8-Substituted O^6-Benzylguanine, Substituted 6(4)-(Benzyloxy)pyrimidine, and Related Derivatives as Inactivators of Human O^6-Alkylguanine-DNA Alkyltransferase[||]

Mi-Young Chae,[†] Kristin Swenn,[§] Sreenivas Kanugula,[§] M. Eileen Dolan,[‡] Anthony E. Pegg,[§] and Robert C. Moschel*,[†]

Carcinogen-Modified Nucleic Acid Chemistry, Chemistry of Carcinogenesis Laboratory, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, Maryland 21702, Departments of Cellular and Molecular Physiology and of Pharmacology, Pennsylvania State University College of Medicine, The Milton S. Hershey Medical Center, P.O. Box 850, Hershey, Pennsylvania 17033, and Division of Hematology-Oncology, The University of Chicago Medical Center, 5841 South Maryland Avenue, Box MC2115, Chicago, Illinois 60637

Received October 7, 1994[⊗]

Several 8-substituted O^6-benzylguanines, 2- and/or 8-substituted 6-(benzyloxy)purines, substituted 6(4)-(benzyloxy)pyrimidines, and a 6-(benzyloxy)-*s*-triazine were tested for their ability to inactivate the human DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT, alkyltransferase). Two types of compounds were identified as being significantly more effective than O^6-benzylguanine (the prototype low molecular weight inactivator) at inactivating AGT in human HT29 colon tumor cell extracts. These were 8-substituted O^6-benzylguanines bearing electron-withdrawing groups at the 8-position (e.g. 8-aza-O^6-benzylguanine and O^6-benzyl-8-bromoguanine) and 5-substituted 2,4-diamino-6-(benzyloxy)pyrimidines bearing electron-withdrawing groups at the 5-position (e.g. 2,4-diamino-6-(benzyloxy)-5-nitroso- and 2,4-diamino-6-(benzyloxy)-5-nitropyrimidine). The latter derivatives were also more effective than O^6-benzylguanine at inactivating AGT in intact HT29 colon tumor cells. Provided these types of purines and pyrimidines do not exhibit undesirable toxicity, they may be superior to O^6-benzylguanine as chemotherapeutic adjuvants for enhancing the effectiveness of antitumor drugs for which the mechanism of action involves modification of the O^6-position of DNA guanine residues.

Inactivation of the human DNA repair protein O^6-alkylguanine-DNA alkyltransferase by exposure to O^6-benzylguanine leads to a dramatic enhancement in the cytotoxic response of human tumor cells and tumor xenografts to chemotherapeutic drugs for which the mechanism of action involves modification of DNA guanine residues at the O6-position.[1–8] In two previous surveys,[9,10] we compared the AGT-inactivating activity of a large number of O^6-benzylguanine analogs with the aim of obtaining information about the types of substituent groups and the sites at which they could be attached to O^6-benzylguanine without significantly lowering its AGT-inactivating activity. While these studies led to the production of a variety of analogs that were as potent or somewhat less potent than O^6-benzylguanine, none of the analogs were more potent than O^6-benzylguanine. In the present report we summarize results of our most recent survey of the AGT-inactivating activity of 21 additional compounds, including 8-substituted O^6-benzylguanines, 2- and/or 8-substituted 6-(benzyloxy)purines, 6(4)-(benzyloxy)pyrimidines, and a 6-(benzyloxy)-*s*-triazine. This survey has revealed that two types of compounds, i.e., 8-substituted O^6-benzylguanine derivatives bearing electron-withdrawing substituents at the 8-position and 5-substituted 2,4-diamino-6-(benzyloxy)pyrimidine derivatives bearing electron-withdrawing substituents at the 5-position, are more effective AGT inactivators than O^6-benzylguanine and may, therefore, be superior to O^6-benzylguanine as adjuvants in enhancing the therapeutic effectiveness of antitumor agents for which the mechanism of action involves modification of the O6-position of DNA guanine residues. The results of this activity survey are summarized below.

Results and Discussion

The 21 compounds surveyed for AGT-inactivating activity in this report are illustrated in Chart 1. Preparation of the 8-substituted O^6-benzylguanine derivatives 8-amino-O^6-benzylguanine (**1a**) and O^6-benzyl-8-methylguanine (**1b**) was accomplished by treating 2,8-diamino-6-chloropurine and 2-amino-6-chloro-8-methylpurine, respectively, with sodium benzyl oxide in benzyl alcohol. O^6-Benzyl-8-oxoguanine (O^6-benzyl-7,8-dihydro-8-oxoguanine, **1c**) was prepared by reacting 1,1′-carbonyldiimidazole with 2,4,5-triamino-6-(benzyloxy)-pyrimidine[11] as recently described.[12] For convenience, the compound is illustrated in the 8-hydroxy tautomeric form although it most probably exists in solution in the 8-keto form with a hydrogen attached to the 7-nitrogen atom. O^6-Benzyl-8-bromoguanine (**1d**) was prepared by bromination of O^6-benzylguanine. O^6-Benzyl-8-(trifluoromethyl)guanine (**1e**) was prepared by reacting 2-amino-6-chloro-8-(trifluoromethyl)purine with sodium benzyl oxide in benzyl alcohol. 8-Aza-O^6-benzylguanine (**2**) was prepared through nitrous acid treatment of 2,4,5-triamino-6-(benzyloxy)pyrimidine. Compound **2** had been prepared previously by another route.[13] With respect to the pyrimidine derivatives (**3a–f**), 4-amino-6-(benzyloxy)-5-nitropyrimidine (**3a**) was prepared by

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

[†] National Cancer Institute-Frederick Cancer Research and Development Center.

[‡] The University of Chicago Medical Center.

[§] Pennsylvania State University College of Medicine.

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1994.

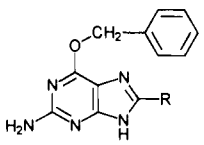
0022-2623/95/1838-0359$09.00/0

Table 1. AGT-Inactivating Activity of 6-(Benzyloxy)purine and 6(4)-(Benzyloxy)pyrimidine Derivatives

compound	ED_{50} (μM)[a]	
	In HT29 cell free extract	In HT29 cells
2,4-diamino-6-(benzyloxy)-5-nitrosopyrimidine (**3d**)	0.06	0.02
2,4-diamino-6-(benzyloxy)-5-nitropyrimidine (**3e**)	0.06	0.02
8-aza-O^6-benzylguanine (**2**)	0.07	0.06
O^6-benzyl-8-bromoguanine (**1d**)	0.08	0.06
O^6-benzylguanine	0.2	0.05
O^6-benzyl-8-methylguanine (**1b**)	0.3	0.1
O^6-benzyl-8-oxoguanine (**1c**)	0.3	0.15
2-amino-4-(benzyloxy)-5-nitropyrimidine (**5a**)	0.4	0.05
2-amino-4-(benzyloxy)-6-methyl-5-nitropyrimidine (**5b**)	0.4	0.06
O^6-benzyl-8-(trifluoromethyl)guanine (**1e**)	0.4	0.25
2,4,5-triamino-6-(benzyloxy)pyrimidine (**3c**)	0.4	0.3
8-amino-O^6-benzylguanine (**1a**)	0.7	2
2,4-diamino-6-(benzyloxy)-5-bromopyrimidine (**3f**)	2	0.8
2,4-diamino-6-(benzyloxy)-*s*-triazine (**6**)	4	1.0
2,4-diamino-6-(benzyloxy)pyrimidine (**3b**)	15	5
O^6-benzyluric acid (**4b**)	25	45
4-amino-6-(benzyloxy)-5-nitropyrimidine (**3a**)	28	8
O^6-benzyl-2-fluorohypoxanthine (**4c**)	48	12
O^6-benzylxanthine (**4a**)	60	35
N^2-acetyl-O^6-benzyl-8-oxoguanine (**4d**)	65	11
O^6-benzyl-N^2-methylguanine (**4e**)	160	60
O^6-benzyl-N^2,N^2-dimethylguanine (**4f**)	200	110

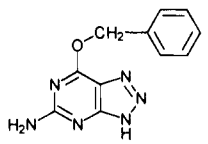
[a] The effective dose required to produce 50% inactivation in cell-free extracts upon incubation for 30 min or in cells upon incubation for 4 h. The values for O^6-benzylguanine were from ref 9. Data for compounds **1c** and **4d** are from ref 12.

Chart 1

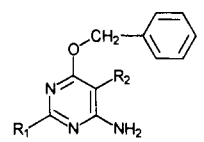


1a-e

1a, R = NH_2
1b, R = CH_3
1c, R = OH
1d, R = Br
1e, R = CF_3

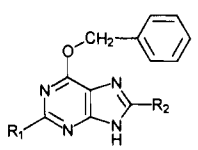


2



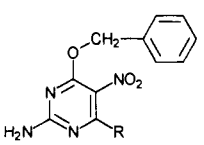
3a-f

3a, R_1 = H; R_2 = NO_2
3b, R_1 = NH_2; R_2 = H
3c, R_1 = NH_2; R_2 = NH_2
3d, R_1 = NH_2; R_2 = NO
3e, R_1 = NH_2; R_2 = NO_2
3f, R_1 = NH_2; R_2 = Br



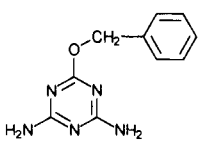
4a-f

4a, R_1 = HO; R_2 = H
4b, R_1 = HO; R_2 = OH
4c, R_1 = F; R_2 = H
4d, R_1 = CH_3CONH; R_2 = OH
4e, R_1 = CH_3NH; R_2 = H
4f, R_1 = $(CH_3)_2N$; R_2 = H



5a,b

5a, R = H
5b, R = CH_3



6

treating 4-amino-6-chloro-5-nitropyrimidine[14] with sodium benzyl oxide in benzyl alcohol. Derivatives **3b–d** were prepared by the method of Pfleiderer et al.[11] 2,4-Diamino-6-(benzyloxy)-5-nitropyrimidine (**3e**) and 2,4-diamino-6-(benzyloxy)-5-bromopyrimidine (**3f**) were prepared previously.[15]

The purines O^6-benzylxanthine (**4a**) and O^6-benzyluric acid (**4b**) were prepared by nitrous acid deamination of O^6-benzylguanine and O^6-benzyl-8-oxoguanine, respectively. N^2-Acetyl-O^6-benzyl-8-oxoguanine (N^2-acetyl-O^6-benzyl-7,8-dihydro-8-oxoguanine) (**4d**) was prepared through acetylation of O^6-benzyl-8-oxoguanine (**1c**).[12] O^6-Benzyl-2-fluorohypoxanthine (**4c**) was prepared previously by Robins and Robins.[16] This material was treated with methylamine and dimethylamine to produce O^6-benzyl-N^2-methylguanine (**4e**) and O^6-benzyl-N^2,N^2-dimethylguanine (**4f**), respectively.

Compounds **5a** (2-amino-4-(benzyloxy)-5-nitropyrimidine) and **5b** (2-amino-4-(benzyloxy)-6-methyl-5-nitropyrimidine) were prepared by treating 2-amino-4-chloro-5-nitropyrimidine and 2-amino-4-chloro-6-methyl-5-nitropyrimidine,[14] respectively, with sodium benzyl oxide in benzyl alcohol. Compound **6** (2,4-diamino-6-(benzyloxy)-*s*-triazine) was prepared previously under similar conditions.[17]

The ability of these compounds to inactivate the AGT protein in HT29 human colon tumor cell extracts and in intact HT29 cells is summarized in Table 1. The data represent the dose of compound required to produce 50% inactivation in cell-free extracts upon incubation for 30 min or in cells upon incubation for 4 h. These ED_{50} values were obtained from plots of the percent alkyltransferase activity remaining as a function of dose of compound administered. Plots for the pyrimidine derivatives **3d** and **3e** are presented in Figure 1. Data for O^6-benzylguanine are included in Figure 1 for comparison.[9]

Of all the compounds tested in these experiments, O^6-benzyl-N^2-methyl- and O^6-benzyl-N^2,N^2-dimethylguanine (**4e** and **4f**) were the least active agents, exhibiting ED_{50} values for inactivation of AGT in HT29 cell extracts of 160 and 200 μM, respectively. For comparison, the ED_{50} value exhibited by O^6-benzylguanine was 0.2 μM (Table 1).[9] Clearly, alkyl substitution at the exocyclic amino group of O^6-benzylguanine is not compatible with efficient alkyltransferase inactivation. Since we showed previously that the absence of an N^2 amino group (as in O^6-benzylhypoxanthine)[9] or an acetylated amino group (as in N^2-acetyl-O^6-benzylguanine)[10] also diminished alkyltransferase inactivating potency, it is

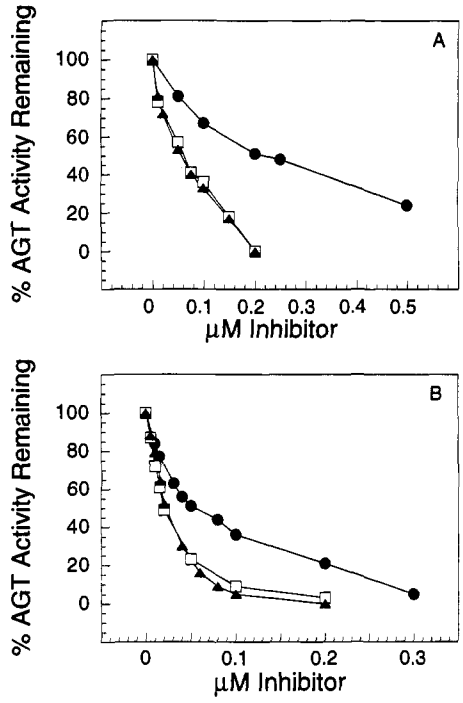


Figure 1. Loss of alkyltransferase activity in HT29 cell-free extracts (A) and HT29 cells (B). (A) Alkyltransferase from extracts was incubated with increasing concentrations of O^6-benzylguanine[9] (closed circles), 2,4-diamino-6-(benzyloxy)-5-nitrosopyrimidine (**3d**) (open squares), or 2,4-diamino-6-(benzyloxy)-5-nitropyrimidine (**3e**) (closed triangles) for 30 min. (B) HT29 cells in exponential growth were exposed to increasing concentrations of O^6-benzylguanine[9] (closed circles), **3d** (open squares), or **3e** (closed triangles) for 4 h. The results are expressed as percentage of alkyltransferase activity remaining after drug addition relative to zero drug addition.

clear that a free exocyclic amino group at the 2-position on an O^6-benzylated purine derivative is required for optimal activity.

The other 2- and/or 8-substituted 6-(benzyloxy)purines, N^2-acetyl-O^6-benzyl-8-oxoguanine (**4d**), O^6-benzylxanthine (**4a**), O^6-benzyl-2-fluorohypoxanthine (**4c**), and O^6-benzyluric acid (**4b**), together with the substituted pyrimidines 4-amino-6-(benzyloxy)-5-nitropyrimidine (**3a**) and 2,4-diamino-6-(benzyloxy)pyrimidine (**3b**), comprised a group of increasingly more active AGT-inactivating agents exhibiting intermediate ED_{50} values for cell-free extracts in the range of 65-15 μM. 2,4-Diamino-6-(benzyloxy)-*s*-triazine (**6**) and 2,4-diamino-6-(benzyloxy)-5-bromopyrimidine (**3f**) were considerably more active than **3b**, indicating that electron-withdrawing groups at the 5-position of a 2,4-diamino-6-(benzyloxy)pyrimidine derivative are positive contributors to efficient AGT inactivation. This is further emphasized by the very high activity exhibited by 2,4-diamino-6-(benzyloxy)-5-nitrosopyrimidine (**3d**) and 2,4-diamino-6-(benzyloxy)-5-nitropyrimidine (**3e**) (Figure 1), which contain strongly electron-withdrawing nitroso and nitro substituents, respectively. These two derivatives are at least 3.3 times more active than O^6-benzylguanine and are the most active alkyltransferase inactivators tested to date (Table 1). The observation that 2-amino-4-(benzyloxy)-5-nitropyrimidine (**5a**) is much more active than **3a** indicates that a 2-amino group is critical for high activity for a 6(4)-(benzyloxy)-5-nitropyrimidine derivative. This parallels the requirement for a free 2-amino group for maximal activity of O^6-benzylated purines, as noted above. An additional alkyl group at the 4(6)-position (e.g. as in **5b**) does not enhance activity significantly over that for **5a** although an amino group at the 4(6)-position (as in **3e**) clearly does. Consequently, AGT-inactivating activity increases substantially over the series **5a** = **5b** < **3d** = **3e**. With these considerations in mind, the activity of 2,4,5-triamino-6-(benzyloxy)pyrimidine (**3c**) seems anomalous, and the reasons for its relatively high activity are unclear at present. It is also significant that pyrimidine **5a** and **5b** are quite active in cells, which is not totally predicted by their corresponding activity in HT29 cell extracts.

All the O^6-benzylguanine analogs **1a**–**e** and **2** were much more active than the purines in the series **4a**–**f**, the activity differences among **1a**–**e** and **2** also reflect enhancements due to introduction of electron-withdrawing groups. Thus, activity increased in the series 8-amino-O^6-benzylguanine (**1a**) < O^6-benzyl-8-(trifluoromethyl)guanine (**1e**) < O^6-benzyl-8-oxoguanine (**1c**) < O^6-benzyl-8-methylguanine (**1b**) < O^6-benzyl-8-bromoguanine (**1d**) < 8-aza-O^6-benzylguanine (**2**). Indeed, derivatives **1d** and **2** were essentially as active as pyrimidines **3d** and **3e** in cell-free extracts although **1d** and **2** were somewhat less active in cells than expected from their activity in cell-free extracts. This reduced activity in cells may be a consequence of their inefficient uptake since these latter derivatives are probably partially anionic under physiological conditions as a result of the electronegativity of their 8-substituents.[18] The enhanced activity exhibited by **1d** and **2** or in the pyrimidine series by **3d** and **3e** is probably a result of their more ready displacement by the alkyltransferase active-site cysteine since the weaker basicity of the anionic forms of 8-bromo- and 8-azaguanine or 2,4-diamino-6-hydroxy-5-nitroso- and 2,4-diamino-6-hydroxy-5-nitropyrimidine relative to guanine anion should make the former heterocycles better leaving groups. On this basis, however, it is unclear why **1e** is less active than **1d** or **2**, unless the 8-(trifluoromethyl) substituent somehow interferes sterically with alkyltransferase inactivation by **1e**. Further experiments will be required to explain its relative inactivity.

The low ED_{50} values exhibited by **1d**, **2**, **3d**, and **3e** suggest that they, or some derivative thereof, may be superior to O^6-benzylguanine for use as chemotherapeutic adjuvants.[1-8] Additional support for the possible superiority of the pyrimidine derivatives has recently been obtained through studies with mutant human alkyltransferases[19] that are resistant to inactivation by O^6-benzylguanine. For example, mutant alkyltransferase derived by replacing proline^{140} or both proline^{138} and proline^{140} by alanines or glycine^{156} by an alanine are 20–240-fold more resistant to inactivation by O^6-benzylguanine than the normal human protein. Therefore, the usefulness of O^6-benzylguanine may be limited when combined with mutagenic chloroethylating or methylating antitumor drugs that could produce point mutations leading to resistant alkyltransferases. However, the pyrimidine derivative **3d** is at least 60 times better than O^6-benzylguanine at inactivating these mutant proteins (Crone *et al.*, unpublished observations), suggesting that this or related inactivators could be used to advantage in adjuvant chemotherapy situations involving drugs for which the mechanism of action involves reaction at the O^6-position of DNA guanine residues.

Experimental Section

Materials and Methods. ^{1}H-NMR spectra were recorded on a Varian VXR 500S spectrometer equipped with Sun 2/110 data stations or a Varian XL 200 instrument interfaced to an Advanced data system. Samples were dissolved in DMSO-d_6 with Me_4Si as an internal standard. EI mass spectra were obtained on a reversed geometry VG Micromass ZAB-2F spectrometer interfaced to a VG 2035 data system. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Most reagents and solvents were from Aldrich Chemical Co., Inc., Milwaukee, WI. O^6-Benzyl-8-oxoguanine (**1c**),[12] N^2-acetyl-O^6-benzyl-8-oxoguanine (**4d**),[12] 8-aza-O^6-benzylguanine (**2**),[13] 2,4-diamino-6-(benzyloxy)pyrimidine (**3b**),[11] 2,4,5-triamino-6-(benzyloxy)pyrimidine (**3c**),[11] 2,4-diamino-6-(benzyloxy)-5-nitrosopyrimidine (**3d**),[11] 2,4-diamino-6-(benzyloxy)-5-nitropyrimidine (**3e**),[15] 2,4-diamino-6-(benzyloxy)-5-bromopyrimidine (**3f**),[15] O^6-benzyl-2-fluorohypoxanthine (**4c**),[16] and 2,4-diamino-6-(benzyloxy)-*s*-triazine (**6**)[17] were prepared previously. Alternative synthetic methods are provided below for some of these compounds together with spectroscopic data not provided previously. AGT inactivation experiments were carried out as described in ref 9.

2,8-Diamino-6-chloropurine. A suspension of 8-aminoguanine[20] (3.0 g, 18.1 mmol) in phosphorus oxychloride (90 mL) and *N,N*-dimethylaniline (3 mL) was refluxed for 30 min and the excess phosphorus oxychloride was evaporated under reduced pressure. Ice (20 g) was added slowly to the resulting solution and the pH was adjusted to 6 with a concentrated aqueous sodium hydroxide solution. A yellow solid formed and was collected by filtration, washed with water, and dried to give a green solid. Crystallization from water with charcoal treatment produced 2,8-diamino-6-chloropurine as a white solid: yield, 2.11 g (63%); mp >275 °C dec; ^{1}H NMR δ 6.09 (s, 2 H, NH_2, exchange with D_2O), 6.71 (s, 2 H, NH_2, exchange with D_2O); MS (EI) calcd *m/z* for $C_5H_5N_6{}^{35}Cl$ 184.0264, found 184.0266; calcd *m/z* $C_5H_5N_6{}^{37}Cl$ 186.0235, found 186.0237.

8-Amino-O^6-benzylguanine (1a). 2,8-Diamino-6-chloropurine (0.9 g, 4.9 mmol) was added to a solution of sodium (0.22 g, 10 mmol) in benzyl alcohol (9.0 mL). The solution was heated in a 130 °C oil bath for 5 h and was poured into water (100 mL) with constant stirring for 10 min. Undissolved solid was removed by filtration and the filtrate was neutralized with glacial acetic acid. The solution was mixed with methanol (100 mL), and half of the aqueous methanol solution was loaded on a 3 × 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm, and fractions (10 mL) were collected. The remainder of the reaction mixture in MeOH/H_2O was chromatographed separately under identical conditions. The desired product eluted in fractions 100–130. Evaporation of solvent from the pooled fractions 100–130 from both chromatographic runs afforded analytically pure **1a**: yield, 0.26 g (21%); mp 269–271 °C dec; UV (pH 1) λ_{max} 241 nm (ϵ = 0.699 × 10^4), 300 (1.109 × 10^4); (pH 6.9) 250 (sh) (0.447 × 10^4), 292 (1.027 × 10^4); (pH 13) 255 (sh) (0.355 × 10^4), 295 (0.932 × 10^4); ^{1}H NMR δ 5.41 (s. 2 H, $ArCH_2$), 5.70 (s, 2 H, NH_2, exchange with D_2O), 6.18 (s, 2 H, NH_2, exchange with D_2O), 7.25–7.55 (m, 5 H, ArH), 11.1 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{12}H_{12}N_6O$ 256.1072, found 256.1059. Anal. ($C_{12}H_{12}N_6O$) C, H, N.

2-Amino-6-chloro-8-methylpurine. A suspension of 8-methylguanine[21] (1.0 g, 6.1 mmol) in phosphorus oxychloride (30 mL) and *N,N*-diethylaniline (1 mL) was refluxed for 3 h. The excess phosphorus oxychloride was evaporated under reduced pressure. The resulting brown oil was dissolved in ice/water and was neutralized with a concentrated aqueous NaOH solution. After evaporation of the solvent, the solid residue was suspended in 70 mL of H_2O. Undissolved solid was filtered off, and the filtrate was loaded on a 3 × 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm, and fractions (10 mL) were collected. Evaporation of pooled fractions 50–60 produced 2-amino-6-chloro-8-methylpurine as a crude solid. Crystallization from ethanol/water with charcoal treatment afforded 2-amino-6-chloro-8-methylpurine as a white solid: yield, 0.57 g (51%); mp > 265 °C dec; ^{1}H NMR δ 2.39 (s, 3 H, CH_3), 6.62 (s, 2 H, NH_2, exchange with D_2O), 12.56 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_6H_6N_5{}^{35}Cl$ 183.0312, found 183.0309; calcd *m/z* for $C_6H_6N_5{}^{37}Cl$ 185.0283, found 185.0286.

O^6-Benzyl-8-methylguanine (1b). Sodium (0.1 g, 4.4 mmol) was stirred in 4.1 mL of benzyl alcohol until all the sodium had reacted. 2-Amino-6-chloro-8-methylpurine (0.41 g, 2.2 mmol) was added, and the reaction mixture was heated in a 130 °C oil bath for 5 h. After cooling of the mixture to room temperature, 40 mL of ether was added to remove excess benzyl alcohol. The sticky precipitate that formed was collected by filtration and was dissolved in water (50 mL). The pH of the yellow solution was adjusted to 5–6 with glacial acetic acid. The solution was mixed with methanol (50 mL) and was loaded on a 3 × 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm, and fractions (10 mL) were collected. Evaporation of pooled fractions 78–93 afforded analytically pure **1b**: yield, 0.25 g (44%); mp 214–216 °C; UV (pH 1) λ_{max} 238 nm (sh) (ϵ = 0.648 × 10^4), 290 (1.136 × 10^4); (pH 6.9) 242 (0.758 × 10^4), 284 (0.897 × 10^4); (pH 13) 240 (sh) (0.495 × 10^4), 286 (0.932 × 10^4); ^{1}H NMR δ 2.33 (s, 3 H, CH_3), 5.46 (s, 2 H, $ArCH_2$), 6.17 (s, 2 H, NH_2, exchange with D_2O), 7.34–7.51 (m, 5 H, ArH), 12.18 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{13}H_{13}N_5O$ 255.1120, found 255.1125. Anal. ($C_{13}H_{13}N_5O \cdot {}^1/_4H_2O$) C, H, N.

O^6-Benzyl-8-bromoguanine (1d). Bromine (0.26 mL, 5.1 mmol) was added slowly to a solution of O^6-benzylguanine (1.205 g, 5.0 mmol) in anhydrous DMF (10 mL) under argon. The resulting deep green solution was stirred at room temperature overnight. The solution was mixed with water (70 mL) to precipitate the crude product. This product was collected by filtration and was dissolved in 50% aqueous methanol (100 mL). The solution was loaded on a 3 × 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm, and fractions (10 mL) were collected. The desired product eluted in fractions 110–190. Evaporation of solvent from the pooled fraction 110–190 afforded **1d** as a pale yellow solid. Crystallization from ethanol/water (1:1) produced analytically pure **1d**: yield, 0.166 g (10%); mp 135–137 °C dec; UV (pH 1) λ_{max} 236 nm (sh) (ϵ = 0.517 × 10^4), 294 (1.429 × 10^4); (pH 6.9) 244 (0.666 × 10^4), 287 (1.043 × 10^4); (pH 13) 245 (sh) (0.544 × 10^4), 289 (1.030 × 10^4); ^{1}H NMR δ 5.45 (s, 2 H, $ArCH_2$), 6.35 (s, 2 H, NH_2, exchange with D_2O), 7.34–7.52 (m, 5 H, ArH), 13.08 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{12}H_{10}N_5O^{79}Br$ 319.0068, found 319.0069; calcd *m/z* for $C_{12}H_{10}N_5O^{81}Br$ 321.0048, found 321.0048. Anal. ($C_{12}H_{10}N_5OBr \cdot {}^3/_2H_2O$) C, H, N, Br.

8-Aza-O^6-benzylguanine (2). Glacial acetic acid (1 mL) was added into the mixture of 2,4,5-triamino-6-(benzyloxy)-pyrimidine[11] (0.231 g, 1.0 mmol) and sodium nitrite (0.069 g, 1.0 mmol) in acetone (5 mL). The resulting mixture was stirred at room temperature for 2 h. The solution was poured in water (100 mL) with stirring to precipitate a crude solid. The solid was collected by filtration and air-dried. Crystallization from ethanol/water (1:1) with charcoal treatment produced **2** as a white solid: yield, 105 mg (43%); mp 191–192 °C (lit.[13] mp 192–193 °C); ^{1}H NMR δ 5.56 (s, 2 H, $ArCH_2$), 7.00 (s, 2 H, NH_2, exchange with D_2O), 7.41–7.58 (m, 5 H, ArH); MS (EI) calcd *m/z* for $C_{11}H_{10}N_6O$ 242.0916, found 242.0924.

4-Amino-6-(benzyloxy)-5-nitropyrimidine (3a). 4-Amino-6-chloro-5-nitropyrimidine[14] (1.5 g, 8.6 mmol) was added to a solution of sodium (0.23 g, 9.9 mmol) in benzyl alcohol (14 mL). The solution was heated in a 130 °C oil bath for 3.5 h and was poured into benzene (50 mL). A yellow solid was collected by filtration and washed with benzene. Crystallization from benzene/ether afforded an analytically pure sample of **3a**: yield, 0.71 g (34%); mp 149–150 °C; UV (pH 1) λ_{max} 284 nm (ϵ = 0.368 × 10^4), 333 (0.488 × 10^4); (pH 6.9) 284 (0.329 × 10^4), 336 (0.470 × 10^4); (pH 13) 290 (0.344 × 10^4), 333 (0.494 × 10^4); ^{1}H NMR δ 5.50 (s, 2 H, $ArCH_2$), 7.33–7.49 (m, 5 H, ArH), 8.12–8.24 (br d, 2 H, NH_a and NH_b, exchange with D_2O), 8.24

(s, 1 H, H-2); MS (EI) calcd *m/z* for $C_{11}H_{10}N_4O_3$ 246.0752, found 246.0751. Anal. ($C_{11}H_{10}N_4O_3$) C, H, N.

2,4-Diamino-6-(benzyloxy)-5-nitropyrimidine (3e). 2,4-Diamino-6-chloro-5-nitropyrimidine[22] (1.0 g, 5.28 mmol) was added to a solution of sodium (0.14 g, 6.08 mmol) in benzyl alcohol (9 mL). The solution was heated in a 160 °C oil bath for 3.5 h and the solvent was evaporated under reduced pressure to provide a yellow solid. This solid was washed with water and air-dried. Crystallization from benzene/ether gave a pale yellow filamentous solid: yield, 0.69 g (50%); mp 194–195 °C (lit.[15] mp 171 °C); UV (pH 1) λ_{max} 236 nm (sh) ($\epsilon = 1.452 \times 10^4$), 264 ($0.522 \times 10^4$), 321 ($1.294 \times 10^4$); (pH 6.9) 242 (sh) ($0.965 \times 10^4$), 337 ($1.493 \times 10^4$); (pH 13) 242 (sh) ($0.952 \times 10^4$), 338 ($1.479 \times 10^4$); ^{1}H NMR δ 5.43 (s, 2 H, $ArCH_2$), 7.26 (br s, 2 H, NH_2, exchange with D_2O), 7.33–7.51 (m, 5 H, ArH), 7.93 (br s, 2 H, NH_2, exchange with D_2O); MS (EI) calcd m/z for $C_{11}H_{11}N_5O_3$ 261.0861, found 261.0866. Anal. ($C_{11}H_{11}N_5O_3 \cdot {}^1/_5H_2O$) C, H, N.

O^6-Benzylxanthine (4a). A suspension of O^6-benzylguanine (0.83 g, 3.4 mmol) in acetone (15 mL) was poured into a solution of sodium nitrite (5 g) in 15 mL of H_2O. Acetic acid (8 mL) was added to the suspension with stirring. Minimum amounts of acetone were added as necessary to dissolve any suspended solid. The resulting pale yellow-green solution was stirred for 3 h. A pale green precipitate that formed was collected by filtration and washed with water (200 mL). Recrystallization of the air-dried solid from ethanol/water (1:1) afforded analytically pure **4a**: yield, 0.43 g (52%); mp 145–147 °C dec; UV (pH 1) λ_{max} 270 nm ($\epsilon = 0.749 \times 10^4$); (pH 6.9) 286 ($1.143 \times 10^4$); (pH 13) 290 ($0.914 \times 10^4$); ^{1}H NMR δ 5.49 (s, 2 H, $ArCH_2$), 7.36–7.54 (m, 5 H, ArH), 8.02 (s, 1 H, H-8), 11.8 (br s, 1 H, NH, exchanges with D_2O), 13.2 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{12}H_{10}N_4O_2$ 242.0803, found 242.0828. Anal. ($C_{12}H_{10}N_4O_2 \cdot H_2O$) C, H, N.

O^6-Benzyluric Acid (4b). Sodium nitrite (1.5 g, 43 mmol) dissolved in water (5 mL) was added to a suspension of O^6-benzyl-8-oxoguanine (**1c**) (0.257 g, 1.0 mmol) in acetone (5 mL). Glacial acetic acid (3 mL) was added to the suspension with stirring. After stirring for 3 h at room temperature a bright yellow precipitate formed. The suspension was mixed with water (150 mL) and undissolved solid was filtered off. Saturated aqueous sodium carbonate solution was added to the filtrate to adjust the pH to approximately 5. A yellow precipitate (130 mg) was collected and washed with water. This solid was crystallized from 50% aqueous ethanol to give an analytically pure sample of **4b**: yield, 75 mg (29%); mp >230 °C; UV (pH 1) λ_{max} 236 nm (sh) ($\epsilon = 0.972 \times 10^4$), 299 ($1.427 \times 10^4$); (pH 6.9) 240 (sh) ($0.821 \times 10^4$), 304 ($2.134 \times 10^4$); (pH 13) 245 (sh) ($0.846 \times 10^4$), 297 ($1.861 \times 10^4$); ^{1}H NMR δ 5.43 (s, 2 H, $ArCH_2$), 7.35–7.51 (m, 5 H, ArH), 10.76 (s, 1 H, NH, exchanges with D_2O), 11.23 (s, 1 H, NH, exchanges with D_2O), 11.39 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{12}H_{10}N_4O_3$ 258.0752, found 258.0753. Anal. ($C_{12}H_{10}N_4O_3 \cdot {}^5/_2H_2O$) C, H, N.

O^6-Benzyl-2-fluorohypoxanthine (4c). O^6-Benzylguanine (1.21 g, 5 mmol) was added to 100 mL of 48% fluoboric acid at −20 °C. Sodium nitrite (1.23 g, 35 mmol) was dissolved in water (5 mL) and 2.5 mL of this sodium nitrite solution was added slowly to the cold fluoboric acid solution. The resulting mixture was stirred for 1 h at or below −15 °C. Additional fluoboric acid (25 mL) was added followed by an additional 2.5 mL of the aqueous sodium nitrite solution. After stirring for an additional 1 h below −15 °C, fluoboric acid (25 mL) was again added and stirring was continued for 1 h. The resulting solution was neutralized with saturated aqueous sodium carbonate solution at −20 °C and was allowed to warm to room temperature. A white precipitate that formed was collected by filtration and was washed with water and dried under vacuum to afford crude **4c**: yield, 0.52 g, 43%. An analytical sample was prepared by chromatography on a Sephadex LH-20 column (3 × 80 cm) eluted with methanol/water (1:1) at 1 mL/min. The desired **4c** eluted in fractions 66–77: mp 182–183 °C (lit.[16] mp 184–185 °C); UV (pH 1) λ_{max} 256 nm ($\epsilon = 1.117 \times 10^4$); (pH 6.9) 257 ($1.078 \times 10^4$); (pH 13) 264 ($1.063 \times 10^4$); ^{1}H NMR δ 5.60 (s, 2 H, $ArCH_2$), 7.37–7.57 (m, 5 H, ArH), 8.40 (s, 1 H, H-8), 13.60 (s, 1 H, NH, exchanges with D_2O), ^{19}F NMR δ 23.54 downfield from trifluoroacetic acid standard; MS (EI) calcd *m/z* for $C_{12}H_9FN_4O$ 244.0760, found 244.0756. Anal. ($C_{12}H_9FN_4O \cdot {}^2/_3H_2O$) C, H, N.

O^6-Benzyl-N^2-methylguanine (4e). Fluoboric acid (48%, 30 mL) was cooled to −20 °C in a dry ice/acetone bath. O^6-Benzylguanine (0.362 g, 1.5 mmol) was added with stirring. Sodium nitrite (0.369 g, 10.5 mmol) was dissolved in water (1 mL) and 0.5 mL of this solution was added slowly to the cold fluoboric acid solution. The resulting solution was stirred at or below −15 °C for 1 h. More fluoboric acid (5 mL) was then added, followed by 0.5 mL of the sodium nitrite solution. After stirring for 1 h at or below −15 °C, fluoboric acid (5 mL) was again added and stirring was continued for an additional 1 h. Methylamine (40% in water, 60 mL) was then added at −20 °C, and the resulting basic solution was stirred at room temperature for 2 days. The solvent was evaporated under reduced pressure to produce a white solid. The solid was suspended in 50 mL of H_2O with stirring for 10 min. Undissolved material was collected by filtration and washed with water. This solid was dissolved in 40 mL of methanol/water (1:1) to which was added 1.2 mL of 28% aqueous ammonia solution. The solution was loaded on a 3 × 80 cm Sephadex LH-20 column eluted with $MeOH/H_2O/NH_4OH$ (30:70:3) at 1 mL/min. Column eluent was continuously monitored at 280 nm, and fractions (10 mL) were collected. Evaporation of the pooled fractions 106–127 gave an analytically pure sample of **4e**: yield, 85 mg (22%); mp 189–190 °C; UV (pH 1) λ_{max} 238 nm (sh) ($\epsilon = 0.665 \times 10^4$), 297 ($0.904 \times 10^4$); (pH 6.9) 246 ($0.898 \times 10^4$), 290 ($0.676 \times 10^4$); (pH 13) 240 (sh) ($0.615 \times 10^4$), 294 ($0.674 \times 10^4$); ^{1}H NMR δ 2.30 (d, 3 H, CH_3), 5.50 (s, 2 H, $ArCH_2$), 6.75 (m, 1 H, MeNH, exchanges with D_2O), 7.31–7.53 (m, 5 H, ArH), 7.82 (s, 1 H, H-8), 12.53 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{13}H_{13}N_5O$ 255.1120, found 255.1107. Anal. ($C_{13}H_{13}N_5O \cdot {}^1/_2H_2O$) C, H, N.

O^6-Benzyl-N^2,N^2-dimethylguanine (4f). Fluoboric acid (48%, 40 mL) was cooled to −20 °C in a dry ice/acetone bath. O^6-Benzylguanine (0.482 g, 2.0 mmol) was added with stirring. Sodium nitrite (0.492 g, 14.0 mmol) was dissolved in water (2 mL) and 1 mL of this solution was added slowly to the cold fluoboric acid solution. The resulting solution was stirred at or below −15 °C for 1 h. More fluoboric acid (10 mL) was added followed by the addition of 1 mL of the sodium nitrite solution. After stirring for 1 h at or below −15° C, additional fluoboric acid (10 mL) was added with stirring for 1 h. Dimethylamine (40% in water, 60 mL) was then added to the solution at −20 °C, and the resulting mixture was allowed to warm to room temperature. The suspension became a clear solution and a precipitate formed within 10 min. After standing overnight at room temperature the precipitate was collected by filtration and was washed with water. The solid was crystallized from 50% aqueous ethanol to give an analytically pure sample of **4f**: yield, 0.25 g (46%); mp 220–221 °C dec; UV (pH 1) λ_{max} 248 nm (sh) ($\epsilon = 0.512 \times 10^4$), 303 ($0.908 \times 10^4$); (pH 6.9) 251 ($1.152 \times 10^4$), 299 ($0.686 \times 10^4$); (pH 13) 248 (sh) ($0.766 \times 10^4$), 299 ($0.710 \times 10^4$); ^{1}H NMR δ 3.12 (s, 6 H, CH_3), 5.54 (s, 2 H, $ArCH_2$), 7.36–7.51 (m, 5 H, ArH), 7.84 (s, 1 H, H-8), 12.56 (s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{14}H_{15}N_5O$ 269.1276, found 269.1254. Anal. ($C_{14}H_{15}N_5O$) C, H, N.

2,4-Diamino-6-(benzyloxy)-5-bromopyrimidine (3f). 2,4-Diamino-5-bromo-6-chloropyrimidine[23] (2.3 g, 10 mmol) was added to a solution of sodium (0.29 g, 12.5 mmol) in benzyl alcohol (10 mL) under argon. The solution was heated in a 130 °C oil bath for 3 h and the benzyl alcohol was evaporated under reduced pressure to give a white solid. This solid was washed with water and air-dried. Crystallization from 50% aqueous ethanol gave white crystalline needles of **3f**: yield, 2.32 g (76%); mp 165–166 °C (lit.[15] mp 136 °C); UV (pH 1) λ_{max} 236 nm ($\epsilon = 0.873 \times 10^4$), 291 ($1.388 \times 10^4$); (pH 6.9) 236 ($0.850 \times 10^4$), 277 ($0.835 \times 10^4$); (pH 13) 234 ($0.869 \times 10^4$), 277 ($0.827 \times 10^4$); ^{1}H NMR δ 5.30 (s, 2 H, $ArCH_2$), 6.15 (s, 2 H, NH_2, exchange with D_2O), 6.32 (s, 2 H, NH_2, exchange with D_2O), 7.31–7.45 (m, 5 H, ArH); MS (EI) calcd *m/z* for $C_{11}H_{11}N_4O^{79}Br$ 294.0115, found 294.0127; calcd *m/z* for

$C_{11}H_{11}N_4O^{81}Br$ 296.0094, found 296.0083. Anal. ($C_{11}H_{11}N_4OBr$) C, H, N, Br.

2-Amino-4-chloro-5-nitropyrimidine. A suspension of 2-amino-4-hydroxy-5-nitropyrimidine (5.0 g, 32.1 mmol) in phosphorus oxychloride (100 mL) was refluxed overnight, and the excess phosphorus oxychloride was evaporated under reduced pressure. The residue was mixed with ice (100 g) in an ice bath, and the mixture was neutralized with concentrated aqueous sodium carbonate solution. A yellow precipitate was collected by filtration and washed with water: yield, 1.39 g (25%); mp 191–194 °C dec; ^{1}H NMR δ 8.45 (br s, 2 H, NH_2, exchange with D_2O), 9.03 (s, 1 H, H-6); MS (EI) calcd *m/z* for $C_4H_3N_4O_2{}^{35}Cl$ 173.9944, found 173.9934; calcd *m/z* for $C_4H_3N_4O_2{}^{37}Cl$ 175.9915, found 175.9916.

2-Amino-4-(benzyloxy)-5-nitropyrimidine (5a). 2-Amino-4-chloro-5-nitropyrimidine (0.70 g, 4.0 mmol) was added to a solution of sodium (0.12 g, 5.2 mmol) in benzyl alcohol (8 mL) under argon. The solution was heated in a 130 °C oil bath for 3 h, and approximately half of the benzyl alcohol was evaporated under reduced pressure. The residue was poured into water (50 mL) with constant stirring for 10 min. After neutralization with glacial acetic acid, a brown precipitate formed which was collected by filtration and washed with water. This solid was crystallized from benzene to give **5a** as a golden crystalline solid: yield, 126 mg (13%); mp 164–167 °C; UV (pH 1) λ_{max} 262 nm ($\epsilon = 0.879 \times 10^4$), 295 (sh) ($0.571 \times 10^4$); (pH 6.9) 235 (sh) ($0.448 \times 10^4$), 273 ($0.360 \times 10^4$), 326 ($1.085 \times 10^4$); (pH 13) 273 ($0.404 \times 10^4$), 327 ($1.055 \times 10^4$); ^{1}H NMR δ 5.51 (s, 2 H, $ArCH_2$), 7.35–7.54 (m, 5 H, ArH), 8.05 (d, 2 H, NH_2, exchange with D_2O), 8.92 (s, 1 H, H-6); MS (EI) calcd *m/z* for $C_{11}H_{10}N_4O_3$ 246.0752, found 246.0758. Anal. ($C_{11}H_{10}N_4O_3$) C, H, N.

2-Amino-4-(benzyloxy)-6-methyl-5-nitropyrimidine (5b). 2-Amino-4-chloro-6-methyl-5-nitropyrimidine[14] (1.24 g, 6.58 mmol) was added to a solution of sodium (0.21 g, 9.13 mmol) in benzyl alcohol (14 mL) under argon. The solution was heated in a 135 °C oil bath for 3.5 h and was poured into water (70 mL) with constant stirring for 10 min. After neutralization with glacial acetic acid, a yellow precipitate formed which was collected by filtration and washed with water. This solid was crystallized from benzene to give **5b** as a bright yellow crystalline solid: yield, 0.57 g (33%); mp 159–160 °C; UV (pH 1) λ_{max} 268 nm ($\epsilon = 0.783 \times 10^4$), 345 (sh) ($0.104 \times 10^4$); (pH 6.9) 282 ($0.564 \times 10^4$), 345 (sh) ($0.338 \times 10^4$); (pH 13) 282 ($0.549 \times 10^4$), 345 (sh) ($0.332 \times 10^4$); ^{1}H NMR δ 2.35 (s, 3 H, CH_3), 5.44 (s, 2 H, $ArCH_2$), 7.34–7.46 (m, 5 H, ArH), 7.64 (br s, 2 H, NH_2, exchange with D_2O); MS (EI) calcd *m/z* for $C_{12}H_{12}N_4O_3$ 260.0908, found 260.0913. Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

2,4-Diamino-6-(benzyloxy)-*s*-triazine (6). 2,4-Diamino-6-chloro-*s*-triazine (2.25 g, 15.0 mmol) was added to a solution of sodium (0.43 g, 18.8 mmol) in benzyl alcohol (30 mL) under argon. The suspension was heated in a 130 °C oil bath for 3.5 h. The excess benzyl alcohol was removed under vacuum and the resulting solid was collected with the aid of benzene and washed with water (100 mL). The solid was crystallized from EtOH/H_2O (1:1): yield, 1.83 g (56%); mp 184–185 °C (lit.[17] mp 186–188 °C); UV (pH 1) λ_{max} 233 nm (sh) ($\epsilon = 0.589 \times 10^4$); (pH 6.9) 238 (sh) ($0.111 \times 10^4$); (pH 13) 240 (sh) ($0.073 \times 10^4$); ^{1}H NMR δ 5.25 (s, 2 H, $ArCH_2$), 6.63 (s, 4 H, NH_2, exchange with D_2O), 7.30–7.42 (m, 5 H, ArH); MS (EI) calcd *m/z* for $C_{10}H_{11}N_5O$ 217.0963, found 217.0955.

2-Amino-6-chloro-8-(trifluoromethyl)purine. A suspension of 8-(trifluoromethyl)guanine[18] (2.0 g, 9.1 mmol) in phosphorus oxychloride (20 mL) was refluxed for 3 h. Excess phosphorus oxychloride was evaporated under reduced pressure. The resulting residue was mixed with ice/water (100 g), and the pH was adjusted to 3–4 with a concentrated aqueous NaOH solution. The resulting solution was mixed with MeOH (100 mL) and approximately 100 mL of the aqueous methanol solution was loaded on a 3 × 80 cm Sephadex LH-20 column eluted with methanol/water (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm, and fractions (10 mL) were collected. The remainder of the reaction mixture in MeOH/H_2O was chromatographed separately under identical conditions. The desired product eluted in fractions 73–85. Evaporation of solvent from the pooled fractions 73–85 from both chromatographic runs afforded analytically pure 2-amino-6-chloro-8-(trifluoromethyl)purine: yield, 0.94 g (43%); mp >225 °C dec; UV (pH 1) λ_{max} 245 nm ($\epsilon = 0.501 \times 10^4$), 314 ($0.746 \times 10^4$); (pH 6.9) 270 ($0.265 \times 10^4$), 315 ($0.612 \times 10^4$); (pH 13) 272 ($0.269 \times 10^4$), 314 ($0.612 \times 10^4$); ^{1}H NMR δ 7.19 (s, 2 H, NH_2, exchange with D_2O), 14.25 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_6H_3N_5F_3{}^{35}Cl$ 237.0029, found 237.0011; calcd *m/z* for $C_6H_3N_5F_3{}^{37}Cl$ 239.0000, found 238.9987. Anal. ($C_6H_3N_5F_3Cl \cdot {}^1/_4H_2O$) C, H, N, F.

***O*^{6}-Benzyl-8-(trifluoromethyl)guanine (1e).** Sodium (0.10 g, 4.3 mmol) was stirred in 5 mL of benzyl alcohol until all of it had reacted. 2-Amino-6-chloro-8-(trifluoromethyl)purine (0.475 g, 2.0 mmol) was added, and the reaction mixture was heated in a 135 °C oil bath for 3.5 h. The benzyl alcohol was removed by vacuum distillation, yielding a brown oil. The oil was dissolved in water (50 mL) and was acidified with glacial acetic acid to produce a pale yellow precipitate. The precipitate was collected by filtration and washed with water. The crude product was loaded on a 2.5 × 35 cm silica gel column (Davisil grade 633, 200–425 mesh, 60 Å). Elution was carried out with 5% EtOH in $CHCl_3$ to provide analytically pure *O*^{6}-benzyl-8-(trifluoromethyl)guanine (**1e**): yield, 0.42 g (67%); mp 214–216 °C dec; UV (pH 1) λ_{max} 291 nm ($\epsilon = 1.229 \times 10^4$); (pH 6.9) 244 ($0.470 \times 10^4$), 289 ($1.023 \times 10^4$); (pH 13) 247 (sh) ($0.393 \times 10^4$), 290 ($0.923 \times 10^4$); ^{1}H NMR δ 5.51 (s, 2 H, $ArCH_2$), 6.82 (s, 2 H, NH_2, exchange with D_2O), 7.38–7.55 (m, 5 H, ArH), 13.75 (br s, 1 H, NH, exchanges with D_2O); MS (EI) calcd *m/z* for $C_{13}H_{10}N_5OF_3$ 309.0837, found 309.0827. Anal. ($C_{13}H_{10}N_5OF_3 \cdot {}^1/_5H_2O$) C, H, N, F.

Acknowledgment. This research was sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-46000 with ABL (R.C.M., M.-Y.C.) and Grant CA57725 (A.E.P., M.E.D.).

References

(1) Dolan, M. E.; Moschel, R. C.; Pegg, A. E. Depletion of mammalian *O*^{6}-alkylguanine-DNA alkyltransferase activity by *O*^{6}-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5368–5372.

(2) Dolan, M. E.; Mitchell, R. B.; Mummert, C.; Moschel, R. C.; Pegg, A. E. Effect of *O*^{6}-benzylguanine analogs on sensitivity of human tumor cells to the cytotoxic effects of alkylating agents. *Cancer Res.* **1991**, *51*, 3367–3372.

(3) Dolan, M. E.; Stine, L.; Mitchell, R. B.; Moschel, R. C.; Pegg, A. E. Modulation of mammalian *O*^{6}-alkylguanine-DNA alkyltransferase in vivo by *O*^{6}-benzylguanine and its effect on sensitivity of a human glioma tumor to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea. *Cancer Commun.* **1990**, *2*, 371–377.

(4) Mitchell, R. B.; Moschel, R. C.; Dolan, M. E. Effect of *O*^{6}-benzylguanine on the sensitivity of human tumor xenografts to 1,3-bis(2-chloroethyl)-1-nitrosourea and on DNA interstrand cross-link formation. *Cancer Res.* **1992**, *52*, 1171–1175.

(5) Friedman, H. S.; Dolan, M. E.; Moschel, R. C.; Pegg, A. E.; Felker, G. M.; Rich, J.; Bigner, D. D.; Schold, S. C. Enhancement of nitrosourea activity in medulloblastoma and glioblastoma multiforme. *J. Natl. Cancer Inst.* **1992**, *84*, 1926–1931.

(6) Felker, G. M.; Friedman, H. S.; Dolan, M. E., Moschel, R. C.; Schold, C. Treatment of subcutaneous and intracranial brain tumor xenografts with *O*^{6}-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea. *Cancer Chem. Pharmacol.* **1993**, *32*, 471–476.

(7) Dolan, M. E.; Pegg, A. E.; Biser, N. D.; Moschel, R. C.; English, H. F. Effect of *O*^{6}-benzylguanine on the response to 1,3-bis(2-chloroethyl)-1-nitrosourea in the Dunning R3327G model of prostatic cancer. *Cancer Chem. Pharmacol.* **1993**, *32*, 221–225.

(8) Dolan, M. E.; Pegg, A. E.; Moschel, R. C.; Grindey, G. B. Effect of *O*^{6}-benzylguanine analogs on the sensitivity of human colon tumor xenografts to BCNU. *Biochem. Pharmacol.* **1993**, *46*, 285–290.

(9) Moschel, R. C.; McDougall, M. G.; Dolan, M. E.; Stine, L.; Pegg, A. E. Structural features of substituted purine derivatives compatible with depletion of human *O*^{6}-alkylguanine-DNA alkyltransferase. *J. Med. Chem.* **1992**, *35*, 4486–4491.

(10) Chae, M.-Y.; McDougall, M. G.; Dolan, M. E.; Swenn, K.; Pegg, A. E.; Moschel, R. C. Substituted *O*^{6}-benzylguanine derivatives and their inactivation of human *O*^{6}-alkylguanine-DNA alkyltransferase. *J. Med. Chem.* **1994**, *37*, 342–347.

(11) Pfleiderer, W.; Lohrmann, R. Synthesis of 2-amino-4-alkoxypteridine. *Chem. Ber.* **1961**, *94*, 12–18.
(12) Dolan, M. E.; Chae, M.-Y.; Pegg, A. E.; Mullen, J. H.; Friedman, H. S.; Moschel, R. C. Metabolism of O^6-benzylguanine, an inactivator of O^6-alkylguanine-DNA alkyltransferase. *Cancer Res.* **1994**, *54*, 5123–5130.
(13) Shealy, Y. F.; Clayton, J. D.; O'Dell, C. A.; Montgomery, J. A. *v*-Triazolo[4,5-*d*]pyrimidines. II. *O*-Substituted derivatives of 8-azaguanine and 8-azahypoxanthine. *J. Org. Chem.* **1962**, *27*, 4518–4523.
(14) Boon, W. R.; Jones, W. G. M.; Ramage, G. R. Pteridines. Part I. An unambiguous synthesis of 7,8-dihydro-6-hydroxypteridines. *J. Chem. Soc.* **1951**, 96–102.
(15) Kosary, J.; Diesler, E.; Matyus, P., Kasztreiner, E. Preparation of pyrimidine derivatives with potential cardiotonic activity. *Acta Pharm. Hung.* **1989**, *49*, 241–247.
(16) Robins, M. J.; Robins, R. K. Purine nucleosides. XXIV. A new method for the synthesis of guanine nucleosides. The preparation of 2′-deoxy-α- and -β-guanosines and the corresponding N^2-methyl derivatives. *J. Org. Chem.* **1969**, *34*, 2160–2163.
(17) Wakabayashi, K.; Okuzu, M. Inhibitory effects of *s*-triazines on the nitrification in soil. III. Diamino-*s*-triazines. *Nippon Dojo-Hiryogaku Zasshi* **1970**, *41*, 193–200.
(18) Pfleiderer, W.; Shanshal, M. Synthesis and properties of 8-methyl- and 8-trifluoromethylguanine. *Liebigs Ann. Chem.* **1969**, *726*, 201–215.
(19) Crone, T. M.; Pegg, A. E. A single amino acid change in human O^6-alkylguanine-DNA alkyltransferase decreasing sensitivity to inactivation by O^6-benzylguanine. *Cancer Res.* **1993**, *53*, 4750–4753.
(20) Beaman, A. G.; Jones, J. W.; Robins, R. K. 2,8-Diaminopurine-6-thiol. In *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W. W., Tipson, R. S., Eds.; John Wiley & Sons: New York, 1968; Vol. 1, pp 41–43.
(21) Daves, G. D.; Noell, C. W.; Robins, R. K.; Koppel, H. C.; Beaman, A. G. Potential purine antagonists. XXII. The preparation and reactions of certain derivatives of 2-amino-6-purinethiol. *J. Am. Chem. Soc.* **1960**, *82*, 2633–2640.
(22) O'Brien, D. E.; Cheng, C. C.; Pfleiderer, W. Pyrimidines. XVIII. 2,4-Diamino-5-nitro-6-arylaminopyrimidines. Nitration study of 2,4-diamino-6-chloropyrimidine and a literature correction. *J. Med. Chem.* **1966**, *9*, 573–575.
(23) Phillips, A. P.; Mehta, N. B.; Strelitz, J. Z. Syntheses of 1,3-diazaphenothiazines. I. *J. Org. Chem.* **1963**, *28*, 1488–1490.

JM940652W