Electronic Supporting information (ESI)

Tuning of pH Enables Carbon-13 Hyperpolarization of Oxalates by SABRE

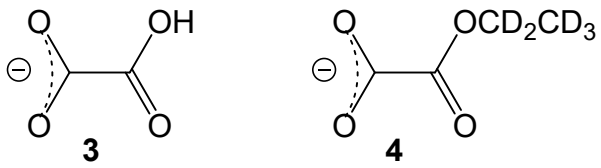
Contents

S1. Experimental procedures

S1.1. Materials

The catalyst precursor [Ir(IMes)(COD)Cl] (**1**) employed in this work was synthesized by established procedures according to literature methods.[1] Methanol-d_4 (CD_3OD), dimethyl sulfoxide-d_6 (DMSO-d_6), deuterium oxide (D_2O), sodium oxalate and sodium oxalate-$^{13}C_2$ (**2**) were purchased from Sigma Aldrich and used as supplied.

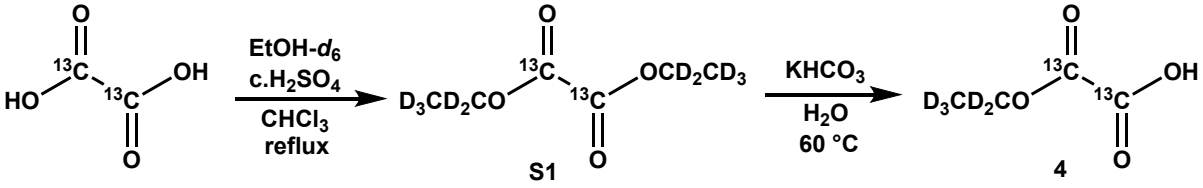
S1. Substrate preparation



S1.A Preparation of oxalate samples at different pH values

Oxalic acid was dissolved in water to form a concentration of 0.2 M. The pH value of each sample was adjusted by step-wise addition of aqueous solutions of 0.2 M NaOH (for pH values of 1.8, 2.8, 4.3 and 6.0) or 1.0 M H_2SO_4 (for pH 0.0). The preference of H_2SO_4 over HCl stems from the fact that SO_4^{2-} is a counter ion and it will not compete with DMSO which has much higher affinity to coordinate to the iridium metal centre. However, adding HCl as acid increases the amount of Cl^- ion which can stabilize the initial iridium DMSO complex [IMesIr(Cl)$(DMSO)_2$]. In each instance, the pH values were estimated by using a pH meter equipped with a Hamilton SpinTrode probe (www.hamiltoncompany.com), suitable for pH measurements inside NMR tubes. After that, water was evaporated from each sample and the resulting solid was used for the NMR experiments. Additional pH readings were recorded inside the NMR tubes using the aforementioned pH meter.

S1.B Synthesis of 2-(ethoxy-d_5)-2-oxoacetic-$^{13}C_2$ acid (**4**):



To oxalic acid-$^{13}C_2$ dihydrate (30 mg, 0.23 mmol) in anhydrous $CHCl_3$ (0.5 mL) was added ethanol-d_6 (40 µL, 0.69 mmol, 3.0 equiv.) and conc. H_2SO_4 (27 µL, 0.506 mmol, 2.2 equiv.), and the reaction heated under gentle reflux for 2 h. The reaction mixture was purified directly by silica gel column chromatography eluting with Et_2O:pentane (1:4) to give bis(ethyl-d_5) oxalate-$^{13}C_2$ (**S1**) as a colourless oil (34.8 mg, 0.22 mmol, 96 %).

To bis(ethyl-d_5) oxalate-$^{13}C_2$ (**S1**) (28 mg, 0.177 mmol) and $KHCO_3$ (17.7 mg, 0.177 mmol, 1.0 equiv.) was added H_2O (1 mL) and the reaction heated at 60 °C for 2 h. The reaction was allowed to cool to rt, acidified to pH 3-4 with 3 N HCl, and extracted with EtOAc (3 x 6 mL). The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. After filtration, the solution was carefully concentrated to give 18.2 mg (0.145 mmol, 82%) of 2-(ethoxy-d_5)-2-oxoacetic-$^{13}C_2$ acid (**4**) as a colorless solid. ^{13}C NMR (101 MHz; $CDCl_3$): 159.42, 158.47, 158.32, 157.37 (ABq, 2C, J_{AB} = 95.3 Hz). ^{2}H NMR (76.8 MHz; $CHCl_3$): 4.36 (s, 2D), 1.35 (s, 3D). LC-MS (ESI) *m/z* calcd. for $C_2{}^{13}C_2D_5O_4$: 124.06 (M-H) ; found: 124.06.

S1.2 Sample preparation

All samples have been prepared in standard 5 mm NMR tubes equipped with Young taps.

Sample 1 with sodium oxalate-$^{13}C_2$ (2). A solution of 5 mM of [IrCl(COD)(IMes)], 20 mM of DMSO-d_6 were prepared in CD_3OD (0.5 mL). 40 mM of **2** in 0.1 mL of D_2O was then added to the resulting solution. The sample was degassed by three cycles at high vacuum line. Subsequently, 50% parahydrogen (pH_2) at a pressure of ca. 4 bar was added. Sample was then shaken for 10 s in the optimum magnetic field inside a microtesla (μT) field before being rapidly transported into the magnet for subsequent interrogation by NMR spectroscopy.

Sample 2 with mono-protonated oxalate (3). A solution of 5 mM of [IrCl(COD)(IMes)], 20 mM of DMSO-d_6 and 40 mM of **3** were prepared in CD_3OD (0.6 mL). The sample was degassed by three cycles at high vacuum line. Subsequently, 50% parahydrogen (pH_2) at a pressure of ca. 4 bar was added. Sample was then shaken for 10 s in the optimum magnetic field of hyperpolarized nuclei (0.4 μT field for ^{13}C) before being rapidly transported into the magnet for subsequent interrogation by NMR spectroscopy.

Sample 3 with mono-esterificated oxalate (4). A solution of 5 mM of [IrCl(COD)(IMes)], 20 mM of DMSO-d_6 and 40 mM of **4** were prepared in CD_3OD (0.6 mL). The sample was degassed by three cycles at high vacuum line. Subsequently, 50% parahydrogen (pH_2) at a pressure of ca. 4 bar was added. Sample was then shaken for 10 s in the optimum magnetic field of hyperpolarized nuclei (0.4 μT field for ^{13}C) before being rapidly transported into the magnet for subsequent interrogation by NMR spectroscopy.

S2. Enhancement factor estimation

The enhancement factors are calculated by simply taking the ratios of the integrals of hyperpolarized signals to their respective thermal equilibrium signal of non-hyperpolarized solutions. In the cases of labelled compounds, no signal averaging was needed to get sufficient thermal equilibrium signal. Whereas, in the cases of unlabeled substrates, signal averaging was essential, and the following formula was used to estimate the enhancement factors.

$$\varepsilon = \left(\frac{S_{Hyp}}{S_{Th}}\right) * N_{Th}$$

where S_{Hyp} and S_{Th} represent signal amplitudes of the hyperpolarized and thermal equilibrium sample respectively. N_{Th} signifies the number of scans of the thermal equilibrium samples.

S3. ^{1}H NMR characterization

All NMR experiments are carried out in a 400 MHz Bruker Avance III spectrometer equipped with a BBO probe at an ambient temperature of 298 K. Figure S1 – S3 show the ^{1}H NMR spectra of samples 1 – 3 under SABRE and thermal equilibrium conditions. The -OH of oxalate is acidic in nature, labile and fast exchanges with the deuterium of the solvent MeOD. Consequently, no observable ^{1}H enhancement is seen from the protonated oxalate species (free or bound).

- S3.1. Sample 1 with sodium oxalate-$^{13}C_2$ (**2**).

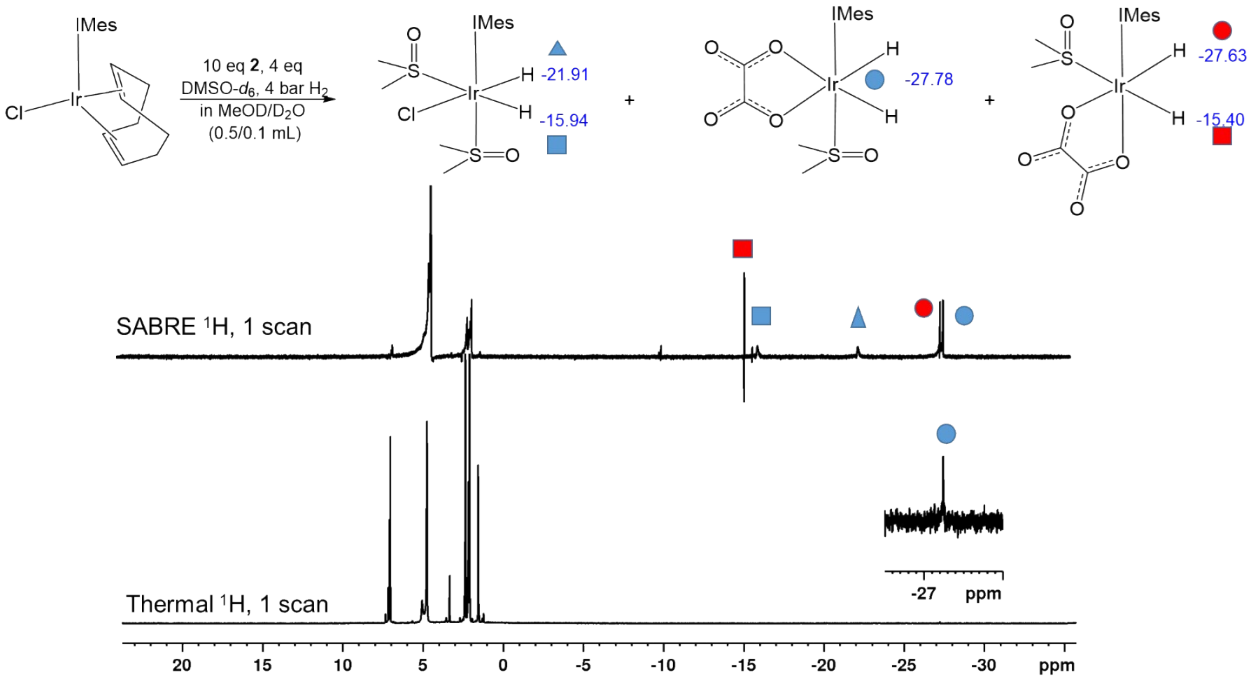


Figure S1. Plausible SABRE active species with ^{1}H hydride ligands chemical shifts in sample 1.

- S3.2. Sample 2 with mono-protonated oxalate (**3**).

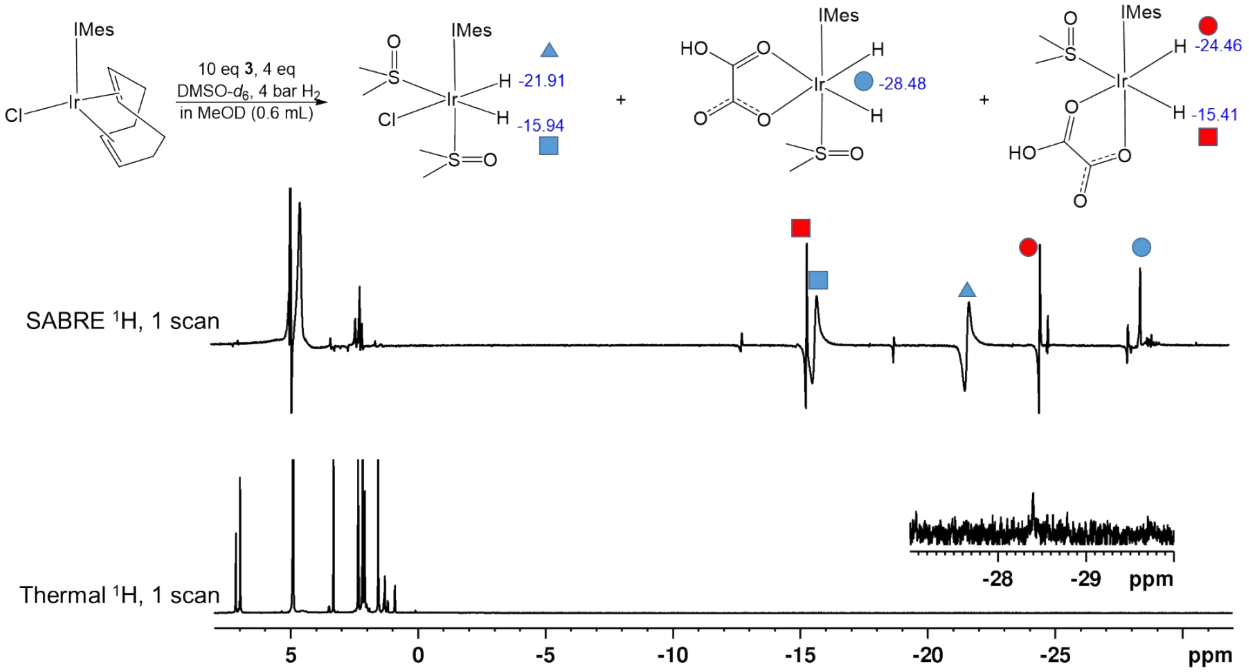


Figure S2. Plausible SABRE active species with ^{1}H hydride ligands chemical shifts in sample 2.

- S3.2. Sample 3 with mono-esterificated oxalate (**4**).

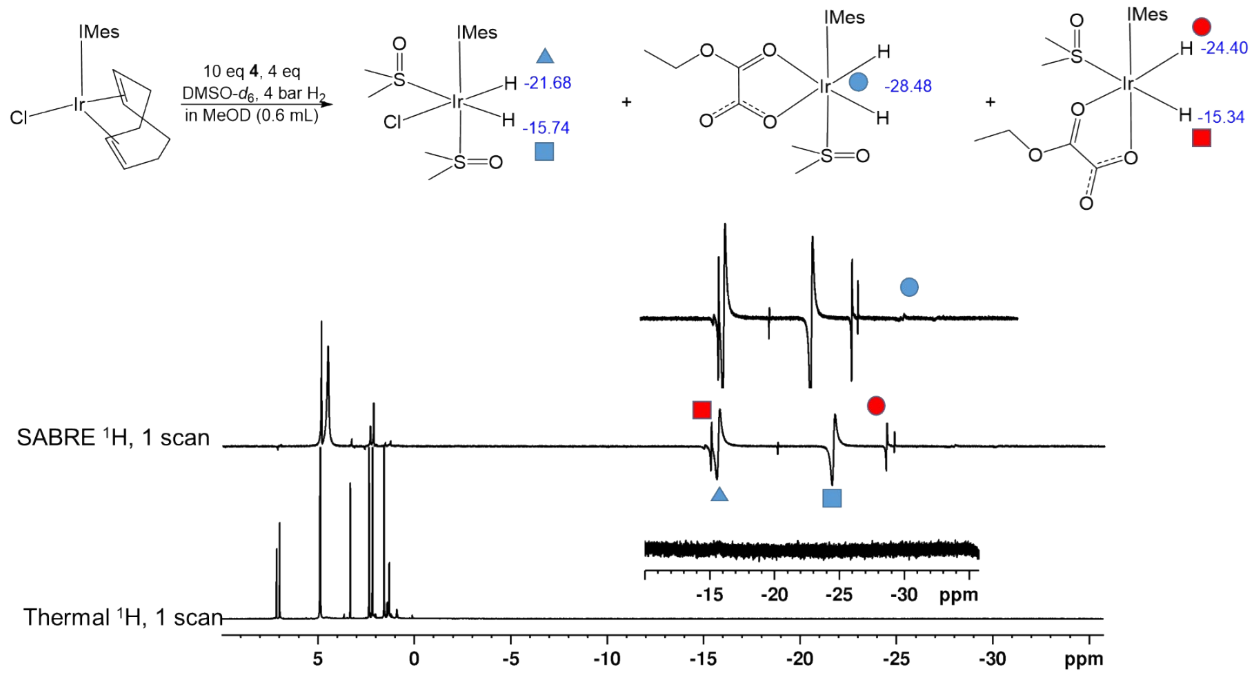


Figure S3. Plausible SABRE active species with ^{1}H hydride ligands chemical shifts in sample 3.

S4. NMR spectra

- S4.1 ^{13}C NMR spectra

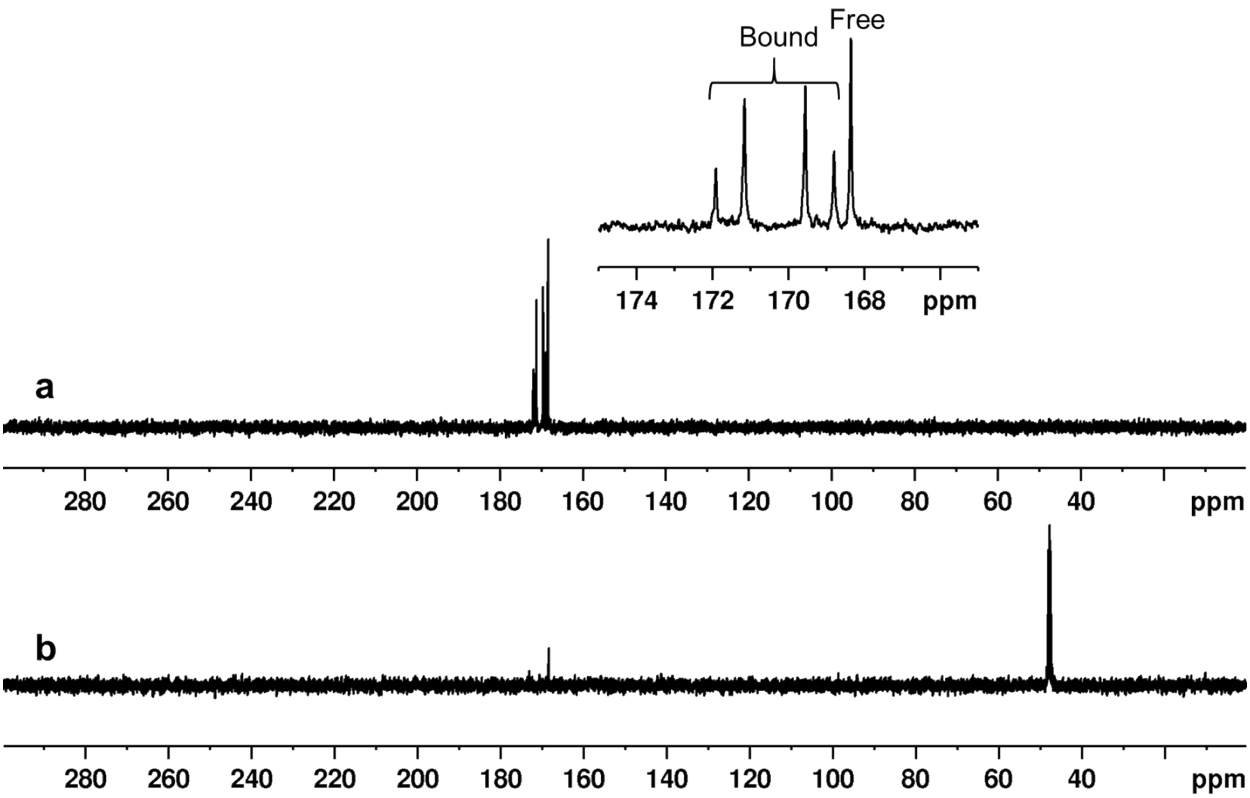


Figure S4. (a) SABRE-hyperpolarized ^{13}C NMR spectra of sample 1. ^{13}C of free/bound sodium oxalate-$^{13}C_2$ enhanced with the use of 50% parahydrogen. The bound oxalate is enhanced significantly compared to the free because of the strong bonding affinity of dianionic oxalate to the iridium that makes the exchange with free very slow. (b) A single scan ^{13}C signal of thermal equilibrium sample.

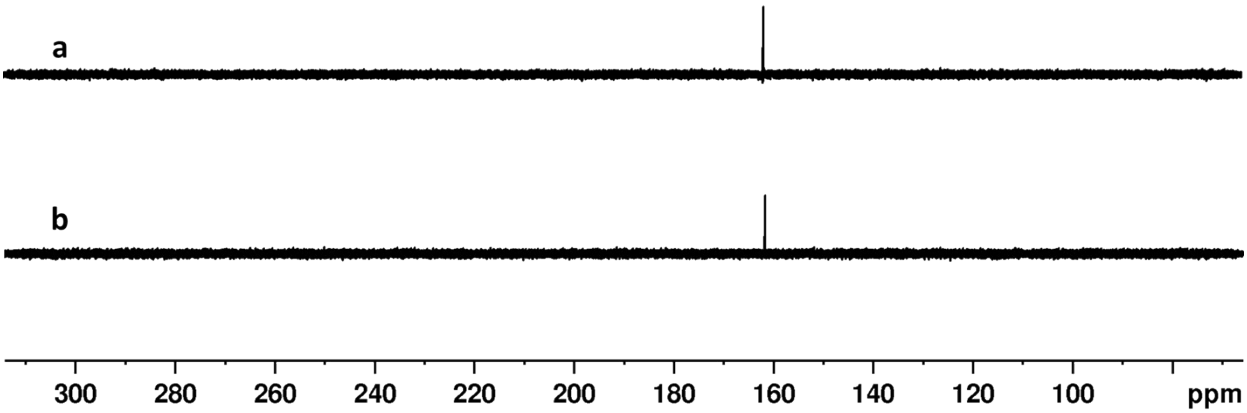


Figure S5. (a) SABRE-hyperpolarized ^{13}C NMR spectra of sample 2. (b) 2000 scans ^{13}C thermal equilibrium spectra of the non-hyperpolarized solution (vertically scaled down to have similar baseline to the SABRE spectrum).

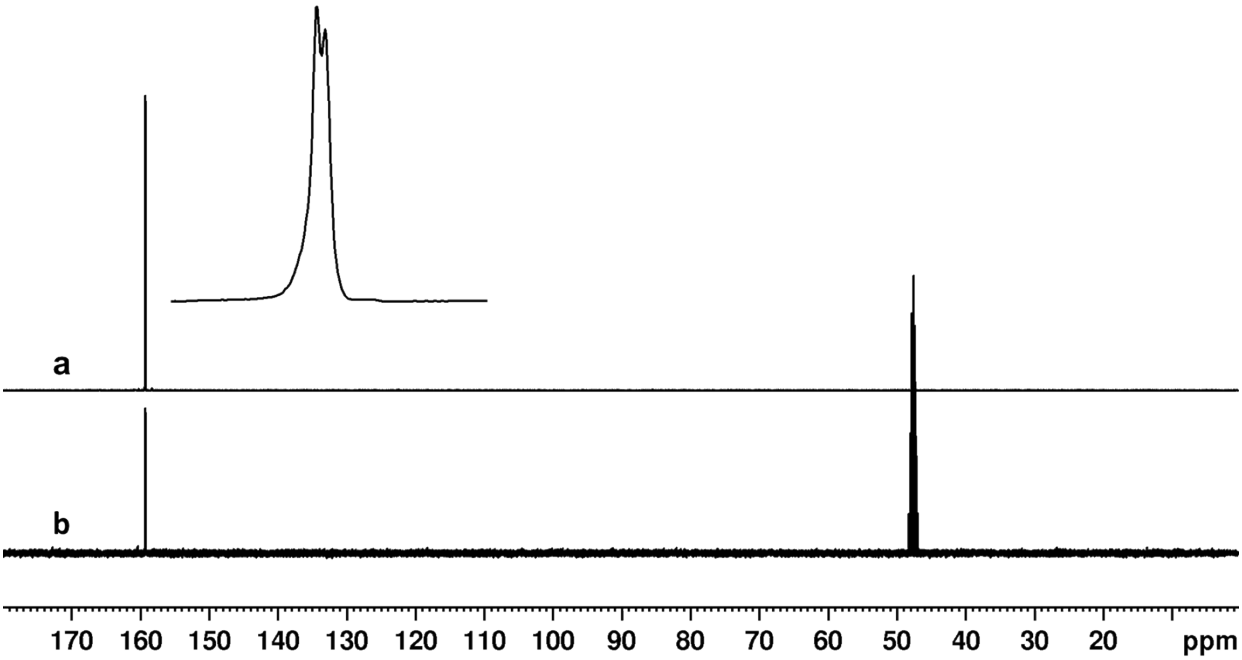


Figure S6. (a) SABRE-hyperpolarized ^{13}C NMR spectra of sample 3. ^{13}C of free Ethyl-d_5 oxalate-$^{13}C_2$ **(4)** enhanced with the use of 50% parahydrogen. (b) A single scan ^{13}C Boltzmann equilibrium conditions vertically expended by 32 times.

S5. Hyperpolarized Singlet Sates

Generating hyperpolarized spin states in singlet order can offer significant advantages toward further applications by preserving hyperpolarized spin order for an extended duration of time. There are several ways that one could prepare singlet states in conjunction with the hyperpolarization but effective symmetry breaking mechanism is the essence of the technique. In this work we employed two different approaches. We synthesized an oxalate ester in order to break the symmetry between the carboxylate carbons. After carbon-13 enrichment, the molecule (2-(ethoxy-d_5)-2-oxoacetic-$^{13}C_2$ acid (**4**)) now represents a strongly coupled spin-1/2 pair with a chemical shift difference between them as 0.16 ppm (~16Hz at 9.4 T) and a *J*-coupling constant of 96 Hz.

(i) First method looks at the indirect way of hyper-singlet population.[1,2] Briefly, the method follows by acquiring magnetization build up to the targets by SABRE and then applying suitable rf pulses to convert it into singlet order. After a period of storage, the singlet needs to be converted back to the magnetization for detection. The M2S-S2M pulse sequence is used to manipulate the spin orders.[3] The singlet order, however, found to be disappointing with singlet lifetime much lower than the T_1. Figure S7 portrays the results of **4** highlighting SABRE-SHEATH optimization, hyperpolarized magnetization decay, and hyper-singlet order decay. The T_1 was measured to be 13.97 ± 0.19 s whilst the T_S (singlet lifetime) as 1.74 ± 0.27 s at 9.4 T.

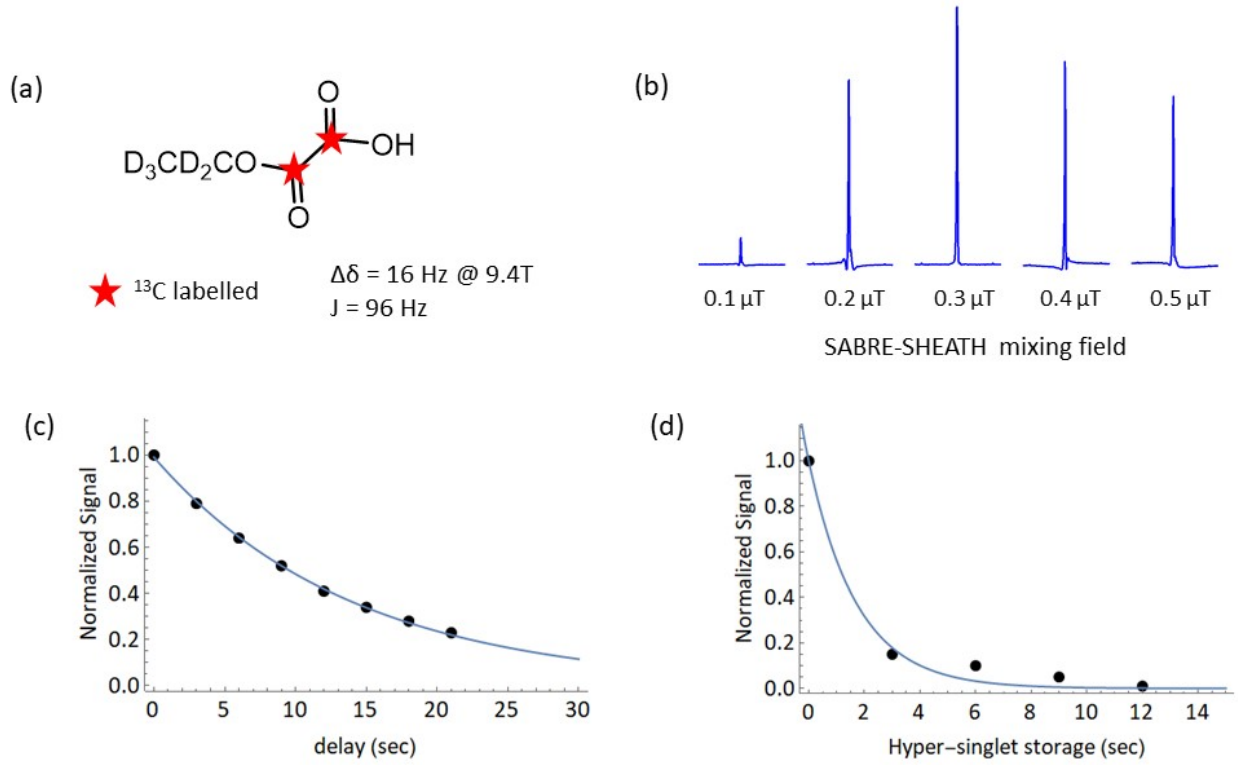


Fig. S7 (a) Chemical structure of 2-(ethoxy-d_5)-2-oxoacetic-$^{13}C_2$ acid (**4**) (b) SABRE-SHEATH hyperpolarized spectra of 4 for a range of magnetic mixing fields with maximum enhancement achieving up to 80-folds; (c) Hyperpolarized magnetization decay at 9.4 T with a time constant of 13.97 ± 0.19 sec; (d) Hyperpolarized singlet order decay at 9.4 T with a time constant of 1.74 ± 0.27 sec.

(ii) By the second method we tried to access the hyperpolarized order directly in the singlet form similar to the cases of diazirin and pyruvate.[4,5] One primary criterion to achieve such direct singlet to singlet transfer is to have matching hydride *J*-coupling values with the target pair's *J*-value. In this case, we have a large mismatch (J_{CC} = 96 Hz whereas J_{HH} ≈ -10 Hz) in terms of the absolute *J* values. Despite that we observe the spectral hint of singlet transfer, albeit with a very poor efficiency. Spindynamica simulations confirms the pattern of a hyper-singlet order of the system.[6] Due to the very low SNR, it was not possible to measure any meaningful singlet lifetime by this method. Figure S8 shows the simulated and experimentally achieved NMR spectra of **4** under such condition.

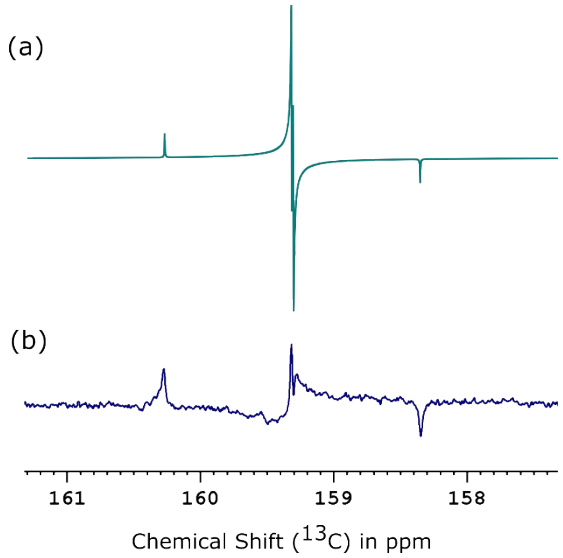


Fig. S8: (a) *Spindynamica* simulations[6] of the resulting ^{13}C NMR signal following a direct singlet-to-singlet transfer and (b) experimentally achieved NMR spectra of **4** by following the 'direct' transfer protocol.

S6. References

(1) Roy, S. S.; Norcott, P.; Rayner, P. J.; Green, G. G. R.; Duckett, S. B. A Simple Route to Strong Carbon-13 NMR Signals Detectable for Several Minutes. *Chem.- Eur. J.* **2017,** *23* (44), 10496.

(2) Roy, S. S.; Rayner, P. J.; Norcott, P.; Green, G. G. R.; Duckett, S. B. Long-lived states to sustain SABRE hyperpolarised magnetisation. *Phys. Chem. Chem. Phys.* **2016,** *18* (36), 24905.

(3) Tayler, M. C. D.; Levitt, M. H. Singlet nuclear magnetic resonance of nearly-equivalent spins. *Phys. Chem. Chem. Phys.* **2011,** *13* (13), 5556.

(4) Theis, T.; Ortiz, G. X.; Logan, A. W.; Claytor, K. E.; Feng, Y.; Huhn, W. P.; Blum, V.; Malcolmson, S. J.; Chekmenev, E. Y.; Wang, Q.et al. Direct and cost-efficient hyperpolarization of long-lived nuclear spin states on universal 15N2-diazirine molecular tags. *Sci. Adv.* **2016,** *2* (3), e1501438.

(5) Iali, W.; Roy, S. S.; Tickner, B. J.; Ahwal, F.; Kennerley, A. J.; Duckett, S. B. Hyperpolarising Pyruvate through Signal Amplification by Reversible Exchange (SABRE). *Angew. Chem. Int. Ed.* **2019,** *58* (30), 10271.

(6) Bengs, C.; Levitt, M. H. SpinDynamica: Symbolic and numerical magnetic resonance in a Mathematica environment. *Magnetic Resonance in Chemistry* **2018,** *56* (6), 374.