# Chemical Synthesis of a Bay-Region Polycyclic Aromatic Hydrocarbon Tetrahydroepoxide-Deoxyadenosine Adduct and Its Site-Specific Incorporation into a DNA Oligomer

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

*Contribution from the Section on Oxidation Mechanisms, Laboratory of Bioorganic Chemistry, NIDDK, The National Institutes of Health, Bethesda, Maryland 20892.*
*Received December 18, 1990*

**Abstract:** The bay-region tetrahydrophenanthrene-3,4-epoxide represents a prototype for the carcinogenic bay-region diol epoxides of polycyclic aromatic hydrocarbons. We describe herein the chemical synthesis of the trans N-6 amino adduct of 2'-deoxyadenosine (dA) with the tetrahydroepoxide at the benzylic 4-position. The adduct was prepared by coupling of the trans C-4 aminolysis product of the epoxide with the 6-fluoro analogue of dA in which the sugar hydroxyl groups were protected as silyl ethers. The resulting pair of diastereomers, after chromatographic separation, were assigned absolute configurations through the use of optically pure epoxide of known configuration. Stereoisomerically identical trans adducts as well as the corresponding pair of diastereomers resulting from cis addition are formed on reaction of the racemic epoxide with 2'-deoxyadenosine 5'-phosphate. The (3*S*,4*S*)-diastereomer, after appropriate derivatization, has been incorporated in high yield into the deoxypentamer TpGpApGpT to document the utility of the nucleotide coupling as well as the blocking/deblocking procedures in the synthesis of a hydrocarbon-adducted oligomer. Synthetic accessibility of DNA containing single, specific adducts of carcinogenic bay-region diol epoxides will be of immense value to an understanding of how such modified sequences are enzymatically processed within the cell and how such processing results in cell transformation.

## Introduction

Carcinogenic polycyclic aromatic hydrocarbons (PAHs) are metabolized to bay-region diol epoxides (Figure 1), which are their ultimate carcinogenic forms.[1,2] It is thought that these metabolically formed, electrophilic carcinogens exert their effects by covalent bonding to DNA bases.[3-9] Although the mechanism(s) by which these reactions occur remains a subject of active investigation (see ref 9), it is clear that the major DNA adducts formed by such covalent interactions result from the attachment of the exocyclic amino groups of deoxyadenosine (dA) and deoxyguanosine (dG) to the benzylic carbon of the diol epoxides through both cis and trans ring opening.[3-11] Since mammals form bay-region diol epoxides from *trans*-dihydrodiols, two enantiomeric pairs of diastereomeric diol epoxides are possible in which the benzylic hydroxyl group and epoxide oxygen are either cis or trans (diol epoxides-1 and -2, respectively, Figure 1). Although the (*R*,*S*,*S*,*R*)-stereoisomer (Figure 1) is generally the most tumorigenic, wide variations in biological activity have been observed for the sets of bay-region diol epoxides from a given hydrocarbon.[1,2,12-15] Given these four stereoisomers, 16 adducts are possible upon both cis and trans ring opening by the exocyclic amino groups of dA and dG. All 16 adducts are formed to varying degrees upon in vitro reaction of these sets of four isomers from several PAHs with purified DNA.[9]

There is an obvious need to understand the relationship between these adducts and cellular transformation. In one approach, attempts to correlate gross chemical reactivity as exemplified by either (i) extent of bonding to DNA versus formation of biologically inactive solvolysis products, (ii) ratio of dA to dG adducts, or (iii) ratio of adducts resulting from cis versus trans opening of the epoxide ring with relative tumorigenic activity of the individual diol epoxides have thus far been unrewarding. In a second approach, biological experiments to detect either chemical or mutational "hot spots" in DNA after exposure to diol epoxides have also not yielded any positive correlation between biological response and diol epoxide structure.[16,17] These studies have indicated that purine-rich sequences in DNA are preferred targets for the diol epoxides.[9] Synthesis and study of specifically adducted DNA constitutes a third approach. Direct reaction of single-stranded DNA oligomers with diol epoxides is impractical due to poor yield and difficulties in separation of products and is limited since only sequences containing a single dA or dG residue can

(1) 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 *Polycyclic Aromatic Hydrocarbons and Carcinogenesis*; Harvey, R. G., Ed.; ACS Symposium Series 283, American Chemical Society: Washington, DC, 1985; pp 63-84.
(2) Thakker, D. R.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Jerina, D. M. In *Bioactivation of Foreign Compounds*; Anders, M. W., Ed.; Academic Press: New York, 1985; pp 177-242.
(3) (a) Meehan, T.; Straub, K.; Calvin, M. *Nature (London)* **1977**, *269*, 725-727. (b) Meehan, T.; Straub, K. *Nature (London)* **1979**, *277*, 410-412.
(4) Dipple, A.; Pigott, M. A.; Agarwal, S. K.; Yagi, H.; Sayer, J. M.; Jerina, D. M. *Nature (London)* **1987**, *327*, 535-536.
(5) 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.
(6) Cheng, S. C.; Prakash, A. S.; Pigott, M. A.; Hilton, B. D.; Roman, J. M.; Lee, H.; Harvey, R. G.; Dipple, A. *Chem. Res. Toxicol.* **1988**, *1*, 216-221.
(7) 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.
(8) Sayer, J. M.; Chadha, A.; Agarwal, S. K.; Yeh, H. J. C.; Yagi, H.; Jerina, D. M. *J. Org. Chem.* **1991**, *56*, 20-29.
(9) 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.
(10) Reardon, D. B.; Prakash, A. S.; Hilton, B. D.; Roman, J. M.; Pataki, J.; Harvey, R. G.; Dipple, A. *Carcinogenesis* **1987**, *8*, 1317-1322.
(11) Vericat, J. A.; Cheng, S. C.; Dipple, A. *Carcinogenesis* **1989**, *10*, 567-570.
(12) 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 Compound Metabolism*; Caldwell, J., Paulson, G. D., Eds.; Taylor and Francis Ltd.: London, 1984; pp 257-266.
(13) Yagi, H.; Sayer, J. M.; Thakker, D. R.; Levin, W.; Jerina, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 838-846. Chang, R. L.; Wood, A. W.; Conney, A. H.; Yagi, H.; Sayer, J. M.; Thakker, D. R.; Jerina, D. M.; Levin, W. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8633-8636.
(14) Levin, W.; Wood, A. W.; Chang, R. L.; Ittah, Y.; Croisy-Delcey, M.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1980**, *40*, 3910-3914.
(15) 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.
(16) Reardon, D. B.; Bigger, C. A. H.; Strandberg, J.; Yagi, H.; Jerina, D. M.; Dipple, A. *Chem. Res. Toxicol.* **1989**, *2*, 12-14.
(17) Bigger, C. A. H.; Strandberg, J.; Yagi, H.; Jerina, D. M.; Dipple, A. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2291-2295. Yang, J.-L.; Maher, V. M.; McCormick, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3787-3791. Carothers, A. M.; Urlaub, G.; Mucha, J.; Harvey, R. G.; Chasin, L. A.; Grunberger, D. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5464-5468.

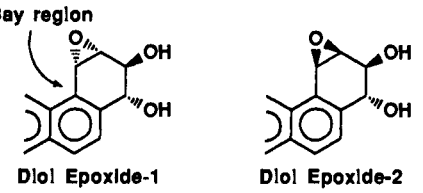
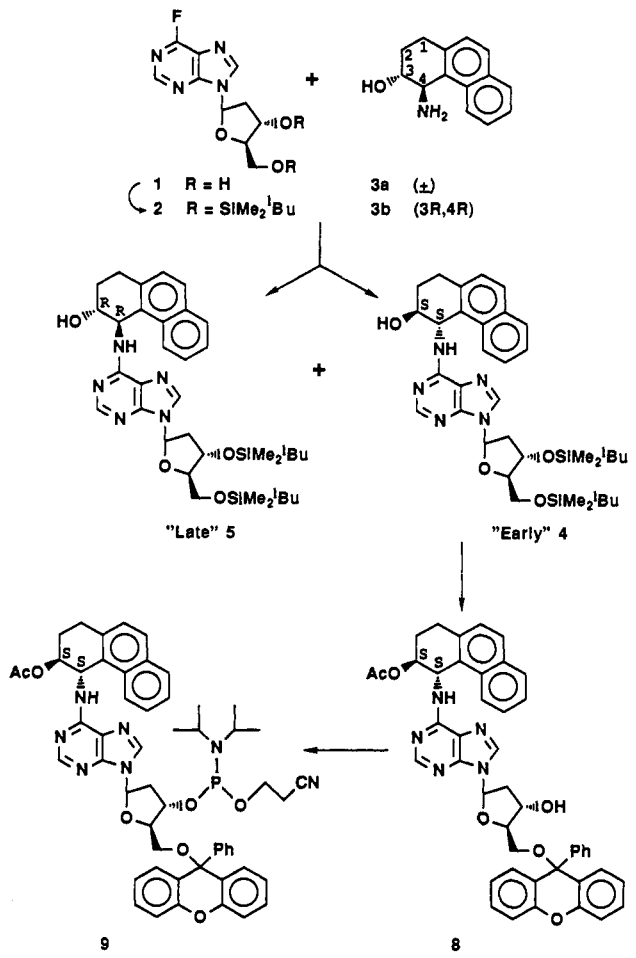



**Figure 1.** Partial structures of an (*R,S,R,S*)-diol epoxide-1 diastereomer in which the benzylic hydroxyl group and epoxide oxygen cis and an (*R,S,S,R*)-diol epoxide-2 diastereomer in which these groups are trans. Absolute configurations are designated from the benzylic hydroxyl group to the benzylic epoxide carbon.

realistically be considered.[18] Reaction of nucleosides or mononucleotides with diol epoxides for further incorporation into DNA oligomers is also impractical due to the low nucleophilicity of the exocyclic amino groups which results in poor yields. Furthermore, the reaction is not stereospecific, in that both cis and trans adducts may be formed. A more promising strategy for synthesis of nucleoside–diol epoxide adducts for incorporation into DNA oligomers involves coupling of a nucleophilic amino derivative of the hydrocarbon with an electrophilic derivative of the nucleoside. Although the construction of DNA oligomers in which the exocyclic amino group of a purine base is attached to the methyl group of a methyl-PAH has been described,[19,20] such adducts are not thought to be responsible for cell transformation nor does the synthetic route address the coupling and blocking problems associated with diol epoxide adducts. Specifically, the unique conformational features of the tetrahydrobenzo ring as well as the hydroxyl groups are not present in such methyl-PAH derivatives. Synthesis of a naphthalene diol epoxide-2 adduct of dC has been described.[21]

Herein, we describe methodology suitable for the site-specific incorporation of a bay-region PAH diol epoxide *trans*-dA adduct into DNA oligomers. Our studies have used the readily accessible bay-region 3,4-epoxide of 1,2,3,4-tetrahydrophenanthrene as starting material in order to conserve synthetically more valuable diol epoxides. Briefly, the epoxide was converted to the *trans*-4-amino-3-hydroxy derivative **3** by direct aminolysis. Since the amino group is sterically restrained in the axial orientation due to its presence in the bay region and is hence less nucleophilic than comparable equatorial benzylic amino groups on tetrahydro PAH derivatives, the relatively reactive 6-fluoro analogue of dA was selected for the coupling reaction.[22] Although the 6-chloro analogue has found limited use in the synthesis of methyl PAH nucleoside adducts,[20,26] anticipated low reactivity of the present bay-region amine suggested that the 6-fluoro derivative of dA would be a more effective reactant. Prior to coupling with the amine, the 3′- and 5′-hydroxyl groups of the nucleoside were protected as silyl ethers. Blocking of the sugar hydroxyl groups at this stage is key to the synthesis in that this allows subsequent discrimination between the secondary 3-hydroxyl group of the hydrocarbon (or three secondary hydroxyl groups in the case of a diol epoxide from a PAH) and the secondary 3′-hydroxyl group of the sugar. As the starting amino alcohol was racemic and the fluoronucleoside was optically pure, the coupling mixture consisted of a diastereomeric pair of nucleoside adducts which were readily separated chromatographically. This is of synthetic advantage in that optically pure products derived from both enantiomers of a given diol epoxide, as required for physical, biochemical, and biological studies of the adducted DNA oligomers, can be isolated from racemic diol epoxides. Acetylation of the hydrocarbon hydroxyl group, removal of the silyl ethers on the sugar hydroxyl groups, blocking of the primary 5′-hydroxyl of the sugar as a 9-phenyl-9-xanthenyl (pixyl) ether, and phosphitylation of the remaining sugar 3′-hydroxyl group provided the dA–hydrocarbon adduct in a form ready for incorporation into an oligomer.

Scheme I




## Results and Discussion

Initial attempts to protect the sugar 3′- and 5′-hydroxyl groups of the known 6-fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine (6-FP)[23] **1** by silylation with imidazole *tert*-butyldimethylsilyl (TBDMS) chloride in the usual fashion proved unsatisfactory in that a 1:1 mixture of the desired disilyl compound **2** and a product resulting from the replacement of the 6-fluoro substituent by imidazole was produced. During the course of this work, it was observed that pyridine does not cause replacement of the fluorine in 6-FP at 0 °C. Thus, 6-FP and TBDMS trifluoromethanesulfonate were stirred in pyridine at 0 °C. Reaction was complete within 1 h, and the desired product was obtained in excellent yield.

Both racemic and optically pure 1,2,3,4-tetrahydrophenanthrene-3,4-epoxide were synthesized from 1,2-dihydrophenanthrene-4(3*H*)-one as described.[24,25] Reaction of the epoxide with ammonia in a Parr high-pressure reactor at 100 °C for 22 h provided the desired racemic 4-amino-3-alcohol **3a** in good yield and high purity. Optically active (3*R*,4*R*)-amino alcohol **3b** was obtained from the (3*R*,4*S*)-epoxide in a similar fashion.

(18) Benasutti, M.; Ezzedine, Z. D.; Loechler, E. L. *Chem. Res. Toxicol.* **1988**, *1*, 160–168. Cosman, M.; Ibanez, V.; Geacintov, N. E.; Harvey, R. G. *Carcinogenesis* **1990**, *11*, 1667–1672.
(19) Casale, R.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1990**, *112*, 5264–5271.
(20) Stezowski, J. J.; Stigler, R. D.; Joos-Guba, G.; Kahre, J.; Lösch, G. R.; Carrell, H. L.; Peck, R. M.; Glusker, J. P. *Cancer Res.* **1984**, *44*, 5555–5566. Stezowski, J. J.; Joos-Guba, G.; Schönwälder, K. H.; Straub, A.; Glusker, J. P. *J. Biomol. Struct. Dyn.* **1987**, *3*, 615–637.
(21) Smith, C. A.; Harper, A. E.; Coombs, M. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2745–2750.
(22) Lakshman, M.; Lehr, R. E. *Tetrahedron Lett.* **1990**, *31*, 1547–1550.
(23) Robins, M. J.; Basom, G. L. *Can. J. Chem.* **1973**, *51*, 3161–3169.
(24) Boyd, D. R.; Greene, R. M. E.; Neill, J. D.; Stubbs, M. E.; Yagi, H.; Jerina, D. M. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1477–1482.
(25) Balani, S. K.; Boyd, D. R.; Cassidy, E. S.; Devine, G. E.; Malone, J. F.; McCombe, K. M.; Sharma, N. D. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2751–2756.
(26) Lee, H.; Hinz, M.; Stezowski, J. J.; Harvey, R. G. *Tetrahedron Lett.* **1990**, *31*, 6773–6776.

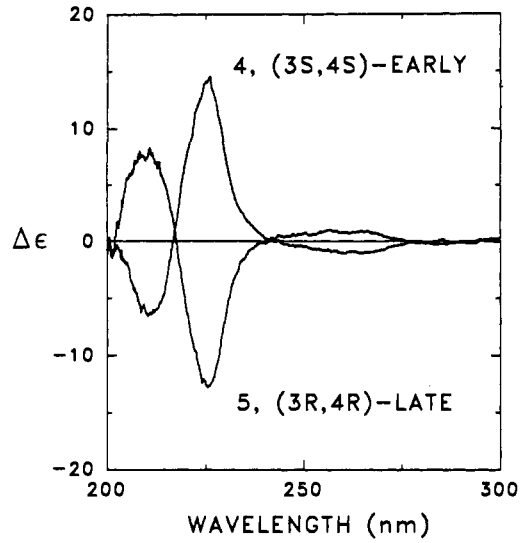



**Figure 2.** CD spectra in methanol of the early (3*S*,4*S*-**4**) and late (3*R*,4*R*-**5**) eluting dA adducts as their 3′,5′-disilyl ethers. Preparation of the dA adduct from the (3*R*,4*S*)-epoxide produced only the late eluting (3*R*,4*R*)-diastereomer **5** with a CD spectrum identical with that shown.

Coupling of the 9-(benzoyloxy)-10-amino-derivative of 7,8,9,10-tetrahydrobenzo[*a*]pyrene with 6-FP has previously been described.[22] The solvent of choice for this reaction was DMF.[22] However, similar reaction of disilyl 6-FP **2** with the tetrahydrophenanthrene amino alcohol **3a** resulted in loss of one silyl group (possibly 5′-) from the desired adduct. Addition of hexamethyldisiloxane (HMDS) to the reaction mixture as a potential "fluoride sponge" effectively prevented loss of the silyl blocking groups. Separation of the resulting diastereomeric adducts **4** and **5** (Scheme **I**) formed from the racemic amino alcohol was readily achieved by HPLC. The *late* eluting diastereomer **5** had the same HPLC retention time and CD spectrum as that produced from the (3*R*,4*R*)-amino alcohol.

Absolute configurations of the two diastereomeric adducts are consistent with their CD spectra (Figure 2). Nucleoside adducts of diol epoxides at the exocyclic amino groups of the purine bases display strong exciton coupling bands due to electric transition dipole interactions between the hydrocarbon and purine chromophores. Chiral carbinol centers contribute little to the observed CD spectra as their intensities are far weaker than the exciton bands. Examination of the CD spectra for the 16 dA and dG bay-region diol epoxide adducts from four hydrocarbons has allowed an empirical correlation between absolute configuration at the N-substituted benzylic carbon of the hydrocarbon and their spectra.[5,8,9] In dA adducts, two major CD bands of opposite sign are generally observed. For adducts with *R* absolute configuration at the N-substituted benzylic carbon atom, the shorter wavelength band is negative, whereas the longer wavelength band is positive. In the case of the adducts reported here, these CD bands appear at shorter wavelengths than previously observed due to the shorter wavelength absorption of the naphthalene chromophore in tetrahydrophenanthrene compared to the other hydrocarbons studied. For example, the $^1B_b$ transition, parallel to the long axis of the aromatic chromophore, that appears at 252 nm in anthracene is shifted to 220 nm in naphthalene. Thus, when the short wavelength shift due to the naphthalene chromophore is taken into account, the negative Cotton effect at 225 nm for the (3*R*,4*R*)-adduct **5** is consistent with our correlation for other hydrocarbons.

NMR spectra of the adducts were temperature dependent. Although the signals are quite sharp at +50 °C, there is a general broadening of all the signals on cooling to −45 °C (500 MHz, acetonitrile-$d_3$) at which point the H-1′ resonance splits into two signals indicating an approximately 5:1 ratio of two conformers. In addition, the CD band at 225 nm increased 17% on cooling the sample from +25 °C to −19 °C. Previous temperature-dependent NMR studies suggested that the present NMR effects may result from changes in the rate and/or equilibrium for interconversion of conformers resulting from either inversion or hindered rotation at the N-6 amino group of the adenine.[22] A rapid sharpening of the purine proton signals at C-2 and C-8 was observed on increasing temperature.[22]

Unequivocal structure assignment for the adducts was achieved by reaction of the racemic epoxide with 2′-deoxyadenosine 5′-monophosphate at pH 7.1 and 37 °C for 20 h. After workup and enzymatic removal of the phosphate group, four $N_6$-adducts (~30% yield based on starting epoxide) were isolated by HPLC (ratio 1:1:1:0.5 in order of elution on reverse phase). The high yield of adducts relative to hydrolysis products is noteworthy. NMR spectra of the adducts as their acetates (particularly the $J_{2ax,3}$ coupling constant) established that the two early eluting isomers arose by trans addition of the $N_6$-amino group to $C_4$ of the epoxide, whereas the two late eluting isomers arose by cis addition. Use of the optically active (−)-tetrahydrophenanthrene-(3*R*,4*S*)-epoxide produced only the second- and third-eluting adducts (*trans*-(3*R*,4*R*) and *cis*-(3*R*,4*S*), respectively). As anticipated, the $^1H$ NMR spectrum of compound **4** after desilylation and acetylation of the sugar hydroxyl groups was identical with that determined for the acetate of the first eluting *trans*-(3*S*,4*S*)-adduct. As discussed above for the trans diastereomers **4** and **5**, the two cis diastereomers formed here have CD spectra which are consistent (the last eluting *cis*-(3*S*,4*R*)-isomer has a negative CD band at 225 nm) with our empirical correlation for PAH adducts.[5,8,9]

Prior to incorporation into an oligomer, the free 3-hydroxyl groups of the disilyl-protected adducts **4** and **5** were acetylated, and the TBDMS protecting groups on the sugar were cleaved with fluoride ion. Both of these steps proceeded in high yield. Protection of the 5′-hydroxyl functionality with the pixyl group followed by phosphitylation of the 3′-hydroxyl group with 2-(cyanoethyl)-*N*,*N*-diisopropyl chlorophosphoramidite gave the desired fully blocked nucleoside adducts. The pixyl blocking group was selected over the more usual 4,4′-dimethoxytrityl (DMT) group as it is considered to be smaller and thus less likely to interfere with the hydrocarbon portion of the molecule.

Utility of our approach to the synthesis of PAH-adducted deoxyoligonucleotides was evaluated by the preparation of TpGpA*pGpT, where A* represents the modified base. The solid-phase synthesis (0.5 μmol scale) was done manually with standard phosphoroamidite chemistry. Reaction time for the coupling step with the blocked and activated (3*S*,4*S*)-diastereomer (24 μmol) was 1 h. Under these conditions, coupling of the modified base and subsequent reactions appeared to occur nearly quantitatively since manual synthesis of TpGpApGpT produced an identical amount of pentamer. Presence of the (3*S*,4*S*)-adducted dA in the modified pentamer was evident when its UV spectrum (increased absorption at 225 and ~275 nm due to the naphthalene chromophore) and CD spectrum (strong positive ellipticity at 225 nm) were compared to the normal pentamer (Figure 3). Additional proof of structure for the adducted pentamer was obtained by negative ion FAB mass spectrometry (*m*/*z* 1712, M − 1). In a second experiment with the blocked and activated (3*R*,4*R*)-diastereomer (3 μmol) and a coupling time of 10 min, the yield for coupling of the adducted dA was reduced to <40%. In this case, the pentamer was constructed with an automated DNA synthesizer with the exception that the central adducted base was introduced manually. The CD spectrum of the oligonucleotide containing the (3*R*,4*R*)-adducted dA also showed an intense CD band at 225 nm but of opposite sign.

## Conclusions

We believe that the present methodology will be generally applicable for the synthesis of oligonucleotides containing *trans*-dA adducts of bay-region PAH diol epoxides. Preliminary studies have indicated that the trans aminolysis reaction proceeds in excellent yield with the bay-region phenanthrene 1,2-diol 3,4-epoxide-2 (100 °C, 21 h)[27a] and benzo[*a*]pyrene 7,8-diol 9,10-

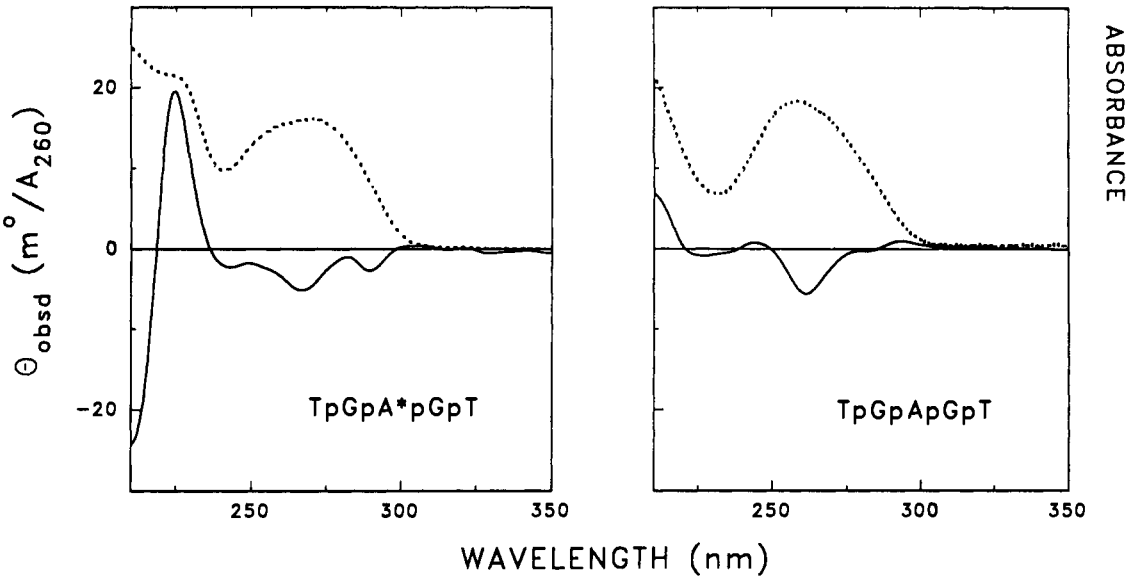



**Figure 3.** CD spectra (normalized to $A_{260}$ = 1.0 in water; solid lines) and UV spectra (water; broken lines) of the (3*S*,4*S*)-adducted deoxypentamer TpGpA*pGpT and of deoxy TpGpApGpT.

epoxide-2 (60 °C, 22 h)[27b] as well as with the more hindered fjord-region benzo[*c*]phenanthrene 3,4-diol 1,2-epoxide-2 (100 °C, 24 h).[27c] In the case of the phenanthrene diol epoxide, the product of coupling between disilyl 6-FP **2** and racemic amino triol provided a pair of diastereomers which were separable by HPLC.[28] Thus, the procedure has the distinct advantage of being able to utilize racemic diol epoxides. In addition, the absolute configuration of the diastereomers produced should be readily assignable from their CD spectra. Studies with the phenanthrene diol epoxide indicate that the blocking and deblocking protocol used here for the tetrahydrophenanthrene epoxide is equally applicable to adducts from diol epoxides.[28]

## Experimental Section

Proton NMR spectra were obtained at 300 MHz in $CDCl_3$, unless otherwise indicated. Chemical shifts ($\delta$) are reported in ppm and coupling constants in Hz. Chemical ionization mass spectra ($NH_3$ gas) were measured either on a Finnigan MAT 4500 quadrupole mass spectrometer or on a JEOL JMS-SX102 spectrometer with a direct exposure probe. FAB mass spectra and the high-resolution data were obtained on a JEOL JMS-SX102 instrument. The numbering system for adducts and their derivatives uses singly primed numbers for carbons of the sugar (i.e., 1′ through 5′) and doubly primed numbers for the purine ring (i.e., the purine protons are 2″ and 8″).

**6-Fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (1).** Synthesis of this compound was essentially identical with that reported[22] except for isolation. The reaction mixture after heating the ammonium salt (0.71 g, 2.2 mmol) with anhydrous KF (1.3 g, 22 mmol) in DMF (18 mL) for 45 min at 40 °C was cooled to room temperature and suction filtered. The residue was washed with dry EtOAc. To the filtrate, 4 g of 70–230 mesh silica gel, and some EtOAc was added. This mixture was evaporated to dryness under high vacuum. The powder was loaded onto a column dry packed with 20 g of silica gel. The column was eluted with dry EtOAc with suction. The first approximately 25 mL was discarded, and the subsequent 1500 mL on evaporation gave 0.42 g (77%) of a white solid. This product was of high purity, and its NMR spectrum and mp (149–152 °C) were consistent with published data.[23]

**6-Fluoro-9-(2-deoxy-3,5-bis(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl)purine (2).** 6-FP **1** (40 mg, 0.16 mmol) was stirred in 1,2-dimethoxyethane (1.0 mL) and then cooled to 0 °C. Pyridine (200 μL, 2.5 mmol) was added, and the stirring was continued for a few minutes. *tert*-Butyldimethylsilyl trifluoromethanesulfonate (108 μL, 0.47 mmol) was added, and the mixture was stirred at ice temperature for 45 min. EtOAc and brine, in that order, were added with stirring. The organic layer was dried and evaporated under reduced pressure. The crude product was chromatographed on a silica column packed in $CHCl_3$ and eluted with 7% MeOH in $CHCl_3$ to provide a pale yellow oil (70 mg, 92%): $^1$H NMR 8.60 (s, 1 $H_8$), 8.46 (s, 1 $H_2$), 6.51 (t, 1 $H_{1'}$, $J$ = 6.4), 4.60 (dt, 1 $H_{3'}$, $J$ = 3.9, 5.5), 4.01 (q, 1 $H_{4'}$, $J_{app}$ = 3.3), 3.86 (dd, 1 $H_{5'}$, $J$ = 2.9; 11.3), 3.76 (dd, 1 $H_{5'}$, $J$ = 3.7, 11.3), 2.62 (quint, 1 $H_{2'}$, $J_{app}$ = 6.9), 2.46 (ddd, 1 $H_{2'}$, $J$ = 4.2, 6.1, 13.1), 0.88 (18 H, *t*-Bu), 0.07 (12 H, Me); MS, DCI ($NH_3$) 483 (M + 1); HR MS, 467.2312 (−0.2 mmu, M − Me), 425.1482 (−1.4 mmu, M − *t*-Bu). Anal. Calcd $C_{22}H_{39}N_4O_3Si_2F$ (Found): C, 54.74 (54.72); H 8.15 (8.06); F 3.94 (3.71).

**(±)-4-Amino-3-hydroxy-1,2,3,4-tetrahydrophenanthrene (3a).** Tetrahydrophenanthrene-3,4-epoxide (120 mg, 0.61 mmol) was heated at 100 °C with ammonia for 16 h in a Parr high-pressure reactor. The reactor was cooled to dry-ice temperature before opening. The ammonia was carefully evaporated under a stream of nitrogen. The residue was dissolved in $CH_2Cl_2$, and the solution was extracted once with water. The organic layer was dried and evaporated under reduced pressure. An off-white solid (100 mg, 78%, mp 142–145 °C) was obtained: $^1$H NMR 8.15 (d, 1 $H_5$, $J$ = 8.5), 7.81 (d, 1 $H_8$, $J$ = 8.1), 7.67 (d, 1 $H_9$, $J$ = 8.4), 7.54 (t, 1 $H_6$, $J$ = 8.5), 7.44 (t, 1 $H_7$, $J$ = 8.1), 7.25 (d, 1 $H_{10}$, $J$ = 8.4), 4.56 (d, 1 $H_4$, $J$ = 3.2), 4.20 (m, 1 $H_3$), 3.12 (m, 1 $H_1$), 2.91 (m, 1 $H_1$), 2.28 (m, 1 $H_2$), 2.02 (m, 1 $H_2$); HR MS, $C_{14}H_{15}NO$ (213.1148, −0.6 mmu).

**(3*R*,4*R*)-4-Amino-3-hydroxy-1,2,3,4-tetrahydrophenanthrene (3b).** Optically active (3*R*,4*S*)-tetrahydroepoxide (30 mg, 0.15 mmol) was allowed to react with ammonia as described for the racemic compound (24 mg, 75%): $^1$H NMR spectrum identical with **3a**.

**$N_6$-(4-(3-Hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (4 and 5).** Disilyl 6-FP **2** (113 mg, 0.23 mmol) and the amino alcohol **3a** (50 mg, 0.23 mmol) were stirred in dry pyridine (70 μL, 0.82 mmol)/DMF (1.4 mL)/HMDS (2.2 mL, 10.4 mmol). The mixture was heated at 65 °C for 24 h, cooled to room temperature, and diluted with $CH_2Cl_2$. The organic phase was washed once with brine. The organic layer was dried and evaporated under reduced pressure. The crude product was chromatographed on a 2-mm silica plate with 1.7% MeOH in $CHCl_3$. The adduct fraction was collected and chromatographed on an Axxiom silica HPLC column (5 μ, 10 × 250 mm), with 2.5% MeOH in $CH_2Cl_2$ and a flow rate of 5.0 mL/min. The *early* and *late* eluting adducts had retention times of 5.60 and 6.45 min. Both adducts were obtained in 51% yield (40 mg of each diastereomer): $^1$H NMR for *early* adduct (500 MHz) 8.55 (s, 1 $H_{8''}$), 8.05 (s, 1 $H_{2''}$), 7.8–7.2 (6 H, aromatic), 6.46 (t, 1 $H_{1'}$, $J$ = 6.2), 5.89

(27) Characterized as their tetraacetates: (a) mp 138–141 °C; $^1$H NMR (300 MHz, $(CD_3)_2CO$) 7.8–8.0, 7.55–7.65 (4 H, aromatic and N*H*), 7.30 (d, $H_{10}$, $J$ = 8.7), 6.45 (d, $H_1$, $J$ = 9.1), 5.78 (dd, $H_4$, $J_{NH,4}$ = 8.2, $J_{3,4}$ = 3.2), 5.68 (app t, $H_3$, $J_{app}$ = ~2.8), 5.56 (dd, $H_2$, $J_{1,2}$ = 9.1, $J_{2,3}$ = 2.3), 1.95–2.25 (4 OAc); FAB MS, 414 (M + 1). (b) mp 248–252 °C (changes appearance above 200 °C); $^1$H NMR (300 MHz, $(CD_3)_2CO$) 8.1–8.4 (8 H, aromatic), 7.97 (d, N*H*, $J$ = ~8), 6.76 (d, $H_7$, $J$ = 9.5), 6.10 (dd, $H_{10}$, $J_{NH,10}$ = 8, $J_{9,10}$ = 3.4), 5.82 (app t, $H_9$, $J_{app}$ = ~3), 5.72 (dd, $H_8$, $J_{7,8}$ = 9.5, $J_{8,9}$ = 2.3), 2.0–2.3 (4 OAc); FAB MS, 488 (M + 1). (c) mp 236–240 °C; $^1$H NMR (300 MHz, $(CD_3)_2CO$) 8.56 (d, $H_{12}$, $J$ = 8.8), 7.5–8.1 (8 H, aromatic and N*H*), 6.46 (d, $H_4$, $J$ = 8.7), 5.99–6.01 (m, $H_1$ and $H_2$), 5.75 (dd, $H_3$, $J_{2,3}$ = 2.1, $J_{3,4}$ = 8.7), 1.9–2.2 (4 OAc); FAB MS, 464 (M + 1).

(28) Lakshman, M. K.; Yagi, H.; Sayer, J. M.; Jerina, D. M. Manuscript in preparation.

(br s, 1 $H_4$), 4.61 (br s, 1 $H_{3'}$), 4.46 (br s, 1 $H_3$), 4.01 (q, 1 $H_{4'}$, $J_{app}$ = 3.7), 3.85 (dd, 1 $H_{5'}$, $J$ = 2.9, 11.0), 3.77 (dd, 1 $H_{5'}$, $J$ = 3.7, 11.0), 3.11 (m, 1 $H_1$), 2.97 (dt, 1 $H_1$, $J$ = 5.5, 17.2), 2.63, 2.43 (m, 1 $H_{2'}$ each), 2.25, 2.01 (m, 1 $H_2$ each), 0.80 (18 H, *t*-Bu), 0.08 (12 H, Me); *late* adduct (500 MHz) 8.55 (s, 1 $H_{8''}$), 8.05 (s, 1 $H_{2''}$), 7.8–7.2 (6 H, aromatic), 6.46 (t, 1 $H_{1'}$, $J$ = 5.9), 5.89 (br s, 1 $H_4$), 4.61 (br s, 1 $H_{3'}$), 4.46 (br s, 1 $H_3$), 4.01 (q, 1 $H_{4'}$, $J_{app}$ = 3.3), 3.87 (dd, 1 $H_{5'}$, $J$ = 4.4, 11.4), 3.77 (dd, 1 $H_{5'}$, $J$ = 3.3, 11.4), 3.11 (m, 1 $H_1$), 2.95 (dt, 1 $H_1$, $J$ = 5.5, 17.2), 2.64 (quint, 1 $H_{2'}$, $J_{app}$ = 6.2), 2.44 (m, 1 $H_{2'}$), 2.26, 2.02 (m, 1 $H_2$ each), 0.80 (18 H, *t*-Bu), 0.08 (12 H, Me); HRMS for *early* and *late* adducts $C_{36}H_{53}N_5O_4Si_2$ (675.3655, 1.6 mmu and –0.1 mmu, respectively); FAB MS 676 (M + 1) for both adducts.

**(3*R*,4*R*)-$N_6$-(4-(3-Hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (5).** Reaction was performed in a manner identical with that for the racemic amino alcohol. The amounts of reactants and reagents were fluoro compound **2** (4.6 mg, 9.6 μmol), amino alcohol **3b** (2.0 mg, 9.6 μmol), dry pyridine (6.5 μL, 0.076 mmol), DMF (130 μL), and HMDS (203 μL, 0.96 mmol). The reaction was carried out for 24 h, and the product after workup was directly subjected to HPLC. The diastereomerically pure adduct (3.6 mg) was obtained in 57% yield. Its retention time on HPLC and CD spectrum were identical with that of the *late* eluting adduct **5**: UV (MeOH) $\lambda_{max}$ 224 nm (ε 9611) and $\lambda_{max}$ 278 nm (ε 2988).

**(3*R*,4*R*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine.** The *late* eluting adduct **5** (14 mg, 0.02 mmol) was acetylated in dry pyridine (300 μL) with acetic anhydride (99 μL, 1.1 mmol), in the presence of 4-(*N*,*N*-dimethylamino)pyridine (2.5 mg, 0.02 mmol). The mixture was evaporated to dryness after 24 h. Chromatography of the crude product on a silica column packed in $CHCl_3$, with 3% MeOH in $CHCl_3$, gave pure monoacetylated adduct (11 mg, 79%): $^1$H NMR 8.58 (s, 1 $H_{8''}$), 8.03 (s, 1 $H_{2''}$), 7.9–7.7, 7.4, 7.3 (6 H, aromatic), 6.47 (t, 1 $H_{1'}$, $J$ = 6.2), 6.12 (br s, 1 $H_4$), 5.57 (br s, 1 $H_3$), 4.61 (q, 1 $H_{3'}$, $J_{app}$ = 3.7), 4.01 (br d, 1 $H_{4'}$, $J$ = 3.3), 3.86 (dd, 1 $H_{5'}$, $J$ = 4.0, 11.2), 3.76 (dd, 1 $H_{5'}$, $J$ = 3.2, 11.2), 2.2–3.2 (6 H, $H_{1,2,2'}$), 2.03 (s, 3 H, OAc), 0.80 (18 H, *t*-Bu), 0.08 (12 H, Me); FAB MS, 718 (M + 1).

**(3*S*,4*S*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (6).** The *early* eluting adduct **4** (14 mg, 0.20 mmol) was acetylated as in the case of the (3*R*,4*R*)-isomer. After chromatography (14 mg, 95%), a pale yellow foam was obtained: $^1$H NMR 8.57 (s, 1 $H_{8''}$), 8.02 (s, 1 $H_{2''}$), 7.9–7.7, 7.4, 7.3 (6 H, aromatic), 6.46 (t, 1 $H_{1'}$, $J$ = 6.4), 6.10 (br s, 1 $H_4$), 5.55 (br s, 1 $H_3$), 4.58 (q, 1 $H_{3'}$, $J_{app}$ = 3.6), 3.99 (br d, 1 $H_{4'}$, $J$ = 3.4), 3.85 (dd, 1 $H_{5'}$, $J$ = 3.8, 11.0), 3.74 (dd, 1 $H_{5'}$, $J$ = 2.9, 11.0), 2.2–3.2 (6 H, $H_{1,2,2'}$), 2.02 (s, 3 H, OAc), 0.80 (18 H, *t*-Bu), 0.08 (12 H, Me); FAB MS, 718 (M + 1).

**(3*R*,4*R*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine.** The monoacetoxy disilyl adduct (10 mg, 0.014 mmol) was stirred with *n*-$Bu_4N^+F^-$ (1 M solution, 31 μL, 2.2 equiv) in anhydrous THF (200 μL) for 1 h at room temperature. The solvent was evaporated, and the product was loaded onto a 250μ (10 × 20 cm) preparative TLC plate. Elution with 4% MeOH in $CHCl_3$ gave the free nucleoside adduct (6.0 mg, 88%): $^1$H NMR 8.53 (s, 1 $H_{8''}$), 7.57 (s, 1 $H_{2''}$), 7.9–7.7, 7.4, 7.3 (6 H, aromatic), 6.22 (br m, 1 $H_{1'}$), 6.17 (br s, 1 $H_4$), 5.51 (s, 1 $H_3$), 4.79 (d, 1 $H_{3'}$, $J$ = 4.4), 4.20 (s, 1 $H_{4'}$), 4.00 (d, 1 $H_{5'}$, $J$ = 12.7), 3.90 (br t, 1 $H_{5'}$, $J$ = 12.7), 2.2–3.8 (6 H, $H_{1,2,2'}$), 2.02 (s, 3 H, OAc); FAB MS, 490 (M + 1).

**(3*S*,4*S*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-2′-deoxyadenosine (7).** The acetate **6** (13 mg, 0.018 mmol) of the *early* eluting disilyl adduct **4** was cleaved as above. After chromatography a pale yellow solid (8.5 mg, 96%) was obtained: $^1$H NMR 8.56 (s, 1 $H_{8''}$), 7.55 (s, 1 $H_{2''}$), 7.9–7.8, 7.4, 7.3 (6 H, aromatic), 6.22 (br m, 1 $H_{1'}$), 6.14 (br s, 1 $H_4$), 5.54 (s, 1 $H_3$), 4.82 (d, 1 $H_{3'}$, $J$ = 4.7), 4.21 (s, 1 $H_{4'}$), 4.01 (d, 1 $H_{5'}$, $J$ = 12.8), 3.82 (br t, 1 $H_{5'}$, $J$ = 10.6), 2.2–3.8 (6 H, $H_{1,2,2'}$), 2.04 (s, 3 H, OAc). Addition of a few drops of MeOH-$d_4$ resulted in the sharpening of the signals at δ 6.22 ppm (to a double doublet) and 3.82 ppm (to a doublet). Exchangeable proton resonances at δ 6.7 and 6.2 ppm disappear: FAB MS, 490 (M + 1).

**(3*R*,4*R*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-5′-(9-phenylxanthen-9-yl)-2′-deoxyadenosine.** The nucleoside adduct resulting from the acetylation and desilylation of **5** (6.0 mg, 0.013 mmol) and pixyl chloride (4.0 mg, 0.014 mmol) were individually dissolved in dry pyridine and evaporated to dryness three times. The adduct was dissolved in dry pyridine (100 μL), and pixyl chloride in pyridine (100 μL) was added to the stirred adduct solution. The mixture was evaporated under oil pump vacuum, and 50 μL of dry pyridine was added. The mixture was stirred in the dark for 45 min, quenched with MeOH/saturated aqueous $NaHCO_3$, and extracted with $CH_2Cl_2$. The organic layer was dried and evaporated. Chromatography of the crude product on a 250μ silica gel preparative TLC plate (10 × 20 cm) with 1% MeOH/1% $Et_3N$/98% $CH_2Cl_2$ gave the 5′-protected nucleoside (4.3 mg, 48%): $^1$H NMR ($CD_3CN$) 8.3, 7.9–7.8, 7.5–6.9 (21 H, aromatic), 6.30 (br t, 1 $H_{1'}$), 6.1 (br, 1 $H_4$), 5.4 (s, 1 $H_3$), 4.54 (br s, 1 $H_{3'}$), 4.0 (br s, 1 $H_{4'}$), 2.7–3.3 (8 H, $H_{1,2,2',5'}$), 2.0 (s, 3 H, OAc).

**(3*S*,4*S*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-5′-(9-phenylxanthen-9-yl)-2′-deoxyadenosine (8).** The nucleoside adduct **7** (32 mg, 0.069 mmol) was dissolved in anhydrous pyridine (100 μL). Pixyl chloride (41 mg, 0.14 mmol) in pyridine (100 μL) was added over 15 min. The mixture was stirred in the dark for 1 h. The pyridine was evaporated with a 40 °C water bath. Fresh pyridine was added, and the process was repeated 4–5 times. TLC (1% MeOH/1% $Et_3N$/98% $CH_2Cl_2$) indicated predominantly the monopixyl product and a minor amount of possibly the 3′,5′-bispixyl derivative. The reaction, on quenching and chromatography as described above, provided 22 mg (56%) of **8** along with 7.3 mg of unreacted nucleoside.

**(3*R*,4*R*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-5′-(9-phenylxanthen-9-yl)-2′-deoxy-3′-(*N*,*N*-diisopropyl-2-(cyanoethyl))-adenosine Phosphoramidite.** The 5′-protected adduct nucleoside (4.3 mg, 5.7 μmol) was stirred in dry $CH_2Cl_2$ (100 μL). Triethylamine (6.4 μL, 46 μmol) was added, and the mixture was transferred to a glove bag filled with $N_2$. 2-(Cyanoethyl)-*N*,*N*-diisopropyl chlorophosphoramidite (2.6 μL, 11.6 μmol) was added to the mixture in the glove bag, and the mixture was stirred for 2 h. The reaction was quenched with MeOH and evaporated to dryness. Chromatography of the crude product on a 250μ silica preparative TLC plate (10 × 20 cm) with 1% $Et_3N$ in $CH_2Cl_2$ gave the phosphoramidite product (3.0 mg, 50%): $^{31}$P NMR ($CD_3CN$) 148.99 relative to 0.1 M phosphoric acid as external standard.

**(3*S*,4*S*)-$N_6$-(4-(3-Acetoxy-1,2,3,4-tetrahydrophenanthrenyl))-5′-(9-phenylxanthen-9-yl)-2′-deoxy-3′-(*N*,*N*-diisopropyl-2-(cyanoethyl))-adenosine Phosphoramidite (9).** The 5′-protected nucleoside **8** (21 mg, 0.028 mmol) was phosphitylated as in the case of the *late* adduct with $Et_3N$ (33 μL, 0.24 mmol) and 2-(cyanoethyl)-*N*,*N*-diisopropyl chlorophosphoramidite (13 μL, 58 μmol) in $CH_2Cl_2$ (0.5 mL) over 2 h. Workup and chromatography as before provided 23 mg (86%) of the activated nucleoside as a clear glass.

**Synthesis of Oligodeoxynucleotides Containing Either dA or the PAH Adducted dA.** Manual synthesis of the normal deoxyoligomer TpGpApGpT was done on a 0.5 μmol column. The phosphoramidites and tetrazole were dried in a desiccator under vacuum overnight and were stored under argon. The column was treated with 700 μL of a 1:1 mixture of $Ac_2O$/THF (3.0 mL/6.6 mL) and 4-(*N*,*N*-dimethylamino)-pyridine/pyridine/THF (350 mg/3.0 mL/3.0 mL) for 4 min prior to the first DMT cleavage and for 2 min at each end capping. DMT cleavage was done with a 3% solution of dichloroacetic acid in $CH_2Cl_2$ for 1.5 min. Coupling of each nucleoside was performed with a 0.14 M solution of the phosphoramidite (200 μL) in $CH_3CN$ containing 0.5 M tetrazole. Coupling time for each nucleoside was 4 min. Oxidation was performed with 1.0 mL of $I_2$/THF/2,6-lutidine/water (0.26 g/8.0 mL/2.0 mL/0.20 mL) for 45 s. After unpacking the column, the completed DMT oligomer was removed from the controlled pore glass support by treatment with a 3:1 solution of ammonia/EtOH for 5 h. The solution was filtered, heated at 55 °C for 8 h, and lyophilized to half its volume. Synthesis of the adducted oligonucleotide TpGpA*pGpT, where A* represents adducted dA resulting from the *early* eluting (3*S*,4*S*)-diastereomer, was done in an identical fashion with the exceptions that (i) coupling time for the adducted base was extended from 4 min to 1 h and (ii) time for removal of the 5′-pixyl protecting group was 2 min rather than the 1.5 min used to remove DMT.

Solutions of the normal and adducted DMT pentamers were purified by gradient elution (2.5 mL/min) on a Hamilton PRP-1 column (10 μ, 7.0 × 305 mm). The gradient was ramped from 90% A/10% B to 100% B over 40 min, and elution was continued at 100% B for 5 min. Solvents A and B consisted of 0.1 M triethylammonium carbonate (TEAC) and 50% $CH_3CN$ in 0.1 M TEAC, respectively, both adjusted to pH 7.5. The profiles for the normal and adducted DMT oligomers (28.0 and 32.3 min, respectively) were free of other significant peaks exhibiting the nucleotide chromophore. Collected oligomer fractions were lyophilized, treated with 80% $CH_3COOH$ in water for 30 min to remove the DMT group, and again lyophilized. Each product was dissolved in 0.5 mL of water and extracted twice with EtOAc. The EtOAc layers were extracted with 0.5 mL of water, and the combined aqueous layers from each product were lyophilized to half their volumes. Products were chromatographed on the Hamilton PRP-1 column as above except that the gradient was ramped from 100% A to 65% A/35% B over 20 min and then to 100% B over 5 min and maintained at 100% B for 5 min. The profiles for the normal oligomer (13 $A_{260}$ units, 15.5 min) adducted oligomer (12 $A_{260}$ units, 25.7 min) were free of any contaminating peaks. On negative ion FAB mass spectrometry, the normal deoxypentamer (TpGpApGpT, MW 1517) showed an ion at 1516 (M – 1) and the adducted deoxypentamer (TpGpA*pGpT, MW 1713) showed an ion at 1712 corresponding to M

- 1 for the desired, adducted pentamer. CD and UV spectra of the normal and adducted pentamers are shown in Figure 3.

**Formation of $N_6$ Adducts from 1,2,3,4-Tetrahydrophenanthrene-3,4-epoxide and 2'-Deoxyadenosine 5'-Monophosphate.** Procedures previously described[7,8] for analytical scale preparation of adducts from analogous PAH diol epoxides were used. The racemic epoxide (20 mg, 100 μmol) was dissolved in 5 mL of acetone and added in two equal portions, at time zero and 1 h, to 50 mL of an aqueous solution containing 1.0 g of 2'-deoxyadenosine 5'-monophosphate at pH 7.1 and 37 °C. After ca. 20 h at 37 °C, the reaction mixture was washed with 3 approximately equal volumes of EtOAc followed by 1 volume of ether to remove hydrolysis products of the epoxide. After removal of organic solvents by purging with $N_2$, the aqueous solution was divided into two portions and passed through two $C_{18}$ Sep-paks (Waters Associates), which were washed with water to remove most of the unreacted nucleotide, prior to elution of the modified nucleotides with MeOH. The MeOH eluate was evaporated to dryness. The residue was dissolved in 50 mM Tris-HCl buffer (pH 8.8) and treated with *E. coli* alkaline phosphatase (40 units in two equal portions at time zero and 3 h) over a period of 5.5 h. No change in the composition of the crude enzymatic reaction mixture between 3 and 5.5 h of reaction time was observed upon reverse-phase HPLC. The mixture was filtered and adsorbed on a $C_{18}$ Sep-pak, which was washed with water and then eluted with ca. 20 mL of MeOH. The methanolic solution containing the nucleoside adducts was separated by HPLC on a Beckman Ultrasphere ODS column (5μ, 10 × 250 mm), eluted at 3 mL/min with 58% MeOH in water for 10 min followed by a linear gradient that increased the MeOH composition to 75% over 10 min. Adducts were formed in a ratio of 1:1:1:0.5 (by integration at 285 nm) with retention times as follows: *trans*-(3*S*,4*S*), 10.5 min; *trans*-(3*R*,4*R*), 12.0 min; *cis*-(3*R*,4*S*), 18.2 min; *cis*-(3*S*,4*R*), 19.3 min. Assignment of relative configuration was based on the NMR spectra of the adducts as their triacetates (vide infra), whereas assignment of absolute configuration was based on exclusive formation of the second- and third-eluting adducts from optically active (-)-tetrahydrophenanthrene-(3*R*,4*S*)-epoxide. CD spectra of the adducts prepared from this enantiomer were identical with those of the corresponding adducts derived from the racemic epoxide.

The four adducts from the racemic epoxide were characterized by their CD spectra in MeOH as well as by the $^1$H NMR spectra (500 MHz, $(CD_3)_2CO$) of their triacetates (overnight reaction at room temperature with pyridine/$Ac_2O$), which were purified when necessary by HPLC on a Du Pont Golden Series SIL column (6.2 × 80 mm) eluted at 2.5 mL/min with $CH_2Cl_2$/EtOAc/MeOH (95/5/2): $k'$ (*trans*-(3*S*,4*S*)-adduct), 3.5; $k'$ (both *cis*-adducts), 2.8. Yields and concentrations of the four adducts were estimated from their UV spectra in MeOH by use of $\epsilon_{278\,nm}$ = 2988, as determined for the analogous compound **5**.

**(3*S*,4*S*)-$N_6$-(4-(3-Hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-2'-deoxyadenosine (9 μmol, 9%)**: CD Δε 260 nm, -1.2; 225 nm, 13.8; 210 nm, -7.6; $^1$H NMR of the triacetate 8.47 (br s, $H_{8''}$), 8.12 (br s, $H_{2''}$), 7.8-7.9, 7.3-7.4 (aromatic), 7.20 (br s, N*H*), 6.47 (app. t, $H_{1'}$, $J_{1',2'}$ = 6.4), 6.18 (br s, $H_4$), 5.50 (dt, $H_{3'}$, $J_{2',3'}$ = 2.2, 6.4, $J_{3',4'}$ = 2.2), 5.45 (dt, $H_3$, $J_{2,3}$ = ~4, $J_{3,4}$ = 2.4), 4.38 (dd, $H_{5'}$, $J_{4',5'}$ = 6.7, $J_{gem}$ = 13.6), 4.31 (m, $H_{4',5'}$), 2.2-3.2 ($CH_2$), 2.10, 2.00, 1.98 (3 s, 3 OAc); FAB MS of the triacetate 574 (M + 1). The $^1$H NMR spectrum of compound **4** after desilylation and acetylation of the sugar hydroxyl groups was identical with that determined for this adduct.

**(3*R*,4*R*)-$N_6$-(4-(3-Hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-2'-deoxyadenosine (8 μmol, 8%)**: CD Δε 258 nm, 1.1; 225 nm, -12.1; 210 nm, 7.9; $^1$H NMR of the triacetate 8.45 (br s, $H_{8''}$), 8.10 (br s, $H_{2''}$), 7.8-7.9, 7.3-7.4 (aromatic), 7.15 (br s, N*H*), 6.47 (app., t, $H_{1'}$, $J_{1',2'}$ = 6.4), 6.19 (br s, $H_4$), 5.51 (dt, $H_{3'}$, $J_{2',3'}$ = 2.1, 6.4, $J_{3',4'}$ = 2.1), 5.45 (dt, $H_3$, $J_{2,3}$ = ~4, $J_{3,4}$ = ~2.5), 4.39 (dd, $H_{5'}$, $J_{4',5'}$ = 6.4, $J_{gem}$ = 13.4), 4.31 (m, $H_{4',5'}$), 2.2-3.2 ($CH_2$), 2.10, 2.00, 1.98 (3 s, 3 OAc); FAB MS of the triacetate 574 (M + 1).

**(3*R*,4*S*)-$N_6$-(4-(3-Hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-2'-deoxyadenosine (9 μmol, 9%)**: CD Δε 266 nm, -1.3; 225 nm, 15.5; 210 nm, -8.6; $^1$H NMR of the triacetate 8.45 (s, $H_{8''}$), 8.10 (s, $H_{2''}$), 7.8-7.95, 7.3-7.4 (aromatic), 7.10 (br d, N*H*, $J_{NH,4}$ = 9), 6.71 (m, $H_4$, $J_{NH,4}$ = 9, $J_{3,4}$ = 4.0), 6.45 (dd, $H_{1'}$, $J_{1',2'}$ = 6.1, 7.9), 5.49 (dt, $H_{3'}$, $J$ = 2.2, 6.4), 5.34 (dt, $H_3$, $J_{2eq,3}$ = $J_{3,4}$ = 3.7, $J_{2ax,3}$ = 13.4), 4.35 (dd, $H_{5'}$, $J_{4',5'}$ = 6.4, $J_{gem}$ = 13.1), 4.30 (m, $H_{4',5'}$), 2.2-3.3 ($CH_2$), 2.10, 2.00, 1.77 (3 s, 3 OAc); FAB MS of the triacetate 574 (M + 1).

**(3*S*,4*R*)-$N_6$-(4-(3-Hydroxy-1,2,3,4-tetrahydrophenanthrenyl))-2'-deoxyadenosine (4 μmol, 4%)**: CD Δε 266 nm, 0.9; 225 nm, -16.6; 210 nm, 8.8; $^1$H NMR of the triacetate 8.45 (s, $H_{8''}$), 8.10 (s, $H_{2''}$), 7.8-7.95, 7.3-7.4 (aromatic), 7.10 (br d, N*H*, $J_{NH,4}$ = 9), 6.71 (m, $H_4$, $J_{NH,4}$ = 9, $J_{3,4}$ = 3.7), 6.45 (dd, $H_{1'}$, $J_{1',2'}$ = 6.4, 7.9), 5.49 (dt, $H_{3'}$, $J$ = 2.1, 6.1), 5.34 (dt, $H_3$, $J_{2eq,3}$ = $J_{3,4}$ = 3.7, $J_{2ax,3}$ = 13.1), 4.38 (dd, $H_{5'}$, $J_{4',5'}$ = 6.3, $J_{gem}$ = 13.3), 4.30 (m, $H_{4',5'}$), 2.2-3.3 ($CH_2$), 2.10, 2.00, 1.78 (3 s, 3 OAc); FAB MS of the triacetate 574 (M + 1).

**Acknowledgment.** We expressly thank Dr. K. Lesiak, NIDDK, NIH for advice on the manual synthesis of oligonucleotides on solid supports, Dr. W. M. Egan and C. A. Ronald at the Center for Biologics Evaluation and Research, FDA for assistance in the use of their automated oligonucleotide synthesizer, and Dr. L. K. Pannell and Y. Murata, NIDDK, NIH for determining FAB mass spectra of the oligonucleotides.

# Diastereofacial Selectivity with Optically Active α-Substituted β-Silyl-(*E*)-hexenoates. Enantioselective Construction of Homoallylic Ethers via Reaction with Aryl Acetals

**James S. Panek* and Michael Yang**

*Contribution from the Department of Chemistry, Metcalf Center for Science and Engineering. Boston University, Boston, Massachusetts 02215.* *Received December 24, 1990*

**Abstract:** The Lewis acid catalyzed reactions of optically active methyl α-methoxy- and α-methyl-β-(dimethylphenylsilyl)-(*E*)-hexenoates **1a-d** with aryl acetals are described. These reagents function as effective carbon nucleophiles in highly diastereo- and enantioselective addition reactions to activated acetals. The reaction constitutes a one-step construction of functionalized hexenoic acid derivatives **3**, containing three stereocenters, an *E*-double bond, and a terminal carbomethoxy group. The two new stereocenters (5,6-syn) have emerged with excellent levels of absolute stereochemical control with ee's reaching 95%.

Over the past several years many laboratories have focused on the development of chiral allyl- and crotylmetal reagents as propionate enolate equivalents for enantioselective carbon-carbon bond formation in the aldol-like construction of homoallylic al-

0002-7863/91/1513-6594$02.50/0 