Published in final edited form as:

Chemistry. 2011 September 19; 17(39): 10825–10827. doi:10.1002/chem.201102037.

BDPA: An Efficient Polarizing Agent for Fast Dissolution Dynamic Nuclear Polarization NMR

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Keywords

dynamic nuclear polarization; radical; NMR spectroscopy; relaxation; hyperpolarization

Fast dissolution-dynamic nuclear polarization (DNP)^[1] is the latest and most successful attempt to solve the problem of low sensitivity in liquid-state nuclear magnetic resonance (NMR). The most prominent applications of this technology are *in vitro* and *in vivo* NMR spectroscopy and imaging with hyperpolarized ¹³C which has been used to study biochemical/metabolic activities in various tissues (heart, liver, tumors) in *real time*.^[2–4] Non-biological applications of this technique include the monitoring of transient chemical kinetic reactions and fast sample characterization via one- and two-dimensional high resolution NMR spectroscopy.^[5,6]

The choice of free radicals is critical in DNP. Mounting evidence^[7–9] show that higher nuclear polarization levels can be achieved when radicals with narrower EPR linewidths *D* are used. Currently, the water soluble trityl-based stable free radicals (Figure 1) are the most frequently used polarizing agents in ¹³C and ¹⁵N dissolution DNP because their EPR linewidth is among the narrowest (*D* = 60–70 MHz).^[9] Although the physical principles of DNP have been described in detail,^[10] the chemical ramifications of the nature of the radical are not well understood yet. This research was initiated to explore other stable radicals as DNP polarizing agents. Here we demonstrate that the well-known 1,3-bisdiphenylene-2-phenylallyl (BDPA) radical may offer a viable alternative to trityl radicals in dissolution DNP. BDPA (Figure 1), first synthesized by Koelsch in 1957,^[11] is one of the most stable carbon-centered free radicals. It has a comparable *D* to that of trityl radicals and has been used in polarized target experiments and solid-state DNP.^[12–14] However, this radical has never been tried in dissolution DNP, most likely because it is practically insoluble in water and therefore, cannot be used in conventional water-based glassing mixtures such as a glycerol-water matrix. Our preliminary experiments have shown that BDPA is reasonably soluble (over 100 mM) in sulfolane, a dipolar aprotic solvent that can dissolve a wide range

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of compounds. Sulfolane (tetramethylene sulfone) is a crystalline solid at room temperature (m.p. = 27.5 °C); however, it forms a glass at low temperature when mixed with other solvents. To demonstrate the general applicability of BDPA as a DNP polarizing agent, we have polarized a number of compounds in the presence of this radical using the HyperSense commercial polarizer. The DNP experiments were performed as previously described.^[1,2]

The data in Table 1 show that a variety of spin $I = \frac{1}{2}$ nuclei with a gyromagnetic ratio ranging from $\gamma = -2.086$ MHz/T (^{89}Y) to 17.24 MHz/T (^{31}P) and an $I=1$ nucleus ^6Li ($\gamma = 6.2655$ MHz/T) could be polarized with an efficiency comparable to or higher than what has been achieved with trityl radicals. The glassing mixtures usually consisted of sulfolane-diethylene glycol monobenzyl ether mixtures (Supporting Information). Samples containing water-soluble compounds were dissolved with 4 mL water after DNP. The use of water for dissolution offered a very simple way to remove the BDPA radical from the hyperpolarized sample: the radical naturally precipitated out from the solution and could be completely removed by a simple and rapid filtration (Supporting Information). The dissolution of hydrophobic substrates was carried out with methanol, in which the BDPA remained in solution.

Due to its biological importance and favourably long longitudinal relaxation time T_1 , hyperpolarized $[1-^{13}\text{C}]$ pyruvic acid is frequently used to study metabolism in heart, liver, kidney, and tumors.^[2-4] Here we demonstrate that BDPA in sulfolane is capable of producing large NMR enhancements of ^{13}C pyruvic acid samples. The ^{13}C DNP microwave spectra of $[1-^{13}\text{C}]$ pyruvic acid recorded in sulfolane in the presence BDPA and trityl OX063 almost overlap with a separation of 60–70 MHz between the positive and negative polarization peaks (Figure 2a). This close similarity of microwave ^{13}C DNP spectra is the result of the comparable EPR linewidths of the two radicals, indicating the same dominant DNP mechanism (thermal mixing). The optimal concentration of BDPA in pyruvic acid-sulfolane (1:1) samples that would afford maximum polarization in approximately 1 hour of DNP irradiation time was 40 mM (Figures S1 and S2, Supporting Information). Figure 2b shows comparable solid-state ^{13}C polarization P of $[1-^{13}\text{C}]$ pyruvic acid:sulfolane samples doped with the optimum concentrations of trityl OX063 (15 mM)^[15] [$P=12\%$] and BDPA (40 mM) [$P=14\%$]. We note that DNP of neat $[1-^{13}\text{C}]$ pyruvic acid (self-glassing) doped with 15 mM trityl OX063 in the HyperSense yielded $P=15\%$ while TEMPO-doped (40 mM) ^{13}C pyruvate samples in glycerol:water matrix produced lower polarization ($P=5.5\%$) (Supporting Information).

The average liquid-state NMR enhancement ϵ of BDPA-doped pyruvic acid:sulfolane sample was about 14,000 [$P=11\%$] times the thermal signal at 298 K in a 9.4 T magnet whereas the same sample doped with trityl yielded $\epsilon = 12,000$ [$P=9.7\%$]. The slight polarization decrease from solid-state to liquid-state is attributed mainly to T_1 decay and field gradients experienced by the sample during dissolution transfer^[16] (in our case, the transfer time is 8 s). Upon dissolution, the BDPA separated out and could be removed from the hyperpolarized pyruvate solution by filtration (Figures S4 and S5, Supporting Information), while the trityl radical stayed in solution. The absence of BDPA radical in the dissolution liquid after filtration, an important consideration for the production of injectable liquid samples for *in vivo* experiments, was verified by UV-vis spectrophotometry (Figure S6, Supporting Information). The filtered and unfiltered dissolution liquids of samples polarized with BDPA had practically identical T_1 values while the ^{13}C T_1 value was slightly shorter in the sample polarized with the trityl radical due to the paramagnetic effect of the dissolved radical (Figure 2c).

We managed to polarize several other water soluble compounds (maleic acid, choline, pyridine, trimethyl phosphate, and LiCl) to achieve high liquid-state NMR enhancements

of ^{13}C , ^{15}N , ^{31}P and ^6Li nuclei (Table 1) which further demonstrate that BDPA works well with water-soluble substrates.

As one would expect, BDPA in sulfolane is extremely well suited for the hyperpolarization of water-insoluble aromatic and aliphatic hydrocarbons as well as other hydrophobic compounds (Table 1). While usually ^{13}C -enriched substrates are used in *in vivo* hyperpolarized experiments, unlabeled compounds can also be polarized to produce high signal enhancement as demonstrated by the ^{13}C DNP-NMR spectrum of nonlabeled tetramethylallene (Figure 3).

In summary, we have shown that carbon-centered stable free radical BDPA is an efficient polarizing agent in dissolution DNP experiments not only for hydrophobic compounds but also for a number of hydrophilic substrates. Large signal enhancements of ^{13}C , ^{15}N , ^{31}P , ^6Li , ^{29}Si , and ^{89}Y nuclei have been achieved with BDPA using sulfolane-based glassing matrices. After the DNP of water-soluble compounds such as $[1-^{13}\text{C}]$ pyruvic acid or ^{15}N -choline, when water is used as the dissolution solvent, the hydrophobic BDPA radical can conveniently be removed from the hyperpolarized solution by a simple and rapid mechanical filtration process. In addition, water-soluble derivatives of BDPA^[17] may allow the use of water-containing glassing mixtures, thus widening the range of compounds that could be polarized with BDPA-based radicals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by NIH grant numbers 1R21EB009147 and RR02584.

References

1. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Lerche MH, Servin R, Thaning M, Golman K. Proc Natl Acad Sci USA. 2003; 100:10158–10163. [PubMed: 12930897]
2. Merritt ME, Harrison C, Storey C, Mark Jeffrey F, Dean Sherry A, Malloy Craig R. Proc Natl Acad Sci USA. 2007; 104:19773–19777. [PubMed: 18056642]
3. Gallagher FA, Kettunen MI, Brindle KM. Prog Nucl Mag Res Sp. 2009; 55:285–295.
4. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, DeBerardinis RJ, Green GG, Leach MO, Rajan SS, Rizi RR, Ross BD, Warren WS, Malloy CR. Neoplasia. 2011; 13:81–97. [PubMed: 21403835]
5. Bowen S, Hilty C. Angew Chem Int Ed. 2008; 120:5313–5315.
6. Frydman L, Blazina D. Nature Phys. 2007; 3:415–419.
7. Lumata L, Jindal AK, Merritt ME, Malloy CR, Sherry AD, Kovacs Z. J Am Chem Soc. 2011; 133:8673–8680. [PubMed: 21539398]
8. Goertz ST, Harmsen J, Heckmann J, Heb Ch, Meyer W, Radtke E, Reicherz G. Nuc Instr And Meth. 2004; 526:43–52.
9. Heckmann J, Meyer W, Radtke E, Reicherz G, Goertz S. Phys Rev B. 2006; 74:134418.
10. Abragam A, Goldman M. Rep Prog Phys. 1978; 41:395–467.
11. Koelsch CF. J Am Chem Soc. 1957; 22:4439–4441.
12. de Boer W. J Low Temp Phys. 1976; 22:185–212.
13. Hu JZ, Zhou J, Yang B, Li L, Qiu J, Ye C, Solum MS, Wind RA, Pugmire RJ, Grant DM. Sol St Nuc Magn Res. 1997; 8:129–137.
14. Becerra LR, Gerfen GJ, Temkin RJ, Singel DJ, Griffin RG. Phys Rev Lett. 1993; 71:3561–3564. [PubMed: 10055008]

15. Wolber J, Ellner F, Fridlund B, Gram A, Johannesson H, Hansson G, Hansson LH, Lerche MH, Mansson S, Servin R, Thaning M, Golman K, Ardenkjaer-Larsen JH. *Nuc Instr And Meth.* 2004; 526:173–181.
16. Mievile P, Jannin S, Bodenhausen G. *J Magn Reson.* 2011; 210:137–140. [PubMed: 21393034]
17. Dane EL, Swager TM. *J Org Chem.* 2010; 75:3533–3536. [PubMed: 20420445]

Figure 1.
Structure of free radical polarizing agents discussed in this work.

Figure 2.

a) Microwave DNP spectra of 1:1 $[1-^{13}\text{C}]$ pyruvic acid:sulfolane doped with BDPA (40 mM) (●) and trityl OX063 (15 mM) (○). The up and down arrows denote the positive $[P(+)=94.06 \text{ GHz}]$ and negative $[P(-)=94.13 \text{ GHz}]$ polarization peaks, respectively. **b)** Polarization buildup curves of ^{13}C pyruvic acid doped with BDPA (●) and OX063 (○) taken at P(+). **c)** Liquid-state T_1 decay of ^{13}C pyruvic acid samples: BDPA filtered (□), unfiltered (▲) and OX063 (○).

Figure 3. Hyperpolarized (1 scan) and thermal (64 scans) ^{13}C NMR spectra of tetramethylallene after dissolution with methanol at 9.4 T and 298 K. The C4,5,6,7 NMR signal at 21 ppm (Figure S7, Supporting Information) is not shown here because of the lower intensity due to short T_1 .

Table 1

Liquid-state NMR enhancements ($N=3$) measured 8 s after dissolution of various compounds polarized with BDPA (40 mM) and trityl OX063 (15 mM) in sulfolane-based glassing matrices (Supporting Information). The liquid-state T_1 values and NMR enhancements were measured at 9.4 T and 298 K in unfiltered dissolution liquids.

Nucleus/Compound	Dissolution solvent	T_1 (s)	Enhancement BDPA (OX063)
^{13}C -pyruvic acid ^{a,c}	water	42	14,000 (12,000)
^{13}C -urea ^a	water	46	10,010 (10,160)
^{13}C -maleic acid ^a	water	30	8,250
^{13}C -benzene ^a	methanol	33	8,840
^{13}C -tetramethylallene ^b	methanol	59	9,120
^{15}N -choline ^a	water	215	8,000 (8,560)
^{15}N -pyridine ^a	water	40	4,920
^{15}N -nitrobenzene ^a	methanol	100	7,880
^{31}P -triMe phosphate ^b	water	20	5,550
^{29}Si -tetraethylsilane ^b	methanol	88	6,330
$^6\text{LiCl}$ ^a	water	273	6,480
$^{89}\text{Y}(\text{acac})_3$ ^b	methanol	287	3,740
$^{89}\text{Y}(\text{OTf})_3$ ^b	water ^d	460	7,420

^a enriched

^b natural abundance

^c the dissolution liquid consisted of 15 mM NaHCO_3

^d the dissolution liquid consisted of 10-fold excess of diethylenetriaminepentaacetic acid solution (pH 7.8).