# Expedited Articles

## Substituted $O^6$-Benzylguanine Derivatives and Their Inactivation of Human $O^6$-Alkylguanine-DNA Alkyltransferase

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Several new $O^6$-benzylguanine analogs bearing increasingly bulky substituent groups on the benzene ring or at position 9 were tested for their ability to inactivate the human DNA repair protein, $O^6$-alkylguanine-DNA alkyltransferase. Substitution on the benzene ring was well tolerated although activity varied considerably with structural changes in groups attached to position 9. For this site, activity was preserved with large or small lipophilic groups while introduction of non-carbohydrate polar groups generally reduced activity regardless of their size.

Inactivation of the human DNA repair protein, $O^6$-alkylguanine-DNA alkyltransferase (alkyltransferase, AGT), leads to a significant enhancement in the cytotoxic response of human tumor cells and tumor xenografts to chemotherapeutic drugs whose mechanism of action involves modification of DNA guanine residues at the $O^6$-position.[1,2] We demonstrated that $O^6$-benzylguanine is a very effective inactivator of the alkyltransferase, and we have used this compound as an adjuvant to enhance the therapeutic effectiveness of several chloroethylating antitumor drugs.[3-10] In our previous survey of other modified purines that might also inactivate the AGT protein,[11] we showed that either an allyl or a benzyl group attached through exocyclic oxygen at the 6-position of a 2-aminopurine derivative was required for efficient inactivation. Furthermore, substitution on $O^6$-benzylguanine derivatives with fairly unobtrusive groups on either the benzyl residue or at position 9 was compatible with AGT inactivation, although substitution at the 7-position eliminated inactivating activity.[11] In the present study, we compare the AGT-inactivating activity of 20 additional $O^6$-benzylguanine analogues, most of which bear increasingly bulky substituents on the benzene ring or at position 9. These experiments provide further information on the types of groups and the location at which they may be attached to $O^6$-benzylguanine without significantly lowering its AGT-inactivating activity. Such sites could then be used to introduce substituents that alter the pharmacokinetics of $O^6$-benzylguanine or a related AGT inactivator by changing its water solubility or by altering its entry into cells. These properties might then be exploited to direct an AGT-inactivating agent more selectively to tumor cells in preference to normal cells of a host.

### Results and Discussion

The 20 compounds tested as AGT inactivators are illustrated in Chart 1. The majority of derivatives substituted on the benzene ring of the benzyl group [i.e., $O^6$-(*p*-bromobenzyl)- (**1a**), $O^6$-(*p*-hydroxymethylbenzyl)- (**1b**), $O^6$-(*p*-isopropylbenzyl)- (**1d**), $O^6$-(*p*-*n*-butylbenzyl)- (**1e**), $O^6$-(*p*-phenylbenzyl)- (**1f**), $O^6$-(3,5-dimethylbenzyl)- (**2**), and $O^6$-(2-pyridylmethyl)guanine (**6**)] were prepared by reacting the respective sodium benzyl oxide with 2-amino-6-chloropurine.[3,11,12] $O^6$-(*p*-Formylbenzyl)guanine (**1c**) was prepared by oxidation of **1b** with pyridinium chlorochromate. $O^6$-Benzyl-9-methyl- (**3b**) and $O^6$-benzyl-7-methylguanine (**5**), $O^6$-benzyl-9-((pivaloyloxy)methyl)-guanine (**3c**), $O^6$-benzyl-9-((3-oxo-4-androsten-17$\beta$-yloxycarbonyl)methyl)guanine (**3h**) and $O^6$-benzyl-9-((3-oxo-5$\alpha$-androstan-17$\beta$-yloxycarbonyl)methyl)guanine (**3i**) were isolated from reactions between the anion of $O^6$-benzylguanine with methyl iodide, chloromethyl pivalate, 17$\beta$-(chloroacetoxy)-4-androsten-3-one,[13] and 17$\beta$-(chloroacetoxy)-5$\alpha$-androstan-3-one,[14] respectively, in *N*,*N*-dimethylformamide (DMF). $O^6$-Benzyl-9-(2-hydroxy-3-isopropoxypropyl)guanine (**3g**) and $O^6$-benzyl-9-(3-chloro-2-hydroxypropyl)guanine (**3d**) were prepared by reaction of neutral $O^6$-benzylguanine with an excess of neat glycidyl isopropyl ether and epichlorohydrin, respectively. The reaction of **3d** with an excess of isopropylamine or *tert*-butylamine in dioxane produced $O^6$-benzyl-9-(2-hydroxy-3-(isopropylamino)propyl)guanine (**3e**) and $O^6$-benzyl-9-(3-(*tert*-butylamino)-2-hydroxypropyl)guanine (**3f**), respectively. $N^2$-Acetyl-$O^6$-benzylguanine (**4**)[15] and 9-acetyl-$O^6$-benzylguanine (**3a**) were prepared by reacting $O^6$-benzylguanine with acetic anhydride in toluene and pyridine, respectively. A sample of $O^6$-phenylguanine (**7**)[16] was kindly supplied to us by Dr. C. Schell, Institute of Hygiene and Occupational Medicine, University Medical Center, Essen, FRG.

The ability of these various compounds to inactivate the AGT protein in HT29 human colon tumor cell extracts and in intact HT29 cells is summarized in Table 1. The

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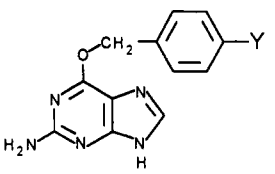
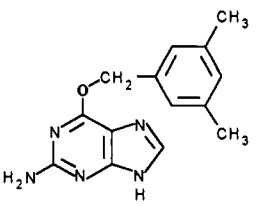
**Chart 1**

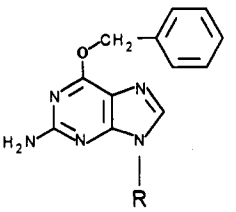


**1**

**1a**, Y=Br
**1b**, Y=$CH_2OH$
**1c**, Y=CHO
**1d**, Y=$CH(CH_3)_2$
**1e**, Y=$(CH_2)_3CH_3$
**1f**, Y=$C_6H_5$

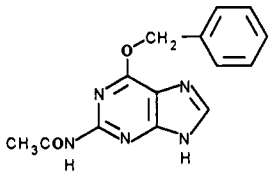


**2**

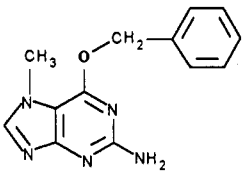


**3**

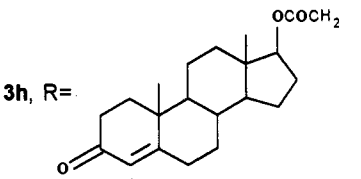
**3a**, R=$COCH_3$
**3b**, R=$CH_3$
**3c**, R=$CH_2OCOC(CH_3)_3$
**3d**, R=$CH_2CH(OH)CH_2Cl$
**3e**, R=$CH_2CH(OH)CH_2NHCH(CH_3)_2$
**3f**, R=$CH_2CH(OH)CH_2NHC(CH_3)_3$
**3g**, R=$CH_2CH(OH)CH_2OCH(CH_3)_2$



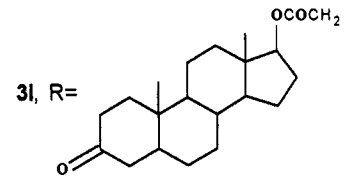
**4**



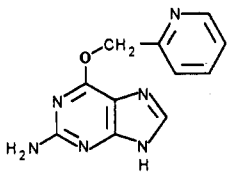
**5**



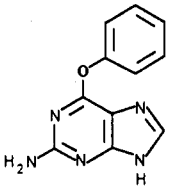
**3h**, R=



**3i**, R=



**6**



**7**

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. Compounds are arranged in descending order of activity. As indicated, compounds **1a**, 1b, and **1f** exhibited similar activity both in cell-free extracts and in HT29 cells and were the most active of the compounds tested. Interestingly, the fairly large *p*-phenyl substituent on $O^6$-(*p*-phenylbenzyl)guanine did not diminish the inactivating ability of **1f** relative to **1a**, 1b. 9-Acetyl-$O^6$-benzylguanine (**3a**) was the next most active compound. However, this material hydrolyzes readily in aqueous medium to liberate $O^6$-benzylguanine, which contributed to the higher than expected activity exhibited by **3a**. With the remaining compounds bearing substituents on the benzene ring, a modest decrease in AGT inactivation was observed in the order $O^6$-(*p*-isopropylbenzyl)- (**1d**) > $O^6$-(*p*-formylbenzyl)- (**1c**) > $O^6$-(3,5-dimethylbenzyl)- (**2**) > $O^6$-(*p*-*n*-butylbenzyl)guanine (**1e**) although, overall, substitution on the benzene ring, even with rather bulky substituents, was very well tolerated.

In contrast, substituent changes at the 9-position of $O^6$-benzylguanine significantly influenced AGT inactivation. As indicated in Table 1, we observed a decrease in the ability to inactivate AGT as a function of 9-substituent in the order 9-methyl [i.e., as in $O^6$-benzyl-9-methylguanine (**3b**)] > 9-((pivaloyloxy)methyl)- (as in **3c**) > ((3-oxo-5α-androstan-17β-yloxycarbonyl)methyl)- (as in **3i**) ≥ ((3-oxo-4-androsten-17β-yloxycarbonyl)methyl)- (as in **3h**) > (2-hydroxy-3-isopropoxypropyl)- (as in **3g**) > (3-chloro-2-hydroxypropyl)- (**3d**) ≫ (2-hydroxy-3-(isopropylamino)-propyl)- (**3e**) = (3-(*tert*-butylamino)-2-hydroxypropyl)- (**3f**). Within this series of 9-substituted $O^6$-benzylguanines, it is of particular interest that the very bulky steroid substituent on analogs **3h** and **3i** were quite compatible with activity. Also of interest is the observation that **3g** was so much more active than **3e** or **3f**. These latter three 9-substituents are very similar in size although the secondary amino groups on compounds **3e** and **3f** would be expected to be protonated at physiological pH. These data suggest that large polar groups are not as well tolerated as large nonpolar groups. Of the remaining compounds tested, $N^2$-acetyl-$O^6$-benzylguanine (**4**), $O^6$-benzyl-7-methylguanine (**5**), and $O^6$-(2-pyridylmethyl)guanine (**6**) exhibited intermediate activity. Compound **5** was the only 7-substituted $O^6$-benzylguanine derivative that we have tested that exhibits AGT inactivation activity. Groups larger than methyl at the 7-position were shown previously to completely eliminate activity.[11] Also inactive at concentrations as high as 300 μM was $O^6$-phenylguanine (**7**).[16] This inactivity is consistent with our earlier observations that only allyl or benzyl substituents at the $O^6$-position of guanine efficiently inactivated AGT.[11]

In our previous study,[11] we compared the AGT-inactivating activity of several other 9-substituted $O^6$-benzylguanine derivatives. Those with either a 2′-deoxyribose or ribose residue were quite active, while for a series of non-carbohydrate substituents, we noted a decrease in activity as a function of 9-substituent structure over the

Table 1. AGT-Inactivating Activity of $O^6$-Benzylguanine Derivatives

| compound | $ED_{50}$ ($\mu$M)[a] in HT29 cell-free extract | $ED_{50}$ ($\mu$M)[a] in HT29 cells |
|---|---|---|
| $O^6$-(*p*-bromobenzyl)guanine (**1a**) | 0.3 | 0.09 |
| $O^6$-(*p*-(hydroxymethyl)benzyl)guanine (1b) | 0.3 | 0.09 |
| $O^6$-(*p*-phenylbenzyl)guanine (**1f**) | 0.3 | 0.1 |
| 9-acetyl-$O^6$-benzylguanine (**3a**) | 0.4[b] | 0.1[b] |
| $O^6$-(*p*-isopropylbenzyl)guanine (**1d**) | 0.5 | 0.6 |
| $O^6$-(*p*-formylbenzyl)guanine (1c) | 0.5 | 0.7 |
| $O^6$-(3,5-dimethylbenzyl)guanine (**2**) | 1.0 | 0.4 |
| $O^6$-benzyl-9-methylguanine (**3b**) | 2.6 | 0.4 |
| $O^6$-benzyl-9-((pivaloyloxy)methyl)guanine (**3c**) | 3.1 | 0.3[c] |
| $O^6$-(*p*-*n*-butylbenzyl)guanine (1e) | 4.0 | 1.0 |
| $O^6$-benzyl-9-((3-oxo-5$\alpha$-androstan-17$\beta$-yloxycarbonyl)methyl)guanine (**3i**) | 4.0 | 0.5 |
| $O^6$-benzyl-9-((3-oxo-4-androsten-17$\beta$-yloxycarbonyl)methyl)guanine (**3h**) | 5.0 | 0.5 |
| $O^6$-benzyl-9-(2-hydroxy-3-isopropoxypropyl)guanine (**3g**) | 7.0 | 0.8 |
| $O^6$-benzyl-9-(3-chloro-2-hydroxypropyl)guanine (**3d**) | 18 | 2 |
| $N^2$-acetyl-$O^6$-benzylguanine (**4**) | 24 | 2 |
| $O^6$-benzyl-7-methylguanine (**5**) | 52 | 17 |
| $O^6$-(2-pyridylmethyl)guanine (**6**) | 58 | 16 |
| $O^6$-benzyl-9-(2-hydroxy-3-(isopropylamino)propyl)guanine (**3e**) | 106 | 23 |
| $O^6$-benzyl-9-(3-(*tert*-butylamino)-2-hydroxypropyl)guanine (**3f**) | 106 | 26 |
| $O^6$-phenylguanine (**7**) | inactive | not tested |

[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 corresponding values for $O^6$-benzylguanine are 0.2 and 0.05, respectively.[11] [b] Converted to $O^6$-benzylguanine by hydrolysis in vitro and in cultures. [c] Converted to $O^6$-benzylguanine by esterases in cell cultures. The compound is stable in vitro.

series $CH_2CN > CH_2CH(OH)CH_2CH_3 > CH_2CO_2CH_2CH_3 > CH_2CONH_2 > CH_2CO_2^-Na^+$. Thus, even fairly small polar substituents reduced AGT-inactivating activity. These observations together with those in this report indicate that either large or small lipophilic substituents attached to the 9-position of $O^6$-benzylguanine are compatible with AGT inactivation while more polar substituent groups generally reduce activity regardless of their size. The finding here that lipophilic groups as large as steroids attached to the 9-position of $O^6$-benzylguanine only marginally diminish its AGT inactivating efficiency suggests that derivatives such as **3h** and **3i** or related analogs might be useful in selectively inactivating AGT in steroid responsive tumors. The presence of steroid receptors in rat mammary carcinoma cells has been exploited to facilitate transfer of chloroethylating agents into these tumor cells and dramatically enhance their therapeutic effectiveness.[17,18] Chloroethylating agents derived from linkage to dihydrotestosterone and estradiol were found to be most effective. It is likely then, that interaction with steroid receptors on tumor cells could serve to concentrate a derivative such as **3i** in tumor cells to selectively deplete AGT activity in these cells and produce an even greater enhancement in the therapeutic response to steroid-linked chloroethylating agents.[17,18] Experiments to test these possibilities are currently in progress.

## Experimental Section

**Materials and Methods.** $^1H$ NMR and $^{13}C$ 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. When analyses for certain elements differed from the calculated value by more than 0.4%, the complete elemental analysis is reported.

Most reagents and solvents were from Aldrich Chemical Co., Inc., Milwaukee, WI. Testosterone and dihydrotestosterone were obtained from Sigma Chemical Co., St. Louis, MO. $N^2$-Acetyl-$O^6$-benzylguanine (**4**) was prepared essentially as described by Bowles et al.[15] AGT-inactivation studies were carried out as described previously.[11]

**$O^6$-(*p*-Bromobenzyl)guanine (1a).** Sodium hydride (21 mmol), 4-bromobenzyl alcohol (21 mmol), and 1,4-dioxane (50 mL) were stirred at room temperature for 2 h and at 40 °C until all NaH had reacted. 2-Amino-6-chloropurine (10 mmol) was then added, and the mixture was refluxed for 20 h. The solution was evaporated to dryness, and the residue was suspended in 100 mL of $H_2O$. Insoluble material was filtered and washed with 50 mL of 1 N NaOH. The pH of the combined filtrates was adjusted to pH 6 with glacial acetic acid, and water was added to a final volume of 200 mL. The suspension was heated to boiling, treated with charcoal, and filtered. On cooling a tan precipitate of crude product was filtered. This material was crystallized from ethanol/$H_2O$ (1:3) and dried to afford analytically pure **1a**: yield 0.43 g (13%); mp 235–240 °C dec; UV (pH 1) $\lambda_{max}$ 226 nm (sh) ($\epsilon = 2.141 \times 10^4$), 288 nm ($\epsilon = 1.144 \times 10^4$), (pH 6.9) 226 nm (sh) ($\epsilon = 1.794 \times 10^4$), 244 nm (sh), ($\epsilon = 0.765 \times 10^4$), 282 nm ($\epsilon = 0.877 \times 10^4$), (pH 13) 248 nm (sh) ($\epsilon = 0.462 \times 10^4$), 285 nm ($\epsilon = 0.879 \times 10^4$); $^1H$ NMR $\delta$ 5.47 (s, 2 H, $BzlCH_2$), 6.28 (s, 2H, $NH_2$), 7.47 (d, 2 H, ArH), 7.59 (d, 2 H, ArH), 7.84 (s, 1 H, H-8), 12.48 (s, 1 H, > NH); $^{13}C$ NMR $\delta$ 65.81 ($ArCH_2$), 121.08 (*p*-Ar), 128.49 (C-5), 130.50 (2 Ar), 130.83 (*ipso*-Ar), 131.25 (2 Ar), 136.22 (C-8), 138.4 (C-4), 159.3 (C-2), 159.52 (C-6); MS (EI) calcd *m/z* for $C_{12}H_{10}N_5O^{79}Br$ 319.0068, found 319.0067; calcd *m/z* for $C_{12}H_{10}N_5O^{81}Br$ 321.0048, found 321.0066. Anal. ($C_{12}H_{10}N_5OBr$) C, 45.02; H, 3.15; N, 21.88; Br, 24.96. Found: C, 45.05; H, 3.25; N, 21.30; Br, 24.86.

**Preparation of $O^6$-(*p*-Isopropylbenzyl)guanine (1d), $O^6$-(*p*-*n*-Butylbenzyl)guanine (1e), $O^6$-(*p*-Phenylbenzyl)guanine (1f), $O^6$-(3,5-Dimethylbenzyl)guanine (2), and $O^6$-(2-Pyridylmethyl)guanine (6).** Compounds **1d**, **1e**, **1f**, **2**, and **6** were prepared by reacting the sodium salts of 4-isopropylbenzyl oxide, 4-*n*-butylbenzyl oxide, 4-phenylbenzyl oxide, 3,5-dimethylbenzyl oxide, and 2-pyridylmethoxide (derived in each case by reaction of NaH with the respective alcohol) with 2-amino-6-chloropurine in 1,4-dioxane essentially as described for preparation of **1a**. After removal of the dioxane by evaporation, the solid residues were dissolved either in $H_2O$ (50 mL) (in the case for **6**), 200 mL of EtOH/$H_2O$ (1:1) (for the case of **1d**), 125 mL of EtOH/$H_2O$ (4:6) (for the case of **1e**), or 35 mL of EtOH/$H_2O$ (3:7) (for the case of **2**). Crude product **1f** was dissolved in aqueous 1 N NaOH and was extracted with $CH_2Cl_2$. The pH of the resulting solutions of all crude products was then adjusted to pH 6 with glacial acetic acid. Crude **1f** was recovered by filtration. The neutral suspensions of products **1d**, **1e**, **2**, and **6** were heated to boiling and treated with charcoal and filtered. After cooling or standing at room temperature overnight the precipitated products were collected by filtration. Compound **1d** was crys-

tallized from 50 mL of EtOH/$H_2O$ (1:1): yield 1.2 g (45.5%); mp 99–102 °C; UV (pH 1) $\lambda_{max}$ 239 nm (sh) ($\epsilon = 0.787 \times 10^4$), 287 nm ($\epsilon = 1.219 \times 10^4$), (pH 6.9) 240 nm ($\epsilon = 0.913 \times 10^4$), 282 nm ($\epsilon = 0.964 \times 10^4$), (pH 13) 250 nm (sh) ($\epsilon = 0.466 \times 10^4$), 286 nm ($\epsilon = 0.889 \times 10^4$); $^1$H NMR $\delta$ 1.20 (d, 6 H, $CH_3$, $^3J$ = 7.0 Hz), 2.89 (septet, 1 H, CH, $^3J$ = 6.9 Hz), 5.44 (s, 2 H, $ArCH_2$), 6.27 (s, 2 H, $NH_2$), 7.26 (d, 2 H, Ar-H), 7.43 (d, 2 H, Ar-H), 7.83 (s, 1 H, H-8), 12.45 (s, 1 H, > NH); $^{13}$C NMR $\delta$ 23.84 (2 $CH_3$), 33.19 (CH), 66.61 ($ArCH_2$), 110.25 (C-5), 126.25 (2 Ar), 127.02 (*p*-Ar), 128.88 (2 Ar), 134.12 (*ipso*-Ar), ~138 (br s, C-8), 148.29 (C-4), 159.61 (C-2, C-6); MS (EI) calcd *m/z* for $C_{15}H_{17}N_5O$ 283.1431, found 283.1434. Anal. ($C_{15}H_{17}N_5O$) C, 63.59; H, 6.05; N, 24.72. Found: C, 64.14; H, 6.03; N, 24.40. Product **1e** was purified by crystallization from 50 mL of EtOH/$H_2O$ (1:1): yield 0.7 g (28%); mp 122–125 °C; UV (pH 1) $\lambda_{max}$ 239 nm (sh) ($\epsilon = 0.615 \times 10^4$), 288 nm ($\epsilon = 0.791 \times 10^4$), (pH 6.9) 240 nm ($\epsilon = 0.752 \times 10^4$), 285 nm ($\epsilon = 0.657 \times 10^4$), (pH 13) 247 nm (sh) ($\epsilon = 0.423 \times 10^4$), 287 nm ($\epsilon = 0.650 \times 10^4$); $^1$H NMR $\delta$ 0.89 (t, 3 H, $CH_3$, $^3J$ = 7.4 Hz), 1.30 (sextet, 2 H, Me-$CH_2$, $^3J$ = 7.4–7.5 Hz), 1.55 (m, 2 H, $CH_2$), 2.58 (t, 2 H, Ar-$CH_2$, $^3J$ = 7.7 Hz), 5.43 (s, 2 H, $ArCH_2O$), 6.28 (s, 2 H, $NH_2$), 7.21 (d, 2 H, Ar-H), 7.40 (d, 2 H, Ar-H), 7.80 (s, 1 H, H-8), 12.40 (s, 1 H, > NH); $^{13}$C NMR $\delta$ 13.72 ($CH_3$), 21.67 ($CH_2$), 33.09 ($CH_2$), 34.51 (Ar-$CH_2$), 66.59 ($ArCH_2O$), 113.48 (C-5), 128.25 (2 Ar), 128.58 (2 Ar), 133.96 (*p*-Ar), 137.70 (*ipso*-Ar), 142.19 (C-8), 155.13 (C-4), 159.63 (C-2, C-6); MS (EI) calcd *m/z* for $C_{16}H_{19}N_5O$ 297.1589, found 297.1576. Anal. ($C_{16}H_{19}N_5O$) C, H, N. Compound **1f** was purified by crystallization from EtOH/$H_2O$ (1:1) with charcoal treatment: yield 0.8 g (47%); mp 219–220 °C dec; UV (pH 1) $\lambda_{max}$ 254 nm ($\epsilon = 1.870 \times 10^4$), 285 nm (sh) ($\epsilon = 1.154 \times 10^4$), (pH 6.9) 250 nm ($\epsilon = 1.518 \times 10^4$), 285 nm (sh) ($\epsilon = 0.921 \times 10^4$), (pH 13) 252 nm ($\epsilon = 1.965 \times 10^4$), 285 nm (sh) ($\epsilon = 1.062 \times 10^4$); $^1$H NMR $\delta$ 5.54 (s, 2, $ArCH_2$), 6.31 (s, 2 H, $NH_2$), 7.37 (t, 1 H, ArH), 7.47 (t, 2 H, ArH), 7.59 (d, 2 H, ArH), 7.68 (t, 4 H, ArH), 7.82 (s, 1 H, H-8), 12.43 (s, 1 H, > NH); $^{13}$C NMR $\delta$ 66.34 ($ArCH_2$), 110.17 (C-5), 127.46 (*p*-Ar), 128.88 (4 Ar), 128.99 (4 Ar), 135.93 (*ipso*-Ar), 137.8 (C-8), 139.76 (2 *ipso*-Ar), 139.82 (C-4), 159.58 (C-2, C-6); MS (EI) calcd *m/z* for $C_{16}H_{15}N_5O$ 317.1276, found 317.1261. Anal. ($C_{16}H_{15}N_5O$) C, H, N. Compound **2** was purified by crystallization from EtOH/$H_2O$ (1:3): yield 0.8 g (29%); mp 175–178 °C (waxes), >230 °C dec; UV (pH 1) $\lambda_{max}$ 237 nm (sh) ($\epsilon = 0.670 \times 10^4$), 288 nm ($\epsilon = 1.174 \times 10^4$), (pH 6.9) 241 nm ($\epsilon = 0.763 \times 10^4$), 282 nm ($\epsilon = 0.879 \times 10^4$), (pH 13) 247 nm (sh) ($\epsilon = 0.494 \times 10^4$), 285 nm ($\epsilon = 0.980 \times 10^4$); $^1$H NMR $\delta$ 2.28 (s, 6 H, $CH_3$), 5.40 (s, 2 H, $ArCH_2$), 6.29 (s, 2 H, $NH_2$), 6.97 (s, 1 H, ArH), 7.09 (s, 2 H, ArH), 7.81 (s, 1 H, H-8), 12.42 (s, 1 H, > NH); $^{13}$C NMR $\delta$ 20.84, (2 $CH_3$), 66.74 ($ArCH_2$), 113.48 (C-5), 126.06 (2 *o*-Ar), 129.30 (*p*-Ar), 136.49 (2 *m*-Ar), 137.35 (C-8), 137.2 (*ipso*-Ar), 155.1 (C-4), 159.59 (C-2), 159.8 (C-6); MS (EI) calcd *m/z* for $C_{14}H_{15}N_5O$ 269.1276, found 269.1264. Anal. ($C_{16}H_{19}N_5O \cdot {}^1/_2H_2O$) C, H, N. Compound **6** was purified by crystallization from EtOH/$H_2O$ (3:7) containing ammonium hydroxide: yield 1.18 g (53%); mp 165–166 °C; UV (pH 1) $\lambda_{max}$ 236 nm (sh) ($\epsilon = 0.642 \times 10^4$), 263 nm ($\epsilon = 0.929 \times 10^4$), 292 nm ($\epsilon = 1.063 \times 10^4$), (pH 6.9) 245 nm ($\epsilon = 0.818 \times 10^4$), 262 nm ($\epsilon = 0.659 \times 10^4$), 268 nm ($\epsilon = 0.727 \times 10^4$), 282 nm ($\epsilon = 0.838 \times 10^4$), (pH 13) 261 nm ($\epsilon = 0.754 \times 10^4$), 268 nm ($\epsilon = 0.787 \times 10^4$), 285 nm ($\epsilon = 0.960 \times 10^4$); $^1$H NMR $\delta$ 5.58 (s, 2 H, $ArCH_2$), 6.32 (s, 2 H, $NH_2$), 7.35 (dd, 1 H, ArH), 7.51 (d, 1 H, ArH), 7.83 (dd, 1 H, ArH), 7.86 (s, 1 H, H-8), 8.59 (d, 1 H, ArH), 12.48 (s, 1 H, > NH); $^{13}$C NMR $\delta$ 67.29 ($ArCH_2$), 113.46 (C-5), 121.75 (*m*-Ar), 122.92 (*m*-Ar), 136.90, (*p*-Ar), 137.96 (C-8), 149.11 (*o*-Ar), 155.33 (*ipso*-Ar), 156.42 (C-4), 159.63 (C-2, C-6); MS (EI) calcd *m/z* for $C_{11}H_{10}N_6O$ 242.0912, found 242.0920. Anal. ($C_{16}H_{19}N_5O \cdot {}^1/_2H_2O$) C, H, N.

**$O^6$-(*p*-(Hydroxymethyl)benzyl)guanine (1b).** 1,4-Benzenedimethanol (10 g) was melted under argon in a 100-mL round-bottom flask in a 130 °C oil bath. Sodium (13 mmol) was added in portions. When all sodium had reacted with the 1,4-benzenedimethanol, the bath temperature was lowered to 110 °C and 2-amino-6-chloropurine (6.4 mmol) was added. The suspension was stirred for 24 h at 110 °C. At this time the solution was poured into $H_2O$ (250 mL) with constant stirring for 30 min. Undissolved solid was removed by filtration, and the filtrate was neutralized with glacial acetic acid. The precipitate that formed was collected by filtration and crystallized from 100 mL of MeOH/$H_2O$ (1:1) to produce analytically pure **1b**: yield 0.96 g (60%); mp 229–231 °C dec; UV (pH 1) $\lambda_{max}$ 240 nm (sh) ($\epsilon = 0.481 \times 10^4$), 287 nm ($\epsilon = 1.165 \times 10^4$), (pH 6.9) 240 nm ($\epsilon = 0.761 \times 10^4$), 282 nm ($\epsilon = 0.864 \times 10^4$), (pH 13) 240 nm (sh) ($\epsilon = 0.474 \times 10^4$), 285 nm ($\epsilon = 0.919 \times 10^4$); $^1$H NMR $\delta$ 4.50 (d, 2 H, $CH_2OH$), 5.19 (t, 1 H, OH, exchanges with $D_2O$), 5.46 (s, 2 H, $ArCH_2$), 6.28 (br s, 2 H, $NH_2$, exchange with $D_2O$), 7.31–7.48 (m, 4 H, ArH), 7.80 (s, 1 H, H-8), 12.41 (br s, 1 H, > NH, exchanges with $D_2O$); MS (EI) calcd *m/z* for $C_{13}H_{13}N_5O_2$ 271.1069, found 271.1063. Anal. ($C_{13}H_{13}N_5O_2$) C, H, N.

**$O^6$-(*p*-Formylbenzyl)guanine (1c).** $O^6$-(*p*-(Hydroxymethyl)benzyl)guanine (**1b**) (3.6 mmol), sodium acetate (1.3 mmol), and pyridinium chlorochromate (5.6 mmol) were stirred in 15 mL of anhydrous pyridine under argon for 20 h at room temperature. Methanol (30 mL) was then added, and the resulting mixture was stirred for 4 h. Water (30 mL) was added, and the deep brown precipitate that formed was collected by filtration. The filtrate was loaded on a 3- × 80-cm Sephadex LH-20 column eluted with methanol/$H_2O$ (1:1) at 1 mL/min. Column eluent was continuously monitored at 280 nm, and fractions (10 mL) were collected. That portion of the brown precipitate (see above) which was soluble in 60 mL of MeOH/$H_2O$ (1:1) was chromatographed separately under identical conditions. The desired product together with some unreacted **1b** eluted in fractions 80–110. Crystallization from methanol/$H_2O$ (1:1) with charcoal treatment of the material recovered from pooled fractions 80–110 afforded analytically pure **1c**: yield 0.45 g (45%); mp >247 °C dec; UV (pH 1) $\lambda_{max}$ 254 nm ($\epsilon = 1.989 \times 10^4$), 287 nm (sh) ($\epsilon = 1.455 \times 10^4$), (pH 6.9) 250 nm ($\epsilon = 2.121 \times 10^4$), 277 nm (sh) ($\epsilon = 1.357 \times 10^4$), (pH 13) 256 nm ($\epsilon = 1.959 \times 10^4$), 279 nm (sh) ($\epsilon = 1.293 \times 10^4$); $^1$H NMR $\delta$ 5.61 (s, 2 H $ArCH_2$), 6.29 (s, 2 H, $NH_2$, exchange with $D_2O$), 7.71 (d, 2 H, ArH), 7.86 (s, 1 H, H-8), 7.94 (d, 2 H, ArH) 10.02 (s, 1 H, CHO), 12.46 (br s, 1 H, > NH, exchanges with $D_2O$); MS (EI) calcd *m/z* for $C_{13}H_{11}N_5O_2$ 269.0913, found 269.0934. Anal. ($C_{13}H_{11}N_5O_2 \cdot {}^1/_5H_2O$) C, H, N.

**9-Acetyl-$O^6$-benzylguanine (3a).** To 0.38 mL (4 mmol) of acetic anhydride in 0.65 mL of pyridine under argon at 0 °C was added 0.241 g (1 mmol) of $O^6$-benzylguanine. The reaction mixture was stirred at 0 °C for 30 min, at which time the ice bath was removed and the reaction was allowed to proceed overnight at room temperature. At this point, a water–ice mixture (~5 mL) was added to the flask with vigorous stirring for 5 min. Compound **3a** was collected as a white solid which was washed with $H_2O$ and dried under vacuum: yield 0.262 g (92.5%); mp 176–177 °C; UV (pH 1) $\lambda_{max}$ 262 nm (sh) ($\epsilon = 0.723 \times 10^4$), 286 nm ($\epsilon = 0.996 \times 10^4$), (pH 6.9) 227 nm (sh) ($\epsilon = 1.117 \times 10^4$), 278 nm ($\epsilon = 1.174 \times 10^4$), (pH 13) (decomposes to $O^6$-benzylguanine); $^1$H NMR $\delta$ 2.83 (s, 3 H, $CH_3$), 5.51 (s, 2 H, $ArCH_2$), 6.84 (s, 2 H, $NH_2$), 7.36 (m, 1 H, ArH), 7.41 (m, 2 H, ArH), 7.51 (m, 2 H, ArH), 8.31 (s, 1 H, H-8); $^{13}$C NMR $\delta$ 24.59 ($CH_3$), 67.09 ($ArCH_2$), 114.47 (C-5), 128.11 (*p*-Ar), 128.41 (2 *m*-Ar), 128.46 (2 *o*-Ar), 136.32 (*ipso*-Ar), 136.66 (C-8), 153.32 (C-4), 160.35 (C-2), 160.42 (C-6), 168.25 (CO); MS (EI) calcd *m/z* for $C_{14}H_{13}N_5O_2$ 283.1069, found 283.1052. Anal. ($C_{14}H_{13}N_5O_2$) C, H, N.

**$O^6$-Benzyl-7-methylguanine (5) and $O^6$-Benzyl-9-methylguanine (3b).** To 0.24 g (1 mmol) of $O^6$-benzylguanine under argon was added 1 mL of a 1 M solution of sodium ethoxide in ethanol. The solution was stirred 10 min, and the ethanol was removed under vacuum. The remaining solid was dissolved in 2 mL of dry DMF. Iodomethane (1.2 mmol) was added to the stirring solution by syringe, producing a slightly exothermic reaction that was allowed to proceed overnight. At this point, the reaction mixture was diluted with approximately 50 mL of $H_2O$, and the aqueous layer was washed with $CH_2Cl_2$. An oily residue was obtained by evaporation of the combined organic washes after drying with $MgSO_4$. Separation of the two isomers was achieved by dissolving the oil in a minimum volume of 10% ethanol in $CHCl_3$ and loading this onto a silica gel column (Davisil grade 633, 200-425 mesh, 60 Å, 2.5 × 35 cm). The 9-isomer eluted from the column with 10% EtOH in $CHCl_3$, while the 7-isomer eluted later with 15% EtOH in $CHCl_3$. The 7-isomer (**5**) was obtained analytically pure upon drying at 110 °C under vacuum. The 9-isomer (**3b**) was further purified by precipitation from $CH_2Cl_2$ with hexane and drying at 110 °C under vacuum. $O^6$-Benzyl-7-methylguanine (**5**): yield 86 mg (33.6%); mp 175–177 °C; UV (pH 1) $\lambda_{max}$ 240 nm (sh) ($\epsilon = 0.550 \times 10^4$), 290 nm ($\epsilon =$

1.060 × $10^4$), (pH 6.9) 241 nm (sh) (ε = 0.748 × $10^4$), 291 nm (ε = 0.900 × $10^4$), (pH 13) 241 nm (sh) (ε = 0.607 × $10^4$), 291 nm (ε = 0.715 × $10^4$); $^1$H NMR δ 3.83 (s, 3 H, $CH_3$), 5.50 (s, 2 H, $ArCH_2$), 6.13 (s, 2 H, $NH_2$), 7.35 (m, 1 H, ArH), 7.41 (m, 2 H, ArH), 7.52 (m, 2 H, ArH), 8.01 (s, 1 H, H-8); $^{13}$C NMR δ 33.43 ($CH_3$), 66.77 ($ArCH_2$), 106.46 (C-5), 127.81 (2 *o*-Ar), 127.92 (*p*-Ar), 128.44 (2 *m*-Ar), 136.55 (*ipso*-Ar), 145.83 (C-8), 156.66 (C-6), 159.47 (C-2), 163.90 (C-4); MS (EI) calcd *m/z* for $C_{13}H_{13}N_5O$ 255.1120, found 255.1124. Anal. ($C_{13}H_{13}N_5O \cdot {}^1/_8H_2O$) C, N, O.

***O*$^6$-Benzyl-9-methylguanine (3b)**: yield 75 mg (29.3%); mp 149–151 °C; UV (pH 1) $\lambda_{max}$ 241 nm (ε = 0.740 × $10^4$), 291 nm (ε = 0.907 × $10^4$), (pH 6.9) 250 nm (ε = 0.753 × $10^4$), 282 nm (ε = 0.951 × $10^4$), (pH 13) 250 nm (ε = 0.829 × $10^4$), 282 nm (ε = 1.056 × $10^4$); $^1$H NMR δ 3.59 (s, 3 H, $CH_3$), 5.50 (s, 2 H, $ArCH_2$), 6.45 (s, 2 H, $NH_2$), 7.35 (m, 1 H, ArH), 7.40 (m, 2 H, ArH), 7.50 (m, 2 H, ArH), 7.82 (s, 1 H, H-8); $^{13}$C NMR δ 29.18 ($CH_3$), 66.76 ($ArCH_2$), 113.19 (C-5), 127.97 (*p*-Ar), 128.36 (2 *m*-Ar), 128.38 (2 *o*-Ar), 136.69 (*ipso*-Ar), 140.39 (C-8), 154.75 (C-4), 159.67 (C-2), 159.94 (C-6); MS (EI) calcd *m/z* for $C_{13}H_{13}N_5O$ 255.1120, found 255.1113. Anal. ($C_{13}H_{13}N_5O$) C, H, N.

***O*$^6$-Benzyl-9-((pivaloyloxy)methyl)guanine (3c).** *O*$^6$-Benzylguanine (0.24 g, 1 mmol) was dissolved in 1 mL of a 1 M solution of sodium ethoxide in ethanol under argon. After 10 min the ethanol was removed under vacuum. The resulting solid was dissolved in 2 mL of dry DMF. Chloromethyl pivalate (1 mmol) was added dropwise to the stirring solution by syringe, resulting in an exothermic reaction. The mixture was allowed to react for 30 min at room temperature. The DMF was then removed under vacuum overnight. The product was purified by treating the recovered solid wtih a small volume of 10% ethanol in $CHCl_3$ and loading the soluble material on a 2.5- × 17-cm silica gel column (Davisil grade 633, 200–425 mesh, 60 Å). Elution was carried out with 10% ethanol in $CHCl_3$ to provide analytically pure *O*$^6$-benzyl-9-((pivaloyloxy)methyl)guanine (**3c**): yield 0.26 g (72.3%); mp 161–162 °C; UV (pH 1 ) $\lambda_{max}$ 245 nm (ε = 0.711 × $10^4$), 289 nm (ε = 0.912 × $10^4$), (pH 6.9) 249 nm (ε = 0.930 × $10^4$), 282 nm (ε = 0.869 × $10^4$), (pH 13) (decomposes to *O*$^6$-benzylguanine); $^1$H NMR δ 1.09 (s, 9 H, $(CH_3)_3$), 5.48 (s, 2 H, $CH_2N$ <), 5.96 (s, 2 H, $ArCH_2$), 6.64 (s, 2 H, $NH_2$), 7.34 (m, 1 H, ArH), 7.39 (m, 2 H, ArH), 7.50 (m, 2 H, ArH), 7.92 (s, 1 H, H-8); $^{13}$C NMR δ 26.51 (3 $CH_3$), 38.20 $CMe_3$), 65.13 ($CH_2N$ <), 66.95 ($ArCH_2$), 113.11 (C-5), 128.05 (*p*-Ar), 128.38 (2 *m*-Ar), 128.47 (2 *o*-Ar), 136.50 (*ipso*-Ar), 139.96 (C-8), 154.30 (C-4), 160.11 (C-2), 160.18 (C-6), 176.97 (OCO); MS (EI) calcd *m/z* for $C_{18}H_{21}N_5O_3$ 355.1644, found 355.1660. Anal. ($C_{18}H_{21}N_5O_3$) C, H, N.

***O*$^6$-Benzyl-9-(3-chloro-2-hydroxypropyl)guanine (3d).** *O*$^6$-Benzylguanine (2 mmol) was dissolved in 10 mL of neat epichlorohydrin. The reaction mixture was heated nearly to boiling. When the solution began to turn yellow, the heating was discontinued and the solution was allowed to cool to room temperature. The excess epoxide was removed under vacuum. The crude product was dissolved in a minimum of 10% ethanol in $CHCl_3$ and loaded on a 2.5- × 18-cm silica gel column (Davisil grade 633, 200–425 mesh, 60 Å). The product was eluted from the column with 10% ethanol in $CHCl_3$. The recovered **3d** was analytically pure after drying at 110 °C under vacuum: yield 240 mg (57%); mp 183–185 °C dec; UV (pH 1) $\lambda_{max}$ 244 nm (ε = 0.681 × $10^4$), 292 nm (ε = 0.928 × $10^4$), (pH 6.9) 250 nm (ε = 0.768 × $10^4$), 282 nm (ε = 0.943 × $10^4$), (pH 13) 249 nm (ε = 0.950 × $10^4$), 282 nm (ε = 1.095 × $10^4$); $^1$H NMR δ 3.56 (dd, 1 H, $H_aCHCl$, $^2J$ = 11.4 Hz, $^3J$ = 5.4 Hz), 3.65 (dd, 1 H, $H_bCHCl$, $^2J$ = 11.3 Hz, $^3J$ = 4.7 Hz), 4.00 (dd, 1 H, $H_aCH$<N, $^2J$ = 13.5 Hz, $^3J$ = 8.0 Hz), 4.11 (m, 1 H, C*H*OH), 4.16 (dd 1 H, $H_bCH$<N, $^2J$ = 13.5 Hz, $^3J$ = 3.8 Hz), 5.50 (s, 2 H, $ArCH_2$), 5.64 (d, 1 H, OH, $^3J$ = 5.4 Hz), 6.45 (s, 2 H, $NH_2$), 7.35 (t, 1 H, ArH), 7.40 (t, 2 H, ArH), 7.50 (d, 2 H, ArH), 7.79 (s, 1 H, H-8); $^{13}$C NMR δ 46.32 ($CH_2Cl$), 47.31 ($CH_2N$<), 66.76 ($ArCH_2$), 68.17 (CHOH), 113.57 (C-5), 127.99 (*p*-Ar), 128.37 (2 *m*-Ar), 128.42 (2 *o*-Ar), 136.70 (*ipso*-Ar), 140.49 (C-8), 154.52 (C-4), 159.58 (C-2), 159.97 (C-6); MS (EI) calcd *m/z* for $C_{15}H_{16}N_5O_2{}^{35}Cl$ 333.0992, found 333.0979; calcd *m/z* for $C_{15}H_{16}N_5O_2{}^{37}Cl$ 335.0963, found 335.0939. Anal. ($C_{15}H_{16}N_5O_2Cl$) C, 53.98; H, 4.83; N, 20.98; Cl, 10.62. Found: C, 53.92; H, 4.90; N, 20.76; Cl, 11.28.

***O*$^6$-Benzyl-9-(2-hydroxy-3-(isopropylamino)propyl)guanine (3e).** To a suspension of 100 mg (0.3 mmol) of **3d** in 10 mL of dioxane was added 1 mL of isopropylamine. The resultant slurry was heated to reflux. The progress of the reaction was monitored by TLC (silica, 10% ethanol in chloroform). After 20 h of reflux, 1 mL more isopropylamine was added to the reaction mixture. Refluxing was continued. When the reaction was judged complete by TLC, solvent was removed under vacuum and the resulting solid was washed with 10% aqueous methanol to remove a yellow contaminant. Recrystallization of the remaining solid from 10 mL of 50% aqueous ethanol containing a few drops of isopropylamine afforded analytically pure **3e**: yield 90 mg (82%); mp 206–208 °C; UV (pH 1) $\lambda_{max}$ 244 nm (ε = 0.690 × $10^4$), 292 nm (ε = 0.886 × $10^4$), (pH 6.9) 250 nm (ε = 0.772 × $10^4$), 282 nm (ε = 0.939 × $10^4$), (pH 13) 250 nm (ε = 0.826 × $10^4$), 282 nm (ε = 1.048 × $10^4$); $^1$H NMR δ 0.96 (d, 6 H, $(CH_3)_2$, $^3J$ = 6.2 Hz), 1.58 (br s, 1 H), 2.45 (m, 2 H, $CH_2NH$), 2.65 (septet, 1 H, $CHMe_2$, $^3J$ = 6.2 Hz) 3.87 (m, 1 H, C*H*OH), 3.94 (dd, 1 H, $H_aCH$<N, $^2J$ = 13.8 Hz, $^3J$ = 7.7 Hz), 4.08 (dd, 1 H, $H_bCH$<N, $^2J$ = 13.7 Hz, $^3J$ = 3.8 Hz), 5.08 (br s, 1 H, OH), 5.48 (d, 1 H, $ArH_aCH$, $^2J$ = 13.3 Hz), 5.51 (d, 1 H, $ArH_bCH$, $^2J$ = 13.5 Hz), 6.43 (s, 2 H, $NH_2$), 7.35 (t, 1 H, ArH), 7.40 (t, 2 H, ArH), 7.50 (d, 2 H, ArH), 7.77 (s, 1 H, H-8); $^{13}$C NMR δ 22.88 ($CH_3$), 22.93 ($CH_3$), 47.07 ($CH_2N$<), 48.22 ($CH_2NH$), 50.54 ($CHMe_2$), 66.74 ($ArCH_2$), 68.13 (CHOH), 113.46 (C-5), 127.97 (*p*-Ar), 128.35 (2 *m*-Ar), 128.40 (2 *o*-Ar), 136.69 (*ipso*-Ar), 140.61 (C-8), 154.55 (C-4), 159.47 (C-2), 159.90 (C-6); MS (EI) calcd *m/z* for $C_{16}H_{24}N_6O_2$ 356.1960, found 356.1970. Anal. ($C_{16}H_{24}N_6O_2 \cdot {}^1/_2H_2O$) C, H, N.

***O*$^6$-Benzyl-9-(3-(*tert*-butylamino)-2-hydroxypropyl)guanine (3f).** To a 5-mL microflex flask fitted with a magnetic stirrer and sealed pressure value was added 80 mg (0.24 mmol) of **3d**, 1 mL of dioxane, 1 mL of *tert*-butylamine, and a few milligrams of potassium carbonate. The resulting suspension was heated in a 90 °C oil bath for 22 h. Solvent was removed under vacuum, and the resulting solid was washed with 10% aqueous methanol to remove a yellow contaminant. *O*$^6$-Benzyl-9-(3-(*tert*-butylamino)-2-hydroxypropyl)guanine (**3f**) was purified by crystallization from 10 mL of 50% aqueous ethanol containing a few drops of *tert*-butylamine: yield 84 mg (95%); mp 169–170.5 °C; UV (pH 1) $\lambda_{max}$ 243 nm (ε = 0.686 × $10^4$), 292 nm (ε = 0.917 × $10^4$), (pH 6.9) 250 nm (ε = 0.782 × $10^4$), 282 nm (ε = 0.952 × $10^4$), (pH 13) 250 nm (ε = 0.766 × $10^4$), 282 nm (ε = 0.974 × $10^4$); $^1$H NMR δ 1.00 (s, 9 H, $CH_3$), 2.44 (m, 2 H, $CH_2N$-*tert*-Bu), 3.84 (m, 1 H, C*H*OH), 3.95 (dd, 1 H, $H_aCH$<N, $^2J$ = 14.1 Hz, $^3J$ = 7.7 Hz), 4.09 (dd, 1 H, $H_bCH$<N, $^2J$ = 13.9 Hz, $^3J$ = 4.1 Hz), 5.06 (m, 1 H, OH), 5.48 (d, 1H, $ArH_aCH$, $^2J$ = 13.7 Hz), 5.50 (d, 1H, $ArH_bCH$, $^2J$ = 13.4 Hz), 6.43 (s, 2 H, $NH_2$), 7.35 (t, 1 H, ArH), 7.40 (t, 2 H, ArH), 7.50 (d, 2 H, ArH), 7.77 (s, 1 H, H-8); $^{13}$C NMR δ 28.70 (3 $CH_3$), 45.70 ($CH_2NH$), 47.03 ($CH_2N$<), 49.66 ($CMe_3$), 66.74 ($ArCH_2$), 68.54 (CHOH), 113.45 (C-5), 127.96 (*p*-Ar), 128.34 (2 *m*-Ar), 128.39 (2 *o*-Ar), 136.68 (*ipso*-Ar), 140.63 (C-8), 154.55 (C-4), 159.45 (C-2), 159.90 (C-6); MS (EI) calcd *m/z* for $C_{19}H_{26}N_6O_2$ 370.2117, found 370.2122. Anal. ($C_{19}H_{26}N_6O_2$) C, H, N.

***O*$^6$-Benzyl-9-(2-hydroxy-3-isopropoxypropyl)guanine (3g).** *O*$^6$-Benzylguanine (1.25 mmol) and glycidyl isopropyl ether (3 mL, 24 mmol) were heated under argon in a 110 °C oil bath for 2 h. The excess glycidyl isopropyl ether was evaporated under vacuum. The resulting brown solid was dried under vacuum overnight and was purified by silica gel column chromatography (Davisil grade 633, 200–425 mesh, 60 Å) using 7% EtOH in $CHCl_3$ as eluent to give a pale yellow solid. It was further purified by dissolving in dichloromethane (3 mL) and precipitating with hexane (6 mL). The white precipitate was collected by filtration to provide analytically pure **3g**: yield 90 mg (20%); mp 193–194 °C; UV (MeOH/$H_2O$, 1:1) $\lambda_{max}$ 251 nm (ε = 0.787 × $10^4$), 283 (ε = 0.940 × $10^4$); $^1$H NMR δ 1.08 (d, 6 H, $CH_3$), 3.25–3.40 (m, 2 H, $OCH_2$), 3.52 (septet, 1 H, $CH(CH_3)_2$), 3.86–4.02 (m, 2 H, C*H*OH + $NCH_aH$), 4.05–4.22 (m, 1 H, $NCH_bH$), 5.18 (br d, 1 H, OH, exchanges with $D_2O$), 5.50 (s, 2 H, $ArCH_2$), 6.41 (br s, 2 H, $NH_2$, exchange with $D_2O$), 7.32–7.54 (m, 5 H, ArH), 7.77 (s, 1 H, H-8); MS (EI) calcd *m/z* for $C_{18}H_{23}N_5O_3$ 357.1800, found 357.1797. Anal. ($C_{18}H_{23}N_5O_3$) C, H, N.

**17β-(Chloroacetoxy)-4-androsten-3-one.** Testosterone (5 mmol) was dissolved in anhydrous methylene chloride (7.0 mL) and triethylamine (5 mmol) under argon. The solution was stirred for 30 min in an ice bath. Chloroacetyl chloride (10 mmol) dissolved in anhydrous methylene chloride (3 mL) was added at 0 °C, and stirring was continued for 30 min. The reaction was

then allowed to warm to room temperature and was stirred overnight. The solution was washed with 0.05 N aqueous HCl solution (2 × 10 mL) and water (2 × 10 mL) and dried over sodium sulfate. It was concentrated under reduced pressure to give a brown solid. It was purified by silica gel column chromatography (Davisil grade 633, 200–425 mesh, 60 Å) using chloroform/petroleum ether (2:1) as eluent to give a white solid: yield 1.23 g (67%); mp 125–126 °C (lit.[13] mp 124–125 °C); UV (MeOH/$H_2O$, 1:1) $\lambda_{max}$ 246 nm ($\epsilon = 1.521 \times 10^4$); $^1H$ NMR $\delta$ 0.81 (s, 3 H, $CH_3$-19), 1.15 (s, 3 H, $CH_3$-18), 0.82–2.50 (m, 19 H), 4.38 (d, 2 H, $CH_2Cl$), 4.63 (t, 1 H, H-17), 5.63 (s, 1 H, H-4); MS (EI) calcd $m/z$ for $C_{21}H_{29}O_3{}^{35}Cl$ 364.1805, found 364.1804; calcd $m/z$ for $C_{21}H_{29}O_3{}^{37}Cl$ 366.1776, found 366.1776.

**$O^6$-Benzyl-9-((3-oxo-4-androsten-17$\beta$-yloxycarbonyl)methyl)guanine (3h).** $O^6$-Benzylguanine (3.0 mmol) was dissolved in 3 mL of a 1 M solution of sodium ethoxide in ethanol under argon with stirring for 30 min. Ethanol was removed under reduced pressure, and the resulting solid was redissolved in anhydrous DMF (4 mL). 17$\beta$-(Chloroacetoxy)-4-androsten-3-one (3.0 mmol) dissolved in anhydrous DMF (6 mL) was added to the solution with stirring for 1 h at room temperature. The solvent was removed under reduced pressure to produce a brown hard foam. The product was purified by silica gel column chromatography (Davisil grade 633, 200–425 mesh, 60 Å) using 5% ethanol in $CHCl_3$ as eluent to give **3h** as a pale yellow hard foam: yield 0.946 g (55%); mp 125–127 °C; UV (MeOH/$H_2O$, 1:1) $\lambda_{max}$ 247 nm ($\epsilon = 2.495 \times 10^4$), 282 ($\epsilon = 0.959 \times 10^4$); $^1H$ NMR $\delta$ 0.61 (s, 3 H, $CH_3$-19′), 1.14 (s, 3 H, $CH_3$-18′), 0.8–2.40 (m, 19 H), 4.56 (t, 1 H, H-17′), 4.93 (d, 2 H, $CH_2N$), 5.51 (s, 2 H, $ArCH_2$), 5.62 (s, 1 H, H-4′), 6.50 (br s, 2 H, $NH_2$, exchange with $D_2O$), 7.30–7.55 (m, 5 H, ArH), 7.85 (s, 1 H, H-8); MS (positive ion fast atom bombardment) calcd $m/z$ for $C_{33}H_{39}N_5O_4$ 569.3001, found 569.3006. Anal. ($C_{33}H_{39}N_5O_4 \cdot H_2O$) C, H, N.

**17$\beta$-(Chloroacetoxy)-5$\alpha$-androstan-3-one.** Dihydrotestosterone (2.6 mmol) was dissolved in anhydrous methylene chloride (3 mL) and triethylamine (2.6 mmol) under argon. The solution was stirred at 0 °C for 30 min, at which time chloroacetyl chloride (3.75 mmol) dissolved in anhydrous methylene chloride (2 mL) was added. The resulting mixture was stirred for an additional 30 min at 0 °C and at room temperature for 4 h. The solution was diluted with $CH_2Cl_2$ (30 mL) and was washed with aqueous 0.05 N hydrochloric acid (2 × 30 mL) and water (2 × 30 mL). The organic layer was dried over sodium sulfate and evaporated under vacuum to give crude product as a white solid. The product was purified by silica gel column chromatography (Davisil grade 633, 200–425 mesh, 60 Å) using $CHCl_3$ as eluent: yield 0.38 g (41%); mp 135–136 °C (lit.[14] mp 138 °C); $^1H$ NMR $\delta$ 0.78 (s, 3 H, $CH_3$-19), 0.98 (s, 3 H, $CH_3$-18), 0.78–2.49 (m, 22 H), 4.37 (d, 2 H, $CH_2Cl$), 4.62 (t, 1 H, H-17); MS (EI) calcd $m/z$ for $C_{21}H_{31}O_3{}^{35}Cl$ 366.1961, found 366.1932; calcd $m/z$ for $C_{21}H_{31}O_3{}^{37}Cl$ 368.1932, found 368.1911.

**$O^6$-Benzyl-9-((3-oxo-5$\alpha$-androstan-17$\beta$-yloxycarbonyl)methyl)guanine (3i).** $O^6$-Benzylguanine (1.0 mmol) was dissolved in 1 mL of a 1 M solution of sodium ethoxide in ethanol was stirring for 30 min at room temperature. The ethanol was evaporated under reduced pressure. The solid residue was dissolved in anhydrous DMF (2 mL), and 17$\beta$-(chloroacetoxy)-5$\alpha$-androstan-3-one (1.0 mmol) dissolved in anhydrous DMF (4 mL) was added. The solution was stirred for 2 h at room temperature. The solvent was evaporated under the vacuum to give a brown hard foam. The product was purified by silica gel column chromatography (Davisil grade 633, 200–425 mesh, 60 Å) using 10% EtOH in $CHCl_3$ as eluent: yield 0.36 g (62%); mp 168–170 °C; UV (MeOH/$H_2O$, 1:1) $\lambda_{max}$ 247 nm ($0.843 \times 10^4$), 284 ($0.997 \times 10^4$); $^1H$ NMR $\delta$ 0.57 (s, 3 H, $CH_3$-19′), 0.96 (s, 3 H, $CH_3$-18′), 0.57–2.47 (m, 22 H), 4.53 (t, 1 H, H-17′), 4.92 (d, 2 H, $CH_2N$), 5.51 (s, 2 H, $ArCH_2$), 6.49 (br s, 2 H, $NH_2$, exchange with $D_2O$), 7.30–7.55 (m, 5 H, ArH), 7.85 (s, 1 H, H-8); MS (positive ion fast atom bombardment) calcd $m/z$ for $C_{33}H_{41}N_5O_4$ 571.3158, found 571.3192. Anal. ($C_{33}H_{41}N_5O_4 \cdot {}^1/_4H_2O$) C, H, N.

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