Synthesis of ^{131}I-Labeled Glucose-Conjugated Inhibitors of O^6-Methylguanine-DNA Methyltransferase (MGMT) and Comparison with Nonconjugated Inhibitors as Potential Tools for in Vivo MGMT Imaging

Ute Mühlhausen,[†] Ralf Schirrmacher,*[,‡] Markus Piel,[†] Bernd Lecher,[§] Manuela Briegert,[§] Andrea Piee-Staffa,[§] Bernd Kaina,[§] and Frank Rösch*[,†]

Institute of Nuclear Chemistry, University of Mainz, Fritz Strassmann-Weg 2, D-55128 Mainz, Department of Nuclear Medicine, University of Mainz, Langenbeckstr. 1, D-55131 Mainz, and Department of Toxicology, University of Mainz, Obere Zahlbacher Strasse 67, D-55131 Mainz, Germany

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O^6-Substituted guanine derivatives are powerful agents used for tumor cell sensitization by inhibition of the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT). To provide targeted accumulation of MGMT inhibitors in tumor tissue as well as tools for in vivo imaging, we synthesized iodinated C_8-alkyl-linked glucose conjugates of 2-amino-6-(5-iodothenyl)-9*H*-purine (O^6-(5-iodothenyl) guanine, ITG) and 2-amino-6-(3-iodobenzyloxy)-9*H*-purine (O^6-(5-iodobenzyl) guanine, IBG). These compounds have MGMT inhibitor constants (IC_{50} values) of 0.8 and 0.45 μM for ITGG and IBGG, respectively, as determined in HeLa S3 cells after 2-h incubation with inhibitor. To substantiate that the ^{131}I−(hetero)arylmethylene group at the O^6-position of guanine is transferred to MGMT, both the glucose conjugated inhibitors ITGG and IBGG and the corresponding nonglucose conjugated compounds ITG and IBG were labeled with iodine-131. The radioiodinations of all compounds with [^{131}I]I^- were performed with radiochemical yields of >70% for the destannylation of the corresponding tri-*n*-butylstannylated precursors. The binding ability of [^{131}I]-ITGG, [^{131}]IBGG, [^{131}I]ITG, and [^{131}I]IBG to purified MGMT was tested. All radioactive compounds were substrates for MGMT, as demonstrated using a competitive repair assay. The newly synthesized radioactive inhibitors were utilized to study ex vivo biodistribution in mice, and the tumor-to-blood ratio of tissue uptake of [^{131}I]IBG and [^{131}I]IBGG was determined to be 0.24 and 0.76 after 0.5 h, respectively.

Introduction

Many cytostatic drugs used in cancer chemotherapy provoke cytotoxic effects through their covalent reaction with DNA. Apart from platinium-containing drugs, most of these are alkylating agents that mostly target purines in DNA.[1] A biologically relevant site is the O^6-position of guanine.[2] However, the therapeutic effectiveness of alkylating agents can be significantly attenuated by the ability of tumor cells to repair DNA damage. Enzymes responsible for removal of DNA lesions act to protect cells from the cytotoxic effects of alkylating drugs. An important player in alkylating drug resistance is the DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT), which transfers alkyl groups from the O^6-position of guanine to an internal cysteine residue.[3] The reaction is stoichiometric in the sense that each MGMT molecule can accept only one alkyl group. Following the reaction, the MGMT protein is inactivated and degradated. Since MGMT is one of the most important factors determining resistance to methylating and chloroethylating agents, also causing acquired resistance of tumor cells against these chemotherapeutics, strategies have been developed to suppress MGMT activity in tumors by means of MGMT pseudosubstrates. A great variety of different MGMT inhibitors have been synthesized and evaluated as to their MGMT inhibitory potency.[4−7] The chemistry of these MGMT inhibitors has been reviewed recently.[8]

The structural requirements of O^6-substituted guanines for efficient depletion of human MGMT were demonstrated by Moschel et al.[9] They showed that O^6-benzylguanine (O^6-BG) and O^6-(*p*-chlorobenzyl)guanine are alternative substrates for the enzyme, causing rapid depletion of MGMT in human tumor cell extracts and intact cells.[10] They synthesized and tested a series of O^6- and S^6-substituted guanine derivatives for their ability to deplete the human MGMT in cell-free extracts of tumor cells as well as in intact cells. The order of potency for inactivation of the enzyme was shown to be strongly dependent on structure. In 1998, McElhinney et al. synthesized novel O^6-substituted guanine derivatives with heterocyclic moieties, and among them was one of the most active inhibitors to date, O^6-(5-bromothenyl)guanine (O^6-5-BTG), which was about 10 times more effective than O^6-BG.[11]

Although most of these compounds showed encouraging in vitro results and also no systemic side effects in patients, a phase II trial combining O^6-BG and carmustin reported no beneficial outcome on patients with glioblastoma.[12] The therapeutic failure might be attributed to the fact that the dose level required for effective inhibition of MGMT could not be achieved within the tumor due to nonselective accumulation of the drug in nontumor tissue. This lack of selectivity of MGMT inhibitors for neoplastic tissue was presumably responsible for the systemic sensitization of all tissues, while the increase in systemic sensitivity forced the use of lower doses of carmustine during therapy.

A potential strategy for selective transport of MGMT inhibitors is synthesizing glucose-conjugated MGMT antagonists in order to target glucose transporters that are often overexpressed in tumor cells.[13] O^6-BG and O^6-(4-bromothenyl) guanine (O^6-4-BTG) were linked to β-D-glucose at the N^9-position of

* Corresponding authors. Tel: +49 6131 17 6736. Fax: +49 6131 17 2386. E-mail: schirrmacher@nuklear.klinik.uni-mainz.de (R.S.). Tel: +49 6131 39 25302. Fax: +49 6131 39 24510. E-mail: Frank.Roesch@uni-mainz.de (F.R.).

[†] Institute of Nuclear Chemistry.

[‡] Department of Nuclear Medicine.

[§] Department of Toxicology.

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the guanine moiety by means of alkyl spacers of different length and were evaluated as to their MGMT inhibition. The targeting concept, i.e., "Will the glucose linked MGMT inhibitor be targeted to the tumor?" still needs to be addressed.

In the context of cancer therapy, a noninvasive method for determining the MGMT status of a given tumor might be helpful for individualized cancer therapy. Thus, if the tumor MGMT level is high, cancer therapy using O^6-alkylating chemotherapeutics would not be advisable and these therapeutics should be replaced by alternative therapeutic strategies. Efforts during the past several years to synthesize radioactively labeled MGMT inhibitors for the in vivo mapping of MGMT concentrations in humans have been unable to quantify the MGMT level.[14−19] It has been demonstrated recently with ^{131}I-iodinated O^6-BG that a tracer with significantly improved selectivity is required for the in vivo mapping of MGMT.[20] The major drawback was a low tumor-to-blood ratio that hindered the noninvasive determination of tumor MGMT status due to high background radioactivity. Therefore, a tracer with in vivo pharmacological properties imparting greater blood clearance and improved tumor targeting is required. Glucosidation is a general strategy for improving both drug solubility and targeting via glucose transporters. Thus, conjugation of MGMT inhibitors with glucose might facilitate targeted depletion of MGMT activity in neoplastic tissue, while the uptake in normal tissue might be minimized. A ^{18}F-labeled MGMT−glucose conjugate based on 2-amino-6-(2-[^{18}F]fluoropyridine-4-ylmethoxy)-9*H*-purine has already been synthesized, but low radiochemical yields and a suboptimal IC_{50} value of 1.8 μM make this compound ineffective as a tracer for quantifying MGMT in vivo.[21] It has also been demonstrated recently that iodinated glucose conjugates, namely, 2-amino-6-(3-iodobenzyloxy)-9-(octyl-β-D-glucosyl)-purine (IBGG) and 2-amino-6-(5-iodothiophen-2-ylmethoxy)-9-(octyl-β-D-glucosyl)purine (ITGG), displayed better in vitro characteristics than the corresponding fluorinated compounds.[22] To evaluate radioiodinated MGMT inhibitor−glucose conjugates, we synthesized iodine-131-labeled derivatives of O^6-BG and O^6-(5-iodothenyl)guanine (ITG) and their corresponding glucose conjugates. Here we report on the synthesis strategy and properties of the compounds, and we verify that these inhibitors are active by showing transfer of the radioactively labeled ^{131}I-(hetero)arylmethylene group to MGMT. In biodistribution studies with the iodobenzyl derivatives in nude mice containing a tumor xenograft, we also determined the tumor-to-blood ratio, giving evidence that the radioactively labeled MGMT ligands are suitable for in vivo imaging of MGMT status.

Results and Discussion

Chemistry. The syntheses of the nonradioactive standard compound 2-amino-6-(5-iodothenyl)-9-(octyl-β-D-glucosyl)purine (ITGG) (**6**) (Scheme 1, Table 5) for determining in vitro and in vivo IC_{50} values and the tri-*n*-butylstannylated precursor 2-amino-9-(octyl-β-D-glucosyl)-6-(5-tri-*n*-butylstannylthenyl)-purine (SnTGG) (**8**) (Scheme 1, Table 5) for the radioiodination were performed starting from the trimethylammonia precursor 2-aminopurin-6-yltrimethylamonium chloride[24] (**1**) and 5-iodothenyl alcohol (**2**) (Schemes 1 and 2). D'Auria et al. synthesized 5-iodo-2-thiophenecarbaldehyde by reacting thenyl alcohol with yellow mercury oxide and iodine.[23] As an intermediate product they obtained 5-iodothenyl alcohol.

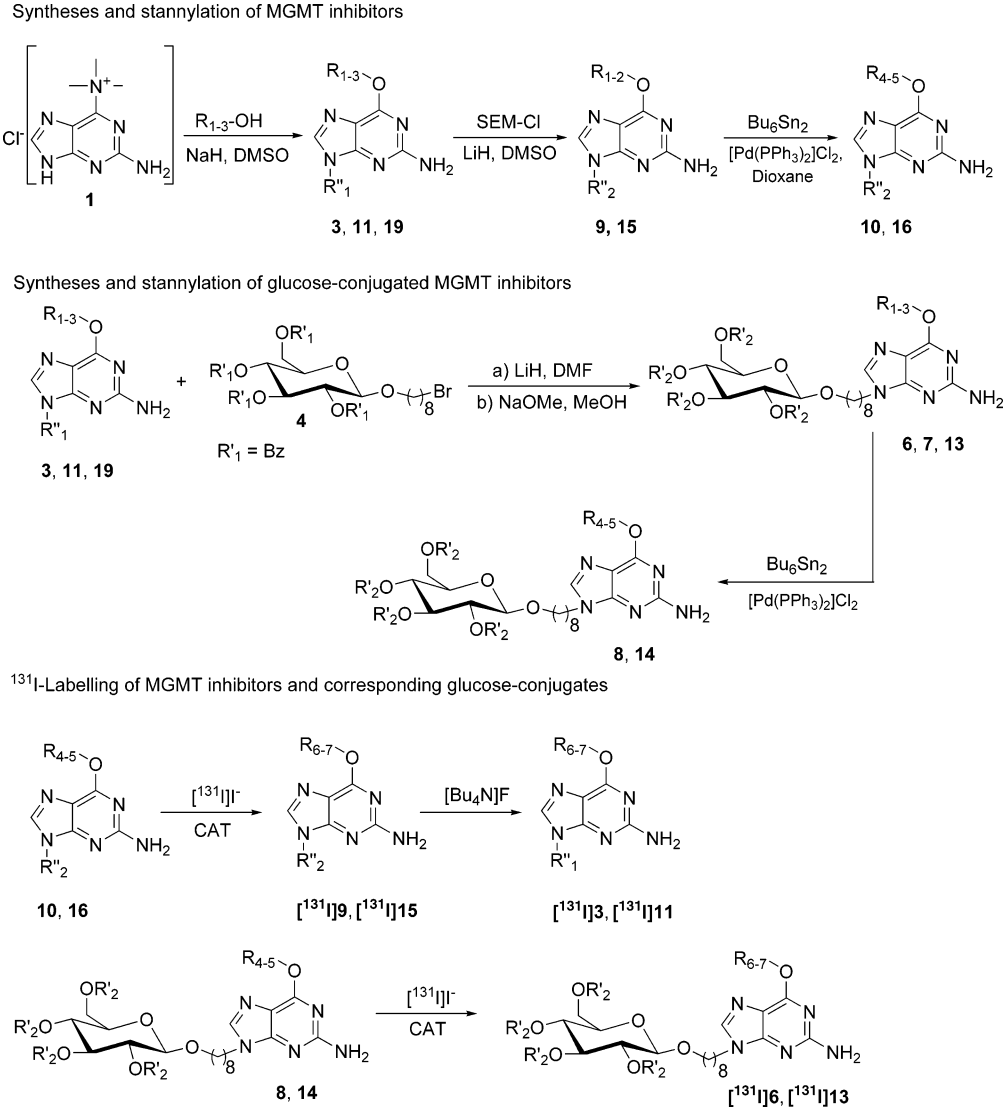
For coupling **2** to the O^6-position of guanine, an adequate leaving group at the O^6-position of guanine is needed. The trimethylammonium salt **1**, which was first described by Kilburis and Lister,[24] has been proven to be a suitable precursor for the syntheses of O^6-substituted guanines in general.[11] Recently, a more convenient synthesis of **1** has been described that avoids highly volatile trimethylamine as a reactant.[25] Despite trimethylammonia being an excellent leaving group, the coupling of alcohols with **1** (Scheme 1) can be difficult, depending on the nature of the alcohol to be coupled. Generally, an excess of the alcohol is deprotonated with sodium hydride, **1** is added, and the mixture is stirred at room temperature.[11] For the reaction of 5-iodothenyl alcohol (**2**) and **1**, basically the procedure of McElhinney et al.[11] was applied with additional precautions such as shielding from light and the use of significantly longer reaction times up to 74 h. 2-Amino-6-(5-iodothenyl)-9*H*-purine (O^6-(5-iodothenyl)guanine, ITG) (**3**) (Scheme 1, Table 5) was obtained in 40% yield.

For the synthesis of the glucose-conjugated derivative ITGG (**6**) for in vitro and in vivo evaluation, the reactive benzoyl-protected glucose-linker 8-bromooctyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **4** had to be prepared (Scheme 2).[13] Preparation of **4** was optimized with boron trifluoride etherate as a catalyst,[30] resulting in a 57% yield. As starting material for this reaction, 2,2,2-trichloroacetimidate 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (**18**) was needed, which was previously synthesized from 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (**17**) (Scheme 2). The synthesis of the benzoyl-protected ITGG derivative **5** (ITGGG) was carried out at room temperature, with light exclusion and by increasing the reaction time up to 23 h, in 53% yield. Cleavage of the benzoyl-protecting groups was achieved by transesterfication under Zémplen conditions (0.1 M sodium methanolate) to give the nonradioactive standard compound ITGG (**6**) for biological evaluation in 56% yield (Scheme 1, Table 5).

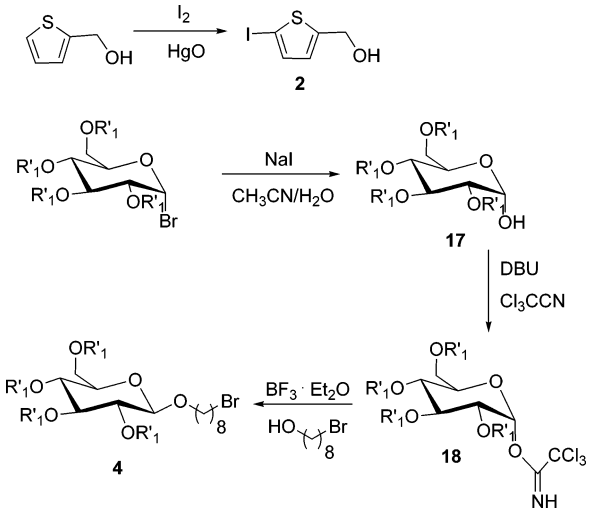
A common method for radioiodination is the radioiododestannylation of the corresponding tri-*n*-butylstannylated precursor. A first attempt to obtain 2-amino-9-(octyl-β-D-glucopyranoside)-6-(5-tri-*n*-butylstannylthenyl)purine (SnTGG) (**8**) started from the iodinated compound **6**, which was dissolved in triethylamine and treated with hexabutylditin and tetrakis-(triphenylphosphine)palladium(0) according to a similar literature procedure.[26] HPLC workup did not yield compound **8**, probably due to the insolubility of **6** in triethylamine. Another procedure for stannylation uses dichlorobis(triphenylphosphine)-palladium(II) as a catalyst and dioxane as a solvent.[19] Although ITGG (**6**) was readily soluble in dioxane, the relatively harsh reaction conditions of 104 °C led to an unwanted deiodination of **6** without formation of the tri-*n*-butylstannylated precursor. As an alternative, the more stable bromo derivative[13] **7** (Table 5) was reacted with hexabutylditin and dichlorobis(triphenylphosphine)palladium(II) as a catalyst (Scheme 1, step E). The desired product SnTGG (**8**) (Table 5) was formed and could be isolated by column chromatography in 74% yield.

For comparing the in vitro and in vivo properties of glucose-conjugated inhibitor ITGG (**6**) to its nonglycosylated form ITG (**3**), radioiodination of ITG (**3**) from the corresponding precursor SnTGSi (**10**) (Table 5) had to be performed. According to a modified literature procedure[19] the first step of preparing **10** was the protection of ITG (**3**) at the N^9-position of guanine with trimethylsilylethoxymethyl chloride (Scheme 1). For the preparation of this protected ITG analogue **9**, LiH as a base was used instead of potassium *tert*-butoxide for the deprotonation of the N^9-proton,[19] yielding the N^9-isomer exclusively in 36% yield. With the silyl-protected derivative **9** the stannylation reaction was performed as described.[19] To obtain larger amounts of SnTGSi (**10**), we reacted ITGSi (**9**) with hexabutylditin and palladium(II) as a catalyst on a larger scale, and column

Scheme 1. Syntheses of MGMT Inhibitors and Corresponding Glucose Derivatives



Scheme 2. Syntheses of 5-Iodothenyl Alcohol and the Glucose Linker



chromatography on silica gel was used for isolation instead of preparative TLC.[19] Column chromatography started with 100% of *n*-hexane to remove nonpolar impurities and continued with *n*-hexane:ethyl acetate (3:2) to elute SnTGSi (**10**) in 18% yield. The deprotection to ITG at the N^9-position was performed after the radioactive labeling with ^{131}I, which is unproblematic due to the long half-life of 8 d and a reaction time for the deprotection of only 25 min.

The preparations of the 3-iodobenzyl derivatives IBG (**11**) and its glucose-conjugated analogue IBGG (**13**) were performed similarly as described for the ITG derivatives **3** and **6** (Scheme 1, Table 5). 3-Iodobenzyl alcohol is commercially available and IBG (**11**) was synthesized as previously described[13] by introducing optimizations such as a longer reaction time for the alcoholysis, which resulted in better yields (97% vs 60%[13]). The conjugation of IBG (**11**) with the glucose linker **4**, performed analogously to the synthesis of ITGGG (**5**), resulted in low yields of IBGGG (**12**) of only 15%. The deprotection of the glucose unit with sodium methanolate (0.1 M) yielded IBGG (**13**) in 64%. In contrast to ITGG (**6**), the stannylation of IBGG (**13**) did not cause any problems and SnBGG (**14**) was obtained in 97% yield. The preparation of the corresponding tri-*n*-butylstannylated precursor SnBGSi (**16**) was performed in the same way as the synthesis of SnTGSi (**10**). After protection with trimethylsilylethoxymethyl chloride at the N^9-position, the final stannylation of IBGSi (**15**) yielded SnBGSi (**16**) in 45%.

Radioactive Labeling. Both electrophilic and nucleophilic aromatic substitutions are common methods for obtaining radioiodinated compounds. The nucleophilic approach requires

high temperatures of about 140 °C for the exchange of bromine with radioiodine, even when a copper(I)-salt is used as a catalyst.[27] For the synthesis of [^{131}I]ITGG (**[^{131}I]6**) the electrophilic labeling method was chosen to avoid possible decomposition and deiodination at higher temperatures. The electrophilic ^{131}I-iodination of the corresponding precursor SnTGG (**8**) was performed by regioselective destannylation under mild reaction conditions using [^{131}I]iodide and chloramine-T (CAT) or Iodogen as oxidizing agents. These oxidizing agents form electrophilic iodine species for subsequent reaction with the aromatic or heteroaromatic system. A first attempt to label SnTGG (**8**) with CAT under acidic conditions (2 N HCl) resulted in an almost quantitative cleavage of the ^{131}I-iodinated iodothenyl alcohol at the O^6-position of guanine. Interestingly, contrasting reports about the acid sensitivity of O^6-substituted guanines were found in the literature. Safadi et al. reported on the instability of O^6-benzylguanine to acidic conditions,[28] whereas Vaidyanathan et al.[19] did not detect any cleavage of the 3-iodobenzyl group from the O^6-position of guanine using TFA for the deprotection of the N^9-position, which is explained to be due to a lack of water. Furthermore, they did not detect any decomposition of [^{131}I]IBG (**[^{131}I]11**) during HPLC purification using 0.1% TFA in water as a cosolvent in contrast to our results. Our next approach using CAT, [^{131}I]I^-, and a phosphate buffer at pH 7.0 yielded the desired product **[^{131}I]6** (Scheme 1) in radiochemical yields (RCY) of up to 93% and a radiochemical purity of >99.9% after HPLC separation. The solvent of the HPLC fractions containing **[^{131}I]6** could be easily removed using a micro rotary evaporator under reduced pressure.

The radioiodination of SnTGSi (**10**) to [^{131}I]ITGSi (**[^{131}I]9**) (Scheme 1) was performed similarly using the CAT/phosphate buffer system with radiochemical yields of 88%. Obviously, the amount of CAT used in the synthesis plays a critical role in obtaining high radiochemical yields. A relatively low concentration of CAT (2.5 μL, c = 1.5 mg CAT/mL) prevented cleavage of the O^6-ethereal linkage that occurred to some extent at higher CAT concentrations. The deprotection[19] of **[^{131}I]9** to [^{131}I]ITG (**[^{131}I]3**) (Scheme 1) was performed in a single step without HPLC separation of the intermediate **[^{131}I]9**. Removal of the trimethylsilylethoxymethyl protecting group was carried out with tetrabutylammonium fluoride solution in THF instead of TFA,[19] due to the instability of **[^{131}I]3** to acidic conditions as observed in our laboratory. After the deprotection, isolation was performed by reverse-phase HPLC and [^{131}I]ITG (**[^{131}I]3**) was obtained in an overall RCY of 71% and a radiochemical purity of >99%. In our experience, it is therefore not necessary to isolate **[^{131}I]9** previous to the deprotection step, making the synthesis more convenient and minimizing the handling of radioactivity. Using normal-phase HPLC and the single-step approach, Vaidyanathan et al.[19] detected an unlabeled side product coeluting with **[^{131}I]-11** which was attributed to the unreacted and deprotected at the N^9-position labeling precursor. This observation could not be confirmed in our laboratory, probably because reverse-phase HPLC was used for purification, preventing coelution.

For the radioiodination of SnBGG (**14**) to [^{131}I]IBGG (**[^{131}I]-13**) (Scheme 1), the CAT/phosphate buffer system at pH 7 was used. Optimization of the reaction conditions such as the amount of CAT and reaction time gave **[^{131}I]13** in 89% RCY with a radiochemical purity of >99.2%. The specific activity of **[^{131}I]-13** was 38 GBq/μmol, as determined by HPLC using a UV calibration curve.

The radioactive synthesis of [^{131}I]IBG (**[^{131}I]11**) was performed analogously to that of [^{131}I]ITG (**[^{131}I]3**). Using a small amount of CAT (2.5 μL, c = 1.5 mg/mL) only, no cleavage of the O^6-benzyl group was observed during the synthesis of the silyl-protected [^{131}I]IBGSi (**[^{131}I]15**). The compound was obtained in 95% RCY and was successfully deprotected to yield final **[^{131}I]11** in 82% RCY and a radiochemical purity of >99.4%.

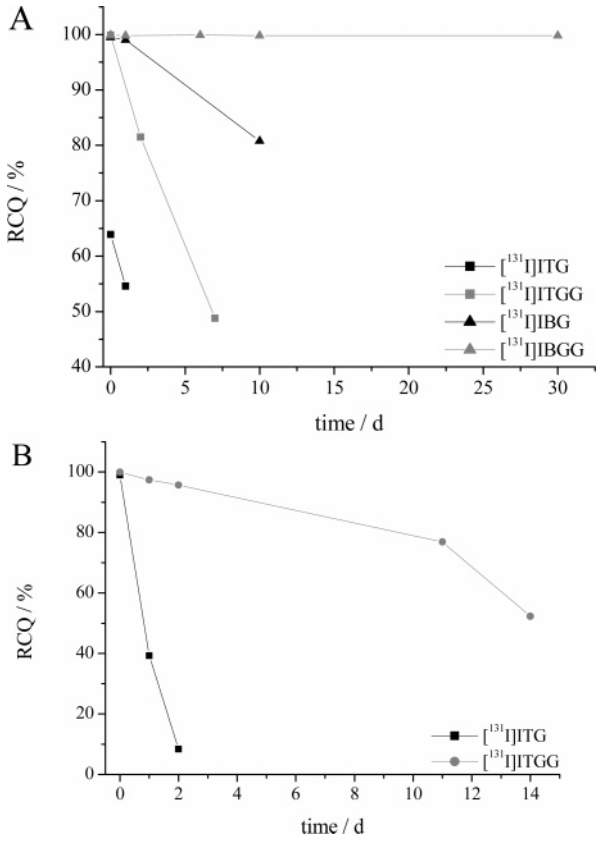


Figure 1. Radiochemical purity of [^{131}I]IBG, [^{131}I]IBGG, [^{131}I]ITG, and [^{131}I]ITGG in physiological saline containing 1% DMSO (A) and of [^{131}I]ITG and [^{131}I]ITGG in phosphate buffer containing 1% DMSO (B).

For further studies, such as in vitro testing in cell cultures and animal studies, the radioiodinated compounds had to be dissolved in physiological saline or in a suitable buffer system. All radioiodinated compounds are sparingly soluble in water. Therefore, we added 1% DMSO to the physiological saline as a cosolvent. Unexpectedly, [^{131}I]ITG and [^{131}I]ITGG (**[^{131}I]3**, **[^{131}I]6**) displayed decomposition in this solvent system, in contrast to the ^{131}I-iodobenzylated compounds **[^{131}I]11** and **[^{131}I]-13** (Figure 1A). No decomposition of the iodothenyl compounds **[^{131}I]3** and **[^{131}I]6** could be observed after 1-h incubation using phosphate buffer (pH 7.0) with 1% DMSO as a cosolvent (Figure 1 B), which was sufficient to start the in vivo and in vitro studies. Different solvents for the longtime storage of radioiodinated compounds, such as physiological saline/1% DMSO (Figure 1A), phosphate buffer/1% DMSO (Figure 1B), and pure ethanol were investigated. Among them, pure ethanol was proven to be the most suitable. All compounds were stable at 5 °C, and even after 1 month a radiochemical purity of >99% was observed. Removing the ethanol directly prior to the application by evaporation and dissolving the radioiodinated compound in phosphate buffer or physiological saline with 1% DMSO or diluting the ethanolic solution directly to an appropriate concentration of ethanol for the given purpose make these compounds suitable for in vitro and in vivo applications.

MGMT Inhibition by ITG (3), ITGG (6), IBG (11), and IBGG (13). We determined the compounds' ability to inactivate MGMT in HeLa S3 cell extracts.[22] As shown in Table 1, the

Table 1. In Vitro IC_{50} Values of Selected MGMT Inhibitors[22]

conjugate	$IC_{50}/\mu M$	conjugate	$IC_{50}/\mu M$
O^6-BG	0.3	ITG	0.75
IBG	0.1	ITGG	0.8
IBGG	0.45		

effective dose to produce 50% inactivation of MGMT (IC_{50}) for the nonglucose conjugated ITG (**3**) and IBG (**11**) was comparable or even better than the IC_{50} value of O^6-BG, an inhibitor used in phase II clinical trials.[12] To obtain tumor-targeting, the glucose-conjugated derivatives were synthesized, and their ability to inhibit MGMT was evaluated. Glucose conjugation reduced the inhibition of MGMT in the in vitro experiments by a factor of 4.5 for IBGG (**13**) when compared to IBG (**11**). For ITG (**3**) and the corresponding ITGG (**6**), the glucose conjugation did not lead to significantly different IC_{50} values for both compounds, which seems to be exceptional because the aforementioned factor of 4.5 for the difference in IC_{50} values was confirmed previously by Reinhard et al.[13] Most importantly, the IC_{50} values of all compounds were in the lower micromolar range and therefore suitable for the validation of the proposed targeting concept via glucose transporters. Furthermore, these novel radioiodinated glucose conjugates might thus serve as potential tracers for the quantification of MGMT in neoplastic tissue, if the appropriate iodine isotope (e.g. [123]I-iodine or [124]Iiodine) is used for labeling.

Binding of [131]ITG ([131]I3), [131]ITGG ([131]I6), [131]I-IBG ([131]I11), and [131]IBGG ([131]I13) to Purified MGMT. To determine the effect of accumulation of the glucose conjugates via overexpressed glucose transporters present on the cell surface of various tumors as well as to determine noninvasively the MGMT level in neoplastic tissue, it is essential that the radiolabel is transferred to MGMT. This is described for [131]IBG (**[131]I11**)[19] but is not proven for the new compounds [131]IBGG (**[131]I13**), [131]ITG (**[131]I3**), and [131]I-ITGG (**[131]I6**). To determine the transfer rate of radioactivity to the purified MGMT, the radiolabeled inhibitor was incubated with purified MGMT (hMGMT), by adding increasing concentrations of the unlabeled compound. At a concentration of 1 μM of unlabeled IBG, 97% of [131]IBG was bound to MGMT. At concentrations higher than 1 μM, a decrease of input activity which bound to MGMT was observed (Figure 2A). For [131]I-IBGG, only 20% bound to MGMT at a concentration of 1 μM of IBGG. The bound activity also decreased with increasing concentrations of unlabeled IBGG (Figure 2A). This difference of the transfer of the radiolabeled groups of the two compounds under the same in vitro conditions reflects their different abilities to inhibit MGMT. IBGG is not as potent as IBG and, therefore, only 20% of [131]IBGG binds to MGMT versus 97% of [131]I-IBG at a concentration of 1 μM unlabeled competitor. The observed binding of both compounds to purified MGMT was specific. This was proven by the fact that only a small amount of radioactivity was bound to bovine serum albumin (BSA), which was included as a negative control, and by the competition with the unlabeled compound.

For [131]ITG the maximum binding to MGMT was 80% at a concentration of 0.1 μM of ITG, and the activity bound to hMGMT was also decreasing with increasing concentration of the unlabeled compound (Figure 2B). At the same concentration (0.1 μM), 58% of [131]ITGG bound to MGMT, decreasing also with increasing concentration of ITGG (Figure 2B). The difference in the percentage of bound radioactivity for these two compounds is not so pronounced, reflecting that their ability to inhibit MGMT is not as different as for IBG and IBGG, respectively. In this case, too, the binding was specific to

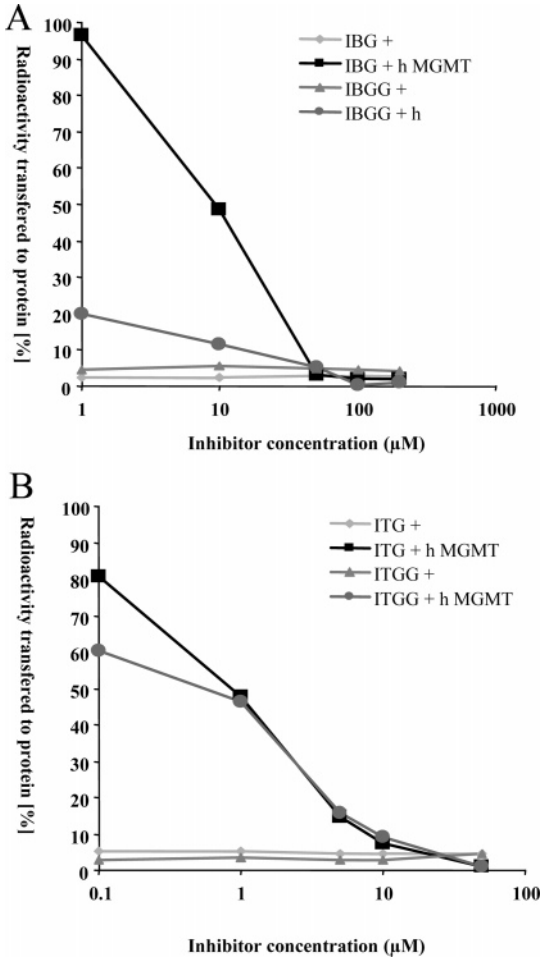


Figure 2. Binding of [131]IBG, [131]IBGG (A) and [131]ITG, [131]I-ITGG (B) to purified human MGMT as a function of unlabeled inhibitor concentration, respectively. As a control for unspecific binding, the assays were performed with BSA as well. The binding activity (% of input radioactivity that was found to be bound to MGMT) was 90% for [131]IBG, 18% for [131]IBGG, 80% for [131]ITG and 63% for [131]ITGG.

MGMT. With these assays it could be demonstrated that the radiolabeled group of each inhibitor is transferred specifically to MGMT, which was a basic requirement for our further investigations.

In Vivo Studies. For the non-glucose-conjugated compound [131]IBG, the tissue distribution of 131I has been described recently.[20] Although the uptake of 131I into the tumor (TE-671 rhabdomyosarcoma xenograft) was lower than that into several normal tissues, this does not necessarily pose a problem for the intended use of [131]IBG as a tracer to quantify MGMT in tumors. But nevertheless it would be highly desirable to provide a tracer that is more selective for the tumor, more metabolically stable, and is cleared more rapidly from the blood. To possibly achieve this, we conjugated IBG with a glucose linker.

In these studies, we compared the biodistribution of the 131I-labeled compounds [131]IBG and [131]IBGG in blood, tumor, and thyroid in nude mice bearing subcutaneous HeLa S3 xenografts. The distribution pattern in the selected tissues of [131]IBG and [131]IBGG measured 0.5, 1, and 4 h postinjection is shown in Figure 3 and Tables 2 and 3.

An important route of metabolism for all iodinated compounds is deiodination, which leads to free iodine, which can be accumulating in the thyroid. Comparing the uptake of [131]I-iodide into the thyroid, it is interesting to note that this uptake

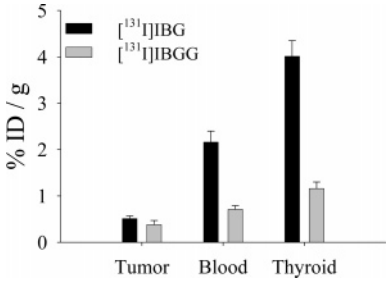


Figure 3. Comparison of ^{131}I uptake in selected tissues in nude mice bearing HeLa S3 xenografts after treatment with nca [^{131}I]IBG or nca [^{131}I]IBGG, as measured 0.5 h after iv administration of the compounds.

Table 2. Tissue Distribution of No-Carrier-Added [^{131}I]IBG in Nude Mice Bearing HeLa S3 Xenografts

	% ID/g (mean ± SD; $n = 3-7$)		
tissue	0.5 h	1 h	4 h
tumor	0.51 ± 0.06	0.83 ± 0.04	0.44 ± 0.03
blood	2.16 ± 0.24	1.81 ± 0.12	0.98 ± 0.06
thyroid	4.01 ± 0.34	6.55 ± 0.42	5.95 ± 1.36

Table 3. Tissue Distribution of No-Carrier-Added [^{131}I]IBGG in Nude Mice Bearing HeLa S3 Xenografts

	% ID/g (mean ± SD; $n = 4-10$)		
tissue	0.5 h	1 h	4 h
tumor	0.54 ± 0.10	0.34 ± 0.02	0.17 ± 0.01
blood	0.71 ± 0.08	0.90 ± 0.13	0.83 ± 0.26
thyroid	1.16 ± 0.14	1.57 ± 0.22	2.83 ± 0.57

Table 4. Tumor to Blood Ratio of No-Carrier-Added [^{131}I]IBG and [^{131}I]IBGG in Nude Mice Bearing HeLa S3 Xenografts

	tumor/blood ratio		
	0.5 h	1 h	4 h
[^{131}I]IBGG	0.76	0.38	0.20
[^{131}I]IBG	0.24	0.46	0.45

is at all time points much less for the glucose conjugate [^{131}I]-IBGG compared to [^{131}I]IBG (Figure 3), e.g. 1.57% ± 0.22% ID/g compared to 6.55% ± 0.42% ID/g at 1 h postinjection. Because deiodination of the molecule in the body is not desirable, the glucose conjugate appears to be more suitable as a tracer. Another desirable property for a potential tracer is rapid blood clearance. As anticipated, blood clearance was found to occur faster for [^{131}I]IBGG than for [^{131}I]IBG. The uptake of [^{131}I]IBGG into the tumor reached a maximum of 0.54% ± 0.10% ID/g at 0.5 h postinjection, decreasing at 1 and 4 h.

This is even less than the uptake of [^{131}I]IBG into the tumor (maximum 0.83% ± 0.04% ID/g 1 h postinjection). Since clearance from blood was more rapid for the glucose conjugate, the tumor to blood radioactivity ratio was clearly better for [^{131}I]-IBGG (0.76 and 0.24 for [^{131}I]IBGG and [^{131}I]IBG at 0.5 h postinjection, respectively; Table 4). The high tumor-to-blood ratio is a very important factor for the potential use of an inhibitor as a tracer for the in vivo imaging of MGMT in tumor tissue.

Conclusions

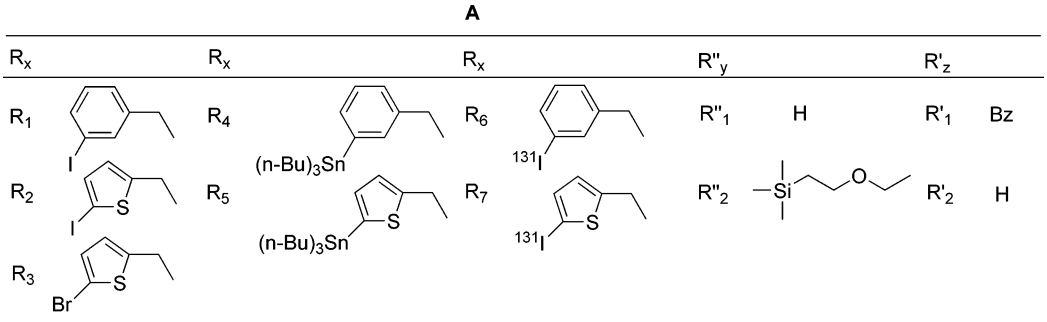
We report on the synthesis of four inhibitors for MGMT, two of them conjugated with glucose via an alkyl spacer. Their no-carrier-added (nca) radioiodination with ^{131}I using the tri-*n*-butylstannylated precursors was performed with good to excellent yields. The stability in aqueous solution, which is a prerequisite for further investigations, was examined for all labeled ^{131}I-iodinated compounds extensively. Absolute ethanol was found to be superior to physiological saline and phosphate buffer, which guaranteed high stability at 5 °C over a long period. In vitro testing with the unlabeled compounds IBG (**11**), IBGG (**13**), ITG (**3**), and ITGG (**6**) revealed IC_{50} values for the glucose conjugates slightly lower than the corresponding non-conjugated derivatives. Nevertheless, they are still eligible for the purpose of investigation of tissue and tumor targeting and determining MGMT status noninvasively. For all ^{131}I-iodinated compounds, we were able to show that the radioactively labeled (hetero)arylmethylene group is transferred to MGMT, which is a basic requirement for their use as potential tracers for the investigation of mechanisms of targeting, action, and inactivation of MGMT in situ. Further experiments with mice bearing tumors are being planned for glucose conjugates [^{131}I]IBGG, [^{131}I]ITGG and their nonconjugated counterparts [^{131}I]IBG and [^{131}I]ITG in order to elucidate tumor uptake, protein binding, and organ uptake as well as blood clearance. Initial studies with [^{131}I]IBG and [^{131}I]IBGG administered to nude mice bearing HeLa S3 xenografts showed less deiodination and a more rapid clearance from the blood of the glucose-conjugated tracer. This resulted in a significantly better tumor-to-blood ratio of radioactivity (as determined 30 min postinjection). Therefore, the glucose-conjugate IBGG, when labeled with an iodine isotope suitable for a 3D-imaging technique such as single-photon emission computed tomography (SPECT) or positron emission tomography (PET), might become a more suitable tracer for determination of MGMT status of tumors than the corresponding IBG.

Experimental Section

Chemistry. Materials and Methods. The chemicals were analytical grade or better and used without further purification. Sodium [^{131}I]iodide (IBS30) was purchased from Amersham Bioscience (Braunschweig, Germany). Analytical TLC was performed on aluminum-backed sheets (Silica gel 60 F_{254}) and normal-phase column chromatography was performed using silica gel 60, both from Merck (Darmstadt, Germany). High performance liquid chromatography (HPLC) was performed using two different systems: (1) a Waters 1525 binary HPLC pump, a Waters 2487 dual wavelength absorbance detector (254 nm), a NaI(Tl) radioactivity-detector M2102 (Messelektronik Dresden), injection valve from Rheodyne (type 8125), and analysis of the HPLC data with Breeze software (Waters), and (2) a Dionex P680A HPLC pump, a Dionex UVD170U UV/vis detector (254 nm), a GABI NaI(Tl) radioactivity detector with GINA STAR software (Raytest), a Dionex injection valve P680, and analysis of HPLC data with CM-PCS-1 software (Dionex). Reverse-phase HPLC was carried out using a LiChrospher 100 RP 18 5 μm EC (250 mm × 4 mm) column from CS-Chromatographie Service GmbH (Langerwehe, Germany). For the separation of ^{131}I-iodinated compounds **[^{131}I]3**, **[^{131}I]6**, **[^{131}I]11**, and **[^{131}I]13**, two different conditions were used: (I) isocratic elution with acetonitrile and water 33:67 at a flow of 1 mL/min and (II) gradient elution using acetonitrile and water (starting with 10% acetonitrile the gradient was increased to 100% acetonitrile over 20 min and held there for 5 min at a flow of 1 mL/min). Radio-TLCs were analyzed using an Instant Imager (Packard Canberra). Activity of tissue samples was counted in a high-purity germanium detector system (Ortec) using GammaVision 5.0 software (Ortec) for analysis. NMR spectra (^{1}H, 400 MHz; ^{13}C, 100 MHz; ^{119}Sn, 149 MHz) were obtained on a DRX400 spectrometer (Bruker Analytik GmbH). Chemical shifts are reported in parts per million, and solvent peaks were referenced appropriately. For identifying the abbreviations used in characterization of the compounds cf. Figure 4. FD mass spectra were obtained on a MAT90 spectrometer (Finnigan) and ESI mass spectra on a Navigator instrument (ThermoQuest).

5-Iodothenyl Alcohol (2). 5-Iodothenyl alcohol was prepared similarly to a previously described procedure.[23] A solution of thenyl alcohol (5.10 g, 44.5 mmol) in toluene (30 mL) was treated alternately with portions of iodine (12.01 g, 47.3 mmol) and yellow mercuric oxide (10.02 g, 46.2 mmol). The resulting mixture was

Table 5. Synthesized Compounds



abbreviation	x	y	compound Nr.
IBG	1	1	11
ITG	2	1	3
BTG	3	1	19
IBGSi	1	2	15
ITGSi	2	2	9
SnBGSi	4	2	16
SnTGSi	5	2	10
[^{131}I]IBGSi	6	2	[^{131}I]15
[^{131}I]ITGSi	7	2	[^{131}I]9
[^{131}I]IBG	6	1	[^{131}I]11
[^{131}I]ITG	7	1	[^{131}I]3

abbreviation	x	z	compound Nr.
IBGGG	1	1	12
ITGGG	2	1	5
BTGGG	3	1	20
IBGG	1	2	13
ITGG	2	2	6
BTGG	3	2	7
SnBGG	4	2	14
SnTGG	5	2	8
[^{131}I]IBGG	6	2	[^{131}I]13
[^{131}I]ITGG	7	2	[^{131}I]6

stirred for 3 h, filtered, and adsorbed on silica gel. Silica gel chromatography using 6:2 *n*-hexane:ethyl acetate afforded **2** (10.10 g, 42.1 mmol) in 95% yield as a yellow liquid.

2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranoside (17). 2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (10.01 g, 15.2 mmol) was dissolved in acetone (35 mL) and water was added until the solution remained cloudy. After addition of sodium iodide (0.45 g, 3.0 mmol) the mixture was stirred at room temperature for 80 h before removing the solvents in vacuo. The residue was resuspended in water (25 mL) and the resulting water phase extracted with ethyl acetate (3 × 100 mL). The mixture was concentrated in vacuo and purified on a silica gel column using 5:1 *n*-hexane:ethyl acetate to give 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**17**) (6.90 g, 11.6 mmol) in 76% yield as a foamy solid.

2,2,2-Tricholoroacetimidate 2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranoside (18). 2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranoside (**17**) (1.90 g, 3.2 mmol) was dissolved in dry dichloromethane (19 mL) under an atmosphere of argon. The solution was cooled to 0 °C, and trichloroacetonitrile (1.37 g, 9.5 mmol) and DBU (48 μL, 0.3 mmol) were added. The mixture was stirred for 2 h until no 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**17**) could be detected (TLC, 3:1, *n*-hexane:ethyl acetate). The solvent was evaporated and the product dried under vacuo to give 2,2,2-trichloroacetimidate 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**18**) (2.2 g, 3.0 mmol) in 95% yield as a foamy yellow solid. The product is sensitive to air and should be stored under argon at −20 °C.

8-Bromooctyl 2,3,4,6-Tetra-*O*-benzoyl-*β*-D-glucopyranoside (4). The synthesis of this compound was performed similarly to a

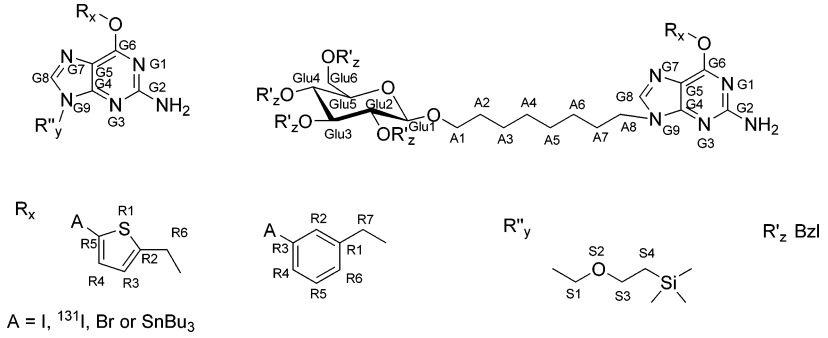


Figure 4. Abbreviations for NMR spectra.

previously described procedure.[30] To a solution of 2,2,2-trichloroacetimidate 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**18**) (3.31 g, 4.5 mmol) and 1-bromooctanol (0.58 g, 2.8 mmol) in dry dichloromethane (3.5 mL) was added boron trifluoride etherate (0.34 mL, 4.5 mmol) under argon at 0 °C. The mixture was allowed to warm to room temperature, stirred for 4 h, and poured into an ice-cold saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with diethyl ether (3 × 50 mL) and washed with water (3 × 60 mL). After adsorbing on silica gel, column chromatography (2:1 *n*-hexane:ethyl acetate) gave 8-bromooctyl 2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranoside (**4**) (2.03 g, 2.6 mmol) in 57% yield as an oil.

2-Amino-6-(5-iodothenyl)-9*H*-purine (3; ITG). To a solution of 5-iodothenyl alcohol (**2**) (4.51 g, 18.7 mmol) in dry DMSO (2.1 mL) under argon in the dark was carefully added sodium hydride (0.30 g, 60% in mineral oil, 7.5 mmol). After stirring for 1 h at room temperature, (2-aminopurin-6-yl)trimethylammonium chloride **1**[25] (0.77 g, 3.4 mmol) was added and the mixture was stirred for another 73 h. The reaction was terminated by the addition of acetic acid (0.58 mL) and diethyl ether (101 mL). After 2 d at 5 °C the resulting precipitate was collected, washed with diethyl ether (20 mL) and water (15 mL), and recrystallized from methanol. 2-Amino-6-(5-iodothenyl)-9*H*-purine (**3**) was obtained as a brownish solid (0.51 g, 1.3 mmol) in 40% yield.

2-Amino-6-(5-iodothenyl)-9-(octyltetra-*O*-benzoyl-*β*-D-glucopyranoside)purine (5). Compound **3** (0.41 g, 1.1 mmol) was dissolved in dry DMF (10 mL) containing 4-Å molecular sieves under argon in the dark, and lithium hydride (8 mg, 0.1 mmol) was added. This mixture was stirred for 1 h at room temperature before adding **4** (0.81 g, 1.03 mmol) in dry DMF (6.5 mL). After stirring for 23 h the reaction mixture was adsorbed on silica gel and purified by silica gel chromatography (1:1 *n*-hexane:acetone) to give **5** (0.61 g, 0.56 mmol) in a yield of 53% as a yellow oil.

2-Amino-6-(5-iodothenyl)-9-(octyl-*β*-D-glucopyranoside)purine (6; ITGG). 2-Amino-6-(5-iodothenyl)-9-(octyl-*β*-D-glucopyranoside)purine (**6**) was prepared similar to a previously described procedure.[13] 2-Amino-6-(5-iodothenyl)-9-(octyltetra-*O*-benzoyl-*β*-D-glucopyranoside)purine (**5**) (0.59 g, 0.55 mmol) was dissolved in dry methanol (13 mL) and treated with a freshly prepared sodium methanolate solution (0.45 mL, 0.1 M). After stirring for 4 h at room temperature the solution was neutralized with Dowex (H^+ 50WX2, 100−200 mesh) ion exchanger. After filtration, the solvent was evaporated and the product purified by column chromatography (5:1 chloroform:methanol) to give ITGG (**6**) (0.21 g, 0.30 mmol) in 56% yield as a foamy solid.

2-Amino-9-(octyl-*β*-D-glucopyranoside)-6-(5-tri-*n*-butylstannylthenyl)purine (8; SnTGG). The preparation of the tin precursor **8** was carried out with some modifications of a procedure described by Vaidyanathan et al.[19] Compound **7**[13] (108 mg, 0.17 mmol) was dissolved in dioxane (5.3 mL) under argon. Hexabutylditin (649 mg, 1.1 mmol) and dichlorobis(triphenylphosphine)palladium(II) (61 mg, 0.09 mmol) were added, and the mixture was heated under reflux for 4 h. After cooling the reaction mixture was filtered (porewidth 1.0 μm) and concentrated in vacuo. Purification by silica gel chromatography (4.5:1 chloroform:methanol) gave **8** (107 mg, 0.13 mmol) in 74% as a white foamy solid.

2-Amino-6-(5-iodothenyl)-9-(2-(trimethylsilanyl)ethoxymethyl)purine (9; ITGSi). 2-Amino-6-(5-iodothenyl)-9*H*-purine (**3**) (440 mg, 1.18 mmol) was dissolved in dry DMF (6.0 mL) under argon in the dark. Lithium hydride (93 mg, 11.8 mmol) was added slowly and the mixture was stirred for 1 h at room temperature. After cooling to 0 °C, 2-trimethylsilylethoxymethyl chloride (198 mg, 1.19 mmol) was added dropwise and the suspension was stirred for another 24 h while warming up to room temperature. After addition of ice-cold water (60 mL), the crude product was extracted with ethyl acetate (3 × 50 mL) and the organic phase was dried over Na_2SO_4. Final purification was performed by column chromatography (3:2 *n*-hexane:ethyl acetate) after adsorbing the crude product on silica gel to give **9** (210 mg, 0.42 mmol) in 36% yield as a yellow oil.

2-Amino-9-(2-(trimethylsilanyl)ethoxymethyl)-6-(5-tri-*n*-butylstannylthenyl)purine (10; SnTGSi). 2-Amino-6-(5-iodothenyl)-9-(2-trimethylsilanylethoxymethyl)purine (100 mg, 0.21 mmol) was dissolved in dioxane (6.0 mL) under argon. Hexabutylditin (742 mg, 1.28 mmol) and dichlorobis(triphenylphosphine)palladium(II) (70 mg, 0.10 mmol) were added, and the resulting suspension was heated under reflux for 140 min. After cooling to room temperature, the crude reaction mixture was adsorbed on silica gel. Final workup was achieved by column chromatography. To remove lipophilic impurities, the first elution occurred with *n*-hexane, and for isolating the product, *n*-hexane:ethyl acetate (3:2) was used as an eluent to give **10** (24 mg, 0.02 mmol) in 18% yield as a yellow oil.

2-Amino-6-(3-iodobenzyloxy)-9*H*-purine (11; IBG). The preparation of 2-amino-6-(3-iodobenzyloxy)-9*H*-purine followed the synthesis of ITG (**3**), using 3-iodobenzyl alcohol (5.01 g, 21.4 mmol), dry DMSO (2.5 mL), sodium hydride (0.35 g, 60% on mineral oil, 8.8 mmol), and **1** (0.90 g, 3.9 mmol). After washing the precipitate with diethyl ether (25 mL) and water (20 mL), **11** (1.41 g, 3.8 mmol) was obtained as a white solid in 97% yield.

2-Amino-6-(3-iodobenzyloxy)-9-(octyltetra-*O*-benzoyl-*β*-D-glucopyranoside)purine (12). The preparation of **12** followed the synthesis of **5**, using 2-amino-6-(3-iodobenzyloxy)-9*H*-purine (0.52 g, 1.4 mmol) in dry DMF (13.3 mL), lithium hydride (11 mg, 1.3 mmol), and 8-bromooctyl 2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranoside (**4**) (1.10 g, 1.4 mmol) in dry DMF (8.6 mL) to give **12** (230 mg, 0.21 mmol) in 15% yield as a foamy yellow solid.

2-Amino-6-(3-iodobenzyloxy)-9-(octyl-*β*-D-glucopyranoside)-purine (13; IBGG). The deprotection of **12** was performed as described for **5**. The protected sugar-conjugate **12** (180 mg, 0.17 mmol) was dissolved in dry methanol (5 mL) and treated with freshly prepared sodium methanolate solution (0.15 mL, 0.1 M). Purification by column chromatography (5:1 chloroform:methanol) gave **13** (70 mg, 0.11 mmol) in 64% yield as a foamy white solid.

2-Amino-9-(octyl-*β*-D-glucopyranoside)-6-(3-tri-*n*-butylstannylbenzyloxy)purine (14; SnBGG). 13 (87 mg, 0.13 mmol) was reacted with hexabutylditin (487 mg, 0.83 mmol) and dichlorobis-(triphenylphosphine)palladium(II) (46 mg, 0.07 mmol) in dioxane (3.9 mL) under reflux. After purification by column chromatography (4.5:1 chloroform:methanol), **14** (110 mg, 0.13 mmol) was obtained in 97% yield as a yellow oil.

2-Amino-6-(3-iodobenzyloxy)-9-(2-(trimethylsilanyl)ethoxymethyl)purine (15; IBGSi). The N^9-protected compound **15** was prepared as described for **9**, using **11** (440 mg, 1.09 mmol) in dry DMF (5.5 mL), lithium hydride (86 mg, 10.9 mmol), and trimethylsilylethoxymethyl chloride (183 mg, 1.09 mmol). After purification by column chromatography (3:2 *n*-hexane:ethyl acetate), **15** (190 mg, 0.38 mmol) was obtained in 35% yield as a yellow oil.

2-Amino-9-(2-(trimethylsilanyl)ethoxymethyl)-6-(3-tri-*n*-butylstannylbenzyloxy)purine (16; SnBGSi). The tri-*n*-butylstannylated precursor **16** was prepared as described for **10**, using IBGSi (100 mg, 0.20 mmol), hexabutylditin (742 mg, 1.28 mmol), and dichlorobis(triphenylphosphine)palladium(II) (70 mg, 0.10 mmol) in dioxane (6.0 mL). After reaction and purification by column chromatography (starting with *n*-hexane, then 3:2 *n*-hexane:ethyl acetate), **16** (59 mg, 0.09 mmol) was obtained in 45% yield as a white crystalline solid.

2-Amino-6-(5-[^{131}I]iodothenyl)-9*H*-purine ([^{131}I]3, [^{131}I]ITG). To a solution of precursor **10** in absolute ethanol (25 μL, 1 μg/μL) was added 10 μL of [^{131}I]I^- (20 MBq) diluted in water followed by 10 μL of phosphate buffer (pH 7.0) and 2.5 μL of CAT (1.5 mg/mL in phosphate buffer, pH 7.0). The reaction was allowed to proceed for 5 min at room temperature. Monitoring by radio-TLC (4:1 toluene:methanol) showed a RCY of 88.0% ± 7.3% for [^{131}I]-ITGSi after 5 min. For removing the 2-(trimethylsilanyl)ethoxymethyl group, the solvent was removed in vacuo, and after addition of 50 μL of tetrabutylammonium fluoride (1 M in THF), the mixture was heated to 68 °C for 25 min.[19] The solvent was evaporated, and the residue was dissolved in absolute ethanol (30 μL) and injected on to a reverse-phase HPLC (condition I). Elution of [^{131}I]-ITG occurred at t_R = 9.4 min. Isolation afforded the labeled compound with an overall RCY of 71.5% ± 5.0% and a radio-

chemical purity of >99%. For in vitro and in vivo testing, the HPLC fraction was evaporated and **[^{131}I]3** was dissolved in phosphate buffer (pH 7.0; 3 mL) with 1% of DMSO.

2-Amino-6-(4-[^{131}I]iodothenyl)-9-(octyl-β-D-glucopyranoside)-purine ([^{131}I]6, [^{131}I]ITGG). To a solution of SnTGG (**8**) in absolute ethanol (25 μL, 1 μg/ μL) was added 10 μL of [^{131}I]I$^-$ (19 MBq) diluted in water. This mixture was treated with 10 μL of phosphate buffer (pH 7.0) and 7.5 μL of CAT [1.5 mg/mL in phosphate buffer (pH 7.0)], and the reaction was allowed to proceed for 5 min at room temperature. The reaction mixture was injected on to a reverse-phase HPLC (condition II) where [^{131}I]ITGG eluted at t_R = 10.9 min with a RCY of 93.6% ± 0.8% and a radiochemical purity of >99.9%. For further studies the solvent of the HPLC fraction was evaporated and the activity was dissolved in phosphate buffer (pH 7.0; 3 mL) with 1% of DMSO.

2-Amino-6-(3-[^{131}I]iodobenzyloxy)-9*H*-purine ([^{131}I]11, [^{131}I]-IBG). The radiochemical synthesis of this compound was accomplished as described for [^{131}I]ITG. The initial reaction afforded [^{131}I]IBGSi (**[^{131}I]15**) after 5 min with a RCY of 95.5% ± 1%. After deprotection with tetrabutylammoniun fluoride (1 M in THF) [^{131}I]IBG could be isolated by reverse-phase HPLC (condition I) at t_R = 11.6 min in 82% ± 6.9% radiochemical yield with a radiochemical purity of >99.4%. For further studies the HPLC fraction was evaporated under vacuo and the activity reconstituted in physiological saline (3 mL) containing 1% of DMSO.

2-Amino-6-(3-[^{131}I]iodobenzyloxy)-9-(octyl-β-D-glucopyranoside)purine ([^{131}I]13, [^{131}I]IBGG). To a solution of SnBGG in absolute ethanol (25 μL, 1 μg/μL) were added 10 μL of [^{131}I]I$^-$ (20 MBq) diluted in water, and after that 10 μL of phosphate buffer (pH 7.0) and 10 μL of CAT [1.5 mg/mL in phosphate buffer (pH 7.0)]. The reaction was allowed to proceed for 5 min at room temperature before injecting the mixture on to a reverse-phase HPLC (condition II). [^{131}I]IBGG could be isolated at t_R = 11.1 min in 89.1% ± 0.9% radiochemical yield with a radiochemical purity of >99.2%. For further studies, the HPLC fraction was evaporated under vacuo and **[^{131}I]13** was dissolved in physiological saline (3 mL) containing 1% of DMSO.

In Vitro Studies. Preparation of Cell Extracts. Exponentially growing HeLa S3 cells expressing MGMT extracts were prepared as described previously.[22] The HeLa S3 cells used by us expressed MGMT at a level of 588 ± 86 fmol/mg protein (mean of 13 determinations). As a negative background control an extract of HeLa MR cells deficient of MGMT served in each assay.

Determination of MGMT Activity. The inactivation of MGMT in cell extracts was determined as described previously.[22] After 30 min incubation of cell extracts with different concentrations of the inhibitors IBG, IBGG, ITG, and ITGG at 37 °C the MGMT activity was measured by assaying the loss of [^{3}H]-*O*^{6}-methylguanine from a [^{3}H]methylated calf thymus DNA substrate as reported.[22] Each assay was performed at least three times.

In Vitro Binding of the ^{131}I-Iodinated Inhibitors by MGMT. Purified human MGMT was prepared as described from *Escherichia coli* transformed with MGMT expression vector according to standard techniques. The activity of the purified MGMT was assayed as described above. The following protocol was the same for all ^{131}I-iodinated compounds. Similar to a previously reported method,[19] nearly identical activities of the ^{131}I-labeled compounds were added in the presence or absence of increasing amounts of the corresponding nonradioactive IBG, IBGG, ITG, or ITGG, respectively, to 10 μg of purified MGMT. The inhibitor compounds were dissolved and stored at −20 °C in ethanol. Immediately before the experiment, they were diluted 1:100 with distilled water and were added to the reaction mixture. The final ethanol concentration in the assay did not exceed 0.1%. To control for unspecific binding, the assay was performed with BSA instead of MGMT. The incubation was done in 100 μL of 50 mM Tris-Cl (pH 7.4), 5 mM DTT, and 0.1 mM EDTA. After incubation for 30 min at 37 °C, precipitation of the protein was accomplished by adding 200 μg of carrier BSA and 1 mL of cold TCA (12%). The precipitated proteins were collected on GF/C filters (Whatman) and washed extensively with cold TCA (5%). The activity remaining on the filter was measured, and the results are expressed as the percentage of input activity. The assay was performed twice for each concentration and twice for all ^{131}I-iodinated compounds.

In Vivo Studies. Biodistribution of [^{131}I]IBG and [^{131}I]IBGG in Nude Mice Bearing Mex(+) Xenografts. All biodistribution studies were performed on male athymic nude mice (Charles River; 4−6 weeks old), which had access to food and water ad libitum. Ten to 13 days prior to the study they were injected subcutaneously with Mex(+) HeLa S3 cells in the right side (10^6 cells in 50 μL of PBS). For the injection of the ^{131}I-iodinated compounds via the tail vein the mice were anesthetized with Ketanest S. No-carrier-added [^{131}I]IBG or [^{131}I]IBGG (400 kBq/mouse) was injected in a volume of 250 μL of physiological saline containing 1% of DMSO. The accumulation of activity in different tissues was evaluated 0.5, 1, and 4 h postinjection of the tracer. For each time-point four to eight mice were used. The animals were euthanized by cervical dislocation, and the tissues of interest were removed, dried of blood, weighed, and homogenized in potassium hydroxide solution. For each sample the activity accumulated in the respective organ was counted in a high-purity germanium detector along with injection standards. The tissue uptake of ^{131}I-activity is expressed as the percentage of injected dose per gram tissue (% ID/g).

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Supporting Information Available: NMR, HPLC, and MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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