Supplementary Material

Gd(III) and Ga(III) complexes with a new tris-3,4-HOPO ligand towards new imaging probes: complex stability, magnetic properties and biodistribution

Silvia Chaves,[a] Karolina Gwizdała,[a,b] Karam Chand,[a] Lurdes Gano,[c] Agnès Pallier,[d] Éva Tóth[d] and M. Amélia Santos[*,a]

[a.] *Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001, Lisboa, Portugal.*
[b.] *Faculty of Chemistry, Gdansk University of Technology, Gabriela Narutowicza 11/12, 80-233 Gdansk, Poland.*
[c.] *Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, 2695-066 Bobadela LRS, Portugal.*
[d.] *Centre de Biophysique Moléculaire, CNRS UPR 4301, Université d'Orléans, rue Charles Sadron, 45071 Orléans, France.*

Equations used for the analysis of the ^{17}O NMR and NMRD data[i]

(a) ^{17}O NMR spectroscopy

From the measured ^{17}O NMR relaxation rates of the paramagnetic solutions, $1/T_2$ and ω, and from the acidified water reference, $1/T_{2A}$ and ω_A, it is possible to calculate the reduced relaxation rates, $1/T_{2r}$ (Equation S1), where $1/T_{2m}$ is the relaxation rate of the bound water and $\Delta\omega_m$ is the chemical shift difference between bound and bulk water, τ_m is the mean residence time or the inverse of the water exchange rate k_{ex} and P_m is the mole fraction of the bound water.

$$\frac{1}{T_{2r}} = \frac{1}{P_m}\left[\frac{1}{T_2} - \frac{1}{T_{2A}}\right] = \frac{1}{\tau_m}\frac{T_{2m}^{-2} + \tau_m^{-1}T_{2m}^{-1} + \Delta\omega_m^2}{(\tau_m^{-1} + T_{2m}^{-1})^2 + \Delta\omega_m^2} + \frac{1}{T_{2os}} \qquad [S1]$$

The outer sphere contribution to the ^{17}O relaxation rates $1/T_{2OS}$ can be neglected and equation S1 can be further simplified into Equation S2:

$$\frac{1}{T_{2r}} = \frac{1}{T_{2m} + \tau_m} \qquad [S2]$$

The exchange rate is assumed to be described by the Eyring equation (Equation S3), where $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are the entropy and enthalpy of activation for the water exchange process, and k_{ex}^{298} is the exchange rate at 298.15 K. R is the usual gas constant whereas h and k_B are the Planck and Boltzmann constants, respectively:

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_B T}{h} exp\left\{\frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{RT}\right\} = \frac{k_{ex}^{298} T}{298.15} exp\left\{\frac{\Delta H^{\ddagger}}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right\} \quad [S3]$$

In the transverse relaxation the scalar contribution, $1/T_{2sc}$, is the most important. In Equation S4, $A/\hbar$ is the scalar coupling constant and $1/\tau_{si}$ is the sum of the exchange rate and the electron spin relaxation rate constants (Equation S5).

$$\frac{1}{T_{2m}} \cong \frac{1}{T_{2sc}} = \frac{S(S+1)}{3}\left(\frac{A}{\mathsf{h}}\right)^2\left(\tau_{s1} + \frac{\tau_{s2}}{1 + \omega_S^2 \tau_{s2}^2}\right) \quad [S4]$$

$$\frac{1}{\tau_{si}} = \frac{1}{\tau_m} + \frac{1}{T_{ie}} \quad [S5]$$

The longitudinal and transverse electronic relaxation rates, $1/T_{1e}$ and $1/T_{2e}$ are expressed by Equations S6-S7, where τ_V is the correlation time for the modulation of the zero-field-splitting interaction, E_V the corresponding activation energy and Δ^2 is the mean square zero-field-splitting energy. We assumed a simple exponential dependence of τ_V versus $1/T$.

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25}\Delta^2 \tau_v \left\{4S(S+1) - 3\right\}\left(\frac{1}{1 + \omega_S^2 \tau_v^2} + \frac{4}{1 + 4\omega_S^2 \tau_v^2}\right) \quad [S6]$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2 \tau_v \left(\frac{5.26}{1 + 0.372\omega_S^2 \tau_v^2} + \frac{7.18}{1 + 1.24\omega_S \tau_v}\right) \quad [S7]$$

$$\tau_v = \tau_v^{298} exp\left\{\frac{E_v}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right\} \quad [S8]$$

(b) ^{1}H NMRD

The measured longitudinal proton relaxation rate, R_1^{obs}, is the sum of the paramagnetic and diamagnetic contributions as expressed in Eq. S9, where r_1 is the proton relaxivity:

$$R_1^{obs} = R_1^d + R_1^p = R_1^d + r_1 \times c_{Gd} \qquad \text{[S9]}$$

The relaxivity can be divided into terms of inner and outer sphere, as follows:

$$r_1 = r_{1is} + r_{1os} \qquad \text{[S10]}$$

The inner sphere term is obtained in Equation S11, where q is the number of inner sphere water molecules.

$$r_{1is} = \frac{1}{1000} \times \frac{q}{55.55} \times \frac{1}{T_{1m}^H + \tau_m} \qquad \text{[S11]}$$

The longitudinal relaxation rate of inner sphere protons, $1/T_{1m}^H$ is expressed by Equation S12, where r_{GdH} is the effective distance between the electron charge and the ^1H nucleus, ω_I is the proton resonance frequency and ω_S is the Larmor frequency of the Gd^{III} electron spin. The correlation time, τ_d, is dependent on the water residence time, τ_m, the electron spin relaxation times, T_{ie}, and the rotational correlation time, τ_R.

$$\frac{1}{T_{1m}^H} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{h^2 \gamma_I^2 \gamma_S^2}{r_{GdH}^6} S(S+1) \times \left[3J(\omega_I;\tau_{d1}) + 7J(\omega_S;\tau_{d2})\right] \qquad \text{[S12]}$$

For slowly rotating species, the spectral density functions are described the Lipari-Szabo approach.[ii] In this model we distinguish two statistically independent motions; a rapid local motion with a correlation time τ_l and a slower global motion with a correlation time τ_g. Supposing the global molecular reorientation is isotropic, the relevant spectral density functions are expressed as in Equations (S13-S17), where the general order parameter S^2 describes the degree of spatial restriction of the local motion. If the local motion is isotropic, $S^2 = 0$; if the rotational dynamics is only governed by the global motion, $S^2 = 1$.

$$J(\omega_I; \tau_{d1}) = \left(\frac{S^2 \tau_{d1g}}{1 + \omega_I^2 \tau_{d1g}^2} + \frac{(1 - S^2)\tau_{d1}}{1 + \omega_I^2 \tau_{d1}^2} \right)$$

(S13)

$$J(\omega_S; \tau_{d2}) = \left(\frac{S^2 \tau_{d2g}}{1 + \omega_S^2 \tau_{d2g}^2} + \frac{(1 - S^2)\tau_{d2}}{1 + \omega_S^2 \tau_{d2}^2} \right)$$

(S14)

$$\frac{1}{\tau_{dig}} = \frac{1}{\tau_m} + \frac{1}{\tau_g} + \frac{1}{T_{ie}} \qquad i = 1, 2$$

(S15)

$$\frac{1}{\tau} = \frac{1}{\tau_g} + \frac{1}{\tau_l}$$

(S16)

$$J_i(\omega_I) = \left(\frac{S^2 \tau_g}{1 + i^2 \omega_I^2 \tau_g^2} + \frac{(1 - S^2)\tau}{1 + i^2 \omega_I^2 \tau^2} \right) \qquad i = 1, 2$$

(S17)

The rotational correlation times, τ_g and τ_l are assumed to have simple exponential temperature dependence with an E_R activation energy as given in Equation (S18).

$$\tau_v = \tau_v^{298} \, exp\left\{ \frac{E_v}{R}\left(\frac{1}{T} - \frac{1}{298.15} \right) \right\}$$

(S18)

The outer-sphere contribution can be described by Equation S19 where N_A is the Avogadro constant, and J_{os} is its associated spectral density function.

$$r_{1os} = \frac{32 N_A \pi}{405}\left(\frac{\mu_0}{4\pi} \right)^2 \frac{h^2 \gamma_S^2 \gamma_I^2}{a_{GdH} D_{GdH}} S(S + 1)[3J_{os}(\omega_I, T_{1e}) + 7J_{os}(\omega_S, T_{2e})] \quad [S19]$$

$$J_{os}(\omega, T_{je}) = Re\left[\frac{1 + \frac{1}{4}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2}}{1 + \left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2} + \frac{4}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right) + \frac{1}{9}\left(i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{3/2}} \right]$$

$$j = 1, 2 \quad [\text{S20}]$$

The diffusion coefficient for the diffusion of a water proton away from a Gd^{III} complex, D_{GdH}, is assumed to obey an exponential law versus the inverse of the temperature, with activation energy E_{GdH}, as given in Equation S21. D_{GdH}^{298} is the diffusion coefficient at 298.15 K.

$$D_{\text{GdH}} = D_{\text{GdH}}^{298} \exp\left\{ \frac{E_{\text{GdH}}}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right\} \quad [\text{S21}]$$

---

[i] Tóth, É.; Helm, L.; Merbach, A.E. Relaxivity of Gadolinium(III) Complexes (Chapter 2) in "The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging", 2nd edition, Eds. Merbach, A.E.; Helm L.; Tóth É., John Wiley & Sons, 2013 pp: 25-82.

[ii] Dunand, F.A.; Tóth, E.; Hollister, R., Merbach, A.E. Lipari-Szabo approach as a tool for the analysis of macromolecular gadolinium(III)-based MRI contrast agents illustrated by the [Gd(EGTA-BA-(CH$_2$)$_{12}$)]$_n^{n+}$ polymer. *J. Biol. Inorg. Chem.* **2001**, *6*, 247–255.
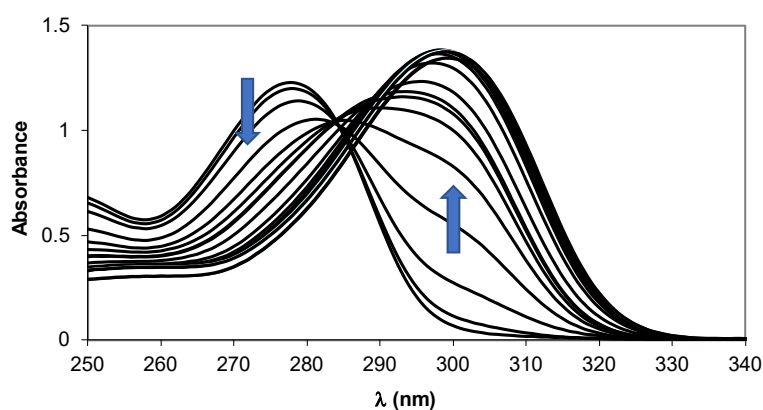
Figure S1. Spectrophotometric absorption spectra of the 1:1 Ga^{III}/H$_3$**L2** system recorded for $0.8 < \text{pH} < 6.95$ ($C_L = 5.3 \times 10^{-5}$ M).
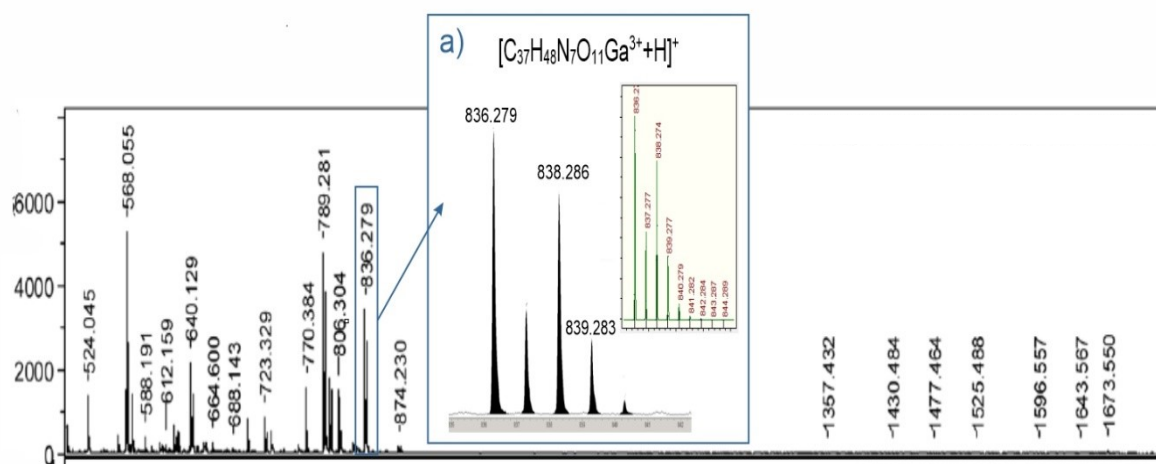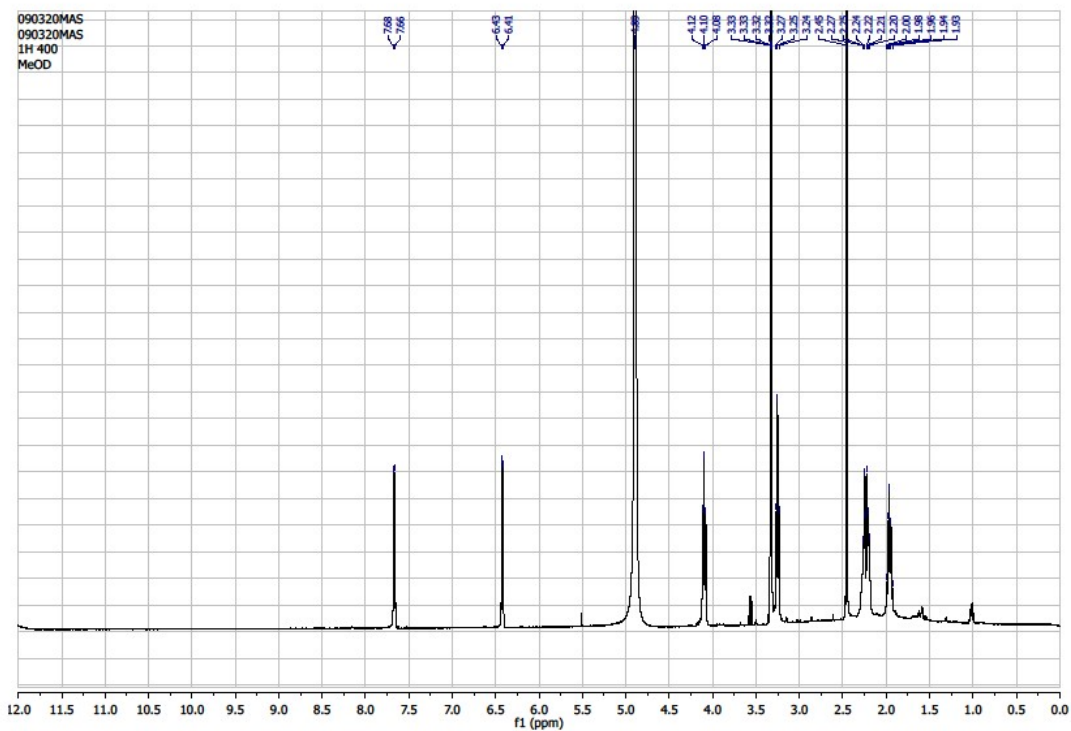
Figure S2. MALDI-TOF mass measurement spectrum of a solution of Ga^{III}/H_3**L2** (pH= 4.18) with matrix CHCA. The group of peaks at *m/z* 836/838 displays an isotopic distribution pattern that agrees well with that calculated for $[Ga^{III}(C_{37}H_{48}N_7O_{11}) + H]^+$ as shown in insert a).

¹HNMR

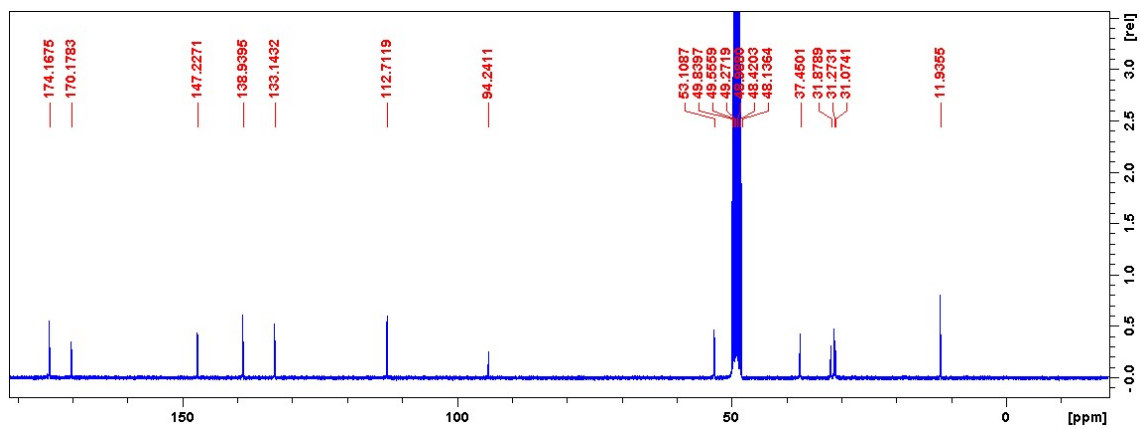## ¹³CNMR

Figure S3. NMR plots of H₃L2.