4.37 (AB x, J = 5, 14 Hz, C-5, 2 H), 5.51 (m, C-2, 1 H), 7.20–7.33 (m, Ph, 5 H); ^{13}C NMR 16.48 (CH_3), 17.20 (CH_3), 33.42 ((CH_3)$_2$CH), 37.13 (CH_2Ph), 74.80 (C-5), 110.66 (C-2), 126.87 (Ph), 128.64 (Ph), 135.26 (Ph), 170.88 (C-4); MS (EI), m/e (relative intensity) 203 (M, 8), 112 (M - PhCH$_2$, 48), 97 (20), 82 (4), 76 (2), 57 (100).

Formation of Triazoline Intermediates. Azido allyl ether **2b** (40 mg, 0.26 mmol) in 0.5 mL of $CDCl_3$ in an NMR tube was heated at 50 °C, and the reaction was monitored by ^{1}H NMR. After 1.0 h the heating was stopped, and the mixture was analyzed by ^{1}H and ^{13}C NMR; 80% of the starting material had cyclized to the triazolines *trans*- and *cis*-**8b**, while only traces of 2,5-dihydrooxazole **6b** had formed.

***trans*-8b (major isomer)**: ^{1}H NMR 1.05 and 1.06 (2 d, J = 6.7 Hz, 2 CH_3), 1.85 (d, sept, J = 7.2, 6.7 Hz, C-7, 1 H), 3.11 (d, d, J = 7.4, 7.4 Hz, C-6, 1 H), 3.76 (m, C-4, 1 H), 3.90 (d,d, J = 7.2, 7.4 Hz, C-6, 1 H), 4.17 (d, d, J = 8.8, 9.0 Hz, C-5, 1 H), 4.25 (d, d, J = 6.7, 8.8 Hz, C-5, 1 H), 5.33 (d, J = 7.2 Hz, C-2, 1 H); ^{13}C NMR 17.70 (CH_3), 17.87 (CH_3) 32.47 (C-7), 55.10 (C-4), 68.69 (C-6), 71.29 (C-5), 98.61 (C-2).

***cis*-8b (minor isomer)**: ^{1}H NMR 1.11 and 1.34 (2 d, J = 6.5 Hz, 2 CH_3), 2.49 (d, sept, J = 2.2, 6.5 Hz, C-7, 1 H), 3.41 (d, d, J = 4.0, 7.8 Hz, C-6, 1 H), 3.75 (m, C-6, 1 H), 3.77 (m, C-4, 1 H, 4.46 (d, J = 2.2 Hz, C-2, 1 H), 4.47 (d, d, J = 2.5, 5.5 Hz, C-5, 1 H), 4.52 (d, d, J = 2.3, 5.5 Hz, C-5, 1 H); ^{13}C NMR 18.91 (CH_3), 19.75 (CH_3), 29.67 (C-7), 54.75 (C-4), 69.80 (C-6), 74.48 (C-5), 99.97 (C-2).

Triazoline Decomposition on Silica Gel. The triazoline mixture in $CDCl_3$ from the above experiment was transfered to a small flask, 10 mg of 230–400 mesh silica gel (E. Merck) was added and the mixture stirred at room temperature for 2 h. Evolution of nitrogen was observed. The contents were filtered into an NMR tube, washing with 0.2-mL of $CDCl_3$. The NMR showed the quantitative decomposition of triazolines to aziridines *trans*- and *cis*-**7b**.

***trans*-7b (major isomer)**: ^{1}H NMR 0.93 and 1.03 (2 d, J = 7.2 Hz, CH_3), 1.37 (d, J = 3.5 Hz, C-6, 1 H), 1.67 (d, sept. J = 7.5, 7.2 Hz, C-7, 1 H), 1.76 (d, d, J = 3.5, 1.2 Hz, C-6, 1 H), 2.60 (m, C-4, 1 H), 3.76 (d, d, d, J = 1.2, 3.2, 8.2 Hz, C-5, 1 H), 3.98 (d, J = 8.2 Hz, C-5, 1 H), 4.12 (d, J = 7.5 Hz, C-2, 1 H); ^{13}C NMR 18.11 (CH_3), 18.49 (CH_3), 28.31 (C-6), 32.74 (C-4), 38.36 (C-7), 65.00 (C-5), 102.11 (C-2).

***cis*-7b (minor isomer)**: ^{1}H NMR 1.01 and 1.13 (2 d, J = 7.2 Hz, 2 CH_3), 1.48 (d, d, J = 3.5, 1.5 Hz, C-6, 1 H), 1.52 (d, J = 3.5 Hz, C-6, 1 H), 1.65 (d, sept, J = 8.0, 7.2 Hz, C-7, 1 H), 2.54 (m, C-4, 1 H), 3.70 (d, d, d, J = 1.5, 3.0, 8.6 Hz, C-5, 1 H), 3.99 (d, J = 8.0 Hz, C-2, 1 H), 4.02 (d, J = 8.6 Hz, 1 H); ^{13}C NMR 17.80 (CH_3), 19.39 (CH_3), 30.50 (C-6), 33.64 (C-4), 36.78 (C-7), 66.31 (C-5), 100.30 (C-2).

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Registry No. **2a**, 111209-39-3; **2b**, 111209-40-6; **2c**, 111209-41-7; **2d**, 111209-42-8; **2e**, 111209-43-9; **2f**, 111209-44-0; **2g**, 111209-45-1; **6a**, 111209-46-2; **6b**, 111209-47-3; **6c**, 111209-48-4; **6d**, 111209-49-5; **6e** (isomer 1), 111209-50-8; **6e** (isomer 2), 111209-51-9; **6f**, 111209-52-0; **6g**, 111209-53-1; *trans*-**7b**, 111209-56-4; *cis*-**7b**, 111209-57-5; *trans*-**8b**, 111209-54-2; *cis*-**8b**, 111209-55-3; allyl alcohol, 107-18-6; propanal, 123-38-6; isobutryaldehyde, 78-84-2; phenylacetaldehyde, 122-78-1; dihydrocinnamaldehyde, 104-53-0; 2-cyclohexenol, 822-67-3; diphenylacetaldehyde, 947-91-1; (*E*)-cinnamyl alcohol, 4407-36-7.

Formation of Six Cyclic 1,N^2-Hydroxybromopropanodeoxyguanosine Isomers upon Reaction of 2-Bromoacrolein with 2′-Deoxyguanosine

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As a preliminary study in the investigation of reactions of the genotoxin 2-bromoacrolein (2-BA) with DNA, we treated the aldehyde with 2′-deoxyguanosine (2′-dG). Six isomeric cyclic 1,N^2-propano-2′-deoxyguanosine adducts were isolated and characterized by UV, LSIMS, and ^{1}H NMR spectral techniques. The adducts **1a–1d** were identified as diastereomeric 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-hydroxy-7-bromopyrimido[1,2-*a*]purin-10(3*H*)-ones. Adducts **2a** and **2b** were regioisomeric 7-bromo-8-hydroxy diastereomers. At physiological pH (7.4) and temperature (37 °C), adducts **1a–1d** are hydrolyzed to 6,7-dihydroxypropano-2′-deoxyguanosines. These can be transformed to stable 6,7-dihydroxypropanoguanines by removal of the deoxyribose moiety. The resulting bases can be used as standards for further investigations of reactions of 2-BA with DNA.

2-Bromoacrolein (2-BA) is a genotoxic metabolite of the flame retardant tris(2,3-dibromopropyl) phosphate (Tris-BP) that is formed in incubations of Tris-BP with mammalian microsomes.[1,2] As a preliminary study in the investigation of reactions of the genotoxin 2-BA with DNA, we treated the aldehyde with 2′-deoxyguanosine (2′-dG).

Reaction of carcinogens can take place at many sites in DNA. Unstable metabolites of carcinogenic arylamines and arylamides predominately react with the C-8 atom of deoxyguanosine, but reaction with the O^6 and N^2 atoms of deoxyguanosine and the N^6 atom of deoxyadenosine also takes place.[3] The N-7 of deoxyguanosine is a major target atom of reactive metabolites formed from a variety of other carcinogens, e.g., aflatoxin B$_1$, 1,2-dibromoethane, and *N*-nitroso compounds.[4–6] However, minor reactions can take place at other atoms as well, and the mutagenic and

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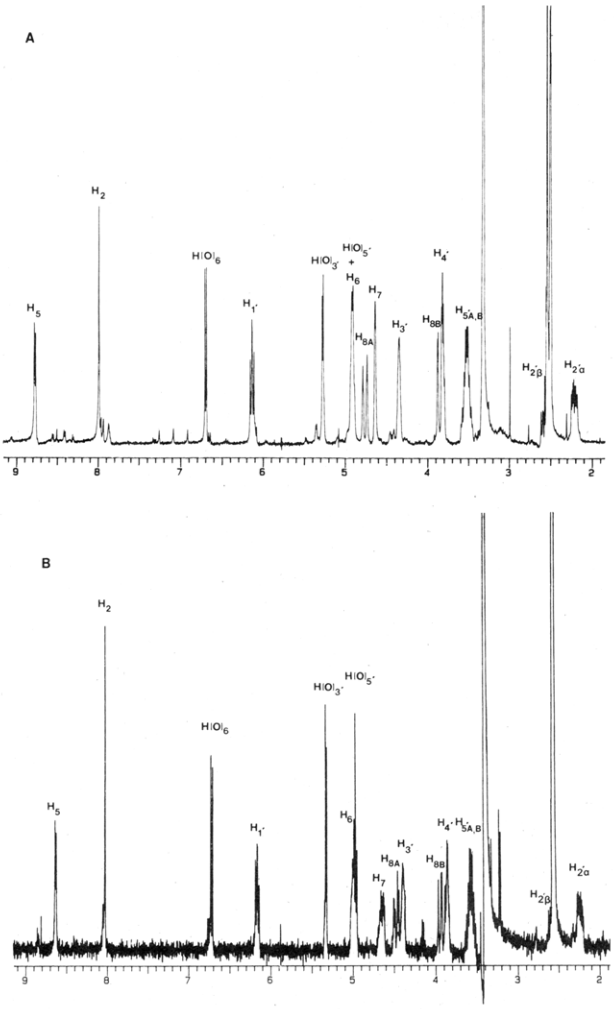


Figure 1. The 300-MHz ^{1}H NMR spectra of **1a** (A) and **1c** (B) in Me_2-SO-d_6. Chemical shifts are relative to Me_4Si.

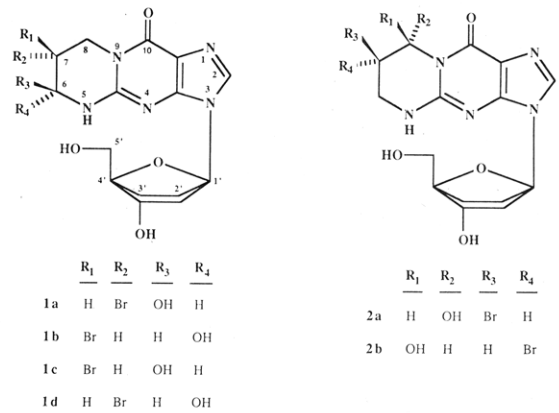
carcinogenic effects of a number of *N*-nitroso compounds might be due to the formation of O-alkylated bases rather than N-7-alkylation.[7,8] Reactive epoxides of some carcinogenic polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene, benz[*a*]anthracene, and 7,12-dimethylbenz[*a*]anthracene predominantly react with exocyclic nitrogen atoms in DNA (the N^2 of 2′-dG, the N^6 of 2′-deoxyadenosine).[4,5,9] In general, chemical carcinogenesis seems not to be related to substitution of one particular site in DNA.[4,5] Because a large number of reaction variables (addition vs substitution, S_N1 vs S_N2 type, "hardness" of the leaving group, etc.) are important in determining the site of reaction in DNA,[4,5] it is still difficult to predict the type of adduct that might be formed in the reaction of a particular carcinogen and DNA.

Cyclic 1,N^2-deoxyguanosine adducts have been isolated after the reaction of the bifunctional compounds glycidaldehyde and chloroacetaldehyde with guanosine, 2′-dG, or DNA. Thus, reaction of chloroacetaldehyde, a metabolite of the carcinogen vinyl chloride, yields 1,N^2-ethenoguanosine,[10] and reaction of the epoxide derivative glycidaldehyde yields 1,N^2-ethenoguanosine and its 7-hydroxymethyl derivative.[11,12] Similarly, reactions of some mutagenic α,β-unsaturated carbonyls[13] yield cyclic 1,N^2-guanosine derivatives. Thus, reaction of acrolein yields 6-hydroxy[1,N^2]propano- and 8-hydroxy[1,N^2]propano-2′-deoxyguanosine and reaction of crotonaldehyde yields 6-methyl-8-hydroxy[1,N^2]propanoguanosine.[14,15]

In this study, we have treated 2-BA with 2′-dG and investigated whether similar cyclic 1,N^2-adducts are formed in this reaction, and we have studied the behavior of several of these adducts under conditions employed for an acid-catalyzed hydrolysis of DNA.

Results and Discussion

Reaction of 2-BA with 2′-dG was accomplished in an aqueous phosphate buffer (pH 7.4) at 37 °C. Products were separated by Sephadex LH20 column chromatography and reversed-phase HPLC. The yield of **1a–d** was 27% and that of **2a** and **2b** was 9%. A large percentage of the remaining 2-BA appeared to have formed polymeric products. The ratio of peak heights of **1a:1b:1c:1d:2a:2b** in the HPLC profile was 19:21:5:5:4:3.



	R_1	R_2	R_3	R_4
1a	H	Br	OH	H
1b	Br	H	H	OH
1c	Br	H	OH	H
1d	H	Br	H	OH

	R_1	R_2	R_3	R_4
2a	H	OH	Br	H
2b	OH	H	H	Br

The identities of **1a–1d** were established by spectral analysis. The UV spectra of **1a–d** were similar to those of the cyclic 1,N^2-propanodeoxyguanosine adducts formed by reaction of acrolein with 2′-dG, indicating a substituted guanine moiety.[15] The LSIMS of a mixture of **1a** and **1b** showed a $[M + Na]^+$ at m/z 424/426 and a $[M + H]^+$ at m/z 402/404, which is consistent with the addition of one molecule of 2-BA to 2′-dG and is consistent with the isotope pattern for bromine. The fragment at m/z 286/288 corresponds with the loss of a deoxyribose moiety. A similar LSIMS was obtained for a mixture of **2a** and **2b**.

Information on the substitution pattern and the conformation of **1a–d** and **2a/2b** was obtained by ^{1}H NMR spectroscopy. Compound **1b** showed a ^{1}H NMR spectrum similar to that of **1a** (Figure 1a); chemical shifts differed by not more than 0.01 ppm (except for the combined signals of $H(O)_5$, and H_6). Coupling constants differed by not more than 0.1–0.2 Hz. Similarly, the ^{1}H NMR spectra of **1c** (Figure 1b) and **1d** were identical with each other (±0.02 ppm). The ^{1}H NMR spectra of **1a–d** and **2a/2b**

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Table I. Chemical Shifts and Coupling Constants of the Non-Deoxyribofuranosyl Protons of 1a–1d

	chemical shift,[a] ppm							coupling constant, Hz					
	H_2	H_5	H_6	$H(O)_6$	H_7	H_{8A}	H_{8B}	$J_{5,6}$	$J_{6,(O)6}$	$J_{6,7}$	$J_{7,8A}$	$J_{7,8B}$	$J_{8A,8B}$
1a	8.00	8.78	4.97	6.70	4.64	3.85	4.76	4.9	5.4		2.6		15.3
1b	8.00	8.79	4.94	6.71	4.64	3.87	4.76	5.0	5.5		2.5	1.7	15.4
1c	7.98	8.58	4.97	6.67	4.60	3.88	4.43	3.8	6.1	2.5	10.6	4.7	13.5
1d	7.97	8.56	4.96	6.66	4.60	3.88	4.42	3.9	5.8	2.8	10.5	4.5	13.5

[a] Chemical shifts of protons (ppm) are relative to those of tetramethylsilane.

all showed the presence of the 2′-dG protons at the same chemical shift (±0.01 ppm), except for $H_{2'\alpha}$ and $H(O)_5$, in **1c/1d** and **2a/2b**, which showed a small upfield shift of 0.03–0.07 ppm compared to the corresponding signals in **1a/1b**. Assignment of the deoxyribose protons was made by comparison of the chemical shifts reported previously[16–18] and suggests that **1a–d** and **2a** and **2b** existed in the anti form because deshielding of $H_{2'\beta}$ by N^4 would have caused a downfield shift of approximately 0.4 ppm to δ 3.0 in the syn conformation.[19] All ^1H NMR spectra showed a signal for H_2 at δ 7.95–8.00, close to the signal of the corresponding H_8 in 2′-dG at δ 7.93 and in other N^2-substituted 2′-dG's.[15,16] The absence of a signal corresponding to H_1 in 2′-dG at δ 10–10.5[17] and the absence of a signal for the exchangeable protons of the exocyclic NH_2 near δ 6.4[17,18] suggested that reaction had taken place at these atoms.

Signals of six additional protons were present in the spectra of **1a–d** (Table I) and **2a/2b**. The six non-deoxyguanosyl signals of **1a** and **1b** were assigned as follows: the exchangeable H_5 appears at δ 8.78 (d, J = 4.9 Hz), 0.33 ppm downfield from the corresponding signal in the unbrominated analogue[15] due to deshielding by the 7-bromine; H_6 appears at δ 4.94–4.97 (m), 0.03–0.06 ppm upfield from the corresponding signal in the unbrominated analogue;[15] $H(O)_6$ appears at δ 6.70 (d, J = 5.4 Hz), 0.81 ppm downfield compared to the unbrominated analogue;[15] H_7 appears at δ 4.64 (br s); the H_8 protons appear as an AB pattern at δ 3.85 and 4.76 (J = 15.3 Hz), 0.5 ppm downfield compared to the unbrominated analogue.[15] The two-dimensional J-correlated (COSY) spectrum of **1b** showed all the expected proton interactions for **1a/1b**. Assignment of the six non-deoxyguanosyl signals in **1a** and **1b** was confirmed by homonuclear decoupling experiments with **1b**. A small $J_{7,8A}$ of 2.5–2.6 Hz and a $J_{7,8B}$ of 1.7 Hz in **1a** and **1b** were observed (Table I). The Karplus equation predicts dihedral angles of 55°/125° and 65°/115° from these values; this prediction is only compatible with H_7 in an equatorial position. Hence, the bromine at C-7 must be in the axial position. A conformational change that puts H_7 in the axial position obviously does not occur since the coupling constants of H_7 with the H_8 protons would be the average value of the J values in both conformations, and this would yield a J value greater than 4.5 Hz for coupling between H_7 and H_8. Because the differences between **1a** and **1b** and **1c** and **1d** are not just changes in conformation (their interconversion requires acid catalysis; see below), the 6-hydroxyl group in **1a** must be in an axial position.

Both C-6 and C-7 are centers of chirality. Additional chiral centers are present in the deoxyribose moiety and are fixed because the 2′-dG was obtained from a natural source. Thus, the identity of **1a** and **1b** was established as a pair of 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-hydroxy-7-bromopyrimido[1,2-*a*]-purin-10(3*H*)-one diastereomers with the 6-hydroxy and 7-bromo substituents in a trans diaxial relationship in each diastereomer.

The assignment of the six non-deoxyguanosyl signals in **1c** and **1d** (Table I) was as follows: H_5 appeared at δ 8.56 (d, J = 3.8 Hz, exchangable with D_2O), H_6 at δ 4.97 (m), the exchangable $H(O)_6$ at δ 6.67 (d, J = 6.1 Hz), H_7 appeared at δ 4.60 (ddd, $J_{6,7}$ = 2.5 Hz, $J_{7,8A}$ = 4.7 Hz, $J_{7,8B}$ = 10.6 Hz), and the H_8 protons appeared as an AB pattern at δ 3.88 and 4.43 (dd, $J_{7,8A}$ = 4.6 Hz, $J_{7,8B}$ = 10.5 Hz, $J_{8A,8B}$ = 13.5 Hz). The expected proton correlations were observed in a COSY spectrum of **1c**. These assignments were confirmed by homonuclear decoupling experiments. The Karplus equation predicts dihedral angles between H_7 and H_8 of 45°/135° and 180°. This is only consistent with H_7 in a constrained axial conformation, and thus the C-7 bromine must be equatorial. The small $J_{6,7}$ value of 2.5 Hz precludes the possibility of a diaxial conformation for these protons; therefore, H_6 is equatorial. Hence, the C-6 hydroxyl group is axial. The major differences in chemical shift between corresponding protons in **1a** and **1b** and **1c** and **1d** are an upfield shift of H_5 (0.20–0.21 ppm) for the **1c/1d** pair and upfield shift for the downfield half of the AB signal of the two H_8 protons (0.33 ppm) observed in the spectra of **1c** and **1d**. The latter is consistent with the C-7 bromine being in an equatorial position in **1c** and **1d**, resulting in less shielding of one of the C-8 protons. Thus, the identity of **1c** and **1d** is established as a pair of 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-6-hydroxy-7-bromopyrimido[1,2-*a*]purin-10(3*H*)-one diastereomers with the 6-hydroxy and 7-bromo substituents in a cis axial–equatorial relationship in each diastereomer. The finding that in all isomers the 6-hydroxy group is an axial configuration suggests that there is a substantial anomeric effect between this group and the N-5 atom, as is observed for the pyran or furan oxygen and its α-hydroxy group in sugars. The compounds **1a–d** most likely are formed by reaction of the exocyclic NH_2 of deoxyguanosine with the 2-BA aldehyde function and addition of deoxyguanosine N-1 to the 1,2-unsaturated bond of 2-BA. The order of events cannot be determined from the available data.

The identity of **2a** and **2b** was established by ^1H NMR spectroscopy as a pair of 8-hydroxy-7-bromo diastereomers. The signal for H_7 appeared at δ 4.68, only 0.04 ppm downfield to the H_7 signal in **1a** and **1b**. The AB pattern of the protons at C-6 appear 0.4 and 0.8 ppm upfield from the C-8 protons in **1a** and **1b**. The $H(O)_8$ and H_8 signals appear 0.82 and 1.24 ppm downfield, respectively, when compared to the $H(O)_6$ and H_6 signals in **1a** and **1b**, probably because in **2a** and **2b** these protons are deshielded by the 9-amide nitrogen. H_8 has almost the same shift as reported for the 8-hydroxy analogue.[15] Irradiation of one of the H_6 protons at δ 3.42 caused the other H_6 and N_5 signals to collapse to a broad singlet. This is only com-

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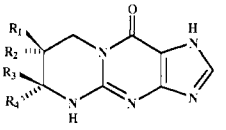
patible with the proposed 7-bromo-8-hydroxy substitution. The absence of a large coupling constant between H_6 and H_7 or H_8 suggests that the 7- and 8-substituents are diaxial. Thus, **2a** and **2b** are a pair of 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-7-bromo-8-hydroxypyrimido[1,2-*a*]purin-10-(3*H*)-one diastereomers. They most likely are formed by reaction of deoxyguanosine N-1 with the 2-BA aldehyde function (instead of the 2,3-unsaturated bond as in the reaction to yield **1a–d**) and addition of the exocyclic NH_2 of deoxyguanosine to the unsaturated bond of 2-BA. The formation of a pair of axial–equatorial 7-bromo-8-hydroxy diastereomers cannot be excluded; several minor unidentified peaks in the HPLC profile of the reaction mixture of 2-BA and 2′-dG were observed.

The compounds **1c** and **1d** were unstable; HPLC analysis showed that a sample of **1c** had been converted upon storage at 0–4 °C into a mixture of **1c** and **1b**, while a sample of **1d** contained **1d** and **1a**. Similarly, a sample that contained **1a** and **1b** contained **1a–d** after storage at 0–4 °C for 1 week. Upon heating of the sample of **1a–d** at 37 °C in aqueous 25 mM sodium phosphate buffer, pH 5.5, the conversion proceeded further, and after 4 h, an equilibrium was reached; the ratio of **1a**/**1d** was 5.6:1 and of **1b**/**1c** was 6.1:1. These data show that the axial–equatorial isomers **1c** and **1d** are less stable than the diaxial isomers **1a** and **1b**. Because the conversion of **1c** and **1d** into **1b** and **1a** respectively, was more rapid at pH <1.0 and 25 °C than at pH 5.5 and 45 °C (data not shown), the interconversion of the different isomers probably is an acid-catalyzed process.

The stabilities of **1a–d** were studied under conditions normally employed for DNA hydrolysis. Therefore, **1a** and **1b** were dissolved in 25 mM sodium phosphate buffer (pH 7.4) and incubated at 37 °C. Aliquots (5 μL) were analyzed by HPLC after 0, 35, 75, 105, and 210 min. The signals of **1a** and **1b** decreased with time and the values for $t_{1/2}$, calculated from the peak heights, of **1a** and **1b** were 62 and 53 min, respectively. At pH 8.5, **1a** and **1b** were less stable; after 5 min at 37 °C, they could no longer be detected. When **1a** and **1b** were dissolved in D_2O and $NaDCO_3$ was added (final concentration 75 mM), the ^1H NMR spectrum recorded after 5 min showed signals also observed with **3a** (except for the H_3 proton) and of a 1-substituted 2′-deoxyribofuranose. The ^1H NMR spectra of **3a** and **3b**



	R_1	R_2	R_3	R_4
3a	H	OH	OH	H
3b	OH	H	H	OH

(prepared by alkaline hydrolysis of a sample of **1a–d** and subsequent removal of the 2′-deoxyribofuranosyl moiety by acid hydrolysis at pH <1.0) showed seven nonguanine signals. The signals of the ^1H NMR spectra were assigned by comparison with the spectra of **1a–d** and 5,6,7,8-tetrahydropyrimido[1,2-*a*]purin-10(3*H*)-one.[15] The assignments were consistent with the results of homonuclear decoupling experiments. The slight upfield shifts in **3a** and **3b** of H_5, H_6, $H(O)_6$, H_7, and $H_{8A,B}$ compared to the corresponding protons in **1a**/**1b** and **1c**/**1d**, combined with the appearance of a new, exchangable signal at δ 5.34, suggest that the C-7 bromine was substituted by a hydroxyl group. The LSIMS data for **3a** and **3b** displayed $[M + H]^+$ and $[M + Na]^+$ isotopic singlets at m/z 224 and 246, respectively, confirming the loss of Br. Since the $J_{7,8A}$, $J_{7,8B}$, and $J_{6,7}$ coupling constants in **3b** were close to those observed in **1c** and **1d**, H_6, H_7, and $H_{8A,B}$ most likely have the same orientations as in **1c** and **1d**. Therefore, the 6- and 7-hydroxyl groups in **3b** would be in axial and equatorial orientations, respectively. Hence, the 6- and 7-hydroxyl groups in **3a** are diaxial. Because C-6 and C-7 are centers of chirality, **3a** and **3b** each exist as racemates.

After removal of the Me_2SO-d_6 from the samples of **3a** and **3b** by lyophilization, a small amount of the residue was dissolved in H_2O and separated by HPLC on a 0.46 × 15 cm Microsorb 3 μm C18 column, which was eluted with MeOH/aqueous 100 mM Et_3N solution, adjusted to pH 5.25 with formic acid (2:98), at a flow of 1.0 mL/min. Two compounds were present in the sample of **3b** that eluted at 2.23 min and 4.83 min. The ratio of their peak heights was 6.4:1. The sample of **3a** almost exclusively contained a compound that eluted at 2.23 min. This suggests that **3b** has been converted into the more stable diaxial **3a**, similar to the (partial) conversion of **1c** and **1d** into the diaxial diastereomers **1a** and **1b**.

In summary, 2-BA reacts with 2′-dG to produce six cyclic 1,N^2-substituted propano-2′-deoxyguanosine adducts: one pair of diaxial 6-hydroxy-7-bromo diastereomers, one pair of axial–equatorial 6-hydroxy-7-bromo diastereomers, and one pair of 7-bromo-8-hydroxy diastereomers. The unstable 6-hydroxy-7-bromo isomers could be transformed into stable 6,7-dihydroxypropanoguanine diastereomeric racemates by alkaline hydrolysis followed by removal of the 2′-deoxyribofuranosyl moiety at pH <1.0. The stable 6,7-dihydroxy derivatives may be used as standards in an assay for the formation of 2-BA/2′-dG adducts in DNA in vivo.

The diaxial 6-hydroxy-7-bromopropano-2′-deoxyguanosine diastereomers as well as the 6,7-dihydroxypropanoguanine diastereomers are more stable than their axial–equatorial analogues. The interconversion of the diaxial and axial–equatorial diastereomers probably is an acid-catalyzed loss and readdition of water, similar to the interconversion of the 6-hydroxypropano-2′-deoxyguanosine analogues.[15]

Experimental Section

The ^1H NMR data were recorded on a Varian VXR-300 spectrometer operating at 300 MHz. Chemical shifts were measured in Me_2SO-d_6, unless indicated otherwise, relative to tetramethylsilane. Coupling constants were obtained from resolution-enhanced spectra. COSY spectra were aquired with 1024 data points in F2 and 256 points in F1 and a spectral width of 2165.9 Hz.

Liquid secondary-ion mass spectrometry (LSIMS) was performed on a Kratos MS-50S mass spectrometer, equipped with a 23 kG magnet and a postacceleration detector; the latter was operated at −10 kV, and spectra were recorded on a Gould ES-1000 electrostatic recorder. Samples were dissolved in a glycerol matrix containing HCl, and ionization was achieved by bombardment with a 1.0-μA primary beam of Cs^+ ions.[20]

The ultraviolet data were taken on a Hewlett-Packard 8451A diode array spectrophotometer. HPLC separations were performed with two Waters 6000A pumps (Waters Assoc., Milford, MA) controlled by a Waters Model 660 solvent programmer. The eluent was monitored with a Waters Model 440 absorbance detector operating at 254 nm.

Preparation of 2-BA. 2-BA was prepared by the method of Wells and Strahl[21] and was obtained in 22% yield: bp 45–47 °C (5 mmHg) [lit.[10] bp 80–84 °C/(123 mmHg)]; ^1H NMR ($CDCl_3$) δ 9.28 (s, 1 H), 6.94 (AB, 1 H, J = 2.5 Hz), 6.93 (AB, 1 H, J = 2.4 Hz).

Preparation and Purification of 1a–d, 2a, and 2b. A solution of 0.56 mmol of 2′-dG (Sigma Chemical Co., St. Louis, MO) in 17 mL of 25 mM phosphate buffer was adjusted with NaOH

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to pH 7.4, flushed with argon, and agitated in a 37 °C water bath. Five 480-μL aliquots of a 0.45 M solution of 2-BA in Me_2SO were added every 30 s. The mixture was held at 37 °C for 20 min after the last addition. It was then extracted with an equal volume of Et_2O, and the aqueous phase was frozen at −70 °C (dry ice/acetone). The sample volume was reduced to 5 mL by lyophilization, and the residue was applied to a 1.5 × 30 cm Sephadex LH20 column (Pharmacia Fine Chemicals, Uppsala, Sweden). The column was eluted first with 120 mL of EtOH/H_2O (1:9), followed by elution with EtOH/H_2O (2:8). Fractions of 20 mL were collected, and 0.5–2 μL of each fraction was analyzed by HPLC on a 0.39 × 30 cm μ Bondapak-C18 column (Waters) assoc. and were eluted with MeOH/aqueous 100 mM Et_3N solution, adjusted to pH 5.5 with formic acid (2:8), at a flow of 1.5 mL/min. Compound **1a** elutes at 5.5 min, **1b** at 6.3 min, **1c** at 4.0 min, **1d** at 4.3 min, **2a** at 6.1 min, and **2b** at 6.7 min. Fraction 3 of the Sephadex LH20 column contained mainly **1c** and **1d**. Fractions 4, 5, and 6 contained **1a**, **1b**, **2a**, and **2b** and were pooled. The samples were lyophilized, and the residues were dissolved in 1–2 mL of H_2O. The residue of fraction 3 was applied to a second Sephadex LH20 column (1.8 × 100 cm) and was eluted with 30 mL of EtOH/H_2O (1:9) at a flow of 1.0 mL/min, followed by elution with a linear gradient starting with 10% (v/v) EtOH in H_2O. After 150 mL, the final concentration of 40% EtOH in H_2O was reached, and isocratic elution was continued. Fractions of 15 mL were collected, and 15–75 μL of each fraction was analyzed by HPLC. Fractions that contained only **1c** and **1d** (16, 17, and 18) were pooled and lyophilized. The residue was dissolved in 1 mL of H_2O, and 200-μL samples were separated by HPLC on a 1 × 25 cm Dynamax-60A 8 μm C18 column (Rainin Instrument Co., Woburn, MA) and were eluted with MeOH/H_2O (12:88) at a flow of 5.0 mL/min. Fractions that contained either **1c** or **1d** were collected and lyophilized.

1c: UV (H_2O) λ_{max} 258 nm; ^1H NMR δ 2.20 (m, 1 H, $H_{2'\alpha}$), 2.53 (m, 1 H, $H_{2'\beta}$, partially obscured by Me_2SO-d_5), 3.51 (m, 2 H, $H_{5'A,B}$), 3.81 (m, 1 H, $H_{4'}$), 3.88 (d AB, 1 H, J = 10.5, 13.4 Hz, H_{8A}), 4.34 (m, 1 H, $H_{3'}$), 4.43 (d AB, 1 H, J = 4.6, 13.5 Hz, H_{8B}), 4.60 (ddd, 1 H, J = 10.6, 4.7, 2.5 Hz, H_7), 4.92 (m, 1 H, $H(O)_{5'}$, partially obscured by H_6, exchanges with D_2O), 4.97 (m, 1 H, H_6, partially obscured by $H(O)_{5'}$), 5.28 (d, 1 H, J = 3.9 Hz, $H(O)_{3'}$, exchanges with D_2O), 6.13 (m, 1 H, $H_{1'}$), 6.67 (d, 1 H, J = 6.1 Hz, $H(O)_6$, exchanges with D_2O), 7.98 (s, 1 H, H_2), 8.58 (d, 1 H, J = 3.8 Hz, H_5, exchanges with D_2O).

1d: UV (H_2O) λ_{max} 258 nm; ^1H NMR δ 2.19 (m, 1 H, $H_{2'\alpha}$), 2.55 (m, 1 H, $H_{2'\beta}$, partially obscured by Me_2SO-d_5), 3.52 (m, 2 H, $H_{5'A,B}$), 3.81 (m, 1 H, $H_{4'}$), 3.88 (d AB, 1 H, J = 10.4, 13.6 Hz, H_{8A}), 4.34 (m, 1 H, $H_{3'}$), 4.42 (d AB, 1 H, J = 4.4, 13.3 Hz, H_{8B}), 4.60 (ddd, 1 H, J = 10.6, 4.6, 2.8 Hz, H_7), 4.91 (m, 1 H, $H(O)_{5'}$, exchanges with D_2O), 4.96 (m, 1 H, H_6, partially obscured by $H(O)_{5'}$), 5.27 (d, 1 H, J = 3.9 Hz, $H(O)_{3'}$, exchanges with D_2O), 6.12 (m, 1 H, $H_{1'}$), 6.66 (d, 1 H, J = 5.8 Hz, $H(O)_6$, exchanges with D_2O), 7.97 (s, 1 H, H_2), 8.56 (d, 1 H, J = 3.9 Hz, H_5, exchanges with D_2O).

Fractions 4, 5, and 6 (containing **1a**, **1b**, **2a**, and **2b** were pooled, concentrated and dissolved in 2 mL of H_2O, and 200-μL aliquots were separated by HPLC on a 1 × 25 cm Altex Ultrasphere ODS column (Rainin). The column was eluted with a MeOH/aqueous 100 mM Et_3N solution, adjusted to pH 5.25 with formic acid (16:84), at 4.5 mL/min. Fractions that contained **1a** or **1b** were collected and lyophilized. The ^1H NMR spectra of the residues showed that residual Et_3N was present; therefore, the solutions of **1a** and **1b** in Me_2SO-d_6 were lyophilized, dissolved in H_2O, and lyophilized a second time. The residues were dissolved in 1–1.5 mL of H_2O, and 200-μL fractions were separated on a 1 × 25 cm Dynamax-60A 8 m C18 column and were eluted with MeOH/H_2O (17:83) at 4.5 mL/min. Fractions that contained **1a** or **1b** were collected and lyophilized.

1a: UV (H_2O) λ_{max} 258 nm; ^1H NMR δ 2.23 (ddd, 1 H, J = 13.3, 5.8, 2.9 Hz, $H_{2'\alpha}$); 2.60 (m, 1 H, $H_{2'\beta}$), 3.52 (m, 2 H, $H_{5'A,B}$), 3.82 (m, 1 H, $H_{4'}$, partially obscured by H_{8A}), 3.85 (d AB, 1 H, J = 2.6, 15.3 Hz, H_{8A}), 4.35 (m, 1 H, $H_{3'}$), 4.64 (br s, 1 H, H_7), 4.76 (AB, 1 H, J = 15.4 Hz, H_{8B}), 4.97 (m, 2 H, $H(O)_{5'}$ and H_6, simplifies to a br s upon addition of D_2O), 5.28 (d, 1 H, J = 3.9 Hz, $H(O)_3$, exchanges with D_2O), 6.13 (m, 1 H, $H_{1'}$), 6.70 (d, 1 H, J = 5.4 Hz, $H(O)_6$, exchanges with D_2O), 8.00 (s, 1 H, H_2), 8.78 (d, 1 H, J = 4.9 Hz, H_5, exchanges with D_2O).

1b: UV (H_2O) λ_{max} 258 nm; ^1H NMR δ 2.23 (ddd, 1 H, J = 13.2, 6.0, 3.0 Hz, $H_{2'\alpha}$), 2.60 (m, 1 H, $H_{2'\beta}$, partially obscured by Me_2SO-d_5), 3.52 (m, 2 H, $H_{5'A,B}$), 3.82 (m, 1 H, $H_{4'}$, partially obscured by H_{8A}), 3.87 (d AB, 1 H, J = 2.5, 15.4 Hz, H_{8A}), 4.35 (m, 1 H, $H_{3'}$), 4.64 (br s, 1 H, H_7), 4.76 (d AB, 1 H, J = 1.7, 15.4 Hz, H_{8B}), 4.94 (m, 2 H, $H(O)_5$, and H_6, simplifies to a br s upon addition of D_2O), 5.29 (d, 1 H, J = 3.8 Hz, $H(O)_3$, exchanges with D_2O), 6.14 (m, 1 H, $H_{1'}$), 6.71 (d, 1 H, J = 5.5 Hz, $H(O)_6$, exchanges with D_2O), 8.00 (s, 1 H, H_2), 8.79 (d, 1 H, J = 5.0 Hz, H_5, exchanges with D_2O).

LSIMS of a sample of **1a** and **1b**: MS, m/z (relative intensity) 426 (7.8), 424 (6.9), 404 (38.8), 402 (39.7), 288 (97.4), 286 (100).

The reaction of 2-BA with 2′-dG was carried out a second time, with 0.27 mmol of 2′-dG and 1.04 mmol of 2-BA. After extraction with Et_2O, the aqueous layer was lyophilized, and the residue was dissolved in H_2O and applied to a 1.8 × 90 cm Sephadex LH20 column and was eluted at 2.5 mL/min with a linear gradient starting with 5% (v/v) EtOH in H_2O. After 300 mL, the final concentration of 40% (v/v) EtOH in H_2O was reached, and elution was continued with a mixture of this composition. Fractions of 15-mL volume were collected, and 25 μL of each was analyzed by HPLC on a 0.39 × 15 cm Novapak C18 column (Waters) and was eluted with MeOH/H_2O (2:8) at 1.0 mL/min. Fractions 18–22 contained **1a–d**, and they were pooled. Fractions 23 and 24 contained **2a** and **2b** and were pooled. The pooled fractions were lyophilized; the yield of **1a–d** was 30 mg (0.075 mmol, 27%) and that of **2a** and **2b** was 10 mg (0.025 mmol, 9%).

Spectral data for the mixture of **2a** and **2b**: LSIMS, m/z (relative intensity), 404 (51.3), 402 (70.0), 288 (98.8), 286 (100); calculated for the most abundant ($[M + H]^+$) isotope 402.04131, found: 402.04149; ^1H NMR δ 2.21 (m, 1 H, $H_{2'\alpha}$), 2.56 (m, 1 H, $H_{2'\beta}$, partially obscured by Me_2SO-d_5), 3.44 (d AB, 1 H, J = 5.0, 15.0 Hz, H_{6b}), 3.52 (m, 2 H, $H_{5'A,B}$), 3.81 (m, 1 H, $H_{4'}$), 3.92 (AB, 1 H, J = 15.0 Hz, H_{6a}, partially obscured by $H_{5'A,B}$), 4.35 (m, 1 H, $H_{3'}$), 4.68 (m, 1 H, H_7), 4.93 (m, 2 H, $H(O)_{5'}$, exchanges with D_2O), 5.29 (d, 1 H, J = 4.0 Hz, $H(O)_{3'}$, exchanges with D_2O), 6.12 (m, 1 H, H_1<< spm), 6.21 (m, 1 H, H_8), 7.53 (d, 1 H, J = 5.3, $H(O)_8$, exchanges with D_2O), 7.95 (s, 1 H, H_2), 8.23 (m, 1 H, H_5, exchanges with D_2O).

Preparation of 3a and 3b. The sample of **1a–b** from the second reaction of 2-BA with 2′-dG was dissolved in 1.0 mL of H_2O, and 8.4 mg of $NaHCO_3$ was added. The mixture was heated to 37 °C for 2 h. Subsequently, 0.2 mL of an aqueous 1 M HCl solution was added to adjust the pH to <1, and the mixture was heated for 45 min at 90 °C. The pH was adjusted to approximately 5 with 10 μL of an aqueous 58% (w/v) NH_4OH solution, and 200-μL samples were separated by HPLC on a 1 × 25 cm Dynamax-60A 8 μm C18 column and were eluted with MeOH/H_2O (1:99) at a flow of 5.0 mL/min. The column was washed after every three injections with MeOH/H_2O (55:45); **3a** eluted after 4.0 min, and **3b** eluted after 6.4 min. Fractions that contained either **3a** or **3b** were collected and lyophilized. The yield of **3a** was 8 mg (48%), and the yield of **3b** was 2 mg (12%).

3a: UV (H_2O) λ_{max} 252 nm; ^1H NMR δ 3.40 (d AB, J = 14.4 Hz, H_{8A}, partially obscured by HDO), 3.86 (br s, 1 H, H_7), 4.44 (d AB, 1 H, J = 14.2 Hz, H_{8B}), 4.62 (br s, 1 H, H_6), 5.35 (d, 1 H, J = 3.3 Hz, $H(O)_7$, exchanges with D_2O), 6.05 (d, 1 H, J = 5.0 Hz, $H(O)_6$, exchanges with D_2O), 7.64 (s, 1 H, H_2), 8.27 (d, 1 H, J = 5.0 Hz, H_5, exchanges with D_2O), 12.31 (s, 1 H, H_3, exchanges with D_2O); ^1H NMR (D_2O) δ 3.75 (d AB, J = 14.5 Hz, H_{8A}), 4.30 (m, 1 H, H_7), 4.65 (d AB, 1 H, J = 14.6 Hz, H_{8B}), 5.06 (m, 1 H, H_6), 7.88 (br s, 1 H, H_2), LSIMS, m/z (relative intensity), 246 (3.5), 224 (100); calculated $[M + H]^+$ 224.07837, found 224.0783.

3b: UV (H_2O) λ_{max} 252 nm; ^1H NMR δ 3.29 (AB, J = 12.5 Hz, H_{8A}, partially obscured by HDO), 3.86 (br s, 1 H, H_7), 4.13 (d AB, 1 H, J = 5.2, 12.8 Hz, H_{8B}), 4.72 (br s, 1 H, H_6, simplifies to a d with a J = 2.8 Hz upon addition of D_2O), 5.34 (d, 1 H, J = 5.9 Hz, $H(O)_7$, exchanges with D_2O), 5.93 (br s, 1 H, $H(O)_6$, exchanges with D_2O), 7.65 (s, 1 H, H_2), 8.20 (d, 1 H, H_5, exchanges with D_2O), 12.33 (s, 1 H, H_3, exchanges with D_2O); LSIMS, m/z (relative intensity), 246 (12.3), 224 (100).

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Registry No. **1a**, 111189-86-7; **1b**, 111265-75-9; **1c**, 111265-76-0; **1d**, 111265-77-1; **2a**, 111189-87-8; **2b**, 111265-78-2; **3a**, 111189-88-9; **3b**, 111408-40-3; 2'-dG, 961-07-9; 2-BA, 14925-39-4.

Pteridines. 53. A Convenient Synthetic Approach to 10-Deazaaminopterin and 10-Deazafolic Acid[1]

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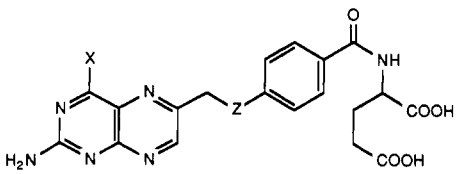
An unambiguous approach to the preparation of 10-deazaaminopterin is described which involves, as its key step, a palladium-catalyzed reaction of 2-amino-3-cyano-5-bromopyrazine with *tert*-butyl 4-ethynylbenzoate. A similar strategy utilizing 2-pivaloyl-6-chloropterin leads to a key intermediate for the preparation of 10-deazafolic acid.

Since its introduction into the clinic in 1953, methotrexate (MTX, 1) has become one of the most widely used chemotherapeutic agents, useful either alone or in combination therapy for the treatment of acute lymphocytic leukemia, choriocarcinoma, breast carcinoma, head and neck cancer, oat cell carcinoma, mycosis fungoides, and osteogenic sarcoma.[2] MTX acts by inhibition of dihydrofolate reductase, the enzyme responsible for the reduction of dihydrofolic acid to tetrahydrofolic acid. The latter is a precursor to a series of enzyme cofactors critical for a variety of one-carbon transfer reactions, which in turn are essential for the biosynthesis of purines and pyrimidines and hence DNA.[3-5] Unfortunately, the cytotoxicity resulting from a blockade of the biosynthesis of these crucial coenzymes is nonspecific, and rapidly proliferating normal cells as well as cancer cells are affected. As a consequence, MTX therapy is underscored by many serious side effects and, with higher doses, extreme toxicity. Intensive research programs over the past decade have been aimed at modifying the MTX structure in such a way as to increase its selectivity of action. Among the most promising of such MTX analogues are 10-deazaaminopterin (**2**)[6-12] and its 10-ethyl derivative (**3**).[8,9,13] The latter appears to be exceptionally promising; its substantially greater activity compared with MTX itself against a variety of murine tumors is attributed to a more favorable membrane transport and to a greater degree of polyglutamation in tumor as contrasted to normal cells. An exhaustive review of the biological and biochemical properties of these and other deaza analogues of MTX has recently appeared.[14]



1. X = NH_2, Z = NCH_3
2. X = NH_2, Z = CH_2
3. X = NH_2, Z = $CHCH_2CH_3$
4. X = OH, Z = CH_2

In light of the promising activity of **2** and its 10-substituted analogues, considerable effort has been devoted to the development of alternative syntheses. The principal strategies that have been utilized thus far for the synthesis of **2** are the following: (1) A Boon–Leigh condensation of 2,4-diamino-5-nitro-6-chloropyrimidine with an appropriate α-amino ketone (usually protected) to give a 6-substituted aminopyrimidine, which is then reductively cyclized following appropriate deprotection of the carbonyl group.[7,9,15] (2) Wittig condensation of the triphenylphosponium ylide derived from 2,4-diamino-6-(bromomethyl)pteridine with diethyl *N*-(4-formylbenzoyl)-L-glutamate followed by catalytic reduction, oxidation of the intermediate 5,6,7,8-tetrahydro derivative, and final saponification.[10,11] (3) Condensation of 2,4,5,6-tetraaminopyrimidine with an α-bromoaldehyde already substituted with the eventual C-6 substituent.[7,8,12] This latter synthesis suffers from the inherent regiochemical ambiguity of such cyclization procedures, which has been discussed extensively elsewhere.[16]

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