CREATED USING THE RSC COMMUNICATION TEMPLATE (VER. 3.1) - SEE WWW.RSC.ORG/ELECTRONICFILES FOR DETAILS

ARTICLE TYPE www.rsc.org/xxxxxx | XXXXXXXX

Highly Stable and Soluble Bis-Aqua Gd, Nd, Yb Complexes as Potential Bimodal MRI/NIR Imaging Agents

Gaylord Tallec, Daniel Imbert, Pascal H. Fries, and Marinella Mazzanti*

Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

Laboratoire de Reconnaissance Ionique et Chimie de Coordination, SCIB, (UMR-E 3 CEA-UJF), Laboratoire Institut Nanosciences et Cryogénie, INAC, CEA-Grenoble, 17 rue des Martyrs, 38054 Grenoble Cedex 09, France.

Table of contents

1. General Details

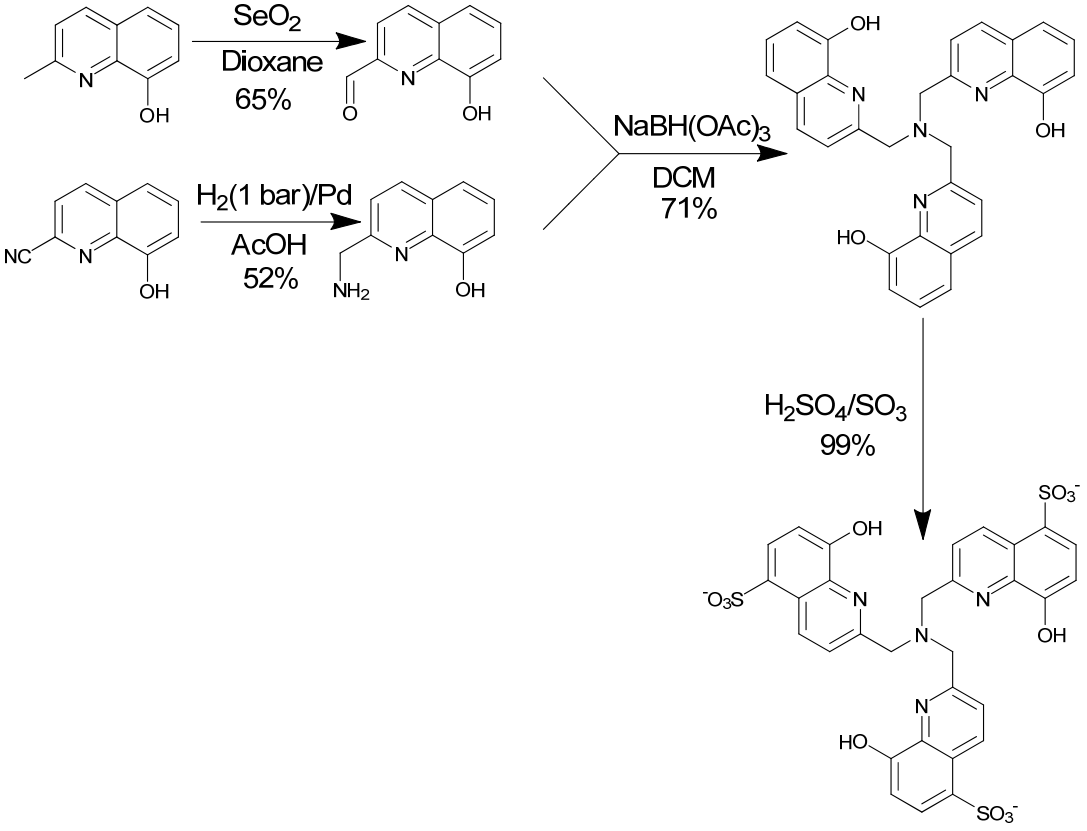
Solvents and starting materials were obtained from Aldrich, Fluka, Acros, and Alfa. They were used without further purification unless otherwise stated. Solvents were dried over the appropriate drying agents when required. Water and H_2O refer to high purity water with resistivity value of 18 MΩ·cm, obtained from the "Millipore/MilliQ" purification system. Lanthanide triflate salts were purchased from Aldrich. The precise metal ion content was titrated by colorimetry in acetate buffer (pH = 4.5) using standardized H_2Na_2edta solution (Merck) and Xylenol orange indicator.

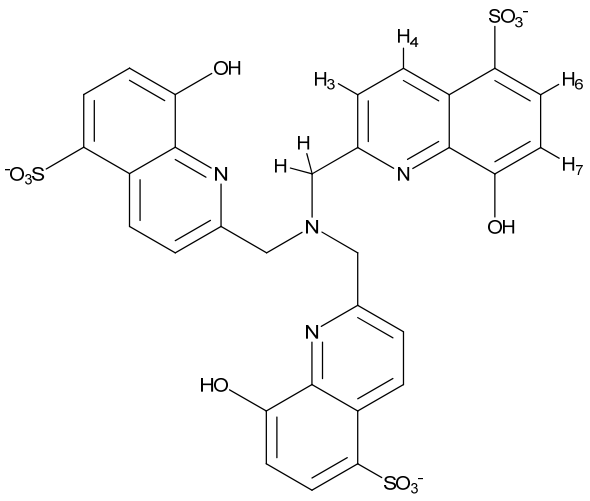
^{1}H NMR spectra were recorded on 200 MHz and 500 MHz Bruker spectrometers. Chemical shifts are reported in ppm with solvent as internal reference.

2. Synthesis

2.1. Synthesis of 2,2',2''-nitrilotris(methylene)tris(8-hydroxyquinoline-5-sulfonic acid)

The ligand H_3thqN-SO_3 was synthesized in 3 steps starting from commercially available 8-hydroxy-2-methyl-quinoline





H_3thqNSO$_3$

8-hydroxyquinoline-2-carbaldehyde: Commercially available 8-hydroxy-2-methyl-quinoline (5.0g, 31 mol) was added to a suspension of selenium oxide (4.35g, 39 mmol, 1.25eq) in dioxane (100ml) under argon. The mixture was heated at 80 °C during 24h. After cooling at room temperature, the mixture was filtered on celite. The filtrate was concentrated under vacuum. Purification of the resulting crude product (on silica column chromatography with dichloromethane) afforded the desired aldehyde as a yellow solid.
Yield : 65%

^{1}H NMR ($CDCl_3$) δ (ppm): 10.22 (s, 1H, CHO), 8.32 (d, 1H, H4 , 8.6Hz), 8.05 (d, 1H, H3, 8.9Hz), 7.62 (t, 1H, H6, 8.2Hz), 7.42 (dd, 1H, H7, 8.3Hz, 1.3Hz), 7.28 (dd, 1H, H5, 8.3Hz, 1.3Hz).

2-(aminomethyl)quinolin-8-ol : 8-hydroxyquinoline-2-carbonitrile (1.5g, 8.8 mmol) was dissolved in acetic acid (60mL). Palladium/C 10% (330mg, 3.1 mmol, 0.35 eq) was added and the mixture placed under dihydrogen (1 bar), and stirred at room temperature for 1 night. The mixture was filtered on celite. The solution was concentrated under vacuum. The product was precipitated in a mixture of $CHCl_3$/ Et_2O, filtered, and washed with Et_2O. The solid obtained was dissolved in $CHCl_3$, washed with saturated K_2CO_3, and then extracted with $CHCl_3$ (3x50mL). The organic layer was dried on Na_2SO_3, filtered, and concentrated under vacuum to yield a light brown solid.
Yield : 52%

^{1}H NMR ($CDCl_3$) δ (ppm): 8.11 (d, 1H, H4 , 8.4Hz), 7.43 (s, 1H, H3), 7.39 (s, 1H, H6), 7.33 (dd, 1H, H7, 8.3Hz, 2Hz), 7.29 (dd, 1H, H5, 8.3Hz, 2Hz), 4.18 (s, 2H, CH2).

2,2',2''-nitrilotris(methylene)triquinolin-8-ol : 2-(aminomethyl)quinolin-8-ol (300 mg, 1.7mmol) and 8-hydroxyquinoline-2-carbaldehyde were mixed in extra dry dichloromethane (25mL) under argon. $NaBH(OAc)_3$ (2.2g, 10.4mmol) was added and the mixture was stirred at room temperature during 48h. The reaction was quenched with HCl (1M, 10mL) and then neutralised with a solution of NaOH and extracted with dichloromethane. The organic layer was dried on Na_2SO_4, filtered, and concentrated under vacuum. The product was purified on column (Sephadex LH20) in MeOH to give a light brown solid.

Yield : 71%

^{1}H NMR ($CDCl_3$) δ (ppm): 8.18 (d, 1H, H4, 8.5Hz), 7.43 (d, 1H, H3, 8.3Hz), 7.37 (t, 1H, H6, 7.8Hz), 7.03 (dd, 1H, H7, 8.0Hz, 1.2Hz), 6.83 (dd, 1H, H5, 8.0Hz, 1.2Hz), 4.84 (s, 2H, CH2).
ES-MS**:** m/z (%): 489.3 $[M + H]^+$

2,2',2''-nitrilotris(methylene)tris(8-hydroxyquinoline-5-sulfonic acid)

2,2',2"-nitrilotris(methylene)triquinolin-8-ol (130 mg, 0.26mmol) was dissolved in the minimum amount of oleum (5mL) and stirred for one night at room temperature. The mixture was then poured on crushed iced. The resulting yellow solid was collected, washed with cold water (2x10mL), cold ethanol (2x10mL), cold diethylether (2x10mL) and then dried to give a brow solid.
Yield = 99%

^{1}H NMR (D_2O) δ (ppm): 9.23(d, 1H, H4, 8.9Hz), 8.05(d, 1H, H6, 8.2Hz), 7.79(d, 1H, H3, 8.9Hz), 7.20(d, 1H, H7, 8.2Hz), 4.98(s, 2H, CH2)
Elemental Anal. Calcd. for H_7thqN-$SO_3 \cdot 5.5H_2O \cdot 1.5H_2SO_4$; (%) $C_{30}H_{38}N_4O_{23.5}S_{4.5}$ (974.93): C 36.96, H 3.93, N 5.75; found: C 36.99, H 3.93, N 4.56.

3. Luminescence measurements

Low-resolution luminescence measurements (spectra and lifetimes) were recorded on a Fluorolog FL 3-22 spectrometer from Spex-Jobin-Yvon-Horiba with double grating emission and excitation monochromators, and a R928P photomultiplier. For the measurements in the NIR spectral range, the spectrometer was fitted with a second measuring channel equipped with a FL-1004 single grating monochromator. The light intensity was measured by two Jobin-Yvon solid state InGaAs detectors (i) DSS-IGA020L, cooled to 77 K (range 800-1600 nm) and (ii) DSS-IGA020A (range 800-1700 nm) working at room temperature and inserted into a LN2 housing including an elliptical mirror (90° beam path) and coupled to a Jobin Yvon SpectrAcq2 data acquisition system. The equipment and experimental procedures for luminescence measurements in the visible and NIR range have been published previously by Comby et al.[1] All spectra were corrected for the instrumental functions. The lifetimes were measured in the time-resolved mode and are averages of three independent measurements, which were made by monitoring the decay at the maxima of the emission spectra. The mono-exponential decays were analyzed with Origin 7.0®. The quantum yields of the complexes in solution at pH 7.4 and in solid state were determined using a home-modified integrating sphere from Oriel and the previously described procedure.[2] Spectra were corrected for the instrumental function with an absolute method with an integrations sphere.

4. Determination of pGd via spectrophotometric competition batch

Gd^{3+} binding of Gd(thqN-SO_3) versus the benchmark ligand dtpa = diethylenetriaminepentaacetate (pGd = 19.11) was monitored by spectrophotometry. Batch titration techniques were employed. Varying volumes of dtpa stock solution were added to the samples. All solutions were brought to pH = 7.4 by using TRIS buffer (0.1M). The molar ratio for Gd : Ligand was fixed to 1:1 whereas the molar ratio for Ligand:dtpa varied from 1: 0.01 up to 1:100. This method allows a precise measurement of the pGd ((pGd= -log $[Gd_{aq}]$ for a total concentrations of $[Gd]_{tot} = 10^{-6}$ M and $[L]_{tot} = 10^{-5}$ M)). The plot directly gives the difference in pM between the ligand and dtpa (log([DTPA]/[L] when log([Gd-dtpa]/[GdL]=0.

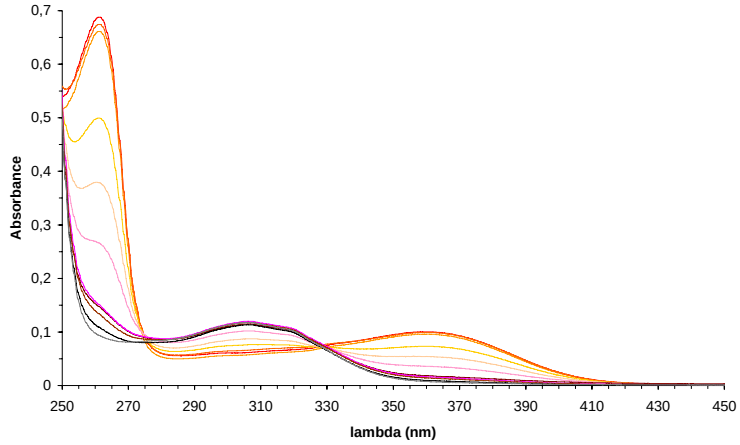


Figure S1 Uv-visible spectra from competition batch titration of thqN-SO_3 versus dtpa for Gd(III) complexation at pH = 7.4 in 0.1 M KCl

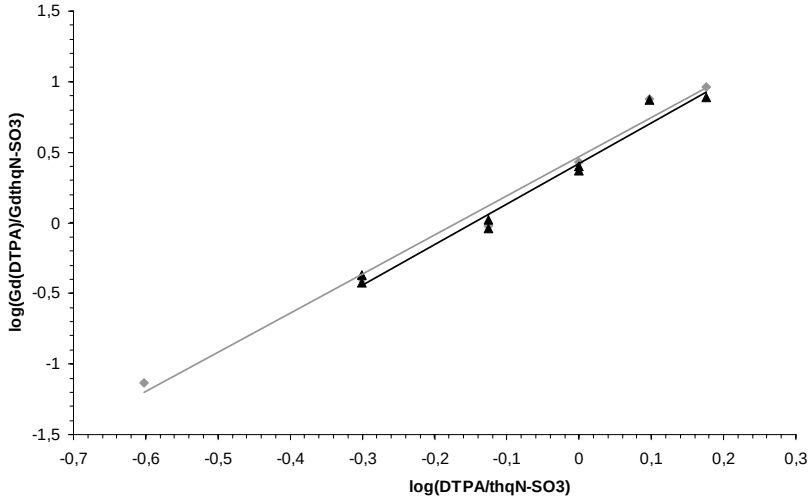


Figure S2 : Competition titration log/log plots against dtpa

at pH 7.4
ΔpM = -0.3 (3) **pGd**= 19.6-0.3 = 19.3 (3)
log β_{GdL} 101 = 23.05 (5)

at pH=4.5,
ΔpM = -0.3 (3) **pGd**= 13.0-0.3 = 12.7 (3)

5. Potentiometric Titrations

Ligand protonation constants of H_7thqN-SO_3 were determined by potentiometric titrations. For that purpose, 20 mL solutions of H_7thqN-SO_3 ($1.037\ 10^{-3}$ M) acidified to pH~2.5 were titrated in a thermostated cell (25.0°C +/- 0.1°C) under a stream of argon with a 0.1 M KOH solution added by means of a 5 mL piston burette (Metrohm). The return titration was done under the same conditions with a 0.1M HCl solution. The ionic strength μ = 0.1 M was fixed with KCl. Titrations were carried out with a Metrohm 751 GPD Titrino potentiometer equipped with a combined pH glass electrode (Metrohm). Calibration of the electrode system was performed prior to each measurement. The electromotrive force is given by $E = E° + s$ pH and both $E°$ and s were determined by titrating a known amount of HCl by 0.1 M KOH at μ=0.1 M (KCl), using the acid range of the titration. The value used for the ion product of water is log K_w = 13.77.[1] More than 200 data points were collected for each experiment.
The data were mathematically treated by the program HYPERQUAD2000.[2,3] All values and errors represent the average of at least three independent experiments.
The protonation constants of H_7thqNSO_3 defined as $K_{ai} = [H_iL]/[H_{i-1}L][H^+]$ were determined by potentiometric titration. The ligand was titrated in a thermostated cell (25° C) under a stream of argon with a 0.1M KOH The ionic strength was fixed with KCl (I=0.1M). The pKa's were found to be:

pKa1+pKa2 = 6.36 (9)
pKa3 = 4.08 (8)
pKa 4 = 7.66 (6)
pKa 5+6 = 17.36 (5)
pKa 7 = 9.16 (5)

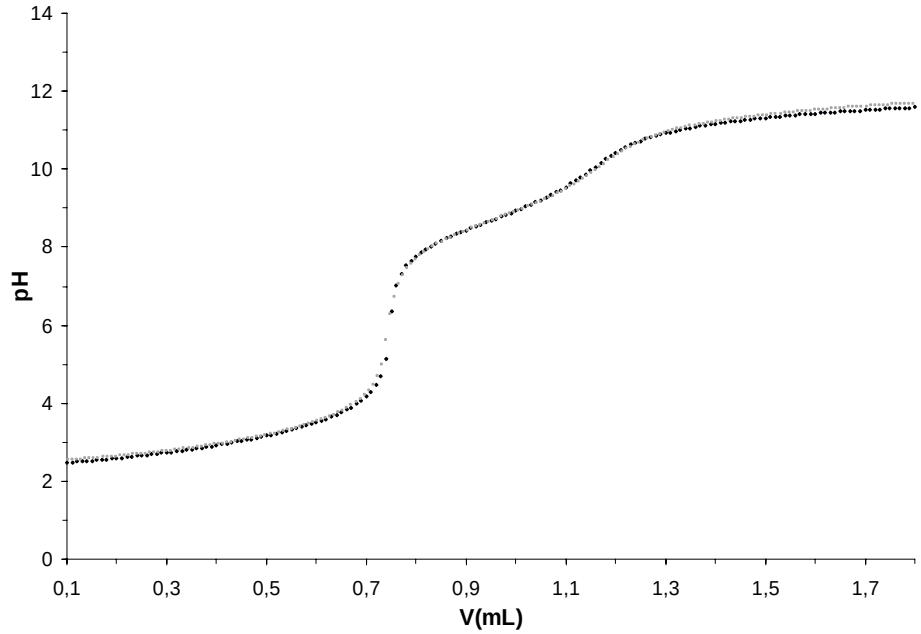


Figure S3 : Potentiometric titration curves for H_7thqN-SO3 (1.037mM) NaOH 0.1M (■) added HCl 0.1M added (■), T=25 °C, I = 0.1M (KCl).

The 3 lowest pKa values are assigned to the deprotonation of the pyridinium nitrogen. They are in agreement with the values reported for 5-sulfo-8-hydroxyquinoline (pKa = 3.92)[3]

The next pKa value can be assigned to the protonation of the nitrogen atom of the tertiary amine (7.66). In comparison, the pKa of the central nitrogen atom is for dpaa 7.33, and 7.3 for dpa

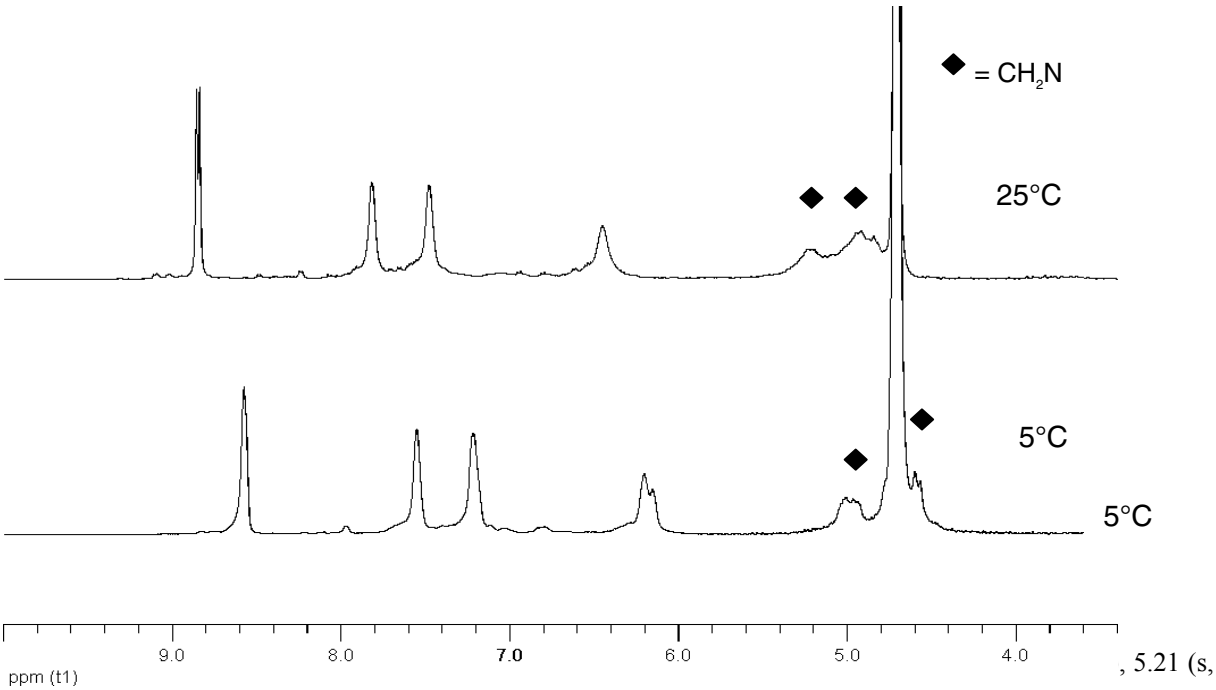
The next three pKa's can be assigned to the deprotonation of the hydroxyl groups of the three quinolinate groups and are similar to the values reported for 5-sulfo-8-hydroxyquinoline (average pKa value of 8.42)

We were unable to determine the stability constant of the complex by potentiometric titration. Even at low pH, the complex is formed as soon as Gd^{3+} and the ligand are mixed together in solution.

6. Lanthanide Complexes of H3thqN-SO3

[Ln(thqN-SO_3)]. (Ln = La, Lu). Complexes for ^{1}H NMR and luminescence measurements were prepared *in situ* by mixing stoechiometric amounts of the ligand H_3thqN-SO_3 and of the appropriate Ln(OTf)$_3$. ^{1}H NRM was performed in D_2O after adjustment of the pD value with a KOD solution.

Figure S4 : ^{1}H 500 MHz NMR spectra of [Lu(thqNSO3)] at 25°C and 5°C in D_2O at pD = 7.4



, 5.21 (s, 1H, CH2) 4.92 (s, 1H, CH2)

^{1}H NMR (D2O) δ (ppm) (278K) : 8.57 (s, 1H, H4), 7.55 (s, 1H, H6), 7.22(s, 1H, H3), 6.17(d, 1H, H7), 4.98 (d, 1H, CH2), 4.58 (d, 1H, CH2)

^{1}H NMR of [La(thqN-SO3)] 500 MHz, 25°C and 5°C in D2O at pD = 7.4, 500 MHz

^{1}H NMR (D2O) δ (ppm) (298K) : 8.98 (s, 1H, H4), 8.04 (s, 1H, H6), 7.75 (s, 1H, H3), 7.16 (s, 1H, H7), 4.98 (s, 2H, CH2)

^{1}H NMR (D2O) δ (ppm) (278K) : 8.70 (s, 1H, H4), 7.77 (s, 1H, H6), 7.50(s, 1H, H3), 6.90(s, 1H, H7), 4.71 (s, 1H, CH2)

7. Relaxivity measurements

7.1. NMRD Profiles

The samples were prepared by dissolving the appropriate amount of ligand and $GdCl_3.6H_2O$ in water followed by the adjustment of pH at different values (4.1, 6.0, and 7.4). The absence of free gadolinium was checked by the xylenol orange test.[6] The NMRD profiles were measured at 298K in the range 0.1 and 35Mz, by using a Spinmaster FFC (fast field cycling) NMR relaxometer (Stelar, Italy).

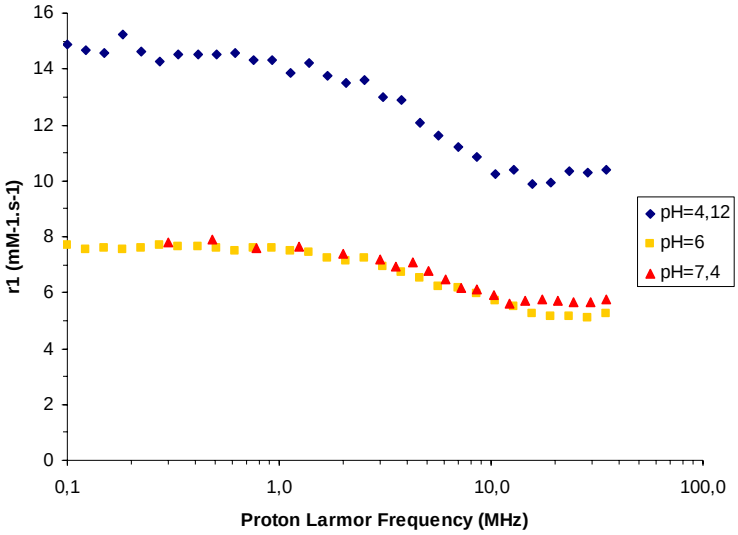


Figure S5 : NMRD profile for [Gd(thqN-SO3)] in H_2O at 298 K.

7.2. pH titration and affinity with carbonates

The samples were prepared by dissolving the appropriate amount of ligand and $GdCl_3.6H_2O$ in water followed by the adjustment at different pH values (4.2 to 8.7) under inert atmosphere. The absence of free gadolinium was checked by the xylenol orange test.[6]
The $1/T_1$ measurement were performed on a Brucker Avance 200 spectrometer (200MHz)

For the affinity with carbonates, a stock solution (1,6M) of potassium bicarbonate was prepared by dissolving the appropriate amount of salt and adjusting the pH to 7.4 with NaOH and HCl solution. Then, the samples were prepared by mixing appropriates volumes of $Gd(thqN\text{-}SO_3)(H_2O)_2$ and stock solution of carbonates, in order to

have 200eq of anions for 1 eq of complex. The relaxivity was measured at 200 MHz and compared with the value obtained in pure H_2O at the same pH value.

r1 = 5.16 $mM^{-1}.s^{-1}$ (pure H_2O)

r1 = 4.87 mM-1.s^{-1} (200 eq of carbonates)

8. References

1. Comby, S.; Imbert, D.; Chauvin, A.-S.; Bünzli, J.-C. *Inorg. Chem.* **2006,** 45, 732-743.

2. deMello, J. C.; Wittmann, H. F.; Friend, R. H. *Adv. Mater.* **1997,** 9, 230-&.

3. Martell, A. E.; Smith, R. M., *Critical Stability Constants*. Plenum Press: New York, 1976; Vol. 4.

4. Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996,** 43, 1739-1753.

5. Gans, P.; Sabatini, A.; Vacca, A. *HYPERQUAD2000*, University of Leeds and University of Florence: Leeds, UK and Florence, Italy, **2000.**

6. G. Brunisholz, M. Randin, *Helv. Chim Acta* **1959**, 42,1927-1938