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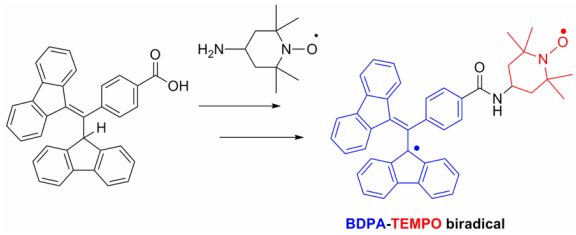
Synthesis of a BDPA-TEMPO Biradical

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, MA 02139

Eric L. Dane: ; Thorsten Maly: ; Galia T. Debelouchina: ; Robert G. Griffin: ; Timothy M. Swager: tswager@mit.edu

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 towards 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-^{1}H 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 amyloid[4] and membrane[5] proteins.

In previous work, we demonstrated that bis-nitroxide biradicals, which contain two tethered TEMPO moieties, provide ^{1}H/^{13}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) mechanism[3] that involves three spins, two dipolar coupled electrons and a nuclear spin (usually ^{1}H), denoted by S_1 and S_2 and I, with electron and nuclear Larmor frequencies, ω_{0S_1}, ω_{0S_2}, and ω_{0_I}, 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, $\omega_{0S_1} - \omega_{0S_2} = \omega_{0_I}$. The high ^{1}H polarizaton is then 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 (>5T) where contemporary NMR experiments are performed, only a few known radicals

exhibit narrow spectra. Among them are two stable species – trityl radical derivatives[7] and the BDPA radical[8] (Scheme 1), which have similar isotropic g-values (g_{iso}(trityl)= 2.00307,[1] g_{iso}(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 linewidth 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. Due to 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 investiging how BDPA was previously made. Koelsch's original method[9] (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 Neugebauer[10] (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] Kuhn[10] reported halogenated BDPA derivatives, and Fox[12] 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 state, 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] Based on this fact, we chose to connect TEMPO through an amide linkage[14] 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 recrystallization 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 t-butoxide in dimethylacetamide (DMA), yields a deep blue solution of the stabilized carbanion. This intermediate is quenched with 2M HCl and purified to give the functionalized BDPA precursor **7**, which is isolated as a white, air-stable powder.

The X-ray crystal structure of **7** (Figure 1) shows its preferred geometry, which explains its unique ^{1}H-NMR spectrum. In solution, one ^{1}H-nucleus resonates at 5.90 ppm. The upfield shift is caused by the nearby phenyl group's local magnetic field. In contrast, the ^{1}H-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 slighly more acidic than that of the protonated BDPA precursor (pKa = 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 ^{1}H- 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 due to the presence of the BDPA radical and the interaction between the two paramagnetic centers. Additionally, several new resonances are observed 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). Based on 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 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 ^{1}H 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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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.
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16. See Supporting Information figure 1 and figure 2.
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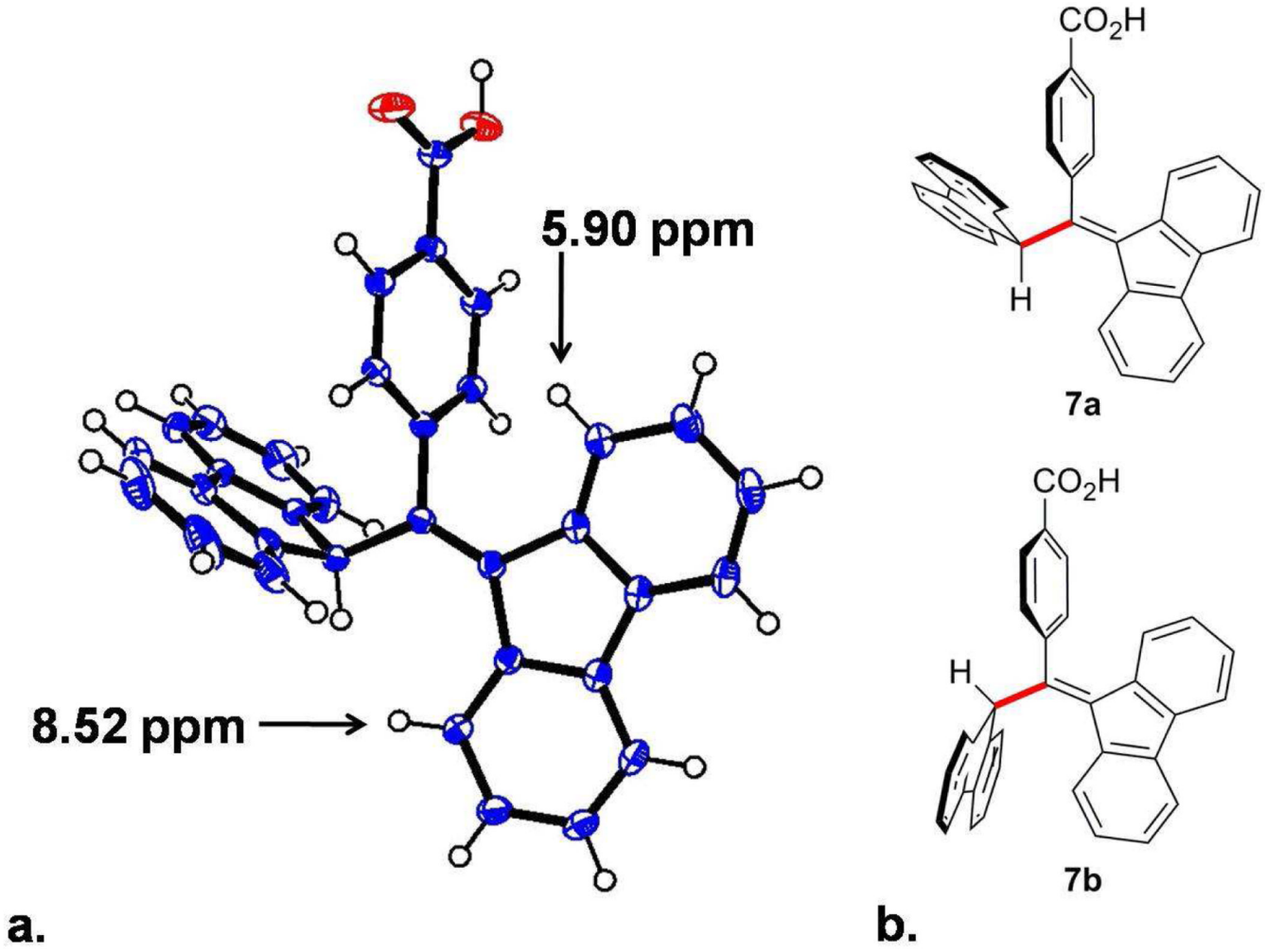


Figure 1.
(a) X-ray ORTEP representation of **7** (50% probability level). (b) Rotamers of **7**.

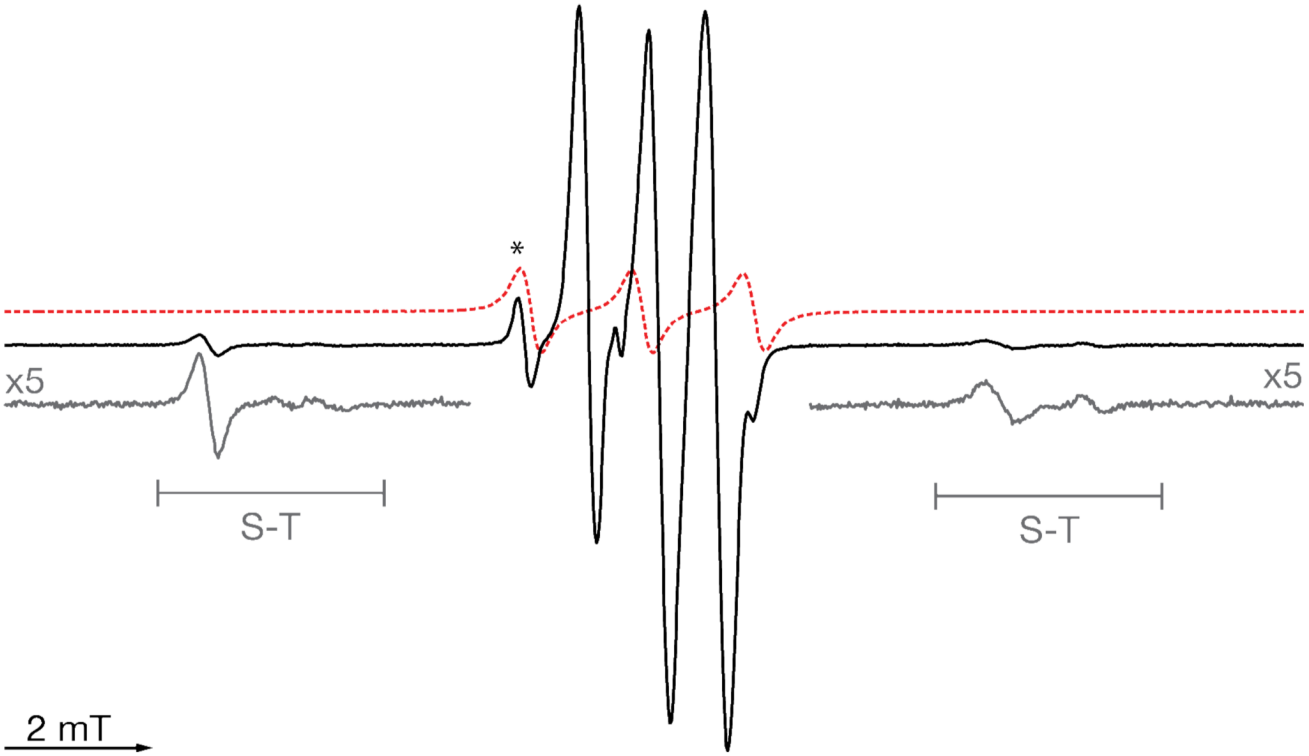


Figure 2.
9 GHz liquid-state EPR of **9** (black) and **8** (red) in toluene. The regions with enlarged vertical scales (grey) show the forbidden singlet-triplet transitions. Experimental parameters: rt, microwave power 2 mW, sweep width 20 mT, modulation amplitude 10 mT.

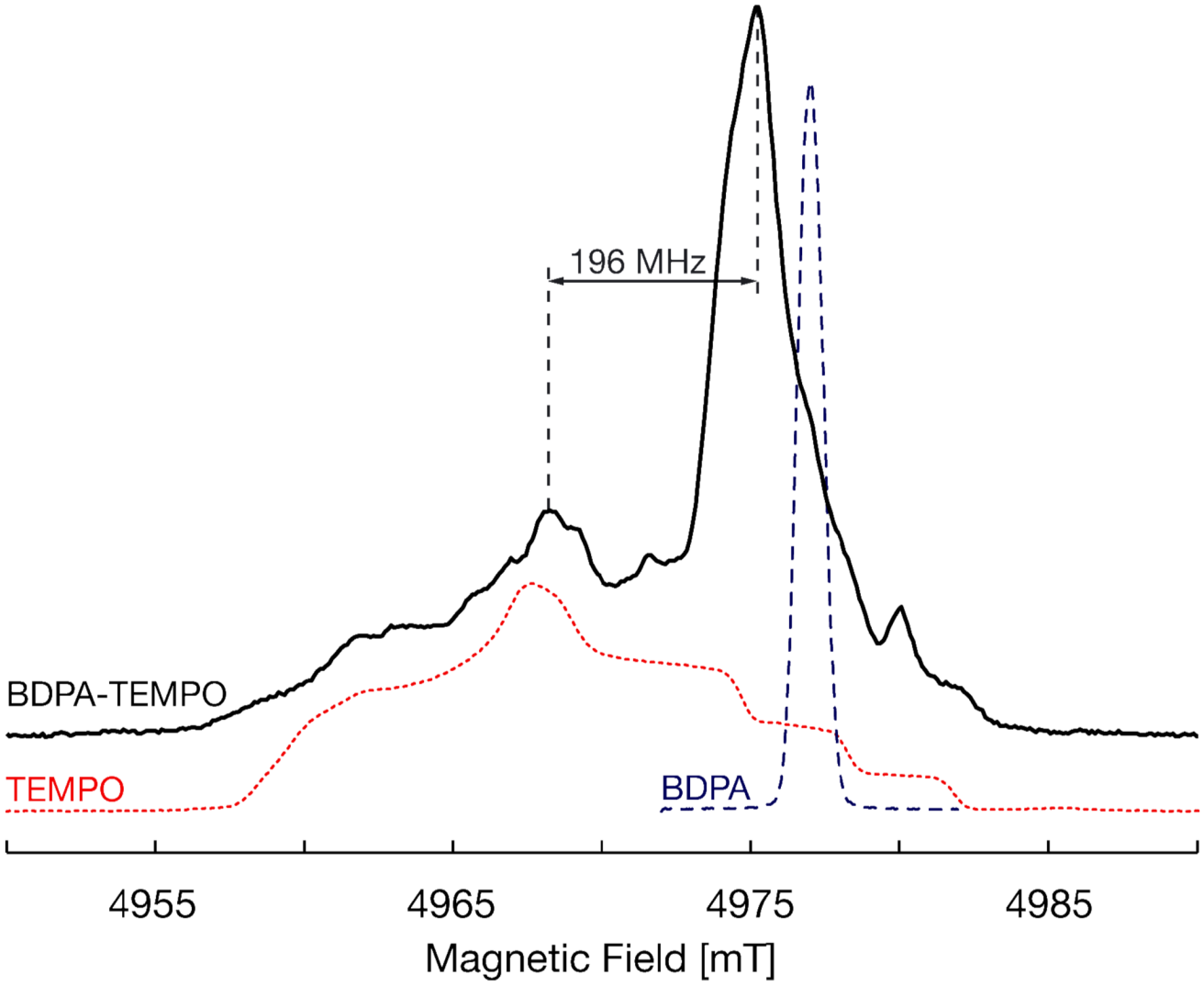
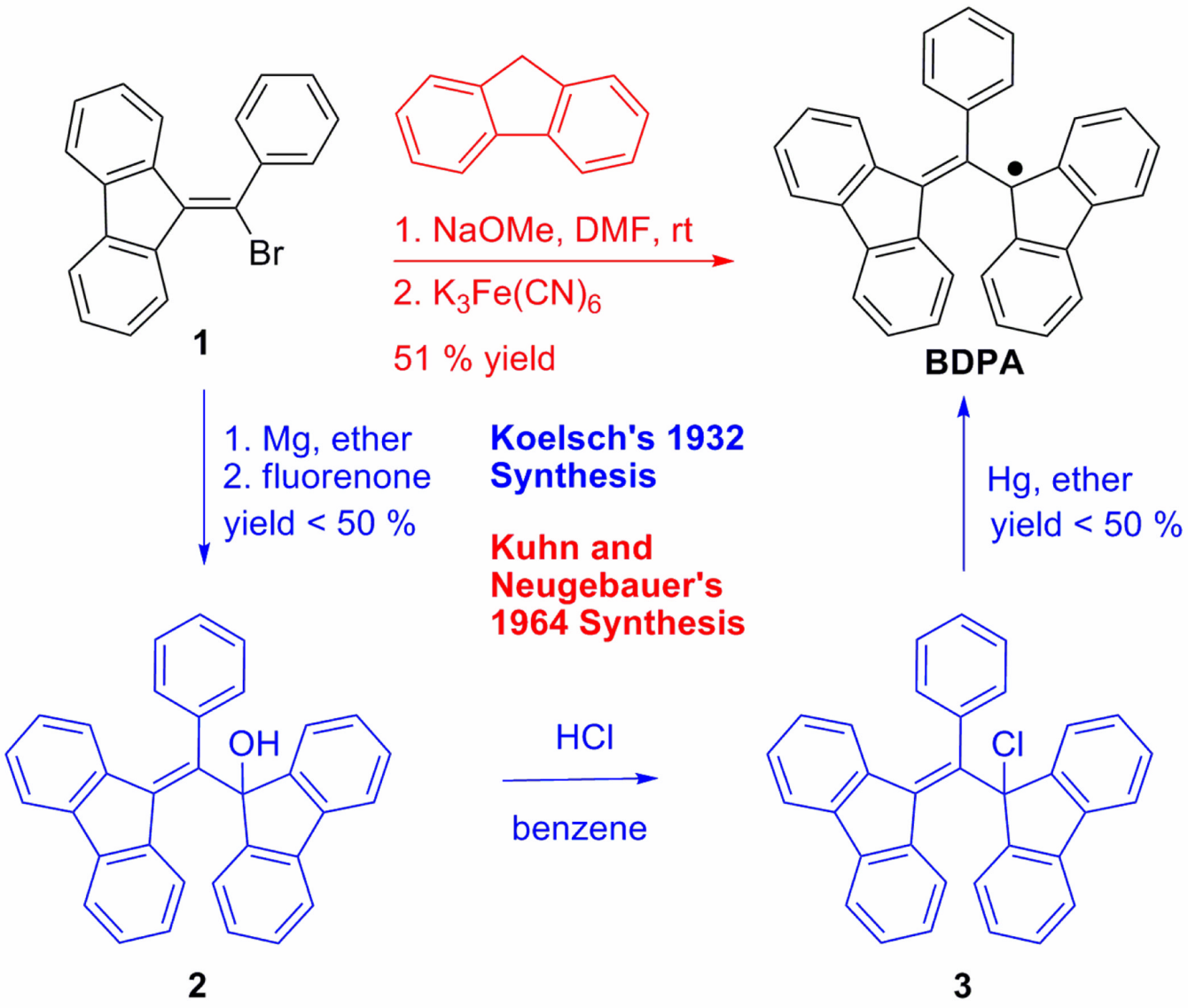
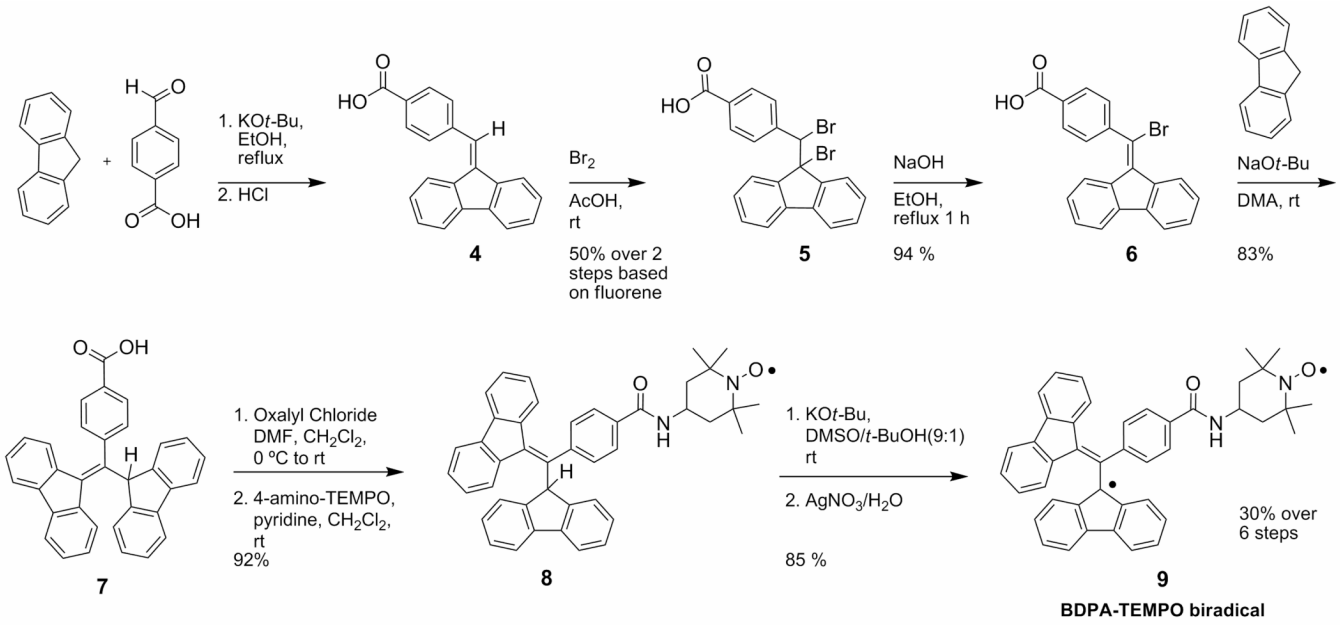


Figure 3.
140 GHz echo-detected solid-state EPR spectra of **9** (black), TEMPO (red), and BDPA (blue) in toluene. Experiments are performed at 20 K, with $t_p(\pi/2)$ = 44 ns, and typically 100 scans are averaged for each point.



Scheme 1.
BDPA radical syntheses



Scheme 2.
Synthesis of BDPA-TEMPO biradical.