Extra Supporting Information (ESI)

Experimental Procedures

General

All chemicals were purchased from Sigma-Aldrich and used as received, unless otherwise specified. 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-NHS ester) was purchased from CheMatech.

Preparation of Aminated Mesoporous Silica Nanoparticles (MSNs) by Delayed Co-condensation Technique

MSNs were prepared using a hydrolysis and condensation reaction in the presence of a templating surfactant. Briefly, cetyl trimethylammonium bromide (CTAB, 0.64 g, 1.77 mmol) was dissolved by vigorous stirring into a mixture of Ultrapure water (16.02 mL, 0.89 mol) and ethanol (1.84 mL, 0.04 mol). The solution was brought to 80 ^{o}C. Triethanol amine (1.03 g, 6.9 mmol) was added and mixed until dissolved. Tetraethylorthosilicate (TEOS, *varied*) was added dropwise. The solution was stirred at 80 ^{o}C. A solution containing a 1:1 molar ratio of 3-aminopropyltriethoxysilane (APTES, *varied*) and TEOS (*varied*) was prepared and added to the solution dropwise after 10 minutes ('short delay' co-condensation) or 1 hour ('long delay' co-condensation); the reaction was then continued at 80 ^{o}C for a total time period of 2 h. After this time, an opaque white solution remained. The nanoparticles were centrifuged and redispersed into an acidified ethanol solution to remove the surfactant. The nanoparticles were then washed three times with ethanol and retained in a concentrated ethanol suspension (Figure S1). The relative amounts of TEOS and APTES were varied depending on the desired loading of amine groups required, as summarised in Table S1.

Preparation of Aminated MSNs by post-grafting technique

CTAB (0.64 g, 1.77 mmol) was dissolved by vigorous stirring into a mixture of Ultrapure water (16.02 mL, 0.89 mol) and ethanol (1.84 mL, 0.04 mol). The solution was brought to 80 ^{o}C. Triethanol amine (1.03 g, 6.9 mmol) was added and mixed until dissolved. TEOS (1.454 mL, 6.5 mmol) was added dropwise. The solution was reacted at 80 ^{o}C for 2 h. The nanoparticles were centrifuged and redispersed into an acidified ethanol solution to remove the surfactant. The nanoparticles were then washed three times with ethanol and retained in a concentrated ethanol suspension (Figure S1). The nanoparticles were aminated by dispersing the MSNs (100 mg) into a mixture of Ultrapure water (5 mL) and ethanol (10 mL) and adding APTES (0.585 µL, 2.6 µmol) and stirring at room temperature for 24 hours. The particles were washed 3 times with ethanol using centrifugation (Figure S1).

Preparation of Aminated Non-porous SiO_2 Nanoparticles

Non-porous silica nanoparticles were prepared using a modified Stöber technique.[1, 2] Briefly, ammonium hydroxide (0.1365 mL, 4.5 mmol, 28 v/v%) was added dropwise to a stirring solution of TEOS (5.585 mL, 0.025 mol), Ultrapure water (1.8 mL, 0.1 mol) and ethanol (11.678 mL, 0.2 mol) at 40 ^{o}C and reacted at this temperature for 2 h. The resulting white precipitate was washed with water 3 times using centrifugation. The nanoparticles were post-grafted with amine groups by dispersing non-porous nanoparticles (90 mg) into a mixture of Ultrapure water (5 mL) and ethanol (10 mL) and

adding APTES (0.5 µL, 2.14 µmol) dropwise and stirring at room temperature for 24 hours. The particles were washed with ethanol 3 times using centrifugation.

Loading of Aminated Nanoparticles with Gd-DOTA Contrast Agent

Aminated MSNs and non-porous silica nanoparticles were functionalised with 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-NHS ester) and subsequently with Gd-ions. Briefly, aminated MSNs (50 mg) were dispersed into anhydrous dimethylformamide (DMF, 15 mL) and stirred vigorously at room temperature for 24 h with DOTA-NHS-ester (*varied*) and triethylamine (150 µL). After this time, the nanoparticles were washed three times with ethanol using centrifugation and redispersed into ethanol (10 mL). $GdCl_3$ (*varied*) was added and stirred vigorously at room temperature for 24 h. The nanoparticles were washed 8 times with ethanol and retained for characterisation (Figure S2). Note: the amount of DOTA-NHS-ester and corresponding $GdCl_3$ was varied depending on the NH_2 loading on the aminated particles used (see Table S1). The weight percentages of Gd on Gd-DOTA-MSNs were calculated and confirmed using ICP-MS to be 1.37 wt% (1470 Gd^{3+} ions per particle), 2.07 wt% (2870 Gd^{3+} ions per particle) and 3.82 wt% (4420 Gd^{3+} ions per particle).

Table S1 Total amounts of TEOS and APTES used in co-condensation amination experiments; amount of DOTA-NHS-ester and $GdCl_3$ used in contrast agent loading experiments.

Sample (mol% amination)	APTES (µmol)	Total TEOS (mmol)	DOTA-NHS-ester (µmol)	$GdCl_3$ (µmol)
0.15	10	6.5	4.7	9.4
0.23	15	6.5	7.2	14.4
0.5	32.5	6.5	15.6	31.2

Stability Testing

The presence of free Gd ions was determined by using arsenazo (III), a dye which forms a coloured complex when chelated with gadolinium. A 10^{-5} M aqueous gadolinium solution was prepared by dissolving $GdCl_3$ (3 mg, 0.11 µmol) into Ultrapure water (1 L). A 10^{-6} M aqueous arsenazo (III) solution was prepared by dissolving arsenazo (III) (0.8 mg, 1.03 µmol) into Ultrapure water (1 L). A series of mixtures of Gd-solution and arsenazo solution were prepared and their UV-vis absorption spectra were collected which assessed the limit of detection of the arsenazo (III) to be 10 nmol $GdCl_3$. After washing with Ultrapure water by centrifugation, the supernatant was removed from the nanoparticles and mixed with arsenazo to assess the presence of free Gd ions. The supernatant of the nanoparticles was tested for free Gd ions after different time periods to assess the long-term stability of the Gd-DOTA chelate on the MSNs in Ultrapure water suspensions (Figure S4). A positive control containing $GdCl_3$ was tested. To confirm that the washing procedure was adequate for removal of free Gd-ions, aminated nanoparticles (NH_2-MSNs) were mixed with equimolar $GdCl_3$ ions and washed using centrifugation; the supernatant was then tested for Gd ions.

Biomodification of Gd-DOTA-MSNs.

Gd-DOTA-MSNs (40 mg) were mixed with silanized PEG5000 (3.5 nmol) in 1:2 H_2O:ethanol (ratio by volume) mixture and reacted at room temperature for 24h. After PEGylation, the nanoparticles were

washed twice with ethanol using centrifugation and then redispersed in anhydrous DMF (20 mL) with succinic anhydride (7 nmol) to convert the amine group to a carboxyl group. The carboxylation reaction was carried out at room temperature overnight. The resulting modified Gd-DOTA-MSNs were coupled with biotin-BSA (3.5nmol) by reacting with N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) and N-Hydroxysuccinimide (NHS). Nanoparticles were washed twice in ice-cold water using centrifugation.

Characterisation Techniques

Transmission Electron Microscopy (TEM)

TEM images were obtained on a Jeol JEM-2100, 200 kV, LaB_6 instrument, operated at 120 kV with a beam current of about 65 mA. Samples for TEM were prepared by deposition and drying of a drop of an aqueous colloidal suspension of nanoparticles onto a formvar-coated 300-mesh copper grid. Diameters were measured using the ImageJ version 1.40 software program; average values were calculated by counting a minimum of 100 particles.

Dynamic Light Scattering (DLS) and Zeta Potential

DLS measurements were carried out using Malvern Zetasizer Nano operated at 25 ^{o}C. A 532 nm laser was used as the light source and the measurements were recorded at a detection angle of 173^{o} (backscatter). PDI indicated the polydispersity index of the suspensions. Zeta potential measurements were executed using a disposable capillary cell. Hydrodynamic diameters (d_{hyd}) and zeta potential (ζ pot.) values were calculated as an average of 5 measurements. Suspensions of nanoparticles (~1mg/mL) were measured in Ultrapure water.

^{1}H MRI Relaxivity Measurements

Samples for MRI measurements were prepared by dispersing a known amount of nanoparticles or Gd-complex into Ultrapure water. Several different concentrations were prepared and measured for each sample. r_1 relaxivity values were calculated from curves plotted of R_1 ($1/T_1$, s^{-1}) vs. [Gd] concentration (mM) and analysis of the slope of the line of best fit for each sample, with error measured from the average of a minimum of 2 measurements for each concentration point. The water ^{1}H longitudinal relaxation rates of these aqueous samples were measured at 283 K at 300 MHz (7 T) using a Varian Inova (Varian Inc., Palo Alto, CA) and 128 MHz (3 T) using a Siemens Verio (Siemens AG, Munich, Germany). For the measurement of the relaxation rates, the standard inversion-recovery method was employed with a typical 90^{o} pulse calibration of 250 µs. The temperature was controlled at 20 ^{o}C. T_1-weighted phantom images were captured on aqueous samples with [Gd] concentrations of 0.04 mM using *TR* = 30 ms; *TE* = 5 ms; a slice thickness of 2 mm and a matrix of 256x256.

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

[Gd] concentrations were calculated and verified using ICP-MS analysis. Analysis of [Gd] concentration was carried out using ICP-MS and samples were measured by $LiBO_2$ fusion by Viridian Partnership, Surrey.

UV-visible Spectroscopy

UV-visible spectroscopy (200-800 nm) was carried out using a Shimadzu UV PC-2401.

Nitrogen Adsorption-Desorption

Nitrogen adsorption-desorption analyses were carried out on a CE Instruments Sorptomatic 1990 instrument using 100 mg of dried powder samples which were degassed at 200°C for at least 12 hours before running adsorption measurements.

Figures and Tables

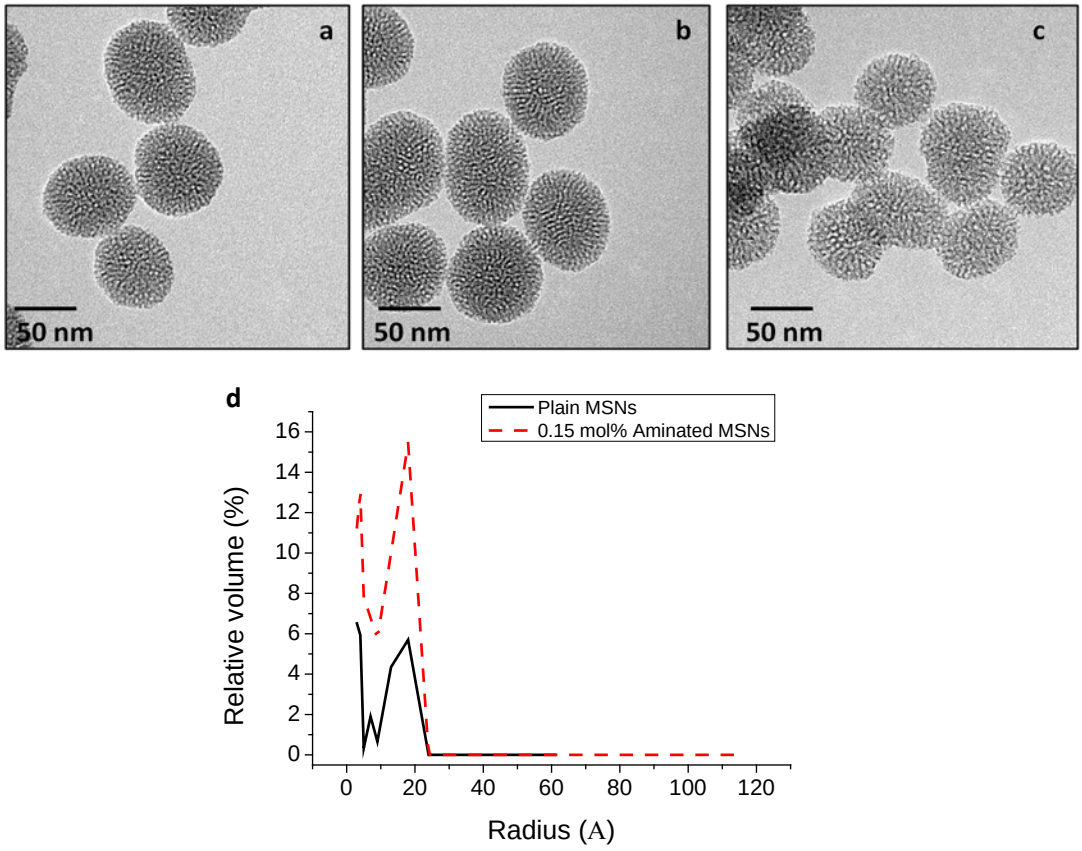

Fig. S1 Transmission Electron Microscope (TEM) images of a) plain MSNs (67.8 ± 7.1 nm; d_{hyd} 143.9 nm, PDI 0.111, ζ pot. -29±6 mV); and 0.15 mol% aminated MSNs prepared by b) delayed co-condensation (68.3 ± 7.2 nm; d_{hyd} 179.3 nm, PDI 0.096, ζ pot. -26±4.3 mV) and c) post-grafting techniques (64.3 ± 6 nm; d_{hyd} 162.3 nm, PDI 0.129, ζ pot. -22±7.1 mV). d) Pore size distribution graph of volume *vs.* radius of plain nanoparticles and 0.15% aminated MSNs indicating nitrogen adsorption inside mesopores of radii 1.6±0.65 nm, which did not change with amination.

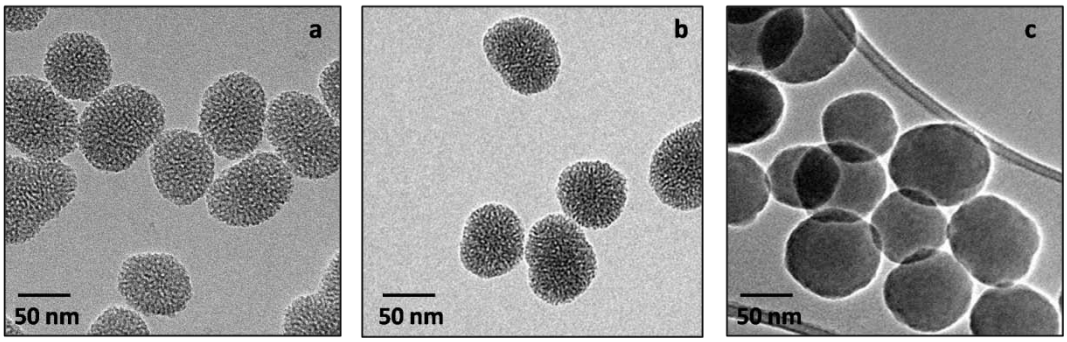

Fig. S2 TEM images of Gd-DOTA-MSNs prepared by a) delayed co-condensation (68.3 ± 7.2 nm; d_{hyd} 138.9 nm, PDI 0.084, ζ pot. -11±6.3 mV), b) post-grafting (64.3 ± 6 nm; d_{hyd} 146.2 nm, PDI 0.129, ζ pot.-11.1±5.2 mV) and c) Gd-DOTA-non-porous silica nanoparticles (81±7.7 nm; d_{hyd} 115.5, PDI 0.076, ζ pot.-43±8.7 mV).

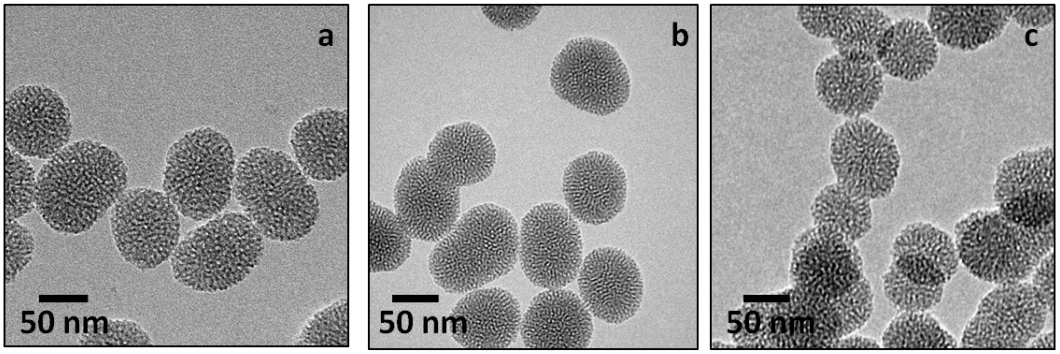

Fig. S3 TEM images of Gd-DOTA-MSNs prepared by using delayed co-condensation technique with a) 1.37 wt% Gd (62±4.9 nm; d_{hyd} 139 nm, PDI 0.084, ζ pot. -11±6.4 mV); b) 2.07 wt% Gd (68±7.2 nm; d_{hyd} 169 nm, PDI 0.085, ζ pot. -11.4±6.1 mV); and c) 3.82 wt% Gd (64±6 nm; d_{hyd} 169.9 nm, PDI 0.128, ζ pot.-10.2±5.5 mV).

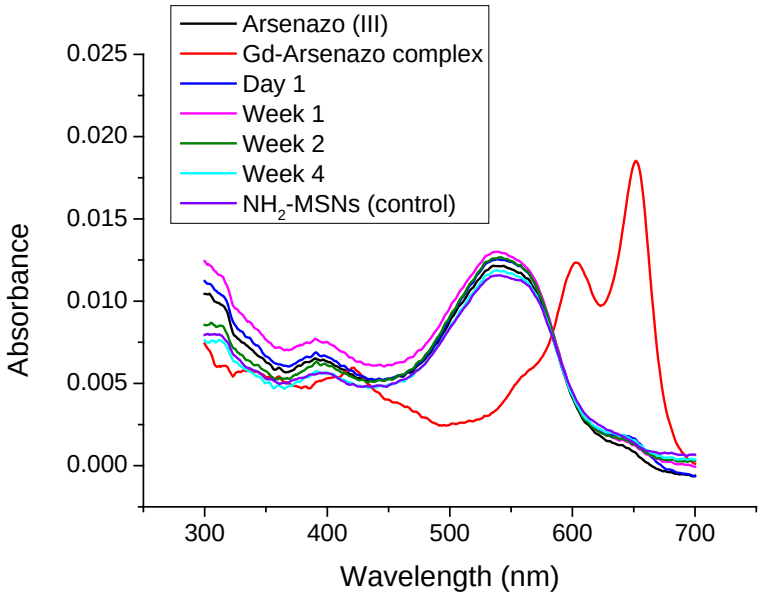

Fig. S4 UV-vis spectra of aqueous arsenazo (III) with washed Gd-DOTA-MSN and NH_2-MSN supernatant solutions (Ultrapure water) and a $GdCl_3$ solution control. Gd-DOTA-MSNs demonstrated excellent stability and no leakage of Gd ions over a 4 week period.

Table S2 Comparison of relaxivities with prior reports of Gd-chelate-loaded nanomaterials.

Nanocomposite (percentage Gd loading)	**Average particle size (nm)**	**Magnetic field (T)**	**Relaxivity (r_1) per Gd ($mM^{-1}s^{-1}$)**
Gd-DOTA complex	n/a	7	2.01
Delayed co-condensation Gd-DOTA-MSNs (1.37 wt% Gd) – this work	70	3 7	**39.26** **33.57**
Immediate co-condensation Gd-DTTA-MSNs[3] (15.2 wt% Gd)	75	3	6.2
Post-grafting Gd-DTTA-MSNs[4] (15.7-20.1 wt% Gd)	75	3 9.4	28.8 10.2
Post-grafting Gd-DTTA-MSNs[5] (4.3 wt% Gd)	40	3	19.7

Chelates described include gadolinium (1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Gd-DOTA); gadolinium diethylenetriaminetetraacetic acid (Gd-DTTA).

References

1. W. Stöber, A. Fink and E. Bohn, *Journal of Colloid and Interface Science*, 1968, **26**, 62.
2. G.-L. Davies, A. Barry and Y. K. Gun'ko, *Chemical Physics Letters*, 2009, **468**, 239-244.
3. K. M. L. Taylor-Pashow, J. D. Rocca and W. Lin, *Nanomaterials*, 2011, **2**, 1-14.
4. K. M. L. Taylor, J. S. Kim, W. J. Rieter, H. An, W. Lin and W. Lin, *Journal of the American Chemical Society*, 2008, **130**, 2154-2155.
5. W. J. Rieter, J. S. Kim, K. M. L. Taylor, H. An, W. Lin, T. Tarrant and W. Lin, *Angewandte Chemie International Edition*, 2007, **46**, 3680-3682.