reverse direction. This is, to our knowledge, the first report of the remarkable cooperation of CyD catalysis with salt effect for highly selective reactions.

The present success in simultaneous improvement of both regioselectivity and reaction rate should be significant for the further development of artificial ribonuclease.

Acknowledgment. We express our sincere thanks to Professors Jinsai Hidaka and Kenichi Okamoto at the Institute of Chemistry, University of Tsukuba, for their assistance in circular dichroism spectroscopy, and to Professor Edward M. Eyring at the Department of Chemistry, University of Utah, for valuable comments on the spectroscopic determination of the K_{Cl} value of the β-CyD-Cl^- complex. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

Registry No. α-CyD, 10016-20-3; β-CyD, 7585-39-9; γ-CyD, 17465-86-0; A>p, 634-01-5; U>p, 606-02-0; G>p, 634-02-6; C>p, 633-90-9; NaCl, 7647-14-5; KCl, 7447-40-7; RbCl, 7791-11-9; CsCl, 7647-17-8; KBr, 7758-02-3; KF, 7789-23-3; LiCl, 7447-41-8; KI, 7681-11-0; $MgCl_2$, 7786-30-3; $CaCl_2$, 10043-52-4; adenosine 2′-phosphate, 130-49-4; adenosine 3′-phosphate, 84-21-9; uridine 3′-phosphate, 84-53-7.

Formation of Etheno Adducts of Adenosine and Cytidine from 1-Halooxiranes. Evidence for a Mechanism Involving Initial Reaction with the Endocyclic Nitrogen Atoms

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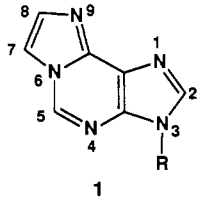
Abstract: The etheno derivatives of nucleic acid bases contain an additional ring and are of interest because of their useful fluorescence properties and their potential as mutagenic lesions in DNA. The mechanism of formation from 2-haloacetaldehydes is known to involve initial Schiff base formation at an exocyclic nitrogen; however, mechanisms of formation from the more relevant 1-substituted oxiranes have not been established. The reaction of N^6-methyladenosine (**5**) with 1-chlorooxirane yielded the stable carbinolamine 7,8-dihydro-8-hydroxy-9-methyl-3-β-D-ribofuranosylimidazo[2,1-*i*]purinium species (**10**), consistent with initial attack of the N^1 atom of adenine at the methylene of 1-chlorooxirane. No products indicative of initial reaction at the N^6 atom of adenine were found. Reaction of 2,2-dibromoethanol with adenosine or cytidine at pH 9.2 yielded 1,N^6-ethenoadenosine (**1**) or 3,N^4-ethenocytidine (**2**), respectively, presumably via the base-catalyzed formation of 1-bromooxirane from the bromohydrin. When reactions were done with 2,2-dibromo[1-^{13}C]ethanol, **1** contained label only at C-7 and **2** contained label only at C-3. A role for 2-bromoacetaldehyde in these reactions was ruled out by the lack of incorporation of deuterium from ^2H_2O into **1** under conditions where the exchange of the methylene protons of 2-bromoacetaldehyde with the solvent was relatively rapid. The collective results are most consistent with a mechanism in which the basic endocyclic nitrogen (N^1 of adenine or N^3 of cytosine) reacts with the methylene carbon of the 1-halooxirane, and, after ring opening and loss of the leaving group, the resulting aldehyde reacts with the exocyclic nitrogen to form the additional ring.

Introduction

Etheno-substituted nucleosides were discovered as products of the reactions of nucleosides with 2-haloacetaldehydes.[1] The 1,N^6-ethenoadenosine (**1**) and 3,N^4-ethenocytidine (**2**) derivatives have been studied the most extensively, and the fluorescence properties of **1** have been of utility in studying the interactions of enzymes with their cofactors.[2] 1,N^2-Ethenoguanosine (**3**), N^2,3-ethenoguanosine (**4**), and a number of other derivatives of nucleosides containing the additional etheno ring have now been prepared.[2,3] These nucleoside derivatives are also of biological interest because of their formation in nucleic acids after exposure of experimental animals to pro-carcinogens that may be enzymatically activated to reactive electrophiles capable of forming these derivatives (e.g., vinyl chloride, vinyl bromide, acrylonitrile, vinyl carbamate, and ethyl carbamate).[4] These compounds have all been demonstrated to yield **1** in vitro (in the presence of adenosine) after oxidation by cytochrome P-450 enzymes.[5] In general the N^7-(2-oxoethyl)guanosine adducts are formed in considerably greater abundance,[6] but the etheno adducts show



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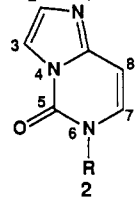

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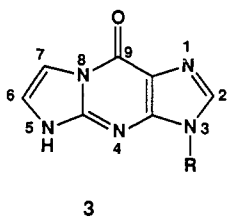

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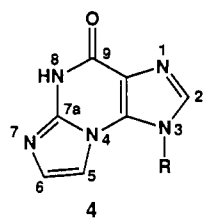

4

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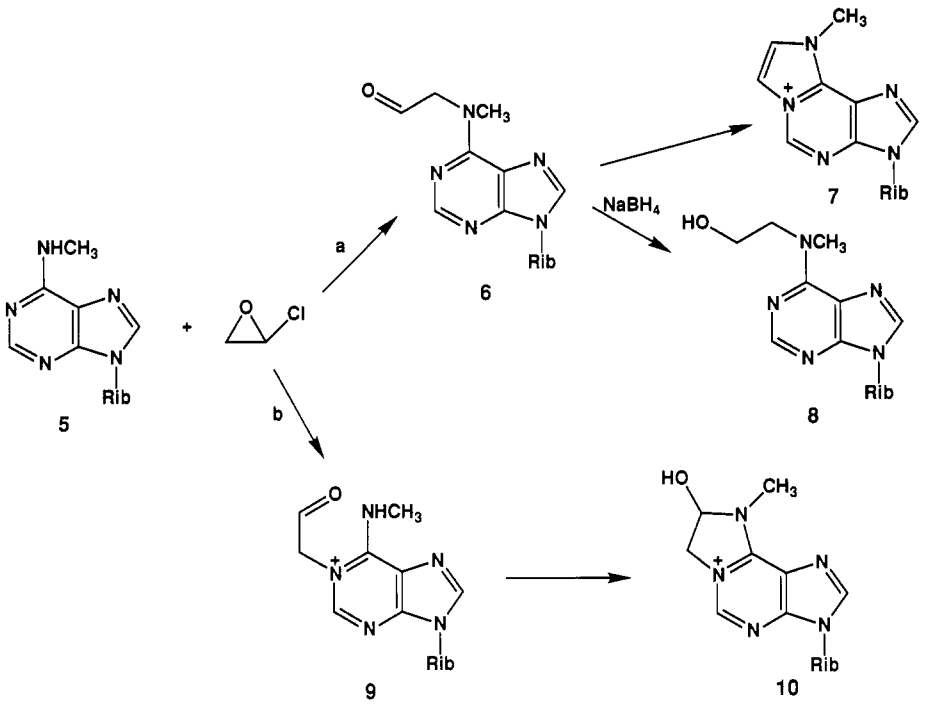
Scheme I. Possible Reactions of **5** with 1-Chlorooxirane and the Expected Products



considerably greater miscoding properties in vitro.[4b,7] Further, in vivo studies indicate that these etheno adducts are extremely persistent in DNA (i.e., not repaired).[6a] Therefore, these etheno adducts may be considered the most likely candidates for tumor-initiating lesions.

The chemistry involved in the formation of these etheno adducts is not yet completely understood. Leonard and his associates have clearly shown that the reaction of 2-haloacetaldehydes with adenosine or N^6-methyladenosine (**5**) proceeds by initial formation of a Schiff base at the N^6 nitrogen and subsequent facilitated displacement of the halogen by attack of the N^1 nitrogen.[2,8,9] However, there is evidence that the initial oxidation products of the olefins (e.g., 1-chlorooxirane, etc.) are more important in forming etheno adducts in biological systems than are the 2-haloacetaldehydes.[10] The reasons are that the reaction of adenosine with 2-haloacetaldehydes appears to be relatively slow and that the 2-haloacetaldehydes are readily conjugated with thiol moieties.[10] The mechanism by which oxiranes bearing good leaving groups undergo reaction with nucleosides has not been directly addressed.

Two approaches toward the mechanism of formation of **1** and **2** from 1-halooxiranes were used in this work. **5** can form a stable carbinolamine[2,8,9] when it reacts with 1-halooxiranes or 2-haloacetaldehydes, and the formation of this adduct was considered along with a product which could be derived from an alternative mechanism. In the second approach 2,2-dibromo[1-^{13}C]ethanol was used as precursor of 1-bromooxirane, and the labeling patterns of the derived **1** and **2** were elucidated by ^1H and ^{13}C NMR spectroscopy.

Results

Reactions of N^6-Methyladenosine (5). The strategy is outlined in Scheme I and takes advantage of the stability of the carbinolamine **10** which would be formed in route *b*.[9,11] If route *a* were favored, the product N^6-methyl,N^6-(2-oxoethyl)adenosine (**6**) might conceivably close to form the 9-methyl derivative of ethenoadenosine (**7**) or, upon treatment with $NaBH_4$, form the stable N^6-methyl,N^6-(2-hydroxyethyl)adenosine (**8**). All of the putative final products were prepared and high-performance liquid chromatography (HPLC) systems were set up for their separation.[12] When 1-chlorooxirane (105 mM) was mixed with **5** (35 mM) in 100 mM potassium phosphate buffer (pH 7.7) at 23 °C, the only product detected (A_{267}) after 5 min was **10** (~2 mM). When the reaction mixture was treated with excess $NaBH_4$, this product disappeared, and no new major peaks were detected.

1-Halooxiranes are known to rearrange to 2-haloacetaldehydes;[13] the $t_{1/2}$ for 1-chlorooxirane under these conditions (23 °C, pH 7.7) is known to be ~1 min.[14] The possibility was considered that the rearrangement product 2-chloroacetaldehyde might have been formed and reacted with **5** to give the identified product. The $t_{1/2}$ of 1-chlorooxirane, as measured using 4-(*p*-nitrobenzyl)pyridine reagent,[14] was increased to 20 min when the temperature was lowered to 5 °C. When the formation of **10** was measured at 5 °C, the product was formed two orders of magnitude more rapidly with 1-chlorooxirane than with 2-chloroacetaldehyde, in the period before the epoxide hydrolyzed (Figure 1). When aliquots of this particular reaction were analyzed by HPLC (during the first 10 min of the reaction), no other products were detected.

Prolonged treatment of synthetic **10** with $NaBH_4$ in 0.01 N NaOH formed **5** as the only major product (~20% yield) detected using gradient elution in the HPLC system. The product was identified by co-HPLC with standard **5** and by the mass, ^1H NMR, and UV spectra of the isolated material, all of which were indistinguishable from those of the authentic material (data not presented).

Modification of Adenosine with 2,2-Dibromo[1-^{13}C]ethanol. A procedure was sought for the synthesis of an oxirane with a single

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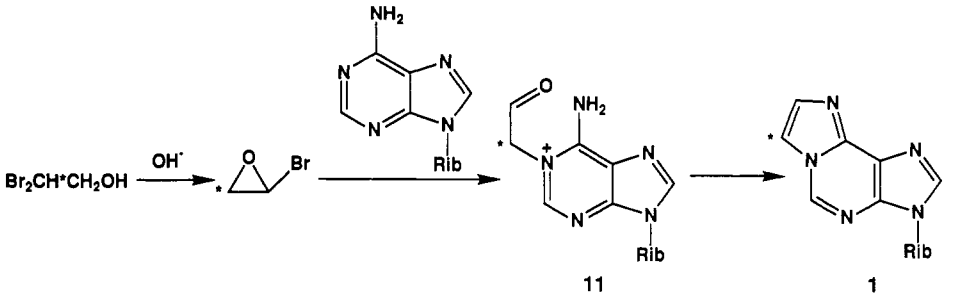
Scheme II. Postulated Mechanism of Reaction of Adenosine with 2,2-Dibromo[1-^{13}C]ethanol To Yield **1**



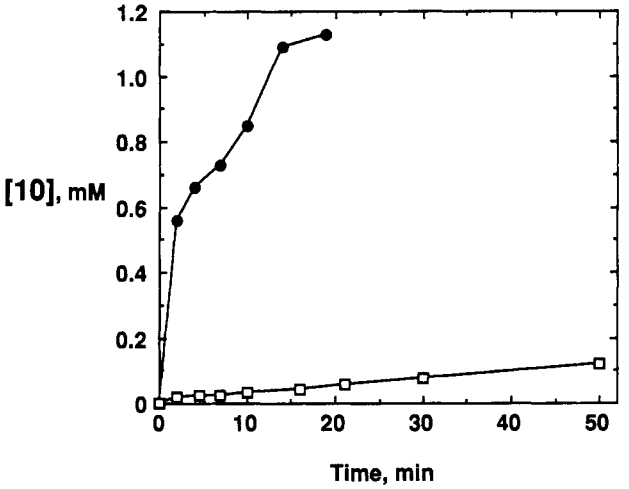


Figure 1. Formation of **10** from **5**. The reactions were done at 5 °C using 35 mM **5** with either 105 mM 1-chlorooxirane (●) or 2-chloroacetaldehyde (□) in 200 mM potassium phosphate, pH 7.7.

carbon labeled with ^{13}C and bearing a good leaving group. Treatment of 2,2-dibromoethanol with NaH in dry $(C_2H_5)_2O$ gave 1-bromooxirane in very low yield as judged by the 4-(*p*-nitrophenyl)pyridine reaction,[10] but the material was difficult to concentrate without destruction. Moreover, in reaction with adenosine the yield of **1** was low (~1%) (in aqueous incubations)—the reaction could not be allowed to proceed beyond the $t_{1/2}$ of the epoxide, because the rearrangement product 2-bromoacetaldehyde begins to react with the adenosine (Figure 1). Indeed, even the reactions of more stable epoxides such as 1-carbamoyloxirane with adenosine are known to give only low yields of **1**.[15] When excess 2,2-dibromoethanol was mixed with adenosine at pH 9.2 for 5 days at 37 °C, the yield of **1** was 12–15% in several different experiments (based upon adenosine). The yield of product obtained under similar conditions with 2,2-dichloroethanol was <1%, and a similar low yield was obtained when the reaction with 2,2-dibromoethanol was carried out at pH 7.7. These results are consistent with the view that slow, base-catalyzed deprotonation of the alcohol leads to 1-bromooxirane formation. In the presence of adenosine this process leads to formation of **1**, in a yield considerably higher than that which could be achieved in a direct reaction with 1-bromooxirane (Scheme II).

2,2-Dibromo[1-^{13}C]ethanol was prepared from 2-bromo[1-^{13}C]acetic acid by extended Hell–Volhard–Zelinsky bromination followed by $LiAlH_4$ reduction of the resulting 2,2-bromoacetic acid. Reaction of the 2,2-bromo[1-^{13}C]ethanol with adenosine yielded **1**, which was isolated by HPLC and analyzed by NMR spectroscopy.

The resonances of the atoms of interest were identified by analysis of standard **1**. The proton at δ 7.77 ppm (*d*, $J_{7,8}$ = 1.3 Hz) was identified as H-7 on the basis of strong nuclear Overhauser effects (NOEs) with the protons at δ 8.87 ppm (s, H-5) and 7.39 ppm (d, H-8, *J* = 1.3 Hz) (Figure 2, Table I). These

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Table I. NOE Values for **1** and **13**[a]

compd	proton irradiated	proton affected	NOE,[b] %
1	H-5	H-7	21
	H-2	H-1′	12[c]
	H-7	H-5	29
	H-7	H-8	16
	H-8	H-7	16
	H-1′	H-2′	26
13	H-1′	H-7	4
	H-8	H-7	16
	H-8	NCH_3	3
	H-2	H-3	9
	H-2	NCH_3	2
	H-3	H-2	8
	H-7	H-8	27
	H-7	H-1′	14

[a] NMR spectra were recorded in ^2H_2O at 200 MHz. [b] All NOEs were negative. [c] The NOE was partial due to exchange of H-2 with the solvent.

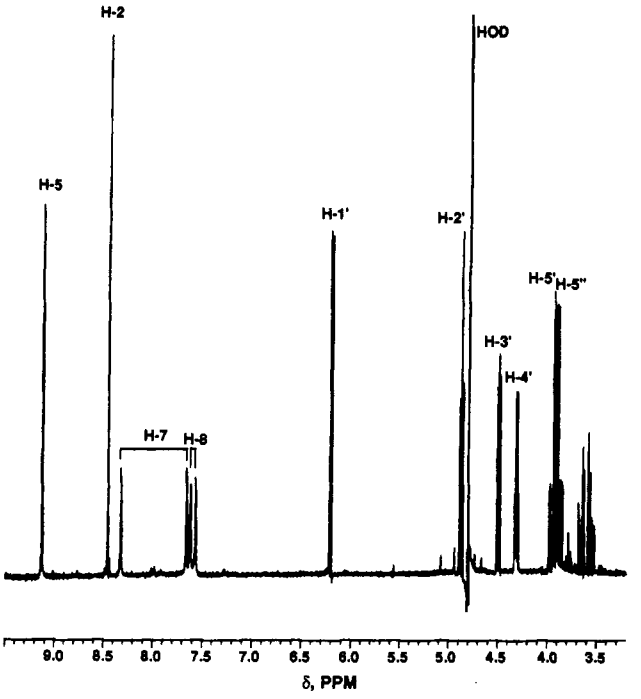
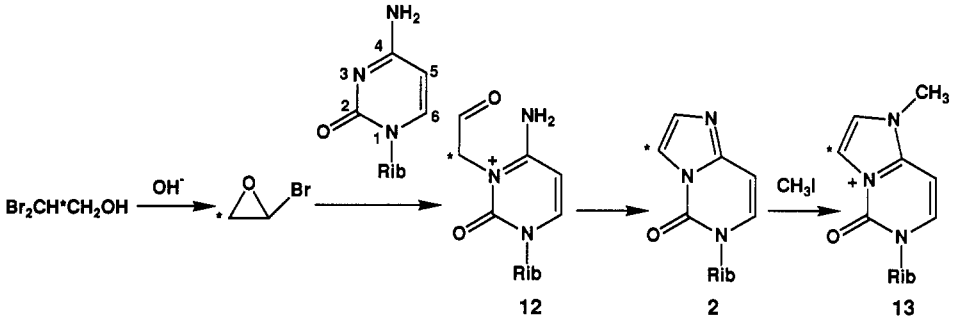


Figure 2. ^1H NMR spectra (300 MHz) of ^{13}C-labeled **1** isolated from reaction of 2,2-dibromo[1-^{13}C]ethanol with adenosine. Assignments with unlabeled **1** were made in ^2H_2O on the basis of the literature[8] and NOE measurements (Table I): δ 3.72 (dd, 1 H, H-5″, $J_{4',5''}$ = 4.1 Hz, $J_{5',5''}$ = 12.8 Hz), 3.80 (dd, 1 H, H-5′, $J_{4',5'}$ = 3.1 Hz, $J_{5',5''}$ = 12.8 Hz), 4.15 (coalesced ddd, 1 H, H-4′, J_{app} = 3.7 Hz), 4.33 (coalesced dd, 1 H, H-3′, J_{app} = 4.6 Hz), 4.70 (coalesced dd, 1 H, H-2′, J_{app} = 5.5 Hz), 6.02 (d, 1 H, H-1′, $J_{1',2'}$ = 5.4 Hz), 7.39 (d, 1 H, H-8, $J_{7,8}$ = 1.3 Hz), 8.00 (d, 1 H, H-7, $J_{7,8}$ = 1.3 Hz), 8.25 (s, 1 H, H-2), 8.87 (s, 1 H, H-5). The peaks in the region δ 3.5–3.7 ppm are due to minor impurities. The shifts of some of the ring protons were shifted slightly in the ^{13}C substituted sample, but all assignments were confirmed by NOE measurements. A mixture of the ^{13}C labeled sample and authentic **1** yielded a single set of resonances.

Scheme III. Postulated Mechanism of Reaction of Cytidine with 2,2-Dibromo[1-^{13}C]ethanol To Yield **2**



assignments are consistent with those of Secrist et al.[8] Off-resonance measurements established that the H-7 proton was attached to a carbon at 115 ppm (C-7); the C-8 carbon signal appears at 135 ppm. Analysis of **1** prepared from 2,2-dibromo[1-^{13}C]-ethanol showed a single ^{13}C peak at 115 ppm (C-7) (Figure 3). In the ^1H spectrum only the H-7 proton (δ 8.00 ppm) shows strong splitting (J = 199 Hz) due to its attachment to a ^{13}C atom (Figure 2).

The possibility was considered that the 1-bromooxirane, formed from 2,2-bromo[1-^{13}C]ethanol, might have rearranged to 2-bromoacetaldehyde to slowly form **1**. The reaction of unlabeled 2,2-dibromoethanol and adenosine was carried out in 90% ^2H_2O under the same conditions—the recovered **1** did not contain any deuterium as judged by ^1H NMR analysis. If 2-bromoacetaldehyde had formed, it would have been expected to have exchanged the methylene protons with the solvent before reacting with adenosine. Indeed, in ^2H_2O at pH 7.6 (37 °C) the methylene protons (δ 3.48) of 2-bromoacetaldehyde or 2-chloroacetaldehyde were exchanged within 1 h.

Modification of Other Nucleosides with $Br_2CH^{13}CH_2OH$. The ^{13}C labeling approach was extended to cytidine (Scheme III). The yield of **2** formed from cytidine and 2,2-dibromo[1-^{13}C]ethanol at pH 9.2 and 37 °C was ~4% after 6 days. The isolated 3,N^4-ethenocytidine was treated with CH_3I to form the N^1-methyl derivative **13** for NMR analysis (Scheme III). The N-methyl protons (δ 4.00) showed NOEs to the protons at δ 7.14 ppm (d, H-8, $J_{7,8}$ = 8.0 Hz) and δ 7.80 ppm (d, H-2, $J_{2,3}$ = 2.2 Hz) (Table I). The ^1H assignments are consistent with those of Barrio et al.[16] Heteronuclear NMR correlation spectroscopy (COSY) experiments with the N-methyl derivative (**12**) indicated that ^{13}C was present only at C-3, as indicated by the large splitting of the proton signal (Figure 4) and the appearance of a single ^{13}C signal at 117 ppm (Figure 5).

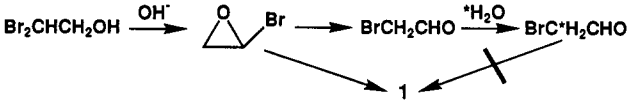
2,2-Dibromo[1-^{13}C]ethanol was also reacted with guanosine and with O^6-ethylguanosine [in 0.2 M N-ethylmorpholine acetate, pH 9.2, containing 50% $(CH_3)_2SO$, v/v] for 7 days at 37 °C. However, HPLC analysis of the reaction mixture indicated that neither **3**, **4**, nor the O^6-ethyl derivative of **4** was formed in >0.5% yield and analysis was precluded.

Discussion

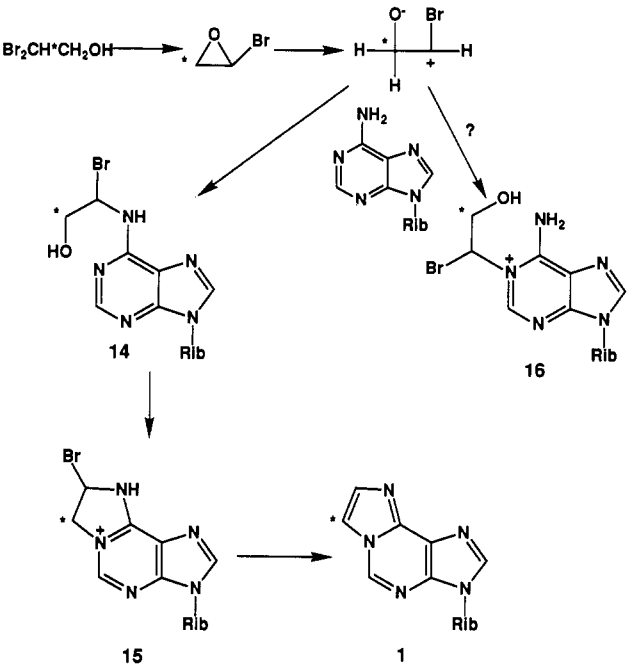
The results collectively support a mechanism in which the most basic endocyclic nitrogen atom of adenosine (N^1) or cytidine (N^3) attacks the methylene of a substituted oxirane to form an N-(2-oxoethyl) adduct, which subsequently closes by Schiff base formation to form the etheno adduct (Schemes Ib, II, and III). Evidence for this view comes from the demonstrated conversion of **5** to **10** and the lack of alternative products resulting from attack of the N^6 atom on 1-chlorooxirane (Scheme I). In addition, the results of specific ^{13}C, labeling experiments are most consistent with such a mechanism (Schemes II and III).

Alternative mechanisms may be considered further. Although the reaction of N^3-methyldeoxycytidine with 1-chlorooxirane has been reported to yield a product in which the exocyclic N^4 atom attacks the oxirane,[17] we found no evidence for an attack of the N^6 atom of adenosine on 1-chlorooxirane (Scheme I), as **8** could not be detected after $NaBH_4$ treatment. The possibility can also be considered that adenosine attacks 2,2-dibromoethanol directly; however, such a reaction (with N^1) would clearly lead to a ^{13}C labeling pattern opposite to that which was observed (Figures 2–5). If 2-bromoacetaldehyde were formed from 2,2-dibromoethanol by direct dehydrohalogenation or if residual 2-bromoethanol in the 2,2-dibromoethanol preparation were to react with adenosine, the opposite labeling pattern would also have resulted. The role of 2-bromoacetaldehyde in the formation of **1** in the studies presented here can be ruled out in two ways. The reaction of **5** to form **10** occurred (at 5 °C) within less than the first $t_{1/2}$ of 1-chlorooxirane (Figure 1). In the labeling studies, the methylene protons of 2-bromoacetaldehyde (and also 2-chloroacetaldehyde) were found to exchange rapidly with ^2H_2O, but no deuterium was detected in **1** when the reaction was done in 90% ^2H_2O (Scheme IV). Another possibility that can be considered is that the initial product of the reaction of 1-bromooxirane with adenosine, **11**,

Scheme IV. Pathways for Direct (1-Bromooxirane) and Indirect (2-Bromoacetaldehyde) Reactions Forming **1**



Scheme V. Possibilities for Formation of **1** from 2,2-Dibromoethanol by an Unfavored S_N1 Mechanism[13]



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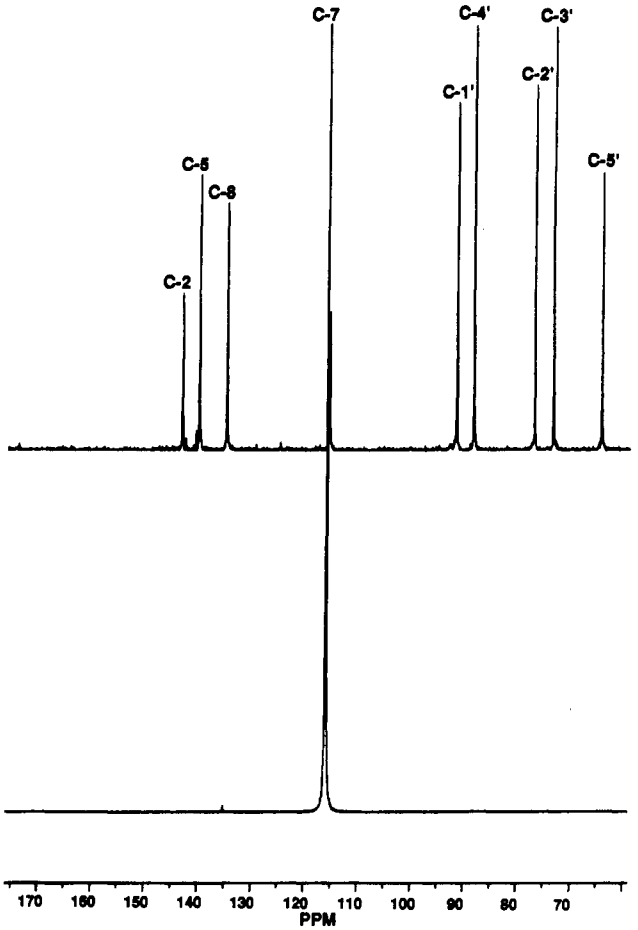
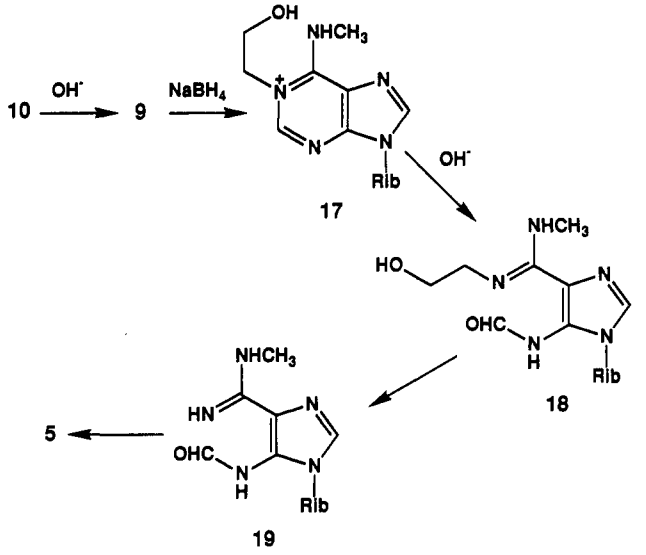


Figure 3. ^{13}C NMR spectra (50 MHz, ^2H_2O) of standard **1** (upper spectrum, natural abundance) and **1** prepared from 2,2-dibromo[1-^{13}C]ethanol (lower spectrum). Carbon assignments in unlabeled **1** were made by running a series of off-resonance spectra with the O2 parameter positioned at different points throughout the ^1H spectrum. Coupling constants were measured for each carbon atom at each O2 value, and plots of residual J values vs O2 values were constructed for each carbon atom. Linear plots were obtained and the $J = 0$ intercepts were used to establish the O2 values of the protons attached to individual carbon atoms. The quarternary carbons did not give signals strong enough to be observed. Assignments (ppm): 65 (C-5′), 73 (C-3′), 77 (C-2′), 88 (C-4′), 91 (C-1′), 115 (C-7), 135 (C-8), 140 (C-5), 143 (C-2).

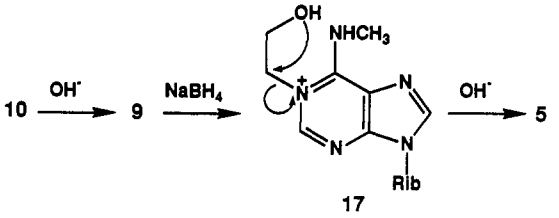
might undergo a Dimroth rearrangement to N^6-(2-oxoethyl)-adenosine. However, any **1** formed from this pathway would also have had the opposite labeling pattern. Apparently the closure of **11** to the carbinolamine must be sufficiently rapid to preclude both base-catalyzed Dimroth rearrangement and exchange of the methylene protons (of **11**) with the solvent (vide supra). One other possibility involves an S_N1-like mechanism (Scheme V)—such an oxirane ring opening is the unfavored mode and can occur in high salt solution.[13] If such a mechanism were operative, then the formal carbonium would have to react with the exocyclic nitrogen. This possibility cannot be unambiguously ruled out by the ^{13}C labeling experiments, but the work with **5** provided no evidence for such a pathway (i.e., no formation of **7**). Consistent with this view that no (S_N1 or S_N2) attack of the adenosine occurs at the halo-substituted carbon is the observation that no major difference was observed between the rates of reaction of adenosine with 1-chloro- and 1-bromooxirane to form **1**.[10]

Precedent exists for the mechanistic conclusions reached here about initial attack of the exocyclic nitrogen atoms of adenosine and cytidine on the methylene of a 1-halooxirane. Reactions of nucleophiles with such oxiranes tend to be dominated by S_N2 attack on the methylene.[13] S_N2-type electrophiles such as C_2H_5I can be used to prepare N^1-alkyladenosine species,[18] and the reaction of ethylene oxide with adenosine yields N^1-(2-hydroxyethyl)adenosine.[18c] 1-(2-Haloethyl)nitrosoureas alkylate cytidine

Scheme VI. Hypothetical Mechanism for Formation of **5** from **7** in Alkaline $NaBH_4$



Scheme VII. Postulated Mechanism of Formation of **5** from **7** in Alkaline $NaBH_4$ via Anchimeric Assistance



at the N^3 atom and the product N^3-(2-chloroethyl)cytidine can form 3,N^4-ethanocytidine or cross-link DNA.[19] Thus, the basicity of the endocyclic nitrogens of adenosine and cytidine appears to drive their reaction with S_N2-type electrophiles. We did not recover sufficient amounts of the guanosine derivatives **3** and **4** to establish the ^{13}C labeling patterns. However, on the basis of the known basicity and other parameters, N^3 might be expected to be the atom which attacks the methylene of the substituted oxirane to form **4**. The mechanism for formation of **3** is more difficult to predict, as the exocyclic amine group and an amide nitrogen must compete, unless the minor enol tautomer of guanosine ($pK_a \sim 9$) is involved.

When **10** was treated with alkaline $NaBH_4$, the major product recovered was **5**. Presumably **10** opened to yield **9** which was reduced to **17** (Scheme VI). One possible mechanism for the retrograde formation of **5** might involve opening of the pyrimidine ring—Dimroth rearrangement would be blocked because the N^6 position is already alkylated. However, this mechanism requires a rationale for the cleavage of a formal glyoxal moiety, and there seems to be no good basis for this. A preferred mechanism is presented in Scheme VII, where the base-catalyzed conversion of **17** to **5** occurs via anchimeric assistance. Fujii et al.[18d] reported that **17** undergoes a facile hydrolytic deamination to N^1-(2-hydroxyethyl)inosine and invoked an electronic effect. No evidence for deamination was particularly apparent in the case with the derivative used here. Anchimeric assistance and the cleavage of adenine is also offered as a reason to explain the facile conversion of *S*-[2-(N^1-adenyl)ethyl]glutathione to adenine under the conditions of Raney Ni treatment.[20]

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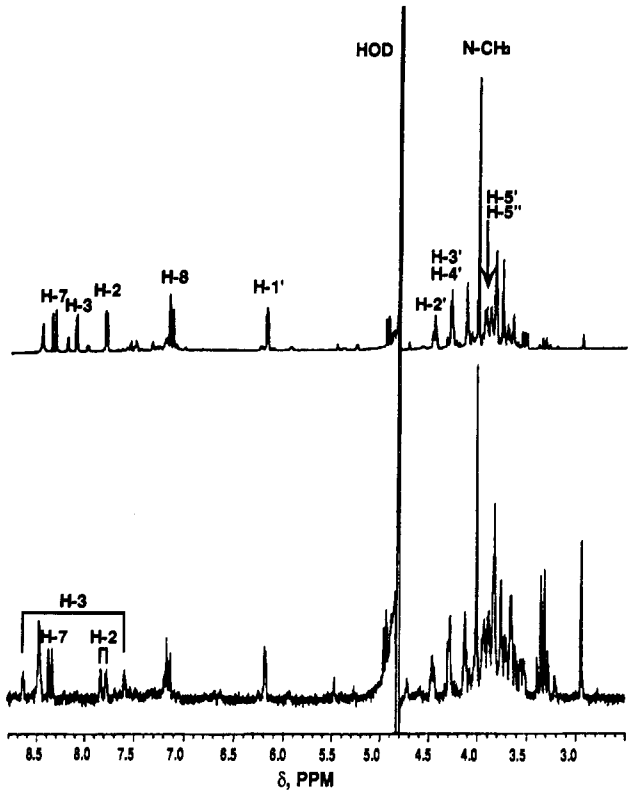

Figure 4. ^1H NMR spectra of **13**. The upper spectrum was recorded (^2H_2O, 300 MHz) with unlabeled material and assignments were made on the basis of the literature[16] and NOE measurements (Table I): δ 3.90 (coalesced dd, 1 H, H-5′, $J_{5',5''}$ = 12.4 Hz, $J_{4',5''}$ = 3.5 Hz), 4.03 (coalesced dd, 1 H, H-5″, $J_{5',5''} \sim$ 14.6 Hz, $J_{4',5'}$ = 1.7 Hz), 4.00 (s, 3 H, NCH_3), 4.28 (coalesced dd, 1 H, H-3′ and coalesced ddd, 1 H, H-4′, $J_{3',4'}$ = 3.9 Hz), 4.45 (dd, 1 H, H-2′, J = 3.6, 4.0 Hz), 6.17 (d, 1 H, H-1′, $J_{1',2'}$ = 3.5 Hz), 7.14 (d, 1 H, H-8, $J_{7,8}$ = 8.0 Hz), 7.80 (d, 1 H, H-2, $J_{2,3}$ = 2.2 Hz), 8.11 (d, 1 H, H-3, $J_{2,3}$ = 2.4 Hz), and 8.35 (d, 1 H, H-7, $J_{7,8}$ = 8.1 Hz). The peaks in the region of δ 2.9–3.85 and at 4.03, 4.9, and 8.47 are minor impurities. The lower panel shows the spectrum of the methylated product isolated from the reaction of cytidine with 2,2-dibromo[1-^{13}C]ethanol. Assignment of the H-2 and H-3 protons was confirmed by the use of NOE and hetero COSY measurements.

Finally, the point should be made that the use of 2,2-dibromoethanol at mild alkaline pH may provide a useful approach of generating 1-bromooxirane in situ. This method may be of use in specific labeling studies or in other applications.

Experimental Section

Adenosine, N^6-methyladenosine (**5**), guanosine, and cytidine were purchased from the Sigma Chemical Co., St. Louis, MO. 2-Chloroacetaldehyde was obtained from the Aldrich Chemical Co., Milwaukee, WI. Bromo[1-^{13}C]acetic acid (99% atomic excess) was purchased from Cambridge Isotope Laboratories, Woburn, MA. 2,2-Dibromoethanol was prepared by reduction of 2,2-dibromoacetic acid.[21] 7,8-Dihydro-8-hydroxy-9-methyl-3-β-D-ribofuranosylimidazo[2,1-*i*]purinium chloride (**10**) and 9-methyl-β-D-ribofuranosylimidazo[2,1-*i*]purinium chloride (**7**) were prepared as described by Sattsangi et al.[9] **3** and the O^9-ethyl derivative of **4** were prepared as described.[4] O^6-Ethylguanosine was prepared as described.[22] N^7-(2-Oxoethyl)guanine was prepared by modification of guanosine, deribosylation, and periodate treatment.[23] All of these chemicals showed the NMR and mass spectral properties expected from the literature references. 1-Chlorooxirane was prepared in ~25% yield by photochemical chlorination of ethylene oxide with *tert*-butylhypochlorite as described previously;[14] this product also contained *tert*-butyl alcohol and acetone as judged by ^1H NMR and was used without attempts at further purification. 2-Bromoacetaldehyde was prepared by hydrolysis of the dimethyl acetal (Aldrich) with 9 M H_2SO_4 in H_2O.[8,10] All other chemicals were reagent grade unless specified otherwise.

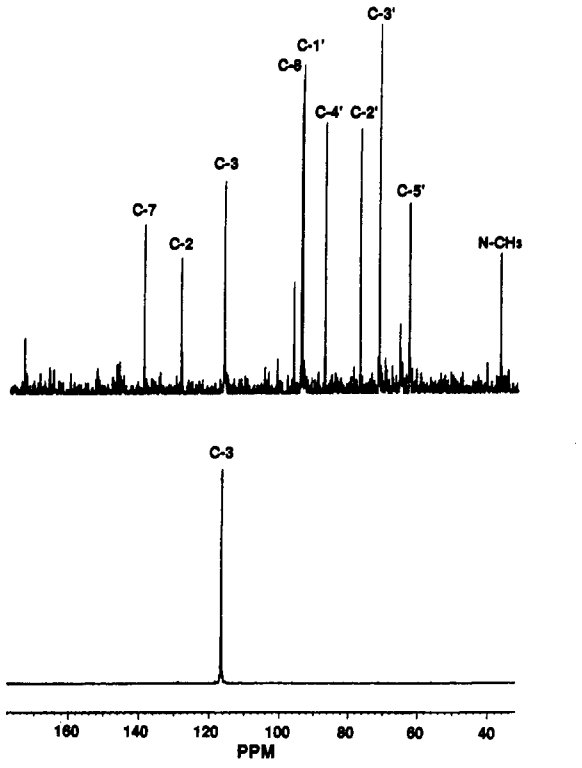

Figure 5. ^{13}C NMR spectra (50 MHz, ^2H_2O) of authentic **13** (upper spectrum, natural abundance) and **13** prepared from 2,2-dibromo[1-^{13}C]ethanol (lower spectrum). Carbon assignments (ppm) in the unlabeled material were made by heteronuclear COSY techniques: 37.4 ($N-CH_3$), 63.6 (C-5′), 72.3 (C-3′), 77.8 (C-2′), 88.1 (C-4′), 94.8 (C-1′), 95.1 (C-8), 117 (C-3), 130 (C-2), 140 (C-7). The peaks at 97.3 and 175 ppm are due to trace impurities.

NMR spectra were obtained with either a Bruker AM-400-NB (400.13 MHz for ^1H), an IBM AC-300 (300.10 MHz), or an IBM NR-200 (200.07 MHz) spectrometer in the Vanderbilt facility. Chemical shifts are reported in ppm; either sodium 2,2-dimethyl-2-silapentane-5-sulfonate or $(CH_3)_4Si$ was used as an internal or external standard. Fast atom bombardment mass spectra (FAB MS) were recorded by Brian Nobes in the Vanderbilt facility on a VG 70-250 system having extended geometry, a standard VG FAB ion source, a standard Ion-Tech saddle field FAB gun producing xenon atoms of 8 kV energy, and a VG 11/250 data system. Glycerol was used as the matrix in all cases. Elemental analyses were done by Galbraith Laboratories (Knoxville, TN).

N^6-Methyl-N^6-2-(hydroxyethyl)adenosine (8). 6-Chloropurine riboside (Aldrich, 285 mg, 1.0 mmol) was mixed with 2-(methylamino)-ethanol (Aldrich, 750 mg, 10 mmol) in 5 mL of distilled $(CH_3)_2NCHO$ and incubated at 37 °C. Analysis of the reaction using HPLC indicated that a new product had formed quantitatively within 1 h. The solvent was removed in vacuo at 50 °C, and the residue was dissolved in C_2H_5OH and crystallized to yield 250 mg of white crystals, mp 164–170 °C. The sample was dried under vacuum at 100 °C in an Aberhalden pistol for 4 days and the mp was 172–173 °C—this material appears to be the monohydrate of the free base: (+)FAB MS m/z (assignment, relative abundance) 348 (M + K, 29), 326 (M + H, 100), 194 (M-ribose, 73); UV (10 mM potassium phosphate, pH 7.4) ϵ_{266} 11 400 M^{-1} cm^{-1}; ^1H NMR (^2H_2O) δ 3.44 (sb, 3 H, NCH_3), δ 3.84 and 3.92 (both dd, 2 H, 5′-CH_2, J = 12.9 and 3.4 Hz for δ 3.84 and J = 12.9 and 2.7 Hz for δ 3.92), 3.90 (dd, 2 H, $-CH_2CH_2OH$, J = 6.4 and 6.4 Hz), 4.17 (sb, 2 H, $-NCH_2-$), 4.30 (ddd, 1 H, H-4′, J = 3.4 Hz, 3.4 Hz, 2.7 Hz), 4.43 (dd, 1 H, H-3′, J = 5.3 and 3.4 Hz), 4.80 (dd, 1 H, H-2′, under ^2HOH), 6.07 (d, 1 H, H-1′, J = 6.2 Hz), 8.22 (s, 1 H, H-8), 8.37 (s, 1 H, H-2). Anal. Calcd for $C_{13}H_{19}N_5O_5 \cdot H_2O$: C, 45.28; H, 6.12; N, 20.41. Found: C, 45.71; H, 6.18; N, 20.56.

2-Dibromo[1-^{13}C]ethanol. 2-Bromo[1-^{13}C]acetic acid (5 g, 36 mmol, 99% atomic excess) was mixed with 11.5 g of Br_2 (72 mmol, washed with concentrated H_2SO_4) and PBr_3 (9.8 g, 36 mmol) and heated at 110 °C

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with stirring for 60 h. The course of the reaction was monitored by ^1H NMR. An additional 36 mmol of Br_2 was added, and heating was continued at 110 °C for an additional 12 h. The reaction was cooled, and excess Br_2 was removed by sweeping with N_2. A 5% aqueous Na_2CO_3 solution (w/v) was added carefully with stirring, and 8 N NaOH was added until the pH was basic. The alkaline solution was washed 3 times with equal volumes of $(C_2H_5)_2O$ and the pH of the aqueous phase was carefully lowered to <2 with 6 N HCl. The solution was extracted 4 times with equal volumes of $(C_2H_5)_2O$, and the combined organic extracts were dried with $MgSO_4$ and concentrated in vacuo to give 4.65 g of the product $Br_2CH^{13}CO_2H$ (21 mmol, 59% yield). The acid reduced with $LiAlH_4$ (1.20 g, 32 mmol) in 100 mL of dry $(C_2H_5)_2O$ under N_2 using the procedure of Sroog and Woodburn[21] to give the product $Br_2CH^{13}CH_2OH$ in 47% yield. ^1H and ^{13}C NMR spectroscopy indicated that the product was contaminated with ~10% $BrCH_2{}^{13}CH_2OH$; only the carbinol carbon was labeled in each case (^{13}C signal at 70 ppm for $Br_2CH^{13}CH_2OH$, 63 ppm for $BrCH_2{}^{13}CH_2OH$). The J value for the ^{13}C induced splitting of the Br_2CHCH_2OH proton doublets in the ^1H NMR spectrum was 140 and 1.4 Hz for the Br_2CH-CH_2OH triplet members.

5,6-Dihydro-1-methyl-5-oxo-6-β-D-ribofuranosylimidazo[1,2-c]pyrimidine Chloride (3,N^4-Etheno-N^4-methylcytidinium Chloride) (13). The procedure was adopted from Barrio et al.[16] N^3,4-Ethenocytidine·HCl (2) (25 mg, 82 μmol) was stirred in 1 mL of distilled $(CH_3)_2NCHO$ with $(C_2H_5)_3N$ (160 μmol) and CH_3I (320 μmol) overnight at 23 °C under Ar. HPLC analysis (Ultrasphere 5 μm octadecasilyl, 10 × 250 mm, 5% CH_3OH in 25 mM NH_4HCO_2, pH 5.5) (Beckman, San Ramon, CA) indicated that the reaction was ~90% complete as judged by A_{267} measurements (formation of a major new polar peak). A portion of the total preparation was purified using the same HPLC column and eluant in the absence of CH_3OH: ^1H and ^{13}C NMR assignments are presented in the legends for Figures 4 and 5.[16] UV (25 mM aqeuous NH_4HCO_2, pH 5.5): apparent broad λ_{max} at 292 nm, second derivative analysis showed peaks at 248, 257, 274, 285, 295, and 307 nm.

Preparation of ^{13}C-Labeled Etheno Derivatives. The general procedure involved mixing $Br_2CH^{13}CH_2OH$ (200 mM), N-ethylmorpholine acetate (200 mM, pH 9.2), and either cytidine (200 mM), adenosine (35 mM), guanosine (50 mM), or O^6-ethylguanosine (50 mM) and shaking at 37 °C under Ar in a Teflon-sealed amber glass vial for 5-7 days. When guanosine or O^6-ethylguanosine was used, 50% (v/v) $(CH_3)_2SO$ was added to improve solubility. The solution was washed four times with an equal volume of $(C_2H_5)_2O$ and concentrated by lyophilization prior to separation of components by HPLC (vide infra).

HPLC. Most HPLC was done using a Beckman octadecasilyl semi-prep column (5 μm, 10 × 250 mm) and mixtures of CH_3OH in 25 mM NH_4HCO_2 (pH 5.5) as modified from the literature.[12] Some of the analytical work on the formation of products of the reaction of N^6-methyladenosine was done using a Zorbax octadecasilyl column (3 μm, 6.2 × 80 mm, Mac-Modd, Chadds Ford, PA). All of the compounds under consideration could be separated using either isocratic or gradient conditions, e.g., see indicated references for examples of separations.[12,24]

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Communications to the Editor

Cooperative Binding of Distamycin-A to DNA in the 2:1 Mode

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The preference of distamycin-A for A-T rich binding sites has been recognized for many years.[1] NMR was first used to show unambiguously that the closely related drug netropsin bound in the minor groove of B-form DNA.[3a] Both NMR and crystallographic studies of complexes of distamycin-A,[2] netropsin,[3] Hoechst 33258,[4] and SN-6999[5] have been carried out. In all of these studies to date, the drugs are bound deep in the minor groove of an A-T segment. Where the drug is bound, the structure of the minor groove closely matches the shape and width of the drug molecule. In several of the DNAs studied crystallographically, the groove is found to be equally narrow without the drug present.[6] NMR studies have shown that the sequences CGCAAATTGGC[7] and CGCAAATTTGCG[8] bind a single drug at low drug ratios (analogous to complexes characterized crystallographically). At higher amounts of added drug, new complexes were formed with two distamycin molecules bound side by side in the same region of the minor groove. This indicates a significant degree of adaptability in the minor groove width, since an increase in groove width of about 3.5 Å is required to accommodate the second drug. It was found that the second drug bound somewhat less tightly than the first for AAATT, but was somewhat tighter than the first for AAATTT.

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