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SYNTHESIS OF THREE NO-CARRIER-ADDED O^6-4-[^{125}I] IODOBENZYLGUANOSINE DERIVATIVES, NEW REAGENTS FOR THE ASSAY OF O^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE ACTIVITY

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SUMMARY

O^6-alkylguanine-DNA alkyltransferase (AGT) is mainly responsible for tumour resistances observed in chemotherapeutic treatments by chloroethylnitrosoureas (CENUs). Measurement of AGT activity is thereby essential to predict the response of the patients to therapy with CENUs. In order to develop a sensitive and easy new assay for AGT, previously undescribed O^6-4-[^{125}I]iodobenzyl-2'-deoxyguanosine, O^6-4-[^{125}I]iodobenzyl-N-acetylguanosine and O^6-4-[^{125}I] iodobenzylguanosine labelled with high specific activity were prepared. The most convenient synthetic route appeared to be a rapid and high yield iododestannylation of a tri-n-butylstannyl derivative with no-carrier-added sodium [^{125}I] iodide. Final HPLC separation from the excess of precursor and unreacted [^{125}I] iodides afforded the radioiodinated guanosine derivatives in yields ranging from 70 to 77 %, chemical and radiochemical purities averaging 99%.

Key words : O^6-4-[^{125}I]iodobenzyl-2'-deoxyguanosine, O^6-4-[^{125}I]iodobenzyl-N-acetylguanosine, O^6-4-[^{125}I] iodobenzylguanosine, iododestannylation, assay for AGT activity.

INTRODUCTION

Alkylating agents, such as chloroethylnitrosoureas (CENUs), used for cancer chemotherapy are known to generate O^6-alkylguanine adducts leading to DNA damage mainly responsible for their

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cytotoxic activity (1-3). Such lesions can be repaired by the action of a protein called O^6-alkylguanine-DNA alkyltransferase (AGT) which transfers the O^6-alkyl group to an internal cysteine residue. By this irreversible reaction, the AGT is inactivated and native guanine restored in DNA (4-7).

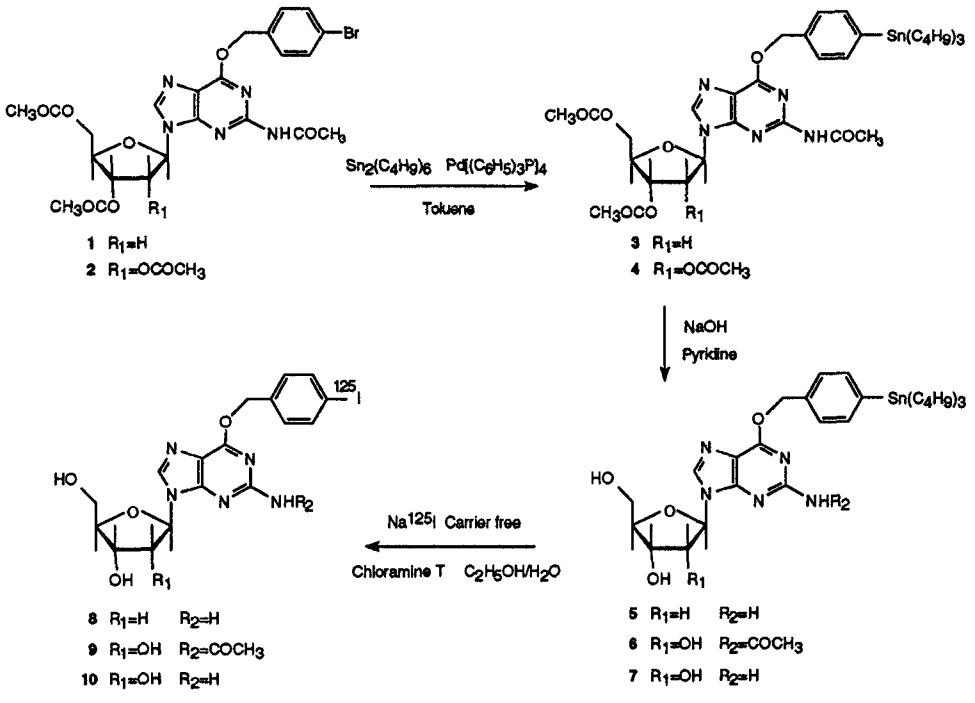
Therefore, the capacity of cells to repair O^6-alkylguanine adducts, resulting in tumour cell resistance to alkylating agents, is directly correlated to AGT content of cells (8). As the constitutive level of AGT varies considerably with the species and cell type, measurement of AGT activity is of fundamental importance for clinical applications to predict whether a chemotherapeutic treatment by chloroethylating agents will be effective.

Many different methods have already been developed for assaying AGT activity. The commonest procedure measures the difference between the amount of initial and residual O^6-[^{3}H] methylguanine DNA substrate when incubated with AGT (9). Another similar approach uses [^{32}P] or [^{35}S] labelled oligodeoxynucleotide containing O^6-methylguanine as a substrate for AGT (10, 11). Nevertheless, these methods raise substantial problems : time consuming preparation of the substrate and separation after incubation unsuitable for large predictive tests, rapid degradation of the substrate and short half-life of the radionuclide requiring frequent resynthesis, semi-quantitative assays.

For the purpose of developing a rapid, easy and sensitive method for direct measurement of AGT activity, we have considered a new approach. The theoretical basis of our assay lies in the AGT mechanism of action. Since AGT repairs O^6-alkylguanine lesions, it could irreversibly transfer an O^6-4-[^{125}I] iodobenzyl group from an O^6-4-[^{125}I] iodobenzylguanosine derivative, substrate for AGT, to its cystein residue resulting in its radiolabelling, making its quantification possible. Also, the use of O^6-4-[^{125}I] iodobenzylguanosine derivatives, as free nucleosides or incorporated in DNA, with high specific activity should lead to a very sensitive and direct detection of the protein.

In this paper we report a rapid and straight forward preparation of the O^6-4-[^{125}I]iodobenzyl-2'-deoxyguanosine (**8**), O^6-4-[^{125}I]iodobenzyl-N-acetylguanosine (**9**) and O^6-4-[^{125}I] iodobenzylguanosine (**10**), all of them substrates for AGT, labelled with high specific activity from their O^6-4-(tri-n-butylstannyl)benzyl precursors.

SYNTHESIS



Scheme 1 : Synthesis of O^6-4-[^{125}I] iodobenzylguanosine derivatives by iododestannylation.

The reaction pathway followed to prepare the O^6-4-[^{125}I] iodobenzylguanosine derivatives **8**, **9**, **10**, is outlined in Scheme 1. O^6-4-bromobenzylperacetyl-2'-deoxyguanosine **1** and guanosine **2**, as starting materials, were synthesized by O^6-4-bromobenzylation of peracetyldeoxyguanosine and guanosine respectively according to a method previously described (12). The reaction of these aromatic bromides with excess hexabutylditin in the presence of tetrakis(triphenylphosphine)palladium as catalyst, in refluxing toluene, afforded compounds **3** and **4** in 51% and 58% yields (13). The tri-n-butylstannyl intermediates **3** and **4** were completely or partially de-acetylated in pyridine under alkaline conditions (NaOH) within hours or minutes respectively to obtain stable O^6-4-(tri-n-butylstannyl)benzyl-2'-deoxyguanosine (**5**), O^6-4-(tri-n-butylstannyl)benzylguanosine (**7**) and O^6-4-(tri-n-butylstannyl)benzyl-N-acetylguanosine (**6**) in good yields. These precursors undergo ready iododestannylation upon treatment with no-carrier-added

sodium [^{125}I] iodide in the presence of chloramine T in aqueous ethanol, for 30 minutes at room temperature, yielding the desired O^6-4-[^{125}I] iodobenzylguanosine derivatives **8**, **9**, **10** (14). Iodinated product isolation from the tri-n-butylstannyl precursor and unreacted [^{125}I] iodides, followed by determination of chemical and radiochemical purities were performed respectively by semi-preparative and analytical reverse phase high performance liquid chromatography (HPLC) monitored by both UV and gamma detection using a gradient of methanol/water as mobile phase (Figure 1). Isolated radiochemical yields ranged from 70% to 77%, chemical and radiochemical purities averaging 99%.

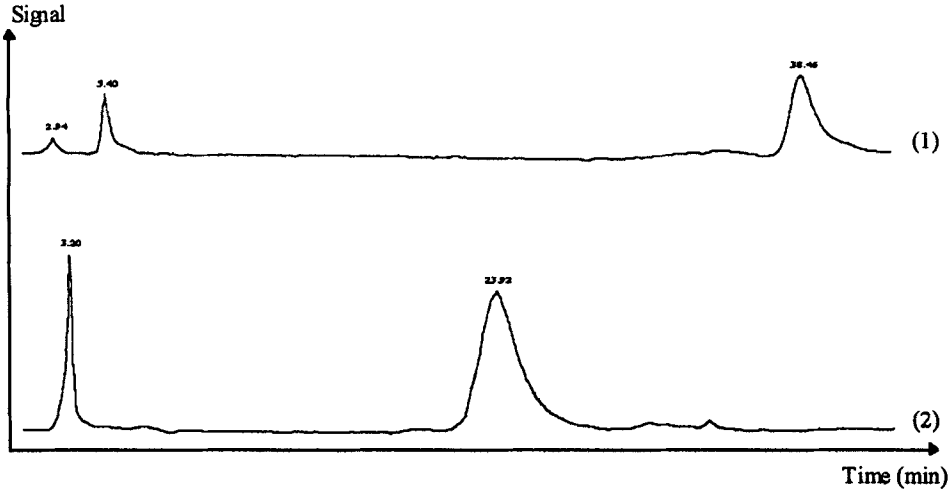


Figure 1 : HPLC elution profile of a crude O^6-4-[^{125}I] iodobenzylguanosine derivative. Chromatogram (1) UV detection : Rt = 38.46 tri-n-butylstannyl precursor ; (2) radioactivity detection : Rt = 3.20 unreacted [^{125}I] iodides, Rt = 23.92 [^{125}I] iodinated product. Operating conditions (system A) are reported in Experimental Section.

All the reactions were initially carried out using non-radioactive sodium iodide. The final compounds were characterized by proton nuclear magnetic resonance, mass spectrometry and infrared spectroscopy. Each non-radioactive compound was used as an authentic standard in the HPLC analysis to identify and confirm the product of interest.

In addition, a radioanalytical quality control has was performed by HPLC over 3 months. Each iodinated guanosine derivative proved to be stable. No significant deiodination was detected.

DISCUSSION

The results reported here suggest that iododestannylation is a suitable method for the preparation of O^6-4-[^{125}I] iodobenzylguanosine derivatives labelled with high specific activity. The major advantages of this approach are that the labelling procedure may be effected quickly, in the last step of the synthetic route, with good yields and under mild conditions. Furthermore, the final radiolabelled product can be cleanly separated from the starting materials and its specific activity depends only on the activity of the iodinating species, allowing high specific activity labelling.

EXPERIMENTAL

General comments. Sodium [^{125}I] iodide as no-carrier-added solution in reductant-free aqueous sodium hydroxide was purchased from CIS bio international. All chemicals were from commercial suppliers and used as received. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were performed on Brücker AM 200 (4.5T) spectrometer. Chemical shifts (δ) are reported in parts per million relative to the internal tetramethylsilane standard. Electron impact mode (EI) mass spectra (MS) were obtained on a Hewlett Packard 5989 A instrument. Infrared (IR) spectra were recorded on a Perkin Elmer 398 spectrometer. Melting points (mp) were determined on an Electrothermal digital apparatus. Analytical thin layer chromatography (TLC) was conducted on precoated silca gel plates (Merck 60F_{254}, 0.2 mm thick and Merck RP 18 $F_{254}S$, 0.25 mm thick) with both detection by ultra violet light at 254 nm and visualization by iodine. Silica gel 60 (Chromagel, 230-400 mesh, SDS) was used for medium pressure chromatography using the indicated solvent mixture expressed as volume/volume ratios. HPLC purification was performed on a Shimadzu HPLC system (LC6A pump, SCL6B system controller, CR5A integrator) equipped with a semi-preparative reverse phase column (Lichroprep RP 18, 25-40 μm, 200x12 mm), connected to a Shimadzu SPD6AV UV spectrophotometric detector (254 nm) in series with a Raytest NaI (Tl) gamma detector (system A). The HPLC analytical system consisted of a Chromatem 800, Pye Unicam PU 4020 (Philips) UV detector and a Radiomatic A200 radioactivity flow one detector employing a reverse phase column (Spherisorb WC 18, 5 μm, 250x4,6 mm) (system B). The solvent mixture indicated later are expressed as volume/volume ratios. Radioactive samples were measured using a Packard 5530 auto gamma counter.

General procedure for the preparation of O^6-4-(tri-n-butylstannyl)benzylperacetyl - 2'-deoxyguanosine (3) and O^6-4-(tri-n-butylstannyl)benzylperacetylguanosine (4).

To a solution of O^6-4-bromobenzylperacetyl-2'-deoxyguanosine **1** (1.8 g, 3.2 mmol) or guanosine **2** (2.0 g, 3.2 mmol) in toluene (32 mL) was added hexabutylditin (3.7 g, 16.1 mmol) and a catalytic amount of tetrakis(triphenylphosphine)palladium (42 mg). The mixture was stirred at reflux temperature for 24 h under nitrogen atmosphere, filtered, and the filtrate evaporated under reduced pressure. The crude product was purified by chromatography on silica gel eluting with ethyl acetate/hexane (70/30).

O^6-4-(tri-n-butylstannyl)benzylperacetyl-2'-deoxyguanosine (3).

Yield : 51%;

TLC (ethyl acetate/hexane, 80/20) *Rf* = 0.50;

IR (KBr) ν : 2850–3000 cm^{-1} (CH, CH_2, CH_3), 1755 cm^{-1} (C=O, ester), 1700 cm^{-1} (C=O, amide), 1230 cm^{-1} (=C-O);

^{1}H-NMR ($CDCl_3$) δ : 0.87 (t, 3H, CH_3), 1.00–1.08 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 1.22-1.39 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 1.45-1.60 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 2.08, 2.15 (2s, 6H, 2 $COCH_3$), 2.53 (s, 3H, $NCOCH_3$), 2.55-2.62 and 2.94-3.05 (2m, 2H, $H_{2'}$), 4.33-4.44 (m, 3H, $H_{4'}$, $H_{5'}$), 5.42-5.45 (m, 1H, $H_{3'}$), 5.58 (s, 2H, $CH_2C_6H_4$), 6.35 (d, 1H, $H_{1'}$), 7.44 (s, 4H, C_6H_4), 7.97 (s, 1H, H_8), 7.99 (s, 1H, NH, exchanges with D_2O).

O^6-4-(tri-n-butylstannyl)benzylperacetylguanosine (4).

Yield : 58%; mp 70-72°C ;

TLC (ethyl acetate/hexane, 80/20) *Rf* = 0.43;

IR (KBr) ν : 2850–3000 cm^{-1} (CH, CH_2, CH_3), 1740 cm^{-1} (C=O, ester), 1670 cm^{-1} (C=O, amide), 1230 cm^{-1} (=C-O);

^{1}H-NMR ($CDCl_3$) δ : 0.87 (t, 3H, CH_3), 1.04 (t, 2H, $SnCH_2CH_2CH_2CH_3$), 1.26-1.37 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 1.45-1.61 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 2.09 and 2.14 (2s, 6H, 2

$COCH_3$), 2.51 (s, 3H, $NCOCH_3$), 4.42-4.45 (m, 3H, $H_{4'}$, $H_{5'}$), 5.58 (s, 2H, $CH_2C_6H_4$), 5.87-6.00 (m, 1H, $H_{3'}$), 5.90-5.92 (m, H, $H_{2'}$), 6.03 (d, 1H, $H_{1'}$), 7.45 (s, 4H, C_6H_4), 7.90 (s, 1H, H_8), 7.94 (s, 1H, NH, exchanges with D_2O).

General procedure for the preparation of O^6-4-(tri-n-butylstannyl)benzyl-2'-deoxyguanosine (5) and O^6-4-(tri-n-butylstannyl)benzylguanosine (7).

A solution of the relevant compound 3 (1.5 g, 2 mmol), 4 (1.7 g, 2 mmol) in pyridine (9 mL) was treated with NaOH 2.5 M (3.6 mL, 9 mmol for 2'-deoxyguanosine derivative and 4.8 mL, 12.0 mmol for guanosine derivative) and stirred for 2 h at 30°C. Acidic cation exchanger (Dowex 50x8 resin, 12 mL) was added with vigorous stirring, filtered, washed with a minimum amount of pyridine. The solution was evaporated under reduced pressure. The residue was then crystallized in hexane to give a white solid.

O^6-4-(tri-n-butylstannyl)benzyl-2'-deoxyguanosine (5).

Yield : 95%; mp 147-149°C;

TLC (dichoromethane/ethanol, 90/10) *Rf* = 0.38, TLC (ethanol/water, 80/20) *Rf* = 0.17;

IR (KBr) ν : 2850–3000 cm^{-1} (CH, CH_2, CH_3), 1230 cm^{-1} (=C-O);

^{1}H-NMR ($CDCl_3$) δ : 0.85 (t, 3H, CH_3), 1.00–1.05 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 1.23-1.34 (m, 2H, $SnCH_2CH_2CH_2CH_3$) , 1.43-1.54 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 2.24-2.30 (m, 2H, $OH_{3'}$, $H_{2'}$), 2.90-3.05 (m, 1H, $H_{2'}$), 3.75, 4.00 (dd, 2H, $H_{5'}$), 4.19 (m, 1H, $H_{4'}$), 4.75 (d, 1H, $H_{3'}$), 5.18 (br s, 2H, NH_2, exchange with D_2O), 5.51 (s, 2H, $CH_2C_6H_4$), 6.18-6.23 (m, 1H, $H_{1'}$), 6.75 (m, 1H, $OH_{5'}$), 7.41 (s, 4H, C_6H_4), 7.63 (s, 1H, H_8).

O^6-4-(tri-n-butylstannyl)benzylguanosine (7).

Yield : 98%; mp 75-77°C ;

TLC (dichoromethane/ethanol, 90/10) *Rf* = 0.38, TLC (ethanol/water, 80/20) *Rf* = 0.30;

IR (KBr) ν : 2850–3000 cm^{-1} (CH, CH_2, CH_3), 1230 cm^{-1} (=C-O);

^{1}H-NMR (DMSO-d_6) δ : 0.85 (t, 3H, CH_3), 1.03 (t, 2H, $SnCH_2CH_2CH_2CH_3$), 1.23-1.38 (m, 2H, $SnCH_2CH_2CH_2CH_3$) , 1.46-1.55 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 3.57-3.62 (m, 2H, $H_{5'}$), 3.88-3.90 (m, 1H, $H_{4'}$), 4.11 (t, 1H, $H_{3'}$), 4.45 (t, H, $H_{2'}$), 5.12 (m, 2H, $OH_{3'}$, $OH_{5'}$), 5.47 (s, 3H,

$OH_{2'}$, $CH_2C_6H_4$), 5.80 (d, 1H, $H_{1'}$), 7.45 (s, 4H, C_6H_4), 6.49 (s, 2H, NH_2, exchange with D_2O), 8.12 (s, 1H, H_8).

Synthesis of O^6-4-(tri-n-butylstannyl)benzyl-N-acetylguanosine (6).

A solution of 4 (1.7 g, 2 mmol) in pyridine (9 mL) was treated with NaOH 2.5 M (2.4 mL, 6 mmol) and stirred for 6 min. The mixture was neutralized by adding acidic cation exchanger (Dowex 50x8 resin, 8 mL) with vigorous stirring, filtered, washed with a minimum amount of pyridine. The solution was evaporated under reduced pressure. The residue was chromatographed on silica gel with a gradient of dichloromethane and dichloromethane/ethanol (98/2, 95/5, 90/10) as eluent to give a white solid.

Yield : 83%; mp 75-77°C ;

TLC (dichoromethane/ethanol, 90/10) *Rf* = 0.54, TLC (ethanol/water, 80/20) *Rf* = 0.39;

IR (KBr) ν : 2850–3000 cm^{-1} (CH, CH_2, CH_3), 1230 cm^{-1} (=C-O);

^{1}H-NMR (DMSO-d_6) δ : 0.92 (t, 3H, CH_3), 1.12 (t, 2H, $SnCH_2CH_2CH_2CH_3$), 1.25-1.38 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 1.52-1.63 (m, 2H, $SnCH_2CH_2CH_2CH_3$), 2.25 (s, 3H, $NCOCH_3$), 3.51-3.63 (m, 2H, $H_{5'}$), 3.88-3.93 (m, 1H, $H_{4'}$), 4.18 (t, 1H, $H_{3'}$), 4.57 (t, H, $H_{2'}$), 4.96 (t, 1H, $OH_{5'}$), 5.16 (d, 1H, $OH_{3'}$), 5.45 (d, 1H, $OH_{2'}$), 5.57 (s, 2H, $CH_2C_6H_4$), 5.88 (d, 1H, $H_{1'}$), 7.42-7.52 (m, 4H, C_6H_4), 8.44 (s, 1H, H_8), 10.47 (s, 2H, NH, exchanges with D_2O).

General procedure for the preparation of O^6-4-iodobenzyl-2'-deoxyguanosine (non-radioactive 8), O^6-4-iodobenzyl-N-acetylguanosine (non-radioactive 9) and O^6-4-iodobenzylguanosine (non-radioactive 10).

To a solution of the relevant tri-n-butylstannyl precursor 5, 6 or 7 (0.6 mmol, 0.4 g) in ethanol was added sodium iodide (1.3 mmmol, 0.2 g) and chloramine T (1.3 mmol, 0.2 g) in aqueous solution (15mL). After 30 min at room temperature, the excess oxidizing agent was reduced by the addition of 5% aqueous sodium metabisulfite (20.3 mL).

O^6-4-iodobenzyl-2'-deoxyguanosine (non-radioactive 8).

Yield : 60% ; mp 127-129°C;

TLC(dichloromethane/ethanol, 90/10) *Rf* = 0.52, TLC (ethanol/water, 80/20) *Rf* = 0.75;

IR (KBr) ν : 3440, 3320 cm^{-1} (NH_2), 1230 cm^{-1} (=C-O);

^{1}H-NMR (DMSO-d_6) δ : 2.20, 2.53 (m, 2H, $H_{2'}$), 3.50-3.55 (m, 2H, $H_{5'}$), 3.80 (m, 1H, $H_{4'}$), 4.34 (m, 1H, $H_{3'}$), 4.99 (t, 1H, $OH_{5'}$, exchanges with D_2O), 5.27 (d, 1H, $OH_{3'}$, exchanges with D_2O), 5.43 (s, 2H, $CH_2C_6H_4I$), 6.19 (t, 1H, $H_{1'}$), 6.49 (s, 2H, NH_2, exchange with D_2O), 7.27-7.76 (dd, 4H, C_6H_4I), 8.08 (s, 1H, H_8);

MS *m/z* : : 483 (M), 367 (B + H), 241 (B + 2H -I), 240 (B + H - I), 217 ($CH_2C_6H_4I$), B = O^6-4-iodobenzylguanine - H).

O^6-4-iodobenzyl-N-acetylguanosine (non-radioactive 9).

Yield : 75% ; mp 146-148°C ;

TLC(dichloromethane/ethanol, 90/10) *Rf* = 0.28, TLC (ethanol/water, 80/20) *Rf* = 0.75;

IR (KBr) ν : 3500–3200 cm^{-1} (OH), 1670 cm^{-1} (C=O amide), 1230 cm^{-1} (=C-O);

^{1}H-NMR (DMSO-d_6) δ : 2.24 (s, 3H, CH_3CO), 3.53-3.64 (m, 2H, $H_{5'}$), 3.90-3.94 (m, 1H, $H_{4'}$), 4.13-4.19 (m, 1H, $H_{3'}$), 4.50-4.59 (m, 1H, $H_{2'}$), 4.96 (t, 1H, $OH_{5'}$, exchanges with D_2O), 5.17 (d, 1H, $OH_{3'}$, exchanges with D_2O), 5.46 (d, 1H, $OH_{2'}$, exchanges with D_2O), 5.56 (s, 2H, $CH_2C_6H_4I$), 5.88 (d, 1H, $H_{1'}$), 7.33-7.76 (dd, 4H, C_6H_4I), 8.44 (s, 1H, H_8), 10.47 (s, 1H, NH, exchanges with D_2O);

MS *m/z* : 541 (M), 452 (B + 44), 409 (B + H), 367 (B + 2H - $COCH_3$), 240 (B + 2H - $COCH_3$-I), 217 ($CH_2C_6H_4I$). B = O^6-4-iodobenzyl-N^2-acetylguanine - H).

O^6-4-iodobenzylguanosine (non-radioactive 10).

Yield : 80% ; mp 207-209°C ;

TLC(dichloromethane/ethanol, 90/10) *Rf* = 0.30, TLC (ethanol/water, 80/20) *Rf* = 0.82;

IR (KBr) ν : 3440, 3320 cm^{-1} (NH_2), 1230 cm^{-1} (=C-O);

^{1}H-NMR (DMSO-d_6) δ : 3.56-3.61 (m, 2H, $H_{5'}$), 3.90 (m, 1H, $H_{4'}$), 4.10 (m, 1H, $H_{3'}$), 4.44 (m, 1H, $H_{2'}$), 5.13 (m, 2H, $OH_{3'}$, $OH_{5'}$, exchange with D_2O), 5.43 (m, 3H, $OH_{2'}$, exchanges with D_2O,

$CH_2C_6H_4I$), 5.78 (d, 1H, $H_{1'}$), 6.50 (s, 2H, NH_2, exchange with D_2O), 7.28-7.77 (dd, 4H, C_6H_4I), 8.11 (s, 1H, H_8);

MS *m/z* : : 499 (M), 367 (B + H), 241 (B + 2H - I), 240 (B + H - I), 217 ($CH_2C_6H_4I$), B = O^6-4-iodobenzylguanine - H).

General procedure for the preparation of O^6-4-[^{125}I]iodobenzyl-2'-deoxyguanosine (8), O^6-4-[^{125}I]iodobenzyl-N-acetylguanosine (9) and O^6-4-[^{125}I] iodobenzylguanosine (10).

To a 10 mg/mL solution of the relevant tri-n-butylstannyl precursor **5**, **6** or **7** (3 µmol, 200 µL) in ethanol placed in a conical reaction vial (Wheaton, 1mL) fitted with a septum was added sodium [^{125}I] iodide (50 µL, 500 µCi, 18.5 MBq) followed by a 10 mg/mL chloramine T aqueous solution (2 µmol, 50 µL). After 30 min at room temperature, the excess oxidizing agent was reduced by the addition of a 15 mg/mL sodium metabisulfite aqueous solution (4 µmol, 50 µL). The material was purified by reverse phase HPLC at a 5 mL/min flow rate using as mobile phase a gradient of ethanol/water mixture beginning at 36/64, increasing to 40/60 from 5 min to 25 min, and then to 80/20 over 55 min (system A).

Determination of chemical and radiochemical final purities was carried out by analytical HPLC at a 1.5 mL/min flow rate using as mobile phase a gradient methanol/water mixture at 40/60 (system B). Each radioiodinated product **8**, **9**, **10** co-migrated with non radioactive standard and showed chemical and radiochemical purities averaging 99%.

O^6-4-[^{125}I]iodobenzyl-2'-deoxyguanosine (8).

Yield based on starting sodium [^{125}I] iodide : 78% (390 µCi, 14.5 MBq);

Rt (system A) = 23.9 min, Rt (system B) = 8.6 min .

O^6-4-[^{125}I]iodobenzyl-N-acetylguanosine (9).

Yield based on starting sodium [^{125}I] iodide : 70% (350 µCi, 13.0 MBq);

Rt (system A) = 20.0 min, Rt (system B) = 5.6 min.

O^6-4-[^{125}I] iodobenzylguanosine (10).

Yield based on starting sodium [^{125}I] iodide : 73% (365µCi, 13.5 MBq);

Rt (system A) = 22.4 min, Rt (system B) = 6.6 min.

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