Conformation and Dynamics of the 8-Substituted Deoxyguanosine 5'-Monophosphate Adduct of the Carcinogen 2-(Acetylamino)fluorene

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Abstract: The conformation and dynamics of the major nucleotide adduct formed from the carcinogen 2-(acetylamino)fluorene (AAF) has been investigated by high-field NMR spectroscopy in a temperature range of -50 to 52 °C. At -50 °C, the ^1H and ^{13}C NMR spectra of 8-(*N*-fluoren-2-ylacetamido)-2'-deoxyguanosine 5'-monophosphate were composed of four subspectra, while at the most elevated temperatures time-averaged parameters were obtained. Barriers to internal rotation of approximately 12-15 kcal/mol are associated with the amide bond and the guanyl-nitrogen bond due to amide conjugation and steric interactions. Minimum energy conformations about the guanyl-nitrogen bond are characterized by a near orthogonal orientation between the planes of the acetamido moiety and the guanine ring. The four torsional diastereomers resulting from restricted rotation about the amide and guanyl-nitrogen bonds interconvert through a cyclic pathway. The results on the conformation of the nucleotide moiety indicated a glycosyl torsion angle in the syn range, a C2'-endo (^2E) conformation of the sugar ring, and a gauche-trans/trans-gauche (*gt/tg*) conformation about the C4'-C5' bond. Both the ^2E and *gt/tg* populations approached 100% at -50 °C. The results were compared to theoretical calculations reported for the base displacement and Z-DNA conformations of modified DNA. The torsion angles from the solution studies are in agreement with the global energy minimum predicted from theoretical studies on base displacement. The torsion angles also fit the Z-DNA conformation, except that the sugar ring is ^2E in solution instead of C3'-endo (^3E). It is proposed that the binding of AAF may alter normal helix base stacking in modified DNA due in part to overcrowding between the 8-substituent and the adjacent 3'-nucleotide.

The carcinogen 2-(acetylamino)fluorene covalently binds to nucleic acids in vivo.[1-3] Two acetylated and one unacetylated adduct have been identified, each of which is bound to a guanine ring. Aside from structure, these adducts differ in the local conformational changes that they induce in DNA,[4-8] as well as in their rate of repair.[2,9,10] Adduct formation is thought to be related to the mutagenic and carcinogenic properties of AAF[2,11] (Kriek and Westra, 1979, and references cited therein). A detailed characterization of the conformation and dynamics is needed in order to explore possible structure-function relationships.

There have been intensive investigations utilizing a variety of techniques on the conformational alterations in AAF-modified DNA[12] (Hingerty and Broyde, 1982, and references cited therein). The adduct with AAF bound via the nitrogen to the 8-position of guanine (8-AAF-dGMP) has received most attention since it is the major adduct formed in in vitro reactions, when the model carcinogen *N*-AcO-AAF is used.[1] In the present work, we have investigated the solution conformation of 8-AAF-dGMP by high-field ^1H, ^{13}C, ^{15}N, and ^{31}P NMR spectroscopy at low temperatures. The low temperatures are used to slow the rate of exchange between energy minimum conformations. Attention is focused on the conformation and dynamics associated with the nitrogen at the site of attachment of the carcinogen to the nucleotide. The results are discussed in terms of possible alterations in DNA conformation. A preliminary account of this work has been published.[13]

Experimental Section

Synthesis of 8-AAF-dGMP was carried out by reaction of *N*-AcO-AAF with dGMP (Sigma, St. Louis, MO) in a methanol-water solution.[1] The product was purified by column chromatography using a DEAE-Sephadex A-25 (bicarbonate form) resin and a linear gradient of 0-0.7 M ammonium bicarbonate for elution. The product, with an absorbance maximum of 275 nm, was pooled and lyophilized to a white powder. The *N*-AcO-AAF was prepared as described previously.[14,15] The selectively ^{15}N-enriched 8-AAF-dGMP was synthesized by reaction of ^{15}N-AcO-AAF (contract, Robert L. Roth, Midwest Research Institute, Kansas City, MO) with dGMP.

Nuclear magnetic resonance spectra were recorded in the ^1H configuration on a Bruker WM 500 spectrometer and in the ^{13}C and ^{31}P configurations on a Bruker WH 270 spectrometer. The ^{15}N measurements were carried out on a Bruker WH 400 located at the National Science Foundation facility in NMR spectroscopy at the University of South Carolina. Samples were dissolved in deuterated methanol. Me_4Si was added as an internal standard for the ^1H and ^{13}C measurements and chemical shifts are reported on the δ scale. The ^{31}P chemicals shifts are referenced to external phosphoric acid. The ^{15}N chemical shifts are reported in ppm downfield from anhydrous liquid ammonia by assigning the resonance from external dimethylformamide to 103.8 ppm. The coupling constants from the ^1H NMR spectra were measured by spectral simulation utilizing standard software from Bruker Instruments.

Typical data acquisition conditions were as follows: for 67.89-MHz ^{13}C spectra, data size, 16K; sweep width, 15 151 Hz; flip angle, 60°; pulse spacing, 2.5 s; number of scans, 10 000 except for the coupled spectrum, which was 38 000 scans; for 40.55-MHz ^{15}N spectra, data size, 8K; sweep width, 2000 Hz; flip angle, 33°; pulse spacing, 11 s; number of scans, 3800; for 109.3-MHz ^{31}P spectra, data size, 16K; sweep width, 5000 Hz; flip angle, 60°; pulse spacing, 1.6 s; number of scans, 80; for 500-MHz ^1H spectra, data size, 32K; sweep width, 6024 Hz; flip angle, 80°; pulse spacing, up to 6 s; number of scans, 200. The NOE difference spectra were obtained by alternating the proton irradiation on and off resonance and substracting the data. The duration of the presaturation was varied as described in the figures, and the pulse spacing was 4.7 s plus the

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(11) Abbreviations used are as follows: 8-AAF-dGMP, 8-(*N*-fluoren-2-yl-acetamido)-2'-deoxyguanosine 5'-monophosphate; 8-AAF-dG, 8-(*N*-fluoren-2-ylacetamido)-2'-deoxyguanosine [frequently called *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene]; AAF, 2-(acetylamino)fluorene; *N*-HO-AAF, *N*-hydroxy-2-(acetylamino)fluorene; *N*-AcO-AAF, *N*-acetoxy-2-(acetylamino)fluorene; Me_4Si, tetramethylsilane; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect.
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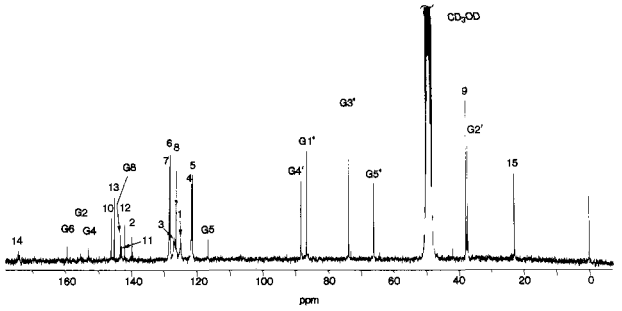

Figure 1. The 67.89-MHz ^{13}C NMR spectrum of 8-AAF-dGMP in methanol-d_4 (30 mg/mL) recorded at 52 °C with broad-band proton decoupling is shown with resonance assignments. Resonance numbering is according to structure **1** in the text, with G referring to the guanine resonances.

Table I. ^1H NMR Chemical Shifts in ppm of 8-(*N*-Fluoren-2-ylacetamido)-2-deoxyguanosine 5'-Monophosphate at Elevated Temperature and at Low Temperature[a]

		−50 °C subspectrum			
assignment	52 °C	I	II	III	IV
F1	7.68	7.78	7.62	7.62	7.80
F3	7.48	7.60	7.40	7.44	ND[d]
F4	7.85	7.94	7.87	7.85	7.95
F5	7.81	7.87	7.82	7.82	7.87
F6	7.36		7.3–7.4		
F7	7.30		7.3–7.4		
F8	7.55	7.59	7.56	7.55	ND
F9	3.92	3.97	ND	ND	ND
F14	2.17	2.17	2.19	2.09	2.18
G1'	6.24	6.28	6.24	6.33	6.38
G2'[b]	3.29	3.58	3.37	3.56	3.32
G2''[b]	2.08	2.17	1.66	2.02	2.23
G3'	4.76	4.72	4.57	4.68	4.66
G4'	4.12	4.17	4.10	ND	ND
G5'[c]	4.34	4.34	4.25	ND	ND
G5''[c]	4.00		3.8–4.0		

[a] Sample dissolved in methanol-d_4 (2 mg/mL) with Me_4Si added as the internal standard. The F refers to the fluorene resonances and G to the guanine resonances. [b] The 2'-proton is on the same side of the sugar ring as the glycosyl bond, according to standard nomenclature. [c] Assignment may be reversed. [d] Not detected.

presaturation time. The X-nuclei spectra were obtained with broad-band proton decoupling and/or gating as appropriate. All spectra were processed with zero-filling and exponential filtering, except for the ^1H spectra for which a Lorentzian to Gaussian resolution enhancement was utilized.

Resonance assignments in the ^1H NMR spectrum of 8-AAF-dGMP at elevated temperature were made by comparison to those for 8-AF-dG,[3,16] from homonuclear decoupling experiments and from the NOE of the F1 and F8 resonances resulting from saturation of the adjacent methylene protons at F9. In addition, saturation transfer experiments and additional decoupling studies were used to assign the corresponding resonances between the four subspectra at low temperatures.

^{13}C NMR resonance assignments of 8-AAF-dGMP were determined mainly from the ^1H assignments, from extensive selective heteronuclear decouplings, and from comparisons to results on dGMP,[17] *N*-AcO-AAF, and AAF.[15]

Results

Temperature Dependence of NMR Spectra. The ^1H, ^{13}C, ^{15}N, and ^{31}P NMR spectra of 8-AAF-dGMP (**1**) in methanol were temperature dependent. At 52 °C, the ^{13}C spectral data were characteristic for normal time averaging, except for residual broadening of several resonances. In particular, the resonances of carbons ortho and para to the acetamido moiety were broadened (Figure 1). Analogous results were obtained by ^1H NMR for

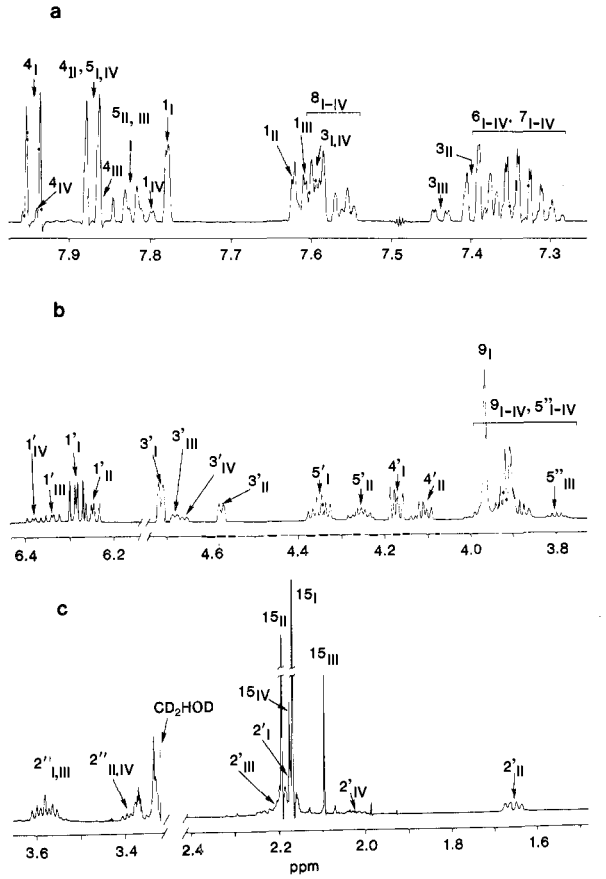

Figure 2. The 500-MHz ^1H NMR spectrum of 8-AAF-dGMP in methanol-d_4 (2 mg/mL) at −50 °C is presented for (a) the aromatic region, (b) the aliphatic region between 3.8 and 6.4 ppm, and (c) the aliphatic region between 1.5 and 3.6 ppm. The resonances are assigned according to structure **1** in the text with subspectra I–IV indicated by subscripts. The chemical shift scale for the aromatic region (a) differs by 0.2 ppm from that previously presented[13] due to an error in the earlier figure.

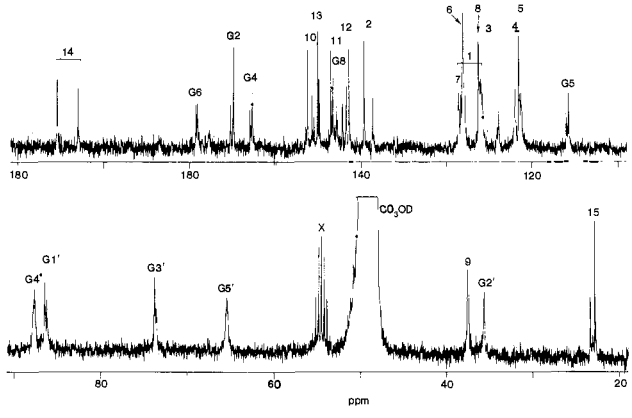

Figure 3. The 67.89-MHz ^{13}C NMR spectrum of 8-AAF-dGMP in methanol-d_4 (30 mg/mL). See legend of Figure 1 for assignments.

which the ortho proton resonances were broadened at 52 °C.[13] Complete assignments for the ^1H and ^{13}C NMR spectra are given in Tables I and II. The results demonstrate the complete ^{13}C assignments for an arylamine nucleotide adduct.

Lowering the temperature resulted in line broadening of all resonances followed by the appearance at −50 °C of four subspectra of differing intensities (Figures 2 and 3). These were labelled I–IV according to descending intensity. The four subspectra are most easily observable for the G1' and G3' proton resonances, since these resonances are adequately spaced and free from overlap with resonance of other protons (Figure 2b). Several resonances could not be detected or assigned due to the low signal

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Table II. ^{13}C NMR Chemical Shifts in ppm of 8-(*N*-Fluoren-2-ylacetamido)-2′-deoxyguanosine 5′-Monophosphate at Elevated Temperature and at Low Temperature[a]

		−50 °C subspectrum[c]		
assignment	52 °C	I	II	III
F1	124.9	125.8	123.9	124.1
F2	139.5	139.6	138.6	138.5
F3	126.8	127.9	125.8	ND[d]
F4	121.5	121.9	121.4	ND
F5	121.2	121.4	121.2	ND
F6	128.0	128.0	128.0	ND
F7	128.3	128.5	128.3	ND
F8	126.1	126.1	126.1	ND
F9	37.7	37.5	37.5	ND
F10	146.0	146.2	145.7	145.5
F11	143.0	143.5	142.2	142.3
F12	141.8	141.4	141.7	141.4
F13	145.1	145.0	144.9	144.8
F14	174.2	175.5	173.0	ND
F15	22.7	22.7	23.2	23.0
G2	155.2	155.0	155.3	155.3
G4	152.9	152.8[b]	153.0[b]	ND
G5	116.4	115.6	115.9	115.8
G6	159.3	159.3	159.2	ND
G8	143.2	143.3	142.9	ND
G1′	86.4	86.5	86.3	86.1
G2′	37.1	35.7	35.7	ND
G3′	73.7	73.9	73.6	ND
G4′	88.1	87.8	87.6	ND
G5′	66.0	65.5	65.5	ND

[a] Sample dissolved in methanol-d_4 (30 mg/mL) with Me_4Si added as the internal standard. The F refers to fluorene resonances and G refers to the guanine resonances. [b] Assignments may be reversed. [c] The following resonances were detected for subspectrum IV: F10, 146.4 ppm; F14, 175.3 ppm. [d] Not detected.

Table III. ^{15}N and ^{31}P Chemical Shifts of 8-(*N*-Fluoren-2-ylacetamido)-2′-deoxyguanosine 5′-Monophosphate[a]

	subspectrum			
nucleus	I	II	III	IV
^{15}N (F2)	175.4	176.7	176.5	ND[b]
^{31}P	2.64	2.50	ND[b]	ND[b]

[a] Sample dissolved in methanol-d_4 (30 mg/mL) with measurements at −50 °C. The ^{15}N and ^{31}P reference was anhydrous liquid ammonia and phosphoric acid, respectively. [b] Not detected due to resonance overlap with subspectrum I or II.

and resonance overlap. This was especially true for subspectrum IV. The chemical shifts and resonance assignments for each of the four ^1H and ^{13}C subspectra from low-temperature measurements are presented in Tables I and II. The ^{15}N chemical shifts of N2-labeled 8-AAF-dGMP as well as the ^{31}P measurements at low temperature are listed in Table III. These results are indicative of slow rates of exchange between four conformers having different populations. Relative populations as measured from the ^1H subspectra were as follows: I, 53%; II, 26%; III, 13%; IV, 8%.

The ^{13}C subspectra obtained at −50 °C contained unusual broadening (Figure 3). This can be partly attributed to not completely attaining conditions of slow exchange. However, most of the residual broadening was present in the resonances of the carbons with a directly attached proton, and these resonances exhibited additional broadening at lower temperatures. This is likely due to dipole–dipole relaxation, which is becoming a significant line broadening mechanism due to the slower tumbling times associated with the lower temperatures and increased solvent viscosity. The broadening begins with the C–H carbons primarily because of the strong distance dependence of the relaxation mechanism. The temperature of −50 °C was selected as the best compromise for resolving the four subspectra.

Aside from the appearance of subspectra at low temperatures, there was little temperature dependence of chemical shifts. The weighted average of the chemical shifts between subspectra I, II, III, and IV was similar to the time-averaged chemical shifts measured at elevated temperature (Tables I and II). Exceptions were the ^1H and ^{13}C resonances of the G2′ position. In the low-temperature spectra, the average G2′ proton and carbon resonances were shifted 0.21 ppm downfield and 1.4 ppm upfield, respectively. In so far as the 2′-carbon and 2′-proton resonances of nucleosides and nucleotides are known to be sensitive to conformation,[18-20] this chemical shift data may suggest a temperature-dependent conformational change of the nucleotide moiety of 8-AAF-dGMP.

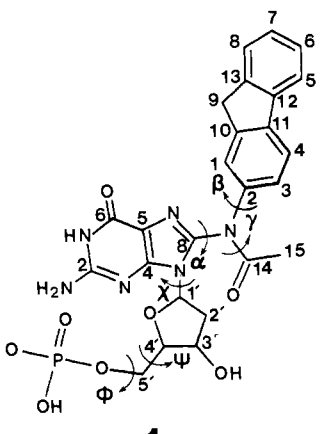
Table IV. Vicinal Coupling Constants in Hertz of the Deoxyribose Moiety of 8-(*N*-Fluoren-2-ylacetamido)-2′-deoxyguanosine 5′-Monophosphate at Elevated Temperature and at Low Temperature[a]

J	52 °C	−50 °C[b]
H1′–H2′	8.0	9.0
H1′–H2″	6.4	6.1
H2′–H2″	−13.3	−13.3
H2′–H3′	5.3	5.3
H2″–H3′	1.1	1.1
H3′–H4′	2.3	1.1
H4′–H5′	7.6	9.3
H4′–H5″	5.4	5.8
H5′–H5″	−10.7	−10.4
H5′–P	7.3	5.5
H5″–P	5.9	4.8
C4′–P	8.3	
H1′–C4 (guanine)	5.6	
H1′–C8 (guanine)	2.8	

[a] Sample dissolved in methanol-d_4 (2 mg/mL) except for the ^{13}C measurements for which sample was 30 mg/mL. [b] At 52 °C, time-averaged values were measured; at −50 °C, the values for the most populated subspectrum (I) were measured.

Selected coupling constants from the spectra of 8-AAF-dGMP were measured at elevated and at low temperature (Table IV). Significant differences in vicinal J_{H-H} and J_{H-P} couplings of the sugar moiety are indicative of a temperature dependence of the sugar conformation. No temperature dependence was detected between the vicinal J_{C-P} and J_{C-H} couplings (Table IV), but due to signal to noise and resolution limitations in the ^{13}C spectra, the negative results do not necessarily indicate an absence of changes.

Conformation and Dynamics Associated with the Site of Attachment of AAF to dGMP. There are three bonds associated with the nitrogen atom that links AAF to the nucleotide. As shown in structure **1**, these are the guanyl–nitrogen (α), the fluorenyl–



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nitrogen (β), and the amide bond (γ). Conformational nomen-

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clature for AAF adducts, which has previously been reported in theoretical studies,[12] is utilized in the present investigation to describe the torsion angles about these bonds.[21]

The conformation about the amide bond of 8-AAF-dGMP was investigated by analysis of the chemical shift differences between subspectra. Several trends were observed in the magnitude and direction of the chemical shift changes. For most cases, the ^1H and ^{13}C resonances of the fluorene moiety of subspectra II and III were upfield of I and IV (Tables I and II). The chemical shift differences for the proton and carbon resonances were among the largest for the positions ortho and para to the acetamido moiety. The magnitudes were as follows: F1, 1.9 ppm; F3, 2.1 ppm; F11, 1.3 ppm for the carbons; F1, 0.16 ppm; F3, 0.20 ppm for the protons. The trends resembled those reported for the *s*-cis and *s*-trans isomerism in *N*-AcO-AAF.[15] It is concluded that the amide bond of 8-AAF-dGMP also exhibits restricted internal rotation, which can account for two of the four subspectra. On the basis of the upfield shifts of the fluorene resonances in the cis form of *N*-AcO-AAF, the upfield shifts in subspectra II and III of 8-AAF-dGMP are attributed to the cis ($\gamma = 0°$) orientation between the fluorene ring and amide oxygen. Likewise, the downfield shifts of I and IV are the basis for assigning these subspectra to conformers with a trans ($\gamma = 180°$) conformation about the amide bond. The ^{15}N chemical shift data on 8-AAF-dGMP and *N*-AcO-AAF[15] is also consistent with these conclusions.

The conformation and dynamics associated with the fluorenyl-nitrogen bond (β) was determined from the ^1H chemical shift data. We have previously reported that the torsion angle β in other N-substituted AAF compounds strongly affects the ortho (F1 and F3) proton chemical shifts due mainly to the anisotropy of the acetamido bond system.[15] In 8-AAF-dGMP the anisotropy of the guanine ring may also affect the chemical shifts of the ortho protons. The observation that the F1 and F3 resonances of the subspectra (Table I) exhibit chemical shift changes in the same direction and of similar magnitude indicates rapid rotation about the fluorenyl–nitrogen bond of 8-AAF-dGMP. If there was restricted rotation, the chemical shifts of F1 and F3 should have moved in opposite directions, due to the anisotropy of the acetamido moiety and the guanine ring. Further, the similarity in the chemical shift differences between F1 and F3 also indicate little differences in populations between the preferred torsion angle β and a 180° rotation about the long axis of the fluorene ring.

Information on the time-averaged torsion angle β was obtained from the ^{13}C chemical shifts of F1, F3, and F11. These resonances in N-substituted AAF compounds are known to be highly sensitive to delocalization of π-electron density from the nitrogen to the fluorene ring.[15] Theoretically, the resonance energy due to conjugation varies by a $\cos^2\beta$ function.[23] Hence, the most downfield shifts can be expected for an orthogonal orientation between the acetamido and fluorene planes ($\gamma = 0°, 180°$; $\beta = 90°, 270°$). The downfield shifts of the F1, F3, and F11 carbon resonances as compared to other AAF compounds[15] indicate the presence of a relatively small amount of π-electron delocalization from the nitrogen into the fluorene ring in 8-AAF-dGMP. It is concluded that the preferred torsion angle (β) is in a range midway between coplanar ($\beta = 0, 180°$), and orthogonal ($\beta = 90°, 270°$) with a probable bias toward the orthogonal orientation. The ^{13}C NMR data also indicate that there is more orthogonal character (β close to 90° or 270°) in the trans conformers (I, IV; $\gamma = 180°$) than in the cis conformers (II, III; $\gamma = 0°$).

The ^{15}N chemical shift difference between the cis and trans conformers about the amide bond (Table III) are roughly comparable in magnitude but opposite in direction to the ^{13}C chemical shift differences of the ortho and para resonances (Table II). Thus, a delocalization of π-electron density from the nitrogen atom to the fluorene ring correlates to a downfield shift of the ^{15}N resonance and an upfield shift of the ^{13}C resonances. Similar results were previously obtained for N-HO-AAF and *N*-AcO-AAF.[15] It is of interest to compare these results on arylamides with time-averaged ^{13}C and ^{15}N chemical shifts reported for a series of arylamines at elevated temperatures.[24] It may be concluded that the ^{15}N chemical shifts of arylamides (Table III) are less sensitive to orientation about the aryl–nitrogen bond than the arylamines. In ancillary measurements, the ^{31}P chemical shifts were found to be relatively insensitive to the rotational isomerism (Table III).

The presence of the second set of subspectra is attributed to the restricted rotation about the guanyl–nitrogen bond (α). Inspection of space-filling models of 8-AAF-dGMP, constructed with a range of different torsion angles α, reveals steric crowding when the acetamido moiety is coplanar with the guanine ring. Most crowding exists between the acetamido moiety and the deoxyribose substituent at G9 and possibly the fluorene ring. Rotation about the guanyl–nitrogen bond by roughly 90° minimizes the steric interactions. Therefore, it is proposed that the coplanar orientation between the amido moiety and the guanine ring represents the transition state and the near orthogonal orientations (α near 90°, 270°) are the energy minima for rotation about the guanyl–nitrogen bond. Indirect support for this conclusion is the relatively small carbon chemical shift differences between subspectra for the various guanine resonances (0.1–0.4 ppm) and the relatively large differences between the G2′-proton resonances (0.6 ppm). The former result can be attributed to torsion angles for α of approximately 90° and 180° since there would be little delocalization of π-electron density from the nitrogen to the guanine ring. The 0.6-ppm upfield shift is a large change by ^1H NMR standards. The magnetic anisotropy of the fluorene ring can cause such a large perturbation. Inspection of molecular models with α at 90° shows that the G2′ proton shifts could be positioned in the strong ring current field of the fluorene ring.

Saturation transfer experiments were used to obtain qualitative information on some of the rates of interconversion between conformers I, II, III, and IV and to aid in the assignments of subspectra I–IV. Selective saturation of the G2′ proton resonance of subspectrum II caused an initial buildup in the intensities of both III and IV followed by a buildup in I, with I eventually gaining higher intensity than either III or IV (Figure 4). The initial rates of transfer to III and IV from II were not noticeably different. In another related experiment, the F15 proton resonance of subspectrum III was saturated (Figure 5). This caused initial rates of increase of I and II, which were roughly comparable in magnitude. No other selective saturation transfer experiments could be performed due to resonance overlap. The results are consistent with a cyclic interconversion between torsional diastereomers I–IV (Figure 6).

The difficulty of performing the saturation transfer measurements prevented an accurate determination of the magnitudes of the barriers to rotation by this method. On the basis of comparisons of the coalescence temperatures between 8-AAF-dGMP, *N*-AcO-AAF, and *N*-HO-AAF (unpublished data) and the known barriers to rotation for the latter two compounds,[15] we estimate that the average barrier to rotation about the guanyl–nitrogen bond and the amide bond is 12–15 kcal/mol in 8-AAF-dGMP.

Conformation and Dynamics of the dGMP Moiety of 8-AAF-dGMP. It is generally regarded that 5′-β-nucleotides are in a rapid state of equilibrium in aqueous solution between various conformations about the glycosyl bond (χ), about the C4′–C5′ bond (ψ), about the C5′–O5′ bond (ϕ) and of the sugar ring.[25] NMR methods have been reported for determining the preferred conformations from the relevant vicinal coupling constants.[25] In the present study we have utilized these procedures in an attempt to estimate the conformer populations of 8-AAF-dGMP in methanol.

(21) Rotations about the dihedral angle A–B–C–D are considered positive for clockwise rotation of the far bond relative to the near bond (0° = eclipsed). Definitions for structure **1** are as follows: α, N9–C8–N–C2; β, C8–N–C2–C1; γ, C8–N–C14–C15;[12] χ, O1′–C1′–N9–C8; ψ, C3′–C4′–C5′–O5′; ϕ, C4′–C5′–O5′–P.[22]

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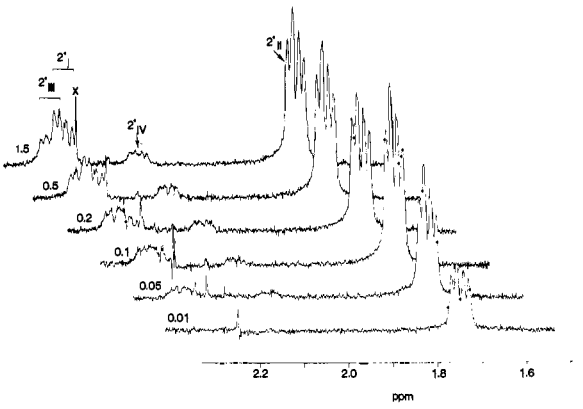

Figure 4. The 500-MHz ^{1}H NMR difference spectra of 8-AAF-dGMP (2 mg/mL) at −40 °C for the region 1.5–2.3 ppm were recorded with selective saturation of the 2′-proton of subspectrum II. The time intervals shown are the durations in seconds of the saturations. Measurements were made at −40 °C in order to obtain faster transfer and, hence, more signal.

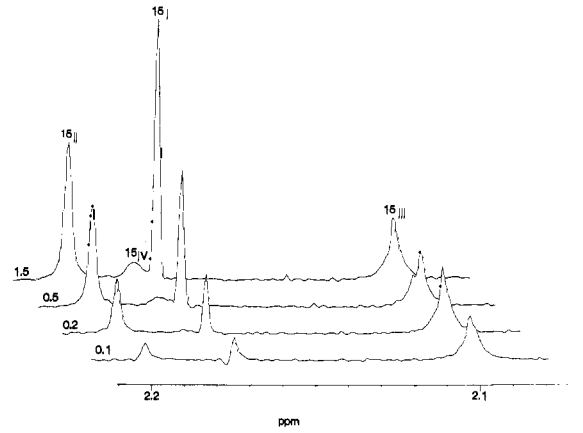

Figure 5. The 500-MHz ^{1}H NMR difference spectra of 8-AAF-dGMP (2 mg/mL) at −40 °C for the region 2.0–2.3 ppm are presented for selective saturation of the methyl protons (F15) of subspectrum III. The time intervals shown are the durations in seconds of the saturations.

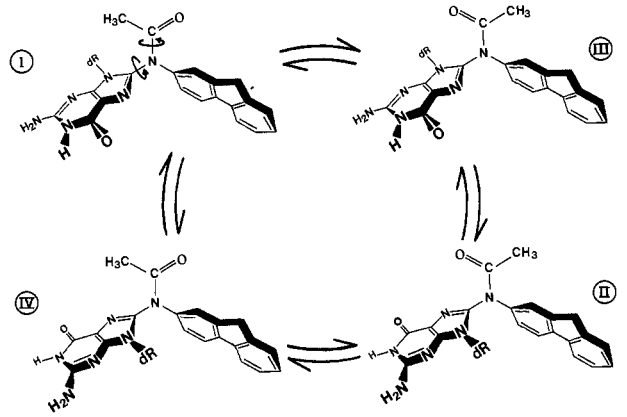

Figure 6. Representation of the cyclic interconversion between torsional diastereomers in 8-AAF-dGMP due to restricted internal rotation about the amide bond and about the guanyl–nitrogen bond. The deoxyribose moiety (dR) is above (II, IV) or below (I, III) the plane of the amide bond system. The torsion angles α and γ are as follows: I, 90°, 180°; II, 270°, 0°; III, 90°, 0°; IV, 270°, 180°.

The possibility of detectable restricted rotation in the nucleotide moiety of 8-AAF-dGMP at low temperatures is ruled out based on the absence of significant differences in vicinal couplings between subspectra. Thus, each of the subspectra may be regarded as a time average with respect to the conformation of the nucleotide moiety.

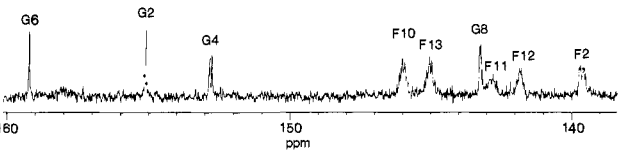

Figure 7. Proton-coupled 67.89-MHz ^{13}C NMR spectrum of 8-AAF-dGMP in methanol-d_4 (30 mg/mL) at 52 °C in the region between 140 and 160 ppm shows the vicinal J_{CH} splittings between the sugar 1′-proton and carbons 4 and 8 of guanine.

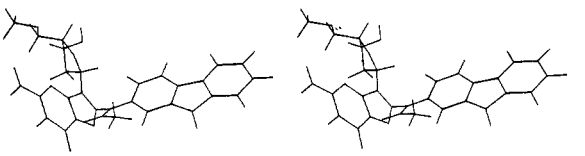

Figure 8. Stereoview of 8-AAF-dGMP is shown in conformation I (α = 90°, γ = 180°) with other torsion angles as follows: β = 60°; χ = 210°; ψ = 180°; ϕ = 180°; sugar ring, ^{2}E.

The conformation of the deoxyribose moiety in solution may be treated as an equilibrium between C2′-endo (^{2}E) and C3′-endo (^{3}E) forms. The magnitude of the proton–proton coupling constants $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$ (Table IV) are consistent with this approximation and indicate a predominant ^{2}E conformation. The ^{2}E population was estimated from $J_{3'4'}$ to be 77% at 52 °C and 89% at −50 °C. No difference in population was detected between conformers I, II, III, and IV at −50 °C.

The dihedral angle about the C4′–C5′ bond and the C5′–O5′ bond of 5′-β-nucleotides can be inferred from the magnitude of the coupling constant sums $J_{4'5'} + J_{4'5''}$ and $J_{5'P} + J_{5''P}$, respectively. The standard method is to estimate populations of predicted energy minimum conformers based on Karplus-like equations for the dependence of vicinal couplings on the dihedral angle. According to these procedures, the combined gauche–trans and trans–gauche populations about the C4′–C5′ bond are 93% at 52 °C and near 100% at −50 °C. The calculations for the conformation about the C5′–O5′ bond indicate more flexibility with gauche′–gauche′ the most stable conformer (57% at 52 °C and 71% conformer I at −50 °C).

Information on the glycosyl torsion angle χ can be obtained from the coupling constant $J_{H1'C4'}$.[18,26,27] The value of 5.6 Hz measured from the ^{13}C spectrum of 8-AAF-dGMP (Figure 7) is typical of the syn conformation and is significantly larger than that the 1.9 Hz measured for dGMP in aqueous solution. On the assumption of a single predominant torsion angle for 8-AAF-dGMP, the dependence of the H–C–N–C bond network on dihedral angle leads to a prediction of either 210° or 260° for χ. In view of the uncertainties associated with this computation, the estimates are in reasonable agreement with theoretical calculations.[12] The range is characteristic of syn nucleosides and nucleotides.[22,28] The ^{13}C NMR spectra are not of sufficient resolution to determine whether χ differs between the torsional diastereomers I–IV. An upfield shift of the G2′ carbon resonance and a downfield shift of the G2′ proton resonance in 8-AAF-dGMP (Tables I and II) compared to dGMP[17,29] are also characteristic of the syn conformation. A stereo view of the minimum energy conformation of 8-AAF-dGMP is illustrated in Figure 8.

Discussion

This investigation is an attempt to determine the detailed conformation and dynamics of an arylamine carcinogen–nucleotide adduct in solution. The principal findings are the large barriers to internal rotation about the guanyl–nitrogen bond (α) and the amide bond (γ), as well as a cyclic interconversion pathway

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between the torsional diastereomers.

The barrier to rotation about the amide bond of 8-AAF-dGMP results in observable cis ($\gamma = 0°$)–trans ($\gamma = 180°$) isomerism due to amide conjugation. The classical example of this is dimethylamide, which has a barrier to rotation of 20 kcal/mol.[30] This phenomenon has been difficult to observe in arylamides by NMR spectroscopy because the equilibrium is usually shifted far in favor of one conformer. Exceptions are *N*-HO-AAF and *N*-AcO-AAF for which cis and trans forms were easily detected at low temperatures.[15] Barriers of 14.4 and 13.7 kcal/mol, respectively, were computed from complete band shape analysis. These values are roughly comparable to the estimate for 8-AAF-dGMP in the present study. X-ray crystallography data on N-HO-AAF,[31] and AAF,[32,33] are also indicative of amide conjugation as evidenced by values γ of 16.7° and 4.2°, respectively. No X-ray crystallography data have been reported for AAF-modified nucleosides or nucleotides. Theoretical studies on AAF-modified dinucleosides predicted values γ deviating 0–16° from planarity in various proposed energy minimum conformations.[12]

The partial double bond character of the amide bond of 8-AAF-dGMP appears to be a dominant factor in determining the barrier to rotation about the guanyl–nitrogen bond. An analogy can be made between the steric interactions in structure **1** and the well-known case of ortho-substituted biphenyls.[34] In 8-AAF-dGMP, the ortho substituents would be the deoxyribose moiety and either the oxygen atom or the methyl group of the acetamido moiety. The analogy is closest when the acetamido moiety is planar. Hence, an interrelationship between α and γ may be predicted.

The combined solution populations of the cis (II plus III) and trans (I plus IV) conformers indicate that on the average the trans ($\gamma = 180°$) orientation is a little lower in energy than the cis ($\gamma = 0°$) form. The conformer populations for I plus III compared to those for II plus IV indicate that the torsion angle $\alpha = 270°$ is on the average lower in energy than for $\alpha = 90°$. Though populations may differ somewhat in an aqueous solvent, the primary conclusion of large barriers to rotation is expected to apply to aqueous solution. Similar measurements were not made in aqueous solution because temperatures below 0 °C would have been required.

The rapid rotation about the fluorenyl–nitrogen bond of 8-AAF-dGMP is analogous with the situation for *N*-HO-AAF and *N*-AcO-AAF.[15] However, in 8-AAF-dGMP the preferred torsion angle β is such that the fluorene ring and the acetamido moiety deviate more from coplanarity. The range for the torsion angle β of 8-AAF-dGMP in methanol solution is consistent with values predicted for AAF-modified dCpdG.[12]

The conformers associated with the nucleotide moiety of 8-AAF-dGMP are rapidly interconverting at all temperatures utilized. No distinction could be made between the nucleotide conformations of torsional diastereomers I–IV. The predominant conformation about the glycosyl bond is determined to be the syn conformer, which is in agreement with previous NMR studies that were based on chemical shift analysis[16,35,36] and theoretical calculations,[12,37] on various AAF adducts. The solution conformation of the C4′–C5′ bond has previously been reported for the nucleoside 8-AAF-dG[16] but for any AAF–nucleotide adducts. The present investigation indicates a significant destabilization of the gauche–gauche (gg, $\psi = 60°$) forms as compared to the nucleoside.

The conformer populations for the combined gt/tg forms as well as the ^2E form for the sugar ring approach 100% at −50 °C. This is a consequence of the low temperature utilized as well as the relatively large free energy difference between conformers. It demonstrates an approach to 100% ^2E as well as gt/tg conformers in a modified nucleotide from low-temperature NMR studies. The large value of $J_{4'5'} + J_{4'5''}$ indicate that the gt/tg population is somewhat overestimated by the computation procedure, under the conditions of low temperature and a methanolic solvent.

The large barriers to rotation about α and γ may be relevant to the conformation in modified DNA. We have attempted to incorporate torsional diastereomers I–IV into models proposed for modified DNA. There is agreement between the minimum energy conformer I in solution and the theoretically computed global minimum for fluorene–cytidine stacking in AAF-modified dCpdG.[12] Not only are the torsion angles α and γ in accord but also values of β, χ, ψ, and the sugar puckers are also in agreement. These data are consistent with the well-known base displacement or insertion/denaturation model[4,6,12,35] of how binding of AAF may interfere with normal base pairing.

The theoretical calculations of Hingerty and Broyde also predicted energy minimum conformations for a Z-DNA duplex in which the AAF moiety is located on the outside of the helix.[12] Again the experimentally derived energy minimum torsion angles are in accord with conformer I, except that the sugar pucker of 8-AAF-dGMP is ^2E in solution but ^3E for Z-DNA. Experimental results indicated that AAF binding in poly(dC-dG) promotes formation of Z-DNA.[38-40]

An attempt has been made to construct space-filling models of AAF-modified DNA without perturbation of the helix, thereby retaining the stabilization from normal hydrogen bonding and base stacking interactions. The constraint is placed that the adduct in DNA assumes one of the four energy minimum conformations for rotation about the guanyl–nitrogen and amide bonds. In order to be properly incorporated into the helix, the model is oriented into the anti conformation about the glycosyl bond, which, though not a favored conformation, has been predicted to be accessible in a modified dinucleoside.[12] Attempts to then incorporate the model into helical DNA (B form) having normal base pairing fail due to steric crowding between the 8-substituent and the adjacent 3′-nucleotide (dXpdG). In conformers I and III, there is overcrowding between the acetyl moiety of AAF and the sugar ring of the adjacent nucleotide, while, in conformers II and IV, the overcrowding involves the fluorene ring. It is suggested that unfavorable steric interactions between the 8-substituent and the adjacent 3′-nucleotide may be relevant to local disruption of helical structure in AAF-modified DNA.

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