

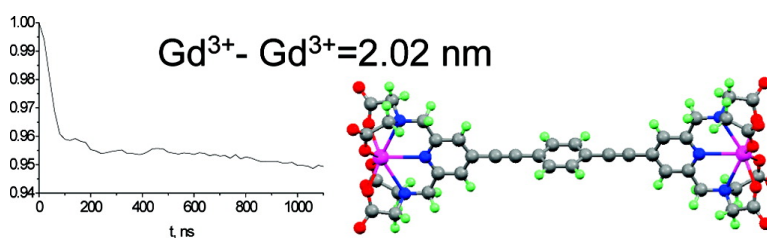
Communication

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Gd³⁺ Complexes as Potential Spin Labels for High Field Pulsed EPR Distance Measurements

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Distance measurements between spin labels by means of pulse EPR have attracted considerable attention because of the applications to biological systems.^{1,2} These determine the dipolar interaction between two electron spins, which for two $S = 1/2$ spins is

$$\nu_{\text{DD}}(\theta, r) = \frac{g_1 g_2 \beta^2 \mu_0}{4\pi h r^3} (3 \cos^2 \theta - 1) \quad (1)$$

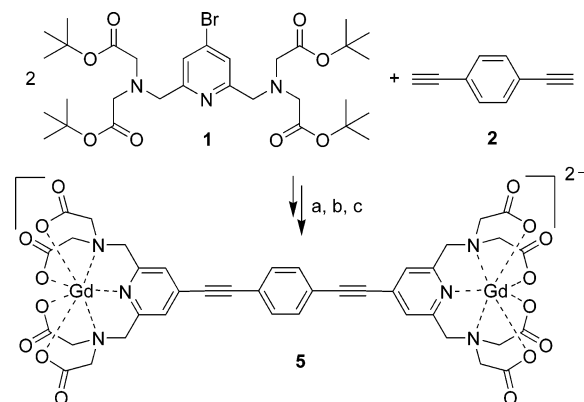
In eq 1, g_1 and g_2 are the g values, r is the interspin distance, and θ is the angle between r and the external magnetic field, B_0 . The accessible distance range, 1.5–6.0 nm, allows one to obtain structural information on biomolecules such as proteins, DNA and RNA, and their complexes.^{3,4} Two nitroxide spin labels (NSL) are introduced in the biomolecule, and the measurements are usually carried out at X-band frequencies (~ 9.5 GHz) on samples of ~ 50 μL of 0.1–0.2 mM solutions (~ 12 h accumulation time). Distance measurements, however, are not limited to organic radicals, and measurements involving metal ions were reported as well.^{5–7}

In principle, high field (HF)/high microwave frequency (HM-WF)^{8,9} measurements can offer improvement in sensitivity over X-band. However, due to the g anisotropy, the width of the NSL EPR spectrum increases at HF, leading to a decrease in the spectral density and of the *flip probability* of the pumped spins. Therefore, excluding cases where the relative orientation of the g frames of the NSLs is of prime interest, HF measurements provide only moderate gain (if any).

For HF distance measurements, a different class of spin labels, the spectrum of which *narrows* with increasing B_0 , such as high spin half integer systems (HSS) like Gd³⁺ ($S = 7/2$) may be attractive. While the width of the entire spectrum of these ions is determined by the crystal field interaction (cfi), which is magnetic field independent, the sub-spectrum of the central $| -1/2 \rangle \rightarrow | 1/2 \rangle$ transition (CT) narrows as B_0 increases. Here we present first results on the synthesis and pulse double electron–electron resonance (DEER) measurements between two Gd³⁺ ions connected via a rigid linker, shown in Scheme 1.

The bis-Gd³⁺ complex comprises two units of a pyridine-based tetracarboxylate gadolinium complex, developed as an MRI contrast agent.¹⁰ Double Sonogashira coupling of the pyridine tetracarboxylate ester **1** and 1,4-diethynylbenzene (**2**) provided the corresponding octa-ester¹¹ (Scheme 1). Deprotection of the octa-ester¹¹ and subsequent complexation of the octa-acid with excess of an aqueous solution of GdCl₃·3H₂O gave the desired bis-Gd³⁺ complex **5** in 86% yield. The paramagnetic complex **5** was characterized by IR, MS (ESI), HRMS, and magnetic moment ($\mu = 15.63(2) \mu_B$) analyses.¹¹

Scheme 1. Synthesis of the Bis-Gd³⁺ Complex^a



^a Conditions: (a) *trans*-PdCl₂(PPh₃)₂, CuI, dry ²Pr₂NH, rt, 40 h, 67%; (b) CF₃CO₂H/CH₂Cl₂, rt, 12 h, 82%; (c) pH 5–7, GdCl₃·3H₂O, rt, 5 h, 86%.

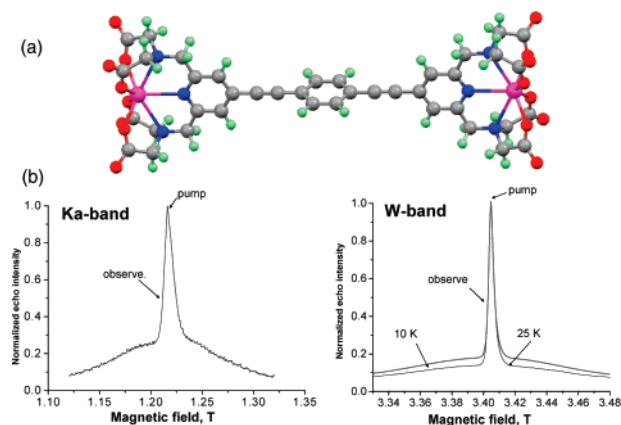


Figure 1. (a) The geometry of the bis-Gd³⁺ complex with a total charge of -2 and 14 unpaired electrons, optimized at the PBE0/sdd level of theory with D_{2h} symmetry constrain.¹¹ (b) Two-pulse ED-EPR spectra of the bis-Gd³⁺ complex recorded at Ka-band (~ 1.5 mM, 13 K), W-band (0.2 mM, 10, 25 K). The positions of the pump and observed pulses are shown on the figure.

The DEER measurements were carried out at Ka- (33.78 and 29.6 GHz) and W- (94.9 GHz) bands.^{12,13} The corresponding echo-detected (ED) EPR spectra are shown in Figure 1b. The width of the W-band CT is about 0.4 of that at Ka-band, as expected.¹⁴

The broad background the CT is superimposed on is due to all other transitions, and their relative intensities are a function of the temperature and spectrometer frequency. The relative populations, $P(M_S)$, of the $M_S = \pm 1/2$ energy levels for the frequencies and temperatures at which the experiments were carried out are listed in Table 1. The four-pulse,¹⁵ time domain DEER traces, shown in Figure 2a, exhibit a steep initial decay and shallow but clear

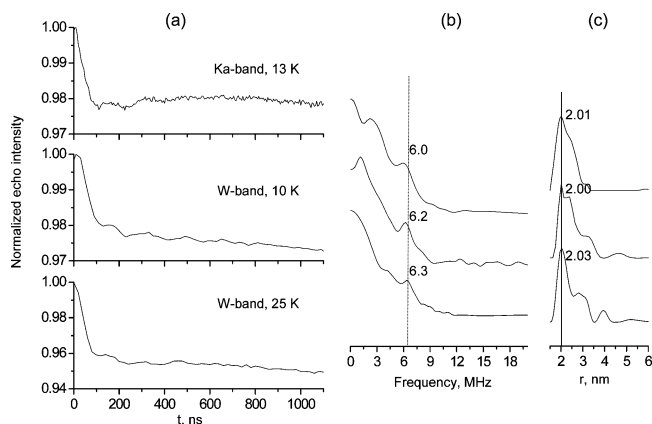
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Table 1. The Relative Populations of the $M_S = \pm 1/2$ levels and the DEER Modulation Depth, $\lambda_{\text{ef}} = \lambda[P(1/2) + P(-1/2)]$

| | $P(-1/2)$ | $P(1/2)$ | λ | λ_{ef} |
|-----------------|-----------|----------|-----------|-----------------------|
| 33.78 GHz, 13 K | 0.128 | 0.113 | 0.19 | 4.6% |
| 94.9 GHz, 10 K | 0.096 | 0.061 | 0.36 | 5.6% |
| 94.9 GHz, 25 K | 0.126 | 0.105 | 0.36 | 8.3% |

**Figure 2.** (a) The four-pulse DEER traces of the bis-Gd³⁺ complex recorded at (top) Ka-band, 13 K; data shown are after removal of the background (observer pulse 15/25/25 ns, pump 12 ns, $\Delta\nu = 160$ MHz; see Figure S1), (middle) W-band, 10 K (observer pulse 20/40/40 ns, pump 16 ns, $\Delta\nu = 83$ MHz, accumulation time 5 h), (bottom) W-band, 25 K (observer pulse 16/32/32 ns, pump 16 ns, $\Delta\nu = 83$ MHz, accumulation time 9 h). (b) Fourier transform of the time domain traces after background removal. (c) The corresponding distance distributions. Peak positions are indicated on the figure.

modulations. This behavior is characteristic of a short distance with some distribution. The corresponding magnitude real Fourier transforms (FT) are shown in Figure 2b, and the distance distributions obtained using DeerAnalysis2006¹⁶ are shown in Figure 2c. All FT spectra show a peak at 6.0–6.4 MHz, which corresponds to a distance of 2–2.05 nm. The distance was derived from eq 1, neglecting exchange interaction and cfi effects on ν_{DD} . The latter depends on ratio of the cfi parameter D and B_0 . For $D \sim 37$ mT (see simulations in Supporting Information (SI) and Figure S3), the deviation of ν_{DD} from the nominal one was found minor even at Ka-band.^{11,17} Density functional theory (DFT) calculations carried out for bis-Gd³⁺ complex **5** (Figure 1a) gave $r = 2.213$ nm.¹¹ Calculations performed on the monocomplex (Figure S2) showed that solvation and counterion effects should decrease r and increase its distribution by relaxing the D_{2h} symmetry constrain used, in agreement with the experimental results.¹¹

In addition to the clear peak at ~ 6.2 MHz, all FT spectra show low-frequency features, which we attribute primarily to artifacts due to some uncertainty in the background removal. The shallow modulation depth and the fast initial decay characteristic of short distances make the shape of the FT spectrum highly susceptible to small variations in background removal. The possibility that the low-frequency features are also related to some intrinsic properties of the HSS, however, cannot be excluded and needs further investigation.

Figure 2a shows that a deeper modulation and a narrower distance distribution around 2 nm are observed at W-band, 25 K, where the contributions of the background signal from the other transitions are the smallest and the intensity of the central transition is the highest. Hence, a higher frequency and a higher temperature are desired while performing distance measurements on Gd³⁺ pairs.

Besides the modulation frequency, ν_{DD} , the DEER traces are characterized by modulation depth λ which for $S = 1/2$ is the flip probability of the “pumped” spins. The observed modulation depth, λ_{ef} , as seen from Figure 2 is smaller than would be expected for S

$= 1/2$. This is a consequence of the selection of the CT which excludes the detection of all pairs with $M_S = \pm 1/2$ for one Gd³⁺ but $|M_S| > 1/2$ for the other. Therefore, λ_{ef} depends on λ , determined from the sub-spectrum of the CT, and the populations of the $\pm[1/2]$ states, $P(\pm 1/2)$. The estimated λ ¹¹ and λ_{ef} values are listed in Table 1. The experimental values are nonetheless smaller by a factor of about 2. This could be due to some distortion of the initial part of the decay caused by the finite duration of the pumping pulse and B_1 inhomogeneity along the sample.

To conclude, this work shows that Gd³⁺ complexes have a potential of being used as spin labels for HF distance measurements in biological systems, provided that suitable labeling techniques, similar to those used for NSL, are developed. They feature high sensitivity, requiring only 2 μL of 0.1 mM solutions, affording high effective B_1 values, allowing for very high repetition rates due to short spin–lattice relaxation times (< 300 μs), and have negligible orientation selectivity. More work is certainly needed to optimize the modulation depth and to understand the exact contribution of the transitions other than the CT.

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Supporting Information Available: Simulations of the Ka- and W-band ED-EPR spectra and brief discussion of its effect of cfi on ν_{DD} , description of the calculations of the modulation depth, detailed synthesis and characterization of the bis-Gd³⁺ complex, and results of DFT calculations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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