Supplemental Information for:

Dynamic Nuclear Polarization with a Water-soluble Rigid Biradical

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All chemicals for synthesis were purchased from Aldrich and used as received unless stated otherwise. Tetraacetyl pentaerythrithiol was made by established procedures.[1] Dichloromethane (DCM) was dried on a solvent purification system (Innovative Technologies).
All spin-labeled compounds for spectroscopy were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA) and were used without further purification.

1: Following a modified version of a literature procedure,[2] tetrahydro-4*H*-pyran-4-one (9.9767 g; 99.77 mmol) was added to a round bottom flask containing 1,2,2,6,6-pentamethylpiperidin-4-one 5.6846 g; 33.64 mmol). A stir bar was added along with 50 mL DMSO followed by ammonium chloride (10.6718 g; 201.36 mmol). The flask was equipped with a reflux condenser, and the mixture was heated to 60 ºC for 6 h under ambient atmosphere. The solution was cooled, and 20 mL water was added. The solution was taken to pH 5 (determined by pH paper) by the addition of 1 M HCl solution. The organics were extracted 3x ethyl ether (~20 mL per wash), and the aqueous layer was taken to pH 9 (pH paper) with saturated sodium bicarbonate solution. The organics were extracted with 4x ethyl acetate (~20 mL per wash), and the combined ethyl acetate fractions were washed with brine, dried with $MgSO_4$, filtered and solvent removed under reduced pressure. The resulting light orange oil is treated with methanol (~5 mL), which produces an off white precipitate that was isolated by suction filtration. Yield: 1.864 g; 23%. Characterization matches the literature.[2]

2: To a round bottom flask equipped with a stir bar was added **1** (1.180 g; 4.94 mmol) and tetraacetyl pentaerythrithiol (1.0 g; 2.71 mmol). Concentrated HCl solution (60 mL) was added in a single aliquot, a reflux condenser attached, and the mixture was brought to reflux while stirring. After 45 min, a second portion of tetraacetyl pentaerythrithiol (0.7070 g; 1.92 mmol) was added in a single addition. The reaction was refluxed for 2.25 h more. The solution was taken to pH 8-9 (pH paper) by the addition of saturated sodium bicarbonate solution. The aqueous layer was extracted with chloroform (5x ~50 mL), dried with $MgSO_4$, filtered and solvent removed under reduced pressure. The resulting oil was treated with acetone, which produces pure **2**, a light yellow powder that was isolated by suction filtration. Yield: 1.11 g; 70%. ESI-HRMS: found: 643.2714; theory: 643.2726. ^{1}H-NMR (δ, $CDCl_3$): 3.88 (m, 8H); 3.64 (m, 8H); 3.00 (s, 8H); 2.06 (s, 8H); 1.75 (m, 16H). ^{13}C-NMR (δ, $CDCl_3$): 64.5; 50.9; 49.3; 47.6; 42.1; 35.8; 24.6.

3: A round bottom flask was loaded with a stir bar, **2** (0.2025 g; 0.32 mmol), methanol (1.0 mL) and water (1.4 mL). The mixture was cooled to 0ºC, and Oxone® (47% $KHSO_5$; 2.6275 g; 8.11 mmol) was added in a single portion. The slurry was stirred for 6 h at room temperature. Sodium bicarbonate (0.5383 g; 6.41 mmol), water (1 mL), methanol (4 mL) and acetone (1 mL) were added. The pH of the solution was maintained at pH 8-9 for the remainder of the reaction by the addition of saturated sodium bicarbonate solution. After 50 min, the pink-orange mixture was diluted with 10 mL water and extracted with DCM (3x ~10 mL) and chloroform (3x ~10 mL). The combined organics were dried over $MgSO_4$, filtered, and removed of volatiles under reduced pressure. Yield: 0.176 g; 70%. X-ray crystals were grown by dissolving material (~20 mg) in acetonitrile/benzene 1:1 with one drop DMSO and evaporating at room temperature over the course of 4 days. ESI-HRMS (negative mode): found: 799.1925; theory for $[C_{31}H_{48}N_2O_{14}S_4{}^{2\bullet}\text{-}H]$: 799.1916. Reduction of **3** with excess phenyl hydrazine allowed for NMR analysis: ^{1}H-NMR (δ, $CDCl_3$): 3.74 (s, 8H); 3.42 (d, 8H); 3.17 (t, 8H); 2.24 (s, 8H); 1.81 (t, 8H); 1.23 (d, 8H). ^{13}C-NMR (δ, $CDCl_3$): 64.3; 59.0; 55.3; 39.6; 32.4; 31.7; 22.7.

bTbtk-py: A flame dried round bottom flask loaded with **2** (0.8967 g; 1.395 mmol) and a stir bar was fitted with a rubber septum and purged with Ar for 15 min. Dry DCM (120 mL) was added via a syringe. In a single aliquot, *m*-CPBA (assayed at 66%; 1.5546 g; 5.93 mmol) was added by briefly removing the septum. The solution was stirred under Ar atmosphere for 18 h. The orange DCM solution was extracted with saturated sodium bicarbonate (2x ~40 mL) and 0.1 M HCl (2x ~40 mL). The DCM layer was stirred vigorously under 60 mL of 0.5 M NaOH solution containing $K_3[Fe(CN)_6]$ (0.4674 g; 1.42 mmol) for 10 min. The organics were retained and washed with brine, dried over Na_2SO_4, and volatiles removed under reduced pressure. The residue was taken up in 6 mL DCM and treated with hexanes, causing an orange precipitate that was collected by decanting. Solid was removed of volatiles under high vacuum; yield based on 8 oxygen atoms (4 in THP moieties, 4 from oxidation): 0.2464 g; 25%. bTbtk-py was confirmed to be a biradical by measuring the electron-electron coupling constant, see DEER experiment. Elemental analysis: found: C, 52.91; H, 6.95; N, 3.67; S, 17.59; expected for $C_{31}H_{48}N_2O_8S_4{}^{2\bullet}$: C, 52.81; H, 6.86; N, 3.97; S, 18.19. HRMS: expected for $C_{31}H_{48}N_2O_8S_4{}^{2\bullet}$: 704.2296; found: see Figure S1. Biradicals are known to exhibit a $[M+2H]^+$ peak in positive mode ESI.[3] Reduction of biradical with excess phenyl hydrazine allowed for NMR analysis: ^{1}H-NMR (δ, DMSO): 4.00 (s, 8H); 3.75 (d, 8H); 3.44 (t, 8H); 2.51 (s, 8H); 2.06 (t, 8H); 1.54 (d, 8H). ^{13}C-NMR (δ, $CDCl_3$): 64.3; 57.8; 50.9; 40.8; 36.8; 32.4; 28.9.

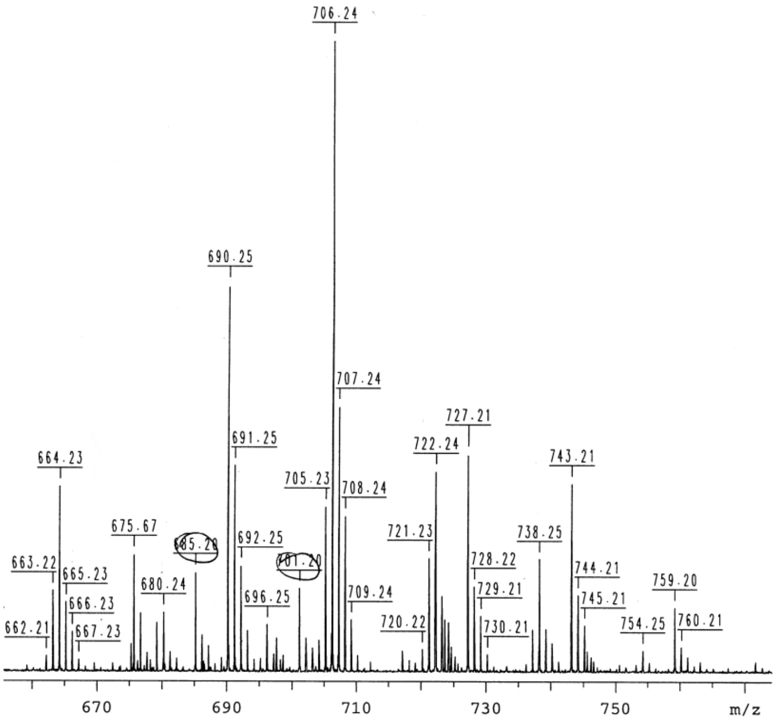

Figure S1. ESI of biradical mixture bTbtk-py. The signals at ~664 m/z are the lowest molecular weight products, accounted for by the various Na+ adducts of the un-oxidized compound: theoretical mass for $C_{31}H_{49}N_2NaO_4S_4^+$: 664.25 m/z.

DNP experiments were performed using a custom-built instrument, consisting of a 140 GHz gyrotron[4] and a 212 MHz (^{1}H frequency) MAS-NMR spectrometer (courtesy of D. Ruben). The spectrometer utilizes a custom-built cryogenic MAS-DNP probe with triple-channel (^{1}H, ^{13}C, ^{15}N) rf resonant circuit and 4 mm rotor diameter Revolution NMR stator. Microwaves are guided to the sample via circular overmoded waveguides whose inner volume has been corrugated to reduce mode conversion from HE_{11} mode into higher order modes and to reduce ohmic losses. The sample is contained within sapphire rotors (Inasco, Inc) with 0.7 mm wall thickness within a volume of ~4 mm height. Samples were prepared by dissolving biradicals in a 60:30:10 (v/v) d_8-glycerol/D_2O/H_2O mixture with a concentration of 10 mM. 1 M ^{13}C-urea was added to provide ^{13}C for NMR detection.

Experiments were performed under MAS with a spinning frequency of $\omega_r/2\pi = 4$ kHz at a temperature of ~82 K, measured directly outside the MAS stator. The field dependent DNP enhancement profile was recorded by detecting the ^{1}H FID intensity during a Bloch decay while sweeping the external magnetic field value. At each field point the spectrometer frequency was re-set, and the probe circuit was retuned. The enhancement values were subsequently scaled to the maximum ε obtained via cross-polarization to ^{13}C because a significant non-enhanceable ^{1}H background signal caused by the stator material, spacers, etc. impedes any direct measurement of ε by comparison of ^{1}H signal intensities recorded with and without microwave irradiation. All spectra were recorded using a 16-pulse presaturation train (phase alternating in $+x$ and $+y$) of 108° flip angle and $\gamma B_1 = 50$ kHz separated by 5 ms each on both ^{1}H and ^{13}C channels. ^{1}H polarization was allowed to build up during a variable recovery time and polarization was then read out by a Bloch decay or cross-polarization to ^{13}C. Build-up curves have been obtained at the field of maximum ε by varying the recovery time between presaturation and detection, and enhancement values were obtained after mono-exponentially fitting by dividing the pre-exponential factor of the on-signal by that of the off-signal. For the microwave power dependence study, only one on- and one off-signal was recorded at each power level with a fixed recovery time corresponding 1.26×T_B; enhancement factors were obtained by dividing the amplitude of on- and off-signals.

DEER experiments were performed on a commercial Bruker ELEXSYS E580 spectrometer at X-band frequency at temperature of 80 K. A typical 4-pulse dead-time free DEER sequence was applied.[5] The field positions representing the pump and probe frequencies are shown in Figure S2 for bTbtk-py and bTbk. Biradicals were dissolved in d_8-toluene with concentrations of 0.5 mM (bTbk) and 0.2 mM (bTbtk-py). bTbtk-py demonstrated poor solubility in toluene. However, solubility was sufficient to achieve a homogeneous distribution of radicals in solution without causing fast decay of echo modulation due to intermolecular couplings.

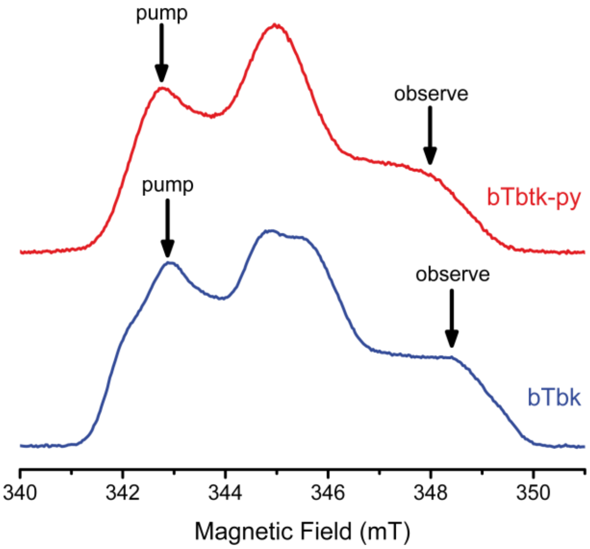

Figure S2. Field-swept echo detected X-Band EPR spectra of bTbtk-py and bTbk in d_8-toluene recorded at 80 K with pump and probe (observe) positions for DEER experiments.

With expected dipole coupling constants on the order of 30 MHz and higher, care has to be taken that the excitation bandwidth of EPR pulses exceeds the apparent coupling at least two-fold in order to create coherence on the probe spin regardless of the m_S state of the dipolar coupled electron spin and also to invert the magnetization of the pump spin regardless of the m_S state of the probe spin. However, this potentially leads to experimental complications since (i) excitation bandwidth of pump and probe frequencies may not overlap (otherwise causing reduced modulation depth and interference due to ESEEM effects) and (ii) significant instrumental requirements arise due to the very short and strong microwave pulses that are required. (i) was overcome by separating pump and probe frequencies as far as possible, preferring lower S/N and lower modulation depth in favor of less excitation overlap. In order to achieve high γB_1 (and thus short pulse lengths), the maximum overcoupling of the TE_{011} resonator normally applied in pulsed EPR experiments was reduced to the point where resonator ringing started to be visible after the detector protection pulse, yielding pulse lengths for π/2 and π pulses of 6 ns and 12 ns, respectively. It should be noted that significant ringing not only compromises dead time but also deviates the pulse shape, thus leading to longer effective pulse lengths and in turn reduced excitation bandwidth. Pulse delays were optimized for highest echo intensity due to ESEEM for each radical, yielding observe channel pulse sequences ($\pi/2-\tau_1-\pi-\tau_2-\pi$) of 6−374−12−1230−12 for bTbk and 6−370−12−1268−12 for bTbtk-py (in ns). The pump pulse was iterated by 2 ns. A shot repetition time of 1 μs was used with 1000 shots per point. DEER curves were averaged over 12 traces in case of bTbk and 16 traces for bTbtk-py.

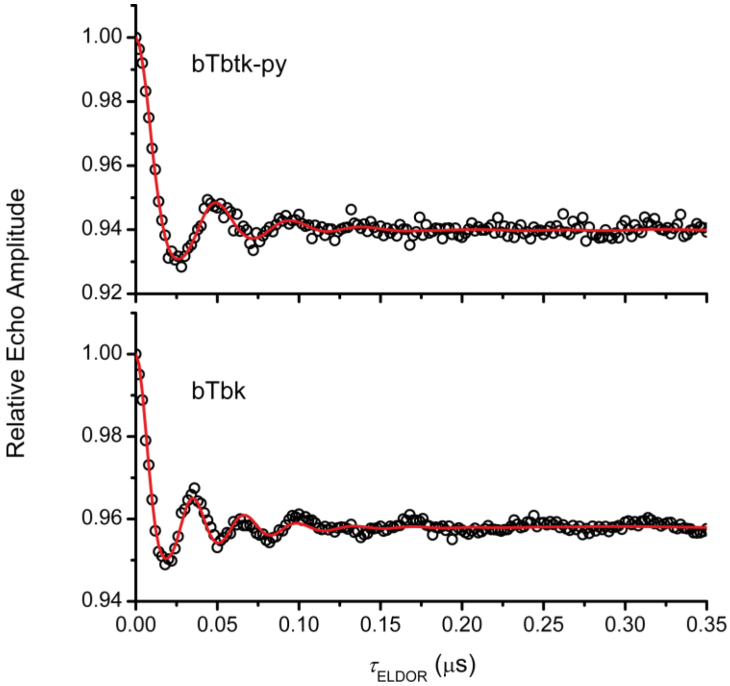

Figure S3. DEER traces and fits using Tikhonov regularization.

DEER traces after background subtraction are shown in Fig. S3. The data for bTbk shows some interference due to ^{1}H ESEEM. This interference, however, did not caused any further problems in data analysis. Data was analyzed using DEERAnalysis 2006.[6] A fit using Tikhonov regularization yielded the distance distribution shown in Figure S4.

The distance distribution is dominated by a single peak for each radical with several much smaller peaks between ~8 and 25 A. These small peaks are considered artifacts due to small S/N and non-ideal DEER conditions due to the large couplings. The mean intermolecular electron-electron distances are 11.8 Å for bTbk and 13.2 Å for bTbtk-py with errors of ~0.1 Å.

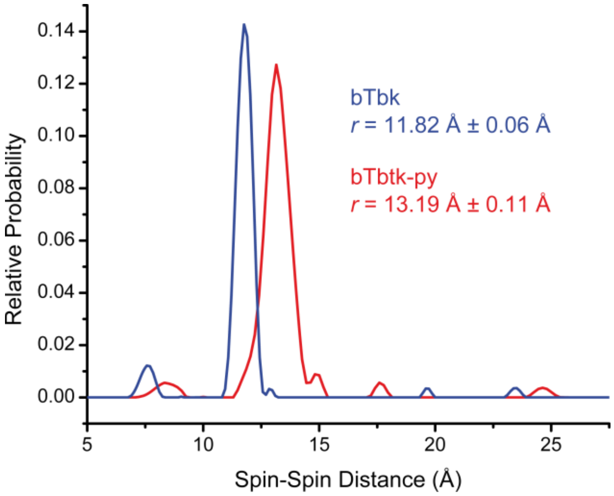

Figure S4. Intramolecular electron-electron distances determined by DEER experiments.

Supplemental Information References:

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