Reprint
Dynamic Nuclear Polarization

On the Limited Stability of BDPA Radicals

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Abstract: 1,3-Bis(diphenylene)-2-phenylallyl (BDPA)-based radicals are of interest as polarizing agents for dynamic nuclear polarization (DNP). For this purpose, a BDPA-nitroxide biradical, employing a phosphodiester linkage, was synthesized. Contrary to what is commonly assumed, BDPA-derived radicals were observed to have limited stability. Hence, the effects of various factors on the stability of BDPA radicals were investigated. Solvent polarity was found to play a significant role on degradation; a polar BDPA radical was observed to degrade faster in a non-polar solvent, whereas non-polar radicals were more unstable in polar solvents. The rate of decomposition was found to increase non-linearly with increasing radical concentration; a 2-fold increase in concentration led to a 3-fold increase in the rate of degradation. Collectively, these results indicate that the dimerization is a significant degradation pathway for BDPA radicals and, indeed, a dimer of one BDPA radical was detected by mass spectrometry.

Introduction

NMR spectroscopy is a versatile technique for elucidating the structures of molecules by utilizing nuclear magnetic moments. However, a shortcoming of NMR spectroscopy is its inherently low sensitivity due to low nuclear spin polarization, i.e., the small difference between the numbers of nuclear spins that are aligned parallel or anti-parallel to an external magnetic field. This is especially a challenge when trying to measure analytes with natural isotopic abundance or at low concentrations. In contrast, electrons possess a much larger spin polarization. For example, at 100 K and 9.4 T, proton spin-polarization is about 0.01%, whereas the electron spin-polarization is approximately 10%. Spin polarization of electrons is transferable to nuclei of interest through a technique called dynamic nuclear polarization (DNP), which subsequently enhances the NMR signal-to-noise ratio. In theory, 600- and 2600-fold enhancement can be achieved for 1H and 13C, respectively, with continuous-wave irradiation. In DNP experiments, stable organic radicals at mm concentration are generally included in the sample as the source of unpaired electrons and microwave irradiation is used to facilitate polarization transfer from the electrons to the nuclei.

There are three principal mechanisms through which polarization transfer can be accomplished in solid-state NMR using magic angle spinning (MAS), namely the Overhauser Effect (OE), the Solid Effect (SE), and the Cross Effect (CE). The CE is the most efficient mechanism for MAS-DNP, especially at higher magnetic fields (> 5 T). The ideal polarizing agent for the CE should have an electron paramagnetic resonance (EPR) spectrum consisting of two sharp lines, separated by the Larmor frequency of the nucleus to be polarized. However, there is no radical or radical pair known to possess such an EPR spectrum. Nitroxide biradicals are frequently used as polarizing agents in MAS-DNP as their EPR linewidth is approximately three-fold the proton Larmor frequency, and thus, fulfill the frequency condition for polarizing protons via the CE. The H-polarization can subsequently be transferred to 13C or 15N via cross-polarization. Carbon-centered radicals, such as the Finland trityl and 1,3-bis(diphenylene)-2-phenylallyl (BDPA) (Figure 1A) possess a narrow EPR linewidth. Since their linewidths are narrower than the proton Larmor frequency, they cannot be used alone to polarize protons. However, when a carbon-centered radical is connected to a nitroxide, the EPR spectrum of such a heterobiradical consists of one sharp line and another much broader line, approximately separated by the proton Larmor frequency. Thus, such biradicals are closer to an ideal polarizing agent for MAS-DNP experiments that utilize the CE. Another advantage is that lower microwave power can be used for efficient CE, due to presence of the narrow-line, slower relaxing radical. Such heterobiradicals also lead to significantly lower depolarization under MAS-DNP conditions, compared to most bis-nitroxides. Trityl-nitroxide or BDPA-nitroxide biradicals work especially well at magnetic fields higher than 10 T, where bis-nitroxides give lower enhancements. A trityl-nitroxide biradical and a BDPA-nitroxide biradical have been shown to yield an enhancement of 65 and 64, respectively at 18.8 T using a 3.2 mm rotor. The enhancement of the latter was tripled using an 1.3 mm MAS-DNP rotor. However, this enhancement is still far from the theoretical maximum and thus, there is still need for new polarizing agents with increased efficiency for DNP.

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In this paper, we describe synthesis of a new BDPA-nitroxide biradical using a phosphodiester linker. The biradical allowed us to readily quantify the amount of biradical relative to a nitroxide monoradical by EPR spectroscopy, as previously described for the BDPA-TEMPO biradical 3 (Figure 1B). While preparing and working with BDPA radicals, it became clear that they had limited stability. This was unexpected since BDPA radicals are usually referred to as stable radicals. However, as stated in the excellent review of Griller and Ingold on persistent carbon-centered radicals: “There is no doubt that the lax use of the word “stable” has introduced a freedom into discussion such that an author almost has the possibility, like Humpty Dumpty, to make “stable” mean just what he chooses.” IUPAC considers the stability of a radical to be a thermodynamic property and for a carbon-centered radical, it can be defined as the energy difference between the C–H bond strength of the radical and a suitable alkane (primary, secondary or tertiary). However, IUPAC recommends the use of ‘persistance’, which refers to a kinetic property, to describe a radical with a half-life greater than several minutes in diluted inert solvents. Keeping in mind that the notion of stable radicals could be ambiguous, Griller and Ingold advised to use the term “stable” only for a radical which is highly unreactive to air, moisture etc., under ambient conditions and can be handled without further precautions similar to most organic compounds.

Understandably, many papers refer to the original report of the BDPA radical when discussing its persistence, where a footnote states that “A sample kept in air 23 years is unchanged in appearance and shows a high free-radical content.” More recent reports indicate that BDPA may not be as persistent as once thought. For example, in the supporting information of a paper from 2017, it states that “We realized that after being stored in the freezer for 6 months the EPR intensity signal of sample 5 decreases in 15%.” Another paper from 2018 on BDPA-derived biradicals reported that “The radicals are stable for 3 months as powders and stable in TCE solution for 2 weeks, when stored at –18°C in both cases”, which also points to instability. Hence, we carried out a systematic investigation of the persistence of BDPA radicals under various conditions. The reaction conditions for generating BDPA radicals were also evaluated as their yields varied greatly with the methods that have been described in the literature.

Results and Discussion

Synthesis of the BDPA-nitroxide biradical

The BDPA-nitroxide biradical was prepared utilizing a phosphodiester linker. First, BDPA alcohol 4 was phosphitylated to give phosphoramidite 5, which was subsequently reacted with 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), followed by an oxidation to give phosphodiester 7 (Scheme 1). The corresponding BDPA-nitroxide biradical 8 was generated by treating 7 sequentially with tBuOK and K$_3$Fe(CN)$_6$.

Figure 2 shows EPR spectra of three different radicals. A single peak (Figure 2, top) was observed for the carbon-centered BDPA monoradical 2, whereas nitroxide monoradical 7 shows the characteristic three peaks of nitroxides (Figure 2, middle). The spectrum of 8 (Figure 2, bottom) shows the presence of two components. Biradical 8 has three peaks in its


The amount of biradical dropped drastically to ca. 30 %, when the reaction was carried out in a polar solvent (DMF, DMSO). Reactions in the polar aprotic solvents, DMF, DMSO and for different bases (tBuOK, NaOH, DBU), use AgNO₃ as the oxidizing agent.

Persistence of BDPA radicals

The rapid decomposition of BDPA radicals that we unexpected-ly observed prompted us to investigate the effect of various factors, such as solvents, temperature and oxygen, on the persistence of the BDPA radicals. As demonstrated above, EPR spectroscopy can be readily used to quantify the amount of biradical 8 relative to the nitroxide monoradical present in the sample. Hence, EPR was used to determine the rate of degradation of 8 under different conditions; this method was corroborated by UV-vis spectroscopy, following the absorption signal of the BDPA radical around 490 nm (Figure S7). Chromatographic purification of biradical 8 was carried out prior to these studies. However, the „purified“ samples contained only about 70 % of the biradical, relative to the nitroxide monoradical, due to decomposition of the BDPA moiety during the purification process. Therefore, a pure sample of the biradical could not be obtained.

First, the solvent-dependent persistence of biradical 8 at 23 °C was investigated (Figure 4A). Solutions of 8 were prepared in 1,2-dichloroethane (DCE), MeOH and DMSO (5 mm) and their EPR spectra were recorded at various time intervals. These solvents were chosen to probe the effect of the solvent polarity. The initial rate of degradation of biradical 8 in different solvents was determined by plotting the amount of the biradical as a function of time (Supporting Information) and found to increase with decreasing polarity of the solvent (Figure 4A). The highest rate of decomposition was observed in DCE, with an initial rate of (1.79 ± 0.35)·10⁻⁷ Ms⁻¹. The degradation was significantly slower in MeOH and DMSO, (1.79 ± 0.03)·10⁻⁸ Ms⁻¹ and (1.50 ± 0.09)·10⁻⁸ Ms⁻¹, respectively.

The rate of degradation of biradical 8 in solution was also studied at different concentrations. Figure 5 shows the initial rates of degradation of 8 in DMSO for a series of solutions
was not found (Figure S12). Instead, masses (DCE). DCE is 1,2-di-
chloroethane. (1.50 ± 0.09) ⋅ 10^{-8} (DMSO), (1.79 ± 0.03) ⋅ 10^{-8} (MeOH),
(1.79 ± 0.35) ⋅ 10^{-7} (DCE). B. Biradical 3; rates (Ms^{-1}): (2.28 ± 0.15) ⋅ 10^{-8}
(DMSO), (7.18 ± 0.64) ⋅ 10^{-8} (MeOH), (2.24 ± 0.23) ⋅ 10^{-8} (DCE). DCE is 1,2-di-
chloroethane.

Figure 4. Rate of degradation of two different BDPA-nitroxide biradicals in
various solvents (5 mm at 23 °C). The percentage of each biradical relative to
a nitroxide monoradical, determined by double integration of its EPR spec-
trum, was plotted as a function of time and the initial rates of decomposi-
tion were calculated accordingly (see the Supporting Information). A. Biradi-
cal 8; rates (Ms^{-1}): (1.50 ± 0.09) ⋅ 10^{-8} (DMSO), (1.79 ± 0.03) ⋅ 10^{-8} (MeOH),
(1.79 ± 0.35) ⋅ 10^{-7} (DCE). B. Biradical 3; rates (Ms^{-1}): (2.28 ± 0.15) ⋅ 10^{-8}
(DMSO), (7.18 ± 0.64) ⋅ 10^{-8} (MeOH), (2.24 ± 0.23) ⋅ 10^{-8} (DCE). DCE is 1,2-di-
chloroethane.

Figure 5. Concentration-dependent initial rates of biradical 8 in DMSO at
23 °C.

with varying concentration at 23 °C. The rate of degradation
was found to increase non-linearly with increasing radical con-
centration. A 2-fold increase in the concentration led to a 3-
fold increase in the rate of degradation, which suggests a reac-
tion order of ca. 1.6 with respect to biradical 8. This result indi-
cates that the well-known reaction with oxygen^{18} may not be
the only degradation pathway since the oxidation is presum-
ably a first/pseudo-first order reaction with respect to the biradical.
One plausible explanation is that dimerization of BDPA radicals,
which follows a second order kinetics, is also a pathway for degradation. Analysis of the decomposed products of bi-
radical 8 by electrospray ionization (ESI) mass spectrometry indi-
cated formation of multiple products, including the oxygenated
products as previously described by Breslin and Fox^{18}, but the dimer of 8 was not found (Figure S12). Instead, masses
approximately 1.8-fold the monomeric mass of 8 were ob-
erved which might be formed from a short-lived dimer. Howev-
ner, we were able to detect a BDPA dimer in the mass spec-
trum of decomposed products of BDPA radical S1, obtained
from compound 4 (Figure S13). This is, to our knowledge,
the first reported indication that dimer formation is a significant
pathway for decomposition of BDPA radicals. However, the fact
that BDPA radicals dimerize is not surprising, since dimerization
of the carbon-centered Gomberg’s radical^{24} (triphenylmethyl/
trityl) is well-known^{23}. In case of the trityl, introduction of sub-
stituents into the aromatic rings has been shown to prevent
dimerization, with the Finland trityl being a good example^{13}.

To compare the persistence of biradical 8 with other BDPA
radicals, the previously reported BDPA-TEMPO biradical 3^{17}
and unsubstituted BDPA radical 2 (Figure 1) were synthesized.
These radicals are less polar than 8. Figure 4B shows the sol-
vent-dependent rate of degradation of biradical 3 in solution
at 23 °C. It was found to be similar to that of 8, although the
solvent-dependence on the rates of decomposition was differ-
ent. Both 3 and 8 have similar initial rate of degradation in
DMSO (2.28 ± 0.15) ⋅ 10^{-8} Ms^{-1}) but different rates in DCE and
MeOH. In DCE, biradical 3 was considerably more persistent
than 8 with an initial rate of degradation (2.24 ± 0.23) ⋅ 10^{-8} Ms^{-1}), whereas the opposite trend was observed in
MeOH ((7.18 ± 0.64) ⋅ 10^{-8} Ms^{-1}). The unsubstituted BDPA (2)
showed a similar rate of degradation in solution (Figure S8) as
the biradicals and the same solvent dependence as 3 (3.71 ± 0.12) ⋅ 10^{-8} Ms^{-1}) and (4.64 ± 0.18) ⋅ 10^{-8} Ms^{-1} for DCE and DMSO,
respectively. In light of the concentration dependence of
BDPA decomposition, a possible explanation for this com-
ound-specific solvent effect is aggregation, since the polar
biradical 8 degrades faster in a non-polar solvent and non-
polar 2 and 3 are more unstable in a polar solvent. Aggrega-
tion would increase the rate of decomposition through dimer
formation.

When the temperature was decreased, the degradation of 8
became slower, as expected. The rates decreased by ca. 6-fold
for both DCE and MeOH by lowering the temperature from
23 °C to −18 °C (Figure S9A). On the other hand, the rate of degradation of 8 in DMSO at −18 °C was unexpectedly ob-
served to be higher ((3.86 ± 0.62) ⋅ 10^{-8} Ms^{-1}) than at 23 °C
((1.50 ± 0.09) ⋅ 10^{-8} Ms^{-1}). One plausible explanation is that the
radical aggregates due to accumulation of solutes at the crys-
tal boundaries of the frozen DMSO. Quick-freezing in liquid ni-
trogen followed by incubation at −18 °C, in an attempt to pre-
vent possible aggregation, gave the same result. When the temperature of the DMSO solution was further lowered to 
−80 °C, a significant increase in the persistence was observed; very little decomposition was observed after a month and
after six months ca. 55% biradical remained (Figure S9B). Similar decomposition was observed in DCE after six months at
−80 °C but ca. 40% biradical remained in MeOH under the same condition. Biradical 2 was found to be stable in the solid
state at −80 °C, with no degradation for six months (Figure S9B). However, only ca. 38% biradical was intact after a
month in the solid state at 23 °C (Figure S10A). BDPA radical 2 was also observed to decompose in the solid state under ex-
posure to air (Figure S10B), contrary to a previous report. This degradation was due to oxidation since no decomposition
was observed when the solid samples were kept under vacuum for two weeks (Figure S10B). Hence, BDPA radicals
cannot be termed ‘stable’, as once considered. However, the BDPA radicals showed no detectable decomposition when
kept at −80 °C in the solid state for longer periods of time (six months).

It is notable that exclusion of light, which has been reported to limit the reaction of BDPA radicals with oxygen, had
noticeable effects on the rate of decomposition. Formation of the same products was observed in presence and absence of
light, both by ESI mass spectrometry and high-performance liquid chromatography (HPLC) (Figure S11B). Removal of oxygen
by saturation with Ar and keeping the solution under a positive pressure of Ar, decreased the rate of degradation
only by 1.5-2-fold (Figure S11A).

Conclusions

In conclusion, we have shed some light on the instability of BDPA radicals, both in solution and the solid state, which pro-
vides guidelines of how to handle BDPA radicals that are prepared for MAS-DNP NMR experiments. The strong electronic
coupling between the BDPA and nitroxide radicals of biradical 8 enabled quantification of its rate of decomposition by EPR
spectroscopy. The BDPA radicals reported here were found to be stable as solids at −80 °C with no noticeable decomposition for
decisively six months, but under all other conditions we observed degradation in this time-frame. The radical concentration was
found to significantly affect the rate of decomposition non-linearly; a 2-fold increase in the concentration resulted in a 3-fold increase in the rate of decomposition. A dimer of BDPA radical S1 was detected by mass spectrometry, indicating that dimer-
ization of BDPA radicals is one pathway of decomposition. The polarity of the solvent affected the rate of BDPA decomposi-
tion: a polar radical degraded faster in a non-polar solvent whereas a non-polar radical was less persistent in a polar sol-
vent. In light of the concentration-dependent persistence of BDPA radicals, the solvent-dependent increase in the rate of
decomposition is likely due to aggregation of the radicals, which would facilitate decomposition through dimer forma-
tion. When synthesizing new BDPA radicals that are more persistent as polarizing agents for DNP, it might be advisable to
focus on structures that prevent dimer formation. Synthesis of

more stable BDPA radicals, keeping this design principle in mind, is underway in our laboratory and will be reported in
due course.

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Conflict of interest

The authors declare no conflict of interest.

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