SUPPLEMENTARY INFORMATION

Gd-XO: a colourimetric probe for the complexation of Gd^{3+} with DO3A-type ligands

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General Methods

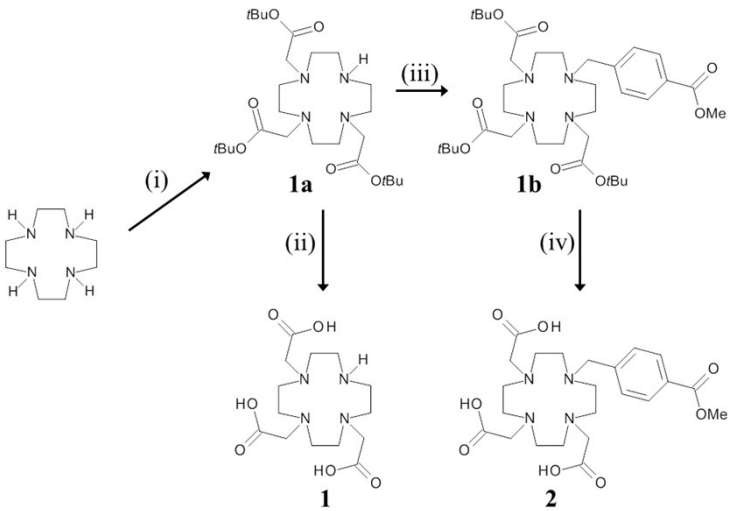
Gadolinium triflate (Sigma, USA), methyl 4-(bromomethyl)benzoate (Sigma, China), *tert*-butyl bromoacetate (Sigma, France), Xylenol Orange (Sigma, Japan), cyclen (Strem Chemicals, USA), reagent grade sodium bicarbonate (Scharlau, Spain), anhydrous sodium sulfate (RCI Labscan, Thailand) and sodium hydroxide (Macron Chemicals, Sweden) were purchased as dry solids. Trifluoroacetic acid (Merck, Germany) was procured as a liquid. Solvents used were diethyl ether (RCI Labscan, Thailand) dichloromethane (J.T. Baker, USA), HPLC grade acetonitrile (Duksan Pure Chemicals , Korea) HPLC grade methanol (Macron, USA). The NMR solvents used for analysis were deuterated chloroform (Merck, USA) and deuterated water (Merck, Switzerland).

Synthesis of PAC ligands was carried out with A.R. grade solvents. Reaction progress was monitored using Silica gel 60 TLC plates (Merck), and spots visualized by UV lamp illumination or permanganate staining.

Purification of crude products was carried out with either gravity column chromatography using Silica Gel 60 (Merck) or semi-preparative HPLC with a C18 reverse-phase column (Phenomenex 5µ 100A 250 x 10 mm). RP-HPLC profiles were obtained using an analytical C18 reverse-phase column (Phenomenex 5µ 100A 250 x 4.6 mm) with a linear elution gradient of mobile phase from 0 to 95% ACN-H_2O with 0.1% TFA in 30 minutes (1.0 mL/min flow rate) with UV-Vis detector set at λ_{max} of the products.

^1H NMR and ^{13}C NMR spectra were recorded on a 500 (^1H 500 MHz, ^{13}C 125 MHz) Agilent (Varian) NMR spectrometer with a 5 mm one-probe. The infrared spectra were generated through diffused reflectance method using a DRS accessory in IR Prestige-21 from Shimadzu. The molecular masses were analyzed through ESI-MS using positive ion mode on a Micromass Quattro II mass spectrometer (Micromass, UK) equipped with an electrospray ionization source (desolvation temperature = 150 ^oC, cone voltage = 40 V).

Synthesis



Scheme 1. Synthesis of ligands: (i) *t*Bu-bromoacetate, ACN, $NaHCO_3$, 0 °C; (ii) TFA, DCM, RT; (iii) 4-(bromomethyl)benzoate, ACN, $NaHCO_3$, Δ; (iv) TFA, DCM, RT.

Synthesis of ligand **1**.

Ligand **1** was synthesized as previously described[8] with slight modifications. In brief, cyclen (1.78 g, 10 mmol) and $NaHCO_3$ (2.78 g , 33 mmol) were dissolved/suspended in freshly distilled ACN (60 mL). *Tert*-butyl bromoacetate (6.45 g, 33 mmol) was added dropwise to the reaction under N_2 atmosphere over 30 min over an ice bath. After addition, the reaction was allowed to equilibrate to room temperature and stirred under N_2 for 48 hours. The reaction was then filtered, and the filtrate evaporated under reduced pressure producing a beige residue, which was recrystallized from hot toluene and washed with diethyl ether to produce a white powder (**1a**). A small amount (0.50 g) was dissolved in DCM (5 mL), to which trifluoroacetic acid (5 mL) was added. The reaction mixture was stirred overnight and then evaporated under reduced pressure. The residue was redissolved in DCM and evaporated under reduced pressure thrice, followed by redissolved in MeOH and evaporation thrice, to remove residual TFA. Finally, the residue was dissolved in minimal MeOH and the ligand precipitated out with diethyl ether as a white solid (0.31 g, 94%). ES^+ MS (H_2O) *m/z* 347 $\{M+H\}^+$; ^1H NMR (500 MHz, D_2O) δ 3.88 – 2.68 (m, 22H). ^{13}C NMR (126 MHz, D_2O) δ 174.63, 170.01 (C=O), 55.91, 53.13, 51.56, 48.89, 47.68, 42.23 (CH_2N, CH_2COO); C=O stretch 1726 cm^{-1}.

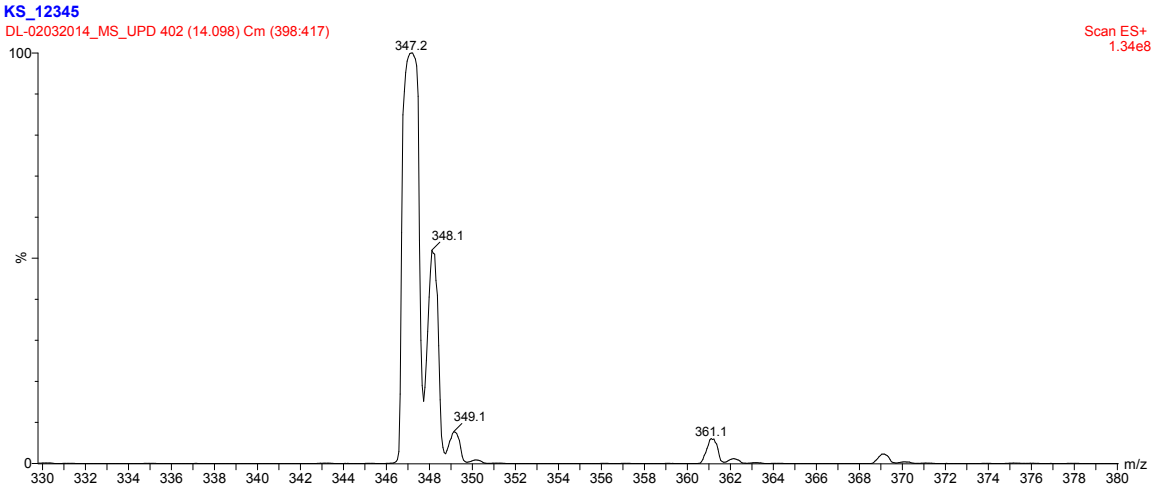


Figure S1. ES^+ mass spectrum of **1**: $\{M+H\}^+$ = 347.2 m/z

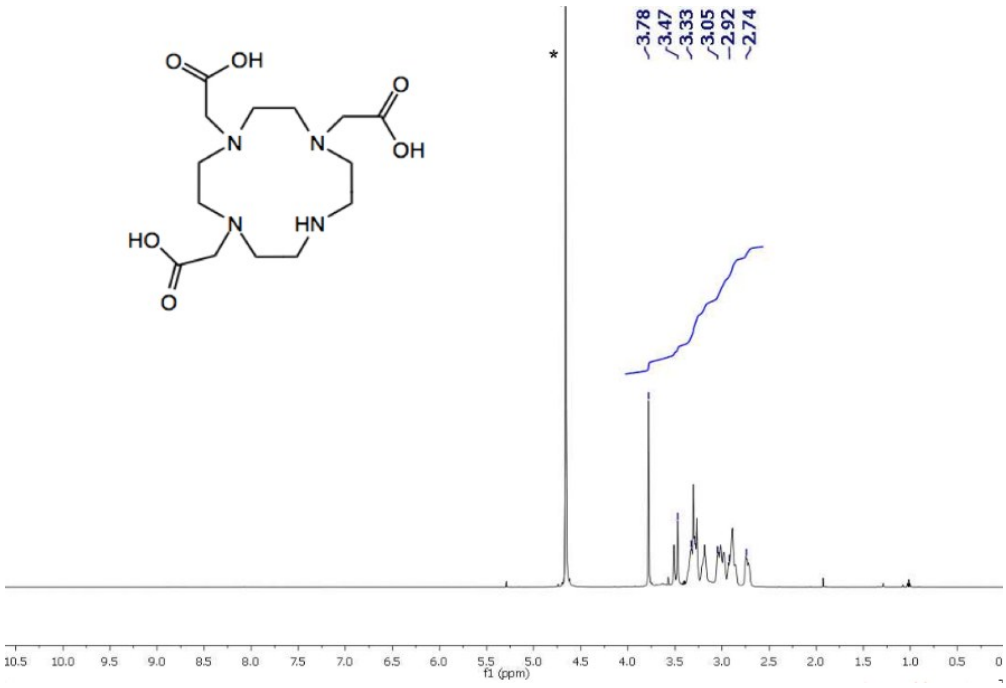


Figure S2. ^1H-NMR spectrum of **1** (500 MHz, D_2O). [*solvent peak]

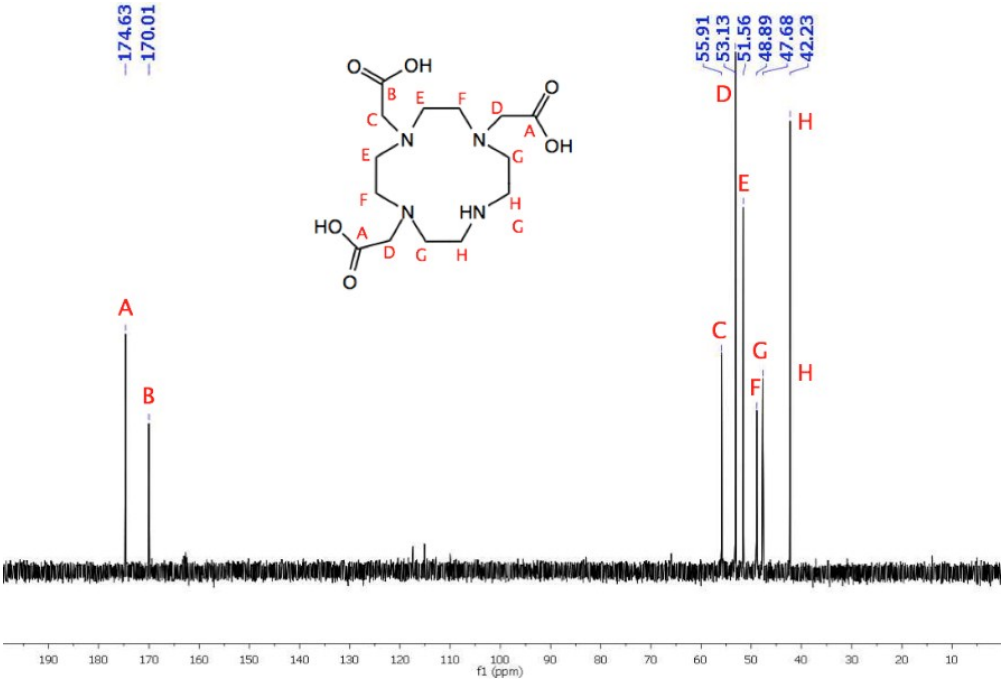


Figure S3. ^{13}C NMR spectrum of **1** (500 MHz, D_2O).

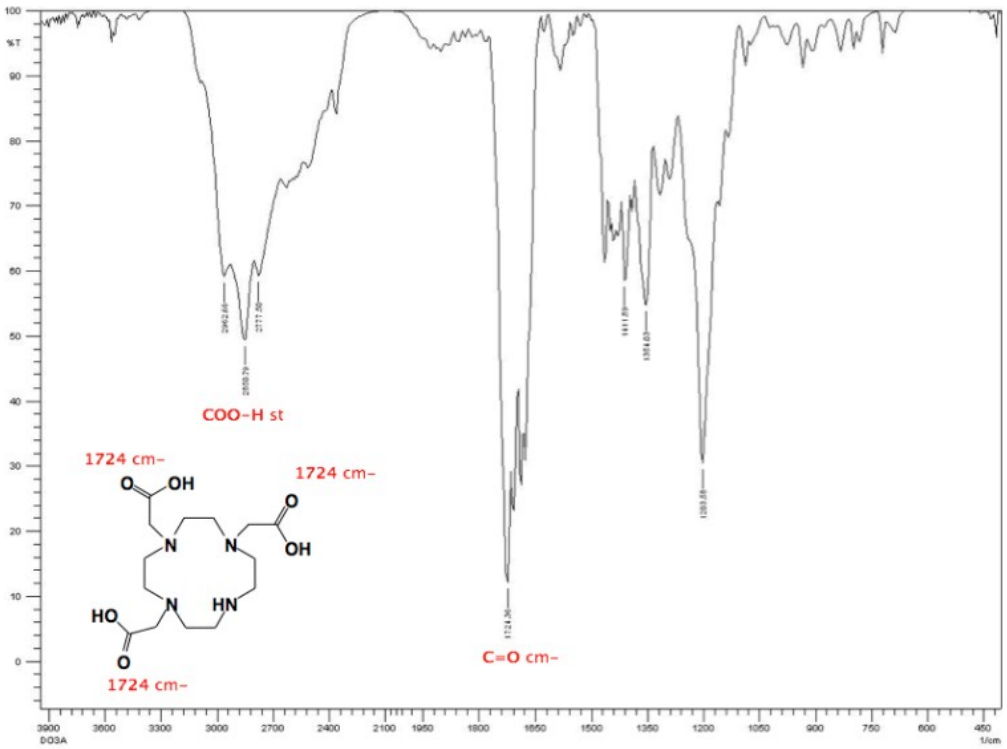


Figure S4. FT-IR spectrum of **1**.

Synthesis of ligand **2**.

Ligand **2** was synthesized as previously described[8] with slight modifications. Compound **1a** (0.50 g, 0.97 mmol) was dissolved in minimal ACN, to which $NaHCO_3$ (0.25 g, 2.97 mmol) was added. 4-(bromomethyl)benzoate (0.22 g, 0.96 mmol) was added to the reaction with stirring. The reaction was heated under reflux overnight, filtered, and the filtrate evaporated under reduced pressure. The residue was purified by silica gel chromatography with gradient elution of DCM to DCM/MeOH (9:1). The purified fractions containing the compound were pooled together and evaporated under reduced pressure. Trituration of the residue in hexane produced a pale yellow powder (**1b**). A small

amount (0.195 g, 0.29 mmol) was dissolved in DCM (5 mL), to which was added TFA. The reaction was stirred overnight, and then all solvents evaporated under reduced pressure. In a similar fashion, DCM was added to the residue and evaporated thrice, followed by MeOH and evaporated thrice. The residue was then dissolved in minimal MeOH and the product precipitated by addition of ether to yield a white powder (0.15 g, 97%). RP-HPLC (H_2O/ACN 5-100% ACN in 0.1% TFA in 30 min at 236 nm) t_R = 8.293 min at λ = 236 nm; ES^+ MS (H_2O) *m/z* 495 $\{M+H\}^+$. 517 $\{M+Na\}^+$, 518 $\{M+H+Na\}^+$; ^1H NMR (500 MHz, D_2O) δ 7.845 (d, *J* = 6.5 Hz, 2H), δ 7.475 (d, *J* = 6.5 Hz, 2H), 3.77 (s, 1H), 3.66 – 2.58 (m, 24H);); ^{13}C NMR (126 MHz, D_2O) δ 168.56, 162.98, 162.70 (C=O) 130.81, 130,37 117.41, 115.08 (Ar), 57.01, 53.38, 52.74, 48.80 (CH_2N, CH_2COO and OCH_3); IR C=O stretch 1687, 1695, 1714 cm^{-1}.

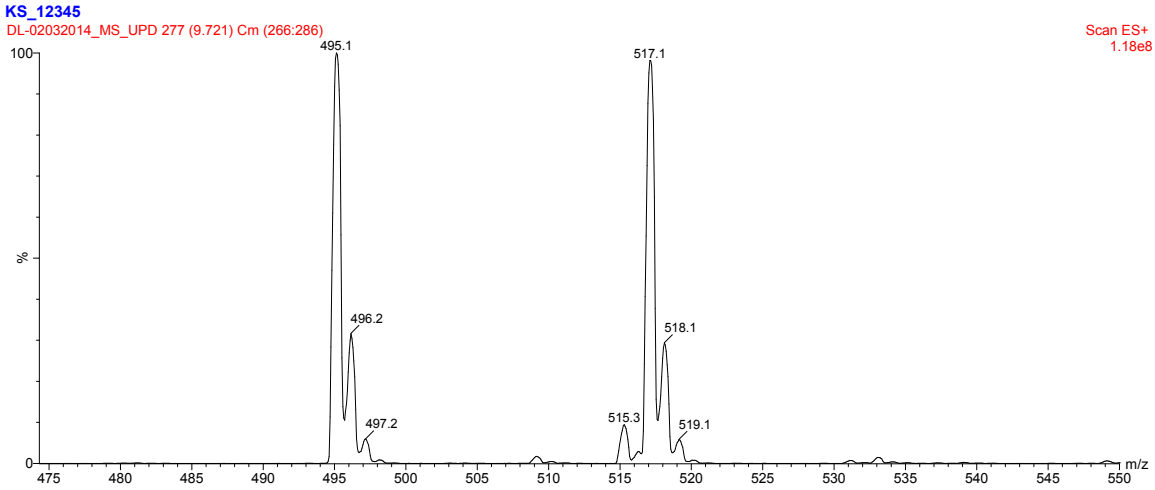


Figure S5. ES^+ mass spectrum of **2**: $\{M+H\}^+$ = 495.1 m/z, $\{M+Na\}^+$ = 517.1 m/z

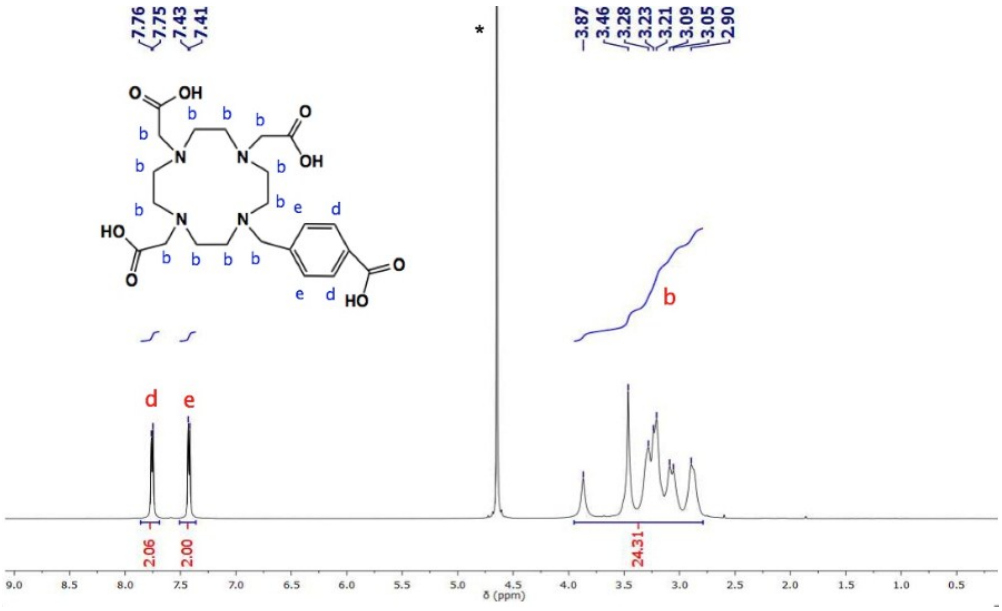


Figure S6. ^1H NMR spectrum of **2** (500 MHz, D_2O). [*solvent peak]

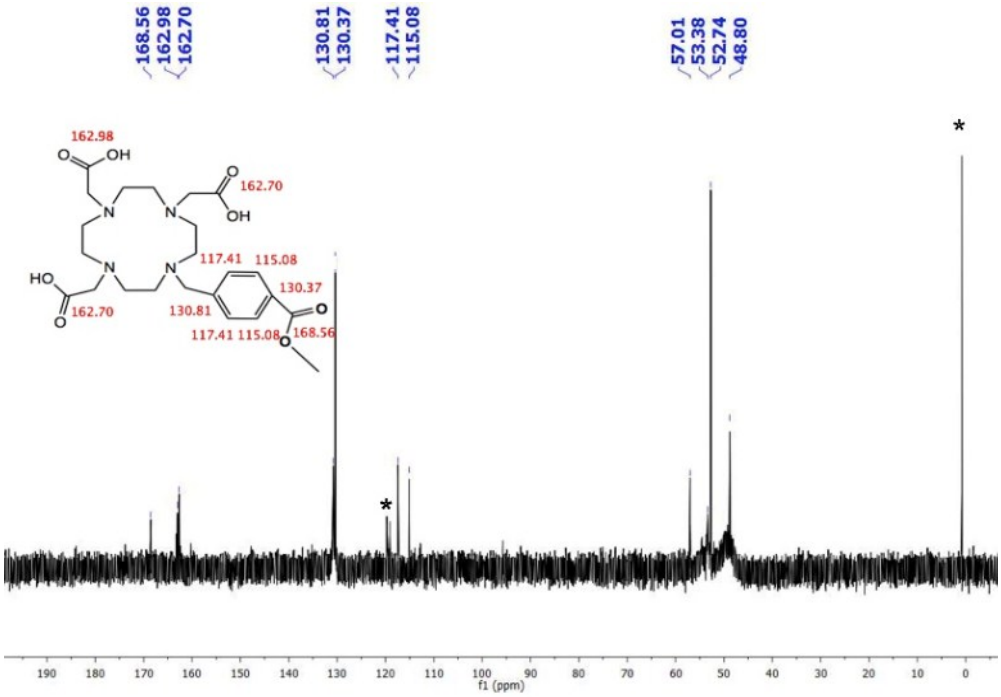


Figure S7. ^{13}C NMR spectrum of **2** D_2O **(**ACN*).

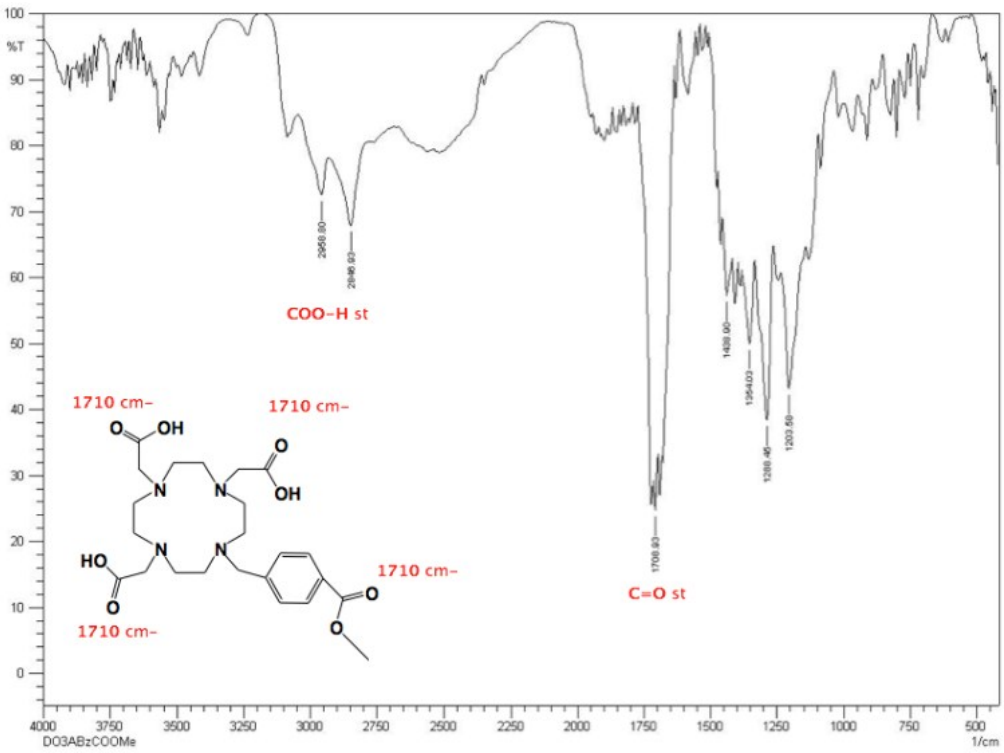


Figure S8. IR spectrum of **2**.

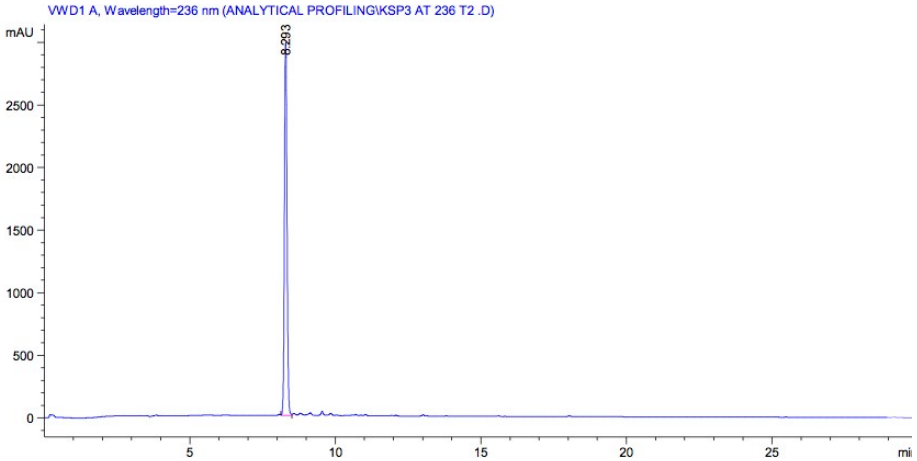


Figure S9. C18 RP-HPLC (H_2O/ACN 5-100% ACN in 0.1% TFA in 30 min at 236 nm) chromatogram.

UV-Vis Kinetic Assay

A double beam UV-Vis spectrophotometer equipped with temperature controller was used and the blank solution that served as reference was the 50 mM HOAc-OAc buffer at pH 5.8. The 750 µL volumes of 100 µM Gd(III), 100 µM XO and the buffer were equilibrated in a quartz cuvette and allowed to equilibrate with the cell temperature for 5 minutes. A 750-µL aliquot of 2000 µM of ligand solutions (i.e. ligand in 20-fold excess) was added to the Gd(III)-XO system and changes in the absorption spectra were monitored after addition with a lag time of 5 sec. The reactions were carried out at 25, 30, 35 and 40 ^{O}C. Three (3) trials were performed per temperature. The rate constants of each ligand exchange system were estimated using the least-squares approximation method from the equation $A_t = A_f + (A_0 - A_f)\exp(-k_{obs}t)$, and activation energy (Ea) was calculated from the Arrhenius equation $k_{obs} = A\exp(-E_a/RT)$.

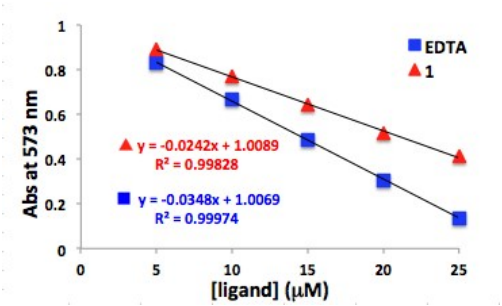


Figure S10. Decrease in absorbance at 573 nm with increasing concentrations of EDTA (O) and **1** (□) (50 mM acetate buffer pH 5.8).

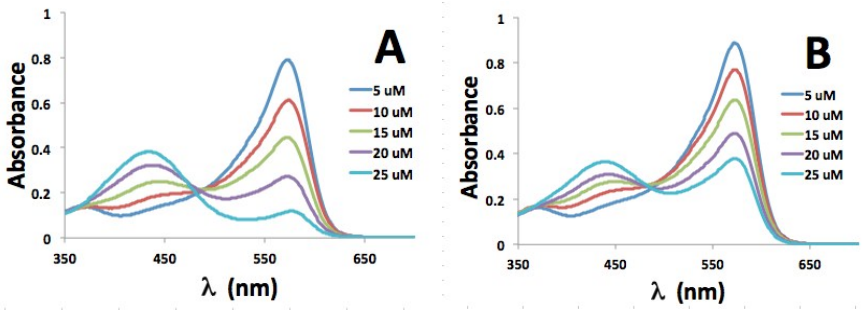


Figure S11. Overlay of spectra of 25 µM Gd-XO solutions with increasing concentrations of (**A**) EDTA and (**B**) **1**. (50 mM acetate buffer pH 5.8).

References

[8] S. Faulkner, B.P. Burton-Pye, *Chem. Commun*. 2005, 259.