The Strength of the Anomeric Effect in Adenosine, Guanosine, and in Their 2′-Deoxy Counterparts is Medium-Dependent

Ingrid Luyten, Christophe Thibaudeau, and Jyoti Chattopadhyaya*

Department of Bioorganic Chemistry, Box 581, Biomedical Centre, University of Uppsala, S-751 23 Uppsala, Sweden

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In nucleosides, the anomeric effect (AE) (i.e. stereoelectronic n(O4′)→$\sigma^*_{C1'-N9}$ interactions) places the aglycon in the pseudoaxial orientation in the N-type conformation (2′-*exo*-3′-*endo*), whereas the inherent steric effect of the nucleobase opposes the AE by its tendency to take up pseudoequatorial orientation in the S-type conformation (2′-*endo*-3′-*exo*). This means that the actual energetic contribution of the AE of an *N*- or a *C*-aglycon in a nucleoside can be determined by subtracting the steric effect of the *N*- or *C*-aglycon from the total effect of the aglycon on the drive of N ⇄ S pseudorotational equilibrium. The $\Delta G°$ of N ⇄ S pseudorotational equilibrium among a set of various neutral *C*- and *N*-nucleosides showed that the relatively most thermodynamically stabilized S-type conformer is found in 9-deazaadenosine in which 9-deazaadenin-9-yl at C1′ takes up the relatively most favored pseudoequatorial orientation between pH 8.8−12.0 ($\Delta H° = -14.2$ kJ/mol) as a result of the exclusive steric control for the drive ($\Delta H°$) of N ⇄ S pseudorotational equilibrium. 9-Deazaadenin-9-yl at C1′ therefore serves as the best reference point for subtraction of the steric effect of the adenin-9-yl or guanin-9-yl in adenosine (A), guanosine (G), and in their 2′-deoxy counterparts (dA and dG). Since the electronic character of adenin-9-yl or guanin-9-yl changes from the neutral to the protonated (or deprotonated in case of guanin-9-yl) state as the pH of the medium changes (refs 1p, 1s), the work reported here shows for the first time that the intrinsic AE of A, G, dA, and dG are indeed pD-dependent. The tunable strength of the AE can vary from 23.4 to 17.7 kJ/mol in A from pD 1.2 to 7.0, 37.5 to 15.6 kJ/mol in G from pD 0.6 to 11.6, 18.0 to 14.8 kJ/mol in dA from pD 0.9 to 7.0, 20.7 to 13.8 kJ/mol in dG from pD 0.9 to 11.6.

Introduction

(I) The Stereoelectronic Forces Drive the Sugar Conformation in Nucleosides. The effect of the *N*-aglycon ($\Delta H°$) on the N ⇄ S pseudorotational equilibrium in *N*-nucleosides consists of two counteracting contributions from (i) the anomeric effect (stereoelectronic interactions between furanose O4′ and the nucleobase nitrogen at C1′), which places the aglycon in the pseudoaxial orientation in the N-type conformation, and (ii) the inherent steric effect of the nucleobase, which opposes the anomeric effect by its tendency to favor pseudoequatorial orientation in the S-type conformation.[1−3] The drive of the two-state N ⇄ S pseudorotational equilibrium of the sugar moiety of β-D-ribofuranosyl-*N*-nucleosides (Figure 1) in solution is energetically ($\Delta G°$) controlled[1] by various stereoelectronic gauche (GE) and anomeric effects (AE) represented by the enthalpy term ($\Delta H°$), as well as by the entropy of the whole system ($\Delta S°$).

Recently, we have quantified[1] the $\Delta H°$ contribution to $\Delta G°$ for the sum of the AE and steric effect of the *N*-aglycon in adenosine (A), guanosine (G), cytidine (C), uridine (U), ribothymidine (rT), and in their 2′-deoxy counterparts (dA, dG, dC, T, and dU) using a comparative set of $\Delta H°$ values for the N ⇄ S pseudorotational equilibrium of (*S*)-tetrahydrofurfuryl alcohol, 1-deoxy-D-ribofuranose, 1,2-dideoxy-D-ribofuranose, 2′,3′-dideoxy-β-D-ribofuranosyl-, 2′-deoxy-β-D-ribofuranosyl-, and β-D-ribofuranosylpurine and -pyrimidine nucleosides.

This study showed quantitatively that the strength of the effect of the *N*-aglycon is indeed nucleobase-dependent,[1a,c] namely, adenin-9-yl < guanin-9-yl < thymin-1-yl < uracil-1-yl < cytosin-1-yl.[1a,m,r]

(II) Our Original Estimate of the AE in Purine Nucleosides. We have subsequently quantified[1k] the AE of adenin-9-yl in A and guanin-9-yl in G based on the subtraction of the steric effect from the stereoelectronic anomeric effect using eq 1:

$$\text{AE of adenin-9-yl in A or guanin-9-yl in G} = \Delta H°_{(\text{A or G})} - [\Delta H°_{(\mathbf{9})} + \Delta H°_{\text{GE(O2′−N)}} + \Delta\Delta H°_{(\mathbf{1}\text{ and }\mathbf{2})}] \quad \text{(eq 1)}$$

where, $\Delta H°_{(\text{A or G})}$ represents the experimental $\Delta H°$ values of A (**5**) and G (**7**) in the neutral state. $\Delta H°_{(\mathbf{9})}$ corresponds to the experimental pD-independent $\Delta H°$ value of 1-deoxy-D-ribofuranose (**9**) (i.e. 0.4 kJ/mol[1a]) and it is used to calculate the sum of the GE of 2′-OH and 3′-OH with O4′

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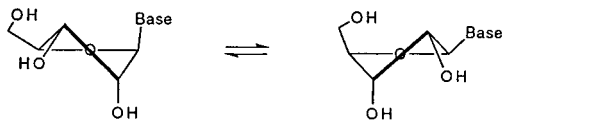


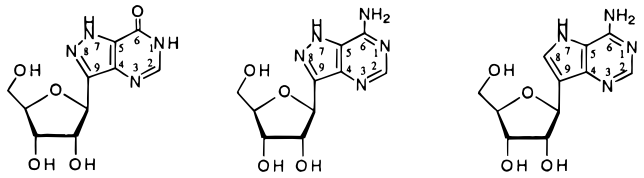
North sugar (C_3-*endo*-C_2-*exo*) South sugar (C_2-*endo*-C_3-*exo*)

Phase angle (P) = 0° ≤ P ≤ 36°; Puckering amplitude (Ψ_m) = 38.6° ± 3°

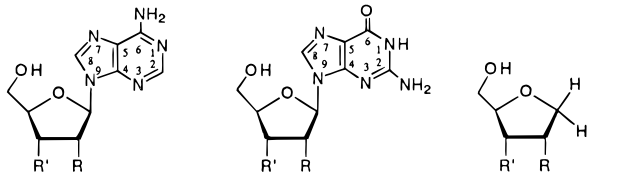
Phase angle (P) = 144° ≤ P ≤ 190°; Puckering amplitude (Ψ_m) = 38.6° ± 3°

Figure 1. Dynamic two-state equilibrium of N ⇄ S pseudorotamers of β-D-ribofuranosyl-nucleosides in solution.

as well as the effect of 4′-CH_2OH in A or G. $\Delta H°_{GE(O2'-N)}$ is the GE of [O2′−C2′−C1′−N9] fragment, which has been considered in our earlier work[1k] as pD-independent and determined from the regression analysis of the thermodynamics of the N ⇄ S equilibrium in nucleosides and abasic sugars (see ref 7a in ref 1k). $\Delta\Delta H°_{(1) \text{ and } (2)}$ represents the substituent effect of the *C*-aglycon in formycin A (**2**) and B (**1**), which is obtained from the subtraction of $\Delta H°$ of the reference compound **9** from the experimental $\Delta H°$ values in the neutral state for formycin A (**2**) and B (**1**).[1k]



1 **2** **3**



4: R = H; R' = OH
5: R = R' = OH
11: R = R' = H

6: R = H; R' = OH
7: R = R'= OH
12: R = R' = H

8: R = H; R' = OH
9: R = R'= OH
10: R = R' = H

We argued[1k] that the $\Delta H°$ of the N ⇄ S equilibrium in formycin A (**2**) and B (**1**) can be attributed mainly to the steric effect exerted by their *C*-aglycon because in them we found the most preference (average $\Delta H° = -7.4$ kJ/mol) for the S-type conformation. Since these *C*-aglycons are isosteric to adenin-9-yl in A and guanin-9-yl in G, they could be used as the reference point for the subtraction of the steric effect from the total effect that adenin-9-yl or guanin-9-yl exerts on the drive of their pentose sugar conformation.

(III) Why the Modification of the Original Estimation of AE is Required. After the completion of this work,[1k] it has emerged[1s] from our recent studies that, since the GE of (O4′−C4′−C3′−O3′) [GE(O4′−O3′)], (O3′−C3′−C2′−O2′) [GE(O3′−O2′)], (O4′−C1′−C2′−O2′) [GE(O4′−O2′)], and (O5′−C5′−C4′−O4′) [GE(O5′−O4′)] within the sugar component of all *C*-nucleosides are identical, it is therefore possible[1s] to correlate the pD-dependent energetics of the drive of the N ⇄ S equilibrium in various *C*-nucleosides (such as formycin A, formycin B, 9-deazaadenosine, Ψ-isocytidine, Ψ-uridine, 1-methyl-Ψ-uridine, and 1,3-dimethyl-Ψ-uridine) directly with the chemical nature of their constituent *C*-aglycon.

A careful review of our old work[1k] on the selection of the reference compound in eq 1, in the light of the information from our latest work,[1p,s] has revealed some serious problems: (i) Our failure[1k] to realize that the protonation or deprotonation of the nucleobase in *C*-nucleosides results in an increased or decreased shift of the N ⇄ S equilibrium toward N or S conformations, which was recently discovered in our laboratory.[1p,s] (ii) It is now clear[1p,s] that the $\Delta H°$ of the N ⇄ S equilibrium in any nucleoside is directly dependent upon whether the aglycon is in the neutral, protonated, or deprotonated state,[1p,s] which is directly related to the pK_a of the constituent aglycon. (iii) We found that the pD-dependent change (protonated ⇄ neutral ⇄ deprotonated states) of the aromatic character of the aglycon is responsible for the change in the strength of the AE. (iv) The $\Delta H°$ of the protonation ⇄ deprotonation equilibrium is transmitted to drive the N ⇄ S pseudorotational equilibrium either through stereoelectronic n(O4′)→$\sigma^*_{C1'-N}$ interactions between furanose O4′ and the nucleobase nitrogen at C1′ in *N*-nucleosides,[1p] or through the stereoelectronic n(O4′)→$\sigma^*_{C1'-C(sp2)}$ interactions between O4′ and the sp^2 hybridized *C*-aglycon at the C1′ of the pentose sugar in *C*-nucleosides.[1s] (v) It has also emerged[1p,s] that the protonation of the aglycon enhances the AE, whereas its deprotonation reduces its strength, compared to the neutral state. (vi) The relative strength of the AE ($\Delta H°$) over a pD range can be monitored by observing the $\Delta H°$ of the N ⇄ S equilibrium; an increase of the N-type sugar in the N ⇄ S equilibrium in the protonated state shows a relative increase of the pseudoaxial orientation of the constituent aglycon, owing to the enhanced strength of the AE, whereas an increase of the S-type sugar population in the deprotonated state shows an enhanced preference for the pseudoequatorial aglycon as a result of weakened AE. (vii) Since we are interested in the determination of $\Delta H°$ for the relatively most pseudoequatorially oriented purine *C*-aglycon in purine *C*-nucleosides **1**−**3**, it is clear[1p,s] that we should examine the $\Delta H°$ of the

(4) In our search for the most pseudoequatorially oriented aglycon, we have quantified the thermodynamics of a few other unnatural *C*-nucleosides, and they all show less pronounced pseudoequatorial *C*-aglycon than 9-deazaadenin-9-yl in 9-deaazaadenosine: 1-Deoxy-1-phenyl-β-D-ribofuranose ($\Delta H° = -5.4$ kJ/mol, $\Delta G° = -2.8$ kJ/mol), 1-Deoxy-1-naphthyl-β-D-ribofuranose ($\Delta H° = 0.4$ kJ/mol, $\Delta G° = -0.4$ kJ/mol), 1-Deoxy-1-(*p*-aminophenyl)-β-D-ribofuranose ($\Delta H° = -7.3$ kJ/mol, $\Delta G° = -3.4$ kJ/mol), 5-(β-D-ribofuranosyl)-2-bromopyridine ($\Delta H° = -4.4$ kJ/mol, $\Delta G° = -3.1$ kJ/mol), 3-(β-D-ribofuranosyl)-2-fluoropyridine ($\Delta H° = 0.4$ kJ/mol, $\Delta G° = -1.1$ kJ/mol), 5-(β-D-ribofuranosyl)pyridin-2-one ($\Delta H° = -5.6$ kJ/mol, $\Delta G° = -3.4$ kJ/mol),), 3-(β-D-ribofuranosyl)pyridin-2-one ($\Delta H° = -0.1$ kJ/mol, $\Delta G° = -0.3$ kJ/mol).

(5) DAISY, Spin Simulation Program, provided by Bruker, was used with 7 spins systems.

(6) For references to the PSEUROT v.5.4 program, see (a) Altona, C.; Francke, R.; de Haan, R.; Ippel, J. H.; Daalmans, G. J.; Westra, Hoekzema, A. J. A.; van Wijk, J. *Magn. Res. Chem.* **1994**, *32*, 670. (b) Donders, L. A.; de Leeuw, F. A. A. M.; Altona, C. *Magn. Reson. Chem.* **1989**, *27*, 556. (c) Altona, C.; Ippel, J. H.; Erkelens, C.; Groesbeek, G.; Donders, L. A. *Magn. Res. Chem.* **1989**, *27*, 564.

(7) Our modification of the PSEUROT v.5.4 program (ref 6) is intended to make it possible to evaluate and assess the propagation of errors from the experimental J_{HH} throughout the PSEUROT calculations as well as throughout subsequent treatment of the obtained data. Our modified program has retained all features of the original PSEUROT program; all changes are additions. The estimated error, expressed as standard deviation (σ), for each J_{HH} is entered in the program as well as the desired number of sets of randomly varied J_{HH}s to be generated and subsequently analyzed by pseudorotational analyses. Typically, 1000 data sets are generated and individually analyzed. Each set of "experimental data" will contain randomly varied J_{HH}s, but over all the data sets, each J_{HH} is normally distributed around its experimental value with the given σ. The output from our modified program consists of statistical data (average, σ, and skew of the calculated geometrical parameters and mole fractions as well as of the generated J_{HH}s) and results from all the individual pseudorotational analyses (the calculated geometrical parameters and mole fractions). It is also possible to discard results which fall outside given ranges ($J_{calc} - J_{exp}$, rms in J_{HH}, P_N, P_S, Ψ_m(N), and Ψ_m(S)).

Table 1. The pD-Dependent Estimation of $\Delta H^{\circ a}$ and $\Delta\Delta H^{\circ b}$ Values of Some Purine *C*-nucleosides and Their Comparison with Those of Adenosine, Guanosine, and Their 2′-Deoxy Counterparts

	formycin B		formycin A		9-deazaadenosine		adenosine (A)		2′-deoxyadenosine (dA)		guanosine (G)		2′-deoxyguanosine (dG)	
pD	ΔH°	$\Delta\Delta H^\circ$	ΔH°	$\Delta\Delta H^\circ$	ΔH°*	$\Delta\Delta H^\circ$	ΔH°	$\Delta\Delta H^\circ$	ΔH°	$\Delta\Delta H^\circ$	ΔH°	$\Delta\Delta H^\circ$	ΔH°	$\Delta\Delta H^\circ$
0.6	−1.3	−1.7	−2.4	−2.8	−7.4	−7.8	−0.2	−0.6	−0.7	−1.1	5.3	4.9	2.0	1.6
0.9	−2.0	−2.4	−2.4	−2.8	−7.4	−7.8	−0.2	−0.6	−0.7	−1.1	5.2	4.8	2.0	1.6
1.2	−3.0	−4.3	−2.4	−2.8	−7.4	−7.8	−0.2	−0.6	−0.8	−1.2	5.0	4.6	1.9	1.5
1.4	−3.9	−6.3	−2.4	−2.8	−7.4	−7.8	−0.2	−0.6	−0.8	−1.2	4.8	4.4	1.8	1.4
1.9	−5.9	−7.2	−2.4	−2.8	−7.4	−7.8	−0.3	−0.7	−0.8	−1.2	3.8	3.4	1.4	1.0
2.2	−6.8	−7.7	−2.4	−2.8	−7.4	−7.8	−0.3	−0.7	−0.8	−1.2	2.7	2.3	0.9	0.5
2.4	−7.3	−7.8	−2.4	−2.8	−7.4	−7.8	−0.4	−0.8	−0.9	−1.3	1.8	1.4	0.5	0.1
2.5	−7.4	−8.1	−2.5	−2.9	−7.4	−7.8	−0.5	−0.9	−0.9	−1.3	1.3	0.9	0.2	−0.2
2.8	−7.7	−8.3	−2.5	−2.9	−7.4	−7.8	−0.8	−1.2	−1.0	−1.4	−0.2	−0.6	−0.6	−1.0
3.1	−7.9	−8.4	−2.6	−3.0	−7.4	−7.8	−1.2	−1.6	−1.3	−1.7	−1.4	−2.0	−1.4	−1.8
3.4	−8.0	−8.5	−2.8	−3.2	−7.4	−7.8	−1.8	−2.2	−1.6	−2.0	−2.2	−2.6	−2.0	−2.4
4.3	−8.1	−8.5	−4.6	−5.0	−7.5	−7.9	−3.7	−4.1	−3.1	−3.5	−3.1	−3.5	−2.7	−3.1
5.3	−8.1	−8.5	−7.3	−7.7	−7.9	−8.3	−4.3	−4.7	−3.8	−4.2	−3.3	−3.7	−2.8	−3.2
7.0	−8.1	−8.5	−8.1	−8.5	−12.8	−13.2	−4.4	−4.8	−3.9	−4.3	−3.3	−3.7	−2.8	−3.2
8.8	−8.5	−8.9	−8.1	−8.5	−14.2	−14.6	−4.4	−4.8	−3.9	−4.3	−3.9	−4.3	−3.2	−3.6
9.4	−8.7	−9.1	−8.1	−8.5	−14.2	−14.6	−4.4	−4.8	−3.9	−4.3	−5.0	−5.4	−3.8	−4.2
9.8	−8.7	−9.1	−8.1	−8.5	−14.2	−14.6	−4.4	−4.8	−3.9	−4.3	−5.9	−6.3	−4.3	−4.7
10.4	−8.8	−9.2	−8.1	−8.5	−14.2	−14.6	−4.4	−4.8	−3.9	−4.3	−7.0	−7.4	−4.7	−5.1
11.0	−8.8	−9.2	−8.1	−8.5	−14.2	−14.6	−4.4	−4.8	−3.9	−4.3	−7.4	−7.8	−4.8	−5.2
11.6	−8.8	−9.2	−8.1	−8.5	−14.2	−14.6	−4.4	−4.8	−3.9	−4.3	−7.6	−8.0	−4.9	−5.3
12.0	−8.8	−9.2	−8.1	−8.5	−14.2	−14.6	−4.4	−4.8	−3.9	−4.3	−7.6	−8.0	−4.9	−5.3

[a] ΔH° values (kJ/mol) of the N ⇄ S pseudorotational equilibrium at various pDs. [b] $\Delta\Delta H^\circ$ values (kJ/mol) are the result of subtraction of the ΔH° values of individual N ⇄ S pseudorotational equilibrium of 1-deoxy-β-D-ribofuranose from the ΔH° values of individual N ⇄ S pseudorotational equilibrium of various purine *N*- and *C*-nucleosides at various pDs.

Table 2. The pD-Dependent Estimation of $-T\Delta S^{\circ a}$ and $\Delta G^{\circ a}$ Values of Some Purine *C*-Nucleosides and Their Comparison with Those of A, G, and Their 2′-Deoxy Counterparts

	formycin B		formycin A		9-deazaadenosine		adenosine (A)		2′-deoxyadenosine 2′-dA		guanosine (G)		2′-deoxyguanosine 2′-dG	
pD	$-T\Delta S^\circ$	ΔG°	$-T\Delta S^\circ$	ΔG°	$-T\Delta S^\circ$	ΔG°	$-T\Delta S^\circ$	ΔG°	$-T\Delta S^\circ$	ΔG°	$-T\Delta S^\circ$	ΔG°	$-T\Delta S^\circ$	ΔG°
0.6	−0.3	−1.5	0.4	−2.0	3.7	−3.6	−0.4	−0.5	−0.4	−1.1	−4.1	1.3	−2.2	−0.0
0.9	0.1	−1.8	0.4	−2.0	3.7	−3.6	−0.4	−0.5	−0.4	−1.1	−4.1	1.2	−2.2	−0.1
1.2	0.7	−2.1	0.4	−2.0	3.7	−3.6	−0.4	−0.5	−0.4	−1.1	−4.0	1.1	−2.1	−0.2
1.4	1.3	−2.3	0.4	−2.0	3.7	−3.6	−0.4	−0.5	−0.4	−1.1	−3.9	0.9	−2.1	−0.2
1.9	2.9	−2.8	0.4	−2.0	3.7	−3.6	−0.3	−0.6	−0.3	−1.1	−3.3	0.3	−1.9	−0.6
2.2	3.6	−3.0	0.4	−2.0	3.7	−3.6	−0.3	−0.6	−0.3	−1.1	−2.7	−0.1	−1.6	−0.9
2.4	4.0	−3.1	0.5	−2.0	3.7	−3.6	−0.2	−0.6	−0.3	−1.1	−2.1	−0.5	−1.3	−1.1
2.5	4.2	−3.2	0.5	−2.1	3.7	−3.6	−0.2	−0.6	−0.3	−1.2	−1.8	−0.6	−1.2	−1.2
2.8	4.5	−3.2	0.5	−2.1	3.7	−3.6	0.0	−0.7	−0.2	−1.2	−0.8	−1.0	−0.7	−1.4
3.1	4.6	−3.3	0.5	−2.1	3.7	−3.6	0.3	−0.8	−0.1	−1.4	0.2	−1.2	−0.1	−1.6
3.4	4.7	−3.3	0.7	−2.1	3.7	−3.6	0.8	−1.0	0.2	−1.5	0.9	−1.4	0.4	−1.7
4.3	4.8	−3.3	1.7	−2.6	3.7	−3.6	2.1	−1.6	1.2	−1.9	1.7	−1.5	1.0	−1.8
5.3	4.8	−3.3	3.9	−3.2	4.0	−4.0	2.5	−1.8	1.8	−2.0	1.8	−1.5	1.1	−1.8
7.0	4.8	−3.3	4.7	−3.3	7.9	−4.9	2.6	−1.8	1.9	−2.0	1.8	−1.5	1.1	−1.8
8.8	5.0	−3.4	4.7	−3.3	9.2	−5.0	2.6	−1.8	1.9	−2.0	2.1	−1.7	1.3	−1.9
9.4	5.1	−3.4	4.7	−3.3	9.2	−5.0	2.6	−1.8	1.9	−2.0	2.8	−2.1	1.7	−2.2
9.8	5.2	−3.5	4.7	−3.3	9.2	−5.0	2.6	−1.8	1.9	−2.0	3.5	−2.4	1.9	−2.4
10.4	5.3	−3.5	4.7	−3.3	9.2	−5.0	2.6	−1.8	1.9	−2.0	4.3	−2.7	2.1	−2.6
11.0	5.3	−3.5	4.7	−3.3	9.2	−5.0	2.6	−1.8	1.9	−2.0	4.7	−2.8	2.1	−2.7
11.6	5.3	−3.5	4.7	−3.3	9.2	−5.0	2.6	−1.8	1.9	−2.0	4.8	−2.8	2.1	−2.7
12.0	5.3	−3.5	4.7	−3.3	9.2	−5.0	2.6	−1.8	1.9	−2.0	4.8	−2.8	2.1	−2.7

[a] $-T\Delta S^\circ$and ΔG° values (kJ/mol) of the N ⇄ S pseudorotational equilibrium at various pDs are given at room temperature (298 K). For pD-dependent ΔH° values, see Table 1.

purine *C*-nucleosides **1**−**3** in the alkaline pD range, where we expect[1p,s] to find the relatively most pseudoequatorially oriented aglycon.

(IV) The New Revised Procedure for the Estimation of the AE. To calculate the pD-dependent AE, the following points should be taken into consideration: (i) The determination of the energetically most preferred pseudoequatorially oriented aglycon (this means that most of the contribution of the C1′ substituent to the N ⇄ S equilibrium comes from the steric effect of the substituent) among all known[4] *N*- and *C*-nucleosides as the new reference point, which will reflect the steric contribution to the total effect of adenin-9-yl or guanin-9-yl. (ii) The pD-dependent ΔH° of the N ⇄ S conformational drive of the sugar moiety in *N*- and *C*-nucleosides is included as well as the pD-dependent GE(O2′−N) in *N*-nucleosides in order to calculate the pD-dependent AE.

Results

(A) The Effect [Stereoelectronic and Steric] of the *N*-Aglycone is Medium-Dependent in Nucleosides. Figures 2A and 2B and Table 1 show the pD dependent ΔH° of A, G, and their 2′-deoxy counterparts as well as their comparison with all purine *C*-nucleosides, whereas Figures 2C-F and Table 2 document the pD-dependent $-T\Delta S^\circ$ (kJ/mol) and ΔG° (kJ/mol). The following observations can be clearly made from these figures and tables: (i) All three thermodynamic terms show sigmoidal pD-dependence, which can be directly correlated to the pD-dependent chemical shift change (see also refs 1p, 1s). (ii) The inflection points of these sigmoidal pD-dependent thermodynamic plots give the pK_a of the *N*- and *C*-nucleobase as does the pD-dependent ^{1}H chemical shifts (see refs 1p, 1s). (iii) Whereas the pD-dependent ΔH° can be attributed to the tunable strength of the stereo-

Table 3. the Pairwise Comparison[a−h] of $\Delta H°$ Values of the N ⇄ S Pseudorotational Equilibria of Compounds 4−12 in Their Protonated, Neutral, and Deprotonated State (see Figure 3)

energy	adenine		guanine		
(kJ/mol)	protonated	neutral	protonated	neutral	deprotonated
$\Delta\Delta H°_1$	8.8	3.1	22.9	3.0	1.0
$\Delta\Delta H°_2$	3.3	0.2	6.1	1.3	−0.8
$\Delta\Delta H°_{3''}$	−9.9	−7.4	−21.3	−6.2	−5.1
$\Delta\Delta H°_4$	−0.6	−4.8	4.9	−3.7	−8.0
$\Delta\Delta H°_{5''}$	0.5	−0.5	3.3	−0.5	−2.7

[a] $\Delta\Delta H°_1 = \Delta H°$ of (**11,12**) − $\Delta H°$ of **10** signifies the total effect of the *N*-aglycon (i.e. both stereoelectronic and steric). Noteworthy is the fact that this effect is pD-dependent. This effect has been assumed to be the same in 2′,3′-dideoxynucleosides, 2′-dNs, and riboNs. [See in Figure 4, panels A for A (**5**), C for G (**7**), E for 2′-dA (**4**), and G for 2′-dG (**6**)]. [b] $\Delta\Delta H°_2 = \Delta H°$ of (**4,6**) − $\Delta H°$ of **8** signifies the total effect of the *N*-aglycon influenced by the gauche effect (GE) of (O4′−C4′−C3′−O3′) (GEO4′−O3′),[1r] assuming that (GEO4′−O3′) is the same in 2′-dNs **4**, **6** and in abasic sugar **8**. [c] $\Delta\Delta H°_{3'} = \Delta H°$ of **8** − $\Delta H°$ of **10** = −4.5 kJ mol^{-1} is the strength of the GE(O4′−O3′) [1r] in abasic sugar **8**, which is constant. [d] $\Delta\Delta H°_{3''} = \Delta H°$ of (**4,6**) − $\Delta H°$ of (**11,12**) represents the GE(O4′−O3′) tuned by the effect of the specific *N*-aglycon in dNs (**4,6**), i.e. *N*-aglycon-dependent GE(O4′−O3′). $\Delta\Delta H°_{3''}$ is taken as the GE(O4′−O3′) by assuming that the pD-dependent effect of the *N*-aglycon of 2′-dN (**4,6**) is the same as that of 2′,3′-ddN (**11,12**). [See in Figure 4, panels B for A (**5**), D for G (**7**), F for 2′-dA 4, and H for 2′-dG (**6**)]. [e] $\Delta\Delta H°_4 = \Delta H°$ of (**5,7**) − $\Delta H°$ of **9** signifies the sum of both the total effect of the *N*-aglycon and the GE of [O2′−C2′−C1′−N] influenced by the GE [O4′−C1′−C2′−O2′] and [O4′−C4′−C3′−O3′] on the drive of the N ⇄ S equilibrium in riboNs (**5,7**).[1r] [f] $\Delta\Delta H°_{5'} = \Delta H°$ of **9** − $\Delta H°$ of **8** = 4.5 kJ mol^{-1} signifies the strength of the GE(O4′−O3′), (O4′−C1′−C2′−O2′) and (O3′−C3′−C2′−O2′) fragments.[1r] It has been assumed that the GE of (O3′−C3′−C2′−O2′) is negligible because of the identical thermodynamics of the abasic sugars **9** and **10**. [g] $\Delta\Delta H°_{5''} = \Delta H°$ of (**5,7**) − $\Delta H°$ of (**4,6**) signifies the mutual dependence of GEs of 2′- and 3′-OH on the drive of the two-state N ⇄ S equilibrium in riboNs (**5**,**7**). It is assumed here that the pD-dependent effect of the *N*-aglycon of the riboN (**5,7**) is the same as that of 2′,3′-ddN (**11,12**). [h] The GE of (O2′−C2′−C1′−N) [GE(O2′−N)] can be obtained by subtracting the GE of (O4′−C1′−C2′−O2′), [GE(O4′−O2′)] from $\Delta\Delta H_{5''}$. The GE of (O4′−C1′−C2′−O2′) is assumed to be identical in strength to the GE of (O4′−C4′−C3′−O3′), but of opposite sign, because the $\Delta H°$ of **9** and **10** is the same (i.e. 0.4 kJ/mol).

Table 4. The Estimation of the pD-Dependent Gauche Effect of (O2′−C2′−C1′−N) [GE(O2′-N)] from the Pairwise Comparison of $\Delta H°$ of the N/S Equilibrium for 4−12 (see footnote of Table 3) of Adenosine, Guanosine

pD of the medium	adenosine GE (O2′−N)	guanosine GE (O2′−N)
0.6	−9.4	−18.1
0.9	−9.4	−17.9
1.2	−9.4	−17.6
1.4	−9.3	−17.3
1.9	−9.3	−15.7
2.2	−9.2	−14.1
2.4	−9.1	−12.9
2.5	−9.0	−12.2
2.8	−8.8	−10.3
3.1	−8.5	−8.8
3.4	−8.2	−7.9
4.3	−7.8	−6.9
5.3	−7.9	−6.7
7.0	−7.9	−6.7
8.8	−7.9	−7.0
9.4	−7.9	−7.5
9.8	−7.9	−8.0
10.4	−7.9	−8.6
11.0	−7.9	−8.9
11.6	−7.9	−9.0
12.0	−7.9	−9.0

electronic forces, the modulation of $-T\Delta S°$ as a function of pD is presumably owing to the change of either solvation shell of the ionized nucleobase or/and change

Table 5. The pD-Dependent Anomeric Effect (AE)[a] of Adenin-9-yl and Guanin-9-yl in Adenosine and Guanosine, Respectively, and in Their 2′-Deoxy Counterparts

pD	AE of A (**6**) $\Delta H°$	AE of G (**7**) $\Delta H°$	AE of 2′-dA (**4**) $\Delta H°$	AE of 2′-dG (5) $\Delta H°$
0.6	23.4	37.5	18.0	20.7
0.9	23.4	37.3	18.0	20.7
1.2	23.4	36.8	17.9	20.6
1.4	23.3	36.3	17.9	20.5
1.9	23.2	33.7	17.9	20.1
2.2	23.1	31.1	17.9	19.6
2.4	22.9	28.9	17.8	19.2
2.5	22.8	27.7	17.8	18.9
2.8	22.3	24.3	17.7	18.1
3.1	21.5	21.7	17.4	17.3
3.4	20.5	19.9	17.1	16.7
4.3	18.3	17.9	15.6	16.0
5.3	17.8	17.6	14.9	15.9
7.0	17.7	17.6	14.8	15.9
8.8	17.7	17.3	14.8	15.5
9.4	17.7	16.7	14.8	14.9
9.8	17.7	16.3	14.8	14.4
10.4	17.7	15.8	14.8	14.0
11.0	17.7	15.7	14.8	13.9
11.6	17.7	15.6	14.8	13.8
12.0	17.7	15.6	14.8	13.8

[a] The $\Delta H°$ of the anomeric effect derived from eq 2 for 2′-dA and 2′-dG or eq 3 for A and G.

of the electrostatic forces or/and to possible increase of the effective size of the nucleobase.[8]

Table 1 also shows the $\Delta\Delta H°$ values (kJ/mol), which are the result of subtraction of the $\Delta H°$ of the N ⇄ S pseudorotational equilibrium of 1-deoxy-β-D-ribofuranose (**9**) from the $\Delta H°$ of the N ⇄ S pseudorotational equilibrium of the purine *C*-nucleosides **1**−**3** at various pDs. It is noteworthy that the $\Delta\Delta H°$ values for natural nucleosides represents the contribution of the steric and sterioelectronic effects of the nucleobase and of the GE of [O2′−C2′−C1′−N] fragment of *N*-aglycon, which is absent in *C*-nucleosides.

The perusal of $\Delta H°$ and $\Delta\Delta H°$ values shown in Table 1 leads to the following conclusions: (i) The $\Delta H°$ values of the N ⇄ S equilibrium of all purine *N*- and *C*-nucleosides are pD-dependent. (ii) The purine *C*-nucleosides have a more thermodynamically preferred S-type conformer compared to adenosine, guanosine, and their 2′-deoxy analogues. (iii) Finally, among the purine *C*-nucleosides, it is 9-deazaadenosine which has the most thermodynamically stable S-type conformer with a pseudoequatorial aglycon ($\Delta H°$ = −14.2 kJ/mol) in the pD range from 8.8 to 12.0. Hence, the $\Delta H°$ of 9-deazaadenosine in the pD range of 8.8−12.0 can be used as the optimal reference point in the calculation of the AE of A, G, and 2′-deoxy derivatives.

(B) The Quantitation of the AE of Adenin-9-yl in 2′-dA and Guanin-9-yl in 2′-dG. Having identified 9-deaazadenin-9-yl in 9-deaazadenosine (**3**) as the most pseudoequatorially oriented C1′ aglycon, with a minimal AE, among all known *C*- and *N*-nucleosides, its $\Delta H°$ can be used as the reference point for the subtraction of the inherent steric component from the total effect of the *N*-aglycon in purine *N*-nucleosides, so as to give the pD-dependent strength of the AE. Hence eq 1 can be

(8) (a) Fabian, M. A., Perrin, C. L, Sinnott, M. L. *J. Am. Chem. Soc.* **1994**, *116*, 8398. (b) Perrin, C. L.; Fabian, M. A.; Armstrong, K. B. *J. Org. Chem.* **1994**, *59*, 5246. (c) Perrin, C. L. *Pure Appl. Chem.* **1995**, *67*, 719. (d) Vaino, A. R.; Chan, S. C.; Szarek, W. A.; Thatcher, G. R. J *J. Org. Chem.* **1996**, *61*, 4514.

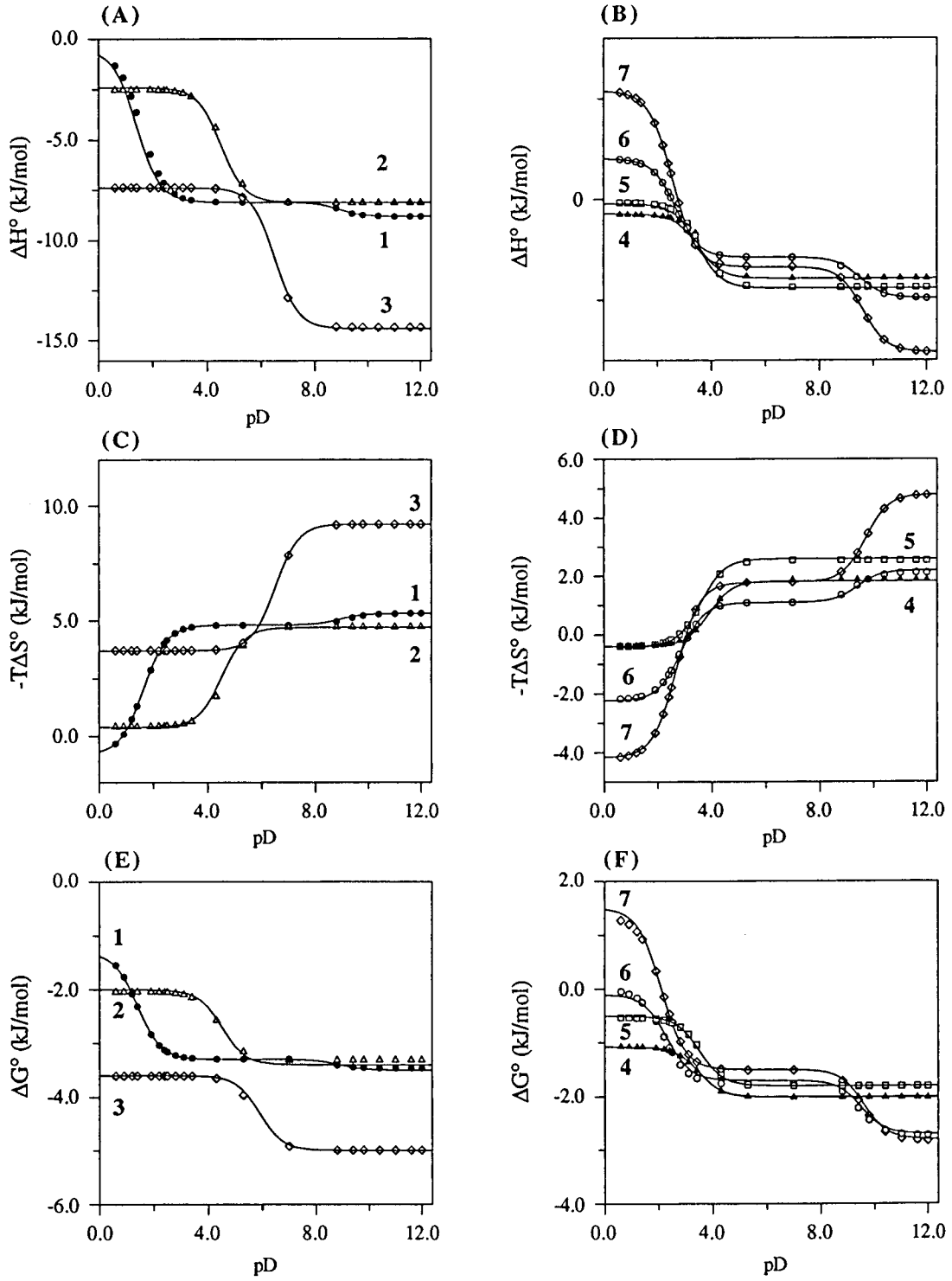


Figure 2. Panels A, C and E show the pD-tunable experimental $\Delta H°$, $-T\Delta S°$, and $\Delta G°$ values, respectively, for the N ⇄ S pseudorotational equilibrium determined from van't Hoff plots of formycin B (**1**), formycin A (**2**), and 9-deazaadenosine (**3**), whereas panels B, D and F show $\Delta H°$, $-T\Delta S°$ and $\Delta G°$ values for adenosine (**5**), 2′-deoxyadenosine (**4**), guanosine (**7**), and 2′-deoxyguanosine (**6**) (see also Table 1 for $\Delta H°$ values and Table 2 for $-T\Delta S°$ and $\Delta G°$ values at each pD). A comparison of the pD-tunability of all these *C*- and *N*-nucleosides shows that the S-type sugar conformation with the pseudoequatorial aglycon is most preferred in 9-deazaadenosine (**3**) and hence can be used as the reference point.

rewritten as eq 2:

$$\text{AE of adenin-9-yl in 2′-dA or guanin-9-yl in 2′-dG at a specific pD} = \Delta H°_{\text{pD(2′-dA or 2′-dG)}} - [\Delta H°_{(8)} + (\Delta H°_{\text{D(3)}} - \Delta H°_{(9)})] \quad \text{(eq 2)}$$

where, $\Delta H°_{\text{pD(2′−dA or 2′−dG)}}$ represents the experimental $\Delta H°$ of 2′-dA or 2′-dG at that particular pD (see Table 1). $\Delta H°_{(8)}$ is simply the $\Delta H°$ of 1,2-dideoxy-β-D-ribose (**8**) (i.e. −4.1 kJ/mol)[1r] and corresponds to the sum of two effects in 2′-dA or 2′-dG, i.e. the GE of 3′-OH with O4′ and the effect of 4′-CH_2OH. $\Delta H°_{\text{D(3)}}$ is the $\Delta H°$ value for the pseudorotational equilibrium in 9-deazaadenosine (**3**) in the pD range from 8.8 to 12.0 (i.e. −14.2 kJ/mol).[1s] $\Delta H°_{(9)}$ is simply $\Delta H°$ of 1-deoxy-β-D-ribofuranose (**9**) (i.e. 0.4 kJ/mol).[1a]

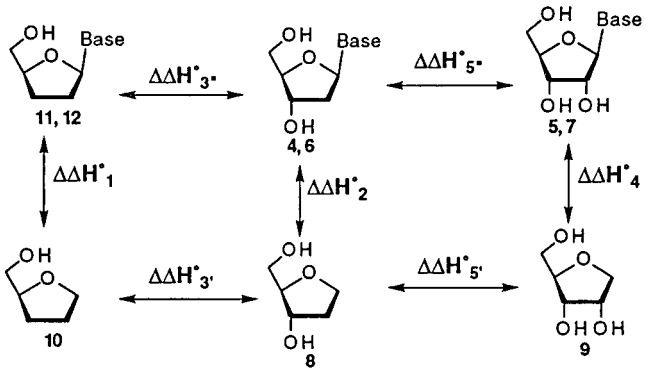


Figure 3. Pairwise comparison of the thermodynamics of the N ⇄ S pseudorotational equilibrium between nucleosides, and between a nucleoside and a sugar, gives a dissection of various combined stereoelectronic (gauche and AEs) and steric effects (see Table 3 for the results of the dissection).

Thus the use of the correct pD-dependent $\Delta H°$ values from Table 1 gives the pD-tunable AE, which varies for adenin-9-yl in 2′-dA from 18.0 to 14.8 kJ/mol from pD 0.9 to 7.0, and for guanin-9-yl in 2′-dG from 20.7 to 13.8 kJ/mol from pD 0.9 to 11.6 (see Table 5 and Figure 5).

(C) The Quantitation of the Strength of the AE of Adenin-9-yl in A and Guanin-9-yl in G. Since the electronic character of the pyrrole-nitrogen depends on the pD-dependent aromatic character of the *N*-aglycon (i.e. protonation ⇄ deprotonation equilibrium), we have added a correction term for the pD-dependent GE of (O2′−C2′−C1′−N) [GE(O2′−N)] in eq 2 to give eq 3. This allows an estimate of the pD-dependent AE of adenin-9-yl in adenosine and guanin-9-yl in guanosine. Hence, in *N*-nucleosides, the strength of the total effect of the *N*-aglycon (steric and stereoelectronic) will be quite different within the pair of ribo and 2′-deoxyribofuranose counterparts, depending upon the pD of the medium; the GE(O2′−N) and GE(O4′−O2′) are present in the former, but not in the latter.

$$\text{AE of adenin-9-yl inA or guanin-9-yl in G at a specific pD} = \Delta H°_{\text{pD(A or G)}} - [\Delta H°_{(9)} + \Delta H°_{\text{pD-GE(O2′-N)}} + (\Delta H°_{\text{D(3)}} - \Delta H°_{(9)})] \quad \text{(eq 3)}$$

where, $\Delta H°_{\text{pD(A or G)}}$ represents the experimental $\Delta H°$ of adenosine or guanosine at that particular pD (see Table 1). $\Delta H°_{(9)}$ is simply $\Delta H°$ of 1-deoxy-β-D-ribofuranose (**9**) (i.e. 0.4 kJ/mol).[1a] $\Delta H°_{\text{pD−GE(O2′−N)}}$ represents the pD-dependent strength of the GE of [O2′−C2′−C1′−N9] fragment (*see below*). $\Delta H°_{\text{D(3)}}$ is the $\Delta H°$ value for the pseudorotational equilibrium in 9-deazaadenosine (**3**) in the pD range from 8.8 to 12.0 (i.e. −14.2 kJ/mol).[1s]

The quantitation of the pD-Dependent GE(O2′−N) in Ribonucleosides. The sum of the GE(O4′−O3′), GE(O3′−O2′), and GE(O4′−O2′) fragments and the effect of the 4′-CH_2OH group is a constant factor in both β-D-ribofuranosyl-*N*- and -*C*-nucleosides. The strength of the GE(O2′−N) fragment in *N*-ribonucleosides is *N*-aglycon dependent[1m,r] (Figures 3 and 4, Tables 3 and 4); it is absent both in 2′-deoxy-*N*-nucleosides and *C*-nucleosides. Thus, the change of the pD-dependent ΔH° of the N ⇄ S equilibrium reflects the change of mainly two intramolecular stereoelectronic interactions, namely, the AE, (O4′−C1′−N), and the GE(O2′−N) in ribonucleosides (Figure 3, Tables 3 and 4).

The pairwise comparison of the thermodynamics of the N ⇄ S pseudorotational equilibrium between nucleosides, and between a nucleoside and a sugar (Figure 3 and Table 3), gives a dissection of various combined stereoelectronic (gauche and AEs) and steric effects (Table 3 and Figure 4). These are the intramolecular forces responsible for the drive of the sugar conformation. From Figure 3, one can directly extract $\Delta\Delta H_1$, the total effect of the N-aglycon, as well as $\Delta\Delta H_{3''}$, representing the GE(O4′−O3′). The pD-dependent changes of $\Delta\Delta H_1$ and $\Delta\Delta H_{3''}$ are shown in Figure 4. The $\Delta\Delta H_{5''}$ signifies the sum of the GE(O4′−O2′) and GE(O2′−N), assuming that the GE of (O3′−C3′−C2′−O2′) is negligible because of the identical thermodynamics of the abasic sugars **9** and **10**. Since the $\Delta H°$ of **9** and **10** are identical, it can be safely assumed that the strength of GE(O4′−O2′) is identical with the strength of GE(O4′−O3′) but of opposite sign. This means that the pD-dependent GE(O2′−N) can now be obtained by simply subtracting the GE(O4′−O2′) from $\Delta\Delta H_{5''}$ (Figure 3). The exact procedure for the extraction of the GE(O2′−N) has been further illustrated in the legend of Table 3, and the result of this dissection is presented in Table 4.

Using the estimates for the gauche effect of (O2′−C2′−C1′−N), presented in Table 4 and eq 3, we have found that the AE in ribonucleosides is tunable by the medium and varies for adenosine from 23.4 to 17.7 kJ/mol from pD 1.2 to 7.0, and for guanosine it changes from 37.5 to 15.6 kJ/mol from pD 0.6 to 11.6 (see Table 5 and Figure 5).

Conclusion

(1) We have identified a new reference point (i.e. 9-deazaadenosine) to quantify the medium-dependent AE for adenosine, guanosine, and their 2′-deoxy derivatives: the $\sigma^*_{\text{C1′−C9(sp2)}}$ orbital of 9 deazaadenin-9-yl has a negligible stereoelectronic interaction with the lone pairs of the constituent furanose-O4′ in the alkaline pD range. Hence, 9-deazaadenin-9-yl as the aglycon at C1′ serves as the best reference point for subtraction of the steric effect for the adenin-9-yl or guanin-9-yl substituent in A, G, and in their 2′-deoxy counterparts to yield the actual AE.

(2) The strengths of the AEs of A, G, and of their 2′-deoxy derivatives are tunable, depending upon the composition of the medium, and we expect the same for cytidine, uridine, ribothymidine, and 2′-deoxy analogues. One sees that the $\Delta H°$ of the N ⇄ S equilibrium of all *N*- and *C*-nucleosides is flexible, depending upon the protonation ⇄ deprotonation equilibrium at various pDs, suggesting that the electronic nature of the aglycon modulates the AE in a pD dependent manner. This change ($\Delta G°$) of the electronic nature of the base is then transmitted to drive the energetics of the N ⇄ S equilibrium.

(3) It is clear that any local N ⇄ S equilibrium of any particular nucleotide in a oligo- or polynucleotide chain will also change upon specific complexation with a metal ion, depending upon its hardness or softness in a manner similar to that found for protonation or deprotonation. We expect a similar change of local structure in a polynucleotide chain upon complexation with any ligand mimicking that of a Lewis acid or Lewis base.

(4) This paper presents the first experimental data on the magnitude of the medium-dependent tunability of the

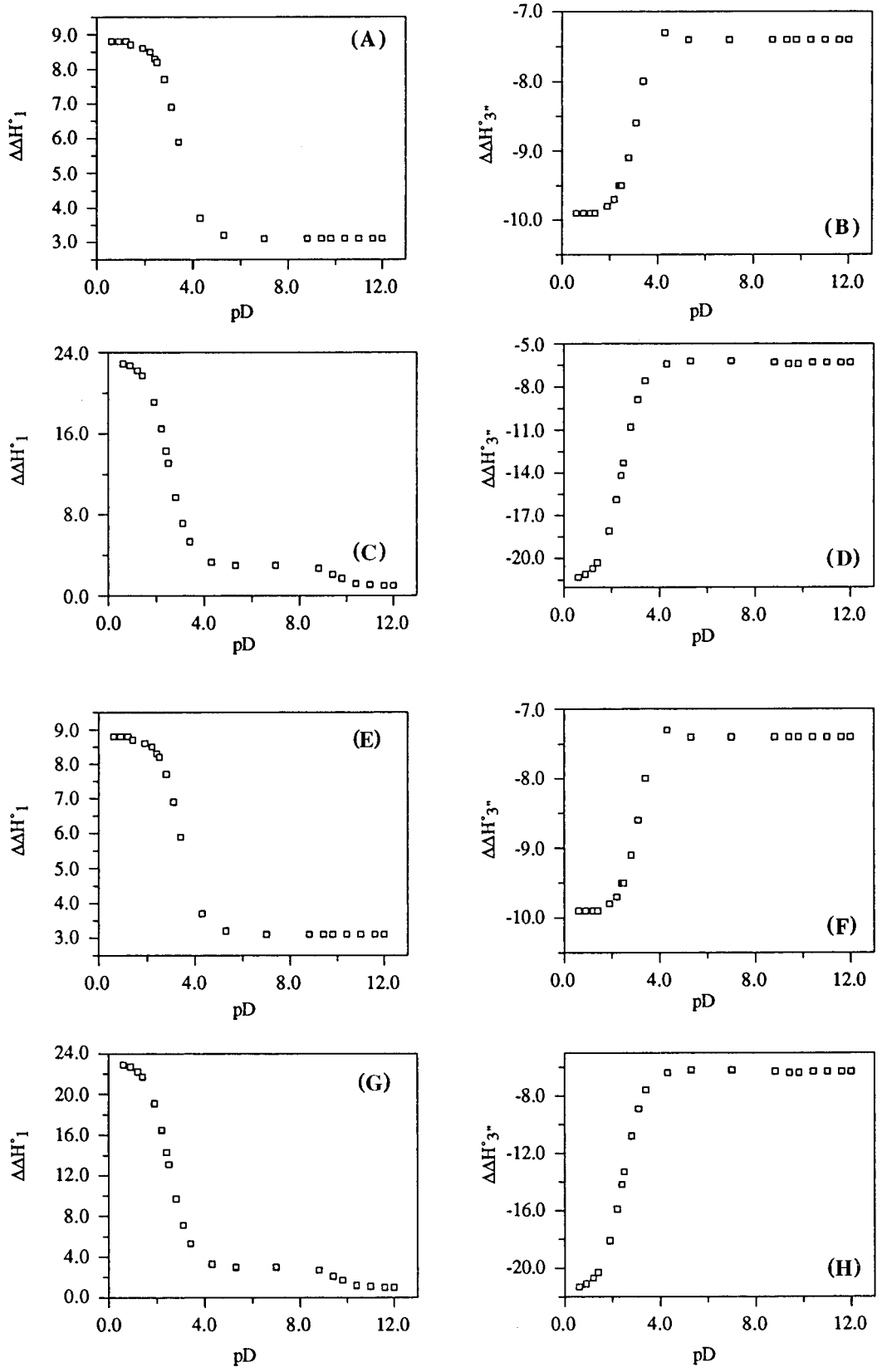


Figure 4. Panels A, C, E and G show the pD-dependent variation of $\Delta\Delta H°_1$ for adenosine (**5**), guanosine (**7**), and their 2′-deoxy counterparts (**4** and **6**) (see Figure 3 for pairwise dissection and Table 3 for the results), giving the total effect of the *N*-aglycon (stereoelectronic and steric). Panels B, D, F and H show the pD-dependent variation of $\Delta\Delta H°_{3"}$ for adenosine (**5**), guanosine (**7**), and their 2′-deoxy counterparts (**4** and **6**) (see Figure 3 and Table 3), yielding the pD-dependent GE(O4′−O3′).

AE in adenosine, guanosine, and their 2′-deoxy derivatives. Hence, these data will be useful for the further improvement of the algorithm used for the theoretical quantum chemical calculations as well as for the parametrization of the molecular mechanics force field used to predict nucleic acids structure in general by introducing the terms that account for the experimentally observed energetics of the GE and AE.

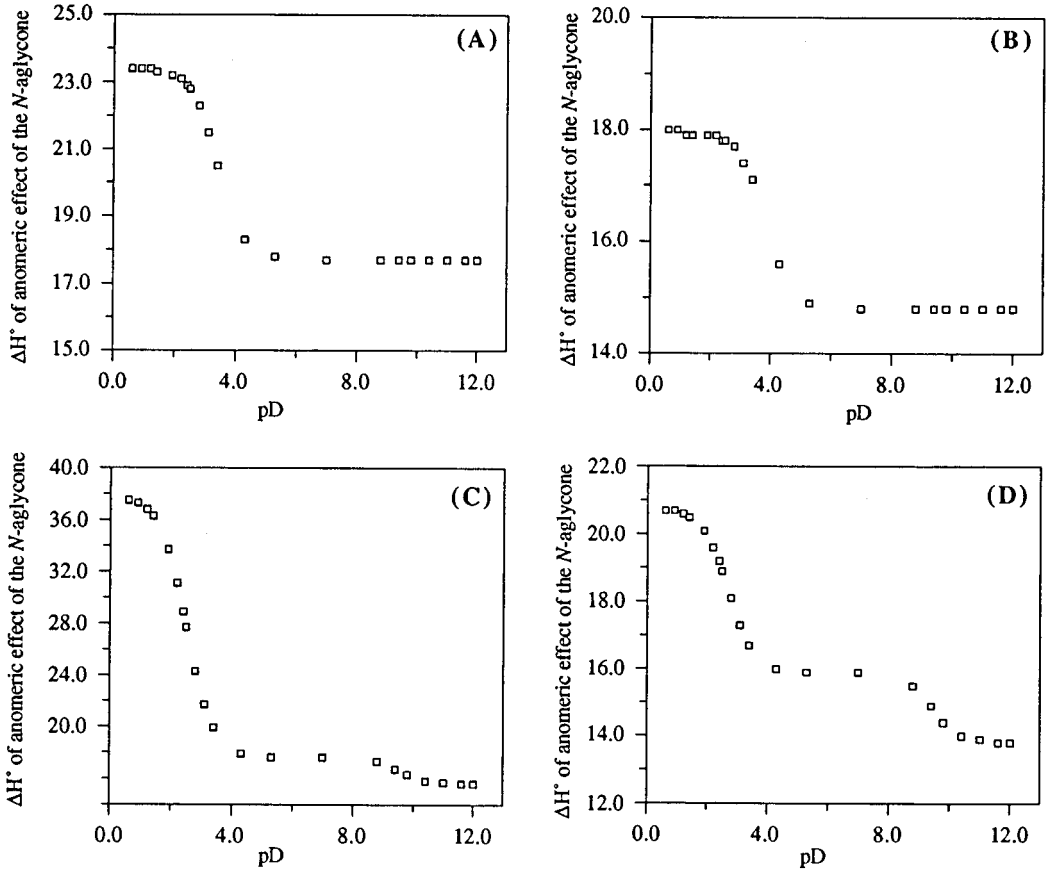


Figure 5. The pD-tunable AEs of the *N*-aglycons are presented in panels A for adenosine (**5**), B for 2′-deoxyadenosine (**4**), C for guanosine (**7**), and D for 2′-deoxyguanosine (**6**) using eqs 2 and 3 (Table 5).

Experimental Section

(A) ^{1}H NMR Spectroscopy. ^{1}H NMR spectra were recorded at 500 MHz (Bruker DRX 500) in D_2O solution [1 mM for all compounds, $\delta_{CH_3CN} = 2.00$ ppm as internal reference] between 278 and 358 K at 10 K intervals in different pD ranges. See ref 1s for compounds **1**−**3**, see ref 1p for **4**−**7**, see Tables S1 and S2 in the Supporting Information for **11** and **12**. The pD values correspond to the reading on a pH meter equipped with a calomel electrode calibrated with pH 4 and 7 standard buffers in H_2O and are not corrected for the deuterium isotope effect. The pD of the samples has been adjusted by the simple addition of microliter volumes of concentrated D_2SO_4 or NaOD solutions. All spectra were recorded using 64 K data points (spectral width of 10 ppm) and 16 or 32 scans. For the pD-dependent accurate $^3J_{HH}$, see ref 1s for **1**−**3**; see ref 1p for **4**− **7**, see ref 1a for **8**−**10**, and see Tables S1 and S2 in the Supporting Information for **11** and **12**. They were obtained through spin simulation and iteration using DAISY program package[5] and have been used for the pseudorotational analyses.

(B) Conformational Analysis with PSEUROT. The conformational analyses of nucleosides **1**−**12** were performed with the program PSEUROT (version 5.4).[6] The results of the PSEUROT analyses for **1**−**3** are given in ref 1s, for **4**−**7** in ref 1p, for **8**−**10** in ref 1a.

The generalized Karplus equation used in the PSEUROT[6] program links coupling constants between vicinal protons to corresponding proton−proton torsion angles. The following λ substituent parameters were used for the substituents on H−C−C−H fragments: λ(C1′) = λ(C4′) = 0.62; λ(C3′) = λ(C2′) = 0.67 (group 13); λ(C5′) = 0.68; λ(O4′) = 1.27; λ(OH) = 1.26 and λ(*N*-aglycon) = 0.58. The PSEUROT analyses of temperature dependent $^3J_{HH}$ (274−358 K, Tables S3 and S4 in the Supporting Information) of the sugar moieties of **11** and **12** at different pDs were performed in either one or two steps in order to carefully examine the conformational hyperspace accessible to the N and S conformers: (i) The puckering amplitude of N and S conformers, Ψ_m(N) and Ψ_m(S), were assumed to be identical, and they were kept fixed during the PSEUROT calculations, while the phase angles of N and S conformers, P_N and P_S, were optimized freely. (ii) When either the N-type or the S-type conformer was preferred by more than 65%, the geometry of the minor conformer was fixed while P and Ψ_m of the major conformer were optimized freely. Typically 5−10 separate PSEUROT calculations were performed in step i and 10 calculations in step ii. To incorporate the error in the coupling constants (σ = 0.1 or 0.2 Hz), 1000 sets of randomly varied coupling constants (Gaussian distribution) were generated and analyzed with a locally modified[7] version of the PSEUROT program[6] (Tables S3 and S4 in the Supporting Information, and their legends for specific description of the conformational spaces covered by the analyses). Typically, a total of 5000−20000 individual pseudorotational analyses were performed for **11** or **12** at each pD. Some of the results were discarded due to (i) too large a difference between a J_{calc} and J_{exp} (ΔJ_{max} = 1.2 Hz), (ii) too large an overall rms in J_{HH} (rms_{max} = 0.8 Hz). (iii) Conformers with the following P (←40° or >40°, and >180° or <120°) and Ψ_m (<29° or >45) were excluded because no crystal structures are found in such conformational hyperspace (see legends of Tables S3 and S4 in the Supporting Information). The total numbers of pseudorotational results used in the subsequent calculations of thermodynamic parameters are given in column 2 of Tables S3 and S4 in the Supporting Information. The mole fractions from the accepted pseudorotational analyses were used to construct van't Hoff plots. The averages of the slopes and the intercepts (Tables S3 and S4 in the Supporting Information) from the 5000−20000 van't Hoff plots were used to calculate $\Delta H°$ and $\Delta S°$ (and their errors) of the N ⇄ S sugar equilibrium of **11** and **12** (Tables S3 and S4 in the Supporting Information).

The free-energy, ΔG^{288}, values were calculated in two ways. (i) By taking the sum of $\Delta H°$ and $-T\Delta S°$. The standard deviation of ΔG^{288} is derived from the standard deviations of the $\Delta H°$ and $-T\Delta S°$ values by the formula $\sigma = (\sigma^2_{\Delta H^2} +$

$\sigma^2_{-T\Delta S})^{1/2}$, giving a rather high error for ΔG^{288} because of the error propagation (and amplification) by the multistep procedure. (ii) From the average of the 5000−20000 individual $\ln(x_S/(1 - x_S))$ at 288 K. This we refer to as $\ln_{av}(x_S/(1 - x_S))$ with its standard deviation $[\sigma \ln_{av}(x_S/(1 - x_S))]$. The free energies at 288 K, obtained by using the formula $\Delta G^{288} = -R(0.288)\ln_{av}(x_S/(1 - x_S))$, are presented in the last column of Tables S3 and S4 in the Supporting Information with their corresponding standard deviations in parentheses. The error of ΔG^{288} is then directly calculated using the formula $\sigma_{\Delta G^{288}} = -R(0.288)\sigma \ln_{av}(x_S/(1 - x_S))$, rather smaller than the one obtained with the first method. However, the value of ΔG^{288} is not changed compared to the first approach.

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Supporting Information Available: The temperature-dependent vicinal coupling constants as a function of pD are available in Table S1 for β-D-2′,3′-ddA (**11**), Table S2 for β-D-2′,3′-ddG (**12**). The thermodynamics of the N/S equilibrium as well as the geometries of the N and S pseudorotamers optimized with PSEUROT are available in Table S3 for **11**, S4 for **12** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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