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NMR Assignments and Conformational Studies of Two Diastereomeric Oxidation Products of 2′-Deoxycytidine

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ABSTRACT: Structural assignment and conformational features of two diastereomeric oxidation products of 2′-deoxycytidine, the 5*S** and 5*R** diastereomers of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin were achieved by combining NMR studies and restrained molecular dynamics. Interproton distances were measured from NOESY and ROESY experiments. The absolute configuration of these two compounds was unambiguously determined and their conformation properties in solution were also studied. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NOE measurement; conformational analysis; ROESY; NOESY; 2′-deoxycytidine derivatives

INTRODUCTION

The carcinogenic and mutagenic effects of ozone are now established.[1] Ozone was found to oxidize 2′-deoxycytidine, giving rise to fragmentation and rearrangement products of the pyrimidine ring. The main ozonolysis products of 2′-deoxycytidine were recently isolated and characterized in the laboratory.[2] Two of these modified nucleosides were assigned as the 5*S** and 5*R** diastereomers of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin (**1** and **2**) (Fig. 1). These oxidation products, which may also be generated by OH· radicals and one-electron oxidation reactions,[3,4] are abbreviated as 5*S*- and 5*R*-hydantoin. These two molecules differ only by the configuration at C-5 of the aglycone and differ slightly in their NMR properties. No x-ray data are currently available for nucleosides **1** and **2**. Moreover, the conformation of such molecules, especially in solution, is of importance for a better assessment of their biological role in living systems (DNA repair, mutagenesis).[4,5]

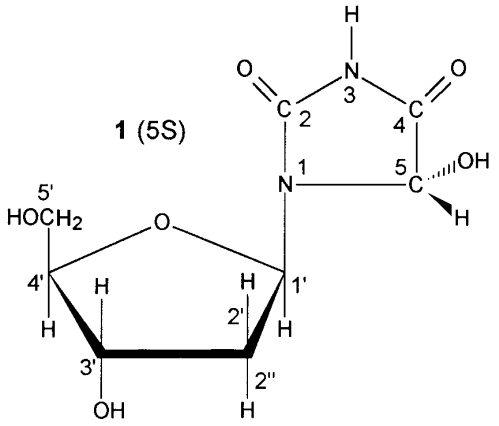

Figure 1. Structure **1** of the 5*S* diastereomer of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin. Structure **2** corresponding to the 5*R* configuration is deduced by inverting the hydroxyl group and the proton at C-5.

EXPERIMENTAL

Oxidation products of 2′-deoxycytidine

Oxidation products **1** and **2** were prepared and purified according to Giraud *et al.*[2] The isolation and separation of the two oxidized nucleosides **1** and **2** were achieved by high-performance liquid chromatography (HPLC) on a Waters Delta Pak C_{18} 100A octadecylsilyl silica gel column (19 mm × 30 cm) using water as the mobile phase at a flow-rate of 5 ml min^{-1}. Typically, nucleoside **1** was eluted at $k' = 4$ and diastereomer **2** at $k' = 3.5$. The corresponding HPLC fractions were collected and the resulting aqueous solutions were evaporated to dryness, yielding homogeneous **1** and **2**. Detection of **1** and **2** was achieved with a Waters R401 refractometer.

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NMR spectroscopy

All 1D and 2D NMR spectra were recorded on a Varian Unity 500 spectrometer at a constant temperature of 283 K using a 5 mm indirect detection probe. The spectrometer was operated at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The compounds were lyophilized twice from D_2O and then dissolved in 99.9% D_2O. Proton and carbon chemical shifts were referenced to 3-(trimethylsilyl)propionate-2,2,3,3-d_4 as external reference. The 1D and 2D experiments were performed using the standard Varian pulse sequences. The conditions for ^{1}H–^{1}H DQF COSY, ^{1}H–^{13}C HMQC, ^{1}H–^{13}C HMBC and NOESY experiments were identical with those reported in detail in a previous paper.[6] The conditions for recording ROESY spectra are indicated below.

ROESY spectra. The standard pulse sequence D_1–90°–t_2–90°–SL–90°–FID acquisition was used to perform the ROESY experiment. The relaxation delay D_1 was 2 s and the sweep width in both dimensions was 4775 Hz. A moderate continuous spin-lock pulse was used with a field strength of 1890 Hz to obtain quantitative ROESY spectra. To minimize the unwanted HOHAHA coherence transfer, the transmitter frequency was set to the low-field side of the spectra. Spectra were recorded and displayed in the phase-sensitive mode. Typical spectra were obtained from 16 scans and 512 increments and processed using a Gaussian weighting function. ROESY spectra with spin lock duration of 0.3, 0.6 and 1.0 s were recorded. Also, residual water suppression, if necessary, was applied successfully by presaturation of its signal for 1.5 s.

Extraction of conformationally relevant parameters

The determination of the proton–proton distances r_{ij}, from the NOESY experiment, was performed by using an approach suggested by Bodenhausen and Ernst[7] and applied later by several other groups.[8,9] It was shown that the distance r_{ij} can be calculated from the intensity ratio between cross and diagonal peaks, denoted a_{ij} and a_{ii}, respectively, using the following equation:

$$r_{ij} = \left[\frac{-2q\tau_{mix}}{\ln\left(\frac{a_{ii} + a_{ij}}{a_{ii} - a_{ij}}\right)} \left(\frac{6\tau_c}{1 + 4\omega^2 {\tau_c}^2} - \tau_c \right) \right]^{1/6} \quad (1)$$

where $q = 0.1\gamma^4 h^2 (\mu_0/4\pi)^2$, τ_{mix} is the mixing time and τ_c is the correlation time. Assuming a molecule with isotropic tumbling, Eqn (1) can be rewritten with a constant term

$$c = \left[-2q\tau_{mix} \left(\frac{6\tau_c}{1 + 4\omega^2 {\tau_c}^2} - \tau_c \right) \right]$$

where c depends only on either the molecule or a part of it (for a large molecule).

This constant term c can be calculated if a known distance, r_{ref}, between two protons of the molecule is used as a reference. In the present work, a distance of 1.78 Å between the two methylenic protons H-2′ and H-2″ of the sugar moiety was used as a reference for the different calculations. The main advantage of the method is that it is independent of the initial rate approximation and, theoretically, only one experiment is needed. Therefore, a mixing time as long as 1 s can be used. The 2D spectra which are thus obtained exhibit a better signal-to-noise ratio than those recorded with usual mixing times (i.e. around 0.3 s for such molecules), fulfilling the linear approximation condition.

To measure interproton distances from ROESY experiments, the data were processed as follows. The data collected (volume integration of the cross and diagonal peaks) from the ROESY spectrum were subsequently corrected for the offset differences between the correlation peaks and the spin locked pulse offset, ω_0.[10,11] The corrected data were used directly in Eqn (1) as for the data from NOESY experiments.

For a correlation between spins at frequencies ω_i and ω_j, the correction factor, c_{ij}, is given by

$$c_{ij} = \frac{1}{(\sin^2 \theta_i \sin^2 \theta_j)}$$

with

$$\tan \theta_i = \frac{\gamma B_1}{\omega_i - \omega_0}$$

The comparison of the distances obtained on the basis of NOESY and ROESY experiments can be a valuable procedure for cross-checking the results since the artefacts in each of those experiments are known to have different origins. The method has been reported and its applications discussed elsewhere.[12]

Molecular modeling studies

The classical approaches which were used are the following. The energy minimization and the molecular dynamic studies were carried out on a Silicon Graphics Indigo 2 computer using the consistent valence forcefield (cvff) of INSIGHT/DISCOVER software (Biosym Technologies, San Diego, CA, USA). The starting structures were built using the functional groups available in the software. First, the energies of the molecules were minimized with atom charges included, using the conjugate gradient method until the derivatives of the minimization were less than 0.001 kcal Å^{-1} After minimization, the molecular dynamic analyses were performed. Typically, a dynamic run consists of 100 steps of equilibration followed by 1000 steps of dynamic analyses at a temperature of 300 K. The time step was 1 fs and every tenth step was saved for analysis. The data obtained from dynamics were evaluated using graphs, in which the root mean square (RMS) deviations between different structures were compared.[13] Some randomly selected structures with low RMS difference were studied further, starting with a similar minimization procedure as at the beginning. For the local minimum structures which were found, torsion angle analyses

were performed. Torsion angles were defined by clockwise rotation by 5° steps around appropriate bonds according to the sign convention of Klyne and Prelog. Two torsion angles, χ (glycosidic) and γ, were considered.[14] The former involved the O-4, C-1′, N-1 and C-2 atoms and the O-5′, C-5′, C-4′ and C-3′ atoms defined the latter angle. The effect on the energy minimization of both torsion angles was studied using graphs in which total energy was correlated with either intermolecular distances or time. Again, a few representative structures were selected, usually near the energy minimum, but also in such a way that none of the maximum or ninimum distances between the H-5 proton of the base on the one hand and the sugar protons H-1′ or H-3′ on the other were violated. These structures were minimized once again and the calculated energies are reported in Table 4. The calculated energy values are not absolute. However, the data for the individual structures are comparable to each other.

Another procedure was also applied to determine the structures. Molecules **1** and **2** were built according to the proton distances calculated from the NMR experiments. An extra energy term, in the form of interproton distance constraints, was included to force the built molecules to fulfil distance requirements in the energy minimization.

RESULTS AND DISCUSSION

Assignment of the proton and carbon resonances

The proton spectra of both nucleosides **1** and **2** were assigned using a combination of 1D and 2D NMR techniques. Data reported in the literature for similar compounds were considered as the starting point.[15,16] The H-1′ sugar proton is known to resonate within the 5.5–6.5 ppm range. The H-1′ pattern represents the X part of an ABMX spin system. The singlet appearing for **1** and **2** at 5.47 and 5.54 ppm, respectively (Fig. 2), is assigned to the H-5 of the base. The connectivities between the different protons of the sugar are assigned by COSY experiments, which allow their assignment in a straightforward way. Finally, the reliability of assignments was checked by spin simulation[17] of the proton spectra, starting with the chemical shifts and coupling constants. Moreover, this procedure allows the extraction of accurate coupling constants, which are reported in Table 2. These assignments are required for the evaluation of the sugar conformation features of **1** and **2**.

On the basis of coupling constant arguments it is possible to assign unambiguously the 2′ and 2″ methylenic protons, since H-2′ is by definition antiperiplanar to H-1′ in a 2-deoxy-β-D-*erythro*-pentofuranosyl structure. This leads to $^3J_{H1',H2'}$ and $^3J_{H2',H3'}$ larger than $^3J_{H1',H2''}$ and $^3J_{H2'',H3'}$, respectively, when the sugar moiety adopts a preferencial C-2′-*endo* puckered conformation. Therefore, the signal at lower field is assigned to H-2′ and that at higher field to H-2″. The coupling constants of H-4′ to H-5′ or H-5″ vary from 1 to 5.6 Hz for both nucleosides **1** and **2**; hence this indicates that the CH_2OH group rotates fairly freely around the C-4′—C-5′ bond. Therefore, it is not possible to assign the H-5′ and H-5″ protons on the basis of coupling constants. The assignment was achieved using the fact that H-5′ usually resonates at lower field than H-5″ in nucleosides.[18]

Starting from the previously assigned ^{1}H chemical shifts, all the protonated carbons of both compounds can be easily identified through their $^1J_{CH}$ connectivities provided by the HMQC experiment. The assignment of the two quaternary carbons of the base which resonate at 177.0 (176.5) and 160.0 (159.3) ppm was achieved by HMBC experiments. The carbon resonating at low field exhibits two long-range connectivities with H-5 of the base and H-1′ of the sugar moiety. On the other hand, the high-field carbon shows only one long-range connectivity with H-5. Therefore, C-2 and C-4 are assigned to the resonances around 160 and 170 ppm, respectively, in agreement with published data on ureids.[19,20] Complete assignments of the ^{1}H snd ^{13}C NMR signals of **1** and **2** are reported in Table 1.

The data in Table 1 show that diastereomers **1** and **2** exhibit only slight differences in their NMR chemical shifts. For the ^{1}H NMR spectra, the largest chemical shift difference $\Delta\gamma$, 0.1 ppm, concerns H-1′. The other

Table 1. ^{1}H and ^{13}C NMR data for hydantoins **1** and **2** in D_2O at room temperature

	^{1}H/^{13}C NMR chemical shifts[a] (ppm)		T_1 proton		
Position	5*S*	5*R*	5*S*	5*R*	^{13}C–^{1}H long range correlations[b]
1′	5.98/84.7	5.87/84.7	2.92	2.95	H-5, H-4′, H-3′, H-2′, H-2″
2′	2.55	2.53	0.82	0.72	
2″	2.18/39.2	2.16/37.6	0.86	0.85	
3′	4.38/73.6	4.41/74.1	2.06	2.14	H-5′, H-5″, H-4′, H-2, H-2′
4′	3.91/88.3	3.91/88.5	2.57	2.74	H-5′, H-5″, H-2′, H-2″
5′	3.73/64.5	3.68/64.7	0.76	0.85	
5″	3.63/64.5	3.63/64.7	0.75	0.83	
5	5.54/79.9	5.47/80.8	4.34	3.08	H-1′
C-2	—/160.0	—/159.3			H-1′, H-5
C-4	—/177.0	—/176.5			H-5

[a] Based on the HMQC spectrum.
[b] Based on the HMBC spectrum.

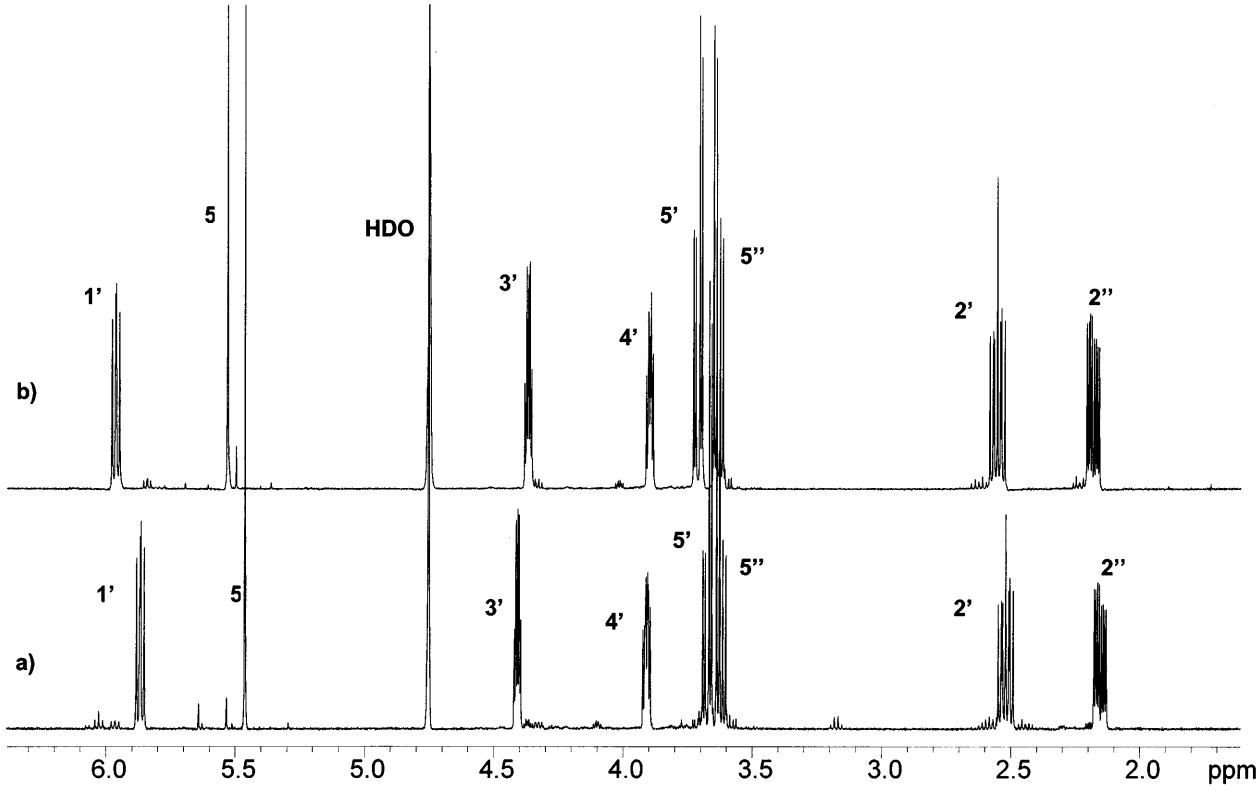

Figure 2. 500 MHz proton spectra of the (a) 5*R* and (b) 5*S* diastereomers of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin in D_2O.

significant $\Delta\gamma$ involved H-5′ of the sugar and H-5 of the base, for which 0.05 and 0.07 ppm differences, respectively, were measured. None of the remaining protons exhibited significant differences in their chemical shifts. This also applies to the ^{13}C chemical shifts. The largest change, $\Delta\gamma$, is observed for C-2′, 1.6 ppm. The other differences between the carbon chemical shifts within the sugar ring are less than 1.0 ppm: 0.5, 0.2 and 0.2 ppm for C-3′, C-4′ and C-5′, respectively. For C-5, C-2 and C-4 of the base, $\Delta\gamma$ is small, 0.9, 0.7 and 0.5 ppm, respectively.

Estimation of sugar pucker from $^3J_{HH}$ analysis

The data obtained on the basis of proton–proton coupling constant analyses, mainly $^3J_{HH}$, are reported in Table 2. The coupling constants appear to be very similar for both diastereomers **1** and **2**. The only observable differences are 0.48, 0.33 and 0.23 Hz between the proton pairs H-4′–H-5′, H-2″–H-3′ and H-3′–H-4′, respectively. The similarity of the coupling constants indicates that irrespective of the aglycone configuration, the sugar moieties of **1** and **2** exhibit similar conformation features. The largest difference concerns $^3J_{HH}$ between protons H-4′ and H-5′, which is nearly 0.5 Hz. This is indicative of a slight difference in the orientation of the 4′-hydroxymethyl group around the C-4′—C-5′ bond.

The conformation of the sugar moiety of nucleosides is rationalized in terms of a dynamic equilibrium between two puckered forms: the S type (C-2′-*endo*, C-3′-*exo*) and the N type (C-2′-*exo*, C-3′-*endo*). On the basis of the coupling constants of the sugar moiety, reported in Table 2, the percentages of the main conformer, S or N type, can be estimated. These calculations were performed using the following semi-empirical equation proposed by Haasnoot *et al.*:[21]

$$f_{\text{C-2′-}endo} = J_{1'2'}/(J_{1'2'} + J_{3'4'})$$
$$f_{\text{C-3′-}endo} = J_{3'4'}/(J_{1'2'} + J_{3'4'}) \quad (2)$$

The results obtained for the two diastereomers **1** and **2** are given in Table 3. The proportions of the S type (C-2′-*endo*) conformer, which is the predominant form, are 71 and 72%, respectively.

Table 2. Proton coupling constants (J, ±0.1 Hz) for hydantoins **1** and **2** in D_2O

	J_{ij}(Hz)	
ij	5*S*	5*R*
1′2′	8.1	8.2
1′2″	6.3	6.4
1′3′	0.6	0.6
2′2″	−13.9	−13.9
2′3′	6.1	6.2
2″3′	3.5	3.1
3′4′	3.4	3.1
4′5′	4.1	4.6
4′5″	5.5	5.6
5′5″	−12.2	−12.1

Table 3. Conformational features of the sugar ring and hydroxymethyl group of hydantoins **1** and **2**

Molecule	2′-*endo*-3′-*exo* (%)	*gauche*$^+$ (%)[a]	*trans* (%)[a]	*gauche*$^-$ (%)[a]
1(5*S*)	71	34	40	26
2(5*R*)	72	29	41	30

[a] % *gauche*$^+$ $= [13.75 - (J_{4'5'} + J_{4'5''})]/10.65$, % *trans* $= (J_{4'5''-1.5})/10$, % *gauche*$^-$ = % *gauche*$^+$ − % *trans* (see Ref. 3).

Internuclear distances from NOESY and ROESY spectra

The distances between the different sugar protons and the H-5 proton of the base are of major importance in determining the absolute configuration about C-5. They are printed in bold type in Table 4. In order to obtain quantitative data on internuclear distances between relevant protons, the results of both ROESY and NOESY experiments (Fig. 3) were exploited. This appears to be a suitable procedure to cross-check the results since the artefacts which may appear in each of these experiments are known to have different origins.[22] The data obtained from either NOESY or ROESY experiments are very similar, as illustrated in Table 4. From these quantitative data, several conclusions can be drawn. The distances between the protons of the sugar moiety are virtually identical for both diastereomers **1** and **2**. Interestingly, the shortest distances between the H-5 of the base and any of the protons H-3′, H-4′, H-5′ and H-5″ were found for the 5*S*-hydantoin **1**. In fact, the distances between either proton H-5′ or H-5″ and H-5 of the base, are nearly beyond the limit of detection for diastereomer **2**. On the other hand, the longest distances between H-5 and the protons H-1′, H-2′ and H-2″ are found for the hydantoin **1**.

Energy minimization and molecular dynamics

For both the 5*S*- and 5*R*-hydantoins, one can build correlation graphs which give the RMS deviations between backbone atoms for the structures generated from the dynamic studies involving the atoms of the sugar, with the exception of the 4′-hydroxymethyl group. In such graphs for either the 5*R*- or 5*S*-hydantoin, two structural families can be distinguished, corresponding to two different puckered modes for the sugar, ^{3}E(C-3′-*endo*) and ^{2}E(C-2′-*endo*). These results are consistent with the interpretation of the proton coupling constants of the sugar moiety which shows the occurrence of the two puckered conformers in dynamic equilibrium (Table 3).

The two conformations found for the sugar moiety were further used as starting structures to find the preferential orientation of the base with respect to the sugar moiety. This was achieved by varying the torsion angle

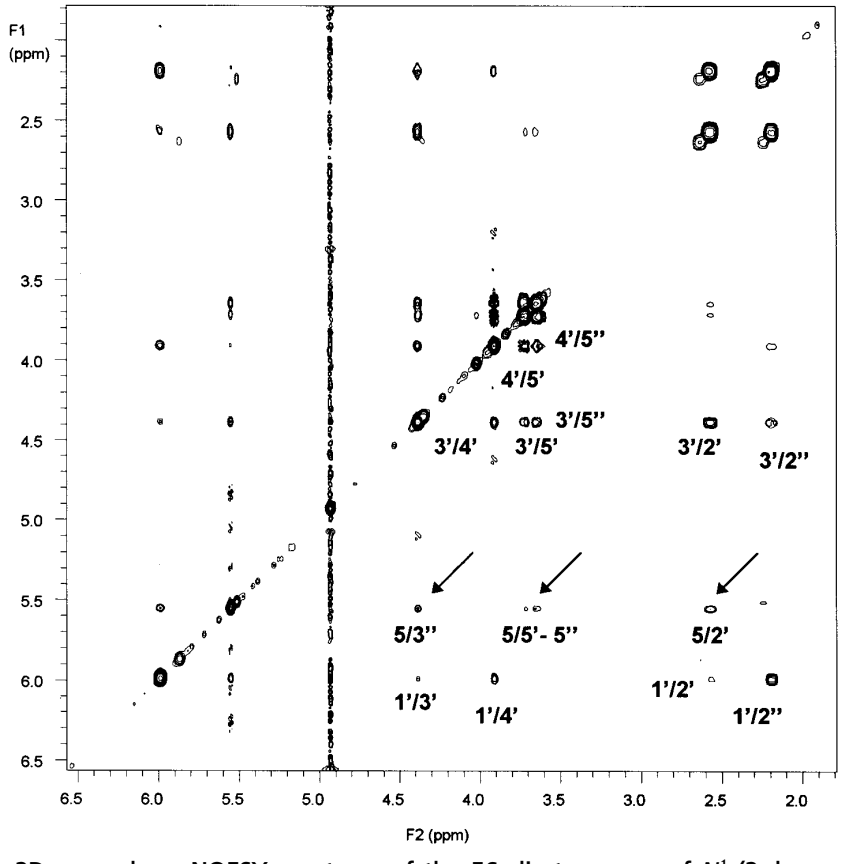

Figure 3. ^{1}H 500 MHz 2D pure-phase NOESY spectrum of the 5*S* diastereomer of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin. Arrows indicate the correlations appearing in the NOESY (or in the ROESY) but not in the ^{1}H–^{1}H COSY.

Table 4. Interproton distances[b] (Å) from NOESY or ROESY and from restrained molecular dynamics (rMD) calculations of 5*S* and 5*R* hydantoins

	5*S* hydantoin (**1**)					5*R* hydantoin (**2**)				
Protons	From NOESY	From ROESY	Average	rMD on 5*S* configuration, $E = -30.5$[a], $X = -100.7°$	rMD on reversed configuration, $E = -34.4$[a], $X = -75.5°$	From NOESY	From ROESY	Average	rMD on 5*R* c/configuration, $E = -48$[a], $X = -65.6°$	rMD on reversed configuration, $E = -49.7$, $X = -20.7°$
1′2′		3.50	3.50	3.06	2.97	3.52	3.23	3.22	2.86	2.89
1′2″	2.29	2.47	2.38	2.40	2.37	2.40	2.44	2.42	2.34	2.34
1′3′	4.37	3.80	4.09	3.91	3.79	3.84	3.81	3.86	3.92	3.92
1′4′	3.08	3.08	3.08	2.82	3.31	3.00	3.02	3.01	3.33	3.32
1′5′		4.51/6.00[c]		4.53/4.98	4.64/5.29	5.41		5.41	4.41/5.17	4.39/5.13
1′5″		4.94/6.91[c]		—	—	5.71	3.78/6.33[c]	5.27	—	—
1′5	**3.09**	**3.34**	**3.21**	**3.28**	**3.31**	**3.02**	**3.10**	**3.06**	**3.12**	**3.09**
2′2″	1.78	1.78	1.78	1.79	1.77	1.78	1.78	1.78	1.74	1.75
2′3′	2.33	2.32	2.32	2.26	2.37	2.32	2.33	2.33	2.29	2.26
2′4′	5.16	3.67/5.41[c]	3.68/5.29[c]	3.86	3.71	4.24/5.25[c]	4.21	4.24/5.23[c]	3.99	3.99
2′5′	3.51	3.32	3.41	4.42/4.85	4.77/4.99	3.14	3.26	3.20	4.31/4.74	4.28/4.68
2′5″	2.72	3.21	3.36	—	—	3.18	3.21	3.19	—	—
2′5	**2.58**	**2.90**	**2.74**	**3.12**	**2.47**	**2.27**	**3.38**	**2.33**	**2.24**	**2.20**
2″3′	2.72	2.85	2.79	3.01	3.03	2.81	2.89	2.85	3.01	3.00
2″4′	3.22	3.16	3.19	3.19	2.60	3.39	3.43	3.43	3.22	3.23
2″5′		4.40	4.40	4.92/4.95	4.62/4.71				4.72/4.81	4.71/4.78
2″5″				—	—				—	—
2″5	**4.23**	**4.10**	**4.17**	**4.23**	**3.81**	**4.23**	**3.55**	**4.23/3.55[c]**	**3.46**	**3.31**
3′4′	2.80	2.80	2.80	2.96	2.99	2.81	2.79	2.80	3.06	3.07
3′5′	2.87	2.86	2.87	2.67/2.81	2.69/3.02	2.72	2.81	2.76	2.65/.272	2.59/2.61
3′5″	2.85	2.75	2.80	—	—	2.65	2.87	2.72	—	—
3′5	**3.24**	**3.31**	**3.27**	**3.32**	**3.38**	**3.92**	**4.12**	**4.02**	**3.97**	**4.07**
4′5′	2.59	2.59	2.59	2.53/2.98	2.52/2.99	2.64	2.61	2.59	2.56/2.91	2.55/2.91
4′5″	2.66	2.74	2.70	—	—	2.60	2.64	2.61	—	—
4′5	**4.27**	**4.40**	**4.33**	**4.24**	**5.27**	**6.03**	**4.43**	**6.03/4.43**	**5.50**	**5.52**
5′5″	1.90	2.10	2.00	1.77	1.77	2.07	2.03	2.05	1.76	1.75
5′5	**2.99**	**3.60**	**3.29**	**3.27/4.72**	**5.25/6.19**	**4.64**			**5.21/6.23**	**5.37/6.31**
5″5	**2.84**	**3.28**	**3.06**	—	—	**4.14/6.14[c]**			—	—

[a] In kcal mol^{-1} (1 kcal = 4.184 kJ).
[b] Averaged value calculated from data recorded with 0.3, 0.6 and 1 s mixing times.
[c] Standard deviations are <5% except for those marked [c], for which data both minimum and maximum interproton distances are reported.

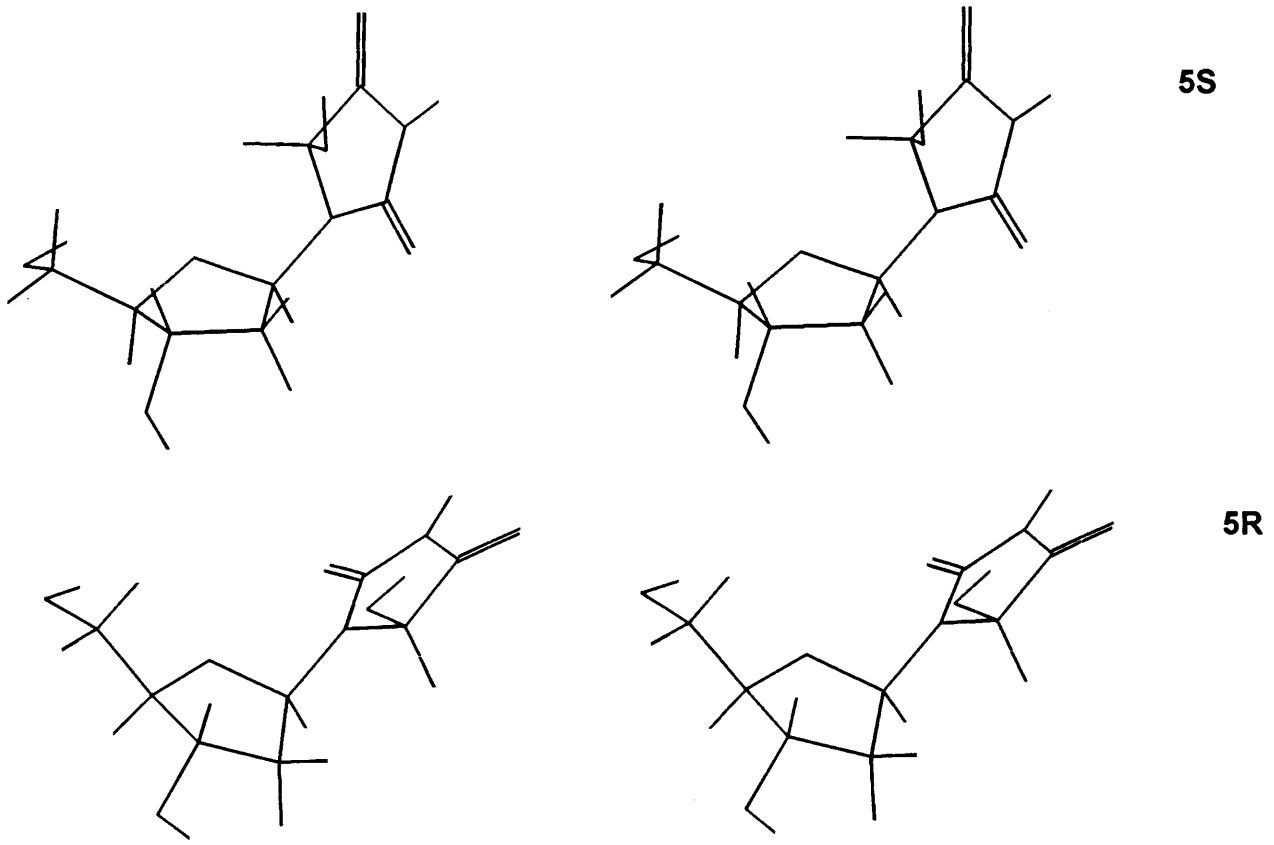

Figure 4. Stereoview of the conformations of the 5*R* and 5*S* diastereomers of N^1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-hydroxyhydantoin, which were obtained by energy minimization and restrained molecular dynamics.

(O-4—C-1′—N-1—C-2) around the *N*-glycosidic bonds in increments of 5°. For both the 5*S*- and 5*R*-hydantoins, two stable structures were found around +60° and −175° for the 5*S*- and around +32° and −141° for the 5*R*-hydantoin, irrespective of the ^{2}E(C-2′-*endo*) or ^{3}E(C-3′-*endo*) conformation of the sugar moiety. These conformations correspond to the expected *syn* and *anti* conformations for such nucleosides.

Similar calculations were performed to determine the conformation of the 4′-hydroxymethyl group by rotating it around the C-4′—C-5′ bond. Usually the conformational features of the 4′-hydroxymethyl group are depicted in terms of dynamic equilibrium between three staggered rotamers named *gg*, *gt* and *tg*. No preferential conformation can be observed on the basis of energy values among the expected *gauche*$^+$, *gauche*$^-$ and *trans* positions.

From the different structures that were generated above, interproton distances can be measured (not reported in this paper). They were compared with the interproton distances measured by ^{1}H NMR spectroscopy. However, it was not possible to find one calculated structure the interproton distances of which matched perfectly those determined by ^{1}H NMR. These calculated structures describe only limit conformations which are averaged in solution on the time-scale of NMR. This may reflect the mobility of the base around the *N*-glycosidic bond, together with a dynamic equilibrium between the ^{3}E(C-3′-*endo*) and ^{2}E(C-2′-*endo*) puckered forms. The latter equilibrium is well illustrated by the similarity of the distances between the different protons of the sugar moiety of **1** and **2** as calculated from ^{1}H NMR experiments. In contrast, the distances between H-5 of the base on one hand and the different protons H-5′, H-5″, H-3′ and H-2′ on the other of the sugar moiety of **1** and **2** are very different. This strongly supports the assumption that the absolute configuration of C-5 exerts an influence on the averaged structure of hydantoins adopted in solution. This may be used as a key parameter to assign the absolute configuration of C-5.

Starting from the above observations, the NOE measurements were included as constraints for the molecular minimization calculations. For both sets of experimental data obtained by NMR, the restrained molecular dynamics calculations were performed for the two possible *R* and *S* configurations of C-5. The resulting proton distances measured from the restrained and minimized structures are given in Table 4. It appears that for the 5*S*-hydantoin, the calculations performed with the NMR constraints lead to a structure which is consistent with the experimental measurements. Such agreement could not be achieved if the same experimental data were used to calculate the restrained minimized structure with a 5*R* configuration (noted in Table 4: rMD on reversed configuration) as the starting point. This does not apply to the experimental data corresponding to the 5*R*-hydantoin. In fact, the calculations performed using either the 5*R* or the 5*S*

configuration led to two different minimized structures which could not be distinguished using the available data. Indeed, these two refined structures, with equivalent energies, simply differ by a *ca.* 180° rotation around the glycosidic bond.

The above data allow one to assign unambiguously the *S* configuration of C-5 to molecule **1**. Consequently, an *R* configuration was inferred for C-5 of molecule **2**. Their averaged structures in solution resulting from the rMD calculations (Table 4), are shown in Fig. 4.

The use of distance data from NOESY (or ROESY) as constraints in the energy minimization calculations appeared to be a valuable approach for the structural analysis of modified nucleosides. In the present study, this allowed us to assign the absolute configuration of the two diastereomers while providing relevant information on their conformation properties in aqueous solution.

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