

An Assessment of the Potential Relationship between the Charge of Gd–DTPA Complexes and the Exchange Rate of the Water Coordinated to the Metal

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The residence time of coordinated water (τ_M), a parameter of paramount importance for relaxivity, is compared in a series of Gd–DTPA derivatives carrying different global charges. The importance of the charge in the first coordination sphere of the metal and in its vicinity is reported. Neutral bisester and bisamide complexes have significantly different τ_M values. The paradoxical similarity of the exchange rates of bisester complexes and Gd–DTPA, on the one hand, and the large difference between neutral bisester and bisamide complexes, on the other hand, suggest that the charge inside the first coordination sphere is not the factor dominating the water exchange rate, which seems therefore more sensitive

to the steric congestion. The study of Gd–(S)-N₆-carboxymethyl–DTPA, Gd–DTPA–BHydroxamide at basic pH, and Gd–DTPA–BAlaA clearly shows that anionic groups located outside the first coordination sphere have a beneficial influence on the value of τ_M , but that this effect strongly decreases with an increasing distance between the anionic group and the coordinated water. These conclusions underline the influence of steric hindrance and of the charged groups present in close proximity of the first coordination sphere on the exchange rate of the coordinated water. (© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2008)

Introduction

The relaxivity of the paramagnetic gadolinium complexes used as MRI contrast agents can be influenced by the residence time of the coordinated water molecule(s) τ_M .^[1,2] It has been suggested that this parameter could be dependent on the effective charge of the gadolinium complex.^[3] Indeed, if the positive charge of the gadolinium ion is more than compensated by the negative charges of the ligand, the interaction of the gadolinium ion with the oxygen atom of the coordinated water could be weaker and consequently could result in a faster water exchange rate, if it is assumed that the transition states have approximately the same energy. Furthermore, repulsive electrostatic interaction between oxygen and the negative charges of the ligands could also favor the expulsion of the water outside the coordination sphere. This could explain the slower water exchange rate of “neutral” complex Gd–DTPA–BMA (4) [DTPA–BMA: diethylenetriaminepentaacetic bis(methylamide)] and other bisamide complexes as compared to that of the negatively charged Gd–DTPA (1).^[4,5] The positively charged macrocyclic DOTA tetraamide [DOTA: 1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecane] has been reported by Aime et al. to have an exchange rate approximately 30 to 70 times slower than that of Gd–

DOTA.^[6] The same group has shown that negatively charged complexes like the triphosphonate macrocycle Gd–PCTP–[12] {PCTP: 3,6,9,15-tetraazabicyclo[9.3.1]pentadecan-1(15),11,13-triene-3,6,9-tris(methanephosphonic) acid} presented a very fast exchange rate.^[7] Kotek et al.^[8] and Leb-duskova et al.^[9] reported that Gd–DTPA derivatives with a phosphorus containing arm on the central nitrogen atom have shorter τ_M values than Gd–DTPA. Corsi et al. have studied Gd–(S,S,S,S)-THP [THP: 1,4,7,10-tetrakis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane] and found that, contrary to other tricationic complexes, it exhibits a faster water exchange rate than Gd–DOTA.^[10] Woods et al. have shown that the diastereoisomers of Gd–(tetracarboxyethyl)-1,4,7,10-tetraazacyclododecane with the same charge have different exchange rates.^[11] The water molecule is exchanged much faster in twisted square-antiprismatic (TSA) isomer than in square antiprismatic (SA) isomer. Very recently,^[12] Hermann et al. suggested that the faster water exchange in the TSA isomer as compared to the SA isomer is related to the greater flexibility of the TSA structure.

It appears, thus, that the influence of the charge of the complex on the water exchange rate is not a trivial matter and should be further investigated. In this study, we report more data on Gd–DTPA derivatives and try to shed some light on the problem. Our strategy relies on two complementary approaches. Firstly, we evaluate the importance of the charge of the complex inside the metal coordination sphere by studying (i) “neutral” bisester complexes [Gd–DTPA–BME (2) and Gd–DTPA–BBnE (3)] and bisamide analogues [Gd–DTPA–BMA (4) and Gd–DTPA–BiPA

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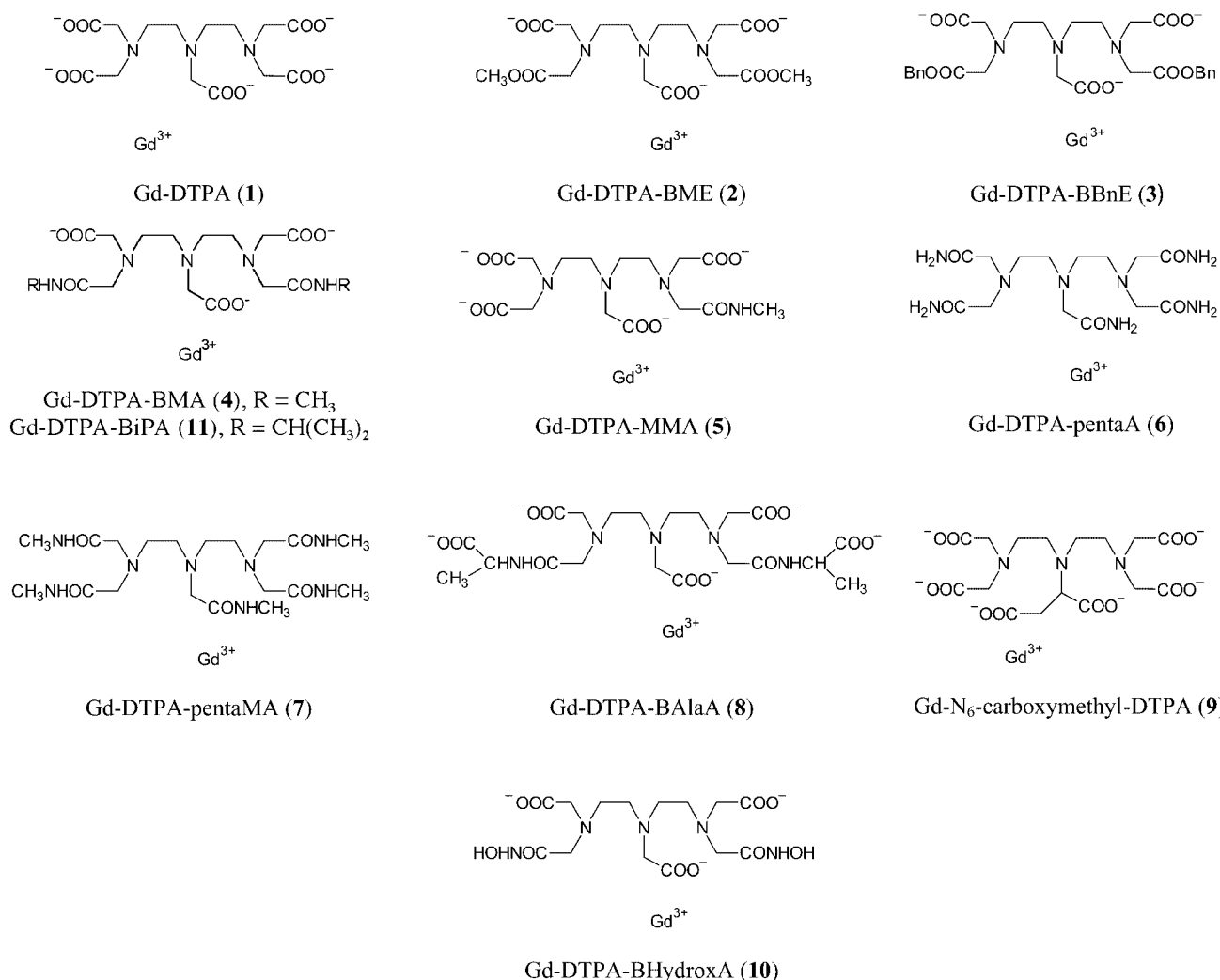


Figure 1. Structures of Gd-DTPA derivatives 1–11.

(11)], (ii) a negatively charged monoamide derivative [Gd-DTPA-MMA (5)], and (iii) positively charged pentamide complexes [Gd-DTPA-PentaA (6) and Gd-DTPA-PentaMA (7)]. Secondly, we investigate the influence of charged groups located close to the first coordination sphere of the metal through the study of Gd-DTPA-BalaA (8), Gd-N₆-carboxymethyl-DTPA (9), and Gd-DTPA-BHydroxA (10) (Figure 1). [BME: bis(methyl ester), BBnE: bis(benzyl ester), MMA: mono(methylamide), pentaA: pentaamide, pentaMA: pentakis(methylamide), BalaA: bis(alanineamide), BHydroxA: bis(hydroxyamide), BiPA: bis(isopropylamide)]

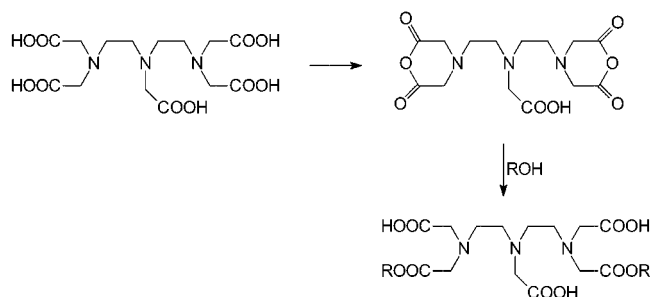
Results and Discussion

Synthesis of the DTPA Ligands

Synthesis of DTPA-Bisesters

DTPA-bisesters can be synthesized either by treating DTPA-bisanhydride with alcohol^[13] (Scheme 1) or by activation of the carboxyl groups of DTPA with isobutyl-

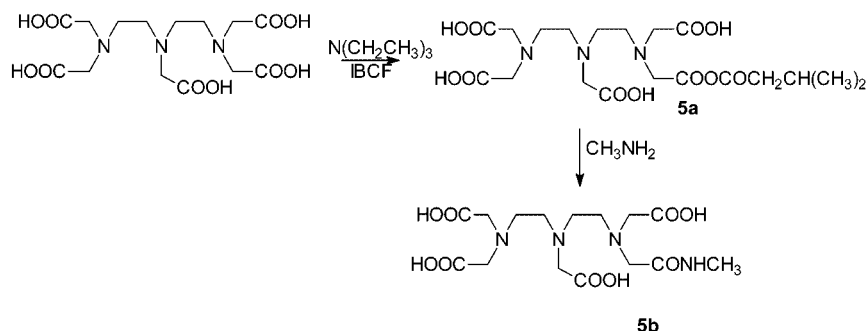
chloroformate.^[14] In previous work, we reported the synthesis of Gd-DTPA-BBnE (3) by reaction between DTPA-bisanhydride and benzyl alcohol.^[15] The same experimental conditions were used to obtain Gd-DTPA-BME (2).



Scheme 1. Synthesis of the DTPA-bisesters 2a and 3a.

Synthesis of DTPA-Monoamides

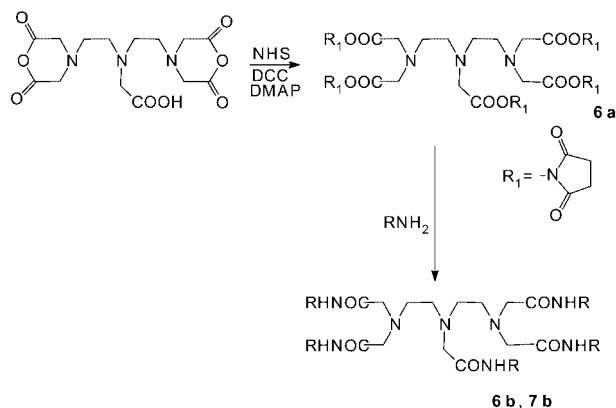
DTPA was treated with triethylamine in anhydrous acetonitrile as described by Krejcarek^[16] and Sherry.^[14] Iso-

Scheme 2. Synthesis of DTPA–MMA **5b**.

butyl chloroformate was then added to pentatriethylammonium–DTPA just before the addition of an excess amount of methylamine (Scheme 2).

Synthesis of DTPA–Pentamides

Pentamide derivatives can be prepared from the activated pentaester [obtained *in situ* by action of 4-dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS)] and an excess amount of the amine (Scheme 3).

Scheme 3. Synthetic scheme of pentaamide ligands **6b** ($R = \text{CH}_3$) and **7b** ($R = \text{H}$).

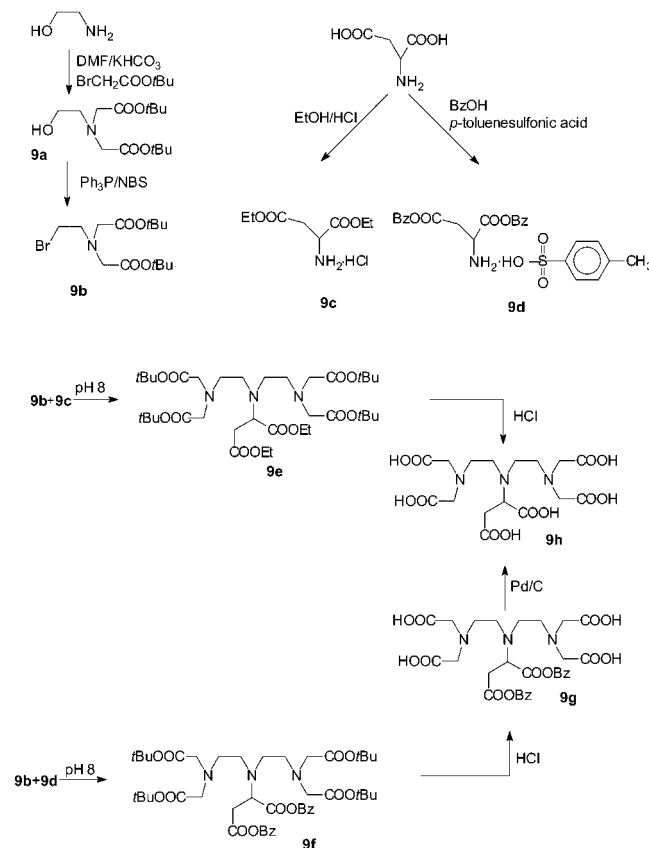
Synthesis of DTPA–BAlaA

The main difficulty in the synthesis of Gd–DTPA–bisamides from amino acids is the low solubility of amino acids in organic solvents. The addition of an aqueous amino acid solution to the DTPA–bisanhidride leads to a mixture containing DTPA, DTPA–bisamide, and DTPA–monoamide. Alanine being slightly soluble in ethanol, a mixture of solvents that limits hydrolysis was used as well as a large excess of amino acid in order to reduce the production of the monosubstituted derivative. The purification was carried out by precipitation and ion exchange chromatography.

Synthesis of (S)-N₆-Carboxymethyl–DTPA

The synthesis of the DTPA derivatives substituted on the α position of the central carboxyl group has been described by Williams et al.^[17] Amedio et al. have reported the synthesis of (S)-N₆-carboxymethyl–DTPA ester from L-as-

partic acid.^[18] To synthesize Gd-(S)-N₆-carboxymethyl–DTPA (**9**; Scheme 4), ethanolamine was dialkylated with butyl bromoacetate. Intermediate **9a** was then converted into its halogenated equivalent **9b** with *N*-bromosuccinimide. L-Aspartic acid was transformed into diethyl ester **9c** or into dibenzyl ester **9d** to increase the solubility in apolar media.

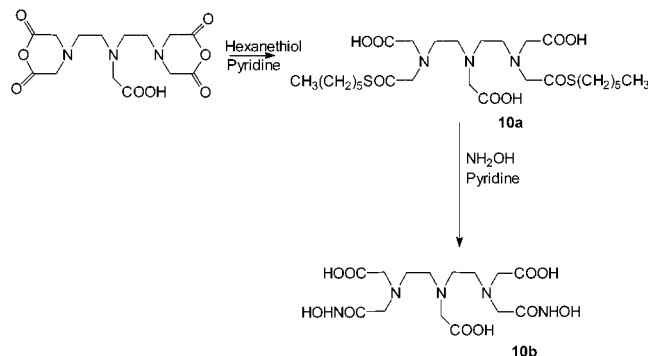
Scheme 4. Synthetic scheme of (S)-N₆-carboxymethyl–DTPA (**9h**).

Intermediates **9e** or **9f** were obtained from intermediates **9b** and **9c** or **9b** and **9d**, respectively, and were purified by chromatography on silica. (S)-N₆-carboxymethyl–DTPA (**9h**) was obtained by simple cleavage of ester **9e** in acidic medium. Benzyl ester **9f** can theoretically be obtained by selective cleavage of the *tert*-butyl ester in hydrochloric acid at 25 °C, but actually, a mixture of partially hydrolyzed

compounds **9f** and **9g** was obtained. This mixture was submitted therefore to catalytic hydrogenation to obtain compound **9h**.

Synthesis of DTPA-BHydroxA

Hydroxamic acid was synthesized from the activated ester or from the chloride derivative, which reacts with the hydroxylamine in alkaline or alcoholate medium.^[19] EDTA bishydroxamide was synthesized from the EDTA ethyl ester.^[20] Turowski et al. synthesized a DTPA-BHydroxA derivative from the bisanhydride.^[21] Under the same conditions, the reaction between hydroxylamine and DTPA-bisester or DTPA-bisanhydride led to a mixture containing DTPA. The complete separation of DTPA-BHydroxA (**10b**) and DTPA was not possible by usual purification techniques. Therefore, DTPA-bis(hexylthio)ester (**10a**) was used. This compound has the advantage of being soluble in DMF and being sufficiently reactive to provide the desired product (Scheme 5). DTPA-BHydroxA (**10b**) precipitates during the reaction, because of its low solubility in DMF.



Scheme 5. Synthetic scheme of DTPA-BHydroxA (**10b**).

Synthesis of the Gd Complexes

All complexes were prepared by treating the ligands with gadolinium chloride, and the absence of uncomplexed Gd ions was confirmed by using the Arsenazo test. However, this test was not useful for Gd-DTPA-pentamide complexes **6** and **7**. Indeed, the replacement of two DTPA carboxylate groups by methylamide functions is known to decrease the stability constant of the gadolinium complex by five orders of magnitude ($K_{\text{stability}} = 10^{17} \text{ M}^{-1}$).^[22] By comparing the stability constants of Gd-DTPA-bispropylamide and Gd-DTPA-monopropylamide, Sherry et al. reported that the replacement of one carboxylate by an amide function decreases the stability constant by three orders of magnitude approximately.^[14] Accordingly, the stability constant of the gadolinium DTPA-pentamide complexes can be estimated to be approximately 10^7 to 10^8 M^{-1} . This value is close to the stability constant of Gd^{3+} -Arsenazo III or Gd^{3+} -xylenol orange ($\approx 10^6 \text{ M}^{-1}$) reported by Sherry et al.^[14] and Barge et al.^[23] and explains why the xylenol orange or Arsenazo III tests were positive for Gd-DTPA-pentamide complexes **6** and **7**. These procedures were thus inadequate

to test for the absence of free gadolinium ions in these two complexes. Therefore, the decrease in the proton relaxation rate was measured after successive additions of minute amounts of ligand to a Gd solution until it reached a plateau typical of the gadolinium complex, testifying so to the complete complexation.

Proton Relaxivity of the Gadolinium Complexes at 20 MHz and Various Temperatures

Proton relaxivity, defined as the proton relaxation rate increase induced by one millimole per liter of contrast agent, reflects the efficacy of MRI contrast agents. It arises mainly from the contributions of short distance interactions between the paramagnetic ion and the coordinated water molecule(s) exchanging with the bulk (the so-called inner-sphere interaction) and from the long-distance interactions related to the diffusion of water molecules near the paramagnetic center (the outer-sphere interaction). The first contribution can be limited by the water residence time and this situation can be easily detected by the qualitative analysis of the proton relaxivity at 20 MHz on decreasing the temperature. Indeed, when temperature is decreased, the outer-sphere contribution always increases, whereas the inner-sphere contribution can either increase if the water residence time (τ_M) is smaller than the relaxation time of the hydrogen nuclei of these bound water molecule(s) (T_{1M}) or decrease if τ_M is larger than T_{1M} .

The evolution of the relaxivity of bisester complexes **2** and **3** as a function of temperature demonstrates their similarity with Gd-DTPA (**1**; Figure 2a): the continuous increase in the relaxivity when temperature decreases shows that the exchange rate is not limiting the relaxivity even at the lower temperatures. The absence of hydrolysis of bisester **2** has been thoroughly investigated by mass spectrometry. The relaxivity of complex **9** presents a similar temperature evolution, but the absolute values are larger than those of the parent complex **1** (Figure 2c). This could be related to the slightly larger molecular weight and hence to a slower tumbling of the complex. The relaxivity of Gd-DTPA-MMA (**6**) shows a slight limitation at low temperatures suggesting some quenching of the relaxivity by the water residence time (Figure 2b). This type of phenomenon, but to a larger extent, is observed for bisamide derivatives **4**, **8**, and **11** and leads to a plateau at temperatures lower than 293 K (Figure 2b). Both pentamide complexes **6** and **7** present a particular behavior: no change in the relaxivity is observed between 318 and 293 K, whereas below 293 K an increase in the relaxivity appears as the temperature is decreased (Figure 2b). This behavior can be explained by a very slow water exchange rate and thus a limited inner-sphere contribution at all temperatures. At temperatures lower than 293 K, the limitation of the inner-sphere contribution is so important that its contribution to the global relaxivity is negligible and the outer-sphere contribution becomes predominant. Consequently, the relaxivity increases as the temperature decreases due to slower translational motions of

water and of the complexes. At temperatures greater than 293 K, both inner-sphere and outer-sphere contributions are active but their temperature evolution is opposite. As a result, the total relaxivity is invariant in the temperature range extending from 293 to 318 K.

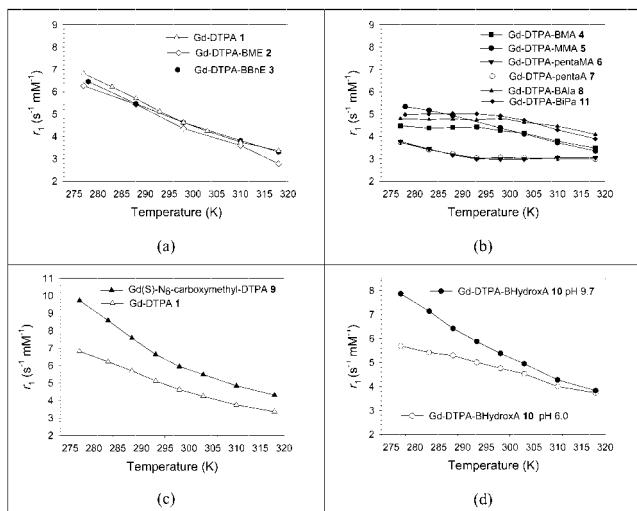


Figure 2. Proton longitudinal relaxivity vs. temperature for the Gd complexes ($B_0 = 0.47$ T).

The dependence of the relaxivity of complex **10** as a function of temperature shows different behaviors at pH 6.0 and 9.7 (Figure 2d). At pH 9.7 the water exchange rate does not limit the relaxivity, whereas at pH 6.0 a limitation at low temperatures is observed. This difference could be attributed to a prototropic exchange at basic pH. This behavior has already been observed for tetraamide macrocyclic complexes^[24] but also for the DTPA bisamide derivatives.^[25] The estimated pK_a of the hydroxamic acid of ligand **10b** gives a value of 9.85 (program ACD/ pK_a DB software developed by Advanced Chemistry Development), a value close to that published for EDTA–DX, a compound with two hydroxamic functions ($pK_{a1,2} = 9.93$ and 9).^[22] According to this estimated pK_a value, at pH 9.7, the partial deprotonation of the hydroxyl function of the hydroxamic group near the coordination sphere could also influence the water residence time.

^{17}O NMR Relaxometry at Variable Temperature

The transverse relaxivity of water oxygen-17 in gadolinium complex aqueous solutions allows for the determination of the water residence time of coordinated water molecules. The theoretical fitting of the experimental data gives the following parameters: A/h , the hyperfine coupling constant between the oxygen nucleus of bound water molecules and the Gd^{3+} ion; τ_v , the correlation time modulating the electronic relaxation of Gd^{3+} ; E_v , the activation energy related to τ_v ; B , related to the mean-square of the zero field splitting energy ($B = 2.4\Delta^2$); and ΔH^\ddagger and ΔS^\ddagger the enthalpy and entropy, respectively, of activation of the water exchange process.

The evolution of the transverse relaxation rate versus temperature of bisester complexes **2** and **3** are very similar to that of Gd–DTPA (**1**; Figure 3a). It is worth mentioning that the absence of hydrolysis of compound **3** has been carefully verified. Consequently, the theoretical adjustment from experimental points provides comparable water residence times for the three complexes (Table 1). In contrast, the experimental data of pentamide complexes **6** and **7** differ markedly, as only a slow exchange zone is observed in the range of temperatures investigated (Figure 3b). The fitted values of τ_M^{310} ($\tau_M^{310} > 2.5$ μs) agree with a very slow water exchange rate. The τ_M^{310} value of the monoamide Gd–DTPA–MMA (**5**) is greater relative to that of Gd–DTPA (**1**), but it is significantly smaller than that of bisamide Gd–DTPA–BMA (**4**) and Gd–DTPA–BiPa (**11**) (Figure 3b,d). The shape of the curve obtained for Gd(S)- N_6 -carboxymethyl–DTPA (**9**) suggests a fast exchange rate (Figure 3e) in agreement with the calculated value of τ_M^{310} , which is much shorter than that of Gd–DTPA (**1**). This faster water exchange rate has already been noticed for macrocyclic equivalents of Gd(S)- N_6 -carboxymethyl–DTPA (**9**).^[26]

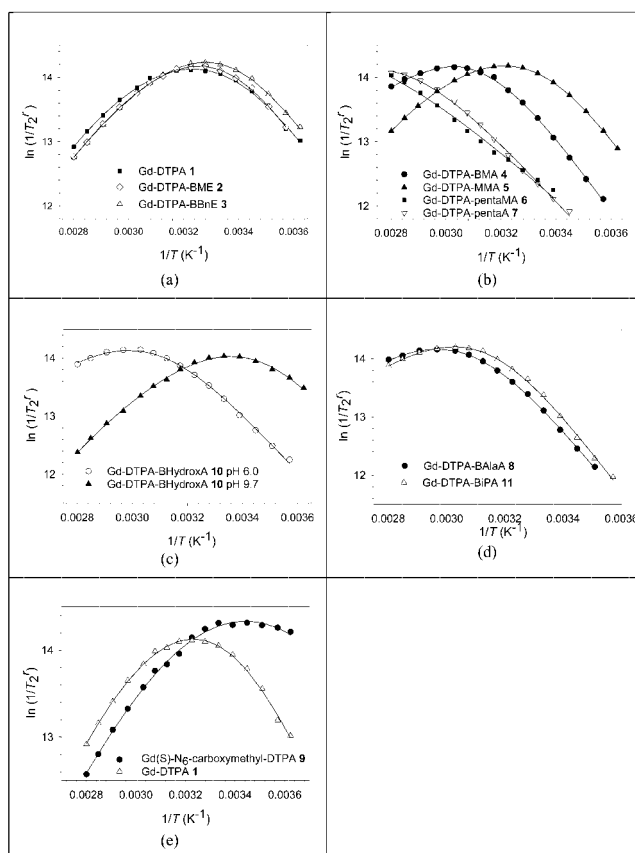


Figure 3. Reduced transverse relaxation rate of oxygen-17 $\{1/T_2^r = 55.55/[T_2^{\text{para}} \cdot (\text{Gd complex})]\}$ as a function of the reciprocal of the temperature for aqueous solutions of the Gd complexes.

The comparison of the water exchange rate of pentamide complexes to that of Gd–DTPA (**1**) seems to indicate that positive charges strongly decrease the water exchange rate. However, neutral bisester complexes **2** and **3** have similar

Table 1. Parameters obtained by the theoretical adjustment of the O-17 transverse relaxation rates vs. the reciprocal of the temperature.

Complexes	τ_M^{310} [ns]	ΔH^\ddagger [kJ mol ⁻¹]	ΔS^\ddagger [J mol ⁻¹ K ⁻¹]	A/h [10 ⁶ rad s ⁻¹]	B [10 ²⁰ s ⁻²]	τ_V^{298} [ps]	E_V [kJ mol ⁻¹]
Gd-DTPA (1) ^[a]	143 ± 25	51.5 ± 0.3	52.1 ± 0.6	-3.4 ± 0.1	2.6 ± 0.1	12.3 ± 0.3	4.5 ± 4.2
Gd-DTPA-BME (2)	150 ± 11	53.0 ± 0.1	56.5 ± 0.2	-3.2 ± 0.1	2.5 ± 0.1	16.4 ± 0.9	11.3 ± 2.4
Gd-DTPA-BBnE (3)	114 ± 11	52.2 ± 0.2	56.3 ± 0.3	-3.5 ± 0.1	2.3 ± 0.1	12.2 ± 0.6	8.7 ± 2.2
Gd-DTPA-BMA (4) ^[a]	967 ± 36	48.0 ± 0.1	24.9 ± 0.2	-3.2 ± 0.1	2.0 ± 0.1	21.2 ± 0.6	15.0 ± 11
Gd-DTPA-MMA (5)	178 ± 14	50.0 ± 0.1	45.6 ± 0.2	-3.6 ± 0.5	2.1 ± 0.1	10.3 ± 0.5	0.9 ± 1.9
Gd-DTPA-pentaMA (6)	2882 ± 323	25.2 ± 0.2	-57.7 ± 0.5	-4.5 ± 1.4	0.6 ± 0.3	8.0 ± 2.8	0.9 ± 14.3
Gd-DTPA-pentaA (7)	2521 ± 83	35.8 ± 0.1	-22.6 ± 0.1	-4.5 ± 1.3	1.0 ± 0.1	12.5 ± 1.6	20.0 ± 12.8
Gd-DTPA-BAlaA (8)	841 ± 64	52.7 ± 0.1	41.1 ± 0.3	-3.6 ± 0.4	2.0 ± 0.3	10.5 ± 1.5	4.9 ± 0.6
Gd(S)-N ₆ -carboxymethyl-DTPA (9)	32 ± 2	42.4 ± 0.6	35.2 ± 0.2	-4.5 ± 0.2	0.9 ± 0.2	26.4 ± 0.6	0.9 ± 9.2
Gd-DTPA-BHydroxA (10) (pH = 6)	901 ± 69	40.0 ± 0.1	-0.3 ± 0.3	-2.5 ± 1.8	0.7 ± 0.3	19.8 ± 7.6	20.0 ± 19.0
Gd-DTPA-BHydroxA (10) (pH = 9.7)	115 ± 5	43.7 ± 0.2	28.7 ± 0.3	-3.5 ± 0.1	3.3 ± 0.1	14.6 ± 0.5	19.1 ± 1.7
Gd-DTPA-BiPA (11)	648 ± 123	45.2 ± 0.3	19.2 ± 0.8	-3.3 ± 1.3	2.3 ± 0.3	25.4 ± 3.7	13.6 ± 0.7

[a] From ref.^[29]

exchange rates to negatively charged Gd-DTPA (**1**) and faster exchange rates than neutral Gd-DTPA-BMA (**4**). It seems thus that other factors have to be taken into account. When carboxylate groups are replaced by amides, the decreased congestion within the coordination sphere due to the weakening of the chelation could result in a slowing down of the water exchange rate. This could explain the τ_M^{310} increase in the series of amide complexes Gd-DTPA-MMA (**5**), Gd-DTPA-BMA (**4**), and Gd-DTPA-PentaMA (**7**). Similarly, the exchange rate of Gd-EGTA, a complex in which two nitrogen atoms are replaced by two oxygen atoms, is 10 times faster than for Gd-DTPA (**1**).^[27] This difference can be related to the steric congestion resulting from the coordination between Gd³⁺ and the two additional oxygen atoms. As shown in a previous study on bisamide derivatives,^[28] the presence of a hydrogen atom on the amide group could also lead to hydrogen bonds and consequently to a decrease in the water exchange rate.

So far, the influence of the ligands on the water exchange rate can thus be summarized as follows: $k_{ex}(\text{COO}^- \approx \text{COOR}_1) > k_{ex}(\text{CONHR}_1)$ (see Table 1). The present comparative study shows that the charge of the complex inside the first coordination sphere is not the factor dominating the exchange. Other factors like the steric congestion or the presence of hydrogen bonds seem to be very important.

The evolution of the transverse relaxation rate of ¹⁷O versus temperature for Gd-DTPA-BHydroxA (**10**) is significantly different at pH 6.0 and 9.7 (Figure 3c). At pH 6.0, this neutral complex has a slower water exchange rate than the negatively charged complex present at basic pH (Table 1) in agreement with the data obtained by proton relaxometry (Figure 2d). The possible influence of the coordination of Gd³⁺ by the hydroxamic group has been rejected, as it would imply the absence of the water molecule in the first coordination sphere, a fact that is contradictory with proton relaxometric results (Figure 2d). In conclusion, the higher proton relaxivity of Gd-DTPA-BHydroxA at basic pH and low temperatures is explained by a faster water exchange rate (Figure 2d) and not by a prototropic exchange. It seems therefore that the negative charges facilitate the expulsion of the water molecule by simple electrostatic repulsion.

Bisamide Gd-DTPA-BAlaA (**8**) and Gd-DTPA-BiPA (**11**) differ only by the presence of a carboxylate group on the side chain of **8** as compared to a methyl group in **11** (Figure 1). The analysis of the ¹⁷O NMR spectroscopic data shows a larger water residence time for the anionic complex (Figure 3d and Table 2). This result, which could appear contradictory with our previous observations, can be explained, as previously reported for bisamide complexes,^[28]

Table 2. Proton longitudinal relaxivity at 310 K and 20 MHz and parameters obtained by the theoretical fitting of the proton NMRD profiles.

Complexes	r_1 [s ⁻¹ mM ⁻¹]	$\tau_M^{310[b]}$ [ns]	τ_R^{310} [ps]	τ_{SO}^{310} [ps]	τ_V^{310} [ps]	r [nm]
Gd-DTPA (1) ^[a]	3.8	143 ± 25	54 ± 14	87 ± 3	25 ± 3	0.31
Gd-DTPA-BME (2)	3.6	150 ± 11	52 ± 0.4	82 ± 2	10 ± 1	0.31
Gd-DTPA-BBnE (3)	3.8	114 ± 11	54 ± 2	79 ± 3	18 ± 2	0.31
Gd-DTPA-BMA (4) ^[a]	3.8	967 ± 36	65 ± 2	95 ± 3	18 ± 3	0.31
Gd-DTPA-MMA (5)	3.8	178 ± 14	58 ± 2	82 ± 3	24 ± 3	0.31
Gd-DTPA-pentaMA (6)	3.0	2882 ± 323	60 ± 3	48 ± 1	7 ± 1	0.31
Gd-DTPA-pentaA (7)	3.0	2521 ± 83	57 ± 3	55 ± 2	7 ± 1	0.31
Gd-DTPA-BAlaA (8)	4.5	841 ± 64	96 ± 5	76 ± 3	16 ± 2	0.31
Gd(S)-N ₆ -carboxymethyl-DTPA (9)	4.8	32 ± 2	85 ± 1	174 ± 7	25 ± 1	0.31
			76 ± 1	162 ± 5	25 ± 1	0.305
Gd-DTPA-BHydroxA (10) (pH 6.0)	4.0	901 ± 69	76 ± 2	102 ± 3	25 ± 3	0.31
Gd-DTPAGd-DTPA-BiPA (11)	4.3	648 ± 123	81 ± 2	95 ± 3	25 ± 1	0.31

[a] From ref.^[29] [b] τ_M was set to the values obtained by ¹⁷O NMR (see Table 1).

by the fact that the alkyl substituents of bisamide complexes are outside the first coordination sphere. Consequently, the carboxyl group of Gd-DTPA-BAlaA (**8**) has no direct influence on the exchange rate. However, contrary to the methyl group of the isopropyl substituent, the carboxylate function favors the formation of a hydration layer as well as of hydrogen bonds that are unfavorable to a fast exchange.

Nuclear Magnetic Resonance Dispersion (NMRD) Profiles

Figure 4 shows the proton NMRD profiles of the various gadolinium complexes at 310 K. The NMRD curves were fitted according to the classical inner-sphere and outer-sphere theories^[30,31,32] and independently from O-17 data except for the τ_M value. Parameters obtained by the theoretical adjustment of the NMRD profiles are summarized in Table 2 (fixed parameters: $q = 1$, $d = 0.36$ nm, $D = 3.3 \times 10^{-9}$ m²s⁻¹,^[33] $r = 0.31$ nm, τ_M^{310} fixed to the value determined by ¹⁷O relaxometry).

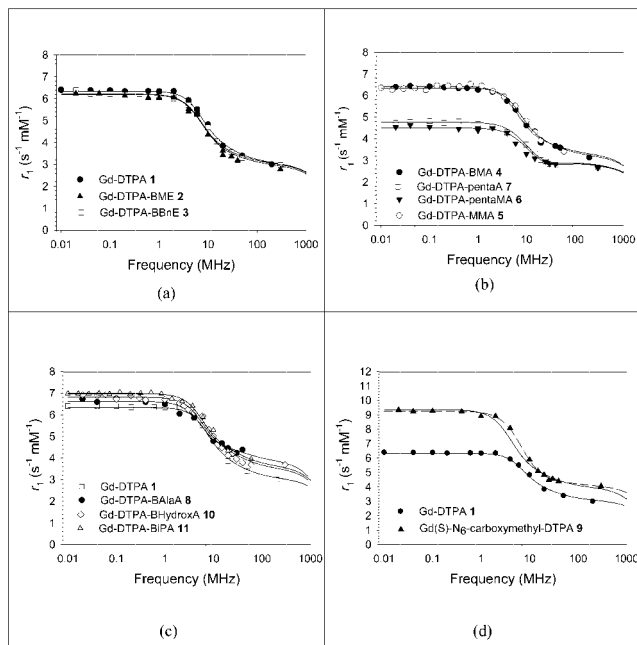


Figure 4. ¹H NMRD relaxivity profiles of water solutions of (a) Gd complexes **1–3**; (b) Gd complexes **4, 5, 6, and 7**; (c) Gd complexes **1, 8, 10, and 11**; and (d) Gd complexes **1 and 9**. The plain lines correspond to the theoretical fittings of the data points using the classical outer-sphere and inner-sphere theories. The dotted line in (d) corresponds to the fitting taking into account the second sphere water molecules [$r = 0.31$ nm, $d = 0.4$ nm, $D = 3.3 \times 10^{-9}$ m²s⁻¹, $\tau_R = (55 \pm 1)$ ps, $\tau_{SO} = (106 \pm 5)$ ps, $\tau_V = (13.5 \pm 2)$ ps, two water molecules in the second sphere at a distance of 0.36 nm, $\tau_{SS} = (47 \pm 3)$ ps].

At 310 K, the proton relaxivities of bisester and monoamide complexes are similar to those of Gd-DTPA (**1**) and Gd-DTPA-BMA (**4**) but the relaxivities of pentamide derivatives **6** and **7** are clearly smaller (Figure 4a,b). This difference is not related to significantly different rotational correlation times, as the complexes have a quite sim-

ilar molecular weight. It is rather explained by the very slow water exchange rate of the pentamide complexes. Indeed, the evolution of the relaxivity at 20 MHz versus temperature for Gd-DTPA-pentamides **6** and **7** and their τ_M values obtained from ¹⁷O relaxometry have shown a limitation of their inner-sphere contribution at 310 K by the water residence time and, consequently, the total relaxivity is decreased at all magnetic fields.

The NMRD profiles of Gd-DTPA-BAlaA (**8**), Gd-DTPA-BiPA (**11**), and Gd-DTPA-BHydroxA (**10**) at pH 6 (Figure 4c) are close to that of Gd-DTPA (**1**) confirming that a water residence time lower than 1 μ s at 310 K does not influence the relaxivity of small gadolinium complexes. The NMRD profile of Gd(S)-N₆-carboxymethyl-DTPA (**9**) is different at all fields (Figure 4d). By using the classical inner-sphere and outer-sphere theories, the theoretical adjustment of the Gd(S)-N₆-carboxymethyl-DTPA (**9**) profile needs high values of the electronic relaxation time at low field (τ_{SO}) and of the rotational correlation time. A reduction of the distance between the Gd³⁺ ion and the coordinated water hydrogen atoms to 0.305 nm does not significantly improve the data (Table 2). Because the presence of the additional carboxylate group could be responsible of a second hydration sphere whose molecules are exchanging very fast with the bulk, the effect of these second sphere water molecules was included in the theoretical model.^[34] The following parameters were obtained: $\tau_R = (55 \pm 1)$ ps, $\tau_{SO} = (106 \pm 5)$ ps, $\tau_V = (13.5 \pm 2)$ ps, two water molecules in the second sphere at a distance of 0.36 nm, and a correlation time for these second sphere molecules (τ_{SS}) equal to (47 ± 3) ps.

When parameters characterizing the electronic relaxation [B , τ_V , and τ_{SO} with $\tau_{SO} = (5B\tau_V)^{-1}$] are compared (Tables 1 and 2), it appears that some values are different. This may be related on the one hand to the fact that for complexes with large τ_M values, the O-17 data are typical of slow and intermediate regimes. Consequently, T_{2M} and thus A/h and τ_{S1} values cannot be calculated very accurately. On the other hand, the experimental conditions used in both methods are different. O-17 measurements are performed at high magnetic field ($B_0 = 7.05$ T), where τ_{S1} values are large (of the order of 10 ns), whereas the proton relaxometric data of small Gd complexes depend on the electronic parameters mainly at magnetic fields lower than 0.5 T (where τ_{S1} values are of the order of 100 ps) but not at high fields where they strongly depend on τ_R .

Conclusions

The water exchange rate depends on several factors whose relative importance varies with the chemical structure of the complex. The complex charge can have an influence on the exchange rate, although its importance is strongly dependent on the location of the charged groups as compared to the coordinated water molecule.

Indeed, the similarity of the exchange rates of bisester complexes **2** and **3** and that of the parent compound Gd-

DTPA (**1**), as well as their significant difference with Gd–DTPA–BMA (**4**) or Gd–DTPA–BiPA (**11**), suggests that the charge is not the factor dominating the exchange rate. This rate seems to be more sensitive to the steric congestion inside the first coordination sphere than to the charge, a fact already invoked to explain the faster exchange rate of water in the C-4 derivatives of Gd–DTPA (MS-325, Gd–EOB–DTPA).

However, the study of Gd(*S*)-N₆-carboxymethyl–DTPA (**9**) and Gd–DTPA–Bhydrox (**10**) at basic pH has clearly shown that anionic groups located close to the first coordination sphere have a very strong influence on the coordinated water exchange rate. These results suggest that this beneficial effect is due to an electrostatic repulsion, the extent of which strongly depends on the distance between the anionic group and the coordinated water. In addition, the behavior of Gd–DTPA–BHydrox (**10**) at different pH values clearly confirms the possibility of conceiving MRI contrast agents whose water exchange rate varies as a function of pH.

Experimental Section

Chemical Products: All chemicals were purchased from Aldrich (Bornem, Belgium) and used without further purification. Gd–DTPA and Gd–DTPA–BMA were respectively provided by Bayer Schering Pharma (Berlin, Germany) and GE Healthcare (Amersham, United Kingdom). Gd–DTPA–BiPA was synthesized as described previously.^[28]

Instrumentation: Mass spectra were recorded with a Q-tof-2 (Micromass, Manchester, UK). Samples (0.1 mg) were dissolved in a mixture of methanol/water (50:50) and infused at a rate of 5 $\mu\text{L min}^{-1}$. Proton and carbon-13 NMR spectra were obtained with an AMX-300 spectrometer (Bruker, Karlsruhe, Germany). For ¹³C NMR, *tert*-butyl alcohol was used as the internal standard (methyl signal at $\delta = 31.2$ ppm). The abbreviations used were: “s” for singlet, “d” for doublet, “t” for triplet, “q” for quartet, “m” for multiplet, and “br.” for broad. Variable-temperature ¹⁷O NMR measurements were recorded with a Bruker AMX-300 spectrometer (7.05 T). The temperature was controlled by a BVT-2000 unit. ¹⁷O NMR measurements at natural abundance were performed on 2-mL samples contained in 10 mm o.d. tubes, and the concentration of the solutions was lower than 30 mM. ¹⁷O diamagnetic transverse relaxation times of water were measured by using a CPMG sequence (90 and 180° pulse lengths were 25 and 50 μs , respectively). All ¹⁷O NMR spectra were proton decoupled. ¹⁷O transverse relaxation times of water in solutions containing Gd complexes were calculated from the spectral linewidth. The data are presented as the reduced transverse relaxation rate ($1/T_2^R = (1/T_2^{\text{obs}} - 1/T_2^{\text{dia}}) \times 55.55 / [\text{Gd complex}]$) vs. the reciprocal of the temperature. The analysis of the data was performed as described previously.^[34,35,36] NMRD curves were obtained with Stelar (PV, Mede, Italy) or IBM (Field Cycling Systems, Honesdale, PA, USA) relaxometers. Additional relaxation rates at 0.47, 1.4, and 7.05 T were obtained with Minispec P-120, Mq-60, and Bruker AMX-300 spectrometers, respectively (Bruker, Karlsruhe, Germany). Fitting of the 1-H NMRD profiles was performed with a data processing software that uses the theoretical models describing the nuclear relaxation phenomena observed (Minuit, CERN Library).^[37,38]

6-Carboxymethyl-3,9-bis(methyloxycarbonylmethyl)-3,6,9-triazaundecanedioic Acid [DTPA–BME (2a**)]:** DTPA–bis-anhydride (3 g,

8.4 mmol)^[39] was mixed in dry methanol (100 mL) and dry dimethylformamide (DMF, 50 mL). Distilled pyridine (2 mL, 25 mmol) in anhydrous DMF (5 mL) was added dropwise under an inert atmosphere. The suspension was heated at 65 °C for 12 h. The solution was evaporated under reduced pressure. Yield: 95% (3.54 g) ¹H NMR (300 MHz, D₂O, 25 °C): $\delta = 3.8$ (s, 6 H, 2 \times CH₃), 3.4 (s, 2 H, CH₂), 3.35 (s, 4 H, 2 \times CH₂), 3.2 (s, 4 H, 2 \times CH₂), 2.9 (t, 4 H, 2 \times CH₂), 2.8 (t, 4 H, 2 \times CH₂) ppm. ¹³C NMR (75 MHz, D₂O, 25 °C): $\delta = 173.1, 171.0, 170.9, 60.5, 55.8, 55.1, 51.9, 50.4$ ppm. HRMS (ESI+): calcd. for C₁₆H₂₇N₃NaO₁₀ [M + Na]⁺ 444.1594; found 444.1592.

1-Methylamino-1-oxo-3,6,9-triaza-3,6,9,9-tetra(carboxymethyl)-undecane [DTPA–MMA (5b**)]:** DTPA (1 g), triethylamine (12.7 mmol), and dry acetonitrile (50 mL) were heated in a water bath while stirring until complete dissolution of DTPA. The penta-triethylammonium–DTPA solution was cooled to room temperature and isobutylchloroformate (2.54 mmol) was added dropwise. An excess amount of methylamine in methanol was added immediately, and the solution was stirred for 30 min. The acetonitrile was subsequently evaporated, and the residue was dissolved in water (3 mL), the pH was adjusted to 2 and the product was poured on a Dowex anionic exchange column in the formate form. The ammonium salts were first eluted with water and then a linear 0–7 M formic acid gradient was initiated. The fractions containing the monoamide were dialyzed (cutoff membrane of 100). Yield: 47% (0.49 g). ¹H NMR (300 MHz, D₂O, 25 °C): $\delta = 3.9$ (s, 4 H, 2 \times CH₂), 3.7 (s, 2 H, CH₂), 3.6 (s, 2 H, CH₂), 3.5 (s, 2 H, CH₂), 3.2 (t, 4 H, 2 \times CH₂), 3 (t, 4 H, 2 \times CH₂), 2.8 (s, 3 H, CH₃) ppm. ¹³C NMR (75 MHz, D₂O, 25 °C): $\delta = 178.2, 177.0, 172.8, 170.6, 59.4, 57.8, 57.1, 56.4, 53.0, 52.9, 52.6, 49.3, 31.1$ ppm. HRMS (ESI+): calcd. for C₁₅H₂₇N₄O₉ [M + H]⁺ 407.1778; found 407.1785.

3,9-Bis(methylcarbamoylmethyl)-6-methylcarbamoylmethyl-3,6,9-triazaundecane-N-methyldiamide [DTPA–PentaMA (6b**)]:** DTPA–bis-anhydride (3 g, 8.4 mmol), DMAP (0.205 g, 1.68 mmol), and NHS (2.92 g, 25.2 mmol) were suspended in anhydrous DMF (100 mL) under an inert atmosphere. The suspension was stirred for 12 h at ambient temperature. NHS (4.87 g, 42 mmol) and DCC (5.2 g), dissolved in anhydrous DMF (20 mL), were added to the solution. The mixture was shaken for a period of 4 d at room temperature. When the dicyclohexyl urea precipitated, the mixture was filtered. The filtrate was evaporated under reduced pressure. The obtained oil was dissolved in DMF (50 mL). A solution of methylamine (2 M in THF, 55 mL) was added dropwise under agitation and inert atmosphere. The solution was stirred for 48 h, then filtered, and the filtrate was evaporated. The residue was dissolved in water (50 mL); the solution was neutralized with a NaHCO₃ saturated solution, filtered, and concentrated by evaporation. The pH of the sample was adjusted to 8 with NaOH (3 M). The sample was deposited on an anionic exchange resin Dowex AG 1X8–400 (HCOO[−], 15 \times 2.4 cm). The column was washed with demineralized water to recuperate DTPA–PentaMA. The solution was treated several times with water and evaporated to eliminate formic acid. The pH of the sample was adjusted to 2, and the solution was deposited on a cationic exchange resin Dowex AG 50W-X8 (H⁺, 15 \times 2.4 cm). The column was washed with water until reaching a pH close to 6. The DTPA–pentaMA was eluted with a gradient of 0 to 2 M NH₄OH. Fractions containing the product were evaporated and lyophilized. Yield: 38% (1.46 g). ¹H NMR (300 MHz, D₂O pH \approx 10, 25 °C): $\delta = 3.3$ –3.0 (m, 10 H, 5 \times CH₂), 2.65 (br. s, 15 H, 5 \times CH₃), 2.5 (m, 8 H, 4 \times CH₂) ppm. ¹³C NMR (75 MHz, D₂O pH \approx 10, 25 °C): $\delta = 169.5, 169.0, 58.5, 57.6, 52.8, 52.5, 25.8, 24.8$ ppm. HRMS (ESI+): calcd. for C₁₉H₃₉N₈O₉ [M + H]⁺ 459.3043; found 459.3059.

3,9-Bis(carbamoylmethyl)-6-carbamoylmethyl-3,6,9-triazaundecanediamide [DTPA–PentaA (7b)]: The synthesis of DTPA–PentaA was similar to that of DTPA–PentaMA. The amidation was obtained by bubbling dry ammonia in the activated DTPA–Pentaester solution for 2 h. Yield: 30% (1.03 g). ^1H NMR (300 MHz, D_2O pH \approx 6, 25 °C): δ = 3.45 (s, 2 H, CH_2), 3.2 (s, 8 H, $4 \times \text{CH}_2$), 2.95 (t, 4 H, $2 \times \text{CH}_2$), 2.8 (t, 4 H, $2 \times \text{CH}_2$) ppm. ^{13}C NMR (75 MHz, D_2O pH \approx 6, 25 °C): δ = 176.7, 176.4, 57.9, 56.0, 53.2, 51.8 ppm. HRMS (ESI $^+$): calcd. for $\text{C}_{14}\text{H}_{28}\text{N}_8\text{O}_5\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 411.2080; found 411.2098.

DTPA–BALaA (8a): L-alanine (4.45 g, 50 mmol) was suspended in ethanol (100 mL) at 60 °C. The pH of the ethanolic solution was adjusted to 11 with NaOH (5 M). DTPA–Bisanhydride (3 g, 8.4 mmol) was added in small amounts under vigorous agitation. The suspension was stirred overnight at 60 °C. The solution was evaporated under reduced pressure, the residue was dissolved in hot methanol, and the solution was filtered. The filtrate was cooled in an ice bath, and the ethanol was added to obtain a white precipitate. The solid was filtered, dissolved in a minimum of water, and the pH of the solution was adjusted to 8. The sample was deposited on an anionic resin Dowex AG 1X8–400 (HCOO^- , 15×2.4 cm). The column was washed with water until a pH close to 6 was reached, and then eluted with HCOOH (0.1 M, 500 mL) to discard byproducts. DTPA–BALaA was then eluted with HCOOH (0.5 M, 500 mL). The solution was evaporated under reduced pressure. The residue was dissolved in water (3 mL) and the solution was dialyzed to eliminate formic acid (membrane cut-off: 100). Yield: 21% (0.94 g). ^1H NMR (300 MHz, D_2O pH \approx 3, 25 °C): δ = 4.25 (quad, 2 H, CH_2), 3.8 (s, 4 H, $2 \times \text{CH}_2$), 3.7 (s, 4 H, $2 \times \text{CH}_2$), 3.6 (s, 2 H, CH_2), 3.25 (t, 4 H, $2 \times \text{CH}_2$), 3.15 (t, 4 H, $2 \times \text{CH}_2$), 1.3 (d, 6 H, $2 \times \text{CH}_3$) ppm. ^{13}C NMR (75 MHz, D_2O pH \approx 3, 25 °C): δ = 174.4, 170.1, 170.0, 166.4, 57.5, 54.6, 49.7, 49.1, 47.0, 14.2 ppm. HRMS (ESI $^+$): calcd. for $\text{C}_{20}\text{H}_{34}\text{N}_5\text{O}_{12}$ [$\text{M} + \text{H}$] $^+$ 536.2203; found 536.2221.

***N,N*-Bis[(*tert*-butoxycarbonyl)methyl]-2-aminoethanol (9a):** KHCO_3 (20.62 g, 205.97 mmol) was added to a solution of *tert*-butyl bromoacetate (29.76 mL, 184.32 mmol) in DMF (130 mL). The suspension was cooled to 0 °C and ethanolamine (4.92 mL, 81.86 mmol) dissolved in DMF (10 mL) was added dropwise under an inert atmosphere. The mixture was stirred at 0 °C for 30 min and at room temperature for 22 h. The mixture was filtered, and then ether (150 mL) and a NaHCO_3 saturated solution (100 mL) were added to the filtrate. The organic phase was washed with a NaHCO_3 saturated solution (100 mL). Aqueous phases were extracted with diethyl ether (100 mL). Organic phases were extracted with brine (100 mL), dried with MgSO_4 , and evaporated under reduced pressure to give an oil that was purified by chromatography on silica (petroleum ether/ether, 2:1). Yield: 84% (19.9 g). ^1H NMR (300 MHz, CDCl_3 , 25 °C): δ = 3.7 (br. s, 1 H, OH), 3.35 (t, 2 H, CH_2), 3.25 (s, 4 H, $2 \times \text{CH}_2$), 2.7 (t, 2 H, CH_2), 1.3 (s, 18 H, $6 \times \text{CH}_3$) ppm. ^{13}C NMR (75 MHz, CDCl_3 , 25 °C): δ = 171.3, 81.1, 59.4, 57.0, 56.6, 27.8 ppm.

***N,N*-Bis[(*tert*-butoxycarbonyl)methyl]-2-bromoethylamine (9b):** Compound **9a** (19.97 g, 69 mmol) was dissolved in dichloromethane (100 mL). Triphenylphosphane (4.36 g, 54.75 mmol) was added under agitation. The solution was cooled to 0 °C and *N*-bromosuccinimide (9.74 g, 54.75 mmol) was added in small portions over 5 min. The solution was shaken at 0 °C for 90 min and became violet. The solution was evaporated, and the oil obtained was mixed with diethyl ether (200 mL). The suspension was filtered and the filtrate was concentrated, deposited on a small column of silica, and eluted with ether. The evaporation of the solution gave a yellow

oil that was purified by chromatography on silica (hexane/ether, 5:1). Yield: 60% (11.57 g). ^1H NMR (300 MHz, CDCl_3 , 25 °C): δ = 3.5 (s, 4 H, $2 \times \text{CH}_2$), 3.45 (t, 2 H, CH_2), 3.15 (t, 2 H, CH_2), 1.45 (s, 18 H, $6 \times \text{CH}_3$) ppm. ^{13}C NMR (75 MHz, CDCl_3 , 25 °C): δ = 170.7, 81.3, 56.9, 56.7, 29.2, 28.4 ppm.

Diethyl-L-Aspartate Hydrochlorhydrate (9c): L-Aspartic acid (5 g, 37.56 mmol) was dissolved whilst stirring in anhydrous ethanol saturated in HCl (100 mL). The solution was shaken for 18 h at room temperature. The solution was evaporated under reduced pressure and the residue was filtered and washed with diethyl ether. The solid white was dried and maintained under P_2O_5 . Yield: 97% (4.84 g). ^1H NMR (300 MHz, D_2O , 25 °C): δ = 4.5 (t, 1 H, CH), 4.3 (quad, 2 H, CH_2), 3.1 (dd, 1 H, CH), 4.2 (quad, 2 H, CH_2), 3.22 (dd, 1 H, CH), 1.9 (t, 3 H, CH_3), 1.65 (t, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, D_2O , 25 °C): δ = 171.4, 169.1, 64.2, 63.0, 34.2, 13.6, 13.5 ppm.

Compound 9d: L-Aspartic acid (2 g, 15.03 mmol) and *p*-toluenesulfonic acid monohydrate (3.43 g, 18.04 mmol) were dissolved in benzyl alcohol (16 mL) and benzene (24 mL). The mixture was heated (T = 160–165 °C) for 10 h and water (ca. 2 mL) was obtained (Dean–Stark). The yellow solution was cooled overnight in a freezer and ether was added until a white precipitate appeared. The solid was filtered and washed with diethyl ether. The product was purified by chromatography on silica (ethyl acetate/methanol, 9:1). R_f = 0.76 (ethyl acetate/methanol, 9:1). Yield: 62% (5.43 g). ^1H NMR (300 MHz, CDCl_3 , 25 °C): δ = 8.45 (br. s, 2 H, NH_2), 7.7 (d, 2 H, Arom), 7.0–7.3 (m, 10 H, $2 \times \phi$), 6.9 (d, 2 H, CH Arom), 4.7–5.1 (m, 4 H, $2 \times \text{CH}_2$), 4.5 (t, 1 H, CH), 3.1 (dd, 2 H, CH_2), 2.2 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, CDCl_3 , 25 °C): δ = 170.3, 168.5, 142.0, 140.6, 135.7, 135.1, 129.3, 128.7–128.9 (br.), 126.6, 68.7, 67.5, 50.0, 34.2, 21.8 ppm. MS (ESI): m/z = 314 [M – tosylate] $^+$.

***tert*-Butyl 3,9-[(*tert*-Butyloxycarbonyl)methyl]-6-[(*S*)-2-ethyloxycarbonylmethyl]ethyloxycarbonylmethyl-3,6,9-triazaundecanedioate (9e):** Compound **9b** (6.7 g, 19.02 mmol) and **9c** (2.06 g, 9.14 mmol) were dissolved in acetonitrile (100 mL) and phosphate buffer (1 M, pH 8, 100 mL). The solution was shaken vigorously for 3 h at room temperature. The aqueous phase was separated and extracted with acetonitrile (3×30 mL). Organic phases were treated with phosphate buffer (1 M, pH 8, 150 mL). The solution was stirred for 48 h. The organic phase was dissolved in ethyl acetate, filtered, and extracted with NaCl saturated solution (2×50 mL). It was then dried on MgSO_4 , filtered, and evaporated under reduced pressure. The residue was deposited on a small column of silica and eluted with ether. The solution was evaporated and the residue purified by chromatography on silica with a gradient 10 to 40% (petroleum ether/ethyl acetate). The product was identified by thin layer chromatography (petroleum ether/ethyl acetate, 1:1). Fractions containing the compound were evaporated. Yield: 45% (3.01 g). ^1H NMR (300 MHz, CDCl_3 , 25 °C): δ = 4.2 (quad, 2 H, CH_2), 4.1 (quad, 2 H, CH_2), 3.65 (t, 1 H, CH), 3.4 (s, 8 H, $s, 4 \times \text{CH}_2$), 2.5–2.8 (m, 10 H, $5 \times \text{CH}_2$), 1.4 (s, 36 H, $12 \times \text{CH}_3$), 1.3 (t, 3 H, CH_3), 1.25 (t, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, CDCl_3 , 25 °C): δ = 173.3, 170.9, 170.7, 80.8, 60.9, 60.6, 57.8, 55.8, 53.8, 45.6, 37.9, 28.1, 14.2, 14.1 ppm. MS (ESI): m/z = 732 [$\text{M} + \text{H}$] $^+$, 754 [$\text{M} + \text{Na}$] $^+$

***tert*-Butyl 3,9-[(*tert*-Butyloxycarbonyl)methyl]-6-[(*S*)-2-benzyloxycarbonylmethyl]benzyloxycarbonylmethyl-3,6,9-triazaundecanedioate (9f):** Compound **9b** (7.03 g, 19.97 mmol) and **9d** (4.66 g, 9.6 mmol) were dissolved in acetonitrile (100 mL) and phosphate buffer (1 M, pH 8, 100 mL). The solution was shaken vigorously for 3 h at room temperature. The aqueous phase was separated and

extracted with acetonitrile (3 × 30 mL). Organic phases were gathered and added to new buffer (150 mL). The solution was stirred for 48 h. The organic phase was dissolved in ethyl acetate, filtered, and extracted with a NaCl saturated solution (2 × 50 mL). It was then dried with MgSO₄, filtered, and evaporated under reduced pressure. The residue was purified by chromatography on silica with a gradient 20 to 80% (petroleum ether/ethyl acetate). Product **9f** was detected by UV spectroscopy at 254 nm. Fractions were evaporated. Yield: 50% (4.10 g). ¹H NMR (300 MHz, CDCl₃, 25 °C): δ = 7.35 (m, 10 H, 2 × φ), 5.0–5.2 (m, 4 H, 2 × CH₂), 4.05 (t, 1 H, CH), 3.4 (s, 8 H, 4 × CH₂), 2.6–2.9 (m, 10 H, 5 × CH₂), 1.5 (s, 36 H, 12 × CH₃) ppm. ¹³C NMR (75 MHz, CDCl₃, 25 °C): δ = 172.2, 171.5, 171.0, 138.2, 138.1, 128.6–129 (br.), 81.2, 66.9, 66.8, 61.3, 56.5, 54.2, 51.5, 36.2, 28.6 ppm. MS (ESI): *m/z* = 856 [M + H]⁺, 878 [M + Na]⁺

(S)-N₆-Carboxymethyl-DTPA (**9h**)

From Intermediate 9f: Compound **9f** (1.82 g, 2.13 mmol) was dissolved in methanol (20 mL). HCl (6 M, 10 mL) was added, and the solution was stirred for 2 h at room temperature. Pd/C (10%, 150 mg) was added, and the solution was hydrogenated (25 psi) for 20 h. The catalyst was extracted by filtration through Celite, and the filtrate was evaporated under reduced pressure. The residue was dissolved in a minimum amount of water, and the pH of the sample was adjusted to 2. The sample was deposited on a cationic exchange resin Dowex AG 50W-X8 (H⁺, 15 × 2.4 cm). The column was washed with water until a pH close to 6 was reached. The product was eluted by a gradient 0 to 2 M NH₄OH. Fractions containing **9h** were identified by thin-layer chromatography (ethanol/ammonia water, 4:1), and then evaporated and lyophilized. Yield: 50% (0.48 g).

From the Intermediate 9e: Compound **9e** (1.96 g, 2.68 mmol) was dissolved in methanol (20 mL). HCl (6 M, 25 mL) was added, and the solution was stirred for 6 h at room temperature. The solution was evaporated, and the residue dissolved in a minimum of water. The pH of the sample was adjusted to 2. The sample was deposited on a cationic exchange resin Dowex AG 50W-X8 (H⁺, 15 × 2.4 cm). The column was washed with water until the pH was close to 6. The product was eluted by a gradient 0 to 2 M NH₄OH. Fractions containing **9h** were identified by thin-layer chromatography (ethanol/ammonia water, 4:1), and then evaporated and lyophilized. Yield: 73% (0.88 g). ¹H NMR (300 MHz, D₂O pH > 10, 25 °C): δ = 4.3 (br. s, 1 H, CH), 4.1 (s, 8 H, 4 × CH₂), 3.9 (t, 4 H, 2 × CH₂), 3.6 (br. s, 2 H, CH₂), 3.2 (t, 4 H, 2 × CH₂) ppm. ¹³C NMR (75 MHz, D₂O pH > 10, 25 °C): δ = 179.8, 179.3, 168.5, 59.6, 54.6, 58.3, 54.1, 45.7 ppm. HRMS (ESI⁺): calcd. for [M + H]⁺ C₁₆H₂₆N₃O₁₂ 452.1516; found 452.1519.

6-Carboxymethyl-3,9-bis(hydroxycarbonylmethyl)-3,6,9-triazaundecanedioic Acid [DTPA-bishydroxamide (10b**):** The first step was to synthesize 6-carboxymethyl-3,9-bis(hexylthiocarbonylmethyl)-3,6,9-triazaundecanedioic acid [DTPA-BHTE (**10a**)]. For this, DTPA-bisanhydride (3 g, 8.4 mmol) was added to anhydrous DMF (50 mL). Hexan-1-thiol (42 mmol, 7.06 mL) and distilled pyridine (17 mmol, 1.36 mL) were added under an inert atmosphere. The mixture was heated at 50 °C for 12 h. The yellow solution was evaporated and the oil treated with water (50 mL). The solution was neutralized with a NaHCO₃ saturated solution, filtered, and extracted with ethyl acetate (5 × 30 mL). The aqueous phase was lyophilized. Yield: 79% (1.97 g). ¹H NMR (300 MHz, D₂O pH ≈ 6, 25 °C): δ = 3.6 (s, 4 H, 2 × CH₂), 3.2 (s, 4 H, 2 × CH₂), 3.0 (s, 2 H, CH₂), 2.8 (t, 4 H, 2 × CH₂), 2.6 (t, 4 H, 2 × CH₂), 2.4 (t, 4 H, 2 × CH₂), 1.0–1.5 (m, 16 H, 8 × CH₂), 0.7 (t, 6 H, 2 × CH₃) ppm. ¹³C NMR (75 MHz, D₂O pH ≈ 6, 25 °C): δ = 201.7, 177.8, 172.4,

63.9, 57.6, 55.8, 52.4, 49.9, 30.9, 28.9, 28.3, 28.0, 22.1, 13.5 ppm. MS (ESI): *m/z* = 616 [M + Na]⁺, 638 [M + 2 Na]⁺. Then, **10a** (1 g, 1.684 mmol) was dissolved in anhydrous DMF (25 mL) under an inert atmosphere. Pyridine (4.21 mL, 5.22 mmol) was added to the solution. Hydroxylamine hydrochloride (dried at 120 °C, 351 mg, 5.052 mmol) was added to the solution. The suspension became clear and, after 10 min, a precipitate appeared. The suspension was shaken overnight at 50 °C, filtered, and the precipitate was suspended in ethanol and shaken for 2 h. The suspension was filtered and the precipitate was washed abundantly in ether until the characteristic odor of thiols disappeared. Product **10b** was isolated as a white solid and dried overnight at 50 °C. Yield: 45% (0.32 g). ¹H NMR (300 MHz, D₂O pH ≈ 6, 25 °C): δ = 3.55 (s, 2 H, CH₂), 3.35 (s, 4 H, 2 × CH₂), 3.2 (s, 4 H, 2 × CH₂), 3.1 (t, 4 H, 2 × CH₂), 2.9 (t, 4 H, 2 × CH₂) ppm. ¹³C NMR (75 MHz, D₂O pH ≈ 6, 25 °C): δ = 177.6, 172.6, 169.4, 58.6, 57.0, 56.3, 53.8, 51.5 ppm. HRMS (ESI⁺): calcd. for C₁₄H₂₆N₅O₁₀ [M + H]⁺ 424.1679; found 424.1654.

Complexation: Complexes were prepared by mixing aqueous solutions of ligand and gadolinium chloride. The pH was kept at 6.5–7. The Arsenazo test was used to verify the absence of free gadolinium ions. The identity of the Gd complexes was confirmed by ESI: Gd-DTPA-BME **2**: 575 [M + H]⁺ and 597 [M + Na]⁺; Gd-DTPA-BBnE **3**: 727 [M + H]⁺; Gd-DTPA-MMA **5**: 561 [M + H]⁺ and 583 [M + Na]⁺; Gd-DTPA-pentaMA **6**: 614 [M]⁺ and 459 (trace of ligand); Gd-DTPA-pentaA **7**: 545 [M]⁺, 567 [M + Na]⁺, and 411 (trace of ligand + Na); Gd-DTPA-BAlaA **8**: 761 [M + H]⁺; Gd(S)-N₆-carboxymethyl-DTPA **9**: 770 [M + Na]⁺, 792 [M + 2Na]⁺, and 814 [M + 3Na]⁺; Gd-DTPA-Bhydrox (pH = 6) **10**: 578 [M + H]⁺ and 600 [M + Na]⁺.

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