# *Water-Soluble Narrow-Line Radicals for Dynamic Nuclear Polarization*

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## SUPPORTING INFORMATION

## Materials

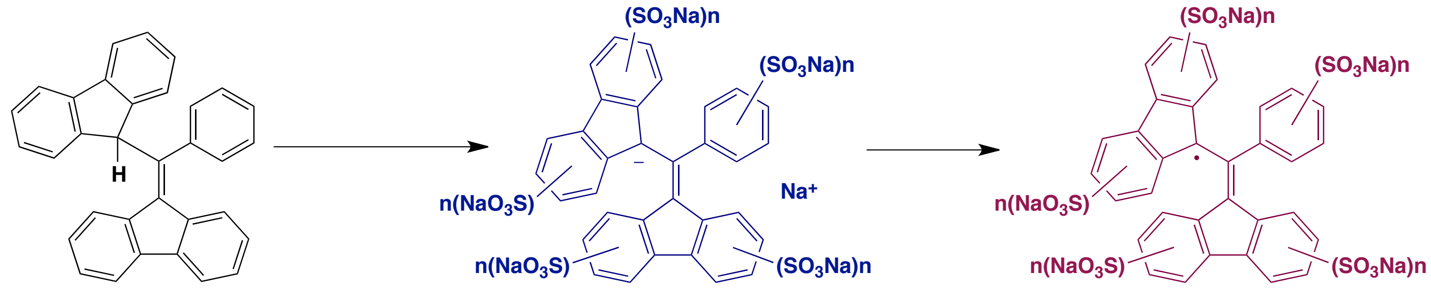
All chemicals, reagents, and solvents were used as received from commercial sources (Aldrich, Acros, Fluka) without further purification. Ion exchange resins were washed with MeOH and deionized water prior to use.

## Instrumentation

Proton nuclear magnetic resonance ($^1H$ NMR) spectra and carbon nuclear magnetic resonance ($^{13}C$ NMR) spectra were recorded on a Bruker Avance-400 (400 MHz) NMR spectrometer. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent. Chemical shifts for carbon are reported in parts per million downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent. The mass spectrometry data were obtained using Waters LCT Premier TOF equipped with an ESI probe. IR spectra were recorded using Nicolet 6700 FT-IR (Thermo Scientific) equipped with ATR probe (Ge window). Uv/Vis spectra were collected on Agilent 8453 spectrophotometer.

## Synthesis

**BDPAH (1)** was prepared according to the published procedures.[1]



**1**
**BDPAH**

**2**
**SA-BDPA$^-$**

**3**
**SA-BDPA**

**SA-BDPA (3):** 100 mg (0.239 mmol, 1 equiv) of BDPAH (**1**) and 1 mL fuming sulfuric acid were heated at 85 °C while stirring for 5 min. The resulting brown solution was carefully diluted with 20 mL of deionized water. The resulting salmon-colored solution was stirred at room temperature for 16 h, and then filtered to remove any insoluble material. The filtrate was treated with 16 mL of 10% NaOH and concentrated. The resulting deep blue powder was extracted with 125 mL of DMSO. The resulting sodium salt SA-BDPA$^-$ (**2**) ($\lambda_{max}$= 628 nm) was precipitated from the DMSO solution with 75 mL of benzene, and washed with DCM. The dark blue powder was dissolved in 3 mL of water and 102 mg (0.598 mmol, 2.5 equiv) of $AgNO_3$ in 2 mL of water was added while stirring. The resulting red mixture stirred at room temperature for 3 h and then was filtered and concentrated to give 200 mg red powder. This was chromatographed on C18 reverse phase column eluting with 5% $H_2O$-$CH_3CN$ to give 189 mg (85%) of deep red glassy solid. EPR (9 GHz, RT, water) g=2.0027. UV-Vis (water): $\lambda_{max}$= 508(13000), 881(900) nm (ε). This material (a mixture of sulfonation products $BDPA(SO_3Na)_{4-7}$ ) was used in DNP experiments.

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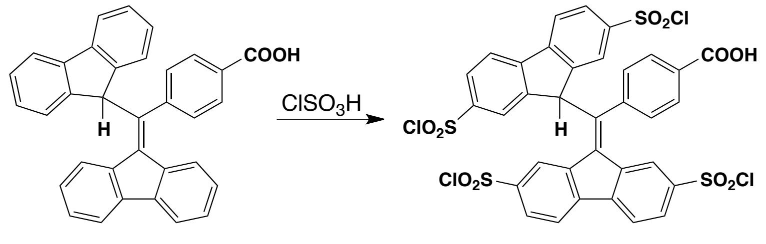
[1] Plater, M.; Kemp, S.; Lattmann, E. *J. Chem. Soc. Perkin Trans. 1* **2000**, 971–979.

Alternatively, 0.53 g (1.27 mmol, 1 equiv) of BDPAH (**1**) and 1 mL fuming sulfuric acid were heated at 85 °C while stirring for 6 h. The resulting black solution was carefully diluted with 10 mL of deionized water, and the pH of resulting salmon-colored solution was brought to 9 by addition of aqueous $Ba(OH)_2$. $BaSO_4$ was filtered out and the blue filtrate was treated with DOWEX HRC-W2 Na+ form. The resulting violet-blue solution was shaken with 5 g of $PbO_2$ until the color changed to deep red (ca 30 min), and then filtered onto Amberlite IRC-50 (-COOH form). The ion exchange resin was separated, and the deep red solution was adsorbed onto RP C18 silica gel in vacuo at 50 °C. This was dry-loaded onto C18 reverse phase column and eluted with 200 proof ethanol followed by 20% $H_2O$-EtOH to give 400 mg (28%) of a deep red glassy solid (BDPA$(SO_3Na)_7$). IR (ATR, Ge) 3449, 1640, 1390, 1184, 1112, and 1041 $cm^{-1}$. $ESI^-$ was obtained after ion exchange on DOWEX HRC-W2 (H+ form) ESI HRMS (*m*/*z*) $[M-2H]^{2-}$ calcd for $C_{33}H_{21}O_{21}S_7^{\bullet}$ 487.4237, found 487.4225. Further elution with 40% $H_2O$-EtOH yields additional material, which consists of a mixture of sulfonation products BDPA$(SO_3Na)_{4\text{-}7}$

Reaction time and temperature may be adjusted to increase or reduce the amount of sulfonation. The sulfonation can be carried out at r.t. over 3 days to produce completely water-soluble product mixture.

**HOOC-BDPAH (4)** was prepared according to the published procedures.[2]



**4**
**HOOC-BDPAH**

**5**

**HOOC-BDPAH$(SO_2Cl)_4$ (5):** 0.50 g (1.08 mmol, 1 equiv) of finely powdered HOOC-BDPAH (**4**) was stirred with 2.0 mL of chlorosulfonic acid in a sealed pressure-release vial at room temperature for 24 h. The resulting deep green solution was slowly poured over 50 g of ice. Ice was allowed to melt, and the mixture was filtered, washing with DI water. The resulting pale pink powder was dried at RT, 100 mTorr to give 0.68 g (74%) This was precipitated from benzene-THF to give a rosy powder. IR (ATR, Ge) 3420, 3077, 1719, 1602, 1405, 1373, 1344, 1166, 1071 and 1004 $cm^{-1}$. $^1H$ NMR (400 MHz, THF-$d_8$) δ 9.51 (s, br, 1H), 9.22 (d, J = 1.5 Hz, 1H), 8.55 (d, 8.3 Hz, 1H), 8.53 (s, 2H), 8.42 (dd, J = 8.3, 1.5 Hz, 1H), 8.39 (d, J = 8.3 Hz, 1H), 8.29 (d, J = 8.2 Hz, 2H), 8.25 (dd, J = 8.2, 1.5 Hz, 2H), 8.10 (dd, J = 8.3, 1.7 Hz, 1H), 7.81 (d, J = 8.3 Hz, 2H), 6.95 (s, 1H), 6.88 (d, J = 8.3 Hz, 2H), 6.53 (d, J = 1.7 Hz, 1H); $^{13}C$ NMR (100 MHz, THF-$d_8$) δ 166.2 (sp2, CO), 147.7, 147.1, 147.0, 145.7, 145.6, 145.4, 144.9, 144.1, 140.9, 140.5, 135.8, 132.3, 130.8, 129.0, 128.8, 128.5, 127.7, 125.7, 125.0, 124.3, 124.1, 123.9, 123.1 and 53.9 (sp3, CH); ESI (*m*/*z*): $[M-H]^-$ calcd for $C_{34}H_{17}Cl_4O_{10}S_4$, 852.846; found 852.837.

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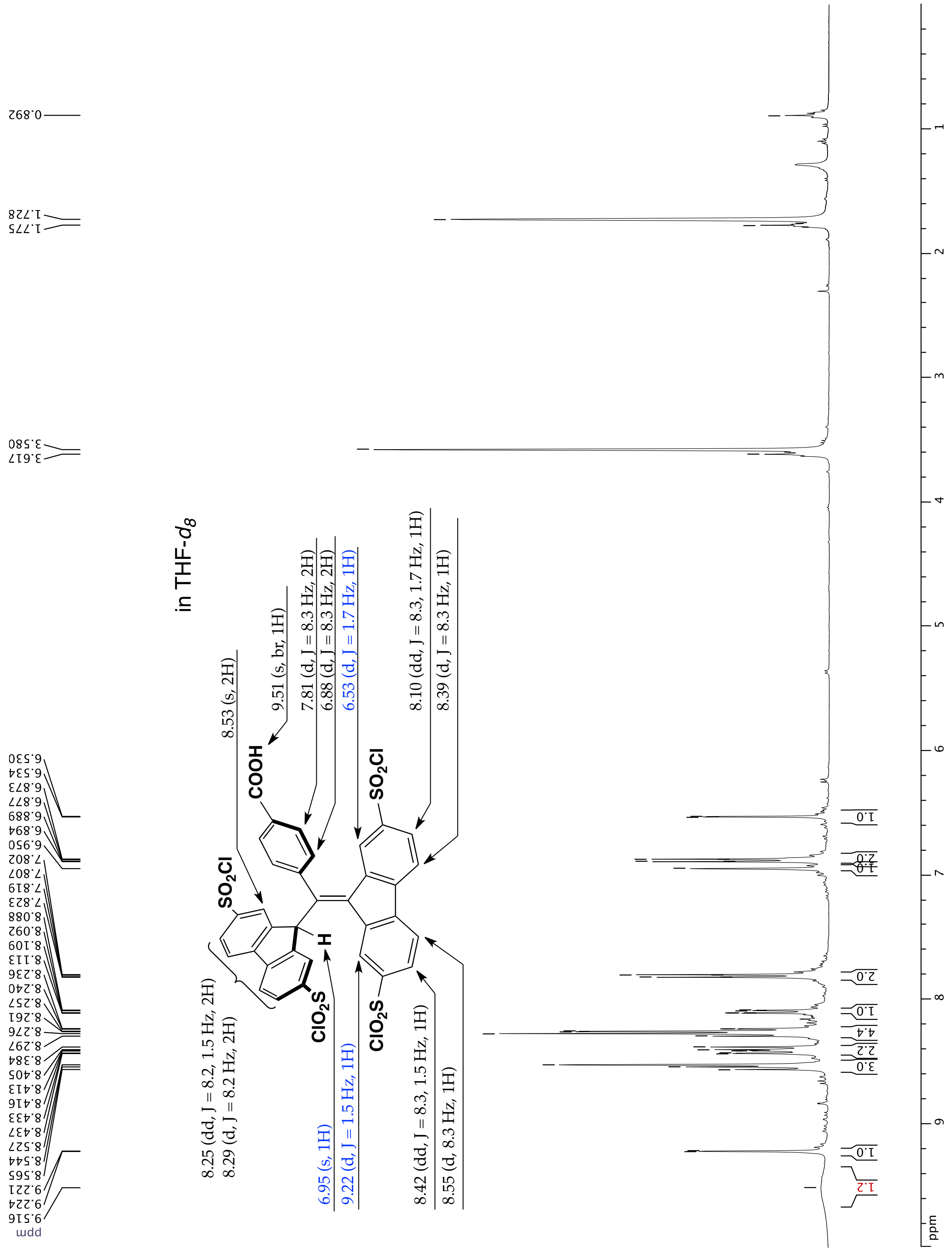
[2] Mi, Q., Ph.D. Thesis, Northwestern University, Evanston, IL, **2009**. Dane, E. L.; Maly, T.; Debelouchina, G. T.; Griffin, R. G.; Swager, T. M. *Org. Lett.* **2009**, *11*, 1871.


in THF-$d_8$
8.25 (dd, J = 8.2, 1.5 Hz, 2H)
8.29 (d, J = 8.2 Hz, 2H)
8.53 (s, 2H)
9.51 (s, br, 1H)
7.81 (d, J = 8.3 Hz, 2H)
6.88 (d, J = 8.3 Hz, 2H)
6.53 (d, J = 1.7 Hz, 1H)
6.95 (s, 1H)
9.22 (d, J = 1.5 Hz, 1H)
8.10 (dd, J = 8.3, 1.7 Hz, 1H)
8.39 (d, J = 8.3 Hz, 1H)
8.42 (dd, J = 8.3, 1.5 Hz, 1H)
8.55 (d, 8.3 Hz, 1H)
SO2Cl
ClO2S
COOH
H
SO2Cl
ClO2S
0.892
1.728
1.775
3.580
3.617
6.530
6.534
6.873
6.877
6.889
6.894
6.950
7.802
7.807
7.819
7.823
8.088
8.092
8.109
8.113
8.236
8.240
8.257
8.261
8.276
8.297
8.384
8.405
8.413
8.416
8.433
8.437
8.527
8.544
8.565
9.221
9.224
9.516
ppm
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2.0
1.0
2.0
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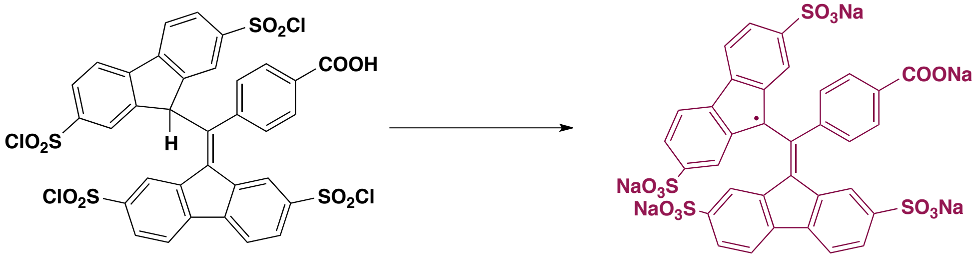
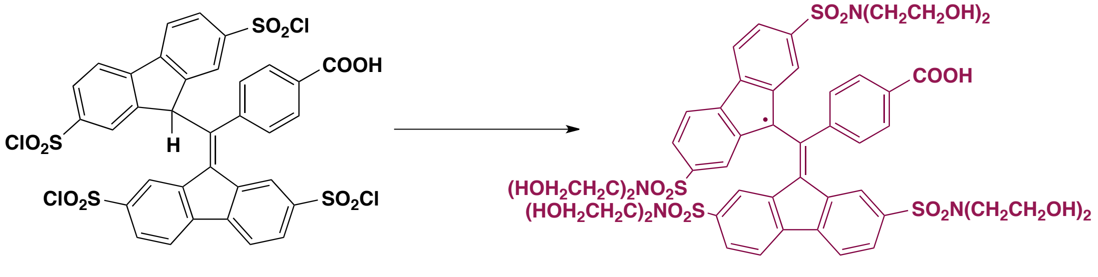
7
8
9
ppm



**5** **6a**

**CA-BDPA (6a):** 0.10 g (0.12 mmol, 1 equiv) of 5 was boiled in 20 mL of deionized water for 15 minutes until a light red homogeneous solution was obtained. NaOH was then added to pH 10, and the resulting deep blue solution (anion $\lambda_{max}$= 611 (20500) nm (ε) ) was shaken with 0.3 g of $PbO_2$ for 15 min (consumption of the anion was monitored by UV/Vis) and filtered onto Amberlite IRP-64 (COOH form) ion exchange resin. The deep red mixture (pH 6) was filtered and concentrated. Chromatography on RP C18 silica gel eluting with 20 % $H_2O$-$CH_3CN$ gave 0.09 g (84%) of deep red powder. UV-Vis (water): $\lambda_{max}$= 496 (13200), 865 (960) nm (ε). EPR (9 GHz, RT, water) g=2.0027.



**5** **6b**

**SN-BDPA (6b):** Diethanolamine (0.5 mL, 5 mmol, 40 equiv) was added to the solution of **5** (0.108 g, 0.126 mmol, 1 equiv) in 12 mL of THF. The resulting deep blue solution stirred at RT. Deep blue oil separated at the bottom of the flask. The remaining clear supernatant was decanted, the oil was washed with 20 mL THF, and then, diluted with 20 mL of nanopure water. Anion UV-Vis (water): $\lambda_{max}$= 639(5000), 834(4000) nm (ε).

Silver nitrate (64.2 mg, 0.384 mmol, 3 equiv) in 3 mL of water was added to the above solution, and the resulting red mixture stirred at RT for 0.5 h. The mixture was acidified to pH 2.5 with 1 M HCl and filtered. The filtrate was washed with 0.1 M HCl, and dried to give 0.136 g (96 %) of **6b** as a deep red powder. EPR (9 GHz, RT, water) g=2.0027. ESI HRMS (m/z): $[M–H]^-$ calcd for $C_{50}H_{58}N_4O_{18}S_4^{\bullet}$, 1129.2556; found 1129.2461. UV-Vis (water): $\lambda_{max}$= 514 (3300) nm (ε).

## EPR

9 GHz EPR experiments (RT) were performed on Bruker EMX spectrometer, equipped with an ER 4199HS cavity and a Gunn diode microwave source operating at 9.8565 GHz. Aqueous samples were measured using Wilmad WG-808-Q Suprasil Low Temperature Aqueous Cell. A microwave power of 5 mW was used in conjunction with 100 kHz field modulation with 0.1 mT amplitude. 1024 field points were recorded over a width of 5 mT; the conversion time was 20.48 ms with an integration time constant of 5.12 ms. The spectrum was recorded in a single sweep.

The 9 GHz EPR (frozen) field profile was performed on a Bruker Elexsys E580 pulsed-EPR spectrometer, equipped with a MD-5-W1 probe with $TE_{011}$ mode cavity operating at 9.745 GHz. The field profile was acquired at 80 K using a Hahn echo pulse sequence using 16 ns $\pi/2$- and $\pi$-pulses, with a 100 ns delay between pulses. 80 shots were acquired at 1024 field points, without phase cycling.

140 GHz EPR experiments were performed on a custom-built 140 GHz EPR spectrometer at 80 K.[3] ~300 nL of sample was contained in a 0.40 mm inner diameter/0.55 outer diameter quart capillary. The spectrum in Figure 3, C is obtained with a Hahn echo using $\pi/2$ and $\pi$-pulse lengths of 30 ns and 60 ns, and a pulse separation of 200 ns. 200 transients were recorded at 131 field points, using a 4-step phase cycle. The spectrum in Figure S1, A was acquired with the same pulse sequence, using 400 transients at 201 field points, and a 2-step phase cycle. Spin-lattice relaxation times of SA-BDPA and trityl (OX063) in Figure S2 were obtained by applying a 3 ms saturating pulse at the center of the EPR spectrum. After a recovery delay, a Hahn echo was used to measure the polarization recovery. The length of the recovery delay is varied to determine the spin-lattice relaxation time. $\pi/2$ and $\pi$ pulses were 30 ns and 60 ns for SA-BDPA, and 35 ns and 70 ns for trityl. The pulse separation was 200 ns in both cases. 100 transients were recorded at each point for SA-BDPA, and 200 transients at each point for trityl, using a 4-step phase cycle in both cases. Nutation curves in Figure S1, B were obtained using a pulse with variable length, followed by a 200 ns delay and $\pi$-pulse of 60 ns. 400 transients were recorded at each point, with a 2-step phase cycle.

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[3] Smith, A. A.; Corzilius, B.; Bryant, J. A.; DeRocher, R.; Woskov, P. P.; Temkin, R. J.; Griffin, R. G. *J. Magn. Reson.* **2012,** submitted.

## EPR of SN-BDPA (6b)

The EPR spectrum of a frozen 40 mM solution of SN-BDPA in glycerol-$d_8$/$D_2O$/$H_2O$ (60/30/10 v/v) is shown in Figure S1.

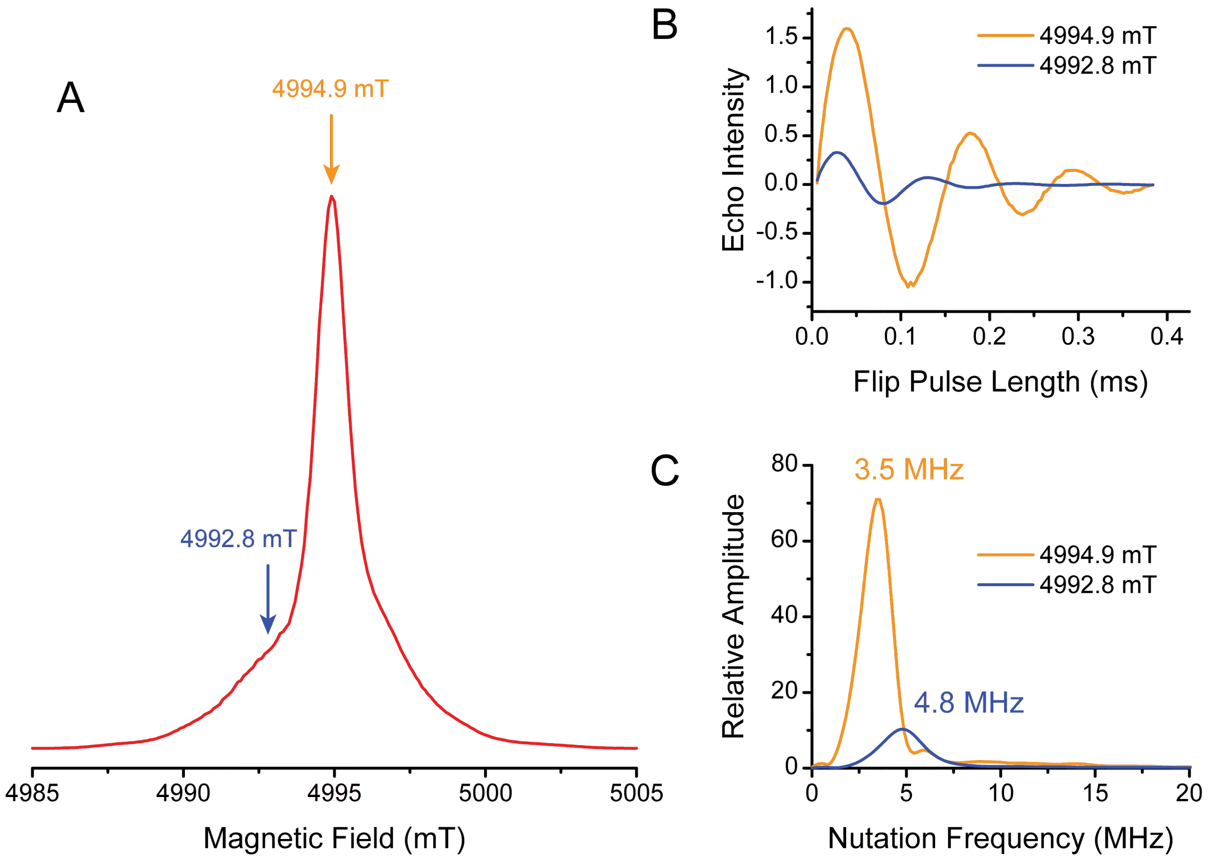

Figure S1. A: 140 GHz field-swept electron-spin echo detected EPR spectrum of a frozen 40 mM solution of SN-BDPA in glycerol-$d_8$/$D_2O$/$H_2O$ (60/30/10 v/v/v). B: Nutation curve acquired by varying the length of the flip-pulse in the Hahn echo sequence. Nutation curves are shown for two field positions, indicated by arrows in A. C: Distribution of nutation frequencies obtained by Fourier transformation of curves shown in B.

Nutation curves were obtained at magnetic fields corresponding to the center of the spectrum and to the distinct shoulder (satellite) of the central line. Nutation frequencies of 3.5 and 4.8 MHz are obtained for the center and the satellite, respectively. The ratio of these frequencies is ~0.7, corresponding to the expected ratio between an $S = 1/2$ and an $S = 1$ spin system.[4] The absence of the 4.8 MHz peak in the frequency distribution of the central line is explained by a collapse of the high-spin properties under the strong microwave field for vanishing electron-electron interactions.

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[4] Astashkin, A.V.; Schweiger, A. *Chem. Phys. Lett.*, **1990,** *174*, 595.

## Spin-Lattice Relaxation

In order to asses potential electron spin saturation during SE DNP experiments, electron spin-lattice relaxation time constants of frozen 1 mM SA-BDPA and trityl OX063 solutions in 60/40 (v/v) glycerol-$d_8$/$D_2O$ have been measured using saturation recovery at 140 GHz. After a sufficiently long presaturation pulse the electron polarization is read out via a Hahn echo sequence. Experiments are performed at the magnetic field corresponding to the respective maximum of the EPR spectrum. Relaxation curves are shown in Figure S2.

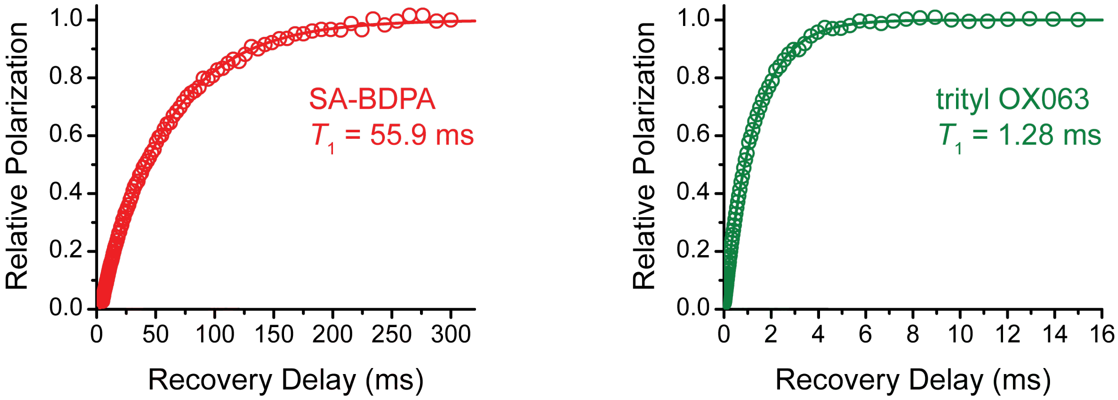

Figure S2. Relaxation curves of SA-BDPA (left) and trityl OX063 (right). Open circles represent experimental data while the solid line is a monophasic exponential fit.

Monophasic exponential fitting following

$$I(t) = I_0\left(1 - e^{-\frac{t}{T_1}}\right)$$

yielded relaxation time constants $T_1$ of 55.9 and 1.28 ms, respectively.

## DNP

DNP experiments were performed using a custom-built instrument, consisting of a 139.65 GHz gyrotron[5] 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 radicals in a 60/30/10 (v/v) glycerol-$d_8$/$D_2O$/$H_2O$ mixture to give a concentration of 10 mM. For standard experiments 1 M $^{13}$C-urea was added to provide $^{13}$C for NMR detection. Further experiments were performed using 0.1 mM $^{13}C_5$-proline; in this case the sample was fully-packed in a rotor, so that the entire active NMR volume was used.

Experiments were performed under MAS with a spinning frequency of $\omega_r/2\pi = 5$ 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 $\varepsilon$ 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 $\varepsilon$ by comparison of $^1$H signal intensities recorded with and without microwave irradiation. All one-dimensional spectra were recorded using a 16-pulse presaturation train (phase alternating in +x and +y) of 108° flip angle and $\gamma B_1/2\pi = 50$ 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. For all pulses (incl. CP and TPPM decoupling) $\gamma B_1/2\pi = 100$ kHz has been used. Build-up curves have been obtained at the field of maximum $\varepsilon$ 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.

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[5] Becerra, L. R.; Gerfen, G. J. ; Temkin, R. J.; Singel, D. J.; Griffin, R. G. *Phys. Rev. Lett.* 1993, *71*, 3561.

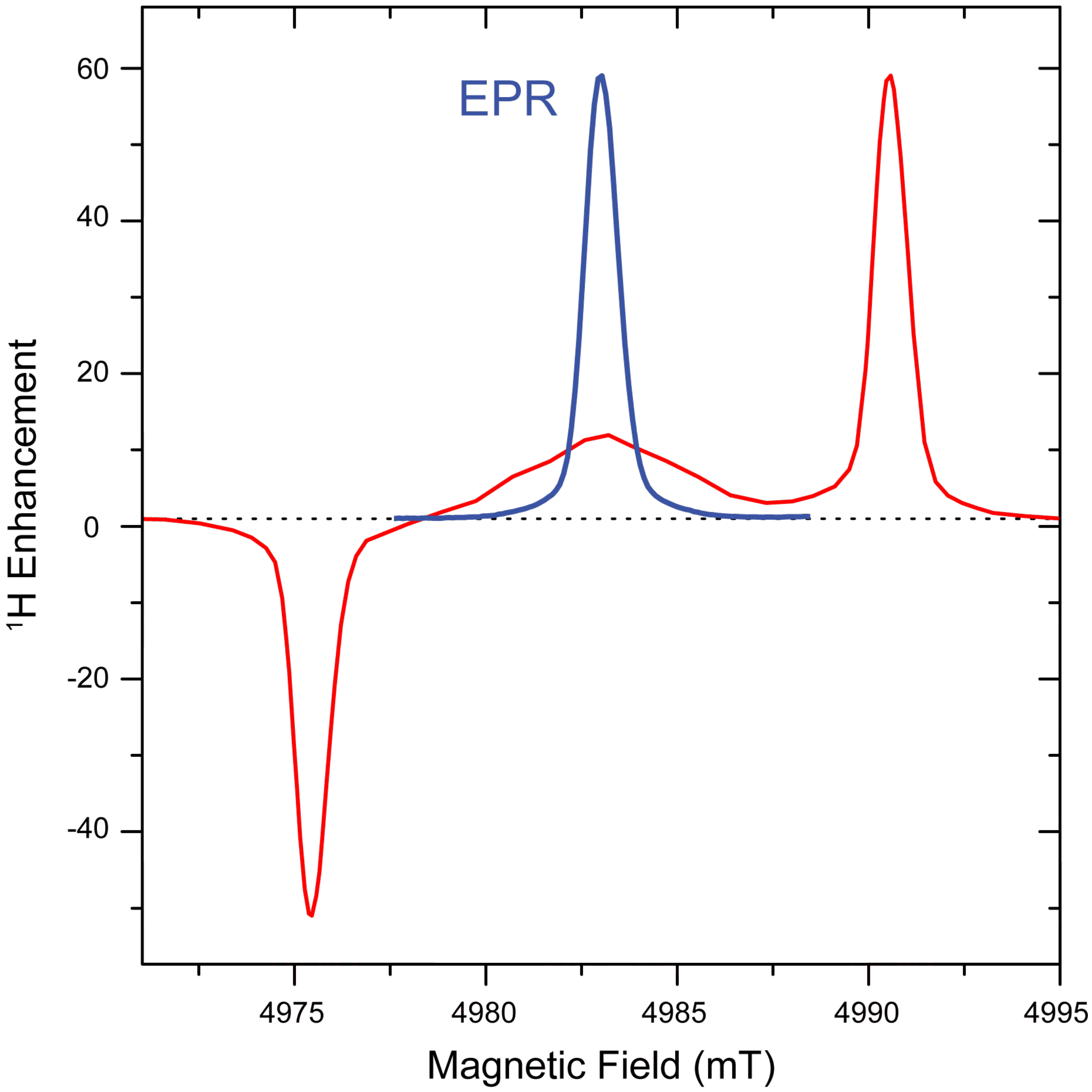

Figure S3. Comparison between DNP field profile (red) and EPR spectrum (blue) of SA-BDPA. Data taken from Figures 3C and 4. The difference in applied microwave frequency between the MAS DNP experiment (139.65 GHz) and the EPR experiment (140.0 GHz) has been corrected for by shifting the EPR spectrum to the resonance field under MAS DNP conditions.