Supporting information

A novel Albumin-binding macrocyclic Gd-HPDO3A complex bearing a deoxycholic acid residue. The role of hydration state, water exchange and local dynamics on the observed relaxivity

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Fitting models

- *^{17}O water transversal relaxation rate vs temperature*

The exchange lifetime (τ_M) of the metal bound water molecule in a paramagnetic chelate may be accurately assessed by measuring the temperature dependence of the paramagnetic contribution (R_{2p}^O) to the observed water ^{17}O transverse relaxation rate (R_{2obs}^O).

R_{2p}^O is related to τ_M through the values of $\Delta\omega_M^O$ (i.e. the ^{17}O chemical shift difference between coordinated and bulk water molecule) and R_{2M}^O (which represents the transverse relaxation rate of the coordinated water oxygen) according to the Swift and Connick equation (eq. 2 and 3).[1] In the presence of two isomers/species (namely A and B) characterized by different water exchange dynamics, two contributions to R_{2p}^O can be defined:

$$R_{2p}^O = R_{2obs}^O - R_{2dia}^O = R_{2pA}^O + R_{2pB}^O \qquad (1)$$

$$R_{2pA}^O = \frac{qC_A}{55.6}\tau_{MA}^{-1}\frac{{R_{2MA}^O}^2 + \tau_{MA}^{-1}R_{2MA}^O + {\Delta\omega_{MA}^O}^2}{\left(R_{2MA}^O + \tau_{MA}^{-1}\right)^2 + {\Delta\omega_{MA}^O}^2} \qquad (2)$$

$$R_{2pB}^O = \frac{qC_B}{55.6}\tau_{MB}^{-1}\frac{{R_{2MB}^O}^2 + \tau_{MB}^{-1}R_{2MB}^O + {\Delta\omega_{MB}^O}^2}{\left(R_{2MB}^O + \tau_{MB}^{-1}\right)^2 + {\Delta\omega_{MB}^O}^2} \qquad (3)$$

where R_{2dia}^O is the contribution to R_{2obs}^O measured for a solution lacking the Gd(III) complex, q is the number of inner sphere water molecules, C_A and C_B are the concentrations of A and B isomers, respectively (with $C_A+C_B=C_{tot}$), τ_{MA} and τ_{MB} are the exchange lifetimes of coordinated water molecule in A and B.

For relatively small-sized Gd(III) chelates, R_{2M}^O is essentially dominated by the electron-nucleus scalar interaction, thus for A and B we have:

$$R_{2MA}^O = \frac{1}{3}\left(\frac{A}{\hbar}\right)^2 S(S+1)\left(\tau_{E1A} + \frac{\tau_{E2A}}{1+\omega_s^2\tau_{E2A}^2}\right) \qquad (4)$$

$$R_{2MB}^{O} = \frac{1}{3}\left(\frac{A}{\mathrm{h}}\right)^2 S(S+1)\left(\tau_{E1B} + \frac{\tau_{E2B}}{1+\omega_s^2\tau_{E2B}^2}\right) \qquad (5)$$

Where S is the electronic spin quantum number (7/2 for Gd(III)), $\frac{A}{\mathrm{h}}$ is the Gd-^{17}O scalar coupling constant (we used a value of - 3.8×10^{6}rad s^{-1})^2 and τ_{EiA} and τ_{EiB} (with i=1, 2) represent the correlation times modulating the scalar interaction. This modulation may occur either through the longitudinal and transverse electronic relaxation times (T_{1E} and T_{2E}) or the mean residence lifetime τ_M of the water molecule at the paramagnetic site, i.e.

$$\tau_{EiA}^{-1} = T_{iEA}^{-1} + \tau_{MA}^{-1} \qquad (6)$$

$$\tau_{EiB}^{-1} = T_{iEB}^{-1} + \tau_{MB}^{-1} \qquad (7)$$

The temperature dependence of R_{2M}^{O} is then expressed by the temperature effect on τ_M and $\Delta\omega_M^O$ according to the following equations:

$$\Delta\omega_{MA}^{O} = \frac{g_e\mu_B S(S+1)B_0}{3k_B T}\frac{A}{\mathrm{h}} \qquad (8)$$

$$\Delta\omega_{MB}^{O} = \frac{g_e\mu_B S(S+1)B_0}{3k_B T}\frac{A}{\mathrm{h}} \qquad (9)$$

where B_0 is the magnetic field strength, k_B is the Boltzmann constant, g_e is the g factor for the free electron, μ_B is the Bohr magneton.

$$(\tau_J)_T = (\tau_j)^{298,15}\exp\left[\frac{E_j}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right] \qquad (10)$$

where *j* refers to the two different dynamic processes involved (*j* = *v*, *M*).

-^{1}H water longitudinal relaxation rate as a function of magnetic field

The relaxivity of a Gd(III) complex results from contributions arising mainly from water molecules in the inner- and the outer-coordination spheres:

$$r_1 = r_1^{His} + r_1^{Hos} \qquad (11)$$

r_I^{His} refers to the contribution from the exchange of the water protons in the first coordination sphere of the paramagnetic metal ion:

$$r_1^{His} = \frac{q[GdL]}{55.56 \times (T_{1\mathrm{M}}^{\mathrm{H}} + \tau_M)} \qquad (12)$$

where *q* is the inner sphere hydration number, [GdL] is the molar concentration of the Gd-complex, T_{IM}^H is the longitudinal relaxation time of the inner-sphere water protons and τ_{M} is their residence lifetime. The classical Solomon-Bloembergen theory [3,4] provides the magnetic field dependence of T_{IM}^H and was applied to fit NMRD profiles of **Gd-HIBDO3A-DCA, Gd-HPDO3A-DCA and Gd-HIBDO3A in PBS**:

$$\frac{1}{T_{1M}^H} = \frac{2}{15} \frac{\gamma_{\mathrm{H}}^2 g_e^2 \mu_B^2 S(S+1)}{r_{\mathrm{H}}^6} \left[\frac{3\tau_{\mathrm{c1}}}{1+\omega_{\mathrm{H}}^2 \tau_{\mathrm{c1}}^2} + \frac{7\tau_{\mathrm{c2}}}{1+\omega_{\mathrm{S}}^2 \tau_{\mathrm{c2}}^2} \right] \qquad (13)$$

where *S* is the electron spin quantum number (7/2 for Gd(III), γ_H is the proton nuclear gyromagnetic ratio, μ_B is the Bohr magneton, g_e is the Landè factor for the free electron, r_H is the distance between the metal ion and the inner-sphere water protons; ω_H and ω_S are the proton and electron Larmor frequencies (ω_S = 658.21× ω_H), respectively and τ_{ci} (*i* =1,2) are the correlation times related to the modulation of the dipolar electron-proton coupling. Such an interaction may be modulated by the reorientation of the paramagnetic species, τ_R, by the residence lifetime, τ_M and by the electronic relaxation times, T_{iE} .

$$\tau_{ci}^{-1} = \tau_R^{-1} + \tau_M^{-1} + T_{iE}^{-1} \qquad (14)$$

Experimental data relative of the NMRD profile of **Gd-HIBDO3A-DCA in human serum** were fitted with the Solomon-Bloembergen-Morgan equations modified according to the Lipari–Szabo model-free approach.[5] This model considers both a local internal rotation, characterized by a correlation time τ_{RL}, and a global motion described by τ_{RG}. The correlation between these two motions is quantified by the order parameter K^2, which varies in the range 0-1, where a value of zero indicates complete independence between the motions, and a value of 1 suggests immobilization in the absence of local fluctuations. Equations 13-14 were modified as follows:

$$\frac{1}{T_{1M}^{H}} = \frac{2}{15}\frac{\gamma_{\mathrm{H}}^2 g_e^2 \mu_B^2 S(S+1)}{r_{\mathrm{H}}^6}\left[\frac{3K^2\tau_{\mathrm{c1g}}}{1+\omega_{\mathrm{H}}^2\tau_{\mathrm{c1g}}^2} + \frac{3(1-K^2)\tau_{\mathrm{c1}}}{1+\omega_{\mathrm{H}}^2\tau_{\mathrm{c1}}^2} + \frac{7K^2\tau_{\mathrm{c2g}}}{1+\omega_{\mathrm{S}}^2\tau_{\mathrm{c2g}}^2} + \frac{7(1-K^2)\tau_{\mathrm{c2}}}{1+\omega_{\mathrm{S}}^2\tau_{\mathrm{c2}}^2}\right] \qquad (15)$$

$$\tau_R^{-1} = \tau_{RG}^{-1} + \tau_{RL}^{-1} \qquad (16)$$

$$\tau_{ci}^{-1} = \tau_R^{-1} + \tau_M^{-1} + T_{iE}^{-1} \qquad (17)$$

$$\tau_{cig}^{-1} = \tau_{RG}^{-1} + \tau_M^{-1} + T_{iE}^{-1} \qquad (18)$$

Analogously to the nuclear relaxation time, the electronic relaxation times depend on the magnetic field strength. For Gd(III) complexes T_{iE} is determined by the modulation of the transient zero field splitting (ZFS) of the electronic spin states caused by the dynamic distortions of the ligand field and, according to the Blombergen-Morgan theory, their magnetic field dependence is given by the following equations:

$$T_{1E}^{-1} = \frac{1}{25}\Delta^2\tau_{\mathrm{v}}\left[4S(S+1)-3\right]\left(\frac{1}{1+\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2} + \frac{4}{1+4\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2}\right) \qquad (19)$$

$$T_{2E}^{-1} = \frac{1}{50}\Delta^2\tau_{\mathrm{v}}\left[4S(S+1)-3\right]\left(3 + \frac{5}{1+\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2} + \frac{2}{1+4\omega_{\mathrm{S}}^2\tau_{\mathrm{v}}^2}\right) \qquad (20)$$

where Δ^2 is the square of the transient ZFS energy and τ_v is the correlation time related to its modulation.

The outer sphere term, r_I^{Hos}, describes the contribution from water molecules which diffuses around the paramagnetic complex and, according to the model developed by Hwang and Freed,[3] may be related to the minimum distance between the metal and the outer-sphere water protons, *a*, the relative solute-solvent diffusion coefficient, *D*, and, again, the electronic relaxation times, T_{iE} :

$$r_1^{Hos} = C^{os}\left(\frac{1}{aD}\right)\left[7\mathrm{J}(\omega_\mathrm{S}) + 3J(\omega_H)\right] \qquad (21)$$

where C^{OS} is a constant ($5.8\cdot10^{-13}$ $s^{-2}M^{-1}$) and the dependence on the electronic relaxation times is expressed in the non-Lorentzian spectral density functions $J(\omega_i)$.

-***Proton Relaxation Enhancement (PRE) for the determination of binding affinity to macromolecules***

The binding parameters involved in the non-covalent interaction between a paramagnetic chelate and a macromolecular system may be conveniently performed through the well-consolidated PRE technique.

When a paramagnetic complex interacts with a macromolecule the following equilibrium is established:

$$\mathrm{GdL} + \mathrm{M} \leftrightarrow \mathrm{GdL/M}$$

The affinity constant K_A is the equilibrium constant and is given by the equation:

$$K_A = \frac{[GdL/M]}{[GdL][nM]} \qquad (22)$$

in which [nM] indicates the concentration of the equivalent and independent binding sites and GdL represents the Gd^{III} chelate.

The measured longitudinal proton relaxation rate (R_{1obs}) is given by the sum of the contributions arising from the unbound and the bound species as well as the diamagnetic contribution of the host (R_{1M}):

$$R_{1obs} = \left(r_1[GdL] + r_1^b[GdL/M]\right)1000 + R_{1M} \qquad (23)$$

Where r_1 and r_1^b are the millimolar relaxivity values of the unbound and bound GdL respectively.

Combination of the equations 22 and 23, allows to correlate the measured R_{1obs} to the binding parameters K_A and n, as follows:

$$R_{1obs} = \frac{(K_A GdL_T + nK_A M_T + 1) - \sqrt{(K_A GdL_T + nK_A M_T + 1)^2 - 4K_A^2 GdL_T nM_T}}{2K_A}$$
$$-(r_1^b - r_1 + r_1 GdL_T)1000 + R_{1M} \qquad (24)$$

Where GdL_T and M_T are the total molar concentrations of the **GdL** and the host macromolecule, respectively.

References

1) T. J. Swift, R. E. J. Connick, *J. Chem. Phys.* 1962, *37*, 307
2) D. H. Powell, O. M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer, A. E. Merbach, J. Am. Chem. Soc. 1996, 118, 93332 9346.
3) L. Banci, I. Bertini, C. Luchinat, Nuclear and Electronic Relaxation,VCH, Weinheim, 1991, p. 91.
4) N. Bloembergen, L. O. Morgan, J. Chem. Phys. 1961, 34, 8422 850.
5) Ref 45 of the main text

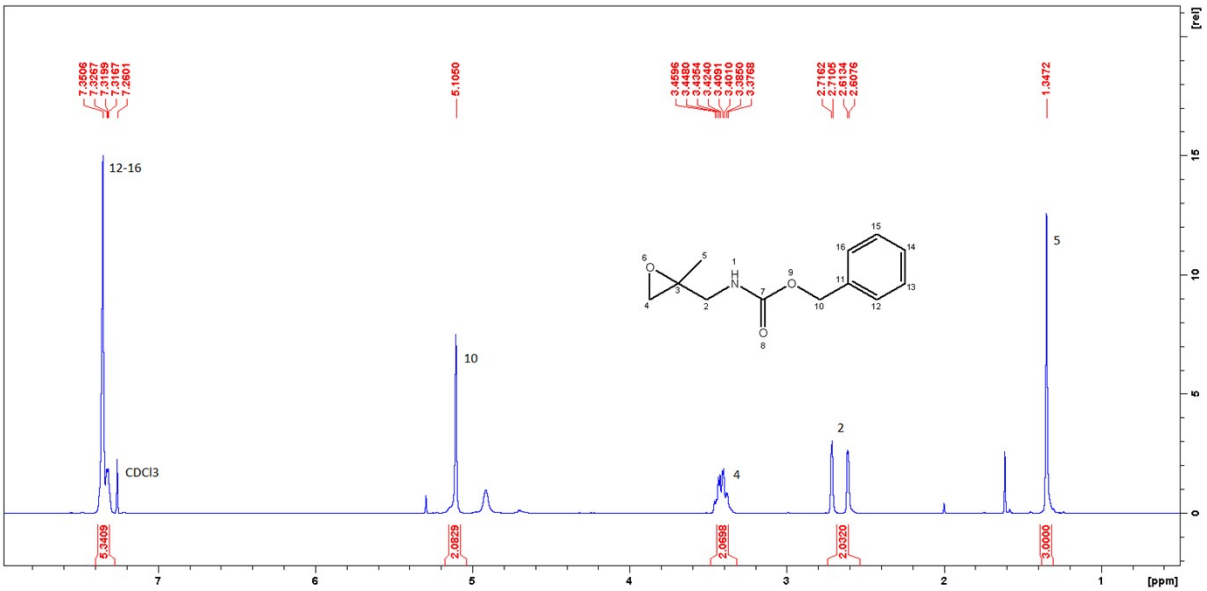


Figure S1: ^{1}H-NMR spectrum of product **2** in $CDCl_3$ at 600 MHz and 298 K.

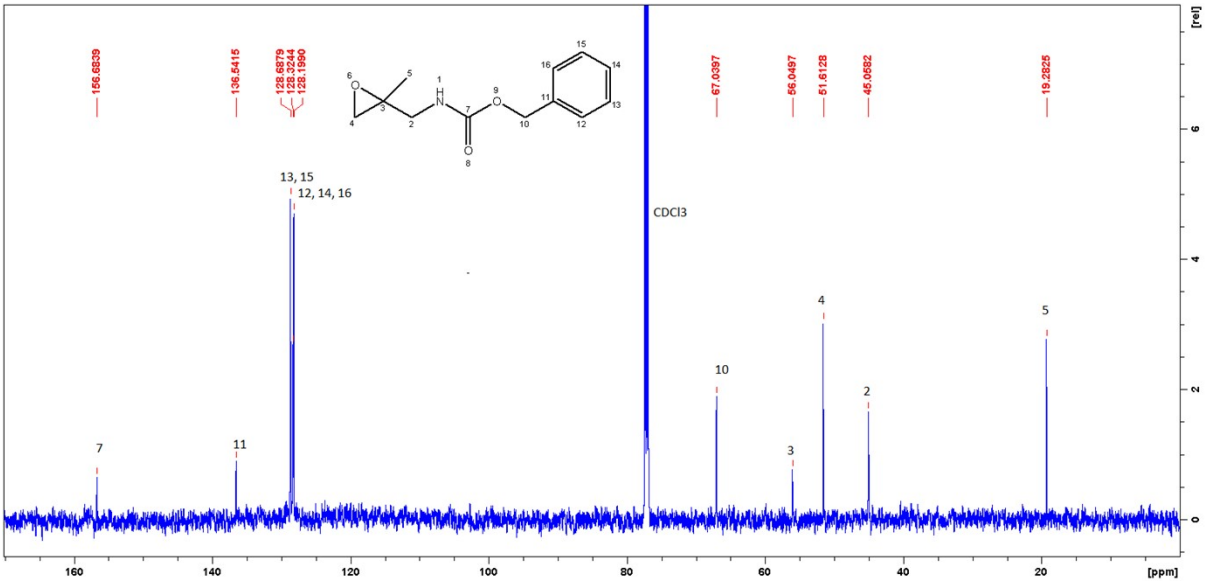


Figure S2: ^{13}C-NMR spectrum of product **2** in $CDCl_3$ at 600 MHz and 298 K.

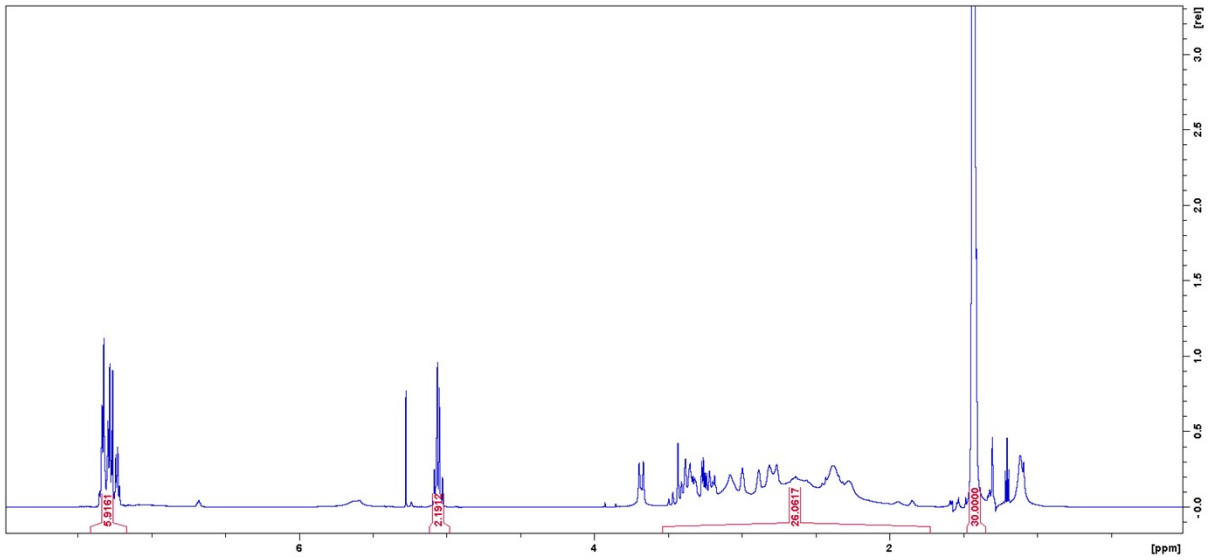


Figure S3: ^{1}H-NMR spectrum of product **3** in $CDCl_3$ at 600 MHz and 298 K.

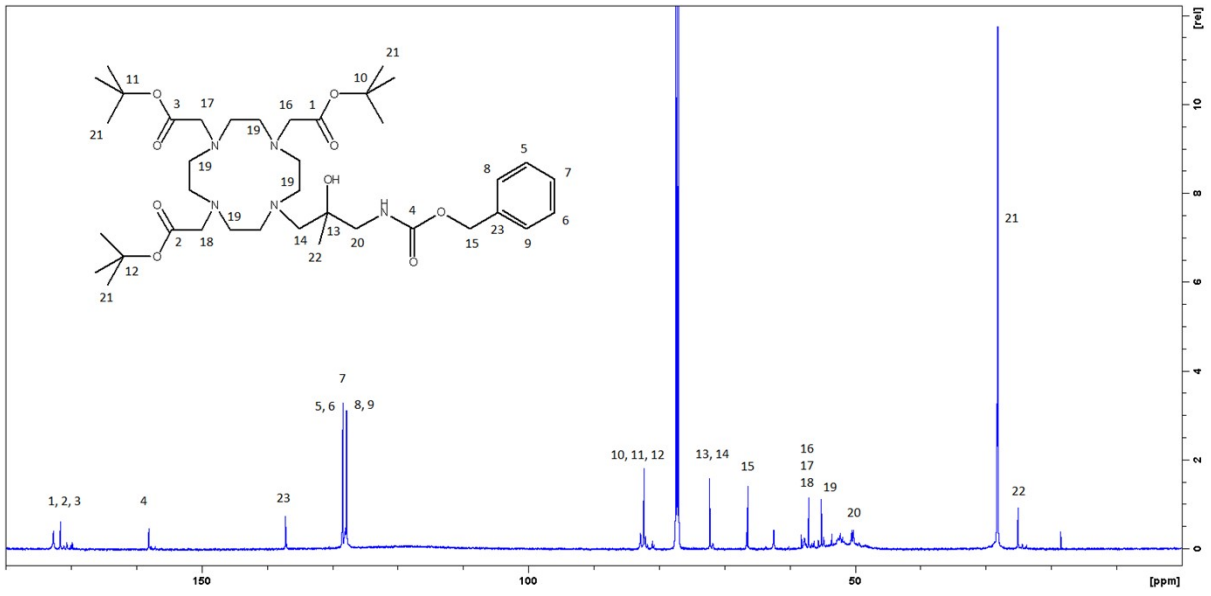


Figure S4: ^{13}C-NMR spectrum of product **3** in $CDCl_3$ at 600 MHz and 298 K.

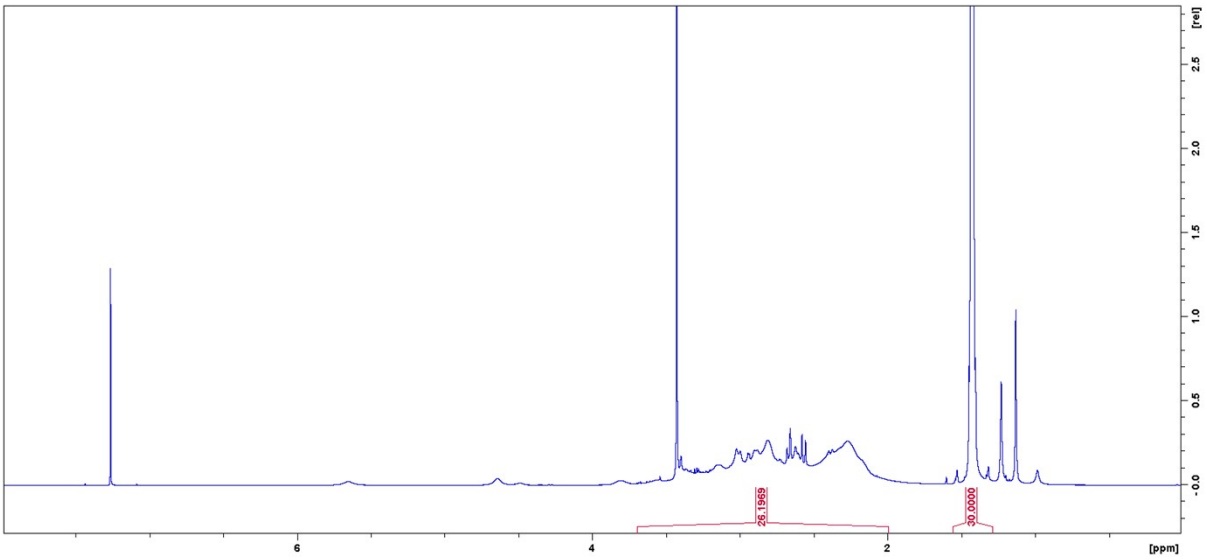


Figure S5: ^{1}H-NMR spectrum of product **4** in $CDCl_3$ at 600 MHz and 298 K.

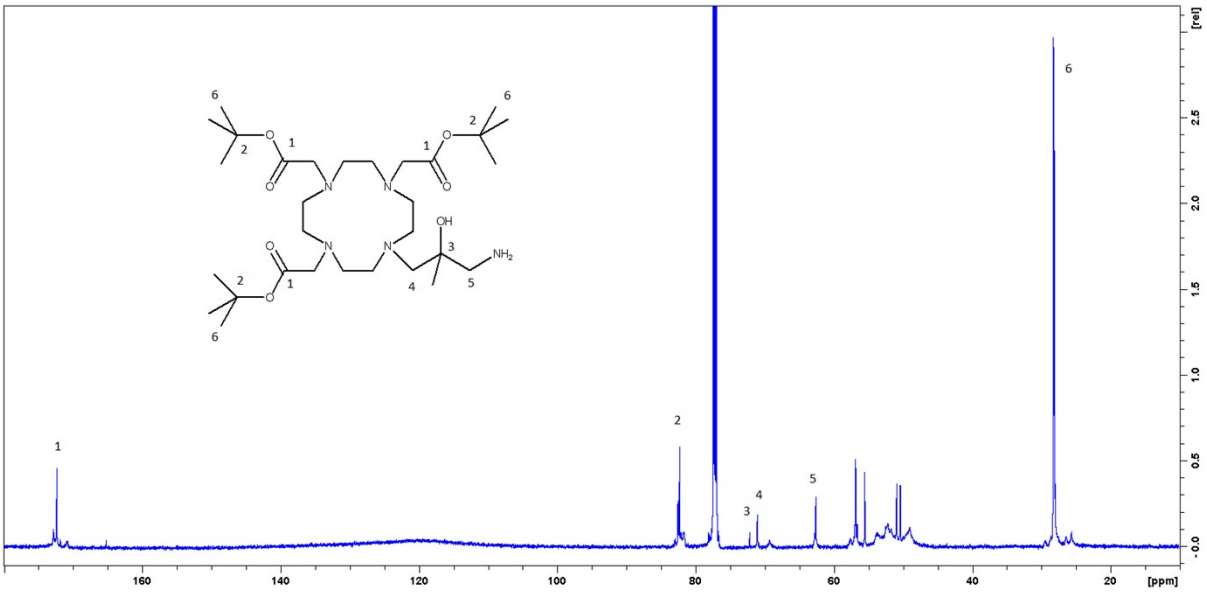


Figure S6: ^{13}C-NMR spectrum of product **4** in $CDCl_3$ at 600 MHz and 298 K.

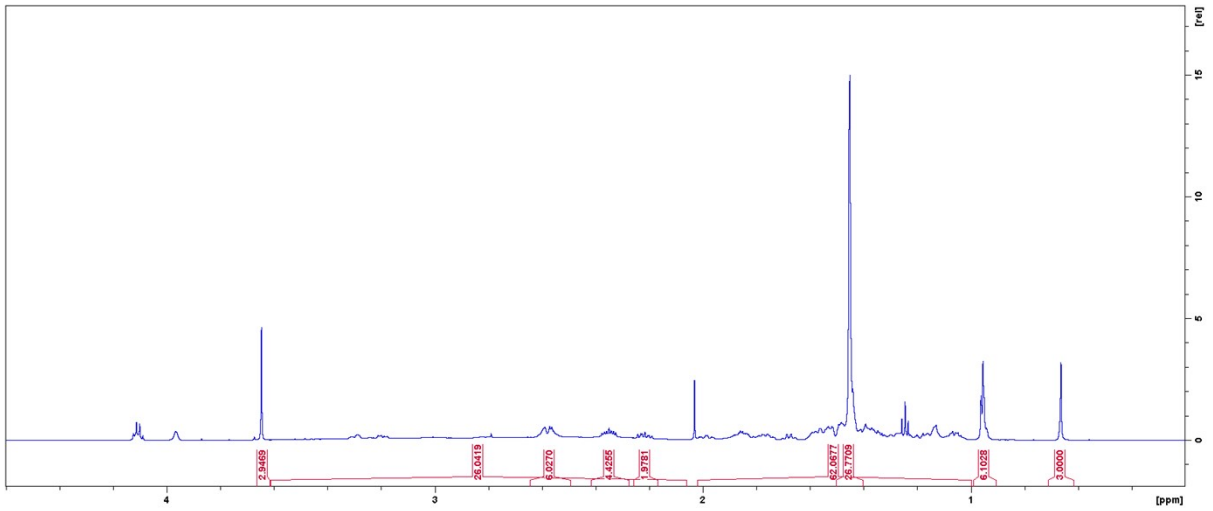


Figure S7: ^{1}H-NMR spectrum of product **5** in $CDCl_3$ at 600 MHz and 298 K.

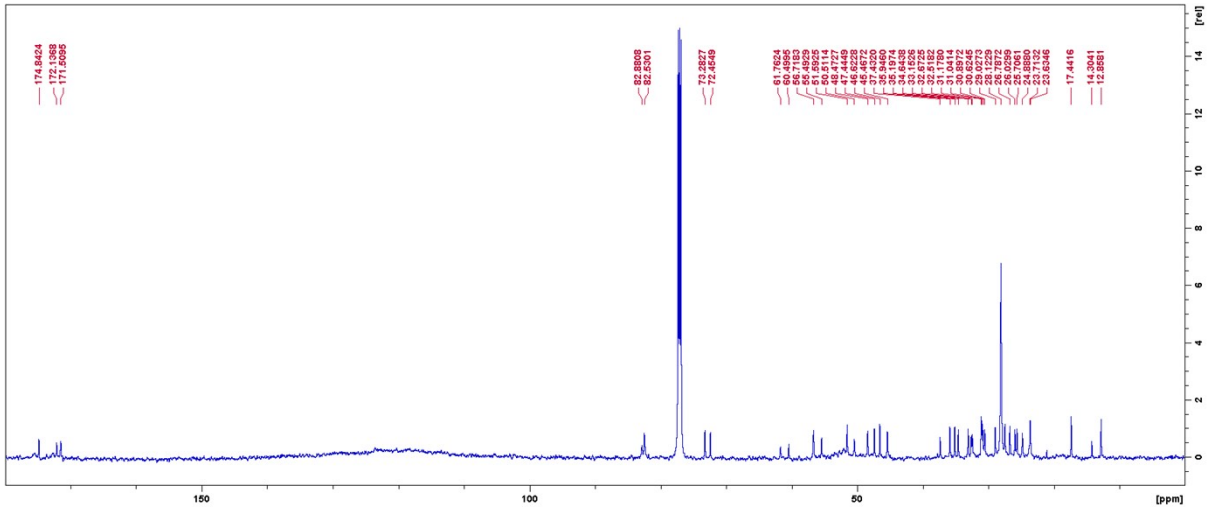
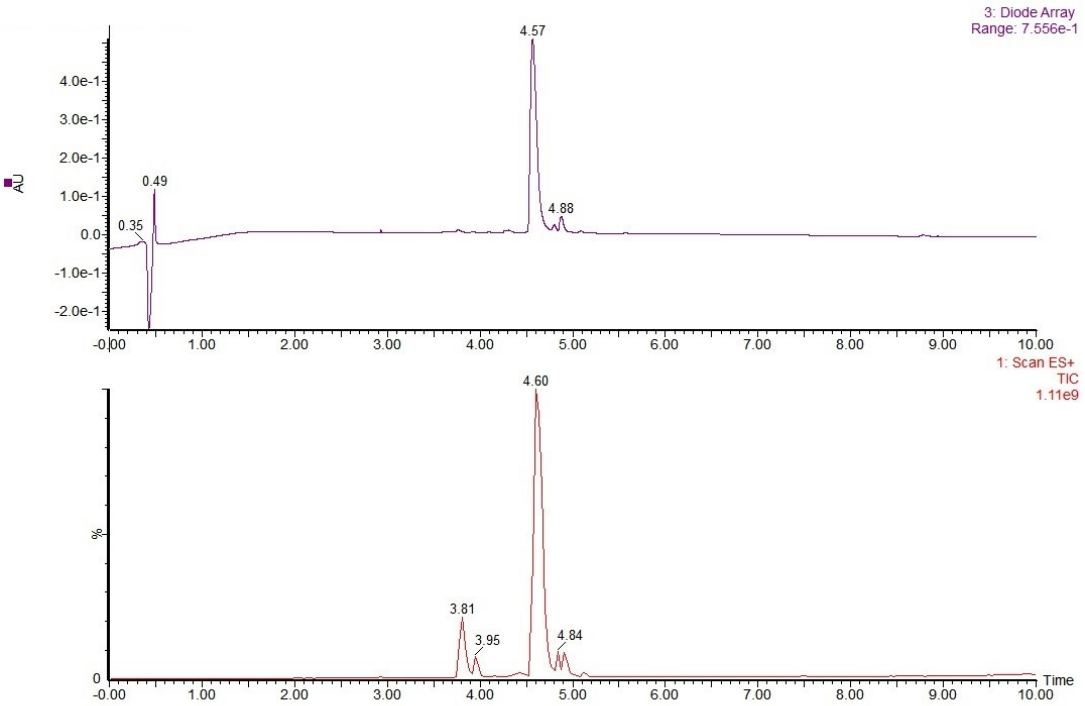


Figure S8: ^{13}C-NMR spectrum of product **5** in $CDCl_3$ at 600 MHz and 298 K.

a)



b)

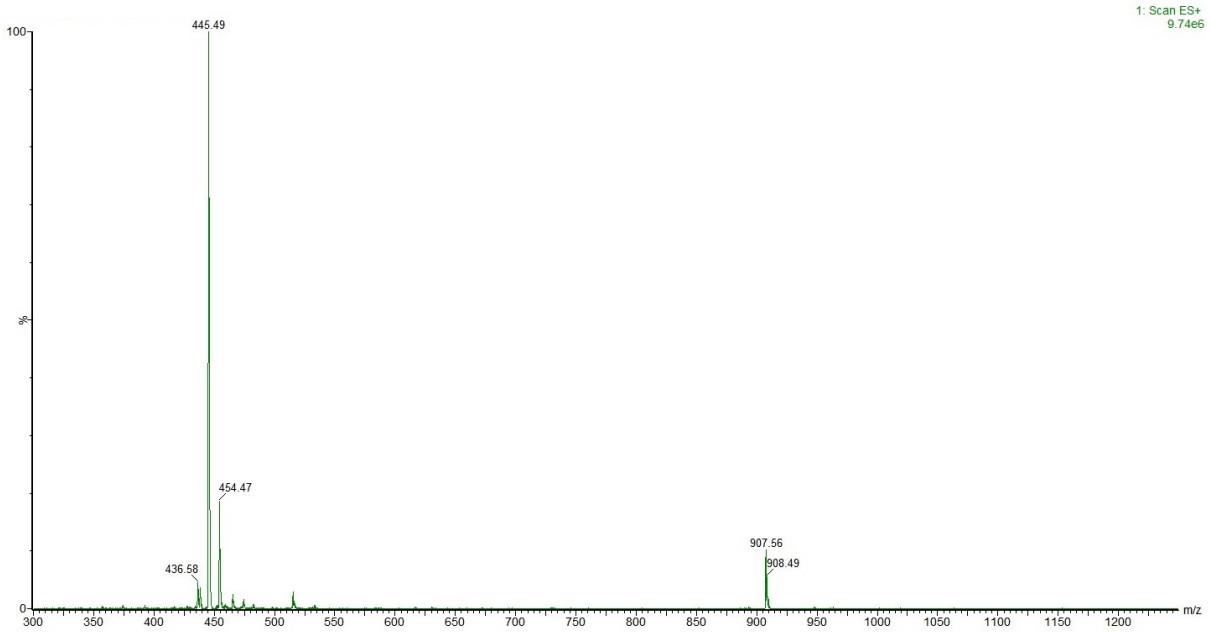


Figure S9: a) Chromatogram (210 nm and ESI+), purity >91% and b) Mass spectrum in ESI+ of peak at 4.6 min of product **6**.

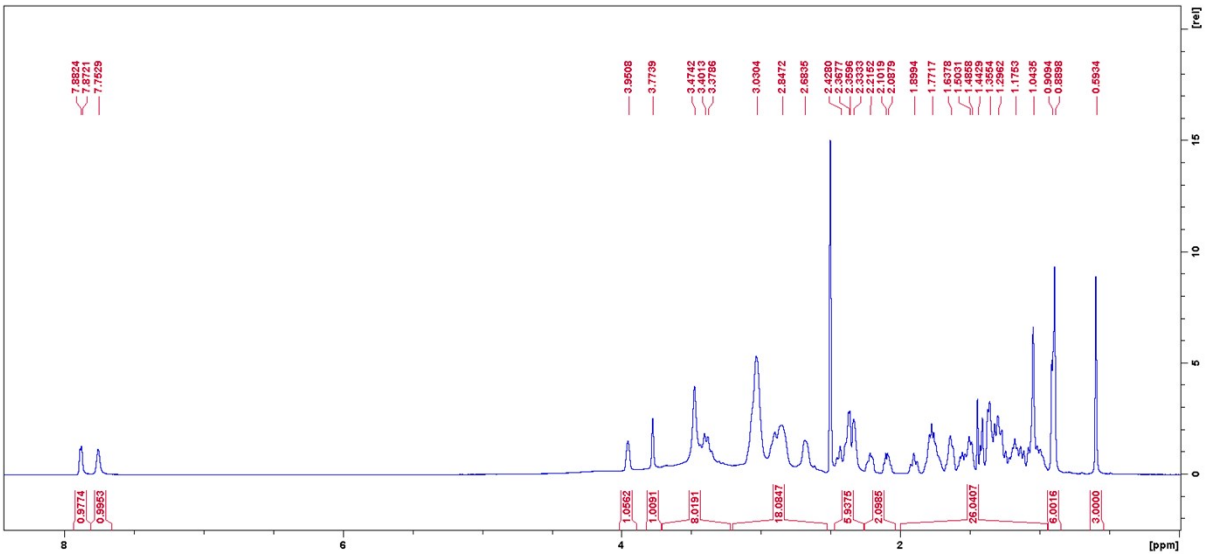


Figure S10: [1]H-NMR spectrum of product **6** in DMSO d6 at 600 MHz and 298 K.

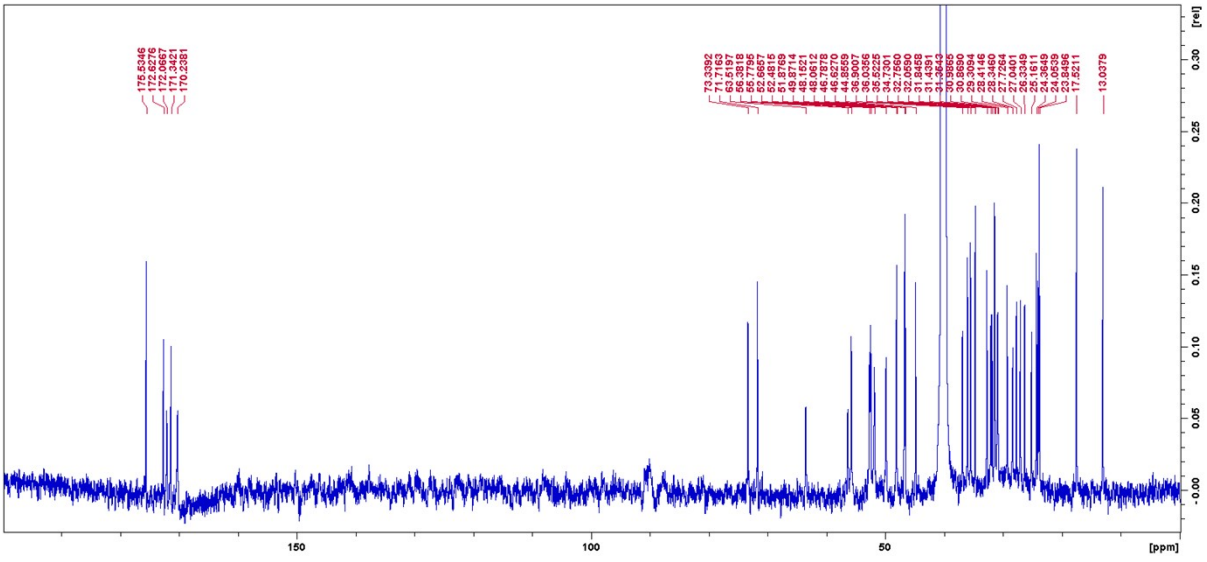
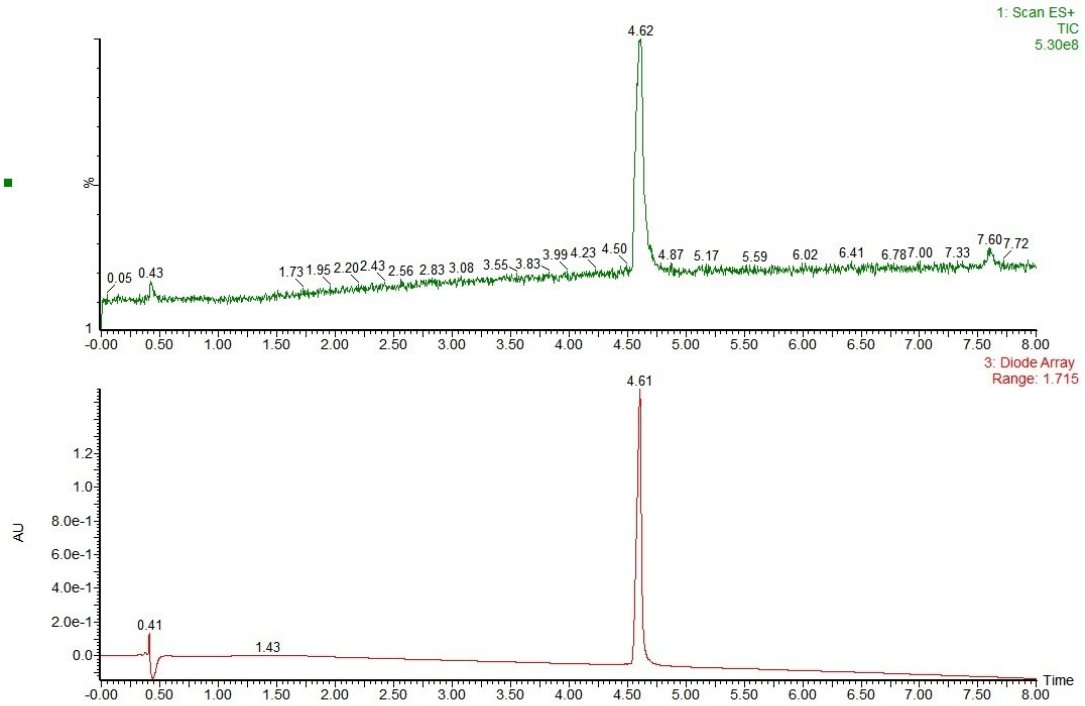


Figure S11: [13]C-NMR spectrum of product **6** in DMSO d6 at 600 MHz and 298 K.

a)



b)

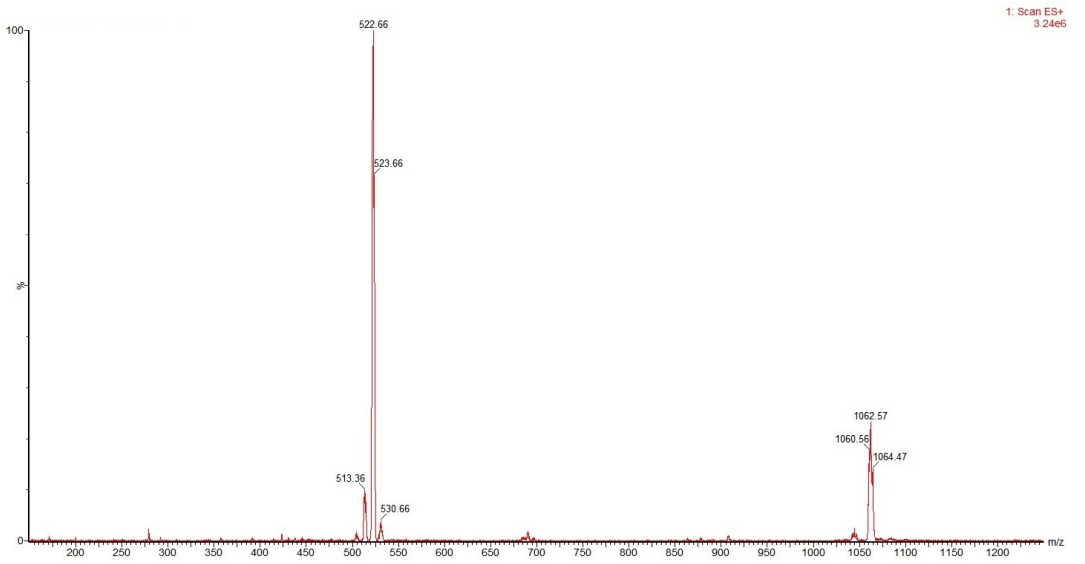


Figure S12: a) Chromatogram (210 nm and ESI+), purity >99% and b) Mass spectrum in ESI+ of peak at 4.61 min of the Gd complex.

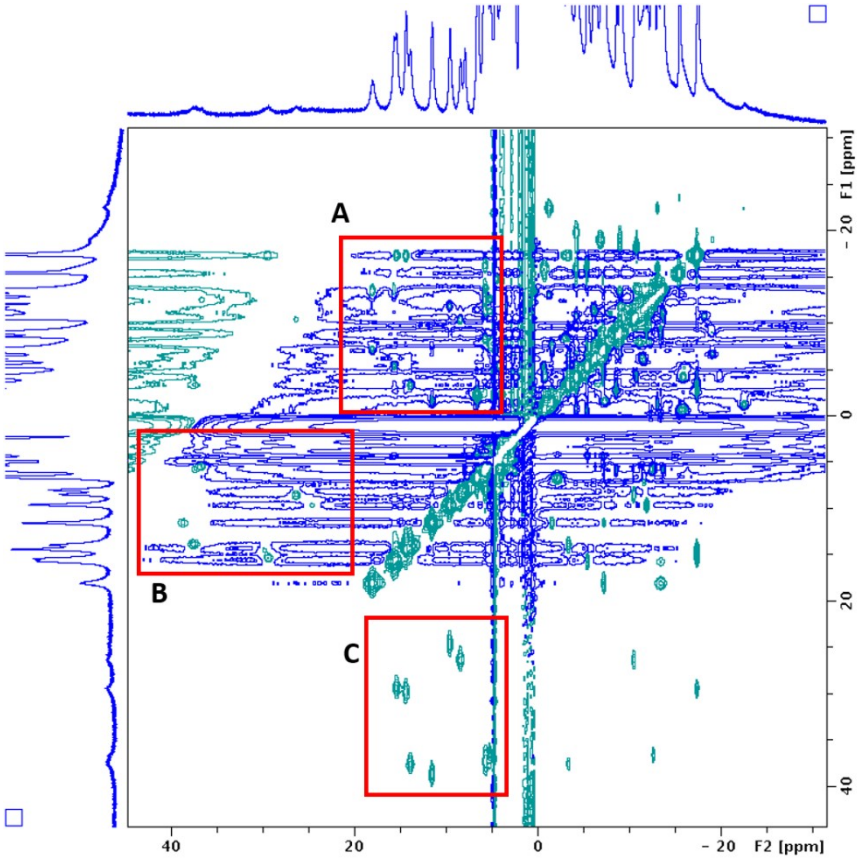


Figure S13: 2D-EXSY 1H NMR of Eu-HIBDO3A-DCA (20 mM) in D_2O at pD 7.4, 298 K and 14.1 T with a mixing time of 5 ms. Box (A) indicates ring inversion and boxes (B) and (C) indicate arm rotation between SAP and TSAP isomers.

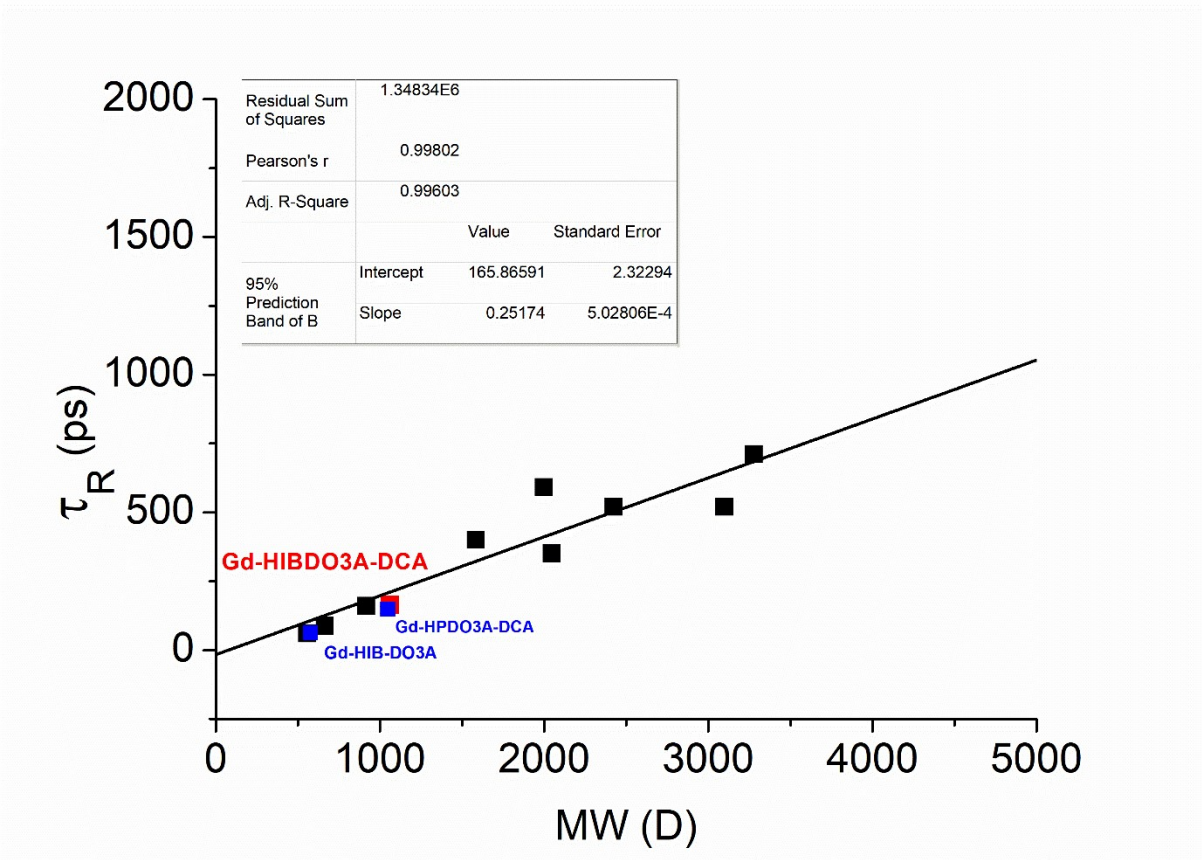


Figure S14: Linear dependence of the τ_R values obtained from fitting the NMRD profiles reported in figure 6A and the molecular weight of the corresponding Gd-complexes. Data relative to Gd-HIBDO3A, Gd-HPDO3A-DCA and Gd-HIBDO3A-DCA are compared to those obtained for other q=1 Gd-complexes reported in the literature.

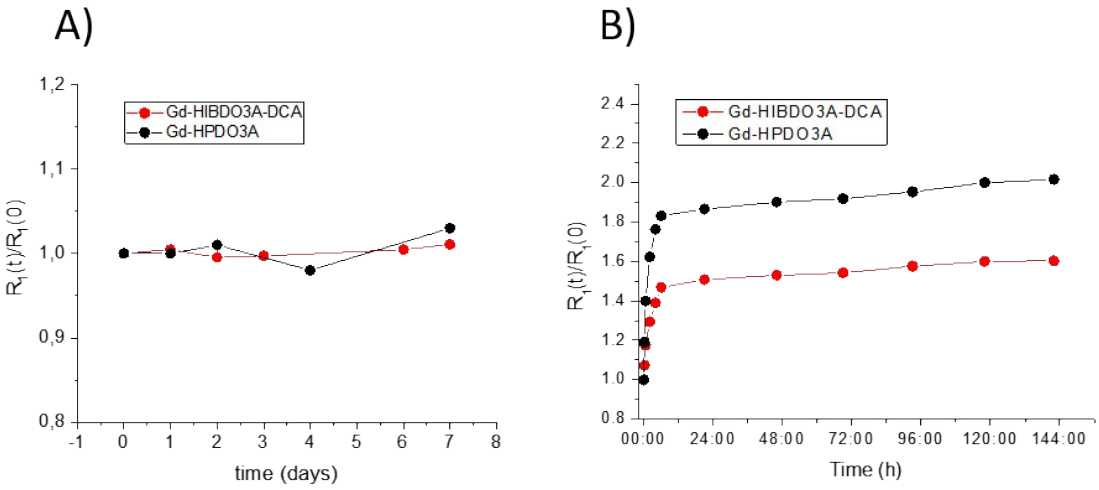


Figure S15: A) Transmetalation of Gd-complexes with 1 eq. Zinc in 50 mM phosphate buffer at 310 K and pH 7.4, measured at 0.47 T; B) Evaluation of inertness of Gd-complexes in 1 M HCl solution at 298K, measured at 0.47T.

room temperature.

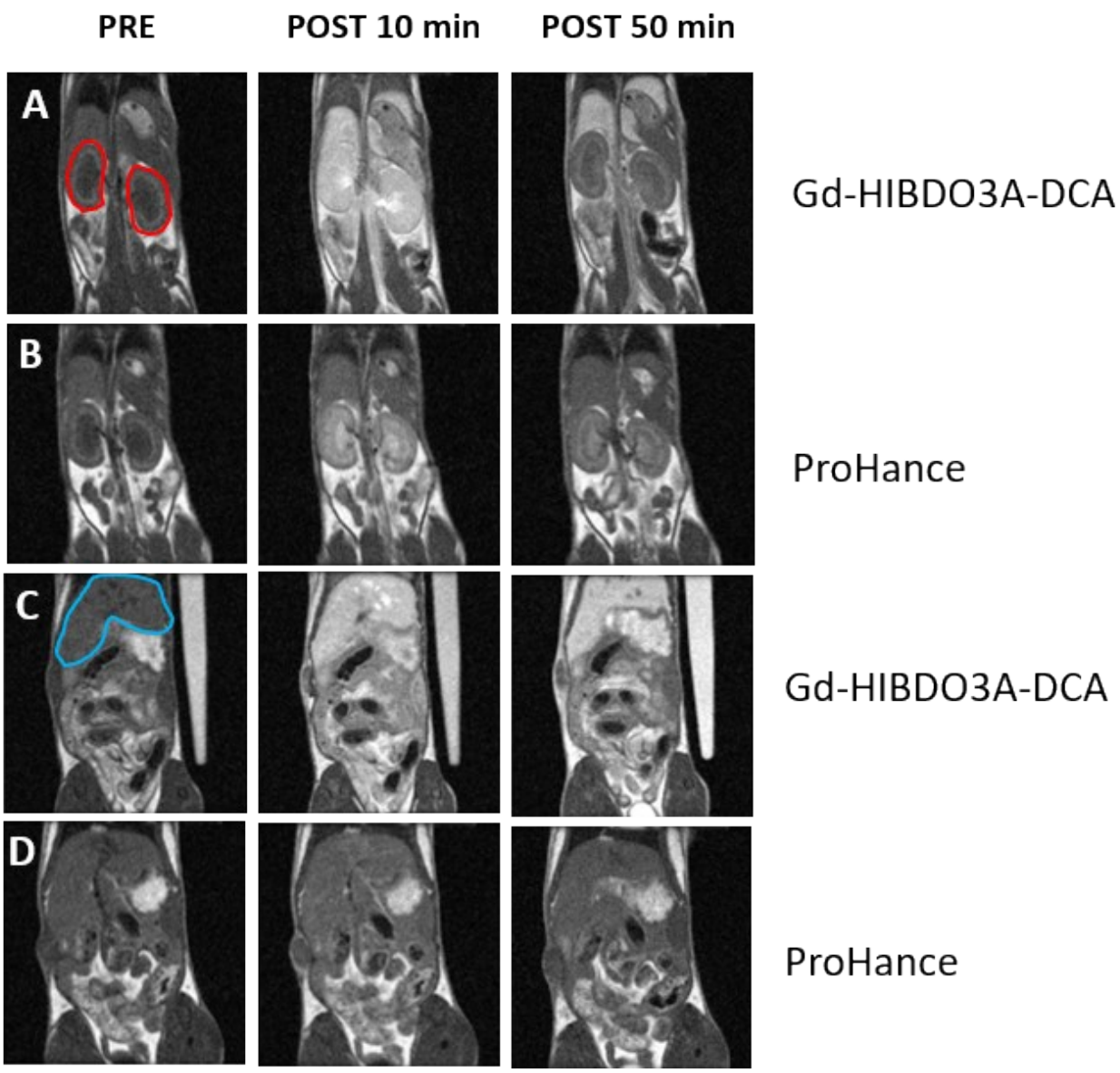


Figure S16: Representative T1-weighted MSME acquired at 1T for healthy tissues: kidneys (A, B – red line) and liver (C,D-blue line) obtained pre and after injection with Gd-HPDO3A (ProHance) and Gd-HIBDO3A-DCA. Images show left to right pre-contrast, and post-contrast at 10 and 50 min.

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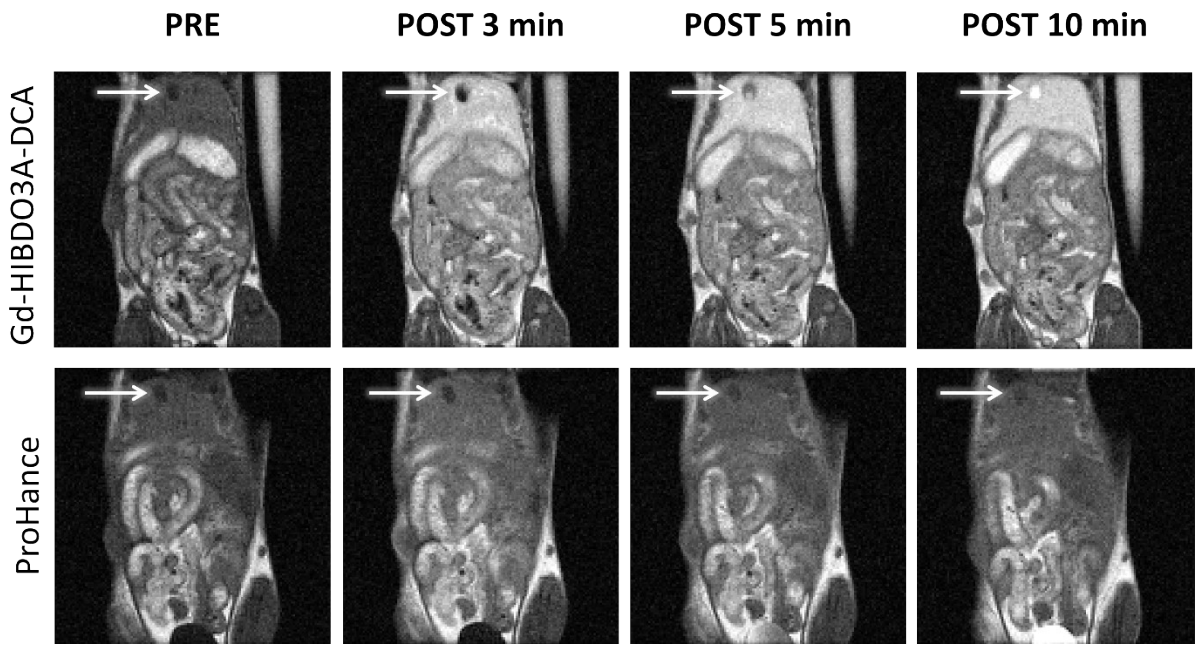

Figure S17: Representative T1-weighted MSME images acquired at 1T showing the enhancement of bile accumulated in the gallbladder (indicated by white arrows) obtained pre- and post-injection of Gd-HPDO3A (ProHance) and Gd-HIBDO3A-DCA. Images display, from left to right, pre-contrast and post-contrast at different time points.

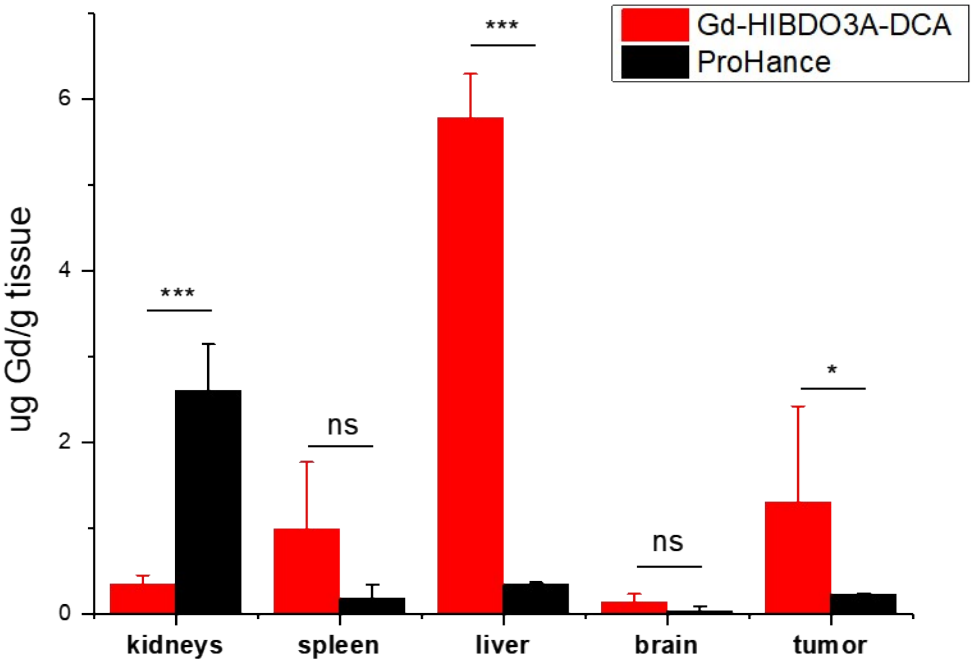

Figure S18: Biodistribution of Gd-HIBDO3A-DCA and ProHance in healthy BALB/c mice 4 hours after the administration of 0.05 mmol/Kg of contrast agent as determined by ICP-MS analysis. The Gd^{3+} contents are presented as µg per gram of tissue and given as mean ± SD from 3 different mice.

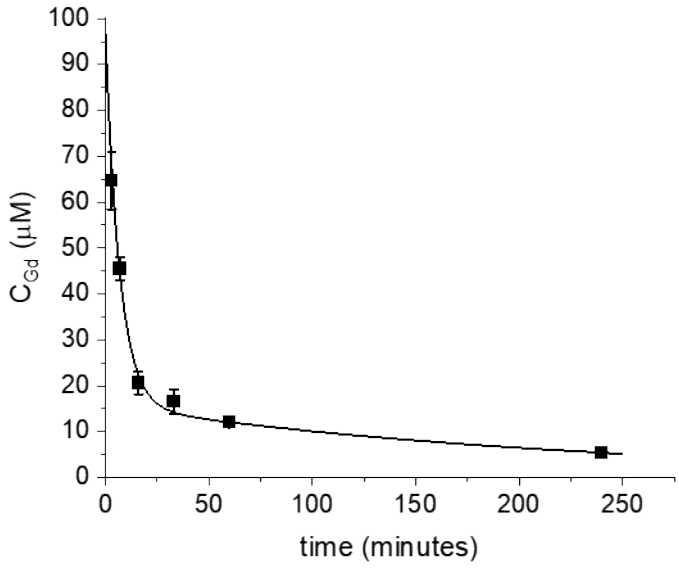

Figure S19: Blood elimination curve of Gd-HIBDO3A-DCA upon the intravenous administration of 0.05 mmol/kg of contrast agent in healthy mice. Data are reported as mean± SD from 3 different mice.

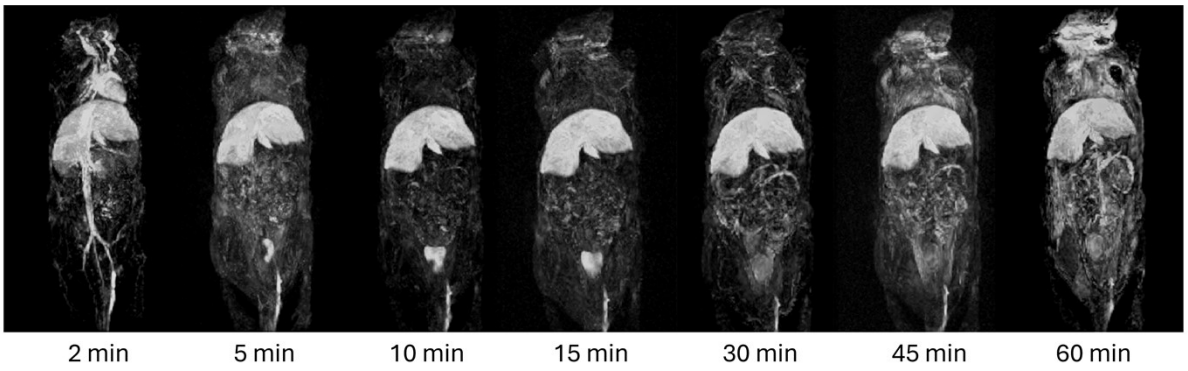

Figure S20: Whole-body coronal maximum intensity projection (MIP) of 3D FLASH images obtained following intravenous administration of Gd-HIBDO3A-DCA to a mouse at a dose of 0.05 mmol Gd/kg. Post-contrast images at 2, 5, 10, 15, 30, 45, and 60 minutes are presented here after subtraction of the corresponding pre-contrast images.