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TmDOTA$^-$: A Sensitive Probe for MR Thermometry *in Vivo*

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The lanthanide complex, thulium 1,4,7,10-tetraazacyclododecane–1,4,7,10-tetraacetic acid (TmDOTA$^-$), has been investigated as an agent for MR thermometry *in vivo***. The chemical shifts of the TmDOTA$^-$ protons were highly sensitive to temperature at a clinically relevant field strength, yet insensitive to pH and the presence of Ca^{2+}. Given the excellent stability of lanthanide–DOTA complexes and high thermal sensitivity, TmDOTA$^-$ is expected to be a good candidate for MR thermometry** *in vivo***.** © 2001 Academic Press

INTRODUCTION

MR methods that measure temperature *in vivo* have been the subject of active investigation in recent years due to the potential of hyper- and hypothermia-related medical procedures (*1–9*). While MR thermal mapping techniques based on the endogenous water proton chemical shift or relaxivity have shown promise for applications such as laser surgery or RF ablation, their application in hyperthermia procedures has been limited due to the high thermal resolution requirement ($\sim\pm0.5^\circ$C), the weak thermal sensitivity of water proton chemical shift ($\sim$0.008 ppm/$^\circ$C), and other factors that degrade the MR phase map (*1, 2, 10*).

Paramagnetic lanthanide complexes have recently been investigated as potential exogenous MR thermometric probes for hyperthermia due to their stronger temperature-dependent chemical shifts (*10–14*). For example, the temperature and pH sensitivity of the proton and phosphorus nuclei of TmDOTP^{5-} allow a simultaneous measure of both parameters (*14*), information valuable in cancer research and treatment (*15–17*). Although the addition of adjuvant Ca^{2+} does not alter the temperature sensitivity of these nuclei (*14, 18, 19*), binding of Ca^{2+} to this highly charged complex could potentially limit its *in vivo* use.

1,4,7,10-Tetraazacyclododecane–*N*,*N*′-*N*″,*N*‴-tetraacetic acid (DOTA) is known to form inert and stable complexes with lanthanide cations (*20–22*). Studies have shown that GdDOTA$^-$, a widely used clinical MR contrast agent, does not alter the concentration of metal ions in plasma (*21*). To take advantage of the large thermal sensitivity of Tm^{3+}, the smaller charge on the LnDOTA$^-$ versus LnDOTP^{5-} complexes, and the proven safety of GdDOTA$^-$ *in vivo*, we have been evaluating the suitability of TmDOTA$^-$ for NMR thermometry *in vivo*. In this report, we demonstrate proof of concept by showing that the chemical shift of the TmDOTA$^-$ protons has a large temperature coefficient that is insensitive to pH and the presence of Ca^{2+}. Our initial results in phantoms and animals indicate that TmDOTA$^-$ is a promising MR thermometric agent for *in vivo* use.

MATERIALS AND METHODS

(1) Preparation of TmDOTA$^-$

TmDOTA$^-$ was synthesized from Tm_2O_3 (Sigma Chemical Co., Milwaukee, WI, USA) and DOTA (Macrocyclics, Dallas, TX, USA) using standard procedures. After conversion of Tm_2O_3 to $TmCl_3$ and standardization, an equivalent amount of Tm^{3+} was added to a solution of DOTA at pH 10. This was warmed to $\sim$80°C and stirred for $\sim$3 days to assure complete complexation. The resulting mixture was added to a cation exchange column (Bio-Rex 70, sodium form) to remove any excess unreacted Tm^{3+} from the complex. Pure TmDOTA$^-$ was eluted from the column using distilled water, concentrated, and filtered just prior to use *in vivo*.

(2) NMR Measurements

(a) Phantoms. A 12 mM TmDOTA$^-$ aqueous solution (concentration was estimated from the amount of DOTA consumed in the complexation reaction) was used in the phantom study. All MR measurements were performed on a 2-T animal system (Bruker Instruments, Billerica, MA) using a 2.5-cm surface coil. The temperature dependence of the proton chemical shifts of TmDOTA$^-$ was measured during cooling of an aqueous sample of TmDOTA$^-$ from 45°C to room temperature. The temperature of the phantom was monitored with a copper-constantan thermocouple (TT-T-36, Omega Eng. Inc., Stamford, CT). Proton free induction decays (FID) were selectively collected using a 1-ms Gaussian pulse, a repetition time (TR) of 60 ms, 256 signal averages, and a spectral bandwidth of 10 kHz. The TmDOTA$^-$ proton chemical shifts were referenced to bulk water set to 0 ppm. Water suppression was used for data

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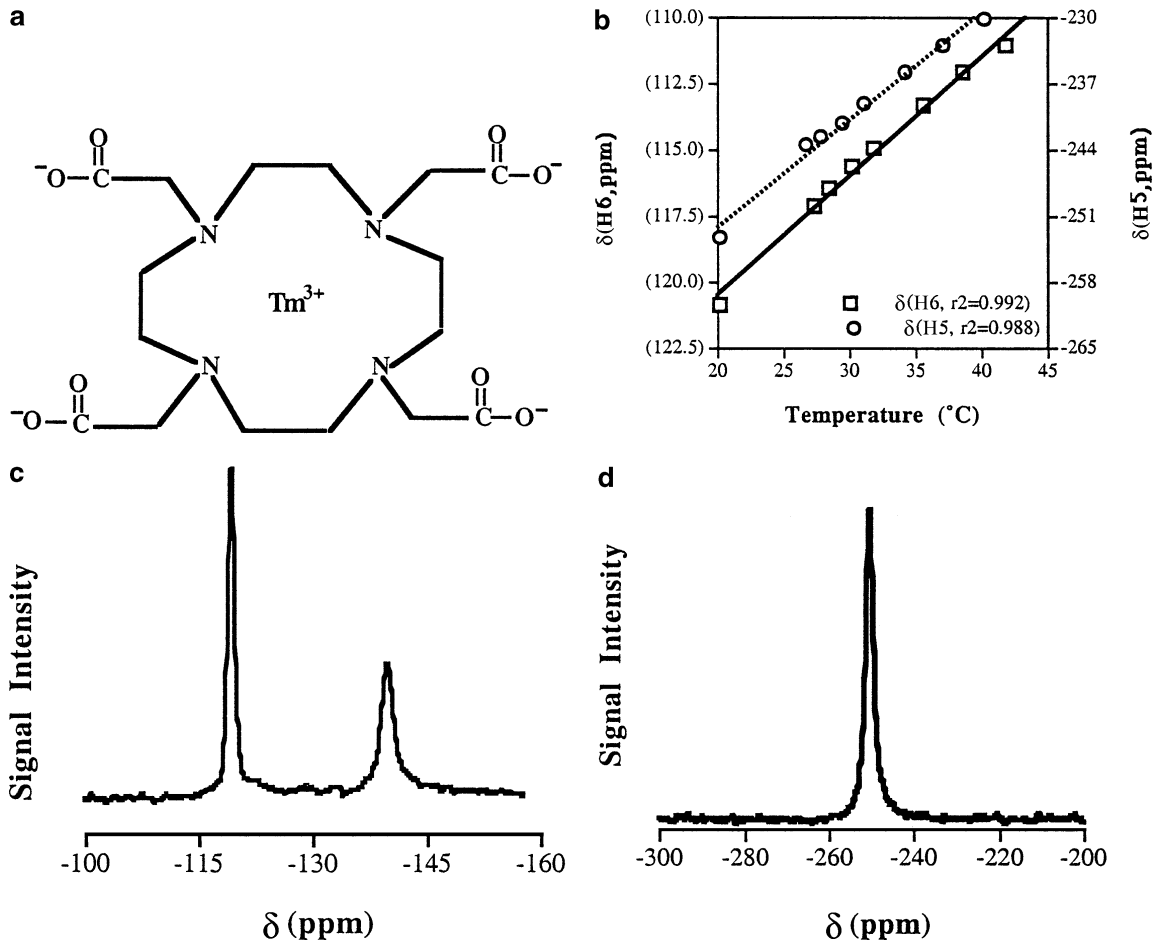

FIG. 1. Molecular structure of $TmDOTA^-$ and portions of proton spectra of $TmDOTA^-$. (a) Structure of $TmDOTA^-$ contains six nonequivalent protons (H1–H6). H1–H4 correspond to ethylene protons, and H5 and H6 correspond to methylene–acetate protons. (b) Chemical shifts of H5 and H6 in $TmDOTA^-$ versus temperature at 2 T. (c) $TmDOTA^-$ H1 and H6 signals in plasma acquired at 2 T at room temperature. (d) $TmDOTA^-$ H5 signal in plasma acquired at 2 T at room temperature.

acquisition of the H2 and H3 signals approximately 5 kHz away from water resonance (Fig. 1) (*23*).

The chemical shifts of the protons in $TmDOTA^-$ were measured at different pH values from pH 5 to 9 at room temperature to determine their chemical shift pH dependencies. The solution pH value was measured with an AR 15 pH meter (Fisher Scientific Co, Pittsburgh, PA, USA) and adjusted with HCl and NaOH. The measurement accuracy of the pH meter is approximately ± 0.1 pH unit.

To determine if Ca^{2+} alters the chemical shift of the $TmDOTA^-$ protons, a 50 mM $CaCl_2$ solution was used to increase the Ca^{2+} concentration in the $TmDOTA^-$ phantom from 0 to approximately 10 mM.

The chemical shifts of the $TmDOTA^-$ protons were measured in bovine plasma at room temperature (23°C) (Sigma Chemical Co.) to see if the chemical shifts of $TmDOTA^-$ protons were affected by the presence of macromolecules in plasma. The chemical shifts of the $TmDOTA^-$ protons were measured weekly up to 2 months post-synthesis to document the stability of $TmDOTA^-$. To investigate the feasibility of imaging one or more of those protons, a $TmDOTA^-$ phantom was imaged using a 3D gradient echo sequence in conjunction with selective RF excitation. Other imaging parameters included TE/TR = 3.6 ms/50 ms, field of view (FOV) 6 × 6 cm, acquisition matrix 32 × 32 × 32, and number of excitations (NEX) 16.

(b) Animals. *In vivo* measurements were also performed on the 2T system in three healthy rats (body weight approximately 250 g). After the rats were anesthetized with halothane vapor, a copper-constantan thermocouple and an 18-gauge plastic tube were implanted beneath the abdominal muscle. The readings of the thermocouple were obtained using an Omega thermometer (DT41, Omega Eng. Inc., Stamford, CT) with a measurement accuracy of ±0.5°C. Approximately 2 cc of ~12 mM $TmDOTA^-$ in normal saline was injected intraperitoneally into the abdominal cavity via the plastic tube after the rat was placed

into the magnet. A 2.5-cm surface coil was positioned above the abdomen and proton signals of TmDOTA$^-$ were selectively acquired (1024 averages) after the themocouple readings were stabilized. A small bag filled with cold or warm water was used to change the temperature in the abdominal region.

RESULTS

(1) Phantom Studies

The high-resolution ^{1}H NMR spectrum of TmDOTA$^-$ appears largely as six resonances due to the fourfold symmetry of the complex (Fig. 1a, H1–H4 correspond to the ethylene protons, H5 and H6 correspond to the acetate protons (*29*), similar to the assignment of TmDOTP^{5-} (*23*)). Although the LnDOTA$^-$ complexes are known to exist as a mixture of two coordination isomers (*28*), only one dominates the spectrum of TmDOTA$^-$. The chemical shifts of the protons in TmDOTA$^-$ ranged from -247 to $+382$ ppm at room temperature referenced with the water resonance frequency (Table 1). The protons of TmDOTA$^-$ had strong chemical shift temperature dependencies ranging from -1.4 to 1.1 ppm/°C (see Table 1, Fig. 1b). The resonace areas were approximately 1 : 1 : 1 for H4, H5, and H6, and 0.9 : 0.8 : 0.8 for H1, H2, and H3. The deviation from unity of H1, H2, and H3 was likely due to integrated signal loss in the broader wings near the base line (Figs. 1c, 1d).

At room temperature, the chemical shifts of protons H1, H2, H3, and H6 did not show noticeable pH dependency ($\sim$0.1 ppm/pH unit) over the physiological pH range (pH 6–9; Table 1) within the accuracy of our measuring devices (pH detection, $\pm$0.1 pH unit; frequency detection, $\pm$0.1 ppm; temperature, $\pm$0.5°C). The H4 and H5 resonances did display a weak pH dependence over this same range (0.1–0.2 ppm/pH unit). The proton chemical shifts were not sensitive to addition of Ca^{2+} nor was any precipitation of a Ca^{2+} complex detected (Table 1). This is consistent with previous observations that GdDOTA$^-$ does not form complexes with Ca^{2+} or Mg^{2+} (*21, 24*). Furthermore, the ^{1}H spectrum of TmDOTA$^-$ was unaffected by the addition of saline or bovine plasma (Table 1).

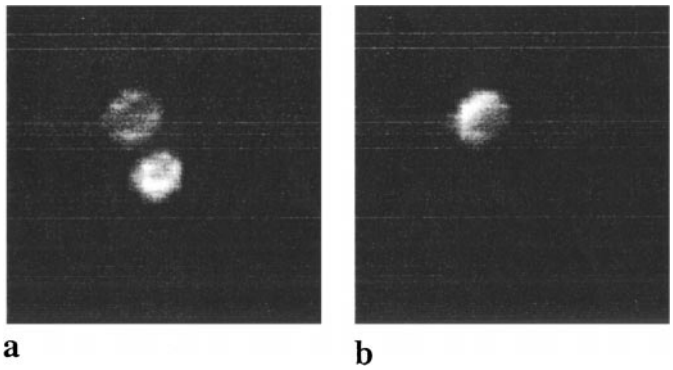

FIG. 2. Gradient echo images of a TmDOTA$^-$ phantom and a saline phantom acquired on a 2-T animal system using a nonselective hard pulse at water frequency (a) and a 1-ms Gaussian RF pulse at the resonance frequency of H5 in TmDOTA$^-$. (b) The region-of-interest (ROI) signal ratio of the TmDOTA$^-$ phantom to the saline was approximately 37 : 1 with the 1-ms Gaussian pulse selective excitation.

Figure 2 shows gradient echo images of a TmDOTA$^-$ aqueous phantom and a saline phantom acquired with (a) a nonselective hard pulse at the water frequency or (b) a 1-ms Gaussian RF pulse at the resonance frequency of the TmDOTA$^-$ H5 resonance. Comparison of (a) with (b) indicates that selective excitation at the H5 resonance alone may be sufficient for TmDOTA$^-$ imaging. The mean signal intensity ratio of the TmDOTA$^-$ phantom to that of the saline in (b) was approximately 37 : 1 with a 1-ms Gaussian pulse selective excitation.

(2) Animal Studies

Spectra of individual TmDOTA$^-$ protons were acquired over a temperature range of 23 to 30°C by selective excitation using a 1-ms Gaussian pulse. One minute of signal averaging yielded *in vivo* spectra with SNR of $\sim$34 for H5 and H6 (Figs. 3a, 3b). The resonances appeared to be broadened due to a distribution of temperatures in the animal, especially when the temperature in the region of interest was much higher or lower than the animal

TABLE 1
Chemical Shifts of Protons in TmDOTA$^-$ and Their Sensitivities to Temperature, Ca^{2+}, and pH

	δ (ppm)							
H*n*	Day 1	Day 7	Pre-Ca^{2+}	Post Ca^{2+}	Preplasma[a]	Postplasma[a]	C_{pH} (ppm/unit)	C_T (ppm/°C)
H1	-140	-139.9	-141.1	-140.9	-139.8	-139.8	—	0.50
H2	59.2	59.3	59.6	59.7	59.4	59.3	—	-0.20
H3	44.8	44.1	44.7	44.4	44.6	44.7	—	-0.27
H4	375.8	376.7	377	377	373.2	374.8	$+0.2$	-1.45
H5	-250.6	-251.8	-252	-252.1	-249.2	-249.3	-0.1	1.13
H6	-119.7	-119.7	-120.4	-120.3	-119.3	-119.3	—	0.45

Note. Shifts are relative to water resonance at 0 ppm for experimental convenience. All measurements were performed at 2*T* at room temperature ($\sim$21°C) unless specified otherwise (see Footnote *a*).

[a] Measurements were performed at 23°C.

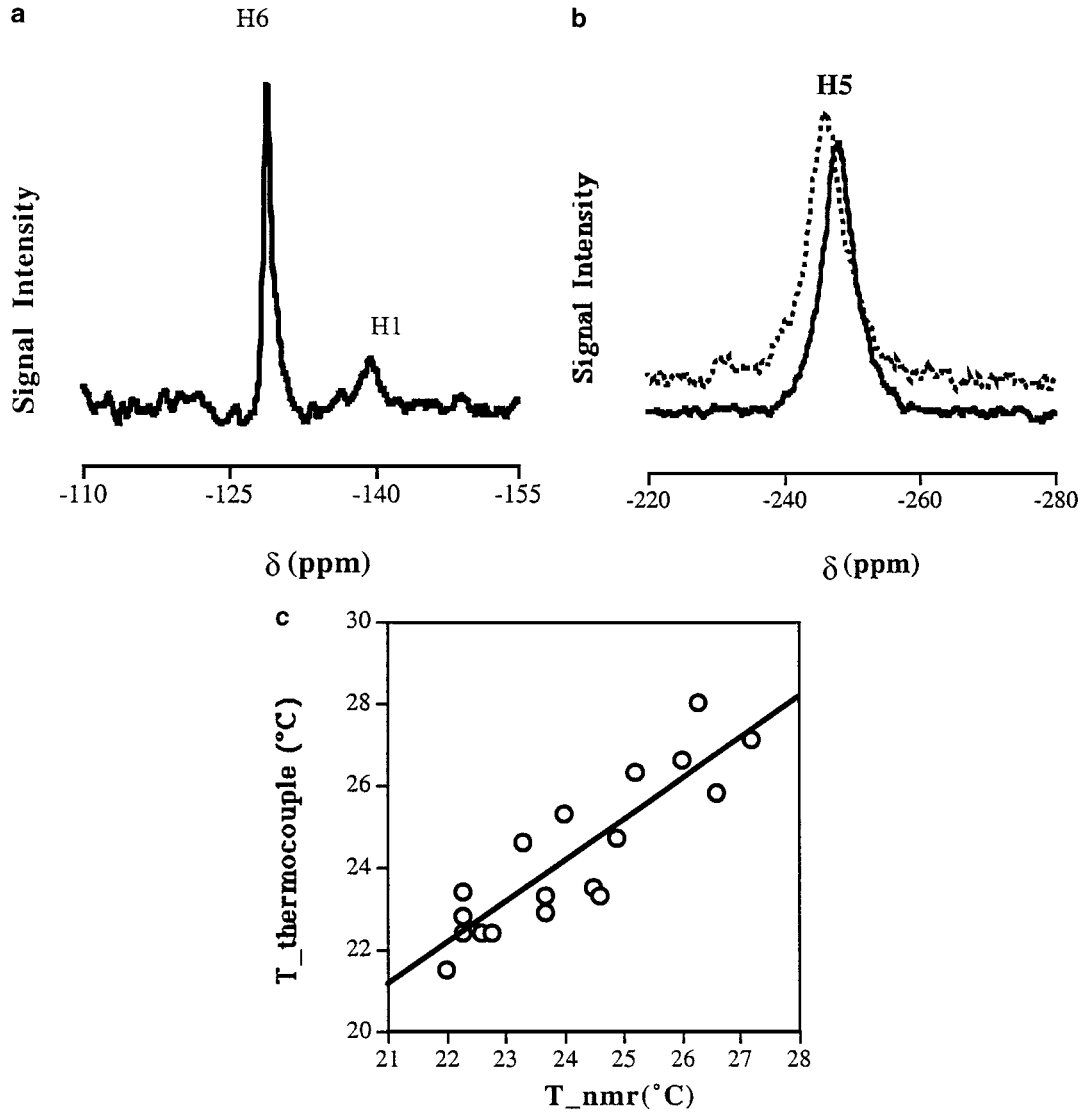

FIG. 3. *In vivo* proton spectra of H1, H5, and H6 (a,b) acquired at 2 T and the correlation between the TmDOTA NMR scheme (T_nmr) and the copper-constantan thermocouple (T_thermocouple) measurements in animals (c). (a) H1 and H6 signals acquired at 2 T at 24°C. The excitation pulse was centered closer to H6 resonance frequency. (b) H5 signal acquired using a 1-ms Gaussian pulse centering at its resonance frequency at 26 (dashed line) and 23°C (solid line). (c) Thermocouple readings versus temperature values based on $TmDOTA^-$ NMR scheme. Measurement reading errors were ±0.5°C for the thermocouple and approximately ±0.3°C for the NMR scheme in the animal experiments. T_thermocouple = 0.999T_nmr + 0.151. $r^2 = 0.78$.

body temperature. The resonance frequency of three different $TmDOTA^-$ protons (each excited separately using a selective pulse) was measured as a function of temperature and plotted versus temperature as sensed by a thermocouple (Fig. 3c).

DISCUSSION

The ideal MR thermometric probe should have an NMR-active nucleus with a chemical shift or relaxation rate that is highly temperature sensitive over the temperature range of interest, should be chemically inert *in vivo*, and is capable of providing absolute temperature measurements. The protons of $TmDOTA^-$ have near ideal characteristics; they have chemical shift temperature dependencies ranging from −1.4 to 1.1 ppm/°C, considerably larger than other paramagnetic complexes proposed for thermometric measurements (*11, 12, 25*) and approximately two orders of magnitude more sensitive than the chemical shift of water protons (*2*). With the larger chemical shift temperature dependence of this complex, it may be possible to measure small differences in local tissue temperatures. For instance, the scattered distribution of *in vivo* data shown in Fig. 3c was likely due to the temperature discrepancy between the location of the thermocouple and the region of the NMR measurement during the experiment (*12*).

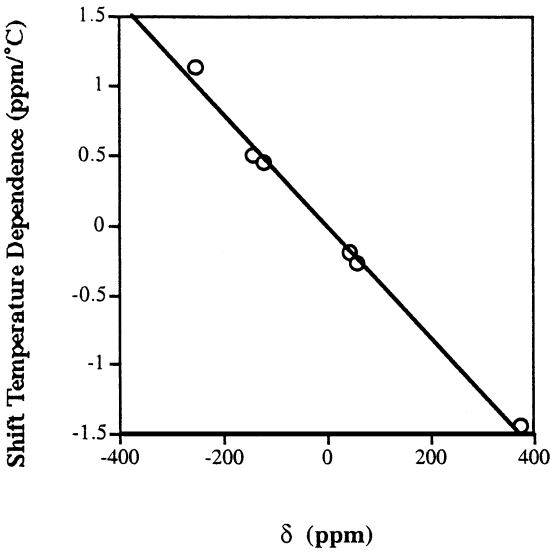

FIG. 4. Chemical shift of TmDOTA$^-$ protons (δ) at room temperature ($\sim$21°C) versus their shift temperature dependence (C_T). The data in the 2nd and 9th columns of Table 1 were used in this plot. The slope and intercept are -0.00401/°C and -0.00600 ppm/°C, respectively ($r^2 = 0.994$).

Both the chemical shift and its temperature dependence appear to be dominated by components of the paramagnetic shielding term (*14*). Figure 4 shows the C_r values measured for each of the six protons of TmDOTA$^-$ plotted versus the chemical shift of those six protons (data from Tables 1 and 2). The intercept of this plot is approximately 0.00600 ppm/°C, a value that is similar in magnitude to the temperature dependence measured for diamagnetic compounds like water (*2*). This figure implies that compounds with the largest absolute chemical shifts are likely to be the most promising candidates for MR thermometry, and strengthens recent conclusions based on data from several different lanthanide complexes (*13, 14*). Figure 4 also allows calculation of the chemical shift range over which proton C_T values can be assumed to remain constant. For instance, if a C_T variation of ± 0.05 ppm/°C is acceptable, the chemical shift may vary by approximately ± 14 ppm. This corresponds to a temperature change of about ± 30°C for H6. Accurate temperature measurement outside this range would probably require an adjusted value for C_T or use of the expression $C_T = -0.00401\delta_t - 0.00600$ (*14*).

TABLE 2
Chemical Shifts of Protons in TmDOTP^{5-} and TmDOTA$^-$ and Their Sensitivities to Temperature

H*n*	δ (TmDOTA$^-$)	δ (TmDOTP^{5-})	C_T,TmDOTA$^-$	C_T,TmDOTP^{5-}
H1	−140	−198.4	+0.50	+1.08
H2	+59.2	+88.1	−0.20	−0.54
H3	+44.8	+68.0	−0.27	−0.42
H4	+375.8	+508.9	−1.45	−2.88
H5	−250.6	−403.6	+1.13	+2.19
H6	−119.7	−160.4	+0.45	+0.87

Note. Shifts are relative to water resonance at 0 ppm for experimental convenience.

Thermal measurements may be performed on the chemical shift of one of the TmDOTA$^-$ protons (compared to water as an internal standard) or on the shifts of two TmDOTA$^-$ protons that have C_T values with opposing signs (*14*). In addition to spectroscopic schemes, the gradient echo image of H5 of TmDOTA$^-$ shows that it is possible to image TmDOTA$^-$ with selective excitation in addition to a spectroscopic scheme (Fig. 2). Due to its large thermal sensitivity of chemical shift, phase images of TmDOTA$^-$ are likely to be more accurate in the mapping of temperature distribution than those based on less thermally sensitive substances such as water protons.

The difference in C_T values of the magnetically equivalent protons of TmDOTA$^-$ versus TmDOTP^{5-} is notable. Clearly, the more highly charged ligand DOTP^{8-} produces a complex having a magnetic susceptibility greater than that of the complex formed with the ligand of lower charge, DOTA^{4-}. The net result is that the protons of TmDOTP^{5-} demonstrate shifts greater than that of the magnetic equivalent proton in TmDOTA$^-$ (the ratio of shifts averages about 2.0 ± 0.1 except for H2, which is 2.7 times larger in TmDOTP^{5-}). Although this characteristic translates into larger C_T values for the protons of TmDOTP^{5-} (*14*), there are other characteristics of this complex that make it a less favorable thermometric probe. The chemical shifts of the TmDOTP^{5-} protons are sensitive to both pH and Ca^{2+} and this complicates the temperature measurement. The chemical shifts of the protons of TmDOTA$^-$ however are largely pH independent, except for small pH dependencies found for H4 and H5. The chemical shifts of TmDOTA$^-$ are also not sensitive to the presence of Ca^{2+} or plasma proteins.

Both TmDOTA$^-$ and TmDOTP^{5-} have chemical shifts and corresponding temperature dependencies larger than those of other recently reported thermally sensitive paramagnetic complexes, PrMOE-DO3A and YbDOTMA$^-$ (*11–14*). All these complexes might be expected to display similar tissue biodistributions, but it is possible that the neutral complex, PrMOE-DO3A, might distribute into more hydrophobic spaces and hence report temperature variations from different regions. Furthermore, the methyl groups in both PrMOE-DO3A and YbDOTMA$^-$ could have T2 relaxation advantages. The methoxy protons in PrMOE-DO3A appeared to have a 0.5-ppm linewidth *in vivo* (a similar linewidth is expected for YbDOTMA$^-$ methyl protons) (*11, 12*), about 0.3 ppm narrower than that of H6 in TmDOTA$^-$. This advantage may make them more suitable for fast imaging schemes requiring long readouts at moderate thermal sensitivities.

Based on previous studies of GdDOTA$^-$ (*21*), one would anticipate that TmDOTA$^-$ would not alter the concentration of other metal ions *in vivo*. Based upon the known thermodynamic stability of TmDOTA$^-$ (*20*) and the recorded *in vivo* safety of GdDOTA$^-$ (*21*), one can safely assume that TmDOTA$^-$ would also be safe for *in vivo* use at the GdDOTA$^-$ dosage levels (*21, 30*).

In summary, we have evaluated the feasibility of using $TmDOTA^-$ for MR thermometry in phantoms and rodents. Our results indicate that protons in $TmDOTA^-$ are highly sensitive to temperature and can be imaged. Compared to $TmDOTP^{5-}$ this complex does not sequester Ca^{2+} and has very little pH dependence in the pH range of 6 to 9. These results, in conjunction with previous studies on lanthanide–DOTA complexes, indicate that $TmDOTA^-$ is likely to be safe for *in vivo* use and provides a much simpler and reliable temperature measurement scheme for *in vivo* applications requiring high thermal resolution.

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