Electronic Supplementary Information (ESI)

Paramagnetic Gd-based gold glyconanoparticles as probes for MRI: tuning relaxivities with sugars

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Experimental Section

General: ^{1}H NMR spectra were acquired on a Bruker DRX-300 spectrometer. TEM examination was carried out at 200 KeV with Philips CM200 microscope. Elemental analyses were carried out in a LECO CHNS-932 apparatus. ICP analyses were performed in an Iris Advantage apparatus (Termo Jarrell Ash, Franklin, MA, USA). UV spectra were obtained with a UV/vis Perkin-Elmer Lambda 12 spectrophotometer.

The glycoconjugate *glc*C_2S was synthesized as previously reported.[1S] *Glc*C_5S and *gal*C_5S were obtained by methanolysis of the corresponding peracetylated derivatives, in turn prepared using their *n*-pentenyl glycosides as precursors.[2S] *Lac*C_5S was prepared by glycosidation of 5-(*S*-acetyl) mercaptopentan-1-ol with the perbenzoylated lactose, using the trichloroacetimidate method.[3S] DO3AC_5S and DO3AC_{11}S were synthesized by alkylation of commercial DO3A-^{t}Bu with 5-bromo-1-(*S*-trityl) mercaptopentane or with 11-bromo-1-(*S*-trityl) mercaptoundecane, and subsequent deprotection of both *t*-butyl esters and trityl group in acidic conditions by using a modification of a reported procedure.[4S]

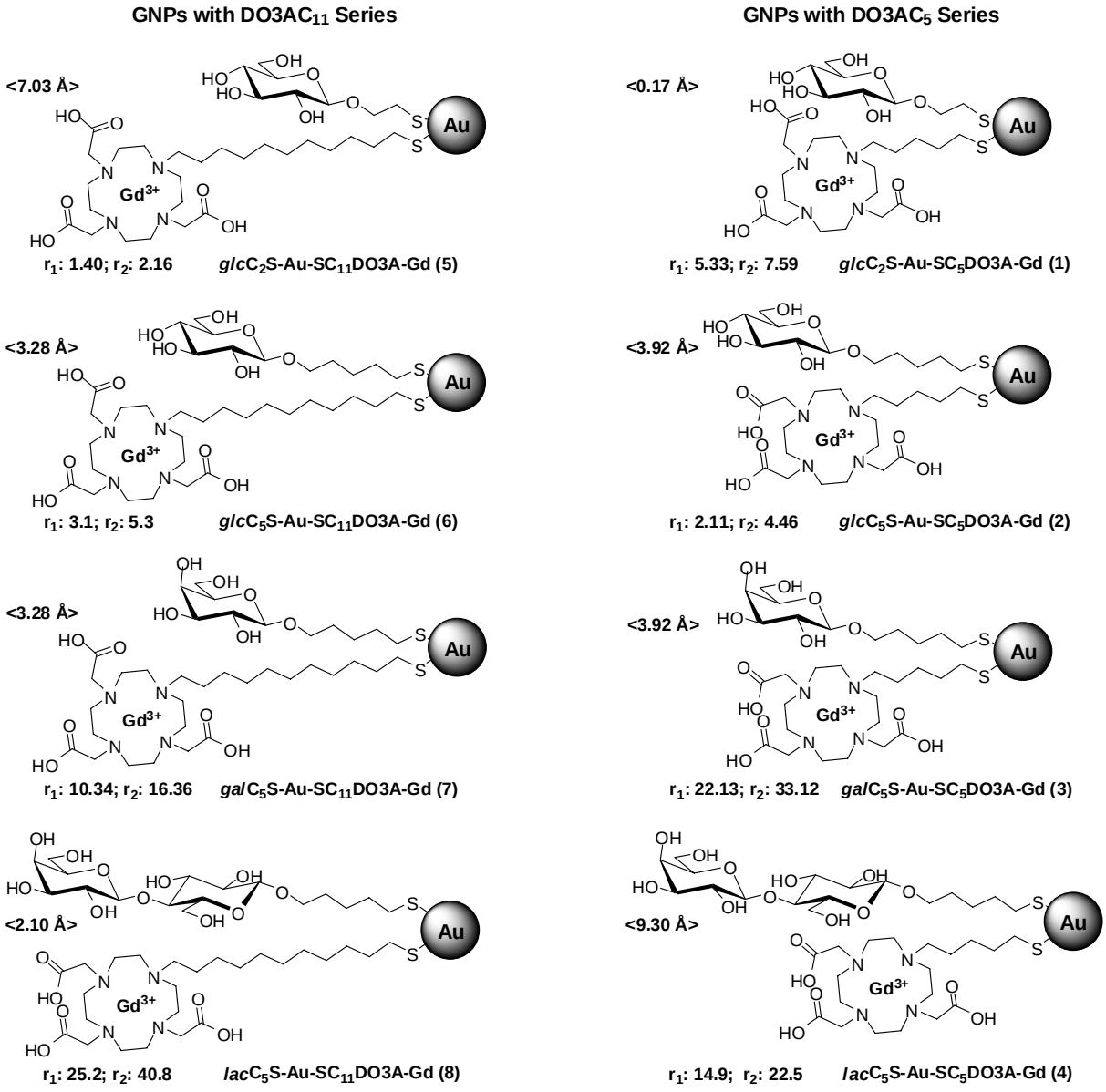

Figure 1S. Structures of the paramagnetic Gd-based gold glyconanoparticles synthesized in this work and changes in the relaxivity values depending on the difference in length ($|\Delta l|$, in Å) between the DO3A and the sugar ligands.

Preparation of the paramagnetic Gd-based gold glyconanoparticles: The gold glyconanoparticles (GNPs) incorporating DO3A derivatives were prepared following the procedure reported in references 1S and 3S. Gd(III) complexation was carried out by adding an excess of aqueous solution of $GdCl_3$ (0.1 M) to a solution of GNPs in HEPES buffer (0.1 M, pH = 7.4). The mixture was orbital shaken in dark for 24 h. A solution of EDTA (0.1 M, pH = 7.4, same excess of $GdCl_3$) was then added and the mixture was shaken for 1 h. The mixture was centrifugal filtered (Centricon, MW=10000, 3000g) and the residue was washed with water (MilliQ, 18 μM) until free Gd(III) was no more detected by xylenol orange

colorimetric assay (pH~7).[5S] The residue on top of the filter was dissolved in water and lyophilized to obtain the corresponding Gd(III)-GNPs as a black powder.

lacC_5*S-Au-SC*$_{11}$*DO3A*: 7.3 mg were obtained as a black powder from DO3AC_{11}S (5.0 mg, $4.7\cdot10^{-3}$ mmol, 0.3 eq) in H_2O (391 µL) and *lac*C_5S (37.5 mg, 0.0422 mmol, 2.7 eq) in MeOH (3.52 mL). ^{1}H NMR (300 MHz, D_2O, δ, detected signals): 4.35 (bs, 2H, H-1, H1´ lactose), 4.1-3.0 (bm, 16H), 2.0-1.0 (bm, 10H); IR (KBr): ν = 3423 (s), 2923 (w), 2852 (w), 1637 (m), 1406 (w), 1076 cm^{-1} (s); UV-vis (water): λ_{max} = 515 nm (surface plasmon band); TEM: 3.2 ± 0.4 nm; Anal. calcd for $Au_{1289}(C_{17}H_{31}O_{11}S)_{469}(C_{25}H_{47}N_4O_6S)_{109}$: C 24.71; H 3.89; N 1.17; S 3.56; found: C 24.57; H 4.22; N 1.17; S 3.74.

lacC_5*S-Au-SC*$_{11}$*DO3A-Gd* (**8**): *lac*C_5S-Au-SC$_{11}$DO3A (202.2 mg, $3.89\cdot10^{-4}$ mmol) in 15 mL of 0.1 M HEPES buffer was first treated with 1.44 mL of $GdCl_3$ 0.1 M (3.4 eq) and then with 1.44 mL of EDTA 0.1 M. After washing, the Gd-complexed GNP (188.6 mg, $3.61\cdot10^{-4}$ mmol) was obtained as black powder. ^{1}H NMR signals of the paramagnetic GNPs are too wide to be assigned; TEM: 3.1 ± 0.8 nm; IR (KBr): ν = 3419 (s), 2923 (w), 2854 (w), 1635 (m), 1406 (w), 1072 cm^{-1} (s); UV-vis (water): λ_{max} = 515 nm (surface plasmon band); Anal. calcd for $Au_{1289}(C_{17}H_{31}O_{11}S)_{469}(C_{25}H_{47}N_4O_6S)_{109}Gd_{17}$: Gd 0.51; found (ICP): Gd 0.5.

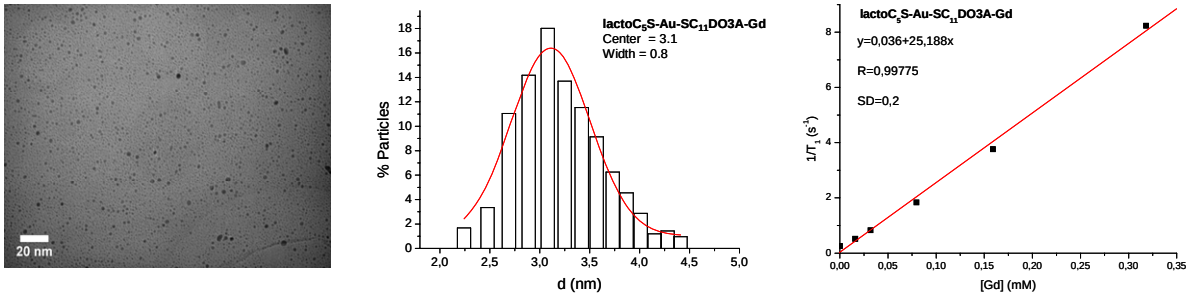

Figure 2S. TEM, size distribution, and relaxivity (r_1) of ***lac*C$_5$S-Au-SC$_{11}$DO3A-Gd** (**8**).

glcC_2*S-Au-SC*$_{11}$*DO3A-Gd* (**5**): *glc*C_2S-Au-SC$_{11}$DO3A (146.8 mg, $4.08\cdot10^{-4}$ mmol) in 15 mL of HEPES buffer was first treated with 587 µL of $GdCl_3$ 0.1 M (1.8 eq) and then with 587 µL of EDTA 0.1 M. After washing, the Gd-complexed GNP (129.4 mg, $3.52\cdot10^{-4}$ mmol) was obtained as black powder. TEM: 3.0 ± 0.6 nm; IR (KBr): ν = 3442 (s), 2923 (w), 2852 (w), 1635 (m), 1458 (w), 1076 cm^{-1} (s); UV-vis (water): λ_{max} = 525 nm (surface plasmon band); Anal. calcd for $Au_{1289}(C_8H_{15}O_6S)_{262}(C_{25}H_{47}N_4O_6S)_{81}Gd_{47}$: Gd 2.01; found (ICP): Gd 2.0.

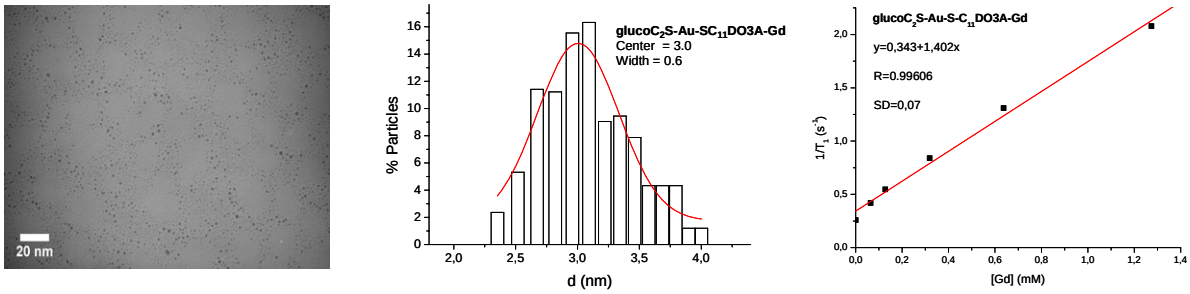

Figure 3S. TEM, size distribution, and relaxivity (r_1) of ***glc*C$_2$S-Au-SC$_{11}$DO3A-Gd** (**5**).

glcC$_2$S-Au-SC$_5$DO3A-Gd (**1**): *glc*C$_2$S-Au-SC$_5$DO3A (17.7 mg, $6.1\cdot10^{-5}$ mmol) in 1.7 mL of 0.1 M HEPES buffer was first treated with 69 µL of $GdCl_3$ 0.1 M (3.8 eq) and then with 69 µL of EDTA 0.1 M. After washing, the Gd-complexed GNP (9.8 mg, $3.2\cdot10^{-5}$ mmol) was obtained as black powder. TEM: 3.0 ± 0.5 nm; IR (KBr): ν = 3444 (s), 2925 (w), 2856 (w), 1632 (m), 1381 (w), 1074 cm^{-1} (s); UV-vis (water): λ_{max} = 520 nm (surface plasmon band); Anal. calcd for $Au_{976}(C_8H_{15}O_6S)_{355}(C_{19}H_{35}N_4O_6S)_{30}Gd_{75}$: Gd 3.90; found (ICP): Gd 3.90.

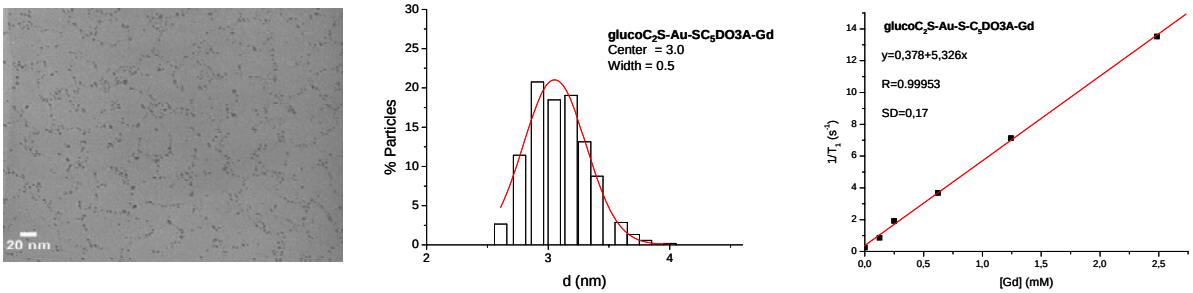

Figure 4S. TEM, size distribution and relaxivity (r_1) of ***glc*C$_2$S-Au-SC$_5$DO3A-Gd** (**1**).

Table 1S. Average molecular formula and molecular weight (MW) calculated on the basis of the gold core size (determined by TEM), elemental analysis and ICP-AES

GNPs	Average Molecular Formula	Average MW
*glc*C$_2$S-Au-SC$_{11}$DO3A-Gd (**5**)	$Au_{1289}(C_8H_{15}O_6S)_{262}(C_{25}H_{47}N_4O_6S)_{81}Gd_{47}$	367 KDa
*glc*C$_5$S-Au-SC$_5$DO3A-Gd (**2**)	$Au_{1289}(C_{11}H_{21}O_6S)_{400}(C_{19}H_{35}N_4O_6S)_{80}Gd_{58}$	411 KDa
*glc*C$_5$S-Au-SC$_{11}$DO3A-Gd (**6**)	$Au_{976}(C_{11}H_{21}O_6S)_{482}(C_{25}H_{47}N_4O_6S)_{65}Gd_{52}$	372 KDa
*glc*C$_2$S-Au-SC$_5$DO3A-Gd (**1**)	$Au_{976}(C_8H_{15}O_6S)_{355}(C_{19}H_{35}N_4O_6S)_{30}Gd_{75}$	302 KDa
*gal*C$_5$S-Au-SC$_{11}$DO3A-Gd (**7**)	$Au_{201}(C_{11}H_{21}O_6S)_{115}(C_{19}H_{35}N_4O_6S)_9Gd_3$	77 KDa
*lac*C$_5$S-Au-SC$_5$DO3A-Gd (**4**)	$Au_{976}(C_{17}H_{31}O_{11}S)_{328}(C_{19}H_{35}N_4O_6S)_{27}Gd_9$	351 KDa
*gal*C$_5$S-Au-SC$_5$DO3A-Gd (**3**)	$Au_{201}(C_{11}H_{21}O_6S)_{93}(C_{19}H_{35}N_4O_6S)_5Gd_3$	68 KDa
*lac*C$_5$S-Au-SC$_{11}$DO3A-Gd (**8**)	$Au_{1289}(C_{17}H_{31}O_{11}S)_{469}(C_{25}H_{47}N_4O_6S)_{109}Gd_{17}$	523 KDa

Relaxation times measurements and phantom imaging: ^{1}H NMR relaxation times T_1 and T_2 were measured at 1.41 Tesla in a Bruker Minispec NMR spectrometer at different concentrations of paramagnetic GNPs (10, 5, 2.5, 1, 0.5 mg mL^{-1}) in HEPES (10 mM, pH = 7.2) and NaCl 155 mM at 37 ºC. T_1 and T_2 values were determined by the inversion-recovery method and by the Carr-Purcell-Maiboom-Gill sequence, respectively. Relaxivities $r_{1(2)}$ were obtained from the slopes of the curves $1/T_{1(2)}$ *vs.* the concentration of Gd(III) expressed in mM. T_1-weighted magnetic resonance images were performed at 7 Tesla (in NaCl 155 mM, at 37 ºC) at different concentrations of Gd(III) (0.05, 0.1, 0.2, 0.4 mM) and contrast enhancement was controlled by running a standard spin-echo sequence with 800 ms or 400 ms repetition time and 8.5 ms echo time.

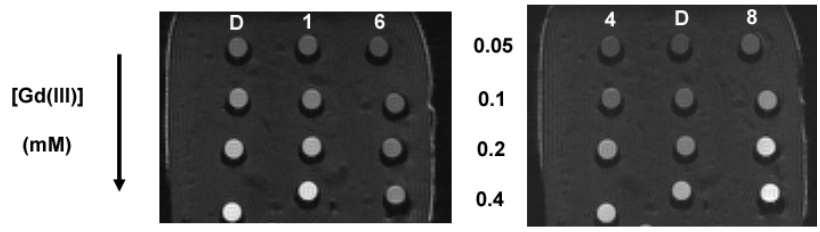

Figure 5S. T_1-weighted MR images of the paramagnetic GNPs **1**, **4**, **6**, and **8** (in NaCl 155 mM, at 37 ºC) at 7 T (repetition time 800 ms, left; 400 ms, right; standard spin echo sequence, echo time 8.5 ms) for four Gd concentrations; **D** stands for Dotarem®.

Cell lines and culture conditions: The C6 mouse glioma cell line was obtained from the American Type Culture Collection (Clonetech Laboratories, Inc., USA) and maintained in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Inc), supplemented with 5% fetal bovine serum (FBS), streptomycin (0.1 mg mL^{-1}, Sigma, St. Louis, MO), penicillin (100 U mL^{-1}, Sigma), and gentamicin (25 μg mL^{-1}, Sigma). The GL261 glioma cell line was obtained from the Tumor Bank Repository (NCI, Frederick, USA) and maintained in DMEM, supplemented with 10% FBS, streptomycin (0.1 mg mL^{-1}), and penicillin (100 U mL^{-1}). All cell cultures were grown in a humidified 37 ºC incubator with 5% CO_2 and 95% humidity (Thermo forma water jacketed CO_2 incubator, Thermo Electron Corporation, Marietta, USA).

Cytotoxicity tests: The viability of cells was determined by using the classical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[6S] Briefly, 104 cells/well were seeded into 96-well plates in 100 μL complete medium and incubated at 37 ºC in 5 % CO_2 atmosphere. After 24 h, the medium was replaced with a fresh one containing nanoparticles at different concentrations (0-20 μM). After a 24 h incubation period, 20 μL of MTT (5 mg mL^{-1} in phosphate buffer pH 7.4) was added to each well. After 4 h of incubation at 37 ºC and 5% CO_2 for exponentially growing cells and 15 min for steady-state confluent

cells, the medium was removed, formazan crystals were solubilized with 200 μL of DMSO, and the solution was vigorously mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader (Dynatech MR7000 instruments) at 550 nm. The spectrophotometer was calibrated to zero absorbance, using culture medium without cells. The relative cell viability (%) related to control wells containing cell culture medium without nanoparticles was calculated by [A]test/[A]control x 100.

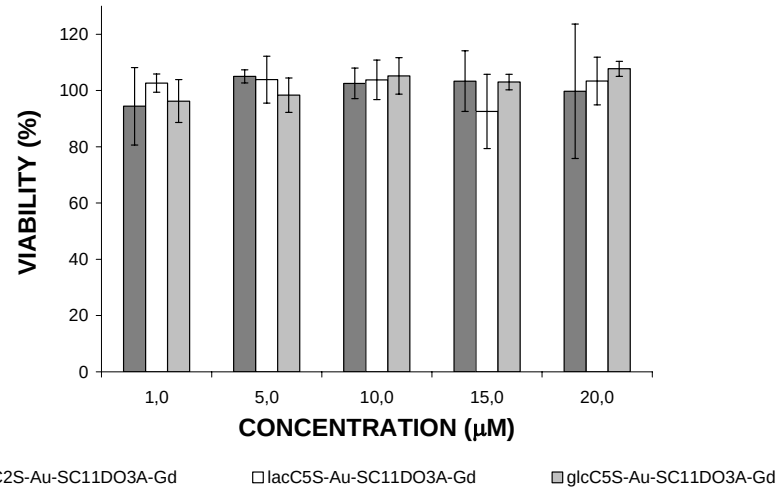

Figure 6S. Evaluation of cytotoxicity of selected paramagnetic GNPs on C6 glioma cells.

Molecular modelling: Theoretical calculations were performed with Sybyl7.3, using Tripos force field and Gasteiger-Huckel charges for the glycoconjugates. Energy minimizations were performed in a process of 1000 runs reaching gradient convergence (0.05 Kcal/(mol.Å)) before exhausting the maximum number of iterations. A dielectric constant of 80 was employed. To simplify the calculations, *trans-trans* conformation was used for the aliphatic linkers and the glycosidic bonds were put in an exo-anomeric conformation. The length of the molecules are taken from the OH-4 to the SH group for the sugars and from a carboxyl to the SH groups for the DO3A derivatives.

In vivo imaging of brain tumors in mice: C57BL/6 line mice (20-25 g weight range) were used following the international ethical guidelines and as by Spanish Law enacted. Mice GL261 glioma cells were used for the stereotactic implantation of the tumor in the mice brain. MRI experiments (three mice for each compound tested) were run with a Bruker Pharmascan (Bruker Medical Gmbh, Ettlingen, Germany) at 7 Tesla. The contrast agents were injected by coil vein at the same Gd(III) concentration (0.1 mmol Kg^{-1} of mouse) in NaCl 155 mM, at 37 ºC. T_2-weighted images (RARE sequence) were run before the contrast agents' injection in

order to localize the tumor. The T_1-weighted images were registered with repetition time 300 ms and echo time 9.8 ms.

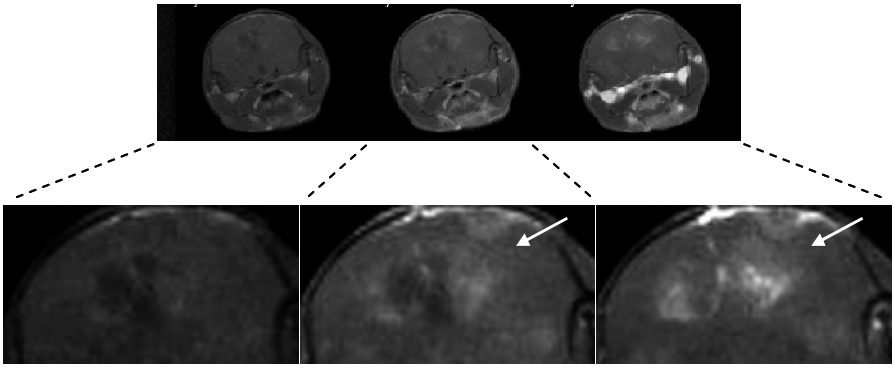

Figure 7S. TOP *Left*: T_1-weighted image of a GL261 generated tumor; *Middle*: T_1-weighted image after injection of Magnevist®; *Right*: T_1-weighted image after injection of *glc*C_5S-Au-SC_{11}DO3A-Gd (**6**). BOTTOM: Zoom of the TOP images with arrows indicating the tumoral zones.

^{17}O NMR experiments: The number of water molecules directly coordinated to the Gd(III) ion (q) was determined by measurement of Gd(III)-induced shifts of the water ^{17}O NMR resonance similarly to the procedure reported by Djanashvili and Peters.[7S]

^{17}O NMR spectra of *glc*C_5S-Au-SC_{11}DO3A-Gd (**6**) were recorded at natural abundance in D_2O as the solvent on a Bruker Avance-500 spectrometer at 67.80 MHz with the deuterium frequency lock. All samples were placed in 3 mm tubes with a total volume of 180 µL. Experiments were performed using 0.06 s acquisition time, 0.2 s acquisition delay and 512 scan averages in a Bruker broadband inverse probe. ^{17}O chemical shift was obtained from peak position. *Glc*C_5S-Au-SC_{11}DO3A was used as a blank.

glcC_5S-Au-SC_{11}DO3A-Gd (**6**) was measured at 25 ºC at 6.5 mM and 20.2 mM (Gd (III) concentrations) to give $q = 2.9 \pm 0.2$ and $q = 3.0 \pm 0.2$, respectively. At 60 ºC, **6** (6.5 mM in Gd(III)) gave $q = 2.7 \pm 0.2$.

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