A Benzyl Alcohol Derivative of the BDPA Radical for Fast Dissolution Dynamic Nuclear Polarization NMR Spectroscopy

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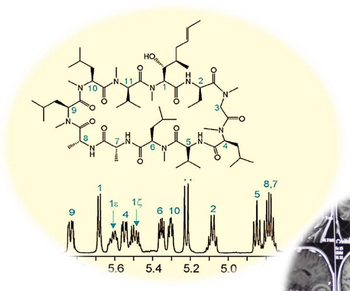
INTRODUCTION

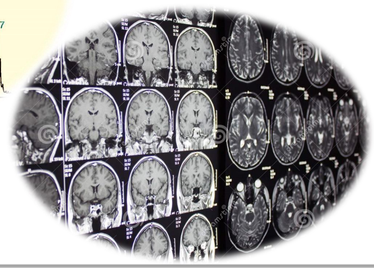
✓ NMR Spectroscopy is a fundamental analytical technique widely used for the identification and characterization of chemical and biochemical molecules

✓ MRI is one of the best non-invasive clinical imaging methods currently used in medicine

The main drawback…

✘ **Poor sensitivity**





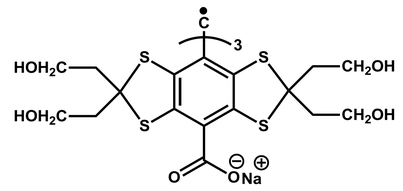
How to enhance NMR sensitivity?

❑ Hardware development:
- Cryogenically cooled probes
- Higher magnetic fields

❑ Hyperpolarization methods:
- Optical pumping
- *para*-hydrogen induced polarization (PHIP)
- **Dynamic Nuclear Polarization (DNP)**

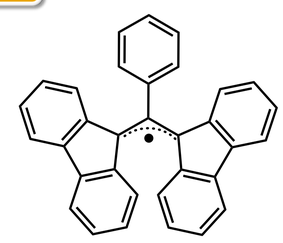
Dissolution DNP

OX63



✓ Narrow EPR linewidth
✓ Soluble into [1-^{13}C]pyruvic acid
✘ Difficult to remove form the hyperpolarization solution
✘ Expensive

BDPA



✓ Comparable EPR linewidth to OX63
✘ Non soluble into [1-^{13}C]pyruvic acid
✘ The use of sulfolane as glassing agent is required

Sample preparation:
- Dissolve the analyte into a glassing solvent
- Add **the polarizing agent**
- Put 10-200 µl into a sample cup

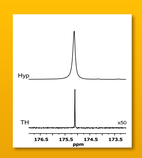
- Load the sample cup into the HyperSense™

Sample conditioning: The sample is cooled to ~1.4 K into a strong magnetic field (Bo = 3.35 T)

Polarizing: Irradiation the polarizing agent free electrons at the appropriate microwave frequency (~ 94 GHz) to transfer its polarization to the nearby nuclei

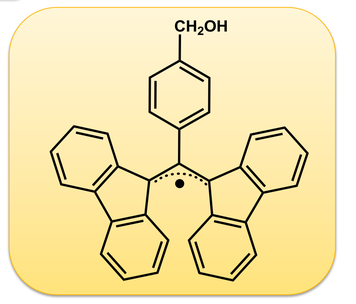
Dissolution Transfer: The sample is dissolved by the injection of 3-5 ml of hot solvent and rapidly transferred to a 600 MHz NMR spectrometer

NMR Data acquisition: Hyperpolarized and thermal equilibrium ^{13}C-DNP-NMR spectra acquired after dissolution

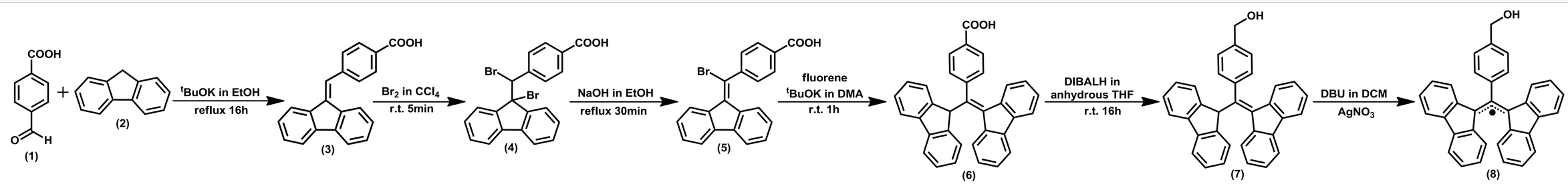


New Polarizing Agent: BA-BDPA

✓ Narrow EPR linewidth
✓ Soluble into [1-^{13}C]pyruvic acid
✓ Insoluble into the transfer solvent
✓ Easy removing by *in-line* filtration



SYNTHESIS



This multistep synthesis consisted of condensation between fluorene and 4-carboxybenzaldehyde followed by a double bond bromination to obtain compound **4**. This bormination step was improved by changing the previously reported reaction conditions enhancing the yield until 88%. The following steps were the elimination of hydrogen bromine, which generates compound **5**, and, finally, the allylic substitution which adds the final fluorene ring that completes the BDPA structure. The reduction reaction of the carboxylic group of **6** to the corresponding benzyl alcohol **7** was performed with diisobutylaluminium hydride (DIBALH) in excess. The acid reduction without anion formation was possible because of the big size of this reducing agent. This process could be naked-eye controlled by the absence of the typical blue colour corresponding to the BDPA anion. Finally, radical BA-BDPA **8** was isolated by the treatment of the benzilic alcohol derivative with 1,8-diazabicycloundec-7-ene (DBU) followed by AgNO3 as an oxidant agent in dichloromethane. The total yield of the synthesis of **8** was 40%.

RESULTS

Microwave Sweep

The microwave sweep shows the ^{13}C NMR signal intensity against the microwave irradiation frequency of 40 mM solutions of BDPA and BA-BDPA radical **8**. The optimum positive P(+) and negative P(−) peaks were determined for both samples by scanning from 94.000 to 94.200 GHz for 3 minutes at each frequency. The optimum P(+) was set at 94.077 and 94.087 GHz for BDPA and **8**, respectively. The separation between positive and negative polarization peaks |P(+) − P(−)| for BDPA and **8** was 50 and 30 MHz, respectively. These close values and the comparable EPR linewidths for both radicals extracted from the microwave DNP spectra, suggest that the same dominant thermal mixing polarization transfer mechanism is operative in both cases.

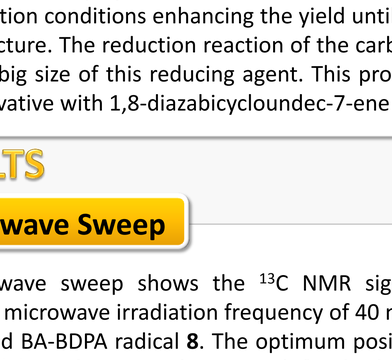


Fig. 1. ^{13}C microwave DNP spectra of 50 µl [1-^{13}C] pyruvic acid doped with 40 mM BA-BDPA **8** (solid circles) at 3.35 T and 1.4 K . The arrows indicate P(+) = 94,087 GHz and P(-) = 94,117 GHz polarization peaks, respectively. The microwave sweep spectra of 50 µl of 1:1 (v/v) [1-^{13}C]pyruvic acid:sulfolane doped with 40 mM BDPA (open circles) is also shown.

Optimum Radical Concentration

In order to determine the optimum concentration of radical **8** to be dissolved in [1-^{13}C]pyruvic acid, 20 µl sample aliquots with concentrations of 20, 40, 60 and 80 mM were polarized at an optimal frequency of 94.087 GHz, for approximately 90 minutes and automatically transferred to the Bruker 600 MHz NMR spectrometer through a home-made *in-line* filtration system that consists of a high pressure adapter connected to a syringe filled with cotton. This filter completely removed the radical from the dissolution down to 10^{-7} M, as confirmed by UV-Vis and EPR spectroscopy. The optimum concentration of radical **8** found was 40 mM.

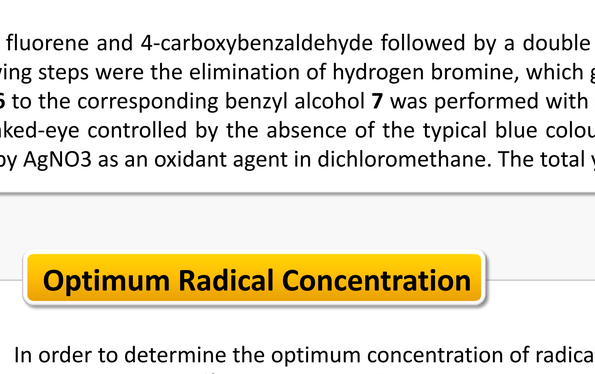


Fig. 2. a) ^{13}C polarization build-up curves for samples of 20 µl [1-^{13}C]pyruvic acid doped with BA-BDPA 20 mM (solid circles), 40 mM (open triangles), 60 mM (open squares) and 80 mM (open diamonds). The curve of 20 µl 1:1 (v/v) [1-^{13}C]pyruvic acid:sulfolane doped with BDPA 40 mM (open circles) is also shown for comparison. **b)** Filters used in the transfer process from the DNP to the NMR to remove the residual radical traces from the hyperpolarized solution

Radical Comparison

The 40 mM solution of **8** provides better solid-state polarization levels with an important reduction in the polarization time with respect to the BDPA radical. This can be related to the reduction in the build-up time constant (T_c) which is three times lower. It can also be observed that comparable polarization levels are achieved between **8** and the OX63 trityl radical.

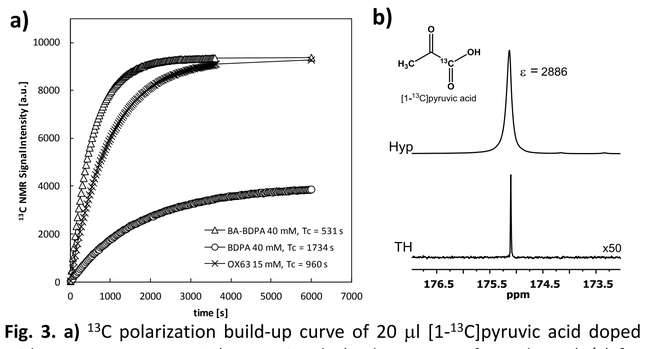


Fig. 3. a) ^{13}C polarization build-up curve of 20 µl [1-^{13}C]pyruvic acid doped with 40 mM BA-BDPA **8** (open triangles). The curves of 20 µl 1:1 (v/v) [1-^{13}C]pyruvic acid:sulfolane doped with 40 mM BDPA (open circles) and 20 µl [1-^{13}C]pyruvic acid doped with 15 mM OX63 (crosses) are also shown for comparison. **b)** Hyperpolarized (Hyp: θ = 90°, 1 scan) and thermal equilibrium (TH: θ = 30°, 32 scans) ^{13}C DNP-NMR spectra of [1-^{13}C]pyruvic acid after filtration.

Radical concentration (mM)	Liquid-state enhancement (ε)
BA-BDPA 20 mM	2191
BA-BDPA 40 mM	**2886**
BA-BDPA 60 mM	2726
BA-BDPA 80 mM	1387
BDPA 40 mM	**2454**
OX63 15 mM	**2658**

Table 1. Liquid-state NMR enhancements (ε) measured 10 s after dissolution of [1-^{13}C]pyruvic acid samples doped with different concentrations of **8**, BDPA and OX63 radicals

SUMMARY

A benzyl alcohol derivative of BDPA, **8**, has been synthesized, characterized and tested as a polarizing agent for fast dissolution DNP. This radical shows some advantages with respect to the commercially available BDPA, such as:

a) Sulfolane is avoided as the glassing agent in the sample preparation
b) Better polarization levels are achieved under solid state DNP conditions
c) Better ^{13}C signal NMR enhancements are reached under liquid NMR conditions.

Moreover, the required polarization time has been substantially reduced by a factor of 2 when [1-^{13}C]pyruvic acid is polarized. Additionally, it has been shown that radical **8** can be easily removed from the hyperpolarized solution in the transfer process, minimizing the loss of hyperpolarization for the presence of the paramagnetic free radical in the solution.

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