Symmetry-Based Singlet-Triplet Excitation in Solution Nuclear Magnetic Resonance

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Coupled pairs of spin-1/2 nuclei support one singlet state and three triplet states. In many circumstances the nuclear singlet order, defined as the difference between the singlet population and the mean of the triplet populations, is a long-lived state which persists for a relatively long time in solution. Various methods have been proposed for generating singlet order, starting from nuclear magnetization. This requires the stimulation of singlet-to-triplet transitions by modulated radiofrequency fields. We show that a recently described pulse sequence, known as PulsePol (Schwartz et al., Science Advances, 4, eaat8978 (2018)), is an efficient technique for converting magnetization into long-lived singlet order. We show that the operation of this pulse sequence may be understood by adapting the theory of symmetry-based recoupling sequences in magic-angle-spinning solid-state NMR. The concept of riffling allows PulsePol to be interpreted using the theory of symmetry-based pulse sequences, and explains its robustness. This theory is used to derive a range of new pulse sequences for performing singlet-triplet excitation and conversion in solution NMR. Schemes for further enhancing the robustness of the transformations are demonstrated.

I. INTRODUCTION

Long-lived states are configurations of nuclear spin state populations which, under suitable circumstances, are protected against important dissipation mechanisms and which therefore persist for unusually long times in solution1–42. The seminal example is the singlet order of spin-1/2 pair systems, which is defined as the population imbalance between the spin $I = 0$ nuclear singlet state of the spin pair, and the spin $I = 1$ triplet manifold7,13. Nuclear singlet order may be exceptionally long-lived, with decay time constants exceeding 1 hour in special cases16. The phenomenon of long-lived nuclear spin order has been used for a variety of purposes in solution nuclear magnetic resonance (NMR), including the study of slow processes such as chemical exchange4,26, molecular transport25–30, and infrequent ligand binding to biomolecules31–34, as well as quantum information processing41,42. The dynamics of nuclear singlet states is also central to the exploitation of parahydrogen spin order in hyperpolarized NMR experiments36–38,43–47. Singlet NMR has also been applied to imaging and in vivo experiments23,25,35,48–56, and related techniques such as spectral editing57,58 and low-field spectroscopy12,59–61.

Several methods exist for converting nuclear magnetization into singlet order in the “weak coupling” regime, meaning that the difference in the chemically shifted Larmor frequencies greatly exceeds the J-coupling between the members of the spin pair2–4. Methods for the “near equivalent” and “intermediate coupling” regimes (where the chemical shift frequency difference is weaker or comparable to the J-coupling), include the magnetization-to-singlet (M2S) pulse sequence5,6 and variants such as gM2S24 and gc-M2S23, the spin-lock-induced crossing (SLIC) method9–12, and slow passage through level anticrossings17,18.

Recently, a new candidate sequence has emerged, namely the PulsePol sequence, which was originally developed to implement electron-to-nuclear polarization transfer in the context of diamond nitrogen-vacancy magnetometry62–64. PulsePol is an attractively simple repeating sequence of six resonant pulses and four interpulse delays. The PhD thesis of Tratzmiller63 reports numerical simulations in which PulsePol is used for magnetization-to-singlet conversion in the near-equivalent regime of high-field solution NMR. These simulations indicate that PulsePol could display significant advantages in robustness over some existing methods such as M2S and its variants. In this article we report the following: (i) the confirmation of Tratzmiller’s proposal by experimental tests; (ii) the use of symmetry-based recoupling theory, as used in magic-angle-spinning solid-state NMR65–68, for elucidating the operation of this pulse sequence and predicting new ones; (iii) the PulsePol sequence and its variants may be used to excite singlet-triplet coherences; (iv) the robustness of the singlet-triplet transformation may be enhanced further by using composite pulses.

The PulsePol sequence was originally derived using average Hamiltonian theory with explicit solution of analytical equations62. In this article we demonstrate an alternative theoretical treatment of PulsePol derived from the principles of symmetry-based recoupling in magic-angle-spinning solid-state NMR65–68. This theoretical relationship is surprising since singlet-to-triplet conversion in solution NMR appears to be remote from recoupling in rotating solids. Nevertheless, as shown below, the problem of singlet-triplet conversion may be analysed in a time-dependent interaction frame in which the nuclear spin operators acquire a periodic time-dependence through the action of the scalar spin-spin coupling. The time-dependent spin operators in the interaction frame may be treated in similar fashion to the anisotropic spin interactions in rotating solids, in which case the periodic time-dependence is induced by the mechanical rotation of the sample. In both contexts, selection rules for the average Hamil-
tonian terms may be engineered by imposing symmetry constraints on the applied pulse sequences.

One common implementation of PulsePol corresponds to the pulse sequence symmetry designated \(R4_1\), using the notation developed for symmetry-based recoupling\(^{65-68}\). As shown below, the spin dynamical selection rules associated with \(R4_1\) symmetry explain the main properties of the PulsePol sequence. Furthermore this description immediately predicts the existence of many other sequences with similar properties. Some of these novel sequences are demonstrated below.

PulsePol deviates from the standard construction procedure for symmetry-based recoupling sequences in solids. The deviation is subtle but invests PulsePol with improved robustness. Incorporating composite pulses can increase the robustness further.

II. THEORY

A. Spin Hamiltonian

The rotating-frame spin Hamiltonian for a homonuclear 2-spin-1/2 system in high-field solution NMR may be written as

\[
H(t) = H_{CS} + H_J + H_{\Omega}(t),
\]

where the chemical shift Hamiltonian is given by

\[
H_{CS} = H_{\Sigma} + H_{\Delta}
\]

and the individual Hamiltonian terms are:

\[
H_{\Sigma} = \frac{1}{2} \omega_{\Sigma}(I_{1z} + I_{2z}),
\]

\[
H_{\Delta} = \frac{1}{2} \omega_{\Delta}(I_{1z} - I_{2z}),
\]

\[
H_J = \omega_J I_1 \cdot I_2.
\]

Here, \(\omega_{\Sigma}\) is the sum of the chemically shifted resonance offsets for the two spins, \(\omega_{\Delta}\) is their difference, and \(\omega_J = 2\pi J\) is the scalar spin-spin coupling (\(J\)-coupling).

The interaction of the spin pair with resonant radiofrequency fields is represented by the Hamiltonian term \(H_{\Omega}(t)\). The rotating-frame Hamiltonian for the interaction of the nuclei with a resonant time-dependent field is given by

\[
H_{\Omega}(t) = \omega_{\text{nat}}(t) \left\{ \cos \phi(t)(I_{1x} + I_{2x}) + \sin \phi(t)(I_{1y} + I_{2y}) \right\},
\]

where the nutation frequency \(\omega_{\text{nat}}\) is proportional to the radiofrequency field amplitude.

The terms \(H_{\Sigma}, H_J\) and \(H_{\Omega}\) all mutually commute. The term \(H_{\Delta}\), on other hand, commutes in general with neither \(H_J\) nor \(H_{\Omega}\). We consider here the case of “near-equivalent” spin pairs\(^{5,6,8}\), for which \(|\omega_{\Delta}| \ll |\omega_J|\). In this case, the term \(H_{\Delta}\) may be treated as a perturbation of the dominant terms \(H_J\) and \(H_{\Omega}\).

B. Propagators

The propagator \(U_A(t)\) generated by a Hamiltonian term \(H_A\) is a unitary time-dependent operator solving the differential equation

\[
\frac{d}{dt} U_A(t) = -i H_A(t) U_A(t)
\]

with the boundary condition \(U_A(0) = 1\). Since \(H_{\Omega}\) and \(H_J\) commute, the propagator \(U(t)\) under the total Hamiltonian of equation 1 may be written as follows:

\[
U(t) = U_J(t) U_{\Omega}(t) U_{CS}(t),
\]

where the propagator \(U_{CS}(t)\) solves the differential equation

\[
\frac{d}{dt} U_{CS}(t) = -i H_{CS}(t) U_{CS}(t)
\]

with the boundary condition \(U_{CS}(0) = 1\). The interaction-frame chemical shift Hamiltonian \(H_{CS}(t)\) is defined as follows:

\[
H_{CS}(t) = U_{\Omega}(t)^\dagger U_J(t)^\dagger H_{CS} U_J(t) U_{\Omega}(t).
\]

Equation 8 shows that the chemical shift terms acquire a double modulation in the interaction frame: first from the action of the \(J\)-coupling, and secondly from the action of the applied rf field.

Since the \(J\)-coupling is time-independent, the propagator \(U_J\) has the following form:

\[
U_J(t) = \exp \{-i H_J t\} = \exp \{-i \omega_J t I_1 \cdot I_2\}.
\]

The singlet and triplet states of the spin-1/2 pair are defined as follows:

\[
|S_0\rangle = 2^{-1/2}(|\alpha\beta\rangle - |\beta\alpha\rangle),
\]

\[
|T_+\rangle = |\alpha\alpha\rangle,
\]

\[
|T_0\rangle = 2^{-1/2}(|\alpha\beta\rangle + |\beta\alpha\rangle),
\]

\[
|T_-\rangle = |\beta\beta\rangle.
\]

Since the singlet and triplet states are eigenstates of \(H_J\), with eigenvalues \(-3\omega_J/4\) and \(+\omega_J/4\) respectively, the propagator \(U_J\) may be written as follows:

\[
U_J(t) = \exp\{+i\frac{t}{2} \omega_J t\} |S_0\rangle \langle S_0| + \exp\{-i\frac{t}{2} \omega_J t\} \sum_M |T_M\rangle \langle T_M|.
\]

The rf propagator \(U_{\Omega}(t)\) corresponds to a time-dependent rotation in three-dimensional space, described by three Euler angles:

\[
U_{\Omega}(t) = R(\Omega_{\Omega}(t)) = R_x(\alpha_{\Omega}(t)) R_y(\beta_{\Omega}(t)) R_z(\gamma_{\Omega}(t)),
\]

with

\[
R_x(\theta) = \exp\{-i \theta I_x\}.
\]
The action of the modulated radiofrequency field on the spin system may therefore be described in terms of a time-dependent set of three Euler angles $\Omega_{lt}(t) = \{\alpha_{lt}(t), \beta_{lt}(t), \gamma_{lt}(t)\}$.

In general, it is possible to modulate the amplitude $\phi_{ml}(t)$ and phase $\phi(t)$ of the rf field in time, in order to generate any desired trajectory of Euler angles $\Omega_{lt}(t)$.

C. Spherical Tensor Operators

It is convenient to define two spherical tensor spin operators of rank-1, denoted $T^g_{1m}$ and $T^u_{1m}$, where the superscripts denote their parity under exchange of the two spin-1/2 particles:

$$
(12) T^g_{1m} (12)^\dagger = T^g_{1m},
$$

$$
(12) T^u_{1m} (12)^\dagger = -T^u_{1m},
$$

where $m \in \{+1, 0, -1\}$ and (12) denotes the particle exchange operator. The gerade spherical tensor operator is constructed from the total angular momentum and shift operators for the spin system:

$$
T^g_{1+1} = -2^{-1/2}(I^+_1 + I^+_2),
$$

$$
T^g_{10} = I^+_1 + I^-_2,
$$

$$
T^g_{1-1} = 2^{-1/2}(I^-_1 + I^-_2).
$$

The ungerade spherical tensor operator of rank-1 plays a prominent role in the current theory. It has the following components:

$$
T^u_{1+1} = \ket{T^+_{11}}\bra{S_0},
$$

$$
T^u_{10} = \ket{T^0_0}\bra{S_0},
$$

$$
T^u_{1-1} = \ket{T^-_{11}}\bra{S_0}.
$$

Each component is given by a shift operator between the singlet state and one of the three triplet states. The adjoint operators are given by

$$
T^u_{1+1} = \bra{T^+_{11}}\ket{S_0},
$$

$$
T^u_{10} = \bra{T^0_0}\ket{S_0},
$$

$$
T^u_{1-1} = \bra{T^-_{11}}\ket{S_0}.
$$

Both sets of operators $T^g_{1m}$ and $T^u_{1m}$ transform irreducibly under the three-dimensional rotation group:

$$
R(\Omega)T^g_{1\mu}(\Omega)^\dagger = \sum_{\mu'=-1}^{+1} T^g_{1\mu} D^g_{\mu\mu'}(\Omega),
$$

$$
R(\Omega)T^u_{1\mu}(\Omega)^\dagger = \sum_{\mu'=-1}^{+1} T^u_{1\mu} D^u_{\mu\mu'}(\Omega).
$$

Here, $D_{\mu\mu'}(\Omega)$ represents an element of the rank-\(\lambda\) Wigner rotation matrix\(^69\).

The gerade spherical tensor operator $T^g_{1\mu}$ obeys the standard relationship between its components under the adjoint transformation\(^69\):

$$
T^g_{1\mu} = (-1)^\mu T^g_{1-\mu}.
$$

However, the analogous relationship does not apply to the components of the ungerade spherical tensor operator $T^u_{1\mu}$.

D. Interaction frame Hamiltonian

The chemical shift Hamiltonian terms, given in equation 3, may be written in terms of the $m = 0$ spherical tensor operator components as follows:

$$
H_\Sigma = \frac{1}{2} \omega_0 T^g_{10},
$$

$$
H_\Delta = \frac{1}{2} \omega_\Delta (T^u_{10} + T^{u\dagger}_{10}).
$$

From equation 11, these operators transform as follows under the propagator $U_J$:

$$
U_J(t)H_\Sigma U_J(t) = \frac{1}{2} \omega_0 T^g_{10},
$$

$$
U_J(t)H_\Delta U_J(t) = \frac{1}{2} \omega_\Delta (T^u_{10} \exp\{-i\omega_\Delta t\} + T^{u\dagger}_{10} \exp\{+i\omega_\Delta t\}).
$$

This may be combined with equations 8, 12 and 18 to obtain the following expression for the interaction-frame chemical shift Hamiltonian:

$$
\tilde{H}_{CS}(t) = \sum_{m=-1}^{+1} \sum_{\mu=-1}^{+1} \tilde{H}_{1m1\mu}(t),
$$

where each term has the form

$$
\tilde{H}_{1m1\mu}(t) = \omega_{m1\mu} d^\dagger_{10}(\beta(t)) \exp\{i(m\omega_\gamma + \mu\gamma_{lt}(t))\} Q_{1m1\mu},
$$

and $d^\dagger_{10}(\beta)$ is an element of the rank-1 reduced Wigner matrix. The amplitudes $\omega_{m1}\mu$ and spin operators $Q_{1m1\mu}$ take the following values:

$$
\omega_{+11\mu} = \frac{1}{2} \omega_\Delta, \quad Q_{+11\mu} = T^g_{1\mu},
$$

$$
\omega_{01\mu} = \frac{1}{2} \omega_0, \quad Q_{01\mu} = T^u_{1\mu},
$$

$$
\omega_{-11\mu} = \frac{1}{2} \omega_\Delta, \quad Q_{-11\mu} = (-1)^\mu T^{u\dagger}_{1-\mu},
$$

where $\mu \in \{+1, 0, -1\}$. Note that the singlet-triplet excitation terms have quantum number $m = \pm 1$, while the resonance off-set term has $m = 0$.

For the terms $\omega_{m3\lambda}$ and $Q_{m3\lambda}$ above, the rank of the interaction under rotations of the spins is specified as $\lambda = 1$. The “pseudo-space-rank” $\ell = 1$, on the other hand, has no physical meaning and is introduced to establish a correspondence with the notation used in magic-angle-spinning solid-state NMR\(^65-68\).
The propagator for a complete $R_{n\nu}$ sequence is given from equation 6 by

$$U(T) = U_{J}(T)U_{rf}(T) \tilde{U}_{CS}(T).$$  

(29)

A complete $R_{n\nu}$ sequence has duration $T = n\tau_{J}$, and is cyclic, in the sense that the net rotation induced by the rf field over the complete sequence is through an even multiple of $\pi$.

The symmetry numbers $N$, $n$ and $\nu$ take integer values. In the case of $R_{n\nu}$ sequences, $N$ must be even, while $n$ and $\nu$ are unconstrained. As discussed below, the symmetry numbers define the selection rules for the spin dynamics under the pulse sequence.

The $R_{n\nu}$ Euler angle symmetries in equation 25 do not define the pulse sequence uniquely. Nevertheless, there is a standard procedure\textsuperscript{65–68} for generating these Euler angle symmetries, which is sketched in figure 1. The procedure is as follows:

- Select a rf pulse sequence known as a basic $R$-element, designated $\mathcal{R}^{0}$. This sequence may be arbitrarily complex, but must induce a net rotation of the resonant spins by an odd multiple of $\pi$ about the rotating-frame x-axis. If the duration of the basic element $\mathcal{R}^{0}$ is denoted $\tau_{R}$, this implies the condition

$$U_{rf}(\tau_{R}) = R_{s}(p\pi),$$

(26)

where $p$ is an odd integer.

- The duration of the basic element $\tau_{R}$ is given by $\tau_{R} = (n/N)J^{-1}$, where $n$ and $N$ are the symmetry numbers of the $R_{n\nu}$ sequence.

- Reverse the sign of all phases in $\mathcal{R}^{0}$. This leads to the conjugate element designated $\mathcal{R}^{0'}$.

- Give all components of the basic element $\mathcal{R}^{0}$ a phase shift of $+\pi\nu/N$. This gives the phase-shifted basic element, denoted $\mathcal{R}^{0}_{+\pi\nu/N}$.

- Give all components of the conjugate element $\mathcal{R}^{0'}$ a phase shift of $-\pi\nu/N$. This gives the element $\mathcal{R}^{0'}_{-\pi\nu/N}$.

- The complete $R_{n\nu}$ sequence is composed of $N/2$ repeats of the element pair, as follows:

$$R_{n\nu} = \left\{ \mathcal{R}^{0}_{+\pi\nu/N}, \mathcal{R}^{0'}_{-\pi\nu/N} \right\}^{N/2}.$$  

(27)

The complete $R_{n\nu}$ sequence has an overall duration of

$$T = N\tau_{R} = nJ^{-1}.$$  

(28)

E. Symmetry-Based Sequences

Symmetry-based pulse sequences\textsuperscript{65–68} were originally developed for magic-angle-spinning solid-state NMR, where the sample is rotated mechanically with the angular frequency $\omega_{J}$, such that its rotational period is given by $\tau_{R} = |2\pi/\omega_{J}|$. In the current case of singlet-triplet excitation in solution NMR, the $J$-coupling plays the role of the mechanical rotation. The relevant period is therefore given by $\tau_{J} = |2\pi/\omega_{J}| = |J^{-1}|$.

In the current context, a sequence with $R_{n\nu}$ symmetry is defined by the following time-symmetry relationship of the rf Euler angles $\beta_{rf}(t)$ and $\gamma_{rf}(t)$, which applies for arbitrary time points $t$\textsuperscript{65–68}:

$$\beta_{rf}(t + n\tau_{J}/N) = \beta_{rf}(t) \pm \pi,$$

$$\gamma_{rf}(t + n\tau_{J}/N) = \gamma_{rf}(t) - 2\pi\nu/N.\quad(25)$$

FIG. 1. Standard implementation of a $R_{n\nu}$ sequence for singlet-triplet conversion. (a) A basic $R$-element denoted $\mathcal{R}^{0}$ is selected. This element induces a rotation about the rotating-frame x-axis through an odd multiple of $\pi$. In the current case, the element $\mathcal{R}^{0}$ is given by the composite pulse $90_{\phi}180_{\phi}90_{\phi}$ with delays $\tau_{R}$ between the pulses, such that its overall duration is $\tau_{R} = n/(NJ)$. The conjugate sequence $\mathcal{R}^{0'}$ is generated from $\mathcal{R}^{0}$ by a change in sign of all phases. (b) The sequence $\mathcal{R}^{0}$ is given a phase shift of $+\phi$, while the sequence $\mathcal{R}^{0'}$ is given a phase shift of $-\phi$, where $\phi = \pi\nu/N$. (c) The pair of sequences $(\mathcal{R}^{0})_{\phi}$ and $(\mathcal{R}^{0'})_{-\phi}$ is repeated $N/2$ times, to give the standard implementation of a $R_{n\nu}$ sequence (d).
From the definition of a $RN^n_\nu$ sequence, the complete sequence propagators $U_J(T)$ and $U_{rf}(T)$ are both proportional to the unity operator and may be ignored. The operator $\tilde{U}_{CS}(T)$ corresponds to propagation under a time-independent effective Hamiltonian:

$$\tilde{U}_{CS}(T) = \exp\{-i\tilde{H}_{CS}T\}. \quad (30)$$

In the near-equivalence limit ($|\omega_J| \gg |\omega_3|, |\omega_2|$), the effective Hamiltonian $\tilde{H}_{CS}$ may be approximated by the first term in a Magnus expansion $^{70-72}$:

$$\tilde{H}_{CS} \simeq \tilde{H}_{CS}^{(1)}, \quad (31)$$

where

$$\tilde{H}_{CS}^{(1)} = \sum_{m=-1}^{+1} \sum_{\mu=-1}^{+1} \tilde{H}_{lm1\mu}(t) dt, \quad (32)$$

In common with many recent papers $^{65-68}$, this article uses a numbering of the Magnus expansion terms which differs from the older literature $^{70-72}$ by one.

The individual average Hamiltonian terms are given by

$$\tilde{H}_{lm1\mu}(t) = T^{-1} \int_0^T \tilde{H}_{lm1\mu}(t) dt, \quad (33)$$

where the interaction frame terms $\tilde{H}_{lm1\mu}(t)$ are given in equation 23.

The Euler angle symmetries in equation 25 lead to the following selection rules for the first-order average Hamiltonian terms of $RN^n_\nu$ sequences $^{65-68}$:

$$\tilde{H}_{lm1\mu}(t_0) = 0 \quad \text{if} \quad mn - \mu \nu \neq N/2 Z_\lambda, \quad (34)$$

where $Z_\lambda$ is any integer with the same parity as $\lambda$. This selection rule may be visualised by a diagrammatic procedure $^{66,67}$.

In the current case, $\lambda = 1$ for all relevant interactions, so that $Z_\lambda$ is any odd integer. Hamiltonian components for which $mn - \mu \nu$ is an odd multiple of $N/2$ are symmetry-allowed and may contribute to the effective Hamiltonian. A symmetry-allowed term with quantum numbers $\{m, \mu\}$ and ranks $\ell = \lambda = 1$ is given in general by

$$\tilde{H}_{lm1\mu}^{(1)} = \kappa_{lm1\mu} \omega_{lm1\mu} Q_{lm1\mu}, \quad (35)$$

where the amplitudes $\omega_{lm1\mu}$ and spin operators $Q_{lm1\mu}$ are given in equation 24.

The scaling factor $\kappa_{lm1\mu}$ of a symmetry-allowed term is given by

$$\kappa_{lm1\mu} = \exp(-i \mu \frac{\pi \nu}{N}) K_{m1\mu}, \quad (36)$$

where $K_{m1\mu}$ is defined with respect to the basic element $\beta^0_{\nu}$:

$$K_{m1\mu} = \tau_R^{-1} \int_0^{\tau_R} d\mu_0 (-\beta^0_{\nu}(t)) \exp\{i(n \mu \nu(t) + m \omega_1\}) dt. \quad (37)$$

![FIG. 2. Energy levels and approximate eigenstates of a J-coupled two-spin-1/2 system in the near-equivalence limit.](image)

(a) $RN^n_\nu$

| $T_{-1}$ | $\infty$ |

| $T_{0}$ |

| $T_{+1}$ |

(b) $RN_{-\nu}$

| $T_{-1}$ |

| $T_{0}$ |

| $T_{+1}$ |

$|S_0\rangle$

Here $\beta^0_{\nu}$ and $\gamma^0_{\nu}$ represent the Euler angles describing the rotation induced by the rf field under the basic element $^{65-68}$.

Symmetry-based pulse sequences are designed by selecting combinations of symmetry numbers $N, n$ and $\nu$ such that all desirable average Hamiltonian terms $\tilde{H}_{lm1\mu}^{(1)}$ are symmetry-allowed while all undesirable terms are symmetry-forbidden. In most cases, the basic element $\beta^0_{\nu}$ is selected such that the scaling factors $\kappa_{lm1\mu}$ are maximised for the desirable symmetry-allowed terms.

G. Transition-selective singlet-triplet excitation

Table I shows some sets of symmetry numbers $\{N, n, \nu\}$ under which the average Hamiltonian terms with quantum numbers $\{\ell, m, \lambda, \mu\} = \{1, \pm 1, 1, \pm 1\}$ are symmetry-allowed, while all other terms are symmetry-forbidden and are suppressed in the average Hamiltonian. In particular, all resonance-offset terms, which have $m = 0$, are symmetry-forbidden in the first-order average Hamiltonian, for the symmetries in table I.

For example, consider the symmetry $R4^1_3$. The term $\{\ell, m, \lambda, \mu\} = \{1, 1, 1, 1\}$ is symmetry-allowed since the ex-
proportion proportional to the transverse operators $I^{(+)}_x$ and $I^{(+)}_y$, indicating the excitation of singlet-triplet states.

Hamiltonian is given through equations (24) by

$$H = \sum_{mK\mu} \text{terms with quantum numbers } \ell, m, \lambda, \mu \text{ given by } \{1, \pm 1, 1, \pm 1\} \text{. Changing the sign of } \nu \text{ selects the terms } \{1, \pm 1, 1, \mp 1\} \text{ instead. Scaling factors } \kappa_{1111} \text{ are given for the basic } R \text{-element in equation 48, in the limit of radiofrequency pulses with negligible duration.}

$$nm - \nu \mu \text{ evaluates to } 3 \times 1 - 1 \times 1 = 2\text{, which is an odd multiple of } N/2 = 2\text{. The term } \{\ell, m, \lambda, \mu \} = \{1, 1, 1, -1\} \text{, on the other hand, is symmetry-forbidden, since } nm - \nu \mu \text{ evaluates to } 3 \times 1 - 1 \times (-1) = 4\text{, which is an even multiple of } 2\text{. Similarly, the resonance-offset term } \{\ell, m, \lambda, \mu \} = \{1, 0, 1, -1\} \text{ is symmetry-forbidden, since } nm - \nu \mu \text{ evaluates to } 3 \times 0 - 1 \times (-1) = 1\text{, which is not an integer multiple of } 2\text{.}

All symmetries in table I select Hamiltonian components with quantum numbers $\{\ell, m, \lambda, \mu \} = \{1, \pm 1, 1, \pm 1\}$, while suppressing all other terms. In this case the first-order average Hamiltonian is given through equations 24 by

$$H^{(1)}_{CS} = \kappa_{1+1+1} \omega_{1+1+1} Q_{1+1+1}
+ \kappa_{-1-1-1} \omega_{-1-1-1} Q_{-1-1-1}
+ \frac{1}{2} \omega_\Delta \{ \kappa_{1+1+1} Q_{1+1+1} + \kappa_{-1-1-1} Q_{-1-1-1} \}.$$  (38)

The first-order average Hamiltonian therefore generates a selective rotation of the transition between the singlet state $|S_0\rangle$ and the lower triplet state $|T_{+1}\rangle$, as shown in figure 2(a):

$$H^{(1)}_{CS} = \frac{1}{2} \omega^{ST}_{\text{mut}} \{ e^{-i\phi_{ST}} |S_0\rangle \langle T_{+1}| + e^{+i\phi_{ST}} |T_{+1}\rangle \langle S_0| \}$$  (39)

The singlet-triplet nutation frequency and phase depend upon the scaling factors as follows

$$\omega^{ST}_{\text{mut}} = \omega_\Delta |\kappa_{1+1+1}| = \omega_\Delta |\kappa_{-1-1-1}|,$$  (40)

$$\phi_{ST} = \text{arg}(\kappa_{1-1-1}) = \text{arg}(-\kappa_{1111}^*)$$  (41)

If a set of symmetry numbers $\{N, n, \nu\}$ selects the terms $\{\ell, m, \lambda, \mu \} = \{1, \pm 1, 1, \pm 1\}$, then the set of symmetry numbers $\{N, n, -\nu\}$ selects the terms $\{\ell, m, \lambda, \mu \} = \{1, \pm 1, 1, \mp 1\}$. As indicated in figure 2b, the change in sign of $\nu$ leads to a selective rotation of the singlet state and the upper triplet state.

In either case the dynamics of the system may be described by a two-level treatment. Define the single-transition operators $T^{(\pm)}$ for the transitions between the singlet state and the outer triplet states:

$$T^{(\pm)} = \frac{1}{2} \{ |T_{+1}\rangle \langle S_0| + |S_0\rangle \langle T_{+1}| \},$$  (42)

These operators have the cyclic commutation relationships:

$$[T^{(\pm)}_x, T^{(\pm)}_y] = i T^{(\pm)}_z.$$  (43)

For the symmetries in table I, the first-order average Hamiltonian in equation 39 may be written as follows:

$$H^{(1)}_{CS} = \omega^{ST}_{\text{mut}} \{ T^{(+)}_x \cos \phi_{ST} + T^{(+)}_y \sin \phi_{ST} \}.$$  (44)

Assume that the density operator of the spin ensemble is prepared with a population difference between the lower triplet state and the singlet state. This arises, for example, if the system is in thermal equilibrium in a strong magnetic field. This state corresponds to a density operator term of the form

$$\rho(0) \sim T^{(+)}_z$$  (45)

omitting numerical factors and orthogonal operators. Suppose that an integer number $p$ of complete $R_N^{(\nu)}$ sequences is applied, with symmetry numbers selected from table I. The excitation interval is given by $\tau = p T$, where $T = N T_R$ is the duration of a complete $R_N^{(\nu)}$ sequence. From the cyclic commutation relationships in equation 43, the density operator at the end of the sequence is given by

$$\rho(\tau) \sim \omega^{ST}_{\text{mut}} \{ T^{(+)}_x \cos \omega^{ST}_{\text{mut}} \tau \cos \phi_{ST} + T^{(+)}_y \sin \omega^{ST}_{\text{mut}} \tau \sin \phi_{ST} \}.$$  (46)

This suggests the following phenomena:

1. Excitation of Singlet-Triplet Coherence. If the interval $\tau$ is chosen such that $\omega^{ST}_{\text{mut}} \tau$ is approximately an odd multiple of $\pi/2$, the resulting density operator contains terms proportional to the transverse operators $T^{(+)}_x$ and $T^{(+)}_y$, indicating the excitation of singlet-triplet...

| $R_N^{(\nu)}$ | $\kappa_{1111}$ |
|---------|-------------|
| $R_4^{-1}$ | -0.264 |
| $R_4^{+1}$ | 0.512 |
| $R_4^0$ | 0.307 |
| $R_4^{-1}$ | 0.038 |
| $R_4^{+1}$ | -0.029 |
| $R_6^{-2}$ | -0.104 |
| $R_6^{+2}$ | 0.291 |
| $R_6^{0}$ | 0.360 |
| $R_6^{-2}$ | -0.253 |
| $R_6^{+2}$ | 0.068 |

TABLE I. A selection of $R_N^{(\nu)}$ symmetries that are appropriate for symmetry-based singlet-triplet conversion in solution NMR. These symmetries select $H^{(1)}_{CS}$ terms with quantum numbers $\{\ell, m, \lambda, \mu \}$ given by $\{1, \pm 1, 1, \pm 1\}$. Changing the sign of $\nu$ selects the terms $\{1, \pm 1, 1, \mp 1\}$ instead. Scaling factors $\kappa_{1111}$ are given for the basic $R$-element in equation 48, in the limit of radiofrequency pulses with negligible duration.
coherence\textsuperscript{21}. In practice, the evolution time $\tau^*$ is restricted to integer multiples of the basic element duration $\tau_F$. In the absence of dissipative effects, the excitation of a singlet-triplet coherence is optimized by completing the following number of R-elements:

$$n^* \approx \text{round}(\pi/(4\omega_{\text{nut}}^{\text{ST}} \tau_F))$$

(ST coherence excitation)

2. Generation of Singlet Order. If the interval $\tau$ is chosen such that $\omega_{\text{nut}}^{\text{ST}} \tau$ is approximately an even multiple of $\pi/2$, the term $I_z^{\text{ST}(\pm)}$ is inverted in sign. This indicates that the populations of the singlet state and the outer triplet state are swapped. This leads to the generation of singlet order, which is a long-lived difference in population between the singlet state and the triplet manifold\textsuperscript{1–42}. In the absence of relaxation, the conversion of magnetization into singlet-order is optimised by completing the following number of R-elements:

$$n^* \approx \text{round}(\pi/(2\omega_{\text{nut}}^{\text{ST}} \tau_F))$$

(SO generation) (47)

It follows that the application of a $R_N^\nu$ sequence to a near-equivalent 2-spin-1/2 system in thermal equilibrium leads either to the excitation of singlet-triplet coherences, or to the generation of singlet order, depending on the number of R-elements that are applied. Experimental demonstrations of both effects are given below.

There are technical complications if the number of applied R-elements does not correspond to an integer number of complete $R_N^\nu$ sequences. In such cases the operators $U_j$ and $U_q$ in equation 6 lead to additional transformations. If the total number of completed R-elements is even, the main consequence is an additional phase shift of excited coherences, which is often of little consequence. If the number of applied R-elements is odd, on the other hand, then the propagator $U_R$ swaps the $|T_{+1}\rangle$ and $|T_{-1}\rangle$ states, exchanging the $I_z^{\text{ST}(\pm)}$ operators.

H. Implementation

1. Standard Implementation

The standard implementation of a $R_N^\nu$ sequence is sketched in figure 1 and described by equation 27.

There is great freedom in the choice of the basic element $\delta^\nu$ upon which the sequence is constructed. In this paper we concentrate on the implementation shown in figure 1, in which the basic element is a three-component composite pulse\textsuperscript{73}, with two $\tau$ delays inserted between the pulses:

$$\delta^0 = (90_90 - \tau - 180_0 - \tau - 90_90)$$

with $\tau$ degrees used here for the flip angles and the phases. This composite pulse generates an overall rotation by $\pi$ around the rotating-frame x-axis\textsuperscript{76}, and hence is an eligible basic element $\delta^0$ for the construction of a $R_N^\nu$ sequence.

The scaling factor $\kappa_{111}$, and hence the nutation frequency of the singlet-triplet transition, depends on the choice of basic element. In the case of the basic element in equation 48, the scaling factor is readily calculated in the limit of “$\delta$-function” pulses, i.e. strong rf pulses with negligible duration. The scaling factors $\kappa_{111\pm}$ are given for general $N$, $n$ and $\nu$ by

$$\kappa_{111\pm} = 2^{1/2} \frac{N}{n\pi} (-1)^{N^2 - (n - \nu)} / (2N) \sin^2(n\pi/2N).$$

(49)

Scaling factors for a set of $R_N^\nu$ symmetries appropriate for singlet-triplet excitation are given in table I. Scaling factors with the largest magnitude are offered by sequences with the symmetries $R_4^1$, $R_5^1$, $R_8^3$, and $R_{10}^7$.

Since the scaling factors in equation 49 are real, the effective nutation axis of the singlet-triplet transition has a phase angle of zero, $\phi_{ST} = 0$. This result applies to the basic-R element in equation 48, in the $\delta$-function pulse limit.

The implementation of a $R_N^\nu$ sequence by the procedure in figure 1 provides selective excitation of the transition between the singlet state of a near-equivalent spin-1/2 pair and one of the outer triplet states. However, the sequence performance is not robust with respect to rf field errors. It is readily shown that a deviation of the rf field from its nominal value induces a net rotation around the $z$-axis which accumulates as the sequence proceeds. This causes a degradation in performance in the case of radiofrequency inhomogeneity or instability.

2. Riffled Implementation

In magic-angle-spinning NMR, error compensation is often achieved by the use of supercycles, i.e. repetition of the entire sequence with variations in the phase shifts, or in some cases, cyclic permutations of the pulse sequence elements\textsuperscript{77–81}. PulsePol achieves very effective compensation for rf pulse errors by a much simpler method, namely a phase shift of just one pulse by 180°. This simple modification may be interpreted as a modified procedure for constructing sequences with $R_N^\nu$ symmetry, but with built-in error compensation.

Consider two different basic elements, denoted here $\delta_A^0$ and $\delta_B^0$, as shown in figure 3a. In the depicted case, the two basic elements differ only in that the central 180° pulse is shifted in phase by 180°:

$$\delta_A^0 = (90_90 - \tau - 180_0 - \tau - 90_90)$$

$$\delta_B^0 = (90_90 - \tau - 180_{180} - \tau - 90_90)$$

(50)

Under ideal conditions, both of these basic elements provide a net rotation by an odd multiple of $\pi$ about the rotating-frame x-axis, and hence are eligible starting points for the $R_N^\nu$ construction procedure. Furthermore, in the $\delta$-function pulse limit, the Euler angle trajectories generated by these sequences are identical. This implies that, in the case of ideal, infinitely short pulses, the elements $\delta_A^0$ and $\delta_B^0$ are completely interchangeable. The modified $R_N^\nu$ construction procedure sketched in figure 3 exploits this freedom by alternating the phase shifted “A” basic element $(\delta_A^0 + \pi\nu/N)$ with the phase-shifted conjugate “B” element $(\delta_B^0 - \pi\nu/N)$. 


The alternation of two different basic elements, as shown in figure 3, resembles the “riffling” technique for shuffling a pack of cards, in which the pack is divided into two piles, and the corners of the two piles are flicked up and released so that the cards intermingle. The procedure in figure 3 therefore leads to a riffled RN^ν_n sequence.

Under ideal conditions, and for pulses of infinitesimal duration, the “standard” and “riffled” construction procedures have identical performance. However, an important difference arises in the presence of rf field amplitude errors. The errors accumulate in the “standard” procedure, but cancel out in the “riffled” procedure. Hence the procedure shown in figure 3 achieves more robust performance with respect to rf field errors than the standard procedure of figure 1. However, it should be emphasised that this form of error compensation does not apply to all basic R-elements, and that even in the current case, strict RN^ν_n symmetry is only maintained in the limit of δ-function pulses. Nevertheless, within these caveats and restrictions, this error-compensation procedure is powerful and useful. As discussed below, error-compensation by riffling is responsible for the robust performance of PulsePol.

To see how a PulsePol sequence arises from the riffled RN^ν_n construction procedure, start with the pair of basic R-elements given in equation 50. Consider the symmetry R4^1, which is appropriate for transition-selective singlet-triplet excitation, as shown in table I. This symmetry implies that each R-element has duration τ_R = (3/4)J^-1, and hence that the delays between the pulses are given by τ = τ_R/2 = (3/8)J^-1, in the δ-function pulse limit.

The phase shifts ±πν/N are equal to ±45° in the case of R4^1 symmetry. Hence the pair of phase-shifted elements is given by

\[
\begin{align*}
R^0_A + 45 & = (90_{135} - τ - 180_{45} - τ - 90_{135}) \\
R^0_B - 45 & = (90_{-135} - τ - 180_{-225} - τ - 90_{-135})
\end{align*}
\]

(51)

This pair of elements may be concatenated, and the pair of elements repeated, to complete the riffled implementation of R4^1:

\[
R4^1[^{\text{riffled}}] = (A^0_R) + 45 (B^0_B) - 45 (R^0_A) + 45 (R^0_B) - 45
\]

(52)

If the riffled R4^1 sequence is given a −45° phase shift, we get:

\[
\begin{align*}
[ (A^0_R) + 45 (B^0_B) - 45 ] - 45 & = (A^0_R)0 (B^0_B) - 90 \\
& = (90_{90} - τ - 180_{90} - τ - 90_{90} - τ - 180_{90} - τ - 90_{90})
\end{align*}
\]

(53)

which is PulsePol. The −45° phase shift is of no consequence for the interconversion of singlet order and magnetization.

The riffled construction procedure may be deployed for the other symmetries in table 1. For example, the riffled implementation of R8^3, using the basic elements in equation 50, is as follows:

\[
R8^3[^{\text{riffled}}] = \left[ (A^0_R) + 67.5 (B^0_B) - 67.5 \right]^4
\]

\[
= [90_{157.5} - τ - 180_{122.5} - τ - 180_{122.5} - τ - 90_{157.5}]
\]

(54)

where the superscript indicates 4 repetitions and the interpulse delays are given by τ = τ_R/2 = (7/8)J^-1, in the δ-function pulse limit. Some sequences of this type have been proposed in the form of “generalised PulsePol sequences”. Some examples are demonstrated below.
TABLE II. Chemical structure of $^{13}$C$_2$-DAND (1,2,3,4,5,6,8-heptakis(methoxy-d$_3$)ap-(propan-2-yl-d$_7$)oxy)naphthalene-4a,8a-[$^{13}$C$_2$] with its relevant NMR parameters in a magnetic field of 9.39 T. The singlet-triplet mixing angle is defined as $\theta_{ST} = \tan^{-1}(\omega_{ST}/2\pi\nu)$ [24].

| Parameter | Value |
|-----------|-------|
| $J_C$ (Hz) | 54.39 ± 0.10 |
| $\Delta\delta$ (ppm) | 75.0 ± 2.0 |
| $\omega_{ST}$ (2π)/Hz | 7.50 ± 0.20 |
| $\theta_{ST}/^\circ$ | 7.85 ± 0.22 |

III. EXPERIMENTAL

A. Sample

Experiments were performed on a solution of $^{13}$C$_2$-labelled deuterated alkoxy naphthalene derivative ($^{13}$C$_2$-DAND), whose molecular structure with its relevant NMR parameters is shown in table II. Further details of the synthesis of ($^{13}$C$_2$-DAND) are given in the reference by Hill-Cousins et al [85]. This compound exhibits a very long $^{13}$C$_2$ singlet lifetime in low magnetic field [16]. The current experiments were performed on 30 mM of $^{13}$C$_2$-DAND dissolved in 500 µL isopropanol-d$_8$. The two $^{13}$C sites have a $J$-coupling of 54.39±0.10 Hz and a chemical shift difference of 7.50±0.2 Hz in a magnetic field of 9.39 T. The solution was doped with 3 mM of the paramagnetic agent (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) in order to decrease the $T_1$ relaxation time, allowing faster repetition of the experiments, and was contained in a standard Wilmad 5 mM sample tube.

B. NMR Equipment

All spectra were acquired at a magnetic field of 9.39 T. A 10 mm NMR probe was used, with the radiofrequency amplitude adjusted to give a nutation frequency of $\omega_{\text{nut}}/(2\pi)$ ~12.5 kHz, corresponding to a 90° pulse duration of 20 µs.

C. Pulse Sequences

1. Singlet-Triplet Excitation

The excitation of coherences between the singlet state and the outer triplet states of $^{13}$C$_2$-DAND was demonstrated using the pulse sequence in figure 4a. On each transient, a singlet destruction block is applied followed by a waiting time of $\sim 2T_1$ to establish thermal equilibrium. This ensures an initial condition free from interference by residual long-lived singlet order left over from the previous transient. After thermal equilibration in the magnetic field, a $R_N^\nu$ symmetry-based singlet-triplet excitation sequence of duration $\tau_{\text{exc}}$ is applied and the NMR signal detected immediately afterwards. Fourier transformation of the signal generates the $^{13}$C NMR spectrum.

2. Singlet Order Generation

The generation of singlet order is assessed by the pulse sequence scheme in figure 4b. After destruction of residual singlet order and thermal equilibration, a $M_2$S or $R_N^\nu$ sequence of duration $\tau_{\text{exc}}$ is applied to generate singlet order. This is followed by a $T_{00}$ singlet-order filtering sequence [8,86]. This consists of a sequence of rf pulses and pulsed field gradients that dephase all signal components not associated with nuclear singlet or-

| Parameter | Value |
|-----------|-------|
| $\omega_{\text{nut}}/(2\pi)$ | 12.5 kHz |
| $\tau_{00}$ | 20 µs |
| $\tau_R$ | 13800 µs |
| $\tau$ | 6860 µs |
| $\nu_R^{\text{exc}}$ | 4 |
| $\tau_{\text{exc}}$ | 55.2 ms |

TABLE III. Experimental parameters for the $R_4$ sequence used to obtain the results in figure 5(c,d). The parameters have the following meaning: $\omega_{\text{nut}}$ is the radiofrequency pulse amplitude, expressed as a nutation frequency; $\tau_{00}$ is the duration of a 90° pulse; $\tau_R$ is the duration of a single R-element; $\tau$ is the interval between pulses within each R-element (see figure 1); $\nu_R^{\text{exc}}$ is the number of R-elements in the excitation sequence; $\tau_{\text{exc}}$ is the duration of the excitation sequence.
FIG. 5. Enhanced singlet-triplet coherent excitation. (a) Conventional $^{13}$C spectrum of $^{13}$C$_2$-DAND using a single 90° pulse for excitation, showing strong signals from the triplet-triplet coherences; (b) Vertical expansion (by a factor of 12) of the conventional $^{13}$C spectrum. Additional signals are visible from minority isotopomers, with the outer peaks barely visible. The strong central peak is truncated. (c) Spectrum obtained by applying four elements of a riffled R$_4$ sequence, showing a strongly enhanced outer peak. The construction procedure in figure 3 was used, starting from the basic elements in equation 50. (d) Spectrum obtained by applying four elements of a R$_4$ sequence, showing the enhancement of the other outer peak. All spectra were obtained with a total of 256 transients and the same processing parameters. No line broadening is applied.

The singlet order is reconverted to z-magnetization by a second RN$_n^y$ sequence of equal duration to the first, or by a S2M sequence (time-reverse of the M2S sequence). The recovered z-magnetization is converted to transverse magnetization by a composite 90° pulse and the NMR signal detected in the following interval. The signal amplitude serves as a measure of the singlet order generated by the excitation sequence, and the efficiency of recovering magnetization from the singlet order. The maximum theoretical efficiency for passing magnetization through singlet order is $2/3$.

The RN$_n^y$ sequences may be constructed by either the standard or the riffled procedures. M2S and S2M sequences may be substituted for the first and last RN$_n^y$ sequences, respectively. The 90° readout pulse in figure 4b was implemented as a symmetrized BB1 composite pulse. Details of the composite pulse, the SOD sequence, and the T$_{00}$ pulse sequence modules are given in the Supporting Information.

FIG. 6. $^{13}$C spectra obtained after (a) a single 90° pulse, or (b-f) after filtering the $^{13}$C NMR signal through singlet order, using the scheme in figure 4b. (a) Standard $^{13}$C spectrum obtained with a single 90° pulse. (b) Singlet-filtered spectrum obtained with M2S for singlet order excitation and S2M for reconversion to magnetization. (c) Singlet-filtered spectrum obtained with a pair of R$_4$ sequences. (d) Singlet-filtered spectrum obtained with a pair of R$_8$ sequences. Both (c) and (d) use the standard implementation of RN$_n^y$ sequences, as in figure 1, using the basic element in equation 48. (e) Singlet-filtered spectrum obtained with a pair of riffled R$_4$ sequences. (f) Singlet-filtered spectrum obtained with a pair of riffled R$_8$ sequences. Both (e) and (f) use the riffled implementation of RN$_n^y$ sequences, as in figure 3, using the basic elements in equation 50. All pulse sequence parameters are given in the Supporting Information.

IV. RESULTS

A. Transition-selective singlet-triplet excitation

In systems of near-equivalent spin-1/2 pairs, the chemical shift difference induces a slight mixing of the singlet state $|Sangle$ with the central triplet state $|T_0\rangle$. This effect lends signal intensity to the single-quantum coherences between the singlet state and the outer triplet states $|T_{\pm 1}\rangle$, which generate the outer lines of the AB quartet. These peaks are feeble for two independent reasons: (i) the coupling of the singlet-triplet coherences to observable transverse magnetization is weak in the near-equivalence limit, and (ii) the singlet-triplet coherences are excited only weakly by conventional single-pulse excitation. The first of these factors is an intrinsic property of a singlet-triplet coherence. The second factor, on the other hand, may be overcome by using a suitable excitation sequence to generate the desired coherence with full amplitude. Many such schemes have been devised. This effect is useful since the frequencies of these peaks provide an accurate estimate of the internuclear J-coupling, which can be difficult to estimate in the near-equivalence regime.

Figure 5a shows the $^{13}$C NMR spectrum of the $^{13}$C$_2$-DAND solution. The strong central doublet is due to the two triplet-triplet coherences. The outer peaks of the AB quartet, which correspond to the weakly allowed singlet-triplet coherences, are barely visible in the spectrum, even after vertical expansion (figure 5b).
Greatly enhanced excitation of the outer AB peaks is achieved by the pulse sequence in figure 4a, using an excitation sequence of symmetry $R4^1_n$ constructed by the riffled procedure (figure 3), and with the number of $R$-elements satisfying equation 47. The strong enhancement of the outer AB peaks, relative to the spectrum induced by a single 90° pulse, is self-evident in figure 5c. Note that changing the sign of the symmetry number $ν$ switches the excitation to the opposite singlet-triplet transition (figure 5d). The experimental pulse sequence parameters are given in table III.

### B. Magnetization-to-singlet conversion

The experimental performance of some magnetization-to-singlet conversion schemes was tested on a TEMPO-doped solution of $^{13}$C$_2$-DAND using the pulse sequence protocol in figure 4b. A selection of singlet-filtered NMR spectra is shown in figure 6(b-f). In all cases the pulse sequence parameters were optimised for the best performance. The optimised parameters are given in the Supporting Information.

Figure 6a shows the unfiltered $^{13}$C NMR spectrum of $^{13}$C$_2$-DAND. Figure 6b shows the spectrum obtained by applying a M2S sequence to generate singlet order, suppressing other spin order terms, and regenerating magnetization from singlet order by applying a S2M sequence. Approximately 50% of the spin order is lost by this procedure, as may be seen by comparing the spectra in figure 6a and b. The theoretical limit on passing magnetization through singlet order is $2/3 ≈ 67%$.

The results obtained by using $RN^ν_n$ sequences with different sets of symmetry numbers are shown in figure 6c and d. The standard $RN^ν_n$ construction procedure in figure 1 was used. The number of $R$-elements was selected according to equation 47. The results are slightly inferior to the M2S sequence. Some of these spectra exhibit perturbed peak intensities. This is unexplained.

Riffling $RN^ν_n$ sequences constructed by the procedure in figure 3 display an improved performance, which is distinctly superior to M2S, as shown in figure 6e and f. The improvement is attributed to the increased robustness of the riffled procedure with respect to a range of experimental imperfections, as discussed further below.

Note that the riffling $R4^1_n$ sequence only differs from PulsePol by an overall phase shift (equations 52 and 53). The increased robustness of PulsePol with respect to M2S/S2M in the context of singlet/triplet conversion has been anticipated by the simulations of Tratzmiller.

The single order relaxation time $T_2$ is readily estimated by introducing a variable delay before the second $RN^ν_n$ sequence in figure 4b. Some results are shown in the Supporting Information. Although $T_2$ is found to be much greater than $T_1$, the value of $T_2$ is considerably shorter than that found in previous experiments. This is attributed to the TEMPO doping of the solution in the current case.

Figure 7 shows the dependence of the singlet-filtered NMR signals on the number of $R$-elements $n_R$, used for both the excitation and reconversion sequence. The corresponding total sequence durations $τ_{exc} = τ_{recon} = n_R τ_R = n_R (n/N) J^{-1}$ are also shown. Clear oscillations of the singlet order are observed, as predicted by equation 46. The singlet order oscillations induced by $R8^3_n$ are slightly slower than those for $R4^1_n$, as expected from the theoretical scaling factors reported in table I. The $R10^3_2$ sequence induces a relatively slow oscillation, corresponding to the small value of $κ_{11,11}$ for this symmetry. In all cases, numerical simulations by SpinDynamica software show qualitative agreement with the experimental results.

The improved robustness of the riffled implementation of $RN^ν_n$ with respect to rf amplitude variations is illustrated by the experimental results in figure 8. These plots show the singlet-filtered signal amplitudes as a function of rf field amplitude, using the protocol in figure 4b. Two different pulse sequence symmetries are explored: $R4^1_n$ (blue, left column) and $R8^3_n$ (red, right column). The horizontal axis represents the rf field amplitude, expressed as a nutation frequency $ω_{nut}$. The horizontal coordinates are given by the ratio $ω_{nut}/ω_{nut}^0$, where the nominal nutation frequency $ω_{nut}^0$ is used to calculate the pulse durations, which are kept fixed. Row (a) shows that the $R4^1_n$ and $R8^3_n$ sequences are both fairly narrowband with respect to rf field amplitude when the standard $RN^ν_n$ protocol is used (figure 1). Row b shows that their robustness with respect to rf amplitude errors is greatly improved by the riffling variant of the $RN^ν_n$ protocol, inspired by PulsePol (figure 3). Their tolerance of rf amplitude errors is increased further when the central 180° pulses of the basic $R$-elements are replaced by ASBO-11 composite pulses (row c). The use of 600°/180°/240°/180°/240°/180°/600° composite pulses provides less improvement (row d). For comparison, the experimental performance of the M2S/S2M protocol is shown by the grey lines in row d. The performance of M2S/S2M is clearly inferior to that of the riffling $RN^ν_n$ sequences.

Another important characteristic of pulse sequences for the generation and reconversion of singlet order is their robustness with respect to resonance offset, defined here as $Δω = ½ ω_0$, where $ω_0$ is the sum of the chemically shifted offset frequencies, see equation 3. A robust performance with respect to resonance offset is usually desirable, since it renders the sequence less sensitive to inhomogeneity in the static magnetic field, which can be particularly important in low-field applications.

Figure 9 compares the resonance-offset dependence of several pulse sequences, for the generation and reconversion of $^{13}$C$_2$ singlet order in the solution of $^{13}$C$_2$-DAND. The left column compares different schemes which have $R4^1_n$ symmetry. The right column compares different schemes which have $R8^3_n$ symmetry. All experimental parameters are given in the Supporting Information.

Figure 9a shows the resonance-offset dependence of $RN^ν_n$ sequences constructed by the standard protocol of figure 1, using the basic $R$-element of equation 48. The resulting sequences have a strong dependence on resonance offset, with the $R8^3_n$ sequence displaying a particularly undesirable offset dependence.

Figure 9b shows the resonance-offset dependence of riffling $RN^ν_n$ sequences, using the pair of basic $R$-elements in equation 50. Riffling clearly stabilises the resonance offset dependence, with the improvement being particularly striking for
Figures 9c and d explore the effect of substituting the central 180° pulse of the basic R-elements by composite pulses. Although ASBO-11 composite pulses do not change the performance of R4 much, they do lead to a significant increase in the bandwidth of R8 (figure 9c). An even more pronounced effect is observed upon replacing all single 180° pulses by 7-element composite pulses (figure 9d). The resonance-offset bandwidth of R8 with 7-element composite pulses is particularly impressive.

The grey lines in figure 9d show the experimental offset dependence of the M2S/S2M protocol. All ruffled RNν sequences have a clearly superior performance to M2S/S2M. To put this in context, even the M2S/S2M protocol is regarded as relatively robust with respect to resonance offset, being first demonstrated on a sample in an inhomogeneous low magnetic field. Some other techniques, such as SLIC, are far more sensitive to resonance offset than M2S.

Results for the dependence of the singlet order conversion on the pulse sequence intervals are given in the Supporting Information.

V. DISCUSSION

The results shown in this paper indicate that PulsePol is a very attractive addition to the arsenal of pulse sequences for the manipulation of nuclear singlet order. The PulsePol sequences provide a high degree of robustness with respect to common experimental imperfections, which is found to be superior to existing methods such as M2S/S2M, especially when combined with composite pulses. This robustness is likely to be particularly important for applications to imaging and in vivo experiments.

In addition, PulsePol is a relatively simple repeating sequence of six pulses. This structure has many advantages over M2S, which performs the magnetization-to-singlet-order transformation in four consecutive steps. For example, the PulsePol repetitions may be stopped at any time, in order to achieve a partial transformation of spin order. This is more difficult to achieve for M2S and its variants.

The theoretical relationship between PulsePol and symmetry-based recoupling sequences in solid-state NMR is unexpected. Nevertheless, this theoretical analogy immediately allows the considerable body of average Hamiltonian theory developed for symmetry-based recoupling to be deployed in this very different context. This immediately allows the use of symmetry-based selection rules for analysing existing PulsePol sequences and for designing new variants.

All of the work reported in this paper uses the same set of basic elements, given in equations 48 and 50. There is clearly scope for using different basic elements within the RNν symmetry framework.

As discussed above, PulsePol may interpreted as a variant implementation of RNν symmetry, involving the alternation of two different basic elements, which compenstate each others’ imperfections. Such ruffled RNν sequences are more robust with respect to a range of experimental imperfections. The same principle might be applied to symmetry-based recoupling sequences in magic-angle-spinning solids. Extensions are also possible, involving more complex interleaved patterns of multiple basic elements. We intend to explore such “riffled supercycles” in future work.

In magic-angle-spinning solid-state NMR, symmetry-based pulse sequences have been used to address a wide variety of spin dynamical problems, including multiple-channel sequences for the recoupling of heteronuclear systems. Such extensions should be possible in the solution NMR context as well.

Variants of M2S/S2M sequences have been applied to heteronuclear spin systems. This has important applications in parahydrogen-induced polarization. It is likely that ruffled RNν sequences are also applicable to this problem.
FIG. 8. Experimental $^{13}$C signal amplitudes of $^{13}$C$_2$-DAND solution, obtained by the protocol in figure 4b, as a function of relative nutation frequency $\omega_{\text{nut}}/\omega^0_{\text{nut}}$, where $\omega^0_{\text{nut}}$ represents the nominal nutation frequency used for the calculation of pulse durations. The traces correspond to the experimental amplitudes for converting magnetization into singlet order and back again, normalized with respect to the signal generated by a single $90^\circ$ pulse. Left column (blue): R$_{4\frac{1}{3}}$ sequences; Right column (red): R$_{8\frac{2}{3}}$ sequences. (a) Standard R$_{N\nu}^V$ sequences using the basic element in equation 48. (b) Riffled R$_{N\nu}^V$ sequences using the basic elements in equation 50. (c) Riffled R$_{N\nu}^V$ sequences with all central $180^\circ_0$ pulses replaced by an ASBO-11 composite pulse$^{84}$. (d) Riffled R$_{N\nu}^V$ sequences with all central $180^\circ_0$ pulses replaced by a $60^\circ_0180^\circ_0240^\circ_0180^\circ_060^\circ_0$ composite pulse$^{83}$. The grey lines in (d) show the experimental response of the M2S/S2M protocol. All experimental details are given in the SI.

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AUTHOR DECLARATIONS

Conflict of interest

The authors have no conflicts to disclose.

In summary, the PulsePol sequence$^{62-64}$ is an important innovation that has potential applications in many forms of magnetic resonance. It sits at the fertile intersection of diamond magnetometry, quantum information processing, solid-state NMR, parahydrogen-induced hyperpolarization, and singlet NMR in solution.

The theory of symmetry-based recoupling in magic-angle-spinning solids was originally formulated using average Hamiltonian theory, as sketched above. It is also possible to obtain the key results using Floquet theory$^{91,92}$, which may have advantages in certain circumstances. Floquet theory should also be applicable to the current context.

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The authors have no conflicts to disclose.
DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Symmetry-Based Singlet-Triplet Excitation in Solution Nuclear Magnetic Resonance

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I. PULSE SEQUENCE DETAILS

A. Composite pulses

1. BB1 composite pulse

The BB1 family of composite pulses originally defined by Wimperis\(^1\) achieves broadband compensation of pulse strength errors. In the time-symmetric version\(^2\), which we designate BB1(\(\beta\)), a composite implementation of a simple \(\beta_0\) pulse with generic flip angle \(\beta\) takes the following form:

\[
BB1(\beta) = (\beta/2)_0 180\theta_W(\beta) 360\theta_W(\beta) 180\theta_W(\beta)(\beta/2)_0
\]  

(1)

The angle \(\theta_W\) in the phases of the error correcting block depends on the desired flip angle \(\beta\), and is given by:

\[
\theta_W(\beta) = \arccos(-\beta/(4\pi)) = \arccos(-\beta/(720^\circ))
\]  

(2)

For a 90° and 180° pulse respectively:

\[
\theta_W(\pi/2) = \arccos(-1/8) \approx 97.18^\circ
\]  

(3)

\[
\theta_W(\pi) = \arccos(-1/4) \approx 104.48^\circ
\]  

(4)

Accordingly, in all our singlet-filtered experiments, the 90\(_0\) readout pulses at the end are replaced by the equivalent composite rotation 45\(_0\)180\(_{97.18}\)360\(_{291.54}\)180\(_{97.18}\)45\(_0\). Additionally, a two step [0,180] phase cycle is implemented on the readout pulse and receiver channel.

2. ASBO-11 composite pulse

ASBO-11 is a closely related infinite family of dual-compensated composite inversion pulses which achieves simultaneous compensation of pulse strength errors and resonance offset/detuning errors. It replaces a single 180\(_0\) pulse with 11 180 pulses with phases arranged in a so-called antisymmetric (i.e. the time reverse inverts all phases) form such as:

\[
\pi_{\text{ASBO}}^{11} = 180-\phi_1 180-\phi_2 180-\phi_3 180-\phi_4 180-\phi_5 180_0 180+\phi_5 180+\phi_4 180+\phi_3 180+\phi_2 180+\phi_1
\]  

(5)

In general, the phases of the 11 pulses are given by:
\[
\phi_1 = \frac{2}{3}\pi - 5\phi \\
\phi_2 = \frac{4}{3}\pi - \theta_W(\pi) - 4\phi \\
\phi_3 = \frac{4}{3}\pi - 2\theta_W(\pi) - 3\phi \\
\phi_4 = \frac{4}{3}\pi - \theta_W(\pi) - 2\phi \\
\phi_5 = \frac{2}{3}\pi - \phi
\]

In this context, \(\phi\) is a free variable which may be tailored for the compensation of resonance offset errors, pulse strength errors, or both.

We have found that the choice \(\phi = \frac{4}{3}\pi - \theta_W(\pi)/2 \approx 187.8^\circ\) works well for dual-compensation. This choice of phase appears to correspond to "ASBO-11(B1)" described by Odedra et al. (they give \(\phi = 188^\circ\)) which was found by a numerical search over \(\phi\) in 1° increments for the ASBO-11 sequence with the largest bandwidth with respect to pulse strength errors.

For \(\phi = \frac{4}{3}\pi - \theta_W(\pi)/2\), we obtain the set of solutions:

\[
(\phi_1, \phi_2, \phi_3, \phi_4, \phi_5) = \left(\frac{5}{2} \theta_W(\pi), \theta_W(\pi), \frac{4}{3}\pi - \theta_W(\pi)/2, \frac{2}{3}\pi, \frac{4}{3}\pi + \theta_W(\pi)/2\right)
\]

Accordingly, this leads to the ASBO-11 composite pulse tested in our experiments:

\[
180_{98.81}180_{255.52}180_{172.24}180_{240}180_{0}180_{67.76}180_{0}180_{292.24}180_{120}180_{187.76}180_{104.45}180_{261.19}
\]

**B. \(T_{00}\) filter**

The \(T_{00}\) filter is a common block in singlet NMR experiments. It consists of a series of pulsed field gradients and radiofrequency pulses which are designed to dephase unwanted operators i.e. those not corresponding to the \(T_{00}\) symmetry of the nuclear singlet order operator. A typical implementation consists of three gradients sandwiched by two radiofrequency pulses:

\[
G_1 - 90_0 - G_2 - \beta_m - G_3
\]

Here, the angle \(\beta_m\) is the magic angle arctan \(\sqrt{2} \approx 54.74^\circ\). In order to ensure the optimal performance of the \(T_{00}\) filter, all pulses were replaced by the corresponding BB1 composite pulses as described in the previous subsection; the 90° pulse is implemented as 45018097.18360291.5418097.18450

3
while the \((\beta_m)_{0}\) pulse is implemented as 27.370\text{18094.36}360283.0818094.3627.370.

The parameters used in our experiments are shown in Table SI. In practice, due to hardware limitations, rest delays \(\tau_r\) follow each pulsed field gradient.

Table SI. Experimental parameters for the \(T_{00}\) filter used in the experiments. The gradient strengths are given by \(G_1\), \(G_2\), and \(G_3\) respectively. The gradient durations are given by \(\tau_{G}^1\), \(\tau_{G}^2\), and \(\tau_{G}^3\) respectively. The recovery delay after each gradient is given by \(\tau_{rest}^1\), \(\tau_{rest}^2\), and \(\tau_{rest}^3\).

| Parameter | Value |
|-----------|-------|
| \(G_1\) [G/cm] | 16.08 |
| \(G_2\) [G/cm] | -9.94 |
| \(G_3\) [G/cm] | -6.14 |
| \(\tau_{G}^1\) [\(\mu s\)] | 8000.000 |
| \(\tau_{G}^2\) [\(\mu s\)] | 4944.272 |
| \(\tau_{G}^3\) [\(\mu s\)] | 3055.728 |
| \(\tau_{rest}^1\) [ms] | 20.4 |
| \(\tau_{rest}^2\) [ms] | 15.4 |
| \(\tau_{rest}^3\) [ms] | 17.3 |
C. Singlet order destruction (SOD) element

In standard NMR experiments, the waiting delay between scans is typically set to be on the order of $\times 5$ the longitudinal relaxation constant $T_1$, which is usually enough to fully equilibrate a spin system for most practical purposes. However, in experiments which excite nuclear singlet order - which relaxes with a time constant $T_S$, often orders of magnitude larger than $T_1$ - this approach is problematic.

In order to ensure the quality of experimental data, a singlet order destruction (SOD) element was incorporated in all experiments.

The SOD element consists of a $T_{00}$ filter followed by a train of J-synchronized spin echoes repeated $m_1$ times.

The J-synchronized block is a building block of M2S, and similar to the M2S sequence has a total echo duration $\tau_e$ ideally set to:

$$\tau_e = 1/(2J)$$

For optimal singlet order destruction, the number of repetitions should roughly accomplish a $2\pi/3$ rotation in the $|S_0\rangle$-$|T_0\rangle$ Bloch sphere:

$$m_1 \approx \text{round}(\pi/(3\theta_{ST}))$$

The SOD element may be repeated $m_2$ times. Previous work suggests $m_2 \approx 1 - 3$ is sufficient for singlet order destruction. Out of an abundance of caution, we set $m_2 = 7$ in our experiments.

The SOD element is illustrated in Figure S2.

Figure S2. Illustration of the SOD filter implemented in the experiments. The $T_{00}$ filter has the same meaning as the previous section. $\tau_e$ is the total spin echo duration. $m_1$ is the number of times the spin echo is repeated within a single SOD element. $m_2$ is the total number of SOD elements. $\tau_r$ is the relaxation delay.

The parameters used in the SOD element in the main text are given in Table SII.
Table SII. Experimental parameters for the SOD block used in the experiments in the main text. The parameters have the same meaning as Figure 2.

| Parameter | Value |
|-----------|-------|
| $m_1$     | 7     |
| $m_2$     | 7     |
| $\tau_e$ [$\text{ms}$] | 9.24 |
| $\tau_r$ [$\text{s}$]   | 30    |

II. EXPERIMENTAL DETAILS FOR FIGURES 6-9

A. Description of M2S/S2M sequences

The M2S sequence is prototypical hard-pulse sequence for generating singlet order from longitudinal magnetization in the near-equivalence regime$^{4-6}$. In general, M2S takes the form:

$$90_x - (\tau_1 - 90_y 180_x 90_y - \tau_1)^{n_1} - 90_y - \tau_2 - (\tau_1 - 90_y 180_x 90_y - \tau_1)^{n_2}$$ (16)

Here, $\tau_1$ and $\tau_2$ are interpulse delays, while $n_1$ and $n_2$ denote the number of repetitions.

Figure S3. Illustration of the M2S sequence in this work. $\tau_1$ is the interval between pulses in the spin echoes (of total duration $\tau_e$), and $\tau_2$ is the interval after the $90_y$ pulse.

Unlike the simple presentation of an R-sequence, M2S consists of five distinct blocks: (i) a $90^\circ$ excitation pulse; (ii) a train of $n_1$ J-modulated spin echoes of total duration $\tau_e \approx 1/(2J)$; (iii)
another 90° pulse with a phase in quadrature with the initial excitation pulse; (iv) a $\tau_e/2 \approx 1/(4J)$ refocusing delay; (v) a train of $n_2 \approx n_1/2$ J-modulated spin echoes.

The pulse sequence which reconverts singlet order to magnetization is the emphtime reverse, denoted S2M.

To ensure maximum error compensation, the 180° pulses in the echo trains are implemented with the standard MLEV-4 four-step $[0,0,180,180]$ supercycle$^{4,6,7}$.

### B. Parameters for sequences in Figure 6

The experimental parameters for the $\text{RN}_n^\nu$ and M2S sequences that appear in Figure 6 are shown in Table SIII.

| $\omega_{\text{nut}}/(2\pi)$ | 12.5 kHz |
|-----------------------------|-----------|
| $\tau_{00}$ | 20 $\mu$s |

| Sequence | $\tau_R$ | $\tau$ | $\nu_R^\text{exc}$ | $\tau_{\text{exc}}$ |
|----------|----------|--------|--------------------|-------------------|
| R4$_3^1$ (riffled) | 13800 $\mu$s | 6860 $\mu$s | 9 | 124.2 ms |
| R4$_3^1$ (standard) | 13400 $\mu$s | 6660 $\mu$s | 9 | 120.60 ms |
| R8$_7^3$ (riffled) | 16000 $\mu$s | 7960 $\mu$s | 9 | 144.00 ms |
| R8$_7^3$ (standard) | 15560 $\mu$s | 7740 $\mu$s | 9 | 140.04 ms |
| M2S | 9240 $\mu$s | 4580 $\mu$s | 4600 $\mu$s | 11 | 5 | 152.46 ms |

Table SIII. Experimental parameters for the M2S and $\text{RN}_n^\nu$ sequences used to obtain the results in Figure 6(b,c,d,e,f) in the main text. The parameters for the $\text{RN}_n^\nu$ sequences have the same meaning as in Table III in the main text. The parameters are given separately for R4$_3^1$ sequences (used in Figure 6(c,e)), the R8$_7^3$ sequences (used in Figure 6(d,f)), and the M2S sequence (used in Figure 6(b).)
### C. Parameters for sequences in Figure 7

| Parameter | Value |
|-----------|-------|
| $\omega_{nut}/(2\pi)$ | 12.5 kHz |
| $\tau_{90}$ | 20 $\mu$s |
| $R_{41/3}$ (riffled) | $\tau_R$ 13800 $\mu$s, $\tau$ 6860 $\mu$s |
| $R_{83/7}$ (riffled) | $\tau_R$ 16000 $\mu$s, $\tau$ 7960 $\mu$s |
| $R_{102/3}$ (riffled) | $\tau_R$ 5560 $\mu$s, $\tau$ 2720 $\mu$s |

Table SIV. Experimental parameters for the $R_{n}^{\nu}$ sequences used to obtain the results in Figure 7 in the main text. The parameters for the $R_{n}^{\nu}$ sequences have the same meaning as in Table SIII. The parameters are given separately for the $R_{41/3}$ sequence (used in Figure 7(a), the $R_{83/7}$ sequence (used in Figure 7(b), and the $R_{102/3}$ sequence (used in Figure 7(c)).
D. Parameters for sequences in Figures 8 and 9

| $\omega_{\text{nut}}/(2\pi)$ | 12.5 kHz | 20 $\mu$s |
|-----------------------------|----------|----------|
| $\tau_90$                   | $\tau_R$ | 13400 $\mu$s |
|                            | $\tau$   | 6660 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 120.60 ms |

| R4$_3^1$ (standard)         | $\tau_R$ | 13800 $\mu$s |
|                            | $\tau$   | 6860 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 124.2 ms |

| R4$_3^1$ (riffled)         | $\tau_R$ | 13800 $\mu$s |
|                            | $\tau$   | 6460 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 124.2 ms |

| R4$_3^1$ (ASBO-11)         | $\tau_R$ | 13800 $\mu$s |
|                            | $\tau$   | 6593 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 124.2 ms |

| R4$_3^1$ (SP7)             | $\tau_R$ | 15560 $\mu$s |
|                            | $\tau$   | 7740 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 140.04 ms |

| R8$_7^3$ (standard)        | $\tau_R$ | 16000 $\mu$s |
|                            | $\tau$   | 7960 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 144.00 ms |

| R8$_7^3$ (riffled)        | $\tau_R$ | 16000 $\mu$s |
|                            | $\tau$   | 7560 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 144.00 ms |

| R8$_7^3$ (ASBO-11)        | $\tau_R$ | 16000 $\mu$s |
|                            | $\tau$   | 7693 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 144.00 ms |

| R8$_7^3$ (SP7)            | $\tau_R$ | 16000 $\mu$s |
|                            | $\tau$   | 7693 $\mu$s |
|                            | $\nu_{\text{exc}}^R$ | 9 |
|                            | $\tau_{\text{exc}}$ | 144.00 ms |

Table SV. Experimental parameters for the $R_N^w$ sequences used to obtain the results in Figures 8(a,b,c,d) and 9(a,b,c,d) in the main text. The parameters for the $R_N^w$ sequences have the same meaning as in Tables SIII-IV. The parameters are given separately for R4$_3^1$ and R8$_7^3$ sequences in the standard implementation (Figures 8(a) and 9(a)); the riffled implementation (Figures 8(b) and 9(b)); the riffled implementation with the ASBO-11 composite pulse (Figures 8(c) and 9(c)); and the riffled implementation with the 7-element Shaka-Pines$^8$ (SP7) composite pulse (Figures 8(d) and 9(d)).
III. RELAXATION EXPERIMENTS

A. $T_1$ measurement

The time constant for the relaxation of longitudinal magnetization is typically denoted $T_1$ in NMR.

We have used a standard inversion recovery experiment to measure $T_1$, as shown in Figure S4.

Figure S4. Illustration of the inversion recovery sequence used to measure $T_1$. After a relaxation delay of 30 seconds, the longitudinal magnetization is inverted with a composite pulse, allowed to evolve, and then read out with a 90 degree pulse.

The time evolution of magnetization following inversion, $M(t)$, may be fitted to the simple equation:

$$M(t) = A(1 - 2 \exp \left( -t/T_1 \right))$$  \hspace{1cm} (17)

B. $T_S$ measurement using PulsePol

The singlet relaxation time $T_S$ can be measured using the sequences described in the main text. The time evolution of nuclear singlet order may be fitted to the simple equation:

$$M(t) = A \exp \left( -t/T_S \right)$$  \hspace{1cm} (18)
Figure S5. Longitudinal relaxation of spin magnetization in $^{13}$C$_2$-DAND@ 9.4 T and 25 °C, following the experiment in Figure 3. Black circles: experimental data. Dashed line: fit using Equation (16), with the parameters $A = 0.984 \pm 0.006$ and $T_1 = 3.41 \pm 0.05$ s.

Figure S6. Illustration of the inversion recovery sequence used to measure $T_S$. After the SOD filter, and generation of nuclear singlet order using the $R_{4_{31}}^1$ sequence, the singlet order is allowed to evolve, filtered, and then read out with another $R_{4_{31}}^1$ sequence and a 90 degree pulse. The $R_{4_{31}}^1$ sequence is performed as per the PulsePol implementation, and has the parameters described in the main text.
Figure S7. Singlet relaxation in TEMPO-doped $^{13}$C$_2$-DAND solution @ 9.4 T and 25° C following the experiment in Figure 5. Black circles: experimental data. Dashed line: fit using Equation (17), with the parameters $A = 1.03 \pm 0.01$ and $T_S = 89.4 \pm 4.3$ s.
IV. ADDITIONAL PERFORMANCE COMPARISONS

A. Dependence on delay mismatch

Figure S8. Experimental $^{13}$C signal amplitudes (white dots) for (a) R4$^1_3$, (b) R8$^2_7$ and (c) M2S as a function of the relative inter-pulse delay mismatch $\Delta \tau / \tau^0$, where $\tau^0$ represents the nominal inter-pulse delay. For the M2S sequence the nominal inter-pulse delay is given by $\tau^0 = 1/(4J)$, whereas for R-based sequences the nominal inter-pulse delay is given by $\tau^0 = n/(NJ)$. The R-sequences have been implemented according to the PulsePol procedure. The final $^{13}$C signal amplitudes were referenced with respect to a single $^{13}$C-pulse-acquire spectrum. Light blue trajectories represent numerical simulations with the pulse sequence parameters given in Tables I-II. Relaxation was neglected in all cases.
V. REFERENCES

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