Synthesis and Properties of an Oligodeoxynucleotide Containing a Polycyclic Aromatic Hydrocarbon Site Specifically Bound to the N^2 Amino Group of a 2'-Deoxyguanosine Residue†

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Abstract: A 2'-deoxyguanosine derivative has been prepared in which the amino group at the 2-position has been modified via the exocyclic methyl group of the polycyclic aromatic hydrocarbon 9-methylanthracene. In order to prepare this derivative, 2'-deoxyguanosine was reacted with bromoacetone to produce 2'-deoxy-4-desmethylwyosine (**1**), which in the presence of potassium carbonate could be alkylated with 9-(chloromethyl)anthracene at the N5 position yielding **2**. Treatment of 5-(anthracen-9-ylmethyl)-2'-deoxy-4-desmethylwyosine (**2**) with *N*-bromosuccinimide followed by aqueous ammonia hydrolyzed the tricyclic base yielding 2'-deoxyguanosine with 9-methylanthracene covalently bound to the exocyclic nitrogen (compound **3**). With this substitution it was not necessary to protect the exocyclic amino group prior to chemical DNA synthesis. The modified nucleoside was converted to its 5'-*O*-(9-phenylxanthen-9-yl) (pixyl) derivative **4**, which was used to prepare the 3'-*O*-(β-cyanoethyl *N,N*-diisopropylphosphoramidite) **5**. This guanosine derivative carrying a covalently bound polycyclic aromatic hydrocarbon was incorporated into oligodeoxynucleotides in order to study the properties of double-stranded DNA containing this potentially carcinogenic lesion located in the minor groove. The presence of the aromatic moiety results in significant destabilization of duplex DNA as determined from thermal melting studies. Thermodynamic parameters characterizing the helix-to-coil transition have been derived from $1/T_m$ vs log ($c_T/4$) plots. The enthalpy changes ($\Delta H°$) upon helix formation for the native and modified sequences are, within experimental error, the same. The 3.2 kcal/mol difference in the free energy ($\Delta\Delta G°$) of helix formation between the two DNA fragments (native sequence, $\Delta G°_{37} = -15.2$ kcal/mol; modified sequence, $\Delta G°_{37} = -12.0$ kcal/mol) appears to be largely the result of a 21 cal/mol·K difference in entropy ($\Delta\Delta S°$). Circular dichroism spectra indicate the presence of an essentially B-form DNA. The fluorescence of the anthracene moiety suggests that the polycyclic aromatic hydrocarbon is not intercalated or significantly stacked with the nucleobases but is more likely nestled within the minor groove of an essentially native although slightly distorted B-DNA helix.

Polycyclic aromatic hydrocarbons are ubiquitous in our environment and many (if not all) are thought to be either directly carcinogenic or result in carcinogenic compounds. In many cases metabolic activation is necessary to generate a reactive electrophilic species[1] (e.g., benzo[*a*]pyrenediol epoxide from benzo[*a*]pyrene[2]), which is often the ultimate carcinogen. However, in other cases, for example the bromomethyl or chloromethyl polycyclic aromatic hydrocarbons,[3] such activation is unnecessary. These latter derivatives can function directly as electrophiles, bind to DNA, and produce carcinogenic effects.[3] The occurrence of carcinogenic or mutagenic effects has been correlated with the ability of the corresponding electrophilic species to modify chromosomal DNA[4] and, presumably, interfere with normal biochemical events involved in cell replication.[5]

Although a number of modified nucleoside derivatives have been isolated from DNA treated with preactivated polycyclic aromatic hydrocarbons[6] such as benzo[*a*]pyrenediol epoxide[7] or similar halomethyl derivatives,[3c] the present body of evidence suggests that the exocyclic amino group of guanine and to a lesser extent those of adenine and cytosine are the primary sites of attachment.[6-8] These functional groups generally exhibit such low reactivity that modification at these positions is not observed to a significant extent in the presence of simple alkylating agents such a dimethyl sulfate, methylnitrosourea, or iodomethane.[9] The preference for reaction at the exocyclic amino groups by this class of compounds has been suggested to be a result of optimal positioning of the electrophile, possibly as a result of initial binding within one of the DNA grooves[10] or by transient intercalation of the aromatic ring system within the DNA double helix.[11] A number of related studies[10,11] have attempted to confirm or negate intercalation or groove binding prior to alkylation, but the evidence at present for binding solely by either mode is not conclusive.

† Abbreviations used: Pixyl, 9-phenylxanthen-9-yl; dT, 2'-deoxythymidine; dC, 2'-deoxycytidine; dA, 2'-deoxyadenosine; dG, 2'-deoxyguanosine; dG^A, N^2-(anthracen-9-ylmethyl)-2'-deoxyguanosine; 2'-deoxy-4-desmethylwyosine, 5,9-dihydro-6-methyl-9-oxo-3-(D-2-deoxyribofuranosyl)-5*H*-imidazo[1,2-*a*]-purine; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography.

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0002-7863/90/1512-5264$02.50/0

Much of the work to date associated with the interaction of electrophiles derived from polycyclic aromatic hydrocarbons with nucleic acids has suffered from the lack of well-characterized modifications at known sites within a sequence. It has often been necessary to work with the complex mixtures that arise after the treatment of DNA from natural sources with the electrophile of interest.[5,8] With some carcinogens it has been possible to design an appropriate DNA sequence, treat it with the derivative of interest, and isolate a complex containing a single modification,[12] but this approach has not been particularly successful with the electrophiles derived from polycyclic aromatic hydrocarbons. This is in part because multiple sites are alkylated by these electrophiles. A second approach involves the preparation of a well-characterized nucleoside derivative with a specific modification for incorporation into the desired sequence using chemical DNA synthesis techniques.[12] Two derivatives have been prepared in this manner in which the exocyclic amino group (N^6) of 2'-deoxyadenosine was modified with a polycyclic aromatic hydrocarbon.[13] The derivative containing a 7,12-dimethylbenz[*a*]anthracene substituent[13b] was subsequently incorporated into a number of oligodeoxynucleotide fragments.[14]

We report the synthesis of N^2-(anthracen-9-ylmethyl)-2'-deoxyguanosine by a procedure that may have general applicability for the preparation of a variety of modified deoxyguanosine derivatives substituted at the N^2 positon by polycyclic aromatic hydrocarbons. After preparation of the modified nucleoside and its conversion to a suitable building block for solid-phase DNA synthesis, it has been employed to synthesize three site specifically modified oligodeoxynucleotides. We have examined modification of the N^2 position since it appears to be one of the predominant biologically relevant sites for alkylation by polycyclic aromatic hydrocarbons (or their metabolically activated intermediates) and have chosen a derivative that could result from alkylation by the carcinogen 9-[bromo(chloro)methyl]anthracene.[3f] Double-stranded DNA fragments alkylated at the N^2 amino group will have the bulky aromatic residue in the minor groove of a B-DNA helix. Of the three sequences prepared with this modified guanosine derivative, a double-stranded 13-mer containing a single anthracen-9-ylmethyl substituent has been studied most extensively.

Experimental Section

Materials. The four common 2'-deoxynucleosides were obtained from American Bionetics (Hayward, CA). Bromoacetone used in the synthesis of 2'-deoxy-4-desmethylwyosine was obtained from Columbia Chemicals and distilled from $CaCl_2$ prior to use. Analytical thin-layer chromatography (TLC) was performed on 5 × 10 cm silica gel 60 F-254 glass-backed plates with fluorescent indicator (E. M. Science); preparative TLC employed glass-backed plates (Analtech) containing a 20 × 20 cm × 0.1 mm layer of silica gel GF; for column chromatography silica gel 60 finer than 230 mesh (E. M. Science) was used. HPLC was performed on a Beckmann chromatograph composed of two Model 114 pumps and a Model 163 variable-wavelength detector. Gradients were controlled with a Model 421A controller. ^1H and ^{31}P NMR spectra were obtained with a Varian XL-300 multinuclear spectrometer. High-resolution mass spectrometry was performed by Dr. Douglas A. Gage, at the Michigan State University—NIH Mass Spectrometry Facility.

Methods. The four common 2'-deoxynucleosides were protected[15] and converted to the phosphoramidite derivatives as described in the relevant references.[16]

5-(Anthracen-9-ylmethyl)-2'-deoxy-4-desmethylwyosine (2). To 2.5 g (8.19 mmol) of **1**[17] dissolved in 60 mL of anhydrous dimethylformamide were added 2.13 g (9.42 mmol) of 9-(chloromethyl)anthracene and 1.70 g (12.3 mmol) of potassium carbonate. The reaction mixture was stirred under argon for 6 h. The resulting suspension was filtered through Celite and rinsed with dimethylformamide. The solution was evaporated to dryness, then dissolved in 1:1 methanol–dichloromethane, and adsorbed onto 5 g of silica gel. After removal of the solvent the dried silica gel containing the reaction mixture was added to the top of a 50-g column packed in dichloromethane. The product was eluted from the column with a 0–10% gradient of methanol in dichloromethane and fractions containing product were evaporated to dryness, redissolved in 25% methanol–dichloromethane, and precipitated into petroleum ether. The product collected by vacuum filtration was of suitable purity for the next synthetic step. An analytical sample was further purified by preparative TLC using silica gel and developed with dichloromethane–methanol (9:1) and crystallized from methanol: yield 3.24 g (80%); mp 203–204 °C dec; UV λ_{max} (methanol) 256, 249 (sh), 276, 348, 366, 385 nm; ^1H NMR (DMSO-d_6) δ 1.54 (s, 3 H, 6-CH_3), 2.35 (m, 2 H, H2' or H2''), 2.50 (m, DMSO), 2.84 (m, 2 H, H2'' or H2'), 3.32 (s, H_2O), 3.59 (m, 2 H, H5', H5''), 3.88 (m, 1 H, H4'), 4.46 (m, 1 H, H3'), 4.93 (t, 1 H, 5'-OH, J = 10.5 Hz), 3.31 (d, 1 H, 3'-OH, J = 3.7 Hz), 6.36 (t, 1 H, H1', J = 13.5 Hz), 6.43 (s, 2 H, N^5 CH_2), 7.39 (s, 1 H, H7), 7.55 (m, 4 H, Anth-H2, -H3, -H6, -H7), 8.17 (m, 2 H, Anth-H1, -H8 or -H4, -H5), 8.22 (s, 1 H, H2), 8.53 (m, 2 H, Anth-H4, -H5, or -H1, -H8), 8.75 (s, 1 H, Anth-H10); high-resolution mass spectrum calcd for $C_{28}H_{25}N_5O_4$ 495.1907, found 495.1889.

N^2-(Anthracen-9-ylmethyl)-2'-deoxyguanosine (3). To 2.0 g (4.04 mmol) of **2** in 90 mL of 20% aqueous 0.2 M acetate buffer (pH 5.0)–dimethyl sulfoxide was added 1.01 g (4.44 mmol) of *N*-bromosuccinimide. After stirring in the dark for 15 min, 75 mL of concentrated ammonia was added and the resultant mixture stirred an additional 15 min. The solution was concentrated by rotary evaporation to 20 mL and poured into 500 mL of water. The precipitate was filtered, redissolved in 50% methanol–dichloromethane and added to 5 g of silica gel. The solvent was removed from the suspension by rotary evaporation, and the dried silica gel was added to the top of a column containing 50 g of silica gel packed in dichloromethane. The column was developed with a methanol–dichloromethane gradient and the product eluted with 15% methanol–dichloromethane. Fractions containing product were evaporated to dryness and recrystallized from methanol in an ether desiccator: yield 480 mg (26%); mp 232–234 °C dec; UV λ_{max} (methanol) 254, 281 (sh), 345, 364, 384 nm; ^1H NMR (DMSO-d_6) δ 2.38 (m, 1 H, H2' or H2''), 2.50 (m, DMSO), 2.73 (m, 1 H, H2'' or H2'), 3.35 (s, H_2O), 3.62 (m, 2 H, H5', H5''), 3.91 (m, 1 H, H4'), 4.46 (m, 1 H, H3'), 4.99 (t, 1 H, 5'-OH, J = 10.6 Hz), 5.40 (d, 1 H, 3'-OH, J = 3.9 Hz), 5.50 (d, 2 H, Anth-CH_2, J = 4.5 Hz), 6.40 (t, 1 H, H1', J = 13.9 Hz), 6.99 (br, 1 H, N^2 H), 7.57 (m, 2 H, Anth-H2, -H7 or -H3, -H6), 7.66 (m, 2 H, Anth-H3, -H6 or -H2, -H7), 8.00 (s, 1 H, H8), 8.14 (d, 2 H, Anth-H1, -H8 or -H4, -H5, J = 8.4 Hz), 8.50 (d, 2 H, Anth-H4, -H5 or -H1, -H8, J = 8.8 Hz), 8.67 (s, 1 H, Anth-H10), 10.17 (s, 1 H, N^1 H). A two-dimensional COSY spectrum showed a cross-peak between the N^2 H and the Anth-CH_2; high-resolution mass spectrum, calcd for $C_{25}H_{23}N_5O_4$ 457.1750, found 457.1754.

N^2-(Anthracen-9-ylmethyl)-5'-*O*-(9-phenylxanthen-9-yl)-2'-deoxyguanosine (4). To 0.25 g (0.54 mmol) of **3**, which was evaporated from

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dry pyridine (2×) and then dissolved in 10 mL of dry pyridine, was added dropwise over 20 min 0.176 mg (0.60 mmol) of 9-chloro-9-phenylxanthene (pixyl-Cl) dissolved in 2 mL of dry pyridine. The reaction mixture was flushed with argon and allowed to stir for 2 h in the dark. Analysis by TLC (dichlormethane–methanol–triethylamine 90:9.9:0.1) at this time indicated that the transformation was complete, and a few drops of methanol were added to stop the reaction. The reaction mixture was poured into 50 mL of dichloromethane, washed with an equivalent volume of 5% sodium bicarbonate and water, and then dried with sodium sulfate. The solvent was removed by rotary evaporation and the residue dissolved in dichloromethane and added to 5 g of silica gel packed in dichloromethane containing 0.1% triethylamine. The column was developed with a gradient of methanol in dichloromethane, the product eluted in dichloromethane–methanol–triethylamine (95:4:1), and fractions containing product were evaporated to dryness. The residue was dissolved in a small quantity of dichloromethane and precipitated into petroleum ether. The precipitate was filtered and dried under vacuum: yield 0.20 g (52%); ^{1}H NMR (DMSO-d_6 + trace D_2O) δ 2.39 (m, 1 H, H2′ or H2″), 2.50 (m, DMSO), 2.82 (m, 1 H, H2″ or H2′), 3.32 (m, 2 H, H5′, H5″), 3.39 (s, HOD), 4.04 (m, 1 H H4′), 4.53 (m, 1 H, H3′), 5.23 (br, 1 H, 3′-OH), 5.44 (d, 2 H, Anth-CH_2, J = 4.2 Hz), 6.34 (t, 1 H, H1′, J = 13.2 Hz), 6.85 (br, 1 H, N^2 H), 6.91–7.34 (m, 13 H, pixyl), 7.58 (m, 4 H, Anth-H2, -H3, -H6, -H7), 7.80 (s, 1 H, H8), 8.16 (d, 2 H, Anth-H1, -H8 or -H4, -H5, J = 8.0 Hz), 8.38 (d, 2 H, Anth-H4, -H5 or -H1, -H8, J = 8.5 Hz), 8.68 (s, 1 H, Anth-H10).

(±)-N^2-(Anthracen-9-ylmethyl)-5′-*O*-(9-phenylxanthen-9-yl)-3′-*O*-[(*N*,*N*-diisopropylamino)(β-cyanoethoxy)phosphinyl]-2′-deoxyguanosine (5). To 150 mg of **4** (0.21 mmol) dissolved in 2.5 mL of dry dichloromethane containing 0.11 mL (0.64 mol) of diisopropylethylamine was added dropwise by syringe over 20 min 71 mg (0.30 mmol) of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite. The solution was stirred for an additional hour under argon and then quenched with methanol. Analysis by TLC (dichloromethane–methanol–triethylamine 90:9.9:0.1) indicated that the reaction was complete. The reaction mixture was reduced in volume by rotary evaporation and product was isolated by preparative TLC (dichloromethane–acetone–triethylamine 49.5:49.5:1), then precipitated into petroleum ether, filtered, and dried under high vacuum: yield 101 mg (53%); R_f (dichloromethane–methanol–triethylamine 90:9.9:0.1) = 0.71; ^{31}P NMR ($CDCl_3$) δ 147.2.

DNA Synthesis and Isolation. The oligodeoxynucleotide sequences were synthesized by using phosphite triester chemistry,[18] 9-phenylxanthen-9-yl-derivatized β-cyanoethylphosphoramidites, and an Applied Biosystems 381A DNA synthesizer. For coupling reactions, the modified phosphoramidite (**5**) was dissolved in dichloromethane–acetonitrile (3:2). Three sequences containing the anthracen-9-ylmethyl-modified 2′-deoxyguanosine residue (G^A) were prepared: d(CGCG^{A}AATTCGCG), d(GTTATCCG^{A}CTCAC), and d(CCGAGCTCG^{A}AATTCATGGCCGTCG). For the acid-deprotection step in each synthetic cycle, 2% dichloroacetic acid in dichloroethane was employed for 90 s at a flow rate of approximately 1.6 mL/min. The coupling time for the modified nucleoside was varied with a maximum time of 60 min employed for each of three additions of the phosphoramidite. The oligodeoxynucleotides were deprotected in concentrated aqueous ammonia for 6 h at 50 °C and subsequently purified by reversed-phase HPLC (9.4 × 250 mm ODS-Hypersil, 50 mM triethylammonium acetate, pH 7.0, and a gradient of acetonitrile) with the Pixyl group still attached to the 5′-terminus. Isolated peaks were treated with 20% aqueous acetic acid for 30 min at 0 °C, concentrated, desalted on Sephadex G-10 columns, and finally lyophilized to dryness.

Nucleoside Analysis. Oligodeoxynucleotides were treated with snake venom phosphodiesterase (SVDP) and bacterial alkaline phosphatase (BAP). The resulting mixture was analyzed by reversed-phase HPLC (4.6 × 250 mm ODS-Hypersil column (elution with 20 mM KH_2PO_4, pH 5.5, with 0–80% linear methanol gradient in 40 min). Retention times under these conditions: dC, 7.5 min; dG, 11.2 min; dT, 12.2 min; dA, 14.6 min; and dG^A, 49.2 min. Presence of the modified nucleoside in the hydrolysis mixture could be confirmed by comparison with a standard (**3**). Most of the alkaline phosphatase preparations we have used exhibit small amounts of adensosine deaminase activity, which results in small quantities of 2′-deoxyinosine eluting just before dG.

T_m Determinations and Thermodynamic Parameters from Absorbance vs Temperature Plots. All T_m's were taken in a buffer containing 10 mM KH_2PO_4, pH 7.0, and 1 M NaCl and solution temperature was measured directly with an immersible probe and a Telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Absorbance and temperature data were collected and stored after analog to digital conversion (DT-2800, Data Translations, Marlboro, MA) using an IBM-XT personal computer employing the ASYST (version 1.53) scientific package (MacMillian Software, New York). From absorbance vs temperature plots, first and second derivatives were calculated to determine T_m values.

Thermodynamic parameters were derived from the T_m values at a number of concentrations from plots of $1/T_m$ vs $c_T/4$ as has been described elsewhere.[26,27] The values for $\Delta H°$ and $\Delta S°$ are related to the T_m and single-strand DNA concentrations according to $1/T_m = 2.3R(\log c_T/4)/\Delta H° + \Delta S°/\Delta H°$. $\Delta H°$ values can also be derived from individual absorbance vs temperature plots from the slope of the curve at the T_m; where $\Delta H° = (2 + 2n)\, RT_m^2\, (df/dT)_{T_m}$ and $f = 0.5$ (the fraction of single strands in a duplex state) at $T = T_m$.[26,27]

Circular Dichroism Experiments. CD spectra were measured in 10 mM KH_2PO_4 pH 7.0, and 1 M NaCl with the Auto-dichrograph Mark V (Jobin Yvon through Instruments S. A., Edison, NJ). Solution temperature was maintained with a refrigerated circulating water bath and monitored with an immersible probe and a Telethermometer. All spectra collected were an average of three cycles (300–220 nm) to reduce noise. Temperature fluctuation over this period was not in excess of ±0.5 °C of the temperature recorded. Spectra were collected and stored by use of an Apple IIe computer and plotted with an Hewlett-Packard 7574 plotter.

Fluorescence Data. Fluorescence spectra were collected on a Shimadzu RF5000U fluorescence spectrophotometer complemented by a Shimadzu DR-15 microprocesser and graphics display terminal. All samples were dissolved in 10 mM KH_2PO_4, pH 7.0, and 1 M NaCl. Temperature control studies were performed with a temperature-controlled cell block and a refrigerated circulating water bath. Temperature of the solution was monitored directly with an immersible probe and a Telethermometer.

Results

Synthesis of the N^2-Modified 2′-Deoxyguanosine Derivative. We considered a number of approaches to the preparation of the desired N^2-alklyated guanosine derivative: The exocyclic amino group of 2′-deoxyguanosine cannot be directly alkylated to any significant extent by a variety of methylating and ethylating agents; reaction at the N7 position predominates for many reagents although in some cases significant amounts of the O^6-alkylation products can be obtained.[8] In the presence of a base, such as potassium carbonate, alkylation by methyl iodide occurs preferentially at the N1 position.[19] N^2-aryl derivatives have been prepared by reaction of arylamines with 2-halohypoxanthine derivatives[20] and N^2-methylguanosine has been prepared by a similar procedure but in low overall yield,[21] but the preparation of the necessary 2-halogenated nucleobases or nucleosides is a somewhat lengthy process. N^2-methylguanosine has also been prepared by the action of diazomethane on O^6-mesyl-N^2-benzoyl-2′,3′,5′-tri-*O*-benzoylguanosine[22] or by the reaction of formaldehyde, thiocresol, and acetic acid with a suitably protected guanosine derivative followed by reduction with sodium borohydride or Raney nickel.[23] However, neither of these methods appeared general enough for the present synthesis. It has been reported that upon treatment of 2′-deoxy-4-desmethylwyosine (or 4-desmethylwyosine) derivatives with diazomethane[17,24] or dimethyl sulfate[25] that N5 alkylation is the predominant product. Boryski and Ueda[26] have additionally shown that these derivatives can be alkylated at the N5 position with methyl or ethyl iodide in the presence of potassium carbonate and that the alkylated products can be converted to N^2-methyl(ethyl)-2-deoxyguanosine

(18) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859–1862.
(19) Broom, A. D.; Townsend, L. B.; Jones, J. W.; Robins, R. K. *Biochemistry* **1964**, *3*, 494–500.
(20) (a) Wright, G. E.; Baril, E. F.; Brown, V. M.; Brown, N. C. *Nucleic Acids Res.* **1982**, *10*, 4431–4440. (b) Wright, G. E.; Dudyz, L. W. *J. Med. Chem.* **1984**, *27*, 175–181.
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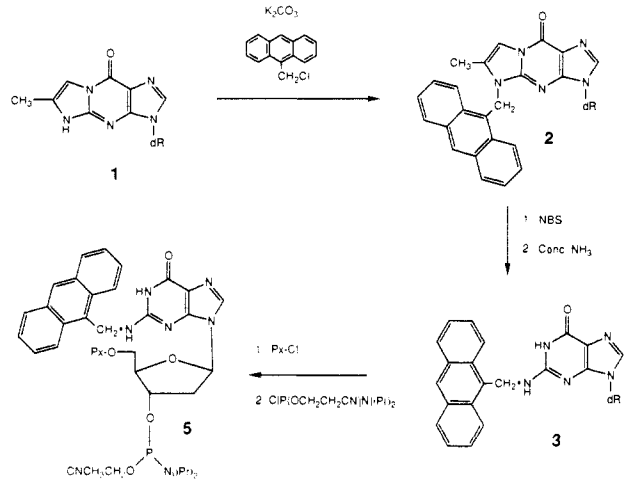
(22) Bridson, P. K.; Markiewicz, W.; Reese, C. B. *J. Chem. Soc., Chem. Commun.* **1977**, 447–448.
(23) Bridson, P. K.; Reese, C. B. *Bioorg. Chem.* **1979**, *8*, 339.
(24) (a) Golankiewica, B.; Folkman, W. *Nucleic Acids Res.* **1983**, *11*, 5243. (b) Boryski, J.; Ueda, T. *Nucleosides Nucleotides* **1985**, *4*, 595–606.
(25) Boryski, J.; Bolankiewicz, B. *Nucleosides Nucleotides* **1984**, *3*, 287–294.
(26) Boryski, J.; Ueda, T. *Nucleosides Nucleotides* **1985**, *4*, 595–606.
(27) (a) Wang, A. H.-J.; Quigley, G. J.; Kolpak, F. J.; Crawford, J. L.; van Boom, J. H.; van der Marel, G.; Rich, A. *Nature* **1979**, *282*, 680–686. (b) Wing, R.; Drew, T.; Takano, C.; Broka, S.; Itakura, K.; Dickerson, R. E. *Nature* **1980**, *287*, 755–758.

Scheme I



(or corresponding guanosine derivative) by a relatively simple series of reactions. This latter approach appeared as though it might provide a direct yet general route to N^2 alkylation and be suitable for the present synthesis.

We prepared 2′-deoxy-4-desmethylwyosine (**1**) (Scheme I) as described by Kasai et al.[17] and then treated it with 9-(chloromethyl)anthracene in the presence of potassium carbonate (essentially as described by Boryski and Ueda[26] for methylation) and produced the N5-alkylated 2′-deoxy-4-desmethylwyosine derivative **2** (Scheme I) in high yield. Regeneration of the 2′-deoxyguanosine nucleoside required some modification of the previously described procedure. Because of the poor solubility of **2** in aqueous solution it was necessary to perform the reaction in a largely organic solvent mixture instead of aqueous buffer. We employed a solution of 20% aqueous dimethyl sulfoxide for reaction with *N*-bromosuccinimide and then proceeded to hydrolyze the putative bromo intermediate with concentrated aqueous ammonia. The workup of this reaction mixture and the isolation (primarily via column chromatography) of N^2-(anthracen-9-ylmethyl)-2′-deoxyguanosine (**3**) was complicated by its poor solubility in organic solvents, but sufficient quantities could be isolated and recrystallized from methanol. We could confirm that the anthracen-9-ylmethyl moiety of **3** (Scheme I) was bound to the N^2 amino group of 2′-deoxyguanosine (indicating alkylation of the N5 nitrogen of the 4-desmethylwyosine in the previous step) by the presence of proton coupling between the guanine N–H and the methylene group bridging the bound polycyclic aromatic hydrocarbon (J_{HH} = 4.5 Hz). A two-dimensional COSY spectrum also exhibited the expected cross-peak. After protection of the 5′-hydroxyl as the 9-phenylxanthen-9-yl (pixyl) derivative, subsequent reaction with 2-cyanoethyl *N,N*-diisopropylchlorophophoramidite afforded the nucleoside phosphoramidite **5** suitable for incorporation of the N^2-modified guanine base into DNA by routine chemical synthesis techniques.

Oligodeoxynucleotide Synthesis. Prior to the chemical synthesis of the DNA fragments, N^2-(anthracen-9-ylmethyl)-2′-deoxyguanosine was treated with base (ammonia, 6 h, 50 °C) and acid (2% dichloroacetic acid–dichloromethane, 2 min, ambient temperature, and 80% aqueous acetic acid, 30 min, 0 °C). These were typical of the conditions the modified nucleoside would experience during chemical assembly and deprotection of DNA fragments by standard phosphoramidite (phosphite triester) synthesis techniques. No significant degradation of the 2′-deoxyguanosine derivative was observed (as monitored by HPLC) under these conditions.

We prepared three DNA sequences containing the anthracen-9-ylmethyl group attached to the exocyclic amino group of the 2′-deoxyguanosine residue (dG^A). The dodecamer d(CGCG^{A}AATTCGCG) was chosen since there is extensive structural information available for the native sequence.[27a] The 25-mer d(CCGAGCTCG^{A}AATTCACTGGCGTCG), corresponding to positions 6277–6301 of the single-stranded circular

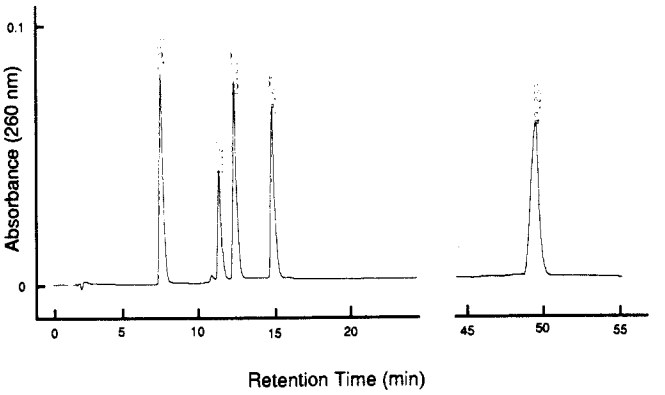


Figure 1. HPLC analysis of the nucleosides resulting from treatment of d(GTTATCCG^{A}CTCAC) with snake venom phosphodiesterase and bacterial alkaline phosphatase. HPLC conditions are described in the Experimental Section. Retention times: 7.5 min, dC; 11.2 min, dG; 12.2 min, dT; 14.7 min, dA; and 49.3 min, dG^A.

DNA from bacteriophage M13mp19, was prepared as a possible template for studies involving in vitro DNA replication. The 13-mer d(GTTATCCG^{A}CTCAC) is complementary to the sequence from positions 6183 through 6195 of the DNA from the bacteriophase M13mp19 and could be used for in vivo studies of DNA replication and repair. Initial attempts at DNA synthesis indicated poor coupling yields when the modified nucleoside was introduced into the growing oligodeoxynucleotide chain at a concentration of 0.05 M (30 equiv). We could in part compensate for the poor coupling yield by increasing the reaction time from the few minutes, sufficient for reaction with the four common 2′-deoxynucleoside phosphoramidites, to 60 min or more. Under these conditions we could achieve a coupling yield at this step of 65–70%. This is far below the yields obtained for coupling of the common nucleoside phosphoramidites (~98%) but allowed acceptable syntheses of the desired sequences. After deprotection of the oligodeoxynucleotides in aqueous ammonia, each partially protected product (still containing the 5′-terminal pixyl group) was isolated by HPLC. By not hydrolyzing the 5′-terminal pixyl group attached to the product sequences during the initial deprotection procedure, a C_{18} reversed-phase column could be employed to isolate only those sequences that still contained the pixyl group and represented complete syntheses. After purification in this manner the acid-labile pixyl group was removed and the DNA fragment desalted and finally lyophilized to dryness.

The oligodeoxynucleotides were judged from HPLC analysis and by polyacrylamide gel electrophoresis (after 5′-terminal radioisotopic labeling) to be better than 95% pure. The presence of the N^2-(anthracen-9-ylmethyl)-2′-deoxyguanosine residue was confirmed by nucleoside analysis (Figure 1) and comparison of the peak eluting at 49 min with that obtained from an authentic standard, compound **3**.

Properties of the Modified Oligodeoxynucleotides. We examined the thermal stability of the self-complementary dodecamer d-(CGCG^{A}AATTCGCG)$_2$, containing two anthracenylmethyl residues at pH 7.0 and 1.0 M sodium chloride, but could not obtain a cooperative transition (based upon UV hyperchromicity) reflecting a well-characterized helix-to-coil transition. We therefore prepared the DNA fragment complementary to the 13-mer in order to study a double-stranded fragment containing only a single anthracen-9-ylmethyl moiety. The double-stranded 13-mer d-(GTTATCCG^{A}CTCAC)·d(GTGAGCGGATAAC), containing a single modified 2′-deoxyguanosine residue, under the same buffer conditions produced a cooperative helix-to-coil transition with a T_m of 50.6 °C (3.06 μM total single-strand concentration) (Figure 2). By comparison, the unmodified 13-mer helix under the same buffer and salt conditions exhibited a T_m of 56.6 °C (3.58 μM total single-strand concentration) (Figure 2). From the two curves of Figure 2 it can be observed that the introduction of a single anthracene residue into the double-stranded 13-mer not only reduces the T_m but also significantly alters the character of the transition as evidenced by the broadening of the absorbance vs

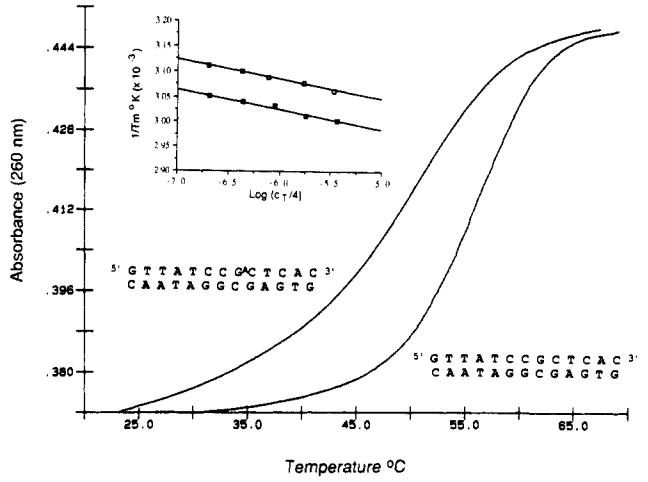


Figure 2. Absorbance vs temperature plots for the native and anthracen-9-ylmethyl-modified 13-mers. Buffer conditions are described in the Experimental Section. Inset: Plots of $1/T_m$ vs log $c_T/4$) (c_T, total single-strand concentration) for the native (■) and anthracen-9-ylmethyl-modified (□) double-stranded 13-mers.

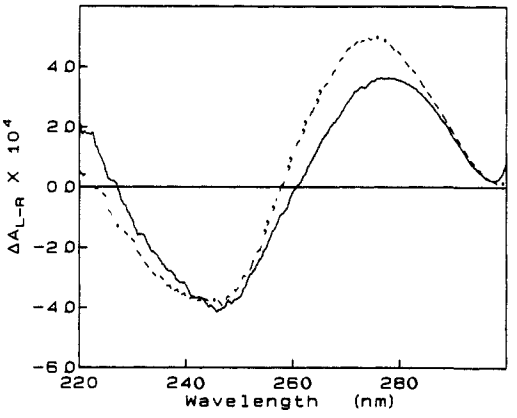


Figure 3. CD spectra for the native (—) and anthracen-9-ylmethyl-modified (---) double-stranded 13-mers. Buffer conditions are described in the Experimental Section.

temperature curve. Thermodynamic parameters characterizing the helix-to-coil transition for both the modified and unmodified 13-mers were obtained from T_m measurements over the concentration range 0.8–15 μM (total single-strand concentration) from plots of $1/T_m$ vs log ($c_T/4$) (c_T, total single-strand concentration)[28] (inset, Figure 2). From such plots thermodynamic parameters for helix formation can be derived, but are considered valid only if the transition is two state (i.e., no intermediates containing partially paired strands are present).[28,29] The native sequence exhibited a change in enthalpy ($\Delta H°$) of −115 kcal/mol, an entropy change ($\Delta S°$) of −323 cal/mol·deg, and a free energy change at 37 °C ($\Delta G°_{37}$) of −15.2 kcal/mol for helix formation. The corresponding values for the modified 13-mer were $\Delta H°$ = −118 kcal/mol, $\Delta S°$ = −344 cal/mol·deg, and $\Delta G°_{37}$ = −12.0 kcal/mol. Enthalpy changes can also be calculated from the slope of individual absorbance vs temperature plots at the T_m.[28,29] This type of analysis gave $\Delta H°$ values of −109 and −87 kcal/mol for the native and modified 13-mers, respectively. The $\Delta H°$ values calculated by these two procedures for the native sequence differed by only 6%, indicating that the melting process was a two-state transition.[28,29] A 36% difference in $\Delta H°$ values was obtained for

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(29) (a) Petersheim, M.; Turner, D. H. *Biochemistry* **1983**, *22*, 256–263. (b) Hickey, D. R.; Turner, D. H. *Biochemistry* **1985**, *24*, 2086–2094. (c) Breslauer, K. J.; Frank, R.; Bloecker, H.; Marky, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3746–3750.

Table I. Fluorescence Characteristics of Anthracen-9-ylmethyl Derivatives[a]

compound	excitation, nm	emission, nm
9-anthracenemethanol	255	391, 413, 437
3	255	392, 415, 438
5'GTTATCCG^{A}CTCAC3'	255	399, 423, 446[b]
5'GTTATCCG^{A}CTCAC3' CAATAGGCGAGTG	255	398, 422, 444[b]

[a] With 10 mM sodium phosphate, pH 7.0, 1.0 M sodium chloride. [b] Estimated value; emission was a shoulder on lower wavelength emission (see Figure 4).

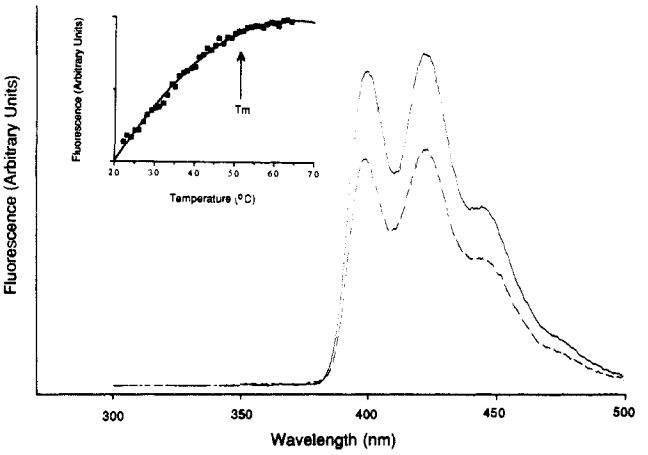

Figure 4. Fluorescence emission spectra (λ_{ex} = 255 nm) for the single-stranded (—) and double-stranded (---) anthracen-9-ylmethyl-modified 13-mer. Buffer conditions are described in the Experimental Section. Inset: Fluorescence of the anthracen-9-ylmethyl-modified helix at varying temperatures (corrected for thermal quenching; see text).

the anthracene-modified 13-mer, suggesting that this fragment did not exhibit a two-state transition.

Analysis of both the native and modified 13-mers by CD spectropolarimetry at pH 7.0 (10 mM KH_2PO_4) and 1.0 M sodium chloride at 22 °C indicated that both sequences adopted an essentially B-form helix (Figure 3). With increasing temperature, loss of the duplex structure could be observed (decreasing intensity of the negative peak near 250 nm and a bathochromic shift to shorter wavelength in the positive maxima).

The anthracene ring system exhibits fluorescent properties that could be employed to monitor local changes in the environment of the polycyclic aromatic hydrocarbon. The emission characteristics for some of the anthracene derivatives prepared have been compared with those resulting from 9-anthracenemethanol in Table I. Although there is some shift of emission maxima between either 9-anthracenemethanol or the anthracen-9-ylmethyl nucleoside and the modified oligodeoxynucleotide (single or double stranded), no significant shift was observed when comparing the single- with the double-stranded form.

We have also monitored the fluorescence yield of the anthracene moiety bound to the single-stranded and double-stranded 13-mer (Figure 4). Approximately a 30% decrease in yield was obtained when the complementary sequence was added to a solution of the single-stranded 13-mer containing the anthracene moiety. We measured the thermal quenching of the fluorescence from the single-stranded 13-mer over the temperature range 20–65 °C and observed a linear decrease in fluorescence (slope of fluorescence vs temperature plot was −1.89). The double-stranded 13-mer exhibited less thermal quenching below its T_m (slope of the fluorescence vs temperature plot was −0.32 from 20 to 40 °C). Thermal quenching increased with helix-to-coil transition and corresponded to that for the single-stranded 13-mer at higher temperatures (>60 °C). We have attempted to partially correct the fluorescence change during helix-to-coil transition by examining the difference between the temperature vs fluorescence curves for both the single- and double-stranded fragments (Figure 4, inset).

Discussion

Alkylation of 2'-deoxy-4-desmethylwyosine in the presence of potassium carbonate afforded the N^5-(anthracen-9-ylmethyl)-4-desmethylwyosine derivative in high yield in agreement with previous work involving methylation (ethylation).[26] Conversion of the wyosine compound to the 2'-deoxyguanosine nucleoside proceeded with a much poorer reaction yield than reported for the methylated derivative. However, TLC analysis indicated that N^2-(anthracen-9-ylmethyl)-2'-deoxyguanosine was the major product of the reaction and the low isolated yield (26%) was in part due to the poor solubility of the product during isolation procedures. Higher yields in this conversion might be obtained if the sugar moiety was suitably substituted (e.g., 3',5'-bis(*O*-*tert*-butyltrimethylsilyl) or similar derivatization) to enhance solubility of the nucleoside in organic solvents. However, this approach would require two additional steps in the synthetic procedure (the addition and, subsequently, the removal of the derivatizing groups). At this time we decided that it was more expedient to employ fewer synthetic steps, albeit with lower overall reaction yield. After conversion to the corresponding pixylated phosphoramidite, we had no solubility difficulties in preparing the appropriate solution (in dichloromethane–acetonitrile 3:2) for solid-phase DNA synthesis.

Coupling yields during solid-phase DNA syntheses for the modified nucleoside (**5**) were 65–70% with sufficient reaction time (60 min). While this is far from ideal, such problems have been reported previously, for example, with the building block used to incorporate the cis–syn thymine dimer into short DNA fragments.[30] However, even with the poor coupling yield during the introduction of the anthracen-9-ylmethyl-modified 2'-deoxyguanosine residue, we were still able to obtain, in purified form, 42 A_{260} units (0.17 μmol) of the longest fragment prepared to date (a 25-mer) from an initial 1 μmol of polymer-bound nucleoside. The identical chromatographic (HPLC) characteristics for the modified nucleoside obtained from analysis of the DNA fragments (i.e., the peak at 49 min in Figure 1) and compound **3** confirmed that the N^2-(anthracen-9-ylmethyl)-2'-deoxyguanosine residue survived the chemical synthesis procedures necessary to prepare the DNA sequence without significant hydrolysis or further modification. It is noteworthy that we were able to obtain complete enzymatic hydrolysis of the modified sequences using snake venom phosphodiesterase. A previous report describing the preparation of sequences containing the 7,12-dimethylbenzo[*a*]-anthracene bound to the exocyclic amino group of adenine indicated some resistant to such enzymatic hydrolyses.[14]

Thermodynamic Parameters. The thermodynamic parameters for helix formation derived from $1/T_m$ vs log ($c_T/4$) plots helped to characterize the native and modified double-stranded 13-mers. The enthalpy change calculated in this fashion agrees with that obtained from individual absorbance vs temperature plots for the native fragment but the two values are quite different for the modified fragment. One interpretation of this result is that the melting process is not an "all-or-none" two-state model. In this case the enthalpy change calculated from the plot of Figure 2 for the modified sequence may be of questionable validity. The differences in melting characteristics (see plots of Figure 2) could also implicate the presence of partially paired intermediates during the helix-to-coil transition of the modified sequence that are not present in the transition of the native sequence. The change in the slope of the absorbance vs temperature transition (Figure 2) would then tend to indicate a change in the cooperativity of the transition rather than reflecting differing enthalpies. The disruption of the helical structure for the modified 13-mer (as evidenced in part from the lower T_m value) by the presence of the bound polycyclic aromatic hydrocarbon could be expected to result in a significant reduction in cooperativity. The thermally induced helix-to-coil transition would then begin relatively early in that portion of the sequence near the anthracene moiety and then continue in a cooperative fashion in sections of the sequence containing a series of "normal" dA-dT and dG-dC base pairs. The result of such a process would broaden the overall helix-to-coil transition as is observed in Figure 2.

The change in enthalpy for helix formation is generally interpreted to reflect the formation of interstrand hydrogen bonds between complementary sequences. The minor difference in enthalpies (−115 kcal/mol for the native 13-mer and −118 kcal/mol for the anthracenyl-modified 13-mer) obtained from the plots of Figure 2 (inset), with a $\Delta\Delta H°$ of approximately 3 kcal/mol, argues for interbase hydrogen-bonding interactions that are essentially identical in both helices (3 kcal/mol is within the estimated error, ±5%, for the derived enthalpies). The anthracene derivative is attached to the exocyclic amino group of dG; this amino group normally forms a hydrogen bond in the minor groove of a B-form DNA with the O^2 carbonyl of dC. Since the amino group is alkylated by only a single polycyclic aromatic hydrocarbon, one N–H functionality would still be available for hydrogen bonding; the measured enthalpy change is consistent with such bonding. However, the microscopic details of helix stabilization in the presence of a hydrophobic substituent are likely to be more complex than this simplified interpretation. A more negative enthalpy obtained for a sequence containing a bound polycyclic aromatic hydrocarbon could implicate the presence of additional van der Waals contacts between the hydrocarbon adduct and the carbohydrate surfaces available in the minor groove. But in the present case it is difficult to argue for additional stabilizing van der Waals contacts since the difference in $\Delta H°$ values (3 kcal/mol) *is* within the experimental error for the analysis. However, the nearly identical $\Delta H°$ values may also be serendipitous in that the presence of the bulky aromatic substituent could result in significant disruption of interstrand hydrogen bonding while compensating hydrophobic (van der Waals) interactions might mask any significant change in the derived enthalpy.

The difference in helix stability for the native ($\Delta G°_{37} = -15.2$ kcal/mol) and modified ($\Delta G°_{37} = -12.0$ kcal/mol) sequences as reflected by a difference in free energy ($\Delta\Delta G°$) of 3.2 kcal/mol (and approximately a 6 °C difference in T_m) results largely from the 21 cal/mol·deg difference in entropy ($\Delta\Delta S°$). Although the entropy term is typically not as well correlated with structure, the less favorable entropy term may, in the present case, reflect some additional order (or loss in flexibility) in a helical DNA structure attempting to accommodate the large polycyclic aromatic hydrocarbon.

It is also important to note that nearest-neighbor interactions are important in determining overall helix stability,[28e] and in the 13-mer examined, the modified dG residue has two dG-dC base pairs as its nearest neighbors. In order to completely assess the helix destabilizing effects of polycyclic aromatic hydrocarbons covalently bound to the exocyclic amino group of dG residues, it will be necessary to compare modified sequences containing other nearest-neighbor base pairs.

The observed helix destabilization after alkylation of the exocyclic amino group of dG is somewhat similar to that observed with fragments containing a dC residue carrying an aliphatic linker bound to the exocyclic amino group.[31] This modified deoxycytidine residue resulted in significant helix destabilization and produced a biphasic absorbance vs temperature curve that was difficult to interpret. In the present case, the anthracenyl-modified deoxyguanosine residue tended to broaden the melting curve of the 13-mer helix but a single cooperative transition still characterized its melting. In previous reports, the attachment of one or two pyrene residues to a thymine base (via an 8–10 atom linker) resulted in significant stabilization of a double-stranded octamer,[31] presumably as a result of intercalative binding by the pyrene moiety. Oligodeoxynucleotides carrying intercalators bound to terminal or internal phosphate groups (via appropriate linkers) have also been shown to increase the T_m for helix-to-coil transitions

(30) Taylor, J.-S.; Brockie, I. R.; O'Day, L. *J. Am. Chem. Soc.* **1987**, *109*, 6735–6742.

(31) (a) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 6966–6976. (b) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L.; Chan, C.-K. *J. Am. Chem. Soc.* **1989**, *111*, 7226–7232.

(and associated $\Delta G°$ values) in a number of reports.[32] In the present study, destabilization of the double-stranded DNA containing the planar (and potentially intercalating) anthracenylmethyl substituent, as well as the lack of extensive quenching in the fluorescence emission spectrum (see below), argues against binding of the anthracene derivative by an intercalative mode.

Circular Dichroism Spectra. Circular dichroism spectra are commonly used to analyze the helical structure, or related conformational changes, of nucleic acids. However, the presence of chromophoric adducts or ligands complicates the interpretation of the observed Cotton effects. Achiral chromophores positioned rigidly in a chiral environment will give rise to an induced CD effect. This has been observed with a number of ligands that bind either covalently or noncovalently to DNA.[33] With one of the most notable cases, binding of mitomycin C to DNA resulted in an inverted CD pattern,[33b] suggesting a conformational change (B to Z) in the DNA. Subsequent experiments[34] confirmed that the B-to-Z transition was actually inhibited by the presence of mitomycin cross-links and the inverted CD spectra may have simply resulted from the superposition of the induced CD of the mitomycin C chromophore on that for a double-stranded B-form DNA.

The CD spectra of the native and anthracenyl-modified 13-mers do not suggest the presence of gross changes in the helical structure of the DNA and are largely characteristic of a B-form structure. The slight differences in the two spectra may reflect small changes in base stacking in the area of the anthracene moiety or the superposition of an induced CD from the rigidly bound polycyclic aromatic hydrocarbon in the minor groove of a B-form helix. Detailed structural analysis of the helical structure containing the covalently bound adduct must await high-field NMR or single-crystal X-ray diffraction techniques.

Fluorescence Spectra. Substantial quenching of fluorescence emission spectra in the presence of double-stranded DNA has been used to implicate intercalative binding within double-stranded DNA (or at least the intimate association of planar chromophores with the heterocyclic bases). The binding of polycyclic aromatic hydrocarbons, such as the benzo[*a*]pyrenediol epoxide, to duplex DNA to form "type I" adducts[35] has been characterized by a red shift in the fluorescence emission spectrum and strong quenching of the emitted irradiation (the fluorescence yield is reduced between 1 and 2 orders of magnitude).[2b,11,36] These observations are consistent with intercalative complexes,[11] and similar fluorescence properties have also been observed for duplexes with acridine labels attached to a terminal phosphorus[32a-c,e,f] and pyrene labels bound to an internal phosphorus[32d] or bound (via a seven-atom linker) to an internal thymine residue,[31] which have also been interpreted to reflect intercalation. The benzo[*a*]pyrenediol epoxide also forms "type II" adducts[10,35] with little or no red shift, moderate quenching of emitted fluorescence, and a positive linear dichroism. Type II adducts are suggested to represent binding exterior to the stacked heterocyclic bases and lie in the minor groove.[10]

The fluorescence emission maxima of the anthracen-9-ylmethyl-modified single-stranded 13-mer exhibits no red shift upon conversion to the duplex structure. There is a decrease in fluorescence yield, but the observed 30% reduction is far less than typically observed with intercalative binding.[11,36] These observations suggest that intercalation by the covalently bound anthracenylmethyl moiety between heterocyclic bases is unlikely. The smaller thermal quenching effects observed with the double-stranded 13-mer (in comparison to the single-stranded derivative) is consistent with the anthracene moiety nestled within the minor groove and partially protected from collisional decay processes. Model building studies show that such minor groove binding would allow hydrogen bonding between the modified-dG and corresponding dC residues but still result in some destabilization of the structure.

Alkylation of DNA by Electrophilic Polycyclic Aromatic Hydrocarbons. A number of previous studies have attempted to correlate spectroscopic observations with the mode of binding between polycyclic aromatic hydrocarbons and double-stranded DNA.[10,11] There is substantial evidence which indicates that derivatives such as benzo[*a*]pyrenediol epoxide bind by intercalation (type I adduct)[11] and by exterior groove binding (type II adduct).[10] Unfortunately, it has been difficult to interpret whether the adduct responsible for the observed spectroscopic evidence is also the covalently bound adduct. The evidence presented in this report indicates that after covalent alkylation (at the guanine N^2 amino group by a (halomethyl)anthracene, the chromophore is positioned in the minor groove in an exterior binding mode. This suggests that intercalation prior to alkylation may not be a required event, and exterior groove binding may be responsible for the initial association and/or positioning of the hydrocarbon for subsequent alkylation of duplex DNA.

Noncovalent binding in the minor groove followed by selective alkylation is not unique and has been proposed for other agents including the benzo(1,4)diazepines,[37] the saframycins,[38] naphthridinomycin,[39] and CC-1065.[37c,40] The benzo(1,4)diazepines, like the electrophilic polycyclic aromatic hydrocarbons, bind to the exocyclic amino group of 2′-deoxyguanonsine residues. CC-1065 (a trimer of dihydropyrroloindole subunits) binds in the minor groove in dA-dT-rich regions, and it may be best represented as a selective alkylating agent superimposed upon a rigid heterocyclic skeleton[41] (in the absence of the alkylating functionality high-affinity minor groove binding is still present[42]). Although the site of groove binding (dA-dT-rich sequences) and alkylation (the N3 position of dA) by CC-1065 is quite different from that

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observed with the benzo(1,4)diazepines and polycyclic aromatic hydrocarbons, this variation in sequence preference partly reflects differences in molecular size and the requirement for CC-1065 to penetrate deeply into the minor groove (via van der Waals contacts) prior to alkylation. The presence of the N^2 amino group of dG residues prevents such penetration. It has been suggested that the benzo(1,4)diazepines such as anthramycin also take advantage of van der Waals contacts in the minor groove in order to alkylate the exocyclic amino group of guanine;[37c] however, the projection of this same amino group out into the minor groove is generally thought to interfere with noncovalent groove binding.

Polycyclic aromatic hydrocarbons may also rely upon preferential binding and positioning in the minor groove, but in this case at dG-dC-rich sequences. Noncovalent binding at such sequences might occur in a "side-stacking" mode.[43] Such side stacking appears to be preferred in the minor groove of A-form rather than B-form helices.[44] In fact, the X-ray-derived crystal structures for a number of DNA sequences that are rich in dG-dC base pairs indicates that such sequences tend to adopt an A-form geometry,[43,45] and in each of these cases, packing in the crystals involves side stacking of the hydrophobic surfaces of the terminal base pairs onto the minor grooves of adjacent molecules (this is quite different from the end-to-end stacking of B-form and Z-form helices[27]). Computer modeling studies have suggested that external binding by polycyclic aromatic hydrocarbons in the minor groove would prefer (may even require) A-like DNA helices.[44] Photolysis experiments have demonstrated that the 16 base pair oligodeoxyguanylate tract in chicken β-globin gene is the predominate target for modification by benzo[*a*]pyrene diol-epoxide.[46] Preferential binding in the minor groove of dG-dC-rich sequences could optimally position the electrophilic halomethyl (or epoxide) functionality for alkylation of the normally unreactive exocyclic amino groups of deoxyguanosine residues.

External groove binding prior to alkylation does not eliminate intercalation as an important noncovalent mode of binding related to carcinogenic effects, since a number of intercalators are known to result in frame shift mutations.[47] Additionally, it is premature to generalize upon the importance of (or lack of) intercalative modes of binding associated with covalent alkylation for the entire class of polycyclic aromatic hydrocarbons on the basis of the present information for a single anthracen-9-ylmethyl adduct. Other regioisomers of (halomethyl)anthracenes or some of the metabolically produced epoxides of polycyclic aromatic hydrocarbons (such as the benzopyrenes) may allow covalent alkylation of guanine in concert with intercalative binding.

Conclusion

We have prepared a 2′-deoxyguanosine derivative that contains an anthracen-9-ylmethyl moiety covalently bound to the exocyclic amino group at the 2-position. Conversion to the appropriate phosphoramidite allowed the preparation of oligodeoxynucleotides site-specifically modified with this polycyclic aromatic hydrocarbon. The chemistry described is simple, straightforward, and only requires a few steps. We anticipate that the reactions illustrated in Scheme I should provide a general approach to the synthesis a number of modified dG derivatives incorporating a variety of covalent adducts reflecting alkylation of the guanine N^2 amino group by carcinogenic halomethyl polycyclic aromatic hydrocarbons. It is also an enticing prospect that alkylation of 2′-deoxy-4-desmethylwyosine by the diol epoxide carcinogens obtained from such compounds as benzo[*a*]pyrene,[8b] benzo[*c*]-phenanthrene,[6b] and dibenz[*aj*]anthracene[6c] may provide efficient synthesis of the corresponding alkylated 2′-deoxyguanosine derivative for site-specific incorporation into DNA sequences.

In the present report a self-complementary dodecamer containing two anthracen-9-ylmethyl residues did not appear to form a well-characterized helical structure, but a double-stranded 13-mer containing a single polycyclic aromatic residue could be studied in some detail. The T_m for the modified 13-mer (50.6 °C) was reduced from that measured for the native sequence (56.6 °C) although thermodynamic parameters suggest that hydrogen bonding in the modified 13-mer may not be significantly altered from that in the native sequence ($\Delta\Delta H° \sim 3$ kcal/mol). The observed CD spectra may be complicated by the presence of an induced CD from a rigidly oriented anthracene chromophore, but still indicated the presence of an essentially normal B-form DNA. The fluorescence of the anthracene moiety decreased with duplex formation but the changes observed do not suggest intercalation. The lack of substantial quenching of the anthracene fluorescence and the observed destabilization of the double-stranded 13-mer argue against and intercalative mode of binding by the covalently bound adduct. The polycyclic aromatic hydrocarbon appears to be bound exterior to the stacked heterocyclic bases of duplex DNA, nestled within the minor groove of a largely B-form helix.

Acknowledgment. We thank Dr. Douglas A. Gage at the Michigan State University—NIH Mass Spectrometry Facility (Grant DRR00480) for performing the mass spectral analyses and Prof. Douglas H. Turner, Dept. of Chemistry, University of Rochester, for his comments concerning the manuscript.

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