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Water-soluble BDPA radicals with improved persistence†

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1,3-Bis(diphenylene)-2-phenylallyl (BDPA) radicals are promising polarizing agents for increasing the sensitivity of NMR spectroscopy through dynamic nuclear polarization (DNP), but have low persistence and solubility in aqueous media. New tetraalkyl/aryl-ammonium derivatives of BDPA are soluble in polar solvents and are highly persistent, with 5–20-fold lower initial rates of degradation than BDPA.

Solid-state nuclear magnetic resonance (ssNMR) spectroscopy with magic angle spinning (MAS), is a valuable technique to acquire high-resolution structural information of heterogeneous systems, such as amyloid fibrils,[1] membrane proteins[2] and heterogeneous catalysts.[3] However, a major challenge of NMR spectroscopy is its low sensitivity due to the small population difference of nuclear spins in the ground state *vs.* the excited state under an applied magnetic field. For instance, this difference is only ~0.01% for protons at 9.4 T (400 MHz) and 100 K. This low nuclear polarization can, however, be significantly increased using dynamic nuclear polarization (DNP), leading to enhanced NMR signals. DNP enables the transfer of polarization to nuclei from unpaired electrons, for which the polarization in a magnetic field is more than two to three orders of magnitude higher than that of the nuclear spins, using microwave irradiation.[5] Persistent organic radicals are commonly introduced into samples during DNP experiments as the source of unpaired electrons.

Nitroxides are the most common choice of radicals for use as polarizing agents, due to their high persistence, relative ease of synthesis and electron paramagnetic resonance (EPR) properties. In particular, the broad EPR linewidth of the nitroxides makes them highly effective for the cross-effect (CE), which is the most efficient polarization transfer mechanism for MAS–DNP NMR.[6] CE is a three-spin mechanism involving two electrons and a nucleus, and requires a polarizing agent that has an EPR linewidth broader than the nuclear Larmor frequency.[6] Therefore, biradicals that have two strongly coupled electron spins are more effective than monoradicals.[8] Nitroxide biradicals with improved DNP performance have been prepared in years past, mostly by trial and error.[9] More recently, advanced simulations have been applied for the design of more efficient polarizing agents.[4] An example is **AsymPolPOK** (Fig. 1), which is currently one of the most efficient nitroxide biradicals for DNP; the two nitroxides are linked by a short tether and have an orthogonal orientation relative to each other, resulting in large *J*-coupling and dipolar interactions between the two electron spins.[4]

Recent technological advancements have enabled DNP NMR at high magnetic fields (≥18.8 T).[10] However, the efficiency of nitroxides, as polarizing agents, decreases substantially with increasing magnetic field, due to a considerable broadening of their EPR spectra.[6b] In contrast, the carbon-centered Finland trityl[11] and 1,3-bis(diphenylene)-2-phenylallyl (**BDPA**)[7] (Fig. 1) radicals have significantly narrower EPR linewidths than nitroxides[12] and longer electron relaxation rates,[13] making them promising polarizing agents at high magnetic fields. When connected to a nitroxide, they give significantly higher signal enhancements than nitroxide biradicals,[14] because the EPR transition of the narrow-line, slow-relaxing carbon radical can be efficiently saturated while the dipolar-coupled fast-relaxing nitroxide ensures multiple polarization transfers to the nuclei.[14b,15] Another advantage of these carbon-based heteromeric biradicals

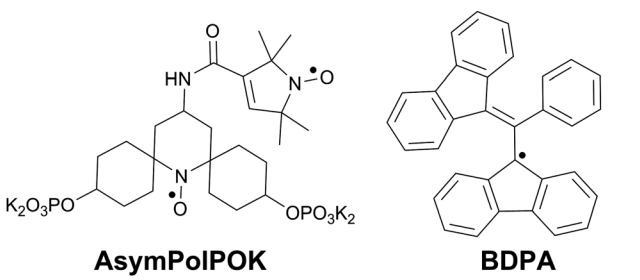

Fig. 1 Structures of **AsymPolPOK**[4] and **BDPA**[7] radicals.

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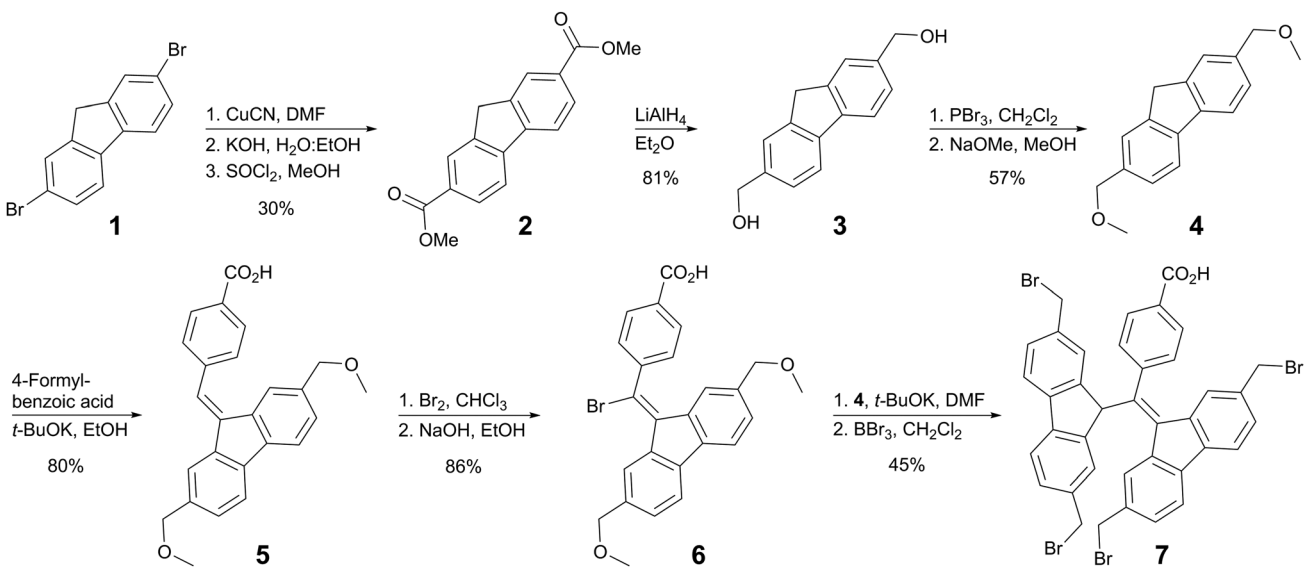
over most nitroxide biradicals is that they produce lower nuclear depolarization, a process that decreases the effective gain in sensitivity.[4,16]

An advantage of the BDPA over the Finland trityl radical is its relative ease of synthesis.[11,17] However, BDPA radicals have seen limited use for DNP,[14c,18] due to two major obstacles. First, the lack of solubility of BDPA radicals in aqueous media limits their applications for biomolecules. A BDPA derivative that contains two carboxylates has been prepared but still has insufficient solubility in aqueous media for DNP applications,[19] while persulfonated BDPA, a mixture of radicals with different degrees of substitution, cannot be readily conjugated to nitroxides.[20] Second, BDPA radicals have limited persistence in solution, primarily due to dimerization, as we have recently reported.[21] Here we describe a new class of BDPA radicals that addresses these two shortcomings of BDPA radicals for DNP. These BDPA radicals contain four positively charged ammonium groups that enhance solubility in aqueous solutions, in addition to increasing their persistence. We also demonstrate that these new BDPA radicals can be used to prepare water-soluble BDPA–nitroxide biradicals.

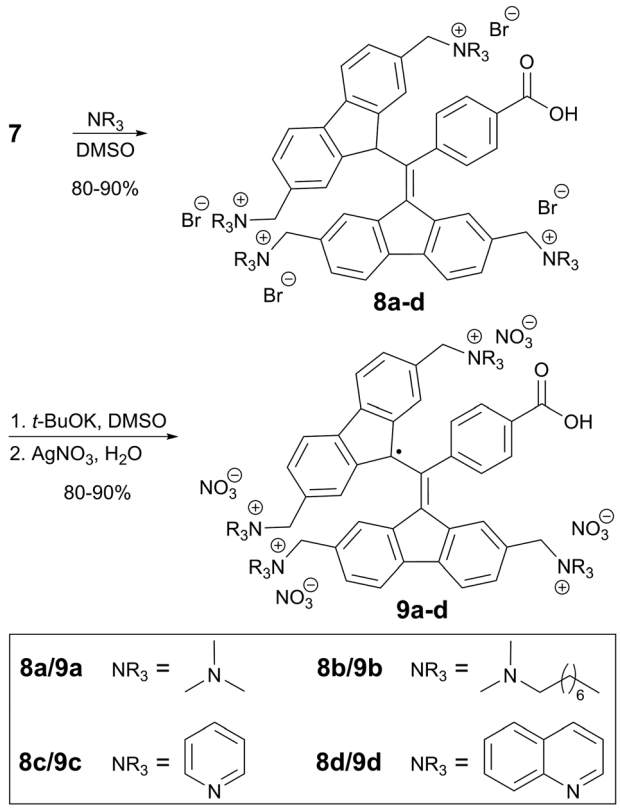
The strategy for the preparation of water-soluble BDPA-based radicals was to attach charged functional groups to BDPA that should also enhance their persistence by preventing decomposition through dimerization.[21] The 2,7-positions of fluorene were selected for incorporation of tetraalkyl/arylammonium groups. These positions can be readily functionalized and enable incorporation of four charged groups into the BDPA radical. We chose to incorporate an isolating methylene linker between the fluorene ring and these functional groups to prevent their interaction with the BDPA radical, which is delocalized in the fluorene rings.[22]

The synthesis started with the incorporation of cyano groups by the Sandmeyer reaction of 2,7-dibromofluorene (**1**), followed by their hydrolysis (Scheme 1).[23] Purification of the resulting fluorene-2,7-dicarboxylic acid was not feasible due to its extremely limited solubility. Consequently, the carboxylates were converted to esters to yield fluorene derivative **2** and subsequently reduced to afford diol **3** in good yields. The hydroxyl groups were protected as methoxy ethers to provide **4**, since the presence of the unprotected hydroxyl groups led to very low yields in the following steps. Condensation of **4** with 4-formylbenzoic acid afforded **5** and bromination, followed by elimination gave **6** in excellent yields. Compound **6** was further coupled with another unit of 2,7-dimethoxymethylfluorene (**4**) with subsequent conversion of the methoxy ethers to bromides to yield tetra-(bromomethyl)–BDPA derivative **7**.

The benzylic bromides of **7** could be readily substituted with nucleophiles. Hence, compound **7** was used as a building block to synthesize a series of BDPA radicals with different tetraalkylammonium groups, to demonstrate the generality of this approach for the preparation of derivatives with custom-made physical properties (Scheme 2). In addition to trimethylamine, the more lipophilic *N*,*N*-dimethyloctylamine was selected, along with the aromatic amines pyridine and quinoline. Reaction of these amines with **7** afforded compounds **8a–d** in good yields. It is noteworthy that the products could be easily isolated from the reaction mixture by precipitation. These compounds had good solubility in "DNP juice"[24] (glycerol : water, 6 : 4), a solvent commonly used for DNP–NMR, and their relative variance in solubility reflected the nature of the substituents on the tetraalkylammonium groups; **8a** had the highest solubility (>150 mM) and the more lipophilic **8b** the lowest (20 mM), while the pyridinium and the quinolinium derivatives had intermediate solubility (**8c**, 65 mM; **8d**, 45 mM). Sequential treatment of compounds **8a–d** with *t*-BuOK and $AgNO_3$ yielded BDPA radicals **9a–d**; again the products were isolated from the reaction mixture by precipitation. As is common for such reactions,[21] *ca.* 85% of the products were radicals, as determined by spin-counting using EPR spectroscopy (ESI†).



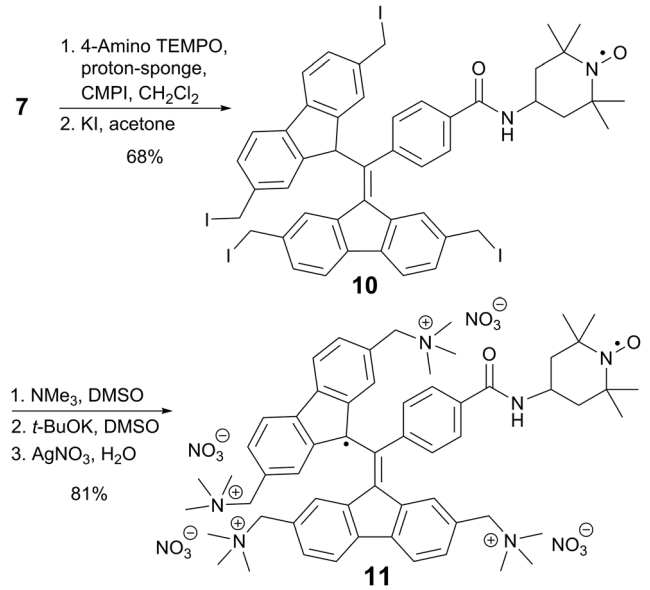
Scheme 1 Synthesis of tetra-(bromomethyl)–BDPA carboxylic acid **7**.



Scheme 2 Synthesis of tetraalkyl/aryl-ammonium BDPA derivatives **9a–d**.

The carboxylic acid group present in the *para*-position on the benzene ring of BDPA derivative **7** was included for the conjugation of other radicals to BDPA, for the purpose of preparing water-soluble BDPA-based biradicals. To demonstrate the synthesis of a BDPA–nitroxide biradical, compound **7** was coupled with 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (4-amino TEMPO) and the resulting compound **10** was treated sequentially with trimethylamine, *t*-BuOK and $AgNO_3$ to give **11** (Scheme 3). This is, to our knowledge, the first reported example of a water-soluble BDPA–nitroxide biradical. The EPR spectrum of **11** shows three sharp lines due to a strong interaction between the BDPA and the nitroxide radical (ESI†); a *J*-coupling of 140 MHz between the two paramagnetic centers has been determined for a BDPA–TEMPO biradical with the same core structure.[17]

Having demonstrated the improved solubility of the BDPA radicals in a polar solvent, we investigated their persistence by monitoring their absorbance[25] at 503 nm. Fig. 2 shows a plot of the persistence of **9a** as a function of time in four different solvents: DMSO, DNP juice, MeOH and water. Radical **9a** could also be quantified by EPR spectroscopy;[21] the data for DNP juice are shown in Fig. S25B (ESI†). The persistence was remarkably high in DMSO (Fig. 2), with an initial rate of degradation *ca.* 20-fold less than what we had previously observed for BDPA radicals.[21] After two weeks in DMSO at 23 °C, only *ca.* 10% of the radical had degraded; this is an unprecedented degree of persistence for BDPA radicals.



Scheme 3 Synthesis of the water-soluble BDPA–TEMPO biradical **11**.

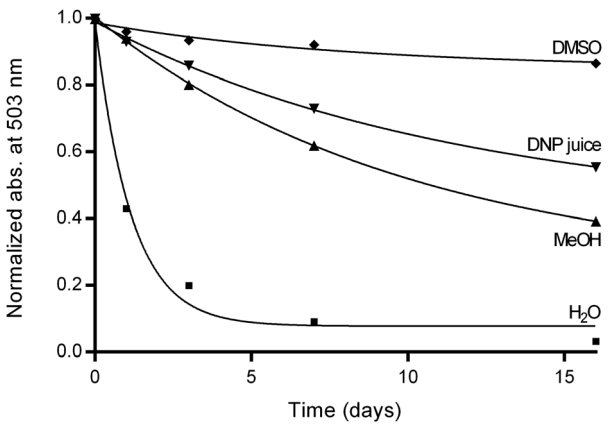


Fig. 2 Persistence of the water-soluble BDPA radical **9a** in solution (10 mM) at 23 °C, monitored by UV-vis spectroscopy at 503 nm. The initial rates of degradation are as follows: $\sim 2.0 \times 10^{-9}$ M s^{-1} (DMSO, ♦), $\sim 5.8 \times 10^{-9}$ M s^{-1} (DNP juice, ▼), $\sim 8.9 \times 10^{-9}$ M s^{-1} (MeOH, ▲), $\sim 92.1 \times 10^{-9}$ M s^{-1} (H_2O, ■).

A large variation in solvent-dependent persistence was observed for **9a** (Fig. 2), **9b**, **9d** and **11** (ESI†). The persistence of **9a** was markedly lower in polar protic solvents than it was in DMSO; *ca.* 40% and 50% of the radicals had degraded in DNP juice and MeOH, respectively after two weeks. However, the rate of degradation in MeOH was still at least 5-fold lower than what has been previously observed with BDPA radicals.[21] The data shown in Fig. 2 were collected at 23 °C, negligible degradation was observed in DNP juice at −80 °C after one month (Fig. S28, ESI†).

The persistence of **9a** was much lower in water than in other solvents (Fig. 2), with *ca.* 50% decomposition within 48 h. Since dimerization is a major decomposition pathway for BDPA radicals in solution,[21] we determined the concentration dependence of the initial rate of degradation for a series of aqueous solutions of **9a**.

Indeed, we observed a non-linear concentration-dependence,[21] with a reaction order of *ca.* 1.6 with respect to the radical (Fig. S29, ESI†). Moreover, we observed a major peak in the mass spectrum of **9a** in water after 48 h corresponding to the dimer of **9a** (Fig. S31, ESI†).

It may seem counterintuitive that **9a** would aggregate in water. However, there have been reports of aggregation of tetraalkylammonium salts in water.[26] It has been postulated that this may be due to the electrostatic attraction between the positively charged nitrogen and the counter anion, as well as hydrophobic interactions between the alkyl substituents.[26] An attempt to reduce aggregation of **9a** in water by increasing the ionic strength resulted in a decrease of its persistence; the initial rate of degradation increased *ca.* 2-fold in the presence of 100 mM NaCl (Fig. S30, ESI†). DMSO has been shown to reduce the aggregation of ammonium–functionalized polythiophene relative to water,[27] which is consistent with our results.

In summary, we have described the synthesis and characterization of a series of water-soluble tetraalkyl/aryl-ammonium BDPA radicals, including a BDPA–nitroxide biradical. These BDPA radicals can be readily used to prepare heterobiradicals as polarizing agents for investigation of biomolecules by MAS–DNP NMR spectroscopy at high magnetic fields. The radicals show significantly improved persistence in solution,[21] presumably due to a reduced tendency to aggregate. Although the persistence of these radicals in water was substantially less than in DMSO, the tetrabromo intermediate **7** can be used to prepare a wide variety of derivatives with tailor-made properties, including limited aggregation in water.

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Conflicts of interest

There are no conflicts to declare.

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