High Relaxivity for Monomeric Gd(DOTA)-Based MRI Contrast Agents, Thanks to Micellar Self-Organization

João P. André,[a,c] Éva Tóth,[b] Holger Fischer,[d] Anna Seelig,[d] Helmut R. Mäcke,*[a] and André E. Merbach*[b]

Abstract: With the aim of obtaining a high-relaxivity MRI contrast agent, we have designed a new amphiphilic Gd^{III} chelate that is capable of self-organization by forming micelles in aqueous solution. The synthesis of the $[GdL(H_2O)]^-$ complex is straightforward and offers an easy way for modification (L = 1,4,7,10-tetraazacyclododecane-1-(1′-carboxy-1′-dodecyl(methyl)amino-oxoethyl)-4,7,10-triacetic acid). Surface-pressure measurements have proved that the compound indeed behaves as an anionic surfactant, and the critical micellar concentration (CMC) was found to be 3.5×10^{-4} M. A variable temperature ^{17}O NMR, EPR, and NMRD study has been performed on the $[GdL(H_2O)]^-$ complex in order to determine the different factors that influence proton relaxivity. The parameters that describe the water exchange are not affected by the micellar structure, since in the aggregates the Gd^{III} chelates point towards the hydrophilic exterior, and the access from the bulk water to the paramagnetic center is not limited. Hence the rate of the water exchange is identical to that of $[Gd(DOTA)(H_2O)]^-$ ($k_{ex}^{298} = 4.8 \times 10^6\ s^{-1}$). The micellar aggregates formed in solution have a long rotational correlation time, as calculated from ^{17}O and ^1H longitudinal relaxation rates, which results in a high proton relaxivity ($R_1 = 18.01\ mM^{-1}s^{-1}$ in saline at 20 MHz proton Larmor frequency; 25 °C). This value is in the order of the relaxivities attained so far only with macromolecular Gd^{III} chelates.

Keywords: amphiphiles · contrast agents · gadolinium complexes · micelles · MRI

Introduction

Nowadays, the majority of the potential MRI contrast agents proposed to obtain high proton relaxivities involve macromolecular Gd^{III} chelates, since the slow rotational motion of the macromolecule may result in an increased proton relaxivity (MRI = magnetic resonance imaging). Several approaches have been tested in recent years, such as binding the Gd^{III} complex to dendrimers,[1, 2] linear polymers[3–6] or proteins.[7–9] In most cases the synthesis of these polymeric species requires complicated multi-step procedures. Moreover, the relaxivity gain obtained by increasing the molecular size is often far less than expected, as a result of internal flexibility or nonrigid attachment of the chelate to the macromolecule.[2, 10]

The macrocyclic DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is an excellent ligand for the complexation of Gd^{III}: the high thermodynamic stability and kinetic inertness of the Gd^{III} chelate ensures nontoxicity for the MRI contrast agent. Previous studies have demonstrated that the substitution of an acetate of the DOTA by the less strongly coordinating amide group, which is often used in coupling the complex to macromolecules, results in an undesirable decrease of the water exchange rate on the Gd^{III} chelate. Recently we published the synthesis of a new DOTA derivative that offers the possibility to easily couple the intact $[Gd(DOTA)(H_2O)]^-$ unit to other molecules without any decrease in the stability and in the water exchange rate.[11]

Here we report the synthesis and physicochemical characterization of a new potential MRI contrast agent whose design was based on the following concepts: i) maintenance the

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Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/chemistry/ or from the author. It contains reduced transverse and longitudinal ^{17}O relaxation rates and chemical shifts as a function of temperature (Table S1), variable temperature transverse electronic relaxation rates (Table S2), and variable temperature proton relaxivities (Table S3).

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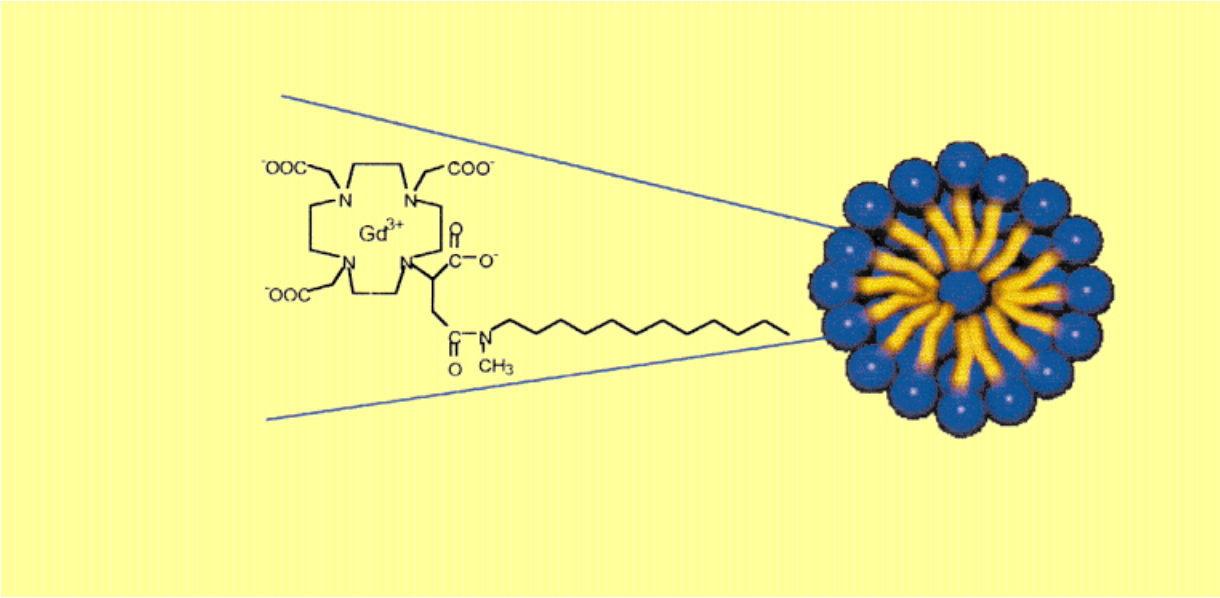

Scheme 1. Schematic representation of the micellar structure formed in aqueous solution of the $[GdL(H_2O)]^-$ complex.

thermodynamic and kinetic stability as well as the relatively fast water exchange of the $[Gd(DOTA)(H_2O)]^-$ and ii) preparation by an easy synthetic route a monomeric complex that is capable of "self-organization", which results in a macromolecular assembly. The amphiphilic $[GdL(H_2O)]^-$ complex fulfills both criteria (L = 1,4,7,10-tetraazacyclododecane-1-[1′-carboxy-1′-dodecyl(methyl)amino-oxoethyl]-4,7, 10-triacetic acid; for structure see Scheme 1). It behaves as an anionic surfactant, forming micelles in aqueous solution. If the micelles are large and rigid enough one can expect a long rotational correlation time and consequently a high proton relaxivity. Relatively high relaxivities have already been attained with DTPA-bisamide-$(CH_2)_n$ copolymers ($n = 6$, 10 or 12), which were attributed to rigid micelle-like structures formed in solution.[3, 10] However, the synthesis of those linear polymers is not a very straightforward procedure. More recently perfluoroalkyl-substituted macrocyclic Gd^{III} complexes have been proposed for blood pool and lymphographic imaging.[12] These agents are present in solution as molecular aggregates. The structure, and hence their relaxivity, strongly depends on the degree of fluorination.

A further possibility to obtain high relaxivities with the $[GdL(H_2O)]^-$ complex is to incorporate it into liposome membranes through its long hydrophobic chain. Previous studies have demonstrated that Gd^{III} chelates entrapped in liposomes have a decreased proton relaxivity relative to the free complex.[13] However, if the chelate is fixed on the exterior of the liposome so that bulk water has an easy access to the Gd^{III} chelate, that is, the relaxivity is not limited by slow water exchange through the membrane, one can take full advantage of the slow rotation of the liposome to attain high relaxivities.

The objective of the present study was twofold: i) to demonstrate that simple, monomer Gd^{III} complexes can also have relatively high proton relaxivities (as high as reported so far only for macromolecular agents), provided they are capable of self-organization and ii) to get information on all factors that influence the proton relaxivity of the amphiphilic $[GdL(H_2O)]^-$ complex, namely, water exchange rate, rotational correlation time, and electronic relaxation rates. To determine the influencing parameters we have performed a variable temperature ^{17}O NMR, EPR, and NMRD study on $[GdL(H_2O)]^-$ (NMRD = nuclear magnetic relaxation dispersion).

Results

Determination of the critical micellar concentration (CMC): In order to prove that $[GdL(H_2O)]^-$ behaves as an anionic surfactant in aqueous solution we have determined its CMC value by means of surface-activity measurements. Figure 1 shows the Gibbs adsorption isotherm ($\pi - \log c$) of the Gd^{III}-complex. The surface pressure (π) of the solution increases

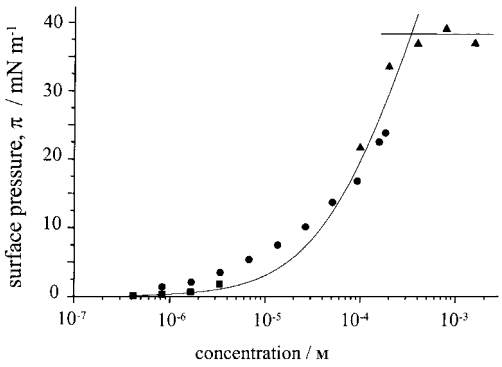

Figure 1. Gibbs adsorption isotherm of $[GdL(H_2O)]^-$ measured in a buffer solution containing 50 mM TRIS and 114 mM NaCl at pH 7.4. The intersection between the horizontal line and the fitted curve defines the CMC (3.5×10^{-4} M). Measurements were performed with stock solutions of 2.48×10^{-4} M (■), 4.95×10^{-4} M (●), and 5.97×10^{-2} M (▲). The temperature was 23 ± 1 °C.

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with increasing $[GdL(H_2O)]^-$ concentration up to a maximal surface pressure of approximately 40 mN m^{-1}, which indicates that the compound is highly surface active. At higher concentrations the surface pressure becomes constant owing to the formation of micelles. Data points were fitted by means of the Szyskowski equation.[14] The intersection between the linear part of the curve and the horizontal line through the points with maximal surface pressure (π_{max}) defines the critical micellar concentration (CMC), which is found to be 3.5×10^{-4} M. The large difference between the onset of surface activity, defined as concentration at a surface pressure of 0.1 mN m^{-1},[15] and the CMC reveals that the compound is highly amphiphilic.[16]

^{17}O NMR, EPR, and NMRD spectroscopy: The treatment used for analyzing ^{17}O NMR, EPR, and NMRD data have been previously described in detail[17] (all relevant equations are given in the Appendix). Since a relatively long rotational correlation time can be expected for a micelle, the longitudinal ^{17}O relaxation rates were analyzed with the inclusion of non-extreme narrowing conditions as well. The complex concentration used in the ^{17}O NMR study was five times higher than that in the NMRD measurements (^{17}O NMR spectroscopy requires relatively high concentrations, whereas in field-cycling relaxometry lower Gd^{III} concentrations have to be used owing to the limitation in the measurable relaxation rates). Previous studies on anionic surfactants, typified by SDS (sodium dodecyl sulphate) showed that the size of the micelles may considerably change with the concentration of the surfactant.[18, 19] Hence, the size and, consequently, the rotational correlation time that can be obtained from ^{17}O or ^{1}H longitudinal relaxation rates may not be the same as a result of the different experimental conditions applied in the two techniques. Generally, the greatest changes in micelle size occur at concentrations close to the CMC.[20] Although the concentrations used in ^{17}O NMR and NMRD spectroscopy are far beyond the CMC—thus one cannot expect big changes in size—we have analyzed the ^{17}O NMR and EPR data separately from the ^{1}H NMRD data.

The reduced ^{17}O transverse and longitudinal relaxation rates and reduced chemical shifts, as well as the transverse electronic relaxation rates measured for $[GdL(H_2O)]^-$ are presented in Figure 2. We have performed a simultaneous least-squares fit of the EPR and ^{17}O NMR data to Equations (1)–(14) in the Appendix with the following fitted parameters: k_{ex}^{298} (or $\Delta S^{\neq}$), $\Delta H^{\neq}$, $A/\hbar$, C_{os}, τ_R^{298}, E_R, τ_v^{298}, Δ^2, δg^2 (for the meaning of the symbols see Appendix). The resulting curves are shown in Figure 2 and the fitted parameters are given in Table 1. The NMRD profiles measured at four different temperatures were fitted to Equations (15)–(21) (see Appendix) by fixing the values for the water exchange rate and the activation enthalpy as obtained from ^{17}O NMR measurements. These two parameters are accurately determined by transverse ^{17}O relaxation rates and they do not depend on the surfactant concentration. The experimentally measured proton relaxivities as well as the fitted NMRD curves are presented in Figure 3.

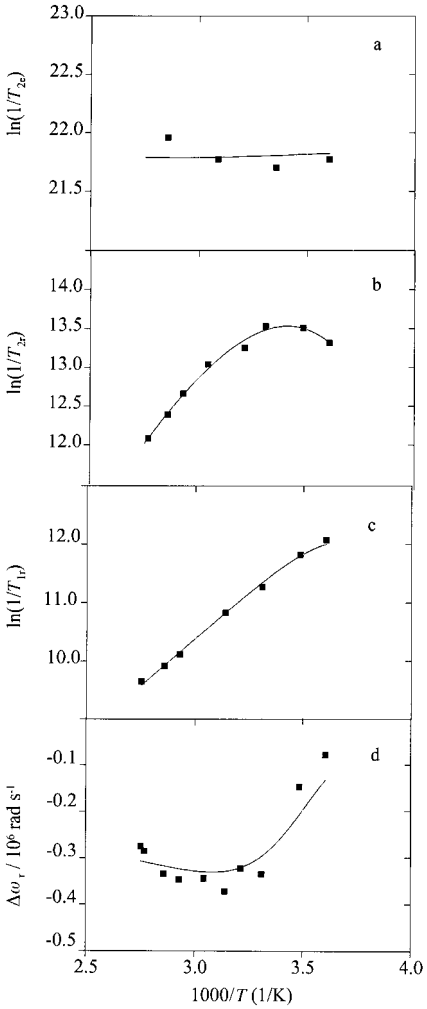

Figure 2. Temperature dependence of a) the reduced transverse electronic relaxation rates at 0.34 T (X-band), b) the transverse ^{17}O relaxation rates, c) the longitudinal ^{17}O relaxation rates, and d) the ^{17}O chemical shifts at $B = 4.7$ T for a $[GdL(H_2O)]^-$ solution. The lines represent the simultaneous least-squares fit to all data points as described in the text.

Table 1. Parameters obtained from the simultaneous fitting of EPR and ^{17}O NMR data.

	$[Gd(DOTA)(H_2O)]^{-[a]}$	$[Gd(L)(H_2O)]^-$
k_{ex}^{298} [10^6 s^{-1}]	4.8 ± 0.4	4.8 ± 0.3
$\Delta H^{\neq}$ [kJ mol^{-1}]	48.8 ± 1.6	42.7 ± 3.0
$\Delta S^{\neq}$ [J mol^{-1} K^{-1}]	$+47 \pm 6$	$+27 \pm 6$
$A/\hbar$ [10^6 rad s^{-1}]	-3.8 ± 0.2	-3.1 ± 0.2
C_{os}	0.13 ± 0.06	0.1[b]
τ_R^{298} [ps]	90 ± 15	920 ± 40
E_R [kJ mol^{-1}]	17 ± 3	27.1 ± 1.0
τ_v^{298} [ps]	0.11 ± 0.01	8 ± 1
E_v [kJ mol^{-1}]	6 ± 4	1.0[b]
Δ^2 [10^{20} s^{-2}]	0.12	0.39 ± 0.04
δg_L^2 [10^{-2}]	1.9 ± 0.3	1.5 ± 0.1

[a] From ref. [25]. [b] The underlined parameters were fixed in the fitting procedure.

Discussion

The synthesis of $[Gd(DOTASA)]^{2-}$ provides a synthon which can be easily modified due to the fact that the four carboxylate groups involved in a five-membered chelate ring are protect-

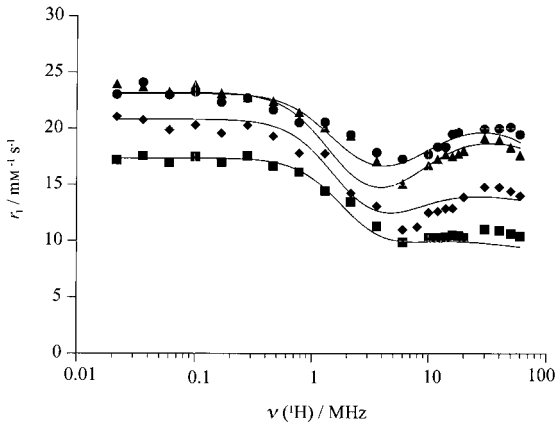

Figure 3. NMRD profiles of $[GdL(H_2O)]^-$ at 5 °C (●), 25 °C (▲), 37 °C (◆), and 50 °C (■). The lines represent the least-squares fit to the data points by fixing k_{ex}^{298} and ΔH^{+} to the values determined from ^{17}O NMR measurements (Table 1). The parameters obtained in the fit are: $\tau_R^{298} = 640$ ps, $E_R = 25.9\ kJ\,mol^{-1}$, $\Delta^2 = 0.08 \times 10^{20}\ s^{-2}$, $\tau_v^{298} = 34$ ps, $E_v = 1.0\ kJ\,mol^{-1}$, $D_{GdH}^{298} = 20 \times 10^{-10}\ m^2\,s^{-1}$, $E_{GdH} = 27\ kJ\,mol^{-1}$.

ed, whereas the β-carboxylate can be modified (H_5DOTASA = 1,4,7,10-tetraazacyclododecane-1-succinic-4,-7,10-triacetic acid). We have previously demonstrated the feasibility of this approach in the field of Ga-radiopharmaceuticals.[21] Now we have extended the method of "protection by the metal" to Gd^{III} complexes by coupling *N*-methyldodecylamine to $[Gd(DOTASA)]^{2-}$. A similar synthetic approach has been recently applied to prepare the phenanthridyl derivative of a monoamide-triphosphinate-cyclen ligand.[22]

The $[GdL(H_2O)]^-$ complex has a strong amphiphilic character and, thus, behaves as an anionic surfactant in aqueous solution. Owing to its micellar structure in solution, the proton relaxivity of this compound is very high ($R_1 = 18.01\ mM^{-1}s^{-1}$ at 20 MHz proton Larmor frequency; 25 °C). It is comparable to relaxivities obtained for dendrimers[1, 2] or Gd^{III} chelates with noncovalent protein binding.[8, 9] Since the CMC as well as the ^{17}O NMR, EPR, and NMRD measurements were carried out in a saline solution (0.114 M NaCl) at pH = 7.4, which corresponds to physiological conditions, this high relaxivity will probably be maintained in blood as well.

In the micelles the Gd^{III} chelates point towards the hydrophilic exterior; therefore, there is easy access from the bulk water to the paramagnetic center (Scheme 1). Although the degree to which water is present in the micelle interior has been the subject of some controversy, it is generally accepted that water molecules penetrate one or two CH_2 groups toward the center and the head group is always fully hydrated.[23] Consequently, the parameters describing water exchange cannot be much influenced by the micellar structure. Indeed, the k_{ex}^{298} is identical and the activation enthalpy is very similar to that of $[Gd(DOTA)(H_2O)]^-$ (Table 1); this was expected on the basis of previous studies that showed no difference in the exchange rate for Gd^{III} complexes containing the same chelating unit.[2, 10] Although no variable pressure measurements have been performed in order to determine the water exchange mechanism on $[Gd(L)(H_2O)]^-$, a dissociative activation mode is highly probable. This is also supported by the positive value of the activation entropy (Table 1).

The rotational correlation time obtained from the longitudinal ^{17}O relaxation rates (Table 1) is ten times higher than that for $[Gd(DOTA)(H_2O)]^-$; this clearly indicates the presence of micellar aggregations in solution. The τ_R^{298} obtained from the NMRD data is lower than the one determined from ^{17}O NMR measurements (640 and 920 ps, respectively). This discrepancy is general for any monomer or macromolecular system. It has been previously explained by the noncompatibility of the Gd–O and Gd–H distances that are usually used in the calculation of the τ_R values from the ^{17}O and ^1H relaxation data.[17, 24] (The exact Gd–H and Gd–O distances for a Gd^{III} complex in solution are not known, only assumptions can be made.) Owing to this problem, it is hard to draw any quantitative conclusion concerning the change in τ_R and, consequently, in micellar size with the concentration of the $[GdL(H_2O)]^-$. However, the ratio of the rotational correlation times obtained from ^{17}O and ^1H relaxation rates is similar to the ratio found for previously studied systems;[17, 25] this gives a good indication that the micellar size is constant in the concentration range covered by NMRD and ^{17}O NMR spectroscopy. This assumption is also supported by the fact that the concentrations used in both the NMRD and ^{17}O NMR studies are far above the critical micellar concentration close to which the changes in micelle size usually occur.[20]

It has to be noted that the rotational correlation time calculated either from ^{17}O or from ^1H relaxation rates represents an average value from several points of view. Firstly, micellar systems are generally polydisperse, though there are examples for fairly monodisperse micelles as well.[23] Secondly, in principle this rotational correlation time could be decomposed into a global τ_R value, which describes the overall tumbling of the entire micelle, and into a local τ_R, which characterizes the local motion of the hydrophilic head group of the molecule. This analysis has been previously applied using the Lipari–Szabo approach for micelle-like structures formed by linear polymers.[10] However, such an interpretation requires longitudinal relaxation measurements at several magnetic fields.

Certainly, there is a considerable flexibility of the head groups relative to the overall motion of the aggregate, which will result in a cut-back in the proton relaxivity. A multiple field relaxation study demonstrated this flexibility for micelles formed by the anionic surfactants sodium octyl-benzene sulphonate or sodium 4-dodecyl-benzene sulphonate.[26] The flexibility of the micelles is also evidenced by the much higher value of the rotational correlation time, $\tau_R \approx 5$ ns, calculated with the Debye formula,[27] with $r_{eff} = 18$ Å estimated from simulations.

The τ_R value in Table 1 could be considered as an average value also owing to the fact that a small fraction of the molecules (the exact quantity is given by the CMC) is always in monomeric form. In principle, it would be possible to take this into account in the analysis of the relaxation data; however, this contribution is negligible since the monomer, present in less than 1% for the ^{17}O NMR and 4% for the NMRD measurements, has a much shorter rotational correlation time.

A possible variation of the micelle size with temperature might also concern the analysis of the variable temperature

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longitudinal relaxation rates. A detailed study has been carried out on temperature effects on the shape and size of sodium dodecyl sulphate micelles at different NaCl concentrations.[28] It has been shown that at NaCl concentrations less than 0.3 M the mean hydrodynamic radius of the micelles does not considerably change with temperature in the range 10–90 °C. Therefore, based on this result, we assume in our treatment that the micelle size remains constant throughout the temperature interval applied. If the micelle size decreased with increasing temperature the only observable consequence would be an unusually low activation energy for the rotation. However, the E_R obtained in the fit is rather high (Table 1), which is also in favor of a constant size in the temperature range studied.

The temperature variation of the NMRD curves (Figure 3) clearly shows that, despite the relatively long rotational correlation time, the proton relaxivity is still limited by fast rotation. An attempt has been made to increase the rigidity of the micelles by adding cholesterol to the solution of $[GdL(H_2O)]^-$. Cholesterol is generally known to incorporate into micelles formed by other surfactants provided the size of the two molecules matches; the resulting mixed micelles are usually more rigid than the original one. The proton relaxivities measured at 25 °C in the presence of cholesterol are about 10% superior to those obtained without cholesterol (experimental points are not shown), which corresponds to a 15% increase in the rotational correlation time. In fact, cholesterol may affect the rotational correlation time, as seen by proton relaxivity, in two different ways. On the one hand, it makes the hydrophobic part of the micelle more rigid. On the other hand, as the cholesterol molecules intercalate between the $[GdL(H_2O)]^-$ molecules, there might be more space for the head-groups thus their flexibility may be increased.

Although the relaxivities of this micellar system are not higher than those of certain macromolecules, there is one more favorable point to be mentioned. The R_1 values are almost constant in the frequency range 20–60 Mhz, which is important for biomedical applications, whereas many macromolecular chelates have a sharp relaxivity peak around 20 MHz followed by an abrupt drop at higher frequencies. This constancy of the relaxivity is a consequence of a favorable interplay between the rotational correlation time and the water exchange rate.

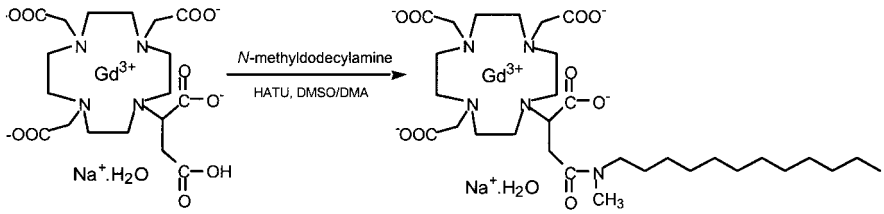
Conclusion

We have prepared a new, amphiphilic Gd^{III} chelate by a synthetic route that offers easy modification. The complex behaves as an anionic surfactant and, hence, it is capable of forming micelles in aqueous solution. The presence of micellar aggregates has been proved in saline at physiological pH. This self-organization gives rise to a slow rotation and, consequently, to very high proton relaxivities that are comparable to those for macromolecular contrast agents.

Experimental Section

Synthesis of NaH[Gd(DOTASA)·H$_2$O]: The ligand DOTASA was synthesized according to a procedure published elsewhere[11] (H_5DOTASA = 1,4,7,10-tetraazacyclododecane-1-succinic-4,7,10-triacetic acid). The NaH[Gd(DOTASA)·H_2O] complex was obtained as its sodium salt by mixing stoechiometric amounts of the ligand H_5DOTASA, Gd_2O_3, and NaOH in water, and heating the magnetically stirred solution at 85 °C for several days until a clear solution was obtained with pH around 7. The obtained solution was cooled down to room temperature, filtered, and centrifugated. After evaporating the water to dryness under reduced pressure the complex was recovered as a white solid in an almost quantitative yield (>95%). MS (ESI$^-$): *m/z* (%): 616.1 (100) $[M-H]^-$.

Coupling of NaH[Gd(DOTASA)·H$_2$O] to *N*-methyldodecylamine: NaH[Gd(DOTASA)]·H_2O (158 mg, 2.4×10^{-4} mol, 1 equiv) in DMSO/DMA (*N*,*N*-dimethylacetamide) (40 mL; 2:1), DIPEA (*N*-ethyldiisopropylamine; 123 μL, 7.2×10^{-4} mol, 3 equiv), and HATU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate; 275 mg, 7.2×10^{-4} mol, 3 equiv] were incubated for 10 minutes (the pH was checked with pH paper and it showed to be 7–8) prior to the addition of *N*-methyldodecylamine (180 μL; 7.2×10^{-4} mol, 3 equiv; Scheme 2). After several hours at room temperature, the reaction mixture was lyophilized in order to remove the solvents. The solid residue thus obtained was dissolved in methanol and purified through a silica-gel column (27×2 cm) with methanol as the eluant. The fractions (5 mL) containing the product (checked by TLC) were evaporated to dryness on a rotary evaporator. The compound thus obtained was recrystallized from acetonitrile/ethanol (9.5:0.5) to afford a white solid (109 mg; yield: 51%). The absence of free Gd^{3+} was checked by use of xylenol orange in urotropine solution.[29] R_f (SiO_2, isopropanol/NH_3(aq), 7:3) = 0.63; IR: $\tilde{\nu}$ = 2924 (C–H), 1617 (N–C=O, COO^-), 1400 cm^{-1} (COO^-); MS (ESI$^-$): *m/z* (%): 799.2 (100) $[M-H]^-$; m.p. >300 °C; $C_{31}H_{53}N_5O_9NaGd\cdot4H_2O$ (892.09): calcd C 41.74, H 6.89, N 7.85; found: C 41.53, H 6.90, N 7.98.



Scheme 2. Coupling of the long-chain amine to $[HGd(DOTASA)]^-$ to obtain $[GdL(H_2O)]^-$.

Sample preparation: For surface-pressure measurements stock solutions of $[GdL(H_2O)]^-$ were prepared by dissolving the solid complex in a buffer solution of 50 mM TRIS, 114 mM NaCl, pH = 7.4 adjusted with HCl (TRIS = tris(hydroxymethyl)amino-methane). The same buffer solution was also used for the preparation of aliquots from the stock solution. Water used for buffers and stock solutions was nanopure. Measurements were performed with stock solutions of 2.48×10^{-4} M, 4.95×10^{-4} M, and 5.97×10^{-2} M.

For ^{17}O NMR and EPR measurements a solution of 0.05 mol kg^{-1} Gd concentration and for NMRD measurements a solution of 0.009 mol dm^{-3} Gd concentration (all in 50 mM TRIS buffer, 114 mM NaCl, pH = 7.4) was used. To improve sensitivity in ^{17}O NMR measurments, ^{17}O-enriched water (10% $H_2{}^{17}O$, Yeda R&D, Israel) was added to the $[GdL(H_2O)]^-$ solution to yield in 2% ^{17}O enrichment. All solutions used in the ^{17}O NMR, EPR, and NMRD studies were completely transparent. Proton relaxivities of $[GdL(H_2O)]^-$ were also measured in the presence of 10% (mol/mol) of cholesterol, at 25 °C (50 mM TRIS, 114 mM NaCl, pH = 7.4). This solution was prepared by 2 hours of sonification.

Measurement of surface activity: Surface-activity measurements were performed with the procedure described by Fischer et al. and Seelig et al.[15, 16] In brief, the surface pressure (π), which is the difference of the

surface tension of the pure buffer solution (γ_0) and the surface tension of the buffer solution containing the Gd^{III} complex (γ), was measured as a function of concentration (c) to yield the Gibbs adsorption isotherm. The temperature was $23 \pm 1\,^{\circ}C$. The surface pressure was measured in a 3 mL home-built teflon trough with the Wilhelmy plate method. Substrate concentration was corrected for evaporation and solvent addition.

^{17}O NMR spectroscopy: Transverse and longitudinal relaxation rates and chemical shifts were measured as a function of temperature at 4.7 T (27.1 Mhz) with a Bruker AC200 spectrometer. The technique used for the variable temperature ^{17}O NMR measurements has been previously described in detail.[30]

EPR spectroscopy: The spectra were recorded on a Bruker ESP300E spectrometer (X-band; 0.34 T). The overall transverse electronic relaxation rates, $1/T_{2e}$, were obtained from the measured peak-to-peak EPR line widths of the derivative spectrum.[31]

NMRD spectrsocopy: The $1/T_1$ nuclear magnetic relaxation dispersion (NMRD) profiles of the solvent protons at 5, 25, 37 and 50 °C were obtained on a Spinmaster FFC (fast field cycling) NMR relaxometer (Stelar), which covered a continuum of magnetic fields from 7×10^{-4} to 0.47 T (corresponding to a proton Larmor frequency range 0.03 – 20 MHz). Proton relaxivities in the range 20 – 60 MHz were measured on a Bruker electromagnet connected to a AC200 console.

In all ^{17}O NMR, NMRD, and EPR studies the temperature was measured by a substitution technique.[32]

Data analysis: The least-squares fitting of the ^{17}O NMR, NMRD, and EPR data was performed by the program Scientist for Windows by Micromath, version 2.0. The reported errors correspond to one standard deviation obtained by the statistical analysis.

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Appendix

EPR spectroscopy: The electron spin relaxation rates for metal ions in solution with $S \geq ½$ are mainly governed by a transient zero-field-splitting mechanism (ZFS). The ZFS terms can be expressed by Equations (1) and (2),[33, 34] in which Δ^2 is the trace of the square of the transient zero-field-splitting tensor, τ_v is the correlation time for the modulation of the ZFS with the activation energy E_v [Eq. (3)], and ω_s is the Larmor frequency of the Gd^{3+} electron spin. The contribution arising from spin rotation is given by Equation (4),[35] in which δg_L^2 is the deviation from the free electron g_L value and τ_R is the rotational correlation time [Eq. (5)]

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25}\Delta^2\tau_V\{4S(S+1)-3\}\left(\frac{1}{1+\omega_S^2\tau_v^2}+\frac{4}{1+4\,\omega_S^2\tau_v^2}\right) \quad (1)$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2\tau_V\left[\frac{5.26}{1+0.372\,\omega_S^2\tau_v^2}+\frac{7.18}{1+1.24\,\omega_S\tau_v}\right] \quad (2)$$

$$\tau_v = \tau_v^{298}\exp\left[\frac{E_V}{R}\left(\frac{1}{T}-\frac{1}{298.15}\right)\right] \quad (3)$$

$$\left(\frac{1}{T_{ie}}\right)^{SR} = \frac{\delta g_L^2}{9\tau_R} \qquad i = 1,2 \quad (4)$$

$$\tau_R = \tau_R^{298}\exp\left[\frac{E_R}{R}\left(\frac{1}{T}-\frac{1}{298.15}\right)\right] \quad (5)$$

^{17}O NMR spectroscopy: From the measured ^{17}O NMR relaxation rates and angular frequencies of the paramagnetic solutions, ($1/T_1$, $1/T_2$, and ω) and of the acidified water reference, ($1/T_{1A}$, $1/T_{2A}$, and ω_A) one can calculate the reduced relaxation rates and chemical shift ($1/T_{1r}$, $1/T_{2r}$ and $\Delta\omega_r$), which are given in Equations (6) – (8),[36] in which $1/T_{1m}$ and $1/T_{2m}$ are the relaxation rates of the bound water and $\Delta\omega_m$ is the chemical shift difference between bound and bulk water. $\Delta\omega_m$ is determined by the scalar coupling constant, $A/\hbar$, according to Equation (9), in which B represents the magnetic field. The outer sphere contribution to the ^{17}O chemical shift is proportional to $\Delta\omega_m$, in which C_{os} is an empirical constant [Eq. (10)].

$$\frac{1}{T_{1r}} = \frac{1}{P_m}\left[\frac{1}{T_1}-\frac{1}{T_{1A}}\right] = \frac{1}{T_{1m}+\tau_m} \quad (6)$$

$$\frac{1}{T_{2r}} = \frac{1}{P_m}\left[\frac{1}{T_2}-\frac{1}{T_{2A}}\right] = \frac{1}{\tau_m}\,\frac{T_{2m}^{-2}+\tau_m^{-1}T_{2m}^{-1}+\Delta\omega_m^2}{(\tau_m^{-1}+T_{2m}^{-1})^2+\Delta\omega_m^2} \quad (7)$$

$$\Delta\omega_r = \frac{1}{P_m}(\omega-\omega_A) = \frac{\Delta\omega_m}{(1+\tau_m T_{2m}^{-1})^2+\tau_m^2\Delta\omega_m^2}+\Delta\omega_{os} \quad (8)$$

$$\Delta\omega_m = \frac{g_L\mu_B S(S+1)BA}{3\,k_B T\,\hbar} \quad (9)$$

$$\Delta\omega_{os} = C_{os}\Delta\omega_m \quad (10)$$

Longitudinal ^{17}O relaxation is caused by dipolar and quadrupolar mechanisms and gives information on rotational dynamics. Since the micelles formed are expected to have slow rotation, the treatment we use also includes non-extreme narrowing conditions. The dipolar contribution is given by Equations (11).[37] The quadrupolar contributions is given by Equation (12).[38] In the transverse relaxation the scalar contribution ($1/T_{2sc}$) is the most important one [Eq. (13)].[25] In Equation (13) $1/\tau_{sj}$ is the sum of the exchange rate constant and the electron spin relaxation rate. The binding time (or exchange rate, k_{ex}) of water molecules in the inner sphere is assumed to obey the Eyring equation [Eq. (14)], where $\Delta S^{\neq}$ and $\Delta H^{\neq}$ are the entropy and enthalpy of activation for the exchange process, and k_{ex}^{298} is the exchange rate at 298.15 K.

$$\frac{1}{T_{1dd}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\hbar^2\gamma_I^2\gamma_S^2}{r_{GdO}^6}S(S+1)\times\left[7\,\frac{\tau_d}{1+\omega_S^2\tau_d^2}+3\,\frac{\tau_d}{1+\omega_I^2\tau_d^2}\right] \quad (11)$$

$$\frac{1}{\tau_d} = \frac{1}{\tau_R}+\frac{1}{T_{1e}}+\frac{1}{\tau_m} \quad (11a)$$

$$\frac{1}{T_{1q}} = \frac{3\pi^2}{10}\,\frac{2I+3}{I^2(2I-1)}\,\chi^2(1+\eta^2/3)\times\left[0.2\,\frac{\tau_R}{1+\omega_I^2\tau_R^2}+0.8\,\frac{\tau_R}{1+4\omega_I^2\tau_R^2}\right] \quad (12)$$

$$\frac{1}{T_{2m}} \cong \frac{1}{T_{2sc}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left(\tau_{s1}+\frac{\tau_{s2}}{1+\omega_s^2\tau_{s2}^2}\right) \quad (13)$$

$$\frac{1}{\tau_{sj}} = \frac{1}{\tau_m}+\frac{1}{T_{je}} \qquad j = 1,2 \quad (13a)$$

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_B T}{h}\exp\left[\frac{\Delta S^{\neq}}{R}-\frac{\Delta H^{\neq}}{RT}\right] = \frac{k_{ex}^{298}T}{298.15}\exp\left[\frac{\Delta H^{\neq}}{R}\left(\frac{1}{298.15}-\frac{1}{T}\right)\right] \quad (14)$$

NMRD spectroscopy: The measured proton relaxivities (normalized to 1 mM Gd^{III} concentration) contain both inner sphere and outer sphere contributions [Eq. (15)]. The inner sphere term is given by Equation (16),

$$r_1 = r_{1is}+r_{1os} \quad (15)$$

$$r_{1is} = \frac{1}{1000}\times\frac{q}{55.55}\times\frac{1}{T_{1m}^H+\tau_m} \quad (16)$$

in which q is the number of inner sphere water molecules. The longitudinal relaxation rate of inner sphere protons ($1/T_{1m}^H$) can be expressed as in Equation (17)[39, 40] In Equation (17) r_{GdH} is the effective distance between the Gd^{III} electron spin and the water protons, ω_I is the proton resonance frequency, and τ_{di} is given by Equation (18)

$$\frac{1}{T_{1m}^H} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\hbar^2\gamma_S^2\gamma_I^2}{r_{GdH}^6}S(S+1)\left[\frac{3\tau_{d1}}{1+\omega_I^2\tau_{d1}^2}+\frac{7\tau_{d2}}{1+\omega_S^2\tau_{d2}^2}\right] \quad (17)$$

$$\frac{1}{\tau_{di}} = \frac{1}{\tau_m}+\frac{1}{\tau_R}+\frac{1}{T_{ie}} \qquad i = 1,2 \quad (18)$$

The outer-sphere contribution can be described by Equations (19) and (20) ($j = 1,2$),[41, 42] in which N_A is the Avogadro constant and J_{os} is a spectral density function. For the temperature dependence of the diffusion coefficient for the diffusion of a water proton away from a Gd^{III} complex (D_{GdH}) we assume an exponential temperature dependence, with an activation energy E_{DGdH} [Eq. (21)]

$$r_{1os} = \frac{32\,N_A\pi}{405}\left(\frac{\mu_0}{4\pi}\right)^2\frac{\hbar^2\gamma_S^2\gamma_I^2}{a_{GdH}D_{GdH}}S(S+1)\,[3J_{os}(\omega_I,T_{1e})+7J_{os}(\omega_S,T_{2e})] \quad (19)$$

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$$J_{os}\left(\omega, T_{je}\right) = R_c \left[\frac{1 + \frac{1}{4}\left(i\,\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2}}{1 + \left(i\,\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2} + \frac{4}{9}\left(i\,\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right) + \frac{1}{9}\left(i\,\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{3/2}} \right] \quad (20)$$

$$D_{GdH} = D_{GdH}^{298} \exp\left[\frac{E_{DGdH}}{R} \left(\frac{1}{T} - \frac{1}{298.15} \right) \right] \quad (21)$$

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