Syntheses of 7-(2-Hydroxy-1-phenylethyl)- and 7-(2-Hydroxy-2-phenylethyl)guanine, DNA Adducts Derived from Styrene 7,8-Oxide

Jan Novák,[a] Igor Linhart,*[a] and Hana Dvořáková[b]

Keywords: DNA adducts / Alkylation / Heterocycles / Protecting groups / Guanine derivatives

7-(2-Hydroxy-1-phenylethyl)- and 7-(2-hydroxy-2-phenylethyl)guanines are important DNA adducts which can be used as markers of exposure to styrene. Two synthetic routes leading to these compounds are presented using allyl-protected bromohydrins as synthetic equivalents of styrene oxide to alkylate 2-amino-6-chloropurine or 7-methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine as precursors to guanine.

Introduction

7-[Hydroxy(phenyl)ethyl]guanines are important DNA adducts formed as a result of exposure to styrene, a large-scale industrial monomer.[1,2] Styrene is metabolised to styrene 7,8-oxide (**1**) an electrophilic intermediate capable of attacking nucleophilic centres in nucleic acids.[3] The main site of the attack in DNA is at N-7 of guanine. When oxirane **1** binds to this site, two guanine adducts can be formed — 7-(2-hydroxy-1-phenylethyl)guanine (**2a**) and 7-(2-hydroxy-2-phenylethyl)guanine (**2b**).[4] The former is formed by nucleophilic attack at the oxirane α-carbon atom and is therefore often denoted as the α adduct, whereas the latter arises from attack at the β-carbon atom and is therefore called the β adduct. Consequently, 7-alkylguanines are the main types of DNA adducts excreted in urine and are therefore important markers of DNA damage caused by alkylating agents.[5] Therefore, 7-alkylated guanines derived from an industrially important compound such as styrene are needed as analytical standards for the development of diagnostic methods to detect and quantitate specific types of DNA damage.

Oxiranes can be used to alkylate purine derivatives mainly at the most reactive position N-9.[6,7] However, in guanine nucleosides, nucleotides and DNA the N-9 position is substituted by a sugar, so the N-7 position becomes the most reactive nucleophilic centre of the molecule. In fact, the most reactive alkylating agents such as methyl iodide, dimethyl sulfate and benzyl bromide can alkylate guanosine effectively and regioselectively to give the corresponding 7-alkylated products.[8] Similarly, reaction of guanosine with ethylene oxide followed by acidic hydrolysis leads to 7-(2-hydroxyethyl)guanine.[9] However, alkylations of nucleosides and nucleotides with less reactive alkylating agents generally proceed with low regioselectivity. In particular, oxirane **1**, as a weak alkylating reagent, gives low regioselectivity in alkylations.[10] To improve both reactivity and regioselectivity we searched for suitable synthetic equivalents of **1** and for a possible precursor of guanine giving a better regioselectivity of alkylation. Several precursors to guanine with enhanced selectivity of alkylation have been reported in the literature, such as 6,7-diacetoxy-5-acetyl-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (**3**),[11] 5-acetyl-6,7-bis(2-methylpropanoyloxy)-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (**4**),[11] 7-methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine (**5**)[12] and 2-amino-6-chloropurine (**6**).[6]

In our preliminary report we described a synthetic route to compounds **2a** and **2b** based on the alkylation of 2-amino-6-chloropurine (**6**) with allyl-protected bromohydrins as synthetic equivalents to **1**.[13] In this paper we report two possible ways to protect guanine in order to improve the regioselectivity of the alkylation. The results are compared with those obtained by alkylation of the purine **6**.

Results and Discussion

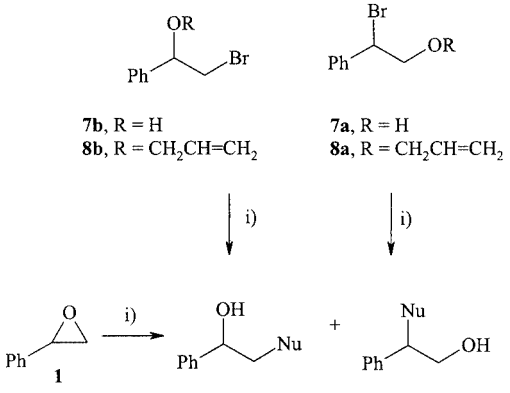
Synthetic Equivalents of Styrene 7,8-Oxide (1)

Two isomeric bromohydrins, 2-bromo-2-phenylethanol (**7a**) and 2-bromo-1-phenylethanol (**7b**) can be derived from **1**, each giving potentially one type of alkylation product — the α and β adduct, respectively (Scheme 1). Bromohydrin **7b** reacted with purine **6** to give a 1:6 mixture of 2-amino-6-chloro-7-(2-hydroxy-2-phenylethyl)purine (**10b**) and 2-amino-6-chloro-9-(2-hydroxy-2-phenylethyl)purine (**11b**).[13] In contrast, bromohydrin **7a** did not alkylate **6** but under

[a] Department of Organic Chemistry, Institute of Chemical Technology Prague,
Technická 5, 16628 Prague, Czech Republic
[b] Central Laboratories, Institute of Chemical Technology Prague, Technická 5, 16628 Prague, Czech Republic

  DOI: 10.1002/ejoc.200300811

the reaction conditions applied (dimethylformamide with potassium carbonate as a base) underwent a base-catalysed condensation reaction. Protection of the hydroxy group by a suitable protective group was therefore required to prevent elimination of HBr from bromohydrins **7a** and **7b**. Allyl has proved to be an effective OH protective group not only to prevent base-catalysed side-reactions of the bromohydrins but also to enhance reactivity in the nucleophilic substitution. This latter effect can be explained by a participation of allylic π-electrons in the nucleophilic substitution.[14] Therefore, allyl-protected bromohydrins, namely allyl 2-bromo-2-phenylethyl ether (**8a**) and allyl 2-bromo-1-phenylethyl ether (**8b**), as well as the allyl-protected tosylate 2-allyloxy-2-phenylethyl tosylate (**9b**), were studied as alkylating agents. Allyl-protected bromohydrin **8a**, a synthetic equivalent of **1** affording α-adduct-type products on alkylation, was prepared from the corresponding alcohol, i.e., allyl 2-hydroxy-2-phenylethyl ether, upon reaction with phosphorus tribromide. In contrast, an analogous reaction of allyl 2-hydroxy-1-phenylethyl ether gave a low yield of **8b**. Therefore, compound **8b** was prepared from 2-allyloxy-2-phenylethyl tosylate (**9b**)[15] by nucleophilic substitution with lithium bromide. Both the bromide **8b** and the tosylate **9b** are synthetic equivalents of **1** giving the β adducts. All our attempts to prepare isomeric 2-allyloxy-1-phenylethyl tosylate (**9a**), which should give the corresponding α-adduct-type products on alkylation, were unsuccessful. Data summarising the results of the alkylation of **6** with synthetic equivalents of **1** are shown in Table 1. Both reactivity and selectivity were improved by introducing the allyl protective group. As expected, benzylic alkyl bromide **8a** showed a better reactivity (a higher yield) than the phenethylic bromide **8b**. In all cases, the position N-9 in purine **6** was the main site of alkylation.



Scheme 1. Synthetic equivalents of styrene 7,8-oxide: i) Nu^-, for allyl-protected compounds **7b** and **8b** followed by deallylation[18]

Precursors of Guanine and Their Alkylation

2-Amino-6-chloropurine (6)

Guanine itself is not a suitable substrate for alkylation reactions due to its low reactivity and solubility. Purine **6** was therefore used as a precursor to guanine that can be converted into a guanine moiety by simple hydrolysis of chlorine in the 6-position.[16] This approach allowed us to prepare 7-[hydroxy(phenyl)ethyl]guanines **2a** and **2b**[13] but the yields of the N-7 isomer required in the alkylation step were rather low (Scheme 2, Table 1). The main problem was the regioselectivity of the alkylation step. Position N-7 in purine **6** is less reactive than N-9, so that the required 7-alkylated product was always the minor one. Bromohydrin **7b**, but not its isomer **7a**, yielded a mixture of alkylated purines **10b** and **11b** which could be converted into corresponding guanine derivatives by hydrolysis with aqueous NaOH. Allyl-protected alkylating agents **8a**, **8b** and **9b** gave better yields of the alkylated products, which were then converted into **2a** and **2b** by a two-step procedure (Scheme 2). The overall yields of **2a** and **2b** for allyl-protected alkylating agents are, however, better than those for unprotected bromohydrins.

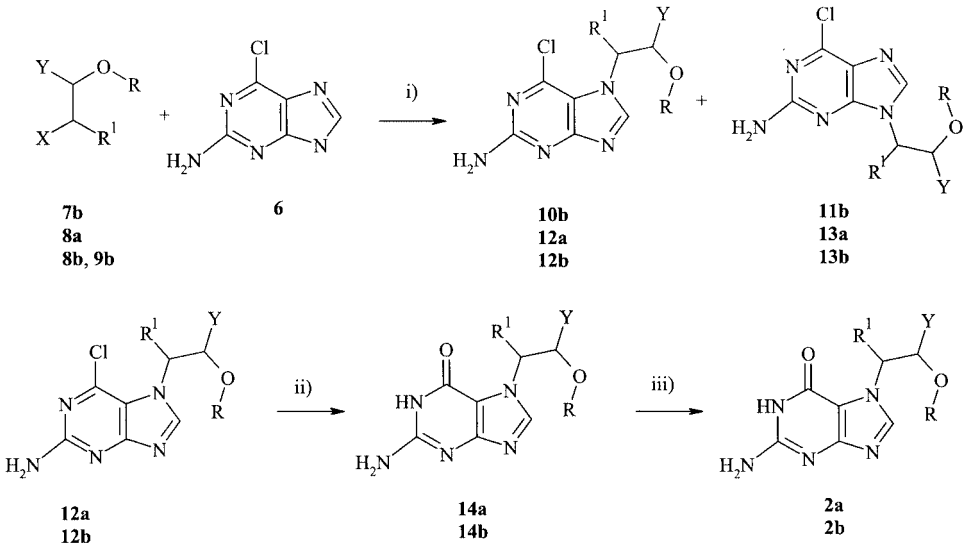
Table 1. 7-(2-Hydroxy-1-phenylethyl)- and 7-(2-hydroxy-2-phenylethyl)guanines by alkylation of 2-amino-6-chloropurine (**6**)

Reagent	Yield of compound			
	Alkylation	Hydrolysis	Deallylation	Overall yield
7b	48% **11b**			
	8% **10b**	95% **2b**		7.6% **2b**
8b	51% **13a**			
	13% **12a**	95% **14b**	90% **2b**	11% **2b**
8a	62% **13b**			
	22% **12b**	97% **14a**	92% **2a**	20% **2a**
9b	62% **13b**			
	21% **12b**		90% **2b**	18% **2b**

The reactivity of the N-7 position can be increased when modified guanine derivatives (guanine precursors) are used which can be easily converted into the corresponding guanine derivatives after alkylation.

Imidazopurine Derivatives

Imidazopurines, namely 6,7-diacetoxy-5-acetyl-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (**4**) and 5-acetyl-6,7-bis(2-methylpropanoyloxy)-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (**5**), were introduced by Kjellberg and Johansson as precursors to guanine for regioselective alkylations.[11] Although the original aim of the application of these compounds was to prepare N-9 guanine derivatives such as buciclovir selectively, the regioselectivity of alkylation was strongly dependent on the reaction conditions, mainly on the base used. When sodium hydride in DMF was used, the N-7 derivative predominated (e.g., with 4-bromobutyl acetate as the alkylating reagent the N-7/N-9 isomer ratio was 18:1).[11a] However, we were not able to alkylate compounds **3** or **4** with either of the three allyl-protected synthetic equivalents of styrene oxide **8a**, **8b** or **9b**. In all cases the reaction proceeded slowly yielding a mixture of degradation products rather than the expected products of alkylation. On the other hand, alkylation of both **3** and **4** with benzyl bromide yielded a nearly 3.5:1 mixture of 1-/3-alkylated products. This product ratio did not change when potassium carbonate was used as a base in-



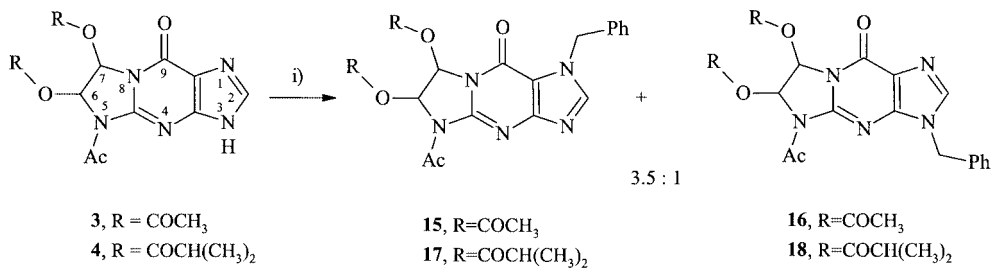
	2a	2b	7b	8a	8b	9b	10b	11b	12a	12b	13a	13b	14a	14b
X	-	-	Br	Br	Br	OTs	-	-	-	-	-	-	-	-
Y	H	Ph	Ph	H	Ph	Ph	Ph	Ph	H	Ph	H	Ph	H	Ph
R	H	H	H	allyl	allyl	allyl	H	H	allyl	allyl	allyl	allyl	allyl	allyl
R^1	Ph	H	H	Ph	H	H	H	H	Ph	H	Ph	H	Ph	H

Scheme 2. Alkylation of 2-amino-6-chloropurine: i) K_2CO_3/DMF, 60 °C; ii) aqueous NaOH; iii) $[Pd(PPh_3)_4]$, PMHS, $ZnCl_2$, DMF[18]

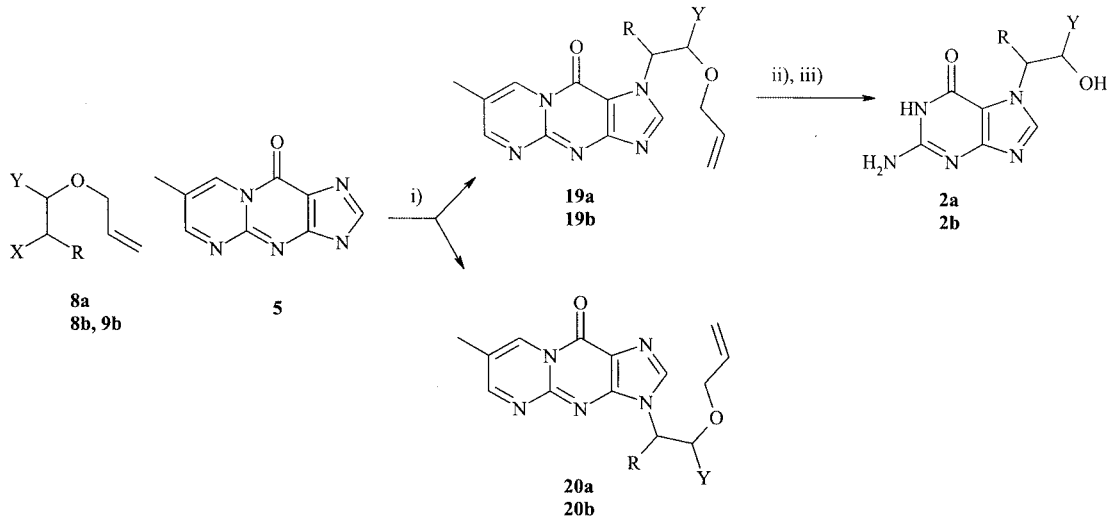
stead of sodium hydride. Acetyl derivative **3** gave 53% of 6,7-diacetoxy-5-acetyl-1-benzyl-5,6,7,9-tetrahydro-9-oxo-1*H*-imidazo[1,2-*a*]purine (**15**) and 15% of 6,7-diacetoxy-5-acetyl-3-benzyl-5,6,7-tetrahydro-9-oxo-1*H*-imidazo[1,2-*a*]purine (**16**). The former is a precursor of the desired N-7 guanine derivative while the latter is a precursor of the N-9 isomer. A very similar product ratio was obtained when diisobutyryl derivative **4** was alkylated (Scheme 3). In this case, however, the two isomers obtained could not be separated by column chromatography and their ratio was determined by integrating signals in the ^{1}H NMR spectrum of their mixture. Although imidazopurine derivatives **3** and **4** showed a much better selectivity for desired positional isomers in the alkylation than purine **6**, they could not be used to prepare guanine derivatives **2a** and **2b** due to low reactivity with synthetic equivalents of **1** and to the degradation of the starting material under these reaction conditions.

Methylmalondialdehyde Condensate of Guanine

Pyrimidopurine **5** was introduced by Kjellberg et al.[12] as a more stable, if not more reactive, guanine precursor for alkylation at N-7 and N-9 positions than both imidazopurines **3** and **4**. It can be prepared by condensation of guanine with methylmalondialdehyde as described by Moschel and Leonard.[17] To prepare compound **5** we used this procedure with the following modification: methylmalondialdehyde was liberated in situ by aqueous HCl from its tetraethyl diacetal, so that its isolation and purification is avoided.



Scheme 3. Alkylation of imidazopurines **3** and **4** by reaction with benzyl bromide: i) K_2CO_3/DMF, room temperature



	2a	2b	8a	8b	9b	19a	19b	20a	20b
X	-	-	Br	Br	OTs	-	-	-	-
Y	H	Ph	H	Ph	Ph	H	Ph	H	Ph
R	Ph	H	Ph	H	H	Ph	H	Ph	H

Scheme 4. Alkylation of pyrimidopurine: i) NaH or K_2CO_3 in DMF, 60 °C, overnight; ii) aqueous NaOH, reflux, 4−6 h; iii) PMHS, $[Pd(PPh_3)_4]$, $ZnCl_2$ in DMF[18]

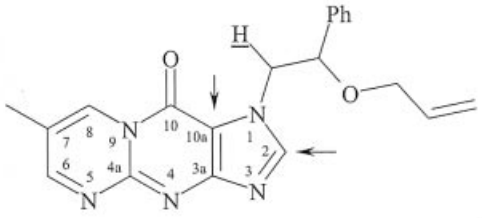
Alkylation of compound **5** with allyl-protected synthetic equivalents of **1** gave two products — the N-1 and N-3 derivatives — in a ratio of nearly 1:1. This ratio did not significantly change upon changing the base used (sodium hydride or potassium carbonate). A similar observation was reported earlier for alkylation of **5** with 4-bromobutyl acetate in the presence of different bases such as sodium hydride, potassium carbonate, triethylamine, thallium(I) ethoxide and lithium diisopropylamide.[12] The three alkylating agents used (**8a**, **8b** and **9b**) showed a similar reactivity but compound **8a** gave somewhat lower yields with benzylic bromide (Scheme 4, Table 2). The products of alkylation were separated and pure N-1 isomers **19a** or **19b** were deprotected by hydrolytic cleavage followed by palladium-catalysed deallylation[18] to yield the N-7 guanine derivatives **2a** and **2b**, respectively.

Table 2. 7-(2-Hydroxy-1-phenylethyl)- and 2-hydroxy-2-phenylethyl)guanines by alkylation of pyrimidopurine (**5**)

Reagent	Yield of compound			
	Alkylation	Hydrolysis	Deallylation	Overall yield
8b	43% **20b**			
	37% **19b**	91% **14b**	90% **2b**	30% **2b**
8a	37% **20a**			
	35% **19a**	81% **14a**	68% **2a**	19% **2a**
9b	41% **20b**			
	39% **19b**	91% **14b**	90% **2b**	32% **2b**

Identification of Products and Determination of the Site of Substitution

All products and intermediates were characterised by usual spectral methods such as ^1H and ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. UV spectra at neutral, acidic and alkaline pH were also recorded. Positional isomers such as 7- and 9-substituted guanines and their precursors show little or no differences in the mass and UV spectra. Based on consideration of NMR spectroscopic data for a series of purine derivatives, including substituted pyrimidopurines (derivatives of **5**) and imidazopurines (derivatives of **3** and **4**), Kjellberg and Johansson formulated general rules for ^1H and ^{13}C NMR chemical shifts to distinguish between N-7 and N-9 derivatives.[19] They observed that 8-H signals for the N-9 isomer are shifted upfield relative to the corresponding signals for the N-7 isomer. Similarly, the peaks for C-8 and C-1′ of the N-9 isomer are shifted upfield relative to the corresponding values for the N-7 isomer. On the contrary, the signals of C-5 are deshielded relative to those of the N-7 isomers.[13] The ring numbering relates to the purine moiety, and may differ from the systematic numbering of the compounds concerned. These empirical rules can be applied also to compounds prepared in this study. However, recent development in 2D NMR techniques and instrumentation, namely HMQC and HMBC experiments, allowed us to obtain unequivocal and much more direct evidence of the site of substitution. HMQC and HMBC spectra were recorded for compounds **12a**,**b**, **13a**,**b**, **15**, **16**, **19a**,**b** and **20a**,**b**. Examples of the HMBC spectrum of purine derivatives **19b** and **20b**



19b

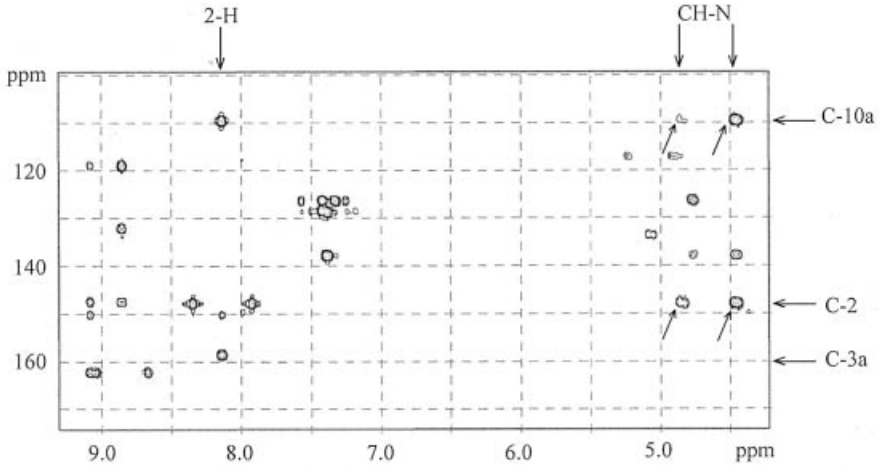


Figure 1. HMBC spectrum of **19b**; cross-peaks between NCH_2 signals and both C-2 and C-10a are highlighted, indicating that the corresponding atoms are separated by three bonds

are shown in Figures 1 and 2; cross-peaks indicating the position of the substituent — the 2-hydroxy-2-phenylethyl moiety — are highlighted. Once the site of substitution was unequivocally determined, subsequent steps converting an alkylation product to the target guanine derivatives could be accomplished without the need to measure an HMBC spectrum at each stage. It is reasonable to expect that no isomerisation (transalkylation from one nitrogen atom to another) will occur in the course of reactions such as hydrolysis and deallylation.

For the benzyl derivatives **17** and **18** of diisobutyrylimidazopurine, which were not separated from each other, 2D spectra were not measured. In this special case, the position of the benzyl group was determined from the ^{1}H NMR spectrum by analogy with corresponding diacetyl derivatives. There are characteristic differences in the ^{1}H NMR spectra between 1-benzyl and 3-benzyl derivatives. The signals of 8-H follow the rule of Kjellberg and Johansson.[19] Moreover, the benzyl CH_2 protons of the N-1 derivatives are magnetically non-equivalent and appear as an AB system, whereas the corresponding protons of the N-3 derivatives are magnetically equivalent and appear as a singlet. The singlet of the latter protons is shifted upfield by nearly 0.2 ppm. These effects can be explained by a hindered rotation in the N-1 derivatives due to the proximity of the benzyl and carbonyl group and by shielding of the carbonyl π-electrons, respectively.

Comparison of Synthetic Approaches Leading to 7-(2-Hydroxy-1- or -2-phenylethyl)guanines 2a and 2b

The most straightforward way of preparing compounds **2a** and **2b** appears to be an alkylation of guanosine or 2′-deoxyguanosine with **1**. In fact, this approach has been used by several groups to obtain small quantities of a whole range of 2-hydroxy-1-phenylethyl- and 2-hydroxy-2-phenylethyl derivatives of guanosine, 2′-deoxyguanosine and 2′-deoxyguanosine phosphates.[4,10] Although guanosine and deoxyguanosine show good selectivity of alkylation at N-7 for some reactive alkylating agents,[8,9] both the reactivity and selectivity of their reaction with **1** was rather low, so that only small amounts of samples could be obtained after HPLC separation.[10] Compounds **5** and **6** as precursors of guanine showed much greater reactivity with the synthetic equivalents of **1** used in this study. This is achieved by deprotonation of the imidazole ring of **5** and **6** which is not possible when the N-9 position of the guanine moiety is occupied by a ribosyl or 2-deoxyribosyl substituent.

A major limitation of the proposed synthetic methods lies in the regioselectivity. In the case of compound **6**, the N-9 position is the major site of electrophilic attack, the N-7 position being the minor one. For compound **5** the reactivity of positions N-1 and N-3 is nearly equal, leading eventually to the formation of 7- and 9-substituted guanine derivatives in a 1:1 ratio. The major advantage of purine **6**

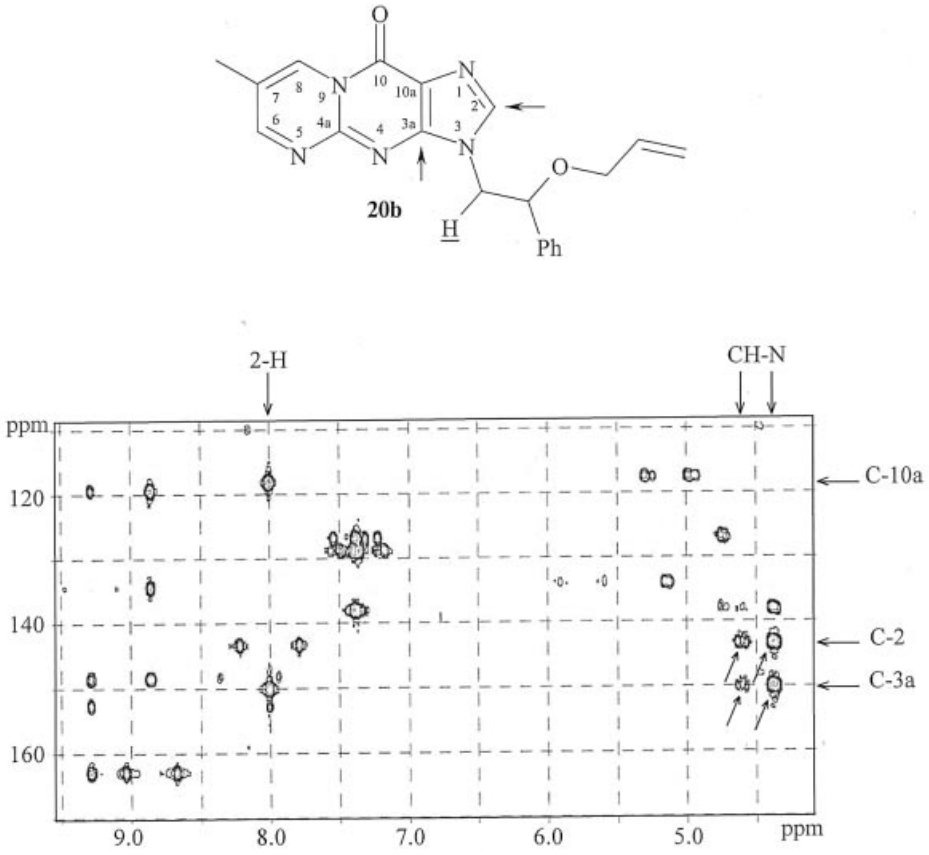


Figure 2. HMBC spectrum of **20b**; cross-peaks between NCH_2 signals and both C-2 and C-3a are highlighted, indicating that the corresponding atoms are separated by three bonds

as a precursor to guanine is the relative simplicity of the reaction pathway. Compound **6** is commercially available and its alkylation products can be easily separated by column chromatography and converted into the final products in two simple steps — hydrolysis and deallylation. On the other hand, pyrimidopurine **5**, which has a better regioselectivity for 7-substituted guanines, is not commercially available. In both cases the alkylation of guanine precursor with synthetic equivalents of **1** is the key reaction step.

Conclusion

Allyl-protected bromohydrins **8a** and **8b** as well as tosylate **9b** are suitable synthetic equivalents of styrene oxide for synthesis of 7-[hydroxy(phenyl)ethyl]guanines, namely 7-(2-hydroxy-1-phenylethyl)guanine (**2a**) and 7-(2-hydroxy-2-phenylethyl)guanine (**2b**), which are important markers of DNA damage. Two guanine precursors, pyrimidopurine **5** and chloropurine **6** can be used as starting material. In both cases the 7-[hydroxy(phenyl)ethyl]guanines required are obtained by a two-step process including alkaline hydrolysis and palladium-catalysed deallylation. Alkylation of purine **6** by **8a** is the process of choice for the preparation of **2a** whereas the alkylation of **5** by **9b** is the best choice for the preparation of **2b**.

Experimental Section

General: Column chromatography was performed on silica gel 60 obtained from Fluka, particle size 0.063−0.200 mm or 0.035−0.070 (flash chromatography). For thin-layer chromatography Merck Silica gel 60 F_{254} plates were used. Dimethylformamide was dried by azeotropic distillation with benzene, followed by distillation from phosphorus pentoxide under vacuum, 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. HMQC and HMBC experiments were used to facilitate the assignment of carbon atoms and to determine the site of substitution in the alkylated products. In HMBC, 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 ionization or on an ion-trap GC-MS system Varian Saturn 2000.

Allyl 2-Bromo-1-phenylethyl Ether (8b): This compound was prepared by reaction of 2-(allyloxy)-2-phenylethyl tosylate (**9b**)[15] with LiBr in analogy with Dickman et. al.[20] and identified by comparison of its NMR spectra with published data.[15] A mixture of tosylate **9b** (3 g, 9.42 mmol) and LiBr (4.1 g, 47.2 mmol) was refluxed in acetone for 20 h. The solvent was removed by evaporation under vacuum and the residue was diluted with 50 mL of water and washed twice with dichloromethane. The organic layer was dried

with $MgSO_4$. The crude product (2.17 g, 96%) obtained by evaporation of the solvent was purified by column chromatography on silica gel. A yellowish liquid (2.0 g, 88%) was obtained.

Allyl 2-Bromo-2-phenylethyl Ether (8a): Allyl 2-hydroxy-2-phenylethyl ether[21] (6 g, 33.7 mmol) was added dropwise to phosphorus tribromide (3.84 g, 14.2 mmol, 26% excess) under dry nitrogen whilst stirring and with external cooling by an ice/water mixture. The reaction mixture was then stirred for 1 h, left overnight at room temperature and thereafter diluted with 60 mL of diethyl ether, washed twice with cold water, dried with anhydrous $MgSO_4$ and filtered. Evaporation of ether yielded 8.1 g (99%) of the crude product. Its vacuum distillation yielded 5.2 g (64%) of colourless liquid, b.p. 88−92 °C at 0.4 mbar. ^{1}H NMR (300 MHz, $CDCl_3$), δ = 3.92 (dd, *J* = 10.8, 6.7 Hz, 1 H, OC*H*$_2$CHBr), 3.97 (dd, 1 H, *J* = 10.8 and 8 Hz, OC*H*$_2$CHBr), 4.05 (m, 2 H, OC*H*$_2$CH=CH$_2$); 5.07 (t, *J* = 7 Hz, 1 H, C*H*Br), 5.19 (dd, *J* = 10.3, 1.3 Hz, 1 H, C*H*$_2$=CH), 5.27 (dq, *J* = 17.2, 1.3 Hz, 1 H, C*H*$_2$=CH), 5.87 (m, 1 H, CH$_2$=C*H*), 7.35 (m, 3 H, Ph) and 7.43 (m, 2 H, Ph) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ$_C$ = 51.9 (*C*HBr), 72.2 and 74.3 (*C*H$_2$O), 117.5 (*C*H$_2$=CH), 127.9, 128.6 and 128.7 (*Ph*), 134.2 (CH$_2$=*C*H), 139.2 (*Ph*) ppm. EI-MS: *m*/*z* (%) = 183 and 185 (25) [M − CH$_2$=CHCH$_2$O]$^+$, 169 and 171 (50) [M − CH$_2$=CHCH$_2$OCH$_2$]$^+$, 161 (65) [M − Br]$^+$, 143 (80) [M − Br − H$_2$O]$^+$, 104 (100) [M − Br − CH$_2$=CHCH$_2$O]$^+$, 91 (85). $C_{11}H_{13}BrO$ (231.13): calcd. C 54.8, H 5.4 Br 33.1; found C 54.9, H 5.7, Br 32.7.

7-Methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine (5): Compound **5** was prepared by a modified reaction described by Moschel and Leonard[17] and identified by comparison of its NMR spectra with published data.[12] Methylmalondialdehyde tetraethyldiacetal[22,23] was used instead of free methylmalondialdehyde. Methylmalondialdehyde tetraethyldiacetal (3.5 equiv.) was added to a solution of guanine hydrochloride (4.6 g, 23 mmol) in 288 mL of 1 M HCl and the resulting solution was stirred at 45 °C for 24 h. The product was obtained as a hydrochloride. Free base was recovered by cautious neutralization of a hot acid solution of the hydrochloride. After cooling, the yellow precipitate was collected by filtration to give 2 g (43%) of **5**.

6,7-Diacetoxy-5-acetyl-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (3) and 5-Acetyl-6,7-bis(2-methylpropanoyloxy)-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (4): These compounds were prepared by a previously described procedure from guanine.[11,12] Compound **3**, m.p. 212−214 °C; compound **4**, m.p. 177−179 (ref.[11b] m.p. 169−170 °C). ^{1}H NMR spectra of both **3** and **4** are in agreement with published data.[6c]

Alkylation of 6,7-Diacetoxy-5-acetyl-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine with Benzyl Bromide: Sodium hydride (0.390 g, 1.64 mmol) or K_2CO_3 (0.227 g, 1.64 mmol) was added to a solution of imidazopurine **3** (0.550 g, 1.64 mmol) in 10 mL of dry DMF. After 15 min, benzyl bromide (1.27 g, 7.45 mmol) was added and the reaction mixture was stirred at room temperature for 3−4 h. The solvent was evaporated to dryness and the resulting oily residue purifed by column chromatography on silica gel. Two products were obtained: 6,7-Diacetoxy-5-acetyl-1-benzyl-5,6,7-tetrahydro-9-oxo-1*H*-imidazo[1,2-*a*]purine (**15**), white powder (374 mg, 54%). UV: λ$_{max}$ = 264 nm (pH = 6.5), 260 nm (pH = 1) 264 nm (pH = 12). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.10 (s, 3 H, C*H*$_3$COO), 2.14 (s, 3 H, C*H*$_3$COO), 2.80 (s, 3 H, C*H*$_3$CON), 5.46 (d, *J* = 15 Hz, C*H*$_2$Ph), 5.67 (d, *J* = 15 Hz, C*H*$_2$Ph), 6.82 (s, 1 H, C*H*O), 6.86 (s, 1 H, C*H*O), 7.34 (m, 5 H, Ph), 7.82 (s, 1 H, 2-H) ppm. ^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 20.5 (*C*H$_3$COO), 25.2 (*C*H$_3$CON), 50.7 (*C*H$_2$Ph), 77.3 and 80.9 (*C*HOAc), 111.4 (*C*-9a), 128.1, 128.7 and 129.1 (phenyl-*C*H), 135.0 (phenyl-*C*), 143.4 (*C*-2), 147.6 (*C*-3a), 151.5 (*C*-4a), 158.0 (*C*-9), 167.6, 168.2 and 168.7 (*C*O) ppm; HMBC shows cross-peaks between C*H*$_2$Ph (δ$_H$ = 5.46 and 5.67 ppm) and both *C*-9a (δ$_C$ = 111.4 ppm) and *C*-2 (δ$_C$ = 143.4 ppm). $C_{20}H_{19}N_5O_6\cdot 1/2H_2O$ (434.42): calcd. C 55.3, H 4.6, N 16.1; found C 55.0, H,4.7, N 16.1. 6,7-Diacetoxy-5-acetyl-3-benzyl-5,6,7-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (**16**), white powder (107 mg, 15%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.12 (s, 6 H, C*H*$_3$COO), 2.70 (s, 3 H, C*H*$_3$CON), 5.27 (s, 2 H, C*H*$_2$Ph), 6.85 (s, 1 H, CHO), 6.86 (s, 1 H, CHO), 7.28 (m, 2 H, Ph), 7.39 (m, 3 H, Ph), 7.74 (s, 1 H, 2-H) ppm. ^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 20.4 (*C*H$_3$COO), 25.0 (*C*H$_3$CON), 47.9 (*C*H$_2$Ph), 78.0 and 81.0 (*C*HOAc), 120.5 (*C*-9a), 127.6, 128.7 and 129.1 (*Ph*), 134.9 (*Ph*), 139.2 (*C*-2), 148.4 and 148.6 (*C*-3a and *C*-4a), 153.5 (-*C*9), 167.8, 167.9 and 168.0 (*C*O) ppm; HMBC shows cross-peaks between C*H*$_2$Ph (δ$_H$ = 5.27 ppm) and both *C*-3a (δ$_C$ = 148.4 ppm) and *C*-2 (δ$_C$ = 139.3 ppm). UV: λ$_{max}$ = 262 nm (pH = 6.5), 260 nm (pH 1) 260 nm (pH = 12). $C_{20}H_{19}N_5O_6\cdot 1/2H_2O$ (434.42): calcd. C 55.3, H 4.6, N 16.1; found C 55.5, H 4.8, N 16.4.

Alkylation of 5-Acetyl-6,7-bis(2-methylpropanoyloxy)-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (5) with Benzyl Bromide: Potassium carbonate (0.71 g, 0.51 mmol) was added to a solution of **5** (0.200 g, 0.51 mmol) in 3 mL of dry DMF followed, after 15 min, by benzyl bromide (288 mg, 1.68 mmol) and the reaction mixture was stirred for 3−4 h. The solvent was evaporated under vacuum and the oily residue was purified by column chromatography on silica gel. The product obtained (150 mg, 61%) was a mixture of two compounds, 5-acetyl-1-benzyl-6,7-bis(2-methylpropanoyloxy)-5,6,7,9-tetrahydro-9-oxo-1*H*-imidazo[1,2-*a*]purine (regioisomer a) and 5-acetyl-3-benzyl-6,7-bis(2-methylpropanoyloxy)-5,6,7,9-tetrahydro-9-oxo-3*H*-imidazo[1,2-*a*]purine (regioisomer b), which were not separated (a/b = 3.5:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.10^{a+b} (m, 12 H, C*H*$_3$CHCO), 2.48^{a+b} (sept, *J* = 6.9 Hz, 2 H, CH$_3$C*H*CO), 2.50^a (s, 3 H, C*H*$_3$CON) 2.52^b (s, 3 H, C*H*$_3$CON), 5.41^a (d, *J* = 14.9 Hz, 1 H, C*H*$_2$Ph), 5.45^a (d, *J* = 14.9 Hz, 1 H, C*H*$_2$Ph), 5.18^b (s, 2 H, C*H*$_2$Ph), 6.70^a (s, 1 H, CHO), 6.75^a (s, 1 H, CHO), 6.71^b (s, 1 H, CHO), 6.72^b (s, 1 H, CHO), 7.22^{a+b} (m, 5 H, Ph), 7.77^a (s, 1 H, 2-H), 7.65^b (s, 1 H, 2-H) ppm.

Alkylation of 7-Methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine (5): Potassium carbonate (206 mg, 1.49 mmol) was added to a solution of pyrimidopurine **5** (300 mg, 1.49 mmol) in 15 mL of dry DMF and the mixture was stirred for 15−30 min. An excess of alkylating reagent (3.94 mmol) was then added and the reaction mixture was stirred under dry nitrogen at 60 °C overnight. The solvent was evaporated under vacuum and the oily residue was separated by column chromatography on silica gel (eluent Me$_2$CO/EtOAc, 1:1).

3-[2-(Allyloxy)-2-phenylethyl]-7-methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine (20b): Yellow powder obtained by alkylation of **5** (above procedure) with bromide **8b** (yield 230 mg, 43%) or with tosylate **9** (yield 219 mg, 41%), m.p. 185−187 °C, R_F (Me$_2$CO/EtOAc, 1:1) = 0.56. UV: λ$_{max}$ = 258, 320 and 356 nm (pH = 6.5), 250, 314 and 344 nm (pH = 1), 258, 320 and inflex at 356 nm (pH = 11). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.47 (s, 3 H, 7-C*H*$_3$), 3.74 (dd, *J* = 6, 12.7 Hz, 1 H, OC*H*$_2$CHCH$_2$), 3.98 (dd, *J* = 4.9, 12.7 Hz, 1 H, OC*H*$_2$CHCH$_2$), 4.37 (dd, *J* = 8.7, 14.3 Hz, 1 H, NCH$_2$), 4.59 (dd, *J* = 3.6, 14.3 Hz, 1 H, NCH$_2$), 4.72 (dd, *J* = 3.6, 8.7 Hz, 1 H, NCH$_2$C*H*Ph), 5.08 (d, *J* = 10.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.13 (d, *J* = 17.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.74 (m, 1 H, OCH$_2$C*H*CH$_2$), 7.34 (m, 5 H, Ph), 8.00 (s, 1 H, 2-H), 8.85 (d, *J* = 2.4 Hz, 1 H, 6-H), 9.27 (s, 1 H, 8-H) ppm. ^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 15.3 (*C*H$_3$) 49.5 (N*C*H$_2$), 69.8

(O*C*H$_2$CHCH$_2$), 79.2 (NCH$_2$*C*HPh), 117.4 (OCH$_2$CH*C*H$_2$), 118.0 (*C*-10a), 119.2 (*C*-7), 126.5, 126.7, 128.6, 128.8 and 137.9 (*Ph*), 133.9 (OCH$_2$*C*HCH$_2$), 134.3 (*C*-8), 143.2 (*C*-2), 148.3 (*C*-3a), 149.9 (-*C*4a), 152.5 (*C*-10), 162.8 (*C*-6) ppm; HMBC shows cross-peaks between NC*H*$_2$ (δ_H = 4.37 and 4.59 ppm) and both *C*-3a (δ_C = 148.3 ppm) and *C*-2 (δ_C = 143.2 ppm). ESI-MS: *m*/*z* = 400 [M + K]$^+$, 384 [M + Na]$^+$, 362, 340, 242, 130 [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 65.6, H 5.3, N 18.9.

1-[2-(Allyloxy)-2-phenylethyl]-7-methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine (19b): Yellow needles obtained by alkylation of **5** with bromide **8b** (yield 198 mg, 37%), or tosylate **9** (yield 219 mg, 41%), m.p. 213−215 °C, R_F (Silica gel Merck; Me$_2$CO/EtOAc, 1:1) = 0.24. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 2.47 (s, 3 H, 7-C*H*$_3$); 3.68 (dd, *J* = 6, 12.8 Hz, 1 H, OC*H*$_2$CHCH$_2$), 3.97 (dd, *J* = 4.9, 12.7 Hz, 1 H, OC*H*$_2$CHCH$_2$), 4.46 (dd, *J* = 8.9, 14.0 Hz, 1 H, NCH$_2$), 4.78 (dd, *J* = 3.0, 8.9 Hz, 1 H, NCH$_2$), 4.84 (dd, *J* = 3.0, 14.0 Hz, 1 H, C*H*Ph), 5.03 (d, *J* = 10.2 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.07 (d, *J* = 17.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.66 (m, 1 H, OCH$_2$C*H*CH$_2$), 7.38 (m, 5 H, Ph), 8.14 (s, 1 H, 2-H), 8.86 (d, *J* = 2.5 Hz, 1 H, 6-H), 9.08 (s, 1 H, 8-H) ppm. ^{13}C NMR (125.8 MHz, CDCl$_3$): δ = 15.4 (*C*H$_3$), 53.3 (N*C*H$_2$), 70.0 (O*C*H$_2$CHCH$_2$), 80.0 (*C*HPh), 110.3 (*C*-10a), 117.3 (OCH$_2$CH*C*H$_2$), 119.1 (*C*-7), 126.5, 128.5, 128.8 and 138.0 (*Ph*), 132.3 (OCH$_2$*C*HCH$_2$), 133.8 (*C*-8), 147.6 (*C*-3a), 148.0 (*C*-2), 150.3 (*C*-4a), 158.7 (*C*-10), 162.3 (*C*-6) ppm; HMBC showed cross-peaks between NC*H*$_2$ (δ_H = 4.46 and 4.78 ppm) and both *C*-10a (δ_C = 110.3 ppm) and *C*-2 (δ_C = 148.0 ppm). ESI-MS: *m*/*z* = 400 [M + K]$^+$, 384 [M + Na]$^+$, 362 [M + H]$^+$. UV: λ_{max} = 260 and inflex at 322 nm (pH = 6.5), 250, 314 and 342 nm (pH = 1), 260 and 320 nm (pH = 11). $C_{20}H_{19}N_5O_2\cdot 1/4H_2O$ (365.91): calcd. C 65.6, H 5.37, N 19.14; found C 65.8; H 5.4, N 19.1. Reaction of **5** with tosylate **9b** under the same conditions yielded 41% of **20b** and 39% of **19b**.

3-[2-(Allyloxy)-1-phenylethyl]-7-methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine (20a): A yellow foam (186 mg, 35%), obtained by alkylation of **5** by bromide **8a**, R_F (Me$_2$CO/EtOAc, 1:1) = 0.60, UV: λ_{max} = 258 and 356 nm (pH = 6.5), 250, 312 and 344 nm (pH = 1), 258 and 320 nm (pH = 12). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 2.38 (s, 3 H, 7-C*H*$_3$), 3.96 (dd, *J* = 0.9, 5.6 Hz, 2 H, OC*H*$_2$CHCH$_2$); 4.00 (dd, *J* = 4.4, 10.6 Hz, 1 H, OC*H*$_2$CHN), 4.25 (dd, *J* = 6.4, 10.6 Hz, 1 H, NCHC*H*$_2$), 5.09 (dd, *J* = 0.9, 10.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.12 (dd, *J* = 0.9, 17.9 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.72 (m, 1 H, OCH$_2$C*H*CH$_2$), 6.08 (dd, *J* = 4.4, 6.4 Hz, 1 H, NC*H*Ph), 7.23 (m, 5 H, Ph), 8.04 (s, 1 H, 2-H), 8.78 (d, *J* = 2.6 Hz, 1 H, 6-H), 9.10 (s, 1 H, 8-H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 15.5 (*C*H$_3$), 57.8 (Ph*C*HN), 70.8 (O*C*H$_2$CHN) and 72.5 (O*C*H$_2$), 118.1 (OCH$_2$CH*C*H$_2$), 118.4 (*C*-10a), 119.5 (*C*-7), 127.5, 128.7, 129.1, and 136.9 (*Ph*), 134.0 (*C*-8), 136.9 (OCH$_2$*C*HCH$_2$), 141.7 (*C*-2), 148.5 (*C*-4a), 150.1 (*C*-3a), 152.7 (*C*-10), 163.0 (*C*-6) ppm; HMBC shows cross-peaks between between NC*H*Ph (δ_H = 6.08 ppm) and both *C*-3a (δ_C = 150.1 ppm) and *C*-2 (δ_C = 141.7 ppm). ESI-MS: *m*/*z* = 384 [M + Na]$^+$, 362 [M + H]$^+$. $C_{20}H_{19}N_5O_2\cdot 3/4H_2O$ (374.92): calcd. C 64.1, H 5.5, N 18.7; found C 64.0, H 5.2, N 18.5.

1-[2-(Allyloxy)-1-phenylethyl]-7-methyl-10-oxo-9,10-dihydropyrimido[1,2-*a*]purine (19a): A yellow foam (0.180 g, 34%) obtained by alkylation of **5** by bromide **8a**, R_F (Me$_2$CO/EtOAc, 1:1) = 0.25. UV: λ_{max} = 258, 356 and 320 nm (pH = 6.5), 250, 314 and 342 nm (pH = 1), 260 and 322 nm (pH = 12). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 2.40 (d, *J* = 0.9 Hz, 3 H, 7-C*H*$_3$), 4.02 (dm, *J* = 4.2 Hz, 2 H, OC*H*$_2$CHCH$_2$), 4.12 (dd, *J* = 4.1, 10.9 Hz, 1 H, OC*H*$_2$CHN), 4.26 (dd, *J* = 7.3, 10.9 Hz, 1 H, OC*H*$_2$CHN), 5.18 (dd, *J* = 1.3, 9.1 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.22 (dd, *J* = 1.3, 17.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.82 (m, 1 H, OCH$_2$C*H*CH$_2$), 6.42 (dd, *J* = 4.1, 7.3 Hz, 1 H, NC*H*), 7.53 (m, 5 H, *Ph*), 8.42 (s, 1 H, 2-H), 9.80 (d, *J* = 1.3 Hz, 1 H, 6-H), 9.00 (m, 1 H, 8-H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 15.3 (*C*H$_3$) 60.9 (N*C*HPh), 71.1 and 72.6 (O*C*H$_2$), 110.3 (*C*-10a), 118.3 (OCH$_2$CH*C*H$_2$), 119.3 (*C*-7), 127.4, 128.8, 129.1 and 137.2 (*Ph*), 132.7 (OCH$_2$*C*HCH$_2$), 133.9 (*C*-8), 146.2 (*C*-2), 147.9 (*C*-3a), 150.4 (*C*-4a), 158.8 (*C*-10), 162.6 (*C*-6) ppm; HMBC shows cross-peaks between between NC*H*Ph (δ_H = 6.42 ppm) and both *C*-10a (δ_C = 110.3 ppm) and *C*-2 (δ_C = 146.2 ppm). ESI-MS: *m*/*z* = 384 [M + Na]$^+$, 362 [M + H]$^+$. $C_{20}H_{19}N_5O_2\cdot 3/4H_2O$ (374.92): calcd. C 64.1, H 5.5, N 18.7; found C 64.0, H 5.2, N 18.5.

Hydrolytic Cleavage of the Alkylated Pyrimidopurines: Pyrimidopurine derivative **19a** or **19b** (0.170 g, 0.47 mmol) was refluxed in 47 mL of 0.1 M NaOH for 4−6 h. After neutralization with 2 M hydrochloric acid, the white precipitate formed was collected by filtration.

7-[2-(Allyloxy)-2-phenylethyl]-2-aminopurin-6-one (14b): A white powder (0.134 g, 91%), obtained by hydrolytic cleavage of **19b**, m.p. >250 °C, R_F (CHCl$_3$/MeOH, 20:1) = 0.20. UV: λ_{max} = 286 nm (pH = 6.5), 252 nm (pH = 1), 282 nm (pH = 12). ^{1}H NMR (500 MHz, [D$_6$]DMSO + CF$_3$COOH): δ = 3.71 (dd, *J* = 5.1, 13.4 Hz, 1 H, OC*H*$_2$CHCH$_2$), 3.86 (dd, *J* = 4.0, 13.4 Hz, 1 H, OC*H*$_2$CHCH$_2$), 4.50 (d, *J* = 6.2 Hz, 2 H, NC*H*$_2$CHPh), 4.80 (t, *J* = 6.2 Hz, 1 H, NCH$_2$C*H*Ph), 5.03 (d, *J* = 10.4 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.07 (d, *J* = 17.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.66 (m, 1 H, OCH$_2$C*H*CH$_2$), 7.41 (m, 5 H, Ph), 8.77 (s, 1 H, 8-H) ppm. ^{13}C NMR (125.8 MHz, [D$_6$]DMSO + CF$_3$COOH): δ = 53.0 (N*C*H$_2$CHPh), 69.2 (O*C*H$_2$CHCH$_2$), 78.6 (NCH$_2$*C*HPh), 114.1 (*C*-5), 116.5 (OCH$_2$CH*C*H$_2$), 126.6, 128.6, 128.8 and 137.9 (*Ph*), 134.5 (OCH$_2$*C*HCH$_2$), 140.2 (*C*-8), 149.7 (*C*-2), 153.4 (*C*-4), 154.3 (*C*-6) ppm. ESI-MS: *m*/*z* = 350 [M + K]$^+$, 334 [M + Na]$^+$, 312 [M + H]$^+$, 242 [M + H − CH$_2$CHCH$_2$OCH$_2$]$^+$. $C_{16}H_{17}N_5O_2$ (311.35): calcd. C 61.7, H 5.5, N 22.5; found C 61.7, H 5.5, N 22.4.

7-[2-(Allyloxy)-1-phenylethyl]-2-aminopurin-6-one (14a): A white powder (0.158 g, 81%) obtained by hydrolytic cleavage of **19a**, m.p. >250 °C, R_F (CHCl$_3$/MeOH, 20:1) = 0.18. UV: λ_{max} = 286 nm (pH = 6.5), 250 nm (pH = 1), 282 nm (pH = 12). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 4.00 (m, 3 H, NCHPhC*H*$_2$, OC*H*$_2$CHCH$_2$), 4.37 (t, *J* = 9.7 Hz, 1 H, NCHPhC*H*$_2$), 5.10 (dd, *J* = 1.5, 10.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.16 (dd, *J* = 1.7, 17.3 Hz, 1 H, OCH$_2$CHC*H*$_2$), 5.82 (m, 1 H, OCH$_2$C*H*CH$_2$), 6.10 (dd, *J* = 4.7, 9.7 Hz, 1 H, NC*H*PhCH$_2$), 6.20 (s, 2 H, NH$_2$), 7.30 (m, 5 H, Ph), 8.28 (s, 1 H, 8-H), 10.76 (s, 1 H, 6-OH) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 59.1 (N*C*HPhCH$_2$), 69.9 (O*C*H$_2$CHCH$_2$), 70.7 (NCHPh*C*H$_2$), 107.7 (*C*-5), 116.6 (OCH$_2$CH*C*H$_2$), 126.3, 127.5, 128.1 and 137.7 (*Ph*), 134.3 (OCH$_2$*C*HCH$_2$), 141.6 (*C*-8), 152.3 (*C*-2), 154.2 (*C*-4), 159.2 (*C*-6) ppm. ESI-MS: *m*/*z* = 334 [M + Na]$^+$, 312 [M + H]$^+$, 152 [M + H − CH$_2$CHCH$_2$OCHCHPh]$^+$. $C_{16}H_{17}N_5O_2\cdot 1/2H_2O$ (320.36): calcd. C 60.0, H 5.7, N 21.9; found C 59.8, H 5.4, N 21.4.

Deallylation: The reaction conditions were analogous to those described by Chandrasekhar et al.[18] Poly(methylhydrosiloxane) (PMHS) (162 mg), tetrakis(triphenylphosphane)palladium (14 mg) and ZnCl$_2$ (34 mg) were added to a stirred solution of the allyl-protected purine derivative (1.35 mmol) in 9 mL of DMF under argon. The reaction mixture was stirred at 60 °C for 2−3 h. After evaporation of the solvent under vacuum, the crude product was purified by column chromatography on silica gel (chloroform/

methanol, 6:1) and crystallized from *i*PrOH/H_2O (4:1) with the addition of charcoal as a decolourising agent.

2-Amino-7-(2-hydroxy-2-phenylethyl)purin-6-one (2b): Obtained as a greyish powder (80%) by deallylation of **14b**. Crystallisation from *i*PrOH/H_2O (8:2) with the addition of charcoal as a decolourising agent yielded a white powder (36%), m.p. > 250 °C, R_F (Silica gel Merck; $CHCl_3$/MeOH, 6:1) = 0.21. UV: λ_{max} = 286 nm (pH = 6.5), 252 nm (pH = 1), 282 nm (pH = 12). ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 4.15 (dd, *J* = 7, 13.5 Hz, 1 H, NCH_2), 4.37 (dd, *J* = 3.5, 13.5 Hz, 1 H, NCH_2), 4.93 (m, 1 H, C*H*OH), 5.68 (d, *J* = 5.0 Hz, 1 H, OH), 6.14 (s, 2 H, NH_2), 7.34 (m, 5 H, Ph), 7.73 (s, 1 H, 8-H), 10.78 (s, 1 H, 6-OH) ppm. ^{13}C NMR (125.8 Hz, [D_6]DMSO): δ = 53.7 (NCH_2), 71.6 (*C*HO), 108.0 (*C*-5), 125.8, 127.3, 128.2 and 142.5 (*Ph*), 143.7 (*C*-8), 152.7 (*C*-2), 154.8 (*C*-4), 160.0 (*C*-6) ppm. ESI-MS: *m*/*z* = 310 $[M + K]^+$, 294 $[M + Na]^+$, 272 $[M + H]^+$, 152 $[M + H - CH_2CHOHPh]^+$, 135 $[M + H - CH_2CHOHPh - OH]^+$. $C_{13}H_{13}N_5O_2 \cdot 1/5\ H_2O$ (274.89): calcd. C 56.8, H 4.90, N 25.5; found C 57.1, H 5.0, N 25.2.

2-Amino-7-(2-hydroxy-1-phenylethyl)purin-6-one (2a): Obtained as a grey powder (68%) by deallylation of **14a**. Crystallisation from *i*PrOH/H_2O (8:2) with the addition of charcoal as a decolourising agent yielded a white powder (40%), m.p. >250 °C, R_F ($CHCl_3$/MeOH, 6:1) = 0.19. UV: λ_{max} = 286 nm (pH = 6.5), 250 nm (pH = 1), 282 nm (pH = 12). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 3.97 (m, 1 H, CH_2OH), 5.30 (t, *J* = 5.5 Hz, 1 H, CH_2OH), 5.90 (dd, *J* = 3.85, 4.95 Hz, 1 H, PhCH), 6.26 (s, 2 H, NH_2), 7.27 (m, 5 H, Ph), 8.23 (s, 1 H, 8-H), 10.82 (s, 1 H, 6-OH) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 62.2 (Ph*C*H), 62.4 (*C*H_2OH), 108.0 (*C*-5), 126.7, 127.6, 128.4 and 138.6 (*Ph*), 141.5 (*C*-8), 152.6 (*C*-2), 154.3 (*C*-4), 159.5 (*C*-6) ppm. ESI-MS: *m*/*z* = 272 $[M + H]^+$, 152 $[M + H - CH_2OHCHPh]^+$. $C_{13}H_{13}N_5O_2 \cdot 1/5\ H_2O$ (274.89): calcd. C 56.9, H 4.90, N 25.5; found C 56.8, H 4.9, N 25.9.

Acknowledgments

The authors are grateful to Dr. A. Holý for valuable advice given at the initial stage of this study. The financial support by grants No. 310/03/0437 from the Grant Agency of the Czech Republic and No. 1553/2003 from the Ministry of Education of the Czech Republic is gratefully acknowledged.

[1] *Monographs on the Evaluation of Carcinogenic Risks to Humans*, International Agency for Research on Cancer, Lyon, France, **1994**, vol. 60.
[2] K. Hemminki, M. Koskinen, H. Rajaniemi, C. Zhao, *Regul. Toxicol. Pharmacol.* **2000**, *32*, 246−275.
[3] K. C. Leibman, E. Ortiz, *J. Pharmacol. Exp. Therap.* **1970**, *173*, 242−246.
[4] P. Vodicka, K. Hemminki, *Carcinogenesis* **1988**, *9*, 1657−1660.
[5] D. E. G. Shuker, P. B. Farmer, *Chem. Res. Toxicol.* **1992**, *5*, 450−460.
[6] [6a] J. Jindřich, H. Dvořáková, A. Holý, *Coll. Czech. Chem. Commun.* **1992**, *57*, 1466−1482. [6b] A. Holý, *Coll. Czech. Chem. Commun.* **1993**, *58*, 649−674. [6c] M. Spassova, H. Dvořáková, A. Holý, M. Budešínský, M. Masojídková, *Coll. Czech. Chem. Commun.* **1994**, *59*, 1153−1174. [6d] O. L. Aceveda, R. S. Andrews, *Tetrahedron Lett.* **1996**, *37*, 3931−3934.
[7] H. Dvořáková, A. Holý, I. Rosenberg, *Coll. Czech. Chem. Commun.* **1994**, *59*, 2069−2094.
[8] [8a] J. W. Jones, P. K. Robins, *J. Am. Chem. Soc.* **1963**, *85*, 193−201. [8b] K.-J. Moon, R. C. Moschel, *Chem. Res. Toxicol.* **1998**, *11*, 696−702.
[9] J. L. Sessler, D. Magda, H. Furuta, *J. Org. Chem.* **1992**, *57*, 818−826.
[10] [10a] K. Hemminki, A. Hesso, *Carcinogenesis* **1984**, *5*, 601. [10b] K. Savela, A. Hesso, K. Hemminki, *Chem.-Biol. Interact.* **1986**, *60*, 235−246. [10c] P. Vodicka, R. Štetina, R. Kumar, K. Plná, K. Hemminki, *Carcinogenesis* **1996**, *17*, 801−808. [10d] M. Koskinen, E. K. H. Schweda, K. Hemminki, *J. Chem. Soc., Perkin Trans. 2* **1999**, 2441−2445. [10e] M. Koskinen, P. Vodicka, K. Hemminki, *Chem.-Biol. Interact.* **2000**, *124*, 13−27.
[11] [11a] J. Kjellberg, N. G. Johansson, *J. Heterocycl. Chem.* **1986**, *23*, 625−627. [11b] J. Kobe, J. Kjellberg, N. G. Johansson, *Acta Chem. Scand., Ser. B* **1987**, *41*, 546−568.
[12] J. Kjellberg, C. E. Hagberg, A. Malm, J. O. Noren, N. G. Johansson, *Acta Chem. Scand., Ser. B* **1986**, *40*, 310−312.
[13] J. Novák, I. Linhart, H. Dvořáková, V. Kubelka, *Org. Lett.* **2003**, *5*, 637−639.
[14] J. Shorter, *Org. React. Mech.* **1993**, 283−311.
[15] O. Masami, T. Masaru, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1498−1508.
[16] [16a] A. Holý, I. Rosenberg, H. Dvořáková, *Coll. Czech. Chem. Commun.* **1990**, *55*, 809−818. [16b] M. E. Jung, N. Rhee, *Tetrahedron Lett.* **1993**, *34*, 4449−4452.
[17] R. C. Moschel, N. J. Leonard, *J. Org. Chem.* **1976**, *41*, 294−300.
[18] S. Chandrasekhar, C. Raji Reddy, R. Jagadeeshwar Rao, *Tetrahedron* **2001**, *57*, 3435−3438.
[19] J. Kjellberg, G. Johansson, *Tetrahedron* **1986**, *42*, 6541−6544.
[20] M. Dickman, J. B. Jones, *Bioorg. Med. Chem.* **2000**, *8*, 1957−1968.
[21] L. M. Stephenson, D. L. Mattern, *J. Org. Chem.* **1976**, *41*, 3614−3619.
[22] R. M. Coates, B. D. Rogers, S. J. Hobbs, D. R. Peck, D. P. Curran, *J. Am. Chem. Soc.* **1987**, *109*, 1160−1170.
[23] P. Zeller, F. Bader, H. Lindlar, M. Montavon, P. Müller, R. Rüegg, G. Ryser, G. Saucy, S. F. Schaeren, U. Schwieter, K. Stricker, R. Tamm, P. Zürcher, O. Isler, *Helv. Chim. Acta* **1959**, *92*, 841−847.

Received December 30, 2003

