Electronic Supplementary Information for:

Dendritic MRI Contrast Agents: An Efficient Pre-labeling Approach Based on CuAAC

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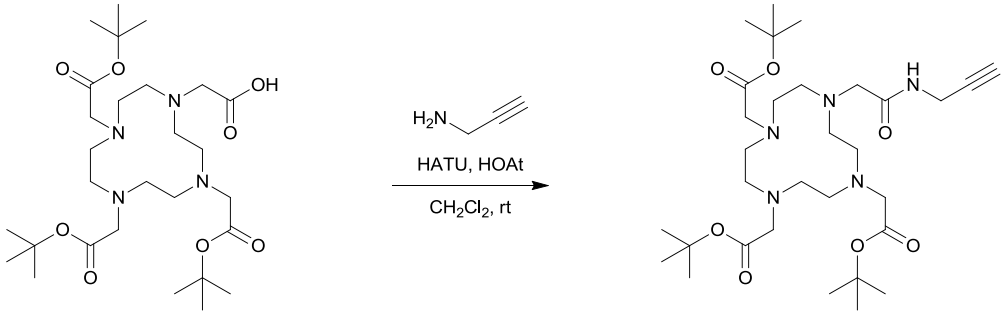
Synthesis of Alk-DO3A-Gd

A modified procedure for the preparation of Alk-DO3A-Gd was adapted so that higher yields for the first intermediate (1) could be achieved without the need of chromatographic purifications in any of the steps.[1,2] The purity of the compounds was confirmed by analytical HPLC/MS, while spectroscopic data was identical to that of the reported compounds.

Analytical HPLC was run using a Waters Spherisorb OSD2 LiChrospher 100 RP-18 (Φ 10 mm, L 250 mm, 0.5 µm particle size). A gradient of H_2O/CH_3CN (TFA 0.1%) form 0% H_2O (5 min) to 75% H_2O in 30 min was employed. ESI-MS was performed by using an Agilent 1100 series LC/MSD model in positive scan mode with direct injection of purified compounds.

- {4,10-Bis-tert-butoxycarbonylmethyl-7-[(2-propynylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1- yl}-acetic acid tert-butyl ester (**1**)

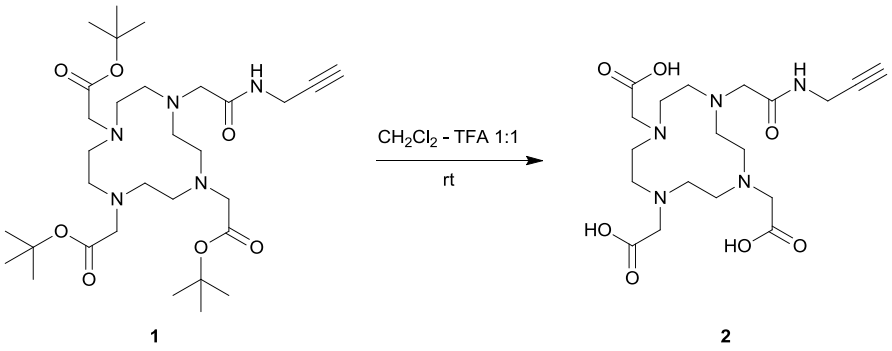


1

Scheme S1

To a suspension of (*t*BuO)$_3$-DOTA-OH (390 mg, 0.68 mmol), HATU (297 mg, 0.77 mmol) and HOAt (109 mg, 0.80 mmol) in CH_2Cl_2 (4 mL) was added propargyl amine (70 µL, 60 mg, 1.07 mmol) and the mixture was left to stirr overnight. The crude was diluted with $CHCl_3$ (16 mL) and washed with H_2O (4 x). The organic phase was concentrated, diluted with EtOAc (20 mL), and washed with H_2O (4 x). The organic phase was concentrated to give 394 mg (95%) of **1** as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$, 60ºC) δ 4.04 (dd, *J* = 5.3, 2.5 Hz, 2H, CH_2-C≡C), 3.89–3.75 (br s, 2H, CH_2), 3.62 (s, 2H, CH_2-C=O), 3.42 (s, 4H, CH_2-C=O), 3.22 (br s, 4H, CH_2), 3.08–2.96 (m, 6H, CH_2), 2.96–2.89 (m, 6H, CH_2), 2.19 (t, *J* = 2.5 Hz, 1H, ≡CH), 1.49 (s, 27H, CH_3). ESI-MS: m/z 610.42 (MH^+, 100%), 632.40 (MNa^+, 80). HR-MS Calcd for $C_{31}H_{56}N_5O_7^+$ (MH^+): 610.4174, found: 610.4170.

- {4,10-Bis-carboxymethyl-7-[(2-propynylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetic acid (**2**)

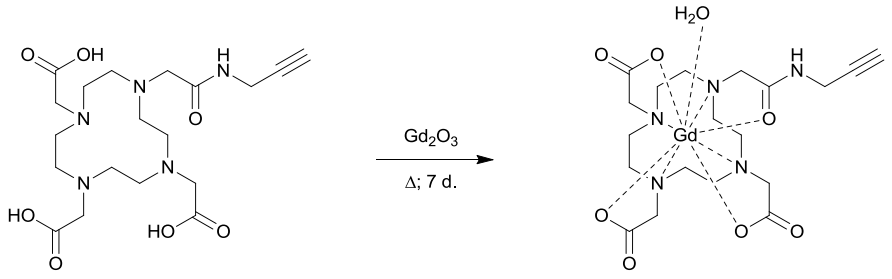


1 **2**

Scheme S2

Following the procedure reported by Finn and coworkers,[2] from 385 mg (0.63 mmol) of **1**, 273 mg (98%) of **2** were isolated as an off-white solid. ^{1}H-NMR (300 MHz, D_2O, 80ºC) δ 3.89 (d, *J* = 2.3 Hz, 2H, CH_2-C≡C), 3.71 (s, 4H, CH_2-C=O), 3.67–3.58 (m, 4H, CH_2-C=O), 3.28–3.18 (m, 8H, CH_2), 3.19–3.07 (m, 8H, CH_2), 2.53 (t, *J* = 2.3 Hz, 1H, ≡CH). ESI-MS: m/z 442.23 (MH^+, 100%), 464.21 (MNa^+, 12), 480.18 (MK^+, 13). HR-MS Calcd for $C_{19}H_{32}N_5O_7^+$ (MH^+): 442.2296, found: 442.2302.

- {4,10-Bis-carboxymethyl-7-[(2-propynylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododec-1-yl}-acetate gadolinium (III) complex (Alk-DO3A-Gd)



2 **Alk-DO3A-Gd**

Scheme S3

Following the procedure reported by Finn and coworkers,[2] from 90 mg (0.2 mmol) of **2**, 113 mg (100%) of Alk-DO3A-Gd were isolated as an off-white solid. ESI-MS: m/z 594.13 (40), 595.13 (74), 596.13 (68), 596.63 (6), 597.13 (MH^+, 100), 598.13 (19), 599.13 (70), 600.14 (10); HR-MS Calcd for $C_{19}H_{29}GdN_5O_7^+$ $[MH]^+$: 597.1308, found: 597.1316.

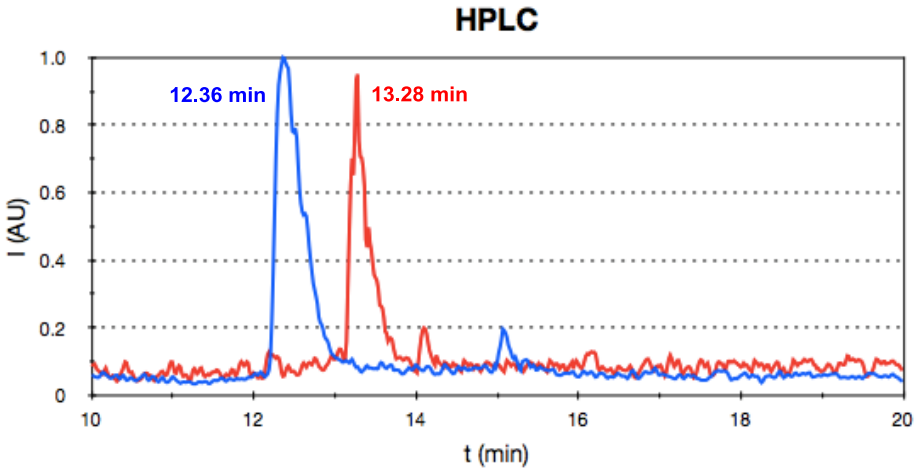


Figure S1: Normalized HPLC elugrams of **2** (blue) and Alk-DO3A-Gd (red).

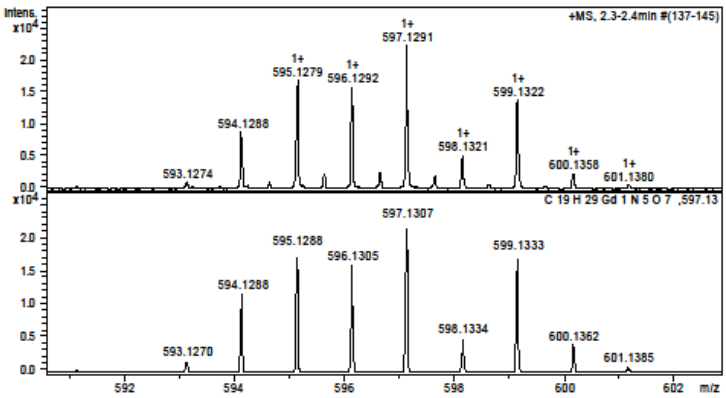


Figure S2: Calculated and found ESI-MS for Alk-DO3A-Gd showing the characteristic Gd isotopic pattern.

Characterization of PEG-[Gn]-(DO3A-Gd)

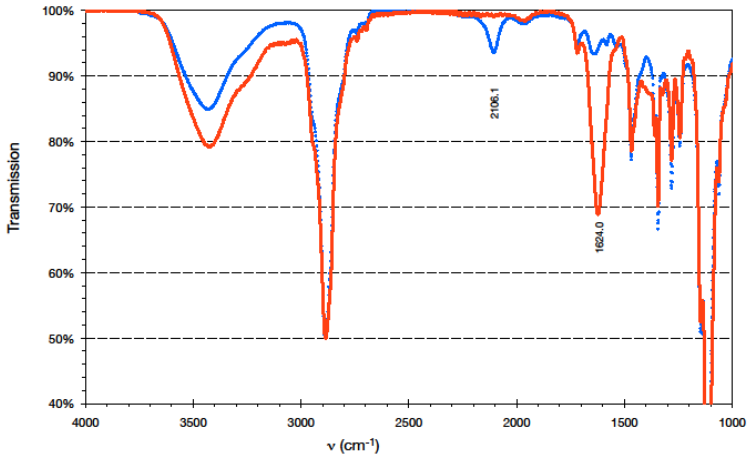


Figure S3: IR spectra of PEG-[G1]-N_3 (blue) and PEG-[G1]-(DO3A-Gd) (red) showing the complete disappearance of the azide signal (2106 cm^{-1}) and appearance of a carbonyl stretch band (1624 cm^{-1}).

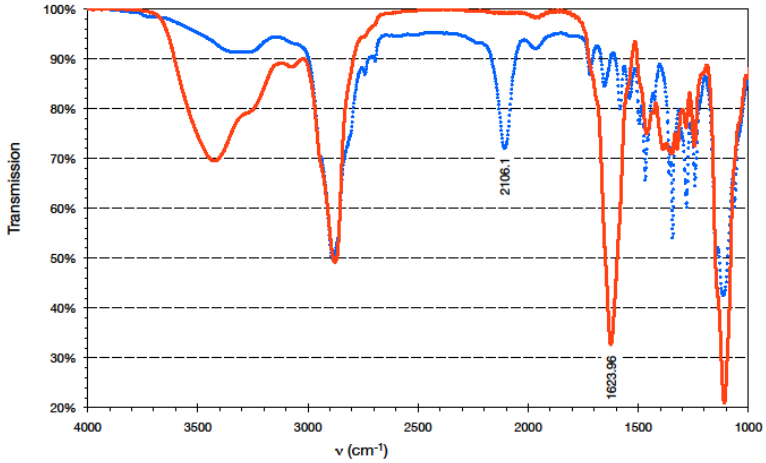


Figure S4: IR spectra of PEG-[G2]-N_3 (blue) and PEG-[G2]-(DO3A-Gd) (red) showing the complete disappearance of the azide signal (2106 cm^{-1}) and appearance of a carbonyl stretch band (1624 cm^{-1}).

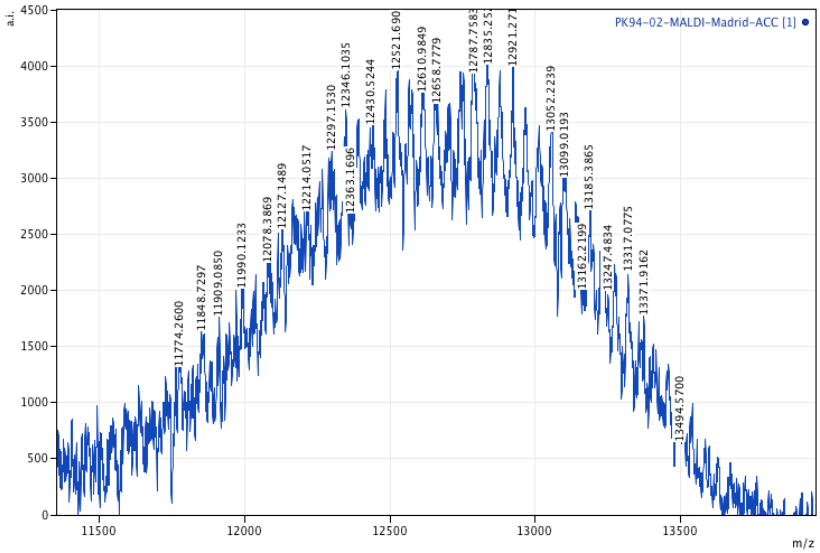


Figure S5: MALDI-TOF spectrum of PEG-[G2]-(DO3A-Gd).

Cytotoxicity tests

The viability of C6 glioma cells was determined by using the classical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Sigma Aldrich MTT kit TOX-1).[3] Briefly, 10^4 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 the dendrimers at different concentrations. After a 24 h incubation period, 20 μL of MTT (15 mg/vial in serum) was added to each well. After 4 h of incubation at 37 ºC and 5% CO_2, the medium was removed, formazan crystals were solubilized with 100 μL of MTT solubilization solution (10% Triton X-100 plus 0.1N HCl in anhydrous isopropanol) and the solution was gently mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader (Dynatech MR7000 instruments) at 570 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 dendrimers was calculated by [A]test/[A]control x 100.

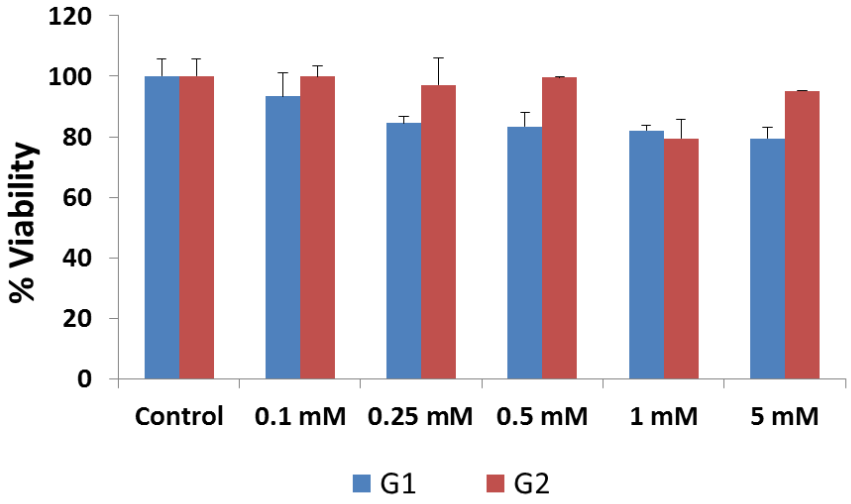


Figure S6: Evaluation of cytotoxicity of PEG-[G1]-(DO3A-Gd) and PEG-[G2]-(DO3A-Gd) on C6 glioma cells relative to Gd concentration.

Magnetic Resonance studies

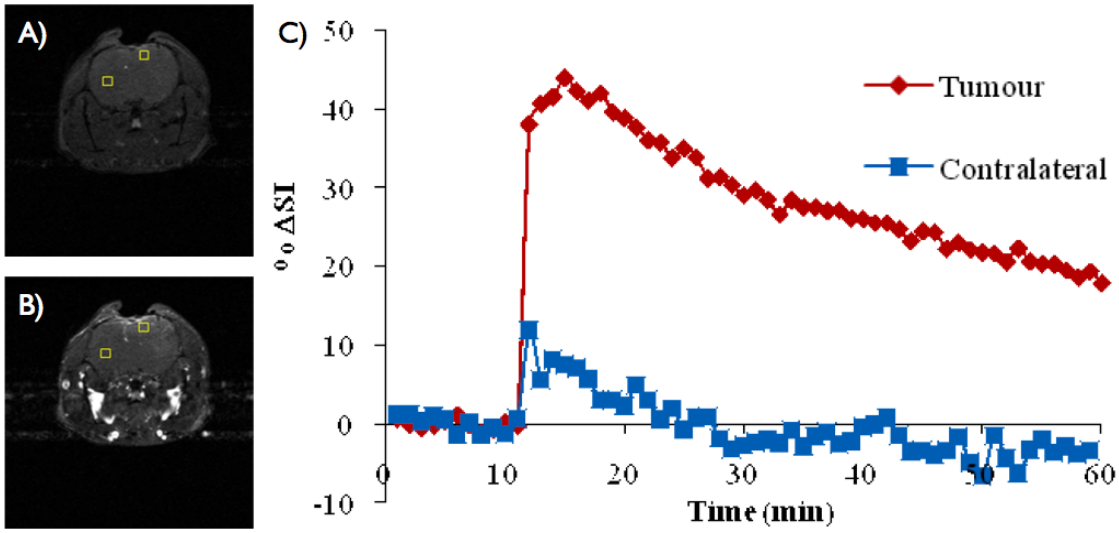


Figure S7: T_1-weighted images of a mouse brain before **A)** and immediately after **B)** the administration of PEG-[G1]-(DO3A-Gd). Right and left squares show the tumor and contralateral hemisphere ROI analyzed. **C)** Normalized ΔSI in tumor (red) and in contralateral hemisphere (blue).

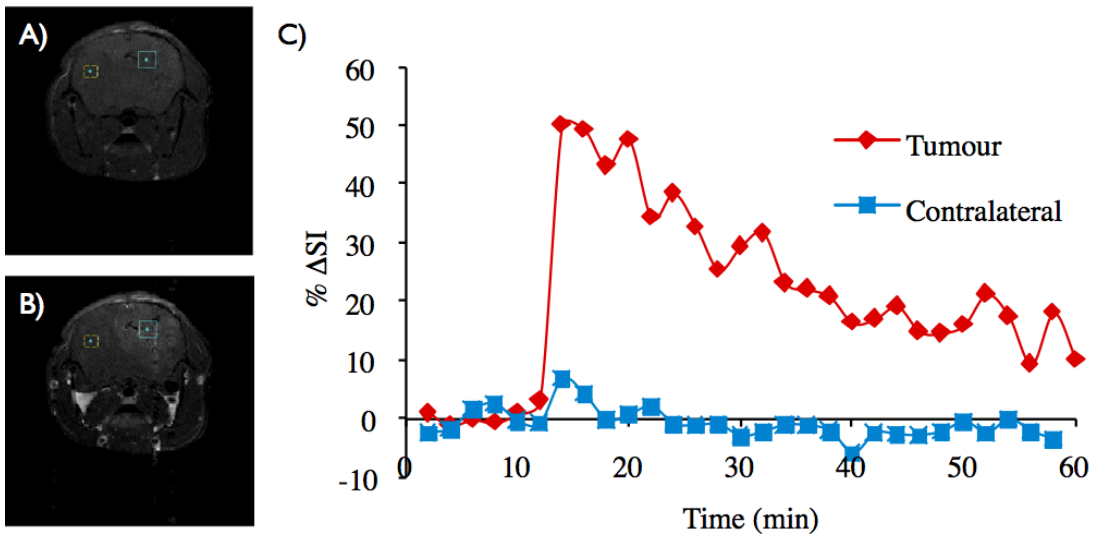


Figure S8: T_1-weighted images of a mouse brain before **A)** and immediately after **B)** the administration of PEG-[G2]-(DO3A-Gd). Right and left squares show the tumor and contralateral hemisphere ROI analyzed. **C)** Normalized ΔSI in tumor (red) and in contralateral hemisphere (blue).

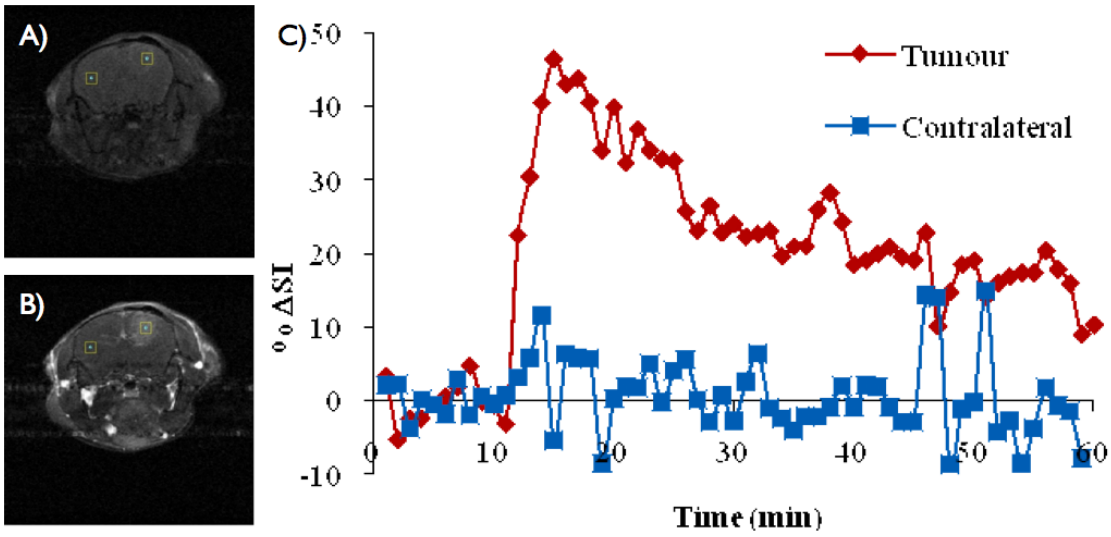


Figure S9: T_1-weighted images of a mouse brain before **A)** and immediately after **B)** the administration of Gadomer-17®. Right and left squares show the tumor and contralateral hemisphere ROI analyzed. **C)** Normalized ΔSI in tumor (red) and in contralateral hemisphere (blue).

References

1 Prasuhn Jr., D. E.; Yeh, R. M.; Obenaus, A.; Manchester, M.; Finn, M. G. *Chem. Commun.* **2007**, 1269.

2 Viguier, R. F. H.; Hulme, A. N. *J. Am. Chem. Soc.* **2006**, *128*, 11370.

3 Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.