Supplementary Material (ESI) for Chemical Communications

A DNA-binding Gd chelate for the detection of cell death by MRI

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Supplementary Information.

Experimental Procedure.

Synthesis of GadoTO.

2: In a sealed tube, 1′-(3′-iodopropyl)-3-methyl-oxa-4′-cyanine iodide **1** [1,2] (200 mg, 341.1 μmol) was suspended and dissolved into 10 mL anhydrous ethanol. A solution of ethyl *N*,*N*-dimethyl-6-aminohexanoate (320 mg, 1.709 mmol, 5 equiv.) in 2 mL anhydrous ethanol was added. The reaction mixture was stirred overnight at 145°C. The dark purple solution was cooled down to room temperature. The precipitate formed was separated by filtration, washed with ether and dried under vacuum. The purple solid powder was dissolved in 8 mL of a mixture of acetonitrile/water (50:50). A solution of NaOH 1M (4 mL) was added. The reaction mixture was stirred at room temperature for 1 h and purified by RP-HPLC (UV monitoring at 500 nm, 0-100% eluant B in 30 min gradient, eluant A: 0.1% TFA, eluant B: 0.1% TFA, 90% acetonitrile, 21 mL.min^{-1} flow rate). The fraction collected (t_R=13 min) was lyophilized. Compound **2** was obtained as an orange solid powder (94.5 mg, 156.5 μmol, 45.9% yield). ($C_{29}H_{36}N_3O_2S^+$, $CF_3CO_2^-$). Calc. exact mass: 490.25+112.99; found m/z: $[M+H]^{2+}$=245.7. ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 1.28 (quintet, 2H, J=8 Hz); 1.56 (quintet, 2H, J=8Hz); 1.69 (m, 2H); 2.30 (m, 4H); 3.05 (s, 6H); 3.29 (m, 2H); 3.50 (m, 2H); 4.07 (s, 3H); 4.65 (t, 2H, J=8 Hz); 7.0 (s, 1H); 7.41 (d, 1H, J=8 Hz); 7.46 (t, 1H, J=8 Hz); 7.65 (t, 1H, J=8 Hz); 7.79 (t, 1H, J=8 Hz); 7.84 (d, 1H, J=8 Hz); 8.03 (t, 1H, J=8 Hz); 8.10 (d, 1H, J=8 Hz); 8.22 (d, 1H, J=8 Hz); 8.65 (d, 1H, J=8 Hz); 8.85 (d, 1H, J=8 Hz).

3: Compound **2** (94.5 mg, 156.5 μmol) was dissolved in 1.3 mL anhydrous DMF. Anhydrous DIPEA (70 μL, 401.9 μmol, 2.6 equiv.) and di(*N*-succinimidyl) carbonate (101.2 mg, 395.1 μmol, 2.5 equiv.) were added. The reaction mixture was stirred at room temperature for 1 h. Precipitation from ether afforded the succinimidyl ester intermediate as a red solid. In a polypropylene vial, *p*-NH_2-Bn-DTPA (70.4 mg, 141.2 μmol) was dissolved in 706 μL anhydrous DMF containing DIPEA (295 μL, 1.694 mmol, 12.0 equiv.). A solution the succinimidyl ester intermediate (78.3 μmol, 0.55 equiv.) in 330 μL anhydrous DMF was added. The reaction mixture was stirred overnight at 60°C and purified by RP-HPLC (UV monitoring at 500 nm; 0-50% eluant B in 30 min gradient; eluant A: ammonium acetate buffer (50 mM); eluant B: acetonitrile; 21 mL.min^{-1} flow rate). The fraction collected (t_R=15 min) was lyophilized. Compound **3** was obtained as a red solid (31.4 mg, 32.4 μmol, 20.7% yield). ($C_{50}H_{63}N_7O_{11}S$). Calc. exact mass: 969.43; found m/z: $[M+2H]^{2+}$=486.0, $[M+3H]^{3+}$=324.2.

4: Compound **3** (23.8 mg, 24.5 μmol) was dissolved in 900 μL of a solution of citric acid 0.1 M pH 5.7. $GdCl_3$,$6H_2O$ (37.0 mg, 99.5 μmol, 4.1 equiv.) dissolved in 1.8 mL citric acid 0.1 M pH 5.7 was added. The reaction mixture was stirred for 2 h at room temperature and purified by RP-HPLC (UV monitoring at 500 nm; 0-50% eluant B in 30 min gradient; eluant A: ammonium acetate buffer 50 mM; eluant B: acetonitrile; 21 mL.min^{-1} flow rate). The fraction collected (t_R=17 min) was lyophilized. Compound **4** (GadoTO) (27.2 mg, 24.2 μmol, quant. yield) was obtained as an orange solid powder. ($C_{50}H_{60}GdN_7O_{11}S$). Calc. exact mass: 1124.33; found m/z: $[M+2H]^{2+}$=563.5.

Relaxation Time Measurements.

Relaxation times were measured at 0.47 T and 40°C using a Bruker Minispec mq20 (Bruker Optics Inc., Woodlands, TX). For T_1 relaxation times, an inversion-recovery sequence was used, which consisted of 12 data points with pulse separations ranging from 5 to 1000 ms and 4 scans each. For T_2 relaxation times, a spin-echo pulse sequence was used, which consisted of 200 data points with a pulse separation of 0.5 ms and 8 scans. Five independent measurements of T_1 and T_2 were recorded.

Preparation of plasmid DNA. Plasmid DNA (6.4 kb total size) was obtained from DH10B E. Coli cells cultured overnight in the presence of ampicillin (100 ug/ml; Mediatech Inc, Manassas, VA) using a Plasmid Maxi preparation kit (Qiagen). The resulting plasmid DNA was quantified by gel electrophoresis.

Determination of the relaxivities of GadoTO.
Solutions of the GadoTO conjugate **4** (concentration range from 0.05 to 2 mM) were prepared in ultrapure water in the presence or in the absence of plasmid DNA at 0.25 μg/μL ([Deoxyribonucleotide]=0.77 mM, assuming an average MW per deoxyribonucleotide of 325 $g.mol^{-1}$). The solutions were transferred into capillary tubes and T_1 and T_2 measured on the Minispec. Molar relaxivities were the slopes of the plots of 1/Ti (i=1,2) versus GadoTO **4** concentrations. R_1 and R_2 were obtained by a linear least-squares fit of the data, with coefficients of correlation greater than 0.99 obtained in all cases. Values are the mean and standard deviation of five independent Ti measurements. Molar relaxivities R_1 and R_2 of Gd-DTPA (Schering, Berlin) in ultrapure water were determined similarly.

Relaxation assay of GadoTO interacting with DNA.
GadoTO **4** at 1 mM was employed with increasing concentrations of plasmid DNA (DNA 0.025 to 0.3 μg/μL). Relaxation times measured on the Minispec as above. T_1 values were fit to a non-linear sigmoidal dose-response regression, GraphPad Prism) to determine the half-maximal concentration (EC_{50}=0.089 mM, 95% confidence interval 0.078-0.101)

Fluorescence assay of GadoTO interacting with DNA.
GadoTO at 2.35 μM was employed with increasing concentrations of DNA. Absorption spectra were measured on a Varian Cary 50 Bio UV-Visible spectrophotometer. The absorbance at 511 nm was measured. Fluorescence emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophometer. Fluorescence intensities were measured between 510 and 700 nm and corrected, when necessary, for matched absorbances at 511 nm. Fluorescence intensities were plotted versus DNA concentrations. Fluorescence values were fit to a non-linear sigmoidal dose-response regression (GraphPad Prism) to determine the half-maximal concentration (EC_{50}=0.179 mM, 95% confidence interval 0.149-0.214).

Cell culture.
All experiments described used Jurkat T cells (Clone E6-1, ATCC #TIB-152). Cells were grown in RPMI 1640 medium (ThermoFisher Scientific, Hampton, NH) with 10 % fetal bovine serum (Valley Biomedical, Winchester, VA), 1% L-Glutamine (Mediatech, Manassas, VA), 1% Penycillin-Streptomycin (Mediatech, Manassas, VA). Media was changed every two or three days. Cells were maintained at 37°C, 5% CO_2.

Flow cytometry experiments.
Cells were treated with camptothecin (CPT, 24 hrs, 10 μM). After removal of the medium, cells were resuspended into 100 μL of binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, 1% FBS). Cells were stained at 37˚C for 15 min with 1 μL Annexin-APC (Invitrogen) and 1 μL propidium iodide (BD Pharmingen) or 1 μL of GadoTO (0.1 mM stock solution in binding buffer). Cells were diluted with 200 μL of binding buffer and analyzed by flow cytometry (FACSCalibur, Becton Dickinson). Fluorescence from the APC fluorochrome was measured in FL4, PI in FL3, and GadoTO in FL1.

Fluorescence microscopy.
Cells were treated (10 μM CPT, 24 h) and, after removal of the medium, resuspended in 200 μL of binding buffer. Cells were stained at 37˚C for 15 min with 4 μL Hoescht 33342 (Invitrogen, stock solution diluted to 10 $\mu g.mL^{-1}$ in binding buffer) and 1 μL of GadoTO (0.1 mM stock solution in binding buffer). The cell suspension (50 μL) was placed on a microscope slide covered with a coverslip imaged immediately at 40x on a Nikon Eclipse 80i equipped with a Cascade Photometrics (512B) camera.

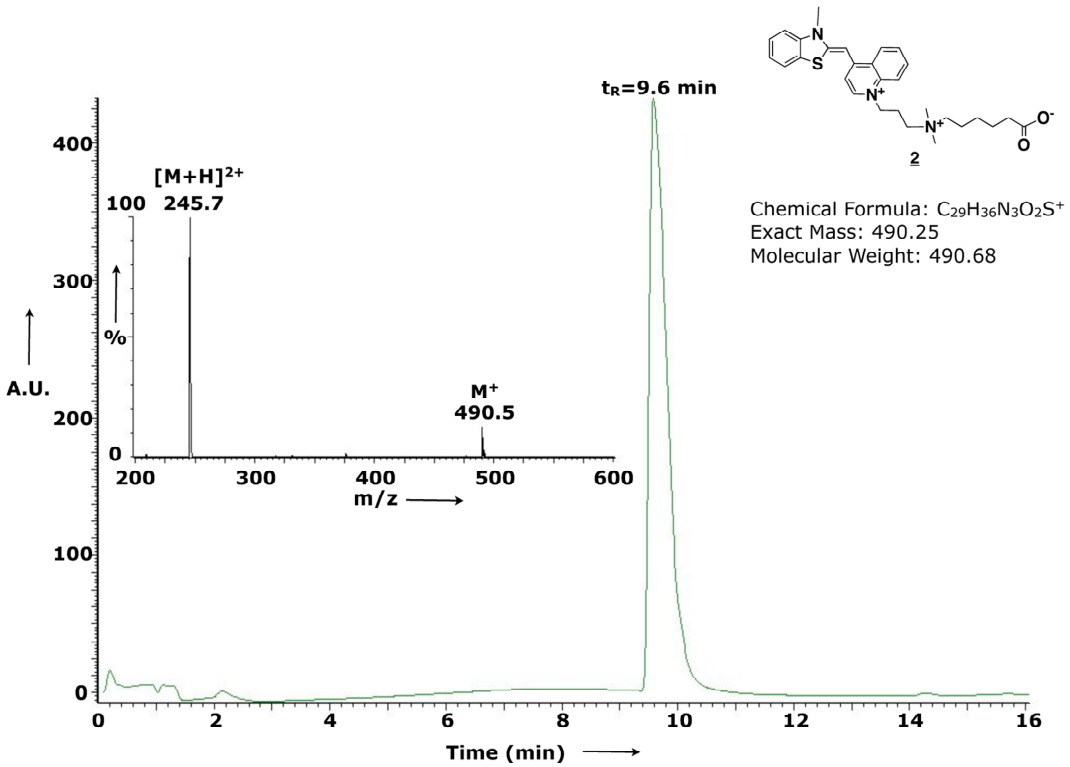
Relaxation assay for cell uptake of GadoTO.
On day 0, cells were seeded at identical and high density into two T75 culture flasks. Cells were cultured for 24 hrs in the absence or in the presence of 10 μM CPT. On day 1, the culture of each flask was numbered with trypan blue and evenly distributed into three assay tubes. The medium was removed and replaced by 100 μL of binding buffer containing either no Gd, 5 mM GadoTO or 5 mM Gd-DTPA. Cells were incubated (45 min, 37°C) and rinsed once with 3 mL binding buffer. Cells were lyzed with 60 μL of 1% Triton X-100 with relaxation times measured as above.

Magnetic Resonance Imaging (MRI).
Cells were treated (10 μM CPT, 24 h), washed with media, and incubated (1h, 37°C) with the Gd complexes in binding buffer. Cells were rinsed twice with 3 mL binding buffer and resupended with liquid Matrigel (BD Biosciences, Bedford, MA) at 4°C. Matrigel solutions were transferred into capillary tubes and allowed to solidify according to manufacturer's intstructions. The tubes were immersed in a 50-mL Falcon tube filled with tap water and imaged on a 9.4-T horizontal bore scanner (Biospec, Bruker, Billerica, MA) equipped with a 1500 mT/m gradient (Resonance Research, Billerica MA). Gradient echo images were acquired with the following parameters: FOV=3 cm, slice thickness 1 mm, matrix 128 x 128, TR 50ms, flip angle 80 degrees, TE= 1.4 ms, and signal averages = 4.

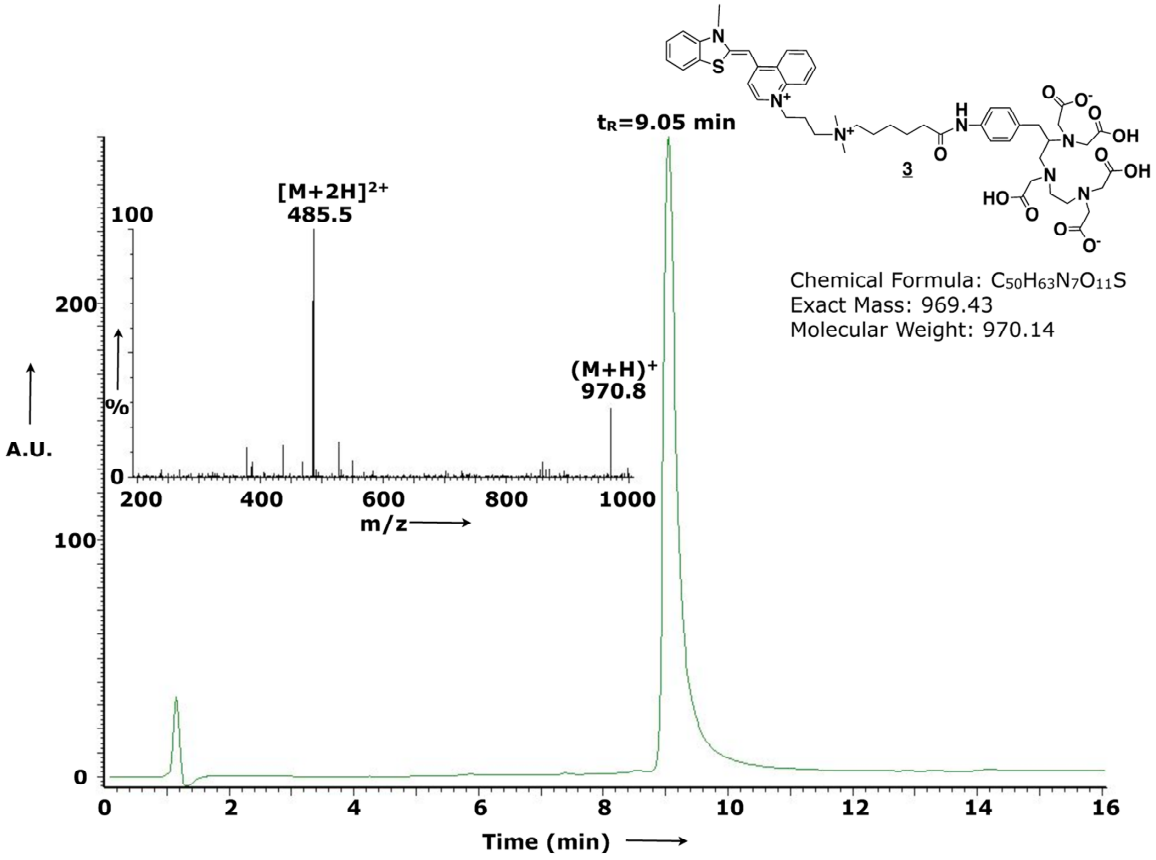
Fluorescence Reflectance Imaging (FRI).
Capillary tubes prepared as described above for MRI were imaged with a 12-bit CCD camera (Kodak, Rochester, NY) equipped with FITC filters, suitable for the detection of TO.

(1) Brooker, L. G. S.; Keyes, G. H.; Williams, W. W. *J Am Chem Soc* **1942**, *64*, 199-210.
(2) Nygren, J.; Svanvik, N.; Kubista, M. *Biopolymers* **1998**, *46*, 39-51.

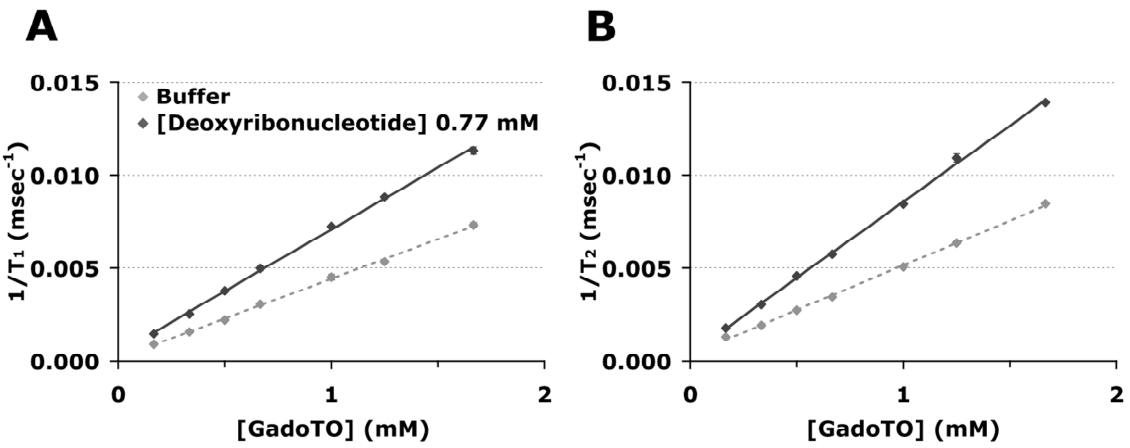
LC-MS of intermediate 2.



LC-MS of intermediate 3.



Determination of molar relaxivities of GadoTO and Gd-DTPA.



		R_1 ($mM^{-1}.msec^{-1}$)	R_2 ($mM^{-1}.msec^{-1}$)
GadoTO	Buffer	$(4.23 \pm 0.06).10^{-3}$ *(r^2=0.999)*	$(4.80 \pm 0.08).10^{-3}$ *(r^2=0.999)*
	DNA ([Deoxyribonucleotide]=0.77mM)	$(6.5 \pm 0.2).10^{-3}$ *(r^2=0.996)*	$(8.2 \pm 0.1).10^{-3}$ *(r^2=0.998)*
Magnevist®	Buffer	$(3.96 \pm 0.02).10^{-3}$ *(r^2=0.999)*	$(4.35 \pm 0.03).10^{-3}$ *(r^2=0.999)*

(0.47 T, 40°C)