evaporated, and subjected to reversed-phase HPLC. The product then crystallized spontaneously to give 3: mp 149–152 °C; ^1H NMR δ 6.18 (d of d, J = 4.8 and 7.2 Hz; H1′), 7.88 (s, H8); MS m/z 291.1193 ($M^+[C_{10}H_{13}N_9O_2]$ = 291.1192); UV (H_2O) max 282 and 256 nm (ε 10 200 and 10 300). Anal. ($C_{10}H_{13}N_9O_2 \cdot 0.35H_2O$) C, H, N. A second analysis was consistent with increasing amounts of H_2O being absorbed by the sample after drying.

X-ray Crystallography of 4. A suitable colorless crystal (0.25 × 0.35 × 0.50 mm) of 4 prepared by method A was mounted on

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Table III. Crystal and Experimental Data

formula	$C_{10}H_{13}N_9O_2$
formula weight	291.3
$F(000)$	608
crystal size, mm	0.25 × 0.35 × 0.50
μ, cm^{-1}	1.07
space group	$P2_12_12_1$
a, Å	7.665 (2)
b, Å	9.244 (3)
c, Å	17.954 (7)
V, Å^3	1272.0 (6)
Z	4
d, g/cm^3	1.52
$(\sin\theta)/\lambda$	0.70
total data	2239
unique obsd data	1686
unobsd data	454
data/parameters	7.9
max shift/esd	0.09
R	0.054
R_w	0.048
extinction parameter	2.0×10^{-5}
GOOF	1.61
largest peak in Δ map	0.27, −0.25

a Nicolet R3 diffractometer with monochromated Mo Kα (λ = 0.71073 Å) radiation. Lattice parameters and the orientation matrix of the crystal were calculated by means of a least-squares procedure involving 25 carefully centered reflections with $6.91 < 2\theta < 24.86$. The lattice parameters of the orthorhombic unit cell were a = 7.665 (2), b = 9.244 (3), and c = 17.954 (7). Systematic extinctions established the space group to be $P2_12_12_1$. Intensity data were collected to a $(\sin\theta)/\lambda$ limit of 0.70. A total of 2239 data collections were measured with a variable-speed (4–30°/min) θ–2θ scan procedure. These data included 75 standard reflections and 24 systematic extinctions ($h00$, $h = 2n + 1$, $0k0$, $k = 2n + 1$, and $00l$, $l = 2n + 1$). The data set was merged to 2140 unique data of which 1686 were observed and 454 were unobserved at $F < 3\sigma(F)$. In addition to the Lorentz and polarization corrections, an empirical extinction correction was applied to the data. Crystal and experimental data are summarized in Table III.

The trial structure was obtained by using direct methods. All non-hydrogen atoms were located in the initial E-map. Subsequent difference maps revealed that the azide group was disordered. The disorder of N23 and N33 was resolved, and the fractional atoms are labeled N23A and N23B, and N33A and N33B. However, the disorder of the proximal N13A atom was not resolved. Population parameters of the atoms were assigned from consideration of peak heights in difference maps and resulting thermal parameters. Occupancy factors for the more populated "A" atom sites were fixed at 0.70 and those of the less populated "B" sites at 0.30. The final R values were R = 0.054 and R_w = 0.048 with the weights based on counting statistics.

Tables of atomic parameters, bond lengths and bond angles, and hydrogen bonds are deposited as supplementary material (see paragraph at end of paper).

2,6-Diamino-9-(2-*O*-*p*-toluenesulfonyl-β-D-ribofuranosyl)purine (6). A stirred mixture of DAPR (**5**, 2.82 g, 10 mmol) and dibutyltin oxide (2.5 g, 10 mmol) in 250 mL of MeOH was refluxed for 2 h and cooled to ambient temperature. Triethylamine (21 mL) and *p*-toluenesulfonyl chloride (28 g) were added, and the mixture was stirred for 15 min. Solvent was evaporated, and the residue was partitioned between H_2O (250 mL) and Et_2O (250 mL). The aqueous layer was concentrated to 100 mL and stored overnight in a refrigerator. Crystalline **6** (1.42 g, 30%) was collected by filtration. TLC indicated that the filtrate contained essentially pure **6** that failed to crystallize, but which could be utilized after further purification. Crystalline **6** had mp 134 °C (softening): ^{1}H NMR δ 2.31 (s, CH_3), 5.42 (dd, J = 4.8 and 7.5 Hz, H2′), 5.93 (d, J = 7.5 Hz, H1′), 7.76 (s, H8); MS m/z 436 (M^+); UV (MeOH) max 282 and 257 nm (ε 10 400 and 10 300). Anal. ($C_{17}H_{20}N_6O_6S\cdot 2H_2O$) C, H, N.

2,6-Diamino-9-(2-deoxy-β-D-*threo*-pentofuranosyl)purine (7). To a solution of **6** (2 g, 4.6 mmol) in 40 mL of dry dimethyl sulfoxide was added a solution of lithium triethylborohydride in tetrahydrofuran (50 mL, 1 M), and the resulting solution was allowed to stand at ambient temperature overnight. H_2O (10 mL) was added **cautiously** and the solution was concentrated to a syrup. The mixture was applied to a column of Dowex 1 × 2 (OH^-) resin in H_2O, and elution was begun with H_2O followed by MeOH/H_2O (1:1). The appropriate fractions were combined and concentrated in vacuo until crystallization began. Cooling overnight and filtration gave 1.15 g (94%) of **7**: mp 175–176 °c; ^{1}H NMR δ 2.05–2.35 (m, H2′), 2.45–2.97 (m, H2″), 6.08 (m, J = 2.2 and 8.8 Hz, H1′ and OH3′), 7.98 (s, H8); MS m/z 266.1128 (M^+ = 266.1127); UV (MeOH) max 280 and 256 nm (ε 10 800 and 10 000). Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

Tritylation of 7. A mixture of **7** (1.15 g, 4.3 mmol) and triphenylmethyl chloride (3.6 g, 12.9 mmol) in 50 mL of dry pyridine was stirred at ambient temperature for 3 days, and 5 mL of MeOH was added. The solution was evaporated, the residue was dissolved in $CHCl_3$, and this solution was washed with H_2O (2 × 100 mL), dried (Na_2SO_4), evaporated, and coevaporated with toluene. Column chromatography (silica gel, MeOH/$CHCl_3$, 2.5:97.5) gave 1.5 g (47%) of a *N*,5′-*O*-ditrityl compound (**8a**) and 2 g (47%) of a *N*,*N*,5′-*O*-tritrityl compound (**8b**) as indicated by chromatographic migration and integration of the ^{1}H NMR signals corresponding to the NH_2/NH/[$C(C_6H_5)_3$]$_2$ and NH/NH/[$C(C_6H_5)_3$]$_3$ protons at δ 5.20, 5.95, 7.00–7.57; and 5.30, 6.60, 6.95–7.62; respectively.

2,6-Diamino-9-(2-deoxy-3-*O*-methanesulfonyl-β-D-*threo*-pentofuranosyl)purine (9). A mixture of the *N*,*N*,5′-*O*-tritrityl derivative (**8b**, 2 g, 2 mmol) and methanesulfonyl chloride (0.47 mL, ~6 mmol) in 15 mL of dry pyridine was stirred for 2 days at ambient temperature, and then 1 mL of MeOH was added. The solution was evaporated in vacuo, and the oily residue was dissolved in $CHCl_3$ (50 mL), washed with H_2O (2 × 50 mL), dried (Na_2SO_4), evaporated, and coevaporated with toluene. The residue was treated with 20 mL of 80% aqueous acetic acid and heated for 30 min at 80 °C. The solution was evaporated and the residue purified by column chromatography (silica gel, MeOH/$CHCl_3$, 10:90) to give 390 mg (57%) of **9** with ^{1}H NMR δ 3.27 (s, CH_3SO_3), 6,15 (dd, J = 2.8 and 7.9 Hz, H1′), 7.77 (s, H8); UV (MeOH) max 280 and 256 nm (ε 10 800 and 10 000). Anal. ($C_{11}H_{16}N_6O_5S$) C, H, N.

2,6-Diamino-9-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)purine (4). Method B. A stirred solution of **9** (260 mg, 0.75 mmol) and lithium azide (370 mg, 7.5 mmol) in 10 mL of DMF was heated for 2 h at 100 °C, cooled, and evaporated and the residue purified by column chromatography (silical gel, MeOH/$CHCl_3$, 8:92). Crystallization of the product from Et_2O/MeOH gave **4** (186 mg, 85%) with mp ~222°C dec; NMR and MS data, see under method A above; UV (H_2O) max 280 and 256 nm (ε 10 800 and 10 200); IR (KBr) 2100 cm^{-1} (N_3). Anal. ($C_{10}H_{13}N_9O_2$) C, H, N.

Determination of the Lipophilicity of Nucleoside Analogues. To evaluate the relative lipid solubilities of the test compounds, partitioning between 1-octanol and 10 mM aqueous potassium phosphate buffer, pH 7.4, was measured as described.[7] Briefly, a 50 μM solution of the nucleoside analogue in the aqueous buffer was thoroughly mixed with an equal volume of octanol for 30 min. The mixture was then allowed to equilibrate for 1 h at ambient temperature, UV absorption was measured for the aqueous and alcohol phases, and the percentage of compound in each phase was calculated from the absorbance ratios.

Cells. MT-4 cells were a gift from Dr. N. Yamamoto, Yamaguchi University, Yamaguchi, Japan.

Viruses. HIV was obtained from the culture supernatant of an H9 cell line persistently infected with HTLV-III$_B$ kindly provided Dr. R. C. Gallo, National Cancer Institute, Bethesda, MD.[18] MSV was prepared from tumors induced upon intramuscular inoculation of 2–3-day-old NMRI mice.[19]

Antiviral Assays. The method for transformation of mouse embryo (C3H) cells by MSV has been described.[6a] Briefly, confluent monolayers of C3H cells in 1-mL wells of Tissue Culture

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Cluster plates (48 wells) were infected with 80 focus-forming units of MSV during 90 min. Medium was then replaced by 1 mL of fresh culture medium containing different concentrations of the test compounds. After 6 days of incubation at 37 °C, transformation of the cell cultures was monitored microscopically.

Determination of the cytopathic effect of HIV in human T-lymphocyte MT4 cells has been described.[6a,20] Briefly, MT-4 cells, subcultured 1 day before the start of the experiment, were adjusted to 5×10^5 cells/mL and infected with HIV (HTLV-III_B) at 400 $CCID_{50}$/mL. Then, 100 μL of the infected cell suspension was transferred to wells of a microtiter tray containing 100 μL of varying dilutions of the test compounds. After 5 days of incubation at 37 °C, the number of viable cells was recorded microscopically in a hematocytometer following the trypan blue exclusion procedure.

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Acknowledgment. We thank Danuta Madej, Ann Absillis, and Lizette van Berckelaer for excellent technical assistance and Mrs. M. C. Samano for evaluating the transfer glycosylation procedure. These investigations were supported by the American Cancer Society (Grant CH-405), Brigham Young University Development Funds, the AIDS Basic Research Programme of the European Community, Beligan Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Projects 3.0040.83 and 3.0097.87), and Belgian Geconcerteerde Onderzoeksacties (Project 85/90-79).

Registry No. 1, 30516-87-1; 2, 34079-37-5; 3, 121231-92-3; 4, 114753-52-5; 5, 2096-10-8; 6, 121231-93-4; 7, 116002-29-0; 8a, 121231-96-7; 8b, 121231-95-6; 9, 121231-94-5.

Supplementary Material Available: Tables of atomic parameters, bond lengths and bond angles, and hydrogen bonds (3 pages). Ordering information is given on any current masthead page.

^{31}P NMR Studies of the Kinetics of Bisalkylation by Isophosphoramide Mustard: Comparisons with Phosphoramide Mustard

Jila H. Boal,[†] Margaret Williamson,[§] Victoria L. Boyd,[†] Susan M. Ludeman,*[,†,‡] and William Egan*[,§]

Department of Chemistry, The Catholic University of America, Washington, D.C. 20064, Department of Chemistry, Trinity College, Washington, D.C. 20017, and Biophysics Laboratory, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892. *Received January 25, 1988*

^{31}P nuclear magnetic resonance spectroscopy was used to measure the pK_a (4.28 ± 0.2) of isophosphoramide mustard (IPM) at 20 °C and to study the kinetics and products of the decomposition of IPM at a solution pH value of ca. 7.4 and at temperatures between 20 and 47 °C in the presence of nucleophilic trapping agents. At 37 °C, the half-life for the first alkylation was ca. 77 min and ca. 171 min for the second alkylation; these data may be compared with those for phosphoramide mustard (Engle, T. W.; Zon, G.; Egan, W. *J. Med. Chem.* **1982**, *25*, 1347), wherein the half-lives for the first and second alkylations are approximately the same (18 min). The rate of fragmentation of aldoifosfamide to IPM and acrolein was also studied by NMR spectroscopy (pH 7.0; 37 °C; 0.07 M phosphate); under the noted conditions, the half-life of aldoifosfamide was found to be ca. 60 min.

Cyclophosphamide (**1a**) is a bisalkylating agent that has been shown to be therapeutically useful against a broad range of human cancers. Of the multitude of structural analogues arising from **1a**, ifosfamide (**1b**) is among the most notably effective.[1-6] It is generally accepted that Scheme I includes the metabolic transformations of **1a** and **1b** which are of cytotoxic significance.[2,3] Oxidation (activation) of **1a** or **1b** leads to 4-hydroxycyclophosphamide/aldophosphamide (**2a**) or 4-hydroxyifosfamide/aldoifosfamide (**2b**), respectively. Fragmentation of these aldehydic metabolites (**2a** and **2b**) produces acrolein and the ultimate DNA alkylating agents, phosphoramide mustard (**3a**) and isophosphoramide mustard (**3b**).

Although the metabolic transformations of cyclophosphamide and ifosfamide are similar, structural differences between the two isomers affect the relative rates of the corresponding transformations, resulting in differences in pharmacokinetics, toxicities, and therapeutic efficacies.[5,6] In certain circumstances, ifosfamide is the superior antitumor agent. For example, while cyclophosphamide shows little activity against soft tissue sarcomas, ifosfamide, as a single drug or in combination chemotherapy, shows promise; moreover, ifosfamide is at times useful for the treatment of tumors that have become resistant to cyclophosphamide.[6-12]

The present study focuses on the comparative alkylating abilities of phosphoramide mustard and isophosphoramide mustard. Since the oncostatic effects of **3a** and **3b** are related to the extent to which they cross-link DNA (i.e., bisalkylate DNA), we considered it necessary to study, in detail, their respective alkylation kinetics. Half-lives for **3a** and **3b** have been reported;[13-16] however, bisalkylation

[†] The Catholic University of America.
[‡] Trinity College.
[§] Food and Drug Administration.

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