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Supplementary information for

Gd-Complexes of Macrocyclic DTPA Conjugates of 1,1'-Bis(amino)ferrocenes as High Relaxivity MRI Blood-Pool Contrast Agents (BPCAs)

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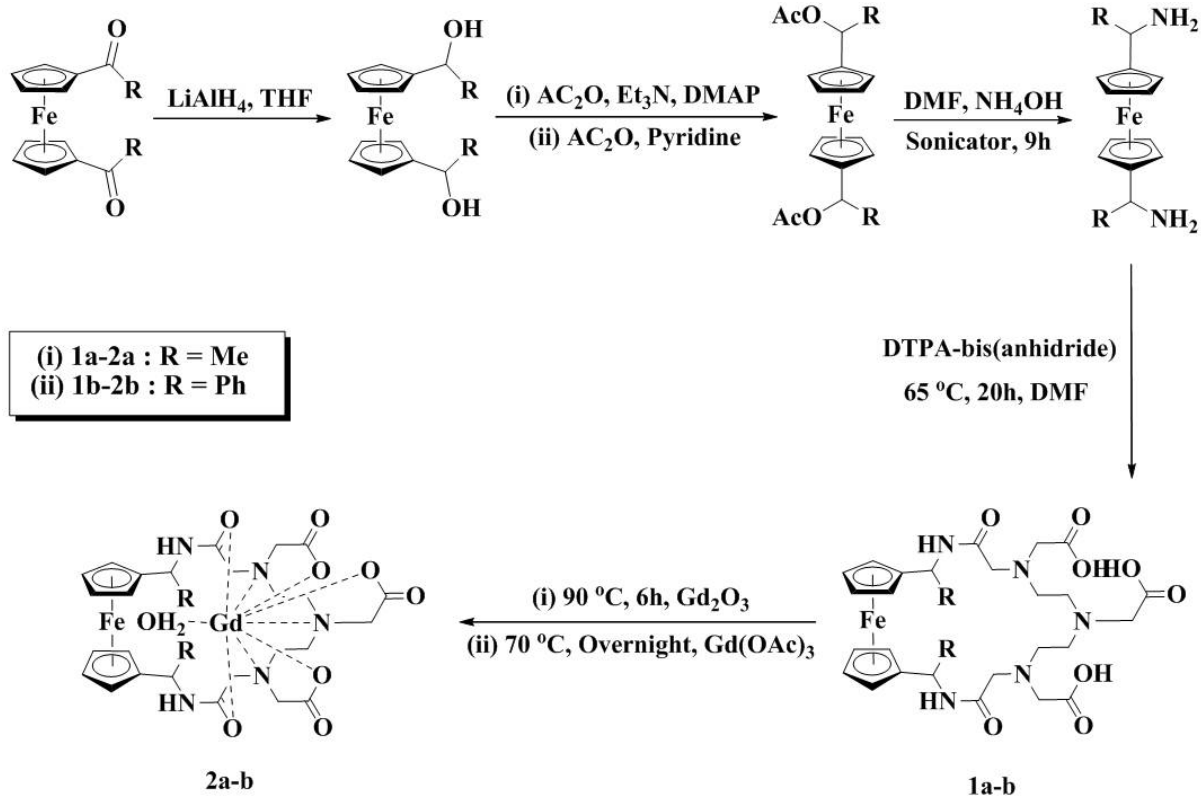
Experimental

General remarks. All reactions were carried out under an atmosphere of dinitrogen using the standard Schlenk techniques. Solvents were purified and dried using standard procedures. DTPA were obtained from TCI and used without further purification. The *N*,*N*-bis(anhydride) of DTPA was prepared according to the literature methods.[S1] All other commercial reagents were purchased from Aldrich. Deionized water was used for all experiments. The ^{1}H NMR experiment was performed on a Bruker Advance 400 Spectrometer by Center for Instrumental Analysis, Kyungpook National University (KNU). Chemical shifts were given as d values with reference to tetramethylsilane (TMS) as an internal standard. Coupling constants are in Hz. Elemental analyses were performed by Center for Instrumental Analysis, KNU and Catholic University in Daegu. FAB-mass spectra were obtained by using a JMS-700 model (Jeol, Japan) mass spectrophotometer by Korea Basic Science Institute (KBSI). Viscosity measurement was performed on a Brookfield DV-2+Pro/LVDV-2+Pro by KNU. Osmolality measurement was performed on a Precision Systems, Inc. 5004 MICRO-OSMETTE^{TM} by Korea Institute of Radiological Medical Science in Seoul.



Scheme S1.

Synthesis and characterization

1,1'-Diacetylferrocene. 1,1'-Diacetylferrocene was prepared according to the literature methods.[S2] From ferrocene (5.0 g, 26.9 mmol), acetyl chloride (4.6 mL, 64.5 mmol) and aluminum(III) chloride (8.6 g, 64.5 mmol), a yield of 88% (6.4 g) was obtained after chromatography (HEX:EA = 87.5:12.5). Red crystalline solid; ^{1}H NMR ($CDCl_3$): δ = 4.77 (s, 4H, Fc-H), 4.51 (s, 4H, Fc-H), 2.36 (s, 6H, -COCH$_3$). Anal. Calcd. for $C_{14}H_{14}FeO_2$: C, 62.25; H, 5.22. Found: C, 62.21; H, 5.22.

1,1'-bis(hydroxyethyl)ferrocene. 1,1'-Diacetylferrocene (5.0 g, 18.5 mmol) in dry THF (50 mL) was dripped slowly into $LiAlH_4$ (1.7 g, 44.4 mmol) in dry THF (100 mL) with stirring at 0 °C. After being sirred for three hours at room temperature, the reaction mixture was quenched by the addition of deionized water and ethyl acetate. After filter, two phases seperated. The aqueous phase was extracted with ethyl

acetate, the organic phases combined and washed with saturated NaCl. The organic phases were combined, dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by recrystallization (HEX/CH_2Cl_2) to yield 79% (3.9 g) as yellow-orange crystals. ^{1}H NMR ($CDCl_3$): δ = 4.60-4.67 (q, 2H, -C<u>H</u>CH$_3$), 4.15-4.19 (m, 8H, Fc-<u>H</u>), 3.76 (s, 2H, O<u>H</u>), 1.38-1.40 (d, 6H, -CHC<u>H</u>$_3$). Anal. Calcd. for $C_{14}H_{18}FeO_2$: C, 61.34; H, 6.62. Found: C, 61.30; H, 6.63.

1,1'-bis(acetoxyethyl)ferrocene. Following general procedure,[S3] a mixture of 1,1'-bis(hydroxyethyl)ferrocene (5.0 g, 18.2 mmol), Acetic anhydride (3.7 mL, 45.6 mmol), Et_3N (5.5 mL, 45.6 mmol) and DMAP (0.18 mmol) gave a orange oil. The crude product was purified by recrystallization (HEX) to yield 91.9% (6.52 g) as yellow crystals. ^{1}H NMR ($CDCl_3$): δ = 5.78-5.84 (q, 2H, -C<u>H</u>CH$_3$), 4.23-4.25 (m, 2H, Fc-<u>H</u>), 4.19 (m, 2H, Fc-<u>H</u>), 4.13-4.15(m, 4H, Fc-<u>H</u>), 2.05 (s, 6H, -COC<u>H</u>$_3$), 1.53-1.56 (two d merge, 6H, -CHC<u>H</u>$_3$). Anal. Calcd. for $C_{18}H_{22}FeO_4$: C, 60.35; H, 6.19. Found: C, 60.61; H, 6.20.

1,1'-bis(aminoethyl)ferrocene. 1,1'-bis(acetoxyethyl)ferrocene (2.0 g, 5.7 mmol) in 100 mL DMF was added 300 mL NH_4OH. The mixture was sonicated for 9 h (30 mL of NH_4OH was added after 4 h) with cooling circulator. The orange mixture was seperated between MTBE and 2 N HCl. The aqueous phase was brought to pH > 9 with 2 N NaOH and extracted with MTBE. The combined organic layers were washed wih saturated NaCl and dried with $MgSO_4$.[13] The compound was sufficiently pure for the next step without special purifications. A yield of compound was 78% (1.2 g) as a yellow-orange oil. ^{1}H NMR ($CDCl_3$): δ = 4.09-4.16 (m, 8H, Fc-<u>H</u>), 3.79-3.84 (m, 2H, -C<u>H</u>CH$_3$), 1.66-1.69 (br m, 4H, -N<u>H</u>$_2$), 1.32-1.34 (d, 6H, -CHC<u>H</u>$_3$).

1,1'-Dibenzoylferrocene. 1,1'-Dibenzoylferrocene was prepared according to the literature methods.[S2] From ferrocene (5.0 g, 26.9 mmol), benzoyl chloride (12.5 mL, 107.5 mmol) and aluminum(III) chloride (14.3 g, 107.5 mmol), a yield of 72.3% (7.5 g) was obtained after chromatography (HEX:EA = 9:1). Red crystalline solid; ^{1}H NMR ($CDCl_3$): δ = 7.77-7.79 (d, 4H, Ph-<u>H</u>), 7.52-7.56 (t, 2H, Ph-<u>H</u>), 7.40-7.44 (t, 4H, Ph-<u>H</u>), 4.92 (s, 4H, Fc-<u>H</u>), 4.58 (s, 4H, Fc-<u>H</u>). Anal. Calcd. for $C_{24}H_{18}FeO_2$: C, 73.12; H, 4.60. Found: C, 73.02; H, 4.56.

1,1'-bis(hydroxybenzyl)ferrocene. 1,1'-Dibenzoylferrocene (3.0 g, 7.8 mmol) in dry THF (100 mL) was dripped slowly into $LiAlH_4$ (11.8 g, 310.9 mmol) in dry THF (150 mL) with stirring at 0 °C. After being sirred for overnight at room temperature, the reaction mixture was quenched by the addition of deionized water and ethyl acetate. After filter, two phases seperated. The aqueous phase was extracted with ethyl acetate, the organic phases combined and washed with saturated NaCl. The organic phases were combined, dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by recrystallization (MTBE) to yield 87.4% (2.7 g) as yellow solid. ^{1}H NMR (DMSO): δ = 7.09-7.28 (m, 10H, Ph-<u>H</u>), 5.79 (d, 1H, -O<u>H</u>), 5.70-5.71 (d, 1H, -O<u>H</u>), 5.36-5.37 (t, 2H, -C<u>H</u>-Ph), 4.20-4.21 (m, 1H, Fc-<u>H</u>), 4.12-4.14 (m, 1H, Fc-<u>H</u>), 4.04-4.05 (m, 1H, Fc-<u>H</u>), 3.95-4.00 (m, 5H, Fc-<u>H</u>). Anal. Calcd. for $C_{24}H_{22}FeO_2$: C, 72.38; H, 5.57. Found: C, 72.44; H, 5.59.

1,1'-bis(acetoxybenzyl)ferrocene. Following general procedure,[S3] a mixture of 1,1'-bis(hydroxybenzyl)ferrocene (2.0 g, 5.0 mmol), Acetic anhydride (12.8 mL, 135.6 mmol), pyridine (60.6 mL, 502.3 mmol) gave a orange oil. The compound was sufficiently pure for the next step without special purifications. A yield of compound was 83.4% (1.65 g). ^{1}H NMR ($CDCl_3$): δ = 7.19-7.30 (m, 10H, Ph-<u>H</u>), 6.54-6.56 (d, 2H, -C<u>H</u>-Ph), 4.21-4.22 (m, 1H, Fc-<u>H</u>), 4.14 (m, 1H, Fc-<u>H</u>), 3.99-4.02 (m, 2H, Fc-<u>H</u>), 3.94-3.97 (m, 2H, Fc-<u>H</u>), 3.89-3.90 (m, 1H, Fc-<u>H</u>), 3.81-3.82 (m, 1H, Fc-<u>H</u>), 2.00-2.01 (d, 6H, COC<u>H</u>$_3$).

1,1'-bis(aminobenzyl)ferrocene. Following procedure of 1,1'-bis(aminoethyl)ferrocene, 1,1'-bis(acetoxybenzyl)ferrocene (0.5 g, 2.1 mmol) in 80 mL DMF was added 150 mL NH_4OH. The mixture was sonicated for 9 h (50 mL of NH_4OH was added after 4 h) with cooling circulator. The orange mixture was seperated between MTBE and 2 N HCl. The aqueous phase was brought to pH > 9 with 2 N NaOH and extracted with MTBE. The combined organic layers were washed wih saturated NaCl and dreid with

$MgSO_4$.[S4] The compound was sufficiently pure for the next step without special purifications. The product was obtained as a yellow solid (0.6 g, 78%). ^{1}H NMR (DMSO): δ = 7.14-7.35 (m, 10H, Ph-H), 4.76 (s, 2H, -CH-Ph), 4.31-4.33 (d, 2H, Fc-H), 4.08-4.10 (m, 6H, Fc-H), 2.24 (s, 4H, -NH_2). Anal. Calcd. for $C_{24}H_{24}FeO_2$: C, 72.74; H, 6.10; N, 7.07. Found: C, 72.31; H, 6.03; N, 6.40.

1a. To a stirred solution of 1,1'-bis(aminoethyl)ferrocene (1.0 g, 3.6 mmol) in dry DMF (20 mL) was added DTPA-bis(anhydride) (1.3 g, 3.6 mmol) in dry DMF (30 mL). The mixture was stirred at 65 °C for 20 h. The solvent was removed from the reaction mixture under reduced pressure, and the residue dissolved in methanol (2 mL). The solution was passed through a short column of silica gel (60 mesh) with methanol as an eluent. The residue obtained after removal of the solvent from the eluate was triturated with a mixture of acetone and diethyl ether (30:70 v/v, 150 mL). The solid product was isolated by filtration, washed with acetone (3×30 mL), and dried *in vacuo* at 70 °C for 8 h. The product was obtained as a yellow powder (1.0 g, 43.3%). (^{1}H NMR (DMSO): δ = 8.08-8.10 (d, 2H, -NH), 4.74 (s, 2H, -CHCH_3), 4.10-4.22 (m, 8H, Fc-H), 3.26-3.39 (m, 10H, -NHCOCH_2- (4H), -NCH_2COO- (6H)), 2.81-2.99 (m, 8H, -NCH_2CH_2N-), 1.26-1.32 (d, 6H, -CHCH_3). Anal. Calcd. for $C_{28}H_{39}FeN_5O_8 \cdot 4H_2O$: C, 47.94; H, 6.75; N, 9.98. Found: C, 47.56; H, 6.65; N, 9.32. HR-FABMS (m/z): calc. for $C_{28}H_{40}FeN_5O_8$, 630.2227 ($[MH]^+$), $C_{28}H_{39}FeN_5NaO_8$, 652.2046($[MNa]^+$). Found: 630.2229 ($[MH]^+$), 652.2048 ($[MNa]^+$)

1b. To a stirred solution of 1,1'-bis(aminobenzyl)ferrocene (0.8 g, 2.0 mmol) in dry DMF (20 mL) was added DTPA-bis(anhydride) (0.8 g, 2.2 mmol) in dry DMF (30 mL). The mixture was stirred at 65 °C for 20 h. The solvent was removed from the reaction mixture under reduced pressure, and the residue dissolved in methanol (2 mL). The solution was passed through a short column of silica gel (60 mesh) with methanol as an eluent. The residue obtained after removal of the solvent from the eluate was triturated with a mixture of acetone and diethyl ether (30:70 v/v, 150 mL). The solid product was isolated by filtration, washed with acetone (3×30 mL), and dried *in vacuo* at 70 °C for 8 h. The product was obtained as a yellow powder (1.3 g, 86.7%). (^{1}H NMR (DMSO): δ = 8.82-8.89 (d, 2H, -NH), 7.19-7.33 (m, 10H, Ph-H), 5.85-5.95 (m, 2H, -CH-Ph), 4.02-4.27 (m, 8H, Fc-H), 3.39-3.49 (m, 10H, -NHCOCH_2- (4H), -NCH_2COO- (6H)), 2.73-3.16 (m, 8H, -NCH_2CH_2N-). Anal. Calcd. for $C_{38}H_{43}FeN_5O_8 \cdot 3H_2O$: C, 56.51; H, 6.12; N, 8.67. Found: C, 56.49; H, 6.17; N, 8.87. HR-FABMS (m/z): calc. for $C_{38}H_{44}FeN_5O_8$, 754.2540 ($[MH]^+$), $C_{38}H_{43}FeN_5NaO_8$, 776.2359 ($[MNa]^+$). Found: 754.2543 ($[MH]^+$), 776.2363 ($[MNa]^+$).

2a. Gd_2O_3 (0.3 g, 0.8 mmol) in deionized water (20 mL) under pH 7.2 was placed in a 50 mL round-bottom flask, and **1a** (0.5 g, 0.8 mmol) was added under reflux. The reaction mixture was further stirred for 6 h at 90 °C after which any solid impurities were removed by filtration through celite. The residue was triturated with acetone (100 mL). The solid was isolated by filtration, washed with acetone (3×30 mL), and dissolved in a minimum amount of methanol to be passed through a short silica gel column. The solvent was removed, and the pale yellow solids taken up in a mixture of methanol and acetone (2:1, v/v) for crystallization at -4 ^{o}C. The product was obtained as pale yellow powder (0.6 g, 96%). Anal. Calcd. for $C_{28}H_{36}FeGdN_5O_8 \cdot 7H_2O$: C, 36.96; H, 5.54; N, 7.70. Found: C, 36.92; H, 5.32; N, 7.38. HR-FABMS (m/z): calc. for $C_{28}H_{37}FeN_5O_8Gd$, 785.1243 ($[MH]^+$). Found: 785.1230.

2b. $Gd(OAc)_3$ (0.3 g, 0.8 mmol) in pyridine (20 mL) and deionized water (10 mL) was placed in a 50 mL round-bottom flask, and **1b** (0.5 g, 0.7 mmol) was added. The mixture was stirred for overnight at 70 °C. Solvent from the reaction mixture was removed and the residue was taken in ehtanol (70 mL). The resulting solution was refluxed for 2 h. The reaction mixture was cooled and passed through a celite column to remove any solid impurities. The volume of the resulting solution was reduced to ~20 mL to be passed through a short silica gel column, and diethyl ether (100 mL) added to precipitate out yellow powder. This was dissolved in a minimum amount of methanol for crystallization at -4 ^{o}C. The product was obtained as yellow crystals (0.6 g, 94%). Anal. Calcd. for $C_{38}H_{40}FeGdN_5O_8 \cdot 6H_2O$: C, 44.92; H, 5.16; N, 6.89. Found: C, 44.34; H, 5.11; N, 6.79. HR-FABMS (m/z): calc. for $C_{38}H_{41}FeGdN_5O_8$, 909.1558 ($[MH]^+$), $C_{38}H_{41}FeGdN_5NaO_8$, 931.1365. Found: 909.1549 ($[MH]^+$), 931.1354 ($[MNa]^+$).

Potentiometric measurements and Computational method. Potentiometric titrations were performed with an automatic titrator to determine the protonation constants of **1a** and the stability constants of corresponding metal complexes. The autotitrating system consists of a 798 MPT Titroprocessor, a 728 stirrer and a PT-100 combination pH electrode (Metrohm). The pH electrode was calibrated using standard buffer solutions. All calibrations and titrations were carried out under a CO_2-free nitrogen atmosphere in a sealed glass vessel (50 cm^3) thermostatted at 25 ± 0.1 ^oC at an ionic strength of 0.10 mol/dm^3 KCl. The concentrations of the metal-ion and the amide solutions were maintained at approximately 0.5 mmol/dm^3. A CO_2-free KOH solution (0.100 mol/dm^3) was used as a titrant to minimize the changes in ionic strength during the titration. Dioxygen and carbon dioxide were excluded from the reaction mixtures by maintaining a positive pressure of purified nitrogen in the titration cell. The electromotive force of the cell is given by $E = E'^o + Q \log[H^+] + E_j$, and both E'^o and Q were determined by titrating a solution with a known hydrogen-ion concentration at the same ionic strength, using the acid range of the titration. The liquid-junction potential (E_j) was found to be negligible under the experimental conditions employed. The protonation constants of the ligands and the overall stability constants of various metal complexes formed in aqueous solutions were determined from the titration data using the computer program HYPERQUAD. The accuracy of this method was verified by measuring the protonation and the stability constants for Ca(II), Zn(II), Cu(II), and Gd(III) complexes of $[DTPA\text{-}BMA]^{3-}$. The results were compared with literature values.[15(b)] The protonation constants of **1a** was determined by potentiometric titration in the pH range 2–11. The protonation constants (K_i^H) are defined in equation (1), where i = 1, 2, . . .

$$K_i^H = [H_iL] / [H_{i-1}L][H^+] \quad (1)$$

The calculated protonation constants are presented in Table S1 along with those for DTPA-BMA, DTPA and DOTA for comparative purposes. It is known that for the DTPA-bis(amide) ligands the first protonation takes place at the central nitrogen atom, while the second and the third at the terminal amine nitrogen atoms.[S4]

The thermodynamic stability constants ($K_{ML(therm)}$) are described by equation (2), where M is the free unhydrolyzed aqua metal ion, L the totally deprotonated free ligand, and ML the nonprotonated and nonhydrolyzed complex.

$$K_{ML(therm)} = [ML] / [M][L] \quad (2)$$

The direct potentiometric titration method cannot be applied for the measurement of the stability constants of Gd(III) complexes of bis(amides) since they are formed at low pH. Instead, they were determined by employing the method of ligand–ligand competition potentiometric titration between EDTA and bis(amide) ligands (L) for Gd(III) ion.[S5] Yet, the stability constants of Cu(II), Zn(II), and Ca(II) complexes were measured by direct titration of the ligands.

The conditional stability constants reveal the extent of complexation at the given pH. To understand the stability of the complexes at the physiological pH, the stability constants of the complexes have been evaluated at pH 7.4 using equation (3),[S6] where K_n^H (n = 1,2,3, . . .) are the stepwise protonation constants of the ligands.

$$K_{ML(cond)} = K_{ML(therm)}(1 + K_1^H [H] + K_1^H K_2^H [H]^2 + K_1^H K_2^H K_3^H [H]^3 + \cdots)^{-1} \quad (3)$$

The modified selectivity constants (K'_{sel}) can be calculated using the following equations (4)-(8) and $K_{ML(therm)}$ values in equation (4). Here α's are the side reaction coefficients.

$$K'_{sel} = K_{ML(therm)} (\alpha_H^{-1} + \alpha_{CaL}^{-1} + \alpha_{CuL}^{-1} + \alpha_{ZnL}^{-1})^{-1} \quad (4)$$

$$\alpha_H^{-1} = 1 + K_1^H [H] + K_1^H K_2^H [H]^2 + K_1^H K_2^H K_3^H [H]^3 + \cdots \quad (5)$$

$$\alpha_{CaL}^{-1} = 1 + K_{CaL}[Ca^{2+}] \quad (6)$$

$$\alpha_{CuL}^{-1} = 1 + K_{CuL}[Cu^{2+}] \quad (7)$$

$$\alpha_{ZnL}^{-1} = 1 + K_{ZnL}[Zn^{2+}] \quad (8)$$

The K'_{sel} values at pH 7.4 for the Gd(III) complex of **1a** was calculated using the in vivo concentrations of

[Cu(II)] = 1.0 × 10^{-3} mM, [Ca(II)] = 2.5 mM, and [Zn(II)] = 5.0 × 10^{-2} mM.[S6(a)] All of stability constants collected in Table S1.

Transmetallation kinetics. This experiment was prepared according to the literature method.[S7] It is based on experiment of the evolution of the water proton relaxation rate (R_1^P) of a buffered solution (phosphate buffer, pH 7.4) containing 2.5 mmol/L gadolinium complex and 2.5 mmol/L $ZnCl_2$. 10 μL of a 250 mmol/L solution of $ZnCl_2$ is added to 1 mL of a buffered solution of the paramagnetic complex. The mixture is vigorously stirred, and 300 μL is taken up for the relaxometric study. A control study, run on Omniscan® and Dotarem® with zinc acetate, has given results identical to those obtained in the presence of $ZnCl_2$. The R_1^P relaxation rate is obtained after subtraction of the diamagnetic contribution of the proton water relaxation from the observed relaxation rate $R_1 = (1/T_1)$. The measurements were performed on a 3 T whole body system (Magnetom Tim Trio, siemens, Germany) at room temperature.

Determination of kinetic constants. These constants were fitted according to the equations (9)-(12). [S7]

$$GdL + Zn^{2+} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} Gd^{3+} + [ZnL]^{1-} \quad (9)$$

,where k_f and k_r are the rate constants for the forward and reverse transmetallation reactions, respectively. The rate of transmetallation can be written by equation (10). Here $[ZnL] = [GdL]_0 - [GdL]$ with $[GdL]_0$ being the initial concentration of the gadolinium complex.

$$\frac{d[GdL]}{dt} = -k_f'[GdL] + k_r'[ZnL] \quad (10)$$

The concentration of [GdL] at a time t can be written by equation (11).

$$[GdL] = [GdL]_\infty + ([GdL]_0 - [GdL]_\infty)e^{-k_1 t} \quad (11)$$

$k_1 = k'_f + k'_r$ and $[GdL]_\infty$ is the equilibrium concentration of [GdL]. Thus, k_f can be calculated as

$$k_f' = k_1 \frac{[GdL]_0 - [GdL]_\infty}{[GdL]_0} \quad (12)$$

The k'_f value is obtained by fitting the experimental data.

Relaxivity. T_1 measurements were carried out using an inversion recovery method with a variable inversion time (TI) at 1.5 T (64 MHz). The magnetic resonance (MR) images were acquired at 35 different TI values ranging from 50 to 1750 msec. T_1 relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each TI value. For T_2 measurements the CPMG (Carr-Purcell-Meiboon-Gill) pulse sequence was adapted for multiple spin-echo measurements. Thirty four images were acquired with 34 different echo time (TE) values ranging from 10 to 1900 msec. T_2 relaxation times were obtained from the non-linear least squares fit of the mean pixel values for the multiple spin-echo measurements at each echo time. Relaxivities (R_1 and R_2) were then calculated as an inverse of relaxation time per mM. The determined relaxation times (T_1 and T_2) and relaxivities (R_1 and R_2) are finally image-processed to give the relaxation time map and relaxivity map respectively.

Serum stability assay. Instead of adopting the traditional method for the serum stability test consisting of the HPLC measurement of relative amount of the free Gd^{3+} ion,[S8] we have developed a qualitative method of determining the $[Gd^{3+}]$ concentration by measuring the ratio of relaxivities, $R_1^p(t)/R_1^p(0)$, as a function of incubation time for the serum solution of GdL (**2a**). The typical procedure is as follows: To the solution of aqueous human serum (Innovative research) (180 μL) was added an aqueous solution of **2a** (20 μL, 5 mM) at 4 °C. The mixture was incubated at 37 °C at different intervals (0 min, 30 min, 1 h, 2 h, 4 h, 48 h, and 96 h), after

which time aqueous TCA (20%, 100 μL) was added to precipitate out free human serum. The supernatant solution, after removal of the white precipitates of human serum by centrifugation at 4 °C for 10 min, was taken to the relaxivity measurement at a 3 T whole body system at RT. In this way the serum stability assay can be qualitatively accomplished, since any changes in R_1 relaxivities (an increase, in fact) of the solutions prepared before and after incubation would indicate the release of free Gd ion from GdL (**2a**). Plots of serum stabilities, $R_1^p(t)/R_1^p(0)$, of Omniscan®, Magnevist® and **2a** are illustrated as a function of incubation time in Fig S6.

In vitro **cell toxicity**. COS-7, monkey kidney cells and IMR-90, human embryonic lung fibroblasts were used. Cells were maintained in DMEM (Gibco®) supplemented with heat-inactivated FCS (10%), penicillin (100 IU/mL), streptomycin (100 mg/mL), and gentamicin (200 mg/mL) (all purchased from Gibco®). The medium was replaced every 2 days, and cells split into a 96-well plate (1×10^4 cells/well/200 μL). Various Gd(III) concentrations (COS-7 : 0.01 – 0.5 mM, IMR-90 : 0.01 – 2.0 mM) of the contrast agent were added into the culture serum free media and incubated for 24 h. CCK-8 (10 μL) was then added to each well to evaluate cell viability. The solution was removed after 4 h at 37 °C. The O. D. (Optical Density) read at 450 nm using a microplate reader (Molecular Device, USA Bio-rad 550 Reader) to determine the cell viability/toxicity.

In vivo **MR Imaging**. All animal experiments were performed in accordance with the rules of the animal research committee of Kyungpook National University. Six-week male ICR mice with weights of 29-31 g were used for the MRI. Ten-week male SD rats with weight 350-400 g were used for the CE-MRA. The mice (n=4) and rats (n=4) were anesthetized by 1.5% isoflurane in oxygen. Measurements were made before and after injection of **2a** via tail vein. The amount of CA per each injection is 0.1 mmol [Gd]/kg for MR and CE-MRA images. After each measurement the mouse was revived from anesthesia, and placed in the cage with free access to food and water. During these measurements, the animals were maintained at approximately 37 °C using a warm water blanket. MR images were taken with a 1.5 Tesla (T) MR unit (GE Healthcare, Milwaukee, WI, USA) equipped with a home-made small animal RF coil. The coil was of the receiver type with its inner diameter being 50 mm. The imaging parameters for 3D fast SPGR (spoiled GRASS images) are as follows: repetition time (TR) = 9.2 ms; echo time (TE) = 2.1 ms; 12 mm field of view (FOV); 256×192 matrix size; 0.8 mm slice thickness; number of acquisition (NEX) = 8. Images were obtained for 110 min after injection. CE-MRA was carried out with a 3.0 Tesla (T) MR unit (Magnetom Tim Trio, Siemens Medical Solution, Erlangen, Germany) equipped with 8HR wrist coil. The imaging parameters for CE-MRA vibe are as follows: repetition time (TR) = 3.3 ms; echo time (TE) = 1.3 ms; 17 mm field of view (FOV); 0.9 mm slice thickness; 192×65 matrix size; number of acquisition (NEX) = 1. Each image was taken at the interval of 11 seconds.

Image Analysis. The anatomical locations with enhanced contrast were identified with respect to heart, kidney and liver on post-contrast MR images. For quantitative measurement, signal intensities in specific regions of interest (ROI) measured using Advantage Window software (GE Medical, USA). The CNR (Contrast to Noise Ratio) was calculated using equation 13, where SNR is the signal to noise ratio.

$$CNR = (SNR_{post} - SNR_{pre}) \quad (13)$$

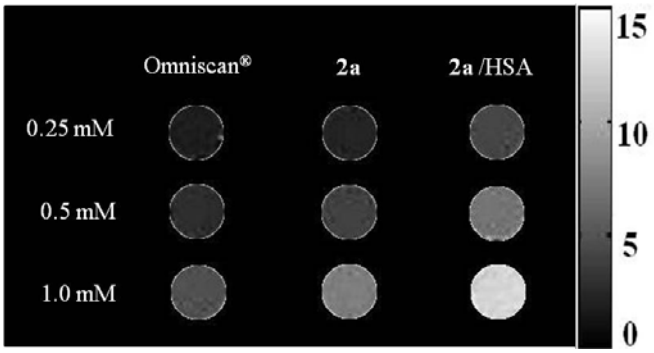


Fig. S1 R_1 map on **2a, 2a**/HSA, and Omniscan®

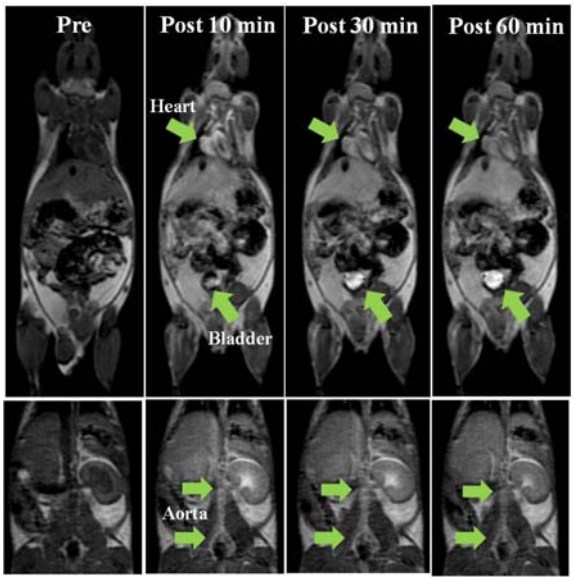


Fig. S2 *In vivo* MR coronal images of mice obtained with **2a** (0.1 mmol/kg)

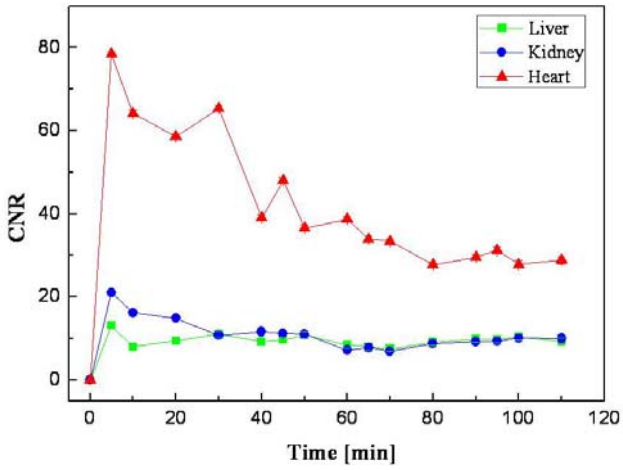


Fig. S3 *In vivo* CNR profiles as a function of time from mice

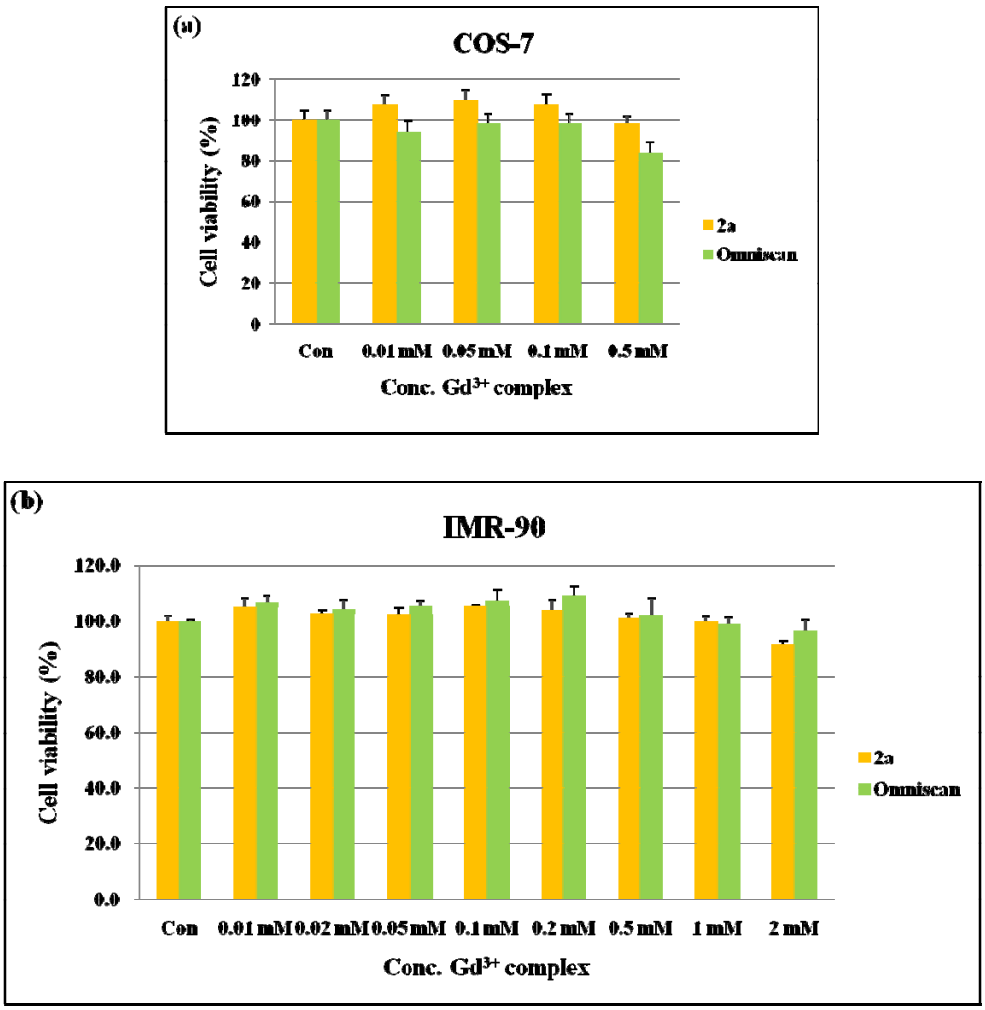


Fig. S4 (a) COS-7 (b) IMR-90 cell viability for **2a** and Omniscan® at various concentrations

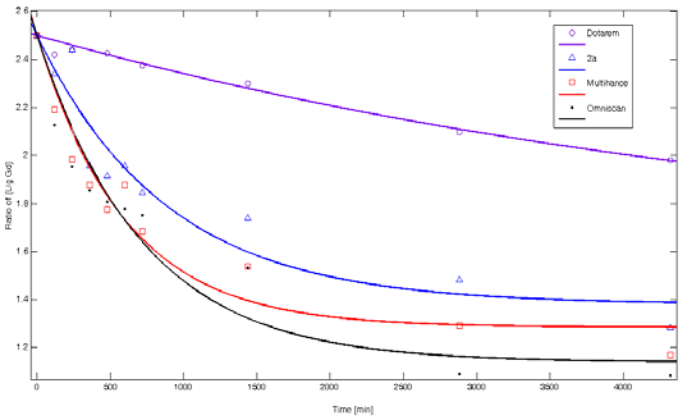


Fig. S5 Experimental data and data fitting with the use of Equation 12 for Dotarem®, **2a**, Multihance® and Omniscan®

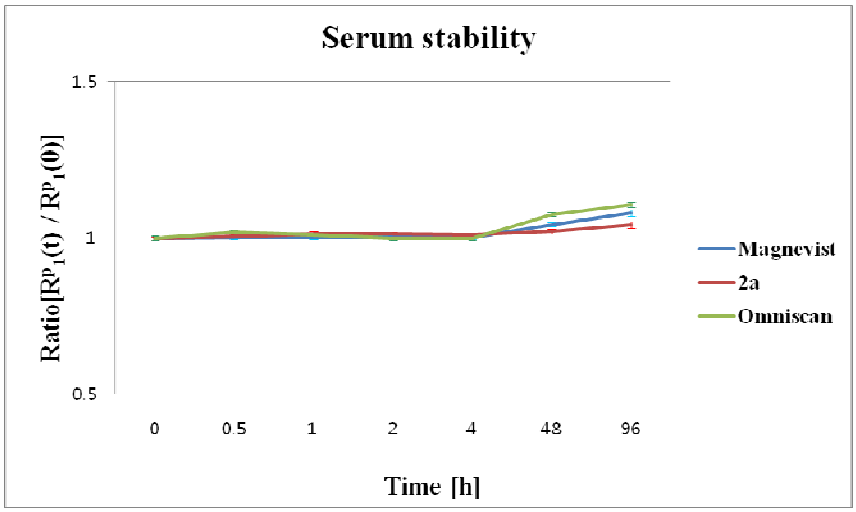


Fig. S6 Human serum stability for Omniscan®, Magnevist® and **2a**

Table S1 Protonation constants (K_i^H), stability and selectivity constants of ML (I=0.10 mol/dm^3), and the pM[a] values of the complexes of Gd^{3+}, Ca^{2+}, Zn^{2+} and Cu^{2+} at pH 7.4

Equilibrium	log K (25 °C, μ = 0.10 M (KCl))			
	L = **1a**	DTPA-BMA[b]	DTPA[c]	DOTA[d]
[HL]/[L][H]	8.27	9.37	10.49	12.09
[H_2L]/[HL][H]	4.46	4.38	8.60	9.68
[H_3L]/[H_2L][H]	3.47	3.31	4.28	4.55
[H_4L]/[H_3L][H]	-	-	2.64	4.13
$\sum pK_a$	16.20	17.06	26.01	30.45
[GdL]/[Gd][L]	22.12	16.85	22.46	25.30
{logK_{GdL}(pH 7.4)}	21.19	14.84	18.14	18.33
[CaL]/[Ca][L]	6.71	7.17	10.75	17.23
{logK_{CaL}(pH 7.4)}	5.78	5.11	6.43	10.26
[ZnL]/[Zn][L]	10.19	12.04	18.70	21.05
{logK_{ZnL}(pH 7.4)}	9.26	10.02	14.38	14.08
[CuL]/[Cu][L]	10.46	13.03	21.38	22.63
{logK_{CuL}(pH 7.4)}	9.53	11.06	17.06	15.66
[logK_{sel}(Gd/Ca)]	15.41	9.68	11.71	8.07
[logK_{sel}(Gd/Zn)]	11.93	4.81	3.76	4.25
[logK_{sel}(Gd/Cu)]	11.66	3.82	1.08	2.67
logK'_{sel}	16.22	9.03	7.04	8.30
pGd	20.15	13.88	17.14	19.20
pCa	4.75	4.19	5.45	-
pZn	8.28	9.06	13.39	15.19
pCu	8.52	10.05	16.06	14.05

[a]pM = -log[M^{n+}]$_{free}$ at pH 7.4; [M^{n+}]$_{total}$ = 1 μmol/dm^3; [L]$_{total}$ = 1.1 μmol/dm^3. [b]Data obtained from ref. S9(a). [c]Data obtained from ref. S9(b). [d]Data obtained from ref. S9(c) and S9(d).

Table S2 Kinetic constants of Dotarem®, **2a**, Multihance® and Omniscan®

Complex	k_1 (min^{-1})	$[Lig\ Gd]_\infty$ (mol/L)	k'_f (min^{-1})
Dotarem®	1.81×10^{-4}	1.53×10^{-3}	0.69×10^{-4}
2a	1.19×10^{-3}	1.35×10^{-3}	5.46×10^{-4}
Multihance®	1.65×10^{-3}	1.24×10^{-3}	8.32×10^{-4}
Omniscan®	1.41×10^{-3}	1.09×10^{-3}	7.96×10^{-4}

Table S3 Osmolality and Viscosity data of Magnevist®, Omniscan®, MS-325 and **2a** at 310 K

Complex	Osmolality[a] (mOsm/kg H_2O)	Vicosity[a] (mPa.s)
Magnevist®[b]	1980	2.90
Omniscan®[b]	645	1.40
MS-325[c]	825	2.10
2a	469	1.48

[a]All complex concentrations are equal to 500 mM except for MS-325 (250 mM)
[b]Data obtained from ref. S10.
[c]Data obtained from ref. S11.

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