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T_1 and T_2 Relaxations of the ^{13}C Nuclei of Deuterium-Labeled Nucleosides

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ABSTRACT: The effect of ^2H on ^{13}C longitudinal (T_1) and transverse (T_2) relaxation parameters was determined for the first time for diastereospecifically deuterium-labeled nucleosides, which are used as the building blocks for non-uniform isotope labeling for the solution NMR structure determination of the large biologically functional oligo-DNA and -RNA ('NMR window' approach, ref. 7). It emerged that the T_1 and T_2 of the deuterated methine carbon in the diastereospecifically deuterium-labeled nucleoside **9** could be used as the correction term to give the monoexponential decay of ^{13}C longitudinal and transverse magnetization of the constituent ^1H–^{13}C–^2H group. The correlation time derived from this corrected T_1 of the methylene carbon corresponds well with the correlation time obtained from deuterium relaxation study. The extreme narrowing limit ($\omega\tau_c \ll 1$) where dipole–dipole (DD) relaxation of ^{13}C and quadrupole (Q) relaxation of ^2H are related by $T_1^{DD}/T_2^{DD} \approx 1$ and $T_1{}^Q/T_2^Q \approx 1$ was used to demonstrate the above conclusion. The difference in the observable T_1 and T_2 in various methylene and methine-type carbons with either fully protonated or diastereospecifically deuterated nucleosides **1**–**14** allowed the estimation of the contribution of the alternative relaxation pathways other than DD relaxation. It was found by comparison of the T_1 relaxation of the quaternary carbon with the methine carbon (^{13}C–^2H) or (^{13}C–^1H) in compound **2** that the contribution of the intermolecular and intramolecular relaxations of ^{13}C with protons that are two bonds away is larger than DD(^{13}C–^2H), and the sum of all these contributions define the T_1 of the methine carbon (^{13}C–^2H). The observed difference between the experimental T_1 and T_2 of the methine carbon is attributed to the cross-correlation between DD(^{13}C–^2H) and Q(^2H) relaxation, which is consistent with recent theoretical predictions. For T_2 measurement, the decoupling of deuterium with 0.6–2.5 kHz power during the echo period by WALTZ does not effectively eliminate the DD(^{13}C–^2H)–Q(^2H) cross-correlation for the methine carbon. The suppression of this DD(^{13}C–^2H)–Q(^2H) cross-correlation was, however, more effective by applying a 180° deuterium pulse in the middle of the short (0.5 ms) echo period (compare T_2 of 3.91s and 0.3s, respectively, at 294 K using these two different decoupling procedures). The comparison of the observed T_1 and T_2 relaxations of the methylene carbon shows that they are indeed very close. The various contributions of the methine carbon relaxation such as DD(^{13}C–^2H), intermolecular and cross-correlation, DD(^{13}C–^1H)–Q(^2H), to the relaxation of the methylene carbon were ca. 15% in T_1 and *ca.* 25% in T_2. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{13}C NMR; ^2H NMR; selective deuteration; deuterated nucleosides; T_1 and T_2 relaxations

INTRODUCTION

The nature of the folded structure of DNA and RNA dictates their selective kinetic accessibility for specific interaction and recognition with various ligands, which culminate in specific biological functions. Clearly, a study of the time-scale and amplitude of the internal motion of DNA, RNA and their complexes will provide a complete physical description of the dynamics of these molecules.[1] It has been shown[1a] for oligo-DNA, for example, that the motion of the nucleobase across a glycosidic bond in a nucleotide is more restricted than that of the sugar component, and that the degree of spatial restriction of the motion,[2] i.e. the motional order parameter (S), for the sugar carbon and correlation time of the internal motion (τ_C) in a nucleotide vary widely from 0.6 to 0.8 and from 30 to 300 ps, respectively.

^{13}C NMR relaxation parameters are often used in studies of dynamics because the relaxation of protonated carbons is dominated by dipolar interaction with the attached proton(s), and this relaxation mechanism may be readily interpreted in terms of molecular dynamics.[3] The ratio of T_1 to T_2 contains information for understanding the molecular dynamics and structure.[4,5] In particular, T_2 may yield information on the chemical exchange rate for dynamic systems.[6]

The contribution of a particular proton to the DD relaxation of another proton[7a] or ^{13}C can be conveniently measured by replacing the proton by deuteron.[8] In these cases, the spatial structure of the deuterated molecule remains unchanged compared with the isosteric parent compound. The specific deuterium substitution, however, uniquely changes the relaxation

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properties of the neighboring nuclei, which can be engineered in a predictable manner,[9] and can be conveniently used as a tool for understanding the local motion and kinetics or for improving the sensitivity of two- or three-dimensional experiments.[9] This can be exemplified by a series of recent studies on proteins in which it was shown[9d–f,10] that the replacement of ^{1}H by ^{2}H causes a decrease in the relaxation rate of the ^{13}C nuclei.

Since the ^{13}C–^{2}H or (^{1}H)C–^{2}H vectors are nearly constant at 1.09 Å, the use of ^{13}C relaxation parameters in newly developed NMR methods[11] may be applied to the recently synthesized diastereospecifically labeled nucleosides[7b–d,12a–f] and also in the non-uniformly deuterium-labeled oligo-DNA[7e] or -RNA,[7f] which should make it possible to ascertain the relationship of how local structure is correlated by the internal motion.

This is the first report of the measurement of T_1 and T_2 relaxation times of ^{13}C nuclei on a series of deuterium-labeled nucleoside derivatives[7b–d,12a–f] in order to examine how the nature of the deuterium substitution affects these systems. For the T_1^{obs} measurement, we applied a conventional inversion–recovery experiment[12] with proton and deuterium decoupling to compare the influence of deuterium on T_1 and T_2 of methylene ^{1}H–^{13}C–^{2}H and methine ^{13}C(^{2}H) carbon to estimate the contribution of alternative relaxation mechanisms other than DD relaxation. For the T_2^{obs} measurement, a series of spin–echo experiments[14] were tested and modified to obtain T_2, which fulfils $T_1^{obs}/T_2^{obs} \approx 1$ for a rigid molecule with overall rotational correlation time τ_c under the extreme narrowing limit with $\omega\tau_c \ll 1$.[2b] We also discuss the underlying mechanisms for cases which showed deviations from the ratio $T_1^{obs}/T_2^{obs} \approx 1$.

EXPERIMENTAL

Compounds

1,2-*O*-Isopropylidene-α-D-allofuranose (**1**) and its C-3-deuterated analogue **2** were synthesized through oxidation of 1,2:5,6-bis-*O*-isopropylidene-α-D-glucofuranose[12a] with pyridinium dichromate–acetic anhydride[12b] in dry dichloromethane followed by stereoselective reduction of the resulting ketone with $LiAlH_4$ or $LiAlD_4$, respectively, and subsequent treatment with 80% aqueous acetic acid at room temperature overnight. Compound **2** was cleaved by $NaIO_4$ in ethanol–water followed by reduction of the resulting aldehyde with $NaBD_4$ in ethanol[12c] to give the 1,2-*O*-isopropylidene-3,5(*R*/*S*)-d_2-α-D-ribofuranose derivative. This was first deprotected with 80% aqueous acetic acid, and then converted into 1-[3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-3′,5′(*R*/*S*)-d_2-β-D-ribofuranosyl]thymine according to standard procedures.[7b] From this compound, 1-[2′,3′,5′(*R*/*S*)-d_3-β-D-ribofuranosyl]–thymine (**4**) was obtained upon oxidation of the 2′-OH group with pyridinium dichromate–acetic anhydride[12b] followed by reduction with $LiAlD_4$,[12d] inversion of the configuration at C-2 via displacement of a 2′-*O*-trifluoromethanesulfonate with cesium propionate[12e] in dry dimethylformamide, and subsequent deprotection with 1.0 M *n*-tetrabutylammonium fluoride in dry tetrahydrofuran. The deuterated thymidine analogue **9** was prepared[7c] from compound **4**. Compounds **5**,[7b] **7**,[12f] **8**,[12f] **10**,[7b] **11**[7d] and **13**[7d] were prepared by our published procedures.

NMR Experiments

Compounds **1**–**14** (Fig. 1) were dissolved in H_2O–D_2O (90:10) to a final concentration of 30 mg in 0.6 ml. The NMR experiments were carried out on a Jeol JNM-GX270 spectrometer at magnetic field strength 6.3 T operating at 67.80 MHz for ^{13}C, on a Bruker DRX 500 spectrometer at 11.7 T operating at 125.74 MHz for ^{13}C and on a Bruker DRX 600 spectrometer at 14.1 T operating at 150.90 MHz for ^{13}C. Bruker DRX 500 and DRX 600 spectrometers were equipped with a Bruker digital lock and triple-resonance probehead and the switching ^{2}H lock–^{2}H pulse device. The probe power after the switching block was 16.1 W for the 90° ^{2}H pulse of 2631 Hz. ^{1}H broadband decoupling was accomplished with the WALTZ16 sequence using a 2500 Hz r.f. field. ^{2}H decoupling utilized a WALTZ16 sequence using a 625 Hz r.f. field (0.625 W) or 2500 Hz. No ^{2}H decoupling was possible on the Jeol JNM-GX270 spectrometer.

The errors in the data in all tables represent the difference between two data sets obtained by fitting intensities or areas. The random errors (±10%) in all experiments were estimated using independent data sets with the same sample and magnetic field strength but with different Bruker triple resonance probes in the inverse (the TXI probe) or selective (selective for ^{13}C and ^{2}H in the TXO probe) mode.

To avoid the spinning artifacts, all spectra were measured on non-spinning samples. For comparison, the T_1 and T_2 measurements for **2** were performed both on spinning and non-spinning samples, and the difference between data was within the experimental error (±10%).

The various proton and carbon pulse lengths were calibrated before each experiment for each sample. A π/2 pulse for ^{13}C was in the range 14.0–14.8 μs for both 14.1 and 11.7 T spectrometers, but on the 11.7 T spectrometer the T_1 and T_2 experiments for **2** were performed using both the inverse TXI probe with a ^{13}C pulse length of 14.5 μs and the TXO probe with a ^{13}C pulse length of 6.25 μs. To evaluate the offset effect on T_1 and T_2 measurements, we performed experiments applying either homospoil pulses on ^{13}C or 180° ^{13}C pulses with on-resonance and off-resonance to 600 Hz. The results are summarized in Tables 1 and 2. The differences in the results from these experiments are well within the limit of the experimental error of ±10%.

The time between any two acquisitions (D1 delay) was 30 s. This delay is 10 times greater than the T_1 of ^{13}C(^{1}H) nuclei, 5–6 times greater than that of ^{13}C(^{2}H) nuclei and only three times greater than that of the quaternary carbon, Q_c (in **2** in Fig. 1). This is why we compared the data for **2** with the data obtained from experiments with a recycle delay of 60 s (Tables 1 and 2): for ^{13}C(^{1}H) and ^{13}C(^{2}H) nuclei the observable T_1 and T_2 were within the experimental error for D1 = 30 s and 60 s, but for Q_c, the T_2s measured with D1 = 60 s were *ca.* 25% longer than for D1 = 30 s (with a longer D1 of 100 s the T_1 and T_2 for Q_c did not change). Hence

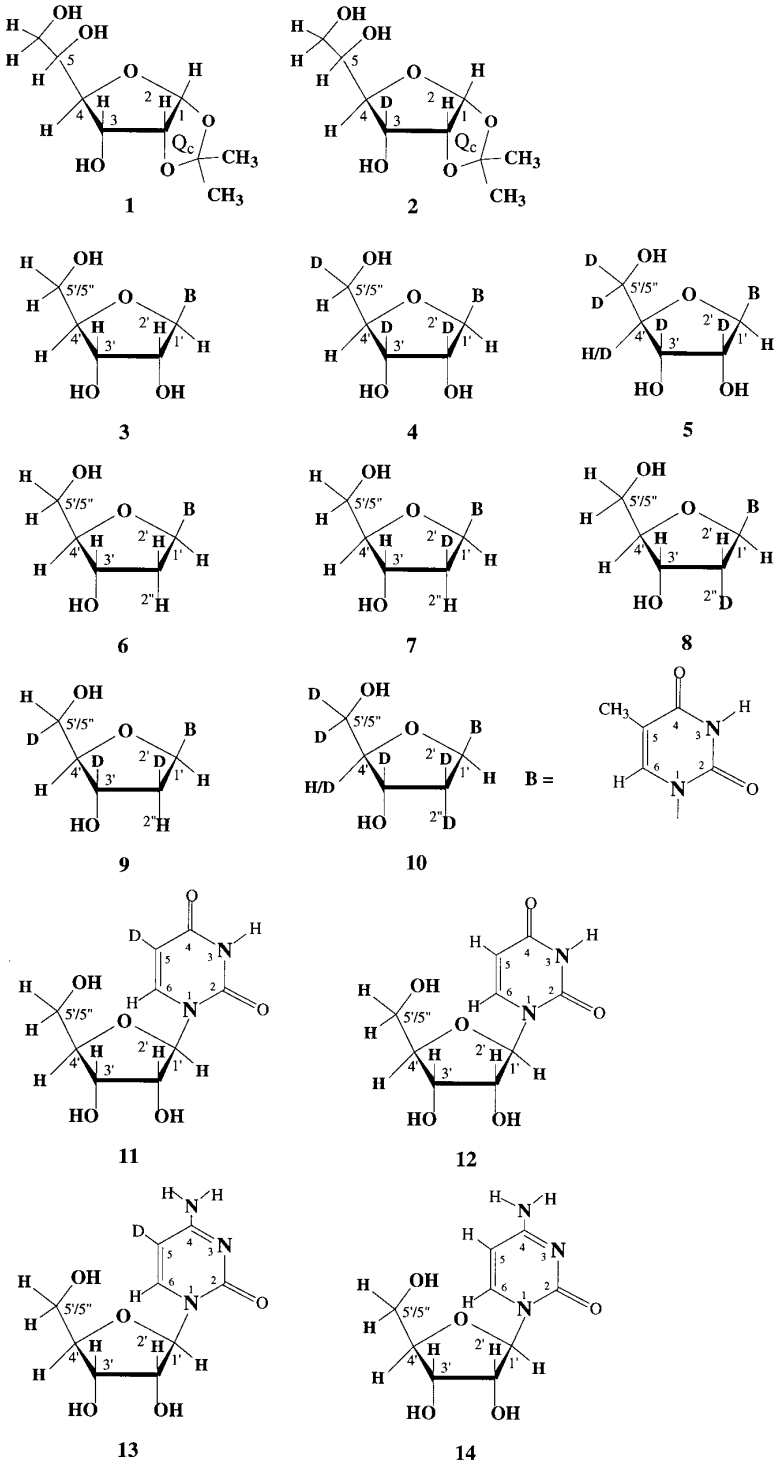

Figure 1. The natural and the stereospecifically labeled deuterated analogues.

the data with D1 = 60 s were used for the analysis and discussion of Q_c.

Proton decoupling during acquisition and saturation of the proton magnetization before inversion and during recovery of the ^{13}C magnetization were applied in the ^{13}C NMR T_1 measurements by the conventional inversion–recovery method[13] to eliminate effects from cross-relaxation and from intramolecular dipolar and anisotropic chemical shift (CSA) cross-correlation[15] (Table 1). The influence of dipolar cross-correlation in cases with the methylene carbon attached to two protons was minimized by deriving the T_1 values from fitting the recovery data to approximately $3T_1$ according to the literature procedure.[16] We performed 19–21 experiments for each sample with relaxation delays between 0.008 and 40 s for the T_1 estimation.

The T_2s were measured on a DRX 500 or DRX 600 spectrometers with experiments presented in Fig. 2. The following parameters were used: 2τ delay between 0.000 25 and 0.002 s, recycle delay 30 s with 128 scans,

Table 1. Comparison of the longitudinal relaxation times T_1^{obs} (s) for ^{13}C of compound 2 at 11.7 T in different experiments at 294 K (TXO probe)

No.[a]	Periods when waltz decoupling has been applied[b]	Pre-scan delay (D1)	C-1′	C-2′	C-3′ deut.	C-4′	C-5′	Q	CH_2
1	^{1}H-dec(all) ^{2}H-dec(AQ)	CP[c] D1 = 30 s	1.26 ± 0.05	1.26 ± 0.05	8.21 ± 0.23 ~300 Hz off-reson.	1.24 ± 0.05	1.29 ± 0.00	12.06 ± 0.02 ~300 Hz off reson.	0.77 ± 0.02
2	^{1}H-dec(all) ^{2}H-dec(AQ)	CP D1 = 60 s	1.28 ± 0.01	1.26 ± 0.03	8.21 ± 0.23 on-reson.	1.26 ± 0.03	1.30 ± 0.04	12.20 ± 0.20 ~600 Hz off-reson.	0.77 ± 0.02
3	^{1}H-dec(all) ^{2}H-dec(AQ)	CP D1 = 60 s	1.25 ± 0.05	1.30 ± 0.05	8.59 ± 0.30 ~600 Hz off-reson.	1.24 ± 0.03	1.30 ± 0.05	12.30 ± 0.30 on-reson.	0.77 ± 0.02
4	^{1}H-dec(all) ^{2}H-dec(AQ)	No CP D1 = 30 s	1.30 ± 0.02	1.28 ± 0.02	7.70 ± 0.40 ~300 Hz off-reson.	1.22 ± 0.03	1.30 ± 0.02	12.08 ± 0.80 ~300 Hz off-reson.	0.77 ± 0.02
5	^{1}H-dec(D1) ^{1}H-dec(AQ) ^{2}H-dec(AQ) No ^{1}H-dec (VD), no ^{2}H-dec(VD)	No CP D1 = 30 s	0.88 ± 0.02	0.87 ± 0.02	6.75 ± 0.05 ~300 Hz off-reson.	0.88 ± 0.02	0.88 ± 0.00	11.21 ± 0.15 ~300 Hz off-reson.	0.49 ± 0.02
6	^{1}H-dec(all) ^{2}H-dec(VD) ^{2}H-dec(AQ)	No CP D1 = 30 s	1.28 ± 0.03	1.25 ± 0.03	8.12 ± 0.30 ~300 Hz off-reson.	1.24 ± 0.02	1.28 ± 0.02	11.75 ± 0.20 ~300 Hz off-reson.	0.77 ± 0.02
7	^{1}H-dec(VD) ^{1}H-dec(AQ) ^{2}H-dec(VD) ^{2}H-dec(AQ)	No CP D1 = 30 s	1.26 ± 0.03	1.30 ± 0.03	8.00 ± 0.60 ~300 Hz off-reson.	1.23 ± 0.05	1.26 ± 0.02	11.45 ± 0.10 ~300 Hz off-reson.	0.77 ± 0.02

[a] Number of experiment.
[b] ^{1}H-dec(all), decoupling of ^{1}H during all experiment; ^{1}H-dec(AQ) and ^{2}H-dec(AQ), decoupling of ^{1}H and ^{2}H during acquisition time; ^{1}H-dec(D1), saturation of ^{1}H during pre-scan delay (D1); ^{1}H-dec(VD) and ^{2}H-dec(VD), decoupling of ^{1}H and ^{2}H during relaxation time VD.
[c] CP, homospoil pulse instead of 180° ^{13}C pulse was applied.

SW for ^{13}C 180 ppm and TD 64K. We performed 19–21 experiments for each sample with relaxation delays (2τ) during the CPMG period of the T_2 experiment between 0.004 and 40.0 s.

The systematic error associated with the off-resonance effect in T_2 relaxation has been discussed recently in the literature,[17] and could reach a value of ±25%. Under our experimental conditions, all ^{13}C

Table 2. Comparison of the transverse relaxation times T_2^{obs} (s) for ^{13}C of compound 2 at 11.7 T for different experiments at 294 K (TXO probe)

No.[a]	Carrier frequency of ^{13}C (Hz)	Prescan delay (D1) (s)	C-1′	C-2′	C-3′ deut.	C-4′	C-5′	Q	CH_2
1	11 600	30	1.30 ± 0.15	1.10 ± 0.10	5.00 ± 0.50 ~300 Hz off-reson.	1.05 ± 0.10	1.00 ± 0.10	7.30 ± 0.50 ~300 Hz off-reson.	0.67 ± 0.70
2	8790	60	1.10 ± 0.10	1.05 ± 0.10	5.00 ± 0.50 on-reson.	1.10 ± 0.10	1.10 ± 0.10	10.0 ± 0.70 ~600 Hz off-reson.	0.66 ± 0.05
3	8790 (^{1}H and ^{2}H pulses are equal to 125°)	60	1.20 ± 0.10	1.00 ± 0.10	4.70 ± 0.50 on-reson.	1.10 ± 0.10	1.10 ± 0.10	10.1 ± 0.30 ~600 Hz off-reson.	0.75 ± 0.05
4	14 311	60	1.15 ± 0.15	1.05 ± 0.10	5.00 ± 0.50 ~600 Hz off-reson.	1.10 ± 0.10	1.10 ± 0.10	10.4 ± 0.7 on-reson.	0.77 ± 0.10

[a] Number of experiment.

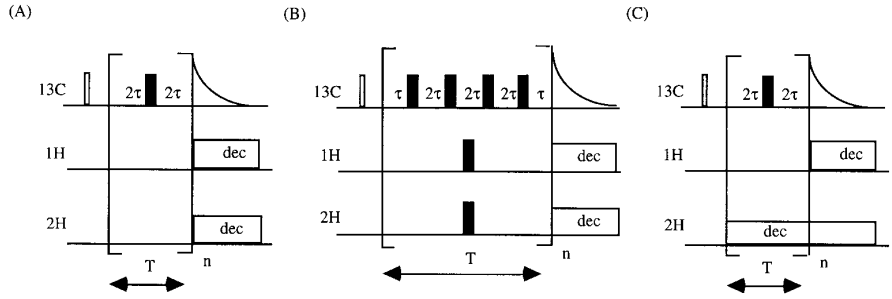

Figure 2. CPMG pulse sequences used for T_2 measurements. (A) Standard pulse sequence with delay in echo block 2τ. (B) Modified pulse sequence to remove cross-correlation effects suggested by Kay *et al.*[34] for both ^{2}H and ^{1}H. (C) Standard pulse sequence with ^{2}H decoupling during T period. Thin bars represent $\pi/2$ pulses and thick bars represent π pulses. In all experiments the decoupling of both proton and deuterium was used during the acquisition period.

resonances in the sugar moiety were located within ± 300 Hz from the carrier. For example, for the Q_c in **2** we could expect the maximum offset effect because it resonates in the most downfield region of amongst all sugar carbons, but the experiments performed with different carrier frequencies showed that the discrepancy is well within the experimental error ($\pm 10\%$) (See Table 2).

T_1 and T_2 values for ^{13}C were calculated with the aid of Bruker software. The deuterium relaxation rate was measured on a Bruker selective triple resonance probe with fluorine lock. In order to obtain the lock signal, 18 μl of CF_3CH_2OH were added to samples containing 30 mg of compounds **7** and **8** dissolved in 0.6 ml of D_2O.

RESULTS AND DISCUSSION

Deuterium Relaxation Rate

In this study, stereospecifically deuterium-labeled nucleosides (**7** and **8**) were used (Fig. 1) to take advantage of the dominance of the quadrupolar relaxation pathway in the longitudinal relaxation rate ($1/T_1^Q$) for the deuterium.[18–20] T_1^Q can be directly related to the molecular motion (under the extreme narrowing limit with $\omega\tau_{eff} \ll 1$ for isotropic motion, where τ_{eff} is effective rotational correlation time[3,5,18–20] and ω is the Larmor frequency) via the equation

$$\frac{1}{T_1^Q} \approx \frac{1}{T_2^Q} \approx \frac{3\pi^2}{2}\left(\frac{e^2qQ}{h}\right)^2 \tau_{eff} \qquad (1)$$

where e^2qQ/h is the quadrupole coupling constant[5,21,22] and $Q = 165$ kHz.[20]

The effective correlation time governing deuterium relaxation defines the reorientation of the ^{13}C–^{2}H vector of the methylene group. It can be directly compared with τ_c for ^{13}C DD relaxation of the partially deuterated carbon, i.e. ^{1}H–$^{13}C(^{2}H)$, which is dependent upon the reorientation of the ^{13}C–^{1}H vector[3,18–20] Thus the following equation describes DD relaxation, $1/T_1^{DD}$, for ^{13}C under extreme narrowing limits ($\omega\tau_c \ll 1$):[3,18,21,23]

$$\frac{1}{T_1^{DD}} = (N\gamma_C^2\gamma_H^2\hbar^2/r_{CH}^6)\tau_c \qquad (2)$$

where N is the number of directly bonded hydrogens, τ_c is the rotational correlation time for the reorientation of the relaxation vector (r_{ij}) and γ_H and γ_C are the proton and carbon gyromagnetic ratios, respectively. For **7** and **8**, the relaxation times T_1 of ^{2}H at different temperatures are presented in Table 3 along with the correlation times obtained using Eqn (1), which shows that the deuterium relaxation times are *ca.* 20 times shorter than those of the methylene carbon at 25 °C (Table 4). This is to be expected if the main longitudinal relaxation mechanism of ^{13}C is DD with ^{1}H, and the main longitudinal relaxation pathway for deuterium is quadrupolar.[20] Note that deuterium relaxation times are in the 25–200 ms range between 278 and 358 K, which is in agreement with the measurements made on a number of organic compounds of varying molecular weights and viscosities.[18–20] Table 3 also shows that the correlation

Table 3. Longitudinal relaxation times T_1^Q and correlation times τ_{eff} [a] of ^{2}H of compounds **7** and **8** measured at 11.7 T at different temperatures, T (K) (TXO probe)

	Compound **7** (2″–^{2}H)		Compound **8** (2′–^{2}H)	
T (K)	T_1^Q (ms)	τ_{eff} (ps)	T_1^Q (ms)	τ_{eff} (ps)
278	24.5	101.2	24.6	101.0
283	29.9	82.9	29.7	83.4
288	38.5	64.4	37.2	66.6
293	44.4	55.9	44.3	56.0
298	55.1	45.0	53.6	46.3
303	61.6	40.3	61.9	40.0
308	72.3	34.3	70.6	35.1
313	81.9	30.3	82.5	30.0
318	94.9	26.1	93.9	26.4
323	106.9	23.2	107.8	23.0
328	121.5	20.4	121.9	20.3
333	136.0	18.2	133.8	18.5
338	151.6	16.4	149.1	16.6
343	167.2	14.8	162.6	15.3
348	186.6	13.3	179.6	13.8
353	201.0	12.3	199.2	12.4
358	220.5	11.2	216.5	11.5

[a] τ_{eff} (ps) was calculated using equation

$$\frac{1}{T_1^Q} = \frac{3\pi^2}{2}\left(\frac{e^2qQ}{h}\right)^2 \tau_{eff}$$

where the quadrupole coupling constant (e^2qQ/h) is 165 kHz.

Table 4. Longitudinal relaxation times T_1 (s) of ^{13}C of compounds **1–14** measured at 6.3, 11.7 and 14.1 T (TXI probe)[a]

Compound[b]	B (T)[c]	C-1′	C-2′	C-3′	C-4′	C-5′	C-2	C-4	C-5	C-6
1	6.3	1.35	1.25	1.18	1.25	1.35	—[d]	—[d]	—[d]	—[d]
	11.7	1.21	1.10	1.05	1.20	1.20				
	14.1	1.22 ± 0.01	1.20 ± 0.01	1.22 ± 0.01	1.16 ± 0.01	1.12 ± 0.01				
2	6.3	1.31	1.30	Deut.[e]	1.30	1.37	—[d]	—[d]	—[d]	—[d]
	11.7	1.22	1.18	7.12	1.21	1.22				
	14.1	1.23 ± 0.01	1.22 ± 0.01	7.12 ± 0.04	1.18 ± 0.01	1.24 ± 0.01				
3	6.3	0.87	0.99	1.02	0.84	0.47	7.63	8.59	7.20	0.78
	11.7	0.79	0.87	0.92	0.76	0.45	5.88	5.99	5.23	0.64
	14.1	0.79 ± 0.01	0.93 ± 0.01	0.96 ± 0.01	0.80 ± 0.01	0.47 ± 0.01	5.54 ± 0.38	5.61 ± 0.20	4.57 ± 0.18	0.67 ± 0.01
4	6.3	1.56	Deut.	Deut.	1.42	Deut.	8.62	7.54	8.53	1.29
	11.7	0.89	6.95	6.95	0.87	0.92	6.29	6.76	5.94	0.73
	14.1	0.92 ± 0.01	6.97 ± 0.01	7.26 ± 0.28	0.8 ± 0.02	0.90 ± 0.01	5.93 ± 0.27	5.51 ± 0.28	5.20 ± 0.42	0.73 ± 0.01
5	6.3	0.82	Deut.	Deut.	0.89	Deut.	7.88	8.25	8.90	0.77
	11.7	0.85	6.62	7.20/7.8[f]	0.84/7.70[g]	5.03	5.91	6.26	5.38	0.65
	14.1	0.84 ± 0.01	7.13 ± 0.03	7.66 ± 0.32[f]	0.86 ± 0.02[g]	4.66 ± 0.30	5.70 ± 0.10	5.18 ± 0.13	4.70 ± 0.03	0.67 ± 0.00
				7.77 ± 0.10	6.93 ± 0.33					
6	6.3	1.01	0.69	1.39	1.03	0.57	7.62	8.42	7.71	0.83
	11.7	0.96	0.66	1.33	0.94	0.50	6.50	7.43	6.21	0.73
	14.1	0.99 ± 0.01	0.72 ± 0.02	1.42 ± 0.01	1.02 ± 0.02	0.58 ± 0.01	6.23 ± 0.03	6.87 ± 0.05	5.07 ± 0.25	0.77 ± 0.02
7	6.3	1.00	Deut.	1.45	1.06	0.60	7.35	8.46	7.48	0.83
	11.7	0.99	1.00	1.21	0.86	0.52	6.51	7.17	6.40	0.79
	14.1	0.95 ± 0.01	1.27 ± 0.03	1.32 ± 0.02	0.97 ± 0.00	0.54 ± 0.01	5.39 ± 0.01	6.20 ± 0.01	5.41 ± 0.11	0.76 ± 0.02
8	6.3	0.91	Deut.	1.38	0.99	0.54	7.74	7.50	7.67	0.81
	11.7	0.90	1.11	1.25	0.93	0.52	6.13	6.81	6.02	0.71
	14.1	0.93 ± 0.03	1.21 ± 0.00	1.30 ± 0.05	0.95 ± 0.03	0.54 ± 0.02	7.02 ± 1.08	6.91 ± 1.67	6.07 ± 0.91	0.77 ± 0.01
9	6.3	0.95	Deut.	Deut.	0.95	Deut.	7.93	7.38	6.56	0.77
	11.7	0.93	1.18	7.65	0.97	0.99	6.56	7.36	6.07	0.72
	14.1	0.93 ± 0.03	1.22 ± 0.01	8.25 ± 0.05	0.97 ± 0.00	1.00 ± 0.01	5.74 ± 0.14	6.28 ± 0.14	5.30 ± 0.16	0.74 ± 0.01
10	6.3	1.04	Deut.	Deut.	1.08	Deut.	7.47	8.74	6.82	0.84
	11.7	0.95	6.47	9.80[h]	0.98/9.04[g]	5.6	6.15	6.79	6.14	0.72
	14.1	0.99 ± 0.02	6.57 ± 0.35	10.66 ± 1.37[h]	0.97 ± 0.02[g]	6.26 ± 0.07	5.97 ± 0.15	6.46 ± 0.07	5.23 ± 0.02	0.73 ± 0.01
					9.28 ± 0.01					
11	11.7	0.95	1.09	1.09	0.94	0.55	7.36	8.66	5.54	0.81
	14.1	0.97 ± 0.01	0.99 ± 0.08	1.11 ± 0.01	0.97 ± 0.01	0.53 ± 0.01	6.02 ± 0.16	6.37 ± 0.61	4.77 ± 0.01	0.76 ± 0.01
12	11.7	0.94	1.04	1.06	0.92	0.52	7.49	7.92	0.91	0.77
	14.1	1.02 ± 0.01	1.18 ± 0.07	1.24 ± 0.09	0.99 ± 0.04	0.57 ± 0.02	6.03 ± 0.09	6.97 ± 0.05	0.91 ± 0.01	0.76 ± 0.02
13	11.7	0.88	0.97	0.97	0.84	0.51	7.05	3.76	4.24	0.75
	14.1	0.87 ± 0.01	0.98 ± 0.01	0.99 ± 0.01	0.85 ± 0.01	0.50 ± 0.01	5.66 ± 0.18	3.05 ± 0.06	4.00 ± 0.13	0.73 ± 0.02
14	11.7	0.83	0.88	0.91	0.76	0.45	7.93	3.60	0.76	0.67
	14.1	0.88 ± 0.03	0.99 ± 0.02	1.01 ± 0.05	0.85 ± 0.03	0.50 ± 0.01	5.77 ± 0.29	3.33 ± 0.19	0.81 ± 0.02	0.70 ± 0.02

[a] The value of the spin–lattice relaxation time T_1 is presented as an average obtained by using the intensity fit and areas fits, which showed a deviation of not more than 5% as shown for experiments at 14.1 T.
[b] Compound numbers according to Fig. 1.
[c] Magnetic field strength.
[d] This nucleus does not exist.
[e] Deut: Could not be measured on the JNM-GX270 spectrometer.
[f] In the case of the isotopic mixture of ^1H and ^2H in the 4′-position, two signals of ^{13}C in C-3′ could be distinguished and both signals were used to measure T_1 relaxation time.
[g] The isotopic mixture of ^1H and ^2H leads to two types of ^{13}C resonances for C-4′, one in proximity to ^1H and the other to ^2H. In this case two ^{13}C signals were observed, and two values are presented.
[h] Two ^{13}C signals in C-3′ could not be used separately for measurement.

times of 2′-^2H or 2″-^2H in the nucleosides **7** and **8**, obtained using the T_1 relaxation time, are similar.

Hence the use of small monodeuterated nucleoside blocks with a relatively small quadrupolar relaxation [see Eqn (1)] allowed us to work under the extreme narrowing limit, $\omega\tau_c \ll 1$, which in turn helped us to obtain a sharp, well resolved deuterium resonance to give us a straightforward determination of the overall correlation time using Eqn (1); this correlation time was subsequently used as a reference to evaluate the T_1 and T_2 relaxations of the methylene carbon with stereospecifically labeled deuterium.

Influence of Deuterium on the T_1 of ^{13}C

Upon deuterium substitutions in the sugar moiety or directly in the aromatic aglycone, the methine and methylene carbons in natural nucleosides may give four different types of spin systems, ^{13}C–H, ^{13}C–^2H, ^1H–^{13}C–^2H and ^{13}C–$^2H_2$. In this work, we did not consider any compound with deuterium in the methyl group of thymidine because we wanted to measure the relaxation properties of only those carbons which involve sugar–phosphate backbone conformation, and are present in all nucleotide moieties in DNA or RNA.

Keeping in view the extreme narrowing limit when $T_1^{DD} \approx T_2^{DD2b}$ for our deuterated substances, we attempted to evaluate overall contributions, other than DD relaxation pathways on the observed T_1^{obs} and T_2^{obs}, such as chemical shift anisotropy (CSA) relaxation[5,24] and other cross-correlation phenomena including DQ(^{13}C–^{2}H, ^{2}H),[25,26] D-CSA(^{13}C–^{2}H, ^{13}C),[27] CSA-Q(^{2}H, ^{2}H)[21] and scalar relaxation of the second kind.[5,22]

The data on the T_1 of ^{13}C for compounds **1–14** in Table 4 show that the T_1 of sugar carbons is weakly dependent upon the frequency of the spectrometer compared with the constituent nucleobase. It can be seen that owing to the dependence of the CSA contribution on the square of the magnetic field[5,24] the contribution of the chemical shift anisotropy to T_1 relaxation of sugar carbons is small, which is in agreement with the data for the aliphatic carbon nuclei.[1,2b,28,29] Moreover, for aromatic unprotonated carbons, i.e. C-2, C-4 and C-5 (Table 4), there is a noticeable dependence of T_1 on the strength of the magnetic field (10–20%) (compare data from the 6.3 and 14.1 T spectrometers). These data show that T_1^{csa} and T_2^{csa} should be taken into consideration for the T_1 of aromatic carbons under narrowing limit when $T_1^{csa}/T_2^{csa} \approx 7/6$.

The experimentally obtained T_1 for **1–14** were as follows (Table 4).

Deuteration in Sugars 1 and 2. The T_1 of sugar protons at C-1′, C-2′, C-3′, C-4′ and C-5′ for **1** are *ca.* 1.2 s which, according to Eqn (1), show that the correlation times are similar (3.81^{-11} s). In **2**, the replacement of the H-3′ by ^{2}H enhances the T_1 of methine C-3′ only by a factor of 7 (see discussion below). Noteworthy is the fact that replacement of 3′-^{2}H in **2** does not affect the relaxation times of the neighboring C-2′ and C-4′.

Deuteration in the Ribonucleosides 3–5. The deuteration enhances T_1 by a factor of 6–7 for the methine carbons in **4** and **5** in the same way as for the sugar **2**. No influence of ^{2}H labeling in **4** and **5** on the T_1 relaxation of the neighboring C-1′ and C-4′ was detectable, however. In the 5′-methylene group in **4**, the replacement of one of the protons by deuterium enhances the relaxation time by a factor of 2, while the replacement of both protons in **5** by deuterium causes an increase in the relaxation time by a factor of 10.

Deuteration in the 2′-Deoxyribonucleosides 6–10. For the C-2′ and C-5′ methylene groups, the replacement of a single proton by deuterium causes an increase in the relaxation time by a factor of 2 (for C-2′, compare **7** and **8** with **6**, and for C-5′ compare **6** with **9**), while the replacement of both protons at methylene C-2′ by deuterium increases the relaxation time by a factor of 10 in **10**. The relaxation times of methylene C-2′ are similar both for 2′-^{2}H in **7** and 2″-^{2}H in **8**, which is consistent with the data obtained from deuterium relaxation studies. The replacement of ^{2}H at methine C-3′ as in **9** and **10** causes an increase in the relaxation time of C-3′ by a factor of 7 and 8, respectively, because there is only one deuterium atom at C-2′ in the former and two deuterium atoms in the latter in the proximity of 3′-^{2}H.

Deuteration in the Nucleobases 11–14. Substitution of a proton by ^{2}H in C-5 for uracil and cytidine nucleobases increases T_1 sixfold, as is evident from the comparison of **11** and **12** and of **13** and **14**.

The following general conclusions can be drawn from the above T_1 data for deuterated nucleosides **1–14**.

The Relaxation of the Methine Carbon. The theoretically expected ratio of DD relaxation rates of ^{13}C-with ^{1}H ($1/T_{1,\,^{13}C-^{1}H}^{DD}$) or ^{2}H ($1/T_{1,\,^{13}C-^{2}H}^{DD}$) is dependent on the gyromagnetic ratio of ^{1}H and ^{2}H ($\gamma_H/\gamma_D \approx 6.514$). This DD relaxation rate ratio is defined as follows:

$$\frac{1/T_{1,\,^{13}C-^{1}H}^{DD}}{1/T_{1,\,^{13}C-^{2}H}^{DD}} = \frac{\gamma_H^2 I(I+1)}{\gamma_D^2 S(S+1)} = \frac{3\gamma_H^2}{8\gamma_D^2} \approx 16 \qquad (3)$$

where I and S are spin quantum numbers for ^{1}H and ^{2}H. In our measurements, the observable $(1/T_{1,\,^{13}C-^{1}H}^{obs})/(1/T_{1,\,^{13}C-^{1}H}^{obs})$ ratio is 6–7 for the methine carbon substituted with ^{2}H and it is *ca.* 10 times for the dideutero-substituted methylene carbon. Noteworthy is the fact that for the Q_c in **2** (Table 1), the T_1 value is *ca.* 12 ± 0.5 s. The observed longitudinal relaxation rate ($1/T_1^{obs}$) is a result of sum of contributions, $1/T_1^{dd} + 1/T_1^{csa} + 1/T_1^{other}$, of the DD interaction ($1/T_1^{dd}$), chemical shift anisotropy ($1/T_1^{csa}$), which is negligible (see above), and the other terms ($1/T_1^{other}$). This means that for the methine carbon, $1/T_1^{obs} = 1/T_1^{dd} + 1/T_1^{other}$.

The $1/T_1^{other}$ term arises from events such as translational diffusion ($1/T_1^{trans}$), spin rotation ($1/T_1^{sr}$), inhomogeneity of magnetic field, paramagnetic impurities including the absorbed oxygen in the NMR sample solution and intramolecular contribution of various DD relaxations resulting from interactions between ^{13}C and all other adjacent protons which are two bonds away (all interactions between ^{13}C and protons that are three bonds away are neglected).

Translational diffusion. To evaluate the intermolecular DD relaxation through translation diffusion, T_1 measurements for **2** were performed at 323, 313, 294 and 278 K, which showed (Table 5) that T_1 for Q_c in **2** were closely related to $1/T_1^{obs} \sim \eta/T$ (K), where η is the viscosity of water at a given temperature, T (K), and the ratio $T\,(K)^{(294)}\eta^{313}/T\,(K)^{(313)}\eta^{294}$ is 0.616, where η at 294 and 313 K are 0.01 and 0.00654 P, respectively. Because the ratio of T_1^{trans} at two different temperatures (294 and 313 K) is related[5,30] by $T_1^{trans(294)}/T_1^{trans(313)} = T\,(K)^{(294)}\eta^{313}/T\,(K)^{(313)}\eta^{294}$, we estimated the value for $T_1^{trans(313)}$ to be *ca.* 20 ± 1.0 s, which is close to the experimentally obtained value, $T_1^{obs(313)} \approx 22 \pm 1.0$ s. The estimated T_1^{trans} values for other temperatures are presented in Table 5 (in parentheses). These data suggest that the translation diffusion term could be one of the most dominant sources of relaxation under the

Table 5. Comparison of the longitudinal T_1^{obs} (s) and transverse relaxation times T_2^{obs} (s)[a] of ^{13}C of compound **2** at 11.7 T at different temperatures (TXO probe)

T (K)	Parameter	C-1′	C-2′	C-3′ deut.	C-4′	C-5′	Q	CH_2
323	T_1	2.80 ± 0.10	2.70 ± 0.10	18.6 ± 0.1 (17.0 ± 1.7)[b]	2.75 ± 0.10	2.70 ± 0.10	26.00 ± 0.05 (24.2 ± 2.0)[b]	1.80 ± 0.10
	T_2	2.60 ± 0.10	2.30 ± 0.30	7.7 ± 1.4 (10.8 ± 1.0)	2.30 ± 0.10	2.40 ± 0.20	14.50 ± 1.50	1.40 ± 0.20
313	T_1	2.20 ± 0.10	2.10 ± 0.10	16.0 ± 1.5 (14.0 ± 1.4)	2.20 ± 0.20	2.25 ± 0.20	22.00 ± 1.00 (20.0 ± 1.0)	1.30 ± 0.10
	T_2	2.00 ± 0.20	1.70 ± 0.30	5.0 ± 0.5 (7.6 ± 0.7)	2.00 ± 0.20	1.80 ± 0.2	10.0 ± 1.500	1.00 ± 0.20
294	T_1	1.21 ± 0.02	1.18 ± 0.01	8.0 ± 0.8 (7.4 ± 0.7)	1.21 ± 0.01	1.22 ± 0.01	12.06 ± 0.02	0.74 ± 0.01
	T_2	1.20 ± 0.10	1.10 ± 0.10	5.0 ± 0.5 (6.5 ± 0.6)	1.10 ± 0.10	1.10 ± 0.10	10.10 ± 0.90	0.70 ± 0.07
278	T_1	0.67 ± 0.01	0.66 ± 0.01	4.2 ± 0.1 (4.0 ± 0.4)	0.64 ± 0.01	0.68 ± 0.01	6.40 ± 0.50 (7.3 ± 0.8)	0.40 ± 0.01
	T_2	0.55 ± 0.01	0.55 ± 0.01	2.1 ± 0.1 (3.7 ± 0.4)	0.55 ± 0.01	0.57 ± 0.02	5.00 ± 0.50	0.33 ± 0.01

[a] See Table 4.
[b] In parenthesis the theoretically calculated values are presented (see text).

conditions of the experiment for non-protonated Q_c in **2**.

Influence of ^{13}C relaxation by adjacent protons which are two bonds away. We also estimated the influence of the DD interaction between ^{13}C and protons that are two bonds away.[31] $1/T_1^{DD}{}_{[C-X-H]}$. For example, 3′-^{13}C has H-4′, 3′-OH and H-2′ in **3** that are two bonds away, and in **6** there are four adjacent protons that are two bonds away. The distances (*ab initio* calculations, Gaussian 94, HF-6–31G* basis set) between ^{13}C and a proton that is two bonds away were found to be 2.15 Å on average. This allowed us to calculate the contribution of three adjacent protons to the T_1 of 3′-^{13}C in **3** or of four adjacent protons to the 3′-^{13}C in **6**, which is between 5 and 7% compared with straight one-bond 3′-^{13}C–^{1}H DD relaxation. This becomes clearly evident when one compares the T_1 values in compounds **6** (four protons two bonds away and one straight proton to C-3′), **9** (three protons two bonds away and one straight deuterium to C-3′ and **10** (one proton two bonds away and all deuterium to C-3′), the T_1 values are 1.33, 6.75 and 9.8, respectively [note DD(^{13}C–^{2}H) is 16 times less than DD(^{13}C–^{1}H), therefore the influence of deuterium is negligible in the two-bond ^{13}C and ^{2}H DD relaxation]. This means that the differences in T_1 between **9** and **10** is due to the absence of two two-bond ^{13}C–^{1}H relaxation. For **10**, the T_1 of 3′-^{13}C is *ca.* 10 s (the theoretically expected T_1 is *ca.* 16 s) despite the fact that only one two-bond proton exists, which means that there is a relaxation leakage through intermolecular dipole–dipole interactions such as translational diffusion as discussed above (i.e. 3′-OH). It is noteworthy that a similar situation also exists for all sugar carbons in nucleosides, which have between three and four protons that are two bonds away. These contributions are expected to be similar, and their influence would be proportional to η/T (K) in the same way as in translational diffusion.

Spin rotation. Relaxation by a spin–rotation mechanism is most effective for small symmetrical molecules and for methyl groups. This relaxation only becomes significant for molecules with fast rotation speeds.[30] To eliminate the spin–rotation term, the experiment should be performed much below 0 °C, which is certainly not a condition of interest for relaxation studies on oligo-DNA or -RNA. Moreover, $1/T_1^{sr} \sim T$ (K)$/\eta$ is opposite to the mechanism of the relaxation by DD interaction, which means that T_1^{sr} decreases with increasing temperature. As pointed above, our estimation of the longitudinal relaxation time of Q_c nuclei (Table 5) has shown that $1/T_1^{obs} \sim \eta/T$ (K), hence the spin–rotation term contribution can be safely neglected.

This shows that $1/T_1^{other}$ for the Q_c carbon in **2** is maximum for any carbons in the nucleoside and nucleotide family since it has six protons that are two bonds away. This means that $1/T_1^{other} \sim 1/T_1^{trans} + 1/T_1^{DD}{}_{[C-X-H]}$, which in turn is approximately equal to T_1^{obs} for the Q_c carbon in **2**. Hence, we have taken into account of this $1/T_1^{other}$ term in correcting the T_1 in **3**–**14** of the methylene and methine carbons by using an upper limit of 12 ± 1 s for the T_1^{other} term (i.e. $1/T_1^{other} \approx 0.083 \pm 0.007$ s^{-1}) at 294 K.

It is easy to see for compound **2** (Table 5) that the experimentally observed longitudinal relaxation rate of the deuterium-substituted methine carbon ($1/T_1^{obs} \approx 0.125 \pm 0.01$ s^{-1}) at 294 K is the sum of the theoretically expected value (0.052 ± 0.005 s^{-1}) for DD ^{13}C–^{2}H relaxation, $1/T_1^{DD\ theory}$ [which is estimated as $1/T_{1,CH}^{DD}$ multiplied by 16; see Eqn (3)], and the $1/T_1^{other}$ term, which is 0.083 ± 0.007 s^{-1} derived from the Q_c in **2**.

The estimated rate of the $1/T_1^{obs}$ of the deuterium-substituted methine carbon at other temperatures, given in parentheses in Table 6, also follows the experimentally observed data.

The Relaxation of the Methylene Carbon. The above data allow us to estimate quantitatively the contribution of the cross-correlation term in the relaxation of the methylene-type carbon using the rate of longitudinal relaxation of deuterated methine carbon (Table 4) in the same sugar moiety. In the present investigation this contribution was found to be less than 15%. After taking into account the effect of 'other' longitudinal relaxation terms, the replacement of one of the protons in the methylene group by deuterium causes a *ca.* twofold change in the T_1 of the methylene carbon, suggesting the dominance of the DD relaxation of the residual proton with carbon. This shows that under our experimental conditions only a minor influence of the DD cross-correlation term in the relaxation behaviour of the carbon attached to two protons or the DD cross-correlation between ^{13}C–^{1}H and ^{13}C–^{2}H. As mentioned in the Experimental section, proton decoupling during acquisition and saturation of the proton magnetization before inversion and during recovery of the ^{13}C magnetization eliminates effects from cross-relaxation and from dipolar-CSA cross-correlation[15] but not DD cross-correlation effects.[16] Moreover, the replacement of ^{1}H by ^{2}H should reduce the effect of DD cross-correlation between ^{13}C–^{1}H and ^{13}C–^{2}H because the gyromagnetic ratio of ^{1}H and ^{2}H is correlated[16] by $\gamma_H/\gamma_D \approx 6.514$. It is noteworthy that the influence of DD cross-correlation has been assumed to be small by several workers in this field and has been neglected.[26b,32]

Comparison of Correlation Time Obtained by Carbon and Deuterium Relaxation Measurements. The correlation time obtained from the T_1 relaxation time of mono-deuterium-substituted methylene carbon corrected for the 'other' relaxation term strictly corresponds to the correlation time obtained from deuterium relaxation study, which again shows the dominance of the single DD pathway for ^{13}C relaxation and the quadrupolar pathway for the deuterium relaxation.

Influence of deuterium on the T_2 of ^{13}C

For the T_2 of in-phase magnetization of sugar carbons, it is expected that $T_1 \approx T_2$ because of the extreme narrowing limit ($\omega\tau_m \ll 1$), $T_1^{DD}/T_2^{DD} \approx 1$ and the CSA contribution is negligible (see above). Variations from this $T_1 \approx T_2$ relationship indicate either an incomplete elimination of the antiphase magnetization[33] in the measurement of T_2 or the involvement of another relaxation mechanism. Decreasing the delay (2τ) in the echo block (T) of the standard CPMG experiment [Fig. 2(A)] to 0.0005 s did not help in reaching the desired $T_2 \approx T_1$ for ^{13}C attached to proton (see Table 6 for compound **2**) but it increased the T_2 value 2–2.5 fold for the ^{13}C attached to deuterium. These data are again in agreement with an earlier observation[34] that the decrease in the delay of the echo block in the routine CPMG experiment did not eliminate either the cross-correlation effect or the antiphase magnetization satisfactorily. What it is important to note is that the T_2 of ^{13}C attached to proton behaved differently to the T_2 of ^{13}C attached to deuterium. Kay *et al.*[34] have shown how to eliminate the cross-correlation effect between dipolar and CSA relaxations in the T_2 measurement of ^{13}C attached to proton by modifying the CPMG pulse sequence by inserting a proton 180° pulse between every second carbon refocusing pulse. We examined Kay *et al.*'s[34] procedure to see how it works for the T_2 measurement of ^{13}C attached to deuterium. Figure 2(B) shows the modified Kay *et al.*'s experiment with triple resonance with 180° pulses on ^{1}H and ^{2}H in the middle of the echo block.

Using this modified pulse sequence for ^{13}C attached to proton, T_2 was found to be closely similar to T_1 when a 2τ delay of 0.002 s was applied, and T_2 even stayed in the same range upon decreasing the delay to 0.000 25 s (Table 6). It is different, however, for ^{13}C attached to deuterium where the T_2 gradually increased with a

Table 6. Transverse relaxation times T_2^{obs} (s) for ^{13}C of compound 2 at 11.7 T measured in different experiments (TXI probe)

No. of expts	2τ (ms)	C-1′	C-2′	C-3′ deut.	C-4′	C-5′	CH_2
1[a]	2.0	0.56 ± 0.01	0.57 ± 0.01	1.15 ± 0.02	0.54 ± 0.01	0.46 ± 0.01	0.28 ± 0.01
1[a]	1.0	0.38 ± 0.03	0.58 ± 0.01	2.06 ± 0.02	0.50 ± 0.01	0.31 ± 0.01	0.28 ± 0.01
1[a]	0.5	0.37 ± 0.03	0.64 ± 0.03	2.56 ± 0.07	0.55 ± 0.01	0.40 ± 0.01	0.26 ± 0.01
5[b]	0.5	0.43 ± 0.04	0.48 ± 0.02	0.31 ± 0.02	0.45 ± 0.03	0.37 ± 0.02	0.26 ± 0.02
2[c]	2.0	1.04 ± 0.05	1.05 ± 0.05	1.61 ± 0.10	0.99 ± 0.05	0.76 ± 0.05	0.42 ± 0.07
2[c]	1.0	1.11 ± 0.05	1.17 ± 0.00	2.30 ± 0.12	1.07 ± 0.06	0.94 ± 0.02	0.53 ± 0.01
2[c]	0.5	1.13 ± 0.05	1.04 ± 0.05	3.91 ± 0.12	1.09 ± 0.01	1.04 ± 0.01	0.54 ± 0.01
2[c]	0.25	0.91 ± 0.11	0.97 ± 0.09	4.14 ± 0.29	1.20 ± 0.03	1.07 ± 0.01	0.60 ± 0.03

[a] Standard CPMG experiment, presented in Fig. 2(A).
[b] Standard CPMG experiment, with ^{2}H decoupling during T delay. The pulse sequence is presented in Fig. 2(C).
[c] Modified CPMG experiment, with ^{2}H and ^{1}H 180° pulses in the middle of the T delay [see Fig. 2(B)].

change in the 2τ delay to 0.0005 s (Table 6), and shows only a minor change with a decrease in 2τ to 0.000 25 s. These data allowed us to conclude that the modified CPMG echo block as shown in Fig. 2(B) completely eliminates, as expected,[34] the influence of the antiphase component for $^{13}C(^{1}H)$ and improves the T_2 value at short 2τ delays for $^{13}C(^{2}H)$.

Changing the power of deuterium decoupling from 625 to 2500 Hz was also tested to observe the effect of deuterium on the T_2 of carbon in the CPMG approach [Fig. 2(C)]. As can be seen in Table 6, WALTZ16 decoupling during the period T of ^{2}H was less effective in the pulse sequence outlined in Fig. 2(C) than the pulse sequence in Fig. 2(B) (compare the data in Table 6).

We also performed experiments on the 11.7 T spectrometer with TXI and TXO probes with 90° pulse lengths of ^{2}H of 95 and 22 μs, respectively, and with 90° pulse lengths of ^{13}C of 14.5 and 6.25 μs, respectively (see the last row of Tables 6 and 2), and the T_2 values were 4.14 ± 0.29 s and 5.00 ± 0.50 s, which are the same within experimental accuracy, showing that the 180° pulse length of ^{2}H in the pulse sequence in Fig. 2(B) plays only a minor role.

We subsequently measured the T_2 relaxation times of the carbons in **1–14** using the modified CPMG pulse sequence, as shown in Fig. 2(B), using a 2τ delay of 0.000 25 s, and the results are presented in Table 7. The data show that under these measurement conditions there is only a modest difference, within the experimental error of *ca.* 10%, between the T_1 and T_2 relaxation times for the $^{13}C(^{1}H)$ vector, whereas the difference between T_1 and T_2 is around 20–45% for $^{13}C(^{2}H)$.

The mechanism responsible for the shorter T_2 of ^{13}C attached to deuterium could perhaps be attributed either to scalar relaxation of the second kind[3,5,22] or cross-correlation between $D(^{13}C–^{2}H)–Q(^{2}H)$ relaxation.[25,26] It has been shown that scalar relaxation of the second kind plays an essential role mainly in the transverse relaxation of nuclei with spin 1/2 attached to quadrupolar nuclei with spin 1.[22] This mechanism could be significant when the Q relaxation rate $(T_1^{Q})^{-1}$ is greater than or of a similar magnitude to that of $2\pi J_{^{13}C–^{2}H}$ resulting from the $J_{^{13}C–^{2}H}$ coupling constant between ^{13}C and ^{2}H.[3,22] The contribution of this mechanism to the transverse relaxation could be estimated using $1/T_2^{sc} \approx S(S+1)J_{^{13}C–^{2}H}\ T_1^{Q}/3$. For molecules with correlation times less than a few nanoseconds, the contribution of scalar relaxation of the second kind to the transverse relaxation of carbon can become significant. It is known that this effect could be eliminated by deuterium decoupling with much larger field strengths (600–2500 Hz)[5,9c] than the scalar coupling constant $J_{^{13}C–^{2}H}$. As has been mentioned above in our experiments, T_2 relaxation measurements with continuous deuterium decoupling during all the T period (see Fig. 2C) with field strengths of 625 Hz (TXI probe) (Table 4) or 2500 Hz (TXO probe) (data not shown) or 180° pulsing (2.5 kHz TXI probe or 12.5 kHz TXO probe) (Tables 4, 5 and 7) on the ^{2}H channel at a rate $\gg T_1^{Q}$ were performed. The data show that the final $1/T_2^{obs}$ value of 0.000 25 s for the methine carbon at the 2τ delay does not depend on the strength of decoupling power, which allows us to conclude that this mechanism of scalar relaxation of the second kind is not responsible for the shorter T_2 value of the methine carbon compared with T_1.

The other mechanism which even at short correlation times[25,26a] could induce line broadening is cross-correlation between $D(^{13}C–^{2}H)–Q(^{2}H)$ relaxation. However, it has been shown[26a,35] that, compared with scalar relaxation of the second kind, the cross-correlation $DD(^{13}C–^{2}H)$ and $Q(^{2}H)$ could be only partly suppressed by high-power deuterium decoup-

Table 7. Transverse relaxation times T_2^{obs} (s) for ^{13}C of compounds **1–14** measured at 11.7 T using the modified CPMG [Fig. 2(B)] experiment with a delay 2τ of 0.000 25 s (TXI probe)

Compound[a]	C-1′	C-2′	C-3′	C-4′	C-5′
1	1.10 ± 0.09	0.99 ± 0.04	0.99 ± 0.01	1.04 ± 0.04	0.99 ± 0.09
2	0.91 ± 0.11	0.97 ± 0.09	4.14 ± 0.29	1.20 ± 0.03	1.07 ± 0.01
3	0.62 ± 0.02	0.73 ± 0.01	0.79 ± 0.09	0.64 ± 0.04	0.40 ± 0.02
4	0.56 ± 0.06	4.32 ± 0.29	2.95 ± 0.14	0.71 ± 0.05	0.75 ± 0.10
5	0.66 ± 0.01	4.16 ± 0.63	5.47 ± 0.14	0.59 ± 0.10	3.07 ± 0.41
			4.79 ± 0.82	4.04 ± 0.29	3.07 ± 0.41
6	0.68 ± 0.05	0.59 ± 0.02	1.05 ± 0.06	0.79 ± 0.04	0.39 ± 0.07
7	0.80 ± 0.02	1.19 ± 0.15	1.10 ± 0.11	0.80 ± 0.02	0.42 ± 0.01
8	0.70 ± 0.01	0.85 ± 0.01	1.20 ± 0.13	0.73 ± 0.03	0.44 ± 0.01
9	0.65 ± 0.03	0.86 ± 0.06	5.31 ± 0.27	0.79 ± 0.01	0.74 ± 0.03
10	0.76 ± 0.04	4.37 ± 0.22	7.73 ± 0.61	1.03 ± 0.20	3.04 ± 0.27
				3.94 ± 0.46	
11	0.76 ± 0.04	0.93 ± 0.05	0.87 ± 0.03	0.80 ± 0.04	0.57 ± 0.08
12	0.77 ± 0.01	0.89 ± 0.03	1.00 ± 0.01	0.87 ± 0.02	0.44 ± 0.04
13	0.57 ± 0.05	0.88 ± 0.03	0.85 ± 0.06	0.66 ± 0.02	0.39 ± 0.03
14	0.64 ± 0.04	0.80 ± 0.02	0.86 ± 0.02	0.71 ± 0.01	0.49 ± 0.01

[a] Compound numbers as in Fig. 1.

ling.[26a,35] The theory of this phenomenon (known as dynamic frequency shift) has already been described.[25,26] This effect could be clearly observed on the unusual multiplet structure of the methine carbon attached to ^{2}H at correlation times longer than those with T_1^Q at the minimum.[25b,f,26a,b] However, in the extreme narrowing limit ($\omega\tau_c \ll 1$), it increases the line broadening with complete collapse of the triplet structure when T_1^Q is minimum. We have observed this effect experimentally on the ^{13}C methine carbon in **2** at low temperature (data not shown). The difference between the theoretically calculated transverse relaxation, $1/T_2^{expect}$ (given in parentheses in Table 6), and the experimentally observed $1/T_2^{obs}$ increased by 0.037, 0.069, 0.089 and 0.219 s^{-1} with decrease in temperature (323, 313, 294 and 278 K), or with increase in the correlation time in the range 0.02–0.1 ns. It may be pointed out that at higher temperatures (compare the data at 313 and 323 K with those at 294 and 278 K, Table 5) the difference between the T_1 and T_2 values for the Q_c carbon in **2** is dramatically increased. Just because the DD inter- and intramolecular relaxation contributions to the observable T_1 and T_2 values are equal in the narrowing limit, the shorter T_2 is attributed to the conformational exchange contribution ($1/T_{ex}$), which is accelerated at higher temperatures. In this case the value $1/T_2^{expect}$ was estimated using the correction for the observable T_2 of the Q_c carbon in **2**.

CONCLUSION

To elucidate high-resolution solution structures of large biologically functional oligo-DNA and -RNA by NMR, we have developed[7,12] synthetic chemistry to construct non-uniformly labeled oligo-DNA and -RNA in which most of the nucleotide residues are stereo- and regiospecifically deuterium labeled, leaving only a small stretch of the oligonucleotide which contains natural proton isotope that is NMR visible.[7] As a continuation of this work, we have shown[7c] the usefulness of the diastereospecifically C-2′ and C-5′ deuterium-labeled nucleotide block which enabled us to assess the local conformational changes by collecting specific spin-diffusion free NOE and coupling constant information from these deuterium-labeled blocks in a large oligo-DNA.

The present work constitutes a preliminary study to assess the dynamic characteristics of deuterium-labeled DNA. The methylene groups such as ^{1}H–$^{13}C_{2'}$–^{2}H and ^{1}H–$^{13}C_{5'}$–^{2}H in the nucleotide residues are particularly good targets for performing dynamic studies of DNA simply because of their uniform distribution throughout the sugar–phosphate backbone of the whole molecule. These methylene groups are also very useful because they allow the assessment of the internal mobility through two different vectors, ^{13}C–^{1}H and ^{13}C–^{2}H. It is expected that for the first vector the relaxation is dominated by DD interaction between ^{13}C and ^{1}H [DD(^{13}C–^{1}H)], and for the second vector involving the deuterium the relaxation is dominated by the quadrupolar relaxation of ^{2}H[Q(^{2}H)].

One of the bottlenecks in this approach is that the relaxation of ^{13}C nuclei attached to deuterium is affected by the quadrupolar deuterium relaxation through scalar interaction (relaxation of the second kind) and cross interference between DD(^{13}C–^{2}H) and Q(^{2}H) relaxation. It has been shown[5,26a,35] that compared with the suppression of relaxation of the second kind, the cross-correlation could not be suppressed even by a higher power of deuterium decoupling.

We initiated this investigation to estimate the contributions of all other alternative relaxation pathways other than DD(^{13}C–^{1}H) and Q(^{2}H) to the ^{13}C relaxation of methylene groups in the sugar moiety of oligo-DNA using ^{13}C–^{1}H and ^{13}C–^{2}H vectors.

A suitable model for the above investigation should fulfil at least the following conditions. (1) It should not have extra relaxation pathways induced by neighboring ^{13}C nuclei[36] between two carbons in methylene and methine fragments in the sugar moiety. This means that the model system should have only natural ^{13}C abundance. (2) For simplicity of the comparison of correlation times obtained from ^{2}H and ^{13}C relaxations, the molecule with a isotropic correlation time is preferable. It is noteworthy that even for a medium-sized oligo-DNA (10–12 nucleobases) the approximation of isotropic correlation time is at best ambiguous. (3) The experimental conditions and intermolecular relaxation of ^{13}C nuclei should be tunable to those prevalent in a real oligo-DNA sample by changing the measurement conditions.

In diastereospecifically deuterium-labeled nucleosides (compounds **4**, **5**, **7**, **8** and **9**) there are deuterated carbon nuclei of the methylene type, $^{1}H^{13}C(^{2}H)$ or/and methine-type, $^{13}C(^{2}H)$. This gives us a unique experimental opportunity to evaluate different relaxation mechanisms in T_2 of ^{13}C nuclei in an *additive* manner based on the relaxation behavior of a quarternary carbon (i.e. Q_c carbon in **1** and **2**). The understanding and the use of the relaxation behavior of the Q_c carbon involving the DD intermolecular relaxation and intramolecular relaxation through two bonds allowed us to understand the relaxation of the methine carbon (compound **2**) where the DD(^{13}C–^{2}H) relaxation, relaxation of the second kind and DD(^{13}C–^{2}H)–Q(^{2}H) cross-correlation relaxation theoretically should be involved. Finally, based on our understanding of the relaxation data on the methine carbon, it was possible to estimate the contribution of all relaxations other than DD(^{13}C–^{1}H) in the methylene group in the nucleosides.

We chose the condition of narrowing limit ($\omega\tau_c \ll 1$) because under this condition the contribution of the relaxation pathways such as DD(^{13}C–^{1}H), DD(^{13}C–^{2}H), DD(intermolecular) and quadrupolar (^{2}H) to T_1 should be approximately equal to T_2. Our results can be summarized as follows.

1. It has been found from the comparison of ^{13}C T_1 data at three different frequencies of the NMR spectrometer that the CSA term is small for the ^{13}C sugar

moiety, and its contribution could be neglected, which is consistent with reports in the literature,[1,2b,28,29] which is not the case for the aromatic ^{13}C nuclei. The cross-correlations between DD(^{13}C–^{1}H)–(CSA) and DD(^{13}C–^{2}H)–(CSA) in the T_1 and T_2 relaxation measurements were suppressed by applying ^{1}H and ^{2}H decoupling or a train of 180° pulses in the relaxation period. For the present condition of measurement ($\omega\tau_c \ll 1$), this term was negligible, but special care may be warranted for $\omega\tau_c \geq 1$ for a large oligo-DNA.

2. It has been found by comparison of the T_1 relaxation of the Q_c carbon with that of the methine carbon (^{13}C–^{2}H) or (^{13}C–^{1}H) in **2** that the contribution of the intermolecular and intramolecular relaxations of ^{13}C with protons that are two bonds away is larger than DD(^{13}C–^{2}H), and the sum of all these contributions defines the T_1 of the methine carbon (^{13}C–^{2}H).

3. The decoupling of deuterium with 0.6–2.5 kHz power by applying WALTZ16 during the echo period for T_2 measurement does not allow efficient elimination of the DD(^{13}C–^{2}H)–Q(^{2}H) cross-correlation relaxation in the methine carbon.

4. It was shown that the decoupling of the DD(^{13}C–^{2}H)–Q(^{2}H) cross-correlation relaxation was more effective by applying a 180° deuterium pulse in the middle of the short (0.5 ms) echo period in the T_2 measurement of the methine carbon than applying WALTZ16 (0.6–2.5 kHz) to deuterium nuclei during this period. Since the literature suggests that the decoupling power should completely suppress relaxation of the second kind in methine ^{13}C relaxation, we attributed the observed difference between the experimental T_1 and T_2 relaxation of the methine carbon to the cross-correlation between DD(^{13}C–^{2}H) and Q(^{2}H) relaxation. This difference increased with decrease in temperature which amounts to an increase in the correlation time of the molecule. This correlates with recent theoretical predictions.[25,26]

5. Comparison of observed T_1 and T_2 relaxations of methylene ^{13}C nuclei shows that they are indeed very close. The various contributions of the methine ^{13}C relaxation mechanism, such as DD(^{13}C–^{2}H), intermolecular relaxation and the cross-correlation relaxation term DD(^{13}C–^{1}H)–Q(^{2}H) relaxation to the methylene ^{13}C relaxation were *ca.* 25% in T_2 and *ca.* 15% in T_1. In this case, to obtain a relevant correlation time, the observed longitudinal relaxation rate of methylene ^{13}C in nucleosides should be corrected for the overall value of the longitudinal relaxation rate of methine ^{13}C. The correlation time obtained in this way strictly corresponds to the correlation time obtained from deuterium relaxation study.

6. It has been shown that in diastereospecifically deuterium-labeled nucleosides with methylene groups such as ^{1}H–$^{13}C_{2'}$–^{2}H, ^{1}H–$^{13}C_{5'}$–^{2}H and methine carbon with $^{13}C_{3'}$–^{2}H, the T_1 and T_2 for methine carbon, $^{13}C_{3'}$–^{2}H, could be used for correction to obtain the decay of the ^{13}C longitudinal and transverse magnetization of the ^{1}H–^{13}C–^{2}H group as monoexponential induced by DD interaction in ^{13}C–^{1}H to a good approximation.

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