Purine Nucleoside Analogues; 9:[1] Benzylation of N^2-Acetyl-8-bromoguanine[2]

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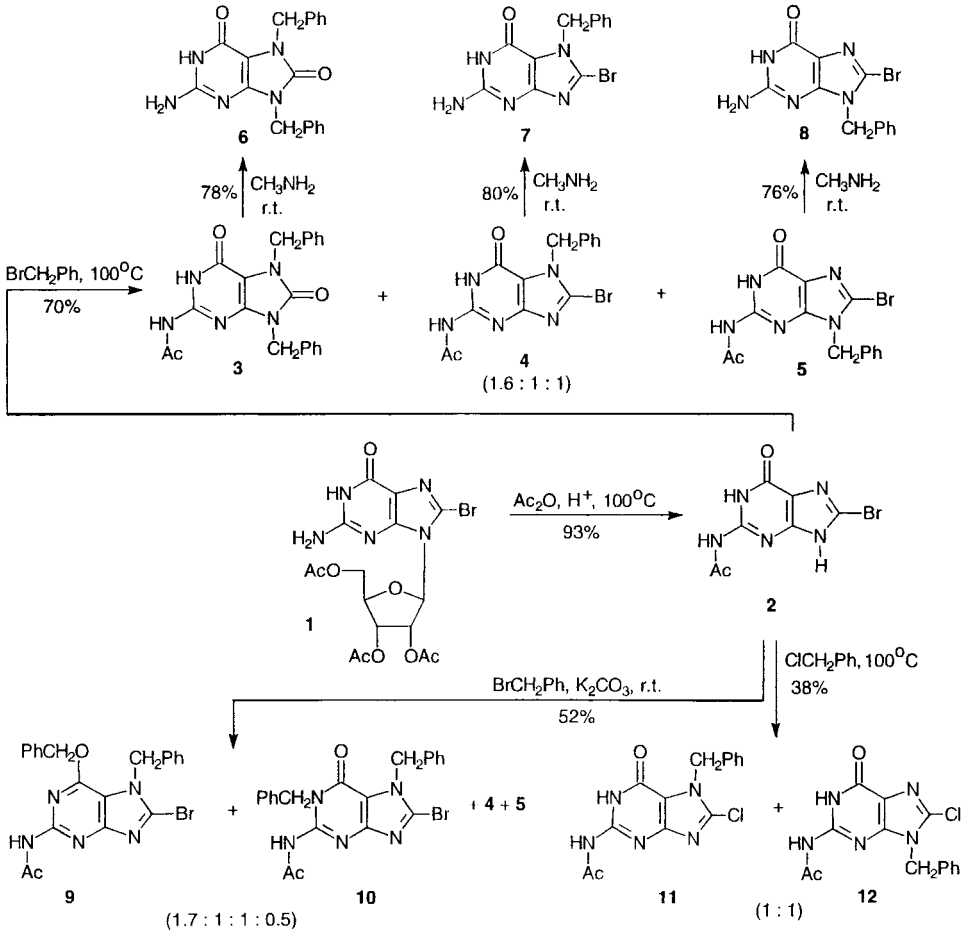
Abstract: A one-step synthesis of regioisomeric *N*- and *O*-benzylated 8-bromoguanines **4, 5, 9, 10** via N^2-acetyl-8-bromoguanine (**2**) is described. The possibility to prepare 8-oxo- and 8-chloroguanine derivatives **3**, **11, 12** from the same compound **2** is demonstrated. ^{1}H and ^{13}C NMR spectra are used to locate the sites of aralkylation.

Key words: N^2-acetyl-8-bromoguanine, benzylation, regioisomers

The biological activity of 8-substituted derivatives of guanosine and modified guanines has been well established. These compounds have been reported to stimulate the humoral immune system[3,4] and to terminate leukemia cell proliferation through conversion to end-stage differentiated cells.[5] Antiviral compounds,[6,7] potent purine nucleoside phosphorylase (PNP, EC 2.4.2.1) inhibitors,[8] and effective AGT protein (EC 2.1.1.63) inactivators[9] have been found among them. A systematic biological evaluation of 8,9- and particularly of 7,8-disubstituted guanines has been limited by the difficulties of their preparation. A commonly used route to 8,9-disubstituted guanines involves the introduction of the corresponding functional group into the 8 position of 9-substituted derivatives that are usually obtained in multi-step procedures starting from such substrates as 2-amino-6-chloropurine or appropriately derived pyrimidines.[6, 9–12]



Scheme

The objective of the present work was to develop an alternative synthetic procedure for the preparation of 8,9- and 7,8-disubstituted guanines, based on the *N*-alkylation of 8-bromoguanine. It is generally established that the direct alkylation of guanine or N^2- acetylguanine provides a mixture of 7- and 9-alkylated products in poor overall yields. The presence of the electron withdrawing bromine atom in the ring increases purine reactivity towards electrophiles as it was observed in the case of 8-bromoadenine[13,14] and 8-bromoisoguanine.[15]

We have investigated in detail the reaction of N^2-acetyl-8-bromoguanine (**2**) with benzyl halides under various reaction conditions. Substrate **2** was obtained by treatment of 9-(2', 3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-8-bromoguanine (**1**)[16] with acetic anhydride in the presence of phosphoric acid (Scheme). The N^2-acetyl group in derivative **2** was expected to increase the solubility of the reaction products, thus facilitating their purification and analysis. When **2** was reacted with benzyl bromide (2 equiv) in dimethylformamide at 100 °C, three products, namely, N^2-acetyl-7,9-dibenzyl-7,8-dihydro-8-oxoguanine (**3**), N^2-acetyl-7-benzyl-8-bromoguanine (**4**) and N^2-acetyl-9-benzyl-8-bromoguanine (**5**) were isolated in 32, 18 and 20% yields, respectively (Scheme). The structures of derivatives **4** and **5** were apparent from their elemental analyses as well as ^{1}H and ^{13}C NMR spectra (Table). The location of the benzylation sites in compounds **4** and **5** was based on the chemical shifts of the methylene protons (NCH_2) in ^{1}H NMR spectra[17] and C-5 carbon peaks in ^{13}C NMR spectra [18]. For product **3** the structure of 8-oxo-7,9-dibenzyl derivative was assigned (8-oxo-1,9-dibenzyl was proposed erroneously in Ref. 2). ^{13}C NMR spectra indicated the attachment of both the benzylic carbons to nitrogen[19] and the shifts of N^1H and N^2H protons

Table Benzylated 8-Haloguanines **3**-**12** Prepared

Product[a]	Yield (%)	mp (°C)[b]	Rf[c]	^{1}H NMR (DMSO-d_6/TMS), δ	^{13}C NMR (DMSO-d_6/TMS)		
					NCH_2	C-5	$NCOCH_3$
3	32	216–218	0.79	2.14 (s,3 H, CH_3), 4.93 (s, 2 H, NCH_2), 5.09 (s, 2 H, NCH_2), 7.28 (m,10 H, H_{arom}), 11.67 (s, 1 H, NH) 12.09 (s, 1 H, NH)	42.95 44.85	103.00	173.33
4	18	275	0.70	2.18 (s, 3 H, CH_3), 5.56 (s, 2 H, NCH_2), 7.28 (m, 5 H, H_{arom}), 11.64 (s, 1 H, NH), 12.18 (s, 1 H, NH)	49.53	113.69	173.44
5	20	285–287	0.53	2.18 (s, 3 H, CH_3), 5.27 (s, 2 H, NCH_2), 7.28 (m, 5 H, H_{arom}), 11.71 (s, 1 H, NH), 12.08 (s, 1 H, NH)	46.63	120.16	173.17
6	78	136–138 (dec.)	0.86	4.81 (s, 2H, NCH_2), 4.99 (s, 2 H, NCH_2), 6.49 (s, 2 H, NH_2), 7.26 (m, 10 H, H_{arom}), 10.64 (s, 1 H, NH)	–	–	–
7	80	> 270 (dec.)	0.80	5.41 (s, 2 H, NCH_2), 6.21 (s, 2 H, NH_2), 7.22 (m, 5 H, H_{arom}), 10.80 (s, 1 H, NH)	–	–	–
8	76	> 290 (dec.)[d]	0.78	5.16 (s, 2 H, NCH_2), 6.58 (s, 2 H, NH_2), 7.29 (m, 5 H, H_{arom}), 10.67 (s, 1 H, NH)	–	–	–
9	20	193–196	0.92	2.23 (s, 3 H, CH_3), 5.51 (s, 2 H, OCH_2), 5.57 (s, 2 H, NCH_2), 7.31 (m,10 H, H_{arom}), 10.36 (s, 1 H, NH)	50.36 67.99[e]	111.09	168.91
10	12	201–204	0.76	2.11 (s, 3 H, CH_3), 5.38 (s, 2 H, NCH_2), 5.53 (s, 2 H, NCH_2), 7.34 (m, 10 H, H_{arom}), 13.45 (s, 1 H, NH)	46.37 49.70	110.83	184.16
11	21	277–279	0.69	2.23 (s, 3 H, CH_3), 5.59 (s, 2 H, NCH_2), 7.36 (m, 5 H, H_{arom}), 11.39 (s, 1 H, NH)	–	–	–
12	17	284–287	0.49	2.24 (s, 3H, CH_3), 5.28 (s, 2H, NCH_2), 7.37 (m, 5 H, H_{arom}), 11.32 (s, 1 H, NH)	–	–	–

[a] All new compounds gave satisfactory microanalyses: C ± 0.4, H ± 0.2, N ± 0.4.
[b] Crystallization solvent was EtOH for compounds **3**, **9**, **10**, EtOH/H_2O (5:1) for compounds **4**, **5**, **11**, **12** and EtOH/H_2O (2:1) for compounds **6**-**8**.
[c] $CHCl_3$/EtOH (10:1) for compounds **3**-**5**, **9**-**12** and *i*-PrOH/NH_4OH/H_2O (7:1:2) for compounds **6**-**8**.
[d] Lit. [10] mp 334–335°C, Lit.[12] mp < 300 °C.
[e] OCH_2.

corresponded to the same signals in **4** and **5** thus excluding N^1-benzylation. An extra carbonyl group (ν = 1677 cm^{-1}) was present in the IR spectrum. *N*-Deacetylation of **3–5** with aqueous methylamine afforded 7,9-dibenzyl-7,8-dihydro, 8-oxo-, 7-benzyl-8-bromo- and 9-benzyl-8-bromoguanines (**6–8**).

The reaction of **2** with benzyl bromide in the presence of anhydrous potassium carbonate followed a different course. In this case the aralkylation proceeded at room temperature, affording four benzylated products, i.e. two bis- and two mono-alkylated ones. The rise in the reaction temperature up to 100 °C did not substantially influence either the overall yield or regioselectivity of benzylation. The mono-benzylated products were identical to compounds **4** and **5**. Bis-substituted derivatives **9**, **10** were identified as N^2-acetyl-O^6,7-dibenzyl-8-bromoguanine and N^2-acetyl-1,7-dibenzyl-8-bromoguanine, respectively, due to the chemical shifts of NCH_2-group protons and C-5 carbon in their ^1H and ^{13}C NMR spectra (Table). We found that the benzyl group at the N^1-position of the heterocycle in compound **10** influenced the adjacent exocyclic acetamido group that was reflected both in ^1H and ^{13}C spectra, i.e. considerable downfield shifts of NH-proton singlet (δ = 13.45) and the carbon peak (δ = 184.16) in comparison to N^1-unsubstituted derivatives.

Treatment of substrate **2** with benzyl chloride in dimethylformamide (100 °C) containing potassium carbonate gave N^2-acetyl-7-benzyl-8-bromo- and N^2-acetyl-9-benzyl-8-bromoguanines (**4, 5**) in poor yield. If this reaction was carried out without the base, N^2-acetyl-7-benzyl-8-chloro- and N^2-acetyl-9-benzyl-8-chloroguanines (**11, 12**) were isolated. Such halogen exchange to produce 8-chloro derivatives from 8-bromo ones has been observed by us in all the cases when the alkylating agents with chlorine as a leaving group were used for the alkylation of 8-bromoguanine in the absence of base.[20]

Our results show that the introduction of a bromine atom in position 8 of guanine ring substantially increases the yield of alkylation as compared with guanine. At room or elevated temperature in the presence of base the preferable site of aralkylation is N^7, although the reaction may also proceed at the ring nitrogens N^1 or N^9 and at the exocyclic oxygen atom. No N^3-substituted products mentioned in References 13–15 were traced in our experiments. If the reaction was carried out at 100 °C without added potassium carbonate the formation of an unanticipated product, i.e. N^2-acetyl-7,9-dibenzyl-7,8-dihydro-8-oxoguanine, was observed. A similar compound, namely, 6-chloro-7,8-dihydro-7,9-dimethyl-8-oxopurine, was isolated in the methylation of 6,8-dichloropurine with methyl iodide.[21] Two reaction pathways have been proposed to explain this phenomenon.[21] On the one hand, the initial alkylation at N^7 or N^9 may increase the susceptibility of the halogen atom at the 8 position of the heterocycle towards nucleophiles and facilitate its hydrolysis to 8-oxopurine. As a result the remaining imidazole nitrogen atom, now protonated, also becomes available for alkylation. This scheme seems feasible enough in our case, particularly taking into account the fact that hydrogen bromide, liberated in the benzylation reaction, could catalyze the hydrolysis of bromine atom. On the other hand, the hydrolysis of the C-8 halogen might be initiated by the formation of 7,9-dialkylated quaternary intermediate. Although charged structures of this type have been demonstrated with related purines[20] we have failed so far to synthesize such 7,9-disubstituted 8-bromo derivatives by alkylation of 8-bromoguanosine or 9-alkoxyalkyl-8-bromoguanines under neutral reaction conditions.

To sum up, this investigation allowed us to develop a preparative one-step route to N^9-benzyl-8-bromoguanines as well as to a series of new *N*- and *O*-benzylated derivatives of 8-bromo, 8-chloro and 8-oxoguanine. The inhibitory effect of these compounds on MOLT-4 leukemia cells is under investigation and the results will be published elsewhere.

All reagents were of commercial grade. Reagent grade solvents were used without further purification. The solvent mixtures are in volumes. Mps were determined on a Boetius hot-stage microscope and are uncorrected. ^1H NMR spectra were recorded on a Bruker WH-90/DS or on a Bruker AC 400 spectrometer. The latter instrument was also used for ^{13}C NMR spectra. IR spectra were recorded on a Perkin-Elmer IFS28 spectrometer in KBr. Column chromatography was performed on silica gel L 100/400 (Czech Republic). Reactions were monitored by TLC on Merck DC-Alufolien Kieselgel 60 F254 plates, which were visualized under UV radiation.

8-Bromo-N^2-acetylguanine (2)

A mixture of **1** (12.60 g; 25.8 mmol), Ac_2O (130 mL) and H_3PO_4 (0.5 mL) was stirred at 100 °C for 1 h and cooled to r.t. The precipitate was separated by filtration and washed thoroughly with $CHCl_3$ to yield 6.53 g (93%) of product **2**, which was used further without any additional purification. An analytical sample was obtained by recrystallization from EtOH/H_2O 3:1, mp > 300 °C (dec).

^1H NMR (90 MHz, DMSO-d_6) δ = 2.13 (s, 3 H, CH_3), 11.56 (s, 1 H, NH), 12.00 (s, 1 H, NH), 13.76 (s, 1 H, NH).

Benzylation of 2 in the Absence of K_2CO_3

(a) with Benzyl Bromide: A solution of **2** (1.69 g, 6.2 mmol) and benzyl bromide (2.12 g, 1.47 mL, 12.4 mmol) in DMF (50 mL) was stirred at 100 °C for 20 h. The mixture was cooled to r. t., adjusted to pH 8 with concd aq ammonia and evaporated to dryness in vacuo. The residue was suspended in $CHCl_3$ (200 mL), the insoluble material was filtered off, the filtrate was concentrated and loaded onto a column with silica gel (20 × 350 mm). Elution was performed with $CHCl_3$ (for compounds **3** and **4**) followed by $CHCl_3$/H_2O (40:1) (for compound **5**) . Chromatographically identical fractions were pooled and evaporated to dryness. Recrystallization from EtOH afforded 0.76 g of compound **3**. Products **4** and **5** were recrystallized from EtOH/H_2O (5:1) to give 0.40 g of **4** and 0.45 g of **5.**

(b) with Benzyl Chloride: A solution of **2** (1.00 g, 3.7 mmol) and benzyl chloride (0.93 g, 0.85 mL, 7.4 mmol) in DMF (20 mL) was stirred at 100 °C for 24 h. Further workup of the reaction mixture was carried out as described above affording 0.24 g of compound **11** and 0.19 g of compound **12**.

Benzylation of 2 with Benzyl Bromide in the Presence of K_2CO_3

A mixture of compound **2** (2.01 g, 7.4 mmol), benzyl bromide (2.51 g, 1.74 mL, 14.7 mmol) and K_2CO_3 (2.03 g, 14.7 mmol) in DMF (50 mL) was stirred at r.t. for 24 h. The mixture was filtered, the filtrate

evaporated to dryness in vacuo and the residue was suspended in $CHCl_3$ (200 mL). The insoluble material was filtered off, the filtrate was concentrated and loaded onto a column with silica gel (20 × 350 mm). Elution was performed with $CHCl_3$ (for compounds **9** and **10**) followed by $CHCl_3$/EtOH (40:1) (for compounds **4, 5**). Recrystallization from EtOH afforded 0.67 g of **9** and 0.40 g of **10**. Products **4** and **5** were recrystallized from EtOH/H_2O (5: 1), giving 0.32 g of **4** and 0.16 g of **5.**

N-Deacetylation of Guanine Derivatives 3–5; General Procedure

A solution of compound **3, 4** or **5** (0.60 mmol) in 25% aq methylamine (20 mL) was stirred at r.t. for 3 h and evaporated to dryness in vacuo. The residue was recrystallized from EtOH/H_2O (2:1), affording derivatives **6–8**.

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