

Synthesis of a BDPA-TEMPO Biradical

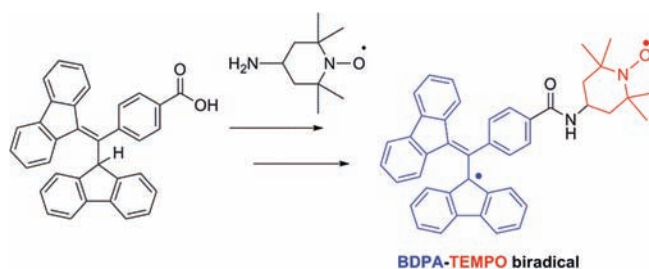
Eric L. Dane, Thorsten Maly, Galia T. Debelouchina, Robert G. Griffin, and Timothy M. Swager*

Massachusetts Institute of Technology and Francis Bitter Magnet Laboratory,
Cambridge, Massachusetts 02139

tswager@mit.edu

Received January 24, 2009

ABSTRACT



The synthesis and characterization of a biradical containing a 1,3-bisdiphenylene-2-phenylallyl (BDPA) free radical covalently attached to a 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) free radical are described. The synthesis of the biradical is a step toward improved polarizing agents for dynamic nuclear polarization (DNP).

Biradicals are of considerable interest as polarizing agents for microwave-driven dynamic nuclear polarization (DNP) NMR experiments.^{1,2} Irradiation of the biradical's electron paramagnetic resonance (EPR) drives electron-nuclear transitions that transfer the large polarization of the electrons to the nuclear spins and thereby enhances the signal-to-noise ratio in NMR experiments. The enhancement factors can reach a theoretical maximum of ~ 660 for electron- ^1H polarization transfer and ~ 2600 when ^{13}C is the nuclear spin of interest.³ Thus, optimized biradical polarizing agents can dramatically decrease acquisition times. These signal enhancements are important for a variety of applications involving solid-state NMR, particularly for systems that are not amenable to crystallographic studies, such as amyloid⁴ and membrane⁵ proteins.

In previous work, we demonstrated that bis-nitroxide biradicals, which contain two tethered TEMPO moieties, provide $^1\text{H}/^{13}\text{C}$ signal enhancements ~ 200 -fold in solid-state magic-angle-spinning (MAS) NMR spectra.² We are interested in molecules that can produce DNP by a cross effect (CE) mechanism³ that involves three spins, two dipolar coupled electrons, and a nuclear spin (usually ^1H), denoted by S_1 and S_2 and I , with electron and nuclear Larmor frequencies, ω_{0S1} , ω_{0S2} , and ω_{0I} , respectively. In a DNP experiment, microwave irradiation of the biradical's EPR spectrum induces the two electrons to undergo a spin flip-flop process, during which a nuclear spin is polarized if the electron Larmor frequencies are separated by the nuclear Larmor frequency and therefore satisfy the matching condition, $\omega_{0S1} - \omega_{0S2} = \omega_{0I}$. The high ^1H polarization is then

(1) Hu, K.-N.; Bajaj, V. S.; Rosay, M. M.; Griffin, R. G. *J. Chem. Phys.* **2007**, *126*, 044512.

(2) (a) Hu, K.-N.; Yu, H.-h.; Swager, T. M.; Griffin, R. G. *J. Am. Chem. Soc.* **2004**, *126*, 10844. (b) Song, C.; Hu, K.-N.; Swager, T. M.; Griffin, R. G. *J. Am. Chem. Soc.* **2006**, *128*, 11385.

(3) (a) Maly, T.; Debelouchina, G. T.; Bajaj, V. S.; Hu, K.-N.; Joo, C.-G.; Mak-Jurkauskas, M. L.; Sigirhi, J. R.; van der Wel, P. C. A.; Herzfeld, J.; Temkin, R. J.; Griffin, R. G. *J. Chem. Phys.* **2008**, *128*, 052211. (b) Barnes, A. B.; Paëpe, G. D.; Wel, P. C. A. v. d.; Hu, K.-N.; Joo, C.-G.; Bajaj, V. S.; Mak-Jurkauskas, M. L.; Sirigiri, J. R.; Herzfeld, J.; Temkin, R. J.; Griffin, R. G. *Appl. Magn. Reson.* **2008**, *34*, 237.

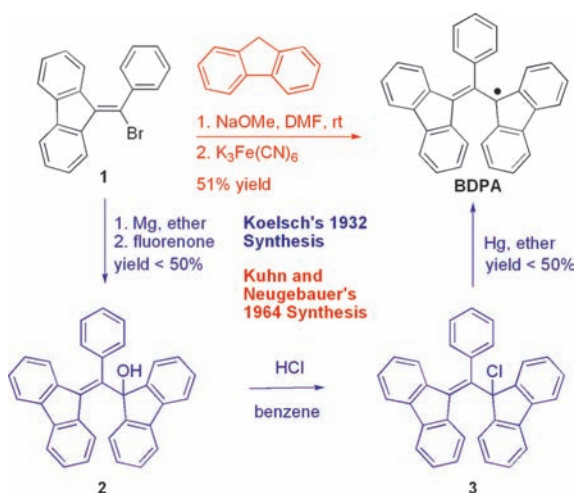
(4) (a) Jaroniec, C. P.; MacPhee, C. E.; Bajaj, V. S.; McMahon, M. T.; Dobson, C. M.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 711. (b) van der Wel, P. C. A.; Hu, K.-N.; Lewandowski, J. R.; Griffin, R. G. *J. Am. Chem. Soc.* **2006**, *128*, 10840. (c) van der Wel, P. C. A.; Lewandowski, J. R.; Griffin, R. G. *J. Am. Chem. Soc.* **2007**, *129*, 5117.

(5) (a) Bajaj, V. S.; Hornstein, M. K.; Kreischer, K. E.; Sirigiri, J. R.; Woskov, P. P.; Mak-Jurkauskas, M. L.; Herzfeld, J.; Temkin, R. J.; Griffin, R. G. *J. Magn. Reson.* **2007**, *189*, 251. (b) Mak-Jurkauskas, M. L.; Bajaj, V. S.; Hornstein, M. K.; Belenky, M.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 883.

transferred via cross-polarization to ^{13}C or ^{15}N , resulting in an enhanced MAS NMR spectrum.⁶

The efficiency of the CE mechanism depends on how many pairs of electrons satisfy the matching condition. Thus, the ideal CE polarizing agent would be a biradical with an EPR spectrum consisting of two sharp lines separated by ω_{0I} . However, at the high magnetic fields (>5 T) where contemporary NMR experiments are performed, only a few known radicals exhibit narrow spectra. Among them are two stable species, trityl radical derivatives⁷ and the BDPA radical⁸ (Scheme 1), which have similar isotropic g -values

Scheme 1. BDPA Radical Syntheses



($g_{\text{iso}}(\text{trityl}) = 2.00307$,¹ $g_{\text{iso}}(\text{BDPA}) = 2.00264$). If trityl or BDPA serves as one of the lines in the EPR spectrum of a polarizing agent, then to satisfy the CE matching condition it is necessary to introduce another radical with a line separated from the first by ω_{0I} . There are no known stable radicals that provide a narrow line and meet this condition; however, TEMPO derivatives have a broad line with significant spectral density at a frequency separation matching ω_{0I} .

Recently, one of us showed that the enhancements observed with a trityl-TEMPO mixture are a factor of ~ 4 larger than those obtained with TEMPO alone.¹ The success of this experiment provides the rationale for the synthesis of a biradical that covalently combines a narrow- and broad-line radical, therefore increasing the dipolar coupling. BDPA was chosen as the narrow-line species, because it has greater stability and a narrower line width than trityl at high magnetic fields. Currently, DNP can only be effectively performed on

biradicals in aqueous solutions that form a rigid glass at $T < 90$ K. Because of the challenges of making the nonpolar BDPA and TEMPO radicals water-soluble, we first chose to synthesize a hydrophobic BDPA-TEMPO biradical as a model compound. As described in this communication, we have developed synthetic methods to join the two sensitive functionalities and studied the biradical's EPR properties.

In attempting to synthesize a BDPA-TEMPO biradical (**9**), our work began by investigating how BDPA was previously made. Koelsch's original method⁹ (Scheme 1, blue) involves the formation of alcohol **2**, the displacement of the hydroxyl group with a chloride to form **3**, and the removal of a chlorine radical with mercury. The method developed by Kuhn and Neugebauer¹⁰ (Scheme 1, red) involves a conjugate addition of the fluorene anion to **1**, followed by a one-electron oxidation of the stable carbanion intermediate. The latter method was pursued to synthesize a functionalized BDPA derivative because it requires fewer steps and provides higher yields.

Efforts to synthesize BDPA derivatives have been limited.¹¹ Kuhn¹⁰ reported halogenated BDPA derivatives, and Fox¹² reported the synthesis of BDPA derivatives with methoxy, cyano, and nitro groups at the 4-position of the phenyl ring. Previously synthesized biradicals containing BDPA have been limited to molecules containing two BDPA radicals linked through the phenyl ring.¹⁰

The reactivity of the BDPA radical complicates its incorporation in biradicals. Although the radical is remarkably stable to oxygen in the solid state⁹ and has been reported to be indefinitely stable to oxygen in solution with the exclusion of light, its photoreactivity produces a variety of oxidation products in solution.¹² Additionally, solutions of the radical are reduced to give the corresponding carbanion when exposed to strong bases, such as hydroxide or alkoxide, and BDPA also reacts with strong acids.⁹

The unpaired electron of BDPA is delocalized throughout the fluorenyl blades, but it is not appreciably delocalized into the phenyl blade.¹³ On the basis of this fact, we chose to connect TEMPO through an amide linkage¹⁴ at the *para* position of the phenyl ring to minimize the disruption of the radical's stability and its propeller-like geometry.

The synthesis of the BDPA-TEMPO biradical is shown in Scheme 2. Compound **4** was prepared by a condensation of fluorene and 4-carboxybenzaldehyde. Purification of **4** was inefficient as a result of the presence of 4-methylbenzoic acid, which is difficult to remove from commercial 4-carboxybenzaldehyde.¹⁵ Nevertheless, pure acid **4** was obtained by recryst-

(9) Koelsch, C. F. *J. Am. Chem. Soc.* **1957**, 79, 4439.

(10) Kuhn, R.; Neugebauer, F. A. *Monatsh. Chem.* **1964**, 95, 3.

(11) (a) Plater, M. J.; Kemp, S.; Lattmann, E. *J. Chem. Soc., Perkin Trans. 1* **2000**, 971. (b) Nishide, H.; Yoshioka, N.; Saitoh, Y.; Gotoh, R.; Miyakawa, T.; Tsuchida, E. *J. Macromol. Sci. Pure Appl. Chem.* **1992**, A29, 775.

(12) Breslin, D. T.; Fox, M. A. *J. Phys. Chem.* **1993**, 97, 13341.

(13) Azuma, N.; Ozawa, T.; Yamauchi, J. *Bull. Chem. Soc. Jpn.* **1994**, 67, 31.

(14) Sosnovsky, G.; Lukszo, J.; Brasch, R. C.; Eriksson, U. G.; Tozer, T. N. *Eur. J. Med. Chem.* **1989**, 24, 241.

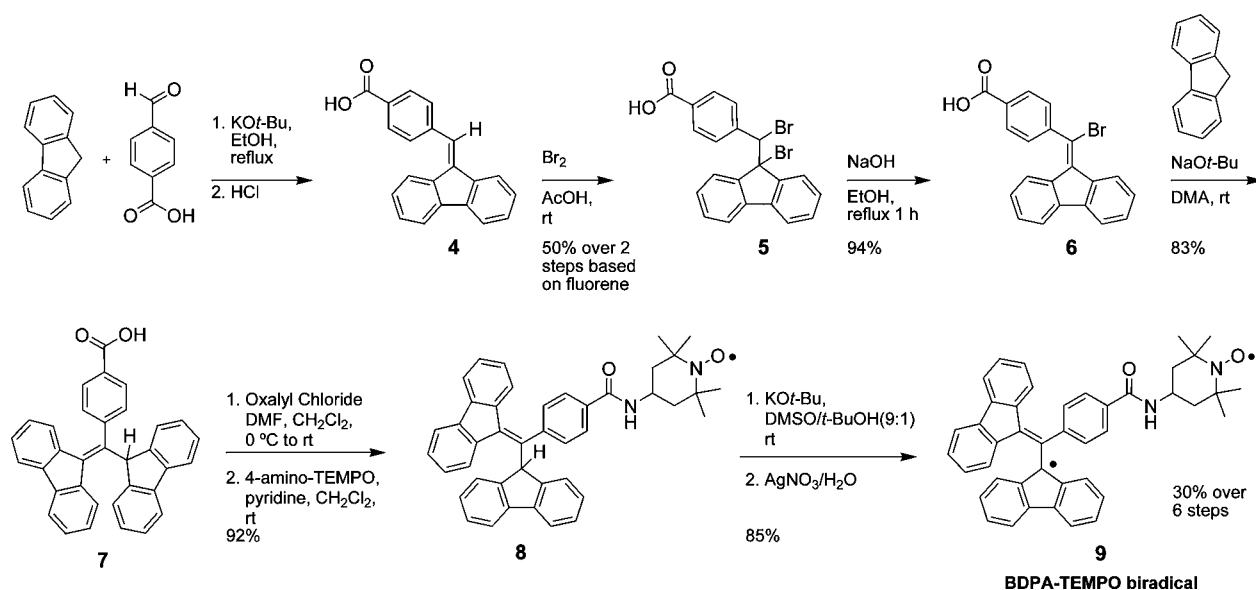
(15) The impurity was identified by ^1H NMR and in our hands could not be removed from the commercial material through recrystallization, extraction, or vacuum sublimation.

(6) (a) Pine, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1972**, 56, 1776. (b) Bennett, A. E.; Rienstra, C. M.; Auger, M.; Lakshmi, K. V.; Griffin, R. G. *J. Chem. Phys.* **1995**, 103, 6951.

(7) (a) Reddy, T. J.; Iwama, T.; Halpern, H. J.; Rawal, V. H. *J. Org. Chem.* **2002**, 67, 4635. (b) Bowman, M. K.; Mailer, C.; Halpern, H. J. *J. Magn. Reson.* **2005**, 172, 254. (c) Liu, Y.; Villamena, F. A.; Sun, J.; Xu, Y.; Dhimitruka, I.; Zweier, J. L. *J. Org. Chem.* **2008**, 73, 1490.

(8) 1,3-Bisdiphenylene-2-phenylallyl (BDPA) free radical and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) free radical will be referred to as simply BDPA and TEMPO throughout the text.

Scheme 2. Synthesis of BDPA-TEMPO Biradical



tallization (tetrahydrofuran/acetic acid), but with a significant loss of material. Carrying forward the impure material proved a better option, because bromination of acid **4** gives compound **5**, which has lower solubility and allows isolation of the pure solid by simple filtration. Dibromide **5** was refluxed in ethanol with sodium hydroxide to eliminate hydrogen bromide and produce the conjugate acceptor **6**. We found this reaction to be concentration sensitive. At higher concentrations, bromine (Br_2) instead of hydrogen bromide was eliminated. Subsequent reaction of **6** with the fluorene anion, which is generated by deprotonation with sodium *tert*-butoxide in dimethylacetamide (DMA), yields a deep blue solution of the stabilized carbanion. This intermediate is quenched with 2 M HCl and purified to give the functionalized BDPA precursor **7**, which is isolated as a white, air-stable powder.

spectrum. In solution, one ^1H -nucleus resonates at 5.90 ppm. The upfield shift is caused by the nearby phenyl group's local magnetic field. In contrast, the ^1H -nucleus on the opposite side of the fluorene moiety resonates at 8.52 ppm and appears to interact with the proximate proton on the sp^3 -hybridized carbon. This interaction has been confirmed by the observation of a 20% NOE enhancement in solution. In the process of characterizing **7** by NMR, we observed a second rotamer, **7b**, that appears stable in the solid state but which slowly converts to the more stable form, **7a**, in solution (Figure 1). Rotamer **7b** is observed in the proton NMR of nonrecrystallized **7**. The conversion of **7b** to **7a** was monitored by NMR and was complete within a week in d_8 -tetrahydrofuran at room temperature. Additionally, **7b** can be regenerated from samples of pure **7a** by deprotonation followed by an acid quench.¹⁶

To synthesize **9**, carboxylic acid **7** was converted to the acid chloride with oxalyl chloride and catalytic DMF and then reacted with 4-amino TEMPO. The proton on the sp^3 -hybridized carbon of **7** is presumed to be slightly more acidic than that of the protonated BDPA precursor ($\text{p}K_{\text{a}} = 14$, DMSO).¹⁷ To avoid side reactions, the weak base pyridine is used to accelerate amide formation and scavenge protons. A portion of **8** was reduced with ascorbic acid, converting the TEMPO radical to the hydroxylamine, for characterization using ^1H and ^{13}C NMR. The biradical **9** was generated by deprotonating **8**, followed by one-electron oxidation.

The 9 GHz liquid-state EPR spectrum of **9** and of precursor **8** are shown in Figure 2. A typical spectrum of a nitroxide radical consists of three sharp lines. The spectrum of the biradical differs as a result of the presence of the BDPA radical and the interaction between the two paramagnetic centers. Additionally, several new resonances are observed

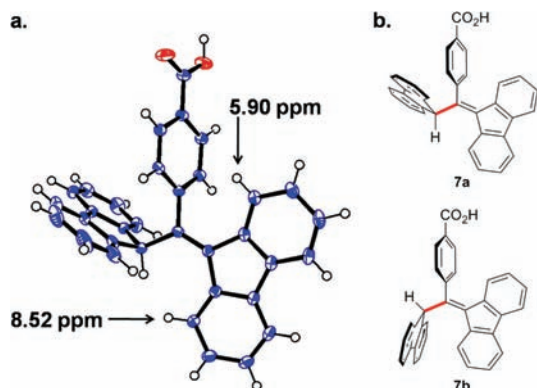


Figure 1. (a) X-ray ORTEP representation of **7** (50% probability level). (b) Rotamers of **7**.

The X-ray crystal structure of **7** (Figure 1) shows its preferred geometry, which explains its unique ^1H NMR

(16) See Supporting Information Figures 1 and 2.

(17) Kuhn, R.; Rewicki, D. *Liebigs Ann. Chem.* **1967**, 706, 250.

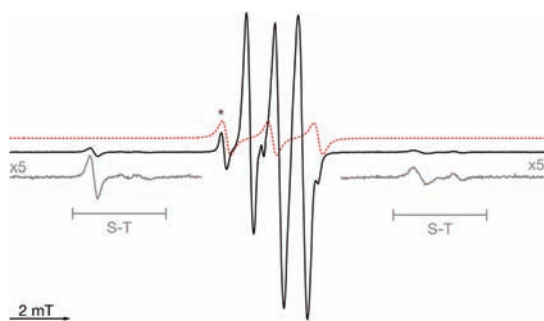


Figure 2. Liquid-state 9 GHz EPR of **9** (black) and **8** (red) in toluene. The regions with enlarged vertical scales (gray) show the forbidden singlet–triplet transitions. Experimental parameters: rt, microwave power 2 mW, sweep width 20 mT, modulation amplitude 10 mT.

well separated from the central region of the spectrum. These features arise from forbidden singlet–triplet transitions (S-T) and are common in spectra of biradicals that feature an intermediate J -coupling. A J -coupling of 140 MHz was measured based on the separation of the peaks from the central region.¹⁸

Present in the spectrum is an impurity with an EPR signal characteristic of a TEMPO radical attached to a diamagnetic fragment (Figure 2, indicated by asterisk), which we attribute to unreacted **8**. We have evaluated the efficiency of the conversion of **8** to **9** using several pieces of evidence. First, we obtain nearly quantitative mass recovery (95%) and see no indication of amide-bond cleavage under the reaction conditions in TLC. Additionally, the TEMPO moiety is unaffected by the reaction conditions. Integration of the EPR signal indicates that on a per molecule basis there is less than 10% of the monoradical impurity present (see Supporting Information). On the basis of this evidence, we estimate the efficiency of the reaction to be 85%.

For high-field (HF) DNP experiments, the HF-EPR spectrum in the solid state is of particular interest (Figure 3). The spectrum of the biradical shows features characteristic of both BDPA and TEMPO, as well as new features that

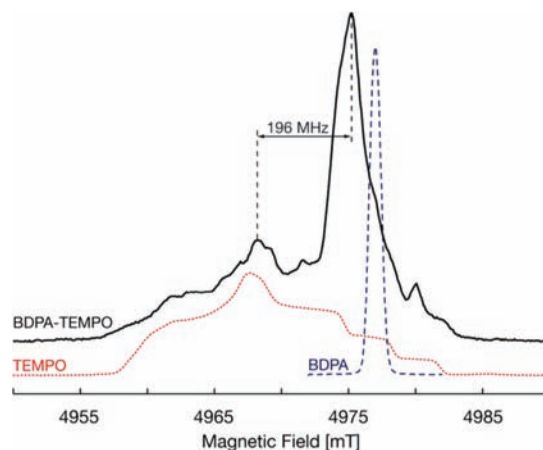


Figure 3. Echo-detected 140 GHz solid-state EPR spectra of **9** (black), TEMPO (red), and BDPA (blue) in toluene. Experiments are performed at 20 K, with $t_p(\pi/2) = 44$ ns, and typically 100 scans are averaged for each point.

indicate the presence of an electron–electron interaction. Most notable is the shift of the BDPA line to lower field.

In summary, we have developed a general method to functionalize BDPA by synthesizing a BDPA precursor (**7**) with a carboxylic acid functional group. We have used this precursor to synthesize a BDPA-TEMPO biradical (**9**) in good yield and studied the resulting EPR spectrum. The HF-EPR spectrum, with two peaks, one narrow and one broad, separated by a value close to the ^1H Larmor frequency, has the desired characteristics for DNP. Future work will focus on adding water-solubilizing groups so that the biradical can be tested for DNP-enhancements in aqueous solutions.

Acknowledgment. We thank Dr. Peter Mueller (MIT) for providing X-ray crystal structures. This work was funded in part by the National Science Foundation DMR-0706408. R.G.G. acknowledges the support of the National Institute of Biomedical Imaging and Bioengineering (EB-002804 and EB-002026).

Supporting Information Available: Experimental and spectral data for all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL9001575

(18) (a) Eaton, S. S.; DuBois, D. L.; Eaton, G. R. *J. Magn. Reson.* **1978**, 32, 251. (b) Eaton, G. R.; Eaton, S. S. *Spin Labelling, Theory and Applications*; Berliner, L. J., Reuben, J., Eds.; Plenum Press: New York, NY, 1989; pp 339–397.