Relaxivity Studies on Dinitroxide and Polynitroxyl Functionalized Dendrimers: Effect of Electron Exchange and Structure on Paramagnetic Relaxation Enhancement

Ashok J. Maliakal,† Nicholas J. Turro,*† Anton W. Bosman,‡ Jeroen Cornel,§ and E. W. Meijer†

Department of Chemistry, Columbia University, 3000 Broadway, New York, New York 10027, and Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, PO Box 513, NL-5600 MB, Eindhoven, The Netherlands

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The 1H NMR relaxivity of o- and p-dinitroxide-substituted phthalate esters and a series of nitroxyl-functionalized poly(propylene imine) dendrimers has been measured in acetonitrile and methanol. Studies of dinitroxide relaxivity indicate that the electron exchange rate has only a small effect on relaxivity. Outer-sphere relaxivity has been measured using benzene as a probe molecule. In studies on dendritic polynitroxides, the per-nitroxide-based outer-sphere relaxivity nearly doubles for the generation 5 nitroxyl-functionalized dendrimer as compared to a mononitroxide model. This relaxivity enhancement may be due to crowding of dendrimer surface groups in higher generation dendrimers. Water relaxivity has been measured for these polynitroxides as well, and a significant inner-sphere contribution to relaxivity is found. Dendritic polynitroxides exhibit higher per-nitroxide-based water relaxivity as compared to a mononitroxide model. This relaxivity enhancement is attributed to an increase in rotational correlation time (τr) for the dendritic polynitroxides.

Introduction

Paramagnetic relaxation enhancement is of fundamental importance to the field of magnetic resonance imaging (MRI) and NMR spectroscopy. Typically, the magnitude of paramagnetic relaxation enhancement is proportional to the concentration of paramagnetic species. The proportionality constant has been defined as the relaxivity of the species and is reported in the literature in units of M−1 s−1 or mM−1 s−1.1−4 The most commonly used paramagnetic relaxation enhancers (PREs) employed for MRI applications have been gadolinium chelates (PREs) which exhibit high relaxivities due to the high spin (7/2) of this metal center.1,2 For years, the use of stable nitroxide free radicals has been evaluated as a potential alternative to gadolinium.6,7 Stable nitroxides have been shown to be more amenable to in vivo use than gadolinium complexes7 and may not require the same extensive chelation protocols required to minimize the toxicity of free gadolinium. However, the intrinsically low paramagnetic relaxivity of nitroxides (in part due to their low spin of 1/2) has prevented their widespread application as MRI contrast agents.8 Attempts have been made to enhance the relaxivity of nitroxides by using nitroxides which have functional groups capable of binding proteins.8 Such protein binding could enhance the inner-sphere contribution to relaxivity and thus improve the profile of nitroxides as relaxation enhancers.

Dendrimer scaffolds have been used to enhance paramagnetic relaxation in the case of gadolinium-based PREs8,10 and nitroxide-labeled systems.11−13 In the case of gadolinium-based dendritic relaxation enhancers, the dendrimer is used to modify the rotational correlation time of the system and thereby enhance inner-sphere relaxivities.9,10 In the case of nitroxide-based dendrimers, relaxivity enhancements in aqueous solution are not observed.13 However, aggregation effects14 in aqueous solution could be complicating the interpretation of relaxivity measurements made in aqueous solution. The dendrimer framework has been shown to be advantageous in decreasing the bioreduction rate of nitroxide in vivo, an extremely rapid process which destroys the relaxation enhancer.12 The potential of dendritic PREs in targeting specific organs has been investigated.10,13,15

Our lab has studied stable nitroxide radicals for several years as ESR probes for surfaces and supramolecular assemblies.16 In the course of our studies we chose to investigate dinitroxides 1a and 1b illustrated in Scheme 117,18 ESR studies18 demonstrated that 1b exhibits fast intramolecular electron exchange on the ESR time scale.19 Furthermore, polarization transfer studies indicated that interactions between polynitroxides and photoexcited triplets occurred in the strong exchange limit [by strong exchange, it is meant that on average the exchange integral (J) is much greater than the hyperfine interaction (a)].18,20,21 Thus, the polarization was transferred from the photoexcited triplet to the entire polynitroxide array, rather than to one particular nitroxide unit. These results suggested that the polynitroxides might be functioning as high-spin species.20,22−24

Since the theory for paramagnetic relaxation25 predicts relaxivity to be proportional to S(S + 1) (see eqs 1−4 and 6−8 below), where S is the spin on the paramagnetic species, we decided to investigate the relaxivity of a series of small molecule (see Scheme 1) and dendrimeric polynitroxides (see Scheme 2) as a function of the number of nitroxides per molecule, structure, and the rate of electronic exchange in these species. We used benzene as a probe molecule for outer-sphere relaxivity, since it does not form complexes with nitroxides. MRI applications depend on relaxation enhancers which exhibit high water relaxivity.1−4 However, poly(propylene imine) dendrimers modified with nonpolar organic groups such as nitroxide

† Columbia University.
‡ Eindhoven University of Technology.
derivatives are highly aggregated and exhibit low solubility in aqueous solution. To measure the water relaxivity of $4N$ in the absence of aggregation, measurements were performed in acetonitrile, with water as the probe molecule, which is expected to display significant inner-sphere relaxivity.7

Our results indicate that electronic exchange rates have only a weak effect on relaxivity. An increase in relaxivity on a per nitroxide basis for higher generation dendrimers is observed for both water and benzene probes. The larger dendrimers (generations 3 and 5) have relaxivities exceeding those of gadolinium-(III) chloride (on a per molecule basis). Increases in outer-sphere relaxivity, as measured using benzene as a probe, are attributed to crowding of dendrimer surface groups in higher generation dendrimers. Modeling of water relaxivity using the Solomon-Bloemberg equations suggests that larger rotational correlation times ($\tau_r$) are responsible for the enhanced inner-sphere relaxivity of dendritic polynitroxides.

**Experimental Section**

**Materials.** Compound 3 was purchased from Aldrich and used as received. Compounds 1a, 1b, 2, and $4N$ ($N = 4, 16,$ and 64) were synthesized as has been reported previously in the literature.11,19

**Electron Spin Resonance.** ESR spectra were recorded on a Bruker ESP 300 spectrometer interfaced to a computer with Bruker ESP1600 system software.

**Relaxivity Measurements.** $T_1$ measurements were made on a 300 MHz Bruker NMR using a standard inversion recovery sequence (180°–t–90). Experiments were performed in methanol-$d_4$ and acetonitrile-$d_3$ at a concentration of ~0.085 M probe molecule (benzene or water) and specified concentration of nitroxide quencher.

**Results**

**ESR of Dinitroxides.** ESR data on dinitroxides 1a, 1b, and 2 are presented in Figure 1. Compound 1b exhibits a five-line ESR signal in both methanol and acetonitrile, which means that each electron is coupled to both nitrogens of the dinitrooxide. These ESR spectra have been attributed to fast conformational interconversion between conformers exhibiting weak ($J \sim 0$)
and strong (\( J \gg a \)) exchange interactions.\(^{19,21}\) Compound 1a, on the other hand, exhibits the three-line pattern typical of a TEMPO-type mononitroxide. In the case of 1a, the para arrangement of the nitroxide esters prevents their close approach as can be appreciated upon inspection of molecular models. Molecular mechanics calculations using the Materials Studio Software package and COMPASS force field estimate the average distance of the two nitroxide moieties to be roughly 13 Å. In the case of 1b, we estimate the average distance between the two nitroxide moieties in the minimized geometry to be roughly 9 Å; however, in the case of 1b, inspection of molecular models reveals that closer approach of the nitroxide moieties can be achieved through C−C and C−O single bond rotations. Compound 2 exhibits intermediate behavior which can be affected by solvent. In acetonitrile, a predominantly five-line pattern is present in the ESR spectra of 2. However, in methanol, the ESR spectrum shows a mostly three-line signal and some evidence of exchange based on the broad intervening signals.

Analogue ESR spectra have been observed in compounds of structure similar to 2, and temperature-dependent studies\(^ {26}\) as well as quantum mechanical calculations\(^ {20}\) are consistent with the hypothesis of 2 undergoing faster conformational interconversion in acetonitrile than in methanol.\(^ {19}\) The slower exchange in methanol could be due to the larger viscosity of methanol (viscosity at 25 °C = 0.544 cP) as compared to acetonitrile (viscosity at 25 °C = 0.369 cP),\(^ {27}\) or is due to hydrogen bonding in methanol.\(^ {11}\) The exchange rate for 2 in acetonitrile (>3.6 \times 10^4 s\(^{-1}\)) can be estimated to be approximately 20 times faster than that for methanol (>2 \times 10^4 s\(^{-1}\)) based on the changes observed in the ESR spectra.\(^ {20}\)

ESR of DAB-\(dendr-(NH-3-CO-PROXYL)-n\) dendrimers. ESR spectra for compounds 4, 4, 16, and 464 in methanol and acetonitrile are presented in Figure 2. Coupling of the free electrons to all 4 nitrogens in 44 is visible in acetonitrile, as is evidenced by the nine-line ESR signal. This result indicates fast exchange on the ESR time scale for this compound. The larger dendrimers show broadened signals as has been observed previously.\(^ {11}\)

**Outer-Sphere Relaxivity Measurements Using Benzene as a Probe.** To isolate outer-sphere relaxivity, we sought to avoid complex formation between the PRE and the probe molecule being relaxed, since complexation between probe molecule and relaxation enhancer introduces inner-sphere contributions to relaxivity.\(^ {2}\) Outer-sphere relaxivities measured using benzene as a probe are expected to provide an estimate of the outer-sphere contribution to water relaxivities (vide infra). The longitudinal relaxation time (\( T_1 \)) for the protons of benzene were measured as a function of nitroxide concentration using a standard inversion recovery experiment. Relaxivities were measured in acetonitrile and methanol, both good solvents for the dinitroxides and dendritic polynitroxides. The results of these measurements are presented in Figure 3 for the dinitroxides 1a and 1b and in Figure 4 for the dendrimeric polynitroxides 4N. The data are presented as Stern–Volmer plots; and slopes, measured by linear fit of the data, are the relaxivity for these species (in units of \( \text{M}^{-1} \text{s}^{-1} \)). The relaxivity data derived from these plots are presented in Tables 1 and 2. The ortho-substituted 1b exhibits a slightly higher relaxivity than the para-substituted 1a.

The results presented in Table 2 indicate an increase in the relaxivity on a per nitroxide basis as a function of dendrimer generation. This information is plotted in Figure 5 for both methanol and acetonitrile. The relaxivity/nitroxide roughly doubles for 464 (generation 5) as compared with the model compound 3 in both solvents, suggesting that the dendritic
framework enhances the outer-sphere relaxivity of these species. The relaxivity/nitroxide vs nitroxides/molecule data are fit using eqs 1–4 with interaction distance \( d \) decreasing with dendrimer generation in methanol and acetonitrile, respectively; (B and D) data fit with fixed interaction distance \( d \) in methanol and acetonitrile, respectively. See text for details.

### Table 3: 

<table>
<thead>
<tr>
<th>solvent</th>
<th>viscosity (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol-( d_3 ) (exchange rate)</td>
<td>( 5.47 \times 10^{-3} )</td>
</tr>
<tr>
<td>acetonitrile-( d_3 ) (exchange rate)</td>
<td>( 3.45 \times 10^{-3} )</td>
</tr>
</tbody>
</table>

### Table 2: Benzene Relaxivities at 300 MHz for 3, 4, 4, 16, and 4,64 As a Function of Solvent for \(^1\text{H}\) and \(^{13}\text{C}\)

<table>
<thead>
<tr>
<th>compound</th>
<th>solvent</th>
<th>relaxivity (^1\text{H}) (M(^{-1})s(^{-1}))</th>
<th>relaxivity (^{13}\text{C}) (M(^{-1})s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>acetonitrile-( d_3 )</td>
<td>46 ± 7</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>4</td>
<td>methanol-( d_3 )</td>
<td>68 ± 7</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>4</td>
<td>acetonitrile-( d_3 )</td>
<td>206 ± 6</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>4,64</td>
<td>methanol-( d_3 )</td>
<td>300 ± 15</td>
<td>75 ± 4</td>
</tr>
<tr>
<td>4,64</td>
<td>acetonitrile-( d_3 )</td>
<td>1000 ± 9</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>4,64</td>
<td>methanol-( d_4 )</td>
<td>1423 ± 14</td>
<td>89 ± 1</td>
</tr>
<tr>
<td>4,64</td>
<td>acetonitrile-( d_3 )</td>
<td>5200 ± 60</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>4,64</td>
<td>methanol-( d_4 )</td>
<td>7100 ± 100</td>
<td>111 ± 2</td>
</tr>
<tr>
<td>GdCl(_3)</td>
<td>methanol-( d_4 )</td>
<td>875 ± 11</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Water relaxivity measurements were performed in acetonitrile-\( d_3 \), using water as a probe and compounds 1a and 1b as PREs. The relaxation rates are presented in Figure 6, and the relaxivities are presented in Table 3. Note that within experimental error, the relaxivity measurements for the two isomeric dinitroxides 1a and 1b are equivalent.

Relaxation rates were measured for poly(propylene imine) dendrimers and model mononitroxide (3, 4, 4, 16, 4,64). The results for these relaxation rate measurements are presented in Figure 7. Water relaxivities are presented in Table 4. There is a dramatic increase of relaxivity from 154 to over 50 000 M\(^{-1}\)s\(^{-1}\) going from the mononitroxide 3 to the generation 5 dendrimer 4,64.

Water relaxivities are presented in Table 4. There is a dramatic increase of relaxivity from 154 to over 50 000 M\(^{-1}\)s\(^{-1}\) going from the mononitroxide 3 to the generation 5 dendrimer 4,64.

### Table 4: 

<table>
<thead>
<tr>
<th>compound</th>
<th>relaxivity (M(^{-1})s(^{-1})) per nitroxide relaxivity (M(^{-1})s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>154 ± 2</td>
</tr>
<tr>
<td>4</td>
<td>780 ± 10</td>
</tr>
<tr>
<td>4,64</td>
<td>5360 ± 80</td>
</tr>
<tr>
<td>4,64</td>
<td>50500 ± 4700</td>
</tr>
</tbody>
</table>

7. Water relaxivities are presented in Table 4. There is a dramatic increase of relaxivity from 154 to over 50 000 M\(^{-1}\)s\(^{-1}\) going from the mononitroxide 3 to the generation 5 dendrimer 4,64.

On a per nitroxide basis, this corresponds to a roughly 5-fold increase in relaxivity (see Table 4 and Figure 8). As can be seen in Figure 8, the relaxivity as measured using water as a probe behaves quite differently than that measured using benzene as a probe. Even for the monomeric nitroxide 3, relaxivity for water is 3.3 times greater than that for benzene.
Relaxivity Studies on Dendrimers

\[ T_1^{-1} = \frac{32\pi^2}{405}[\gamma_1^2\gamma_5^2\hbar^2S(S + 1)] \times \left[ \frac{N_A}{1000} \right] \frac{[J(\omega_S - \omega_1) + 3J(\omega_1) + 6J(\omega_S + \omega_1)]}{(1 + 5\lambda/8 + \lambda^2/8)} \] \]

In comparing compounds 1a and 1b, we see that nearly all the parameters for these two species are identical; both these isomers should exhibit similar diffusional properties. However, one major difference in these two species is their electronic exchange rates, as demonstrated by ESR studies (see Figure 1) which may manifest themselves in the relaxivity through the spin number \( S \) in eq 2. By using ESR, 1b exhibits the five-line pattern which indicates that 1b is in the strong exchange limit \( J \gg \alpha \).20,21,23 The ESR spectra 1b can be explained based on the interaction between the unpaired electrons of 1b and the two \(^{14}\text{N} \) \((I = 1)\) of the molecule. In the strong exchange limit, each electron interacts equally with each \(^{14}\text{N} \) nuclei, and so the multiplicity of the hyperfine lines is \( 2I + 1 \), where \( I = 2 \).21 In the case of 1a, the ESR spectra exhibit a three-line pattern indicative of negligible electronic exchange. Our data indicate a slight (~10%) increase in relaxivity for 1b as compared to 1a, which is lower than what might be expected if 1b were acting as a spin 1 species in relaxing the protons of benzene \( T_1^{-1}(S = 1)/[2T_1^{-1}(S = 1/2)] = 1.33 \). In the case of 1a, the two spin-1/2 contributions effectively increase the concentration of paramagnetic species to double that of a similar spin 1 species, hence the factor of 2 in the denominator. These results suggest that the exchange rate may not be fast enough, compared with \( \tau_D \) \((estimated to be \sim 10 – 20 \text{ ps})\), to have a significant effect on \( T_1^{-1} \) in these systems. Water relaxivity results \( \text{see Table 3 and Figure 6}\) also show no significant effect of exchange rate on relaxivity. The ESR spectra for compound 2, which shows fast exchange behavior in acetonitrile but slower exchange behavior in methanol \( \text{see Figure 1}\) can be used to estimate the exchange rate in this system to be close to the hyperfine frequency \( (2.2 \times 10^7 \text{ s}^{-1})\).19,20

In the case of dendrimers, 4a, we can further probe the relaxivity of polynitroxides as we increase the number of nitroxides per molecule. In the event that a high-spin ensemble is generated, we would expect dramatic increases in relaxivity on a per nitroxide basis due to the \( S(S + 1) \) dependence from eq 2. The ESR spectrum for 4a in acetonitrile \( \text{see Figure 2}\) exhibits a nine-line pattern \( (2I + 1 \text{ lines}, \text{with } I = 4)\), indicating strong exchange and suggesting the possibility of a \( S = 2 \) state. Higher generation dendrimers show broad ESR spectra, and splittings are no longer resolvable, but if the trend continues, the possibility exists that spin states higher than \( S = 2 \) could result. The maximum possible spin state for 4a would be \( S = 32 \). On the basis of our relaxivity measurements, this result was not observed, as seen in Figure 4 and Table 2. The relaxivity of 4a is only ~2 times greater than that of 3 on a per nitroxide basis, as opposed to the 22 times greater relaxivity \( \{T_1^{-1}(S = 32)/[64T_1^{-1}(S = 1/2)] = 22\} \) expected if all 64 nitroxides were exchanging on a time scale which was fast with respect to \( \tau_D \). Although this dramatic result is not observed, it is interesting to note that the per nitroxide relaxivity does increase as a
SCHEME 3: Determining Distance of Closest Approach (d in Eq 1) for (a) Small Molecule Case and (b) Dendrimer Case

![Diagram of Scheme 3](image)

The function of generation by roughly a factor of 2 going from 3 to 4₄ in both acetonitrile and methanol (see Figure 5 and Table 2).

This effect is not entirely understood, but some insight can be gained by modeling the experimental data (Figure 5) using the outer-sphere diffusional model (eqs 1–4). To model the relaxivity for the dendrimers, the dendrimer diffusion coefficients D_PRE must be determined. Using data for the radii (a) for the parent poly(propylene imine) dendrimers and eq 5, the values for D_PRE can be estimated ($k_{B}T/6\eta a$). Actual values for $a$ are expected to be slightly higher, since the nitroxide labels are expected to increase the size of the dendrimers.

$$D_{\text{PRE}} = \frac{k_{B}T}{6\eta a} \quad (5)$$

Using these estimated diffusion constants, the experimental data can be modeled and fit using a reasonable estimate for $d$ (the distance of closest approach; see eq 2). The estimated used for $d$ is 1.4 Å which has been used previously for nitroxides. These fits are presented as the dashed lines in Figure 5. Although the relaxivity does increase slightly based on the slower diffusion of the dendrimeric nitroxides, this effect is not sufficient to explain the experimental results.

As the dendrimer generation increases, the end groups in dendrimers are forced into closer and closer proximity, especially when secondary interactions such as hydrogen bonding between amides are present. For higher generation dendrimers, the periphery of the dendrimer may be presenting nitroxides in a more orderly fashion as a result of this crowding effect. The result might be a smaller effective radius of collision $d$ for the relaxation interaction in larger dendrimers. Scheme 3 illustrates this possibility. The dashed regions in Scheme 3 represent the 3-dimensional volume in which a front-side collision would occur with the correspondingly lower value of $d$. As can be seen from Scheme 3b, this volume would be a larger percentage of the molecule’s volume for the dendritic polynitroxides, than for the mononitroxide.

In the small molecule case, the distance of closest approach would be the average of collisions from the back and collisions from the front of the mononitroxide, with the distance being greater for collisions from the backside (Scheme 3a). In the dendrimer case, the likelihood of hitting the nitroxide on the front face is enhanced (see Scheme 3b), and thus the effective value of $d$ might be smaller. By systematically decreasing the value of $d$ from 1.4 Å for the model compound 3 to 1.06 Å for the generation 5 dendrimer 4₄₅, a better fit of the data is achieved for methanol (see solid line in Figure 5). Although this method of fitting the data is somewhat ad hoc, using the $d$ values obtained from methanol, this model can be used to account for the experimental data measured for acetonitrile, providing some generality to this hypothesis. (The only parameter that was changed going from methanol to acetonitrile is the solvent viscosity parameter $\eta$.) Although this represents a viable possibility to explain the results, more studies are required to fully understand this effect.

Enhanced relaxivities in gadolinium–chelate-based dendritic PRE’s are attributed to an increased rotational correlation time due to slower rotation of these macromolecular gadolinium chelates.10 This effect manifests itself when inner-sphere relaxation mechanisms are dominant, which is found to be the case when water relaxivity is considered (vide infra). However, this possibility for the benzene/polynitroxide system is considered unlikely based on the lack of strong interactions required to invoke an inner-sphere mechanism.

Another hypothesis to explain the enhanced relaxivity is the onset of faster electronic exchange in the sterically crowded larger dendrimers. In this hypothesis, the possibility exists that, in the larger generation dendrimers, subgroups of nitroxides are exchanging rapidly and thereby acting as high-spin ensembles. The data from compounds 1a and 1b suggest that, in the event of pairwise exchange, only a ~10% enhancement is expected. Also comparing 4 with the model 3 (see Table 2), we see only a ~10% enhancement of relaxivity on a per nitroxide basis, even though the ESR data (Figure 2) indicate fast exchange for 4. So although exchange effects may play a role in enhanced relaxivity in these dendrimers, the magnitude of exchange effects appears to be too small to explain the almost 2-fold increase in relaxivity in generation 5 over the model compound 3.

**Water Relaxivity for Dendritic Polynitroxides.** Measurement of water relaxivity was of interest due to its relevance to MRI applications. Water relaxivity is the sum of both inner-sphere and outer-sphere contributions. To properly model inner-sphere relaxivity, the outer-sphere contribution should be subtracted from the total measured relaxivity.

<table>
<thead>
<tr>
<th>compound</th>
<th>$r_{\text{TOT}}$ (M⁻¹ s⁻¹)</th>
<th>$r_{\text{OUTER}}$ (M⁻¹ s⁻¹)</th>
<th>$r_{\text{INNER}}$ (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>154 ± 2</td>
<td>55 ± 7</td>
<td>99 ± 9</td>
</tr>
<tr>
<td>4₄</td>
<td>195 ± 3</td>
<td>59 ± 2</td>
<td>136 ± 5</td>
</tr>
<tr>
<td>4₁₆</td>
<td>335 ± 5</td>
<td>71 ± 1</td>
<td>264 ± 6</td>
</tr>
<tr>
<td>4₆₄</td>
<td>786 ± 73</td>
<td>86 ± 1</td>
<td>700 ± 74</td>
</tr>
</tbody>
</table>

Subtracting these values from the measured water relaxivities
for these PREs ($r_{\text{TOTAL}}$), an estimate of the inner-sphere relaxivity contribution ($r_{\text{INNER}}$) is achieved, as reported in Table 5 and Figure 7.

The Solomon-Bloemberg equations (eqs 6–8) have been developed to model inner-sphere relaxivity. In general, two types of inner-sphere relaxations are possible, i.e., contact and dipole–dipole mechanism. However, the contact interaction is not important in the relaxivity of nitroxides. The dipolar relaxation mechanism is the dominant inner-sphere relaxation mechanism and the magnitude of relaxivity ($r_{\text{INNER}}$) is determined by eqs 6 and 7. \( g \) is the coordination number of the PRE, \( [C] \) is the molar concentration of the PRE, \( (1/T_{1P})_{\text{DIPROLE}} \) is the dipolar relaxation rate within the PRE–probe complex, \( r_m \) is the residence time of the probe in the complex, \( \gamma_1 \) is the gyromagnetic ratio for the nuclei being relaxed (\(^1_H \) in our case), \( g \) is the electronic g-factor, \( \mu_B \) is the Bohr magneton, \( r \) is the distance between the proton and the paramagnetic center in the complex, \( \omega_1 \) and \( \omega_S \) are the nuclear and electronic Larmor frequencies, and \( \tau_r \) is the global correlation time (see eq 8). \(^1\) \( ^2\)

\[
r_{\text{INNER}} = \frac{q}{[C](1/T_{1P})_{\text{DIPROLE}} + \tau_m)
\]

\[
\left(\frac{1}{T_{1P}}\right)_{\text{DIPROLE}} = \frac{2}{15} \left\{ \gamma_1^2 g^2 \mu_B^2 3(S + 1) r^6 \right\} \left[ \frac{3 \tau_c}{1 + \omega_1^2 \tau_c^2} + \frac{7 \tau_c}{1 + \omega_2^2 \tau_c^2} \right] \tag{7}
\]

The value of \( q \) is taken to be 1, which means one hydrogen bond can be formed to one nitroxide at a time. \(^3\) The global correlation time \( \tau_r \) takes into account all processes which modulate the inner-sphere dipolar interaction. These processes include electronic relaxation, chemical exchange in the complex, and rotation. The global correlation time is calculated from the correlation times for each of these processes as defined by eq 8. \(^1\) \( ^2\)

\[
\frac{1}{\tau_c} = \frac{1}{T_{1e}} + \frac{1}{\tau_m} + \frac{1}{\tau_r}
\]

The electronic relaxation time \( T_{1e} \) is slow for nitroxides and is on the order of 200 ns. \(^3\) This turns out to be several orders of magnitude larger than the chemical exchange correlation time \( \tau_m \) and the rotational correlation time \( \tau_r \), and thus contributes very little to the global correlation time \( \tau_c \). Although one may consider the possibility that the electronic relaxation time could decrease in the dendritic nitrolyoxides as a function of generation, this effect would be accompanied by a broadening in the ESR line width for these compounds. However, line broadening is not observed in comparison of 1a and 1b, and also in dendrimer 4a (see Figures 1 and 2). Furthermore, since the rotational correlation times are of the order of tens of picoseconds to a maximum of \( \sim 1 \) ns for 4a, a reduction of \( T_{1e} \) by about 2 orders of magnitude would be required in order for this correlation time to begin contributing significantly to the global correlation time \( \tau_c \). Although this possibility cannot be rigorously excluded at this point, the existing data and previous studies of di-, tri-, and tetranitroxides in the literature\(^19\) suggest that this possibility is unlikely.

Correlation times for chemical exchange of water at nitroxides have been estimated to be between 10 and 50 ns. \(^7\) Again these values are much larger than the values of \( \tau_r \) for small molecules, and are still an order of magnitude larger than the maximum value of \( \tau_r \) of \( \sim 1 \) ns for 4a (vide infra). Also the values

### Table 6: Radii and Calculated Rotational Correlation Times for Dendritic Nitrolyoxides

<table>
<thead>
<tr>
<th>Compound</th>
<th>$r_{\text{TOTAL}}$ ($\AA^{16}$)</th>
<th>$r_r$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.9 $\times$ 10$^{-11}$</td>
<td>119</td>
</tr>
<tr>
<td>4</td>
<td>3.2 $\times$ 10$^{-11}$</td>
<td>183</td>
</tr>
<tr>
<td>4$_6$</td>
<td>3 $\times$ 10$^{-10}$</td>
<td>1600</td>
</tr>
<tr>
<td>4$_6$</td>
<td>1 $\times$ 10$^{-9}$</td>
<td>4850</td>
</tr>
</tbody>
</table>

### Table 7: Radii and Calculated Rotational Correlation Times for Dendritic Nitrolyoxides (Parameters Used: $T_{1e}$ $= 200 \text{ ns}$; $r_m$ $= 10 \text{ ns}$; $R$ $= 2.5 \text{ Å}$)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$r_r$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>calcd</td>
<td>measd</td>
</tr>
</tbody>
</table>

\((1/T_{1P})_{\text{DIPROLE}}\) tend to be much greater than 10–50 ns. The result is that $r_{\text{INNER}}$ tends to be very weakly dependent on \( r_m \) at values which are reasonable for the chemical exchange correlation time.

The interaction distance \( r \) has been estimated through ESR and relaxivity measurements. \(^3\) For 2,2,6,6-tetramethyl-1-piperidinoloxo (TEMPO), this distance is measured to be 2.2 $\pm$ 0.3 Å. In fitting the experimental small molecule data, the distance dependence is quite strong due to the $r^6$ term in eq 7. The value for \( r \) was fit using the mononitroxide 3, and a value of 2.5 Å was found to best fit the data for this small molecule. This value was held constant in evaluating the dendritic effects on the assumption that water is a small molecule and steric crowding of end groups in the dendrimer should have a minimal effect on the water–nitroxide hydrogen bond distance.

The rotational correlation time \( \tau_r \) is by far the fastest of the three correlation times in eq 8 for small molecules. In methanol, \( \tau_r \) has been evaluated to be \( \sim 30 \) ps for a small molecule nitroxide. \(^3\) Adjusting for solvent viscosity, this value is estimated to be 19 ps in acetonitrile. For small molecules, the rotational correlation time dominates the global correlation time and hence has a strong influence on inner-sphere relaxivity as has been observed experimentally. \(^7\) This effect on the rotomyraxyl nitrolyoxides, the rotational correlation time is expected to increase.

Small-angle neutron-scattering studies have been used before to measure the size of poly(propylene imine) dendrimers. These radii are presented in Table 6, and they can be used to estimate the rotational correlation time for these molecules using eq 9 (\( a \) is the dendrimer radius, \( \eta \) is the solvent viscosity, \( k \) is Boltzmann’s constant, and \( T \) is the temperature). \(^1\) \( ^2\) The rotational correlation times calculated for acetonitrile (viscosity, \( \eta = 0.369 \) cP), \(^3\) are also presented in Table 6.

\[
\tau_r = \frac{4\pi a^3 \eta}{3kT}
\]

Using these values for \( \tau_r \), an estimate for the inner-sphere relaxivity can be calculated, and these estimates are presented in Table 7. As can be seen from Table 7, using the rotational correlation times derived from experimental measurements of dendrimer size does not give inner-sphere relaxivities that agree with experimentally measured values. Attempts to improve agreement between the model and experimental data using different values of \( r_m \), varying from 100 ps to 20 $\mu$s, failed (see Supplemental Information for details). The discrepancy between the experimental data and estimates derived using the experimental dendrimer radii argues against whole-dendrimer rotation being responsible for the relaxivity enhancements observed for the poly(propylene imine) dendritic nitrolyoxides.
Since the rotational correlation times calculated using the experimentally measured dendrimer radii do not correspond to measured relaxivity, it suggests that the rotational correlation time for the nitroxide in the dendrimer is not determined by the motion of the entire dendrimer molecule. An alternative possibility is that the nitroxide can rotate through segmental motion faster than through rotation of the entire dendrimer. If this is the case, an effective rotational correlation time which is faster than that for whole molecule rotation will result. Similar conclusions have been drawn for gadolinium-based dendritic PREs. Using the experimental data and assuming that changes in rotational correlation time are responsible for the change in inner-sphere relaxivity, one can calculate an effective rotational correlation time, and these are presented in Table 8.

As can be seen from Table 8, the increase in $r_e$ required to account for the experimental data is only a factor of 9 going from the mononitroxide 3 to the generation 5 $4_{64}$, which contrasts with the ~50 times increase in $r_e$ expected if $r_e$ reflected rotation of the entire dendrimer molecule. This suggests that if $r_e$ is the determining factor in the dendritic relaxivity increase, then the dendrimer structure is primarily increasing the segmental rotational correlation time of the terminal nitroxide groups, possibly through increased steric crowding in the larger dendrimers.

Other possibilities must also be considered. In all calculations performed thus far, the spin state of the nitroxide is assumed to be $1/2$, that is to say no high-spin states have been invoked to account for the data. However, with water as the probe, the residence time $r_{m}$ (10–50 ns)$^7$ is on the order of the electronic exchange time scale. This suggests the possibility that the water probe molecule could possibly experience relaxation from a high-spin state in the fast exchanging nitroxides.

The data, however, argue against this possibility. The water relaxivities of 1a and 1b are identical within experimental error. As was seen in the case of outer-sphere relaxivity, the exchange rate does not appear to be an important parameter. This further suggests that although electronic exchange occurs rapidly in the ortho isomer 1b, it does not imply the presence of high-spin ($S = 1$) states in any significant concentration.

As was the case with outer-sphere relaxivities, the measured $r_{INNER}$ values are too small to be consistent with high-spin species in the case of dendritic polynitroxides. Alternative to extremely high-spin states formed through exchange of as many as 64 nitroxide spins, the possibility exists for exchange among subgroups of spins in the dendritic polynitroxides. To consider this possibility, the spin state $S$ is calculated from the experimentally derived inner-sphere relaxivities. In these calculations, the rotational correlation time is held constant for the different generations of nitroxides and the entire relaxation enhancement is considered to be due to an increase in the effective spin $S$. These results are presented in Table 9.

This hypothesis offers an alternative explanation to that of rotational correlation time determining the inner-sphere relaxivity of dendritic polynitroxides. In this hypothesis, the polynitroxides exist in an ensemble of high and low spin states, which results in an effective spin $S$ that is greater than 0.5 for these polynitroxides. In the case of the generation 5 polynitroxide $4_{64}$, the effective spin required to fit the data is 1.65, suggesting that, on average, groups of roughly three nitroxides may interact to form high-spin states through strong exchange interactions.

Although the possibility exists that steric crowding of end groups could push the dendrimers into a regime of strong exchange and higher effective spin state, the results for the model dinitroxides 1a and 1b do not agree with this hypothesis, since no difference is observed in relaxivities of these two isomers regardless of the fact that ESR indicates 1b to be in fast exchange, whereas 1a shows a negligible exchange rate.

Ultimately, magnetic susceptibility measurements may answer the question of the effective spin state of these dendritic polynitroxides. However, the more likely cause of the enhanced inner-sphere relaxivities of these dendritic polynitroxides is that the rotational correlation time increases with increasing dendrimer generation.

Interestingly, in a previous study of dendrimer-linked polynitroxides in aqueous solution, the authors reported constant relaxivity on a per nitroxide basis. The apparent conflict between this result and the results of our study can be explained on the basis of known aggregation effects of nitroxide-labeled dendrimers in aqueous solution. Aggregation of nitroxide-labeled dendrimers could render some surface nitroxide groups inaccessible, and this is consistent with a small decrease (~10%) in relaxivity on a per nitroxide basis observed for generation 4 dendrimers as compared to model compounds in this previous aqueous study.

**Conclusions**

Fully nitroxide functionalized poly(propylene imine) dendrimers show higher outer-sphere relaxivities on a per nitroxide basis than do model monomeric nitroxides as measured using the probe molecule, benzene, in both acetonitrile and methanol. The relaxivity is observed to increase as a function of dendrimer generation. This enhancement may be related to the congestion of dendrimer end groups in higher generation dendrimers permitting a more ordered presentation of nitroxide ends on the periphery of the dendrimer. This crowding may facilitate collisions between our probe benzene molecules and the active nitroxide end of the PROXYL moiety. In the small molecule case, the potential exists for less productive collisions with the alkyl ring back portion of the PROXYL moiety. The greater likelihood of front face collisions in the larger dendrimers would be expected to lower the effective distance of closest approach $d$ (see eq 1) and thereby enhance relaxivity. Although this is a viable hypothesis as demonstrated by modeling experimental data in two solvents, other possibilities may contribute.

Water relaxivity for these PREs has been measured in acetonitrile. Although eventual applications are more likely to be in an aqueous environment, solubility and aggregation issues complicate the study of the basic relaxivity parameters of the dendritic polynitroxides $4_{64}$ in aqueous solution. The dendrimeric
polynitroxides have been shown to aggregate in aqueous solution,\textsuperscript{14} and these aggregation effects obscure\textsuperscript{12} any correlation between measured relaxivity and the parameters we sought to probe.

Water relaxivity measurements show even more dramatic enhancements of relaxivity as a function of dendrimer generation than do measurements with benzene. Significant inner-sphere contributions to relaxivity are responsible for enhanced water relaxivity. The inner-sphere contribution to relaxivity has been modeled using the Solomon–Bloemberg equations, and slower rotational correlation times \( \tau_s \) in the larger dendritic polynitroxides appears to be responsible for larger per nitroxide relaxivities. The increase in \( \tau_s \) appears to be due to a reduction in segmental motion in higher generation dendrimers rather than to global rotation of the dendrimer. This conclusion is analogous to findings for gadolinium-based dendritic PREs.\textsuperscript{30}

The evaluation of compounds \( 1a \) and \( 1b \) indicates that both inner- and outer-sphere relaxivities are not strongly dependent on exchange rate in these dinitroxides.

The higher generation polynitroxide dendrimers exhibit relaxivities exceeding those for gadolinium(III) chloride and thus represent an exciting class of potential paramagnetic relaxation enhancers for application in the magnetic resonance imaging area. Furthermore, chemical modification of the substrate dendrimer could possibly overcome problems associated with aqueous phase aggregation and in addition open up the possibility of targeting specific tissues or organs in imaging applications. Finally, although enzymatic processes for reductive degradation of nitroxides reduce their effectiveness in vivo, the steric constraints of a dendritic polynitroxide framework have been shown to render these species less active toward enzymatic destruction, thereby increasing their effective lifetimes in vivo.\textsuperscript{12}

In summary, the advantages of polynitroxide dendrimers (i.e., high relaxivity, low toxicity, and potential for specific targeting) make them promising candidates for further study as paramagnetic relaxation enhancers for magnetic resonance imaging applications. However, the significant challenge of removing aqueous phase aggregation is yet to be overcome.

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Supporting Information Available: Text discussing the calculation of inner-sphere relaxivities using Solomon–Bloemberg equations, \( r_k \) as calculated from experimentally measured dendrimer radii, and varying \( r_{\text{s2}} \) between 100 ps and 19 \( \mu \text{s} \) and two figures plotting these calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


