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Synthesis of 7-Hydroxy(phenyl)ethylguanines by Alkylation of 2-Amino-6-chloropurine with Allyl-Protected Bromohydrins

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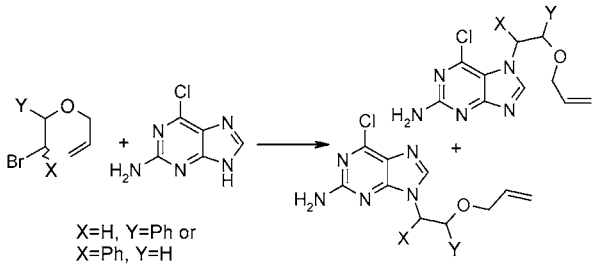
ABSTRACT



Protecting the hydroxyl group in both 2-bromo-2-phenylethanol and 2-bromo-1-phenylethanol enhanced the alkylation of 2-amino-6-chloropurine to give corresponding 7- and 9-alkylated products. Subsequent hydrolysis and deprotection led to 7- and 9-hydroxy(phenyl)ethylguanines. 7-Hydroxy(phenyl)ethylguanines are major guanine adducts formed by interaction of styrene 7,8-oxide with DNA.

Styrene 7,8-oxide (**1**), an electrophilic metabolic intermediate of styrene, reacts with nucleophilic centers in nucleic acids, the main site of the attack being position 7 at guanine. 7-Hydroxy(phenyl)ethylguanine derivatives formed by this process were found in DNA of animals and humans exposed to styrene and can be therefore used as markers of exposure for early detection of damage to DNA.[1] 7-Alkylguanines and 3-alkyladenines are the main types of DNA adducts excreted in urine.[2] The reaction of alkylating agents such as oxirane **1** with nucleotides proceeds with low regioselectivity and low conversion of the nucleotide. The resulting nucleotide adducts are isolated from the reaction mixture using HPLC.[3] The aim of the present study was to develop a synthetic procedure for 7-hydroxy(phenyl)ethylguanines derived from **1**. Oxirane **1** itself is not a suitable alkylating reagent due to its low reactivity and lack of selectivity in alkylation reactions.[3] Therefore, we searched for a suitable synthetic equivalent. Two types of DNA adducts with respect to the site of attack on the oxirane ring are formed. Under acidic conditions, the attack on the α-carbon predominates, leading to 2-hydroxy-1-phenylethyl derivatives (α-adducts), whereas under basic conditions, the attack on the *β*-carbon predominates, yielding 2-hydroxy-2-phenylethyl derivatives (*β*-adducts). Therefore, 2-bromo-2-phenylethanol (**2a**) and 2-bromo-1-phenylethanol (**2b**) are possible synthetic equivalents of **1**, which should yield α- and *β*-adducts, respectively. In fact, bromohydrin **2b** alkylated 2-amino-6-chloropurine (**3**) to yield a 6:1 mixture of 2-amino-6-chloro-9-(2-hydroxy-

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(1) (a) *Monographs on the Evaluation of Carcinogenic Risks to Humans*; International Agency for Research on Cancer: Lyon, France, 1994; Vol. 60. (b) Hemminki, K.; Koskinen, M.; Rajaniemi, H.; Zhao, Ch. *Regul. Toxicol. Pharmacol.* **2000**, *32*, 246.

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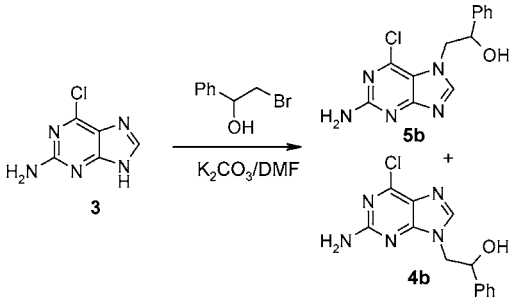
2-phenylethyl)purine (**4b**) and 2-amino-6-chloro-7-(2-hydroxy-2-phenylethyl)purine (**5b**) (Scheme 1). However, the

Scheme 1



regioisomeric bromohydrin **2a** failed to alkylate **3** under the same conditions, i.e., in DMF solution with potassium carbonate as a base. In the presence of base, **2a** underwent dehydrobromination and subsequent condensation reactions leading to a mixture of products and leaving purine **3** unreacted. Therefore, a protection of the hydroxyl group in **2a** was apparently needed to prevent these condensation reactions. We used several protective groups such as acetyl, tetrahydropyranyl, and allyl to protect hydroxyl function in **2a**. Whereas both the acetyl and tetrahydropyranyl groups were insufficiently stable under the conditions of alkylation, so that attempted alkylations resulted in complicated product mixtures, the allyl group effectively prevented formation of byproducts. Moreover, when attached to the vicinal hydroxyl, the allyl group facilitates loss of the leaving group. Participation of allylic π-electrons can effectively stabilize both the intermediary carbocation in S_N1 reaction and the transition state of S_N2 reaction, so that nucleophilic substitution on both benzylic (in **2a**) and phenylethylic carbons (in **2b**) is facilitated. Bromide (**6a**,**b**) or tosylate (**7b**) was used as a leaving group. Alkylation reactions were carried out in DMF solution using potassium carbonate as a base.[4] Alkylating reagents used are shown in Figure 1. Substitution on both

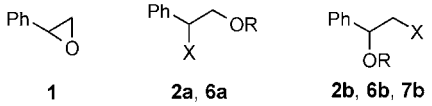
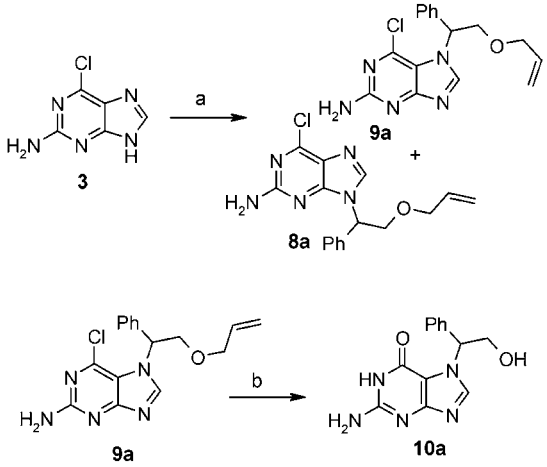


Figure 1. Structures of styrene-7,8-oxide and its synthetic equivalents used as alkylating reagents. In **2a**/**b**, X = Br and R = H; in **6a/b**, X = Br and R = $CH_2-CH=CH_2$; and in **7b**, X = OTs and R = $CH_2-CH=CH_2$.

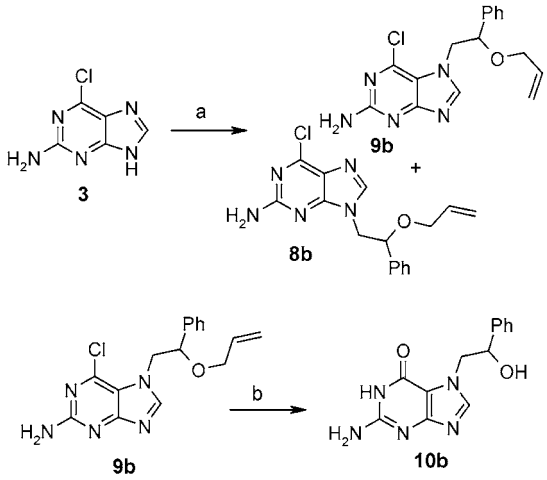
imidazol ring nitrogens occurred, yielding a mixture of 7- and 9-alkylated products with the 9-substituted purine predominating. So, the reaction of **3** with bromide **6a** yielded a mixture of 9-(2-allyloxy-1-phenylethyl)-2-amino-6-chloropurine (**8a**) and 7-(2-allyloxy-1-phenylethyl)-2-amino-6-chloropurine (**9a**). The isomer **9a** was converted to the corresponding guanine derivative by alkaline hydrolysis (3 h of reflux with 1 M NaOH). The protective group was then removed reductively using a palladium complex catalyst[5] to yield 7-(2-hydroxy-1-phenylethyl)guanine (**10a**) as shown in Scheme 2. Regioisomeric 7-(2-hydroxy-2-phenylethyl)guanine (**10b**) was prepared by an analogous procedure using **6b** or **7b** (Scheme 3). Bromide **6b** showed lower reactivity

Scheme 2[a]



[a] Reaction conditions: (a) K_2CO_3/DMF, **6a**, 60 °C. (b) (i) Aqueous NaOH; (ii) $(PPh_3)_4Pd$, PMHS, $ZnCl_2$, DMF.

Scheme 3[a]



[a] Reaction conditions: (a) K_2CO_3/DMF, **6b** or **7b**, 80 °C. (b) (i) Aqueous NaOH; (ii) $(PPh_3)_4Pd$, PMHS, $ZnCl_2$, DMF.

than its regioisomer **6a** with a benzyl bromide moiety, so complete conversion of **3** to alkylated products **8b** and **9b** could not be achieved. Unlike bromide **6b**, tosylate **7b** gave complete conversion of **3** in the alkylation reaction and a higher proportion of the desired regioisomer **9b**.

The results of the alkylation reaction are summarized in Table 1.

Table 1. Alkylation of 2-Amino-6-chloropurine (**3**)

reagent	product ratio (9-N:7-N)	yield in % 9-N	7-N
2b	**4b**:**5b** = 6:1	48	8
6a	**8a**:**9a** = 3:1	62	22
6b	**8b**:**9b** = 4:1	51	13
7b	**8b**:**9b** = 7:2	62	21

Protection of the hydroxyl group in the alkylating reagents improved both the reaction rate and the yield of the products (Table 1). It also increased the proportion of desired 7-N-alkylated product as compared to the alkylation with unprotected bromohydrin **2b**. In all cases, the 9-substituted products were the major ones, while the 7-substituted isomers required were the minor ones. Nevertheless, the procedure described seems to be a promising way for preparation of 7-hydroxy(phenyl)ethylguanines as analytical standards for research on DNA adducts derived from styrene and for early detection of damage to DNA.

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Supporting Information Available: Detailed description of experimental procedures for preparation of alyklating reagents **6a**,**b** and compounds **8a**, **9a**, **10a**, **8b**, **9b**, and **10b** and spectra of newly prepared compounds (UV, NMR, MS). This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) An equimolar mixture of **3** and K_2CO_3 with 2 molar equiv of the alkylating agent (**6a**, **6b**, or **7b**) was stirred in dry DMF at 60−80 °C under an atmosphere of dry nitrogen for 3−4 h. The solvent was removed by evaporation in a vacuum, and the residue was separated by column chromatography on silica gel (50:1 chloroform−methanol). The two formed products were characterized by NMR, MS, and UV spectra and identified as corresponding 7-N- and 9-N-derivatives of **3**. The position of the substituent was determined by HMQC and HMBC two-dimensional NMR experiments. Alkylation of **3** by unprotected **2b** was performed in a similar way at 110 °C overnight.

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