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Long-Lived Nuclear Spin States in Monodeuterated Methyl Groups

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Abstract

It is possible to access long-lived nuclear singlet order in monodeuterated methyl groups, in the case that a significant chemical shift difference exists between the CH_2D protons. This occurs when the local environment is chiral, and the CH_2D rotamers have different populations. An experimental demonstration is presented for the case of N-CH_2D-2-methylpiperidine. The ratio of the singlet relaxation time constant T_S to the longitudinal relaxation time constant T_1 is found to be equal to 3.1 ± 0.1, over a wide range of temperatures, solvents, and magnetic fields. The longest observed value of T_S approaches 1 minute. The relaxation mechanisms of the long-lived state are discussed, and a modified model of the CH_2D geometry is proposed to explain the observed ratio of T_S to T_1.

Keywords: long-lived states, singlet state, methyl group, rotamer

1. Introduction

Long-lived states (LLS) are configurations of nuclear spins which are protected against relaxation in nuclear magnetic resonance (NMR) experiments [1–22]. Long-lived state lifetimes exceeding the conventional relaxation time T_1 by a factor of 50 have been observed, with a lifetime exceeding 1 hour in room-temperature solution in one case [22]. The combination of LLS phenomena with hyperpolarization techniques is particularly promising. Techniques such as dissolution-DNP (dynamic nuclear polarization) [23] offer

large NMR signal enhancements, with wide-ranging implications, including the characterization of cancer in human patients [24]. However, the range of hyperpolarization applications is restricted by the finite lifetime of the enhanced magnetization, which is normally limited by T_1. The use of LLS offers a promising means to transcend this limitation [9, 25–28].

In systems of spin-1/2 pairs, the LLS is called *singlet order*. This consists of the population imbalance between the spin-0 singlet state and the spin-1 triplet states [1, 2]. The decay time constant of singlet order is denoted T_S. Access to nuclear singlet order is provided by a chemical shift difference between the participating spins, or by differences in spin-spin couplings to spins outside the pair [13].

LLS have also been observed in the 3-spin-1/2 systems of rapidly rotating methyl groups in solution [29–31]. In this case the LLS is given by the imbalance in populations between spin states spanning different irreducible representations of the C_3 permutation group [32]. Some materials, such as γ-picoline, display quantum-rotor induced polarization (QRIP) effects, in which a large polarization of the methyl LLS is induced by dissolution of the material from cryogenic conditions [29, 33–35]. However, the hyperpolarized LLS only gives rise to observable NMR signals through an incoherent cross-relaxation mechanism involving a fourth nuclear spin, which greatly reduces the available signal enhancement [29–31].

Since methyl groups are ubiquitous in nature, the exploitation of methyl LLS is potentially attractive. In this communication we show that in certain cases, it is possible to achieve coherent access to a methyl LLS with a high conversion efficiency into observable NMR signals. The conditions are: (i) the methyl group is monodeuterated, and therefore contains a proton pair; (ii) the local environment is chiral, and (iii) the three methyl rotamers have sufficiently different populations, so that a small chemical shift difference is induced between the CH_2D protons after averaging over all populated states. We show that the small chemical shift difference allows coherent (and therefore efficient) access to the long-lived singlet order of the proton spin pair, using known radiofrequency pulse techniques [11, 12, 19, 20].

Chemical inequivalence of CH_2D protons has been observed in several molecular systems, most of which contain a N-CH_2D group in a chiral environment [36–38]. In this communication we report the observation of a proton LLS in the N-CH_2D group of N-CH_2D-2-methylpiperidine (Figure 1). We observe singlet lifetimes T_S with values between 20 and 55 seconds (dependent on temperature). The ratio of singlet relaxation time T_S to longi-

tudinal relaxation time T_1 is found to be remarkably constant over a wide range of conditions, and equal to 3.1 ± 0.1. This result shows that is feasible to exploit methyl LLS without relying on weak cross-relaxation effects, in suitable cases. We provide evidence that singlet relaxation in this system is dominated by the dipolar interactions between the CH_2D protons and the CH_2D deuteron, and show that the singlet relaxation times cannot be explained by a model in which the three hydrogen nuclei are localised at the vertices of an equilateral triangle. We propose a modified geometrical model which is consistent with the experimental data.

2. Experiments

2.1. Proton Spectra

The methyl region of the proton NMR spectrum of (N-CH_2D)-2-methylpiperidine is shown in Figure 1. This spectral region has a quartet-like appearance in the absence of deuteron decoupling (Figure 1a). The characteristic AB spectral pattern of an inequivalent proton pair appears when a deuteron decoupling field is used to remove the $^2J_{\mathrm{HD}}$ splittings (Figure 1b). This spectrum is consistent with a J-coupling of $\left|^2J_{\mathrm{HH}}\right| = 11.7 \pm 0.2$ Hz and a chemical shift difference of $\Delta\delta = 13.5 \pm 0.4$ ppb between the CH_2D protons, as reported previously [36].

The existence of a small chemical shift difference has been attributed to (i) hyperconjugation between the nitrogen lone pair and the *anti*-methyl C-H(D) σ-bond, which allows the zero-point vibrational energies of the *anti* CH and CD bonds to influence the rotamer energies; as a result, the rotamer with the CD bond *anti* to the nitrogen lone pair is less populated than the other two rotamers in thermal equilibrium, and (ii) the chiral environment associated with the neighbouring methyl group, which causes a significant chemical shift difference between the two protons in each rotamer [36, 38]. In these circumstances, there remains a significant chemical shift difference between the CH_2D protons after averaging over all methyl rotamers. The observed chemical shift difference decreases as the temperature is increased, as shown in Figure 2a. This is consistent with the Boltzmann populations of the three rotamers becoming more similar at higher temperatures.

2.2. Singlet NMR

The small chemical shift difference allows access to the long-lived singlet order between the CH_2D protons by using the M2S [11, 12] or SLIC [19] pulse

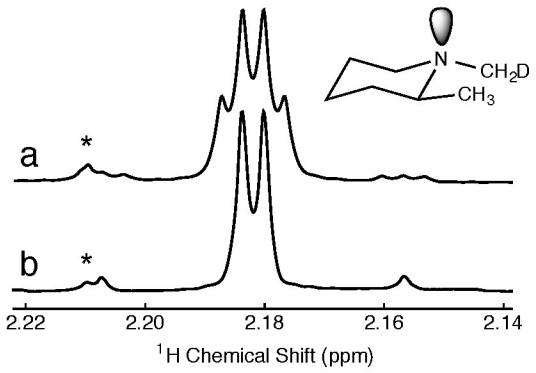

Figure 1: Part of the experimental ^{1}H spectrum of (N-CH_2D)-2 methylpiperidine in CD_2Cl_2 solution acquired at 11.7 T (500 MHz) with a single transient (for the full proton spectrum, see the Supporting Information). (a) Spectrum without deuteron decoupling; (b) Spectrum with deuteron decoupling (deuteron nutation frequency 500 Hz). The asterisk indicates a small signal from a non-deuterated N-methyl-2-methylpiperidine impurity, shifted in frequency by a secondary isotope effect. Inset: dominant di-equatorial conformation [36] of N-CH_2D-2-methylpiperidine in solution, showing the nitrogen lone pair which is implicated in the inequivalence of the CH_2D protons.

sequences, and their variants [20, 39]. In the current study, we used a variant of the SLIC (Spin-Lock-Induced Crossing) method, as shown in Figure 3.

This pulse sequence operates as follows: after the initial 90° pulse, a radiofrequency field is applied with a 90° phase shift. The amplitude of this field is selected so that the nutation frequency $\omega_{\mathrm{SLIC}}/2\pi$ matches the J-coupling $^2J_{\mathrm{HH}}$. This establishes a resonance which causes the spin-locked magnetization to be converted into singlet order through the action of the chemical shift difference, with the conversion complete in a time $\tau_{\mathrm{SLIC}} \simeq 2^{-1/2}\Delta\nu^{-1}$, where $\Delta\nu$ is the chemical shift difference in Hertz, neglecting relaxation and other complications [19]. The singlet order is allowed to evolve for a variable interval τ_{EV} under a larger-amplitude "spin-locking" rf field (nutation frequency $\omega_{\mathrm{LOCK}}/2\pi$), which suppresses singlet-triplet mixing [8].

A sequence of radiofrequency pulses and pulsed field gradients (known as a "T_{00} filter") suppresses NMR signals that do not pass through singlet order [40, 41]. A second SLIC sequence converts the singlet order into transverse proton magnetization, and the induced NMR signal is detected. The maximum amplitude of the singlet-filtered ^{1}H NMR signal, relative to that induced by a single 90° pulse, was found to be 0.43, somewhat lower than the

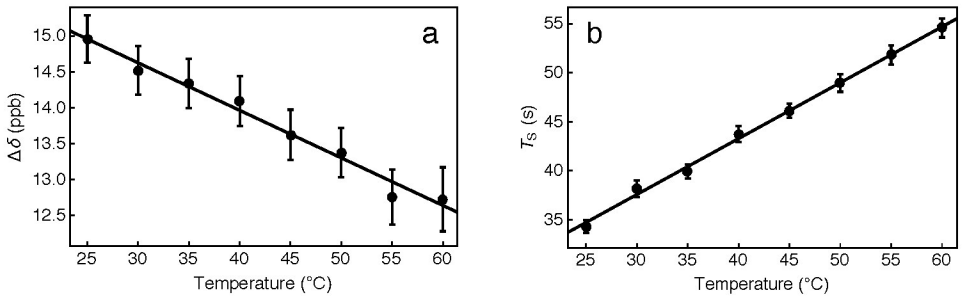

Figure 2: (a) Chemical shift difference $\Delta\delta$ between the CH_2D protons, and (b) singlet relaxation time T_S of 0.1 M N-CH_2D-2-methylpiperidine in degassed C_6D_6 solution, as a function of temperature. Experiments were performed at fields of (a) 11.7 T (500 MHz), and (b) 14.1 T (600 MHz).

theoretical maximum of 2/3 [42], the loss being attributed to radiofrequency field imperfections and relaxation. The decay of the long-lived singlet order is tracked by repeating the pulse sequence with different values of the singlet evolution interval τ_{EV}. Experimental parameters and details of sample preparation are found in the Supporting Information (SI).

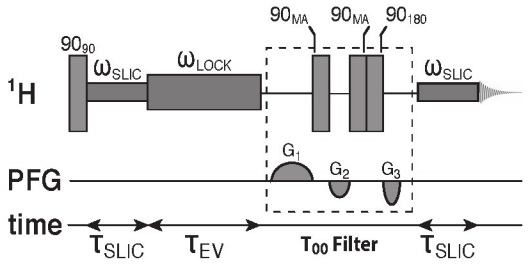

Figure 3: Pulse sequence used for accessing long-lived singlet order in monodeuterated methyl groups and measuring its decay. The experiments used the following parameters: nutation frequencies $\omega_{\mathrm{SLIC}}/2\pi = 11.7$ Hz and $\omega_{\mathrm{LOCK}}/2\pi = 300$ Hz. The duration of the SLIC pulse was 100 ms in the 500 MHz experiments and 73 ms in the 600 MHz experiments. The "T_{00} filter" sequence suppresses signals that do not pass through singlet order, as described in the Supporting Information. "MA" denotes the "magic angle" (54.7°). An interval of 90 s was used between successive transients.

A typical decay curve for singlet order is shown in Figure 4. This shows a single exponential decay with time constant $T_S = 27.0 \pm 0.6$ s. This is approximately three times longer than the relaxation time for longitudinal

magnetization $T_1 = 8.7 \pm 0.1$ s, as estimated from the inversion-recovery curve, also shown in Figure 4.

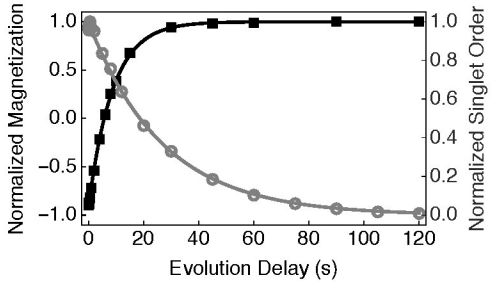

Figure 4: Experimental relaxation curves for 0.1 M N-CH_2D-2-methylpiperidine in degassed CD_2Cl_2 solvent (proton frequency 500 MHz, temperature 25°C). Open symbols, grey line, and right-hand axis: Decay of singlet order measured by the pulse sequence in Figure 3. Filled symbols, black line, and left-hand axis: Spin-lattice relaxation measured by inversion-recovery. All signal amplitudes were normalized to the first point. The fitted curves have a single-exponential form.

The singlet relaxation time constants T_S increase with increasing temperature, as shown in Figure 2b. Measured relaxation time constants T_1 and T_S are presented for a variety of solvents, temperatures and magnetic fields in the Supporting Information. Figure 5 shows a plot of T_1^{-1} against T_S^{-1} for this disparate data set. The fit to a straight line with zero intercept and slope $(3.1)^{-1} = 0.324$ is remarkably good. The ratio of T_S to T_1 is remarkably consistent and given by 3.1 ± 0.1 over a wide range of conditions.

The data shown in Figure 5 were all obtained for (N-CH_2D)-2-methylpiperidine, except for a single point which was obtained for a compound with complete deuteration of the second methyl group, i.e. (N-CH_2D)-2-CD_3-piperidine (purple triangle). Clearly, deuteration of the second methyl group in N-CH_2D-2-methylpiperidine does not have a strong effect on the relaxation behaviour.

2.3. Carbon-13 and Deuterium NMR

^{13}C and ^{2}H spectra of N-CH_2D-2-methylpiperidine, and T_1 values for the ring ^{13}C sites, are shown in the Supporting Information, for the case of degassed CD_2Cl_2 solution at 11.7 T (500 MHz) and 25°C. The ring CH_2 sites have similar ^{13}C relaxation time constants T_1 of 6.5 ± 0.3 s, with the ring CH site displaying a longer ^{13}C relaxation time constant of 10.2 ± 0.3 s.

Under the same conditions, the ^{2}H relaxation time constant for N-CH_2D-2-methylpiperidine is given by $T_1(^2\mathrm{H}) = 0.75 \pm 0.01$ s.

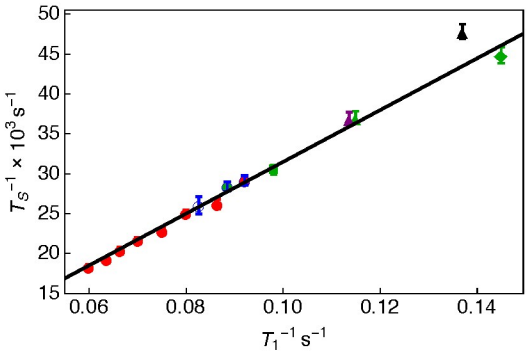

Figure 5: Longitudinal relaxation rate constants (T_1^{-1}) plotted against singlet relaxation rate constants (T_S^{-1}) for 0.1 M N-CH_2D-2-methylpiperidine over a wide range of solvents (degassed), temperatures and magnetic fields. The experimental conditions are as follows: (●) C_6D_6 solution, at temperatures increasing from 25°C (rightmost point) to 60°C (leftmost point) in 5°C increments, at a field of 14.1 T (600 MHz); (○) C_6D_6 solution, at magnetic fields increasing from 9.4 T (400 MHz, leftmost point) to 14.1 T (600 MHz, rightmost point) in 100 MHz increments, at a temperature of 25°C; (▲) CH_2Cl_2 solution at 25°C and 11.7 T (500 MHz); (●) C_6D_6 solution at 25°C and 11.7 T (500 MHz); (■) CD_3CN solution at 25°C and 11.7 T (500 MHz); (▲) CD_2Cl_2 solution at 25°C and 11.7 T (500 MHz); (◆) CD_3OD solution at 25°C and 11.7 T (500 MHz); (▲) CD_2Cl_2 solution of (N-CH_2D)-2-(CD_3)-piperidine at 25°C, 11.7 T (500 MHz). A constant ratio $T_S/T_1 = 3.1 \pm 0.1$ is observed over a wide range of experimental conditions.

3. Relaxation Theory

The data show that long-lived singlet order may be accessed coherently, and with high efficiency, for the proton pair of the monodeuterated methyl group in N-CH_2D-2-methylpiperidine. The singlet lifetime T_S is proportional to T_1 of the same protons, with the proportionality constant given by 3.1 ± 0.1, over a wide range of experimental conditions. In this section we propose an explanation for the constant ratio of T_S to T_1, and investigate the value of the proportionality constant.

3.1. Relaxation Mechanisms

A large variety of relaxation mechanisms may contribute to the proton T_S and T_1 relaxation of the CH_2D protons. As well as the magnetic dipole-dipole

interactions between the three magnetic nuclei of the CH_2D group, there are also chemical shift anisotropy contributions, magnetic dipole-dipole interactions with other nuclei in the same molecule, and intermolecular dipole-dipole interactions. The quadrupolar relaxation of the deuterium nuclei might also contribute to proton relaxation through mechanisms such as scalar relaxation of the second kind [43, 44]. Furthermore, spin-rotation relaxation is known to be significant for many rotating methyl groups [45–48], and contributes to the LLS relaxation of γ-picoline in solution [29, 30]. Singlet relaxation may also be caused by state mixing due to a finite chemical shift difference between the participating protons, or by asymmetric J-couplings to other magnetic nuclei ("singlet-triplet leakage") [14].

The excellent correlation between the T_S and T_1 values shown in Figure 5 strongly supports the hypothesis that the longitudinal and singlet relaxation of the CH_2D proton pair is driven by a common mechanism, with a common correlation function. This suggests that the mechanisms that dominate the T_1 and T_S relaxation of the CH_2D protons are internal to the CH_2D group. This conclusion is supported by the following observations: (i) Deuteration of the 2-methyl group leads to only a small change in the relaxation times even though the protons of the 2-methyl group approach to within 229 pm of the CH_2D protons; (ii) a large change in the deuteration level of the solvent only has a small influence on the relaxation times (see Figure 5 and the data in the Supporting Information).

In addition, one may discount major contributions from the spin-rotation, scalar relaxation, chemical shift anisotropy and singlet-triplet leakage mechanisms, for the following reasons:

1. *Spin-rotation.* The experimental finding that T_S increases with increasing temperature (Figure 2b) speaks strongly against a significant contribution from spin-rotation, since that mechanism usually increases in strength with increasing temperature [45–48]. Presumably, in the current case, the methyl rotation is too strongly hindered to permit a significant spin-rotation relaxation contribution.
2. *Scalar relaxation of the second kind.* The existence of a resolved deuteron splitting in the proton spectrum (Figure 1a), as well as direct measurements of deuteron relaxation (see section 2.3) indicate that deuteron relaxation is too slow to induce significant scalar relaxation of the coupled protons. Furthermore, the two $^2J_{\mathrm{HD}}$ couplings are identical to a good approximation, which precludes a scalar contribution to singlet

relaxation [44].

3. *Chemical shift anisotropy.* The weak dependence of T_1 and T_S on magnetic field indicates a relatively small contribution from CSA.
4. *Singlet-triplet leakage.* Singlet-triplet leakage induced by the small chemical shift difference between the CH_2D protons can be a significant contribution to the singlet relaxation rate constant T_S^{-1} [14]. However, in the current experiments, this contribution is suppressed very effectively by the application of a resonant radiofrequency field during the singlet relaxation interval (see the Supporting Information).

We therefore postulate that both the singlet and the longitudinal relaxation of the proton pair in the monodeuterated methyl group of N-CH_2D-2-methylpiperidine are dominated by the ^{1}H-^{1}H and ^{1}H-^{2}D dipole-dipole interactions within the methyl group itself, modulated by the internal rotation of the methyl group with respect to the rest of the molecule, and by the rotation of the molecule as a whole. A relaxation model is therefore constructed based on (i) a simplified description of the motion of the methyl group and the molecule as a whole, and (ii) a description of the vibrationally-averaged spin-spin interactions within the rotating methyl group.

3.2. Motional Model

A plausible motional model for the monodeuterated methyl group in N-CH_2D-2-methylpiperidine consists of a hindered 3-fold rotor attached to a sphere undergoing isotropic rotational diffusion (Figure 6). Although the existence of a finite chemical shift difference between the CH_2D protons implies that the methyl rotamers have slightly unequal populations, we ignore this effect in the relaxation analysis, for the sake of simplicity. The thermally activated jumps between the methyl rotamers are assumed to be described by a rate constant κ, while the overall rotational diffusion of the molecule is described by a correlation time τ_c. Both κ and τ_c are in general temperature-dependent. We also assume that the correlation time τ_c is short enough relative to the nuclear Larmor period to invoke the extreme narrowing approximation [43].

3.3. Dipole-Dipole Interactions

The three magnetic nuclei in the CH_2D group interact by the magnetic dipole-dipole interaction. In general these dipole-dipole interactions are described by traceless second-rank tensors, and are subjected to averaging over

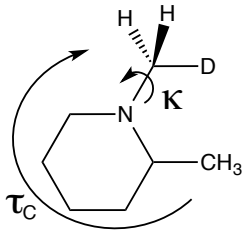

Figure 6: The relaxation of the CH_2D protons in N-CH_2D-2-methylpiperidine is governed by the three-fold jump rate κ and overall rotational correlation time τ_c.

local molecular vibrations and librations on a timescale fast compared to the methyl 3-fold jumps or the molecular tumbling. Spin relaxation is caused by motional modulation of the vibrationally-averaged dipole-dipole interaction tensors.

In general, the vibrationally-averaged tensors differ, both in magnitude and in orientation, from tensors derived from a naive geometrical model, for which point-like nuclei are located at the vertices of an equilateral triangle, with the magnitudes of the dipole-dipole interactions in exact proportion to the product of the gyromagnetic ratios, since the internuclear distances are all equal. As discussed below, this naive equilateral model of the CH_2D group is inconsistent with the experimental results.

To maintain high generality, the relaxation theory is developed using three different interaction tensors for the vibrationally-averaged dipole-dipole interactions, leaving the magnitudes and orientations of the tensors as adjustable parameters. The two ^{1}H-^{2}D interaction tensors are assumed to have the same principal values, by symmetry. For the sake of simplicity, the vibrationally-averaged interaction tensors are assumed to be axially symmetric, with the unique principal axes perpendicular to the N-C rotor axis. The angle between the unique principal axes of the two vibrationally-averaged ^{1}H-^{2}D interaction tensors is denoted 2θ. This angle defines the cross-correlation of the two ^{1}H-^{2}D interaction tensors, and is therefore important for the proton singlet relaxation. By symmetry, the unique principal axis of the vibrationally-averaged ^{1}H-^{1}H interaction tensor is perpendicular to the bisector of the two ^{1}H-^{2}D principal axes (see the Supporting Information). A point-nucleus equilateral geometry model would lead to the angle $2\theta = 60°$, but this value is not

assumed in the following discussion.

3.4. Rate Expressions

As shown in the Supporting Information, the motional and intra-methyl interaction models described above lead to the following expressions for the proton longitudinal and singlet relaxation rate constants:

$$T_1^{-1}(\mathrm{CH_2D}) = \frac{(4+3\kappa\tau_\mathrm{c})}{24\,(1+3\kappa\tau_\mathrm{c})}\left(16\omega_\mathrm{HD}^2 + 9\omega_\mathrm{HH}^2\right)\tau_\mathrm{c}, \tag{1}$$

$$T_S^{-1}(\mathrm{CH_2D}) = \frac{8\sin^2 2\theta}{1+3\kappa\tau_\mathrm{c}}\omega_\mathrm{HD}^2\tau_\mathrm{c}. \tag{2}$$

The following expressions apply to the relaxation of the ^{13}C nuclei in the CH_N groups on the six-membered ring (with $N = 1$ or 2), and for the ^{2}H relaxation of the deuteron in the monodeuterated methyl group:

$$T_1^{-1}(^{13}\mathrm{CH}_N) = N\omega_\mathrm{CH}^2\tau_c, \tag{3}$$

$$T_1^{-1}(^2\mathrm{H}) = \frac{3\left(32+33\kappa\tau_\mathrm{c}+9\kappa\tau_\mathrm{c}\left(4\cos(2\theta_\mathrm{Q})+3\cos(4\theta_\mathrm{Q})\right)\right)}{64\,(1+3\kappa\tau_\mathrm{c})}\omega_\mathrm{Q}^2\tau_\mathrm{c}. \tag{4}$$

Here ω_CH is the dipole-dipole coupling constant for the interaction between the proton and carbon nuclei, ω_Q is the quadrupole coupling frequency of the deuteron, and θ_Q is the angle between the 3-fold jump axis and the dominant principal axis of the ^{2}D electric field gradient (EFG) tensor (assumed to be along the CD bond vector). For a tetragonal geometry of the $\mathrm{CH_2D}$ group:

$$T_1^{-1}(^2\mathrm{H}) = \frac{(3+\kappa\tau_\mathrm{c})}{2\,(1+3\kappa\tau_\mathrm{c})}\omega_\mathrm{Q}^2\tau_\mathrm{c}. \tag{5}$$

These equations assume rigid-body rotational diffusion of the whole molecule (including the ring) and 3-fold jumps of the methyl group, with ^{13}C-^{1}H dipolar and quadrupolar relaxation dominating the ^{13}C and ^{2}D relaxation, respectively.

For the simple case of point nuclei (ignoring vibrational averaging), the coupling constants are defined as follows:

$$\omega_\mathrm{CH} = -\left(\mu_0/4\pi\right)\gamma(^1\mathrm{H})\gamma(^{13}\mathrm{C})\hbar r_\mathrm{CH}^{-3} \tag{6}$$

$$\omega_Q = \frac{e^2qQ}{2\hbar} \tag{7}$$

where r_{HC} is the internuclear distance, Q is the electric quadrupolar moment of the deuterium nucleus, and eq is the electrical field gradient at the deuterium nucleus [49].

This relaxation model leads to the following expression for the ratio of T_S to T_1:

$$\frac{T_S}{T_1} = (4 + 3\kappa\tau_c)\,\frac{16\omega_{\mathrm{HD}}^2 + 9\omega_{\mathrm{HH}}^2}{192\omega_{\mathrm{HD}}^2 \sin^2 2\theta}. \tag{8}$$

In general the ratio T_S/T_1 depends on the jump rate κ and rotational correlation time τ_c, and is expected to depend on temperature, solvent, and other factors. However, in the "slow-jump regime" $\kappa\tau_c \ll 1$, the ratio of T_S to T_1 becomes independent of κ and τ_c, and only depends on interaction parameters within the CH_2D group:

$$\frac{T_S}{T_1} \simeq \frac{16\omega_{\mathrm{HD}}^2 + 9\omega_{\mathrm{HH}}^2}{48\omega_{\mathrm{HD}}^2 \sin^2 2\theta} \simeq \frac{3\omega_{\mathrm{HH}}^2}{16\omega_{\mathrm{HD}}^2 \sin^2 2\theta} \tag{9}$$

The fact that ^{1}H-^{1}H dipolar couplings are about 6 times stronger than ^{1}H-^{2}D dipolar couplings (at equal distances) has been invoked in the last approximation. The observed direct proportionality of T_S to T_1 is consistent with the validity of the slow-jump regime over the explored range of experimental conditions.

3.5. Relaxation analysis

The ^{13}C and ^{2}H relaxation data allow an estimate of the rotational correlation time τ_c and 3-fold jump rate κ. The following analysis refers to data obtained on 0.1 M N-CH_2D-2-methylpiperidine in degassed CD_2Cl_2 solution, at 500 MHz and 25°C.

The overall rotational correlation time τ_c was estimated by analysing the experimental $T_{1\mathrm{C}}$ relaxation time constants for ^{13}C nuclei in the CH_N groups on the six-membered ring, using Equation 3 which applies for extreme-narrowing isotropic rotational tumbling, dominated by the ^{13}C-^{1}H dipolar relaxation mechanism [50]. By assuming an internuclear ^{13}C-^{1}H distance of 108.9 pm, which corresponds to a dipole coupling constant of $\omega_{\mathrm{CH}}/2\pi = -23.4$ kHz, we obtain the following estimate of the rotational correlation time: $\tau_c = 3.8 \pm 0.6$ ps.

The 3-fold jump rate constant κ may be estimated from the ^{2}D T_1 relaxation time constant, by using Equation 5. The deuteron quadrupole coupling constant $\omega_Q/2\pi = 83.5$ kHz has been estimated by solid-state NMR [51, 52].

The unique principal axis of the deuteron quadrupole coupling tensor is assumed to be along the C-D bond, at an angle of 70.5° with respect to the 3-fold jump axis. From comparing the experimental relaxation time $T_1(^2\mathrm{H}) = 0.75 \pm 0.01\,\mathrm{s}$ with Equation 4, which was derived for the case of a hindered 3-fold rotor attached to a molecule undergoing isotropic rotational diffusion, and assuming that the quadrupolar mechanism dominates the deuteron relaxation, we estimate an upper limit on the 3-fold jump rate: $\kappa \lesssim 3.2 \times 10^{10}\,\mathrm{s}^{-1}$. The product of the rotational correlation time and the 3-fold jump rate constant is therefore given by $\kappa\tau_c \lesssim 0.12$, which supports the validity of the slow-jump regime and hence Equation 9.

Now consider the case where the three hydrogen nuclei of the CH_2D group are considered to be points, located at the vertices of an equilateral triangle. In this case $2\theta = 60°$, the internuclear distances are all equal, and the dipolar couplings are in the ratio of the magnetogyric ratios $\omega_{\mathrm{HH}}/\omega_{\mathrm{HD}} = \gamma(^1\mathrm{H})/\gamma(^2\mathrm{D}) = 6.51$. This "equilateral triangle model" predicts the following relaxation time ratio: $\left(T_S/T_1\right)_{\triangle} = 10.5$. However, the observed value is quite different: $\left(T_S/T_1\right)_{\mathrm{obs}} = 3.1 \pm 0.1$.

How can this discrepancy be explained? One approach would be to call into question the intra-methyl relaxation model: However, as discussed above, the evidence for the dominance of intra-CH_2D interactions in the ^{1}H and ^{2}D relaxation is very strong. An alternative approach is to maintain the local intra-methyl relaxation model, but to modify the relative magnitudes and geometries of the dipole-dipole interactions within the rotating CH_2D group, to take into account differential vibrational averaging on a faster timescale than the molecular rotation or 3-fold jumps.

A detailed analysis of the effect of rapid vibrational motions on the dipolar interaction tensors in the CH_2D group would be a major project. For the sake of simplicity we employ a naive picture, in which the nuclei are still regarded as localized points, but with the ^{2}D nucleus displaced from its original position, in order to account for differential vibrational averaging of the ^{2}D and ^{1}H interactions. In order to maintain symmetry, we consider a model in which the ^{2}D nucleus is moved in the CH_2D plane along the line bisecting the ^{1}H-^{1}H vector (Figure 7a). This adjustment changes the internuclear distances as well as the angle θ. The dependence of the theoretical relaxation rate ratio T_S/T_1 on the deuterium displacement is shown in Figure 7b. There is a strong sensitivity to the deuterium displacement, with the observed ratio $T_S/T_1 = 3.1$ being consistent with a displacement of 38.7 pm towards the

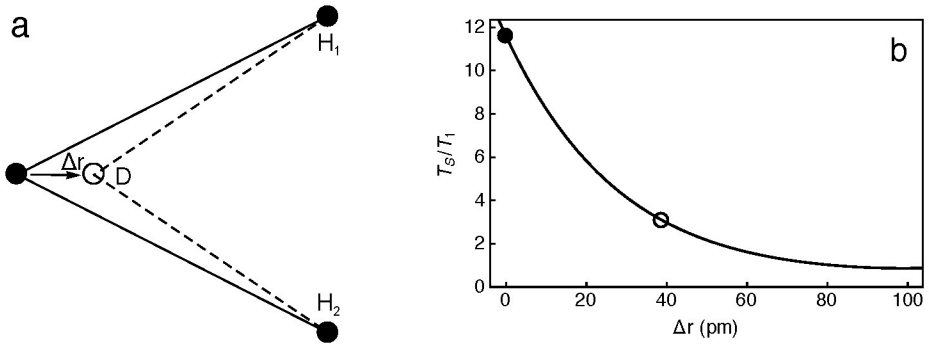

Figure 7: (a) Adjustment of the effective geometry of the CH_2D group to account for the observed relaxation rate ratio $T_S/T_1 = 3.1$. The three nuclei are initially at the vertices of an equilateral triangle of side length 179.6 pm (black circles). The deuterium nucleus is displaced towards the original centre of the triangle (open circle) by a distance Δr. (b) Dependence of the relaxation rate ratio T_S/T_1 on the displacement Δr. The open circle shows the displacement needed for consistency with the observed rate ratio $T_S/T_1 = 3.1$. The black circle shows the predicted rate ratio from an equilateral triangle geometry.

original centre of the equilateral triangle. This represents a contraction in both ^{1}H-^{2}D distances by about 18%, and a change in the angle 2θ from 60° to 75.1°. A sketch of the adjusted geometry is shown in Figure 7a.

This degree of geometrical distortion is probably unrealistic. It is more likely that differential vibrational averaging of the ^{1}H-^{1}H and ^{1}H-^{2}D dipolar interactions is responsible for the observed T_S/T_1 ratio. Vibrational motion out of the CH_2D plane is likely to be particularly effective. For example, the larger vibrational amplitudes of the ^{1}H nuclei relative to the more massive ^{2}D nucleus would reduce the ^{1}H-^{1}H dipolar interaction more than the ^{1}H-^{2}D dipolar interactions. This effect would lead to a correction in the right direction. We have not attempted a more sophisticated analysis of vibrational effects on the interaction parameters.

4. Discussion

It is possible to populate long-lived nuclear singlet order in the proton pairs of monodeuterated methyl groups, under suitable conditions. This requires non-uniformity in the rotamer populations as well as a local chiral environment in order to induce a small isotropic shift difference between the CH_2D protons. Both conditions are fulfilled for the CH_2D group in N-CH_2D-

2-methylpiperidine, where a hyperconjugation effect involving the nitrogen lone pair perturbs the vibrational energies and hence the rotamer populations, while the neighbouring methyl group provides a chiral environment.

In this system, the ratio of singlet relaxation time T_S to longitudinal relaxation time T_1 is found to be 3.1 ± 0.1 over a wide range of conditions, with the longest observed value of T_S approaching 1 minute at elevated temperature. The observation of a constant ratio of T_S to T_1 supports a relaxation model in which dipolar interactions between the CH_2D protons and deuteron dominate the singlet relaxation. However, a naive model in which the ^1H and ^2D nuclei of the CH_2D group are viewed as point-like magnetic dipoles fixed at the vertices of an equilateral triangle predicts a much larger ratio of T_S to T_1. We explore the effects of a geometric distortion in which the ^2D nucleus is displaced towards the centre of the triangle and find that a displacement of 38.7 pm is needed to explain the experimental data. This adjustment should not be viewed as a realistic structural proposal, but as a crude attempt to represent the differential effects of vibrational averaging and nuclear wavefunction delocalization within a simplistic point-nucleus geometric model.

The observed relaxation time ratio of $T_S/T_1 \simeq 3.1$ is probably too small for most feasible applications. However, it should be noted that equation 8 permits a much larger ratio T_S/T_1 in the case of fast 3-fold jumps, i.e. $\kappa\tau_c \gtrsim 1$. This does not occur in N-CH_2D-2-methylpiperidine, where the rotational barrier appears to be relatively large. Nevertheless it may arise in other compounds displaying inequivalence of the CH_2D proton pair. It remains to be seen whether it is possible to maintain a sufficient differential between rotamer populations while still having a sufficiently low barrier to permit fast 3-fold jumps. We are currently exploring some other chemical candidates.

We have also attempted to observe long-lived singlet states in other chiral CH_2D systems, such as derivatives of 3-^{2}D-lactic acid, and also (N-CH_2D)-3-methylpiperidine (similar to the substance used above, but with a more remote CH_3 group). We failed to access the CH_2D singlet state in both of these cases: the chemical inequivalence of the CH_2D protons is presumably too small to exploit. Apart from unusual circumstances [53], hyperconjugation between a lone pair on a neighbouring atom (such as N) and the methyl C-H/D bonds seems to be a requirement for obtaining a sufficient chemical shift difference of a few ppb or more. At the time of writing we have not attempted these experiments on other chiral compounds containing a X-CH_2D moiety, where X is an atom other than N possessing a long pair, such as P, O or S.

It should be noted that many interesting chiral molecules do contain a tetrahedral N-methyl group of suitable type. Examples include the psychoactive agents lysergic acid, codeine, morphine, cocaine, heroin, and methamphetamine. It is not yet known whether the methyl-monodeuterated versions of these systems possess an accessible long-lived singlet state.

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