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Macrocyclic polyaminocarboxylate complexes of lanthanides as magnetic resonance imaging contrast agents

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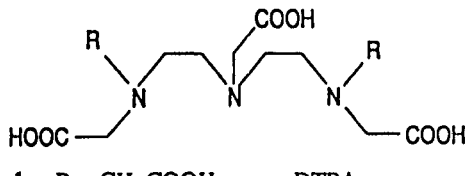
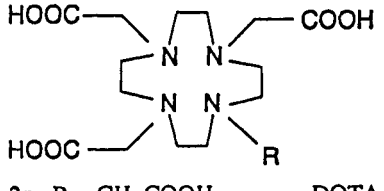
Abstract. Macrocyclic aminocarboxylates form thermodynamically stable and kinetically inert complexes of lanthanides e.g. Gd^{3+}, which are clinically useful as MRI contrast agents. Solubility, relaxivity, thermodynamics and kinetics, crystal structure, and tolerance studies are presented here.

INTRODUCTION

MRI is a diagnostic imaging technique (ref. 1-4) which relies upon detection of spatially localized NMR signals of water protons; the most sensitive and abundant nuclei in vivo. The signal intensity in the image is governed by the values of the T_1 and T_2 relaxation times of the water protons. Paramagnetic metal ions (e.g. Mn^{2+}, Mn^{3+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, and Gd^{3+}) containing one or more unpaired electrons catalyze water proton relaxation by reducing the magnitude of T_1 and T_2 of the bulk water protons (ref. 1-4). Based on its large magnetic moment (S=7/2), and labile Gd-water coordination ($k_{ex} = 9 \times 10^8\ s^{-1}$) gadolinium has been found to be ideal for this use. However, free or unchelated gadolinium is undesirable because: (1) it hydrolyzes under physiological conditions, (2) it precipitates in vivo with inorganic phosphate and carbonate, and (3) it has very poor acute tolerance and its long term toxicity effects are unknown. To overcome these problems multidentate ligands with strong donor atoms were developed. Four Gd^{3+} complexes of linear and macrocyclic polyaminocarboxylates are in clinical use. The ligands used are: DTPA (1a) (diethylenetriaminepentaacetic acid), DTPA-BMA (1b) (bis methyl amide of DTPA), DOTA (2a) (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), and HP-DO3A (2b) (10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid).



1a R = CH_2COOH DTPA
1b R = $CH_2CONHCH_3$ DTPA-BMA



2a R = CH_2COOH DOTA
2b R = $CH_2CH(CH_3)OH$ HP-DO3A
2c R= H DO3A

Desirable properties of these agents are high water solubility, relaxivity, thermodynamic stability, in vivo and in vitro kinetic inertia, efficient formulation, and high tolerance. Low osmolality, low viscosity, and rapid excretion encourage high tolerance of this class of agents. In this lecture review these properties will be discussed.

SYNTHESIS

A general synthetic route to synthesize DO3A and its derivatives has been published (ref. 5). The procedure involves the protection of one nitrogen by forming a novel intermediate, 1,4,7,10-tetrazacyclo- dodecane-1-carboxaldehyde. From DO3A, and its triester, a wide range of R-DO3A derivatives are possible. The procedure is amenable to ton scale production.

SOLUBILITY

The intravenous dose in humans is 0.1-0.3 mmol/kg, or >10g, delivered in some cases as a bolus. High solubility (0.5-1.0 M) of gadolinium based MRI contrast agents is therefore required. Anions, such as **1a** and **2a** are solubilized with cations such as sodium and N-methylglucamine. The neutral molecules are relatively unpredictable as a class. **1b**, **2b**, and **2c** have solubilities >0.5 M.

RELAXIVITY

The catalysis of the relaxation process is governed by a second-order process called relaxivity ($r_{1,2}$). The T_1 relaxivity, r_1, of a paramagnetic metal ion is the sum of the inner- and outer-sphere components. Both components depend on the magnitude of dipole-dipole (through space) interaction between the electron spin on closely associated water molecules. The inner-sphere relaxivity is described by the Solomon-Bloembergen-Morgan (SBM) theory. A highly simplified expression is: $^{20}r_1 = C\, q\, \mu_{eff}^2\, \tau_c / a^6$, where C is a constant, q is the number of coordinated water molecules, μ_{eff} is the effective magnetic moment, a is the internuclear distance between the metal ion and the protons of the coordinated water molecules, and τ_c is a correlation time with at least three components: $\tau_c^{-1} = \tau_r^{-1} + \tau_s^{-1} + \tau_m^{-1}$, where τ_r is the rotational correlation time, τ_s is the electronic correlation time, and τ_m is the lifetime of the coordinated water on the metal ion. Relaxivity is dependent on magnetic field, temperature, and viscosity. The relaxivity of Gd(HP-DO3A) was determined at 20 MHz and 40°C by T_1 measurements of solutions with variable concentrations. The slope of the plot of $1/T_1$ vs [Gd(HP-DO3A) (Figure 1a) gave the value of relaxivity ($^{20}r_1$, $mM^{-1}\, s^{-1}$) as 3.6.

For simple Gd^{3+} complexes, the value of relaxivity at high field is controlled by q and τ_r, as other parameters are similar for these complexes. To estimate the contributions of outer- and inner-sphere relaxivity, a correlation of $^{20}r_1$ with q for some stable gadolinium complexes of linear and macrocyclic polyaminocarboxylates at pH 7.4 (bis-tris buffer) is shown in Figure 1b (ref. 6). The q values were determined by Horrock's method (ref. 7). A least-squares analysis gave an intercept of 2.0±0.3 $mM^{-1}s^{-1}$ (a measure of total outer-sphere relaxivity) and a slope of 1.7±0.1 $mM^{-1}s^{-1}$ (a measure of inner-sphere relaxivity per water molecule). The intercept value of the plot is in excellent agreement with the measured relaxivity of $Gd(TTHA)^{2-}$, which is known to have q ~ 0. Although a good correlation between $^{20}r_1$ and q exists, the thermodynamic stability of the complexes with higher q values is lower. When the pH of the gadolinium complexes is decreased, the value of $^{20}r_1$ and q is increased (ref. 6). This is due to significant dissociation of these complexes at lower pH which gives free Gd^{3+}, with higher relaxivity and hydration. Complexes with q > 1 at pH 7 have yet to be developed for clinical use.

Large molecules have slower tumbling rates; longer τ_r and τ_c. The effect of the size/molecular weight/τ_r on the relaxivity was studied by Weinman and coworkers (ref 8). In this study they synthesized monomers, a dimer, a trimer, a polymer, and conjugates of $Gd(DTPA)^{2-}$ with proteins (q =1). The relaxivity increased with increasing size, but leveled off at 14 $mM^{-1}s^{-1}$. Intramolecular motional contributions were not addressed because the structures of the ligands were not revealed.

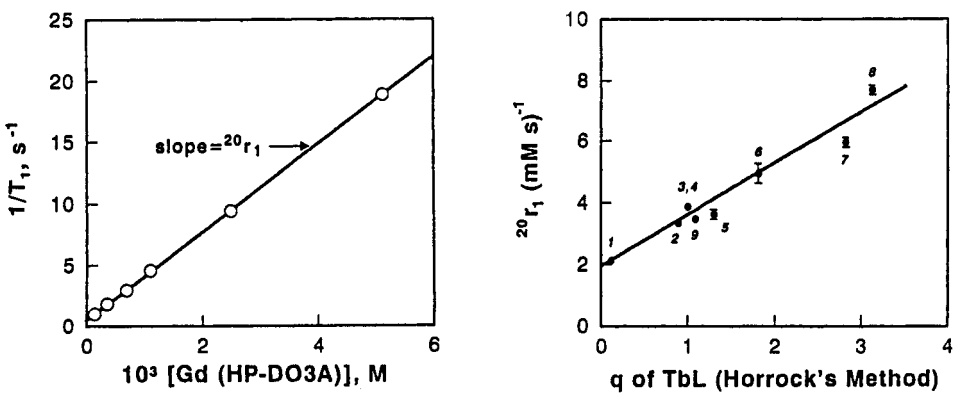


Figure 1. (a) Plot of $1/T_1$ vs. [Gd(HP-DO3A)] for determination of the relaxivity ($^{20}r_1$) at 20 MHz and 40°C. (b) Correlation of relaxivity with number of coordinated water molecules for some Gd(III) complexes.

THERMODYNAMIC AND KINETIC STUDIES

For development of gadolinium based contrast agents, the thermodynamic stability and kinetic inertia are very important because free ligand and metal are poorly tolerated. In vitro thermodynamic and kinetic parameters are also important for optimizing conditions for synthesis of gadolinium chelates, and to understand reactivity and coordination chemistry of lanthanides. Understanding factors such as the charge density on the metal ion, rigidity, internal cavity size, conformation, and preorganization of the free ligand may be important in controlling equilibrium and rates of the formation and dissociation of gadolinium chelates.

The stability constants for Ln complexes of EDTA increase regularly with increasing cationic radius while those of Ln complexes of DOTA and NOTA show little change from La to Sm, then increase sharply from Eu to Tb and show little change through the heavier (>Tb) members of the lanthanide family. This can be interpreted as reflecting the fit of the cavity of the macrocyclic ring to the diameter of Sm-Tb cations in the NOTA and DOTA complexes (ref. 9). While kinetic studies of acid-assisted dissociation of lanthanide complexes of CyDTA showed a strong dependence on the size of lanthanide ion, the complexes of macrocyclic polyoxapolyaminocarboxylate demonstrated an unusual charge density and cavity size dependence of stability and dissociation rates (ref. 10,11). Brucher and Sherry (ref. 12) reported on dissociation kinetics of NOTA complexes of Ce^{3+}, Gd^{3+}, and Er^{3+}finding that rates were slower for heavier lanthanides. NOTA is smaller than the 12-membered macrocyclic polyaminocarboxylates, e.g. DO3A, HP-DO3A, and DOTA, and the effect of charge density or ligand variation on dissociation rates is not known.

Consistent with previous work on linear and macrocyclic polyaminocarboxylates two protonation constants in the basic region were assigned for the protonation of nitrogens in the macrocyclic ring. These are more basic than those of the parent macrocyclic amine due to the presence of additional negative charge on the deprotonated carboxylates (ref. 13). The first protonation constant of the macrocyclic polyaminocarboxylates was lower in NaCl medium than in TMACl medium due to the binding of Na^+. Binding is supported by the crystal structure study on the sodium complex of the t-butyl ester of DOTA (ref 13). Two more protonation constants related to the carboxylate oxygens were observed in the acidic region. To determine the thermodynamic stability and the importance of ligand variation and charge density, the stability constants for Ce^{3+}, Gd^{3+}, and Lu^{3+} complexes of DO3A, HP-DO3A, and DOTA were determined by a competitive spectrophotometric titration method (ref. 9). The calculated thermodynamic stability constants of the complexes are given in Table I. A comparison of the stability constants of the lanthanide complexes of macrocyclic polyaminocarboxylates demonstrates the order DOTA > HP-DO3A > DO3A, which reflects the difference in number and type of donor atoms. To demonstrate preferential ionic binding of lanthanides, an excellent correlation of log K_{GdL} vs. pKa of the neutral form of the ligand was presented (ref. 13) for the complexes which form one or more 5-membered chelate rings (Figure 2a). However, some ligands do not follow the trend: ligands with fewer coordination sites (EDDA, MEDTA ref. 14), size (NOTA ref 11), ligands with mismatched metal ion cavity size (PEPA, HEHA ref 16), and the non-preorganized ligand (TETA ref 16).

For physiologic application, a knowledge of the conditional formation constants of Gd(III) complexes under physiological conditions (pH 7.4) is more valuable than the thermodynamic stability constants. At pH 7.4 there is a significant proton competition depending on the basicity of the ligand. A plot of log K_{GdL} vs. pH is shown for Gd(HP-DO3A) in Figure 2b.

Table I. Thermodynamic Stability Constants and Pseudo-First-Order Rate Constants For Acid-Assisted Dissociation in 0.1 M HCl for Some Lanthanide Complexes of Macrocyclic Polyaminocarboxylates[a].

	log K_{LnL}			10^3k_{obsd}, s^{-1}		
Ligand	Ce	Gd	Lu	Ce	Gd	Lu
DO3A	19.7	21.0	23.0	8.4	2.4	1.56
HP-DO3A	21.2	23.8	23.3	1.97	0.31	not obsd.
DOTA	23.0	25.3	25.5	0.1[b]	8.4×10^{-4}[c]	not obsd

[a] at 25°C and μ = 1.0 (NaCl), [b] E. Brucher, G. Laurenczy, Z. Makara, Inorg. Chim. Acta, 139, 141 (1987), [c] X. Wang, J. Tianzhu, V. Comblin, A. Lopez-Mut, E. Merciny, J. F. Desreux, Inorg. Chem. 31, 1095, (1992).

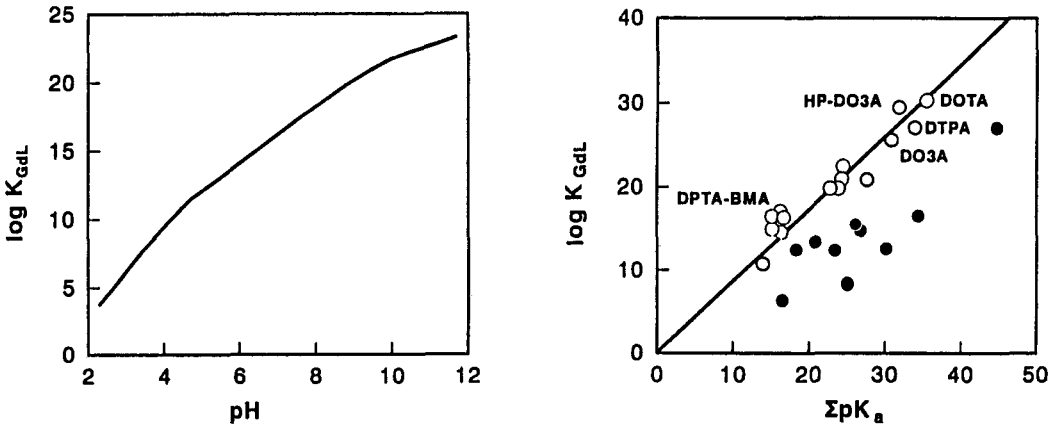


Figure 2 (a) Correlation of log K_{GdL} with ΣpKa of the neutral ligand. (b) Plot of log K_{GdL} vs. pH for HP-DO3A.

The lanthanide complexes of DO3A, HP-DO3A, and DOTA are remarkably inert under physiological conditions. However, in strong acidic conditions (0.01-1.0 M) they form MLH complexes (probably with the proton on oxygen) which dissociate to free metal and ligand (ref 17). A plot of k_{obsd} vs. $[H^+]$ is shown for Gd(HP-DO3A)H in Figure 3a. The saturation kinetic behavior suggests the formation of a diprotonated species, Gd(HP-DO3A)H_2, prior to the rate determining step. Different kinetic behaviors were observed for other lanthanide complexes and a direct comparison of the rate and equilibrium constants is not possible. However, from the resolved values of rate and equilibrium constants one can compare calculated values of k_{obsd}, s^{-1} (in 0.1 M HCl concentration) (Table I). The kinetic inertness of gadolinium complexes follows the order: DOTA>HP-DO3A>DO3A. The effect of charge density on the rate of acid-assisted dissociation and stability constants is shown in the case of DO3A complexes of Ce^{3+}, Gd^{3+}, and Lu^{3+} (Figure 3b). The stability constants (log K) and kinetic inertia (k_{obsd} in 0.1 M HCl) of lanthanide complexes increase with increasing charge density.

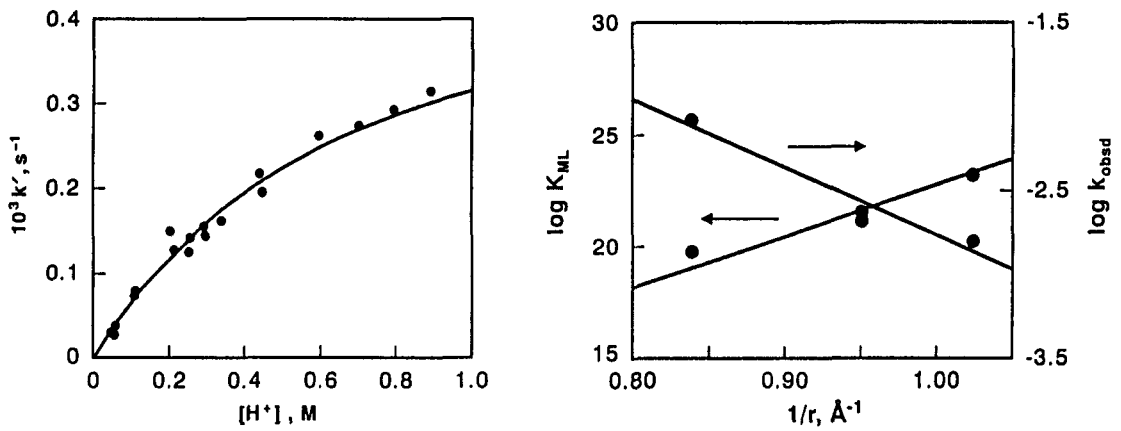


Figure 3. (a) Plot of k_{obsd} vs. $[H^+]$ for acid-assisted dissociation of Gd(HP-DO3A) (b) Plot of log K_{ML} and log k_{obsd} vs. 1/r for lanthanide complexes of DO3A.

Dissociation of the protonated lanthanide complexes seems to follow the mechanism proposed by Brucher and Sherry (ref. 12), and is shown in Figure 4. Depending on the lanthanide ion, ML(H) could convert to $ML(H)^*$ via an acid-independent path in the rate determining step, followed by the rapid formation and dissociation of $ML(H_2)^*$ to the product. The reactant ML(H) could also convert to $ML(H_2)$ by a parallel acid-assisted pathway in the equilibrium step followed by a rate determining conversion to $ML(H_2)^*$, which dissociates rapidly to the product. The second proton in $ML(H_2)$ presumably associates with the second oxygen of the carboxylate. As proposed previously for ML(H) species (ref 12), in the diprotonated species, the metal ion moves out of the

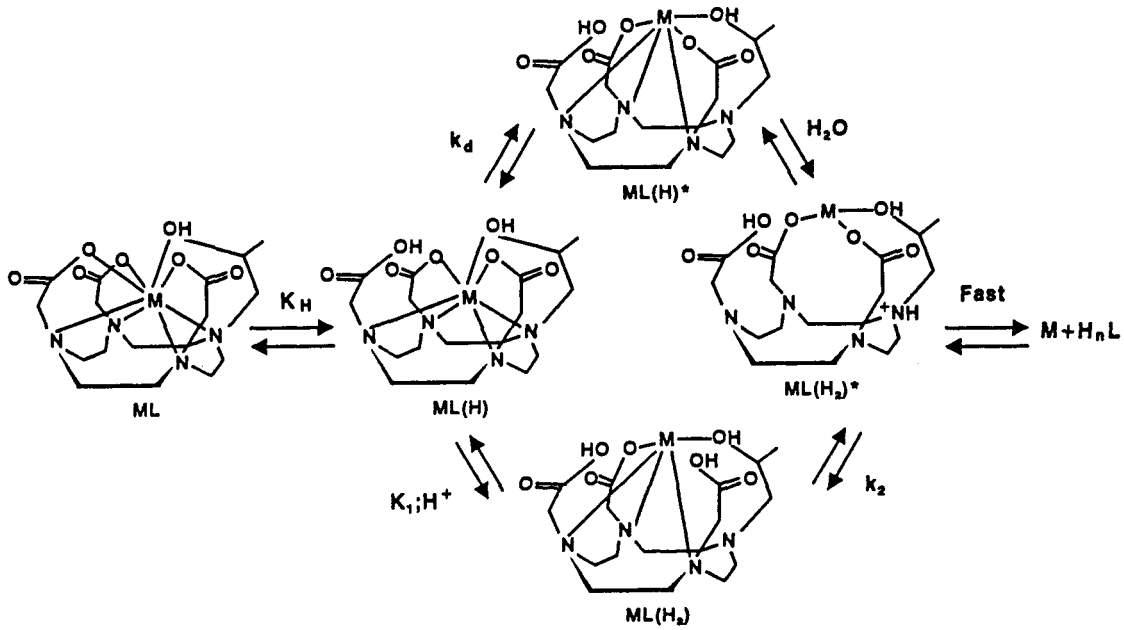


Fig. 4. Proposed mechanism of acid-assisted dissociation of lanthanide complexes of macrocyclic polyaminocarboxylates.

macrocyclic cavity to a position where three oxygens and fewer nitrogens are coordinated. The slow rate-determining step in the dissociation of ML(H) is the acid-independent or acid-dependent rearrangement which includes metal-nitrogen bond breakage and probably nitrogen inversion before protonation. Proton transfer from these two oxygens to the nitrogen results in electrostatic repulsion, and assists removal of the metal ion from the macrocyclic cavity.

The extent of the reaction of endogenously available ions e.g. Cu^{2+}, Zn^{2+}, Ca^{2+}, and Fe^{3+} in the presence of phosphate and carbonate is another way to demonstrate in vitro stability of potential MRI contrast agents. Although in vivo concentration of these metal ions is relatively low, we established our stress test protocols at higher concentration (25 mM). The reactions of Ca^{2+} and Fe^{3+} were not attempted due to very small driving force in the former case and hydrolysis of Fe^{3+} in the later. The reactions of Cu^{2+} and Zn^{2+} with GdL (where L= DTPA, DTPA-BMA, DO3A, HP-DO3A, and DOTA) in the presence of phosphate were studied at room temperature. The percentage of the reaction (metal ions and percentages are given in the parenthesis) after 10 minutes is as follows: $Gd(DTPA)^{2-}$ (Cu 25, Zn 21), Gd(DTPA-BMA) (Cu 35, Zn 25) , Gd(DO3A) (Cu <1, Zn <1), Gd(HP-DO3A) (Cu <1, Zn <1) , and $Gd(DOTA)^{-}$ (Cu <1, Zn <1) (ref. 18).

In vivo dissociation of gadolinium chelates was measured by detecting the residual gadolinium in mice after 14 days upon injection of radiolabeled gadolinium chelates (ref. 19). Significant amounts of residual gadolinium were found in the cases of $Gd(EDTA)^{-}$, Gd(NP-DO3A), Gd(DTPA-BMA), Gd(DO3A), and to a lesser extent for $Gd(DTPA)^{2-}$. The macrocyclic, $Gd(DOTA)^{-}$ and Gd(HP-DO3A), had much lower residual gadolinium. The amount of residual gadolinium at 14 d did not correlate well with log K' (conditional stability constant at pH 7.4). However data did parallel in vitro dissociation rates of these complexes in 0.1 M HCl (ref. 19).

CRYSTAL STRUCTURE STUDIES

The macrocyclic ligand, HP-DO3A in Gd(HP-DO3A) adopts a preorganized quadrangular [3333] conformation in which the 4N and 4 O atoms are coordinated to the embedded Gd(III) atom (ref. 13) which is consistent with previous work (ref. 20-22). The ninth coordination site is occupied by a water molecule. Nitrogens and oxygens of the molecule define two different planes, which are nearly parallel, and the metal is sandwiched between these two planes. Gd(III) is 1.65 A above the nitrogen plane and 0.76 A below the oxygen plane. The coordination polyhedron is defined in terms of a distorted capped square antiprism. In accordance with the preferred ionic binding of lanthanides M-O bonds are shorter (2.34 A) than M-N bonds (2.64 A).

TOLERANCE STUDIES

MRI contrast agents (0.5 M solution) are administered via the intravenous route. Administration of a hyperosmolar drug relative to blood (Osmolality =0.3 Osmol/kg) increases their potential to cause adverse reactions such as pain. Similarly a highly viscous drug is more difficult to inject. The osmolality and viscosity of ionic compounds is high. The values of osmolality and viscositiy of 0.5M formulations of non ionic chelates e.g Gd(HP-DO3A) and Gd(DTPA-BMA) are 35-70% lower than ionic chelates, e.g. $Gd(DOTA)^-$ and $Gd(DTPA)^{2-}$.

SUMMARY

(1) The relaxivity ($^{20}r_1$) of Gd(III) chelates of linear and macrocyclic polyaminocarboxylates increases with increasing q and the size of the molecule.
(2) Gadolinium chelates with well matched macrocyclic cavity and size of the metal ion are the most dissociation inert in vitro and in vivo.
(3) A crystal structure study of Gd(HP-DO3A) shows two planes of oxygens and nitrogens, with gadolinium sandwiched between these two planes.
(4) Non ionic Gd(III) chelates have lower osmolality and viscosity and are well tolerated.

Acknowledgement

We thank our collaborators at Bristol-Myers Squibb whose preliminary and published results are included in this review.

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1. ***Pure & Appl. Chem.*, Vol. 65, No. 3, pp. 515-520, 1993**

The corrected Figure 2, given hereunder, may please be pasted over the incorrect one on page 518.

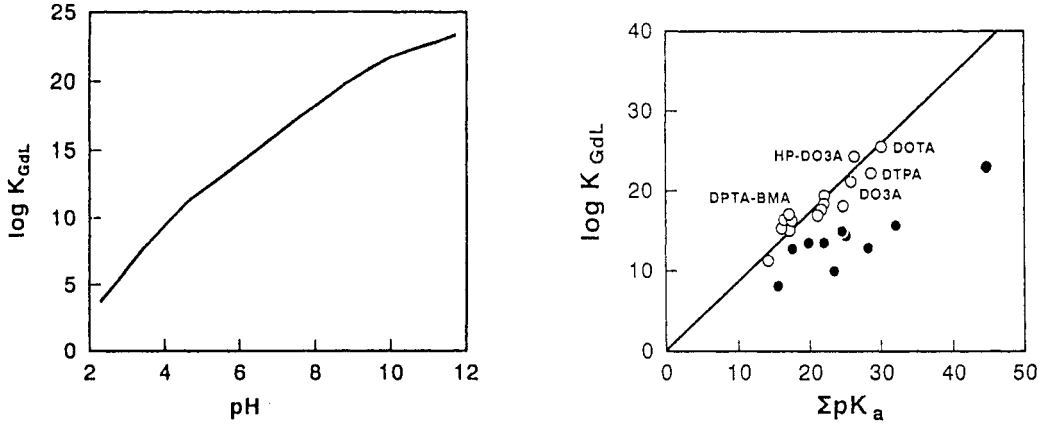

Figure 2 (a) Correlation of log K_{GdL} with ΣpKa of the neutral ligand. (b) Plot of log K_{GdL} vs. pH for HP-DO3A.

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2. ***Pure & Appl. Chem.*, Vol. 65, No. 8, pp. 1745-1750, 1993**

The corrected pages 1748 and 1749, which follow, may please be pasted over the incorrect ones.