Supplementary Informations

Ho-L characterization

- Synthesis of Ho(III)-L complex

The preparation of Holmium complex has been carried out by mixing equimolar quantities of $HoCl_3$ and DO3A-based ligand at neutral pH and room temperature. The solution was stirred at room temperature for 2 hours. The formation of the Ho(III)-L complex has been followed by acquiring the ^{1}H-NMR spectra of the solution at 7.1T and 298K. The disappearance of the ligand resonances was complete after two hours. Ho(III)-L yields a very complex ^{1}H-NMR spectrum that is indicative of the presence of more isomers. The eventual excess of uncomplexed Ho(III) ions was removed by filtration of the solution brought to pH 10 to form insoluble $Ho(OH)_3$; The Orange Xylenol method (Barge A. Et al Contrast Media ans Molecular Imaging, 1, 184-188, 2006) was used to assess the absence of free Ho(III) ions from the solution of Ho(III)-L at pH 7.

- Ho(III)-L relaxivity pH dependence

Even if Ho(III) ion is not a relaxation agent and so it is expected to be characterized by very low relaxivity values, the pH dependence of its relaxivity has been determined at 298K and 7.1T. It is reported in Fig. S1. The exact concentration of Ho(III)-L complex has been determined through the Evans's method as explained in the experimental procedures paragraph.
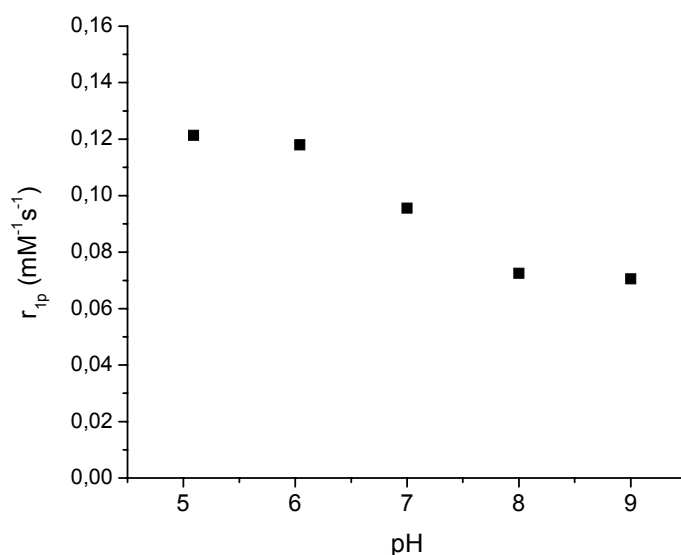

Fig. S1: Relaxivity pH dependence of Ho(III)-L complex measured at 7.1 T and 298K.

- Calibration lines for the determination of Ho(III) concentration via γ-counting experiments

The unknown concentrations of Ho-probe in the different capillaries have been calculated by the use of two different calibration lines obtained by measuring γ-rays emission at 80.6 Kev and 1.38

MeV as a function of the Ho-complex concentration (Fig. S2). The exact concentration of Ho(III)-L complex for each experimental point has been determined through the Evans's method as explained in the experimental procedures paragraph.
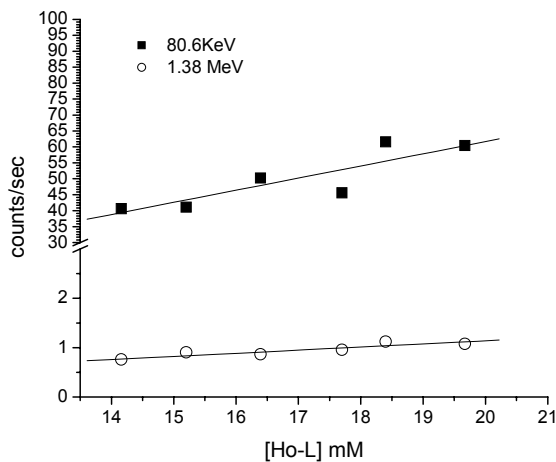
Fig. S2: γ-rays emission at 80.6 Kev (■ linear regression Y = -14.54778+ 3.8094 * X ; R= 0.852) and 1.38 MeV (○ linear regression Y = -0.13553 + 0.06372 * X ; R=0.934) as a function of the Ho-complex concentration.

Experimental Procedures

- <u>Determination of Gd(III)-L concentration through relaxometric procedure</u>

The concentration of Gd-complexes solutions was determined by mineralizing a given quantity of sample solution by the addition of HCl 37% at 120°C overnight: from the measurement of the observed relaxation rate (R_1^{obs}) at 20MHz and 298K of the acidic solution and knowing the relaxivity (r_{1p}) of Gd^{III} aquaion in acidic conditions (13.5 mM^{-1} s^{-1}), it was possible to calculate the exact Gd^{III} concentration according to the following linear correlation (this method was calibrated using standard ICP solutions, and the accuracy was determined to be 1%):

$$R_1^{obs} = 0.5 + 13.5[Gd]$$

The longitudinal water proton relaxation rates (R_1^{obs}) were measured by using a Stelar Spinmaster (Stelar, Mede, Pavia, Italy) spectrometer operating at 20 MHz, by means of the standard inversion-recovery sequence. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper constantan thermocouple (uncertainty 0.1°C).

- <u>Determination of Ho(III)-L concentration through Evans Method</u>

The Evans method is a simple technique for accurate determination of Ln(III)-complexes concentration in solution.[1] It utilizes the bulk magnetic susceptibility shift (BMS) of ^{1}H-NMR

resonance signal of an inert compound (e.g. *tert*-butyl alcohol) caused by the presence of the paramagnetic solute.

A D_2O (1% **tert**-butyl alcohol) solution of the Ln(III) compound was placed in a 5 mm NMR tube. The BMS was determined from the frequency shift of the *tert*-butyl alcohol signal with respect to a diamagnetic sample (1% *tert*butyl alcohol in D_2O) placed in a inner co-axyal tube. ^{1}H-NMR spectra were recorded at 25 °C on a Bruker Avance600 spectrometer operating at the Proton frequency of 600 MHz. Two resonance signals from the methyl protons of the *tert*-butyl alcohol, originating from the inner and outer tubes, are observed. The frequency shift of the two *tert*-butyl alcohol signals in the NMR spectrum represents the bulk magnetic susceptibility shift.

To a good approximation, the BMS shift ($\Delta\chi$) may be expressed by the following equation:

$$\Delta_\chi = \frac{4\pi CS}{T}\left(\frac{\mu_{eff}}{2.84}\right)^2 \times 10^3$$

where C is the concentration of paramagnetic solute in mol/ l^{-1}, **S** is dependent on the shape of the sample and its position in the magnetic field (**S**= 1/3,1/6 and 0 for a cylinder parallel to the main field, a cylinder perpendicular to the main field and a sphere, respectively) and in this case is equal to 1/3, **T** is the temperature (in K) and μ_{eff} is the effective magnetic moment for a particular lanthanide ion (For Ho(III) μ_{eff}= 10.6).

1. D. M. Corsi, C. Platas-Iglesias, H. van Bekkum and J.A. Peters, *Magn. Reson. Chem.* 2001; 39: 723–726

Instrumental

MRI experiments

For recording MR images, the solutions were transferred into glass capillaries and placed in an agar phantom. MR images were acquired at 298K on a Bruker Avance300 spectrometer operating at 7.1 T equipped with a microimaging probe, using a standard T_1 weighted multislice multiecho sequence (TR = 250 ms, TE = 3.3 ms, NEX = 6, FOV = 1.15x1.15 cm^2, 3 slices, slice thickness = 1 mm). T_1 values were measured by using a Saturation Recovery Spin Echo sequence (TE = 2.6 ms, 16 variable TR ranging from 40 to 5000 ms, NEX = 1, FOV = 1.1x1.1 cm^2, 3 slices, slice thickness = 1 mm)

Neutron Irradiation

For neutron irradiation, a cyclotron-driven neutron activator, realized on a modification of the ARC method, has been installed on a beam line of the Scanditronix MC40 Cyclotron (K=40) of the Joint Research Centre (JRC, Ispra, Italy). The prototype is made up of four basic components: Be target, a water cooling system, a lead buffer and a graphite reflector. The scope of the Be target is to produce secondary neutrons by proton bombardment. Neutrons are then thermalised and confined in the lead-graphite assembly. This neutron flux produced with the activator is particularly efficient for the production of radioisotopes with short-medium half-lives and high neutron capture cross sections in the thermal and epithermal region. Targets to be activated are placed into the irradiation channels where they undergo neutron irradiation.

Activated samples, removed from the insertion rods, are put in individual holders for activity measurements by γ-ray spectrometry with HPGe detectors. For the data acquisition the samples were placed at a distance of around 10 cm from the crystal. All HPGe detectors are calibrated in energy and efficiency for different geometries with certified standard radioactive sources (ENEA Italy, DAMRI and CERCA France). For activity measurement confidence, two γ-ray spectrum analysis software packages are used, the Gamma Vision acquisition system (Model A66-B32, Version 5.10, EG&G ORTEC USA), and Genie 2000 (CANBERRA, USA). The statistical counting uncertainty for the acquired γ-peaks of the different radioisotopes is maintained less than 5%.

For this study, the produced radioisotope was ^{166g}Ho from the reaction ^{165}Ho(n,γ)^{166g}Ho. ^{165}Ho has an isotopic abundance of 100%. ^{166g}Ho decays by E.C. and β- emission to the stable isotope ^{166}Er with a half-life of 26.88 h; The specific activity calculated for sample no.2 was 3 kBq/(μA.h.mg) at the end of the beam (36 MeV protons, 18 μA, 2 h).