1. Introduction

Nuclear magnetic resonance spectroscopy (NMR) and imaging (MRI) are unrivalled tools for the determination of molecular structure and dynamics. However, some applications of NMR and MRI such as hyperpolarization [1–3], diffusion [4, 5] and measurements of slow processes [6, 7] would ultimately prosper from an increased time period in which nuclear spin order is preserved. Such capabilities would allow the study of dynamic processes which are relatively slow compared to the spin-lattice relaxation time $T_1$. Long-lived states (LLS) provide an opportunity to maintain nuclear spin order for extended times [8, 9]. For systems of spin-1/2 pairs, the long-lived state is immune to in pair dipolar relaxation, with other symmetric decay mechanisms strongly attenuated [10–18]. LLS have relaxation time constants denoted $T_{LLS}$, which often transcend $T_1$ by a large factor. Potential applications of LLS include hyperpolarized imaging and transport [19, 20], and the study of enzymatic reactions and ligand binding [21–23].

A number of molecular structures exhibiting large $T_{LLS}/T_1$ ratios have previously been demonstrated. An asymmetric cis-fumarate diester supports a proton long-lived state of $\sim$10 minutes and a $T_{LLS}/T_1$ ratio of $\sim$50 at high field [24, 25]. A $\sim$77 minute long-lived state is provided by a $^{13}$C labelled naphthalene derivative in room temperature solution [26], and a $^{15}$N labelled diazirine spin pair exhibits a long-lived state of $\sim$23 minutes at low field [27]. The long-lived state of $^{15}$N-nitrous oxide has been recorded utilizing field-cycling equipment and surpasses 26 minutes in solution [28].

In this paper we present a $^{15}$N labelled molecular structure (Figure 1) exhibiting a long-lived state lifetime exceeding 40 minutes in solution. This system ($^{15}$N$_2$-$\text{I}$) is constructed by a Diels-Alder reaction of ($S$)-(4-(1-phenylethyl)-3H-1,2,4-triazole-3,5(4H)-dione-1,2-$^{15}$N$_2$ and a fully deuterated cyclopentadiene. Although the spin-lattice and long-lived state relaxation times are relatively short at high field, impressive relaxation times are unveiled at low field. The decay of the long-lived state is biexponential, with the slowly relaxing component having a time constant $\sim$21 times longer than $T_1$. Experiments at low field make use of a dedicated two-field NMR spectrometer which allows fast transfer of a sample shuttle between two magnetic field centres operating at different magnetic fields (14.1 T and 0.33 T), and with radiofrequency irradiation at low magnetic field. The dipolar relaxation mechanisms of the long-lived state are discussed.

2. Experimental Methods

The molecular system selected for study was 2-((($S$)-1-phenylethyl)-5,8-dihydro-1H-5,8-methano[1,2,4]triazolo[1,2-α]pyridazine-1,3(2H)-dione-5,6,7,8,10,10-d$_6$-4,9-$^{15}$N$_2$ ($^{15}$N$_2$-$\text{I}$) and was prepared by...
a rapid room temperature Diels-Alder cycloaddition of (S)-4-(1-phenylethyl)-3H-1,2,4-triazole-3,5(4H)-dione-1,2-15N2 and fully deuterated cyclopentadiene. Freshly prepared cyclopentadiene was perdeuterated (95 atom % D) by five cycles of proton-deuterium exchange using NaOD in DMSO/D2O [29]. (S)-4-(1-phenylethyl)-1,2,4-triazolidine-3,5-dione-1,2-15N2 was synthesised from 15N2 hydrazine and (S)-α-methylbenzyl isocyanate by adaptation of the procedure described by Cookson et al. [30]. For further details of the synthetic route to 15N2-I see the Supporting Information (SI). Sample preparation for two-field NMR experiments: 29.1 mg of 15N2-I was dissolved in 106 µL of CD2Cl2 solvent at a concentration of 1 M. The solution was transferred to a glass shuttling tube which was sealed with glue. Samples were prepared under an argon atmosphere to displace the majority of molecular oxygen.

The relevant portion of the experimental 15N NMR spectrum of 15N2-I is shown in Figure 2. The characteristic AB spectral pattern of the inequivalent 15N2 spin pair is well-resolved, and indicates a chemical shift difference which is a similar magnitude to the in pair J-coupling. The spectrum may be simulated using a J-coupling of \(|J_{NN}| = 11.7 ± 0.1\) Hz and an isotropic chemical shift difference of \(\Delta\delta_{NN} = 199 ± 3\) ppm between the labelled 15N sites, corresponding to 12.1 ± 0.2 Hz at the 14.1 T magnetic field.

The small chemical shift difference is due to: (i) a chiral centre three bonds away from the 15N2 spin pair; and (ii) an out-of-plane carbon-bridge structure which creates a conformational population bias in the rotation of the chiral group. These factors combine to generate a small chemical shift difference between the 15N2 sites after averaging over all conformations.

The small isotropic chemical shift difference between the 15N labelling sites of 15N2-I is reproduced remarkably well by quantum chemistry computations [32-34]. The 15N chemical shift difference was estimated to be 176 ppm after averaging over the rotation of the chiral group (Figure 1a, *), see the Supporting Information (SI) for details.
Experimental parameters: $\Delta = 20.0$ ms, $n_1 = 2$ and $n_2 = 1$. $180^\circ$ COMP denotes a composite $180^\circ$ pulse. 90s, $180^\circ$ COMP 90s. The S2M sequence is a magnetization-to-singlet (M2S) pulse sequence. The long-lived state of $1\text{M}^{15}\text{N}_2\text{-I}$ in $\text{CD}_2\text{Cl}_2$ solvent was prepared at $14.1\text{T}$ (107.5 MHz) and 22°C. The $T_1$ of the $2\text{H}$ spins $D_A$ and $D_B$ (Figure 1a) is $T_1(2\text{H}) = 106 \pm 6$ ms.

4. Discussion

Evaluation of the dipolar coupling contributions to the long-lived state relaxation time $T_{1\text{LS}}$ requires an estimate of the rotational correlation time $\tau_C$. The $2\text{H}$ relaxation data allow an estimate of $\tau_C$. The following analysis refers to data obtained on $1\text{M}^{15}\text{N}_2\text{-I}$ in $\text{CD}_2\text{Cl}_2$ solution, at $14.1\text{T}$ (107.5 MHz) and 22°C. The rotational correlation time $\tau_C$ was estimated by analysing the experimental relaxation curve for the long-lived state at LF. The data allow an estimate of $\tau_C$. Use of the pulse sequence described in Figure 3. All signal amplitudes were normalized to the first data point. The decay curve was fitted using a bi-exponential decay function $A \exp(-t/T_{1A}^F) + B \exp(-t/T_{1B}^F)$. Fit parameters: $A = 0.105$, $T_{1A}^F = 155 \pm 12$ s, $B = 0.060$, $T_{1B}^F = 2437 \pm 374$ s. We attribute the longer relaxation time constant to the decay of the long-lived state, giving $T_{1\text{LS}}^F = 2437 \pm 374$ s. This is $\sim 21$ times longer than the LF relaxation time for the longitudinal magnetization of the $15\text{N}$ sites $T_{1H}^F = 117 \pm 12$ s, as estimated from a separate inversion-recovery measurement. The time constants $T_{1A}^F$ and $T_{1B}^F$ (the initial portion of the LLS decay curve) are in approximate agreement.

The $2\text{H}$ longitudinal relaxation times for $15\text{N}_2\text{-I}$ were measured in $\text{CD}_2\text{Cl}_2$ solvent at $14.1\text{T}$ (107.5 MHz) and 22°C. The $T_1$ of the $2\text{H}$ spins $D_A$ and $D_B$ (Figure 1a) is $T_1(2\text{H}) = 106 \pm 6$ ms.

3. Results

A decay curve for the $15\text{N}$ long-lived state at LF is shown in Figure 4. The experimental decay (black data points) is well fitted with a bi-exponential decay function (black solid line) using two relaxation time constants, denoted $T_{1A}^F$ and $T_{1B}^F$. Bi-exponential decay function: $A \exp(-t/T_{1A}^F) + B \exp(-t/T_{1B}^F)$. Fit parameters: $A = 0.105$, $T_{1A}^F = 155 \pm 12$ s, $B = 0.060$, $T_{1B}^F = 2437 \pm 374$ s. We attribute the longer relaxation time constant to the decay of the long-lived state, giving $T_{1\text{LS}}^F = 2437 \pm 374$ s. This is $\sim 21$ times longer than the LF relaxation time for the longitudinal magnetization of the $15\text{N}$ sites $T_{1H}^F = 117 \pm 12$ s, as estimated from a separate inversion-recovery measurement. The time constants $T_{1A}^F$ and $T_{1B}^F$ (the initial portion of the LLS decay curve) are in approximate agreement.

The $2\text{H}$ longitudinal relaxation times for $15\text{N}_2\text{-I}$ were measured in $\text{CD}_2\text{Cl}_2$ solvent at $14.1\text{T}$ (107.5 MHz) and 22°C. The $T_1$ of the $2\text{H}$ spins $D_A$ and $D_B$ (Figure 1a) is $T_1(2\text{H}) = 106 \pm 6$ ms.

Figure 4: Experimental relaxation curve (black data points) for the long-lived state of $1\text{M}^{15}\text{N}_2\text{-I}$ in $\text{CD}_2\text{Cl}_2$ solution acquired at 0.33 T and 22°C with 2 transients per data point. The decay of the long-lived state was measured by using the pulse sequence described in Figure 3. All signal amplitudes were normalized to the first data point. The decay curve has a multi-exponential form, and was fitted with a bi-exponential decay function $A \exp(-t/T_{1A}^F) + B \exp(-t/T_{1B}^F)$. Fit parameters: $A = 0.105$, $T_{1A}^F = 155 \pm 12$ s, $B = 0.060$, $T_{1B}^F = 2437 \pm 374$ s. Data point error bars were estimated from the standard deviation of 10 integrated noise regions outside of the $15\text{N}_2$ peak area.

The sample is subsequently returned to HF by using the pneumatic shuttle, and a chronologically-reversed M2S sequence (S2M) is applied. The induced NMR signal is detected at HF. In the current study, the parameters of the M2S pulse sequence were chosen to maximise the detected signal intensity of the long-lived state: $\Delta = 20.0$ ms, $n_1 = 2$ and $n_2 = 1$. The maximum amplitude of the $15\text{N}$ NMR signal, relative to that induced by a single $90^\circ$ pulse, was found to be 10.15%. The theoretical performance of the M2S sequence for the experimental parameters given is 19.7%. The loss relative to the theoretical maximum of 2/3 [46] is not yet fully understood but could be associated with a breakdown of the strong-coupling regime, nonadiabatic magnetic field variation during sample shuttling, radiofrequency field imperfections and relaxation.

Figure 3: a) Pulse sequence used for preparing a long-lived state in $15\text{N}_2\text{-I}$ and monitoring its decay. The decay of the long-lived state is tracked by repeating the pulse sequence for different values of the evolution delay $\tau_{\text{EV}}$. Continuous wave (CW) irradiation (nutation frequency $= 500$ Hz) is applied to the $15\text{N}$ spins at low magnetic field throughout the duration of $\tau_{\text{EV}}$. A two-step phase cycle is used to remove spurious signals generated by longitudinal magnetization accrued during the M2S sequence. An interval of 900 s was used between successive transients. b) Qualitative magnetic field profile used during field-cycling experiments. Sample switching from high magnetic field (HF, 14.1 T) to low magnetic field (LF, 0.33 T) takes 280 ms, and from LF to HF takes 500 ms. c) Magnetization-to-singlet (M2S) pulse sequence. The long-lived state was prepared and reconverted at HF by using the following experimental parameters: $\Delta = 20.0$ ms, $n_1 = 2$ and $n_2 = 1$. $180^\circ$ COMP denotes a composite $180^\circ$ pulse. 90s, $180^\circ$ COMP 90s. The S2M sequence is a magnetization-to-singlet (M2S) pulse sequence.

Application of resonant radiofrequency irradiation at the low-field $15\text{N}$ resonance frequency.
where e\(Q\) is the electric quadrupolar moment of the deuterium nucleus, and e\(q\) is the electrical field gradient at the deuterium nucleus [48].

The structure of \(^{15}\text{N}_2\)-I was optimized by the quantum chemistry package Gaussian 09 [33]. Computations employed the DFT/B3LYP/6-31G+(d,p) level of theory. The deuteron quadrupole coupling constant \(\omega\) was estimated by including the keyword “pickett” in a NMR calculation engaging the GIAO-DFT/B3LYP/6-31G(df,dp) level of theory [49]. The computations revealed two predominant rotamers for the chiral group (Figure 1a, *), but each possesses similar NMR parameters and only one structure (Figure 1b) is used to calculate the relaxation dynamics, see the Supporting Information (SI) for details.

From comparing the experimental relaxation time \(T_1(\text{H}) = 106 \pm 6\) ms with equation 1, which was derived for the case of a molecule undergoing isotropic rotational diffusion [50], and assuming that the quadrupolar mechanism dominates the deuteron relaxation, we estimate a correlation time for the overall tumbling of the molecule in solution: \(\tau_C = 17.8 \pm 1.0\) ps. The contribution of dipolar interactions to \(^2\text{H}\) longitudinal relaxation is found to be negligible and is neglected.

LLS are immune to the motional modulation of the impair dipolar coupling, but the contribution to long-lived state relaxation from dipolar couplings originating outside the spin pair is expected to be important. Long-lived state relaxation of this kind is highly dependent on molecular geometry, and is given by [51, 52]:

\[
T_{\text{LLS}}^{-1}(\text{DD}) = \frac{4}{3} \sum_k I_k (I_k + 1) \times \left[ \omega_{ik}^2 + \omega_{jk}^2 - 2\omega_{ik} \omega_{jk} P_2 \left( \cos(\theta_{ikj}) \right) \right] \tau_C,
\]

where the sum runs over all spins \(k\) external to the spin pair \(ij\), \(\theta_{ikj}\) is the angle subtended by the \(ik\) and \(jk\) internuclear vectors, \(I_k\) is the angular momentum quantum number of the external spin \(k\), and \(P_2(x) = (3x^2 - 1)/2\) is a second-rank Legendre polynomial. The dipolar coupling constant \(\omega_{ik}\) is defined as follows:

\[
\omega_{ik} = - \left( \mu_0 / 4\pi \right) \gamma_i \gamma_k h r_{ik}^{-3},
\]

where \(\gamma_i\) is the magnetogyric ratio of spin \(i\) and \(r_{ik}\) is the internuclear distance between spins \(i\) and \(k\).

The internuclear separations \(r_{ik,j}\) and subtended angles \(\theta_{ikj}\) for the geometry optimized structure of \(^{15}\text{N}_2\)-I for all magnetic sites \(k\) which are external to the \(^{15}\text{N}_2\) spin pair \(ij\) are shown in Table 1. The deuteron labels at the base of the carbon-bridge structure (Figure 1a, \(\text{DA}\) and \(\text{DB}\)) are the closest to the \(^{15}\text{N}\) spins.

Using equation 3 which applies for extreme-narrowing isotropic rotational tumbling [47], and assuming dipolar coupling constants of \(\omega_{ND_{\text{A}}}/2\pi = 52.8\) Hz and 192.9 Hz, we obtain the following estimate of the dipolar contribution to the LLS relaxation rate constant from the deuteron \(\text{DA}\): \(T_{\text{LLS}}^{-1}(\text{ND}_{\text{A}}) = (0.043 \pm 0.002) \times 10^{-3} \text{s}^{-1}\). A similar dipolar contribution is calculated for the deuteron \(\text{DB}\) (Figure 5, black columns).

The predicted time constant for the slowly relaxing component of the biexponential decay is given by the sum of dipolar contributions within the spin system: \(T_{\text{LLS}}^{-1}(\text{DD}) = (0.151 \pm 0.009) \times 10^{-3} \text{s}^{-1}\) (Figure 5, blue column). The combined contribution from deuterons \(\text{DA}\) and \(\text{DB}\) is found to provide \(\sim 57\%\) of the predicted dipolar LLS relaxation rate constant for the \(^{15}\text{N}\) spins. The combined estimate was found to be insufficient as to explain the experimental time constant: \((T_{\text{LLS}}^{-1})^{-1} = (0.54 \pm 0.07) \times 10^{-3} \text{s}^{-1}\). At such long relaxation times, the discrepancy between estimated and experimental LLS decay rate constants could be attributed to a large number of relaxation mechanisms including: attenuated chemical shift anisotropy and singlet-triplet leakage [52] at LF, dipolar couplings with molecules in the solvent, and spin-rotation or spin-internal-motion
couplings [26, 53]. It is also plausible that molecular diffusion could influence the measurement of the long-lived state decay, as translation of the $^{15}$N$_2$ spin pair outside of the radiofrequency coil region (at long evolution delays after spin encoding) would manifest as an apparent attenuation of the nuclear singlet lifetime [40].

The biexponential nature of the LLS decay is not currently understood, but is thought to be associated with non-scalar orders which decay on the $T_1$ timescale and are unaffected by the change in magnetic field induced by fast sample shuffling. It is possible that the build up of such terms is related to the use of the M2S sequence. Pulsed methods to target a singlet precursor order are currently under development in our laboratories.

5. Conclusions

In summary, we have presented a $^{15}$N labelled molecular system with a long-lived state exhibiting a biexponential decay. The time constant for the slowly decaying component of the biexponential decay $T_{LLS}$ exceeds 40 minutes in solution. The ratio of $T_{LLS}$ to $T_1$ was ~21. The long-lived state was studied at low magnetic field by using sample shuffling apparatus housed inside a dedicated two-field NMR spectrometer. The dipolar relaxation pathways of the long-lived state were explored, and were found to be too weak to explain the experimental data. The reason for this discrepancy is not currently understood. These results are encouraging for the future construction of core molecular units which may support long-lived states, and demonstrate that $^{15}$N$_2$ systems house a suitable target spin pair. We are currently investigating other molecular candidates of this kind in our laboratories.

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