STUDIES OF LIQUID PHASE INTERMOLECULAR INTERACTIONS UTILIZING $^1$H AND $^{13}$C DYNAMIC NUCLEAR POLARIZATION AND NUCLEAR MAGNETIC RESONANCE TECHNIQUES

by

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(ABSTRACT)

Liquid phase $^{13}$C DNP experimental data were collected in a flow transfer system for different organic molecules, such as acetone, acetaldehyde, diethyl malonate, ethyl acetoacetate, diphenylmethane, and triphenylmethane. These molecules represent a wide range of functional groups with different acidities of the respective carbon-hydrogen bonds. The $^{13}$C DNP results demonstrated that the scalar dominated enhancement is sensitive to the acidity of carbon-hydrogen bonds as well as to the correlation times of the sample molecules. A hydrogen bonding spin polarization model is used, for the first time, to interpret the scalar components induced by the nitroxide free radical at the carbon sites of the acidic carbon-hydrogen bonds.

Three aromatic molecules: nitrobenzene, 1, 2-dichlorobenzene, and toluene, are studied by the solution $^{13}$C DNP technique. The scalar components for the ring carbons are sensitive to the electronic environment of these carbon sites. A spin delocalization
model is used, for the first time, to explain the scalar contributions for the $^{13}$C DNP enhancements of the ring carbons.

Both $^1$H and $^{13}$C DNP experiments are performed for the Taxol/TEMPO (2, 2, 6, 6-tetramethyl-1-piperidinyloxy) system. The different $^1$H enhancements for the hydrogens in the two acetyl groups indicate the different accessibility of these groups to the free radical. The $^{13}$C DNP results for the skeleton carbons of Taxol show the different accessibility of these carbon sites to the free radical.

The solution $^{13}$C DNP result of adamantane indicates that the DNP enhancements and thus the correlation times for the two different carbon sites are very close under the high free radical concentration.

The $^{13}$C DNP study of C$_{70}$ empty cage fullerene suggests that the endcap carbons are more accessible than those at the center of the cage, and that the scalar coupling between the cage carbons and the free radical is very weak.
This dissertation is dedicated to

my parents and my wife.
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CHAPTER 1: INTRODUCTION

1.1 Background

Dynamic nuclear polarization (DNP) is a double magnetic resonance technique in which a nuclear magnetic resonance (NMR) signal is observed while the electron resonance transition is simultaneously saturated. The system subjected to a DNP analysis generally consists of at least two non-identical spins, for example, a nuclear spin system and an unpaired electron spin system. In solutions, due to the random molecular motion, the unpaired electron spins interact with the nuclear spins. This interaction is called nuclear-electron coupling. When the sample solution is placed in a static magnetic field, the Zeeman effect causes the splitting of the spin levels of both the nuclear spins and the unpaired electron spins, therefore, a Boltzmann distribution of the spin populations in the spin levels is established. The relative population difference of the spins is called polarization. In order to develop a qualitative understanding of the DNP phenomenon, an energy level diagram for the nuclear-electron spin coupled system is shown in Figure 1.1. In this diagram, the single quantum transitions between the energy levels 1-2, 3-4, 1-3, and 2-4 are theoretically allowed. Among these transitions, 1-2 and 3-4 are the nuclear transitions which can be observed in the NMR spectrum. The transitions 1-3 and 2-4 are the electron transitions which can be saturated by the microwave radiation. The
Figure 1.1 Energy Level Diagram for the Nucleus-Electron Spin Coupled System with $I=1/2$ and $S=1/2$: (a) Equilibrium Distribution of the Electron and Nuclear Spins; (b) Spin Distribution after the Saturation of the Electron Transitions. (The hatched area in the diagram represents electron spin population which are transferred).
transitions 4-1 and 3-2 are zero quantum and double quantum transitions, respectively. These transitions are spectroscopically forbidden and cannot be excited by electromagnetic radiation. However, both transitions (zero and double) are allowed in relaxation. In Figure 1.1, the thickness of the "bars" indicate the relative populations occupying the different energy levels, $N_1$ to $N_4$ represent the level populations of the coupled nuclear-electron spins under the equilibrium conditions, $N_1$ to $N_4$ correspond to the level populations after the saturation of the electron transitions. The NMR signal is proportional to the population differences between energy levels 1-2 and 3-4. The initial equilibrium population distribution is shown in Figure 1.1 (a). If the electron transitions are saturated, the electron populations at levels 1 and 3 are equal. The electron populations at levels 2 and 4 also become equal. In Figure 1.1 (b), the electron populations transferred from the low to high energy levels are indicated by the black bars. If the single quantum relaxations were the only ways to reach a new equilibrium, the equalization of the electron populations would have no effect on the total intensity of the nuclear signal since the nuclear populations are not affected by the saturation of the electron transitions. Due to the existence of the allowed relaxation process labeled $W_0$ and $W_3$, the relative nuclear populations between levels 1-2 and 3-4, and therefore the NMR signal intensity, are changed in the relaxation process. Specifically, since $N_4 > N_4'$ and $N_1 > N_1'$, the relaxation process labeled $W_3$ tries to increase $N_4'$ at the expense of $N_1'$ so that a new equilibrium can be established. This causes an increase in the nuclear population differences between levels 1-2 and 3-4 which determine the intensity of the
NMR signal. This, in fact, is an NMR signal enhancement. On the other hand, the relaxation process labeled $W_2$ tries to increase $N_3$, at the expense of $N_2$. This reduces the nuclear population differences between levels 1-2 and 3-4 thereby tending to reduce the NMR signal intensity. Depending on the magnitudes of the contributions from $W_0$ and $W_2$, the combination of these two relaxation processes determines the sign and magnitude of the enhanced NMR signal. The phenomenon that the equalization of the unpaired electron spin populations in the spin levels induces an increase of the nuclear spin population difference is normally called dynamic nuclear polarization. The theoretical signal enhancement is proportional to the magnetogyric ratio $(\gamma)$ of the electron and nuclear spin. Since $\gamma$ of the electron spin is two to three orders larger in magnitude than that of the nuclear spin, tremendous sensitivity improvements for the observed NMR signals can be predicted. In practice, however, only part of the ultimate enhancement can be observed because of the limitations of the experimental methods and the instruments.

In contrast to NMR methods, DNP techniques are more informative about the molecular motions of small molecules. NMR methods are very effective for studying the systems with long correlation times, such as the case of large molecules, high viscosity sample solutions, or long complexation times between the solvent and solute molecules.

To investigate molecular motions of small solvent molecules, a technique that is sensitive to short correlation times ($t_c < 10^{-11}$ seconds) is needed, and the solution DNP technique satisfies this requirement. Because of this sensitivity nature, DNP techniques have been utilized to characterize the transient intermolecular interactions between a free
radical (one kind of the unpaired electron spin system) and the nuclei of interest in a molecule. The free radical employed in a DNP experiment works as a molecular probe which interacts with the nuclei of sample molecules by two different mechanisms. One is dipolar coupling, which is the direct interaction between the unpaired electron magnetic dipole and the nuclear magnetic dipole through the interspin space. This interaction occurs whenever an unpaired electron and a magnetic nucleus are present in the system. The other coupling mechanism is called scalar interaction, which occurs when an excess of electron spin density is transferred from the paramagnetic molecule to the nuclei of the sample molecule during the intermolecular collisions. Scalar coupling is also called contact interaction because the interaction involves the overlapping of the wavefunctions of the molecular orbitals of the electron and nuclear spins. The intermolecular dipolar and scalar interactions are monitored as enhanced NMR signals in a DNP experiment. A detailed discussion of the dipolar and scalar coupling mechanisms is given in Chapter 2.

1.2 Review of Solution $^1$H and $^{13}$C DNP

The DNP phenomenon was first predicted by Overhauser in 1953 for the conducting electrons in a metal.$^2$ Other early DNP studies were performed by Jeffries$^3$ and Abragam.$^4$

The first two DNP experiments were reported by Carver and Slichter in 1953.$^5$ They successfully polarized the $^7$Li nucleus in metallic lithium.
From 1956 to 1961, several nuclei were studied by DNP techniques using different paramagnetic molecules. The experiments were performed by changing the magnetic fields from 1 to 13,000 gauss and the temperature from 1° to 350° K. Three different detection methods were used.

The coupling between the nuclear spin and an unpaired electron spin is essential for obtaining the dynamic nuclear polarization effect. The paramagnetic species for the DNP experiments can be in the following forms: the conduction electrons in metals or metal ammonia solutions; the donor or acceptor electrons in semi-conductors; paramagnetic ions in a diamagnetic solid; paramagnetic ions in solution; free radicals; and color centers.

The DNP effects may be detected by at least three different methods: enhancement of the NMR signal; the shift in the EPR frequency; and the β asymmetry or γ anisotropy from a polarized radioisotope. Among the three detection techniques, the detection of the enhanced NMR signal is commonly adopted.

Two important applications of the DNP technique have been in the studies of weak intermolecular interactions,6-9 and in the detection of NMR signals which cannot be monitored with an ordinary high resolution NMR spectrometer.10

Among the two coupling mechanisms, scalar coupling is sensitive to the chemical and electronic environment of the receptor molecules. One of the most significant applications of DNP techniques in recent years has been in investigations of the dynamics
of molecular interactions which involve the scalar coupling between solvent and free radical molecules.\textsuperscript{1,11}

In this review, \textsuperscript{1}H and \textsuperscript{13}C DNP investigations of the dipolar and scalar interactions between some solvent molecules and free radicals are summarized.

\textbf{\textsuperscript{1}H DNP}

Kramer \textit{et al.}, Muller-Warmuth, and Richards \textit{et al.} investigated the interactions between protons in diamagnetic molecules and free radicals.\textsuperscript{12-16} It was found that the interactions are dipolar dominated and modulated by translational motion, little scalar contribution can be observed. In addition, the ultimate enhancement of the proton has little or no association with its chemical and electronic environment in the molecule because of the absence of significant scalar contribution.

For some special systems, scalar dominated proton enhancements have been observed. One of the examples was aqueous solutions of manganese ions (Mn\textsuperscript{2+}).\textsuperscript{17} The water molecule in the hydration sphere of the manganese ion experiences a scalar interaction with the unpaired manganese electrons. This interaction is modulated either by the rapid flipping of the electron spins or by the chemical exchange of the water molecules in the hydration sphere, depending on the temperature. Dwek \textit{et al.}\textsuperscript{18,19} reported positive \textsuperscript{1}H enhancements for the t-butyl groups of 2, 4, 6-tri-t-butylphenol in solutions of 2, 4, 6-tri-t-butyl phenoxy (TTBP) radical. The positive enhancements were
explained as a result of hydrogen atom exchange between TTBP and the tri-t-butyl phenol. For the solutions of alkali metals in liquid ammonia or hexamethyl phosphoramid, positive $^1$H DNP enhancements are also found. In these solutions, the metal is completely dissociated into $M^+$ and "free electrons". The "free electrons" reside in cavities formed by solvent molecules, and their wave functions overlap with those of the solvent molecules, resulting in a spin density transfer from the electron to the solvent nucleus and a scalar coupling.

Bates et al. observed small scalar components in the enhancement of the CHCl$_3$:DTBN (di-tert-butyl nitro oxide) system. This result indicated that unpaired electron spin density was transferred from the free radical to the hydrogen of CHCl$_3$. Bates stated in his review: "The CHCl$_3$-DTBN study is of significance because it showed the importance of DNP detection of hydrogen bonding in systems other than those in which the H is bonded to a N or an O atom. It also demonstrated the value of using replicate samples of CHCl$_3$:C$_6$D$_6$ and CDCl$_3$:C$_6$H$_6$ with the same nitro oxide concentration to determine $^1$H enhancements by the ratio method at low magnetic fields where chemical shifts are too small to resolve chemically different nuclei." Several free radicals for $^1$H and $^{13}$C DNP experiments are shown in Figure 1.2.

Helbert et al. studied the interactions between trifluoroacetic acid and several radicals by the $^1$H DNP technique. Positive proton enhancements were observed for trifluoroacetic acid. They also studied the hydrogen bonding tendency between
Figure 1.2. Molecular Structure of Some Free Radicals for $^1$H and $^{13}$C DNP Experiments
imidazoline nitroxides and several different solvents (acetone, chloroform, and trifluoroacetic acid). For acetone, the proton enhancements always reach the dipolar limit, while for chloroform, the enhancement is smaller than the dipolar limit, suggesting the existence of a scalar component.

Dorn et al. have developed a flow transfer DNP instrument and have used it to study the liquid-liquid and solid-liquid intermolecular transfer $^1$H DNP of several organic solvents, such as benzene, chloroform, dichloroform, acetonitrile, 1-chlorobutane, n-hexane, ethylbenzene, and n-propylbenzene.\textsuperscript{1,24} With this novel approach, relatively large dipolar $^1$H DNP enhancements were observed. They also used the flow transfer instrument to study the conformation of taxol in organic solutions.\textsuperscript{25}

Recently, Grucker et al. investigated the $^1$H DNP parameters in biological fluids by dissolving several different nitroxide free radicals in the fluids.\textsuperscript{26} The goal of their study was to obtain the $^1$H DNP parameters for the biological/nitroxides systems in order to provide guidelines for the design of new nitroxides which are suitable for the biological applications of the DNP technique. It was found that nitroxides with a long side chain resulted in poor enhancement possibly because of the formation of micelles when the nitroxides were dissolved in water. Another conclusion of this work is that the nitroxides which have narrower EPR linewidth exhibit larger enhancement. The $^1$H DNP enhancements for the whole blood/nitroxide systems were lower than the other biological solutions studied, such as albumin/nitroxide solutions, serum/nitroxide solutions. This is mainly attributed to the decrease of the $f$ factor in the whole blood/nitroxide solutions. In
order to improve the performance of the nitroxides in the DNP studies of biological systems, the authors suggested the use of $^{15}$N and $^2$D-substituted nitroxides to bind the nitroxides either to a macromolecule or to a long side chain so that the saturation factor $s$ and the leakage factor $f$ can be improved.

Another research work completed by Grucker et al. on $^1$H DNP of water/nitroxide system involved the saturation of the $\sigma$ EPR transitions of nitroxides in low magnetic fields. Although theoretical predications have indicated that the DNP effect could not be produced by saturation of $\sigma$ transitions, the experimental work by these authors has shown that DNP with $\sigma$ EPR irradiation is allowed at low magnetic fields. This work is significant because it develops a promising method that can be used to overcome the overheating problem in the biological applications of DNP. It is also an important approach to having a better understanding of the relaxation mechanisms of nitroxides.

$^2$H DNP

Very limited DNP results have been reported for $^2$H because of a low natural abundance and a low magnetogyrlic ratio. Bates et al. observed enhancements for $\text{C}_6\text{D}_6$ with GALV (galvinoxyl) and BDPA (bis(diphenylene)-phenylallyl) radicals. The estimated ultimate enhancements are $-2210\pm950$ for $\text{C}_6\text{D}_6$/BDPA and $-2090\pm290$ for $\text{C}_6\text{D}_6$ GALV. No evidence of scalar coupling was observed.\textsuperscript{28}
\textsuperscript{13}C DNP

Hausser \textit{et al.} reported the first \textsuperscript{13}C DNP result for the solution of benzene and BDPA. It was found that the interaction is dipolar dominated.\textsuperscript{29} Other experiments with radical TTBP (2, 4, 6-tri-t-butylphenoxide) in benzene also show dipolar dominated enhancements, but in other solvents a positive enhancement was found.\textsuperscript{30-32} The positive enhancements are observed in compounds containing sp\textsuperscript{3} hybridized carbon atoms bonded to halogen atoms, such as chloroform. It seems that halogen atoms facilitate the transfer of electron density from the radical to the \textsuperscript{13}C nucleus.\textsuperscript{33-37} For sp\textsuperscript{2} hybridized \textsuperscript{13}C atoms with halogen atoms attached to it,\textsuperscript{38-39} only dipolar dominated enhancements are observed. For Cl\textsubscript{2}C(1)=C(2)HCl, it was found that the C(1) is more negatively enhanced than the C(2) doublet.\textsuperscript{30} This was interpreted as the closer contact of C(2) with the free radical than that of C(1).

High resolution \textsuperscript{13}C DNP spectra for several hydrocarbons and fluorocarbons were obtained by Imbaud in 1965.\textsuperscript{31} No enhancement was calculated because the static NMR spectra could not be obtained.

Dorn \textit{et al.} have used flow transfer \textsuperscript{13}C DNP techniques to study the intermolecular interaction mechanisms and DNP enhancements of some small organic molecules (benzene, dichloromethane, chloroform, cyclohexane, 1-chlorobutane, chlorobenzene, 1,3,5-trichlorobenzene, phenylacetylene, acetonitrile, n-butanol,
isoctane, and bromocamphor) with TEMPO (2, 2, 6, 6-tetramethyl-1-piperidinyloxy) in
the liquid phase and at the solid-liquid interface. Large scalar enhancements were
observed at carbon atoms which have weakly acidic hydrogens.\textsuperscript{1,32,24,40}

Recently, Dorn \textit{et al.} studied the dynamics of fullerene $C_{60}$/TEMPO system by the
flow transfer $^{13}$C DNP techniques.\textsuperscript{41} The $^{13}$C DNP enhancement and the dynamic
parameters were measured in a deuterated benzene solvent ($C_{6}$D$_{6}$). The deuterated
benzene was used as the solvent mainly for the purpose of reducing the three-spin effect
which can cause an error in the DNP enhancement. Both flow liquid-liquid
intermolecular transfer (LLIT) and solid-liquid intermolecular transfer (SLIT) techniques
were employed for this study. For the LLIT $^{13}$C DNP experiment, the dipolar dominated
ultimate enhancement was measured as $-250\pm20$ in the solution of $C_{6}$D$_{6}$ and TEMPO. By
assuming a rotational modulation for the time-dependence of the interaction between $C_{60}$
and TEMPO, the rotational correlation time was estimated as 65 ps. For a translational
model, the closest approach between the nuclear spin and the electron spin was in the
range of 0.16-0.35 nm. The translational correlation time was estimated as 6-28 ps. For
the solid-liquid intermolecular transfer (SLIT) $^{13}$C DNP experiment on the same system,
an enhancement curve as a function of the low magnetic field was obtained. This curve
was comparable with the EPR spectrum of the $C_{60}$/TEMPO system, indicating the
expected nitrooxide triplet structure modulated by an anisotropic interaction.

In a more recent study, Dorn \textit{et al.} reported a novel LC-$^{13}$C DNP technique.\textsuperscript{42} In
their experiment, the nitrooxide radical was immobilized on the silica gel in the low-field
flow cell and the cell was placed in the EPR cavity. As stated by the authors: “solid-liquid intermolecular transfer (SLIT) $^{13}$C DNP technique was employed for the following reasons: (1) high efficiency for transfer of the flowing polarized bolus, (2) wide range of accessible flow rates, (3) favorable detection of scalar-dominated $^{13}$C DNP signals, and (4) absence of scalar $^{13}$C contact shifts (or spectral line broadening) in the high magnetic field volume, since the radical is not a part of the solvent system.” The sample solution (a mixture of several chlorinated organic molecules) was first pumped through a HPLC column then was polarized and detected by the DNP instrument. The significant aspect of this work is that the sample molecules, which could not be resolved by the HPLC output, were clearly resolved by the scalar-dominated $^{13}$C DNP signals. The main drawback of the SLIT technique is the difficulty in observing dipolar dominated $^{13}$C DNP signals.$^{41,43}$

In summary, to the date of this work, only a very limited amount of $^{13}$C DNP data has been published. Although some of the $^1$H and $^{13}$C DNP results$^{1,21,23}$ have indicated that the scalar contribution for the DNP enhancements is associated with the weak hydrogen bonding between the acidic C-H bond and the nitrooxide free radicals, the mechanism that the electron spin density transfers from the free radical to the carbon or hydrogen nucleus through the hydrogen bond has not been resolved.
1.3 Objectives of the Present Research

As noted in the background section 1.2, only a limited number of $^1$H and $^{13}$C DNP measurements have been reported to date. Thus, the major objectives of this work are: (1) to further reveal the association between the acidic C-H bond of organic molecules and the scalar dominated $^{13}$C DNP enhancements; (2) to study the electron spin density transfer mechanisms between TEMPO and the carbon nuclei in different chemical and electronic environments; (3) to investigate the structure and time dependence of organic molecules in colliding with TEMPO by liquid phase $^1$H and $^{13}$C DNP and NMR techniques.
CHAPTER 2. BASIC THEORY OF DNP IN SOLUTIONS

2.1 Concepts and Basic Equations for Solution DNP

In general, the nuclear spins in a static magnetic field follow the Boltzmann distribution among the spin energy levels. In most cases, the distribution is not uniform, the number of spins aligned with the magnetic field \( B_0 \) exceeds that anti-parallel to the field. A quantity to measure this uneven spin distribution is called the nuclear polarization. If the nuclear spin quantum number is \( I \), the nuclear spins distribute among \( 2I \) different energy levels, and the nuclear polarization is defined as \(^{44}\)

\[
P_I = \frac{1}{I} \frac{\sum m N_m}{\sum N_m} = \frac{1}{I} \langle I_z \rangle, \quad (2.1)
\]

where \( m \) is the magnetic quantum number corresponding to a distribution energy level in the magnetic field; \( N_m \) is the number of nuclear spins on the energy level \( m \); \( \langle I_z \rangle \) is the expectation value of the nuclear spin operator \( \hat{I}_z \). For a nuclear spin of \( I = 1/2 \), the nuclear polarization is given as

\[
P_I = \frac{N_+ - N_-}{N_+ + N_-}, \quad (2.2)
\]
where \( N_+ \) and \( N_- \) are the nuclear spins aligned with and aligned against the magnetic field, respectively. The polarization is normally very small. For the \( ^1\text{H} \) nucleus in the magnetic field of 1 Tesla and at 293 \(^\circ\)K, \( P_1 \) is about \( 3.4 \times 10^{-6} \). The larger the nuclear polarization, the stronger the corresponding NMR signal. There are three means by which the polarization can be increased. One is to increase the magnetic field, another is to lower the sample temperature, and the third one is to use the DNP technique, that is, to saturate the electron spin transition when the nuclear spin is coupled to the electron spin.

In a normal DNP experiment, a stable free radical is added into the sample solution usually at a concentration of 0.1 - 0.001 M. In the solution, the free radical molecules interact with the sample molecules through diffusional collisions. For such a coupled nucleus-electron spin system, the Hamiltonian operator can be expressed as\(^{45}\)

\[
\hat{H} = -\gamma_s \hbar \hat{S}_Z B_0 - \gamma_1 \hbar \hat{I}_Z B_0 + \hat{H}_{sl}(t) + \hat{H}_{nt}(t) + \hat{H}_{ss}(t), \quad (2.3)
\]

In this formula, the first two terms are the respective electron and nuclear Zeeman terms; the last three terms represent the time-dependent electron-nuclear, nucleus-nucleus, and electron-electron interaction terms, respectively; \( \hat{S}_Z \) and \( \hat{I}_Z \) are the electron and nuclear spin operators, respectively; \( \gamma_s \) and \( \gamma_1 \) are the respective electron and nuclear spin gyromagnetic ratios. Among the three inter-spin coupling terms, \( \hat{H}_{sl}(t) \) is the only term
which can significantly induce DNP effects in liquids. \( \hat{H}_{sl} \) normally consists of two terms, one is the classic dipolar interaction term, which can be expressed as

\[
\hat{H}_{sl}^D(t) = \gamma_s \gamma \hbar \left\{ \frac{3(\hat{I} \cdot \hat{r})(\hat{S} \cdot \hat{r})}{r^5} - \frac{\hat{I} \cdot \hat{r}}{r^3} \right\}, \quad (2.4)
\]

The time dependence of the dipolar interaction arises either from the fluctuations in the orientation of \( \hat{r} \) with respect to the external magnetic field, i.e., rotational motion, or more commonly from the fluctuations of \( \hat{r} \), i.e., translational motion. Another term of \( \hat{H}_{sl} \) is the scalar interaction term, which is in the following form:

\[
H_{sl}^S(t) = -A_{sl} \cdot \hat{I} \cdot \hat{S}, \quad (2.5)
\]

where \( A_{sl} \) is the isotropic hyperfine coupling constant, which can be expressed as

\[
A_{sl} = -\left( \frac{8 \pi}{3} \right) \gamma_s \gamma_s |\psi(0)|^2, \quad (2.6)
\]

where \( |\psi(0)|^2 \) is the amplitude of the electronic wave function at the nucleus of the sample molecule. The energy level diagram of the nucleus-electron pairs is shown in Figure 2.1.
Figure 2.1. Energy Level Diagram of the Nucleus-Electron Spin Coupled System

with $i=1/2$ and $S=1/2$ (Cited from Ref. 1).
For an electron-nucleus spin coupling system, the nuclear polarization is proportional to \( \langle I_z \rangle \) (Eq. 2.1), which is related to the transition probabilities of the nuclear spins in between the energy levels of the coupled system. The detailed derivation of \( \langle I_z \rangle \) in the form of nuclear transition probabilities has been done by Solomon,\(^{46}\) and the final result can be expressed as

\[
\langle I_z \rangle = I_0 \left[ 1 + \frac{W_2^0 - W_0^0 - W_{0c}^0}{W_0^0 + W_0^{Sc} + 2W_1^D + W_2^D} \times \frac{W_0^D + W_0^{Sc} + 2W_1^D + W_2^D}{(W_0^D + W_0^{Sc} + 2W_1^D + W_2^D) + 2W_{10}} \times \frac{S_0 - \langle S_z \rangle}{S_0} \times \frac{S_0}{I_0} \right]
\]

(2.7)

Among the four multiplications of this formula, the first three terms have specific physical meaning, and have been defined as follows:

\[
\rho = \frac{W_2^0 - W_0^0 - W_{0c}^0}{W_0^0 + W_0^{Sc} + 2W_1^D + W_2^D}, \quad (2.8)
\]

\[
f = \frac{W_0^D + W_0^{Sc} + 2W_1^D + W_2^D}{(W_0^D + W_0^{Sc} + 2W_1^D + W_2^D) + 2W_{10}}, \quad (2.9)
\]

\[
s = \frac{S_0 - \langle S_z \rangle}{S_0}, \quad (2.10)
\]
Substituting Eq. 2.8-2.10 into Eq. 2.7, and using the relation $S_0/I_0 = \gamma_s/\gamma_I$, the $\langle I_Z \rangle$ can be expressed as:

$$\langle I_Z \rangle = I_0 \left( 1 + \rho fs \frac{\gamma_s}{\gamma_I} \right), \quad (2.11)$$

This formula can be expressed in another form:

$$\frac{\langle I_Z \rangle - I_0}{I_0} = \rho fs \frac{\gamma_s}{\gamma_I}, \quad (2.12)$$

By defining the observed DNP enhancement as $A_{\text{obs}} = \frac{\langle I_Z \rangle - I_0}{I_0}$, the theoretical expression for the observed DNP enhancement is:

$$A_{\text{obs}} = \rho fs \frac{\gamma_s}{\gamma_I}, \quad (2.13)$$

Since $\langle M_Z \rangle = N\hbar \gamma_I \langle I_Z \rangle$, the observed DNP enhancement can also be expressed as:

$$A_{\text{obs}} = \frac{\langle M_Z \rangle - M_0}{M_0}, \quad (2.14)$$
where $M_Z$ is the longitudinal magnetization with DNP enhancement, while $M_0$ is the normal longitudinal magnetization of static NMR, $N$ is the number of nuclear spins. Since the longitudinal magnetization can be obtained either from the peak heights of the DNP spectrum, or from those of the NMR spectrum, the observed DNP enhancement $A_{obs}$ can be experimentally measured. Because the observed DNP enhancement is system and experimental condition dependent, it needs to be converted to the ultimate enhancement before it can be used to evaluate the experimental results. The ultimate DNP enhancement is defined as follows:

$$A_u = \rho \frac{\gamma_s}{\gamma_I}$$  \hspace{1cm} (2.15)

The way to calculate the ultimate DNP enhancement is to divide the observed DNP enhancement in Eq. 2.13 by the $f$ factor and $s$ factor. From Figure 2.1 and Equation 2.8, it can be seen that if the scalar relaxation (labeled $W_0^{(s)}$) is a dominant relaxation process, a scalar dominated enhancement will be observed. On the other hand, if the dipolar relaxations (labeled $W_1^{(D)}$, $W_0^{(D)}$, and $W_2^{(D)}$) dominate, a dipolar dominated enhancement will be observed.

The leakage factor $f$ measures the nuclear relaxation time percentage induced by the free radical among the total nucleus relaxation time. Experimentally, the $f$ factor can be determined by measuring the nuclear relaxation times of the sample molecules in the
presence and the absence of the free radical molecule in the solution. The calculation formula for the $f$ factor is as follows:

$$f = 1 - \frac{T_1}{T_{10}}, \quad (2.16)$$

where $T_1$ and $T_{10}$ are the relaxation times of the sample nucleus measured with and without the free radical in the sample solution.

Although the $f$ factor can range from 0 to 1 in the formula 2.13, for the proton or fluorine coupled nucleus of interest (such as $^{13}$C), the DNP enhancement for the nucleus of interest can only be observed when the $f$ factor value is close to 1. When the $f$ factor is much less than 1, it means that the nucleus relaxation is significantly caused by the surroundings other than by the unpaired electron of the free radical, such as by the oxygen molecules in the sample solution or by the three-spin effect which results from the dipolar coupling between the nucleus of interest and the proton or fluorine nucleus attached to the nucleus of interest. The nuclear relaxation induced by the dissolved oxygen can be eliminated through extensively degassing the sample solution. The three-spin effect, however, is free radical concentration dependent, when the free radical concentration is high enough (usually higher than 0.1 M), the three-spin effect is negligible, when the free radical concentration is too low, the three-spin effect can be quite large, and a correction needs to be made to the observed DNP enhancement of the nucleus in question because the three-spin effect can contribute either positively or negatively to the observed DNP
enhancement of the nucleus depending on the sign of the gyromagnetic ratio of the nucleus of interest. The final form of the observed DNP enhancement after the three-spin effect correction is as follows:\textsuperscript{47,48}

\[ A = \left( \rho_N^s f_N^s - \rho_N^H f_N^H \rho_H^s f_H^s \right) \frac{\gamma_N^s}{\gamma_N^s}, \quad (2.17) \]

In this formula, if \( N \) represents the \(^{13}\)C nucleus,

then \[ \rho_C^H = \frac{1}{2}, \quad f_C^H = \frac{\eta_{NOE} - 1}{\gamma_H / \gamma_C}, \quad \rho_H^s = \frac{A_m^H}{\gamma_S / \gamma_H}, \quad f_S^H \approx 1. \]

Where \( \eta_{NOE} \) is the NOE factor.

The three-spin effect correction can also be made directly to the experimental ultimate enhancement in the following formula:

\[ A_C^s(\text{exp}) = \left( \rho_C^s - \rho_C^H f_C^H f_S^H \right) \frac{\gamma_S}{\gamma_C}, \quad (2.18) \]

The true \(^{13}\)C DNP ultimate enhancement corrected for the three spin effects is therefore given by the following formula:

\[ A_C^s(\text{true}) = \rho_C^s \frac{\gamma_S}{\gamma_C} = A_C^s(\text{exp}) + \rho_C^H f_C^H \rho_H^s \frac{\gamma_S}{\gamma_C}, \quad (2.19) \]
An illustration of the nucleus-electron two and three spin coupling systems is shown in Figure 2.2. The $s$ factor defined in Eq. 2.10 is a measure for the degree of saturation of the electron spin transition of the free radical molecules by the microwave power. The longitudinal expectation value of the electron spin moment is given by the following formula:

$$\langle S_z \rangle = \frac{S_0}{1 + \gamma_s^2 B_{is}^2 T_{is} T_{2s}}, \quad (2.20)$$

Therefore, the $s$ factor in Eq. 2.10 can be expressed as:

$$s = \frac{\gamma_s^2 B_{is}^2 T_{is} T_{2s}}{1 + \gamma_s^2 B_{is}^2 T_{is} T_{2s}}, \quad (2.21)$$

where $T_{is}$ and $T_{2s}$ are the electron spin-lattice and spin-spin relaxation rates, respectively; $B_{is}$ is the inductance of the microwave field, the square of this quantity is proportional to the microwave power $P$. From Eq. 2.13 and Eq. 2.21,

$$\frac{1}{A} = \left( \frac{\gamma_s^2 B_{is}^2}{\gamma_I} \right)^{-1} \left( 1 + \frac{1}{\gamma_s^2 B_{is}^2 T_{is} T_{2s}} \right), \quad (2.22)$$

Since $B_{is} \ll P$, the $s$ factor can be obtained by making a plot of $1/A$ versus $1/P$. The reciprocal of the intercept of this plot is the extrapolated enhancement when $s = 1$, that is,
Figure 2.2. Nucleus-Electron Coupling Systems: (a) Two Spin Coupling System; (b) Three Spin Coupling System (Cited from Ref. 1).
\[ A_{\text{obs}}^{\text{obs}} = \rho f \frac{Y_2}{Y_1} \text{ Intercept Value, } \quad (2.23) \]

By making a ratio of the observed enhancement \( A_{\text{obs}}^{\text{obs}}(P) \) measured under certain microwave power \( P \) to the \( A_{\text{obs}}^{\text{obs}}(P \to \infty) \), the \( s \) factor corresponding to the \( A_{\text{obs}}^{\text{obs}}(P) \) is obtained, that is,

\[ s = \frac{A_{\text{obs}}^{\text{obs}}(P)}{A_{\text{obs}}^{\text{obs}}(P \to \infty)}, \quad (2.24) \]

The stable free radicals employed in this work were nitroxide radicals TEMPO and TEMPOL (4-hydroxy TEMPO). These free radicals are very stable; the unpaired electron is localized on the N-O group; and they are commercially available. Some of them are water soluble, such as TEMPOL.

The major drawback of these free radicals is that there are three electron spin transitions (Figure 2.3), which means the possible incomplete saturation of these electron spin transitions by the microwave power during the DNP experiment, and thus an error occurs in the ultimate DNP enhancement.

The influence of the triple resonance of the nitroxide free radical on the DNP enhancement depends on the free radical concentration. When the free radical concentration is high enough (>0.08 M), fast unpaired electron spin exchange (called electron-electron exchange effect, or briefly, e-e effect) may occur which results in a rapid spreading of the electron spin transitions among the free radicals so that the three triple electron spin transition peaks average into a broad single transition peak (Figure 2.3),
which is easier to saturate, and the electron-electron exchange has little effect on the DNP enhancement. The EPR spectra of TEMPO in different concentration are given in Figure 2.3.

When the free radical concentration is lower than 0.005 M, the e-e effect can not be ignored. The estimation of the e-e effect on the DNP ultimate enhancement can be made by the following formula:\(^{40,50}\)

\[
\frac{A_\infty}{G_\infty} = 3 \frac{p + \kappa}{p + 3\kappa}. \tag{2.25}
\]

where \(A_\infty\) is the true DNP enhancement with nitroxides as the free radical, while \(G_\infty\) is the extrapolated enhancement with the e-e effect; \(\kappa\) is the e-e exchange rate, which is proportional to the free radical concentration, \([R^-]\), and can be estimated from the EPR linewidth in the following formula:

\[
\frac{1}{T_{1S}} \approx \left( \frac{1}{T_{1S}} \right)_{[R^-]=0} + \kappa. \tag{2.26}
\]

\(p\) is the electron transition probability, which is estimated from the EPR linewidth when the free radical concentration is zero,
Figure 2.3. EPR Spectra of TEMPO: (a) 0.001 M; (b) 0.1 M
(Cited from Ref. 1).
\[ p \approx \left( \frac{1}{T_{1S}} \right)_{\left[ R \right]=0}, \quad (2.27) \]

where \( \frac{1}{T_{1S}} \) and \( \left( \frac{1}{T_{1S}} \right)_{\left[ R \right]=0} \) are the linewidth of the EPR signals of the nitroxide free radicals at certain and zero concentrations, respectively.

From the experimentally measurable data \( G_\infty \), \( \kappa \), and \( p \), the true enhancement \( A_\infty \) can be obtained.

Another possible approach to eliminate the e-e effect is to saturate the triple electron transitions of the nitroxide radicals by a pulsed EPR technique. This technique may also be a promising method for accurately measuring the \( s \) factor in the DNP experiment.

### 2.2 General Form of the Coupling Factor \( p \)

From Eq. 2.15, it can be seen that the ultimate DNP enhancement is determined by the gyromagnetic ratio between the electron and the nucleus, and by the coupling constant \( p \). Since the gyromagnetic ratio is the intrinsic property of the electron and the nucleus, the intermolecular interaction information, such as the coupling intensity and the coupling mechanism, is solely contained in the coupling constant.
nucleus, the intermolecular interaction information, such as the coupling intensity and
the coupling mechanism, is solely contained in the coupling constant.

According to Abragam, the Hamiltonian term in Eq. 2.8 for the nuclear-electron
coupling pair can be expressed as

\[
\hat{H}_j(t) = \sum_{i=2}^{3} \hat{U}_i F_i(t). \quad (2.28)
\]

where \( F_j(t) \) are the random functions of only the relative positions of the two spins of
interest and the \( \hat{U}_j \) are operators acting only on the spin variables of the nuclear-electron
system, with the convention \( F_j = F_j^* \), and \( \hat{U}_j = \hat{U}_j^* \).

The functions \( F_j \) are given by

\[
F_0 = -\sqrt{\frac{48\pi}{15}} Y_2^0(\theta, \phi) \cdot r^{-3}; \quad F_1 = -\sqrt{\frac{8\pi}{15}} Y_2^1(\theta, \phi) \cdot r^{-3}.
\]

\[
F_2 = \sqrt{\frac{32\pi}{15}} Y_2^{1*}(\theta, \phi) \cdot r^{-3}; \quad F_3 = \Lambda(t) \quad (2.29)
\]

The functions \( Y_2^l \) are the second order normalized spherical harmonics. The angles \( \theta \)
and \( \phi \) are the polar coordinates of the interspin vector \( \vec{r} \) in the laboratory frame. The
quantity \( \Lambda(t) \) represents the intermolecular hyperfine coupling between the nucleus and
the electron. According to the nature of the dipolar and scalar coupling, \( F_0, F_1, \) and \( F_2 \) are
the random functions for describing the dipolar coupling, while $F_2$ is for the scalar coupling.

From Eq. 2.8, it can be seen that the coupling constant is determined by the transition probabilities of the nucleus-electron coupling system. The energy level and the corresponding transitions are shown in Figure 2.1. With the help of perturbation theory, the transition probability $W_{km}$ between the states $|k\rangle$ and $|m\rangle$ in the coupling system can be expressed as\textsuperscript{51}

$$W_{km} = \frac{1}{\hbar^2} \sum_{\nu,\lambda} \left| \langle k | \hat{U}_j | m \rangle \right|^2 J_j(\omega_j), \quad (2.30)$$

where $J_j(\omega_j)$ are the spectral density functions whose form are determined by the specific nucleus-electron coupling mechanisms. By calculating out the $W_{km}$ for each term in formula 2.8, the coupling constant $\rho$ can be expressed as a function of the spectral density functions, which contain the intensity and the interacting mechanism information of the coupling process. The derivation of the coupling constant $\rho$ is given below.

The operators $\hat{U}_j$ are given by\textsuperscript{51}

$$\hat{U}_0 = \alpha \left\{ -\frac{2}{3} \hat{I}_x \hat{S}_z + \frac{1}{6} (\hat{I}_y \hat{S}_z + \hat{I}_z \hat{S}_y) \right\},$$

$$\hat{U}_1 = \alpha \left\{ \hat{I}_x \hat{S}_z + \hat{I}_z \hat{S}_y \right\},$$
\[
\hat{U}_z = \frac{1}{2} \alpha \mathbf{i}_z \mathbf{\hat{S}}_z, \tag{2.31}
\]

\[
\hat{U}_r = \mathbf{i}_r \mathbf{\hat{S}}_r + \frac{1}{2} \{ \mathbf{\hat{S}}_+ \mathbf{i}_- + \mathbf{\hat{S}}_- \mathbf{i}_+ \}.
\]

Where \( \alpha = -\frac{3 \gamma \gamma' h'}{8 \pi \mu_n} \). \hspace{1cm} (2.32)

Using the relation \( \hat{U}_r = \hat{U}_r \), the following formula can be derived:

\[
\hat{U}_{r1} = \hat{U}_r
\]
\[
= \alpha \left\{ (\mathbf{\hat{S}}_+ \mathbf{\hat{S}}_-) + (\mathbf{\hat{S}}_- \mathbf{\hat{S}}_+) \right\}
\]
\[
= \alpha \left\{ (\mathbf{\hat{S}}_+ \mathbf{\hat{i}_-}) + (\mathbf{\hat{S}}_- \mathbf{\hat{i}_+}) \right\}
\]
\[
= \alpha \left\{ (\mathbf{\hat{S}}_+ \mathbf{\hat{i}_-}) + (\mathbf{\hat{S}}_+ \mathbf{\hat{i}_-}) \right\}
\] \hspace{1cm} (2.33)

Similarly,

\[
\hat{U}_{r2} = \hat{U}_r
\]
\[
= \frac{1}{2} \alpha \mathbf{i}_z \mathbf{\hat{S}}_z \tag{2.34}
\]

\[
\hat{U}_{r3} = \mathbf{i}_z \mathbf{\hat{S}}_z + \frac{1}{2} \{ \mathbf{\hat{S}}_+ \mathbf{i}_- + \mathbf{\hat{S}}_- \mathbf{i}_+ \}, \tag{2.35}
\]

By applying the Eqs 2.31-2.35 to Eq. 2.30, the transition probabilities in Eq. 2.8 can be calculated as follows:
\[ W_0 = W_1 \]
\[ = \frac{1}{\hbar} \sum_{j=0}^{\infty} \left| \langle 1 | \hat{U}_j | 4 \rangle \right|^2 J_j \left( \omega_j \right) \]  \hspace{1cm} (2.36)

where \( J_j \left( \omega_j \right) = \int g_j(\tau) e^{\omega_j \tau} d\tau \), and \( g_j(\tau) = \frac{F_j^*(\tau)F_j(\tau + \tau)}{F_j^*(\tau)F_j(\tau + \tau)} \).

Since \( g_j(\tau) = g_{-j}(\tau) \), and \( \omega_j = \omega_{-j} \), \( J_j(\omega_j) = J_{-j}(\omega_{-j}) \). Therefore,

\[ W_0 = W_1 \]
\[ = \frac{1}{\hbar} \left\{ \left| \langle 1 | \hat{U}_{-0} | 4 \rangle \right|^2 J_{-0} \left( \omega_{-0} \right) + \left| \langle 1 | \hat{U}_{-1} | 4 \rangle \right|^2 J_{-1} \left( \omega_{-1} \right) \right. \\
+ \left. \left| \langle 1 | \hat{U}_{0} | 4 \rangle \right|^2 J_{0} \left( \omega_{0} \right) + \left| \langle 1 | \hat{U}_{1} | 4 \rangle \right|^2 J_{1} \left( \omega_{1} \right) \right\} \]  \hspace{1cm} (2.37)

According to Figure 2.1 and the properties of the raising- and lowering- operators, the following results are obtained:

\[ \left| \langle 1 | \hat{U}_{-0} | 4 \rangle \right| = \frac{1}{2} \alpha \left| \langle 1 | \hat{S}_z | 4 \rangle \right| \]
\[ = \frac{1}{2} \alpha \left| \langle 1 | \hat{S}_z | 3 \rangle \right| \]
\[ = 0 \]

\[ \langle 1 | \hat{U}_{0} | 4 \rangle = \frac{1}{2} \alpha \langle 1 | \hat{S}_z | 4 \rangle, \]  \hspace{1cm} (2.38)
\[ = 0 \]
\begin{align*}
\langle 1| \hat{\mathcal{U}}_s | 4 \rangle &= \frac{1}{2} \{ \langle 1| \hat{\mathcal{S}}_x | 3 \rangle + \langle 1| \hat{\mathcal{S}}_y | 4 \rangle + \langle 1| \hat{\mathcal{S}}_z | 4 \rangle \} + \frac{1}{2} \{ \langle 1| \hat{\mathcal{S}}_x | 4 \rangle + \langle 1| \hat{\mathcal{S}}_y | 3 \rangle + \langle 1| \hat{\mathcal{S}}_z | 4 \rangle \} \\
&= 0 + \frac{1}{2} \{ \langle 1| \hat{\mathcal{S}}_x | 3 \rangle + 0 \} \\
&= \frac{1}{2} \langle 1| 1 \rangle \\
&= \frac{1}{2}
\end{align*}

\therefore \quad W_0 = \frac{1}{\hbar^2} \left\{ \left( -\frac{1}{2} \alpha \right)^2 J_5 (\omega_0) + \left( \frac{1}{2} \alpha \right)^2 J_2 (\omega_2) \right\}, \quad (2.39)

W_i = W_{i_2}
= \frac{1}{\hbar^2} \sum_{\beta = 0}^{2} \left\{ \langle 1| \hat{\mathcal{U}}_s | 2 \rangle + J_3 (\omega_3) \right\}
= \frac{1}{\hbar^2} \left\{ \langle 1| \hat{\mathcal{U}}_s | 2 \rangle \right\} + J_3 (\omega_3)
+ \left\{ \langle 1| \hat{\mathcal{U}}_s | 2 \rangle \right\} + J_3 (\omega_3)
+ \left\{ \langle 1| \hat{\mathcal{U}}_s | 2 \rangle \right\} + J_3 (\omega_3)
+ \left\{ \langle 1| \hat{\mathcal{U}}_s | 2 \rangle \right\} + J_3 (\omega_3)
\right\}
= \frac{1}{\hbar^2} \left( -\frac{1}{2} \alpha \right)^2 J_1 (\omega_1)
\right\}, \quad (2.41)

W_i = W_{i_4}
= \frac{1}{\hbar^2} \sum_{\beta = 0}^{2} \left\{ \langle 3| \hat{\mathcal{U}}_s | 4 \rangle \right\} + J_3 (\omega_3)
= \frac{1}{\hbar^2} \left( -\frac{1}{2} \alpha \right)^2 J_3 (\omega_3)
\right\}, \quad (2.42)

2W_i = W_{i_2} + W_{i_4}
= \frac{2}{\hbar^2} \left( -\frac{1}{2} \alpha \right)^2 J_1 (\omega_1). \quad (2.43)
\[ W_z = W_{33} \]
\[ = \frac{1}{h^2} \sum_{\nu=1}^{3} |\langle 2 \mid \hat{U}_{\nu} \mid 3 \rangle|^2 J_z(\omega_z), \quad (2.44) \]
\[ = \frac{1}{h^2} \left( \frac{1}{2} \alpha \right)^2 J_z(\omega_z) \]

\[ \therefore \rho = \frac{W_z - W_0}{W_0 + 2W_z + W_2} \]
\[ = \frac{1}{h^2} \left( \frac{1}{2} \alpha \right)^2 J_z(\omega_z) - \frac{1}{h^2} \left\{ \left( \frac{1}{6} \alpha \right)^2 J_z(\omega_z) + \left( \frac{1}{2} \alpha \right)^2 J_z(\omega_z) \right\} \]
\[ = \frac{1}{h^2} \left[ \left( \frac{1}{6} \alpha \right)^2 J_z(\omega_z) + \left( \frac{1}{2} \alpha \right)^2 J_z(\omega_z) \right] + \frac{1}{h^2} \left[ \left( \frac{1}{2} \alpha \right)^2 J_z(\omega_z) + \frac{1}{h^2} \left( \frac{1}{2} \alpha \right)^2 J_z(\omega_z) \right] \]
\[ = 9J_z(\omega_z - \omega) - J_z(\omega_z + \omega) - \left( 8 \pi \mu_0 \gamma_3 \gamma_z h^2 \right)^2 J_z(\omega_z + \omega) \]
\[ 9J_z(\omega_z - \omega) + J_z(\omega_z + \omega) + 18J_z(\omega) + \left( 8 \pi \mu_0 \gamma_3 \gamma_z h^2 \right)^2 J_z(\omega_z + \omega) \]

where \( \omega \) and \( \omega \) denote the nuclear and electron resonance frequency. The specific form of the spectral density function in this formula depends on the model chosen for the interspin coupling. Different modulation models for the nucleus-electron coupling will be discussed in the next section. Since \( W_0 \) is a sum of two terms which involve the spectral density functions of \( J_n(\omega_n) \), which is related to the dipolar coupling, and \( J_z(\omega_z) \), which is related to the scalar coupling, this transition probability can be written as:

\[ W_0 = W_0^d + W_0^s, \quad (2.46) \]

Therefore, the coupling factor can be further written into the following form:
\[ \rho = \frac{W_0^D - W_0^S - W_0^\infty}{W_0^D + W_0^\infty + 2W_1^D + W_2^D}, \quad (2.47) \]

2.3 Dipolar and Scalar Coupling

The interaction between the nuclear spin and the electron spin in the sample solution can be classified into two mechanisms: the dipolar coupling and the scalar coupling. The former is a classical magnetic interaction between the magnetic dipoles of the nuclear spin and that of the electron spin. The dipolar interaction is effective through the interspin space and is always present in the solution. The corresponding spectral density function is inversely proportional to either the third power or the sixth power of the interspin distance, depending on whether the coupling model is translational or rotational diffusion, respectively. The scalar coupling is a quantum mechanical interaction which involves the electron spin density transfer from the unpaired electron to the \textit{ns} orbital of the sample nucleus. The transferred electron spin density in the \textit{ns} orbital interacts with the nuclear spin and causes the spin relaxation labeled $W_0^{Sc}$ in Figure 2.1. Since the electron spin density transfer requires a close contact and overlapping of the wave functions of the nuclear and electron spins, the scalar interaction is a short range interaction and is also called “contact coupling”.
pair. For dipolar coupling, two modulation models have been proposed. One is the relative translational diffusion model.\textsuperscript{54} In this model, the nuclear and electron spins are in the center of the molecular moieties, which are assumed to be spherical. The dipolar coupling is modulated by fluctuations in the interspin distance, and the free radical and the sample molecule do not form a complex. The much more general case of off-center spins cannot be solved completely. For this model, the spectral density function is given by

\[ J_1(\omega) = \frac{1}{1 + 0.9(\omega, \tau)^{\frac{1}{2}} + 1.5(\omega, \tau)^{\frac{3}{2}}}, \quad (2.48) \]

where \( \omega_l \) is the Larmor frequency for the coupled nuclear-electron pair, and takes the values of \( \omega_n, \omega_s + \omega_n, \omega_s - \omega_n \) for the transitions of single, double, and zero quantum, respectively. \( \tau \) is the translational correlation time, which is defined by the following equation:\textsuperscript{48}

\[ \tau = \frac{D_i^2}{\frac{3}{2}(D_i + D_s)}, \quad D = \frac{kT}{6\pi\eta r}, \]

\[ = \frac{12\pi d_i^2}{5k} \left( \frac{r_r}{r_i + r_s} \right) \frac{\eta}{T} \quad (2.49) \]
where $D$ is the diffusion constant, $d_i$ is the distance of the closest approach between the free radical and the receptor molecule, $r_i$ and $r_s$ are the radii of the sample molecule and of the free radical molecule, and $\eta$ is the solution viscosity.

The dipolar transition probabilities for this model are given by\textsuperscript{54}

$$2W_i^{\circ} = 3W_0^{\circ} = W_i^{\circ}/2 = \frac{2\pi}{5} \gamma_3^N \gamma_i^N \hbar^2 \varepsilon_i N_c d_i^{-3} J_i(\omega_i),$$ \hfill (2.50)

where $N_c$ is the unpaired electron concentration.

Another modulation mechanism for the dipolar coupling is the rotational diffusion model.\textsuperscript{55} This model applies to the situation where the free radical and the sample molecule form a tight bound complex, or when the nuclear and electron spins are in the same molecule. The spectral density function for this model is given by

$$J(\omega) = \frac{1}{1 + \omega^2 \tau^2},$$ \hfill (2.51)

where $\tau$ is the rotational correlation time and is defined as:

$$\tau = \frac{4\pi b^3 \eta}{3kT},$$ \hfill (2.52)

$b$ is the effective tumbling radius of the molecular complex.
The transition probabilities are given by

\[ 2W_1^D = 3W_0^D = W_2^D / 2 = \frac{3}{10} \gamma_2^2 \gamma_2^2 \hbar^2 \tau_r \left( \frac{n_p N_e}{N_r} \right) d_r^{-6} J_r(\omega_j), \quad (2.53) \]

where \( n_p \) is the number of the receptor nuclei bound near each electron, \( N_p \) is the total nuclear concentration, \( d_r \) is the average pair radius of the rotating adduct.

When the sample molecule and the free radical form a weak complex, such as a weak hydrogen bond between a proton of the sample molecule and the free radical, the dipolar transition may be modulated by the mixed translational and rotational diffusion. The spectral density function for the mixed modulation is in the following form:\(^56\)

\[ J_p(\omega_i) = \frac{2 n_e \tau_r}{3}\left\{ f_i(\omega, \tau) + R \frac{\tau_r}{\tau_i} f_r(\omega, \tau) \right\}, \quad (2.54) \]

where \( n_e \) is the number of the unpaired electrons per unit volume, \( f_i(\omega, \tau) \) and \( f_r(\omega, \tau) \) are the reduced spectral density functions, and are defined by

\[ f_i(\omega, \tau) = \frac{J_p(\omega_i)}{J_p(0)}; \quad f_r(\omega, \tau) = \frac{J_p(\omega, \tau)}{J_p(0)}, \quad (2.55) \]

The quantity \( R \) measures the relative influence of the rotational diffusion to the mixed modulation of the dipolar coupling. It is given by
\[ R = \frac{3md^3}{4\pi b^3 n_i} \]  
(2.56)

where \( n_i \) is the number of the nuclei per unit volume, \( m \) is the average number of nuclei associated with a radical molecule.

The coupling constant for the case of a mixture of translational and rotational diffusion can be derived by assuming that \( \omega_i << \omega_s \), and by employing the following formula:

\[ J_0(\omega_i) = J(\omega_i)/C_j, \]  
(2.57)

where \( J(\omega_i) \) is the same spectral density function used in Eq.2.44, \( \omega_i \) can be \( \omega_t, \omega_s-\omega_t \) or \( \omega_s+\omega_t \) \( C_j \) is a constant. By substituting the \( J(\omega_i) \) into Eq.2.45, the coupling factor can be written as

\[ \rho = \frac{1}{2} \frac{\left\{ f_s(\omega_s \tau) + R \frac{\tau}{\tau_s} f_s(\omega_s \tau) \right\} + 0.3 \left\{ f_s(\omega_s \tau) + R \frac{\tau}{\tau_s} f_s(\omega_s \tau) \right\}}{0.7 \left\{ f_s(\omega_s \tau) + R \frac{\tau}{\tau_s} f_s(\omega_s \tau) \right\} + 0.3 \left\{ f_s(\omega_s \tau) + R \frac{\tau}{\tau_s} f_s(\omega_s \tau) \right\}}, \]  
(2.58)

This equation can be used for interpreting the DNP results of nuclei that have a mixed translational and rotational diffusion dipolar coupling mechanism, such as the case of protons without any scalar contribution.
As mentioned before, the Hamiltonian for the scalar interaction between the nuclear spin and the unpaired electron spin can be expressed as

\[ \hat{H}_{SI}(t) = -A_{SI} \hat{I} \cdot \hat{S}, \quad (2.5) \]

The time dependence of the scalar coupling may arise either from the time dependence of the hyperfine coupling constant \( A_{SI} \), or from the time dependence of the orientation of the electron spin \( \hat{S} \). These two mechanisms are denoted as scalar relaxation of the first kind and the second kind, respectively.\(^{57}\) The second kind of the scalar relaxation has been found to be important in systems such as MnCl\(_2\) in aqueous solutions. The unpaired electron of Mn\(^{2+}\) couples with the water protons. At low magnetic field, large positive enhancements were observed, indicating a scalar dominated coupling mechanism.\(^{60}\) This is due to the rapid flipping of the electron spin \( S \) since the transition metal ions have very short relaxation times (order of magnitude \( 10^{-9} \) s). For the free radicals (nitrooxides) employed in the present work, the relaxation time is much longer than that of the transition metal ions (order of magnitude of \( 10^{-6} \) s), the time dependence of the scalar coupling is dictated by the first kind of relaxation mechanism.

For the first kind of the scalar relaxation mechanism, two modulation models have been introduced. One is called the sticking model, another is called the diffusion model. In the sticking model, there is a finite scalar interaction only when the nuclear spin and the unpaired electron spin are "stuck" together, such as in the case of forming a weak
radicals (nitroxides) employed in the present work, the relaxation time is much longer than that of the transition metal ions (order of magnitude of $10^{-6}$ s), the time dependence of the scalar coupling is dictated by the first kind of relaxation mechanism.

For the first kind of the scalar relaxation mechanism, two modulation models have been introduced. One is called the sticking model, another is called the diffusion model. In the sticking model, there is a finite scalar interaction only when the nuclear spin and the unpaired electron spin are "stuck" together, such as in the case of forming a weak complex between the sample molecule and the free radical. When the complex falls apart, the hyperfine coupling constant becomes zero, no scalar interaction will occur. The sticking time is a random variable. This model leads to a scalar spectral density function given by

$$J_{1s} (\omega_s) = \frac{m A_{\gamma\gamma}^2}{n_1 h^2} \tau_s \frac{\omega_s}{1 + \omega_s^2 \tau_s^2}, \quad (2.59)$$

where $\tau_s$ is the scalar correlation time, and it is assumed that $\omega_s >> \omega_1$. The scalar transition probability is given by

$$W_{s}^{\infty} = \frac{1}{2} \frac{A_{\gamma\gamma}^2}{h^2} \frac{n_1 N_e}{N_s} \tau_s \frac{\omega_s}{1 + (\omega_s + \omega_1)^2 \tau_s^2}, \quad (2.60)$$
In the diffusion model, the scalar interaction is assumed to be a function of the interspin distance $r$ of $I$ and $S$. The time dependence of the interaction is due to the variation of $r$ with time. Since the scalar interaction may involve the overlap of the atomic orbitals between the free radical and the sample molecules, the interspin distance can be very short. The hyperfine coupling constant was assumed to be a steep function of the interspin distance in the following form

$$A_{ij} = A_0 \frac{d}{r_{is}} \exp \{-\lambda (r_{is} - d)\}, \quad (2.61)$$

where $d$ is the distance of the closest approach between $S$ and $I$, $r_{is}$ is the interspin distance between $S$ and $I$, $A$ and $\lambda$ are constants.

The scalar transition probability for the diffusion model is given by

$$W_d^{\infty} = \frac{N \tau_s A_0 d^4}{2 \lambda D (\omega \tau_s)^{1/2}} \left\{1 + \exp(\omega \tau_s)^{1/2} \left[\sin(\omega \tau_s)^{1/2} - \cos(\omega \tau_s)^{1/2}\right]\right\}, \quad (2.62)$$

where $\omega = \omega_s + \omega_r$, $\tau_s$ is the scalar correlation time, the other parameters are the same as before.

For some nuclei, such as $^{13}$C and $^{31}$P, the interaction with the free radical is modulated by a mixture of scalar and dipolar coupling. The dipolar coupling is usually modulated by a mixture of translational and rotational diffusion. The coupling factor for these nuclei should take into account both the nuclear-electron interactions and the
\[ J_3(\omega) = J_{A_0}(\omega) \]
\[ = \int_{-\infty}^{+\infty} \frac{A(t)A(t+\omega)}{A(t)A(t+\omega)} e^{-i\omega \tau} d\tau, \quad (2.64) \]

In order to simplify the expression of \( \rho \), the spectral density function for scalar coupling is defined as

\[ J_{Sc}(\omega) = \frac{64 \pi^2 \mu_0^2}{C^2 \gamma_s^2} J_{A_0}(\omega), \quad (2.65) \]

Further, the reduced scalar intensity function is defined as

\[ f_{Sc}(\omega, \tau_s) = \frac{J_{Sc}(\omega, \tau_s)}{J_{Sc}(0)}, \quad (2.66) \]

The coupling factor is obtained as\(^{11}\)

\[ \rho = \frac{0.5 \left\{ f_1(\omega, \tau_s) + R \frac{\tau}{\tau_s} f_2(\omega, \tau_s) - K f_{Sc}(\omega, \tau_s) \right\}}{0.7 f_1(\omega, \tau_s) + 0.3 f_1(\omega, \tau_s) + R \frac{\tau}{\tau_s} \left\{ 0.7 f_1(\omega, \tau_s) + 0.3 f_1(\omega, \tau_s) + 0.5 K f_{Sc}(\omega, \tau_s) \right\}}, \quad (2.67) \]

where \( K \) measures the importance of the scalar interaction relative to the dipolar interaction, both at zero frequency. \( K \) is defined as
\[
K = \frac{2}{15} \frac{J_{\omega}(0)}{C_{\sigma}f'(0)}, \quad (2.68)
\]

Eq. 2.67 can be used for interpreting the DNP results for \(^{13}\)C and \(^{31}\)P atoms. Under certain assumptions the quantity \(K\) and the reduced intensity spectrum \(f_{\omega}(\omega, \tau_{\omega})\) can be obtained from the measurements. By fitting these data into Eq. 2.67, information concerning complexation tendencies and collision times can be obtained.

2.4 NMR Contact Shift

It has been recognized for many years that when a relaxation agent such as a free radical is present in a NMR sample solution, the NMR chemical shifts of the sample nuclei change either upfield or downfield relative to that of the sample solution without the free radical. Since this change in chemical shift is due to the interaction between the unpaired electron and the nucleus, it is called the NMR contact shift.\(^58\) In general, the contact shift is the result of two kinds of intermolecular interactions between the unpaired electron and the nucleus. One is the Fermi contact interaction which involves an excess of electron spin density induced by the unpaired electron at the nucleus. If the excess electron is in a spin state of \(S_z=1/2\), the spin density is defined as negative and is designated by \(\downarrow\). Since the spin state of \(S_z=1/2\) is anti-parallel to the applied magnetic field, an upfield contact shift will be induced for the nucleus. If the excess electron spin is
in a state of $S_z=-1/2$ (i.e., positive spin density $\uparrow$), a downfield shift will be observed for
the nucleus of interest. Another source of contribution to the NMR contact shift is the
dipolar or pseudocontact interaction which acts through space.\textsuperscript{58} The dipolar interaction is
inversely proportional to the third power of the interspin distance, i.e., $r_{ij}^{-3}$, and is only
effective when the local magnetic field produced by the unpaired electron at the nucleus
does not average to zero. The Fermi contact interaction arises from the close contact
between the two spins, such as the formation of a hydrogen bond ($\sigma$-type interaction), or a
direct overlap of the molecular orbitals ($\pi$-type interaction). In solutions, if the free
radical used is one of the nitroxides, the dipolar interaction between the two spins is
averaged to zero due to the rapid tumbling of the spins. The only significant contribution
to the contact shift comes from the Fermi contact interaction. For nitroxide free radicals
with $S = 1/2$, the contact shift $\Delta\delta_f$ is given by

$$\Delta\delta_f = -A_{SI} \frac{\gamma_S}{\gamma_I} \frac{1}{4kT}, \quad (2.69)$$

where $A_{SI}$ is the coupling constant, $k$ is the Boltzmann constant, and $T$ is the absolute
temperature. In the calculation of $\Delta\delta_f$, cyclohexane is usually used as an internal
reference.
CHAPTER 3: EXPERIMENTAL SECTION

3.1 Instrumentation

3.1.1 The Flow Transfer DNP Instrument

Since its first invention, the flow transfer DNP experimental system has been improved and utilized both for the liquid phase and for the solid-liquid interface DNP studies of various nuclei, such as $^1$H, $^{13}$C, $^{15}$N and $^{29}$Si.\textsuperscript{22,59-61}

The DNP instrument used in this work, which consists of several parts, is shown in Figure 3.1. As can be seen in this figure, there are two magnetic fields, one is at the lower part of the experimental set-up, which is a variable electromagnet, serving as a low field in the experiment with a range of 0 to 0.6 T. Above the low field is a 4.7 T superconducting high magnetic field. The two fields are 1.2 meter apart and are orthogonal. At the separating distance (1.2 m), the influence of the low field on the homogeneity of the high field is small. In the low magnetic field, there is a microwave TE$_{102}$ cavity arranged in such a way that the microwave field is perpendicular to the low magnetic field. Inside the cavity is the sample cell. The microwave power for saturating the unpaired electron transition of the free radical is provided by the Klystron of an EPR spectrometer and is amplified to 2 to 20 W using a Varian "K" series TWT amplifier. The resonance frequency of the unpaired electrons is around 9.30 GHz and is changeable for
Figure 3.1. Low to High Magnetic Field Flow Transfer DNP Experimental Apparatus
different samples. In this work, the low magnetic field strength was controlled at 0.33 T. The high magnetic field consists of two detecting channels, one is the proton channel with the resonance frequency of 199.5 MHz, and the broad band $^1$H decoupling was provided by this channel. The other one is the $^{13}$C channel, which has a resonance frequency of 50.2 MHz, and is used for the sampling purpose. The NMR probe inside the high field was self-made using the tuning and matching capacitors and the Helmholtz coils. The spectrum resolution for the static $^1$H NMR was about 5 Hz (~ 0.025 ppm). Inside the Helmholtz coil is a homemade sample cell. The two sample cells in the low and high magnetic fields were connected by PEEK (polyetheretherketone, from Upchurch Scientific) tubing with a 0.007” OD. A HPLC pump was used to cycle the sample solution through the two fields at the flow rate of 1.00 mL/min to 9.00 mL/min. All the DNP experiments were performed at room temperature and under non-spinning, non-locking conditions.

3.1.2 Sample Cells for the EPR Cavity and the NMR Probe

The sample cell used for the non-aqueous solution in the low field is a ceramic cell bought from Omega Engineering, Inc.. This kind of cell has a low dielectric constant, which means that the cell can be easily tuned in the microwave field, and high mechanical strength, which is suitable for the high back pressures arising from the high flow rates and
from the thin PEEK tubing. The volume of the cell is about 160 μL. The flow cell in the
NMR probe is a self-made glass cell with a volume of 100 μL and a size of about 2.5 cm
long which is a little bit longer than the coil of the probe. The structure and the
dimensions of these cells are shown in Figure 3.2.

3.2 Materials

The stable free radical TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, 98% ) was
purchased from Aldrich and was used as received. The reagents and solvents were
obtained commercially. The following organic solutions for DNP experiments were
prepared with 0.1 M TEMPO in each solution unless specified: acetone/carbon
tetrachloride/cyclohexane (20/70/10, v/v%); acetaldehyde/benzene (20/80, v/v%);
cyclopentanone/carbon tetrachloride/cyclohexane (20/70/10, v/v%); diethyl
malonate/benzene/cyclohexane (20/70/10, v/v%); ethyl acetoacetate/benzene/cyclohexane
(20/70/10, v/v%); 2, 4-pentanedione/benzene/cyclohexane (20/70/10, v/v%) with 0.5 M
TEMPO; nitromethane/benzene (20/80, v/v%); nitrobenzene/cyclohexane (10/90, v/v%);
toluene/cyclohexane (40/60, v/v%); diphenyl methane/cyclohexane (40/60, v/v%); 1 M
triphenyl methane in cyclohexane; 0.5 M adamantane in benzene. Deuterated chloroform
(HPLC grade) was purchased from Aldrich and was used as the solvent for Taxol. Taxol
powder (99%) was kindly provided by Dr. Kingston at Virginia Tech, and was used
Figure 3.2. Low and High Field Flow Cells
without further purification. For the $^1$H and the $^{13}$C DNP experiments of Taxol, the following respective sample solutions were prepared: 0.0230M Taxol in CDCl$_3$ with $3.7 \times 10^{-3}$ M TEMPO, 0.0459M Taxol in CDCl$_3$ with $3.7 \times 10^{-3}$ M TEMPO. All the sample solutions were degassed by bubbling N$_2$ gas through the solutions during the DNP experiments.

3.3 Methods of the Liquid Phase DNP Experiment

3.3.1 General Description

During a liquid phase flow transfer DNP experiment, the sample solution, conducted by the PEEK tubing and nitrogen degassing, was pumped through the low and high magnetic fields at flow rates of 2-8 mL/min by the HPLC pump. The enhanced DNP signals were obtained by saturating the electron transitions with microwave power in the low magnetic field and by detecting the polarized NMR signals in the high magnetic field. For the $^{13}$C DNP experiment, the $^1$H broad band decoupling technique was used. The spin-lattice relaxation times $T_1$'s of the sample nuclei were measured using a Bruker 200 NMR spectrometer utilizing the standard inverse-recovery ($180^\circ-\tau-90^\circ-T$) method.$^{62}$
3.3.2 Determination of the Leakage Factor $f$

As can be seen from Eq. 2.16, the leakage factor can be obtained by measuring the spin-lattice relaxation rates of the sample solution in the presence and the absence of the free radical at the low magnetic field where the DNP effect is developed. In this study, however, the relaxation rates measured in the high magnetic field were used to approximate those in the low field because of the sensitivity and the spectral resolution limitations in the low field. The high field spin-lattice relaxation times were measured at 4.7 T using a standard inverse recovery sequence ($180^\circ - \tau - 90^\circ - T$). The samples were sufficiently degassed before the $T_1$ measurements. The free radical concentration for most of the sample solutions was 0.1 M, which was high enough to eliminate the three-spin effect. The relative standard deviation for the $T_1$ measurements was between 1-6%. A sample curve of the $T_1$ measurement is illustrated in Figure 3.3.

3.3.3 Determination of the Saturation Factor $s$

The $s$ factor can be determined by Eq. 2.24. In this equation, the extrapolated DNP enhancement $A_{inc}(P \to \infty)$ can be obtained from the intercept of the plot of $[A_{obs}(P)]^{-1}$ versus $P^{-1}$ (DNP power plot) at a optimized flow rate (usually 6mL/min). The $A_{inc}(P)$ term can be obtained from the power plot by substituting the microwave power $P$, which is the microwave power under which the DNP flow plot is made, into the linear equation.
Figure 3.3. $^1$H 200 MHz Spin-Lattice Relaxation Time Plot for 0.0460M Taxol/3.70x10$^{-3}$ M TEMPO in CDCl$_3$
of the power plot. In the calculation of $A_{\text{obs}}(P) = \frac{M_z^*(P) - M_{z^L}^H}{M_0^H}$ for each power $P$, the flow magnetization $M_{z^L}^H$ is the one that corresponds to the optimized flow rate in the flow plot, which will be discussed in the next section. A sample DNP power plot is shown in Figure 3.4.

3.3.4 Determination of the Ultimate DNP Enhancement $A_{\infty}$ by the Exhaustive Method

The exhaustive method has been used to calculate the ultimate DNP enhancement. The advantage of this method is that it is closely correlated to the flow transfer DNP system used in this work, and takes into account as many variable factors that may cause an error to the final result as possible, such as the low to high magnetic field strength change, $f$ and $s$ factors, so that the calculated result matches the true value more closely. The disadvantage of this method is that it requires the instrument to be stable and it is quite time consuming since the data have to be obtained for a whole range of flow rates. This approach can be simplified for data collected at low flow rates where the observed polarization is dominated by decay of the low field magnetization and is not dependent on build-up of the magnetization in the low field. In this simplified approach, the first step for calculating the ultimate DNP enhancement is to make a linear plot of $\ln|A_{\text{obs}}|$ versus $1/F$ under certain microwave power $P$, such $P = 15$ W. From the
Figure 3.4. $^{13}$C Power Plot of Benzene at 128.50 ppm
intercept of this plot, the extrapolated $A_{obs}$ is obtained. The ultimate DNP enhancement is determined by dividing the extrapolated $A_{obs}$ by $f$ and $s$ factors, and then multiplied by the high to low magnetic field ratio (i.e. 14.2) as is shown in the following equation:\(^1\)

$$A_\infty = 14.2 \times \frac{A_{obs}^{extrap}}{f \cdot s}$$

where $A_{obs}^{extrap}$ is the extrapolated DNP enhancement obtained from the intercept of the flow plot. A sample DNP flow plot is shown in Figure 3.5. The sample calculations by the exhaustive method can be found in Ref. 1.

3.3.5 Determination of $A_\infty$ by the Ratio Method

Most of the DNP data in this work were collected for and processed by the ratio method mainly because some of the sample solutions cannot be readily studied by the exhaustive method which is more demanding either to the sensitivity and stability of the instrument or to the experimental time. The ultimate DNP enhancement $A'_\infty$ determined by the ratio method is given by\(^6\)

$$A'_\infty = A''_\infty \left[ A_s(P) / A_{ref}(P) \right] \left[ f_{ref} / f_s \right], \quad (3.1)$$
Figure 3.5. $^{13}$C Flow Plot of Benzene at 128.50 ppm
where $A_u$ is the ultimate DNP enhancement of the reference nucleus, such proton or other well characterized nuclei; $A_f(P)$ and $A_{ref}(P)$ are the observed DNP enhancements for the respective nucleus $I$ and the reference nucleus measured under microwave power $P$: $f_{ref}$ and $f_I$ are the $f$ factors of the reference nucleus and the nucleus $I$, respectively. By measuring the observed DNP enhancements under several microwave powers, and by obtaining the $f$ factors for the reference nucleus and the nucleus $I$, the averaged ultimate DNP enhancement is determined by Eq.3.1. No flow plot or power plot has to be made in this method. The $s$ factor is assumed to be the same for all the nuclei in a given experiment.

A sample calculation of the $^{13}$C DNP ultimate enhancements for the 0.5 M adamantane/benzene/0.1 M TEMPO system by the ratio method is given below.

**Step 1.** To obtain the peak height data from the $^{13}$C static NMR, flow NMR, and DNP spectra for the 0.5 M adamantane/benzene/0.1 M TEMPO system. The peak heights are listed in Table 3.1.
Table 3.1 Peak Height Data for 0.5 M Adamantane/Benzene/0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$M_0^{H}$ a</th>
<th>$M_{ZL}^{HL}$ b</th>
<th>$-M_Z^c$ (P=5W)</th>
<th>$-M_Z^c$ (P=15W)</th>
<th>$-M_Z^c$ (P=20W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>28.66</td>
<td>168</td>
<td>53</td>
<td>642</td>
<td>710</td>
<td>740</td>
</tr>
<tr>
<td>C-2</td>
<td>37.91</td>
<td>289</td>
<td>115</td>
<td>768</td>
<td>826</td>
<td>904</td>
</tr>
<tr>
<td>C₆H₆</td>
<td>128.50</td>
<td>5718</td>
<td>2211</td>
<td>17562</td>
<td>19070</td>
<td>20488</td>
</tr>
</tbody>
</table>

Note:

a. $M_0^H$ represents the peak height of the carbon nucleus in the static NMR spectrum.

b. $M_{ZL}^{HL}$ represents the peak height of the carbon nucleus in the flow NMR spectrum.

c. $M_Z^c$ represents the peak height of the carbon nucleus in the DNP spectrum.
Step 2. To calculate the observed $^{13}$C DNP enhancement from the peak height data by the following formula:

$$A_{obs}^L(P) = \frac{M_x^L(P) - M_0^L}{M_0^L}, \quad (3.2)$$

For the carbon nucleus at C-1 position of adamantane, the observed enhancement can be calculated as:

$$A_{obs}^{C-1}(P = 5W) = \frac{-642 - 53}{168} = -4.14$$

The calculated observed $^{13}$C DNP enhancements at different microwave powers are listed in Table 3.2.
<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$-\Delta \tilde{A}_{obs}^t (P=5\text{W})$</th>
<th>$-\Delta \tilde{A}_{obs}^t (P=10\text{W})$</th>
<th>$-\Delta \tilde{A}_{obs}^t (P=15\text{W})$</th>
<th>$-\Delta \tilde{A}_{obs}^t (P=20\text{W})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>28.66</td>
<td>4.14</td>
<td>4.54</td>
<td>4.72</td>
<td>4.38</td>
</tr>
<tr>
<td>C-2</td>
<td>37.91</td>
<td>3.06</td>
<td>3.26</td>
<td>3.53</td>
<td>3.30</td>
</tr>
<tr>
<td>C_6H_6</td>
<td>128.50</td>
<td>3.46</td>
<td>3.72</td>
<td>3.97</td>
<td>3.79</td>
</tr>
</tbody>
</table>
Step 3. To convert the observed $^{13}$C DNP enhancement ($A'_{\text{obs}}$) obtained from the flow transfer system to the value for the static DNP system ($A_f$). The formula for converting $A'_{\text{obs}}$ to $A_f$ is as follows:\(^1\)

$$A_f(P) = \frac{K \cdot A'_{\text{obs}}(P)}{1 - e^{-\frac{\nu_a}{T_{1a} F}} \cdot e^{\left(\frac{\nu_a + \nu_c}{T_{lb} F}\right) - \left(\frac{\nu_c}{T_{lc} F}\right)}}$$

(3.3)

where $T_{1a}$, $T_{1b}$, and $T_{1c}$ are the nuclear relaxation times in the regions a, b, and c, respectively (Figure 3.1); $V_a$, $V_b$, and $V_c$ are the sample solution volumes in the regions a, b, and c, respectively; F is the flow rate ($\mu$L/sec.) of the sample solution; K is the high to low magnetic field strength ratio which is equal to 14.2. By making the following approximation:

$$T_{1a} \approx T_{1c}, \quad T_{1b} \approx T_{1c},$$

$$V_a \approx 160 \ \mu$L, $V_b + V_c \approx 120 \ \mu$L,

The converted DNP enhancement $A_f$ can be expressed by the experimental measureable quantities in the following formula:

$$A_f(P) = \frac{K \cdot A'_{\text{obs}}(P) \cdot e^{\frac{120}{160} F}}{1 - e^{-\frac{120}{T_{lc} F}}}$$

(3.4)

For the carbon nucleus at C-1 position, the converted $^{13}$C DNP enhancement can be calculated as follows:
\[
A_{C-1}(P = 5W) \cong \frac{14.2 \times (-4.14) \times e^{\frac{120}{160}}}{1 - e^{\frac{2.64 \times 5.00 \times 16.7}{160}}} = -196
\]

The converted DNP enhancements for the 0.5 M adamantane/benzene/0.1 M TEMPO system are listed in Table 3.3.
Table 3.3 The Converted $^{13}$C DNP Enhancements for 0.5 M Adamantane/Benzene/0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_1c^a$ (Sec.)</th>
<th>$-A_f^b$ (P=5W)</th>
<th>$-A_f$ (P=10W)</th>
<th>$-A_f$ (P=15W)</th>
<th>$-A_f$ (P=20W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>28.66</td>
<td>2.64</td>
<td>196</td>
<td>215</td>
<td>223</td>
<td>207</td>
</tr>
<tr>
<td>C-2</td>
<td>37.91</td>
<td>2.25</td>
<td>153</td>
<td>163</td>
<td>177</td>
<td>165</td>
</tr>
<tr>
<td>C$_6$H$_6$</td>
<td>128.50</td>
<td>2.46</td>
<td>175</td>
<td>188</td>
<td>206</td>
<td>192</td>
</tr>
</tbody>
</table>

Note:

a. $T_1c$ was measured at 4.7 T magnetic field for the 0.5 M adamantane/benzene/0.1 M TEMPO system.

b. The $^{13}$C DNP enhancements was measured at the flow rate (F) of 5.00 mL/min.
**Step 4.** To calculate the ultimate $^{13}$C DNP enhancements for the 0.5 M adamantane/benzene/0.1 M TEMPO system by the ratio method.

Equation 3.1 is used for the calculation of the $^{13}$C DNP enhancements of a static DNP system:

$$A_{\infty}^{\prime} = A_{\infty}^{\text{ref}} \left[ A_i(P)/A_{\text{ref}}(P) \right] \cdot \left[ f_{\text{ref}}/f_i \right], \quad (3.1)$$

With $A_{\infty}^{\text{ref}} = A_{\infty}^{\text{benzene}} = -200.1$, $f_{\text{ref}} = f_{\text{benzene}} = 1 - \frac{2.46}{29.9} = 0.918$, the $^{13}$C ultimate DNP enhancements for the 0.5 M adamantane/benzene/0.1 M TEMPO system can be obtained. The calculated ultimate enhancements are listed in Table 3.4.
Table 3.4 Ultimate $^{13}$C DNP Enhancements and Relaxation Data for 0.5 M Adamantane/Benzene/0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_{1c}$ (Sec.)</th>
<th>f</th>
<th>$-A_e$ (P=5W)</th>
<th>$-A_\infty$ (P=10W)</th>
<th>$-A_\infty$ (P=15W)</th>
<th>$-A_\infty$ (P=20W)</th>
<th>$-A_\infty$ b</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>20.3</td>
<td>2.64</td>
<td>0.870</td>
<td>236</td>
<td>259</td>
<td>269</td>
<td>250</td>
<td>254±14</td>
</tr>
<tr>
<td>C-2</td>
<td>13.0</td>
<td>2.25</td>
<td>0.827</td>
<td>194</td>
<td>207</td>
<td>225</td>
<td>209</td>
<td>209±13</td>
</tr>
</tbody>
</table>

Note:

a. $T_{10}$ is the relaxation time for the 0.5 M adamantane/benzene system.

b. $A_\infty$ is the average value of the four ultimate $^{13}$C DNP enhancements obtained at different microwave powers. The standard deviation was calculated from the ultimate enhancements ($A_\infty$) measured at the four microwave power.
For the carbon nucleus at C-1 position, the calculation of the ultimate enhancement at one microwave power is as follows:

$$A_{C-1}^e = (\sim 200) \times \frac{196}{175} \times \frac{0.918}{0.870} = -236$$

The final propagated error in this measurement is estimated to be on the order of 15-20%.
CHAPTER 4. RESULTS AND DISCUSSION

4.1 Solution $^{13}$C DNP Studies of Proton Acidity in Carbon-Hydrogen Bonds of Organic Molecules

4.1.1 Introduction

One of the most significant applications of the DNP technique is to study transient solvent-solute interactions.\textsuperscript{24,40,43,45,48,59-61,63} In order to accomplish this goal, a stable free radical is introduced into the solvent system as a solute probe, and the dominant coupling mechanism(s) (either dipolar or scalar) is readily observed by negatively or positively enhanced NMR signals. Several researchers have found that when a weak hydrogen bond is formed between the receptor protons in an organic molecule and the free radicals, a scalar component among the $^1$H DNP enhancement was observed.\textsuperscript{20,21,23,65} Previous $^{13}$C DNP studies of some organic molecules have shown that scalar dominated enhancements are observed at the proton bound carbon nucleus. A transient hydrogen bond between the free radical and the hydrogen at the carbon nucleus has been suggested to explain these scalar dominated enhancements.\textsuperscript{1,64} However, the mechanism that the weak hydrogen bonding transfers the electron spin density from the free radical to the proton and carbon nuclei has not been resolved to explain the DNP results. In another respect, since the weak hydrogen bonding is associated with the weak acidity of the bridging protons, the scalar dominated $^{13}$C DNP enhancement may provide a new method of measuring the
weak proton acidity in organic molecules. By this means, the potential weak hydrogen bonding sites in a receptor molecule can also be identified.

In this study, the liquid phase $^{13}$C DNP experiment was performed on several organic compounds which contain either the carbonyl or nitro functional groups because the methyl or methylene groups adjacent to these functional groups have acidic carbon-hydrogen bonds. None of these molecules has been measured by the flow transfer $^{13}$C DNP technique before. In addition, no scalar dominated $^{13}$C DNP enhancements have been observed for these molecules. To the date of the present work, only a few (less than ten) small organic molecules usually containing halogen atoms, such as chloroform, carbon tetrachloride, etc. have been reported to have scalar $^{13}$C DNP enhancements.$^{33-37}$

The purpose of this work is to investigate the influence of the carbonyl and nitro functional groups on the observed DNP enhancement of the carbon site adjacent to them, and to study the mechanism that electron spin density transfers from TEMPO to the carbon nucleus of an acidic C-H bond through the weak hydrogen bonding.

4.1.2 Solution $^{13}$C DNP Studies of Acetone and Acetaldehyde with the Free Radical TEMPO

For acetone, the $^{13}$C DNP ultimate enhancement at the $\alpha$-carbon position exhibits negative peaks, which means a dipolar dominated interaction with TEMPO (Figure 4.1). From Table 4.1, it can be seen that the ultimate enhancement of the $\alpha$-carbons of this
Figure 4.1. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Acetone/Carbon tetrachloride/Cyclohexane (20/70/10, v/v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6mL/min, 100 Scans).
Table 4.1. Liquid Phase $^{13}$C DNP Enhancement and Relaxation Data for Acetone/Carbon tetrachloride/Cyclohexane (20/70/10, v/v%)/0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}^c$ (Sec.)</th>
<th>$T_1^c$ (Sec.)</th>
<th>$f^d$</th>
<th>$A_{\infty}^{a,e,f,g}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>30.40</td>
<td>21.7</td>
<td>4.09</td>
<td>0.811</td>
<td>-84.2±12.6</td>
</tr>
<tr>
<td>C-2</td>
<td>203.91</td>
<td>23.4</td>
<td>3.94</td>
<td>0.831</td>
<td>-744±112</td>
</tr>
<tr>
<td>C_6H_{12}</td>
<td>26.90</td>
<td>27.2</td>
<td>3.81</td>
<td>0.860</td>
<td>-270±40</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Cyclohexane was used as the reference compound with $A_{\infty}^{ref} = -270±40$.

(g) The ultimate $^{13}$C DNP enhancement $A_{\infty}$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_{\infty}$ is about 15%.
compound is far away from the dipolar limit of carbon-13 (-1310). The small dipolar enhancement is attributed to the existence of a fairly large scalar component which contributes to the ultimate enhancement. This interpretation is based on the NMR contact shift study of this molecule conducted by Morishima et al. According to their results, a sizable downfield \(^{13}\)C contact shift has been observed for the methyl group when acetone interacted with the nitroxide free radical DTBN. As mentioned in Chapter 2, when a electron spin density is transferred from the free radical to the nucleus of interest through the contact interaction (or scalar interaction), a NMR contact shift can be observed. Therefore, the observed \(^{13}\)C NMR contact shift for the \(\alpha\)-carbon of acetone in the presence of nitroxide free radical suggests that a sizable scalar component may exist in the \(^{13}\)C DNP enhancement of the acetone/TEMPO system. Much larger dipolar dominated \(^{13}\)C DNP enhancement is observed for the carbonyl group of acetone (Table 4.1), indicating a much smaller scalar component in the enhancement. This result is also consistent with the \(^{13}\)C NMR contact shift result observed by Morishima et al. for the carbonyl group of acetone, that is, a smaller \(^{13}\)C contact shift was observed for the carbonyl group of acetone.

For acetaldehyde, a positive DNP enhancement was observed at the \(\alpha\)-carbon site, indicating a scalar dominated interaction with TEMPO (Figures 4.2, Table 4.2). Obviously, in comparison with acetone, the methyl group of acetaldehyde has a much larger scalar component in the \(^{13}\)C DNP enhancement. The \(^{13}\)C NMR contact shifts have
(a) Static NMR Spectrum

(b) DNP Spectrum

Figure 4.2. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Acetaldehyde/Benzene (20/80, v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (860 Scans); (b) Flow Transfer DNP Spectrum (6mL/min, 300 Scans).
Table 4.2. Liquid Phase $^{13}$C DNP Enhancement and Relaxation Data for Acetaldehyde/Benzene (20/80, v/v%) /0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak$^b$ Position (ppm)</th>
<th>$T_1^{10}$ $^c$ (Sec.)</th>
<th>$T_1^{c}$ (Sec.)</th>
<th>$f^d$</th>
<th>$A_{\infty}^{a,e,f,g}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>198.26</td>
<td>15.1</td>
<td>1.75</td>
<td>0.884</td>
<td>-236±35</td>
</tr>
<tr>
<td>C-2</td>
<td>30.26</td>
<td>13.1</td>
<td>1.41</td>
<td>0.892</td>
<td>96.2±14</td>
</tr>
<tr>
<td>C$_6$H$_6$</td>
<td>128.50</td>
<td>19.2</td>
<td>1.97</td>
<td>0.898</td>
<td>-200±30</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements.

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Benzene was used as the reference compound with $A_{\infty}^{ref} = -200 \pm 30$.

(g) The ultimate $^{13}$C DNP enhancement $A_{\infty}$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_{\infty}$ is about 15%.
also shown the same trend for the methyl groups of these two molecules. Specifically, with the same free radical concentration, the $^{13}$C contact shift for the methyl group is about two times larger than that of acetone. In comparison with acetone, a smaller dipolar dominated enhancement is observed for the carbonyl group of acetaldehyde (Table 4.2). This is interpreted as the presence of larger scalar contribution for the DNP enhancement. The supporting evidence for this interpretation comes from the larger $^{13}$C contact shift of the carbonyl group of acetaldehyde.

Previous $^1$H and $^{13}$C DNP studies of several protic organic molecules have indicated that weak acidity of the C-H bond in the protic molecules can be sensed by the scalar component in the DNP enhancement. Transient hydrogen bonding between the acidic C-H bond and the free radical was believed to be able to promote the electron spin density transfer from the free radical to the carbon or hydrogen nuclei. However, up to this date, a clear mechanism has not been reported to describe the electron spin transfer process through the transient hydrogen bonding between the acidic C-H bond and the nitroxide free radical for $^{13}$C DNP experiments. Morishima et al have proposed a spin polarization mechanism to interpret their $^1$H and $^{13}$C NMR contact shift results of a series of protic organic molecules which interact with the nitroxide free radical through weak hydrogen bonding. The spin polarization mechanism has proven to be helpful for interpreting most of the NMR contact shift results and has been supported by some experimental evidence. According to Morishima's model, weak hydrogen bonding between the acidic hydrogen and the free radical, which has a positive
unpaired electron spin density, will induce an excess negative electron spin on the hydrogen nucleus, and a positive excess electron spin density on the carbon nucleus. As mentioned in Chapter 2, an excess positive electron spin density at the nucleus will cause a downfield NMR contact shift, an excess negative electron spin density will cause an upfield NMR contact shift. The $^{13}$C NMR contact shift results obtained by Morishima et al. have shown that the stronger the hydrogen bonding between the acidic C-H bond and the nitrooxide free radical, the larger the carbon-13 contact shift.\textsuperscript{66-71} Conceptually, the Fermi interaction in the NMR contact shift experiment is the same as the scalar interaction of the DNP experiment except that the magnitude of the scalar interaction in a DNP experiment depends on the correlation time of the sample molecule and the applied magnetic field strength. But this dependence does not affect the electron spin transfer mechanism. Therefore, in this work, the spin polarization model is used, for the first time, to explain the $^{13}$C DNP results. An illustration of the spin polarization mechanism for interpreting the $^{13}$C DNP results of this work is shown in Figure 4.3.

By comparing the $pK_a$ values of the $\alpha$-H of acetone and acetaldehyde (Table 4.3),\textsuperscript{74,75} it was found that the $\alpha$-H of acetaldehyde is more acidic than that of acetone, which suggests a stronger hydrogen bond can be formed between TEMPO and the protons at the $\alpha$-carbon site of the acetaldehyde. According to the electron spin polarization mechanism, a larger amount of positive excess electron spin density can be induced at the carbon nucleus of the methyl group of acetaldehyde than that of acetone.
Acetone/TEMPO

Methyl Hydrogen Site:

[Chemical structure image]

Acetaldehyde/TEMPO

Formyl Hydrogen Site:

[Chemical structure image]

Methyl Hydrogen Site:

[Chemical structure image]

Figure 4.3 Intermolecular Electron Spin Polarization Mechanism for Acetone/TEMPO and Acetaldehyde/TEMPO Systems
Table 4.3. $^{13}$C DNP Enhancements and the pKa Values of C-H Bond for Acetone and Acetaldehyde with 0.1 M TEMPO

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Carbon sites$^a$</th>
<th>Peak positions (ppm)</th>
<th>$A_\infty$</th>
<th>pKa values$^b$ of C-H bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>-CH$_3$</td>
<td>30.40</td>
<td>-84.2±12.6</td>
<td>20.0</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>-CH$_3$</td>
<td>30.26</td>
<td>96.2±14</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Note:

a. The carbon sites refer to the $\alpha$-carbon positions.

b. Reference 74, 75.
Therefore, a larger scalar component can be observed for the α-carbon of acetaldehyde than that of acetone. This prediction is consistent with the $^{13}$C DNP results for the α-carbon of these two molecules. For the carbonyl group of acetone, the smaller scalar component is explained as the reduction of the induced spin density at that carbon site as the spin polarization propagates along the covalent bonds. For the carbonyl group of acetaldehyde, the larger scalar component is attributed to the direct interaction between both the methyl and formyl hydrogens and TEMPO, which jointly induces larger electron spin densities at the carbon nucleus of the carbonyl group.

Although the magnitude of the scalar interaction increases with the hydrogen bonding strength in a NMR contact shift experiment, it may not be true for a DNP experiment because the DNP enhancement depends on the correlation time of the sample molecule as well as the applied field strength. Under certain field strength, if the hydrogen bonding is too strong between the free radical and the proton of a carbon site, the scalar component may decrease rapidly because of a drop-off of the corresponding spectral density function at long correlation time range (Figure 4.18), and the C-H bond acidity may not be reflected by the dominated DNP enhancement.
4.1.3 $^{13}$C DNP Studies of Diethyl malonate, Ethyl acetoacetate, Nitromethane, and 2, 4-Pentanedione in TEMPO/Benzene Solutions

It is well known that the hydrogen atoms in the methylene (−CH$_2$−) group between two carbonyl groups in an organic molecule show considerable acidity because of the stabilized tautomeric form.$^{75}$ The DNP experiments at this carbon site of the diethyl malonate, ethyl acetoacetate, and 2, 4-pentanedione exhibit scalar dominated DNP enhancements (Figure 4.4-4.6, Tables 4.4-4.6). The DNP results for diethyl malonate and ethyl acetoacetate show that when the acidity of the hydrogens at the methylene site increases (Figure 4.7, Table 4.7),$^{76}$ the corresponding DNP enhancement also shows an increasing trend (Table 4.5, 4.6). For 2, 4-pentanedione, the hydrogens at the methylene site are more acidic than the other two molecules; however, the DNP enhancement at this carbon site provides a lower enhancement (Table 4.7). This can be explained in terms of the rapid exchange between the keto and the enol tautomeric forms of this molecule at room temperature. The pK$_a$ value of the methylene group in 2, 4-pentanedione reflects the proton acidity in the keto form, but the DNP enhancement corresponds to the time average of the keto and the enol forms at that carbon site. Since the carbon nucleus at the −CH= site of the enol form has a dipolar dominated enhancement (negative value), the time averaged $^{13}$C DNP enhancement at the methylene group thus reflects a reduced scalar enhancement. Although the ethyl acetoacetate also
Figure 4.4. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Diethyl malonate/Benzene/Cyclohexane (20/70/10, v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6mL/min, 100 Scans).
Table 4.4. Liquid Phase $^{13}$C DNP Enhancements and Relaxation Data for Diethyl malonate/Benzene/Cyclohexane (20/70/10, v/v%)/0.1 M TEMPO

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_1$ (Sec.)</th>
<th>$f$</th>
<th>$A_\infty$ a,e,f,g</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>165.76</td>
<td>15.8</td>
<td>3.75</td>
<td>0.763</td>
<td>-642±96</td>
</tr>
<tr>
<td>C-2</td>
<td>41.69</td>
<td>6.60</td>
<td>1.50</td>
<td>0.773</td>
<td>401±60</td>
</tr>
<tr>
<td>C-1´</td>
<td>60.96</td>
<td>5.97</td>
<td>2.24</td>
<td>0.625</td>
<td>86.4±13.0</td>
</tr>
<tr>
<td>C-2´</td>
<td>13.86</td>
<td>6.12</td>
<td>2.55</td>
<td>0.583</td>
<td>-96.8±14.5</td>
</tr>
<tr>
<td>C$_6$H$_6$</td>
<td>128.17</td>
<td>11.2</td>
<td>3.14</td>
<td>0.720</td>
<td>-200±30</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OCCH}_2\text{COCH}_2\text{CH}_3
\end{align*}

\[2' \\ 1' \\ 12\]

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements.

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Benzene was used as the reference compound with $A_\infty^{ref} = -200 \pm 30$.

(g) The ultimate $^{13}$C DNP enhancement $A_\infty$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_\infty$ is about 15%.

84
(a) Static NMR Spectrum

100 scans

(b) DNP Spectrum

100 scans

Figure 4.5. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Ethyl acetoacetate/Benzene/Cyclohexane (20/70/10, v/v/v)/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6mL/min, 100 Scans).
Table 4.5. Liquid Phase $^{13}$C DNP Enhancements and Relaxation Data for Ethyl acetoacetate/Benzene/Cyclohexane (20/70/10, v/v/v%)/0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_{1}$ (Sec.)</th>
<th>$f$</th>
<th>$A_{\infty}$ a,e,f,g</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>166.54</td>
<td>22.4</td>
<td>2.90</td>
<td>0.870</td>
<td>-711±107</td>
</tr>
<tr>
<td>C-2</td>
<td>49.87</td>
<td>3.75</td>
<td>1.31</td>
<td>0.651</td>
<td>440±66</td>
</tr>
<tr>
<td>C-3</td>
<td>198.65</td>
<td>52.0</td>
<td>2.60</td>
<td>0.950</td>
<td>-720±108</td>
</tr>
<tr>
<td>C-4</td>
<td>29.33</td>
<td>14.2</td>
<td>1.47</td>
<td>0.896</td>
<td>72.5±10.9</td>
</tr>
<tr>
<td>C-1'</td>
<td>60.67</td>
<td>3.98</td>
<td>2.26</td>
<td>0.432</td>
<td>250±38</td>
</tr>
<tr>
<td>C-2'</td>
<td>13.86</td>
<td>8.89</td>
<td>2.54</td>
<td>0.714</td>
<td>-117±18</td>
</tr>
<tr>
<td>C_{6}H_{6}</td>
<td>128.30</td>
<td>12.7</td>
<td>2.75</td>
<td>0.784</td>
<td>-200±30</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Benzene was used as the reference compound with $A_{\infty}^{ref} = -200 ± 30$.

(g) The ultimate $^{13}$C DNP enhancement $A_{\infty}$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_{\infty}$ is about 15%.
Figure 4.6. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for 2, 4-Pentanedione/Benzene/Cyclohexane (20/70/10, v/v%)/0.05 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6mL/min, 100 Scans).
Table 4.6. Liquid Phase \(^{13}\)C DNP Enhancements and Relaxation Data for 2, 4-Pentanedione / Benzene / Cyclohexane (20/70/10, v/v/v\%) / 0.05 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type (\text{^1})</th>
<th>Peak Position (\text{ppm})</th>
<th>(T_{10}) (Sec.)</th>
<th>(T_1) (Sec.)</th>
<th>(f)</th>
<th>(A_{\infty}) (^a,^e,^f,^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1 (e)</td>
<td>24.08</td>
<td>13.1</td>
<td>5.92</td>
<td>0.548</td>
<td>8.34±1.25</td>
</tr>
<tr>
<td>C-1 (k)</td>
<td>29.92</td>
<td>34.4</td>
<td>2.07</td>
<td>0.940</td>
<td>61.3±9.2</td>
</tr>
<tr>
<td>C-2 (e)</td>
<td>190.77</td>
<td>39.1</td>
<td>4.64</td>
<td>0.881</td>
<td>-644±97</td>
</tr>
<tr>
<td>C-2 (k)</td>
<td>200.79</td>
<td>41.4</td>
<td>4.71</td>
<td>0.886</td>
<td>-300±45</td>
</tr>
<tr>
<td>C-3 (e)</td>
<td>99.98</td>
<td>26.1</td>
<td>3.43</td>
<td>0.869</td>
<td>-54.0±8.1</td>
</tr>
<tr>
<td>C-3 (k)</td>
<td>57.94</td>
<td>20.3</td>
<td>2.64</td>
<td>0.870</td>
<td>88.0±13.2</td>
</tr>
<tr>
<td>(\text{C}_6\text{H}_6)</td>
<td>128.20</td>
<td>25.6</td>
<td>4.62</td>
<td>0.820</td>
<td>-200±30</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{CH}_3\text{C}=\text{CHCCH}_3 & \quad \xrightarrow{K=29} \quad \text{CH}_3\text{CCH}_2\text{CCH}_3 \\
1 & 2 3 & 1 2 3
\end{align*}
\]

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements
(b) \(^1\)H broad-band decoupling was employed to eliminate proton-carbon coupling.
(c) \(T_1\) and \(T_{10}\) are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.
(d) The leakage factor \(f\) was calculated based on \(T_1\) measurements at 4.7 T magnetic field.
(e) The 0.33 T \(^{13}\)C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.
(f) Benzene was used as the reference compound with \(A_{\infty}^{ref} = -200 \pm 30\).
(g) The ultimate \(^{13}\)C DNP enhancement \(A_{\infty}\) is an average value of four measurements at different power levels. The estimated relative standard deviation for the \(A_{\infty}\) is about 15%.
(i) "e" and "k" in the carbon type column represent the enol and the keto form of the 2, 4-pentanedione, respectively.
Figure 4.7. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Nitromethane/Benzene (20/80, v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 100 Scans).
Table 4.7. Liquid Phase $^{13}$C DNP Enhancement and Relaxation Data for CH$_3$NO$_2$/Benzene (20/80, v/v%) with 0.1 M TEMPO

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_1$ (Sec.)</th>
<th>$f$</th>
<th>$A_\infty$ $^a,e,f,g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$NO$_2$</td>
<td>61.93</td>
<td>10.4</td>
<td>0.879</td>
<td>0.915</td>
<td>996±149</td>
</tr>
<tr>
<td>C$_6$H$_6$</td>
<td>128.44</td>
<td>27.7</td>
<td>2.43</td>
<td>0.912</td>
<td>-200±30</td>
</tr>
</tbody>
</table>

CH$_3$NO$_2$

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Benzene was used as the reference compound with $A_{eff}^{ref} = -200 \pm 30$.

(g) The ultimate $^{13}$C DNP enhancement $A_\infty$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_\infty$ is about 15%. 

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has the keto and enol equilibrium at room temperature, the enolization equilibrium constant \( K_{\text{enol}} = 0.3 \) is much smaller than that of the 2, 4-pentanedione \( K_{\text{enol}} = 29 \). This may be the reason that the \( pK_a \) value of the former can be more closely related to the dominant DNP enhancement. The \(^{13}\text{C} \) DNP spectrum of nitromethane is shown in Figure 4.7. Due to the electron attracting effect, the hydrogens in the methyl group of the nitromethane have an acidity close to that of the hydrogens in the methylene group of ethyl acetoacetate (Table 4.8). The corresponding scalar dominated DNP enhancements (Table 4.5, 4.7) of these two molecules, however, are not close. This may due to the enolization of ethyl acetoacetate which causes a decrease of the scalar dominance for the carbon nucleus of the methylene group.

The \(^{13}\text{C} \) DNP enhancements for the above molecules indicate that the acidity of carbon-hydrogen bonds can be detected in a qualitative way by the \(^{13}\text{C} \) DNP technique. The \(^{13}\text{C} \) DNP results and the \( pK_a \) values are summarized in Table 4.8.

The large \(^{13}\text{C} \) scalar enhancements for the methylene groups between the carbonyl groups can be understood with the help of the electron spin polarization model. The stronger the acidity of the C-H bond of the methylene group, the larger amount of electron spin density can be induced an the carbon site. Therefore, a larger scalar enhancement can be expected for the carbon sites of this molecules except for the special case of 2, 4-pentanedione. For the carbonyl groups of these molecule, a smaller amount of electron spin density can be induced at the
Table 4.8. $^{13}$C DNP Enhancements and the pKa Values of C-H Bond for Diethyl malonate, Ethyl acetoacetate, 2, 4-Pentanedione, and Nitromethane with 0.1 M TEMPO

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Carbon site$^a$</th>
<th>Peak position (ppm)</th>
<th>$A_{\infty}^b$</th>
<th>pK$_a$ value$^c$ of C-H bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl malonate</td>
<td>-CH$_2$</td>
<td>41.69</td>
<td>401±60</td>
<td>13</td>
</tr>
<tr>
<td>Ethyl acetoacetate</td>
<td>-CH$_2$</td>
<td>49.87</td>
<td>440±66</td>
<td>11</td>
</tr>
<tr>
<td>2, 4-Pentanedione (keto form)</td>
<td>-CH$_2$</td>
<td>57.94</td>
<td>88.0±13.2</td>
<td>9</td>
</tr>
<tr>
<td>2, 4-Pentanedione (enol form)</td>
<td>-CH=</td>
<td>99.98</td>
<td>-54.0±8.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Nitromethane</td>
<td>-CH$_3$</td>
<td>61.93</td>
<td>996±149</td>
<td>10</td>
</tr>
</tbody>
</table>

Note:

a. The carbon site for the β-dicarbonyl derivatives refers to the carbon nucleus in between the two carbonyl groups. The carbon site for the nitromethane means the α-carbon position.

b. The $^{13}$C DNP ultimate enhancement of benzene ($A_{\infty}^{bm} = -200$) was used as the reference for the calculation of the ultimate enhancements. The estimated standard deviation of the calculated ultimate enhancement is 15%.

c. Reference 74.
Figure 4.8. Acetaldehyde, Ketones, Esters, and Nitromethane / TEMPO Systems
$^{13}$C Scalar and Dipolar DNP Enhancements ($A_\omega$) and the pKa Values
carbon nucleus due to the decrease of the spin polarization effect along the covalent bonds. When the scalar contribution is small, the DNP enhancement is dominated by the dipolar interaction because dipolar coupling is always present in the system and is independent of the chemical environment of carbon nuclei. However, dipolar enhancement depends on the correlation time of the sample molecule as well as the applied magnetic field strength. Since the molecular sizes of the β-dicarbonyl molecules studied in this work are very close, the correlation times for these molecules can be expected to be close. Therefore, the dipolar dominated $^{13}$C DNP enhancements for the carbonyl groups are close except for 2, 4-pentanedione. The $^{13}$C DNP enhancements for other carbon sites of these molecules can be understood by using the spin polarization model and by considering the net effect of the scalar and dipolar contributions for the $^{13}$C DNP enhancement.
4.2 $^{13}$C DNP Studies of Toluene, 1, 2-Dichlorobenzene, and Nitrobenzene in TEMPO/Cyclohexane Solutions

4.2.1 Introduction

A flow transfer $^{13}$C DNP study of the substituted aromatic compounds has been conducted for mono- and tri- chlorobenzenes, such as, 1-chlorobenzene and 1, 3, 5-trichlorobenzene.\textsuperscript{1} For 1-chlorobenzene, the carbon nucleus attached to the chlorine exhibited a large negative dipolar dominated enhancement, whereas the carbon adjacent to the C-1 showed a large scalar dominated enhancement. Similarly, for 1, 3, 5-trichlorobenzene, the carbon sites of 1, 3, 5 exhibited a large negative dipolar dominated DNP enhancement, whereas, the carbons at 2, 4, 6 showed a large positive scalar dominated enhancement. The large scalar enhancement at the carbon sites adjacent to the chlorinated carbon position was interpreted as the possible weak complexation of TEMPO with the hydrogen attached to the carbons of interest. The driving force for the hydrogen-TEMPO complexation was attributed to the presence of the electron attracting chlorine atom on the benzene ring.

Although the transient hydrogen bonding was believed to play an important role in the $^{13}$C DNP scalar enhancements of aromatic molecules,\textsuperscript{1} as in the case of $^{13}$C DNP studies of aliphatic molecules, no electron spin transfer mechanism has been reported to clarify the spin transfer process through the weak hydrogen bonding between the aromatic
molecule and the free radical for the $^{13}$C DNP experiments. Also, very limited $^{13}$C DNP data is available for the aromatic molecules to verify a proposed spin transfer model.

In this work, 1, 2-dichlorobenzene was studied by $^{13}$C DNP, and the result was compared with those of the other two chlorobenzenes investigated before. Since chlorine is an unusual scalar enhancement functional group whose working mechanism has not yet been resolved,$^{77-79}$ it was desired to replace this electron attracting group with other functional groups, e.g., nitro groups which is inert to nitroxide free radicals. Toluene was also investigated in order to see the electron releasing effect of the methyl group on the DNP enhancement of the carbons on the benzene ring.

4.2.2 $^{13}$C DNP Studies of Nitrobenzene, 1, 2-Dichlorobenzene, and Toluene in TEMPO/Cyclohexane Solutions

As can be seen from Figure 4.9 and Table 4.9, the carbon site attached to the nitro group shows a large dipolar dominated DNP enhancement, whereas the carbons adjacent to it exhibit a large scalar dominated enhancement. In addition, the C-3 and C-4 positions also exhibit large scalar dominated enhancements. These results indicate that the electron attracting groups on the benzene ring do promote the scalar enhancement of the aromatic carbons except for the carbon directly connected to the functional group.

Although the acidity of the aromatic hydrogens of nitrobenzene ($pK_a\sim30-40$) is much weaker than that of acetone ($pK_a=20$), the scalar component for the hydrogen
Figure 4.9. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Nitrobenzene/Cyclohexane (10/90, v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 100 Scans).
Table 4.9. Liquid Phase $^{13}$C DNP Enhancement and Relaxation

Data for Nitrobenzene/Cyclohexane(10/90, v/v%)/0.1 M TEMPO

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_1$ (Sec.)</th>
<th>$f^d$</th>
<th>$A_\infty$ $^e,f,g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>148.54</td>
<td>45.7</td>
<td>1.45</td>
<td>0.968</td>
<td>-572±86</td>
</tr>
<tr>
<td>C-2</td>
<td>123.43</td>
<td>12.4</td>
<td>1.62</td>
<td>0.869</td>
<td>587±88</td>
</tr>
<tr>
<td>C-3</td>
<td>129.37</td>
<td>13.7</td>
<td>1.03</td>
<td>0.925</td>
<td>198±30</td>
</tr>
<tr>
<td>C-4</td>
<td>134.30</td>
<td>8.25</td>
<td>0.807</td>
<td>0.902</td>
<td>366±55</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>26.90</td>
<td>12.8</td>
<td>1.60</td>
<td>0.875</td>
<td>-270±40</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Cyclohexane was used as the reference compound with $A_{\infty}^{ref} = -270 \pm 40$.

(g) The ultimate $^{13}$C DNP enhancement $A_\infty$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_\infty$ is about 15%.
attached aromatic carbons is much larger than that of acetone. If the hydrogen bonding spin polarization model is used to predict the scalar component for the hydrogen attached aromatic carbons, dipolar dominated $^{13}$C DNP enhancements would be expected for these carbon sites. However, this is not true. The scalar components difference between nitrobenzene and acetone cannot arise from the correlation time difference because the molecular sizes of these two molecules are very close. From the above comparison, it is clear that the electron spin polarization model cannot be used to explain the $^{13}$C DNP result of nitrobenzene. Another model need to be used to reveal the electron spin density transfer process for the nitrobenzene/TEMPO system.

The $^{13}$C DNP results (Figure 4.10, Table 4.10) of 1, 2-dichlorobenzene was obtained via the exhaustive method, and the calculated ultimate DNP enhancement forms a reasonable trend with the previous results of mono- and tri-chlorobenzenes (Table 4.12). As shown in Figure 4.9, the chlorine substituted aromatic carbons shows dipolar dominated enhancement, whereas, the carbons adjacent to the substituted carbons exhibit strongest scalar enhancement. The carbons at C-4 and C-5 show weaker scalar dominance (Table 4.10). As in the case of nitrobenzene, the large scalar components for the hydrogen attached carbons of the chlorine substituted benzenes cannot be explained by the spin polarization model.

In contrast to 1, 2-dichlorobenzene and nitrobenzene, all the carbon sites of toluene show negative dipolar dominated enhancements as illustrated by Figure 4.11 and Table 4.11. As expected, the DNP enhancements at the positions of the methyl group and
Figure 4.10. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for 1, 2-Dichlorobenzene (ODCB)/Cyclohexane (90/10, v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (200 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 200 Scans).
Table 4.10. Liquid Phase $^{13}$C DNP Enhancement and Relaxation Data for 1, 2 - Dichlorobenzene (ODCB)/Cyclohexane (90/10, v/v%) / 0.1 M TEMPO

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_1$ (Sec.)</th>
<th>$f$</th>
<th>$s$</th>
<th>$A_{\text{obs}}$</th>
<th>$A_\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1, 2</td>
<td>131.78</td>
<td>32.6</td>
<td>2.19</td>
<td>0.932</td>
<td>0.353</td>
<td>-2.77</td>
<td>-120±18</td>
</tr>
<tr>
<td>C-3, 6</td>
<td>129.84</td>
<td>10.5</td>
<td>0.845</td>
<td>0.920</td>
<td>0.353</td>
<td>4.06</td>
<td>178±27</td>
</tr>
<tr>
<td>C-4, 5</td>
<td>127.02</td>
<td>5.99</td>
<td>0.819</td>
<td>0.863</td>
<td>0.353</td>
<td>1.50</td>
<td>69.9±10</td>
</tr>
</tbody>
</table>

Note:

(a) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(b) $A_{\text{exp}}$ was obtained from the intercept of $\ln(A_{\text{obs}})$ versus inverse flow rate (2.00 - 9.00 ml/min).

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field. The saturation factor $s$ was calculated from the plot of $1/A_{\text{obs}}$ versus $1/P$, where $P$ is the microwave power in watts.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) The ultimate $^{13}$C DNP enhancement $A_\infty$ is the value of one measurement. The estimated relative standard deviation for $A_\infty$ is about 15%.
Figure 4.11. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Toluene/Cyclohexane (40/60, v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 100 Scans).
Table 4.11. Liquid Phase \(^{13}\text{C}\) DNP Enhancement and Relaxation Data for Toluene/Cyclohexane (40/60, v/v\%)/0.1 M TEMPO

<table>
<thead>
<tr>
<th>Carbon Site</th>
<th>Peak(^\text{b}) Position (ppm)</th>
<th>(T_{10})(^\text{c}) (Sec.)</th>
<th>(T_1)(^\text{c}) (Sec.)</th>
<th>(f)(^d)</th>
<th>(A_\infty)(^a,\text{ef})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>137.25</td>
<td>41.4</td>
<td>3.39</td>
<td>0.918</td>
<td>-423±63</td>
</tr>
<tr>
<td>2</td>
<td>128.88</td>
<td>17.2</td>
<td>2.54</td>
<td>0.852</td>
<td>-185±28</td>
</tr>
<tr>
<td>3</td>
<td>128.10</td>
<td>25.0</td>
<td>1.54</td>
<td>0.938</td>
<td>-196±29</td>
</tr>
<tr>
<td>4</td>
<td>125.28</td>
<td>11.7</td>
<td>3.04</td>
<td>0.740</td>
<td>-192±29</td>
</tr>
<tr>
<td>-CH(_3)</td>
<td>21.06</td>
<td>17.0</td>
<td>1.23</td>
<td>0.928</td>
<td>-209±31</td>
</tr>
<tr>
<td>C(<em>6)H(</em>{12})</td>
<td>26.90</td>
<td>33.3</td>
<td>3.48</td>
<td>0.895</td>
<td>-270±40</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements.

(b) \(^1\text{H}\) broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) \(T_1\) and \(T_{10}\) are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor \(f\) was calculated based on \(T_1\) measurements at 4.7 T magnetic field.

(e) The 0.33 T \(^{13}\text{C}\) DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Cyclohexane was used as the reference compound with \(A_\infty^{\text{ref}} = -270 ± 40\).

(g) The ultimate \(^{13}\text{C}\) DNP enhancement \(A_\infty\) is an average value of four measurements at different power levels. The estimated relative standard deviation for \(A_\infty\) is about 15%.
C-1 show dipolar dominance. The aromatic carbons at C-2, C-3, and C-4; however, also show a strong dipolar enhancements which is contrary to that of the nitrobenzene.

Although the spin polarization model can be used to explain the $^{13}\text{C}$ DNP enhancements of the ring carbons of toluene, a model that can be used for other benzene derivatives will be applied to toluene.

In 1968, Potenza et al. investigated several fluorine substituted benzenes by the solution $^{19}\text{F}$ DNP technique. In their effort of interpreting the large scalar $^{19}\text{F}$ enhancement for the fluorobenzene, they proposed three types of intermolecular collisions between fluorobenzene and planner free radicals. These three collisions are: $\pi$ collisions, where the solvent and radical molecules are coplanar; $\sigma$ collisions, where the contact axis passes through nuclei at the edge of solvent and radical molecules and is perpendicular to the radical plane; and, for aromatic molecules, plane-plane collisions, where the molecular planes are eclipsed. The three collisions are illustrated schematically in Figure 4.12. In their study, they found that plane-plane collisions between aromatics were the most effective attitude in producing spin density at solvent nuclei. The similar studies of fluorinated benzenes were also performed by Muller-Warmuth et al. later on. They showed that the scalar mechanism for the substituted fluorobenzenes tends to occur through electron delocalization into the lowest unoccupied $\pi$ orbital and then $\pi/\sigma$ polarization to the ring fluorines.

Up to the date of this work, no report has been found using the spin delocalization model to interpret $^{13}\text{C}$ DNP results.
Figure 4.12. Generalized Collision Attitudes for Fluorobenzene and Benzoquinone (Cited from Ref. 6)
For the purpose of interpreting the $^{13}$C scalar components for the ring carbons of the aromatic molecules studied in this work, a spin delocalization model is used. The reason for using such a model is because the $^{13}$C DNP results for the aromatic molecules studied in this work can be explained by this model. A schematic illustration of this model is shown in Figure 4.13. In this model, $\sigma$ collisions are assumed to be the most efficient way for the spin delocalization by considering the largest molecular orbital (LUMO) overlapping and the steric hindrance of the methyl groups of TEMPO. According to this model, the positive electron spin density from TEMPO is transmitted into the antibonding orbital $\pi^*$ (i.e., LUMO) of the aromatic molecule. The transmitted electron spin density correlates with the $\sigma$ orbitals around the carbon nucleus and induces an excess positive electron spin density near the carbon nucleus and excess negative spin densities at the adjacent carbon nuclei and the hydrogen nucleus.\textsuperscript{58} Similar to the intermolecular spin polarization, the intensity of the intramolecular polarization also decreases along the covalent bonds.\textsuperscript{58} Therefore, the carbon site where the collision happens has the largest induced electron spin density. Compared with the intermolecular spin polarization mechanism, spin delocalization mechanism can transmit the electron spin density more efficiently due to the direct overlapping of the molecular orbitals between the ring carbons and the free radical.
Figure 4.13. The Electron Spin Delocalization Mechanism: (a) $\sigma$ Contact; (b) Spin Delocalization; (c) Electron-Electron Correlation.
With the help of the spin delocalization model, the $^{13}$C DNP enhancements for the aromatic carbons of nitrobenzene can be understood. Specifically, the collision between the ortho carbon (C-2) and TEMPO results in an excess positive electron spin density at the other carbon nucleus. The excess electron spin couples with the carbon nucleus and induces a scalar component. The negative spin density induced at the meta carbon (C-3) is smaller than the positive spin density arising from the direct collision with TEMPO. Therefore, a net positive spin density is produced at the meta carbon nucleus and a scalar component is induced. The same analysis can be applied to the para carbon. Since the nitro group is a strong electron attracting functional group, the benzene ring of nitrobenzene is an electron poor ring, or a good electron acceptor. Therefore, a large amount of electron spin density can be expected to transmit to the ring carbons and causes large scalar dominated DNP enhancements except for the junction carbon (C-1) which will be discussed later on. Since the positive spin density at the carbon nucleus causes a downfield $^{13}$C NMR contact shift, the $^{13}$C NMR contact shift result observed by Morishima et al. can also be explained with the spin delocalization model. It should be mentioned in here that Morishima et al. used the hydrogen bonding intermolecular spin polarization mechanism to interpreted the $^{13}$C NMR contact shift results of aromatic molecules. Their model is successful in interpreting their NMR contact shift results, however, the spin polarization model fails to explain the $^{13}$C DNP results for nitrobenzene and the substituted chlorobenzenes studied in this work.
From Table 4.9, it can be seen that the scalar enhancements for the ortho, meta, and para carbons of nitrobenzene change in the order of ortho>para>meta. This scalar enhancement difference may due to the different $\pi$-electron spin density distribution at these carbon sites as can be seen in Figure 4.14. Since the $\pi$-electron spin density at the ortho and para carbons is lower than that of the meta carbon, the ortho and para carbons are more acceptable to the transmitted electron spin density than the meta carbon. Therefore, a scalar enhancement difference is observed. Although the $\pi$-electron spin density at the ortho and para carbons are almost the same (Figure 4.14), the scalar enhancement of ortho carbon is larger than that of the para carbon. This may due to the larger collision probability at the ortho carbons with the free radical than that of the para carbon.

For the junction carbon of nitrobenzene, a large dipolar dominated $^{13}$C DNP enhancement is observed. This may due to the steric hindrance and the electrostatic repulsion of the nitro group to the free radical. The close contact between the junction carbon and the free radical, which is essential for spin delocalization, cannot be formed. The upfield $^{13}$C NMR contact shift observed by Morishima et al.\textsuperscript{66-71} for the junction carbon is attributed to the small negative spin density induced by the positive spin density at the ortho carbons. Since only a small scalar component is present at the junction carbon site, a dipolar dominated $^{13}$C DNP enhancement is observed at this carbon site of nitrobenzene.
π-Electron Densities

Toluene and Nitrobenzene $^{13}$C DNP Enhancements

Figure 4.14. Total π-electron Densities and $^{13}$C DNP Enhancements for Toluene and Nitrobenzene (Cited from Ref. 82)
Compared with nitrobenzene, both the dipolar and the scalar dominated $^{13}$C DNP enhancements for 1, 2-dichlorobenzene (ODCB) show smaller values. This may due to the higher solution viscosity of the ODCB/TEMPO system, which has a high ODCB concentration (Table 4.10), than that of the nitrobenzene/TEMPO system, which has a much lower nitrobenzene concentration (Table 4.9). The higher viscosity of the ODCB/TEMPO system results in a longer correlation time for 1, 2-dichlorobenzene. Therefore, the reduction of the spectral density functions, and thus the DNP enhancements, can be observed. Another possible reason for the smaller $^{13}$C DNP enhancements for the ring carbons of ODCB compared with those of nitrobenzene is that the chlorine is a weak electron attracting group which makes the benzene ring a weak electron acceptor. Therefore, less electron spin density is transmitted to the carbon sites of C-3 to C-6, and the smaller scalar enhancements are induced at these carbon sites. The small dipolar dominated $^{13}$C DNP enhancement for the junction carbons (C-1 and C-2) may due to the electron spin delocalization from the free radical into the LUMO of the chlorine atoms. The transmitted spin density further delocalizes into the s orbital, which is symmetry compatible with the LUMO of the chlorine atom, of the substituted ring carbons and produces a sizable scalar component there. The dipolar dominance at C-1 and C-2 is attributed to the steric hindrance of the chlorine atoms to the free radical so that the direct spin delocalization from the free radical to the junction carbons cannot happen.
Compared with the $^{13}$C DNP enhancements at C-4 and C-5 carbon positions (Table 4.10), the larger scalar enhancements for the ring carbons adjacent to the junction carbons of ODCB are attributed to the larger electron attracting effect of the substituted chlorines at these carbon sites. Due to the inductive effect of the substituted chlorines, a larger amount of electron spin density is expected to transmit to the carbons at C-3 and C-6 during a collision than to the carbons at C-4 and C-5 through the spin delocalization process.

From Table 4.12 and Figure 4.15 it can be seen that the scalar enhancement at the carbon site adjacent to the substituted carbon increases with the number of the substituted chlorine. This is attributed to the electron withdrawing effect becomes stronger when the degree of the chlorination increases so that the electron spin density can be more easily transmitted to the adjacent carbon site. For 1-chlorobenzene, the small dipolar dominated $^{13}$C DNP enhancements\textsuperscript{1} for the carbons at C-3 to C-5 are attributed to the smaller scalar components induced at these carbon positions because of the weaker electron attracting effect of chlorine at these carbons. The dipolar dominated $^{13}$C DNP enhancements for the substituted carbons of these chlorinated benzenes also show an increasing trend from 1-chlorobenzene to 1, 3, 5-trichlorobenzene. This may be the reason that the dipolar coupling probability increases at the junction carbon site as the number of the substitution increases.

For toluene, the dipolar dominated $^{13}$C DNP enhancements for all of the ring carbons are attributed to the weak scalar interaction at these carbon sites. Since the
Table 4.12. $^{13}$C Ultimate DNP Enhancements of 1-Chlorobenzene/CCl$_4$, 1, 2-Dichlorobenzene/C$_6$H$_{12}$, and 1, 3, 5-Trichlorobenzene/CCl$_4$ with 0.1 M TEMPO

<table>
<thead>
<tr>
<th>Chlorobenzenes$^a$</th>
<th>C-1$^b$</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Chlorobenzene</td>
<td>-100±15$^c$</td>
<td>140±21$^d$</td>
<td>-17±3</td>
<td>-23±3</td>
<td>-17±3</td>
<td>140±21</td>
</tr>
<tr>
<td>1,2-Dichlorobenzene</td>
<td>-120±18</td>
<td>-120±18</td>
<td>178±27</td>
<td>69.9±10</td>
<td>69.9±10</td>
<td>178±27</td>
</tr>
<tr>
<td>1,3,5-Trichlorobenzene</td>
<td>-330±50</td>
<td>290±44</td>
<td>-330±50</td>
<td>290±44</td>
<td>-330±50</td>
<td>290±44</td>
</tr>
</tbody>
</table>

Note:

a. Liquid phase $^{13}$C DNP experiments for 1-Chlorobenzene and 1,3,5-trichlorobenzene were finished by Dr. K. H. Tsai in 1990 (Ref. 1).

b. Carbon positions on the benzene ring of chlorine substituted benzenes.

c. Ultimate $^{13}$C DNP enhancements of the chlorobenzenes.

d. Highlighted scalar dominated enhancements at the carbon sites adjacent to the substituted carbon positions.
Figure 4.15. $^{13}$C Ultimate DNP Enhancements of 1-Chlorobenzene/CCl$_4$, 1, 2-Dichlorobenzene/C$_6$H$_{12}$, and 1, 3, 5-Trichlorobenzene/CCl$_4$ with 0.1 M TEMPO
methyl group is an electron donor for the benzene ring. Compared with nitrobenzene (Figure 4.14), the ring carbons of toluene are much poorer electron acceptor. Therefore, much less electron spin density can transmit to the ring carbons through the spindelocalization mechanism. The smaller dipolar enhancements observed at the carbon sites of C-2 to C-4 than that of the junction carbon are explained as the presence of larger scalar components at these positions where the direct spin delocalization may occur more efficiently than at the junction carbon site. The smaller $^{13}$C dipolar enhancement for the methyl group suggests that a larger scalar component exists at this carbon site. The intramolecular and the intermolecular spin polarization may jointly contribute to the spin density induced at this carbon site.

4.3 $^{13}$C DNP Studies of Diphenylmethane and Triphenylmethane in TEMPO/Cyclohexane Solutions

4.3.1 Introduction

As mentioned before, DNP enhancements depends on the correlation time of the sample molecules. Although the acidity of a C-H bond can affect the scalar enhancement of the carbon nucleus of the acidic carbon-hydrogen bond, molecular correlation time may also influence the magnitude of the scalar component. In order to see the correlation time effect on the $^{13}$C scalar enhancements of weak hydrocarbon acids, triphenylmethane
and diphenylmethane, which have larger molecular size and thus longer correlation times than those of the carbonyl compounds studied before, are studied by the liquid phase $^{13}$C DNP technique. Up to this date, no $^{13}$C DNP results have been reported for these molecules.

### 4.3.2 $^{13}$C DNP Studies of Diphenylmethane and Triphenylmethane in TEMPO/Cyclohexane Solutions

The $^{12}$C DNP spectra of toluene, diphenylmethane, and triphenylmethane are shown in Figure 4.11, 4.16, 4.17. As can be seen from these spectra and Tables 4.11, 4.13, 4.14, the ultimate DNP enhancement for toluene is dipolar dominated, but becomes a small scalar dominance for diphenyl methane, and further changes into a fairly large scalar enhancement for triphenylmethane. This trend is consistent with the acidity change as indicated by the corresponding pKa values of these molecules (Figure 4.18). Also, this trend can be explained by the hydrogen bonding spin polarization mechanism, that is, the more acidic C-H bond of the methylene group in triphenylmethane can have a stronger hydrogen bonding with the free radical and more electron spin density can be induced at the carbon site.

Although the DNP ultimate enhancements of these three molecules show the same trend as that of the pKa values, the DNP results in this section are not comparable with the acidities of the carbonyl molecules characterized in this work, such as acetaldehyde.
Figure 4.16. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for Diphenyl methane/Cyclohexane (40/60, v/v%)/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 100 Scans).
Table 4.13. Liquid Phase $^{13}$C DNP Enhancement and Relaxation Data for Ph$_2$CH$_2$/Cyclohexane (40/60, v/v%) / 0.1 M TEMPO

<table>
<thead>
<tr>
<th>Carbon Site</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_1$ (Sec.)</th>
<th>$f$</th>
<th>$A_\infty$ a,e,f,g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>140.75</td>
<td>38.7</td>
<td>2.64</td>
<td>0.932</td>
<td>-429±64</td>
</tr>
<tr>
<td>2</td>
<td>128.69</td>
<td>13.2</td>
<td>1.91</td>
<td>0.855</td>
<td>-15.8±2.37</td>
</tr>
<tr>
<td>3</td>
<td>128.10</td>
<td>9.95</td>
<td>1.85</td>
<td>0.814</td>
<td>-9.96±1.49</td>
</tr>
<tr>
<td>4</td>
<td>125.77</td>
<td>7.64</td>
<td>2.22</td>
<td>0.709</td>
<td>3.82±0.57</td>
</tr>
<tr>
<td>-CH$_2$</td>
<td>41.98</td>
<td>6.43</td>
<td>1.29</td>
<td>0.800</td>
<td>67.2±10.1</td>
</tr>
<tr>
<td>C$<em>6$H$</em>{12}$</td>
<td>26.90</td>
<td>27.2</td>
<td>3.14</td>
<td>0.885</td>
<td>-270±40</td>
</tr>
</tbody>
</table>

\[ \text{\includegraphics[width=1in]{diagram.png}} \]

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements.

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Cyclohexane was used as the reference compound with $A_\infty^\infty = -270 \pm 40$.

(g) The ultimate $^{13}$C DNP enhancement $A_\infty$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_\infty$ is about 15%.
Figure 4.17. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for 1 M Triphenylmethane/Cyclohexane/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 100 Scans).
Table 4.14. Liquid Phase $^{13}$C DNP Enhancement and Relaxation Data for 1 M (Ph)$_2$CH/Cyclohexane(40/60, v/v-%)/0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Site</th>
<th>Peak Position (ppm)</th>
<th>$T_\text{10}$ (Sec.)</th>
<th>$T_\text{1}$ (Sec.)</th>
<th>$f$</th>
<th>$A_\infty$^{a,e,f,g}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>144.84</td>
<td>29.9</td>
<td>1.92</td>
<td>0.936</td>
<td>-425±63.8</td>
</tr>
<tr>
<td>2</td>
<td>129.17</td>
<td>3.99</td>
<td>1.22</td>
<td>0.694</td>
<td>96.7±14.5</td>
</tr>
<tr>
<td>3</td>
<td>127.8i</td>
<td>4.41</td>
<td>1.05</td>
<td>0.762</td>
<td>83.0±12.5</td>
</tr>
<tr>
<td>4</td>
<td>125.86</td>
<td>4.78</td>
<td>0.722</td>
<td>0.849</td>
<td>88.4±13.2</td>
</tr>
<tr>
<td>$\text{CH}^-$</td>
<td>56.97</td>
<td>4.06</td>
<td>0.907</td>
<td>0.777</td>
<td>129±19</td>
</tr>
<tr>
<td>C$<em>6$H$</em>{12}$</td>
<td>26.90</td>
<td>27.9</td>
<td>2.76</td>
<td>0.901</td>
<td>-270±40</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Cyclohexane was used as the reference compound with $A_{\infty}^{ref} = -270 \pm 40$.

(g) The ultimate $^{13}$C DNP enhancement $A_{\infty}$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_{\infty}$ is about 15%.
Figure 4.18. $^{13}$C DNP Enhancements ($A_{\infty}$) and pKa Values of Toluene, Diphenylmethane, Triphenylmethane, and Acetaldehyde with 0.1 M TEMPO
This result may suggest that the transient hydrogen bonding between the acidic C-H bond and the free radical may not be the only factor of influencing the scalar interaction. It should be noted that the DNP enhancement is closely related to the correlation time ($\tau_c$) of the sample molecules because the spectral function $J(\omega)$ is a function of $\tau_c$. A picture of $J(\omega)$ as a function of $\tau_c$ is given in Figure 4.19. It can be seen from this Figure that at medium to low resonance frequencies (a frequency region for the DNP experiment), molecules with long correlation time have larger spectral density function values than the molecules with short correlation times. Since the correlation time of large molecules is longer than that of the small molecules, it can be expected that the spectral density function of large molecules has a higher value than that of small molecules. Since the DNP enhancement is proportional to the spectral density function, large molecules, such as, triphenylmethane are expected to have a higher $^{13}$C DNP enhancement than the smaller molecules, such as acetaldehyde.

The $^{13}$C DNP enhancements for the ring carbons of toluene, diphenylmethane, and triphenylmethane change from the large dipolar dominated enhancements to the considerable large scalar dominated enhancements except for the junction carbons, respectively. Three major factors may account for this trend. The first one is the electron spin transfer efficiency difference to the phenyl groups of these three compounds through the spin delocalization process. From toluene to triphenylmethane, the electrostatic repulsion of the phenyl group to the free radical can be expected to become weaker. Therefore, more electron spin density can be transmitted to the ring carbons of
Figure 4.19. Variation of Spectral Density Function $J(\omega)$ with $\omega$ (log scale) as a Function of Correlation Time ($\tau_c$) (Cited from Ref. 1).
triphenylmethane than to those of toluene, and larger scalar components can be induced for the ring carbons of triphenylmethane. The second factor that may contribute to this trend is the correlation time effect. From toluene to triphenylmethane, the molecular correlation time increases. The scalar spectral density function value for trphenylmethane, which has a longer correlation time, may be larger than that of toluene at the experimental resonance frequency and results in a larger scalar component. The third factor can be the collision probability difference for the ring carbons of these three molecules. Compared with toluene, the collision probability with the free radical for a ring carbon of triphenylmethane is about three times larger than that of toluene. This may result in a larger amount of electron spins transmitting to the carbon site of triphenylmethane, and causes a larger scalar component at that site. The $^{13}$C DNP enhancement trend for the ring carbons (except for the junction carbon) of these three molecules may be the result of the joint contributions of above three factors.

The dipolar dominated enhancements for the junction carbons of the phenyl groups are very close for the three molecules. The steric hindrance of the methyl (or methylene) group may be the main factor that causes the dipolar dominated enhancement at this carbon site of these molecules. The closeness of the dipolar dominated enhancements for the junction carbons of these molecules may due to the competition result between the dipolar component and the scalar contribution at the junction carbons.
4.4 Solution $^1$H and $^{13}$C DNP Studies of Taxol: A Regio-selectivity Study

4.4.1 Introduction

Taxol (Figure 4.20), an important anti-cancer drug, has been extensively studied in recent years. One major research effort on Taxol is the conformational analysis of this complex molecule. In solutions, the conformation of Taxol and the related compounds has been studied by NMR and molecular modeling. By comparing the NMR and the molecular modeling conformational analysis results of Taxol in solutions with the x-ray analysis results of Taxotere, an analog compound of Taxol, it was found that the A-ring side chain conformation of Taxol changes slightly in different phases, and even in solutions the conformations were different between that in non-aqueous solvent and that in aqueous solvent. Also, due to the limitation of the NMR and molecular modeling techniques, some of the conformational character of Taxol in solutions has not been clearly resolved.

As mentioned in Chapter 2, dipolar interaction between the sample molecule and the free radical depends on the intermolecular distance. When the scalar component in a DNP enhancement is negligible, the DNP enhancement can be related to the interaction distance between the sample molecule and the free radical molecule. Since the interaction distance is a measure of the accessibility of the receptor nucleus to the free radical probe,
Figure 4.20. Molecular Structure of Taxol.
the dipolar dominated DNP enhancement, in which the scalar contribution is negligible, thus reflect the degree of openness of the receptor nucleus towards the outside molecules, such as the free radical molecule. If the scalar contribution is large, the DNP enhancement will be sensitive to the chemical and electronic environment of the receptor nuclei in the sample molecule.\textsuperscript{1,20,21,65} In this case, a clear correlation between the DNP enhancement and the interaction distance can not be clearly obtained. Previous investigations on \textsuperscript{1}H DNP have indicated that in most cases, the ultimate enhancements for protons are close to the dipolar limit,\textsuperscript{45} which means that the scalar contribution is close to zero, and that the \textsuperscript{1}H DNP enhancements is only sensitive to the interaction distance. Former studies also showed that the \textsuperscript{13}C DNP enhancement is more sensitive to the scalar contribution when the chemical and electronic environment of the receptor nucleus is favorable to the scalar interaction mechanism.\textsuperscript{1,64,94} In most situations, when a carbon atom is not attached or adjacent to an electron attracting functional group, the scalar contribution is relatively small. the \textsuperscript{13}C DNP enhancement is dipolar dominated and is sensitive to the interacting distance.\textsuperscript{1,94} Based on the above considerations, the strategy of this work is to investigate the "scalar coupling insensitive" proton and carbon nuclei in Taxol molecules by solution \textsuperscript{1}H and \textsuperscript{13}C DNP experiments in order to find the relationship between the interaction distance and the DNP enhancements of the receptor nuclei.

The purpose of this study is to obtain the conformational information of Taxol in non-aqueous solutions by \textsuperscript{1}H and \textsuperscript{13}C DNP techniques.
4.4.2 A $^1$H DNP Study of Acetyl Side Chains of Taxol in the TEMPO/Chloroform Solution

Due to the limitation of the resolution of the NMR spectrometer, only certain $^1$H DNP signals were clearly resolved (e.g., acetyl hydrogens, Figure 4.21). It can be seen from Table 4.15 that the $^1$H DNP enhancement of the acetyl protons at C-10 is larger than that at C-4. This enhancement difference could not result from the $T_1$ difference of these two groups of protons because the flow transfer time (~0.19 second) was shorter than either of the $T_1$ values (Table 4.15) of the protons. Since the scalar component among the $^1$H DNP enhancement of the acetyl protons is negligible, the enhancement difference of these receptor protons can only be ascribed to the dipolar coupling intensity difference resulting from the interacting distance difference of these two groups of protons with TEMPO. Therefore, the acetyl protons at C-10 are more accessible to the TEMPO probe than those at C-4. This result is consistent with the NMR and molecular modeling conformational analysis results.\textsuperscript{83-92} The small dipolar dominated enhancements of the acetyl protons relative to the dipolar enhancement limit may arise from the large molecular size of Taxol which results in a too long correlation time and thus a reduced spectral density function value in the low magnetic field (Figure 4.19).
Figure 4.21. Liquid Phase $^1$H NMR (199.5 MHz) and DNP Spectra for 0.023 M Taxol/CDCl$_3$/3.7 x $10^{-3}$ M TEMPO: (a) Static NMR Spectrum (300 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 300 Scans).
Table 4.15. Liquid Phase \(^1\text{H}\) DNP Enhancement and Relaxation Data for 0.023 M Taxol/CDCl\(_3\)/TEMPO (3.70 x 10\(^{-3}\) M)

<table>
<thead>
<tr>
<th>Proton Type</th>
<th>Peak Position (ppm)</th>
<th>(T_{10}) (^b) (Sec.)</th>
<th>(T_1) (^b) (Sec.)</th>
<th>(f) (^c)</th>
<th>(s) (^d)</th>
<th>(A_{\text{obs}}) (^{a})</th>
<th>(A_\infty) (^{a,e,f})</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-OAc</td>
<td>2.23</td>
<td>2.23</td>
<td>0.602</td>
<td>0.730</td>
<td>0.381</td>
<td>-0.658</td>
<td>-33.6±5.0</td>
</tr>
<tr>
<td>4-OAc</td>
<td>2.37</td>
<td>1.35</td>
<td>0.304</td>
<td>0.775</td>
<td>0.381</td>
<td>-0.204</td>
<td>-9.81±1.47</td>
</tr>
</tbody>
</table>

Note:

(a) Exhausted method was used to calculate the DNP ultimate enhancements

(b) \(T_1\) and \(T_{10}\) are the spin-lattice relaxation times of the proton nuclei with and without TEMPO in the sample solution, respectively.

(c) The leakage factor \(f\) was calculated based on \(T_1\) measurements at 4.7 T magnetic field.

(d) The saturation factor was obtained from the \(^1\text{H}\) DNP power plot.

(e) The 0.33 T \(^{1}\text{H}\) DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) The ultimate \(^{13}\text{C}\) DNP enhancement \(A_\infty\) is the value of one measurement. The estimated relative standard deviation for \(A_\infty\) is about 15%.
4.4.3 A $^{13}$C DNP Study of Skeleton Carbon Sites of Taxol in the TEMPO/Chloroform Solution

Due to the low sensitivity of the $^{13}$C static NMR of Taxol on the flow transfer system, only the $^{13}$C DNP spectrum was obtained. For this reason, the ultimate $^{13}$C DNP enhancement could not be calculated. Only a qualitative discussion was given in this study. The dipolar dominated $^{13}$C DNP enhancements can be used to evaluate the interaction distance between the receptor carbon nuclei and the free radical probe as long as there is no or, only a small amount of scalar contribution among the enhancements at the receptor nuclei. The most favorable situation is the dipolar dominated $^{13}$C DNP enhancement at the carbon nuclei of hydrocarbon groups which are not adjacent to any electron withdrawing or resonance structure inducing functional groups, otherwise, a large scalar contribution or even a scalar dominance will be observed at the receptor nuclei, and the DNP enhancements may not be uniquely correlated to the interaction distance. Therefore, the most reliable comparison of the accessibility of the skeleton sites and the side groups on the rings of Taxol will be among the hydrocarbon groups which are isolated from the scalar coupling inducing environments. These carbon nuclei are as follows: C-6, C-3, C-14, C-16, C-17, C-15, C-19, C-8, C-18, C-11 (signal overlapped), and C-12 (Figure 4.22). Other carbon nuclei are either adjacent to the oxygen, nitrogen, or to a carbonyl group which are favorable for the scalar coupling. The carbon nuclei on the phenyl ring are not evaluated because of the signal overlapping. By examining the peak
Figure 4.22. Liquid Phase $^{13}$C DNP Spectra for 0.046 M Taxol/CDCl$_3$/3.7 x 10$^{-3}$ M TEMPO (7 mL/min, 175,371 scans).
heights and the number of the carbons corresponding to each peak, the following relative height sequence was obtained from high to low: C-6 and/or C-14 (the two signals overlapped) > C-16 ≥ C-3 > C-17 > C-19 > C-18 ≈ C-8 ≈ C-15 > C-12 > C-11. Since the flow transfer time (at 7mL/min, ~ 0.17 s) is shorter than the spin-lattice relaxation times (Table 4.17), the above peak height sequence can be recognized as the relative enhancement sequence, and reflects the accessibility of these carbon nuclei. This result is also closely coincident with the accepted Taxol conformation as can be seen from Figure 4.23, which illustrates the intermolecular interaction between Taxol and the TEMPO in the liquid phase.

Due to the resolution limitation of the NMR spectrometer employed in this work, the important conformational feature of the side chains containing carbonyl groups and phenyl groups on the Taxol rings (the A-ring side chain) was not clearly resolved. Further instrumental improvements and research work need to be done in this respect.
<table>
<thead>
<tr>
<th>Carbon Position</th>
<th>Peak Position (ppm)</th>
<th>T&lt;sub&gt;1&lt;/sub&gt; (Second)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-19</td>
<td>9.08</td>
<td>0.454</td>
</tr>
<tr>
<td>C-18</td>
<td>14.44</td>
<td>0.893</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-COO-10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.36</td>
<td>0.931</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-COO-4</td>
<td>22.18</td>
<td>0.863</td>
</tr>
<tr>
<td>C-17</td>
<td>26.36</td>
<td>0.651</td>
</tr>
<tr>
<td>C-6/C-14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.16</td>
<td>0.452</td>
</tr>
<tr>
<td>C-15</td>
<td>42.80</td>
<td>1.604</td>
</tr>
<tr>
<td>C-3</td>
<td>45.16</td>
<td>0.445</td>
</tr>
<tr>
<td>C-8</td>
<td>58.14</td>
<td>2.302</td>
</tr>
<tr>
<td>C-7</td>
<td>71.66</td>
<td>0.271</td>
</tr>
<tr>
<td>C-13</td>
<td>71.85</td>
<td>0.388</td>
</tr>
<tr>
<td>C-2</td>
<td>75.06</td>
<td>0.343</td>
</tr>
<tr>
<td>C-10</td>
<td>75.30</td>
<td>1.265</td>
</tr>
<tr>
<td>CDCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>76.33</td>
<td>14.37</td>
</tr>
<tr>
<td>CDCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>76.94</td>
<td>3.183</td>
</tr>
<tr>
<td>CDCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>77.61</td>
<td>10.82</td>
</tr>
<tr>
<td>C-4</td>
<td>80.64</td>
<td>2.634</td>
</tr>
<tr>
<td>C-5</td>
<td>83.91</td>
<td>0.418</td>
</tr>
<tr>
<td>o-NHCOBz/o-NHCHBz&lt;sup&gt;c&lt;/sup&gt;</td>
<td>126.61</td>
<td>0.747</td>
</tr>
<tr>
<td>o-NHCHBz/o-NHCOBz</td>
<td>126.73</td>
<td>0.625</td>
</tr>
<tr>
<td>p-NHCOBz</td>
<td>127.88</td>
<td>0.524</td>
</tr>
<tr>
<td>m-NHBz/m-OCOPh&lt;sub&gt;-2&lt;/sub&gt;</td>
<td>128.24</td>
<td>0.557</td>
</tr>
<tr>
<td>m-OCOPh&lt;sub&gt;-2&lt;/sub&gt;/m-NHBz</td>
<td>128.55</td>
<td>0.761</td>
</tr>
<tr>
<td>o-OCOPh&lt;sub&gt;-2&lt;/sub&gt;</td>
<td>129.70</td>
<td>0.339</td>
</tr>
<tr>
<td>o-OCOPh&lt;sub&gt;-2&lt;/sub&gt;</td>
<td>129.94</td>
<td>0.400</td>
</tr>
<tr>
<td>p-NHCHBz</td>
<td>131.52</td>
<td>0.396</td>
</tr>
<tr>
<td>C-11</td>
<td>132.67</td>
<td>2.852</td>
</tr>
<tr>
<td>p-OCOPh&lt;sub&gt;-2&lt;/sub&gt;</td>
<td>133.22</td>
<td>0.679</td>
</tr>
<tr>
<td>CO-NHCOBz/OCOPh&lt;sub&gt;-2&lt;/sub&gt;</td>
<td>166.57</td>
<td>3.443</td>
</tr>
<tr>
<td>CO-OAc-10</td>
<td>170.75</td>
<td>2.729</td>
</tr>
<tr>
<td>C-9</td>
<td>203.14</td>
<td>2.181</td>
</tr>
</tbody>
</table>
(Table 4.16 Continues)

Note:

a. The highlighted carbon is the one that the peak position and the $T_1$ value are assigned.

b. These two peaks overlap, the peak position and $T_1$ value can not be unambiguously assigned.

c. The carbon nucleus of interest is the carbon of benzene; two substituted benzene ring carbons have the same or almost the same peak position which can not be resolved by the NMR instrument.
Figure 4.23. Taxol/TEMPO Model (Cited from Ref. 25) and the Molecular Structure of Taxol
4.5 $^{13}\text{C}$ DNP Studies of Adamantane and Fullerene $C_{70}$ in TEMPO/Benzene Solutions

4.5.1 Introduction

Adamantane as a standard reference material for solid state NMR has been well characterized by NMR techniques.\textsuperscript{95-101} Solution NMR studies of this molecule have shown that the NOE effect at C-1, which has one attached proton, is the same as that at C-2 with two protons on it.\textsuperscript{101} This result indicated that the NOE effect is independent of the number of the protons attached to the carbon atoms of interest and that the correlation times for the two different carbon sites are the same. Up to this date, no $^{13}\text{C}$ DNP study of this compound has been done to reveal the difference of the correlation times at the two different carbon sites. In this work, the $^{13}\text{C}$ DNP experiment was performed on adamantane to compare the DNP enhancements at the two different carbon sites. Since the free radical concentration for the adamantane/TEMPO system is high enough (0.1 M TEMPO) to eliminate the three-spin effect, the observed $^{13}\text{C}$ DNP enhancements for the two different carbons of adamantane can be related to the correlation times at these carbon sites.

$^{13}\text{C}$ DNP investigation of fullerene $C_{60}$ has been published.\textsuperscript{41} It was found that the interaction of this molecule with the free radical is dipolar dominated with little tendency
to form a scalar interaction. The NMR contact shift experiment further confirmed the
negligible component of the scalar interaction. The short correlation time (∼ 10⁻¹¹ sec.) of
C₆₀ molecules in solution was also characterized by the ¹³C DNP experiment.

C₇₀ as the second simplest isolated fullerene molecule has been well characterized
by NMR,¹⁰²-¹⁰⁷ but no DNP results have been reported. In this work, a liquid phase ¹³C
DNP study of C₇₀ was carried out for the purpose of revealing the structural and the
dynamic feature of this molecule probed by the free radical.

4.5.2 A ¹³C DNP Study of Adamantane in the TEMPO/Benzene Solution

The ¹³C DNP result of adamantane is shown in Figure 4.24 and Table 4.17. It can
be seen from the DNP data that the enhancements for C-1 and C-2 are very close and that
the corresponding T₁ values are different by about a factor of two. This means that in the
¹³C DNP experiment of adamantane, the relaxation of the two different carbon nuclei is
induced by the direct coupling with the nitroxide free radical (very small contribution
from the attached protons) and that the correlation time is the same at the two different
carbon sites. This result is in good agreement with the NOE result of adamantane. This is
also an indirect evidence that the three spin effect is negligible at high free radical
concentration.
Figure 4.24. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for 0.5 M Adamantane/Benzene/0.1 M TEMPO: (a) Static NMR Spectrum (100 Scans); (b) Flow Transfer DNP Spectrum (5 mL/min, 100 Scans).
Table 4.17. Liquid Phase $^{13}$C DNP Enhancement and Relaxation Data for 0.5 M Adamantane/Benzene/0.1 M TEMPO

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_1$ (Sec.)</th>
<th>$f^d$</th>
<th>$A_\infty^{a,e,f,g}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>28.66</td>
<td>20.3</td>
<td>2.64</td>
<td>0.870</td>
<td>-254±14</td>
</tr>
<tr>
<td>C-2</td>
<td>37.91</td>
<td>13.0</td>
<td>2.25</td>
<td>0.827</td>
<td>-209±13</td>
</tr>
<tr>
<td>C_6H_6</td>
<td>128.50</td>
<td>29.9</td>
<td>2.46</td>
<td>0.918</td>
<td>-200±30</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements

(b) $^1$H broad-band decoupling was employed to eliminate proton-carbon coupling.

(c) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.

(d) The leakage factor $f$ was calculated based on $T_1$ measurements at 4.7 T magnetic field.

(e) The 0.33 T $^{13}$C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(f) Benzene was used as the reference compound with $A_\infty^{\text{ref}} = -200±30$.

(g) The ultimate $^{13}$C DNP enhancement $A_\infty$ is an average value of four measurements at different power levels. The estimated relative standard deviation for $A_\infty$ is about 15%.
4.5.3 A $^{13}$C DNP and NMR Study of the C$_{70}$/TEMPO System

The interaction between all the carbon sites of the C$_{70}$ molecule and the free radical is dipolar dominated as shown in Figure 4.25 and Table 4.18. The DNP data shown in Table 4.18 indicate that the carbons at the polar positions of this molecule (i.e. C-a, C-b) exhibit larger dipolar enhancements than those at the equatorial positions (i.e. C-d, C-e), and from the poles to the equatorial, the enhancements decrease gradually. An NMR contact shift experiment for the C$_{70}$/TEMPO system (Table 4.19) indicates that the scalar contribution to enhancements is very small and negligible. Therefore, the observed enhancement trend can be recognized as the intensity trend of the dipolar interaction.

A previous theoretical calculation on the C$_{70}$ molecule has shown that the polar carbon atoms experience larger angle strain than those at the equatorial sites (Table 4.19, Figure 4.26), and that the polar carbons are more exposed to the outside than the equatorial carbons.$^{108}$ This geometric feature suggests that the polar carbons are more accessible to the free radical than the equatorial carbons.

$^{13}$C DNP investigation of the C$_{60}$/C$_{6}$D$_{6}$/TEMPO system has been completed by Gu et al.$^{41}$ The ultimate $^{13}$C DNP enhancements for C$_{60}$ and C$_{6}$D$_{6}$ were -250±20 and 200±20, respectively. The average molar NMR contact shift for C$_{60}$ was very small even in comparison with aromatic hydrocarbons. Both the $^{13}$C DNP enhancement and the NMR contact shift results for C$_{60}$/TEMPO system suggested that the scalar interaction between C$_{60}$ and the TEMPO is very weak and negligible. The reduction in the $^{13}$C DNP
Figure 4.25. Liquid Phase $^{13}$C NMR (50.1 MHz) and DNP Spectra for 0.024 M $C_{79}/C_{6}D_{6}/0.1$ M TEMPO: (a) Static NMR Spectrum (24000 Scans); (b) Flow Transfer DNP Spectrum (6 mL/min, 5000 Scans).
Table 4.18. Liquid Phase \(^{13}\)C DNP Enhancements and Relaxation Data
for 0.024 M C\(_{70}\)/C\(_{6}D_6\)/0.1 M TEMPO System

<table>
<thead>
<tr>
<th>Carbon Type</th>
<th>Peak Position (ppm)</th>
<th>(T_{10}) (Sec.)</th>
<th>(T_1) (Sec.)</th>
<th>(f)</th>
<th>(\frac{1}{n} \sum (\theta_{\sigma n} - \pi/2)^2) (rad(^2))</th>
<th>(A_{\infty})</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - a</td>
<td>151.08</td>
<td>57.8</td>
<td>2.51</td>
<td>0.957</td>
<td>0.0435</td>
<td>-281±42</td>
</tr>
<tr>
<td>C - b</td>
<td>147.85</td>
<td>54.9</td>
<td>2.44</td>
<td>0.956</td>
<td>0.0436</td>
<td>-273±41</td>
</tr>
<tr>
<td>C - c</td>
<td>148.52</td>
<td>57.6</td>
<td>2.67</td>
<td>0.954</td>
<td>0.0400</td>
<td>-244±37</td>
</tr>
<tr>
<td>C - d</td>
<td>145.75</td>
<td>59.7</td>
<td>2.33</td>
<td>0.940</td>
<td>0.0308</td>
<td>-182±27</td>
</tr>
<tr>
<td>C - e</td>
<td>131.25</td>
<td>47.7</td>
<td>1.31</td>
<td>0.964</td>
<td>0.0235</td>
<td>-160±24</td>
</tr>
<tr>
<td>C(_{60})</td>
<td>143.60</td>
<td>37.1</td>
<td>3.59</td>
<td>0.903</td>
<td>0.0413</td>
<td>-250±38</td>
</tr>
</tbody>
</table>

Note:

(a) Ratio method was used to calculate the DNP ultimate enhancements.
(b) \(^1\)H broad-band decoupling was employed to eliminate proton-carbon coupling.
(c) \(T_1\) and \(T_{10}\) are the spin-lattice relaxation times of the carbon nuclei with and without TEMPO in the sample solution, respectively.
(d) The leakage factor \(f\) was calculated based on \(T_1\) measurements at 4.7 T magnetic field.
(e) The 0.33 T \(^{13}\)C DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.
(f) C\(_{60}\) was used as the reference compound with \(A_{\infty}^{C_{60}} = -250±38\).
(g) The angle strain of the C\(_{70}\) carbons is assessed with the parameter \(\frac{1}{n} \sum (\theta_{\sigma n} - \pi/2)^2\) by Haddon\(^{108}\), where \(n\) is the identical carbons, \(\theta_{\sigma n}\) is the angle between the carbon \(\sigma\) orbital and the \(p_\pi\) orbital.
(h) The ultimate \(^{13}\)C DNP enhancement \(A_{\infty}\) is an average value of four measurements at different power levels. The estimated relative standard deviation for \(A_{\infty}\) is about 15%.
Carbon atoms are numbered from the apical to the equatorial carbons.

Angle strain is assessed with the parameter \( \frac{1}{n} \sum (\theta_{\text{act}} - \pi/2)^2 \).
Table 4.19. NMR Contact Shift of the C$_{70}$/TEMPO/ODCB/C$_6$H$_{12}$/System

<table>
<thead>
<tr>
<th>?TEMPO Conc. (M)</th>
<th>-$\Delta\delta_a$ (ppm)</th>
<th>-$\Delta\delta_b$ (ppm)</th>
<th>-$\Delta\delta_c$ (ppm)</th>
<th>-$\Delta\delta_d$ (ppm)</th>
<th>-$\Delta\delta_e$ (ppm)</th>
<th>-$\Delta\delta_{C60}$ (ppm)</th>
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<tbody>
<tr>
<td>0.1</td>
<td>0.15</td>
<td>0.12</td>
<td>0.19</td>
<td>0.20</td>
<td>1.21</td>
<td>0.15</td>
</tr>
<tr>
<td>0.2</td>
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<td>0.26</td>
<td>0.33</td>
<td>0.30</td>
<td>1.35</td>
<td>0.29</td>
</tr>
<tr>
<td>0.3</td>
<td>0.50</td>
<td>0.51</td>
<td>0.49</td>
<td>0.51</td>
<td>1.53</td>
<td>0.48</td>
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<td>0.4</td>
<td>0.58</td>
<td>0.51</td>
<td>0.58</td>
<td>0.59</td>
<td>1.65</td>
<td>0.58</td>
</tr>
<tr>
<td>0.5</td>
<td>0.80</td>
<td>0.80</td>
<td>0.84</td>
<td>0.84</td>
<td>N/A$^H$</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Note:

I. The contact shift $\Delta\delta_i$ was obtained by subtracting the chemical shift of the nucleus of the undoped sample from that of the doped sample using cyclohexane as the internal reference.

II. The e-band of C$_{70}$ was overlapped by the solvent peak at this free radical concentration.
enhancements of $C_{60}$ was attributed to the presence of small scalar component and the long correlation time.

For the $C_{70}$/TEMPO system, the molecular size of $C_{70}$ is larger than that of $C_{60}$, an even longer correlation time can be expected than that of $C_{60}$. This means that the spectral density function and thus the $^{13}$C DNP enhancement will show a smaller value than that of the $C_{60}$/TEMPO system. On the other hand, it can be seen from Table 4.18 that the curvatures for the apical carbons (i.e. C-a and C-b) are smaller than that of $C_{60}$. This suggests that the free radical may have a shorter interaction distance with these carbons than with the carbons of $C_{60}$ as well as the equatorial carbons of $C_{70}$. As the result of the shorter interaction distance, stronger dipolar couplings can be expected for the apical carbons. The stronger dipolar coupling at the apical carbons may overweigh the dipolar enhancement reduction at these carbon sites caused by the longer correlation time and makes the dipolar dominated enhancements slightly larger than that of the $C_{60}$ carbons. The smaller dipolar enhancements for the $C_{70}$ carbons of C-c to C-e is attributed to the smaller curvatures of these carbon sites and the longer correlation time of the $C_{70}$ molecule than those of $C_{60}$.

Therefore, the trend for the dipolar dominated $^{13}$C DNP enhancements of $C_{70}$ is attributed to the longer correlation time of this molecule and the curvature difference for the different carbons of $C_{70}$. 

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The small scalar components for the $C_{70}$ carbons may be due to the nonplanar conjugation character of this molecule. It has been known that for the nonplanar conjugated organic molecules, [2, 2] paracyclophepane, the $\pi$-electron density shifts to the outside faces of the molecule. If this is also true for the $C_{70}$ molecule, a large electrostatic repulsion of the $\pi$-electron density at the outside face of $C_{70}$ to the unpaired electron spin density of TEMPO can be expected. As a result, little electron spin density can be transmitted to the $C_{70}$ carbons.
CHAPTER 5. CONCLUSIONS AND FUTURE DEVELOPMENTS

5.1 Conclusions

Liquid phase $^{13}$C DNP results obtained in this work for several organic molecules containing acidic carbon-hydrogen bonds indicate that the $^{13}$C DNP technique is sensitive to the weak acidity of the hydrogen in a C-H bond. An electron spin density polarization mechanism is used for the first time to interpret the scalar component induced at the carbon-13 nucleus of the acidic C-H bond through the weak hydrogen bonding between the C-H bond and the nitrooxide free radical TEMPO.

$^{13}$C DNP studies of three aromatic molecules with electron attracting and releasing functional groups indicate that transient hydrogen bonding is not the only way to transmit electron spin density from the free radical to the carbon nuclei of organic molecules. For the aromatic carbons, a spin delocalization model is used for the first time to explain the $^{13}$C DNP results of aromatic carbons. This is the most important contribution of this work. $^{13}$C DNP results for the aromatic molecules also show that the $^{13}$C DNP technique is sensitive to the electronic environment of the substituted benzene ring. Specifically, the transmitted electron spin density seems to be sensitive to the electron distribution in the $\pi$ orbital of the benzene ring.
For the first time, the $^{13}$C DNP technique is used to study the conformation of Taxol in solutions. Due to the limitation of the instrument employed in this work, the important conformational information of the A-ring side chain, which has not been clearly resolved by other experimental means, is not obtained in this work, either. However, novel information about the accessibility of the ring carbon sites and the acetyl side chains of Taxol toward the molecular probe TEMPO is obtained in this work.

The $^{13}$C DNP study of adamantane indicates that the carbon nuclear relaxation of this molecule is solely induced by the free radical and that the two different carbon sites have the same correlation times. This experiment also shows that the three spin effect is negligible under the high free radical concentration.

The $^{13}$C DNP investigation of C$_{70}$/TEMPO system provides some qualitative structural and dynamic information of the C$_{70}$ molecule probed by the free radical in solutions. The larger dipolar enhancements at the apical carbons of this molecule confirms the "football shape" molecular structure of C$_{70}$ and is consistent with the theoretical calculation. The smaller dipolar dominated enhancements for the equatorial carbons indicates the longer correlation time of this molecule than that of the C$_{60}$/TEMPO system. The $^{13}$C NMR contact shift study of the C$_{70}$/TEMPO system indicates that the scalar contribution for the $^{13}$C DNP enhancements of the C70 molecule is negligible. This may result from the electrostatic repulsion of the $\pi$-electron density of the C$_{70}$ molecule to the unpaired electron spin of the free radical.
5.2. Future Developments

Some future research projects can be planned based on the studies completed in this work. In addition, some experiments which are not included in this dissertation but have been tried unsuccessfully may also be considered in the future.

5.2.1. $^{13}$C DNP Study of Aromatic Molecules and the Corresponding Deuterated Aromatic Molecules

Although the $^{13}$C DNP results for the aromatic molecules can be well explained by the spin delocalization mechanism. More experimental evidence need to be collected to verify this model. One direct experiment is to measure the $^{13}$C DNP enhancements of some aromatic molecules and the corresponding deuterated molecules. If the spin delocalization model is valid, the isotopic substitution will not have a large effect on the $^{13}$C DNP enhancements of the ring carbons. If an obvious isotopic effect is observed, the spin polarization mechanism may be the real mechanism.

5.2.2. $^{89}$Y DNP Studies of Y(NO$_3$)$_3$/H$_2$O/TEMPO System

An NMR probe has been prepared and tuned for the $^{89}$Y DNP investigation of some organic yttrium compounds. No $^{89}$Y NMR signal has been observed for these
systems with this probe. One possible reason for the unsuccessful experiment is the low solubility of the yttrium compounds in the organic solvents. By the time of that experiment, a low field flow cell which is tunable for the aqueous solutions in the microwave field has not been designed and built. Therefore, the inorganic yttrium compounds which have a large solubility in water were not tried.

Further experiments can be carried out on the Y(NO₃)/H₂O/TEMPOL system. If this experiment is successful, a series of nuclei with low magnetogyric ratios can be studied by this probe because by changing the capacitor of this probe, a fairly wide range of low NMR resonance frequency can be covered.

5.2.3. A Solution $^{13}$C DNP Study of Taxol in Different Solvents

As mentioned in this dissertation, the A-ring side chain conformation of Taxol changes in different solvents. Also it is believed that the conformation of the A-ring side chain has an important influence on the drug effect of Taxol. However, due to the limitation of the instrument, $^{13}$C DNP signals for the A-ring side chain has not been observed. In order to perform this experiment successfully, large effort need to be made to improve the instrument. Two parts of the instrument need to be improved. One is the $^{13}$C NMR probe. A high resolution $^{13}$C NMR probe need to be built. For this purpose, a good quality coil with high Q value need to be made. Another part of work need to be done on the flow transfer system. Taxol is a large molecule which has a very short
relaxation time (very long correlation time). In order to reduce the transfer loss of the nuclear polarization, a faster transfer system, which does not cause a large build-up loss of the nuclear polarization, is needed. A shuttle transfer system can ideally fulfill this requirement. In order to build up such a system, a lot of electronic and mechanic work need to be done. A detailed proposal for the shuttle transfer system can be found from Ref. 1.

5.2.4. A $^{13}$C DNP Study of the C$_{70}$/TEMPO System at Different Magnetic Resonance Frequencies

The $^{13}$C DNP result for the C$_{70}$/TEMPO system at certain frequencies has demonstrated that the coupling between the C$_{70}$ molecule and the free radical is dipolar dominated. The trend shown by the $^{13}$C DNP enhancements of this system is attributed to the interaction distance difference between the carbons of the C$_{70}$ molecule and the free radical. One method for verifying the validity of this assumption is to calculate the closest approaching distance of the free radical to the different carbon sites of the C$_{70}$ molecule in the solution. In order to perform such a calculation, the diffusion mechanism which modulates the coupling between the C$_{70}$ molecule and TEMPO needs to be determined by measuring the frequency dependence of the $^{13}$C DNP enhancements of the C$_{70}$/TEMPO system since for the different modulation mechanism (translational or rotational diffusion modulation), the formula for the calculation of the closest approaching distance are
different (Chapter 2). For the translational modulated dipolar coupling, the spectral density function has a frequency dependence which is quite different from the spectral density function modulated by the rotational mechanism.\textsuperscript{22}
Reference


5. T. R. Carver and C. P. Slichter, Phys. Rev. 92, 212 (1953); 102, 975 (1956).


APPENDIX

A $^1$H DNP Study of the Water/TEMPOL Solution

Introduction

The $^1$H DNP of aqueous solution or pure water is difficult to perform because of the high dielectric constant of water which causes significant heating of the sample solution in the microwave field if the regular low field sample cell is used. Thus, the water solution cannot be sharply tuned in the microwave field, and a small or no DNP enhancement is usually obtained. The way to circumvent this problem is to use a specially designed sample cell in the low field so that the heating effect can be largely reduced. In this study, a glass sample cell for the proton DNP of water is built as is shown in Figure A. The detailed description is given in the next section.

The significance of the $^1$H DNP experiment of water is that water is an important biological solvent, any attempt to apply $^1$H DNP techniques to the aqueous biological solutions have to deal with its solvent. Also the hydrogen bonding between the water proton and the biological molecules may be used to transfer the electron polarization to the nuclei of biological molecules so that the structural or dynamic information of the biological molecules can be enhanced and probed. In addition to the medium function in
the DNP experiments, $^1$H DNP enhancement of water alone may also provide information about biological systems, such as the technique of magnetic resonance imaging.

Design of the Low Field Flow Cell for the $^1$H DNP of Water

In the design of the low field flow cell for water, three factors have been considered: firstly, the geometric structure of the cell should satisfy two criteria, one is that the shape of the cell should be as flat as possible so that as much $\vec{B}$ microwave field flux as possible will pass through the flat part (i.e. the wide part) of the cell, and as little $\vec{E}$ field flux, which causes the heating effect, as possible will pass through the narrow part of the cell, the other criterion is that the volume of the cell should be as large as possible relative to that of the high field flow cell, so that the optimized DNP enhancement can be observed; secondly, the cell material should be able to be processed into the desired shape and has a small dielectric constant; lastly, the cell built should be able to stand fairly high back pressure of the flow system. In this work, Pyrex glass was used to made the low field flow cell with a volume of 117 $\mu$L. The volume of the high field glass flow cell is 44 $\mu$L.
Figure A. Low Field Flow Cell for the $^1$H DNP Experiment of H$_2$O/TEMPOL System
A $^{1}H$ DNP Study of the Water/TEMPO Solution

The $^{1}H$ DNP spectrum of water, which is the first DNP spectrum obtained in this work was unfortunately not saved. But the peak height data were recorded and the ultimate DNP enhancement was calculated as shown in Table A. As can be seen, a small dipolar dominated enhancement was observed for the H$_2$O/TEMPOL system. The predicted ultimate dipolar enhancement limit of proton is -330. It is unusual that the water proton enhancement is much less than the dipolar limit since in most of the cases, the $^{1}H$ DNP is close to the dipolar enhancement limit. Three possible reasons may account for the small dipolar enhancement: one is the proton exchange effect that reduced the DNP enhancement, another is the electron-electron exchange effect that resulted from the low free radical concentration; the third one may arise from the possible instrumentation problem that either causes poor saturation of the electron transition, or large build-up and transfer loss of the nuclear polarization. After further improvement of this work, the observed enhancement of water proton may become larger and more understandable. But the present work is an important step toward the goal of this study.
Table A. Liquid Phase $^1$H DNP Enhancement and Relaxation Data for H$_2$O/ TEMPO (7.00 x $10^{-3}$ M) System

<table>
<thead>
<tr>
<th>H$_2$O</th>
<th>Peak Position (ppm)</th>
<th>$T_{10}$ (Sec.)</th>
<th>$T_{1}$ (Sec.)</th>
<th>$f$</th>
<th>$s$</th>
<th>$A_{obs}$ a</th>
<th>$A_{\infty}$ d,e</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>4.80</td>
<td>4.00</td>
<td>0.819</td>
<td>0.795</td>
<td>0.595</td>
<td>-1.03</td>
<td>-30.9±4.6</td>
</tr>
</tbody>
</table>

H$_2$O

Note:

(a) $A_{obs}$ was obtained from the intercept of ln$(A_{obs})$ versus inverse flow rate (2.00 - 9.00 ml/min).

(b) $T_1$ and $T_{10}$ are the spin-lattice relaxation times of the proton with and without TEMPO in the sample solution, respectively.

(c) The leakage factor $f$ was calculated based on $T_1$ measurements at 9.4 T magnetic field. The saturation factor $s$ was calculated from the plot of $1/A_{obs}$ versus $1/P$, where P is the microwave power in watts.

(d) The 0.33 T $^1$H DNP enhancements were monitored at 4.7 T. All data was obtained at room temperature.

(e) The ultimate $^{13}$C DNP enhancement $A_{\infty}$ was the value of one measurement. The estimated relative standard deviation for $A_{\infty}$ is about 15%.
VITA

Ziqi Sun was born on October 21, 1962 in Changchun, China. He graduated with a B.S. degree in Chemistry from Nankai University, Tianjin, China in July 1984. Since then, he had been working in the area of chemistry for the period 1984-1991. In the Fall of 1991, he enrolled in the Chemistry Department of Virginia Polytechnic Institute and State University (VPI & SU) as a graduate student in Physical Chemistry. In 1993, he joined Dr. Harry C. Dorn's research group and started his Ph.D. research in the area of dynamic nuclear polarization studies of organic molecules which led to the completion of his Ph.D. work in September, 1996.