Supplementary Information

For

DTTA-Ligated Uridine-Quantum Dot Conjugate as a Bimodal Contrast Agent for Cellular Imaging

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Materials, methods and instrumentations: *tert*-Butyl bromoacetate (Aldrich), *N*-bromosuccinimide (Aldrich), 2-aminoethanol (Fluka), sodium azide (Aldrich), triphenylphosphine (TCI, Japan), carbon tetrabromide (Aldrich), uridine (Aldrich), lipoic acid (Aldrich), gadolinium chloride(III) (Aldrich), Quantum Dot (0.9 to 1 μM/mL) (Invetrogen) were received and were used without further purification. 5-azido-5'-deoxyuridine,[1] 5-amino-5'-deoxyuridine,[2] *N,N*-bis[(*tert*-butoxycarbonyl)methyl]-2-bromoethylamine[3] were synthesized according to a published procedures. Column chromatography was performed using silica gel 60 (70 ~ 230 mesh) as stationary phase. Analytical thin layer chromatography was performed using 60 silica gel (precoated sheets with 0.25 mm thickness). The mass spectra were obtained on an IonSpec HiResESI mass spectrometer. NMR spectra were collected on a 300MHz, 400MHz spectrometer (AS400,300, Varian, USA) and relaxivity measurements were carried out on NMR (Minispec mq60, Bruker, Germany), and 200 MHz animal MRI (Biospec 47/40, Bruker, Germany) systems.

Experimental Section

Compounds **1- 3** have been synthesized by adaptation of procedures reported earlier.[1-4]

Synthesis of 4: To compound **3** (1.0 g, 1.27 mmol) dissolved in anhydrous DMF (30 mL) *N*-3-(dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (609 mg, 3.18 mmol) and 4-dimethylaminopyridine (311 mg, 2.54 mmol) was added and cooled to 0 ^{o}C. (±)-Lipoic acid (655 mg, 3.17 mmol) as slowly added to the solution then the temperature was raised to room temperature and continued to stir for 24 hr. The reaction mixture was diluted with water and extracted with ethyl acetate (2 × 100 mL). The ethyl acetate phase was dried over anhydrous magnesium sulfate and concentrated *in vacuo*, and chromatography on a column of silica gel using ethyl acetate (30-50%) in hexane as eluent and dried to afford 1.02 g (69 %). ^{1}H-NMR (300 MHz, $CDCl_3$)**:** δ 7.77 (d, 1H, *J* = 9.99 Hz, Ar-H); 6.09 (br, 1H, Ar-H); 5.78 (d, 1H, *J* = 8.92 Hz, -OCH-Ar); 5.51 (m, 2H, 2-C<u>H</u>OH); 4.20 (m, 1H, -CH-O-); 3.42 (s, 8H, 4-NCH_2-); 3.14 (m, 6H, -NCH_2-, -CH_2CH_2-); 2.84 (m, 10H, 2(-NCH_2-CH_2N-), 2-CHS-); 2.40 (m, 4H, 2-SCH_2CH_2S-); 2.28 (m, 2H, -CH_2-); 1.92(m,2H, -CH_2-); 1.62(m, 8H, 2-CH_2CH_2-); 1.44(s, 40H, 4Me_3C-, 2-CH_2-). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.28, 171.88, 170.85,163.42, 150.79, 140.89, 103.41, 86.21, 81.13, 72.09, 56.47, 52.92, 51.09,40.42, 38.70, 34.84, 34.76, 33.71, 29.03, 28.83, 28.38, 24.56ppm. FAB-MS m/z (M+H) calcd 1162.54, found 1162.57.

Synthesis of 5: Compound **4** (1.0 g, 0.86 mmol) was dissolved in methanol and cooled to 0 ^{o}C. Sodium borohydride (130 mg, 3.44 mmol) was added little by little and stirred at 0 ^{o}C for 3 hr. The solution was neutralized with 1*N* AcOH solution, extracted with ethyl acetate then dried over anhydrous magnesium sulfate. After ethyl acetate was removed *in vacuo*, the crude compound was purified by column chromatography using silica gel with methanol (5 %) in methylene chloride as eluent to yield 409 mg (40 %). ^{1}H-NMR (400 MHz, $CDCl_3$)**:** δ 7.76 (d, 1H, *J* = 8.14 Hz, Ar-H); 6.11 (d, 1H, *J* = 5.51 Hz, Ar-H); 5.78 (d, 1H, *J* = 8.07 Hz, -OCH-Ar); 5.51 (m, 2H, -2C<u>H</u>(OH)-); 4.38 (t, 1H, *J* =7.95 Hz, -CH-O-); 4.20 (m, 1H, -CH_2-); 3.70 (t, 1H, *J* = 8.01 Hz, -CH_2-); 3.42 (s, 4H, 2-NCH_2-); 3.12 (S, 2H, NCH_2-); 2.77 (m, 16H, 2NCH_2CH_2-,-NCH_2-, 2-CHS-,2SCH_2CH_2-); 1.92 (m,4H, 2CH_2CO-); 1.46(m, 40H, 4Me_3C-, 2-CH_2-). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.84,171.23,169.34, 167.72,153.21,141.22, 103.34, 81.11, 81.10, 62.75,62.71, 56.78, 52.90, 51.10, 46.23, 45.12, 42.95,

40.44, 39.63, 39.18, 38.62, 35.05, 32.73, 29.84, 29.3028.35, 26.97, 25.77, 25.65, 14.33 ppm. FAB-MS m/z (M+H) calcd 1166.57, found 1166.50.

Synthesis of 6: Compound **5** (400 mg, 0.34 mmol) was dissolved in methylene chloride then cooled to 0 ^{o}C. Trifluoroacetic acid (7.0 mL, 9.14 mmol) was added in the solution dropwise and continued to stir overnight. After trifluoroacetic acid was removed *in vacuo*, the residue was washed with ethyl ether (2 × 100 mL) and filtered to give a pale yellow solid of 220 mg (68 %). ^{1}H-NMR (300 MHz, $CDCl_3$)**:** δ 7.76 (d, 1H, *J* =8.96 Hz, Ar-H); 5.93 (d, 1H, *J* = 5.86 Hz, Ar-H); 5.73 (d, 1H, *J* = 9.09 Hz, Ar-CH-; 5.51 (t, 1H, *J* = 6.26 Hz, 2-CHOH-); 5.24 (d, 1H, *J* = 5.86 Hz, -CHOH-);4.54(m,1H, CH-O) 3.83(m, 2H, -CH_2O-); 3.51 (s, 8H, -N-CH_2-); 3.02 (m, 4H, -NCH_2CH_2-); 2.84 (br, 4H, -NCH_2CH_2-);); 2.61 (m, 4H, 2-SCH2-); 2.34 (m,8H, 2-SCH_2-,2-SCH-,-CH_2-); 1.52(m, 16H, alp-H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.65163.66, 150.98, 140.81, 103.22, 84.21, 71.49, 71. 56.72, 55.09, 52.11, 49.23, 43.06, 42.49, 39.50, 38.79, 38.43, 33.67, 32.06, 31.45, 28.44, 26.55, 26.04, 24.67, 22.27 ppm. FAB-MS m/z (M+H) calcd 941.29, found 940.70.

Preparation of gadolinium complex (6-Gd^{3+}): Compound **6** (100 mg, 0.11 mmol) was in ultrapure water (10 mL) and the solution was adjusted to ~pH 7 with sodium bicarbonate. Gadolinium chloride hexahydrate (32 mg, 0.08 mmol) was dissolved in 3.0 mL of ultrapure water and added to the solution of **6**. Upon completion of the addition, the pH was adjusted back to between 6.5-7.0 using 0.1 M potassium carbonate solutions. The solution was allowed to stir for additional 30 min to allow for Gd^{3+} chelation to complete, dialyzed against ultrapure water overnight, and lyophilized to yield respective complex **6-Gd^{3+}**. FAB-MS m/z (M+H) calcd 1095.18, found 1095.10. Isotopice MS: **6-**$^{154}Gd^{3+}$:1091.1; **6-**$^{156}Gd^{3+}$:1093.1; **6-**$^{158}Gd^{3+}$:1095.1

Synthesis of 6-Gd^{3+}-QDs: To 1.0 mL of QDs in *n*-decane 100 mg of **6**-Gd^{3+} in 5.0 mL aqueous ethanol (90%) were added. The mixture was stirred at 60 ^{o}C for 12 hr. In between, another 5 mL of ethanol were added. **6**-Gd^{3+}-QD was separated by precipitation by washing with ethanol.

Measurement of MR properties: Longitudinal T_1 relaxivity, r_1, of **6**-Gd^{3+}-QD solution was measured at 60 MHz (0.47 T) and 200 MHz (4.7 T) at 36 ^{o}C on NMR (mq60, Bruker, Germany), and animal MRI

(Biospec 47/40, Bruker, Germany) systems. T_1 relaxation times of water solution with several different Gd^{3+} concentration were measured with inversion recovery pulse sequence. Relaxivity value of r_1 was calculated through the linear fitting of measured relaxation rates ($R_1 = 1/T_1$, s^{-1}) *vs.* the Gd^{3+} concentration (mM). Gd^{3+} content in a start solution was measured by an inductively coupled plasma atomic emission spectrophotometer (ICP-AES; Optima 4300DV, Perkin Elmer, MA, USA).

***In vitro* labeling of 6-Gd^{3+}-QD:** Murine macrophage cells (RAW 264.7) were maintained in culture dishes with RPMI 1640 medium containing 10 % fetal bovine serum and 1.0 % antibiotics in a humidified atmosphere of 5.0 % CO_2 in air at 37 °C. The cells were incubated with **6**-Gd^{3+}-QD at different Gd^{3+} concentration lower than 11.2 μM for 24 hr. The labeled macrophages were washed 3 times with Dulbecco's phosphate-buffered saline (DPBS) and collected in 200 μl polymerase chain reaction (PCR) tubes. Labeling efficiency was determined by measuring iron content per cell using an ICP-AES system. Cell cytotoxicity was also measured for Gd^{3+} concentration by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method. To visualize the intracellular delivery of **6**-Gd^{3+}-QD, various concentrations of **6**-Gd^{3+}-QD was treated to macrophage cells and the fluorescence images of them were obtained. Macrophage cells were plated in μ-slide 8 well microscopy chamber at a density of 3×10^3 cells in culture medium. Cells were allowed to seed for 24 hr, and then incubated with **6**-Gd^{3+}-QD at 37 °C for 24 hr in culture medium. Unbounded **6**-Gd^{3+}-QD was removed by washing with phosphate buffered saline (PBS, Gibco). Cells were fixed with cytofix/cytoperm solution (BD PharMingen, San Diego, CA, USA) for 10 min, and blocked for 30 min in PBS containing 1% bovine serum albumin (BSA; Sigma, St Louis, MO, USA). T_1-weighted (T_1-W) MR images with spin-echo pulse sequence were measured from the **6**-Gd^{3+}-QD labeled macrophages (10^7 cells/mL) in 200-μl PCR tubes. The following MRI parameters were used: field of view (FOV) = 5 × 4 cm^2; matrix = 256 × 256; slice thickness = 1 mm; echo time (TE) = 10 ms; repetition time (TR) = 200 ms.

ELLS spectra of QD by TEM Observation: Transmission electron microscopy (TEM) images were taken with a JEOL JEM-2100 F instrument operated at 150 kV. Images were recorded on 2k CCD (Gatan Inc. USC 1000).

Dynamic Light Scattering (DLS). Static light scattering was performed with home-built equipment using a He-Ne laser as a light source (632.8 nm, 10 mW). The logarithm of scattering intensity data was plotted against the scattering vector $k = 4\pi n \sin(\theta/2)/\lambda$, where n is the solvent refractive index, θ is the scattering angle, and λ is the wavelength in vacuum.

An average number of 6-Gd^{3+} complexes per QD particle: To determine an average number of **6-Gd^{3+}** complexes per QD particle, we estimated the number of **6-Gd^{3+}** binding at a QD surface. Since **6-Gd^{3+}** has UV absorption around 262 nm that is arising from its uridine groups, the concentrations of **6-Gd^{3+}** bound to QDs can be determined by comparison of its absorption spectrum before and after the conjugation reaction. We reacted 5.0 mL (aqueous, pH 7) of **6-Gd^{3+}** (20 mg/mL, 90 µM) with 1.0 ml of QDs (1.0 µM). The amount of unreacted **6-Gd^{3+}** was determined from the concentration of the aqueous solution of separated by dialysis. Fig. S8 shows the UV spectra used for the conjugation reaction and **6-Gd^{3+}** unreacted. Applying the Beer–Lambert's law; $A = \varepsilon c l$ where A = absorbance, ε = extinction coefficient, c = concentration of substrate, l = path length. Here, before conjugation concentration of **6-Gd^{3+}** was 90 µM, and numerical value of A =1.43; ε is constant for a given solution of substrate and l = 1 (for all UV-Vis instrument). From the UV spectrum of unreacted **6-Gd^{3+},** it is found that numerical value of A=0.54. The calculated unreacted **6-Gd^{3+}** is to be 33.98 µM. Hence, 56.02 µM of **6-Gd^{3+}** complexes were anchor on the surface of QD particles. From the UV spectral data, an average number of complexes **6-Gd^{3+}** per QD particle was calculated to be 56±6 (±6 is within the error range)

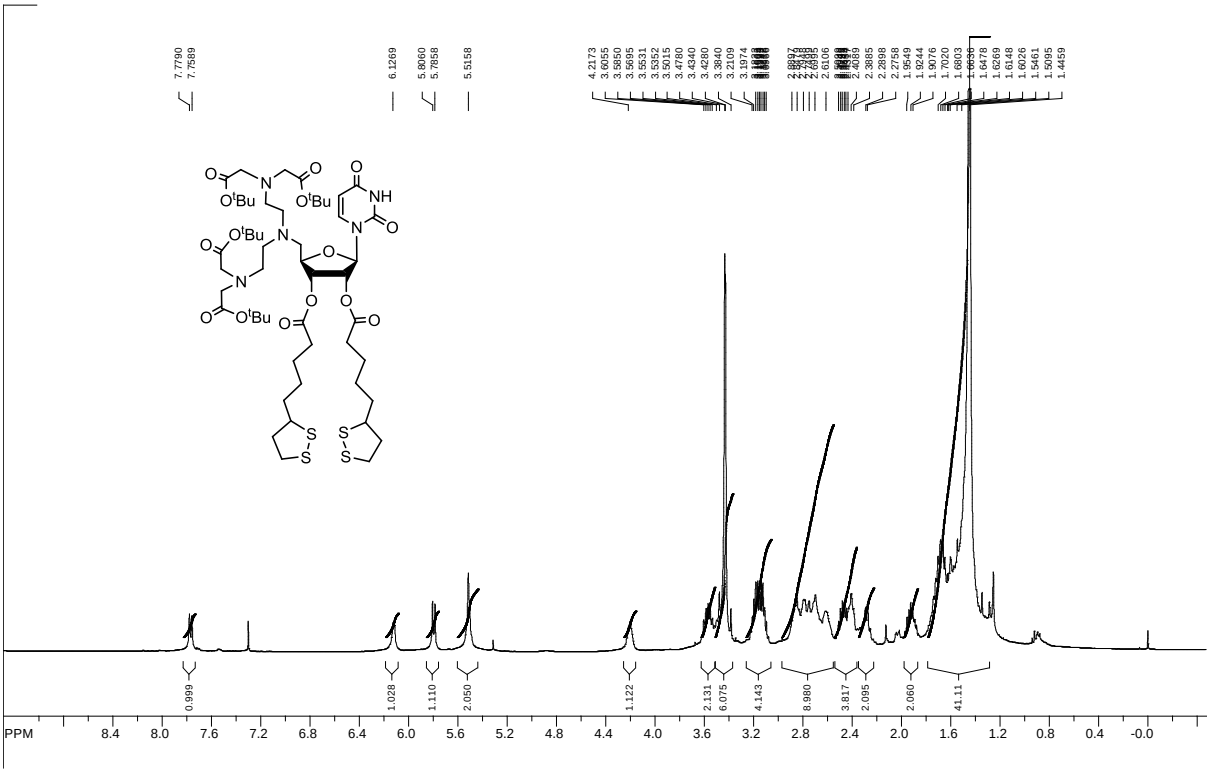

Figure S1. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of **4**.

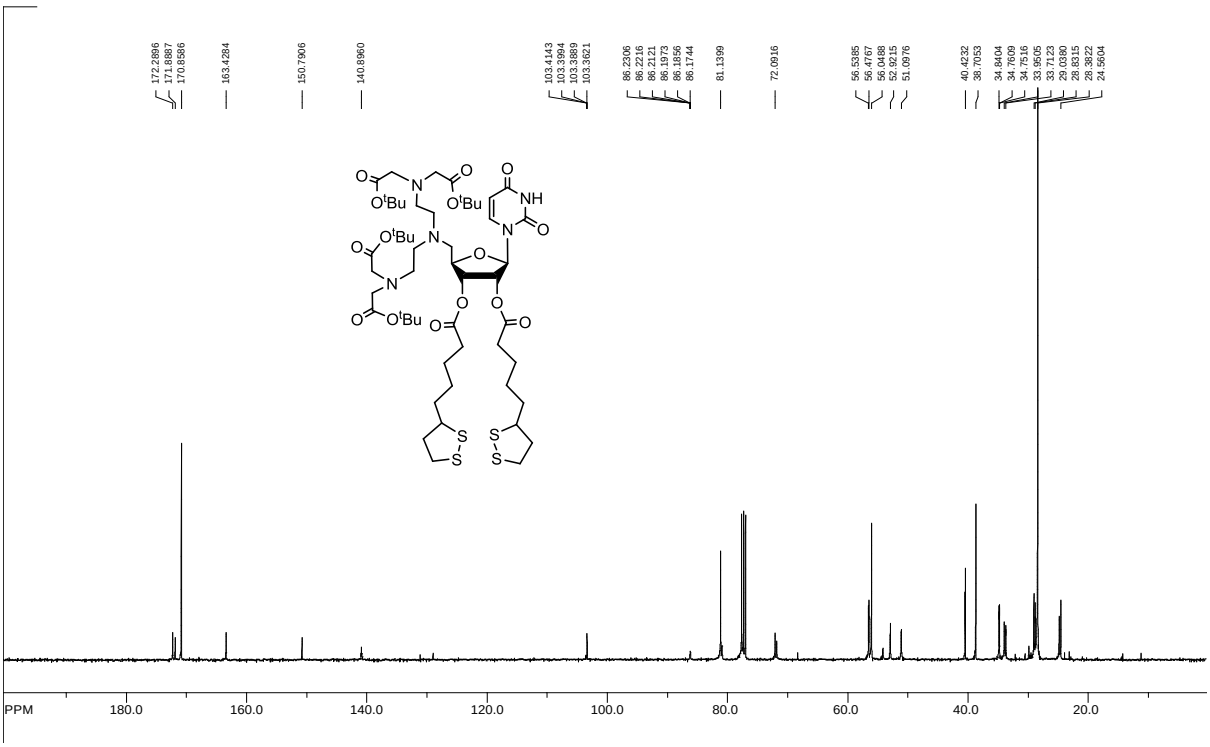

Figure S2. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of **4**.

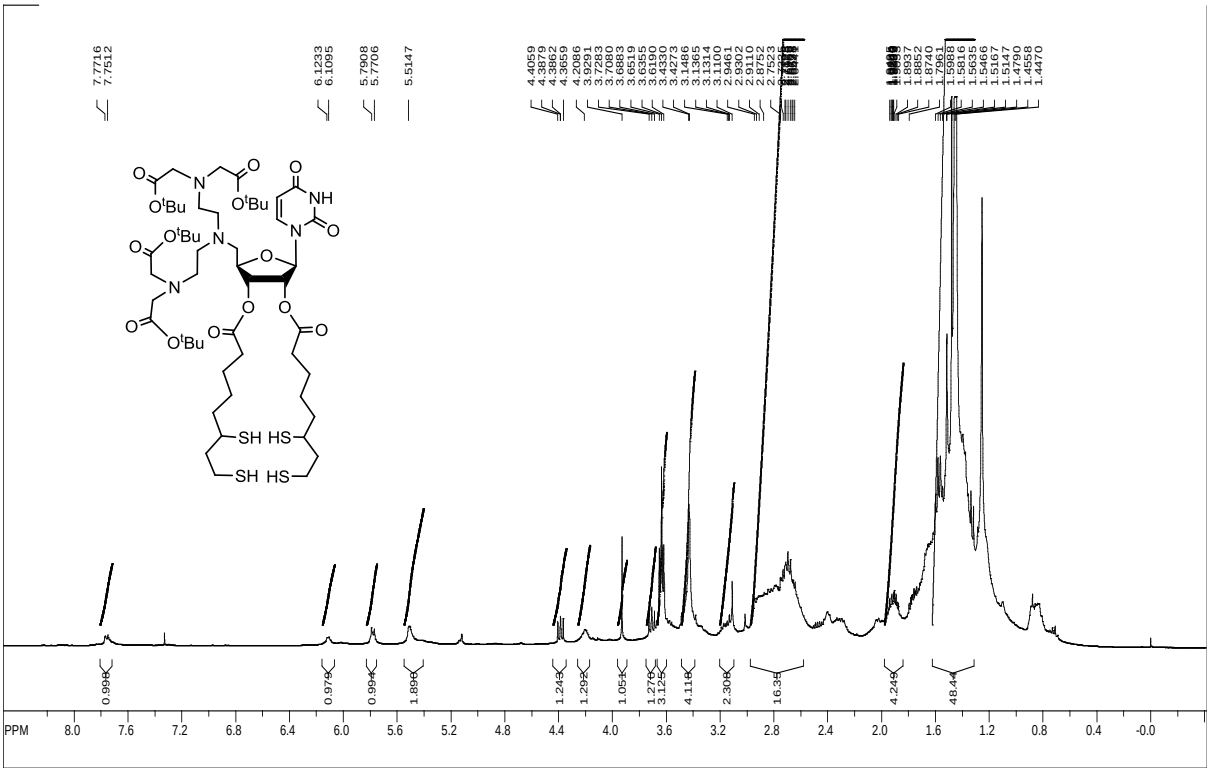

Figure S3. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of **5**.

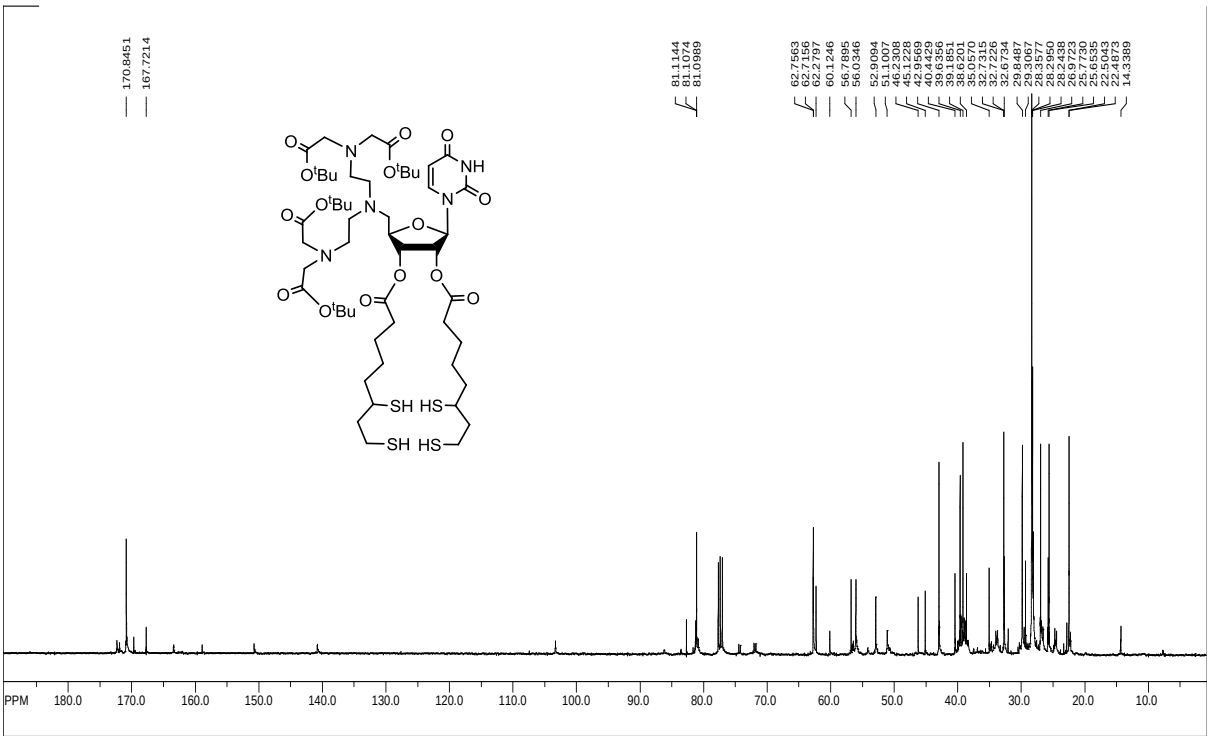

Figure S4. ^{13}C NMR ($CDCl_3$, 100 MHz) spectrum of **5**.

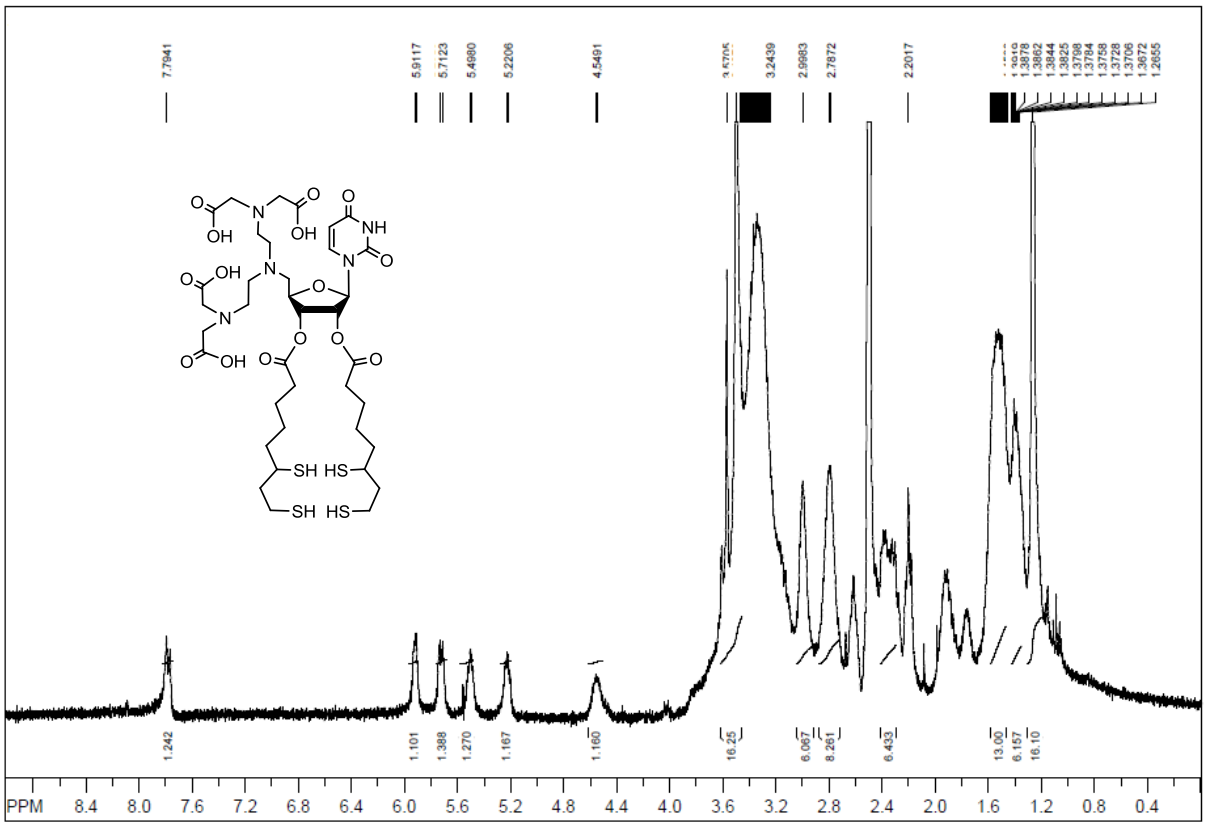

Figure S5. ^{1}H NMR (DMSO, 300 MHz) spectrum of **6**.

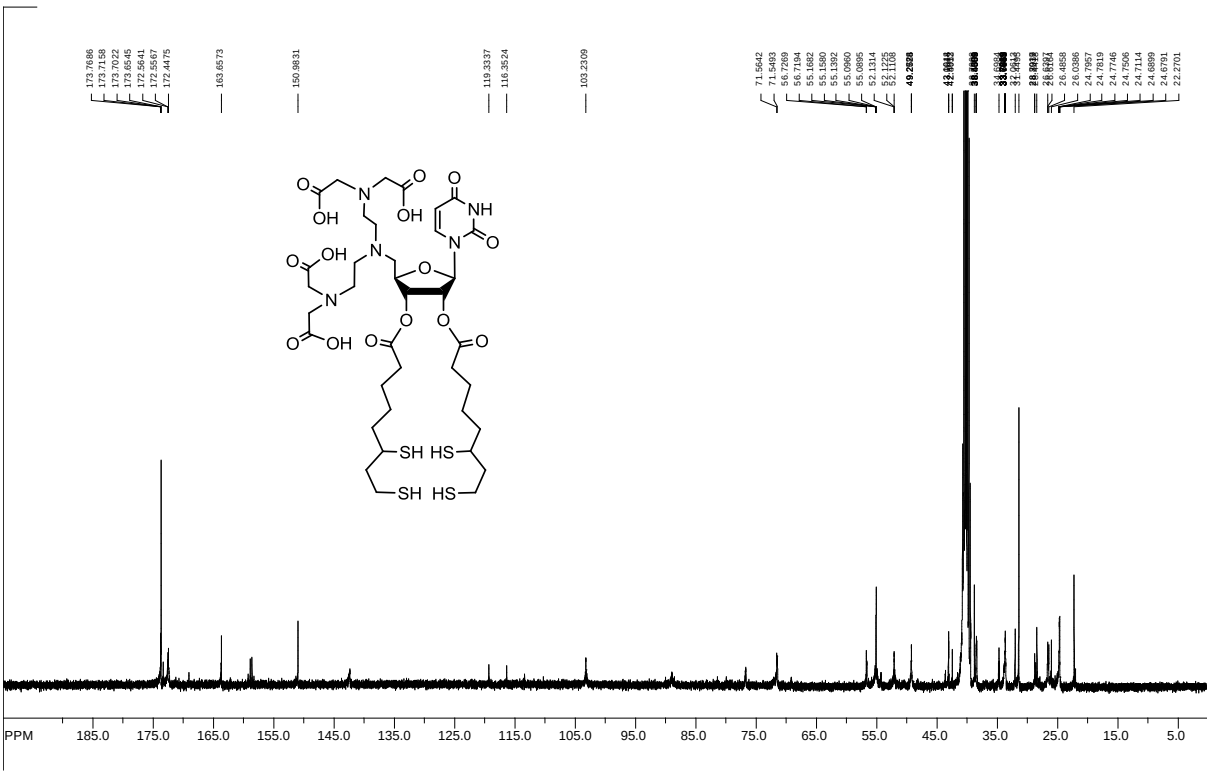

Figure S6. ^{13}C NMR (DMSO, 100 MHz) spectrum of **6**.

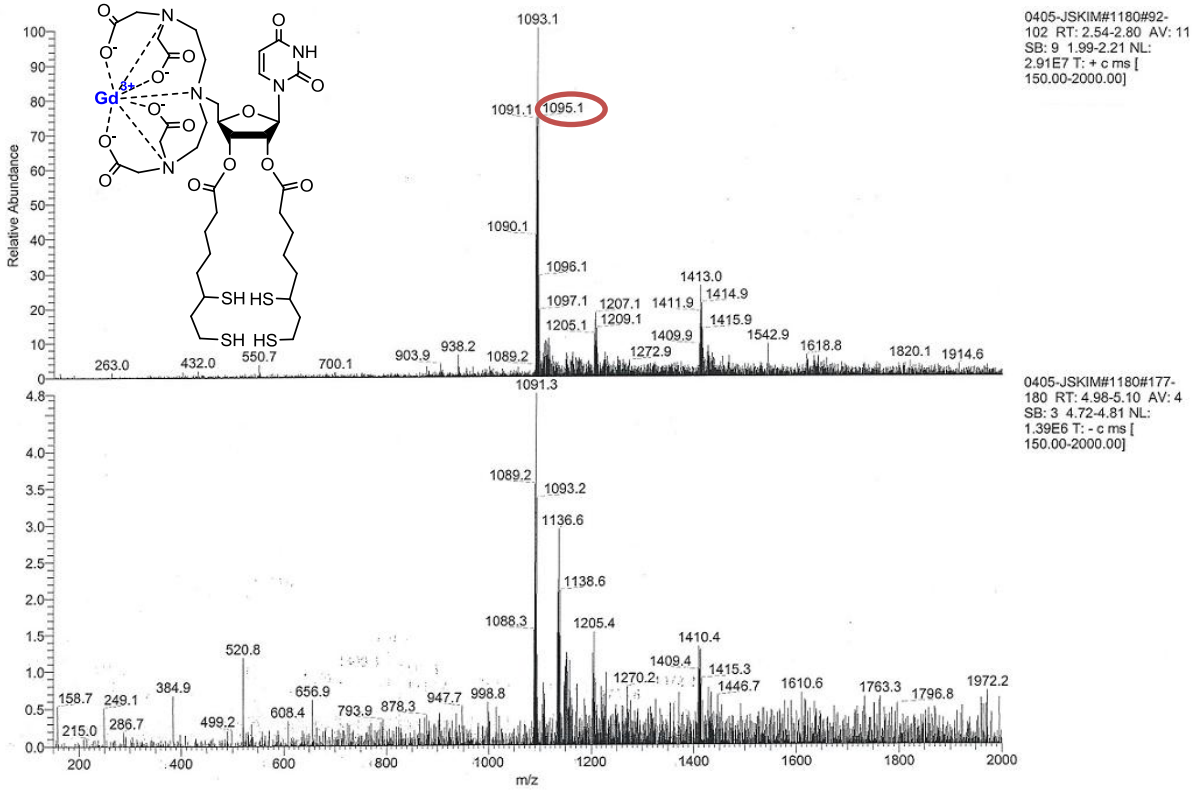

Figure S7. ESI-Mass spectrum of **6**-Gd^{3+}.

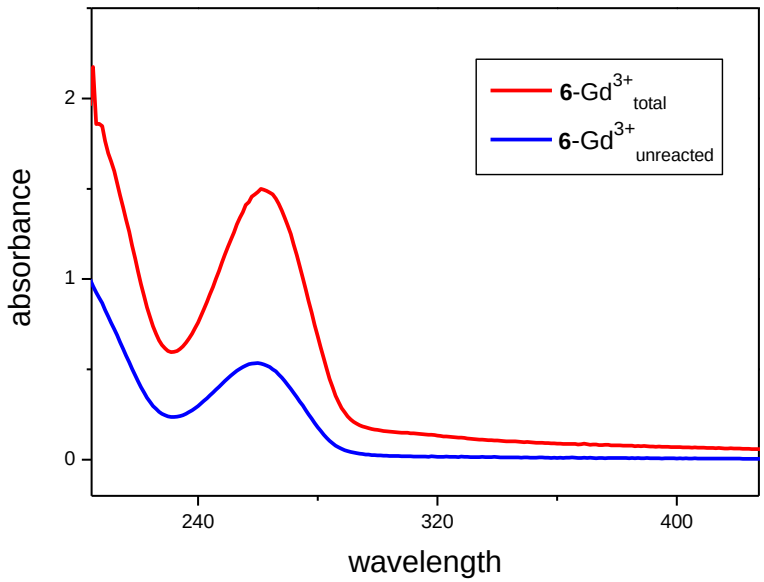

Figure S8. UV-vis spectra of **6**-Gd^{3+} and **6**-Gd^{3+}-QD.

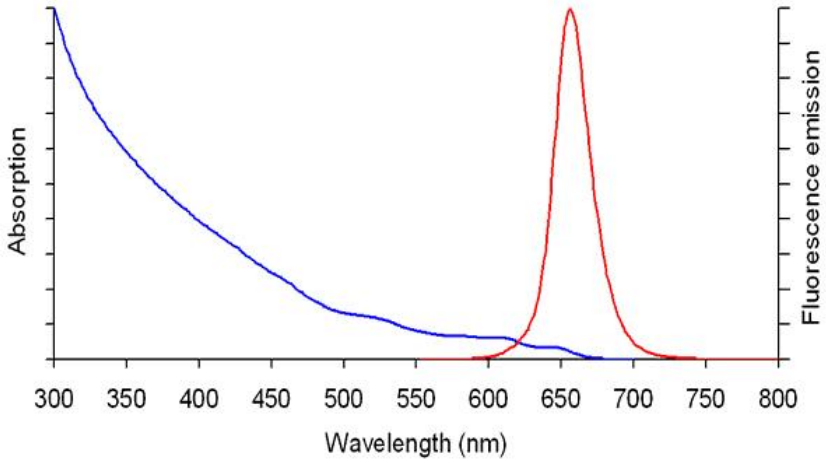

Figure S9. UV-vis and emission spectra of QD (1.0 μM/mL)

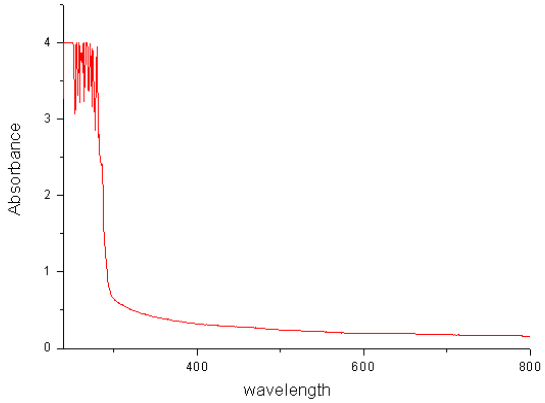

Figure S10. UV-vis spectrum of **6**-Gd^{3+}-QD

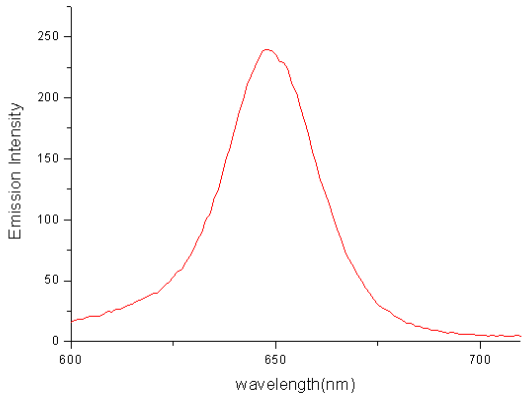

Figure S11. Emission spectrum of **6**-Gd^{3+}-QD (1.0 μM/mL) (λ_{ex} = 490 nm)

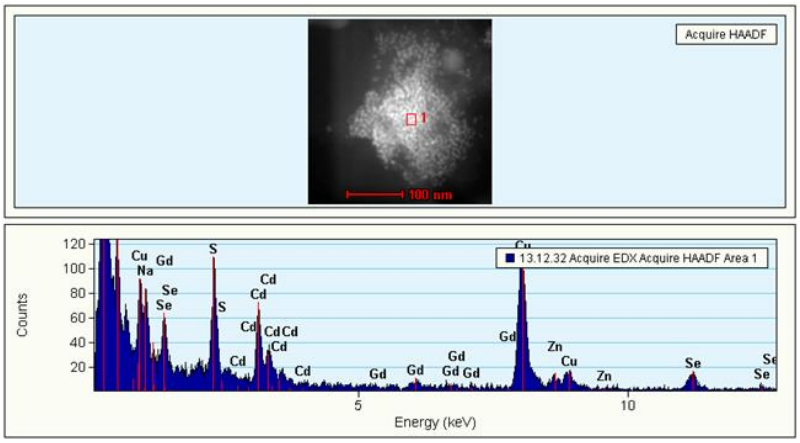

Figure S12. EDX spectrum measured from **6**-Gd^{3+}-QD, showing elemental signals from the CdSe/ZnS quantum dots and **6**-Gd^{3+}.

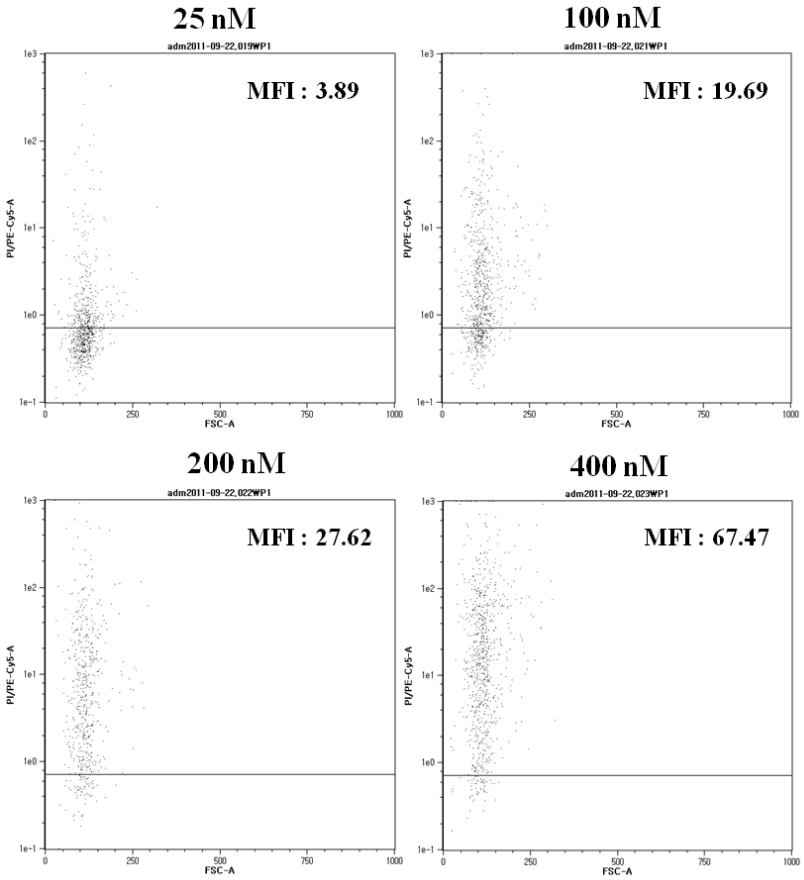

Figure S13. Flow cytometry analysis of RAW264.7 cells labeled with **6**-Gd^{3+}-QD depending on QD concentration in cell culture media. Mean fluorescence intensity (MFI) from **6**-Gd^{3+}-QD-labeled raw264.7 cells increased with QD concentration in cell culture media. Four concentrations (25, 100, 200, and 400 nM) **6**-Gd^{3+}-QDs were added to each well and incubated for 24h. After washing with PBS, the cells were analysis by using a FACS calibur (Becton Dickinson, Mountain View, CA, USA).

References:

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