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Approaching the Kinetic Inertness of Macrocyclic Gd³⁺-based MRI Contrast Agents with Highly Rigid Open-Chain Derivatives

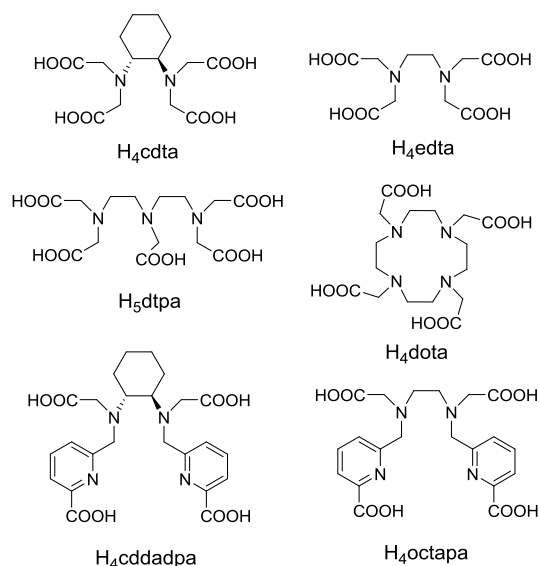
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This paper is dedicated to Professor Imre Tóth in occasion of his 65th birthday.

Abstract: A highly rigid open-chain octadentate ligand (H₄cddadpa), containing a diaminecyclohexane unit to replace the ethylenediamine bridge of 6,6'-((ethane-1,2-diylbis((carboxymethyl)azanediyl))bis(methylene))dipicolinic acid (H₄octapa), was synthesized. This structural modification slightly improves the thermodynamic stability of the Gd³⁺ complex (logK_{GdL} = 20.68 vs 20.23 for [Gd(octapa)]⁻), while other MRI-relevant parameters remain practically unaffected (number of coordinated water molecules, q=1; relaxivity, r₁ = 5.73 mm³s⁻¹ at 20 MHz and 295 K). Most remarkably, the kinetic inertness of the complex is improved to a great extent by the rigidifying effect of the diaminecyclohexane unit in the ligand skeleton: the half-life of dissociation calculated for physiological conditions is 6 orders of magnitude higher for [Gd(cddadpa)]⁻ (t_{1/2} = 1.49 × 10⁵ h) than for [Gd(octapa)]⁻. As compared to non-macrocyclic MRI contrast agents approved for clinical use ([Gd(dtpa-bma)], [Gd(dtpa-bmea)], [Gd(dtpa)]²⁻ and [Gd(bopta)]²⁻), the kinetic inertness of this novel chelate is superior by 2-3 orders of magnitude, and it is the highest among all linear Gd³⁺ chelates known so far.

The application of gadolinium(III) complexes as contrast agents in magnetic resonance imaging (MRI) requires stable complexation of the metal ion under physiological conditions to avoid the release of the toxic free metal ion.^[1,2] Several pathways leading to the potential dissociation of Gd³⁺ complexes *in vivo* have been identified: i) Acid-catalysed dissociation, which is often responsible for the dissociation of complexes with macrocyclic ligands such as dota⁴⁻ (Scheme 1);^[3] ii) Dissociation catalyzed by endogenous metal ions such as Zn²⁺ and Cu²⁺, which contributes to the dissociation of contrast agents with non-

macrocyclic ligands such as dtpa⁵⁻.^[4] iii) Dissociation assisted by endogenous ligands like citrate, phosphate or bicarbonate, which is also important for non-macrocyclic contrast agents.^[5] Complexes of linear ligands generally present faster dissociation kinetics than the macrocyclic counterparts, although some macrocyclic Gd³⁺ complexes were shown to undergo rather fast dissociation.^[6] Low basicity of the Gd³⁺ complex has been identified as an important factor to improve its kinetic inertness. For instance, dota-tetraamide Gd³⁺ complexes were shown to be considerably more inert than [Gd(dota)]⁻, which has been attributed to the low basicity of the amide oxygen atom that makes proton transfer to the ring nitrogen very unlikely.^[7] Another important factor is rigidity, which is likely responsible for the higher kinetic inertness of macrocyclic complexes. For instance, cdta⁴⁻, which contains a rigid diaminecyclohexane unit, forms considerably more inert Ln³⁺ and Mn²⁺ complexes than the edta⁴⁻ analogues.^[8,9] The rigidity of the backbone in numerous bishydrated Gd³⁺ complexes of ligands derived from cdta⁴⁻ and 2,6-bis(aminomethyl)pyridine was also found to play an important role; however these systems were either similar or just slightly better than the non-macrocyclic MRI contrast agents available in the market.^[10, 11] The beneficial effect of alpha-C-substitution on the thermodynamic and kinetic properties of Y³⁺ complexes (Y³⁺ behaves very similarly to Gd³⁺) formed with dtpa-like ligands is also well documented in the literature.^[12] On the other hand, a rigid cross-bridge macrocyclic ligand was recently shown to provide exceptionally inert Ln³⁺ complexes, highlighting again the impact of ligand rigidity on the kinetic inertness of macrocyclic complexes as well.^[13]



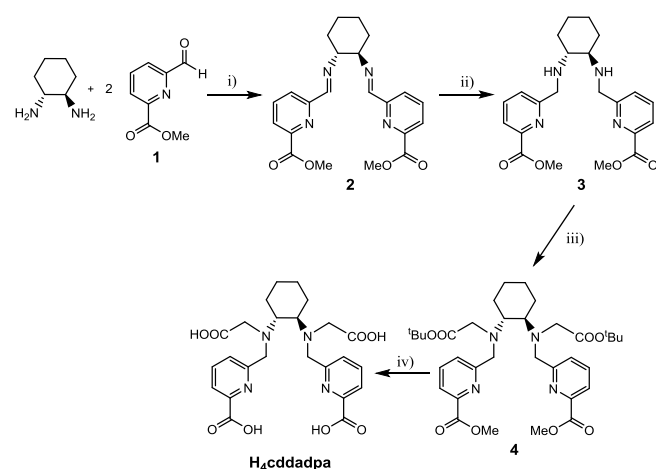
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Scheme 1. Ligands discussed in the present work.

The non-macrocyclic octadentate ligand 6,6'-((ethane-1,2-diylbis((carboxymethyl)azanediyl))bis(methylene))dipicolinic acid ($H_4octapa$, Scheme 1) forms Ln^{3+} complexes with high thermodynamic stability ($\log K = 19.9 - 20.5$ depending on the Ln^{3+} ion).^[14,15] Furthermore, $[Gd(octapa)]^-$ contains a coordinated water molecule that results in 1H relaxivities similar to those of commercially available contrast agents such as $[Gd(dota)]^-$ or $[Gd(dtpa)]^{2-}$.^[16] However, kinetic studies showed that $[Gd(octapa)]^-$ is considerably more labile than the $edta^{4-}$ and $dtpa^{5-}$ counterparts, which is unacceptable for practical applications.^[15] In this work, we report the octadentate ligand $H_4cddadpa$, which incorporates a rigid diaminocyclohexane unit instead of the ethylenediamine linker of $H_4octapa$. The corresponding Gd^{3+} complex retains a coordinated water molecule while providing improved relaxivities and thermodynamic stability with respect to $[Gd(octapa)]^-$. Most importantly, $[Gd(cddadpa)]^-$ displays an unprecedented kinetic inertness for an open-chain system.

Ligand $H_4cddadpa$ was synthesized in four steps starting from methyl 6-formylpicolinate (**1**), which was obtained in three steps from commercially available pyridine-2,6-dicarboxylic acid.^[15] Reaction of **1** with (1*R*,2*R*)-diaminocyclohexane gave Schiff base **2**, which was reduced with $NaBH_4$ to give amine **3** (Scheme 2). *N*-alkylation of **3** with *tert*-butyl-2-bromoacetate at room temperature in acetonitrile solution and subsequent deprotection of the methyl and *tert*-butyl esters with 6 M HCl gave the $H_4cddadpa$ ligand, which was isolated with an overall yield of 37% over the four steps (the experimental details of synthesis of **2** and **3** are included in the Supporting Information). The $[Ln(cddadpa)]^-$ complexes ($Ln = Eu$ or Gd) were prepared in aqueous solution by reaction of equimolar amounts of the ligand and $Ln(OTf)_3$ followed by adjustment of the pH to ~ 7 with aqueous NaOH. The HR-ESI⁺ mass spectra of the complexes present a peak due to the $[Ln(cddadpa)]^-$ entities that confirms the formation of the complexes (Figures S5 and S6, Supporting Information). The absorption and CD spectra of the Gd complex confirm the formation of the optically active complex (Figure S7, Supporting Information).

**Scheme 2.** Synthesis of $H_4cddadpa$. Reagents and conditions: i) MeOH, reflux, 4 h, 83% (see SI for experimental details); ii) $NaBH_4$, MeOH, 92% (see SI for

experimental details); iii) *tert*-butyl-2-bromoacetate (2.1 equiv), K_2CO_3 , acetonitrile, room temperature, 4 d and 45 °C, 3 d, 65%; iv) 6 M HCl, reflux, 24 h, 75%.

Equilibrium studies to obtain the ligand protonation constants and the stability constant of the Gd^{3+} complex were carried out by pH-potentiometry (Figures S8 and S9, Supporting Information). The first protonation constant (Table 1) of $cddadpa^{4-}$ is ca. 0.8 logK unit higher than that of the related $octapa^{4-}$.^[15] The small differences in the basicity of these ligands may be attributed to the structural rigidity brought by the cyclohexyl bridge compared to the more flexible ethylene bridging unit. A similar increase in the basicity of the nitrogen atom was also evidenced for $cdta^{4-}$ when comparing its $\log K_1^H$ to that of $edta^{4-}$.^[17] Similarly to the $[Gd(octapa)]^-$ complex, it was not possible to determine the stability constant of $[Gd(cddadpa)]^-$ by using solely pH-potentiometry since the complex is nearly quantitatively formed even at pH=1.8. Therefore, the pH-potentiometric titration data were complemented by 1H -relaxometric titrations (the relaxivity pH profile is included in the Supporting Information). The relaxivity of $[Gd(cddadpa)]^-$ remains constant in the pH range ~ 12.0 -2.0, and increases below ~ 2.0 due to the dissociation of the complex (Figure S9, Supporting Information). By knowing the relaxivities of the Gd^{3+} aqua-ion and $[Gd(cddadpa)]^-$, the stability constant of the complex could be determined (Table 1). Owing to the increased basicity of $cddadpa^{4-}$, the stability of its Gd^{3+} complex is increased by ca 0.4 logK units in comparison to $[Gd(octapa)]^-$ and it is close to that of $[Gd(dtpa)]^{2-}$ (Table 1), which is a commercial open-chain MRI contrast agent used in clinical practice.^[18] Moreover, the stability of $[Gd(cddadpa)]^-$ is comparable to or slightly lower than those of the Gd^{3+} complexes formed with macrocyclic $do3a^{3-}$ and its derivatives used as commercial contrast agents (*hp*- $do3a^{3-}$ and *do3a*-butrol³⁻).^[19] Given the considerably lower basicity of $cddadpa^{4-}$, the conditional stability constant of $[Gd(cddadpa)]^-$ will be higher near physiological conditions, despite the comparable stability constants of the complexes. This is demonstrated by comparing the pGd values calculated as proposed by K. N. Raymond and co-workers (Table 1).^[20] The pGd value obtained for $[Gd(cddadpa)]^-$ is slightly higher than that determined for $[Gd(dtpa)]^{2-}$ (the increase is only 0.2 pGd units) but clearly higher than those of complexes with macrocyclic ligands such as $[Gd(do3a)]^-$ (~ 3.8 pGd units). This proves that $[Gd(cddadpa)]^-$ has the highest stability among the complexes compared in Table 1 at physiological pH.

Kinetic inertness is a key parameter for safe application of a Gd^{3+} complex as a contrast agent, since the complexes injected to the body must remain intact. In the light of the encouraging stability data discussed above, the kinetic inertness of $[Gd(cddadpa)]^-$ was characterized by studying the rate of the metal exchange reaction occurring with Cu^{2+} . Cu^{2+} is typically the most efficient among the physiologically relevant metal ions to promote transmetalation reactions with Gd^{3+} complexes.^[4,5] The pseudo-first-order rate constants determined for the exchange reaction between $[Gd(cddadpa)]^-$ and Cu^{2+} ion are shown in Figure 1. The k_{obs} values increase with increasing concentrations of both H^+ and Cu^{2+} ions, which can be rationalized by

considering three dissociation pathways: spontaneous, proton-assisted (via the formation of a protonated $[\text{GdH}(\text{cddadpa})]$ intermediate) and metal-assisted (through the formation of a dinuclear reaction intermediate). These dissociation pathways are characterized by rate constants k_0 (s^{-1}), k_1 ($\text{M}^{-1}\text{s}^{-1}$) and k_3 ($\text{M}^{-1}\text{s}^{-1}$), respectively. The overall map of dissociation pathways, the expression used for the pseudo-first-order rate constant (k_{obs}) and the equations employed for data refinement are included in the Supporting Information.

Table 1. Protonation constants of cddadpa^{4-} and related ligands and stability constants and pGd values of their Gd^{3+} complexes (25 °C, 0.15 M NaCl).

	cddadpa^{4-}	octapa^{4-} [a]	dtpa^{5-} [b]	do3a^{3-} [c, d]
$\log K_1^{\text{H}}$	9.35(2)	8.52	9.93	11.99
$\log K_2^{\text{H}}$	5.66(3)	5.40	8.37	9.51
$\log K_3^{\text{H}}$	4.20(3)	3.65	4.18	4.31
$\log K_4^{\text{H}}$	3.72(3)	2.97	2.71	3.63
$\log K_5^{\text{H}}$	2.62(4)	1.66	2.00	1.84
$\Sigma \log K_i^{\text{H}}$	25.55	22.20	27.19	31.26
$\log K_{\text{GdL}}$	20.68(9) ^[e]	20.23 ^[f]	22.03	21.56
$\log K_{\text{GdHL}}$	2.38(2) ^[e]	-	1.96	-
pGd	19.66	19.23	19.44	15.81

[a] Ref. [15]; [b] Ref. [18]; [c] Ref. [21]; [d] Protonation and stability constants were determined by using 0.1 M KCl ionic strength. All these constants are expected to be considerably lower in 0.15 M NaCl, but the pGd value will be very likely similar to the one calculated by using the data corresponding to 0.1 M KCl ionic strength; [e] Determined by simultaneous fitting of relaxometric and pH-potentiometric data; [f] Determined by relaxometric titration.

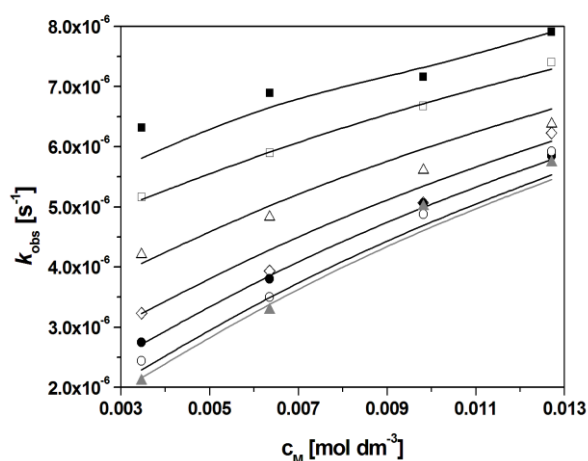


Figure 1. Plot of the pseudo-first-order rate constants measured as function of Cu^{2+} ion concentration and pH (50 mM DMP, 25 °C, 0.15 M NaCl, pH=3.38, 3.51, 3.70, 3.96, 4.21, 4.64 and 4.94 downwards).

The rate constants are compared with those of the Gd^{3+} complexes formed with octapa^{4-} , dtpa^{5-} and do3a^{3-} in Table 2. The rate constant of the proton-assisted dissociation (k_1) of $[\text{Gd}(\text{cddadpa})]^-$ decreases by nearly 3 orders of magnitude when compared to the corresponding value for $[\text{Gd}(\text{octapa})]^-$. Moreover, k_1 is more than 30 times smaller for $[\text{Gd}(\text{cddadpa})]^-$ than that for $[\text{Gd}(\text{dtpa})]^{2-}$, and very similar to that of $[\text{Gd}(\text{do3a})]$. On the other hand, the rate constant characterizing the direct attack of the Cu^{2+} ion on the complex, k_3 , is more than 3 orders of magnitude smaller for $[\text{Gd}(\text{cddadpa})]^-$ than for $[\text{Gd}(\text{dtpa})]^{2-}$ (endogenous metal ions are known not to affect the dissociation of Ln^{3+} complexes of do3a^{3-} or dota^{4-} ligands).^[19] A comparison of the dissociation half-lives, $t_{1/2}$, calculated near physiological conditions (pH=7.4, $c_{\text{Cu}^{2+}}=1 \mu\text{M}$; Table 2) confirms the remarkable kinetic inertness of $[\text{Gd}(\text{cddadpa})]^-$, resulting from the integration of a highly rigid diaminocyclohexane unit in the ligand skeleton. The deceleration of the dissociation is a consequence of the strained structure of the chelate, as demetallation requires structural rearrangements that will proceed more slowly for complexes with non-flexible ligands.^[10,11,12,22] Indeed, the ^1H NMR spectrum of the $[\text{Eu}(\text{cddadpa})]^-$ complex recorded in D_2O solution evidences a very rigid structure (Figure 2). It shows a total of 22 paramagnetically shifted signals in the range $\sim +28$ to -14 ppm (at 25 °C), which corresponds to a C_1 symmetry of the complex. This is in contrast with the more flexible structure of $[\text{Ln}(\text{octapa})]^-$ complexes ($\text{Ln} = \text{La}, \text{Ce}, \text{Pr}, \text{Nd}$ or Sm) reflected by an effective C_2 symmetry.^[16]

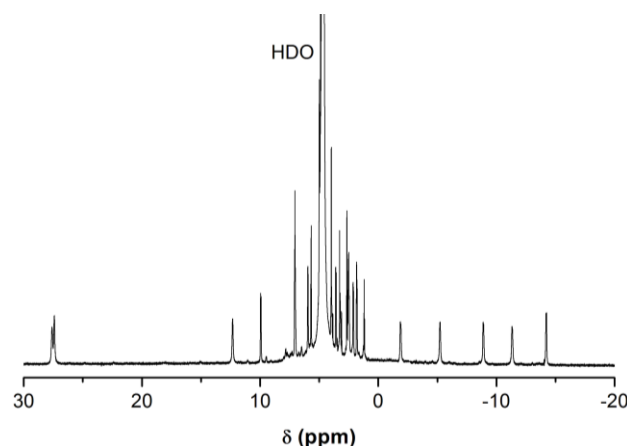


Figure 2. ^1H NMR spectrum of $[\text{Eu}(\text{cddadpa})]^-$ recorded in D_2O solution [300 MHz, 25 °C, pD 7.0].

Table 2. Rate and equilibrium constants characterizing the dissociation of the Gd^{3+} complexes of cddadpa^{4-} , octapa^{4-} , dtpa^{5-} and do3a^{3-} (25 °C).

	cddadpa^{4-}	octapa^{4-} [a]	dtpa^{5-} [b]	do3a^{3-} [c]
k_1 ($\text{M}^{-1}\text{s}^{-1}$)	0.016 ± 0.002	11.8	0.58	$0.023, 0.025$ [f]
k_2 ($\text{M}^{-2}\text{s}^{-1}$)	–	2.5×10^4	9.7×10^4	–
k_3^{Cu} ($\text{M}^{-1}\text{s}^{-1}$)	$6.8 \pm 0.4 \times 10^{-4}$	22.5	0.93	–

$k_{\text{G}}^{\text{Cu}} (\text{M}^{-2}\text{s}^{-1})$	–	5.0×10^9	–	–
$K_{\text{H}}^{[\text{d}]}$	737 ± 435	2.6	1.3	–
K_{CuGdL}	48 ± 8	–	13	–
$t_{1/2} (\text{h})^{[\text{e}]}$	1.49×10^5	0.15	202	$\frac{2.10 \times 10^5}{1.93 \times 10^5}^{[\text{f}]}$

[a] Ref. [15]. [b] Ref. [4]. [c] Ref. [21]. [d] A $\log K_{\text{H}}$ value of 2.38(2) was determined by pH-potentiometry. [e] $t_{1/2} = \ln 2 / k_{\text{obs}}$ where k_{obs} was calculated by using $\text{pH}=7.4$ and $C_{\text{Cu}^{2+}}=1 \mu\text{M}$, [f] Ref. [23].

The emission spectra of $[\text{Eu}(\text{cddadpa})]^-$ recorded in H_2O and D_2O solutions show the $^5\text{D}_0 \rightarrow ^7\text{F}_J$ transitions expected for this metal ion, with maxima at 580 ($J=0$), 593 ($J=1$), 615 ($J=2$), 652 ($J=3$) and 685 nm ($J=4$) (Figure S10, Supporting Information). The emission lifetimes of the $^5\text{D}_0$ excited state recorded in H_2O and D_2O are 0.58 and 2.15 ms, respectively. These values provide hydration numbers of $q=1.20$ and 1.05 using the methodologies developed by Beeby^[24] and Horrocks,^[25] respectively, which points to the presence of one water molecule coordinated to the Ln^{3+} ion.

The relaxivity ($r_{1\rho}$) measured for $[\text{Gd}(\text{cddadpa})]^-$ at 20 MHz and 25 °C ($\text{pH}=7.4$) amounts to $5.6 \text{ mm}^{-1}\cdot\text{s}^{-1}$, a value that is ca. 12% higher than that measured under the same conditions for $[\text{Gd}(\text{octapa})]^-$, and ca. 19% higher than those reported for the commercially available contrast agents $[\text{Gd}(\text{dota})]^-$ and $[\text{Gd}(\text{dtpa})]^-$ (Figure 3). The relaxivity is somewhat lower at 37 °C ($4.5 \text{ mm}^{-1}\cdot\text{s}^{-1}$), which indicates that it is limited by fast rotation. However, the $r_{1\rho}$ value obtained at 37 °C is still higher than those of $[\text{Gd}(\text{octapa})]^-$ ($3.9 \text{ mm}^{-1}\cdot\text{s}^{-1}$), $[\text{Gd}(\text{dota})]^-$ ($3.8 \text{ mm}^{-1}\cdot\text{s}^{-1}$) and $[\text{Gd}(\text{dtpa})]^-$ ($4.0 \text{ mm}^{-1}\cdot\text{s}^{-1}$). This is likely related to the rigid nature of $[\text{Gd}(\text{cddadpa})]^-$, which leads to a slightly longer rotational correlation time.

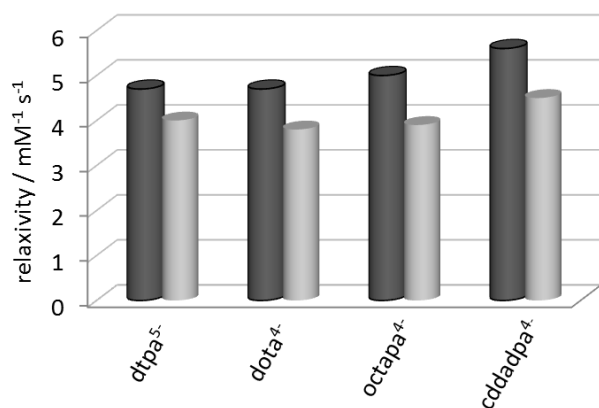


Figure 3. Plot of the relaxivity, $r_{1\rho}$, for selected Gd^{3+} complexes at 20 MHz and 25 °C (dark grey) and 37 °C (light grey).

In conclusion, we have shown that the strained, open-chain ligand cddadpa^{4-} forms a Gd^{3+} complex with high thermodynamic stability and unprecedented kinetic inertness for a linear chelate, which becomes comparable to those of macrocyclic complexes. The incorporation of the rigid diaminocyclohexane unit does not alter the hydration number of

the complex and increases slightly its ^1H relaxivity. The $[\text{Gd}(\text{cddadpa})]^-$ complex is a very promising candidate for further development of safer MRI contrast agents. Our results highlight the importance of rigidification in the ligand backbone to design Gd^{3+} -based contrast agents with improved features. "The $[\text{Gd}(\text{cddadpa})]^-$ complex is a very promising candidate for further development of safer MRI contrast agents"

Experimental Section

General: Chemicals were purchased from commercial sources and used without further purification. SiO_2 (Fluka, pore size 60 Å, 70-230 mesh) was used for preparative column chromatography. Compound **3** was synthesized as described previously.^[26] ^1H and ^{13}C NMR spectra were recorded at 25 °C on Bruker Avance 500 MHz and Bruker Avance 300 MHz spectrometers. High resolution ESI-TOF mass spectra were recorded using a LC-Q-q-TOF Applied Biosystems QSTAR Elite spectrometer both in the positive and negative modes. Elemental analyses were carried out on a ThermoQuest Flash EA 1112 elemental analyzer. IR spectra were recorded using a Bruker Vector 22 spectrophotometer equipped with a Golden Gate Attenuated Total Reflectance (ATR) accessory (Specac). Excitation and emission spectra were recorded on a Perkin-Elmer LS-50B spectrometer. Luminescence lifetimes were calculated from the monoexponential fitting of the average decay data, and they are averages of at least 3 – 5 independent determinations. Hydration numbers, q , were obtained using equation (1), where $\tau_{\text{H}_2\text{O}}$ and $\tau_{\text{D}_2\text{O}}$ respectively refer to the measured luminescence decay lifetimes (in ms) in water and deuterated water:

$$q_{\text{Eu}} = A \left(\frac{1}{\tau_{\text{H}_2\text{O}}} - \frac{1}{\tau_{\text{D}_2\text{O}}} - B \right) \quad [1]$$

Where A and B are empirical constants that take values of $A=1.2$ and $B=0.25$ ^[23] or $A=1.11$ and $B=0.31$.^[25]

Equilibrium measurements: The concentration of the $\text{H}_4\text{cddadpa}$ ligand as well as its protonation constants were determined by using pH-potentiometric titrations. Metrohm 785 DMP Titrimo equipped with a Metrohm 6.0233.100 combined electrode was used to measure the pH in titration experiments. For the pH-calibration of the electrode KH-phthalate ($\text{pH}=4.005$) and borax ($\text{pH}=9.177$) buffers were used. The calculation of $[\text{H}^+]$ from the measured pH values was performed with the use of the method proposed by Irving et al.^[27] by titrating a 0.01 M HCl solution ($I=0.15 \text{ M NaCl}$) with a standardized NaOH solution. The differences between the measured and calculated pH values were used to obtain the $[\text{H}^+]$ concentrations from the pH-data collected in the titrations. The ion product of water was determined ($\text{p}K_w=13.820$) from the same experiment in the pH range 11.40–12.00. The ionic strength in the titrated and thermostated (at 25 °C) samples of 6.00 mL was kept constant and set to 0.15 M NaCl. The samples were stirred by a mechanical stirrer and kept under inert gas atmosphere (N_2) to avoid the effect of CO_2 . The protonation constants of the ligand were determined by direct pH-potentiometric titration by titrating 2.28 mM ligand solutions with a standardized NaOH solution in the pH range of 1.80–12.00. The protonation constants of the ligand and the stability constant of its Gd^{3+} complex are defined as $K_{\text{HIL}}=[\text{H}_i\text{L}]/[\text{H}_{i-1}\text{L}][\text{H}^+]$ and $K_{\text{GdL}}=[\text{GdL}]/[\text{Gd}^{3+}][\text{L}]$. The determination of the stability constant of $[\text{Gd}(\text{cddadpa})]^-$ complex was carried out using the ^1H -relaxometric method by measuring the longitudinal relaxation times of the samples acquired in a wide pH range ($\text{pH}=1-12$ for the samples with $\text{pH}<1.8$ the $\text{pH}=-\log C_{\text{H}^+}$). 34 samples were prepared for this purpose containing Gd^{3+} and the cddadpa^{4-} ligand at 1

mm concentration ($I=0.15$ M). The samples were equilibrated for one day and their T_1 relaxation times were then recorded. The measurements were performed with a Bruker Minispec MQ20 NMR analyzer (20 MHz, 25 °C) using the inversion recovery method ($180^\circ - \tau - 90^\circ$) at 14 different τ values. The data were fitted by using the molar relaxivities of $[\text{Gd}(\text{cddadpa})]^-$ and Gd^{3+} determined independently (5.73 and 13.27 mm^2s^{-1} at 25 °C and 20 MHz, respectively) by using literature protocols. These data were fitted simultaneously with the pH-potentiometric titration data for the sample containing equimolar amounts of the ligand and Gd^{3+} . The protonation and stability constants were calculated from the titration data with the PSEQUAD program.^[28] The $\text{pGd}=-\log[\text{Gd}]_{\text{free}}$ values of the complexes were calculated by using the protonation constants of the ligands and stability constants of the complexes at physiological pH=7.4 using 10 μM ligand and 1 μM Gd^{3+} ion concentrations as suggested by K. N. Raymond and co-workers.^[20]

Kinetic measurements: The rates of the metal exchange reactions involving the $[\text{Gd}(\text{cddadpa})]^-$ complex and the Cu^{2+} ion were studied by using UV-Vis spectrophotometry following the formation of the $[\text{Cu}_2(\text{cddadpa})]$ complex. The conventional UV-vis spectroscopic method was applied to follow the decomplexation reactions of $[\text{Gd}(\text{cddadpa})]^-$ as these reactions were very slow even at relatively low pH. The absorbance vs. time kinetic curves were acquired by using a Jasco V-670 UV-vis spectrophotometer equipped with Peltier thermostatted multicell holder. The temperature was maintained at 25 °C and the ionic strength of the solutions was kept constant by using 0.15 M NaCl. For keeping the pH constant, 50 mM dimethylpiperazine (dmp) buffer was used ($\log K_2^{\text{H}} = 4.15$ (0.03) as determined by using pH-potentiometry). The exchange reactions were followed at 310 nm in the pH range 3.38–4.96 for 4–5 days continuously (60–90% conversion) while absorbance readings at equilibrium were determined by allowing the reactions to react for 3–4 weeks depending on the pH of the samples (8–10 times longer than the half-life of the reaction). The concentration of the $[\text{Gd}(\text{cddadpa})]^-$ complex was 0.311 mM, while the Cu^{2+} ion was applied at high excess (11.1 to 40.9 fold) in order to guarantee pseudo-first order conditions. The absorbance vs. time reaction profiles could perfectly fitted by using the monoexponential function (Eq. 2):

$$A_t = (A_0 - A_e)e^{-k_{\text{obs}}t} + A_e \quad [2]$$

where A_t , A_0 , and A_e are the absorbance at time t , at the start and at equilibrium, respectively. The pseudo-first-order rate constants were fitted with the computer program Micromath Scientist, version 2.0 (Salt Lake City, UT, USA) by using a standard least-squares procedure.

Dimethyl 6,6'-(((1*R*,2*R*)-cyclohexane-1,2-diylbis((2-*tert*-butoxy)-2-oxoethyl) azanediyl))bis(methylene)dipicolinate (4). A mixture of **3** (1.00 g, 2.42 mmol obtained as described in the Supporting Information) and K_2CO_3 (2.95 g, 21.3 mmol) in acetonitrile (100 mL) was stirred for 30 min, and then *tert*-butyl-2-bromoacetate (0.99 g, 5.08 mmol) was added. The mixture was stirred at room temperature for 4 days under an inert atmosphere (Ar), and later at 45 °C for a period of 3 days. The excess K_2CO_3 was filtered off, the filtrate was concentrated to dryness and the yellow oil was extracted with a 1:3 mixture of H_2O and CHCl_3 (200 mL). The organic phase was evaporated to dryness giving an oily residue that was purified by column chromatography on SiO_2 with a $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 5% mixture as the eluent to give 1.01 g of **4** as a pale yellow oil. Yield 65%; ^1H NMR (500 MHz, CDCl_3 , 25 °C, TMS): δ = 8.05 (d, $^3J = 7.8$ Hz, 2H), 7.94 (d, $^3J = 7.8$ Hz, 2H), 7.58 (t, $^3J = 7.8$ Hz, 2H), 3.98 (s, 6H), 3.92 (d, $^2J = 15.1$ Hz, 2H), 3.80 (d, $^2J = 15.1$ Hz, 2H), 3.38 (d, $^2J = 16.8$ Hz, 2H), 3.27 (d, $^2J = 16.8$ Hz, 2H), 2.64 (m, 2H), 2.13 (m, 2H), 1.74 (m, 2H), 1.43 (s, 18H), 1.11 ppm (m, 4H); ^{13}C NMR (125.8 MHz, CDCl_3 , 25 °C, TMS): δ = 171.3, 166.0, 161.7, 146.7, 136.9, 127.6, 123.5, 80.4, 61.6, 56.0, 52.8, 52.6, 28.1, 26.2, 25.9 ppm; IR (ATR): ν = 1721 cm^{-1} (C=O); HR-MS (ESI⁺, $\text{MeOH}:\text{CH}_3\text{CN}:\text{H}_2\text{O}$ 9:1:1): m/z calcd for $[\text{C}_{34}\text{H}_{49}\text{N}_4\text{O}_8]^{+}$:

641.3544; found: 641.3554; elemental analysis calcd (%) for $\text{C}_{34}\text{H}_{48}\text{N}_4\text{O}_8$: C 63.73, H 7.55, N 8.74; found: C 64.02, H 7.54, N 8.86.

6,6'-(((1*R*,2*R*)-cyclohexane-1,2-diylbis((carboxymethyl)azanediyl))bis(methylene)dipicolinic acid ($\text{H}_4\text{cddadpa} \cdot 3\text{HCl} \cdot 2\text{H}_2\text{O}$). A solution of compound **4** (1.01 g, 1.58 mmol) in 6 M HCl (50 mL) was heated to reflux for 24 h, and then the solvent was removed in a rotary evaporator to give a yellow oil. A small amount of H_2O was added (~20 mL) and the mixture evaporated to dryness. This process was repeated once with addition of H_2O and twice with addition of diethyl ether (~20 mL) returning 0.77 g of the desired ligand as a dark yellow solid. Yield 75%; ^1H NMR (500 MHz, D_2O , pD 2.5, 25 °C, TMS): δ = 8.10–7.86 (m, 6H), 4.26–3.35 (m, 10H), 2.22 (m, 2H), 1.83 (m, 2H), 1.45 (m, 2H), 1.29 ppm (m, 2H); ^{13}C NMR (125.8 MHz, D_2O , pD 2.5, 25 °C, TMS): δ = 171.4, 164.9, 152.3, 145.2, 129.4, 125.7, 125.6, 62.4, 52.7, 50.5, 23.8 ppm; IR (ATR): ν = 1714, 1627 cm^{-1} (C=O); MS (ESI⁺, $\text{MeOH}:\text{CH}_3\text{CN}:\text{H}_2\text{O}$ 9:1:1): m/z 539 $[\text{C}_{24}\text{H}_{28}\text{KN}_4\text{O}_8]^{+}$; elemental analysis calcd (%) for $\text{C}_{24}\text{H}_{28}\text{N}_4\text{O}_8 \cdot 3\text{HCl} \cdot 2\text{H}_2\text{O}$: C 44.63, H 5.46, N 8.67; found: C 44.46, H 5.41, N 8.49.

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Keywords: gadolinium • NMR imaging • contrast agents • coordination compounds • lanthanides

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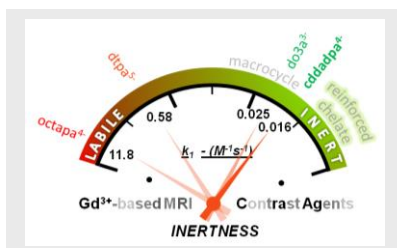
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Entry for the Table of Contents

Headline: Rigid=inert

COMMUNICATION

The Gd^{3+} complex of a rigidified non-macrocyclic octadentate ligand (cddadpa^{4-}) shows favourable properties as a magnetic resonance imaging (MRI) contrast agent: high thermodynamic stability, relatively high relaxivity thanks to the presence of a coordinated water molecule and a remarkable kinetic inertness comparable to those typically shown by complexes with macrocyclic ligands.



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Approaching the Kinetic Inertness of Macrocyclic Gd^{3+} -based MRI Contrast Agents with Highly Rigid Open-Chain Derivatives