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Kinetics of formation and dissociation of lanthanide(III) complexes with the 13-membered macrocyclic ligand TRITA^{4-}

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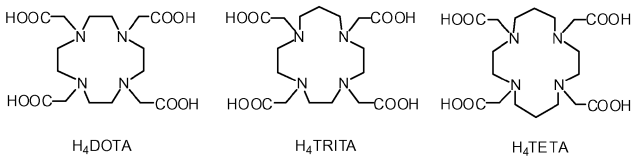
The tetraazamacrocyclic ligand TRITA^{4-} is intermediate in size between the widely studied and medically used 12-membered DOTA^{4-} and the 14-membered TETA^{4-}. The kinetic inertness of GdTRITA^{-} was characterized by the rates of exchange reactions with Zn^{2+} and Eu^{3+}. In the Zn^{2+} exchange, a second order [H$^+$] dependence was found for the pseudo-first-order rate constant ($k_0 = (4.2 \pm 0.5) \times 10^{-7}$ s^{-1}; $k' = (3.5 \pm 0.3) \times 10^{-1}$ M^{-1}s^{-1}, $k'' = (1.4 \pm 0.4) \times 10^{3}$ M^{-2}s^{-1}). In the Eu^{3+} exchange, at pH <5 the rate decreases with increasing concentration of the exchanging ion, which can be accounted for by the transitional formation of dinuclear GdTRITAEu^{2+} species. At physiological pH, the kinetic inertness of GdTRITA^{-} is considerably lower than that of GdDOTA^{-} ($t_{1/2} = 444$ h (25 °C) *vs.* 3.8×10^5 h (37 °C), respectively). However, GdTRITA^{-} is still kinetically more inert than GdDTPA^{2-}, the most commonly used MRI contrast agent ($t_{1/2} = 127$ h). The formation reactions of LnTRITA^{-} complexes (Ln = Ce, Gd and Yb) proceed *via* the rapid formation of a diprotonated intermediate and its subsequent deprotonation and rearrangement in a slow, OH^{-} catalyzed process. The stability of the LnH$_2$TRITA* intermediates (log $K_{\mathrm{LnH2L*}} = 3.1$–$3.9$) is lower than that of the DOTA-analogues. The rate constants of the OH^{-} catalyzed step increase with decreasing lanthanide ion size, and are about twice as high as for DOTA-complexes.

Introduction

In the last two decades there has been a growing interest in the synthesis and complexation properties of functionalized macrocyclic ligands. This has been motivated by both the peculiar chemical properties and the successful biomedical application of these chelating agents. The most important and widely studied representative of the group is H$_4$DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) which forms lanthanide complexes of exceptionally high thermodynamic stability. Kinetically, LnDOTA^{-} complexes are very slow to form and to dissociate, as compared to linear poly(amino carboxylate) ligands like EDTA^{4-} or DTPA^{5-} (EDTA^{4-} = ethylenediamine-tetraacetate; DTPA^{5-} = diethylenetriamine-pentaacetate). High thermodynamic stability and kinetic inertness are both indispensable for safe medical application of metal chelates. Gadolinium(III) poly(amino carboxylates) are routinely used today to enhance contrast in Magnetic Resonance Imaging (MRI). Among the commercialized Gd^{III} chelates, Gd(DOTA)$^{-}$ has by far the highest thermodynamic and kinetic stability. The same features also allow for successful application of DOTA-type complexes of the β-emitter ^{90}Y in cancer treatment. The conjugation of ^{90}YDOTA^{-} to monoclonal antibodies or to other receptor targeting moieties (*e.g.* somatostatin-analogues) presents a huge potential in selective internal radiotherapy.[1–3]

The efficiency of a Gd^{III} chelate as an MRI contrast agent is given by its proton relaxivity. Proton relaxivity is influenced by several molecular parameters, involving the rotation of the complex, the electron spin relaxation of the metal center, and the exchange rate between coordinated and bulk water. The development of highly efficient contrast agents requires parallel optimization of all these parameters. The water exchange on currently used Gd^{III} complexes is too slow. Consequently, when such chelates are attached to macromolecules with the objective of optimizing rotation, the relaxivity gain is limited by the non-optimal water exchange rate. Recently we have shown that the water exchange can be considerably accelerated on Gd^{III} complexes by inducing steric compression around the water binding site, a concept that proved to be valid for both linear DTPA-type, and macrocyclic DOTA-type chelates. Steric crowding is induced by elongation of the amine backbone of the ligand, or by replacing a carboxylate arm with a propionate.[4,5] The water exchange on the Gd^{III} complex of the 13-membered macrocyclic TRITA^{4-} is 65 times faster as compared to the 12-membered DOTA^{4-}, which makes GdTRITA^{-} a potential synthon for the development of high relaxivity, macromolecular MRI contrast agents (Scheme 1).



Scheme 1

Non-toxicity is primordial for *in vivo* application of Gd^{III} (or other metal) complexes as MRI contrast agents. In the recent years it has become evident that competitive equilibria based on plasma models cannot solely explain the *in vivo* behavior of Gd^{III} complexes. The excretion of low molecular weight Gd^{III} chelates from the body is very rapid ($t_{1/2} = 1.6$ h for Gd(DTPA)$^{2-}$), whereas the dissociation and transmetallation of the Gd^{III} complexes is relatively slow. Therefore, the system is far from equilibrium, and kinetic factors must be considered.[6] On administration of the contrast agent into the body fluids, the Gd^{III} chelate is surrounded by various endogenous metal ions and ligands. The kinetic stability of the Gd^{III} complex depends on exchange reactions that take place in plasma. The most important is probably the displacement of Gd^{3+} by the endogenous metals Zn^{2+} and Cu^{2+}.[7] This can occur *via* the direct attack of the endogenous metal on GdL, or *via* the proton-assisted dissociation of the complex, followed

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by the fast recombination of the ligand with the scavenging metal ion. Ligand exchange reactions between GdL and ligands present in the blood plasma are usually considered to be of low probability. The dissociation kinetics of macrocyclic and linear lanthanide chelates differ substantially, the rigidity of macrocycles leading to considerably slower dissociation.[8,9] For the linear $Gd(DTPA)^{2-}$, which is probably the most widely used MRI contrast agent, a kinetic model has been established to describe the fate of the complex in body fluids.[10] The excretion and the dissociation of $Gd(DTPA)^{2-}$ were regarded as parallel first-order processes, and by using the kinetic data obtained *in vitro* for exchange reactions with Cu^{2+} and Zn^{2+}, the amount of the complex dissociated in body fluids was estimated at any time after intravenous administration.

The formation kinetics of lanthanide complexes is also remarkably different with linear or macrocyclic ligands. Macrocyclic ligands tend to form complexes much more slowly.[6,11] This slow formation led to significant discrepancies in complex stability constants *e.g.* for $LnDOTA^-$ chelates. From the practical point of view, slow formation kinetics can be a drawback when macrocyclic ligands are used to complex radioactive lanthanides (^{153}Sm, ^{90}Y) in radiopharmaceutical applications.

Crystal structures of lanthanide DOTA complexes and the non-complexed $DOTA^{4-}$ itself indicate similar configurations of the complexed and free ligand, with the four carboxylate groups in *syn* position with respect to the macrocycle plane.[12,13] On metal complexation the ligand undergoes little reorganization, *i.e.* it has a preorganized stucture. Contrary to $DOTA^{4-}$, the structurally similar, but larger macrocycle size $TETA^{4-}$ has the adjacent carboxylate arms in *anti* configuration.[14] Consequently, complex formation requires a drastic ligand rearrangement in order to achieve the *syn* stucture of the complex. It has been postulated that preorganized ligands like $DOTA^{4-}$ form more stable and kinetically more inert lanthanide complexes than ligands without, such as $TETA^{4-}$.[11] With regard to formation kinetics, $LnTETA^-$ complexes seem to show a greater pH and temperature dependency as compared to DOTA analogues and the reaction mechanism also appears to be more complex.[11]

The objective of the present study was to complete the picture on formation and dissociation kinetics of macrocyclic lanthanide(III)–DOTA and TETA complexes with data on the intermediate macrocycle size $LnTRITA^-$ chelates. This contributes not only to the general understanding of kinetic properties of Ln-poly(amino carboxylates), but, given the fast water exchange on $GdTRITA^-$, it has also importance with regard to a potential application of this chelate or its macromolecular derivatives as an MRI contrast agent. The dissociation kinetics of $GdTRITA^-$ has been described by the rates of exchange reactions with Zn^{2+} and Eu^{3+}. For direct comparison, dissociation of $GdTETA^-$ has also been studied. The formation kinetics has been followed with three Ln^{3+} ions, Ce^{3+}, Gd^{3+} and Yb^{3+}, which allow for investigating the effect of the lanthanide size on the rate of complex formation.

Results and discussion

Dissociation kinetics of $GdTRITA^-$ and $GdTETA^-$

To characterize the kinetic stability of $GdTRITA^-$, we have studied the following metal exchange reactions between the complex and Zn^{2+} or Eu^{3+} as exchanging metal ions (charges are omitted for simplicity):

$$\mathrm{GdTRITA} + \mathrm{M} \rightleftharpoons \mathrm{MTRITA} + \mathrm{Gd} \qquad \mathrm{M} = \mathrm{Zn}^{2+}, \mathrm{Eu}^{3+} \qquad (1)$$

Zn^{2+} is the endogenously most abundant metal ion, with a concentration estimated to $\sim 10^{-5}$ M in the plasma, while the second most abundant metal ion Cu^{2+}, is about 10 times less concentrated.[15] The stability constant is known for $GdTRITA^-$ ($\log K_{GdL} = 19.17$, $\log K_{GdHL} = 3.2$),[16] but not for $EuTRITA^-$. However, we can assume very similar complex stability for the two neighbouring lanthanides. The stability of $ZnTRITA^{2-}$ ($\log K_{ZnL} = 19.42$, $\log K_{ZnHL} = 4.07$, $\log K_{ZnH2L} = 2.60$) is also comparable to that of $GdTRITA^-$. Consequently, in the presence of 10–60 fold excess of the exchanging Eu^{3+} or Zn^{2+}, the exchange reactions (1) are quantitative or close to quantitative.

In the presence of metal ion excess, the reaction is of pseudo-first-order and the exchange rate is directly proportional to the total concentration of the Gd^{3+} complex at a given time, $[GdL]_t$:

$$-\frac{\mathrm{d}[\mathrm{GdL}]_t}{\mathrm{d}t} = k_{obs}[\mathrm{GdL}]_t \qquad (2)$$

where k_{obs} is a pseudo-first-order rate constant. The rates of the metal exchange reactions have been determined for varying concentrations of the exchanging metal ion (5–30 mM; $c_{GdTRITA} = 0.5$ mM) and for different pH values (4.1–5.2). Figs. 1 and 2 show the pseudo-first-order rate constants, k_{obs}, for the reactions with Zn^{2+} and Eu^{3+}, respectively.

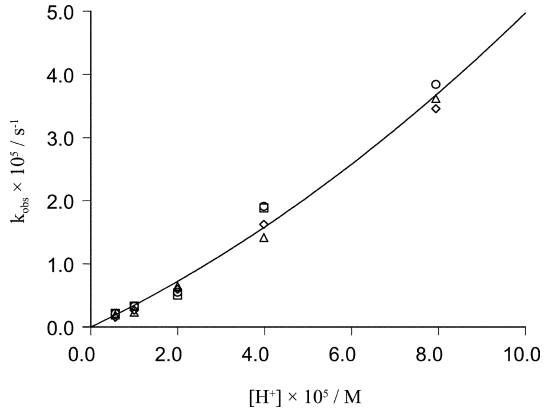


Fig. 1 Pseudo-first-order rate constants, k_{obs}, as a function of the proton concentration in the reaction of $GdTRITA^-$ with Zn^{2+}. $c_{Zn} = 0.005$ M (□); 0.01 M (○); 0.02 M (◇) and 0.03 M (△) (25 °C; 0.1 M KCl).

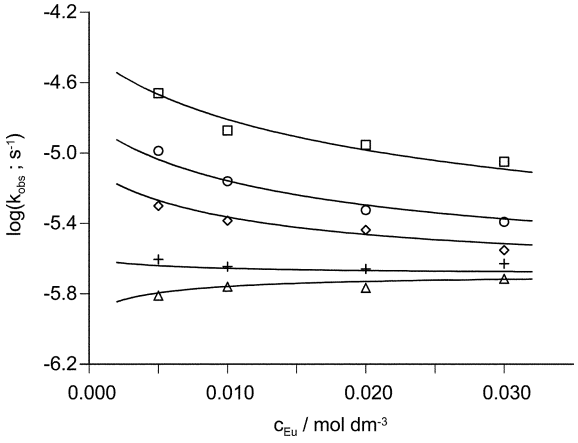


Fig. 2 Pseudo-first-order rate constants, k_{obs}, as a function of the Eu^{3+} concentration in the reaction of GdTRITA with Eu^{3+}. pH = 4.11 (□); 4.28 (○); 4.58 (◇), 5.02 (+) and 5.33 (△) (25 °C; 0.1 M KCl).

The variation of the k_{obs} values with pH and with the concentration of the exchanging metal ion is fundamentally different for Eu^{3+} and Zn^{2+}. The rate constants are independent of $[Zn^{2+}]$, but dependent upon $[H^+]$, as was previously found for exchange reactions between Zn^{2+} or Cu^{2+} and macrocyclic Ln^{III} chelates such as LnNOTA complexes†.[17] This means that the rate determining dissociation of $GdTRITA^-$ is followed by rapid formation of $ZnTRITA^{2-}$. The dependence of the

† H_3NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid.

Table 1 Dissociation rate constants for some Ln^{III} complexes; 25 °C

	k_0/s^{-1}	$k'/M^{-1}s^{-1}$	$k''/M^{-2}s^{-1}$
$GdDOTA^{-a}$	5.0×10^{-10}	2.0×10^{-5}	—
$GdTRITA^{-}$	$(4.2 \pm 0.5) \times 10^{-7}$	$(3.5 \pm 0.3) \times 10^{-1}$	$(1.4 \pm 0.4) \times 10^{3}$
$EuTETA^{-b}$	8.3×10^{-4}	1.76	—
$GdNOTA^{c}$	8.3×10^{-6}	2.3×10^{-2}	—

[a] 37 °C; Ref. 8 [b] Ref. 18 [c] Ref. 17

experimentally obtained rate constants on the proton concentration can be expressed by a second-order function of $[H^+]$:

$$k_{obs} = k_0 + k' [H^+] + k''[H^+]^2 \quad (3)$$

This pH-dependence implies the equilibrium formation of the protonated complex, GdHTRITA, which dissociates faster then the non-protonated complex:

$$GdL^- + H^+ \overset{K_{GdHL}}{\rightleftharpoons} GdHL \xrightarrow{k_{GdHL}} Gd^{3+} + HL^{3-} \quad (4)$$

The quadratic term in eqn. (3) shows that, in addition to eqn. (4), the proton-assisted dissociation of the monoprotonated species has also to be considered:

$$GdHL + H^+ \xrightarrow{k^H_{GdHL}} Gd^{3+} + H_2L^{2-} \quad (5)$$

The pseudo-first-order rate constants in Fig. 1 were fitted to eqn. (3) and the rate constants obtained are shown in Table 1. Within the tetraazamacrocycles, the kinetic stability remarkably increases with decreasing macrocycle size from L = TETA to TRITA and DOTA. In comparison to $EuTETA^-$, the rate constant k_0, characterizing the spontaneous dissociation of $GdTRITA^-$ is three orders of magnitude, while k', which describes the proton-assisted dissociation, is one order of magnitude lower. For $GdDOTA^-$, the corresponding rate constants are again several orders of magnitude lower. The proton-assisted dissociation of the protonated complex was not detected for $EuTETA^-$ or GdNOTA in studying exchange reactions with Zn^{2+} or Cu^{2+}.[17,18] In the protonated GdHTRITA complex, the proton is attached to a carboxylate oxygen, however, the dissociation of the complex will only occur when the proton is transferred to a macrocycle nitrogen. This rearrangement of the complex is probably the rate-controlling step of the dissociation, as was previously stated for various Ln^{III} macrocyclic chelates.[8,17]

Although the spontaneous dissociation of these Gd^{III} complexes is very slow, at physiological pH (7.4), important for biomedical application, it is the k_0 value that essentially determines the overall dissociation rate (for $GdTRITA^-$, the proton-assisted pathway represents ~3%). Assuming the validity of eqn. (3) at pH 7.4, the half-live of the dissociation is 444 h for $GdTRITA^-$ *vs.* 0.23 h for $EuTETA^-$, 23.2 h for GdNOTA (25 °C) and 3.8×10^5 h for $GdDOTA^-$ (37 °C). This clearly shows the exceptional kinetic inertness of $GdDOTA^-$, linked to the perfect size match between the metal ion and the coordination cage. The coordination cage of the NOTA-macrocycle is too small for Gd^{3+}, while that of $TRITA^{4-}$ is too large, which in both cases leads to a considerable decrease of the kinetic stability of the Gd^{3+} complexes. It should be noted that for the spontaneous dissociation of $^{111}InTRITA^-$ in human serum (37 °C), a value of $k_{obs} \approx 4 \times 10^{-8}$ s^{-1} was estimated.[19] For the linear $GdDTPA^{2-}$, a half-life of $t_{1/2} = 127$ h was calculated (pH = 7.4; $c_{Zn^{2+}} = 10^{-5}$ M, $c_{Cu^{2+}} = 10^{-6}$ M; 25 °C).[20] In addition to the proton-assisted dissociation, this $t_{1/2}$ takes into account the Zn^{2+} and Cu^{2+}-assisted pathways, which are very efficient in the case of linear, DTPA-derivative Gd^{3+} complexes.

Contrary to Zn^{2+} exchange, in the reaction of $GdTRITA^-$ with Eu^{3+} the pseudo-first-order rate constants change with the Eu^{3+} concentration. Moreover, they show different trends depending on the pH: at lower pH the k_{obs} values strongly decrease, while at higher pH they increase with increasing $[Eu^{3+}]$ (Fig. 2). Such a dependence on the exchanging metal concentration appears surprising, however, similar results have been already reported for metal exchange reactions of different linear poly(amino carboxylate) complexes. Margerum observed that in the reaction of Cd(CyDTA) with Pb^{2+} or Cu^{2+} the exchanging metal ion suppresses its own rate of exchange‡.[21] He explained this anomaly by steric requirements of $CyDTA^{4-}$ which prevent the direct exchange of metal ions. Instead, the exchange rate is controlled by a hydrogen ion reaction with $CdCyDTA^{2-}$. Lead(II) forms a weak complex with the acetate groups of $CdCyDTA^{2-}$ which blocks the protonation of the complex, thus the excess Pb^{2+} or Cu^{2+} slows down the reaction. More recently, similar results have been found for the metal exchange between $GdDTPA^{2-}$ and Eu^{3+}[10] or GdEOB–$DTPA^{2-}$ and Y^{3+}.[22]

The rate decrease with increasing Eu^{3+} concentration in Fig. 2 can therefore be interpreted with the transitional formation of the dinuclear species, $[GdTRITA]Eu^{2+}$. In the case of $DTPA^{5-}$, such Ln_2 dinuclear species were not only assumed from kinetic studies,[23] but experimentally detected by NMR.[24] For macrocyclic Ln^{III} complexes, we are not aware of similar data. Therefore it appeared interesting to directly compare the behaviour of $GdTRITA^-$ to a related, macrocyclic chelate. For this comparative study we have chosen the Gd^{III} complex of the 14-membered analogue $TETA^{4-}$.

Fig. 3 shows the pseudo-first-order rate constants obtained for the exchange reaction between $GdTETA^-$ and Eu^{3+} as a function of the exchanging metal ion concentration at variable pH values. At all pHs studied, the k_{obs} values diminish with increasing $[Eu^{3+}]$, indicating that, similarly to $GdTRITA^-$, the transitional formation of dinuclear complexes has to be taken into account. The formation of such $GdLEu^{2+}$ species becomes increasingly important with increasing Eu^{3+} concentration, and will result in a decrease of the concentration of the monoprotonated GdHTRITA or GdHTETA by shifting the protonation equilibrium in eqn. (4) to the left. Since the protonated complex contributes actively to the observed dissociation rate, this latter can decrease with increasing Eu^{3+} concentration.

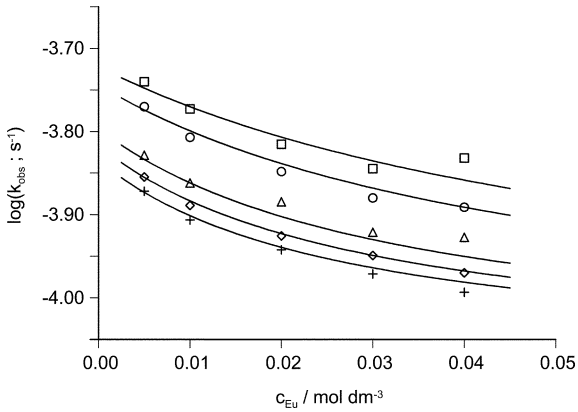
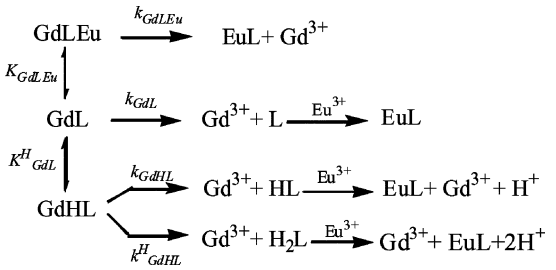


Fig. 3 Pseudo-first-order rate constants, k_{obs}, as a function of the Eu^{3+} concentration in the reaction of GdTETA with Eu^{3+}. pH = 4.51 (□); 4.70 (○); 5.10 (△), 5.30 (◇) and 5.55 (+) (25 °C; 0.1 M KCl).

Based on considerations on the metal-assisted dissociation of Ln^{III} poly(amino carboxylates) as previously reported,[10,22,23] the following possible reaction pathways are assumed to describe the experimental rate data in the exchange of $GdTRITA^-$ or $GdTETA^-$ with Eu^{3+} (Scheme 2): According to this reaction scheme, the rate of the exchange reaction can be given as in eqn. (6):

$$-\frac{d[GdL]_t}{dt} = k_{GdL}[GdL] + k_{GdHL}[GdHL] + k^H_{GdHL}[GdHL][H^+] + k_{GdLEu}[GdLEu] \quad (6)$$

‡ H_4CyDTA = *trans*-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid.



Scheme 2

In eqn. (6), the first term refers to the spontaneous, the second and third terms to the proton-assisted dissociation of the complex, while the last contribution describes the metal-assisted dissociation. The equilibrium constants for the protonated and the dinuclear species are given by eqns. (7) and (8):

$$K^{H}_{GdL} = \frac{[GdHL]}{[GdL][H^+]} \quad (7)$$

$$K_{GdLEu} = \frac{[GdLEu]}{[GdL][Eu]} \quad (8)$$

The total concentration of the complex can be expressed as:

$$[GdL]_t = [GdL] + [GdHL] + [GdLEu] \quad (9)$$

Thus for the pseudo-first-order rate constant one derives:[23]

$$k_{obs} = \frac{k_0 + k_1[H^+] + k_2[H^+]^2 + k_3[Eu^{3+}]}{1 + K^{H}_{GdL}[H^+] + K_{GdLEu}[Eu^{3+}]} \quad (10)$$

with $k_0 = k_{GdL}$, $k_1 = k_{GdHL} \times K^{H}_{GdL}$, $k_2 = k^{H}_{GdHL} \times K^{H}_{GdL}$ and $k_3 = k_{GdLM} \times K_{GdLM}$. The protonation constants for GdTRITA$^-$ and GdTETA$^-$ have been previously determined by pH-potentiometry (logK^{H}_{GdL} = 3.2 and 4.5, respectively).[16] The pseudo-first-order rate constants in Figs. 2 and 3 were fitted to eqn. (10) by fixing logK^{H}_{GdL} to the above values, and the rate constants k_0, k_1, k_2 and k_3, as well as the stability constant of the dinuclear complex, K_{GdLEu} were calculated. All constants obtained are listed and compared to those for GdDTPA^{2-} in Table 2.

In some of the exchange reactions of Ln^{III} DTPA- or DTPA-derivative complexes with different metal ions (M = Ln^{3+}, Cu^{2+} or Zn^{2+}), the proton assisted dissociation of the dinuclear complex LnLM had also to be taken into account in order to describe the experimental reaction rates.[10,23] This reaction pathway, usually characterized by a rate constant k_4, does not seem to be operative in our case (a value of k_4 = 40 M^{-2}s^{-1} was reported for the GdDTPA–Eu^{3+} exchange[10]). In fact, including this reaction route in the analysis, the fit of the data did not improve for the GdTRITA$^-$/GdTETA$^-$ + Eu^{3+} systems, and small negative k_4 values were obtained with large errors.

By using the rate constants presented in Table 2, one can derive the individual rate constants for each reaction pathway of Scheme 2: k_{GdHL} = (1.3 ± 0.2) × 10^{-4} s^{-1}, k^{H}_{GdHL} = 2.6 ± 0.6 s^{-1}M^{-1}; k_{GdLEu} = (2.1 ± 0.5) × 10^{-6} s^{-1} for GdTRITA$^-$ and k_{GdHL} = (2.3 ± 0.5) × 10^{-4} s^{-1}, k^{H}_{GdHL} = 0.055 ± 0.021 s^{-1}M^{-1} and k_{GdLEu} = (8.2 ± 1.5) × 10^{-5} s^{-1} for GdTETA$^-$. k_0 could be determined only with a large error, since at pHs of this study it represents a small contribution in the numerator of eqn. (10). However, k_0 values for both GdTRITA$^-$ and GdTETA$^-$ are in the same order of magnitude as those reported in Table 1.

Table 2 Rate constants obtained for the exchange reaction of GdL complexes with Eu^{3+}; 25 °C

	TRITA	TETA	DTPA[10]
k_0/s^{-1}	≤6 × 10^{-7}	(1.3 ± 0.5) × 10^{-4}	—
k_1/M^{-1}s^{-1}	0.21 ± 0.04	10.8 ± 2.1	0.58
k_2/M^{-2}s^{-1}	(4.1 ± 1.0) × 10^3	(2.6 ± 1.5) × 10^3	9.7 × 10^4
k_3/M^{-1}s^{-1}	(3.7 ± 1.1) × 10^{-4}	(4.7 ± 1.3) × 10^{-3}	4.9 × 10^{-4}
K_{GdLEu}	180 ± 44	57 ± 20	20
log K^{H}_{GdL}	3.2	4.5	2.0

Formation kinetics of LnTRITA$^-$ complexes

The kinetics of formation of LnTRITA$^-$ complexes has been studied for three lanthanide ions, Ce, Gd and Yb, representing the early, middle and late lanthanides, respectively. In the pH range studied, the formation is slow enough to be followed by classical UV-Vis spectrophotometry. For CeTRITA$^-$, we followed the change in the absorbance of the complex, while for Gd- and YbTRITA$^-$ the formation was studied in weakly buffered solutions by monitoring the pH decrease with bromocresol green as a dye. In the presence of Ln^{3+} excess, the complex formation is a pseudo-first-order process and its rate can be expressed as in eqn. (11):

$$\frac{d[LnTRITA]}{dt} = k_{obs}[TRITA]_0 \quad (11)$$

where [TRITA]$_0$ is the total concentration of the free ligand and k_{obs} is the pseudo-first-order rate constant. The formation reactions were investigated at different pHs with varying concentration of the lanthanide ions. For all systems and at each pH, the k_{obs} values show a saturation curve against Ln^{3+} concentration (Fig. 4). Such saturation kinetics can be ascribed to the rapid formation of an intermediate that rearranges to the product in

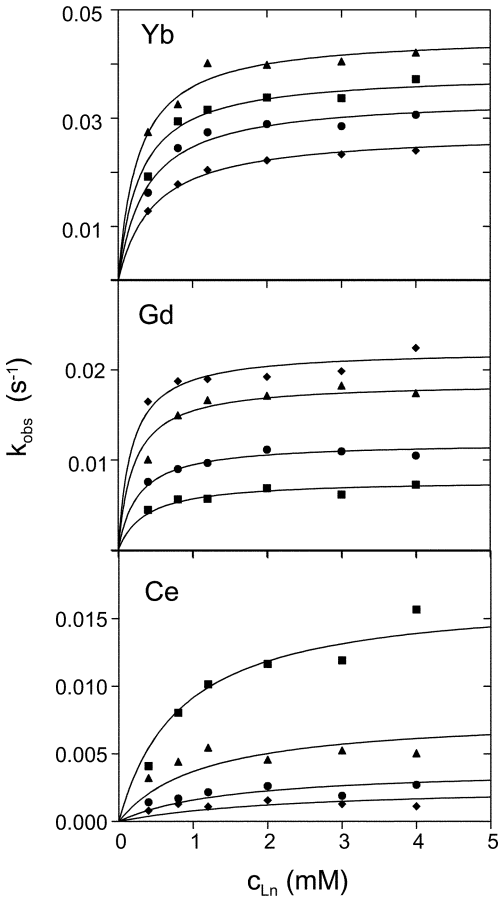


Fig. 4 Pseudo-first-order rate constants, k_{obs}, as a function of the Ln^{3+} concentration in the formation reaction of LnTRITA complexes. Yb: pH = 4.45 (◆); 4.65 (●); 4.87 (■), 5.00 (▲); Gd: pH = 4.49 (■); 4.66 (●); 4.89 (▲), 5.08 (◆); Ce: pH = 4.40 (◆); 4.70 (●); 5.00 (▲), 5.80 (■) (25 °C: 1.0 M KCl). The solid lines represent the simultaneous fit of the data at all pHs to eqn. (18).

Table 3 Protonation constants of H_4TRITA

	log K_1	log K_2	log K_3	log K_4
H_4TRITA 0.1 M TMACl (this work)	11.03(0.04)	9.47(0.02)	4.62(0.07)	3.74(0.07)
H_4TRITA 0.1 M KCl (this work)	10.91(0.08)	9.48(0.08)	4.52(0.13)	4.06(0.09)
H_4TRITA 0.1 M KCl[16]	11.79	9.20	4.00	2.57
H_4DOTA 0.1 M KCl[16]	11.14	9.69	4.85	3.95

a slow, rate controlling process. Similar formation behaviour has been reported for a series of macrocyclic chelates, such as $CaDOTA^{2-}$,[25] $LnDOTA^-$ and LnDOTA-derivatives,[8,26–28] or LnNOTA complexes.[17]

The dependence of the k_{obs} values on the metal ion concentration is given by eqn. (12):

$$k_{obs} = \frac{k\,K^*[\mathrm{Ln}]}{1 + K^*[\mathrm{Ln}]} \tag{12}$$

where K^* is the equilibrium constant (conditional stability constant) characterizing the formation of the intermediate and k is the rate constant of the deprotonation and rearrangement of the intermediate to the product.

We propose that the intermediate is the diprotonated species, LnH_2TRITA*. This is supported on one hand by previous data on the formation of Ln^{3+}–DOTA analogues, for which the presence of diprotonated intermediates has been experimentally proved.[8,26] In the formation of $CeDOTA^-$, the intermediate could be directly detected by UV-Vis spectroscopy. At pH < 8, the intermediate was found to be diprotonated, while above this pH it was monoprotonated, however, they both had the same spectrum, thus the same structure.[29] For $CeTRITA^-$, the isosbestic point in the UV-Vis spectrum does not overlap with the spectrum of the aqua ion, thus points to the formation of an intermediate (Fig. 5). The protonation constants of $TRITA^{4-}$ and $DOTA^{4-}$ are similar (Table 3). The formation of the diprotonated intermediates is a consequence of the large difference between the first two and the third-fourth protonation constants of the ligands. The LnH_2TRITA* intermediate is likely to be similar in structure to LnH_2DOTA*, where the two protons are attached to the ring nitrogens and the lanthanide ion has not yet entered the coordination cage.

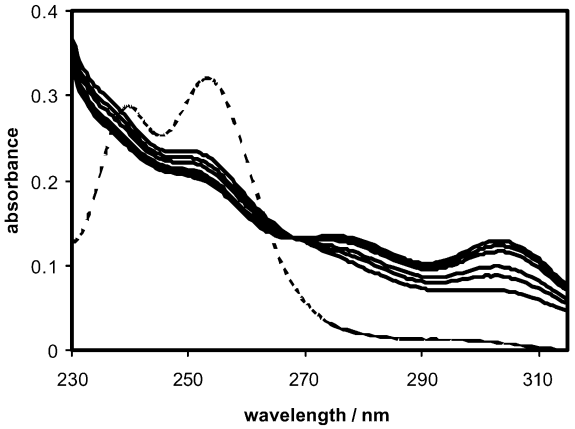


Fig. 5 UV-Vis spectra recorded at different t times after mixing Ce^{3+} and TRITA (solid lines) at pH = 4.30 (0.02 M N-methylpiperazine; $c_{Ce} = c_{TRITA} = 2 \times 10^{-4}$ M; 0.1 M KCl, 25 °C); t = 10, 20, 30, 60, 90 and 150 min (from bottom to top). The dotted line shows the spectrum of Ce^{3+} aq.

The composition of the LnH_2TRITA* intermediates has also been evidenced by monitoring the pH decrease, thus the quantity of the protons released in the first rapid, and the second slow step of the formation reaction.[8,17] On mixing slightly buffered solutions (0.025 M N-methylpiperazine) of Ce^{3+} and TRITA ($c_{Ce} = 2.5 \times 10^{-3}$M; $c_{TRITA} = 5 \times 10^{-4}$M; pH 4.40; V = 10.0 ml), one observes an immediate pH drop to 4.36 (within ~30 s), which is followed by a slow decrease of the pH to an equilibrium value of 4.25 (measured after ~1 day). The exact amount of protons released in each of the two steps could be determined from an independent titration of a solution of the same N-methylpiperazine concentration with 0.1 M HCl, by measuring the amount of HCl necessary for a pH decrease corresponding to that observed above (0.054 ml and a further 1.080 ml 0.1 M HCl had to be added to reach pH 4.36 and 4.25, respectively). The amount of protons released in the first 30 s which leads to the intermediate is half of that liberated in the slow step (formation of the complex from the intermediate). According to the protonation constants, at the starting pH (4.40), the ligand is in $H_{2.98}$TRITA form. Therefore this experiment proves that the intermediate is the diprotonated GdH_2TRITA* complex.

The conditional equilibrium constant of the intermediate, K^*, as given in eqn. (12) can be related to the thermodynamic stability constant, $K_{LnH2TRITA*}$, defined in eqn. (13):

$$K_{LnH2TRITA^*} = \frac{[LnH_2TRITA^*]}{[Ln]\,[H_2TRITA]} \tag{13}$$

If we assume that the rate determining step of the formation reaction is the proton release from the intermediate and the rearrangement of the complex, the rate of this first-order process can be expressed as in eqn. (14):

$$\frac{d[LnTRITA]}{dt} = k_{obs}[TRITA]_0 = k[LnH_2TRITA^*] \tag{14}$$

The total ligand concentration is given by eqn. (15)

$$[TRITA]_0 = [LnH_2TRITA^*] + [TRITA]_{free} \tag{15}$$

where $[TRITA]_{free}$ is the total concentration of the non-complexed ligand.

$$[TRITA]_{free} = [H_4TRITA] + [H_3TRITA^-] + [H_2TRITA^{2-}] + [HTRITA^{3-}] + [TRITA^{4-}] \tag{16}$$

The concentration of the fully deprotonated and the monoprotonated ligand is practically zero at the pH of the complex formation study. Using the protonation constants one obtains:

$$[TRITA]_{free} = [H_2TRITA]\left(1 + K_3[H^+] + K_3K_4[H^+]^2\right)$$
$$[TRITA]_{free} = [H_2TRITA]\cdot\alpha_H \tag{17}$$

From eqns. (13), (14) and (17), one derives eqn. (18), with $K^* = K_{LnH2TRITA*}/\alpha_H$.

$$k_{obs} = \frac{k(K_{LnH2TRITA^*}/\alpha_H)[Ln]}{1 + (K_{LnH2TRITA^*}/\alpha_H)[Ln]} \tag{18}$$

The pseudo-first-order rate constants at the various pHs and metal concentrations (Fig. 4) were simultaneously fitted to eqn. (18), and the rate constants k and the stability constant of the intermediate, $K_{LnH2TRITA*}$, were calculated for each of the three lanthanides. (By fitting the k_{obs} rate constants separately for each pH, the $K_{LnH2TRITA*}$ values did not show any tendency with pH).

The stability constants of the LnH_2TRITA* intermediates, presented in Table 4, are comparable to those obtained for

Table 4 Rate constants, k_{OH}, characterizing the formation of a selection of LnL complexes, stability constants of the reaction intermediates, log K_{LnH2L*}, and of the complexes, log K_{LnL}

Complex	k_{OH} /$M^{-1}s^{-1}$	log K_{LnH2L*}	log K_{LnL}
$CeTRITA^-$	$(6.9 \pm 0.3) \times 10^6$	3.11 ± 0.05	[e]
$GdTRITA^-$	$(2.6 \pm 0.1) \times 10^7$	3.89 ± 0.05	19.17
$YbTRITA^-$	$(5.0 \pm 0.2) \times 10^7$	3.73 ± 0.04	
$CeDOTA^-$	3.5×10^6 [a]	4.5[a]	21.6[f]
$EuDOTA^-$	1.1×10^7 [a]	4.4	23.7[f]
$YbDOTA^-$	4.1×10^7 [a]	4.3	24.0[f]
CeDO3AB[b]	2.1×10^6	2.4	17.8
CeDO3AME[b]	6.7×10^6	3.1	18.8
$CeDO2A^{+}$ [b]	2.8×10^5	1.98	11.3
CeNOTA[c]	6.3×10^7	3.2[d]	

[a] Ref 8; [b] Ref 28; [c] Ref 17; [d] log K_{LnHL*}; [e] log K_{LnL} = 17.02 for $LaTRITA^-$, Ref 16; [f] Ref 31

NOTA- or DO3AME§-intermediates, but lower than the stability constants of the LnH_2DOTA* analogues. A correlation between the stability constant of the formation intermediate and the thermodynamic complex stability constant of the final product has already been observed for different Ln^{3+} cyclen-derivatives.[28] LnH_2DOTA* intermediates were found to be the most stable among all macrocyclic complexes, while the intermediate stability is progressively reduced for macrocycles that possess three or two carboxylates (DO3A- and DO2A-derivatives). This was rationalized by the coordination of four carboxylates in the DOTA-intermediates, compared to only three or two coordinated carboxylates for the DO3A- and DO2A-derivatives. Moreover, even within the DO3A- or DO2A-derivatives, the intermediate stability could be related to the complex stability. Indeed, by plotting the log K_{LnHxL*} stability constant of the intermediate *versus* the log K stability constant of the complex, the data for the various systems reported in the literature seem to show a linear tendency, though with large scattering (Fig. 6). The $LnH_2TRITA*$ intermediates also fit into this picture. As the Ln^{3+} complexes are less stable for $TRITA^{4-}$ than for $DOTA^{4-}$, the stability of the intermediates is also decreased by about one order of magnitude, even if in both cases all four carboxylates are likely coordinated to the metal in the intermediate stage. On the other hand, the three different lanthanides studied do not show any trend with respect to the intermediate stability, as it was also observed for cyclen derivatives studied so far.

The rate constants, k, characterizing the formation of the product from the intermediate are inversely proportional to the proton concentration (eqn. (19); Fig. 7).

$$k = k' \frac{1}{[H^+]} = k_{OH}[OH^-]; \quad k_{OH} = \frac{k'}{K_w} \tag{19}$$

Similar rate laws have been obtained for Ln^{3+} complexes of all cyclen derivatives or NOTA.[8,17,28] The k_{OH} values determined are presented and compared to different cyclen derivative complexes in Table 4.

As for the DOTA complexes,[28] the inverse proportionality between the formation rates and the H^+ ion concentration can be explained in terms of the equilibrium deprotonation of the LnH_2L* intermediate, which is followed by the release of a second proton in a rate-controlling step and the subsequent, fast rearrangement of the complex. The concentration of the monoprotonated complex, which will then deprotonate in the rate determining step, is inversely proportional to the $[H^+]$, which thus accounts for the observed rate law (eqn. (19)). The validity of a general base catalysis was proved for CeDO3AB which supports the rate controlling role of the deprotonation

§ H_3DO3AME = 1-methoxyethyl-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane.

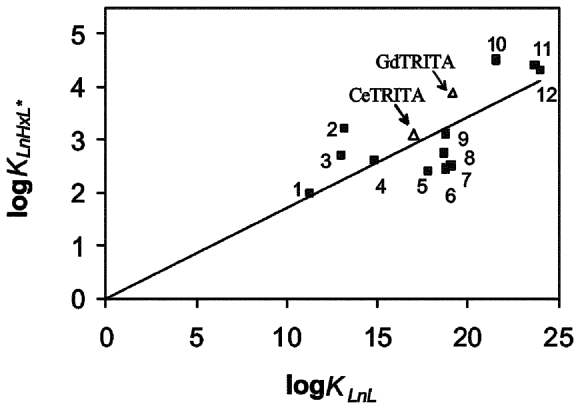


Fig. 6 Correlation between the stability constant of the formation intermediate, log K_{LnHxL*}, and the stability constant of the final complex, log K_{LnL} for various macrocyclic lanthanide(III) complexes reported in the literature. 1. $CeDO2A^{+}$;[28] 2. $CeDOTAGl^{3+}$*;[30] 3. CeNOTA;[17] 4. $EuDOTAGl^{3+}$¶;[30] 5. CeDO3AB*;[28] 6. YbDO3AB;[28] 7. EuDO3AB;[28] 8. YbDO3AMe;[28] 9. CeDO3AMe;[28] 10. $CeDOTA^-$;[8] 11. $EuDOTA^-$;[8] 12. $YbDOTA^-$.[8] For $CeTRITA^-$, we used the log K value of $LaTRITA^-$.[16] The solid line represents a linear fit to the data points only to show the tendency ($R^2 = 0.78$).

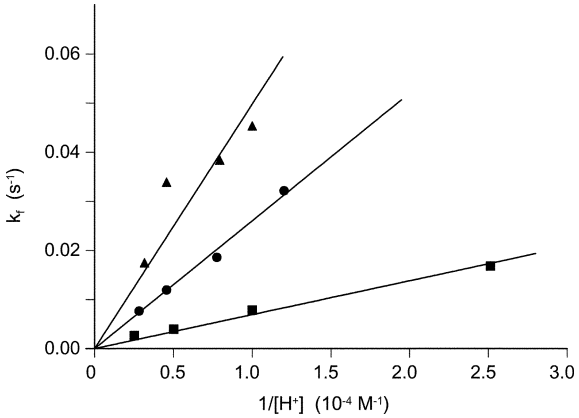


Fig. 7 Formation rates of $LnTRITA^-$ complexes *vs.* the inverse H^+ concentration for Ln = Ce (■), Gd (●) and Yb (▲). 0.1 M KCl, 25 °C.

of the monoprotonated intermediate.[28] Since the $TRITA^{4-}$ ligand is close in structure to these cyclen derivatives, the same considerations are likely to apply for the formation of $LnTRITA^-$ complexes as well.

For $LnTRITA^-$ formation, the k_{OH} rate constants increase with decreasing lanthanide size (Table 4). This is not surprising, since the OH^- catalyzed deprotonation is faster for a late lanthanide with a greater charge density which leads to a stronger electrostatic repulsion between the metal ion and the proton. This tendency of the k_{OH} values in the lanthanide series seems to be general, as it has been reported for all cyclen derivatives with acetate pendant arms, however, not for LnNOTA complexes.[9,17,28] Interestingly, in the formation reaction of $CeTETA^-$ a second-order $[OH^-]$ dependence was found for the k constants, indicating the contribution a di-hydroxide ion assisted pathway.[11] This was explained with the hydroxide catalyzed rearrangement of the non-pre-organized TETA ligand.

Conclusion

We have described the kinetic stability of $GdTRITA^-$ by determining the rates of exchange reactions with the endogenously most abundant Zn^{2+} and with Eu^{3+} ions. In the first case, the

¶ DOTAGl = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetylglycine.

* H_3DO3AB = 1-butriol-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane.

rate is independent of the exchanging metal concentration, and shows a quadratic dependence on [H^+]. In contrast to this, the dissociation of $GdTRITA^-$ (and $GdTETA^-$) in the presence of Eu^{3+} can be slowed down by the exchanging metal ion, due to a competition between the formation of dinuclear GdLEu species and the protonation of the GdL complex, this latter leading to an efficient pathway for dissociation. The kinetic inertness of Ln^{3+} tetraazamacrocyclic complexes gradually decreases by several orders of magnitude from the 12-membered DOTA to the 13-membered TRITA and to the 14-membered TETA. The formation of $LnTRITA^-$ complexes is very similar in mechanism to that of the DOTA analogues (or other cyclen derivatives), but proceeds about twice as fast as for $LnDOTA^-$ complexes.

Given the fast water exchange rate on $GdTRITA^-$, these results may have implications for the future design of contrast agents for Magnetic Resonance Imaging. In particular, they show that with regard to kinetic inertness $GdTRITA^-$ is better than $GdDTPA^{2-}$, the most commonly used contrast agent.

Experimental

H_4TRITA was synthesized according to procedures described in the literature.[4,32] H_4TETA was purchased from Fluka and used without further purification. The concentration of TRITA and TETA solutions was determined by pH-potentiometry, on the basis of titration curves of the ligands obtained in the absence and presence of at least 50 fold excess of $CaCl_2$. The difference in the inflection points between the two titration curves corresponds to 2 equivalents of the ligand.

Preparation of the stock solutions

The stock solution of $GdCl_3$ and $EuCl_3$ were prepared by dissolving Gd_2O_3 and Eu_2O_3 (Fluka) in a slight excess of conc. HCl in doubly distilled water. The excess of HCl was evaporated off. The $ZnCl_2$ and $CeCl_3$ solutions were made from chloride salts in doubly distilled water. The concentrations were determined by complexometric titrations with Na_2H_2EDTA solution using xylenol orange as an indicator.

The solutions of the GdL complexes were prepared by mixing equimolar amounts of $LnCl_3$ and the ligand, the pH was adjusted to about 6.0 by adding 0.1 M HCl or 0.1 M KOH.

Equilibrium measurements

The protonation constants of H_4TRITA were determined with pH-potentiometry by titrating 2.0 mM H_4TRITA solutions with standardized KOH or $(CH_3)_4NOH$ (TMAOH) solution using a Metrohm Dosimat 665 automated burette. A combined glass electrode (C14/02-SC, reference electrode Ag/AgCl in 3 M KCl, Moeller Scientific Glass Instruments, Switzerland) connected to a Metrohm 692 pH/ion-meter was used to measure pH. The titrated samples (3 ml) were stirred and N_2 was bubbled through the solutions. The titrations were carried out at 25 °C and at a constant ionic strength of 0.1 M KCl or 0.1 M TMACl. The H^+ concentration was obtained from the measured pH values according to the method proposed by Irving *et al.*[33] The protonation constants were calculated from 3 parallel titrations with the program PSEQUAD.[34] The errors given correspond to one standard deviation.

Kinetic studies

Dissociation kinetics. The rates of the exchange reactions between the complex GdL and Eu^{3+} or Zn^{2+} have been studied by measuring the longitudinal relaxation rates ($1/T_1$) of water protons by inversion recovery at 200 MHz on a Bruker spectrometer. The temperature in the sample holder was maintained at 25 °C with an air stream and was measured by a substitution technique. The Eu^{3+} and Zn^{2+} concentration varied between 5×10^{-3} and 3×10^{-2} M, while the concentration of $Gd(TRITA)^-$ and $Gd(TETA)^-$ was 5×10^{-4} M. 0.02 M *N*-methylpiperazine was used as buffer and the ionic strength was 0.1 M KCl. The pH varied between 4.1–5.3.

The relaxivities, r_1, were calculated from the measured $1/T_{1obs}$ water proton relaxation rates according to eqn. (20), where $1/T_{1w}$ is the relaxation rate of water at the given temperature, and [Gd] is the Gd^{3+} concentration in mM.

$$\frac{1}{T_{1obs}} = \frac{1}{T_{1w}} + \frac{1}{T_{1p}} = \frac{1}{T_{1w}} + r_1 \times [\mathrm{Gd}] \tag{20}$$

The pseudo-first-order rate constants (k_{obs}) were calculated by fitting the relaxation rate data to eqn. (21),

$$r_{1t} = (r_{10} - r_{1e}) \exp(-k_{obs} t) + r_{1e} \tag{21}$$

where r_{1t}, r_{10} and r_{1e} are the relaxivity values at time t, time zero and at equilibrium, respectively.

Formation kinetics. The formation rates of $Ce(TRITA)^-$, $Gd(TRITA)^-$ and $Yb(TRITA)^-$ were studied at 25 °C and 0.1 M KCl ionic strength by direct spectrophotometry for Ce^{3+} and by an indicator method for Gd^{3+} and Yb^{3+}, on a Perkin-Elmer Lambda 19 UV-Vis spectrometer. In the indicator method, bromocresol green was used and the pH was allowed to change 0.05–0.1 unit in slightly buffered solutions. The measurements were performed in tandem cuvettes (HELLMA, optical path length 2×4.38 mm) in a cuvette holder capable of being thermostatted. The metal concentrations varied between 2×10^{-4}–2×10^{-3} M, while the concentration of TRITA was 2×10^{-4} M. *N*-Methylpiperazine was used as buffer, and its suitable concentration was determined in each experiment. The pH varied between 4.1–5.5.

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