Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

GdDOTAGA(C_{18})$_2$: an efficient amphiphilic Gd(III) chelate for the preparation of self-assembled high relaxivity MRI nanoprobes

M. Filippi,[a] D. Remotti,[b] M. Botta,[b] E. Terreno,[a] and L. Tei[b]*

[a] *Dipartimento di Biotecnologie Molecolari e Scienze della Salute, Centro di Imaging Molecolare e Preclinico, Università degli Studi di Torino, Via Nizza 52, Torino, 10126, Italia.*

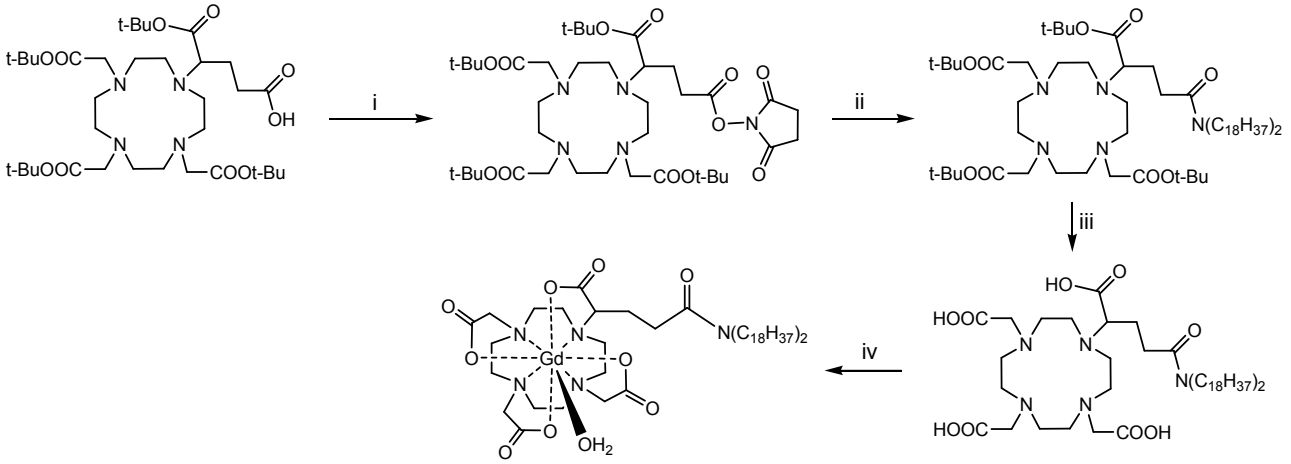
[b] *Dipartimento di Scienze ed Innovazione Tecnologica, Università del Piemonte Orientale "Amedeo Avogadro", Viale T. Michel 11, Alessandria, 15121, Italia.*

** E-mail: lorenzo.tei@uniupo.it, Telephone: +39 0131 360 208, Fax: +39 0131 360250.*

Contents:

1. Synthesis:

General. All chemicals were purchased from Sigma-Aldrich or Alfa Aesar unless otherwise stated and were used without further purification. DOTAGA(tBu)$_4$ was synthesized as reported in the literature.[1] ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance III or on JEOL ECP 400 spectrometers operating at 11.74 T and 9.4 T, respectively. Chemical shifts are reported relative to TMS and were referenced using the residual proton solvent resonances. Electrospray ionization mass spectra (ESI MS) were recorded on a SQD 3100 Mass Detector (Waters), operating in positive or negative ion mode, with 1% v/v formic acid in methanol as the carrier solvent. ESI MS spectra on Gd-complex was acquired on a high resolving power mass spectrometer LTQ Orbitrap (Thermo Scientific, Rodano, Italy), equipped with an atmospheric pressure interface and an ESI ion source. Elemental analyses were carried out with a EA3000 CHN Elemental Analyzer (EuroVector, Milano, Italy).



Scheme S1. Synthesis of GdDOTAGA(C_{18})$_2$: i: NHS, EDC, DCM, RT; ii: NH(C_{18})$_2$, pyridine, 70°C; iii: TFA/DCM 1:1 *v*/*v*, RT; iv: $GdCl_3$, MeOH, 50°C.

DOTAGA(tBu)$_4$NHS

DOTAGA(tBu)$_4$ (530 mg; 0.76 mmol) and EDC (217 mg; 1.13 mmol) were dissolved in 10 ml of dichloromethane under N_2. *N*-hydroxysuccinimide (217 mg; 1.13 mmol) was added in portion and the mixture was stirred for 4 hours at room temperature. Then the organic phase was washed with water (2 x 10 mL) and brine (2 x 10 mL) and dried over Na_2SO_4. After filtration the solvent was removed by evaporation under reduced pressure to obtain a white solid (472 mg, 0.59 mmol, 77.4% yield) . ESI-MS$^+$(m/z): 798.48 [M + H$^+$]; calc for [(DOTAGA(tBu)$_4$NHS) + H$^+$]: 798.48. ^{1}H-NMR

($CDCl_3$) 500 MHz δ_H = 3.73 (m, 1H, C*H*N), 3.5-3.0 (6H, m, CH_2COO*t*Bu), 2.95-2.8 and 2.6-2.2 (16H, m, NCH_2-CH_2N), 2.87 (4H, s, CH_2CON, NHS), 2.13 (CH_2COONHS), 1.84 (2H, m, CHCH_2) 1.49, 1.44 (36H, s, C(CH_3)$_3$). ^{13}C-NMR ($CDCl_3$) 126 MHz δ_C = 174.5, 173.3, 173.0, 168.4 (C=O), 83.1, 82.0 (*C*(CH_3)$_3$), 59.9, 56.0, 55.8, 55.7 (*C*H_2COO*t*Bu, *C*HCOO*t*Bu), 53.5, 52.9, 52.7, 52.5, 48.8, 48.7, 48.3, 47.4 (NCH_2CH_2N), 44.4 (*C*H_2COONHS), 29.6 (*C*H_2CH_2COONHS), 28.1, 28.0 (C(*C*H_3)$_3$), 25.8 (*C*H_2CON, NHS).

DOTAGA(*t*Bu)$_4$(C_{18})$_2$

DOTAGA(tBu)$_4$-NHS (352 mg; 0.44 mmol) and dioctadecylamine (232 mg; 0.44 mmol) were dissolved in 5 ml of pyridine under N_2, and the mixture was stirred for 24 hours at a constant temperature of 70 °C. Then, the solvent was removed by evaporation under vacuum. The crude was dissolved in dichloromethane (10 mL) and washed with water (2 x 10 mL) and brine (2 x 10 mL). The organic phase was then dried over Na_2SO_4, filtered and evaporated to obtain a white solid (451 mg, 0.37 mmol, 85% yield). ESI-MS$^+$ (m/z): 1205.78 [M + H$^+$]; calc for [DOTAGA(*t*Bu)$_4$(C_{18})$_2$ + H$^+$]: 1205.88. ^{1}H-NMR ($CDCl_3$) 400 MHz δ_H = 3.4 – 3.0 (7H, m, C*H*N CH_2COO*t*Bu), 2.6 - 2.0 (18H, m, CH_2CON, NCH_2CH_2N), 2.78 (4H, t, *J* = 9.0 Hz, CONCH_2), 1.66 (2H, m, NCHCH_2), 1.44, 1.42 (36H, s, C(CH_3)$_3$), 1.22 (30H, s, CH_2 C_{18} chains), 0.84 (6H, t, *J* = 8.5 Hz, CH_3 C_{18} chains). ^{13}C-NMR ($CDCl_3$) 100 MHz δ_C = 175.1, 173.0, 172.9, 171.1 (C=O), 82.3, 82.0 (C(CH_3)$_3$), 60.2, 55.9, 55.6, 55.2 (*C*H_2COO*t*Bu, *C*HCOO*t*Bu), 52.9, 52.8, 52.6, 48.5, 48.1, 48.0, 47.3, 46.3, 44.2, 42.2 (NCH_2CH_2N, CONCH_2, *C*H_2CON), 32.0 (*C*H_2CH_2CON), 29.8, 29.7, 29.4, 27.2, 22.8 (*C*H_2, C_{18}), 28.0, 27.9 (C(*C*H_3)$_3$), 14.2 (CH_3, C_{18}).

DOTAGA(C_{18})$_2$

DOTA(tBu)$_4$GA(C_{18})$_2$ (451 mg; 0.37 mmol) was dissolved in 2 mL of dichloromethane. Trifluoroacetic acid (4 mL) was added to the solution and the mixture was stirred for 2 hours at room temperature. The solution was evaporated under vacuum and the residue was recovered and evaporated thrice with HCl 2M (3 x 3 mL) and finally lyophilized. The crude was purified by crushing the solid with a solution 1:3 vol/vol (HCl (6N): EtOH). The suspension was centrifuged and the solid phase was washed with ethanol (5 x 10 ml). and dried under vacuum to obtain an off-white solid (214 mg, 0.22 mmol, 58% yield). ESI-MS$^+$ (m/z): 980.82 [M + H$^+$]; calc for [DOTAGA(C_{18})$_2$ + H$^+$]: 980.79. ^{1}H-NMR (CD_3OD) 500 MHz δ_H = 4.4 – 4.0 (6H, m, CH_2COO*t*Bu), 4.22 (1H, m, NC*H*COOH), 3.9 – 3.1 (16H, m, NCH_2-CH_2N), 2.93 (4H, m, CONCH$_2$), 2.48 (2H, m, CH_2CON), 2.10, 1.93 (2H, m, NCHCH_2CH$_2$CO), 1.30 (26H, s, CH_2 C_{18}), 0.91 (6H, t, J = 7.0 Hz, CH_3 C_{18}). ^{13}C-NMR (CD_3OD) 126 MHz δ_C = 176.0, 175.4, 174.8, 172.3 (C=O), 60.8, 56.5, 56.4,

56.3 (*C*H_2COOH, *C*HCOOH), 54-50 broad signals, 50.1, 47.9, 46.9. 43.6 (N*C*H_2*C*H_2N, CON*C*H_2, *C*H_2CONH), 36.5 (*C*$H_2$$CH_2$CON), 33.0, 30.7, 30.4, 30.3, 29.7, 23.7 (CH_2, C_{18}), 14.4 (CH_3, C_{18}).

GdDOTAGA(C_{18})$_2$

49.0 mg (0.05 mmol) of DOTAGA(C_{18})$_2$ and 18.6 mg (0.05 mmol) of $GdCl_3$ were suspended in H_2O (1 mL). The pH was brought to 6.5 by addition of few drops of a 1 M solution of NaOH and then the solution was rotavaporated. The residue was taken up in CH_3OH (10 mL) and stirred at 50 °C overnight. After evaporation of the solvent, the complex was dissolved in $CHCl_3$ (10 mL) and washed six times with H_2O (10 mL each). The final complex was obtained after evaporation of the complex (34 mg, 0.03 mmol, 60% yield).

Elemental analysis: found C 56.07% H 8.71% N 5.79%; Calc. for $C_{55}H_{101}GdNaN_5O_9 \cdot H_2O$ C 56.24%, H 8.84% N 5.96%.

ESI-HRMS$^-$(m/z): 1133.6805 [M^-]; calc for [GdDOTAGA(C_{18})$_2^-$]: 1133.6835. The isotope pattern is consistent with a Gd(III) complex.

2. Nanosystems

2.1 Nanosystem preparation

Nanosystems were prepared by the thin film hydration method.[2] Briefly, appropriate amounts of amphiphilic material (phospholipids for liposomes, and Janus Dendrimers "JDs" for dendrimersomes), DSPE-PEG2000-COOH, and the amphiphilic complexes Gd-GOTAGA(C_{18})$_2$ or Gd-GOTAMA(C_{18})$_2$, were weighted and dissolved into chloroform. For dendrimersome preparation, the JD 3,5-C12-EG-$(OH)_4$ was used to create the vesicles.[3] The vesicular membrane composition was envisaged to contain phospholipids or JDs, DSPE-PEG2000-COOH and the paramagnetic complexes always in the following molar ratio: 75:5:20. After dissolution, the organic solvent was slowly removed by means of a rotary evaporator, so that the amphiphilic components were distributed in a thin homogeneous film. After two hours under vacuum, film hydration was performed at 50°C. Amphiphilic films were hydrated with a 300 mOsm HEPES-buffered solution at pH 7.4. Vesicular suspensions were then extruded through polycarbonate filters (Lipex extruder, Northern Lipids Inc.) with pore diameters decreasing from 1 μm to 200 nm. After the extrusion, a dialysis process against isotonic buffer was performed at 4°C to remove any non-incorporated material. The mean hydrodynamic diameter of vesicles and their polydispersity were assessed by the Dynamic Light Scattering (DLS), with a Malvern Instruments particle sizer (Zetasizer Nano 90 ZS, Malvern, UK). Nanosystems provided with a mean hydrodynamic diameter of around 150 nm

and values of polydispersity index (PDI) lower than 0.2 were used for *in vitro* and *in vivo* experiments.

3. Relaxometry

3.1. *^{1}H NMR relaxation measurements*

The observed water protons longitudinal relaxation rate (R_1^{obs}) values were measured on a Stelar SpinMaster Spectrometer (Stelar Snc, Mede (PV), Italy) operating at 20 MHz by using the standard inversion recovery pulse sequence with 4 scans for each acquired data point. A precise control of the temperature was operated during the measurements by means of a Stelar VTC-91 airflow heater equipped with a calibrated copper constantan thermocouple (uncertainty of ±0.1°C). Furthermore, the real temperature inside the probe head was additionally monitored by a Fluke 52 k/j digital thermometer (Fluke, Zürich, Switzerland). The concentration of Gd^{3+} in the samples was determined by a relaxometric procedure (at 20 MHz) after mineralization. Briefly, the samples were diluted 1:2 into concentrated HCl (37%) and stored overnight at high temperature (120°C) in a sealed glass ampoule, to effectively remove the metal ions from the complexes and to obtain a solution containing free Gd^{3+} under the form of aqua-ion. The R_1^{obs} measurement (at 25°C) allowed for the accurate estimation of the Gd^{3+} concentration through a calibration line obtained by using standard solutions of $GdCl_3$.

3.2. *^{1}H NMRD profiles:*

The Nuclear Magnetic Resonance Dispersion profiles (^{1}H NMRD profiles) were recorded on a field cycling relaxometer (Stelar Spinmaster FFC 2000; Stelar Snc, Mede, Pavia, Italy) equipped with a silver magnet and able to switch under computer control the magnetic field strength to values of Proton Larmor frequencies ranging from 0.01 to 20 MHz (corresponding to: 2.4×10^{-4} to 0.47 T range). For each field strength, sixteen experiments of two scans each were used for the T_1 determination and each R_{1obs} value was automatically acquired with an absolute uncertainty of ±1%. On the other hand, water proton T_1 measurements at a field strength varying from 0.00024 to 1.65 T (corresponding to Proton Larmor frequencies between 20 and 70 MHz) were operated singularly at fixed frequency on a Stelar SpinMaster spectrometer (Stelar Snc, Mede-Pavia, Italy) by means of the inversion-recovery method.

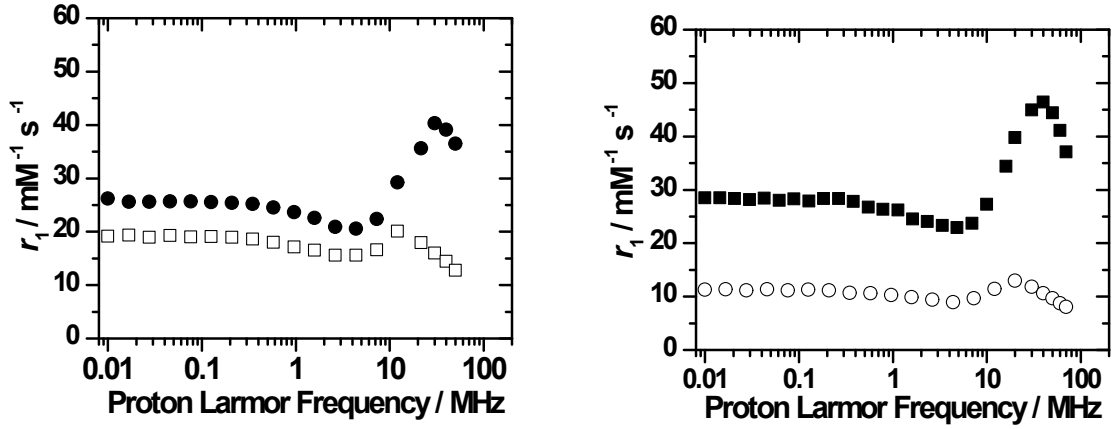


Figure S1. ^{1}H NMRD profiles at 310 K of paramagnetic dendrimerosomes (left) and liposomes (right) incorporating GdDOTAGA(C_{18})$_2$ (● and ■) and GdDOTAMA(C_{18})$_2$ (□ and ○).

4. Cell viability

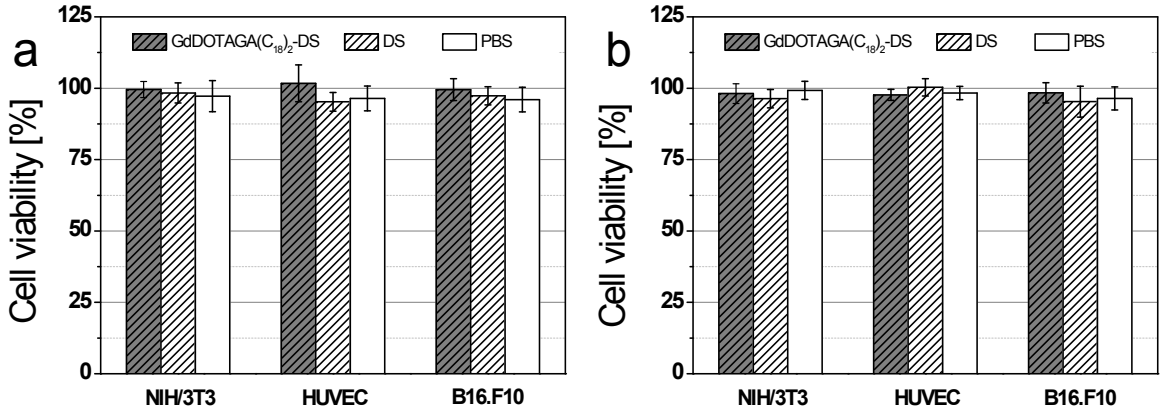
4.1 Cells

All cell lines were obtained from American Type Culture Collection (ATCC, Manassass, VA, USA) and resulted negative for mycoplasma test (MycoAlert™ Mycoplasma Detection Kit, Lonza Sales AG, Verviers, Belgium). Murine fibroblasts (NIH/3T3), melanoma (B16.F10) and human umbilical vein endothelial (HUVEC) cells were cultured as monolayers at 37°C in a 5% CO_2-containing humidified atmosphere in Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% (v/v) of heat-inactivated fetal bovine serum, 2 mM of Glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin. At 80% confluency, cells were trypsinized with 0.01% trypsin and 0.02% EDTA in Phosphate-buffered Saline (PBS). All cells employed for cellular tests were at passages included between 5 and 10. Culture medium, fetal bovine serum, penicillin-streptomycin mixture and trypsin were all purchased from Lonza (Lonza Sales AG, Verviers, Belgium).

4.2 *Cell incubation and viability assessment*

The cell viability was assessed on murine fibroblasts (NIH/3T3), melanoma (B16.F10) and human umbilical vein endothelial (HUVEC) cells after incubation with medium containing the

paramagnetic dendrimersomes (GdDOTAGA(C_{18})$_2$-DS), the vehicle (DS), or an equivalent volume of PBS, for the time ranges of 1 h (a), 6 h (b) or 24 h (c), at a concentration of 40 mg/ml of amphiphilic material. Before being added to cell culture, vesicles were diluted into culture medium and filtered by sterile 0.4 μm sized filters. The viability was assessed by a fluorimetric assay, based on the cell metabolic activity (CellTiter-Blue® Cell Viability assay, Promega, Madison, USA). The CellTiter-Blue® reagent contains the indicator dye Resazurin that is reduced to highly fluorescent Resofurin by viable cells retaining metabolic capacities, so that the fluorescent signal generated from the assay is proportional to the number of living cells in the sample. Briefly, NIH/3T3, HUVEC and B16.F10 cells were plated in 96-well multiwell plate at a concentration of 1.0×10^4, 1.0×10^4 and 5.0×10^3 cells per well, respectively. After 24 h, cells were exposed to incubation medium for 1, 6 and 24 h. Next, the incubation medium was removed and three washes were performed with sterile PBS. The CellTiter-Blue® reagent was added following manufacturer's instructions (20 μl of reagent diluted 1:5 into culture medium for each well). After 4 h, supernatants were collected and placed in a black 96-well multiwell plate for fluorescence detection (excitation wavelength: 530-560 nm, emission wavelength: 590 nm). Five independent experiments were performed to test each single condition, with cells plated in triplicate and fluorescence measurements repeated three times. The reported results represent the mean ± SD of the percentage of viable cells, normalized with respect to the control samples obtained by incubating cells with PBS-containing medium.



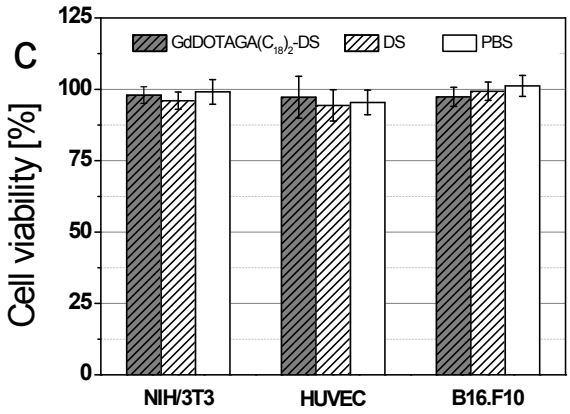


Figure S2. Cell viability assessed by Resazurin/Resofurin assay on murine fibroblasts (NIH/3T3), melanoma (B16.F10) and human umbilical vein endothelial (HUVEC) cells after incubation with medium containing the paramagnetic dendrimersomes (GdDOTAGA(C_{18})$_2$-DS), the vehicle (DS), or an equivalent volume of PBS, for the time ranges of 1 h (a), 6 h (b) or 24 h (c), at a concentration of 40 mg/ml of amphiphilic material.

5. *In vivo* experiments

To confirm *in vitro* data, the *in vivo* performance of the Gd-DOTAGA(C_{18})$_2$ complex was investigated on experimental tumour models by an MRI scanner operating at 1 T (40 MHz). Among the previously studied nanosystems, dendrimersomes (DS) were selected to perform the *in vivo* experiments: vesicular suspensions loaded with the new Gd-agent were systemically administered to mice bearing subcutaneous syngeneic B16 melanomas (0.05 mmol Gd kg^{-1} body weight) and compared to analogous DS bearing the same percentage of Gd-DOTAMA(C_{18})$_2$.

5.1 Cells

Melanoma cells (B16-F10) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and resulted negative for mycoplasma test (MycoAlert™ Mycoplasma Detection Kit, Lonza Sales AG, Verviers, Belgium). Cells were cultured as monolayers at 37°C in a 5% CO_2-containing humidified atmosphere in Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% (v/v) of heat-inactivated fetal bovine serum, 2 mM of Glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin. At 80% confluency, cells were

detached with 0.01% trypsin and 0.02% EDTA in PBS. Culture medium, fetal bovine serum, penicillin-streptomycin mixture and trypsin were all purchased from Lonza (Lonza Sales AG, Verviers, Belgium).

5.2 Animals

Female C57/BL6 mice with same age (8 weeks) and similar weight (around 20-22 g) were used for the *in vivo* experiments, with at least three animals per condition in all performed experiments. All mice involved in the study were obtained from Charles River Laboratories (Calco, LC, Italy) and maintained in standard housing conditions, with water and rodent chow ad libitum and 12 h light/dark cycle. Animal manipulation and all experimental procedures were performed in accordance with the European Community guidelines and approved by the Ethical Committee of the University of Torino.

5.3 In vivo MRI

The performance of the two Gd-complexes (Gd-DOTAGA(C_{18})$_2$ or Gd-DOTAMA(C_{18})$_2$) was investigated in xenograft mouse models bearing a melanoma B16 syngeneic tumour. Gd-complexes were incorporated into the membrane of the DSs, as described above. Tumours were generated by subcutaneous injection of 2.5×10^5 melanoma cells (in 150 ul of PBS) on the flank of the animal. After 10 days, mice developed solid tumours of 2-4 mm in diameter and were then administered with GdDOTAMA(C_{18})$_2$ or GdDOTAGA(C_{18})$_2$–bearing dendrimersomes, so that they receive the same dose of Gd^{III} (0.05 mmol/kg). The administration was performed via tail vein injection. For the MRI experiments, mice were anesthetized by intramuscular injection of a combination of tiletamine/zolazepam (zoletil 100; Virbac, Milan, Italy) 20 mg/kg and xylazine (Rompum; Bayer, Milan, Italy) 5 mg/kg.

The animals were subjected to MRI investigation before the injection, at several time points within 2 h post-injection, and finally after 6 and 24 h post-injection in order to define the early evolution of the contrast enhancement in *in vivo* imaging. Then, images were also acquired at different time points (each two days: day 2, 4, 6, 8, 10 ,12, 14) along two weeks.

MRI experiments were performed on a 1 T Imaging Scanner (Aspect M2 High Performance System, Aspect Magnet Technologies Ldt, Letanya, Israel), consisting of NdFeB Magnet equipped

with a solenoid Tx/Tr coil of inner diameter of 35 mm. The system is equipped with a fast gradient (gradient strength 450 mT m^{-1} at 60 A, ramp time 250 us at 160 uV), with a field homogeneity of 0.2-0.5 gauss. Images were acquired by using a standard T_1-weighted spin echo sequence, setting the following parameters: TR = 250 ms, TE = 8.9 ms, matrix size = 128 × 128, slice thickness = 1.5 mm, NA = 8, FOV = 35 mm. Multislice T_2-weighted images were used as anatomical reference to draw Regions of Interest (ROIs) on T_1-weighted images, and were acquired by using a Fast Spin Echo sequence with the following parameters: TR = 2500 ms, TE = 49.4 ms, matrix size = 160 × 152, slice thickness = 1.5 mm, NA = 6, FOV = 40 mm.

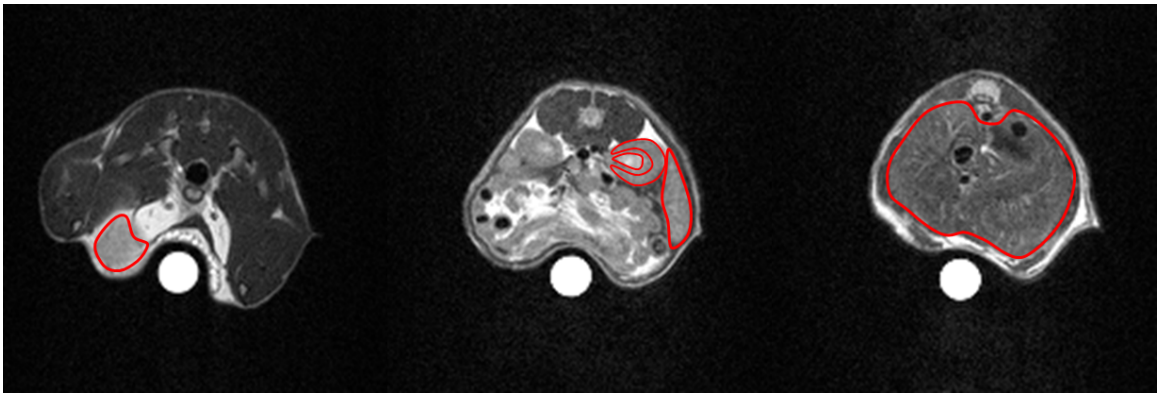


Figure S3. Representative anatomical T_{2w} axial images of the subcutaneous melanoma tumour, spleen, kidney (renal pelvis and cortex), and liver, highlighted by the red contour.

The mean Signal Intensity (SI_0) was calculated on the manually drawn ROIs on T_1-weighted (T_{1w}) images. To take into accounts differences among images due to the animal repositioning in the MRI scanner, the SI_0 was normalized with respect to an external reference (0.5 mM of Gadoteridol aqueous solution, ProHance® (Gd–HPDO3A) provided by Bracco Imaging, Milan, Italy) to obtain the normalized Signal Intensity, SI_n.

The percentage T_1 contrast enhancement (T_1-CE) was calculated with respect to the signal measured before the injection of vesicles (SI_{nPRE}), by applying the following expression:

$$T_1\text{-CE [\%]} = \frac{SInPOST - SInPRE}{SInPRE} \times 100$$

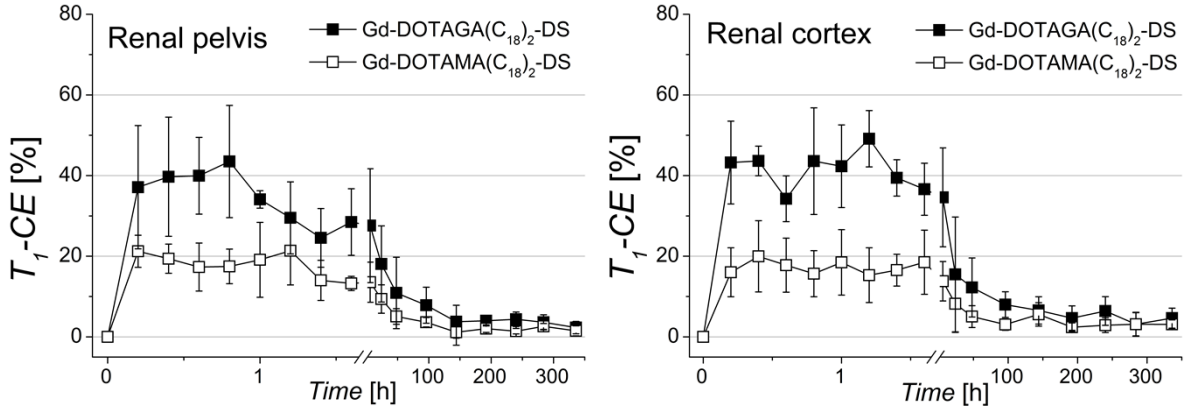


Figure S4. Time evolution of the percentage T_1 contrast enhancement (T_1-CE) calculated on T_{1w} images of kidneys after the systemic administration of GdDOTAGA$(C_{18})_2$ or GdDOTAMA$(C_{18})_2$-loaded dendrimersomes (black squares and white squares, respectively).

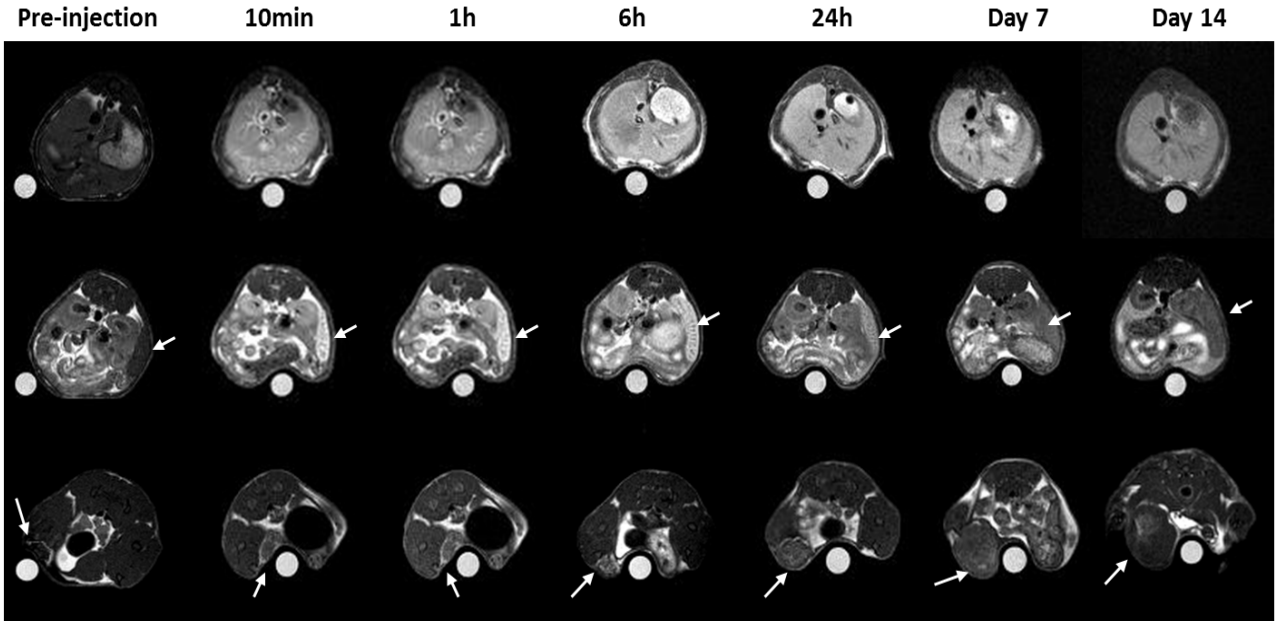


Figure S5. Representative axial T_{1w} images showing the evolution of T_1 contrast over 14 days of MRI monitoring, after the administration of GdDOTAGA$(C_{18})_2$-loaded dendrimersomes, on liver (top), spleen (middle) and tumor (bottom).

5.4 Biodistribution

After 6 h or 14 days from the injection, animals were sacrificed to obtain the organs (kidneys, liver, spleen, and tumour), and the content of Gd^{3+} was quantified by ICP-MS measurement after digestion. Briefly, the weights of all organs were accurately registered. Then, the biological

specimens were treated with concentrated HNO_3 (70%) and digested by applying microwave heating (Milestone MicroSYNTH, Microwave labstation equipped with an optical fiber temperature control and HPR-1000/6M six position high-pressure reactor, Bergamo, Italy). After digestion, the remaining material was diluted in ultrapure water (final volume of 3 mL) and further filtered with 0.2 µm filters. Finally, samples were analysed by ICP-MS (Thermo Scientific ELEMENT 2 ICP-MS-Finnigan, Rodano, Milano, Italy) to measure the Gd^{3+} content. The content of Gd^{3+} was expressed with respect to the organ mass.

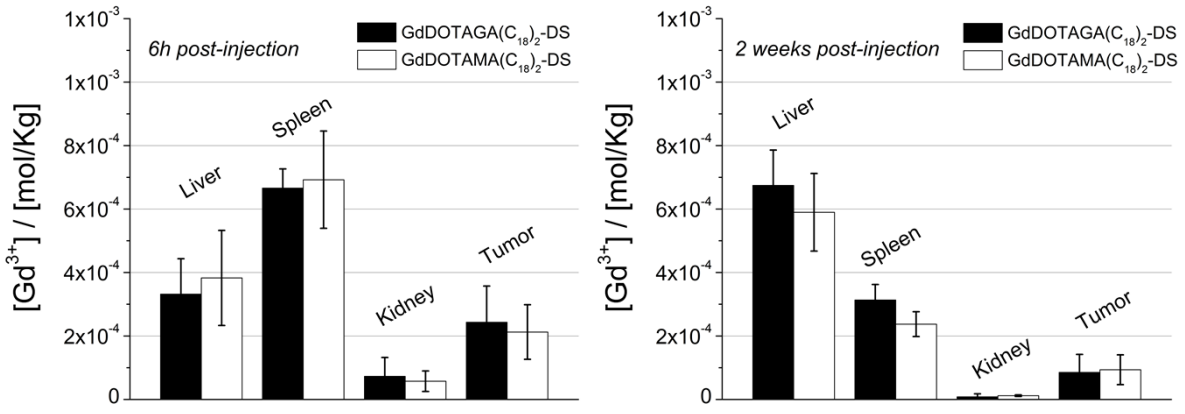
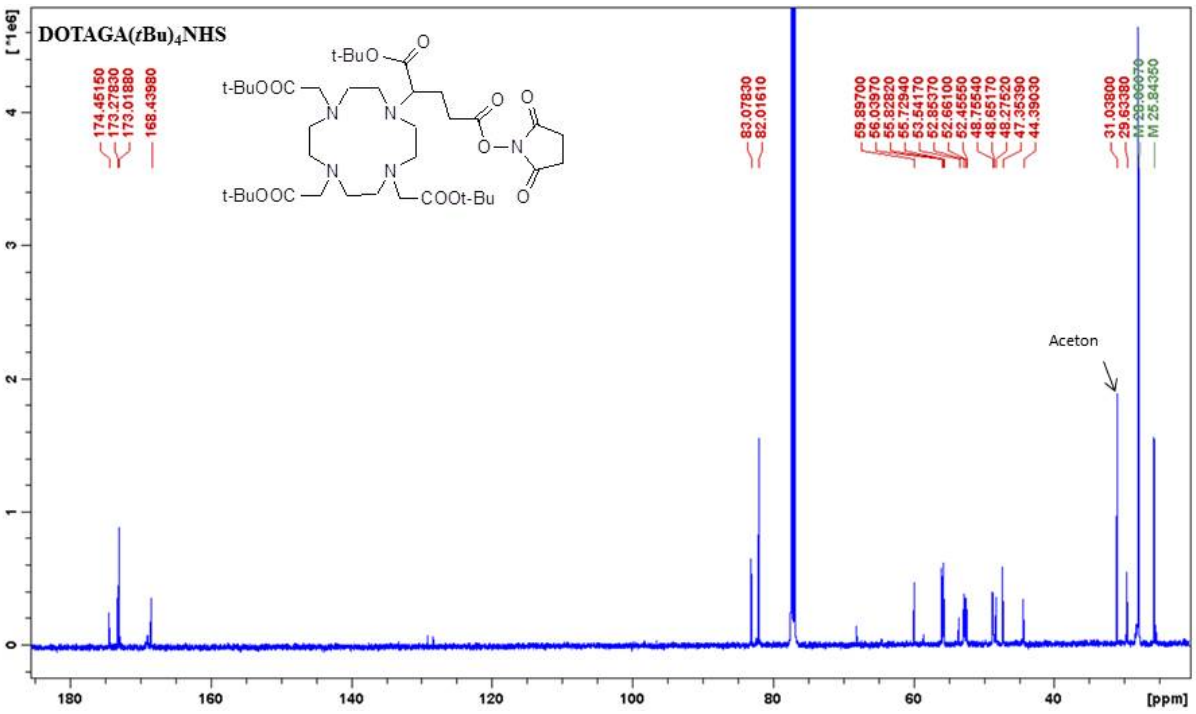
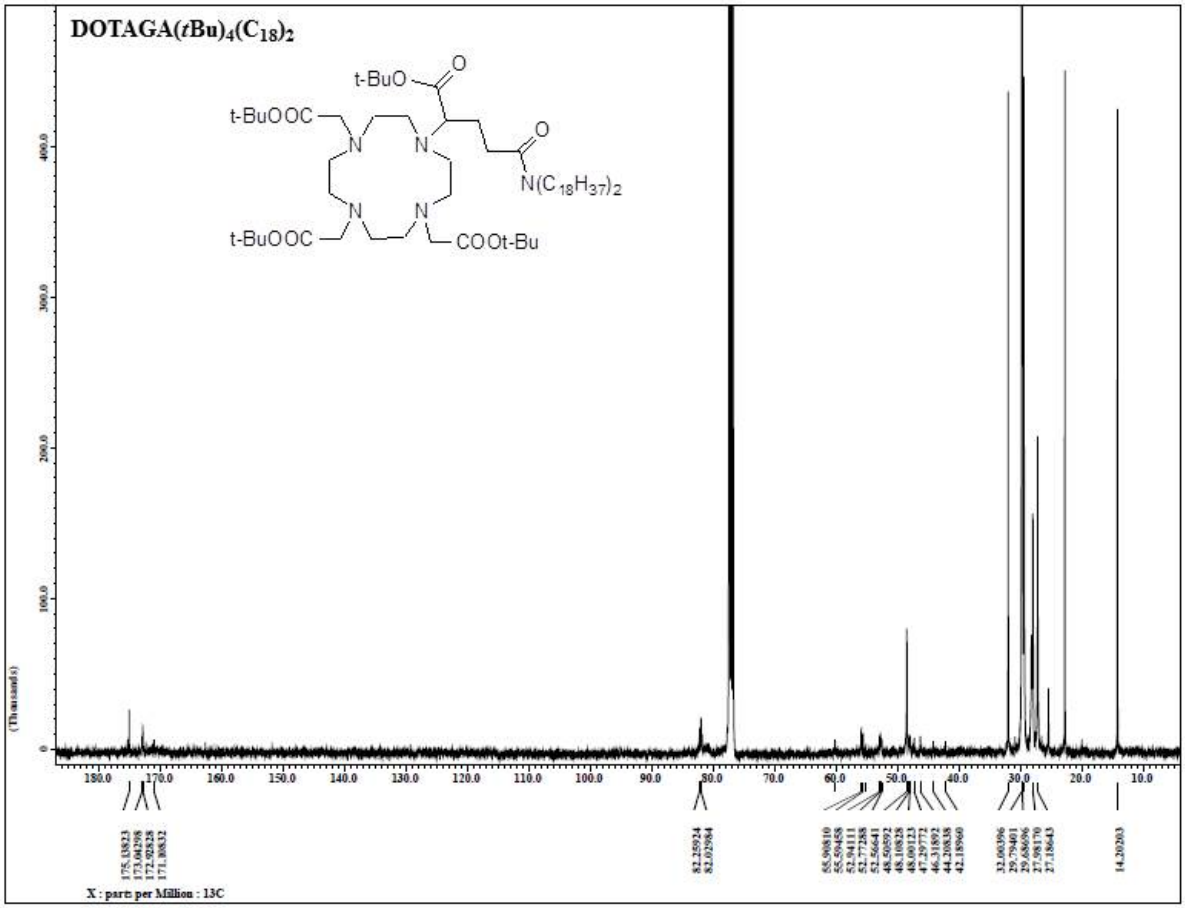
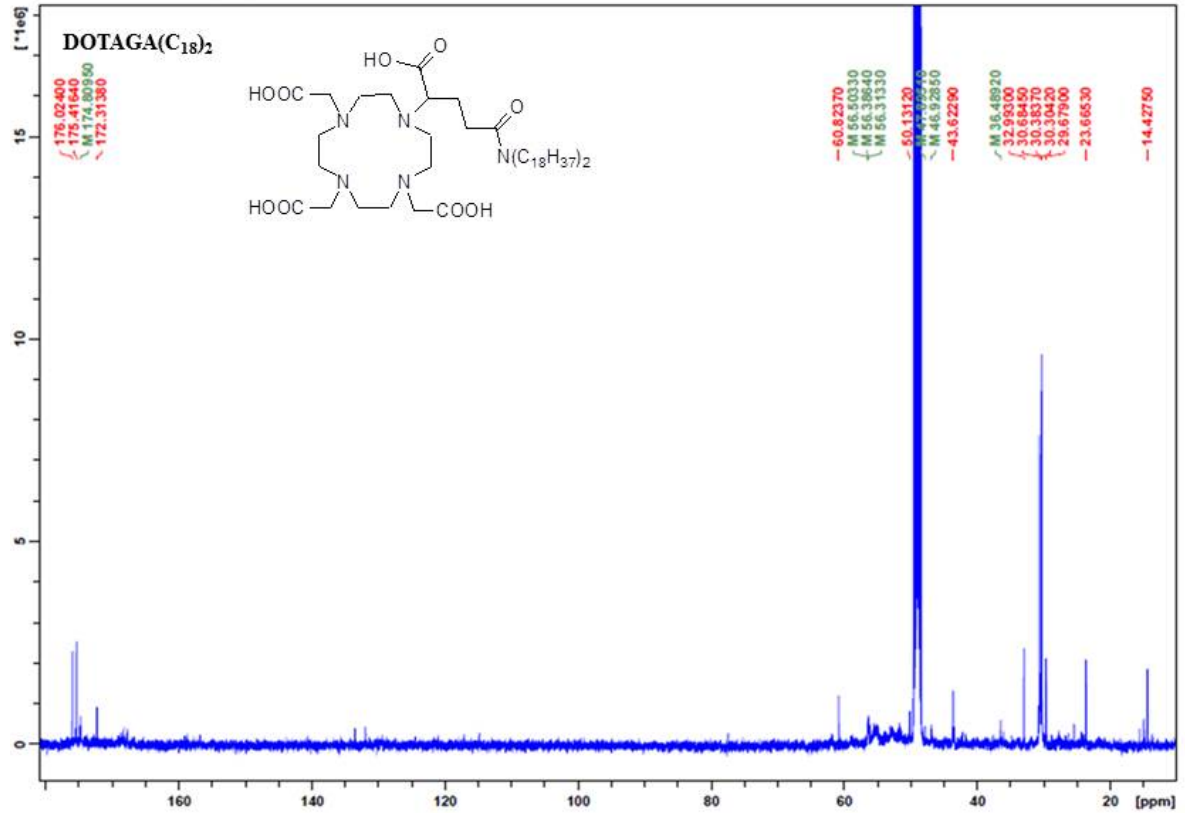


Figure S6. Amount of gadolinium normalized to the organ weight, measured by ICP-MS for the different organs (liver, spleen, kidney, tumour) excised from mice 6 h or 14 days after the administration of GdDOTAGA(C_{18})$_2$ or GdDOTAMA(C_{18})$_2$-loaded dendrimersomes (black bars and white bars, respectively).

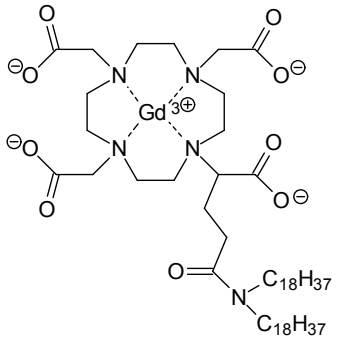
6. ^{13}C NMR spectra of DOTAGA(tBu)$_4$NHS, DOTAGA(tBu)$_4$(C_{18})$_2$, DOTAGA(C_{18})$_2$




DOTAGA(tBu)4(C18)2
t-BuO
t-BuOOC
t-BuOOC
COOt-Bu
N(C18H37)2
(Thousands)
X : parts per Million : 13C


DOTAGA(C18)2
HO
HOOC
HOOC
COOH
N(C18H37)2
[*1e6]
[ppm]

7. Mass spectrum of GdDOTAGA(C_{18})$_2$



$C_{55}H_{101}GdN_5O_9$
1133.684041

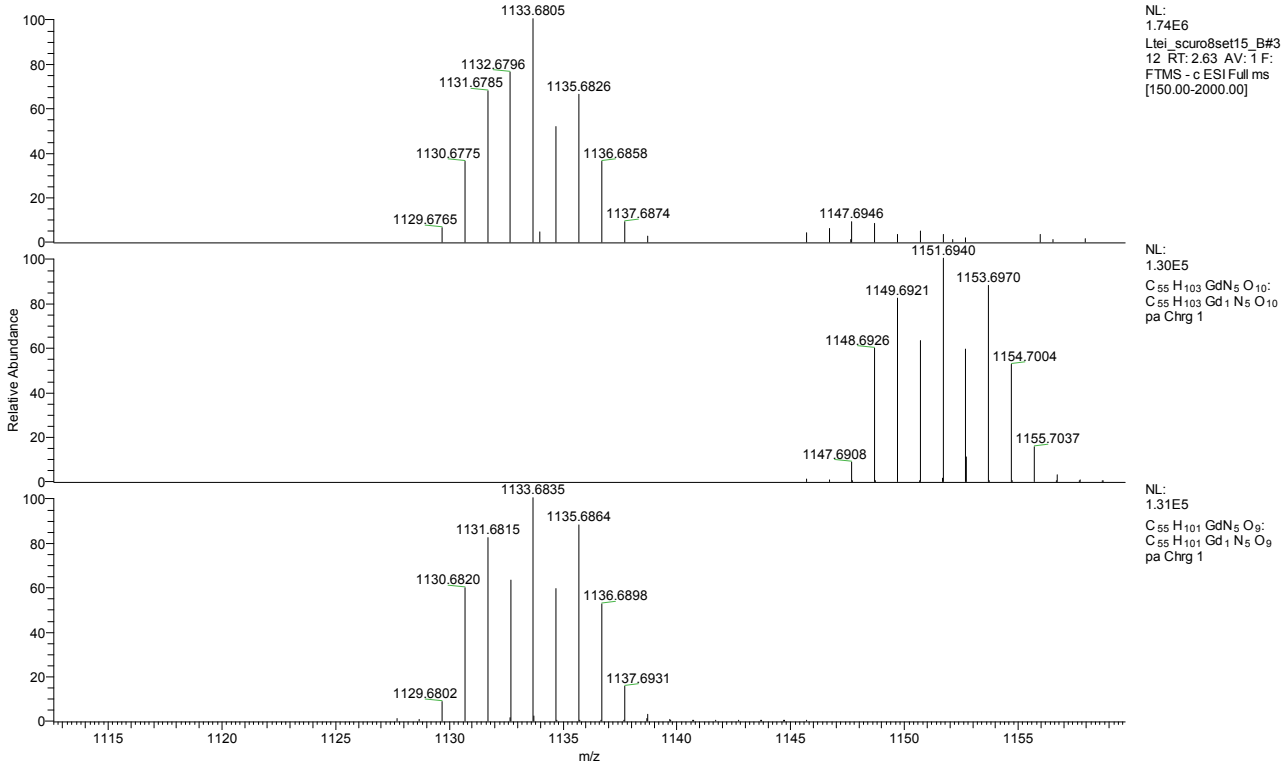


Figure S7: HRMS (ESI$^-$) of [GdDOTAGA(C_{18})$_2$]$^-$ (top) and the simulated spectra of [GdDOTAGA(C_{18})$_2$(H_2O)]$^-$ (centre) and of [GdDOTAGA(C_{18})$_2$]$^-$ (bottom).

8. References:

1 S. G. Levy, V. Jacques, K. L. Zhou, S. Kalogeropoulos, K. Schumacher, J. C. Amedio, J. E. Scherer, S. R. Witowski, R. Lombardy and K. Koppetsch, *Org Proc Res Dev*, 2009, **13**, 535.

2 M.R. Mozafari, *Cell Mol Biol Lett,* 2005, **10**, 711.

3 M. Filippi, D. Patrucco, J. Martinelli, M. Botta, P. Castro-Hartmann , L. Tei and E. Terreno, *Nanoscale*, 2015, **7**, 12943.