SUPPORTING INFORMATION

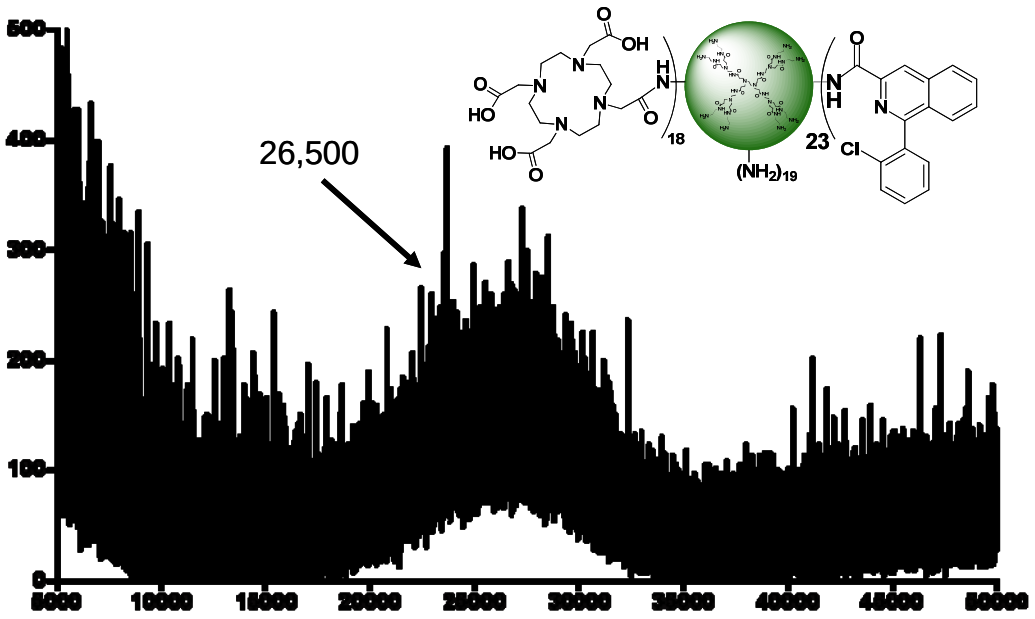

Figure S1. MALDI-TOF Spectrum of ClPhIQ-PAMAM-DO3A.

Table S.1 Relaxivities of PAMAM-Gd compounds.

Compound	T1 Relaxation per Gd^{3+} ($mM^{-1}s^{-1}$)	T1 Relaxation per PAMAM ($mM^{-1}s^{-1}$)	T2 Relaxation per Gd^{3+} ($mM^{-1}s^{-1}$)	T2 Relaxation per PAMAM ($mM^{-1}s^{-1}$)
Magnevist™	4.8±0.044	-	5.6±0.13	-
PAMAM-Gd	7.8±0.65	180	7.7±0.27	177
ClPhIQ-PAMAM-Gd (**6**)	7.7±0.76	177	8.0±1.2	184
ClPhIQ-PAMAM-Gd-Liss (**7**)	4.0±1.04	92	3.8±0.40	87

Table S.2 Relaxation rates of Dye-PAMAM-Gd compounds obtained on a Maran 0.5T NMR scanner.

Compound	T1 Relaxation per Gd^{3+} ($mM^{-1}s^{-1}$)	T1 Relaxation per PAMAM ($mM^{-1}s^{-1}$)	T2 Relaxation per Gd^{3+} ($mM^{-1}s^{-1}$)	T2 Relaxation per PAMAM ($mM^{-1}s^{-1}$)
Magnevist™	4.8±0.044	-	5.6±0.13	-
Cy5.5-PAMAM-Gd	5.56±0.10	128	5.95±0.17	137
Liss-PAMAM-Gd (Gd attached first)	4.34±0.24	100	4.04±0.33	93
PAMAM-Gd (reacted under dye conjugation conditions)	7.33±0.25	169	7.19±0.18	165
Liss-PAMAM-Gd (Liss attached first)	5.83±0.79	134	6.10±0.86	140

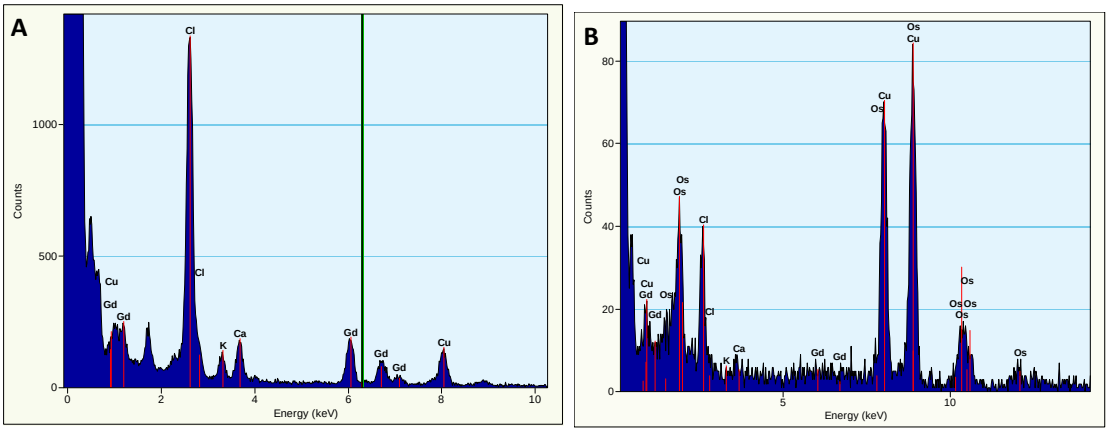

Figure S2. EDX spectrum of $ClPhIQ_{23}$-PAMAM-Gd_{18} on a grid and within C6 rat glioma cells. **A:** 83.3 µM $ClPhIQ_{23}$-PAMAM-Gd_{18} on Formvar coated, 300 mesh Copper grid (0.5 µL of solution added 1.915 mM in Gd) **B:** $ClPhIQ_{23}$-PAMAM-Gd_{18} (32µM, 18 Gadolinium/DOTA's, 23 conPK's) incubated for 10 hrs with C6 Glioma Cells, 250nm thick, Carbon-Coated (No U or Pb stain)

Experimental Procedures

General Methods: G(4)-PAMAM™ dendrimer in a 10% w/w solution of methanol was purchased from Fischer Scientific and pipetted into a pre-weighed vial immediately before use. The methanol was evaporated under a stream of $Ar_{(g)}$ and the resulting viscous oil was dissolved in water, frozen and lyophilized to give a fluffy white powder. The mass of the dendrimer was determined by re-weighing the vial and calculating the mass difference. 1-(2-chlorophenyl)isoquinoline-3-carboxylic acid ClPhIQ Acid was synthesized in our laboratory as previously reported[36-37]. All 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and DOTA derivatives were purchased form Macrocyclics and used as arrived. All other chemicals were purchased from Fischer Scientific and used as is unless otherwise indicated.

MALDI-TOF MS were obtained on a PerSeptive Biosystems Voyager-DE STR mass spectrometer. Freshly recrystalized trans-indole acrylic acid (IAA) was used as the matrix in a 10 mg/mL solution of DMSO. The plate was spotted with 1 μL of a 10:1 solution of matrix to analyte. NMR spectra were obtained on a 400 MHz Bruker AV-400 instrument with a 5 mm Z-gradient broadband inverse probe. NMR spectra were obtained in d6-dimethyl sulphoxide. UV-vis spectra were obtained on a Shimadzu 1700 UV-vis spectrophotometer. Fluorescence spectra were obtained using a ISS PCI spectrofluorometer at room temperature. MR relaxivities were obtained using a Maran 0.5T NMR scanner.

$ClPhIQ_{23}$-PAMAM™-DOTA$^tBu_{18}$ (1): $ClPhIQ_{23}$-PAMAM™ (202 mg, 9 μmoles) and DOTA^{t}Bu (179 mg, 97 μmols) were dissolved in 2 and 1 mL of DMSO respectively. The 1,4,7,10-Tetraazacyclododecane-1,4,7-tris(t-butyl acetate)-10-acetic acid mono(N-hydroxysuccinimide ester) (DOTA^{t}Bu-NHS) solution was added dropwise to a stirring solution of $ClPhIQ_{23}$-PAMAM™ over a 10 minute period. The reaction was stirred overnight and purified with the Amicon Centrifugation filters (5kDa. MWCO) following the procedure used for $ClPhIQ_{23}$-PAMAM purification and lyophilized to give 163.2 mg (100% yield) of $ClPhIQ_{23}$-PAMAM-DOTA$^tBu_{18}$. The molecular weight was determined using MALDI-TOF MS to be 31,000 a.m.u. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.8 (1H, s), 8.6 (1H, s), 8.2-8.5 (20H, m), 7.5-8.0 (9H, multi), 2.9 (3H, bs) and 3.1-3.5 (208H, m), 1.4 (108, s) ppm.

$ClPhIQ_{23}$-PAMAM-$DO3A_{18}$: $ClPhIQ_{23}$-PAMAM-DOTA$^tBu_{18}$ (256.0 mg, 9 μmoles) was dissolved in 3 mL of neat trifluoroacetic acid (TFA) and stirred overnight. The solution was diluted 10 fold with water and purified with Amicon Centrifugation filters (5kDa. MWCO) following the procedure used for $ClPhIQ_{23}$-PAMA purification and lyophilized to give 120 mg (100% yield). The molecular weight was determined using MALDI-TOF MS to be 27,000. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.8 (1H, s), 8.6 (1H, s), 8.2-8.5 (5H, m), 7.5-8.0 (20H, multi), 2.9 (3H, bs) and 3.1-3.5 (73H, m) ppm.

$ClPhIQ_{23}$-PAMAM-$DO3A_{18}$-Lissamine: $ClPhIQ_{23}$-PAMAM-$DO3A_{18}$ (4.8 mg, 148 nmoles) was dissolved in 1 mL of dimethylformamide (DMF). Next, 12.8 μL of a 17.3 mM solution of lissamine rhodamine B sulfonyl chloride™ (221 nmoles) was added to the stirred dendrimer solution. The reaction was stirred overnight followed by purification in Amicon Centrifugation filters (5kDa. MWCO) as described above and lyophilized to give 2.8 mg (57% yield) of a pink fluffy solid. The presence of the fluorophor was confirmed with UV-vis (max absorbance at 551 nm) and Fluorescence (ex 551 nm, em. 586 nm) spectroscopies.

$ClPhIQ_{23}$-PAMAM-Gd_{18}: $ClPhIQ_{23}$-PAMAM-$DO3A_{18}$ (22 mg, 679 nmoles) was dissolved in water. Next, 1 mL of a 9.1 mg/mL solution of $GdCl_3$ (9.1 mg, 25 μmoles) was added to the dendrimer solution. The reaction was stirred at room temperature for 24 hours and purified with Amicon Centrifugation filters (5kDa. MWCO) following the

procedure used for $ClPhIQ_{23}$-PAMAM purification and lyophilized to give 24.7 mg (100% yield). The molecular weight was determined using MALDI-TOF MS to be 30,500 Da.

$ClPhIQ_{23}$-PAMAM-Gd_{18}-Liss (3): $ClPhIQ_{23}$-PAMAM-Gd_{18} (5.3 mg, 145 nmoles) was dissolved in 1 mL of dimethylformamide (DMF). Next, 12.7 μL of a 17.3 mM solution of lissamine rhodamine B sulfonyl chloride™ (220 nmoles) was added to the stirred dendrimer solution. The reaction was stirred overnight followed by purification in Amicon Centrifugation filters (5kDa. MWCO) as described above and lyophilized to give 3.7 mg (69% yield) of a pink fluffy solid. The presence of the fluorophor was confirmed with UV-vis (max absorbance at 551 nm) and Fluorescence (ex 551 nm, em. 586 nm) spectroscopies.

Cell Internalization Experiments: PAMAM-Liss derivatives: Either 20,000 C6 rat glioma or 40,000 MDA-MB-231 breast cancer cells were plated into collagen coated glass bottom microscopy (MatTeck™) dishes and allowed to grow for two days in cell media. After two days, the cells had good morphology and were attached, however were not confluent. PAMAM-Liss compounds (with or without other moieties) were diluted to 1 μM with media from a 10 mg/mL stock solution in DMSO. The media over the cells was replaced with the media containing the fluorophor and incubated for 6-12 hours. The media was then poured off and the cells were carefully rinsed 3-4 times with Dulbecco's Phosphate Buffered Saline (DPBS). The cells were imaged live. Both white light and fluorescence pictures were obtained.

For fixed cells, the rinsed cells were incubated at room temperature for 30 minutes with a 4% Para formaldehyde solution. The cells were carefully washed 4 times with DPBS and stored under DPBS at 4 °C until imaged (not longer than 1 week, typically overnight). Immediately before imaging, the fixed cells were incubated with a 25 nM solution of mitotracker green (MTG) for 10 minutes. Excess MTG was removed by 4 washings with DPBS and the cells were imaged.

Radiobinding Assay: To complete this assay, C6 rat glioma cells (cultured in Dulbecco's modified Eagle medium (DMEM)-F12 medium (Gibco/Invitrogen) supplemented with 0 5% FBS and 2.5% horse serum (HS) at 3.7% CO_2) were scraped from 150 mm culture dishes into 5 mL of phosphate buffered saline (PBS), dispersed by trituration, and centrifuged at 500 x*g* for 15 min. Cell pellets were resuspended in PBS and assayed to determine protein concentration. The binding studies with [^{3}H]PK11195 on 30 μg of protein from cell suspensions were performed as previously described [176]. The data was analyzed using PRISM software (vs 4.0, GraphPad, Inc., San Diego, CA). In PRISM, the one binding site competition assay wizard was used, which incorporates the equation: Y=Bottom + (Top-Bottom)/(1^10(X-LogEC_{50})). For the ClPhIQ-PAMAM-Gd dendrimer, the IC50 was found to be 510 nM on a per ClPhIQ ligand basis with a goodness to fit error of 0.9394.

Preparation of TEM Samples: C6 cells were plated in a 4 cm culture dish, allowed to propagate to near 75% confluency and treated with a 32 μM $ClPhIQ_{23}$-PAMAM-Gd_{18} solution in Dulbecco's Modified Eagle Medium. After incubation at 37 °C for 10 hours, the C6 cells were then washed three times with a 0.1 M solution of sodium cacodylate buffer to remove any extracellular $ClPhIQ_{23}$-PAMAM-Gd_{18}. The cells were subsequently fixed for one hour using a 4% paraformadehyde solution. The fixed cells were post-fixed using a 1% Osmium Tetroxide solution, dehydrated using ethanol, pelletized and sliced into 80 nm thick sections. The sections were placed on a 300 mesh copper grid and imaged on a Phillips CM-12 Electron Microscope.

Relaxivity of Gd Dendrimers: Samples were prepared at 5 concentrations (in Gd) from 1 μM to 1 mM, depending on the availability of sample. Pre-written pulse sequences

were used to obtain the relaxation at each concentration for both T1 and T2 which varied the time intervale between the pluse and detecting the population that had relaxed. The data was graphed and fitted to a three parameter exponential rise to max curve to give the relaxation at each concnetration. Each sample was run 3 times with 15 minute intervals between trials. The relaxivity was determined by graphing the relaxation versus concentration and fit to a line. The slope of the line is the relaxivity.