Synthesis and Physicochemical Characterization of New C-Functionalized Derivatives of the Gadolinium(III) Complex with 3,6,10-Tris(carboxymethyl)-3,6,10-triazadodecanedioic Acid (H_5ttda) Exhibiting Fast Water Exchange – Potential Paramagnetic Reporters for Molecular Imaging

by **Sophie Laurent**, **Luce Vander Elst**, **Antoine Vroman**, and **Robert N. Muller***

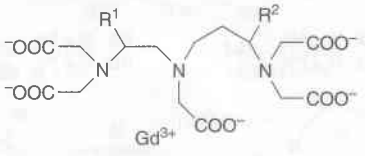
Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, B-7000 Mons
(phone/fax: +32-65-373520; e-mail: robert.muller@umh.ac.be)

To confirm the observation that [Gd(ttda)] derivatives have a significantly shorter residence time τ_M of the coordinated H_2O molecule than [Gd(dtpa)], four new C-functionalized [Gd(ttda)] complexes, [Gd(4-Me-ttda)] (**1**), [Gd(4-Ph-ttda)] (**2**), [Gd(9-Me-ttda)] (**3**), and [Gd(9-Ph-ttda)] (**4**), were prepared and characterized (H_5ttda = 3,6,10-tris(carboxymethyl)-3,6,10-triazadodecanedioic acid; H_5dtpa = 3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic acid). The temperature dependence of the proton relaxivity for these complexes at 0.47 T and of the ^{17}O transverse relaxation rate of $H_2{}^{17}O$ at 7.05 T confirm that the proton relaxivity is not limited by the H_2O-exchange rate. The residence time of the H_2O molecules in the first coordination sphere of the gadolinium complexes at 310 K, as calculated from ^{17}O-NMR data, is 13, 43, 2.9, and 56 ns for **1**, **2**, **3**, and **4**, respectively. At 310 K, the longitudinal relaxivity of **2** is higher than for the parent compound [Gd(ttda)] and the other complexes of the series. The stability of the new compounds was studied by transmetallation with Zn^{2+} ions. All the new complexes are more stable than the parent compound [Gd(ttda)].

Introduction. – Current research on tracers for magnetic resonance molecular imaging (MRMI) is devoted to the development of contrast agents with a high relaxivity and high specificity towards molecules overexpressed under some pathological conditions. One of the factors limiting the proton relaxivity of the gadolinium complexes is the exchange rate of the coordinated H_2O with bulk. An optimal H_2O residence time τ_M is comprised between 10 and 50 ns depending on the field strength [1]. Previous studies have shown that the C(4)-functionalized derivatives of [Gd(dtpa)] (H_5dtpa = 3,6,9-tris(carboxymethyl)-3,6,9-triazaundecanedioic acid) are characterized by a H_2O residence time τ_M that is shorter than that of the parent compound [2–4] and have a higher stability towards transmetallation by zinc, contrarily to the bis-amides that have longer τ_M and lower stability [5]. [Gd(ttda)] (H_5ttda = 3,6,10-tris(carboxymethyl)-3,6,10-triazadodecanedioic acid) has been shown to have a much faster H_2O-exchange rate than [Gd(dtpa)] [6][7], but its stability towards transmetallation by Zn^{2+} ions is very low. Similarly, [Gd(ttda)]-derived bis-amides are characterized by short τ_M values (20–30 ns at 310 K) but also show a very poor stability towards transmetallation [8].

In this work, four new C-functionalized [Gd(ttda)] complexes, [Gd(4-Me-ttda)] (**1**), [Gd(4-Ph-ttda)] (**2**), [Gd(9-Me-ttda)] (**3**), and [Gd(9-Ph-ttda)] (**4**) were

synthesized with the objective to combine the beneficial effects of the C-substitution previously observed, *i.e.*, to maintain a high-H_2O-exchange rate, and to increase the stability towards transmetallation by Zn^{2+} ions.



		R^1	R^2
1	[Gd(4-Me-ttda)]	Me	H
2	[Gd(4-Ph-ttda)]	Ph	H
3	[Gd(9-Me-ttda)]	H	Me
4	[Gd(9-Ph-ttda)]	H	Ph
5	[Gd(ttda)]	H	H

The new complexes were characterized by ^1H- and ^{17}O-NMR relaxometry at various temperatures with the objective to determine the H_2O residence time and its influence on the ^1H relaxivity. The ^1H-NMRD profiles were recorded at 310 K and analyzed by means of the classical model of the inner- and outer-sphere theories. The stability towards Zn^{2+} transmetallation was tested by a previously described procedure [5].

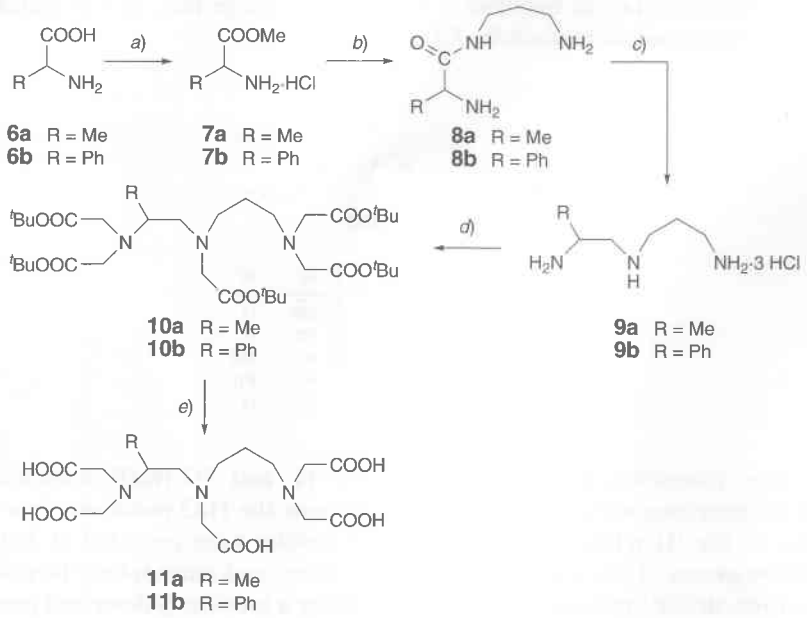
1. Results and Discussion. –1.1. *Syntheses.* Four C-functionalized H_5ttda derivatives were synthesized, two of them carrying a Me or Ph group at the ethane-1,2-diyl moiety (*Scheme 1*), and two of them carrying a Me or Ph group at the propane-1,3-diyl moiety (*Scheme 2*) bridge. The synthetic scheme is inspired from the literature [9].

C(4)-Substituted Derivatives **11**. The methyl ester hydrochloride **7** of the commercial α-amino acid **6** is treated with propane-1,3-diamine to give the corresponding amide **8** (*Scheme 1*). This amide is reduced, the obtained amine **9** alkylated with *tert*-butyl bromoacetate, and the pentaester **10** hydrolyzed to give the polyaminocarboxylic acid ligand **11**.

C(9)-Substituted Derivatives **17**. The methyl ester hydrochloride **13** of the commercial β-amino acid **12** is treated with ethane-1,2-diamine and the product **14** reduced. The final ligand **17** is obtained after alkylation of the 1,4,8-triazaoctane **15**, with *tert*-butyl bromoacetate (→**16**), followed by hydrolysis (*Scheme 2*).

1.2. *Physicochemical Characterization.* 1.2.1. *Proton Relaxivity.* The variation of the proton relaxivity r_1 [s^{-1} mM^{-1}] as a function of temperature reflects the temperature dependence of the inner- and the outer-sphere relaxation mechanisms. While the outer-sphere relaxivity always increases when the temperature is decreased, the inner-sphere relaxivity may either increase or decrease: if the H_2O exchange between the first coordination sphere and the bulk is very fast (*i.e.*, τ_M is smaller than the relaxation time of the bound nuclei T_{1M} over the whole temperature range), the inner-sphere relaxivity increases when temperature decreases. By contrast, the inner-sphere relaxivity decreases when the temperature is lowered if a slow-exchange regime is reached (τ_M is larger than T_{1M}).

Scheme 1. *Synthesis of H_5(4-Me-ttda)* (**11a**; R = Me) *and H_5(4-Ph-ttda)* (**11b**; R = Ph)



a) MeOH, HCl. *b*) Et_3N, Et_2O, propane-1,3-diamine. *c*) $BH_3 \cdot THF$. *d*) $BrCH_2COO^tBu$, $^iPr_2EtN$. *e*) HCl.

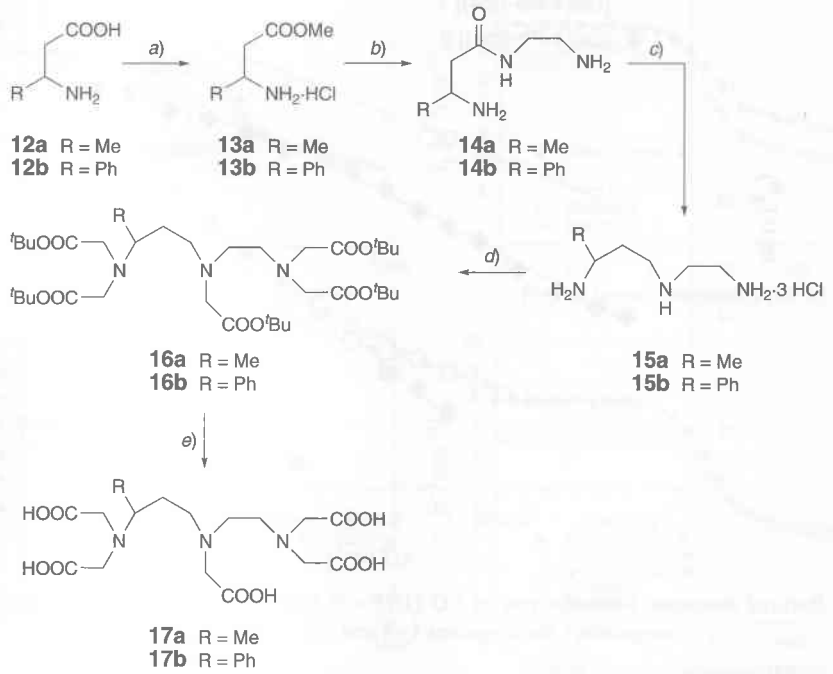
As a result, when the exchange rate is not limiting, the global relaxivity increases on decreasing the temperature. But if the exchange rate becomes limiting, the relaxivity tends to reach a plateau or even to decrease at low temperatures. Clearly, the relaxivity evolution observed for the four [Gd(ttda)] derivatives **1**–**4** shows that τ_M does not limit the proton relaxivity (*Fig. 1*).

1.2.2. *^{17}O-NMR Relaxometry.* The H_2O residence time τ_M can be calculated from the temperature dependence of the ^{17}O water transverse relaxation rate of the gadolinium complex solutions [10–14]. The theoretical adjustment of the experimental data depends on six parameters: ΔS^{+} and ΔH^{+}, the entropy and enthalpy of activation, respectively; $A/\hbar$, the hyperfine-coupling constant between the O-nucleus and the gadolinium; τ_V, the correlation time describing the electronic relaxation times; B, related to the amplitude of the zero field splitting energy; and E_V, the activation energy relative to τ_V.

The curves representing $\ln(1/T_2^R)$ obtained for the four C-functionalized [Gd(ttda)] derivatives **1**–**4**, as well as for the parent compound [Gd(ttda)] (**5**), do not reach a maximum in the temperature range investigated and are thus characteristic of a water exchange that is faster than that for [Gd(dtpa)] (*Fig. 2*).

The theoretical adjustment of the experimental data shows that the methyl derivatives [Gd(4-Me-ttda)] (**1**) and [Gd(9-Me-ttda)] (**3**) have τ_M values ranging between 3 and 13 ns at 310 K, whereas larger values (43 and 56 ns, resp.) are obtained for the phenyl derivatives [Gd(4-Ph-ttda)] (**2**) and [Gd(9-Ph-ttda)] (**4**) (*Table 1*). In these fittings, the number of coordinated H_2O-molecules was fixed to one to achieve a 9

Scheme 2. *Synthesis of H_5(9-Me-ttda)* (**17a**; R = Me) *and H_5(9-Ph-ttda)* (**17b**; R = Ph)



a) MeOH, HCl. *b*) Et_3N, Et_2O, ethane-1,2-diamine. *c*) $BH_3 \cdot THF$. *d*) $BrCH_2COO^tBu$, $^iPr_2EtN$. *e*) HCl.

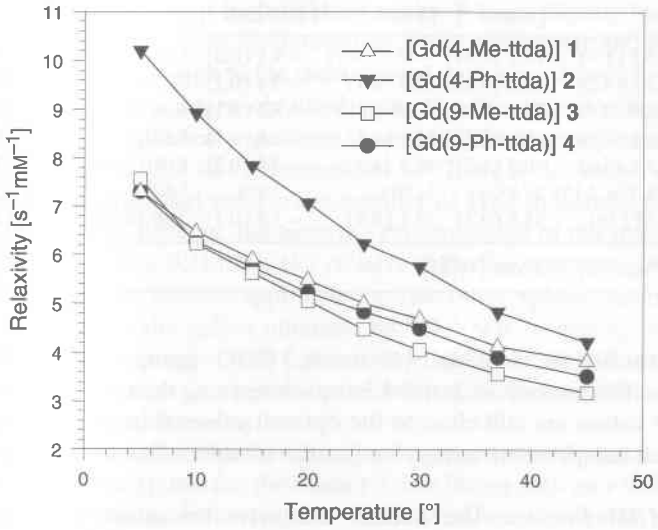


Fig. 1. *Temperature dependence of the proton longitudinal relaxivity* r_1 *of the [Gd(ttda)] derivatives* **1**–**4** ($B_0 = 0.47$ T)

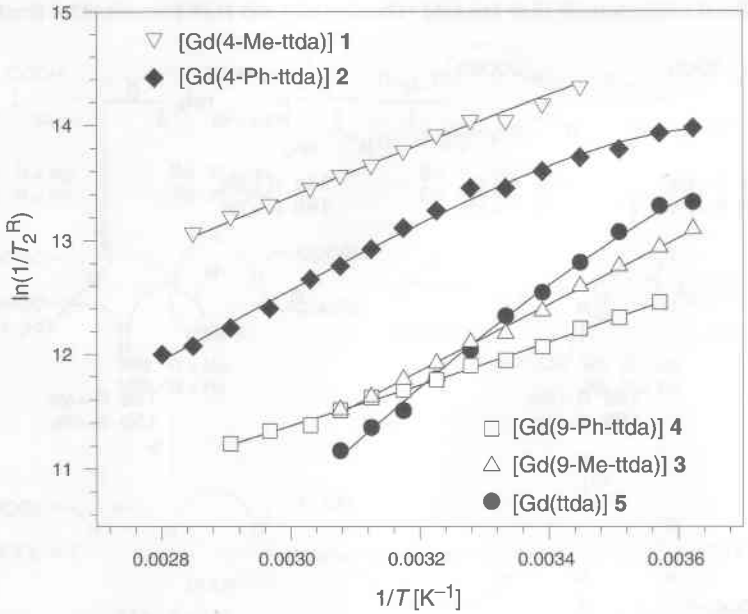


Fig. 2. *Reduced transverse relaxation rate of ^{17}O ($1/T_2^R = 55.55/(T_2^p * [\text{Gd-complex}])$) as a function of temperature for complexes* **1**–**4** *and the parent complex*

Table 1. *Parameters of the Theoretical Adjustment of the ^{17}O-NMR Transverse Relaxation Rate Evolution with Temperature.* Errors in parentheses.

	τ_M^{310} [ns]	ΔH^+ [kJmol^{-1}]	ΔS^+ [J mol^{-1} K^{-1}]	$A/\hbar$ [10^6 rads^{-1}]	B [10^{20} s^{-2}]	τ_V^{298} [ps]	E_V [kJ mol^{-1}]
1	13.3 (1.9)	15.3 (0.44)	−45.1 (0.9)	−4.1 (0.3)	1.26 (0.81)	26.2 (6.4)	20.0 (15.4)
2	43.3 (4.2)	35.6 (0.13)	10.8 (0.4)	−3.4 (0.1)	6.27 (0.1)	12.2 (0.2)	19.8 (0.4)
3	2.9 (0.5)	22.4 (0.03)	−9.6 (0.1)	−3.3 (0.1)	4.99 (0.6)	21.6 (2.7)	20.0 (19.0)
4	56.1 (22.8)	42.6 (0.22)	31.1 (3.3)	−2.8 (0.1)	5.57 (0.21)	2.4 (0.2)	12.6 (1.0)
[Gd/ttda)]	3.6 (3.6); 6.3[a]), 2.1[b])	40.3 (1.3); 27.9[b])	46.4 (4.0); 11.0[b])	−2.8 (0.3); −3.9[b])	4.01 (0.38); 1.82[b])	19.5 (1.7); 25[a]), 22.4[b])	0.9 (14.9); 1.6[a]), 1.0[b])
[Gd(ttda)][c])	143 (26)	51.5 (0.3)	52.1 (0.6)	−3.4 (0.1)	2.60 (0.06)	12.3 (0.3)	4.5 (4.2)

[a]) From [6]. [b]) From [7]. [c]) From [4][15].

coordination shell of the Gd^{3+} ion (3 N-atoms, 5 COO^- groups, and 1 H_2O molecule). It seems that the Ph derivatives **2** and **4** have a longer τ_M than the parent compound **5**. However, the values are still close to the optimal value; thus covalent or noncovalent binding of such complexes to a macromolecular structure should result in very efficient complexes.

1.2.3. *NMRD Profiles.* The nuclear magnetic relaxation dispersion (NMRD) profiles of aqueous solutions of the complexes are shown in *Fig. 3*. At low fields, [Gd(4-Me-ttda)] (**1**), [Gd(9-Me-ttda)] (**3**), and the parent compound [Gd(ttda)] (**5**) have

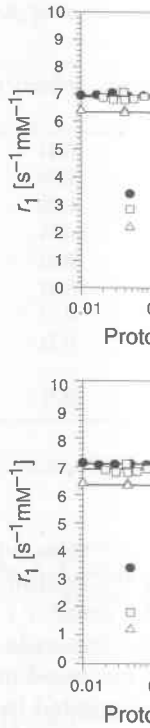


Fig. 3. *^1H-NMR Me-ttda)]* (3

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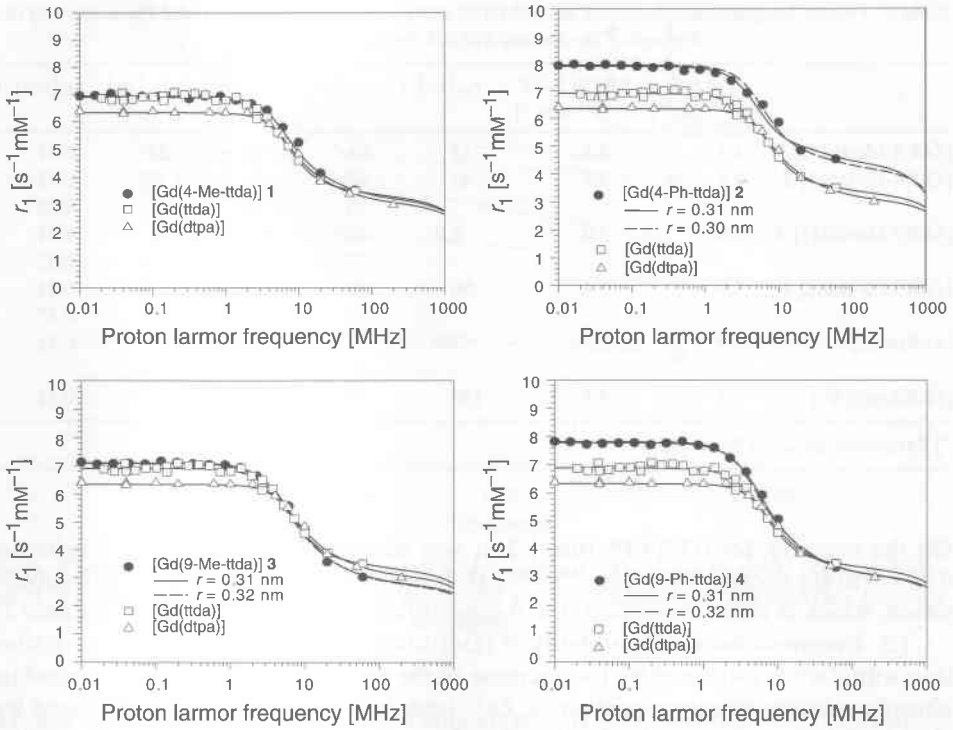

Fig. 3. *^{1}H-NMRD Profiles of aqueous solutions of [Gd(4-Me-ttda)]* (**1**), *[Gd(4-Ph-ttda)]* (**2**), *[Gd(9-Me-ttda)]* (**3**), *and [Gd(9-Ph-ttda)]* (**4**), *each compared to those of [Gd(ttda)] and [Gd(dtpa)]*

rather similar relaxivities, but at high fields, complex **3** has a slightly lower relaxivity. The relaxivity at low field of both Ph derivatives **2** and **4** is similar and larger than for the parent compound, but at high fields, the 4-phenyl derivative **2** has a larger relaxivity than [Gd(ttda)]. The parameters obtained from the theoretical adjustment of the NMRD curves with the classical equations describing the inner-sphere [16–17] and outer-sphere relaxations [18] are summarized in *Table 2*. In these fittings, some parameters were fixed to usual values: the number of H_2O molecules coordinated to the Gd^{3+} ion ($q = 1$), the distance between the proton nuclei of the inner-sphere H_2O molecule and the Gd^{3+} ($r = 0.31$ nm), the relative diffusion constant ($D = 3.3\ 10^{-9}$ m^2 s^{-1}), and the distance of closest approach for the outer sphere contribution ($d = 0.36$ nm). τ_M was fixed to the values obtained by ^{17}O-NMR. τ_V and τ_{SO} (the electronic relaxation time at zero field $\tau_{SO} = 5B\tau_V$), describing the electronic relaxation times, and τ_R (the rotational correlation time) were optimized for the outer-sphere and the inner-sphere contributions simultaneously.

For complex **1**, the τ_R value obtained is in good agreement with those characterizing [Gd(ttda)] and [Gd(dtpa)]; but for the isomer **3**, the fitting with an r value of 0.31 nm resulted in an unrealistic τ_R value ($\tau_R{}^{310} < 50$ ps). A more reasonable value ($\tau_R{}^{310} = 57$ ps) was obtained by increasing r to 0.32 nm. Similarly, for the 9-phenyl derivative **4**, a larger r value ($r = 0.32$ nm) had to be used to get an acceptable τ_R ($\tau_R{}^{310} = 68$ ps).

Table 2. *Proton Longitudinal Relaxivity at 0.47 T* (20 MHz) *and 1.4 T* (60 MHz) *and Parameters of the Fittings of the Proton NMRD Profiles* (*T* 310 K)

	r_1 at 0.47 T [s^{-1} mM^{-1}]	r_1 at 1.4 T [s^{-1} mM^{-1}]	τ_M [ns][a])	τ_R [ps]	τ_{SO} [ps]	τ_V [ps]	r [nm][a])
[Gd(4-Me-ttda)] **1**	4.1	3.5	13	61	96	23	0.31
[Gd(4-Ph-ttda)] **2**	4.8	4.5	43	97	91	22	0.31
				75	83	20	0.30
[Gd(9-Me-ttda)] **3**	3.6	3.0	2.9	48	148	14	0.31
				57	171	20	0.32
[Gd(9-Ph-ttda)] **4**	3.9	3.4	56	56	171	23	0.31
				68	196	38	0.32
[Gd(ttda)] **5**	3.9	3.5	3.6	64	90	11	0.31
				57			
[Gd(dtpa)] **6**[b])	3.8	3.4	143	54	87	25	0.31

[a]) Parameter fixed. [b]) From [15].

On the contrary, for [Gd(4-Ph-ttda)] **2**, it was necessary to decrease the r value to 0.30 nm to get a reasonable τ_R ($\tau_R^{310} = 75$ ps). It seems thus that contrary to the 4-substitution, which is rather beneficial, the 9-substitution negatively affects the distance r.

1.3. *Transmetallation.* The stability of [Gd(ttda)] derivatives towards transmetallation with Zn^{2+} is estimated by the decrease of the proton relaxation rate measured in phosphate-buffer solutions containing Zn^{2+} ions. When Gd^{3+} ions are substituted by Zn^{2+} ions, Gd^{3+} ions are indeed released and precipitate as phosphates. Consequently, the relaxation rate decreases according to the amount of released Gd^{3+} ions. In a first step, the stability of the complexes has to be tested in the phosphate buffer in the absence of Zn^{2+} ions. For [Gd(dtpa)] or [Gd(dtpa-bma)] (dtpa-bma = 6-(carboxymethyl)-3,9-bis[2-(methylamino)-2-oxoethyl]-3,6,9-triazaundecanedioic acid), no marked change of the relaxation rate is observed after several days, but for [Gd(ttda)] derivatives, the relaxation rates decrease markedly indicating a rather poor stability in phosphate buffer (*Fig. 4*). This decrease is larger for complex **1** than for the parent complex and smaller for complexes **2**–**4**.

After addition of Zn^{2+} ions, the relaxation-rate decrease is very fast for the methyl derivatives **1** and **3** and for the parent compound [Gd(ttda)] (*Fig. 5*) indicating a fast and large transmetallation process. On the other hand, the Ph substitution has a beneficial effect on stability. Among the two isomers, the 9-substituted derivative **4** is the best regarding stability. It is more stable than [Gd(dtpa-bma)], and its stability is only slightly lower than the one of [Gd(dtpa)]. Although markedly more stable than the parent compound, [Gd(4-Ph-ttda)] (**2**) is still less stable than the commercial [Gd(dtpa-bma)]. Thus, the Ph group seems to stabilize the [Gd(ttda)] structure, particularly when the substitution is at C(9).

2. Conclusions. – Four new [Gd(ttda)] derivatives have been synthesized, [Gd(4-Me-ttda)] (**1**), [Gd(4-Ph-ttda)] (**2**), [Gd(9-Me-ttda)] (**3**) and [Gd(9-Ph-(ttda)] (**4**), with the objective to obtain a paramagnetic reporter characterized by a very short τ_M in order to preserve a high relaxivity after coupling to the vector. From the analysis of the

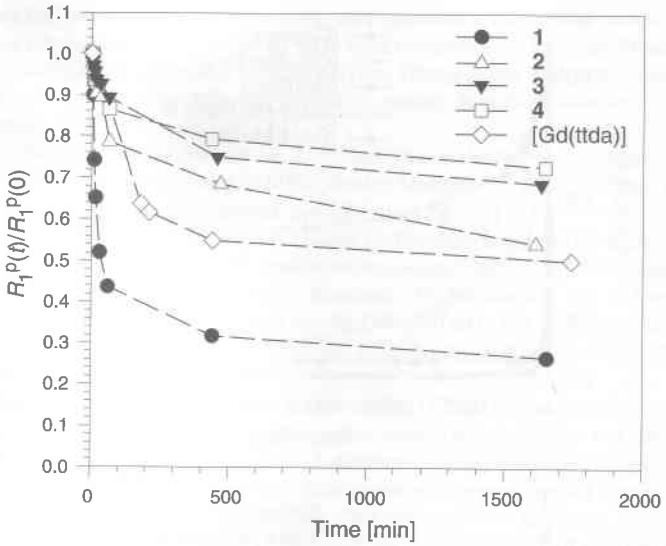

Fig. 4. *Evolution of the proton relaxation rate of [Gd(4-Me-ttda)]* (**1**), *[Gd(4-Ph-ttda)]* (**2**), *[Gd(9-Me-ttda)]* (**3**), *and [Gd(9-Ph-ttda)]* (**4**) *in phosphate-buffer solution.* The data for [Gd(ttda)] are given for comparison.

^{1}H- and ^{17}O-NMR relaxometric data, it appears that: *i*) All C-functionalized derivatives **1**–**4** have a τ_M value close to the optimal value in the imaging field range. *ii*) The Me derivatives [Gd(4-Me-ttda)] (**1**) and [Gd(9-Me-ttda)] (**3**) have a lower low-field relaxivity than the Ph derivatives [Gd(4-Ph-ttda)] (**2**) and [Gd(9-Ph-ttda)] (**4**). *iii*) Complexes **1**, **3**, and **4** have a high-field relaxivity similar to the parent complex [Gd(ttda)], whereas [Gd(4-Ph-ttda)] (**2**) has a relaxivity increased by *ca.* 30% at 1.4 T as compared to [Gd(ttda)] or [Gd(dtda)]. *iv*) The stability in the phosphate buffer solution is significantly increased for the two Ph derivatives **2** and **4** but not for the Me derivatives. *v*) The presence of Ph substituents has a beneficial effect on the transmetallation process by Zn^{2+} ions, with [Gd(9-Ph-ttda)] **4** showing the best stability. Indeed, 36% of the relaxivity of [Gd(9-Ph-ttda)] **4** is preserved after 4 days.

It turns out that both Ph derivatives [Gd(4-Ph-ttda)] (**2**) and [Gd(9-Ph-ttda)] (**4**) are interesting complexes since their relaxivity could reach very high values after inclusion in slowly tumbling systems. Actually, it can be assumed that the longitudinal relaxivity of [Gd(4-Ph-ttda)] (**2**) and [Gd(9-Ph-ttda)] (**4**) included in supramolecular structures with τ_R values ranging between 20 and 30 ns could reach values larger than 50 and 70 s^{-1} mM^{-1}, respectively, in the imaging field region and that their r_2 values could be larger than 150 s^{-1} mM^{-1}.

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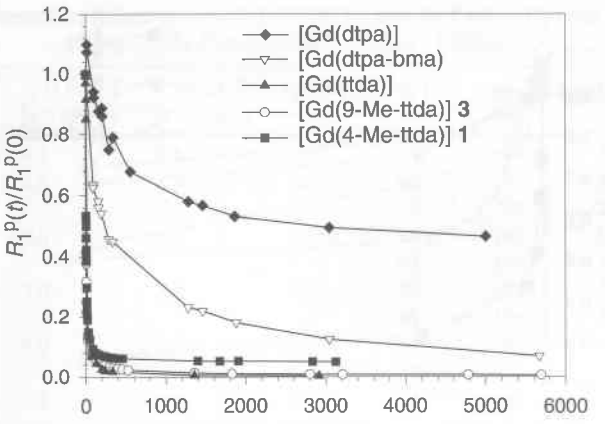

Fig. 5. *Stability with respect to transmetallation.* Data for [Gd(ttda)], [Gd(dtpa)], and [Gd(dtpa-bma)] are given for comparison.

Experimental Part

1. *General.* H- and ^{13}C-NMR Spectra: *Bruker-AMX-300* (*Bruker*, Karlsruhe, Germany), in D_2O or $CDCl_3$, for δ(C), t_{BuOH} as internal standard (Me at δ(C) 31.2). MS: *Q-Tof 2* mass spectrometer (*Micromass*, Manchester, UK); samples in MeOH/H_2O 1:1; injection rate 5 µl/min.

2. *^{17}O-NMR Spectroscopy.* ^{17}O-NMR Measurements of solns. were performed with 2-ml samples in 10-mm external diameter tubes and a *Bruker-AMX-300* spectrometer. The temp. was regulated by air or N_2 flow controlled by a *BVT-2000* unit. ^{17}O Transverse relaxation times of distilled water (pH 6.5–7) were measured by using a CPMG sequence and a subsequent two-parameter fit of the data points. The 90° and 180° pulse lengths were 25 and 50 µs, resp. The ^{17}O T_2 of water in a complex soln. was obtained from linewidth measurement. Broadband proton decoupling was applied during the acquisition of all ^{17}O-NMR spectra. Concentration of the samples was lower than 25 mM.

3. *^{1}H-NMRD Profiles.* Proton nuclear magnetic relaxation dispersion (NMRD) profiles were measured on a *Stelar Spinmaster FFC*, fast field cycling NMR relaxometer (*Stelar*, Mede (PV), Italy) over a range of magnetic fields extending from 0.24 mT to 0.35 T and corresponding to ^{1}H *Larmor*

frequencies from 0.01 to 15 MHz. Measurements were performed with 0.6-ml samples in 10-mm o.d. tubes. Additional relaxation rates at 20 and 60 MHz were obtained with a *Bruker-Minispec-PC-120* and *mq-60* spectrometer (*Bruker*, Karlsruhe, Germany), resp. Fitting of the ^{1}H-NMRD was adjusted with a data-processing software that uses different theoretical models describing observed nuclear-relaxation phenomena (*Minuit*, CERN Library) [19][20].

4. *Transmetallation.* Transmetallation by Zn^{2+} ions was evaluated by the decrease of the water longitudinal relaxation rate at 310 K and 20 MHz (*Bruker Minispec PC 20*) of buffered phosphate solns. (pH 7) containing 2.5 mM of the Gd-complex and 2.5 mM of Zn^{2+} [6].

5. *Synthesis of the Ligands* **11** *and* **17**. 5.1. *(S)-3,6,10-Tris(carboxymethyl)-4-methyl-3,6,10-triazadodecane dioic Acid* = N-*{(2S)-2-[Bis(carboxymethyl)amino]propyl}*-N-*{3-[bis(carboxymethyl)amino]propyl}glycine*; **11a**; H_5(4-Me-ttda)). *Methyl* L-*Alaninate Hydrochloride* **7a**. L-Alanine (**6a**; 10 g, 122.2 mmol) was suspended in HCl-saturated dry MeOH (200 ml). The soln. was stirred overnight at r.t. Then MeOH was evaporated: **7a** (98%). ^{1}H-NMR ($CDCl_3$): 4.2–4.1 (*m*, H–C(2)); 3.8 (*s*, MeO); 2.2 br. *s*, NH_2); 1.8 (*d*, *J* = 7, Me(3)).

N^1-*(3-Aminopropyl)*-L-*alaninamide* (**8a**). A soln. of **7a** (17.72 g) in MeOH (36 ml) was treated with Et_3N (26 ml) and Et_2O (390 ml) to liberate the amino ester. The precipitate was filtered off and the filtrate evaporated. To the residual oil, propane-1,3-diamine (147 ml) was added dropwise. The soln. was stirred for 19 h at r.t. The excess of propane-1,3-diamine was evaporated, MeOH (20 ml) was added, and the soln. again evaporated. These last 2 operations were repeated 3 × : **8a** (48%). Yellow oil which was used without further purification. ^{1}H-NMR ($CDCl_3$): 7.7 (br. *s*, NH, 2 NH_2); 3.7–3.6 (*m*, H–C(2)); 3.5 (*t*, *J* = 7, 1 CH_2); 2.8 (*t*, *J* = 7, 1 CH_2); 1.6–1.5 (*m*, 1 CH_2); 1.4 (*d*, *J* = 7, Me(3)).

(S)-2-Methyl-1,4,8-triazaoctane Trihydrochloride (2S)-N^1*-(3-Aminopropyl)propane-1,2-diamine Trihydrochloride*; **9a**). A soln. of **8a** (7.6 g) in THF (100 ml) was stirred under Ar at −10° for 30 min. Then 1M borane in THF (330 ml) was added dropwise, and the soln. was maintained at −10° for 1 h. Stirring was continued for 20 h under reflux. The mixture was cooled at −10° and anh. MeOH (33 ml) was injected to destroy the excess of borane. The mixture was allowed to warm up to r.t., and solvents were evaporated. Anh. MeOH (33 ml) was added and the mixture evaporated. The residue was recovered in anh. MeOH (132 ml) sat. with gaseous HCl and heated to reflux for 6 h. The mixture was cooled and left for 68 h at 0°. The precipitate was filtered, and the filtrate was distilled under reduced pressure. The residue was dissolved in H_2O and extracted with Et_2O. The org. phase was discarded and the aq. phase evaporated: **9a** (52%). ^{1}H-NMR (D_2O, pH *ca.* 6): 3.6 (*t*, *J* = 7, 1 CH_2); 3.4 (*t*, *J* = 7, 1 CH_2); 3.1–2.9 (*m*, 1 CH_2, CH); 1.9–1.85 (*m*, 1 CH_2); 1.2 (*d*, *J* = 7, Me).

*Di(*tert*-butyl) (S)-3,6,10-Tris[(2-*tert*-butoxy)-2-oxoethyl]-4-methyl-3,6,10-triazadodecanedioate* (= N-*{(2S)-2-{Bis[2-(*tert*-butoxy)-2-oxoethyl]amino}propyl}*-N-*{3-{bis[2-(*tert*-butoxy)-2-oxoethyl]amino}propyl}glycine* tert-*Butyl Ester*; **10a**). To a soln. of **9a** (6.55 g) and ^{i}Pr$_2$EtN (50 ml) in DMF (200 ml) at r.t., *tert*-butyl bromoacetate (26 ml) was added under N_2. Stirring was continued for 14 h at r.t. After filtration, the solvents were evaporated. The oil was dissolved in AcOEt (400 ml) and H_2O (150 ml). The aq. phase was extracted with AcOEt (3 × 50 ml). The org. phases were extracted with H_2O (50 ml) and sat. $NaHCO_3$ (50 ml). The org. phase was dried ($MgSO_4$) and concentrated and the residue purified by column chromatography (silica gel (*Merck 60*), AcOEt): **10a** (10%). ^{1}H-NMR ($CDCl_3$): 3.5–3.1 (*m*, 8 CH_2, CH); 2.8–2.7 (*m*, CH_2); 1.4 (*s*, 5 ^{t}Bu); 1.3 (*d*, *J* = 7, 1 Me).

Ligand **11a** (H_5(4-Me-ttda)). The pentaester **10a** was hydrolyzed with conc. HCl soln. (25 ml) for 24 h. The precipitate was discarded and the soln. washed with Et_2O (2 × 50 ml). The aq. phase was then evaporated and the product isolated by lyophilization: **11a** (52%). ^{1}H-NMR (D_2O): 4.0–3.9 (*m*, CH); 3.8 (*s*, 4 CH_2); 3.75 (*s*, 1 CH_2); 3.1–3.05 (*m*, 3 CH_2); 1.8–1.7 (*m*, 1 CH_2); 1.2 (*d*, *J* = 7, Me). ^{13}C-NMR (D_2O): 173.0; 171.3; 169.0; 168.9; 168.4; 63.8; 61.5; 59.9; 55.7; 54.5; 52.8; 52.6; 50.1; 49.3; 30.1; 13.4. EI-MS: 444 (40, $[M+Na]^+$), 422 (100, $[M+H]^+$).

5.2. *(S)-3,6,10-Tris(carboxymethyl)-4-phenyl-3,6,10-triazadodecane dioic Acid* = N-*{(2S)-2-[Bis(carboxymethyl)amino]-2-phenylethyl}*-N-*{3-[bis(carboxymethyl)amino]propyl}glycine*; **11b**; H_5(4-Ph-ttda). As described for **11a** (*Sect. 5.1*), from (2*S*)-2-phenylglycine methyl ester.

(2S)-N^1*-(3-Aminopropyl)-2-phenylglycinamide* (**8b**). Yield 98%. ^{1}H-NMR (D_2O): 7.4–7.2 (*m*, Ph); 4.4 (*s*, H–C(2)); 3.1 (*t*, *J* = 7, 1 CH_2); 2.5 (*t*, *J* = 7, 1 CH_2); 1.5–1.4 (*m*, 1 CH_2).

(S)-2-Phenyl-1,4,8-triazaoctane Trihydrochloride (= N^1-*{(2S)-2-Amino-2-phenylethyl}propane-1,3-diamine Trihydrochloride*; **9b**): Yield 54%. ^{1}H-NMR ($CDCl_3$): 7.4–7.2 (*m*, Ph); 4.0 (*t*, *J* = 7, CH); 3.9 (br. *s*, 2 NH_2, NH); 3.7 (*t*, *J* = 7, CH_2); 3.2 (*t*, *J* = 7, CH_2); 3.1 (*dd*, *J* = 13, 4, CH_2); 1.8–1.7 (*m*, CH_2).

Di(tert-*Butyl*) *(S)-3,6,10-Tris{2-*(tert-*butoxy)-2-oxoethyl}-4-phenyl-3,6,10-triazadodecanedioate* (= N-*{(2S)-2-{Bis{2-*(tert-*butoxy)-2-oxoethyl}amino}-2-phenylethyl}*-N-*{3-{bis{2-*(tert-*butoxy)-2-oxoethyl}amino}propyl}glycine* tert-*Butyl Ester*; **10b**): Yield 25%. ^{1}H-NMR ($CDCl_3$): 7.25–7.15 (*m*, 3 H, Ph); 7.1–7.0 (*m*, 2 H, Ph); 5.1 (*t*, *J* = 6, CH); 3.9 (*s*, 2 CH_2); 3.8 (*s*, 1 CH_2); 3.7 (*s*, 2 CH_2); 3.0 (*dd*, *J* = 13, 4, 1 CH_2); 2.5 (*t*, *J* = 7, 1 CH_2); 2.4 (*t*, *J* = 7, 1 CH_2); 2.2–2.1 (*m*, 1 CH_2).

Ligand **11b** (H_5(4-Ph-ttda)): Yield 67%. ^{1}H-NMR (D_2O): 7.5–7.3 (*m*, Ph); 5.1 (*t*, *J* = 6, CH); 4.1 (*s*, 1 CH_2); 4 (*d*, *J* = 7, 2 CH_2); 3.9 (*s*, 2 CH_2); 3.5–3.3 (*m*, 2 CH_2); 3.1 (*t*, *J* = 7, 1 CH_2); 2.1–2.0 (*m*, CH_2). ^{13}C-NMR (D_2O): 175.4; 172.9; 172.4; 169.1; 168.3; 139.4; 134.4; 133.3; 131.6; 60.1; 58.0; 55.8; 53.1; 52.1; 51.7; 50.2; 49.2; 48.3; 30.6. EI-MS: 508 (30, $[M + Na]^+$), 486 (100, $[M + H]^+$).

5.3. *(RS)-3,6,10-Tris(carboxymethyl)-9-methyl-3,6,10-triazadodecanedioic Acid* (= N-*{(3RS)-3-[Bis(carboxymethyl)amino]butyl}*-N-*{2-[bis(carboxymethyl)amino]ethyl}glycine*; **17a**; H_5(9-Me-ttda)). As described for **11a** (*Sect. 5.1*), from (3*RS*)-3-aminobutanoic acid, but with ethane-1,2-diamine instead of propane-1,3-diamine in the second step.

(3RS)-3-Aminobutanoic Acid Methyl Ester Hydrochloride (**13a**): Yield 98%. ^{1}H-NMR (D_2O): 3.8 (*s*, MeO); 3.1–2.7 (*m*, 1 CH_2); 2.5–2.4 (*m*, CH); 1.5 (*d*, *J* = 7, Me(4)).

(3RS)-3-Amino-N-*(2-aminoethyl)butanamide* (**14a**): Yield 92%. ^{1}H-NMR (D_2O): 3.2–3.1 (*m*, CH); 3.1–2.9 (*m*, 1 CH_2); 2.5 (*t*, *J* = 7, 1 CH_2); 2.1 (*t*, *J* = 7, 1 CH_2); 0.8 (*dd*, *J* = 10, 4, Me(4)).

(RS)-7-Methyl-1,4,8-triazaoctane Trihydrochloride = *(3RS)*-N^1-*(2-Aminoethyl)butane-1,3-diamine Trihydrochloride*; **15a**): Yield 58%. ^{1}H-NMR (D_2O): 3.7–3.0 (*m*, 3 CH_2, CH); 1.9–1.7 (*m*, 1 CH_2); 1.2 (*d*, *J* = 7, Me).

Di(tert-*butyl* (RS)-*3,6,10-Tris{2-*(tert-*butoxy)-2-oxoethyl}-9-methyl-3,6,10-triazadodecanedioate* (= N-*{(3RS)-3-{Bis{2-*(tert-*butoxy)-2-oxoethyl}amino}butyl}*-N-*{2-{bis{2-*(tert-*butoxy)-2-oxoethyl}amino}ethyl}glycine* tert-*Butyl Ester*; **16a**): Yield 30%. ^{1}H-NMR ($CDCl_3$): 3.6 (*s*, 1 CH_2); 3.5–3.1 (*m*, CH, 5 CH_2); 2.9–2.7 (*m*, 2 CH_2); 1.7–1.4 (*m*, CH_2, 5 ^{t}Bu); 1.0 (*dd*, *J* = 10, 4, Me).

Ligand **17a** (H_5(9-Me-ttda)): Yield 55%. ^{1}H-NMR (D_2O): 3.7 (*s*, 1 CH_2); 3.2–2.5 (*m*, CH, 5 CH_2); 2.5 (*t*, *J* = 7, 1 CH_2); 2.4 (*t*, *J* = 7, 1 CH_2); 1.8–1.7 (*m*, 1 CH_2); 1 (*dd*, *J* = 10, 7, Me). ^{13}C-NMR (D_2O): 176.7; 173.0; 171.2; 169.1; 168.4; 63.9; 63.7; 60.0; 56.3; 55.7; 54.7; 53.4; 53.2; 49.3; 32.2; 13.5. EI-MS: 466 (26, $[M + 2 Na]^+$), 444 (42, $[M + Na]^+$), 422 (100, $[M + H]^+$).

5.4. *(RS)-3,6,10-Tris(carboxymethyl)-9-phenyl-3,6,10-triazadodecanedioic Acid* (= N-*{2-[Bis(carboxymethyl)amino]ethyl}*-N-*{(3RS)-3-[bis(carboxymethyl)amino]-3-phenylpropyl}glycine* (**17b**; H_5(9-Ph-ttda)). As described for **11a** (*Sect. 5.1*), from (3*RS*)-3-amino-3-phenylpropano acid but with ethane-1,2-diamine instead of propane-1,3-diamine in the second step.

(3RS)-3-Amino-3-phenylpropanoic Acid Methyl Ester Hydrochloride (**13b**): Yield 98%. ^{1}H-NMR ($CDCl_3$): 8.7 (br. *s*, NH_2); 7.5–7.4 (*m*, 2 H, Ph); 7.3–7.1 (*m*, 3 H, Ph); 4.6 (*t*, *J* = 7, CH); 3.8 (*s*, MeO, 3 H); 3.3 (*dd*, *J* = 10, 4, 1 CH_2).

(3RS)-3-Amino-N-*(2-aminoethyl)-3-phenylpropanamide* (**14b**): Yield 98%. ^{1}H-NMR ($CDCl_3$): 7.4–7.2 (*m*, Ph); 4.3 (*t*, *J* = 7, CH); 3.2–3.1 (*m*, 1 CH_2); 2.9–2.8 (*m*, 2 CH_2); 1.7 (br. *s*, 2 NH_2, NH).

7-Phenyl-1,4,8-triazaoctane Trihydrochloride (= *(1RS)*-N^3-*(2-Aminoethyl)-1-phenylpropane-1,3-diamine*; **15b**): Yield 58%. ^{1}H-NMR ($CDCl_3$): 7.5–7.4 (*m*, 2 H, Ph); 7.3–7.2 (*m*, 3 H, Ph); 5 (br. *s*, 2 NH_2, NH); 4.3 (*t*, *J* = 7, CH); 3.1–2.8 (*m*, 2 CH_2); 1.65–1.55 (*m*, 1 CH_2); 1.5–1.4 (*m*, 1 CH_2).

Di(tert-*Butyl*) (RS)-*3,6,10-Tris{2-*(tert-*butoxy)-2-oxoethyl}-9-phenyl-3,6,10-triazadodecanedioate* (= N-*{2-{Bis{2-*(tert-*butoxy)-2-oxoethyl}amino}ethyl}*-N-*{(3RS)-3-{bis{2-*(tert-*butoxy)-2-oxoethyl}amino}-3-phenylpropyl}glycine* tert-*Butyl Ester:* Yield 27%. ^{1}H-NMR ($CDCl_3$): 7.3 (*s*, Ph); 3.7 (*t*, *J* = 6, CH); 3.5 (*s*, 2 CH_2); 3.45 (*s*, 2 CH_2); 3 (*s*, CH_2); 2.8–2.5 (*m*, 4 CH_2); 1.4 (*s*, 5 ^{t}Bu).

Ligand **17b** (H_5(9-Ph-ttda)): Yield 92%. ^{1}H-NMR (D_2O): 7.5–7.4 (*m*, Ph); 4.4 (*t*, *J* = 6, CH); 3.8–3.5 (*m*, 5 CH_2); 3.3–3.0 (*m*, 3 CH_2); 2.5–2.4 (*m*, 1 CH_2). ^{13}C-NMR (D_2O): 176.5; 170.7; 169.7; 168.2; 167.9; 137.2; 130.8; 130.0; 128.4; 61.4; 60.7; 59.6; 55.2; 55.1; 53.9; 51.6; 50.8; 49.8; 31.4. EI-MS: 530 (30, $[M + 2 Na]^+$), 486 (100, $[M + H]^+$).

6. *Synthesis of the Corresponding Gd-Complexes.* The Gd^{3+} complexes were prepared by mixing aq. solns. of equimolar amounts of hexahydrated $GdCl_3$ and one of the ligands **11a**,**b** or **17a**,**b**. The pH was

adjusted to 6.5–7
The mass of the c
{N-{(2S)-2-{
carboxymethyl)ar
$[M + 2 Na]^+$).
amino]propyl}gly
{N-{(3RS)-3-[(B
gadolinate(2–) (
no]ethyl}-N-*{(3R*
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adjusted to 6.5–7 with NaOH. The absence of free Gd^{3+} ions was checked with arsenazo(III) indicator. The mass of the complexes was confirmed by ES-MS.

*{*N*-{(2*S*)-2-[Bis(carboxymethyl)amino]propyl}*-N*-{3-[bis(carboxymethyl)amino]propyl}glycinato(5−)}gadolinate(2−)* (**1**; [Gd(4-Me-ttda)]): ES-MS: 620 (100, $[M+2\,Na]^+$). *{*N*-{(2*S*)-2-[Bis(carboxymethyl)amino]-2-phenylethyl}*-N*-{3-[bis(carboxymethyl)amino]propyl}glycinato(5−)}gadolinate(2−)* (**2**; [Gd-4-Ph-ttda)]): ES-MS: 682 (100, $[M+2\,Na]^+$). *{*N*-{(3*RS*)-3-[(Bis(carboxymethyl)amino]butyl}*-N*-{2-[bis(carboxymethyl)amino]ethyl}glycinato(5−)}gadolinate(2−)* (**3**; [Gd(9-Me-ttda)]): ES-MS: 620 100, $[M+2\,Na]^+$). *{*N*-{2-[Bis(carboxymethyl)amino]ethyl}*-N*-{(3*RS*)-3-[bis(carboxymethyl)amino]-3-phenylpropyl}glycinato(5−)}gadolinate(2−)* (**4**; [Gd(9-Ph-ttda)]): ES-MS: 682 (100, $[M+2\,Na]^+$).

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