Magnetic Resonance Imaging

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High-Relaxivity MRI Contrast Agents: Where Coordination Chemistry Meets Medical Imaging

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contrast agents · lanthanides · ligand design · magnetic resonance imaging · O ligands

The desire to improve and expand the scope of clinical magnetic resonance imaging (MRI) has prompted the search for contrast agents of higher efficiency. The development of better agents requires consideration of the fundamental coordination chemistry of the gadolinium(III) ion and the parameters that affect its efficacy as a proton relaxation agent. In optimizing each parameter, other practical issues, such as solubility and in vivo toxicity, must also be addressed, making the attainment of safe, high-relaxivity agents a challenging goal. This Minireview presents recent advances in the field, with an emphasis on gadolinium(III) hydroxypyridinone chelate complexes.

1. Introduction

Magnetic resonance imaging (MRI) has become an important technique in modern diagnostic medicine, providing high-quality three-dimensional images of soft tissue without the need for harmful ionizing radiation.[1] Signal intensity in MRI is related to the relaxation rate of in vivo water protons and can be enhanced by the administration of a contrast agent prior to scanning. These agents utilize paramagnetic metal ions and are evaluated on the basis of their ability to increase the relaxation rate of nearby water proton spins in dependence on the concentration of agent administered (i.e. relaxivity). Gadolinium(III), with its high magnetic moment and long electron spin relaxation time, is an ideal candidate for such a proton relaxation agent and is the most widely used metal center for such purposes.[2,3] Free Gd^{III} is toxic ($LD_{50} = 0.2$ mmol kg^{-1} in mice)[4] and must therefore be administered in the form of stable chelate complexes that will prevent the release of the metal ion in vivo. For these reasons, the development of ligands that are suitable for production of high-relaxivity agents with favorable properties for imaging applications remains an important goal.

This Minireview provides a brief summary of changes in the contrast-agent field with an emphasis on the hydroxypyridinone (hopo) class of compounds developed by our research group. Principles governing contrast-agent efficacy will be discussed with regard to the underlying coordination chemistry of the Gd^{III} ion. For a more detailed account of the theory, the reader is referred to several reviews on the subject.[1–3,5] While this is not intended to be a comprehensive report, several recent attempts to improve agent efficiency through structural modification of the commercially used aminocarboxylate ligands are also presented.

1.1. MRI Contrast Agents

Paramagnetic contrast agents enhance the contrast in an MR image by positively influencing the relaxation rates of water protons in the immediate surroundings of the tissue in which they localize.[2,6] The first experiments to demonstrate the feasibility of such a concept employed manganese(II) salts and achieved tissue discrimination in animal studies.[7,8] Since these early reports, Gd^{III} has become the most widely used metal center for the production of paramagnetic contrast agents. The seven unpaired electrons in Gd^{III} combined with a relatively long electronic relaxation time make this lanthanide an effective proton relaxation agent. Gd^{III} was utilized in the first approved contrast agent in 1988, and while other systems based on iron oxide particles and manganese(II) have been approved, gadolinium-based agents are by far the most commonly used agents in the clinic.[2,5] It is worth noting that while contrast agents containing Gd^{III} increase both the longitudinal and transverse relaxation rates, the percentage

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change in tissue is much greater for the longitudinal rate ($1/T_1$). As a result, such agents are best visualized with T_1-weighted scans.[2]

The most commonly used commercial contrast agents (Figure 1) utilize polyaminocarboxylate ligands, which incorporate nitrogen and oxygen donor atoms to coordinate the Gd^{III} center. The first six complexes shown in Figure 1 act as nonspecific extracellular agents. Following intravascular injection, these compounds distribute rapidly between plasma and interstitial spaces and are ultimately eliminated through the renal route with half-lives of about 1.6 h.[9,10] The remaining three dtpa derivatives, $[Gd(eob\text{-}dtpa)(H_2O)]^{2-}$, MS-325, and $[Gd(bopta)(H_2O)]^{2-}$, are designed specifically as targeted agents. The bopta complex MultiHance is known to target the hepatobiliary system and acts as a liver imaging agent,[2,11,12] while MS-325 interacts noncovalently with the abundant blood protein human serum albumin (HSA). Once bound to HSA, the proton relaxation efficiency of MS-325 increases, and the longer in vivo retention times present opportunities for MR angiography.[13–15] Common to all the aminocarboxylate-based commercial agents is the octadentate ligand motif, a chelate design that leaves only one open coordination site for an inner-sphere water molecule. This low hydration number ($q = 1$) limits the potential effectiveness of these complexes as relaxation agents (see Section 1.2).

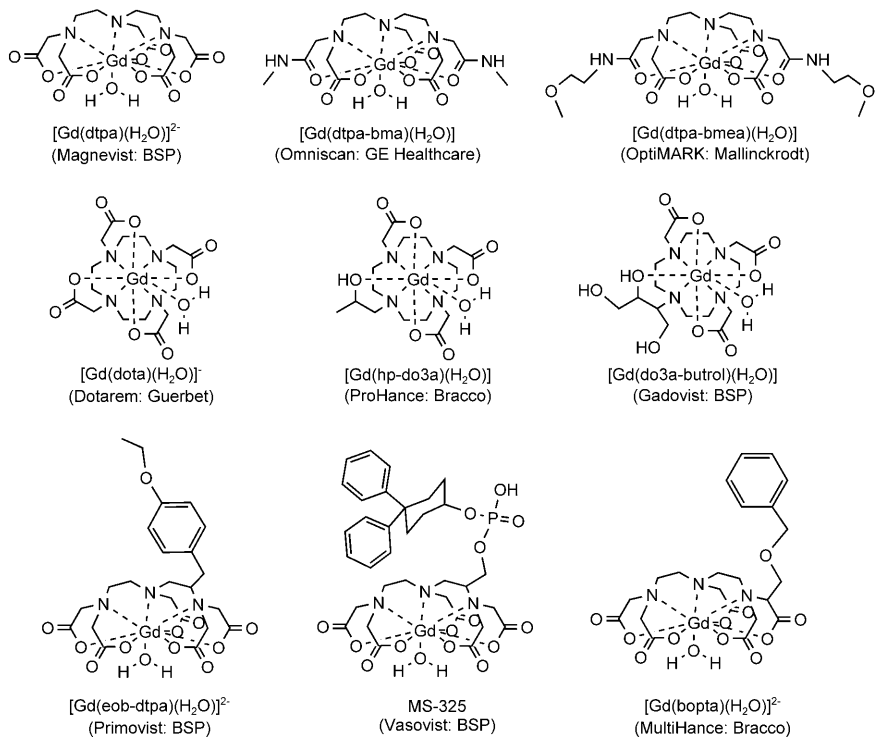

Figure 1. Commercial aminocarboxylate-based MRI contrast agents (BSP = Bayer Schering Pharma AG).

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Ankona Datta grew up in Kharagpur, India. She received her B.Sc. and M.Sc. degrees in chemistry from the Indian Institute of Technology, Kharagpur, India in 2000. She did her graduate work on chiral water-soluble porphyrins for catalysis and recognition with Prof. John T. Groves at Princeton University (Ph.D., 2006). Since then she has been a postdoctoral scholar in Prof. K. N. Raymond's group at the University of California, Berkeley, working on macromolecular MRI contrast agents.

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Professor Kenneth N. Raymond was born in 1942 in Astoria, Oregon. He attended Reed College, where he received a B.A. in 1964. Following his Ph.D. from Northwestern University, he began his faculty appointment at the University of California at Berkeley in 1967. There he has remained, becoming Associate Professor in 1974 and Professor in 1978. He was appointed Chancellor's Professor in 2006.

1.2. Relaxivity and Solomon–Bloembergen–Morgan Theory

Contrast agents are evaluated on the basis of their relaxivity, or how much the relaxation rates of water protons are increased in the presence of the agent at a given concentration. The observed relaxation rate of solvent protons, in this case water protons, is comprised of both diamagnetic and paramagnetic contributions. The paramagnetic contribution is linearly related to the concentration of paramagnetic species present. Relaxivity (r_i, $i=1,2$) is then defined as the concentration-dependent increase in relaxation rate of the paramagnetic agent, or the slope of a plot of $(1/T_i)_{obs}$ versus concentration [Eq. (1)].

$$(1/T_i)_{obs} = (1/T_i)_{dia} + r_i\,[Gd] \quad (1)$$

Paramagnetic relaxation enhancement includes both an inner-sphere component from the proton relaxation of a solvent molecule directly coordinated to the Gd^{III} ion and an outer-sphere component from solvent in the second coordination sphere and the bulk solvent. Current agent design focuses mainly on attaining higher inner-sphere, longitudinal relaxivity r_{1p} from protons of water molecules in the first coordination sphere of the metal. Equation (2) reveals that if water exchange at the Gd^{III} center is fast enough (small values of τ_M, the mean water residence time), the paramagnetic relaxation enhancement experienced by the bulk solvent will come from the relaxation rate $(1/T_{1m})$ increase of the coordinated solvent molecule.

$$(1/T_1) = q\,P_m[1/(T_{1m}+\tau_M)] \quad (2)$$

In Equation (2), $1/T_1$ is the longitudinal relaxation rate, q is the number of bound solvent molecules, and P_m is the mole fraction of water coordinated to the metal center. According to the Solomon-Bloembergen-Morgan (SBM) equations of paramagnetic relaxation theory,[16–20] T_{1m} for the applicable dipole–dipole relaxation mechanism is defined by Equation (3). This equation shows that modulation of the correlation time τ_c [Eq. (4); $i=1,2$] becomes critical if the high relaxivities predicted by theory are to be obtained.[2]

$$\frac{1}{T_{1m}^{DD}} = \frac{2}{15}\,\frac{\gamma^2 g^2 S(S+1)\mu_B^2}{r_{Gd\text{-}H}^6}\left[\frac{3\tau_{C1}}{1+\omega_H^2\tau_{C1}^2} + \frac{7\tau_{C2}}{1+\omega_S^2\tau_{C2}^2}\right] \quad (3)$$

$$1/\tau_{ci} = 1/\tau_R + 1/T_{ie} + 1/\tau_M \quad (4)$$

The relaxivities of current commercial agents based on polyaminocarboxylate scaffolds are small compared to what is theoretically possible, with r_{1p} values of only 4–5 $mM^{-1}s^{-1}$.[2,21] As shown by Equations (2)–(4), theory demonstrates the need to maximize the hydration number q ($q=1$ for all commercial agents) and optimize τ_M (150–1000 ns in commercial agents), the rotational correlation time τ_R (in the picosecond regime for small molecules), and the electronic relaxation times T_{ie} to obtain high relaxivity. These parameters are illustrated pictorially in Figure 2, and their optimization can result in a dramatic increase in relaxivity. At 20 MHz, the relaxivity for a $q=3$ complex can theoretically reach values above 300 $mM^{-1}s^{-1}$, representing a 60-fold increase over the relaxivities of current commercial agents. However, such high relaxivities can only be attained if all relevant parameters are optimized. In particular, optimal values of about 1–30 ns for τ_M (optimal value decreases with increasing magnetic field strength) and nanosecond values of τ_R are required to reach the peak in the relaxivity profile. It is therefore necessary to increase water exchange rates and to slow down molecular tumbling relative to commercial agents while also maintaining long electronic relaxation times with a high number of inner-sphere water molecules to achieve the high relaxivities predicted by theory. While attaining a more favorable combination of these parameters relative to current agents is desirable, it must come without sacrificing chelate stability, so that toxicity arising from free Gd^{III} is avoided. This clearly presents a challenging problem for the coordination chemist!

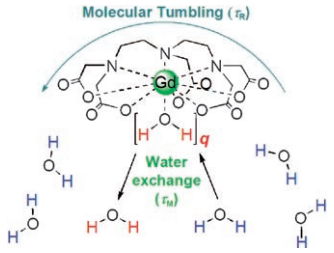

Figure 2. Selected key factors that affect proton relaxivity r_{1p}.

1.3. Designing Gd^{III}-Based Imaging Agents: A Coordination Chemistry Problem

In addition to the favorable electronic properties mentioned above, the general coordination chemistry of the Gd^{III} ion lends itself to its application as a relaxation agent; fast water exchange rates are crucial for attaining high relaxivity, and the ionic radius of Gd^{III} is ideal for fast exchange. Owing to lanthanide contraction,[22–25] lanthanide sizes decrease across the 4f row of the periodic table, resulting in higher coordination numbers for the early lanthanides and smaller coordination numbers for those toward the end of the series. Since the Gd^{III} ion is situated in the middle of the row, a low energy barrier exists between the eight- and nine-coordinate states, favoring fluctuation between the two. However, the rate of water exchange of Gd^{III}, once complexed, is slowed significantly relative to that of the free ion, often to the extent that it is no longer in the optimal range for high relaxivity. Moreover, there is a significant decrease in the number of inner-sphere water molecules as they are replaced by ligating atoms in a chelating ligand.

Related to water exchange, an important trend to consider when designing new contrast agents is relaxation dispersion: the inherent decrease in proton relaxation rates with increasing magnetic field strength.[1,26,27] With the appearance in clinics of new high-field scanners (100 MHz and above) that give better signal-to-noise ratios, this effect becomes significant. Thus, short water residence times (or fast water exchange rates) become increasingly important at high field, with the optimal value for τ_M decreasing to about 1 ns for

2.4 T scanners (100 MHz proton Larmor frequency). To attain high relaxivities at high fields, the coordination chemistry challenge therefore involves the design of ligands that effectively chelate Gd^{III} while limiting the decrease in the water exchange rate and reduction in q once the ion is bound.

2. Recent Strategies in Contrast-Agent Design

The primary developmental focus of next-generation MRI contrast agents has been the synthesis of derivatives of the aminocarboxylate systems used in the clinic. Features of compounds based on ligands such as dtpa and dota include inexpensive streamlined syntheses as well as adequate solubility and toxicological parameters.[1,28,29] The following examples illustrate several approaches toward optimizing the aminocarboxylate system en route to more efficient relaxation agents.

Research efforts in new contrast-agent design are generally directed towards the optimization of one or more of the aforementioned relaxation parameters through ligand structural modification. For example, Merbach and co-workers have reported numerous studies that probe the factors influencing the water exchange rate of aminocarboxylate Gd^{III} complexes.[30–34] The main explanation for increased water exchange rates is steric crowding at the water binding sites, a property that favors the release of the coordinated water molecule in a dissociative exchange process. Derivatives of dtpa, shown in Figure 3, have been synthesized with varying numbers of carbon atoms in the ligand scaffold. As shown by the τ_M values, water exchange is accelerated in the resultant Gd^{III} complexes, with values approaching the optimal range for high relaxivity at higher magnetic fields (60–100 MHz). This rate enhancement is achieved, however, at the cost of thermodynamic stability, as measured by the relatively low pGd[35] values (Figure 3). This phenomenon of decreased stability upon increasing water exchange rates is common for aminocarboxylate ligands and must be addressed when considering these complexes as high-relaxivity agents, particularly at the high magnetic field strengths of future clinical scanners.

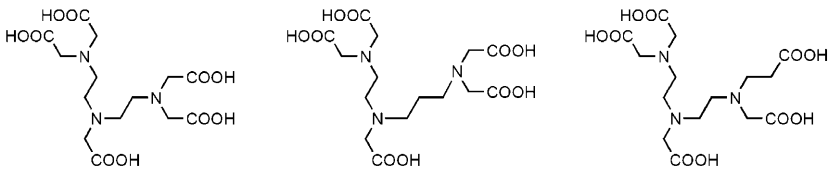

Figure 3. The dtpa ligand and two of its derivatives prepared for water exchange rate studies.[31,32]

Increased water exchange rates for macrocyclic complexes based on $[Gd(dota)(H_2O)]^-$ (Figure 1) have also been reported. The pyridine-*N*-oxide derivative of dota (Figure 4) resulted in a gadolinium complex with a significantly faster water exchange rate than the parent complex ($\tau_M = 39$ vs. 244 ns.[36]). As with the linear aminocarboxylates mentioned above, the rate acceleration was attributed to an increase in steric crowding. A monophosphinic acid derivative (Figure 4) was found to possess an even faster exchange rate, with $\tau_M =$ 16 ns.[37] Steric crowding owing to the bulky phosphinate group is given as a rationale for the increased rate, as well as a possible favorable arrangement of water molecules in a second coordination sphere. The relaxivity of this complex is 6 $mM^{-1}s^{-1}$ (20 MHz, 25 °C), an improvement over commercial agents.

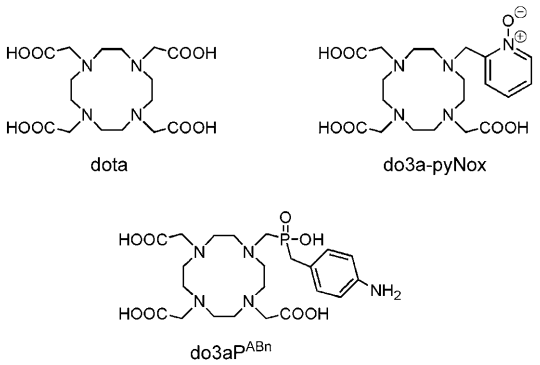

Figure 4. The dota ligand and derivatives prepared to increase water exchange rates.[36,37]

Complexes with $q > 1$ have also been reported for aminocarboxylate systems in efforts to achieve higher relaxivity. As indicated by Equation (2), relaxivity is highly dependent on this parameter, and relaxivity values will always be limited for complexes that possess only one coordinated water molecule ($q = 1$). Two examples of $q = 2$ complexes are depicted in Figure 5. In each case, dtpa complexes are tethered to a central core to produce dinuclear Gd^{III} complexes with increased hydration numbers ($q =$ 2). The relaxivity values of $[Gd_2\{pX\text{-}(dtta)_2\}(H_2O)_4]^{2-}$ and $[Gd_2\{mX(dtta)_2\}\text{-}(H_2O)_4]^{2-}$ are 12.8 and 11.6 $mM^{-1}s^{-1}$ (20 MHz, 37 °C), respectively, and represent significant increases over that of the parent dtpa complex ($r_{1p} = 4.3$ $mM^{-1}s^{-1}$).[2] These values are influenced by the higher q value and (owing to increased molecular weight) by an increase in the rotational correlation time τ_R.[38] A supramolecular approach was used to generate another dinuclear $q = 2$ complex via iron terpyridine complexes derivatized with dtpa (Figure 5). The high relaxivity of $[Fe(tpy\text{-}dtta)_2Gd_2(H_2O)_4]$ (15.7 $mM^{-1}s^{-1}$, 20 MHz, 37 °C) is attributed to an increase in q as well as a long τ_R value resulting from the higher molecular weight and rigidity of the complex.[39]

While the relaxometric properties of such $q = 2$ compounds are improved, the thermodynamic chelate stability suffers greatly in both cases. The increase in q (Figure 5) from one to two is made possible by removing one carboxylate arm

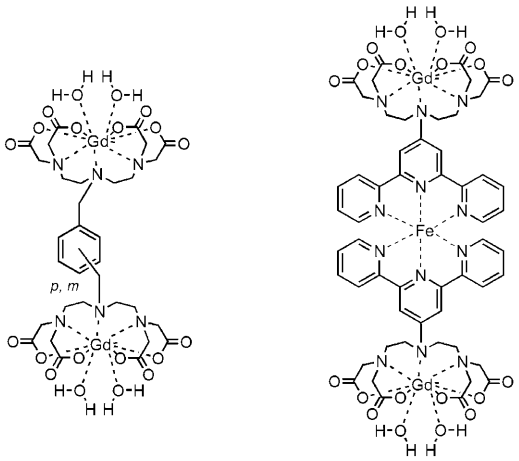

Figure 5. Examples of dinuclear, $q = 2$ dtpa-based Gd^{III} complexes proposed as improved high-relaxivity MRI contrast agents.[38,39]

of the parent dtpa to open up a metal coordination site. As is observed in accelerating water exchange in the aminocarboxylate class of compounds, attaining a higher number of coordinated water molecules by decreasing ligand denticity is accompanied by a dramatic decrease in thermodynamic stability. The pGd values for the *para*- and *meta*-substituted xylene-based complexes (16.2 and 15.1, respectively) are significantly lower than the value of 19.1 for the parent dtpa complex.[28] Even less stable is the iron terpyridine complex; its pGd value of 10.6 represents a decrease by more than five orders of magnitude in stability relative to the dtpa-bma complex (pGd = 15.8, Figure 1), the ligand with the lowest pGd value of all clinically approved agents. This effect of decreased stability resulting from increased q or water exchange rates raises a key concern in contrast agent design: The optimization of one parameter will often hamper that of another, making the goal of high-relaxivity, practical agents a major challenge. To achieve a practical high-relaxivity agent, the optimal combination of all relevant parameters must be accomplished while maintaining solubility and chelate stability.

As an example of a gadolinium-based contrast agent not focused on traditional aminocarboxylate ligand scaffolds, Wilson and co-workers have reported several studies of Gd^{III} encapsulated inside fullerene cages.[40–42] The peripheries of these cages are decorated with solubilizing groups to allow for application of C_{60} in aqueous media. Relaxivities ranging from about 10 to as high as 38.5 $mM^{-1}s^{-1}$ (30 MHz, 26 °C) are due entirely to second and outer-sphere relaxation, as there are no inner-sphere water molecules directly coordinated to the gadolinium ion. In solution, these "gadofullerenes" aggregate, resulting in large assemblies with long rotational correlation times and consequent high relaxivities.[42] However, practical concerns such as in vivo toxicity and deaggregation in the presence of various salts (thereby limiting the effect of long τ_R values on relaxivity)[43] may preclude considering such systems for contrast-agent applications.

3. Hydroxypyridinone-Based Agents

In 1995, Raymond and co-workers reported a Gd^{III} complex **1** that showed promise as a contrast agent (Figure 6).[44] The X-ray crystal structure revealed that the tris(2-aminoethyl)amine (tren)-capped tripodal hydroxypyridinone (hopo) ligand is hexadentate, leaving two open water coordination sites in its overall eight-coordinate complex. The r_{1p} value of this complex, 10.5 $mM^{-1}s^{-1}$ (20 MHz, 37 °C), is more than twice that of commercial agents. This observed increase is due in large part to the value $q = 2$ (vs. $q = 1$ for current commercial agents) combined with a rapid water exchange rate. Even more important and unlike that of previous $q = 2$ complexes, the stability of this complex is higher than that of commercial agents, despite the lower ligand denticity (pGd = 19.2[45]). This effect can be attributed in part to the oxygen-only donor set provided by the hopo chelates (instead of the mixed oxygen/nitrogen donors of the aminocarboxylates), since lanthanide cations prefer hard, anionic oxygen donors to nitrogen.

Since this initial report, a family of hopo-based gadolinium complexes has been developed to explore the potential of this motif in MRI contrast agents.[46] Early studies were hindered by the low aqueous solubility of the parent complex **1**,[47,48] which led to subsequent efforts to improve this important parameter. The replacement of one hopo unit with a terephthalamide (tam) chelator created the negatively charged Gd^{III} complex **2** (Figure 6).[47] A key feature of this ligand design is the second amide functionality of the tam unit, which allows for further derivatization with solubilizing and targeting groups.[49,50] Relaxivities of $Gd(hopo)_3$ and $Gd(hopo)_2(tam)$ complexes are generally in the range of 7–13 $mM^{-1}s^{-1}$ (20 MHz), and high complex stabilities combined with increased q and optimal water exchange rates make these compounds promising as safe, high-relaxivity agents at high field.

The coordination chemistry and relaxometric properties of this class of compounds will be described in the following sections with emphasis on recent work published subsequent to our previous review.[46] Furthermore, a detailed summary of the solution thermodynamic stability and selectivity data collected to date is presented for this class of potential MRI contrast agents.

3.1. Solution Thermodynamics

High stability is essential for gadolinium complexes used in medicine because of toxicity related to the presence of free Gd^{III} in vivo. For example, the metal can precipitate in tissue, and hydrated lanthanide ions are known to block Ca^{2+} binding sites.[28] When other potential interactions with free Gd^{III} with various serum proteins as well as irreversible binding to skeletal tissue are considered,[2] the importance of the chelate staying intact while in the body becomes clear. The known oxophilicity of lanthanides has been exploited to develop oxygen-only donor ligands such as hydroxypyridinone (both the 3,2-hopo and 1,2-hopo isomers), maltol (mam), and terephthalamide (tam) as chelators expected to

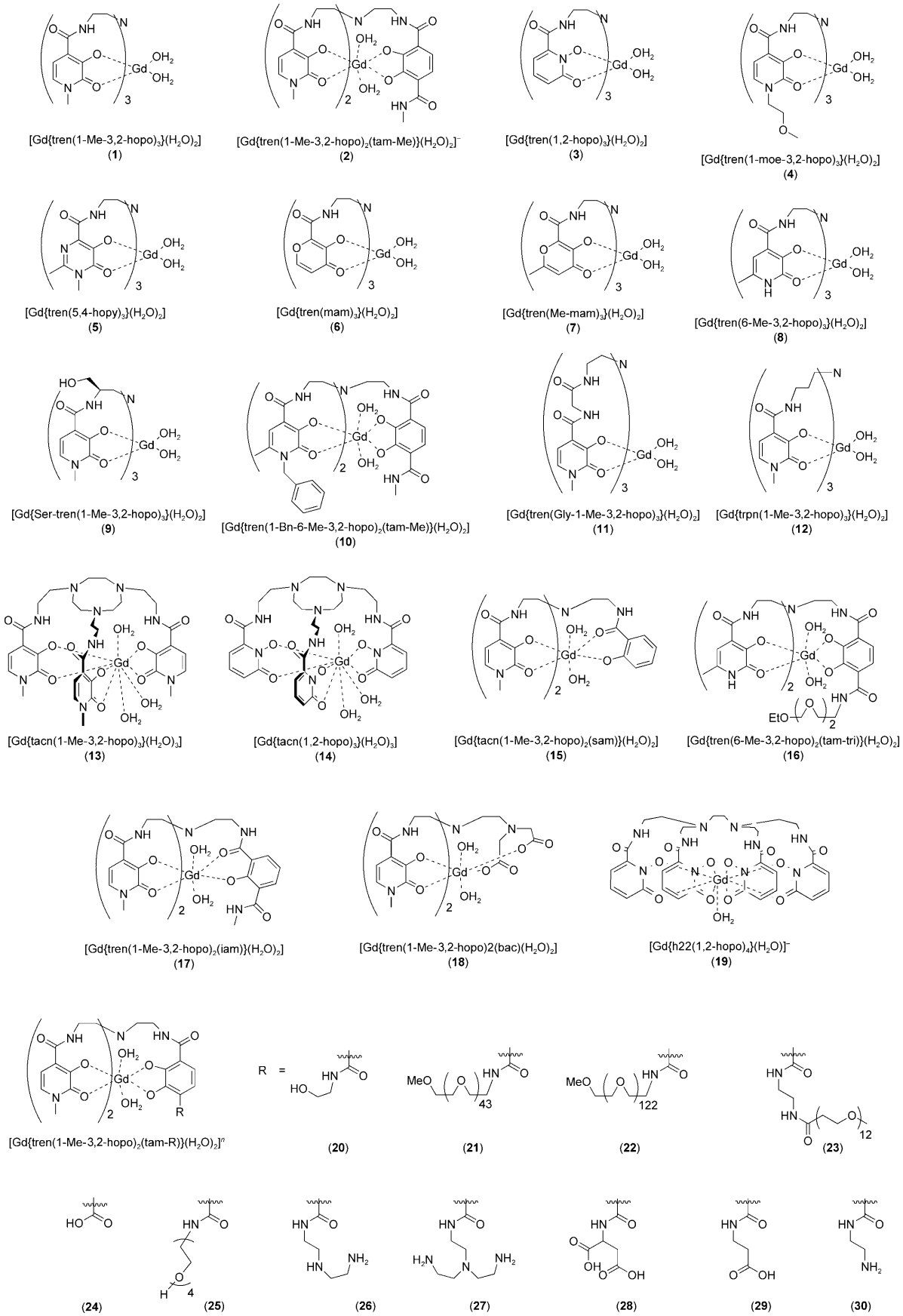

Figure 6. Chemical structures of hopo-based Gd^{III} complexes. For **20**–**30**, $n = +1$ (**27**), 0 (**26**), −1 (**20**–**23**, **25**, **30**), −2 (**24**, **29**), −3 (**28**).

form stable gadolinium complexes. The obtained complexes (with two[46] or three[51] inner-sphere water molecules) have been examined as candidates for next-generation contrast agents. The feasibility of applying these O-donor ligands as practical agents has been demonstrated successfully in vivo.[52] Scaffold and chelate-group variations were also examined to help understand the principles governing stability aspects. The results obtained from these solution thermodynamic studies are summarized in the following sections.

Stability Constants

Thermodynamic stability data of hopo-based chelates have been published. Typically, stabilities of MRI contrast agents are reported as their pGd values, thus providing a convenient way to compare stabilities of chelates with differing protonation behavior.[2,53] These pGd values range from 13.7 to 20.6 (see Table 1) for hexadentate ligands, and

Table 1: Ligand acidity, Gd^{III} binding constants $\log\beta_{110}$ (in 0.1 M KCl at 298 K) and pGd values of various hopo-based chelate systems (see Figure 6).

ΣpK_a	$\log\beta_{110}$	pGd[b]	Compound	Reference
20.70	18.5	19.3	**3**	[69]
21.90[a]	18.5[a]	19.3[a]	**6**	[63]
24.15[a]	18.7[a]	19.0[a]	**7**	[63]
25.69	19.7	19.8	**4**	[48]
25.96	19.2	19.2	**1**	[45]
27.57	18.2	18.0	**5**	[67]
27.61	20.3	19.5	**8**	[45]
37.34	24.1	20.1	**2**	[47]
38.05	24.3	20.3	**20**	[66]
38.64	24.9	20.6	**16**	[62]
24.52	15.9	16.7	**11**	[58]
24.77	17.2	17.7	**9**	[59]
26.59	16.5	16.2	**17**	[47]
26.59	14.8	13.7	**18**	[47]
27.53	16.5	15.6	**12**	[58]
28.13	17.3	16.1	**15**	[47]
37.11	21.5	21.2	**19**	[65]

[a] 0.1 N NaCl. [b] pH 7.4; total concentration of metal: 1 μM; total concentration of ligand: 10 μM.

one octadentate ligand achieves a pGd of 21.2. For comparison, the benchmark compounds dtpa and dota reach 19.1[2,28] and 20.4,[54] respectively, while dtpa-bma has the lowest value of all approved agents at 15.8.

The favorable thermodynamic properties of hopo ligands can be attributed to several effects. First, the Gd^{III} cation is highly oxophilic and will bind more strongly to the six oxygen donors of the hopo-based ligands than to the mixture of nitrogen and oxygen donors offered by hexadentate aminocarboxylate ligands. Second, the two donor atoms on each hopo moiety are predisposed to bind Gd^{III} in a five-membered chelate ring. Such an arrangement of donor atoms is expected to favor larger cations such as Ca^{II} or Gd^{III} over smaller cations such as Zn^{II} and Cu^{II}.[55–57] The final important effect is that the Lewis basicities of the hopo oxygen donor atoms are an optimal match for Gd^{III}, resulting in strong binding.[45,55,56]

Scaffold

A significant factor in chelate stability is the ligand scaffold. The tren scaffold has been found to provide the highest chelate stability for hexadentate hopo ligands. For example, in a series of 1-Me-3,2-hopo ligands, replacing tren[45] with the propylene-bridged cap trpn (as in **12**),[58] extending tren by insertion of a glycine spacer into each of the ligand arms (as in **11**),[58] or variation towards a more sterically crowded serine-funcionalized tren cap (as in **9**)[59] reduces pGd from 19.2 to 16.7, 15.6, and 17.7, respectively. Complex **12** demonstrates the importance of an intramolecular hydrogen-bonding network that preorganizes the ligand for metal complexation in the tren-capped complexes such as **1**.[44,60,61] The extension of the spacer in trpn disrupts these interactions, resulting in a lower pGd value. While most deviations from the tren cap have resulted in significantly decreased stability, an exception is the tacn (triazacyclononane) scaffold (e.g. in **13**), which attains pGd = 18.7.[51]

Acidity

Variations in the chelate groups influence pGd values only to a minor extent in the examined tren-capped hexadentate, homopodal ligands. Complexes **1**, **3**, **4**, **6**, and **8** all have similar pGd values (19.2–19.8) that slightly exceed the benchmark compound $[Gd(dtpa)(H_2O)]^{2-}$ (19.1). The only notable exception is **5**, with a lower pGd of 18.0. Significant differences across the series appear more clearly, however, when considering metal binding ($\log\beta$) values and protonation constants for a series of tren–hopo-based complexes (Table 1 and Figure 7). The $\log\beta$ values for Gd^{III} binding increase with increasing ligand basicity for the series.

The pGd value varies with pH value, and a plot of pGd versus pH can indicate the acid resistance of a given Gd^{III} complex (Figure 8). The complex formed from the more acidic {tren(1,2-hopo)$_3$} ligand (**3**) has greater resistance to acid hydrolysis than the dtpa complex. In comparison, the more basic {tren(1-Me-3,2-hopo)$_3$} ligand forms a gadolinium complex (**1**) that is more acid-sensitive than both $[Gd(dtpa)(H_2O)]^-$ and [Gd{tren(1,2-hopo)$_3$}]. The variation of the pGd value with the pH value for a particular Gd^{III} complex can thus give useful information about the stability of the complex

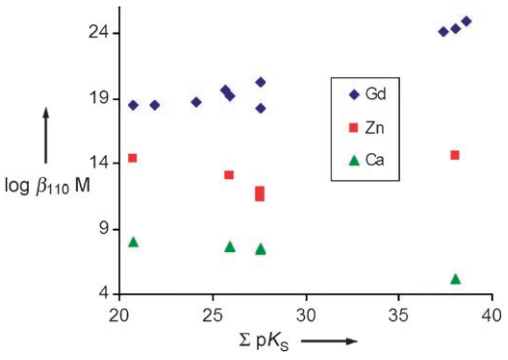

Figure 7. Metal binding constants for Gd^{III}, Zn^{II}, and Ca^{II} versus ligand acidity of a series of tren ligands (Table 1, entries 1–10 for Gd^{III}).

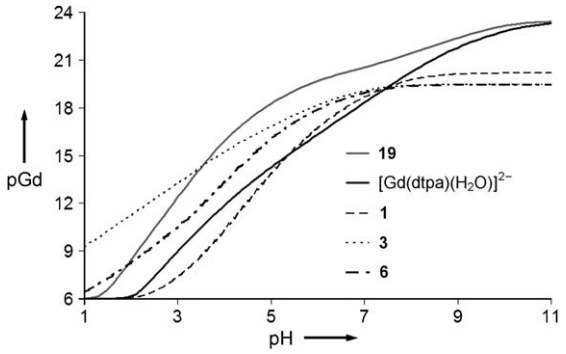

Figure 8. Ligand acidity influences the acid resistance of the complex. Basic 1-Me-3,2-hopo chelates (in **1**) tend to be slightly more sensitive to acid than dtpa, while acidic 1,2-hopo and mam ligands (in **3** and **6**, respectively) form more stable Gd^{III} complexes under acidic conditions than dtpa.

in vivo. In considering targeted imaging, such information can serve as an important guide when designing agents for a specific region of a particular pH value.

Charge

The influence of the overall charge of the Gd^{III} complex on the stability constant pGd is demonstrated in a series of eight {tren(1-Me-3,2-hopo)$_2$(tam)} complexes (**23**–**30**). Varying the substituent on the tam moiety and consequently the complex charge resulted in pGd values ranging from 17.1 (−3 charge) to 19.9 (neutral).[64] Interestingly, the three anionic complexes of −1 charge (**23**, **25**, and **30**) all exhibit the same pGd value. This study demonstrated that, to maximize stability, the charge of the complex should be as close to zero as possible, with the highest pGd value belonging to the neutral amine-substituted complex **26**.

Selectivity

For Ca^{II} and Zn^{II} binding, no clear trend between ligand acidity and binding strength is seen in the data shown in Figure 7. The more basic ligands have a higher selectivity for $M = Gd^{III}$ over Zn^{II} and Ca^{II}, as indicated by the differences in the pM values illustrated in the following examples. The two more basic {tren(1-Me-3,2-hopo)$_2$(tam)} ligands (in **20** and **25**) prefer Gd^{III} by $\Delta p(Gd-Zn) = 8.1$ and 7.0,[64,66,68] while the less basic {tren(1-Me-3,2-hopo)$_3$} (in **1**) achieves a selectivity of $\Delta p(Gd-Zn) = 6.1$,[44,45] all of which exceed that of the dtpa complex ($\Delta p(Gd-Zn) = 4.2$). The Gd^{III} selectivity of the most acidic ligand, {tren(1,2-hopo)$_3$} (in **3**), is the lowest ($\Delta p(Gd-Zn) = 4.1$) among the ligands studied.[69] Increasing denticity from six to eight improves the discrimination behavior for 1,2-hopo to $\Delta p(Gd-Zn) = 6.7$ in the case of {h22(1,2-hopo)$_4$} (in **19**).[65]

Solution Anion Affinity

Solution serum anion affinities of an anionic (**25**) and a cationic (**27**) {tren(1-Me-3,2-hopo)$_2$(tam)} complex ($q = 2$) are comparable to commercial contrast agents ($q = 1$).[64] Phosphate binds weakly with $\log K_A = 1.4$ and 2.4, respectively. Oxalate is the only other physiologically relevant anion that interacts with these complexes ($\log K_A = 1.0$ and 2.9). These affinities are similar to the phosphate binding values of the commercial contrast agents with dtpa ($\log K_A = 2.0$) and dota ligands ($\log K_A = 2.2$), while complexes of aminocarboxylate ligands with $q = 2$, such as do3a, exhibit higher anion affinities ($\log K_A = 4.8$).[6] In comparing the hopo complexes with do3a complexes, this difference in anion binding despite the same hydration number ($q = 2$) illustrates the importance of coordination geometry. In the case of **25** and **27**, it is proposed that the water molecules are in positions *anti* to one another, making it more difficult to displace both water molecules. Interestingly, oxalate binding for the cationic chelate complex **27** increased the coordination number from eight to nine, but no change in coordination number occurred for the anionic chelate complex **25**, since the oxalate anion replaces both bound water molecules.[64] Neutral **3**, which contains the more acidic chelate 1,2-hopo, exhibits a small affinity for oxalate ($\log K_A = 1.5$), and an interaction with a bidentate 3,2-hopo anion could be detected ($\log K_A = 3.5$). For interactions with both the oxalate and the bidentate 3,2-hopo anions, an increase in coordination number was observed by relaxivity and, in the case of oxalate, by luminescence measurements of the Eu^{III} analogue. No phosphate binding, however, could be detected for the complex with the 1,2-hopo ligand.[69] Octadentate **19** does not show any anion binding at neutral pH values, at which the neutral, monoprotonated complex dominates.[65] Thus, the hopo-based ligands examined do not have any appreciable anion binding capacity for the physiologically relevant anions that could affect the in vivo performance of these ligands.

In Vivo Behavior

The biodistribution of several of the hopo-based chelates was tested in mice.[52] Depending on the functionalizing groups of the chelates, different accumulation locations and finely tuned retention times were observed. For example, liver uptake is enhanced upon addition of a short polyethylene glycol (PEG) chain to the chelate, while longer PEG chains favor blood-pool localization. Complex **22**, bearing a relatively long chain of 123 ether units, gave the best MR angiographic results, despite the known decrease in human serum albumin (HSA) affinity with increasing PEG chain length.[50] In this case, the low albumin affinity may enhance the MR angiogram quality by limiting water displacement from the gadolinium center by the protein.

3.2. Tuning q and Water Exchange

As most hopo-based Gd^{III} complexes are eight-coordinate, an associative water exchange mechanism involving a nine-coordinate intermediate species can be predicted. The energy difference between the eight-coordinate ground state and a nine-coordinate intermediate is small, leading to fast water exchange and subsequent high relaxivity (Figure 9).[62,70] This rapid exchange rate was initially supported

by temperature-dependent relaxivity studies and the X-ray crystal structure of the lanthanum complex with {tren(1-Me-3,2-hopo)$_3$}. In this structure both an eight- and a nine-coordinate metal center are observed.[47] The presence of both coordination numbers indicates that the coordination environments of the two lanthanum ions must be similar in energy and that the ligand motif can accommodate both. Indeed, variable-pressure ^{17}O NMR spectroscopy studies have been carried out for **16** (Figure 6) to determine a small, negative value for the volume of activation, a result indicative of associative interchange exchange.[62]

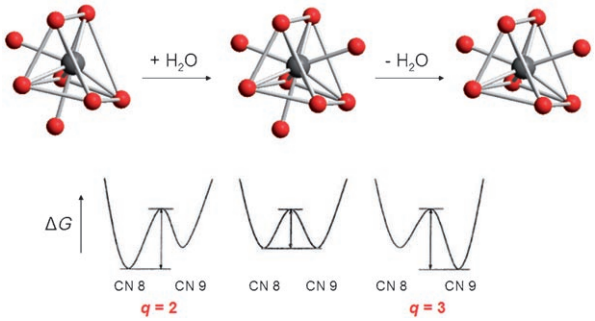

Figure 9. Top: Coordination polyhedra of the gadolinium ion illustrating associative water exchange for hopo-based complexes. Bottom: Free-energy diagrams for water exchange.[47] Most hopo complexes have an eight-coordinate ground state and a nine-coordinate intermediate (left), but studies indicate the two states are close in energy.

Since the eight- and nine-coordinate states are close in energy for gadolinium hopo complexes, small changes in the ligand structure can affect the number of bound water molecules (q) and the rate of water exchange. Importantly, increases in q for hopo-based complexes have been achieved without reducing the denticity of the ligand, resulting in complexes with fast water exchange rates that maintain the favorable thermodynamic stability properties of the parent compound. The following section reviews several examples that demonstrate the effect of both ligand scaffold and substituent on water coordination in the hopo family of Gd^{III} complexes.

Effect of PEG Substituents

Utilization of the {tren(hopo)$_2$(tam)} motif as in complex **2** affords negatively charged gadolinium complexes and a modest increase in aqueous solubility relative to the parent complex **1**. To further enhance solubility of hopo-based complexes, polyethylene glycol (PEG) chains were introduced to the tam moiety. Moreover, it was proposed that the PEG moiety may induce noncovalent interactions with the abundant blood protein HSA to slow tumbling (increase τ_R) and thereby increase relaxivity.[49] The first PEG chains chosen for attachment had 44 (**21**) and 123 (**22**) ether moieties. Analysis of the nuclear magnetic relaxation dispersion (NMRD) profiles of complexes **21** and **22** indicate a reduction in q relative to the parent complex **2** ($q = 2$), with the best refinements obtained by fixing $q = 1$. This finding can be explained by the ether oxygen atoms partially coordinating to the gadolinium center, displacing bound water molecules. Variable-temperature ^{17}O NMR spectroscopy experiments were conducted to yield the water exchange rates of each complex. It was noted that τ_M increases as the PEG chain is lengthened, thus enabling tuning of the rate. A reduction in the hydration number for PEG-substituted complexes has also been observed in subsequent studies.[50] While complexes bearing chains of 11 and 12 ether units (**23**) exhibit relaxometric behavior that suggests $q = 1$,[50,64] q remains 2 as in the parent compound when the chain is reduced to four ether units (**25**). These results indicate that a relatively short PEG chain is necessary to balance a high q value with water solubility.

Effect of Other Solubilizing Substituents

In addition to the PEG-substituted compounds, complexes bearing alcohol, acid, and amine functionalities have been studied with regard to their effects on water coordination. In a recent study, tuning of the coordination number was demonstrated by the addition of pendant amine groups. At physiological pH values, one such substituent forms a hydrogen-bond interaction with a water molecule, thereby favoring its coordination (Figure 10). This interaction results in stabilization of the nine-coordinate $q = 3$ state in the exchange process and in a consequent higher relaxivity ($11.1\ mM^{-1}s^{-1}$; 20 MHz, 298 K, pH 7).[70] Importantly, the complex (**30**) retains high stability (pGd = 19.4, comparable to $[Gd(dpta)(H_2O)]^{2-}$) as well as high relaxivity at relevant magnetic field strengths above 0.5 T.

Further study of other solubilizing substituents demonstrated the abilities of other structures to partake in similar hydrogen-bond interactions to aid in water binding at the metal center. Complexes bearing the ethanolamine moiety (**20**) and various carboxylic acid groups (**28** and **29**) also have relaxometric properties consistent with three bound water molecules, as indicated by NMRD profiles.[64] In all cases, thermodynamic stabilities were determined to be sufficient for consideration of such compounds as clinical agents. Moreover, the water-molecule residence times obtained for the series of complexes are all similarly short, regardless of complex charges or ground-state coordination numbers. This observation provides further support for the close energy of

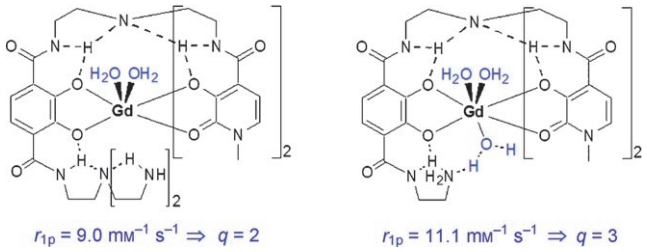

Figure 10. {tren(hopo)$_2$(tam)} complexes **27** (left) and **30** (right) substituted with pendant amines. Complex **30** possesses a substituent capable of forming a hydrogen bond with an additional water molecule to promote its coordination to the Gd^{III} center.

the eight- and nine-coordinate states for hopo-based complexes and reveals how subtle changes in ligand structure can alter the nature of the ground state (Figure 9).

tacn-Capped Complexes

The effect of the ligand-capping structure on water coordination has also been explored. In a recent report, triazacyclononane (tacn) was used as a ligand cap to produce complexes with both the 3,2- or 1,2-hopo units (**13** and **14**).[51] Molecular modelling studies predicted that complexes with a larger cap than tren would accommodate three inner-sphere water molecules, and subsequent relaxometric and luminescent characterization revealed that this design strategy was successful. Relaxivities were found to be 13.1 and 12.5 $mM^{-1}s^{-1}$ (20 MHz, 298 K, pH 7), values that remained high at field strengths above 0.5 T. The stabilization of the $q = 3$ complex in this case is accomplished without the need for the asymmetric $(hopo)_2$tam motif. Use of the tacn cap also results in a dramatic increase in aqueous solubility, possibly owing in part to protonation of the tacn cap to give a charged species near neutral pH values. Furthermore, stability is not significantly affected (pGd = 18.7) upon increasing the hydration number to $q = 3$. These tacn-capped complexes are therefore unique examples of highly soluble tris-hopo Gd^{III} complexes that demonstrate high hydration numbers, fast water exchange rates, and high stabilities.

3.3. Increasing Rotational Correlation Times through Macromolecular Association

Once the basic hydration and water-exchange properties of the gadolinium chelate complex are optimized, further enhancement of the relaxivity can be achieved by grafting the complex to macromolecules to slow molecular tumbling, thereby increasing τ_R. The attachment of commercially available contrast agents based on dota and dtpa ligands to macromolecular constructs has been extensively studied, and in several cases, enhancements have been observed upon slowing of molecular rotation.[71–79] These contrast agents, however, are somewhat restricted, because they do not have the optimal water exchange rates that would lead to large enhancements in relaxivity. The major advantage of these aminocarboxylate-based contrast agents is their high water solubility. For example, in the case of the attachment of multiple contrast agents to dendrimers, the solubility of the resulting macromolecule would decrease if the contrast agent has low solubility. As stated previously, solubility has generally been acknowledged as the major drawback in hopo-based contrast agents.[64] While the choice of ligand scaffold can play a role (see Section 3.2), poor solubility can be alleviated by the use of a more soluble macromolecule or by attaching the contrast agent to the interior of a soluble macromolecular vehicle.

Covalent attachment to macromolecules such as dendrimers, proteins, virus capsids, and inorganic nanoparticles; encapsulation into fullerenes, virus capsids, and liposomes; noncovalent interactions with proteins; and supramolecular self-assembly to form larger constructs are ideas that have been explored to build high-molecular-weight contrast agents.[5,80,81] Construction of macromolecular entities with multiple contrast agents has the advantage of increased ionic (per mM Gd) and molecular (per particle) relaxivity. The ionic relaxivity increase is due to slower molecular tumbling, and the use of multiple attachment sites leads to high molecular relaxivities. Macromolecular agents of size greater than 10 nm also have potential for application as MR angiography agents. These nanoparticles will preferentially accumulate near lesions in vessels but will not cross the healthy endothelial layer.[82] The design of macromolecules with different attachment sites for contrast agents and targeting agents is important for imaging of biological targets that display only low in vivo concentrations and require greater contrast enhancement for visualization. High-relaxivity contrast agents based on hopo can theoretically (according to SBM theory) reach relaxivity values useful for targeted imaging (up to 100 $mM^{-1}s^{-1}$) and are therefore excellent candidates for attachment to macromolecules. The remainder of this section is devoted to a few representative case studies involving the construction of macromolecular hopo-based contrast agents and comparison to their commercial counterparts.

HSA Binding

Noncovalent binding of contrast agents to HSA protein in vivo has been used to obtain contrast enhancements. The commercial agent Vasovist (MS-325) is based on this strategy.[71,76,79] Binding to HSA increases this contrast agent's circulation time in blood and also slows down its tumbling rate, leading to greater contrast enhancements for blood-vessel imaging. The advantage of this concept is that less material has to be injected into the patient, while the concern is the thermodynamic stability of the complex (i.e. the agent remains in the body longer, depending on the binding constant between the agent and HSA).

As illustrated in the previous sections, heteropodal $(hopo)_2$(tam) ligands can be modified by the attachment of various functional groups to the terminal carboxy group of the tam moiety (Figure 6). The increase in relaxivity observed for **22** (to 9.1 from 8.8 $mM^{-1}s^{-1}$ at 20 MHz) upon the attachment of the PEG group is modest considering the large increase in molecular weight; this relativley poor performance is due both to the decrease in q and to the rapid local motions in the PEG chains. The HSA adduct of **22** afforded a relaxivity of (74 ± 14) $mM^{-1}s^{-1}$ at 20 MHz with a formation constant of (186 ± 50) M^{-1}, indicating weak binding that leads to a mixture of HSA-bound and unbound species.[49] Upon attachment of a benzyl group through the hopo nitrogen atom (**10**), the HSA binding affinity increased to (8640 ± 2000) M^{-1}.[50] The number of inner-sphere water molecules, however, is lowered to about zero (owing to closer interaction with the protein), which results in relaxivities in the range of 15–19 $mM^{-1}s^{-1}$. For comparison, the association constant of HSA with MS-325 is (6100 ± 2130) M^{-1}, and the relaxivity is 50 $mM^{-1}s^{-1}$ at 25 MHz.[76] The interactions between HSA and the hydroxypyridonate complexes must be further refined while high q values are maintained and rapid local motion is limited.

Iron–Gadolinium Supramolecular Complexes

Self-assembly to supramolecular species that contain more than one gadolinium center can lead to high-relaxivity agents, owing to the slower tumbling rate of the large construct. This supramolecular approach has not yielded a marketable candidate, however, because of difficulties in synthesis, characterization, and in tailoring of final physiological properties of defined supramolecular gadolinium compounds. Many compounds in this class also do not fulfill stability requirements because of modifications in the gadolinium chelating unit.

Supramolecular lanthanide complexes have been reviewed by Bünzli and Piguet.[83] For supramolecular d-block metals, the concept of "incommensurate coordination number" was developed,[84–88] a term that relates to preferred coordination geometries of metal centers and rigid ligands with a defined angle between chelating groups. Lanthanides are more flexible with regard to preferred coordination geometries than transition metals, which is especially important for gadolinium-based MRI contrast agents (these can favor eight- or nine-coordinate geometries, as discussed above). The concept of incommensurate coordination number thus cannot be transferred directly to lanthanides. Nevertheless, it has been demonstrated that ligands designed along this concept can assemble into supramolecular lanthanide complexes. In most cases, the structure of these supramolecular assemblies cannot be predicted. The dominant example is the La_8L_8 complex **31**,[89] which forms instead of the analogous tetrahedron In_6L_6 (Figure 11). The lanthanum atoms occupy the eight vertices of the polyhedron, while each ligand occupies one of the eight triangular faces. Each lanthanum atom is coordinated to three ligands, and each ligand binds three lanthanum atoms.

Most supramolecular constructs developed as potential MRI contrast agents are based on attaching preformed Gd^{III} chelates to tris-bidentate Fe^{III} complexes. These complexes, commonly referred to as "metallostars", can provide high relaxivity enhancements arising from molecular-weight increases. For example, the *N*,*N*-bis(2-aminoethyl)amine-*N*′,*N*′,*N*″,*N*″-tetraacetate (ttaha)-based metallostar has a relaxivity of 32 $mM^{-1}s^{-1}$ at 20 MHz.[90] To test the approach of true self-assembly, hopo–tam mixed ligands were investigated using a C_3 linker between the tam unit and the hopo chelates (**32**, Figure 11).[68] The assembly reduces the tumbling of the incorporated gadolinium complexes in solution, and the resulting longer rotational correlation time afforded higher relaxivities of 18.7 and 23 $mM^{-1}s^{-1}$ at 20 and 60 MHz, respectively.

Attachment to Dendrimers and Virus Capsids

Covalent attachment of contrast agents to dendrimers has been widely explored because of the advantages presented by the attachment of multiple complex fragments and the deceleration of the tumbling rate arising from an increased molecular weight. However, the molecule has to be rigid enough to prevent vibrational modes that allow for fast tumbling of the gadolinium-containing portions.[91] Gadomer-17, a dendrimer-based contrast agent developed by Schering, affords a 3.5-fold increase in relaxivity relative to the starting complex $[Gd(dota)(H_2O)]^-$ (to 16.5 from 4.7 $mM^{-1}s^{-1}$ at 20 MHz).[91] The relaxivity enhancement, however, is not as high as would be expected for the huge increase in molecular weight (40000 Da), owing to a slow water exchange rate. A single molecule of a {tren(hopo)$_2$tam}-based complex grafted onto an aspartate based dendrimer (**33**, Figure 12, molecular weight 1576 $g\,mol^{-1}$) gave a relaxivity enhancement of 1.6 times over the analogous free complex **2** (to 14.3 from 8.8 $mM^{-1}s^{-1}$ at 20 MHz). The compactness of the system and an optimal water exchange rate allow reasonable relaxivity

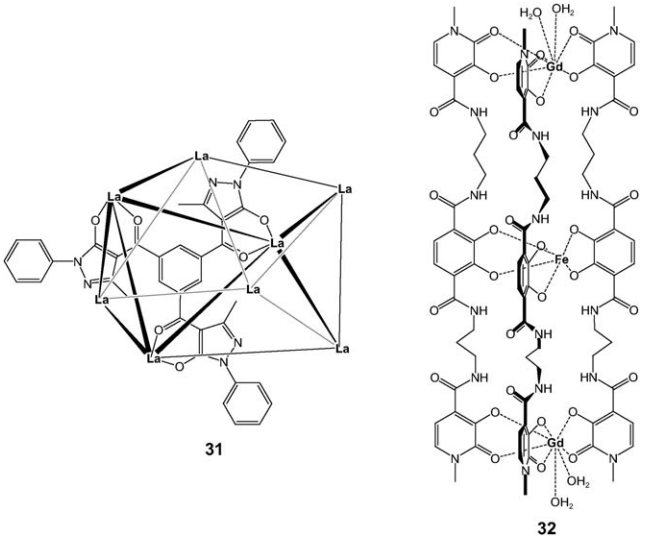

Figure 11. Supramolecular lanthanum-based construct **31** ([La(acac)$_3$]/DMSO (acac = acetylacetonate); the coordinated DMSO molecules are omitted for clarity) and {$FeGd_2$} species **32**.

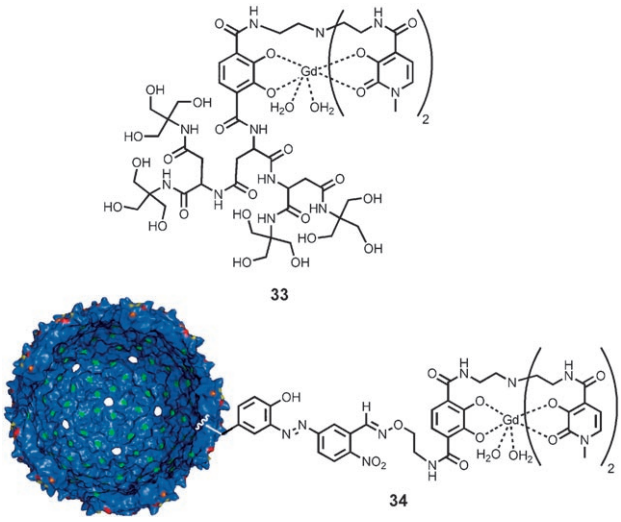

Figure 12. Attachment of hopo-based contrast agents to an aspartate-based dendrimer (**33**) and the internally modified MS2 virus capsid (**34**; modified tyrosine residues in the interior are highlighted in green on the capsid structure[96]). The linker attaching the gadolinium complex is shown in the expanded structure of one of the modified tyrosine residues.

enhancements, despite the fact that the molecular weight increase is not very large.[92]

Virus capsids have recently been explored as potential scaffolds for attachment of gadolinium complexes.[73,77,81,93] Covalent attachment of {tren(hopo)$_2$(tam)}-based chelates onto bacteriophage MS2 capsids (90 Gd complexes per capsid) has led to one of the highest relaxivities reported for these systems to date (Figure 12).[94] The capsid shell consists of 180 copies of the coat protein ($M_r = 13700$) assembled into an icosahedral arrangement (Figure 12). The diameter of this nanosized assembly is 27.4 nm, and its surfaces can be modified through lysine, cysteine, or tyrosine molecules (one per monomer on the interior surface). The interior and exterior surfaces of the capsid shells (devoid of RNA) were modified separately, and relaxivity enhancement depended upon the local motion of the chelate. The interior surface was modified through tyrosine moieties (**34**, relaxivity per Gd 41.6 $mM^{-1}s^{-1}$ and relaxivity per particle 3900 $mM^{-1}s^{-1}$ at 30 MHz, 25 °C), while the exterior surface was modified through lysine residues (relaxivity per Gd 30.7 $mM^{-1}s^{-1}$ and relaxivity per particle 2500 $mM^{-1}s^{-1}$ at 30 MHz, 25 °C). The rigidity of the linker attaching the Gd complex to the macromolecule clearly affects the relaxivity of the complexes, with the more rigid linkers yielding higher relaxivities.[95] Furthermore, the interior attachment strategy has several major advantages, including increased solubility as well as the possibility to develop targeted contrast agents by exterior surface modification.

4. Conclusions

Detailed studies on lanthanide coordination chemistry have yielded exciting possibilities in the production of next-generation MRI contrast agents. While the aminocarboxylate-based agents currently in use provide some contrast enhancement, there is much room for improvement. New ligand designs may be required to attain high relaxivity values at high magnetic field strengths to take advantage of the increased resolution made possible by new high-field instruments. Exploration of the hopo family of Gd^{III} chelate complexes has revealed several promising platforms for consideration as practical agents. These complexes possess the unique combination of high hydration numbers, fast water exchange rates, high stabilities, and high relaxivity values at the fields of interest. Recent work aimed at tethering the hopo compounds to macromolecules is promising, and further optimization of these strategies is ongoing. Regardless of the platform used, it is clear that the development of safe, high-relaxivity MRI contrast agents will remain a challenging task. With the multitude of factors that must be considered, agent design and evaluation requires a creative, multidisciplinary approach. The rewards are great, however, as improved agents would increase the breadth of possible MR applications and enhance the power of MRI as an imaging modality even further.

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