O^6-(Alkyl/aralkyl)guanosine and 2′-Deoxyguanosine Derivatives: Synthesis and Ability To Enhance Chloroethylnitrosourea Antitumor Action

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A series of O^6-(alkyl/aralkyl)guanosines and 2′-deoxyguanosine analogs extended to peracetyl and N^2-acetyl derivatives, potentially water soluble, was synthesized. Each was associated with *N*′-(2-chloroethyl)-N-[2-(methylsulfonyl)ethyl]-*N*′-nitrosourea for *in vitro* evaluation on M4Beu melanoma cells of their ability to enhance the cytotoxic effect of this chloroethylnitrosourea, which is frequently reduced by repairs performed by O^6-alkylguanine-DNA-alkyltransferase. Structure−activity analysis revealed that (i) benzyl and 4-halobenzyl are the O^6-substituents required to afford a significant activity, (ii) 2′-deoxyguanosine derivatives demonstrate greater potency than guanosine analogs, (iii) acetylation, especially at the N^2 position, generally results in compounds with moderate ability but may prevent incorporation of such nucleosides into DNA. Accordingly, O^6-(4-iodobenzyl)-N^2-acetylguanosine (**3b**) and O^6-benzylperacetyl-2′-deoxyguanosine (**2a**), as well as O^6-benzyl-N^2-acetylguanosine (**1b**) and O^6-benzyl-N^2-acetyl-2′-deoxyguanosine (**2b**), by far the most water soluble, exhibit a good profile for further *in vivo* trials by the intravenous route.

Introduction

Therapeutic effectiveness of chemotherapeutic alkylating agents such as chloroethylnitrosoureas (CENUs) is mainly limited by tumor cell resistance resulting from DNA repairs. Hence this repair is a major hindrance in chemotherapeutic treatments by alkylating drugs. Specifically, the cytotoxic effects of CENUs are attributed to the alkylation at the O^6 position of guanine, resulting in the formation of O^6-chloroethylguanine, which undergoes rearrangement leading to a DNA interstrand covalent cross-link.[1,2] However, before the cross-link occurs, the O^6-chloroethyl adduct can be removed from guanine by irreversible transfer on a cystein residue in the active site of a DNA repair protein called O^6-alkylguanine-DNA-alkyltransferase (AGT), which is thereby permanently inactivated. This reaction, by regenerating a native guanine, accounts for the tumor cell resistance observed in CENU treatments.[3,4] Therefore, the sensitivity of various cells to CENUs is directly dependent on intracellular content of AGT;[3] that is, tumors with high AGT activity will be more resistant to O^6-alkylating agents.

Inhibition of AGT activity may therefore offer a means to increase CENU effectiveness. A way to achieve this end is the pretreatment of resistant tumor cells by DNA-methylating agents such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, 2-deoxy-2-(3′-methyl-3′-nitrosoureido)-D-glucopyranose (Streptozotocin), resulting in the saturation of the repair system.[5,6] However, previous phase I clinical trials testing 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and Streptozotocin or Dacarbazine and Fotemustine combinations have demonstrated the high toxicity of such treatments.[7,8] An alternative way is the use of molecules closely related to the AGT substrate such as O^6-alkylguanine derivatives, capable of specifically transferring an alkyl group to the AGT active site. Among this class of compounds, O^6-benzylguanine is known to be an efficient AGT-depleting agent.[4,9] Several combinations of O^6-benzylguanine/CENU have displayed better antitumor activities than CENU alone on human tumor experimental models.[4,10,11] Nonetheless, O^6-benzylguanine has some limitations: first, the molecule is sparingly water soluble and can be difficult to administer intravenously for human clinical trials; second, it may be incorporated into both DNA or RNA strands and induce point mutations.

In this paper, we report the synthesis of a new series of O^6-alkyl/aralkylguanosine and 2′-deoxyguanosine analogs, **1c**−**6c**, as well as related peracetyl and N^2-acetyl derivatives, **1a**−**6a** and **1b**−**11b**, respectively. The presence of a sugar moiety should increase water solubility compared with a purine base, and acetylation may prevent incorporation of the nucleosides into DNA. This second assumption was based on a molecular modeling study. We next examine the ability of each new compound and O^6-benzylguanine (**12**), as a reference, to increase CENU *in vitro* cytotoxicity. On the basis of these results, we suggest a structure−activity relationship according to the nature of the O^6 substituent, the sugar moiety, and the degree of acetylation in this series of nucleosides and, finally, select the most effective molecules meeting the above criteria, for further *in vivo* antitumor evaluation.

Chemistry

The synthesis of 23 mostly new O^6-alkyl/aralkylguanosine and 2′-deoxyguanosine derivatives **1a**−**6a**, **1b**−**11b**, **1c**,[12] **2c**[13]−**6c**, listed in Table 1, was accomplished according to an approach based on a procedure used for the preparation of O^6-methyl-2′-deoxyguanosine, summarized in Scheme 1.[14,15] Guanosine and 2′-deoxyguanosine, as starting materials, were fully acetylated in pyridine by acetic anhydride in the pres-

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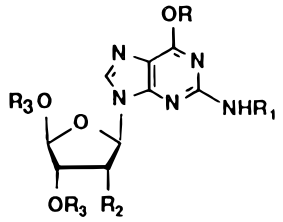
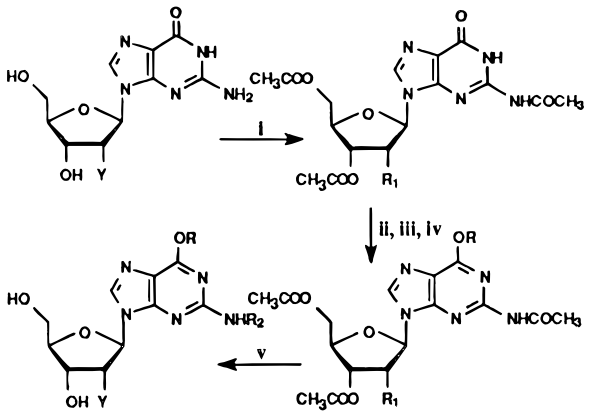
Table 1. *In Vitro* Intrinsic Cytotoxicity and Ability To Enhance Cystemustine Cytotoxicity of Target Compounds: O^6-Alkyl/Aralkylguanosines and 2′-Deoxyguanosine Analogs **1a**−**6a**, **1b**−**11b, 1c**−**6c**, and O^6-Benzylguanine (**12**) on M4Beu Cells



compd	R	R_1	R_2	R_3	cell survival at 300 μM[a] (%)	ED_{50}[b] (μM)
1a	$C_6H_5CH_2$	$COCH_3$	$OCOCH_3$	$COCH_3$	96 ± 8	>400
1b	$C_6H_5CH_2$	$COCH_3$	OH	H	100 ± 16	200
1c	$C_6H_5CH_2$	H	OH	H	99 ± 5	244
2a	$C_6H_5CH_2$	$COCH_3$	H	$COCH_3$	106 ± 5	73
2b	$C_6H_5CH_2$	$COCH_3$	H	H	100 ± 12	165
2c	$C_6H_5CH_2$	H	H	H	101 ± 2	6
3a	4-$IC_6H_4CH_2$	$COCH_3$	$OCOCH_3$	$COCH_3$	101 ± 9	298
3b	4-$IC_6H_4CH_2$	$COCH_3$	OH	H	99 ± 9	82
3c	4-$IC_6H_4CH_2$	H	OH	H	100 ± 7	122
4a	4-$IC_6H_4CH_2$	$COCH_3$	H	$COCH_3$	100 ± 9	200
4b	4-$IC_6H_4CH_2$	$COCH_3$	H	H	102 ± 10	269
4c	4-$IC_6H_4CH_2$	H	H	H	85 ± 10	4
5a	4-$BrC_6H_4CH_2$	$COCH_3$	$OCOCH_3$	$COCH_3$	104 ± 8	400
5b	4-$BrC_6H_4CH_2$	$COCH_3$	OH	H	105 ± 7	>400
5c	4-$BrC_6H_4CH_2$	H	OH	H	99 ± 3	277
6a	4-$BrC_6H_4CH_2$	$COCH_3$	H	$COCH_3$	94 ± 8	216
6b	4-$BrC_6H_4CH_2$	$COCH_3$	H	H	103 ± 3	211
6c	4-$BrC_6H_4CH_2$	H	H	H	96 ± 3	4
7b	2-$IC_6H_4CH_2$	$COCH_3$	OH	H	95 ± 5	NE[c]
8b	CH_3	$COCH_3$	OH	H	101 ± 11	>400
9b	CH_3	$COCH_3$	H	H	98 ± 7	>400
10b	4-pyridylCH_2	$COCH_3$	OH	H	103 ± 4	NE[c]
11b	$(C_6H_5)_2CH$	$COCH_3$	OH	H	106 ± 3	NE[c]
12		O^6-benzylguanine			110 ± 11	2

[a] M4Beu cell survival rate after a 4 h drug exposure (300 μM) at 37 °C, accounting for intrinsic cytotoxicity. Values are the mean of at least three separate determinations. [b] 50% effective dose or concentration of compound, associated with cystemustine (50 μM), required to reduce by 50% cell survival rate determined for a treatment with cystemustine alone (50 μM) (see the Experimental Section). [c] Not significantly effective at 400 μM.

Scheme 1[a]



1b-11b Y = H, OH R_2 = $COCH_3$ **1a-11a** R_1 = H, $OCOCH_3$
1c-6c Y = H, OH R_2 = H
R = $XC_6H_4CH_2$, $(C_6H_5)_2CH$, 4-pyridylCH_2
X = H, Br, I

[a] Reagents: (i) $(CH_3CO)_2O$, 4-(dimethylamino)pyridine (DMAP), Et_3N, pyridine; (ii) mesitylenesulfonyl chloride, DMAP, Et_3N,CH_2Cl_2; (iii) *N*-methylpyrrolidine; (iv) ROH, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU); (v) 2.5 N NaOH, pyridine.

ence of triethylamine. To be readily O^6-alkylated, the resulting peracetylguanosine and peracetyl-2′-deoxyguanosine underwent a two-step *in situ* activation. First, the O^6-(2-mesitylenesulfonyl) derivative was prepared, using 2-mesitylenesulfonyl chloride in the presence of triethylamine and 4-(dimethylamino)pyridine (DMAP) in dichloromethane, and then easily displaced by *N*-methylpyrrolidine to give the unstable O^6-(*N*-methylpyrrolidinium) derivative. A subsequent nucleophilic attack by a large excess of the appropriate alcohol (methyl, benzyl, (2- or 4-halobenzyl), methyl-4-pyridyl, or diphenylmethyl alcohol) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) led to the corresponding O^6-alkylperacetylguanosines and 2′-deoxyguanosines, **1a**−**11a**, in moderate yields. These compounds were partially or completely deacetylated in pyridine under alkaline conditions (NaOH) within a few minutes or hours respectively to afford O^6-alkyl-N^2-acetylguanosines and 2′-deoxyguanosines, **1b**−**11b**, or O^6-alkylguanosines and 2′-deoxyguanosines, **1c**−**6c**.

Physicochemical data, yields, and spectral characterizations of target nucleosides **1a**−**6a**, **1b**−**11b**, and **1c**−**6c** are presented in the Experimental Section.

***In Vitro* Cytotoxicity Evaluation.** This study was carried out using cystemustine (*N*′-(2-chloroethyl)-*N*-[2-(methylsulfonyl)ethyl]-*N*′-nitrosourea), a new CENU being tested in phase II trials for melanoma and glioma treatment, as a bisalkylating agent.[16,17] Tests of colony-forming ability were performed on malignant cells derived from human melanoma (M4Beu). This cell line contains a high constitutive AGT level (918 ± 145 fmol of active AGT/mg of protein), inducing high resistance

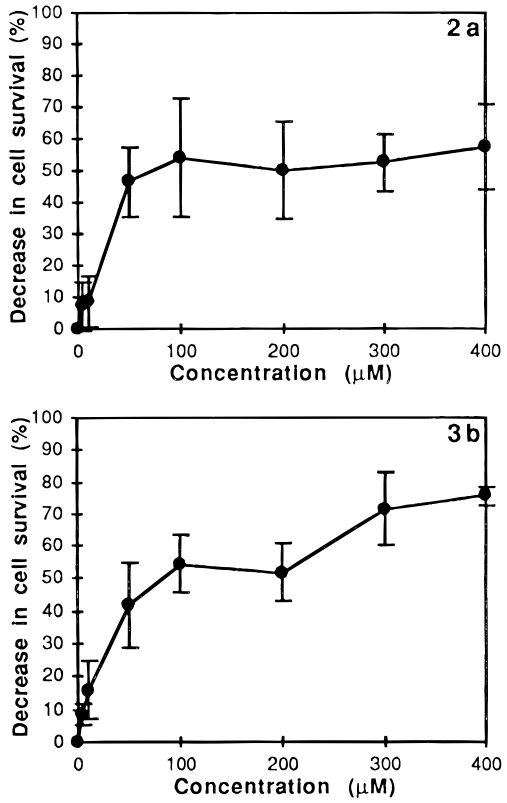


Figure 1. Dose−response curves of compounds **2a** and **3b** on M4Beu cells after a 4 h incubation followed by a 2 h cystemustine (50 μM) exposure. Decrease in cell survival is calculated as the ratio (100 × ((cell survival rate with cystemustine alone (50 μM) − cell survival rate with cystemustine associated with compound (**2a**, **3b**))/cell survival rate with cystemustine alone)). Each point is the average of at least three separate determinations.

to CENUs.[2] M4Beu cell survival rate after treatment by each compound **1a**−**6a**, **1b**−**11b**, **1c**−**6c**, **12**, alone (300 μM), accounted for their intrinsic cytotoxicity. Their ability to enhance cystemustine cytotoxicity was expressed by ED_{50} values (defined in the Experimental Section), determined graphically from plots of the decrease in cell survival rate as a function of compound concentration administered. Figure 1 shows representative curves for nucleosides **2a** and **3b**.

Results and Discussion

Biological results are reported in Table 1. No significant decrease in cell survival rate was noted after treatment with the nucleosides alone except for O^6-(4-iodobenzyl)-2′-deoxyguanosine (**4c**), which tended to be slightly cytotoxic by itself.

Regarding the structure−activity relationship in the series of compounds synthesized, three factors play a major role: the nature of the O^6 substituent, the sugar moiety, and the degree of acetylation. The results shown in Table 1 show that among the series of N^2-acetylated compounds **1b**−**11b**, O^6-benzyl and O^6-4-halobenzyl derivatives **1b**−**6b** generally exhibited a significant capacity to enhance cystemustine cytotoxicity, whereas nucleosides substituted by other groups were not efficient. Moreover, para substitution by halogens on the benzyl group did not appreciably affect the activity. Consequently, the O^6-benzyl and O^6-4-halobenzyl series of compounds was extended to nucleosides **1a**−**6a** and **1c**−**6c** and further investigated.

In contrast, the nature of the sugar moiety greatly influences the ability to enhance cystemustine cytotoxicity. Whatever the O^6 substituent, 2′-deoxyguanosine analogs were markedly more potent than guanosine ones. This trend was marked for nonacetylated compounds (ED_{50} (**2c**, **4c**, **6c**) ≤ 6 μM) but was unexpectedly reversed for O^6-(4-iodobenzyl)-N^2-acetyl derivatives; the corresponding guanosine **3b** was considerably more effective than the 2′-deoxyguanosine analog **4b**.

Likewise, the effect of introducing an acetyl group, whether on the exocyclic amine of guanine or on both the exocyclic amine of the guanine and the hydroxyl groups of the sugar moiety, is also of considerable interest for the modulation of the cytotoxicity enhancement. In the 2′-deoxyguanosine series, acetylation markedly decreased the potency of the nucleosides compared with the nonacetylated congeners (e.g. comparison of **4a** and **4b** with **4c**). On the other hand, in the guanosine series, acetylation had little effect on the potency (e.g. comparison of **1a** and **1b** with **1c**). However, the results obtained with N^2-acetylated derivatives generally approximate to those of peracetylated analogs, whereas nonacetylated 2′-deoxyguanosines are more active. Accordingly, acetylation at the N^2 position specifically is mostly responsible for the loss of activity observed. This trend could be explained by a decrease in the affinity of the AGT active site toward N^2-acetylated nucleosides.

An additional test was performed as a reference in the same experimental conditions with O^6-benzylguanine (**12**). The ED_{50} value determined, 2 μM, showed that O^6-benzylguanine was effective for potentiation of cystemustine, in M4Beu cell line, at a lower dose than the corresponding guanosine and 2′-deoxyguanosine.

To proceed with pharmacological *in vivo* experiments by intravenous administration, closer to clinical conditions, the water solubility of the active compounds **1a**−**6a**, **1b**−**6b**, **1c**−**6c**, and **12** is essential. O^6-Benzyl-N^2-acetyl derivatives **1b** and **2b** were found to be the most water soluble (2 mM at 20 °C) followed by **2c** and **1c**. These nucleosides will be easier to inject intravenously compared with the others, among them O^6-benzylguanine (**12**), at least 10 times less soluble (≤0.2 mM). As expected, the presence of the sugar moiety in the O^6-benzyl series improves water solubility.

Preliminary experiments by computer-assisted molecular modeling provided further data on the possible incorporation of such modified nucleosides into DNA. It is well known that O^6-methylguanine incorporated into DNA is strongly promutagenic and gives rise primarily to guanine (G) to adenine (A) transition mutations by pairing with thymine in place of cytosine.[15,18−20] As a similar misincorporation might also be produced by O^6-benzylguanine derivatives, we evaluated the possibility of this undesirable effect. The pairing of O^6-methyl-, O^6-benzylguanines (O^6-methylG, O^6-benzylG) and O^6-benzyl-N^2-acetylguanine with cytosine (C) and thymine (T) was performed by the molecular modeling software SYBYL 6.03[21] as described in the Experimental Section. All the generated molecule geometries were minimized using the Tripos force field and then optimized using AM1[22] calculations. In the case of O^6-benzylguanine, the free rotation around the two bonds of the OCH_2Ph group generates a

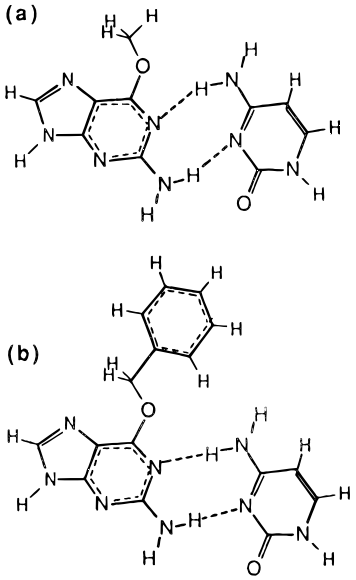


Figure 2. Most stable orientation of (a) O^6-methylG·C and (b) O^6-benzylG·C base pairs.

multiplicity of conformers, among which two minima with close energies were found. The phenyl group was found to be perpendicular to the base plane in one conformer and in the base plane in the other. The pairing between the bases was done using the docking facility of SYBYL. The concerned bases were brought close to each other until a minimum of energy was found. The aggregate so obtained was minimized by Tripos force field and then by AM1 calculations.

The hydrogen-bonding characteristics of the purine base guanine were complementary to those of cytosine with the three usual hydrogen bonds. The O^6-methylG·C pairing presented only two hydrogen bonds and the most stable geometry of the base pair showed a lateral displacement of one base with respect to the other. The same effect was observed for the pairing of O^6-benzylguanine with cytosine, thus creating diagonal hydrogen bonding (Figure 2). The Watson−Crick A·T base pair exhibited two hydrogen bonds. The pairing between O^6-methylguanine or O^6-benzylguanine and thymine also induced a lateral displacement of thymine and the creation of diagonal hydrogen bonds. As reported by Jorgensen,[23] secondary interactions between diagonally partial charges were found to be energetically worth about one-third of the primary hydrogen bonds. These interactions could be energetically favorable for isolated base pairs, but they would probably induce some perturbations of the double helix geometric features of DNA. In our case, this effect was observed to be greater with O^6-benzyl derivatives probably due to steric interactions. Finally, in the case of O^6-benzyl-N^2-acetylguanine, no energy minimum was found and the bases tended to repel each other, i.e. energy is needed to keep the bases together.

These qualitative observations clearly show that the steric bulk of the O^6-benzyl substituent decreases the stability of the association of O^6-benzylguanine either with cytosine or thymine with respect to O^6-methylguanine taken as a reference. The introduction of an N^2-acetyl group further destabilizes this association. Moreover, the lateral displacement observed would also modify the phosphodiester backbone of DNA. Finally, molecular modeling of a heptamer C-G-C-modified G-C-G-C clearly showed a marked perturbation of the geometry of the base pairs close to the modified base. Thus it can be expected that the probability of incorporation of substituted guanosine in DNA and hence the possibility of inducing an undesired mutation strongly decreases going from O^6-methylguanosine to O^6-benzylguanosine, then to O^6-benzyl-N^2-acetylguanosine, showing the importance of the N^2-acetyl group.

On the basis of these results, even if O^6-benzyl-N^2-acetylguanosine (**1b**) exhibits a lower activity than nonacetylated 2′-deoxyguanosine analogs, this nucleoside was selected first for *in vivo* investigations, for its high water solubility and the presence of an N^2-acetyl group that might prevent incorporation into DNA. The pharmacokinetic study showed that compound **1b** is stable in animal models.[24] No N^2-deacetylation was noted. Furthermore, pharmacological tests demonstrated that the combination of **1b**/cystemustine significantly enhances tumor growth inhibition in mice grafted with M4Beu resistant tumor.[25]

Conclusion

Among the series of nucleosides synthesized and evaluated for their ability to enhance cystemustine cytotoxicity, the presence of O^6-benzyl or O^6-4-halobenzyl substituents is essential for any significant activity; 2′-deoxyguanosine derivatives are significantly more potent than guanosine, and acetylation, whether on the exocyclic amine of guanine or on both exocyclic amine of guanine and hydroxyl groups of the sugar moiety, significantly diminishes the effectiveness. All of these results are consistent with the conclusions reported in a previous study on AGT depleting properties of related compounds.[9] Accordingly, O^6-benzyl/4-halobenzyl-2′-deoxyguanosines **2c**, **4c**, and **6c** proved to be the most potent molecules.

However, on the basis of the preliminary molecular modeling study, we expect acetylation at the N^2 position to prevent incorporation of these nucleosides into DNA. Consequently, considering the three criteria stated above (i.e. ability to enhance cystemustine activity, water solubility, and limited incorporation into DNA), nucleosides **1b**, **2a**, **2b**, and **3b** were selected for preclinical studies. Preliminary *in vivo* pharmacological tests demonstrated the ability of **1b** associated with cystemustine to inhibit resistant tumor growth.[25] Biological experiments currently in progress will further investigate these compounds in several pharmacological models and test the potential preventive role played by N^2-acetylation against incorporation of these nucleosides into DNA.

Furthermore, hydroxyl groups of the sugar moiety would provide a point of attachment by which chemical groups showing high affinity toward cancer cells could be appended, to vehicle the whole molecule selectively to the tumor. The synthesis of such derivatives is to be our main concern in future work.

Experimental Section

Chemistry. General Comments. Proton nuclear magnetic resonance (NMR) spectra were performed on a Brücker AM 200 (4.5T) spectrometer. Chemical shifts (δ) are reported in parts per million relative to the internal tetramethylsilane standard. Mass spectra (MS) were obtained on a Hewlett-Packard 5989 A instrument operating in the electron impact mode (EI) unless otherwise stated. Infrared (IR) spectra were

recorded on a Perkin-Elmer 398 spectrometer. Elemental analyses for carbon, hydrogen, nitrogen obtained from CNRS Service Central d'Analyse at Vernaison (France) were within ±0.4% of theory for the formulas given unless otherwise indicated. Melting points (mp), uncorrected, were determined on an Electrothermal digital apparatus. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Water solubility was evaluated at 20 °C according to Vogel's method.[26] Values are stated only for compounds that were completely dissolved at a concentration above 0.2 mM. Analytical thin layer chromatography (TLC) was conducted on precoated silica gel plates (Merck 60 F-254, 0.2 mm thick), with both detection by ultra violet light at 254 nm and visualization by iodine, using the following eluents: A, dichloromethane/ethanol (98/2); B, dichloromethane/ethanol (90/10); C, dichloromethane/ethanol (80/20); D, dichloromethane/ethanol (95/5). Silica gel 60 (Chromagel, 230−400 mesh, SDS) was used for medium-pressure chromatography using the indicated solvent mixture expressed as volume/volume ratios.

Synthesis of Useful Starting Materials and Standard Compounds. Cystemustine was synthesized according to a procedure described by Madelmont *et al.*[27] O^6-Benzylguanine was prepared by the common literature method.[28] 4-Iodobenzyl alcohol, needed for the preparation of O^6-(4-iodobenzyl)-guanosine derivatives, was obtained by reduction of 4-iodobenzoyl chloride (15 g, 56 mmol) in the presence of sodium borohydride (6.3 g, 166 mmol) in 1,4-dioxane (90 mL) and stirred for 10 min at 100 °C and an additional 1 h at 30 °C. The reaction was quenched by adding water. Volatiles were removed under reduced pressure, and the residue was extracted with dichloromethane. Workup of organic layer gave a crude product, purified by chromatography on silica gel, eluting with dichloromethane and dichloromethane/ethanol (98/2) to yield the alcohol (8.3 g, 67%) as a white solid: R_f 0.52 (D); mp 66−68 °C; ^{1}H-NMR ($CDCl_3$) δ 1.77 (br s, 1H, OH, exchanges with D_2O), 4.64 (s, 2H, CH_2), 7.09−7.70 (dd, 4H, C_6H_4I).

N^2,2′,3′,5′-Tetracetylguanosine and N^2,3′,5′-triacetyl-2′-deoxyguanosine were prepared using a procedure previously reported.[14] The crude product was chromatographed on silica gel with dichloromethane and dichloromethane/ethanol (95/5, 90/10) as eluent.

All the other chemicals were from commercial suppliers and used as received unless otherwise mentioned.

General Procedure for O^6-Alkylation/Aralkylation of Guanosine and 2′-Deoxyguanosine. Preparation of 1a−11a. To a stirred solution of N^2,2′,3′,5′-tetracetylguanosine (5.4 g, 12 mmol) or N^2,3′,5′-triacetyl-2′-deoxyguanosine (4.7 g, 12 mmol), as required, in dichloromethane (90 mL) was added triethylamine (6.6 mL, 48 mmol), 2-mesitylenesulfonyl chloride (5.23 g, 24 mmol), and 4-(dimethylamino)pyridine (61 mg, 0.5 mmol) at ambient temperature. The reaction, followed by TLC (R_f 0.6 (D)), was complete 30 min later. *N*-Methylpyrrolidine (12 mL, 115 mmol) was added to the cold solution, at 0 °C. The reaction mixture was stirred for an additional 2 h at 0 °C until the total conversion was accomplished (R_f 0 (D)). The appropriate methyl, benzyl, (2- or 4-halobenzyl), methyl-4-pyridyl, or diphenylmethyl alcohol (61 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (36 mmol) were respectively added at 0 °C. The mixture was then allowed to warm to room temperature, stirred for 3 h, and washed with a saturated KH_2PO_4 solution (pH = 6.5). The organic extract was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, eluting with ether/hexanes (80/20) to remove excess alcohol and ethyl acetate/hexanes (90/10) to afford a yellowish foam.

***N*²,2′,3′,5′-Tetracetyl-*O*⁶-benzylguanosine (1a):** yield 38%; R_f 0.74 (A); mp 58−60 °C; $[\alpha]^{25}_D$ −14.4 (c = 3.8, DMSO); ^{1}H-NMR (DMSO-d_6) δ 1.99, 2.03, 2.11 (3s, 9H, $OCOCH_3$), 2.22 (s, 3H, $NCOCH_3$), 4.30−4.44 (m, 3H, 4′-H, 5′-H), 5.61 (s, 2H, $CH_2C_6H_5$), 5.72−5.77 (m, 1H, 3′-H), 5.87−5.93 (m, 1H, 2′-H), 6.20 (d, J = 4.8 Hz, 1H, 1′-H), 7.37−7.57 (m, 5H, C_6H_5), 8.40 (s, 1H, 8-H), 10.54 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1740 (C=O ester), 1600 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 541 (M), 283 (B + H), 241 (B + 2H − $COCH_3$), 91 ($CH_2C_6H_5$), B = N^2-acetyl-O^6-benzylguanine − H. Anal. ($C_{25}H_{27}N_5O_9\cdot0.5H_2O$) C, H, N.

***N*²,3′,5′-Triacetyl-*O*⁶-benzyl-2′-deoxyguanosine (2a):** yield 54%; R_f 0.44 (B); mp 51−53 °C; $[\alpha]^{25}_D$ −1.6 (c = 1.2, DMSO); $_1$H-NMR (DMSO-d_6) δ 1 .99, 2.09 (2s, 6H, $OCOCH_3$), 2.24 (s, 3H, $NCOCH_3$), 2.50−2.59, 3.19−3.30 (2m, 2H, 2′-H), 4.22−4.40 (m, 3H, 4′-H, 5′-H), 5.44 (m, 1H, 3′-H), 5.61 (s, 2H, $CH_2C_6H_5$), 6.37 (t, J = 7.6, 6.4 Hz, 1H, 1′-H), 7.36−7.57 (m, 5H, C_6H_5), 8.38 (s, 1H, 8-H), 10.44 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1760 (C=O ester), 1600 (C=O amide), 1240 cm^{-1} (=CO); MS m/z 483 (M), 283 (B + H), 241 (B + 2H − $COCH_3$), 91 ($CH_2C_6H_5$), B = N^2-acetyl-O^6-benzylguanine − H. Anal. ($C_{23}H_{25}N_5O_7\cdot0.5H_2O$) C, H, N.

***N*²,2′,3′,5′-Tetracetyl-*O*⁶-(4-iodobenzyl)guanosine (3a):** yield 40%; R_f 0.58 (A); mp 80−82 °C; $[\alpha]^{25}_D$ −14.7 (c = 1.0, DMSO); ^{1}H-NMR (DMSO-d_6) δ 2.02, 2.04, 2.12 (3s, 9H, $OCOCH_3$), 2.21 (s, 3H, $NCOCH_3$), 4.29−4.41 (m, 3H, 4′-H, 5′-H), 5.57 (s, 2H, $CH_2C_6H_4I$), 5.73−5.77 (m, 1H, 3′-H), 5.88−5.93 (m, 1H, 2′-H), 6.20 (d, J = 4.8 Hz, 1H, 1′-H), 7.34−7.79 (dd, 4H, C_6H_4I), 8.41 (s, 1H, 8-H), 10.55 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1735 (C=O ester), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 667 (M), 409 (B + H), 283 (B + 2H − I), 241 (B + 3H − $COCH_3$ − I), 217 ($CH_2C_6H_4I$), B = N^2-acetyl-O^6-(4-iodobenzyl)guanine − H. Anal. ($C_{25}H_{26}N_5O_9I$) C, H, N.

***N*²,3′,5′-Triacetyl-*O*⁶-(4-iodobenzyl)-2′-deoxyguanosine (4a):** yield 60%; R_f 0.51 (A); mp 59−61 °C; $[\alpha]^{25}_D$ −1.9 (c = 2.1, DMSO); ^{1}H-NMR (DMSO-d_6) δ 2.00, 2.09 (2s, 6H, $OCOCH_3$), 2.21 (s, 3H, $NCOCH_3$), 2.50−2.59, 3.15−3.29 (2m, 2H, 2′-H), 4.21−4.32 (m, 3H, 4′-H, 5′-H), 5.43 (m, 1H, 3′-H), 5.57 (s, 2H, $CH_2C_6H_5$), 6.36 (t, J = 7.2, 6.4 Hz, 1H, 1′-H), 7.34−7.78 (m, 5H, C_6H_5), 8.41 (s, 1H, 8-H), 10.47 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1730 (C=O ester), 1590 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 609 (M), 409 (B + H), 367 (B + 2H − $COCH_3$), 283 (B + 2H − I), 241 (B + 2H − $COCH_3$ − I), 217 ($CH_2C_6H_4I$), B = N^2-acetyl-O^6-(4-iodobenzy)-lguanine − H. Anal. ($C_{23}H_{24}N_5O_7I\cdot0.25H_2O$) C, H, N.

***N*²,2′,3′,5′-Tetracetyl-*O*⁶-(4-bromobenzyl)guanosine (5a):** yield 36%; R_f 0.55 (A); mp 85−87 °C; $[\alpha]^{25}_D$ −15.9 (c = 1.9, DMSO); ^{1}H-NMR (DMSO-d_6) δ 1.99, 2.03, 2.11 (3s, 9H, $OCOCH_3$), 2.22 (s, 3H, $NCOCH_3$), 4.30−4.39 (m, 3H, 4′-H, 5′-H), 5.61 (s, 2H, $CH_2C_6H_4Br$), 5.73−5.78 (m, 1H, 3′-H), 5.90−5.93 (m, 1H, 2′-H), 6.20 (d, J = 4.8 Hz, 1H, 1′-H), 7.37−7.57 (m, 5H, C_6H_4Br), 8.40 (s, 1H, 8-H), 10.54 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1740 (C=O ester), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 621, 619 (M), 363, 361 (B + H), 321, 319 (B +2H − $COCH_3$), 283 (B + 2H − Br), 241 (B + 3H − $COCH_3$ − Br), 171, 169 ($CH_2C_6H_4Br$), B = N^2-acetyl-O^6-(4-bromobenzyl)guanine − H. Anal. ($C_{25}H_{26}N_5O_9Br$) C, H, N.

***N*²,3′,5′-Triacetyl-*O*⁶-(4-bromobenzyl)-2′-deoxyguanosine (6a):** yield 57%; R_f 0.45 (A); mp 93−95 °C; $[\alpha]^{25}_D$ −1.4 (c = 2.0, DMSO); ^{1}H-NMR (DMSO-d_6) δ 1.99, 2.09 (2s, 6H, $OCOCH_3$), 2.22 (s, 3H, $NCOCH_3$), 2.50, 3.21 (2m, 2H, 2′-H), 4.25−4.32 (m, 3H, 4′-H, 5′-H), 5.41−5.45 (m, 1H, 3′-H), 5.59 (s, 2H, $CH_2C_6H_4Br$), 6.36 (t, J = 7.4, 6.2 Hz, 1H, 1′-H), 7.49−7.61 (m, 5H, C_6H_4Br), 8.40 (s, 1H, 8-H), 10.47 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν (C=O ester), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 563, 561 (M), 321, 319 (B + 2 H − $COCH_3$), 363, 361 (B + H), 283 (B + 2H − Br), 240 (B + 2H − $COCH_3$ − Br), 171, 169 ($CH_2C_6H_4Br$), B = N^2-acetyl-O^6-(4-bromobenzyl)guanine − H. Anal. ($C_{23}H_{24}N_5O_7Br\cdot0.25H_2O$) C, H, N.

***N*²,2′,3′,5′-Tetracetyl-*O*⁶-(2-iodobenzyl)guanosine (7a):** yield 51%; R_f 0.42 (A); mp 69−71 °C; ^{1}H-NMR (DMSO-d_6) δ 2.01, 2.06, 2.12 (3s, 9H, $OCOCH_3$), 2.24 (s, 3H, $NCOCH_3$), 4.31−4.47 (m, 3H, 4′-H, 5′-H), 5.59 (s, 2H, $CH_2C_6H_4I$), 5.78−5.83 (m, 1H, 3′-H), 5.90−5.96 (m, 1H, 2′-H), 6.22 (d, 1H, 1′-H), 7.14 (t, 1H, $H_{meta}C_6H_4I$), 7.44 (t, 1H, $H_{para}C_6H_4I$), 7.63 (d, J = 4.8 Hz, 1H, $H_{meta}C_6H_4I$), 7.93 (d, 1H, $H_{ortho}C_6H_4I$), 8.40 (s, 1H, 8-H), 10.54 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1740 (C=O ester), 1590 (C=O amide), 1220 cm^{-1} (=CO).

***N*²,2′,3′,5′-Tetracetyl-*O*⁶-methylguanosine (8a):** yield 45%; R_f 0.33 (A); mp 68−71 °C; ^{1}H-NMR (DMSO-d_6) δ 1.99, 2.04, 2.11 (3s, 9H, $OCOCH_3$), 2.24 (s, 3H, $NCOCH_3$), 4.08 (s, 3H, OCH_3), 4.35−4.45 (m, 3H, 4′-H, 5′-H), 5.78−5.80 (m, 1H, 3′-H), 5.90−5.92 (m, 1H, 2′-H), 6.18 (t, J = 4.7 Hz, 1H, 1′-H),

8.36 (s, 1H, 8-H), 10.43 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1740 (C=O ester), 1590 (C=O amide), 1230 cm^{-1} (=CO).

***N*²,3′,5′-Triacetyl-*O*⁶-methyl-2′-deoxyguanosine (9a):** yield 31%; R_f 0.23 (A); mp 46−48 °C; ^{1}H-NMR (DMSO-d_6) δ 2.01, 2.10 (2s, 6H, $OCOCH_3$), 2.26 (s, 3H, $NCOCH_3$), 2.47−2.58, 3.21−3.35 (2m, 2H, 2′-H), 4.09 (s, 3H, OCH_3), 4.24−4.38 (m, 3H, 4′-H, 5′-H), 5.45−5.47 (m, 1H, 3′-H), 6.38 (t, J = 7.3, 6.5 Hz, 1H, H_1'), 8.38 (s, 1H, 8-H), 10.39 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1740 (C=O ester), 1590 (C=O amide), 1230 cm^{-1} (=CO).

***N*²,2′,3′,5′-Tetracetyl-*O*⁶-(methyl-4-pyridyl)guanosine (10a):** yield 25%; R_f 0.36 (B); mp 68−70 °C; ^{1}H-NMR (DMSO-d_6) δ 2.00, 2.05, 2.12 (3s, 9H, $OCOCH_3$), 2.19 (s, 3H, $NCOCH_3$), 4.30−4.46 (m, 3H, 4′-H, 5′-H), 5.68 (s, 2H, $CH_2C_5H_4N$), 5.74−5.79 (m, 1H, 3′-H), 5.88−5.94 (m, 1H, 2′-H), 6.21 (d, J = 4.9 Hz, 1H, 1′-H), 7.51, 8.60 (2d, 4H, C_5H_4N), 8.44 (s, 1H, 8-H), 10.53 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1735 (C=O ester), 1670 (C=O amide), 1230 cm^{-1} (=CO).

***N*²,2′,3′,5′-Tetracetyl-*O*⁶-(diphenylmethyl)guanosine (11a):** yield 15%; R_f 0.35 (D); mp 74−76 °C; ^{1}H-NMR (DMSO-d_6) δ 1.98, 2.02, 2.17 (3s, 9H, $OCOCH_3$), 2.24 (s, 3H, $NCOCH_3$), 4.27−4.44 (m, 3H, 4′-H, 5′-H), 5.74−5.76 (m, 1H, 3′-H), 5.88−5.94 (m, 1H, 2′-H), 6.20 (d, J = 4.9 Hz, 1H, 1′-H), 7.30−7.53 (m, 11H, $CH(C_6H_5)_2$), 8.45 (s, 1H, 8-H), 10.47 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 1730 (C=O ester), 1670 (C=O amide), 1230 cm^{-1} (=CO).

General Procedure for the Preparation of *N*²-Acetyl-*O*⁶-(Alkyl/Aralkyl)guanosine and 2′-Deoxyguanosine 1b−11b. A solution of the relevant compound **1a−11a** (2 mmol) in pyridine (9 mL) was treated with 2.5 M NaOH (3 mL, 7.5 mmol for guanosine derivatives and 2 mL, 5 mmol for 2′-deoxyguanosine derivatives) and stirred for 6 min. The mixture was neutralized by adding acidic cation exchanger (Dowex 50 × 8 resin, 8 mL) with vigorous stirring, filtered, and washed with a minimum amount of pyridine. The solution was evaporated under reduced pressure. The residue was chromatographed on silica gel, eluting with dichloromethane and dichloromethane/ethanol (98/2, 95/5, 90/10) to afford a white foam.

***N*²-Acetyl-*O*⁶-benzylguanosine (1b):** yield 68%; R_f 0.28 (B); mp 146−148 °C; $[\alpha]^{25}_D$ −16.7 (c = 2.3, DMSO); water solubility 2 mM; ^{1}H-NMR (DMSO-d_6) δ 2.22 (s, 3H, $NCOCH_3$), 3.56−3.62 (m, 2H, 5′-H), 3.89−3.94 (m, 1H, 4′-H), 4.12−4.20 (m, 1H, 3′-H), 4.52−4.61 (m, 1H, 2′-H), 4.99 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.19 (d, J = 4.8 Hz, 1H, 3′-OH, exchanges with D_2O), 5.48 (d, 1H, 2′-OH, exchanges with D_2O), 5.61 (s, 2H, $CH_2C_6H_5$), 5.89 (d, J = 5.8 Hz, 1H, 1′-H), 7.30−7.60 (m, 5H, C_6H_5), 8.45 (s, 1H, 8-H), 10.49 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 415 (M), 326 (B + 44), 283 (B + H), 241 (B + 2H − $COCH_3$), 91 ($CH_2C_6H_5$), B = *N*²-acetyl-*O*⁶-benzylguanine − H. Anal. ($C_{19}H_{21}N_5O_6\cdot H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-benzyl-2′-deoxyguanosine (2b):** yield 54%; R_f 0.31 (B); mp 184−186 °C; $[\alpha]^{25}_D$ −8.4 (c = 1.1, DMSO); water solubility: 2 mM; ^{1}H-NMR (DMSO-d_6) δ 2.23 (s, 3H, $NCOCH_3$), 2.28−2.32, 2.64−2.77 (2m, 2H, 2′-H), 3.51−3.63 (m, 2H, 5′-H), 3.83−3.87 (m, 1H, 4′-H), 4.41−4.44 (m, 1H, 3′-H), 4.85 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.26 (d, J = 3.8 Hz, 1H, 3′-OH, exchanges with D_2O), 5.60 (s, 2H, $CH_2C_6H_5$), 6.33 (t, J = 7.0, 6.5 Hz, 1H, 1′-H), 7.35−7.57 (m, 5H, C_6H_5), 8.43 (s, 1H, 8-H), 10.47 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 399 (M), 283 (B + H), 241 (B + 2H − $COCH_3$), 91 ($CH_2C_6H_5$), B = *N*²-acetyl-*O*⁶-benzylguanine − H. Anal. ($C_{19}H_{21}N_5O_5\cdot 0.5H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-(4-iodobenzyl)guanosine (3b):** yield 90%; R_f 0.28 (B); mp 146−148 °C; $[\alpha]^{25}_D$ −16.6 (c = 1.4, DMSO); $_1$H-NMR (DMSO-d_6) δ 2.24 (s, 3H, $NCOCH_3$), 3.53−3.64 (m, 2H, 5′-H), 3.90−3.94 (m, 1H, 4′-H), 4.13−4.19 (m, 1H, 3′-H), 4.50−4.59 (m, 1H, 2′-H), 4.96 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.17 (d, J = 4.6 Hz, 1H, 3′-OH, exchanges with D_2O), 5.46 (d, J = 5.8 Hz, 1H, 2′-OH, exchanges with D_2O), 5.56 (s, 2H, $CH_2C_6H_4I$), 5.88 (d, J = 5.8 Hz, 1H, 1′-H), 7.33−7.76 (dd, 4H, C_6H_4I), 8.44 (s, 1H, 8-H), 10.47 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 541 (M), 452 (B + 44), 409 (B + H), 367 (B + 2H − $COCH_3$), 241 (B + 3H − $COCH_3$ − I), 217 ($CH_2C_6H_4I$), B = *N*²-acetyl-*O*⁶-(4-iodobenzyl)guanine − H. Anal. ($C_{19}H_{20}N_5O_6I\cdot 0.5H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-(4-iodobenzyl)-2′-deoxyguanosine (4b):** yield 53%; R_f 0.35 (B); mp 145−147 °C; $[\alpha]^{25}_D$ −9.4 (c = 4.5, DMSO); ^{1}H-NMR (DMSO-d_6) δ 2.23−2.34 (m, 4H, $NCOCH_3$, 2′-H), 2.65−2.69 (m, 1H, 2′-H), 3.52−3.61 (m, 2H, 5′-H), 3.85−3.87 (m, 1H, 4′-H), 4.43−4.45 (m, 1H, 3′-H), 4.89 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.29 (d, J = 3.9 Hz 1H, 3′-OH, exchanges with D_2O), 5.56 (s, 2H, $CH_2C_6H_5$), 6.33 (t, J = 6.5, 6.8 Hz, 1H, 1′-H), 7.34−7.76 (m, 5H, C_6H_5), 8.42 (s, 1H,8-H), 10.42 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1660 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 525 (M), 452 (B + 44), 409 (B + H), 367 (B + 2H − $COCH_3$), 241 (B + 3H − $COCH_3$ − I), 217 ($CH_2C_6H_4I$), B = *N*²-acetyl-*O*⁶-(4-iodobenzyl)guanine − H. Anal. ($C_{19}H_{20}N_5O_5I\cdot H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-(4-bromobenzyl)guanosine (5b):** yield 72%; R_f 0.43 (B); mp 134−136 °C; $[\alpha]^{25}_D$ −15.8 (c = 1.5, DMSO); $_1$H-NMR (DMSO-d_6) δ 2.22 (s, 3H, $NCOCH_3$), 3.54−3.69 (m, 2H, 5′-H), 3.90−3.93 (m, 1H, 4′-H), 4.17−4.19 (m, 1H, 3′-H), 4.52−4.60 (m, 1H, 2′-H), 4.98 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.20 (d, J = 4.7 Hz, 1H, 3′-OH, exchanges with D_2O), 5.49 (d, J = 5.8 Hz, 1H, 2′-OH, exchanges with D_2O), 5.59 (s, 2H, $CH_2C_6H_4Br$), 5.89 (d, J = 5.8 Hz, 1H, 1′-H), 7.50−7.62 (m, 4H, C_6H_4I), 8.46 (s, 1H, 8-H), 10.50 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 495, 493 (M), 363, 361 (B + H), 321, 319 (B + 2H − $COCH_3$), 241 (B + 3H − $COCH_3$ − Br), 171, 169 ($CH_2C_6H_4Br$), B = *N*²-acetyl-*O*⁶-(4-bromobenzyl)guanine − H. Anal. ($C_{19}H_{20}N_5O_6Br\cdot H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-(4-bromobenzyl)-2′-deoxyguanosine (6b):** yield 45%; R_f 0.43 (B); mp 132−134 °C; $[\alpha]^{25}_D$ −8.3 (c = 1.3, DMSO); ^{1}H-NMR (DMSO-d_6) δ 2.24 (s, 3H, $NCOCH_3$), 2.26−2.28 (m, 1H, 2′-H), 2.67−2.73 (m, 1H, 2′-H), 3.52−3.61 (m, 2H, 5′-H), 3.86−3.88 (m, 1H, 4′-H), 4.43−4.45 (m, 1H, 3′-H), 4.92 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.31 (d, J = 3.9 Hz 1H, 3′-OH, exchanges with D_2O), 5.59 (s, 2H, $CH_2C_6H_5$), 6.35 (t, J = 6.4, 6.9 Hz, 1H, 1′-H), 7.50−7.61 (m, 5H, C_6H_5), 8.44 (s, 1H, 8-H), 10.45 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1660 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 479, 477 (M), 363, 361 (B + H), 321, 319 (B + 2H − $COCH_3$), 241 (B + 3H − $COCH_3$ − Br), 171, 169 ($CH_2C_6H_4Br$), B = *N*²-acetyl-*O*⁶-(4-bromobenzyl)guanine − H. Anal. ($C_{19}H_{20}N_5O_5Br\cdot 0.5H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-(2-iodobenzyl)guanosine (7b):** yield 95%; R_f 0.33 (B); mp 158−160 °C; $[\alpha]^{25}_D$ −14.1 (c = 1.4, DMSO); $_1$H-NMR (DMSO-d_6) δ 2.22 (s, 3H, $NCOCH_3$), 3.50−3.70 (m, 2H, 5′-H), 3.89−3.95 (m, 1H, 4′-H), 4.13−4.19 (m, 1H, 3′-H), 4.56−4.62 (m, 1H, 2′-H), 4.96 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.17 (d, J = 4.6 Hz, 1H, 3′-OH, exchanges with D_2O), 5.48 (d, J = 5.8 Hz, 1H, 2′-OH, exchanges with D_2O), 5.57 (s, 2H, $CH_2C_6H_4I$), 5.88 (d, J = 5.8 Hz, 1H, 1′-H), 7.14 (t, 1H, $H_{meta}C_6H_4I$), 7.44 (t, 1H, $H_{para}C_6H_4I$), 7.63 (d, 1H, $H_{meta}C_6H_4I$), 7.92 (d, 1H, $H_{ortho}C_6H_4I$), 8.46 (s, 1H, 8-H), 10.51 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 541 (M), 409 (B + H), 367 (B + 2H − $COCH_3$), 241 (B + 3H − $COCH_3$ − I), 217 ($CH_2C_6H_4I$), B = *N*²-acetyl-*O*⁶-(2-iodobenzyl)-guanine − H. Anal. ($C_{19}H_{20}N_5O_6I\cdot 1.5H_2O$) C, H; N: calcd, 12.32; found, 11.66.

***N*²-Acetyl-*O*⁶-methylguanosine (8b):** yield 87%; R_f 0.21 (B); mp 122−124 °C; $[\alpha]^{25}_D$ −0.6 (c = 1.4, DMSO); water solubility 5 mM; ^{1}H-NMR (DMSO-d_6) δ 2.24 (s, 3H, $NCOCH_3$), 3.51−3.65 (m, 2H, 5′-H), 3.91−3.96 (m, 1H, 4′-H), 4.08 (s, 3H, OCH_3), 4.15−4.21 (m, 1H, 3′-H), 4.55−4.61 (m, 1H, 2′-H), 4.95 (t, J = 5.4 Hz, 1H, 5′-OH exchanges with D_2O), 5.15 (d, J = 4.5 Hz, 1H, 3′-OH exchanges with D_2O), 5.46 (d, J = 5.9 Hz, 1H, 2′-OH exchanges with D_2O), 5.90 (d, J = 5.8 Hz, 1H, 1′-H), 8.45 (s, 1H, 8-H), 10.45 (s, 1H, NH exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 339 (M), 297 (M + H − $COCH_3$), 250 (B +

44), 207 (B + H), 165 (B + 2H − $COCH_3$), B = N^2-acetyl-O^6-methylguanine − H. Anal. ($C_{13}H_{17}N_5O_6 \cdot 1.5H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-methyl-2′-deoxyguanosine (9b):** yield 56%; R_f 0.23 (B); mp 197−199 °C; $[\alpha]^{25}_D$ −7.2 (c = 2.0, DMSO); water solubility 5 mM; ^{1}H NMR (DMSO-d_6) δ 2.23−2.30 (m, 4H, $NCOCH_3$, 2′-H), 2.63−2.77 (m, 1H, 2′-H), 3.45−3.61 (m, 2H, 5′-H), 3.81−3.87 (m, 1H, 4′-H), 4.06 (s, 3H, OCH_3), 4.40−4.43 (m, 1H, 3′-H), 4.86 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.26 (d, J = 4.0 Hz, 1H, 3′-OH, exchanges with D_2O), 6.32 (t, J = 6.5, 7.0 Hz, 1H, 1′-H), 8.42 (s, 1H, 8-H), 10.40 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 323 (M), 207 (B + H), 165 (B + 2H − $COCH_3$), B = N^2-acetyl-O^6-methylguanine − H. Anal. ($C_{13}H_{17}N_5O_5 \cdot H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-(methyl-4-pyridinyl)guanosine (10b):** yield 88%; R_f 0.28 (C); mp 140−142 °C; $[\alpha]^{25}_D$ −13.0 (c = 1.5, DMSO); $_1$H-NMR (DMSO-d_6) δ 2.18 (s, 3H, $NCOCH_3$), 3.52−3.63 (m, 2H, 5′-H), 3.92−3.94 (m, 1H, 4′-H), 4.17−4.19 (m, 1H, 3′-H), 4.57−4.60 (m, 1H, 2′-H), 4.98 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.21 (d, J = 4.7 Hz, 1H, 3′-OH, exchanges with D_2O), 5.51 (d, 1H, 2′-OH, exchanges with D_2O), 5.68 (s, 2H, $CH_2C_5H_4N$), 5.90 (d, J = 5.5 Hz, 1H, 1′-H), 7.50, 8.59 (2d, 4H, C_5H_4N), 8.50 (s, 1H, 8-H), 10.49 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS m/z 416 (M), 284 (B + H), 242 (B + 2H − $COCH_3$), 92 ($CH_2C_5H_4N$), B = N^2-acetyl-O^6-(methyl-4-pyridinyl)guanine − H. Anal. ($C_{18}H_{20}N_6O_6 \cdot 1.5H_2O$) C, H, N.

***N*²-Acetyl-*O*⁶-(diphenylmethyl)guanosine (11b):** yield 45%; R_f 0.48 (B); mp 148−150 °C; $[\alpha]^{25}_D$ −6.4 (c = 0.9, DMSO); $_1$H-NMR (DMSO-d_6) δ 2.17 (s, 3H, $NCOCH_3$), 3.56−3.61 (m, 2H, 5′-H), 3.91 (m, 1H, 4′-H), 4.15−4.19 (m, 1H, 3′-H), 4.55−5.58 (m, 1H, 2′-H), 4.97 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.17 (d, J = 4.5 Hz, 1H, 3′-OH, exchanges with D_2O), 5.47 (d, J = 5.5 Hz, 1H, 2′-OH, exchanges with D_2O), 5.87 (d, J = 5.8 Hz, 1H, 1′-H), 7.34−7.54 (m, 11H, $CH(C_6H_5)_2$), 8.49 (s, 1H, 8-H), 10.43 (s, 1H, NH, exchanges with D_2O); IR (KBr) ν 3500−3200 (OH), 1670 (C=O amide), 1230 cm^{-1} (=CO); MS (FAB) m/z 492 (M + H); MS (EI) m/z 359 (B + H), 317 (B + 2H − $COCH_3$), 167 ($CH(C_6H_5)_2$), B = N^2-acetyl-O^6-(diphenylmethyl)guanine − H. Anal. ($C_{25}H_{25}N_5O_6 \cdot 1.25H_2O$) C, H, N.

General Procedure for the Preparation of *O*⁶-(Alkyl/Aralkyl)guanosine and 2′-Deoxyguanosine 1c−6c. A solution of the relevant compound **1b**−**6b** (2 mmol) in pyridine (9 mL) was treated with 2.5 M NaOH (4.8 mL, 12 mmol for guanosine derivatives and 3.6 mL, 9 mmol for 2′-deoxyguanosine derivatives) and stirred for 2 h at 30 °C. Acidic cation exchanger (Dowex 50 × 8 resin, 13 mL) was added with vigorous stirring, filtered, and washed with a minimum amount of pyridine. The solution was evaporated under reduced pressure. The residue was chromatographed on silica gel, eluting with dichloromethane and dichloromethane/ethanol (98/2, 95/5, 90/10) to give a white solid.

***O*⁶-Benzylguanosine (1c):** yield 70%; R_f 0.21 (B); mp 159−161 °C; $[\alpha]^{25}_D$ −25.1 (c = 1.7, DMSO); water solubility 0.5 mM; $_1$H-NMR (DMSO-d_6) δ 3.48−3.61 (m, 2H, 5′-H), 3.88−3.92 (m, 1H, 4′-H), 4.11−4.13 (m, 1H, 3′-H), 4.44-4.48 (m, 1H, 2′-H), 5.06−5.15 (m, 2H, 5′-OH, 3′-OH, exchange with D_2O), 5.40 (d, J = 6.0 Hz, 1H, 2′-OH, exchanges with D_2O), 5.49 (s, 2H, $CH_2C_6H_5$), 5.79 (d, J = 6.0 Hz, 1H, 1′-H), 6.50 (s, 2H, NH_2, exchange with D_2O), 7.33−7.51 (m, 5H, C_6H_5), 8.11 (s, 1H, 8-H); IR (KBr) ν 3440, 3320 (OH, NH_2), 1230 cm^{-1} (=CO); MS m/z 373 (M), 241 (B + H), 91 ($CH_2C_6H_5$), B = O^6-benzylguanine − H. Anal. ($C_{17}H_{19}N_5O_5$) C, H, N.

***O*⁶-(4-Benzyl)-2′-deoxyguanosine (2c):** yield 84%; R_f 0.63 (B); mp 144−146 °C; $[\alpha]^{25}_D$ −14.5 (c = 1.1, DMSO); water solubility 0.5 mM; ^{1}H-NMR (DMSO-d_6) δ 2.16−2.24 (m, 1H, 2′-H), 2.54−2.64 (m, 1H, 2′-H), 3.54−3.56 (m, 2H, 5′-H), 3.82−3.84 (m, 1H, 4′-H), 4.35−4.37 (m, 1H, 3′-H), 5.01 (t, J = 5.4 Hz, 1H, 5′-OH, exchanges with D_2O), 5.26 (d, J = 3.7 Hz, 1H, 3′-OH, exchanges with D_2O), 5.50 (s, 2H, $CH_2C_6H_4I$), 6.23 (t, J = 7.6, 6.1 Hz, 1H, 1′-H), 6.46 (s, 2H, NH_2, exchange with D_2O), 7.36−7.52 (dd, 4H, C_6H_4I), 8.08 (s, 1H, 8-H); IR (KBr) ν 3440, 3320 (OH, NH_2), 1250 cm^{-1} (=CO); MS m/z 357 (M), 241 (B + H), 91 ($CH_2C_6H_5$), B = O^6-benzylguanine − H. Anal. ($C_{17}H_{19}N_5O_4 \cdot 0.75H_2O$) C, H, N.

***O*⁶-(4-Iodobenzyl)guanosine (3c):** yield 86%; R_f 0.30 (B); mp 207−209 °C; $[\alpha]^{25}_D$ −22.5 (c = 0.8, DMSO); ^{1}H-NMR (DMSO-d_6) δ 3.56−3.61 (m, 2H, 5′-H), 3.87−3.90 (m, 1H, 4′-H), 4.09−4.11 (m, 1H, 3′-H), 4.42-4.47 (m, 1H, 2′-H), 5.12−5.14 (m, 2H, 3′-OH, 5′-OH, exchange with D_2O), 5.42−5.44 (m, 3H, 2′-OH, exchange with D_2O, $CH_2C_6H_4I$), 5.78 (d, J = 5.8 Hz, 1H, 1′-H), 6.50 (s, 2H, NH_2, exchange with D_2O), 7.28−7.77 (dd, 4H, C_6H_4I), 8.11 (s, 1H, 8-H); IR (KBr) ν 3440, 3320 (OH, NH_2), 1230 cm^{-1} (=CO); MS m/z 499 (M), 367 (B + H), 241 (B + 2H − I), 217 ($CH_2C_6H_4I$), B = O^6-(4-iodobenzyl)-guanine − H. Anal. ($C_{17}H_{18}N_5O_5I$) C, H, N.

***O*⁶-(4-Iodobenzyl)-2′-deoxyguanosine (4c):** yield 90%; R_f 0.52 (B); mp 127−129 °C; $[\alpha]^{25}_D$ −16.6 (c = 1.5, DMSO); ^{1}H-NMR (DMSO-d_6) δ 2.18−2.24, 2.52−2.62 (m, 2H, 2′-H), 3.50−3.55 (m, 2H, 5′-H), 3.80−3.82 (m, 1H, 4′-H), 4.34−4.36 (m, 1H, 3′-H), 4.99 (t, J = 5.3 Hz, 1H, 5′-OH, exchanges with D_2O), 5.27 (d, J = 3.6 Hz, 1H, 3′-OH, exchanges with D_2O), 5.43 (s, 2H, $CH_2C_6H_4I$), 6.19 (t, J = 6.6, 6.9 Hz, 1H, 1′-H), 6.49 (s, 2H, NH_2, exchange with D_2O), 7.27−7.76 (dd, 4H, C_6H_4I), 8.08 (s, 1H, 8-H); IR (KBr) ν 3440, 3320 (OH, NH_2), 1230 cm^{-1} (=CO); MS m/z 483 (M), 367 (B + H), 241 (B + 2H − I), 217 ($CH_2C_6H_4I$), B = O^6-(4-iodobenzyl)guanine − H. Anal. ($C_{17}H_{18}N_5O_4I \cdot 0.25H_2O$) C, H, N.

***O*⁶-(4-Bromobenzyl)guanosine (5c):** yield 73%; R_f 0.48 (B); mp 212−214 °C; $[\alpha]^{25}_D$ −21.4 (c = 1.6, DMSO); ^{1}H-NMR (DMSO-d_6) δ 3.55−3.60 (m, 2H, 5′-H), 3.87−3.89 (m, 1H, 4′-H), 4.09−4.11 (m, 1H, 3′-H), 4.44−4.46 (m, 1H, 2′-H), 5.12−5.14 (m, 2H, 3′-OH, 5′-OH, exchange with D_2O), 5.43−5.45 (m, 2H, 2′-OH, exchange with D_2O, $CH_2C_6H_5Br$), 5.77 (d, J = 5.9 Hz, 1H, 1′-H), 6.52 (s, 2H, NH_2, exchange with D_2O), 7.43−7.61 (dd, 4H, C_6H_5I), 8.12 (s, 1H, 8-H); IR (KBr) ν 3440, 3310 (OH, NH_2), 1250 cm^{-1} (=CO); MS (FAB) m/z 438, 436 (M + H); MS (EI) m/z 321, 319 (B + H), 241 (B + 2H − Br), 171, 169 ($CH_2C_6H_4Br$), B = O^6-(4-bromobenzyl)guanine − H. Anal. ($C_{17}H_{18}N_5O_5Br$) C, H, N.

***O*⁶-(4-Bromobenzyl)-2′-deoxyguanosine (6c):** yield 93%; R_f 0.31 (B); mp >250 °C; $[\alpha]^{25}_D$ −16.8 (c = 1.3, DMSO); ^{1}H-NMR (DMSO-d_6) δ 2.20−2.27, 2.50−2.66 (m, 2H, 2′-H), 3.45−3.62 (m, 2H, 5-H), 3.82−3.84 (m, 1H, 4′-H), 4.34−4.36 (m, 1H, 3′-H), 4.99 (t, J = 5.3 Hz, 1H, 5′-OH, exchanges with D_2O), 5.27 (d, J = 3.6 Hz, 1H, 3′-OH, exchanges with D_2O), 5.47 (s, 2H, $CH_2C_6H_4I$), 6.22 (t, J = 6.5, 7.0 Hz, 1H, 1′-H), 6.53 (s, 2H, NH_2, exchange with D_2O), 7.43−7.61 (dd, 4H, C_6H_4I), 8.13 (s, 1H, 8-H); IR (KBr) ν 3440, 3320 (OH, NH_2), 1230 cm^{-1} (=CO); MS (FAB) m/z 438, 436 (M + H); MS (EI) m/z 321, 319 (B + H), 241 (B + 2H − Br), 171, 169 ($CH_2C_6H_4Br$), B = O^6-(4-bromobenzyl)guanine − H. Anal. ($C_{17}H_{18}N_5O_4Br \cdot 0.5H_2O$) C, H, N.

Biological Studies. *In Vitro* Cytotoxicity Assay. For colony-forming assay, M4 Beu cells were plated into plastic dishes (200 cells/dish) and allowed to adhere for 20 h before treatment. The cells were treated by culture medium containing 0−400 μM guanosine, 2′-deoxyguanosine, or guanine derivatives, previously dissolved at 100 mM in dimethyl sulfoxide, and incubated at 37 °C for 4 h. Medium was then replaced by medium containing 50 μM cystemustine directly dissolved. Further incubation was performed at 37 °C for 2 h. The latter step was omitted for intrinsic cytotoxicity determination at 300 μM. After drug exposure, treated medium was replaced by fresh medium, and the cells were grown for 14 days. The dishes were rinsed with phosphate-buffered saline, fixed with methanol, and stained with 0.2% crystal violet. Colonies that contained more than 50 cells were counted, and cell survival rates (percent cell survival relative to untreated control) were calculated. Plating efficiency of untreated cells was 75.0 ± 11.0%. Each experiment at a fixed dose was carried out at least three times in triplicate. The average of the decrease in cell survival (100 × ((cell survival rate with cystemustine alone − cell survival rate with cystemustine associated with each compound (**1a**−**6a**, **1b**−**11b**, **1c**−**6c**, **12**))/cell survival rate with cystemustine alone)) was graphed for each concentration, and the data obtained were used to calculate the ED_{50} values, effective dose of drug, associated with cystemustine, required to reduce by 50% cell

survival rate determined for a treatment with cystemustine alone (50 μM). Without nucleoside pretreatment, the survival rate of M4 Beu cells to 50 μM cystemustine for 2 h was 87.7 ± 7.7%.

Molecular Modeling Studies. The topographical and electrostatic characterization of the studied molecules was performed using the SYBYL 6.03 software package on a Silicon Graphics Personal IRIS 4D35TG workstation. Structures were built within SYBYL and minimized by MAXIMIN2 with the Tripos force field, *in vacuo* conditions, to provide reasonable standard geometries. Molecules were deemed to be minimized when the minimum energy change of less than 0.021 kJ mol^{-1} for one iteration was obtained. The conjugate gradient method was used for minimization. All AM1 calculations involved the singlet state. Molecules were deemed to be minimized when the gradient fell to less than 0.021 kJ mol^{-1}. The conformational spaces of O^6-substituted guanines and N^2-acetyl-O^6-substituted guanines were explored using the SYBYL search facility. Torsion angles were defined and a grid search was performed allowing chosen bonds to rotate with a 180° revolution by 15° increments. The lowest energy conformers thus obtained were submitted to AM1 calculations (MOPAC version 5.0) to optimize their geometry. The pairing between the bases was done by using the docking facility of SYBYL. The concerned bases were brought close to each other until a minimum of energy was found. The aggregate so obtained was minimized by Tripos force field, then by using AM1 calculations.

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References

(1) Lemoine, A.; Lucas, C.; Ings, R. M. J. Metabolism of the chloroethylnitrosoureas. *Xenobiotica* **1991**, *21*, 775−791.
(2) Godeneche, D.; Rapp, M.; Thierry, A.; Laval, F.; Madelmont, J. C.; Chollet, P.; Veyre, A. DNA Damage induced by a new 2-chloroethylnitrosourea on malignant melanoma cells. *Cancer Res.* **1990**, *50*, 5898−5903.
(3) D'Incalci, M.; Citti, L.; Taverna, P.; Catapano, C. V. Importance of the DNA repair enzyme O^6-alkyl guanine-DNA-alkyltransferase in cancer chemotherapy. *Cancer Treat. Rev.* **1988**, *15*, 279−292.
(4) For a review, see: Pegg, A. E.; Dolan, M. E.; Moschel, R. C. Structure, function and inhibition of O^6-alkylguanine-DNA-alkyltransferase. *Prog. Nucleic Acid Res. Mol. Biol.* **1995**, *51*, 167−223.
(5) Pieper, R. O.; Futscher, B. W. Dong, Q.; Erickson, L. C. Effects of streptozotocin/bis-chloroethylnitrosourea combination therapy on O^6-methylguanine-DNA-methyltransferase activity and mRNA levels in HT-29 cells *in vitro*. *Cancer Res.* **1991**, *51*, 1581−1585.
(6) Zlotogorski, C.; Erickson, L. C. Pretreatment of human colon tumor with DNA methylating agents inhibits their ability to repair chloroethyl monoadducts. *Carcinogenesis* **1984**, *5* (1), 83−87.
(7) Panella, T. J.; Smith, D. C.; Schold, S. C.; Rogers, M. P.; Winer, E. P.; Fine, R.L.; Crawford, J.; Herdon, J. E.; Trump, D. L. Modulation of O^6-alkyltransferase-mediated carmustine resistance using streptozotocin: A phase I trial. *Cancer Res.* **1992**, *52*, 2456−2459.
(8) Gerard, B.; Aamdal, S.; Lee, S. M.; Leyvraz, S.; Bizzari, J. P. Activity and unexpected lung toxicity of the sequential administration of two alkylating agents -Dacarbazine and Fotemustine- in patients with melanoma. *Eur. J. Cancer* **1993**, *29*, 711−719.
(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) Dolan, M. E.; Mitchell, R. B.; Mummert, C.; Moschel, R. C.; Pegg, A. E. Effect of O^6-benzylguanine analogues on sensitivity of human tumor cells to the cytotoxic effects of alkylating agents. *Cancer Res.* **1991**, *51*, 3367−3372.
(11) Gerson, S. L.; Zborowska, E.; Norton, K.; Gordon, N. H.; Wilson J. K. V. Synergistic efficacy of O^6-benzylguanine and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) in a human colon cancer xenograft completely resistant to BCNU alone. *Biochem. Pharmacol.* **1993**, *45*, 483−491.
(12) Gerster, J. F.; Robins, R. K. Purine nucleosides. X. The synthesis of certain naturally occurring 2-substituted amino-9-β-D-ribofuranosylpurin-6(1H)-ones (N^2-substituted guanosines). *J. Am. Chem. Soc.* **1965**, *87*, 3752−3759.
(13) Pauly, G. T.; Powers, M.; Pei, G. K.; Moschel, R. C. Synthesis and properties of H-ras DNA sequences containing O^6-substituted 2′-deoxyguanosine residues at the first, second, or both positions of codon 12. *Chem. Res. Toxicol.* **1988**, *1*, 391−397.
(14) Gaffney, B. L.; Marky, L. A.; Jones, R. A. Synthesis and characterization of a set of four dodecadeoxyribonucleoside undecaphosphates containing O^6-methylguanine opposite adenine, cytosine, guanine, and thymine. *Biochemistry* **1984**, *23*, 5686−5691.
(15) Li, B. F. L.; Swan, P. F. Synthesis and characterization of oligodeoxynucleotides containing O^6-methyl-, O^6-ethyl-, and O^6-isopropylguanine. *Biochemistry* **1989**, *28*, 5779−5786.
(16) Madelmont, J. C. Cystemustine. *Drugs Future* **1994**, *19* (1), 27−30.
(17) Godeneche, D.; Labarre, P.; Cussac, C.; Madelmont, J. C.; Dupuy, J. M.; Fontanon, C.; Tisserrant, A.; Chollet, P.; Baudry, J. P.; Veyre, A. Pharmacokinetics of two new 2-chloroethylnitrosoureas in cancer patients submitted to phase II clinical trials. *Drug Invest.* **1994**, *7*, 234−243.
(18) Loveless, A. Possible relevance of O^6-alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitosamides. *Nature* **1969**, *223*, 206−207.
(19) Singer, B.; Essigmann, J. M. Site-specific mutagenesis: retrospective and prospective. *Carcinogenesis* **1991**, *12*, 949−955.
(20) Brown, T. Mismatches and mutagenic lesions in nucleic acids. *Aldrichimica Acta* **1995**, *28*, 15−20.
(21) Tripos Associates, Inc., 1699 S. Harley Rd, Suite 303, St. Louis, MO 63144.
(22) Merz, K. M.; Besler, B. H. MOPAC version 5.0 ESC, QCPE no. 589, 1990, with AM1 force field parameters.
(23) Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. OPLS potential functions for nucleotide bases. Relative association constants of hydrogen-bonded base pairs in chloroform. *J. Am. Chem. Soc.* **1991**, *113*, 2810−2819.
(24) Cussac, C.; Mounetou, E.; Rapp, M.; Madelmont, J. C.; Maurizis, J. C.; Labarre, P.; Chollet, P.; Chabard, J. L.; Godeneche, D.; Baudry, J. P.; Veyre, A. Disposition and metabolism of O^6-alkylguanine DNA alkyltransferase inhibitor in Nude mice bearing human melanoma. *Drug Metab. Disp.* **1994**, *22*, 637−642.
(25) Cussac, C.; Rapp, M.; Mounetou, E.; Madelmont, J. C.; Maurizis, J. C.; Godeneche, D.; Dupuy, J. M.; Sauziéres, J.; Baudry, J. P.; Veyre, A. Enhancement by O^6-benzyl-N-acetylguanosine derivatives of chloroethylnitrosourea antitumor action in chloroethylnitrosourea resistant human malignant melanocytes. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1353−1358.
(26) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. In *Vogel's textbook of practical organic chemistry*; Longman Group UK Limited: Harlow, UK, 1989; p 1198.
(27) Madelmont, J. C.; Godeneche, D.; Parry, D.; Duprat, J.; Chabard, J. L.; Plagne, R.; Mathe, G.; Meyniel, G. New cysteamine (2-chloroethyl)nitrosoureas. Synthesis and preliminary antitumor results. *J. Med. Chem.* **1985**, *28*, 1346−1350.
(28) Bowles, W. A.; Schneider, F. H.; Lewis, L. R.; Robins, R. K. Synthesis and antitumor activity of 9-(tetrahydro-2-furyl)purine analogs of biologically important deoxynucleosides. *J. Med. Chem.* **1963**, *6*, 471−480.

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