How Does the 2'-Hydroxy Group Drive the Pseudorotational Equilibrium in Nucleoside and Nucleotide by the Tuning of the 3'-Gauche Effect?

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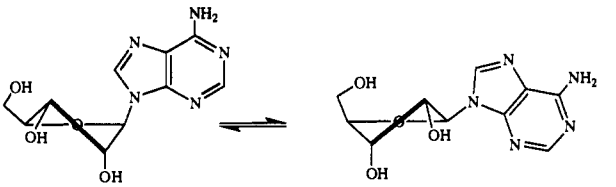
Abstract: The stereochemical environment of 2'-OH in ribonucleoside or ribonucleotide dictates the nature of its stereoelectronic interactions by modulating the strengths of the gauche effect of [O4'-C4'-C3'-O3'H] or [O4'-C4'-C3'-O3'PO_3H^-] fragments to drive the N ⇄ S pseudorotational equilibria toward S. A detailed analysis of $\Delta H°$ values of the N ⇄ S pseudorotational equilibria suggests that the 2'-OH promoted modulation of the [O4'-C4'-C3'-O3'PO_3H^-] gauche effect in 3'-AMP is 3.1 kJ mol^{-1}, whereas the modulation of the [O4'-C4'-C3'-O3'H] gauche effect in adenosine is 2.2 kJ mol^{-1}, which has been attributed to a more free 2'-OH in the former, opposing the driving force of the [O4'-C4'-C3'-O3'PO_3H^-] gauche effect with the full strength, whereas this opposing force of 2'-OH is much reduced in adenosine owing to the nature of intramolecular 3'-OH···O2' H-bonding.

The pseudorotation concept[1a] has been introduced to interpret the spontaneous transitions between indefinite nonplanar geometries of the cyclopentane ring. The phase angle of pseudorotation (P) and the puckering amplitude (Ψ_m) describe the extent of puckering of the pentofuranosyl moiety, which occurs in solution preferentially in a two-state dynamic equilibrium:[2,3] North (N, $0° < P_N < 36°$) ⇄ South (S, $144° < P_S < 190°$) (Scheme 1).[1b,2]

The conformations of nucleosides found in the X-ray crystal structures are considerably influenced by the crystal packing forces[1b] rather than by the intrinsic structural properties. There is no simple correlation between the preference of the N ⇄ S pseudorotational equilibrium in solution and the X-ray structure of a nucleoside. For example, adenosine is predominantly in the S-type (67% S at 298 K) and 2',3'-dideoxynucleosides are predominantly in the N-type conformations (>75% N at 298 K)[4a] in solution, whereas they crystallize in the N-type[1c] and S-type conformations,[1d,e] respectively. In solution various steric and stereoelectronic effects[4] of the sugar skeleton (gauche effect)[5] and the nucleobase (anomeric effect) energetically dictate the pseudorotational equilibrium between the two preferred conformational states of the pentofuranosyl moiety in 2',3'-dideoxynucleosides, 2'-deoxynucleosides, and ribonucleosides. The anomeric effect drives the N ⇄ S equilibrium toward N in order to place the heterocycle in the pseudoaxial orientation for maximal 1,4-interaction of glycosidic nitrogen with one of the O4' lone pairs. The gauche effect of 3'-OH, [O4'-C4'-C3'-O3'H], in 2'-deoxynucleosides has been clearly shown to drive the pseudorotational equilibrium toward S, which is opposed by the anomeric effect.[4] In 2'-deoxynucleosides, this gauche preference cannot result from the attractive H-bonding,[5d] but that cannot be ruled out in ribonucleosides. We have shown for the first time that the $\Delta H°_{GE}$ (gauche effect enthalpy) of the drive of the N ⇄ S pseudorotational equilibria in a series of 3'-substituted 2',3'-dideoxythymidines toward S increases steadily with the increase of the electronegativity of the 3'-substituent (X) (*i.e.*, an increase of the polarization of the C3'-X bond), and in fact they are linearly correlated.[4d] An inspection of a simple molecular model shows that the consequence of an S-type conformation is that the C3'-X bond becomes gauche oriented relative to the C4'-O4' bond (see also Scheme 1). Consistent with the work of Wiberg *et al.*,[5e] this means that the gauche effect in nucleosides is due to destabilizing

Scheme 1. Relative Orientations of Adenine Base and 2'- and 3'-Hydroxy Groups in Adenosine Influencing the Drive of Two-State (North ⇄ South) Pseudorotational Equilibrium (See Refs 2-4) through Anomeric and Gauche Effects



North sugar (C_3-*endo*-C_2-*exo*) South sugar (C_2-*endo*-C_3-*exo*)

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(3) The clear NMR observations of two distinctly identifiable, dynamically interconverting N and S conformations of constituent sugar moieties in B ⇄ Z DNA or A ⇄ Z RNA or A-form ⇄ B-form lariat RNA transformations (see refs 7-9 in our paper: ref 4e) have formed the basis of van't Hoff type analysis to give $\Delta H°$ and $\Delta S°$ of the two-state dynamic equilibrium. Note that no experimental evidence has ever been reported supporting the existence of a third pseudorotamer for the pentofuranose sugar moieties in nucleosides and nucleotides.

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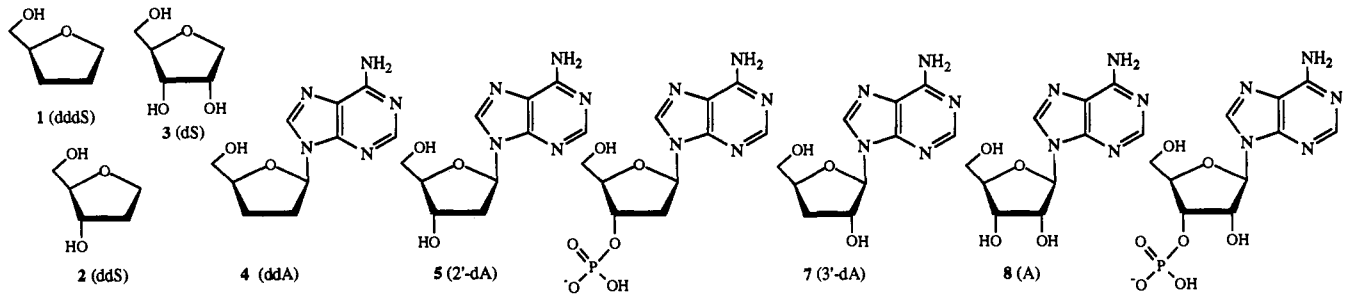
Table 1. $\Delta H°$ and $\Delta S°$ of the N ⇄ S Pseudorotational Equilibria of the Sugar Moieties in **1–9**



compound	$\Delta H°$ [a]	$\Delta S°$ [a]	$-T\Delta S°$ [b]	ΔG^{298} [b]	% S^{278} [c]	% S^{358} [c]	Δ(% S) (358–278 K)
dddS (**1**)	0.4 (0.3)	1.1 (1.0)	−0.3	0.1	49	50	1
ddS (**2**)	−4.1 (0.3)	−5.0 (0.9)	1.5	−2.6	76	68	−8
dS (**3**)	0.4 (1.1)	−5.1 (3.5)	1.5	1.9	31	32	1
ddA (**4**)	4.8 (0.2)	7.4 (2.1)	−2.2	2.6	23	33	10
2'-dA (**5**)	−4.2 (0.1)	−6.9 (0.7)	2.1	−2.1	73	64	−9
dAMP (**6**)	−5.4 (0.3)	−9.9 (0.7)	3.0	−2.4	76	65	−11
3'-dA (**7**)	2.2 (0.2)	0.6 (1.9)	−0.2	2.0	29	34	5
A (**8**)	−4.6 (0.4)	−9.5 (0.6)	2.8	−1.8	70	60	−10
AMP (**9**)	−4.9 (0.4)	−9.1 (0.7)	2.7	−2.2	74	63	−11

[a] $\Delta H°$ (kJ mol^{-1}) and $\Delta S°$ (J mol^{-1} K^{-1}) are average values (their standard deviations are in parentheses) derived from slopes and intercepts, respectively, of various van't Hoff plots and are based on various PSEUROT analyses of $^3J_{HH}$ measured from 278 and 358 K in 10 K steps at pH between 6.6 and 7.3.[4e,7] [b] $-T\Delta S°$ and ΔG^{298} (at 298 K) are in kJ mol^{-1}. [c] The South conformer populations at 278 and 358 K have been back-calculated from the corresponding free energy values as follows: $\%\ S^T = 100[\exp(-\Delta G^T/RT)]/[\exp(-\Delta G^T/RT) + 1]$.

interaction in the trans rotamer due to the formation of severely bent bonds and reduced bond overlap compared to the gauche rotamer. In ribonucleosides, however, the interplay of stereoelectronic effects is much more complex because of the gauche effects of 2'-OH, which can operate through [O4'–C1'–C2'–O2'H], [N–C1'–C2'–O2'H] and [O3'–C3'–C2'–O2'] fragments. The sugar conformation in ribooligonucleotides is greatly influenced by forces such as H-bonding, stacking interactions, and changes across sugar–phosphate backbone torsions,[4e] which mask and tune the intrinsic properties of the constituent ribomononucleotides. Thus the N-type sugars are frequently found in the double-stranded RNA duplex, whereas the S-type sugars have been found in the single-stranded region of the hairpin loop in solution.[10b–d] In contrast, the sugars of the lariat RNAs in solution are known to take up both N- and S-type conformations depending upon the size of the lariat loop.[10e] Therefore, it can be seen that RNA is indeed a flexible molecule and its sugar conformation can undergo fine tuning depending upon the interactions with the local environment. In the present paper we have delineated, for the first time, the various stereoelectronic contributions of 2'-OH in adenosine (A, **8**) in comparison with 2',3'-dideoxyadenosine (ddA, **4**),[4e] 2'-deoxyadenosine (2'-dA, **5**),[4e] 3'-deoxyadenosine (3'-dA, **7**), dAMP (**6**),[4e] AMP (**9**), and the apurinic sugars **1–3** (for all formulas, see Table 1). This set of compounds has been carefully chosen in order to understand the stereoelectronic uniqueness of 2'-OH in A (**8**) in thermodynamic terms compared to its 2'- and 3'-deoxy counterparts (*i.e.*, 2'-dA and 3'-dA), which can be obtained easily by simple subtractions of $\Delta H°$ (Table 1) for each of the above nucleosides.

The $\Delta H°$ values (Table 1) have been obtained by van't Hoff plots of $\ln(X_S/X_N)$ as a function of reciprocal of temperature.[4] The populations of pseudorotamers involved in N ⇄ S equilibria[3,7] in nucleosides and apurinic sugars have been calculated from the analysis of temperature dependent $^3J_{HH}$ (error ± <0.1 Hz) measured at 500 MHz (10 K intervals from 278 to 358 K) in D_2O at pH between 6.6 and 7.3. The following conclusions can be drawn from these studies:

(1) The substraction of $\Delta H°$ values for **1** and **2** gives the estimate of −4.5 kJ mol^{-1} for the strength of the pure gauche effect of [O4'–C4'–C3'–O3'H] fragment in the apurinic sugar **2**. The comparison of $\Delta H°$ in **1** and **3** shows that the respective gauche effects of 2'-OH and 3'-OH with O4' (*i.e.*, [O4'–C1'–C2'–O2'H] and [O4'–C4'–C3'–O3'H]) oppose each other with identical magnitude and are therefore mutually canceled. The subtraction of $\Delta H°$ values in **2** and **3** gives the estimate 4.5 kJ mol^{-1} for the gauche effect of the [O4'–C1'–C2'–O2'H] fragment.

(2) The gauche effect of the [O4'–C4'–C3'–O3'H] fragment drives the N ⇄ S pseudorotational equilibrium in 2'-dA (**5**) toward S, and its strength is −9.0 kJ mol^{-1}, which is obtained by the subtraction of $\Delta H°$ values for ddA (**4**) and 2'-dA (**5**).[4b] That

(7) $^3J_{HH}$ (error ±≤0.1 Hz) at ≈20 mM concentration in D_2O at 278 and 358 K are respectively for 3'-dA (**7**)[4f], $^3J_{1'2'}$ (2.3, 2.7 Hz), $^3J_{2'3'}$ (5.8, 6.2 Hz), $^3J_{2'3''}$ (3.2, 3.4 Hz), $^3J_{3'4'}$ (9.0, 8.7 Hz), $^3J_{3''4'}$ (6.5, 6.7 Hz), $^3J_{4'5'}$ (2.7, 3.2 Hz), $^3J_{4'5''}$ (4.3, 4.9 Hz), $^2J_{3'3''}$ (13.8, 13.8 Hz), and $^2J_{5'5''}$ (12.8, 12.5 Hz); and for AMP (**9**), $^3J_{1'2'}$ (6.6, 5.9 Hz), $^3J_{2'3'}$ (5.1, 5.3 Hz), $^3J_{3'4'}$ (2.8, 3.7 Hz), $^3J_{4'5'}$ (2.7, 3.2 Hz), $^3J_{4'5''}$ (3.1, 4.0 Hz), $^2J_{5'5''}$ (13.0, 12.8 Hz), and $^3J_{3'P}$ (8.0, 7.5 Hz). Almost negligible change in the chemical shift (<0.05 ppm) of all protons over the whole temperature range suggests the absence of aggregation. The interpretation of $^3J_{HH}$ in 3'-dA (**7**), A (**8**, for $^3J_{HH}$ data see ref 4b), and AMP (**9**) has been performed by PSEUROT program[6] with the use of the latest λ electronegativity set (program version 5.4, July 1992). Several PSEUROT analyses[6] were performed for 3'-dA (**7**) in which the geometries of minor S conformers were fixed in the range $140° < P_S < 170°$ in 10° steps ($\Psi_m^S = 32°$, 35°) and alternatively Ψ_m of both N- and S-type pseudorotamers were fixed in the range $30° < \Psi_m < 39°$ in 1° steps (root mean square < 0.3 Hz, ΔJ^{max} < 0.6 Hz). The major N-type pseudorotamers of 3'-dA (**7**) optimized freely in the above PSEUROT analyses were characterized by $-10° < P_N < 20°$ and $30° < \Psi_m < 33°$. For AMP (**9**) the geometries of minor N conformers were fixed in the range $-36° < P_N < 36°$ in 18° steps ($\Psi_m^N = 30°, 26°, 41°$) and alternatively Ψ_m of both N- and S-type pseudorotamers were fixed in the range $30° < \Psi_m < 41°$ in 1° steps, which resulted (root mean square < 0.2 Hz, ΔJ^{max} < 0.3 Hz) in the following geometries of the major S pseudorotamers: $135° < P_S < 165°$ and $30° < \Psi_m < 48°$. The populations of N and S conformers resulting from the above 19 and 27 PSEUROT analyses for 3'-dA (**7**) and AMP (**9**) respectively were used in the individual van't Hoff plots to calculate the average $\Delta H°$ and $\Delta S°$ of N ⇄ S equilibrium and their associated standard deviations (Table 1).

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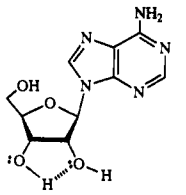
strength is reduced in A (**8**) to –6.8 kJ mol^{-1}, which is evident from the subtraction of $\Delta H°$ values for 3′-dA (**7**) and A (**8**). *Therefore, 2′-OH in A (**8**) reduces the strength of the [O4′–C4′–C3′–O3′H] gauche effect to drive the N ⇄ S equilibrium toward S by 2.2 kJ mol^{-1} compared to 2′-dA (**5**).*

(3) The effect of 2′-OH on the drive of N ⇄ S pseudorotational equilibria can be estimated by the comparison of $\Delta H°$ values in the following pairs of nucleosides: (i) the comparison of ddA (**4**) and 3′-dA (**7**) gives the 2′-OH strength of –2.6 kJ mol^{-1} in 3′-dA (**7**), (ii) the comparison of 2′-dA (**5**) and A (**8**) gives the 2′-OH strength of –0.4 kJ mol^{-1} in A (**8**), and (iii) the comparison of dAMP (**6**) and AMP (**9**) yields the 2′-OH strength of 0.5 kJ mol^{-1} in AMP (**9**). *Therefore the driving force of 2′-OH in 3′-dA (**7**), A (**8**), and AMP (**9**) is strongly dependent on the presence and the nature of the vicinal 3′-substituent.* Thus the influence of 2′-OH (*i.e.*, from S → N drive) in opposing the 3′-gauche effect (*i.e.*, from N → S drive) increases in the following order: 3′-dA < A < AMP. The 2′-OH promoted drive toward N takes place either by intensifying the strength of the gauche effect of the [O4′–C1′–C2′–O2′H] fragment or alternatively by weakening the [N9–C1′–C2′–O2′H] gauche effect.[4b]

(4) The gauche effect of the [O4′–C4′–C3′–O3′PO_3H^-] fragment drives the N ⇄ S pseudorotational equilibrium in dAMP (**6**) toward S, and its strength is –10.2 kJ mol^{-1}, which is obtained by the subtraction of $\Delta H°$ values for ddA (**4**) and dAMP (**6**). Similarly, the substraction of $\Delta H°$ values for 3′-dA (**7**) and AMP (**9**) yields –7.1 kJ mol^{-1}, which is the strength of the 2′-OH-modulated [O4′–C4′–C3′–O3′PO_3H^-] gauche effect contribution to the drive of the pseudorotational equilibrium in AMP (**9**). *This means that 2′-OH in AMP (**9**) reduces the strength of 3′-phosphate to drive the N ⇄ S equilibrium toward S by 3.1 kJ mol^{-1} compared to dAMP (**6**).*

(5) Why does 2′-OH weaken the strength of the [O4′–C4′–C3′–O3′PO_3H^-] gauche effect in AMP (**9**) (by 3.1 kJ mol^{-1}; see conclusion 4) more strongly than the [O4′–C4′–C3′–O3′H] gauche effect in A (**8**) (by 2.2 kJ mol^{-1}; see conclusion 2)? In both A (**8**) and AMP (**9**) 2′-OH is involved in the interactions with O4′ and N9 through the [O4′–C1′–C2′–O2′H] and [N9–C1′–C2′–O2′H] gauche effects and possibly in H-bonding with N3 which can strengthen the latter gauche effect[5d] in the drive of N ⇄ S equilibria. The reason for more effective modulation of the [O4′–C4′–C3′–O3′PO_3H^-] gauche effect by 2′-OH ($\Delta\Delta H°$ = 0.9 kJ mol^{-1}) in AMP (**9**) is that the lone pair of 2′-OH is more free to oppose the driving force of the [O4′–C4′–C3′–O3′PO_3H^-] gauche effect, whereas freedom of 2′-OH in A (**8**) (pK_a = 12.35)[8b] is much reduced because it is known[8a] to be involved in the intramolecular H-bonding with vicinal 3′-OH as shown in the structure below:



The lone pair of the 2′-oxygen is involved as acceptor in the H-bonding[8] with 3′-OH, and therefore the [O4′–C1′–C2′–O2′H] and [N9–C1′–C2′–O2′H] gauche effects are weaker, whereas the lone pairs of 3′-oxygen are still free and the gauche effect of [O4′–C4′–C3′–O3′H] can drive the sugar pseudorotational equilibria toward the S with full strength. These results are consistent with the ^{1}H-NMR observations[9] that there is an intramolecular water bridge between the vicinal 2′-OH and 3′-phosphate in cAMP in which 2′-hydroxyl proton accepts the lone pair of water oxygen, and this makes the 2′-oxygen lone pairs free to steer the gauche effect with O4′ and N9.

Clearly, the results described herein give the first experimental evidence showing how the strength and the nature of the involvement of 2′-OH in an H bond with the vicinal 3′-substituent can successfully modulate the 3′-gauche effect, which in turn thermodynamically drives the N ⇄ S sugar conformational equilibrium [*i.e.*, when 2′-OH is a lone-pair donor, the 3′-gauche effect acts strongly to stabilize the S conformer, whereas when the 2′-OH lone pair is free (acceptor), it stabilizes the N conformer itself]. This means that when the lone pair of a 2′-OH of a particular nucleotide in a folded 3-D structure of an RNA interacts strongly with a vicinal phosphate (for example, due to specific changes of the phosphate backbone torsions) or with any metal ion, it should actively stabilize the N ⇄ S sugar dynamic equilibrium of that particular phosphodiester function among many hundreds or thousands of phosphates in a pre-mRNA molecule to S by ~0.9 kJ mol^{-1}, which would then contribute toward the free energy of activation ("on–off" switch for the energy pump) for the site-specific transesterification reaction found in the splicing reaction in our cell[10] or promote RNA catalysis at a specific point in hammerhead,[10a] hairpin,[10b–d] or lariat RNA loop.[10e]

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