Highly stable and soluble bis-aqua Gd, Nd, Yb complexes as potential bimodal MRI/NIR imaging agents†

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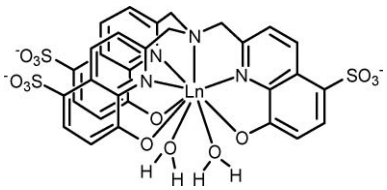
A tripodal ligand based on the 8-hydroxyquinolinate binding unit yields a soluble and highly stable bis-hydrated Gd^{3+} complex in water (pGd = 19.2(3)) with relaxivity change in the pH range 4.5–7.4 and Nd^{3+}, Yb^{3+} analogues with sizeable NIR emission upon excitation at 370 nm providing a new architecture for the development of bimodal agents.

The recent development of high-field instruments for magnetic resonance imaging (MRI) has refocused the search for more efficient contrast agents (CAs) towards paramagnetic Gd^{3+} complexes with a high number of coordinated water molecules.[1,2] The enhancement of the water proton relaxation rate per mmol L^{-1} concentration of dissolved MRI contrast agents is named relaxivity and gauges the contrast efficiency of these agents. The inner sphere relaxivity, which is directly proportional to the number q of water molecules coordinated to Gd^{3+}, can be increased at any magnetic field by reducing the denticity of the chelating ligand. However, ligands of reduced denticity can lead to Gd^{3+} chelates of lower thermodynamic stability, likely to release this toxic ion in physiologic medium. In spite of numerous attempts, there are only very few examples of multi-hydrated Gd^{3+} chelates showing high stability and solubility in physiological conditions.[2–5] In the search for new structural motifs, the preparation of ligand scaffolds suitable to simultaneously sensitize lanthanide Ln^{3+} emission and yield stable multi-hydrated Gd^{3+} complexes is of primary importance because it is a route towards bimodal agents for both optical and MR imaging.[6–11] Only two bimodal MRI/NIR luminescence have been reported recently.[10,12] Obtaining sizeable luminescence emission from NIR emitting lanthanide complexes and high inner sphere relaxivity of the gadolinium complex using the same ligand is challenging because of the unfavourable effect of O–H oscillators.[13] Recent studies have suggested that 8-hydroxyquinolinate based lanthanide complexes are good candidates for the design of near infra-red (NIR) emitting luminescent probes for biomedical application due to their good stability, low cytotoxicity, sizeable quantum yields in water, long excitation wavelength, ability to interact with proteins.[14–19] In spite of these attractive properties, the hydroxyquinolinate groups have never been envisaged for the design of Gd^{3+} CAs. Here, we report on the new tripodal heptadentate ligand H_3thqN-SO_3 containing three 8-hydroxiquinolinate groups connected to a nitrogen anchor (Scheme 1). This ligand yields highly stable (pGd = 19.2(3)) bis-aqua Ln^{3+} complexes. The Gd^{3+} complex shows a relaxivity of



Scheme 1 Structure of the lanthanide complexes [Ln(thqN-SO_3)(H_2O)$_2$].

5.16 mM^{-1} s^{-1} at 200 MHz, pH = 7.4 and 25 °C, while the Nd^{3+} and Yb^{3+} analogues present sizeable NIR-emission.

The H_3thqN-SO_3 ligand was synthesised in 3 steps‡ with an overall yield of 34% by coupling three 2-functionalized 8-hydroxyquinoline followed by a regiospecific sulfonation by using oleum. The pK_a's of the protonated form of the ligand H_7thqN-SO_3^+ were determined (the three sulfonates remain completely deprotonated under the used experimental conditions) as well as the stability constant of the resulting chelate with Gd^{3+}. Analysis of the potentiometric curves allowed the determination of the acidity constants, pKa_{1+2} = 6.36, pKa_3 = 4.08, pKa_4 = 7.66, pKa_{5+6} = 17.36, pKa_7 = 9.16 corresponding to the deprotonation of the pyridinium groups, the central nitrogen and the hydroxyl oxygen atoms, respectively. For pKa_1 and pKa_2, as often described for acidity constants in the same pH range, only the sum could be calculated and a similar situation prevails for pKa_5 and pKa_6. This assignment compares well with that of the previously described ligands TsoxMe and H_3thqtcn-SO_3.[17,18]

The anionic lanthanide complexes [Ln(thqN-SO_3)] (Ln = Nd, Gd, Yb) were prepared *in situ* by reacting the protonated ligand with the appropriate lanthanide triflate salt followed by adjustment of the pH. The lanthanide complexes of thqN-SO_3^{3-} are highly soluble in water (> 35 mM). To evaluate their potential as imaging probes, we investigated the thermodynamic stability of the gadolinium complex at physiological pH (7.4) by spectrophotometric competition batch titration *versus* H_5dtpa (diethylenetriamine-pentaacetic acid).[20,21] The titration yielded a pGd value (pGd = –log [Gd_{aq}] for a total concentration of $[Gd]_{tot}$ = 10^{-6} M and $[L]_{tot}$ = 10^{-5} M) of 19.2(3) at pH = 7.4 and of 12.7(3) at pH = 4.5. At pH = 7.4 the major solution species is the 1 : 1 complex of the deprotonated ligand [Gd(thqN-SO_3)]. A model with one complexation constant ($\beta_{110(LMH)}$) was used to fit the titration data with the Hyss2 software[22] yielding a value of logβ_{110} = 23.05(5). The stability of the complex at physiological pH is very high compared to other bis-aqua complexes of acyclic ligands.[23,24] A strong stability enhancement is also found for these complexes with respect to the previously reported 8-hydroxyquinolinate based podates[15,17] in spite of the decreased ligand denticity. The obtained pGd value is comparable to that of the mono-aqua complex [Gd(dtpa)(H_2O)], a clinically approved MRI contrast agent.[25] Moreover, the high

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Table 1 Metal ion centered lifetimes τ, absolute quantum yields Q_{Ln}^{L}, and number q of water molecules bound in the inner coordination sphere for Nd($^4F_{3/2}$), Yb($^2F_{5/2}$) in the 1 : 1 [Ln(thqN-SO_3)] complexes at pH = 7.4 (TRIS buffer) in H_2O/D_2O solutions

Compound	τ/μs H_2O	Q_{Ln}^{L}/% H_2O	τ/μs D_2O	q
[Nd(thqN-SO_3)]	0.0521(60)	0.00068(4)	0.350(3)	1.7(2)
[Yb(thqN-SO_3)]	0.329(5)	0.00122(8)	1.86(7)	2.3(2)

ligand acidity renders it more resistant than polyaminocarboxylate ligands to protonation and a reasonable stability is maintained after partial ligand protonation (pGd = 12.7(3) at pH = 4.5). The high value of the pGd suggests that decoordination does not occur after protonation. Moreover, solid state and solution studies of the two previously reported lanthanide complexes of partially protonated hydroxyquinoline based nonadentate[26] and tetradentate[27] ligands showed that these ligands can efficiently coordinate lanthanide also in their protonated form. ^{1}H NMR of the diamagnetic La^{3+} and Lu^{3+} at 298 K and pH = 7.4 show 5 signals (see ESI†). In the case of the Lu^{3+} complex, the ^{1}H NMR at 278 K shows two different signals for the diastereotopic protons of the methylene group, which coalesce at room temperature. This suggests the presence of a slow exchange between the two possible helical (Λ and Δ) conformations of the Lu^{3+} complex in which the chelating arms remain coordinated to the metal ion. A faster exchange is observed for the La^{3+} and Nd^{3+} ions. The number q of water molecules bound to the Ln^{3+} ion in water was calculated to be 1.7 ± 0.2 for Nd^{3+} and 2.3 ± 0.2 for Yb^{3+} from the measured luminescence lifetimes of the Nd($^4F_{3/2}$) and Yb($^2F_{5/2}$) excited states which re-emit the near UV light (370 nm) absorbed by the ligand levels (Table 1). A similar number of coordinated molecules is expected for the Gd^{3+} ion since its ionic radius is intermediate between those of Nd^{3+} and of Yb^{3+}. These results indicate that the Gd^{3+} complex of thqN-SO_3 is a rare example of highly stable bis-aqua complex with potential as MRI contrast agent.

The measured relaxivity r_1 of the Gd^{3+} complex of thqN-SO_3 at physiological pH (7.4) and 25 °C is 5.16 mM^{-1} s^{-1} at 200 MHz and 5.73 mM^{-1} s^{-1} at 20 MHz. This value is higher than those found for the mono-aqua complexes $[Gd(dtpa)(H_2O)]^{2-}$ or $[Gd(dota)(H_2O)]^{-}$ (4.3 mM^{-1} s^{-1} at 25 °C), but somewhat low for a bis-aqua complex.[1] It remains unchanged when the relaxivity is measured in anaerobic conditions excluding the possibility of partial water displacement by carbonate.[4] It still keeps unaltered even after addition of 200 equivalents of carbonate. Note that the relaxivity can be limited in this complex by a rather slow exchange rate of coordinated water and preliminary ^{17}O NMR[1,25] results support this interpretation. Besides, the relaxivity value is higher in physiological serum than in water (r_1 = 7.01 mM^{-1} s^{-1} instead of 5.16 mM^{-1} s^{-1} at 200 MHz). The origin of this increase will be the object of future studies. Finally, as shown in Fig. 1, the relaxivity values increase markedly, *e.g.*, from 5.16 to 7.38 mM^{-1} s^{-1} at 200 MHz, when the pH drops from 7.4 to 4.5. Such increase parallels the protonation of the 8-hydroxyquinolinate groups followed by a relaxivity decrease observed after pH 7.5 probably associated to the formation of lanthanide hydroxo complexes.[9,28] [Gd(thqN-SO_3)] is a new example of a pH sensitive complex. While most attention has been directed to the development of sensors in the pH range 6–8 because they can serve to detect pH decreases

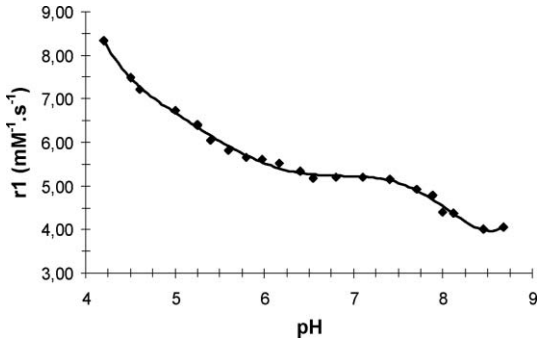

Fig. 1 Relaxivity values of Gd(thqNSO_3) at different pH, in water, 25 °C, 200 MHz.

associated to cancer and iskemic or kidney diseases,[29,30] low pH sensors are potentially attractive to monitor the pH within cells (pH = 5.5–6 in endosomes, 4–5 in lysosomes).[31–34] Further studies will be directed to investigate the origins of the observed pH dependency and the possibility of tuning the pH range.

We also investigated the viability of the Nd^{3+} and Yb^{3+} complexes of H_3thqN-SO_3 to act as NIR-emitting luminescent probes in biomedical applications. Ligand excitation at 370 nm results in sizeable NIR luminescence of the complexed Nd^{3+} and Yb^{3+} ions in water (Fig. 2). The measured absolute quantum yields, which amount to 5×10^{-4} % for Nd^{3+} and 1.2×10^{-3} % for Yb^{3+}, show that the H_3thqN-SO_3 tripod is an efficient sensitizer of the NIR emission of these ions in spite of the presence of two coordinated water molecules. These yields are in the range of values reported for non-hydrated lanthanide complexes.[15–19,35] They are remarkable for a bis-hydrate lanthanide complex and comparable values have only been reported once recently for a bis-aqua Nd^{3+} complex of a pyridine based aminocarboxylate ligand (9.7×10^{-4} %) upon excitation at 266 nm.[36] In conclusion, for the first time, the 8-hydroxyquinolinate chelating unit has been used to prepare very stable Ln^{3+} complexes with potential bimodal imaging properties: a bis-aqua Gd^{3+} complex with a relaxivity of 5.16 mM^{-1} s^{-1} at 200 MHz and its Nd^{3+} or Yb^{3+} analogues showing sizeable NIR emission and long excitation wavelength. These properties indicate that 8-hydroxyquinolinate is a promising complexing motif which can be the starting point of a new class of multimodal reporters. Future work will be directed to the rational optimization of their relaxivity after proper characterization at the molecular level.[37–40]

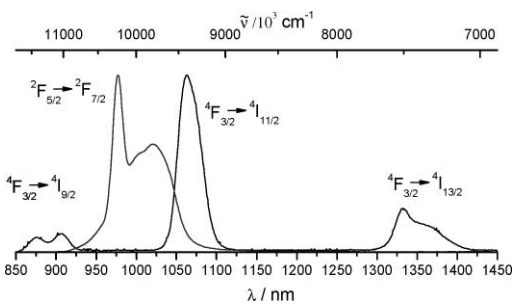

Fig. 2 Normalized emission spectra (λ_{ex} = 370 nm) of water solutions (1 mM) of $[Nd(thqN\text{-}SO_3)]^{3-}$ and $[Yb(thqN\text{-}SO_3)]^{3-}$, Nd($^4F_{3/2}$), Yb($^2F_{5/2}$).

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Notes and references

‡ **Synthesis and characterisation of H_3thqN-SO_3, 2,2′,2″-nitrilotris(methylene)tris(8-hydroxyquinoline-5-sulfonic acid):** 2,2′,2″-nitrilotris(methylene)triquinolin-8-ol (130 mg, 0.26 mmol) (see ESI†) was dissolved in the minimum amount of oleum (5 mL) 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 (2 × 10 mL), cold ethanol (2 × 10 mL), cold diethylether (2 × 10 mL) and then dried to give a brown solid (Yield = 99%). ^{1}H NMR (D_2O) δ (ppm): 9.23 (d, 1H, H4, 8.9 Hz), 8.05 (d, 1H, H6, 8.2 Hz), 7.79 (d, 1H, H3, 8.9 Hz), 7.20 (d, 1H, H7, 8.2 Hz), 4.98 (s, 2H, CH2). Elemental Anal. Calcd. for H_7thqN-SO_3·5.5H_2O·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.

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