Effect of (5′*S*)-5′,8-cyclo-2′-deoxyadenosine on the conformation of di and trinucleotides. A NMR and DFT study

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5′,8-Purine cyclonucleosides constitute an important class of oxidatively generated tandem lesions whose formation involves initial hydroxyl radical-mediated hydrogen atom abstraction from the 5-hydroxymethyl group of 2-deoxyribose followed by intramolecular cyclization. The present study deals with the synthesis of the 5′*S* diastereomer of 5′,8-cyclo-2′-deoxyadenosine containing di- and tri-oligodeoxynucleotides as an attempt to delineate the conformational changes induced in the DNA fragments by the presence of a rigid modified nucleoside. For this purpose, extensive 1D and 2D NMR measurements that were completed by DFT theoretical calculations were performed. As a striking result, it was found that the covalent bond between C(5′) and C(8) in the investigated purine cyclonucleoside induces an unusual West ($_0$T^1) conformation of the furanose ring. Thus it can be postulated that the rigid structure of the tandem lesion would strongly perturb the global geometry of oligonucleotides at the site of the modification and therefore affect the enzymatic activity of DNA polymerases and repair enzymes.

Introduction

Oxidation reactions that may be caused by endogenous and/or exogenous chemical and physical agents are ubiquitous in living cells.[1] Reactive oxygen species (ROS) including the superoxide anion radical, hydrogen peroxide and the •OH radical[2,3] may be produced through respiration burst, water radiolysis or photosensitization reactions. Oxidatively generated lesions to DNA are likely to be involved in the etiology of several human pathologies[4–6] including cancers, Parkinson's and Alzheimer's diseases, to name but a few. In the past 20 years, more than 80 oxidatively generated base and sugar lesions have been isolated and identified as the result of extensive model studies.

The most frequent oxidized bases that have been detected so far in cellular DNA are 8-oxo-7,8-dihydroguanine,[7] 8-oxo-7,8-dihydroadenine,[8] 2,6-diamino-4-hydroxy-5-formamidopyrimidine,[9] 4,6-diamino-5-formamidopyrimidine,[9] 5-hydroxycytosine,[10] 5-hydroxyuracil[11] and 5,6-dihydroxy-5,6-dihydrothymine.[12] These lesions are characterized by the unrestricted flexibility of the modified base about the *N*-glycosidic bond of related nucleosides. In contrast, 5′,8-cyclo-2′-deoxyadenosine (cdA)[13a,b] and 5′,8-cyclo-2′-deoxyguanosine (cdG)[14a,b] that may be generated by •OH radical-mediated hydrogen atom abstraction at C5′ present a rigid structure with limited possibility of conformational changes. Purine 5′,8-cyclonucleosides that exhibit a carbon–carbon linkage between the sugar and the base units may be considered as tandem DNA modifications since both the purine unit and the 2-deoxyribose moiety are altered. It may be added that purine 5′,8-cyclonucleosides exist as pairs of *R* and *S* diastereomers due to the presence of a chiral centre at C(5′) (Fig. 1). There are several lines of evidence showing that the diastereomeric nucleosides behave differently as substrates towards dedicated enzymatic activity. For example (5′*S*)-5′,8-cyclo-2′-deoxyadenosine completely blocks the progression of polymerases involved in oligonucleotide chain extension whereas the 5′*R* diastereomer has a less pronounced effect.[15]

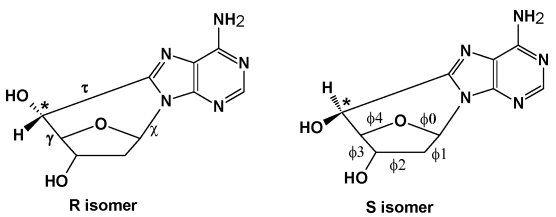

Fig. 1 The 5′*R* and 5′*S* diastereomers of 5′,8-cyclo-2′-deoxyadenosine with indication of the torsional angles in the sugar ring (symbols ϕ_0; ϕ_1; ϕ_2; ϕ_3; ϕ_4) and the glycosidic angle (χ). τ indicates the new torsional angle that is specific for cyclic nucleosides.

Both 5′*R* and 5′*S* diastereomers of cdA that are not substrates for the enzymes of the base excision repair (BER)[16a] pathways are excised from oligonucleotides by proteins acting *via* the nucleotide excision repair (NER) mechanism.[16b] Interestingly, the 5′*R* form was found to be more efficiently removed from cdA containing oligonucleotides than the 5′*S* diastereomer.

This is likely to be the result of different conformational features in the 5′*R* and 5′*S* diastereomers for which the sugar ring adopts an unusual $_0$T^1 puckered form. The present study is aimed at delineating the conformational features of the 5′*S* diastereomer of either free cdA (**1**) or when inserted into two

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short oligonucleotides, namely $T_P$cdA (**2**) and $T_P$cdA$_P$T (**3**). This was achieved by both extensive NMR studies in aqueous solutions and theoretical DFT calculations.

Results and discussion

Synthesis of cdA containing di- and trinucleotides

The (5′*S*)-5′,8-cyclo-2′-deoxyadenosine (**1**) and related suitable derivatives for short oligonucleotide synthesis were prepared by the method described by Romieu *et al.*[13] The $T_P$cdA **2** and $T_P$cdA$_P$T **3** were synthesized by solid phase synthesis on a 1 μmol scale using the classical phosphoramidite strategy[17] with a slight modification, as applied by Brooks *et al.*[18] The synthetic products were purified according to a one-step method that involved the use of reverse phase-HPLC in the trityl off mode.

^{1}H and ^{31}P features of cdA 1, $T_P$cdA 2 and $T_P$cdA$_P$T 3

The ^{1}H NMR and ^{31}P decoupled ^{1}H NMR analysis for each compound was performed at two temperatures, namely 25 and 41 °C. NMR signals collected at 25 °C were used for the determination of the chemical shifts *via* straightforward 2D COSY[19] NMR experiments. The set of data obtained, reported in Table 1, constituted the starting points for further investigations. We expected to observe changes in the sugar conformation associated with two phenomena. First, the nucleoside adjacent to 5′,8-cyclo-2′-deoxyadenosine should adopt a conformation that does not correspond to the global energy minimum. Second, the energy supplied by the temperature increase should facilitate the formation of other more or less probable conformations. In fact, no significant changes in the vicinal proton coupling constants were observed; the largest variation being 0.8 Hz, which corresponds to a change in the dihedral angle by *ca.* 3° (Table 2). Similar effects have been noted for the methylenic protons at C5′ investigated at the same temperature.

Table 1 Chemical shifts [ppm] of sugar and base protons from 500 MHz ^{1}H NMR spectra at 25 °C in D_2O

Chemical shifts of protons						
		2		**3**		
Proton	**1**	T_P	cdA	T_P	cdA	$_P$T
1′	6.47	6.28	6.43	6.27	6.51	6.29
2′	2.63	2.46	2.63	2.43	2.64	2.28
2″	2.23	2.70	2.22	2.67	2.47	2.29
3′	4.74	4.95	4.79	4.96	5.10	4.57
4′	4.76	4.27	4.95	4.28	5.08	4.13
5′		3.87		3.83		4.10
5″	5.32	3.80	5.67	3.79	5.73	4.06
2	8.13		8.10		8.16	
5		1.83		1.64		1.84
6		7.62		7.62		7.65

Sugar moiety conformation

Prior to reporting the results of both experimental and theoretical studies dealing with the effect of the rigid structure of (5′*S*)cdA **1** on the conformation features of $T_P$cdA **2** and $T_P$cdA$_P$T **3**, whose structures are shown in Fig. 2, a few general considerations on the conformational properties of the 2-deoxyribose moiety in either the free nucleoside or DNA are provided. The geometry of double- and single-stranded nucleic acids depends in most cases on the flexibility of the 2-deoxyribofuranose ring and on the type of the phosphate linkage.[20] From NMR measurements, X-ray data and quantum calculations, it is well documented that the 2-deoxyribose five-membered ring preferably adopts the C(2′)-*endo* ^{2}E and C(3′)-*endo* ^{3}E conformations also referred to as S and N, respectively.[21]

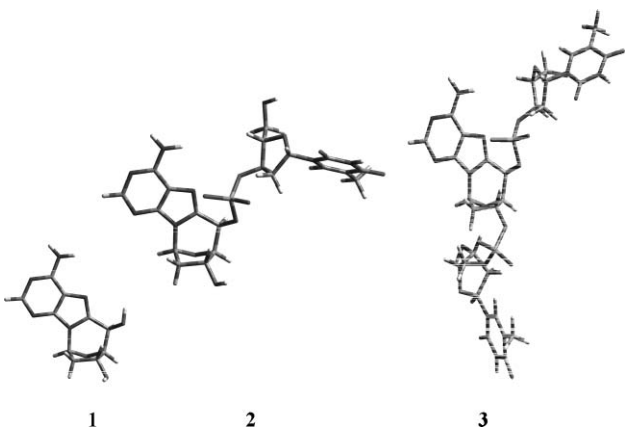

Fig. 2 Three dimensional structure of cdA **1**, $T_P$cdA **2** and $T_P$cdA$_P$T **3** inferred from NMR data.

According to the pseudorotation concept,[22] the N↔S and S↔N interconversions are possible through O(4′)-*endo*; °E, (East-(E)-type) or O(4′)-*exo*; $_0$E(West-(W)-type) conformations. From an energetic point of view, the pathway through °E is much more attractive than the $_0$E one; the energetic barriers are 1.8 and 5.6 kcal respectively.[23a,b,24,25] Moreover, in the $_0$E geometry, both the nucleobase and the 5′-CH_2OH group are in an unfavorable axial

Table 2 Vicinal coupling constants $^3J_{H-H}$ [Hz] obtained at (a) 25 °C; (b) 41 °C for the sugar moieties of compounds **1**, **2** and **3**

a											b								
		Coupling constants at 25 °C									Coupling constants at 41 °C								
Compounds		1′–2′	1′–2′	2′–2″	2′–3′	2″–3′	3′–4′	4′–5′	4′–5″	5′–5″	1′–2′	1′–2′	2′–2″	2′–3′	2″–3′	3′–4′	4′–5′	4′–5″	5′–5″
cdA **1**	cdA	< 0.2	4.9	−13.9	7.5	4.6	< 0.2		6.2		< 0.2	4.9	−13.8	7.6	4.7	< 0.2		6.2	
$T_P$cdA **2**	T_P	6.5	7.3	−14.5	7.1	3.1	3.3	3.5	4.7	−10.2	6.7	6.9	−14.3	7.1	3.2	3.4	3.5	4.8	−12.5
	cdA	< 0.2	4.6	−13.8	7.5	4.5	< 0.2		6.1		< 0.2	4.8	−13.9	7.5	4.4	< 0.2		6.2	
$T_P$cdA$_P$T **3**	T_P	6.4	6.0	−14.1	7.2	2.6	4.0	3.7	4.5	−12.4	6.7	6.0	−13.9	7.0	2.2	3.2	3.2	4.6	−13.0
	cdA	< 0.2	4.7	−14.3	7.5	4.2	< 0.2		6.1		< 0.2	4.4	−13.7	7.3	4.0	< 0.2		6.1	
	$_P$T	6.9	6.9		4.8	4.6	3.2	2.4	2.8	−11.6	7.2	7.2		4.7	4.	3.2	2.6	3.3	−11.5

position (2.9 Å); in contrast, in the °E form both substituents are in more suitable equatorial positions (4.6 Å).[20] Only a few examples of nucleosides that contain the W-type of sugar have been reported in the literature so far.[26,27] It can be assumed, therefore, that if they present in the genome, their unusual structures should play an important role in the biological processing of these unusual cyclic nucleosides.

The conformation of the five-membered furanose ring

In order to further elucidate these results and to verify the proposed working hypothesis, attempts were made to assess the distances between the sugar protons by 2D NOESY[28] experiments at 25 °C (Table 3). From these data and with the help of molecular mechanistic calculations,[29] we have been able to build, separately for the three studied molecules, a three-dimensional structure corresponding to each of the furanose rings. In order to achieve the calculations, the previously determined distances were kept fixed. The conformation of the five-membered ring is described using the pseudorotation concept mentioned previously. Thus, the relationship between the five torsion angles $\phi_{(0-4)}$ (Fig. 1) is described by simple equations (eqn (1), (2)) relating the phase (P) and the amplitude (ϕ_{max})[30] (Table 4). It has been shown that the sugar moiety in (5′*S*)cdA adopts an unusual W-west conformation, ${}_0\mathrm{T}^1$.

$$\tan(P) = [(\phi_4 + \phi_1) - (\phi_3 + \phi_0)]/[2\phi_2\,(\sin(36) + \sin(72))] \quad (1)$$

$$\phi_{max} = \phi_2/\cos(P) \quad (2)$$

Moreover, it may be inferred by considering the ${}^3J_{H-H}$ data over the temperature range investigated that the conformation of the rigid (5′*S*)cdA unit is not affected by the temperature within the wide range studied. In addition, the results thus obtained suggest that the 5′ and 3′ sugars of the nucleosides attached to cdA **1** adopt predominantly a S conformation.

The orientation of the hydroxyl group with respect to the γ-bond

The spatial orientation around the C4′–C5′ bond is expected to have the most pronounced influence on the geometry of the 5′ internucleotide phosphodiester linkage. The distribution frequency of the three possible staggered conformers depicted in Fig. 3: g^+ (*gauche* +), t (*trans*), g^- (*gauche* −) is not equivalent.

Table 4 Value of inter-sugar dihedral angles, sugar conformation phase P and amplitude ϕ_{max} of sugar calculated from 2D NOESY NMR experiments

		cdA 1	$T_P$cdA 2		$T_P$cdA$_P$T 3		
Angle			T_P	cdA	T_P	cdA	${}_P$T
	ϕ_0	45.2	−4.3	44.7	19.4	48.4	−7.3
	ϕ_1	−27.3	30.4	−30.7	4.5	−32.7	31.4
	ϕ_2	1.3	−43.8	6.3	−24.8	7.3	−41.8
	ϕ_3	25.1	43.1	19.6	37.0	20.4	39.8
	ϕ_4	−44.8	−23.8	−40.3	−35.5	−43.8	−20
Phase	P	272	193	278	229	279	189
Amplitude	ϕ_{max}	37.1	45.1	44.4	37.7	47.7	42.4
Puckering		${}_0\mathrm{T}^1$	${}_3\mathrm{T}^2$	${}_0\mathrm{T}^1$	${}^4\mathrm{T}_3$	${}_0\mathrm{T}^1$	${}_3\mathrm{T}^2$

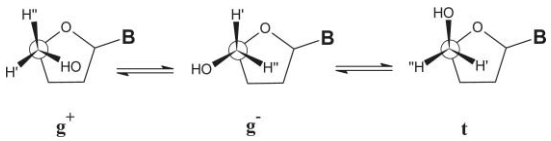

Fig. 3 Graphical view of different staggered rotamers of the 5′-hydroxymethyl group of nucleosides.

Their populations depend on the sugar puckering and the substituents that are present in the nucleobases. The rotameric populations have been estimated from the coupling constants between H(4′) and H(5′,5″) using the method described by Westhof *et al.*[31] (eqn (3), (4), (5)), for different temperatures in the range of 25–41 °C.

$$\%\gamma^{(g+)} = \{1.46 - [{}^3J_{4',5'} + {}^3J_{4',5''}]/8.9\}100 \quad (3)$$

$$\%\gamma^{t} = \{({}^3J_{4',5''}/8.9) - 0.23\}100 \quad (4)$$

$$\%\gamma^{(g-)} = \{({}^3J_{4',5'}/8.9) - 0.23\}100 \quad (5)$$

It has been found that in both **2** and **3**, $\gamma^{(g+)}$ and $\gamma^{(t)}$ rotamers predominate. This means that in the investigated structures, the S-type sugar pucker is the most abundant for the normal nucleosides at both 5′ and 3′ ends (Table 5).

Moreover, these results are in good agreement with the estimation of the rotamers that was achieved considering the values of ${}^3J_{H-H}$ measured by ^{1}H NMR at different temperatures and using eqn (6), (7) (Table 5). The situation is different for **1** because the

Table 3 Selected distances of sugar protons calculated by the [1]DFT B3LYP 6-31G** level and from a [2]2D NOESY NMR experiment. (obs = obscured.)

		Inter molecular distances [Å] between inter-ring protons					
System		**1′–2′**	**1′–2″**	**2′–3′**	**2″–3′**	**3′–4′**	**4′–5′**
cdA **1**	cdA[1]	2.75	2.48	2.43	3.00	2.83	2.45
	cdA[2]	2.95	2.60	2.4	2.99	obs	2.32
$T_P$cdA **2**	T_P[1]	2.84	2.36	2.46	3.0	3.05	
	T_P[2]	3.1	2.3	2.3	2.6	2.4	
	cdA[1]	2.74	2.49	2.42	3.00	2.86	2.46
	cdA[2]	2.8	2.4	2.4	3.1	obs	2.3
$T_P$cdA$_P$T **3**	T_P[1]	2.76	2.37	2.55	3.0	3.0	
	T_P[2]	2.7	2.45	2.4	3.0	2.6	
	cdA[1]	2.87	2.42	2.25	2.85	2.75	2.37
	cdA[2]	2.94	2.5	2.5	3.2	obs	2.4
	${}_P$T[1]	3.05	2.42	2.44	2.73	2.69	
	${}_P$T[2]	obs	2.4	2.25	obs	2.4	

Table 5 Distribution of the rotamers about the C4′–C5′ bond and sugar puckered conformer population inferred from ^{1}H NMR and ^{1} H{^{31}P} NMR coupling constants at 25 °C and 41 °C

		Conformation %γ									Population of conformers			
		dA and cdA			T_P			$_PT$			T_P		$_PT$	
System		$g^{(+)}$	t	$g^{(-)}$	$g^{(+)}$	t	$g^{(-)}$	$g^{(+)}$	t	$g^{(-)}$	S	S/N	S	S/N
dA[32]		59	25	16										
cdA 1														
25 °C	$T_P$cdA			100	55	29	16				69	1.99		
	$T_P$cdA$_P$T			100	56	27	18	87	9	4	69	1.59	74	2.17
41 °C	$T_P$cdA			100	53	30	17				72	2.0		
	$T_P$cdA$_P$T			100	59	29	12	80	14	6	72	2.10	77	2.23

rotation around C4′–C5′ bond is prevented, and only one $\gamma^{(g-)}$ conformer can exist.

$$X_S = 100(J_{1',2'}/9.3) \quad (6)$$

$$K_{eq} = S/N = J_{1',2'}/J_{3',4'} \quad (7)$$

Internucleotide backbone geometry determination

In the final step, the ^{1}H-^{31}P coupling constants have been assigned with the aim of assessing the dihedral angle ε [C(4′)–C(3′)–O(3′)–P] and β [P-O(5′)–C(5′)–C(4′)] (Table 6) using the Karplus equation (eqn (8), (9))[32] parameterized by Mooren *et al.*[33,34]

$$^3J_{H3'-P3'} = 15.3\cos(\varepsilon + 120) - 6.2\cos(\varepsilon + 120) + 1.5 \quad (8)$$

$$^3J_{H5'-P3'} = 15.3\cos(\beta - 120) - 6.2\cos(\beta - 120) + 1.5 \quad \text{for H-5}' - 120 \text{ for H-5}'' + 120 \quad (9)$$

In contrast to the sugar moieties in the 3′ and 5′ terminal positions, which show a significant structural flexibility, the γ [O(5′)–C(5′)–C(4′)–C(3′)] and δ [C(5′)–C(4′)–C(3′)–O(3′)] in cdA are fixed due to the rigidity of the molecule. The remaining two parameters that could be of structural relevance *i.e.* α [C(3′)–P–O(5′)–C(5′)], ξ [C(3′)–O(3′)–P–O(5′)] are not accessible to measurement in this analysis.

Based on the data derived from the above available measurements and calculations, attempts were made to build three dimensional models of **1**, **2** and **3** in which the cdA component appears as an extremely stable element of the structure (Fig. 2).

Molecular structure of cdA 1, $T_P$cdA 2 and $T_P$cdA$_P$T 3 from density functional theory studies

It should be noted that X-ray data are available only for 5′,8-cycloadenosine (cA),[27] the ribofuranose derivative of cdA. We have therefore chosen to use quantum mechanics and a DFT calculation[35] with the goal being to cross-check the experimental data inferred from NMR. We have found that the conformation of the fixed sugar ring of the cyclonucleoside is independent of the substituents, while the torsional angles and the distances in cdA are similar to those measured in cA. Moreover, we could not observe any changes in the structure of (5′*S*)-5′,8-cyclo-2′-deoxyadenosine independently, whether we modelled it as the cdA monomer or as conjugates such as $T_P$cdA or $T_P$cdA$_P$T. Surprisingly, the crystallographic structural data of cA[27] were consistent with those inferred from the quantum chemical calculations for all structures studied that exhibit the $_0T^1$ conformation for the sugar moiety. Another point to be noted from the DFT calculations is that the 5′-furanose ring adopts the ($^3T_4$) N form in **2** and **3**. A different structure is found for the 3′ sugar, which shows the typical S conformation $_3T^2$ as in non modified nucleosides (Table 7).

Conclusions

In conclusion, we have synthesized and studied by NMR and DFT the conformational features of the 5′*S* diastereomer of cdA **1** as a model of tandem base modifications occurring in DNA as the result of exposure to ionizing radiation or an •OH radical. It has been demonstrated that the covalent bond between C(5′) and C(8) in the cyclic nucleoside induces an unusual West ($_0T^1$) conformation for the furanose ring. Moreover, the postulated geometry is independent of the substituents of the furanose

Table 6 Coupling constants $^3J_{H-P}$ [Hz] measured at 25 and 41 °C for the base-linked sugar

Temp. °C	System	^{T}3′-P	P-^{cdA}5′	^{cdA}3′-P	P-^{T}5′ P-^{T}5″
25	$T_P$cdA	obs	10.0		
	$T_P$cdA$_P$T	9.5	9.9	10.7 obs	About 7.7
Dihedral angle calculated at 25 °C		$\alpha_1 = -102.6$ $\alpha_2 = 3.2$	$\alpha_1 = 123.9$ $\alpha_2 = 224.5$	$\alpha_1 = -120$ $\alpha_2 = 6.9$	$\alpha_1 = 149.5$ $\alpha_2 = 237.7$	
41	$T_P$cdA	obs	9.6		
	$T_P$cdA$_P$T	9.5	9.7	12.1 obs	

Table 7 Values of torsion angles and pseudorotation parameters in the sugar moieties calculated by (A) DFT B3LYP method with 6-31G** and (B) crystal structures

		Torsion angle value/°					$^{(A)}T_PcdA$ **2**		$^{(A)}T_PcdA_PT$ **3**		
Angle		$^{(B)}dA$[25a]	$^{(A)}dA$	$^{(B)}cA$[26]	$^{(A)}cdA$	$^{(B)}dT$[24b]	T_P	cdA	T_P	cdA	T_P
$O^{4'}–C^{1'}–N^9–C^2$	χ					−139.5	−118.1		176.3		−119.1
$C^{4'}–O^{4'}–C^{1'}–C^{2'}$	ϕ_0	3.4	19.0	50.6	48.1	−7.4	−9.7	48.8	0.13	47.6	−5.0
$O^{4'}–C^{1'}–C^{2'}–C^{3'}$	ϕ_1	−24.9	8.3	−40.6	−36.0	28.4	−12.6	−38.3	−24.9	−34	27.7
$C^{1'}–C^{2'}–C^{3'}–C^{4'}$	ϕ_2	35.3	−30.0	15.9	10.8	−37.2	28.1	13.4	39.03	8.4	−38.8
$C^{2'}–C^{3'}–C^{4'}–O^{4'}$	ϕ_3	−34.1	41.7	12.6	16.9	33.8	−34.4	14.5	−39.3	19.2	36.4
$C^{3'}–C^{4'}–O^{4'}–C^{1'}$	ϕ_4	19.6	−38.5	−39.1	−40.7	−16.8	28.4	−39.4	25.0	−41.7	−19.8
$C^{3'}–C^{4'}–C^{5'}–C^8$	τ			62.2	68.3			65.3		66.8	
$O^{4'}–C^{1'}–N^9–C^4$	χ	−165.1	−175.7	−161.2	−149.6			−152.5		−147	
Pseudorotation parameters											
Phase	P	13.2	224.5	288.9	283.2	187.4	34.7	286.3	18.1	280.2	191.1
Amplitude	ϕ_{max}	36.2	42.3	49.1	47.4	37.6	34.2	47.7	41.0	47.1	39.55
Puckering		$^3T_2$	$^4T_3$	$^1T_4$	$_0T^1$	$_3T^2$	$^3T_4$	$_0T^1$	$^3T_4$	$_0T^1$	$_3T^2$

ring. An excellent agreement has been reached between available crystallographic data, DFT calculations and NMR measurements. As a result, this converges to essentially the same puckering, amplitude, and distance values between protons in cdA **1**. This clearly indicates that the 5-membered sugar ring adopts a similar conformation in both the 2′-deoxy and ribonucleosides with a rigid geometry that is temperature independent. Within the range of temperatures studied (25–41 °C) no significant changes in the values of $^3J_{H–H}$ have been observed for cdA **1**, similar to the case of d[T_PAP$_P$T] (AP – apurinic/apyrimidinic site).[36] Finally, by combining 2D NOESY experiments with simple molecular mechanics calculations and using fixed inter-proton distances, it has been demonstrated that the 5′ and 3′ sugar residues of nucleosides **2** and **3** adopt predominately S conformations, namely $_3T^2$ and $^4T_3$. It can therefore be postulated that the rigid and fixed structure of cdA **1** can strongly influence the global geometry of oligonucleosides.

Experimental part

Synthesis of cdA 1, $T_P$cdA 2 and $T_P$cdA$_P$T 3

(5′*S*)-5′,8-Cyclo-2′-deoxyadenosine and related derivatives for short oligonucleotide synthesis were obtained by the method described by Romieu *et al.*[13] The short oligonucleotides $T_P$cdA **2** and $T_P$cdA$_P$T **3** were synthesised according to a solid phase synthesis approach on a 1 μmol scale using the classical phosphoramidite strategy[17] with a slight modification applied by Brooks *et al.*[18] This was performed on an Applied Biosystem 392 DNA/RNA synthesizer according to the trityl off mode. The synthesized products were purified in one-step RP-HPLC in the $DMT^{OFF\ mode}$. For this purpose, a Supelco column, Discover® RP-C18 (25 cm x 4.6 mm, 5 μm), was used. The elution was achieved using 0.1M triethylammonium acetate (TEAA) as the buffer with a gradient for compound **1** from 0 to 20% of acetonitrile in 50 min, and for compounds **2**, **3** from 0 to 14% of acetonitrile in 60 min; detection $\lambda = 260$ nm;.

cdA **1**; yield (total yield after 9 steps 30%), RP-HPLC (TEEA–acetonitrile) $R_t = 27.4$ min; UV (H_2O) $\lambda_{max} = 267$ nm; CD (H_2O) negative $\lambda_{max} = 263$ nm; *m/z* (ESI) 250.0 ([M − H]$^+$, requires 250.23), *m/z* 248.2 ([M − H]$^-$, requires 248.23). $T_P$cdA **2**; yield 95% (under HPLC integration), RP-HPLC (TEEA–acetonitrile) $R_t = 54.1$ min; UV (H_2O) $\lambda_{max} = 267$ nm; CD (H_2O) negative $\lambda_{max} = 254$ nm; *m/z* (ESI) 552.1 ([M − H]$^-$, requires 552.42). $T_P$cdA$_P$T **3**; yield 70% (under HPLC integration), RP-HPLC (TEEA–acetonitrile) $R_t = 53.6$ min; UV (H_2O) $\lambda_{max} = 267$ nm; CD (H_2O) negative $\lambda_{max} = 252$ nm, positive $\lambda_{max} = 278$ nm; *m/z* (ESI) 856.3 ([M − H]$^-$, requires 856.61).

Computation methodology

Quantum mechanics study. The molecular structures of cdA **1**, $T_P$cdA **2** and $T_P$cdA$_P$T **3** were calculated using a DFT (density functional theory) approach with B3LYP (Becke's three-parameter exchange functional and the gradient-corrected functional of Lee, Yang and Parr). For cdA **1**, $T_P$cdA **2** and $T_P$cdA$_P$T **3**, the 6-31G** base was used. The calculation for cdA **1** was performed with Gaussian 98 whereas analysis of $T_P$cdA **2** and $T_P$cdA$_P$T **3** was achieved with Gaussian 03 Revision B.05.[37]

Molecular mechanics study. The structures of the sugar moieties of **1**, **2** and **3** were calculated by the CAChe 6.1.12.31, trial version (Fujitsu, Japan) software using CaCheMM3 molecular force files with previous fixed inter-proton bound distances that were previously determined. Values of inter-proton distances were assessed from 2D NOESY NMR experiments. The following are the molecular mechanics geometry optimization parameters:

Optimization method: conjugate gradient.

Iteration control: convergence value 0.001; convergence unit kcal mol^{-1}; maximum updates 300.

NMR parameters

All NMR spectra were recorded on a Brucker Advance 500 spectrometer. ^{1}H spectra was recorded at 25, 30, 35, 41 °C and collected in 65.3 K data points including 34–537 transients; the spectral width was 5482 Hz at an operation frequency of 500.13 MHz.

^{1}H {^{31}P} decoupled spectra were acquired at 25 °C in 20.5 K, over 64 transients with a spectral width of 5000 Hz and an operating frequency of 202.45 MHz.

^{1}H spectra were referenced to the residual HOD of D_2O fixed at 4.80 ppm.

2D COSY spectra and 2D NOESY spectra were obtained by standard pulls-program implemented in the standard Brucker software.

All 1D spectra were processed using XWINNMR version 2.6 and a Gaussian window function (GB = 0 and LB = 0.3). All spectra were processed on a PC computer after accumulation, using the MestReC 4.4.1 (Mestreclab Research, Santiago de Compostela, A Coruna, Spain) software.

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