Formation and Dissociation Kinetics of the Complexes $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^{-}$

László Burai,[a] Róbert Király,[a] István Lázár,[a] and Ernő Brücher*[a]

Keywords: Gadolinium / Imaging agents / Kinetics / Macrocyclic ligands / Phosphonate complexes

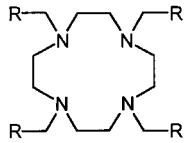
The monobutyl ester of H_8DOTP, the ligand $H_4DOTPMB$, was synthesized, and the protonation constants (K_i^H) and the stability constant of Gd(DOTPMB) were determined by pH-potentiometry (25 °C, 0.1 M Me_4NCl): $\log K_i^H$, (i = 1, 2, and 3) = 10.34(0.02), 7.72(0.025), and 2.42(0.030), respectively, and $\log K_{GdL}$ = 12.19(0.05). The rates of formation of Gd(DOTPMB) and Gd(DOTP) were studied by ^1H relaxometry in the pH range 5.4–7, and also by spectrophotometry in the case of Gd(DOTP) (7 < pH < 8). For both reactions, first-order rate constants were obtained at different concentration ratios of the reactants, which indicated the rapid formation of a reaction intermediate. The compositions of the intermediates are $Gd(H_iDOTPMB)$ (i = 1, 2) and $H_nGd(H_2DOTP)$ (n = 0–4), respectively, where one or two protons are attached to the nitrogen atoms of the ligand. The rate of rearrangement (k_r) of the intermediate $Gd(H_iDOTPMB)$ to the product Gd(DOTPMB) increases with increasing $[OH^-]$: $k_r = k_{OH}[OH^-] + k_{2OH}[OH^-]^2$, where k_{OH} = (1.3±0.25) $\times 10^3$ $M^{-1}s^{-1}$ and k_{2OH} = (7.8±0.2) $\times 10^{11}$ $M^{-2}s^{-1}$. For the formation reaction of Gd(DOTP), only the first term exists and k_{OH} = (7.2±0.1) $\times 10^3$ $M^{-1}s^{-1}$. For the complexation reactions, similar mechanisms were proposed in which deprotonation of the species Gd(HDOTPMB) and $H_nGd(HDOTP)$ plays the rate-determining role. For the deprotonation, general base catalysis was found to be satisfactory. The rate of dissociation of Gd(DOTPMB) in 0.025–1.0 M HCl solution ([HCl] + $[Me_4NCl]$ = 1.0 M) was lower than that of Gd(DOTP) and the first-order rate constants exhibited saturation curves with increasing $[H^+]$. Based on the assumption that the protonated species HGd(DOTPMB) and $H_5Gd(DOTP)$ dissociate, the rate constants (and protonation constants) were found to be (5.4±0.2) $\times 10^{-4}$ $M^{-1}s^{-1}$ (K_{GdL}^H = 1.7±0.1) and (2.1±0.1) $\times 10^{-4}$ $M^{-1}s^{-1}$ (K_{H4GdL}^H = 1.9±0.1), respectively.

Introduction

Complexes of lanthanides with the tri- and tetraacetate derivatives of cyclen (e.g. DOTA; H_4DOTA = 1,4,7,10-tetraazacyclododecanetetraacetic acid) are finding wide-ranging applications in medical diagnosis and therapy. $Gd(DOTA)^-$ is a clinically used contrast agent in magnetic resonance imaging (MRI).[1−5] The radioactive $^{90}Y(DOTA)^-$ attached to proteins or monoclonal antibodies is a potential drug in the therapy of cancer.[4,6] The success of the cyclic tetraazapolycarboxylates in practical use, and also their interesting chemistry, initiated the synthesis and study of the phosphonate analogues. The ligand 1,4,7,10-tetraazacyclododecanetetrakis(methylenephosphonic acid), H_8DOTP, an analogue of H_4DOTA, has also been synthesized.[7−9] The protonation constants of $DOTP^{8-}$ are unusually high because of the large negative charge of the ligand, and the first protonation constant could be determined only by ^1H NMR spectroscopy.[10] The stability constants of the complexes $Ln(DOTP)^{5-}$ [Ln^{3+} = lanthanide(III) ion] are somewhat higher than those of $Ln(DOTA)^-$.[10−12] However, the species $Ln(DOTP)^{5-}$ predominates only at pH > ca. 9, while in the pH range 4−9, protonated complexes are formed: $H_iLn(DOTP)^{(5-i)-}$ (i = 1−4). The successive protonation equilibria have been characterized by determining the protonation constants.[12]

The complex $Gd(DOTP)^{5-}$ cannot be used as a contrasting agent in MRI because of its high negative charge [at physiological pH values, the monoprotonated $GdH(DOTP)^{4-}$ predominates[12]]. However, some complexes $Ln(DOTP)^{5-}$ are used as shift and relaxation reagents in biology.[6,13−16]

The complexes of lanthanides with the monoesters of DOTP are similar to the complexes of DOTA in that they possess a single negative charge, and can be regarded as potential contrast agents in MRI. (The charges of the ligands DOTP and DOTPMB as well as those of the complexes will be written only when absolutely necessary.) The complex formed between Gd^{3+} and the monobutyl ester of



R = COOH	H_4DOTA
R = $PO(OH)_2$	H_8DOTP
R = PO(OBu)OH	$H_4DOTPMB$

Scheme 1

DOTP (= DOTPMB; see Scheme 1) interacts strongly with human serum albumin and is partly excreted through the hepatobiliary system. $Gd(DOTPMB)^-$ can therefore be regarded as a potential liver contrast agent.[17]

It is important for lanthanide complexes used in biological systems to be thermodynamically stable and in particular they should be kinetically inert. Whereas the stability constant of $Gd(DOTP)^{5-}$ is known,[12] that of

[a] Department of Inorganic and Analytical Chemistry, University of Debrecen,
Debrecen 4010, Hungary
Fax: (internat.) + 36-52/489-667
E-mail: ebrucher@delfin.klte.hu

  1434−1948/01/0303−0813 $ 17.50+.50/0

$Gd(DOTPMB)^-$ has not been reported.[17] Practically no quantitative data are available on the formation and dissociation rates of the Ln^{3+} complexes of DOTP and its esters. Kim et al. found that both the formation and dissociation of La(F-DOTPME) {F-DOTPME = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis[methylenephosphonic acid mono-(2′,2′,2′-trifluoroethyl) ester]} were very slow, and that the stability constant of the complex ($\log K_{LaL}$ = 14.9[18]) was much lower than that of $La(DOTP)^{5-}$ ($\log K_{LaL}$ = 27.6[12]). Both this finding and the much lower protonation constants of the ligand F-$DOTPME^{4-}$ indicate that the basicity of the oxygen donors in the phosphonate ester functional groups, −PO(OR)OH, is much lower than that in the phosphonate groups, $-PO(OH)_2$. The values of the protonation constants indicate that the phosphonate ester groups, −PO(OR)OH, similarly to the phosphinate groups, −PO(R)OH, are strongly acidic.[18,19] In spite of the low basicity of the phosphinate oxygen atoms, the kinetic stability of the lanthanide complexes of the tetraazatetraphosphinate derivative ligand is very high. The proton-assisted dissociation rates of the phosphinate complexes in 0.1 M HCl are comparable with those of $Ln(DOTA)^-$.[4,5,19−23] Thus, a high kinetic stability can be expected for the complex $Gd(DOTPMB)^-$.

The rates of formation and dissociation of the complexes Gd(DOTP) and Gd(DOTPMB) were studied in order to obtain useful information about the biological application of the ligands DOTP and DOTPMB, and for a comparison of the kinetic behaviour of complexes formed with DOTP, DOTPMB, and DOTA, which have similar donor atoms but different functional groups and charges.

Results and Discussion

Protonation and Stability Constants

In order to control the purity of the DOTP sample, we re-determined the protonation constants of $DOTP^{8-}$. The K_1^H value was too high to be determined by pH-potentiometry.[10] The log K_i^H values (i = 2−7) obtained were 12.45(0.005), 9.18(0.007), 7.95(0.008), 6.08(0.008), 5.20(0.007), and 1.85(0.15), respectively, which agree well with the data reported earlier by Delgado et al.[10] and Geraldes et al.[8]

Three protonation constants could be determined by pH-potentiometry for the ligand $DOTPMB^{4-}$: $\log K_1^H$ = 10.34(0.020), $\log K_2^H$ = 7.72(0.025) and $\log K_3^H$ = 2.42(0.030). The completely deprotonated $DOTPMB^{4-}$ has only four negative charges, and its first two protonation constants are therefore significantly lower than those of $DOTP^{8-}$. The oxygen donor atoms of the $P(O)(OBu)O^-$ groups are weakly basic, and therefore we obtained only one protonation constant in the low pH range (2 < pH < 5). For the protonation sequence of the 12-membered tetraaza macrocyclic derivatives, which possess methylenephosphonate pendant arms, it was demonstrated that the first two protonations take place on two opposite nitrogen atoms of the ring.[8]

The stability constant of $Gd(DOTPMB)^-$, calculated from the titration data obtained with the "out-of-cell" technique, was found to be $\log K_{GdL}$ = 12.19(0.05). The formation of protonated or dinuclear complexes was not detected

The stability constant of $Gd(DOTPMB)^-$ is much lower than that of $Gd(DOTP)^{5-}$ ($\log K_{GdL}$ = 28.8[12]), because the charge of the $DOTPMB^{4-}$ and the basicity of its donor atoms are much lower than those of $DOTP^{8-}$.

Formation Kinetics of $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^-$

The reactions between Gd^{3+} and DOTP or DOTPMB in the pH range 5.4−7 are relatively slow and can be studied by measuring the longitudinal relaxation rates of the water protons. The relaxivities of Gd^{3+}·aq and the complexes $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^-$ differ sufficiently to furnish reliable rate data. In the presence of excess ligand, the formation rates can be expressed as Equation (1), where k_{obs} is a pseudo-first-order rate constant, and $[GdL]_t$ is the total concentration of the complex formed.

$$\frac{d[GdL]_t}{dt} = k_{obs}[Gd^{3+}] \quad (1)$$

Similarly to the results obtained in the study of the formation of the complexes $Ln(DOTA)^-$,[20−23] first-order rate constants were obtained even at comparable concentrations of the reactants. These findings were interpreted by assuming that there is a rapid formation of a reaction intermediate which rearranges to the product in the rate-determining step, characterized by the rate constant, k_r in Equation (2).

$$Gd^{3+} + H_xL \rightleftharpoons (x\text{-}y)H^+ + Gd(H_yL) \xrightarrow{k_r} GdL + yH^+ \quad (2)$$

The values of the rate constants, k_{obs}, increase with increasing ligand concentration, and at a ligand to Gd^{3+} ratio of approximately 2−3 give rise to a saturation value, k_r. In the presence of a 10-fold excess of ligand, we obtained such values (i.e., $k_{obs} = k_r$), which characterize the rate of rearrangement of the intermediate.

In the formation reactions of $Gd(DOTPMB)^-$, the ligand is present in the form of the species $H_2DOTPMB^{2-}$ and $HDOTPMB^{3-}$ (5.4 < pH < 7), since the protonation constants of $DOTPMB^{4-}$ ($\log K_i^H$) are 10.34, 7.72, and 2.42. The reaction intermediates formed by mixing solutions of Gd^{3+} and DOTPMB are probably mono- and diprotonated species, Gd(HDOTPMB) and $Gd(H_2DOTPMB)^+$, since there is practically no pH change at the start of the reaction. The structures of the intermediates are probably not similar to those of $Ce(H_2DOTA)^+$, Ce(HDOTA), and $Eu(H_2DOTA)^+$,[22,23] in which four carboxylate oxygens are coordinated to the Ln^{3+}, which occupies an "out-of-cage" position, while the protons protonate one or two nitrogen atoms. The structure of the ligand DOTPMB is not known and probably only two phosphonate ester groups of the mono- or diprotonated ligand can be coordinated to the Gd^{3+} in the intermediates.

The rate constants k_r are presented in Figure 1 and display a second-order dependence on the OH^- concentration.

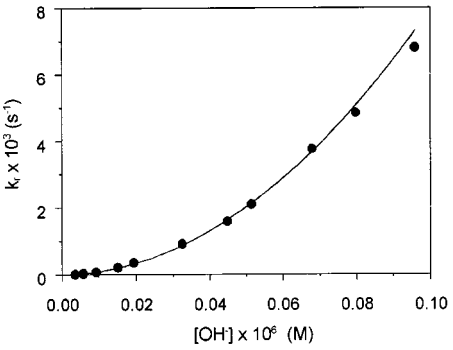


Figure 1. First-order rate constants (k_r) obtained for the formation reaction of $Gd(DOTPMB)^{-}$ {25 °C, 0.1 M $(CH_3)_4NCl$, $[Gd^{3+}]_t = 5\cdot10^{-4}$ M, $[L]_t = 5\cdot10^{-3}$ M}

$$k_r = k_{OH}[OH^-] + k_{2OH}[OH^-]^2 \quad (3)$$

The fit of the k_r data to Equation (3) gave the rate constants k_{OH} and k_{2OH}, presented in Table 1.

Table 1. Rate constants characterizing the formation of complexes with cyclen derivative ligands {25 °C, 0.1 M $(CH_3)_4NCl$}

	Gd(DOTP)	Gd(DOTPMB)	Gd(DOTA)[a]
k_{OH} [$M^{-1}s^{-1}$]	$(7.2\pm0.1)\cdot10^3$	$(1.3\pm0.25)\cdot10^3$	$5.9\cdot10^6$
k_{2OH} [$M^{-2}s^{-1}$]	–	$(7.8\pm0.2)\cdot10^{11}$	–
k_{LnHL} [s^{-1}]	–	$(3.3\pm1.6)\cdot10^{-4}$	18.5[b]
k^{OH}_{LnHL} [$M^{-1}s^{-1}$]	–	$(4.4\pm1.7)\cdot10^5$	$1.9\cdot10^7$[b]

[a] Ref.[26] – [b] Obtained for the formation of Ce(DOTA).[24]

The mechanism of formation of $Gd(DOTPMB)^{-}$ is presumably similar to those of the complexes $Ln(DOTA)^{-}$.[22–24] It is assumed that the diprotonated intermediate is involved in a dissociation equilibrium with the kinetically active monoprotonated intermediate [Equation (4)].

$$Gd(H_2DOTPMB)^+ \rightleftharpoons Gd(HDOTPMB) + H^+ \quad (4)$$

The extent of dissociation is characterized by the protonation constant, $K^H_{GdHL} = [GdH_2L]/([GdHL][H^+])$. With an increase in the pH, the monoprotonated intermediate is produced in increasing amounts in a direct reaction, since $\log K^H_2 = 7.72$. This intermediate undergoes deprotonation in a slow, rate-determining process, presumably with the assistance of an H_2O molecule [Equation (5)] or an OH^- ion [Equation (6)].[24]

$$Gd(HDOTPMB) + H_2O \xrightarrow{k_{GdHL}} Gd(DOTPMB)^- + H_3O^+ \quad (5)$$

$$Gd(HDOTPMB) + OH^- \xrightarrow{k^{OH}_{GdHL}} Gd(DOTPMB)^- + H_2O \quad (6)$$

The slow transfer of a proton is followed by a fast rearrangement of the deprotonated intermediate to furnish the product.

On the basis of the equilibrium reaction [Equation (4)], the concentration of the monoprotonated intermediate is directly proportional to the OH^- concentration, which explains the direct proportionality between the rate constant k_r and the OH^- concentration. The second-order dependence of the k_r values on the OH^- concentration can be interpreted in terms of the OH^--catalyzed deprotonation of the monoprotonated intermediate Gd(HDOTPMB) [Equation (6)].

When Equations (5) and (6) are taken into account, the rate of formation of $Gd(DOTPMB)^{-}$ can be given as in Equation (7).

$$\frac{d[GdL]_t}{dt} = k_{GdHL}[GdHL] + k^{OH}_{GdHL}[GdHL][OH^-] \quad (7)$$

Comparing Equation (1) and Equation (7), and bearing in mind that the formation of the intermediates is practically complete in the presence of excess ligand ($[Gd^{3+}] = [GdHL] + [GdH_2L]$), the concentration of GdH_2L can be expressed in terms of the protonation constant K^H_{GdHL}, when Equation (8) is obtained[24], where K_W ($= 1.66\cdot10^{-14}$) is the ionic product of water. From the fit of the experimental k_r values (Figure 1) to Equation (8), the rate constants k_{GdHL} and k^{OH}_{GdHL}, and the protonation constant K^H_{GdHL} were calculated and are presented in Table 1.

$$k_r = \frac{k_{GdHL}[H^+] + k^{OH}_{GdHL}K_W}{[H^+] + K^H_{GdHL}[H^+]^2} \quad (8)$$

In the postulated reaction mechanisms expressed by Equations (4)–(6), the deprotonation of the monoprotonated intermediate Gd(HDOTPMB) plays an important role. If the reactions according to Equations (5) and (6) are rate-controlling, then general base catalysis must occur in the formation reactions. In this case, deprotonation of the intermediate will take place with the transfer of a proton to any Brönsted base, e.g. to the basic form of the buffer. The rate constants $k_{obs} = k_r$ presented in Figure 2 increase with increasing concentration of the buffer MES, which indicates the validity of the Brönsted equation and the occurrence of general base catalysis.[25] These results demonstrate that the rate-determining step in the formation of $Gd(DOTPMB)^{-}$ is the transfer of a proton from the intermediate Gd(HDOTPMB) to a Brönsted base (H_2O, OH^- or the basic form of the buffer), followed by a fast rearrangement of the deprotonated intermediate, in which the Gd^{3+} enters the coordination cage formed by the four nitrogen and oxygen atoms of the ligand.

The formation kinetics of $Gd(DOTP)^{5-}$ seems simpler than in the case of $Gd(DOTPMB)^{-}$, since the rate of formation is directly proportional to the OH^- concentration in the pH range 5.4–8, as shown in Figure 3. On the basis of the rate constants in Figure 3, the rate law is also simple for the formation of $Gd(DOTP)^{5-}$, since $k_r = k_{OH}[OH^-]$, where $k_{OH} = (7.2\pm0.1)\times10^3$ $M^{-1}s^{-1}$. The k_{OH} values obtained for the formation of Gd(DOTP), Gd(DOTPMB), and Gd(DOTA)[20] are compared in Table 1. It can be seen that the rates of formation of Gd(DOTA) and of some other complexes Ln(DOTA)[20–23] are significantly higher than those of the phosphonate com-

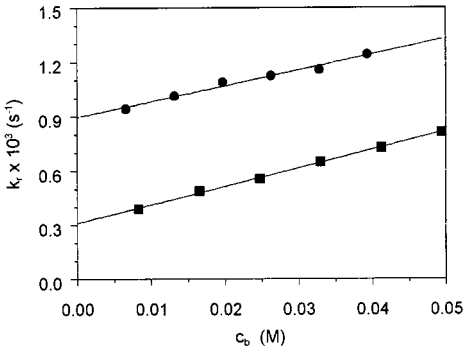


Figure 2. First-order rate constants (k_r) obtained for the formation reaction of $Gd(DOTP)^{5-}$ at pH = 6.82 (■) and of $Gd(DOTPMB)^{-}$ at pH = 6.43 (●) as a function of the concentration of the buffer MES {25 °C, 0.1 M $(CH_3)_4NCl$, $[Gd^{3+}]_t = 5\cdot10^{-4}$ M, $[L]_t = 5\cdot10^{-3}$ M}

plexes, and Gd(DOTP) is formed somewhat faster than Gd(DOTPMB) in this pathway. The simple rate law characterising the formation of Gd(DOTP) is surprising, because the ligand is present in the form of the species H_2L^{6-}, H_3L^{5-}, H_4L^{4-}, H_5L^{3-}, and H_6L^{2-} in the pH range investigated, in which several differently protonated intermediates can be formed in the first stage of the formation reaction.

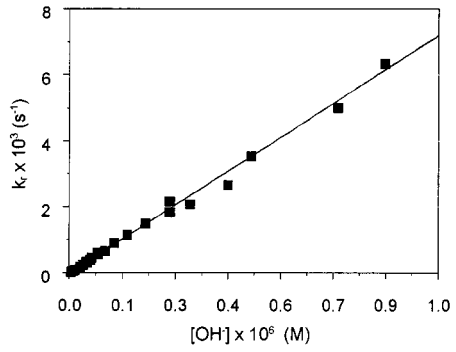


Figure 3. First-order rate constants (k_r) obtained for the formation reaction of $Gd(DOTP)^{5-}$ {25 °C, 0.1 M $(CH_3)_4NCl$, $[Gd^{3+}]_t = 5\cdot10^{-4}$ M, $[L]_t = 5\cdot10^{-3}$ M}

In the reactions of formation of the similar complexes $Ln(DOTA)^{-}$ in the pH range 4−6, a pronounced pH drop was observed at the start of the reactions, which was followed by a slow decrease in the pH value.[20,22] This finding was explained in terms of the formation of the intermediate $Ln(H_2DOTA)^{+}$, which slowly lost the protons.[22,23] In contrast, we did not observe any drop in the pH value when solutions of Gd^{3+} and $H_xDOTP^{(8-x)-}$ were mixed. This indicates that the composition of the intermediate formed is $Gd(H_xDOTP)$, where x is the average number of protons on DOTP at the given pH value. Taking the protonation constants of DOTP into consideration, in the pH range 5.3−8 the average number of protons decreases from $x = 5.7$ to $x = 3.5$. Since two of these protons are attached to two diagonally located nitrogen atoms of the ligand in the pH range investigated, an average of between $n = 3.7$ and 1.5 protons protonate the eight phosphonate oxygen atoms, and thus there are always at least four nonprotonated oxygen atoms $-O^-$ which coordinate to Gd^{3+} in the intermediates $H_nGd(H_2DOTP)$. However, it is not likely that the protonated $H_xDOTP^{(8-x)-}$ is preorganized in a similar way as the ligand $H_xDOTA^{(4-x)-}$. The X-ray crystal structure of H_8DOTP indicates that two diagonally located phosphonate groups are positioned above the plane of the ring nitrogen atoms, while the other two phosphonate groups are orientated away from the ring.[9] It thus seems more probable that only two methylenephosphonate groups are coordinated to Gd^{3+} in the protonated intermediates $H_nGd(H_2DOTP)$. This difference in the structures of the intermediates $H_nGd(H_2DOTP)$ and $Gd(H_2DOTA)$ partly explains the significantly lower rates of formation of the complexes $Ln(DOTP)^{5-}$.

The rearrangement of the intermediate to the product occurs with the loss of protons. The complexes Gd(DOTP) formed are protonated on the noncoordinated oxygen atoms at pH < 9.[12] The protonation constants[12] indicate that the average composition of the protonated complex at pH = 8 is $H_{0.6}Gd(DOTP)$, while at pH = 5.3 it is $H_{2.5}Gd(DOTP)$, i.e. the rearrangement of the intermediate and the entry of Gd^{3+} into the coordination cage occurs with the liberation of 1−2 protons.

The data presented in Figure 2 indicate that the rate constants k_r increase with increasing concentration of the basic form of the buffer, MES, pointing to the validity of general base catalysis. This can be interpreted by assuming the formation of kinetically active intermediates with the dissociation of one of the two protons attached to the nitrogen atoms [Equation (9)] where $n = 0-4$.

$$H_nGd(H_2L) \rightleftharpoons H_nGd(HL) + H^+ \quad (9)$$

The rate-controlling step is presumably the transfer of the proton from the nitrogen atom of $H_nGd(HL)$ to a Brönsted base, e.g. H_2O or the basic form of the buffer. It may be expected that the catalytic effect of OH^- occurs at pH > 8. However, in the formation of $Ce(DOTA)^{-}$, the effect of OH^- catalysis was observed at pH > ca. 7.5.[24] The direct dependence of the formation rates on $[OH^-]$ (Figure 3) is a consequence of the equilibrium according to Equation (9). The concentration of the intermediate $H_nGd(HL)$, which plays a crucial role in the rate-determining step, is directly proportional to $[OH^-]$.

On the basis of the direct proportionality between the rates of formation (k_r) and the concentration of OH^-, it can be assumed that the rate-controlling proton transfer is not influenced by the presence of various numbers of protons attached to the phosphonate oxygen atoms in the intermediates $H_nGd(HL)$. Based on this assumption, the rate of formation of Gd(DOTP) can be expressed as $\Sigma k_{H_nGd(HL)}[H_nGd(HL)]$. To calculate of the rate constants $k_{H_nGd(HL)}$, it is necessary to know the stability constants of the intermediates $H_nGd(H_2L)$. Unfortunately, these stability constants are not available and their pH-potentiometric determination would be difficult, because of the very low solubilities of some protonated species. The rate of

formation of $Gd(DOTP)^{5-}$ can thus be characterized by the rate constant k_{OH} presented in Table 1.

Table 2. Rate constants and protonation constants characterizing the dissociation and protonation of the complexes {25 °C, 1.0 M $(CH_3)_4NCl$ − HCl}

	Gd(DOTP)	Gd(DOTPMB)	Gd(DOTA)[a]
$k_{HGdL} \times 10^4$ [$M^{-1}s^{-1}$]	5.4±0.2	2.1±0.1	0.006
K^H_{HiGdL}[b]	1.7±0.1	1.9±0.1	14[c]

[a] Ref.[20] − [b] $i = 4$ for Gd(DOTP) and $i = 0$ for Gd(DOTPMB) and Gd(DOTA). − [c] Determined by pH-potentiometry.[22]

The k_{OH} values obtained for the formation of $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^{-}$ are significantly lower than the value of the rate of formation of Gd(DOTA) (Table 1). These differences can be interpreted by assuming a more preorganised structure of the ligand DOTA than of DOTP and DOTPMB. The structures of the intermediates $Gd(H_2DOTPMB)$ and $H_nGd(H_2DOTP)$ probably differ more significantly from the structures of the products $Gd(DOTPMB)^{-}$ and $Gd(DOTP)^{5-}$ than in the case of the complexes of DOTA. The deprotonation and rearrangement of Gd(HDOTPMB) with the assistance of an H_2O molecule are particularly slow if we take into account that this intermediate is present in a macroscopic concentration, owing to the low value of $\log K_2^H$. The extremely slow formation of $Gd(DOTPMB)^{-}$ through this pathway is particularly evident if the $k_{Ln(HL)}$ value ($3.3 \cdot 10^{-4}$ s^{-1}) is compared with that obtained for $Ce(DOTA)^{-}$ ($k_{Ln(HL)} = 18.5$ s^{-1}). This large difference in the rate constants is indicative of the difficulties connected with the deprotonation and rearrangement of the intermediate Gd(HDOTPMB).

Kinetic Stability of $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^{-}$

It is known from the literature that the dissociation of the Ln^{III} complexes of tetraaza macrocyclic derivative ligands is very slow, because of their rigid structures.[19−22,26] ^1H, ^{13}C and ^{31}P NMR spectroscopic studies indicate that the structures of the complexes $Ln(DOTP)^{5-}$ are even more rigid than that of $Ln(DOTA)^{-}$.[27] The kinetic stability of $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^{-}$ was therefore studied in the presence of a large excess of HCl. The reaction according to Equation (10) was observed.

$$GdL + xH^+ \rightleftharpoons Gd^{3+} + H_xL \quad (10)$$

The progress of the dissociation was monitored by measuring the changes in the longitudinal relaxation rates of water protons in solutions with different HCl concentrations. The rate of the dissociation reaction is as shown in Equation (11) where $[GdL]_t$ is the total concentration of the Gd^{3+} complex.

$$-\frac{d[GdL]_t}{dt} = k_d[GdL]_t \quad (11)$$

The first-order rate constants (k_d) are shown in Figure 4. The k_d values increase with increasing $[H^+]$, showing saturation curves. Similar results were obtained in the study of the rates of dissociation of some DOTA and DOTA-derivative complexes.[20,22,26] This led us to assume that rapid formation of protonated complexes occurs at the beginning of the reaction. The protonation occurs on the phosphonate oxygen atom(s), but the resulting protonated complex cannot dissociate directly because the Gd^{3+} is in the "coordination cage". For dissociation, at least one proton must be transferred to a ring nitrogen atom, after which the metal ion moves out from the "in-cage" position and the dissociation may take place. Either the transfer of the proton(s) or the structural rearrangement or both processes occur slowly. The protonation equilibria of $Gd(DOTP)^{5-}$, which forms the thermodynamically stable protonated species $HGd(DOTP)^{4-}$, $H_2Gd(DOTP)^{3-}$, $H_3Gd(DOTP)^{2-}$, and $H_4Gd(DOTP)^{-}$ in the pH range 4−8,[12] suggest that species that can dissociate [e.g. $H_5Gd(DOTP)$, etc.] are formed at lower pH values, as a consequence of further protonation. This assumption was supported by the ^1H NMR spectra of $La(DOTP)^{5-}$ solutions, recorded after the addition of increasing amounts of DCl. When 1−4 equiv. of DCl had been added to the solution, the spectrum was similar to that shown in Figure 5C, even after a few weeks. After the addition of 5 equiv. of DCl, the spectrum slowly changed, as shown in Figure 6. The multiplet pattern of the spectrum at the beginning of the reaction (Figure 5C and Figure 6A) was transformed into two broad bands, characteristic of the ligand (Figure 5A). The conversion was almost complete after 4 months. This reveals that the attachment of the fifth proton plays a crucial role in the kinetic stability of the complex. This proton must be attached to a binding $P-O^-$ oxygen atom since the four noncoordinated oxygen atoms are protonated when the rigid, symmetrical structure of the complex is no longer evident. When a noncoordinated phosphonate group is formed, one of the protons can be transferred to the nitrogen atom, after which the Ln^{3+} ion moves out of the coordination cage, and dissociation occurs.

Since the complex $Gd(DOTPMB)^{-}$ involves only four coordinated P−O donor groups, formation of the monoprotonated complex HGd(DOTPMB) results in the breaking

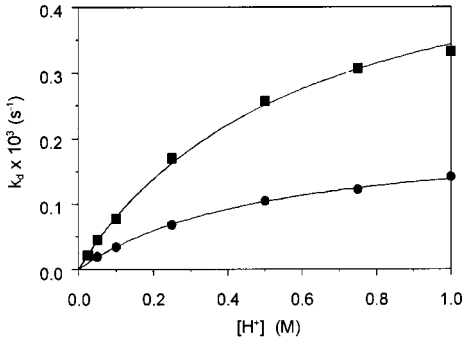


Figure 4. Rate constants of dissociation (k_d) of $Gd(DOTP)^{5-}$ (■) and of $Gd(DOTPMB)^{-}$ (●) as a function of the H^+ concentration {25 °C, 1.0 M $(CH_3)_4NCl$ − HCl, $[GdL]_t = 1 \times 10^{-3}$ M}

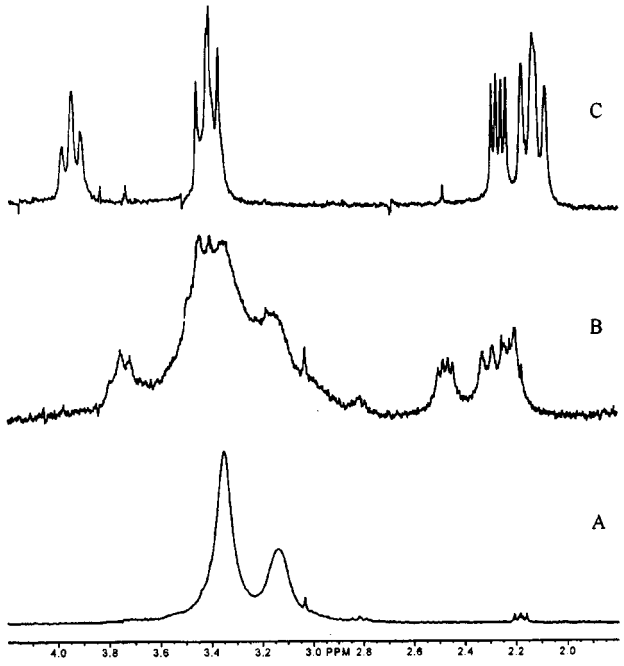


Figure 5. ^{1}H NMR spectra of DOTP at pD = 6 (A), and of La(DOTP)$^{5-}$ at pD = 4.5 (B) and at pD = 10.2 (C)

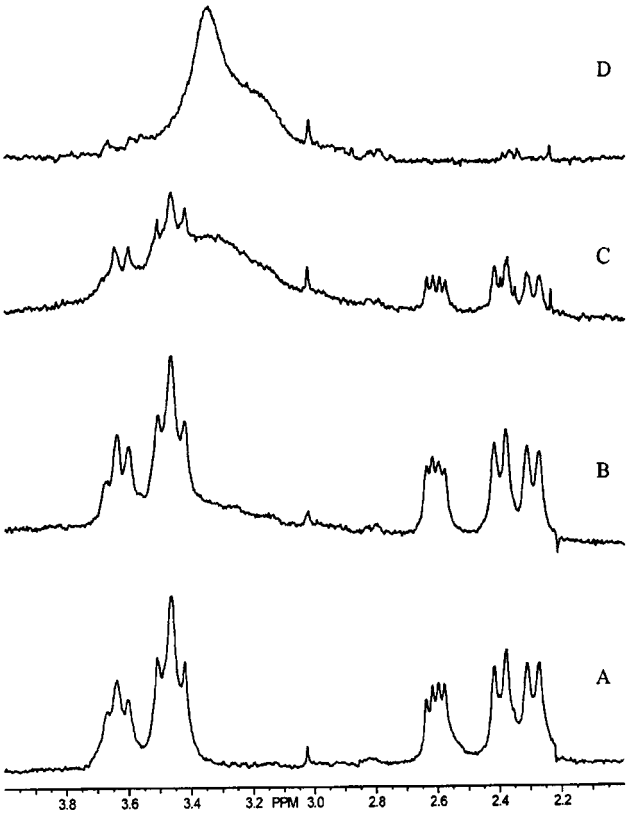


Figure 6. ^{1}H NMR spectra of La(DOTP)$^{5-}$ immediately after the addition of 5 equiv. of DCl (A), 1 d later (B), 1 week later (C), and 4 months later (D)

of a Gd^{3+}−O−P bond, after which a nitrogen atom can be protonated and the complex dissociates.

If dissociation of the protonated species HGd(DOTPMB) and H_5Gd(DOTP) is assumed, the rate of dissociation can be given as Equation (12) where $i = 1$ for Gd(DOTPMB) and $i = 5$ for Gd(DOTP).

$$-\frac{d[GdL]_t}{dt} = k_{HiGdL}[H_iGdL] \quad (12)$$

The total concentration of the complexes is $[GdL]_t = [H_{i-1}GdL] + [H_iGdL]$, since formation of the tetraprotonated H_4Gd(DOTP)$^-$ is practically complete at pH < 3. From Equations (11) and (12), and by using the expression for the total concentration of the complexes, we obtain Equation (13) where K^H_{HiGdL} is the first protonation constant of Gd(DOTPMB) ($i = 0$) and the fifth protonation constant ($i = 4$) of Gd(DOTP): $K^H_{HiGdL} = [H_{i+1}GdL)]/([H_iGdL][H^+])$.

$$k_d = \frac{k_{HiGdL}K^H_{HiGdL}[H^+]}{1 + K^H_{HiGdL}[H^+]} \quad (13)$$

The experimental k_d data (Figure 4) were fitted to Equation (13), and the rate constants k_{HiGdL} and protonation constants K^H_{HiGdL} were calculated (Table 2). The low value of K^H_{H4GdL} for Gd(DOTP)$^{5-}$ indicates that further protonation of H_4Gd(DOTP)$^-$, in which all the oxygen donors are bonded or protonated, is much more difficult than the protonation of e.g. H_3Gd(DOTP)$^{2-}$ ($\log K^H_{H3GdL} = 4.0$[12]). It is surprising that the kinetic stability of Gd(DOTP) is somewhat lower than that of Gd(DOTPMB) (Table 2) in spite of the much higher thermodynamic stability of Gd(DOTP)$^{5-}$ ($\log K_{Gd(DOTP)} = 28.8$[12] and $\log K_{Gd(DOTPMB)} = 12.19$). This unexpected result is probably a consequence of the practical unavailability of protonation sites in Gd(DOTPMB)$^-$. The negatively charged phosphonate oxygen atoms are coordinated to Gd^{3+}, while the oxygen atoms of the ester cannot be protonated under such conditions. Furthermore, the basicity of the P−O$^-$ oxygen atoms is very low; the third protonation constant of DOTPMB is $\log K^H_3 = 2.42$.

These results permit some general conclusions to be made about the kinetic stability of the Gd^{3+} complexes. The kinetic stability depends on the availability of basic protonation sites on the complex. The complex is appreciably inert against proton-assisted dissociation if the basicity of the donor atoms of some functional groups is low. In this case, the stability constant is also low, but the low ligand basicity means that the extent of protonation of the complex is very low, and thus the proton-assisted dissociation occurs slowly. Similar findings on the kinetic stabilities of the complexes of phosphinate and amide derivatives of DOTA and DTPA were reported earlier.[19,28,29]

Owing to its high kinetic stability, Gd(DOTPMB)$^-$ can be regarded as a safe contrast agent (it is more inert than Gd(DTPA)$^{2-}$), in spite of its relatively low stability constant.

Table 2 gives the rate constant k_{HGdL} which characterizes the proton-assisted dissociation of Gd(DOTA)$^-$.[20] On the basis of Equation (13), at low [H$^+$] $k_d = k_{HiGdL}K^H_{HiGdL}[H^+] = k_1[H^+]$. Wang et al.[20] reported the k_1 value and k_{HGdL} was calculated with the use of the protonation constant of Gd(DOTA)$^-$.[22] A comparison of the

data reveals that the rates of dissociation of the Gd^{3+} complexes with the phosphonate and phosphonate ester ligands are larger by about three orders of magnitude. The protonation constant of $Gd(DOTA)^{-}$ is about ten times larger than that of $H_4Gd(DOTP)$ or Gd(DOTPMB). However, $Gd(DOTA)^{-}$ is known to be protonated on a non-coordinated carboxylate oxygen atom[30] and thus even the protonated complex is relatively inert.[30] The formation of $HGd(DOTPMB)$ or $H_5Gd(DOTP)$ occurs at low pH values where the complex is thermodynamically unstable and protonation results in decoordination of a functional group and gradual dissociation of the complex.

Conclusions

The ligand DOTP and its monobutyl ester, DOTPMB, are significantly more sluggish than their tetraacetate analogue, DOTA, in their reactions with Gd^{3+}. However, the proton-assisted dissociation of the complexes $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^{-}$ is approximately one thousand times faster than that of $Gd(DOTA)^{-}$.

The basicities of the nitrogen and particularly of the oxygen donor atoms and the charge of $DOTPMB^{4-}$ are much lower than those of $DOTP^{8-}$, and the stability constants of $Gd(DOTPMB)^{-}$ and $Gd(DOTP)^{5-}$ are $\log K_{GdL} = 12.19$ and $\log K_{GdL} = 28.8$, respectively. In spite of this large difference in stability, the proton-assisted dissociation of $Gd(DOTP)^{5-}$ is somewhat faster than the similar reaction of $Gd(DOTPMB)^{-}$. The high inertness of $Gd(DOTPMB)^{-}$ is a result of the low basicities of the donor atoms. $Gd(DOTP)^{5-}$ forms protonated complexes in neutral media, while $Gd(DOTPMB)^{-}$ is protonated only in acidic solutions.

The complexes $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^{-}$ are formed slowly through the formation of protonated intermediates, $H_nGd(H_2DOTP)$ and $Gd(H_2DOTPMB)$, in which presumably only two functional groups are coordinated to the Gd^{3+} and two nitrogen atoms are protonated. The rearrangement of the intermediates to the product is a general base-catalyzed process, with the release of the last proton from the ring nitrogen atoms as the rate-determining step.

Experimental Section

Synthesis of H_8DOTP: The ligand H_8DOTP was synthesized according to a published method.[9] The purity of the ligand was checked by ^{1}H NMR spectroscopy and was found to be higher than 99.5%.

Synthesis of Na_4DOTPMB: Into a 100-mL round-bottomed flask were placed cyclen (2.66 g, 15.41 mmol), benzene (15 mL), glacial acetic acid (6 mL), paraformaldehyde (3.20 g), and dibutyl phosphite (13.0 g), dissolved in benzene (15 mL), and the mixture was stirred at room temperature for 3 d. From the reaction mixture, an azeotropic mixture of benzene and water (20 mL) was distilled off, then benzene (30 mL) was added to the residue and distilled off again. This latter procedure was repeated once more, and the solution was then concentrated under reduced pressure to give a dark brown viscous oil. This oil was redissolved in diethyl ether (150 mL), and water (50 mL) was added. Solid sodium bicarbonate was added until the evolution of gas had ceased. The organic phase was separated and dried with anhydrous sodium sulfate for 8 h. The drying agent was filtered off and the filtrate was concentrated under reduced pressure, furnishing the dibutyl ester DOTPDB as a viscous, dark brown oil. DOTPDB was suspended in water (50 mL), sodium hydroxide pellets (14 g) were added and the two-phase mixture was vigorously stirred and boiled for 30 min, and then left to stand at room temperature for 2 d. The aqueous layer was removed, water (50 mL) and sodium hydroxide (10 g) were added, and the mixture was boiled with vigorous stirring for 30 min. The mixture was then cooled to room temperature, and the aqueous layer was separated. The semisolid residue was redissolved in boiling water (approx. 28 mL), sodium hydroxide solution (4.8 M, 3 mL) was added and the clear solution was heated at reflux for 3 h. NaOH pellets were then added until a significant amount of solid had precipitated. Additional NaOH (2 g) was added and, after cooling, the precipitate was filtered off, washed thoroughly with acetone and dried to constant weight under reduced pressure, resulting in a brownish hygroscopic powder, which proved to be pure Na_4DOTPMB (8.69 g). – ^{1}H NMR (D_2O, DSS): δ = 3.88 (q, 8 H, O–CH_2), 2.89 (d, 8 H, N–CH_2–P), 2.75 (s, 16 H, ring CH_2), 1.63 (m, 8 H, CH_2), 1.38 (m, 8 H, CH_2), 0.92 (t, 12 H, CH_3).

Equilibrium Measurements: The concentrations of ligand solutions were determined by pH-potentiometry, from the titration curves obtained in the absence and in the presence of excess $CaCl_2$. – $LnCl_3$ (Ln = Gd and La) solutions were prepared by dissolving Ln_2O_3 (99.9%, Fluka) in 6 M HCl, after which the excess HCl was evaporated. The concentrations of $LnCl_3$ solutions were determined by complexometry with standardized K_2H_2EDTA, and xylenol orange was used as indicator. – The pH-potentiometric titrations were carried out in jacketed vessels kept at a constant temperature of 25 °C (thermostat), with a Radiometer PHM85 pH-meter, an ABU80 autoburette and PHG211 glass and K401 calomel electrodes. The ionic strength of the solutions was maintained constant (0.1 M Me_4NCl, $pK_W = 13.90$). For the titrations, an Me_4NOH solution (0.1 M) was used. Hydrogen ion concentrations were calculated from the measured pH values by a known procedure.[24,31] For the determination of the protonation constants of the ligands, the concentrations of the samples were 0.010 M for DOTP and 0.0038 M for DOTPMB. Three parallel titrations (60–90 points) were performed for each ligand in the pH range 2.0–12.2. The protonation constants are defined as $K_i^H = [H_iL]/([H_{i-1}L][H^+])$. – The compositions of the intermediates formed in the reaction between Gd^{3+} and H_xDOTP were studied by measuring the pH changes that occurred after the reactants were mixed. The concentrations of Gd^{3+} and H_xDOTP in the samples were $3\cdot10^{-4}$ M and $5\cdot10^{-3}$ M, respectively. The initial pH values of the samples (which were slightly buffered owing to the presence of a small excess of ligand) were 5.37, 6.07, 6.88, and 7.82. The decrease in the pH value was measured until equilibrium was reached. For the determination of the amount of protons released in the reactions, similar samples containing only H_xDOTP (0.1 M KCl) were titrated with HCl of known concentration until the equilibrium pH was established. – The stability constant of $Gd(DOTPMB)^{-}$ was determined by an "out-of-cell" technique. In the pH range 2.7–5.0, 15 solution samples (5 mL) were prepared in which the concentrations of DOTPMB and $GdCl_3$ were 0.0038 M each. The equilibrium pH values were measured three weeks after the preparation of the samples.

Kinetic Measurements: The rates of formation of the complexes were studied by relaxometry in the pH range 5.4−7.0. The formation of $Gd(DOTP)^{5-}$ was also investigated by spectrophotometry ($7.0 < pH < 8.0$). For the formation reactions, pseudo-first-order conditions were maintained with a 10-fold ligand excess, while the concentration of Gd^{3+} was $5\cdot10^{-4}$ M. All measurements were performed at constant temperature (25±0.2 °C) and constant ionic strength (0.1 M Me_4NCl). − The rates of formation reactions at $pH < 7.0$ were studied by measuring the longitudinal relaxation rates ($1/T_1$) of water protons. The pseudo-first-order rate constants (k_{obs}), were calculated from the values of the proton relaxation rates measured at the start, at time t and at equilibrium of the reactions. The proton relaxation times were measured at 9 MHz with an MRS-4 NMR spectrometer (Institute Jozef Stefan, Ljubljana, Slovenia). The temperature of the sample holder was kept constant with the use of an air stream. The T_1 values were measured by using the inversion recovery method (180° − τ − 90°), with an average of 4−6 measurements at 6−8 different τ values. A constant pH value was maintained by using 0.05 M MES as a buffer. − In the spectrophotometric measurements, the indicator method[32] was applied. In slightly buffered solutions (0.01 M HEPES, pH = 7.0−8.0), an approximate decrease of 0.1 in the pH value was monitored at 573 nm by using cresol red as indicator ($c_{ind} = 2.5\cdot10^{-5}$ M). The measurements were carried out with a Cary 1E UV/Vis spectrophotometer, using tandem cells for mixing the reagents. − The rates of dissociation of the complexes were studied by relaxometry (25 °C) in 0.025−1.0 M HCl. The ionic strength of the solutions was kept constant. The sum of the concentrations of HCl and Me_4NCl was 1.0 M. The concentrations of $Gd(DOTP)^{5-}$ and $Gd(DOTPMB)^{-}$ were each 0.001 M. For calculation of the first-order rate constant, k_d, the relaxation rates were used, measured at the start, at time t and at equilibrium of the reaction. − The ^1H NMR spectra were recorded in D_2O with a Bruker AM 360 spectrometer, DSS being used as internal standard. The pD values were calculated from the measured pH values by using the known equation[33] pD = pH + 0.4. − The protonation constants and the stability constants were calculated from the equilibrium measurement data through the use of the PSEQUAD program.[34] All calculations with kinetic data were made with least-squares fitting by the program SCIENTIST® for WINDOWS™ by MICROMATH®, version 2.0. The reported errors correspond to the standard deviation calculated by the programs.

Acknowledgments

This research was supported in part by the Hungarian Ministry of Education (FKFP 0448/1997) and by the Hungarian Science Foundation (OTKA 23810).

[1] R. B. Lauffer, *Chem. Rev.* **1987**, *87*, 901.
[2] M. F. Tweedle, in: *Lanthanide Probes in Life, Chemical and Earth Sciences* (Eds.: J.-C. G. Bünzli, G. R. Choppin), Elsevier, Amsterdam, **1989**, p. 127.
[3] G. R.Choppin, K. M. Schaab, *Inorg. Chim. Acta* **1996**, *252*, 299.
[4] D. Parker, J. A. G. Williams, *J. Chem. Soc., Dalton Trans.* **1996**, 3613.
[5] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[6] W. A. Volkert, T. J. Hoffmann, *Chem. Rev.* **1999**, *99*, 2269.
[7] M. I. Kabachnik, T. Ya. Medved, F. I. Belskij, S. A. Pisareva, *Izv. Acad. Nauk SSSR, Ser. Khim.* **1984**, 844.
[8] C. F. G. C. Geraldes, A. D. Sherry, W. P. Cacheris, *Inorg. Chem.* **1989**, *28*, 3336.
[9] I. Lázár, D. C. Hrncir, W.-D. Kim, G. E. Kiefer, A. D. Sherry, *Inorg. Chem.* **1992**, *31*, 4422.
[10] R. Delgado, L. C. Siegfried, T. A. Kaden, *Helv. Chim. Acta* **1990**, *73*, 140.
[11] W. P. Cacheris, S. K. Nickle, A. D. Sherry, *Inorg. Chem.* **1987**, *26*, 958.
[12] A. D. Sherry, J. Ren, J. Huskens, E. Brücher, É. Tóth, C. F. G. C. Geraldes, M. M. Castro, W. P. Cacheris, *Inorg. Chem.* **1996**, *35*, 4604.
[13] A. D. Sherry, C. R. Malloy, F. M. H. Jeffrey, W. P. Cacheris, C. F. G. C. Geraldes, *J. Magn. Reson.* **1988**, *76*, 528.
[14] D. C. Buster, M. M. C. A. Castro, C. F. G. C. Geraldes, C. R. Malloy, A. D. Sherry, T. C. Siemers, *Magn. Reson. Med.* **1990**, *15*, 25.
[15] N. Bansal, M. J. Germann, V. Seshan, G. T. Shires, C. R. Malloy, A. D. Sherry, *Biochemistry* **1993**, *32*, 5638.
[16] S. Aime, P. Ascenzi, E. Comoglio, M. Fasano, S. Paoletti, *J. Am. Chem. Soc.* **1995**, *115*, 9365.
[17] C.F.G.C. Geraldes, A. D. Sherry, I. Lázár, A. Miseta, P. Bogner, E. Berényi, B. Sümegi, G. E. Kiefer, K. McMillan, F. Maton, R. N. Müller, *Magn. Reson. Med.* **1993**, *30*, 696.
[18] W. D. Kim, G. E. Kiefer, J. Huskens, A. D. Sherry, *Inorg. Chem.* **1997**, *36*, 4134.
[19] K. P. Pulukkody, T. J. Norman, D. Parker, L. Royle, C. J. Broan, *J. Chem. Soc., Perkin Trans. 2.* **1993**, 605.
[20] X. Wang, T. Jin, V. Comblin, A. Lopez-mut, E. Merciny, J. F. Desreux, *Inorg. Chem.* **1992**, *31*, 1095.
[21] K. Kumar, T. Jin, W. Xiangium, J. F. Desreux, M. F. Tweedle, *Inorg. Chem.* **1994**, *33*, 3823.
[22] É. Tóth, E. Brücher, I. Lázár, I. Tóth, *Inorg. Chem.* **1994**, *33*, 4070.
[23] S. L. Wu, Jr. W. D. Horrocks, *Inorg. Chem.* **1995**, *34*, 3724.
[24] L. Burai, I. Fábián, R. Király, E. Szilágyi, E. Brücher, *J. Chem. Soc., Dalton Trans.* **1998**, 243.
[25] J. W. Moore, R. G. Pearson, *Kinetics and Mechanisms*, 3rd ed., J. Wiley and Sons, New York, **1981**, p. 353.
[26] K. Kumar, C. A. Chang, M. F. Tweedle, *Inorg. Chem.* **1993**, *32*, 587.
[27] C. F. G. C. Geraldes, A. D. Sherry, G. E. Kiefer, *Magn. Reson.* **1992**, *97*, 290.
[28] J. R. Morrow, L. A. Buttrey, V. M. Shelton, K. A. Berback, *J. Am. Chem. Soc.* **1992**, *114*, 1903.
[29] G. L. Jr.Rothermel, E. N. Rizkalla, G. R. Choppin, *Inorg. Chim. Acta* **1997**, *262*, 133.
[30] E. Szilágyi, É. Tóth, E. Brücher, A. E. Merbach, *J. Chem. Soc., Dalton Trans.* **1999**, 2481.
[31] H. M. Irving, M. G. Miles, L. Pettit, *Anal. Chim. Acta* **1967**, *38*, 475.
[32] J. C. Cassat, R. G. Wilkins, *J. Am. Chem. Soc.* **1968**, *90*, 6045.
[33] K. Mikkelsen, S. O. Nielsen, *J. Phys. Chem.* **1960**, *64*, 632.
[34] L. Zékány, I. Nagypál, in: *Computational Methods for Determination of Formation Constants* (Ed.: D. J. Leggett), Plenum Press, New York, **1985**, p. 291.

Received June 16, 2000
[I00236]