Synthesis and Antiviral Activity of 9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]purines

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Alkylation of 2-amino-6-chloropurine with 5-(2-bromoethyl)-2,2-dimethyl-1,3-dioxane (**5**) provided 2-amino-6-chloro-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]purine (**6**) in high yield. This aminochloropurine **6** was readily converted to the antiviral acyclonucleoside 9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]guanine (**1**) and to its 6-chloro (**10**), 6-thio (**11**), 6-alkoxy (**12–17**), 6-amino (**20**), and 6-deoxy (**21**) purine analogues. The guanine derivative **1** was converted to its xanthine analogue **9**. Similarly, alkylation of 6-chloropurine with **5** provided a route to **8**, the hypoxanthine analogue of **1**. Of these 9-substituted purines, the guanine derivative **1** showed the highest activity against herpes simplex virus types 1 and 2 in cell cultures, and in some tests it was more active than acyclovir, with no evidence of toxicity for the cells. A series of monoesters (**30–33**) and diesters (**24–27, 29**) of **1** were prepared, and some of these also showed antiherpes virus activity in cell cultures, the most active ester being the dihexanoate **27**.

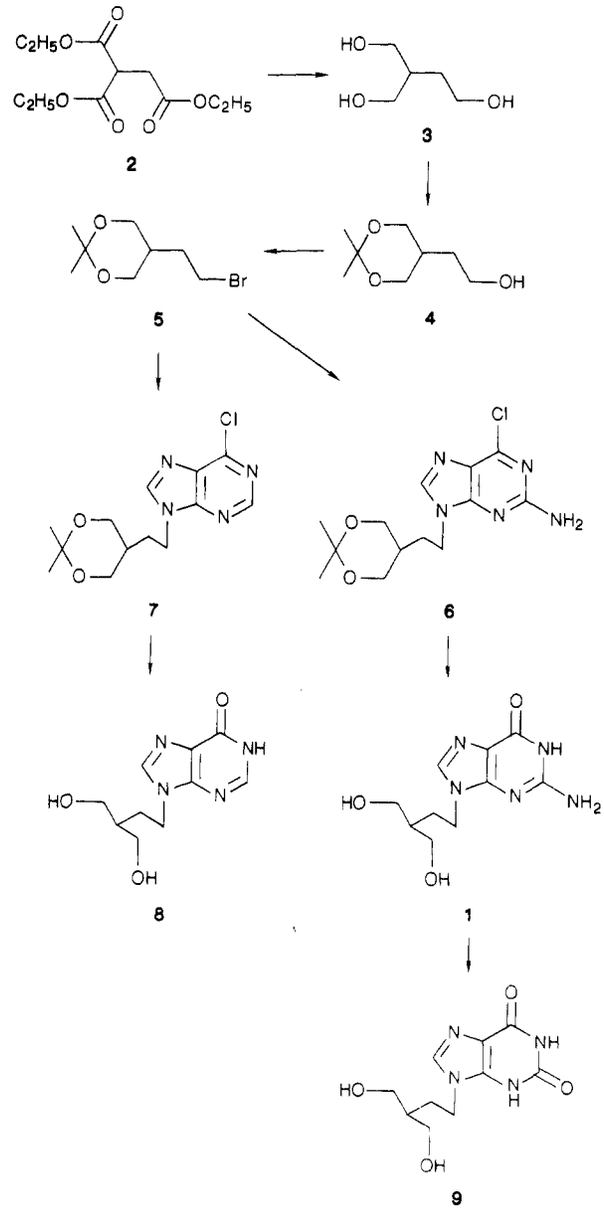
The discovery of the potent and selective antiherpes virus agent 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir)[1-3] has led to extensive research into the preparation and evaluation of novel acyclic analogues of nucleosides. The most potent of these reported to date is 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (ganciclovir, DHPG),[4-6] but in animals[7] and in humans[7,8] this compound has been found to cause adverse toxicological effects. Our interest has been centered on the carba analogue of ganciclovir, 9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]guanine (**1**, BRL 39123).[9] The preparation of **1** was first claimed in 1972,[10] and we have recently reported[11] a short novel synthesis of the compound. In the present paper we report results that we have obtained from antiviral evaluation of **1** and some of its esters, as well as the preparation and properties of a series of 9-[4-hydroxy-3-(hydroxymethyl)-but-1-yl)purines.

Chemistry

Our synthesis of **1** involves selective 1,3-protection of the triol **3** by the isopropylidene group to allow functionalization of the 4-position (**4** → **5**, Scheme I). The isopropylidene group also has a useful stability profile, its stability to nucleophilic, basic, and hydrogenolytic conditions permitting a variety of subsequent transformations on the purine moiety (Scheme II) and its acid lability allowing its removal concomitant with purine 6-chloro to 6-oxo hydrolysis (Scheme I).

6-Oxopurines. Alkylation of 2-amino-6-chloropurine with the bromo compound **5** in DMF afforded predominantly a single isomer, which, from its UV spectrum,[12] was shown to be the 9-substituted purine **6**. Hydrolysis of **6** with refluxing hydrochloric acid gave 9-[4-hydroxy-3-(hy-

Scheme I



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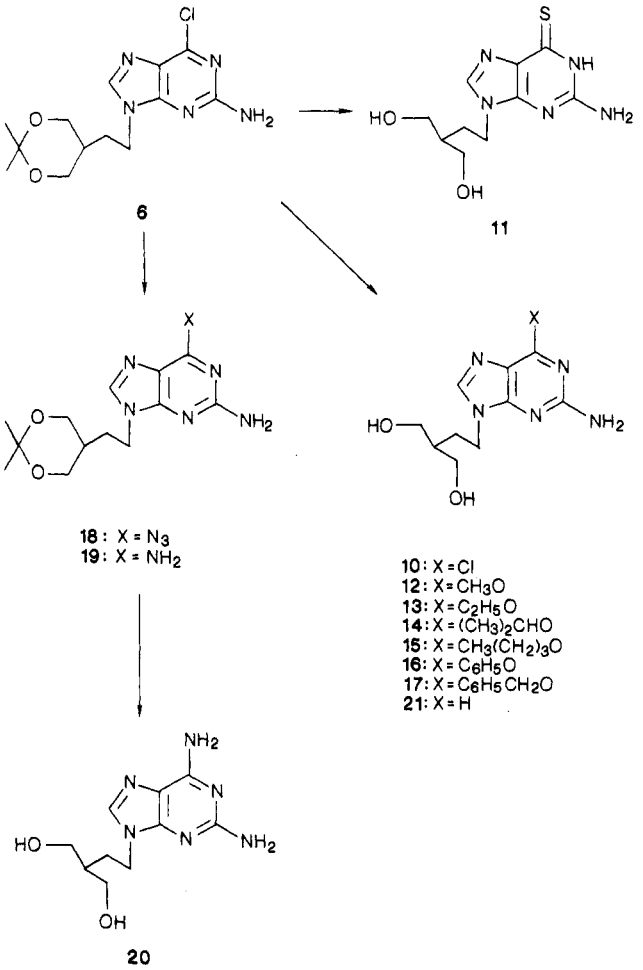
Scheme II



droxymethyl)but-1-yl]guanine (**1**). The product obtained in this way had mp 275–277 °C, about 100 °C higher than that quoted in the earlier publication by Pandit et al.[10] claiming synthesis of **1**. We have repeated[11] the experimental conditions used by these workers[10,13] and did indeed obtain a material that melted at 170–175 °C, but we showed that it was a mixture of **1** and very substantial quantities of its O-benzylated derivatives.

Reaction of **5** with 6-chloropurine and sodium hydride in DMF afforded the 9-alkylated purine **7** in 47% yield and also a small amount of the 7-isomer (9% yield). UV spectral data were again used in the assignment[12] of the position of substitution of these isomers. Deprotection of **7** in refluxing hydrochloric acid gave the hypoxanthine analogue **8** (69% yield). The xanthine analogue **9** was obtained from **1** by treatment with nitrous acid at room temperature.

2-Aminopurines. The 6-chloro compound **6** proved to be a versatile intermediate in the preparation of 2-amino-6-substituted analogues of **1** (Scheme II). Under mild acid conditions (0.2 M hydrochloric acid in aqueous tetrahydrofuran at room temperature), selective removal of the isopropylidene group was achieved, affording the 6-chloro analogue (**10**) of **1** as its hydrochloride salt (63% yield). Reaction of **6** with sodium hydrosulfide in DMF followed by mild acid hydrolysis provided the 6-thio compound **11** in 48% yield. The chloropurine **6** was treated with a number of sodium alkoxides, and, after mild acid hydrolysis, a series of 6-alkoxypurines (**12–17**) were obtained. Reaction of **6** with sodium azide in DMF gave the 6-azido compound **18** in 75% yield. This compound did not show an azido absorption in the IR spectrum, but it would be expected to exist predominantly as the tetrazolo tautomer.[14] The azido group of **18** was, however, readily reduced to amino by catalytic hydrogen transfer from ammonium formate in refluxing methanol, and **19** was obtained in 65% yield. Mild acid hydrolysis of **19** gave the 2,6-diaminopurine **20** in 63% yield. The 6-unsubstituted purine **21** was prepared by hydrogenolysis of **6** followed by acid hydrolysis. Catalytic hydrogen transfer from either cyclohexene or ammonium formate effected this reduction, but the latter reagent was particularly efficient, providing a much quicker and more convenient reaction than conventional hydrogenolysis.[15] Catalytic hydrogen transfer from ammonium formate is therefore a preferred procedure for reduction of both azido-substituted (**18** → **19**) and chloro-substituted (**6** → **21**) purines.

Table I. Antiviral Activity in Plaque Reduction Assays[a] against Herpes Simplex Virus Types 1 and 2

compd[b]	IC_{50},[c] μM HSV-1 (HFEM)	IC_{50},[c] μM HSV-2 (MS)
1	6.4 ± 3.5 (n = 24)[d]	6.4 ± 4.2 (n = 17)
12	>250	236
24	5.8	10
27	4.2	3.6
30	81	31
31	6.0	15
32	33	128
acyclovir	3.8 ± 2.0 (n = 18)	2.5 ± 1.7 (n = 20)

[a] Plaque reduction[20] assays were performed by using a range of concentrations of the compounds incorporated into an agarose overlay on Vero cell monolayers infected with about 50 PFU of HSV-1 or HSV-2. Compounds were present throughout the incubation; 3 days after infection, plaques were counted. The compound concentration required to reduce the plaque count to 50% of that in untreated control cultures was calculated (IC_{50}). [b] Test compounds were prepared as 10 mg/mL solutions in Me_2SO and aliquots further diluted in test medium. [c] None of the compounds for which IC_{50} data is given caused morphological abnormalities in Vero cells at concentrations up to 100 μg/mL. [d] Mean ± standard deviation (n = number of assays).

Esters of 9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]guanine. Selective O-acylation of **1** (Scheme III) was achieved either with a carboxylic acid and dicyclohexylcarbodiimide (diformate **24** in 43% yield and dihexanoate **27** in 45% yield) or by reaction with an acid anhydride (e.g., diacetate **25** in 47% yield). The latter reaction also gives N^2-acylation if left for a longer period, and both the dipropionyl **26** and tripropionyl **28** compounds (41% and 47% yield, respectively) were isolated from reaction with propionic anhydride in DMF. In order to obtain selective O-acylation with an acid chloride or to obtain monoacylation, protection of the N^2-amino group and, in the latter case, of one hydroxyl group was required. The recently developed[16,17] N^2-trityl type protection of guanosine analogues was applied to afford the monomethoxytrityl-protected derivatives **22** and **23**. Reaction of **22** with 2,2-dimethylpropionyl chloride in pyridine, followed by deprotection with 80% acetic acid at 80 °C, gave the diester **29** in 58% yield. Similar reaction of **23** with the

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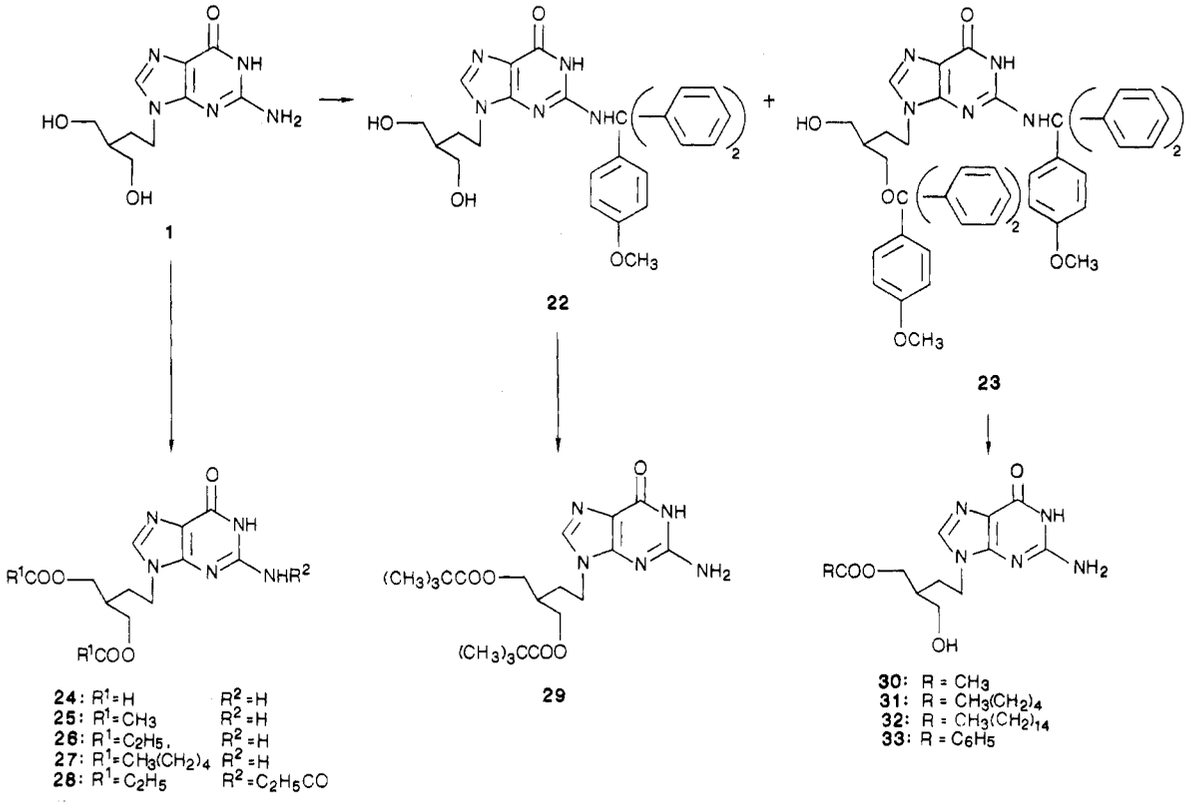
Scheme III



appropriate acyl chlorides afforded the monoesters **30–33**.

Biological Results

The acyclonucleosides **1** and **8–20** prepared in this study were tested in plaque reduction assays for activity against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) in Vero cells, and the results obtained for active compounds (IC_{50} < 250 μM) are given in Table I. The most active compound, 9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]guanine (**1**), was further tested in parallel with acyclovir in virus yield reduction assays in human lung fibroblasts (MRC-5) against three clinical strains and one laboratory strain of both HSV-1 and HSV-2, and the results are shown in Table II.

9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]guanine (**1**) exhibited potent antiviral activity in plaque reduction assays using laboratory strains of both HSV-1 and HSV-2. In these tests it was 1.7-fold less active than acyclovir against the type 1 virus and was 2.6-fold less active than acyclovir against the type 2 virus. In virus yield reduction assays, **1** was generally more active than acyclovir against HSV-1 and of equal activity against HSV-2. In the above tests, no morphological abnormalities were observed in Vero or MRC-5 cells treated with **1** at the highest concentration tested (400 μM). Furthermore, in cell growth experiments in which MRC-5 cells were incubated for 72 h in the presence of the compound, **1** at 400 μM inhibited the increase in cell numbers by only 18%. There was a 17-fold increase in cell numbers in the untreated control cultures. Since the antiviral activity of **1** was seen at concentrations of less than 10 μM, there is thus a very substantial difference between virus inhibitory and cell inhibitory concentrations. The activity of **1** in plaque reduction assays against herpes viruses has been independently noted by others.[18,19] Tippie et al.[18] reported that the IC_{50}'s of **1** against single strains of HSV-1 (F) and HSV-2 (G) were 0.5 and 4.0 μM, respectively.

Table II. Antiviral activity of 9-[4-Hydroxy-3-(hydroxymethyl)-but-1-yl]guanine (**1**) and Acyclovir in Virus Yield Reduction Assays[a] against Clinical and Laboratory Strains of Herpes Simplex Virus Types 1 and 2

virus (strain)	IC_{90}, μM	
	1	acyclovir
HSV-1 (HFEM)	1.3	4.8
HSV-1 (18189)	0.5	0.7
HSV-1 (19407)	0.6	1.4
HSV-1 (20132)	0.6	0.9
HSV-2 (MS)	1.1	1.2
HSV-2 (17409)	0.9	0.7
HSV-2 (20605)	0.8	0.3
HSV-2 (21929)	0.9	0.9

[a] Virus yield reduction assays[20] were performed in MRC-5 cells which were infected at a multiplicity of infection of about 1 PFU/cell. Compounds, in a range of concentrations, were added in culture medium after virus adsorption and were present throughout the incubation period. Supernatants were collected 24 h after infection and titrated on Vero cells. The concentration of compound required to reduce the yield of infectious virus by 90% compared with the yield in untreated control cultures was calculated (IC_{90}).

The mechanism of action of **1** may, like that of acyclovir, involve phosphorylation by a virus-specified thymidine (2′-deoxycytidine) kinase, since at concentrations up to 400 μM it was not active against a TK^- mutant of HSV-1. Additionally, none of the analogues (**8–17, 20, 21**) of **1**, in which the 4-hydroxy-3-(hydroxymethyl)butyl substituent is attached at N-9 of other purine bases, demonstrated significant antiviral activity when tested at concentrations

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up to 400 μM. This stringent requirement for the presence of the guanine moiety for retention of activity suggests that phosphorylated metabolites of **1** may compete with 2′-deoxyguanosine nucleotides at the catalytic center of enzymes involved in viral nucleic acid synthesis. Acyclovir triphosphate is competitive with dGTP at the catalytic center of the herpes virus DNA polymerases, and the triphosphate of **1** could act similarly.

Antiviral data for esters of **1** that were active at concentrations <250 μM are shown in Table I. It seems likely that the activity of the esters is dependent upon their ease of chemical and/or enzymatic hydrolysis to **1**. Thus, from the activity data, it would appear that the hexanoate **31** and diformate **24** are readily hydrolyzed. In contrast, the diacetate **25**, the propionate derivatives **26** and **28**, the bis[pivaloyl ester] **29**, and the benzoate **33** were not active at concentrations <250 μM. Since the solubility of **25** in water is about twice that of **1**, the low activity observed with these esters cannot be entirely attributable to decreased bioavailability and presumably reflects their unusually high stability under the assay conditions.

It is notable that the dihexanoate **27** has the highest activity ($IC_{50} \approx 4$ μM) of all of the compounds reported and in these tests was consistently almost 1.5 times as active as **1** ($IC_{50} \approx 6$ μM). The high activity of **27** may be a consequence of an advantageous combination of more facile cellular uptake due to increased lipophilicity and an alkanoyl moiety that is very susceptible to hydrolysis by intracellular esterases. Whether this combination of properties will also result in increased activity in animals is, however, doubtful, since the conversion of **27** to **1** in vivo is likely to be extremely rapid.

9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]guanine is a potent and highly selective antiherpes virus agent, and we are continuing to investigate its antiviral activity in cell culture and in animal infection models.

Experimental Section

Melting points were determined by using a Reichert Kofler apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 90-MHz or a JEOL GX-270 270-MHz spectrometer. Infrared spectra were recorded with a Perkin-Elmer 580 spectrometer and ultraviolet spectra with a Cary 219 spectrometer. Mass spectra were recorded on a VG 70-70 instrument, and accurate masses were measured on a VG ZAB spectrometer. Microanalyses were performed on a Carlo-Erba Model 1106 analyzer and, where only the symbols for the elements are recorded, were within ±0.4% of the calculated values. Upon TLC of analytical samples using silica gel $60F_{254}$ precoated aluminum sheets (Merck Art. No. 5554), in each case only a single component was detected.

2-(Hydroxymethyl)butane-1,4-diol (3). To a refluxing solution of triethyl 1,1,2-ethanetricarboxylate (46 mL, 200 mmol) and sodium borohydride (20 g) in *tert*-butyl alcohol (400 mL) was added methanol (25 mL) in three aliquots over 0.5 h. The solution was heated under reflux for a further 0.5 h and allowed to cool. Hydrochloric acid (5 M) was added with care until the solution was neutral. The solution was filtered, and the residue was extracted with ethanol (2 × 100 mL). The solutions were combined, and the solvent was removed. The residue was extracted with ethanol (120 mL) and filtered and the solvent removed to afford **3** as a clear colorless liquid (24 g, 100%): ^{1}H NMR (D_2O) δ 1.53 (2 H, q, J = 6 Hz, $CHCH_2$), 1.75 (1 H, m, $CHCH_2$), 3.57 (4 H, d, J = 6 Hz, $CH(CH_2OH)_2$), and 3.64 (2 H, t, J = 6 Hz, CH_2CH_2OH).

5-(2-Hydroxyethyl)-2,2-dimethyl-1,3-dioxane (4). To a solution of **3** (12 g, 100 mmol) in THF (25 mL) and 2,2-dimethoxypropane (13.5 g, 110 mmol) was added *p*-toluenesulfonic acid monohydrate (0.57 g, 3 mmol). The solution was stirred for 0.5 h at room temperature and was then neutralized by addition of triethylamine. The solvent was removed and the residue purified by column chromatography on silica gel eluting with chloroform–methanol mixtures to afford **4** as a clear colorless liquid (6.5 g, 41%): IR (film) ν_{max} 3420, 2940, 1375, 1200, and 1080 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34–1.70 (8 H, m, $C(CH_3)_2$ and CH_2CH_2OH), 1.7–2.1 (1 H, m, CH), 2.15 (1 H, br s, D_2O exchangeable, OH), and 3.5–4.0 (6 H, m, 3 × CH_2O). Anal. ($C_8H_{16}O_3 \cdot 0.25H_2O$) C, H.

5-(2-Bromoethyl)-2,2-dimethyl-1,3-dioxane (5). To an ice-cooled solution of **4** (6.08 g, 38 mmol) and carbon tetrabromide (18.90 g, 57 mmol) in DMF (110 mL) was added triphenylphosphine (14.95 g, 57 mmol), and the solution was stirred at this temperature for 0.5 h. To the solution was added saturated aqueous $NaHCO_3$ (55 mL) followed by water (55 mL), and the mixture was extracted with hexane (2 × 150 mL). The organic layers were combined and dried ($MgSO_4$), and the solvent was removed. The residue was kept in vacuo for 2 h and taken up in hexane. The solution was filtered and the solvent removed to afford **5** as a clear liquid, which crystallized on cooling (7.40 g, 87%): mp 18 °C; IR (film) ν_{max} 2940, 1370, 1270, 1260, 1200, and 1070 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (6 H, s, $C(CH_3)_2$), 1.94 (3 H, m, $CHCH_2CH_2$), 3.43 (2 H, t, J = 7 Hz, CH_2Br), 3.63 (2 H, dd, J = 12 Hz and 6 Hz, 2 × H_{ax}), and 4.00 (2 H, dd, J = 12 Hz and 6 Hz, 2 × H_{eq}). Anal. ($C_8H_{15}BrO_2$) C, H.

2-Amino-6-chloro-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)-ethyl]purine (6). To a solution of **5** (0.75 g, 3.7 mmol) in dry DMF (12 mL) were added 2-amino-6-chloropurine (0.68 g, 4.0 mmol) and anhydrous potassium carbonate (0.83 g, 6.0 mmol). The solution was stirred at room temperature for 5 h and left at 4 °C overnight. The solution was filtered and the solvent removed. The residue was purified by column chromatography on silica gel eluting with chloroform–methanol mixtures (80:1 and 60:1) to afford **6** (0.74 g, 64%): mp 125–126 °C; UV (H_2O) λ_{max} 223 (ε 28 900), 247 (5700), and 310 (7700) nm; IR (KBr) ν_{max} 3450, 3340, 1635, 1615, 1565, 1470, 1410, and 1375 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.26 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 1.45–1.85 (3 H, m, $CHCH_2CH_2N$), 3.51 (2 H, dd, J = 11 Hz and 7 Hz, 2 × H_{ax}), 3.78 (2 H, dd, J = 11 Hz and 4 Hz, 2 × H_{eq}), 4.05 (2 H, t, J = 7 Hz, CH_2N), 6.89 (2 H, s, D_2O exchangeable, 2-NH_2), and 8.38 (1 H, s, 8-H). Anal. ($C_{13}H_{18}ClN_5O_2$) C, H, N.

9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]guanine (1). A solution of **6** (3.74 g, 12 mmol) in hydrochloric acid (2.0 M, 12 mL) was heated under reflux for 75 min. The solution was neutralized by addition of aqueous NaOH (10%) and then allowed to cool. The solution was filtered, and the solid was washed with water to afford **1** (2.18 g, 72%): mp 275–277 °C; UV (H_2O) λ_{max} 253 (ε 11 500) and 270 (sh, 8600) nm; IR (KBr) ν_{max} 3420, 3140, 1690, 1645, and 1605 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.45 (1 H, m, 3′-H), 1.71 (2 H, q, J = 7.1 Hz, 2′-H), 3.39 (4 H, m, D_2O exchange gives AB of ABX, 2 × CH_2O), 4.00 (2 H, t, J = 7.4 Hz, 1′-H), 4.40 (2 H, t, D_2O exchangeable, 2 × OH), 6.42 (2 H, s, D_2O exchangeable, 2-NH_2), 7.67 (1 H, s, 8-H), and 10.52 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{10}H_{15}N_5O_3$) C, H, N.

6-Chloro-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]purine (7) and 6-Chloro-7-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]-purine. To a mixture of 6-chloropurine (0.93 g, 6.0 mmol) and sodium hydride (0.35 g, 7.2 mmol 50% dispersion in oil) in DMF (20 mL) was added **5** (1.34 g, 6.0 mmol), and the mixture was stirred at room temperature for 16 h. The solution was filtered and the solvent removed. The residue was purified by column chromatography on silica gel eluting with chloroform–methanol mixtures (50:1 and 25:1). The first compound to eluate was **7** (0.84 g, 47%): mp 148–150 °C (from ether); UV (EtOH) λ_{max} 265 (ε 8930) nm; IR (KBr) ν_{max} 1595, 1560, 1500, 1440, 1335, and 1195 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.44 (6 H, s, 2 × CH_3), 1.50–1.85 (1 H, m, $CHCH_2CH_2$), 2.04 (2 H, q, J = 7 Hz, $CHCH_2CH_2$), 3.65 (2 H, dd, J = 6 Hz and 12 Hz, 2 × H_{ax}), 3.98 (2 H, dd, J = 4 Hz and 12 Hz, 2 × H_{eq}), 4.36 (2 H, t, J = Hz, CH_2N), 8.37 (1 H, s, 8-H), and 8.77 (1 H, s, 2-H). Anal. ($C_{13}H_{17}ClN_4O_2$) H, N; C: calcd, 52.62; found, 52.02. The second compound to elute was 6-chloro-7-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]purine (0.16 g, 9%): mp 135.5–138 °C; UV (EtOH) λ_{max} 270 (ε 7250) nm; IR (KBr) ν_{max} 3000, 1600, 1540, 1485, 1450, and 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.79 (1 H, m, $CHCH_2CH_2$), 2.05 (2 H, m, $CHCH_2CH_2$), 3.69 (2 H, dd, J = 6.4 Hz and 11.9 Hz, 2 × H_{ax}), 4.03 (2 H, dd, J = 4.0 Hz and 12.1 Hz, 2 × H_{eq}), 4.56 (2 H, m, CH_2N), 8.26 (1 H, s, 8-H), and 8.90 (1 H, s, 2-H), HRMS calcd for $C_{13}H_{17}ClN_4O_2$ 296.1044, found 296.1038.

9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]hypoxanthine (8). A solution of **7** (0.33 g, 1.1 mmol) in hydrochloric acid (1.0 M, 2.2 mL) was heated under reflux for 75 min. The solution was neutralized by addition of aqueous $NaHCO_3$ and allowed to cool. The solvent was removed and the residue purified by column chromatography on silica gel eluting with chloroform–ethanol mixtures (5:1 and 3:1) to afford **8** (0.18 g, 69%): mp 209–211 °C (from MeOH); UV (H_2O) λ_{max} 250 (ϵ 12000) nm; IR (KBr) ν_{max} 3050, 2880, and 1690 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.45 (1 H, m, 3′-H), 1.76 (2 H, q, J = 7 Hz, 2′-H), 3.34 (4 H, d, J = 5 Hz, 2 × CH_2O), 4.17 (2 H, t, J = 7 Hz, 1′-H), 4.4 (2 H, br, D_2O exchangeable, 2 × OH), 8.01 (1 H, s, 8-H), 8.08 (1 H, s, 2-H), and 12.1 (1 H, br, D_2O exchangeable, 1-H). Anal. ($C_{10}H_{14}N_4O_3$) C, H, N.

9-[4-Hydroxy-3-(hydroxymethyl)but-1-yl]xanthine (9). To a solution of **1** in hydrochloric acid (1 M, 11 mL) was added a solution of sodium nitrite (0.12 g, 1.7 mmol) in water (1 mL). The solution was stirred for 30 min, neutralized with aqueous NaOH, and concentrated to 4 mL. The solution was passed down a column of XAD-4 resin and eluted with water and 5% methanol. Product-containing fractions were pooled, and the solvent was removed to give 0.26 g. A portion of this material (0.17 g) was purified by preparative HPLC on a reverse-phase C_{18} μBondapak column eluting with 0–2% acetonitrile in water to give **9** (100 mg, 60%), which was recrystallized from 20% acetic acid in water: UV (H_2O) λ_{max} (pH 1) 236 and 262 nm, (pH 7) 249 and 271 nm, (pH 13) 248 and 279 nm; IR (KBr) ν_{max} 1707 and 1688 cm^{-1}; ^1H NMR (Me_2SO-d_6) 1.47 (1 H, m, 3′-H), 1.66 (2 H, q, J = 7.2 Hz, 2′-H), 3.3–3.5 (4 H, m, 2 × CH_2O), 4.06 (2 H, t, J = 7.6 Hz, 1′-H), 4.5 (2 H, br s, D_2O exchangeable, 2 × OH), 7.66 (1 H, s, 8-H), and 10.70 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{10}H_{14}N_4O_4 \cdot 0.3CH_3CO_2H$) C, H, N.

2-Amino-6-chloro-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]purine Hydrochloride (10). To a solution of **6** (0.46 g, 1.5 mmol) in THF (4.5 mL) was added hydrochloric acid (2.0 M, 0.5 mL). A white precipitate formed, and after 0.5 h, the solution was diluted with further THF and was filtered to give **10**·HCl (290 mg, 63%): decomposed over 165 °C; UV (H_2O, pH 5.5) λ_{max} 223 (ϵ 28400), 245 (4600), and 307 (7600) nm; IR (KBr) ν_{max} 3370, 3330, 3200, 2500, 1650, 1630, 1595, and 1505 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.53 (1 H, m, 3′-H), 1.83 (2 H, q, J = 7 Hz, 2′-H), 3.35 (4 H, d, J = 6 Hz, 2 × CH_2O), 4.19 (2 H, t, J = 7 Hz, 1′-H), 5.85 (9 H, s, D_2O exchangeable, 2 × OH, NH_2, HCl and H_2O), and 8.57 (1 H, s, 8-H). Anal. ($C_{10}H_{15}Cl_2N_5O_4$) C, H, N.

2-Amino-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]-6-thiopurine (11). A solution of **6** (0.31 g, 1.0 mmol) in aqueous sodium hydrosulfide (2 M, 3.0 mL) and ethanol (1.5 mL) was stirred at 70 °C for 1 h. To this solution was added glacial acetic acid (2.5 mL), and the mixture was stirred for a further 1 h at 70 °C. The solution was allowed to cool and filtered and the solvent removed. The residue was recrystallized from water to afford **11** (0.13 g, 48%): mp 260 °C dec; UV (H_2O) λ_{max} 230 (ϵ 16900), 263 (7200), and 341 (25200) nm; IR (KBr) ν_{max} 3310, 3130, 1650, 1610, and 1580 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.45 (1 H, m, 3′-H), 1.72 (2 H, q, J = 7 Hz, 2′-H), 3.3–3.5 (4 H, AB of ABX, J_{AB} = 10.7 Hz, J_{AX} = 5.5 Hz, and J_{BX} = 5.8 Hz, 2 × CH_2O), 4.02 (2 H, t, J = 7.4 Hz, 1′-H), 4.45 (2 H, br s, D_2O exchangeable, 2 × OH), 6.77 (2 H, s, D_2O exchangeable, 2-NH_2), 7.87 (1 H, s, 8-H), and 11.9 (1 H, br s, D_2O exchangeable, 1-H); HRMS calcd for $C_{10}H_{15}N_5O_2S$ 269.0956, found 269.0947. Anal. ($C_{10}H_{15}N_5O_2S$) C, H; N: calcd, 26.00; found, 25.44.

Preparation of 6-Alkoxy Compounds 12–17. Compound **6** (1.0 mmol) was treated with sodium alkoxide (1.1–2.0 mmol) in dioxane or the respective alcohol (2–4 mL) at 50–75 °C for 20–90 min. After cooling, dilute hydrochloric acid (1 mL) was added and the solution was stirred at room temperature until hydrolysis of the acetal was complete. The solution was neutralized by addition of aqueous $NaHCO_3$, and the solvent was removed. The residue was extracted with chloroform–ethanol (2:1) and purified by column chromatography on silica gel eluting with chloroform–methanol mixtures.

2-Amino-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]-6-methoxypurine (12): yield 77%; mp 117–119 °C; UV (H_2O) λ_{max} 213 (ϵ 22100), 249 (6900), and 280 (8400) nm; IR (KBr) ν_{max} 3400, 3240, 3210, 1640, 1610, 1590, 1410, and 1395 cm^{-1}; ^1H NMR (Me_2SO-d_6) 1.47 (1 H, m, 3′-H), 1.74 (2 H, q, J = 7 Hz, 2′-H), 3.40 (4 H, m, 2 × CH_2O), 3.95 (3 H, s, OCH_3), 4.06 (2 H, t, J = 7 Hz, 1′-H), 4.4 (2 H, br s, D_2O exchangeable, 2 × OH), 6.33 (2 H, s, D_2O exchangeable, 2-NH_2), and 7.46 (1 H, s, 8-H); HRMS calcd for $C_{11}H_{17}N_5O_3$ 267.1331, found 267.1340. Anal. ($C_{11}H_{17}N_5O_3$) H, N; C: calcd, 49.43; found, 48.82.

2-Amino-6-ethoxy-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]purine (13): yield 85%; mp 150–152 °C; UV (H_2O) λ_{max} 213 (ϵ 24300), 249 (7400), and 280 (9300) nm; IR (KBr) ν_{max} 3330, 3210, 2900, 1650, 1610, and 1580 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.3–1.6 (4 H, m, 3′-H and CH_3), 1.73 (2 H, q, J = 7 Hz, 2′-H), 3.2–3.6 (4 H, AB of ABX, 2 × CH_2OH), 4.04 (2 H, t, J = 7 Hz, 1′-H), 4.3–4.55 (4 H, m, 2 H, D_2O exchangeable, 2 × OH; D_2O exchange leaves 2 H, q, J = 7 Hz, 6-OCH_2), 6.30 (2 H, s, D_2O exchangeable, 2-NH_2), and 7.84 (1 H, s, 8-H). Anal. ($C_{12}H_{19}N_5O_3$) C, H, N.

2-Amino-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]-6-isopropoxypurine (14): yield 76%; mp 111.5–113.5 °C (from chloroform–carbon tetrachloride); UV (H_2O) λ_{max} 213 (ϵ 24700), 249 (7200), and 280 (9300) nm; IR (KBr) ν_{max} 3400, 3330, 3220, 1640, 1610, 1580, and 1525 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.34 (6 H, d, J = 6.3 Hz, $CH(CH_3)_2$), 1.45 (1 H, m, 3′-H), 1.74 (2 H, q, J = 7.2 Hz, 2′-H), 3.3–3.5 (4 H, m, 2 × CH_2O), 4.06 (2 H, t, J = 7.3 Hz, 1′-H), 4.39 (2 H, t, J = 5.2 Hz, D_2O exchangeable, 2 × OH), 5.49 (1 H, septet, J = 6.3 Hz, $CH(CH_3)_2$), 6.27 (2 H, s, D_2O exchangeable, 2-NH_2), and 7.83 (1 H, s, 8-H); HRMS calcd for $C_{13}H_{21}N_5O_3$ 295.1644, found 295.1649. Anal. ($C_{13}H_{21}N_5O_3 \cdot 0.2H_2O$) C, H, N.

2-Amino-6-butoxy-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]purine (15): yield 87%; mp 154–156 °C (from water); UV (H_2O) λ_{max} 213 (ϵ 24200), 249 (7300), and 280 (9300) nm; IR (KBr) ν_{max} 3470, 3440, 3210, 1655, 1610, and 1585 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.94 (3 H, t, J = 7.4 Hz, CH_3), 1.44 (3 H, m, 3′-H and CH_2CH_3), 1.73 (4 H, m, 2′-H and $CH_2CH_2CH_3$), 3.3–3.5 (4 H, m, 2 × CH_2OH), 4.07 (2 H, t, J = 7.3 Hz, 1′-H), 4.39 (2 H, t, J = 7.2 Hz, 6-OCH_2), 4.44 (2 H, t, J = 5.2 Hz, D_2O exchangeable, 2 × OH), 6.36 (2 H, s, D_2O exchangeable, 2-NH_2), and 7.86 (1 H, s, 8-H). Anal. ($C_{14}H_{23}N_5O_3$) C, H, N.

2-Amino-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]-6-phenoxypurine (16): yield 55%; mp 173–175 °C; UV (MeOH) λ_{max} 246 (ϵ 8500) and 288 (10300) nm; IR (KBr) ν_{max} 3470, 3370, 3330, 3220, 1630, 1615, and 1575 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.48 (1 H, m, 3′-H), 1.78 (2 H, q, J = 7.1 Hz, 2′-H), 3.3–3.5 (4 H, m, 2 × CH_2O), 4.12 (2 H, t, J = 7.4 Hz, 1′-H), 4.42 (2 H, t, J = 5.1 Hz, D_2O exchangeable, 2 × OH), 6.35 (2 H, s, D_2O exchangeable, 2-NH_2), 7.2–7.5 (5 H, m, C_6H_5), and 7.98 (1 H, s, 8-H). Anal. ($C_{16}H_{19}N_5O_3$) C, H, N.

2-Amino-6-(benzyloxy)-9-[4-hydroxy-3-(hydroxymethyl)-but-1-yl]purine (17): yield 50%; mp 146–147.5 °C; UV (EtOH) λ_{max} 212 (ϵ 32300), 250 (8400), and 283 (10100), nm; IR (KBr) ν_{max} 3340, 3220, 1655, 1605, and 1580 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.3–1.6 (1 H, m, 3′-H), 1.72 (2 H, q, J = 7 Hz, 2′-H), 3.38 (4 H, AB of ABX, 2 × CH_2OH), 4.03 (2 H, t, J = 7 Hz, 1′-H), 4.36 (2 H, t, J = 5.5 Hz, D_2O exchangeable, 2 × OH), 5.47 (2 H, s, $PhCH_2$), 6.37 (2 H, s, D_2O exchangeable, 2-NH_2), 7.3–7.6 (5 H, m, C_6H_5), and 7.84 (1 H, s, 8-H); HRMS calcd for $C_{17}H_{21}N_5O_3$ 343.1644, found 343.1642. Anal. ($C_{17}H_{21}N_5O_3$) H; C: calcd, 59.46; found, 58.98. N: calcd, 20.40; found, 19.71.

2-Amino-6-azido-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)-ethyl]purine (18). A solution of **6** (0.47 g, 1.5 mmol) and sodium azide (0.20 g, 3.0 mmol) in DMF (5 mL) was stirred at 100–110 °C for 4 h. The solvent was removed and the residue washed with water to leave **18** (0.36 g, 75%): mp 200 °C dec; UV (MeOH) λ_{max} 272 (ϵ 8200), and 301 (10100) nm; IR (KBr) ν_{max} 1670, 1625, and 1560 cm^{-1}; ^1H NMR ($CDCl_3$–CD_3OD) δ 1.44 (6 H, s, $C(CH_3)_2$), 1.6–2.2 (3 H, m, $CHCH_2CH_2$), 3.5–3.8 (2 H, dd, J = 7 Hz and 11 Hz, 2 × H_{ax}), 3.85–4.15 (2 H, dd, J = 4 Hz and 11 Hz, 2 × H_{eq}), 4.29 (2 H, t, J = 7 Hz, CH_2N), and 7.93 (1 H, s, 8-H). Anal. ($C_{13}H_{18}N_8O_2$) C, H, N.

2,6-Diamino-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]-purine (19). To a solution of ammonium formate in methanol (400 mM, 10 mL) were added **18** (318 mg, 1.0 mmol) and 10% palladium on charcoal (30 mg), and the mixture was heated under reflux for 1 h. The solution was allowed to cool and filtered and the solvent removed. The residue was purified by column chromatography on silica gel eluting with chloroform–methanol mixtures (20:1, 15:1) to give **19** (190 mg, 65%): mp 202–204 °C; IR (KBr) ν_{max} 1670, 1640, 1595, and 1410 cm^{-1}; ^1H NMR (CD-

Cl_3–CD_3OD) δ 1.42 (6 H, s, $C(CH_3)_2$), 1.6–2.0 (3 H, m, $CHCH_2CH_2$), 3.5–4.2 (6 H, m, 2 × CH_2O and CH_2N), and 7.68 (1 H, s, 8-H); HRMS calcd for $C_{13}H_{20}N_6O_2$ 292.1648, found 292.1652. Anal. ($C_{13}H_{20}N_6O_2$) H, N; C: calcd, 53.41; found, 52.88.

2,6-Diamino-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]purine (20). A solution of **19** (180 mg, 0.6 mmol) in 70% acetic acid (10 mL) was stirred for 1 h at room temperature. The solvent was removed, the residue was suspended in methanol, and sodium methoxide was added to neutralize. Column chromatography on silica gel eluting with chloroform–methanol mixtures (6:1 to 3:1) gave **20** (95 mg, 63%): mp 187–190 °C; UV (H_2O, pH 6.5) λ_{max} 215 (ε 25 500), 255 (7290), and 280 (9170) nm; IR (KBr) ν_{max} 3150, 1680, 1650, 1605, 1590, and 1410 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.46 (1 H, m, 3′-H), 1.73 (2 H, q, J = 7.1 Hz, 2′-H), 3.3–3.5 (4 H, AB of ABX, J_{AB} = 10.6 Hz, J_{AX} = 5.5 Hz, and J_{BX} = 5.9 Hz, 2 × CH_2O), 4.01 (2 H, t, J = 7.3 Hz, 1′-H), 4.41 (2 H, br s, D_2O exchangeable, 2 × OH), 5.70 (2 H, s, D_2O exchangeable, NH_2), 6.56 (2 H, s, D_2O exchangeable, NH_2), and 7.69 (1 H, s, 8-H). Anal. ($C_{10}H_{16}N_6O_2$·0.1$CHCl_3$) C, H, N.

2-Amino-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]purine (21). Method A. To a solution of **6** (0.54 g, 1.75 mmol) in ethanol (10 mL) and cyclohexene (20 mL) was added 10% palladium on charcoal (400 mg), and the solution was refluxed for 7 h. A further quantity of catalyst (200 mg) was added, and the solution was refluxed overnight. The solution was filtered and washed through with methanol. To the filtrate were added hydrochloric acid (5 M, 0.3 mL) and water (0.7 mL), and the solution was stirred for 30 min at room temperature. The solution was neutralized by addition of aqueous $NaHCO_3$, and the solvent was removed. The residue was purified by column chromatography on silica gel eluting with chloroform–methanol (5:1, 4:1) to afford **21** (150 mg, 36%): mp 156–158 °C; UV (H_2O) λ_{max} 220 (ε 25 600), 242 (4030), and 303 (6800) nm; IR (KBr) ν_{max} 3320, 3210, 1640, 1610, 1580, and 1430 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.47 (1 H, m, 3′-H), 1.78 (2 H, q, J = 7.2 Hz, 2′-H), 3.3–3.5 (4 H, m, 2 × CH_2O), 4.12 (2 H, t, J = 7.4 Hz, 1′-H), 4.42 (2 H, t, J = 5.2 Hz, D_2O exchangeable, 2 × OH), 6.45 (2 H, s, D_2O exchangeable, 2-NH_2), 8.06 (1 H, s, 8-H), and 8.56 (1 H, s, 6-H). Anal. ($C_{10}H_{15}N_5O_2$) C, H, N.

Method B (Alternative Reduction). To a solution of ammonium formate in methanol (400 mM, 3 mL) were added **6** (90 mg, 0.3 mmol) and 10% palladium on charcoal (28 mg), and the mixture was heated under reflux. After 1.5 h, reduction to 2-amino-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]purine was complete.

9-[4-(Formyloxy)-3-[(formyloxy)methyl]but-1-yl]guanine (24). A mixture of **1** (0.23 g, 0.9 mmol), dicyclohexylcarbodiimide (0.92 g, 4.5 mmol), formic acid (0.17 mL, 4.5 mmol), 4-(dimethylamino)pyridine (20 mg), and DMF (5 mL) was stirred for 40 min at room temperature and then quenched by addition of methanol (1 mL). The solution was filtered and the solvent removed. The residue was purified by column chromatography on silica gel eluting with chloroform–methanol mixtures (7:1, 4:1) to afford **24**, which was crystallized from methanol (0.12 g, 43%): mp 195–198 °C; IR (KBr) ν_{max} 1720, 1680, 1630, 1600, and 1570 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.83 (2 H, q, J = 7.1 Hz, 2′-H), 2.01 (1 H, m, 3′-H), 4.04 (2 H, t, J = 7.1 Hz, 1′-H), 4.13 (4 H, d, J = 5.5 Hz, 2 × CH_2O), 6.39 (2 H, s, D_2O exchangeable, 2-NH_2), 7.70 (1 H, s, 8-H), 8.23 (2 H, s, 2 × HCOO), and 10.52 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{12}H_{15}N_5O_5$) C, H, N.

9-[4-Acetoxy-3-(acetoxymethyl)but-1-yl]guanine (25). A mixture of **1** (253 mg, 1.0 mmol), 4-(dimethylamino)pyridine (25 mg), and acetic anhydride (8.5 mL) was stirred for 4 days at room temperature. The acetic anhydride was removed under reduced pressure. The residue was purified by column chromatography on silica gel eluting with chloroform–methanol mixtures (20:1 and 10:1) to afford **25** (160 mg, 47%): mp 202–205 °C (from MeOH); IR (KBr) ν_{max} 1737, 1690, 1628, 1600, and 1240 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.79 (2 H, q, J = 6.7 Hz, 2′-H), 1.91 (1 H, m, 3′-H), 2.00 (6 H, s, 2 × CH_3) 4.00 (6 H, m, 2 × CH_2O and 1′-H), 6.42 (2 H, s, D_2O exchangeable, 2-NH_2), 7.71 (1 H, s, 8-H), and 10.54 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{14}H_{19}N_5O_5$) C, H, N.

9-[4-(Propionyloxy)-3-[(propionyloxy)methyl]but-1-yl]guanine (26) and N^2-Propionyl-9-[4-(propionyloxy)-3-[(propionyloxy)methyl]but-1-yl]guanine (28). A mixture of **1** (253 mg, 1.0 mmol), 4-(dimethylamino)pyridine (30 mg), propionic anhydride (8 mL), and DMF (15 mL) was stirred at room temperature for 66 h. The solvent was removed and the residue subjected to column chromatography on silica gel eluting with chloroform–methanol mixtures (30:1, 10:1). The first compound to elute was **28** (200 mg, 47%): mp 152–154 °C (from ether–MeOH); IR (KBr) ν_{max} 1740, 1675, 1610, 1560, and 1185 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (6 H, t, J = 7.5 Hz, 2 × $OCOCH_2CH_3$), 1.27 (3 H, t, J = 7.5 Hz, $NCOCH_2CH_3$), 1.88 (2 H, q, J = 6.9 Hz, 2′-H), 2.01 (1 H, m, 3′-H), 2.35 (4 H, q, J = 7.5 Hz, 2 × $OCOCH_2CH_3$), 2.56 (2 H, q, J = 7.5 Hz, $NCOCH_2CH_3$), 4.1–4.3 (6 H, m, 2 × CH_2O and 1′-H), 7.65 (1 H, s, 8-H), 9.14 (1 H, s, 2-NH), and 11.95 (1 H, s, 1-H); HRMS calcd for $C_{19}H_{27}N_5O_6$ 421.1961, found 421.1958. Anal. ($C_{19}H_{27}N_5O_6$) C, H; N: calcd, 16.62; found, 16.19.

The second compound to elute was **26** (150 mg, 41%): mp 204–206 °C (from MeOH); IR (KBr) ν_{max} 3310, 3150, 1740, 1690, and 1190 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.01 (6 H, t, J = 7.4 Hz, 2 × CH_2CH_3), 1.80 (2 H, q, J = 7.0 Hz, 2′-H), 1.91 (1 H, m, 3′-H), 2.29 (4 H, q, J = 7.5 Hz, 2 × CH_2CH_3), 4.01 (6 H, m, 2 × CH_2O and 1′-H), 6.37 (2 H, s, D_2O exchangeable, 2-NH_2), 7.69 (1 H, s, 8-H), and 10.50 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{16}H_{23}N_5O_5$) C, H, N.

9-[4-(Hexanoyloxy)-3-[(hexanoyloxy)methyl]but-1-yl]guanine (27). A mixture of **1** (253 mg, 1.0 mmol), dicyclohexylcarbodiimide (0.83 mg, 4.0 mmol), hexanoic acid (0.38 mL, 3.0 mmol), 4-(dimethylamino)pyridine (20 mg), and DMF (5 mL) was stirred for 64 h at room temperature. The mixture was diluted with water and extracted with chloroform (2×). The combined organic layers were washed with aqueous $NaHCO_3$ and dried ($MgSO_4$), and the solvent was removed. The residue was purified by column chromatography eluting with chloroform–methanol mixtures to afford **27** (200 mg, 45%): mp 198.5–201 °C (from MeOH); IR (KBr) ν_{max} 3340, 3160, 2960, 2930, 1740, 1690, 1650, 1605, and 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (6 H, t, J = 6.9 Hz, 2 × CH_3), 1.28 (8 H, m, 2 × $CH_2CH_2CH_3$), 1.60 (4 H, quintet, J = 7.4 Hz, 2 × $COCH_2CH_2$), 1.90 (2 H, q, J = 6.9 Hz, 2′-H), 2.02 (1 H, m, 3′-H), 2.30 (4 H, t, J = 7.6 Hz, 2 × $COCH_2CH_2$), 4.13 (6 H, m, 2 × CH_2O and 1′-H), 6.42 (2 H, s, D_2O exchangeable, 2-NH_2), 7.70 (1 H, s, 8-H), and 12.16 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{22}H_{35}N_5O_5$) C, H, N.

N^2-(Monomethoxytrityl)-9-[3-(hydroxymethyl)-4-[(monomethoxytrityl)oxy]but-1-yl]guanine (23) and N^2-(Monomethoxytrityl)-9-[4-hydroxy-3-(hydroxymethyl)but-1-yl]guanine (22). A solution of **1** (4.05 g, 16 mmol), monomethoxytrityl chloride (10.9 g, 35 mmol), triethylamine (6.7 mL), and 4-(dimethylamino)pyridine (40 mg) in DMF (50 mL) was stirred for 2 h. The reaction was quenched with methanol, and the solvent was removed. The residue was taken up in ethyl acetate and the solution washed with aqueous $NaHCO_3$ and water. The solution was dried ($MgSO_4$) and the solvent removed. The residue was purified by column chromatography on silica gel eluting with chloroform–methanol mixtures. The first major product to elute was **23** (4.4 g, 34%): mp 142–145 °C; UV (EtOH) λ_{max} 230 (ε sh, 29 900) and 262 (16 000) nm; ^1H NMR (Me_2SO-d_6) δ 1.24 (2 H, m, 2′-H), 1.43 (1 H, m, 3′-H), 2.7–2.9 (2 H, AB of ABX, CH_2OC), 3.1–3.4 (2 H, AB of ABX, CH_2OH), 3.42 (2 H, t, J = 6.7 Hz, 1′-H), 3.66 (3 H, s, CH_3O), 3.74 (3 H, s, CH_3O), 4.35 (1 H, t, J = 4.8 Hz, D_2O exchangeable, OH), 6.7–7.4 (28 H, m, Ar H), 7.44 (1 H, s, 8-H), 7.55 (1 H, s, D_2O exchangeable, 2-NH), and 10.50 (1 H, s, D_2O exchangeable, 1-H).

The second major product to elute was **22** (1.4 g, 17%): mp 205–207 °C; UV (EtOH) λ_{max} 261 (ε 14 500) nm; ^1H NMR (Me_2SO-d_6) δ 1.25 (3 H, m, 2′-H and 3′-H), 3.1–3.3 (4 H, m, 2 × CH_2O), 3.52 (2 H, t, J = 6.6 Hz, 1′-H), 3.72 (3 H, s, CH_3O), 4.28 (2 H, t, J = 5.2 Hz, D_2O exchangeable, 2 × OH), 6.86–7.35 (14 H, m, Ar H), 7.54 (1 H, s, 8-H), 7.56 (1 H, s, D_2O exchangeable, 2-NH), and 10.49 (1 H, s, D_2O exchangeable, 1-H).

9-[4-(Pivalyloxy)-3-[(pivalyloxy)methyl]but-1-yl]guanine (29). To a solution of **22** (0.47 g, 0.9 mmol) in pyridine (4.5 mL) was added pivalyl chloride (0.55 mL, 4.5 mmol), and the solution was stirred for 45 min. The mixture was precipitated in water (45 mL), and the resulting precipitate was stirred in 80% acetic acid (10 mL) at 80 °C for 20 min. The solvent was removed, and the residue was purified by column chromatography on silica gel eluting with chloroform–methanol (10:1) to afford **29** (0.22 g, 58%): mp 239–245 °C; IR (KBr) ν_{max} 3430, 2980, 1730, 1690, and 1620 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.11 (18 H, s, 2 × $C(CH_3)_3$), 1.81 (2 H, q, J = 6.8 Hz, 2′-H), 1.93 (1 H, m, 3′-H), 4.0–4.1 (6 H, m, 1′-H

and 2 × CH_2O), 6.38 (2 H, s, 2-NH_2), 7.69 (1 H, s, 8-H), and 10.58 (1 H, br s, 1-H). Anal. ($C_{20}H_{31}N_5O_5$) C, H, N.

9-[4-(Acyloxy)-3-(hydroxymethyl)but-1-yl]guanines 30–33. To a solution of **23** (0.80 g, 1.0 mmol) in pyridine (4 mL) was added acyl chloride (3.0 mmol), and the solution was stirred for 20–30 min. The mixture was precipitated in water (40 mL) and the resulting precipitate stirred in 80% acetic acid (10 mL) at 80 °C for 0.5–2.0 h. The solvent was removed and the residue purified by column chromatography on silica gel eluting with chloroform–methanol mixtures.

9-[4-Acetoxy-3-(hydroxymethyl)but-1-yl]guanine (30): yield 64%; mp 201–205 °C; IR (KBr) ν_{max} 3330, 3170, 2930, 1730, 1690, 1660, 1610, and 1565 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.6–1.8 (3 H, m, 2′-H and 3′-H), 1.98 (3 H, s, CH_3), 3.39 (2 H, br s, D_2O exchange gives d, J = 5 Hz, CH_2OH), 3.9–4.1 (4 H, m, 1′-H and CH_2OCO), 4.61 (1 H, br t, D_2O exchangeable, OH), 6.44 (2 H, s, D_2O exchangeable, 2-NH_2), 7.68 (1 H, s, 8-H), and 10.59 (1 H, s, D_2O exchangeable, 1-H); FABMS (positive ion, thioglycerol) 318 (MNa^+), 296 (MH^+). Anal. ($C_{12}H_{17}N_5O_4$·0.3CH_3CO_2H) C, H, N.

9-[4-(Hexanoyloxy)-3-(hydroxymethyl)but-1-yl]guanine (31): yield 38%; mp 179–181 °C; IR (KBr) ν_{max} 2960, 2930, 1730, 1690, 1630, and 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.84 (3 H, t, J = 6.9 Hz, CH_3), 1.24 (4 H, m, $CH_3(CH_2)_2$), 1.50 (2 H, quintet, J = 7.3 Hz, CH_2CH_2CO), 1.6–1.8 (3 H, m, 2′-H and 3′-H), 2.26 (2 H, t, J = 7.3 Hz, CH_2CO), 3.40 (2 H, t, J = 5 Hz, D_2O exchange leaves d, CH_2OH), 3.9–4.1 (4 H, m, 1′-H and CH_2OCO), 4.60 (1 H, t, J = 5.1 Hz, D_2O exchangeable, OH), 6.38 (2 H, s, D_2O exchangeable, 2-NH_2), 7.67 (1 H, s, 8-H), and 10.49 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{16}H_{25}N_5O_4$·0.25H_2O) C, H, N.

9-[4-(Hexadecanoyloxy)-3-(hydroxymethyl)but-1-yl]guanine (32): yield 52%; mp 183–191 °C; IR (KBr) ν_{max} 3340, 3160, 2920, 2850, 1740, 1690, and 1605 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.85 (3 H, t, J = 6.6 Hz, CH_3), 1.23 (24 H, m, $CH_3(CH_2)_{12}$), 1.49 (2 H, m, CH_2CH_2CO), 1.6–1.8 (3 H, m, 2′-H and 3′-H), 2.26 (2 H, t, J = 7.3 Hz, CH_2CO), 3.39 (2 H, t, J = 5 Hz, D_2O exchange leaves d, CH_2OH), 3.9–4.1 (4 H, m, 1′-H and CH_2OCO), 4.60 (1 H, t, J = 5.2 Hz, D_2O exchangeable, OH), 6.38 (2 H, s, D_2O exchangeable, 2-NH_2), 7.67 (1 H, s, 8-H), and 10.50 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{26}H_{45}N_5O_4$) C, H, N.

9-[4-(Benzoyloxy)-3-(hydroxymethyl)but-1-yl]guanine (33): yield 25%; mp 160–169 °C; UV (MeOH) λ_{max} 231 (ϵ 15 100) and 254 (13 100) nm; IR (KBr) ν_{max} 1715, 1690, 1625, and 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.75–1.90 (3 H, m, 2′-H and 3′-H), 3.50 (2 H, t, J = 5 Hz, D_2O exchange leaves d, CH_2OH), 4.07 (2 H, t, J = 6.9 Hz, 1′-H), 4.2–4.35 (2 H, AB of ABX, CH_2OCO), 4.68 (1 H, t, J = 5.1 Hz, D_2O exchangeable, OH), 6.39 (2 H, s, D_2O exchangeable, 2-NH_2), 7.5–8.0 (6 H, m, C_6H_5 and 8-H), and 10.53 (1 H, s, D_2O exchangeable, 1-H); FABMS (positive ion, thioglycerol) 380 (MNa^+), 358 (MH^+). Anal. ($C_{17}H_{19}N_5O_4$·1.5H_2O) C, H, N.

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1-Amino-Substituted 4-Methyl-5*H*-pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines: A New Class of Antineoplastic Agents

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In an attempt to find new anticancer agents, a series of pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines were synthesized and evaluated in the standard NCI screening. Among these new compounds, which are structurally related to 9-azaellipticines but differ by deletion of a cycle, those that have a 4-methyl group and a $NHCH_2CH_2CH_2NR_2$ side chain at the 1-position show significant cytotoxicity on L1210 cultured cells and antitumor properties in the in vivo P388 leukemia system. The in vivo antineoplastic activity of the most potent compounds were confirmed on the L1210 leukemia model.

The antineoplastic activity in the ellipticine series and tetracyclic analogues is now well-documented.[1-10] A common feature of this class of compounds is their intercalative binding to DNA. Nevertheless, in various series of closely related derivatives, many compounds having the same apparent affinity for DNA differ in their biological properties.[2,7] It was concluded that a good affinity for DNA is a necessary, but not a sufficient, condition.

Ellipticine treatment of cells in culture causes DNA strand breaks and formation of DNA protein crosslinks probably through the interaction with topoisomerases (Pommier et al.[11,12]). The role of the binding to DNA is currently unknown.

Studies of DNA binding vs. antitumor potency of a series of *m*-AMSA analogues, another family of intercalating antitumor compounds, suggest that these parameters are related, but other parameters are clearly also involved in

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