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# Syntheses of $O^6$-Alkyl- and Arylguanine Derivatives: Nucleobase Adducts Derived from Styrene 7,8- and 3,4-Oxides

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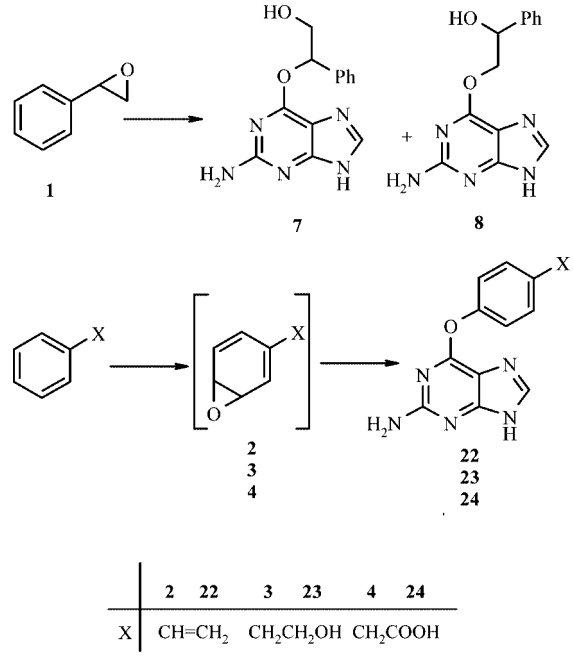
**Keywords:** Alkoxypurines / Aryloxypurines / DNA adducts / DNA damage / Heterocycles

A series of $O^6$-alkyl and -arylguanine derivatives that may be formed in vivo after exposure to styrene has been prepared by reaction of 6-(4-aza-1-azoniabicyclo[2.2.2]octyl)-purine with alkoxides and aryloxides, respectively. The monoprotected diols 2-allyloxy- or 2-benzyloxy-1-phenylethanol and 2-allyloxy- or 2-benzyloxy-2-phenylethanol were used as synthetic equivalents of styrene 7,8-oxide. 4-Vinylphenol, 2-(4-hydroxyphenyl)ethanol and 4-hydroxyphenylacetic acid were used as synthetic equivalents of arene oxide metabolites of styrene, i.e., styrene 3,4-oxide, 4-(2-hydroxyethyl)benzene 1,2-oxide and 4-carboxymethylbenzene 1,2-oxide, respectively.


## Introduction

$O^6$-Alkylguanines are important DNA adducts formed by alkylation with electrophilic mutagens, including some cancer chemotherapeutic agents. Certain $O^6$-alkylguanines are potent inhibitors of alkylguanine-DNA transferase, an enzyme responsible for repair of damage at the guanine $O^6$ position in DNA. Inactivation of this enzyme leads to a significant enhancement of the effect of chemotherapeutic drugs whose mechanism of action involves alkylation at the guanine $O^6$ position.[1,2] Modified guanines are also used as biomarkers of exposure to compounds that are capable of DNA alkylation as they are indicators of exposure and can also serve as indicators of damage to DNA, which is linked to the risk of cancer.[3,4] The residues of modified DNA − DNA adducts − can be determined in various tissues as nucleotide adducts or in urine as modified nucleobases.[5,6] In our previous work we described syntheses of 7-alkylguanines derived from styrene 7,8-oxide (**1**), a carcinogenic metabolite of styrene.[7] To extend the range of nucleobase adducts that can be used as markers of exposure to styrene, we hereby present synthetic routes leading to $O^6$-alkylguanines derived from styrene 7,8-oxide (**1**) and $O^6$-arylguanines derived from arene oxide metabolites of styrene, such as styrene 3,4-oxide (**2**), 4-(2-hydroxyethyl)benzene 1,2-oxide (**3**) and 4-carboxymethylbenzene 1,2-oxide (**4**). A simplified view of the possible formation of $O^6$-guanine adducts from styrene metabolites and metabolic intermediates is shown in Scheme 1.




Scheme 1. $O^6$-Alkyl- and -arylguanines derived from reactive metabolic intermediates of styrene.

$O^6$-Guanine adducts derived from **1** have been detected in DNA samples treated with **1**,[8,9] in experimental animals[10,11] and in humans exposed to styrene.[12,13] On the other hand, no adducts derived from arene oxides **2**–**4** have been reported as yet, although it is assumed that styrene 3,4-oxide (**2**) may contribute significantly to the cytogenetic toxicity of styrene.[14,15]

$O^6$-Alkylguanines (2-amino-6-alkoxypurines) can be prepared by reaction of 2-amino-6-chloropurine (**5**) with sodium alkoxides. The reaction proceeds easily when the cor-

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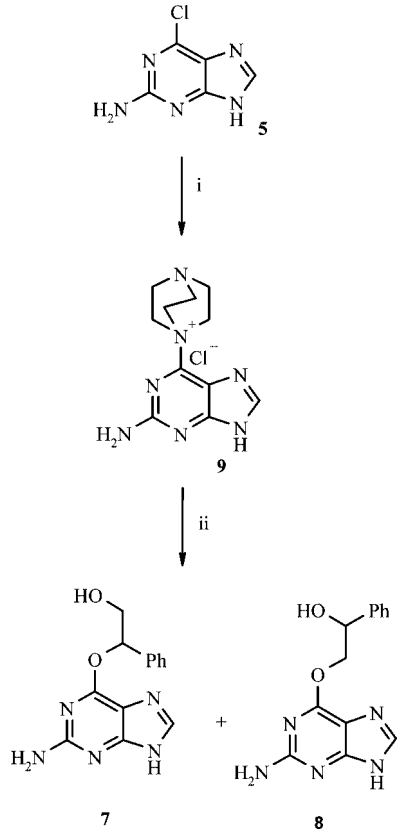
responding alcohol is used as the solvent.[16] However, it is limited to liquid and relatively inexpensive alcohols. Another approach was therefore used to prepare alkoxypurines derived from solid or expensive alcohols. Position 6 in **5** was activated for nucleophilic attack by displacement of chlorine by trimethylamine[17] or 1,4-diazabicyclo[2.2.2]octane (DABCO).[18,19] The ammonium salts formed react readily with alkoxides and aryloxides in polar aprotic solvents.[17–19] Moreover, they can be converted to 6-aryloxypurinyl derivatives when 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is used as a base.[20] We decided to use this synthetic approach to prepare $O^6$-alkylguanine derivatives derived from styrene 7,8-oxide as well as $O^6$-arylguanines derived from styrene 3,4-oxide. The compounds synthesised will be useful as analytical standards for the development of methods for the determination of nucleobase adducts in urine.

Unlike the corresponding guanine derivatives, $O^6$-2′-deoxyguanosine and $O^6$-2′-deoxyguanosine phosphate adducts have been described in the literature. In general, these compounds are prepared by a non-selective alkylation of nucleosides and nucleotides with **1**. The reaction proceeds with low conversion and is non-selective, affording a complex mixture of products containing an excess of unreacted nucleosides or nucleotides. Minor amounts of the adducts can then be obtained by HPLC separation. $O^6$-Substituted 2′-deoxyguanosines[13,21] and deoxyguanosine-3′-phosphates[22,23] derived from **1** have been obtained by this analytical approach. However, this approach is not applicable to arene oxide adducts because arene oxides are not readily available and are unstable in aqueous solutions. $O^6$-Guanosine adducts derived from **1** were prepared by reaction of 6-chloro-9-ribosylpurine with 1-phenylethane-1,2-diol (**6**) in molten sodium. This reaction, which proceeds under rather severe conditions, affords a mixture of two regioisomeric adducts, i.e., 6-(2-hydroxy-1-phenylethyl)-9-ribosylpurine and 6-(2-hydroxy-2-phenylethyl)-9-ribosylpurine, which were separated by HPLC.[24] Similarly, a mixture of regioisomeric adducts was obtained by Mitsunobu reaction of protected 2′-deoxyguanosine-3′-phosphate with a mixture of two isomeric **6** monoacetates.[25]

## Results

### $O^6$-(2-Hydroxy-1-phenylethyl)- (7) and $O^6$-(2-Hydroxy-2-phenylethyl)guanine (8)

Reaction of chloropurine **5** with diol **6** proceeded only after activation of the 6-position of the purine moiety by DABCO and deprotonation of **6** with sodium hydride to yield a mixture of 2-amino-6-(2-hydroxy-1-phenylethoxy)-9*H*-purine [$O^6$-(2-hydroxy-1-phenylethyl)guanine (**7**)] and 2-amino-6-(2-hydroxy-2-phenylethoxy)-9*H*-purine [$O^6$-(2-hydroxy-2-phenylethyl)guanine (**8**)] (Scheme 2). The intermediate in this reaction, 6-(4-aza-1-azoniabicyclo[2.2.2]octyl)-purine [DABCO-purine (**9**)], can be prepared in situ or isolated and reacted in a subsequent step. Conventional heating to 70 °C for 24 h yielded 46% of products (**7** + **8**). Shorter reaction times were achieved by heating in a microwave reactor. Also, the product ratio was affected by reaction temperature and by microwave irradiation (Table 1). The products were separated by semi-preparative HPLC. The two isomers differ in their stability in acidic aqueous solutions. While isomer **8** is stable, **7** decomposes at pH 2 following first-order reaction kinetics with a rate constant, $k$, of $2.7 \times 10^{-5}$ s ($t_{1/2}$ = 42 min). A similar stability and reactivity have been observed by Moschel et al. for corresponding $O^6$-guanosine derivatives.[24]




Scheme 2. Direct synthesis of $O^6$-alkyklguanine adducts derived from **1**: (i) DABCO in DMF; (ii) **6** and NaH in DMF, 70 °C, 24 h or MW, 50–90 °C, 2.5–3.5 h.

Table 1. The yields and isomer ratios obtained by the reaction of DABCO-purine (**9**) with diol **6** induced thermally by conventional heating or by microwaves.

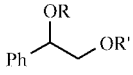
| Heating | Reaction time [h] | Yield (**7** + **8**) | Ratio **8/7**[a] |
|---|---|---|---|
| Conventional, 70 °C | 24 | 46% | 1.50 |
| MW, 50 °C | 3.5 | 39% | 3.55 |
| MW, 70 °C | 2.5 | 45% | 4.26 |
| MW, 90 °C | 2.5 | 43% | 1.00 |

[a] Determined by HPLC with UV detection at 254 nm.

To prepare the adducts **7** or **8** selectively one of the hydroxy groups in diol **6** was protected. Allyl and benzyl protective groups were chosen, which meant that four monoprotected diols, i.e., 2-allyloxy-1-phenylethanol (**10**), 2-allyloxy-2-phenylethanol (**11**), 2-benzyloxy-1-phenylethanol (**12**) and 2-benzyloxy-2-phenylethanol (**13**), were prepared. Their structures are shown in Figure 1. Like with unprotected diol **6**, it was necessary to activate chloropurine **5** with

DABCO. The resulting DABCO-purine **9** reacted readily with the alkoxides derived from alcohols **10**–**13**. Sodium hydride was used for deprotonation of the alcohols. Allyl-protected alcohols **10** and **11** gave $O^6$-(2-allyloxy-1-phenylethoxy)guanine (**14**) and $O^6$-(2-allyloxy-2-phenylethoxy)-guanine (**15**), respectively. Similarly, benzyl-protected alcohols **12** and **13** gave $O^6$-(2-benzyloxy-1-phenylethoxy)-guanine (**16**) and $O^6$-(2-allyloxy-2-phenylethoxy)guanine (**17**), respectively (Scheme 3). The yield of the reaction increased with increasing amount of the base added, as shown in Table 2. The secondary alcohols **10** and **12** gave lower yields than the primary ones (**11** and **13**). The difference between the reactivity of primary and secondary alcohols can be explained by steric hindrance of the secondary hydroxy group.




| | **6** | **10** | **11** | **12** | **13** |
|---|---|---|---|---|---|
| R | H | H | allyl | H | benzyl |
| R' | H | allyl | H | benzyl | H |

Figure 1. Structures of monoprotected diols used as synthetic equivalents of styrene 7,8-oxide.

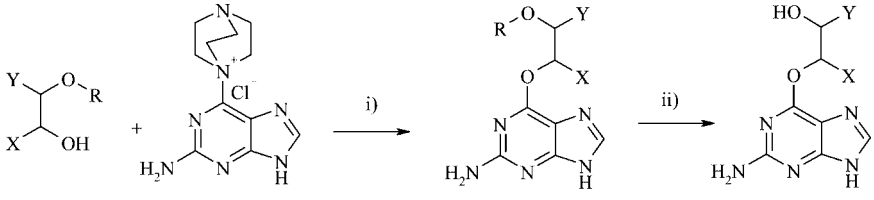
An efficient deprotection of the allyl-protected derivatives **14** and **15** was achieved by tetrakis(triphenylphosphane)palladium-catalysed reductive cleavage according to Chandrasekhar et al.[26] On the other hand, debenzylation of the analogous benzyl-protected products **16** and **17** gave rather poor yields, even with a catalyst (10% Pd-C, Degussa-type containing nearly 50% of water), which was freshly activated by heating under vacuum to 110 °C. The poor yields may be caused, at least in part, by a strong adsorption on the catalyst and the low solubility of the products. In fact, both **7** and **8** are poorly soluble even in dipolar aprotic solvents such as DMF and DMSO. Due to its facile cleavage, allyl is a protective group of choice in this reaction. The yields of both steps and overall yields are summarised in Table 3.

Table 3. The reactions of DABCO-purine (**9**) with monoprotected diols **10**–**13**; the yields of the individual steps and the overall yield.

| Reagent | Yield | | |
|---|---|---|---|
| | Substitution | Deprotection | Overall yield |
| **10** | 54% **14** | 84% **7** | 45% **7** |
| **11** | 94% **15** | 80% **8** | 75% **8** |
| **12** | 45% **16** | 19% **7** | 9% **7** |
| **13** | 79% **17** | 21% **8** | 17% **8** |

### $O^6$-Arylguanines

Four $O^6$-arylguanines (aryloxypurines) derived from hypothetical arene oxide metabolic intermediates of styrene (**2**–**4**) were synthesised from 4-vinylphenol (**18**), 2-(4-hydroxyphenyl)ethanol (**19**), 4-hydroxyphenylacetic acid (**20**) and ethyl 4-hydroxyphenylacetate (**21**). The reaction of DABCO-purine **9** with 4-vinylphenol (**18**) in DMF with sodium hydride as base yielded a complex mixture of prod-




**10**
**11**
**12**
**13**

**9**

**14**
**15**
**16**
**17**

**7**
**8**
**7**
**8**

| | **7** | **8** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **73** |
|---|---|---|---|---|---|---|---|---|---|---|
| X | Ph | H | Ph | H | Ph | H | Ph | H | Ph | H |
| Y | H | Ph | H | Ph | H | Ph | H | Ph | H | Ph |
| R | - | - | allyl | allyl | benzyl | benzyl | allyl | allyl | benzyl | benzyl |

Scheme 3. Synthesis of $O^6$-alkylguanines using monoprotected diols **10**–**13**: (i) NaH in DMF, room temperature, 24 h; (ii) deallylation with PMHS, $[Pd(Ph_3)_4]$, $ZnCl_2$ in DMF[19] for compounds **14** and **15**; debenzylation with $H_2$/Pd-C for compounds **16** and **17**.

Table 2. The reactions of DABCO-purine (**9**) with monoprotected diols **10**–**13**; product yields as influenced by the amount of base added.

| Starting material | Product yield | | |
|---|---|---|---|
| | 2 equiv. of NaH[a] | 2.5 equiv. of NaH[b] | 3 equiv. of NaH[b] |
| **9** + **10** | 48% **14** | 52% **14** | 54% **14** |
| **9** + **11** | 54% **15** | 59% **15** | 94% **15** |
| **9** + **12** | 29% **16** | 32% **16** | 45% **16** |
| **9** + **13** | 49% **17** | 50% **17** | 79% **17** |

[a] Three equivalents of the corresponding monoprotected diol were used. [b] Four equivalents of the corresponding monoprotected diol were used.

ucts. HPLC-MS analysis showed a number of peaks with quasi-molecular ions at *m*/*z* = 254, 366, 374, 484, 494 and 606 corresponding to a purinyl or DABCO-purinyl moiety with one to three vinylphenol molecules added. The expected product, 2-amino-6-(4-vinylphenyloxy)-9*H*-purine [$O^6$-vinylphenylguanine (**22**)], was detected as a minor peak (Figure 2). This strongly suggests the formation of a series of adducts. In these adducts, vinylphenol may be bound to the purine either by its vinyl or its phenolic group. In the latter case, Meisenheimer complexes can be formed (Figure 3).

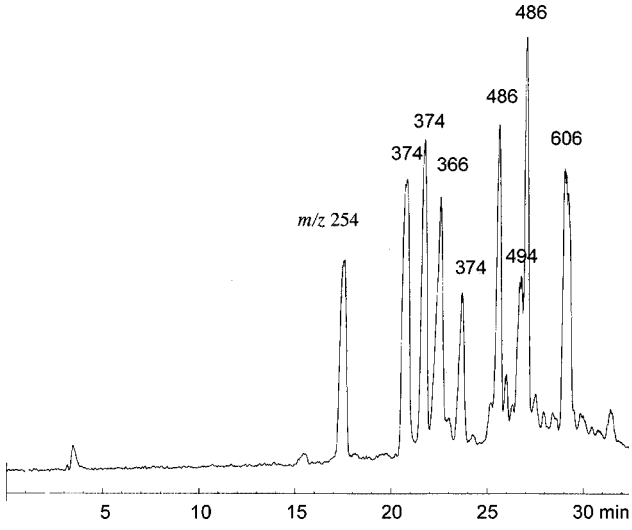
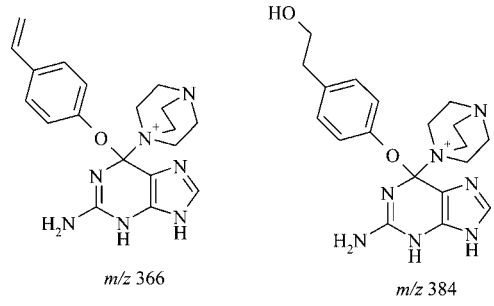



Figure 2. Products of the reaction of DABCO-purine (**9**) with vinylphenol (**18**). HPLC-ESI-MS chromatogram.

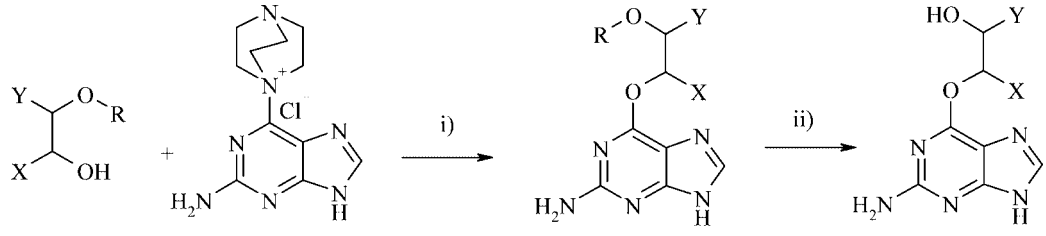
Despite low abundance of arylguanine **22** in the reaction mixture, it could be isolated by column chromatography on silica gel with an acidic eluent ($CHCl_3$/MeOH/AcOH, 7:2:1) followed by crystallisation. Under these separation conditions, at least some of the adducts were unstable and **22** was obtained in a 9% yield.




**26** **27**

Figure 3. Possible structures of Meisenheimer-type complexes corresponding to the main ionic species found by HPLC-ESI-MS of the product mixture arising from the reaction of **9** with **18**.

$O^6$-Arylguanines derived from arene oxides **3** and **4**, namely $O^6$-[4-(2-hydroxyethyl)phenyl]guanine (**23**) and $O^6$-(4-carboxymethylphenyl)guanine (**24**), respectively, were obtained by the treatment of **9** with phenolic alcohol **19** and phenolic acid **20**, respectively, as shown in Scheme 4. Similarly, **21** gave $O^6$-(4-ethoxycarbonylmethylphenyl)guanine (**25**). Potassium carbonate, which was used as a base, can deprotonate both carboxylic and phenolic groups whilst leaving alcoholic hydroxy groups untouched. Nevertheless, one equivalent of DBU was needed to obtain reasonable yields of arylguanines **23**–**25**. The yields of arylguanines **23**, **24** and **25** were significantly better than that of arylguanine **22**, amounting to 35%, 35% and 25%, respectively. A by-product of arylguanine **23** was detected by HPLC/MS at *m*/*z* = 384, which corresponds to a Meisenheimer adduct containing phenol **19** and DABCO attached to the aminopurine moiety. Unlike for the vinylphenylguanine **22** (Figure 3), no by-products containing two or more phenolic molecules attached to the purine moiety were detected. Proposed structures of Meisenheimer-type complexes **26** and **27** derived from phenols **18** and **19**, respectively, are shown in Figure 3.




**10** **11** **12** **13** **9** **14** **15** **16** **17** **7** **8** **7** **8**

| | 7 | 8 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 73 |
|---|---|---|---|---|---|---|---|---|---|---|
| X | Ph | H | Ph | H | Ph | H | Ph | H | Ph | H |
| Y | H | Ph | H | Ph | H | Ph | H | Ph | H | Ph |
| R | - | - | allyl | allyl | benzyl | benzyl | allyl | allyl | benzyl | benzyl |

Scheme 4. Preparation of $O^6$-arylguanines: (i) $K_2CO_3$ and DBU in DMF; 50 °C, 2 d for **21**, 65 °C, 3 d for **22** and **23**.

## Discussion

1-Phenylethane-1,2-diol reacts non-selectively with DABCO-purine in the presence of a base to give a mixture of two $O^6$-guanine adducts **7** and **8**. Each of these products can be obtained selectively by the reaction of monoprotected diols **10**–**13**. In all cases an excess of sodium hydride was required as a base. One equivalent of NaH is required for neutralisation of the acidic NH at position 6 of DABCO-purine **9**, therefore more base is needed for deprotonation of the hydroxy group to obtain a nucleophilic alkoxide capable of the reaction with **9**. For primary alcohols **11** and **13**, the yield of the reaction increases significantly as the amount of the base is increased from two to three equivalents, giving high yields of 94 and 79% for **11** and **13**, respectively. On the other hand, the secondary alcohols **10** and **12** gave rather low yields, even when three equivalents of the base were used. This observation may be explained by a lower reactivity of the alkoxide formed due to steric hindrance. This assumption is also supported by the higher reactivity of allyl-protected diols **10** and **11** as compared to their benzyl-protected analogues **12** and **13**.

Monoprotected diols **10**–**13** react with DABCO-purine **9** exclusively at the unprotected hydroxy group. Thus, compounds **10** and **12** give solely the α-isomers **14** and **16**, respectively, whereas compounds **11** and **13** give the β-isomers **15** and **16**, respectively. The isomers were assigned unequivocally for allyl-protected derivatives **14** and **15** by HMBC NMR experiments. Cross-peaks between purine C-6 signals and the CH or $CH_2$ protons of the side-chain clearly indicate which carbon of the side-chain is attached to $O^6$. Another distinctive feature of the NMR spectra of the α- and β-isomers is a significant downfield proton shift of the CH and $CH_2$ groups, respectively, directly attached to the $O^6$ position. As a consequence, the difference between the chemical shifts values [$\Delta = \delta_H(CH) - \delta_H(CH_2)$] for α-isomers (2.6 ± 0.1 ppm), is markedly greater than that for β-isomers (0.4 ± 0.3 ppm). These data are summarised in Table 4.

While styrene 7,8-oxide (**1**) is a known metabolite of styrene that is capable of DNA adduct formation,[27] the evidence of the formation of arene oxides **2**–**4** is indirect. It has been shown that several phenolic metabolites are formed during the biotransformation of styrene, mainly vinylphenol (**18**),[28] hydroxyphenylethanol (**19**) and hydroxyphenylacetic acid (**20**).[29] A general metabolic pathway from arenes to phenols proceeds via the corresponding arene oxides and an NIH shift.[30] Arylguanines **22**–**24** are therefore expected DNA adducts arising from the arene oxidation of styrene and its metabolites (phenylethanol and phenylacetic acid, respectively). Phenolic metabolites **18**–**20** themselves indicate that arene oxidation occurs during biotransformation of styrene but do not indicate whether or not the corresponding arene oxides are capable of binding to DNA under physiological conditions. On the other hand, arylguanines **22**–**24**, if found in urine, would indicate an arylation of nucleic acids by arene oxides **2**–**4** in vivo.

Vinylphenol (**18**) is not commercially available and was therefore prepared by alkaline hydrolysis of its acetate. This reaction has been described previously by Corson et al.[31] to proceed in aqueous KOH solution. After hydrolysis, the phenolate obtained was acidified with carbon dioxide to liberate phenol **18**. Under these conditions, it was rather difficult to control the pH of the solution and to prevent the product from polymerising, which occurs at slightly acidic pH. Therefore, we modified the reaction conditions using KOH in aqueous ethanol for hydrolysis and an addition of toluene to the reaction mixture during acidification. The product liberated from the phenolate was immediately extracted into the toluene layer and thereby protected from polymerisation.

Phenolates are much weaker nucleophiles than alkoxides. It is therefore not surprising that they react much more slowly and give lower yields than the alkoxides derived from **10**–**13**. On the other hand, a combination of weaker bases (potassium carbonate and DBU instead of sodium hydride) is sufficient to deprotonate phenols and induce their reaction with DABCO-purine (**9**). The structures of the Meisenheimer-type complexes shown in Figure 3 are based solely on ESI-MS data and should therefore be considered tentative. Analogous complexes were to be expected when reacting phenolic compounds **19** and **21** with **9**. In fact, in these reactions only hydroxyphenylethanol (**19**) gave a compound that gives an ionic species in the ESI mass spectrum that could be assigned as a Meisenheimer adduct of DABCO and phenol **19**. The addition of DBU to the reaction mixture favoured the formation of $O^6$-arylguanines **23**–**25**. The ability of DBU to induce elimination reactions is well known, so thigher yields of arylguanines **23**–**25** are consistent with the formation of Meisenheimer complexes, which eliminate the corresponding phenols to give arylguanines as the final products. On the other hand, DBU did not improve the yield of vinylphenylguanine (**22**). Therefore, it is likely that the by-products detected by HPLC-MS at $m/z$ = 374, 494 and 606 are adducts of the aminopurinyl moiety to the vinyl group of **18** rather than Meisenheimer complexes.

Table 4. Comparison of the proton chemical shift values (in ppm) of the $OCH(Ph)CH_2O$ moiety for isomeric $O^6$-guanine adducts.

| | α-Isomers | | | | β-Isomers | | | |
|---|---|---|---|---|---|---|---|---|
| | **7** | **14** | **16** | mean | **8** | **15** | **17** | mean |
| $\delta_H(CH)$ | 6.45 | 6.55 | 6.51 | 6.50 ± 0.05 | 5.17 | 4.85 | 4.87 | 4.96 ± 0.18 |
| $\delta_H(CH_2)$[a] | 3.95 | 3.82 | 3.81 | 3.86 ± 0.08 | 4.49 | 4.50 | 4.67 | 4.55 ± 0.10 |
| $\Delta = \delta_H(CH) - \delta_H(CH_2)$ | 2.50 | 2.73 | 2.70 | 2.64 ± 0.13 | 0.68 | 0.35 | 0.20 | 0.41 ± 0.25 |

[a] Average value of $\delta_A$ and $\delta_B$ (AB system).

Protection of the carboxylic group of phenol **20** as its ethyl ester did not lead to any improvement of the reaction yield. In fact, the reaction of **9** with ethyl ester **21** required a higher reaction temperature, a prolonged reaction time and gave a lower yield than that with unprotected carboxylate **20**.

Unlike $O^6$-alkylguanines **7** and **8** and their nucleoside and nucleotide analogues, $O^6$-arylguanine adducts **22**–**24** have not been prepared as yet and are hardly accessible by the reaction of corresponding oxirane derivative (arene oxide) with guanine

## Experimental Section

**General:** Column chromatography was performed on silica gel 60 purchased from Fluka (particle size 0.063–0.200 mm). Merck Silica gel 60 $F_{254}$ plates were used for thin-layer chromatography. Dimethyl sulfoxide (DMSO) was dried by distillation over calcium hydride and stored over molecular sieves. Other chemicals obtained from commercial sources were of analytical or synthetic grade and were used as received. $^1$H and $^{13}$C NMR spectra were recorded with a Bruker Avance DRX500 (500 MHz for $^1$H) or with a Varian Mercury 300 (300 MHz for $^1$H) Fourier transform NMR spectrometer. In the HMBC experiments, the parameters were set to show cross-peaks for nuclei interacting with a $J_{H,C}$ coupling constant of 7 Hz. Mass spectra were measured with a triple quadrupole HPLC-MS system Varian 1200 equipped with electrospray ionisation (ESI). Positive ions were detected. Microwave-induced reactions were performed in a Synthewave 402 reactor (Synthelabo, France) operating in the controlled-temperature mode.

**Starting Materials:** 2-Allyloxy-1-phenylethanol (**10**) was prepared from methyl mandelate as described in the literature.[32] 2-Benzyloxy-1-phenylethanol (**12**) was prepared in an analogous way and identified by comparing its NMR spectra with those published.[33] Isomeric monoprotected diols, i. e., 2-allyloxy-2-phenylethanol (**11**) and 2-benzyloxy-2-phenylethanol (**13**) were prepared by DDQ-catalysed ring opening of **1** with allyl and benzyl alcohol, respectively.[34] 2-Amino-6-(1-azonia-4-azabicyclo[2.2.2]oct-1-yl)purine (2) (DABCO-purine) was obtained by reaction of 2-amino-6-chloropurine (**5**) with an excess of 1,4-diazabicyclo[2.2.2]octane (DABCO).[18]

**4-Vinylphenol (18):** 4-Vinylphenyl acetate (2 g, 12.3 mmol) was added to a solution of potassium hydroxide (1.72 g, 32 mmol) in 18 mL of aqueous ethanol (1:1 mixture) and the reaction mixture was stirred for 1.5 h under external cooling with crushed ice. Toluene (16 mL) was then added and the reaction mixture was neutralised to pH 7–8 by introduction of carbon dioxide. The layers were separated, the aqueous layer extracted with toluene (8 mL) and the combined organic phases evaporated under vacuum to dryness. Crystallisation of the residue from petroleum ether yielded 880 mg (60%) of white crystals, m.p. 67–70 °C (ref.[31] 68–69 °C) which were identified by comparing their NMR spectra with those published.[35]

**Reaction of 1-Phenylethane-1,2-diol (6) with DABCO-Purine (Conventional Heating):** The reaction conditions were analogous to those of Lembitz et al.[19] A mixture of 2-amino-6-chloropurine (**5**; 0.25 g, 1.48 mmol) and DABCO (0.5 g, 4.45 mmol) was dissolved in 4 mL of DMSO and left standing for 3 h to form a solution of 2-amino-6-(1-azonia-4-azabicyclo[2.2.2]oct-1-yl)purine (**9**; DABCO-purine). The sodium salt of diol **6** was then added [1 g (7.24 mmol) of **6** and 71 mg (2.96 mmol) of NaH] and the reaction mixture stirred at 70 °C for 24 h, then neutralised with acetic acid (17.8 mg, 2.96 mmol) and the DMSO removed under vacuum. The oily residue was purified by column chromatography on silica gel using $CHCl_3$/MeOH (6:1) as an eluent to obtain 182 mg (46%) of a white powder of which a fraction was pure $O^6$-(2-hydroxy-2-phenylethyl)guanine (**8**; ($O^6$-β-isomer, 18% yield) and the rest was a 1:1 mixture of the $O^6$-β-isomer **8** and $O^6$-(2-hydroxy-1-phenylethyl)-guanine (**7**; $O^6$-α-isomer). The mixture was further separated by HPLC using 0.02 M $HCOONH_4$ and methanol at pH 6.0 as a mobile phase.

***O*$^6$-(2-Hydroxy-1-phenylethyl)guanine (*O*$^6$-α-isomer, 7):** M.p. 131–135 °C. $R_f$ ($CHCl_3$/MeOH, 6:1) = 0.35. UV: $\lambda_{max}$ = 242 and 282 nm (pH 6.5, 290 nm (pH 1); 286 nm (pH 12). $^1$H NMR (500 MHz, [D$_7$]DMF): $\delta$ = 3.85 (dd, $J$ = 4.0 and 11.6 Hz, 1 H, C$H_2$O), 4.05 (dd, $J$ = 7.4 and 11.6 Hz, 1 H, C$H_2$O), 6.45 (dd, $J$ = 4.2 and 7.4 Hz, 1 H, C*H*O), 7.32 (m, 3 H, *Ph*), 7.51 (d, $J$ = 7.2 Hz, 2 H, *Ph*), 8.02 (s, 1 H, 8-H) ppm. $^{13}$C NMR (125.8 MHz, [D$_7$]DMF): $\delta$ = 66.0 (*C*$H_2$O), 78.6 (*C*HO), 127.6, 128.2, 140.0 (*Ph*), 138.0 (*C*-8), 160.9 (*C*-6) ppm. ESI-MS: $m/z$ = 294 [M + Na]$^+$, 272 [M + H]$^+$, 152 [M + H – PhCHOHCH$_2$]$^+$, 135 [M + H – PhCHOHCH$_2$OH]. $C_{13}H_{13}N_5O_2$ (271.28): calcd. C 57.6, H 4.8, N 25.8; found C 57.4, H 4.9, N 25.7.

***O*$^6$-(2-Hydroxy-2-phenylethyl)guanine (*O*$^6$-β-isomer, 8):** M.p. 138–141 °C. $R_f$ ($CHCl_3$/MeOH, 6:1) = 0.37. UV: $\lambda_{max}$ = 240 nm and 282 nm (pH 6.5); 288 nm (pH 1); 284 nm (pH 12). $^1$H NMR (500 MHz, [D$_7$]DMF): $\delta$ = 4.42 (dd, $J$ = 8.4 and 10.8 Hz, 1 H, C$H_2$O), 4.56 (dd, $J$ = 3.6 and 10.8 Hz, 1 H, C$H_2$O), 5.17 (dd, $J$ = 3.4 and 8.1 Hz, 1 H, C*H*O), 7.39 (m, 3 H, *Ph*), 7.57 (d, $J$ = 7.2 Hz, 2 H, *Ph*), 8.02 (s, 1 H, 8-H) ppm. $^{13}$C NMR (125.8 MHz, [D$_7$]-DMF): $\delta$ = 71.8 (*C*HO), 72.2 (*C*$H_2$O), 113.2 (*C*-5), 127.0, 127.9, 128.7 and 143.1 (*Ph*), 138.0 (*C*-8), 138.6 (*C*-4), 142.1 (*C*-2), 160.9 (*C*-6) ppm. ESI-MS: $m/z$ = 294 [M + Na]$^+$, 272 [M + H]$^+$, 152 [M + H – PhCHOHCH$_2$]$^+$, 135 [M + H – PhCHOHCH$_2$OH]$^+$. $C_{13}H_{13}N_5O_2$ (271.28): calcd. C 57.6, H 4.8, N 25.8; found C 57.4, H 4.9, N 25.7.

**Reaction of 1-Phenyl-1,2-ethanediol with DABCO-Purine (MW-Induced):** The reaction mixture was prepared as described for the experiment with conventional heating and then irradiated by microwaves in a MW reactor with temperature set to 50, 70 or 90 °C. The reaction time varied from 2.5 to 3.5 h. The products were isolated by column chromatography on silica gel as described above. The mixtures of **7** and **8** obtained were further separated by semi-preparative HPLC to get samples of pure isomers. The yields and ratios of the isomers are shown in Table 1.

**Reaction of DABCO-Purine (9) with Monoprotected Diols 10–13:** A solution of the monoprotected diol (3.18 mmol) in DMF (7 mL) was added to NaH (51 mg, 2.12 mmol) and the mixture was stirred for 30 min at ambient temperature under dry nitrogen. DABCO-purine (**9**; 0.300 g, 1.06 mmol) was added to the alkoxide formed and the reaction mixture was allowed to react for 24 h at room temperature. After neutralisation with acetic acid (121 μL, 127 mg, 2.12 mmol) and evaporation of DMF under vacuum the crude product was purified by column chromatography on silica gel using $CHCl_3$/MeOH (10:1 or 15:1) as eluent for allyl- and benzyl-protected compounds, respectively. In another set of experiments, 4.24 mmol (4 equiv.) of the corresponding monoprotected diol was used, while the amount of NaH was increased to 2.65 mmol (2.5 equiv.) or 3.20 mmol (3 equiv.). The yields are shown in Table 2.

***O*$^6$-[2-(Allyloxy)-1-phenylethyl]guanine (14):** A yellowish powder (0.180 g, 54%) obtained by the reaction of alcohol **10** with **9**. Recrystallisation from $H_2O$/EtOH (10:1) yielded a white powder,

m.p. 160–162 °C. $R_f$ ($CHCl_3$/MeOH, 10:1) = 0.35. UV: $\lambda_{max}$ = 242 and 284 nm (pH 6.5); 290 nm (pH 1); 286 nm (pH 12). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 3.72 (dd, $J$ = 3.5 and 10.7 Hz, 1 H, OCHPhC$H_2$), 3.91 (dd, $J$ = 8.4 and 10.6 Hz, 1 H, OCHPhC$H_2$), 4.00 (m, 2 H, OC$H_2$CHCH$_2$), 5.06 (s, 2 H, NH$_2$), 5.17 (d, $J$ = 10.6 Hz, 1 H, OCH$_2$CHC$H_2$), 5.21 (d, $J$ = 17.3 Hz, 1 H, OCH$_2$CHC$H_2$), 5.81 (m, 1 H, OCH$_2$C$H$CH$_2$), 6.55 (dd, $J$ = 3.5 Hz and 8.1 Hz, 1 H, OC$H$PhCH$_2$), 7.25 (m, 3 H, *Ph*), 7.42 (d, $J$ = 7.1 Hz, 2 H, *Ph*), 7.68 (s, 1 H, 8-H) ppm. $^{13}$C NMR (125.8 MHz, $CDCl_3$): $\delta$ = 72.3 (OCHPh$C$H$_2$), 73.2 (O$C$H$_2$CHCH$_2$), 76.5 (O$C$HPhCH$_2$), 114.3 (*C*-5), 117.4 (OCH$_2$CH$C$H$_2$), 126.8, 128.1, 128.4 and 137.9 (*Ph*), 134.3 (OCH$_2$$C$HCH$_2$), 137.7 (*C*-8), 154.6 (*C*-2), 159.1 (*C*-4), 160.3 (*C*-6) ppm. HMBC shows cross-peaks between *C*-6 ($\delta_C$ = 160.3 ppm) and C$H$O ($\delta_H$ = 6.55 ppm). ESI-MS: $m/z$ = 350 [M + K]$^+$, 334 [M + Na]$^+$, 312 [M + H]$^+$. $C_{16}H_{17}N_5O_2\cdot 1/4H_2O$ (315.85): calcd. C 60.8, H 5.6, N 22.2; found C 60.6, H 5.4, N 22.5.

***O*$^6$-[2-(Allyloxy)-2-phenylethyl]guanine (15):** A yellowish precipitate (0.311 g; 94%) obtained from the reaction of alcohol **11** with purine **9**. Recrystallisation from toluene/ethanol (10:1) gave a white powder, m.p. 195–197 °C. $R_f$ ($CHCl_3$/MeOH, 10:1) = 0.40. UV: $\lambda_{max}$ = 240 and 282 nm (pH 6.5); 288 nm (pH 1); 286 nm (pH 12). $^1$H NMR (500 MHz, [D$_6$]DMSO): $\delta$ = 3.95 (br. s, 2 H, OC$H_2$CHCH$_2$), 4.50 (d, $J$ = 5.5 Hz, 2 H, OC$H_2$CHPh), 4.85 (t, $J$ = 5.5 Hz, 1 H, OCH$_2$C$H$Ph), 5.11 (d, $J$ = 10.5 Hz, 1 H, OCH$_2$CHC$H_2$), 5.27 (d, $J$ = 17.5 Hz, 1 H, OCH$_2$CHC$H_2$), 5.87 (m, 1 H, OCH$_2$C$H$CH$_2$), 6.25 (s, 2 H, NH$_2$), 7.40 (m, 5 H, *Ph*), 7.81 (s, 1 H, 8-H) ppm. $^{13}$C NMR (125.8 MHz, [D$_6$]DMSO): $\delta$ = 69.1 (O$C$H$_2$CHCH$_2$), 69.2 (O$C$H$_2$CHPh), 78.6 (OCH$_2$$C$HPh), 113.4 (*C*-5), 116.3 (OCH$_2$CH$C$H$_2$), 127.0, 128.1, 128.5 and 138.8 (*Ph*), 135.0 (OCH$_2$$C$HCH$_2$), 137.8 (*C*-8), 155.2 (*C*-2), 159.6 (*C*-4), 159.9 (*C*-6) ppm. HMBC shows cross-peaks between *C*-6 ($\delta_C$ = 159.9 ppm) and OC$H_2$CHPh ($\delta_H$ = 4.50 ppm). ESI-MS: $m/z$ = 334 [M + Na]$^+$, 312 [M + H]$^+$, 152. $C_{16}H_{17}N_5O_2\cdot 1/4H_2O$ (315.85): calcd. C 60.8, H 5.6, N 22.2; found C 60.5, H 5.4, N 22.5.

***O*$^6$-[2-(Benzyloxy)-1-phenylethyl]guanine (16):** A white precipitate (0.173 g, 45%) obtained from the reaction of **12** with **9**, m.p. 69–73 °C. $R_f$ ($CHCl_3$/MeOH, 15:1) = 0.26. UV: $\lambda_{max}$ = 242 and 284 nm (pH 6.5); 290 nm (pH 1); 286 nm (pH 12). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.71 (dd, J = 3.8 and 10.6 Hz, 1 H, OCHPhC$H_2$), 3.92 (dd, $J$ = 8.2 and 10.6 Hz, 1 H, OCHPhC$H_2$), 4.47 (d, $J$ = 12.0 Hz, 1 H, OC$H_2$Ph), 4.54 (d, $J$ = 12.0 Hz, 1 H, OC$H_2$Ph), 4.83 (s, 2 H, NH$_2$), 6.51 (dd, $J$ = 3.8 and 7.9 Hz, 1 H, OC$H$PhCH$_2$), 7.19 (m, 8 H, *Ph*), 7.38 (d, $J$ = 6.7 Hz, 2 H, *Ph*), 7.62 (s, 1 H, 8-H) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 73.4, (OCHPh$C$H$_2$), 73.5 (O$C$H$_2$Ph), 76.6 (O$C$HPhCH$_2$), 127.0, 127.8, 127.9, 128.3, 128.5 and 137.8, 138.0 (*Ph*), 159.2 (*C*-6) ppm. ESI-MS: $m/z$ = 400 [M + K]$^+$, 384 [M + Na]$^+$, 362 [M + H]$^+$. $C_{20}H_{19}N_5O_2\cdot 1/4H_2O$ (365.91): calcd. C 65.6, H 5.4, N 19.1; found C 65.8, H 5.5, N 18.9.

***O*$^6$-[2-(Benzyloxy)-2-phenylethyl]guanine (17):** A white precipitate (0.305 g, 79%) obtained from the reaction of **13** with **9**, m.p. 73–76 °C. $R_f$ ($CHCl_3$/MeOH, 15:1) = 0.23. UV: $\lambda_{max}$ = 240 and 282 nm (pH 6.5); 290 nm (pH 1); 286 nm (pH 12). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 4.39 (d, $J$ = 11.7 Hz, 1 H, OC$H_2$Ph), 4.54 (d, $J$ = 11.7 Hz, 1 H, OC$H_2$Ph), 4.59 (dd, $J$ = 3.3 and 11.3 Hz, 1 H, OC$H_2$CHPh), 4.75 (m, 1 H, OC$H_2$CHPh), 4.87 (dd, $J$ = 3.5 and 7.6 Hz, 1 H, OCH$_2$C$H$Ph), 5.33 (s, 2 H, NH$_2$), 7.30 (m, 8 H, *Ph*), 7.43 (d, $J$ = 7.1 Hz, 2 H, *Ph*), 7.76 (s, 1 H, 8-H) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 70.1 (O$C$H$_2$CHPh), 70.7 (O$C$H$_2$Ph), 79.3 (OCH$_2$$C$HPh), 127.1, 127.5, 127.6, 128.2, 128.6 and 137.9, 138.3 (*Ph*), 138.5 (*C*-8), 159.2 (*C*-6) ppm. ESI-MS: $m/z$ = 400 [M + K]$^+$, 384 [M + Na]$^+$, 362 [M + H]$^+$. $C_{20}H_{19}N_5O_2$ (361.41): calcd. C 66.46, H 5.30, N 19.38; found C 66.2, H 5.1, N 19.3.

**Deallylation:** The reaction conditions were analogous to those described by Chandrasekhar et al.[26] Poly(methylhydrosiloxane) (PMHS; 115 mg), tetrakis(triphenylphosphane)palladium (10 mg) and $ZnCl_2$ were added to a stirred solution of the allyl-protected purine derivative (0.300 g, 0.96 mmol) in 8 mL of DMF under argon. The reaction mixture was stirred at 60 °C for 7–10 h. After evaporation of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (chloroform/methanol, 4:1) and crystallised from MeOH with the addition of charcoal as a decolourising agent.

***O*$^6$-(2-Hydroxy-1-phenylethyl)guanine (*O*$^6$-α-isomer, 7):** Obtained as a greyish powder (0.220 g, 84%) by deallylation of **14**. Crystallisation from MeOH with the addition of charcoal as a decolourising agent yielded a white powder (0.084 g, 32%).

***O*$^6$-(2-Hydroxy-2-phenylethyl)guanine (*O*$^6$-β-isomer, ):** Obtained as a greyish powder (0.210 g, 80%) by deallylation of **15**. Crystallisation from MeOH with the addition of charcoal as a decolourising agent yielded a white powder (0.074 g, 28%).

**Debenzylation:** The reaction conditions were analogous to those described by Bieg et al.[36] Palladium (10%) on carbon containing nearly 50% of water, (Degussa type E101 NE/W) (1.484 g) was activated under vacuum at 110 °C. The benzyl-protected derivative (0.300 g, 0.83 mmol) and ammonium formate (0.325 g) in 19 mL of absolute MeOH was added to the catalyst and refluxed for 5–7 h. The total amount of ammonium formate (1.3 g) was added subsequently in four portions. Each portion was added after hydrogen had stopped forming. The catalyst was filtered off and washed with hot aqueous methanol. Finally, the filtrate was evaporated under vacuum.

***O*$^6$-(2-Hydroxy-1-phenylethyl)guanine (*O*$^6$-α-isomer, 7):** Obtained as a white powder (0.043 g, 19%) by debenzylation of **16**.

***O*$^6$-(2-Hydroxy-2-phenylethyl)guanine (*O*$^6$-β-isomer, 8):** Obtained as a white powder (0.045 g, 21%) by debenzylation of **17**.

**Preparation of *O*$^6$-arylguanines Using NaH as a Base [Procedure (i)]:** DABCO-purine (**9**; 300 mg, 1.06 mmol) was added to a solution of sodium phenolate in DMF prepared from the corresponding phenol (3.18 mmol, threefold excess) and sodium hydride (76 mg, 3.17 mmol). The reaction mixture was heated at 60 °C under dry nitrogen for 3 d. After cooling to room temperature, acetic acid (180 μL, 189 mg, 3.15 mmol) was added for neutralisation and the solvent was distilled off under vacuum. The residue was redissolved in $CHCl_3$/MeOH (8:1) and separated by column chromatography on silica gel to obtain a crude product.

**Preparation of *O*$^6$-arylguanines Using $K_2CO_3$ and DBU [Procedure (ii)]:** A mixture of the phenol (3.28 mmol), potassium carbonate (731 mg, 5.30 mmol) and DMF (5 mL) was stirred under dry nitrogen for 30 min. DABCO-purine (**9**; 300 mg, 1.06 mmol) was then added and the reaction mixture was stirred for another 30 min at ambient temperature. Thereafter, DBU (158 μL, 161 mg, 1.06 mmol) was added and the resulting mixture heated for 3 d at 65 °C. The solvent was evaporated under vacuum and the residue separated by column chromatography on silica gel using $CHCl_3$/MeOH (8:1) to obtain a crude product.

***O*$^6$-(4-Vinylphenyl)guanine (22):** Obtained by crystallisation of the crude product of the reaction of **9** with phenol **18** from $CHCl_3$/MeOH/AcOH. Procedures (i) and (ii) yielded 25 mg (9%) and 17 mg (6%), respectively, of a white powder identified as aryloxypurine **22**, m.p. 212–217 °C. $R_f$ ($CHCl_3$/MeOH, 8:1) = 0.25. UV: $\lambda_{max}$ = 244 and 294 nm (pH 1); 246 and 286 nm (pH 6.5); 250 and 292 nm (pH 12). $^1$H NMR (500 MHz, [D$_6$]DMSO): $\delta$ = 1.88 (s, 3

H, $CH_3COO^-$), 5.23 (d, $J$ = 10.7 Hz, 1 H, $CH_2$), 5.80 (d, $J$ = 17.6 Hz, 1 H, $CH_2$), 6.75 (dd, $J$ = 10.7, 17.6 Hz, 1 H, PhC*H*), 7.19 (d; 2 H; $J$ = 8.3 Hz, *Ph*), 7.51 (d, $J$ = 8 Hz, 2 H, 5 Hz, *Ph*), 7.91 (s, 1 H, C8-*H*) ppm. $^{13}$CNMR (75 MHz, [D$_6$]DMSO): $\delta$ = 21.1 ($CH_3COO^-$), 114.0 (CH=$CH_2$), 122.0 and 127.3 (aromatic *C*H), 134.1 and 152.4 (aromatic *C*), 135.9 (*C*H=$CH_2$), 156.2 (*C*4), 159.7 (*C*6). ESI-MS: $m/z$ = 292 [M + K]$^+$, 276 [M + Na]$^+$, 254 [M + H]$^+$. $C_{13}H_{11}N_5O{\cdot}C_2H_4O_2$ (313.32): calcd. C 57.5, H 4.8, N 22.3; found C 57.8, H 5.0, N 22.8.

***O*$^6$-[4-(2-Hydroxyethyl)phenyl]guanine (23):** Obtained by the reaction of **9** with phenol **19**. The crude product was purified by column chromatography on silica gel using $CHCl_3$/MeOH/AcOH, 30:3:1 as an eluent. Procedures (i) and (ii) gave 90 mg (35%) and 27 mg (9%), respectively, of a white powder which was identified as arylguanine **23**, m.p. 232–236 °C. $R_f$ ($CHCl_3$/MeOH/AcOH, 30:3:1) = 0.28. UV: $\lambda_{max}$ = 292 nm (pH 1); 286 nm (pH 6.5); 292 nm (pH 12). $^1$H NMR (300 MHz, [D$_6$]DMSO): $\delta$ = 2.72 (t, $J$ = 6.9 Hz, 2 H, PhC$H_2$), 3.62 (t, $J$ = 6.9 Hz, 2 H, C$H_2$O), 4.66 (s, 1 H, $CH_2$O*H*), 7.10 (d, $J$ = 8.5 Hz, 2 H, *Ph*), 7.24 (d, $J$ = 8.6 Hz, 2 H, *Ph*), 7.91 (s, 1 H, C8-*H*) ppm. $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta$ = 38.0 ($CH_2CH_2OH$), 62.7 ($CH_2OH$), 113.6 (*C*5), 122.0 and 130.6 (aromatic *C*H), 137.0 and 151.4 (aromatic *C*), 140.0 (*C*8), 157.4 (*C*4), 160.0 (C*2*), 160.4 (*C*6). ESI-MS: $m/z$ = 310 [M + K]$^+$, 294 [M + Na]$^+$, 272 [M + H]$^+$. $C_{13}H_{13}N_5O_2{\cdot}H_2O$ (289.30): calcd. C 54.0, H 5.2, N 24.2; found C 53.6, H 4.8, N 22.9.

***O*$^6$-(4-Carboxymethylphenyl)guanine (24):** Obtained by procedure (ii) by reacting **9** with phenolic acid **20**. The crude product was purified by column chromatography on silica gel using $CHCl_3$/MeOH/AcOH (30:3:1) as eluent. Arylguanine **24** (105 mg, 35%) was obtained as a white powder, m.p. > 300 °C, $R_f$ ($CHCl_3$/MeOH/AcOH, 30:3:1) = 0.23. UV: $\lambda_{max}$ = 292 nm (pH 1); 286 nm (pH 6.5); 290 nm (pH 12). $^1$H NMR ([D$_6$]DMSO): $\delta$ = 3.44 (s, 2 H, OC$H_2CO_2$); 6.27 (s, 2 H, N$H_2$), 7.10 (d, $J$ = 8.5 Hz, 2 H, *Ph*), 7.27 (d, $J$ = 8.5 Hz, 2 H, *Ph*), 7.9 (s, 1 H, C8-*H*) ppm. $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta$ = 43.0 (Ph$CH_2$), 113.6 (*C*5), 121.9 and 131.1 (aromatic *C*H), 134.3 and 151.5 (aromatic *C*), 157.5 (*C*4), 160.4 (*C*6), 175.0 (*C*OOH). ESI-MS: $m/z$ = 324 [M + K]$^+$, 308 [M + Na]$^+$, 286 [M + H]$^+$. $C_{13}H_{11}N_5O_3{\cdot}H_2O$ (303.28): calcd. C 51.5, H 4.3, 23.1; found C 51.1, H 4.3, N 22.9.

***O*$^6$-(4-Ethoxycarbonylmethylphenyl)guanine (25):** Obtained by procedure (ii) by reacting **9** with phenol **21**. Crystallisation of the crude product from chloroform afforded 82 mg (25%) of arylguanine **25**. White powder, m.p. 215–217 °C, $R_f$ ($CHCl_3$/MeOH/AcOH, 30:3:1) = 0.56. UV: $\lambda_{max}$ = 292 nm (pH 1); 286 nm (pH 6.5); 290 nm (pH 12). $^1$H NMR ([D$_6$]DMSO): $\delta$ = 1.19 (t, $J$ = 7.15 Hz, 3 H, $CH_2CH_3$), 3.68 (s, 2 H, OC$H_2CO_2$), 4.85 (q, $J$ = 7.15 Hz, 2 H, C$H_2CH_3$), 6.26 (s, 2 H, N$H_2$), 7.17 and 7.30 (d,, $J$ = 8.4 Hz 2+2 H, *Ph*); 7.93 (s, 1 H, C8–*H*) ppm. $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta$ = 14.8 ($CH_2CH_3$), 52.4 ($CH_2COOH$), 61.0 (O$CH_2CH_3$), 114.4 (*C*5), 122.3 and 131.2 (aromatic *C*H), 131.7 (aromatic *C*), 152.2 (aromatic *C*OH), 156.8 (*C*4), 160.4 (*C*6), 172.0 (*C*OOH). ESI-MS: $m/z$ = 352 [M + K]$^+$, 334 [M + Na]$^+$, 314 [M + H]$^+$.

**HPLC-MS Analyses:** The crude products of the reaction of **9** with phenols **18**–**20** were dissolved in aqueous methanol and analysed by HPLC-MS on a Phenomenex Luna 2 C18 column (250 × 2 mm; particle size: 5 μm). The column was eluted with 5 mM ammonium formate, pH 5.0, containing 33% methanol at a rate of 170 μL min$^{-1}$. The concentration of methanol was increased linearly up to 100% within 25 min and than kept unchanged for an additional 5 min. The effluent was introduced into an ESI chamber and the ions formed were detected in the mass range of $m/z$ = 150–700.

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[1] A. E. Pegg, *Cancer Res.* **1990**, *50*, 6119–6129.
[2] C. Mi-Young, M. G. McDougall, M. E. Dolan, K. Swenn, A. E. Pegg, R. C. Moschel, *J. Med. Chem.* **1994**, *37*, 342–347.
[3] M. Otteneder, W. K. Lutz, *Mutat. Res.* **1999**, *424*, 237–247.
[4] K. Hemminki, M. Koskinen, H. Rajaniemi, C. Zhao, *Regul. Toxicol. Pharmacol.* **2000**, *32*, 264–275.
[5] P. Vodicka, L. Vodickova, K. Trejbalova, R. J. Šram, K. Hemminki, *Carcinogenesis* **1994**, *15*, 1949–1953.
[6] D. E. G. Shuker, P. B. Farmer, *Chem. Res. Toxicol.* **1992**, *5*, 450–460.
[7] J. Novák, I. Linhart, H. Dvořáková, *Eur. J. Org. Chem.* **2004**, 2738–2746.
[8] P. Vodicka, K. Hemminki, *Carcinogenesis* **1988**, *9*, 1657–1660.
[9] K. Pongracz, S. Kaur, A. L. Burlingham, W. J. Bodell, *Carcinogenesis* **1989**, *10*, 1009–1013.
[10] M. Otteneder, E. Eder, W. K. Lutz, *Chem. Res. Toxicol.* **1999**, *12*, 93–99.
[11] W. Pauwels, P. Vodicka, M. Severi, K. Plna, H. Veulemans, K. Hemminki, *Carcinogenesis* **1996**, *17*, 2673–2680.
[12] P. Vodicka, K. Hemminki, *IARC Sci. Publ.* **1993**, *127*, 109–118.
[13] P. Vodicka, L. Vodickova, K. Hemminki, *Carcinogenesis* **1993**, *14*, 2059–2061.
[14] P. Manini, L. Buzio, R. Andreoli, M. Goldoni, E. Bergamaschi, M. Jakubowski, P. Vodicka, A. Hirvonen, A. Mutti, *Toxicol. Appl. Pharmacol.* **2003**, *189*, 160–169.
[15] P. Vodicka, R. Stetina, M. Koskinen, P. Soucek, L. Vodickova, P. Hlavac, M. Kuricova, R. Necasova, K. Hemminki, *Int. Arch. Occup. Environ. Health* **2002**, *75* S75–S85(Suppl.).
[16] R. W. Balsinger, J. A. Montgomery, *J. Org. Chem.* **1960**, *25*, 1573–1575.
[17] R. L. Lewis, F. H. Schneider, R. K. Robins, *J. Org. Chem.* **1961**, *26*, 3837–3842.
[18] J. A. Linn, E. D. McLean, J. L. Kelley, *J. Chem. Soc., Chem. Commun.* **1994**, 913–914.
[19] N. K. Lembitz, S. Grant, W. Clegg, R. J. Griffin, S. L. Heath, B. T. Golding, *J. Chem. Soc., Perkin Trans. 1* **1997**, 185–186.
[20] M. K. Lakshman, F. N. Ngassa, J. C. Keeler, Y. Q. V. Dinh, J. H. Himler, L. M. Russon, *Org. Lett.* **2000**, *2*, 927–930.
[21] K. Savela, A. Hesso, K. Hemminki, *Chem.-Biol. Interact.* **1986**, *60*, 235–246.
[22] M. Koskinen, E. K. H. Schweda, K. Hemminki, *J. Chem. Soc., Perkin Trans. 2* **1999**, 2441–2445.
[23] P. Vodicka, K. Hemminki, *Chem.-Biol. Interact.* **1991**, *77*, 39–50.
[24] R. C. Moschel, K. Hemminki, A. Dipple, *J. Org. Chem.* **1986**, *51*, 2952–2955.
[25] K. Pongracz, S. Kaur, A. L. Burlingame, W. Bodell, *Carcinogenesis* **1989**, *10*, 1009–1013.
[26] S. Chandrasekhar, C. R. Reddy, R. J. Rao, *Tetrahedron* **2001**, *57*, 3435–3438.
[27] *Monographs on the Evaluation of Carcinogenic Risks to Humans*; International Agency for Research on Cancer, Lyon, France, **1994**; vol. 60, pp. 272–275.
[28] O. M. Bakke, R. R. Scheline, *Toxicol. Appl. Pharmacol.* **1970**, *16*, 691–700.
[29] I. Linhart, I. Gut, J. Šmejkal, J. Novák, *Chem. Res. Toxicol.* **2000**, *13*, 36–44.
[30] G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop, S. Udenfriend, *Science* **1967**, *157*, 1524–1530.

  

[31] B. B. Corson, W. J. Heintzelman, L. H. Schwartzman, H. E. Tiefenthal, R. J. Lokken, J. E. Nickels, G. R. Atwood, F. J. Pavlik, *J. Org. Chem.* **1958**, *23*, 544–549.
[32] L. M. Stephenson, D. L. Mattern, *J. Org. Chem.* **1976**, *41*, 3614–3619.
[33] C. S. Shiner, T. Tsunoda, B. A. Goodman, S. Ingham, S. Lee, P. E. Vorndam, *J. Am. Chem. Soc.* **1989**, *111*, 1381–1392.
[34] N. Iranpoor, I. M. Baltork, *Tetrahedron Lett.* **1990**, *31*, 735–738.
[35] U. Bettach, Y. Le Bigot, Z. Mouloungui, M. Delmas, A. Gaset, *Synth. Commun.* **1992**, *22*, 513–518.
[36] T. Bieg, W. Szeja, *Synthesis* **1985**, *1*, 76–77.

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