Supplementary Material (ESI) for Analyst

Supplementary Information

Novel 15-Crown-5 Ether or β-Diketone Incorporated Gadolinium Complexes for the Detection of Potassium Ions or Magnesium and Calcium Ions

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Synthesis

(15-Crown-5)-4-benzoic acid 2-tert-butoxycarbonylamino-ethyl ester (1). To a mixture of 4-carboxybenzo 15-crown-5 (0.77 g, 2.5 mmol) and potassium carbonate (0.93 g, 6.7 mmol) in dry *N*,*N*-dimethylformamide (20 mL), a solution of 2-(Boc-amino)ethyl bromide (0.50 g, 2.2 mmol) in dry *N*,*N*-dimethylformamide (8 mL) was added and the mixture was stirred at 50°C for 2 hours under an Ar atmosphere. The reaction mixture was filtered to remove potassium carbonate. To the filtrate was added dichloromethane (150 mL) and then, the organic solution was washed with water six times. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, chloroform : methanol = 30 : 1, v/v) to give compound **1** (1.1 g, 94%) as a yellow crystals. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.44 (9H, s, *t*-Bu), 3.50-3.55 (2H, m, C*H*$_2$NH), 3.75-3.80 (8H, m, crown), 3.90-3.95 (4H, m, crown), 4.15-4.20 (4H, m, crown), 4.35 (2H, t, *J* 5.1, C*H*$_2$CH$_2$NH), 4.85-4.95 (1H, br s, NH), 6.85 (1H, d, *J* 8.3, ArH), 7.53 (1H, s, ArH), 7.67 (1H, d, *J* 8.3, ArH); *m*/*z* (ESI-TOF) 478.21 (M + Na^+. $C_{22}H_{33}NNaO_9$ requires 478.21).

(15-Crown-5)-4-benzoic acid 2-amino-ethyl ester (2). To a solution of compound **1** (0.10 g, 1.1 mmol) in dichloromethane (15 mL), trifluoroacetic acid (5 mL) was added and the mixture stirred at 0°C for 30 minutes, followed by stirring at room temperature overnight. The reaction mixture was evaporated in vacuo and the residue was purified by column chromatography (SiO_2, chloroform : methanol : triethylamine = 10 : 1 : 0.1, v/v) to give compound **2** (70 mg, 90%) as a yellow oil. δ_H (300 MHz; $CDCl_3$; Me_4Si) 3.35-3.45 (2H, m, C*H*$_2$NH$_2$), 3.60-3.80 (8H, m, crown), 3.95-4.20 (2H, m, C*H*$_2$CH$_2$NH$_2$), 4.35-4.60 (8H, m, crown), 6.70 (1H, d, *J* 8.5, ArH), 7.40-7.50 (1H, m, ArH), 7.51 (1H, s, ArH); *m*/*z* (ESI-TOF) 356.87 (M + H^+. $C_{17}H_{26}NO_7$ requires 356.17).

(Bis-(2-(((2-carboxy-(benzo-15-crown-5)-ethylcarbamoyl)-methyl)-carboxymethyl-amino)-ethyl)-amino)-acetic acid (3). To a mixture of diethylenetriaminepentaacetic dianhydride (60 mg, 0.17 mmol) in dry *N*,*N*-dimethylformamide (2 mL), compound **2** (30 mg, 84 μmol) was added and the mixture was stirred at 70°C for 3 hours under an Ar atmosphere. The reaction mixture was evaporated in vacuo. The residue was purified by reverse-phase column HPLC (methanol : water : trifluoroacetic acid = 100 : 66 : 0.5, v/v, flow rate: 3.5 mL/min, retention time: 25 min) to give compound **3** (86 mg, 95%) as a white solid. δ_H (300 MHz; CD_3OD; Me_4Si) 3.35-3.40 (2H, m, NC*H*$_2$COOH), 3.45-3.55 (8H, m, NCH$_2$CH$_2$N), 3.55-3.63 (8H, m, N(C*H*$_2$CO)C*H*$_2$COOH), 3.64 (4H, s, OCH$_2$C*H*$_2$NH), 3.70-3.75 (16H, m, crown), 3.85-3.90 (8H, m, crown), 4.10-4.20 (8H, m, crown), 4.35-4.40 (4H, m, OC*H*$_2$CH$_2$NH), 6.95-7.05 (2H, m, ArH), 7.50-7.60 (2H, m, ArH), 7.65-7.70 (2H, m, ArH); *m*/*z* (ESI-TOF) 546.02 (M + H^+ + Na^+. $C_{48}H_{70}N_5NaO_{22}$ requires 545.72).

KMR-K1. To a solution of compound **3** (10 mg, 9.4 μmol) in water (4 mL), gadolinium(III) chloride hexahydrate (3.1 mg, 8.4 μmol) was added and the mixture stirred at room temperature overnight. During the reaction, the pH of the solution was maintained at pH 6 by addition of dilute aqueous NaOH. The reaction mixture was then evaporated in vacuo and the residue purified by reverse-phase column HPLC (methanol : water = 3 : 2, v/v, flow rate: 3.5 mL/min, retention time: 59 min) to give KMR-K1 (9.9 mg, 86%) as a white solid. *m*/*z* (ESI-TOF) 634.94 (M + 2 × Na^+. $C_{48}H_{66}GdN_5Na_2O_{22}$ requires 634.16).

((2-(Carboxymethyl-(2-(carboxymethyl-(benzo-15-crown-5)-carbamoylmethyl-amino)-ethyl)-amino)-ethyl)-phenylcarbamoylmethyl-amino)-acetic acid (4). To a mixture of diethylenetriaminepentaacetic dianhydride (0.32 g, 0.88 mmol) in dry *N*,*N*-dimethylformamide (4 mL), 4'-aminobenzo-15-crown-5 (0.50 mg, 1.8 mmol) was added and the mixture was stirred at 70°C for 4 hours under an Ar atmosphere. The reaction mixture was evaporated in vacuo. The residue was purified by reverse-phase column HPLC (methanol : water : trifluoroacetic acid = 100 : 66 : 0.5, v/v, flow rate: 3.5 mL/min, retention time: 25 min) to give compound **4**

(0.83 g, 79%) as a gray solid. δ_H (300 MHz; CD_3OD; Me_4Si) 3.20-3.30 (4H, m, C*H*$_2$N(CH_2COOH)C*H*$_2$), 3.45-3.50 (4H, m, C*H*$_2$$CH_2$N($CH_2COOH$)$CH_2$C*H*$_2$), 3.56 (4H, s, NC*H*$_2$COOH), 3.58 (4H, s, $COCH_2N$), 3.65-3.75 (16H, m, crown), 3.80-3.90 (8H, m, crown), 3.91 (2H, s, NC*H*$_2$COOH), 4.05-4.10 (8H, m, crown), 6.77 (2H, d, *J* 8.7, ArH), 7.07 (2H, d, *J* 8.4, ArH), 7.28 (2H, s, ArH); *m*/*z* (ESI-TOF) 924.68 (M + H^+. $C_{42}H_{62}N_5O_{18}$ requires 924.41).

KMR-K2. To a solution of compound **4** (0.10 g, 0.11 mmol) in water (40 mL), gadolinium(III) chloride hexahydrate (36 mg, 97 μmol) was added and the mixture was stirred at room temperature overnight. During the reaction, the pH of the solution was maintained at pH 6 by addition of dilute aqueous NaOH. The reaction mixture was then evaporated in vacuo and purified by reverse-phase column HPLC (methanol : water = 3 : 2, v/v, flow rate: 3.5 mL/min, retention time: 67 min) to give KMR-K2 (97 mg, 83%) as a white solid. *m*/*z* (ESI-TOF) 562.46 (M + 2 Na^+. $C_{42}H_{58}GdN_5Na_2O_{18}$ requires 562.14).

4-Oxo-4*H*-quinolizine-3-carboxylic acid ethyl ester (5). To a solution of diisopropylamine (7.5 g, 74 mmol) in dry THF (40 mL), 1.6 M *n*-butyllithium hexane solution (30 mL, 47 mmol) was added dropwise at -78ºC and the mixture was stirred for 10 minutes. Then, a solution of 2-methylpyridine (5.5 g, 93 mmol) in dry THF (40 mL) was added at -78ºC and stirring was continued for 30 minutes. Finally, a solution of ethoxymethylenemalonic acid diethyl ester (15 g, 68 mmol) in dry THF (40 mL) was added dropwise to the reaction mixture at -78ºC. After stirring for 4 hours at -78ºC, the temperature of the solution was allowed to rise to 0ºC and stirring was continued for further 3 hours. Water was added to the reaction mixture and it was extracted with dichloromethane three times. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give an intermediate as a red oil. The intermediate was dissolved in *p*-xylene (40 mL), and the solution was stirred at reflux for 12 hours. The reaction mixture was extracted with dichloromethane three times and the combined organic phase was washed with water and saturated aqueous $NaHCO_3$. The organic solution was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, dichloromethane : methanol = 20 : 1) to give compound **5** (10 g, 80%) as a yellow solid. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.43 (3H, t, *J* 7.1, CH_2CH_3), 4.43 (2H, q, *J* 7.1, CH_2CH_3), 6.66 (1H, d, *J* 8.6, ArH), 7.17-7.22 (1H, m, ArH), 7.56-7.62 (2H, m, ArH), 8.41 (1H, d, *J* 8.3, ArH), 9.40 (1H, d, *J* 7.3, ArH); *m*/*z* (ESI-TOF) 240.15 (M + Na^+. $C_{12}H_{11}NNaO_3$ requires 240.06).

1-Bromo-4-oxo-4*H*-quinolizine-3-carboxylic acid ethyl ester (6). To a solution of compound **5** (0.50 g, 2.3 mmol) in acetic acid (1.5 mL), a solution of bromine (0.37 g, 2.3 mmol) in acetic acid (1.5 mL) was added dropwise at room temperature and the solution was stirred for 20 minutes. To the reaction mixture was added dichloromethane and the organic phase was washed with water and saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was then purified by column chromatography (SiO_2, dichloromethane : methanol = 50 : 1) to give compound **6** (0.49 g, 72%) as a yellow solid. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.43 (3H, t, *J* 7.0, C CH_2CH_3), 4.43 (2H, q, *J* 7.1, CH_2CH_3), 7.30 (1H, t, *J* 6.6 Hz, ArH), 7.80 (1H, t, *J* 7.4 Hz, ArH), 8.10 (1H, d, *J* 8.8, ArH), 8.63 (1H, s, ArH), 9.49 (1H, d, *J* 7.3, ArH); *m*/*z* (ESI-TOF) 296.17 (M + H^+. $C_{12}H_{11}BrNO_3$ requires 295.99).

1-(3-Amino-phenyl)-4-oxo-4*H*-quinolizine-3-carboxylic acid ethyl ester (7). A mixture of a solution of compound **6** (0.30 g, 1.0 mmol) in toluene (60 mL), a solution of 3-aminophenylboronic acid (0.19 g, 1.3 mmol) in methanol (30 mL), and a solution of sodium carbonate (0.32 g, 3.0 mmol) in water was degassed in vacuo, and then tetrakis(triphenylphosphine) palladium(0) (59 mg, 51 μmol) was added at 70ºC and the resulting mixture was stirred for 2 hours. The reaction mixture was washed with saturated aqueous $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was then purified by column chromatography (SiO_2, hexane : ethyl acetate = 5 : 1) to give compound **7** (0.24 g, 77%) as a yellow solid. δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.37 (3H, t, *J* 7.1, CH_2CH_3), 4.35 (2H, q, *J* 7.2, CH_2CH_3), 6.69 (1H, d, *J* 7.8, ArH), 6.76 (1H, s, ArH), 6.79 (1H, d, *J* 8.3, ArH), 7.22 (1H, t, *J* 7.5, ArH), 7.41 (1H, t, *J* 6.9, ArH), 7.72-7.78 (1H, m, ArH), 7.87 (1H, d, *J* 8.5, ArH), 8.29 (1H, s, ArH), 9.44 (1H, d, *J* 7.1, ArH); *m*/*z* (ESI-TOF) 331.42 (M + Na^+. $C_{18}H_{16}N_2NaO_3$ requires 331.11).

1-(3-Amino-phenyl)-4-oxo-4H-quinolizine-3-carboxylic acid (8). To a solution of compound **7** (0.20 mg, 0.65 mmol) in methanol (10 mL), a 3 N aqueous NaOH solution (2 mL) was added at room temperature and the mixture was stirred for 6 hours. The reaction mixture was evaporated in vacuo. The residue was purified by thin layer chromatography (reverse-phase, methanol : water = 2 : 1) to give compound **8** (0.10 g, 58%) as a yellow solid. δ_H (300 MHz; CD_3OD; Me_4Si) 6.69 (1H, d, *J* 7.8, ArH), 6.76 (1H, s, ArH), 6.75-6.79 (1H, m, ArH), 7.21 (1H, t, *J* 7.5, ArH), 7.31 (1H, t, *J* 7.6, ArH), 7.62 (1H, t, *J* 6.8 Hz, ArH), 7.85 (1H, d, *J* 8.8, ArH), 8.39 (1H, s, ArH), 9.37 (1H, d, *J* 7.1, ArH); *m*/*z* (ESI-TOF) 303.04 (M + Na^+. $C_{16}H_{12}N_2NaO_3$ requires 303.08).

1-(3-(2-((2-((2-(Bis-carboxymethyl-amino)-ethyl)-carboxymethyl-amino)-ethyl)-carboxymethyl-amino)-acetylamino)-phenyl)-4-oxo-4*H*-quinolizine-3-carboxylic acid (9). A solution of compound **8** (0.10 g, 0.36 mmol) and diethylenetriaminepentaacetic dianhydride (0.19 g, 0.54 mmol) in *N*,*N*-dimethylformamide (20 mL) was stirred

at 60ºC for 3 hours. The solvent was evaporated in vacuo and the residue was purified by thin layer chromatography (reverse-phase, methanol : water = 1 :1) to give compound **9** (0.21 g, 91%) as a yellow solid. δ_H (300 MHz; CD_3OD; Me_4Si) 3.10-3.30 (4H, br, $CH_2N(CH_2COOH)CH_2$), 3.40-3.65 (4H, br, $CH_2CH_2N(CH_2COOH)CH_2CH_2$), 3.65-3.90 (10H, br s, CH_2COOH), 7.05-7.15 (3H, br, ArH), 7.20 (1H, s, ArH), 7.30-7.40 (1H, br, ArH), 7.55-7.65 (2H, br, ArH), 7.84 (1H, s, ArH), 9.15-9.20 (1H, br, ArH); *m*/*z* (ESI-TOF) 337.15 ($M - 2\ H^+ + Na^+$. $C_{30}H_{30}N_5NaO_{12}$ requires 337.79).

KMR-Mg. To a solution of compound **9** (0.15 g, 0.23 mmol) in water (5 mL), gadolinium(III) chloride hexahydrate (85 mg, 0.22 mmol) was added and the solution was stirred at room temperature overnight. During the reaction, the pH of the solution was maintained at pH 6 by addition of dilute aqueous NaOH. The reaction mixture was then evaporated in vacuo and purified by reverse-phase column HPLC (methanol : water = 3 :2, v/v, flow rate: 3.5 mL/min, retention time: 250 min) to give KMR-Mg (0.16 g, 88%) as a yellow solid. *m*/*z* (ESI-TOF) 808.98 (M^-. $C_{30}H_{29}GdN_5O_{12}$ requires 809.11).

Longitudinal relaxation time measurement

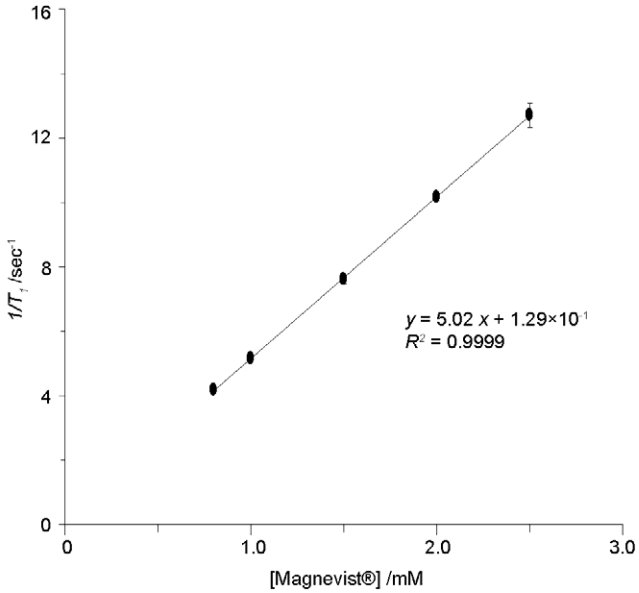

Fig. S1 The longitudinal relaxivity r_1 curve was obtained from solutions of various Magnevist® concentrations (0.8, 1.0, 1.5, 2.0, 2.5 mM) in distilled water at 40°C. These measurements were performed three times on each concentration.

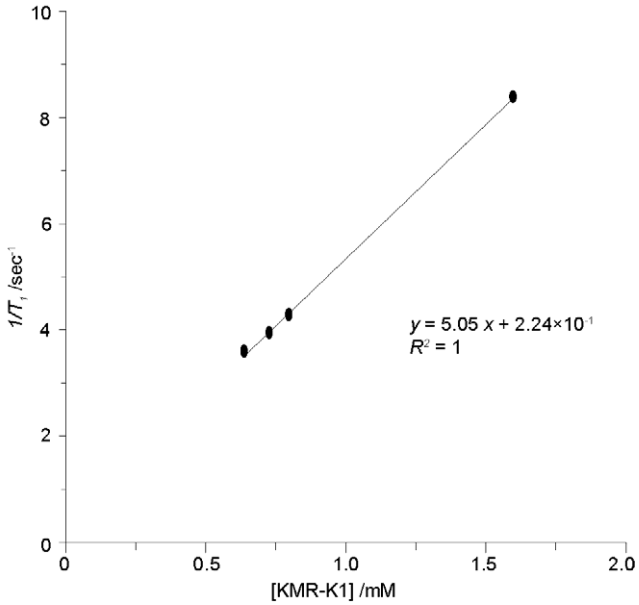

Fig. S2 The longitudinal relaxivity r_1 curve was obtained from solutions of various KMR-K1 concentrations (0.64, 0.73, 0.80, 1.6 mM) in distilled water at 40°C. These measurements were performed three times on each concentration.

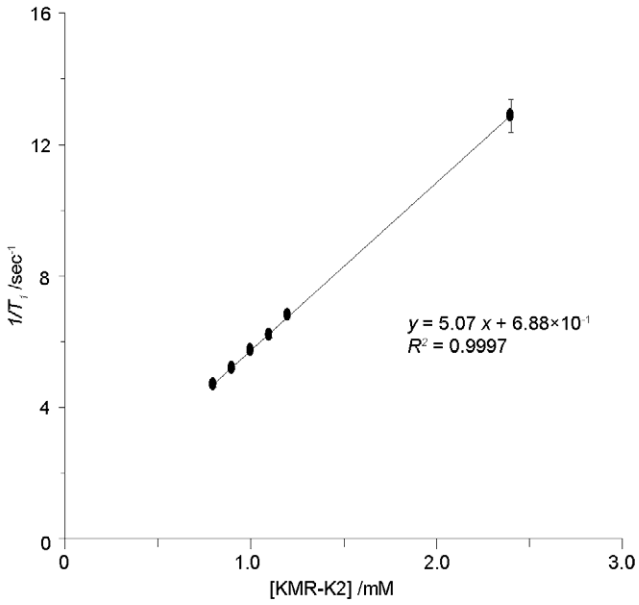

Fig. S3 The longitudinal relaxivity r_1 curve was obtained from solutions of various KMR-K2 concentrations (0.75, 0.90, 1.0, 1.1, 1.2, 2.4 mM) in distilled water at 40°C. These measurements were performed three times on each concentration.

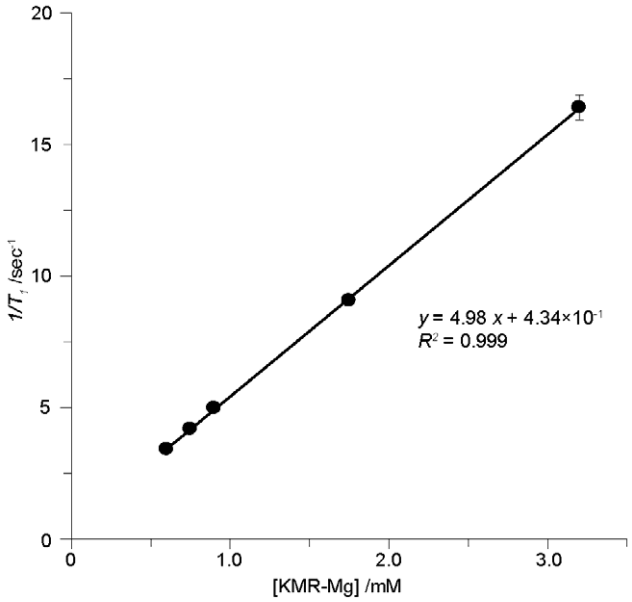

Fig. S4 The longitudinal relaxivity r_1 curve was obtained from solutions of various KMR-Mg concentrations (0.60, 0.75, 0.90, 1.75, 3.20 mM) in distilled water at 40°C. These measurements were performed three times on each concentration.

Absorption measurement

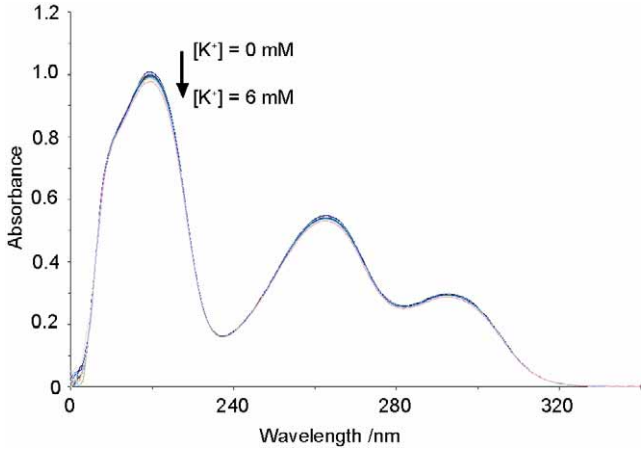

Fig. S5 Absorption spectra of KMR-K1 (60.0 μM) with various concentrations of K^+, from 0 to 6.0 mM, were measured in 0.05 M pH7.4 Tris / HCl buffer at room temperature.

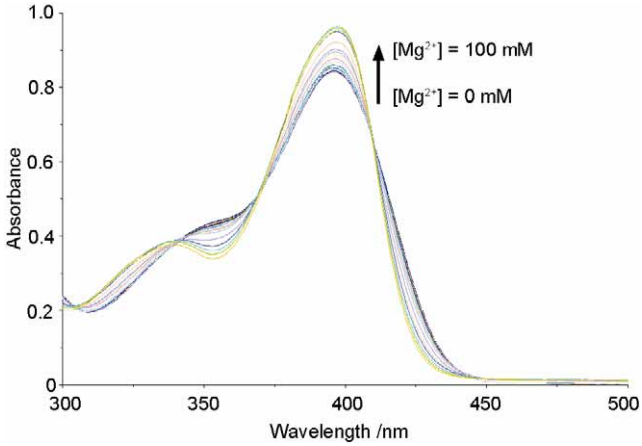

Fig. S6 Absorption spectra of KMR-Mg (100.0 μM) with various concentrations of Mg^{2+}, from 0 to 100.0 mM, were measured in 0.05 M pH7.4 Tris / HCl buffer at room temperature.

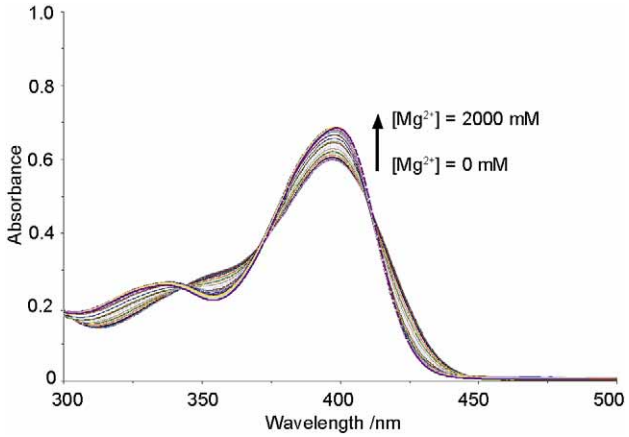

Fig. S7 Absorption spectra of Compound 8 (120.0 μM) with various concentrations of Mg^{2+}, from 0 to 2000 mM, were measured in 0.05 M pH7.4 Tris / HCl buffer at room temperature.

Competitive test

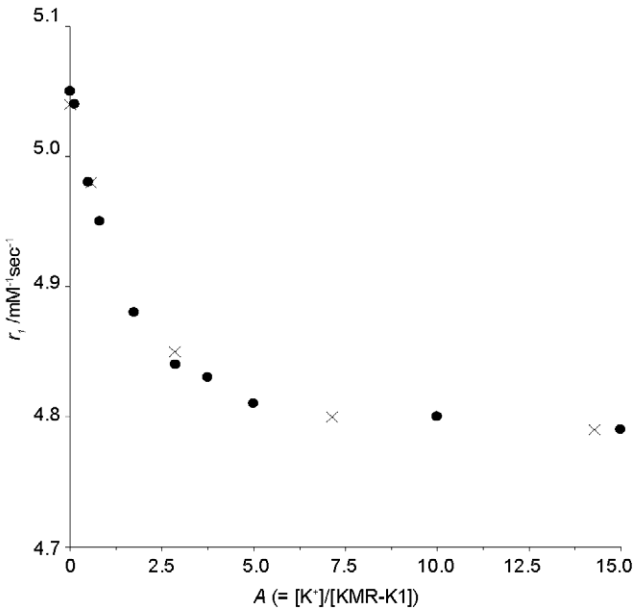

Fig. S8 The longitudinal relaxivity r_1 of KMR-K1 in the presence of various K^+ concentrations only (filled circle), same data as in Figure 2, at various molar ratios A (= [K^+] / [KMR-K1]) in 0.05 M pH7.4 Tris / HCl buffer, and in the presence of K^+ (cross; A=0, 0.6, 2.9, 7.1, 14.3) in 0.05 M pH7.4 Tris / HCl buffer with a constant physiological ion background of 150.0 mM Na^+, 5 mM Ca^{2+} and 5 mM Mg^{2+}. Each relaxivity value r_1 was determined from the slope of the plot of $1/T_1$ versus [KMR-K1].

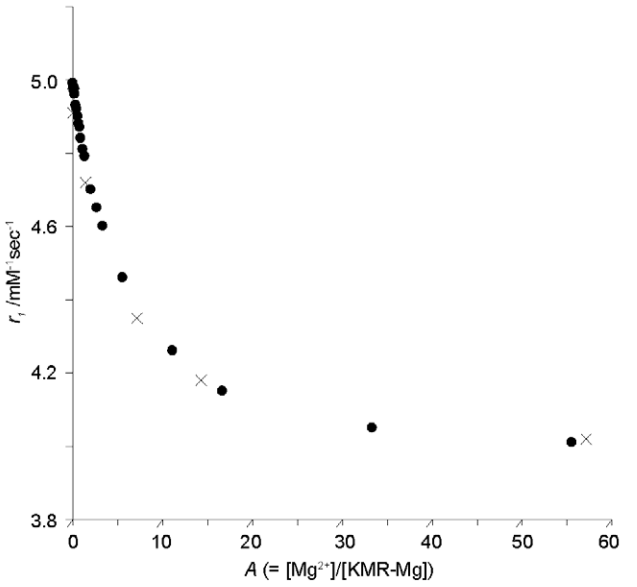

Fig. S9 Longitudinal relaxivity r_1 of KMR-Mg in the presence of various Mg^{2+} concentrations only (filled circle), same data as in Figure 3, at various molar ratios A (= [Mg^{2+}] / [KMR-Mg]) in 0.05 M pH7.4 Tris / HCl buffer, and in the presence of Mg^{2+} (cross; A=0, 1.4, 7.1, 14.3, 57.1) in 0.05 M pH7.4 Tris / HCl buffer with a constant physiological ion background of 150.0 mM Na^+, 5 mM K^+ and 5 mM Ca^{2+}. Each relaxivity value r_1 was determined from the slope of the plot of $1/T_1$ versus [KMR-Mg].

Determination of association constants

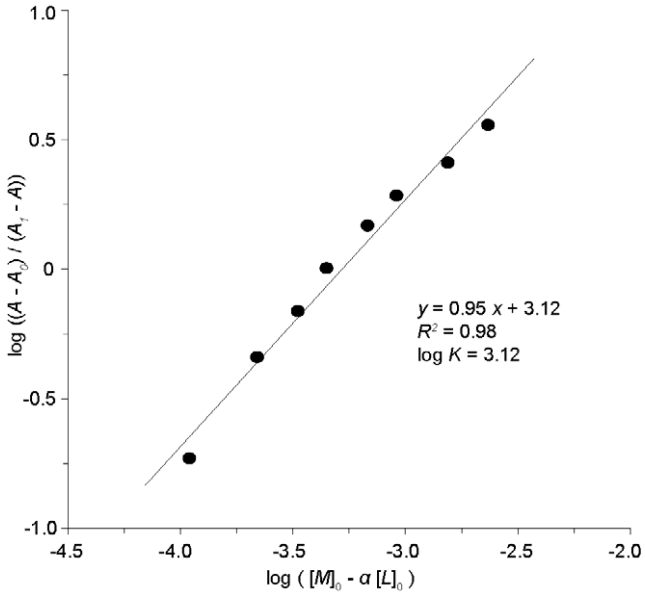

Fig. S10 Hill plot analysis of KMR-K1 with K^+ using absorbance data.

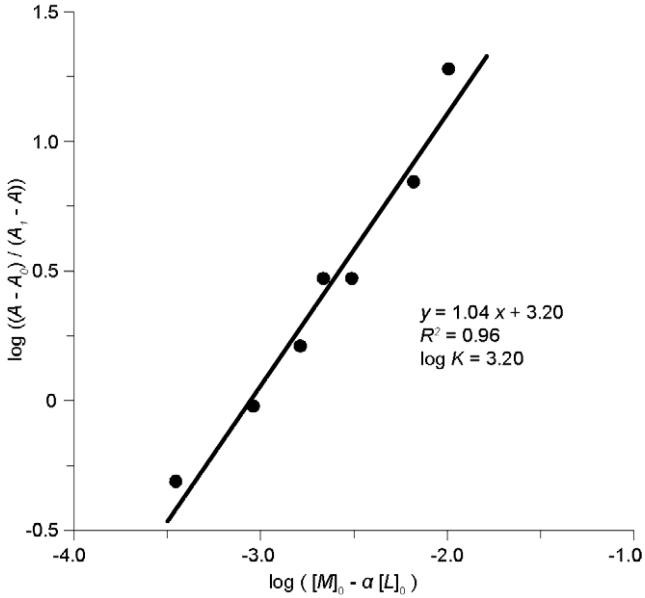

Fig. S11 Hill plot analysis of KMR-K1 with K^+ using longitudinal relaxation time measurement data.

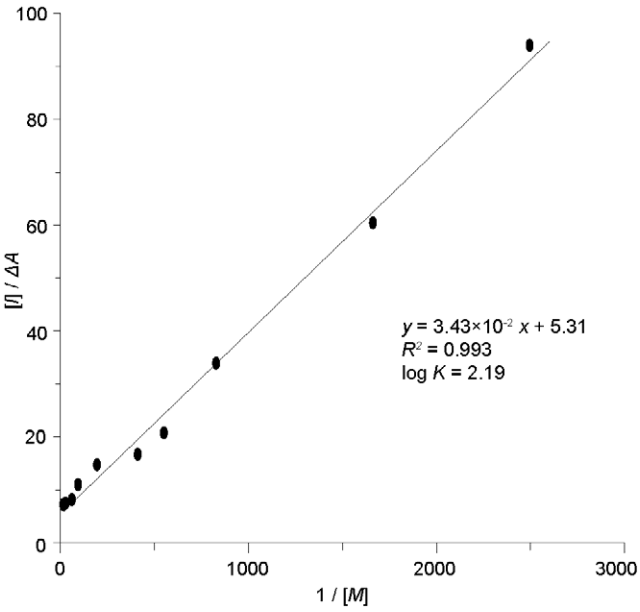

Fig. S12 Benesi-Hildebrand plot analysis of KMR-Mg with Mg^{2+} using absorbance data.

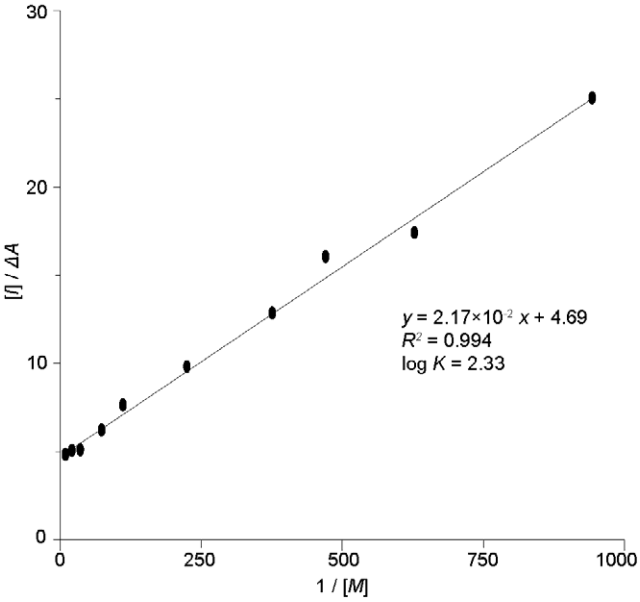

Fig. S13 Benesi-Hildebrand plot analysis of KMR-Mg with Mg^{2+} using longitudinal relaxation time measurement data.

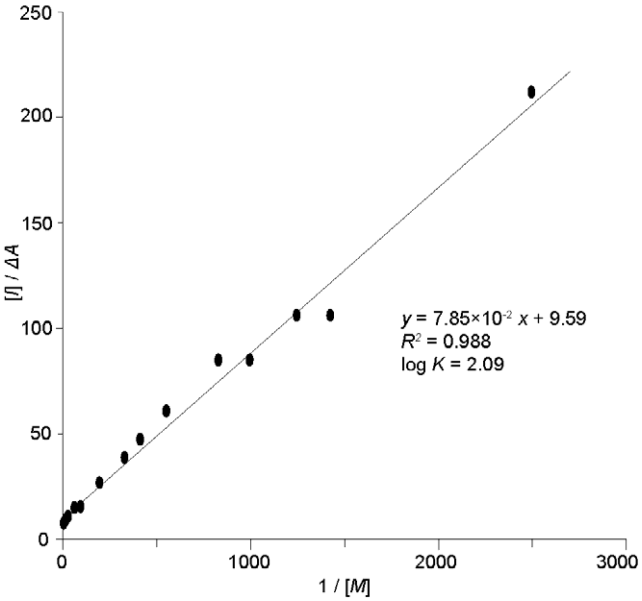

Fig. S14 Benesi-Hildebrand plot analysis of KMR-Mg with Ca^{2+} using absorbance data.

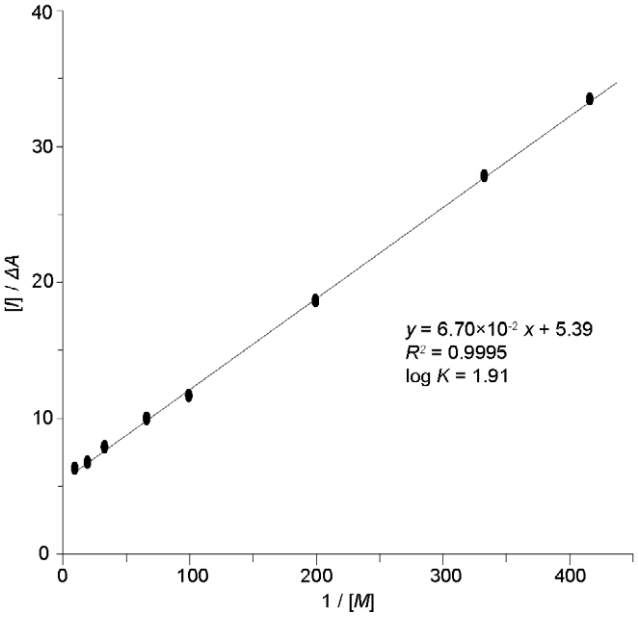

Fig. S15 Benesi-Hildebrand plot analysis of KMR-Mg with Ca^{2+} using longitudinal relaxation time measurement data.

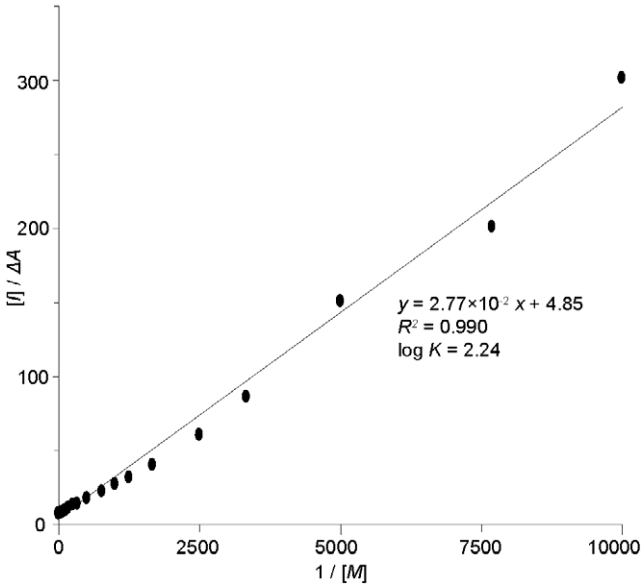

Fig. S16 Benesi-Hildebrand plot analysis of compound 8 with Mg^{2+} using absorbance data.