Dynamic Nuclear Polarization using Polychlorotriphenylmethyl Radicals Reveals Supramolecular Polarization Transfer Effects**

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Dynamic Nuclear Polarization (DNP) is attracting considerable attention as a method to increase NMR sensitivity. [1] Although the basic theory has been known for a long time, [2] the field is rapidly evolving with the introduction of new technologies as well as new radicals that enable new applications. [3]

The radical used as polarizing agent determines the polarization transfer mechanism. Supramolecular interactions take place between the radical, the glassing solvent, and the molecule being polarized. However, the understanding of the first events that occur in DNP experiments has been limited by the lack of structural diversity in the radicals used so far. [4]

Here we introduce polychlorotriphenylmethyl radicals (PTM) as a new class of DNP polarizing agents. [5] The presence of chlorine nuclei allows a different polarization mechanism from that of other commonly used trityl radicals, such as OX63 (Figure 1).

Six chlorine atoms surrounding the central triphenyl-substituted carbon at ca. 3 Å ensure the stability of the radical. Three or six carboxylate substituents in *para* or in *meta* position, in **1** and **2** respectively, provide water solubility. The remaining positions are also occupied by chlorine atoms.

The g values of the EPR spectra of **1** and **2** at 298K in neat pyruvic acid, used in DNP frequency sweeps, are g=2.0027 and 2.0039. The room temperature line widths of the EPR lines in H_2O-DMSO (1:1) of **1** and **2** are 0.4G and 0.65G, respectively.

Optimal DNP enhancement of ^{13}C spins by OX63, occurs at microwave frequencies $\pm\nu_c$ (36 MHz at 3.4T) from the centre of the EPR frequency (results not shown). Instead, the frequencies giving the maximum DNP enhancements for **1** and **2** (15 mM) are separated by 124 MHz and 136 MHz respectively (Figure 2). These values are much larger than $2\nu_c$ and incompatible with a direct DNP to carbon through the solid-effect mechanism.

Chlorine has two isotopes (^{35}Cl, 75.5%; ^{37}Cl 24.5%) with spin 3/2. Quadrupolar couplings of aromatic chlorinated molecules are around -70 MHz for ^{35}Cl, [7] much larger than the Zeeman frequency at 3.4 T (14 MHz). As a result, the quantization axes of chlorine and ½ spins are not necessarily collinear. [8] The effect of the presence of quadrupolar nuclei in radicals used for DNP has not been previously reported.

Preliminary DFT calculations of the chlorine hyperfine Fermi contact couplings were carried out for **1** and **2** as free acids using Gaussian 09 with the B3LYP hybrid functional and a 6-31G(d,p) basis set.[9] The results for the chlorines in **1** are 0.49 MHz (*ortho*) and 0.06 MHz(*meta*). For **2** the values are 0.36 (*ortho*) and 0.92 (*para*). The largest coupling occurs with the *para*-chlorine atom of **2**. More refined calculations, including the carboxylate forms, are under way and will be published elsewhere. Thus, in spite of the identical arrangement around the centre of the radicals, different properties are predicted for **1** and **2** if the chorine atoms mediate the DNP effect to surrounding carbons. This makes these radicals excellent probes for supramolecular interactions present during the polarization transfer step.

2-^{13}C acetone, 1-^{13}C sodium pyruvate and ^{13}C urea were independently polarized under comparable conditions (1.4K, water-DMSO 1:1) with OX63, **1** and **2** and their NMR spectra were recorded following a 90º pulse. Table 1 shows DNP enhancements estimated by comparison with the thermal signal measured with 1024 scans using 20º pulses. The sign of the polarization was confirmed by comparing the signs of the polarized signals and the methanol solvent used for the transfer.

Irradiation of the low frequency microwave maximum of **1** and OX63 causes a positive ^{13}C DNP enhancement. In contrast, **2** induces a negative DNP for acetone and pyruvate, but a positive DNP for urea.

Sign reversal is observed in all cases when the high frequency band is irradiated, thereby confirming that the enhancements observed originate from electron polarization (results not shown).

The sign of the DNP enhancement using radical **2** is substrate-dependent. 2-Phenoxyethylamine (POEA) is positively charged at pH 7.5 but uncharged at pH 9. The carboxylate groups of **2** are negatively charged. The DNP enhancement of the aromatic carbons of POEA is positive at pH 7.5 but negative at pH 9 (Figure 3). The dependency on the charge state of POEA suggests that electrostatic effects between the substrate and the carboxylate groups of **2** are crucial in determining the sign of the enhancement. The differences between urea and acetone may result from variations in the interaction geometries of these two molecules with **2** through hydrogen bond formation with the carboxylate groups.

These results highlight the supramolecular character of polarization transfer, in which both the radical and the substrate affect the outcome of the process.

The chlorinated radicals **1** and **2** can be used to polarize ^{13}C nuclei; however, the microwave frequencies at which DNP occurs do not support a direct solid-effect mechanism. The optimal frequencies for **1** and **2** differ, and **2**, but not **1**, shows negative enhancements for some substrates. Calculations of the Fermi term reveal significant coupling of *para*-chlorine nuclei with the unpaired electron in **2**. The *para*- position is occupied by a carboxylate in the case of **1**.

We propose that the observed ^{13}C-DNP enhancements are mediated by non-equilibrium polarization of chlorine nuclei generated by microwave irradiation in the radicals, followed by NOE-type transfer to the surrounding nuclei (protons or carbons) and detection in carbon. While in **1** calculations suggest that only *ortho*-chlorine atoms are significantly polarized, **2** has two types of chorine atoms that can be efficiently polarized. We speculate that the two types of chlorine atoms in **2** provide two distinct transfer pathways that lead to the polarization of different signs. Accordingly, the sign of the polarization of the ^{13}C nuclei may reflect a closer proximity to one or the other type of chlorine, which would be dependent on the nature of the substrate. Carbon polarization from chlorine should be efficient only at very short distances, because of the low gyromagnetic ratio of chlorine, thus making the observed enhancement very sensitive to local supramolecular contacts. This study opens the way to the design of new functionalized chemospecific radicals for selective DNP enhancement in complex systems.

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Figure Legends

Figure 1. Radicals used in this study. OX63; tri-*para* carboxy PTM (**1**); hexa-*meta* carboxy PTM (**2**). OX63 is commercially available from Oxford Molecular Biotools. **1** and **2** were synthesized as reported in the literature.[6]All radicals were used as sodium salts.

Figure 2. Microwave frequency sweep. Solid-state 13C-NMR signal magnitude of neat 1-^{13}C-pyruvic signal doped with **1** (filled symbols) or **2** (open symbols), at 3.4 T and 1.5K and as a function of the microwave frequency irradiation in a Hypersense polarizer. Radical concentration was 15 mM and the microwave power was 100 mW.

Figure 3. ^{13}C-NMR spectra of 2-phenyloxyethylamine enhanced by DNP at pH 7.5 and pH 9.0 and irradiation at 94.120 GHz. 1M POEA and 15 mM 2 were dissolved in 1:1 mixtures of DMSO and H_2O (final pH 7.5, a) or MOPS buffer (pH 9.0, b). The samples were polarized for 1.5 hours at 1.4 K and transferred with methanol. ^{13}C-NMR spectra were recorded using one 90º pulse after 4 seconds from the start of transfer. Only the aromatic carbons *ipso*, *ortho* and *meta* are observed, probably due to the faster relaxation of the aliphatic carbons. The slowly relaxing *ipso* carbon (ca. 160 ppm) may have acted as polarization reservoir during the transfer.

Table of contents
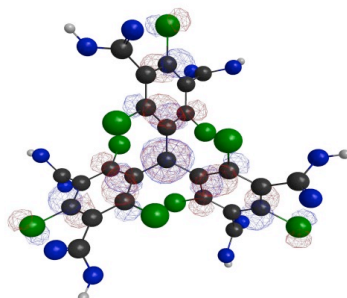
DNP

e⁻   (Cl)   ¹³C

I=1/2   I=3/2   I=1/2

Ups and downs of DNP. Polychlorinated trityl radicals used for Dynamic Nuclear Polarization show a new transfer mechanism involving quadrupolar chlorine nuclei. Positive or negative enhancements are observed depending on the substrate highlighting the supramolecular character of the initial polarization transfer process.

Ups and downs of DNP. Polychlorinated trityl radicals used for Dynamic Nuclear Polarization show a new transfer mechanism involving quadrupolar chlorine nuclei. Positive or negative enhancements are observed depending on the substrate, highlighting the supramolecular character of the initial polarization transfer process.

Keywords

Dynamic Nuclear Polarization (DNP), Polychlorinated trityl radicals,
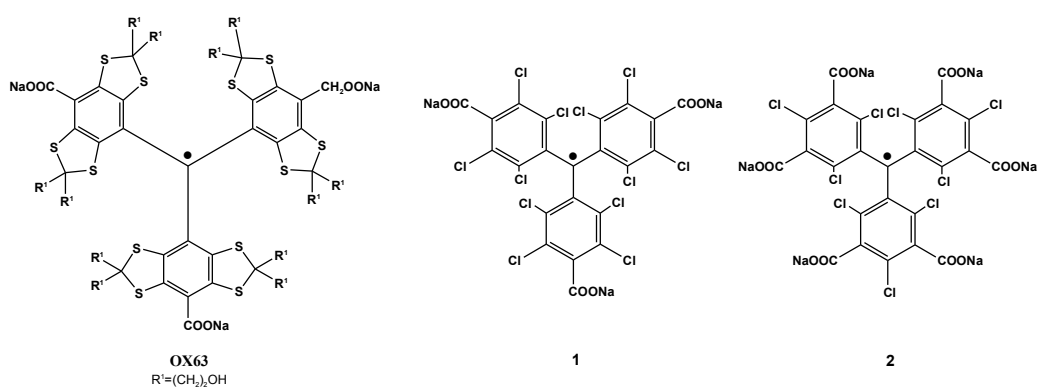
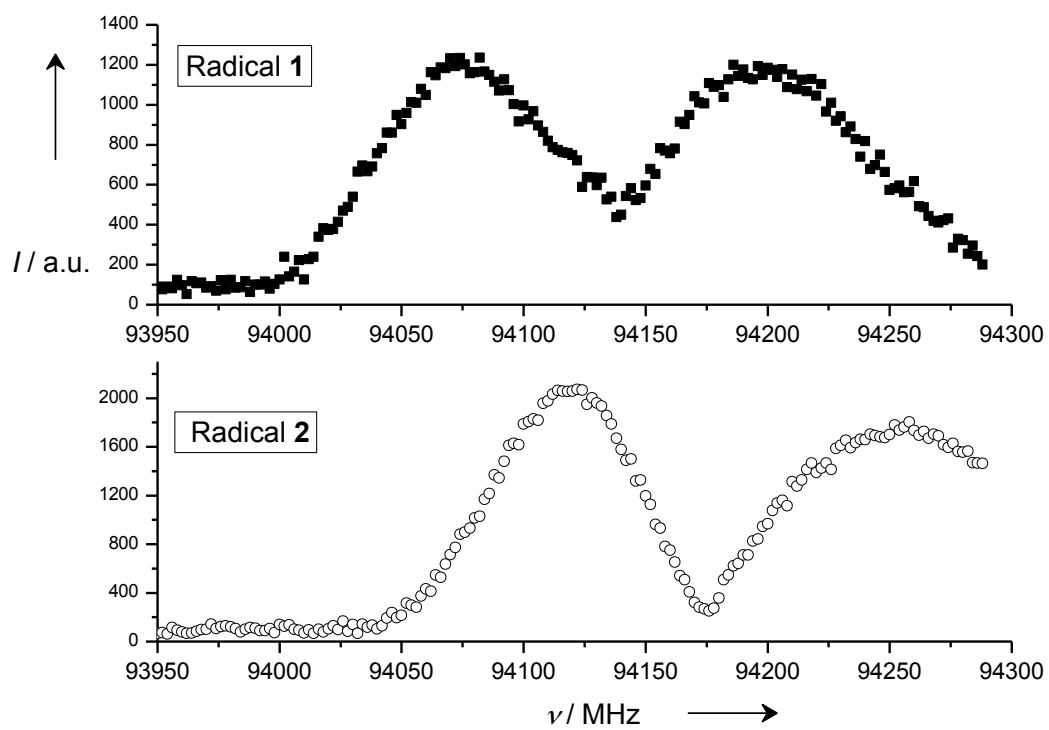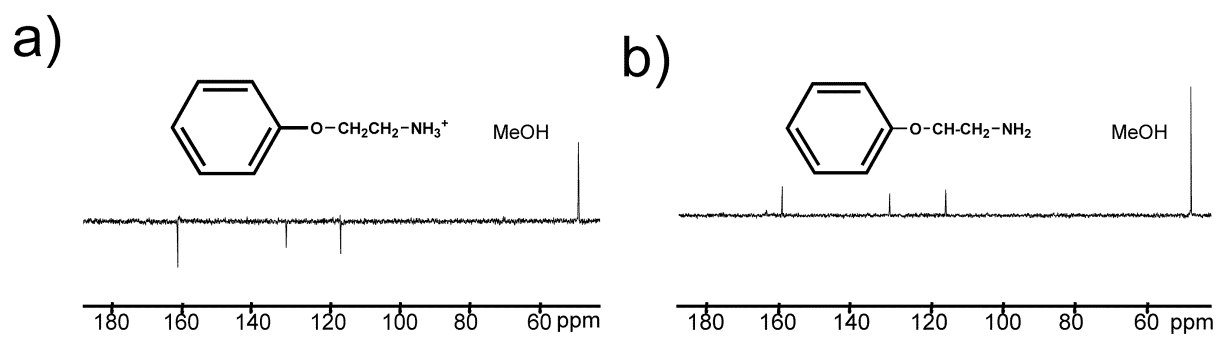**quadrupolar nuclei, supramolecular effects**

OX63
R¹=(CH₂)₂OH

1

2

Figure 1

Figure 2

a)

b)

Figure 3

12

Table 1. Transferred DNP enhancements[a] using distinct radicals.

Radical[b]	1-^{13}C Pyruvate	^{13}C Urea	2-^{13}C Acetone
OX63	39214	12430	10863
1	29925	6392	4061
2	**-3026**	2545	**-901**

[a] Ratio of the intensity of the DNP and thermal spectra multiplied by the square root of the number of scans.

[b] Irradiating at the optimal low frequency band for each radical