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Dynamic Nuclear Polarization with a Water-soluble Rigid Biradical

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Supporting Information Placeholder

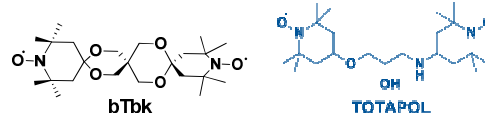
ABSTRACT: A new biradical polarizing agent, bTbtk-py, for dynamic nuclear polarization (DNP) experiments in aqueous media is reported. The synthesis is discussed in light of the requirements of the optimum, theoretical, biradical system. To date, the DNP NMR signal enhancement resulting from bTbtk-py is the largest of any biradical in the ideal glycerol/water solvent matrix, $\epsilon = 230$. EPR and X-ray crystallography are used to characterize the molecule and suggest approaches for further optimizing the biradical distance and relative orientation.

Dynamic nuclear polarization (DNP)¹⁻³ allows for the intrinsically large spin polarization of electrons to be transferred to nuclei for detection in an NMR experiment with a theoretical signal enhancement of 2-3 orders of magnitude over the Boltzmann population of the nuclear spins. In a typical DNP experiment, the electron paramagnetic resonance (EPR) spectrum of a paramagnetic molecule is irradiated with microwaves resulting in the transfer of spin polarization to surrounding nuclei. The particular appeal of DNP lies in its ability to facilitate structural measurements (internuclear distances and torsion angles) that are limited by signal-to-noise in multidimensional biomolecular magic angle spinning (MAS) experiments^{4,5}. In these situations, optimal NMR signal enhancements are usually observed from experiments conducted at cryogenic temperatures^{6,7}. Thus, glassy solvents, particularly those that form a glass regardless of cooling rate, are required to ensure a homogeneous dispersion of polarizing agent and to prevent cold denaturation of proteins^{8,9}.

The proper instrumentation (microwave sources, low temperature MAS probes, etc.)^{7,10-12} is essential to the successful implementation of DNP. However, an equally important aspect of the methodology is the development of polarizing agents from which large signal enhancements, ϵ , are observed. Currently, the most efficient high field *in situ* DNP mechanism, the cross effect (CE), arises from the interaction of three spins, two electrons and one nucleus¹³⁻²¹. Irradiation of the EPR spectrum results in a spin “flip-flop” process between the electrons that leads to transfer of polarization to the nucleus. The process is most efficient when the difference in the Larmor frequencies of the electrons matches that of the nucleus, $\omega_{0S1} - \omega_{0S2} \approx \omega_{0I}$, where ω_{0S} and ω_{0I} are the Larmor frequencies of the electron spin S or nuclear spin I , respectively. Although monomeric nitroxide radicals were among the early cross effect DNP agents^{18,19,22}, tethering the radicals resulted in larger signal enhancements with lower paramagnet concentration²⁰.

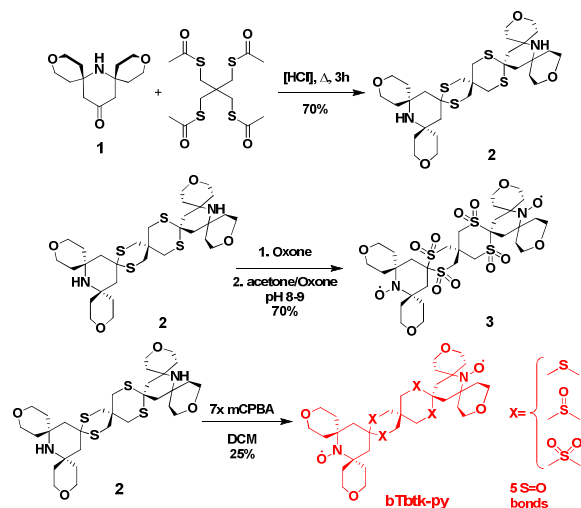
In particular, because the low temperature EPR spectra of nitroxide radicals are anisotropic, the construction of discrete biradical systems permits control of the relative orientation of the g -tensors of the radicals and their separation, hence further augmenting ϵ .

Simulation of field dependent EPR spectra of the biradical TOTAPOL suggests that its nitroxide moieties are, somewhat serendipitously, of near-optimal orientation for DNP²³. Indeed, no other biradical that is soluble in glycerol/water (60/40) has been reported to yield a higher ϵ . DNP from the highly crystalline bTbk in DMSO/water mixtures did yield a larger ϵ than TOTAPOL²⁴. However, this spiro-cyclic biradical is insoluble in glycerol/water mixtures and only sparingly soluble in DMSO/water. This limits the applicability of bTbk as a polarizing agent because glycerol/water serves as a cryoprotectant and is the solvent of choice for DNP of proteins. The near-ideal geometry and experimental results with bTbk and the reported synthesis of **1**²⁵ stimulated us to investigate water soluble analogues of bTbk.



Starting from the modified TEMPone structure, **1**, and drawing upon previous research²⁶, the bis-thioketal was made in moderate yield, Scheme 1. The fully oxidized biradical **3** was generated in a two stage oxidation using potassium peroxy-monosulfate as the oxidant. The low pH of the first oxidation step acts as a protecting group for the amines²⁷, which are subsequently oxidized under basic conditions using dimethyl dioxirane generated *in situ* from the oxidation of acetone^{28,29}. Biradical **3**, however, is effectively insoluble in glycerol/water mixtures (solubility <0.1 mM). Accordingly, we decided to pursue a mixed sulfur oxidation state biradical with the knowledge that a mixture of species should enhance solubility. The partial oxidation of **2** was performed by the reaction with *m*-chloroperoxybenzoic acid (*m*-CPBA). The resulting mixture, bTbtk-py, an extension of the established naming system short for bis-TEMPO-bis-thioketal-tetra-tetrahydropyran²⁴, was characterized by NMR (the reduced form having N-OH groups), CHN analysis and electrospray ionization high resolution mass spectrometry (HRMS-ESI), and it was found to be a biradical possessing a mixture of sulfur oxidation states, 0-5 S=O bonds. The X-band EPR spectrum is typical for a nitroxide radical; the broad 1:1:1 triplet due to hyperfine coupling to a nitrogen, $a_{\text{iso}}(^{14}\text{N}) = 41.8$ MHz, is consistent with a biradical featuring a short, rigid tether with a weak electron-electron J -

coupling (exchange integral), $J < 10 \cdot a(^{14}\text{N})$ ^{23,30}. The biradical exhibited excellent solubility in the desired 60/40 glycerol/water mixture (10.9 mM in glycerol/water (60/40); 3.0 mM in D₂O).



Scheme 1. Synthesis of bTbtk-py.

The DNP-enhanced ¹³C-CPMAS NMR of ¹³C-urea (1M in 60/40 glycerol/water) showed $\epsilon = 230$ versus thermal polarization. Experimentally, ¹³C-urea and bTbtk-py in the glassy glycerol/water matrix is continuously irradiated with microwaves under cryogenic ($T \approx 82$ K) MAS conditions to polarize the ¹H's, and this polarization is transferred to ¹³C for detection via a cross-polarization step. In the absence of microwaves, the thermal equilibrium polarization develops. In either event, the signal intensity as measured after a presaturation sequence versus recovery time shows a mono-exponential polarization build up, Figure 1. The enhancement, ϵ , is given by the ratio of signal intensity with the microwaves on versus microwaves off. Interrogated under identical conditions, TOTAPOL shows a lower enhancement factor, $\epsilon = 191$. The direct comparison with an external standard, TOTAPOL in this case, is crucial in order to determine the relative performance of different polarizing agents. Absolute enhancements are greatly influenced by instrumental (e.g. rotor size, microwave coupling to the sample) and experimental (e.g. MAS frequency, sample temperature, optimum magnetic field) parameters, thus impeding comparison between studies performed under varying conditions. The DNP enhancement derived from bTbtk-py constitutes the largest signal enhancement observed so far for any biradical in a biologically relevant glycerol/water mixture under given conditions³¹⁻³⁴.

Another metric of the efficiency of a polarizing agent is given by measuring ϵ as a function of microwave power and extrapolating the enhancement to infinite power, ϵ_{∞} in Figure 2. This factor, ϵ_{∞} , is independent of microwave power, P_{mw} , and the saturation parameter, a , which depend on microwave transmission efficiency and EPR relaxation properties^{23,24}. However, care must be taken to control sample temperature and rotor size.

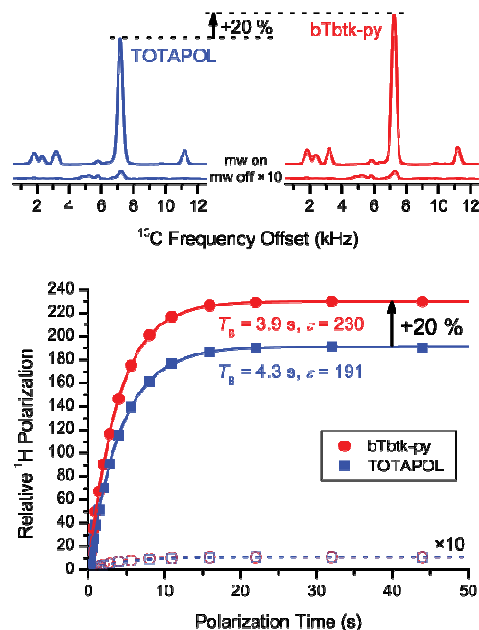


Figure 1. DNP enhanced polarization was measured under microwave irradiation using a gyrotron beam current of 34 mA, which corresponds to ~ 12 W microwave power. (Upper) ¹³C-NMR (CPMAS) of 1M urea-¹³C in 60/30/10 glycerol-d₈/D₂O/H₂O (v/v/v) with 10 mM of the appropriate biradical acquired with and without microwave irradiation. (Lower) Build-up curves were recorded at the respective field yielding the maximum enhancement for each radical (i.e. 4.982 T for bTbtk-py, 4.980 T for TOTAPOL; see Figure 3) and subsequently scaled in order to normalize the off signal intensities at $t = \infty$. Proton longitudinal relaxation time constants were determined as $T_{11} = 3.9$ s for bTbtk-py and $T_{11} = 4.5$ s for TOTAPOL. Note that aside from the radical used and the magnetic field strength, the experimental conditions are the same for each trace.

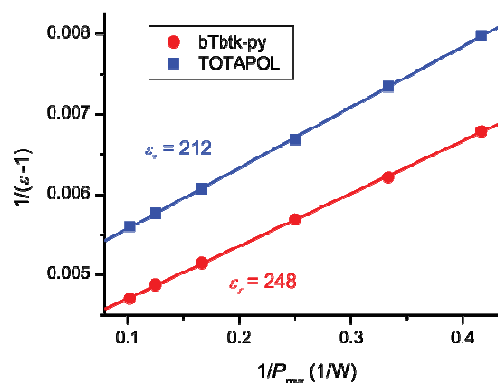


Figure 2. Power dependence of the steady-state DNP enhancement ϵ_{∞} for bTbtk-py and TOTAPOL given by: $1/\epsilon = 1/\epsilon_{\infty}(1+1/aP)$. The experiments with the two polarizing agents were carried out at the field position giving the optimum enhancement for each. Otherwise, the experimental conditions were identical (see supporting information).

The enhancement field profile of a polarizing agent shows the NMR signal intensity as a function of magnetic field and, hence, the electron Larmor frequency and provides the field for optimum enhancement. The breadth and intensity of the enhancement profile of bTbtk-py versus TOTAPOL (Figure 3) suggest that the nitroxide moieties have slightly different rela-

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tive orientation and electron-electron coupling in the two biradicals. Nevertheless, the shape of the enhancement profile for bTbtk-py is qualitatively similar to that of all nitroxide biradicals whose electron-electron dipolar coupling (~ 20 MHz) is small relative to the g -anisotropy and $a(^{14}\text{N})$ ^{20,23,24}, yet the different ϵ , vis-à-vis that of TOTAPOL, corroborates the observation that enhancement is extremely sensitive to electron-electron distance and relative orientation. Further, the inhomogeneous line shape of the EPR spectrum and the field profile, shown in Figure 3, is consistent with bTbtk-py supporting the cross-effect DNP mechanism.

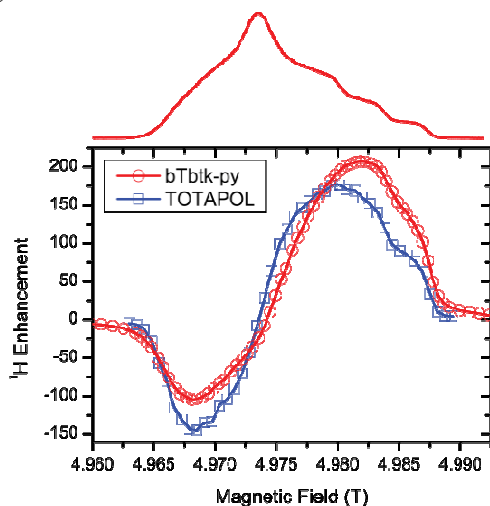


Figure 3. (upper) EPR spectrum of bTbtk-py measured by echo detection at 140 GHz and $T=80$ K and (lower) field enhancement profiles of bTbtk-py and TOTAPOL (see supporting information). The enhancement profile was recorded by directly observing the ^1H polarization under ~ 8 W of microwave power using a Bloch decay and scaling the obtained data with respect to the maximum enhancement determined by cross-polarization to ^{13}C . TOTAPOL field profile adapted from Hu, et al.²³

This study indicates that bTbtk-py is superior to TOTAPOL as a DNP biradical polarizing agent by a factor of ($\epsilon_{\text{bTbtk-py}}/\epsilon_{\text{TOTAPOL}}=$) 1.20 ± 0.02 . However, previous studies in non-ideal solvents have determined this ratio is larger for bTbk, $\epsilon_{\text{bTbk}}/\epsilon_{\text{TOTAPOL}}= 1.41\pm 0.09$. The difference in these numbers belies the similar architecture of the two spiro-biradicals. Therefore, suggesting why bTbtk-py ‘underperforms’ in ϵ may guide the development of the next generation of biradicals for DNP.

Differences in NO-NO distance change the dipolar coupling between the two electrons, with larger distances leading to smaller couplings. The dipolar coupling, in conjunction with the J -coupling, leads to the state mixing that is critical for efficient cross effect.²¹ Note that in the case of TOTAPOL, bTbk, and bTbtk-py the dipolar coupling is the dominant contributor to state mixing. Therefore, the structural differences in bTbk and bTbtk-py, including the NO-NO distance, were examined by measuring the electron-electron dipole coupling with double electron-electron resonance (DEER). The DEER-measured inter-electron distances (r) were determined to be $r_{\text{bTbk}}= 11.82\pm 0.06$ Å and $r_{\text{bTbtk-py}}= 13.19\pm 0.11$ Å. The measured r_{bTbk} agrees quite well with the average NO-NO distance measured from the crystal structure, 11.8 Å; the dihedral angle between the NO vectors, 82° , was also measured. While bTbtk-py would not crystallize in our hands, the fully oxidized

form, **3**, proved highly crystalline, and an X-ray structure was obtained, Figure 4. The average NO-NO distance was measured, 13.33 Å, along with the NO dihedral angle, 77° . In bTbtk-py, the twist-boat conformation of the nitroxide moieties may be acting synergistically with the increased (vis-à-vis bTbk) ketal ring size to produce the larger NO-NO distance and smaller NO dihedral angle. Insofar as structure determines DNP enhancement, the non-ideal NO-NO relationships might be improved by removing the THP moieties or sulfone bridges. Certainly, the lower DNP enhancement of bTbtk-py relative to bTbk suggests avenues for the further exploration of the bTbk structure space.

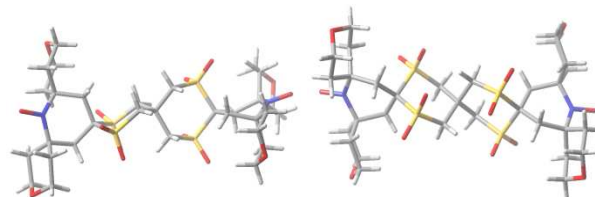


Figure 4. Two views of the X-ray crystal structure of **3**.

In summary, the reported biradical, bTbtk-py, yields the largest enhancement, $\epsilon = 230$, to date of any biradical in the ideal glycerol/water solvent matrix. The spiro-cycle structure is highly crystalline in its fully oxidized form (**3**), but a partially oxidized mixture of species is soluble in the desired medium. The ^{13}C -NMR signal enhancement has been shown to depend with *extreme* sensitivity upon the modulation of the electron-electron distance and relative orientation of the NO moieties of the biradical. This extreme sensitivity suggests that additional synthetic fine tuning of the linkers separating nitroxide radicals can produce superior DNP polarizing agents.

ASSOCIATED CONTENT

Supporting Information. Experimental details, DEER spectra, crystallographic details and a CIF file. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Author Contributions

The manuscript was written through contributions of all authors.

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