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Gallium(III) Complexes of DOTA and DOTA−Monoamide: Kinetic and Thermodynamic Studies

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Given the practical advantages of the ^{68}Ga isotope in positron emission tomography applications, gallium complexes are gaining increasing importance in biomedical imaging. However, the strong tendency of Ga^{3+} to hydrolyze and the slow formation and very high stability of macrocyclic complexes altogether render Ga^{3+} coordination chemistry difficult and explain why stability and kinetic data on Ga^{3+} complexes are rather scarce. Here we report solution and solid-state studies of Ga^{3+} complexes formed with the macrocyclic ligand 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, $(DOTA)^{4-}$, and its mono(*n*-butylamide) derivative, $(DO3AM^{Bu})^{3-}$. Thermodynamic stability constants, log *K*(GaDOTA) = 26.05 and log *K*(GaDO3AM^{Bu}) = 24.64, were determined by out-of-cell pH-potentiometric titrations. Due to the very slow formation and dissociation of the complexes, equilibration times of up to ∼4 weeks were necessary. The kinetics of complex dissociation were followed by ^{71}Ga NMR under both acidic and alkaline conditions. The GaDOTA complex is significantly more inert ($\tau_{1/2}$ ∼12.2 d at pH = 0 and $\tau_{1/2}$ ∼6.2 h at pH = 10) than the GaDO3AM^{Bu} analogue ($\tau_{1/2}$ ∼2.7 d at pH = 0 and $\tau_{1/2}$ ∼0.7 h at pH = 10). Nevertheless, the kinetic inertness of both chelates is extremely high and approves the application of Ga^{3+} complexes of such DOTA-like ligands in molecular imaging. The solid-state structure of the GaDOTA complex, crystallized from a strongly acidic solution (pH < 1), evidenced a diprotonated form with protons localized on the free carboxylate pendants.

Introduction

Positron emission tomography (PET) plays an important role in medical diagnostics. The method uses pharmaceuticals based on positron emitting isotopes. Upon emission, the positron annihilates and generates two collinear γ photons (E = 511 keV) which are detected by the scanner. Due to the simultaneous detection of both photons, the method shows much higher resolution compared to other nuclear imaging techniques. For medical utilization, the isotope has to fulfill certain requirements with respect to specific activity obtained after irradiation of a target, decay modes, half-life, and maximal particle energy and particle range in the tissue. The most studied PET isotopes are ^{11}C and ^{18}F. Beside these, the interest is also focused on suitable metal isotopes due to their easier production. For many metals, one drawback is their toxicity when applied as a free aqua ion. In order to avoid deposition of the metal radioisotopes in body tissues and to suppress their toxic effects, they must be used in the form of stable complexes. For in vivo application of the complexes, both their thermodynamic stability and kinetic inertness play a crucial role.

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Among metal-based PET agents, gallium complexes have attracted much attention due to the excellent properties of the ^{68}Ga isotope ($\tau_{1/2}$ = 67.6 min). Furthermore, a ^{68}Ge/^{68}Ga generator has been commercialized which significantly improved the accessibility and decreased the price of the isotope.[1] The ligands used for the complexation of Ga^{3+} are mostly based on linear or macrocyclic polyamines modified with negatively charged pendant arms.[2] Among them, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, Chart 1) and its derivatives represent one of the most investigated groups of ligands. Gallium(III) complexes of DOTA-like ligands bearing oligopeptides are intensively studied for imaging of several types of tumors.[1,3−5] The results are extremely promising, and some of the complexes have already entered clinical trials. Despite the wide utilization of Ga^{3+} complexes with DOTA-like ligands, information

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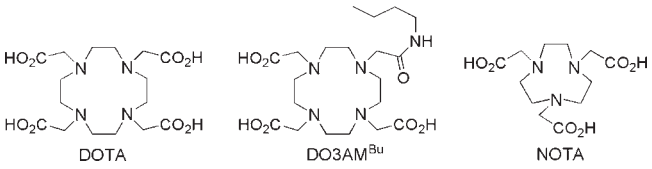
Chart 1. Ligands Discussed



on their solution properties[6−10] and solid-state structures[11−15] is rather scarce, and no data are available on their kinetic behavior.

Here, we report on the thermodynamic stability and kinetic inertness of Ga(III) complexes with DOTA and its monoamide derivative, DO3AM^{Bu} (Chart 1). DO3AM^{Bu} has been chosen as a model compound for DOTA derivatives used in the clinical practice, as the peptide targeting moieties are mostly attached to one carboxylic pendant arm of DOTA through an amide bond.

Results and Discussion

In order to simplify the text, abbreviations GaDOTA and GaDO3AM^{Bu} are used for the complexes regardless of their charge/protonation state, except when the distinction is necessary for comprehension.

Thermodynamic Stability. Thermodynamic stabilities of the complexes were studied by potentiometry. The protonation constants of DOTA were taken from the literature (since the protonation constants are expected to decrease with an increasing number of amide pendants, some other data measured in 1.0 M Me_4NCl media are also shown for comparison in Table 1).[16] For DO3AM^{Bu}, five protonation constants were determined. Similarly to DOTA, DO3AM^{Bu} exhibits two constants in the alkaline region corresponding to the protonation of the macrocyclic amine groups. The other three constants describe protonation of the carboxylate pendant arms. Previously, slightly different constants have been determined for DO3AM^{Bu} by NMR spectroscopy.[17] The differences can be mainly ascribed to the presence of Na^+ (used for preparation of the NMR samples) forming weak complexes with DOTA-like ligands. The formation of the Na^+ complex should result in a noticeable decrease of the first protonation constant.

Table 1. Stepwise Protonation Constants[a] of DO3AM^{Bu} and Related Ligands

	log K_A				
species	DO3AM^{Bu} [b]	DO3AM^{Bu} [c]	DO3AM^{Phe} [d]	DOTA[e]	DOTA[f]
HL	12.22	10.73	11.63	11.9	12.6
H_2L	8.90	9.05	9.28	9.72	9.70
H_3L	4.34	4.53	4.18	4.60	4.50
H_4L	2.49	3.32	2.14	4.13	4.14
H_5L	1.47	2.25	<2	2.36	2.32
log β_4[g]	27.94	27.63	27.23	30.35	30.94

[a] $K_A = [H_hL]/([H] \times [H_{h-1}L])$. [b] This work (25 °C, I = 0.1 M NMe_4Cl). [c] Ref 17; (25 °C, NaOH/HCl, no ionic strength control). [d] DO3AM^{Phe} = DOTA−monoamide bearing D-phenylalanine, ref 12; (25 °C, I = 0.5 M NMe_4Cl). [e] Ref 16; (25 °C, I = 0.1 M NMe_4Cl). [f] Ref 18; (25 °C, I = 0.1 M NMe_4Cl). [g] Overall basicity defined as $\beta_4 = [H_4L]/([H]^4 \times [L])$.

Table 2. Stepwise Protonation (K_A) or Stability (K) Constants of the Studied Gallium(III) Complexes[a]

	equilibrium constants (log K_A or log K)	
equilibrium[b]	GaDOTA	GaDO3AM^{Bu}
[Ga(OH)(L)] + H ⇌ [Ga(L)] + H_2O		8.81[c]
Ga + L ⇌ [Ga(L)]	26.05[c]	24.64[c]
H + [Ga(L)] ⇌ [Ga(HL)]	3.64[c] (*3.52*)[d]	3.12[c] (*3.24*)[d]
H + [Ga(HL)] ⇌ [Ga(H_2L)]	2.43[c] (*2.44*)[d]	2.26[c] (*1.54*)[d]
H + [Ga(H_2L)] ⇌ [Ga(H_3L)]	1.84[c] (*1.57*)[d]	

[a] $K_A = [H_hGaL]/([H] \times [H_{h-1}GaL])$ and $K = [GaL]/([Ga] \times [L])$. [b] Charges are omitted. [c] Constants determined by out-of-cell (equilibrium) titration. [d] Constants determined by titration of the preformed complex.

Gallium(III) forms highly stable hydroxocomplexes. Gallium(III) hydroxide precipitates already at pH ∼3.5 and dissolves back at pH ∼8, forming the tetrahydroxogallate anion. Therefore, contrary to most of the metal ions, the stability of Ga^{3+} complexes could be determined not only from a competition between protons and the metal ion (for the ligand) in acidic media but also from a competition between the ligand and hydroxide ions (for the metal ion) in alkaline media. Due to a slow formation and dissociation of the complexes studied (see below), their stability constants have to be determined by the out-of-cell method. During sample preparation, Ga^{3+} hydroxide precipitated in the pH region ∼3.7−7.0 as a consequence of the slow complexation of Ga^{3+}. To avoid contamination of the alkaline solutions with carbon dioxide, the samples at pH > 7 were stored in flame-sealed glass ampules. We should note that this sample treatment is indispensable for a correct and reproducible determination of the stability constants.

Both complexes show high stability constants (Table 2). The higher value found for GaDOTA as compared to GaDO3AM^{Bu} could be ascribed to the higher overall basicity (see Table 1) of the ligand. The Ga−DOTA system has been previously studied by Martell et al.,[6] and they reported a much lower stability constant (log K(GaDOTA) = 21.33 in 0.1 M KCl). The incomplete description of the experimental conditions in Martell's work makes it difficult to compare and discuss the reported values. The disagreement likely arises from the not fully equilibrated solutions. On the other hand, the stability

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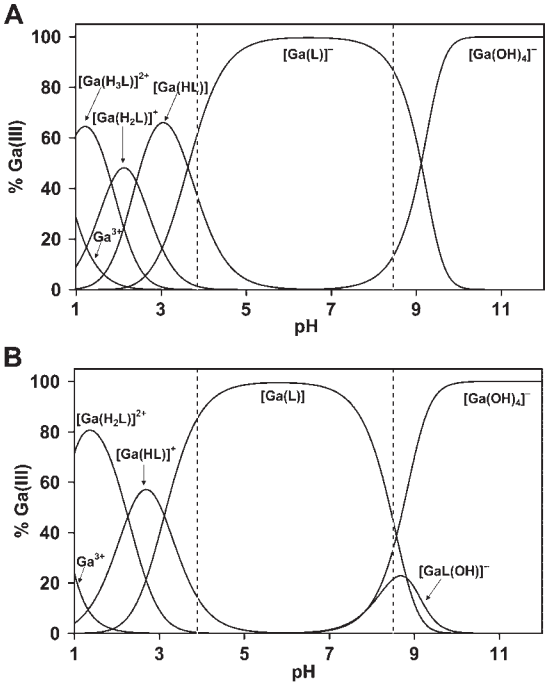

Figure 1. Distribution diagrams for the Ga−DOTA (A) and Ga−DO3AM^{Bu} (B) systems under equilibrium conditions ($c_L = c_{Ga} = 0.004$ M, out-of-cell titrations). The dashed lines indicate the pH range where precipitate was present.

constant recently reported for the Ga^{3+} complex of a DOTA−monoamide bearing a quinazoline moiety (L) is similar to ours (log K(GaL) = 24.5).[9]

For both complexes, several protonation steps were found (Figure 1). In the solid-state structures of GaDOTA, the metal ion is hexacoordinated by the four nitrogen atoms of the cycle and the two trans-located carboxylate groups in a distorted octahedral environment,[11,12] while the two other carboxylate groups remain free. The first two protonations of the complex likely occur on those free carboxylate groups. Based on the X-ray structure of GaDO3AM^{Phe},[13] a similar coordination mode with two coordinated carboxylates and the first protonation occurring on the single free carboxylate pendant could be expected for the GaDO3AM^{Bu} complex. Taking into account the formation mechanism commonly accepted for DOTA-type complexes,[19] the thermodynamically stable protonated species [Ga(H$_3$dota)]$^{2+}$ or [Ga(H$_2$do3am^{Bu})]$^{2+}$ present in acidic solution under equilibrium conditions are expected to hold the proton on ring nitrogen atoms and/or on coordinated carboxylate groups, whereas the Ga^{3+} ion should be located out-of-cage (see also below).

Despite the tendency of gallium(III) to form hydroxocomplexes, a mixed hydroxocomplex was observed only for the Ga^{3+}−DO3AM^{Bu} system but not for the Ga^{3+}−DOTA system. In this hydroxospecies, the hydroxide anion probably replaces one of the coordinated acetate pendant arms of GaDO3AM^{Bu}. The different behavior can be ascribed to the different charge of [Ga(dota)]$^-$ and [Ga(do3am^{Bu})] (the negative charge of [Ga(dota)]$^-$ repulses the negatively charged hydroxide ion).

The knowledge of the full set of protonation constants of the complexes can help in the interpretation of the dissociation kinetic data. Due to the high kinetic inertness of both complexes in acidic media (see below), we could also determine the consecutive protonation constants of the preformed complexes by direct potentiometric titration. The perfect agreement of the highest dissociation constant(s) of GaDOTA or GaDO3AM^{Bu} confirms that the protonation sites are indeed on the free carboxylate(s). In contrast, the lowest dissociation constants show differences between those determined by the out-of-cell (equilibrium) titrations and those originating from in-cell ("non-equilibrium") titrations of the preformed complexes. A comparison of the distribution diagrams from the out-of-cell and in-cell titrations is shown in the Supporting Information (Figures S1 and S2).

The differences are probably related to the different nature of these protonated species. In the preformed complex, the metal ion is entrapped firmly in the macrocyclic cavity, and the corresponding protonation constants describe the proton binding to the oxygen atom(s) of the coordinated pendant arm(s).[20−23] In the case of the "equilibrium" batch titration, the protonation constants can be assigned to the thermodynamically stable "out-of-cage" complex (under the conditions used), where the ligand is coordinated only through carboxylate groups with the proton(s) localized on the ring amine group(s). Such species are commonly considered as kinetic intermediates during the complexation reactions of DOTA-like ligands, but they can be thermodynamically stable under highly acidic conditions. Due to a higher basicity of the ring amine groups, the protonation constants obtained from the out-of-cell titration are higher compared to those determined for the preformed complex where only oxygen atoms are protonated. A formation of thermodynamically stable protonated species in acidic solutions[23,24] as well as an apparent increase of protonation constants due to partial ring nitrogen atom protonation(s)[19,25,26] have been suggested for DOTA-like Ln^{3+} complexes.

The stability constants of the gallium(III) complexes (with six donor atoms forming an octahedral complex) are higher than those of trivalent lanthanide complexes (all eight donor atoms are bound in a square-antiprismatic environment, log K(LnDOTA) ∼22−24),[16] despite the lower number of coordinated donor atoms. The difference can be rationalized by considering the size of these ions and the nature of their interaction with the ligands. The gallium(III) ion is much smaller (by ∼0.3−0.4 Å)[27] than

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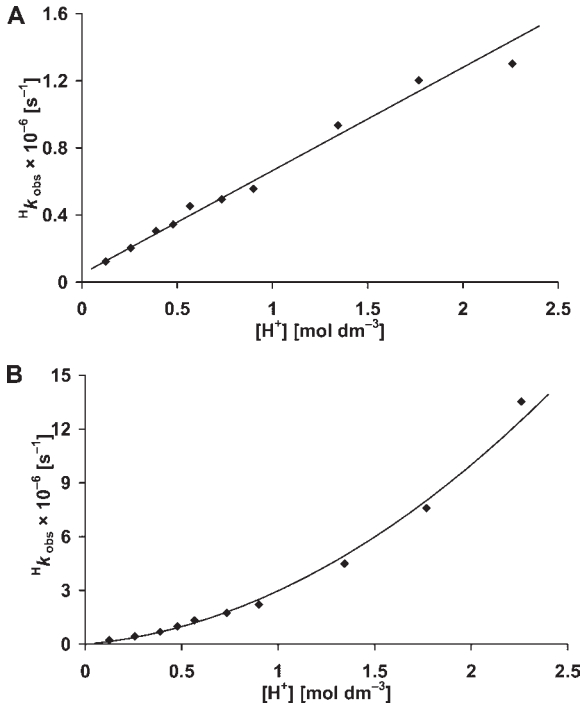

Figure 2. Dependence of the pseudofirst-order rate constants, $^{H}k_{obs}$, of the proton-assisted dissociation of GaDOTA (A) and GaDO3AM^{Bu} (B) on the proton concentration (25 °C, I = 1.0 M (K,H)NO_3).

those of the lanthanide(III) ions. Since the interaction between the polyaminocarboxylates and these trivalent metal ions is mainly of ionic character, the much smaller Ga^{3+} exhibits a stronger electrostatic interaction leading to more stable complexes, despite the lower number of coordinated donor atoms (typical bond lengths observed in GaDOTA and LuDOTA complexes are shown in Table S1, Supporting Information). A comparison of our data and those reported for Ga^{3+} complexes of other DOTA-like ligands shows that the presence of one amide pendant arm in the DOTA skeleton does not remarkably change the thermodynamic stability, as the amide group is not coordinated.[9] The differences are given mainly by the change in basicity of the donor atoms. On the other hand, a significant stability decrease was observed when a nitrogen atom of the cyclen skeleton was substituted by an oxygen or a pyridine ring.[7,8] Therefore, these substitutions might not be suitable when designing new chelators for the trivalent gallium. Ga^{3+} forms thermodynamically less stable complexes with DOTA-like chelators than with NOTA-like ligands (H_3NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid, Chart 1), which have a much lower overall basicity.[28,29] The reason is that the octahedron of the Ga^{3+} complexes of DOTA-like ligands is strongly distorted, while the cavity of the 1,4,7-triazacyclononane-based ligands perfectly matches the size of the trivalent gallium.

Kinetic Inertness. The kinetic inertness of lanthanide(III) complexes has been often studied using transmetalation experiments with zinc(II) or other lanthanide(III) ions.[30] Due to the very high thermodynamic stability of gallium(III) complexes, this method cannot be applied, thus only the proton-assisted decomplexation in strongly acidic media can be studied. Furthermore, as gallium(III) shows an extreme affinity to hydroxide anions, all known Ga(III) complexes decompose in alkaline media forming the tetrahydroxogallate anion, thus the hydroxide-mediated decomplexation can also be monitored. Both decomplexation reactions were followed by ^{71}Ga NMR. The high quadrupole moment of the nucleus results in a significant line broadening, therefore only the signals of very symmetric species, such as $[Ga(H_2O)_6]^{3+}$, $[Ga(OH)_4]^-$, and highly symmetric complexes, can be observed. Both GaDOTA and GaDO3AM^{Bu} show a low symmetry, and their ^{71}Ga NMR signals cannot be detected. Thus, the complex dissociation was quantified by the increasing signal intensity of $[Ga(H_2O)_6]^{3+}$ or $[Ga(OH)_4]^-$ (experiments in acidic or alkaline media, respectively) in comparison to a reference solution contained in an inserted capillary tube.

Table 3. Kinetic Parameters Characterizing the Proton-Assisted Dissociation of the Complexes[a]

parameter	GaDOTA	GaDO3AM^{Bu}
k_{H0} [s^{-1}]	$(5.8 \pm 1.7) \times 10^{-8}$	–
k_{H1} [M^{-1} s^{-1}]	$(6.0 \pm 0.3) \times 10^{-7}$	$(1.0 \pm 0.2) \times 10^{-6}$
k_{H2} [M^{-2} s^{-1}]	–	$(2.0 \pm 0.2) \times 10^{-6}$
$\tau_{1/2}$ (pH = 0) [days]	12.2	2.7
$\tau_{1/2}$ (pH = 1) [days][b]	68	69

[a] Parameters: 25 °C and I = 1.0 M (K,H)NO_3. [b] Extrapolation, since outside of the measured pH region.

Proton-Assisted Dissociation. The decomplexation in acidic media was studied at 25 °C in a range of proton concentrations 0.13−2.5 M. When the pseudofirst-order rate constant, $^{H}k_{obs}$, is plotted against the proton concentration (Figure 2), the obtained curves show different shapes for the two complexes. The simplified rate law corresponding to the experimental results is given by eq 1 (for the elucidation of the complete rate law, see Supporting Information); the results of the fitting are given in Table 3.

$$^{H}k_{obs} = k_{H0} + k_{H1} \cdot [H] + k_{H2} \cdot [H]^2 \qquad (1)$$

The different behavior of the two complexes reflects their different charge and protonation scheme. The suggested reaction mechanism is depicted in Scheme 1.

For GaDOTA (Scheme 1A), only zero- and first-order rate constants were found. In the pH region used, the triprotonated complex is the major species, and the corresponding protonation constant (log K_{H3} = 1.57) was determined by potentiometric titration of the preformed complex (Table 2 and Figure S1, Supporting Information), while the constant corresponding to the tetraprotonated species was too low to be detected. The constant k_{H0} corresponds to the dissociation of the triprotonated species ($k_{H0} = {}'k_{H3}$), whereas k_{H1} corresponds to the dissociation of the tetraprotonated species ($k_{H1} = K_{H4} \times {}'k_{H4}$). For GaDO3AM^{Bu} (Scheme 1B), first- and second-order rate constants could be reliably calculated. Here, the constants k_{H1} and k_{H2} correspond to the dissociation of the tri- and tetraprotonated species, respectively ($k_{H1} = K_{H3} \times {}'k_{H3}$ and $k_{H2} = K_{H3} \times K_{H4} \times {}'k_{H4}$). While K_{H2} for the diprotonated species

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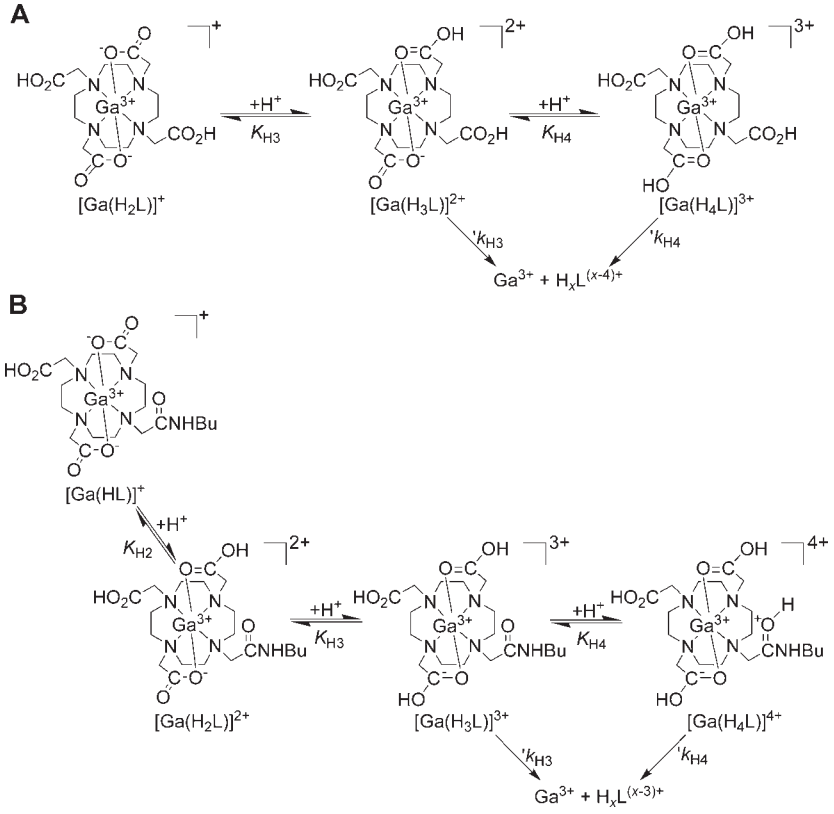
Scheme 1. Mechanism of the Proton-Assisted Dissociation of GaDOTA (A) and GaDO3AM^{Bu} (B)[a]



[a] The protonations sites are tentative.

was determined by potentiometry of the preformed complex (log K_{H2} =1.54, Table 2), the values of K_{H3} and K_{H4} could not be obtained in this way.

For both complexes, only the species which are protonated not only on the noncoordinated but also on the coordinated carboxylate(s) play a role in the dissociation process. The species with protons only on the noncoordinated carboxylates ($[Ga(H_2L)]^+$ for DOTA and $[Ga(HL)]^+$ for DO3AM^{Bu}; Scheme 1) are thermodynamically stable. The binding of an additional proton to a coordinated carboxylate group (below pH ~2.5; Figures S1 and S2, Supporting Information) then initiates the decomplexation process. In the Ga−DOTAM^{Bu} system, the zero-order rate constant describing the dissociation of the $[Ga(H_2L)]^{2+}$ species is probably very low and could not be reliably determined. The corresponding protonation constants, log K_A, are ~1.5 (Table 2), as determined by potentiometric titration of the preformed complexes. The difference in the experimental rate laws (i.e., the molecularity of the reactions) for GaDOTA and GaDO3AM^{Bu} excludes a direct comparison of the particular rate constants. Thus, only the experimental parameters, k_{obs} or $\tau_{1/2}$, can be compared. They show that GaDO3AM^{Bu} dissociates significantly faster (see Figure 2 and Table 3).

To explain these observations, we can consider the generally accepted mechanism for acid-assisted dissociation of DOTA-like complexes. In this mechanism, the dissociation starts with the protonation of a coordinated carboxylate group in a fast equilibrium step. The rate-determining step is then the transfer of this proton from the carboxylate to a ring nitrogen, followed by the fast decomposition of the N-protonated complex. Thus, the faster dissociation of GaDO3AM^{Bu} can be probably rationalized by an easier proton transfer from the protonated amide moiety to the carboxamide-binding ring nitrogen atom due to the different electronic and hydrogen-bonding properties of the carboxamide moiety as compared to the carboxylate group.

As no other acid-assisted dissociation parameters have been reported for Ga^{3+} complexes, the only comparable data available are those for DOTA-like lanthanide(III) chelates, though these complexes have a different structure as all the pendant arms are coordinated. Since both types of complexes are applied in medicine, the comparison might be relevant. For instance, the decomposition half-life can be compared with that of EuDOTA (~40 h at pH = 1, 25 °C)[20] and GdDOTA (~210 h at pH = 1, 25 °C).[31] Both gallium(III) complexes are kinetically much more inert (see Table 3; $\tau_{1/2}$ at pH = 1 can be estimated to ~70 d for both complexes) than the lanthanide(III) analogues despite the lower coordination number of the Ga^{3+} ion. Taking into account the mechanism given above, we can speculate that the difference can be caused by: (i) the smaller ionic radius of the gallium(III) ion leading to a much stronger electrostatic interaction with the hard donor atoms, preventing an efficient proton transfer from the carboxylate oxygens to the ring nitrogens in the rate-determining step and/or (ii) the different molecular structure of the complexes, as an octahedron (Ga), is

(31) Wang, X.; Jin, T.; Comblin, V.; Lopez-Mut, A.; Merciny, E.; Desreux, J. F. *Inorg. Chem.* **1992**, *31*, 1095–1099.

Table 4. Kinetic Parameters Characterizing the Hydroxide-Assisted Dissociation of the Complexes[a]

parameter	GaDOTA	GaDO3AM^{Bu}
k_{OH1} [M^{-1} s^{-1}]	$(1.8 \pm 0.4) \times 10^{-1}$	2.70 ± 0.07
k_{OH2} [M^{-2} s^{-1}]	$(1.33 \pm 0.08) \times 10^{3}$	–
$\tau_{1/2}$ (pH = 10) [h][b]	6.2	0.71

[a] Conditions: 25 °C, 1.0 M KCl, and 0.2 M CAPS buffer. [b] Extrapolated, since outside of the measured pH region.

less flexible than a square antiprism (Ln). The different protonation constants of the complexes might also have an importance. The protonated species which are important in the decomposition of the gallium(III) complexes ($[Ga(H_3dota)]^{2+}$ and $[Ga(H_2do3am^{Bu})]^{2+}$) have stepwise protonation constants of log $K_A \sim 1.5$ and log $K_A \ll 1$, which are different from the corresponding constants of the mono- and diprotonated LnDOTA complexes having both log K_A values close to 1.[20,22]

Hydroxide-Assisted Dissociation. The decomplexation in alkaline media was studied in the pH range 9.7−11.0. The simplified rate law corresponding to the experimental results is given by eq 2 (for the elucidation of the complete rate law, see Supporting Information), and the results of the fitting are compiled in Table 4.

$$^{OH}k_{obs} = k_{OH0} + k_{OH1} \cdot [OH^-] + k_{OH2} \cdot [OH^-]^2 \quad (2)$$

Like in the proton-assisted decomplexation, the dependence of $^{OH}k_{obs}$ on the hydroxide concentration is different for the two complexes (Figure 3), as a result of their different charge and of the formation of thermodynamically stable hydroxido species in the case of GaDO3AM^{Bu}. The suggested reaction mechanism is depicted in Scheme 2.

In both systems, the zero-order rate constant k_{OH0} is very low and could not be determined. For GaDOTA, first- and second-order rate constants were found. The rate constant k_{OH1} is related to monohydroxido species ($k_{OH1} = K_{OH1} \times {}'k_{OH1}$), whereas k_{OH2} is associated with the dihydroxido complex ($k_{OH2} = K_{OH1} \times K_{OH2} \times {}'k_{OH2}$). The potentiometric data indicate negligible abundance for both hydroxido complexes along the pH range studied; thus, the corresponding thermodynamic stability constants K_{OH1} and K_{OH2} could not be determined by potentiometry. For GaDO3AM^{Bu}, only a first-order dissociation rate constant was found, associated with the dihydroxido complex ($k_{OH1} = K_{OH2} \times {}'k_{OH2}$), whereas the monohydroxido complex seems to be thermodynamically stable and kinetically not active (its thermodynamic stability constant was determined by potentiometry). On the other hand, the stability constant K_{OH2} could not be successfully included in the fit of the potentiometric data for the DOTA−monoamide complex.

Similarly to the proton-assisted dissociation, the decomposition rate of the two complexes shows a different dependency on the hydroxide concentration (Figure 3). The decomposition is initiated by a direct coordination of at least one hydroxide anion which replaces an acetate arm (Scheme 2). In the case of the Ga−DO3AM^{Bu} system, the monohydroxido mixed complex is thermodynamically stable and kinetically inert, thus the decomplexation most likely starts only upon the coordination of a second hydroxide anion. Correspondingly, the zero-order

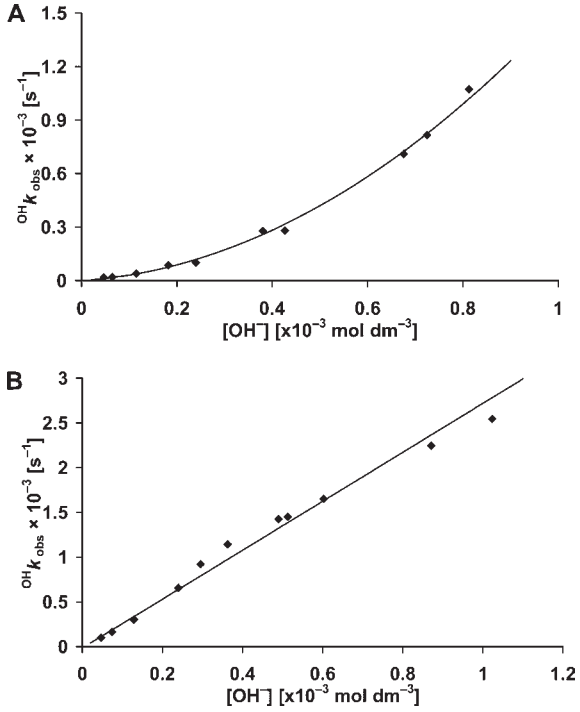


Figure 3. Dependence of the pseudofirst-order rate constants, $^{OH}k_{obs}$, for the hydroxide-assisted dissociation of GaDOTA (A) and GaDO3AM^{Bu} (B) on the hydroxide anion concentration (25 °C, 1.0 M KCl, and 0.2 M CAPS buffer).

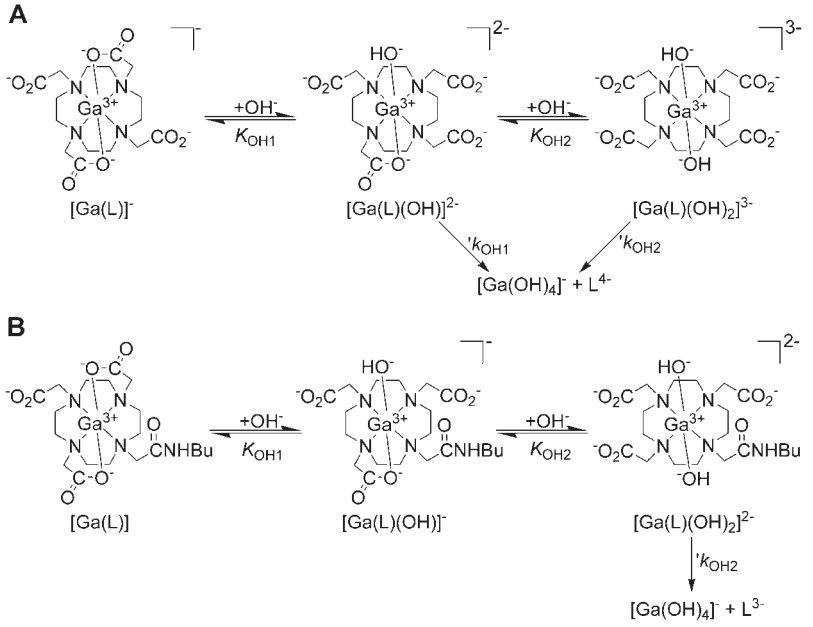
rate constant related to the decomposition of the monohydroxido species could not be included in the fit. For GaDOTA, both the mono- and dihydroxido species are kinetically active. As mentioned above, a direct comparison of the rate constants is not possible as they are connected with a different molecularity of the reactions; thus, only experimental values can be compared. The k_{obs} or $\tau_{1/2}$ values show a significantly slower dissociation for GaDOTA (see Figure 3 and Table 4) in the pH range used for the measurements. It could be rationalized by its negative charge and the corresponding electrostatic repulsion between the complex and the attacking hydroxide anion.

In biological media, the dissociation kinetics is the most important factor for the in vivo stability and nontoxicity of the complexes. The high thermodynamic stability of Ga^{3+} complexes hampers transmetalation with other biogenic metal ions. Transchelation can also be excluded as both ligands studied fully occupy the coordination sphere of the Ga^{3+} ion, thus disfavor the ligand exchange. A direct comparison of the dissociation rates shows that the hydroxide-assisted decomplexation is several orders of magnitude faster than the proton-assisted one in the pH range of the experiments; in addition, the alkaline pH range studied is more close to the physiological one. Thus, one can conclude that the hydroxide-assisted dissociation may play a more important role under in vivo conditions.

Unfortunately, data on the kinetic inertness of gallium(III) complexes are practically missing. The only information for aminocarboxylate ligands is from Parker et al.[32] who have reported an extremely high kinetic inertness for

(32) (a) Craig, A. S.; Parker, D.; Adams, H.; Bailey, N. A. *J. Chem. Soc., Chem. Commun.* **1989**, 1793–1794. (b) Broan, C.; Cox, J. P.; Craig, A. S.; Kataky, R.; Parker, D.; Harrison, A.; Randall, A. M.; Ferguson, G. *J. Chem. Soc., Perkin Trans. 2* **1991**, 87–99.

Scheme 2. Mechanism of the Hydroxide-Assisted Dissociation of GaDOTA (A) and GaDO3AM^{Bu} (B)[a]



[a] Structures of the ternary hydroxospecies are tentative.

GaNOTA, as no dissociation was observed in 6 M HNO_3 (at least for 6 months) or at pH 12 (at least for 10 d). The gallium(III) complex of a phosphinate analog of NOTA exhibits similar kinetic inertness though only qualitative data were reported.[29] The complexes studied in this work are less inert, but they still remain highly resistant to both proton- and hydroxide-assisted dissociations. This approves that DOTA-like ligands are suitable chelators of trivalent gallium for radiopharmaceutical applications.

Crystal Structure of $[Ga(H_2dota)](ClO_4)\cdot 0.5HClO_4\cdot 5.5H_2O$. During the preliminary kinetic experiments, we observed low solubility of GaDOTA in the presence of perchlorate anions in acidic media, while no precipitation was detected in nitrate- or chloride-containing solutions. Single crystals could be obtained by very slow diffusion of perchloric acid into a perchlorate-containing solution of the complex.

In the solid-state structure of the complex, the independent unit is formed by one-half of the macrocycle, one pendant arm placed in a general position (the uncoordinated one, with a full occupancy), two coordinated carboxylates occupying a special position (plane) with a half-occupancy and the gallium(III) ion (also with a half-occupancy). The central Ga^{3+} ion is coordinated in a distorted *cis*-O_2N_4 octahedron formed by all four nitrogen atoms of the macrocycle and two oxygen atoms of "trans" acetate pendants (Figure 4), with the coordination bond lengths laying in the expected range [*d*(N−Ga) ~2.1 Å and *d*(O−Ga) ~1.9 Å, Table 5].[33] Both uncoordinated pendant arms are protonated (protons were located in the difference map of electron density). The protonation state of the complex molecule is also evident from the C−O distances (compare the C21−O211distance in the protonated carboxylate, ~1.3 Å, with distances of

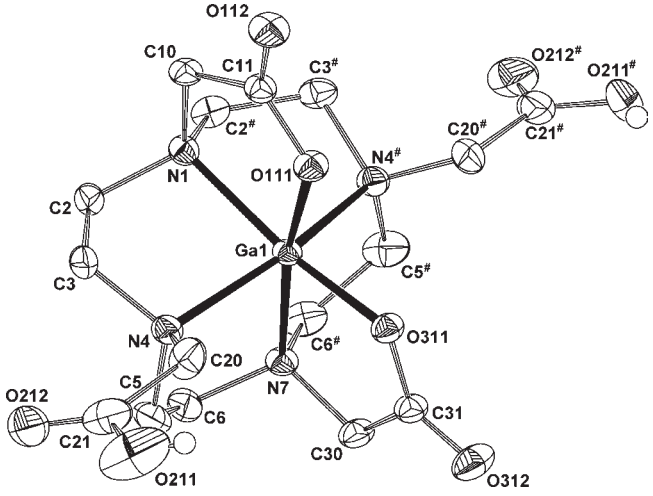


Figure 4. The molecular structure of the $[Ga(H_2dota)]^+$ cation found in the solid-state structure of $[Ga(H_2dota)](ClO_4)\cdot 0.5HClO_4\cdot 5.5H_2O$. Hydrogen atoms bound to carbon atoms are omitted for the sake of clarity.

Table 5. Selected Geometric Parameters Found in the Structure of $[Ga(H_2dota)](ClO_4)\cdot 0.5HClO_4\cdot 5.5H_2O$

distances (Å)		angles (°)	
Ga1−N1	2.094(4)	N1−Ga1−N4	84.13(7)
Ga1−N4	2.153(3)	N1−Ga1−N7	107.18(14)
Ga1−N7	2.111(3)	N1−Ga1−O111	83.34(13)
Ga1−O111	1.946(3)	N1−Ga1−O311	167.66(13)
Ga1−O311	1.924(3)	N4−Ga1−N4[a]	157.66(14)
C11−O111	1.293(5)	N4−Ga1−N7	82.70(7)
C11−O112	1.213(5)	N4−Ga1−O111	98.72(7)
C21−O211	1.322(5)	N4−Ga1−O311	97.76(7)
C21−O212	1.198(6)	N7−Ga1−O111	169.48(14)
C31−O311	1.309(5)	N7−Ga1−O311	85.16(13)
C31−O312	1.212(5)	O111−Ga1−O311	84.32(13)

[a] Symmetrically associated atom.

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formally double-bonded oxygen atoms C11═O112 and C31═O312 ~1.2 Å; Table 5). The proton originating from the excessive perchloric acid is probably bound to a water solvate forming the oxonium ion; however, the huge disorder in the water solvate molecules (see below) prevents the localization of hydrogen atoms. The structure of the diprotonated complex species, $[Ga(H_2dota)]Cl \cdot 5H_2O$, was published;[12] however, a close inspection of the published data showed that the protonation state of the complex in the structure might be incorrect (for more information, see Supporting Information). A very similar coordination mode of the Ga^{3+} ion has been found in the structures of all reported gallium(III) complexes with DOTA-like ligands (see Table S1, Supporting Information).[11−15] In all those structures, the coordination sphere of the central metal ion is distorted in the same way, with trans N−Ga−N bond angles ~157° and trans N−Ga−O angles ~169°. All angles corresponding to *cis*-coordination of the five-membered chelate rings are ~83−85° as a result of a sterical strain of the chelate ring. Similar values were found also for GaNOTA (see Table S1, Supporting Information).[32a] However, in that case, all transannular angles are ~167°, forming a much more regular coordination sphere.

In the crystal structure, there is a large free space between the complex molecules. In this free space, water solvate molecules are present but could not be reliably located from the data. It is probably due to the absence of hydrogen-binding groups in the complex molecule to create a network of hydrogen bonds. Thus, the energy differences between different sites occupied by the water solvent molecules are small, resulting in a huge disorder of water solvate molecules (Figure S4, Supporting Information). Consequently, the electron density belonging to the solvate molecules was squeezed.

Conclusions

The thermodynamic stability and the kinetic inertness of gallium(III) complexes of DOTA and its monoamide, $DO3AM^{Bu}$, were studied. $DO3AM^{Bu}$ was taken as a model compound since most conjugates used in gallium-based radiopharmaceuticals contain a DOTA−monoamide chelating moiety. Both ligands form complexes endowed with a high thermodynamic stability, and several protonated species were detected under equilibrium conditions. The stability constant published previously ($\log K \sim 21$)[6] for GaDOTA was revised to a higher value ($\log K \sim 26$). Such a higher $\log K$ is indeed expected for GdDOTA on the basis of stability constants of DOTA with other trivalent metal ions (Table S2, Supporting Information); trivalent gallium should exhibit a higher stability with the macrocyclic DOTA than with the acyclic ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA). In nuclear medicine, complexation of gallium isotopes with DOTA-like ligands is commonly done at pH = 3−4 and with a brief heating.[34,35] Our equilibrium data confirm the relevance of the experimental conditions as, according to the distribution diagrams (Figure 1), Ga^{3+} should be fully complexed in this pH region. Data on the formation kinetics of these complexes would be also highly interesting. However, the precipitation of $Ga(OH)_3$ above pH = 3 at concentrations necessary for the "chemical" measurements hampers such experiments. The two complexes studied show a different affinity to proton and hydroxide (to form hydroxido species), resulting in different dissociation mechanisms. Kinetic experiments confirmed long half-lives of the complexes both in strongly acidic and slightly alkaline media. Among all DOTA complexes, Ga-DOTA is the most kinetically inert in acidic solutions.[36] The species protonated only on the noncoordinated acetate pendant arm(s) remain kinetically inert. The protonation sites were independently confirmed by the solid-state structure of the diprotonated GaDOTA complex. A direct comparison of the proton- and hydroxide-assisted dissociation rates indicates that at neutral pH, important for in vivo applications, the hydroxide-assisted dissociation may play a more important role. The presence of the amide group in the $GaDO3AM^{Bu}$ complex decreases the kinetic inertness as compared to GaDOTA. Nevertheless, both chelates are extremely inert, and this approves the application of gallium(III) complexes with DOTA-like ligands in human medicine and biomedical molecular imaging.

Experimental Section

Materials and Methods. Commercially available chemicals had synthetic purity and were used as received. DOTA and $t\text{-}Bu_3DO3A \cdot HBr$ (H_3DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) were synthesized according to published procedures.[37,38] *N*-Cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffer was received from Sigma-Aldrich. NMR spectra were recorded on Bruker Avance 500 (^{71}Ga, 152.5 MHz, and 11.75 T; chemical shifts were externally referenced to 0.01 M $Ga(NO_3)_3$ in 0.1 M HNO_3; 0.0 ppm) and Varian ^{UNITY}*PLUS*-300 (^{1}H) spectrometers. For the measurements in D_2O, *t*-BuOH was used as an internal standard with the methyl signal referenced to 1.2 ppm (^{1}H). The kinetic experiments were performed at constant temperature 25.0 °C maintained by the NMR spectrometer and/or by a thermostatted bath. The experimental data of the kinetic experiments were fitted with the Micromath Scientist.[39]

Synthesis of $DO3AM^{Bu}$. In 100 mL flask, a mixture of $t\text{-}Bu_3DO3A \cdot HBr$ (2.0 g, 3.4 mmol), α-bromoacet-(*n*-butyl)amide (0.80 g, 4.1 mmol), and annealed K_2CO_3 (1.2 g, 8.7 mmol) in dry acetonitrile (50 mL) was stirred overnight at room temperature. Then, the solids were filtered, and the volatiles were evaporated under reduced pressure. The resulting oil was dissolved in a mixture of dichloromethane (10 mL) and trifluoroacetic acid (10 mL). The solution was refluxed overnight. Then, the volatiles were removed under reduced pressure. The resulting oil was purified on a strong cation exchange resin Dowex 50 in H^+-form. After the elution of impurities with water, the macrocyclic compounds were eluted off with 5% aq ammonia. The final purification was performed on a weak cation exchange resin Amberlite CG50 in H^+-form using a gradient water−ethanol (100:0−0:100). The fractions containing a pure product (according to ^{1}H NMR) were combined and purified on a strong

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(39) *Scientist for Windows* version 2.01, Micromath Inc., Salt Like City, UT, 1995.

anion exchange resin Dowex 1 in OH^--form to remove remaining ammonia. After washing with water, the product was eluted off with 50% aq acetic acid. After the repeated evaporation with water, the lyophylization yielded 0.55 g (33%) of the product as $DO3AM^{Bu}\cdot 2H_2O$ (based on elemental analysis). Anal. calcd. for $C_{20}H_{37}N_5O_7\cdot 2H_2O$: C, 48.47; H, 8.34; N, 14.13. Found C, 48.61; H, 8.22; N, 14.33. ^{1}H NMR (D_2O, 80 °C, 300 MHz): δ 1.33 (t, 3H, CH_3-CH_2-, $^3J_{HH}$ = 7.2 Hz), 1.82 (sextet, 2H, CH_3-CH_2-, $^3J_{HH}$ = 7.2 Hz), 2.00 (p, 2H, $-CH_2-CH_2-CH_2-$, $^3J_{HH}$ = 7.2 Hz), 3.56 (m, 8H, ring CH_2), 3.70 (t, 2H, $-CH_2-CH_2-CH_2-N-$, $^3J_{HH}$ = 7.2 Hz), 3.88 (m, 8H, ring CH_2), 3.96 (s, 2H, $-CH_2-CO$), 3.98 (s, 2H, $-CH_2-CO$), and 4.23 (s, 4H, $-CH_2-COOH$).

Sample Preparation. The $Ga(NO_3)_3$ solution was prepared by dissolving 99.99% Ga metal in HNO_3 (the final pH was 1.3). The Ga^{3+} concentration was determined by adding excess of Na_2H_2EDTA solution to the $Ga(NO_3)_3$ solution and titrating back the Na_2H_2EDTA with Pb^{2+} at pH 5.8 in the presence of xylenol orange indicator. Stock solutions of the complexes for the kinetic measurements were prepared by mixing solutions of the ligand and the gallium(III) salt to give a final L:M = 1.05:1 molar ratio. Diluted aq KOH was added slowly in small portions (to avoid precipitate formation) while heating (50−70 °C) until the solution pH remained constant at ~6.5.

Dissociation Kinetics in Acidic Media. The dissociation of the Ga(III) complexes was followed by ^{71}Ga NMR at the complex concentration 10 mM. The increasing amount of $[Ga(H_2O)_6]^{3+}$ (signal around 0.0 ppm) was quantified against an insert tube containing $[Ga(OH)_4]^-$ (signal at 220 ppm) as a solution in 0.1 M aq NaOH. The experiments were carried in HNO_3 (0.13−1.0 M) at 1.0 M ionic strength (K,H)NO_3). For higher (1.0−2.5 M) HNO_3 concentrations, the experiments were carried out without controlling the ionic strength. Selected NMR spectra at different time points are given in Supporting Information (Figure S5).

Dissociation Kinetics in Alkaline Media. The dissociation of the Ga(III) complexes was followed by ^{71}Ga NMR at the complex concentration 5 mM. The increasing amount of $[Ga(OH)_4]^-$ complex (signal at 220 ppm) was quantified against an insert tube containing $[Ga(H_2O)_6]^{3+}$ (signal at 0.0 ppm) as solution in 0.1 M aq HNO_3. The experiments were carried out at pH = 9.7−11.0 (0.2 M CAPS buffer in 1.0 M KCl solution). Selected NMR spectra at different time points are given in Supporting Information (Figure S6).

Potentiometric Measurements. Potentiometry was carried out according to the previously published procedure for preparation of solutions and chemicals, equipments, electrode system calibration, titration procedures and data treatment; see refs 23 and 26. The $Ga(NO_3)_3$ stock solution was acidified with aq HNO_3, and the excess of acids in the stock solution was determined independently by acid−base titration. Throughout the paper, pH means $-\log[H^+]$. Protonation and stability constants of DOTA (protonation constants taken from literature)[16] or $DO3AM^{Bu}$ and their complexes were determined in 0.1 M $(NMe_4)Cl$ at 25.0 °C with pK_w = 13.81. Protonation constants of $DO3AM^{Bu}$ ($\log\beta(H_hL)$ = 12.22(1), 21.11(1), 25.45(1), 27.94(1), and 29.41(1); h = 1−5) were determined from data obtained in pH range 1.6−12 with ~40 points per titration and four parallel titrations (c_L = 0.004 M). The stability constants of the Ga(III) complexes were obtained by the out-of-cell "equilibrium" method. The batches (starting volume 1 mL) were prepared under Ar stream in tubes with ground joints (final pH < 4) or in glass ampules (final pH > 7) from ligand, metal ion, and HCl/$(NMe_4)Cl$ stock solutions and water (Ga:L = 0.95:1 molar ratio, c_L = 0.004 M). Then a known amount of $(NMe_4)OH$ standard solution was added under Ar. The tubes were firmly closed with stoppers and ampules were flame-sealed, and the solutions were equilibrated at room temperature for four weeks (some solutions were checked after six weeks and gave the same data). Titrations were performed in the pH ranges of 1.5−3.8 and 7.5−10.0 (final pH values) with around 20 data points per whole titration and three titrations per system. In the pH range of 4−7, some precipitate of $Ga(OH)_3$ was observed due to a slow complexation and much faster hydroxide precipitation; the precipitate is not dissolving upon standing at room temperature. In the case of the out-of-cell titration, the flame-sealing of the ampules is absolutely essential to avoid any contamination of the alkaline samples with atmospheric CO_2 during equilibration. Application of any other method for samples above pH = 7 (using of just firmly closed vials/tubes or sealed Eppendorf tubes secured with parafilm) resulted in low-quality data. The preformed gallium(III) complexes in solution were obtained by mixing a known amount of the ligand (5% molar excess) with $Ga(NO_3)_3$ (as the defined stock solution) in a glass ampule and a slow portion-wise addition (2 h) of standard $(NMe_4)OH$ solution (4 equiv, just to neutralize the ligand amount) under Ar, and the ampule was flame-sealed and left at 80 °C overnight to fully complex the metal ion. The slow addition of $(NMe_4)OH$ minimalizes the formation of $Ga(OH)_3$ during alkalization, the heating and the ligand excess ensure dissolving of any colloidal hydroxide leading to the quantitative formation of the complex. The ampules were opened under Ar, and aliquots of the solutions of the Ga(III) complexes were transferred into a titration vessel. Water and excess of HCl and $(NMe_4)Cl$ solutions were added (to reach a pH ~1.2 and I = 0.1 M (H,NMe_4)Cl in the final solution, starting volume 5 mL, complex concentration ~0.004 M), and the solution was immediately titrated with a standard $(NMe_4)OH$ solution up to pH ~5 at 25.0 °C acquiring around 70 data points per each of three titrations. The determined constants (with their standard deviations given directly by the program) are shown in Supporting Information (Tables S3 and S4). The titration data were treated with OPIUM[40] program, and the presented chemical models were chosen to have a chemical sence and to exhibit the best fitting statistics.

X-ray Crystallographic Analysis. A 2 mL vial was filled with 10 mM solution of the GaDOTA complex dissolved in 0.1 M aq $NaClO_4$ (1 mL). Another 2 mL vial was filled with 1 M aq $HClO_4$ (1 mL). The vials were connected by a capillary (5 cm, diameter < 0.1 mm) filled with water. A slow diffusion of the solutions through the capillary over period of several weeks yielded colorless plate single crystals suitable for X-ray analysis in the vial containing Ga(III) complex. *Caution! Dry powder perchlorate samples are prone to explosion and should be handled with special care, especially in larger quantities.*

A selected crystal of $[Ga(H_2dota)](ClO_4)\cdot 0.5HClO_4\cdot 5.5H_2O$ was mounted on a glass fiber in a random orientation using a silicone fat. Diffraction data were collected with graphite-monochromatized Mo K_α radiation (λ = 0.71073 Å) on an Enraf−Nonius KappaCCD diffractometer at 150(1) K (Cryostream Cooler, Oxford Cryosystem) and analyzed using the HKL DENZO program package. Cell parameters were determined from all collected data with the same program package.[41] The structure was solved by direct methods and refined by full-matrix least-squares techniques (SIR92,[42] SHELXL97)[43]. The scattering factors used for neutral atoms

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were included in the SHELXL97 program. A semiempirical absorption correction was carried out using SORTAV.[44] The remaining difference electronic maxima were interpreted as water solvate molecules; however, the close contact and low intensity of the maxima strongly imply a huge disorder and a nonfull occupancy of the solvate positions. The best fit revealed 2.75 water molecules distributed over 9 positions in the asymmetric unit. Therefore, the SQUEEZE command was applied using PLATON98[45] to subtract the disordered solvate from the data. Electron count corresponds to 5 water molecules per one complex unit, and the remaining highest electronic maximum was interpreted as a water oxygen atom with 0.25 occupancy, leading to total sum 5.5 of water solvate per one complex molecule, which is consistent with previous model without squeezing. The squeezing improved refinement statistics noticeably (drop of R from 0.0640 to 0.0578). All nonhydrogen atoms were refined with anisotropic thermal parameters, and hydrogen atoms were located in the difference map of electronic density, however, they were placed in idealized (C−H) or original (O−H) positions and allowed to refine riding on the parent atoms using $U(H) = 1.2U(X)$. Experimental data are compiled in Table S5, Supporting Information (CCDC number is 782645).

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Supporting Information Available: Distribution diagrams for the Ga^{3+}−DOTA and Ga^{3+}−DO3AM^{Bu} systems. Pseudofirst-order rate constants of proton- and hydroxide-assisted dissociation. CIF file, the crystal packing scheme and the experimental data for $[Ga(H_2dota)](ClO_4)\cdot 0.5HClO_4\cdot 5.5H_2O$. Changes of ^{71}Ga NMR spectra during the kinetic experiments. The elucidation of the complete rate law for the proton- and hydroxide-assisted decomplexation. Comparison of geometrical parameters of Ga^{3+} complexes with DOTA-like ligands. Comparison of stability constants for complexes of polyaminocarboxylate ligands with trivalent metal ions. Overall stability and protonation constants of the studied complexes with standard deviations. Suggested correction of the published solid-state structure of $[Ga(H_2dota)]Cl\cdot 5H_2O$.[12] This material is available free of charge via the Internet at http://pubs.acs.org.