NMR Study of Stacking Interactions and Conformational Adjustments in the Dinucleotide–Carcinogen Adduct 2′-Deoxycytidylyl-(3→5)-2′-deoxy-8-(*N*-fluoren-2-ylacetamido)guanosine

Frederick E. Evans* and Robert A. Levine

Division of Biochemical Toxicology (HFT-110), National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas 72079

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ABSTRACT: The conformation and dynamics of the dinucleotide d-CpG modified at the C(8) position of the guanine ring by the carcinogen 2-(acetylamino)fluorene has been investigated by high-field ^{1}H NMR spectroscopy. A two-state analysis of chemical shift data has enabled estimation of the extent of intramolecular stacking in aqueous solution as a function of temperature. The stacking, which is mostly fluorene–cytosine, is virtually complete in the low-temperature range. The 500-MHz ^{1}H NMR spectrum consists of two subspectra near ambient temperatures due to a 14.3 ± 0.3 kcal/mol barrier to internal rotation about the amide bond in the stacked form. A large barrier to internal rotation about the guanyl–nitrogen bond at C(8) has also been ascertained, but separate NMR subspectra were not detected due to the predominance of one of the torsional diastereomers ($\alpha' = 90°$) in the fully stacked state. Problems of self-association and chemical exchange were identified and overcome to enable analysis of the sugar–phosphate backbone conformation utilizing coupling constants. For the exocyclic C(4′)–C(5′) bond of the deoxyguanosine moiety, there is a high gauche$^+$ ($\gamma = 60°$) conformer population, which is uncommon for a purine nucleotide with a syn orientation about the glycosyl bond. The gauche$^-$ conformation ($\gamma = 300°$), which is normally present in syn purine nucleotides in solution, was not detected. The exocyclic C(5′)–O(5′) torsion of the deoxyguanosine moiety remains near the classical energy minimum ($\beta = 180°$) in the major stacked conformations. The sugar ring of the deoxycytidine moiety is predominantly in the C2′-endo conformation, while the deoxyguanosine ring is a mixture of conformations, one of which appears to be unusually puckered. The results support intercalation models of modified DNA and suggest a looped-out structure, with the modified guanine being the first base in the loop. Such structures could explain the relatively rapid rate of repair and the frame-shift mutations of this type of adduct.

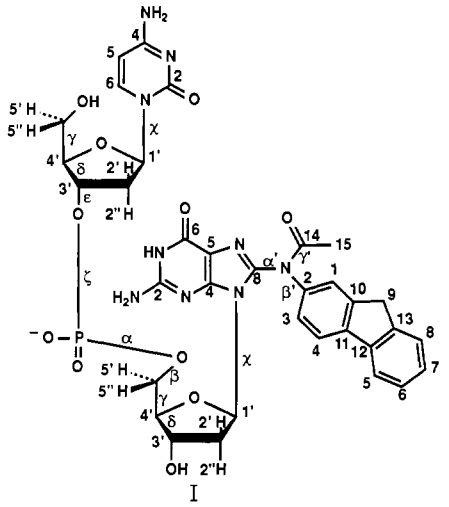
The chemical carcinogen 2-(acetylamino)fluorene (AAF)[1] covalently binds to guanine bases in DNA (Kriek & Westra, 1979; Beland et al., 1980; Hemminki, 1983). Adduct formation is thought to be a possible initial step in chemical carcinogenesis. The adduct in which AAF is substituted at the C(8) position of the guanine ring is an important compound for mechanistic studies. It is formed in large quantities and is more rapidly repaired in vivo than the corresponding unacetylated adduct (Beland et al., 1982; Kriek & Spelt, 1979; Poirier et al., 1982); it causes frame-shift mutations (Fuchs et al., 1981) and can cause conformational changes in DNA (Singer & Grunberger, 1983). AAF has been the most widely studied arylamine or arylamide carcinogen (Hemminki, 1983). A detailed characterization of the conformation and dynamics associated with adduct formation is needed to explore possible structure–function relationships [Shapiro et al. (1986) and references cited therein].

Structural studies on AAF-containing nucleic acid model systems have revealed some of the conformational details and factors affecting the conformation. The existence of stacking interactions involving the fluorene ring has previously been shown (Nelson et al., 1971; Santella et al., 1980; Box et al., 1984; Leng et al., 1980), while for certain sequences, a Z-like DNA structure has been proposed (Sage & Leng, 1980; Santella et al., 1981a,b; Sanford & Krugh, 1985), with the fluorene ring residing on the outside of the helix (Hingerty & Broyde, 1982). NMR and CD studies on short AAF–oligonucleotides have suggested that the stacking interactions of fluorene are greatest for the case of a purine nucleotide on the 5′ side of the modified nucleotide, although quantitation of the stacking was not attempted (Nelson et al., 1971; Santella et al., 1980; Leng et al., 1980; Alderfer et al., 1984). A syn orientation about the glycosyl bond of the modified nucleotide has also been confirmed (Lipkowitz et al., 1982; Evans et al., 1980; Leng et al., 1980; Hingerty & Broyde, 1982). A detailed NMR investigation on the mononucleotide adduct d-p(AAFG) has demonstrated the presence of large barriers to internal rotation about the guanyl–nitrogen bond at C(8) and the amide bond as well as a strong tendency to self-associate in aqueous solution. This resulted in the appearance of subspectra and anomalous line broadening (Evans et al., 1984, 1986). In spite of the importance of the intramolecular stacking interactions in AAF-modified oligonucleotides, very few experimental data on the torsion angles of the stacked form in aqueous solution are available, nor have NMR subspectra previously been observed in the presence of intramolecular stacking.

In this investigation, we have principally utilized 500-MHz ^{1}H NMR spectroscopy in an attempt to quantify the stacking interactions of an AAF-modified dinucleotide in aqueous so-

[1] Abbreviations: d-Cp(AAFG), 2′-deoxycytidylyl-(3→5)-2′-deoxy-8-(*N*-fluoren-2-ylacetamido)guanosine; d-p(AAFG), 8-(*N*-fluoren-2-ylacetamido)-2′-deoxyguanosine 5′-monophosphate; AAF, 2-(acetylamino)fluorene; N-AcO-AAF, *N*-acetoxy-2-(acetylamino)fluorene; 8-Br-AMP, 8-bromoadenosine 5′-monophosphate; NMR, nuclear magnetic resonance; CD, circular dichroism; T_m, melting temperature; NOE, nuclear Overhauser effect; rms, root mean square. The abbreviations Ap(AAFG) and Up(AAFG) are analogous to d-Cp(AAFG), except that the parent dinucleotides are ApG and UpG instead of d-CpG.

lution and to elucidate the conformational adjustments that are coupled to the stacking, including the changes at the site of attachment of the carcinogen to the dinucleotide that are reflected in NMR subspectra. The compound chosen for study is 2′-deoxycytidylyl-(3→5)-2′-deoxy-8-(*N*-fluoren-2-ylacetamido)guanosine [d-Cp(AAFG)]. The structure of d-Cp(AAFG) (I) along with the numbering system and torsion angle symbols is shown. Minimized potential energy calcu-



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lations have previously been reported for this same compound (Hingerty & Broyde, 1982). The results are discussed in terms of modified structures of DNA and its implications to biological function.

Materials and Methods

The d-Cp(AAFG) was synthesized by reaction of N-AcO-AAF with d-CpG (Sigma) according to procedures used previously for the mononucleotide adduct d-p(AAFG) (Evans et al., 1984). The crude reaction mixture was extracted with chloroform. Then a preliminary cleanup was performed by using a gravity flow column containing C_{18} (octadecylsilane) packing material. This packing was prepared by chemically bonding octadecyltrichlorosilane to Woelm silica gel (32–100 μm) and deactivating the unreacted silanol sites with trimethylchlorosilane (Little et al., 1979a,b). The reaction mixture was added to the top of the column, and salts were removed by washing the column with three 200-mL volumes of water. The starting material was eluted with three 100-mL portions of 10% methanol/H_2O. The adduct was collected after elution with three 100-mL portions of 50% methanol/H_2O. This material was adequate for several of the NMR measurements. For cases in which high purity was required, the adduct was subjected to HPLC cleanup by using a Beckman C_3 Ultrapore semipreparative column. The mobile phase was 15% methanol/H_2O flowing at 3.0 mL/min.

NMR spectra were recorded in the ^1H or ^{13}C configurations on Bruker WM 500 and Bruker WH 270 NMR spectrometers. Samples were dissolved in D_2O or in mixtures of D_2O and deuteriated methanol (Cambridge Isotope) with dioxane added as an internal standard for both the ^1H (3.76 ppm) and ^{13}C (67.8 ppm) measurements. Typical NMR data acquisition conditions were as follows: (for 270-MHz ^1H spectra) data size, 16K; sweep width, 3000 Hz; flip angle, 85°; presaturation of HDO peak during relaxation delay, 1 s; (for 500-MHz ^1H spectra) data size, 16K; sweep width, 5000–6000 Hz; flip angle, 85°; relaxation delay, 0 s; (for 67.9-MHz ^{13}C spectra) data size, 32K; sweep width, 16100 Hz; flip angle, 85°; number of scans, 96000; relaxation delay, 1.5 s. The ^{13}C spectra were obtained with broad-band gated decoupling.

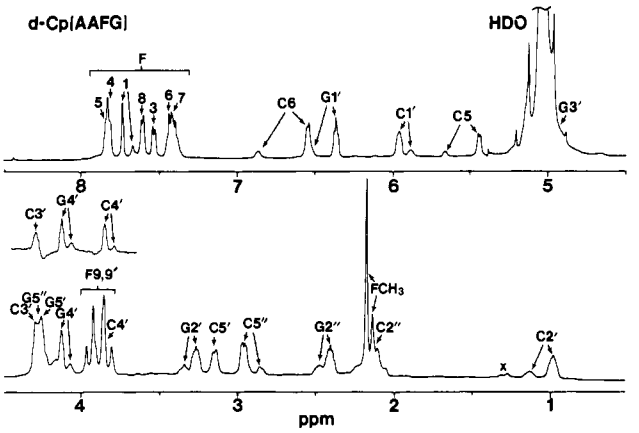

FIGURE 1: The 500-MHz ^1H NMR spectrum of d-Cp(AAFG) in D_2O (0.6 mM) at 1 °C is shown with resonance assignments for the two subspectra. Resonance numbering is according to structure I, with C, G, and F referring to deoxycytidine, deoxyguanosine, and fluorene resonances, respectively. The spectrum was processed with exponential filtering of 1 Hz. The number of scans was 1800. The insert is a rephased inversion–recovery spectrum of the 4 ppm region obtained with a pulse spacing of 0.4 s at a temperature of 10 °C following 690 scans.

Additional information on ^1H data acquisition and processing conditions is given in certain figure legends. The Lorentzian to Gaussian resolution enhancements and the computer simulations were carried out with standard Bruker software on the WM 500 NMR spectrometer.

Resonance assignments in the ^1H NMR spectra were made by comparisons with proton assignments for d-p(AAFG) (Evans et al., 1984, 1986) and the deoxycytidine resonances of d-CpG (Cheng & Sarma, 1977), as well as by homonuclear decoupling experiments and the observation of the temperature dependencies of the resonances. In addition, an inversion–recovery experiment aided in assignments of several methylene protons by elimination of overlap from methine proton resonances. The assignment of the 2′ and 2″ resonances was confirmed by conformational analysis using sugar ring coupling constants (J). This is possible since only one set of assignments is compatible with a C2′-endo–C3′-endo equilibrium. For the estimation of pseudorotational parameters and populations of the 2′-deoxyribose ring, both the program PSEUROT (Haasnoot et al., 1981; de Leeuw et al., 1983) and a modified version of this program (Evans & Levine, 1987) were used. The 5′ and 5″ resonances of the deoxyguanosine moiety (G5′ and G5″) of d-Cp(AAFG) and d-p(AAFG) were assigned from stereochemical considerations. The corresponding protons of the deoxycytidine moiety (C5′ and C5″) have been tentatively assigned according to the Remin–Shugar method (Remin & Shugar, 1972). The ^{13}C resonance assignments for d-Cp(AAFG) are based on chemical shift and spectral comparisons with d-p(AAFG) (Evans et al., 1984, 1986) and other model compounds (Alderfer, 1984).

Results

Temperature Dependence of NMR Spectra. The 500-MHz ^1H NMR spectra of d-Cp(AAFG) in aqueous solution have been recorded as a function of temperature in the range of 1–89 °C. At 1 °C, two subspectra in a ratio of 80:20 are observed (Figure 1). Residual line broadening is also present. Raising the temperature resulted in coalescence at about 50 °C. These data are characteristic of chemical exchange, with an approach to a condition of slow exchange being observed at 1 °C. The temperature dependence of the mononucleotide adduct, d-p(AAFG), has previously been reported (Evans et al., 1984, 1986); however, in that case there were four sub-

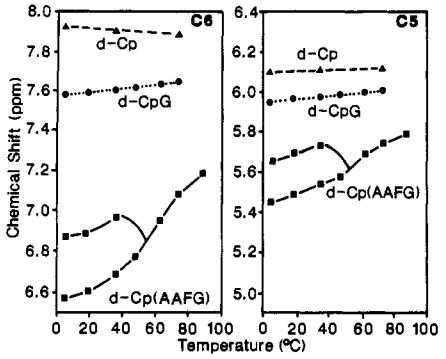

FIGURE 2: Chemical shift versus temperature profiles of the C6 (left) and C5 (right) resonances of d-Cp(AAFG) (0.3 mM), d-CpG (1 mM), and d-Cp (1 mM, pD 5.0) in D_2O are shown. The apparent chemical shifts from the two subspectra of d-Cp(AAFG) are shown in the low-temperature region.

spectra due to restricted internal rotation about the amide bond and the guanyl–nitrogen bond at C(8) instead of the two subspectra detected here for the dinucleotide adduct. The barrier to internal rotation for d-Cp(AAFG) in aqueous solution has been estimated by utilizing complete band-shape analysis and a two-state model. Analysis of the two subspectra from the C6 proton at temperatures of 5 and 19 °C yielded a value for the barrier to internal rotation about the amide bond (γ') of d-Cp(AAFG) (vide infra) of 14.3 ± 0.3 kcal/mol.

The ^1H chemical shift data of d-Cp(AAFG) in aqueous solution at a low concentration (0.3 mM) are highly temperature dependent. Temperature profiles for the C5 and C6 resonances of d-Cp(AAFG) show a greater chemical shift dependence than those of d-Cp and d-CpG (Figure 2). In the low-temperature range, both subspectra of d-Cp(AAFG) are observable and approach a plateau. Chemical shift data for d-Cp(AAFG) at 1 and 89 °C are given in Table I; resonance overlap precluded complete analysis of the minor subspectrum. All resonances of the deoxycytidine moiety of d-Cp(AAFG) shift downfield with increased temperature, with the effect being the largest for the C6, C5, C2′, C5′, and C5″ resonances. Likewise, the fluorene resonances shift downfield, although to a lesser degree, while the deoxyguanosine resonances of the adduct exhibit very small upfield shifts. A relatively large chemical shift difference between the fluorene methylene protons (F9, 9′) is also observed at low temperature (Figure 1). The results indicate that the cytosine base is involved in extensive stacking interactions with the fluorene ring.

Comparison to d-Cp and d-p(AAFG). The chemical shift parameters for d-Cp(AAFG) at 1 °C show several significant chemical shift changes compared to d-Cp and d-p(AAFG) (Table I). The upfield shifts for the major subspectrum are 0.66 ppm for C5 and 1.36 ppm for C6 relative to d-Cp (Table I). Other resonances experiencing large upfield shifts are C2′ (1.39 ppm), C5′ (0.73 ppm), and C5″ (0.83 ppm). Upfield shifts of the fluorene resonances of d-Cp(AAFG) relative to d-p(AAFG) are much less (0–0.2 ppm), and the deoxyguanosine resonances shift downfield by a comparable amount. Since the monomer data can represent the unstacked state, the chemical shift differences compared to d-Cp(AAFG) are an approximation of the effect of stacking. Several significant chemical shift differences also exist between corresponding resonances of the two subspectra of d-Cp(AAFG), especially the deoxycytidine 5, 6, 2′, and 5″ resonances, the major subspectrum being upfield, except for C5″ (Figure 1, Table I). These are the same resonances that exhibit large upfield shifts relative to d-Cp.

Table I: ^1H NMR Chemical Shifts (ppm) of d-Cp(AAFG) and Related Compounds[a]

	d-Cp(AAFG)			d-p(AAFG)	d-Cp
assignment	1 °C, I[b]	1 °C, III[b]	89 °C	52 °C[d]	29 °C
F1	7.74	7.68	7.73	7.72	
F3	7.54	ND[c]	7.52	7.51	
F4	7.83	ND	7.93	8.00	
F5	7.84	ND	7.91	7.96	
F6	7.44	ND	7.49	7.50	
F7	7.41	ND	7.44	7.45	
F8	7.62	ND	7.67	7.70	
F9	3.93	ND	3.99	4.03	
F9′	3.85	ND	3.97	4.03	
FCH_3	2.18	ND	2.21	2.25	
G1′	6.37	6.53	6.30	6.31	
G2′	3.27	3.35	3.23	3.11	
G2″	2.41	2.48	2.31	2.17	
G3′	5.0	ND	4.83	4.81	
G4′	4.13	4.07	ND	4.12	
G5′	4.26	ND	ND	4.00	
G5″	4.29	ND	ND	4.34	
C5	5.45	5.67	5.78		6.11
C6	6.55	6.87	7.18		7.91
C1′	5.96	5.89	6.02		6.29
C2′	0.99	1.14	1.66		2.38
C2″	2.18	ND	2.31		2.62
C3′	4.29	ND	4.48		4.75
C4′	3.86	ND	3.95		4.23
C5′	3.14	ND	3.40		3.87
C5″	2.97	2.85	3.30		3.80

[a] Samples of d-Cp(AAFG) (0.3 mM, pD 7), d-p(AAFG) (0.7 mM, pD 5.4), and d-Cp (0.7 mM, pD 5.4) were dissolved in D_2O with dioxane added as an internal standard (3.76 ppm). Mononucleotides were at a pD such that the phosphate group would be monoanionic for better comparisons with the dinucleotide adduct. The F refers to fluorene resonances, G to deoxyguanosine resonances, and C to deoxycytidine resonances. [b] The major subspectrum is labeled I ($\gamma' = 180°$) and the minor subspectrum is labeled III ($\gamma' = 0°$). [c] Not detected due to resonance overlap by the major subspectrum or the HDO peak. [d] An elevated temperature was utilized in order to approach a condition of rapid exchange.

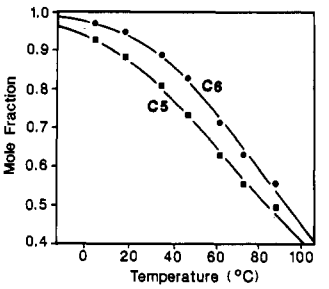

FIGURE 3: The mole fraction of stacking versus temperature was computed from the weighted average chemical shifts of C5 and C6 by utilizing a two-state model. The curves were generated from a least-squares fit of the van't Hoff equation by utilizing the C5 and C6 chemical shifts from d-Cp of 6.11 and 7.91 ppm. The best fit resulted with values of 5.44 and 6.59 ppm for C5 and C6 in the stacked state.

Quantitation of Stacking Interactions. Since the chemical shifts of d-Cp(AAFG) are a weighted average from the stacked and unstacked states, the amount of stacking can in principle be computed from the chemical shift data. The chemical shifts from the monomer d-Cp were employed in the calculations for the unstacked state. The chemical shift profiles of d-Cp(AAFG) should permit determination of chemical shifts in the fully stacked state (Figure 2). This has been accomplished by computing the least-squares fit of the van't Hoff equation for the C5 and C6 resonances over the entire temperature range. For temperatures at which subspectra appeared, the calculations were carried out on the weighted average chemical shifts. The chemical shifts for the stacked

Table II: Melting Temperature (T_m) for d-Cp(AAFG) and Several Dinucleotides

compound	measurement	T_m (°C)
d-Cp(AAFG)	NMR, resonance C6	93
d-Cp(AAFG)	NMR, resonance C5	85
CpC	optical rotation	5[a]
CpC	circular dichroism	24[b]
CpA	circular dichroism	15[b]
CpU	circular dichroism	6[b]

[a] Data are from Davis and Tinoco (1968). [b] Data are from Brahms et al. (1967).

state, which produced the best fit, are 5.44 and 6.59 ppm, respectively. The results showing the mole fraction of stacking at the various temperatures are presented in Figure 3 along with the theoretical curves generated from the van't Hoff equation. There is a reasonably good fit between the data points and the theoretical curves, and in addition, the curves for C5 and C6 are reasonably close. The average melting temperature for the cytosine resonances of d-Cp(AAFG) is 89 °C (Table II). The computations also indicate that d-Cp(AAFG) in aqueous solution is 96% stacked at 1 °C. In view of the uncertainty in applying a two-state model and the experimental error in measurements of chemical shifts due to chemical exchange, the results are considered to be an estimate. Melting temperatures for base stacking that have been reported for several unmodified dinucleotides containing cytosine (Davis & Tinoco, 1968; Brahms et al., 1967) are much lower (Table II). This is additional evidence for stacking that is primarily between the cytosine and fluorene ring systems.

Conformation and Dynamics Associated with the Site of Attachment of AAF to d-CpG under Conditions of Stacking. Dihedral angles[2] have been utilized to describe the conformation at the central nitrogen of nucleotide adducts (Hingerty & Broyde, 1982; Evans et al., 1984; Shapiro et al., 1986). The conformational nomenclature used here (α', β', and γ') is the same as that used previously, except that primes have been added to distinguish these torsions from the nomenclature used for DNA (structure I).

The identification of the two torsional diastereomers detected for d-Cp(AAFG) in aqueous solution was carried out by monitoring the spectra as a function of methanol and temperature. It is anticipated that the addition of methanol will progressively remove the stacking interactions and allow direct comparison to previously reported subspectra of d-p(AAFG) (Evans et al., 1984, 1986). Figure 4 shows the downfield shift of the C6 proton as methanol is added, confirming the destacking. However, the C6 resonance exhibits considerable broadening (Figure 4a–c) and eventually overlaps with the fluorene resonances (data not shown). The G1′ and C1′ resonances are more suitable for monitoring changes in subspectra populations. The addition of 15% methanol appears to show additional subspectra (Figure 4b), while further addition of methanol and lowering of the temperature clearly reveals more subspectra (Figure 4c,d). The changes for the G1′ resonance, which are the best resolved, establish the correspondence between the two subspectra in aqueous solution (Figure 4a) with those in methanolic solution (Figure 4e). The subspectra are labeled I–IV according to descending relative intensity in pure methanolic solution (Evans et al., 1984, 1986).

[2] Rotations about the dihedral angle A–B–C–D are considered positive for clockwise rotation of the far bond relative to the near bond, with the eclipsed conformation being assigned 0°. Definitions for the central nitrogen are as follows: α', N(9)–C(8)–N(2)–C(2); β', C(8)–N(2)–C(2)–C(1); γ', C(8)–N(2)–C(14)–C(15).

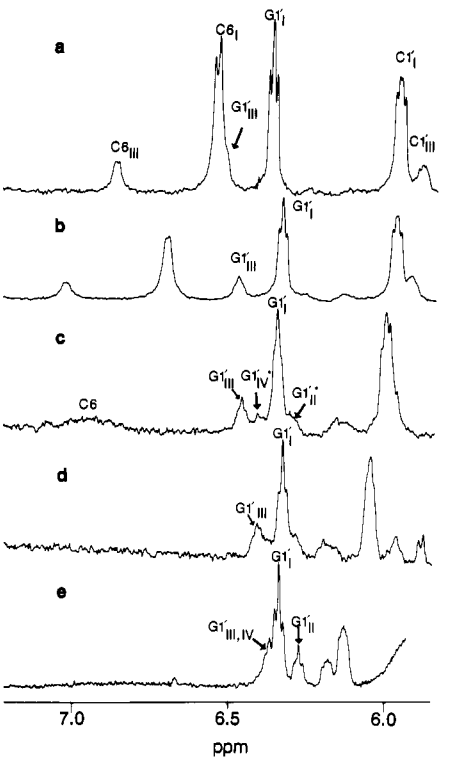

FIGURE 4: The 500-MHz ^{1}H NMR spectra of d-Cp(AAFG) show the effects of temperature and addition of methanol on the G1′ subspectra. The subspectra have been labeled with subscripts in order to show the correspondence between subspectra in aqueous solution and in methanol solution. An asterisk denotes a tentative assignment. Some of the C6 and C1′ subspectra also appear in this spectral region. The solvent and temperature conditions are as follows: (a) aqueous solution at 1 °C (0.6 mM); (b) 16% methanol at 1 °C; (c) 30% methanol at −12 °C; (d) 50% methanol at −12 °C; and (e) 100% methanol at −38 °C. Coalescence occurs at about 50 °C (spectrum not shown). Spectra a–d were obtained with exponential filtering of 1.0 Hz, and spectrum e was obtained with a Lorentzian to Gaussian resolution enhancement using a line broadening of −1 Hz and Gaussian broadening of 0.16 of the acquisition time. Number of scans ranged from 540 to 1800.

The results indicate that conformers I and III are detected in aqueous solution at 1 °C. These subspectra have previously been assigned to specific torsional diastereomers in d-p(AAFG) (Evans et al., 1984). The comparison allows identification of subspectra I and III in d-Cp(AAFG) as arising from cis–trans isomerism about the amide bond, with the amide oxygen being preferentially oriented trans to the fluorene ring (conformer I, $\gamma' = 180°$; conformer III, $\gamma' = 0°$). The comparison also shows that the torsion angle for rotation about the guanyl–nitrogen bond at C(8) (α') is near 90° for both conformers I and III. Thus, the alternate $\alpha' = 270°$ conformer of d-Cp(AAFG) is virtually excluded in the stacked form and as such does not give rise to detectable subspectra.

Information on the time-averaged torsion angle about the fluorenyl–nitrogen bond (β') can be deduced from the chemical shifts of the carbon atoms ortho to the central nitrogen. The chemical shifts of these carbons are known to be highly sensitive to delocalization of π-electron density from the nitrogen to the fluorene ring, which in turn is approximately a $\cos^2 \beta'$ function. We have previously utilized the ^{13}C chemical shift data and NOE measurements to characterize the torsion angle in d-p(AAFG) and several AAF model compounds (Evans & Miller, 1983; Evans et al., 1984, 1986). The ^{13}C data for d-Cp(AAFG) in aqueous solution are presented in Table III. The chemical shifts of F1 and F3 of d-Cp(AAFG) are slightly downfield of values for d-p(AAFG) (Evans et al., 1984, 1986). These results indicate that β' of d-Cp(AAFG) in aqueous is

Table III: ^{13}C NMR Chemical Shifts (δ) of d-Cp(AAFG)[a]

assignment	δ	assignment	δ	assignment	δ
G1′	84.7	C3′	76.9	F5	121.7
G2′	37.7	C4′	86.4	F6	128.3
G3′	71.2	C5′	62.6	F7	128.9
G4′	86.4	C2	158.2	F8	126.4
G5′	66.4	C4	ND[b]	F9	37.7
G2	154.9	C5	97.6	F10	146.6
G4	152.7	C6	141.1	F11	ND
G5	115.9	F1	125.8	F12	ND
G6	160.0	F2	138.8	F13	145.2
G8	143.3	F3	127.8	F14	ND
C1′	86.8	F4	122.2	F15	22.8
C2′	38.8				

[a] Sample dissolved in D_2O (5 mM, pD 7) at 18 °C with dioxane added as internal standard (67.8 ppm). [b] Not detected due to low signal to noise ratio.

close to 90° and/or 270°. As low a concentration as possible (5 mM) was used in this measurement in order to reduce interference from self-association.

Conformation and Dynamics of the Nucleotide Backbone under Conditions of Stacking. The conformational nomenclature utilized for the nucleotide moieties of d-Cp(AAFG) is included in structure I. Of the various sugar–phosphate torsion angles, the ones that are most amenable to NMR analysis utilizing J values are the C(5′)–O(5′) bond (β), the C(4′)–C(5′) bond (γ), the C(3′)–O(3′) bond (ϵ), and the C(3′)–C(4′) bond (δ) (Sarma, 1980). It is generally regarded that nucleotides in aqueous solution are in a rapid state of equilibrium between various conformations which results in time-averaged NMR spectral parameters. This of course is in contrast to the slow exchange that takes place about the amide bond and the guanyl–nitrogen bond at C(8) of d-Cp(AAFG). The analysis of J values has the advantage of providing equal weight to all conformers.

The spectral analysis of d-Cp(AAFG) is hampered not only by chemical exchange but also by the necessity of conducting measurements at submillimolar concentrations. In cases where the detection of minor subspectra is important, data were processed with exponential filtering to improve signal to noise (Figures 1 and 4). For the measurement of J values, such as in Figures 5 and 6, a resolution enhancement function is needed, which has the opposite effect of lowering signal to noise. By combining resolution enhancement with decoupling experiments and spectral simulation under a variety of experimental conditions, it is possible to measure J values and estimate the error in the measurements for the major subspectrum. The utility of combining resolution enhancement with high-field measurement has previously been shown (Evans et al., 1981). NOEs were not detected due to low sensitivity and the likelihood of relatively small NOEs for a compound of this size.

Figures 5 and 6 show the analysis of the most complex resolution-enhanced spectral region of d-Cp(AAFG), comprising the C3′, G4′, G5′, and G5″ protons. The analysis was aided by addition of a trace amount of methanol, which caused a reduction in resonance overlap (Figures 5 and 6). Homonuclear decoupling of G3′ clearly shows the large difference in magnitude between $J_{4'5'}$ and $J_{4'5''}$ of the resolution-enhanced G4′ resonance, and it also confirms the relatively large value of $J_{3'4'}$ observed for G4′ (Figure 6). Likewise, decoupling of C2′ aided in analysis of C3′ (Figure 6), notably the measurement of $J_{3'P}$. All decoupling experiments (data not shown) were consistent with the results of the simulations. The complete set of J values for the sugar moieties of fully stacked d-Cp(AAFG) are presented in Table IV. The accompanying

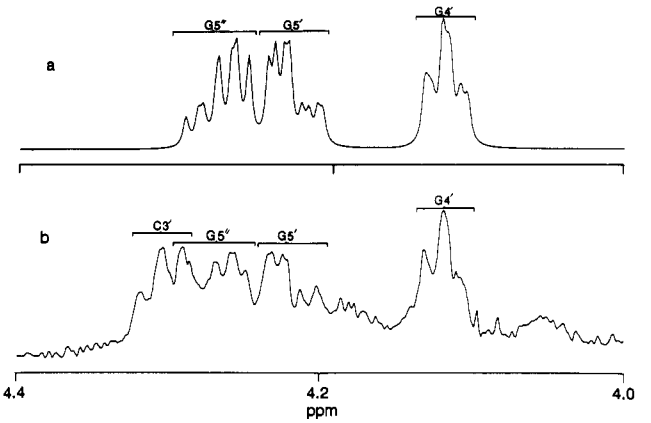

FIGURE 5: (a) The computer-simulated spectrum of the G4′, G5′, and G5″ spectral region is shown with resonance assignments. It matches the major subspectra in the actual resolution-enhanced 500-MHz ^1H NMR spectral region shown in (b), except for the C3′ resonance, which was not included in the simulation. The NMR spectrum was recorded in aqueous solution (0.3 mM) at −1 °C with a trace of methanol (8%) added to aid in the spectral analysis. The spectrum was obtained with 1600 scans. The data were processed with a Lorentzian to Gaussian resolution enhancement using a line broadening of −2 Hz and Gaussian broadening of 0.2 of the acquisition time.

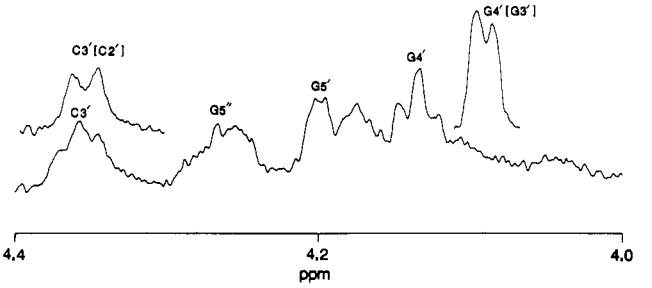

FIGURE 6: Expansion of the resolution-enhanced C3′, G4′, G5′, and G5″ proton resonances of d-Cp(AAFG) (0.3 mM) was obtained at 1 °C with 18% methanol added. These conditions resulted in a downfield shift of C3′ and an increase in the chemical shift difference between G5′ and G5″. The inserts show the effect of decoupling the C2′ (left) and G3′ (right) protons. The 500-MHz spectra were obtained with approximately 1000 scans and were resolution enhanced as described in the legend of Figure 5.

Table IV: Coupling Constants (Hz) of the Sugar Moieties of d-Cp(AAFG) and Related Compounds[a]

	d-Cp(AAFG)			
J	G	C	d-p(AAFG)	d-Cp
1′2′	5.5 ± 0.5	9.3 ± 0.6	7.4 ± 0.6	7.3 ± 0.1
1′2″	7.8 ± 0.5	5.5 ± 0.6	7.0 ± 0.6	6.3 ± 0.1
2′2″	−14.0 ± 0.6	−14 ± 1	ND[b]	−14.4 ± 0.1
2′3′	7.7 ± 1.0	6 ± 1	ND	6.3 ± 0.1
2″3′	5.8 ± 0.6	2 ± 1	ND	3.3 ± 0.1
3′4′	6.0 ± 0.5	2 ± 1	3.3 ± 0.3	3.3 ± 0.1
4′5′	1.9 ± 0.3	3 ± 1	5.9 ± 0.2	3.3 ± 0.1
4′5″	6.0 ± 1.0	5.5 ± 0.6	6.6 ± 0.2	4.8 ± 0.1
5′5″	−11.0 ± 0.3	−11.9 ± 0.6	−11.0 ± 0.2	−12.5 ± 0.1
3′P		8.1 ± 0.6		7.9 ± 0.1
5′P	5.0 ± 1.1		6.3 ± 0.2	
5″P	3.8 ± 0.9		6.6 ± 0.2	

[a] Measurements on d-Cp(AAFG) were conducted at 1 °C. Other sample conditions are given in Table I. The error limits are estimations of the range of J values in computer-simulated spectra that match the NMR spectra. Spectra recorded with a trace of methanol aided in simulation of the methanol-free spectra. [b] Not detected due to excessive line broadening.

parameters for the monomer model compounds (Table IV) reveal the large effect of stacking on certain J values.

The conformation about the C(4′)–C(5′) (γ) bonds of d-Cp(AAFG) has been estimated from the values $J_{4'5'}$ and $J_{4'5''}$

Table V: Estimated Conformer Mole Fractions along the C(4′)–C(5′) Bond of d-Cp(AAFG) and Related Compounds[a]

compound	conformer		
	gauche$^+$	trans	gauche$^-$
d-Cp(AAF<u>G</u>)[b]	0.50	0.50	0[c]
d-p(AAFG)	0.12	0.46	0.42
d-<u>C</u>p(AAFG)[b]	0.50	0.44	0.06
d-Cp	0.55	0.35	0.10

[a] For d-Cp(AAFG), measurements were conducted at 1 °C. Other sample conditions are given in Table I. [b] The underlined nucleotide denotes which C(4′)–C(5′) bond is being described. [c] The initial calculations on the deoxyguanosine moiety of d-Cp(AAFG) produced a negative population (−7%) for the gauche$^-$ form, which could be attributed to the experimental error in J values. These calculations were redone by a best-fit approach with the gauche$^-$ population set to zero. The estimate does not imply that no gauche$^-$ form is actually present.

Table VI: Estimated Sugar Ring Conformer Mole Fractions of d-Cp(AAFG) and Related Compounds[a]

compound	C2′-endo	C3′-endo	rms[c]
d-Cp(AAF<u>G</u>)[b]	0.39	0.61	0.79
d-p(AAFG)	0.70	0.30	NA[d]
d-<u>C</u>p(AAFG)[b]	0.90	0.10	0.26
d-Cp	0.70	0.30	0.37

[a] Measurements on d-Cp(AAFG) were conducted at 1 °C. Details on sample conditions are given in Table I. [b] The underlined nucleotide denotes which sugar ring is being described. [c] The rms error is in Hz. [d] Not applicable since estimated from $J_{3'4'}$ only.

by utilizing a three-state model (Haasnoot et al., 1979). In this method, it is assumed that the torsion angles of the gauche$^+$ (γ = 53°), trans (γ = 180°), and gauche$^-$ (γ = 290°) conformers are equal to the average torsion angles from X-ray crystallographic studies from a large number of nucleoside moieties (Haasnoot et al., 1979). Assignment of the 5′ and 5″ resonances is required. Initially, the conformational calculations were carried out for both possible sets of assignments. The assignment with G5″ being downfield of G5′ (Figures 5 and 6) results in the prediction of nearly equal populations of the gauche$^+$ and trans forms without any detectable gauche$^-$ population (Table V), while the reverse assignment predicts the absence of the trans form. The correct choice was made by comparison to the potential energy calculations on d-Cp(AAFG) that have been listed for the case where fluorene stacks with cytosine [Table II from Hingerty and Broyde (1982)]; one may observe that all seven low-energy conformations are in either the gauche$^+$ or trans forms. Thus, the agreement with the NMR results is very good for the former assignment, while the reversed assignment is totally inconsistent with the theoretical conformations. The predicted conformer populations along C(4′)–C(5′) are listed in Table V. Populations for the deoxycytidine moiety are based on the tentative assignments of C5′ and C5″ according to the Remin–Shugar method (Table V).

The determination of the conformation about the C(4′)–C(5′) bond of the mononucleotide d-p(AAFG) also requires assignment of the 5′ and 5″ resonances. It should be possible to deduce assignments for d-p(AAFG) from those of d-Cp(AAFG) even though the J values and the chemical shifts differ under conditions of stacking. The correspondence can be established by monitoring the spectra of d-Cp(AAFG) as the stacking interactions are gradually reduced. The addition of methanol to the aqueous solution of d-Cp(AAFG) causes an increase in the chemical shift difference between G5′ and G5″ (Figure 6), which is a movement in accord with the large chemical shift difference that is observed for d-p(AAFG) (0.34 ppm, Table I). It is concluded that G5″ is downfield of G5′ for both compounds. The conformer populations along C(4′)–C(5′) for d-p(AAFG) derived from these assignments are given in Table V. In sharp contrast to the stacked dinucleotide adduct, the gauche$^+$ form is virtually absent in the mononucleotide adduct. On the other hand, the conformation along the C(4′)–C(5′) bond of the deoxycytidine moiety is unchanged by stacking, at least to within the experimental error limits of the J values (Table V).

The C(5′)–O(5′) torsion (β) and the C(3′)–O(3′) torsion (ϵ) are reflected in the proton–phosphorus J values (Sarma, 1980). According to the most recent Karplus-like equation for J_{HCOP}, $J = 15.3 \cos^2 \phi - 6.1 \cos \phi + 1.6$ (Lankhorst et al., 1984). The values of $J_{5'P}$ and $J_{5''P}$ of 5.0 and 3.8 Hz demonstrate that the time-averaged proton–phosphorus dihedral angle, ϕ, is close to 60° for both the G5′ and G5″ protons of d-Cp(AAFG), i.e., β near 180°. Furthermore, the magnitude of these J values cannot be accounted for by a single torsion angle β. Rather, a two-state model, at a minimum, is required to account for the J values, and both conformers would then be predicted to lie in the trans domain (180 ± 60°). Alternatively, this can be evaluated as a classical threefold torsion, with the trans conformer predominating (estimated as 81%). The assessment of the C(3′)–O(3′) conformation is more limited since only a single relevant J value is available. The $J_{3'P}$ of 8.1 Hz, according to a one-state model, computes to a time-averaged ϕ value of ±28° or ±119° (ϵ = 121°, 212°, 268°, 359°). The 268° value is very close to one of the two empirically determined values that appear to be associated with a C2′-endo ring pucker (Lankhorst et al., 1985). Since the deoxycytidine ring of d-Cp(AAFG) is predominantly in the C2′-endo form (vide infra), it is suggested that ϵ is near 268°.

The sugar ring pucker of 2′-deoxynucleosides and 2′-deoxynucleotides in solution can in certain cases by determined with good resolution from the five relevant proton–proton J values (Haasnoot et al., 1981; Evans & Levine, 1987). The sugar ring is frequently described in terms of the pseudorotational parameters P and Φ, where P is the phase angle of pseudorotation and Φ the amplitude of pucker. These parameters are related to δ by the formula: $\delta = 120.6° + 1.1\Phi \cos (P + 145.3°)$ (Mellema et al., 1984). Unfortunately, the experimental error in J values (Table IV) is considered to be too large for estimation of the pseudorotational parameters in a conventional two-state analysis. In view of this limitation, we have confined the pseudorotational parameters of both the C2′-endo and C3′-endo forms to be equal to the average values from X-ray crystallography measurements. A survey of 2′-deoxynucleosides and 2′-deoxynucleotides in the solid state has indicated the following average values: P(S), 164.1°; Φ(S), 37.7°; P(N), 9.6°; Φ(N), 35.3° (de Leeux et al., 1980). Our calculations, utilizing a least-squares best-fit analysis in which only the populations of these two conformations were varied, predict C2′-endo mole fractions of 0.39 and 0.90, respectively, for the deoxyguanosine and deoxycytidine rings of d-Cp(AAFG) at 1 °C (Table VI). Similar procedures predict that the C2′-endo populations of the monomers d-p(AAFG) and d-Cp are 0.70 in both cases (Table VI). The large rms error in the calculations (Table VI) for the deoxyguanosine ring of d-Cp(AAFG) may be related to the experimental error in J values. However, calculations allowing for a flattening of either pucker, or a shift of P(N) in the O4′-endo direction, significantly improved the fit.

The occurrence of an O4′-endo conformation in certain modified nucleosides analyzed by X-ray crystallography has been the basis for the suggestion of a three-state model (Birnbaum et al., 1979; Olson, 1981; Evans & Levine, 1987). Thus, it would be of interest to test for this possibility in the

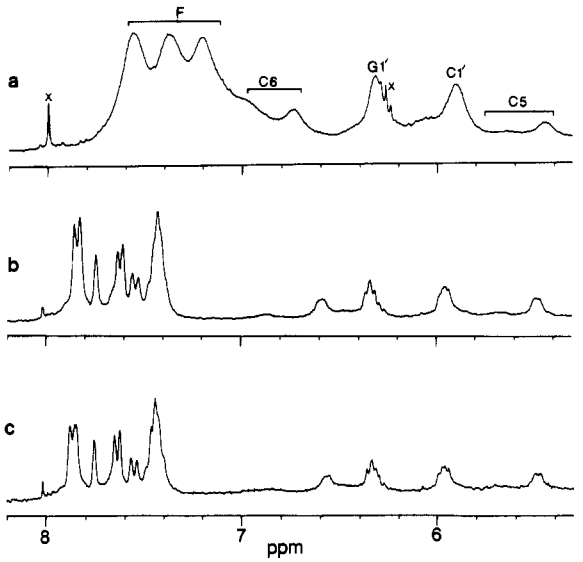

FIGURE 7: 270-MHz ^{1}H NMR spectra of d-Cp(AAFG) in aqueous solution at 20 °C show the effect of concentration on the downfield spectral region. The concentrations were (a) 10 mM, (b) 1 mM, and (c) 0.3 mM. The number of scans ranged from 900 to 19 000, and the spectra were processed with expontential filtering of 0.5 Hz.

modified dinucleotide by three-state calculations. This can be approached by using the same procedure described above for a two-state model. Pseudorotational parameters for the average O4′-endo pucker were obtained from X-ray crystallography data on 2′-deoxyribose structures (Birnbaum et al., 1979; Grand & Cadet, 1978; Young et al., 1974; Konnert et al., 1970). The average phase angle of pseudorotation and amplitude of pucker of the O4′-endo form (four examples) is 87.0° and 36.5°, respectively. Three-state best-fit calculations (Evans & Levine, 1987) incorporating these values yielded the following mole fractions for the deoxyguanosine ring of d-Cp(AAFG) at 1 °C: C2′-endo, 0.22; C3′-endo, 0.46; O4′-endo, 0.32, with rms error of 0.29 Hz. This is a smaller error than that obtained from the confined two-state calculations (Table VI).

Concentration Dependence of NMR Spectra. The 270-MHz ^{1}H NMR spectra of d-Cp(AAFG) in aqueous solution at 20 °C have been recorded as a function of concentration in the range of 0.15–10 mM by utilizing nine evenly spaced data points. Three representative spectra, shown in Figure 7, reveal a conspicuous increase in line broadening concomitant with upfield shifts of the fluorene resonances (0.3 ppm), an upfield shift of C5 (0.05 ppm), and a downfield shift of C6 (0.16 ppm) at the highest concentration. The chemical shift profiles (data not shown) show a leveling off in the 0.3–0.6 mM range at 20 °C. The results demonstrate self-association of a dinucleotide–carcinogen adduct in aqueous solution at relatively low concentration levels.

Discussion

A two-state model has enabled estimation of the extent of intramolecular stacking in d-Cp(AAFG) in aqueous solution as a function of temperature. The stacking, which is mostly fluorene–cytosine, is virtually complete in the low-temperature range. Major conformational adjustments at the central nitrogen and along the sugar–phosphate backbone are necessary to accommodate the stacking interactions. Each of the analyzed bonds was oriented in predominantly one or two conformations with torsions near the following values: α', 90°; β', 90° and 270°; γ', 180°; β, 180°; γ, 60° and 180°; ϵ, 268°; deoxycytidine ring, C2′-endo; deoxyguanosine ring, C3′-endo and an unusually puckered form. These torsions, together with the constraints of fluorene–cytosine stacking, also place limits on the allowable values for the remaining sugar–phosphate backbone torsions α and ζ.

The fluorene–cytosine stacking places restrictions on the conformation at the central nitrogen of d-Cp(AAFG) in aqueous solution. This is evidenced by the presence of only two detectable subspectra in aqueous solution, instead of the possible four, due to the predominance of the $\alpha' = 90°$ conformer along the guanyl–nitrogen bond in the stacked form. It is gratifying to observe that all low-energy conformations for fluorene–cytosine stacking have been predicted from potential energy calculations (Hingerty & Broyde, 1982) possess a torsion angle α' that is near 90°. This also supports our earlier assignment of the subspectra of p-(AAFG) to specific torsional diastereomers (Evans et al., 1984). The subspectra of d-Cp(AAFG) indicate a change at the amide bond as well, although less conspicuous. The $\gamma' = 180°$ conformer population is moderately increased to a level of 80% in the stacked form. A closer approach to the $\beta' = 90°/270°$ region along the fluorenyl–nitrogen bond due to stacking is suggested by the ^{13}C NMR data, which unfortunately needed to be recorded under sample conditions in which considerable self-association occurred.

The 14.3 ± 0.3 kcal/mol barrier to internal rotation about the amide bond (γ') of predominantly stacked d-Cp(AAFG) is significantly higher than the 12.3 ± 0.1 kcal/mol barrier that we have previously measured for the modified base 8-(*N*-fluoren-2-ylacetamido)guanine (Neidle et al., 1984), but within the error limits of the rough estimates for d-p(AAFG) (Evans et al., 1984). The experimental barrier is lower than that incorporated into the energy minimum calculations on d-Cp(AAFG).

The conformation about the C(4′)–C(5′) bond (γ) of the deoxyguanosine moiety of d-Cp(AAFG) is dramatically altered by the stacking interactions. It is widely accepted that, in purine nucleotides with a syn conformation about the glycosyl bond, there is a preference for the gauche$^-$ or trans conformations. Classical examples are the cases of 8-Br-AMP (Sarma et al., 1974) and the deoxyguanosine moiety of a Z-DNA duplex, which exists in a syn-trans form (Wang et al., 1981). Likewise, our results on d-p(AAFG) show the destabilization of the gauche$^+$ conformation about C(4′)–C(5′), the gauche$^+$ form being present in d-p(AAFG) at an estimated mole fraction of 0.12. The 0.50 mole fraction of gauche$^+$ form in stacked d-Cp(AAFG) is believed to be unprecedented for a syn purine nucleotide in solution. The accessibility of the syn-gauche$^+$ form of d-Cp(AAFG) has been successfully predicted from potential energy calculations (Hingerty & Broyde, 1982), although the NMR results indicate this conformer to be more populated. It should be recognized that the determination of the sugar–phosphate torsions is necessary for the ultimate characterization of a nucleic acid duplex. Even with the correct base arrangement, incorrect conclusions about the sugar conformation can lead to misleading inferences about the overall morphology at the macromolecular level (Srinivasan & Olson, 1987).

The *J* values relating to the C(5′)–O(5′) torsion of d-Cp(AAFG) point to a β value that is close to 180°. This result shows that the stacking interactions do not significantly alter β from the classical energy minimum in the main conformations. The potential energy calculations (Hingerty & Broyde, 1982) had predicted more flexibility about this bond.

The stacking interactions in d-Cp(AAFG) significantly alter the sugar ring conformer populations. For the deoxycytidine

moiety, the C2′-endo population is increased and becomes the predominant form. The C2′-endo population of the deoxyguanosine ring decreases to result in a mixture of conformations, but the nature of the mixture is not subject to a unique interpretation. It appears that one, if not more, of the conformers has an unusual pucker. Theoretical calculations on d-Cp(AAFG) predicted a phase angle of pseudorotation [*P*-(N)] of 40–50° for the deoxyguanosine ring (Hingerty & Broyde, 1982). This parameter does yield a better fit of the NMR *J* values when substituted into the otherwise confined two-state analysis.

The chemical shift changes observed for d-Cp(AAFG) can be rationalized in a qualitative manner from anisotropic ring current effects. The large upfield shifts of the cytosine base protons and the much smaller upfield shifts of the fluorene resonances are attributable to the difference in the magnitude of the ring current fields and to extent of overlap by the adjacent ring system in the stack (Evans et al., 1986). The upfield shifts of the C2′, C5′, and C5″ resonances are also compatible with ring current considerations. Examination of molecular models with the conformational constraints acquired from the NMR analysis shows that the C5′ and C5″ protons can be shielded by the nearby fluorene ring when the C(4′)–C(5′) bond is gauche$^+$ and by the guanine ring when the C(4′)–C(5′) is trans. The shielding by guanine can result from the positioning of the deoxycytidine moiety next to the guanine ring even though the guanine ring is not involved in the base–base stacking. Likewise, C2′ can be shielded by either the guanine or fluorene rings, depending to a large extent on γ. In contrast to the deoxycytidine protons, the deoxyguanosine protons exhibit small downfield shifts in the stacked state. This trend can be rationalized on the basis of a change in the conformation at the central nitrogen, since the 8-substituent (AAF moiety) contains an aromatic ring system which would be expected to affect the chemical shifts of the attached sugar protons.

The chemical shift differences between subspectra reveal that the two torsional diastereomers relating to rotation about the amide bond exist in different magnetic environments due to stacking. If this were not the case, one would not expect the large chemical shift differences between subspectra for protons that are far removed from the site of cis–trans isomerism. In addition, the protons that exhibit the largest upfield shifts due to stacking are the same ones that exhibit the most conspicuous chemical shift differences between subspectra (C5, C6, C2″, and C5″). It can be concluded that the mode and/or extent of stacking depends on whether the amide bond is cis or trans. The downfield shift of the cis form ($\gamma' = 0°$) indicates more guanine–cytosine stacking, differences in ring overlap, or less fluorene–cytosine stacking in the cis form. This result, along with the findings on the conformational adjustments about the guanyl–nitrogen bond, shows the importance of the conformation at the central nitrogen to the stacking interactions.

There are several notable chemical shift parameters that may be indicators of the proposed structure. The chemical shift of C2′ at 0.99 ppm is in a unique position for a nucleotide sugar proton, and C5′ and C5″ are also far upfield of the norm. These resonances may be characteristic of the proposed conformation and as such may occur in other oligonucleotide adducts having a similar conformation. Another example is the G2″ chemical shift. We have previously reported a chemical shift of one of the G2″ subspectra at 1.7 ppm, which was identified as being associated with the alternate $\alpha' = 270°$ torsional diastereomer in d-p(AAFG) (Evans et al., 1984, 1986). This sharply contrasts with the 2.4 ppm value observed for G2″ of d-Cp(AAFG), for which only the $\alpha' = 90°$ form is detected in the fully stacked state. Thus, the presence or absence of a G2″ resonance near 1.7 ppm (Figure 1) under conditions of slow exchange may be generally helpful in deducing the conformation about the guanyl–nitrogen bond in AAF adducts.

It is of interest to make comparisons of the stacking interactions between different aryl carcinogens and nucleic acid bases. Rough estimations of the degree of stacking in other AAF-modified dinucleotides may be possible from comparisons of NMR chemical shift data. Upfield shifts of 0.6 ppm have been reported for the adenine protons of d-Ap(AAFG) relative to d-ApG (Nelson et al., 1971; Santella et al., 1980). For d-Cp(AAFG), the average C5 and C6 chemical shifts are approximately 0.5 and 0.9 ppm upfield of those in d-CpG (Figure 2). This suggests that the extent of stacking between fluorene and cytosine rings is comparable to that between fluorene and adenine rings for this dinucleotide sequence, although CD studies on Up(AAFG) have suggested much weaker interactions with a uracil ring (Nelson et al., 1971). The previous studies has also suggested that the stacking interactions of fluorene are greatest for the case of a purine nucleotide on the 5′ side of the modified nucleotide (Nelson et al., 1971; Santella et al., 1980; Leng et al., 1980; Alderfer et al., 1984). The upfield shifts of d-Cp(AAFG) are much greater than those observed for C(8)-substituted 4-aminobiphenyl adducts (Shapiro et al., 1986; Lasko et al., 1987). This comparison strongly suggests that the extent of stacking between the aminobiphenyl ring system and nucleic acid bases is significantly less than that in AAF-modified oligonucleotides.

Most of the NMR measurements on d-Cp(AAFG) were carried out at submillimolar concentrations to minimize self-association. The lack of a strong chemical shift dependence on concentration for the cytosine base protons suggests that the cytosine and fluorene rings continue to be extensively stacked at the intramolecular level in the self-associated form, with the intermolecular interactions being primarily between the fluorene and/or guanine rings. The concentration dependence of d-Cp(AAFG) qualitatively resembles that reported for d-p(AAFG), for which a cooperative self-association has been shown (Evans et al., 1986). There may be a relationship to gel formation that has been reported for GMP (Petersen et al., 1982; Fisk et al., 1982).

The elimination of self-association of d-Cp(AAFG) was needed in order to obtain parameters for the intramolecular state and to reduce the line widths. Lowering the temperature also produced spectra more suitable for characterization of the major subspectrum by narrowing the line widths and apparently increasing the population of the major torsional diastereomer. The NMR measurements on this compound were more difficult than may appear at first inspection. An example is Figure 4c, where, under the experimental conditions, there is nearly a complete loss of the C6 resonance due to chemical exchange, while the G1′ part of the spectrum remained suitable for the intended purpose of the experiment. The difficulty in making NMR measurements may explain why NMR studies on this type of important adduct have been limited in the past. With the judicious choice of experimental conditions, it was possible to assign all the proton resonances, measure the *J* values, and detect and assign subspectra.

Recently, sufficient quantities of oligonucleotide–AAF adducts suitable for duplex formation have been prepared for NMR analysis. One experiment suggested that the AAF moiety is intercalated into a pocket that may convert to other

subsequent structures (Winkle et al., 1987). Another study demonstrated the presence of all nine imino resonances in the ^{1}H NMR spectrum in an AAF-modified 9-mer duplex, with two of the resonances being upfield of the normal imino region (Krugh et al., 1987). This and related experiments were interpreted as showing that the majority of the duplex is in a right-handed conformation in this case. It may be relevant that hairpin loops comprising only two bases have been reported and the imino protons of such structures are upfield of the normal imino region (Haasnoot et al., 1983).

The results on d-Cp(AAFG) show some of the main conformational adjustments at the central nitrogen and in the sugar–phosphate backbone that accommodate stacking between a covalently bound aryl carcinogen and a nucleotide on the 5′ side. The high level of fluorene–cytosine stacking interactions supports intercalation models for DNA–AAF complexes (Shapiro & Klein, 1967; Nelson et al., 1971; Fuchs & Daune, 1972; Drinkwater et al., 1978; Hingerty & Broyde, 1986). However, the conformation of this fragment does not imply that the fluorene moiety will necessarily be intercalated between the neighboring bases in a DNA sequence but, rather, models of modified DNA that conserve the torsion angles for stacked d-Cp(AAFG) would permit looped-out structures for a range of loop sizes. The modified guanine would be expected to be the first base in such a loop. The loop may continue to the complementary strand to allow resumption of normal hydrogen bonding. Looped-out structures are attractive possibilities since they would provide a structural basis for the frame-shift mutations, by a rationale similar to the proposed bulged-base mechanism (Lee & Tinoco, 1978). Deformations in the normal helical structure of the type suggested by d-Cp(AAFG) might also be recognizable sites for certain repair enzymes and account for the relatively rapid rate of repair of this general type of adduct in modified DNA.

A recent theoretical study on the conformation of d-Cp(AAFG) incorporated into oligomers and a miniduplex (Broyde & Hingerty, 1987) has yielded additional complementary findings. Included in that study is the global energy minimum conformation for fluorene–cytosine stacked d-Cp(AAFG) in a model tetramer. The results on the tetramer are in general agreement with our solution studies. The comparison is complicated by the mixture of conformations present in solution. The theoretical predictions for the AAF–base orientation in modified duplexes are in best agreement with our solution studies for the case in which two hydrogen bonds at the center of the duplex are disrupted; the relevant torsions (α', β', and γ') are all within 33° of the solution data on the modified dinucleotide. The authors also described the relevance of the predicted bulges to frame-shift mutations involving two deletions.

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Registry No. I, 80229-05-6.

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