(s, Me); ^{13}C NMR ($CDCl_3$) δ 163.22 (C4), 150.05 (C2), 142.67 (Ar), 134.42 (C6), 128.49 (Ar), 127.82 (Ar), 127.42 (Ar), 112.01 (C5), 87.74 (Ph_3C), 80.91 (C1'), 80.68 (t, C4'), 60.92 (C5'), 40.21 (t, J_{CF} = 23.35 Hz, C2'), 11.87 ppm (CH_3); ^{19}F NMR δ -98.16 (dq), -109.89 ppm (ddt); HRMS (FAB) calculated for (M + H) 505.1939, found 505.1926.

Preparation of 3',3'-Difluoro-3'-deoxythymidine (3). Deprotection of 5'-*O*-trityl-3',3'-difluoro-3'-deoxythymidine was accomplished by heating the compound in 80% acetic acid at 100 °C for 15 min and after prep-TLC (silica gel; chloroform/methanol [95/5] as solvent) and elution through a P-2 Biogel column 18.6 mg (66%) of 3',3'-difluoro-3'-deoxythymidine was isolated: ^1H NMR (DMSO-d_6) 7.65 (s, H6), 6.23 (dd, H1', $J_{1',2''}$ = 7.05 Hz, $J_{1',2'}$ = 8.1 Hz), 5.26 (t, 5'OH, J = 5.3 Hz), 4.11 (m, H4'), 3.68 (m, H5' and H5''), 2.85 (dddd, H2', $J_{1',2''}$ = 7.05 Hz, $J_{2',2''}$ = 14.7 Hz, $J_{2'F3'}$ = 9.0 Hz, $J_{2'',F3''}$ = 14.6 Hz), 2.66 (dddd, H2', $J_{1',2'}$ = 8.1 Hz, $J_{2'F3'}$ = 14.6 Hz, $J_{2',F3''}$ = 18.7 Hz), 1.80 ppm (s, Me); ^{13}C NMR (DMSO-d_6) 163.30 (C4), 150.09 (C2), 135.15 (C6), 127.0 (t, J = 250 Hz, C3'), 109.97 (C5), 80.80 (dd, J = 24.7 and 28.8 Hz, C4'), 80.14 (dd, J = 5.8 and 7.5 Hz, C1'), 58.33 (C5'), 12.15 (CH_3); the C2' resonance was obscured by DMSO; HRMS (FAB) calculated for (M + H) 263.0843, found 263.0842.

Biological Evaluation. Anti-HIV activity was determined by monitoring inhibition of cytopathic effect of HIV for MT-4 cells.[24,25]

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Synthesis and Antiviral Properties of (±)-5'-Noraristeromycin and Related Purine Carbocyclic Nucleosides. A New Lead for Anti-Human Cytomegalovirus Agent Design

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(±)-5'-Noraristeromycin (**3**) has been prepared in three steps beginning with the 2,3-*O*-isopropylidene derivative of (±)-(1α,2β,3β,4α)-4-amino-1,2,3-cyclopentanetriol (**7**). Also prepared from the same starting material were the related hypoxanthine (**4**), guanine (**5**), and 2,6-diaminopurine (**6**) analogues. Compounds **3**-**6** were evaluated for antiviral activity against a large number of viruses with marked activity being observed for **3** towards vaccinia virus, human cytomegalovirus, vesicular stomatitis virus, parainfluenza (type 3) virus, measles virus, respiratory syncytial virus, reovirus (type 1), and the arenaviruses Junin and Tacaribe. None of the compounds showed cytotoxicity to the host cell monolayers used in the antiviral studies. Both **3** and **6** have been found to be inhibitors of *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase), which likely accounts for their antiviral activity. Inhibition of AdoHcy hydrolase represents a new approach to human cytomegalovirus drug design that should be pursued. Also, the activity of **3** should be further scrutinized for the treatment of pox-, rhabdo-, paramyxo-, reo-, and arenavirus infections.

Carbocyclic nucleosides have become increasingly relevant to the design of biologically meaningful agents.[1] Aristeromycin (**1**)[2] is the carbocyclic nucleoside analogue of adenosine that displays antiviral properties as a result of its inhibition of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase.[3] The clinical potential of **1** is, however, limited

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(2) (±)-Aristeromycin was synthesized[2a,b] prior to isolation and structure proof of the biologically active[2c] (-)-enantiomer.[2d,e] (a) Shealy, Y. F.; Clayton, J. D. 9-[β-DL-2α,3α-Dihydroxy-4β-(hydroxymethyl)cyclopentyl]adenine, the Carbocyclic Analog of Adenosine. *J. Am. Chem. Soc.* **1966**, *88*, 3885-3887. (b) Shealy, Y. F.; Clayton, J. D. Synthesis of Carbocyclic Analogs of Purine Ribonucleosides. *J. Am. Chem. Soc.* **1969**, *91*, 3075-3083. (c) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Vanderhaeghe, H. Resolution of Aristeromycin Enantiomers. *J. Med. Chem.* **1985**, *28*, 1385-1386. (d) Kusaka, T.; Yamamoto, H.; Shibata, M.; Muroi, M.; Kishi, T.; Mizuno, K. *Streptomyces Citricolor* Nov. Sp. and a New Antibiotic Aristeromycin. *J. Antibiot. (Tokyo)* **1968**, *21*, 255-263. (e) Kishi, T.; Muroi, M.; Kusaka, T.; Nishikawa, M.; Kamiya, K.; Mizuno, K. The Structure of Aristeromycin. *Chem. Pharm. Bull.* **1972**, *20*, 940-946.

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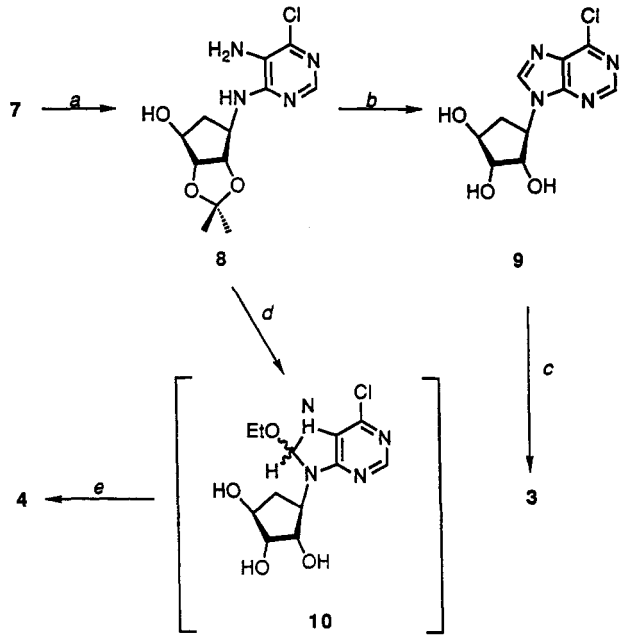
Scheme I[a]

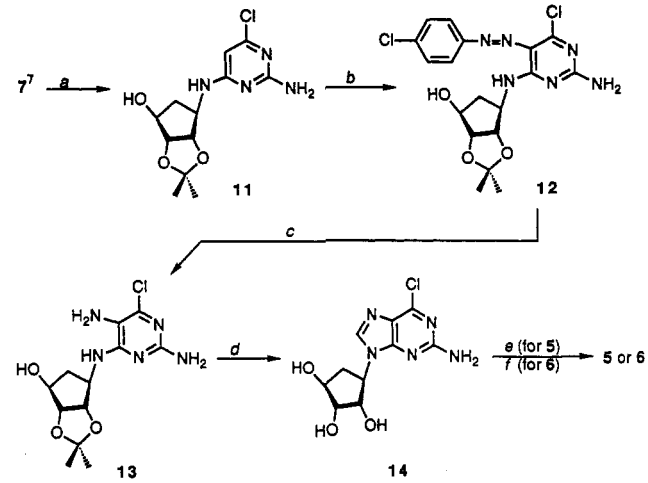


[a] Reaction conditions: (a) 5-amino-4,6-dichloropyrimidine in 1-BuOH containing Et_3N, 120 °C, 40 h; (b) (i) $AcOCH(OEt)_2$, 80–90 °C, 40 h; (ii) 0.5 N HCl in MeOH, room temperature, 30 min; (c) NH_3 in MeOH, 140 °C, 2 days; (d) (i) $(EtO)_3CH$, HCl (cat.), 110 °C, 24 h; (e) 1 N HCl, reflux, 5 h.

by its cytotoxicity, which has been attributed to metabolism to its 5′-phosphates.[4] Thus, the development of aristeromycin-based compounds with greater therapeutic indices requires compounds incapable of phosphorylation. Borchardt and his co-workers have addressed this situation for the dehydro aristeromycin derivative neplanocin A (**2**), which has biological properties similar to **1**, by preparing its derivative lacking the exocyclic hydroxymethyl substituent. They found that this analogue retained the potent antiviral properties of **2** but with less associated toxicity.[5]

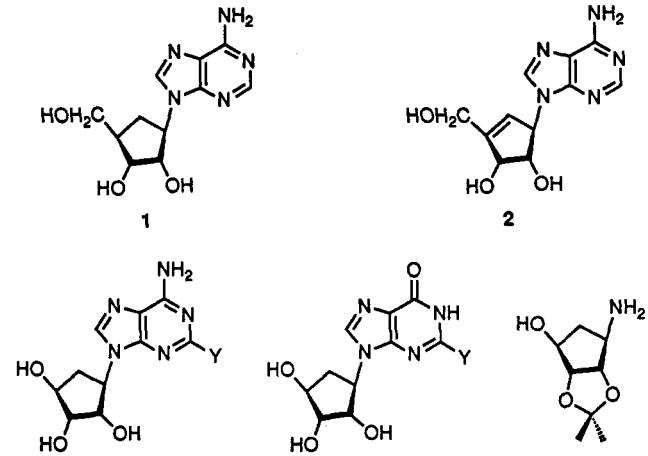
To systematically search for more useful aristeromycin derivatives, it seemed logical to begin with the 5′-nor compound **3** in which the phosphate accepting hydroxyl of **1** is displaced from its normal location. [It is interesting to note that a similar derivative (i) of **2** is not readily available due to the keto–enol tautomerism that would

Scheme II[a]



[a] Reaction conditions: (a) 2-amino-4,6-dichloropyrimidine in 1-BuOH containing Et_3N, reflux, 2 days; (b) 4-chlorobenzenediazonium chloride in H_2O containing $AcONa{\cdot}3H_2O$ and AcOH, room temperature, 18 h; (c) zinc dust–AcOH in $EtOH/H_2O$, reflux, 5 h; (d) (i) $AcOCH(OEt)_2$, room temperature then 80–85 °C; (ii) 0.5 N HCl in MeOH, room temperature, 30 min; (e) 1 N HCl, reflux, 5 h; (f) NH_3 in MeOH, 120–140 °C, 5 days.

exist and (ii) of adenosine is inaccessible since a hemiacetal would result that would break down to adenine and (2*R*,3*S*)-2,3-dihydroxysuccinaldehyde.] This paper reports the synthesis and antiviral properties of (±)-5′-noraristeromycin (**3**). The related hypoxanthine (**4**), guanine (**5**), and 2,6-diaminopurine (**6**) derivatives were also conveniently available during this study and, as a consequence, their synthesis[6] and antiviral properties are similarly described. A correlation of the antiviral activity of **3** and **6** with their ability to inhibit *S*-adenosyl-L-homocysteine hydrolase is also reported.



Chemistry

The 2,3-*O*-isopropylidene derivative of (±)-(1α,2β,3β,4α)-4-amino-1,2,3-cyclopentanetriol (**7**)[7] was

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Table I. Inhibitory Effects of Compounds 3, 4, 5, and 6 on the Replication of DNA Viruses and RNA Viruses

virus[a]	cell	MIC[b] (μg/mL)[c]							
		3	4	5	6	BVDU	ribavirin	C-c^{3}Ado	neplanocin A
HSV-1	E_6SM	200	300	300	300	0.02	>400	>400	>40
TK$^-$ HSV-1	E_6SM	20	200	≥300	>200	40	>400	>400	4
HSV-2	E_6SM	300	300	>400	≥300	10	>400	>400	>40
VZV	HEL	65	200	225	230	0.001	–	–	8
TK$^-$ VZV	HEL	40	250	235	165	>10	–	–	5
HCMV	HEL	0.4	250	250	250	–	–	–	0.4
vaccinia	E_6SM	0.3	≥300	>400	15	7	40	2	0.2
vesicular stomatitis	E_6SM	0.07	≥300	>400	2	>400	70	0.7	0.2
vesicular stomatitis	HeLa	0.07	>400	>400	70	>400	20	2	0.2
coxsackie B4	HeLa	>200	>400	>400	>400	>400	70	>400	>40
coxsackie B4	Vero	>200	>200	>200	>200	>400	100	≥100	>40
polio 1	HeLa	>200	>400	>400	>400	>400	70	>400	>10
respiratory syncytial	HeLa	2	>100	>100	50	–	–	–	0.2
parainfluenza 3	Vero	0.4	>200	>200	20	>400	20	0.7	0.4
measles	Vero	0.4	>100	>100	20	–	–	–	0.2
reo type 1	Vero	0.07	>200	>200	10	>400	70	2	0.4
sindbis	Vero	>200	>200	>200	>200	>400	40	20	>40
semliki forest	Vero	>200	>200	>200	>200	>400	40	>400	>40
Junin	Vero	0.5	>100	>100	85	–	–	2	0.4
Tacaribe	Vero	1.0	>100	>100	100	–	–	2	0.4
influenza A	MDCK	>100	>100	>100	>100	–	–	–	>20
influenza B	MDCK	>100	>100	>100	>100	–	–	–	>20

[a] Strains used: HSV-1 (KOS); TK$^-$ HSV-1 (B2006); HSV-2 (G); VZV (Oka); TK$^-$ VZV (07/1); HCMV (AD169 and Davis); respiratory syncytial virus (Long); parainfluenza 3 (VR-93); measles (Sugiyama); influenza A (Ishikawa); influenza B (Singapore). [b] Minimum inhibitory concentration required to reduce virus-induced cytopathicity by 50%. [c] Average value for three separate experiments.

chosen from among several literature possibilities[7-9] as a useful starting point for the preparation of 3–6. The syntheses are outlined in Schemes I and II and follow standard carbocyclic purine ring construction. Thus, in Scheme I, reaction of 7 with 5-amino-4,6-dichloropyrimidine yielded the common precursor 8, which, upon treatment with diethoxymethyl acetate, produced 9. Conversion of 9 into 3 was readily accomplished with ammonia in methanol. Attempts to prepare 9 from the reaction of 8 with triethyl orthoformate consistently led to the intermediate 10. Compound 10 could not be induced to lose ethanol except when treated with 1 N hydrochloric acid, which resulted in 4 rather than 9. Evidence for 10 as a diastereomeric mixture arose from careful analysis of its ^{1}H NMR spectrum.

As shown in Scheme II, refluxing a mixture of 7 and 2-amino-4,6-dichloropyrimidine in 1-butanol produced the substituted pyrimidine 11. Diazo coupling of 11 with 4-chlorobenzenediazonium chloride gave 12, which was transformed into the triamino pyrimidine 13 using zinc–acetic acid. As a result of the success in forming 9 from 8 using diethoxymethyl acetate, reaction of 13 with the same reagent gave 14. Reaction of 14 with 1 N hydrochloric acid produced 5 and with ammonia in methanol yielded 6.

Antiviral Results

Compounds 3–6 were evaluated against a wide variety of both DNA viruses and RNA viruses (Table I). Marked activity (MIC_{50} values ranging from 0.07 to 2 μg/mL) was observed with 3 against vaccinia virus (in E_6SM cells), vesicular stomatitis virus (in both E_6SM and HeLa cells), respiratory syncytial virus (in HeLa cells), parainfluenza virus type 3 (in Vero cells), measles virus (in Vero cells), reovirus type 1 (in Vero cells), Junin and Tacaribe virus (in Vero cells), and human cytomegalovirus (HCMV) (in HEL cells). The activity spectrum shown by 3 conforms to that of other adenosine analogues which exert their antiviral activity through inhibition of *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase).[3a,5] Some activity was noted with compound 3 against TK$^-$ HSV-1, but not TK$^+$ HSV-1. This is also compatible with an action targeted at AdoHcy hydrolase, as a relatively greater inhibition of TK$^-$ HSV-1 has also been observed with other AdoHcy hydrolase inhibitors such as neplanocin A.[10] In fact, neplanocin A, when tested in parallel with compounds 3–6, showed an activity spectrum quite similar to that of compound 3 (Table I).

Compounds 4 and 5 were virtually inactive as antiviral agents while 6 showed activity against those viruses that were also sensitive to inhibition by 3. However, 6 was considerably less potent than 3 (Table I).

On the basis of a microscopically detectable alteration of normal cell morphology, no cytotoxicity was noted for any of the host cell monolayers (E_6SM, HEL, HeLa, Vero, MDCK) with any of the compounds tested at concentrations up to 400 μg/mL (except for neplanocin A, which was cytotoxic at a concentration of 20–40 μg/mL). When evaluated for their inhibitory effects on proliferation of HEL cells, the 50% inhibitory concentrations (IC_{50}, average values for three separate experiments) of compounds 3, 4, 5, and 6 were 88, >200, >200, and 43 μg/mL, respectively.

Since the antiviral spectrum for 3 and 6 suggested that inhibition of AdoHcy hydrolase may be responsible for their activity, 3 and 6 were directly examined for their

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(10) De Clercq, E.; Bères, J.; Bentrude, W. G. Potent Activity of 5-Fluoro-2′-deoxyuridine and Related Compounds against Thymidine-Kinase Deficient (TK$^-$) Herpes Simplex Virus: Targeted at Thymidylate Synthase. *Mol. Pharmacol.* **1987**, *32*, 286–292.

inhibitory effects on this enzyme. With AdoHcy hydrolase isolated from murine L929 cells,[3b] K_i values of 0.0111 ± 0.0048 μM and 0.85 ± 0.15 μM were found for 3 and 6, respectively. In previous studies,[3b] a close correlation has been found between the inhibitory effects of a series of acyclic and carbocyclic adenosine analogues on murine L929 cell AdoHcy hydrolase and their inhibitory effects on the replication of some target viruses such as vaccinia virus and vesicular stomatitis virus. If the present data obtained for 3 and 6 are interpolated with those obtained previously for related carbocyclic adenosine analogues (that is, carbocyclic 3-deazaadenosine, 3-deazaneplanocin A, and neplanocin A (2) and its derivative lacking the exocyclic hydroxymethyl substituent[5]), it appears that the antiviral potency of compounds 3 and 6 correlates closely with inhibition of AdoHcy hydrolase. This is particularly noteworthy as a new approach to developing new agents for the treatment of HCMV infections.

It would be revealing to synthesize the enantiomer of 3 that is configurationally equivalent to 1 and 2 and to determine the antiviral properties of this enantiomer. This goal will be the subject of future work.

Experimental Section

Melting points were recorded on a Mel-Temp capillary melting point apparatus and are uncorrected. Combustion analyses were performed by M-H-W Laboratories, Phoenix, AZ. ^1H and ^{13}C NMR spectra were recorded on a JEOL FX90Q or a Bruker AMX-360 spectrometer in DMSO-d_6 referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), m (multiplet), and br (broad). Reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm E. Merck silica gel 60-F_{254} precoated silica gel plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Fluka flash chromatography silica gel 60 (particle size 0.035–0.07 mm; 220–440 mesh ASTM) by eluting with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) homogeneous materials.

(±)-(1α,2β,3β,4α)-4-[(5-Amino-6-chloropyrimidin-4-yl)amino]-2,3-*O*-isopropylidene-1,2,3-cyclopentanetriol (8). A solution of the 2,3-*O*-isopropylidene derivative of (±)-(1α,2β,3β,4α)-4-amino-1,2,3-cyclopentanetriol (7)[7] (800 mg, 4.62 mmol), 5-amino-4,6-dichloropyrimidine (946 mg, 5.77 mmol), and Et_3N (5 mL) in 1-butanol (25 mL) was heated at 120 °C under N_2 for 40 h. The mixture was concentrated in vacuo, and the residue was azeotroped with MeOH (2 × 50 mL). The crude product was purified by flash chromatography (MeOH–$CHCl_3$, 5:95) to give a solid that recrystallized from CH_2Cl_2–hexane as white crystals of 8 (1.18 g, 85%): mp 196.5–197 °C; ^1H NMR (DMSO-d_6, 90 MHz) δ 1.21 and 1.36 (2 s, 6 H, 2 × Me), 1.55–2.42 (m, 2 H, H-5), 4.0–4.62 (m, 4 H, H-1, H-2, H-3, and H-4), 4.92 (brs, 2 H, NH_2), 5.23 (d, J = 2.9 Hz, 1 H, OH), 6.47 (d, J = 7 Hz, 1 H, NH), 7.79 (s, 1 H, H-5 of pyrimidine). Anal. ($C_{12}H_{17}ClN_4O_3$) C, H, N.

(±)-(1α,2β,3β,4α)-4-(6-Amino-9*H*-purin-9-yl)-1,2,3-cyclopentanetriol ((±)-5'-Noraristeromycin, 3).[11] A mixture of 8 (400 mg, 1.33 mmol) and diethoxymethyl acetate (25 mL) was stirred at room temperature for 1 h. Then, the resulting clear solution was stirred at 80–90 °C for 40 h under N_2. The excess diethoxymethyl acetate was removed under reduced pressure, and the residue was dissolved in 0.5 N HCl in MeOH (40 mL). This mixture was stirred at room temperature for 30 min. The solvent was evaporated under reduced pressure and the residue azeotroped with MeOH (2 × 50 mL). The new residue was then dissolved in MeOH and the solution neutralized with IRA-400 (basic) resin. The resin was removed by filtration, washed with MeOH, and the filtrate evaporated in vacuo. The resulting residue was azeotroped with MeOH to give a residue that was purified using flash chromatography (MeOH–CH_2Cl_2, 1:9) to give pure (±)-(1α,2β,3β,4α)-4-(6-chloro-9*H*-purin-9-yl)-1,2,3-cyclopentanetriol (9) (105 mg) and an uncyclized product (70 mg) in which the hydroxyl groups were deprotected (NMR). This latter compound was subjected to the cyclization and workup conditions described above to give an additional amount of 9 (55 mg). The products were combined and recrystallized from CH_2Cl_2–MeOH to give colorless crystals of 9 (152 mg, 42%): mp 173–174 °C; ^1H NMR (DMSO-d_6, 90 MHz) δ 1.81–1.92 (m, 1 H, H-5), 2.56–2.69 (m, 1 H, H-5), 3.79 and 3.95 (2 brs, 2 H, H-1 and H-2), 4.51–5.28 (m, 5 H, H-3, H-4, and 3 OH), 8.44 and 8.53 (2 s, 2 H, H-2 and H-8 of purine). This material was homogeneous by TLC (MeOH–$CHCl_3$, 2:8, R_f = 0.44) and was used directly in the next step.

Methanol (20 mL) was placed in a steel bomb and cooled to −10 °C. Anhydrous NH_3 was bubbled into the MeOH for 1 h. To this NH_3/MeOH mixture 9 (100 mg, 0.33 mmol) was slowly added. The mixture was then heated in the bomb at 140 °C for 2 days. Following this, the reaction mixture was evaporated to dryness under reduced pressure to yield a residue that was purified using flash chromatography (MeOH–$CHCl_3$, 1:4) to give a white solid that was recrystallized from MeOH–H_2O to yield 3 (60 mg, 65%) as colorless crystals: mp 274–276 °C; ^1H NMR (DMSO-d_6, 360 MHz) δ 1.77–1.90 (m, 1 H, H-5), 2.50–2.60 (m, 1 H, H-5), 3.75 (brs, 1 H, H-2), 3.89 (brs, 1 H, H-1), 4.40–4.52 (m, 1 H, H-3), 4.62–4.70 (m, 1 H, H-4), 4.87 (d, J = 3.6 Hz, 1 H, C-2 OH), 5.01 (d, J = 6.6 Hz, 1 H, C-3 OH), 5.36 (d, J = 4.8 Hz, 1 H, C-1 OH), 7.22 (s, 2 H, NH_2), 8.11 and 8.15 (2 s, 2 H, H-2 and H-8 of purine); ^{13}C NMR (DMSO-d_6, 90 MHz) δ 36.50 (C-5), 58.81 (C-4), 73.40, 75.56, and 76.44 (C-1, C-2, and C-3), 118.32, 142.47, 145.42, 148.73, and 150.60 (C of purine). Anal. ($C_{10}H_{13}N_5O_3$) C, H, N.

(±)-1,9-Dihydro-9-[(1'α,2'β,3'β,4'α)-(2',3',4'-trihydroxy-1'-cyclopentyl)]-6*H*-purin-6-one (4). A suspension of 8 (500 mg, 1.66 mmol) and triethyl orthoformate (26 mL) was stirred at room temperature for 5 min. Concentrated HCl (0.4 mL) was added to this suspension and the mixture then stirred at 110 °C for 24 h under N_2. The reaction mixture was cooled to room temperature, and then a mixture of concentrated HCl (0.4 mL) and MeOH (10 mL) was added to it. This solution was stirred at room temperature for 1 h and the solvent then removed under reduced pressure. The residue was dissolved in MeOH and neutralized with IRA-400 (basic) resin. The resin was removed by filtration and washed with MeOH. After evaporation of the filtrate, the residue was purified using flash chromatography (MeOH–$CHCl_3$, 2:98) to give an unstable noncrystalline material (540 mg) that was assigned as intermediate 10 by NMR. This compound was refluxed in 1 N HCl (60 mL) for 5 h under N_2. The reaction mixture was then evaporated to dryness under reduced pressure; the residue was azeotroped with EtOH (2 × 50 mL) and the residue was dissolved in H_2O (10 mL). Neutralization of this mixture with 6 N NaOH was followed by cooling overnight in a refrigerator. Following this period, the mixture was evaporated to dryness in vacuo to give a residue that was treated with hot MeOH–CH_2Cl_2 (3:7). A small amount of insoluble material was removed by filtration and the filtrate evaporated to dryness under reduced pressure. The residue was purified using flash chromatography (MeOH–CH_2Cl_2, 1:4) to give a white solid that was recrystallized from MeOH–H_2O to give 4 (230 mg, 55%) as colorless crystals): mp 230–232 °C; ^1H NMR (DMSO-d_6, 360 MHz) δ 1.68–1.80 (m, 1 H, H-5'), 2.52–2.66 (m, 1 H, H-5'), 3.76 (brs, 1 H, H-3'), 3.90 (brs, 1 H, H-4'), 4.40–4.50 (m, 1 H, H-2'), 4.60–4.74 (m, 1 H, H-1'), 4.92 (d, J = 3.4 Hz, 1 H, C-2' OH), 5.04 (d, J = 7.6 Hz, 1 H, C-3' OH), 5.21 (d, J = 4.2 Hz, 1 H, C-4' OH), 8.03 and 8.14 (2 s, 2 H, H-2 and H-8 of purine); ^{13}C NMR (DMSO-d_6, 90 MHz) δ 37.06 (C-5'), 58.56 (C-4'), 73.66, 75.68, and 76.75 (C-1', C-2', and C-3'), 124.36, 149.19, 145.34, 148.69, and 156.79 (C of purine). Anal. ($C_{10}H_{12}N_4O_4$) C, H, N.

(±)-(1α,2β,3β,4α)-4-[(2-Amino-6-chloropyrimidin-4-yl)amino]-2,3-*O*-isopropylidene-1,2,3-cyclopentanetriol (11). A mixture of 7[7] (1.65 g, 9.65 mmol), 2-amino-4,6-dichloropyrimidine (2.37 g, 14.17 mmol) and Et_3N (10 mL) in 1-butanol (50 mL) was refluxed under N_2 for 2 days. The mixture was evaporated to

(11) As part of an NMR analysis of the compounds leading to the synthesis of the "lyxo" isomer of aristeromycin, ref 8a required the 2,3-*O*-isopropylidene derivative of 3. For this purpose, 3 served as a synthetic intermediate and was obtained via a route different than that described herein. However, no preparative details or physical or spectral properties for 3 were given in ref 8a.

dryness under reduced pressure, and the residue was azeotroped with MeOH (2 × 50 mL). The material remaining after this treatment was purified using flash chromatography (MeOH–CH_2Cl_2, 1:9). The solid obtained from the homogeneous fractions was recrystallized from CH_2Cl_2–hexane to give 11 (2.72 g, 95%) as white crystals: mp 223.5–224.5 °C; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.19 and 1.33 (2 s, 6 H, 2 × Me), 1.44–1.65 (m, 1 H, H-5), 2.04–2.17 (m, 1 H, H-5), 3.95–4.43 (m, 4 H, H-1, H-2, H-3, and H-4), 5.35 (brs, 1 H, OH), 5.84 (brs, 1 H, H-5 of pyrimidine), 6.42 (brs, 2 H, NH_2), 6.54 (brs, 1 H, NH); ^{13}C NMR (DMSO-d_6, 90 MHz) δ 24.01 and 26.39 (2 × Me), 35.94 (C-5), 55.58 (C-4), 75.48, 84.87, and 85.75 (C-1, C-2, and C-3), 93.08, 109.69, 157.47, 162.84, and 163.04 (C of isopropylidene and pyrimidine). Anal. ($C_{12}H_{17}ClN_4O_3$) C, H, N.

(±)-(1α,2β,3β,4α)-4-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]pyrimidin-4-yl]amino]-2,3-*O*-isopropylidene-1,2,3-cyclopentanetriol (12). A cold solution of 4-chlorobenzenediazonium chloride was prepared by adding a solution of $NaNO_2$ (0.65 g, 9.5 mmol) in H_2O (5 mL) to a solution of 4-chloroaniline (1.15 g, 9 mmol) dissolved in 12 N HCl (5 mL) and H_2O (15 mL) and cooled in an ice bath. The cold solution of 4-chlorobenzenediazonium chloride was added dropwise, with stirring, to a mixture of 11 (2.35 g, 7.8 mmol), sodium acetate trihydrate (17 g), and glacial AcOH (40 mL) in H_2O (40 mL) at room temperature. After stirring this mixture at room temperature for 18 h, the reaction was found to be incomplete by TLC analysis. Therefore, additional 4-chlorobenzenediazonium chloride (prepared from $NaNO_2$ (0.325 g) and 4-chloroaniline (0.58 g) as described above) was slowly added to the mixture. The stirring was continued for a total of 40 h. The reaction mixture was then cooled, and the resulting yellow solid obtained by filtration, washed with cold H_2O, and dried. This solid was recrystallized from CH_2Cl_2–MeOH to give 12 (3.155 g, 92%) as yellow crystals: mp 264–265 °C dec; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 1.21 and 1.35 (2 s, 6 H, 2 × Me), 1.55–2.40 (m, 2 H, H-5), 3.15–4.95 (m, 5 H, H-1, H-2, H-3, H-4, and OH), 7.14–7.96 (m, 6 H, ArH and NH_2), 10.11 (d, 1 H, NH). Anal. ($C_{18}H_{20}Cl_2N_6O_3$) C, H, N.

(±)-2-Amino-1,9-dihydro-9-[(1′α,2′β,3′β,4′α)-(2′,3′,4′-trihydroxy-1′-cyclopentyl)]-6*H*-purin-6-one (5). A mixture of 12 (2.16 g, 4.93 mmol), Zn dust (3.14 g), and glacial AcOH (1.6 mL) in EtOH (80 mL) and H_2O (80 mL) was refluxed under N_2 until the yellow color of 12 disappeared (5 h). The reaction mixture was filtered hot, and the insoluble material was washed with hot EtOH. The combined filtrates were evaporated under reduced pressure to produce a residue that was first azeotroped with EtOH and, then, purified using flash chromatography (3.5–5% MeOH in CH_2Cl_2) to obtain pure (±)-(1α,2β,3β,4α)-4-[(6-chloro-2,5-diaminopyrimidin-4-yl)amino]-2,3-*O*-isopropylidene-1,2,3-cyclopentanetriol (13) (1.2 g, 77%) as colorless crystals (from CH_2Cl_2–MeOH): mp 212–214 °C; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 1.21 and 1.35 (2 s, 6 H, 2 × Me), 1.47–2.36 (m, 2 H, H-5), 3.97–4.51 (m, 4 H, H-1, H-2, H-3, and H-4), 5.42 (d, J = 2.6 Hz, 1 H, OH), 6.43 (d, 1 H, NH), 6.72 (brs, 2 H, NH_2); ^{13}C NMR (DMSO-d_6, 22.5 MHz) δ 24.01 and 26.40 (2 × Me), 35.61 (C-5), 56.08 (C-4), 75.86, 85.12, and 85.77 (C-1, C-2, and C-3), 109.50, 112.10, 144.77, 156.52, and 156.90 (C of isopropylidene and pyrimidine).

A mixture of 13 (1.12 g, 3.8 mmol) in diethoxymethyl acetate (30 mL) was stirred at room temperature for 1 h. The reaction mixture was then stirred at 80–85 °C for 22 h. The excess diethoxymethyl acetate was removed under reduced pressure, and the residue was dissolved in 0.5 N HCl in MeOH (50 mL). This mixture was stirred at room temperature for 30 min and the solution then evaporated in vacuo to yield a residue that was azeotroped with MeOH. The residue was dissolved in MeOH and the pH adjusted to 7 by treating the solution with IRA-400 (basic) resin. The resin was removed by filtration and washed with MeOH. The combined filtrates were evaporated under reduced pressure to yield a residue that was purified using flash chromatography (MeOH–CH_2Cl_2, 1:4) to give 300 mg of pure (±)-(1α,2β,3β,4α)-4-(2-amino-6-chloro-9*H*-purin-9-yl)-1,2,3-cyclopentanetriol (14) and 400 mg of mostly an uncyclized product in which the hydroxyl functionalities were deprotected (by NMR). This latter material was again subjected to the cyclization conditions described above to produce, after similar workup, an additional 320 mg of 14 (total yield: 620 mg, 57%). The combined crude product was recrystallized from $CHCl_3$–MeOH: mp 166–168 °C; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.61–1.78 (m, 1 H, H-5), 2.50–2.68 (m, 1 H, H-5), 3.25–4.72 (m, 4 H, H-1, H-2, H-3, and H-4), 6.38 (brs, 2 H, NH_2), 7.93 (s, 1 H, H-8 of purine). This material was homogeneous by TLC (MeOH–$CHCl_3$, 2:8, R_f = 0.34) and was used directly in the next step.

Compound 14 (120 mg, 0.42 mmol) was dissolved in 1 N HCl (20 mL), and this solution was refluxed for 5 h under N_2. The solvent was removed under reduced pressure, and the residue was azeotroped with EtOH. The resultant residue was dissolved in H_2O (10 mL), and the solution was neutralized with 6 N NaOH. Even though a precipitate formed immediately, the mixture was refrigerated overnight. The solid material was obtained by filtration and was washed with cold H_2O to give 5 (90 mg, 80%) that was recrystallized from MeOH–H_2O as colorless crystals: mp > 282 °C; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.53–1.66 (m, 1 H, H-5′), 2.51–2.61 (m, 1 H, H-5′), 3.74 (brs, 1 H, H-3′), 3.87 (brs, 1 H, H-4′), 4.35–4.45 (m, 1 H, H-2′), 4.47–4.61 (m, 1 H, H-1′), 4.84 (d, J = 3.3 Hz, 1 H, C-3′ OH), 5.02 (d, J = 6.2 Hz, 1 H, C-2′ OH), 5.19 (d, J = 6.2 Hz, 1 H, C-4′ OH), 6.44 (s, 2 H, NH_2), 7.77 (s, 2 H, H-8 of purine), 10.62 (s, 1 H, H-1 of purine); ^{13}C NMR (DMSO-d_6, 90 MHz) δ 37.01 (C-5′), 57.21 (C-1′), 73.41, 75.28 and 76.55 (C-2′, C-3′, and C-4′), 116.57, 135.81, 151.29, 153.16, and 156.77 (C of purine). Anal. ($C_{10}H_{13}N_5O_4 \cdot 0.75H_2O$) C, H, N.

(±)-(1α,2β,3β,4α)-4-(2,6-Diamino-9*H*-purin-9-yl)-1,2,3-cyclopentanetriol (6). Compound 14 (130 mg, 0.45 mmol) was stirred in MeOH (20 mL) saturated with anhydrous NH_3 in a steel bomb at 120–140 °C for 5 days. The solution was evaporated to dryness under reduced pressure, and the residue was purified using flash chromatography (10–15% MeOH in $CHCl_3$) to yield 18 mg of recovered 14 from the initial fractions. Later fractions gave a white solid that was recrystallized from MeOH–H_2O to provide 6 (70 mg, 67% based on recovered starting material): mp 240–242 °C; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 1.60–1.70 (m, 1 H, H-5), 2.51–2.60 (m, 1 H, H-5), 3.75 (brs, 1 H, H-2), 3.88 (brs, 1 H, H-1), 4.38–4.65 (m, 2 H, H-3 and H-4), 4.81 (brs, 1 H, C-2 OH), 5.05 (brs, 1 H, C-3 OH), 5.45 (brs, 1 H, C-1 OH), 5.77 (s, 2 H, NH_2), 6.73 (s, 2 H, NH_2), 7.76 (s, 1 H, H-8 of purine); ^{13}C NMR (DMSO-d_6, 90 MHz) δ 36.64 (C-5), 57.51 (C-4), 73.60, 75.20 and 76.71 (C-1, C-2, and C-3), 113.47, 136.33, 151.44, 156.07, and 159.70 (C of purine). Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

Antiviral Activity Assays. All antiviral assays were carried out as described in ref 12, except for the anti-VZV assays (see ref 13). The sources of the viruses have also been described in these previous publications. Abbreviations used for the viruses and cells are as follows: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VZV, varicella-zoster virus; HCMV, human cytomegalovirus; TK$^-$, thymidine kinase deficient; E_6SM, embryonic skin-muscle; HEL, human embryonic lung; MDCK, Madin-Darby canine kidney. Abbreviations used for the reference compounds are as follows: BVDU, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine; C-c^{3}Ado, carbocyclic 3-deazaadenosine.

Cytotoxicity Assays. Cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero, MDCK) or inhibition of cell growth (HEL), as has been described previously.[12]

AdoHcy Hydrolase Assays. AdoHcy hydrolase was purified from murine L929 cells to apparent homogeneity by using affinity chromatography; enzymatic activity was measured in the direction of AdoHcy synthesis, as described previously.[3b]

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this publication does not necessarily reflect the views or policies of the Department of Health and Human Services nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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Registry No. 3, 142635-42-5; 4, 142635-43-6; 5, 135700-55-9; 6, 142635-44-7; 7, 88756-83-6; 8, 142635-45-8; 9, 142635-46-9; 10, 142635-47-0; 11, 135700-56-0; 12, 135700-57-1; 13, 135700-58-2; 14, 135700-59-3; 5-amino-4,6-dichloropyrimidine, 5413-85-4; diethoxymethylacetate, 14036-06-7; triethyl orthoformate, 122-51-0; 2-amino-4,6-dichloropyrimidine, 56-05-3; 4-chlorobenzenediazonium chloride, 2028-74-2.

Antitumor Imidazotetrazines. 25.[1] Crystal Structure of 8-Carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one (Temozolomide) and Structural Comparisons with the Related Drugs Mitozolomide and DTIC[†]

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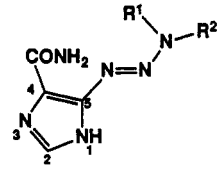
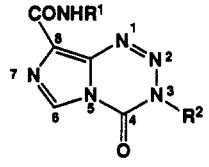
The antitumor imidazotetrazinone, temozolomide (5), $C_6H_6N_6O_2$, forms crystals with unit cell dimensions a = 17.332 (3), b = 7.351 (2), c = 13.247 (1), β = 109.56 (1)° and space group $P2_1/c$. A doubly hydrogen-bonded dimer constitutes the asymmetric unit. One carboxamide group forms an additional intermolecular NH···O hydrogen bond; in both molecules the carboxamide group is coplanar with the heterocycle and its NH_2 group interacts with the imidazole nitrogen atom N(7). Molecular orbital calculations show the carbonyl carbon C(4) to be the most electron deficient atom, with relatively weak N(3)–C(4) and C(4)–N(5) bonds confirming that temozolomide should ring-open at this position in solution. The energy barrier to carboxamide group rotation of approximately 20 kJ mol^{-1} should permit interconversion between rotamers. In temozolomide and the related drug mitozolomide (4), N(7) is more negatively charged than N(1), which favors the formation of hydrogen bonds to the former atom in spite of their poor geometry. The relevance of these structural features to the action of temozolomide as a major-groove-directed prodrug of the alkylating agent MTIC (3) is discussed.

The antitumor drug 5-(3,3-dimethyltriazen-1-yl)-imidazole-4-carboxamide (1; DTIC) is used in the treatment of malignant melanoma,[2] a disease which is increasing in incidence particularly in Northern European races.[3] The bicyclic imidazotetrazinones mitozolomide (4)[4] and temozolomide (5)[5] have progressed to clinical trial as potential alternatives to DTIC based on their activity in in vivo murine[6] and human xenograft[7] tumor screens.





(1) R^1=R^2= Me DTIC
(2) R^1=H, R^2=$(CH_2)_2Cl$ MCTIC
(3) R^1=H, R^2=Me MTIC
(4) R^1=H, R^2=$(CH_2)_2Cl$ Mitozolomide
(5) R^1=H, R^2=Me Temozolomide
(6) R^1=Me, R^2=Me

Clinical trials on 4 were compromised by the emergence in patients of a delayed and profound thrombocytopenia (damage to platelets).[4] This dose-limiting toxicity is probably elicited by a DNA cross-linking lesion which is forged subsequent to monoalkylation at the O(6) position of guanine residues[8] by the active cytotoxic species MCTIC (2), formed by the ring-opening of the prodrug mitozolomide at the electron-deficient C(4) position.[6,9] Clearly DTIC and temozolomide cannot induce DNA cross-linking and this is reflected in a different profile of toxicity with these two agents.

Structure–activity studies of antitumor imidazotetrazinones[10] substituted at the 8-position but retaining the C=O moiety show very good antitumor activity for several N-monosubstituted carboxamides. Aromatic substituents, and bulky substituents in general, are inimical to activity.[10] However, the *N*,*N*-dimethylcarboxamide derivative requires metabolic demethylation to become active, and a

[†] Contribution from the Joint Crystallography Unit, Universities of Aston and Birmingham.

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