

## Mechanism of Formation of *N*<sup>2</sup>-Benzylguanine in the Reaction of 2-Amino-6-chloropurine with Sodium Benzyl Oxide, and Benzylation of Nucleic Acid Bases

Ken-ichi KOYAMA, Kenichi HITOMI, Isamu TERASHIMA, and Kohfuku KOHDA\*

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.

Received December 21, 1995; accepted February 23, 1996

The mechanism of formation of *N*<sup>2</sup>-benzylguanine in the reaction of 2-amino-6-chloropurine with a large excess (12—13 molar eq) of sodium benzyl oxide in benzyl alcohol at 130 °C was studied. *N*<sup>2</sup>,*O*<sup>6</sup>-Dibenzylguanine, a reaction intermediate, was isolated and a possible mechanism for its formation is discussed. Furthermore, using this sodium benzyl oxide system, benzylation at the amino group of nucleic acid bases was facilitated.

**Key words** *O*<sup>6</sup>-benzylguanine; *N*<sup>2</sup>-benzylguanine; *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine; benzylation

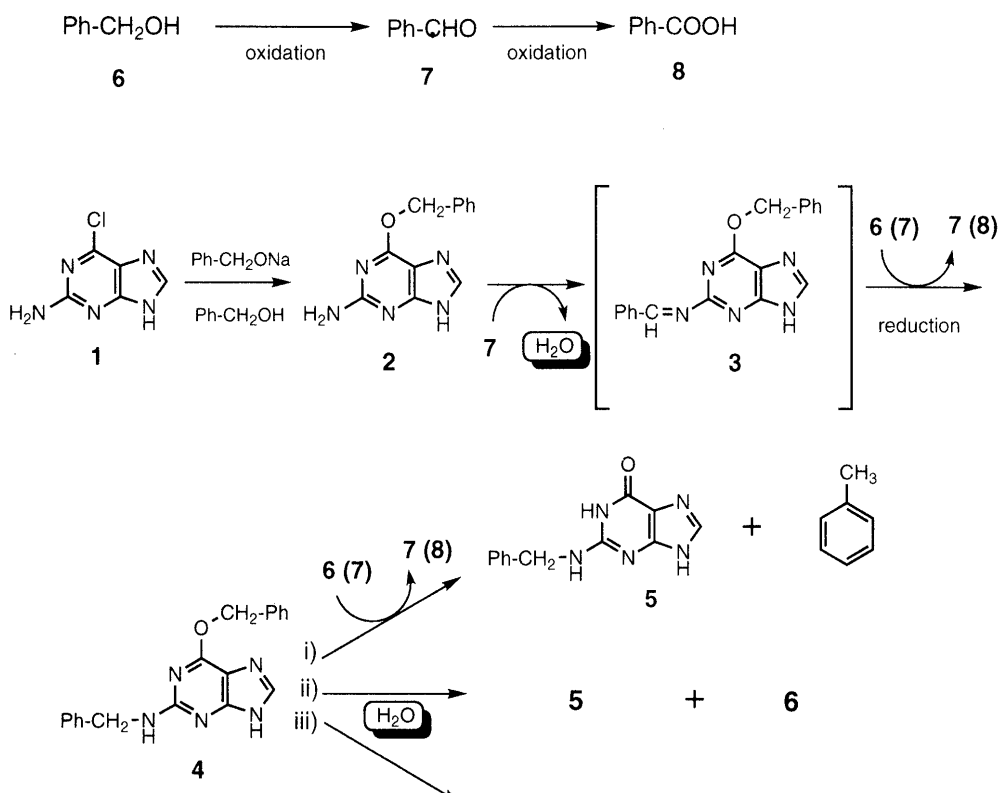
*O*<sup>6</sup>-Benzylguanine (6-Bzl-G) is reported to be an effective depleter of a repair enzyme, *O*<sup>6</sup>-alkylguanine-DNA alkyltransferase (AGT). Pretreatment of cells with 6-Bzl-G resulted in the cytotoxic potentiation of alkylating antitumor agents such as *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) and *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-*N'*-(2-chloroethyl)-*N'*-nitrosourea (ACNU).<sup>1)</sup> Studies of 6-Bzl-G derivatives as AGT depleters have been reported.<sup>2-4)</sup> In previous work, we carried out fundamental studies on the AGT repair mechanism and used these results to try to design a more effective AGT depleter.<sup>5-12)</sup>

6-Bzl-G can be synthesized by the usual method using 2-amino-6-chloropurine and sodium benzyl oxide.<sup>13,14)</sup> When 2 molar eq of sodium benzyl oxide in benzyl alcohol

was used, the desired 6-Bzl-G was obtained, but when 3 molar eq of sodium benzyl oxide was used, the reaction afforded *N*<sup>2</sup>-benzylguanine.<sup>14)</sup> Although a mechanism of formation of *N*<sup>2</sup>-benzylguanine has been proposed,<sup>14)</sup> the details are still unclear. In this study, *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine was isolated as a reaction intermediate and a possible reaction mechanism, shown in Chart 1, is presented. This sodium benzyl oxide system was applied to the benzylation of the amino group of nucleic acid bases.

### Results and Discussion

*O*<sup>6</sup>-Alkylguanines (alkyl=methyl, ethyl, propyl and butyl) are reported to be easily and quantitatively synthesized by reaction of 2-amino-6-chloropurine with a



large excess (10 molar eq) of sodium alkoxide in the corresponding alcohol at reflux temperature for 17 h.<sup>15)</sup> Using this procedure, when we conducted the reaction of 2-amino-6-chloropurine (**1**) with a large excess (13 molar eq) of sodium benzyl oxide in benzyl alcohol at 130 °C for 17 h, instead of the desired *O*<sup>6</sup>-benzylguanine (**2**), we obtained *N*<sup>2</sup>-benzylguanine (**5**), benzoic acid (**8**) and a new compound, **4** (Chart 1). In order to determine whether *N*<sup>2</sup>-benzylguanine is formed *via* *O*<sup>6</sup>-benzylguanine, reaction of *O*<sup>6</sup>-benzylguanine with 13 molar eq of sodium benzyl oxide was carried out under the same conditions described above. The reaction afforded *N*<sup>2</sup>-benzylguanine, indicating that *O*<sup>6</sup>-benzylguanine is initially formed from 2-amino-6-chloropurine and subsequently undergoes a further reaction. Using HPLC, we analyzed the time-dependent formation of products in the reaction of 2-amino-6-chloropurine with 12 molar eq of sodium benzyl oxide in benzyl alcohol (Fig. 1). 2-Amino-6-chloropurine was almost completely consumed within 20 min and was replaced by *O*<sup>6</sup>-benzylguanine. The amount of *O*<sup>6</sup>-benzylguanine thus formed decreased as the reaction proceeded and a new compound, *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (**4**), was concomitantly formed. At the same time, formation of *N*<sup>2</sup>-benzylguanine was observed. The maximum formation of *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine was obtained at around a 5 h reaction time under these conditions.

The effect of the amount of sodium benzyl oxide (2 to 12 molar eq) in the reaction with 2-amino-6-chloropurine on the formation of *O*<sup>6</sup>-benzylguanine, *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine and *N*<sup>2</sup>-benzylguanine was examined at 130 °C for 12 h (Fig. 2). The product ratio changed with the amount of sodium benzyl oxide used. Thus, the main products were *O*<sup>6</sup>-benzylguanine and *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine at 2 molar eq of sodium benzyl oxide. The yield of *O*<sup>6</sup>-benzylguanine decreased with increasing amount of sodium benzyl oxide, and concomitantly, the yield of *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine increased as well. Formation of *N*<sup>2</sup>-benzylguanine was observed at 8 molar eq of sodium benzyl oxide and its formation increased further at 12 molar eq.

Employing suitable reaction conditions, a mixture containing a high yield of *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (**4**) was prepared. After removal of benzyl alcohol, **4** was separated by silica gel column chromatography. After purification, the structure of *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (**4**) was established by NMR, UV and mass spectroscopies and elemental analysis.

We then addressed the question of how *N*<sup>2</sup>-benzylation proceeds. It was reported that the reaction of 6-amino-9-methyl-2-methylsulfonyl-purine with 1 molar eq of sodium benzyl oxide afforded 6-amino-2-benzyloxy-9-methyl-purine, whereas with 3 molar eq, the product was the unexpected 6-benzylamino-2-benzyloxy-9-methyl-purine.<sup>16)</sup> The reaction of 9-ethylguanine (**9**), 2-aminopurine (**11**), adenine (**13**) or cytosine (**15**) was then carried out at 130 °C for 17 h with a large excess (13 molar eq) of sodium benzyl oxide and it was found that each reaction afforded the product benzylation at the amino group (**10**, **12**,<sup>17)</sup> **14**,<sup>18,19)</sup> **16**<sup>20)</sup> in isolated yields of 26%, 63%, 55% and 80%, respectively (Chart 2). These results suggested that

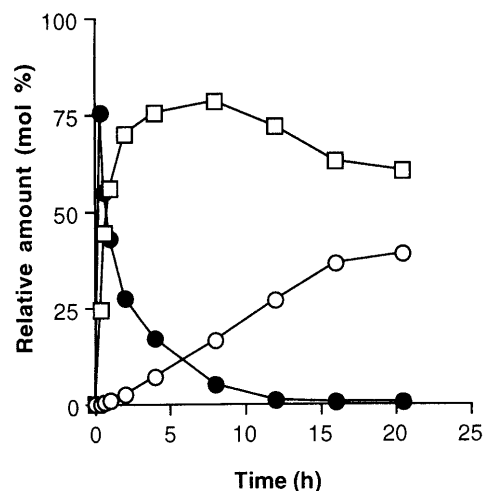


Fig. 1. Time-Dependent Formation of Products in the Reaction of 2-Amino-6-chloropurine with Sodium Benzyl Oxide

2-Amino-6-chloropurine was reacted with sodium benzyl oxide (12 molar eq) at 130 °C and the products formed were analyzed by HPLC. *O*<sup>6</sup>-Benzylguanine (●), *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (□), *N*<sup>2</sup>-benzylguanine (○).

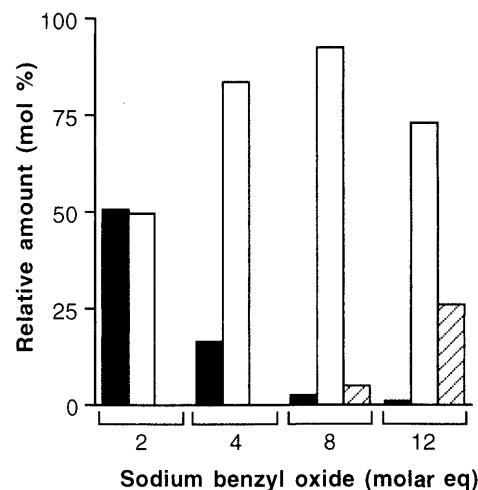


Fig. 2. Effect of the Amount of Sodium Benzyl Oxide on Product Formation

2-Amino-6-chloropurine was reacted with sodium benzyl oxide at 130 °C for 12 h and the products formed were analyzed by HPLC. *O*<sup>6</sup>-Benzylguanine (■), *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (□), *N*<sup>2</sup>-benzylguanine (▨).

*N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine was formed from *O*<sup>6</sup>-benzylguanine by benzylation at the 2-amino group. The evidence for the formation of benzoic acid in the reaction mixture supports the formation of benzaldehyde. The benzaldehyde (**7**) formed may react with *O*<sup>6</sup>-benzylguanine at the 2-amino group to form a Schiff base (**3**), which is subsequently reduced by sodium benzyl oxide<sup>21)</sup> (Meerwein-Ponndorf-type reduction) to give *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (**4**) (Chart 1). Detection and isolation of the Schiff base (**3**) were unsuccessful for reasons to be discussed later.

When the reaction of *O*<sup>6</sup>-benzylguanine (**2**) with 13 molar eq of deuterated sodium benzyl-*d*<sub>7</sub> oxide in benzyl-*d*<sub>7</sub> alcohol was carried out, the products obtained were *N*<sup>2</sup>,*O*<sup>6</sup>-di(benzyl-*d*<sub>7</sub>)guanine (**4'**) and *N*<sup>2</sup>-(benzyl-*d*<sub>7</sub>)guanine (**5'**) (Chart 3). This indicates that the Schiff base (Ph-*d*<sub>5</sub>-CD=N-) formed by the reaction with benzaldehyde-*d*<sub>6</sub> (Ph-*d*<sub>5</sub>-CDO) at the 2-amino group of **2** was reduced to the benzyl form (Ph-*d*<sub>5</sub>-CD<sub>2</sub>-NH-) by sodium

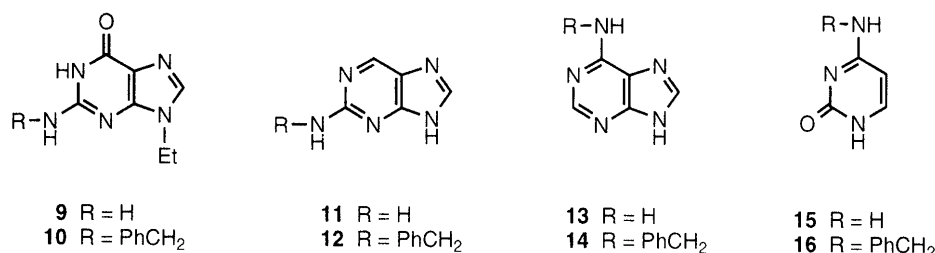


Chart 2

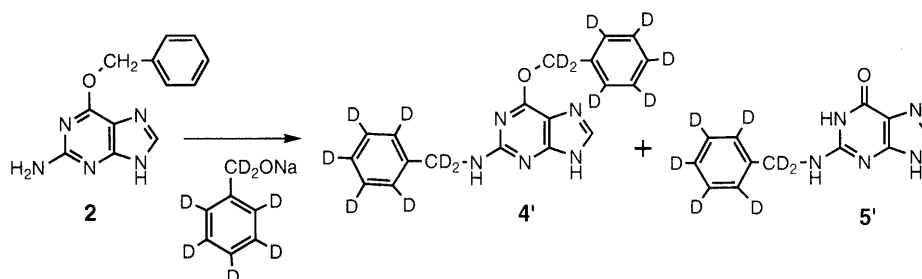


Chart 3

benzyl-*d*<sub>7</sub> oxide, which was simultaneously oxidized to benzaldehyde-*d*<sub>6</sub>. The deuteration of the *O*<sup>6</sup>-benzyloxy group of **4'** was due to the easy replacement of the *O*<sup>6</sup>-benzyloxy group of **2** or of *N*<sup>2</sup>-(benzyl-*d*<sub>7</sub>)-*O*<sup>6</sup>-benzylguanine by alkoxide (benzyl-*d*<sub>7</sub> oxide), as reported.<sup>22)</sup>

The possibility that a reducing agent may quench benzaldehyde was examined using NaBH<sub>4</sub> in the reaction of 6-chloro-2-aminopurine with 13 molar eq of sodium benzyl oxide. In order to avoid the degradation of NaBH<sub>4</sub>, the reaction was carried out at 60 °C for 24 h and products formed were analyzed by HPLC. Under these conditions, without NaBH<sub>4</sub>, the reaction gave *O*<sup>6</sup>-benzylguanine, *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine, benzaldehyde and benzoic acid, and neither the starting material nor *N*<sup>2</sup>-benzylguanine were observed. On the other hand, the addition of NaBH<sub>4</sub> (13 molar eq) prevented the formation of benzaldehyde, benzoic acid and *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine, and only gave *O*<sup>6</sup>-benzylguanine. These results support the idea that benzaldehyde is involved in the reaction. The lack of formation of *N*<sup>2</sup>-benzylguanine without NaBH<sub>4</sub> suggests that the *O*<sup>6</sup>-benzyl group of *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine is stable under these conditions.

The next question addressed was how the *O*<sup>6</sup>-benzyl group was removed from *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (**4**) to form *N*<sup>2</sup>-benzylguanine (**5**). Three possible mechanisms were considered, as shown in Chart 1: i) reductive *O*<sup>6</sup>-debenzylation by the sodium benzyl oxide system,<sup>23)</sup> ii) hydrolysis of the *O*<sup>6</sup>-benzyl ether by the H<sub>2</sub>O that was formed in the process of Schiff base formation, and iii) nucleophilic attack of the benzyl oxide ion at the benzylic carbon of the *O*<sup>6</sup>-benzyl group. In order to examine the effect of H<sub>2</sub>O, *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine was treated with 13 molar eq of sodium benzyl oxide at 130 °C in the presence or absence of 1 molar eq of H<sub>2</sub>O, and the time-dependent formation of *N*<sup>2</sup>-benzylguanine was examined using HPLC. *N*<sup>2</sup>-Benzylguanine formation in the presence of H<sub>2</sub>O was slightly faster during the first 2 h than in its absence, but subsequently the rates became almost the same. These results suggest that 1 molar eq of H<sub>2</sub>O has

only a small effect on *O*<sup>6</sup>-debenzylation (or *O*<sup>6</sup>-debenzyloxylation). Toluene, a reduction product of the benzyl group, was observed in both reaction mixtures. Quantification of toluene was however unsuccessful, because the dibenzyl ether which was formed in the reaction mixture, even in the absence of *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine, also produced toluene due to reduction by the sodium benzyl oxide system. Although it seems unlikely that reaction iii) would produce dibenzyl ether, it can not be ruled out, because quantification of dibenzyl ether formation was impossible for the same reason described above. From the results of these experiments, the most probable mechanism for formation of *N*<sup>2</sup>-benzylguanine (**5**) from *N*<sup>2</sup>,*O*<sup>6</sup>-dibenzylguanine (**4**) is considered to be route i, as shown in Chart 1. In the formation of **5** from 2-amino-6-chloropurine (**1**) by the sodium benzyl oxide system, a small amount of benzaldehyde which may be formed by air oxidation or hydride transfer of benzyl oxide is required as an initiator of the reaction.

With regard to Schiff base formation, aniline (p*K*<sub>a</sub> 4.7) forms a stable Schiff base (*N*-benzylideneaniline) by reaction with benzaldehyde under neutral conditions, but this Schiff base is stable and it did not give *N*-benzylaniline on reaction with 13 molar eq of sodium benzyl oxide at 130 °C for 17 h. Furthermore, the reaction of aniline with 13 molar eq of sodium benzyl oxide did not give *N*-benzylaniline, and aniline was recovered, indicating that under these conditions, the formation of a Schiff base does not proceed. On the other hand, neither 9-ethylguanine (**9**), 2-aminopurine (**11**), adenine (**13**) nor cytosine (**15**) which has an amino group with a lower p*K*<sub>a</sub> value than that of aniline, gave a stable Schiff base upon reaction with benzaldehyde under neutral conditions, but each of them did afford a benzylated product upon reaction with 13 molar eq of sodium benzyl oxide, as already described.

The benzylation rates were obtained from pseudo-first-order kinetics and the results are summarized in Fig. 3 and Table 1. The order of benzylation rates was as follows; 9-ethylguanine > cytosine > 2-aminopurine > adenine. It

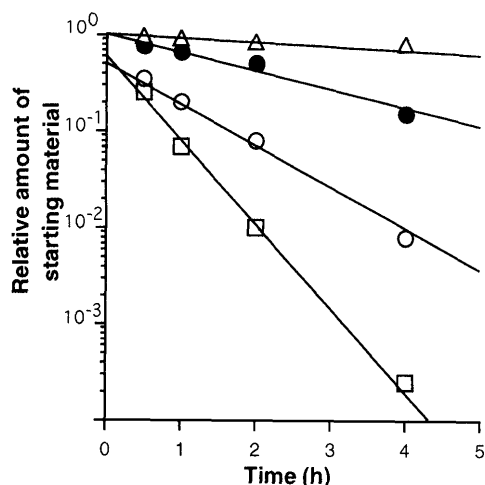


Fig. 3. Pseudo-First-Order Kinetics for the Formation of Benzylated Products

Adenine ( $\Delta$ ), 2-aminopurine ( $\bullet$ ), cytosine ( $\circ$ ) and 9-ethylguanine ( $\square$ ). The experimental conditions employed are described in Experimental.

Table 1. Benzylation Rates and Half Lives

	9-Ethyl- guanine (9)	2-Amino- purine (11)	Adenine (13)	Cytosine (15)
$k_{\text{obs}}$ ( $\text{h}^{-1}$ )	1.93	$4.46 \times 10^{-1}$	$5.75 \times 10^{-2}$	$9.89 \times 10^{-1}$
$T_{1/2}$ (h)	$3.59 \times 10^{-1}$	1.55	$1.21 \times 10^1$	$7.01 \times 10^{-1}$

can be considered that a more electron-rich amino group is more favorable for Schiff base formation, whereas for the reduction of the Schiff base formed, an electron-deficient Schiff base is more favorable. This order may reflect a combination of these factors and/or others.

With regard to the bioactivity of benzylated nucleic acid bases,  $N^2$ -benzylguanine derivatives are reported to be inhibitors of herpes simplex virus type 1 (HSV1) and HSV2 thymidine kinases<sup>24</sup>) and  $N^6$ -benzyladenine derivatives are reported to possess cytokinin activities.<sup>25</sup>) Therefore, the application of this benzylation reaction to the amino group of nucleic acid bases was examined. In order to obtain benzylaminated bases, the following methods are generally available, *i.e.*, i) reaction of a halogenated base with benzylamine,<sup>18,24,26</sup>) ii) reaction of an amino-substituted base with benzoyl chloride and subsequent reduction,<sup>19,26</sup>) and iii) reaction of an amino-substituted base with benzyl halide.<sup>27</sup>) These reactions have limitations in terms of the availability of the halogenated base or the selectivity of the reaction.<sup>27</sup>) Our benzylation method makes possible the selective introduction of a benzyl group at the amino group of normal nucleic acid bases using commercially available substituted benzyl alcohol derivatives. Arylmethylation reactions with several sodium arylmethyl alkoxides were examined in a preliminary trial using 9-ethylguanine as a substrate. The arylmethyl alcohols examined were mono-substituted benzyl alcohol (*o*-Me, *p*-Me, *p*-F, *o*-Cl, or *p*-Cl), 4-pyridylcarbinol, 1-naphthalenemethanol and 1-pyrenemethanol (in the presence of *tert*-butyl alcohol). The yields of  $N^2$ -arylmethylated 9-ethylguanine ranged from 30–60%. Details of this application will be published elsewhere.

## Experimental

$^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded on a JEOL EX 270 or GSX 400 spectrometer (Tokyo, Japan) and chemical shifts are reported in parts per million (ppm) using tetramethylsilane as an internal standard. Mass spectra were obtained with a JEOL DX-300 spectrometer (Tokyo, Japan). UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer (Kyoto, Japan). Melting points were measured with a Yanagimoto micro-melting point apparatus (Kyoto, Japan) without correction. The silica gel used for column chromatography was Merck Silica gel 60 (70–230 mesh, 63–200  $\mu\text{m}$ ). Authentic samples of  $N^2$ -benzylguanine<sup>26</sup>) and  $O^6$ -benzylguanine<sup>14</sup>) were synthesized as reported.

**HPLC Conditions** A Shimadzu LC-9A HPLC apparatus (Kyoto, Japan) equipped with a UV detector and a Merck LiChroCart 250-4 LiChrospher 100 RP-18 (5  $\mu\text{m}$ ) end-capped column (4  $\times$  250 mm) were used. Two solvent systems were employed for elution. Solvent system A: a gradient of 0–90% MeOH in 1/15 M phosphate buffer (pH 6.8) was applied over 40 min. Solvent system B: a programmed gradient of MeOH in 10 mM aqueous  $\text{NaH}_2\text{PO}_4$  solution was applied as follows; 30% from 0–5 min, 30–60% from 5–50 min, and 60% after 50 min. The flow rate was 0.8 ml/min and detection was carried out by measuring UV absorbance at wavelengths of 260 and 280 nm.

Retention times (min) of compounds were as follows. Solvent system A: guanine (3.0), benzoic acid (5.3), benzyl alcohol (15.3),  $N^2$ -benzylguanine (20.5), benzaldehyde (21.0),  $O^6$ -benzylguanine (26.0), toluene (36.2) and  $N^2,O^6$ -dibenzylguanine (39.2). Solvent system B: adenine (4.8),  $N^6$ -benzyladenine (11.3), 2-aminopurine (4.4),  $N^2$ -benzylaminopurine (9.4), cytosine (2.9),  $N^4$ -benzylcytosine (5.1), 9-ethylguanine (7.3),  $N^2$ -benzyl-9-ethylguanine (21.2).

**Product Analyses by HPLC** Na (2, 4, 8 or 12 molar eq) was dissolved in 5 ml of benzyl alcohol. 2-Amino-6-chloropurine (100 mg, 0.59 mmol) was added to the solution and the mixture was heated at 130  $^\circ\text{C}$  for 12 h or another appropriate time with stirring. Products were analyzed by HPLC.

**$N^2,O^6$ -Dibenzylguanine (4)** Na (1.76 g, 76.5 mmol, 13 molar eq) was dissolved in 40 ml of benzyl alcohol. 2-Amino-6-chloropurine (1 g, 5.9 mmol) was added to this solution and the mixture was heated at 130  $^\circ\text{C}$  for 4 h with stirring. The mixture was then cooled to room temperature and neutralized with acetic acid. This solution was poured into diethyl ether (1 l) with vigorous stirring and the precipitates which appeared were collected by filtration. Products were separated on a silica gel column (3.5  $\times$  40 cm) with  $\text{CHCl}_3/\text{MeOH}$  (50/1, 500 ml and then 25/1, 1500 ml). Recrystallization of the crude product from MeOH gave white needles of  $N^2,O^6$ -dibenzylguanine (4) in a 0.67 g (34%) yield. mp 188–190  $^\circ\text{C}$ ; UV  $\lambda_{\text{max}}$  (MeOH) nm ( $\epsilon$ ): 243 (sh) (14500), 248 (16000), 253 (13900), 259 (sh) (7700), 290 (9200);  $^1\text{H}$ -NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 4.51 (d, 2H,  $J=6.3$  Hz,  $N^2$ - $\text{CH}_2$ ), 5.48 (s, 2H,  $O^6$ - $\text{CH}_2$ ), 7.2–7.5 (m, 10H, Ph), 7.42 (br s, 1H, 2-NH), 7.80 (s, 1H, 8-H), 12.50 (s, 1H, 9-NH). *Anal.* Calcd for  $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}$ : C, 68.86; H, 5.17; N, 21.14. Found: C, 68.61; H, 5.22; N, 21.23. MS  $m/z$ : 331 ( $\text{M}^+$ ).

**General Method for the Reaction with an Excess of Sodium Benzyl Oxide** Na (350 mg, 15.2 mmol) was dissolved in 5 ml of benzyl alcohol. The nucleic acid base (1.2 mmol) was added and the mixture was heated at 130  $^\circ\text{C}$  with stirring. For HPLC analysis, an aliquot was removed from the reaction mixture at an appropriate time, neutralized with acetic acid and diluted with MeOH, and this solution was subjected to product analysis. For separation of the products, the reaction mixture was neutralized with acetic acid after 17 h. After this, either one of two methods was employed. Method 1: MeOH (100 ml) was added and insoluble products were removed by filtration, then the mixture was reduced to a small volume and applied to a Sephadex LH20 column (2.0  $\times$  20 cm, eluted with MeOH) to remove benzyl alcohol. The fractions containing products were collected, then the solvent was removed, and the products were separated on a silica gel column (2.8  $\times$  30 cm) with  $\text{CHCl}_3/\text{MeOH}$  (50/1, 300 ml, then 20/1, 1500 ml). Method 2: The mixture was poured into diethyl ether (1 l) with vigorous stirring and the precipitates which appeared were collected by filtration. Products were separated on a silica gel column, as described in Method 1.

$N^2$ -Benzyl-9-ethylguanine: Recrystallization from MeOH afforded white needles in a 26% yield. mp 343–346  $^\circ\text{C}$ . UV  $\lambda_{\text{max}}$  (MeOH) nm ( $\epsilon$ ): 254 (5900), 274 (sh) (3900).  $^1\text{H}$ -NMR ( $\text{Me}_2\text{SO}-d_6$ )  $\delta$ : 1.30 (t, 3H,  $J=7.3$  Hz,  $\text{CH}_3$ ), 3.97 (q, 2H,  $\text{CH}_2$ ), 4.49 (d, 2H,  $J=5.6$  Hz,  $\text{CH}_2$ ), 6.87 (br s, 1H, 2-NH), 7.2–7.4 (m, 5H, Ph), 7.70 (s, 1H, 8-H), 10.56 (s, 1H, 1-NH). *Anal.* Calcd for  $\text{C}_{14}\text{H}_{15}\text{N}_5\text{O}$ : C, 62.43; H, 5.62; N, 26.01. Found: C, 62.63; H, 5.47; N, 26.30.

2-Benzylaminopurine: Recrystallization from MeOH afforded white needles in a 63% yield. mp 194—196 °C [lit.<sup>17)</sup> 194—195 °C]. UV  $\lambda_{\max}$  (MeOH) nm: 241, 310. <sup>1</sup>H-NMR (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$ : 4.53 (d, 2H, *J*=6.1 Hz, CH<sub>2</sub>), 7.2—7.4 (m, 5H, Ph), 7.48 (br s, 1H, 2-NH), 8.04 (s, 1H, 6-H or 8-H), 8.61 (s, 1H, 6-H or 8-H), 12.6 (br s, 1H, 9-NH). MS *m/z*: 225 (M<sup>+</sup>).

*N*<sup>6</sup>-Benzyladenine: Recrystallization from MeOH afforded white needles in a 55% yield. mp 225—229 °C [lit.<sup>18)</sup> 216—218 °C]. UV  $\lambda_{\max}$  (MeOH) nm: 266. <sup>1</sup>H-NMR (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$ : 4.71 (br s, 2H, CH<sub>2</sub>), 7.2—7.4 (m, 5H, Ph), 8.09 (s, 1H, 8-H), 8.16 (br s, 2H, 2-H and 6-NH), 12.91 (br s, 1H, 9-NH). MS *m/z*: 225 (M<sup>+</sup>).

*N*<sup>4</sup>-Benzylcytosine: Recrystallization from MeOH afforded white needles in an 80% yield. mp 210—212 °C [lit.<sup>20)</sup> 213—217 °C]. UV  $\lambda_{\max}$  (MeOH) nm: 232, 275. <sup>1</sup>H-NMR (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$ : 4.48 (d, 2H, *J*=5.6 Hz, CH<sub>2</sub>), 5.66 (d, 1H, 5-H), 7.2—7.4 (m, 5H, Ph), 7.29 (d, 1H, *J*=6.6 Hz, 6-H), 7.99 (t, 1H, 4-NH), 10.28 (br s, 1H, 1-NH). MS *m/z*: 201 (M<sup>+</sup>).

**Reaction Using Sodium Benzyl-*d*<sub>7</sub> Oxide** Na (125 mg, 5.4 mmol) was dissolved in benzyl-*d*<sub>7</sub> alcohol (2 ml). After *O*<sup>6</sup>-benzylguanine (100 mg, 0.42 mmol) was added to the solution, the mixture was heated at 130 °C for 17 h. Products were isolated by a procedure described in general methods. *N*<sup>2</sup>,*O*<sup>6</sup>-Di(benzyl-*d*<sub>7</sub>)guanine (**4'**) and *N*<sup>2</sup>-(benzyl-*d*<sub>7</sub>)guanine (**5'**) was obtained in yields of 12 mg (17%) and 36 mg (70%), respectively. **4'**: <sup>1</sup>H-NMR (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$ : 7.35 (br s, 1H, 2-NH), 7.80 (s, 1H, 8-H), 12.50 (s, 1H, 9-NH). **5'**: <sup>1</sup>H-NMR (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$ : 6.74 (br s, 1H, 2-NH), 7.62 (s, 1H, 8-H), 10.51 (br s, 1H, 1-NH), 12.46 (br s, 1H, 9-NH).

**Reaction with Sodium Benzyl Oxide in the Presence of NaBH<sub>4</sub>** Na (176 mg, 7.67 mmol) was dissolved in 5 ml of benzyl alcohol. 2-Amino-6-chloropurine (100 mg, 0.59 mmol) and NaBH<sub>4</sub> (290 mg, 7.67 mmol) were added to this solution and the mixture was heated at 60 °C for 24 h with stirring. The products formed were analyzed by HPLC.

**Acknowledgement** We would like to thank Professor Y. Kawazoe of Nagoya City University for helpful advice and encouragement. We also thank Dr. T. Aoyama for a critical reading of the manuscript.

#### References

- Dolan M. E., Mitchell R. B., Mummert C., Moschel R. C., Pegg A. E. *Cancer Res.*, **51**, 3367—3372 (1991).
- Moschel R. C., McDougall M. G., Dolan M. E., Stine L., Pegg A. E., *J. Med. Chem.*, **35**, 4486—4491 (1992).
- Chae M.-Y., McDougall M. G., Dolan M. E., Swenn K., Pegg A. E., Moschel R. C., *J. Med. Chem.*, **37**, 342—347 (1994).
- Chae M.-Y., Swenn K., Kanugula S., Dolan M. E., Pegg A. E., Moschel R. C., *J. Med. Chem.*, **38**, 359—365 (1995).
- Kohda K., Yasuda M., Sawada N., Itano K., Kawazoe Y., *Chem.-Biol. Interactions*, **78**, 23—32 (1991).
- Kohda K., Terashima I., Sawada N., Nozaki I., Yasuda M., Kawazoe Y., *Chem. Res. Toxicol.*, **5**, 8—9 (1992).
- Terashima I., Koyama K., Kohda K., *Nucleic Acids Symp. Ser.*, No. 29, 23—24 (1993).
- Terashima I., Yamagata Y., Yamagami N., Kohda K., *Chem. Pharm. Bull.*, **42**, 903—906 (1994).
- Mineura K., Izumi I., Watanabe K., Kowada M., Kohda K., Koyama K., Terashima I., Ikenaga M., *Int. J. Cancer*, **58**, 706—712 (1994).
- Mineura K., Izumi I., Watanabe K., Kowada M., Kohda K., Ikenaga M., *Acta Neurochir.*, **128**, 13—20 (1994).
- Kohda K., Terashima I., Koyama K., Watanabe K., Mineura K., *Biol. Pharm. Bull.*, **18**, 424—430 (1995).
- Mineura K., Fukuchi M., Kowada M., Terashima I., Kohda K., *Int. J. Cancer*, **63**, 148—151 (1995).
- Bowles W. A., Schneider F. H., Lewis L. R., Robins R. K., *J. Med. Chem.*, **6**, 471—480 (1963).
- Frihart C. R., Leonald N. J., *J. Am. Chem. Soc.*, **95**, 7174—7175 (1973).
- Balsiger R. W., Montgomery J. A., *J. Org. Chem.*, **25**, 1573—1575 (1960).
- Andrews K. J. M., Anand N., Todd A. R., Topham A., *J. Chem. Soc.*, **1949**, 2490—2497.
- Okumura S., *Chem. Abstr.*, **62**, P 5286c (1965), Japan. Patent 18048 (1964). Registry No. 1456-46-8.
- Daly J. W., Christensen B. E., *J. Org. Chem.*, **21**, 177—179 (1956).
- Bullock M. W., Hand J. J., Stokstad E. L. R., *J. Org. Chem.*, **22**, 568—569 (1957).
- Fidler W. E., Wood H. C. S., *J. Chem. Soc.*, **1957**, 3980—3984.
- Sprinzak Y., *J. Am. Chem. Soc.*, **78**, 3207—3208 (1956).
- Borowy-Borowski H., Chambers R. W., *Biochemistry*, **26**, 2465—2471 (1987).
- Shriner R. L., Ruby P. R., "Organic Syntheses," Coll. Vol. 4, John Wiley and Sons Inc., New York, 1963, pp. 798—801.
- Hildebrand C., Sandoli D., Focher F., Gambino J., Ciarrocchi G., Spadari S., Wright G., *J. Med. Chem.*, **33**, 203—206 (1990).
- Matsubara S., *Phytochemistry*, **19**, 2239—2253 (1980).
- Shapiro R., Cohen B. I., Shiuey S.-J., Maurer H., *Biochemistry*, **8**, 238—245 (1969).
- Moschel R. C., Hudgins W. R., Dipple A., *J. Org. Chem.*, **44**, 3324—3328 (1979).