

## Substitution of Aminomethyl at the Meta-Position Enhances the Inactivation of *O*<sup>6</sup>-Alkylguanine-DNA Alkyltransferase by *O*<sup>6</sup>-Benzylguanine

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*O*<sup>6</sup>-Benzylguanine is an irreversible inactivator of *O*<sup>6</sup>-alkylguanine-DNA alkyltransferase currently in clinical trials to overcome alkyltransferase-mediated resistance to certain cancer chemotherapeutic alkylating agents. In order to produce more soluble alkyltransferase inhibitors, we have synthesized three aminomethyl-substituted *O*<sup>6</sup>-benzylguanines and the three methyl analogs and found that the substitution of aminomethyl at the meta-position greatly enhances inactivation of alkyltransferase, whereas para-substitution has little effect and ortho-substitution virtually eliminates activity. Molecular modeling of their interactions with alkyltransferase provided a molecular explanation for these results. The square of the correlation coefficient (*R*<sup>2</sup>) obtained between E-model scores (obtained from GLIDE XP/QPLD docking calculations) vs log(ED<sub>50</sub>) values via a linear regression analysis was 0.96. The models indicate that the ortho-substitution causes a steric clash interfering with binding, whereas the meta-aminomethyl substitution allows an interaction of the amino group to generate an additional hydrogen bond with the protein.

### Introduction

*O*<sup>6</sup>-Alkylguanine-DNA alkyltransferase (alkyltransferase<sup>a</sup>) is a unique DNA repair protein that acts in a single step to restore DNA with *O*<sup>6</sup>-alkylguanine adducts by transferring the alkyl group to an acceptor site, which in human alkyltransferase is located at Cys145.<sup>1–3</sup> Alkyltransferase activity in tumors is an important source of resistance to therapeutic alkylating agents such as dacarbazine, temozolomide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) or 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea.<sup>1,2,4–8</sup> *O*<sup>6</sup>-Benzylguanine (BG) is a potent inhibitor of human alkyltransferase, which acts as a pseudosubstrate. After binding in the active site, it leads to the formation of *S*-benzylcysteine at the Cys145 acceptor site, irreversibly inactivating the protein.<sup>9–11</sup> A related compound *O*<sup>6</sup>-(4-bromophenyl)guanine was found to be a slightly more potent inactivator of alkyltransferase<sup>12</sup> and is thought to act in the same way. Both compounds are undergoing clinical trials.<sup>4,7,8,13–18</sup> Although there have been some responses in these trials providing proof of concept for the target, it is clear that improved inhibitors will be needed.

Since the original development of BG, structural and biochemical studies have provided a much greater understanding of the repair reaction catalyzed by alkyltransferase and the binding and reaction of this inhibitor. After binding the substrate DNA via the minor groove using a helix–turn–helix motif, the alkyltransferase protein brings about a change in the DNA

structure that rotates the alkylated guanine deoxynucleoside from the base stack into an active site pocket that contains the Cys145 acceptor residue.<sup>3,19</sup> This places the *O*<sup>6</sup>-alkylguanine in the correct position for repair in a hydrophobic cleft where the Cys145 and Val148 carbonyls accept hydrogen bonds from the exocyclic amine of the guanine, the N of Ser159 donates a hydrogen bond to the substrate guanine-*O*<sup>6</sup>, and the hydroxyl of Tyr114 donates a hydrogen bond to guanine-*N*3. These interactions exactly position the alkyl group for attack by Cys145. This residue is highly reactive by virtue of its activation to a thiolate anion by a Glu172/His146/water/Cys145 hydrogen bond network.<sup>3,11,20</sup> BG binds much more weakly at the active site pocket, since all of the interactions with the alkyltransferase DNA-binding domain are lost, but it is held in a position that allows attack by Cys145 via the interactions with the guanine moiety described above and the interaction of the benzyl group with the side chain of Pro140.<sup>3,11</sup> Mutation of this Pro residue profoundly reduces the ability of BG to inactivate human alkyltransferase.<sup>21,22</sup> Despite the weak binding, the reactivity of benzyl in bimolecular displacement reactions such as that occurring in the alkyltransferase active site facilitates the inactivation reaction. However, the rate constant for the reaction with BG free base is only about 600 M<sup>-1</sup>·s<sup>-1</sup>,<sup>10</sup> which is >10000 times less than that for the repair of *O*<sup>6</sup>-methylguanine in DNA or of oligodeoxyribonucleotides containing BG, which are much more potent inactivators.<sup>23,24</sup> Such oligodeoxyribonucleotides are not ideal for clinical use, and other attempts to improve the binding of low molecular weight pseudosubstrates are needed.

Another serious problem with BG is its poor solubility. In an attempt to make more soluble derivatives, we have now tested *O*<sup>6</sup>-[(aminomethyl)benzyl]guanines (compounds **1–3** shown in Figure 1) for the ability to inactivate human alkyltransferase. It was found that the nature and position of the substitution profoundly affected inhibition and that compound **2**, the

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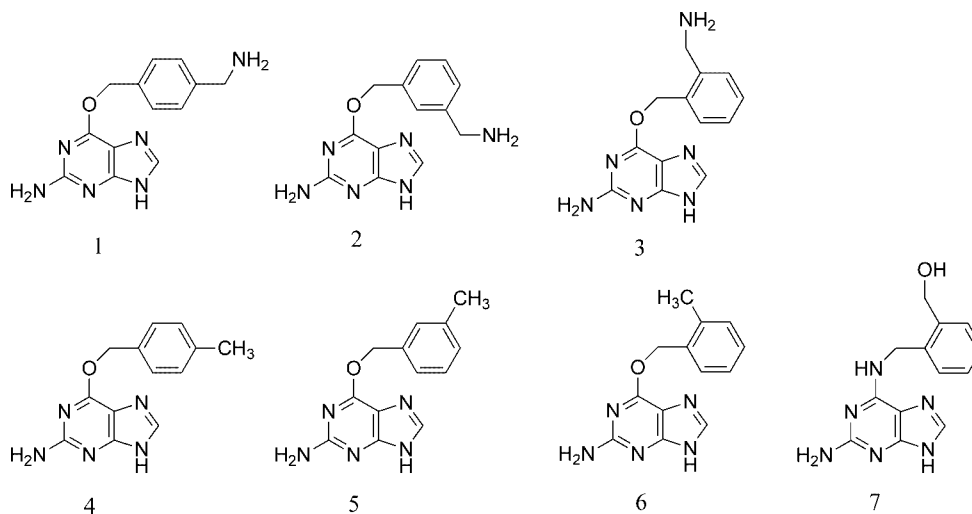
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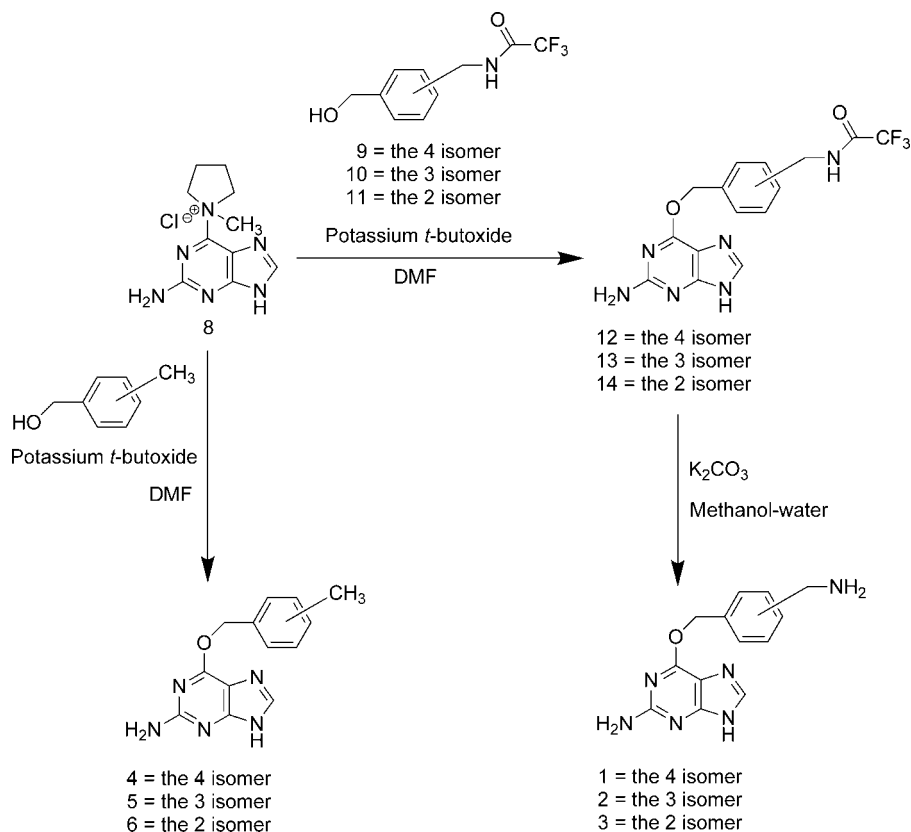
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<sup>a</sup> Abbreviations: alkyltransferase, *O*<sup>6</sup>-alkylguanine-DNA alkyltransferase; BG, *O*<sup>6</sup>-benzylguanine; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; DMSO, dimethyl sulfoxide; DMF, dimethylformamide.



**Figure 1.** Substituted BGs and an *N*<sup>6</sup>-[(hydroxymethyl)benzyl]-2-aminoadenine (**7**) resulting from a rearrangement of **3**.

**Scheme 1.** Synthesis of *O*<sup>6</sup>-[(Aminomethyl)benzyl]guanines and *O*<sup>6</sup>-[(Methylbenzyl)guanines



substitution with an aminomethyl group at the meta- position, provides a more potent and much more soluble inhibitor than BG. Comparison with *O*<sup>6</sup>-(methylbenzyl)guanines (compounds **4–6**) was used to determine if the improved activity was due to stabilization of the transition state by a positional effect of substitution on the benzyl ring or to the ability of the meta-aminomethyl group to bind more tightly to the active site. Compounds **4–6** did not show improvement over BG, suggesting that the latter was the case. Molecular modeling studies using one of the crystal structures available for the protein that involved its interaction with compounds **1–6** and BG were carried out and provide a plausible explanation for these results and show that the meta-substituent on **2** provides additional

interactions with the active site pocket that increase the affinity for binding to the alkyltransferase protein.

## Results and Discussion

### Synthesis of Methyl- and Aminomethyl-Substituted BGs.

Preparation of the three isomeric *O*<sup>6</sup>-[(aminomethyl)benzyl]guanines (**1–3**) was adapted from the method reported by Keppler et al.<sup>25</sup> to make compound **1** as shown in Scheme 1. 2-Amino-6-chloropurine was converted to 1-(2-amino-9*H*-purin-6-yl)-1-methylpyrrolidinium chloride (**8**). The amine groups of the three isomers of (aminomethyl)benzyl alcohol were protected as the corresponding trifluoroacetamides (**9**, **10**, and **11**). Treatment of compound **8** in DMF with one of the three protected (aminomethyl)

**Table 1.** Inactivation of Purified Human Alkyltransferase in Vitro

alkyltransferase tested	inhibitor	ED <sub>50</sub> (μM) <sup>a</sup>	
		no DNA	with DNA
wild type	BG	0.30	0.10
wild type	<b>2</b>	0.017	0.004
wild type	<b>1</b>	0.23	0.053
wild type	<b>3</b>	80	45
wild type	<b>5</b>	0.25	0.16
wild type	<b>4</b>	0.32	0.20
wild type	<b>6</b>	185	90
wild type	<b>7</b>	>1000	>1000
S159A mutant	<b>2</b>	0.013	0.003
S159A mutant	<b>1</b>	0.16	0.028

<sup>a</sup> Results shown are the mean values for three to five assays with different preparations of recombinant alkyltransferase, which agreed within ±20%.

ethyl)benzyl alcohols in the presence of potassium *tert*-butoxide gave the corresponding trifluoroacetyl protected *O*<sup>6</sup>-(aminomethyl)benzylguanines (**12**, **13**, and **14**). Removal of the trifluoroacetate protecting groups with potassium carbonate in methanol–water then gave the three *O*<sup>6</sup>-(aminomethyl)benzylguanines (**1**, **2**, and **3**). Similarly, the treatment of **8** with one of the three isomers of methylbenzyl alcohol in the presence of potassium *tert*-butoxide gave the three methylbenzylguanines **4**, **5**, and **6** (Scheme 1).

During the attempt to recrystallize a sample of *O*<sup>6</sup>-[2-(aminomethyl)benzyl]guanine (**3**) from boiling water, an insoluble precipitate formed. This precipitate was recovered by filtration. The proton NMR spectrum of the precipitated material was not consistent with the original sample of compound **3**, even though MS analysis of the original sample and the precipitate gave similar molecular ions with *m/z* = 271.1 [M + H]<sup>+</sup>. A sample of compound **3** was dissolved in water and heated at 65 °C for 1 week. The precipitate was then recovered for further analysis by NMR. The NMR spectrum of the precipitated material is consistent with *N*<sup>6</sup>-[2-(hydroxymethyl)benzyl]-2-aminoadenine (**7** in Figure 1), which we propose is formed by intramolecular attack of the benzylic amine at the 6-position of the purine, displacing the oxygen. This rearrangement was not observed with **1** and **2**.

#### Inactivation of Purified Human Alkyltransferase in Vitro.

Compound **1**, the para-substituted aminomethyl derivative of BG, was only slightly more active than BG itself, but the meta-substituted derivative **2** was considerably more active with an ED<sub>50</sub> of approximately 4 nM compared to 100 nM for BG when assayed in the presence of DNA and 17 nM compared to 300 nM when assayed in the absence of DNA (Table 1). The inactivation of the S159A mutant alkyltransferase by compounds **1** and **2** differed only slightly from the inactivation of the wild type (Table 1). This rules out the possibility that there is an interaction between the side chain of this serine residue in alkyltransferase and the aminomethyl group of these inhibitors.

The ortho-substituted derivative **3** was much less active than BG or the other substituted BGs with an ED<sub>50</sub> of 45 μM when assayed in the presence of DNA and 80 μM when assayed in the absence of DNA (Table 1). This derivative was assayed immediately after a solution was made up, since as described above it is unstable in aqueous solution, rearranging to form **7** with a half-life of about 50 h at 37 °C. This compound was also tested and was inactive as an alkyltransferase inhibitor (less than 10% inhibition at 1 mM).

The improved activity of an aminomethyl meta-substituent is not mimicked by a simple methyl group. There was no difference between BG itself and compound **4** or **5**, which had ED<sub>50</sub> values of about 0.3 μM in the absence of DNA and about 0.15 μM in the presence of DNA (Table 1). The ortho-methyl

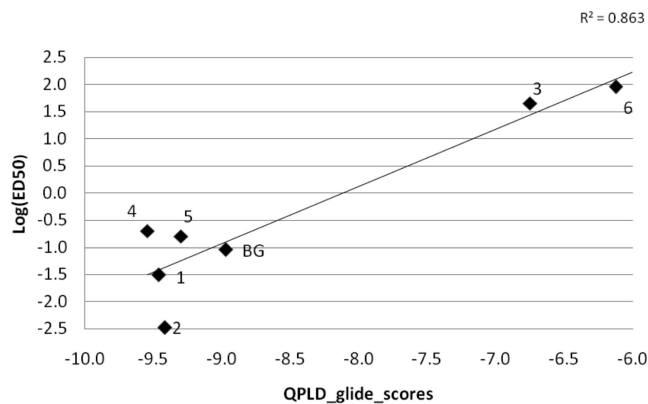
substituted compound **6** was similar to **3** in leading to a large loss of inhibitory potency (ED<sub>50</sub> values of 90 μM and 185 μM in the presence and absence of DNA) (Table 1). These results suggest that the improved activity of **2** was not caused by stabilization of the transition state due simply to a substitution on the benzyl ring at the meta-position.

In contrast to studies with folate ester derivatives of BG where the ED<sub>50</sub> values were affected by the presence of a (His)<sub>6</sub> tag on the alkyltransferase protein,<sup>26</sup> there was no difference between assays with untagged alkyltransferase protein and assays using either a C-terminal or N-terminal (His)<sub>6</sub>-tagged alkyltransferase with any of the compounds listed in Table 1. Some of these folate derivatives are less potent inactivators of a polymorphic form of human alkyltransferase in which Ile143 is changed to a Val and Lys178 to an Arg (I143V/K178R).<sup>26</sup> This variant was only slightly less susceptible than wild type to compound **2** (results not shown).

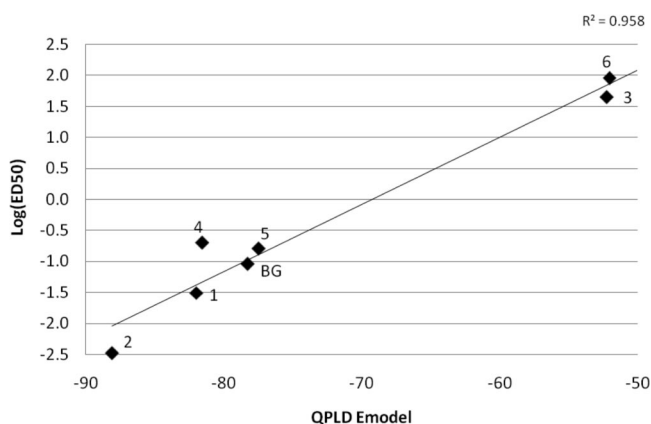
**Molecular Docking of Inhibitors to Human Alkyltransferase.** Computational docking studies were performed using the GLIDE program (version 4.5, Schrödinger, LLC, New York, 2007). The docked structures were chosen for comparison with experimentally determined ED<sub>50</sub> values using either the Glide Score or E-model scoring function. For enhanced docking accuracy, the best docked structures using GLIDE extra precision (XP) mode were used to calculate ligand partial charges in the protein environment and then redocked with XP using Schrödinger's QPLD (quantum polarized ligand docking) method.<sup>27</sup> The Maestro user interface (version 8.0, Schrödinger, LLC, New York, 2007) was employed to set up the GLIDE docking studies and for visualization of the results. The alkyltransferase X-ray crystal structure chosen for our modeling studies was human alkyltransferase bound to DNA containing *O*<sup>6</sup>-methylguanine and Cys145 mutated to Ser to prevent the alkylation reaction from taking place.<sup>19</sup> In our modeling studies, Ser145 was mutated back to Cys. To validate the docking approach, self-docking was performed with XP/QPLD using the partial native ligand *O*<sup>6</sup>-methylguanine. The rms of the docked pose when compared to the crystallographically observed position of the *O*<sup>6</sup>-methylguanine moiety was 0.14 Å, and thus, GLIDE produced a docking mode that closely resembled the X-ray crystal structure. Thus, our hypothesis was that GLIDE would be capable of producing docking poses for the compounds studied that are similar to the position, orientation, and conformation adopted by the ligands prior to nucleophilic attack by Cys145 and that the docking scores obtained would correlate well with the experimentally observed ED<sub>50</sub> values for enzyme inactivation. Although there is a crystal structure available for human alkyltransferase benzylated at Cys145,<sup>11</sup> this structure was not employed to model the BG analogues described in this study, since the GLIDE program is unsuitable for modeling covalent bonds formed between the ligand and protein.

In addition to *O*<sup>6</sup>-methylguanine, compounds **1–6** and BG were docked to human alkyltransferase using the GLIDE XP. Each pose of these compounds was redocked with XP using the QPLD method. The coefficient of determination (i.e., the square of the correlation coefficient, *R*<sup>2</sup>) was calculated between QPLD Glide Scores vs log(ED<sub>50</sub>) values, determined in the presence of DNA, as part of a linear regression analysis. An acceptable *R*<sup>2</sup> value of 0.86 between log(ED<sub>50</sub>) values and XP/QPLD Glide Scores was obtained (Figure 2).

E-model scores were also used for correlation studies. It had been previously observed by Bytheway and Cochran<sup>28</sup> that E-model scores correlated better with log(ED<sub>50</sub>) values than Glide Scores in their particular study. In our study, the



**Figure 2.** Plot of log(ED<sub>50</sub>) values versus QPLD\_Glide\_scores with DNA. Numbering corresponds to compounds shown in Figure 1. The ED<sub>50</sub> values determined in the presence of DNA from Table 1 were used.



**Figure 3.** Plot of log(ED<sub>50</sub>) values versus QPLD\_E-model scores with DNA. Numbering corresponds to compounds shown in Figure 1. The ED<sub>50</sub> values determined in the presence of DNA from Table 1 were used.

coefficient of determination obtained between E-model scores (obtained from XP/QPLD calculations) vs log(ED<sub>50</sub>) values via a linear regression analysis gave an exceptional  $R^2$  of 0.96, shown in Figure 3. It is noteworthy that the E-model is a composite scoring function, derived from a combination of the Glide Score itself coupled with Coulombic energy, van der Waals energy, and ligand strain energy terms, and is used by GLIDE to select the best docking pose for each individual inhibitor regardless of the scoring function subsequently used and can be used to rank order the inhibitors as well.

From examination of the QPLD based docking modes, one can conclude that the binding affinities correlate quite well with the number of hydrogen bonds and good van der Waals contacts formed between the inhibitor and the alkyltransferase binding site. There is insufficient space to adequately accommodate the ortho-substituted benzylguanines. Thus, because of fewer favorable van der Waals contacts and because of their fewer hydrogen bonds, compounds **3** and **6** are the least potent of the inhibitors we tested in the modeling studies. On the other hand, compounds **1** and **2** exhibit three and four hydrogen bonds, respectively, and both form good van der Waals contacts with the alkyltransferase protein. All the values of these docking based binding interactions are summarized in Table 2.

Figure 4 shows the key hydrogen bonding interactions of compound **2** with human alkyltransferase. Interactions with residues Tyr114, Cys145, Ser159, and Asn137 are depicted. All

**Table 2.** Binding Interactions Derived from Docking Compounds **1–6**, BG, and *O*<sup>6</sup>-Methylguanine to Human Alkyltransferase

compd	E-model score	Glide score	H-bonds	good vdw <sup>a</sup> contacts	bad vdw <sup>a</sup> contacts
<b>2</b>	−88.1	−9.42	4	297	8
<b>1</b>	−82.0	−9.46	3	312	8
<b>4</b>	−81.5	−9.54	3	306	7
BG	−78.2	−8.79	3	287	4
<b>5</b>	−77.5	−9.30	3	289	8
<i>O</i> <sup>6</sup> -methylguanine	−56.6	−7.16	4	177	3
<b>3</b>	−52.2	−6.75	2	260	5
<b>6</b>	−52.0	−6.12	1	180	1

<sup>a</sup> vdw, Van der Waals.

except for the interaction with Tyr114 involve interaction with protein backbone rather than the amino acid side chains.

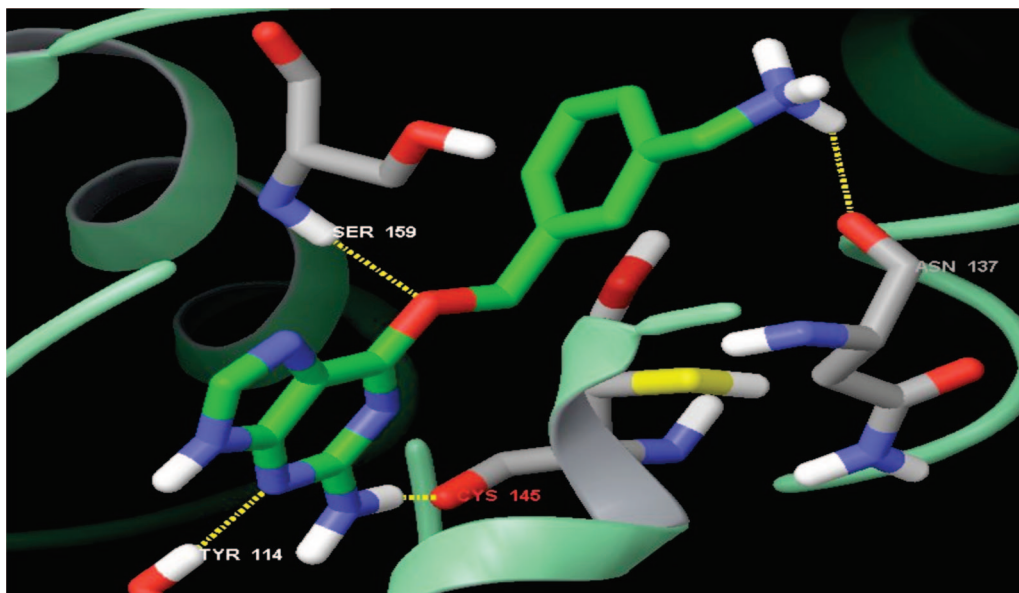
Figure 5 shows an overlay of all of the inhibitor poses in the active site with *O*<sup>6</sup>-methylguanine. It is clearly apparent that compounds **3** and **6** are not oriented in the catalytic site of human alkyltransferase in same way as the other inhibitors.

**Inactivation of Alkyltransferase in HT29 Cells in Culture.** The striking difference between **1** and **2** in the inactivation of alkyltransferase was also seen when the compounds were added to the culture medium of HT29 cells (Figure 6). When cell extracts were prepared from these cells and residual alkyltransferase activity measured, it was found that within 30 min or less of exposure to 0.5–2.5  $\mu$ M compound **2**, the alkyltransferase was almost completely reduced. Compound **1** was clearly less effective; the 1  $\mu$ M dose only produced 20% loss of activity in 30 min (Figure 6A). This difference was also seen when the ability of **1** and **2** to sensitize HT29 cells to BCNU was examined (Figure 6B). Alkyltransferase activity provides resistance to BCNU, and alkyltransferase inhibitors overcome this resistance.<sup>9,12,29–31</sup> Compound **2** was much more effective than compound **1** in sensitizing HT29 cells to the chloroethylating agent (Figure 6B). Previously published studies have shown that BG passes very readily through cell membranes and very rapidly inactivates alkyltransferase in HT29 cells.<sup>9,29,32</sup> Even though **2** produces a comparable degree of alkyltransferase inactivation, the rate of inactivation by **2** seen in Figure 6A is significantly slower than with BG. This is likely to be due to a reduced uptake of **2** due to its positive charge.

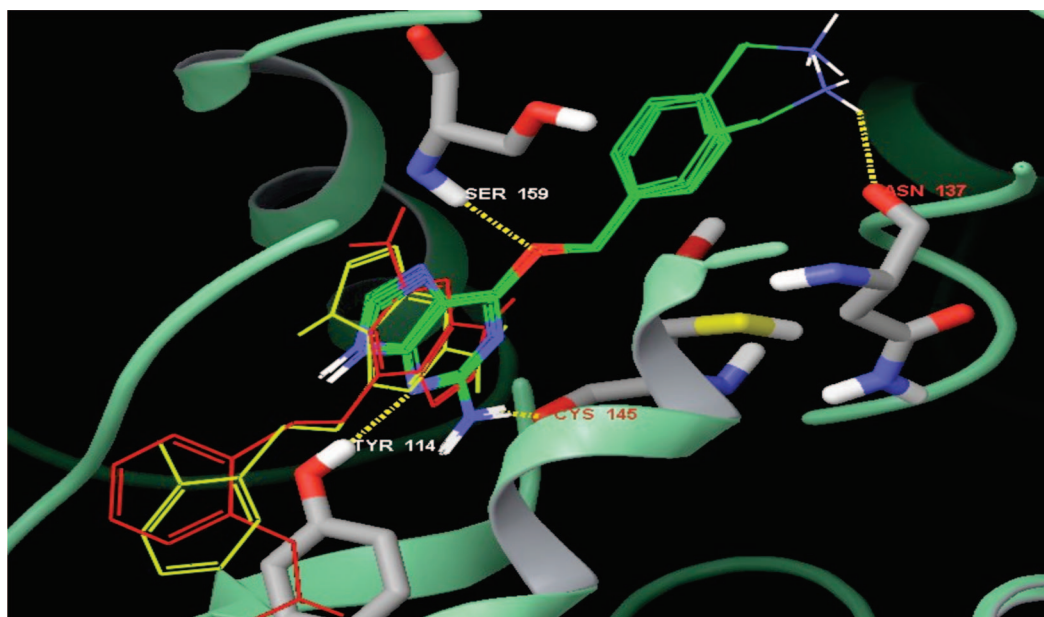
## Conclusions

The addition of a meta-aminomethyl group to BG forming compound **2** not only greatly increases the solubility but results in an approximately 20-fold improvement in the ability to inactivate purified human alkyltransferase. This improvement is seen in assays conducted with or without added DNA. It is noteworthy that the molecular modeling studies showed a similar trend and an  $R^2$  of 0.90 (data not shown) for a linear regression analysis of log(ED<sub>50</sub>), determined in the absence of DNA, vs XP/QPLD Glide Scores, and an  $R^2$  of 0.98 (data not shown) for log(ED<sub>50</sub>), determined in the absence of DNA, vs E-model scores were obtained. As previously noted, inactivation of alkyltransferase by BG<sup>33</sup> is enhanced 3- to 4-fold by the presence of DNA, which stimulates the rate of alkyl transfer.<sup>34</sup> In contrast, some BG derivatives with bulky substituents are much less potent inhibitors in the presence of DNA, since they cannot be accommodated in the active site when DNA is bound there.<sup>34,35</sup> The inactivation of alkyltransferase by compounds **1** and **2** was increased by the presence of DNA, indicating that these compounds, like BG, do not compete with DNA for access to the active site. However compound **1**, the para-aminomethyl derivative, did not show any improvement in inhibitory potency over BG itself. These results are very well explained by the





**Figure 4.** Key hydrogen bonding interactions of compound **2** (*O*<sup>6</sup>-[3-(aminomethyl)benzyl]guanine) with the protein residues represented in stick model. The inhibitor is represented in green for carbon, blue for nitrogen, and red for oxygen. The rest of the protein is shown as pale green ribbon cartoon.

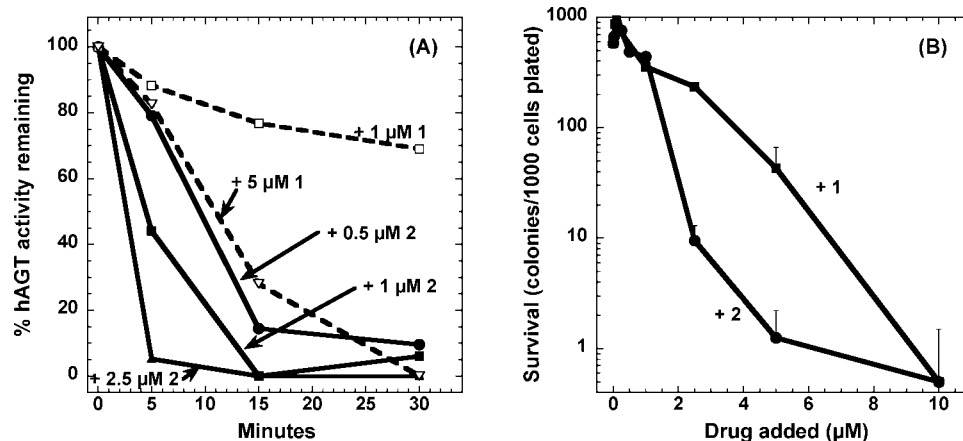


**Figure 5.** Overlay of the position of all of the docked compounds in the human alkyltransferase active site with the native ligand *O*<sup>6</sup>-methylguanine and parent inhibitor BG. All of the potent inhibitor poses in green (compounds **1**, **2**, **4**, **5**, and BG) are oriented in the same way as the native ligand *O*<sup>6</sup>-methylguanine. The ineffective inhibitors *O*<sup>6</sup>-[2-(aminomethyl)benzyl]guanine (**3**) in red and *O*<sup>6</sup>-[2-(methyl)benzyl]guanine (**6**) in yellow are not oriented in the same way as *O*<sup>6</sup>-methylguanine. The rest of the color code is the same as in Figure 4.

molecular modeling studies, which indicate the formation of an additional hydrogen bond when compound **2** is bound in the active site. The aminomethyl group from **2** but not **1** is able to interact with Asn137. This also explains why a simple methyl substitution (compound **5**) was ineffective, since it cannot form this hydrogen bond. The modeling studies also show clearly why the ortho-substituted BG derivatives (**3** and **6**) are much less effective (>200-fold) than BG since, because of steric clashes, they cannot be positioned in the same way as the parent compound.

Our results not only demonstrate that **2** may be a valuable alkyltransferase inhibitor but also show clearly the value of molecular modeling for the design of improved alkyltransferase inhibitors. Even though it has not yet been possible to obtain

actual crystal structures of BG bound to wild type alkyltransferase or its C145S mutant, the remarkable  $R^2$  values obtained using XP/QPLD with E-model scoring for ranking the compounds tested here show that the structure of human alkyltransferase bound to DNA containing *O*<sup>6</sup>-methylguanine and Cys145 mutated to Ser can be used as a starting structure for this process. The mutation of Ser145 back to Cys, as is appropriate for the wild type enzyme, and refinement of the structure obtained with MacroModel were essential in order to obtain the  $R^2$  values we report. The model we have generated should be highly useful in the design of more potent inhibitors by performing additional modeling studies on BG analogues and by virtually screening databases of commercially available compounds to identify potential new lead compounds for further elaboration.



**Figure 6.** Inactivation of alkyltransferase in HT29 cells. Panel A shows the loss of alkyltransferase (hAGT) activity as a function of time after addition of the compound indicated to cultures of HT29 cells. Panel B shows the effect of exposing HT29 cells to the compound indicated for 2 h prior to the addition of 40  $\mu$ M BCNU. Cell survival was then measured using a colony forming assay as described in the Experimental Section.

A major advantage of compound **2** is that it is much more soluble (approximately 1000 times at physiological pH) than BG. The limited solubility of BG requires formulation in a diluent containing polyethylene glycol. Although adequate blood levels for alkyltransferase inactivation in a variety of tumors can be achieved with this route of administration,<sup>4,13–17</sup> the potentiation of the myelotoxicity of the alkylnitrosourea-based and methylating chemotherapies due to inactivation of alkyltransferase in the bone marrow limits the effectiveness of BG. Regional delivery is an obvious way to attempt to circumvent this limitation, but in many cases, only a small volume can be administered, and the poor solubility of BG prevents its use in this way. This could be avoided by using **2**.

In particular, **2** is suitable for testing for protocols involving administration by convection-enhanced therapy to treat brain tumors. Convection-enhanced therapy uses a hydrostatic pressure gradient-dependent direct intracerebral infusion to establish a bulk flow interstitial current that produces a relatively uniform spatial distribution of drugs at concentrations nearer the infused concentration at significant distances from the point of delivery. It is currently under development for use with a variety of agents directed against brain tumors.<sup>36–38</sup> The hydrophilicity of the protonated **2** is also likely to prevent back-loss across the blood–brain barrier. The slower uptake of **2** compared to BG is not likely to prove a significant obstacle to its use, since total alkyltransferase inactivation is easily obtained within 30 min of exposure to 1  $\mu$ M levels. Obviously, the success of the compound in clinical use will depend on its biological stability and bioavailability.

## Experimental Section

**Chemistry.** Chemicals were obtained from Aldrich (Milwaukee, WI) and were used without further purification. UV spectra were determined on a Beckman Coulter DU 7400 spectrophotometer. Molar extinction coefficients for compounds **1–3** were determined by simply dissolving approximately 10 mg of the material in 1.00 L of 0.01 M phosphate buffer, pH 7.0, and recording the UV absorbance spectrum. Compounds **4–7** did not dissolve completely, even at this low concentration. As a result, these four compounds were predissolved in 1 mL of DMSO and then diluted to 1.00 L with phosphate buffer prior to recording their UV absorbance spectra. <sup>1</sup>H NMR spectra were recorded with a Varian INOVA 400 MHz spectrometer. Chemical shifts are reported as  $\delta$  values in parts per million relative to TMS as an internal standard. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, and m = multiplet. Coupling constants are in hertz. Mass spectra

were collected on a Thermo Finnigan TSQ Quantum spectrometer in positive ion electrospray mode scanning from  $m/z = 100$  to  $m/z = 1500$  in 1 s. The electrospray voltage was 3.5 kV, and the transfer tube was at 350 °C. Elemental analysis was performed by Atlantic Microlab, Inc. (Norcross, GA). All silica gel chromatography was carried out using Davisil, grade 633, 200–425 mesh, 60 Å. 4-(Aminomethyl)benzyl alcohol was made according to the procedure reported by Keppler et al.<sup>25</sup> The NMR spectrum of this material was in agreement with the published data. 2-(Aminomethyl)benzyl alcohol was made as described by McCalmont et al.,<sup>39</sup> and the NMR spectrum agreed with the reported data.

**3-(Aminomethyl)benzyl Alcohol.** A sample of 3-cyanobenzoic acid (20 g, 136 mmol) was suspended in 100 mL of dry tetrahydrofuran (THF). Borane–THF complex (400 mL of 1.0 M solution in THF) was added dropwise over 1 h, and the mixture was stirred for an additional 3 h. The reaction was quenched with 220 mL of concentrated HCl/water (1:1 v/v) to cleave the borate ester complex. The THF was then removed under vacuum. Solid NaOH pellets, 70 g total, were added slowly to neutralize the acid and to bring the pH of the solution to 12. The aqueous phase was then extracted 4 times with 500 mL portions of ether. The ether extract was dried over MgSO<sub>4</sub> and concentrated under vacuum to give 18 g (131 mmol, 96%) of 3-(aminomethyl)benzyl alcohol as a light-colored oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>/TMS)  $\delta$  = 1.95 (broad s, 3H, –OH and –NH<sub>2</sub>, exchangeable with D<sub>2</sub>O), 3.86 (s, 2H, –CH<sub>2</sub>–NH<sub>2</sub>), 4.66 (s, 2H, –CH<sub>2</sub>–OH), 7.18–7.35 (m, 4H, Ar) ppm, in agreement with data published by Lee et al.<sup>40</sup> who made this compound by a different route.

**1-(2-Amino-9H-purin-6-yl)-1-methylpyrrolidinium Chloride (8).** This compound was prepared from 2-amino-6-chloropurine as described.<sup>25</sup> The <sup>1</sup>H NMR spectrum of this product was in agreement with the data in that publication.

**General Synthesis of 2,2,2-Trifluoro-N-[(hydroxymethyl)benzyl]acetamides.** Protection of the amine groups of the three aminomethylbenzyl alcohols as trifluoroacetamides was performed according to the procedure used by Keppler et al. to make 2,2,2-trifluoro-N-[(4-(hydroxymethyl)benzyl]acetamide (**9**).<sup>25</sup>

**2,2,2-Trifluoro-N-[(4-(hydroxymethyl)benzyl]acetamide (9).** The yield of this reaction was 89%, giving a white solid with a melting point of 91–92 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>/TMS)  $\delta$  = 1.77 (s, 1H, –OH, exchanges with D<sub>2</sub>O), 4.52 (d,  $J = 6.0$ , 2H, –CH<sub>2</sub>–NH–), 4.70 (s, 2H, –CH<sub>2</sub>–OH), 6.63 (broad s, 1H, –NH–CO–, exchanges with D<sub>2</sub>O), 7.26–7.39 (m, 4H, Ar) ppm, in agreement with the published data.<sup>25</sup>

**2,2,2-Trifluoro-N-[(3-(hydroxymethyl)benzyl]acetamide (10).** The yield of this reaction was 91%, giving a white solid with a melting point of 73–74 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>/TMS)  $\delta$  = 1.84 (s, 1H, –OH, exchanges with D<sub>2</sub>O), 4.52 (d,  $J = 5.6$ , 2H,

—CH<sub>2</sub>—NH—), 4.70 (s, 2H, —CH<sub>2</sub>—OH), 6.65 (broad s, 1H, —NH—CO—, exchanges with D<sub>2</sub>O), 7.20–7.4 (m, 4H, Ar) ppm.

**2,2,2-Trifluoro-*N*-[2-(hydroxymethyl)benzyl]acetamide (11).** The yield of this reaction was 76%, giving a white solid with a melting point of 75–76 °C. <sup>1</sup>H NMR (CDCl<sub>3</sub>/TMS) δ = 2.20 (s, 1H, —OH, exchanges with D<sub>2</sub>O), 4.61 (d, *J* = 5.6, 2H, —CH<sub>2</sub>—NH—), 4.78 (s, 2H, —CH<sub>2</sub>—OH), 7.30–7.44 (m, 4H, aromatic), 7.76 (broad s, 1H, —NH—CO—, exchanges with D<sub>2</sub>O) ppm.

**General Synthesis of the *N*-[2-(2-amino-9*H*-purin-6-ylloxymethyl)benzyl]-2,2,2-trifluoroacetamides (12–14).** The procedure used by Keppler et al.<sup>25</sup> to make *N*-[4-(2-amino-9*H*-purin-6-ylloxymethyl)benzyl]-2,2,2-trifluoroacetamide (12) was used. Compound 8 was suspended in 35–40 mL/g DMF. To this was added between 1.4 and 2.0 equiv of one of the three protected aminomethylbenzyl alcohols (9–11) together with 2 equiv, with respect to the benzyl alcohol, of potassium *tert*-butoxide. After 3–5 h the reactions were quenched with acetic acid in water and the solvents were evaporated. The products were then purified by silica gel chromatography, eluting with methanol/dichloromethane (1:50) followed by the same solvents (1:10).

***N*-[4-(2-Amino-9*H*-purin-6-ylloxymethyl)benzyl]-2,2,2-trifluoroacetamide (12).** This reaction gave a white solid with a melting range of 213–215 °C in 34% yield based on the purine. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 4.40 (s, 2H, —CH<sub>2</sub>—NH—), 5.47 (s, 2H, —O—CH<sub>2</sub>—), 6.27 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.27–7.52 (m, 4H, Ar), 7.83 (s, 1H, guanine H8), 10.02 (broad s, 1H, —NH—CO— exchanges with D<sub>2</sub>O), 12.44 (broad s, 1H, guanine H9, exchanges with D<sub>2</sub>O) ppm. Keppler et al.<sup>25</sup> report the signal at 4.40 ppm as a doublet.

***N*-[3-(2-Amino-9*H*-purin-6-ylloxymethyl)benzyl]-2,2,2-trifluoroacetamide (13).** This reaction gave a white solid which decomposed above 210 °C in 35% yield based on the purine. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 4.42 (s, 2H, —CH<sub>2</sub>—NH—), 5.48 (s, 2H, —O—CH<sub>2</sub>—), 6.27 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.23–7.45 (m, 4H, Ar), 7.83 (s, 1H, guanine H8), 10.03 (broad s, 1H, —NH—CO— exchanges with D<sub>2</sub>O), 12.45 (broad s, 1H, guanine H9, exchanges with D<sub>2</sub>O) ppm.

***N*-[2-(2-Amino-9*H*-purin-6-ylloxymethyl)benzyl]-2,2,2-trifluoroacetamide (14).** This reaction gave a white solid which decomposed slowly above 180 °C in 25% yield based on the purine. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 4.58 (d, *J* = 5.6, 2H, —CH<sub>2</sub>—NH—), 5.57 (s, 2H, —O—CH<sub>2</sub>—), 6.29 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.26–7.60 (m, 4H, Ar), 7.81 (s, 1H, guanine H8), 10.00 (t, *J* = 5.6, 1H, —NH—CO— exchanges with D<sub>2</sub>O), 12.43 (broad s, 1H, guanine H9, exchanges with D<sub>2</sub>O) ppm.

**General Method for the Removal of 2,2,2-Trifluoroacetyl Protecting Groups.** Trifluoroacetyl protecting groups were removed from compounds (9–11) using the procedure described by Keppler et al.<sup>25</sup> to make *O*<sup>6</sup>-[4-(aminomethyl)benzyl]guanine (1).

***O*<sup>6</sup>-[4-(Aminomethyl)benzyl]guanine (1).** 1 was recovered as a white solid which decomposed above 185 °C in 76% yield. UV (0.01 M sodium phosphate pH 7.0), λ<sub>max</sub> = 241 nm (ε = 7400), λ<sub>max</sub> = 282 nm (ε = 8500). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 3.72 (s, 2H, —CH<sub>2</sub>—NH<sub>2</sub>), 5.45 (s, 2H, —O—CH<sub>2</sub>—) 6.26 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.31–7.47 (m, 4H, Ar), 7.82 (s, 1H, guanine H8) ppm, in agreement with the data published by Keppler et al.<sup>25</sup> Neither the guanine H9 proton nor the benzylic amine protons are observed.<sup>25</sup> A sample of this material was recrystallized from water for elemental analysis. Analysis calculated for C<sub>13</sub>H<sub>14</sub>N<sub>6</sub>O<sub>1</sub>•0.2H<sub>2</sub>O (C, H, N). The material was converted to the hydrochloride salt by suspending it in water and adding 1 equiv of HCl. The material was then filtered and lyophilized. Analysis of the hydrochloride salt was calculated for C<sub>13</sub>H<sub>15</sub>N<sub>6</sub>O<sub>1</sub>Cl<sub>1</sub>•0.5H<sub>2</sub>O (C, H, N, Cl).

***O*<sup>6</sup>-[3-(Aminomethyl)benzyl]guanine (2).** 2 was recovered as a white solid with a melting range of 158–160 °C in 68% yield. UV (0.01 M sodium phosphate, pH 7.0), λ<sub>max</sub> = 241 nm (ε = 7500), λ<sub>max</sub> = 282 nm (ε = 8600). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 4.02 (s, 2H, —CH<sub>2</sub>—NH<sub>2</sub>), 5.48 (s, 2H, —O—CH<sub>2</sub>—) 6.30 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.29–7.48 (m, 4H, Ar), 7.83 (s, 1H, guanine H8) ppm. As with compound 1, neither the guanine

H9 proton nor the benzylic amine protons are observed. A sample of this material was recrystallized from water for elemental analysis. Analysis calculated for C<sub>13</sub>H<sub>14</sub>N<sub>6</sub>O<sub>1</sub> (C, H, N). The material was converted to the hydrochloride salt by suspending it in water and adding 1 equiv of HCl. The material was then filtered and lyophilized. Analysis of the hydrochloride salt was calculated for C<sub>13</sub>H<sub>15</sub>N<sub>6</sub>O<sub>1</sub>Cl<sub>1</sub> as the monohydrate (C, H, N, Cl).

***O*<sup>6</sup>-[2-(Aminomethyl)benzyl]guanine (3).** 3 was recovered as a white solid which decomposed above 170 °C in 74% yield. UV (0.01 M sodium phosphate pH 7.0), λ<sub>max</sub> = 241 nm (ε = 7400), λ<sub>max</sub> = 282 nm (ε = 8400). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 3.82 (s, 2H, —CH<sub>2</sub>—NH<sub>2</sub>), 5.55 (s, 2H, —O—CH<sub>2</sub>—) 6.27 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.22–7.50 (m, 4H, Ar), 7.83 (s, 1H, guanine H8) ppm. As with compound 1, neither the guanine H9 proton nor the benzylic amine protons are observed. Elemental analysis calculated for C<sub>13</sub>H<sub>14</sub>N<sub>6</sub>O<sub>1</sub> (C, H, N). This material was not converted to a hydrochloride salt because of the rearrangement observed below.

***N*<sup>6</sup>-[2-(Hydroxymethyl)benzyl]-2-aminoadenine (7).** Upon attempting to recrystallize *O*<sup>6</sup>-[2-(aminomethyl)benzyl]guanine (3) from water, it was observed that the material came out of solution upon heating. The NMR spectrum (<sup>1</sup>H) of the recovered solid material indicated decomposition or rearrangement. The mass spectrum of the original sample of compound 3 and the precipitate contained similar molecular ions at *m/z* = 271.1 [M + H]<sup>+</sup>.

A sample of *O*<sup>6</sup>-[2-(aminomethyl)benzyl]guanine (3) was dissolved in water and heated to 65 °C for 1 week. The insoluble precipitate was then filtered, washed with water, and dried under vacuum. This material with a melting range of 219–221 °C was identified as *N*<sup>6</sup>-[2-(hydroxymethyl)benzyl]-2-aminoadenine (7) based on the NMR spectrum. UV (0.01 M sodium phosphate with 0.1% v/v DMSO, pH 7.0), λ<sub>shoulder</sub> = 250 nm (ε = 8000), λ<sub>max</sub> = 284 nm (ε = 12 800). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 4.64 (singlet with a broad shoulder downfield, 4H, —CH<sub>2</sub>—NH— and —CH<sub>2</sub>—OH), 5.30 (s, 1H, —CH<sub>2</sub>—OH exchanges with D<sub>2</sub>O), 5.67 (s, 2H, purine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.15–7.40 (m, 4H, Ar), 7.47 (broad d, *J* = 7.2, 1H, benzylic NH, exchanges with D<sub>2</sub>O), 7.65 (s, 1H, purine H8), 12.06 (broad s, 1H, purine H9, exchanges with D<sub>2</sub>O) ppm. Elemental analysis calculated for C<sub>13</sub>H<sub>14</sub>N<sub>6</sub>O<sub>1</sub>•0.5H<sub>2</sub>O (C, H, N). The conversion of a very dilute solution of compound 3 to compound 7 was followed by UV spectroscopy in 0.1 M sodium phosphate buffer at pH 7.4 and 37 °C and was found to proceed with a half-life of 51 h with first order kinetics.

**General Synthesis of *O*<sup>6</sup>-[(Methyl)benzyl]guanines (4–6).** To a solution of one of the three isomers of methylbenzyl alcohol (2.5 g, 20 mmol) in 100 mL of dry DMF, potassium *tert*-butoxide (4.5 g, 80 mmol) was added. After 30 min, compound 8 (2.54 g, 10 mmol) was added. The mixture was stirred for 18 h, and then the solvent was evaporated under vacuum. The resulting material was purified over silica gel, loading with dichloromethane/methanol (25:1) and eluting with the same solvents (10:1). The three compounds produced in this way were each recrystallized from ethanol/water (1:1).

***O*<sup>6</sup>-[4-(Methyl)benzyl]guanine (4).** The above procedure gave 2.2 g (8.6 mmol, 43%) of 4 as a white solid with a melting point of 205–207 °C. UV (0.01 M sodium phosphate with 0.1% v/v DMSO, pH 7.0), λ<sub>max</sub> = 241 nm (ε = 7600), λ<sub>max</sub> = 282 nm (ε = 8700). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 2.31 (s, 3H, —CH<sub>3</sub>), 5.45 (s, 2H, —O—CH<sub>2</sub>—), 6.27 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.16–7.44 (m, 4H, Ar), 7.81 (s, 1H, guanine H8), 12.41 (broad s, 1H, guanine H9, exchanges with D<sub>2</sub>O), in agreement with the data published by Dolan et al.<sup>9</sup> who made this compound by a different route. Elemental analysis for C<sub>13</sub>H<sub>13</sub>N<sub>5</sub>O<sub>1</sub> (C, H, N).

***O*<sup>6</sup>-[3-(Methyl)benzyl]guanine (5).** The above procedure gave 2.2 g (8.6 mmol, 43%) of 5 as a white solid with a melting point of 201–203 °C. UV (0.01 M sodium phosphate with 0.1% v/v DMSO, pH 7.0), λ<sub>max</sub> = 241 nm (ε = 7500), λ<sub>max</sub> = 282 nm (ε = 8700). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 2.32 (s, 3H, —CH<sub>3</sub>), 5.44 (s, 2H, —O—CH<sub>2</sub>—), 6.27 (s, 2H, guanine—NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.13–7.37 (m, 4H, Ar), 7.81 (s, 1H, guanine H8), 12.41



(broad s, 1H, guanine H9, exchanges with D<sub>2</sub>O). Elemental analysis for C<sub>13</sub>H<sub>13</sub>N<sub>5</sub>O<sub>1</sub> (C, H, N).

***O*<sup>6</sup>-[2-(Methyl)benzyl]guanine (6).** The above procedure gave 1.8 g (7.0 mmol, 35%) of **6** as a white solid with a melting point of 211–213 °C. UV (0.01 M sodium phosphate with 0.1% v/v DMSO, pH 7.0), λ<sub>max</sub> 241 nm (ε = 7600), λ<sub>max</sub> = 282 nm (ε = 8600). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>/TMS) δ = 2.35 (s, 3H, -CH<sub>3</sub>), 5.48 (s, 2H, -O-CH<sub>2</sub>-), 6.28 (s, 2H, guanine-NH<sub>2</sub>, exchanges with D<sub>2</sub>O), 7.18–7.49 (m, 4H, Ar), 7.82 (s, 1H, guanine H8), 12.43 (broad s, 1H, guanine H9, exchanges with D<sub>2</sub>O). Elemental analysis for C<sub>13</sub>H<sub>13</sub>N<sub>5</sub>O<sub>1</sub>·0.25H<sub>2</sub>O (C, H, N).

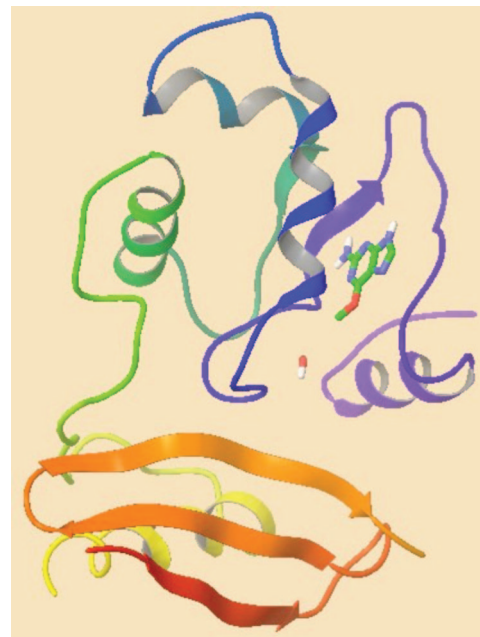
**Inactivation of Purified Recombinant Human Alkyltransferase.** Human alkyltransferase proteins with and without (His)<sub>6</sub> tags were expressed in *E. coli* and purified to homogeneity as previously described.<sup>26</sup> ED<sub>50</sub> values for the inactivation of purified human alkyltransferase in vitro were obtained essentially as previously described.<sup>34,35</sup> Briefly, purified recombinant human alkyltransferase was incubated with different concentrations of potential inhibitors in 0.5 mL of reaction buffer (50 mM Tris-HCl, pH 7.6, 0.1 mM EDTA, 5.0 mM dithiothreitol) containing 50 μg of hemocyanin for 30 min at 37 °C. For assay in the presence of DNA, 10 μg of calf thymus DNA was added to the 0.5 mL of reaction buffer. The remaining alkyltransferase activity was then determined after incubation with [<sup>3</sup>H]methylated calf thymus DNA substrate for 30 min at 37 °C by measuring the [<sup>3</sup>H]methylated protein formed, which was collected on nitrocellulose filters. A graph of the percentage of the alkyltransferase activity remaining against inhibitor concentration was then plotted, and the ED<sub>50</sub> values representing the amount of inhibitor needed to produce a 50% loss of activity were calculated from the equation for the best fit exponential curve (KaleidaGraph, Synergy Software, Reading, PA 19606).

**Cell Culture and Inactivation of Cellular Alkyltransferase.** HT29 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and maintained by seeding at 1 × 10<sup>5</sup> cells/25-cm<sup>2</sup> flask at weekly intervals. Inactivation of cellular alkyltransferase by potential inhibitors was determined in cells that reached about 80% confluence. After different times of exposure to the compounds, cells were harvested by trypsinization and cell pellets were washed with PBS. Cell extracts were prepared as previously described by sonication on ice and centrifugation at 15000g, and supernatant extracts were stored at -70 °C until assayed. Crude cell protein was determined using Bio-Rad protein assay dye (Bio-Rad, Hercules, CA).

The remaining alkyltransferase activity was measured as removal of *O*<sup>6</sup>-[<sup>3</sup>H]methylguanine from [<sup>3</sup>H]methylated calf thymus DNA.<sup>9</sup> Cell extracts were incubated with [<sup>3</sup>H]methylated DNA substrate for 30 min at 37 °C in 1 mL of reaction buffer (50 mM Tris-HCl, pH 7.6, 0.1 mM EDTA, 5.0 mM dithiothreitol). The DNA was precipitated and hydrolyzed, and modified bases were separated by reverse-phase HPLC. The results were expressed as the percentage of the alkyltransferase activity present in cultures to which no compound was added.

**Sensitization of Cells to Killing by BCNU.** The effect of alkyltransferase inactivators on the sensitivity of cells to BCNU was determined using a colony-forming assay. Cells were plated at a density of 10<sup>6</sup> in 25 cm<sup>2</sup> flasks and 24 h later were exposed to different concentrations of potential inhibitors for 2 h as indicated before exposure to 40 μM BCNU for 2 h as previously described.<sup>35,41</sup> After 2 h, the medium was replaced with fresh medium containing the alkyltransferase inhibitor but no BCNU and the cells were left to grow for an additional 16–18 h. The cells were then replated at densities of 250–1000 cells per 25 cm<sup>2</sup> flask and grown for 8 days until discrete colonies had formed. The colonies were washed with 0.9% saline solution, stained with 0.5% crystal violet in ethanol, and counted.

**Molecular Docking Studies.** The computational modeling studies relied upon the GLIDE (Grid-based Ligand Docking from Energetics) program (Glide, version 4.5, Schrödinger, LLC New York, 2007)<sup>42–44</sup> for the docking simulations. These simulations were performed using the X-ray crystal structure of the human



**Figure 7.** Prepared protein of the human alkyltransferase without DNA. *O*<sup>6</sup>-Methylguanine and a bound water (WAT1) are also shown.

alkyltransferase bound to a DNA oligonucleotide and containing *O*<sup>6</sup>-methylguanine determined at 3.2 Å resolution (PDB ID: 1T38).<sup>19,45</sup> For our studies, DNA was removed except for *O*<sup>6</sup>-methylguanine (see Figure 7). Solvent molecules in the protein crystal structure were deleted, except for a water molecule in the active site (WAT 1), Ser145 was mutated to Cys145, and the protein was then prepared for the docking studies by processing it using Schrödinger's protein preparation facility. This procedure minimizes the protein to 0.30 Å rmsd using the OPLS-2001 force field. Subsequent refinement of the protein/ligand complex was performed using the mixed torsional/low-mode conformational sampling method within Schrödinger's MacroModel program. The OPLS2001 force field was used, and a distance dependent dielectric "constant" further attenuated by a factor of 2 was employed. A 5 Å shell of residues surrounding the partial native ligand (*O*<sup>6</sup>-methylguanine), the ligand itself, and a nearby water molecule (WAT 1) were fully flexible during the conformational sampling/energy minimization phase of the conformational search procedure. The rest of protein was frozen at its crystallographically determined position including residues Asn157 and Arg135, which were within the 5 Å shell of mobile residues. The lowest energy conformer that retained the native ligand pose in the X-ray crystal structure of PDB 1T38 was used for subsequent studies. Structures of *O*<sup>6</sup>-methylguanine, BG, and compounds **1–6** were prepared using Schrödinger's LigPrep facility.

The initial docking studies were done with GLIDE (version 4.5, Schrödinger, LLC, New York, 2007) operating in either SP or XP mode.<sup>42–44</sup> Maestro (version 8.0, Schrödinger Suite 2007, Schrödinger, LLC New York, 2007) was employed as the graphical user interface and for generation of the graphics used in the figures. The best docked structures from XP were used to calculate the ligand partial charges in the environment of the protein and then redocked using Schrödinger's QPLD (quantum polarized ligand docking) method.<sup>27</sup> In order to validate the docking approach, self-docking was performed using the partial native ligand *O*<sup>6</sup>-methylguanine using XP/QPLD. The rms of the docked pose when compared to the crystallographically observed position was 0.14 Å, and thus, GLIDE produced a docking mode that closely resembled the X-ray crystal structure.

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**Supporting Information Available:** Elemental analysis results for compounds 1–7. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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