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NMR and FT-IR Conformational Studies of 8-Substituted Guanine Nucleosides and Nucleotides and Their Metal Adducts and Cancer*

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Abstract

NMR and FT-IR Studies of the conformational changes of guanosine and guanosine-5′-monophosphate upon substitution of the H8 of guanine by a heavy, large atom, such as bromine, are presented. The conformational forms, *syn*, *anti*, C2′-*endo* and C3′-*endo* and *gg*, *gt* and *tg* rotamers of the above molecules are compared to those of their metal (Mg^{2+} and Pt^{2+}) adducts, where the metal is fixed to the N7 nitrogen atom of guanine. The antitumor activity of cisplatin is discussed with relation to the conformational form and the effect of cisplatin is compared to the effects of the Mg^{2+} ion and carcinogens.

Introduction

Recently, there have been several NMR studies on 8-substituted purine nucleosides [1–4] and nucleotides [5–8]. These studies addressed the question of the structure and conformational changes of the nucleobase purine with a bulky substituent at the C8-position. It has been reported [9] that substitution of H8 in guanosine (Guo) by bromine or cyclic guanosine-5′-monophosphate ($cGp^{5'}$) changes the molecular conformation of the molecule so much that subsequent metabolic processing by guanosine or cyclic guanosine-5′-monophosphate specific enzyme is minimal. This behavior was in striking contrast to the unbrominated species. Investigations of the conformational alterations of the deoxyguanosine-5′-monophosphate adduct of the carcinogen 2-(acetylamino)fluorene ($AAF\text{-}8dGp^{5'}$) interestingly showed a glycosyl torsional angle in the *syn* range, together with a C2′-*endo* sugar pucker and a *gauche–trans*, *trans–gauche* (*gt*/*tg*) conformation rotation around the C4′–C5′ bond [5]. It has also been reported that bromination at C8 of guanine in Z DNA stabilizes the Z DNA structure [10]. On the other hand, NMR investigations [11] on the conformational changes of the deoxyguanosine-5′-monophosphate adduct of the antitumor drug cisplatin in D_2O solutions showed a glycosyl torsional angle in the *anti* range, together with a 50% C3′ *endo* sugar pucker and a predominantly 70% *gauche–gauche* (*gg*) conformation.

The *anti* orientation in cisplatin is most likely due to the attraction of the negatively charged phosphate (PO_3^{2-}) group by the positively charged $Pt(NH_3)_2^{2+}$ unit attached at N7 together with the intramolecular bonds of the ammonia molecules with the phosphate group, *i.e.*, $NH_3\cdots$phosphate hydrogen bonds.

The *syn–anti*, C2′-*endo*–C3′-*endo* and *gg–gt*/*tg* conformational changes are widespread in DNA. It is known that spontaneous mutations, for instance, could arise from the *syn* conformation [12]. It is also known that enzymes require the *anti* form of the substrate in order to dephosphorylate the nucleotides and that 8-alkyl-substituted nucleotides are resistent to dephosphorylation [13].

In the present work, we have attempted to study the conformational changes of 8-bromo-substituted guanosine-5′-monophosphate by high field ^1H NMR and IR spectroscopy. The data on *syn–anti*, C2′-*endo*, C3′-*endo* and on the rotation about the C4′–C5′ bond are correlated with the substituent at the C8 and/or at the N7 substituent of guanine.

Experimental

Nuclear Magnetic Resonance Spectra

8-Bromoguanosine-5′-monophosphate ($Br^8Gp^{5'}$) disodium salt was purchased from Sigma Chemical Co. The NMR spectra were recorded on a Bruker WH-400 spectrometer. Proton chemical shifts are reported from internal standard sodium 2,2-dimethyl-2-silapentanone-5-sulfonate (DSS) (δ = 0.0 ppm). ^{31}P

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irradiation was carried out using standard Bruker accessories. The solutions to be studied were prepared by dissolving the 8-bromoguanosine (5 mmol) and 8-bromoguanosine-5′-monophosphate (20 mmol) in D_2O (99.996 Kor isotopes).

Fourier Transform Infrared Spectra (FT-IR)

The IR spectra were recorded on a Digilab FTS-15C/D Fourier Transform infrared interferometer equipped with a wide-range HgCdTe detector (Infrared Associates, New Brunswick, NJ), a KBr beam splitter and a global source. Normally, 250 interferograms of 2048 points were recorded with an optical velocity of 1.2 cm s^{-1} and a maximum optical retardation of 0.25 cm, co-added and Fourier Transformed with a resolution of 4 cm^{-1}. Films on KRS-5 windows and KCl pellets were used. The samples in H_2O (0.02 M) were freeze-dried at 78 K (liquid nitrogen) before use.

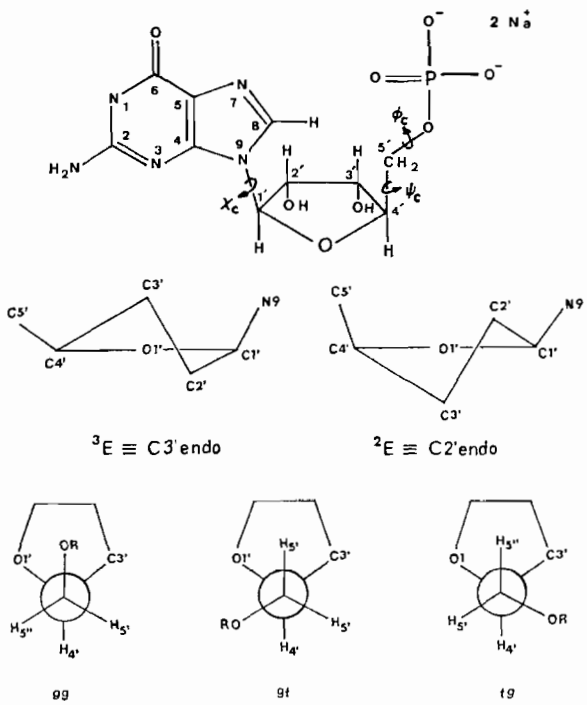

Fig. 1. Chemical structure and numbering of $Gp^{5'}$ together with the sugar pucker conformations:

syn	*anti*	(χ, rotation around the N9–C1′ bond)
$^3E \equiv$ C3′-*endo*	$^2E \equiv$ C2′-*endo*	(flip–flap of the sugar)
gg	*gt/tg*	(ψ, rotation around the C4′–C5′ bond)
g′g′	*g′t′/t′g′*	(ϕ, rotation around the C5′–O5′ bond).

Results and Discussion

The base-sugar conformations are shown in Fig. 1. The bulky substituent at the C8-position of guanosine sterically constrains the molecule to the *syn* conformation which in turn could promote formation of Z DNA. In Fig. 2 are shown the molecular structures of $Br^8Gp^{5'}$ and $AAF^8dGp^{5'}$ in the *syn* conformation. The molecular structures of the adducts, $Mg^7Gp^{5'}$ and $Pt^7Gp^{5'}$ in the *anti* conformation are shown in Fig. 3. These two metal adducts promote and stabilize the *anti* conformation by bringing the negatively charged phosphate group closer to the positive charge of the

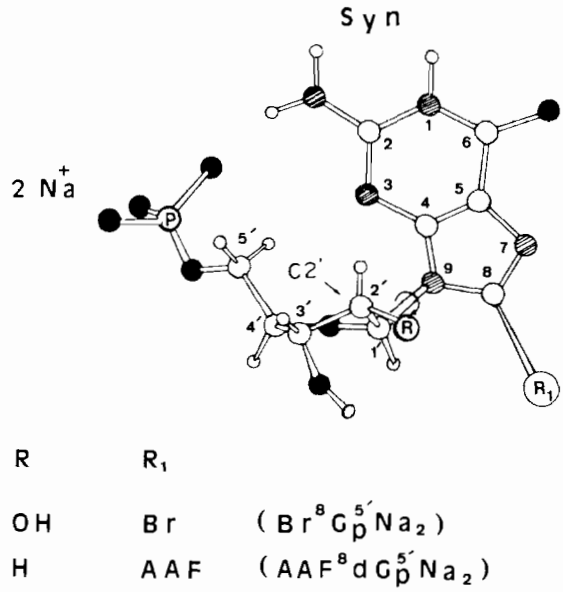

Fig. 2. Molecular structures of $Br^8Gp^{5'}$ and $AAF^8dGp^{5'}$.

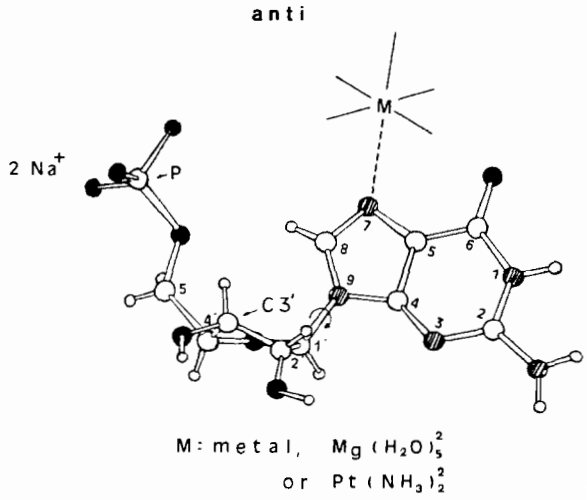

Fig. 3. Molecular structures of $Mg^7Gp^{5'}$ and $Pt^7Gp^{5'}$.

TABLE I. Proton Chemical Shifts and Coupling Constants for Various Purine Nucleosides and Nucleotides

Compound	pD	Chemical shifts, δ (ppm)					
		1′	2′	3′	4′	5′	5″
Guo	7.2	5.895	4.712	4.394	4.215	3.845	3.845
Br^8Guo	7.5	5.922	5.073	4.485	4.223	3.890	3.854
	Δδ	0.027	0.361	−0.009	0.008	0.045	0.009
$Gp^{5'}$	8.3	5.921	4.783	4.481	4.310	3.981	3.981
$Br^8Gp^{5'}$	6.5	5.957	5.295	4.585	4.257	4.140	4.098
	Δδ	0.036	0.512	0.104	−0.053	0.159	0.117

	Coupling constants, J (Hz)							
	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	$J_{5'5''}$	$J_{5'p}$	$J_{5''p}$
Guo	5.9	5.2	3.7	3.2	3.8	−12.5		
Br^8Guo	6.6	5.6	3.1	3.1	4.1	−12.7		
$Gp^{5'}$	6.1	5.2	3.4	3.7	3.7		4.6	4.6
$Br^8Gp^{5'}$	6.2	5.7	3.7	4.5	5.6	−11.5	5.7	5.6

TABLE II. Conformational Parameters for Various Purine Nucleosides and Nucleotides

Compound	T (°C)	^3E	Conformational isomers (%)[c] [17]			
			gg	*gt, tg*	*g′t′*	*g′t′, t′g′*
Guo	20	38	69	31		
$Gp^{5'}$	20	36	65	35	76	24
$dGp^{5'}$	20	33	53	47	71	29
$Br^8Guo^{5'}$	20	33	67	33		
$Br^8Gp^{5'}$	20	40	37	63	66	34
$AAF^8dGp^{5'}$ [a]	52	22	~0	~100	57	43
$PtCl_3{}^7Gp^{5'}$	20	51	36	64	72	28
$Mg^7Gp^{5'}$	20	44	75	25	72	28
cis-$Pt^7Gp^{5'}$ [b]	43	50	69	31	76	24
cis-$Pt^7dGp^{5'}$ [b]	20	40	57	43	82	18

[a]Carcinogen. [b]Antitumor agents. [c]% 3′-*endo*(^3E) = $100J_{3',4}J_{1,2'} + J_{3',4'}$; %*gg* = (13.7 − Σ)/9.7 × 100, where $\Sigma = J_{4',5'} + J_{4',5''}$ and the %*g′g′* = (25 − Σ′)/20.8 × 100, where $\Sigma' = J_{5'p} + J_{5''p}$.

TABLE III. Chemical Shift Changes (Δδ in ppm) of the Sugar Protons and Carbons upon 7 and 8 Substitution for Various Purine Nucleosides and Nucleotides

Compound	$H_{1'}$	$H_{2'}$	$H_{2''}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$
Br^8Guo	0.027	0.361		−0.009	0.008	0.045	0.009
$Br^8Gp^{5'}$	0.036	0.512		0.104	−0.053	0.159	0.117
$AAF^8dGp^{5'}$	−0.060	0.490	−0.410	0.060	−0.080	0.410	0.070
$PtCl_3{}^7Gp^{5'}$	−0.068	−0.053		−0.078	−0.052	0.039	0.039
$Mg^7Gp^{5'}$	0.006	−0.067		−0.003	0.010	0.057	0.014
cis-$Pt^7Gp^{5'}$	−0.015	−0.174		−0.025	0.006	0.097	0.047
cis-$Pt^7dGp^{5'}$	−0.035	−0.123	0.090	0.002	0.019	0.089	0.089

Compound	$C_{1'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	$C_{5'}$
Br^8Guo [a]	3.23	−3.30	0.00	0.65	0.54
$Br^8Gp^{5'}$ [a]	2.97	−3.20	−0.19	−0.35	0.34
$AAF^8dGp^{5'}$ [b]	2.10	−2.60	1.40	1.10	1.20
cis-$Pt^7Gp^{5'}$ [c]	0.78	−1.36	0.63	−0.42	−0.82
cis-$Pt^7dGp^{5'}$ [c]	0.00	0.41	−1.56	0.12	0.56

[a]Ref. 23. [b]Ref. 5. [c]Ref. 11.

metal cation and by forming hydrogen bonds with the coordinated water or ammonia molecules, respectively.

In Table I are given the proton NMR chemical shifts and coupling constants for Guo, Br^8Guo, $Gp^{5'}$ and $Br^8Gp^{5'}$. In Table II are summarized the conformational parameters for guanosine nucleoside and mononucleotide and their derivatives with bulky substituents at the 8-position or N7–metal adducts. The chemical shift (changes (Δδ in ppm) of the sugar protons and carbons upon C8 and N7 substitution are shown in Table III and also illustrated in Fig. 4.

The changes in chemical shifts for H1′, H2′ and H3′ for the various molecules relative to those of free guanosine and free $Gp^{5'}$ are consistent with the molecule being in the *syn* conformation. The changes are clearly due to base orientation with respect to the glycosidic bond. The H1′ chemical shifts are most likely due to an anisotropic interaction with the 8-substituent being very close to H1′. Partial ^1H NMR spectra of the various species are shown in Fig. 5. The proton–proton coupling constants do allow a conformational analysis by using a simple set of Karplus constants [17]. From Δδ values of H2′ it is easy to

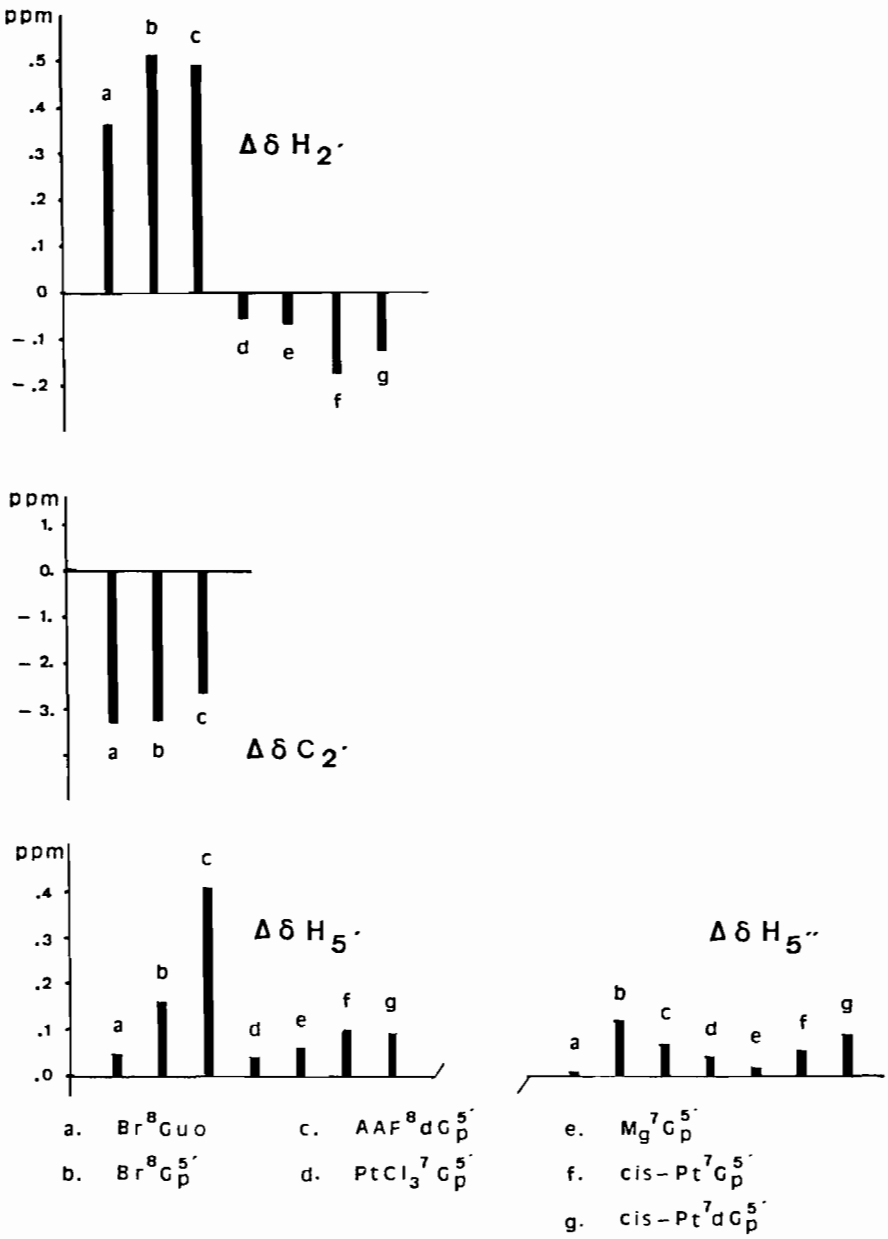

Fig. 4. Significant chemical shift changes (^{1}H and ^{13}C) of 8-bromoguanosine, 8-bromoguanosine-5′-monophosphate, 8-AAF-deoxyguanosine-5′-monophosphate, 7-metalated guanosine-5′-monophosphate and 7-platinated deoxyguanosine-5′-monophosphate.

obtain two types of nucleobases, those with *syn* conformation (*i.e.*, Br^8Guo, $Br^8Gp^{5'}$ and $AAF^8Gp^{5'}$) and those with *anti* conformation (*i.e.*, $Mg^7Gp^{5'}$ and $Pt^7Gp^{5'}$). The former show positive $\Delta\delta$ values (see Table III and Fig. 4), whereas the latter show negative $\Delta\delta$ values. It is of great interest that the carcinogen $AAF^8dGp^{5'}$ shows a C2′-*endo,syn,gt* conformation [5], which is similar to that of $Br^8Gp^{5'}$ (see Fig. 4). Negative $\Delta\delta$ values of H2′ and C2′ are indicative of *anti* conformation.

The percent of *syn* conformation for the various compounds can be estimated from $\Delta\delta H_2'$, if it is assumed a 100% *syn* for *t*-Bu^8Guo (Bu = butyl) and 100% *anti* or 0% *syn* for cisplatin ($Gp^{5'}$). In the case of *t*-Bu^8Guo, because of steric hindrance the imidazole ring is turned away to give the *syn* conformation, whereas for the platinum complex, *cis*-$[Pt(NH_3)_2(Gp^{5'})_2]^{2+}$, the phosphate is attracted by the positive charges of the platinum metal; in addition the ammonia ligands have been shown to form hydrogen

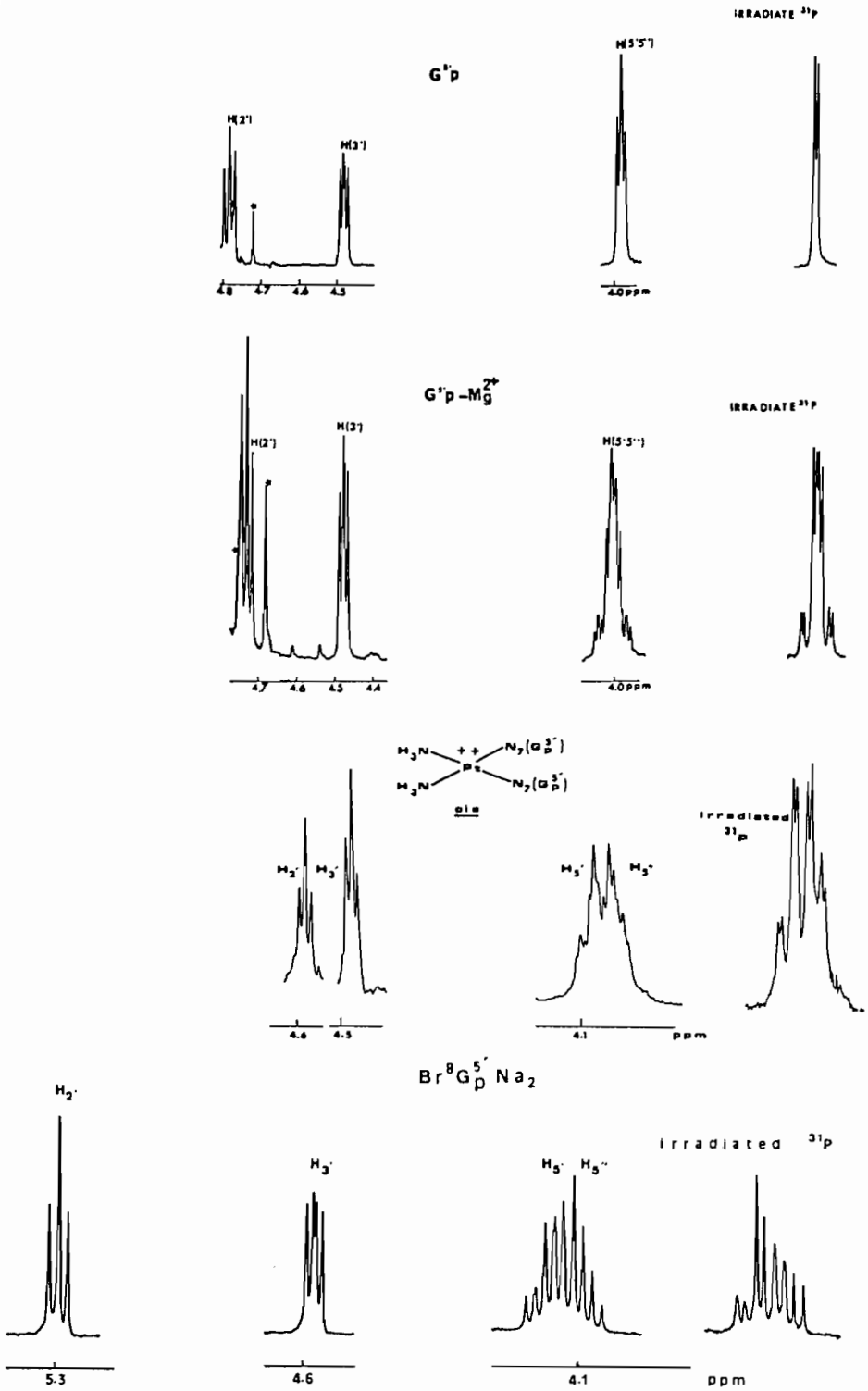

Fig. 5. 400 MHz proton NMR spectra (partial) for $Gp^{5'}$, $Mg^{7}Gp^{5'}$, *cis*-$[Pt^{7}(NH_3)_2(Gp^{5'})_2]^{2+}$ and $Br^{8}Gp^{5'}$ in D_2O solutions (20 mM).

bonds with the phosphate groups. As a result, the nucleobase turns totally to the *anti* conformation. The intramolecular $NH_3\cdots$phosphate hydrogen bond further stabilizes the *anti* orientation in the cisplatin complex. The percentage values for *syn* and *anti* populations are given in Table IV. The

TABLE IV. Estimates of the Percent (%) *syn* Conformation of Guo, $Gp^{5'}$ and $dGp^{5'}$ together with their 8-Substituted Derivatives and N7-Metal Adduct

Compound (X)	$\Delta\delta H_2$	*syn* (%)[a]
t-Bu^8Guo	0.83	100.00
$Br^8Gp^{5'}$	0.69	83.00
$AAF^8dGp^{5'}$	0.67	81.00
Br^8Guo	0.54	65.00
Gp^5	0.18	22.00
$PtCl_3{}^7Gp^{5'}$	0.13	16.00
$dGp^{5'}$	0.12	14.00
$Mg^7Gp^{5'}$	0.11	13.00
cis-$Pt^7(dGp^{5'})_2$	0.06	7.00
cis-$Pt^7(Gp^{5'})_2$	0.00	0.00

[a]The percentages (%) have been calculated from the empirical formula,

$$\%\,syn = \frac{\Delta\delta(\text{ppm})}{0.83} \times 100 \text{ (with an accuracy of } \pm 4.00)$$

where $\Delta\delta(\text{ppm}) = \delta H2'(X) - \delta H2'(\text{cisplatin})$ and $0.83 = \Delta H2'(t\text{-}Bu^8\text{Guo}) - \Delta H2'(\text{cisplatin})$.

differences in $\Delta\delta H_2'$ values are important when a bulky substituent replaces the H8, which may interact with the exocyclic alkylphosphate chain ($-CH_2OPO_3{}^{2-}$) and pushes the sugar into the *syn* orientation. However, N7-addition of a metal with a positive charge, (e.g., the metal species $Mg(H_2O)_5{}^{2+}$ or *cis*-$[Pt(NH_3)_2(Gp^{5'})_2]^{2+}$) favors the *anti* conformation.

Fourier Transform Infrared Spectra (FT-IR)

The FT-IR spectra of $Gp^{5'}$ and $Br^8Gp^{5'}$ in the region of the sugar ring vibrations are shown in Fig. 6. The spectra at liquid nitrogen temperatures show considerable changes in the relative intensities of the bands near 800 cm^{-1}. This region is characteristic of the sugar-phosphate vibrations diagnostic of sugar pucker, C2'-*endo,anti* and C3'-*endo,anti* conformations [18–20].

The infrared spectra were obtained as transparent films containing water of crystallization. Under these conditions and at about room temperatures (27 °C) we observed a large band for $Gp^{5'}$ and a more structured band for $Br^8Gp^{5'}$ which may be an overlap of several bands. The three distinct bands for $Br^8Gp^{5'}$ may be due to the three sugar conformations interacting with water, *i.e.*, 825 cm^{-1} (C2'-*endo,anti*), 812 cm^{-1} (C2'-*endo,syn*) and 803.3 cm^{-1} (C3'-*endo, anti*). The band at 779.2 cm^{-1} is attributed to a pyrimidine breathing mode and can be considered as a calibration band, since it only changes slightly upon metal complexation or C8-substitution. Upon freeze-drying the samples at liquid nitrogen temperatures (77 K), the predominant band for $Gp^{5'}$ is the 801.4 cm^{-1} (C3'-*endo,anti*), but for $Br^8Gp^{5'}$ the predominant band is the 818.8 cm^{-1} which may be assigned to (C3'-*endo,syn*). The low temperature behavior of the nucleobases is an indication of stabilizing the lowest energy conformation of the three isomers. In our case the most stable conformations found by freeze-drying the nucleotides at liquid nitrogen temperature seems to be the C3'-*endo,anti* and C3'-*endo, syn* for $Gp^{5'}$ and $Br^8Gp^{5'}$, respectively. This result is interesting because it is known that by drying the nucleic acids one obtains the A-form of DNA, where the sugar pucker is C3'-*endo,anti*. If however, one has a bulky substituent at the C8-position the *syn* conformation becomes more predominant with a C3'-*endo,syn* sugar pucker.

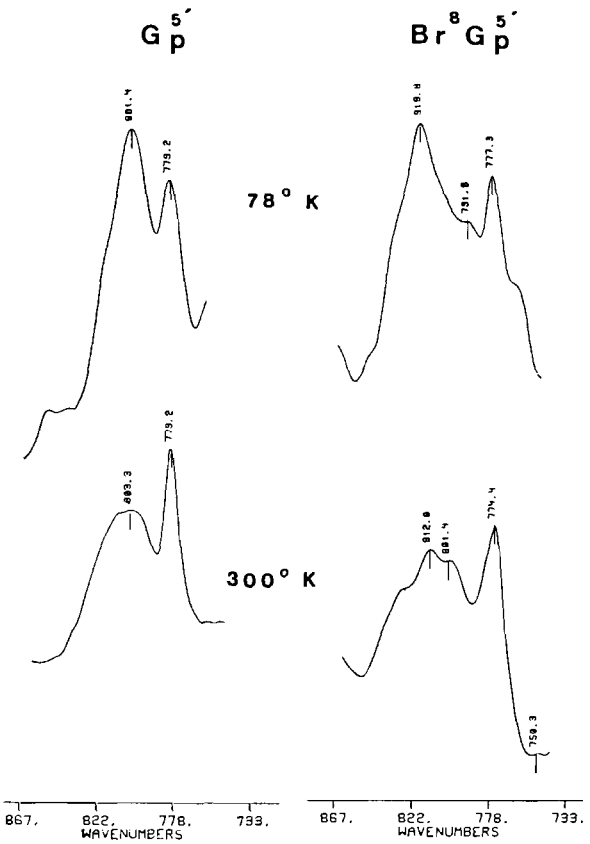

Fig. 6. Fourier Transform infrared spectra of $Gp^{5'}$ and $Br^8Gp^{5'}$ in films obtained from water solutions (0.02 M) at 27 °C and in solid state from freeze-dried water solutions (0.02 M) at 78 K.

Conclusions

In conclusion, it is shown here by ^1H, ^{13}C NMR and FT-IR spectroscopic techniques that important conformational changes do take place in nucleobases upon C8-substitution with bulky substituents and upon N7-metalation. A carcinogen such as 2-acetyl-

aminofluorene (AAF), when substituted at the C8 position of guanine, has a tendency to change the C2′-*endo,anti,gg* (B-DNA) conformation into a C3′-*endo,syn,gt*, which may be the precursor of a Z-DNA conformation. On the other hand, an anticancer drug like cisplatin changes predominantly the C2′-*endo, anti,gg* conformation (B-DNA) into a C3′-*endo,anti,gg* conformation (A-DNA). Similar conformational changes were also observed in intercalating drugs [21–24]. The DNA molecule may change its conformation in several ways because of drug or antibiotic binding by modification of its sugar pucker and/or base orientation. This remodeling of the DNA duplex could be due to a change in the *syn–anti* conformations which may activate genes and induce cancers or show antitumor activity [25].

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