Synthesis of Oligonucleotide Adducts of the Bay Region Diol Epoxide Metabolites of Carcinogenic Polycyclic Aromatic Hydrocarbons

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An efficient method for the site-specific synthesis of adducts between the biologically active diol epoxide metabolites of carcinogenic polycyclic aromatic hydrocarbons (PAHs) and oligonucleotides in which a PAH component of predetermined stereochemistry is linked covalently to the exocyclic amino groups of deoxyadenosine (dA) and deoxyguanosine (dG) is described. The synthetic strategy involves in the key step coupling a protected halopurine derivative with an amino derivative (or an aminotriol derivative) of the PAH. This method was initially employed to prepare the dA and dG adducts of the model PAH 1-methylpyrene. The appropriately protected dA adduct was then incorporated into the oligonucleotide sequence d(GCAGGTCA*AGAG) where A* represents N6-pyrenylmethyl-dA. This methodology was extended to the synthesis of trans adducts of *anti*-diol epoxide metabolites of benzo[*a*]pyrene and 5-methylchrysene linked to the 6-amino function of dA. The parent hydrocarbons are widespread environmental carcinogens. This synthetic approach, dubbed the *total synthesis method*, complements the *direct synthesis method* which involves the direct reaction of PAH diol epoxides with oligonucleotides. The *total synthesis method* is broader in scope than the latter, and it is readily adaptable to the large scale preparation of PAH–oligonucleotide adducts required for structure determination by high resolution NMR and X-ray crystallographic techniques.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants produced in the combustion of fossil fuels and other organic matter.[1] Some PAHs are relatively potent mutagens and carcinogens.[1,2] They are activated enzymatically to bay or fjord region diol epoxide metabolites that bind covalently to DNA, leading initially to mutations and ultimately to tumor induction.[3,4] These reactions occur mainly, though not exclusively, via addition of the exocyclic amino groups of deoxyguanosine (dG) and deoxyadenosine (dA) to the benzylic carbon atoms of the epoxide functions.[5]

The details of the mechanism of PAH carcinogenesis at the molecular-genetic level remain uncertain.[1] It is not established which of the numerous PAH–DNA adducts formed is principally responsible for tumor formation. For each PAH diol epoxide, four stereoisomers are possible, i.e., a pair of *anti* and *syn* diastereomers each of which has a pair of (+) and (−) enantiomers. Each of these stereoisomers may form *cis* and/or *trans* adducts at multiple dG and dA sites on the DNA helix. The ratios of the adducts actually formed vary considerably for different PAHs and does not appear to relate simply to the carcinogenic potencies of the parent hydrocarbons. However, it has been observed by Dipple and co-workers[6] that for diol epoxides derived from planar PAHs, such as benzo[*a*]pyrene, covalent binding occurs predominantly to dG sites of nucleic acids, whereas for diol epoxides derived from PAHs distorted from planarity, such as 7,12-dimethylbenz[*a*]anthracene[6] and benzo[*g*]-chrysene,[7] an equal or greater extent of binding takes place on the dA residues. While it is often assumed that the major DNA-bound adducts are of greatest importance in tumorigenesis, supporting evidence is lacking, and minor adducts may actually play an equal or greater role.

In order to gain greater insight into the relation between the three dimensional structures of the PAH–DNA adducts and the biological consequences of their replication, efficient methods for the site specific synthesis of PAH–oligonucleotide adducts with a PAH diol epoxide-derived component of predetermined stereochemistry attached covalently to the exocyclic amino groups of specific dA and dG bases are required. Convenient synthetic accessibility of adducts of this type

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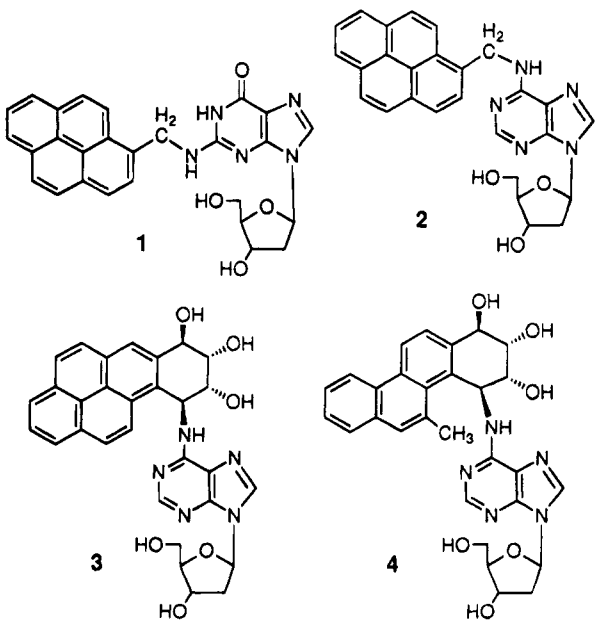
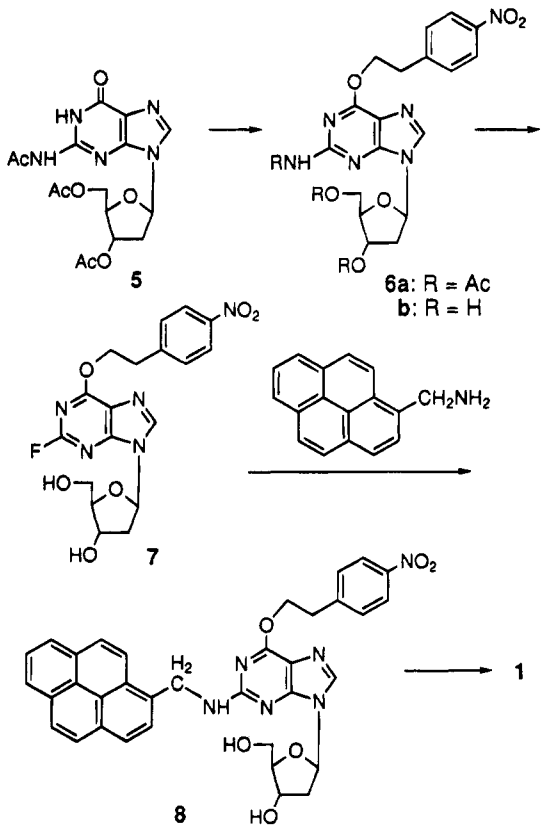
would make it possible, in principle, to relate adduct molecular structures determined by two-dimensional NMR and other techniques to effects on DNA replication and mutation. In our initial studies, an efficient method for the synthesis of the dG and dA adducts of the model compound 1-methylpyrene (**1** and **2**) was developed, and these adducts were incorporated into an oligonucleotide sequence.[8] 1-(Methylpyrenyl)oligonucleotide adducts are convenient models for the corresponding adducts of the *anti*-diol epoxide of benzo[*a*]pyrene because of the obvious structural relation and the lack of the hydroxyl groups expected to complicate their synthesis. 1-Methylpyrene is of interest in its own right because it is a common environmental pollutant which is activated enzymatically to 1-(hydroxymethyl)pyrene, 1-(sulfoxymethyl)pyrene, and 1-(chloromethyl)pyrene.[9] The latter metabolites are potent mutagens capable of alkylation of DNA to form adducts of the type whose synthesis is described herein. In other related studies, syntheses of the diastereomeric cis and trans adducts of 1,2,3,4-tetrahydrophenanthrene-3,4-oxide with dA,[10,11] the trans adducts of 1,2-dihydroaceanthrylene-1,2-oxide and 3,4-dihydrocyclopenta[*cd*]pyrene-3,4-oxide with dA and dG,[12] and the trans adducts of the *fjord* region *anti*-diol epoxide of benzo[*c*]phenanthrene (*trans-3,4*-dihydroxy-*anti-1,2*-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene) with dA[13] were described.

We now report full details of the synthesis of **1** and **2** and application of the methodology to the synthesis of trans adducts of the *anti*-diol epoxide metabolites of benzo[*a*]pyrene and 5-methylchrysene linked to the 6-amino group of dA (**3** and **4**). Although covalent bonding of these diol epoxides to DNA in animal cells takes place principally on deoxyguanosine, significant binding also occurs on deoxyadenosine sites.



Scheme 1



Results

The synthetic strategy involves in the key step coupling an appropriately protected halopurine derivative with an amino derivative of the PAH. In the case of **1** and **2**, the amine component is 1-(aminomethyl)pyrene. This approach reverses the nucleophile–electrophile roles in the alkylation of DNA by PAH metabolites, ensuring that the products are exclusively those in which the PAH moiety is bound to the amino group of the purine component. This is advantageous over the direct alkylation of purine bases or oligonucleotides which generally affords mixtures that contain additional products arising from alkylation on other base sites, e.g., ^{7}N or ^{6}O of dG,[1,4,5] which often present difficult separation problems.

Synthesis of 2′-Deoxy-N^2-(1-pyrenylmethyl)guanosine (1). The synthetic route to **1** is based on 2′-deoxy-N^2,3′,5′-triacetylguanosine (**5**) (Scheme 1). Treatment of **5** with equimolar ratios of diethyl azodicarboxylate, triphenylphosphine, and 2-(*p*-nitrophenyl)ethanol in dioxane at ambient temperature for 24 h furnishes the 2-(*p*-nitrophenyl)ethyl derivative **6a** quantitatively.[14–16] Deacetylation of **6a** takes place smoothly on treatment

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

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(13) (a) Lakshman, M. K.; Yeh, H. J. C.; Yagi, H.; Jerina, D. M. *Tetrahedron Lett.* **1992**, *33*, 7121. (b) Steinbrecher, T.; Becker, A.; Stezowski, J. J.; Oesch, F.; Seidel, A. *Tetrahedron Lett.* **1993**, *34*, 1773.

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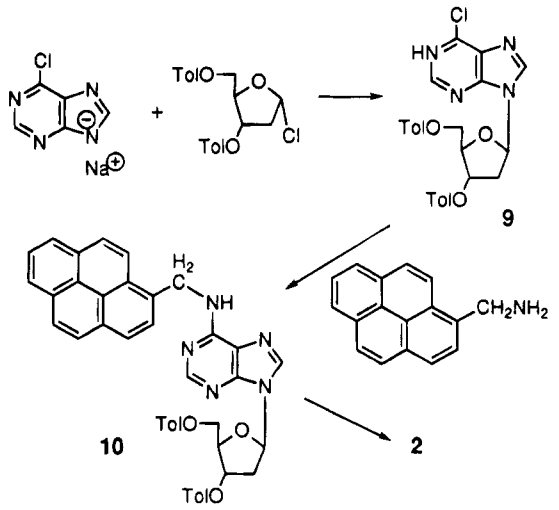
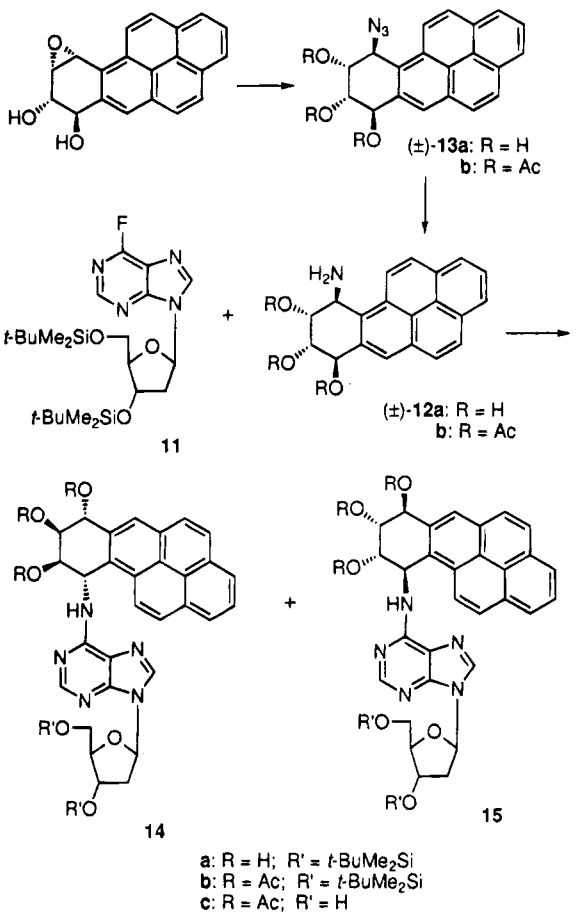
(15) Himmelsbach, F.; Schulz, B. S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron* **1984**, *40*, 59.

(16) In our initial studies, a benzyl group was employed as protecting group for the O^2 function, but it was found that its removal by hydrogenation resulted in partial hydrogen addition to the pyrene ring.

Scheme 2



Scheme 3



with methanolic ammonia to provide pure 2′-deoxy-O^6-((*p*-nitrophenyl)ethyl)guanosine (**6b**) in 87% yield. Deacetylation of the hydroxyl groups is complete in 1 h, while deprotection of the amino group requires 6 days. Conversion of **6b** to 2-fluoro-O^6-((*p*-nitrophenyl)ethyl)-guanosine (**7**) was accomplished by treatment with *tert*-butyl nitrite in 60% anhydrous HF/pyridine at −20 °C using a modification of the procedure of Robins and Uznanski.[17] Strict adherence to the experimental protocol is essential to achieve the optimum yield (~80%). Condensation of **7** with 1-(aminomethyl)pyrene takes place smoothly in DMF at room temperature to furnish the protected adduct **8** in 70% yield.[18] Removal of the (*p*-nitrophenyl)ethyl group by reaction with 0.5 M DBU in pyridine[19] gives **1** essentially quantitatively. Its 500 MHz ^{1}H NMR spectrum is entirely consistent with this assignment.

Synthesis of 2′-Deoxy-N^6-(1-pyrenylmethyl)adenosine (2). The synthetic route to **2** is based on 6-chloro-9-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-purine (**9**) (Scheme 2). Compound **9** is prepared from reaction of the *p*-toluoyl-protected 2′-deoxychloro sugar with the sodium salt of 6-chloropurine by the reported procedure.[20] Reaction of **9** with 1-(aminomethyl)pyrene in DMF furnishes the *p*-toluyl derivative of **2** (**10**) which is converted to **2** by treatment with NaOMe in MeOH/THF at room temperature. The 500 MHz ^{1}H NMR spectrum of **2** is consistent with this assignment.

Synthesis of Deoxyadenosine Adducts of Benzo[*a*]pyrene *anti*-Diol Epoxide (3). The synthetic approach to these adducts entails in the key step coupling between protected derivatives of a halogen-substituted purine analog of deoxyadenosine and an aminotriol derivative of benzo[a]pyrene with the appropriate stereochemistry (Scheme 3). The specific compounds chosen for this purpose were the 6-fluoro analogue of dA in the form of its bis(*tert*-butyldimethylsilyl) derivative (**11**) and the benzo[*a*]pyrene aminotriol derivative **12a**. The more reactive fluoro analogue was selected on the basis of preliminary experiments which showed the chloro analogue to be poorly reactive with the sterically hindered bay region amine *trans*-10-amino-9-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. The *tert*-butyldimethylsilyl group was employed for protection of the ribose hydroxyl groups because of its anticipated greater facility of selective removal at a later stage in the synthesis in comparison with the toluoyl groups used previously in the synthesis of **2**.

The amino triol **12a** was synthesized from the racemic diol epoxide derivative of benzo[*a*]pyrene, (±)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene,[21,22] via reaction with NaN_3 at room temperature (Scheme 3). NMR analysis indicated that epoxide ring opening took place essentially *trans*-stereospecifically to furnish the azidotriol 10β-azido-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (**13a**). Due to the instability of **13a**, its melting point could not be obtained, and it was used directly in the next step. Two methods were employed for conversion of **13a** to **12a**. These were direct reduction by hydrogenation over a Pd/charcoal catalyst and an indirect procedure entailing acetylation of the azidotriol to the triacetate **13b** followed by catalytic reduction to **12b** and deacetylation. Although the indi-

(17) Robbins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2608.
(18) In preliminary experiments the 2-chloro and 2-bromo analogs of **7** were synthesized, but it was found that their reaction with 1-(aminomethyl)pyrene was much less satisfactory, affording very low yields of **8**.
(19) Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1982**, *23*, 2257.
(20) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379.
(21) Fu, P. P.; Harvey, R. G. In *Polycyclic Hydrocarbons and Cancer*; Gelboin, H. V., Ts'o, P. O. P., Eds.; Academic Press: New York, 1978; Vol. 1, pp 133–165. Fu, P. P.; Harvey, R. G. *Tetrahedron Lett.* **1977**, 2059. Beland, F. A.; Harvey, R. G. *J. Chem. Soc., Chem. Commun.* **1976**, 84.
(22) The *anti* diol epoxide is by definition the isomer in which the epoxide function is on the opposite face of the molecule as the benzylic hydroxyl group; the *syn* isomer has these groups on the same face. This nomenclature, although it is not officially sanctioned, is employed by the majority of the investigators in the field.

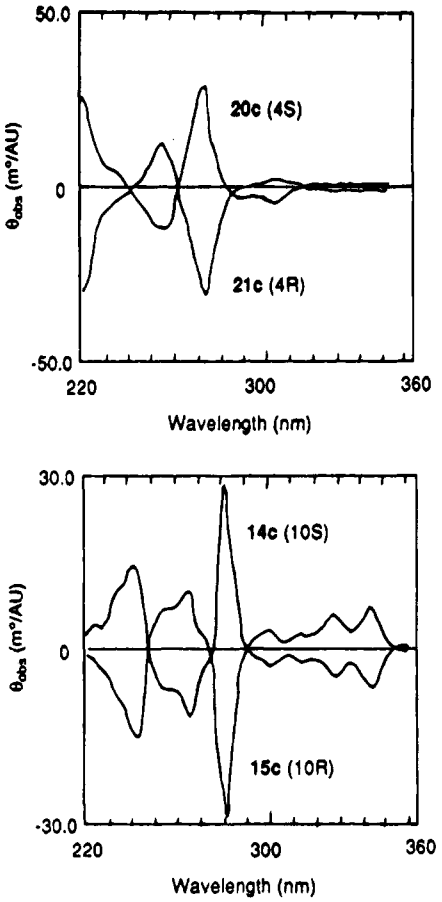
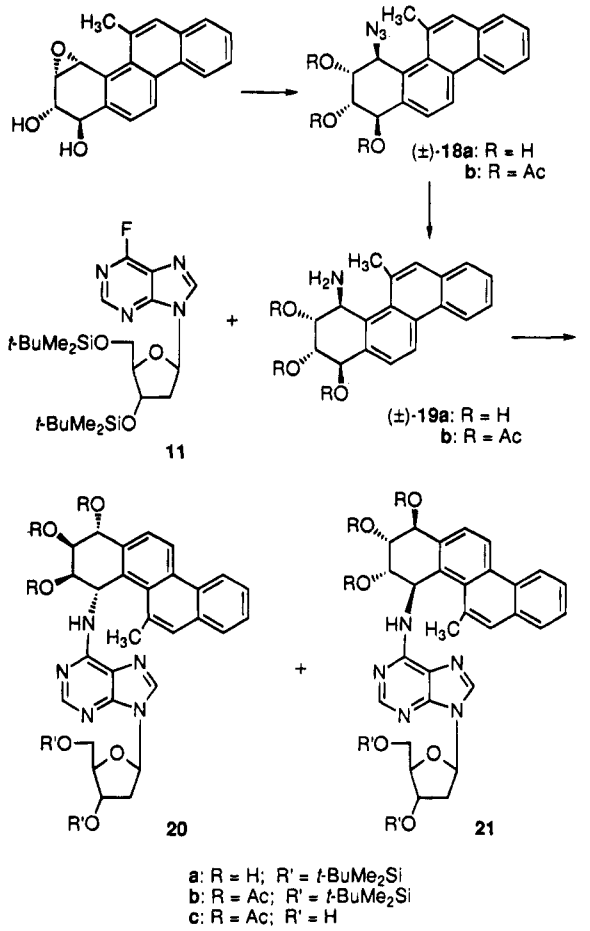


Figure 1. Circular dichroism spectra of the 5-methylchrysene–deoxyadenosine (**20c** and **21c**) and benzo[*a*]pyrene–deoxyadenosine (**14c** and **15c**) adducts in ethanol. Spectra have been normalized to 1 absorbance unit at λ_{max} 256 nm.

rect pathway required additional steps, the reactions were clean, the intermediate compounds were stable crystalline solids, and the overall yield of **12a** was higher via this route.

Coupling of **11** with **12a** was carried out in the presence of trimethylamine and hexamethyldisiloxane (HMDS) to prevent loss of the silyl groups caused by the hydrogen fluoride formed. Reaction was monitored by TLC. In contrast to similar reactions with 1-(aminomethyl)pyrene which were complete in a few hours, reaction was still incomplete after 3 days at 65 °C, but longer reaction time did not enhance the yield. Separation of the pure diastereomeric adducts (**14a** and **15a**) was conveniently accomplished by acetylation of the mixture followed by preparative TLC of the acetylated adducts (**14b** and **15b**) on silica gel plates. For larger scale preparations, separation of **14b** and **15b** was achieved by flash chromatography on a column of silica gel. The absolute configurations of the adducts were assigned on the basis of their circular dichroism CD spectra (Figure 1). Previous correlations have shown that the major CD bands in the region of 244 nm and 279 nm of the deoxyadenosine adducts formed by the *anti*-diol epoxide isomer of benzo[*a*]pyrene with dA are positive in the case of 10*S* adducts and negative in the case of 10*R* adducts.[23] The CD spectrum of the adduct **14c** derived from the early eluting diastereomer **14b** showed positive peaks at 244 nm and 281 nm, allowing assignment of its absolute

Scheme 4



stereochemistry as 7*R*,8*S*,9*R*,10*S*. Therefore, the adduct **15c** derived from the late eluting diastereomer **15b** which showed negative peaks in this region must be assigned the 7*S*,8*R*,9*S*,10*R* absolute stereochemistry.

Synthesis of Deoxyadenosine Adducts of 5-Methylchrysene *anti*-Diol Epoxide (4). The synthetic approach to these adducts was patterned after the sequence employed for the synthesis of related adducts of the benzo[*a*]pyrene *anti*-diol epoxide (Scheme 4). The amino triol derivative of 5-methylchrysene (**19a**) was synthesized from the (±)-*anti*-diol epoxide of 5-methylchrysene[24] via reaction with NaN_3 and direct reduction of the resulting azidotriol (**18a**) with hydrogen over a Pd/C catalyst. In contrast to the corresponding derivative of benzo[*a*]pyrene **13a**, the azidotriol **18a** was stable and readily isolable in pure state. The product of competing nonbenzylic attack by the azide ion was not detected by TLC or NMR analysis of the crude azidotriol product. This is in contrast with a report that a nonbenzylic adduct is detected as a minor product in the analogous reaction of the benzo[*c*]phenanthrenediol epoxide.[13a] The indirect route for the preparation of **19a** via acetylation of **18a** to **18b** followed by reduction to **19b** and deacetylation was also examined, but the overall yield was lower, mainly because of the low yield in the deacetylation step.

The absolute configurations of the nucleoside adducts of 5-methylchrysene (**20c** and **21c**) were also established from their CD spectra (Figure 1). These adducts, like those of benzo[*a*]pyrene, showed strong exciton coupling bands due to the electronic transition dipole interactions

(23) Sayer, J. M.; Chadha, A.; Agarwal, S. K.; Yeh, H. J. C.; Yagi, H.; Jerina, D. M. *J. Org. Chem.* **1991**, *56*, 20.

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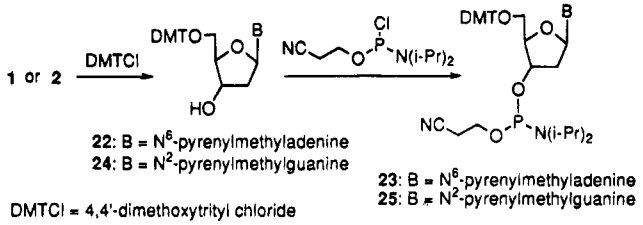
Scheme 5. Conversion of Mononucleoside–PAH Adducts into Suitably Protected Phosphoramidite Adducts for Incorporation into Oligonucleotides



between the polycyclic hydrocarbon and purine chromophores. The two diastereomers exhibited nearly mirror image CD bands. The CD spectrum of the adduct **20c** derived from the early eluting diastereomer **20b** showed positive peaks at 258 and 275 nm, requiring assignment of its absolute configuration as 1*R*,2*S*,3*R*,4*S*. It follows that **20a** and **20b** must have the same configuration.[23] On the other hand, the adduct **21c** derived from the late eluting diastereomer **21b**, which exhibited negative peaks in this region, must have the 7*S*,8*R*,9*S*,-10*R* absolute configuration, as must **21a** and **21b**.

Incorporation of Mononucleoside Adducts into Oligonucleotides. The mononucleoside adduct **2** was used as a model compound for this purpose. In order to protect the 5′-hydroxyl group of the deoxyribose function of **2**, it was converted to the 5′-*O*-(4,4′-dimethoxytrityl) derivative **22** by reaction with 4,4′-dimethoxytrityl chloride (DMTCl)[25] (Scheme 5). Reaction of **22** with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite by the usual procedure[26] furnished the 3′-[*O*-(2-cyanoethyl)-diisopropylphosphordiamidite] derivative **23**. It was then incorporated into an oligonucleotide with the sequence GCAGGTCA*AGAG where A* represents N^6-(1-methylpyrenyl)deoxyadenosine. Synthesis of the oligomer was performed on an automated DNA Synthesizer (15 μmol scale) using 2-cyanoethyl phosphoramidite chemistry. The standard procedure was employed, except that in the critical step involving incorporation of the PAH-adducted purine base the coupling was performed manually by injecting a solution of **23** dissolved in Activator (tetrazole solution) and allowing 1 h reaction time. This modification gave a coupling efficiency of 86%, a considerable improvement over standard machine coupling.[27] The completed 12-mer, still bearing the terminal DMT blocking group, was purified by HPLC, providing a total yield of 52%. The retention time of the 5′-DMT-oligomer was 40.5 min, and that of the detritylated 5′-OH-oligomer, which formed to the extent of 20% during the HPLC analysis conditions, was 27.6 min (Figure 2). The UV spectra of the tritylated and detritylated oligomers had bands (maxima at 332 nm and 348 nm, respectively), indicating the presence of the pyrenyl chromophore in these oligomers (Figure 2). After the removal of the DMT blocking group, the PAH-adducted 12-mer was judged to be 98% pure by HPLC. The UV spectrum of a nonmodified oligomer with the same base sequence showed the absence of any bands in the range of 300–350 nm, further confirming the presence of the pyrenyl moiety in the oligomer containing A*.

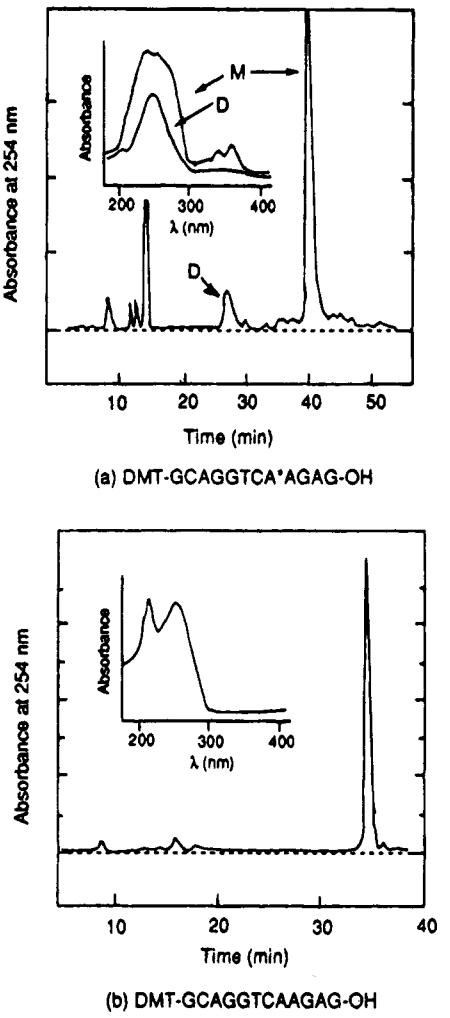


Figure 2. (a) HPLC separation of the modified oligonucleotide 12-mer where A* represents N^6-(1-methylpyrenyl)deoxyadenosine using a DuPont Zorbax ODS column eluted at 3 mL/min with 0.1 M TEAA, pH 6.5 (A) in acetonitrile (B) over 60 min with a gradient of 10–40% B. Under these conditions, the retention time of the modified oligomer (M) was 40.5 min and that of the related detritylated 5′-OH-oligomer (D) which formed to the extent of 20% during the HPLC separation was 27.6 min. The inset shows the photodiode array UV spectrum of the modified tritylated and detritylated oligomers. (b) HPLC separation under the same conditions of the unmodified 12-mer DMT-GCAGGTCAAGAG-OH. The retention time of this oligomer was 34.5 min. The inset shows its photodiode array UV spectrum.

Preparation of the Protected Phosphordiamidites of the Deoxyadenosine Adducts of the Benzo[*a*]pyrene and 5-Methylchrysene *anti*-Diol Epoxides. The procedure used for synthesis of the protected phosphordiamidite of the 1-(pyrenylmethyl)deoxyadenosine adduct (**23**) was also employed for the preparation of the analogous phosphordiamidite derivatives of the benzo[*a*]pyrene and 5-methylchrysene *anti*-diol epoxide adducts (**26** and **27**). It was convenient to employ the triacetoxy derivatives **14c** and **20c** as the starting compounds for this purpose, since it was necessary to protect the hydroxy groups of the PAH moiety in these operations and the acetate functions may be readily removed at a later stage. A similar strategy was successfully employed previously for the preparation of oligonucleotides containing N^6-deoxyadenosine adducts of benzo[*c*]phenanthrene *anti*-diol epoxide.[13b]

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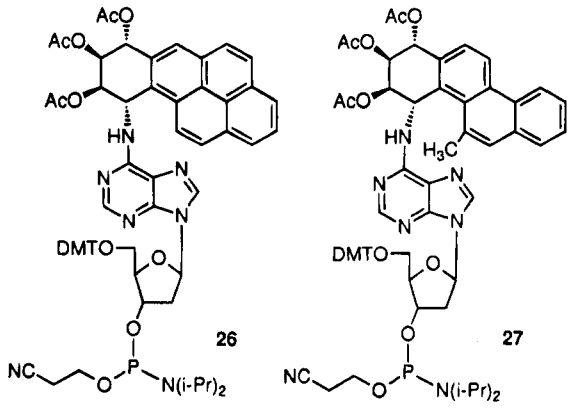

Discussion

The synthetic approach to PAH–oligonucleotide adducts described herein provides, in principle, convenient synthetic access to a wide range of site-specifically alkylated adducts containing a PAH moiety at any preselected base site. This *total synthetic method* contrasts with the *direct synthesis method* described previously[28] in which a short oligonucleotide with a defined base sequence is first synthesized and then modified by reaction with a diol epoxide or other PAH carcinogen metabolite. While the direct synthesis method offers advantages of relative simplicity and convenience, its scope is limited by the relative greater reactivity of dG sites compared to dA sites and the facility of PAH diol epoxides to react relatively indiscriminantly with all the dG sites present in an oligonucleotide. Consequently, the direct method is applicable mainly to the preparation of modified oligomers in which the PAH moiety is covalently bonded to dG in a base sequence with no more than one neighboring dG. It is less conveniently applicable to preparation of modified oligomers containing a PAH molecule attached to dA in a base sequence flanked by dGs or to a sequence having a PAH molecule linked to a single predetermined dG in a sequence of multiple dGs. The total synthesis method which is not subject to these limitations is inherently more powerful. Its potential importance is enhanced by emerging evidence that reactions on nucleic acid base sequences of the types that are not readily accessible by direct synthesis may play an important role in the mechanism of PAH carcinogenesis.[6,7,28,29]

Experimental Section

Materials and Methods. 6-Chloro-9-(2′-deoxy-3′,5′-di-O-*p*-toluoyl-β-D-erythropentafuranosyl)purine (**9**),[17] 6-fluoro-9-(2-deoxy-3,5-bis(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuranosyl)purine (**11**),[11] benzo[*a*]pyrene *anti*-diol epoxide, (±)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene,[21] and 5-methylchrysene *anti*-diol epoxide, (±)-*trans*-1,2-dihydroxy-*anti*-3,4-epoxy-1,2,3,4-tetrahydro-5-methylchrysene,[24] were synthesized by the methods described. Benzene and diethyl ether were purified by distillation from sodium. CH_2Cl_2 was distilled from CaH_2. Acetone was dried over 4 Å molecular sieves, and pyridine was dried over NaOH. Proton NMR spectra were obtained in $CDCl_3$ with tetramethylsilane as internal standard. Integration was consistent with all assignments.

2′-Deoxy-N^2,3′,5′-triacetylguanosine (5). A mixture of 2′-deoxyguanosine (16.7 g, 62.5 mmol) in 70 mL of acetic anhydride and 250 mL of anhydrous pyridine was heated at reflux for 1.5 h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and washed (3×) with aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, the solvent was evaporated, and the residue was recrystallized from water to provide **5** (24.0 g, 94%) as a white solid, mp 226–227 °C: ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3), 2.10 (s, 3), 2.28 (s, 3), 2.48 (m, 1), 2.98 (m, 1), 4.39 (m, 2), 4.79 (dd, 1), 5.21 (d, 1), 6.18 (dd, 1), 7.64 (s, 1), 9.04 (s, 1), 11.85 (s, 1). Anal. Calcd for $C_{16}H_{19}O_7N_5$: C, 48.86; H, 4.87; N, 17.80. Found: C, 48.65; H, 4.86; N, 17.66.

2′-Deoxy-O^6-((*p*-nitrophenyl)ethyl)-N^2,3′,5′-triacetylguanosine (6a) and 2′-deoxy-O^6-((*p*-nitrophenyl)ethyl)guanosine (6b). A mixture of **5** (6.48 g, 16.5 mmol), diethyl azodicarboxylate (5.9 g, 34 mmol), triphenylphosphine (8.9 g, 34 mmol), and (*p*-nitrophenyl)ethanol (5.7 g, 34 mmol) in 100 mL of *p*-dioxane were stirred for 2 h at room temperature then evaporated to dryness. The residue was dissolved in CH_2Cl_2 and purified on a silica gel column (4 × 10 cm). Following initial elution of minor contaminants with CH_2Cl_2 (2 L), slightly impure **6a** (10.0 g, 100%) was eluted with CH_2Cl_2–MeOH (9:1): NMR ($CDCl_3$) δ 2.08 and 2.12 (2s, 6), 2.48 (s, 3), 2.59 and 3.00 (2m, 2), 3.16 (t, 2), 4.37 and 4.43 (m, 2), 4.35 (apparent s, 1), 4.79 (t, 2), 5.41 (m, 1), 6.36 (dd, 1), 7.51 (d, 2), 7.95 (s, 1), 8.12 (d, 2), 8.29 (broad s, 1). A solution of **6a** (16.5 mmol) in MeOH (300 mL) was diluted with 300 mL of concd NH_4OH, stirred at room temperature for 6 days, and then evaporated to dryness. Chromatography of the residue on a silica gel column gave on elution with CH_2Cl_2–MeOH (95:5) **6b** (5.88 g, 87%), mp 189–190 °C (benzene/$CHCl_3$): NMR ($CDCl_3$) δ 2.25 and 2.91 (m, 2), 3.22 (t, 2), 3.87 and 3.90 (m, 2), 4.18 (br s, 1), 4.69 (m, 1), 4.70 (s, 2), 6.21 (dd, 1), 7.43 (d, 2), 7.64 (s, 1), 8.11 (d, 2). Anal. Calcd for $C_{18}H_{20}O_6N_6$: C, 51.92; H, 4.84. Found: C, 51.65; H, 4.86.

2-Fluoro-O^6-((*p*-nitrophenyl)ethyl)-9-(2′-deoxy-β-D-ribofuranosyl)purine (7). To a solution of HF/pyridine (70% by wt, 12 mL) diluted with pyridine (4 mL) and THF (4 mL) was added a solution of **6b** (500 mg, 1.4 mmol) in 8 mL of THF followed by *tert*-butyl nitrite (0.5 mL). Stirring was continued for 5 min at −25 °C, and then the solution was poured into ice–water. The aqueous mixture was extracted with CH_2Cl_2 (5×). The combined organic phase was washed with water (3×) and 5% $NaHCO_3$ solution, dried over $MgSO_4$ for 2 h, filtered, and evaporated to dryness. The resinous product was purified by chromatography on a silica gel column. Elution with CH_2Cl_2–MeOH (9:1) provided pure **7** (466 mg, 79%), mp 76–77 °C: NMR ($CDCl_3$) δ 2.39 and 2.89 (m, 2), 3.30 (t, 2), 3.80 (d of d, 1), 3.94 (d of d, 1), 4.17 (d, 1), 4.77 (d, 1), 4.82 (t, 2), 6.32 (dd, 1), 7.45 (d, 2), 8.02 (s, 1), 8.12 (d, 2). Anal. Calcd for $C_{18}H_{18}O_6N_5F$: C, 51.55; H, 4.33. Found: C, 51.44; H, 4.47.

2′-Deoxy-O^6-((*p*-nitrophenyl)ethyl)-N^2-(1-pyrenylmethyl)guanosine (8). A mixture of **6** (210 mg, 0.5 mmol), 1-(aminomethyl)pyrene (173 mg, 0.75 mmol), and triethylamine (1 mL) in DMF (20 mL) was stirred at room temperature for 72 h. The solvent was evaporated *in vacuo*, and the residue was purified by chromatography on a silica gel column. Elution with $CHCl_3$–MeOH (95:5) provided **8** (236 mg, 75%), mp 131–132 °C (acetone): NMR ($CDCl_3$) δ 2.22 (m, 1), 2.93 (broad peak, 2), 3.05 (m, 1), 3.70 (m, 1), 3.91 (d, 1), 4.13 (br s, 1), 4.49 (t, 2), 4.68 (d, 1), 5.29 (d, 2; J = 5.4 Hz), 6.19 (dd, 1), 6.96 (broad s, 2), 7.58 (s, 1), 7.75 (broad s, 2), 7.9–8.02 (m, 4), 8.05–8.07 (m, 2), 8.15–8.16 (m, 2), 8.25 (d, 1; J = 9.2 Hz), the NH signal, expected to be broad, was apparently hidden under the aromatic peaks; MS-FAB m/e 631 (M + 1). Anal. Calcd for $C_{35}H_{30}O_6N_6$: C, 66.65; H, 4.80; N, 13.33. Found: C, 66.44; H, 5.17; N, 13.14.

2′-Deoxy-N^2-(1-pyrenylmethyl)guanosine (1). A solution of **8** (490 mg, 0.77 mmol) in 20 mL of a 0.5 M solution of DBU in pyridine was stirred at ambient temperature overnight. The solution was neutralized with 1 M AcOH and then diluted with pyridine and evaporated to dryness. Chromatography of the residue on a silica gel column afforded on elution with CH_2Cl_2–MeOH (2:1) a fraction containing **1** plus impurities. Dilution with acetone furnished a precipitate

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which was collected by filtration to afford pure **1** (367 mg, 98%) as a white solid, mp 240–241 °C (MeOH): NMR (DMSO-d_6) δ 2.27 (m, 1), 2.65 (m, 1), 3.55 and 3.58 (m, 2), 3.86 (m, 1), 4.38 (s, 1), 4.91 (t, 1), 5.27 (d, 2; J = 2.8 Hz), 5.30 (s, 1), 6.28 (dd, 1; J = 6.7 Hz), 7.30 (broad peak, 1), 7.95 (s, 1), 8.10 (t, 1), 8.17 (t, 1; J = 8.1 Hz), 8.19 (s, 2), 8.29–8.34 (m, 4), 8.50 (d, 1; J = 9.3 Hz), 10.6 (broad s, 1); MS-FAB m/e 482 (M + 1). Anal. Calcd for $C_{27}H_{23}O_4N_5$: C, 67.35; H, 4.81; N, 14.54. Found: C, 67.44; H, 4.86; N, 14.39.

1-Pyrenecarboxaldehyde Oxime. To a solution of 1-pyrenecarboxaldehyde (10 g, 44 mmol) and hydroxylamine·HCl (4.8 g, 68 mmol) in 200 mL of ethanol and 40 mL of water was added NaOH (8.6 g). The mixture was heated at reflux for 5 min and then stirred at room temperature for 2 h and poured into dilute HCl solution. The precipitate was collected by filtration and crystallized from benzene to yield the oxime (9.5 g, 90%), mp 196–197: NMR ($CDCl_3$) δ 7.9–8.3 (m, 8), 8.5 (d, 1; J = 10.0 Hz), 9.2 (s, 1). Anal. Calcd for $C_{17}H_{11}NO$: C, 83.23; H, 4.53. Found: C, 83.25; H, 4.52.

1-(Aminomethyl)pyrene. A solution of the oxime (10.5 g, 44 mmol) in 200 mL of acetic acid was stirred with zinc dust (40 g) at room temperature for 15 h. The mixture was filtered, the filtrate was neutralized with NaOH, and the resulting precipitate was filtered and washed with water. The solid was dissolved in hot EtOAc/THF and filtered hot. The filtrate was evaporated, and the residue was crystallized from benzene to give the pure amine (8.5 g, 85%) as a white solid, mp 212–213 °C: NMR ($CDCl_3$) δ 4.55 (s, 2), 7.94–7.97 (m, 2), 7.99 (s, 2), 8.07–8.14 (m, 4), 8.27 (d, 1; J = 9.2 Hz); MS-CI m/e 231 (M). Anal. Calcd for $C_{17}H_{13}N$: C, 88.28; H, 5.67. Found: C, 88.25; H, 5.55.

2′-Deoxy-N^6-(1-pyrenylmethyl)-3′,5′-di-*O*-*p*-toluoyladenosine (10). A mixture of **9** (1.466 mg, 2.88 mmol), 1-(aminomethyl)pyrene (666 mg, 2.88 mmol), and triethylamine (2 mL) in 50 mL of DMF was stirred at room temperature for 72 h. The solvent was evaporated, and the residue was purified by chromatography on a silica gel column. Elution with EtOAc/benzene (20:8) furnished pure **10** (1.28 g, 63%), mp 116–117 °C (ethanol/CH_2Cl_2): NMR ($CDCl_3$) δ 2.24 (s, 3), 2.35 (s, 3), 2.62 (m, 1), 2.89 (m, 1), 4.47 (d, 1), 4.55 (m, 2), 5.41 (broad s, 2), 5.64 (d, 1), 6.38 (dd, 1), 6.38 (broad s, 1), 7.07 (d, 2), 7.16 (d, 2), 7.58 (broad s, 1), 7.77 (d, 2; J = 8.1 Hz), 7.85 (d, 2), 7.87–8.06 (m, 8), 8.19 (d, 1; $J_{9,10}$ = 9.2 Hz), 8.33 (broad s, 1); MS-FAB m/e 702 (M + 1). Anal. Calcd for $C_{43}H_{35}O_5N_5$: C, 73.59; H, 5.03; N, 9.98. Found: C, 73.44; H, 5.02; N, 9.79.

2′-Deoxy-N^6-(1-pyrenylmethyl)adenosine (2). A mixture of **10** (1.28 g, 1.82 mmol) in 20 mL of THF was diluted with MeOH (120 mL), and NaOMe (200 mg) was added. The mixture was stirred at ambient temperature for 4 h, and then Dowex (H-form) was added to neutralize the solution. The mixture was filtered, and the filtrate was concentrated to dryness. The residue was purified by chromatography on a silica gel column eluted with CH_2Cl_2–MeOH (9:1) to provide pure **2** (710 mg, 99%), mp 140–141 °C (MeOH): NMR ($CDCl_3$) δ 2.10 (d of d, 1), 2.95 (m, 1), 3.69 (t, 1), 3.90 (d, 1; J = 12.8 Hz), 4.07 (apparent s, 1), 4.68 (d, 1), 5.48 (broad s, 2), 6.03 (dd, 1), 7.44 (s, 1), 7.44–8.14 (m, 8), 8.25 (d, 1; $J_{9,10}$ = 9.2 Hz), 8.39 (broad s, 1); MS-CI m/e 466 (M). Anal. Calcd for $C_{27}H_{23}O_3N_5$: C, 69.66; H, 4.98; N, 15.04. Found: C, 69.41; H, 4.82; N, 14.99.

(±)-10β-Azido-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (13a). To a suspension of NaN_3 (46 g, 71 mmol) in 200 mL of CH_2Cl_2 and 200 mL of MeOH was added the *anti*-diol epoxide of benzo[*a*]pyrene (1.39 g, 5 mmol), and the mixture was stirred at room temperature for 7 h. The mixture was concentrated under vacuum, 80 mL of water was added, and the solid precipitate was filtered and dried. Chromatography on a column of Florisil eluted with CH_2Cl_2–MeOH (10:1) gave a solid which was crystallized from the same solvent to yield **13a** (970 mg, 61%) as a pale yellow solid; a mp could not be obtained due to the instability of the compound; the NMR spectrum was consistent with this assignment.[30] Compound **13a** was used directly in the next step.

(±)-10β-Azido-7β,8α,9α-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (13b). To a solution of **13a** (453 mg, 1.31 mmol) in 5 mL of dry pyridine was added dropwise 5 mL of Ac_2O, and the mixture was stirred at room temperature under N_2 for 3.5 h. The solution was poured onto 150 g of ice, water was added, and the cream-colored precipitate was filtered off. Chromatography on a column of Florisil eluted with CH_2Cl_2 gave **13b** (618 mg, 86%) as a lemon yellow solid. A sample crystallized from acetone–hexane gave light yellow prismatic crystals, mp 213.5–214.5 °C dec: NMR ($CDCl_3$) δ 2.04 (s, 3), 2.13 (s, 3), 2.31 (s, 3), 5.62 (d, 1; J = 3.9 Hz), 5.79–5.85 (m, 2), 6.76 (d, 1; J = 8.4 Hz), 8.05–8.31 (m, 8); MS m/z (rel intensity) 471 (M^+, 1). Anal. Calcd for $C_{26}H_{21}N_3O_6$: C, 66.24; H, 4.49; N, 8.91. Found: C, 66.18; H, 4.48; N, 8.88.

(±)-10β-Amino-7β,8α,9α-triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (12b). To a solution of **13b** (135 mg, 0.29 mmol) in EtOAc (37 mL) and THF (6 mL) was added 50 mg of 10% Pd/C, and the mixture was hydrogenated at 7 psig for 40 min. The mixture was filtered through Na_2SO_4, the solvent was evaporated, and the residue was chromatographed on a column of Florisil eluted with CH_2Cl_2–MeOH (100:1) to furnish pure **12b** (118 mg, 93%) as a pale yellow solid, mp 114 °C dec (ether–hexane): NMR ($CDCl_3$) δ 1.91 (br s, 2), 2.00 (s, 3), 2.15 (s, 3), 2.30 (s, 3), 5.16 (d, 1; J = 3.4 Hz), 5.65 (t, 1), 6.07 (dd, 1; J = 9.2, 2.4 Hz), 6.79 (d, 1; J = 9.0 Hz), and 8.00–8.40 (m, 8); MS m/z (rel intensity) 445 (M^+, 6). Anal. Calcd for $C_{26}H_{23}NO_6$: C, 70.10; H, 5.20; N, 3.14. Found: C, 69.87; H, 5.29; N, 3.13.

(±)-10β-Amino-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (12a). A. From 12b. To a solution of **12b** (200 mg, 0.45 mmol) in 40 mL of MeOH was bubbled a stream of ammonia for 20 min. The solution became warm and transparent. Stirring was continued for 1 h and then the solvent was removed under vacuum and the residue was suspended in CH_2Cl_2 and filtered off to give pure **12a** (110 mg, 77%), mp 234.5–236 °C dec: the NMR spectrum matched that reported.[31] **B. From 13a:** To a solution of **13b** (70 mg, 0.20 mmol) in 10 mL of THF and 13 mL of EtOAc was added 30 mg of 10% Pd/C, and the mixture was hydrogenated at 10 psig for 7 h. The mixture was filtered through Na_2SO_4, the solvent was evaporated, and the residue was chromatographed on a silica gel column eluted with an EtOAc to MeOH gradient to provide **12a** (38 mg, 59%) as a pale yellow solid whose NMR spectrum was identical with that of **12a** obtained by ammonolysis.

(±)-N^6-[10α-(7β,8α,9α-Trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (14a and 15a). A mixture of **11** (50 mg, 0.1 mmol) and **12a** (31 mg, 0.1 mmol), triethylamine (0.3 mL), and hexamethyldisiloxane (0.7 mL) in 4.5 mL of dimethylacetamide and 1 mL of dimethylformamide was stirred at 65 °C for 3 days. The solvent was evaporated, and the residue was chromatographed on a silica gel column. Elution with $CHCl_3$–MeOH (98:2) furnished a mixture of the diastereomeric adducts **14a** and **15a** (35 mg) which were employed directly in the next step. Further elution with 20% MeOH in $CHCl_3$ gave recovered unreacted **12a** (13 mg).

A similar reaction with pyridine in place of triethylamine furnished a lower yield of the mixture of **14a** and **15a** (14 mg, 18%) accompanied by bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**16**) (17 mg) and 10 mg of a benzo[*a*]pyrene derivative of uncertain structure. The structure of **16** was confirmed by comparison with an authentic sample prepared by silylation of 2′-deoxyadenosine: ^{1}H NMR ($CDCl_3$) of **16** δ 0.11 and 0.13 (2 s, 12), 0.94 (s, 18), 2.42–2.66 (m, 2), 3.77–3.89 (m, 2), 4.02 (m, 1), 4.62 (m, 1), 6.24 (s, 2), 6.45 (dd, 1), 8.12 (s, 1), 8.32 (s, 1, H_8); FAB MS, 480 (M).

(7*R*,8*S*,9*R*,10*S*)-N^6-[10-(7,8,9-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (14b) and (7*S*,8*R*,9*S*,10*R*)-Isomer (15b). The mixture of **14a** and **15a** (27 mg) was treated with 1 mL of

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dry pyridine and 1 mL of Ac_2O overnight at room temperature and evaporated to dryness. Preparative TLC of the residue was conducted on silica gel (0.5mm) eluted with 3% MeOH in $CHCl_3$. Visualization of the bands by UV lamp showed good separation of **14b** and **15b**, which were scraped from the plate and recovered by solvent extraction. For larger scale preparations, separation of **14b** and **15b** is more conveniently accomplished by flash chromatography on a silica gel column (200–425 mesh). The pure diastereomers **14b** (8.7 mg) and **15b** (12.5 mg) were isolated in 54% overall yield based on **12a**: 500 MHz ^{1}H NMR ($CDCl_3$) **14b** (early eluting isomer) δ 0.10 and 0.16 (2 d, 12), 0.92 and 0.97 (2s, 18), 2.10, 2.11, 2.35 (3s, 9), 2.39 (m, 2), 3.84 (m, 2), 4.03 (m, 1), 4.62 (m, 1), 5.93 (d, 1; J = 8.9; J = 2.0 Hz), 6.15 (s, 1), 6.50 (t, 1), 6.59 (br s, 1), 6.79 (d, 1), 7.99–8.21 (m, 9), 8.65 (br s, 1); FAB MS, 908 (M + 1). Anal. Calcd for $C_{48}H_{61}O_9N_5Si_2$: C, 63.48; H, 6.77. Found: C, 63.34; H, 6.88. 500 MHz ^{1}H NMR ($CDCl_3$) **15b** (late eluting isomer): δ 0.10 and 0.16 (3s, 12, CH_3), 0.92 and 0.97 (2s, 18), 2.09, 2.11, 2.34 (3s, 9), 2.39 and 2.75 (m, 2), 3.80-3.91 (m, 2), 4.04 (m, 1), 4.65 (m, 1), 5.93 (d, 1), 6.15 (apparent s, 1), 6.46 (dd 1), 6.50 (dd, 1), 6.78 (d, 1), 7.96–8.22 (m, 9), 8.62 (br s, 1); FAB MS 908 (M + 1). Anal. Calcd for $C_{48}H_{61}O_9N_5Si_2$: C, 63.48; H, 6.77. Found: C, 63.66; H, 6.62.

(7*R*,8*S*,9*R*,10*S*)-*N*^{6}-[10-(7,8,9-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-2′-deoxyadenosine (14c). The adduct **14b** (14 mg, 0.015 mmol) was treated with n-Bu_4N^+ F^- (35 μL of 1 M solution, 2.2 equiv) in anhydrous THF (200 μL) and stirred at room temperature for 1 h. Evaporation of the solvent gave a residue which was purified by chromatography on a silica gel column. Elution with 6% MeOH in $CHCl_3$ provided the free nucleoside adduct **14c** (10 mg, 94%): 500 MHz ^{1}H NMR ($CDCl_3$/D_2O) δ 2.06–2.15 (3s, 9), 2.19 (m, 1), 2.95 (m, 2), 3.77–3.93 (dd, 2), 4.12 (apparent s, 1), 4.70 (m, 1), 5.90 (m, 1), 6.01 (m, 1), 6.08 (apparent s, 1), 6.61 (apparent s, 1), 6.76 (d, 1), 8.01–8.23 (m, 10), 8.56 (s, 1); FAB MS, 680 (M + 1). Anal. Calcd for $C_{36}H_{33}O_9N_5$: C, 63.62; H, 4.89. Found: C, 63.34; H, 4.78.

(7*S*,8*R*,9*S*,10*R*)-*N*^{6}-[10-(7,8,9-Triacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-2′-deoxyadenosine (15c). Similar treatment of the adduct **15b** gave **15c** (10 mg, 94%): 500 MHz ^{1}H NMR ($CDCl_3$/D_2O) δ 2.04–2.11 (3s, 9), 2.16 (m, 1), 2.97 (m, 1), 3.80–3.94 (dd, 2), 4.20 (apparent s, 1), 4.70 (m, 1), 5.85 (m, 1), 6.02 (m, 1), 6.12 (apparent s, 1), 6.68 (d, 1), 6.78 (apparent s, 1), 7.98–8.21 (m, 10), 8.60 (s, 1). Anal. Calcd for $C_{36}H_{33}O_9N_5$: C, 63.62; H, 4.89. Found: C, 63.53; H, 4.99.

(±)-4β-Azido-1β,2α,3α-trihydroxy-1,2,3,4-tetrahydro-5-methylchrysene (18a). Reaction of NaN_3 with the *anti*-diol epoxide of 5-methylchrysene (550 mg, 1.91 mmol) was conducted by the procedure employed for **13a** except that reaction time was extended to 48 h. Chromatography of the crude product on silica gel eluted with a gradient of CH_2Cl_2 to CH_2Cl_2–EtOAc (1:1) gave pure **18a** (527 mg, 80%), mp 160–162 °C dec (from CH_2Cl_2–MeOH): NMR (DMSO-d_6 + D_2O) δ 2.98 (s, 3), 3.85 (dd, 1; J = 7.1, 2.1 Hz), 4.11 (dd, 1; J = 5.1 Hz), 4.74 (d, 1), 5.58 (d, 1; J = 5.2 Hz), 7.64 (m, 2), 7.76 (s, 1), 7.88 (d, 2), 8.78 (apparent d, 1; J = 8.9 Hz), 8.93 (d, 1); MS m/z (rel intensity) 335 (M^+ 19), 307 (10), 247 (100), 232 (41); HRMS calcd 335.1270, obsd 335.1305.

(±)-4β-Azido-1β,2α,3α-triacetoxy-1,2,3,4-tetrahydro-5-methylchrysene (18b). Acetylation of **18a** (200 mg, 0.60 mmol) by the procedure employed for **13b** gave **18b** (250 mg, 91%) as a pale yellow solid, mp 187.5–188.0 °C dec: NMR ($CDCl_3$) δ 2.08 (s, 3), 2.12 (s, 3), 2.50 (s, 3), 3.04 (s, 3), 5.60 (dd, 1; J = 4.8, J = 2.7 Hz), 5.69 (dd, 1; J = 7.8 Hz), 5.88 (d, 1; J = 4.9 Hz), 6.54 (d, 1), 7.51 (d, 1; J = 8.8 Hz), 7.64 (m, 2), 7.72 (s, 1), 7.82 (apparent d, 1), 8.62 (apparent d, 1), 8.84 (d, 1); MS m/z (rel intensity) 461 (M^+ 12), 316 (18), 274 (80), 271 (100). Anal. Calcd for $C_{25}H_{23}N_3O_6$: C, 65.07; H, 5.02; N, 9.11. Found: C, 65.11; H, 5.04; N, 9.01.

(±)-4β-Amino-1β,2α,3α-triacetoxy-1,2,3,4-tetrahydro-5-methylchrysene (19b). Reduction of **18b** (300 mg, 0.65 mmol) by the procedure employed for **12b** gave **19b** (250 mg, 88%), a light yellow solid mp 162–163 °C dec (from ether–hexane): NMR ($CDCl_3$) δ 1.62 (br s, 2), 2.08 (s, 3), 2.11 (s, 3), 2.23 (s, 3), 3.10 (s, 3), 5.37 (d, 1; J = 4.4 Hz), 5.46 (dd, 1), 5.91 (dd, 1; J = 2.6 Hz), 6.53 (d, 1; J = 8.3 Hz), 7.45 (d, 1; J = 8.8 Hz), 7.60 (m, 2), 7.67 (s, 1), 7.79 (m, 1), 8.60 (apparent d, 1), 8.76 (d, 1); MS m/z (rel intensity) 435 (M^+, 7), 315 (21), 301 (6), 273 (18), 256 (100); HRMS calcd 435.1682; obs 435.1687.

(±)-4β-Amino-1β,2α,3α-trihydroxy-1,2,3,4-tetrahydro-5-methylchrysene (19a). A. From 19b. Deacetylation of **19b** (240 mg, 0.55 mmol) by the procedure employed for preparation of **12a** from **12b** (reaction time 2 h) gave pure **19a** (105 mg, 62%), mp 208–210 °C dec; analytical sample cream colored crystals, mp 209–211 °C dec: NMR (DMSO-d_6 + D_2O) δ 3.98 (dd, 1; J = 3.9 Hz), 4.05 (dd, 1; J = 7.9, J = 2.3 Hz), 4.71 (d, 1), 5.01 (d, 1), 7.60 (m, 2), 7.68 (s, 1), 7.86 (m, 2), 8.75 (apparent d, 1), 8.82 (d, 1; J = 8.9 Hz); HRMS calcd 309.1365; obs 309.1378. **B. From 18a.** The procedure used for the preparation of **12a** from **13b** was followed (5.3 h) to yield **19a** (81%) as a pale yellow solid whose NMR spectrum was identical with **19a** obtained from method A.

(±)-*N*^{6}-[4β-(5-Methyl-1β,2α,3α-trihydroxy-1,2,3,4-tetrahydro-5-methylchrysenyl)]-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (20a) and Its Triacetate (21a). A mixture of **11** (50 mg, 0.1 mmol) and **19a** (31 mg, 0.1 mmol), triethylamine (0.3 mL), and hexamethyldisiloxane (0.7 mL) in 4.5 mL of dimethylacetamide and 1 mL of dimethylformamide was stirred at 65 °C for 3 days. Work up by the procedure used for the preparation of **14a** and **15a** provided a mixture of **20a** and **21a** (64 mg) which was used directly in the next step. Further elution of the chromatography column with $CHCl_3$–MeOH (4:1) gave **19a** (7 mg). Similar reaction with pyridine in place of triethylamine provided a mixture of **14a** and **15a** (16 mg, 21%), **16** (16 mg), and recovered **19a** (40 mg).

(1*R*,2*S*,3*R*,4*S*)-*N*^{6}-[4-(1,2,3-Triacetoxy-1,2,3,4-tetrahydro-5-methylchrysenyl)]-3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (20b) and (1*S*,2*R*,3*S*,4*R*)-Isomer (21b). Acetylation of the mixture of **20a** and **21a** (64 mg) by the procedure employed for **14b** and **15b** provided an early-eluting adduct **20b** (17 mg) and a late-eluting adduct **21b** (15 mg) in 36% overall yield (47% based on starting material consumed) in two steps. For **20b**: 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.10–0.16 (m, 12), 0.93–0.97 (m, 18), 2.08, 2.12, 2.27 (3s, 9), 2.48–2.50 (m, 2), 2.89 (s, 1), 3.78–3.88 (m, 2), 4.03 (m, 1), 4.42 (m, 1), 5.79–5.81 (m, 2), 6.33 (br s, 1), 6.48 (dd, 1), 6.49–6.54 (m, 1), 6.67 (m, 1), 7.52 (d, 1; J = 9.1 Hz), 7.58 (s, 1), 7.59–7.63 (m, 2) , 7.74 (d, 1; J = 7.3 Hz), 8.05 (s, 1), 8.56 (s, 1), 8.62 (d, 1), 8.86 (d, 1); FAB MS 898 (M + 1). Anal. Calcd for $C_{47}H_{63}O_9N_5Si_2$: C, 62.85; H, 7.07. Found: C, 63.04; H, 6.98. For **21b**: 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.10–0.16 (m, 12, CH_3), 0.92–0.97 (m, 18, *t*-Bu), 2.06, 2.13, 2.27 (3s, 9), 2.45–2.89 (m, 2), 2.91 (s, 1), 3.80–3.93 (m, 2), 4.03 (m, 1), 4.65 (m, 1), 5.78–5.90 (m, 2), 6.32 (br s, 1), 6.43 (dd 1; J = 8.9 Hz), 6.48 (d 1; J = 8.9 Hz), 6.68 (m, 1), 7.53 (d, 1; J = 9.1 Hz), 7.58 (s, 1), 7.57–7.64 (m, 2), 7.75 (d, 1; J = 7.8 Hz), 8.00 (s, 1), 8.55 (s, 1), 8.62 (d, 1; J = 8.5 Hz), 8.85 (d, 1; J = 8.9 Hz); FABMS 898 (M + 1). Anal. Calcd for $C_{47}H_{63}O_9N_5Si_2$: C, 62.85; H, 7.07. Found: C, 62.98; H, 7.18.

(1*R*,2*S*,3*R*,4*S*)-*N*^{6}-[4-(1,2,3-Triacetoxy-1,2,3,4-tetrahydro-5-methylchrysenyl)]-2′-deoxyadenosine (20c). Desilylation of the early-eluting adduct **20b** (17 mg, 0.019 mmol) with n-Bu_4N^+ F^- by the procedure used for **14c** gave the free nucleoside adduct **20c** (12 mg, 99%): 500 MHz ^{1}H NMR ($CDCl_3$/D_2O) δ 2.06, 2.11, 2.24 (3s, 9), 2.91 (s, 3), 2.25–3.05 (m, 2), 3.74–3.94 (2 d, 2), 4.13 (apparent s, 1), 4.76 (m, 1), 5.79 (m, 1), 6.19 (m, 1), 6.24 (dd, 1), 6.48 (d, 1), 6.72 (m, 1), 7.53–7.63 (m, 5), 7.73 (d, 1; J = 8.4 Hz), 8.49 (s, 1), 8.62 (d, 1; J = 8.4 Hz), 8.86 (d, 1; J = 8.9 Hz); FAB MS 670 (M + 1). Anal. Calcd for $C_{35}H_{35}O_9N_5$: C, 62.77; H, 5.27. Found: C, 62.84; H, 5.15.

(1*S*,2*R*,3*S*,4*R*)-*N*^{6}-[4-(1,2,3-Triacetoxy-1,2,3,4-tetrahydro-5-methylchrysenyl)]-2′-deoxyadenosine (21c). Desilylation of the late-eluting adduct **21b** (9 mg) with n-Bu_4N^+ F^- by the same procedure gave the isomeric nucleoside adduct **21c** (7 mg, 100%): 500 MHz ^{1}H NMR ($CDCl_3$/D_2O) δ 2.06, 2.12, 2.26 (3s, 9), 2.89 (s, 3), 3.07 (m, 2), 3.81 and 3.98 (2 d, 2), 4.17 (s, 1), 4.74 (m, 1), 5.79 (m, 1; J = 9.0 Hz), 6.17 (apparent s, 1), 6.25 (dd, 1), 6.47 (d, 1; J = 8.8 Hz), 6.69 (d, 1; J = 3.6 Hz), 7.53 (d, 1; J = 9.0 Hz), 7.55–7.63 (m, 4), 7.75 (d, 1; J = 7.3 Hz), 8.49 (s, 1), 8.61 (s, 1), 8.62 (d, 1; J = 8.3 Hz), 8.86 (d, 1;

J = 8.8 Hz); FAB MS 670 (M + 1). Anal. Calcd for $C_{35}H_{35}O_9N_5$: C, 62.77; H, 5.27. Found: C, 62.54; H, 5.38.

2′-Deoxy-5′-(*p*-dimethoxytrityl)-N^6-(1-pyrenylmethyl)-adenosine (22). Adduct **2** (750 mg, 1.5 mmol) was taken up in anhydrous pyridine (15 mL) and dried by coevaporation with anhyd pyridine three times. Dimethoxytrityl chloride (560 mg, 1.65 mmol) in 4 mL of anhydrous pyridine was added slowly (~1 drop per 20 s) from an addition funnel under N_2, and the course of reaction was monitored by TLC. After addition was complete, the reaction mixture was stirred for 2 h and then poured into ice–water, and the aqueous solution was extracted twice with EtOAc. The combined organic phase was backwashed with water and dried over $MgSO_4$. Following removal of the drying agent by filtration, the solution was concentrated to dryness, and the residue was dissolved in a small volume of $CHCl_3$. This solution was coevaporated with a small volume of toluene several times to remove pyridine. This step improves subsequent chromatographic resolution. The crude tritylated nucleoside gum was dissolved in a small volume of $CHCl_3$ and chromatographed on a column of silica gel (15 g). Initial elution with $CHCl_3$ afforded trityl alcohol. The tritylated nucleoside was then eluted from the column with a 10% solution of MeOH in $CHCl_3$. Evaporation of the eluents provided **22** (1.10 g, 95%) as a solid, mp 146–147 °C: 500 MHz ^{1}H NMR ($CDCl_3$) δ 2.52 and 2.78 (m, 2), 3.37 (m, 2), 3.72 (s, 6), 4.07 (m, 1), 4.63 (m, 1), 5.57 (br s, 2), 6.03 (br s, 1), 6.37 (dd, 1), 6.75 (d, 4), 7.22 (m, 9), 7.78 (s, 1), 7.96–8.33 (m, 9), 8.40 (s, 1, H_2); FAB MS 768 (M + 1).

(±)-N^6-(1-Pyrenylmethyl)-2′-deoxy-3′-(*N,N*-diisopropyl-2-cyanoethyl)-5′-(*p*-dimethoxytrityl)adenosine Phosphoramidite (23). *Important. All reactions with 2-cyanoethyl N,N-diisopropylchorophosphoramidite were conducted in flame-dried glassware with freshly distilled dry degassed solvents under an argon atmosphere. The tritylated adducts were thoroughly dried before use by evaporation with freshly distilled pyridine (3 × 10 mL) followed by removal of traces of pyridine under vacuum (0.1 mm Hg, 50 °C, 24 h).* To the tritylated adduct **22** (100 mg, 0.13 mmol) in a flame-dried flask was added 0.5 mL of freshly distilled THF followed by redistilled diisopropylamine (0.12 mL, 0.52 mmol) under argon. Then 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.058 mL, 0.26 mmol) was added rapidly by syringe to the magnetically stirred solution at room temperature. Analysis by TLC (silica gel–EtOAc/CH_2Cl_2/Et_3N: 5/5/1) indicated the reaction to be complete in 30 min. The amine hydrochloride was removed by filtration, and the filtrate was diluted with EtOAc and a few drops of Et_3N. The solution was extracted with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and filtered. After evaporation of solvent, the residue was dissolved in 4 mL of benzene and poured into hexane at −78 °C. The white precipitate was collected by filtration, giving **23** (127 mg, 99%) as a white solid, mp 94–95 °C dec: 500 MHz ^{1}H NMR ($CDCl_3$) δ 1.09 and 1.14 (2d, 12), 2.41 (t, 2), 2.54–2.76 (m, 2), 3.37 (m, 2), 3.48–3.91 (m, 4), 3.82 (s, 6), 4.11 (m, 1), 4.65 (m, 1), 5.60 (br s, 2), 6.39 (dd, 1), 6.78 (d, 4), 7.20 (m, 9), 7.81 (s, 1), 7.91–8.30 (m, 9), 8.41 (s, 1); ^{31}P NMR ($CDCl_3$) δ 149.74 and 149.94 relative to phosphoric acid as external standard. Anal. Calcd for $C_{57}H_{58}N_7O_6P$ + $1H_2O$: C, 69.42; H, 6.13. Found: C, 69.38; H, 6.12.

2′-Deoxy-5′-(*p*-dimethoxytrityl)-N^2-(1-pyrenylmethyl)-guanosine (24). The guanosine adduct **1** (900 mg, 1.86 mmol) was tritylated as in the case of **2** with 4,4′-dimethoxytrityl chloride (695 mg, 2.05 mmol) in 4.5 mL of pyridine. Workup and chromatography as before provided **24** (1.17 g, 80%) as a white solid, mp 171–172 °C: ^{1}H NMR ($CDCl_3$) δ 2.10 (br m, 2), 3.15 (2 br s, 2), 3.74 (s, 6), 3.92 (br s, 1), 4.20 (br s, 1), 4.79 (br s, 2), 5.70 (br s, 1), 6.55 and 6.90–7.35 (m, 13), 7.60–7.95 (m, 9), 8.08 (br s, 1); FABMS 784 (M + 1).

(±)-N^6-(1-Pyrenylmethyl)-2′-deoxy-3′-(*N,N*-diisopropyl-2-cyanoethyl)-5′-(*p*-dimethoxytrityl)guanosine Phosphoramidite (25). The 5′-protected nucleoside **24** (50 mg, 0.065 mmol) was converted to **25** by treatment with diisopropylethylamine (0.045 mL) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.03 mL) in 0.5 mL of THF by the procedure employed for **22**. Purification on a column of silica gel (2 g) eluted with $CHCl_3$/Et_3N/MeOH (90/5/10) furnished **25** (60 mg, 94%), mp 78–79 °C dec: ^{1}H NMR ($CDCl_3$) δ 1.11, 1.16 (2d, 12), 2.14–2.25 (m, 2), 2.39 (t, 2), 3.13–3.18 (m, 2), 3.51–3.86 (m, 4), 3.96 (br s, 1), 4.20 (br s, 1), 4.81 (br s, 2), 5.74 (br s, 1), 6.40–6.56 (m, 4), 6.98–7.32 (m, 9), 7.64–7.92 (m, 9), 8.11 (s, 1); ^{31}P NMR ($CDCl_3$) δ 149.902 and 150.127 relative to phosphoric acid external standard. Anal. Calcd for $C_{57}H_{58}N_7O_7P$ + $1H_2O$: C, 68.31; H, 6.04. Found: C, 68.30; H, 6.39.

Synthesis and Purification of the modified 12-mer 5′-GCAGGTCA*AGAG-3′ (26). The AGAG-3′ portion of the oligonucleotide was synthesized on the automatic DNA Synthesizer (15 mmol scale) using the 2-cyanoethyl phosphoramidite chemistry to yield 5′-HO-AGAG-3′-linked to the solid support. Detritylation of the last nucleoside AGAG was measured spectrophotometrically at 502 nm. The column was then removed from the machine. A solution of the modified A* representing **23** (116 mg, 115 mmol) was dissolved in 800 mL of acetonitrile and mixed with 1.8 mL of Activator (tetrazole solution). The resulting solution was injected into one end of the column using a gas tight syringe. Another gas tight syringe was fitted to the other end of the column, and the two syringes were used to push the solution in the column back and forth several times. The column was allowed to stand for 0.5 h, and then this procedure was repeated again before reattaching the column to the machine. Then the washing steps were carried out before resuming the normal DNA synthesis program. Spectroscopic measurement of detritylation after the manual coupling of **23** to the tetramer indicated a relative coupling efficiency of 86% at this stage.

At the end of the 12-mer synthesis the DMT group was not removed from the last nucleoside in the sequence. The solid support carrying the oligomer was transferred to a vial containing 10 mL of concd NH_4OH, and the suspension was heated at 55 °C for 5 h. The supernatant was transferred to a small vial and concentrated to dryness using a vacuum pump. The residue was taken up in 1.5 mL of 0.1 M tetraethylammonium acetate (TEAA) (pH 6.5), and the precipitate was removed by filtration through Millex-LCR (Millipore) and washed with water. The filtrate was concentrated down to 900 μL. The residue containing the oligomer was analyzed on a DuPont Zorbax ODS column (4.5 mm × 15 cm) eluted with 0.1 M TEAA (pH 6.5) (A) and acetonitrile (B) with a gradient of 10–40% B over 60 min at 3 mL/min. The retention time of the 5′-DMT-oligomer was 40.5 min, and that of the detritylated 5′-OH-oligomer which formed to the extent of 20% during the analysis conditions was 27.6 min (see Figure 2a). The UV spectrum of each of these peaks on the photodiode array detector (Waters Model 991) confirmed incorporation of **23** by the presence of the pyrene chromophore in the region of 310–360 nm. These peaks were collected by loading 5 × 200 mL portions of the solutions on semipreparative DuPont Zorbax ODS column (9.4 mm × 25 cm) and eluting under the same conditions employed with the analytical column. The recovered oligomers were dried by lyophilization. The second peak collected contained the trityl group which was removed by treatment with HOAc/water (8:2) for 30 min and redried by coevaporation with EtOH. The recovered sample was redissolved in 0.5 mL of H_2O and extracted twice with EtOAc. The EtOAc layer was extracted with 0.5 mL of H_2O, and the combined aqueous layer was lyophilyzed to give **26** (31.2 mg, 52%) as a white solid. The detritylated **26** was chromatographed on the DuPont Zorbax ODS analytical column under the same conditions as above. The retention time was the same as that of the first peak of the crude oligomer. The purified 12-mer was >98% pure by HPLC, and its UV spectrum showed the presence of the pyrenyl chromophore.

Synthesis of the Corresponding Nonmodified 12-mer DMT-GCAGGTCAAGAG. For comparison, the corresponding oligomer containing nonmodified A was synthesized using the standard program on a 0.2 mmol scale on the automatic DNA synthesizer. The UV spectrum of this oligomer showed the absence of any absorption in the region of 300–350 nm as expected (Figure 2b).

Protected Phosphordiamidite of the Deoxyadenosine Adduct of Benzo[*a*]pyrene *anti*-Diol Epoxide 26. The procedure used for synthesis of the protected phosphordia-

midite of the 1-(pyrenylmethyl)deoxyadenosine adduct **23** was employed for the preparation of the analogous phosphordiamidite derivative of the benzo[*a*]pyrene *anti*-diol epoxide adduct **26** from **14c**. Purification on a column of silica gel eluted with $CHCl_3/Et_3N$/MeOH (90/5/10) furnished **26** (88%) as a resinous solid. Anal. Calcd for $C_{66}H_{70}N_7O_{12}P + 1H_2O$: C, 65.93; H, 6.04. Found: C, 65.88; H, 5.98.

Protected Phosphordiamidite of the Deoxyadenosine Adduct of 5-methylchrysene *anti*-Diol Epoxide 27. The foregoing procedure was also employed for the synthesis of the analogous phosphordiamidite derivative of the 5-methylchrysene *anti*-diol epoxide adduct **27** from **20c**. Similar purification gave **27** (82%) as a resinous solid. Anal. Calcd for $C_{66}H_{70}N_7O_{12}P + 1H_2O$: C, 65.70; H, 5.94. Found: C, 65.91; H, 6.08.

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