Supplementary Information

Controlled intracellular self-assembly of gadolinium nanoparticles as smart molecular MR contrast agents

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[4]These authors contributed equally to this work

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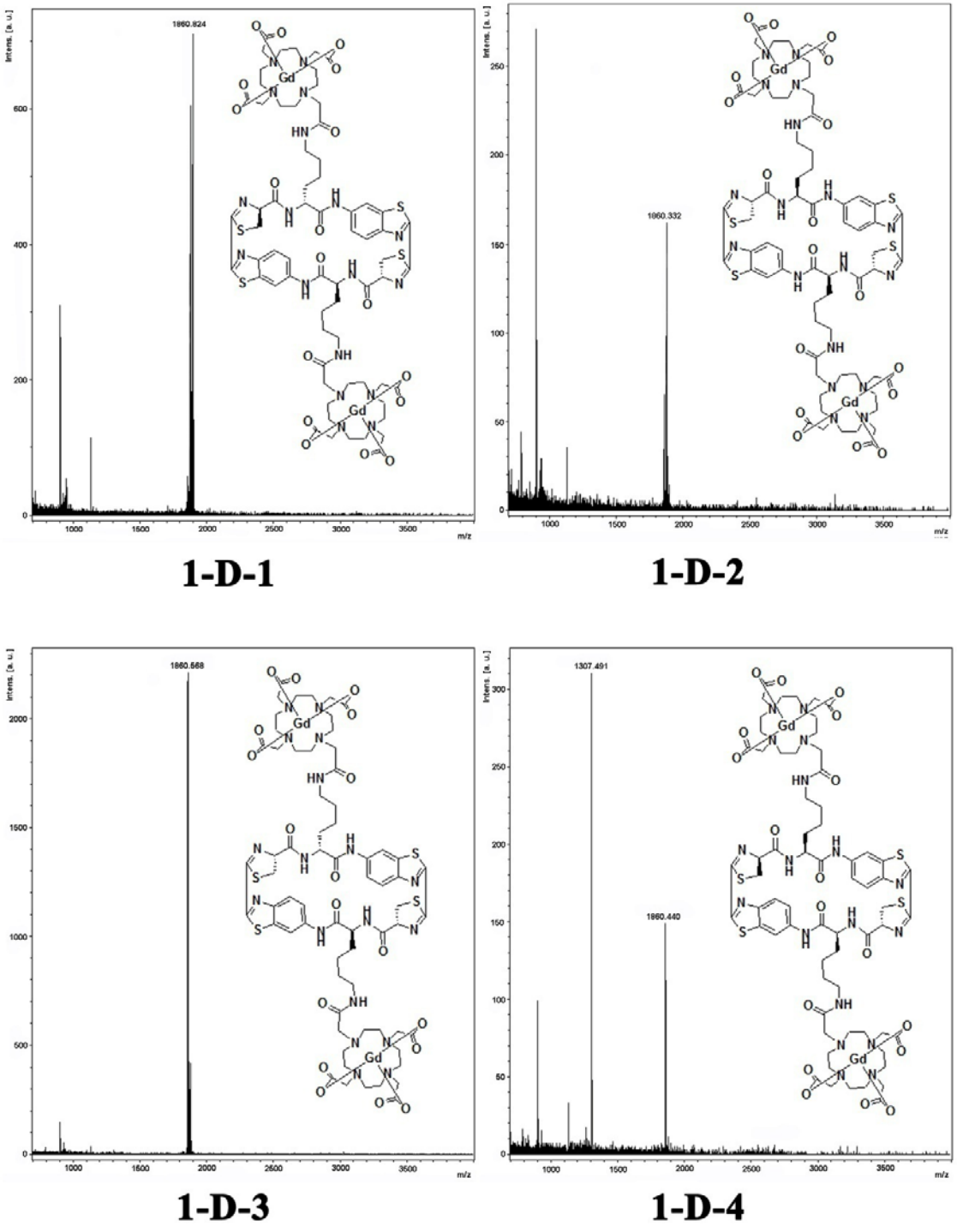

Figure S1. MALDI mass spectra of HPLC peaks at 38.2 min (**1-D-1**), 39.5 min (**1-D-2**), 40.5 min (**1-D-3**), and 42.8 min (**1-D-4**) in Fig. 3a respectively.

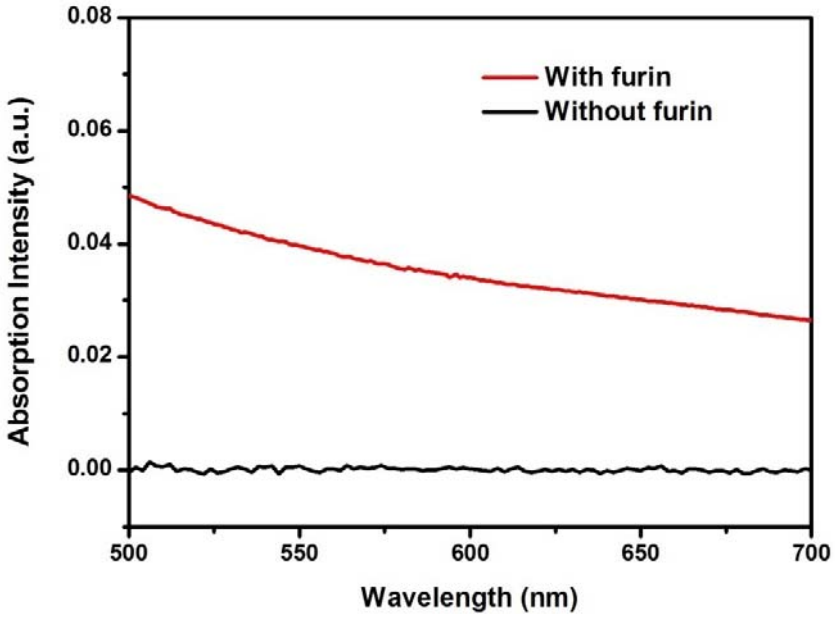

Figure S2. Absorption spectra (500-700 nm due to the light scattering) of **1** at 100 μM without furin (black) or incubated with furin at 1 nmol/U, pH 7.4, and 30 °C for 17 h (red).

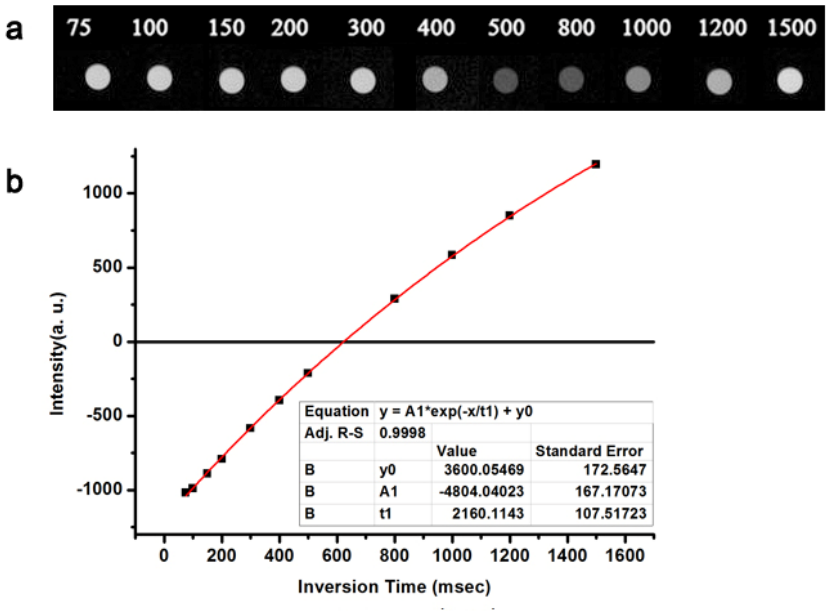

Figure S3. Plotted curves and fitting results of signal intensity versus inversion time of phosphate buffer (pH 7.4, 0.2 M) on a 1.5 T MR scanner.

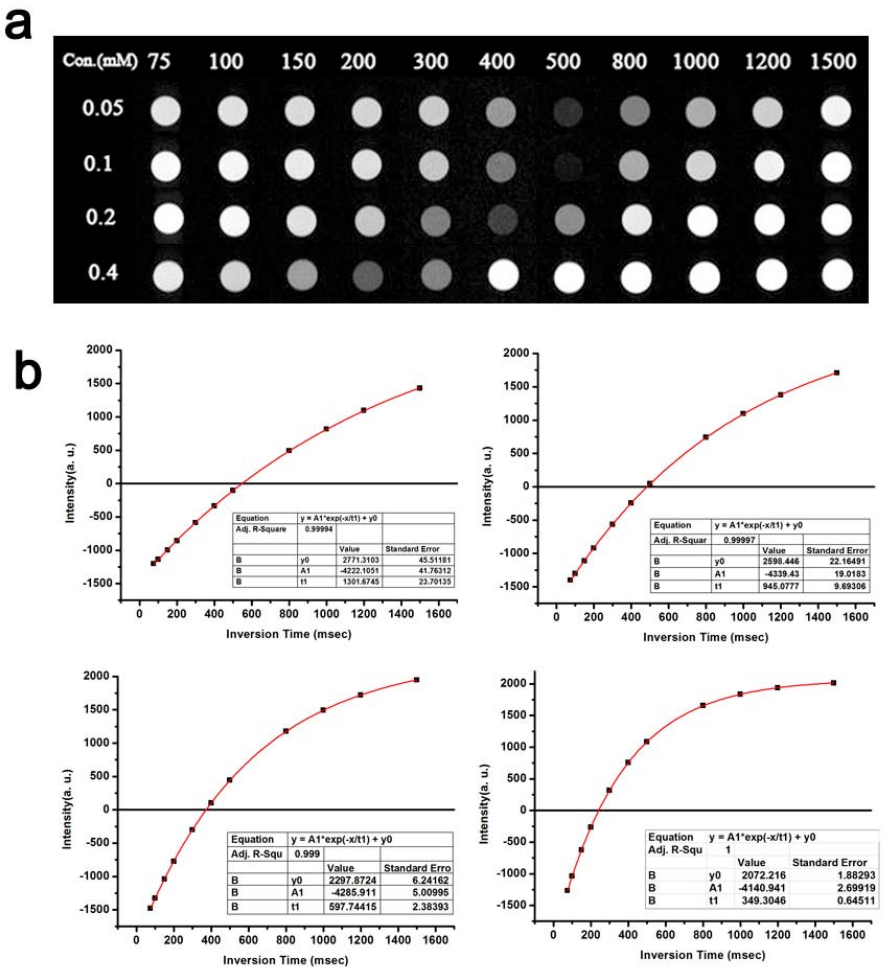

Figure S4. (**a**) T_1-weighted MR phantom images of **1** at 0.05 mM (first lane), 0.1 mM (second lane), 0.2 mM (third lane), 0.4 mM (fourth lane) in 0.2 M PB buffer (pH 7.4). Images were obtained on a 1.5 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S4a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

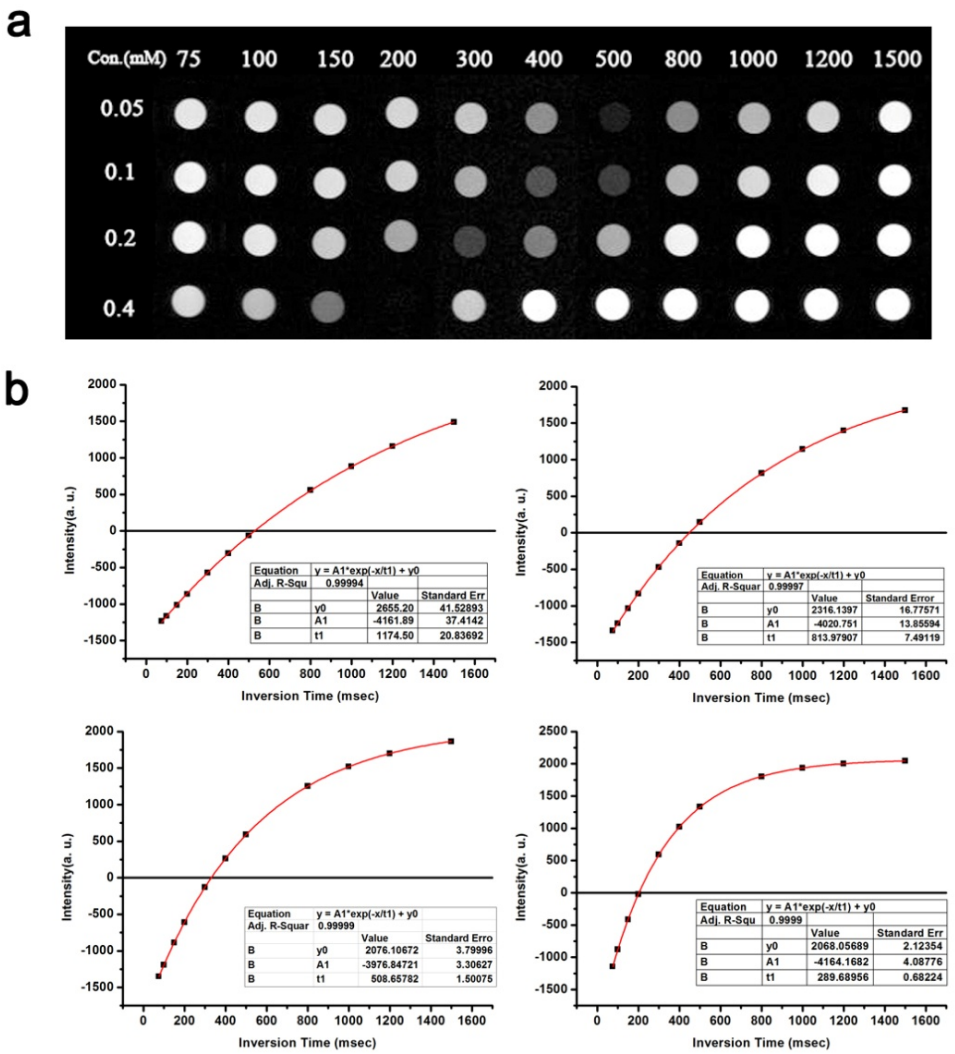

Figure S5. (**a**) T_1-weighted MR phantom images of **1-Scr** at 0.05 mM (first lane), 0.1 mM (second lane), 0.2 mM (third lane), 0.4 mM (fourth lane) in 0.2 M PB buffer (pH 7.4). Images were obtained on a 1.5 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S5a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

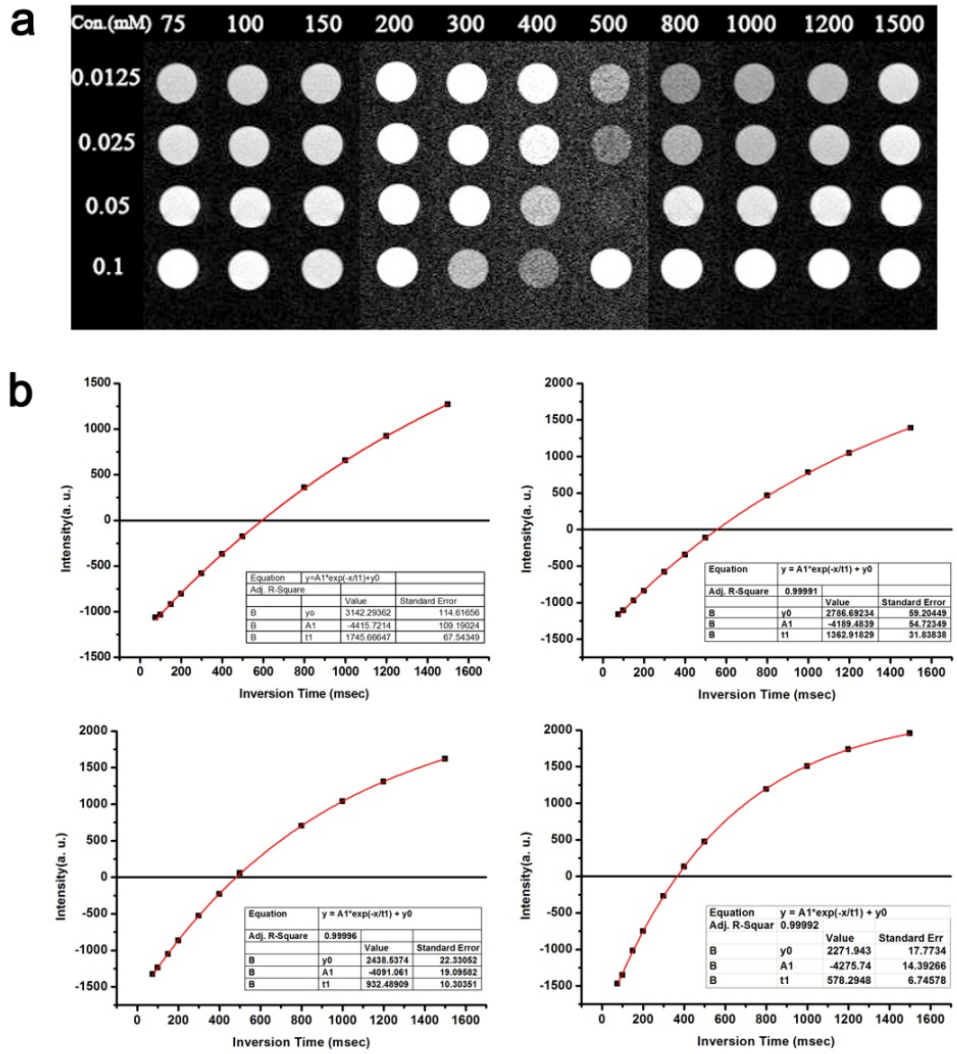

Figure S6. (**a**) T_1-weighted MR phantom images of **1-D** at 0.0125 mM (first lane), 0.025 mM (second lane), 0.05 mM (third lane), 0.1 mM (fourth lane) in 0.2 M PB buffer (pH 7.4, 1.5% DMSO). Images were obtained on a 1.5 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S6a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

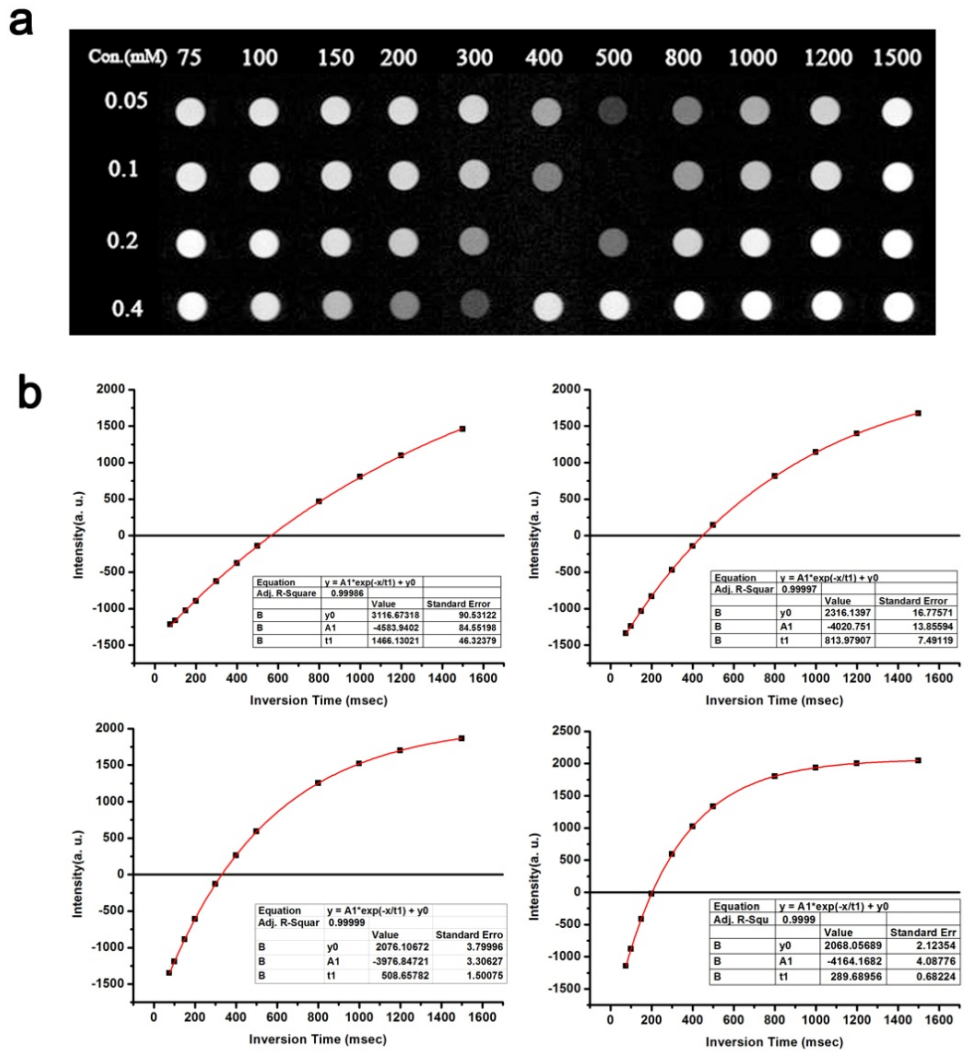

Figure S7. (**a**) T_1-weighted MR phantom images of Gd-DTPA (Magnevist) at 0.05 mM (first lane), 0.1 mM (second lane), 0.2 mM (third lane), 0.4 mM (fourth lane) in 0.2 M PB buffer (pH 7.4). Images were obtained on a 1.5 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S7a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

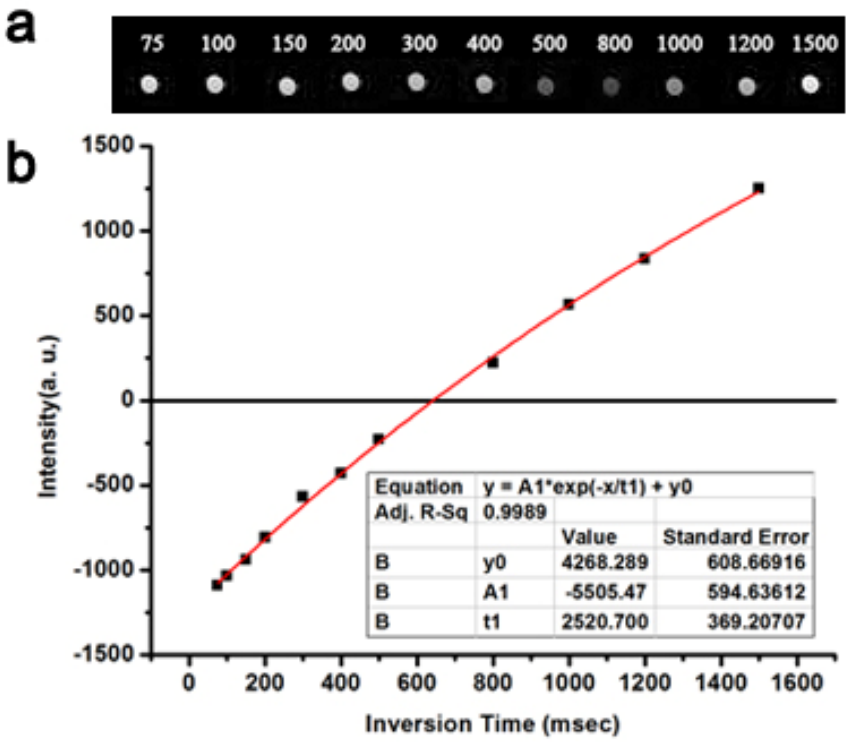

Figure S8. Plotted curves and fitting results of signal intensity versus inversion time of 0.2 M PB buffer (pH 7.4) on a 3 T MR scanner.

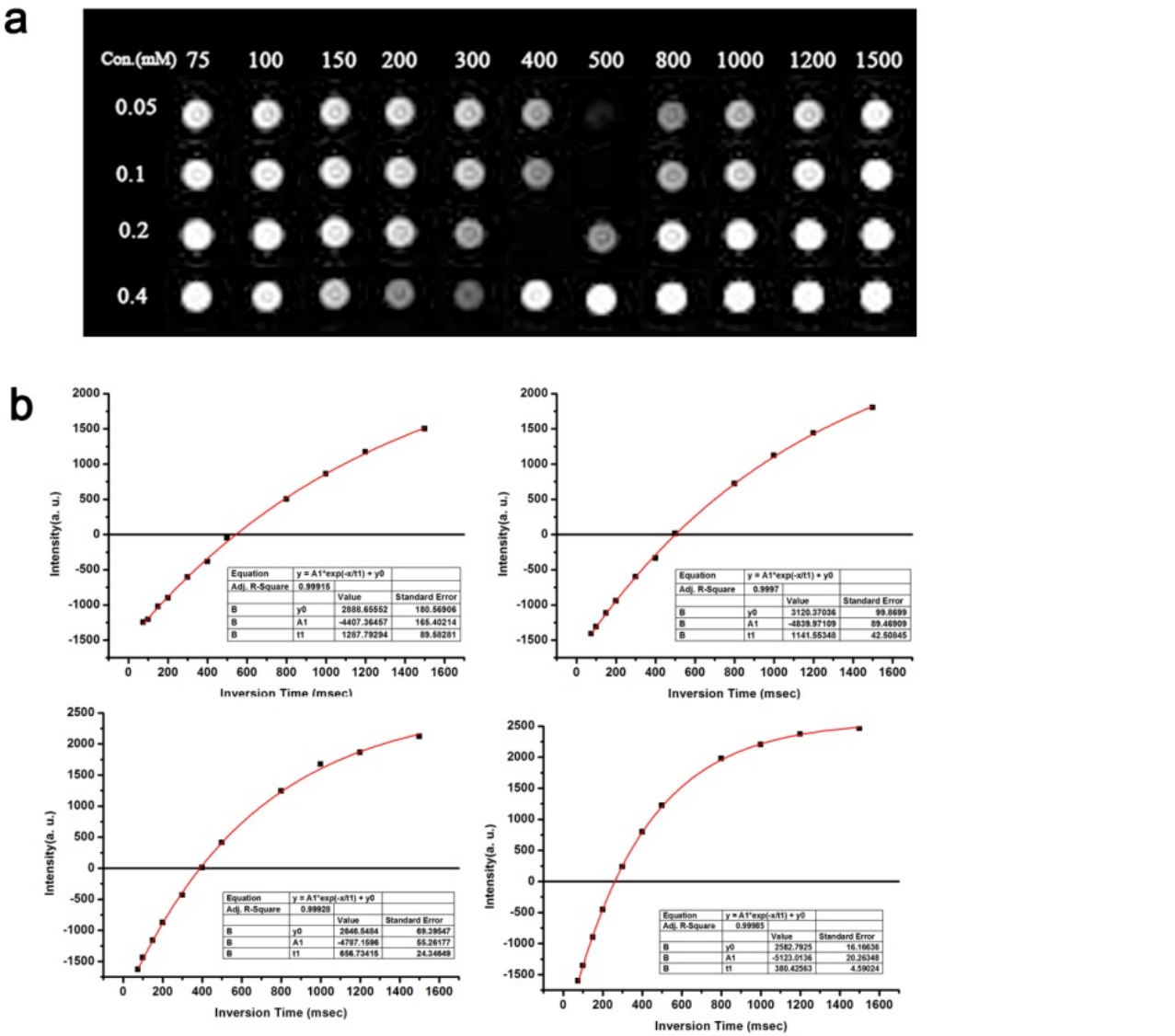

Figure S9. (**a**) T_1-weighted MR phantom images of **1** at 0.05 mM (first lane), 0.1 mM (second lane), 0.2 mM (third lane), 0.4 mM (fourth lane) in 0.2 M PB buffer (pH 7.4). Images were obtained on a 3 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S9a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

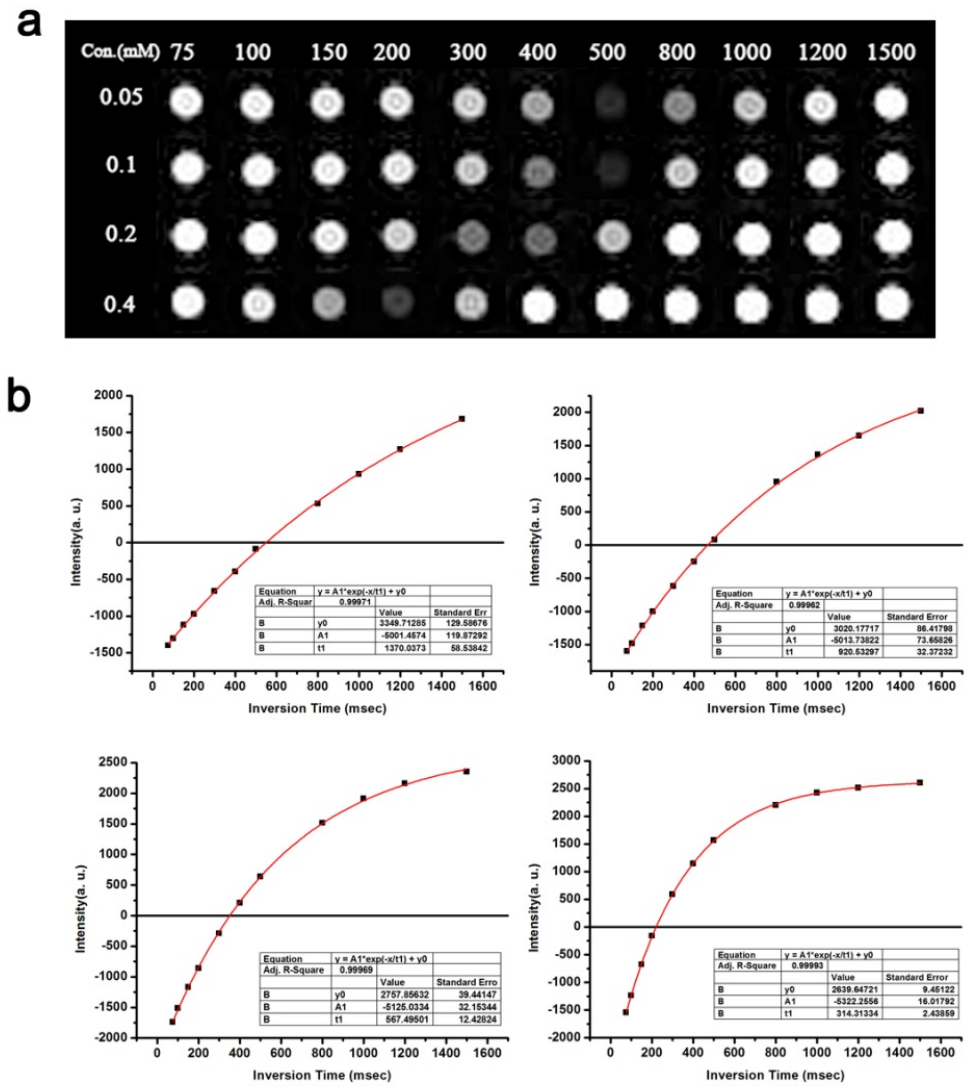

Figure S10. (**a**) T_1-weighted MR phantom images of **1-Scr** at 0.05 mM (first lane), 0.1 mM (second lane), 0.2 mM (third lane), 0.4 mM (fourth lane) in 0.2 M PB buffer (pH 7.4). Images were obtained on a 3 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S10a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

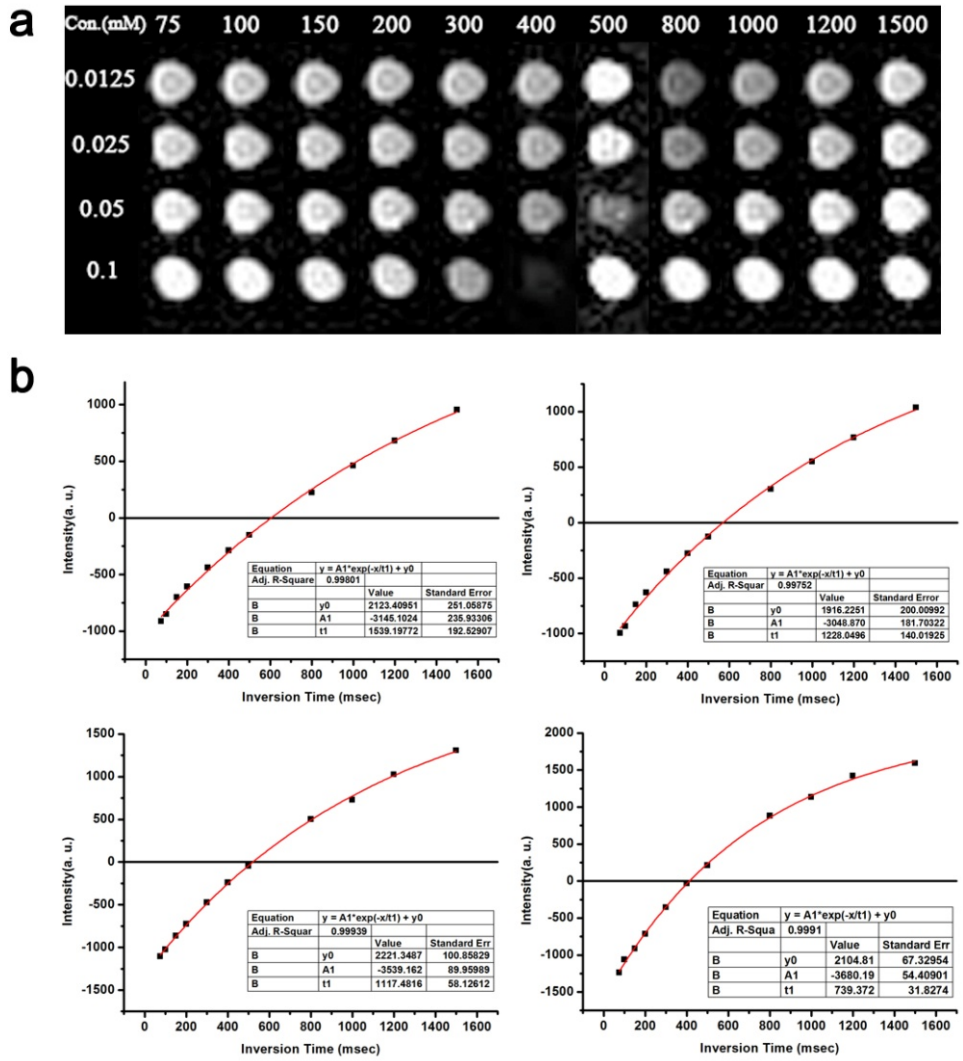

Figure S11. (**a**) T_1-weighted MR phantom images of **1-D** at 0.0125 mM (first lane), 0.025 mM (second lane), 0.05 mM (third lane), 0.1 mM (fourth lane) in 0.2 M PB buffer (pH 7.4, 1.5 % DMSO). Images were obtained on a 3 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S11a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

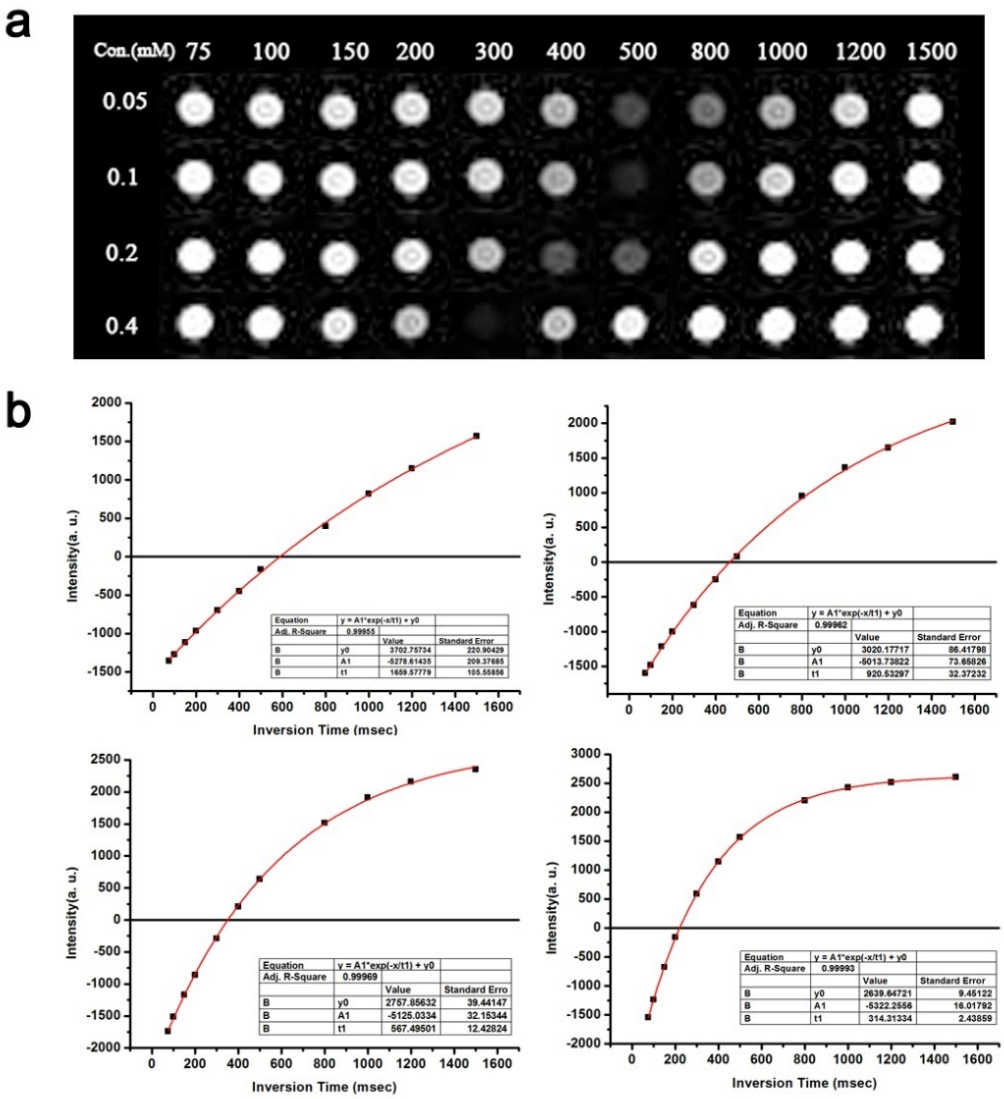

Figure S12. (**a**) T_1-weighted MR phantom images of Gd-DTPA (Magnevist) at 0.05 mM (first lane), 0.1 mM (second lane), 0.2 mM (third lane), 0.4 mM (fourth lane) in 0.2 M PB buffer (pH 7.4). Images were obtained on a 3 T MR scanner and the inversion time (ms) are shown above the phantom images. (**b**) Plotted curves and fitting results of signal intensity versus inversion time from Fig. S12a of different lanes: first lane (upper left), second lane (upper right), third lane (bottom left), fourth lane (bottom right).

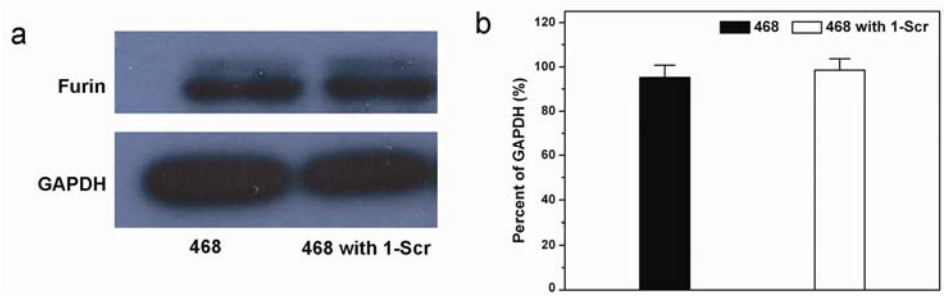

Figure S13. Western blot analysis (**a**) and quantification (**b**) of furin expression in MDA-MB-468 cells before and after incubation with **1-Scr** at 100 μM for 8 h. Expression level of furin in cells treated with **1-Scr** did not show obvious change (98.5% of GAPDH), compared with that in cells untreated (95.2% of GAPDH, $p = 0.631$).

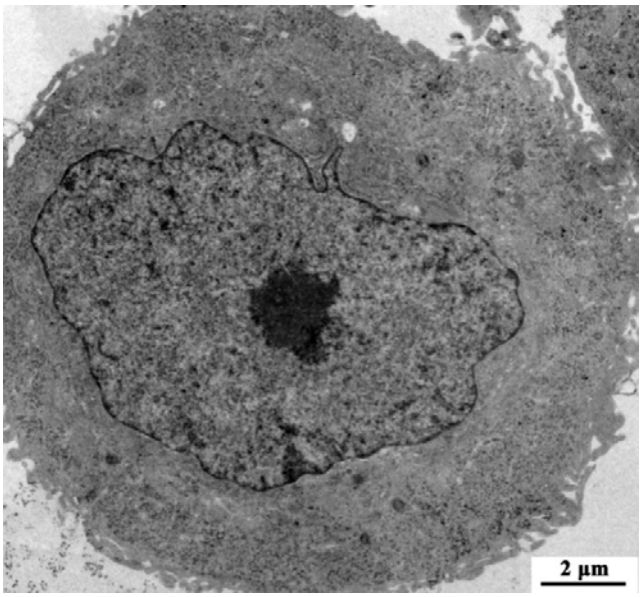

Figure S14. Electron microscopic image of sections of MDA-MB-468 cells incubated with **1-Scr** at 100 μM for 8 h. Scale bar: 2 μm.

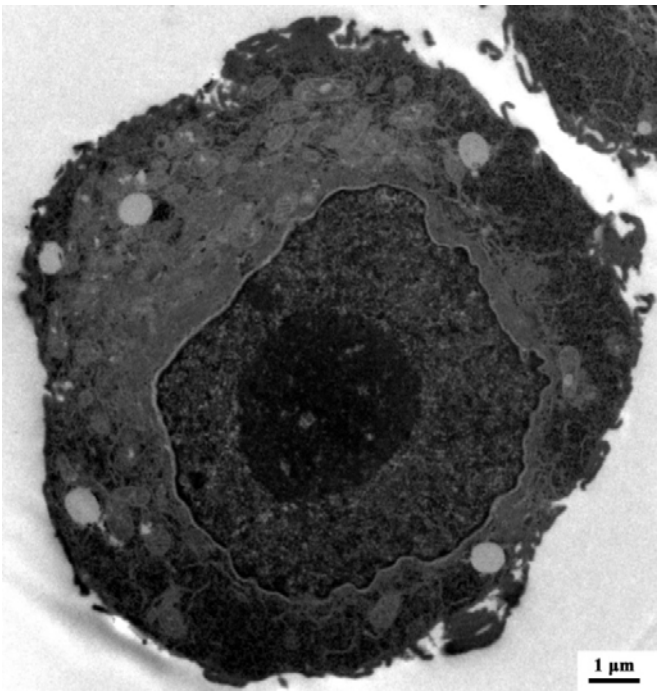

Figure S15. Electron microscopic image of sections of MDA-MB-468 cells untreated as negative control. Scale bar: 1 μm.

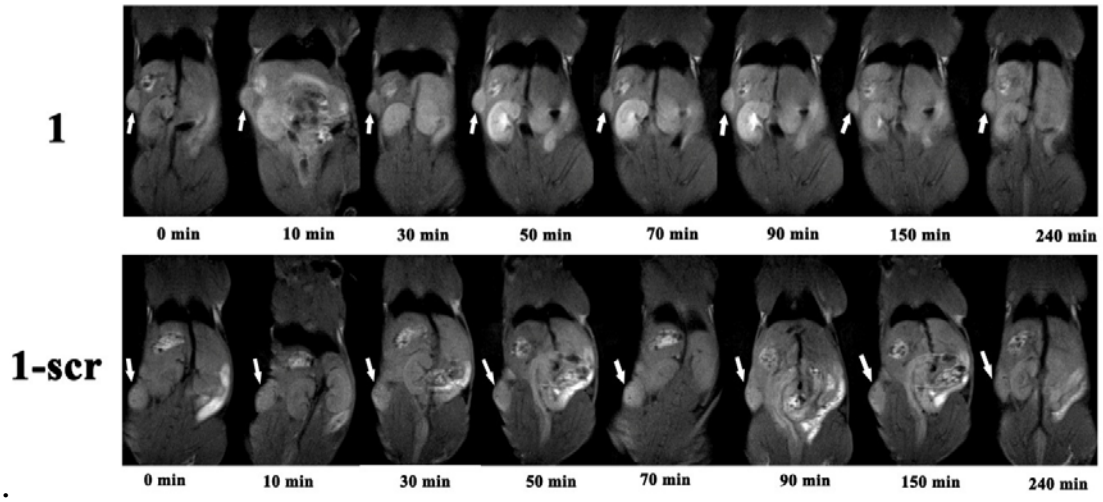

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Figure S16. Representative coronal MR images of mice with subcutaneously xenografted MDA-MB-468 tumors at 0 min (pre-injection), 10 min, 30 min, 50 min, 70 min, 90 min, 150 min, and 240 min after two intravenous injections of **1** (upper) or **1-Scr** (lower) via tail veins (1st injection: 0.15 mmol/kg at 0 min; 2nd injection: 0.15 mmol/kg at 50 min). Tumors are indicated by arrows.

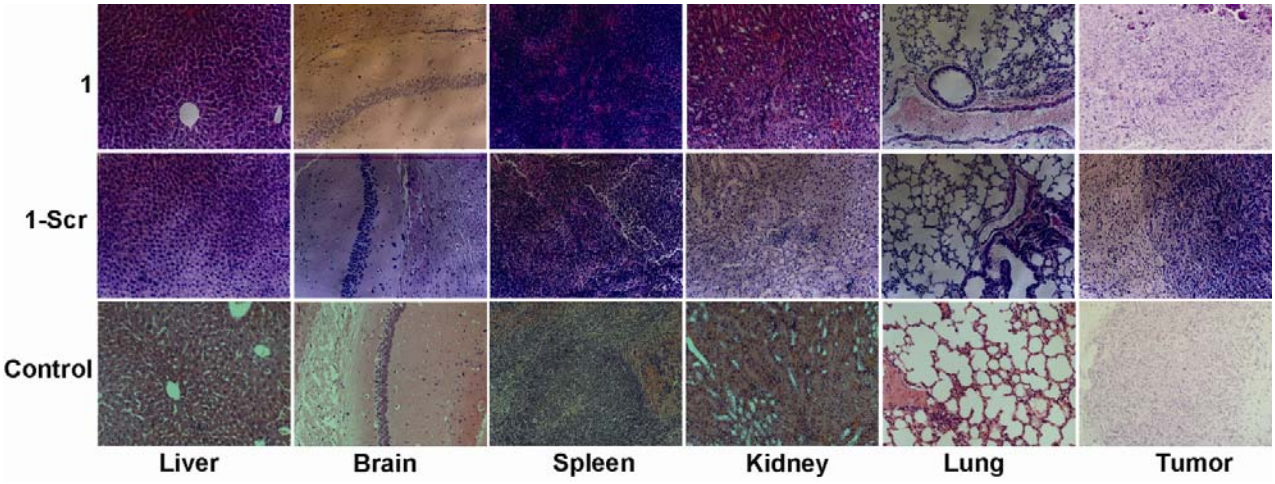


Figure S17. Hematoxylin and eosin (HE) staining of tissue slices of mice untreated (lower, Control), injected with **1** (upper) or **1-Scr** (middle) after 240 min of MRI.

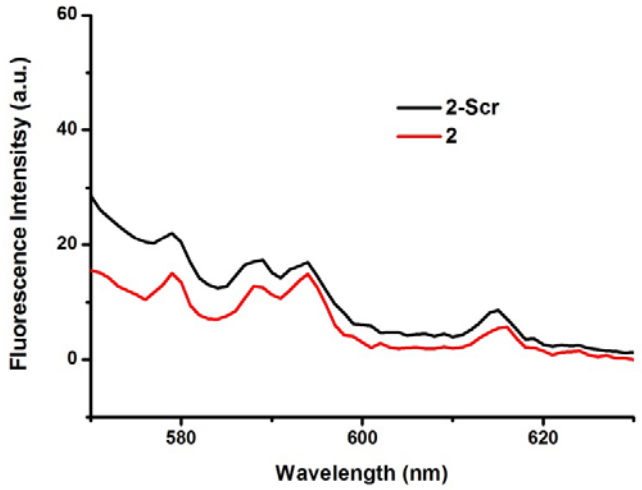


Figure S18. Fluorescence emission spectra of **2** and **2-scr** excited at 355 nm.

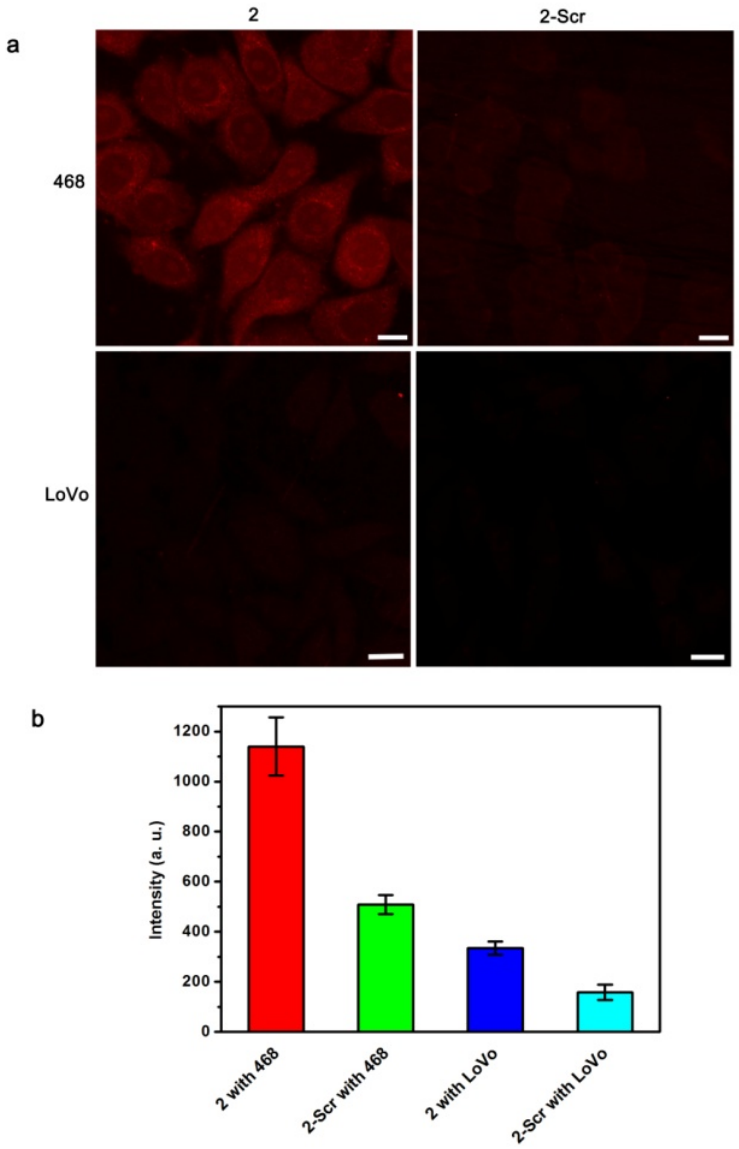


Figure S19. (**a**)TPLM images (λex = 725 nm, λem = 565-636 nm) of MDA-MB-468 cells (upper) and LoVo (bottom) cells incubated with **2** (left) or **2-Scr** (right) at 100 μM for 8 h and then rinsed and fixed prior to imaging. Scale bar: 20 μm. (**b**) Quantification of the average fluorescence intensity of cell images in Figure S19a.

Table S1. The contents of Gd (μg/g, determined with ICP-MS) in different organs of mice injected with **1** or **1-Scr** after 240 min of MRI.

	Lung	Brain	Liver	Spleen	Kidney	Tumor
1-Scr	0.018	0.017	0.043	0.021	0.13	0.046
1	0.26±0.19	0.022±0.018	2.24±0.66	0.93±0.18	0.55±0.28	0.12±0.0007

Table S2. HPLC condition for the purification of compound **1, 1-Scr, 2,** and **2-Scr**.

Time (minute)	Flow (ml/min.)	H_2O %	CH_3OH %
0	12.0	80	20
3	12.0	80	20
35	12.0	20	80
37	12.0	20	80
38	12.0	80	20
40	12.0	80	20

Table S3. HPLC condition for the analysis and purification of **1-D**.

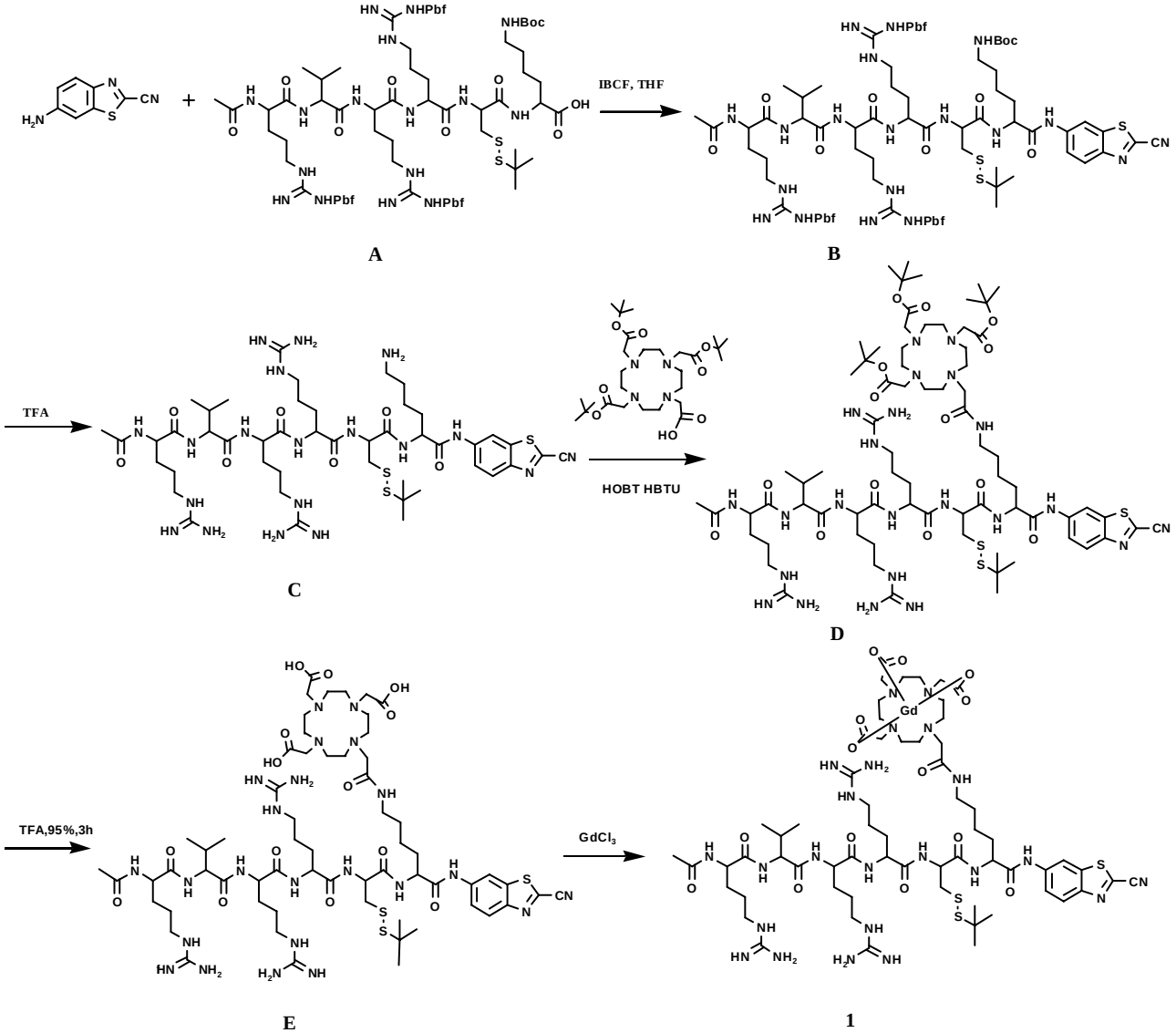
Time (minute)	Flow (ml/min.)	H_2O %	CH_3OH %
0	1.0	90	10
3	1.0	90	10
55	1.0	30	70
57	1.0	30	70
58	1.0	90	10
60	1.0	90	10

Supplementary Methods

Chemical syntheses and characterizations of 1, 1-Scr, 2, 2-Scr, and 1-D.

The preparations of compound **1, 1-Scr, 2, 2-Scr,** and **1-D** was described as below; 2-cyano-6-aminobenzothiazole (CBT) was synthesized following the literature method (White, E. H., Worther, H., Seliger, H. H., McElory, W. D. Amino analogs of firefly luciferin and biological activity thereof. *J. Am. Chem. Soc.* 1966, 88, 2015-2019). Compound **K** was synthesized following the literature method (Liang, G. L., Ronald, J., Chen, Y. X., Ye, D. J., Controlled self-assembling of gadolinium nanoparticles as smart molecular magnetic resonance imaging contrast agents. *Angew. Chem. Int. Ed.* 2011, 123, 6407-6410).

Scheme S1. Synthetic route for **1**.



*Synthesis of compound **B***: The isobutyl chloroformate (82 mg, 0.6 mmol) was added to the mixture of peptide **A** (1024 mg, 0.6 mmol) and MMP (4-methylmorpholine, 101mg, 1.0 mmol) in THF (5.0 mL) at 0 ^{o}C under N_2 and the

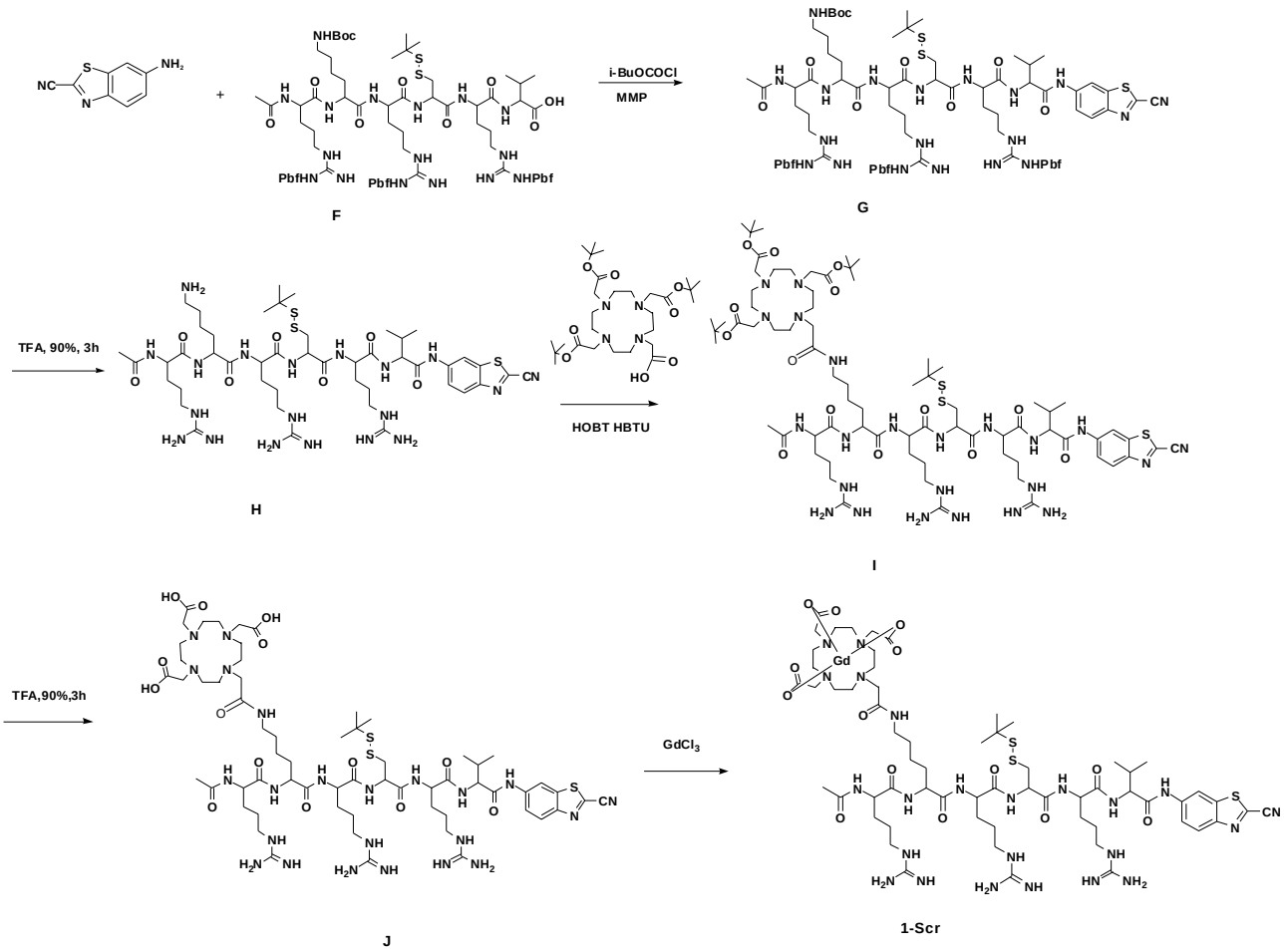
reaction mixture was stirred at this temperature for 20 min. The solution of 2-cyano-6-aminobenzothiazole (105 mg, 0.6 mmol) was added to the reaction mixture and stirred for further 2 h at 0 ^{o}C then overnight at room temperature. Water (30 mL) was added and the reaction mixture was extracted with ethyl acetate (2 × 100 mL). The combined organic phase was dried by Na_2SO_4 and then evaporated. The pure product **B** (yield: 28 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1).

*Synthesis of compound **D***: The protecting groups 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and t-butoxycarbonyl (Boc) of compound **B** were removed with 100 % TFA for 3 h. Compound **C** was obtained after HPLC purification (yield: 70 %). The mixture of **C** (93.2 mg, 0.05 mmol), Tri-*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA(OtBu)$_3$) (28.6 mg, 0.05 mmol) and O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (18.9 mg, 0.05 mmol) in Dimethylformamide (DMF) (5.0 mL) was stirred overnight in presence of N,N-Diisopropylethylamine (DIPEA) (16.7 mg, 0.15 mmol). The pure product of **D** was obtained after HPLC purification (yield: 80 %). MS: calc. M^+ = 1656.9, obsvd. ESI-MS: m/z 1657.1 [M^+].

*Synthesis of **E***: The OtBu protecting groups of **D** were removed with 100% TFA for 3 h, purified with HPLC to yield compound **E**. ^{1}H NMR of compound **E** (CD_3OD, 300 MHz): 9.10 (s, 1 H), 8.58 (d, 1 H), 8.24 (d-d, 1 H, J = 9.0 Hz), 7.31(s, 1 H), 4.83-4.56 (m, 3 H), 4.51 (m , 2 H), 4.38-4.09 (m, 6 H), 4.00 (m, 12 H, J = 21 Hz), 3.83-3.44 (m, 18 H), 3.43-3.24 (m, 6 H), 2.63 (m, 2 H), 2.32-2.14 (m, 6 H), 2.14-2.00 (m, 8 H), 1.93 (s, 1 H), 1.87 (s, 1 H), 1.77 (s, 6 H), 1.70 (m, 3 H), 1.58 (t, 16 H, J =14.1 Hz), 1.37 (m, 6 H). MS: calc. M^+ = 1488.7, obsvd. ESI-MS: m/z 1488.8 [M^+].

*Preparation of compound **1***: Compound **E** (14.9 mg, 0.01 mmol) was dissolved in water and the pH value of this solution was adjusted to 6-7. $GdCl_3 \cdot 6H_2O$ (37.1 mg, 0.1 mmol) was added into above solution and stirred for 3h at room temperature. Pure compound **1** was obtained after HPLC purification (yield: 70 %). MS: calc. M^+ = 1644.1, obsvd. MALDI-MS: m/z 1644.8 [$(M+H)^+$].

Scheme S2: Synthetic route for **1-Scr**



*Synthesis of compound **G***: The isobutyl chloroformate (82 mg, 0.6 mmol) was added to the mixture of peptide **F** (1024 mg, 0.6 mmol) and MMP (4-methylmorpholine, 101mg, 1.0 mmol) in THF (5.0 mL) at 0 ^{o}C under N_2 and the reaction mixture was stirred at this temperature for 20 min. The solution of 2-cyano-6-aminobenzothiazole (105 mg, 0.6 mmol) was added to the reaction mixture and stirred for further 2 h at 0 ^{o}C then overnight at room temperature. Water (30 mL) was added and the reaction mixture was extracted with ethyl acetate (2 × 100 mL). The combined organic phase was dried by Na_2SO_4 and then evaporated. The pure product **G** (yield: 35 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1).

*Synthesis of compound **I***: The protecting groups Pbf and Boc of compound **G** were removed using 100% TFA for 3 h, purified by HPLC to yiled compound **H** (yield: 70 %). The mixture of **H** (93.2 mg, 0.05 mmol), Tri-*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA(OtBu)$_3$) (28.6 mg, 0.05 mmol) and HBTU (18.9 mg, 0.05 mmol) in DMF (5.0 mL) was stirred overnight in the presence of DIPEA (16.7 mg, 0.15 mmol). Pure compound of **I** was obtained after HPLC purification (yield: 75 %). MS: calc. M^+ = 1656.9, obsvd. ESI-MS: m/z 1657.0 [M^+].

*Synthesis of compound **J***: The OtBu protecting groups of **I** were removed with 100% TFA for 3 h and purified with HPLC to yield compound **J**. ^{1}HNMR of compound J (CD_3OD, 300 MHz): (CD_3OD, 300 MHz): 9.10 (s, 1 H), 8.58 (d,

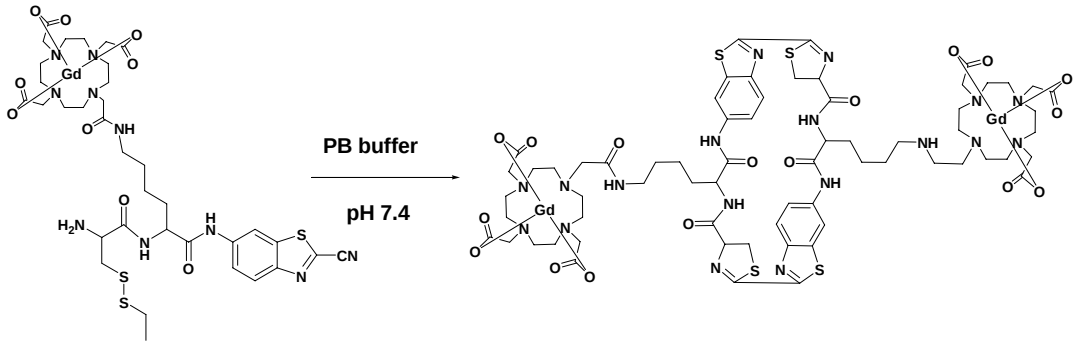
1 H), 8.24 (d-d, 1 H, J = 9.0 Hz), 7.31(s, 1 H), 5.81 (m, 2 H), 4.91-4.80 (m, 1 H), 4.83-4.56 (m, 3 H), 4.51 (m , 2 H), 4.38-4.09 (m, 4 H),4.00 (m, 12 H, J = 21 Hz), 3.82-3.46 (m, 18 H), 3.46-3.35 (m, 2 H), 3.24 (s, 3 H), 2.63 (m, 3 H), 2.32-2.14 (m, 6 H), 2.14-2.00 (m, 8 H), 1.93 (s, 1 H), 1.87 (s, 1 H), 1.77 (s, 5 H), 1.70 (m, 4 H), 1.58 (t, 15 H, J =14.1 Hz), 1.37 (m, 6 H). MS: calc. M^+ = 1488.7, obsvd. ESI-MS: m/z 1488.6 [M^+].

*Preparation of compound **1-scr***: Compound **J** (14.9 mg, 0.01 mmol) was dissolved in 5 ml water and pH value of this solution was adjusted to 6-7. $GdCl_3 \cdot 6H_2O$ (37.1 mg, 0.1 mmol) was added into above solution and stirred for 3h at room temperature. Pure compound **1-Scr** was obtained after HPLC purification (yield: 70 %). MS: calc. M^+ = 1644.1, obsvd. MALDI-MS: m/z 1644.8 [$(M+H)^+$].

*Synthesis of compound **2***: Synthetic route for **2** is similar to that of **1**, except $GdCl_3 \cdot 6H_2O$ at the last step of synthesis of **1** was replaced with $EuCl_3 \cdot 6H_2O$. MS for **2**: calc. M^+ = 1638.7, obsvd. ESI-MS: m/z 1638.4 [M^+].

*Synthesis of compound **2-Scr***: Synthetic route for **2-Scr** is similar to that of **1-Scr**, except $GdCl_3 \cdot 6H_2O$ at the last step of synthesis of **1-Scr** was replaced with $EuCl_3 \cdot 6H_2O$. MS for **2-Scr**: calc. M^+ = 1638.7, obsvd. MS (ESI): m/z 1638.6 [M^+].

Scheme 3: Synthetic route for **1-D**



*Synthesis of compound **1-D***: Compound **K** was dissolved in 0.2 M phosphate buffer to make a 1 mM solution. 4 equiv. of TCEP was added into above solution and stirred for 1 h at room temperature. Pure compound of **1-D** was obtained after HPLC purification. MS: calc. M^+ = 1860.36, obsvd. ESI-MS: m/z 1860.24 [M^+].