# Synthesis and Assignment of Absolute Configuration to the $N^6$-Deoxyadenosine Adducts Resulting from Cis and Trans Ring-Opening of Phenanthrene 9,10-Oxide

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Reaction of calf thymus DNA with phenanthrene 9,10-oxide *in vitro* results in alkylation of the exocyclic amino groups of the purine bases. Adducts result from both cis and trans opening of the epoxide. In the present study, structures of the $N^6$-deoxyadenosine adducts have been unequivocally assigned by synthesis from optically pure *cis*- and *trans*-9-amino-10-hydroxy-9,10-dihydrophenanthrene. Resolution of *trans*-9-azido-10-hydroxy-9,10-dihydrophenanthrene as its acetate was achieved on a chiral HPLC column. The *early*-eluting (−)-enantiomer was assigned (9*R*,10*R*)-absolute configuration based on a characteristic negative CD band at 232 nm due to the helicity of its biphenyl chromophore, in combination with a $^1$H NMR coupling constant that indicated pseudodiaxial orientation of the substituents at C-9 and C-10. Aminolysis of the ester followed by reduction of the azido group provided the desired, optically active trans (9*R*,10*R*) amino alcohol. As a starting material for synthesis of the cis amino alcohol, *trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene was resolved by chiral HPLC. As above, the *early*-eluting (−)-enantiomer was assigned (9*R*,10*R*)-absolute configuration based on a characteristic negative CD band at 234 nm. Displacement of bromine with inversion of configuration by azide, aminolysis of the ester, and reduction provided optically pure *cis*-(9*S*,10*R*)-9-amino-10-hydroxy-9,10-dihydrophenanthrene. Coupling of the optically active amino alcohols with 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (the 6-fluoro analog of dA) yielded the corresponding $N^6$-deoxyadenosine adducts. Comparison of CD spectra and HPLC retention times of the synthetic adducts with those of the adducts obtained from calf thymus DNA make it possible to assign unambiguously the structures of the DNA adducts.

## Introduction

Phenanthrene is the simplest polycyclic aromatic hydrocarbon to contain both a K region and a bay region, structural features commonly associated with carcinogenicity and mutagenicity.[1] Although phenanthrene and its metabolically formed bay-region diol epoxides have little or no tumorigenic activity,[2,3] its K-region 9,10-oxide is a major metabolite (67–84% of total metabolites)[3a,4] and has modest mutagenic activity.[2,5] In separate studies, we have examined the covalent binding of phenanthrene 9,10-oxide to calf thymus DNA *in vitro*.[6] After enzymatic hydrolysis to the nucleoside level and isolation of the hydrocarbon-modified nucleosides by HPLC, we established by a combination of mass spectrometry and p$K_a$ determinations that most of these adducts resulted from alkylation of the exocyclic amino groups of the purine bases: 52% $N^2$-deoxyguanosine (dG) and 43% $N^6$-deoxyadenosine (dA). From the total number of products isolated, it was clear that both cis and trans opening of the arene oxide must have occurred. Although the identity of the DNA base, dG, dA, or dC in each adduct could be determined, it was extremely difficult to determine whether the adducts were cis or trans isomers, as well as their absolute configurations at C-9 and C-10. Since phenanthrene 9,10-oxide is a meso compound due to its plane of symmetry, the cis and trans adducts were each formed as pairs of optically active diastereomers. Although there is an empirical relationship between the sign of the long wavelength exciton CD band and the absolute configuration of the carbon bearing the purine amino group for diol epoxide adducts at the exocyclic amino groups of dA and dG,[7] data are unavailable for comparable nucleoside adducts at K-regions. Alternatively, the adduct CD spectra could be dominated by the presence of the skew biphenyl chromophore.[8] Inspection of the CD spectra of

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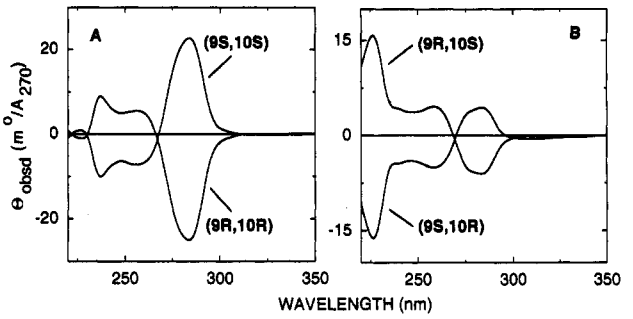



**Figure 1.** CD spectra (methanol, normalized to 1.0 absorbance unit at 270 nm) of the synthetic *early*- and *late*-eluting (ODS column), diastereomeric pairs of $N^6$-(10-hydroxy-9,10-dihydrophenanthren-9-yl)-2′-deoxyadenosine adducts which result from trans (panel A) and cis (panel B) opening of phenanthrene 9,10-oxide. The trans-substituted phenanthrene adducts exhibit CD maxima at 236 and 284 nm whereas the cis-substituted isomers exhibit maxima at 226 and 283 nm. The spectra of the diastereomers appear as mirror image pairs since there is little contribution to the observed ellipticity by the 2-deoxy-β-D-*erythro*-pentofuranose portion of the molecules.

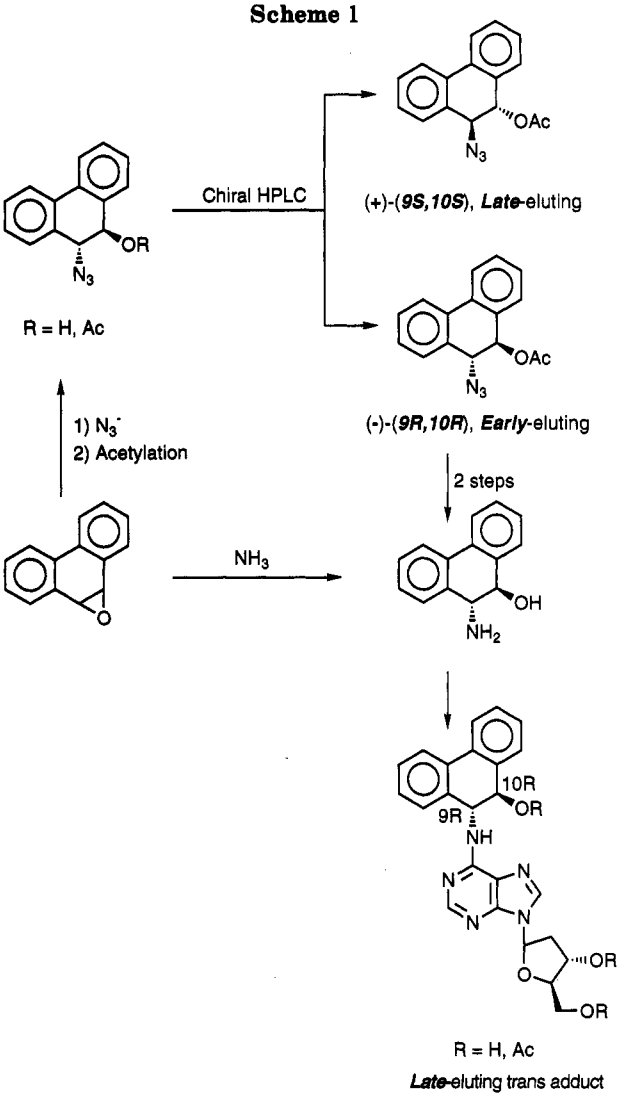
the adducts[6] suggested that neither of these chiroptical methods would provide unequivocal assignment of absolute configuration. Furthermore, assignment of cis vs trans relative stereochemistry of the substituents at C-9 and C-10 requires NMR spectra, and in many cases sufficient quantities of the DNA-derived adducts were unavailable. In the present report, we synthesize and assign relative and absolute configuration to the four possible $N^6$-dA adducts formed on alkylation of DNA by phenanthrene 9,10-oxide.

## Results and Discussion

$N^6$-Alkylpurine adducts have been prepared by coupling amino derivatives of hydrocarbons with 6-fluoropurine 2′-deoxyribosides.[9] An analogous approach to the preparation of the desired $N^6$-dA adducts of phenanthrene 9,10-oxide, taken in this study, involves reaction of the 6-fluoro analog of dA with optically active *cis*- and *trans*-9-amino-10-hydroxy-9,10-dihydrophenanthrene of known absolute configuration.

Previously, *trans*-9-amino-10-hydroxy-9,10-dihydrophenanthrene had been prepared by reduction of the corresponding azido alcohol.[10] Direct aminolysis (liquid ammonia at 90 °C for 24 h) of phenanthrene 9,10-oxide proved to be a considerably easier route to the racemic trans amino alcohol. Reaction of this amino alcohol with 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine at 80 °C for 6 h provided the desired mixture of diastereomeric trans opened phenanthrene 9,10-oxide adducts at the $N^6$-position of dA in ~65% yield after separation on an ODS column (cf. Scheme 1). CD spectra of the separated diastereomers are shown in Figure 1. The spectra are dominated by a strong Cotton effect at ~284 nm that is positive for the *early*-eluting and negative for the *late*-eluting diastereomer. By analogy with spectra of bay-region diol epoxide adducts,[7] these bands may result from exciton interactions between the biphenyl and purine chromophores. With diol epoxide adducts, (*S*) absolute configuration is associated with a positive CD band around 250-280 nm and a negative band around 270-300 nm. The prominent 284-nm band of the phenanthrene 9,10-oxide adducts does not conclusively fall into either region, and with structurally dissimilar adducts the same absolute configuration need not give rise to CD bands with the same signs. The sign of the CD band in the region of 230 nm is diagnostic of the helicity of the biphenyl chromophore and can be used for the assignment of absolute configuration if the conformation (pseudodiaxial or pseudodiequatorial) of the C-9 and C-10 substituents is known. Thus, the observed weak, negative band (M helicity of the biphenyl) is consistent with (9*S*,10*S*) absolute configuration if the substituents are mainly pseudoequatorial but with (9*R*,10*R*) absolute configuration if the opposite conformation predominates. The adducts as their triacetates exhibit a value of $J_{9,10} \sim 7$ Hz that is intermediate between the values expected for predominantly pseudoaxial and predominantly pseudoequatorial conformations and suggests an approximately equal population of the two conformers, consistent with the low circular dichroic extinction coefficient for the biphenyl chromophore.[8] In the absence of a clearly preferred conformation for the trans adducts, it was not possible to

**Scheme 1**

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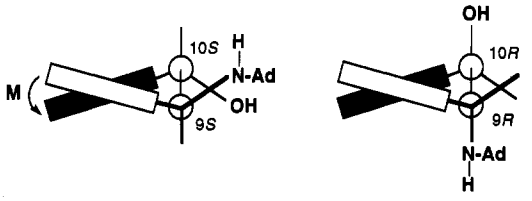


use the sign of this weak CD band for assignment of absolute configuration to these adducts, and thus their synthesis from trans amino alcohol of known absolute configuration was required.

Attempts to separate the enantiomers of the trans amino alcohol on Chiralpak OB and OP chiral HPLC columns were unsuccessful. As an alternative approach, we attempted resolution of *trans*-9-azido-10-hydroxy-9,10-dihydrophenanthrene.[10] Reaction of phenanthrene 9,10-oxide with $NaN_3$–trimethylsilyl azide in MeCN at 60 °C provided the desired azido alcohol. Although the enantiomers of the azido alcohol failed to separate on chiral HPLC columns, the less polar azido acetates could be efficiently separated ($\alpha = 1.41$) on a Chiralpak OP column using 10% 2-propanol in hexane. The enantiomerically pure trans amino alcohols were prepared *via* aminolysis of each ester and reduction of the azide to the amine (Scheme 1). CD spectra of the resolved trans azido acetates showed strong bands at ~230 nm due to the presence of the skew-biphenyl chromophore. The value of 5.4 Hz for $J_{9,10}$ indicates that the 9-azido and 10-acetoxy substituents prefer a pseudoaxial orientation. This conformational information coupled with the presence of a negative CD band at 232 nm (M helicity of the skew-biphenyl) for the *early*-eluting (−)-enantiomer on the chiral column requires (9*R*,10*R*)-absolute configuration as shown in Figure 2. CD spectra in methanol of the (9*R*,10*R*)-trans azido acetate and its derived azido alcohol and amino alcohol are shown in Figure 3. Notably the sign of the skew biphenyl band passes through zero and reverses in this reaction sequence, while the coupling constants $J_{9,10}$ in chloroform-*d* go from 5.4 (azido acetate) to 7.2 (azido alcohol) to 10 Hz (amino alcohol). Similar values of $J_{9,10}$ were obtained in methanol for the azido acetate (4.7) and the amino alcohol (9.0), indicative that the preferred conformation of each compound remains the same in both solvents. Thus the distribution of conformers changes from predominance of the pseudoaxial orientation for the azide and acetoxy substituents, through approximately equal populations of both conformers of the azido alcohol, to a preference for pseudoequatorial substituents in the amino alcohol. This inversion of *preferred conformation* results in the observed change in the helicity of the biphenyl chromophore even though the same (9*R*,10*R*) *configuration* must be retained in all three intermediates.

Upon coupling with the 6-fluoro analog of deoxyadenosine, the (9*R*,10*R*)-amino alcohol produced the *late*-eluting adduct diastereomer on the ODS column. Thus, the *late*-eluting trans adduct with a negative CD band at 284 nm has (9*R*,10*R*)-absolute configuration. The CD spectrum for the (9*S*,10*S*) adduct is a virtual mirror image of the spectrum for the (9*R*,10*R*) adduct (Figure 1). Although the observed CD bands probably result from exciton interactions between the purine and hydrocarbon chromophores, the same considerations that limit the ability to compare CD bands exhibited by diol epoxide adducts with those exhibited by K-region adducts apply, as were discussed above.

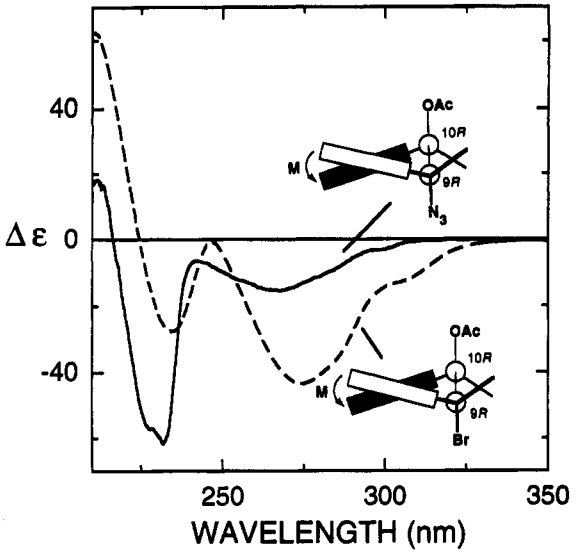


**Figure 2.** CD spectra (methanol) of the *early*-eluting enantiomer of *trans*-9-azido-10-acetoxy-9,10-dihydrophenanthrene and the *early*-eluting enantiomer of *trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene on a Chiralpak OP column eluted with 10% 2-propanol in hexane.

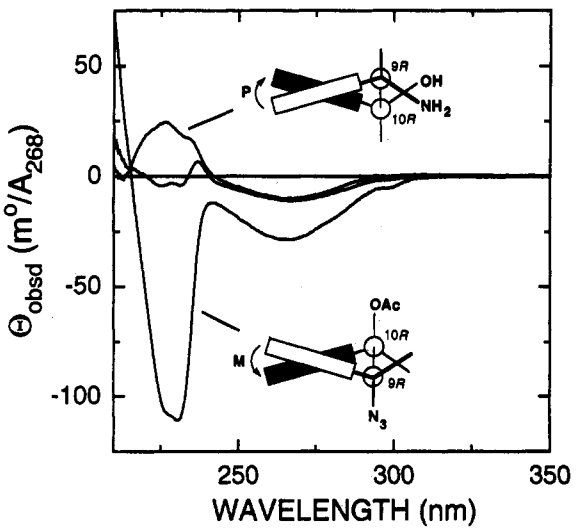


**Figure 3.** CD spectra (methanol) of the (9*R*,10*R*)-trans azido acetate (lower curve) and its derived azido alcohol (middle curve) and amino alcohol (upper curve).

*trans*-9-Bromo-10-acetoxy-9,10-dihydrophenanthrene[11] was selected as precursor for the optically active *cis*-9-amino-10-hydroxy-9,10-dihydrophenanthrene required in the synthesis of the cis opened phenanthrene 9,10-oxide adducts since assignment of absolute configuration should be possible from the CD spectra of the trans enantiomers. Adverse steric interaction between the bromo and acetoxy groups causes these groups to prefer the pseudoaxial orientation ($J_{9,10}$ = 2.8 Hz).[11] Their absolute configurations can thus be assigned from the helicity of the biphenyl chromophore. Nucleophilic displacement of bromine by azide, aminolysis of the acetate, and reduction of the azide to an amino group (Scheme 2) would then provide the desired cis amino alcohols.

Conversion of *trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene to *cis*-9-azido-10-hydroxy-9,10-dihydro-

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**Scheme 2**

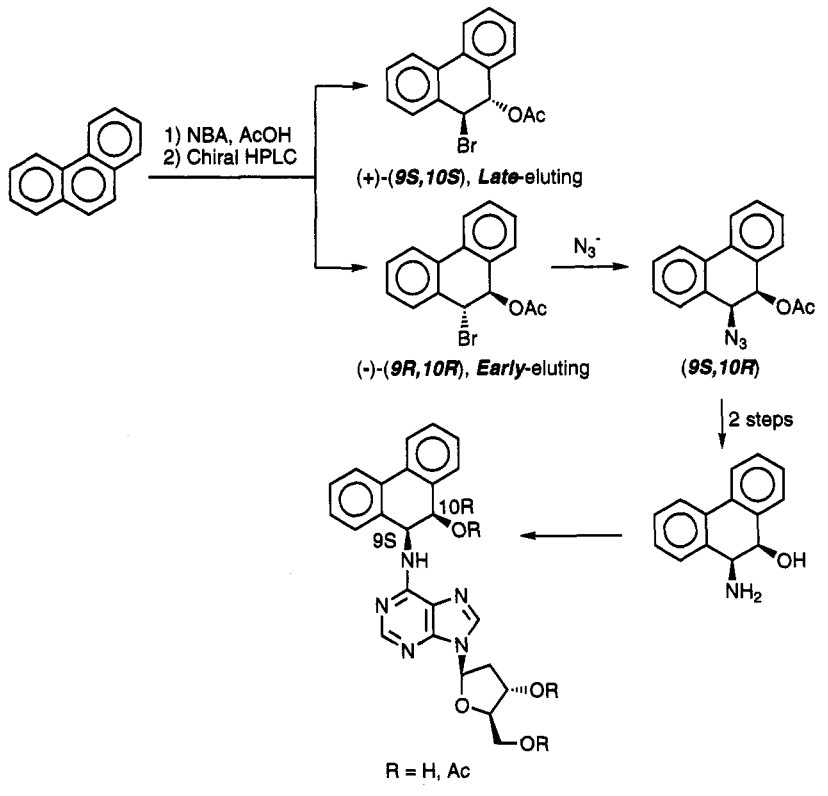



**Table 1. Conditions Examined for Conversion of *trans*-9-Bromo-10-Acetoxy-9,10-Dihydrophenanthrene to *cis*-9-Azido-10-acetoxy-9,10-dihydrophenanthrene[a]**

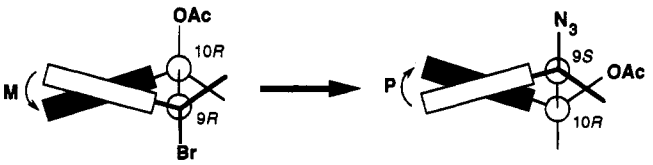
| reagent | conditions | % cis isomer[b] | % yield[c] |
|---|---|---|---|
| $NaN_3$ | 80 °C, 2 h, 1:4 water/DMSO | >90 | 12 |
| $NaN_3$, 18-crown-6 | 80 °C, 12 h, benzene | ~50 | 58 |
| $NaN_3$, $Me_3SiN_3$, 18-crown-6 | 80 °C, 12 h, acetonitrile | ~10 | 85[d] |
| $Me_3SiN_3$, $n$-$Bu_4N^+F^-$ | 60 °C, 5 h, acetonitrile | >99 | 74 |

[a] Reactions were run until TLC indicated complete consumption of starting material. [b] Percent cis product relative to total cis plus trans products as determined from integration of $^1H$ NMR signals at the respective K-regions. [c] Total yield of cis plus trans products based on starting material. [d] When (9*R*,10*R*) trans bromo acetate was used in this reaction, the resulting trans azido acetate was found to be racemic on chiral HPLC.

phenanthrene proved to be more difficult than anticipated (Table 1). Reaction with sodium azide in DMSO–water at 80 °C resulted in substantial decomposition of the starting material. Sodium azide in the presence of 18-crown-6 produced a reasonable yield of azides (58%), but only half was the desired cis isomer. A mixture of $n$-$Bu_4N^+F^-$ and $Me_3SiN_3$ in acetonitrile, which enabled use of a high concentration of organic soluble azide under optimal conditions for nucleophilic displacement, produced the desired cis product exclusively, in 74% yield. The trans azido acetate formed in the presence of 18-crown-6 is presumed to arise *via* a symmetrical acetoxonium ion intermediate, since optically active trans bromo acetate produced racemic trans azido acetate under the same conditions.

Facile resolution ($\alpha = 1.39$) of *trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene was achieved on the Chiralpak OP column again using 10% 2-propanol in hexane. The *early*-eluting (–)-enantiomer has a negative CD band at 234 nm indicative of (9*R*,10*R*)-absolute configuration. However, the CD spectrum also shows a strong, negative band at 275 nm (Figure 2). We are uncertain as to the origin of this band. After displacement of bromide by azide, the *cis*-9-azido-10-acetoxy-9,10-dihydrophenanthrene produced should have (9*S*,10*R*)-absolute configuration. This cis azido acetate and the derived cis azido alcohol and cis amino alcohol all have positive CD bands at ~230 nm. As discussed above, the sign of these bands is indicative of absolute configuration provided the orientation of each substituent is known. Regardless of conformation (oxygen substituent pseudoaxial *vs* pseudoequatorial), all of these cis derivatives should have small and comparable values of $J_{9,10}$. An attempt to determine the conformation of the cis azido acetate on the basis of a NOESY experiment was inconclusive due to weak cross peaks. The chemical shift of the acetoxy group (2.22 ppm), however, strongly indicates that it preferentially resides in a pseudoequatorial orientation.[12] Given that the acetate is pseudoequatorial, a change in the skew sense of the biphenyl chromophore has occurred upon formation of the cis azido acetate, and a positive CD band at ~230 nm is to be expected for (9*S*,10*R*)-absolute configuration. This change in helicity is shown. Reaction of the (9*S*,10*R*)-*cis*-9-amino-10-hydroxy-9,10-dihydrophenanthrene produced from the *early*-eluting (–)-enantiomer of the trans

(12) The chemical shift of benzylic acetate groups can be informative as to their conformation. For example, in *trans*-5,6-diacetoxy-5,6-dihydrochrysene the acetates appear at 1.87 and 1.97 ppm whereas in the cis isomer these signals are at 1.94 and 2.30 ppm.[13a] Because of steric hindrance due to the proximal bay region, both acetates in the trans diacetate are pseudoaxial and appear at higher field. For the cis diacetate, the 5-acetate (1.94) in the bay region is pseudoaxial and is shielded whereas the pseudoequatorial 6-acetate (2.30) is edge deshielded. The 5- (1.92, ax.) and 6- (2.40, eq.) monoacetates show a consistent pattern of chemical shifts. Notably, the pseudoaxial acetate in the *trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene used in the present study appears at 1.90 ppm. Methyl ethers of K-region dihydrodiols show even larger axial *vs* equatorial differences in chemical shifts.[13b]




9-bromo 10-acetate with 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine at 80 °C for 6 h provided the *early*-eluting (HPLC on the ODS column) diastereomeric cis opened phenanthrene 9,10-oxide adduct with a weak, positive Cotton effect at 284 nm. The low intensity of this band precludes its use in assigning absolute configuration to the adduct. However, the above chemical correlation requires the configuration to be (9*S*,10*R*).

Unequivocal syntheses of the optically active $N^6$-dA adducts of phenanthrene 9,10-oxide reported here make possible the assignment of both relative and absolute configuration to the corresponding adducts obtained from DNA. Specifically, comparisons of CD spectra (Figure 1) and HPLC elution order on ODS (trans (9*S*,10*S*), trans (9*R*,10*R*), cis (9*S*,10*R*), cis (9*R*,10*S*)) of the synthetic adducts permit unambiguous identification of the isomeric $N^6$-dA adducts produced from DNA upon reaction with phenanthrene 9,10-oxide followed by enzymatic digestion to nucleosides. Details of the formation and quantitation of these adducts from calf thymus DNA will be published elsewhere.[6]

## Experimental Section

$^1$H NMR spectra were measured at 300 MHz unless otherwise noted. Chemical shifts ($\delta$) are reported in ppm and coupling constants ($J$) are in hertz. Rotations were determined in freshly distilled THF at $c$ = 0.7–0.9 g/100 mL. Circular dichroism spectra were measured in methanol; where concentrations were not determined values of $\Theta_{obsd}$ were normalized to 1.0 absorbance unit at 270 nm (dA adducts) or 268 nm (intermediates). Resolved samples were judged to contain ≤3% of the other enantiomer based on peak areas on chiral HPLC. Chiral products had $^1$H NMR and mass spectra identical to their racemic counterparts. The conventional numbering system for the phenanthrene ring is used. For adducts and related compounds, singly primed numbers are used for the protons on the ribose moiety (1′–5′) and the purine protons are doubly primed (2″ and 8″). TLC $R_f$ values are provided for starting material (SM) and product (P).

**(±)-*trans*-9-Amino-10-hydroxy-9,10-dihydrophenanthrene.** Phenanthrene-9,10-oxide[14] (56 mg, 0.29 mmol) was heated with $NH_3$ at 90 °C in a Parr high pressure reactor for 24 h (~1000 psi). After evaporation of $NH_3$ under a stream of $N_2$, the residue was dissolved in $CH_2Cl_2$. The solution was washed with water, dried over $Na_2SO_4$, and evaporated. (±)-*trans*-9-Amino-10-hydroxy-9,10-dihydrophenanthrene[10] was obtained as a light brown solid (51 mg, 74%): TLC (15% MeOH in $CH_2Cl_2$/$SiO_2$) $R_{f(SM)}$ = 0.85 and $R_{f(P)}$ = 0.3; $^1$H NMR ($CDCl_3$) 7.75 (m, $2H_{4,5}$), 7.66 (m, $1H_8$), 7.54 (m, $1H_1$), 7.39 (m, $4H_{2,3,6,7}$), 4.51 (d, $1H_{10}$, $J_{10,9}$ = 10.0), 3.90 (d, $1H_9$, $J_{9,10}$ = 10.0); HRMS calcd for $C_{14}H_{13}NO$ ($M^+$) 211.0997, found 211.1003.

**(±)-*trans*-9-Azido-10-hydroxy-9,10-dihydrophenanthrene.** Phenanthrene 9,10-oxide[14] (25 mg, 0.126 mmol) was mixed with 18-crown-6 (33 mg, 0.12 mmol), $NaN_3$ (13 mg, 2.52 mmol), and $Me_3SiN_3$ (33 μL, 1.26 mmol) in dry $CH_3CN$ (0.5 mL). The reaction mixture was stirred at 60 °C overnight. The mixture was filtered and the filtrate was evaporated. The product was loaded onto a 1-mm (20 × 20 cm) preparative TLC plate. Elution with PhH gave the desired trans azido alcohol[10] as a white solid (13 mg, 43%): TLC (PhH/$SiO_2$) $R_{f(SM)}$ = 0.7 and $R_{f(P)}$ = 0.3; $^1$H NMR ($CDCl_3$) 7.85 (m, $2H_{4,5}$), 7.45 (m, $6H_{1,2,3,6,7,8}$), 4.80 (d, $1H_{10}$ $J_{10,9}$ = 7.2), 4.68 (d, $1H_9$, $J_{9,10}$ = 7.2); HRMS calcd for $C_{14}H_{11}N_3O$ ($M^+$) 237.0902, found 237.0899.

**(±)-*trans*-9-Azido-10-acetoxy-9,10-dihydrophenanthrene.** (±)-*trans*-9-Azido-10-hydroxy-9,10-dihydrophenanthrene (50 mg, 0.18 mmol) was dissolved in 1:1 pyridine/acetic anhydride (1 mL). The reaction mixture was stirred overnight at rt. The sample was concentrated with a stream of $N_2$. Benzene was added and evaporated twice to remove residual pyridine. An oily product, which was sufficiently pure for the subsequent step, was obtained (56 mg, 92%): $^1$H NMR ($CDCl_3$), 7.85 (m, $2H_{4,5}$), 7.45 (m, $6H_{1,2,3,6,7,8}$), 6.01 (d, $1H_{10}$ $J_{10,9}$ = 5.4), 4.74 (d, $1H_9$, $J_{9,10}$ = 5.4), 2.04 (s, 3H, OAc); HRMS calcd for $C_{16}H_{13}N_3O_2$ ($M^+$) 279.1008, found 279.1010; UV (MeOH) $\lambda_{max}$ 269 nm ($\epsilon$ = 16 330).

**HPLC Resolution of (±)-*trans* 9-azido-10-acetoxy-9,10-dihydrophenanthrene.** Enantiomers of *trans*-9-azido-10-acetoxy-9,10-dihydrophenanthrene were separated by HPLC on a Chiralpak OP column (25 cm × 0.46 cm, void volume 2.54 mL) eluted with 10% 2-propanol in hexane at a flow rate of 1 mL/min. The enantiomers had $T_{R(early)}$ = 9.6 min and $T_{R(late)}$ = 12.5 min ($\alpha$ = 1.41). The *early*-eluting (9*R*,10*R*)-enantiomer has $[\alpha]_D$ −376° and $\Delta\epsilon_{232\,nm}$ −61.7, and the *late*-eluting (9*S*,10*S*)-enantiomer has $[\alpha]_D$ +365° and $\Delta\epsilon_{232\,nm}$ +57.5. See Results and Discussion and Figure 2 for configurational assignment.

***trans*-(9*R*,10*R*)-9-Azido-10-hydroxy-9,10-dihydrophenanthrene.** The *early*-eluting (−)-(9*R*,10*R*)-*trans*-9-azido-10-acetate (10 mg, 0.035 mmol) was stirred in MeOH (1 mL) at 0 °C. $NH_3$ was bubbled through the solution for 15 min at 0 °C. After ~3 h at rt under $NH_3$, the sample was concentrated with a stream of $N_2$. The crude product was dissolved in EtOAc. Standard workup provided the azido alcohol as white crystals (7.8 mg, 95%). Its CD spectrum shows a weak negative band at ~230 nm (Figure 3).

***trans*-(9*R*,10*R*)-9-Amino-10-hydroxy-9,10-dihydrophenanthrene.** The above optically active *trans*-(9*R*,10*R*)-9-azido-10-hydroxy-9,10-dihydrophenanthrene (5 mg, 0.024 mmol) was dissolved in MeOH (1 mL) and ~1 mg of 5% Pd/C was added. The solution was stirred under hydrogen gas for ~2 h. The mixture was centrifuged to remove the catalyst. Chromatography of the product on a 250-μm (10 × 20 cm) preparative TLC plate using 10% MeOH in $CH_2Cl_2$ gave a light brown solid (4 mg, 89%). Its CD spectrum shows a weak positive band at ~230 nm (Figure 3).

**(±)-*trans*-9-Bromo-10-acetoxy-9,10-dihydrophenanthrene.** Phenanthrene (1 g, 5 mmol) was dissolved in ca. 20 mL of glacial acetic acid. About 4–5 equiv of anhydrous lithium acetate (1.7 g, 25 mmol) was added to the solution, and the flask was protected from light. Recrystallized *N*-bromoacetamide (0.85 g, 6.2 mmol) was dissolved in a minimum volume of glacial acetic acid (ca. 4–5 mL). The *N*-bromoacetamide solution was added to the stirred reaction mixture over 1–1.5 h. The reaction was monitored by TLC every 30 min. The reaction is normally complete within 2 h at rt. The reaction mixture was added dropwise to an ice-cold saturated $NaHCO_3$ solution. After standard workup, the product was purified either on a silica gel column eluted with 1:1 PhH/hexane or by trituration with ether/hexane. The bromo acetate[11] was obtained as a white solid (0.42 g, 23%): TLC (1:1 PhH/hexane on $SiO_2$ gel) $R_{f(SM)}$ = 0.9, $R_{f(P)}$ = 0.6; $^1$H NMR ($CDCl_3$) 7.89 (m, $2H_{4,5}$), 7.41 (m, $6H_{1,2,3,6,7,8}$), 6.15 (d, $1H_{10}$ $J_{10,9}$ = 2.8), 5.39 (d, $1H_9$, $J_{9,10}$ = 2.8), 1.90 (s, 3H, OAc); HRMS calcd for ($C_{16}H_{13}BrO_2$ − AcOH) 255.9888, found 255.9877; UV (MeOH) $\lambda_{max}$ 274 nm ($\epsilon$ = 12 600).

**(±)-*cis*-9-Azido-10-acetoxy-9,10-dihydrophenanthrene.** *n*-$Bu_4N^+F^-$ (164 mg, 0.12 mmol) and $Me_3SiN_3$ (52 μL, 0.612 mmol) were dissolved in 2 mL of $CH_3CN$ at 0 °C. The solution was stirred at 0 °C for 5 min, followed by addition of *trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene (40 mg, 0.13 mmol). The reaction mixture was heated at 60 °C for 5 h and evaporated and the crude product purified on a 500-μm (20 × 20 cm) preparative TLC plate with PhH as eluting solvent. An oily product was obtained (26 mg, 74%): TLC (PhH/$SiO_2$) $R_{f(SM)}$ = 0.7, $R_{f(P)}$ = 0.65; $^1$H NMR ($CDCl_3$) 7.81 (m, $2H_{4,5}$), 7.41 (m, $6H_{1,2,3,6,7,8}$), 6.20 (d, $1H_{10}$ $J_{10,9}$ = 3.7), 4.75 (d, $1H_9$, $J_{9,10}$ = 3.7), 2.22 (s, 3H, OAc); HRMS calcd for $C_{16}H_{13}N_3O_2$ ($M^+$) 279.1008, found 279.1001.

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**(±)-*cis*-9-Azido-10-hydroxy-9,10-dihydrophenanthrene.** *cis*-9-Azido-10-acetoxy-9,10-dihydrophenanthrene (20 mg, 0.07 mmol) was stirred in MeOH (2 mL) at 0 °C while a stream of $NH_3$ was bubbled through the solution for 15 min. After ~3 h with stirring under $NH_3$, volatiles were evaporated with a stream of $N_2$. The crude product was dissolved in EtOAc. Standard workup provided cis azido alcohol as white crystals (10.6 mg, 63%): TLC ($PhH/SiO_2$) $R_{f(SM)}$ = 0.6 and $R_{f(P)}$ = 0.3; $^1H$ NMR ($CDCl_3$) 7.85 (m, $2H_{4,5}$), 7.69 (m, $1H_8$), 7.50 (s, $1H_9$), 7.42 (m, $4H_{2,3,6,7}$), 5.01 (d, $1H_{10}$, $J_{10,9}$ = 4.0), 4.72 ppm (d, $1H_9$, $J_{9,10}$ = 4.0); HRMS calcd for $C_{14}H_{11}N_3O$ ($M^+$) 237.0902, found 237.0904.

**(±)-*cis*-9-Amino-10-hydroxy-9,10-dihydrophenanthrene.** *cis*-9-Azido-10-hydroxy-9,10-dihydrophenanthrene (10 mg, 0.041 mmol) was dissolved in MeOH (2 mL), and 5% Pd/C (~2 mg) was added. The solution was stirred under hydrogen gas for 2 h. After removal of the catalyst by centrifugation, the solution was evaporated and the product was loaded onto a 250-μm (10 × 20 cm) preparative TLC plate and eluted with 10% MeOH in $CH_2Cl_2$. The amino alcohol was obtained as a light-brown solid (8.0 mg, 89%): TLC (15% MeOH in $CH_2Cl_2/SiO_2$) $R_{f(SM)}$ = 0.85 and $R_{f(P)}$ = 0.3; white crystals from PhH, mp 138–140 °C dec; $^1H$ NMR ($CDCl_3$) 7.70 (m, $2H_{4,5}$), 7.30 (m, $4H_{1,2,3,6,7,8}$), 4.80 (d, $1H_{10}$, $J_{10,9}$ = 4.5), 3.88 (d, $1H_9$, $J_{9,10}$ = 4.5); HRMS calcd for $C_{14}H_{14}NO$ ($M^+$ + 1) 212.1075, found 212.1079.

**HPLC Resolution of (±)-*trans*-9-Bromo-10-acetoxy-9,10-dihydrophenanthrene.** Enantiomers of *trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene were separated by HPLC on a Chiralpak OP column (25 cm × 0.46 cm, void volume 2.54 mL) eluted with 10% 2-propanol in hexane at a flow rate of 1 mL/min. The optically pure isomers had $T_{R(early)}$ = 11.8 min and $T_{R(late)}$ = 15.4 min ($\alpha$ = 1.39). The *early*-eluting (9*R*,10*R*)-enantiomer has $[\alpha]_D$ −762° and $\Delta\epsilon_{234\ nm}$ −27.7 and $\Delta\epsilon_{275\ nm}$ −43.5; the *late*-eluting (9*S*,10*S*)-enantiomer has $[\alpha]_D$ +741° and $\Delta\epsilon_{234\ nm}$ +27.9 and $\Delta\epsilon_{275\ nm}$ +42.6. See Results and Discussion and Figure 2 for configurational assignment.

***cis*-(9*S*,10*R*)-9-Amino-10-hydroxy-9,10-dihydrophenanthrene.** About 5 mg of *cis*-(9*S*,10*R*)-amino alcohol was obtained from 20 mg of the *early*-eluting (−)-(9*R*,10*R*)-*trans*-9-bromo-10-acetoxy-9,10-dihydrophenanthrene by the procedures described above for the racemic compounds. The cis azido acetate ($\Theta_{225\ nm}$ +30.2), cis azido alcohol ($\Theta_{230\ nm}$ +81.7), and cis amino alcohol ($\Theta_{229\ nm}$ +56.2) had the indicated CD bands.

**Diastereomeric *trans*-$N^6$-(10-Hydroxy-9,10-dihydrophenanthren-9-yl)-2′-deoxyadenosine.** The trans amino alcohol (5 mg, 0.024 mmol) and 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine[9a] (6-FP, 8.8 mg, 0.036 mmol) were stirred in 100 μL of DMF/5.4 μL of 2,6-lutidine (0.048 mmol) at 80 °C for 6 h. The reaction mixture was monitored by HPLC using a 5-μm, 0.46 × 25 cm Beckman Ultrasphere ODS column eluted at 1.5 mL/min with a gradient from 50% 0.1 M $NH_4OAc$ (pH 7.0) and 50% MeOH to 100% MeOH over 20 min. The amino alcohol eluted at ca. 7 min. The retention times of the *trans* adducts were >10 min. The reaction was stopped when the amino alcohol was completely consumed and the mixture was cooled. Solvents were evaporated under reduced pressure. The crude product was redissolved in MeOH/water and purified by HPLC on the above column eluted at 1 mL/min with 60% MeOH and 40% water. The *trans* adducts had $T_{R(early)}$ = 11.3 min and $T_{R(late)}$ = 12.5 min. Both isomers were obtained as white solids (*early* diastereomer: 3.8 mg, *late* diastereomer: 3.2 mg, 65% combined yield). HRMS (*early* diastereomer) calcd for $C_{24}H_{24}N_5O_4$ ($M^+$ + 1): 446.1828, found 446.1840; HRMS (*late* diastereomer) calcd for $C_{24}H_{24}N_5O_4$ ($M^+$ + 1) 446.1828, found 446.1848. The above reaction run with the trans (9*R*,10*R*)-amino alcohol produced the *late*-eluting diastereomer based on retention time and CD spectra. Both diastereomers were acetylated (1:1 pyridine/acetic anhydride overnight at rt) and purified on a Du Pont SIL (5 μm) column eluted at 5 mL/min with 2% MeOH in $CH_2Cl_2$: HRMS (*trans early*-eluting) calcd for $C_{30}H_{30}N_5O_7$ ($M^+$ + 1) 572.2145, found 572.2147. HRMS (*trans late*) calcd for $C_{30}H_{30}N_5O_7$ ($M^+$ + 1) 572.2145, found 572.2145; $^1H$ NMR (500 MHz, acetone-$d_6$) *trans*-(9*S*,10*S*)-*early*-eluting diastereomer 8.33, 8.11 (2s, $2H_{2'',8''}$), 7.96 (d, $2H_{4,5}$), 7.32–7.54 (m, $6H_{1,2,3,6,7,8}$), 6.78 (d, $1H_{NH}$, $J$ = 8.8), 6.45 (t, $1H_{1'}$, $J$ = 6.6), 6.32 (d, $1H_{10}$, $J$ = 7.0), 5.93 (broad, $1H_9$), 5.49 (dt, $1H_{3'}$, $J$ = 2.6, 6.2), 4.25–4.40 (m, $3H_{4',5',5'}$), 3.22 (quint, $1H_{2'}$, $J_{app}$ = 6.6), 2.62 (ddd, $1H_{2'}$, $J$ = 2.6, 6.2, 14.2), 1.95–2.10 (3s, 9H, OAc); and *trans*-(9*R*,10*R*)-*late*-eluting diastereomer 8.33, 8.13 (2s, $2H_{2'',8''}$), 7.96 (d, $2H_{4,5}$), 7.32–7.54 (m, $6H_{1,2,3,6,7,8}$), 6.78 (d, $1H_{NH}$, $J$ = 8.8), 6.45 (t, $1H_{1'}$, $J$ = 6.6), 6.32 (d, $1H_{10}$, $J$ = 6.6), 5.94 (broad, $1H_9$), 5.49 (dt, $1H_{3'}$, $J$ = 2.6, 6.2), 4.25–4.40 (m, $3H_{4',5',5'}$), 3.22 (quint, $1H_{2'}$, $J_{app}$ = 6.2), 2.61 (ddd, $1H_{2'}$, $J$ = 2.6, 6.2, 13.9), 1.95–2.10 (3s, 9H, OAc).

**Diastereomeric *cis*-$N^6$-(10-Hydroxy-9,10-dihydrophenanthren-9-yl)-2′-deoxyadenosine.** Reaction of the cis amino alcohol and 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine[9a] was conducted as above for the trans isomer. The *cis* adducts had $T_{R(early)}$ = 14.3 min and $T_{R(late)}$ = 15.2 min on the ODS column eluted at 1.0 mL/min with 60% MeOH and 40% water as above. Both isomers were obtained as white solids (*early* diastereomer: 3.4 mg, *late* diastereomer: 3.2 mg, 67% combined yield). HRMS (*early* diastereomer) calcd for $C_{24}H_{24}N_5O_4$ ($M^+$ + 1) 446.1828, found 446.1843; HRMS (*late* diastereomer) calcd for $C_{24}H_{24}N_5O_4$ ($M^+$ + 1) 446.1828, found 446.1846. The above reaction run with the *cis*-(9*S*,10*R*)-amino alcohol produced the *early*-eluting diastereomer based on retention time and CD spectra. Both diastereomers were acetylated as above. HRMS (*cis early*) calcd for $C_{30}H_{30}N_5O_7$ ($M^+$ + 1): 572.2145, found 572.2147; HRMS (*cis late*) calcd for $C_{30}H_{30}N_5O_7$ ($M^+$ + 1) 572.2145, found 572.2120; $^1H$ NMR (500 MHz, acetone-$d_6$) *cis*-(9*S*,10*R*)-*early*-eluting diastereomer 8.30, 8.22 (2s, $2H_{2'',8''}$), 7.99 (t, $2H_{4,5}$), 7.34–7.60 (m, $6H_{1,2,3,6,7,8}$), 6.82 (broad, $1H_{NH}$), 6.47 (dd, $1H_{1'}$, $J$ = 6.2, 8.1), 6.18 (broad s, $2H_{9,10}$), 5.50 (m, $1H_{3'}$), 4.25–4.40 (m, $3H_{4',5',5'}$), 3.22 (broad m, $1H_{2'}$), 2.62 (ddd, $1H_{2'}$, $J$ = 2.6, 6.2, 14.3), 1.95–2.10 (3s, 9H, OAc) and *cis*-(9*R*,10*S*)-*late*-eluting diastereomer 8.30, 8.21 (2s, $2H_{2'',8''}$), 7.99 (t, $2H_{4,5}$), 7.34–7.60 (m, $6H_{1,2,3,6,7,8}$), 6.82 (broad, $1H_{NH}$), 6.47 (dd, $1H_{1'}$, $J$ = 6.2, 8.1), 6.18 (broad s, $2H_{9,10}$), 5.49 (m, $1H_{3'}$), 4.25–4.40 (m, $3H_{4',5',5'}$), 3.24 (broad m, $1H_{2'}$), 2.62 (ddd, $1H_{2'}$, $J$ = 2.6, 6.2, 13.9), 1.95–2.10 (3s, 9H, OAc).

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**Supplementary Material Available:** Proton NMR spectra of the *trans*-9-bromo-10-acetoxy-, 9-azido-10-acetoxy-, 9-azido-10-hydroxy-, and 9-amino-10-hydroxy-9,10-dihydrophenanthrenes, the *cis*-9-azido-10-acetate-, 9-azido-10-hydroxy-, and 9-amino-10-hydroxy-9,10-dihydrophenanthrenes, and the acetates of adenine adducts depicted schematically in Schemes 1 and 2 are shown (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.