Stereoelectronic effects of modified purine bases on the sugar conformation of nucleosides: pyrrolo[2,3-\(\alpha\)]pyrimidines

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Steric and stereoelectronic effects of 7- and 8-substituents of 7-deaza-2′-deoxy-adenosine and -guanosine on the dynamic sugar puckering as well as on the conformation about the C(4′)–C(5′) bond have been studied. (i) The higher the electron-attracting effect of the substituents, the more the N=S (North=South) equilibrium of the pentofuranose moiety is biased towards the N-conformation. (ii) The higher the electron-withdrawing effect of the 7-substituents, the higher the \( \gamma^{+} \) (+sc) rotamer population around the C(4′)–C(5′) bond. The interdependencies of the conformational equilibria of 7-deazapurine nucleosides and their influence on oligonucleotide secondary structures are discussed.

Introduction

The base moiety of a nucleoside residue is directly involved in carrying genetic information and its propagation in the replication process of nucleic acids by Watson–Crick or Hoogsteen hydrogen bonded base pairing. Comparatively little is known about how the glycone and the constituent pentofuranose moiety form the steric and stereoelectronic partnership that gives the nucleoside its unique role in storing genetic information, however.

Two dynamic two-state and one three-state equilibrium decide the topology of a nucleoside in solution. These are (i) the \( \text{syn} \rightleftharpoons \text{anti} \) equilibrium of the base about the N-glycosyl bond, (ii) the puckering of the pentofuranosyl moiety (N=S, \( ^{3}\text{t}_{2} \rightleftharpoons ^{2}_{1} \text{t}^{2} \)) and (iii) the rotational equilibrium about the C(4′)–C(5′) bond (\( \gamma^{10} \rightleftharpoons \gamma^{5} \rightleftharpoons \gamma^{0} \)). These equilibria are interdependent, and the energy barrier between the preferred conformational states is usually low.  

![Diagram showing North (N) sugar (\( ^{3}\text{t}_{2} \)) and South (S) sugar (\( ^{1}_{2} \text{t}^{2} \)).](image)

The effect of nucleobases at C(1′) in driving the two-state N=S pseudorotational equilibrium in N-(\( \beta \)-d-ribofuranosyl) nucleosides consists of two counteracting contributions from (i) the anomeric effect [= stereoelectronic interactions between O(4′) and the nucleobase nitrogen at C(1′)], which places the glycone in the pseudooxial orientation and (ii) the inherent steric effect of the nucleobase, which opposes the anomeric effect by its tendency to take up the pseudoequatorial position. The latter is sterically possible only in the S-type conformations.  

The N=S equilibrium of the sugar moiety of N-(\( \beta \)-d-ribofuranosyl) nucleosides in solution is energetically controlled by various gauche effects: the gauche effect of the O(4′)–C(4′)–C(3′)–O(3′) and O(2′)–C(2′)–C(1′)–N fragments bias the pseudorotational equilibrium towards S, whereas it is driven to N by the gauche effect of O(4′)–C(1′)–C(2′)–O(2′). In the case of 2′-deoxy-\( \beta \)-3′-ribofuranosyl-N-nucleosides this latter effect is of course absent—one of the reasons for the generally preferred S-type sugar puckering of 2′-deoxy-\( \beta \)-3′-ribofuranosyl nucleosides.

In an extensive and detailed study Chattopadhyaya and co-workers were able to differentiate for the first time between the gauche and anomeric effects by comparison of the thermodynamics of the pseudorotational equilibrium of the pentofuranose moiety in various regular and modified N- and C-nucleosides. In this manuscript we investigate this phenomenon on 7-deazapurine nucleosides. In particular, the tuning of the sugar puckering and also indirectly the conformation about the C(4′)–C(5′) bond of corresponding 2′-deoxynucleosides is studied and the influence of various substituents on the steric and stereoelectronic (anomeric) effects is investigated. The interdependence of the different conformational equilibria as well as their importance for the secondary structure and stability of the corresponding oligodeoxynucleotides is discussed.

Results and discussion

The pseudorotation concept was introduced to describe the continuous interconversions of the pentofuranose puckering of nucleosides. The geometry of the sugar ring is conveniently described using the Altona–Sundaralingam parameters, namely the phase angle of pseudorotation (\( P \)) and the puckering amplitude (\( \phi_{\text{m}} \)). A survey of nearly 200 X-ray crystal structures of nucleosides and nucleotides found nucleosides in both the north (N) and south (S) conformations, the first of which is centred around \( P = 16.8° \) [C(1′)-endo], whereas the second is around \( P = 18° \) [C(1′)-exo]. For most \( \beta \)-d-ribonucleosides the ratio between N- and S-states is approximately 1:1, and for 2′-deoxy-\( \beta \)-\( \text{d} \)-ribonucleosides 1:3.

Rotation about the C(4′)–C(5′) bond plays a crucial role in positioning the 5′-phosphate group of a nucleotide relative to the sugar and the base. The exocyclic CH2OR group may exist in three staggered conformations designated \( +sc \) [\( \left[ \left( \pm \right) g \right] \), \( -sc \) (\( \tau \)) and \( ap \) [\( \left[ \left( - \right) g \right] \). These three ranges are not uniformly populated because their distribution is dependent on the sugar puckering and on the base.

The preferred puckering conformations of furanoside moieties in nucleosides and the relative proportions of N- and S-conformers are controlled by steric and stereoelectronic effects of substituents. Thus, a systematic survey of conformational pre-
interconversions could be estimated (Me

purine but not systematic pyrrolo-

linearly with the electronegativity of the 2'-substituents. The sugar conformation is, moreover, influenced by modification of the base. Structures 1–19 show 7-deaza-2'-deoxynucleosides (purine but not systematic pyrrolo[2,3-d]pyrimidine numbering is used throughout this manuscript) of which the sugar puckering as well as the conformation about the C(4')-C(5') bond has been evaluated.

Sugar puckering has been studied on the basis of five vicinal \(^1H-^1H\) coupling constants (Table 1) using the PSEUROT 6.2 program.28,29 The populations of the staggered conformations about the C(4')-C(5') bond were calculated according to Westhof et al.,30 using the vicinal \(^1H-^1H\) couplings between H(4') and H(5') as well as H(6'), respectively, and applied to eqns. (1)-(3).

\[
\begin{align*}
% J_{\text{Av}} &= \frac{1.46 - \frac{1}{2} J_{\text{Av}}}{8.9} \times 100 \\
% J' &= \frac{J_{\text{Av}}}{8.9} - 0.23 \times 100 \\
% J'' &= \frac{J_{\text{Av}}}{8.9} - 0.23 \times 100
\end{align*}
\]

All vicinal \(^1H-^1H\) couplings of the sugar protons as well as the evaluated populations of conformers are given in Table 1. From these data some general trends can be deduced. Enhancement of the bulkiness of a substituent in position 8 drives the N=\(\equiv\)S equilibrium of a 7-deaza-2'-deoxyguanosine (10, 12, 16) towards S-type sugar puckers. The conformation generally correlates with the \(\psi\) conformation about the \(\beta\)-glycosyl bond. The nature of this effect seems to be mainly steric because a linear correlation exists between the S-conformer population and the van der Waals radii of the 8-substituents (data not shown).

An interdependence exists between the sugar puckering of the 8-substituted 7-deaza-2'-deoxyguanosines and their conformation about the C(4')-C(5') bond: the higher the S-conformer population, the higher the \(J''\)\(\psi\) rotamer population. Regarding the temperature dependence of sugar conformation only the sterically demanding 7-deaza-8-methyl-2'-deoxyguanosine (16) exhibits a noticeable change between 296 and 343 K.

From van't Hoff plots (data not shown) the thermodynamics of the N=\(\equiv\)S interconversions could be estimated (Me\(^\text{3}C\)Gp, 16: \(\Delta H = -3.3 \pm 0.4\) KJ mol\(^{-1}\), \(\Delta S_{\text{Av}} = -1.7 \pm 0.8\) J K\(^{-1}\) mol\(^{-1}\)). The thermodynamic data of N=\(\equiv\)S interconversion of the 8-methylated compound 16 are similar to those of 2'-deoxyguanosine (\(\Delta H = -2.5 \pm 0.3\) kJ mol\(^{-1}\), \(\Delta S_{\text{Av}} = -0.8 \pm 0.8\) J K\(^{-1}\) mol\(^{-1}\)).

Table 1 \(^1J_{\text{Av}}\) Coupling constants of the sugar moieties and conformer populations of 2'-deoxynucleosides at 303 K*

<table>
<thead>
<tr>
<th>Compound</th>
<th>(^1J_{\text{Av}}/\text{Hz})</th>
<th>Conformation</th>
<th>(% N)</th>
<th>(% S)</th>
<th>(% J'\psi)</th>
<th>(% J''\psi)</th>
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* Solvent, D\(_2\)O; RMS, \(< 0.4\) Hz; \(|\Delta J_{\text{Av}}|\) \(< 0.5\) Hz.

Fig. 1 demonstrates the stereoelectronic influence of 7-substituents on the N=\(\equiv\)S equilibrium of a series of 7-deaza-2'-deoxyadenosines (1–9). The graph shows that the higher the electron-attracting effect of the 7-substituents (expressed by their Hammett constants \(\sigma\)), the more the N=\(\equiv\)S equilibrium of the sugar moieties is biased towards N conformation.

The 7-nitro compound 7 exhibits the highest N-conformer population. Indeed, an X-ray analysis of this nucleoside also shows N-type sugar puckering.24 X-Ray crystal structures of N-nucleosides generally show a shortening of the O(4')-C(1') bond relative to C(4')-O(4') by about 3 pm22 (dA 3.2 pm22) which has been considered to be a manifestation of the anomic effect. In the case of 7-deaza-2'-deoxyadenosine (2'-deoxytubercidin) this relative shortening amounts to only 0.9 pm.26 This value increases to 2.3 pm in the case of the nitro compound 6 indicating the enhanced stereoelectronic effect of

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In case of the 7,8-dichloro-substituted 7-deaza-2'-deoxyguanosine (17) the steric effect of the 8- and the stereoelectronic effect of the 7-substituent compensate each other, so that for this compound the distribution of N- and S-conformers is almost the same as for the unsubstituted 7-deaza-2'-deoxyguanosine (10). An analogous result is obtained if the $\gamma^{(1)}$g population of compound 17 is compared with that of 10; also the conformation at the C(4')-C(5') bond of both compounds is almost identical.

A plot of the $\gamma^{(1)}$g rotamer population of the 7-substituted 7-deaza-2'-deoxyadenosine 1-9 vs. the corresponding $\sigma_m$ constants (Fig. 2) shows a correlation: the more electron-attracting the 7-substituent, the higher the $\gamma^{(1)}$g population. Combining the correlations of Figs. 1 and 2 demonstrates the interdependence of both conformational equilibria, the sugar puckering (N-conformer population) and the rotation about the C(4')-C(5') bond (Fig. 3): electron-withdrawing substituents drive the equilibria towards N as well as to $\gamma^{(1)}$g.

The electronic influence of the different 7-substituents of 7-deaza-2'-deoxyadenosine derivatives on the electron distribution is sensitively monitored by the $^1$H NMR chemical shifts of the corresponding H(8) signals, as the $\delta$ values parallel the Hammett $\sigma_p$ constants of the substituents (Fig. 4).

The deviation of the downfield shift (0.2-0.3 ppm) measured for the terminal alkynyl-substituted compounds 8 and 9 is striking. This may be due to a strong diamagnetic anisotropy of the C≡C triple bond leading to a significant deshielding of the ortho-located H(8) atoms. The fact that such an extraordinary deshielding cannot be observed for the 7-cyano- or 7-nitro-substituted derivatives 6 and 7 is noteworthy. These differences in the electronic effects of the 7-substituents between compounds 8 and 9 on the one hand and compounds 6 and 7 on the other hand are in line with the finding that only the 7-alkynyl-substituted derivatives exhibit strong fluorescence while 6 and 7 do not.

In a previous paper a graphical method for a quantitative determination of syn and anti conformer populations of regular and modified nucleosides based on one-dimensional $^1$H NOE measurements was reported. Applying this method to the 7-substituted 7-deaza-2'-deoxyadenosines 1-5 and 7, their conformation about the N-glycosyl bond was calculated, and the anti-conformer population was plotted vs. their S-conformer population (Fig. 5). From this graph another trend in conformational interdependence can be observed. Enhancement of S-type sugar puckering coincides with a decrease in anti conformers.
The data reported above and their interdependencies fall qualitatively into line with results which have been evaluated for other ribo- and 2'-deoxyribo-nucleosides, in particular for 5-substituted uridine derivatives. Due to the different electron affinities of the donor and acceptor groups in position 7 of the pyrrolo[2,3]pyrimidine bases, the electron distribution within the π system of the heterocycle is altered. Since electron-attracting groups strengthen the bonding interaction between a lone pair of O(4') and the π* orbital of the C(7)-C(8) double bond, they should favour small dihedral angles χ and an increased N-conformer population. Electron-donating groups are expected to exert an opposing influence.35

Substituent influence of 7-deazapurine bases on the oligonucleotide structures

The formation of an oligonucleotide single or double strand from nucleotide units follows a restriction of the conformational flexibilities discussed above. This increment of ΔG for the extension of an oligonucleotide chain counterbalances the formation of extra base stacks and two or three hydrogen bonds.66 The growth of the double helix is spontaneous (cooperative zipper mechanism29,32), due mainly to the demanding geometrical constraints of the sugar–phosphate backbone which are implied by the stereochemistry of the nucleotide unit. This in turn preferred conformation is preserved despite the thermal hypochromicity data (1–3) using S_H^Me and S_H^Me. All measurements were performed twice on a Bruker AMX-500 NMR spectrometer at 303 K unless otherwise stated with a delay time of 1 h to allow temperature stabilisation.

Acknowledgements

Financial support from the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF) is gratefully acknowledged.

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*Paper 7/02501G*

Received 11th April 1997
Accepted 20th June 1997