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^{13}C Chemical Shifts and $^{1}J_{CH}$ Coupling Constants of Cytidine at Different χ Dihedrals Based on DFT Calculations

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A molecular dynamic simulation of cytidine reproduced the dominating ^{3}E-*endo*, the so-called North conformation of the sugar and the *anti* base orientation with $\chi = -120°$. Taken as starting structures for a geometry optimisation, ^{13}C chemical shifts and ^{1}J coupling constants were calculated by DFT [functional: B3LYP, basis set: 6-31G(d,p)]. As for the first time no minimal structural model was used, the results can be interpreted without further approximations except solvent dependence which was not included. The influence of the glycosidic torsion angle was studied. The ^{13}C chemical shifts correlated with a North conformation of the sugar independent of the base orientation when using an empirically derived coordinate analysis. However, the $^{1}J_{CH}$ coupling constants and ^{13}C chemical shifts clearly showed a dependence on the glycosidic torsion which enables the identification of χ. The $^{1}J_{CH}$ analysis showed that the sugar pucker is not the major determinant for $^{1}J_{C1'H1'}$. Instead, the base orientation caused major changes, with a maximal difference of 14 Hz. Additionally, $^{1}J_{C2'H2'}$, $^{1}J_{C3'H3'}$ and $^{1}J_{C4'H4'}$ are differently influenced by the glycosidic torsion which can be exploited for assigning χ. Analysis of electrostatic and steric effects showed that an isolated view is not able to explain all NMR spectroscopic data but gives some useful ideas. A higher charge on C3′ and the $^{1}J_{C6H6}$ coupling constants were explained by through-space effects. Depending on the glycosidic torsion, the base non-planarity changes substantially. The results clearly show that also for ribonucleotides ^{13}C chemical shifts and $^{1}J_{CH}$ coupling constants are dependent on the base orientation which was questioned in the past.

Introduction

A number of biological functions of RNA and nucleotide analogues are related to inherent structural flexibility, e.g. the puckering of the ribose and the orientation of the base through the glycosidic linkage, which have been studied in the past by NMR spectroscopy.[1] Four conformational parameters are required to define the shape of a nucleoside:[2] i. the glycosidic torsion angle χ which determines the *syn* (+60°) or *anti* (–120°) orientation of the base relative to the sugar; ii. the torsion angle γ; iii. the pseudorotation phase angle, *P*, which is 140°–180° around the C-2′-*endo* conformation, the so-called South conformation, and 0°–40° around the C-3′-*endo* conformation, the so-called North conformation; iv. the puckering amplitude ν_{max} = falling mainly in the range of 30°–45°. For the majority of nucleosides, the value of *P* normally falls in a tight range in the vicinity of either one of the North or South extremes. In solution, the two conformations are in a rapid dynamic equilibrium dictated by the balance of stereoelectronic effects, which are in turn influenced by the electronegativity, ionisation state, steric bulk, and relative stereochemistry.

The key for a better operational understanding lies in a thorough description of the factors governing this mobility. To this end quantum mechanical calculations of derivatives of the smallest units, the four bases, were performed due to experimental and computational limitations.[3,4] Consequently, results derived from these studies have to be interpreted cautiously when dealing with experimental results of native RNA bases. To overcome these difficulties we focus in this report on the genuine nucleoside cytidine and its structural features with special emphasis on the torsional angle χ between C1′ of the ribose and N1 of the base. We present calculated data of ^{13}C chemical shifts and $^{1}J_{CH}$ coupling constants of cytidine in relation to rotation of the pyrimidine base.

Results and Discussion

In a molecular dynamics approach, models of cytidine with three values of ν_{max} were built (30°, 38° and 45°). The chemical formula of cytidine is shown in Figure 1. After minimisation no differences in the location of the energy minima were observed. The DFT calculations were then performed with a structural model of the North conformation (energy minimum) derived from ν_{max} = 38° with only one geometrical constraint for the sugar (C4′–O–C1′–C2′

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was set to 0°). A structure of cytidine in the North conformation with a χ dihedral of –120° is shown in Figure 2.

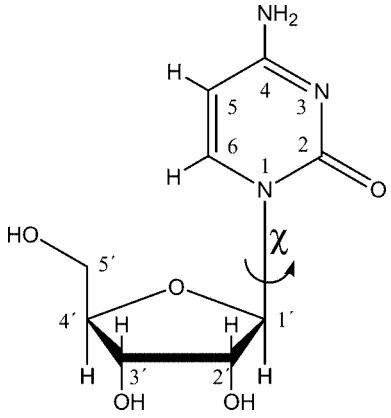

Figure 1. Chemical formula of cytidine. Base torsional angle χ is defined according to Wijmenga et al.[2] as O4′–C1′–N1–C2.

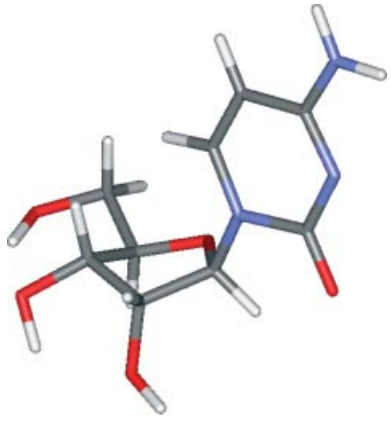

Figure 2. Structure of cytidine in the North conformation and *anti* (χ = –120°) glycosidic orientation of the base.

The importance of a careful treatment of the geometrical data was illustrated by Cloran.[6] During geometry optimisation in their computational study on 2-deoxy-β-D-ribofuranosylamine and analogues spontaneous bond rotations occurred to yield final structures different from starting structures. Consequently, an additional structural constraint was added to fix the desired envelope form. This in turn might influence the geometrical data and subsequently the calculated NMR spectroscopic data (vide infra). The same study showed that conformational preferences and energy barriers to pseudorotation were affected by changes in the C1′ substitution. This clearly advocated the analysis of natural RNA bases instead of model compounds.

The crystal structure of cytidine[7] showed a North conformation, a ν_{max} = of 38.7°, χ of 18.4° and for the hydroxymethyl group a *gg* orientation which is in agreement with the modelling. Similarly, the global energy minimum of methyl β-D-ribofuranoside was assigned to the North conformation.[4] The South conformer was found as a local minimum, 2.4 kcal/mol higher in energy. The east barrier (P = 90°) was estimated 3.7 kcal/mol and the west barrier (P = –90°) 4.4 kcal/mol.[8] In agreement, we found that the North conformer of cytidine is energetically more stable than the South conformer with the same base torsional angle of –120° (Table 1). The local minimum with χ of +60° is only reached by higher energy conformations at +110° and –145°.

In Table 1 the calculated ^{13}C chemical shifts of the North and South conformation of cytidine in relation to different glycosidic torsion angles are shown. First we analysed the reproduction of the North conformation.

^{13}C Chemical Shift and Sugar Pucker

The effects of sugar puckering on the C1′, C4′, and C5′ resonances of the North and South and C3′ ribose conformations were established by Harbison[9] based on empirical, structural, and NMR spectroscopic data. For the first canonical coordinate *can*1 > –6.25 ppm the sugar is in the North conformation, for *can*1 < –6.25 ppm, the sugar adopts a South conformation (*can*1 = 0.179·δC1′ – 0.225·δC4′ – 0.0585·δC5). In a subsequent study they conducted DFT calculations of the four conformations N-*anti*-*gg*, N-*anti*-*gt*, S-*anti*-*gg*, and S-*syn*-*gg* of a model pyrimidine-type base.[10] Computations yielded ^{13}C chemical shifts that were systematically different from experimentally derived values in the range from 4.23 to 6.17 ppm depending on the geometrical approach used. These authors again emphasised the need for geometry optimisation because differences in distances and angles influence the calculated chemical shifts. They concluded that unconstrained minimisation

Table 1. Calculated ^{13}C chemical shifts (in ppm) and relative energies of the North (N) and South (S) conformation of cytidine with restrained glycosidic torsion angles; experimental ^{13}C chemical shifts in ppm in DMSO as solvent.

χ/Atom	N60°	N110°	N180°	N–145°	N–120°	N–60°	S–120°	Experimental
C2	144.8	144.5	147.3	146.4	146.5	144.8	146.3	156.2
C4	152.0	151.4	153.1	151.9	151.7	151.8	150.8	166.4
C5	84.2	86.1	83.9	83.2	84.1	84.7	84.9	94.5
C6	137.5	140.2	134.1	134.8	136.6	133.0	135.5	142.3
C1′	101.2	97.0	93.6	90.8	88.1	91.0	88.6	90.0
C2′	82.5	75.2	75.6	76.8	78.6	75.3	73.9	74.7
C3′	76.1	79.3	76.5	77.4	79.5	79.0	78.2	70.1
C4′	80.8	77.7	80.0	78.0	79.9	79.3	84.6	84.7
C5′	60.8	63.1	64.0	63.3	65.1	64.3	65.6	61.3
Rel. energy[a]	4.5	5.5	7.3	11.4	0	25.1	4.9	–

[a] Relative energies (in kcal/mol) corresponds to the difference to the minimum energy for χ = –120° (absolute value in hartree = –886.061148).

of average crystal-structure coordinates with the B3LYP/6-31+G(d) method gives a reliable starting structure.

Applying an averaged systematic correction (subtraction of 5.2 ppm), our ^{13}C chemical shifts can be assigned to the North and South conformers and confirm the canonical coordinate analysis for the determination of the sugar pucker. For χ = –120° a borderline result is obtained that might be explained by a different approach (empirical vs. calculated, vide infra).

Preliminary ^{13}C data in relation to sugar pucker were presented by Case.[4] They found that the chemical shieldings of the C3′ and C5′ were the most sensitive to the sugar ring pucker. In contrast, our data for cytidine show that the C2′ resonance is sensitive to the sugar pucker and is deshielded by 4.7 ppm in the North conformation whereas C4′ is shielded by 4.7 ppm (Table 1). Furthermore, the C1′, C3′ and C5′ resonances are not much affected by pseudorotation. Additional correlations between sugar pucker and ^{13}C chemical shifts have been found by Tinoco.[11] A 5–6 ppm downfield shift of the C3′ resonance was attributed to a North to South conversion which is not observed in our calculations. The effect was explained by steric interaction between H3′ and O5′: repulsion of the σ-bonding electrons toward the carbon would increase the diamagnetic shielding.[12] It is known that steric strain produces significant shielding in the tensor component perpendicular to the plane of the strain but there is no simple model to quantify this effect.[13]

^{13}C Chemical Shifts and Glycosidic Torsion

In contrast to the large amount of correlation data between the sugar pucker and ^{13}C chemical shifts, at least for model compounds, the glycosidic torsion could not be determined from sugar ^{13}C chemical shift data only.[9] Prestegard[3] calculated a difference of 6 ppm between the two conformational minima (*syn* and *anti*) for a base carbon of a model compound. However, in our analysis of cytidine the calculated carbon chemical shifts and $^1J_{CH}$ coupling constants of the sugar clearly separate the two major conformations *syn* and *anti* around the glycosidic torsion angle (Table 1). The use of this information as a tool in the NMR structure elucidation of RNA and the structural evaluation of existent structural models requires the assignment of the sugar pucker as North conformer, which can be easily done with the above-mentioned analysis of Harbison.[9] A reason why a canonical coordinate for the base conformation was not established might be the different methods applied. Harbison[9] used experimental data of real molecules for which they tried to derive a correlation, whereas in our study the calculated NMR spectroscopic data were used.

Another possibility to derive conformational information is to study the temperature dependence of ^{13}C chemical shifts. In RNA oligomers C3′, C4′, and C5′ showed downfield shifts with increasing temperature.[14,15] This was explained by an increased population of the South conformer with increasing temperatures. C1′ chemical shifts were observed to move upfield with increasing temperature, while C2′ resonances were not affected much.[14,15] By analysing the ^{13}C chemical shifts of cytidine in D_2O, H_2O/D_2O mixture (90:10), H_2O-based phosphate buffer (50 mM, pH 6.7) and cryo mixtures of H_2O/DMSO (70:30) we could not verify this observation. The maximal temperature shift was +0.4 ppm going from 263 K to 298 K. Therefore, we expect no substantial influence on the sugar pucker populations in this temperature region and assign the major conformation to the North conformer.

$^1J_{CH}$ Coupling Constants and Sugar Pucker

In the analysis of cytidine and a model compound it was proposed that the sugar conformation is the major determinant of the $^1J_{C1'H1'}$ in nucleosides.[16] The measured $^1J_{C2'H2'}$ coupling constant (exp. value: 153.4 Hz) should increase with a quasi-equatorial orientation of the proton, which would correspond to the North conformation rather than the South conformation. Similarly, a larger $^1J_{C1'H1'}$ coupling constant (exp. value: 170.3 Hz) would indicate a preference for the North conformer. The calculated data for cytidine show these trends but indicate the influence of the χ dihedral on the $^1J_{CH}$ coupling constants (Table 2). In general, deviations of 10% between experimental and calculated ^1J coupling constants were observed for methyl α-D-xylopyranoside.[17] The difference of $^1J_{C1'H1'}$ coupling constants between purine and pyrimidine bases were attributed in ribonucleotides to a different preference of the North and South conformers.[16] In contrast, for the deoxyribonucleotides studied by Sklenár[18] it was found that only the bases itself are responsible for this difference and it was speculated that this also holds for ribonucleosides. The calculated $^1J_{C1'H1'}$ coupling constants of cytidine in the present study show that the sugar pucker is not the major factor favouring the hypothesis of Sklenár.[18]

Additionally, as several effects are operating in the flexible cytidine, only the sum of all can reproduce experimental data. As an example, a correlation for the $^1J_{CH}$ coupling constants was proposed:[19] $^1J_{CH} = -3432 + 182.2 q_C q_H + 3889/r_{CH}$.

where q_C and q_H are the total atomic charges on carbon and hydrogen from Mulliken population analysis, and r_{CH} is the C–H bond length. Using this formula and the calculated values (Table 3 and Table 4) one obtains for χ = +60°, –120° and –60° $^1J_{C1'H1'}$ coupling constants constants of 125.3 Hz, 139.6 Hz, and 144.2 Hz, respectively. All deviate substantially from the calculated and experimental values indicating that charge and bond length of the directly involved atoms is not sufficient to obtain good ^1J correlations and/or the parametrisation has to be changed for the specific molecule. The latter seems to be appropriate for cytidine. The inclusion of solvent might influence atomic charges due to specific interactions and/or global electrostatic interactions. In a study on Schiff base models using the simple COSMO model of solvent simulation, different

Table 2. Calculated sugar carbon $^{1}J_{CH}$ coupling constants of cytidine with restrained glycosidic torsion angles, experimental $^{1}J_{CH}$ coupling constants in DMSO as solvent.

χ/Atom pair	N60°	N110°	N180°	N–145°	N–120°	N–60°	S–120°	Experimental
C5–H5	143.3	143.4	143.4	143.4	143.2	143.5	146.5	172.4
C6–H6	150.0	153.3	155.3	153.4	150.2	146.7	153.9	180.1
C1′-H1′	164.2	170.3	178.3	175.1	169.5	175.7	169.2	169.0
C2′-H2′	157.3	170.1	159.2	155.2	152.1	154.8	158.1	147.5
C3′-H3′	167.1	163.6	155.5	154.4	152.3	154.3	162.8	146.5
C4′-H4′	150.5	151.7	159.8	159.7	159.1	153.6	155.3	142.4

Table 3. Calculated Mulliken charges of selected atoms with restrained glycosidic torsion angles.

χ/Atom	N60°	N110°	N180°	N–145°	N–120°	N–60°	S–120°
C4′	–0.025	–0.032	–0.017	–0.019	–0.014	–0.023	–0.041
H4′	0.178	0.178	0.228	0.228	0.223	0.186	0.203
O4′	–0.475	–0.484	–0.489	–0.482	–0.476	–0.457	–0.480
C3′	–0.186	0.001	0.002	0.003	0.001	0.002	–0.040
H3′	0.276	0.255	0.229	0.226	0.226	0.225	0.251
O3′	–0.556	–0.551	–0.552	–0.552	–0.553	–0.549	–0.541
C2′	–0.052	–0.073	–0.054	–0.036	–0.042	–0.077	0.014
H2′	0.239	0.274	0.245	0.224	0.216	0.231	0.211
O2′	–0.540	–0.545	–0.563	–0.562	–0.559	–0.544	–0.554
OH2′	0.353	0.351	0.372	0.373	0.371	0.367	0.341
C1′	0.243	0.233	0.262	0.241	0.226	0.242	0.230
H1′	0.210	0.224	0.244	0.253	0.258	0.254	0.237
N1	–0.728	–0.731	–0.742	–0.738	–0.734	–0.738	–0.741
C2	0.833	0.827	0.823	0.816	0.813	0.826	0.825
O2	–0.503	–0.512	–0.540	–0.524	–0.508	–0.497	–0.505
N3	–0.659	–0.658	–0.662	–0.663	–0.663	–0.665	–0.664
C5	–0.287	–0.291	–0.291	–0.291	–0.289	–0.281	–0.293
C6	0.179	0.193	0.171	0.177	0.181	0.170	0.232
H5	0.188	0.189	0.185	0.185	0.187	0.187	0.182
H6	0.233	0.245	0.258	0.247	0.242	0.232	0.232

Table 4. Calculated bond lengths [Å] of cytidine with restrained glycosidic torsion angles.

χ/ Bonds	N60°	N110°	N180°	N–145°	N–120°	N–60°	S–120°
C5′–C4′	1.5218	1.5233	1.5212	1.5216	1.5223	1.5229	1.5342
C4′–O4′	1.4764	1.4727	1.4803	1.4799	1.4742	1.4699	1.4807
C4′–C3′	1.5461	1.5423	1.5374	1.5417	1.5429	1.5406	1.5286
O4′–C1′	1.4526	1.4808	1.4618	1.4633	1.4697	1.4837	1.4793
C3′–H3′	1.0848	1.0892	1.0921	1.0923	1.0921	1.0925	1.0879
C3′–O3′	1.4496	1.4513	1.4518	1.4508	1.4504	1.4486	1.4744
C3′–C2′	1.534	1.5323	1.5313	1.5346	1.5361	1.5385	1.5464
C2′–C1′	1.5522	1.5457	1.5495	1.5437	1.5443	1.5384	1.5511
C1′–H1′	1.0961	1.095	1.0914	1.0907	1.0921	1.0907	1.0932
C1′–N1′	1.4794	1.4742	1.4825	1.4727	1.4655	1.4674	1.4495
N1–C2	1.4642	1.4591	1.451	1.4605	1.4719	1.4803	1.4685
N1–C6	1.3715	1.3738	1.3612	1.3627	1.3667	1.3688	1.3643
C2–N3	1.3712	1.3694	1.3659	1.3677	1.3687	1.3711	1.3712
C5–C6	1.3554	1.3553	1.3599	1.3585	1.3564	1.3565	1.3565

ε values did not influence the calculated charges justifying our gas-phase approach.[20] Although inappropriate taken alone, atomic charges are helpful in explaining conformational preferences as can be shown for the increased negative charge on C3′ of –0.186 at χ = +60° (Table 3).

Chattopadhyaya[21] investigated the electronic transmission from a purine base to the backbone torsions in nucleotides. On protonation of the base, the North conformation is favoured. A decreased *gauche* effect between $\sigma_{C3'H3'}$ and $\sigma^*_{C4'O4'}$ leaves more electron density at the C3′ atom (Figure 3A). In the present study on cytidine the high negative charge of C3′ for the χ = +60° conformer cannot be simply related to the north conformation because this was held constant. Furthermore, the $\sigma_{C3'H3'}$ and $\sigma^*_{C4'O4'}$ interaction is geometrically not changed during rotation around the glycosidic angle. The high positive charge of H3′ (+0.2764) might therefore indicate the influence of O2 on the C3′–H3′ bond (vide infra). The decreased bond length corresponds to a high $^{1}J_{C3'H3'}$ coupling constant (N60°: 167.1 Hz, Table 2).

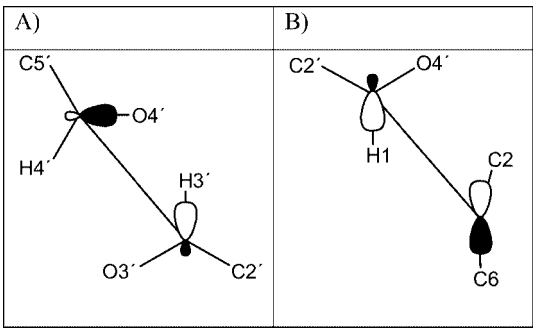

Figure 3. Newman projections of the (A) $\sigma_{C3'H3'}$ and $\sigma^*_{C4'O4'}$ interaction of the North conformer with χ = +60°, and (B) $\sigma_{C1'H1'} \rightarrow \pi^*_{N1C6}$ interaction of the North conformer with χ = –60°.

$^1J_{CH}$ Coupling Constants and χ Torsion

Serianni[16] suggested that the $^1J_{C1'H1'}$ coupling constants are unaffected by N-glycosidic torsion. It was speculated that bond length and orientation influences the $^1J_{C1'H1'}$ coupling. This was tested with a model compound with constrained sugar pucker. Rotation of the χ torsion resulted in a small change of 0.8 Hz in $^1J_{C1'H1'}$. The molecular size of the model compound limited the calculations to the HF level of theory. In contrast, Davies[22] had found a strong dependence on the χ torsion for a cyclic pyrimidine nucleoside.

Supporting this view the $^1J_{C1'H1'}$ coupling constant as a function of χ could be reproduced by a generalized Karplus equation in the analysis of deoxyribonucleotides.[18] For this analysis, the SD (spin-dipolar) term was neglected for *J*-coupling calculations. In the present study, the ribonucleoside cytidine also shows a strong dependence of the $^1J_{C1'H1'}$ coupling on the glycosidic torsion (Table 2) which is in contrast to Serianni.[16]

The most important effect defining the angular dependence of one-bond carbon–proton coupling constants is their sensititivy to the lone-pair orientation belonging to an atom placed α to the C–H bond. The glycosidic nitrogen bears a lone pair perpendicular to the base plan (partially delocalised within the base) whose orientation with respect to the sugar is given by χ. For χ around +30° and –150° the overlap between n_{N1} and C1′–C2′ is maximized. Such hyperconjugative charge transfer into $\sigma^*_{C1'-C2'}$ weakens the C1′–C2′ bond and reduces the absolute value of other-bond contribution to $^1J_{C1'H1'}$. In our calculations the bond length C1′–C2′ for χ = +60° (1.5522 Å) is increased but not for χ = –145° (1.5437 Å) indicating that additional factors are relevant. Furthermore, the $\sigma_{C1'H1'} \rightarrow \pi^*_{N1C6}$ interaction with an overlap at a χ torsion around +60° would weaken the C1′–H1′ bond and its contribution to $^1J_{C1'H1'}$ (Figure 3, B). In agreement, the C1′–H1′ bond length in cytidine was found to be increased at a glycosidic torsion of +60° when compared with the other torsional angles (N60°: 1.0961 Å, N-145°: 1.0907 Å).

Recently, Cuevas[23] hypothesized that delocalisation is not the origin of the decreased $^1J_{CH}$ coupling constants found in cases of the Perlin effect. In their calculation of oxane and acyclic ethers the minimum $^1J_{CH}$ value occurred at an angle near 0°, when the C–H bond is not antiperiplanar to any lone pair of electrons contradicting the convential reasoning of hyperconjugative $n_O \rightarrow \sigma^*_{CH}$ delocalisation discussed above. They suggested a dipolar interaction with the electric field of the oxygen dipole polarizing the electron distribution in the C–H bond. This is supported by a charge distribution at the H atom that can better be correlated with a dipolar interaction than by a delocalisation. These dipolar effects might also operate for H6 of the cytidine base. A C–H·····O contact influencing the charge of the proton could explain the calculated $^1J_{C6H6}$ coupling constants (Table 2 and Table 3). The maximum (155.3 Hz) is found for χ = 180°, concomitantly with the highest positive charge on H6 (+0.258) and with H6 positioned near to O4′.

In addition to electrostatic data describing structural preferences, bond lengths also monitor steric factors. In the deoxyribonucleotides studied by Sklenár[18] a maximum of $r_{C1'H1'}$ vs. χ was found for the *syn* (+60° to +150°) and a minimum for the *anti* orientation (–135° to –105°). The difference between these two was 0.01 Å. As a comparison, the maximal bond-length difference between axial and equatorial C–H bonds in cyclohexane amounts to 0.002 Å, but becomes large for methylene groups adjacent to oxygen and nitrogen (in oxane: 0.011 Å, in azane: 0.013 Å).

For cytidine a maximum of 1.0961 Å was found for χ = +60°, in a region of repulsion between O4′ of the sugar and O2 of the base (Table 4). In contrast to the data of Sklenár[18] this trend reflected the $^1J_{C1'H1'}$ coupling constant dependence on $r_{C1'H1'}$.

Base Planarity and χ Torsion

In deoxyribonucleotides a base nonplanarity was observed in such a way that the N1–C6 bond was moved away

Table 5. Calculated dihedrals related to base planarity and hybridisation of C1′–N1 with restrained glycosidic torsion angles.

χ/Dihedral	N60°	N110°	N180°	N–145°	N–120°	N–60°	S–120°
C6–N1–C2–N3	1.3°	–6.9°	1.4°	5.9°	8.8°	1.8°	6.5°
C2–N1–C6–C5	1.3°	4.5°	–1.0°	–6.7°	–8.7°	–3.5°	–5.8°
N1–C2–N3–C4	–2.5°	4.8°	–0.9°	–1.7°	–3.7°	0.2°	–3.0°
C2–N3–C4–C5	1.1°	–0.3°	0.1°	–1.8°	–1.5°	–0.5°	–1.1°
N3–C4–C5–C6	1.6°	–2.4°	0.3°	1.2°	1.9°	–1.1°	2.1°
C4–C5–C6–N1	–2.7°	0.1°	0.2°	3.0°	3.3°	3.1°	1.5°
Sum of pos. ring dihedrals	5.3°	9.4°	2.0°	10.1°	14.0°	5.1°	10.1°
C1′–N1–C6–C5	172.6°	–179.6°	–179.1°	–174.0°	–171.8°	–166.9°	–177.6°
C1′–N1–C2–N3	–169.8°	177.6°	179.6°	174.1°	172.7°	166.8°	178.6°

from the C2′ atom.[18] The deviation from planar geometry increased for χ between –120° and –60° with decreasing H6–H2′ distance. A base nonplanarity of 8–9° with a χ torsion of –120° corresponded to $r_{H6H2'}$ = 2.4 Å. It was suggested that the driving force is the repulsion between H6 and H2′. In the present study of cytidine we found a different base planarity at different χ values (Table 5). The highest non-planarity was found for +60° and –60°, but in the opposite direction. The bending at χ = –60° can be explained by steric repulsion between H6 and H2′ and electrostatic repulsion between O2 and O4′ (Figure 4). However, for the +60° torsion we hypothesize electrostatic repulsion between O2 and the ring oxygen and steric repulsion between O2 and H3′. As a consequence the aromatic ring remains almost planar but the connecting bond C1′–N1 is bend in the direction of O4′. This is indicated by the low sum of positive ring dihedrals and the deviation from 180° for the hybridisation dihedrals φ_{hyb} (C1′–N1–C6–C5) and ξ_{hyb} (C1′–N1–C2–N3) (Table 5).

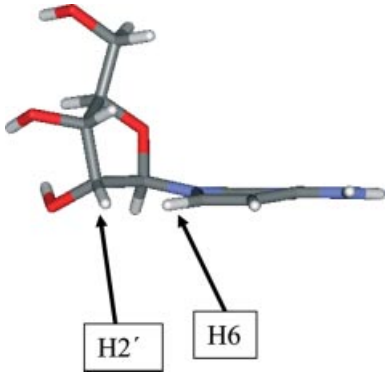

Figure 4. Base bending and repulsion of H6 and H2′ of cytidine in the North conformation with χ = –60°.

Conclusion

The calculated ^{13}C chemical shifts of cytidine correlated with a North conformation of the sugar. Moreover, the $^{1}J_{CH}$ coupling constants and ^{13}C chemical shifts clearly showed a dependence on the glycosidic torsion that enables the identification of χ. The $^{1}J_{CH}$ analysis revealed that the sugar pucker is not the major determinant for $^{1}J_{C1'H1'}$. Instead, the base orientation caused major changes, with a maximal difference of 14 Hz. The calculated base non-planarity gives an important view on the geometrical flexibility of the aromatic bases in RNA and protein-RNA complexes.

Experimental Section

Computational Methods: Molecular modelling of cytidine was done with "Discover" in the Insight program package (Accelrys™).[24] The puckering amplitude of the ribose was set at 30°, 38°, and 45°. Chemical shifts of ^{13}C (isotropic shielding) and $^{1}J_{CH}$ coupling constants (including all four contributions PSO: paramagnetic spin orbit, DSO: diamagnetic spin orbit, FC: Fermi contact and SD: spin dipole) were calculated by DFT methods for geometry optimised cytidine (Gaussian™).[5] The shifts are reported relative to the computed shielding for TMS (tetramethylsilane). The coupling constants are presented as total values of all four contributions. The B3LYP functional and a 6-31G(d,p) basis set[25] were used for geometry optimisation and calculation of NMR parameters. The sum of electronic and thermal free energy was calculated. Different sets of constraints were applied on the ribose dihedrals and the glycosidic torsion angle. One of the five ribose ring dihedrals was set at 0° in order to fix an envelope conformation.

NMR: Solutions of 5 mM and 50 mM cytidine were prepared with the following solvents: D_2O, H_2O/D_2O (90:10), H_2O/D_2O phosphate buffer (50 mM, pH = 6.7), H_2O/DMSO (70:30). ^{13}C NMR spectra were recorded at different temperatures with a Avance Bruker 400 spectrometer with standard techniques and a data size of 16 K. Standard ^{1}H-^{13}C-HSQC spectra without decoupling were performed to obtain $^{1}J_{CH}$ coupling constants.

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