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**PAPER**

# Synthesis, complexation and NMR relaxation properties of $Gd^{3+}$ complexes of $Mes(DO3A)_3$†

**Pascal Miéville,[a] Hugues Jaccard,[a] Felipe Reviriego,[b] Raphaël Tripier[b] and Lothar Helm[a]**

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Medium sized molecules endowed with multiple $Gd^{3+}$ complexes are efficient high magnetic field MRI contrast agents. The novel ligand $Mes(DO3A)_3$, presenting three DO3A (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″-triacetatic acid) units grafted on the methyl positions of a central mesitylene (1,3,5-trimethylbenzene), has been synthesized. Designed as an MRI contrast agent, this ligand is complexed with $Gd^{3+}$ and its efficiency is characterized by variable field $^{1}$H-NMR and variable temperature $^{17}$O-NMR. The evaluation of the relaxation and paramagnetic chemical shift data allowed the identification of an undesired binuclear complex which is obtained by using the classical procedure for complexation as described in the literature. An intramolecular capping mechanism appears to be responsible for the failure to introduce a third $Gd^{3+}$ ion into the ligand. A new alternative method, based on pre-complexation with $Mg^{2+}$ followed by transmetallation is described here and leads to the expected trinuclear $Gd^{3+}$ complex $[Mes\{Gd(DO3A)(H_2O)_2\}_3]$. The rate constants for the water exchange ($k_{ex}^{298} = 32 \times 10^6$ s$^{-1}$) for the bi- and trinuclear complex appeared to be the same, which is surprising in relation to the difference in the charge of the complex and to the difference in the number of coordinated water molecules, one and two per $Gd^{3+}$ for the binuclear and trinuclear complex, respectively.

## Introduction

Currently, all approved gadolinium-based contrast agents (CA) for magnetic resonance imaging (MRI) are based on complexes with chelating poly(amino carboxylate) ligands.[1] These octa-dentate ligands, which are either acyclic like DTPA or DTPA-BMA or macrocyclic like DOTA or HP-DO3A, form extremely stable complexes with lanthanides offering space for the coordination of one water molecule.[2] Tremendous efforts have been made in the last decade to develop new compounds with increased efficiency required for targeted CA and molecular imaging. The enhancement of longitudinal nuclear spin relaxation, commonly expressed as relaxivity ($r_1$) if normalized to 1 mM solution of gadolinium ions, could be increased by more than an order of magnitude, mainly by slowing down the rotational diffusion of the molecules.[3] This increase in relaxivity has been achieved at magnetic fields common to MRI instruments actually used in clinical applications.

However, most highly efficient CA lose nearly all of the gain in relaxivity at high magnetic fields above 3 T. MRI instruments working at 7 T or even above are now successively installed in research institutions, creating a need for contrast agents designed for use at these conditions.[4] Theoretical calculations using the simple Solomon–Bloembergen–Morgan approach[5–8] show that the relaxivity which can be reached at magnetic fields above 3 T is well below the performance that can be achieved between 1 and 1.5 T.[8–9] Staying with chelate complexes of gadolinium, the only way to boost the efficiency of CA is to increase the number ($q$) of water molecules directly bound to $Gd^{3+}$ and the assembly of many chelating units in larger molecules or particles. The theoretical calculations have also shown that the compounds should have a reasonable size leading to rotational correlation times between 500 ps and 1 ns.

Several mid-size molecules assembled around a benzene ring have been synthesized and tested for their relaxation enhancement capabilities (Scheme 1).[10–12] The chelating groups used are either the acyclic DTTA ($H_4$DTTA = diethylenetriaminetetraacetic acid = 2,2′,2″,2‴-[iminobis(ethane-2,1-diylnitrilo)]tetraacetic acid) or the macrocyclic DO3A (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″-triacetate). Both can form $Gd^{3+}$ complexes with two water molecules ($q$ = 2) in the first coordination sphere. Surprisingly it had been found that the

[a]*Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, ISIC, BCH, CH-1015, Lausanne, Switzerland. E-mail:lothar.helm@epfl.ch; Fax: +41 21 693 98 55; Tel: +41 21 693 98 71*

[b]*UMR CNRS 6521, Chimie, Electrochimie Moléculaires et Chimie Analytique, Université de Bretagne Occidentale, C. S. 93837, 6 avenue Victor Le Gorgeu, 29238, Brest Cedex 3, France. E-mail: raphael.tripier@univ-brest.fr; Tel: +33 2 98 01 79 27*

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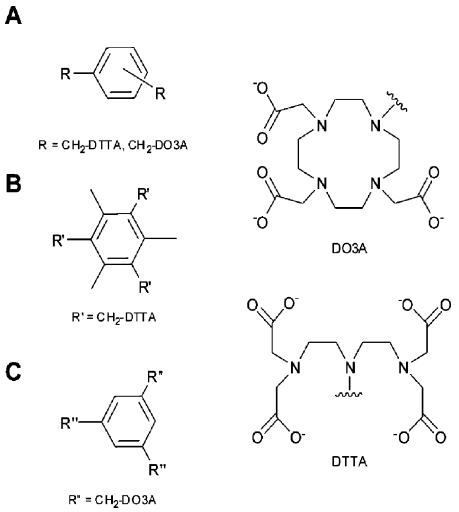



**Scheme 1** Structures of benzene-based molecules which can complex 2 (A) or 3 (B, C) $Gd^{3+}$ ions; A: ref. 10, 11, B: ref. 12, C: this work.

compounds with DO3A[11] had $q = 1$ and formed aggregates in aqueous solution.

To further investigate the behavior of gadolinium–DO3A complexes bound to a benzene ring we decided to synthesize and to determine the relaxivity of the tris-gadolinium complex formed by mesitylene substituted with three DO3A units on the three methyl positions. It would be interesting to see if these compounds form aggregates in aqueous solution. The aromatic part sits in the center of the molecule and aggregation by π-stacking of the benzene rings would be strongly disfavored. A second question we intended to answer concerns the number of inner sphere water molecules. Will we also find $q = 1$ as for the xylene-cored dinuclear Gd chelates[11] and for other DO3A-based dimeric Gd complexes[13–14] or will we find $q \cong 2$ as for the DO3A monomer?[15]

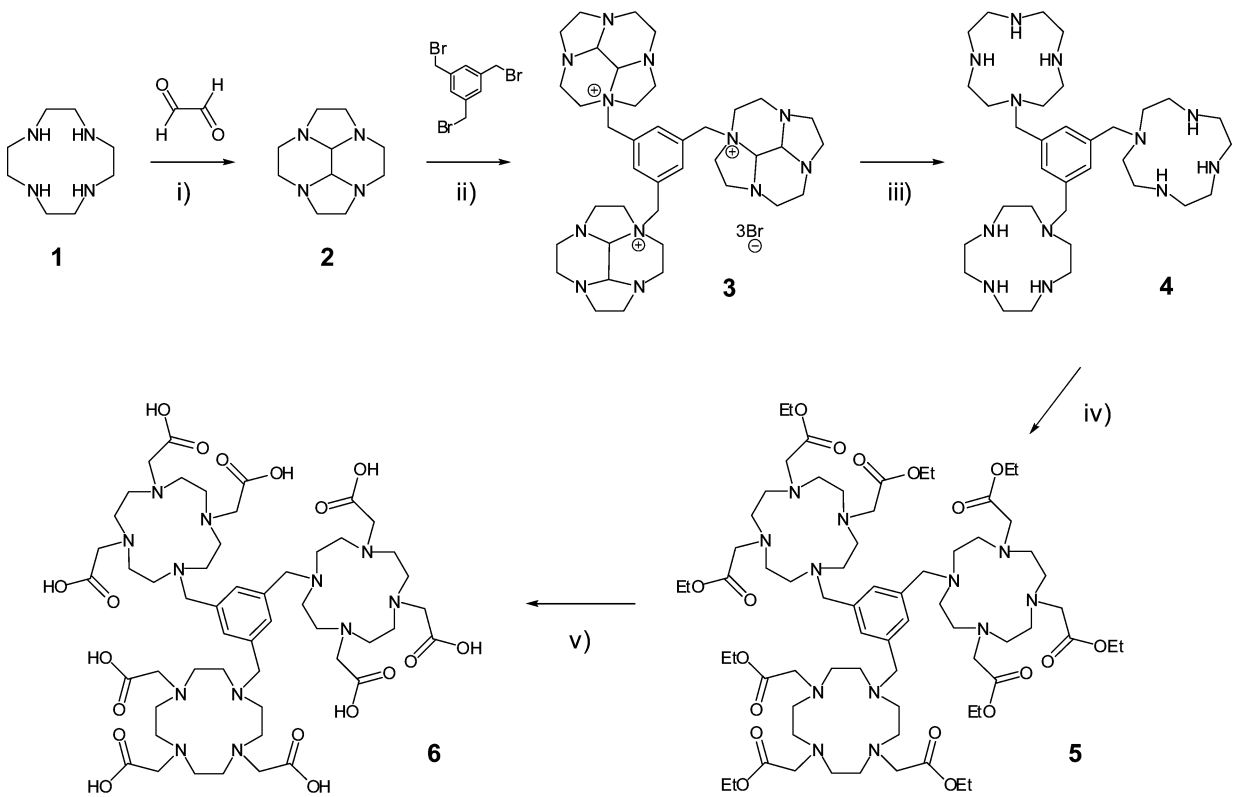
## Results and discussion

### Synthesis of the ligand

Tetraazacycloalkane-based ligands are very attractive compounds for coordination chemistry since they are now easily obtained by selective N-alkylation of the starting macrocycle.[16–19] As shown in Scheme 2, the studied ligand **6** consists in three DO3A moieties linked with a mesitylenyl center and is obtained following an easy route involving the bisaminal methodology of tetraazacycloalkanes.[20–23] In a first step, the macrocyclic bisaminal **2** is obtained by condensation of glyoxal with cyclen **1** as previously described.[24] Reaction of one equivalent of the tris-electrophile 1,3,5-tris(bromomethyl)benzene with **2** leads to the tris-salt **3**, easily deprotected by hydrazine monohydrate to obtain the tris-cyclen **4** in quantitative yield. This step is followed by the alkylation of the three secondary amine functions of each cyclen moiety with ethyl bromoacetate. Finally, the nine-fold ester derivative is hydrolyzed in HCl (6 N) with 78% overall yield.

### Complexation

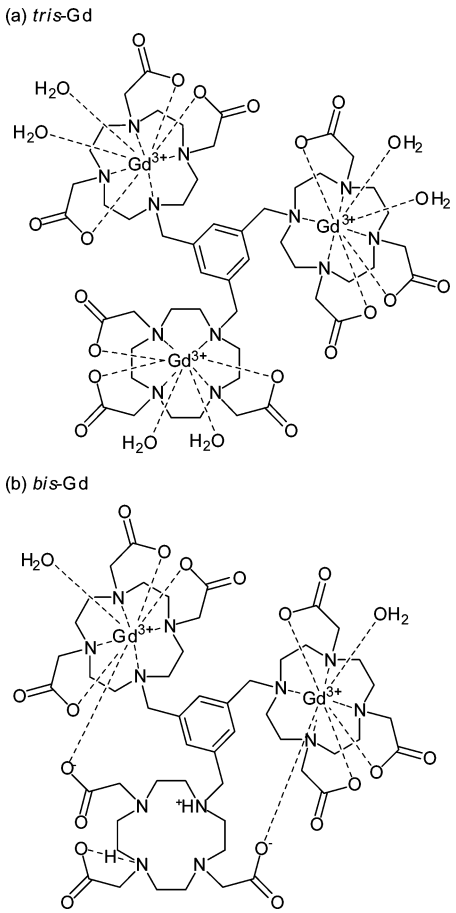
The classical complexation method consists in mixing a ligand solution with stoichiometric quantities of $Gd^{3+}$ calculated for $Gd_3(\mathbf{6})(H_2O)_6$ (see Experimental). In the case of our ligand **6** this leads to a surprising result. From the back titration of free $Gd^{3+}$




**Scheme 2** (i) In MeOH; (ii) 1,3,5-tris(bromomethyl)benzene, $CH_3CN$, r.t.; (iii) $NH_2NH_2{\cdot}H_2O$, Δ, 2 h; (iv) $BrCH_2COOEt$, $CH_3CN$, $K_2CO_3$; (v) HCl 6 N, Δ, 12 h.

it has been found that only 1.98 $Gd^{3+}$ are bound to the ligand instead of the 3 expected. The calculation of this Gd/L ratio has been based on the molar mass established from the elementary analysis ($C_{51}H_{84}N_{12}O_{18}\cdot 12HCl\cdot 2.5H_2O$). This surprising result is confirmed by the Gd/L ratio of 2.17 which is obtained from gadolinium to carbon mass ratios determined by ICP-MS and elementary analysis for Gd and C, respectively.

The observed difficulties to complex a third $Gd^{3+}$ ion by the ligand **6** could arise subsequently from the important pH drop during the complexation reaction (pH ~1.2 after mixing). This pH drop implies the protonation of the carboxylic groups of the third DO3A ring, which prevents the chelation of the third $Gd^{3+}$. After restoring the pH to 5.8, the two acetates of the uncomplexed DO3A, instead of staying deprotonated and free, are suspected to bind immediately to the two chelated $Gd^{3+}$ ions by replacing one water molecule from the first coordination sphere of each of the two paramagnetic centers (Scheme 3).[25–26] We therefore conclude that we synthesized the compound $[Gd_2(\mathbf{6})]^-$, which we named bis-Gd as opposed to $[Gd_3(\mathbf{6})]$ which we named tris-Gd.




**Scheme 3** Proposed structures of (a) $[Mes\{Gd(DO3A)(H_2O)_2\}_3]$ (tris-Gd) and (b) $[Mes\{H_2DO3A\}\{Gd(DO3A)(H_2O)\}_2]^-$ (bis-Gd isomer A).

A preparation of the tris-Gd complex by starting the reaction at much higher pH is not possible due to the formation of gadolinium hydroxide at pH >5.9. We therefore decided to prepare $[Gd_3(\mathbf{6})]$ in two steps. In a first step we complex the ligand **6** with a metal ion forming much weaker complexes than $Gd^{3+}$. A condition is that this metal should not form precipitating hydroxides at a pH at which only one amine of the DO3A is protonated (pH ~9).[26–27] In a second step this first complex is transformed to the final gadolinium compound by transmetallation at pH 5.8. We have chosen the $Mg^{2+}$ ion to perform the first complexation step of **6**. Besides the much lower stability of DO3A complexes with 2+ ions[27] we selected the smallest alkali earth ion to disfavor binding of acetate groups from another DO3A chelate of **6** due to steric crowding around the cation. After adjusting the pH of the solution to 5.8 by adding NaOH a solution containing an excess of $GdCl_3$ was added. The advancement of the reaction has been followed by measuring the water proton relaxation rate $R_1 = 1/T_1$ at 30 MHz (Fig. 1). The relaxation rate drops after mixing and reaches a stable value after ~800 min. The pH of the mixture did not change during the transmetallation reaction. After eliminating the excess of $Gd^{3+}$ and free $Mg^{2+}$ ions by size exclusion chromatography a $Gd^{3+}$/ligand ratio of 2.96 has been determined by Gd/C measurement.

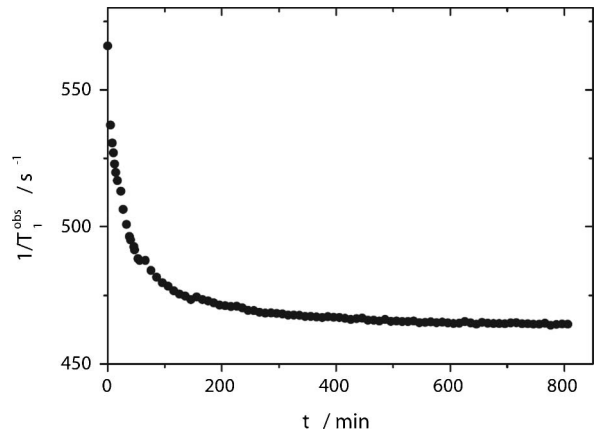



**Fig. 1** Transmetallation reaction from $[Mg_3(\mathbf{6})]^{3-}$ to $[Gd_3(\mathbf{6})]$ followed by $^1H$ NMR relaxation of water at 30 MHz and 25.0 °C.

### Structural transition induced by pH

In a simple experiment we tried to confirm the binding of two acetate groups of the uncomplexed DO3A to the two $Gd^{3+}$ ions bound to the other two chelating groups. By replacing the paramagnetic lanthanide $Gd^{3+}$ by the diamagnetic $Y^{3+}$, which has the same charge and a very similar ionic radius, we are able to measure the $^1H$ NMR spectrum of the bis-Y complex in $D_2O$ solution. The $^1H$ NMR spectra are rather complex due to the presence of different geometrical isomers in slow exchange. At about neutral pH there are two relatively broad signals in the aromatic region at 7.09 ppm and 7.52 ppm corresponding to two main isomers of the bis-Gd complex, called isomer A and isomer B. Varying the pH of the solution by adding 2 M NaOD in $D_2O$ shows an isomer transition, occurring between pH 3 and 5.6 and shifting the equilibrium from isomer B to A (Fig. 2). We attribute the isomer A to the closed, or capped, form of the bis-Y complex, *i.e.* with acetate groups bound to the $Y^{3+}$-ions as presented in Scheme 3(b). Isomer B would correspond to the open form of the bis-Y complex with protonated and unbound acetate groups. This isomer transition would prove the formation of a closed conformation, unable to bind a third metal center at working pH (4 to 5.8).

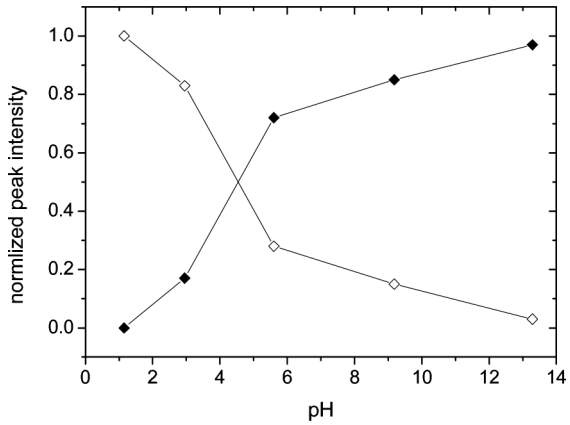



**Fig. 2** Normalized peak area of the aromatic $^1$H NMR signals at 7.09 ppm (isomer A, ◆) and 7.52 ppm (isomer B, ◇) *vs.* pH for $[Mes\{DO3A\}\{Y(DO3A)(H_2O)\}_2]^{3-}$.

## $^1$H NMRD

To characterize the relaxivity of the bis-Gd and tris-Gd complexes nuclear magnetic relaxation dispersion (NMRD) profiles of water protons have been measured at 25.0 °C and 37.0 °C (Fig. 3). Comparison of the relaxation enhancement at low Larmor frequencies ($\nu$ < 1 MHz) induced by 1 mM $Gd^{3+}$ (relaxivity, $r_1$) shows that the relaxivity of the bis-Gd compound (Fig. 3: empty symbols) is about half of that of the tris-Gd compound (Fig. 3: filled symbols). Assuming similar relaxation rates of the $Gd^{3+}$ electron spin for both compounds the only explanation for this difference in relaxivity is a change in the number ($q$) of coordinated water molecules.

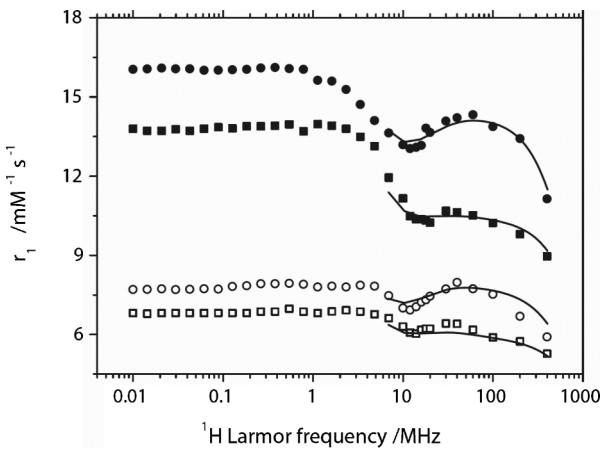



**Fig. 3** NMRD profiles of the tris-Gd complex (filled symbols) and of the bis-Gd complex (empty symbols) at 25 °C (○,●) and at 37 °C (□,■). The lines are calculated from a simultaneous fit of $^1$H NMRD and $^{17}$O data (for parameters see Table 1).

It has been shown that $[Gd(DO3A)(H_2O)_q]$ shows an equilibrium between 8 and 9 coordination and $q$ = 1.8 and 1.9 has been determined by UV-vis spectroscopy and luminescence lifetime of $[Eu(DO3A)(H_2O)_q]$[15] and $[Tb(DO3A)(H_2O)_q]$,[28] respectively. We can therefore conclude that in the tris-Gd complex two water molecules are directly bound to the cation ($q$ = 2). For the bis-Gd complex two structures with two first coordination sphere water molecules per compound are conceivable: the first one having one $Gd^{3+}$ with $q$ = 2 and one with $q$ = 0 and the second one having two $Gd^{3+}$-ions with $q$ = 1. For the first compound the coordination sphere of the $Gd^{3+}$ with $q$ = 0 is completed by two acetate groups from the metal-free DO3A. For the second compound each $Gd^{3+}$ ion binds one acetate group of the free DO3A (Scheme 3(b)). Molecular mechanics calculations (see ESI†) show that the first structure leads to high intramolecular strain when compared to the second structure. This reinforces our assumption of the structure proposed in Scheme 3(b) with coordination of acetate groups of the uncomplexed DO3A to each of the two gadolinium ions in $[Mes\{DO3A\}\{Gd(DO3A)(H_2O)\}_2]^{3-}$.

**Table 1** Parameters obtained from fits of the $^{17}$O NMR and the $^1$H NMRD data, using the Solomon–Bloembergen–Morgan approach[a]

| | bis-Gd | | tris-Gd |
|---|---|---|---|
| $q$ | 1 | | 2 |
| $k_{ex}^{298}/10^6$ s$^{-1}$ | | $32 \pm 3^b$ | |
| $\Delta H^{\ddagger}$/kJ mol$^{-1}$ | | $25.8 \pm 1.2^b$ | |
| $\Delta S^{\ddagger}$/J K$^{-1}$ mol$^{-1}$ | | $-14.7 \pm 4^b$ | |
| $A/\hbar/10^6$ rad s$^{-1}$ | | $-3.1 \pm 0.2^b$ | |
| $\tau_R^{298}$/ps | $193 \pm 4$ | | $201 \pm 2$ |
| $E_R$/kJ mol$^{-1}$ | $18 \pm 1$ | | $20.9 \pm 0.5$ |
| $\tau_v^{298}$/ps | $18.5 \pm 4$ | | $10.9 \pm 1$ |
| $\Delta^2/10^{20}$ s$^{-2}$ | $0.38 \pm 0.04$ | | $0.19 \pm 0.01$ |

[a] Other parameters fixed in the fitting procedure are: $r_{GdO}$ = 2.5 Å, $r_{GdH}$ = 3.1 Å, $a_{GdH}$ = 3.6 Å, $\chi(1+\eta^2/3)^{1/2}$ = 7.58 MHz, $D_{GdH}^{298} = 2.5 \times 10^{-9}$ m$^2$ s$^{-1}$, $E_{GdH}$ = 20 kJ mol$^{-1}$, $E_v$ = 1 kJ mol$^{-1}$; [b] From $^{17}$O NMR data.

## $^{17}$O NMR

The longitudinal and transverse $^{17}$O NMR relaxation enhancements as well as the $^{17}$O NMR chemical shift differences, all with respect to acidified water, have been measured as a function of temperature. The reduced relaxation rates $1/T_{1r}$, $1/T_{2r}$, and the reduced chemical shift differences, $\Delta\omega_r$, are calculated using eqn (1) to (3) and the results are shown in Fig. 4. The mole fraction of bound water, $P_m$, has been calculated for the bis-Gd and the tris-Gd complexes using $q$ = 1 and $q$ = 2, respectively.

$$\frac{1}{T_{ir}} = \frac{1}{P_m}\left(\frac{1}{T_i^{obs}} - \frac{1}{T_i^{ref}}\right); i = 1,2 \quad (1)$$

$$\Delta\omega_r = \frac{1}{P_m} 2\pi\left(\nu^{obs} - \nu^{ref}\right) \quad (2)$$

$$P_m = \frac{q\left[Gd^{3+}\right]}{55.56} \quad (3)$$

The $^{17}$O paramagnetic chemical shift experienced by water molecules directly bound to gadolinium ions is governed by the scalar or Fermi contact term.[29] At high temperatures ($T$ > 322 K, $1000/T$ < 3.1 K$^{-1}$) in the fast exchange regime the reduced chemical shift $\Delta\omega_r$ is directly given by the chemical shift of the bound water molecules, $\Delta\omega_m$.[30] Because $\Delta\omega_m$, which is proportional to the scalar coupling constant $A/h$, is very similar for complexes with the same chelating unit,[11,15] it can be used to estimate the number of coordinated water molecules. From the chemical shift results in Fig. 4 it can be seen that the reduced shifts for bis-Gd and tris-Gd complexes are essentially the same. Because

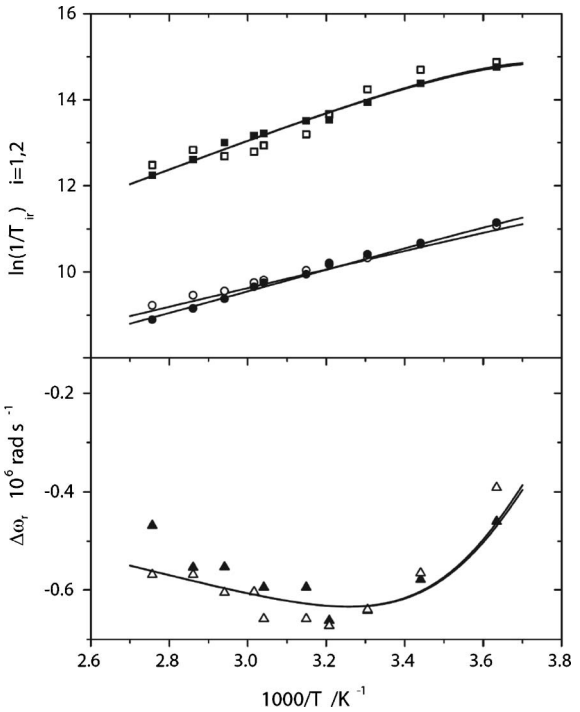



**Fig. 4** Reduced $^{17}$O NMR relaxation rates $1/T_{2r}$ (■, □) and $1/T_{1r}$ (●, ○) and reduced chemical shifts, $\Delta\omega_r$ (▲, △), for the tris-Gd (filled symbols) and for the bis-Gd complex (open symbols); lines are calculated from the fitted parameters (Table 1).

their values have been calculated using $q = 1$ for bis-Gd and $q = 2$ for tris-Gd this confirms that the number water molecules bound to the $Gd^{3+}$ ions is different for the bis-Gd and tris-Gd complexes.

Like the chemical shift differences, the reduced enhancement of transverse ($1/T_{2r}$) and longitudinal ($1/T_{1r}$) relaxation are very similar for bis-Gd and tris-Gd (Fig. 4). The continuous decrease of $1/T_{2r}$ with increasing temperature is a clear indication that the water exchange is in the fast exchange regime.[31] The reduced $^{17}$O-NMR transverse relaxation rates, $1/T_{2r}$, are determined by the water exchange rate constant $k_{ex}$, the scalar relaxation of bound oxygen atoms and the chemical shift difference $\Delta\omega_m$.[31] Because the $1/T_{2r}$ values of bis-Gd- and tris-Gd-complexes are so similar we can conclude that the water exchange rates are the same over the temperature range of the study.

The quantitative analysis of the NMR data has been performed in two steps using the standard Solomon–Bloembergen–Morgan approach.[6] If we are not interested in detailed information about the electron spin relaxation and if we restrict the data analysis to medium to high magnetic fields the SBM approach gives reliable information on dynamic processes like water exchange rate constants and rotational correlation times for small to mid-size complexes.[32] In a first step we fitted the $^{17}$O relaxation rates and chemical shift data of both compounds together. We fixed the distance between $Gd^{3+}$ and the water oxygen, $r_{GdO}$, to 2.5 Å. The nuclear quadrupole coupling constant, $\chi(1 + \eta^2/3)^{1/2}$, has been fixed to the value of neat water, 7.58 MHz. From the fit we obtained for the exchange rate constant $k_{ex}^{298} = 3.2 \times 10^7$ s$^{-1}$ and $\Delta H^{\ddagger} = 25.8$ kJ mol$^{-1}$. A mean rotational correlation time $\tau_R^{298} = 212$ ps ($E_a = 19.7$ kJ mol$^{-1}$) has been calculated from the longitudinal $^{17}$O spin relaxation.

In a second step we fitted the $^{17}$O NMR data together with the high frequency $^1$H relaxivity ($\nu(^1H) > 6$ MHz) in separate fits for bis-Gd and tris-Gd. In these separate fits we fixed the exchange rate constant and activation enthalpy to the values obtained from the $^{17}$O data analysis. The water proton–Gd distance, $r_{GdH}$, and parameters defining the outer sphere contribution to the $^1$H relaxivity have been fixed to common values (Table 1). Rotational correlation times and parameters defining the electron spin relaxation ($\Delta^2$, the amplitude of the transient zero-field splitting, and $\tau_v$, the correlation time for the transient zero-field splitting) are obtained from the two fits (Table 1). The reasonable quality of the fits (calculated curves in Fig. 3 and 4) confirms once again the difference in water coordination numbers ($q$) of the two compounds.

The water exchange rate constant is surprising in two aspects. First of all it is unexpected that the water exchange rates in the bis-Gd- and the tris-Gd compounds are so similar. If we accept the coordination of one of the acetate oxygens to the $Gd^{3+}$ ion as proposed above the coordination number of gadolinium is nine at all coordination sites in the two compounds. The local electric charge is however different since an acetate oxygen is more negatively charged than a water oxygen. This should lead to a marked difference.[30,33–34] As a general trend it has been found that a higher negative overall charge favors the departure of the water molecule in a dissociative process.[34] We expected therefore a faster exchange on the negatively charged $[Mes\{DO3A\}\{Gd(DO3A)(H_2O)\}_2]^{3-}$ with respect to the neutral $[Mes\{Gd(DO3A)(H_2O)_2\}_3]$. This is clearly not observed. The second unexpected result is the fast water exchange due to the low activation enthalpy ($\Delta H^{\ddagger} = 25.8$ kJ mol$^{-1}$). The water exchange rate constant on $[Gd(DO3A)(H_2O)_2]$ has been measured as $11 \times 10^6$ s$^{-1}$, which is about three times slower (Table 2).[15] Terreno *et al.*[35] concluded from two DO3A derivatives with $q = 1$ that the water exchange rate is modulated by the basicity of the macrocyclic nitrogen atom bearing the pendant group: a lower basicity results in a slower water exchange rate. The fastest exchange rate constant they measured ($k_{ex}^{298} = 17.6 \times 10^6$ s$^{-1}$ on $[Gd(NH_2PhDO3A)(H_2O)_2]$) is about 1.8 times slower than our exchange rate. Botta *et al.*[36] measured a 1.7 times faster water exchange on a substituted DO3A complex which has $q = 2$ ($[Gd(B\text{-}DO3A)(H_2O)_2]$). The activation entropy measured on our complex is negative ($\Delta S^{\ddagger} = -14.7$ J K$^{-1}$ mol$^{-1}$) suggesting a change in mechanism from dissociative activation to associative activation. This would mean that for both compounds an incoming water molecule helps the bound water molecule to leave the first coordination sphere.

## Conclusion

In order to develop new high field MRI contrast agents based on small molecules bearing multiple $Gd^{3+}$ complexes, we synthesized the novel ligand $Mes(DO3A)_3$. Its trinuclear complex with $Gd^{3+}$ was characterized and a relaxivity of 10.2 mM$^{-1}$ s$^{-1}$ (13.7 mM$^{-1}$ s$^{-1}$) has been determined at 20 MHz and 37 °C (25 °C). This relaxivity is slightly higher than that measured for similar trimeric compounds (see, for example, Caravan *et al.*[33] Table 21). The complexation of the ligand was however not straightforward since the classical method lead to the undesired binuclear chelate $[Mes\{DO3A\}\{Gd(DO3A)(H_2O)\}_2]^{3-}$. This has been seen through the $^1$H-NMR relaxivities $r_1$ and the reduced

**Table 2** Hydration numbers $q$, water exchange rates $k_{ex}^{298}$, activation enthalpies $\Delta H^{\ddagger}$ and activation entropies $\Delta S^{\ddagger}$ for a selection of DO3A-type Gd chelates

| | $q$ | $k_{ex}^{298}/10^6$ s$^{-1}$ | $\Delta H^{\ddagger}$/kJ mol$^{-1}$ | $\Delta S^{\ddagger}$/J K$^{-1}$mol$^{-1}$ | Ref. |
|---|---|---|---|---|---|
| Mononuclear | | | | | |
| [Gd(DO3A)(H$_2$O)$_q$] | 1.9 | 11 | 33.6 | +2 | 15 |
| [Gd(NO$_2$PhDO3A)(H$_2$O)$_q$] | 1 | 7.4 | 33.8 | (0)[a] | 35 |
| [Gd(NH$_2$PhDO3A)(H$_2$O)$_q$] | 1 | 17.6 | 36.2 | (+15.2)[a] | 35 |
| [Gd(B-DO3A)(H$_2$O)$_q$][b] | 2 | 55 | 40.8 | (+40.1)[a] | 36 |
| Dinuclear | | | | | |
| [pip{Gd(DO3A)(H$_2$O)$_q$}$_2$] | 2 | 1.5 | 34.2 | −12 | 14 |
| [bisoxa{Gd(DO3A)(H$_2$O)$_q$}$_2$] | 2 | 1.4 | 38.5 | +2 | 14 |
| {pX{Gd(DO3A)(H$_2$O)$_q$}$_2$} | 1 | 7.5 | 45.9 | +41 | 11 |
| {mX{Gd(DO3A)(H$_2$O)$_q$}$_2$} | 1 | 11 | 41.0 | +28 | 11 |
| {mX(COOH){Gd(DO3A)(H$_2$O)$_q$}$_2$} | 1 | 12 | 32.7 | +0.3 | 11 |
| [Mes{DO3A}{Gd(DO3A)(H$_2$O)$_q$}$_2$]$^{3-}$ | 1 | 32 | 25.8 | −14.7 | This work |
| Trinuclear | | | | | |
| [Mes{Gd(DO3A)(H$_2$O)$_q$}$_3$] | 2 | 32 | 25.8 | −14.7 | This work |

[a] Calculated from $k_{ex}^{298}$ and $\Delta H^{\ddagger}$; [b] Ligand **5** in ref. 36

$^{17}$O-NMR chemical shifts $\Delta\omega_r$. We had to develop a new alternative method, using pre-complexation with $Mg^{2+}$ and transmetallation, to achieve the complete complexation. Surprisingly, water exchange rate on both complexes, the negatively charged [Mes{DO3A}{Gd(DO3A)(H$_2$O)}$_2$]$^{3-}$ with $q$ = 1 and the neutral [Mes{Gd(DO3A)(H$_2$O)$_2$}$_3$] with $q$ = 2, is very similar, which is quite surprising. The measured rate constant is among the highest found so far on DO3A-type Gd-complexes (Table 2).

## Experimental

### Synthesis and characterization

NMR data: br s = broad signal; vbr s = very broad signal

Reagents were purchased from ACROS Organics and from ALDRICH Chemical Co. Elemental analyses were performed at the Service de Microanalyse, CNRS, 91198 Gif sur Yvette, France. NMR and mass spectrometry were investigated at the "services communs" of the University of Brest.

**Synthesis of 2.** Cyclen-glyoxal **2** was synthesized by direct condensation of glyoxal with cyclen **1** as previously reported.[16–19]

**Synthesis of 3.** To a solution of cyclen-glyoxal **2** (0.97 g, 5 mmol, 3.3 eq.) in anhydrous acetonitrile (5 mL) vigorously stirred at room temperature, 1,3,5-tris(bromomethyl)benzene (0.54 g, 1.5 mmol) in anhydrous acetonitrile (10 mL) was slowly added. When the addition was complete the reaction was allowed to proceed for 2 weeks. The solid was filtered off and dried in vacuum to give compound **3** (90%) as an oil. $^{13}$C NMR (100 MHz, D$_2$O, 298 K): Δ = 141.23 (CAr), 133.13 (CHAr), 86.38, 74.32 (CHaminal), 64.39 (αCH$_2$–Ar), 62.87, 60.02, 54.12, 51.03, 50.87, 50.33 (2), 46.35 (αCH$_2$) ppm. Anal. calcd. for C$_{39}$H$_{63}$Br$_3$N$_{12}$ (939.72): C 49.85, H 6.76, N 17.89; found: C 49.72, H 6.51, N 17.33.

**Synthesis of 4.** Compound **3** was refluxed in 10 mL of hydrazine hydrate for 2 h. After cooling, the solvent was removed to dryness to yield **4** (yield quantitative).$^{13}$C NMR (100 MHz, CDCl$_3$, 298 K): Δ = 138.9 (CAr), 129.4 (CHAr), 59.0 (αCH$_2$Ar), 51.0, 47.2, 46.6, 45.1(αCH$_2$). Anal. calcd. for C$_{33}$H$_{66}$N$_{12}$ (630.97): C 62.82, H 10.54, N 26.64; found: C 62.71, H 10.66, N 26.32. *m/z* (FAB-MS) 631.1 (MH$^+$, 100%).

**Synthesis of 5.** 1.57 g (9.3 eq) of ethyl bromoacetate in acetonitrile (mL) was slowly added to a solution of compound **4** (0.63 g, 1 mol) with K$_2$CO$_3$ in acetonitrile. The reaction was allowed to proceed to reflux for 24 h and the solution was filtered. After solvent evaporation, the residue was dissolved in water (20 mL) and extracted with chloroform (3 × 20 mL). The organic phase was dried with MgSO$_4$ and evaporated to dryness to yield **5** as a solid (93%). $^{13}$C NMR (100 MHz, CDCl$_3$, 298 K): Δ = 171.40 (CO), 138.71 (br s, CAr), 128.25 (br s, CHAr), 61.32 (CH$_2$CAr), 60.09 (*C*H$_2$CH$_3$), 55.24, 51.74, 51.59 (αCH$_2$), 14.03 (CH$_3$) ppm. Anal. calcd. for C$_{69}$H$_{120}$N$_{12}$O$_{18}$ (1405.78): C 58.95, H 8.60, N 11.96; found: C 59.01, H 8.72, N 11.59. *m/z* (FAB-MS) 1406.1 (MH$^+$, 100%). mp: 149 °C.

**Synthesis of 6.** Compound **5** was dissolved in a hydrochloric acid solution (6 N) and stirred at 80 °C for 12 h. After evaporation to dryness the compound was dissolved in water and evaporated (3 times). The product was dissolved in a small amount of water (5 mL) and eluted first through a column packed with a Dowex 50WX8 (H$^+$ form) cation exchange resin with ammonium hydroxide and then eluted through a column with a Dowex 1 × 2–200 (OH$^-$ form) anion exchange resin with hydrochloric acid. The compound was obtained in 93% yield as a maroon solid as an adduct with hydrochloric acid. $^{13}$C NMR (100 MHz, D$_2$O, 298 K): Δ = 176.54, 170.84 (vbr s, CO), 138.03 (vbr s, *C*$_{Arom}$–CH$_2$), 132.10 (vbr s, C$_{Arom}$), 58.93 (br s, *C*H$_2$–C$_{Arom}$), 55.83, 54.52, 51.53 (vbr s, CH$_2$) ppm. Anal. calcd. for C$_{51}$H$_{84}$N$_{12}$O$_{18}$. 12 HCl. 2.5 H$_2$O (1635.86): C 37.45, H 6.22, N 10.27, Cl 26.01; found: C 37.20, H 6.52, N 10.00 Cl 25.98. MS (ESI): *m/z* (%): 577.79 (50) [MH$_2^{2+}$], 385.36 (100) [MH$_3^{3+}$].

**Chromatography.** The purity of the ligand molecule was checked with gas chromatography (HP 6890 with a 20 m FFAP column specific for carboxylic acids). One single and pure compound was detected at 10.55 min.

**Preparation of stock solutions.** A 29.5 mM $Gd^{3+}$ solution in water was prepared from GdCl$_3$ (79.0 mg of GdCl$_3$ (0.3 mmol) in 10.0 mL H$_2$O). The exact concentration of the metal ion was measured by complexometric titration with Na$_2$H$_2$EDTA 5 mM in urotropine/HCl buffer and xylenol orange as metal indicator.

97.3 mg of the solid ligand **6** ($C_{51}H_{84}N_{12}O_{18}$. 12HCl·2.5$H_2O$, $M$ = 1635.86 g $mol^{-1}$, 59.48 μmol) was dissolved in 1.00 mL of water in order to obtain a theoretical 59.5 mM solution. The exact concentration of **6**, determined by complexometric back titration of a $Gd^{3+}$ excess with $Na_2H_2EDTA$ 5 mM in urotropine buffer and xylenol orange as metal indicator, was determined at 58.8 mM based on the formation of the complex $Gd_2$(**6**)$(H_2O)_2$.

**Complexation reactions.** All attempts to prepare the tris-Gd complex by mixing a ligand solution with an adequate amount of $Gd^{3+}$ solution failed. In all cases the bis-Gd complex $Gd_2$(**6**)$(H_2O)_2$ with an excess of free $Gd^{3+}$ was obtained. Finally solutions of $Gd_2$(**6**)$(H_2O)_2$ without free gadolinium ions were prepared by mixing a ligand solution with a $GdCl_3$ stock solution in a 1 : 2 stoichiometric ratio. The pH, which drops spontaneously after mixing to 1.2, was corrected to 5.8 by adding NaOH (0.01 M) (measured with a combined glass electrode on a Metrohm 713 pH meter, calibrated with Metrohm buffers). The solution was stirred overnight and finally heated to 60 °C under argon bubbling in order to remove carbon dioxide. The absence of free $Gd^{3+}$ was verified with the xylenol orange test.

The tris-Gd complex $Gd_3$(**6**)$(H_2O)_6$ was prepared by complexing **6** in a first step with $Mg^{2+}$ followed by transmetallation with $Gd^{3+}$. 11.8 mg of $MgCl_2·2H_2O$ (58 μmol, 3 eq.) in 100 μL $H_2O$ were added to the ligand solution (31.5 mg, 19.3 μmol, 1 eq.). The pH was set to 8.9 with NaOH (2 M) and the solution was stirred overnight. The following day, the pH was adjusted to 5.8 with HCl and the solution was added to 3 mL of a 29.50 mM $Gd^{3+}$ (88.5 μmol, 4.59 eq.) solution, previously adjusted to pH 5.9 with NaOH (0.1 M) and degassed with argon for 15 min. The transmetallation reaction was followed by relaxometry (see below). The excess of $Gd^{3+}$ and the released $Mg^{2+}$ were removed by size exclusion chromatography (Sephadex G-25 resin, eluted with water). The fractions containing the complex were identified by its yellow color and confirmed by UV (254 nm) on a TLC silica plate. The xylenol orange test was performed to indicate the absence of free ions. The collected fractions were dried and the solid complex was recovered.

**Analytics.** Gadolinium and carbon mass contents were measured by ICP-MS (Perkin–Elmer) and by elemental analysis, respectively. Gd/ligand ratios were calculated from Gd/C ratios assuming that carbons are only from the ligand (51 C atoms per ligand). The exact concentrations of the paramagnetic $Gd^{3+}$ were determined by bulk magnetic susceptibility (BMS)[37] at 25 °C on a Bruker DRX-400 NMR spectrometer.

### NMR measurements and data treatment

**Transmetallation.** The transmetallation from $Mg^{2+}$ to $Gd^{3+}$ was followed by NMR relaxometry at 25 °C and 30 MHz using a Bruker Minispec mq40. In a first step the longitudinal relaxation rate of the $GdCl_3$ solution was measured. In a second step the GdCl3 and $[Mg_3$(**6**)$(H_2O)_x]^{3-}$ solutions were mixed (8% excess of $Gd^{3+}$ with respect to the DO3A binding sites) at 25 °C and the solution degassed for 5 min. with argon. The relaxation rates were measured during 800 min after mixing at 10 minute intervals.

**$^1$H relaxometry.** Longitudinal relaxation rates ($R_1 = 1/T_1$) for a full NMRD profile were measured at $^1$H Larmor frequencies from 0.01 to 400 MHz using the following equipment: Stelar Spinmaster FFC relaxometer (0.01 to 20 MHz),[38] Bruker Minispec mq40 (30 and 40 MHz) and mq60 (60 MHz), and Bruker NMR spectrometers working at 100, 200 and 400 MHz. The measurements were made at 25.0 °C and 37.0 °C using $Gd^{3+}$ concentrations of 5.53 and 20.15 mM for $Gd_2$(**6**)$(H_2O)_4$ and 12.79 mM for $Gd_3$(**6**)$(H_2O)_6$.

**$^{17}$O-NMR spectroscopy.** Two $^{17}$O enriched solutions (2% in $^{17}$O obtained by diluting 20% $^{17}$O enriched normalized water, Isotec) were prepared with final concentrations of $Gd^{3+}$ 20.15 mM ([Mes(DO3A){Gd(DO3A)$(H_2O)_2$}$_2$]) and 20.61 mM ([Mes{Gd(DO3A)$(H_2O)_2$}$_3$]). Relaxation measurements ($R_1 = 1/T_1$ by the inversion-recovery method[39–40] and $R_2 = 1/T_2$ by the Carr–Purcell–Meiboom–Gill method[40–41]) and chemical shifts (using spherical samples to avoid susceptibility corrections[42]) were performed on a Bruker ARX-400 spectrometer (9.4 T, 54.2 MHz). Acidified water ($HClO_4$, pH = 3.0) was used as an external reference. In all measurements, the temperature was maintained by a Bruker B-VT 3000 temperature control unit, and was measured by a substitution technique.[43]

**Data treatment.** Solomon–Bloembergen–Morgan (SBM) theory[6–7] has been used for data analysis (for equations see ref. 7). $^1$H NMRD profiles, $^{17}$O relaxation and chemical shifts were fitted in a simultaneous fit using the Visualiseur/Optimiseur 3.5.0 program[44] running on a Matlab® 6.5 platform.

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## Notes and references

1 S. Laurent, L. Vander Elst and R. N. Muller, *Contrast Media Mol. Imaging*, 2006, **1**, 128–137.
2 A. E. Merbach, and É. Tóth, ed., *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, John Wiley & Sons Ltd, Chichester, 2001.
3 P. Caravan, *Chem. Soc. Rev.*, 2006, **35**, 512–523.
4 S. Trattnig, K. Pinker, A. Ba-Ssalamah and I.-M. Nöbauer-Huhmann, *Eur. Radiol.*, 2006, **16**, 1280–1287.
5 É. Tóth, L. Helm, and A. E. Merbach, in *Magnetic Resonance Contrast Agents*, ed. W. Krause, Springer, Heidelberg, 2002.
6 J. Kowalewski, D. Kruk, and G. Parigi, in *Advances in Inorganic Chemistry*, ed. R. Van Eldik and I. Bertini, Elsevier, San Diego, 2005, vol. 57, pp. 42–104.
7 L. Helm, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2006, **49**, 45–64.
8 L. Helm, *Future Med. Chem.*, 2010, **2**, 385–396.
9 P. Caravan, C. T. Farrar, L. Frullano and R. Uppal, *Contrast Media Mol. Imaging*, 2009, **4**, 89–100.
10 J. Costa, É. Tóth, L. Helm and A. E. Merbach, *Inorg. Chem.*, 2005, **44**, 4747–4755.
11 J. Costa, E. Balogh, V. Turcry, R. Tripier, M. Le Baccon, F. Chuburu, H. Handel, L. Helm, E. Tóth and A. E. Merbach, *Chem.–Eur. J.*, 2006, **12**, 6841–6851.
12 J. B. Livramento, L. Helm, A. Sour, C. O'Neil, A. E. Merbach and É. Tóth, *Dalton Trans.*, 2008, 1195–1202.
13 É. Tóth, S. Vauthey, D. Pubanz and A. E. Merbach, *Inorg. Chem.*, 1996, **35**, 3375–3379.

 

14 D. H. Powell, O. M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer and A. E. Merbach, *J. Am. Chem. Soc.*, 1996, **118**, 9333–9346.
15 É. Tóth, O. M. Ni Dhubhghaill, G. Besson, L. Helm and A. E. Merbach, *Magn. Reson. Chem.*, 1999, **37**, 701–708.
16 S. Develay, R. Tripier, M. Le Baccon, V. Patinec, G. Serratrice and H. Handel, *Dalton Trans.*, 2006, 3418–3426.
17 N. Bernier, M. Allali, R. Tripier, F. Conan, V. Patinec, S. Develay, M. Le Baccon and H. Handel, *New J. Chem.*, 2006, **30**, 435–441.
18 S. Develay, R. Tripier, N. Bernier, M. Le Baccon, V. Patinec, G. Serratrice and H. Handel, *Dalton Trans.*, 2007, 1038–1046.
19 A.-S. Delepine, R. Tripier and H. Handel, *Org. Biomol. Chem.*, 2008, **6**, 1743–1750.
20 J. Kotek, P. Hermann, P. Vojtisek, J. Rohovec and I. Lukes, *Collect. Czech. Chem. Commun.*, 2000, **65**, 243–266.
21 M. Le Baccon, F. Chuburu, L. Toupet, H. Handel, M. Soibinet, I. Dechamps-Olivier, J.-P. Barbier and M. Aplincourt, *New J. Chem.*, 2001, **25**, 1168–1174.
22 S. Develay, R. Tripier, F. Chuburu, M. Le Baccon and H. Handel, *Eur. J. Org. Chem.*, 2003, 3047–3050.
23 F. Chuburu, R. Tripier, M. Le Baccon and H. Handel, *Eur. J. Org. Chem.*, 2003, 1050–1055.
24 G. R. Weisman, M. E. Rogers, E. H. Wong, J. P. Jasinski and E. S. Paight, *J. Am. Chem. Soc.*, 1990, **112**, 8604–8605.
25 J. F. Desreux, E. Merciny and M. F. Loncin, *Inorg. Chem.*, 1981, **20**, 987–991.
26 K. Kumar, C. A. Chang, L. C. Francesconi, D. D. Dischino, M. F. Malley, J. Z. Gougoutas and M. F. Tweedle, *Inorg. Chem.*, 1994, **33**, 3567–3575.
27 K. Kumar, M. F. Tweedle, M. F. Malley and J. Z. Gougoutas, *Inorg. Chem.*, 1995, **34**, 6472–6480.
28 X. Zhang, C. A. Chang, H. G. Brittain, J. M. Garrison, J. Telser and M. F. Tweedle, *Inorg. Chem.*, 1992, **31**, 5597–55600.
29 O. V. Yazyev and L. Helm, *J. Chem. Phys.*, 2007, **127**, 084506.
30 É. Tóth, L. Helm, and A. E. Merbach, in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, ed. A. E. Merbach and É. Tóth, John Wiley & Sons, Chichester, 2001, pp. 45–119.
31 L. Helm, G. M. Nicolle and A. E. Merbach, *Adv. Inorg. Chem.*, **57**, 327–379.
32 P. H. Fries and E. Belorizky, *J. Chem. Phys.*, 2005, **123**, 124510.
33 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293–2352.
34 P. Hermann, J. Kotek, V. Kubicek and I. Lukes, *Dalton Trans.*, 2008, 3027–3047.
35 E. Terreno, P. Boniforte, M. Botta, F. Fedeli, L. Milone, A. Mortillaro and S. Aime, *Eur. J. Inorg. Chem.*, 2003, 3530–3533.
36 M. Botta, S. Quinci, G. Pozzi, G. Marzanni, R. Pagliarin, S. Barra and S. G. Crich, *Org. Biomol. Chem.*, 2004, **2**, 570–577.
37 D. M. Corsi, C. Platas-Iglesias, H. Van Bekkum and J. A. Peters, *Magn. Reson. Chem.*, 2001, **39**, 723–726.
38 G. Ferrante, and S. Sykora, in *Advances in Inorganic Chemistry*, ed. R. Van Eldik and I. Bertini, Elsevier, San Diego, 2005, vol. 57, pp. 405–470.
39 R. L. Vold, J. S. Waugh, M. P. Klein and D. E. Phelps, *J. Chem. Phys.*, 1968, **48**, 3831–3832.
40 T. C. Farrar, and E. D. Becker, *Pulse and Fourier transform NMR: introduction to theory and methods*, Academic Press, New York, 1971.
41 S. Meiboom and D. Gill, *Rev. Sci. Instrum.*, 1958, **29**, 688–691.
42 A. D. Hugi, L. Helm and A. E. Merbach, *Helv. Chim. Acta*, 1985, **68**, 508–521.
43 C. Ammann, P. Meier and A. E. Merbach, *J. Magn. Reson.*, 1982, **46**, 319–321.
44 F. Yerly, EPFL, Lausanne, edn, 2003.