Long-Lived Nuclear Singlet Order in Near-Equivalent ^{13}C Spin Pairs

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S *Supporting Information*

ABSTRACT: Molecules that support ^{13}C singlet states with lifetimes of over 10 min in solution have been designed and synthesized. The ^{13}C$_2$ spin pairs in the asymmetric alkyne derivatives are close to magnetic equivalence, so the ^{13}C long-lived singlet states are stable in high magnetic field and do not require maintenance by a radiofrequency spin-locking field. We suggest a model of singlet relaxation by fluctuating chemical shift anisotropy tensors combined with leakage associated with slightly broken magnetic equivalence. Theoretical estimates of singlet relaxation rates are compared with experimental values. Relaxation due to antisymmetric shielding tensor components is significant.

A pair of spin-$\frac{1}{2}$ nuclei may form a nuclear singlet state, which has zero total spin and no magnetic moment, or three components of a triplet state with total spin = 1. Nuclear singlet order (an imbalance in population between the singlet and triplet states) decays with a time constant T_S, which is often many times the ordinary magnetization decay constant T_1.[1−5] Applications to the study of transport, diffusion, protein folding, and ligand binding have been demonstrated.[6−9] A particularly promising set of applications concerns the transport of hyperpolarized spin order, as generated by methods such as dynamic nuclear polarization.[10−14]

In most cases, nuclear singlet order is maintained by transporting the sample to a low magnetic field or by applying a radiofrequency (rf) spin-locking field. Both conditions raise obstacles for potential applications of singlet NMR. However, if the members of the spin pair are in near-equivalent molecular sites, the singlet order is stable in high magnetic field without additional intervention.[15] Specialized techniques are used to access the long-lived singlet order in this case.[15,16]

We have designed and synthesized molecular systems containing nearly equivalent ^{13}C spin pairs in order to achieve long single relaxation times T_S exceeding 15 min. Although not as long as the singlet lifetime of 25 min achieved for ^{15}N$_2$O,[17,18] these lifetimes greatly exceed any previously reported values of ^{13}C or ^{1}H singlet relaxation times in solution.

The molecules discussed here are all derived from ^{13}C$_2$-labeled acetylenes and have the structures shown in Figure 1; the R$_1$ and R$_2$ groups are listed in Table 1. Molecules belonging to class I consist of ^{13}C$_2$-labeled alkynes with deuterated aliphatic substituents containing an ether linkage. Molecules belonging to class II are diesters of ^{13}C$_2$-labeled acetylenedicarboxylic acids. In all cases, the attached R$_1$ and R$_2$ groups are
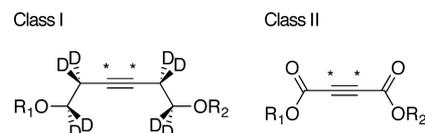

Figure 1. Molecular structures of substituted ethynes. * denotes a ^{13}C label. R$_1$ and R$_2$ are reported in Table 1.

different, which induces a small isotropic chemical shift difference $\Delta\delta_{iso}$ between the two ^{13}C nuclei, providing access to the long-lived singlet order. The synthetic scheme is described in the Supporting Information (SI). Most of the following discussion refers to two specific samples (see Table 1). Sample **1** is a compound of class I with R$_1$ = Me, R$_2$ = Et in degassed CD$_3$OD solution; sample **2** is a compound of class II with R$_1$ = Me-d_3, R$_2$ = Et-d_3, also in degassed CD$_3$OD solution.

The pulse sequences used to measure the decay of nuclear singlet order are shown in Figure 2. A magnetization-to-singlet (M2S) pulse sequence converts transverse magnetization into singlet order in the regime of near magnetic equivalence.[15,16] After a variable delay (which may include transport from high to low field and back again), a singlet-to-magnetization (S2M) sequence converts the singlet order back to observable magnetization. The M2S and S2M sequences use trains of π pulses synchronized with the singlet−triplet splitting.[15,16] The pulse sequence parameters are reported in the SI.

The results for samples **1** and **2** are shown in Figure 3. In panel (a), the sample was kept in a field of 7.04 T during the storage interval T, while in panel (b), the sample was transported to a low magnetic field of 2.2 mT during T. In all cases, the decays were strongly nonexponential because the M2S pulse sequence generated a nonequilibrium population distribution within the triplet manifold as well as singlet order.[15] The triplet populations equilibrated within a time on the order of T_1. The decay of the signal amplitudes at longer times ($T \geq 2T_1$) was approximately exponential with a time constant equal to T_S. Estimated singlet relaxation time constants T_S are reported in Table 1.

There was a strong variation in the singlet relaxation time constants T_S and the response to the external magnetic field. Sample **1** had T_S > 10 min in low field (2.2 mT) and slightly shorter in high field (7.04 T). Sample **2** had a T_S > 15 min in low field but <1 min in high field. In the following discussion, we try to gain insight into these unexpected observations.

Table 1. NMR Parameters and Relaxation Decay Constants of $^{13}C_2$-Labeled Acetylene Derivatives[a]

sample	class	R_1	R_2	solvent	$\Delta\delta_{iso}$ (ppm)[b]	J_{CC} (Hz)	T_1^{LF} (s)	T_S^{LF} (s)	T_1^{HF} (s)	T_S^{HF} (s)
1	I	Me	Et	CD_3OD	0.13 ± 0.01	180 ± 1[c]	51.5 ± 0.8	613 ± 68	26.6 ± 0.8	577 ± 22
2	II	Me-d_3	Et-d_5	CD_3OD	0.62 ± 0.01	185 ± 1[d]	42 ± 0.5	952 ± 15	31 ± 1.0	52 ± 5
	I	Me	THP	CD_3OD	0.26 ± 0.01	177 ± 1[c]	36 ± 2.0	370 ± 16	19.3 ± 0.9	360 ± 10
	I	Me	Et	D_2O	0.08 ± 0.01	178 ± 1[c]	21 ± 2.0	140 ± 30	10.7 ± 0.5	195 ± 20
	II	Me-d_3	Et-d_5	DMSO-d_6	0.41 ± 0.01	185 ± 1[d]	10.2 ± 1.0	365 ± 20	9.1 ± 0.4	20 ± 2
	II	Me-d_3	Et-d_5	CD_3CN	0.63 ± 0.01	185 ± 1[d]	44 ± 4.5[e]	800 ± 43[e]	24 ± 1.0[f]	–

[a]Samples were degassed by bubbling O_2-free N_2 gas for ∼15 min. Me = methyl, Et = ethyl, and THP = tetrahydropyranyl. For all rows but the last one, LF = 2.2 mT and HF = 7.04 T. [b]Measured from the ^{13}C NMR spectrum of the unlabeled compound. [c]Derived from the optimized values of n_1 and τ (see the SI). [d]Measured from the ^{13}C NMR spectrum of the labeled compound. [e]LF = 20 mT. [f]HF = 9.4 T.
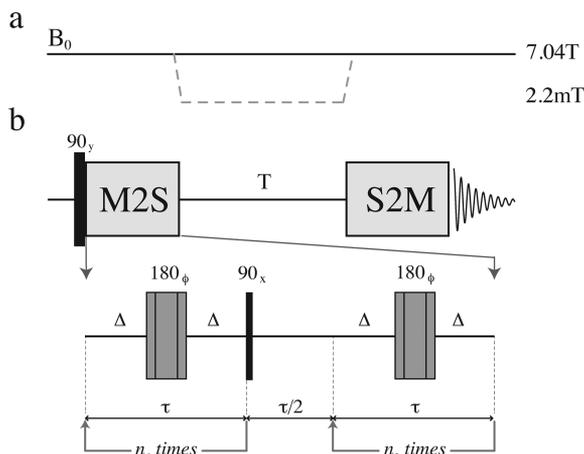

Figure 2. Pulse sequence used to measure singlet order decay. (a) Trajectory of magnetic fields. The magnetic field may be kept at a high value throughout (solid line) or changed to a low field by transporting the sample out of the magnet (dashed line). (b) Radiofrequency pulse sequence using an M2S block to convert magnetization into singlet order and an S2M sequence for the reverse transformation.
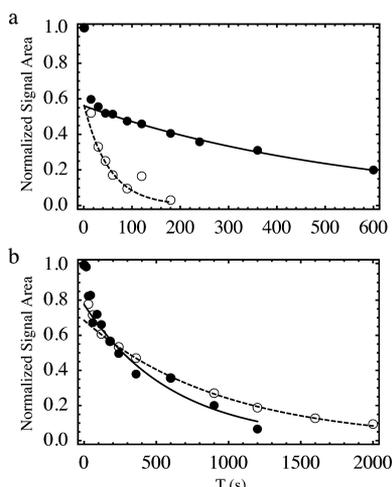

Figure 3. Decays of signal amplitudes for samples **1** (●) and **2** (○) as functions of the interval T between the M2S and S2M blocks. The samples were (a) kept in high magnetic field or (b) transported to low field.

Motional modulation of the dipole−dipole coupling between the members of the spin pair is a significant T_1 mechanism but does not lead to singlet relaxation. The singlet relaxation rate constant T_S^{-1} is expected to be approximately additive over the other contributing mechanisms[3−5,19−24] and may be written as

$$T_S^{-1} = R_S^{\sigma+} + R_S^{\sigma-} + R_S^{leak} + R_S^{DD} + R_S^{other} \quad (1)$$

where $R_S^{\sigma+}$ and $R_S^{\sigma-}$ are rates of relaxation due to motional modulation of the symmetric (rank-2) and antisymmetric (rank-1) components of the chemical shift anisotropy (CSA) tensors, R_S^{leak} is the relaxation rate due to singlet−triplet leakage, R_S^{DD} takes into account dipole−dipole couplings to spins outside the pair, and R_S^{other} includes minor relaxation mechanisms such as spin rotation and scalar relaxation that here are neglected for simplicity.

(1) *CSA relaxation.* The CSA-driven singlet relaxation is caused by rotational modulation of the difference in the 3 × 3 Cartesian shielding tensors of the two nuclear sites, $\Delta\sigma = \sigma_1 - \sigma_2$. If the difference tensor $\Delta\sigma$ is decomposed into a traceless symmetric part $\Delta\sigma^+$ and an antisymmetric part $\Delta\sigma^-$ (i.e, $\Delta\sigma = \Delta\sigma^+ + \Delta\sigma^-$), the theoretical singlet relaxation rate constants are given by

$$R_S^{\sigma+} = \frac{2}{9}\gamma^2 B_0^2 \tau_2 \|\Delta\sigma^+\|^2; \quad R_S^{\sigma-} = \frac{2}{9}\gamma^2 B_0^2 \tau_1 \|\Delta\sigma^-\|^2 \quad (2)$$

assuming rigid isotropic molecular motion in the extreme narrowing limit. Here γ is the magnetogyric ratio, B_0 is the magnetic field, τ_j is the rank-j correlation time, and $\|t\|$ denotes the Frobenius norm of tensor t (i.e., the root-sum-square of all elements of t). The correlation times have the relationship $\tau_1 = 3\tau_2$ for isotropic rotational diffusion.[25] The CSA contribution to singlet relaxation vanishes if the two shielding tensors are identical, since the singlet state is sensitive only to differences in local magnetic fields.

(2) *Singlet−triplet leakage.* In the absence of coupling partners and an applied rf field, singlet−triplet leakage is caused by the small isotropic chemical shift difference $\Delta\delta_{iso}$ between the coupled ^{13}C nuclei, which interconverts the slowly relaxing singlet order with rapidly relaxing triplet order. In the limit $|\Delta\delta_{iso}\gamma B_0/(2\pi J_{CC})| \ll 1$, this gives rise to exponential decay of the singlet order. If the traceless symmetric parts of the two shielding tensors, σ_1^+ and σ_2^+, are approximately uniaxial with the same value $\sigma_{CSA} = \sigma_{ZZ} - \sigma_{iso}$ for the largest eigenvalue, the leakage rate constant for singlet decay is predicted to be

$$R_S^{leak} \approx \frac{\Delta\delta_{iso}^2 \gamma^2 B_0^2 (5b_{CC}^2 + 3\gamma^2 B_0^2 \sigma_{CSA}^2) \tau_2}{60\pi^2 J_{CC}^2} \quad (3)$$

where $b_{CC} = -(4\pi)^{-1}\mu_0\gamma^2\hbar r_{CC}^{-3}$ is the dipole−dipole coupling constant and J_{CC} is the scalar coupling constant within the ^{13}C pair.

(3) *Dipole−dipole relaxation.* The contribution to singlet relaxation from dipole−dipole couplings to spins outside the pair is given by[24]

$$R_S^{DD} = \frac{4}{3} \sum_j I_j(I_j + 1)\left[b_{C_{1j}}{}^2 + b_{C_{2j}}{}^2 \right.$$

$$\left. - 2b_{C_{1j}}b_{C_{2j}}P_2(\cos\theta_{C_{1j}C_2})\right]\tau_2 \tag{4}$$

where the sum runs over all spins j external to the pair, $\theta_{C_{1j}C_2}$ is the angle subtended by the C_1–j and C_2–j internuclear vectors, I_j is the angular momentum quantum number of the external spin, and $P_2(x) = (3x^2 - 1)/2$ is a second-rank Legendre polynomial. In the extreme narrowing limit, the contributions R_S^{leak}, $R_S^{\sigma+}$, and $R_S^{\sigma-}$ are strongly field-dependent but R_S^{DD} is field-independent.

The molecules shown in Figure 1 were designed to have a very small chemical shift difference $\Delta\delta_{iso}$, which minimizes the singlet leakage even in high magnetic field. To estimate the CSA contributions, we performed GIAO DFT[26] calculations to estimate the two ^{13}C CSA tensors as functions of molecular conformation. The molecular torsional angle ϕ defines the conformation of the (class I) X–CD$_2$–C≡C–CD$_2$–Y or (class II) X–CO$_2$–C≡C–CO$_2$–Y moiety. In all cases, the torsional angle $\phi=0$ corresponds to a conformation with X and Y on the same side.

The calculated CSA tensors were approximately symmetric and uniaxial, with average shielding anisotropies σ_{CSA} of approximately −172 and −152 ppm for samples **1** and **2**, respectively, and small biaxialities ($\eta < 0.2$). The orientation of the principal axis depends on the molecular torsional angle, especially for molecules of class II. This generates a relatively large difference tensor $\Delta\sigma$ at most conformations, except in the vicinity of $\phi = \pi$, which has local inversion symmetry.

For sample **1** (class I), the CSA-derived relaxation is weak since the principal axes of the CSA tensors are almost collinear for all molecular conformations. The root-mean-square Frobenius norms for the symmetric and antisymmetric difference tensors, averaged over all torsional angles ϕ with equal probability, were found to be $\langle\|\Delta\sigma^+\|^2\rangle^{1/2} = 13$ ppm and $\langle\|\Delta\sigma^-\|^2\rangle^{1/2} = 15$ ppm. The rank-2 rotational correlation time was estimated to be $\tau_2 = 17.8 \pm 0.3$ ps from the low-field value of ^{13}C T_1, assuming that the low-field relaxation is dominated by the dipole–dipole coupling between the ^{13}C nuclei. This gives the following estimates of the CSA contributions to the singlet relaxation rate constants in high field: $R_S^{\sigma+} = 0.06 \times 10^{-3}$ s^{-1} and $R_S^{\sigma-} = 0.15 \times 10^{-3}$ s^{-1}. Notably, the antisymmetric contribution exceeds the symmetric contribution, mainly because τ_1 is longer than τ_2. The estimated high-field leakage term is $R_S^{leak} = (0.08 \pm 0.01) \times 10^{-3}$ s^{-1}. The dominant singlet relaxation mechanism for sample **1** is the dipole–dipole relaxation to the nearby deuterons, for which $R_S^{DD} = (1.13 \pm 0.02) \times 10^{-3}$ s^{-1}. The total singlet relaxation rate constant estimated by theory is $(1.96 \pm 0.03) \times 10^{-3}$ s^{-1}, which is in rough agreement with the experimental value of ~1.73 × 10^{-3} s^{-1}.

For sample **2** (class II), the CSA-derived relaxation is much larger because the CSA principal axes deviate strongly in orientation. This is presumably due to partial conjugation of the C=O and C≡C π orbitals. The root-mean-square Frobenius norms of the shielding tensors averaged over all torsional angles ϕ were estimated to be $\langle\|\Delta\sigma^+\|^2\rangle^{1/2} = 81$ ppm and $\langle\|\Delta\sigma^-\|^2\rangle^{1/2} = 51$ ppm. The estimated rotational correlation time for sample **2** is 21.8 ± 0.3 ps. The estimated CSA contributions to the high-field singlet relaxation are $R_S^{\sigma+} = 2.4 \times 10^{-3}$ s^{-1} and $R_S^{\sigma-} = 2.9 \times 10^{-3}$ s^{-1}. The antisymmetric contribution again exceeds the symmetric contribution. The

singlet–triplet leakage mechanism contributes with $R_S^{leak} = (1.8 \pm 0.1) \times 10^{-3}$ s^{-1}. The dipole–dipole mechanism is insignificant for compound **2** because there are no magnetic nuclei close to the ^{13}C pair within the same molecule. The total theoretical rate constant for singlet relaxation is $(7.1 \pm 0.1) \times 10^{-3}$ s^{-1}. This indicates much faster singlet relaxation for compound **2** than for compound **1** in high magnetic field, in qualitative agreement with experiment.

The predicted singlet relaxation rate is considerably less than the experimental value of ~19.2 × 10^{-3} s^{-1}. This discrepancy is not yet understood but may be associated with anisotropic molecular motion[25] or inaccuracies in the shielding tensor estimations.

In summary, we have synthesized $^{13}C_2$-labeled compounds with long T_S values. In some cases the long T_S persists in high magnetic field without intervention. We have obtained an improved theoretical understanding of the major relaxation mechanisms, with strong indications that antisymmetric shielding tensors play a significant role. These insights are expected to guide the design of molecular systems with much longer T_S values in high magnetic field, even in the presence of protonated solvents or dissolved oxygen.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Synthetic routes; pulse sequence parameters; correlation time estimates; relaxation rate calculations; and DFT calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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