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Conformational Studies of Thymidine Dimers Containing Sulfonate and Sulfonamide Linkages by NMR spectroscopy

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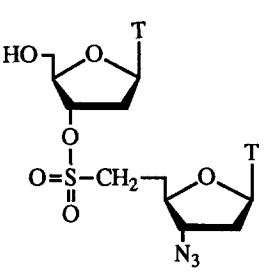
Abstract *The conformations of 3'-azido-terminated-sulfonate-dimer 1 and 3'-amino-terminated-sulfonamide-dimer 2 are characterized by the following features (1) The 5'-terminal nucleoside moiety of 1 has a S-type sugar (87% S), a staggered γ^+ rotamer across the C4'-C5' bond (65 %) and an anti orientation of the base about the glycosidic bond The 5'-terminal nucleoside moiety of 2 has an almost equal population of S and N conformations, a staggered γ^+ rotamer (69 %) and an anti orientation of the base (2) The 3'-terminal nucleoside moieties of 1 and 2 are in ~50% N $\rightleftarrows$ S equilibrium and the γ^t conformer is the most populated A comparison of the conformational properties of 1 and 2 with the natural thymidylyl(3'→5')thymidine [d(TpT)] 3, thymidylyl-(3'→5')-5'-thio-5'-deoxythymidine d(TpST)[5] 4 and thymidinylacetamido-[3'(O)→5'(C)]-5'-deoxythymidine NH_2d(TcmT)[5] 5, show the following characteristics (i) The conformational preference of the sugar ring is partially determined by the gauche effect This means that the more polar the C3'-X bond due to the electronegative character of the 3'-α-X substituent, the more the N $\rightleftarrows$ S equilibrium is biased toward the S-type conformation 3'-O-S > 3'-O-P > 3'-O-H > 3'-N_3 > 3'-NH_2 (ii) The conformation about the C4'-C5' bond (γ) is also influenced by the gauche effect based on the nature of the 5'-substituent and by the ability of the 5'-substituent to form hydrogen bonding with the H6 of thymine Thus, the population of the γ^+ conformer follows the order 5'-O > 5'-N > 5'-S > 5'-C (iii) The C5'-C6' bond has a slight preference for the β^t conformation (56 % β^t in 1 and 58 % β^t in 2), while in natural d(TpT) 3, the C5'-O5' bond accounts for 83% of β^t conformation Upon substitution of the 5'-oxygen by 5'-sulfur, as in d(TpST) 4, the population of β^t conformer was found to decrease to 57 % This decrease in the β^t population in 1, 2 and 4 is a result of the reduced polarity of the 5'-C-X [X = CH_2 in 1 and 2 and X = S in 4] in comparison to the 5'-C-O bond in 3, which weaken the gauche effect*

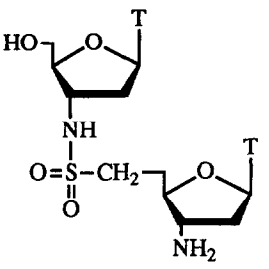
Backbone modified analogues of oligo-DNA may act as antisense repressors at the transcriptional and translational level of gene expression through specific base pairing owing to the information contained in the nucleot(s)ide sequence Such antisense analogues may prove useful for designing specific chemotherapeutic agents against viral, bacterial, and parasitic infections as well as cancer[1] The study of conformational characteristics of these backbone modified analogues of oligo-DNA may also shedlight on how the nucleic acid structures dictate their specific functions[2] For the backbone modified analogues of oligo-DNA to be biologically specific, they should be resistant to chemical and biochemical degradation, must penetrate through cell membranes and be capable of forming stable base-paired duplexes Clearly, the potential utility of such analogues as specific gene-directed agents in biological systems will be related to the degree to which their geometry resembles the geometry of the natural counterpart Some of the important characteristics to be studied in this regard are the chemical changes that are permissible in modified analogues of oligo-DNA that

still allows the formation of a stereoregular backbone, correct geometrical spacing and orientation of the nucleobases for hybridization Only limited data are available[3] on the structural perturbation created by the modification of the phosphate backbone and most of these are concerned with analogues of nucleotides in which one or two of the non-bridging oxygen atoms are substituted[3]

In this work, we have studied the conformational properties of 3'-azido-terminated-sulfonate-dimer[4] **1** and 3'-amino-terminated-sulfonamide-dimer[4] **2** by NMR (^{1}H at 500 MHz) spectroscopy The conformational properties of **1** and **2** have been compared with those of natural thymidylyl(3'→5')thymidine [d(TpT)][5] **3**, and thymidylyl-(3'→5')-5'-thio-5'-deoxythymidine d(TpST)[5] **4** and thymidinylacetamido-[3'(O)→5'(C)]-5'-deoxythymidine NH_2d(TcmT)[5] **5** In this study, we have attempted to understand the effect of the different backbone substituents on the conformation of the sugar ring and on the conformation about the C4'-C5' bond



(1)



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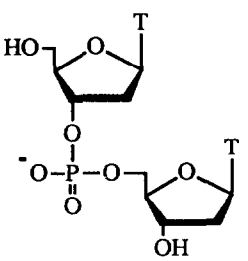

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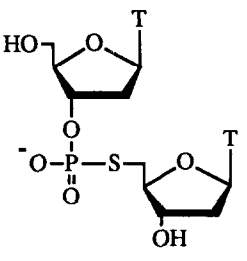

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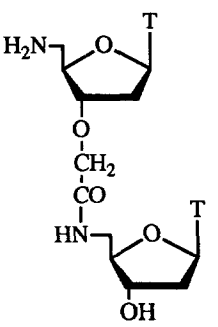

(5)

T = Thymin-1-yl

Assignment of proton resonances The assignment of the non-exchangeable proton resonances was achieved by using two dimensional HOHAHA (Fig 1), DQF-COSY (Fig 2) and NOESY (Fig 3) experiments HOHAHA (Fig 1) was used to identify the two spin systems while DQF-COSY served to assign the H1' to H5'/H5" spin system within each sugar residue NOESY was used to connect the base protons to their own sugar residue and to confirm the assignment of the H2' and H2" protons In compounds **1** and **2**, one 5'-oxygen has been substituted by a CH_2 group which have been assigned C6', H6' and H6" The chemical shifts of the non-exchangeable protons for compounds **1** and **2** are listed in Table 1 The downfield proton at C5' was assigned as the H5' while the upfield proton was assigned as the H5" according to the Remin and Shugar[6] rule Similarly, the lower field proton at C6' was assigned as the H6' while the higher field proton was

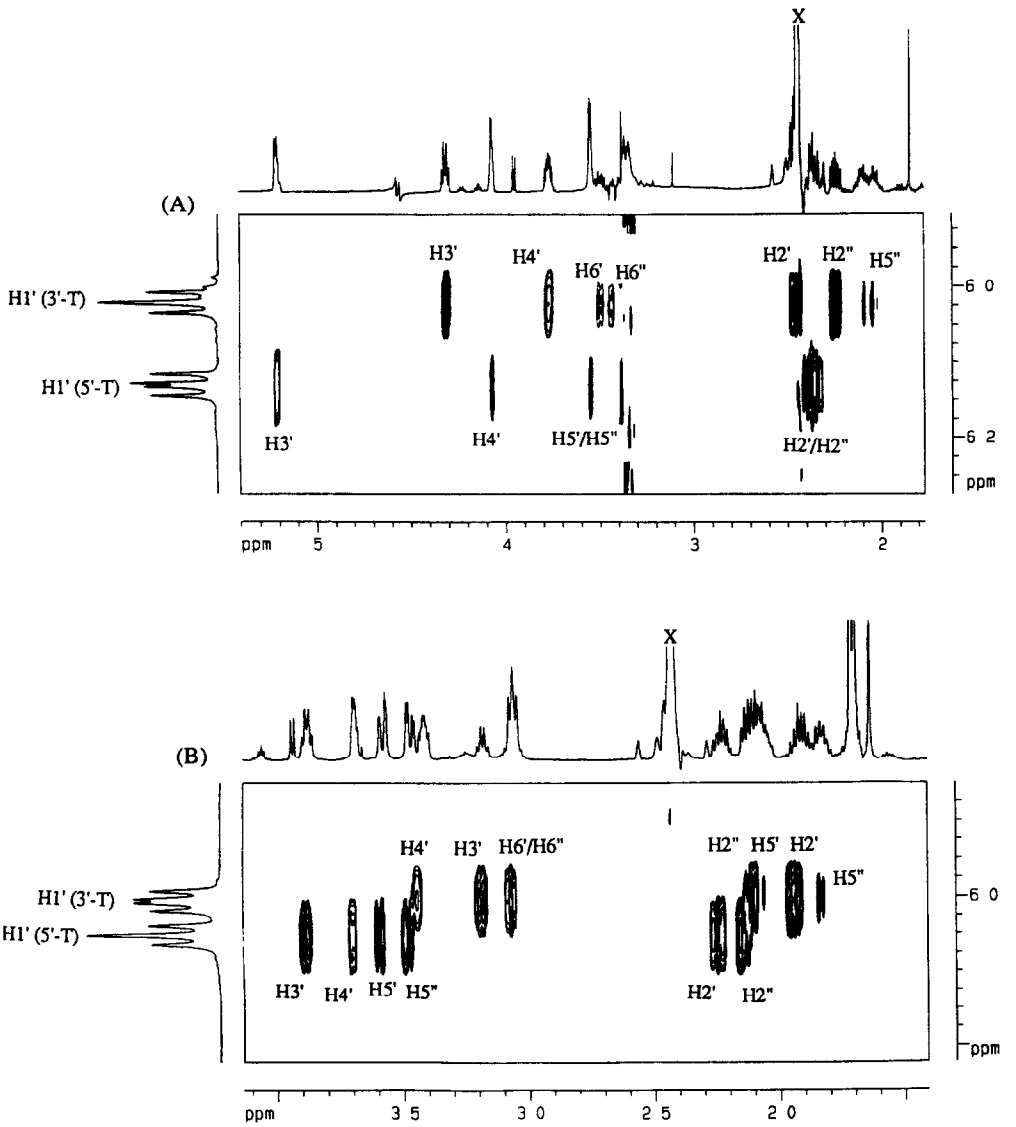

Figure 1 Two-dimensional HOHAHA spectra of (A) **1** at 298K, and (B) **2** at 298K The F1 axis represents the H1' protons, while the F2 axis represents the H2'-H5" protons The H6' and H6" refer to the CH_2 group which replaces the 5'-oxygen (x denotes the DMSO peak)

assigned as the H6" The H5' and H5" of the 3'-terminal nucleotide absorb at higher field (~2 ppm) compared to the H5' and H5" of the 5'-terminal nucleoside due to the replacement of the 5'-oxygen by a CH_2 group in the internucleoside linkage In compound **1**, the H3' of the 5'-terminal residue is slightly deshielded because of the substitution of the phosphorus atom by a sulfur atom in the internucleoside linkage, while in compound **2**, the H3' is slightly shielded due to the substitution of the 3'-oxygen by a NH group The chemical shift of the other protons, H1', H2', H2" and H4' is not affected by the modification of the internucleoside linkage

Determination of vicinal coupling constants The vicinal $^3J_{HH}$ coupling constants were obtained directly from the one dimensional ^1H spectra and/or by measurement of the fine structure of the H1'-H2', H1'-H2", H2'-H3', H2"-H3', H4'-H5' and H4'-H5" cross peaks in a DQF-COSY spectrum The coupling constants were then refined by simulation of the above cross peaks (Fig 2) using the spectral simulation program SMART[7] and are listed in Table 2

Determination of the sugar ring conformation The conformation of the pentofuranose ring in a nucleoside moiety can be fully described in terms of the phase angle of pseudorotation (P) and the puckering amplitude (ϕ)[8] From X-ray studies[9] on nucleosides and nucleotides, it was found that ϕ values range from 35° to 45° For North-type (N) sugars (C3'-*endo*, C2'-*exo*), P ranges from -1° to 34° and for S-type (S) sugars (C2'-*endo*, C3'-*exo*), P ranges from 137° to 194° In solution, the sugar ring exists in an equilibrium of the two rapidly interconverting conformers N $\rightleftarrows$ S The mole fraction of N and S conformer as well as their geometry, expressed by their phase angle of pseudorotation P_N and P_S and puckering amplitude ϕ_N and ϕ_S, can be calculated from the vicinal proton-proton ($^3J_{HH}$) coupling constants $J_{1'2'}$, $J_{1'2"}$, $J_{2'3'}$, $J_{2"3'}$ and $J_{3'4'}$[8,10] These coupling constants (Table 2) were used as an input for the pseudorotational analysis of the sugar using the program PSEUROT[11] (Table 3) When necessary, the PSEUROT program was adapted to take into account the presence of 3'-nitrogen instead of 3'-oxygen[12] Table 3 shows that the two sugar rings in 3'-azido-terminated-sulfonate-dimer **1** are in the S-type conformation, the conformational purity being higher for the 5'-terminal nucleoside (87% S) than for the 3'-azido-terminated nucleoside (56% S) In 3'-amino-terminated-sulfonamide-dimer **2**, the 5'- and 3'-sugar rings show 52 and 49% S-type conformation respectively In natural [d(TpT)] **3**, the sugar ring of the 5'-terminal nucleoside and 3'-terminal nucleoside shows 74% and 66 % of S-type conformation In [d(TpST)] **4** and [NH_2d(TcmT)] **5**, the sugar rings have the comparable population of S-type conformation as in the natural d(TpT) **3** (Table 3) These data suggest that the nature of the 3'-α-substituent has an influence on the conformation of the sugar ring Upon substitution of the 3'-oxygen by a 3'-terminal azido in **1** or 3'-terminal amino in **2**, the N $\rightleftarrows$ S equilibrium is shifted toward smaller population of S-type pseudorotamer 56% S in **1** and 49% S in **2** This effect is also noticeable in the 5'-terminal residue change of the 3'-phosphate function in the 5'-terminal residue in d(TpT) by a sulfonate in **1** or a sulfonamide in **2** changes the pseudorotamer equilibrium from 74% S to 87% S to 52% S, respectively This difference in the solution conformation may be partly explained by the *gauche* effect[13], that is the tendency to adopt the structure in which the O4' and 3'-α substituent are in a *gauche* orientation (Figure 4A) In N-type deoxysugars, the O4' and 3'-α substituents are in a *trans* orientation, while in S-type deoxysugars, the O4' and 3'-α substituents are in a *gauche* orientation, hence the preference for S conformation (Figure 4A) The higher electronegativity of oxygen versus nitrogen leads to a greater preference for a X3'-C3'-C4'-O4' *gauche* orientation For the 5'-terminal residue in **1** - **5**, the *gauche* effect increases with the higher polarisation of the C3'-X bond, and therefore the %S increases in the following order **2** < **3** ≈ **4** ≈ **5** < **1** with the increased electronegativity of the 3'-substituent For the 3'-terminal residue in **1** - **5**, the *gauche* orientation also increases

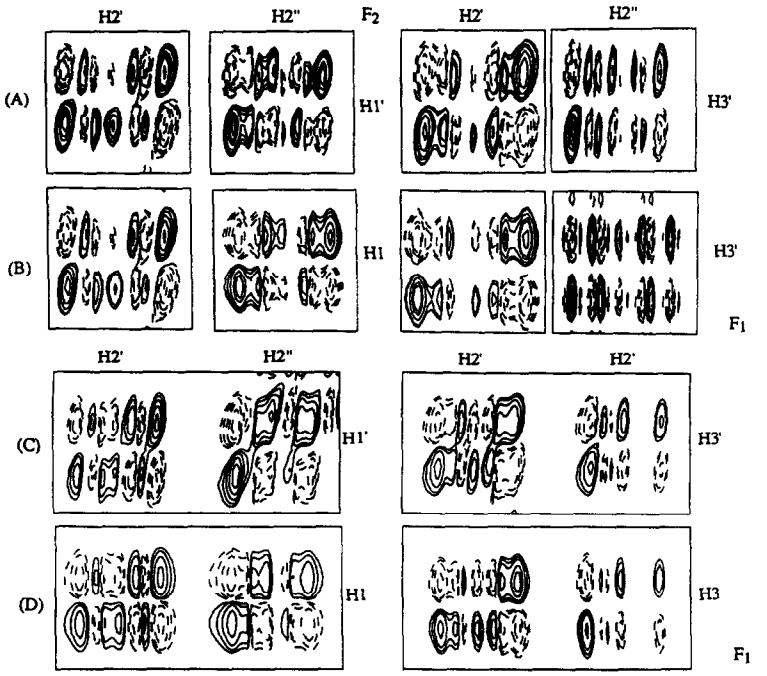

Figure 2 Comparison of (A) experimental DQF-COSY cross peaks and (B) simulated DQF-COSY cross peaks for the 3'-terminal residue of 1 (C) Experimental DQF-COSY cross peaks and (D) simulated DQF-COSY cross peaks for the 5'-terminal residue of 2 The chemical shifts given in Table 1 were used. Line widths of 2 Hz were used

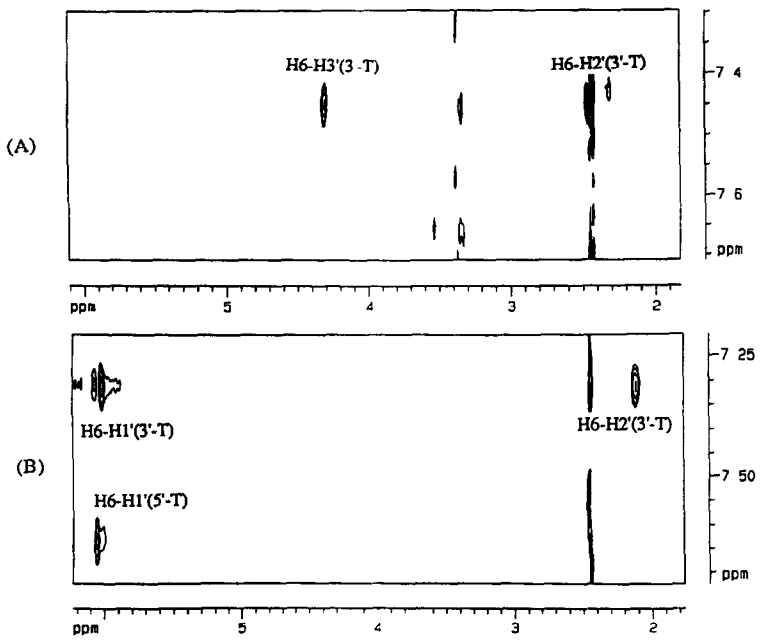

Figure 3 Two-dimensional NOESY spectra of (A) 1 at 298K, and (B) 2 at 298K Mixing time of 800 ms In compounds 1, the nOe cross peaks between H6-H2' and H6-H3' indicate an *anti* conformation In compounds 2, the 3'-terminal residue shows nOe's between H6-H1' and H6-H2' indicating an *anti* conformation

with the higher electronegativity of the 3'-substituent **2 < 1 < 3 ≈ 4 ≈ 5**. This *gauche* effect has been previously observed in 3'-α-substituted-2'-deoxynucleosides such as 3'-α-fluorothymidine and 3'-α-azidothymidine in which the population of S conformer is 90 % and 50% respectively[14] (Table 4)

Table 1: ^{1}H-NMR chemical shifts (ppm) of **1** and **2** at 298K

Compd	Sugar	H1'	H2'	H2"	H3'	H4'	H5'	H5"	H6A	H6B	H6	CH3
1	5'T	6 13	2 33	2 40	5 20	4 06	3 54	3 54			7 60	1 71
	3'T	6 02	2 45	2 25	4 31	3 76	2 11	2 10	3 49	3 43	7 40	1 72
2	5'T	6 05	2 41	2 31	3 88	3 69	3 61	3 50			7 63	1 70
	3'T	6 00	2 10	2 28	3 18	3 42	1 85	1 84	3 06	3 06	7 30	1 71

Table 2: $^3J_{HH}$ (Hz) coupling constants* of **1** and **2** at 298 K

Compd	Sugar	1'2'	1'2"	2'2"	2'3'	2"3'	3'4'	4'5'	4'5"	5'5"	5'H6'	5'H6"	5"H6'	5"H6"
1	5'T	8 3	5 8	-14 7	5 7	2 2	1 9	3 6	3 6	-12 0	-	-	-	-
	3'T	5 6	7 2	-13 0	7 0	5 5	4 4	4 2	8 5	-12 0	5 9	10	10	5 1
2	5'T	6 3	7 0	-14 0	8 2	5 8	5 7	2 9	3 8	-12 1	-	-	-	-
	3'T	5 2	7 5	13 9	6 4	5 9	6 8	4 1	8 2	-12 0	5 4	10 5	10 3	4 9

*Error limit for $^3J_{HH}$ = ± 0 2 Hz for **1** and ± 0 3 Hz for **2**

Table 3: Pseudorotational parameters (P, ϕ) and population (%) of S-type conformer for **1** and **2**

	1		**2**		**3**[d]		**4**[d]		**5**[d]	
	5'T	3'T	5'T	3'T	Tp	pT	Tp	pST	Tcm	cmT
P_N	9[a]	0	26	b						
ϕ_N	36-38[a]	36	37	b						
P_S	170	185	146	b						
ϕ_S	36	32	37	b						
%S	87	56	52	49	74	66	76	64	78	65
error (Hz)[c]	0 9	1 03	0 48							

a P_N and ϕ_N were kept constant while P_S, ϕ_S and %S were redifined
b Not determined due to the 50% N,S equilibrium
c Sum of the differences between the five experimental coupling constants and their calculated values
d taken from ref # 5

2'-α-Substituted nucleosides also show the same behavior An increase of the polarity of 2'C-X bond produce a larger preference for the N-type conformation Thus, the population of the N conformer is lower in 2'-deoxyuridine (40% N) than in uridine (58% N) and 2'-azido-2'-deoxyuridine (58% N), while it is higher in 2'-

deoxy-2'-fluorouridine (87% N)[15] (Table 4) 2'-Amino-2'-deoxyuridine and 2'-amino-2'-deoxyadenosine show more S-type conformation than 2'-deoxyuridine and 2'-deoxyadenosine despite the higher electronegativity of

Table 4: Relation between electronegativity of C3'-α-substituent (X) and C2'-α-substituent (Y) and % S-type conformer in nucleosides

X	Δχ	%S	Ref	Y	Δχ	%S	Ref
2'-dU	0	60	15	3'-dA	0	25	15
2'-NH_2-dU	0 7	75	15	3'-NH_2-dA	0 7	18	15
2'-N_3-dU	1 0	42	15	ribo-A	1 3	64	15
ribo-U	1 3	42	15	3'-dAMP		77	16
2'-F-U	1 7	13	15				
				3'-N_3-dT	1 0	60	14
dA	0	65	15	2'-dT	1 3	70	16
2'-NH_2-dA	0 7	78	15	3'-dTMP		74	16
ribo-A	1 3	64	15	3'-F-dT	1 7	90	14
2'-F-dA	1 7	25	15				

Table 5: Calculated limited coupling constants (Hz) $J_{4'5'}$ and $J_{4'5''}$ for the three conformers γ^+, γ^t and γ^- about the C3'-C4'-C5'-X bond

	X = O			X = CH_2		
	γ^+	γ^t	γ^-	γ^+	γ^t	γ^-
$J_{4'5'}$	2 4	2 6	10 6	3 8	2 2	11 5
$J_{4'5''}$	1 3	10 5	3 8	1 9	11 5	4 1
$\phi_{4'5'}$	-64°	64°	174°	-64°	64°	174°
$\phi_{4'5''}$	55°	-178°	-68°	55°	-178°	-68°

Table 6: Rotamer distribution about the C4'-C5' bond (γ)

Compound		γ^+	γ^t	γ^-
1	5'T	65	20	15
	3'T	17	64	19
2	5'T	69	26	5
	3'T	21	62	17
3[a]	Tp	56	31	13
	pT	75	22	3
4[a]	Tp	53	38	9
	pST	32	47	21
5[a]	Tcm	13	67	20
	cmT	47	38	15

a taken from ref # 5

a nitrogen compared to hydrogen Similarly, a comparison of 3'-deoxyadenosine with 3'-amino-3'-deoxyadenosine is expected to show an increase of % S population but, in fact, one observes a slight decrease

of the S population (18% S) in the latter compared to the former (25% S) This fact suggests that it is primarily the polarity of the C2'-X2' (or C3'-X3') bond which dictates the strength of the *gauche* effect, and the contribution to the polarity to C3'-X or C2'-X by the above polar substituents (X) increases in the following order $NH_2 < H < OH \approx N_3 < F$

Table 7: Correlation between electronegativity and hydrogen bonding ability of 5'-substituent (Z) and population of γ^+ conformer[22]

Comp	Z	$\Delta\chi$	H-bonding	$\%\gamma^+$
3	O	1 3	+++	75
5	N	0 85	+++	47
4	S	0 4	+	32
1	C	0 4	-	17
2	C	0 4	-	21

Table 8: Calculated limiting coupling constants (Hz) $J_{5'6'}$, $J_{5'6''}$, $J_{5''6'}$ and $J_{5''6''}$ for the three conformers β^+, β^t and β^- about the C4'-C5'-C6'-S bond (columns 3, 4 and 5) and population distribution in compounds **1** and **2** (columns 6, 7 and 8) calculated from equation 4 with the J-couplings listed in Table 2

Comp	$^3J_{HH}$	β^+	β^t	β^-	β^+	β^t	β^-
1	$J_{5'6'}$	2 8	2.8	15 5	26	56	18
	$J_{5'6''}$	3 2	15 5	2 8			
	$J_{5''6'}$	2 8	15 5	2 7			
	$J_{5''6''}$	15 5	3 2	3 2			
2	$J_{5'6'}$	2 9	2 9	15 2	21	59	20
	$J_{5'6''}$	3 1	15 2	2 9			
	$J_{5''6'}$	2 8	15 2	2 8			
	$J_{5''6''}$	15 2	3 0	3 0			

The $J_{1'2'}$ and $J_{1'2''}$ coupling constants and the H6 and H1' chemical shifts were measured as a function of temperature (5 ° to 70 °C) The very small changes observed in the N $\rightleftarrows$ S equilibrium, together with the small changes of the H6 and H1' chemical shifts clearly suggest that the intramolecular base-base stacking is most probably not an important structural feature in compounds **1** and **2** It has been suggested[10] that for unstacked dimers, the thermodynamic parameters (ΔH° and ΔS°) controlling the ribose equilibrium depend more on the nature and the stereochemical orientation of the nucleobase and the phosphate backbone than on the conformation adopted by the neighboring ribose unit Thus, the N $\rightleftarrows$ S equilibrium in compounds **1-5** must be significantly determined by the *gauche* effect of the internucleoside linkage The enthalpy difference between the N and S conformers is close to zero with the S conformer having the greater entropy

Conformation of the glycosidic bond The conformation about the glycosidic bond was determined from 2D NOESY experiments (Fig 3) A nucleotide has a *syn* conformation when a strong nOe between the H6 and H1' together with a weak nOe between the H6 and H2' or H3' is observed A nucleotide with a S-type sugar is in *anti* conformation when a strong nOe between the H6 and H2' is observed while a nucleotide with a N-type

sugar has an *anti* conformation when a strong nOe between the H6 and H3' is observed In compound **1**, the H6 of the 5'-terminal nucleoside has an nOe cross peak with the H2' indicating an *anti* conformation The H6 of the 3'-terminal nucleoside shows stronger nOe cross peaks with the H2' and H3' indicating an *anti* conformation around the glycosidic bond In compound **2**, nOe cross peaks between H6-H1', H6-H2' and H6-H3' were found (Fig 3) to be weak, and since the sugars are in a 50% N $\rightleftarrows$ S equilibrium, it is difficult to assess the glycosidic bond conformation

Determination of the backbone conformation C4'-C5' bond (γ) The conformation about the C4'-C5' bond is described in terms of three staggered conformers, γ^+, γ^t and γ^- (Fig 4b) The population distribution between these conformers is calculated from the vicinal proton-proton coupling constants $J_{4'5'}$ and $J_{4'5''}$

$$^3J = (X\gamma^+) \times J'\gamma^+ + (X\gamma^t) \times J'\gamma^t + (X\gamma^-) \times J'\gamma^- \quad (1)$$

$$^3J = (X\gamma^+) \times J''\gamma^+ + (X\gamma^t) \times J''\gamma^t + (X\gamma^-) \times J''\gamma^- \quad (2)$$

$$[\text{when, } X\gamma^+ + X\gamma^t + X\gamma^- = 1]$$

The $J_{4'5'}$ and $J_{4'5''}$ of the individual rotamers have been calculated by Haasnoot et al using the generalized Karplus equation[17]

$$^3J_{HH} = 13\ 22 \cos^2(\phi) - 0\ 99 \cos(\phi) + \Sigma (0\ 87 - 2\ 46 \cos^2(\zeta_1\phi + 19\ 9\ \{\Delta\chi_1\})) \Delta\chi_1 \quad (3)$$

where ϕ is the proton-proton torsion angle, ζ_1 has a value of +1 or -1 depending on the orientation of the substituent, and $\Delta\chi_1$ is the difference in electronegativity between the substituent and the hydrogen

The C4'-C5' bond of the 5'-terminal nucleoside in **1** and **2** shows a preference for the γ^+ conformation, 65 and 69 % respectively (Table 6), which is also observed in naturally occurring nucleotides For the 3'-terminal nucleoside in **1** and **2**, one clearly observes the effect of the C4' linked exocyclic $CH_2^{5'}$-$CH_2^{6'}$ bond on the population of the staggered rotamers across C4'-C5' Inspection of Table 2 shows that the $J_{4'5'}$ and $J_{4'5''}$ are larger for the 3'-terminal nucleoside than for the 5'-terminal nucleoside In order to make such a comparison feasible, it is necessary to take into consideration the dependence of the vicinal proton-proton coupling constants on the electronegativity and orientation of the substituent An electronegativity factor $\Delta\chi_1$ of 0 4[18] was therefore introduced for C6' in equation 3, and the limiting coupling constants for each individual rotamer γ^+, γ^t and γ^- were calculated from this equation using the torsion angles given by Haasnoot *et al* [17] The calculated J-couplings are listed in Table 5 and were employed in equations 1 and 2 to calculate the rotamer distribution about the C4'-C5' bond (Table 6) Table 6 shows that upon substitution of the 5'-oxygen by a 5'-CH_2 group, the γ^+ conformation is substantially less populated (17% γ^+ in **1** and 21 % γ^+ in **2**), while the γ^t conformer becomes the most populated (64 % γ^t in **1** and 62 % γ^t in **2**) The distinction between the γ^t and γ^- conformers requires the unambiguous assignment of the H5' and H5" If the assignment is reversed, the effect is to interchange the γ^t and the γ^- population It is well established however that in nucleosides, there is a marked preference for the γ^+ rotamer, the γ^- rotamer being rarely encountered This preferred γ^+ conformation is determined in part by the *gauche* effect The polar C5'-O5' bond is most stable when it is *trans* to the C4'-H4' bond giving rise to the γ^+ conformation (Figure 4B) The most unfavorable conformation is that in which two vicinal polar C-O bonds are *trans* orientated (γ^-) while the γ^t rotamer is of intermediate stability Since in the absence of base at C1', the population of γ^+ and γ^t conformers are approximatively equal[19], intramolecular hydrogen bonding between pyrimidine H6 and O5' also contributes to the stabilization of the γ^+ conformation[22] In d(TpST) **4**, where the 5'-oxygen is replaced by a 5'-sulfur and in NH_2d(TcmT) **5**, where the 5'-oxygen is replaced by 5'-nitrogen, the population of γ^+ conformer (Table 6) is also reduced (32% γ^+ in **4** and 35% γ^+ in **5**) In Table 7, we have correlated the γ^+ population with the electronegativity and the hydrogen

bonding ability of the 5'-substituent. The γ^+ population decreases primarily with a decrease in the electronegativity of the 5'-substituent (O > N > S ≈ C). A 5'-oxygen which has the higher electronegativity and a good ability to hydrogen bond has the higher γ^+ population A nitrogen is a good hydrogen bond acceptor, but its electronegativity is lower and therefore the γ^+ population is lower The *gauche* effect is weaker with a sulfur because of its lower electronegativity, hence it controls γ^+ population in a much less effective manner Also since the carbon-sulfur bond is longer than a carbon-oxygen bond, the H6 of pyrimidine is further apart and therefore H6-S5' hydrogen bond in **4** is not very important.

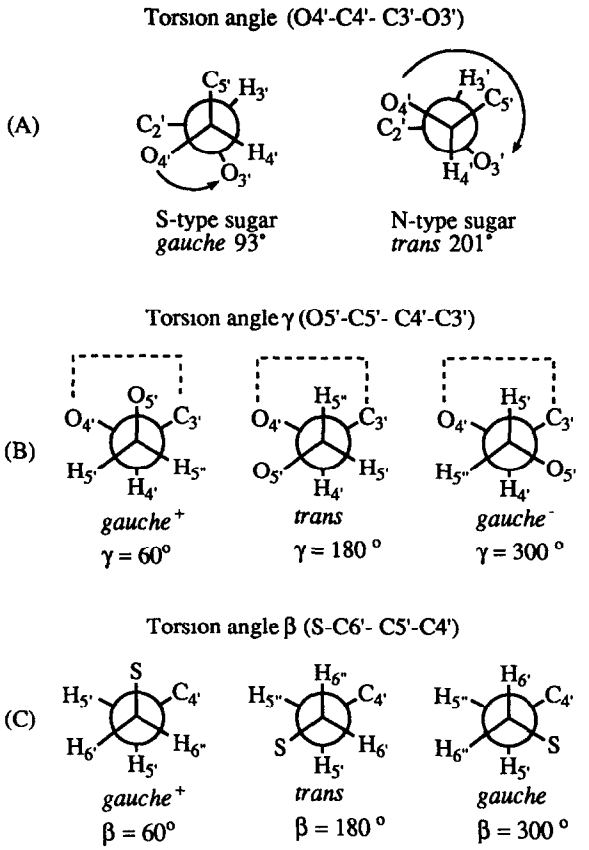

Figure 4. Newman projections along the (A) C3'-C4', (B) C4'-C5' and (C) C5'-C6' bonds

Conformation about the C5'-C6' bond ("β") In analogy with the nomenclature used to define the torsion angles in a nucleotide[20], the torsion angle about the C5'-C6' bond was denoted as β As for the C4'-C5' bond, the conformation about the C5'-C6' bond can be defined as an equilibrium between the three staggered conformation β^+, β^t and β^-, shown in Figure 4C The conformation about this bond is then monitored by the coupling constants $J_{5'6'}$, $J_{5'6''}$, $J_{5''6'}$ and $J_{5''6''}$ Knowing the proton-proton torsion angles ϕ, it should be possible to calculate the limiting $J_{5'6'}$, $J_{5'6''}$, $J_{5''6'}$ and $J_{5''6''}$ for each individual rotamer using the Karplus equation derived by Altona *et al* for the $CH_2^{5'}$-$CH_2^{6'}$ moiety[21]

$$^3J_{HH} = 13\ 89 \cos^2(\phi) - 0\ 98 \cos(\phi) + \Sigma (1\ 02 - 3\ 40 \cos^2(\zeta_1\phi + 14\ 9\ \{\Delta\chi_1\})) \Delta\chi_1 \quad (4)$$

$$\text{with } \Delta\chi_1 = \Delta(\chi_1)^{\alpha} - 0\ 24\ \Sigma\Delta(\chi_1)^{\beta} \quad (5)$$

The limiting coupling constants for each rotamer were calculated from equation 4 Taking into account the electronegativity of the β substituents (equation 5), an electronegativity factor $\Delta\chi_1$ of 0 27 was taken for C4' and an electronegativity factor $\Delta\chi_1$ of -0 54 was taken for the 6'-S substituent Since no data were available for the values of the torsion angles, the proton-proton torsion angles were assumed to be ± 60° and ± 180° The population distribution between the three conformers was then calculated using equations 1 and 2 From Table 8, it can be seen that the C5'-C6' bond has a slight preference for the β^t conformation (56 % β^t in **1** and 59 % β^t in **2**) In d(TpT) **3**, the C5'-O5' bond accounts for 83% of β^t conformation Upon substitution of the 5'-oxygen by 5'-sulfur in d(TpST) **4**, the population of β^t conformer was found to decrease to 57 % Again, as for the conformation of the sugar ring and the conformation around the C4'-C5' bond, this decrease in the β^t population must be due in part to a decrease of the polarity of the 5'-S-P bond compared to the 5'-O-P bond, which weakens the *gauche* effect

Conclusion The conformation of compounds **1** and **2** is characterized by the following features For the 5'-terminal nucleoside of compound **1** An S-type sugar, a C4'-C5' bond in the γ^+ conformation and an *anti* orientation of the base about the glycosidic bond The 3'-terminal nucleosides of compound **1** and compound **2** are in ca 50% N ⇄ S equilibrium and the γ^t conformer is the most populated The 5'-terminal nucleoside of compound **1** shows a 50% N ⇄ S equilibrium, and has the C4'-C5' bond in the γ^+ conformation From the comparison of the conformational properties of **1** and **2** with the natural d(TpT) **3** and with d(TpST) **4** and NH_2d(TcmT) **5**, the following points can be drawn (i) The conformational preference of the sugar ring is partially determined by the *gauche* effect The more polar the C3'-X bond, the more the N ⇄ S equilibrium is biased toward the S-type conformation 3'-OS > 3'-OP > 3'-O-H > 3'-N_3 > 3'-NH_2 (ii) The conformation about the C4'-C5' bond (γ) is influenced by the *gauche* effect (nature of the 5'-substituent) and by the ability of the 5'-substituent to form hydrogen bonding with the H6 of the base[22] Thus, the population of γ^+ conformer follows the order 5'-O > 5'-N > 5'-S > 5'-C Since the conformation of the internucleoside linkage is significantly different from the conformation of the phosphate backbone of natural d(TpT) **3**, one can expect that the formation of duplexes with natural oligonucleotides will be destabilized

Experimental

Compounds **1** and **2** were dissolved in 0 5 ml of a 50 50 ^2H_2O, DMSO and transferred into 5 mm tubes The sample concentration was 3 mM for **1** and **2** For the measurements of the proton chemical shifts, a trace of acetonitrile was added as an internal reference (set at 2 00 ppm) The NMR experiments were performed on a BRUKER AMX-500 MHz spectrometer The two-dimensional NMR spectra, DQF-COSY[23], HOHAHA[24] and NOESY[25], were recorded in pure-phase absorption mode with the time proportional incrementation method (TPPI) and with low power preirradiation of the residual HOD peak during the relaxation delay The DQF-COSY spectra were acquired with 8K data points in t_2 and 512 points in t_1 The data were zero filled to give a 8K x 1K matrix and a sine square window was applied in both directions before Fourier transformation The HOHAHA and NOESY spectra were acquired with 2K data points in t_2 and 256 points in t_1 For the NOESY experiments, a mixing time of 800 ms was used

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$^3J_{HH} = 13\ 22 \cos^2(\phi) - 0\ 99 \cos(\phi) + \Sigma (0\ 87 - 2\ 46 \cos^2(\zeta_i\phi + 19\ 9\ \{\Delta\chi_1\}))\ \Delta\chi_1$

For the 3'-4' coupling constants, the following Karplus equation is used

$^3J_{HH} = 13\ 24\cos^2(\phi) - 0\ 91\cos(\phi) + \Sigma(0\ 53 - 2\ 41\cos^2(\zeta_i\phi + 15\ 5\{\Delta\chi_1\}))\Delta\chi_1$ with $\Delta\chi_1 = \Delta\chi_1^{\alpha} - 0\ 19\ \Sigma\Delta\chi_1^{\beta}$

The electronegativities input for the PSEUROT for a deoxysugar with a 3'-N substituent are

1',2'	0 85	1 3	0 0	0 4
1',2"	0 85	1 3	0 4	0 0
2',3'	0 85	0 4	0 4	0 0
2",3'	0 85	0 4	0 0	0 4
3',4'	1 22	0 32	0 32	0 69

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