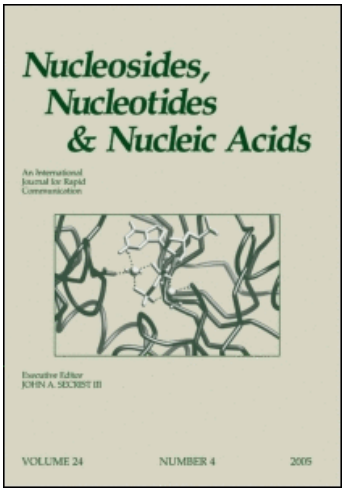

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COMPARATIVE CONFORMATIONAL ANALYSIS OF NUCLEOSIDES BY NMR, X-RAY, AND SEMI-EMPIRICAL (PM3 VS. AM1) METHODS

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▫ *The 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl ortho-aza-purine and -pyrimidine nucleosides manifest an unusually rigid sugar N conformation in solution.*

Keywords *Ortho*-Azanucleosides, Conformational Analysis

INTRODUCTION

The 8-aza-7-deaza analogues of the natural purine 2′-deoxy-β-D-ribonucleosides are of interest as constituents of oligonucleotides as they enhance the stability of DNA duplexes but maintaining the base pairing specificity.[1] In this respect, the most important finding is that analogues carrying a bromo or iodo substituent at the C7 position of the base remarkably strengthen the thermal stability of normal DNA duplexes as well as of DNA-RNA hybrids and of DNA with parallel chain orientation. In order to assess the contribution of the spatial arrangement of these analogues in the observed biophysical properties, we initiated studies of their stereochemistry in the solid state and in solution.[2–6]

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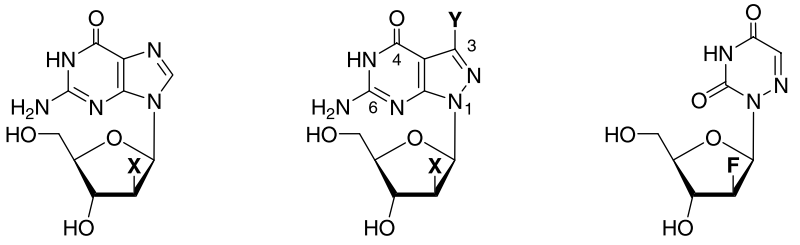
RESULTS AND DISCUSSION

Previously, we have quantitatively analyzed the H,H coupling constants of the 2′-deoxyribofuranose rings using PSEUROT (version 6.3) and found that by going from 2′-deoxyguanosine (dG) to its analogues 8-aza-7-deazaguanosine ($dG^{8N,7C}$) and the C-7 halogenated derivatives ($dG^{8N,7CBr(I)}$), the population of the *S* conformation (%: 71→64→61) and the (+)*g* orientation (%: 53→31→27) are successively diminished.[2] In the solid state, the $dG^{8N,7CBr}$ exists in the *N* type of the 2′-*deoxy*-ribose pucker (P_N = 36.6°; ψ_{max} = 33.8°).[2] Note that the crystal structure of 8-aza-7-deaza-2′-deoxyadenosine ($dA^{8N,7C}$) represents an example of the *ortho*-aza analogues with the *S* type sugar pucker (P_S = 182.2°; ψ_{max} = 41.2°, $-g$ = 178.7°, χ = −106.3°).[3] However, halogenation of $dA^{8N,7C}$ at C-7 gives rise to derivatives characterized by the *N* conformation in the solid state (e.g., $dA^{8N,7CBr}$: P_N = 310.9°; ψ_{max} = 35.0°, $-g$ = 175.2°, χ = −74.1°)[4,5] (Scheme 1).

Now, we have synthesized 8-aza-7-deaza-7-bromo-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine ($dG^{8N,7CBr;2'F}$) and found that its pentofuranose ring exists in an unusually rigid *N* conformation in solution (*N* = 100%; P_N = 345.1°; ψ_{max} = 35.7°) as well as in the solid state (P_N = 346.5°; ψ_{max} = 34.5°, *t* = 290.0°, χ = −108.0°).[6,7] This striking finding prompted us to investigate the stereoelectronic factors, the interplay of which led to such rigidity. Now, we paid our attention to 1) the interaction of the electronegative atoms fluorine, nitrogen-8, and oxygens O-4′ and O-5′ occupying the rather narrow β-area, 2) the possible consequences of the presence of the fluorine and bromine atoms in different parts of the molecule, and 3) to a comparison the NMR and X-ray data with semiempirical calculations using PM3 and AM1 methods.

The calculations (MOPAC 6 program) employing the AM1 method led to the contradictory results with well known data of crystallographic studies, e.g., the bond length of C4′–O4′ was calculated to be slightly shorter than that of C1′–O4′, which is in complete disagreement with the crystallographic data for most of the nucleosides (cf. Ref. [2]). On the contrary, the PM3 method furnished satisfactory



1, X = H (dGuo)
2, X = F ($dG^{2'F}$)

3, X = Y = H ($dG^{8N,7C}$)
4, X = H; Y = Br(I) ($dG^{8N,7CBr(I)}$)
5, X = F; Y = Br ($dG^{8N,7CBr;2'F}$)

6

SCHEME 1 Structure of nucleosides **1**–**6**.

coincidence with the NMR data as well as the bond length C4′–O4′>O4′–C1′ relationship in good agreement with the X-ray data. Thus, a rather good correspondence was found between the NMR and PM3 data for $dG^{8N,7CBr(I)}$. The rigidity of the furanose ring of $dG^{8N,7CBr;2'F}$ is manifested by an excellent coincidence of the relevant NMR and X-ray data. Moreover, the results of PM3 calculations clearly point to the predominant population of the *N* conformation favored by $\Delta\Delta H_f = -1.05$ kcal/mol, which is characterized by stereochemically closely related parameters, P_N and ψ_{max}.

A perusal of the X-ray data revealed that the bond lengths (Å) N9–C1′, C1′–O4′, and O4′–C4′ of $dG^{8N,7CBr}$ (for $dG^{8N,7CI}$ in parentheses) are 1.443 (1.441), 1.432 (1.428), and 1.442 (1.436)[2] whereas the same bond lengths in the solid state of fluoride $dG^{8N,7CBr;2'F}$ are 1.445, 1.415, and 1.448.[6,7] Thus, C2′-*ara*-fluorination resulted in the slight lengthening of the glycosylic bond (0.002 Å), a remarkable shortening of the C1′–O4′ bond (0.017 Å), and a slight lengthening of the O4′–C4′ bond (0.006 Å).

It was unequivocally shown that the pentofuranose ring conformation is strongly influenced by the electronic structure of the heterocyclic base and by electronegative sugar substituent(s) (for a comprehensive review, see Ref. [8]). A detailed conformational analysis of 3′-deoxy-3′-fluoro adenosine and its *xylo* counterpart[9] as well as closely related nucleosides[10,11] by the PSEUROT program showed that the sugar conformation is mainly steered by the R^1,R^2,R^3C-F configuration. On the contrary, in the case of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)purines an influence of a fluorine atom on the conformational behavior of the furanose ring was found to be rather moderate as can be seen from the NMR data for dG and its 2′-*ara*-fluoro counterpart, $dG^{2'F}$.[12] This conformational mobility of 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides obviously imply that the moderate steric and/or electronic alterations may bias the $N \leftrightarrow S$ equilibrium to one of two conformational regions.

It was demonstrated that an increase of the *Hammett* constant σ_m of the C-7 substituent of 7-deaza-2′-deoxyadenosines leads to an enhancement of the *N* conformer population.[13] Thorough analysis of these data led to the conclusion that the O4′–C1′–N9 anomeric effect is responsible for the observed increase of the *N*-type conformation.[8] The changes in the lengths of the N9–C1′, C1′–O4′, and O4′–C4′ bonds are completely compatible with the strengthening of the anomeric effect (the $n_{O4'} \rightarrow \sigma^*_{C1'-N9}$ interactions) in the molecule of $dG^{8N,7CBr;2'F}$ vs. the parent $dG^{8N,7CBr}$ resulting in the dominating population of the *N* conformation in solution and in the solid state.

The nucleoside $dG^{8N,7CBr}$ in the solid state occupies the *high-anti* ($\chi = -92.9°$) conformation about the glycosylic bond characterized by attractive *van der Waals's* interactions resulting from the short intramolecular contacts between the N-8 and the C-2′ and H-2′ of the sugar. Replacement of the 2′-*ara*-proton by an electronegative fluorine atom creates a repulsive *Coulomb* interactions between the N-8 and the F-2′ constraining the $dG^{8N,7CBr;2'F}$ molecule to adopt the C2′-*exo* conformation compared

to the C3′-*endo*/C4′-*exo* arrangement of the parent $dG^{8N,7CBr}$, on the one hand, and to rotate the base from the *high-anti* ($\chi = -92.9°$) to the *anti* ($\chi = -108.0°$) conformation. Note that the PM3 calculations gave very similar atomic distances for N8–O4′ (3.029 Å) and N8-2′F (3.011 Å).

The structure of $dU^{6N;2'F}$ in the solid state was found to be somewhat different from that of $dG^{8N,7CBr;2'F}$, thereby the most essential peculiarities lies in the conformation about the glycosylic bond and the exocyclic C4′–C5′ bond as well. The NMR data for $dU^{6N;2'F}$ clearly point to a dominating population of the *N* conformation of the pentofuranose ring and the PSEUROT analysis of the H,H and H,F coupling constants indicated two populated *N* regions. Of interest is the fact that the most populated *N* region ($P_N = 14.8°$; 77%) is in close vicinity to that found in the solid state ($P_N = 359.2°$). Furthermore, the results of semi-empirical calculations (PM3) are in good agreement with the X-ray data. As might be expected, the N-6 nitrogen, like N-8 of $dG^{8N,7CBr;2'F}$, occupies an intermediary position between the O-4′ (2.906 Å) and 2-′F (2.762 Å) atoms, thereby both distances are shorter vs. the aforementioned *ortho*-azapurine nucleoside. The latter differences reflect the various geometry of the aglycones and different calculated charges on N-6 of $dU^{6N;2'F}$ and on N-2 of $dG^{8N,7CBr;2'F}$ as well. The agreement in the results between three independent methods points to rather rigid spatial arrangement of $dU^{6N;2'F}$ that issues from an interplay of stereoelectronic factors similar to those discussed above for $dG^{8N,7CBr;2'F}$.

In conclusion, the stereochemistry of the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides results from the interplay of steric and stereoelectronic effects of the carbohydrate fragment and the heterocyclic base. The most striking finding is that some of the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl *ortho*-azanucleosides manifest an unusually rigid sugar *N* conformation in solution. Within the *ortho*-azanucleosides, there is a possibility to tune the spatial arrangement of the molecules by introducing different substituents either at C-7 position, or at C-2′ in the *ara* configuration, or combining diverse variants of both modifications.

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