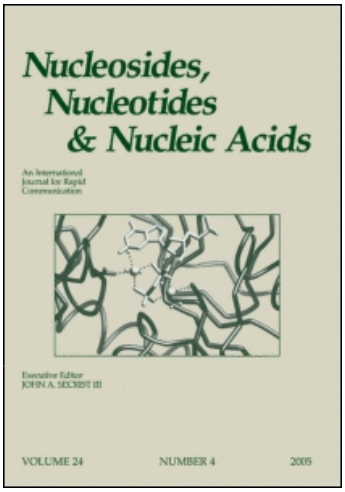

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The Information Transmission from the Nucleobase Drives the Sugar-Phosphate Backbone Conformation in the Nucleotide Wire

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The Information Transmission from the Nucleobase Drives the Sugar-Phosphate Backbone Conformation in the Nucleotide Wire

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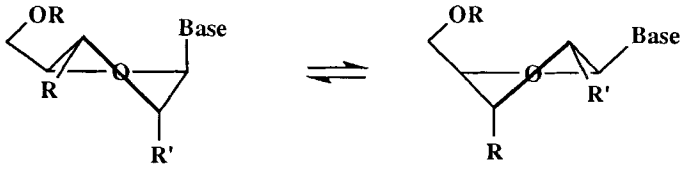
Abstract: We herein present our results showing that a simple mononucleotide block actually acts as a wire. This is evidenced by the fact that any change of the electronic character of the nucleobase is transmitted to drive the sugar conformation, which in turn dictates cooperatively the preferred orientation of the phosphate backbone.

The nucleobase, pentofuranose and phosphodiester moieties are the essential three covalently linked components that contribute to the dynamic and flexible architecture of nucleic acids that is endowed with the storage of the genetic code in the DNA level and the unique catalytic properties in the RNA level[1]. It is well known that the three-dimensional structure of nucleic acids is stabilized by strong intermolecular interactions, such as H-bonds formed between complementary nucleobases and the base-stacking interaction; other forces such as electrostatic and hydrophobic forces between adjacent base pairs and across the nucleotide chain have also been considered to play important role for the self-organization of polynucleotides.

The spontaneous transitions between indefinite nonplanar geometries of pentofuranose ring in DNA and RNA is mediated by pseudorotation[2a], and the pentofuranose moiety reduces its energy by becoming puckered[2b,c] (Scheme 1). Recent quantum chemical studies have shown that the inconversion of the North (N, 3'-*endo*-2'-*exo*) and South (S, 3'-*exo*-2'-*endo*) sugars occurs preferentially through the relatively small O4'-*endo* eastern energy barrier of the pseudorotational cycle [≈ 2 and ≈3 kcal/mol for 2'-deoxyribonucleosides (dNs) and ribonucleosides (rNs), respectively][3] over the west energy barrier (≈ 5 and ≈ 7 kcal/mol for dNs and rNs, respectively)[3]. On the other hand, the interconversion of pyranose conformers requires a much higher energy barrier, which is highly dictated by the position, nature and configuration of various substituents. Our NMR-based experimental data in the aqueous environment shows that the differences between the stabilities of N and S conformers are both nucleobase and sugar-substituent dependent, and in the range from -2.1 to 3.6 kJ/mol[4t,u].



North sugar ($C_{3'}$-*endo*-$C_{2'}$-*exo*)
Phase angle (P) = $0° \leq P \leq 36°$;
Puckering amplitude (Ψ_m) = $38.6° \pm 3°$

South sugar ($C_{2'}$-*endo*-$C_{3'}$-*exo*)
Phase angle (P) = $144° \leq P \leq 190°$;
Puckering amplitude (Ψ_m) = $38.6° \pm 3°$

Base = adenin-9-yl (A), guanin-9-yl (G), cytosin-1-yl (C), thymin-1-yl (T)

Scheme 1: The two-state N⇌S pseudorotational equilibrium in β-D-nucleos(t)ides

We herein present our experimental evidences from the ongoing studies from our laboratory which unequivocally show that there is a clear interdependency between the conformational preferences of the pentofuranose ring and the phosphate moiety, as well as between the electronic character of the nucleobase and the conformation of the sugar ring in nucleosides and nucleotides.

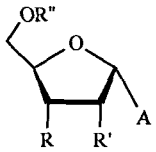
(I) In solution, various steric and stereoelectronic gauche and anomeric effects energetically drive the North (N) ⇄ South (S) sugar pseudorotational equilibrium in nucleos(t)ides

(A) The pseudorotation concept[2a] as a tool to define the pentofuranose conformation in nucleosides.

The geometry of the sugar moiety in nucleosides is defined by (i) The phase angle of pseudorotation (P), indicating which part of the ring is mostly puckered and (ii) the puckering amplitude (Ψ_m), showing the extent of puckering[2]. The hypothesis of a two-state North (N, *C2'-exo-C3'-endo*) ⇄ South (S, *C2'-endo-C3'-exo)* pseudorotational equilibrium in solution, originally based on the analysis of the statistical distribution of the X-ray crystal structures of nucleosides[5] was also further corroborated by the NMR observations of two distinctly identifiable and dynamically interconverting N and S conformations (as evident by their respective chemical shifts and $^3J_{HH}$) of the constituent sugar moieties in oligonucleotides as in B ⇄ Z DNA[6a,b] or A ⇄ Z RNA[6c,d] or in the A-form ⇄ B-form lariat RNA[6e,f].

(B) The fine balance of the stereoelectronic forces and entropy drives the sugar conformation in nucleosides.

Our NMR studies on 59 interrelated analogs including abasic sugars, α and β-D-2',3'-dideoxynucleosides (ddNs), α and β-D/L-2'-deoxynucleosides, β-D-ribonucleosides and their 3'-phosphates as well as several *C*-nucleosides **53 - 59** have uniquely shown[4] that the conformation of the sugar moiety in nucleosides is energetically (ΔH°) controlled by gauche and anomeric effects as well as steric effects. Through the pairwise comparison of the thermodynamics of the

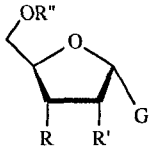


1: α-D-ddA
(R = R' = R" = H)

15: α-D-dA
(R = OH; R' = R" = H)

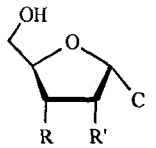
16: 3'-OMe-α-D-dA
(R = OMe; R' = R" = H)

17: 3',5'-diOMe-α-D-dA
(R = OMe; R' = H; R" = Me)



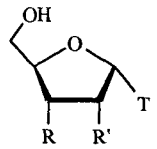
3: α-D-ddG
(R = R' = H)

18: α-D-dG
(R = OH; R' = H)



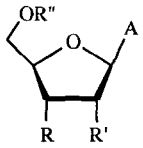
6: α-D-ddC
(R = R' = H)

19: α-D-dC
(R = OH; R' = H)



8: α-D-ddT
(R = R' = H)

20: α-D-T
(R = OH; R' = H)



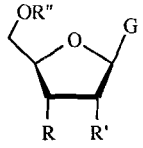
2: β-D-ddA
(R = R' = R" = H)

21: β-D-dA
(R = OH; R' = R" = H)

22: 3'-OMe-β-D-dA
(R = OMe; R' = R" = H)

23: 3',5'-diOMe-β-D-dA
(R = OMe; R' = H;
R" = Me)

28: β-D-A
(R = R' = OH; R" = H)

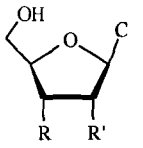


4: β-D-ddG
(R = R' = R" = H)

5: 5'-OMe-β-D-ddG
(R = R' = H; R" = Me)

24: β-D-dG
(R = OH; R' = R" = H)

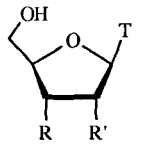
29: β-D-G
(R = R' = OH; R" = H)



7: β-D-ddC
(R = R' = H)

25: β-D-dC
(R = OH; R' = H)

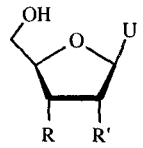
30: β-D-C
(R = R' = OH)



9: β-D-ddT
(R = R' = H)

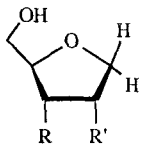
26: β-D-T
(R = OH; R' = H)

31: β-D-rT
(R = R' = OH)



27: β-D-dU
(R = OH; R' = H)

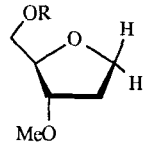
32: β-D-U
(R = R' = OH)



10: R = R' = H: (*S*)-tetrahydrofurfuryl-alcohol (THFA)

11: R = OH; R' = H:
1,2-dideoxy-D-*ribo*furanose

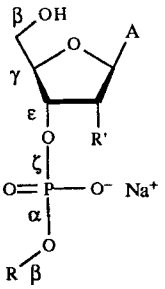
12: R = R' = OH:
1-deoxy-D-ribofuranose



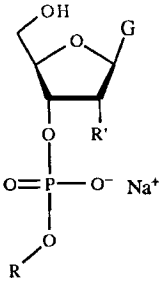
13: R = H: 3'-OMe-tetrahydrofurfuryl-alcohol (3'-OMe-THFA)

14: R = Me: 3',5'-diOMe-tetrahydro-furfurylalcohol (3',5'-diOMe-THFA)

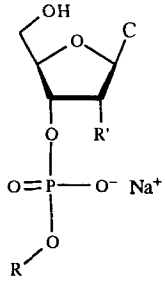
A = adenin-9-yl; G = guanin-9-yl; C = cytosin-1-yl; T = thymin-1-yl; U = uracil-1-yl



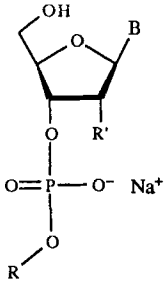
33: dAMP
(R' = R = H)



34: dGMP
(R' = R = H)



35: dCMP
(R' = R = H)



36: TMP (R = R' = H, B = T)
37: dUMP (R = R' = H, B = U)

38: dApEt
(R = Et, R' = H)

39: dGpEt
(R = Et, R' = H)

40: dCpEt
(R = Et, R' = H)

41: TpEt (R = Et, R' = H, B = T)
42: dUpEt (R = Et, R' = H, B = U)

43: AMP
(R = H; R' = OH)

44: GMP
(R = H; R' = OH)

45: CMP
(R = H; R' = OH)

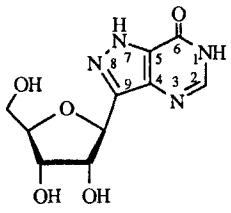
46: rTMP (R = H, R' = OH, B = T)
47: UMP (R = H, R'= OH, B = U)

48: ApEt
(R = Et; R' = OH)

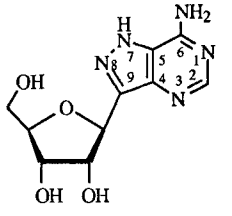
49: GpEt
(R = Et; R' = OH)

50: CpEt
(R = Et; R' = OH)

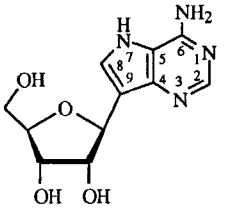
51: rTpEt (R = Et; R' = OH, B = T)
52: UpEt (R = Et; R' = OH; B = U)



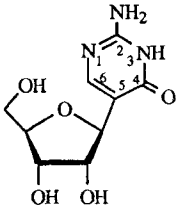
53: Formycin B



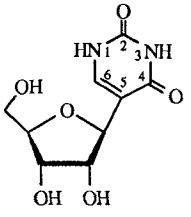
54: Formycin A



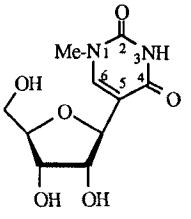
55: 9-deaza-adenosine



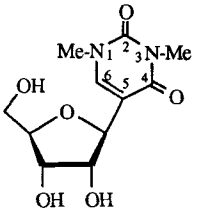
56: ψ-isocytidine



57: ψ-uridine



58: 1-methyl-ψ-uridine



59: 1,3-dimethyl-ψ-uridine

A = adenin-9-yl; G = guanin-9-yl; C = cytosin-1-yl; T = thymin-1-yl; U = uracil-1-yl

pseudorotational equilibrium in these 59 nucleos(t)ides and abasic sugars that differ only in one structural feature or by the configuration of a specific center, it has been possible to quantitate the strength of these stereoelectronic forces and their modulation upon the change of the environment. The result of our pairwise comparison is as follows. (1) The effect of the nucleobase consists of the counteracting anomeric effect[7] [O4'-C1'-N interactions: n(O4')→σ*C1'-N orbital overlap] and the steric effect of the aglycone. It has been shown by us that the strength of the anomeric effect is nucleobase-dependent and increases in the following order: adenine, guanine, thymine, uracil, cytosine[4a,c]. (2) It has been possible to quantify the strength of the anomeric effect in purine *N*-nucleosides by comparing their conformational preferences with those of isosteric purine *C*-nucleosides[4k,v]. (3) The strength of the anomeric and gauche effects is dictated by the nature of the electronic character of the substituent or the nucleobase, which can be modulated by the change of the medium[4a,p,s-v]. (4) The modulation of the sugar conformation is a result of either cooperative or counteractive gauche and anomeric effects[4]. (5) The nucleobase-dependent gauche effects[3a-b,4] of [O4'-C4'-C3-O3'] and [O2'-C2'-C1'-N(nucleobase)] fragments drive the N⇄S equilibrium in β-D-nucleosides toward S-type conformers whereas the [O4'-C1'-C2'-O2'] gauche effect drives it toward N-type sugars. (6) The overall steric and gauche effects of [O5'-C5'-C4'-O4'] fragment are negligible[4a,t]. (7) The strength of both gauche and anomeric effects is directly dependent upon the configuration at a chiral center[4d,t,u]. (8) The strength of the gauche effect of 2'-OH both with O4' and the glycosyl-N is nucleobase-dependent, which also explains their different chemical reactivities[4r]. (9) The *C*-nucleosides, just as in *N*-nucleosides, have unique anomeric effect through orbital mixing of O4' lonepair with the antibonding orbital of C1'-C_{sp2} : n(O4') → σ*[C1'-C(sp2)][4s].

The study of the stereoelectronic effects of nucleosides as a result of the change of the medium by the change of pD is designed to mimic the complexes of various soft and hard metal ions to the nucleobase or the phosphate moiety. A detailed inventory of the stereoelectronic gauche and anomeric effects allows us to predict and design the conformational features of nucleosides and nucleosides analogs.

(II) The interdependency of the conformational preferences of the sugar and phosphate moieties in 2'-deoxynucleosides-3'-ethylphosphates (dNMPEt, 38 - 42) and the corresponding ribonucleotides (rNMPEt, 48 - 52).

In this section, we report the results of our investigations[4g,n] that have adressed the following fundamental questions: Does the sugar conformation dictate the phosphate backbone torsions? Is there any preferred phosphate torsion that steers the sugar conformation in a certain manner? Are there any correlated interdependencies of endocyclic sugar torsions with the preferred phosphate torsions?

In order to further understand the forces that govern the stabilization of the tertiary structure of oligo- and polynucleotides, we have examined and dissected the nature of fundamental intranucleotidyl interactions that contribute to the drive of the sugar-phosphate backbone in DNA and RNA using simple model systems, *i.e.* nucleoside 3'-ethylphosphates, in which the effect of internucleotidyl base-base stacking on the drive of the sugar-phosphate backbone is completely eliminated. The main outcome of our studies[4g,n] can be summarized as follows.

(A) Is there any correlation between the conformation of the sugar and of the phosphate in DNA?

The comparison of the conformational preferences of 3'-monophosphates (dNMP) **33** - **37** and 3'-ethylphosphates (dNMPEt) **38** - **42** of 2'-deoxynucleosides with the respective parent nucleosides (dNs) has shown[4g] that the gauche effects of 3'-monophosphate and 3'-ethylphosphate in dNMPs have nearly the same strength and that they result into the stabilisation of S-type sugar pseudorotamers by $\Delta H^\circ \approx 1.7$ kJ mol^{-1} in comparison with 3'-OH in the dN counterparts. However, as the bias of the two-state N$\rightleftarrows$ S dynamic equilibrium is shifted in the deoxy series from more to less S-type conformers from 278 K to 358 K, we have not observed any concomittant change of the populations of the ε and/or β rotamers of the phosphodiester moiety, suggesting that the mechanisms that govern the conformation of the pentofuranose moiety and of the phosphate backbone are independent in the absence of any base-base stacking interaction in our 2'-deoxy model systems (dNMPEt, **38** - **42**)[4g].

(B) The interaction of 2'-OH with the vicinal phosphate steers the sugar-phosphate backbone into a unique two-state (N,ε^t) $\rightleftarrows$ (S,ε$^-$) conformational equilibrium in rNMPEts (48 - 52).

(1) The sugar conformation in ribonucleotides[4n] (rNMPs) **43** - **47** is driven more towards the South by $-1.3 < \Delta\Delta H^\circ < -0.3$ kJ mol^{-1} in the purine and by $-2.2 < \Delta\Delta H^\circ < -0.9$ kJ mol^{-1} in the pyrimidine nucleotides compared to the corresponding parent ribonucleosides (rNs) **28** - **32**. This is owing to the fact that the gauche effect of [O4'-C4'-C3'-3'-phosphate] in the former is stronger than that of [O4'-C4'-C3'-3'-OH] in the latter.

(2) The S-type sugars are further stabilized in rNMPEts in comparison with the parent rNs by $\Delta\Delta H^\circ \approx -2$ kJ mol^{-1}.

(3) As the temperature is increased from 278 K to 358 K, the population of S-type conformers decreases and the population of ε^t rotamers in rNMPEts **48** - **52** increases[4n] in a coopeative manner. The interdependency of the conformation of the sugar and phosphate moieties is further evidenced by the fact that the free-energies (ΔG^{298}) of the two-state N$\rightleftarrows$ S and ε^t $\rightleftarrows$ ε$^-$ equilibria are the same for all rNMPEts (see Table 1), within the experimental error of our measurements and calculations (±0.5 kJ/mol). This is further evidenced by a simple correlation plot of the temperature-dependent populations of ε$^-$ rotamers as a function of the temperature-dependent population of the S sugar pseudorotamers (see Fig 1) for all rNMPEts **48** - **52**, showing their straight forward correlation with the correlation coefficients from 0.87 - 1.00.

(4) The unique interaction of 2'-OH with a vicinal heteroatom (*e.g.* O3') may be able to act as a molecular switch between (N,ε^t)$\rightleftarrows$ (S,ε$^-$) conformational equilibria in **48** - **52**. This 2'-OH interaction stabilizes the S and ε$^-$ conformers ("On-Off" switch) in a cooperative manner over N and ε^t (in ApEt, GpEt, rTpEt, UpEt). The free-energy stabilization of S and ε$^-$ conformers is however insignificant ($\Delta G^{298} = 0.1$ kJ mol^{-1}) for CpEt because of the strongly opposing anomeric effect.

These data have been the basis for the unique two-state (N,ε^t) $\rightleftarrows$ (S,ε$^-$) conformational equilibrium that exists in ribonucleotides, which we expect to contribute to the overall forces of the self-organization of of RNA.

Table 1. The thermodynamics[4n] of the two-state N ⇄ S and ε^t ⇄ ε^- pseudorotational equilibria in rNMPEt **48 - 52** showing the identical values (within experimental error) for ΔG^{298} of N ⇄ S pseudorotational and ε^t ⇄ ε^-equilibria (see Fig 1 for the correlation plot).

Compound	Estimation of the drive of N ⇄ S pseudorotational equilibria derived from temperature-dependent $^3J_{HH}$						
	ΔH° [a]	ΔS° [a]	$-T\Delta S^\circ$ [b]	ΔG^{298}	$\%S^{278}$ [c]	$\%S^{358}$ [c]	$\Delta\%S$ [d]
ApEt (**48**)	-6.9 (0.8)	-13.6 (1.1)	4.1	**-2.8**	79	66	-13
GpEt (**49**)	-5.8 (0.4)	-12.3 (1.1)	3.7	**-2.1**	74	62	-12
CpEt (**50**)	-1.5 (0.2)	-5.3 (1.0)	1.6	**0.1**	50	47	-3
rTpEt (**51**)	-2.5 (0.3)	-5.3 (1.2)	1.6	**-0.9**	61	55	-6
UpEt (**52**)	-1.6 (0.1)	-3.1 (0.6)	0.9	**-0.7**	58	54	-4
	Estimation of the drive of ε^t ⇄ ε^- conformational equilibria derived from temperature-dependent $^3J_{HP}$ and $^3J_{CP}$						
	$\Delta H_\varepsilon^\circ$ [a]	$\Delta S_\varepsilon^\circ$ [a]	$-T\Delta S^\circ$ [b]	ΔG^{298}	$\%\varepsilon^{-278}$ [c]	$\%\varepsilon^{-358}$ [c]	$\Delta\%\varepsilon^-$ [e]
ApEt (**48**)	-6.6 (0.9)	-14 (3)	4.2	**-2.4**	76	63	-13
GpEt (**49**)	-5.8 (0.9)	-12 (3)	3.6	**-2.2**	74	62	-12
CpEt (**50**)	-2.8 (0.9)	-9 (4)	2.7	**-0.1**	53	46	-7
rTpEt (**51**)	-3.8 (0.8)	-8 (3)	2.4	**-1.4**	66	58	-8
UpEt (**52**)	-3.3 (0.7)	-7 (2)	2.1	**-1.2**	64	57	-7

[a] ΔH° (kJ mol^{-1}) and ΔS° (J mol^{-1} K^{-1}) are the average values (standard deviations are given in brackets) and were calculated from individual van't Hoff plots using populations of N and S pseudorotamers from several individual PSEUROT analyses. $\Delta H_\varepsilon^\circ$ and $\Delta S_\varepsilon^\circ$ were calculated from 15 van't Hoff plots using populations of ε^t and ε^- rotamers. The signs of thermodynamic parameters are arbitrarily chosen in such a way that the positive values indicate the drive of N ⇄ S and ε^t ⇄ ε^- equilibria to N and ε^t, whereas the negative signs describe the drive to S and ε^-, respectively. [b] $-T\Delta S^\circ$ (kJ mol^{-1}) term is given at 298 K. [c] The population of the S and ε^- conformers were calculated using the relation: %S (T) or $\%\varepsilon^-$ (T) = 100 * [exp (- ΔG^T/ RT)] / [exp (- ΔG^T/ RT) +1]. [d] $\Delta\%S = \%S^{358} - \%S^{278}$. [e] $\Delta\%\varepsilon^- = \%\varepsilon^{-358} - \%\varepsilon^{-278}$.

(III) The free-energy of the protonation ⇄ deprotonation equilibrium of the constituent nucleobase is efficiently transmitted to steer the sugar conformation in nucleosides through tunable stereoelectronic forces

In oligonucleotides, the protonation of the nucleobases directly affects their hydrogen-bonding capabilities and therefore induces a change in the overall three-dimensional structure, both in deoxyribo- and ribo- series. We have recently reported[4p,t] how the protonation ⇄ deprotonation equilibrium affects the electronic character of the nucleobase as a result of a change of the environment (pD), inducing a concomitant shift of the bias of the two-state N ⇄ S equilibrium of the pentofuranose sugar through tunable stereoelectronic forces in all β-D-ddNs **2**, **4**, **5**, **7** and **9**[4t] β-D-dNs **21** - **27**[4p] and rNs **28** - **32**[4p].

(A) The thermodynamics of the two-state North ⇄ South pseudorotational equilibrium in nucleosides are pD-dependent:

This is experimentally evidenced by the plots shown in Fig 2(A) for β-D-ddNs **2**, **4**, **5**, **7** and **9**, Fig 2(B) for β-D-dNs **21** - **27** and Fig 2(C) for β-D-rNs **28** - **32**. The shift of the N ⇄ S

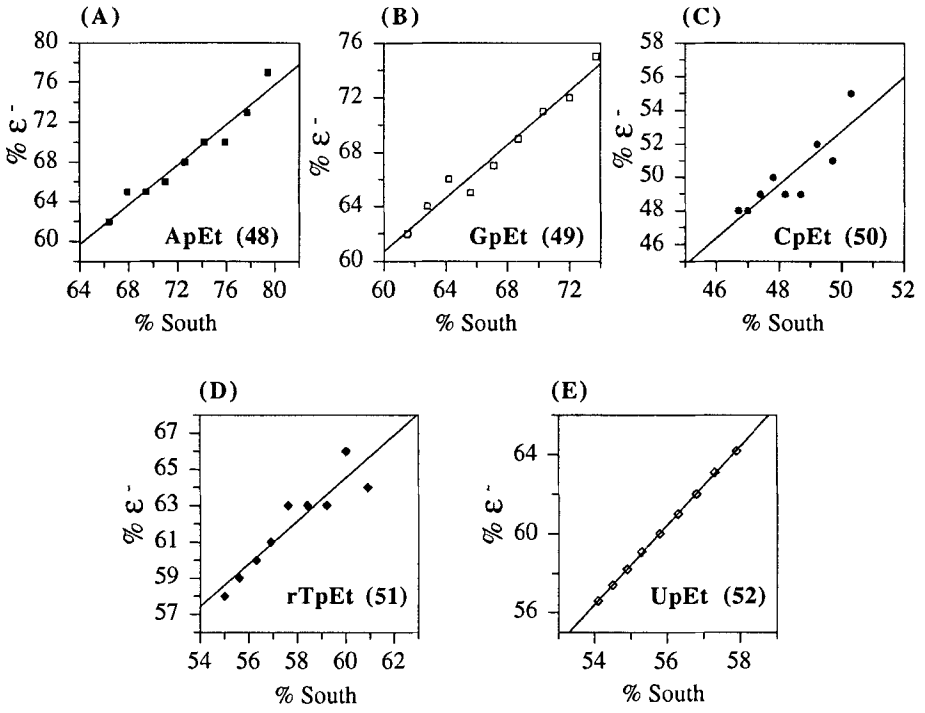


Fig 1: The correlation plots of the temperature-dependent population of the ε⁻ phosphate backbone rotamers with the population of S-type conformers (at 298 K). **(A)** The correlation plot for ApEt (**48**) shows a straight line, with a slope of 1.00, an intercept of -4.83 and a correlation coefficient of 0.97. **(B)** The correlation plot for GpEt (**49**) shows a straight line, with a slope of 0.99, an intercept of 1.58 and a correlation coefficient of 0.98. **(C)** The correlation plot for CpEt (**50**) shows a straight line, with a slope of 1.60, an intercept of -27.3 and a correlation coefficient of 0.87. **(D)** The correlation plot for rTpEt (**51**) shows a straight line, with a slope of 1.18, an intercept of -6.40 and a correlation coefficient of 0.93. **(E)** The correlation plot for UpEt (**52**) shows a straight line, with a slope of 2.00, an intercept of -51.9 and a correlation coefficient of 1.00.

equilibrium toward more N-type (at acidic pDs, upon protonation of the nucleobase) or S-type (at alkaline pDs, upon its deprotonation) pseudorotamers in β-D-ddNs, β-D-dNs and β-D-rNs results from the transmission of the pD-tunable electronic character of the nucleobase to steer the sugar conformation through modulable anomeric and gauche effect[4p,t,v]. The efficiency of the modulation of the anomeric effect by the pD of the medium increases in the order: dNs, rNs, ddNs. The reduced pD-dependent flexibility of the sugar conformation in dNs and rNs series in comparison with ddNs is the result of the competing influence of the gauche effects in deoxyribo- and ribo- nucleosides.

(B) The two-state N ⇄ S equilibrium is a valid model.

This is shown by the fact that the pD values at the inflection points of the plots of the pD-dependent thermodynamics of the pseudorotational equilibrium shown in Fig 2 correspond to the

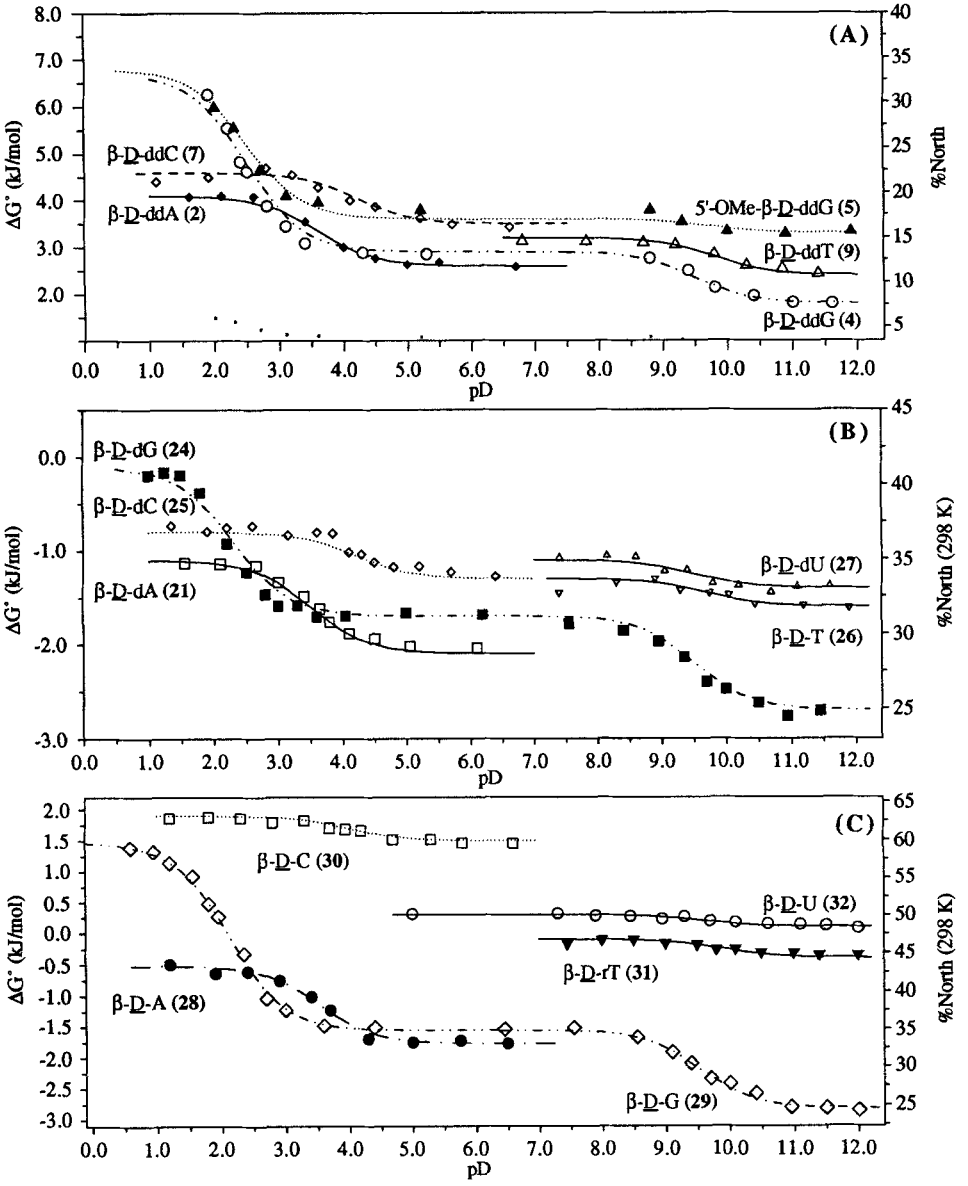


Fig 2: The pD-dependent free-energy [$\Delta G°$ at 298 K for all compounds except β-D-ddA (2), β-D-ddG (4) and 5'-OMe-β-D-ddG (5)] for the two-state N⇄S equilibrium in β-D-nucleosides. (A) For β-D-ddNs **2, 4, 5, 7** and **9**. (B) For β-D-dNs **21, 24 - 27.** (C) For β-D-rNs **28 - 32.** For all nucleosides, $\Delta G°$ shows a sigmoidal dependence on the pD of the medium. The pK_a of each nucleobase remains nearly the same in ddN, dN and rN series.

pK_a values[8] of the constituent nucleobases in the ddN (**2**, **4**, **5**, **7** and **9**)[4t] as well as in dN (**21** - **27**)[4p] and rN (**28** - **32**)[4p] series. Our studies have therefore provided the first experimental evidence of the interdependency of the thermodynamics of the two-state N $\rightleftarrows$ S equilibrium in nucleosides with the electronic state of the constituent nucleobase. This means that the aglycones of natural β-D-nucleosides constitute excellent sensors of the environment, since any change of their electronic character directly dictates the sugar conformation, which in turn dictates the phosphate backbone conformation (vide supra). The transmission of the actual electronic state of the nucleobase to drive the sugar conformation through the glycosidic bond is further evidenced by the correlation plots of the chemical shifts of aromatic and anomeric protons as a function of the free-energy of the N $\rightleftarrows$ S equilibrium, which show straight lines for all nucleosides[4p,t].

(C) What is the expected effect of metallation on the sugar conformation?

From the perusal of these results[4], it is reasonable to expect that the metallation of any basic sites of the aglycone in a nucleotide should also modulate the shift of the N $\rightleftarrows$ S equilibrium of its constituent sugar as well as the constituent phosphodiester moieties in a cooperative and concerted manner, depending upon the nature of the metal ion as well as its site of complexation. This should in general be true for any other acidic or basic ligand involved in the complexation with nucleic acids.

(D) The natural β-D-2'-deoxynucleosides act as better sensors of the environment than pyranosyl nucleosides to steer the conformation of the constituent sugar

It is well known that the barrier of the interconversion between the N and S furanosyl sugars is much reduced in comparison with the energy penalty involved upon conversion of one pyranose conformer to the other, which is highly dictated by the position, nature and the configuration of various substituents as well as the nature of the medium. Our unpublished results[9] show that the actual free-energy of activation for the interconversion of the N and S pseudorotational barrier in the pentofuranose sugar is dictated by the nature of the aglycone as well as by its actual electronic character depending upon the composition of the medium (*i.e.* whether the aglycone is in a protonated, neutral or in a deprotonated state or metallated by a hard or a soft metal ion). On the other hand, it has been shown that the conformation of pyranose ring in tetra-*O*-acetyl glucosylimidazole[10f], tetra-*O*-acetyl mannosylimidazole[10f], and tri-*O*-acetyl xylopyranosyl imidazole[10e] in a lipophilic medium ($CDCl_3$, CCl_4 and Me_2CO-d_6) depends upon whether the imidazole is in the neutral or protonated state as evidenced by the clear change of $^3J_{HH}$ by 2-3 Hz. Hence, in these lipophilic media, what we are observing is a transmission of the free-energy involved in the change of the electronic character of the C1' aglycone from the protonated to the neutral to drive the constituent pyranose conformation. Imidazolium as an aglycone in the above pyranose derivatives prefers a more equatorial orientation than does the corresponding neutral counterpart in the lipophilic medium. The actual origin of the observed modulation of the conformation is still being debated[10a-d].

The above situation in the lipophilic medium however completely changes when the coupling constants of their nonacetylated parent compounds were measured in the *aqueous solution*[10f, g]: no significant change (well within the experimental accuracy) in $^3J_{HH}$ was found in their protonated

versus neutral states, showing that the solvation shell and the electrostatics have profound influence on the actual transmission of the free-energy of protonation/deprotonation equilibrium of the aglycone to drive the conformation of the constituent pyranose sugar through the anomeric or reverse anomeric effects. It has also been shown by Perrin et al that the $\Delta G°$ of protonation/deprotonation equlibrium for *N*-alkylglucopyranosylamines in the aqueous environment is very poorly transmitted to drive the pyranose sugar conformation: $\Delta\Delta G°_{N\rightarrow N^+}$ = -0.2 (± 0.5) to -0.4 (± 0.3) kJ/mol[3d]

Our study[4p,t,u] of $\Delta G°$ of protonation/deprotonation equlibrium with 28 different 2',3'-dideoxy-, 2'-deoxy- and ribonucleosides has shown that the $\Delta G°$ change of N $\rightleftarrows$ S equilibrium ($\Delta\Delta G°_{N\rightarrow N^+}$ or $\Delta\Delta G°_{N\rightarrow N}$) varies for purines from 1 to 4 (±0.1) kJ/mol, whereas for pyrimidines it is from 0.2 to 1 (±0.1) kJ/mol *(Note: Because of the accuracy of these $\Delta G°$ values, we can derive the pK_a of the aglycones of these 28 different nucleosides as accurately as from the potentiometric or UV titration studies!).* This means that the free-energy of the protonation/deprotonation equilibrium in purine nucleosides are transmitted from the aglycone to the sugar more efficiently in case of pentofuranose-based nucleosides compared to the pyranose-based nucleosides. This might also account for the evolutionary choice of the pentofuranose-based nucleosides over the pyranose-based nucleosides.

(IV) The comparison of the conformational flexibility in α-D- versus β-D-dNs and ddNs.

Our latest studies[4t,u] on the quantitation of the competing forces that drive the sugar conformation in α-D- versus β-D-nucleosides have uniquely shown that the pK_as of the nucleobases remain unchanged both in α- and β-nucleosides, but the relative drive of the sugar conformation by their aglycone is different.

(A) The pK_as of the nucleobases remain unchanged[4t,u] iboth in α- and β-nucleosides

For each of the α-D- versus β-D-ddN and α-D- versus β-D-dN pairs, we have estimated the pK_as of the constituent nucleobases from pD-dependent ^1H chemical shifts of the aromatic protons and / or from pD-dependent thermodynamics of the N $\rightleftarrows$ S equilibrium. (For the pD-dependent $\Delta G°$ of the pseudorotational equilibrium, see Fig 2(A) for β-D-ddNs **2**, **4**, **5**, **7** and **9** and Fig 3 for α-D-dNs **15** - **20** and α-D-ddNs **1**, **3**, **6** and **8**). These estimates showed that: (1) The pK_a of a particular nucleobase is the same in α-nucleosides and in their β-counterparts, suggesting that the electronic character of the nucleobase is not influenced by the configuration at the anomeric center, and (2) the pK_a of the nucleobase is the same in ddNs and dNs, showing the lack of influence of 3'-OH.

(B) The sugar moieties in β-D-ddNs and α-D-ddNs preferentially adopt N-type and S-type conformations, respectively, owing to the anomeric effect alone. However, the strength of the anomeric effect is considerably reduced in α-D-ddNs compared to β-D-ddNs, which results in the intrinsic lack of conformational flexibility of the former

This is experimentally evidenced by the fact[4t] that the slopes of the correlation plots of aromatic ^1H chemical shifts of the constituent nucleobase versus $\Delta G°$ of the two-state N $\rightleftarrows$ S equilibrium. They show straight lines both in the case of α-D-ddNs **1**, **3**, **6** and **8** and of their β-D-counterparts **2**, **4**, **5**, **7** and **9**, but the slopes are clearly larger in the α-series than in the β. It is

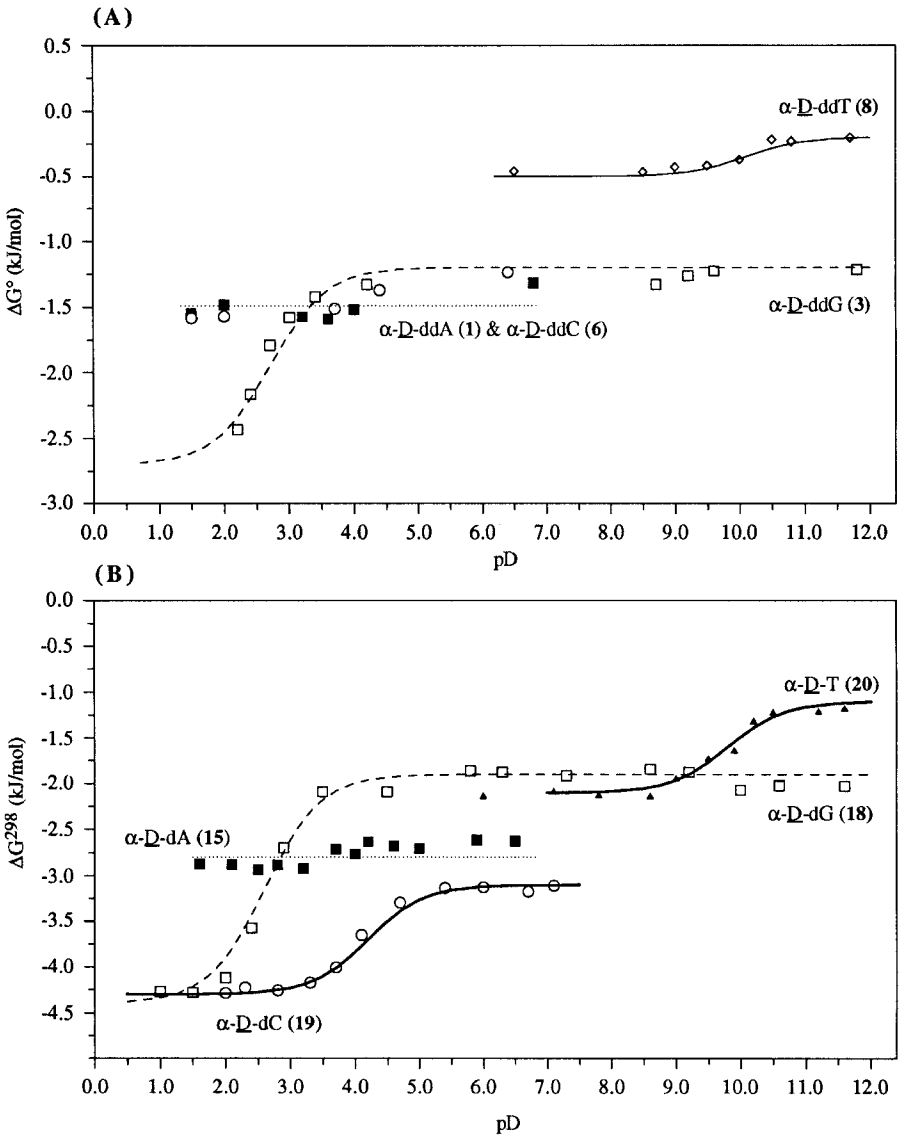


Fig 3: The pD-dependent free-energy, ΔG^{298} (at 298 K), of the two-state N ⇄ S equilibrium in α-D-ddNs **1**, **3**, **6** and **8** (Panel A) and α-D-dNs **15**, **18**, **19** and **20** (Panel B). These plots give the identical pK_a value for each nucleobase, in ddNs and dNs, both in α- and β-series, which shows that (i) the electronic nature of the nucleobased remains the same independently of the configuration at C1' and (ii) 3'-OH has virtually no effect.

noteworthy that the strength of the anomeric effect is much less modulated by the pD of the medium in α-D-ddNs than in β-D-ddNs. This implies that the internal flexibility of the sugar conformation is significantly restricted in α-D-ddNs compared to β-D-ddNs. This also means that the sugar moiety in β-D-ddNs will be much more predisposed to respond to the change of the environment (*i.e.* change of the pH of the solution, metallation or interaction of the nucleobase with any other ligand) than that in α-D-ddNs. Using 1D nOe difference spectroscopy[4t], we have also shown that the preferential *anti* orientation of the nucleobase around the glycosidic torsion is nearly the same within each of the α-D-ddN versus β-D-ddN pairs, and that it remains unchanged in 5'-OMe-β-D-ddG (**5**) in comparison with β-D-ddG (**4**), which leads us to rule out the contribution of any H-bonding interaction to the significant larger pD-dependent flexibility of β-D-ddNs with respect of α-D-ddNs.

(C) The strength of the stereoelectronic forces is pD-independent in all α-D-dNs (except α-D-dG). However, the actual preference of the sugar moiety in α-D-dC and α-D-T for S-type pseudorotamers is MORE affected by the protonation state of the nucleobase than in the β-D-dN counterparts .

Our work[4u] on the comparison of the conformational preferences of α-D-dNs with β-D-dNs and with their ddN counterparts has shown the followings. (1) The nucleobase in all α-D-dNs is mainly in *anti*-type orientations, which eliminates any contribution of an intramolecular H-bond between 3'-OH proton and the nucleobase to the drive of the sugar conformation. (2) For α-D-dA (**15**), α-D-dC (**19**) and α-D-T (**20**), the strength of the anomeric effect is not affected by the pD. (3) Owing to the steric clash induced by the proximity in space of their constituent 3'-OH moieties and nucleobases, the overall entropy of α-D-dNs is changing significantly in pyrimidine α-D-dNs as the pD of the solution is changed. The modulation of ΔG° of the N ⇄ S equilibrium by the pD of the solution is even *greater* in α-D-dC (**19**) and α-D-T (**20**) than in β-D-dC (**25**) and β-D-T (**26**), making the former better sensor of the environment than the latter!

(D) The comparison of the modulation of the strength of the anomeric effect by the pD of the solution in α-D-dG in comparison with α-D-ddG and in β-D-dNs with respect of β-D-ddNs clearly shows the influence of the gauche effect of 3'-OH upon the strength of the anomeric effect in dNs (The 3'-OH effect in dNs compared to ddNs).

The strength of the anomeric effect and its modulation upon the change of the pD is considerably reduced in all β-D-dNs in comparison with β-D-ddNs as well as in α-D-dG (**15**) compared with α-D-ddG (**3**). This is consistent with the presence of 3'-OH moiety in dNs, which either opposes the anomeric effect (in the case of β-D-dNs) or cooperates with it to drive the sugar conformation (in the case of α-D-dNs).

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