Supplementary Information

for

Exploring the intramolecular catalysis of the proton exchange process to modulate the relaxivity of Gd(III) - complexes of HP-DO3A-like ligands

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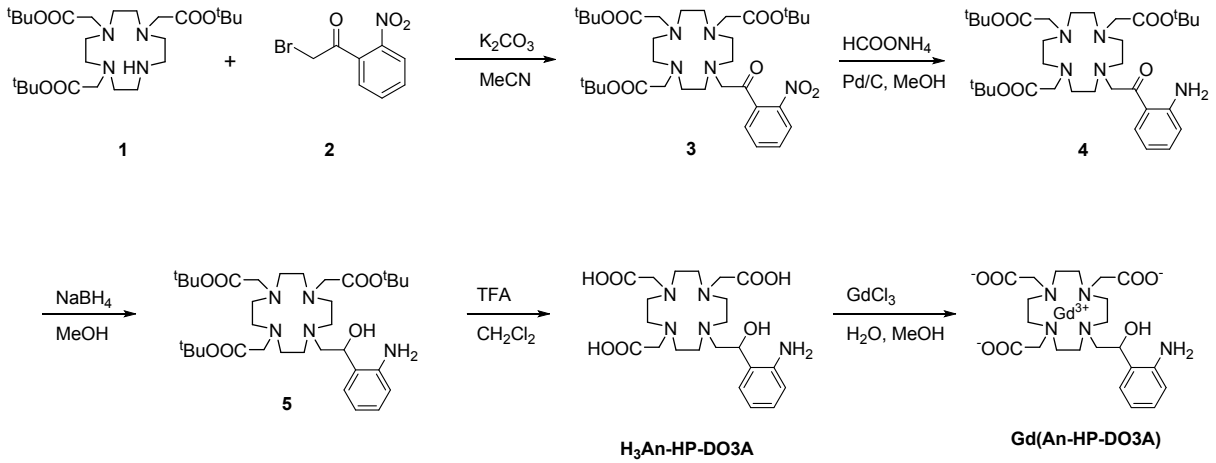
Content:

I. Synthesis of HP-DO3A derivative ligands and those of Gd(III)-complexes

All chemical reagents were obtained from commercial suppliers and used without further purification. 2-Bromo-1-(2-nitrophenyl)ethanone **2** was purchased from TCI. 1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid tris-*t*-butyl ester **1** [1] and 2-(2-bromoacetyl)benzoic acid methyl ester **6** [2] were prepared following the procedures reported in literature. Analytical grade solvents were used without further purification. When needed, solvents were distilled prior to use and dried on 4 Å molecular sieves. Thin layer chromatography (TLC) on silica gel was carried out on 5 x 20 cm plates with 0.25 mm layer thickness. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh ASTM) and employing an automated Biotage Isolera Prime equipment. Gadolinium complexes were purified and desalted with an Akta Purifier Pharmacia using an Amberchrom™ CG161M (Dow) resin column (1.6 x 50 cm) and eluting with a MeCN/H_2O gradient, flow 10 mL/min. HPLC analyses were performed on a HPLC-UV system (MERK-HITACHI), equipped with an auto sampler of 60 µL injection volume (MERK-HITACHI AS-2000A), a binary HPLC pump (MERK-HITACHI L-6200 IP) and a diode array detector (MERK-HITACHI L-4250). The analytical column was Zorbax SB Phenyl Agilent® (4.6x250 mm, 5.0 µm particle size) (Waters). Compounds were dissolved in methanol (1 mg/mL solution) and injected through a 60 µL loop. The mobile phase consisted of methanol/water with 0.1 % trifluoroacetic acid (gradient 0 min, 5% MeOH+0.1% TFA; 30 min, 80% MeOH+0.1% TFA; 35 min, 100 % MeOH+0.1% TFA). HPLC analyses were run at flow rates of 1.0 mL/min, and the column effluent was monitored at 210 nm. HPLC-MS analyses of gadolinium complexes were performed on a Thermo ACCELA HPLC made up of ACCELA Pump, ACCELA Autosampler and ACCELA PDA detector, hyphenated with TSQ QUANTUM ACCESS mass spectrometer with an ESI interface. Data were processed using Xalibur software for qualitative and qualitative analysis. The analytical column was Zorbax SB Phenyl Agilent® (3.0 x 250 mm, 5.0 µm particle size). Elution conditions: phase A: 13 mM $AcONH_4$ in water; phase B methanol; gradient: 0 min, 5% B; 5 min, 5% B; 30 min, 95% B; 35 min, 95% B; T=40°C; flow rate: 0.35 mL/min; sample concentration: 1mg/mL; injection volume: 10 µL; UV detection: 210-400nm. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 instrument or a JEOL ECZR600. Chemical shifts (δ) are given in parts per million (ppm). Electron spray ionization (ESI) mass spectra were obtained on Micromass Quattro API micro (Waters Corporation, Milford, MA, USA) mass spectrometer or Waters Micromass ZQ equipped with an ESCi source for electrospray ionization mass spectra. Electron spray

ionization (ESI) exact mass spectra for gadolinium complexes were obtained on HUPLC Acquity (Waters) system interfaced with Vion IMS Qtof.

I.1 Synthesis of Gd(An-HP-DO3A)



Scheme S1. Synthesis of complex **Gd(An-HP-DO3A)**.

10-[2-(2-Nitro)phenyl-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid tris-t-butyl ester (**3**): A mixture of 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid tris-t-butyl ester **1**[1] (20 g, 39 mmol), 2-bromo-1-(2-nitrophenyl)ethanone **2** (21 g, 86 mmol) and K_2CO_3 (9.7 g, 70 mmol) in acetonitrile (250 mL) was stirred at room temperature for 5 h. The mixture was filtered and evaporated to a residue which was dissolved in CH_2Cl_2 (200 mL), washed with brine (3x200 mL), dried (Na_2SO_4) and evaporated to residue. The crude was purified by flash chromatography on silica gel (eluent: gradient of EtOAc/MeOH = 99:1→8:2) to give compound **3** as an orange solid (17.5 g, 25.8 mmol, 66%). TLC (CH_2Cl_2/MeOH 9:1): R_f = 0.66. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 8.08 (d, J = 8.2 Hz, 1H, Ar), 7.74 (m, 1H, Ar), 7.64-7.69 (m, 2H, Ar), 2.16-3.62 (bm, 24H, CH_2), 1.43 (s, 27H, tBu). ^{13}C-NMR (600 MHz, $CDCl_3$): δ = 202.58, 173.26, 173.04, 146.25, 135.48, 134.43, 131.67, 128.15, 124.74, 82.21, 63.40, 55.88, 55.71, 52.74, 48.51, 28.08, 27.96. MS (ESI) m/z calculated for $C_{34}H_{55}N_5O_9$ 677.83, found 678 $[M + H]^+$, 700 $[M + Na]^+$. HPLC retention time = 30.7 min.

10-[2-(2-Amino)phenyl-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid tris-t-butyl ester (**4**): Compound **3** (17.4 g, 25.7 mmol) was dissolved in dry MeOH (150 mL) then Pd/C (4.78 g, 4.5 mmol) and ammonium formate (26.9 g, 427 mmol) were added and the mixture was stirred for 2 h. The mixture was filtered and evaporated to a residue which was

dissolved in CH_2Cl_2 (400 mL), washed with brine (4x150 mL), dried (Na_2SO_4) and evaporated to residue. The crude was purified by flash chromatography on silica gel (eluent: gradient of EtOAc/MeOH = 99:1→8:2) to give compound **4** as a beige solid (11.7 g, 18 mmol, 70%). TLC (CH_2Cl_2/MeOH 9:1): R_f = 0.57. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.52-7.54 (m, 1H, Ar), 7.18-7.22 (m, 1H, Ar), 6.76 (d, J = 8.4 Hz, 1H), 6.51-6.54 (m, 1H, Ar), 6.36 (bs, 2H, NH_2), 2.15-3.99 (bm, 24H, CH_2), 1.40 (s, 18H, tBu), 1.15 (bs, 9H, tBu). ^{13}C-NMR (600 MHz, $CDCl_3$): δ = 200.62, 172.74, 150.82, 134.96, 129.42, 117.83, 116.37, 115.75, 82.24, 82.06, 60.17, 56.02, 55.54, 52.84, 48.60, 27.96, 27.82. MS (ESI) m/z calculated for $C_{34}H_{57}N_5O_7$ 647.85, found 648 $[M + H]^+$, 670 $[M + Na]^+$. HPLC retention time = 29.2 min.

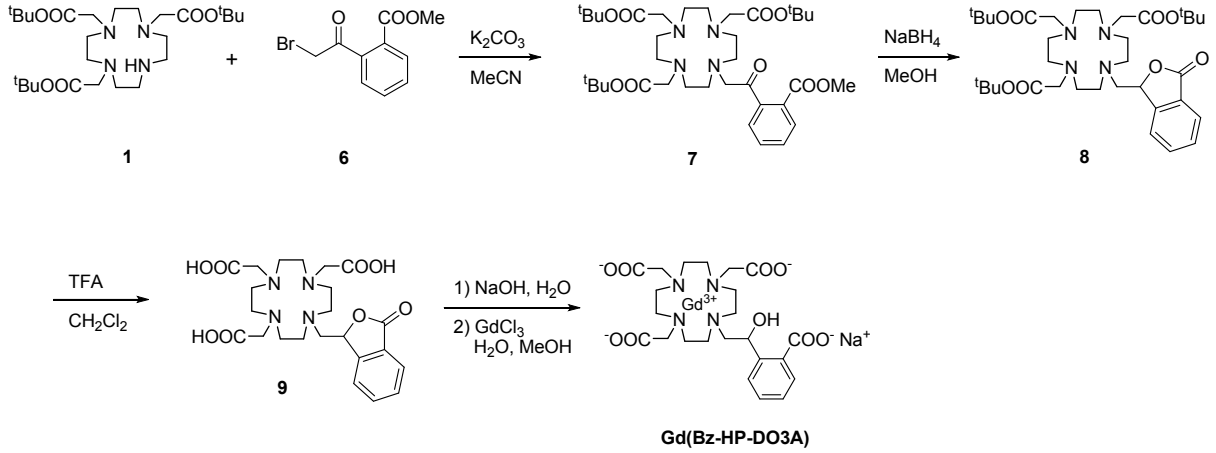
10-[2-(2-Amino)phenyl-2-hydroxyethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid tris-t-butyl ester (**5**): Compound **4** (11.2 g, 17.3 mmol) was dissolved in dry MeOH (60 mL), the solution cooled to 0°C and $NaBH_4$ (14.6 g; 387 mmol) was added. The mixture was stirred at room temperature for 2 h then evaporated. The residue was dissolved in CH_2Cl_2 (300 mL), washed with a saturated solution of NH_4Cl (4x200 mL) then brine (2x200 mL). The organic phase was evaporated to give a residue that was purified by flash chromatography on silica gel (eluent: gradient of EtOAc/MeOH = 99:1→8:2) to give compound **5** as a white solid (7.5 g, 11.5 mmol, 67%). TLC (CH_2Cl_2/MeOH 9:1): R_f = 0.46. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.44 (d, J = 7.5 Hz, 1H, Ar), 6.99 (td, J_1 = 7.8, J_2 = 1.4 Hz, 1H, Ar), 6.64 (td, J_1 = 7.6, J_2 = 0.8 Hz, 1H, Ar), 6.59 (dd, J_1 = 7.9 Hz, J_2 = 0.9 Hz, 1H, Ar), 4.9 (m, 1H, CH), 4.56 (br signal, 2H, NH_2), 2.0-3.9 (bm, 25H, CH_2+OH), 1.52 (s, 9H, tBu), 1.43 (s, 18H, tBu). ^{13}C-NMR (600 MHz, $CDCl_3$): δ = 173.78, 172.32, 171.86, 145.51, 128.35, 127.53, 118.14, 116.82, 82.18, 82.10, 82.07, 65.95, 57.50, 57.03, 56.45, 55.57, 52.78, 52.72, 52.43, 50.58, 48.75, 28.37, 28.04. MS (ESI) m/z calculated for $C_{34}H_{59}N_5O_7$ 649.87, found 650 $[M + H]^+$, 672 $[M + Na]^+$. HPLC retention time = 28.1 min.

10-[2-(2-Amino)phenyl-2-hydroxyethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (**H_3An-HP-DO3A**): Compound **5** (7.5 g, 11.5 mmol) was dissolved in CH_2Cl_2 (70 mL), the solution cooled to 0°C and trifluoroacetic acid (9 mL) was added. The solution was stirred at room temperature for 5 h then evaporated. The residue was dissolved in neat trifluoroacetic acid (45 mL) and the solution stirred for 16 h. The solution was evaporated and the residue

treated with Et_2O (2x200 mL) to give a brown solid that was filtered. The crude product was purified by chromatography on Amberlite XAD 1600 column (eluent: gradient water/MeCN=0% →10%) to give **H_3An-HP-DO3A** as a white solid (3.86 g, 8 mmol, 70%). TLC ($CHCl_3$/MeOH/30%NH_4OH 10:9:2): R_f = 0.28. ^{1}H-NMR (300 MHz, D_2O+KOD): δ = 7.20-7.25 (m, 1H, Ar), 7.09-7.14 (m, 1H, Ar), 6.78-6.85 (m, 2H, Ar), 5.05 (m, 1H, CH), 2.11-3.67 (bm, 24H, CH_2). ^{13}C-NMR (300 MHz, D_2O+KOD): δ = 180.32, 180.27, 179.77, 144.10, 129.05, 128.44, 127.73, 119.86, 118.08, 69.24, 59.90, 59.46, 58.82, 55.63, 53.18, 51.16. MS (ESI) m/z calculated for $C_{22}H_{35}N_5O_7$ 481.54, found 482 $[M + H]^+$, 520 $[M + K]^+$. HPLC retention time = 11.5 min.

[10-[2-(2-Amino)phenyl-2-hydroxyethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(3-)] gadolinium (**Gd(An-HP-DO3A)**). **H_3An-HP-DO3A** (3.24 g, 6.7 mmol) was dissolved in water (25 mL) and the pH was brought to 7 by addition of 1M NaOH (8 mL). Gadolinium chloride hexahydrate (2.5 g, 6.7 mmol) was added and the solution was stirred at room temperature for 16 h. The complex was purified by elution with a MeCN/H_2O gradient on an Amberchrom^{TM} CG161M column (1.6 x 50 cm). The fractions containing the product were pooled, concentrated by evaporation and lyophilized to give complex **Gd(An-HP-DO3A)** as a white solid (3.41 g, 5.4 mmol, 80%). The absence of free Gd^{3+} ion was determined with the xylenol orange test.[3] MS (ESI) m/z calculated for $[M - H]^-$ $C_{22}H_{31}GdN_5O_7$ 635.1465; found 635.1465. Anal. Calcd. for $C_{22}H_{32}GdN_5O_7$: C 41.56, H 5.07, N 11.02, Gd 24.73; Found: C 41.61, H 5.39, N 10.85, Gd 24.70%. HPLC retention time = 17.57 min (at 234-239 nm).

I.2 Synthesis of Gd(Bz-HP-DO3A)



Scheme S2. Synthesis of complex **Gd(Bz-HP-DO3A)**.

10-[2-(2-Methoxycarbonyl)phenyl-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid tris-t-butyl ester (**7**): A mixture of 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid tris-t-butyl ester **1** [1] (12.8 g; 24.8 mmol), 2-(2-bromoacetyl)benzoic acid methyl ester **6** [2] (8.3 g; 32.3 mmol) and K_2CO_3 (5.2 g; 37.6 mmol) in acetonitrile (50 mL) was stirred at room temperature for 40 h. The mixture was filtered and evaporated to a residue which was dissolved in CH_2Cl_2 (150 mL), washed with brine (3x100 mL), dried (Na_2SO_4) and evaporated to residue. The crude was purified by flash chromatography on silica gel (eluent: gradient of EtOAc/MeOH = 98:2→50:50) to give compound **7** as an orange solid (5.1 g, 7.4 mmol, 30%). TLC (CH_2Cl_2/MeOH 9:1): R_f = 0.66. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.91-7.93 (m, 1H, Ar), 7.50-7.55 (m, 2H, Ar), 7.43-7.45 (m, 1H, Ar), 3.89 (s, 3H, CH_3), 2.17-3.43 (bm, 24H, CH_2), 1.43 (s, 27H, tBu). ^{13}C-NMR (600 MHz, $CDCl_3$): δ = 205.76, 173.05, 166.87, 141.91, 132.44, 130.40, 130.27, 126.77, 82.12, 82.10, 63.32, 55.88, 55.77, 52.92, 50.73, 48.54, 28.28, 28.11, 27.98. MS (ESI) m/z calculated for $C_{36}H_{58}N_4O_9$ 690.87, found 691 $[M + H]^+$, 713 $[M + Na]^+$. HPLC retention time = 31.8 min.

10-[(3-Oxo-1H-2-benzofuran-1-yl)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid tris-t-butyl ester (**8**): Compound **7** (4.4 g, 6.3 mmol) was dissolved in dry MeOH (20 mL), the solution cooled to 0°C and $NaBH_4$ (4.82 g; 127 mmol) was added. The mixture was stirred at 0°C for 1 h then evaporated. The residue was dissolved in CH_2Cl_2 (100 mL), washed with a saturated solution of NH_4Cl (4x100 mL) then brine (2x100 mL). The organic phase was dried (Na_2SO_4) and evaporated to give compound **8** as a yellow solid (4.0 g, 6.0 mmol, 95%). TLC (CH_2Cl_2/MeOH 9:1): R_f = 0.43. ^{1}H-NMR (600 MHz, $CDCl_3$): δ = 7.83 (d, J = 7.6 Hz, 1H, Ar), 7.61-7.67 (m, 2H, Ar), 7.48 (t, J = 7.4 Hz, 1H, Ar), 5.97 (bm, 1H, CH), 2.01-3.59 (bm, 24H), 1.43 (s, 18H, tBu), 1.22 (s, 9H, tBu). ^{13}C-NMR (600 MHz, $CDCl_3$): δ = 172.71, 170.66, 169.79, 148.39, 134.44, 129.35, 125.66, 125.55, 122.60, 82.70, 82.46, 82.13, 58.33, 56.02, 55.56, 51.42, 48.60, 48.47, 47.68, 28.06, 28.04, 27.84. MS (ESI) m/z calculated for $C_{35}H_{56}N_4O_8$ 660.85, found 661 $[M + H]^+$, 683 $[M + Na]^+$. HPLC retention time = 29.5 min.

10-[(3-Oxo-1H-2-benzofuran-1-yl)methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (**9**): Compound **8** (4.6 g, 7 mmol) was dissolved in CH_2Cl_2 (20 mL), the solution cooled to 0°C and trifluoroacetic acid (5.4 mL) was added. The solution was stirred at room temperature for 4 h then evaporated. The residue was dissolved in neat trifluoroacetic acid (27

mL) and the solution stirred for 16 h. The solution was evaporated, the residue was dissolved again in neat trifluoroacetic acid (27 mL) and the solution stirred for more 16 h. The solution was evaporated and the residue treated with Et_2O (100 mL) to give an orange solid that was filtered. The crude product was purified by chromatography on Amberlite XAD 1600 column (eluent: gradient water/MeCN=0% →5%) to give compound **9** as a white solid (2 g, 4 mmol, 57%). TLC ($CHCl_3$/MeOH/30%NH_4OH 10:9:2): R_f = 0.46. ^{1}H-NMR (300 MHz, D_2O+KOD): δ = 7.83-7.89 (m, 3H, Ar), 7.62-7.66 (m, 1H, Ar), 5.95 (br signal, 1H, CH), 2.54-4.30 (bm, 24H, CH_2). ^{13}C-NMR (300 MHz, D_2O+KOD): δ = 175.22, 172.99, 170.15, 169.84, 147.88, 135.17, 129.76, 125.22, 124.46, 123.34, 83.95, 57.06, 56.83, 56.06, 53.61, 52.81, 52.27, 50.82, 49.62, 48.93, 48.58, 46.18. MS (ESI) m/z calculated for $C_{23}H_{32}N_4O_8$ 492.52, found 493 $[M + H]^+$, 515 $[M + Na]^+$, 531 $[M + K]^+$. HPLC retention time = 14.5 min.

[10-[2-(2-Carboxy)phenyl-2-hydroxyethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetato(4-)] gadolinium sodium salt (**Gd(Bz-HP-DO3A)**): A solution of compound **9** (1.5 g, 3 mmol) in water (25 mL) was stirred for 18 h while keeping pH=12 by addition of 1M NaOH. The solution was heated at 40°C for 1 h then cooled to room temperature. At this point the HPLC analysis confirmed the complete hydrolysis of the lactone ring of **9**. The pH of solution was brought to 7 by addition of 37% HCl then gadolinium chloride hexahydrate (1.11 g; 3 mmol) was added and the solution stirred at room temperature for 16 h. The complex was purified by elution with a MeCN/H_2O gradient on an Amberchrom™ CG161M column (1.6 x 50 cm). The fractions containing the product were pooled, concentrated by evaporation and lyophilized to give complex **Gd(Bz-HP-DO3A)** as a white solid (1.59 g, 2.3 mmol, 77%). The absence of free Gd^{3+} ion was determined with the xylenol orange test.[3] MS (ESI) m/z calculated for $[M - H]^-$ $C_{23}H_{30}GdN_4O_9$ 664.1254; found 664.1258. Anal. Calcd. for $C_{23}H_{30}GdN_4NaO_9$: C 40.23, H 4.40, N 8.16, Gd 22.90, Na 3.35; Found: C 40.26, H 4.32, N 8.01, Gd 23.28, Na 3.18. HPLC retention time = 10.72 min (at 225-230 nm).

II. Equilibrium measurements

The chemicals used for the experiments were of analytical grade. The concentration of the $LnCl_3$ stock solutions were determined by complexometric titration with standardized Na_2H_2EDTA and xylenol orange as indicator. The concentration of the H_3HP-DO3A, H_3Bz-HP-DO3A, H_3An-HP-DO3A and H_3Ph-HP-DO3A (prepared as described in Ref. 4) ligand solutions was determined by pH-potentiometric titration in the presence and absence of a large (40-fold) excess of $CaCl_2$. The pH-potentiometric titrations were made with

standardized 0.2 M NaOH. Gd(HP-DO3A), Gd(Bz-HP-DO3A), Gd(Ph-HP-DO3A) and Gd(Az-HP-DO3A) solutions were prepared by mixing equivalent amounts of $GdCl_3$ and H_3HP-DO3A, H_3Bz-HP-DO3A, H_3Ph-HP-DO3A or H_3An-HP-DO3A solutions.

The protonation constants of H_3HP-DO3A, H_3Bz-HP-DO3A, H_3Ph-HP-DO3A and H_3An-HP-DO3A ligands and those of the Gd(III)-complexes were determined by pH-potentiometric titration. The concentration of ligands and Gd(III)-complexes was typically 0.002 M. In calculating the protonation constants of the ligands and the Gd(III)-complexes, the best fitting of the NaOH - pH data pairs, were obtained by assuming the formation of L, HL, H_2L, H_3L, H_4L, H_5L and H_6L and GdLH, GdL and $GdLH_{-1}$ complexes in the 1.7-12.0 pH range. For the pH measurements and titrations, a *Metrohm 888 Titrando* titration workstation and *Metrohm-6.0234.110* combined electrode were used. All Equilibrium measurements were carried out at a constant ionic strength (0.15 M NaCl) in 6 mL samples at 25 °C. The solutions were stirred and to avoid the effect of ambient CO_2, N_2 was bubbled through them. The titrations were made in the pH range of 1.7-12.0. KHphthalate (pH=4.002) and borax (pH=9.177) buffers were used to calibrate the pH meter. For the calculation of $[H^+]$ from the measured pH values, the method proposed by Irving et al. was used.[5] A 0.01M HCl solution was titrated with the standardized NaOH solution in the presence of 0.15 M NaCl or ionic strength. The differences (A) between the measured (pH_{read}) and calculated pH ($-\log[H^+]$) values were used to obtain the $[H^+]$ at equilibrium (A=0.040). In general, the pH corresponds to $-\log[H^+]$ thorough this paper. For the equilibrium calculations, the stoichiometric water ionic product (pK_w) was also needed to calculate $[OH^-]$ values under basic conditions. The $V_{NaOH} - pH_{read}$ data pairs of the HCl – NaOH titration obtained in the pH range 10.5 – 12.0 were used to calculate the pK_w value (pK_w=13.85).

The protonation constants of the H_3Bz-HP-DO3A ligand and Gd(Bz-HP-DO3A), Gd(Ph-HP-DO3A) and Gd(Az-HP-DO3A) complexes were determined by UV spectrophotometry at the absorption band of the aromatic moiety in the wavelength range of 210-340 nm. The concentration of H_3Bz-HP-DO3A ligand, Gd(Bz-HP-DO3A), Gd(Ph-HP-DO3A) and Gd(Az-HP-DO3A) complex were 270 μM, 300 μM, 49 μM and 262 μM (0.15 M NaCl, 25°C). The pH was adjusted by stepwise addition of concentrated NaOH or HCl solutions. The spectrophotometric measurements were made with the use of *PerkinElmer Lambda 365* UV-Vis spectrophotometer at 25 °C, using 1.0 cm cells. The equilibrium constants were calculated with the program *PSEQUAD*.[6]

III. ^{1}H NMR relaxometry

The relaxivity values were calculated from the longitudinal relaxation time of water protons (T_1) measured with a Bruker Avance III 400 (9.4 T) NMR spectrometer equipped with BB inverse z gradient probe (5 mm). The temperature of the sample holder was kept constant (298 K) with a thermostated air stream. The longitudinal relaxation times were measured with the "inversion recovery" method (180° - τ - 90°) by using 8 different τ values. The measurements were carried out in a 1 mM non-deuterated aqueous solutions of the Gd(HP-DO3A), Gd(Bz-HP-DO3A), Gd(Ph-HP-DO3A) and Gd(Az-HP-DO3A) complexes, respectively. The relaxivity values were given as $r_1=1/T_{1p} + 1/T_{1w}$ where T_{1p} and T_{1w} are the relaxation times of the bulk water protons in the presence and absence of 1.0 mM Gd^{III}-complex. The pH-dependent relaxivity measurements of Gd(HP-DO3A), Gd(Bz-HP-DO3A), Gd(Ph-HP-DO3A) and Gd(Az-HP-DO3A) complexes were carried out by direct titration of the samples (2.5<pH<13.0) ([GdL]=1.0 mM, 0.15 M NaCl and 25°C). The pH was adjusted by stepwise addition of concentrated NaOH or HCl solution. The relaxivity values of Gd(Bz-HP-DO3A), Gd(An-HP-DO3A) and Gd(Ph-HP-DO3A) complexes were also measured at 0.47 T and 1.41 T (corresponding to a proton Larmor frequency of 20 and 60 MHz) by using a spin analyzer Minispec MQ-20 and MQ-60 (Bruker Biospin, Rheinstetten, Germany) at pH=7.4 and 37°C in saline solution (NaCl 0.9%, Eurospital, Trieste, Italy) and in human plasma (Control Plasma N; Munich, Germany). The temperature was kept constant by thermostatic bath connected to the sample holder of the spectrometer. T_1 values were measured with the "inversion recovery" method by using 15 inversion times from 10 ms to $5\times T_1$. Calculations were performed with the computer program *Micromath Scientist*, version 2.0 (Salt Lake City, UT, USA).

IV. Protonation constants of the H_3HP-DO3A, H_3Bz-HP-DO3A, H_3Ph-HP-DO3A and H_3An-HP-DO3A ligands and those of Gd(III)-complexes

The protonation constants ($\log K_i^H$) of the free ligands H_3HP-DO3A, H_3Bz-HP-DO3A, H_3Ph-HP-DO3A and H_3An-HP-DO3A and those of Gd(III)-complexes ($\log K^H_{GdLHi}$) were determined by pH-potentiometry and UV-spectrophotometry. The UV-spectra of the H_3Bz-HP-DO3A ligand and the Gd(III)-complexes formed with H_3Bz-HP-DO3A, H_3Ph-HP-DO3A and H_3An-HP-DO3A ligands are shown in Figures S1 – S4. The $\log K_i^H$ values of H_3HP-DO3A, H_3Bz-HP-DO3A, H_3Ph-HP-DO3A and H_3An-HP-DO3A ligands and $\log K^H_{GdLHi}$ values of Gd(III)-complexes formed with H_3HP-DO3A, H_3Bz-HP-DO3A, H_3Ph-HP-DO3A and H_3An-HP-DO3A ligands are defined by Eqs. (1) - (3).

$$H_{i-1}L + H^+ \rightleftharpoons H_iL \qquad (1)$$

$$K_i^H = \frac{[H_iL]}{[H_{i-1}L][H^+]} \qquad i=1, 2, 3, \ldots 5$$

$$GdL + H^+ \rightleftharpoons GdLH \qquad (2)$$

$$K^H_{GdLH} = \frac{[GdLH]}{[GdL][H^+]}$$

$$GdLH_{-1} + H^+ \rightleftharpoons GdL \qquad (3)$$

$$K^H_{GdLH_{-1}} = \frac{[GdL]}{[GdLH_{-1}][H^+]}$$

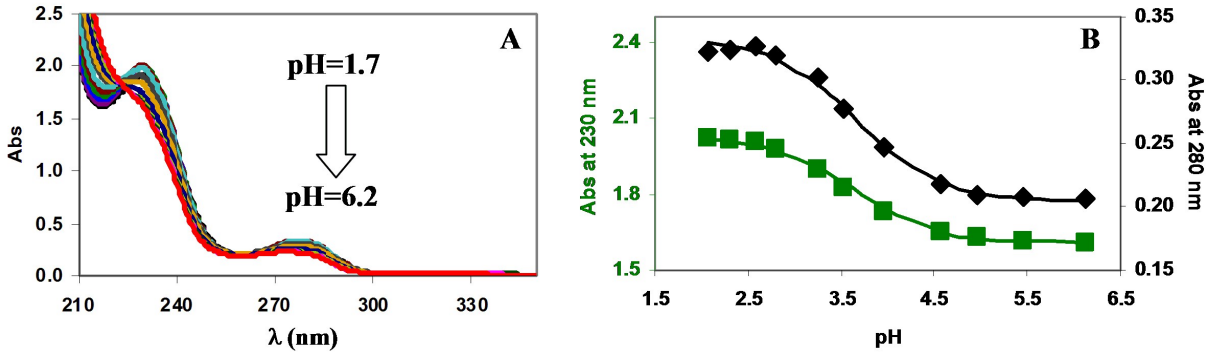


Figure S1. Absorption spectra (**A**) and absorbance values of Bz-HP-DO3A at **230** and **280** nm (**B**) in the pH range 1.7 – 6.2. ([Bz-HP-DO3A]=270 μM, 0.15 M NaCl, 25°C)

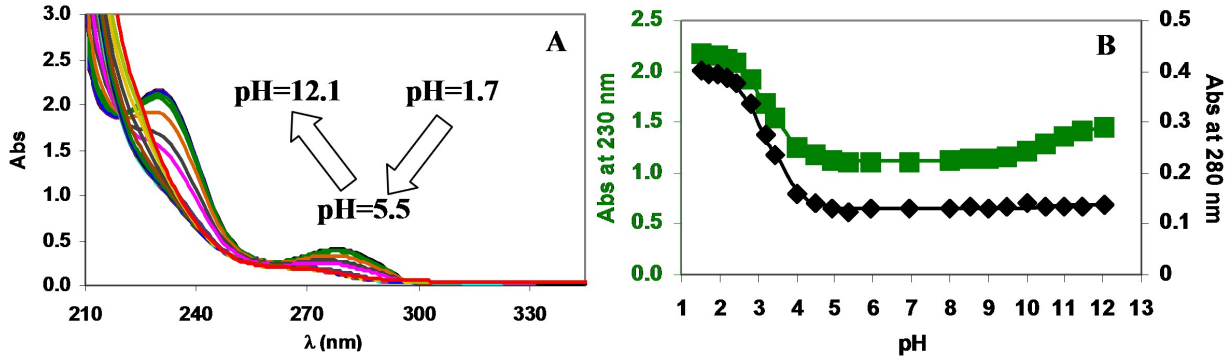


Figure S2. Absorption spectra (**A**) and absorbance values of Gd(Bz-HP-DO3A) at **230** and **280** nm (**B**) in the pH range 1.7 – 12.1. ([Gd(Bz-HP-DO3A)]=300 μM, 0.15 M NaCl, 25°C).

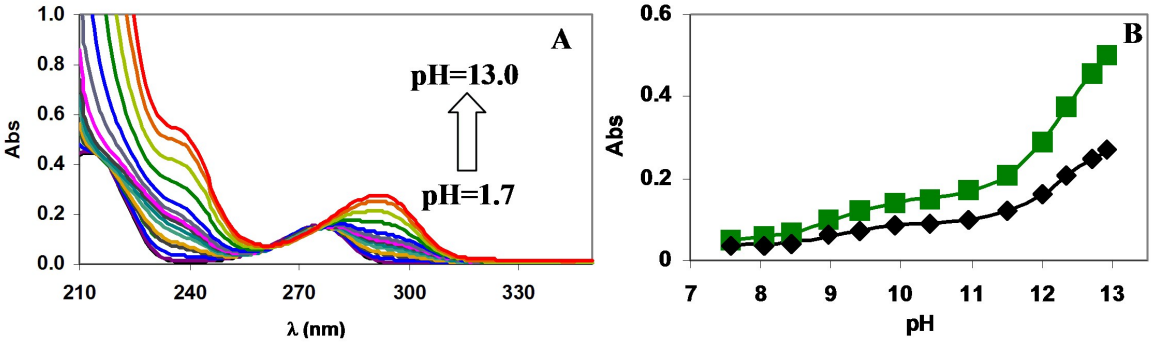


Figure S3. Absorption spectra (**A**) and absorbance values of Gd(Ph-HP-DO3A) at **240** and **294** nm (**B**) in the pH range 1.7 – 13.0. ([Gd(Bz-HP-DO3A)]=49 μM, 0.15 M NaCl, 25°C).

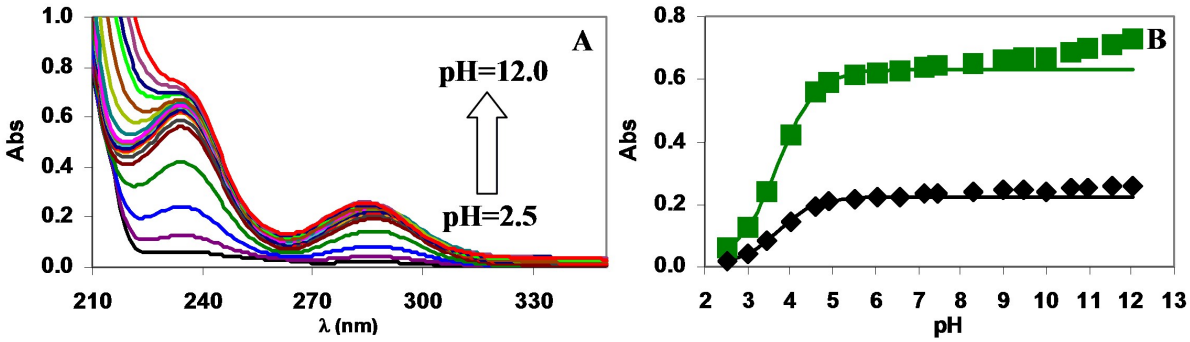


Figure S4. Absorption spectra (**A**) and absorbance values of Gd(An-HP-DO3A) at **235** and **285** nm (**B**) in the pH range 2.5 – 12.0. ([Gd(An-HP-DO3A)]=262 μM, 0.15 M NaCl, 25°C).

The absorbance values at each wavelength can be expressed by the sum of the absorption of each species:

$$A = \sum c_i l \varepsilon_i \qquad (4)$$

where i=0, 1, 2,…,n, c_i , l and ε_i are the concentration, the path length and the molar absorptivity of the involved species, respectively. By taking into account of the total

concentration ($[L]_t$=[L]+[HL]+[H_2L]+…+[H_nL]) and the protonation constants of the Bz-HP-DO3A ligand (α_H = 1+K_1[H^+]+K_1K_2[H^+]^2+…+$K_1K_2...K_n$[H^+]^n), the Eq. (4) can be expressed in the following form (the *l* can be neglected by using same cuvette):

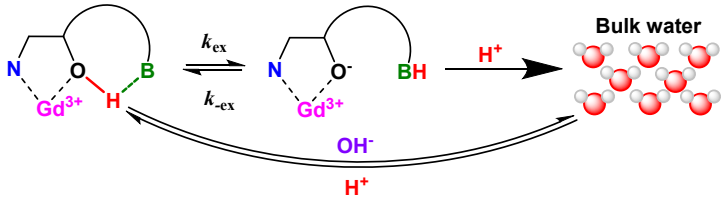
$$A = \left[\frac{\varepsilon_L}{\alpha_H} + \frac{\varepsilon_{HL} K_1 [H^+]}{\alpha_H} + \frac{\varepsilon_{H_2L} K_1 K_2 [H^+]^2}{\alpha_H} + \ldots + \frac{\varepsilon_{H_nL} K_1 K_2 \ldots K_n [H^+]^n}{\alpha_H} \right] [L]_t \quad (5)$$

The protonation constants and the molar absorptivity of each species of the Bz-HP-DO3A ligand were calculated by the fitting of the pH – absorbance data (Figure S1) to Eq. 5. The protonation constant of the Gd(Bz-HP-DO3A), Gd(Ph-HP-DO3A) and Gd(Az-HP-DO3A) complex were also calculated by the fitting the of the pH – absorbance data (Figure S2 – S4) to Eq. (6)

$$A = \left[\frac{\varepsilon_{GdLH_{-1}}}{\alpha_H} + \frac{\varepsilon_{GdL} K^H_{GdLH_{-1}} [H^+]}{\alpha_H} + \frac{\varepsilon_{GdLH} K^H_{GdLH_{-1}} K^H_{GdLH} [H^+]^2}{\alpha_H} \right] [GdL]_t \quad (6)$$

where ε_{GdLH-1}, ε_{GdL}, ε_{GdLH} and $[GdL]_t$ are the molar absorptivity of the $GdLH_{-1}$, GdL and GdLH species and the total concentration of the Gd(III)-complexes, whereas α_H=1+ K^H_{GdLH-1}[H^+]+ K^H_{GdLH-1} K^H_{GdLH}[H^+]^2.

V. Mechanism of the intramolecular proton exchange process of Gd(HP-DO3A) derivatives.



Scheme S1. Mechanism of the intramolecular proton exchange process of Gd(HP-DO3A) derivatives. B represents the basic group in the proximity of the -OH moiety.

VI. References

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