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Synthesis of 8-[^{18}O]hydroxy-2'-deoxyguanosine

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ABSTRACT

8-[^{18}O]Hydroxy-2'-deoxyguanosine was synthesized starting from 8-bromo-2'-deoxyguanosine and the sodium salt of [^{18}O]benzyl alcohol, resulting in the intermediate 8-[^{18}O]benzyloxy-2'-deoxyguanosine. Subsequent reduction by catalytic transfer hydrogenation, yielded the desired product 8-[^{18}O]hydroxy-2'-deoxyguanosine.

8-Bromo-2'-deoxyguanosine was synthesized from 2'-deoxyguanosine by treatment with Br_2. The sodium salt of [^{18}O]benzyl alcohol was prepared by treatment of ethyl benzimidate hydrochloride with $H_2{}^{18}O$ (95%), $LiAlH_4$ and sodium hydride. Starting from $H_2{}^{18}O$ the overall yield of 8-[^{18}O]hydroxy-2'-deoxyguanosine was approximately 4%. The isotopic purity was calculated to be 93.4 atom% based on GC-MS measurements. The chemical purity and the isotopic purity comply with the planned application, namely as internal standard for the determination of 8-hydroxy-2'-deoxyguanosine by GC-MS.

Key words: 8-[^{18}O]hydroxy-2'-deoxyguanosine, synthesis, stable isotope labelling

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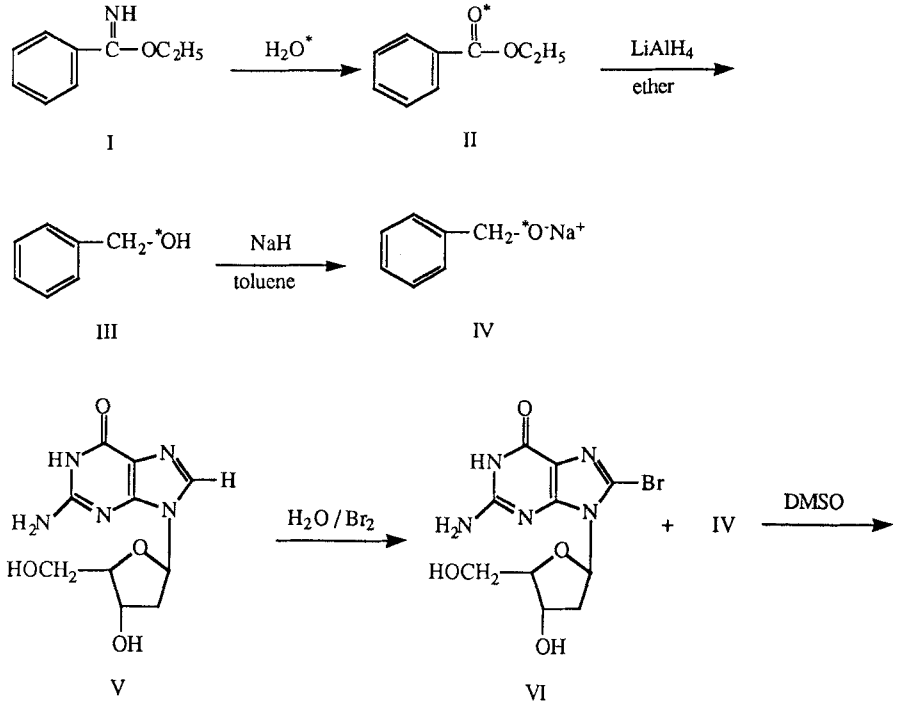
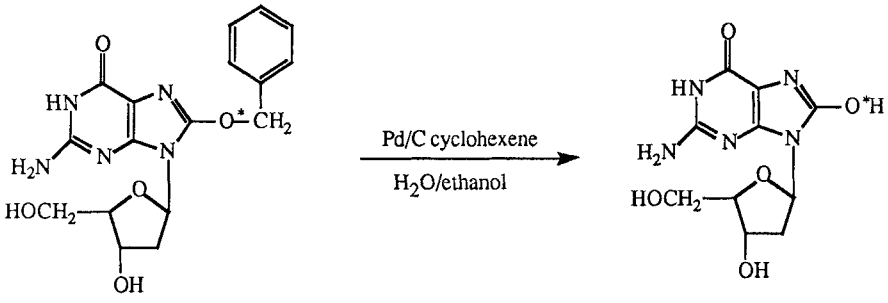
INTRODUCTION

8-Hydroxy-2'-deoxyguanosine (oh^{8}dG) is one abundant form out of approximately twenty identified oxidatively damaged DNA base products (**1**). They are thought to play an important role in carcinogenesis, mutagenesis and toxicology. Oxidatively damaged DNA products, including oh^{8}dG, are generated in vivo as a consequence of normal aerobic metabolism, causing a permanent background level. An increased level is induced by ionising radiation and by exposure to a wide variety of chemicals, some of which are from environmental and occupational sources. We started to develop a method for the quantification of oh^{8}dG extracted from in vivo DNA and from urine (**2**,**3**). For the quantitative analysis of oh^{8}dG by GC-MS, we were in need of a stable isotope-labelled analogue to be used as internal standard. We have chosen to introduce ^{18}O, because stable isotope-labelled ^{15}N, ^{13}C or ^{18}O-2'-deoxyguanosine analogues are not commercially available. The reaction pathway has been essentially described by Holmes *et al* (**4**) and was modified by us as described here.

RESULTS AND DISCUSSION

8-[^{18}O]Hydroxy-2'-deoxyguanosine (VIII) was prepared according to the following scheme:





VII VIII

*=^{18}O

Commonly a route is followed in which the oxidation of 2'-deoxyguanosine is performed with gaseous oxygen or hydrogen peroxide (**5**). However, the use of ^{18}O labelled gaseous oxygen would be rather expensive for the synthesis of labelled oh^{8}dG. Furthermore, labelled hydrogen peroxide is not commercially available and its synthesis would require the use of labelled gaseous oxygen as well. The source of ^{18}O in the method described here is $H_2{}^{18}O$. It is more suitable for the incorporation of the labelled oxygen atom because it uses non-volatile materials.

In order to incorporate an ^{18}O atom, labelled benzyl alcohol (III) was synthesized (**6**) by a reaction between ethyl benzimidate hydrochloride (I) and $H_2{}^{18}O$ (95%), resulting in labelled ethyl benzoate (II), with ^{18}O exclusively in the carbonyl group. Subsequently, labelled ethyl benzoate was reduced by lithium aluminium hydride to labelled benzyl alcohol (III). Finally, benzyl alcohol was converted into its sodium salt by treatment with sodium hydride. Starting with $H_2{}^{18}O$ the overall yield was 65% with an isotopic purity of 92.3 atom%.

8-[^{18}O]Hydroxy-2'-deoxyguanosine (VIII) was synthesized in three steps. First, dG (V) was brominated at the C-8 position, resulting in 8-Br-dG (VI) (**7**). This product was converted into 8-[^{18}O]OBz-dG (VII) by treatment of VI with the sodium salt of labelled benzyl alcohol. Finally, VII was hydrogenated resulting in 8-[^{18}O]hydroxy-2'-deoxyguanosine. From $H_2{}^{18}O$ the overall yield was 4%.

Three major modifications of the published methods had to be introduced. The first modification concerns the purification of 8-[^{18}O]OH-dG (VIII) and 8-[^{18}O]OBz-dG (VII). The published methods (**4,7,8**) apply recrystallization. In our hands this technique did not work, most probably because we used smaller amounts. 8-[^{18}O]OBz-dG was purified by flash chromatography on silica. 8-[^{18}O]OH-dG was purified by semi preparative HPLC. The second modification concerns the introduction of the ^{18}O atom. Three additional reaction steps were required to accomplish this (**6**). These steps were mentioned before. The third modification concerns the debenzylation of (VII). The earlier described method used heterogenous catalytic hydrogenation with hydrogen gas under pressure (**4,7,8**). We changed to catalytic transfer

hydrogenation (**9**) using cyclohexene as the hydrogen donor combined with the use of Pd/C as the catalyst. The reaction is safer because it is conducted at atmospheric pressure. An additional advantage is that the reaction proceeds much faster.

The compounds were characterized by chromatographic (GC and LC) and spectroscopic (IR, NMR and MS) methods. From MS experiments the isotopic purity was calculated to be 93.4 atom %. The labelled material will be used as internal standard for the quantification of oh^{8}dG extracted from in vivo DNA and from urine.

EXPERIMENTAL

NMR-spectra were recorded on a JEOL GSX 270 in DMSO-d_6 or $CDCl_3$. Tetramethylsilan (TMS) was used as an internal standard (∂=0 ppm). IR-spectra were recorded on a Bruker IFS-85 FTIR. GC-MS experiments in Positive Chemical Ionization mode (PCI) were performed on a Finnigan SSQ 710 equipped with a HP5890-gas chromatograph (column: DB 1701 30m*0.25 mm*df=0.15μm (CH_4)) or on a VG70S in Negative Chemical Ionization mode (NCI) equipped with a HP5890-gas chromatograph (column:CPSil5CB 12m*0.25mm*df=0.12μm (CH_4)). HPLC analyses were carried out on a Perkin Elmer Series 4 gradient pump equipped with a HP 1040A diode array detector. The used column was an analytical column (100*4 mm) plus a guard column (30*4 mm) with Macherey-Nagel 100-5 C18 AB as stationairy phase. Semi-preparative HPLC of (VII) was carried out on a Gyncotech 480 gradient pump equipped with a UV detector (Spectroflow 757), using a preparative column (250*10 mm) and a guard column (30*10 mm) with Macherey Nagel Nucleosil 100-5 C18 AB as stationairy phase. A gradient of water and acetonitrile was used as eluent.

Deoxyguanosine (98%) and ethyl benzimidate hydrochloride were purchased from Fluka. Water-^{18}O, normalized, 95 atom % ^{18}O from Isotech inc., USA. The Macherey-Nagel HPLC columns and TLC plates (Macherey-Nagel Polygram^R SIL G/UV-254) were purchased from Macherey-Nagel and Co, Düren, GFR.

Ethyl [^{18}O]carboxy benzoate (II)

To a reaction vial (3 ml with a stirring magnet) ethyl benzimidate hydrochloride (4.5 g 0.032 mol) and $H_2{}^{18}O$ (2.7 g 0.15 mol) was added (**6**). The vial was capped and heated for thirty minutes at a temperature of 80 °C. The produced organic layer was removed with a pasteur pipet and put into a dry test tube. An additional 1 g (0.007 mol) of ethyl benzimidate hydrochloride was added to the reaction mixture, which was treated in the same way for 15 minutes. The produced organic layer was removed and added to the previous one. Hexane was added to the organic layer in order to precipitate the by products. The precipitate was filtered off. Finally, the hexane solution was dried with $MgSO_4$ and removed under reduced pressure. The remaining oily residue was used in the next step without purification.

The yield was 3.9 g (0.026 mol, 81 %).
1. NMR (^{1}H-NMR, chloroform-d_1): δ 1.4 (t, 3H, CH_3), 4.35 (q, 2H, CH_2), 7.35-7.60 (m, 3H, phenyl), 8.05 (d, 2H, phenyl).
2. IR (KBr): C=O (1691 cm^{-1}).
3. The purity was estimated to be 95% based on NMR data.
4. GC-MS (PCI) From the fragments at m/z 151 and m/z 153 (for the unlabelled and labelled analogue, respectively) an isotopic purity of 95.9 atom% was calculated.

[^{18}O]benzyl alcohol (III)

A solution of 3.5 g (0.023 mol) of ethyl [^{18}O]carboxy benzoate in 45 ml of dry diethyl ether was slowly added to a mixture of 1.3 g (0.035 mol) $LiAlH_4$ in 40 ml of dry ether (**11**). After the addition of the ester, the mixture was heated on a oil bath for one hour. After completion of the reduction, checked by TLC (toluene), the excess of $LiALH_4$ was decomposed by cautious addition of ethyl acetate. The reaction mixture was poured out into 500 ml ice-cold diluted sulphuric acid. The ethereal layer was separated from the aqueous layer, extracted 5 times with 8 ml sodium hydroxide solution (0.5 mol/l) and washed with water until the pH of the wash-water was neutral. The obtained product was used in the next reaction without further purification.

The yield was 2g (0.0182mol, 80%).
1. NMR (^{1}H-NMR, chloroform-d_1): δ 1.9 (s, 1H, OH), 4.65 (s, 2H, CH_2), 7.25-7.42 (m, 5H, phenyl).
2. IR (KBr): (C-O) 993 cm^{-1}, (OH) 3319 cm^{-1}.
3. The purity was estimated to be 90% based on the NMR-data.
4. GC-MS (PCI) From the fragments at m/z 108 and m/z 110 (for the unlabelled and labelled analogue, respectively) an isotopic purity of 92.3 atom% was calculated.

8-Bromo-2'-deoxyguanosine (VI)

2'-Deoxyguanosine (V) (5g, 17 mmol) was suspended in 10 ml of water (**7**). Portions of approximately 5 ml of a saturated solution of bromine in water were added under vigorously stirring until the yellow colour of the added bromine solution did no longer disappear . The obtained white solid was filtered immediately and washed with ice water until the pH of the filtrate was neutral. Finally, the solid was washed with 15 ml of cold acetone. The crude product was dried in an oven at a temperature of 50 °C for 20 minutes, and used in the next step without further purification.

The yield was 4.4 g (12.7 mmol, 75%).
1. mp >208 °C dec (210 °C dec (**7**)).
2. NMR (^{1}H-NMR, DMSO-d_6): δ 2.10+3.16 (m, 2H, 2'-H), 3.62 (m, 2H, 5'-H), 3.79 (m, 1H, 3'-H), 4.39 (m, 1H, 4'-H), 4.7-5.3 (b, 2H, 3' and 5'-OH, D_2O exchangeable), 6.14 (t, 1H, 1'-H), 6.49 (s, 2H, 2-NH_2, D_2O exchangeable), 10.77 (s, 1H, 1-NH, D_2O exchangeable).

3. IR (KBr): Compared to dG, the C=N has shifted to a lower frequency (1605 cm^{-1}) due to the inductive effect of Br at the C-8 position. This is in accordance with the structure.
4. The purity was estimated to be 95% based on the NMR-data.

8-[^{18}O]Benzyloxy-2'-deoxyguanosine (VII)

A suspension of 350 mg (14.6 mmol) of sodium hydride in 2 ml of toluene was made. To this suspension 2 ml (9.2 mmol) of [^{18}O]benzyl alcohol (III) were added, dissolved in 3 ml of toluene (7). After the sodium hydride had reacted completely with [^{18}O]benzyl alcohol, the toluene was removed by evaporation under a stream of nitrogen. Then 15 ml of DMSO was added and finally 1.2 g (3.5 mmol) of 8-Br-dG (VI) was dissolved into the reaction mixture. The solution was heated during twelve hours at a temperature of 65 °C. The resulting brown solution was allowed to cool down to room temperature. Glacial acetic acid was added until the pH of the solution was neutral. The solution was then poured out slowly into 300 ml of diethyl ether and the obtained brown oil was separated from the diethyl ether. To the oily residue 300 ml of acetone were added. The precipitate was filtered off and purified by flash chromatography on silica (methanol : chloroform = 1:9).

The yield was 89 mg (0.255 mmol, 9%).
1. mp>187 °C dec (187 °C dec (**7**)).
2. NMR (^{1}H-NMR, DMSO-d_6): δ 2.02+2.87 (m, 2H, 2'-H), 3.4 (m, 2H, 5'-H), 3.69 (m, 1H, 3'-H), 4.24 (m, 1H, 4'-H), 4.80 (t, 1H, OH, D_2O exchangeable), 5.19 (d, 1H, OH, D_2O exchangeable), 5.40 (s, 2H, 8-OCH_2Ar), 6.08 (t, 1H, 1'-H), 6.35 (s, 2H, 2-NH_2, D_2O exchangeable), 7.31-7.52 (m, 5H, 8-$OCH_2C_6H_5$), 10.53-10.76 (br s, 1H, 1-NH, D_2O exchangeable).
3. IR (KBr): 696 cm-1 and 725 cm^{-1} due to monosubstitution of the benzyl group, 1352 (=C-O-benzyl).
4. The purity was estimated to be 98% based on the NMR-data.

8-[^{18}O]Hydroxy-2'-deoxyguanosine (VIII)

A solution of 40 mg (0.11 mmol) of 8-[^{18}O]OBz-dG (VII) in 25 ml of ethanol, 1.5 ml of water and 4 ml of cyclohexene was made (**9**). To this solution 100 mg of palladium (10 %) on active carbon was added. The reaction mixture was heated at 80°C during thirty minutes. The course of the conversion process of 8-[^{18}O]OBz-dG was checked by TLC (chloroform : methanol = 9:1). A few droplets of triethylamine were added to the reaction mixture. The catalyst was removed by filtration. Finally the solvent was evaporated. The remaining yellow product was purified by semi-preparative HPLC.

The yield was 18 mg (0.066 mmol, 60%).
1. The recorded UV spectrum ($\lambda_{max\ 1}$ = 247 nm, $\lambda_{max\ 2}$ = 293 nm) was in accordance with the literature (**10**).
2. NMR (^{1}H-NMR, DMSO-d_6): δ 1.92+2.97 (m, 2H, 2'-H), 3.45 (m, 2H, 5'-H), 3.75 (m, 1H, 3'-H), 4.32 (m, 1H,4'-H), 4.86 (t, 1H, OH D_2O

exchangeable), 5.13 (d, 1H, OH D_2O exchangeable), 6.05 (t, 1H, 1'-H), 6.45 (s, 2H, 2-NH_2),10.40-10.85 (br s, 1H, 1-NH, D_2O exchangeable).
3. IR (KBr): The presence of the C=O stretch (1713 cm^{-1}) proves the occurrence of the keto form. C=O stretch has shifted to a higher frequency due to ring tension. 2677 cm^{-1} and 2492 cm^{-1} points to NH^+.
4. The purity was estimated to be 89%, based on HPLC analysis.
5. GC-MS (NCI): The mixture was triacetylated and dipentafluorobenzylated. From the fragments at m/z 588 and m/z 590 (for the unlabelled and labelled analogue, respectively) an isotopic purity of 93.4% was calculated.

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