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Research Article

Synthesis of radiolabeled O^6-benzylguanine derivatives as new potential PET tumor imaging agents for the DNA repair protein O^6-alkylguanine-DNA alkyltransferase

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Summary

Novel radiolabeled O^6-benzylguanine derivatives, 2-amino-6-O-[^{11}C]-[(methoxymethyl)benzyloxy]-9-benzyl purines ([^{11}C]*p*-O^6-AMBP, **1a**; [^{11}C]*m*-O^6-AMBP, **1b**; [^{11}C]*o*-O^6-AMBP, **1c**), have been synthesized for evaluation as new potential positron emission tomography (PET) tumor imaging agents for the DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (AGT). The appropriate precursors for radiolabeling were obtained in three steps from

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starting material 2-amino-6-chloropurine with moderate to excellent chemical yields. Tracers were prepared by O-[^{11}C]methylation of hydroxymethyl precursors using [^{11}C]methyl triflate. Pure target compounds were isolated by solid-phase extraction (SPE) purification procedure in 45–60% radiochemical yields (decay corrected to the end of bombardment), and a synthesis time of 20–25 min.

Key Words: radiolabeled O^6-benzylguanine derivatives; 2-amino-6-O-[^{11}C]-[(methoxymethyl)benzyloxy]-9-benzyl purines; carbon-11; positron emission tomography; DNA repair protein; O^6-alkylguanine-DNA alkyltransferase

Introduction

DNA repair enzyme O^6-alkylguanine-DNA alkyltransferase (AGT) is a suicide protein as it reverts alkylator cytotoxicity by transferring the O^6-alkyl group from the modified DNA guanine to cysteine-145 in its active site in an irreversible, stoichiometric process, thereby restoring a normal guanine at the site of the modified base.[1] However, the resultant alkylated AGT is inactivated for subsequent dealkylations. Therefore, the ability of tumor cells to be resistant to toxic effects of alkylating agents is dependent on cellular AGT levels; the higher the AGT content, the less effective will be tumor killing by alkylator therapy.[2] Tumor cells frequently express high levels of AGT. Human AGT is encoded by the O^6-methylguanine-DNA methyltransferase (MGMT) gene.[1] Elevated levels of AGT are known to result in resistance of human gliomas to nitrosoureas.[3] AGT plays a critical role in protecting cancer cells from the cytotoxic effects of chemotherapeutic drugs that alkylate the O^6 position of guanine.[4] The inactivation of AGT by administration of substrates such as O^6-benzylguanine (O^6-BG) has been shown to increase the cytotoxicity of alkylators in human tumor cell lines[5,6] and xenografts.[7] O^6-BG provides a means to effectively inactivate the AGT protein and increase the chemotherapeutic effectiveness of alkylating agents.[8]

The overexpression of AGT in tumors indicates that AGT is a suitable target for tumor imaging agent development. Positron emission tomography (PET) is a functional medical imaging modality that can probe tumor cell physiology and gene expression. Using PET and a positron-labeled O^6-BG analog, which binds selectively to AGT, may prove to be a useful tool for monitoring AGT levels in tumor tissues and for evaluating the effectiveness of drug and genetic strategies to

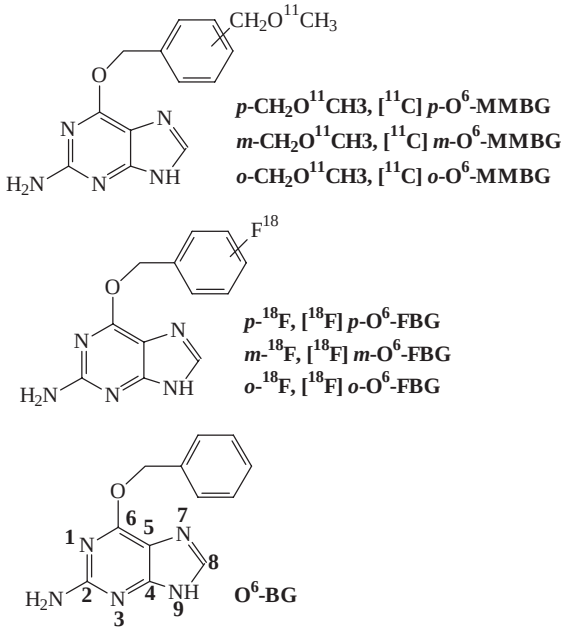


Figure 1. Structure of O^6-BG, [^{11}C]O^6-MMBG and [^{18}F]O^6-FBG

down-regulate AGT in tumors and/or up-regulate its expression in chemotherapy sensitive tissues, such as bone marrow.[9]

A number of carbon-11 and fluorine-18 labeled O^6-BG analogs have been developed in this laboratory.[10] 6-O-([^{11}C]methoxymethylbenzyl)-guanine ([^{11}C]O^6-MMBG) and 6-O-([^{18}F]Fluorobenzyl)guanine ([^{18}F]O^6-FBG) (Figure 1) are our initial target radiotracers; however, the low radiochemical yields, long reaction time and complicated purification methods in their radiochemical syntheses have prevented the reliable routine production and automation of these tracers. In order to explore novel radiolabeled O^6-BG analogs as alternative candidate agents, we have synthesized *p*-, *m*-, and *o*-derivatives of 2-amino-6-O-[^{11}C]-[(methoxymethyl)benzyloxy]-9-benzyl purines ([^{11}C]*p*-O^6-AMBP, **1a**; [^{11}C]*m*-O^6-AMBP, **1b**; [^{11}C]*o*-O^6-AMBP, **1c**).

Results and discussion

O^6-BG is a well-known low-molecular-weight inhibitor of mammalian AGT.[11] The application of O^6-BG and a number of alkylating agents in combination cancer chemotherapy is currently being employed in

clinical trials. Numerous O^6-BG derivatives and their related compounds have been synthesized and tested for their ability to inhibit AGT activity, which include those derivatives modified at the 2-amino group, 7-nitrogen, 9-nitrogen, and the *para* position of the O^6-benzyl group.[12–14] Our objective was to develop radiolabeled O^6-BG analogs as PET tumor imaging agents for AGT. The radiochemistry strategy was to synthesize guanine derivatives modified at the 9-nitrogen position and labeled at the O^6-benzyl group. Here we report the synthesis of radiolabeled O^6-BG derivatives **1a**–**c**.

The affinity of unlabeled standard samples of **1a–c** was evaluated via an *in vitro* AGT oligonucleotide assay.[15–17] The three compounds **1a–c** proved to be potent AGT inhibitors albeit not as effective as the model compound O^6-BG, which was synthesized in our laboratory.[10] The concentration giving $\geq 90\%$ inhibition of AGT activity in HeLa cell extracts were as follows: O^6-BG = 10 μM, *p*-O^6-AMBP (**1a**) = ~150 μM, *m*-O^6-AMBP (**1b**) = > 150 μM, *o*-O^6-AMBP (**1c**) = ≫ 150 μM (Table 1). The ranking is therefore O^6-BG > **1a** ≥ **1b** > **1c**. These results were not unexpected, because none of the O^6-BG derivatives have been reported to be more potent AGT inhibitors than O^6-BG, with the exception of three classes of inhibitors that may have more activity than O^6-BG

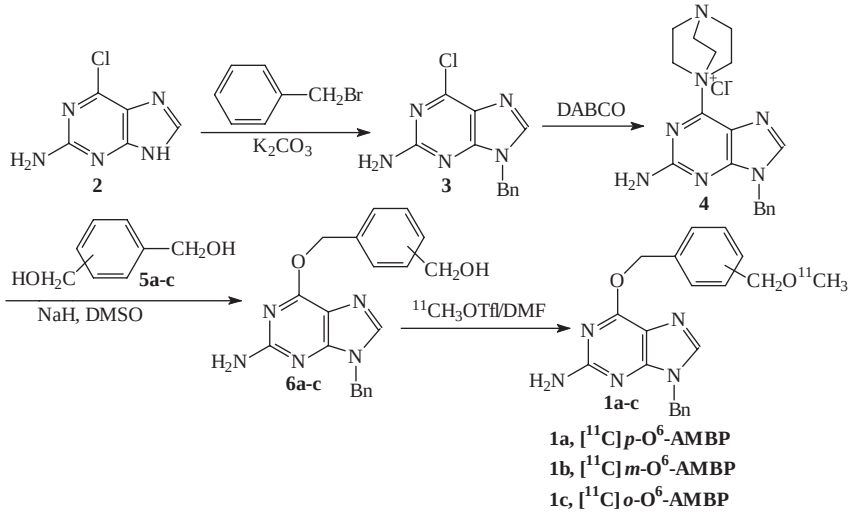
Table 1. AGT-inhibitory activity of O^6-BG, *p*-O^6-AMBP (1a), *m*-O^6-AMBP (1b), and *o*-O^6-AMBP (1c), in fmol O^6-methylguanine (O^6-MeG) removed/mg of protein

	fmol O^6-MeG removed/mg of protein ± S.D. (*n*)
HeLa diluent control	2020 ± 496 (3)
HeLa MR (methylation repair deficient)	48 ± 44 (3)
O^6-BG 0.1 μM	2272 ± 748 (3)
O^6-BG 1 μM	480 ± 792 (4)
O^6-BG 10 μM	29 ± 50.8 (3)
p-O^6-AMBP 1 μM	2044 ± 395 (4)
p-O^6-AMBP 10 μM	1508 ± 1132 (4)
p-O^6-AMBP 50 μM	840 ± 728 (3)
p-O^6-AMBP 150 μM	68 ± 112 (3)
m-O^6-AMBP 1 μM	2096 ± 320 (4)
m-O^6-AMBP 10 μM	2020 ± 748 (4)
m-O^6-AMBP 50 μM	816 ± 608 (3)
m-O^6-AMBP 150 μM	333 ± 416 (3)
o-O^6-AMBP 1 μM	1940 ± 856 (4)
o-O^6-AMBP 10 μM	1612 ± 920 (4)
o-O^6-AMBP 50 μM	1308 ± 1024 (3)
o-O^6-AMBP 150 μM	888 ± 319 (3)

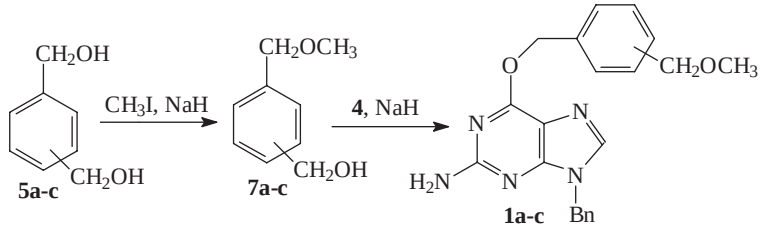
in vitro including: (1) those O^6-BG derivatives bearing electron-withdrawing groups (electronic effect) at the 8-position;[18] (2) some O^6-(hetarylmethyl)guanines;[19] and (3) 2,4-diamino-6-benzylpyrimidines (BP) bearing electron-withdrawing groups at the 5-position.[20] However, these three classes of inhibitors are not ready for *in vivo* studies, since it is difficult to make BG derivatives labeled at the 8-position; the toxicity of O^6-(hetarylmethyl)guanines remains to be investigated; and BP derivatives are not as effective as O^6-BG in mouse liver or spleen due to their short half-life *in vivo*.[21] The potentiation effect order of the substituted O^6-BG analogs is *para* > *meta* > *ortho*,[22,23] most likely because the steric effect at the *ortho*-position plays a more important role than the electronic effect. Preliminary findings from the biological assay of unlabeled **1a**, **1b**, and **1c** warrant further evaluation of radiolabeled **1a**, **1b**, and **1c** as new potential PET tumor imaging agents for the AGT *in vivo*.

The synthetic approaches for radiolabeled **1a–c** and unlabeled **1a–c** were shown in Schemes 1 and 2.

The commercially available starting material 2-amino-6-chloropurine (ACP, **2**) was reacted with benzyl bromide to give the desired 9-isomer, 2-amino-9-benzyl-6-chloropurine (ACP-Bn, **3**), and the byproduct 7-isomer. **3** was reacted with 1,4-diazabicyclo[2.2.2]octane (DABCO)



Scheme 1. Synthetic approach for [^{11}C]*p*-O^6-AMBP, [^{11}C]*m*-O^6-AMBP, and [^{11}C]*o*-O^6-AMBP



Scheme 2. Synthetic approach for unlabeled *p*-O^6-AMBP, *m*-O^6-AMBP, and *o*-O^6-AMBP

to convert into its quaternary ammonium salt, 1-(2-amino-9-benzyl-9H-purin-6-yl)-4-aza-1-azoniabicyclo[2.2.2]octane chloride (**4**).[24] The crude product **4**, without further purification, was reacted with the sodium alkoxide of benzene 1,4-, 1,3-, or 1,2-dimethanols (**5a**, **5b**, or **5c**) to provide the corresponding precursors, *p*-, *m*-, or *o*- substituted 2-amino-6-O-[(hydroxymethyl)benzyloxy]-9-benzyl purine (*p*-O^6-AHBP, **6a**; *m*-O^6-AHBP, **6b**; *o*-O^6-AHBP, **6c**). The overall chemical yields of **6a–c** from ACP were moderate to excellent.

To prepare the standard samples, unlabeled **1a**, **1b**, and **1c**, **5a–c** were methylated by methyl iodide under strong basic condition using sodium hydride to give 1,4-, 1,3-, or 1,2-methoxymethyl-benzyl alcohol (**7a**, **7b**, or **7c**). To obtain the mono-methylated products of benzenedimethanol, the suitable ratio of benzenedimethanol to sodium hydride was 1:5. **7a–c** were then coupled with quaternary ammonium salt **4** under basic condition using sodium hydride to give the standard samples **1a–c**.

The precursors **6a–c** were labeled by [^{11}C]methyl triflate[25] through O-[^{11}C]methylation of hydroxymethyl position under basic conditions.[26–29] The tracers were isolated by solid-phase extraction (SPE) purification[26] to produce pure target compounds with 45–60% radiochemical yields (based on $^{11}CO_2$, decay corrected to end of bombardment), and 20–25 min synthesis time. The large polarity difference between the precursor and the labeled methylated product permitted the use of SPE technique for purification of radioligands from radiolabeling reaction mixture. The reaction mixture was diluted with $NaHCO_3$ and loaded onto C-18 cartridge by gas pressure. The cartridge column was washed with water to remove unreacted precursor and reaction solvent, and then the final labeled product was eluted with ethanol. Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC methods. The chemical purity of

precursors **6a**, **6b**, and **6c**, and standard samples **1a**, **1b**, and **1c** was >97%. The radiochemical purity of target radiotracers **1a–c** was >99%, and the chemical purities of target radiotracers **1a–c** were ~98% (**1a**), ~95% (**1b**), ~93% (**1c**). The average ($n = 5$–10) specific activity of target radiotracers **1a–c** was 0.6–0.8 Ci/μmol at end-of-synthesis (EOS).

Conclusion

We have developed synthetic procedures that provide radiolabeled O^6-BG derivatives **1a**, **1b**, and **1c**. Preliminary findings from biological assay indicate the synthesized analogs were less effective but have similar inhibitory effectiveness on AGT in comparison with O^6-BG. The results warrant further evaluation of these radiotracers as new potential PET tumor imaging agents for the DNA repair protein AGT *in vivo*.

Experimental

All commercial reagents and solvents were used without further purification unless otherwise specified. The [^{11}C]methyl triflate was made according to a literature procedure[25] by the metathetical reaction of [^{11}C]methyl bromide over a hot column of silver triflate supported on porous graphite beads. One silver triflate column was used for 20–40 [^{11}C]methyl triflate runs before replacement. Melting points were determined on an MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal standard TMS (δ 0.0). The low resolution mass spectra were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and the high-resolution mass measurements were obtained using a Kratos MS80 mass spectrometer, in the Department of Chemistry at Indiana University. Chromatographic solvent proportions are expressed on a volume: volume basis. Thin layer chromatography was run using Analtech silica gel GF uniplates ($5 \times 10\,cm^2$). Plates were visualized by UV light. Normal phase flash chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described

below. All moisture-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, $4.6 \times 250\,mm^2$; 3:1:3 CH_3CN: MeOH: 20 mM, pH 6.7 $KHPO_4^-$ (buffer solution) mobile phase, flow rate 1.5 ml/min, and UV (240 nm) and γ-ray (NaI) flow detectors. Semi-prep C-18 silica guard cartridge column $1 \times 1\,cm^2$ was obtained from E. S. Industries, Berlin, NJ, and part number 300121-C18-BD 10 μm. Sterile vented Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.

*2-Amino-9-benzyl-6-chloropurine (ACP-Bn, **3**)*

Benzyl bromide (2.25 ml, 18.7 mmol) was added to a mixture of 2-amino-6-chloropurine (**2**, 2.13 g, 12.5 mmol), potassium carbonate (5.2 g, 37.5 mmol), and anhydrous DMF (60 ml). The mixture was stirred at room temperature overnight with exclusion of moisture. The suspension was filtered and the filtrate was taken down to dryness under vacuum. The residue chromatographed on a silica gel column by 4/1 CH_2Cl_2/EtOAc. Elution and evaporation afforded the title compound **3** (2.27 g, 70%) as a white solid, mp 202–204°C. ^1H NMR (300 MHz, DMSO-d_6): δ 5.31 (s, 2H, $CH_2N<$), 6.96 (s, br, 2H, NH_2, exchange with D_2O), 7.25–7.38 (m, 5H, ArH), 8.24 (s, 1H, 8-H).

*1-(2-Amino-9-benzyl-9H-purin-6-yl)-4-aza-1-azoniabicyclo[2.2.2]octane chloride (**4**)*

ACP-Bn (**3**) (519.4 mg, 2 mmol) was reacted with 1,4-diazabicyclo [2.2.2]octane (DABCO) (1.23 g, 11 mmol) in anhydrous DMF (4 ml) at room temperature overnight. The mixture was filtered, and the resulting solid was washed with acetone and dried under vacuum to afford a quaternary ammonium salt **4** as a light yellow solid. The crude product was used for the next step reaction without further purification.

*2-Amino-6-O-[(4′-hydroxymethyl)benzyloxy]-9-benzyl purine (p-O^6-AHBP, **6a**)*

Sodium hydride (80 mg, 2 mmol, 60% in oil) was added into a solution of 1,4-benzenedimethanol (**5a**) (1.38 g, 10 mmol) in anhydrous DMSO (1.0 ml). The mixture was stirred for 1 h at room temperature.

The quaternary ammonium salt **4** (372 mg, 1 mmol) was added into the mixture, which was stirred overnight. After addition of acetic acid (185 µl), the mixture was adsorbed onto a silica gel column and eluted with 8/1 CH_2Cl_2/MeOH to give a white solid **6a** (260 mg, 72%), mp 179–181°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.52 (d, 2H, $J = 5.89$ Hz, $\underline{CH_2}OH$, when exchanged with D_2O, d changed to s), 5.24 (m, 1H, OH, exchange with D_2O), 5.26 (s, 2H, $CH_2N<$), 5.49 (s, 2H, O^6-CH_2), 6.51 (s, 2H, NH_2, exchange with D_2O), 7.21–7.31 (m, 5H, N^9-ArH), 7.33–7.36 (d, 2H, $J = 7.4$ Hz, O^6-ArH), 7.45–7.48 (d, 2H, $J = 7.4$ Hz, O^6-ArH), 7.97 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{20}H_{19}O_2N_5$ 361.1539, found 361.1521.

2-Amino-6-O-[(3′-hydroxymethyl)benzyloxy]-9-benzyl purine (m-O^6-AHBP, ***6b****)*

Prepared as **6a** starting with 1,3-benzenedimethanol (**5b**) and quaternary ammonium salt **4** on the same scale to give a white solid **6b** (253 mg, 70%), mp 134–136°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.55 (d, 2H, $J = 5.89$ Hz, $\underline{CH_2}OH$, when exchanged with D_2O, d changed to s), 5.17 (t, 1H, $J = 5.1$ Hz, OH, exchange with D_2O), 5.26 (s, 2H, $CH_2N<$), 5.51 (s, 2H, O^6-CH2), 6.24 (s, 2H, NH_2, exchange with D_2O), 7.21–7.47 (m, 7H, ArH), 7.47 (s, 1H, O^6-ArH), 7.84 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{20}H_{19}O_2N_5$ 361.1539, found 361.1526.

2-Amino-6-O-[(2′-hydroxymethyl)benzyloxy]-9-benzyl purine (o-O^6-AHBP, ***6c****)*

Prepared as **6a** starting with 1,2-benzenedimethanol (**5c**) and quaternary ammonium salt **4** on the same scale to give a white solid **6c** (199 mg, 55%), mp 156–158°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.68 (s, 2H, $\underline{CH_2}OH$), 5.22 (m, 1H, OH, exchange with D_2O), 5.27 (s, 2H, $CH_2N<$), 5.58 (s, 2H, O^6-CH_2), 6.40 (s, 2H, NH_2, exchange with D_2O), 7.24–7.36 (m, 7H, ArH), 7.47–7.52 (m, 2H, ArH), 7.93 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{20}H_{19}O_2N_5$ 361.1539, found 361.1528.

*The general method to prepare 1,4-, 1,3-, and 1,2-methyoxymethyl-benzyl alcohol (****7a****,* ***7b****,* ***7c****)*

Sodium hydride (80 mg, 2 mmol, 60% in oil) was added in the solution **5a**, **5b**, or **5c** (1.38 g, 10 mmol) in anhydrous THF (15 ml). The mixture

was stirred at room temperature for 1 h. To this mixture was added neat methyl iodide (123 µl, 2 mmol), and the resulting mixture was stirred overnight. The mixture was evaporated and purified by flash column chromatography to give the desired product **7a**, **7b**, or **7c** as colorless oil at a yield of 60–80%. **7a**, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.85 (s, br, 1H, $ArCH_2\underline{OH}$, exchange with D_2O), 3.34 (s, 3H, OCH_3), 4.41 (s, 2H, $Ar\underline{CH_2}O$), 4.57 (s, br, 2H, $Ar\underline{CH_2}OH$), 7.28 (s, 4H, ArH). **7b**, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (s, br, 1H, $ArCH_2\underline{OH}$, exchange with D_2O), 3.35 (s, 3H, OCH_3), 4.42 (s, 2H, $Ar\underline{CH_2}O$), 4.58 (s, br, 2H, $Ar\underline{CH_2}OH$), 7.23–7.31 (m, 4H, ArH). **7c**, ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.39 (s, 3H, OCH_3), 3.64 (s, br, 1H, $ArCH_2\underline{OH}$, exchange with D_2O), 4.53 (s, 2H, $Ar\underline{CH_2}O$), 4.62 (s, br, 2H, $Ar\underline{CH_2}OH$), 7.28–7.37 (m, 4H, ArH).

*2-Amino-6-O-[(4′-methoxymethyl)benzyloxy]-9-benzyl purine (p-O^6-AMBP, **1a**)*

Sodium hydride (40 mg, 1 mmol, 60% in oil) was added to a solution of **7a** (152 mg, 1 mmol) in anhydrous DMSO (0.5 ml), and the mixture was stirred at room temperature for 1 h. To this mixture was added the quaternary ammonium salt **4** (186 mg, 0.5 mmol), and the mixture was stirred at room temperature for 3 h. The acetic acid (80 µl) was used to neutralize the mixture. The mixture was evaporated and purified by flash chromatography to give the unlabeled standard sample **1a (**152 mg, 81%) as a white solid, mp 129–130°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.35 (s, 3H, OCH_3), 4.43 (s, 2H, CH_2O), 5.07 (s, 2H, H_2N, exchange with D_2O), 5.19 (s, 2H, $CH_2N<$), 5.55 (s, 2H, O^6-CH_2), 7.19–7.21 (m, 2H, ArH), 7.29–7.32 (m, 5H, ArH), 7.46–7.49 (d, 2H, $J = 8.1$ Hz, ArH), 7.55 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{21}H_{21}O_2N_5$ 375.1695, found 375.1695.

*2-Amino-6-O-[(3′-methoxymethyl)benzyloxy]-9-benzyl purine (m-O^6-AMBP, **1b**)*

Prepared as **1a** starting with **7b** and the quaternary ammonium salt **4** on the same scale to give the unlabeled standard sample **1b** (156 mg, 83%) as a white solid, mp 98–99°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.38 (s, 3H, OCH_3), 4.56 (s, 2H, CH_2O), 4.92 (s, 2H, H_2N, exchange with D_2O), 5.23 (s, 2H, $CH_2N<$), 5.57 (s, 2H, O^6-CH_2), 7.22–7.26 (m, 2H, ArH), 7.30–7.36 (m, 5H, ArH), 7.42–7.48 (d, 2H, $J = 8.1$ Hz, ArH), 7.56

(s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{21}H_{21}O_2N_5$ 375.1695, found 375.1684.

2-Amino-6-O-[(2′-methoxymethyl)benzyloxy]-9-benzyl purine (o-O^6-AMBP, ***1c****)*

Prepared as **1a** starting with **7c** and the quaternary ammonium salt **4** on the same scale to give the unlabeled standard sample **1c** (107 mg, 57%) as a light yellow solid, mp 132–133°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.36 (s, 3H, OCH_3), 4.63 (s, 2H, CH_2O), 5.06 (s, br, 2H, NH_2, exchange with D_2O), 5.20 (s, 2H, $CH_2N<$), 5.63 (s, 2H, O^6-CH_2), 7.20–7.39 (m, 9H, ArH), 7.59 (s, 1H, 8-H). HRMS (CI, CH_4): calcd for $C_{21}H_{21}O_2N_5$ 375.1695, found 375.1685.

Typical experimental procedure for the radiosynthesis of 2-amino-6-O-[^{11}C]-[(methoxymethyl)benzyloxy]-9-benzyl purines ([^{11}C] p-O^6-AMBP, ***1a****; [^{11}C]m-O^6-AMBP,* ***1b****; [^{11}C]o-O^6-AMBP,* ***1c****)*

The precursor (*p*-O^6-AHBP, **6a;** *m*-O^6-AHBP, **6b;** or *o*-O^6-AHBP, **1c)** (0.6–1.0 mg) was dissolved in CH_3CN (300 µl). To this solution was added tetrabutylammonium hydroxide (TBAH) (2–3 µl, 1 M solution in methanol). The mixture was transferred to a small volume, three-neck reaction tube. [^{11}C]methyl triflate was passed into the air-cooled reaction tube at −15 to −20°C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until activity reached a maximum (~3 min), then the reaction tube was heated at 70–80°C for 3 min.The contents of the reaction tube were diluted with 0.1 M $NaHCO_3$ (1 ml). This solution was passed onto a C-18 cartridge by gas pressure. The cartridge was washed with 2 × 3 ml H_2O, and the aqueous washing was discarded. The product was eluted from the column with 2 × 3 ml EtOH, and then passed onto a rotatory evaporator. The solvent was removed by evaporation under high vacuum. The labeled product **1a-c** was formulated with 50 mM NaH_2PO_4, whose volume was dependent upon the use of the labeled product **1a-c** in tissue biodistribution studies (~6 ml, 3 × 2 ml) or in micro-PET imaging studies (1–3 ml) of the tumor bearing mice, sterile-filtered through a sterile vented Millex-GS 0.22 µm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis time was ~20 min. The decay corrected yield, from $^{11}CO_2$, was 45–60%, and the

radiochemical purity was >99% by analytical HPLC. Retention times in the analytical HPLC system were: RT**6a** = 3.58 min, RT**6b** = 3.84 min, RT**6c** = 3.98 min; RT**1a** = 5.70 min, RT**1b** = 5.90 min, RT**1c** = 6.18 min.

In vitro AGT (MGMT) oligonucleotide assay

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DME) supplemented with 10% bovine calf serum. Measurement of cellular AGT (MGMT) activity was performed by a modification of the assay described by Wu *et al.*[15] Control and drug treated cells were washed in cold PBS, pH 7.4 and resuspended in cold assay buffer (Tris-HCl, pH 8, 5% glycerol, 1 mM DTT, 1 mM EDTA). The cells were sonicated in 400 µl of assay buffer for 5 s on ice. The lysate was pelleted by centrifugation for 5 min, at 12,000 g, at 4°C. The protein concentrations were determined by the Pierce Coomassie plus protein assay (Pierce, Rockford, IL). 50 µg of total cell protein were then reacted with 0.2 pmol of 5′-Hex-labeled 18mer oligo in assay buffer for 2 h, at 37°C. The reaction was terminated by two phenol:chloroform extractions, one chloroform:isoamyl alcohol extraction, and then ethanol-precipitated in the presence of 1 µg carrier glycogen. Following precipitation, the precipitate was washed with 70% ethanol, dried under vacuum, and reacted with three units of PvuII (Promega, Madison, WI) in a total volume of 20 µl for 2 h at 37°C. The reaction was terminated by the addition of 10 µl of gel loading buffer (96% formamide, 1 mM EDTA, pH 8) and heating at 90°C for 2 min. Samples (**1a**–**c**, and O^6-BG) were chilled on ice and loaded directly on to the gel. The samples were electrophoresed through a 1.5 mm, 20% acrylamide, 7 M urea gel, at 300 V, for approximately 30 min.The gels were then placed on an FMBIO II fluorescent scanner (MiraiBio, South San Francisco, CA) and quantitated using the calculated fluorescent absorbance. AGT specific activity (fmol of O^6-methylguanine removed/mg of protein) was calculated according to the following equation:

fluorescent units of 8-bp fragment/fluorescent units of 18-bp fragment x 200 fmol/mg protein.

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