# Studies of the Mechanisms of Adduction of 2′-Deoxyadenosine with Styrene Oxide and Polycyclic Aromatic Hydrocarbon Dihydrodiol Epoxides

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The mechanism of adduction of 2′-deoxyadenosine by styrene oxide and polycyclic aromatic hydrocarbon dihydrodiol epoxides has been explored using $^{15}N^6$-labeled adenine nucleosides. The extent of reaction at N1 versus $N^6$ was evaluated by $^1H$ NMR of the $N^6$ adducts after allowing Dimroth rearrangement to occur. Products arising from attack at N1 followed by Dimroth rearrangement exhibited a small two-bond $^1H-^{15}N$ coupling constant (N1−H2 $J \sim$ 13 Hz); products from direct attack exhibited a much larger one-bond $^1H-^{15}N$ coupling constant ($J \sim 90$ Hz). In the case of styrene oxide, all of the $N^6$ $\beta$ adduct arose by initial attack at N1, whereas the majority (70−80%) of the $N^6$ α adducts came from direct attack. The styrene oxide reaction was also studied with a self-complementary oligodeoxynucleotide (24-mer) containing nine $^{15}N^6$-labeled adenine residues. NMR examination of the $N^6$ α- and $\beta$-styrene oxide adducts isolated after enzymatic degradation of the 24-mer gave very similar results, indicating that N1 attack can occur readily even with a duplexed oligonucleotide. With the PAH dihydrodiol epoxides, only naphthalene dihydrodiol epoxide exhibited significant initial reaction at N1 (50%). No detectable rearranged product was seen in reactions with benzo[*a*]pyrene dihydrodiol epoxide or non-bay or bay region benz[*a*]anthracene dihydrodiol epoxide; interestingly, a small amount of N1 attack (5−7%) was seen in the case of benzo[*c*]phenanthrene dihydrodiol epoxide. It appears that initial attack at N1 is only a significant reaction pathway for epoxides attached to a single aromatic ring.

## Introduction

The genotoxicity of xenobiotic compounds involves reactions of the compounds or more frequently their metabolic products with DNA. The resulting adducts or lesions can degrade replication fidelity, leading to mutations. In most of the adduction reactions, the xenobiotic species acts as an electrophile. There are well-documented examples of attack on many different sites in the DNA, but the identity of the adduct giving rise to any specific mutation is frequently not known. As a consequence, the reactivity of electrophiles with nucleic acid constituents and the biochemical impact of the resulting adducts continue to be the subject of intense study with the hope of better understanding the structural basis of mutagenesis and carcinogenesis.

A particularly interesting situation exists with adenine. The reactivity of its nucleosides with alkylating agents, including both the alkyl and aralkyl halides and the related epoxides, has been studied extensively, and adducts have been observed at N1, N3, $N^6$, and N7 with the distribution of products depending upon the structure of the electrophile and the reaction conditions. With simple epoxides such as propylene oxide (*1*), butadiene monoepoxide (*2*), and glycidyl ethers (*3*), both N1 and $N^6$ products have been observed. In these cases, the adduction reaction may always involve initial attack at N1 with the $N^6$ adduct arising by a Dimroth rearrangement. The Dimroth rearrangement is a base-catalyzed cleavage of the six-membered ring followed by a recyclization that interchanges the locations of the endocyclic and exocyclic nitrogens (Scheme 1). With the dihydrodiol epoxides of polycyclic aromatic hydrocarbons and other highly conjugated epoxides, N1 adducts have not been observed. Although it has generally been presumed that this is due to reaction occurring directly at $N^6$, the possibility that a very rapid Dimroth rearrangement with bulky N1 derivatives makes the N1 adducts difficult to detect cannot be excluded.

Both the simple epoxides and those of the PAHs cause mutations at adenine sites. For example, butadiene monoepoxide produces a significant fraction of mutations at A·T base pairs but so do the bay region dihydrodiol epoxides of PAHs.[1] The mutagenicity of $N^6$ adducts of the PAHs, styrene oxide, and to a lesser extent the simpler epoxides has been studied using synthetic oligonucleotides containing adducts of unambiguous structure. However, these studies do not preclude the possibility that mutations observed in vivo may be arising from N1 lesions rather than from $N^6$.

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[1] Abbreviations: HRMS-FAB$^+$, high-resolution mass spectrometry-fast atom bombardment positive mode; EI-MS, electron impact mass spectrometry; COSY, correlation spectroscopy; HMQC, heteronuclear correlation through multiple-bond quantum coherence; INEPT, insensitive nuclei enhanced by polarization transfer; PAH, polycyclic aromatic hydrocarbon; SO, styrene oxide; NA, naphthalene; nBA, non-bay region benz[*a*]anthracene; BA, bay region benz[*a*]anthracene; BP, benzo[*a*]pyrene; BC, benzo[*c*]phenanthrene; DE, dihydrodiol epoxide.

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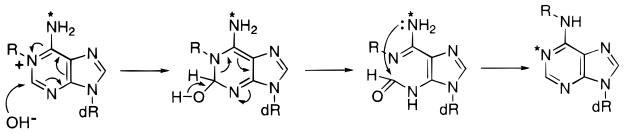
**Scheme 1. Dimroth Rearrangement of N1 Alkyl Adenine Nucleosides**



The situation with regard to styrene oxide adducts is particularly interesting. Styrene oxide appears to have properties intermediate between the extremes represented by propylene oxide and PAH dihydrodiol epoxides (*4*−*6*). Qian and Dipple (*6*) found that styrene oxide reacts with adenosine at N1 and $N^6$ to give both α- and *β*-products. They proposed that the $N^6$ *β*-product arises entirely by Dimroth rearrangement, whereas the $N^6$ α-product is formed through a combination of Dimroth rearrangement from N1 and direct attack at $N^6$ (Scheme 2). The $N^6$ α adducts of styrene oxide have been reported to show negligible mutagenicity in a bacterial system (*7*), but styrene oxide has been reported to be mutagenic in human t-lymphocytes with a significant fraction of these mutations involving A·T base pairs (*8*). These results raise the question of whether transient N1 adducts may be the source of some or all of the AT mutations observed in vivo.

We wanted to probe further the formation and reactivity of adducts of styrene oxide and PAH dihydrodiol epoxides on the N1 position of deoxyadenosine. The relatively rapid rearrangement of the N1 styrene oxide adducts, and possibly even faster rearrangement of as yet unobserved N1 adducts of PAH dihydrodiol epoxides, complicate the investigation of this issue. It occurred to us that by using $^{15}N^6$-labeled deoxyadenosine in such alkylation reactions we would be able to detect rearrangement even if we were unable to isolate the N1 adducts. The inherently low sensitivity of $^{15}N$ NMR spectroscopy even with highly enriched samples is an experimental complication for such a study in that direct observation of $^{15}N$ would require large samples and/or long acquisition times. However, greatly enhanced sensitivity can be obtained by using $^1H$ NMR to detect $^1H-^{15}N$ one- and two-bond coupling so that microgram samples of $^{15}N$-enriched nucleosides would be adequate for assigning the location of $^{15}N$ labels.

In this paper, we report the results of a study of the reactions of $^{15}N^6$-labeled deoxyadenosine with a series of aralkyl epoxides, ranging from styrene oxide to the dihydrodiol epoxides of the highly carcinogenic PAHs benzo[*c*]phenanthrene and benzo[*a*]pyrene. The goal of these experiments was to determine the extent to which the $N^6$ deoxyadenosine derivatives of these compounds arise by initial attack at N1. In addition, the reaction of styrene oxide with a 24-mer oligonucleotide containing $^{15}N^6$-labeled adenine was examined to determine the effect of duplex structure on the relative reactivity of the N1 and $N^6$ sites.

## Experimental Procedures

**Caution:** *The dihydrodiol epoxides of polycyclic aromatic hydrocarbons are potent mutagens and/or carcinogens and should be handled with care, as outlined in National Cancer Institute guidelines.*

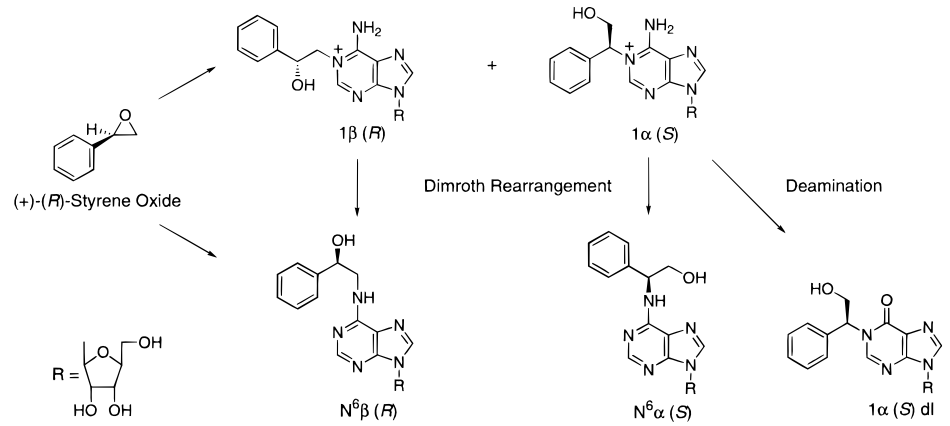
**General.** (±)-3*β*,4α-Dihydroxy-1*β*,2*β*-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene and (±)-3*β*,4α-dihydroxy-1*β*,2*β*-epoxy-1,2,3,4-tetrahydrobenz[*a*]anthracene were purchased from the NCI Chemical Carcinogen Repository (Midwest Research Institute). (*R*)-Styrene oxide was purchased from Aldrich. $^{15}N$-labeled ammonium chloride (>98 at. %) was purchased from Aldrich and $^{15}NH_4OH$ (3.3 M, >98 at. %) from Cambridge Isotopes. $^1H$, one-dimensional (1D) COSY, two-dimensional (2D) COSY, and HMQC NMR spectra were obtained at either 400 or 500 MHz. The spectra were recorded in DMSO-$d_6$ or mixtures of DMSO-$d_6$ with $D_2O$. The $^1H$ spectra were internally referenced to the residual $^1H$ signal in DMSO-$d_6$ ($\delta$ = 2.49 ppm); the $^{15}N$ spectra were referenced externally to 2.9 M $^{15}NH_4Cl$ in 1% HCl /DMSO ($\delta$ = 0 ppm). Mass spectra were obtained in a positive ion FAB mode (glycerol/DMSO/TFA). Reactions were monitored by TLC on silica gel plates (EM Science, Kieselgel 60, F254). TLC plates were visualized by UV and/or anisaldehyde stain. Column chromatography was conducted on silica gel 60 (70−230 mesh) from EM Science. HPLC analyses and isolations of nucleosides were carried out on a gradient HPLC system (Beckman System Gold) equipped with a diode array detector. Analytical runs were performed on a 4.6 mm × 250 mm YMC-ODS-AQ column at a flow rate of 1.0 mL/min; preparative-scale isolations were carried out on a 10 mm × 250 mm YMC-ODS-AQ column at a flow rate of 3.0 mL/min.

**Synthesis of $^{15}N^6$-Labeled Nucleosides. (1) [$^{15}N^6$]-2′-Deoxyadenosine.** 6-Chloropurinyl 2′-deoxyriboside (0.30 g, 1.1 mmol) was dissolved in 100 *μ*L of DMSO and 1 mL of dioxane in a heavy-walled reaction vial. $^{15}NH_4OH$, generated by the reaction of $^{15}NH_4Cl$ (240 mg, 4.4 mmol) and NaOH (4.4 M, 1 mL), was added. The vial was sealed, and the reaction mixture was heated at 50 °C for 62 h, evaporated to dryness, and redissolved in methanol. Precipitated NaCl was filtered off, and the filtrate was passed through an anion exchange column (Bio-Rad; AG1-8X, $Cl^-$ form, 20 mm × 140 mm; eluted with 4:6 MeOH/$H_2O$). The resulting solution was evaporated to dryness and then redissolved in MeOH for purification by silica gel column chromatography (8:2 $CHCl_3$/EtOH) to give [$^{15}N^6$]-2′-deoxyadenosine in 60−80% yield: $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.32 (s, 1H, H8), 8.11 (s, 1H, H2), 7.29 (d, 2H, $^{15}NH_2$, $^1J_{NH}$ = 90.1 Hz, can be exchanged in $D_2O$), 6.33 (dd, 1H, H1′, $J_1 = J_2$ = 7.7 Hz), 5.30 (d, 1H, OH, $J$ = 3.6 Hz, can be exchanged in $D_2O$), 5.22 (t, 1H, OH, $J$ = 1.3 Hz, can be exchanged in $D_2O$), 4.40 (br, 1H, H3′), 3.87 (br, 1H, H4′), 3.59−3.50 (m, 2H, H5′, H5″), 2.76 (m, 1H, H2″), 2.22 (m, 2H, H2′); $^{15}N$ NMR (DMSO-$d_6$) $\delta$ 60.03 ($J$ = 90.1 Hz); HRMS-FAB$^+$ $m/z$ 253.1062, calcd for $C_{10}H_{14}{}^{14}N_4{}^{15}N_1O_3$ ($MH^+$) 253.0989.

**(2) [$^{15}N^6$]Adenosine.** 6-Chloropurinyl riboside (Sigma Chemical Co., 100 mg, 0.35 mmol) was dissolved in 3.3 M $^{15}NH_4OH$ (1.4 mL, Cambridge Isotopes). The mixture was heated (50 °C) with stirring for 24 h in a sealed vial. The reaction mixture was evaporated to dryness and redissolved in water. Silica gel (1.4 g) was added, and the mixture was lyophilized. Purification of the labeled nucleoside was accomplished by column chromatography on silica gel (7:3 $CHCl_3$/EtOH) to give the pure nucleoside in 40% yield (37.5 mg, 0.18 mmol): $R_f$ = 0.3 (7:3 $CHCl_3$/EtOH); $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.34 (s, 1H, adenine H8), 8.12 (s, 1H, adenine H2), 7.35 (d, 1H, $^{15}NH_2$, $^1J_{NH}$ = 90.2 Hz, can be exchanged in $D_2O$), 5.86 (d, 1H, H1′, $J$ = 6.2 Hz), 5.42 (m, 2H, OH, can be exchanged in $D_2O$), 5.16 (br, 1H, OH, can be exchanged in $D_2O$), 4.59 (m, 1H, H2′), 4.13 (br, 1H, H3′), 3.95 (dd, 1H, H4′, $J$ = 6.6 Hz), 3.65−3.52 (m, 2H, H5′, H5″); $^{15}N$ NMR (INEPT, DMSO-$d_6$) $\delta$ 60.48; HRMS-FAB$^+$ $m/z$ 269.1016, calcd for $C_{10}H_{14}{}^{14}N_4{}^{15}N_1O_4$ ($MH^+$) 269.1018.

**Reaction of (*R*)-Styrene Oxide with Unlabeled 2′-Deoxyadenosine.** (*R*)-Styrene oxide (40 *μ*L, 0.17 mmol) and DMSO (40 *μ*L) were added to a solution of deoxyadenosine (20 mg, 0.08 mmol) dissolved in 50 mM Tris-HCl (2 mL, pH 7.2). The mixture was sonicated for 30 min, and then stirred for 72 h at 42 °C. The sonication was repeated every 24 h. The mixture was loaded onto C-18 Sep-Pak cartridges which were eluted with water (40 mL) and finally with 100% MeOH (2 × 2 mL) to elute the more lipophilic styrene oxide adducts. The resulting MeOH fractions were evaporated to dryness and redissolved in MeOH/$H_2O$ (50:50) for HPLC purification. UV absorbance was moni-

**Scheme 2. Reaction of Styrene Oxide with Adenosine**




Qian, *et al. Chem Res. Toxicol.* **1995**, *8*, 389.

tored at 260 and 280 nm. Peaks from multiple chromatographic runs were pooled and concentrated to dryness (combined yield of ~25%). The purity of samples was established by HPLC under the same conditions.

***N*$^6$-[2-Hydroxy-1-(*S*)-phenylethyl]-2′-deoxyadenosine [N$^6$α-(*S*)SOdAdo]:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.35 (s, 1H, adenine H8), 8.13 (s, 1H, adenine H2), 8.01 (d, 1H, NH, $J$ = 8.6 Hz, can be exchanged in $D_2O$), 7.41 (d, 2H, Ar-H, ortho, $J$ = 7.3 Hz), 7.27 (dd, 2H, Ar-H, meta, $J_1$ = 7.3 Hz, $J_2$ = 7.7 Hz), 7.19 (t, 1H, Ar-H, para, $J$ = 7.7 Hz), 6.33 (dd, 1H, H1′, $J_1 = J_2$ = 6.9 Hz), 5.37 (br, 1H, α-CH), 5.27 (d, 1H, 3′-OH, $J$ = 4.0 Hz, can be exchanged in $D_2O$), 5.16 (t, 1H, 5′-OH, $J$ = 5.1 Hz, can be exchanged in $D_2O$), 4.92 (t, 1H, $\beta$-OH, $J$ = 5.8 Hz, can be exchanged in $D_2O$), 4.39 (br, 1H, H3′), 3.86−3.85 (m, 1H, H4′), 3.74−3.70 (m, 2H, $\beta$-$CH_2$), 3.58−3.49 (m, 2H, H5′, H5″), 2.75−2.65 (m, 2H, H2″), 2.30−2.20 (m, 1H, H2′); HRMS-FAB$^+$ $m/z$ 372.1676, calcd for $C_{18}H_{22}N_5O_4$ ($MH^+$) 372.1672.

***N*$^6$-[2-Hydroxy-1-(*R*)-phenylethyl]-2′-deoxyadenosine [N$^6$α(*R*)SOdAdo]:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.35 (s, 1H, adenine H8), 8.13 (s, 1H, adenine H2), 8.01 (d, 1H, NH, $J$ = 8.6 Hz, can be exchanged in $D_2O$), 7.41 (d, 2H, Ar-H, ortho, $J$ = 7.3 Hz), 7.27 (dd, 2H, Ar-H, meta, $J_1$ = 7.3 Hz, $J_2$ = 7.6 Hz), 7.19 (t, 1H, Ar-H, para, $J$ = 7.6 Hz), 6.33 (dd, 1H, H1′, $J_1 = J_2$ = 6.9 Hz), 5.37 (br, 1H, α-CH), 5.27 (d, 1H, 3′-OH, $J$ = 4.0 Hz, can be exchanged in $D_2O$), 5.16 (t, 1H, 5′-OH, $J$ = 5.1 Hz, can be exchanged in $D_2O$), 4.92 (t, 1H, $\beta$-OH, $J$ = 5.8 Hz, can be exchanged in $D_2O$), 4.39 (br, 1H, H3′), 3.86−3.85 (m, 1H, H4′), 3.74−3.70 (m, 2H, $\beta$-$CH_2$), 3.58−3.49 (m, 2H, H5′, H5″), 2.75−2.65 (m, 2H, H2″), 2.30−2.20 (m, 1H, H2′); HRMS-FAB$^+$ $m/z$ 372.1675, calcd for $C_{18}H_{22}O_4N_5$ ($MH^+$) 372.1672.

***N*$^6$-[1-Hydroxy-2-(*R*)-phenylethyl]-2′-deoxyadenosine [N$^6$β(*R*)SOdAdo]:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.33 (br, 1H, adenine H8), 8.21 (br, 1H, adenine H2), 7.57 (br, 1H, NH, can be exchanged in $D_2O$), 7.36 (br, 2H, Ar-H, ortho), 7.31 (t, 2H, Ar-H, meta, $J$ = 7.4 Hz), 7.23 (t, 1H, Ar-H, para, $J$ = 5.2 Hz), 6.34 (t, 1H, H1′, $J$ = 7.6 Hz), 5.57 (br, 1H, α-OH, can be exchanged in $D_2O$), 5.29 (br, 1H, 3′-OH, can be exchanged in $D_2O$), 5.19 (t, 1H, 5′-OH, $J$ = 5.8 Hz, can be exchanged in $D_2O$), 4.87 (m, 1H, α-CH), 4.39 (m, 1H, H3′), 3.87−3.86 (m, 1H, H4′), 3.80−3.70 (m, 1H, $\beta$-$CH_2$), 3.63−3.50 (m, 3H, $\beta$-$CH_2$, H5′, H5″), 2.71−2.65 (m, 1H, H2″), 2.32−2.22 (m, 1H, H2′); HRMS-FAB$^+$ $m/z$ 372.1676, calcd for $C_{18}H_{22}N_5O_4$ ($MH^+$) 372.1672.

The structure and stereochemistry of the styrene oxide adducts were confirmed by independent syntheses from 6-chloropurine 2′-deoxyriboside and (*R*)- and (*S*)-2-amino-2-phenylethanol (*9*) for the α adducts and (*R*)- and (*S*)-2-amino-1-phenylethanol (*10*) for the $\beta$ adducts and also by comparison with literature values (*6*, *11*).

**Reaction of (*R*)-Styrene Oxide with [$^{15}$N$^6$]-2′-Deoxyadenosine.** The reaction conditions described above for the nonlabeled styrene oxide adducts were used to prepare the labeled products, using 2′-deoxyadenosine labeled with $^{15}$N at the N$^6$ position (yield of 28%).

**N$^6$α(*S*)SOd$^{15}$Ado.** The isolated N$^6$α(*S*)SOd$^{15}$Ado was a mixture of rearranged and un-rearranged products: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.36 (s, 1H, adenine H8), 8.13 (s, 1H, adenine H2), 8.01 (dd, 1H, $^{15}$NH, $^1J_{NH}$ = 92.0 Hz, $J_{HH}$ = 8.5 Hz, can be exchanged in $D_2O$), 7.41 (d, 2H, Ar-H, ortho, $J$ = 7.5 Hz), 7.27 (dd, 2H, Ar-H, meta, $J$ = 7.5 and 7.3 Hz), 7.18 (t, 1H, Ar-H, para, $J$ = 7.3 Hz), 6.33 (dd, 1H, H1′, $J_1$ = 7.4 Hz, $J_2$ = 6.5 Hz), 5.38 (br, 1H, α-CH), 5.28 (d, 1H, OH, $J$ = 4.0 Hz, can be exchanged in $D_2O$), 5.16 (t, 1H, OH, $J_1 = J_2$ = 5.7 Hz, can be exchanged in $D_2O$), 4.93 (t, 1H, OH, $J$ = 5.6 Hz, can be exchanged in $D_2O$), 4.39 (br, 1H, OH, $J_1 = J_2$ = 5.7 Hz, can be exchanged in $D_2O$), 3.86−3.85 (m, 1H, H4′), 3.74−3.70 (m, 2H, $\beta$-$CH_2$), 3.58−3.49 (m, 2H, H5′, H5″), 2.75−2.65 (m, 1H, H2″), 2.30−2.20 (m, 1H, H2′). The NMR spectra established that the material was 20% rearranged and 80% un-rearranged: HRMS-FAB$^+$ $m/z$ 373.1646, calcd for $C_{18}H_{22}{}^{14}N_4{}^{15}N_1O_4$ ($MH^+$) 373.1644.

**N$^6$α(*R*)SOd$^{15}$Ado:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.36 (s, 1H, adenine H8), 8.13 (s, 1H, adenine H2), 8.01 (dd, 1H, $^{15}$NH, $^1J_{NH}$ = 92.0 Hz, $J_{HH}$ = 8.5 Hz, can be exchanged in $D_2O$), 7.41 (dd, 2H, Ar-H, ortho, $J$ = 7.5 Hz), 7.27 (dd, 2H, Ar-H, meta, $J_1$ = 7.5 Hz, $J_2$ = 7.3 Hz), 7.18 (t, 1H, Ar-H, para, $J$ = 7.3 Hz), 6.33 (dd, 1H, H1′, $J_1$ = 7.4 Hz, $J_2$ = 6.5 Hz), 5.38 (br, 1H, α-CH), 5.28 (d, 1H, OH, $J$ = 4.0 Hz, can be exchanged in $D_2O$), 5.16 (t, 1H, OH, $J_1 = J_2$ = 5.7 Hz, can be exchanged in $D_2O$), 4.93 (t, 1H, OH, $J$ = 5.6 Hz, can be exchanged in $D_2O$), 4.39 (br, 1H, OH, can be exchanged in $D_2O$), 3.86−3.85 (m, 1H, H4′), 3.74−3.70 (m, 2H, $\beta$-$CH_2$), 3.58−3.49 (m, 2H, H5′, H5″), 2.75−2.65 (m, 1H, H2″), 2.30−2.20 (m, 1H, H2′). The NMR spectra established that the material was essentially un-rearranged: HRMS-FAB$^+$ $m/z$ 373.1646, calcd for $C_{18}H_{22}{}^{14}N_4{}^{15}N_1O_4$ 373.1644 ($MH^+$).

**N$^6$βSOd$^{15}$Ado:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.35 (br, 1H, adenine H8), 8.24 (d, 1H, adenine H2, $^2J_{NH}$ = 16.4 Hz), 7.55 (br, 1H, NH, can be exchanged in $D_2O$), 7.36 (br, 2H, Ar-H, ortho), 7.31 (t, 2H, Ar-H, meta, $J$ = 7.5 Hz), 7.23 (t, 1H, Ar-H, para, $J$ = 7.2 Hz), 6.34 (dd, 1H, H1′, $J_1 = J_2$ = 6.3 Hz), 5.57 (br, 1H, α-OH, can be exchanged in $D_2O$), 5.29 (br, 1H, OH, can be exchanged in $D_2O$), 5.19 (t, 1H, OH, $J_1$ = 5.2 Hz, $J_2$ = 5.8 Hz, can be exchanged in $D_2O$), 4.87 (m, 1H, α-CH), 4.39 (m, 1H, H3′), 3.87−3.86 (m, 1H, H4′), 3.8−3.7 (m, 1H, $\beta$-$CH_2$), 3.63−3.50 (m, 3H, $\beta$-$CH_2$, H5′, H5″), 2.71−2.65 (m, 1H, H2″), 2.32−2.22 (m, 1H, H2′). The NMR spectra established that the material was completely rearranged from N1 to N$^6$: HRMS-FAB$^+$ $m/z$ 373.1646, calcd for $C_{18}H_{22}{}^{14}N_4{}^{15}N_1O_4$ ($MH^+$) 373.1644.

**Reaction of (*R*)-Styrene Oxide with [$^{15}$N$^6$]Adenosine.** [$^{15}$N$^6$]Adenosine (20 mg, 0.07 mmol) was stirred in 2 mL of $H_2O$ at 42 °C to form a homogeneous solution. (*R*)-Styrene oxide (40 $\mu$L, 0.17 mmol) was added. The reaction mixture was stirred at 42 °C for 72 h. The reaction mixture was extracted with EtOAc

(3 × 1 mL), followed by $Et_2O$ (2 × 1 mL), to remove epoxide hydrolysis products. The aqueous solution was then loaded onto C-18 cartridges (Alltech), and most of the unreacted nucleoside was removed by washing the cartridges with water (20 mL). The more lipophilic styrene oxide−nucleoside adducts were subsequently eluted with 100% MeOH (2 × 2 mL). The resulting MeOH fraction was evaporated to dryness and redissolved in MeOH/$H_2O$ (50:50, 1 mL). Aliquots (50 $\mu$L) were injected onto a 10 mm × 250 mm C-18 column (YMC-ODS-AQ) for purification. The peaks of interest were collected over a 20 min linear gradient from 40 to 99% MeOH in $H_2O$ with retention times of 11.7 min (α adduct) and 12.9 min ($\beta$ adduct) at a flow rate of 3.0 mL/min (combined yield of ∼2%). UV absorbance was monitored at 258 and 278 nm.

**N$^6$α(*S*)SO$^{15}$Ado:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.38 (s, 1H, adenine H8), 8.14 (s, 1H, adenine H2), 8.14 (d, adenine H2, $^2J_{NH}$ = 16 Hz), 8.08 (dd, 1H, $^{15}$NH, $^1J_{NH}$ = 92 Hz, $J_{HH}$ = 8.2 Hz, can be exchanged in $D_2O$), 7.41 (d, 2H, Ar-H, ortho, $J$ = 7.5 Hz), 7.28 (dd, 2H, Ar-H, meta, $J_1$ = 7.3 Hz, $J_2$ = 7.5 Hz), 7.19 (t, 1H, Ar-H, para, $J$ = 7.3 Hz), 5.86 (d, 1H, H1′, $J$ = 6.2 Hz), 5.42 (d, 1H, OH, $J$ = 6.0 Hz, can be exchanged in $D_2O$), 5.36 (m, 1H, α-CH), 5.16 (d, 1H, OH, $J$ = 4.6 Hz, can be exchanged in $D_2O$), 4.94 (t, 1H, OH, $J$ = 5.7 Hz, can be exchanged in $D_2O$), 4.61−4.57 (m, 1H, H2′), 4.14−4.11 (m, 1H, H3′), 3.94 (dd, 1H, H4′, $J_1$ = 6.5 Hz, $J_2$ = 3.2 Hz), 3.78−3.50 (m, 4H, $\beta$-$CH_2$, H5′, H5″). The NMR spectra established that the material was 30% rearranged and 70% un-rearranged: HRMS-FAB$^+$ *m/z* 389.1588, calcd for $C_{18}H_{22}{}^{14}N_4{}^{15}N_1O_5$ ($MH^+$) 389.1591. No N$^6$α(*R*) was detected.

**N$^6$$\beta$SO$^{15}$Ado:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.35 (s, 1H, adenine H8), 8.22 (d, 1H, adenine H2, $J$ = 16.5 Hz), 7.63 (s, br, 1H, $^{14}$NH, can be exchanged in $D_2O$), 7.36−7.21 (m, 5H, Ar-H), 5.87 (d, 1H, H1′, $J$ = 6.1 Hz), 5.58 (br, 1H, OH, can be exchanged in $D_2O$), 5.44 (d, 1H, OH, can be exchanged in $D_2O$, $J$ = 6.2 Hz), 5.37 (dd, 1H, OH, can be exchanged in $D_2O$, $J_1$ = 7.0 Hz, $J_2$ = 2.4 Hz), 5.18 (d, 1H, OH, $J$ = 4.7 Hz, can be exchanged in $D_2O$), 4.88 (br, 1H, α-CH), 4.62−4.59 (m, 1H, H2′), 4.15−4.08 (m, 1H, H3′), 3.95 (dd, 1H, H4′, $J_1$ = 6.6 Hz, $J_2$ = 3.2 Hz), 3.69−3.53 (m, 4H, $\beta$-$CH_2$, H5′, H5″). The NMR spectrum established that the material was 100% rearranged: HRMS-FAB$^+$ calcd for $C_{18}H_{22}{}^{14}N_4{}^{15}N_1O_5$, 389.1591 ($MH^+$), found 389.1598.

**Synthesis of the [$^{15}$N$^6$]dAdo 24-mer d(CG*A*TTA*A*TAT-*A*GCTATATTA*A*TCG-3′).** [$^{15}$N$^6$]-2′-Deoxyadenosine was converted to the 5′-*O*-(dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino)-(2-cyanoethyl)phosphinyl derivative by literature procedures (*12*−*15*). The phosphoramidite was used to prepare a self-complementary 24-mer with all adenines labeled at the N$^6$ position. The terminal DMT group was left on, and after removal of the protecting groups by treatment with concentrated $NH_4$-OH (8 h, 60 °C), the oligomer was purified using Nensorb (DuPont) cartridges according to the manufacturer's directions. A portion of the Nensorb-purified material was subjected to additional PAGE purification. Similar results were obtained from both samples.

To the $^{15}$N$^6$-labeled 24-mer (50 $A_{260}$ units) dissolved in 1 mL of 0.05 M potassium phosphate buffer (pH 7.00) was added (*R*)-styrene oxide (3.5 $\mu$L) at 24 h intervals for 30 days. After removal of most of the unreacted SO by ether extraction (5 × 1 mL), the remainder was removed by HPLC (YMC-ODS-AQ C-18 column; gradient, (A) 0.1 M ammonium formate and (B) $CH_3$-CN, from 10 to 90% B over the course of 10 min). All peaks were collected and combined except the last one which was unreacted epoxide. The solvents were removed by centrifugal evaporation in vacuo followed by lyophilization. The dry oligonucleotide mixture was dissolved in 50 mM $Na_2CO_3$ (500 $\mu$L) and heated for 10 h at 55 °C to ensure complete Dimroth rearrangement of N1 adducts. The pH of the reaction was adjusted to pH 8, and the solution was lyophilized. The dry material was enzymatically hydrolyzed [500 $\mu$L of 10 mM $MgCl_2$, 1 unit of snake venom phosphodiesterase (Sigma, P-7027), and 10 units of alkaline phosphatase (Sigma, P-4252), 12 h, 37 °C]. The N$^6$ dAdo adducts [(*S*)-N$^6$-α, (*R*)-N$^6$-α, and (*R*)-N$^6$-$\beta$] were collected and analyzed by $^1$H NMR as before.

**Reactions of PAH Dihydrodiol Epoxides with [$^{15}$N$^6$]-2′-Deoxyadenosine. (1) (+)-3$\beta$,4α-Dihydroxy-1$\beta$,2$\beta$-epoxy-1,2,3,4-tetrahydronaphthalene (NADE).** Racemic *anti*-NADE was synthesized from 1,2-dihydronaphthalene (*16*, *17*). [$^{15}$N$^6$]-2′-Deoxyadenosine (40 mg, 0.16 mmol) was dissolved in 4 mL of 50 mM Tris-HCl (pH 7.2) to give a homogeneous solution at 40 °C. Racemic NADE (20 mg/50 $\mu$L of DMSO, 0.12 mmol) was added. The mixture was stirred for 5 days at 40 °C. The reaction mixture was extracted with water-saturated EtOAc (3 × 2 mL), followed by ethyl ether (2 × 2 mL) to remove diol epoxide hydrolysis products. The aqueous solution was lyophilized to dryness and redissolved in 1 mL of MeOH/$H_2O$ (50:50) for HPLC purification. Aliquots (50 $\mu$L) were injected onto a 10 mm × 250 mm C-18 column (Kromasil, Higgins Analytical, Inc.). The (1*R*)- and (1*S*)-*anti-trans* diastereomers (retention times of 9.0 and 10.7 min, respectively) were collected (combined yield of ∼2%) using a 35 min linear gradient from 3 to 80% MeOH in $H_2O$. UV absorbance was monitored at 258 and 268 nm.

**(1α,2$\beta$,3$\beta$,4α)-2′-Deoxy-[$^{15}$N$^6$](1,2,3,4-tetrahydro-2,3,4-trihydroxynaphthalen-1-yl)adenosine [(+)- and (−)-*anti-trans*].** The $^1$H NMR spectra of the diastereomers were indistinguishable: $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.34 (s, 1H, H8), 8.23 (s, br, 1H, adenine H2), 7.72 (d, 1H, $^3J_{HH}$ = 9.0 Hz, $^{14}$NH, dd, 1H, $^1J_{NH}$ = 93 Hz, $^3J_{HH}$ = 9.0 Hz, $^{15}$NH, can be exchanged in $D_2O$), 7.34 (d, 1H, H8, $J$ = 7.5 Hz), 7.21 (dd, 1H, H7, $J_1$ = 8.0 Hz, $J_2$ = 7.5 Hz), 7.14 (dd, 1H, H6, $J_1$ = 6.5 Hz, $J_2$ = 8.0 Hz), 7.09 (br, 1H, H5), 6.36 (t, 1H, H1′, $J$ = 7.1 Hz), 5.64 (br, 1H, H4), 4.50 (t, 1H, H1), 4.41 (br, 1H, H3′), 4.17 (br, 1H, H3), 3.96 (br, 1H, H2), 3.88 (br, 1H, H4′), 3.62−3.57 (m, 2H, H5′, H5″), 2.75 (m, 1H, H2″), 2.27 (m, 1H, H2′). The NMR spectrum established that the material was 50% rearranged and 50% un-rearranged: HRMS-FAB$^+$ *m/z* 431.1702, calcd for $C_{20}H_{24}{}^{14}N_4{}^{15}N_1O_6$ 431.1697 ($MH^+$). The NMR spectra matched those reported by Kim et al. (*18*) for adducts prepared by an independent pathway.

**(2) (+)-8(*R*),9(*S*)-Dihydroxy-10(*S*),11(*R*)-epoxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (nBADE).** Non-bay region enantiopure (+)-*anti*-nBADE was prepared by Jacobsen oxidation (*19*, *20*).[2] [$^{15}$N$^6$]-2′-Deoxyadenosine (10 mg, 0.04 mmol) was dissolved in 1 mL of 50 mM Tris-HCl (pH 7.2) to give a homogeneous solution at 42 °C. Enantiopure epoxide (20 $\mu$L of a 10 mg/mL solution of epoxide in DMSO) was added to the solution, which was stirred at 42 °C for 72 h. The reaction mixture was extracted with EtOAc (3 × 1 mL), followed by $Et_2O$ (2 × 1 mL) to remove hydrolysis products. The aqueous solution was then loaded onto C-18 Sep-Pak cartridges (Waters), and most of the unreacted nucleoside was removed by washing with $H_2O$ (20 mL). The more lipophilic nBADE−nucleoside adducts were subsequently eluted with 100% MeOH (2 × 2 mL). The resulting methanol fraction was evaporated to dryness and redissolved in MeOH/$H_2O$ (50:50). Aliquots (50 $\mu$L) were fractionated on a 10 mm × 250 mm C-18 column (YMC-ODS-AQ). Using a 30 min linear gradient from 40 to 90% MeOH in $H_2O$, the *anti-trans* adduct (retention time of 21.9 min) was collected (one-isomer yield of ∼1%). The following peak at 23.2 min was most likely the cis adduct, but there was not enough material to identify it by NMR. UV absorbance was monitored at 258 and 300 nm.

**(+)-(8*R*,9*S*,10*R*,11*S*)-2′-Deoxy-[$^{15}$N$^6$](8,9,10,11-tetrahydro-8,9,10-trihydroxybenz[*a*]anthracen-11-yl)adenosine [Non-Bay Region (+)-*anti-trans*]:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.53 (br, 1H, H8), 8.37 (s, 1H, adenine H2), 8.33 (br, 2H, H12, H1), 8.00 (s, 1H, H7), 7.96 (dd, 1H, $^{15}$NH, $^1J_{NH}$ = 92 Hz, $^3J_{HH}$ = 8.9 Hz, can be exchanged in $D_2O$), 7.93−7.91 (m, 1H, H2), 7.82 (dd, 2H, H5, H6, $J_1$ = $J_2$ = 8.9 Hz), 7.76 (d, 1H, H7, $J$ = 8.9 Hz), 7.58−7.55 (m, 2H, H3, H4), 6.40 (t, 1H, H1′, $J_1$ = 6.9 Hz, $J_2$ = 6.7 Hz), 5.88 (br, 1H, H11), 5.32 (d, 1H, OH, $J$ = 4.0 Hz, can be exchanged in $D_2O$), 5.23 (t, 1H, OH, $J$ = 5.7 Hz, can be exchanged in $D_2O$), 5.06 (d, 1H, OH, $J$ = 4.0 Hz, can be

[2] Synthesized by L. N. Nechev and S. Han via chiral epoxidations employing a Jacobsen catalyst.

exchanged in $D_2O$). Because of the very limited amount of sample, the upfield region of the spectrum was obscured by solvent peaks. However, the regions of interest in the spectrum showed that no rearrangement had occurred: HRMS-FAB$^+$ *m/z* 531.2018, calcd for $C_{28}H_{28}{}^{14}N_4{}^{15}N_1O_6$ ($MH^+$) 531.2010. The $^1H$ NMR spectrum was in agreement with that of the adducted nucleoside synthesized independently by McNees et al. (*21*). The non-biomimetic synthesis had been performed with racemic aminotriol, and the stereochemistry of the adducts had been assigned from CD spectra by analogy to other PAH adducts; the CD spectrum of the adduct prepared in this work from chiral nBADE confirmed the earlier assignments.

**(3) (±)-3*β*,4α-Dihydroxy-1*β*,2*β*-epoxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (BADE).** [$^{15}N^6$]-2′-Deoxyadenosine (20 mg, 0.08 mmol) was dissolved in 2 mL of 50 mM Tris-HCl (pH 7.2) to give a homogeneous solution at 42 °C. Racemic BADE (40 μL of 10 mg of epoxide/mL of DMSO) was added to the solution, which was stirred at 42 °C for 72 h. The reaction mixture was extracted three times with EtOAc (1 mL), and then twice with $Et_2O$ (1 mL) to remove hydrolysis products. The aqueous solution was loaded onto C-18 Sep-Pak cartridges, and most of the unreacted nucleoside was removed by washing with $H_2O$ (20 mL). The more lipophilic BA−nucleoside adducts were subsequently eluted with 100% MeOH (2 × 2 mL×). The resulting methanol fraction was evaporated to dryness and redissolved in MeOH/$H_2O$ (50:50). Aliquots (50 μL) were injected onto a 10 mm × 250 mm C-18 column (YMC-ODS-AQ) for purification. The peaks of interest [retention times of 22.0 and 22.7 min corresponding to (1*S*)- and (1*R*)-*anti-trans* isomers, respectively] were collected using a 30 min linear gradient from 40 to 90% MeOH in $H_2O$ (combined yield of ∼2%). UV absorbance was monitored at 258 and 300 nm. The $^1H$ NMR spectra were in accord with literature values (*21*, *22*).

**(±)-(1α,2*β*,3*β*,4α)-2′-Deoxy-[$^{15}N^6$](1,2,3,4-tetrahydro-2,3,4-trihydroxybenz[*a*]anthracen-1-yl)adenosine [Bay Region (+)- and (−)-*anti-trans*].** The $^1H$ NMR spectra of the diastereomers were indistinguishable: $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.58 (s, 1H, adenine H8), 8.53 (s, 1H, H12), 8.43 (s, 1H, H7), 8.28 (s, 1H, adenine H2), 8.03 (dd, 2H, H6, H8, $J_1 = 8.6$ Hz, $J_2 = 8.0$ Hz), 7.70 (d, 1H, H5, $J = 8.6$ Hz), 7.65 (dd, 1H, $^{15}$NH, $^1J_{NH} = 93$ Hz, $^3J_{HH} = 8.3$ Hz, can be exchanged in $D_2O$), 7.65−7.63 (m, 2H, H11), 7.46−7.36 (m, 2H, H9, H10), 6.37 (br, 1H, $J = 5.3$ Hz, H1′), 6.29 (m, 1H, H1), 5.31 (d, 1H, OH, $J = 4.0$ Hz, can be exchanged in $D_2O$), 5.26 (br, 1H, H4), 5.20 (d, 1H, H2, $J = 8.3$ Hz), 5.14 (t, br, 1H, H3′), 4.96 (d, 1H, OH, $J = 5.2$ Hz, can be exchanged in $D_2O$), 4.67 (t, 1H, OH, $J = 8.3$ Hz, can be exchanged in $D_2O$), 4.40 (br, 1H, H3), 4.14 (m, 1H, H4′), 3.62−3.50 (m, 2H, H5′, H5″), 2.75 (m, 1H, H2″), 2.40 (m, 1H, H2′); HRMS-FAB$^+$ *m/z* 531.2002, calcd for $C_{28}H_{28}{}^{14}N_4{}^{15}N_1O_6$ ($MH^+$) 531.2001.

**(4) (+)-7(*R*),8(*S*)-Dihydroxy-9(*S*),10(*R*)-epoxy-7,8,9,10-tetrahydrobenz[*a*]pyrene (BPDE).** [$^{15}N^6$]-2′-Deoxyadenosine (10 mg, 0.04 mmol) was dissolved in 1 mL of 50 mM Tris-HCl (pH 7.2) at 42 °C to give a homogeneous solution. To this was added enantiopure (+)-*anti*-BPDE (20 μL of a solution of 1 mg of epoxide/100 μL of DMSO), itself prepared by Jacobsen oxidation (*19*, *20*), and the mixture was stirred at 42 °C for 72 h. The reaction mixture was extracted three times with ethyl acetate (1 mL), and then twice with $Et_2O$ (1 mL) to remove hydrolysis products. The aqueous solutions were then loaded onto C-18 Sep-Pak cartridges, and most of the unreacted nucleoside was removed by washing with $H_2O$ (20 mL). The more lipophilic BPDE−nucleoside adducts were subsequently eluted with 100% methanol (2 × 2 mL). The resulting MeOH fraction was evaporated to dryness and redissolved in 1 mL of MeOH/$H_2O$ (50:50). Aliquots (50 μL) were injected onto a 10 mm × 250 mm C-18 column (YMC-ODS-AQ) for purification. The peaks of interest, with retention times of 24.2 min (*anti-trans*) and 25.5 min (*anti-cis*), were collected over a 35 min linear gradient from 40 to 100% MeOH in $H_2O$ (combined yield of ∼3%). UV absorbance was monitored at 258 and 333 nm. Structure (cis or trans) and stereochemistry were confirmed by comparison with literature data (*18*, *23*, *24*).

**(7*R*,8*S*,9*R*,10*S*)- and (7*R*,8*S*,9*R*,10*R*)-2′-Deoxy-[$^{15}N^6$](7,8,9,-10-tetrahydro-7,8,9-trihydroxybenzo[*a*]pyren-10-yl)adenosine [(+)-*anti-trans* and (+)-*anti-cis*]. (10*S*)-*anti-trans*:** $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.51 (d, 1H, H11, $J = 8.7$ Hz), 8.50 (s, 1H, adenine H8), 8.29 (s, 1H, adenine H2), 8.25 (d, 1H, H1, $J = 7.5$ Hz), 8.19 (d, 1H, H4, $J = 3.2$ Hz), 8.17 (s, 1H, H6), 8.14−8.08 (m, 3H, H5, H2, H3), 8.10 (dd, 1H, $^{15}$NH, $^1J_{NH} = 95$ Hz, $^3J_{HH} = 7.5$ Hz, can be exchanged in $D_2O$), 8.01 (t, 1H, H2, $J = 7.6$ Hz), 6.42−6.36 (m, 2H, H10, H1′), 5.25 (br, 1H, H7), 4.97 (d, 1H, H8), 4.40 (br, 1H, H3′), 4.24 (m, 2H, H8, H9), 4.02 (m, H4′), 3.54 (m, 2H, H5′, H5″), 2.87 (m, 2Hs, H2′, H2″). The spectrum indicated no rearrangement: HRMS-FAB$^+$ *m/z* 555.2009, calcd for $C_{30}H_{28}{}^{14}N_4{}^{15}N_1O_6$ ($MH^+$) 555.2010.

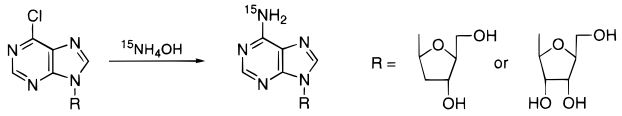
**(10*R*)-*anti-cis*:** $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.81 (d, 1H, H11, $J = 9.4$ Hz), 8.53 (s, 1H, adenine H8), 8.31 (s, 1H, adenine H2), 8.25 (d, 1H, H1, $J = 7.5$ Hz), 8.21 (s, 1H, H6), 8.19 (d, 1H, H3, $J = 4.5$ Hz), 8.15 (d, 2H, H4, H5, $J = 5.4$ Hz), 8.11 (d, 1H, H12, $J = 9.4$ Hz), 8.02 (t, 1H, H2, $J = 7.6$ Hz), 7.45 (dd, 1H, $^{15}$NH, $^1J_{NH} = 92$ Hz, $^3J_{HH} = 10$ Hz, can be exchanged in $D_2O$), 6.69−6.65 (m, 1H, H10), 6.34 (dd, 1H, H1′, $J_1 = 7.4$ Hz, $J_2 = 6.4$ Hz), 5.90 (d, 1H, OH, can be exchanged in $D_2O$), 5.72 (d, 1H, OH, $J = 5.2$ Hz, can be exchanged in $D_2O$), 5.29 (d, 1H, OH, $J = 3.8$ Hz, can be exchanged in $D_2O$), 5.18 (br, 1H, H7), 5.10 (dd, 1H, H9, $J_1 = 7.7$ Hz, $J_2 = 4.0$ Hz), 4.55 (m, 1H, H3′), 4.40 (br, 1H, H8), 3.88 (m, 1H, H4′), 3.61−3.49 (m, 2H, H5′, H5″), 2.74 (m, 1H, H2″), 2.26 (m, 1H, H2′). The spectrum indicated that no rearrangement had taken place: HRMS-FAB$^+$ *m/z* 555.1998, calcd for $C_{30}H_{28}{}^{14}N_4{}^{15}N_1O_6$ ($MH^+$) 555.2010.

**(5) (+)-3*β*,4α-Dihydroxy-1*β*,2*β*-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (BCDE).** $^{15}N^6$-labeled 2′-deoxyadenosine (10 mg, 0.04 mmol) was dissolved completely in 1 mL of 50 mM Tris-HCl (pH 7.2) at 42 °C. An acetone solution of racemic *anti*-BCDE (100 μL of a 1 mg/mL solution) was added to the 2′-deoxyadenosine solution. The reaction mixture was stirred at 42 °C for 72 h. The reaction mixture was extracted twice with EtOAc and $Et_2O$ to remove hydrolysis products. The aqueous solution was loaded onto a C-18 cartridge and treated with the same method described previously. The resulting methanol fractions were evaporated to dryness and redissolved in MeOH/$H_2O$ (50:50). Aliquots (50 μL) were injected onto a YMC-ODS-AQ HPLC column (10 mm × 250 mm). The peaks of interest were collected over a 15 min linear gradient from 50 to 100% MeOH in $H_2O$ (combined yield of ∼2%). UV absorbance was monitored at 250 and 303 nm. The two adducts had retention times of 11.2 and 11.6 min. Peaks were pooled from multiple HPLC runs. These adducts were subjected to NMR, CD, and MS analyses. The structural and stereochemical assignments were confirmed by comparison with literature data (*18*, *25*, *26*).

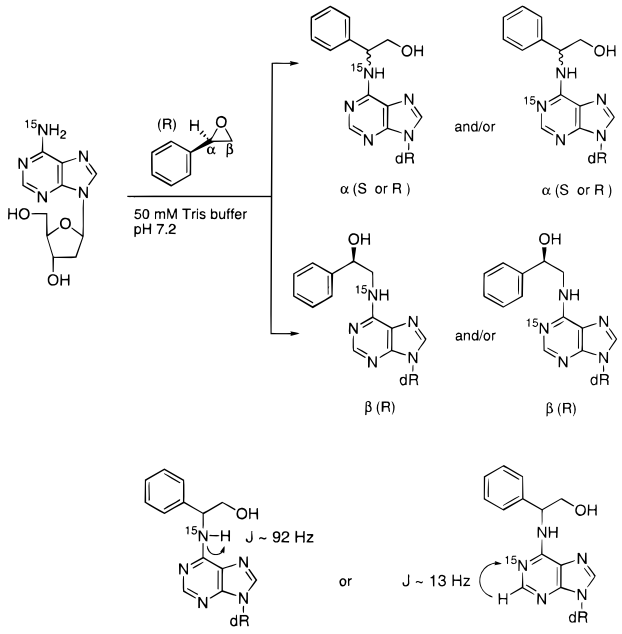
**(±)-(1α,2*β*,3*β*,4α)-2′-Deoxy-[$^{15}N^6$](1,2,3,4-tetrahydro-2,3,4-trihydroxybenzo[*c*]phenanthren-1-yl)adenosine [(+)- and (−)-*anti-trans*]. (1*S*)-*anti-trans*:** $^1H$ NMR (400 MHz, DMSO-$d_6$ with $D_2O$) $\delta$ 8.61 (d, 1H, H12, $J = 8.8$ Hz), 8.53 (s, 1H, adenine H8), 8.35 (s, 1H, adenine H2), 8.01 (d, 1H, H5, $J = 8.3$ Hz), 7.96 (dd, 1H, $^{15}$NH, $^1J_{NH} \sim 93$ Hz, $^3J_{HH} = 9$ Hz, can be exchanged in $D_2O$; calcd from 2D COSY data), 7.94 (d, 1H, H6, $J = 8.3$ Hz), 7.86 (d, 1H, H9, $J = 8.3$ Hz), 7.81 (t, 2H, H7, H8, $J = 5.0$ Hz), 7.49 (t, 1H, H10, $J_1 = J_2 = 8.0$ Hz), 7.12 (t, 1H, H11, $J = 8.8$ Hz), 6.39 (dd, 1H, H1′, $J_1 = 9.2$ Hz, $J_2 = 8.5$ Hz), 6.26 (d, 1H, H1, $J = 4.3$ Hz), 4.60 (d, 1H, H4, $J = 7.5$ Hz), 4.42 (m, 1H, H3′), 4.35 (m, 1H, H2), 4.15 (dd, br, 1H, H3, $J = 2.5$ Hz), 3.89−3.88 (m, 1H, H4′), 3.70−3.55 (m, 2H, H5′, H5″), 2.76 (m, 1H, H2″), 2.30 (m, 1H, H2′). The NMR spectra established that the material was ∼7% rearranged from N1 to $N^6$ (calcd from 2D COSY data): HRMS-FAB$^+$ *m/z* 531.2005, calcd for $C_{28}H_{28}{}^{14}N_4{}^{15}N_1O_6$ ($MH^+$) 531.2010.

**(1*R*)-*anti-trans*:** $^1H$ NMR (400 MHz, DMSO-$d_6$ with $D_2O$) $\delta$ 8.61 (d, 1H, H12, $J = 8.8$ Hz), 8.53 (s, 1H, adenine H8), 8.35 (s, 1H, adenine H2), 8.01 (d, 1H, H5, $J = 8.3$ Hz), 7.96 (dd, 1H, $^{15}$NH, $^1J_{NH} \sim 93$ Hz, $^3J_{HH} = 9$ Hz, can be exchanged in $D_2O$; calcd from 2D COSY data), 7.94 (d, 1H, H6, $J = 8.3$ Hz), 7.86

**Scheme 3. Synthesis of [$^{15}N^6$]Ado and dAdo**



**Scheme 4. Reaction of [$^{15}N^6$]dAdo with (*R*)-Styrene Oxide, with Predicted $^{15}N-^1H$ Coupling Constants**



(d, 1H, H9, $J$ = 8.3 Hz), 7.81 (dd, 2H, H7, H8, $J_1 = J_2$ = 5.0 Hz), 7.49 (dd, 1H, H10, $J_1 = J_2$ = 8.0 Hz), 7.12 (t, 1H, H11, $J$ = 8.8 Hz), 6.39 (dd, 1H, H1′, $J_1$ = 9.2 Hz, $J_2$ = 8.5 Hz), 6.26 (d, 1H, H1, $J$ = 4.3 Hz), 4.60 (d, 1H, H4, $J$ = 7.5 Hz), 4.42 (m, 1H, H3′), 4.35 (m, 1H, H2), 4.15 (dd, br, 1H, H3, $J$ = 2.5 Hz), 3.89−3.88 (m, 1H, H4′), 3.70−3.55 (m, 2H, H5′, H5″), 2.76 (m, 1H, H2″), 2.30 (m, 1H, H2′). The NMR spectra established that the material was ∼7% rearranged from N1 to $N^6$ (calcd from 2D COSY data): HRMS-FAB$^+$ *m/z* 531.2010, calcd for $C_{28}H_{28}{}^{14}N_4{}^{15}N_1O_6$ ($MH^+$) 531.2010.

## Results

The reactions of deoxyadenosine with epoxides were initially carried out with unlabeled nucleoside to establish chromatography conditions and to obtain standards for comparison with the labeled products. The structures of these standards were established by NMR and fast atom bombardment mass spectrometry (FAB-MS). Standards were also prepared independently by reaction of 6-chloro- or 6-fluoropurine 2′-deoxyribonucleoside or ribonucleoside (in the case of the styrene oxide reaction) with the appropriate amino alcohols (*18*, *27*).

**[$^{15}N^6$]Deoxyadenosine and [$^{15}N^6$]Adenosine.** The [$^{15}N^6$]deoxyadenosine and [$^{15}N^6$]adenosine required for these studies were prepared by treatment of the corresponding 6-chloronucleoside with $^{15}NH_4OH$ (Scheme 3). The NMR data for the products were consistent with those reported by Gao and Jones (*28*); the $^{15}N^6-H$ coupling constant was 91 Hz. Integration of the $^1H$ NMR spectrum showed less than 1% contamination with $^{14}N^6$; $^{15}N$ NMR showed that the label was exclusively at $N^6$.

**Reaction of Styrene Oxide with [$^{15}N^6$]Deoxyadenosine and [$^{15}N^6$]Adenosine.** The initial reaction of [$^{15}N^6$]deoxyadenosine with (*R*)-styrene oxide was carried out in 0.05 M Tris-HCl at pH 7.2 (Scheme 4). HPLC

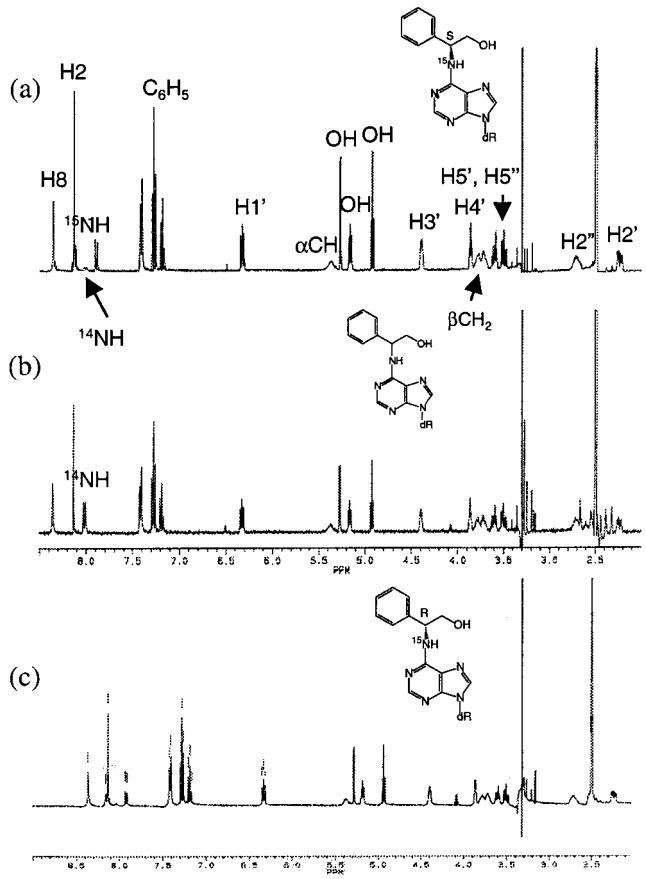



**Figure 1.** 400 MHz $^1H$ NMR spectra (DMSO-$d_6$, 25 °C) of (a) $N^6\alpha$(*S*)-SOd$^{15}$Ado, (b) $N^6\alpha$(*S*)-SOd$^{14}$Ado, and (c) $N^6\alpha$(*R*)-SOd$^{15}$-Ado adducts.

gave three major components, the structures of which were established by comparison with unlabeled standards. Two of them were identified as the $N^6\alpha$ (*R*) and (*S*) adducts. CD spectra were used to confirm the configuration of the α adducts (*11*). The third product was an $N^6\beta$ adduct. The (*R*) and (*S*) $\beta$ diastereomers had identical HPLC retention times; however, it was assumed that the product had the (*R*) configuration since opening of the epoxide at the $\beta$ position does not affect the configuration of the α position. The order of elution from a reversed-phase HPLC column was α(*S*), α(*R*), and $\beta$; the products were found in a ratio of 7:1:8. The reaction conditions were chosen such that all N1 products would be converted to $N^6$, and as a consequence, no N1 products were detected. The N1 adducts can also undergo deamination (*11*, *29*); however, little (<5%) deaminated product was seen.

The $^1H$ NMR spectrum of the $N^6\alpha$(*S*) adduct (Figure 1a) formed from the $^{15}N^6$-labeled deoxyadenosine showed that the product arose from both direct reaction at $N^6$ and reaction at N1 followed by rearrangement. The doublet (8.5 Hz, vicinal coupling to the benzylic proton) for the $N^6$ proton signal seen in the unlabeled sample (Figure 1b) became a doublet of doublets in the isotopically labeled sample with a large coupling constant (∼92 Hz), due to coupling between the proton and $^{15}N$, superimposed on the smaller doublet. There was still a significant $^{14}NH$ doublet centered under the doublet of doublets. This result implies that the product was formed by two pathways. The majority of the material arose by direct reaction at $N^6$ and the remainder by reaction at

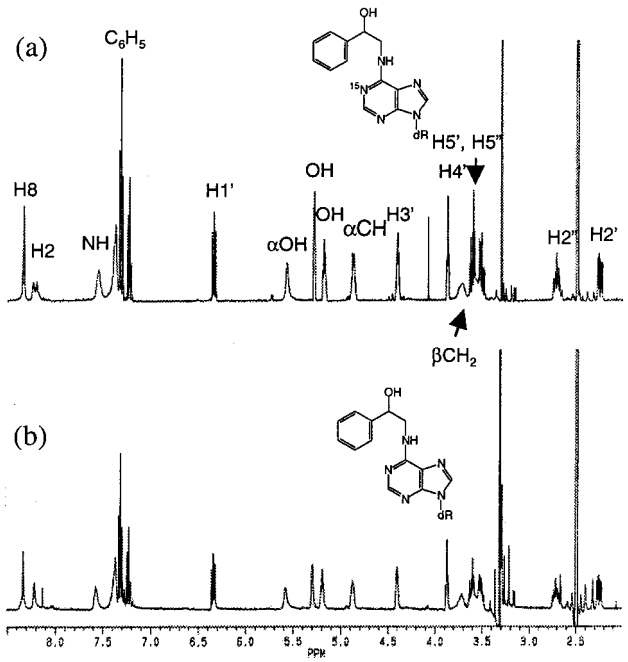



**Figure 2.** 400 MHz $^1H$ NMR spectra (DMSO-$d_6$, 25 °C) of (a) N$^6\beta$SOd$^{15}$Ado and (b) N$^6\beta$SOd$^{14}$Ado adducts.

N1 followed by Dimroth rearrangement. The ratio of the un-rearranged to rearranged products was 4:1. The NMR spectrum of the N$^6\alpha$(*R*) adduct showed that it arose almost entirely by direct reaction at N$^6$ (Figure 1c); the small amount of this isomer that was obtained did not permit an accurate determination of the $^{15}N$ content at N1.

The NMR spectrum of the N$^6\beta$ adduct revealed that it arose entirely by rearrangement (Figure 2a). The proton at C2 of adenine, which is a singlet in the adduct of unlabeled deoxyadenosine (Figure 2b), became a doublet in the labeled material with a $^2J_{NH}$ coupling constant of 16.4 Hz, indicating that because of rearrangement, the labeled nitrogen had moved to the N1 position. In the NMR spectrum, the peaks were broad at room temperature but the lines could be sharpened by raising the temperature to 52 °C such that the doublet could be clearly resolved. The line broadening at lower temperatures is presumed to be the result of slow equilibration among conformers.

Thus, under the conditions of this initial experiment, the N$^6\beta$ product arose exclusively by reaction at N1 followed by rearrangement whereas the N$^6\alpha$ adduct was formed by a combination of N1 and N$^6$ reaction. More than 80% of the α product had arisen by direct reaction at N$^6$. Loss of configurational integrity occurred during formation of the α product. The α product had mainly the inverted configuration, i.e., (*S*); however, 12% of it had been formed with retention.

One possible explanation for partial racemization involves reaction of the epoxide with chloride ion. Formation of an α-chlorohydrin by chloride ion attack on the epoxide such as that reported by Meehan et al. (*30*) with the diol epoxide of benzo[*a*]pyrene would occur with inversion of configuration. A second inversion during a subsequent reaction with the nucleoside would give a net retention of configuration (*30*). As a test of this possibility, the adduction reaction was carried out in the absence of chloride ion using 0.05 M potassium phosphate at pH 7.0 and 25 °C. The reaction still gave net inversion with partial racemization. The mixture of products was very similar to that obtained in the presence of chloride ion. We conclude that 0.05 M chloride ion is insufficient for the chlorohydrin mechanism to play a significant role in the reaction of styrene oxide with deoxyadenosine.

A third reaction was carried out in water without the addition of any buffers or salts. For this reaction, [$^{15}N^6$]-adenosine was used rather than the labeled 2′-deoxyadenosine. The reaction at 40 °C showed no loss of configurational integrity and gave only N$^6\alpha$(*S*) and N$^6\beta$ adducts. The N$^6\alpha$(*S*) adduct was formed in a 70:30 ratio by direct reaction at N$^6$ and rearrangement from N1; the N$^6\beta$ adduct was formed entirely by rearrangement from N1. A small amount, less than 5%, of an α adduct on the N1 position of 2′-deoxyinosine was also found. The (*R*)-styrene oxide adduction reactions of $^{15}N^6$-labeled adenosine and deoxyadenosine are summarized in Table 1.

It is likely that the only noteworthy difference between deoxyadenosine and adenosine is the better solubility of the latter in water. However, there are two significant differences between the adenosine experiment and the previous ones. First, the lack of salts in the adenosine reaction mixture decreased the ability of the medium to support cations involved in $S_N1$ type mechanisms. More subtly, the absence of buffer also means the pH is uncontrolled. Consequently, the reaction mechanism may be shifting toward $S_N2$ type processes.

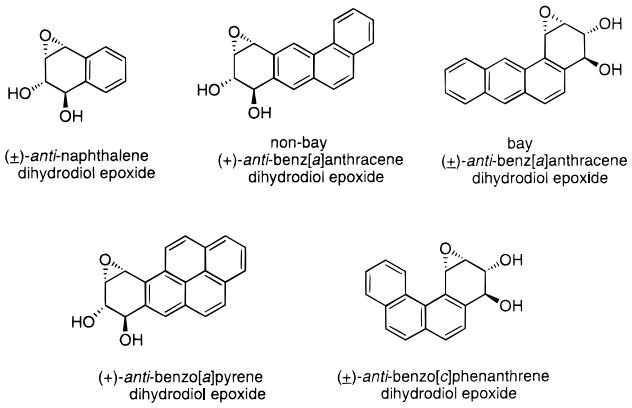
**Reaction of (*R*)-Styrene Oxide with the [$^{15}N^6$]-dAdo 24-mer d(CGATTAATATAGCTATATTAATCG-3′).** A self-complementary 24-mer containing nine $^{15}N^6$-labeled adenines was used to examine the reaction of styrene oxide with deoxyadenosine in duplexed DNA. The oligonucleotide was reacted with (*R*)-styrene oxide at pH 7.0 and 25 °C (well below the $T_M$ of this sequence which was 44 °C) for 30 days, treated with base at 55 °C to effect Dimroth rearrangement of N1 products, and enzymatically hydrolyzed to nucleosides. The deoxyadenosine adducts of styrene oxide were isolated and analyzed by NMR as before. The results, shown in Table 1, are very similar to those found for the nucleosides in terms of relative yields of adducts and the extent of rearrangement, indicating that the existence of duplex structure in which the N1 and N$^6$ of adenine are involved in hydrogen bonding does not significantly change the course of the reaction or the relative reactivity of the two nitrogens. These results support those of Barlow et al. (*11*), who studied the reaction of styrene oxide with DNA containing $^3H$-labeled deoxyadenosine. They found the level of adducts in native DNA decreased 5-fold relative to that in denatured DNA but the ratio of α to β adducts was relatively unchanged. They observed somewhat increased levels of the deaminated β N1-styrene oxide product in the native DNA sample.

**Reaction of [$^{15}N^6$]Deoxyadenosine with PAH Dihydrodiol Epoxides.** The PAH dihydrodiol epoxides with which mechanisms were explored are shown in Scheme 5. Reactions of the epoxides with deoxyadenosine were carried out under conditions previously employed by Canella et al. (*24*), except that 2′-deoxyadenosine was used instead of the 3′-phosphate (Scheme 6). The buffer was 50 mM Tris-HCl (pH 7.2). The nucleoside was stirred in the buffer until the solution became homogeneous before adding the epoxide in a small amount of DMSO solution. After the reaction was complete, the products were separated by HPLC, and NMR spectra were used to identify them and to probe the extent of rearrange-

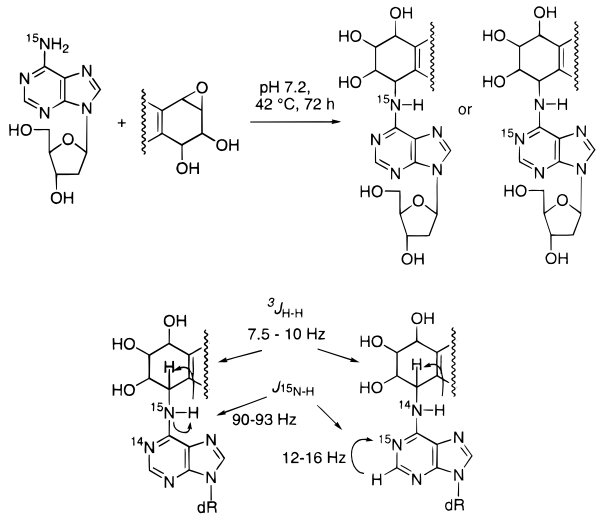
**Table 1. Reaction of [$^{15}N^6$]Adenine Nucleosides and Oligonucleotide with (*R*)-Styrene Oxide**

| nucleoside | reaction conditions | isolated $^{15}N^6$ adduct % rearranged (relative yield) α-(*S*) | α-(R) | *β* |
|---|---|---|---|---|
| Ado | $H_2O$, 40 °C, 72 h | 30 (40−45) | ∼0 | 100 (55−60) |
| 2′-dAdo | 0.05 M Tris-HCl, pH 7.2, 40 °C, 72 h | 20 (44) | not determined (6) | 100 (50) |
| 2′-dAdo | 0.05 M potassium phosphate, pH 7.0, 25 °C, 96 h | 15 (49) | 10 (5) | 100 (46) |
| oligonucleotide d(CGATTAATATAGCTATATTAATCG) | 0.05 M potassium phosphate, pH 7.0, 25 °C, 30 days | 15 (44) | <10 (9) | 100 (47) |

**Scheme 5. PAH Diol Epoxides Used in Reactions with [$^{15}N^6$]dAdo**




**Scheme 6. Reaction of [$^{15}N^6$]dAdo with PAH Dihydrodiol Epoxides, with Predicted Coupling Constants**




ment. As in the studies with styrene oxide, the $^{15}N-^{1}H$ coupling constants were used to distinguish direct reaction at $N^6$ from Dimroth rearrangement of N1 adducts (Scheme 6). In the case of the PAH dihydrodiol epoxides, the major products arose in all cases from trans opening at the benzylic position of the epoxide; only in the case of BPDE were cis products isolated in sufficient quantity for NMR analysis. The structure and stereochemistry of products were confirmed by comparison with literature values and by comparison with samples prepared by independent synthesis.

**(±)-*anti*-Naphthalene Dihydrodiol Epoxide.** The reaction of racemic *anti*-NADE with [$^{15}N^6$]deoxyadenosine was carried out under the conditions described above. The reaction temperature was maintained at 40 °C for 72 h.

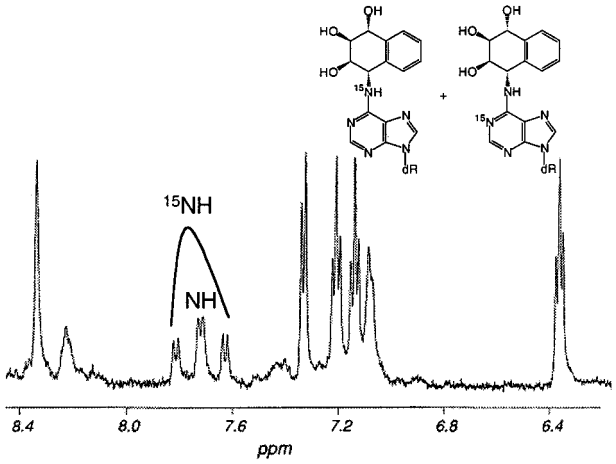



**Figure 3.** 500 MHz NMR spectrum (DMSO-$d_6$, 23 °C) of *anti-trans*-NADE-d$^{15}$Ado adduct.

The two major products, eluting from a reverse-phase HPLC column at 9.5 and 9.8 min, were the diastereomeric $N^6$(*S*) and -(*R*) trans adducts previously synthesized by Kim et al. (*18*). Minor components at 8.7 and 8.9 min were obtained in quantities insufficient to permit identification, but were probably cis adducts.

The diastereomers were collected, and the NMR spectra were analyzed in the manner described for the styrene oxide adducts (Figure 3). The $N^6$ proton signal was used to analyze the reaction. The narrowly spaced doublet for rearranged material was centered within the wide doublet of doublets (93.0 and 9.0 Hz) for material that had arisen by direct reaction at $N^6$. The proton at C2 of adenine should also be useful for analyzing the extent of rearrangement and should appear as a doublet (∼13 Hz) for rearranged and a singlet for un-rearranged material; however, the lines in this region of the spectrum were too broad to permit analysis. The analysis showed that 50% of the product arose by reaction at N1 followed by rearrangement and the remainder by direct reaction at $N^6$.

**Non-Bay Region (11*R*)-*anti*-Benz[*a*]anthracene Dihydrodiol Epoxide.** The 11*R* enantiomer of *anti*-nBADE was used.[2] A mixture of cis and trans adducts was formed in which the trans adduct predominated. The trans adduct, i.e., 8*R*,9*S*,10*R*,11*S*, eluted from the HPLC column at 22 min; the cis adduct, i.e., 8*R*,9*S*,10*R*,11*R*, eluted at 23.3 min. The identity of the trans adduct was confirmed by comparison with independently synthesized material (*21*). The cis adduct was obtained in quantities insufficient for NMR study. The cis and trans tetrols were obtained as byproducts; they eluted at 18 and 19 min, respectively.

Analysis of the NMR spectrum (Figure 4) of the trans adduct showed that essentially the entire reaction had

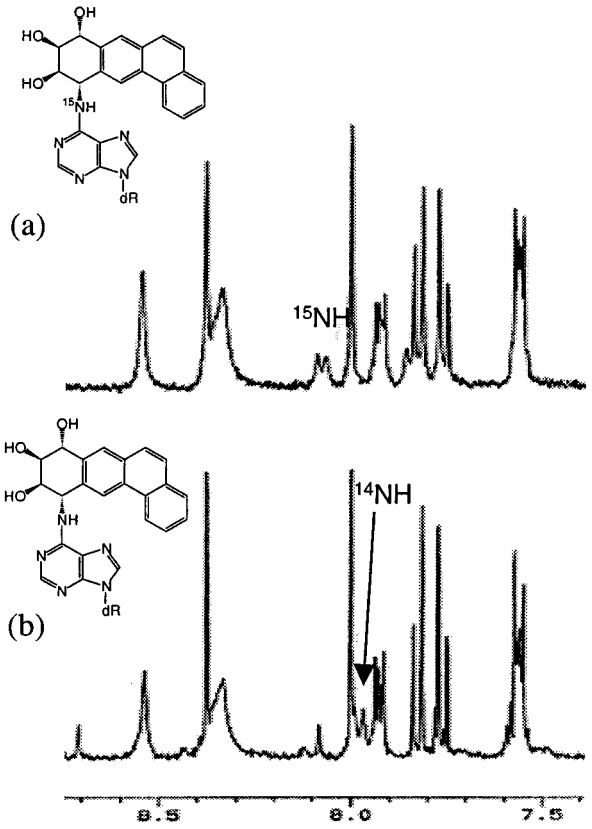



**Figure 4.** 400 MHz $^1$H NMR spectra (DMSO-$d_6$, 25 °C) of *anti-trans*-nBADE dAdo adduct (11*S*) with (a) $^{15}N^6$ and (b) $^{14}N^6$.

occurred directly at $N^6$; i.e., there was no evidence of any rearrangement. In the NMR spectrum of the unlabeled adduct (Figure 4b), the NH signal is seen as a doublet at 7.97 ppm with a vicinal coupling constant of ~8.5 Hz. The adduct from the reaction with [$^{15}N^6$]-2′-deoxyadenosine (Figure 4a) gave a doublet of doublets with a large coupling constant ($^1J_{NH}$ = 92 Hz) accompanied by a vicinal coupling constant (8.5 Hz). Any material arising via Dimroth rearrangement was below the level of detection (<5%). By the addition of two more benzene rings to the naphthalene dihydrodiol epoxide, the electron delocalization in the transition state with this softer electrophile favored direct attack at the $N^6$ position.

**Bay Region (±)-*anti*-Benz[*a*]anthracene Dihydrodiol Epoxide.** Racemic material was used for the study of the BADE reaction. Therefore, diastereomeric products were expected. Two major adducts were detected by HPLC between 21 and 22 min. The second-eluting product overlapped with a following peak which was probably epoxide. Nevertheless, the adduct could be purified by multiple HPLC fractionations. Analysis of the $^1$H NMR spectra of the two adducts showed the two products to be diastereomeric trans adducts (Figure 5). Moreover, the spectra showed they arose by direct adduction at $N^6$. The NH signal for the labeled sample is partially obscured by another signal (Figure 5b). A doublet at 7.89 ppm is half of the $^{15}$NH signal. The other half is under the signal for H11 of the hydrocarbon at 7.67 ppm. When the α-proton of the hydrocarbon (H1) was decoupled (Figure 5a), the doublet at 7.89 ppm became a singlet and the shape of the peak containing H11 and the other arm of the $^{15}$N doublet of doublets changed. The coupling constants were as follows: $^1J_{NH}$ = 93.2 Hz and $^3J_{HH}$ = 8.3 Hz. Comparison of the CD

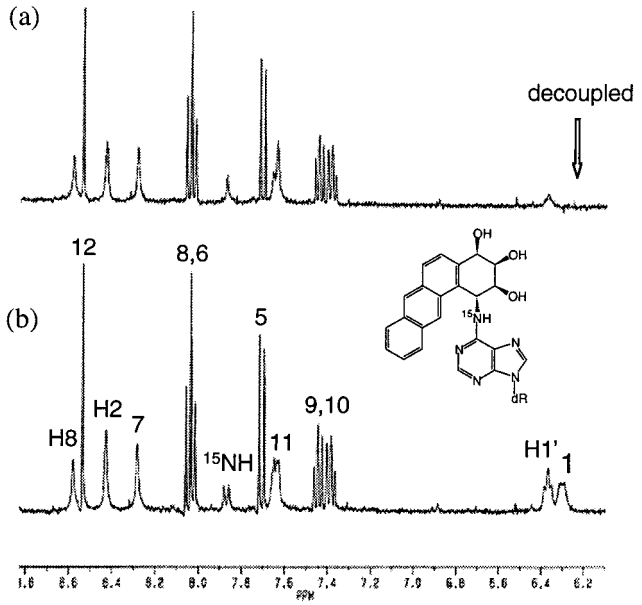



**Figure 5.** 400 MHz $^1$H NMR spectra (DMSO-$d_6$, 25 °C) of (a) *anti-trans*-BADE d$^{15}$Ado adduct (1*R*) with decoupling at 6.3 ppm (H1) and (b) the same adduct without decoupling at 6.3 ppm.

spectra with those of products prepared by Kim et al. (*18*) using an independent method and with literature values (*22*) permitted the first component to be assigned as 1*S* and the second as 1*R*.

In summary, neither the bay region nor the non-bay region benz[*a*]anthracene dihydrodiol epoxide reacted significantly at the N1 position of deoxyadenosine.

**(10*R*)-*anti*-Benzo[*a*]pyrene Dihydrodiol Epoxide.** The reaction of racemic *anti*-BPDE with 2′-deoxyadenosine gave four products, resulting from the trans and cis opening of the enantiomeric *anti*-epoxides at C10. These diastereomers eluted close to one another, which made them difficult to purify by HPLC. The availability of enantiopure (10R)-*anti*-BPDE provided a solution to the problem (*20*). Optically active BPDE was reacted with [$^{15}N^6$]-2′-deoxyadenosine to give two products, the trans (10*S*) and cis (10*R*) forms, in a ratio of 1:1.3. The peak that eluted first on HPLC was the *anti-trans* adduct (10*S*), and the second was the *anti-cis* adduct (10*R*). The cis product was formed to a significantly greater extent than had been seen with the three previous dihydrodiol epoxides. The reaction was carried out in Tris-HCl buffer, and this result is in accordance with Meehan's previous observation (*30*) that chloride ion can react with BPDE to give a chlorohydrin. The reaction competes with direct attack on the nucleoside. The chlorohydrin can in turn alkylate the nucleoside. If both steps involve inversion, the resulting adduct will be cis instead of trans. As discussed previously, the chloride ion effect was not very significant in the case of the styrene oxide reaction. The assignments were made by comparison with materials synthesized by Kim et al. by reaction of the (±)-*anti-trans*-BP aminotriols with 6-fluoropurine deoxyriboside (*18*, *27*) and with other literature values (*23*, *24*). The relative HPLC elution order is consistent with those reported by Harvey and co-workers (*31*).

The trans and cis isomers exhibited distinctly different NMR spectra that are shown in Figures 6 and 7. The NMR spectra of the $^{15}$N-labeled trans isomer (Figure 6b) showed that reaction had occurred directly at $N^6$. The NH signal for the unlabeled trans adduct (Figure 6c)

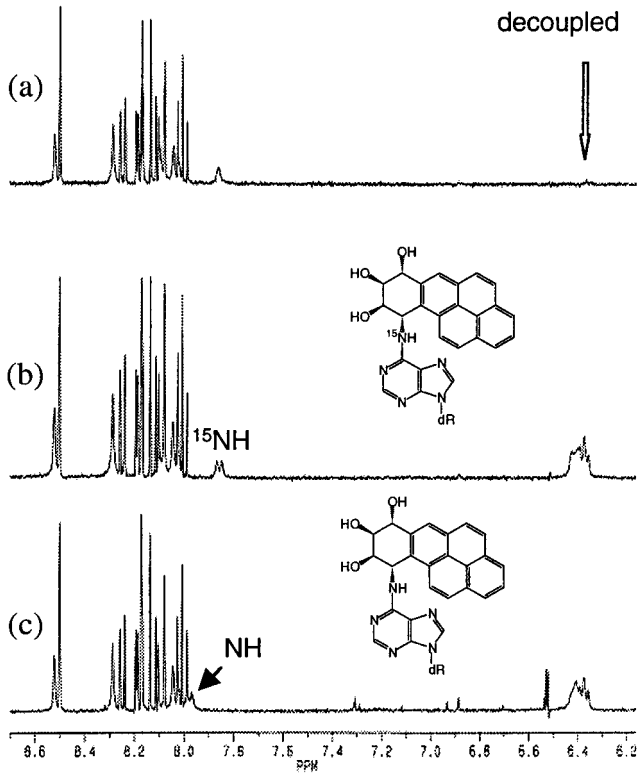



**Figure 6.** 400 MHz $^1$H NMR spectra (DMSO-$d_6$, 25 °C) of the *anti-trans*-BPDE dAdo adduct (10*S*): (a) from the [$^{15}$N$^6$]dAdo spectrum acquired with decoupling at 6.4 ppm (H10), (b) the same adduct without decoupling at 6.4 ppm, and (c) the adduct derived from [$^{14}$N$^6$]dAdo.

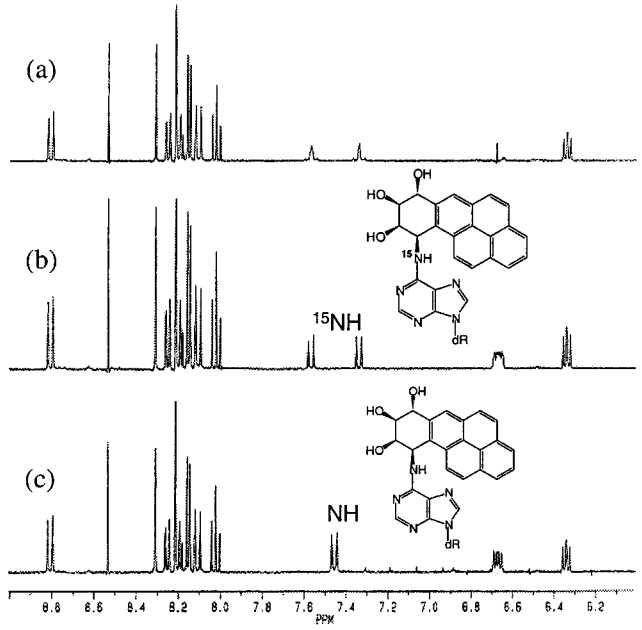



**Figure 7.** 400 MHz $^1$H NMR spectra (DMSO-$d_6$, 25 °C) of the *anti-cis*-BPDE dAdo adduct (10*R*): (a) adduct from [$^{15}$N$^6$]dAdo, where the spectrum was acquired with decoupling at 6.7 ppm (H10), (b) the same adduct without decoupling at 6.7 ppm, and (c) the adduct derived from [$^{14}$N$^6$]dAdo.

appeared as a doublet lying at 7.98 ppm. The NH signal of the product from the reaction with $^{15}$N-labeled 2′-deoxyadenosine appeared as a doublet of doublets (95.4 and 7.5 Hz) which collapsed to a doublet upon irradiation at 6.4 ppm (H10). No N1 label was detected.

The NH signal of the cis adduct was sharper than that of the trans adduct and was 0.5 ppm further upfield (Figure 7). The doublet of the NH in the unlabeled sample (Figure 7c) became a doublet of doublets (91.6 and 10.0 Hz) in the labeled material (Figure 7b) which collapsed to a doublet when the peak at 6.68 ppm (H10) was irradiated.

As seen with the benz[*a*]anthracene adducts, additional benzene rings give more stabilization of charge in the transition state to yield N$^6$ adducts directly.

**(±)-*anti*-Benzo[*c*]phenanthrene Dihydrodiol Epoxide.** Commercially available racemic BCDE was used for this experiment. Two major products, the diastereomeric *anti-trans* adducts, were obtained with retention times of 11.4 and 11.8 min using a gradient of 50 to 100% methanol in water over the course of 15 min. Using a different gradient, 36 to 90% methanol over the course of 30 min, two minor peaks eluting earlier than the trans adducts were also observed. These were provisionally assigned as the cis adducts on the basis of comparison of UV spectra with those reported by Dipple (*32*). The structure of the trans adducts was confirmed by comparison with literature values (*18*, *25*), and the stereochemical assignments were confirmed by CD spectroscopy; the 1*S* isomer eluted before the 1*R*.

An interesting observation was made with the BCDE reaction. The NMR spectra revealed that a small amount of rearrangement (5−7%) had occurred. To quantify this observation, many different NMR and MS techniques were examined. In the one-dimensional $^1$H NMR spectrum, the $^{15}$NH peaks fell on top of the benzo[*c*]phenanthrene aromatic protons. Therefore, they could not be used to quantitate the NH peaks. For $^{15}$N NMR, a prohibitively costly amount of epoxide would have been required to prepare sufficient material to achieve spectra with adequate signal-to-noise ratios.

Proton−carbon HMQC spectra were explored as an indirect route for observation of $^{15}$N spectra. These spectra are more readily acquired than proton−$^{15}$N spectra. Using inverse detection, the natural abundance of $^{13}$C is easier to observe in this way than the 100% isotopically enriched $^{15}$N. It was hoped that the $^1J_{^{13}C-^{15}N}$ coupling (∼20 Hz) would be visible in the $^1$H−$^{13}$C spectra. Spectra with sufficient signal-to-noise levels for seeing all the key $^{13}$C signals were easily obtained. However, it was not possible to achieve sufficient resolution in the $^{13}$C domain to resolve the carbon−nitrogen coupling.

Several mass spectrometric methods were tried without success. Meehan had reported that a permethylated derivative of the BP Ado adduct showed fragmentation between C6 and N$^6$ of adenine with electron impact (EI) ionization (*33*). Attempts to use this method to observe the corresponding fragmentation with the unmethylated and permethylated benzo[*a*]pyrene and benzo[*c*]phenanthrene adducts were unsuccessful using both EI and electrospray methodologies. FAB$^+$ and FAB$^-$ were also investigated. It is possible that the FAB methods gave appropriate fragment ions but the background noise in the spectra was too high for the method to be quantitatively useful.

Finally, this analytical problem was solved with COSY spectra (Figure 8). Cross-peaks were observed for vicinal coupling between the NH and the α-proton of the benzo[*c*]phenanthrene moiety when the spectra were acquired in anhydrous DMSO-$d_6$. In the isotopically labeled sample, the cross-peak was split (∼93 Hz) by $^{15}$N when the

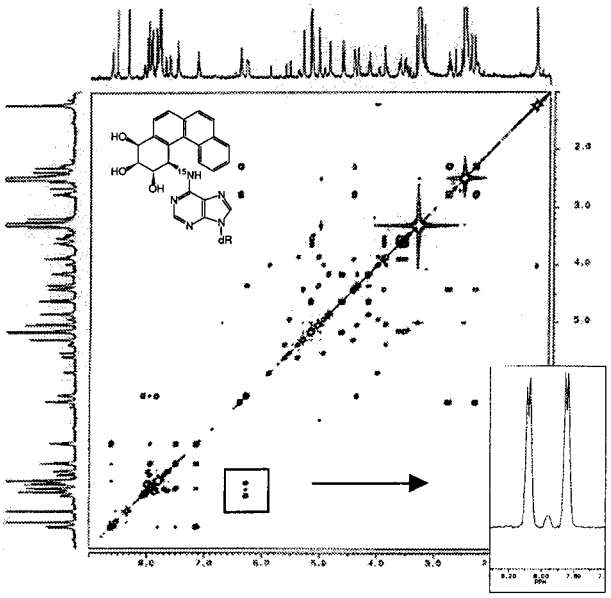


**Figure 8.** 400 MHz NMR spectra (DMSO-$d_6$, 25 °C) of the *anti-trans*-BCDE d$^{15}$Ado adduct (1*R*): 2D COSY and (inset) cross section of the $^{15}N^6$−H region (~8 ppm).

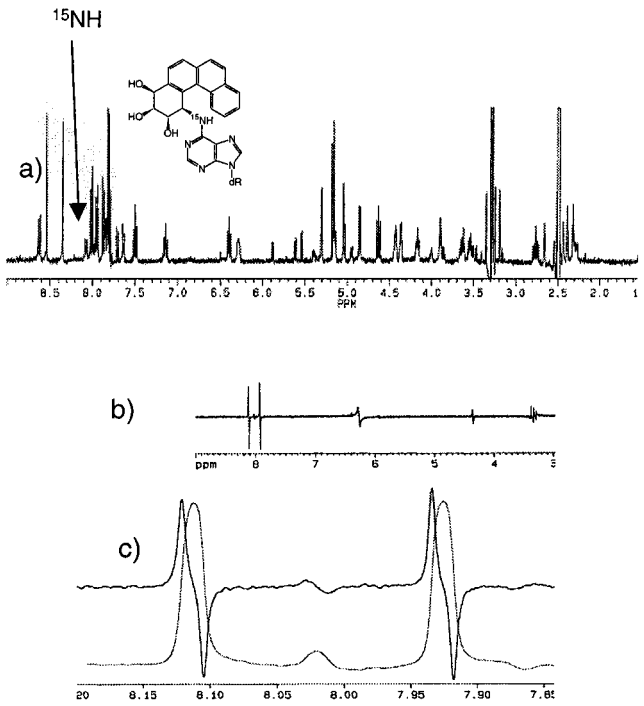


**Figure 9.** 500 MHz NMR spectrum (DMSO-$d_6$, 28 °C) of the *anti-trans*-BCDE d$^{15}$Ado adduct (1*R*): (a) $^1$H 1D spectrum, (b) 1D COSY spectrum, and (c) integration spectrum of the proton attached to $N^6$.

isotopic label lay at $N^6$ but not when it was at N1. The spectra exhibited intense signals for the $^{15}$N-split peak but also a weak signal, centered within the wide doublet due to rearranged material (plus any unlabeled material that might have been present). It was not clear that the spectra could be used to obtain good quantitation due to a number of factors involved in the acquisition and processing of the 2D spectra. In particular, differences in relaxation could lead to biasing of results during spectral apodization. Therefore, a specific 1D COSY spectrum (Figure 9) for the $^{15}$NH region was obtained. By this experiment, it was determined that 6−8% of the

**Table 2. Extent of Dimroth Rearrangement in the Reaction of Certain PAH Dihydrodiol Epoxides with [$^{15}N^6$]Deoxyadenosine as Evaluated by $^1$H NMR[a]**

| *anti*-dihydrodiol epoxide | [$^{15}N^6$]dAdo adduct | Dimroth rearrangement (%) |
|---|---|---|
| (±)-naphthalene | (−)-*anti-trans*<br>(+)-*anti-trans* | 50 |
| non-bay region (+)-benz[*a*]anthracene | (+)-*anti-trans* | ~0 |
| bay region (±)-benz[*a*]anthracene | (±)-*anti-trans* | ~0 |
| (+)-benzo[*a*]pyrene | (+)-*anti-trans*<br>(+)-*anti-cis* | ~0 |
| (±)-benzo[*c*]phenanthrene | (±)-*anti-trans*<br>(±)-*anti-cis* or $\beta$ | 5−7 |

[a] The reactions were carried out in 0.05 M Tris-HCl buffer at pH 7.2 and 42 °C for 60−72 h.

$N^6$ adducts did not contain $^{15}$N at the $N^6$ position. Thus, since $^{15}$N labeling of the deoxyadenosine was at a level of ~99%, it can be concluded that 5−7% of the product had arisen by Dimroth rearrangement. Both diastereomers were analyzed and gave similar results.

The data for the reaction of 2′-deoxyadenosine with PAH dihydrodiol epoxides are summarized in Table 2.

## Discussion

The adduction reaction between (*R*)-styrene oxide and 2′-deoxyadenosine when carried out in Tris-HCl (pH 7.2) yielded $N^6\alpha(S)$, $N^6\alpha(R)$, and $N^6\beta$ adducts in a ratio of 7:1:8. The reaction between the riboside and (*R*)-styrene oxide carried out in the absence of Tris-HCl gave only $N^6\alpha(S)$ and $N^6\beta$ adducts. The product ratio was 1:1.5. In both cases, overall combined yields were 20−30%. Deaminated N1 adducts were seen in minor amounts (<5%). Adduction at N1 followed by Dimroth rearrangement accounted for 20−30% of the $N^6\alpha$ adducts. The $\beta$ adducts arose entirely by this rearrangement. These reactions reflect the relative reactivities of N1 and $N^6$ in nucleosides. The studies with adenine incorporated into duplex DNA by Barlow et al. (*11*) and by us indicate that, although the extent of reaction may be decreased in native DNA, the mechanism of reaction and relative reactivity of the nitrogens is not significantly affected by duplex structure. One must conclude that breathing motions in duplexed DNA lead to disruption of Watson−Crick A·T base pairs a significant portion of the time so that reactions are able to occur at the N1 position.

The reaction of the simplest dihydrodiol epoxide, i.e., that of naphthalene, with deoxyadenosine yielded a significant amount (50%) of $N^6$ adduct via rearrangement. However, in this case, all reaction occurred at the benzylic position of the epoxide; no products from $\beta$ attack were observed. The overall yield was low (3%, one diastereomer); only trace amounts (<1% of the total product mixture) of deaminated products were detected. The polycyclic aromatic hydrocarbon dihydrodiol epoxides reacted almost exclusively at $N^6$. In the reaction of benzo[*c*]phenanthrene dihydrodiol epoxide, a small amount of N1 to $N^6$ rearrangement in the formation of the $N^6$ adducts was detected. The other PAH dihydrodiol epoxides may also have given small quantities of N1 adducts which rearranged to the $N^6$ position, but the amount of adduct formed by this pathway was below the level of detection.

The interpretation of these results is not totally straightforward. If electron delocalization in the elec-

trophile were the sole factor, one would expect the naphthalene dihydrodiol epoxide to attack $N^6$ at least as much as styrene oxide. However, NADE showed an approximately 1:1 ratio of initial attack at N1 versus $N^6$, whereas for the SO α adducts, the ratio is closer to 1:3. The difference may lie in the greater importance that steric hindrance has for the rigidly constrained electrophilic site in NADE. Thus, attack at the sterically more accessible N1 position may be more favored by the naphthalene epoxide than it is for styrene oxide, the reaction of which is less affected by steric considerations. Similarly, the steric hindrance caused by the fjord region of benzo[*c*]phenanthrene dihydrodiol epoxide may produce a higher preference for reaction at N1 than is seen with the less hindered bay region epoxides or the non-bay region epoxide. It should be recognized that benz[*a*]-anthracene and benzo[*c*]phenanthrene are isomeric to each other, and charge in the transition states of the adduction reactions will be stabilized by the same number of aromatic rings. However, as Szeliga and Dipple (*34*) have suggested, the nonplanar benzo[*c*]-phenanthrene fjord region epoxide has less tendency to ionize, as evidenced by its much slower rate of hydrolysis, and hence, it has more opportunity to react at the nucleophilic sites in DNA.

The conclusion to be drawn from the studies reported herein is that, whereas aliphatic epoxides react preferentially at the N1 of adenine, the dihydrodiol epoxides of PAHs react almost entirely by direct reaction with $N^6$ and the epoxides attached to only one aromatic ring give a mixture of initial attack at N1 and at $N^6$. For aliphatic and aralkyl epoxides, it is clear that both N1 and $N^6$ adducts (and their deaminated products) need to be examined carefully for their biological and structural properties.

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