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Water-Soluble Narrow-Line Radicals for Dynamic Nuclear Polarization

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Supporting Information

ABSTRACT: The synthesis of air-stable, highly water-soluble organic radicals containing a 1,3-bis(diphenylene)-2-phenylallyl (BDPA) core is reported. A sulfonated derivative, SA-BDPA, retains the narrow electron paramagnetic resonance linewidth (<30 MHz at 5 T) of the parent BDPA in highly concentrated glycerol/water solutions (40 mM), which enables its use as polarizing agent for solid effect dynamic nuclear polarization (SE DNP). A sensitivity enhancement of 110 was obtained in high-field magic-angle-spinning (MAS) NMR experiments. The ease of synthesis and high maximum enhancements obtained with the BDPA-based radicals constitute a major advance over the trityl-type narrow-line polarization agents.

Dynamic nuclear polarization (DNP) is used to significantly enhance the limited sensitivity of NMR studies of small molecules and complex biological systems.[1] The increased sensitivity and decreased measurement time afforded by DNP allow observation of transient (e.g., photochemical intermediates) and metabolic processes. DNP coupled with multidimensional magic-angle-spinning (MAS) NMR spectroscopy is useful in structural studies of proteins that cannot be crystallized, such as membrane and amyloid proteins.[2]

DNP relies on the transfer of polarization from relatively highly polarized unpaired electron spins to nuclear spins (e.g., ^{1}H or ^{13}C) via microwave irradiation of the electron paramagnetic resonance (EPR) spectrum. Unpaired electrons are supplied by the polarizing agents, which in most cases are persistent radical species added to a glass-forming solvent. The solid effect (SE) DNP[3] is a two-spin process that relies on the polarization transfer between a single electron spin and a nuclear spin. This method requires a polarization agent with both the homogeneous EPR linewidth (δ) and the inhomogeneous spectral breadth (Δ) smaller than the nuclear Larmor frequency (ω_{0I}). Trityl-type[4] and bis(diphenylene)-2-phenylallyl (BDPA)-based[5] radicals satisfy these requirements at high magnetic fields for SE DNP of ^{1}H.

The trityl-type radicals CT-03 and OX063[6] (Figure 1) exhibit narrow EPR linewidths (Δ = 50 MHz at 140 GHz) and have been used successfully in DNP NMR experiments and in vivo EPR imaging of tissue oxygenation and pH.[7] However, a lengthy synthesis[8] contributes to the high cost of these radicals and limits their use as routine polarizing agents.

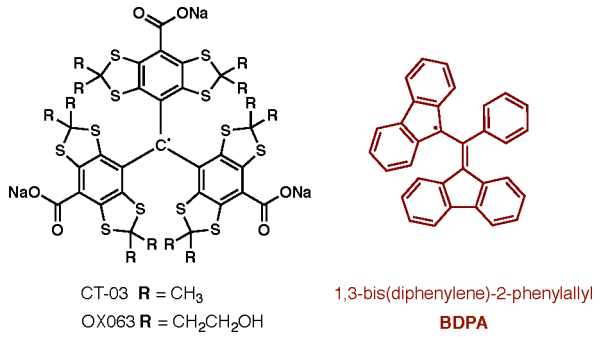
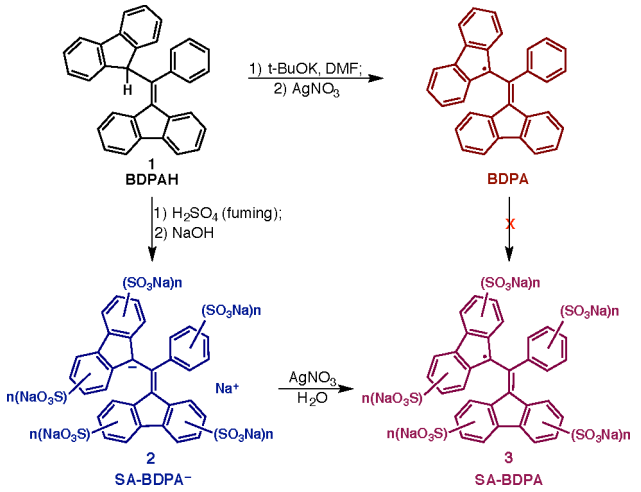


Figure 1. Trityl-type radicals and BDPA.

BDPA is an air-stable persistent radical that shows no dimerization in solid or in solution and has a narrow EPR linewidth[9] ($\Delta \approx 25$ MHz at 140 GHz). The ability of BDPA to transfer polarization was investigated via SE DNP in a polystyrene matrix[10] and recently via dissolution DNP in sulfolane.[11] While BDPA was proven to be an excellent polarization agent, its utility in biological applications is limited by its lack of solubility in aqueous media. Only one water-soluble BDPA derivative has been described.[12] But again, its water solubility is limited, and DNP experiments with this compound were not pursued. We report an efficient synthesis

Scheme 1. Synthesis of Water-Soluble SA-BDPA Radicals



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of highly water-soluble BDPA derivatives that preserve the desirable DNP properties of BDPA and expand its application to aqueous systems.

The BDPA precursor BDPAH[13] (**1**) can be prepared in four steps from fluorene and benzaldehyde. Whereas BDPA radical decomposes in strong acid, treatment of **1** with fuming sulfuric acid followed by oxidation with silver nitrate yielded a mixture of the extremely water-soluble sulfonated radicals SA-BDPA (**3**). (Scheme 1)

The shape of the UV−vis spectrum, the redox behavior, and the EPR spectrum of **3** closely resemble those of BDPA. The sulfonate groups cause a red shift of the absorption maxima of the carbanion **2** and SA-BDPA radical **3** by ∼20 nm relative to those of BDPA anion and BDPA. As shown in Figure 2, SA-BDPA in water has a strong absorption in the visible region (λ_{max} = 508 nm, $D_0 \rightarrow D_2$) and a weak absorption in the near-IR region (λ_{max} = 881 nm, $D_0 \rightarrow D_1$). BDPA in dichloromethane has similar absorption maxima (485 and 859 nm, respectively).[12,14] Similar to BDPA, SA-BDPA is reduced under basic conditions [e.g., aqueous NaOH in the presence of catalytic amounts of acetone, tetrahydrofuran (THF), dimethyl sulfoxide, or ascorbate]. The resulting carbanion **2** can be reoxidized to **3** chemically (e.g., by $AgNO_3$) or electrochemically.

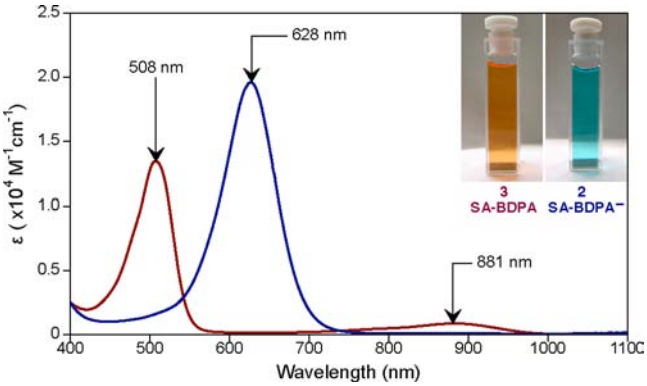

Figure 2. Absorption spectra and (inset) photographs of aqueous solutions of SA-BDPA and its anion.

Also, SA-BDPA is air-stable both in solution and as a solid. Unlike BDPA, however, SA-BDPA does not partition into organic solvents and is soluble in water in all proportions. The EPR spectrum of SA-BDPA (Figure 3) shows no evidence of aggregate formation or radical dimerization in liquid or frozen solution. The solution EPR spectrum features a hyperfine pattern typical of BDPA-type radicals, with nine lines resulting from coupling to eight protons with similar coupling constants of ∼5.1 MHz (Figure 3A). In frozen solution, the linewidth [Δ = 26 MHz at 9 GHz, full width at half-maximum (fwhm)] is dominated by unresolved hyperfine couplings to protons and perfectly matches the envelope of the solution spectrum (Figure 3B). At 140 GHz, the line broadens insignificantly to Δ = 28 MHz as a result of a very small **g** anisotropy (Figure 3C). This small **g** anisotropy qualifies SA-BDPA as an interesting new polarizing agent for SE DNP at even higher fields (e.g., 9.4 T). Trityl's linewidth, on the other hand, increases linearly with the external field because **g** anisotropy is the dominant broadening mechanism in that case.[15]

Encouraged by SA-BDPA's desirable properties, we proceeded to test it as a polarization agent for SE DNP.

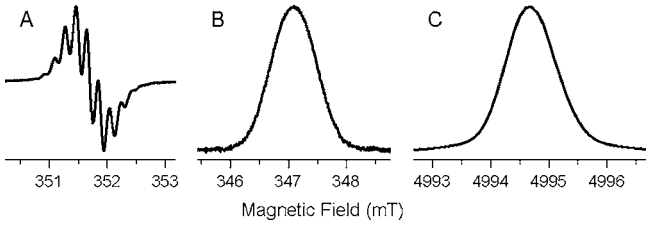

Figure 3. EPR spectra of SA-BDPA: (A) 1 mM solution in water (9.856 GHz, RT, cw EPR); (B) 1 mM frozen solution in 60/40 (v/v) glycerol-d_8/D_2O (9.745 GHz, 80 K, echo-detected); (C) 1 mM frozen solution in 60/40 (v/v) glycerol-d_8/D_2O (140.0 GHz, 80 K, echo-detected).

Figure 4 shows the field-dependent DNP enhancement at 5 T (140 GHz microwave frequency) obtained with a 40 mM frozen solution of SA-BDPA in 60/30/10 (v/v) glycerol-d_8/D_2O/H_2O compared with that of the commonly used trityl polarizing agent OX063. The glass-forming glycerol/water mixture is crucial because it prevents phase separation of the solutes and allows for efficient nuclear spin diffusion. Furthermore, it acts as a cryoprotectant to prevent potential cold denaturation of proteins. ^{1}H signal enhancement by DNP was directly observed via a Bloch decay as function of the external magnetic field. The two radicals gave frequency profiles typical of the well-resolved SE, with a positive peak and a negative peak separated by twice the Larmor frequency of the polarized nucleus and centered around the EPR resonance. As expected, the smaller linewidth of SA-BDPA was retained in the DNP field profile. The enhancement factors were determined with a Hartmann−Hahn cross-polarization (CP) step to ^{13}C at the respective fields of maximum enhancement (1 M [^{13}C]urea was added to provide sufficient ^{13}C for detection of thermal equilibrium polarization). SA-BDPA yielded an NMR signal enhancement (ε) of 61 by comparison of the on and off signals after a time of $1.3T_B$ (where T_B is the time constant of polarization buildup) at a microwave power of 6 W; this is ∼30% higher than the enhancement obtained with OX063 under the same conditions.[16]

In addition to the signals attributed to the SE, another feature centered at the EPR resonance field of 4983 mT was observed for SA-BDPA. Experiments have shown a ^{1}H enhancement of ∼8 independent of the applied microwave power over the range of 2.4−10 W. This flat power dependence together with the symmetric shape and increased width of this feature led us to conclude that the underlying mechanism is solely based on direct saturation of the EPR resonance and that nuclear polarization is induced via cross-relaxation, similar to the Overhauser effect. It is unlikely that the cross effect (CE) or thermal mixing (TM) caused this peak because both mechanisms require a much larger EPR linewidth and typically yield a DNP field profile with regions of positive and negative enhancements and an overall width comparable to the EPR linewidth. For direct comparison, an overlay of the DNP field profile and the EPR spectrum of SA-BDPA is shown in Figure S3 in the Supporting Information. In fact, we attributed the feature in the center of the trityl profile to CE/TM.[16] However, the SA-BDPA profile does not show any sign of CE/TM, in accordance with the significantly reduced linewidth of SA-BDPA compared with trityl (28 vs 50 MHz).

Previous studies of the solid effect have shown that the polarization enhancement is accompanied by a decrease in the time constant at which nuclear longitudinal polarization builds

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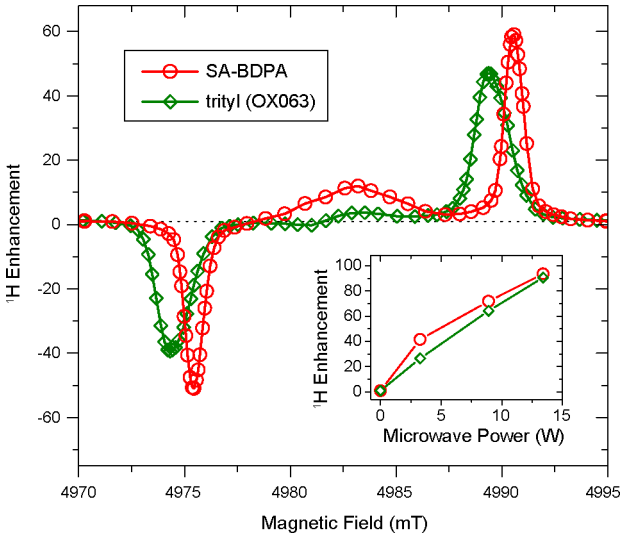

Figure 4. Field-dependent ^{1}H DNP enhancement by 40 mM SA-BDPA (red) in 60/30/10 (v/v) glycerol-d_8/D_2O/H_2O compared with that of trityl OX063 (green) recorded at a microwave power of ~6 W. Inset: power dependence of the ^{1}H enhancements measured at the respective field maxima by ^{1}H–^{13}C CP. The trityl OX063 comparison data (recorded under similar conditions) were taken from ref 16.

up.[16,17] Therefore, careful analysis of the buildup dynamics and extrapolation of the signal intensity at infinite polarization time are crucial to prevent misinterpretation of data (Figure 5). We report enhancement values extrapolated to infinite polarization time.

The buildup time constants T_B were found to be between 43 and 31 s depending on the incident microwave power and obtained enhancements (Figure 5). The reduction of T_B by SE

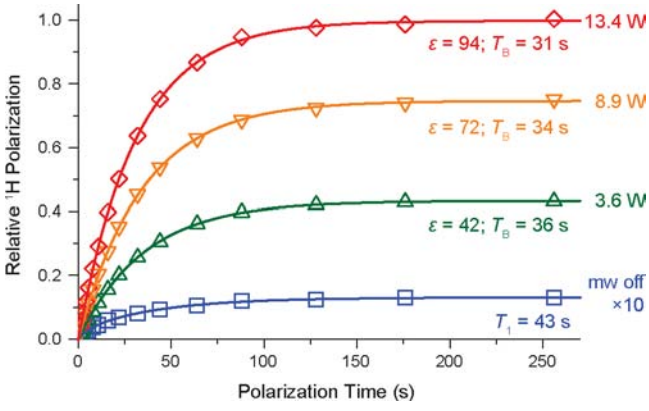

Figure 5. Polarization buildup curves obtained with 40 mM SA-BDPA in 60/30/10 (v/v) glycerol-d_8/D_2O/H_2O at different microwave power levels and without microwave irradiation (multiplied by a factor of 10 to enhance visibility).

DNP compared with T_1 leads to a further increase in sensitivity due to more rapid recycling of NMR experiments.[17] At the highest microwave power, the enhancement factor was $\varepsilon = 94$, while the gain in sensitivity was $\varepsilon(T_1/T_B)^{1/2} = 110$. However, the overall T_B values (including T_1) were ~50% larger in comparison with those of trityl OX063 under similar conditions,[16] which is attributed to less efficient longitudinal relaxation enhancement of protons by the paramagnetic species.

This explanation is further supported by the significantly slower spin–lattice relaxation of the respective electron spins (T_1 = 56 ms in SA-BDPA vs 1.3 ms in trityl; Figure S2). This longer spin–lattice relaxation time constant might also lead to a "saturation effect" observed in microwave-power-dependent measurements of the ^{1}H DNP enhancement: while trityl showed a near-linear power dependence, the enhancements obtained with SA-BDPA were more than 50% larger at the lowest applied power but approached those obtained with trityl at the highest gyrotron output power available (Figure 4 inset).

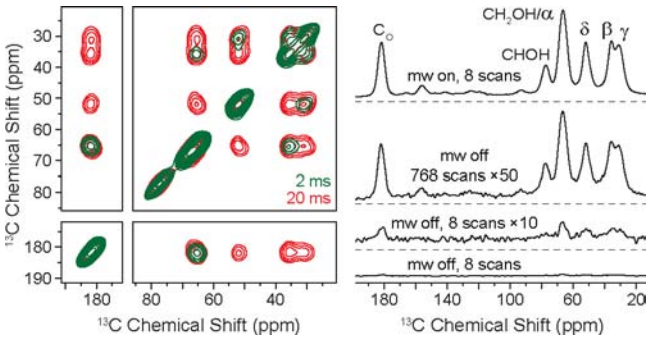

Figure 6. (left) DNP-enhanced 2D ^{13}C–^{13}C correlation spectrum (spin diffusion) using mixing times of 2 ms (green) and 20 ms (red) and (right) ^{13}C CPMAS spectra of 0.1 M [U-$^{13}C_5$]proline polarized by 40 mM SA-BDPA in 60/30/10 (v/v) glycerol-d_8/D_2O/H_2O under 8.9 W microwave irradiation.

In Figure 6 we demonstrate the application of SA-BDPA in 1D DNP-enhanced CPMAS and 2D ^{13}C–^{13}C correlation spectra of a 0.1 M solution of uniformly ^{13}C-labeled proline. ^{13}C–^{13}C mixing was achieved using spin diffusion assisted by a dipolar-assisted rotational resonance (DARR) field applied to ^{1}H.[18] Mixing was either limited to a one-bond distance (Figure 6, green) or allowed to occur among all of the nuclei in the small molecule (Figure 6, red) by allowing spin diffusion for a mixing period of 2 or 20 ms, respectively. In each case all of the expected cross-peaks were present and clearly resolved. The ^{1}H signal enhancement was determined to be $\varepsilon = 50$ by comparison of the 1D signal amplitudes with and without microwave irradiation (Figure 6, right). The slight decrease in the DNP enhancement compared with the experiments using urea could be caused by less efficient irradiation of the entire sample volume (a fully packed NMR rotor was used for proline, while the urea experiments were conducted in a center-packed rotor). Additionally, different ^{1}H relaxation properties induced by alicyclic side chain dynamics could lead to lower equilibrium polarization.

SA-BDPA is an effective narrow-line DNP agent with outstanding water solubility. However, access to a family of peripherally substituted BDPA derivatives would be advantageous because experience has indicated that a single polarization agent may not suffice for all analytes.[19] For example, DNP NMR experiments may be compromised if the polarizing agent binds to the analyte, which would cause significant paramagnetic broadening of the NMR signals of interest. Therefore, we set out to develop a modular route to access differentially functionalized water-soluble BDPA derivatives. The reaction of HOOC–BDPAH (**4**)[20] with chlorosulfonic acid produced chlorosulfonate **5**, which could be hydrolyzed or reacted with nucleophiles prior to oxidation to give both ionic and neutral polarization agents.[21] For example, treatment of **5**

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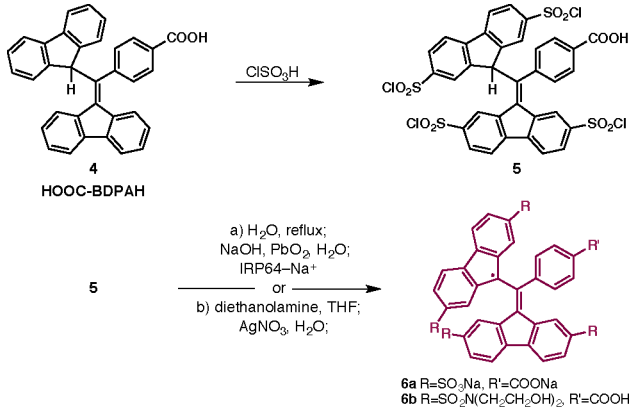
with sodium hydroxide in water followed by $AgNO_3$ oxidation and cation exchange gave **6a**, a highly water-soluble ionic radical similar to SA-BDPA (Scheme 2). Replacing NaOH with

Scheme 2. Modular Approach to Water-Soluble BDPA-Based Radicals



diethanolamine gave neutral radical **6b**. Unfortunately, **6b** formed aggregates in glycerol/water mixtures, as shown by its EPR spectrum at 80 K (Figure S1). However, it may be used to polarize pyruvate via dissolution DNP in metabolic imaging.[22]

In summary, we have developed water-soluble BDPA-based persistent radicals as effective SE DNP agents. Trityl-type EPR probes have been used to measure tissue oxygenation and pH,[23] and we are currently investigating whether water-soluble BDPA derivatives can find application in this field. We anticipate that biradicals and multiradicals incorporating the water-soluble BDPA cores will be useful for cross effect DNP.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and characterization of **3**, **6a**, and **6b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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