Dynamic Nuclear Polarization

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Dynamic Nuclear Polarization with Polychlorotriphenylmethyl Radicals: 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 the polarizing agent determines the polarization-transfer mechanism. Supramolecular interactions take place between the radical, the glassing solvent, and the molecule undergoing polarization. However, 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] Herein we introduce polychlorotriphenylmethyl (PTM) radicals as a new class of DNP polarizing agents.[5] The presence of chlorine nuclei enables a different polarization mechanism to take place from that observed for other commonly used trityl radicals, such as OX63 (Scheme 1).

In the new PTM radicals **1** and **2**, six chlorine atoms surrounding the central triphenyl-substituted carbon atom at a distance of approximately 3 Å ensure the stability of the radical. Three or six carboxylate substituents in *para* or *meta* positions 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 298 K in neat pyruvic acid, which is used in DNP frequency sweeps, are 2.0027 and 2.0039. The linewidths of the EPR lines in H_2O/dimethyl sulfoxide (DMSO; 1:1) at room temperature are 0.4 and 0.65 G for **1** and **2**, respectively.

Optimal DNP enhancement of ^{13}C spins by OX63 occurs at microwave frequencies $\pm\nu_c$ (36 MHz at 3.4 T) from the center of the EPR frequency (results not shown). In contrast, the frequencies that give the maximum DNP enhancement for **1** and **2** (15 mM) are separated by 124 and 136 MHz, respectively (Figure 1). These values are much larger than $2\nu_c$ and incompatible with a direct DNP to carbon atoms 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] and thus much larger than the Zeeman frequency at 3.4 T (14 MHz). As a result, the quantization axes of chlorine and 1/2 spins are not necessarily collinear.[8] The effect of the presence of quadrupolar nuclei in radicals used for DNP has not been reported previously.

Preliminary DFT calculations of the chlorine hyperfine Fermi contact coupling constants were carried out for **1** and **2** as free acids by using Gaussian 09 with the B3LYP hybrid functional and a 6-31G(d,p) basis set.[9] The results for the chlorine atoms in **1** are 0.49 (*ortho*) and 0.06 MHz (*meta*). For **2**, the values are 0.36 (*ortho*) and 0.92 MHz (*para*). The strongest coupling occurs with the *para*-chlorine atom of **2**. More refined calculations of **1** and **2** and their ionized forms are underway. Thus, in spite of the identical arrangement around the center of the radicals, different properties are predicted for **1** and **2** if the chorine atoms mediate the DNP effect to surrounding carbon atoms. These radicals are therefore excellent probes for supramolecular interactions that occur during the polarization-transfer step.

[2-^{13}C]Acetone, [1-^{13}C]sodium pyruvate, and [^{13}C]urea were polarized independently under comparable conditions (1.4 K, water/DMSO 1:1) with OX63, **1**, and **2**, and their NMR spectra were recorded following a 90° pulse. Table 1 shows DNP enhancements estimated by comparison with the thermal signal measured with 1024 scans with 20° 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 at the low-frequency microwave maximum of **1** and OX63 caused a positive ^{13}C DNP enhancement. In contrast, **2** induced a negative DNP enhancement for acetone and pyruvate, but a positive DNP enhancement for urea. Sign reversal was observed in all cases upon irradiation at the high-frequency band; this result

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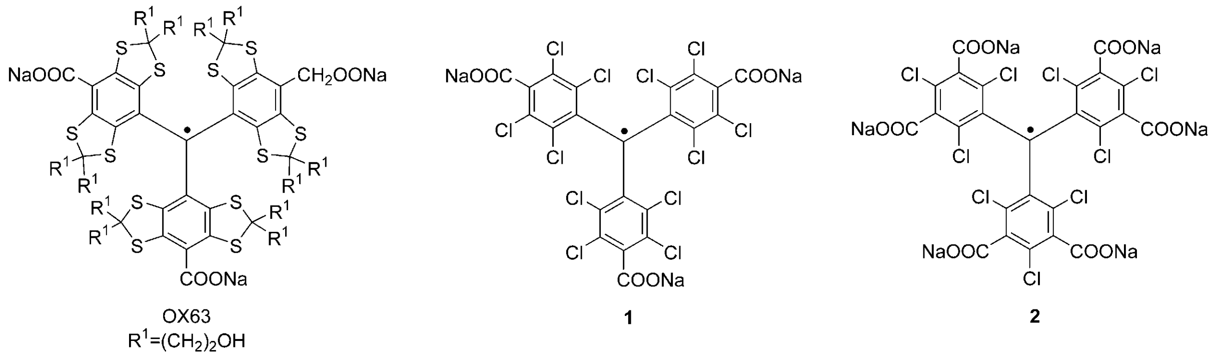

Scheme 1. Radicals used in this study. OX63 is commercially available from Oxford Molecular Biotools. Radicals **1** and **2** were synthesized as described previously.[6] All radicals were used as sodium salts.

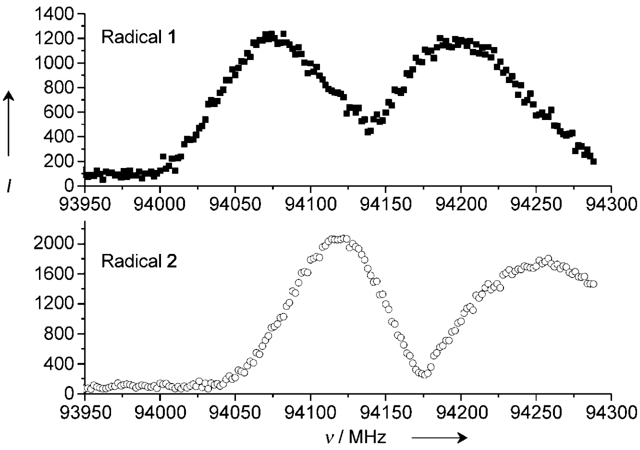

Figure 1. Microwave-frequency sweep. Magnitude of the solid-state ^{13}C NMR signal of neat [1-^{13}C]pyruvic acid doped with **1** (filled symbols) or **2** (open symbols) at 3.4 T and 1.5 K as a function of microwave-frequency irradiation in a HyperSense polarizer. The radical concentration was 15 mM; the microwave power was 100 mW.

Table 1: Transferred DNP enhancements[a] with specific 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] Irradiation at the optimal low-frequency band for each radical.

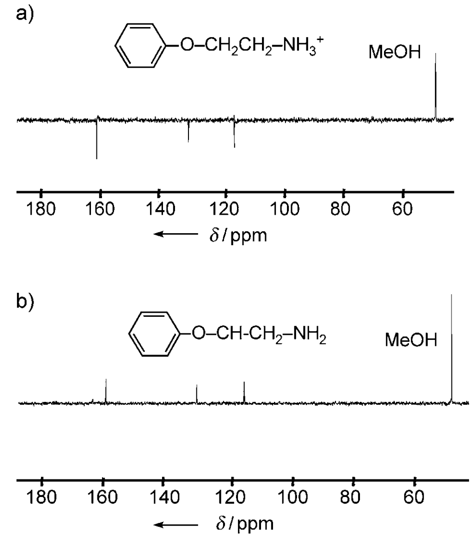

Figure 2. ^{13}C NMR spectra of 2-phenyloxyethylamine enhanced by DNP at pH 7.5 and 9.0 upon irradiation at 94.120 GHz. POEA (1 M) and **2** (15 mM) were dissolved in 1:1 mixtures of a) DMSO and H_2O (final pH 7.5) or b) DMSO and 3-morpholinopropane-1-sulfonic acid buffer (pH 9.0). The samples were polarized for 1.5 h at 1.4 K and transferred with methanol. ^{13}C NMR spectra were recorded by using one 90° pulse 4 s after the start of transfer. Only the aromatic *ipso*, *ortho*, and *meta* carbon atoms are observed, probably as a result of the faster relaxation of the aliphatic carbon atoms. The *ipso* carbon atom ($\delta = 160$ ppm), which relaxes slowly, may act as a polarization reservoir during the transfer.

confirmed that the enhancements observed originate from electron polarization (results not shown).

The sign of the DNP enhancement with radical **2** is substrate-dependent. 2-Phenoxyethylamine (POEA) is positively charged at pH 7.5 but uncharged at pH 9, and the carboxylate groups of **2** are negatively charged. The DNP enhancement of the aromatic carbon atoms of POEA is positive at pH 7.5 but negative at pH 9 (Figure 2). 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 difference in the results for 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 that 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 revealed significant coupling of *para* chlorine nuclei with the unpaired electron in **2**. The *para* position is occupied by a carboxylate group in the case of **1**.

We propose that the observed ^{13}C DNP enhancements are caused by nonequilibrium polarization of chlorine nuclei as a result of microwave irradiation of the radicals, followed by NOE-type transfer to the surrounding nuclei (hydrogen or carbon atoms) and detection in carbon atoms. Whereas calculations suggest that only *ortho* chlorine atoms in **1** are significantly polarized, **2** has two types of chorine atom that can be polarized efficiently. We speculate that the two types of chlorine atom in **2** provide two distinct transfer pathways that lead to polarization with 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 atom, depending on the nature of the substrate. The polarization of carbon atoms from chlorine atoms should be efficient only at very short distances because of the low gyromagnetic ratio of chlorine; thus, the observed enhancement is 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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