Synthesis of a BDPA-TEMPO Biradical

The MIT Faculty has made this article openly available. *Please share* how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1021/ol9001575">http://dx.doi.org/10.1021/ol9001575</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>American Chemical Society</td>
</tr>
<tr>
<td>Version</td>
<td>Author's final manuscript</td>
</tr>
<tr>
<td>Accessed</td>
<td>Sun Apr 24 12:02:45 EDT 2016</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/74224">http://hdl.handle.net/1721.1/74224</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
**Synthesis of a BDPA-TEMPO Biradical**

<table>
<thead>
<tr>
<th>Journal:</th>
<th><em>Organic Letters</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>ol-2009-001575.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Communication</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td></td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Swager, Timothy; Mass. Inst. of Tech., Chemistry Dane, Eric; Massachusetts Institute of Technology, Chemistry Maly, Thorsten; Massachusetts Institute of Technology Griffin, Robert; Massachusetts Institute of Technology, Chemistry Debelouchina, Galia; Massachusetts Institute of Technology</td>
</tr>
</tbody>
</table>
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
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. 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. 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 $^1H$/$^{13}C$-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 $^1H$), denoted by $S_1$ and $S_2$ and $I$, with electron and nuclear Larmor frequencies, $\omega_{0S_1}$, $\omega_{0S_2}$, and $\omega_I$, 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, $\omega_H = n \omega_0$. The high $^1$H polarization is then transferred via cross-polarization to $^1$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 $\omega_0$. 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 derivatives1 and the BDPA radical8 (Scheme 1), which have similar isotropic g-values ($g_{iso}$ (trityl) = 2.00307, $g_{iso}$ (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 $\omega_0$. 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 $\omega_0$.

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 investigating how BDPA was previously made. Koelsch’s original method6 (Scheme 1, blue) involves the formation of alcohol 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.11,12 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 state,9 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 linkage14

---


8 (a) 1,3-bis(phenylene-2-phenyllal) (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.


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. 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 (Br₂) 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 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 ¹H-NMR 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 sp³-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).

(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.
Rotamer 7b is observed in the proton NMR of non-recrystallized 7. The conversion of 7b to 7a was monitored by NMR and was complete within a week in d$_6$-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$^2$-hybridized carbon of 7 is presumed to be slightly 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.

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.

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 electronic electronic coupling. A J-coupling of 140 MHz was measured based on the separation of the peaks from the central region.\(^{18}\)

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 $t_\lambda(\pi/2) = 44$ ns, and typically 100 scans are averaged for each point.

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 watersolubilizing 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-

\(^{16}\) See Supporting Information figures 1 and 2. 
\(^{17}\) Kuhn, R.; Rewicki, D. Liebig Ann. Chem. 1967, 706, 250. 
0706408. RGG 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 is available free of charge via the Internet at http://pubs.acs.org.
Supporting Information for:

**Synthesis of a BDPA-TEMPO Biradical**

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

*Department of Chemistry and Francis Bitter Magnet Lab*

*Massachusetts Institute of Technology*

77 Massachusetts Ave., Cambridge MA 02139 USA
tswager@mit.edu

Table of contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>1</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>2</td>
</tr>
<tr>
<td>Synthetic Procedures</td>
<td></td>
</tr>
<tr>
<td>Compound 4</td>
<td>2</td>
</tr>
<tr>
<td>Compound 5</td>
<td>3</td>
</tr>
<tr>
<td>Compound 6</td>
<td>3</td>
</tr>
<tr>
<td>Compound 7</td>
<td>4</td>
</tr>
<tr>
<td>Compound 8</td>
<td>5</td>
</tr>
<tr>
<td>Compound 9 (BDPA-TEMPO Biradical)</td>
<td>6</td>
</tr>
<tr>
<td>NMR Spectra</td>
<td></td>
</tr>
<tr>
<td>Compound 4 ((^1)H, (^{13})C)</td>
<td>8</td>
</tr>
<tr>
<td>Compound 5 ((^1)H, (^{13})C)</td>
<td>10</td>
</tr>
<tr>
<td>Compound 6 ((^1)H, (^{13})C)</td>
<td>12</td>
</tr>
<tr>
<td>Compound 7 ((^1)H, (^{13})C, NOE, gCOSY)</td>
<td>14</td>
</tr>
<tr>
<td>Compound 8 ((^1)H, (^{13})C, gCOSY)</td>
<td>18</td>
</tr>
<tr>
<td>Figure 1</td>
<td>23</td>
</tr>
<tr>
<td>Figure 2</td>
<td>24</td>
</tr>
<tr>
<td>EPR Spectra</td>
<td></td>
</tr>
<tr>
<td>Compound 9</td>
<td>25</td>
</tr>
<tr>
<td>X-ray Crystal Structure</td>
<td></td>
</tr>
<tr>
<td>Compound 8</td>
<td>27</td>
</tr>
</tbody>
</table>

**Materials.** All chemicals, reagents, and solvents were used as received from commercial sources without further purification except dimethylacetamide,
dimethylformamide, and dimethylsulfoxide, which were dried over oven-activated 4-Å molecular sieves.

**Instrumentation.** Proton nuclear magnetic resonance (\(^1\)H NMR) spectra and carbon nuclear magnetic resonance (\(^{13}\)C NMR) spectra were recorded on an Inova-500 (500 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. Data are represented as follows: chemical shift, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q= quartet, m = multiplet), and coupling constants in Hertz (Hz). The mass spectrometry data were obtained at the MIT mass spectrometry facility, using a Bruker Daltonics APEX II 3T FT-ICR-MS. Elemental analysis was obtained by Columbia Analytical Services, Tucson, AZ.

**Compound 4:** To an oven-dried 500-mL round-bottom flask containing a magnetic stir-bar were added 5.00 g (30.1 mmol, 1.00 equiv) of fluorene and 9.27 g (82.8 mmol, 2.75 equiv) of \(t\)-BuOK, followed by 300 mL of absolute ethanol. The flask was fitted with a water-cooled reflux condenser and heated to reflux with vigorous stirring, at which time 5.50 g (36.7 mmol, 1.20 equiv) of 4-carboxybenzaldehyde was added and the flask was allowed to reflux overnight. After being allowed to cool to room temperature, the reaction mixture was poured into a large flask containing excess 1M HCl and ice. This mixture was extracted with 2 portions of ethyl acetate (approximately 500 mL total), and the organic layer was subsequently washed three times with dilute sodium bicarbonate solution (pH 9) to remove terephthalic acid, which is an impurity in the 4-carboxybenzaldehyde starting material. Finally, the organic layer was washed with brine and dried over sodium sulfate. After removal of the ethyl acetate, the dark yellow material was twice refluxed in 30 mL of toluene, allowed to cool, and then filtered, to obtain 6.25 g of a bright yellow powder which contains a small amount of 4-methylbenzoic acid. This material can be purified by recrystallization from a refluxing mixture of tetrahydrofuran and acetic acid with significant losses of material (3.38 g of material yielded 1.87 g of pure material). Therefore, the impure material was carried through to the next step.

**Compound 4:**
\[^1\]H NMR (500 MHz, \(d_6\)-dimethylsulfoxide): \(\delta\) 13.1 (1H, s), 8.07 (2H, d, \(J = 7.5\) Hz), 7.99 (1H, d, \(J = 7.5\) Hz), 7.96 (1H, s), 7.89 (1H, d, 7.5 Hz), 7.87 (1H, d, 7.5 Hz), 7.73 (2H, d, 8.0 Hz), 7.44 (2H, m), 7.37 (2H, t, \(J=7.0\) Hz), 7.13 (1H, td (\(J = 7.0, 1.0\) Hz).
Compound 5: To an oven-dried 250-mL round-bottom flask equipped with a magnetic stir-bar were added 2.57 g of acid 4 (8.61 mmol, 1.00 equiv) and 65 mL of glacial acetic acid. The resulting suspension was heated to reflux at which time additional acetic acid was added drop-wise until a homogeneous solution was formed. The mixture was cooled to room temperature and a slight excess of bromine (0.47 mL, 1.45 g, 9.06 mmol, 1.10 equiv) was added slowly over 5 minutes. The suspension became a homogeneous red solution and was allowed to stir overnight at room temperature until a white precipitate formed. The resulting suspension was filtered and the filter cake was washed with hexanes to afford 2.85 g (6.22 mmol) of a white powder and a yield of 50% over two steps based on fluorene as the limiting reagent.

Compound 5:
$^{13}$C NMR (125 MHz, d$_6$-dimethylsulfoxide): 167.0, 140.9, 140.8, 138.7, 138.5, 136.5, 135.4, 130.2, 129.6, 129.3, 129.1, 128.7, 127.3, 127.2, 127.0, 123.8, 121.0, 120.3, 119.9.

HRMS (ESI): 297.0924 [calc’d for M-H$: 297.0921$].

FT-IR: $\nu_{max}(KBr)/cm^{-1}$ 1674 s, 1601 s, 1419 m, 1290 s, 722 m.

MP: 250-255 °C (from tetrahydrofuran/acetic acid).

Compound 6: To an over-dried 250-mL round-bottom flask equipped with a stir-bar were added 1.01 g of dibromide 5 (2.21 mmol, 1.00 equiv) and 5.00 g (50 pellets) of
NaOH (125 mmol, 56.6 equiv). To the flask was added 125 mL of absolute ethanol and a water-cooled reflux condenser was attached. The reaction was refluxed for 60 minutes and then allowed to cool to room temperature. The solution was acidified with dilute hydrochloric acid and then extracted with 100 mL of ethyl acetate, which was subsequently washed with brine and dried over sodium sulfate. Removal of the solvent yielded 0.786 g (2.08 mmol, 94 % yield) of a yellow powder.

Compound 6:

$^1$H NMR (500 MHz, d$_8$-dimethylsulfoxide): δ 8.76 (1H, d, J = 7.5 Hz), 8.12 (2H, dd, J = 7.5, 1.5 Hz), 7.94 (1H, d, J = 7.5 Hz), 7.85 (1H, d, J = 7.5 Hz), 7.62 (2H, d, J = 7.5 Hz), 7.52 (1H, t, J = 7.0 Hz), 7.45 (1H, t, J= 7.0 Hz), 7.28 (1H, t, J = 7.0 Hz), 6.92 (1H, t, J = 7.0 Hz), 6.08 (1H, t, J = 7.5 Hz).

$^{13}$C NMR (125 MHz, d$_8$-dimethylsulfoxide): 166.6, 146.0, 140.6, 139.3, 136.9, 136.8, 135.4, 131.5, 130.4, 129.8, 128.8, 128.5, 127.4, 127.2, 125.5(2), 123.0, 120.2, 120.0.

HRMS (EI): 376.0106 [calc'd 376.0093].

FT-IR: $\nu_{\text{max}}$(KBr)/cm$^{-1}$ 1691 s, 1604 w, 1445 m, 728 m.

Compound 7: To an over-dried 100-mL Schlenk flask equipped with a stir-bar and rubber septum were added 0.182 g (1.10 mmol, 1.50 equiv) of fluorene and 0.350 g (3.65 mmol, 5.00 equiv) of NaOt-Bu. The flask was evacuated under high vacuum and refilled with argon three times and 25 mL of dry dimethylacetamide (DMA) was added. The solution appeared dark red and was allowed to stir for 5 minutes. In a separate oven-dried 25-mL schlenk flask, 0.272 g (0.730 mmol, 1.00 equiv) of bromide 6 was added. The flask was evacuated under high vacuum and refilled with argon three times and 10 mL of dry DMA was added. After complete dissolution of bromide 6 in the DMA, the solution was slowly added to the 100-mL Schlenk flask by cannulation over a period of 10 minutes. The reaction immediately turned deep blue and was stirred for 1 hour at room temperature. Next, the flask was cooled in an ice bath and aqueous 1M HCl was added by pipette until the blue color disappeared. The solution was then further diluted with water and extracted into ethyl acetate, which was washed two times with neutral water and once with brine. The organic layer was dried over sodium sulfate and then removed to yield an orange solid. This orange solid was was purified by silica gel flash chromatography eluting with a gradient from pure DCM to 95 DCM : 5 MeOH. The solvent was removed and the solid was dissolved in approximately 1 mL of tetrahydrofuran(THF) and 2 mL of acetic acid with heating. This solution was allowed to cool to room temperature and the THF was allowed to evaporate, yielding, after filtration
and washing with hexanes, 0.280 g (0.605 mmol, 83 % yield) of acid 7 as an off-white powder.

**Compound 7:**

\(^1\)H NMR (500 MHz, d\(_8\)-tetrahydrofuran): \(\delta\) 8.52 (1H, dd, J = 7.5, 1.0 Hz), 7.92 (1H, dq, J = 7.5, 0.50 Hz), 7.77 (1H, dq, J = 7.5, 0.50 Hz), 7.68 (2H, dt, J = 6.5, 1.0 Hz), 7.63 (4H, m), 7.45 (1H, td, J = 7.5, 1.0 Hz), 7.36 (1H, td, J = 7.0, 1.0 Hz), 7.31 (2H, tt, J = 7.0, 1.0 Hz), 7.25 (2H, td, J = 7.5, 1.0 Hz), 7.18 (1H, td, J = 7.5, 1.0 Hz), 7.18 (2H, dt, J = 6.5, 1.0 Hz), 6.77 (2H, d, J = 7.5 Hz), 6.75 (1H, m), 6.56 (1H, s), 5.90 (1H, dt, J = 8.0, 1.0).

\(^13\)C NMR (125 MHz, d\(_8\)-tetrahydrofuran): 167.3, 145.1, 145.0, 144.3, 143.2, 142.5, 141.1, 140.2, 139.6, 137.2, 130.8, 130.1, 129.6, 129.3, 128.6, 128.5, 128.4, 127.9, 127.4, 127.0, 126.3, 125.2, 121.0, 120.8, 120.6, 120.1, 53.6.

HRMS (ESI): 461.1552 [calc’d for M\(^-\)H\(^-\): 461.1547].

FT-IR: \(\nu_{\text{max}}\) (KBr)/cm\(^{-1}\) 1692 s, 1606 m, 1445 m, 1290 m, 730 m.

MP: >280 °C with decomposition (from tetrahydrofuran/acetic acid).

**Compound 8:** To an oven-dried 100-mL Schlenk flask equipped with a magnetic stir-bar and rubber septum was added 0.200 g (0.432 mmol, 1.00 equiv) of acid 7. The flask was evacuated under high vacuum and refilled with argon three times and then 10 mL of dry dichloromethane (DCM) was added along with three drops of dry dimethylformamide (DMF). The reaction flask was cooled to 0 °C in an ice-bath and 0.080 mL (0.86 mmol, 2.0 equiv) of oxalyl chloride was added. The flask was slowly warmed to room temperature. After 1 hour at room temperature, the solvent was removed under high vacuum to obtain a yellow solid, which was then redissolved in 20 mL of dry DCM. In a separate flask, 0.149 g (0.864 mmol, 2.00 equiv) of 4-amino-TEMPO was added and the flask was evacuated and refilled with argon three times. To the flask was added 10 mL of dry DCM and 0.14 mL of dry pyridine (4 equiv) and the resulting solution was stirred in an ice-bath. The solution containing the acid chloride of 7 was slowly added to the amine containing flask, after which the flask was allowed to warm to room temperature overnight with stirring. The solution was washed three times with dilute acid and once with brine. It was dried over sodium sulfate and the solvent was removed. The material was purified by silica gel flash chromatography eluting with a gradient from pure DCM to 90 DCM : 10 ethyl acetate. After removing the solvent, 0.246 g (0.399 mmol, 92 % yield) of amide 8 was obtained as a pink powder. To characterize by NMR, 20 mgs of material was dissolved in 2 mL methanol and excess ascorbic acid was added to reduce the TEMPO radical to a hydroxylamine. After 10 minutes, the solvent was removed and the reduced compound was taken up in 2 mL of
chloroform. After removal of the chloroform, the resulting white solid was dissolved in 0.7 mL of deuterated acetone. The material still contained some ascorbic acid and its oxidation products.

**Compound 8:**

HRMS (ESI): 616.3067  [calc'd for M+H: 616.3084].


FT-IR: $\nu_{\text{max}}$(KBr)/cm$^{-1}$  1652 s, 1607 m, 1539 s, 1498 m, 1446 s, 1322 m, 1242 m, 732 s.

**Compound 8H:**

$^1$H NMR (500 MHz, d$_6$-acetone): $\delta$ 8.57 (1H, dd, J = 7.5, 1.0 Hz), 8.01 (1H, dd, J = 7.5, 1.0 Hz), 7.87 (1H, d, J = 7.5, 0.50 Hz), 7.76 (2H, dt, J = 7.0, 1.0 Hz), 7.69 (2H, d, J = 7.0 Hz), 7.56 (2H, d, J = 7.0 Hz), 7.52 (1H, t, J = 7.0 Hz), 7.45 (1H, t, J = 7.0 Hz), 7.35 (5H, m), 7.25 (1H, t, J = 7.0 Hz), 6.79 (1H, m), 6.78 (2H, d, J = 7.5 Hz), 6.54 (1H, s), 5.85 (1H, d, J = 8.0), 4.28 (1H, m), 1.82 (2H, dd, J = 12.0, 3.0 Hz), 1.50 (2H, t, J = 12.0 Hz), 1.18 (6H, s), 1.12 (6H, s).

$^{13}$C NMR (125 MHz, d$_6$-acetone): 166.1, 146.0, 145.8, 143.8, 143.5 (2), 143.0, 141.8, 140.4, 140.2, 137.8, 135.4, 130.3, 130.3, 129.6, 129.5 (2), 128.9, 128.5, 128.3, 127.8, 127.2, 127.0, 121.9, 121.8, 121.1, 53.3, 45.4, 41.9, 32.3, 20.3.

HRMS (ESI): 617.3142  [calc’d for M+H: 617.3163].

**BDPA-TEMPO Biradical (Compound 9):** To an over-dried Schlenk flask equipped with a magnetic stir-bar, 0.0800 g (0.130 mmol, 1.00 equiv) of 8 and 0.0440 g (0.390 mmol, 3.00 equiv) of potassium t-butoxide were added and the flask was evacuated and refilled with argon three times. To the flask was added 80 mL of a dry 9 to 1 solution of dimethylsulfoxide and tert-butyl alcohol. The flask was vigorously stirred for 20 minutes and then 0.0660 g (0.390 mmol, 3.00 equiv) silver nitrate was dissolved in 1.5 mL of water and added to the reaction. After stirring for 1 minute, additional water was added and the solution was extracted with 50 mL of diethyl ether four times for a total volume of 200 mL. The ether was washed once with both water and brine, and then dried over sodium sulfate. The ether solution was filtered through a small plug of silica on a fritted funnel to ensure that any remaining silver particles were removed. Removal of the
ether yielded 0.0760 g (0.124 mmol, 95 % mass recovery) of a dark brown material with a slight metallic luster. Based on EPR measurements, we estimate the purity of material to be at least 90 %, with the major impurity being the starting material, 8. Therefore, taking into account mass recovery and purity, we estimate the yield of the reaction to be at least 85 %.

FT-IR: $\nu_{\text{max}}$(KBr)/cm$^{-1}$ 1646 s, 1607 m, 1539 s, 1497 m, 1444 s, 1324 m, 1242 m, 731 s. HRMS (ESI): 614.2938  [calc'd for M$: 614.2939$].

$^1$H-NMR, 500 MHz d$_6$-Dimethylsulfoxide
$^{13}$C-NMR, 125 MHz
d$_6$-Dimethylsulfoxide

$^1$H-NMR, 500 MHz, d$_6$-Dimethylsulfoxide.

$^1$H-NMR, 500 MHz
$\text{d}_8$-Tetrahydrofuran

Page 14 of 27

\[ \text{13}^C\text{-NMR, 125 MHz} \]

\[ \text{d}_8\text{-Tetrahydrofuran} \]
Measurement of NOE enhancement between the proton at 6.56 ppm and the proton at 8.52 ppm (in d$_8$-tetrahydrofuran, room temperature, 500 MHz).

Peak assignment for compound 7 in ppm based on NOE-measurement and gCOSY (next page).
$^1$H-gCOSY-NMR, 500 MHz (see previous page for assignment of peaks).

d$_8$-Tetrahydrofuran
$^{1}H$-NMR, 500 MHz
d$_{6}$Acetone

gCOSY NMR, 500 MHz
d$_6$-Acetone
gCOSY NMR, 500 MHz
d$_6$Acetone

![Chemical Structures](image)
Figure 1. Spectrum a shows the aromatic portion of the proton NMR of compound 7 after chromatography but before recrystallization. Both rotamers are present in a ratio of 83:17 (7a:7b) based on integration of the signals. After being left in the NMR-tube, which was stored at or below room temperature, for two days, the proportion of 7a increases with a concurrent decrease in 7b, using the integration of both the DCM signal and an impurity present at slightly above 5.00 ppm as standards. In b, the rotamers are in a ratio of 95:5 (7a:7b). After a week under the same conditions, 7b can no longer be detected.
Figure 2. Spectrum a shows the aromatic portion of the proton NMR of compound 7 after the pure compound, displaying only the major rotamer in proton-NMR, was deprotonated in DMA with sodium t-butoxide and quenched with acid. As can be seen, the minor rotamer has reappeared. Spectrum b shows that after three days the minor rotamer is no longer visible.
9 GHz EPR of 9 is shown above. The peak between 3450-3455 G, which is attributed to the monoradical impurity, is integrated and given the arbitrary value 1.00. The rest of the middle portion, which contains most of the biradical signal and two-thirds of the impurity signal, integrates to 84.89. The smaller integral represents one-third of the impurity's signal because it represents one of the three peaks of the triplet. Therefore, the portion of the larger integral that is due to the biradical is 82.89 (84.89 – 2.00). Each biradical has two spins and therefore gives twice the signal compared to the monoradical impurity. Therefore, to compare on a molar basis the biradical's signal must be divided by 2, giving 41.45. The percentage of monoradical impurity is therefore (3.00/(3.00 + 41.45)) x 100 = 6.7 % and percentage of the biradical is (41.45/(3.00 + 41.45)) x 100 = 93.3 % based on integration of the EPR signal. Experimental parameters: rt, microwave power 2 mW, sweep width 20 mT, modulation amplitude 10 mT.
Below is the 9 GHz solution EPR spectrum of 9H, which is formed when the BDPA-TEMPO biradical is treated with excess ascorbic acid. Ascorbic acid reduces TEMPO, but does not reduce BDPA. The spectrum shown matches that of BDPA, a narrow linewidth with hyperfine coupling to protons on the fluorene rings.
X-ray ORTEP representation of 8 drawn with displacement ellipsoids to the 50% probability level.