Synthesis and Site-Specific Incorporation of a Bay-Region Cis Ring-Opened Tetrahydro Epoxide–Deoxyadenosine Adduct into a DNA Oligomer

Mahesh K. Lakshman, Jane M. Sayer, and Donald M. Jerina*

Section on Oxidation Mechanisms, Laboratory of Bioorganic Chemistry, NIDDK, The National Institutes of Health, Bethesda, Maryland 20892

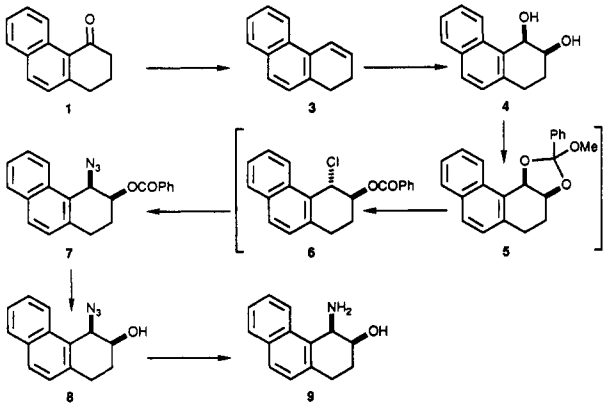
Received December 30, 1991

Chemical synthesis of the 1,2,3,4-tetrahydrophenanthrene 3,4-epoxide adducts resulting from benzylic, cis ring-opening of the epoxide by the exocyclic amino group of 2′-deoxyadenosine (dA) is described. The approach taken consists of coupling (±)-*cis*-3-hydroxy-4-amino-1,2,3,4-tetrahydrophenanthrene with a 6-fluoro analogue of dA in which the furanose hydroxyl groups are protected. The required amino alcohol was obtained by reaction of 1,2-dihydrophenanthrene with osmium tetraoxide to form the cis 3,4-diol, conversion to the trans chlorohydrin benzoate via its orthobenzoate, displacement of the benzylic chloride by azide, hydrolysis to the cis azido alcohol, and reduction to the racemic cis amino alcohol. Coupling of the amino alcohol with the 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl) derivative of 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine results in a pair of diastereomers that are readily separated by HPLC on silica gel. Replacement of the previously used pyridine by 2,6-lutidine significantly improved the yield for the coupling step. Both adducts were acetylated on the hydroxyl group of the hydrocarbon and then desilylated on the sugar. Absolute configurations were assigned to the adducts on the basis of the shapes of their CD spectra. The 3*S*,4*R* diastereomer (derived from the more polar, *late*-eluting adduct) was blocked at the 5′-sugar hydroxyl group with the 4,4′-dimethoxytrityl group and allowed to react with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite to produce the desired activated nucleoside. Incorporation into the deoxynucleotide TpGpA*pGpT as the central base proceeded in good yield with minor modifications to the standard DNA synthesizer protocol.

Introduction

Alternant polycyclic aromatic hydrocarbons (PAH), many of which are cytotoxic, mutagenic, and carcinogenic, are ubiquitous environmental contaminants. The carcinogenic PAH are metabolized to bay-region diol epoxides,[1] which are known to be ultimate carcinogens.[2] These metabolically formed diol epoxides exert their carcinogenic and other genotoxic effects through covalent bonding to DNA bases.[3] Although the exact mechanism(s) by which these adducts cause cell transformation remains a subject of active investigation, there is ample evidence that the major covalent adducts formed involve bonding of the diol epoxides to the exocyclic amino groups of the purine bases dA and dG. Reaction occurs by both cis and trans addition of the amino group to the benzylic carbon of the epoxide group.[3,4] Synthesis of such purine–diol epoxide adducts and their site-specific incorporation into DNA oligomers has great potential for biochemical and biological studies of the mechanism of cell transformation.

Scheme I



In a recent report,[5] we have described the synthesis of the diastereomeric trans adducts of 1,2,3,4-tetrahydrophenanthrene 3,4-epoxide at the exocyclic amino group of dA and their site-specific incorporation into a DNA oligomer, with use of a blocking–deblocking protocol that is also applicable to the incorporation of trans dA adducts of bay-region diol epoxides into DNA oligomers. The diastereomers were prepared by coupling (±)-*trans*-3-hydroxy-4-amino-1,2,3,4-tetrahydrophenanthrene with 6-fluoro-9-(2-deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl)purine. The trans amino alcohol, as well as related trans amino triols from bay-region diol epoxides of phenanthrene, benzo[*c*]phenanthrene, and benzo[*a*]pyrene, was prepared by direct aminolysis[5,6] of the epoxide or diol epoxide. The present report describes an approach by which cis dA adducts of diol epoxides can be prepared and incorporated into DNA oligomers. In closely related studies, Smith et al.[7] have reported the synthesis

(1) Jerina, D. M.; Yagi, H.; Thakker, D. R.; Sayer, J. M.; van Bladeren, P. J.; Lehr, R. E.; Whalen, D. L.; Levin, W.; Chang, R. L.; Wood, A. W.; Conney, A. H. In *Foreign Compounds Metabolism*; Caldwell, J., Paulson, G. D., Eds.; Taylor and Francis Ltd.: London, 1984; pp 257–266.

(2) See: Lehr, R. E.; Kumar, S.; Levin, W.; Wood, A. W.; Chang, R. L.; Conney, A. H.; Yagi, H.; Sayer, J. M.; Jerina, D. M. In *Polycylic Aromatic Hydrocarbons and Carcinogenesis*; Harvey, R. G., Ed.; ACS Symposium Series 283, American Chemical Society: Washington, DC, 1985; pp 63–84. Jerina, D. M.; Sayer, J. M.; Agarwal, S. K.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Pruess-Schwartz, D.; Baird, W. M.; Pigott, M. A.; Dipple, A. In *Biological Reactive Intermediates III*; Kocsis, J. J., Jollow, D. J., Witmer, C. M., Nelson, J. O., Snyder, R., Eds.; Plenum Press: New York, 1986; pp 11–30.

(3) Jerina, D. M.; Chadha, A.; Cheh, A. M.; Schurdak, M. E.; Wood, A. W.; Sayer, J. M. In *Biological Reactive Intermediates IV*; Witmer, C. M., Snyder, R., Jollow, D. J., Kalf, G. S., Kocsis, J. J., Sipes, I. G., Eds.; Plenum Press: New York, 1991; pp 533–553.

(4) A total of 16 adducts can form by cis and trans addition of dA and dG to each enantiomer of the two bay-region diol epoxide diastereomers (epoxide oxygen either cis or trans to the benzylic hydroxyl group of the trans diol). See: Agarwal, S. K.; Sayer, J. M.; Yeh, H. J. C.; Pannell, L. K.; Hilton, B. D.; Pigott, M. A.; Dipple, A.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 2497–2504. Cheng, S. C.; Prakash, A. S.; Pigott, M. A.; Hilton, B. D.; Roman, J.; Lee, H.; Harvey, R. G.; Dipple, A. *Chem. Res. Toxicol.* **1988**, *1*, 216–221. Sayer, J. M.; Chadha, A.; Agarwal, S. K.; Yeh, H. J. C.; Yagi, H.; Jerina, D. M. *J. Org. Chem.* **1991**, *56*, 20–29. Chadha, A.; Sayer, J. M.; Yeh, H. J. C.; Yagi, H.; Cheh, A. M.; Pannell, L. K.; Jerina, D. M. *J. Am. Chem. Soc.* **1989**, *111*, 5456–5463.

(5) Lakshman, M. K.; Sayer, J. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1991**, *113*, 6589–6594.

(6) In a subsequent study Kim et al. [Kim, S. J.; Harris, C. M.; Jung, K.-Y.; Koreeda, M.; Harris, T. M. *Tetrahedron Lett.* **1991**, 6073–6076] also report aminolysis of benzo[*c*]phenanthrene and benzo[*a*]pyrene diol epoxides as well as naphthalene-1,2-diol 3,4-epoxide.

(7) Smith, C. A.; Harper, A. E.; Coombs, M. M. *J. Chem. Soc., Perkin Trans. I* **1988**, 2745–2750. Smith, C. A. *Carcinogenesis* **1991**, *12*, 631–636.

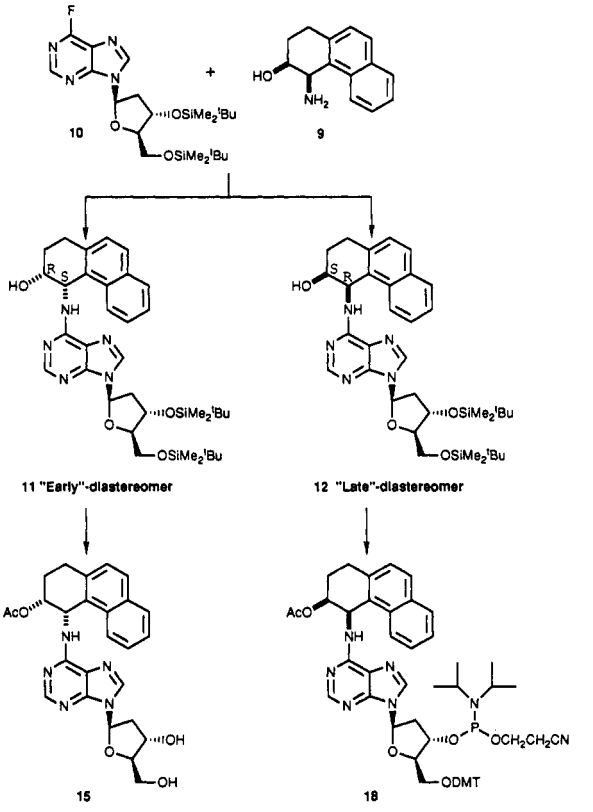
of trans adducts of dC with naphthalene-1,2-diol 3,4-epoxide and their incorporation into oligonucleotides.

Results and Discussion

The present synthesis (Scheme I) required (±)-*cis*-3-hydroxy-4-amino-1,2,3,4-tetrahydrophenanthrene for the coupling step. This was obtained through reduction ($NaBH_4$ in methanol, 87%) of 1,2-dihydrophenanthren-4(3*H*)-one (1) to the corresponding alcohol 2, dehydration (*p*-toluenesulfonic acid in refluxing benzene, quantitative) to 1,2-dihydrophenanthrene (3), and oxidation (OsO_4 in pyridine, 75%) to (±)-*cis*-3,4-dihydroxy-1,2,3,4-tetrahydrophenanthrene (4). The cis diol 4 was converted to the trans chlorohydrin benzoate 6 via treatment of the mixed orthobenzoates 5 with Me_3SiCl. As expected from the work of Newman and Chen,[8] product 6 was exclusively the desired *trans*-3-(benzoyloxy)-4-chloro-1,2,3,4-tetrahydrophenanthrene. Chloride efficiently opened the acylium ion in a trans fashion at the more reactive benzylic C-4 center. Three methods were examined for the displacement of chloride by azide: the N_3^- form of Amberlite resin in acetonitrile[9] (70 °C, 24 h), Me_3SiN_3 and anhydrous n-$Bu_4N^+F^-$ in acetonitrile (50 °C, overnight), and NaN_3 in DMF (60 °C, overnight). Overall yields for the three-step (one-pot) conversion of 4 to *cis*-3-(benzoyloxy)-4-azido-1,2,3,4-tetrahydrophenanthrene (7) were 60, 76, and 78%, respectively. In the ^1H NMR spectrum of the azido benzoate 7, the H-3 and H-4 proton resonances are superimposed, so that the assignment of each of these resonances as well as the relative stereochemistry was difficult at this stage. The azido acetate was also readily prepared through this route[10] but offered no particular advantage over the benzoate.

Direct catalytic reduction of the azido benzoate 7 resulted in the exclusive formation of the undesired *N*-benzoyl amino alcohol by migration of the benzoyl group. This was evidenced by the upfield shift of the nonbenzylic H-3 from 5.47 ppm in the azido benzoate to 4.29 ppm.[11] Reduction of the azide functionality should have caused an upfield shift of the H-4 resonance from 5.47 ppm. Instead, this proton is shifted downfield to 6.11 ppm, and only a single exchangeable proton (6.30 ppm) was present in the product. Such migrations have been observed on catalytic reduction of vicinal, trans azido acetates but not benzoates of related polycyclic hydrocarbon derivatives.[12] Prior hydrolysis of the azido benzoate 7 to the azido alcohol 8 and subsequent reduction produced the desired 3-hydroxy-4-amino-1,2,3,4-tetrahydrophenanthrene (9) in which the resonance for H-4 has shifted upfield and that of H-3 remains essentially unchanged compared to that

Scheme II



of 8. Assignment of cis relative stereochemistry to azido alcohol 8 was established unequivocally by the synthesis of *trans*-3-hydroxy-4-azido-1,2,3,4-tetrahydrophenanthrene by direct ring opening of the tetrahydro epoxide with NaN_3 in DMF.[13] Differences in the ^1H NMR spectra between the cis and trans derivatives are quite substantial, the most characteristic feature being the appearance of the H-3 resonance. Since steric hindrance in the bay region forces the substituent at C-4 to be pseudoaxial regardless of cis or trans substitution at C-3, H-4 is pseudoequatorial in both series and H-3 is therefore pseudoequatorial in the trans derivatives and pseudoaxial in the cis derivatives. Thus, for example, H-3 of the trans azido alcohol appears as a narrow multiplet at 4.37 ppm since all three vicinal coupling constants are small. In contrast, in the cis isomer 8 this proton appears as a doublet of triplets at 4.08 ppm as a result of the large coupling with the pseudoaxial proton at C-2. The latter pattern is typical of all compounds in the cis tetrahydro series.

Coupling of the 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl) derivative of 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (10) with the cis amino alcohol 9, in the presence of hexamethyldisiloxane (HMDS) as a fluoride sponge[5] in DMF/2,6-lutidine resulted in the formation of the desired diastereomeric adducts (Scheme II). Replacement of pyridine by 2,6-lutidine as the added base

(8) Newman, M. S.; Chen, C. H. *J. Am. Chem. Soc.* **1973**, *95*, 278–279.
(9) Hassner, A.; Stern, M. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 478–479.
(10) *cis*-3-Acetoxy-4-azido-1,2,3,4-tetrahydrophenanthrene: ^1H NMR ($CDCl_3$) 8.05 (d, 1 H_5, J = 8.5); 7.87 (d, 1 H_8, J = 8.1); 7.81 (d, 1 H_9, J = 8.5); 7.64 (t, 1 H_6, J = 7.1); 7.54 (t, 1 H_7, J = 7.0); 7.26 (d, 1 H_{10}, J = 8.5); 5.47 (d, 1 H_4, J = 3.5); 5.30 (dt, 1 H_3, J = 3.5, 12.8); 3.18 (m, 2 H_1); 2.38 (m, 1 H_2); 2.27 (s, 3 H, OAc); 2.16 (m, 1 H_2).
(11) *cis*-3-Hydroxy-4-(benzoylamino)-1,2,3,4-tetrahydrophenanthrene. The cis azido benzoate 7 (19 mg, 55.5 μmol) was reduced with 10% Pd on carbon (10 mg) in 2 mL of 1:1 THF/MeOH for 10 h. The mixture was filtered and evaporated under reduced pressure. Chromatography of the product on a 250-μm (10 × 20 cm) silica gel plate using 10% MeOH in CH_2Cl_2 gave the *N*-benzoyl compound (8 mg, 47%) as a white solid. ^1H NMR ($CDCl_3$): 7.20–7.90 (11 H, aromatic); 6.30 (d, N*H*, J = 7.4); 6.11 (dd, 1 H_4, J = 4.3, 7.4); 4.29 (dt, 1 H_3, J = 4.3, 12.4); 3.08 (dd, 2 H_1, J = 3.9, 9.1); 2.11 (m, 1 H_2); 1.96 (m, 1 H_2). The signal at 6.30 ppm disappears after addition of a few drops of MeOH-d_4 and warming the sample at ca. 40 °C for a few hours. This exchange results in the signal at 6.11 ppm in becoming a doublet. MS (EI) m/e: 317, 299, 196, 178, 167, 105, 77.
(12) Lakshman, M.; Nadkarni, D. V.; Lehr, R. E. *J. Org. Chem.* **1990**, *55*, 4892–4897.
(13) *trans*-3-Hydroxy-4-azido-1,2,3,4-tetrahydrophenanthrene: 1,2,3,4-tetrahydrophenanthrene 3,4-oxide (10 mg, 51 μmol) and NaN_3 (33.1 mg, 0.51 mmol) were stirred at 65 °C in 1 mL of DMF overnight. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. Chromatography of the crude product on a 250-μm (10 × 20 cm) silica gel plate gave two bands. The more polar band (5.8 mg, 48%) was the azido alcohol and the less polar band was phenanthrene. ^1H NMR ($CDCl_3$): 8.04 (d, 1 H_5, J = 8.5); 7.80 (d, 1 H_8, J = 8.1); 7.72 (d, 1 H_9, J = 8.5); 7.54 (dt, 1 H_6, J = 1.3, 7.0); 7.45 (dt, 1 H_7, J = 0.9, 7.0); 7.25 (d, 1 H_{10}, J = 9.5); 4.88 (d, 1 H_4, J = 3.2); 4.37 (m, 1 H_3); 3.10 (ddd, 1 H_1, J = 6.6, 10.4, 17.8); 2.92 (ddd, 1 H_1, J = 3.5, 5.8, 17.8); 2.13 (m, 2 H_2).

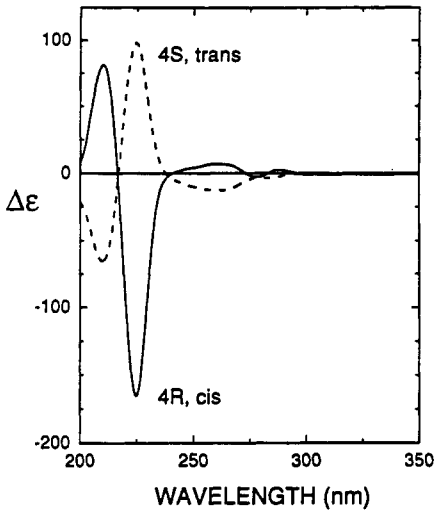


Figure 1. CD spectra (methanol) of (3*S*,4*R*)-N^6-(4-(3-acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine (**16**) and the trans isomer (3*S*,4*S*)-N^6-(4-(3-acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine. Note that because of an error in the reported[5] extinction coefficient of the analogous compound (3*R*,4*R*)-N^6-(4-(3-hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (see Experimental Section of the present paper), the values of Δε for all the deoxyadenosine adducts reported in ref 5 should be multiplied by a factor of 6.9 for trans adducts and 8.6 for cis adducts.

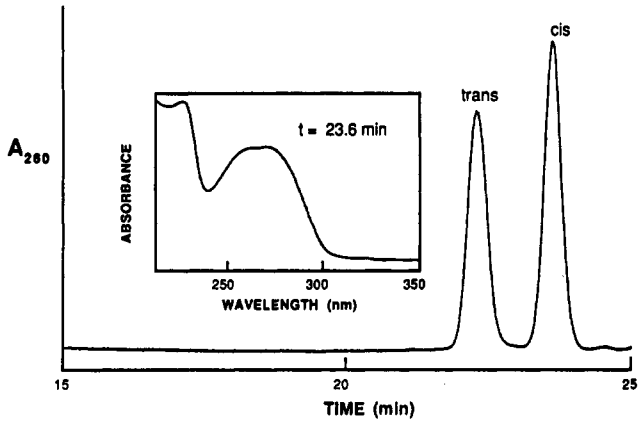


Figure 2. HPLC separation (Hamilton PRP-1 column eluted at 2.5 mL/min with a gradient ramped from 100% A (0.1 M ammonium carbonate, pH 7.5) to 65% A:35% B (50% CH_3CN in 0.1 M ammonium carbonate, pH 7.5) over 20 min and then to 100% B over 10 min) of the oligonucleotide pentamers TpGpA*pGpT, where A* represents the *cis*-(3*S*,4*R*)-N^6-(4-(3-hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine adduct (23.6 min) or the diastereomeric trans N^6-(4-(3-hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine adduct (22.3 min). Under these conditions, the adducted pentamers derived from the two diastereomeric trans dA adducts were not separated. The unsubstituted pentamer TpGpApGpT elutes at 12.3 min. The inset shows the diode-array UV spectrum of the cis-adducted pentamer, which was virtually identical to that of the trans-adducted pentamer (cf. ref 5).

in the reaction mixture results in a somewhat improved yield (66%) and a much less colored product. Pyridine is known to displace leaving groups from the C-6 position of purines, and the resulting pyridinium salts slowly decompose.[14] 2,6-Lutidine seems superior to pyridine in this reaction due to its nonnucleophilic character. The separated adducts **11** and **12** (HPLC on an Axxiom silica gel column) were acetylated at their 3-hydroxyl groups (**13** and **14**, respectively), and the sugar hydroxyl groups were deprotected by cleavage of the silyl groups with *n*-$Bu_4N^+F^-$ (**15** and **16**, respectively).

Absolute configurations of the nucleoside adducts **15** and **16** at C-4 were established from their circular dichroism (CD) spectra. As with other nucleoside adducts of polycyclic hydrocarbon epoxides and diol epoxides at the exocyclic amino groups of purine bases, the present adducts exhibit strong exciton coupling bands due to electric transition dipole interactions between the hydrocarbon and purine chromophores. The two diastereomers have nearly mirror image CD bands (not shown) since the chiral centers of the sugar and the saturated benzo ring contribute little to the observed spectra. The CD spectrum of **16** (Figure 1), derived from the *late*-eluting diastereomer **12**, shows a strong negative band at 225 nm (Δε −165) along with a positive band at 210 nm (Δε +81). On the basis of our empirical correlation between absolute configuration at the benzylic carbon attached to the purine base and the sign and shape of adduct CD spectra,[3-5] 3*S*,4*R* absolute configuration is required for **16** and thus for **12** and **14**. The CD spectrum of the trans adduct, (3*S*,4*S*)-N^6-(4-(3-acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine,[5] is shown for comparison. As expected, this CD is very similar but opposite in sign. In our previous study,[5] we had prepared the diastereomeric cis and trans adducts formed on reaction of (±)-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene with 2′-deoxyadenosine 5′-monophosphate. After removal of the phosphate group the resultant cis (3*R*,4*S*)- and (3*S*,4*R*)-N^6-(4-(3-hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine diastereomers had CD spectra nearly identical to those of **15** and **16**, respectively.

Adduct **16** from the late-eluting diastereomer **12** was selectively blocked at the 5′-hydroxyl group with the 4,4′-dimethoxytrityl group (DMT) using 4,4′-dimethoxytritylium tetrafluoroborate[15] in 2,6-lutidine. In our previous report,[5] we had used the sterically smaller 9-phenyl-9-xanthenyl (pixyl) protecting group.[16] Subsequently, we have concluded that the DMT tetrafluoroborate salt has the desired reactivity and is better able to discriminate between the 5′- and 3′-hydroxyl groups. Reaction of **17** with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite produced the activated nucleoside **18**, ready for incorporation into an oligonucleotide.

The activated nucleoside **18** was incorporated into the oligodeoxynucleotide TpGpA*pGpT as the central base (A*). Reactions (2 μmol scale) were run on an automated DNA synthesizer with the exception that the adducted base (3-fold excess) was added manually. The resulting pentamer, still bearing the terminal DMT blocking group, was purified by HPLC. Compared to fully automated synthesis of the normal pentamer TpGpApGpT (DMT on), the yield of adducted pentamer was 44%. After removal of the terminal DMT blocking group, the adducted pentamer was judged to be ≥98% pure by HPLC and capillary zone electrophoresis (detection at 260 nm). It was well separated on HPLC from the previously reported[5] trans-adducted pentamer (Figure 2). CD spectra of the cis- and trans-adducted pentamers (both with benzylic 4*R* configuration) are compared in Figure 3.

Concluding Remarks

In this report, we describe a strategy for the site-specific incorporation of a cis-opened, bay-region diol epoxide

(14) Fathi, R.; Goswami, B.; Kung, P.-P.; Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1990**, *31*, 319–322.

(15) Beasdale, C.; Ellwood, S. B.; Golding, B. T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 803–805.

(16) Chattopadhyaya, J. B.; Reese, C. B. *J. Chem. Soc., Chem. Commun.* **1978**, 639–640.

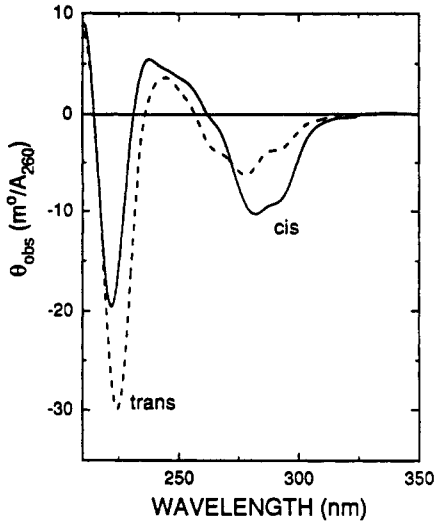
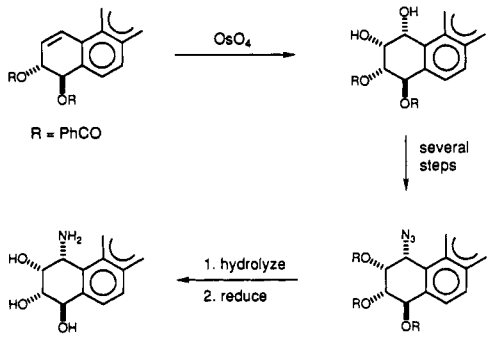


Figure 3. CD spectra (normalized to A_{260} = 1.0 in water) of the cis (solid line) and trans (dashed line) adducted deoxyoligonucleotides TpGpA*pGpT, where A* represents N^6-substituted deoxyadenosine. The substituents, both with 4*R* absolute configuration, are those which would result from cis and trans opening of (3*S*,4*R*)- and (3*R*,4*S*)-3,4-epoxy-1,2,3,4-tetrahydrophenanthrene, respectively, at the benzylic C-4 position. The strongest CD band of the nonadducted pentamer is negative and occurs at about 263 nm.[5]

Scheme III



adduct into a DNA oligomer. This method, we believe, is generally applicable for the preparation of cis ring-opened adducts of diol epoxides in which the epoxide oxygen and benzylic hydroxyl group are trans. In this approach (Scheme III), the diester of a benzo ring trans dihydro diol would be converted to a tetrol diester with osmium tetraoxide, and the sequence of reactions described here would be utilized. The osmium tetraoxide reaction is known to occur predominantly from the face of benzo ring trans dihydro diols and diesters that bears the allylic substituent provided the hydroxyl groups or diesters are not locked in a pseudodiaxial orientation.[17]

In the present approach several modifications have been made to our previous methodology. Use of the hindered base 2,6-lutidine in place of pyridine improves both the yield and quality of the product in the coupling step between the hydrocarbon and the 6-fluoro analogue of dA. Previously, pixyl chloride had been used to block the 5'-hydroxyl group on the sugar of the adducted nucleoside due to its smaller size and higher reactivity relative to DMT-Cl. In the present study, DMT tetrafluoroborate was found to be quite reactive with the adducted nucleoside and was better able to discriminate between the 3'- and 5'-hydroxyl groups than was pixyl chloride. We have used a combination of automated and manual synthesis in which anhydrous *tert*-butyl hydroperoxide[18] is employed as the oxidant in place of the aqueous iodine reagent. With a threefold excess of the critical phosphoramidite we obtained a ~40% yield of product relative to the support-bound oligonucleotide chain, as measured both by the recovery of DMT cation and by the overall yield of adducted relative to normal DMT pentamer.

Experimental Section

^{1}H NMR spectra were measured at 300 MHz. Chemical shifts are reported in ppm and coupling constants are in hertz. 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'), whereas doubly primed numbers are used for the purine protons (2'' and 8''). 1,2-Dihydrophenanthren-4(3*H*)-one (1) was prepared as described[19] and is also available commercially (Aldrich Chemical Co., Milwaukee, WI).

(±)-*cis*-3,4-Dihydroxy-1,2,3,4-tetrahydrophenanthrene (4). Reduction of ketone 1 to alcohol 2 and dehydration of alcohol 2 were essentially as described[19] except that dehydration was effected with *p*-toluenesulfonic acid refluxing in benzene for 45 min. In the present case, the yield in the dehydration step was essentially quantitative. ^{1}H NMR ($CDCl_3$): 8.05 (d, 1 H_5, J = 8.5); 7.74 (d, 1 H_8, J = 7.5); 7.60 (d, 1 H_9, J = 8.2); 7.43 (dt, 1 H_6, J = 1.4, 8.2); 7.36 (dt, 1 H_7, J = 1.4, 8.1); 7.20 (2 $H_{4,10}$); 6.20 (dt, 1 H_3, J = 4.6, 9.9); 2.88 (t, 2 H_1, J_{app} = 8.2); 2.34 (m, 2 H_2). MS (EI) *m/e*: 180, 165. To a stirred solution of 1,2-dihydrophenanthrene (3) (1.8 g, 10 mmol) in 50 mL of pyridine was added OsO_4 (3.0 g, 12 mmol in 3 mL of pyridine), and stirring was continued at rt for 4 h in the dark. The reaction mixture was slowly poured into 300 mL of saturated, aqueous $NaHSO_3$ with stirring. After stirring overnight, the mixture was extracted twice with ethyl acetate. The combined organic layer was washed with 1 L of 25% aqueous HCl, washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting white solid was crystallized from benzene (1.6 g, 75%): mp 160–161 °C. ^{1}H NMR ($CDCl_3$): 8.24 (d, 1 H_5, J = 8.2); 7.81 (dd, 1 H_8, J = 1.1, 8.1); 7.73 (d, 1 H_9, J = 8.2); 7.56 (dt, 1 H_6, J = 1.1, 8.2); 7.46 (dt, 1 H_7, J = 1.2, 8.1); 7.21 (d, 1 H_{10}, J = 8.2); 5.39 (d, 1 H_4, J = 3.9); 4.00 (dt, 1 H_3, J = 3.9, 11.8); 3.04 (m, 2 H_1); 1.90–2.12 (m, 2 H_2). Anal. Calcd for $C_{14}H_{14}O_2$: C, 78.47; H, 6.59. Found: C, 78.51; H, 6.53. MS (EI) *m/e*: 214, 196, 170, 141. Previously, optically active tetrahydro diol 4 had been obtained by reduction of the bacterial metabolite (+)-*cis*-3,4-dihydroxy-3,4-dihydrophenanthrene[20] and by synthesis.[21]

(±)-*cis*-3-(Benzoyloxy)-4-azido-1,2,3,4-tetrahydrophenanthrene (7). The cis diol 4 (0.04 g, 0.19 mmol), trimethyl orthobenzoate (48 μL, 0.28 mmol), and a trace of benzoic acid were refluxed in 2 mL of anhydrous benzene for 3 h. The mixture was cooled and treated with solid K_2CO_3, filtered and evaporated. The product was dissolved in 2.5 mL of CH_2Cl_2 and cooled to 4 °C. Et_3N (5.2 μL, 37 μmol) and Me_3SiCl (48 μL, 0.38 mmol) were added, and the mixture was stirred at 4 °C for 1.5 h. Another portion of Me_3SiCl (24 μL, 0.19 mmol) was added, stirring was

(17) For references to the facial selectivity of osmium tetraoxide toward dihydro diols, see: Vyas, K. P.; van Bladeren, P. J.; Thakker, D. R.; Yagi, H.; Sayer, J. M.; Levin, W.; Jerina, D. M. *Mol. Pharmacol.* **1983**, *24*, 115–123. Bushman, D. R.; Grossman, S. J.; Jerina, D. M.; Lehr, R. E. *J. Org. Chem.* **1989**, *54*, 3533–3544. Sayer, J. M.; Lehr, R. E.; Kumar, S.; Yagi, H.; Yeh, H. J. C.; Holder, G. M.; Duke, C. C.; Silverton, J. V.; Gibson, C.; Jerina, D. M. *J. Am. Chem. Soc.* **1990**, *112*, 1177–1185. Similar results are obtained with diesters of dihydro diols (unpublished). The opposite facial selectivity, necessary for the other series of diol epoxide diastereomers, can also be achieved. See: Woodward, R. B.; Brutcher, F. V., Jr. *J. Am. Chem. Soc.* **1958**, *80*, 209–211.

(18) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191–4194. Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691–1696.

(19) Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1975**, *97*, 3185–3192.

(20) Jerina, D. M.; Selander, H.; Yagi, H.; Wells, M. C.; Davey, J. F.; Mahadevan, V.; Gibson, D. T. *J. Am. Chem. Soc.* **1976**, *98*, 5988–5996. Koreeda, M.; Akhtar, M. N.; Boyd, D. R.; Neill, J. D.; Gibson, D. T.; Jerina, D. M. *J. Org. Chem.* **1978**, *43*, 1023–1027.

(21) Boyd, D. R.; Greene, R. M. E.; Neill, J. D.; Stubbs, M. E.; Yagi, H.; Jerina, D. M. *J. Chem. Soc., Perkin Trans. I* **1981**, 1477–1482.

continued an additional 1.5 h, and the mixture was evaporated. To the residue were added 0.5 mL DMF and NaN_3 (120 mg, 1.85 mmol), and the mixture was heated overnight at 60 °C. The mixture was cooled, diluted with water, and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. Chromatography of the product on a silica gel column using CH_2Cl_2 gave **7** (50.2 mg, 78% after three steps) as an oil. ^1H NMR ($CDCl_3$): 8.19 (d, 2 H, ortho protons of the benzoyl group, J = 8.6); 8.03 (d, 1 H_5, J = 8.5); 7.84 (d, 1 H_8, J = 7.7); 7.78 (d, 1 H_9, J = 8.5); 7.55–7.64 (m, 2 H); 7.45–7.52 (br t, 3 H); 7.25 (d, 1 H_{10}, J = 8.5); 5.47 (m, 2 $H_{3,4}$); 3.18 (dd, 2 H_1, J = 4.2, 9.1); 2.45 (m, 1 H_2); 2.25 (m, 1 H_2). MS (FAB) m/e: 343 (M^+); HRMS calcd for $C_{21}H_{17}N_3O_2$ (M^+) 343.1321, found 343.1306.

(±)-*cis*-3-Hydroxy-4-azido-1,2,3,4-tetrahydrophenanthrene (8). The azido benzoate **7** (96 mg; 0.28 mmol) was stirred at rt in 1:1 THF/MeOH (2 mL) containing NaOMe (75 mg, 1.4 mmol) for 2 h. The mixture was diluted with water and extracted twice with ethyl acetate. The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure, and dried under vacuum to remove residual methyl benzoate. The waxy solid (62 mg, 93%) was of sufficiently high purity, based upon its ^1H NMR, to be used in the subsequent step without further purification. ^1H NMR ($CDCl_3$): 8.06 (d, 1 H_5, J = 8.4); 7.82 (d, 1 H_8, J = 8.1); 7.74 (d, 1 H_9, J = 8.4); 7.57 (dt, 1 H_6, J = 1.3, 8.4); 7.46 (t, 1 H_7, J = 8.1); 7.22 (d, 1 H_{10}, J = 8.4); 5.24 (d, 1 H_4, J = 3.8); 4.08 (dt, 1 H_3, J = 3.8, 11.7); 3.06 (m, 2 H_1); 2.08 (m, 2 H_2). MS (FAB) m/e: 239 (M^+); HRMS calcd for $C_{14}H_{13}N_3O$ (M^+) 239.1059, found 239.1052.

(±)-*cis*-3-Hydroxy-4-amino-1,2,3,4-tetrahydrophenanthrene (9). The azido alcohol **8** from the previous step (62 mg, 0.26 mmol in 2.0 mL of methanol) and 5% Pd on carbon (5 mg) were stirred at rt for 4 h in a hydrogen atmosphere. The reaction mixture was centrifuged to remove the catalyst, which was washed twice with methanol by centrifugation. The MeOH solution from the reaction mixture was pooled with the MeOH washings and evaporated under reduced pressure. The resulting solid was loaded onto a silica gel column packed in CH_2Cl_2 and sequentially eluted with CH_2Cl_2 followed by 10% MeOH in CH_2Cl_2. Pure amino alcohol (41 mg, 74%) was obtained as a white solid after crystallization from benzene: mp 238–239 °C. ^1H NMR ($CDCl_3$): 8.08 (d, 1 H_5, J = 8.5); 7.84 (d, 1 H_8, J = 7.8); 7.68 (d, 1 H_9, J = 8.3); 7.56 (dt, 1 H_6, J = 1.3, 8.5); 7.46 (t, 1 H_7, J = 7.8); 7.22 (d, 1 H_{10}, J = 8.3); 4.61 (d, 1 H_4, J = 4.2); 3.95 (dt, 1 H_3, J = 4.2, 12.1); 3.01 (dd, 2 H_1, J = 3.9, 8.9); 2.05 (m, 1 H_2); 1.91 (m, 1 H_2). MS (FAB) m/e: 214 (M^+ + 1); HRMS calcd for $C_{14}H_{15}NO$ (M^+) 213.1154, found 213.1155.

***cis*-N^6-(4-(3-Hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (11 and 12).** The 6-fluoro dA derivative **10** (54 mg, 0.11 mmol) and racemic amino alcohol **9** (12 mg, 0.056 mmol) were stirred with hexamethyldisiloxane (0.60 mL, 2.8 mmol) and 2,6-lutidine (13 μL, 0.11 mmol) in 0.5 mL of DMF at 90 °C. After 4 h, complete consumption of the amino alcohol was observed by TLC. The reaction mixture was cooled, and the volatiles were evaporated under reduced pressure. The mixture was diluted with CH_2Cl_2 and extracted with water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to provide a white solid which was chromatographed on an Axxiom silica HPLC column (5 μm, 10 × 250 mm) eluted with 2% MeOH in CH_2Cl_2 at a flow rate of 5 mL/min. The early- and late-eluting diastereomers had retention times of 4.9 and 5.8 min, respectively. Early-eluting diastereomer (12 mg): ^1H NMR (acetone-d_6) 8.42, 8.09 (2 s, 2 $H_{8'',2''}$); 7.98 (d, 1 H_5, J = 7.9); 7.85 (d, 1 H_8, J = 8.8); 7.78 (d, 1 H_9, J = 8.5); 7.32–7.40 (m, 2 $H_{6,7}$); 7.28 (d, 1 H_{10}, J = 8.5); 6.90 (br, N*H*); 6.45 (br, 2 $H_{1',4}$); 4.77 (br, 1 $H_{3'}$); 4.26(dt, 1 H_3, J = 3.8, 12.5); 3.91–3.98 (m, 2 $H_{4',5'}$); 3.78 (dd, 1 $H_{5'}$, J = 3.0, 10.6); 3.09 (br, 2 H_1); 2.98 (m, 1 $H_{2'}$); 2.44 (m, 1 $H_{2'}$); 2.30 (m, 1 H_2); 1.98 (m, 1 H_2); 0.9–1.0 (18 H, *t*-Bu); 0.05–0.17 (12 H, CH_3). HRMS calcd for $C_{36}H_{54}N_5O_4Si_2$ (M^+ + 1): 676.3714 found 676.3722. Late-eluting diastereomer (13 mg): ^1H NMR (acetone-d_6) 8.42, 8.07 (2 s, 2 $H_{8'',2''}$); 8.00 (d, 1 H_5, J = 8.1); 7.85 (d, 1 H_8, J = 9.1); 7.78 (d, 1 H_9, J = 8.5); 7.32–7.42 (m, 2 $H_{6,7}$); 7.28 (d, 1 H_{10}, J = 8.5); 6.88 (br, N*H*); 6.45 (br, 2 $H_{1',4}$); 4.78 (quint, 1 $H_{3'}$, J = ~3.3); 4.26 (dt, 1 H_3, J = 3.6, 12.4); 3.92–3.98 (m, 2 $H_{4',5'}$); 3.80 (dd, 1 $H_{5'}$, J = 3.3, 10.3); 3.08 (br, 2 H_1); 2.98 (quint, 1 $H_{2'}$, J_{app} = 6.0); 2.42 (br m, 1 $H_{2'}$); 2.28 (m, 1 H_2); 1.96 (m, 1 H_2); 0.9–1.0 (18 H, *t*-Bu); 0.05–0.17 (12 H, CH_3). HRMS calcd for $C_{36}H_{54}N_5O_4Si_2$ (M^+ + 1): 676.3714, found 676.3733. Total yield of the two diastereomers in pure form was 66%. See Results and Discussion for assignment of absolute configuration.

(3*R*,4*S*)-N^6-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (13). The early-eluting diastereomer (12 mg, 18 μmol) was stirred in 200 μL of anhydrous pyridine with 200 μL of acetic anhydride at rt overnight. The solution was concentrated to dryness, and benzene was added and evaporated from the flask three times to remove residual pyridine. The crude monoacetoxy compound was dried further under vacuum, loaded onto a 250-μm (10 × 20 cm) silica gel plate, and chromatographed with 1% MeOH in CH_2Cl_2. Pure **13** (11 mg, 87%) was obtained as a white solid. ^1H NMR (acetone-d_6): 8.42, 7.90 (2 s, 2 $H_{8'',2''}$); 7.97 (d, 1 H_5, J = 8.2); 7.85 (d, 1 H_8, J = 7.7); 7.80 (d, 1 H_9, J = 8.5); 7.29–7.44 (m, 2 $H_{6,7}$); 7.28 (d, 1 H_{10}, J = 8.5); 7.14 (br d, N*H*, J = 9.3); 6.72 (dd, 1 H_4, J = 3.9, 9.3); 6.41 (br t, 1 $H_{1'}$); 5.34 (dt, 1 H_3, J = 3.9, 13.2); 4.78 (m, 1 $H_{3'}$, J = ~3); 3.89–3.96 (m, 2 $H_{4',5'}$); 3.79 (dd, 1 $H_{5'}$, J = 3.9, 10.7); 3.17 (br, 2 H_1); 2.98 (quint, 1 $H_{2'}$, J_{app} = 6.9); 2.60 (m, 1 H_2); 2.42 (ddd, 1 $H_{2'}$, J = 3.6, 6.3, 13.2); 2.00 (m, 1 H_2); 1.77 (s, 3 H, OAc); 0.9–1.0 (18 H, *t*-Bu); 0.05–0.17 (12 H, CH_3). HRMS calcd for $C_{38}H_{56}N_5O_5Si_2$ (M^+ + 1) 718.3820, found 718.3784.

(3*S*,4*R*)-N^6-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (14). The late-eluting diastereomer **12** (13 mg, 19 μmol) was acetylated in a manner identical to that described for the preparation of **13**. Chromatography as before provided **14** (11 mg, 81%) as a white solid. ^1H NMR (acetone-d_6): 8.43, 7.96 (2 s, 2 $H_{8'',2''}$); 7.95 (d, 1 H_5, J = 7.8); 7.84 (d, 1 H_8, J = 7.6); 7.80 (d, 1 H_9, J = 8.5); 7.12–7.42 (m, 2 $H_{6,7}$); 7.30 (d, 1 H_{10}, J = 8.5); 7.12 (br d, N*H*, J = 8.9); 6.71 (dd, 1 H_4, J = 3.9, 8.9); 6.43 (t, 1 $H_{1'}$, J = 6.7); 5.34 (dt, 1 H_3, J = 3.9, 13.3); 4.76 (m, 1 $H_{3'}$, J = ~3); 3.88–3.96 (m, 2 $H_{4',5'}$); 3.79 (dd, 1 $H_{5'}$, J = 4.1, 10.9); 3.17 (br, 2 H_1); 2.96 (quint, 1 $H_{2'}$, J_{app} = 7.1); 2.60 (m, 1 H_2); 2.42 (ddd, 1 $H_{2'}$, J = 3.6, 6.7, 13.2); 2.00 (m, 1 H_2); 1.78 (s, 3 H, OAc); 0.9–1.0 (18 H, *t*-Bu); 0.05–0.17 (12 H, CH_3). HRMS calcd for $C_{38}H_{56}N_5O_5Si_2$ (M^+ + 1) 718.3820, found 718.3815.

(3*R*,4*S*)-N^6-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine (15). The disilyl monoacetoxy compound **13** resulting from the early-eluting diastereomer (11 mg, 15 μmol in 0.10 mL of THF) was stirred at rt for 1.5 h with *n*-$Bu_4N^+F^-$ (34 μL of a 1.0 M solution in THF, 2.2 equiv), and the mixture was evaporated to dryness. Chromatography of the resulting product on a 250-μm (10 × 20 cm) silica gel plate using 4% MeOH in CH_2Cl_2 provided the free mononucleoside **15** (6.7 mg, 89%) as a white solid. ^1H NMR (acetone-d_6): 8.41, 7.86 (2 s, 2 $H_{8'',2''}$); 7.96 (d, 1 H_5, J = 8.0); 7.86 (d superimposed on a purine signal, 1 H_8); 7.82 (d, 1 H_9, J = 8.5); 7.33–7.44 (m, 2 $H_{6,7}$); 7.30 (d, 1 H_{10}, J = 8.5); 6.70 (br, 1 H_4); 6.38 (dd, 1 $H_{1'}$, J = 5.7, 8.8); 5.34 (dt, 1 H_3, J = 3.8, 13.2); 4.63 (d, 1 $H_{3'}$, J = 5.2); 4.09 (br d, 1 $H_{4'}$, J_{app} = 1.7); 3.80 (dd, 1 $H_{5'}$, J = 2.5, 12.3); 3.68 (dd, 1 $H_{5'}$, J = 2.3, 12.3); 3.15 (m, 2 H_1); 2.88 (ddd, 1 $H_{2'}$, J = 5.4, 8.8, 13.1); 2.57 (m, 1 H_2); 2.30 (ddd, 1 $H_{2'}$, J = 1.7, 5.7, 13.1); 2.00 (br, 1 H_2); 1.80 (s, 3 H, OAc). On addition of a few drops of MeOH-d_4, the signal at 6.70 ppm sharpens, suggesting an exchangeable proton at this position. MS (EI) m/e: 489 (M^+), 429, 312, 178; HRMS calcd for $C_{26}H_{28}N_5O_5$ (M^+ + 1) 490.2090, found 490.2108.

(3*S*,4*R*)-N^6-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine (16). The disilyl monoacetoxy compound **14** resulting from the late-eluting diastereomer (11 mg, 15 μmol) was treated with *n*-$Bu_4N^+F^-$ as above for **13**. Workup and chromatography as in the case of 15 produced **16** (6.8 mg, 88%) as a white solid. ^1H NMR (acetone-d_6): 8.41, 7.96 (2 s, 2 $H_{8'',2''}$); 7.96 (d superimposed on a purine signal, 1 H_5); 7.84 (dd, 1 H_8, J = 2.2, 7.3); 7.80 (d, 1 H_9, J = 8.5); 7.32–7.44 (m, 2 $H_{6,7}$); 7.30 (d, 1 H_{10}, J = 8.5); 6.71 (br, 1 H_4); 6.38 (dd, 1 $H_{1'}$, J = 5.8, 8.8); 5.34 (dt, 1 H_3, J = 3.8, 13.3); 4.64 (br d, 1 $H_{3'}$, J = 4.9); 4.08 (br d, 1 $H_{4'}$, J_{app} = 1.7); 3.80 (dd, 1 $H_{5'}$, J = 2.3, 12.4); 3.68 (dd, 1 $H_{5'}$, J = 2.0, 12.4); 3.17 (m, 2 H_1); 2.92 (ddd, 1 $H_{2'}$, J = 5.4, 8.8, 13.1); 2.60 (m, 1 H_2); 2.35 (ddd, 1 $H_{2'}$, J = 1.6, 5.8, 13.1); 2.00 (br, 1 H_2); 1.80 (s, 3 H, OAc). On addition of a few drops of MeOH-d_4, the signal at 6.71 ppm sharpens, suggesting an ex-

changeable proton at this position. MS (EI) m/e: 489 (M^+), 429, 312, 178; HRMS calcd for $C_{26}H_{28}N_5O_5$ ($M^+ + 1$) 490.2090, found 490.2110. UV (MeOH): λ_{max} 225 (ϵ_{max} 86 500), 278 (25 600). CD spectrum: Figure 1. The substantial difference between these extinction coefficients and CD intensities and those previously reported by us[5] for the closely related trans adduct, (3*R*,4*R*)-*N*^{6}-(4-(3-hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine led us to redetermine the extinction coefficients for the latter compound: corrected values of 68 600 (224 nm) and 20 600 (278 nm) were obtained. Since estimation of the quantities and concentrations of several compounds in the previous report depended on the use of ϵ_{278}, the following corrections are required: (1) values of $\Delta\epsilon$ for CD spectra in ref 5 should be corrected as described in the legend of Figure 1 and (2) the reported[5] yields of the four diastereomeric deoxyadenosine adducts from reaction of 1,2,3,4-tetrahydrophenanthrene 3,4-epoxide with 2′-deoxyadenosine 5′-monophosphate should be divided by 6.9 (for the trans adducts) or 8.6 (for the cis adducts).

(3*S*,4*R*)-*N*^{6}-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosine (17). The mononucleoside **16** (6.8 mg, 14 μmol), $DMT^+BF_4^-$ (5.4 mg, 14 μmol) and Li_2CO_3 (2 mg, 27 μmol) were dried in a cone-vial under vacuum for 20 min. 2,6-Lutidine (20 μL) was added, and the mixture was stirred at rt for 1 h under argon. Since TLC indicated the presence of starting nucleoside, another 5.4 mg of $DMT^+BF_4^-$ was added, and stirring was continued for an additional 2 h. A third portion (2.7 mg) of $DMT^+BF_4^-$ was added to the reaction mixture after cooling to 0 °C, and the mixture was warmed to rt over 15–20 min. At this time TLC indicated that very little **16** remained. The mixture was diluted with CH_2Cl_2 and filtered into a few drops of MeOH. The filtrate was concentrated, and the residue was chromatographed on a 250-μm (10 × 20 cm) silica gel plate using 98% CH_2Cl_2:1% MeOH:1% Et_3N. The product **17** was obtained as a white solid (7.6 mg, 69% based upon 1.8 mg of recovered **16**). ^1H NMR (acetone-d_6): 8.26, 7.90 (2 s, 2 $H_{8'',2''}$); 7.96 (d, 1 H_5, J = 8.3); 7.83 (d, 1 H_8, J = 7.9); 7.78 (d, 1 H_9, J = 8.6); 6.80–7.50 (aromatic, 16 H); 6.70 (dd, 1 H_4, J = 4.0, 9.0); 6.43 (t, 1 $H_{1'}$, J = 6.6); 5.34 (dt, 1 H_3, J = 4.0, 13.4); 4.70 (br, 1 $H_{3'}$); 4.13 (q, 1 $H_{4'}$, J_{app} = 4.8); 3.74 (s, 6 H, OCH_3); 3.35 (br, 2 $H_{5'}$); 3.16 (br, 2 H_1); 2.94 (quint, 1 $H_{2'}$, J_{app} = 6.2); 2.60 (m, 1 H_2); 2.46 (ddd, 1 $H_{2'}$, J = 3.8, 6.2, 13.2); 1.90 (m, 1 H_2); 1.75 (s, 3 H, OAc). The mass spectrum (EI) of the product only shows a signal for the DMT cation.

(3*S*,4*R*)-*N*^{6}-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-[(*N*,*N*-diisopropylamino)(β-cyanoethoxy)phosphinyl]-2′-deoxyadenosine (18). The 5′-DMT-protected nucleoside **17** (7.6 mg, 9.6 μmol) was stirred in anhydrous CH_2Cl_2 (0.10 mL) and Et_3N (11 μL, 77 μmol). The mixture was transferred to a glove bag filled with argon, and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (4.3 μL, 19 μmol) was added. The reaction mixture was capped under argon, stirred at rt for 1 h, quenched with a few drops of MeOH, and evaporated to dryness. Chromatography of the product on a 250-μm (10 × 20 cm) silica gel plate using 98% CH_2Cl_2:1% MeOH:1% Et_3N gave the desired phosphoramidite **18** (5.9 mg, 62%), which was judged homogeneous by ^{31}P NMR (Varian XL-300, 121.4 MHz, in acetone-d_6): 149.39 ppm with reference to 0.10 M phosphoric acid as external standard. The ^{31}P signals for the two diasteromers which comprise phosphoramidite **18** were unresolved.

Synthesis of an Oligodeoxynucleotide Containing the PAH-Adducted dA. Synthesis of the adducted DMT pentamer TpGpA*pGpT, where A* represents adducted dA derived from the 3*S*,4*R* diastereomer **18** was carried out using 2 μmol of T-substituted controlled pore glass (CPG) packed in a 1-μmol size column. Automated steps were done on an Applied Biosystems DNA synthesizer with the following modifications of the synthesizer program (Model 392/394, System Software Version 1.01) to compensate for the larger scale of the synthesis: (1) solvent delivery times were increased by a factor of approximately 2, (2) delivery times for tetrazole and tetrazole plus phosphoramidite were increased by a factor of 5, (3) the endcapping procedure was performed twice per cycle with increases in the delivery time (twofold) and residence time (fourfold) of the capping reagent on the column, (4) delivery time of the detritylation reagent was increased by 1.5 and residence time of this reagent on the column increased by a factor of 2, and (5) an additional solvent wash and reverse flush of the column were introduced following the final treatment with the detritylation reagent. To eliminate contact of the column with water and thus minimize potential hydrolysis of the valuable phosphoramidite **18**, the aqueous iodine reagent normally used for oxidation of the CPG-bound phosphite triester intermediate was replaced by a solution of 1.1 M *tert*-butyl hydroperoxide[18] in 4:1 methylene chloride–2,2,4-trimethylpentane. Delivery and residence times on the column for this reagent were 15 and 40 s, respectively. Before beginning the synthesis the column was washed with MeCN, dried under argon, endcapped for 2.5 min with 1.0 mL of a 1:1 mixture of Ac_2O/THF (1.5 mL/3.3 mL) and 4-(*N*,*N*-dimethylamino)pyridine/pyridine/THF (350 mg/3.0 mL/3.0 mL), washed with MeCN, and purged with argon. Addition, oxidation, and deblocking of the first dG residue were carred out on the synthesizer, after which the column was removed from the synthesizer and purged with argon. Phosphoramidite **18** (5.9 mg, 5.9 μmol, in ~0.1 mL of dry MeCN) was manually added to the column followed by ~0.15 mL of 0.5 M tetrazole in dry MeCN, and reaction was allowed to proceed for 1.5 h in an argon atmosphere. Following reaction, the column was purged of reagents, washed with MeCN, subjected to the manual endcapping procedure as described for initial preparation of the column, and returned to the synthesizer for automated addition of the final dG and T residues. The yield for introduction of the modified residue, relative to the growing oligonucleotide chain, as measured by recovery of the DMT cation after the manual coupling step, was 37%.

The ammonia solution of the modified oligonucleotide eluted from the support was heated overnight at 55 °C, concentrated, and made up to a volume of 2.0 mL with water. A 100-μL portion was subjected to gradient HPLC at a flow rate of 2.5 mL/min on a Hamilton PRP-1 column (10 μm, 7.0 × 305 mm). The gradient was ramped from 80% A/20% B to 100% B over 15 min, and elution was continued at 100% B for 5 min. Solvents A and B consisted of 0.1 M triethylammonium carbonate and 50% MeCN in 0.1 M triethylammonium carbonate, respectively, both at pH 7.5. A solution of the unmodified pentamer prepared as described, but entirely on the synthesizer, was analyzed at the same time. The HPLC peaks corresponding to the normal (13.3 min) and adducted (15.2 min) pentamers were collected and diluted to known volume for estimation of yield: 65 and 28.5 A_{260} units for the normal and adducted DMT oligonucleotides, respectively. Upon detritylation (30 min, 80% acetic acid in water), removal of dimethoxytrityl alcohol by extraction into ethyl acetate, and lyophilization, the modified nucleotide TpGpA*pGpT (23 A_{260} units) was obtained. Both the normal and adducted pentamers were essentially (≥98%) free of contaminants absorbing at 260 nm on capillary zone electrophoresis (0.2% ammonium carbonate, 20 kV over 15 min, capillary length 70 cm). On negative ion FAB mass spectrometry the adducted pentamer gave m/e 1712 (M − 1).