



Cite this: DOI: 10.1039/c8dt03621g

# Eu(III) and Tb(III) complexes of 6-fold coordinating ligands showing high affinity for the hydrogen carbonate ion: a spectroscopic and thermodynamic study†

Fabio Piccinelli,<sup>a</sup> Chiara De Rosa,<sup>a</sup> Andrea Melchior,<sup>a</sup> Georgina Faura,<sup>b</sup> Marilena Tolazzi<sup>b</sup> and Marco Bettinelli<sup>a</sup>

In the present contribution, four classes of Ln(III) complexes (Ln = Eu and Tb) have been synthesized and characterized in aqueous solution. They differ by charge, **Ln(bpcd)<sup>+</sup>** [bpcd<sup>2-</sup> = *N,N'*-bis(2-pyridylmethyl)-*trans*-1,2-diaminocyclohexane *N,N'*-diacetate] and **Ln(bQcd)<sup>+</sup>** (bQcd<sup>2-</sup> = *N,N'*-bis(2-quinolinmethyl)-*trans*-1,2-diaminocyclohexane *N,N'*-diacetate) being positively charged and **Ln(PyC3A)** (PyC3A<sup>3-</sup> = *N*-picolyl-*N,N',N'*-*trans*-1,2-cyclohexylenediaminetriacetate) and **Ln(QC3A)** (QC3A<sup>3-</sup> = *N*-quinolyl-*N,N',N'*-*trans*-1,2-cyclohexylenediaminetriacetate) being neutral. Combined DFT, spectrophotometric and potentiometric studies reveal the presence, under physiological conditions (pH 7.4), of a couple of equally and highly stable isomers differing by the stereochemistry of the ligands (*trans*-N,N and *trans*-O,O for bpcd<sup>2-</sup> and bQcd<sup>2-</sup>; *trans*-O,O and *trans*-N,O for PyC3A<sup>3-</sup> and QC3A<sup>3-</sup>). Their high log  $\beta$  values (9.97 < log  $\beta$  < 15.68), the presence of an efficient antenna effect and the strong increase of the Ln(III) luminescence intensity as a function of the hydrogen carbonate concentration in physiological solution, render these complexes as very promising optical probes for a selective detection of HCO<sub>3</sub><sup>-</sup> *in cellulo* or in extracellular fluid. This particularly applies to the cationic **Eu(bpcd)<sup>+</sup>**, **Tb(bpcd)<sup>+</sup>** and **Eu(bQcd)<sup>+</sup>** complexes, which are capable of guesting up to two hydrogen carbonate anions in the inner coordination sphere of the metal ion, so that they show an unprecedented affinity towards HCO<sub>3</sub><sup>-</sup> (log *K* for the formation of the adduct in the 4.6–5.9 range).

Received 7th September 2018,  
Accepted 7th November 2018

DOI: 10.1039/c8dt03621g

rsc.li/dalton

## Introduction

Eu(III) and Tb(III) complexes have been broadly exploited as efficient optical probes in the field of bioimaging and sensing<sup>1–6</sup> due to the peculiar properties of their *f* ↔ *f* transitions, such as long luminescence lifetime and large energy difference between the absorbing and emissive states. These advantageous properties allow the mitigation of the interference of background fluorescence originating from the biological sample and the removal of self-absorption issues, respectively. Thanks to both the lack of self-absorption and the usual low concentration of the optical probe, which ensures

absorbance below 0.1 at the excitation wavelength, the intensity of the luminescence signal of the complexes is proportional to their concentration over a wide range of values. With this in mind, lanthanide-based molecular probes have been employed for the detection of the pH<sup>7</sup> and intracellular analytes such as ATP.<sup>8</sup> The optical properties of the complexes are strongly dependent on the nature of the ligand; the luminescence stemming from the metal ion can be conveniently sensitized if the ligand is capable of strongly absorbing and efficiently transferring the UV excitation to the metal ion (antenna effect). Furthermore, solvent molecules usually give rise to competitive non-radiative mechanisms such as the multiphonon relaxation process. On the basis of the “energy gap law”, if the gap between the emitting level and the one below is bridged by less than four vibrational quanta, the multiphonon relaxation process significantly works and the luminescence quantum yield will be low.<sup>9,10</sup> The high energy of the O–H vibrations in water molecules is particularly efficient in the non-radiative quenching of the emitting level and this phenomenon is especially relevant for applications in biomedicine, where aqueous media are commonly employed.

<sup>a</sup>Laboratorio Materiali Luminescenti, DB, Università di Verona, and INSTM, UdR Verona, Strada Le Grazie 15, 37134 Verona, Italy.

E-mail: fabio.piccinelli@univr.it

<sup>b</sup>Dipartimento Politecnico di Ingegneria e Architettura, Laboratorio di Tecnologie Chimiche, Università di Udine, via Cotonificio 108, 33100 Udine, Italy.

E-mail: andrea.melchior@uniud.it

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c8dt03621g

Nevertheless, Eu(III) and mainly Tb(III) are less affected by the multiphonon relaxation process as the energy gap between the emitting level and the lower lying ones is relatively large [about  $12\,400\text{ cm}^{-1}$  for Eu(III) and  $14\,800\text{ cm}^{-1}$  for Tb(III)].<sup>11</sup>

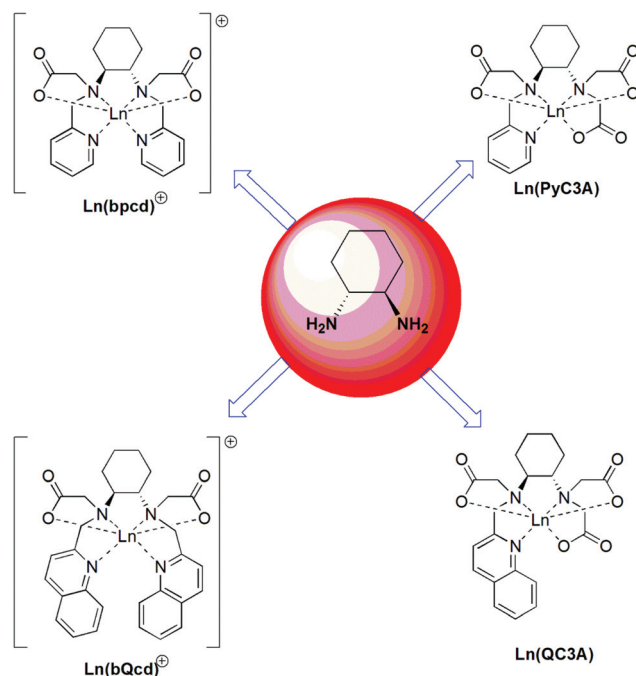
As far as optical sensing experiments based on luminescent lanthanide complexes are concerned, the displacement of water molecules from the inner coordination sphere of the metal ion by the target molecule is often employed. This displacement gives rise to an increase in the quantum yield and the concomitant increase in the luminescence intensity could be linked to the concentration of the analyte in solution.

Another important factor that must not be neglected when the compounds are used in *in vitro* experiments is the impact of the hydrophobicity and charge of lanthanide metal complexes on cell viability and cell association,<sup>12</sup> including their membrane permeability.<sup>13–17</sup> The *in vitro* localization of the optical probe affects the type of analyte that can be detected. For example, a probe with an extracellular location is particularly suitable to detect analytes such as group I ions, polysaccharides, hormones, or other signaling molecules.<sup>12</sup>

Another crucial aspect to consider is the selectivity of the optical response towards a particular analyte. In this context, the modulation of the structure of the side pendants within the DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetate)-based complexes gave the best results when serum proteins and biologically relevant ions are taken into account.<sup>18–21</sup>

In summary, the charge and lipophilicity of the probe and selectivity towards a particular target analyte are crucial properties that must be considered when designing a complex with potential application in optical sensing of biologically relevant species. As a natural extension of a recent structural and spectroscopic study, performed by some of us, on a promising chiro-optical probe based on a Tb(III) complex of a polyaminocarboxylate ligand [*N,N'*-bis(2-pyridylmethyl)-*trans*-1,2-diaminocyclohexane-*N,N'*-diacetic acid ( $\text{H}_2\text{bpcd}$ )],<sup>18,19</sup> we propose a new library of ligands and their relative Eu(III) and Tb(III) complexes, based on the chiral diaminocyclohexane (DACH) motif (Fig. 1).<sup>22–25</sup>

The proposed water-soluble complexes differ: (i) by charge, since  $\text{Ln}(\text{bpcd})^+$  and  $\text{Ln}(\text{bQcd})^+$  are cationic whilst  $\text{Ln}(\text{PyC3A})$  and  $\text{Ln}(\text{QC3A})$  are neutral, (ii) by steric hindrance at the metal ion, which is large in the case of  $\text{Ln}(\text{bQcd})^+$  and small in the case of  $\text{Ln}(\text{PyC3A})$  and (iii) by lipophilicity, the molecules containing the quinoline fragment being more hydrophobic than the relative pyridine-based ones. Total charge and steric hindrance are expected to have a strong impact on the stability of both the complexes and their adducts with target analytes so as to enable the opportunity of a selective probe–target interaction. In addition, in light of the lower number of donating atoms (6-fold coordination) for ligands shown in Fig. 1 than in the case of ligands commonly employed for Ln(III)-based luminescence anion sensing (*i.e.* DOTA-like possessing 8-fold coordination), we expect a higher number of target analyte molecules bound to the metal ion and a concomitant higher affinity towards them. This should be particularly true when non-sterically demanding anions are considered. Together with monoatomic anions, hydro-



**Fig. 1** Library of the complexes presented in this contribution:  $\text{Ln}(\text{bQcd})^+$  and  $\text{Ln}(\text{bpcd})^+$  are cationic complexes;  $\text{Ln}(\text{QC3A})$  and  $\text{Ln}(\text{PyC3A})$  are neutral complexes. The solvent molecules bound to the metal ion are omitted for the sake of clarity.

gen carbonate ( $\text{HCO}_3^-$ ) also meets this requirement and, in addition, plays a crucial role in many physiological processes<sup>26</sup> including intracellular pH homeostasis, kidney function and sperm maturation, and therefore must be considered an important target in probe development. Moreover, the  $\text{HCO}_3^-$  concentration is critical in assessing metabolic acidosis, that is abnormally increased hydrogen ion concentration. Patients with chronic kidney disease due to metabolic acidosis show low serum hydrogen carbonate concentrations.<sup>27,28</sup> For all these reasons, in the present contribution we present the synthesis, optical spectroscopy, the thermodynamic and structural characterization in aqueous solution of the Eu(III) and Tb(III) complexes as shown in Fig. 1. The good performance of these molecules in the optical detection of hydrogen carbonate anions under physiological conditions is also documented and analyzed in relation to the aforementioned properties of the complexes (*i.e.* total charge, steric hindrance, *etc.*).

## Experimental section

All commercially available reagents were used as received from their respective suppliers. Solvents (Sigma-Aldrich) were dried when required using an appropriate drying agent. Reactions requiring anhydrous conditions were carried out using Schlenk-line techniques under an atmosphere of dry argon. Water and  $\text{H}_2\text{O}$  refer to high purity water obtained from the 'Millipore Elix 10' purification system.  $\text{Eu}(\text{CF}_3\text{SO}_3)_3$  and  $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$  (Aldrich, 98%) were stored under vacuum for

several days at 80 °C and then transferred to a glove box. All other chemicals were purchased from Alfa Aesar.

Thin-layer chromatography was carried out on neutral alumina plates (Fluka Analytical) or silica plates (Sigma-Aldrich) and visualized under a UV lamp (254 nm). Cationic exchange chromatography was performed on SCX cartridges (1 g) purchased from "Agilent Technologies-sample Prep solutions".

***N,N'*-Bis(2-quinolinmethyl)-*trans*-1,2-diaminocyclohexane  
*N,N'*-*tert*-butyl diacetate (1*R*,2*R*) (2)**

Ligand **1** (1.8 g, 4.54 mmol) was dissolved in a mixture of anhydrous acetonitrile (80 mL) and anhydrous potassium carbonate under inert conditions (argon). Then, a solution of *tert*-butyl 2-bromoacetate (1.68 mL, 11.4 mmol) in anhydrous acetonitrile (15 mL) was added dropwise over ten minutes. After stirring for 12 h at room temperature dichloromethane was added and the reaction mixture was washed with brine solution. The organic phase was evaporated under reduced pressure to give 3.3 g of a yellowish oil. The crude product was purified by chromatography on activated neutral alumina (Al<sub>2</sub>O<sub>3</sub>, Cy:AcOEt from 9:1 to 1:9) giving 2.50 g of a yellowish oil (yield: 88%). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm) 8.07–8.04 (m, 4H, quinoline), 7.92 (d, *J* = 7.76 Hz, 2H, quinoline), 7.75 (d, *J* = 7.10 Hz, 2H, quinoline), 7.69 (t, *J* = 7.68 Hz, 2H, quinoline), 7.50 (t, *J* = 7.40 Hz, 2H, quinoline), 4.16 (m, 2H, methylene-ester), 3.86 (d, *J*<sub>GEM</sub> = 13.75 Hz, 2H, methylene-ester), 3.49 (d, *J*<sub>GEM</sub> = 17.22 Hz, 2H, methylene-quinoline), 3.31 (d, *J*<sub>GEM</sub> = 17.22 Hz, 2H, methylene-quinoline), 2.71 (m, 2H, methylene-cyclohexane), 2.18–1.12 (m, 8H, cyclohexane), 1.46 (s, 18H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm) 173.0, 159.3, 148.5, 135.3, 129.4, 129.0, 127.1, 125.8, 125.0, 122.0, 73.5, 57.8, 55.1, 53.7, 29.2, 26.7, 22.0. Elemental anal. calc. for C<sub>38</sub>H<sub>48</sub>N<sub>4</sub>O<sub>4</sub> (MW 624.8): C, 73.05; H, 7.74; N, 8.97; O, 10.24 found: C, 72.89; H, 7.51; N, 9.03; O, 10.36.

***N,N'*-Bis(2-quinolinmethyl)-*trans*-1,2-diaminocyclohexane  
*N,N'*-diacetic acid (1*R*,2*R*) (H<sub>2</sub>bQcd, ligand 3 as ammonium salt)**

**2** (1.20 g, 1.92 mmol) was dissolved in aq HCl (6 M, 30 mL); the obtained reaction mixture was stirred for 12 h at 80 °C. The reaction mixture was washed with ethyl acetate and the aqueous phase was evaporated under reduced pressure. The obtained brownish oil (2.47 g) was suspended in 10 mL of methanol, aqueous ammonia solution was added until pH 8–9 was reached and 1.05 g of a yellowish solid were obtained after chromatography [C18 column; eluent H<sub>2</sub>O:acetonitrile 4:6 + 0.1% aq NH<sub>4</sub>OH 30% w/w (50 mL)]. This solid was further purified by trituration in DCM:AcOEt:EtOH 1:2:2 at 80 °C for 30 minutes, obtaining 548 mg of a yellowish solid (ligand **3**, yield: 52%). UV-Vis absorption spectroscopy (water: methanol 9:1): ε (316 nm): 6728 M<sup>−1</sup> cm<sup>−1</sup>. <sup>1</sup>H-NMR (CD<sub>3</sub>OD) δ (ppm) 8.05–7.48 (m, 12H, quinoline), 3.74–3.55 (m, 8H, methylene-ester/quinoline), 2.39 (m, 2H, cyclohexane), 1.98 (m, 2H, cyclohexane), 1.67–1.28 (m, 6H, cyclohexane). <sup>13</sup>C-NMR (CD<sub>3</sub>OD) δ (ppm) 171.4, 160.1, 146.6, 137.3, 129.7, 127.9, 127.6, 126.7, 126.3, 121.2, 61.6, 56.1, 53.4, 24.5, 24.1. UV-Vis spectroscopy: ε (316 nm) = 6248 M<sup>−1</sup> cm<sup>−1</sup> (methanol). Elemental anal. calc.

for C<sub>30</sub>H<sub>38</sub>N<sub>6</sub>O<sub>4</sub> (MW 546.7): C, 65.91; H, 7.01; N, 15.37; O, 11.71 found: C, 65.79; H, 7.09; N, 15.27; O, 11.59.

The triflate of the cationic complex Eu(bQcd) (complex **4**) has been synthesized as follows: ligand **3** (100 mg, 0.182 mmol) was dissolved in hot (60 °C) 2-propanol (7 mL). Upon cooling, 98% europium(III) trifluoromethanesulfonate (109 mg, 0.182 mmol) was added portion-wise, and a yellowish suspension was formed. After neutralization with 2 M aq KOH (pH ≈ 7), the reaction mixture was stirred at room temperature for 2 h. The suspension was centrifuged, and the solid collected was suspended in methanol (5 mL). The resulting solid was removed by centrifugation, and the filtrate was concentrated under reduced pressure to give 44 mg of the desired product as a beige solid (yield: 30%). UV-Vis spectroscopy: ε (319 nm): 8808 M<sup>−1</sup> cm<sup>−1</sup> (water). Elemental anal. calc. for C<sub>31</sub>H<sub>30</sub>EuF<sub>3</sub>N<sub>4</sub>O<sub>7</sub>S·(H<sub>2</sub>O)<sub>2</sub> (MW 847.6): C, 43.93; H, 4.04; N, 6.61; O, 16.99; S, 3.78 found: C, 43.87; H, 4.00; N, 6.48; O, 17.04; S, 3.89.

**{2-[(Pyridyl-2-ylmethyl)-amino]-cyclohexyl}-carbamic acid *tert*-butyl ester **6** and {2-[(quinolyl-2-ylmethyl)-amino]-cyclohexyl}-carbamic acid *tert*-butyl ester **10****

Compound **5** (0.670 g, 3.13 mmol) was added to a solution of 2-quinolinecarboxaldehyde or 2-pyridinecarboxaldehyde (3.13 mmol) in ethanol (35 mL) and stirred at room temperature for 12 h. Sodium borohydride was slowly added to the mixture. The reaction was monitored by TLC (SiO<sub>2</sub>, cyclohexane:ethyl acetate 7:3 + NH<sub>4</sub>OH 30% w/w) and after 4 h the mixture was extracted twice with dichloromethane and the solvent was removed under reduced pressure to give respectively compounds **6** and **10** as yellowish oils, in quantitative yield, which were used in the next step without further purification.

***N*-Pyridyl-2-ylmethyl-cyclohexane-1,2-diamine (**7**) and  
*N*-quinolyl-2-ylmethyl-cyclohexane-1,2-diamine (**11**)**

Compound **6** or **10** (3.13 mmol) was added to a trifluoroacetic acid 98% w/w (13 mL) and dichloromethane (40 mL) solution and stirred at room temperature for 12 h. The solvent was removed under reduced pressure, and the obtained trifluoroacetate salt (≈3 g) was purified by cationic exchange chromatography (eluent: NH<sub>3</sub> 3 M in MeOH) to give 398 mg (yield 61%) of ligand **11**. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm) 8.13 (d, *J* = 8.33 Hz, 1H), 8.08 (d, *J* = 8.56 Hz, 1H), 7.81 (d, *J* = 8.33 Hz, 1H), 7.71 (t, *J* = 7.74 Hz, 1H), 7.55–7.48 (m, 2H), 4.25 (dd, *J*<sub>GEM</sub> = 14.55 Hz, 1H), 4.07 (dd, *J*<sub>GEM</sub> = 14.55 Hz, 1H), 3.71 (m, 1H), 2.50 (m, 1H), 2.18 (m, 2H), 1.92 (m, 1H), 1.75 (m, 1H), 1.70 (m, 1H), 1.35–1.04 (m, 5H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ (ppm) 160.2, 148.2, 135.4, 129.0, 128.8, 127.3, 126.1, 125.1, 122.1, 57.1, 53.2, 52.1, 31.4, 28.9, 22.3. Elemental anal. calc. for C<sub>16</sub>H<sub>21</sub>N<sub>3</sub> (MW 255.4): C, 75.26; H, 8.29; N, 16.46 found: C, 75.21; H, 8.22; N, 16.39.

**Compound 7:** Yield 38%. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm) 8.52 (m, 1H), 7.62 (t, *J* = 7.54 Hz, 1H), 7.34 (d, *J* = 7.70 Hz, 1H), 7.13 (t, *J* = 5.90 Hz, 1H), 4.03 (dd, *J*<sub>GEM</sub> = 14.09 Hz, 1H), 3.83 (dd, *J*<sub>GEM</sub> = 14.09 Hz, 1H), 3.64 (m, 1H), 2.43 (m, 1H), 2.18 (m, 2H), 1.88 (m, 1H), 1.75 (m, 1H), 1.70 (m, 1H), 1.35–1.04 (m, 5H).

$^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm) 159.5, 149.0, 136.0, 123.5, 120.6, 57.8, 52.1, 31.5, 29.4, 22.3, 21.5. Elemental anal. calc. for  $\text{C}_{12}\text{H}_{19}\text{N}_3$  (MW 205.3): C, 70.20; H, 9.33; N, 20.47 found: C, 70.15; H, 9.19; N, 20.44.

***N*-Picoly-*N,N,N'*-*trans*-1,2-cyclohexylenediamine-*tert*-butyl triacetate (8) and *N*-quinolyl-*N,N,N'*-*trans*-1,2-cyclohexylenediamine-*tert*-butyl triacetate (12)**

Under an inert atmosphere, compound 7 or 11 (1.94 mmol, 1.20 mmol, respectively) was dissolved in an anhydrous acetonitrile (40 ml or 25 mL) solution of *N,N*-diisopropylethylamine (6.8 mmol or 4.19 mmol). Then, *tert*-butyl 2-bromoacetate (6.8 mmol or 4.19 mmol) in anhydrous acetonitrile (10 mL or 5 mL) was added dropwise. The reaction was monitored using TLC ( $\text{SiO}_2$ ,  $R_f$ : 0.47, DCM:MeOH 95:5 + 0.5%  $\text{NEt}_3$ ) and after 12 h, water (approx. 25 mL) was added and the reaction mixture was extracted twice with dichloromethane. The combined organic phases were dried on anhydrous  $\text{Na}_2\text{SO}_4$  and the solvent was evaporated under reduced pressure to give 0.580 g of the crude product which was purified by chromatography (on silica gel, DCM/MeOH 95:5 + 0.5% triethylamine,  $R_f$ : 0.47) giving rise to compounds 8 (yield 58%) and 12 (yield = 55%).

Compound 8:  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm) 9.97 (d,  $J$  = 6.56, 1H), 8.79 (d,  $J$  = 7.91, 1H), 8.34 (t,  $J$  = 7.91, 1H), 7.94 (t,  $J$  = 6.56, 1H), 6.30 (dd,  $J_{\text{GEM}}$  = 17.64 Hz, 1H), 5.94 (dd,  $J_{\text{GEM}}$  = 17.64 Hz, 1H), 4.55 (dd,  $J_{\text{GEM}}$  = 16.93 Hz, 2H), 3.53 (m, 2H), 3.44 (dd,  $J_{\text{GEM}}$  = 16.93 Hz, 2H), 2.66 (m, 3H), 2.08 (m, 2H), 1.78 (m, 2H), 1.45 (s, 27H), 1.12 (m, 3H).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm) 172.4, 171.0, 170.5, 159.4, 149.4, 136.0, 121.3, 123.0, 72.9, 73.3, 73.5, 57.3, 55.5, 55.3, 54.0, 54.2, 30.5, 30.1, 29.4, 27.3, 26.9, 22.2. Elemental anal. calc. for  $\text{C}_{30}\text{H}_{49}\text{N}_3\text{O}_6$  (MW 547): C, 65.78; H, 9.02; N, 7.67; O, 17.53 found: C, 65.70; H, 8.95; N, 7.73; O, 17.41.

Compound 12:  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm) 8.12 (m, 2H), 8.07 (d, 1H), 7.81 (d,  $J$  = 7.92, 1H), 7.67 (t,  $J$  = 7.71, 1H), 7.51 (t,  $J$  = 7.29, 1H), 4.32 (dd,  $J_{\text{GEM}}$  = 13.79 Hz, 1H), 3.94 (dd,  $J_{\text{GEM}}$  = 13.79 Hz, 1H), 3.60 (m, 2H), 3.52 (m, 4H), 2.81 (m, 1H), 2.67 (m, 1H), 2.15 (m, 1H), 2.09 (m, 1H), 1.74 (m, 2H), 1.45 (s, 27H), 1.14 (m, 4H).  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  (ppm) 173.1, 172.9, 172.1, 160.2, 148.0, 134.0, 130.3, 127.5, 126.0, 124.7, 123.0, 74.5, 74.1, 73.3, 58.2, 57.4, 56.0, 55.3, 55.0, 54.1, 33.0, 32.3, 32.1, 28.1, 27.3, 22.8, 22.1. Elemental anal. calc. for  $\text{C}_{34}\text{H}_{51}\text{N}_3\text{O}_6$  (MW 597.8): C, 68.31; H, 8.60; N, 7.03; O, 16.06 found: C, 68.27; H, 8.51; N, 7.00; O, 15.97.

***N*-Picoly-*N,N,N'*-*trans*-1,2-cyclohexylenediaminetriacetic acid ( $\text{H}_3\text{PyC3A}$ , 9 as ammonium salt) and *N*-quinolyl-*N,N,N'*-*trans*-1,2-cyclohexylenediaminetriacetic acid ( $\text{H}_3\text{QC3A}$ , 13 as ammonium salt)**

Compound 8 or 12 (1.12 mmol, 0.652 mmol, respectively) was added to an aqueous HCl (6 M, 22 ml or 13 ml) solution and stirred at  $\approx 80^\circ\text{C}$  for 12 h. After neutralization with NaOH, extraction with DCM was performed and the resulting aqueous solution was evaporated under reduced pressure. The solid was washed with ethanol for 1 h at  $80^\circ\text{C}$ . Upon cooling, the suspension was filtered to remove all the insoluble inorganic salts and the resulting solution was evaporated under reduced

pressure and the crude product was purified by ionic exchange chromatography to give the corresponding product 9 or 13 (yield = 24% for 9 and 40% for 13). Compound 9: ESI-MS (scan ES+;  $m/z$ ): 468 (100%); 469 (20%) ( $[\text{Na}_4(\text{PyC3A})]^{4+}$ ). Elemental anal. calc. for  $\text{C}_{18}\text{H}_{34}\text{N}_6\text{O}_6$  (MW 430.5): C, 50.22; H, 7.96; N, 19.52; O, 22.30 found: C, 50.18; H, 7.90; N, 19.47; O, 22.18.

Compound 13: ESI-MS (scan ES+;  $m/z$ ): 513 (100%); 514 (25%) ( $[(\text{NH}_4)\text{Na}_3(\text{QC3A})]^{4+}$ ). Elemental anal.  $\text{C}_{22}\text{H}_{36}\text{N}_6\text{O}_6$  (MW 480.6): C, 54.99; H, 7.55; N, 17.49; O, 19.98 found: C, 54.90; H, 7.46; N, 17.42; O, 20.01.

$\text{Eu}(\text{PyC3A})$  (14a) [and  $\text{Tb}(\text{PyC3A})$  (14b)] has been synthesized as follows: ligand 9 (60 mg, 0.140 mmol) was partially dissolved in a mixture of 2-propanol:ethanol 1:1 (4 mL) by heating at  $\approx 60^\circ\text{C}$ . Then, 98% europium(III) trifluoromethanesulfonate (83.6 mg, 0.140 mmol) was added portion-wise and the pH of the solution was carefully adjusted to 7 by the addition of 2 M aq KOH. The obtained suspension was stirred at room temperature for 12 h. The collected solid ( $\approx 94$  mg) was re-crystallized in methanol ( $\approx 10$  mL) and  $\text{Et}_2\text{O}$  ( $\approx 30$  mL) solution to yield 70 mg (yield 95%) of a whitish solid (14a). UV-Vis spectroscopy:  $\epsilon$  (265 nm):  $3390\text{ M}^{-1}\text{ cm}^{-1}$  (water). ESI-MS (scan ES+;  $m/z$ ): 552 (100%); 550 (90%) ( $[\text{NaEu}(\text{PyC3A})]^{2+}$ ). Elemental anal. calc. for  $\text{C}_{18}\text{H}_{22}\text{EuN}_3\text{O}_6 \cdot (\text{H}_2\text{O})_2$  (MW 564.4): C, 38.31; H, 4.64; N, 7.45; O, 22.68 found: C, 38.28; H, 4.54; N, 7.40; O, 22.51.

Ligand 9 (27 mg, 0.063 mmol) was dissolved in water (3 mL), then terbium(III) chloride hexahydrate (23.5 mg, 0.063 mmol) was added portion-wise and the pH of the solution was carefully adjusted to 7 by the addition of 2 M aq KOH. The obtained solution was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was re-crystallized in methanol ( $\approx 2$  mL) and  $\text{Et}_2\text{O}$  ( $\approx 15$  mL) solution yielding a white solid after centrifugation (34 mg of 14b, quantitative yield). UV-Vis spectroscopy:  $\epsilon$  (266 nm):  $4008\text{ M}^{-1}\text{ cm}^{-1}$  (water). ESI-MS (scan ES+;  $m/z$ ): 558 (100%) ( $[\text{NaTb}(\text{PyC3A})]^{2+}$ ). Elemental anal. calc. for  $\text{C}_{18}\text{H}_{22}\text{TbN}_3\text{O}_6 \cdot (\text{H}_2\text{O})_2$  (MW 571.3): C, 37.84; H, 4.59; N, 7.35; O, 22.40 found: C, 37.78; H, 4.50; N, 7.30; O, 22.37.

$\text{Eu}(\text{QC3A})$  (15) has been synthesized as follows: compound 13 (60 mg, 0.125 mmol) was partially dissolved in a mixture of 2-propanol:ethanol 8:2 (6 mL) at  $\approx 60^\circ\text{C}$ . Then, 98%  $\text{Eu}(\text{CF}_3\text{SO}_3)_3$  (75 mg, 0.125 mmol) was added portion-wise followed by 2 M aq KOH until pH  $\approx 7$ . The obtained suspension was stirred at room temperature for 12 h. The solid was removed under centrifugation, and the solution was dried under reduced pressure to give  $\approx 112$  mg of a white solid, which was re-crystallized in ethanol ( $\approx 10$  mL) and  $\text{Et}_2\text{O}$  ( $\approx 40$  mL) solution, yielding a white solid after centrifugation (55 mg; yield 76%). UV-Vis spectroscopy:  $\epsilon$  (319 nm) =  $3725\text{ M}^{-1}\text{ cm}^{-1}$  (water). ESI-MS (scan ES+;  $m/z$ ): 602 (100%); 600 (92%) ( $[\text{NaEu}(\text{QC3A})]^{2+}$ ). Elemental anal. calc. for  $\text{C}_{22}\text{H}_{24}\text{EuN}_3\text{O}_6 \cdot (\text{H}_2\text{O})_2$  (MW 614.4): C, 43.00; H, 4.59; N, 6.84; O, 20.83 found: C, 42.97; H, 4.54; N, 6.74; O, 20.76.

**$^1\text{H}$ -NMR spectroscopy**

Nuclear magnetic resonance (NMR) experiments were performed at 298.15 K using a 600 MHz Bruker Avance III spectro-



meter equipped with a triple resonance TCI cryogenic probe. Spectra were usually recorded in  $\text{CDCl}_3$  and, unless otherwise noted, chemical shifts are expressed as ppm and referenced to the internal standard tetramethylsilane (TMS). One dimensional NMR spectra were recorded with 8 or 16 scans and a spectral width of 12 019 Hz. All spectra were manually phased and baseline corrected using TOPSPIN 3.2 (Bruker, Karlsruhe, Germany). Chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; b, broad), coupling constants and integration area are reported.

### Elemental analysis

Elemental analyses were carried out by using an EACE 1110 CHNOS analyzer.

### Potentiometric titrations

The protonation constants of the ligands (bQcd (**3**), PyC3A (**9**) and QC3A (**13**)) were determined by acid–base potentiometric titrations. The titration cell was maintained at constant temperature ( $298.15 \pm 0.1$  K) using a circulatory bath. A computer-controlled potentiometer (Amel Instruments, 338 pH meter) collected the electromotive force (emf) values measured by means of a combined glass electrode (Metrohm Unitrode 6.0259.100). Before each titration the electrode was calibrated by an acid–base titration with standard HCl and NaOH solutions and the carbon dioxide contamination in solution was checked by Gran's method.<sup>29</sup> Titrations were performed in duplicate on solutions containing the ligand (typical concentration around 0.9 mM for **3**, 0.6 mM for **9** and 0.7 mM for **13**) and an excess of standard HCl by adding standard NaOH solution. The pH range was varied from an initial approximate value of 2.3 to about pH 11.5. All the solutions were prepared with ultrapure water ( $>18$  M $\Omega$  cm) from a Milli-Q system (ELGA Purelab Option-Q) and the ionic strength ( $\mu$ ) was adjusted to 0.1 M by using appropriate amounts of NaCl (Sigma-Aldrich). Among 50–70 points were collected in each titration and processed with Hyperquad.<sup>30</sup>

### Spectrophotometric titrations

The formation constants of all the L–Ln(III) complexes (L = **3**, **9**, **13**; Ln = Eu, Tb) were determined by UV-Vis spectrophotometric acid–base titrations.<sup>31</sup> A Varian Cary 50 instrument equipped with a fibre optic (optical path of 10 mm) was used. The wavelength range investigated was 240–300 nm for **9** and 275–355 nm for **3** and **13** in the same pH range and  $\mu$  as in the potentiometry. The titration cell was maintained at  $T = 298.15 \pm 0.1$  K by means of a circulatory bath, and contained both the ligand (ligand **3**, 0.08 mM with Eu(III), 0.03 mM with Tb(III); ligand **9**, 0.13 mM with Eu(III), 0.15 mM with Tb(III); ligand **13**, 0.09 mM with both, Eu(III) and Tb(III)) and the Ln(III) (1 : 1 L : Ln(III) ratio, with a slight metal excess). The NaOH and HCl stock solutions were the same as those used during the potentiometric titrations. The stock solutions of Eu(III) and Tb(III) were prepared by dissolving their chloride hexahydrate salts (Sigma-Aldrich). The lanthanide content in the stock solutions was determined by EDTA titration, using xylenol

orange as an indicator.<sup>32</sup> Free acid concentrations in lanthanide solutions were checked by Gran's method.<sup>29</sup> Formation constants were calculated by a simultaneous fit of the absorbance values at several wavelengths using HypSpec.<sup>30</sup>

### ESI-MS

Electrospray ionization mass spectra (ESI-MS) were recorded with a Finnigan LXQ Linear Ion Trap (Thermo Scientific, San Jose, CA, USA) operating in positive ion mode. The data acquisition was under the control of Xcalibur software (Thermo Scientific). A MeOH solution of the sample was properly diluted and infused into the ion source at a flow rate of 10  $\mu\text{L min}^{-1}$  with the aid of a syringe pump. The typical source conditions were a transfer line capillary at 275 °C; ion spray voltage at 4.70 kV; sheath, auxiliary and sweep gas ( $\text{N}_2$ ) flow rates at 10, 5 and 0 arbitrary units, respectively. Helium was used as the collision damping gas in the ion trap set at a pressure of 1 mTorr.

### DFT calculations

As the paramagnetic Eu(III) and Tb(III) complexes are rather difficult to model computationally, the analogues of the diamagnetic Y(III) ion were studied. It has been shown that Y(III) complexes may serve as suitable models for the Eu(III) analogues,<sup>33–36</sup> consistent with the fact that their ionic radii differ from those of Eu(III) ions by about 0.05 Å [and less for Tb(III)]. Geometry optimizations of the  $[\text{Y}(\text{L})(\text{H}_2\text{O})_n]$  complexes were carried out at the DFT level under vacuum using the B3LYP exchange–correlation functional.<sup>37,38</sup> The 6-31+G(d) basis set was employed for the ligand atoms, while the Y(III) ion was described by the quasi-relativistic small core Stuttgart–Dresden pseudopotential and the relative basis set.<sup>39</sup> This level of theory was previously demonstrated to provide correct geometries and thermochemical properties, also maintaining the calculation feasible with similar complex systems.<sup>23,33</sup> All final geometries were checked to be minima by vibrational analysis. Solvent effects were included by means of the PCM model.<sup>40</sup> All calculations were carried out with Gaussian16.<sup>41</sup>

The complexes studied were the *trans*-O,O and *trans*-N,N isomers of the Y(III) complexes  $[\text{Y}(\text{trans-O,O-bQcd})(\text{H}_2\text{O})_5]^+$  and  $[\text{Y}(\text{trans-N,N-bQcd})(\text{H}_2\text{O})_5]^+$ , as the *cis*-O,O, *cis*-N,N isomer was demonstrated to be much less stable in the case of bpcd.<sup>33</sup> As previously done<sup>33</sup> for the analogues with bpcd, five water molecules were initially placed near the metal ion. During geometry optimization only two of them were retained in the first coordination sphere. Also in the case of complexes with triacetate ligands  $[\text{Y}(\text{PyC3A})(\text{H}_2\text{O})_3]$  and  $[\text{Y}(\text{QC3A})(\text{H}_2\text{O})_3]$  one water molecule was always expelled from the starting structure to provide a final complex with only two inner sphere water molecules. Since the final number of coordinated water molecules was 2 in all cases, the  $[\text{Y}(\text{L})(\text{H}_2\text{O})_2]$  complexes were considered for structural comparisons. The calculations have been performed on the two possible coordination geometries of the ligands (Fig. S1†) giving rise to  $[\text{Y}(\text{trans-O,O-L})(\text{H}_2\text{O})_2]$  and  $[\text{Y}(\text{trans-N,O-L})(\text{H}_2\text{O})_2]$  isomeric complexes.

## Luminescence and decay kinetics

Room temperature luminescence was measured with a Fluorolog 3 (Horiba-Jobin Yvon) spectrofluorometer, equipped with a Xe lamp, a double excitation monochromator, a single emission monochromator (mod. HR320) and a photomultiplier in photon counting mode for the detection of the emitted signal. All the spectra were corrected for the spectral distortions of the setup.

In decay kinetics measurements, a xenon microsecond flashlamp was used and the signal was recorded by means of a multichannel scaling method. True decay times were obtained using the convolution of the instrumental response function with an exponential function and the least-squares-sum-based fitting program (SpectraSolve software package). The total quantum yields ( $\Phi_{\text{Tot}}$ ) have been obtained by secondary methods described in the literature<sup>42</sup> by measuring the visible emission spectrum of quinine bisulfate in 1 N H<sub>2</sub>SO<sub>4</sub> solution, a fluorescence quantum yield reference sample ( $\Phi = 54.6\%$ ).  $\Phi_{\text{Tot}}$  for the complexes has been calculated by the  $[(A_s \cdot F_u \cdot n^2)/(A_u \cdot F_s \cdot n_0^2)] \cdot \Phi_s$  equation; where: the u subscript refers to unknown and s to the standard and other symbols have the following meanings:  $\Phi$  is quantum yield,  $A$  is the absorbance at the excitation wavelength,  $F$  is the integrated emission area across the band and  $n$ 's are respectively indexes of refraction of the solvent containing the unknown ( $n$ ) and the standard ( $n_0$ ) at the sodium D line and the temperature of the emission measurement (see ESI, Fig. S15–S20†).

## Luminescence sensing of HCO<sub>3</sub><sup>−</sup>

The binding interactions between hydrogen carbonate and the Eu(III) complexes were studied using the double reciprocal plot

following the Benesi–Hildebrand equation<sup>43</sup> adapted to the values of the asymmetry ratio ( $R$ ) of the Eu(III) emission spectra:

$$\frac{R_0}{R - R_0} = \frac{R_0}{R_\infty - R_0} + \frac{R_0}{\{K(R_\infty - R_0)[\text{HCO}_3^-]^n\}}$$

where  $R_0$ ,  $R$ , and  $R_\infty$  are the asymmetry ratios of Eu(III) in the complexes considered in the absence of hydrogen carbonate, at an intermediate hydrogen carbonate concentration and at a concentration of complete interaction, respectively. In the above equation,  $K$  is the binding constant and  $n$  is the number of hydrogen carbonate anions bound to the metal center and  $[\text{HCO}_3^-]$  is the hydrogen carbonate concentration. The models have been validated by the statistical tests present in the MS-Excel cEST program here adapted to treat fluorescence data.<sup>44</sup>

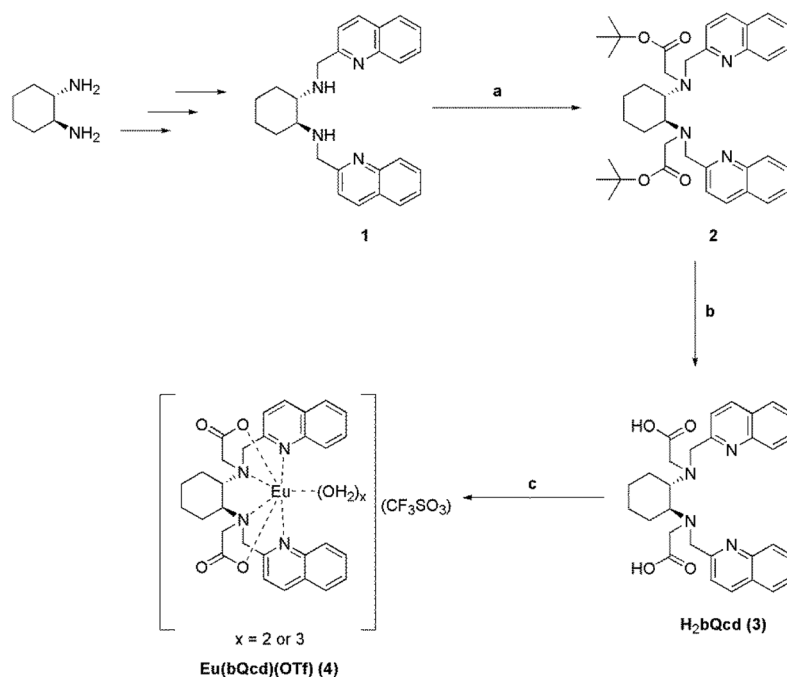
## Results and discussion

### Synthesis

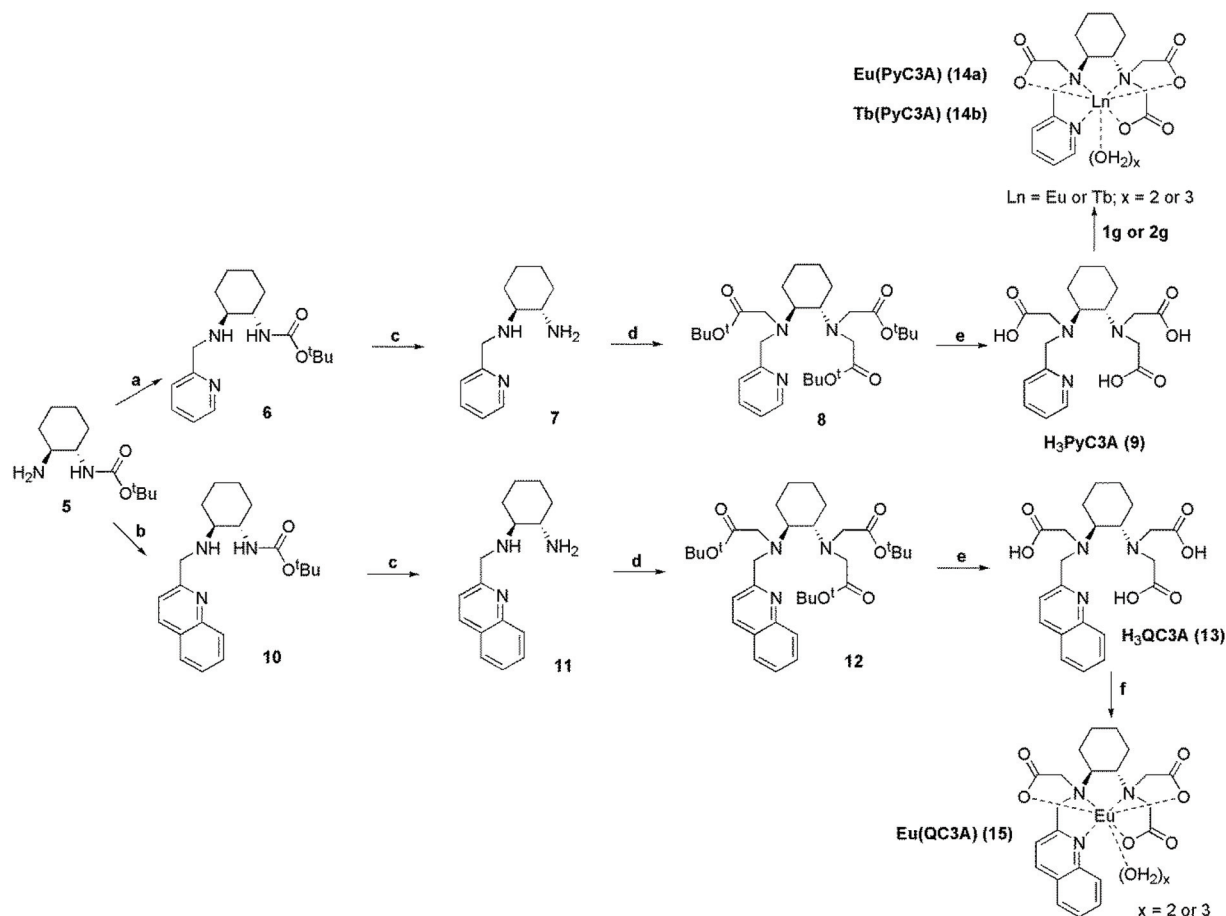
The synthesis of the ligands and the relative Ln(III) complexes discussed in this paper are presented in Schemes 1 and 2.

For the synthesis of C<sub>1</sub>-symmetric ligands **9** and **13**, we exploit the straightforward chemistry of the *t*-butoxycarbonyl (BOC) protective group. In this context, the derivative **5** (Scheme 2) can be obtained in good yield as previously reported.<sup>45</sup> All the ligands (**9** and **13**) and the relative Ln complexes (**14** and **15**) have been obtained in good yield and with a high degree of purity (see the Experimental section for details).

The chlorides of the cationic complexes Eu(bpcd) and Tb(bpcd) were synthesized as reported previously.<sup>33</sup> The synthesis



**Scheme 1** Synthetic protocol for the synthesis of H<sub>2</sub>bQcd (**3**) and Eu(bQcd)(CF<sub>3</sub>SO<sub>3</sub>) (**4**). (a) 3 eq. *tert*-Butyl bromoacetate, 3.2 eq. K<sub>2</sub>CO<sub>3</sub>, MeCN, room temperature, 12 h; (b) 6 M aq. HCl, 80 °C, 12 h; (c) 1 eq. Eu(OTf)<sub>3</sub>, 2-propanol, room temperature, 12 h.



**Scheme 2** Synthetic protocol for the synthesis of the ligands H<sub>3</sub>PyC3A (**9**), H<sub>3</sub>QC3A (**13**), the Eu(III) complexes Eu(PyC3A) (**14a**), Eu(QC3A) (**15**) and the Tb(III) complex Tb(PyC3A) (**14b**). (a) 1 eq. pyridine-2-carbaldehyde, absolute ethanol, room temperature, 12 h; 1.2 eq. NaBH<sub>4</sub>, MeOH, room temperature, 12 h; (b) 1 eq. quinoline-2-carbaldehyde, absolute ethanol, room temperature, 12 h; 1.2 eq. NaBH<sub>4</sub>, MeOH, room temperature, 12 h (c) trifluoroacetic acid : dichloromethane (1 : 3), room temperature, 12 h; (d) 3.5 eq. *tert*-butyl bromoacetate, 3.5 eq. *N,N*-diisopropylethylamine, MeCN, room temperature, 12 h; (e) 6 M aq. HCl, 80 °C, 12 h; (f) 1 eq. Eu(OTf)<sub>3</sub>, 2-propanol : ethanol (8 : 2), room temperature, 12 h; (1 g) 1 eq. Eu(OTf)<sub>3</sub>, 2-propanol : ethanol (1 : 1), room temperature, 12 h; (2 g) 1 eq. TbCl<sub>3</sub>·6H<sub>2</sub>O, water, room temperature, 12 h.

of **1** (Scheme 1) has already been reported previously,<sup>24</sup> as well as the synthesis of **5**.<sup>45</sup>

### Protonation constants

The best fit of the potentiometric data was obtained for all three ligands (**3**, **9** and **13**) when four protonated species were considered. The obtained log *K<sub>n</sub>* values are reported in Table 1 along with the constants for similar ligands containing the

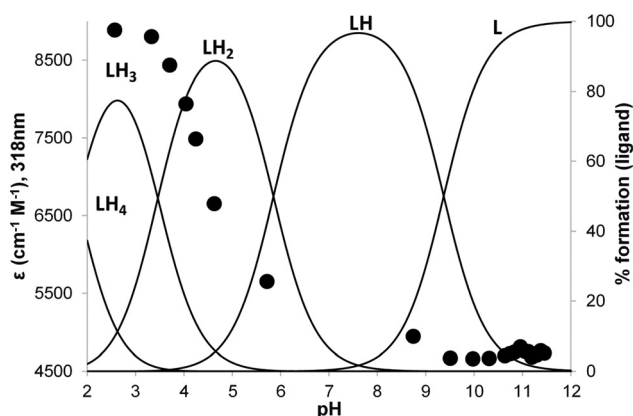
chiral DACH backbone: bpdc,<sup>33</sup> PyC3A (**9**)<sup>46</sup> and CDTA<sup>46</sup> (1,2-cyclohexanediaminetetraacetic acid). The titration curves for ligands **3**, **9** and **13** are displayed in Fig. S2, S3 and S4† respectively, together with the speciation plots calculated by using the protonation constants shown in Table 1.

The log *K* values reported in Table 1 indicate that two fairly strong acidic and two weakly acidic sites are present. In particular, the values for the first protonation constant of the

**Table 1** Protonation constants (log *K<sub>n</sub>*, *K<sub>n</sub>* = [LH<sub>*n*</sub>]/([H]<sup>+</sup>[LH<sub>*n*-1</sub>])) of the ligands **3**, **9** and **13** with their confidence intervals (*T* = 298.15 K and *μ* = 0.1 M NaCl). Additional protonation data for similar ligands are also reported. Charges are omitted for clarity

	bQcd ( <b>3</b> )	PyC3A ( <b>9</b> )	QC3A ( <b>13</b> )	bpdc <sup>a</sup>	PyC3A <sup>b</sup>	CDTA <sup>b</sup>
log <i>K</i> <sub>1</sub>	9.37 ± 0.03	10.26 ± 0.02	10.53 ± 0.03	9.72 ± 0.02	10.16 ± 0.02	9.43 ± 0.02
log <i>K</i> <sub>2</sub>	5.85 ± 0.07	6.33 ± 0.07	6.29 ± 0.09	5.87 ± 0.07	6.39 ± 0.04	6.01 ± 0.02
log <i>K</i> <sub>3</sub>	3.46 ± 0.10	3.67 ± 0.11	3.60 ± 0.16	2.94 ± 0.12	3.13 ± 0.03	3.68 ± 0.02
log <i>K</i> <sub>4</sub>	1.79 ± 0.31	2.01 ± 0.14	2.81 ± 0.16	2.22 ± 0.17	—	2.51 ± 0.05

<sup>a</sup> Ref. 33. <sup>b</sup> Ref. 46, *μ* = 0.15 M NaCl.



**Fig. 2** Species distribution of ligand **3** (L) along with the molar absorbance values at  $\lambda = 318$  nm obtained by acid–base spectrophotometric titration ( $T = 298.15$  K,  $\mu = 0.1$  M NaCl). The speciation was calculated by using the fitted protonation constants (Table 1) and the concentration of **3** ( $[L] = 0.09$  mM dm<sup>-3</sup>). Charges are omitted for clarity.

ligands **3**, **9** and **13** (Table 1) are in agreement with those reported for tertiary amines ( $\log K \sim 6.9$ – $10.7$ , depending on the substituents).<sup>47</sup> This suggests that the first protonation constant can be assigned to an aliphatic amino group, as previously reported for bpdc<sup>33</sup> and CDTA.<sup>48</sup>

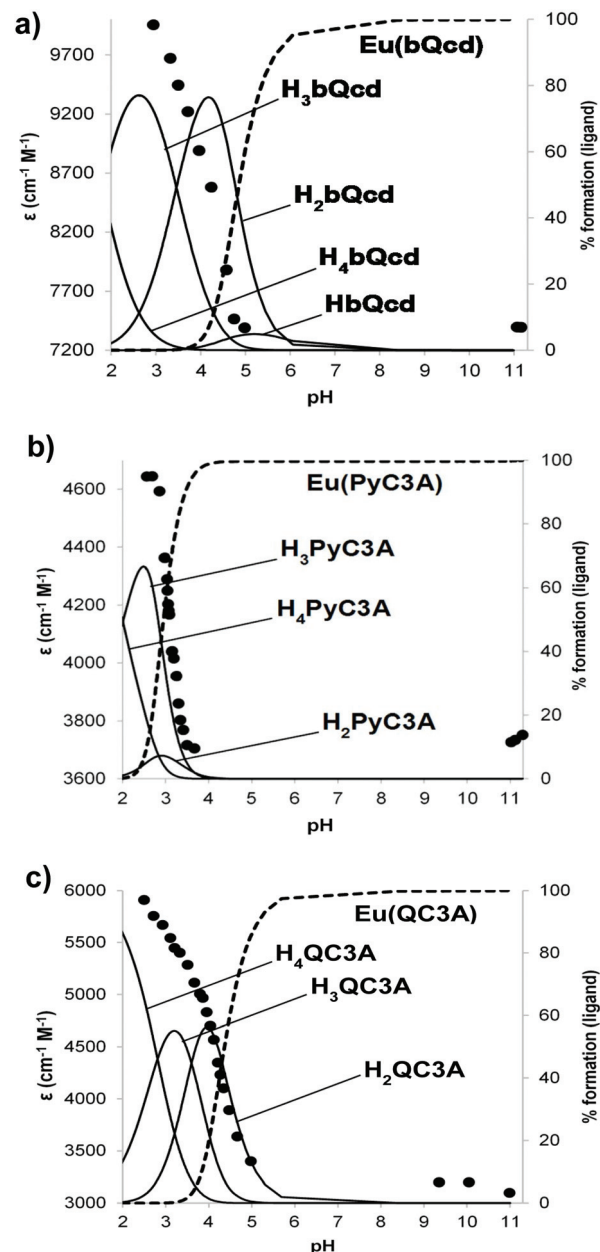
Spectrophotometric acid–base titrations were performed in order to study the species distribution of the ligand as a function of the pH. The molar absorbance ( $\epsilon_\lambda$ ) variations are reported in Fig. 2, for ligand **3**.

Similar plots are reported for ligands **9** and **13** in Fig. S5 and S6.† For ligand **3** (Fig. 2),  $\epsilon_\lambda$  is nearly constant in the range of 11–8.5 and then increases below pH  $\sim 8.5$  with the formation of the bi- and tri-protonated species ( $\log K_2 = 5.85$ ,  $\log K_3 = 3.46$ ). This change is related to the protonation of the quinoline moieties, and is compatible with the protonation constant of quinoline ( $\log K = 4.97$ ).<sup>49</sup> The  $\epsilon_\lambda$  values of **9** and **13** increase below pH  $\sim 8$  together with the formation of LH<sub>2</sub> species, presumably related to the protonation of the pyridine and quinoline moieties. The associated protonation values ( $\log K_2 = 6.33$  for **9**,  $\log K_2 = 6.29$  for **13**) are in line with the protonation constants of 2-methylpyridine (picoline) and quinoline ( $\log K = 6.06$  for picoline,  $\log K = 4.97$  for quinoline).<sup>49,50</sup> The remaining protonation constants ( $\log K_4$  for **3**,  $\log K_3$  and  $\log K_4$  for **9** and **13**) could be ascribed to acetate moieties.<sup>51</sup>

### Ln(III) complex formation

The formation constants of the Eu(III) and Tb(III) complexes with ligands **3**, **9** and **13** were determined by acid–base spectrophotometric titration. In Fig. S7† the absorbance changes upon the addition of a base to equimolar solutions of Eu(III) and ligands **9** and **13** are shown. The spectra for the complexes containing the same chromophore are very similar (Fig. S8†).

In Fig. 3, the speciation diagrams for each studied Eu(III) complex along with the  $\epsilon_\lambda$  changes for quinoline- ( $\lambda = 318$  nm) and pyridine- ( $\lambda = 260$  nm) based ligands are reported as a



**Fig. 3** Species distribution of the complexes for the ligands (a) **3** (0.08 mM), (b) **9** (0.13 mM) and (c) **13** (0.09 mM) with Eu(III) (ratio 1 : 1 M : L, with a little excess of metal), along with the molar absorbance values at  $\lambda = 318$  nm (for the ligands **3** and **13**) and  $\lambda = 265$  nm (for ligand **9**) obtained by acid–base spectrophotometric titration at  $T = 298.15$  K and  $\mu = 0.1$  M. Charges and negligible species (below 5%) are omitted for clarity.

function of pH. The same plots for the Tb(III) complexes are very similar to their Eu(III) analogues and are reported in Fig. S9.† In all plots, the formation of the complex is accompanied by a steep decrease of  $\epsilon_\lambda$ .

The best fit of the spectrophotometric data has been obtained when only the ML species was considered and the formation constants obtained for Eu(III) and Tb(III) are reported in Table 2 along with those available for similar ligands for



**Table 2** Formation constant ( $\log \beta$ ) complexes of the ligands **3**, **9** and **13** with Eu(III) and Tb(III) at  $T = 298.15$  K and  $\mu = 0.1$  M NaCl. Other similar complexes have been added for comparison. Charges are omitted for clarity

Complex	bQcd ( <b>3</b> )	PyC3A ( <b>9</b> )	QC3A ( <b>13</b> )	bpcd <sup>a</sup>	bped <sup>b</sup>	PEDTA <sup>c</sup>	CDTA <sup>d</sup>
Eu(III)L	9.97 ± 0.08	15.682 ± 0.009	12.55 ± 0.16	11.19 ± 0.32	—	—	19.6
Eu(III)L(OH)	—	—	—	2.18 ± 0.57	—	—	—
Tb(III)L	9.80 ± 0.13	15.70 ± 0.02	12.08 ± 0.28	11.36 ± 0.15	—	—	20.0
Tb(III)L(OH)	—	—	—	2.04 ± 0.33	—	—	—
Gd(III)L	—	—	—	—	12.37	15.56	19.6
Gd(III)L(OH)	—	—	—	—	2.1	—	—

<sup>a</sup> Ref. 33. <sup>b</sup> Ref. 52,  $\mu = 0.16$  M NaCl. <sup>c</sup> Ref. 53. <sup>d</sup> Ref. 54.

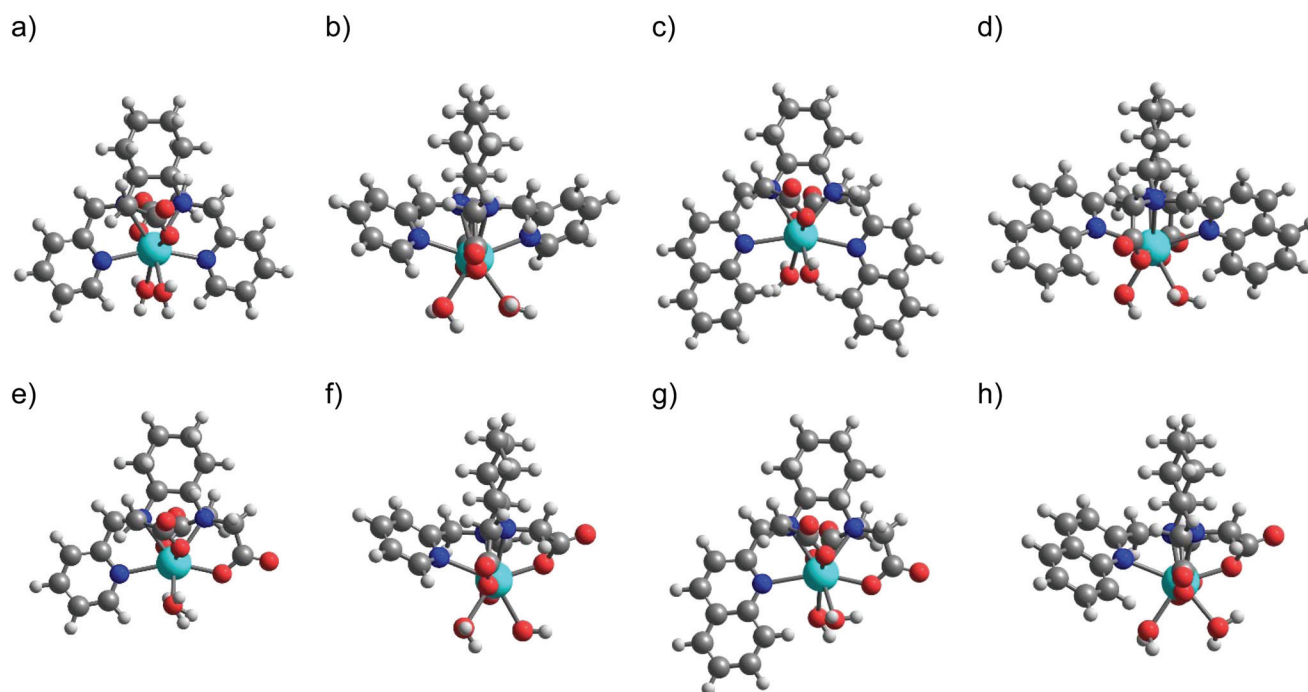
comparison. According to this model, at pH = 7.4 the ML species is largely predominant in all cases (>99%).

As expected on the basis of the strong oxophilicity of Ln(III) ions,<sup>55</sup> the stability constants for the triacetate ligands [PyC3A (**9**) and QC3A (**13**)] are higher than their diacetate analogues [bpcd and bQcd (**3**) respectively]. Besides, the stability constants of the Ln(III) complexes with the quinoline-substituted ligands (**3** and **13**) are lower than for their pyridine analogues (bpcd and **9**, respectively). This result could be due to a weaker interaction of the quinoline moieties with respect to the pyridine ones and also to the increased steric hindrance. In the perspective of *in vitro* application experiments, the values of these formation constants appear promising, in particular for triacetate-based ligands (**9** and **13**) whose stability is close to that of macrocyclic ligands possessing similar coordination ability and already employed in molecular imaging appli-

cations (*i.e.* DO3A derivatives with  $\log \beta$  values in the 18–21 range).<sup>56</sup>

Molecular models obtained by DFT calculations show that Y(III) is 8-fold coordinated in all cases and additional water molecules were expelled in the second-sphere as can be seen from the minimum energy structures in Fig. S10.† On this basis, further geometry optimizations were performed on the [Y(L)(H<sub>2</sub>O)<sub>2</sub>] complexes in the presence of PCM water. The increase of steric crowding when passing from pyridine- to the quinoline-substituted ligands can be clearly seen in Fig. 4.

From the obtained bond distances (Table 3) it emerges that the substitution of pyridine by quinoline has nearly no effect on the Y(III)–O<sub>acetate</sub> bonds (average variation,  $\Delta \text{Py} \rightarrow \text{Q} \sim -0.001$  and  $+0.005$  Å for the di- and tri-acids, respectively), and also the Y(III)–N<sub>amine</sub> distances are marginally affected ( $\Delta \text{Py} \rightarrow \text{Q} \sim -0.019$  and  $-0.006$  Å). It can be also noted that



**Fig. 4** Minimum energy structures of (a) [Y(*trans*-O,O-bpcd)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>; (b) [Y(*trans*-N,N-bpcd)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>; (c) [Y(*trans*-O,O-bQcd)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>; (d) [Y(*trans*-N,N-bQcd)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>; (e) [Y(*trans*-O,O-PyC3A)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>; (f) [Y(*trans*-N,N,O-PyC3A)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>; (g) [Y(*trans*-O,O-QC3A)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>; (h) [Y(*trans*-N,N,O-QC3A)(H<sub>2</sub>O)<sub>2</sub>]<sup>+</sup>.

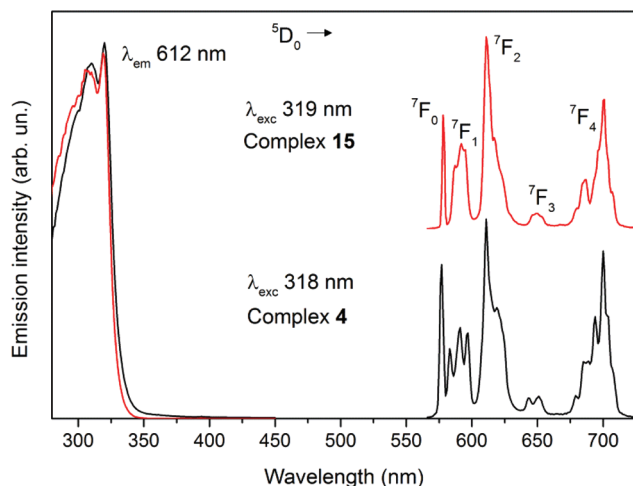
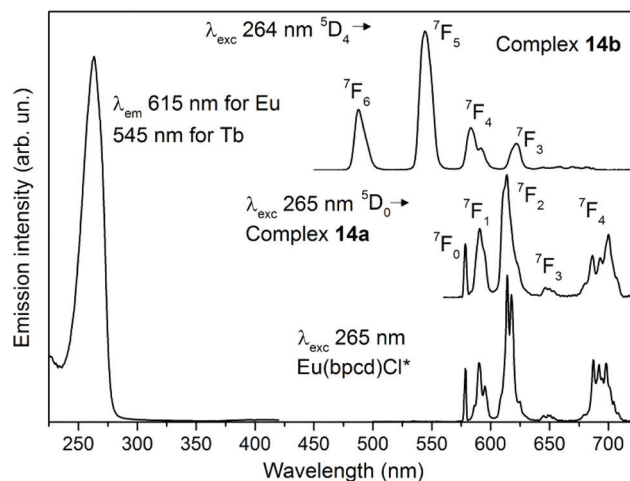
**Table 3** Selected bond distances (Å) of the complexes in Fig. 4

Complex	Y–O <sub>acetate</sub>	Y–N <sub>amine</sub>	Y–N <sub>heterocycle</sub>	Y–O <sub>water</sub>
[Y( <i>trans</i> -O,O bpdc)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+</sup>	2.262	2.550	2.525	2.448
[Y( <i>trans</i> -N,N bpdc)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+</sup>	2.292	2.610	2.503	2.492
[Y( <i>trans</i> -O,O bQcd)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+</sup>	2.268	2.557	2.661	2.464
[Y( <i>trans</i> -N,N bQcd)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+</sup>	2.284	2.567	2.594	2.482
[Y( <i>trans</i> -O,O PyC3A)(H <sub>2</sub> O) <sub>2</sub> ]	2.286	2.568	2.550	2.474
[Y( <i>trans</i> -N,O PyC3A)(H <sub>2</sub> O) <sub>2</sub> ]	2.300	2.595	2.546	2.539
[Y( <i>trans</i> -O,O QC3A)(H <sub>2</sub> O) <sub>2</sub> ]	2.286	2.574	2.654	2.458
[Y( <i>trans</i> -N,O QC3A)(H <sub>2</sub> O) <sub>2</sub> ]	2.290	2.576	2.642	2.478

Y(III)–O<sub>water</sub> bonds are slightly longer in the pyridine triacid isomers as it could be expected on the basis of the decreased charge on the metal ion, while in the quinoline complexes they are only slightly affected. However, the most remarkable finding is that the average Y(III)–N<sub>heterocycle</sub> bond distance increases significantly ( $\Delta\text{Py} \rightarrow \text{Q} \sim +0.11$  Å), indicating the weaker interaction of the quinoline with respect to pyridine ligands with the metal ion; this possibly contributes to the drop of stability of the quinoline complexes with respect to the pyridine analogues (on average  $\sim 1.4$  and  $3.4$  log units for the di and tri-acetate ligands). However, it is expected that quinoline also has a notable impact on the solvation properties of the complex which often have a strong influence on the stability.

### Luminescence

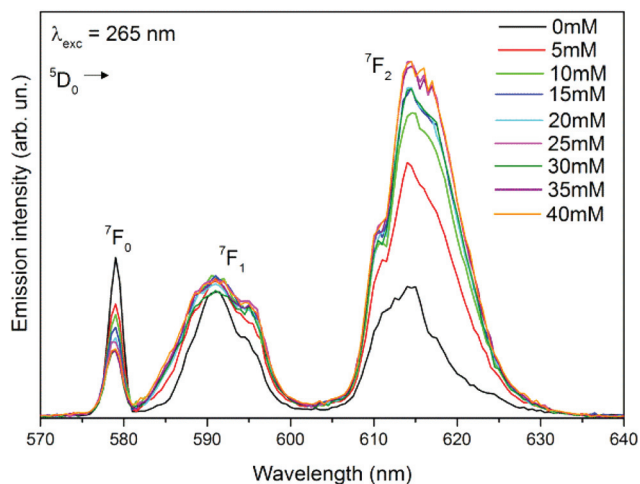
Excitation spectra of the complexes **4**, **14a**, **15** and Eu(bpcd)Cl dissolved in water upon monitoring the  $^5\text{D}_0 \rightarrow ^7\text{F}_2$  transition of Eu(III) ( $\lambda_{\text{em}} = 612\text{--}615$  nm) are shown in Fig. 5 and 6 (left). In the case of Tb(III) complex **14b**, the excitation spectrum has been recorded monitoring the  $^5\text{D}_4 \rightarrow ^7\text{F}_5$  transition of Tb(III) ( $\lambda_{\text{em}} = 545$  nm, Fig. 6). As all the spectra are superimposable with the corresponding absorption ones, a ligand to metal energy transfer mechanism works in all the complexes under investigation. As already observed<sup>33</sup> the pyridine ring is

**Fig. 5** Luminescence excitation spectra (left) and emission spectra (right) of Eu(III) complexes **4** and **15** in water solution ( $10^{-4}$  M) at 298 K.**Fig. 6** Luminescence excitation spectra (left) and emission spectra (right) of Eu(III) complex **14a** and Eu(bpcd)Cl and Tb(III) complex **14b** in water solution ( $10^{-4}$  M) at 298 K. \*Data from ref. 33.

capable of sensitizing both Eu(III) and Tb(III) luminescence. On the other hand, the quinoline ring effectively sensitizes only the Eu(III) ion.

As far as the luminescence emission spectra are concerned (Fig. 6 and 7, right), the typical Eu(III) or Tb(III) luminescence originating from f–f transitions is clearly detected for all the complexes. Upon excitation of the pyridine ring ( $\lambda_{\text{exc}} = 265$  nm) complexes **14a** and Eu(bpcd)Cl showed a visual red luminescence while complex **14b** showed a green one. Upon excitation of the quinoline ring ( $\lambda_{\text{exc}} = 319$  nm) a red luminescence is detected for complexes **4** and **15**. In all the Eu(III) emission spectra, the hypersensitive  $^5\text{D}_0 \rightarrow ^7\text{F}_2$  transition dominates the spectrum and one strong  $^5\text{D}_0 \rightarrow ^7\text{F}_0$  band is also detected (in particular in the case of quinoline-based complexes). All this is compatible with the presence of emitting species where the point symmetry of Eu(III) deviates from the inversion symmetry and is characterized by an axial character.<sup>57</sup> The  $C_n$ ,  $C_{nv}$  or  $C_s$  are the only possible point symmetries in the presence of sizeable intensity of the  $^5\text{D}_0 \rightarrow ^7\text{F}_0$  transition<sup>57</sup> even though in our case, the  $C_s$  symmetry can be ruled out due to the presence of the chiral ligand.

The luminescence decay curves of the  $^5\text{D}_0$  and  $^5\text{D}_4$  excited states of Eu(III) and Tb(III), respectively were recorded in aqueous solution for all the complexes under investigation; in



**Fig. 7** Eu(III) luminescence emission spectra of the complex **14a** (limited to 570–640 nm range) upon addition of hydrogen carbonate ions. The concentration of the anion is reported.

Fig. S12† we report only a representative selection. All the curves are well fitted by a single exponential function and the observed lifetimes, in water and deuterium oxide, are reported in Table 4, together with the values of the hydration number ( $q$ ), the radiative lifetime ( $\tau_{\text{rad}}$ ), the intrinsic ( $\Phi_{\text{Ln}}$ ), the total ( $\Phi_{\text{Tot}}$ ) quantum yields and the efficiency of the sensitization of the lanthanide luminescence by the ligand ( $\eta_{\text{sens}}$ ). The hydration number is the number of water molecules in close proximity of the metal ion and can be calculated by means of the Horrocks equation<sup>58–60</sup> that is based on the values of  $\tau_{\text{obs}}$  in H<sub>2</sub>O and D<sub>2</sub>O.

It is worth noting that the number of water molecules in the inner coordination sphere of the metal ion in each complex is, in practice, the same (around 2.5). This result seems to be in partial agreement with the DFT structures reported in Fig. 4 where always two water molecules are retained in the first coordination sphere of the metal ion. Nevertheless, as already observed for the complexes Eu(bpcd)Cl and Tb(bpcd)Cl, this is compatible with the presence of an equilibrium in solution interconverting two species having

one 6-fold coordinating ligand molecule and a different number of water molecules (2 or 3) in the inner coordination sphere of the metal ion.<sup>33</sup> In addition, it has to be reminded that the hydration number ( $q$ ), calculated by the Horrocks equation, is also slightly sensitive to the presence of water molecules in the outer coordination sphere.<sup>58</sup>

The intrinsic quantum yield of the lanthanide ion ( $\Phi_{\text{Ln}}$ ), defined by a number of emitted/absorbed photons, when lanthanide ions are directly excited, is around 10% and 16% for the Eu(III) and Tb(III) complexes, respectively (Table 4). The higher values of the intrinsic quantum yield for the Tb(III)-based complexes are due to the energy gap between the emitting level and the lower lying ones, that is bigger in the case of Tb(III) so as to limit the multiphonon relaxation process. On the other hand, to estimate  $\eta_{\text{sens}}$ , we need to know the total quantum yield ( $\Phi_{\text{Tot}}$ ), that is defined by the number of photons emitted by the lanthanide ion/number of photons absorbed by the ligand. Since,  $\Phi_{\text{Tot}} = \eta_{\text{sens}} \cdot \Phi_{\text{Ln}}$ , then  $\eta_{\text{sens}} = \Phi_{\text{Tot}}/\Phi_{\text{Ln}}$ . The values of  $\Phi_{\text{Tot}}$  for all the complexes have been determined by using a reference standard of known quantum yield (quinine bisulfate;  $\Phi = 54.6\%$ ; see the Experimental section for details). The obtained  $\eta_{\text{sens}}$  is in the 60–70% range for all Eu(III) and Tb(III) complexes containing the pyridine chromophore, whilst the Eu(III) complexes containing the quinoline fragment show a significantly lower sensitization efficiency (in particular for Eu(bQcd)OTf,  $\eta_{\text{sens}} = 29\%$ ). This seems to be related to the longer Y–N<sub>heterocycle</sub> bond distances found by DFT calculations, in the case of quinoline-based complexes. In this context, it is useful to remember that the probability of the energy transfer from an antenna ligand (S = sensitizer) to a metal ion (A = acceptor) is strongly dependent on the S–A distance, for both the most common energy transfer processes taking place in lanthanide-based complexes (dipole–dipole and exchange mechanisms).<sup>62</sup> In particular, the longer the distance, the lower is the energy transfer probability and the sensitization efficiency. The seemingly low total quantum yields ( $\Phi_{\text{Tot}}$  in the 3–11% range) must be reassessed in light of the following statements: (i) the quantum yield of many lanthanide and d-block compounds used for cellular imaging is in the 4–10% range;<sup>63,64</sup> (ii) the total quantum yield

**Table 4** Observed and radiative excited state lifetimes (ms) for Eu(III) and Tb(III) complexes along with the number of water molecules ( $q$ ) obtained from data fitting. Intrinsic ( $\Phi_{\text{Ln}}$ ), total ( $\Phi_{\text{Tot}}$ ) quantum yields and  $\eta_{\text{sens}}$  are also reported

Complex	$\tau_{\text{obs}}$		$\tau_{\text{rad}}$	$q$	$\Phi_{\text{Ln}}$ (%)	$\Phi_{\text{Tot}}$ (%)	$\eta_{\text{sens}}$ (%)
	H <sub>2</sub> O	D <sub>2</sub> O					
Eu(bpcd)Cl	0.30(1)	1.70(1)	3.00 <sup>a</sup>	2.7(1)	10.0 <sup>c</sup>	6.1	61
Eu(bQcd)Cl	0.29(1)	1.68(1)	3.22 <sup>a</sup>	2.8(1)	9.0 <sup>c</sup>	2.6	29
Eu(PyC3A)	0.33(1)	3.56(1)	3.66 <sup>a</sup>	2.7(1)	9.0 <sup>c</sup>	5.67	63
Eu(QC3A)	0.33(1)	2.15(1)	3.34 <sup>a</sup>	2.5(1)	9.9 <sup>c</sup>	4.0	40
Tb(bpcd)Cl	0.94(1)	2.15(1)	5.98 <sup>b</sup>	2.6(5)	15.7 <sup>d</sup>	10.0	64
Tb(PyC3A)	1.16(1)	3.53(1)	6.86 <sup>b</sup>	2.4(5)	16.9 <sup>d</sup>	11.2	66

<sup>a</sup> Estimated from the analysis of the Eu(III) emission spectra by using the formula reported by Werts *et al.*<sup>61</sup> <sup>b</sup> Calculated by  $\tau_{\text{rad}} = \tau_{\text{obs}}/\Phi_{\text{Ln}}$ ;  $\tau_{\text{obs}}$  and  $\Phi_{\text{Ln}}$  have been determined in H<sub>2</sub>O. <sup>c</sup> Estimated in aqueous solution thanks to the formula  $\tau_{\text{obs}}/\tau_{\text{rad}}$ . <sup>d</sup> Determined by using the reference standard.

of our complexes is expected to grow upon interaction of the complex with a target analyte thanks to the concomitant displacement of water molecules from the metal ion. For these reasons, we believe that the class of complexes under investigation can be considered a promising family of optical probes for sensing application.

### Sensing of $\text{HCO}_3^-$

As can be seen from the inspection of Fig. 7, the intensity of the  $^5\text{D}_0 \rightarrow ^7\text{F}_0$  and  $^5\text{D}_0 \rightarrow ^7\text{F}_2$   $\text{Eu}(\text{III})$  transitions is significantly affected by the addition of the hydrogen carbonate ion to complex **14a** (chosen as a representative example). As it is reasonable to assume that the  $\text{HCO}_3^-$  anion is capable of coordinating the  $\text{Eu}(\text{III})$  ion, on displacing the water molecules from the inner coordination sphere, we can claim that the interaction between  $\text{Eu}(\text{III})$  and the target anion produces an increase in the intensity of  $^5\text{D}_0 \rightarrow ^7\text{F}_2$  transition and a decrease in the intensity of the  $^5\text{D}_0 \rightarrow ^7\text{F}_0$  one. This means that during

the titration with  $\text{HCO}_3^-$ , the geometry of the  $\text{Eu}(\text{III})$  environment is undergoing a change in symmetry. In fact, the values of the asymmetry ratio:

$$R = \frac{I(^5\text{D}_0 \rightarrow ^7\text{F}_2)}{I(^5\text{D}_0 \rightarrow ^7\text{F}_1)}$$

indicative of the degree of asymmetry of the coordination polyhedron around the  $\text{Eu}(\text{III})$  ion, increase upon addition of the anion.

The value of  $R$  during the titration with hydrogen carbonate also increases for all the  $\text{Eu}(\text{III})$  complexes under investigation (Fig. 8).

In all cases, a logarithmic-like trend is observed, and an asymptote is reached after the addition of 10 mM of anion for the  $\text{Eu}(\text{III})$  complex with a bpcd ligand (Fig. 8). On the other hand, the asymptotic value is reached only after the addition of hydrogen carbonate at 15–20 mM, in the case of all the other complexes [Eu(bQcd)OTf, Eu(PyC3A) and Eu(QC3A)]. The sensitivity of the optical response to the  $\text{HCO}_3^-$  concentration can be qualitatively evaluated by analyzing the slope of the graph in the range of biological interest where the hydrogen carbonate concentration is related to serious metabolic acidosis (0–10 mM). As can be seen in Fig. 8, Eu(bpcd)Cl and Eu(QC3A) (**15**) complexes show the best sensitivity. The binding interactions between hydrogen carbonate and the  $\text{Eu}(\text{III})$  complexes were studied using the Benesi–Hildebrand equation adapted to the values of the asymmetry ratio, as described in the Experimental section. Since there is linearity in the plot of  $R_0/(R - R_0)$  vs.  $[\text{HCO}_3^-]^{-2}$  for Eu(bpcd)Cl and Eu(bQcd)OTf (Fig. 9 and S13<sup>†</sup>) and in the plot of  $R_0/(R - R_0)$  vs.  $[\text{HCO}_3^-]^{-1}$  for Eu(PyC3A) and Eu(QC3A) (Fig. 10), the stoichiometry of the hydrogen carbonate adducts is 1 : 1 for Eu(PyC3A) and Eu(QC3A) complexes and 1 : 2 for Eu(bpcd)Cl and Eu(bQcd)OTf ones.

Since the affinity of the anion for the  $\text{Ln}(\text{III})$  centre is mainly determined by coulombic attraction it is not surprising that the cationic complexes Eu(bpcd)Cl and Eu(bQcd)OTf can coordinate the hydrogen carbonate anion with high affinity con-

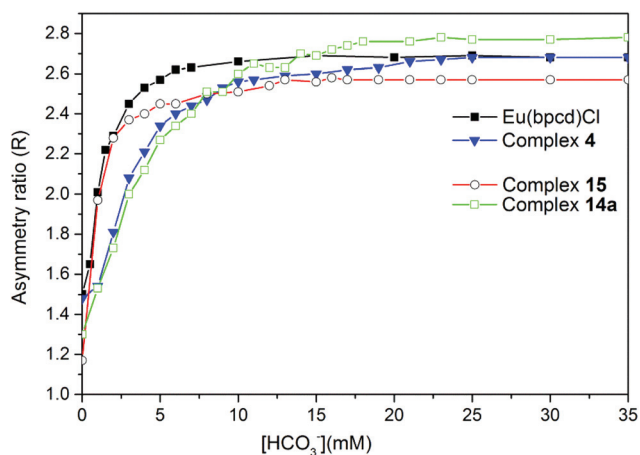


Fig. 8 Asymmetry ratio ( $R$ ) for the  $\text{Eu}(\text{III})$  complexes vs.  $[\text{HCO}_3^-]$  concentration plots. The employed ligands are reported in each plot (reference labels in Fig. 1).

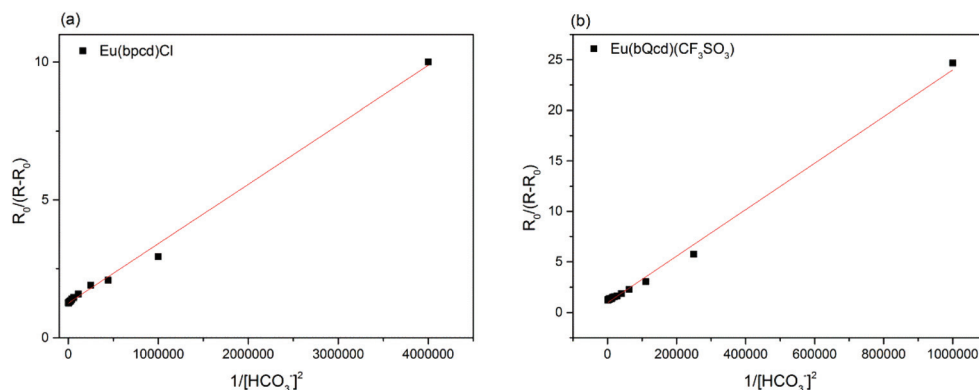
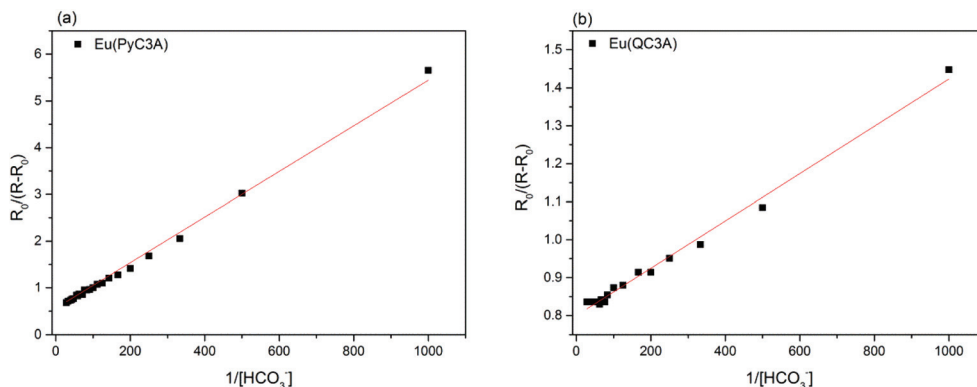


Fig. 9 Benesi–Hildebrand plot vs.  $[\text{HCO}_3^-]^{-2}$  ( $\text{M}^{-2}$ ) for (a)  $[\text{Eu}(\text{bpcd})]^+$  and (b)  $[\text{Eu}(\text{bQcd})]^+$  complexes.  $R_0$  is the asymmetry ratio of the starting complex;  $R$  is the asymmetry ratio after each addition of the analyte.  $R$  and  $R_0$  have been calculated from the relative  $\text{Eu}(\text{III})$  luminescence emission spectrum.





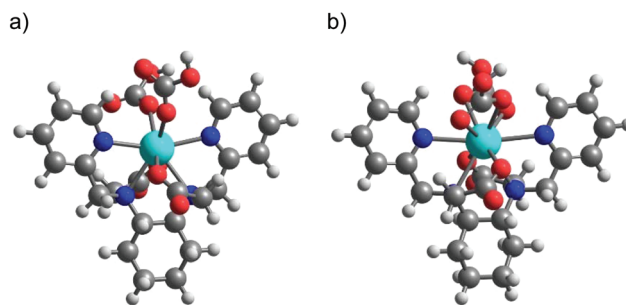
**Fig. 10** Benesi–Hildebrand plot vs.  $[\text{HCO}_3^-]^{-1}$  ( $\text{M}^{-1}$ ) for (a) Eu(PyC3A) and (b) Eu(QC3A) complexes.  $R_0$  is the asymmetry ratio of the starting complex;  $R$  is the asymmetry ratio after each addition of the analyte.  $R$  and  $R_0$  have been calculated from the relative Eu(III) luminescence emission spectrum.

**Table 5** Apparent equilibrium constants ( $\log K$ ) for the formation of the adducts with hydrogen carbonate ( $\text{HCO}_3^-$ ),  $[\text{complex}] + n\text{-hydrogen carbonate} \rightleftharpoons [\text{complex}(\text{hydrogen carbonate})_n]$  ( $T = 298 \text{ K}$ ,  $\text{pH } 7.40 (\pm 0.05)$ ,  $I = 0.1 \text{ M NaCl}$ ,  $40 \mu\text{M complex}$ ), determined through fluorimetric titration. Charges are omitted for clarity

Complex	$n$	$\log K$
Eu(bpcd)Cl/Tb(bpcd)Cl	2	5.76(8)/5.94(8)
Eu(bQcd)(CF <sub>3</sub> SO <sub>3</sub> )	2	4.62(8)
Eu(PyC3A)	1	2.06(8)
Eu(QC3A)	1	3.11(8)

stants (calculated from the ratio of intercept/slope of the Benesi–Hildebrand plot, Table 5). To the best of our knowledge, a value of  $\log K$  higher than 4, in the case of the hydrogen carbonate ion, is unprecedented in the literature and this is probably related to the unusual number of target anions bound to the metal center. The Eu(III) in these complexes can bind up to 2 hydrogen carbonate units, likely due, as discussed in the Introduction, to the lower number of donating atoms (6-fold coordination) than in the case of ligands commonly employed for Ln(III)-based luminescence anion sensing (NOTA and DOTA-like possessing 7-fold coordination). The possible structures of the 1 : 2 hydrogen carbonate adducts to  $[\text{Y}(\text{trans-O,O-bpcd})(\text{H}_2\text{O})_2]^+$  obtained by DFT calculations (Fig. 11) show two possible coordination modes. However, the bis-monodentate hydrogen carbonate seems to be the only possible isomer being  $10.3 \text{ kcal mol}^{-1}$  more stable than the bis-bidentate one (also the latter presents an imaginary vibrational mode corresponding to the opening of two Y–O bonds). The optimization of the 1 : 2 adduct with both hydrogen carbonate coordination modes (one bi- and one mono-dentate) led always to a bis-monodentate structure.

Due to the neutral charge of Eu(PyC3A) and Eu(QC3A) complexes, it is not surprising to find in their adducts with hydrogen carbonate lower binding constants and a 1 : 1 stoichiometry. It is also reasonable to assume that the negative charge of the 1 : 1 adduct hampers the formation of the bis-anionic



**Fig. 11** Minimum energy structures of the  $[\text{Y}(\text{trans-O,O-bpcd})(\text{HCO}_3)_2]^+$  complexes obtained in PCM water with (a) bis-monodentate and (b) bis-bidentate hydrogen carbonate coordination modes.

1 : 2 species. Furthermore, it is worth underlining that the presence of the quinoline ring affects the stability of the adduct with hydrogen carbonate. In the case of cationic complexes, the adduct with quinoline is less stable compared to the one with pyridine. In contrast, an opposite trend is observed for the neutral complexes. The reasons of such behavior in the case of the diacid ligand could be found in the high steric hindrance of the heteroaromatic ring which, at least for the *trans*-O,O-bQcd isomer (Fig. 4c), presents two hydrogen atoms pointing towards the inner coordination sphere, thus likely to hinder the coordination of hydrogen carbonate and maybe giving rise to an affinity constant one order of magnitude lower than for the pyridine-based analog. Here, we demonstrate how, thanks to a modulation of the steric hindrance at the metal ion using the different ligands shown in Fig. 1, it is possible to tune the affinity (and the selectivity) of the complexes towards  $\text{HCO}_3^-$ .

As far as the affinity of the analogous Tb(III) complexes towards hydrogen carbonate is concerned, we expect a behavior similar to the one observed for Eu(III) derivatives, since Eu(III) and Tb(III) complexes are often isostructural due to the similarity of their ionic radii.<sup>55</sup> As expected, the calculated affinity constant for the Tb(bpcd)Cl complex, chosen as a

representative example, is similar to the one of Eu(bpcd)Cl (Table 5 and Fig. S14†).

The most efficient and selective optical probes for hydrogen carbonate, capable of detecting this anion *in cellulo* or in extracellular fluid, are based on charged and neutral Eu(III) and Tb(III) complexes of heptadentate ligands.<sup>21,65–67</sup> As far as their affinity towards hydrogen carbonate under physiological conditions is concerned, they show log *K* values in the 2.6–3.85 range. The surprising higher affinity of Eu(bQcd)(CF<sub>3</sub>SO<sub>3</sub>) and Eu(bpcd)Cl (log *K* 4.62 and 5.76, respectively) promises a better selectivity towards HCO<sub>3</sub><sup>–</sup>. In this context, since both the enantiomers of the analog Tb(bpcd)Cl weakly interact with L-lactate (log *K* = 1.3–1.45),<sup>34</sup> a strong selectivity for hydrogen carbonate is expected in a solution containing both analytes.

## Conclusions

The cationic Ln(bpcd)<sup>+</sup> and Ln(bQcd)<sup>+</sup> complexes and the neutral Ln(PyC3A) and Ln(QC3A) ones are highly stable in aqueous solution (9.97 < log β < 15.68) and they exist as a couple of isomeric compounds differing by the ligand stereochemistry (*trans*-N,N and *trans*-O,O for bpcd<sup>2–</sup> and bQcd<sup>2–</sup>; *trans*-O,O and *trans*-N,O for PyC3A<sup>3–</sup> and QC3A<sup>3–</sup>).

For all the complexes, the Ln(III) luminescence intensity increases as a function of the hydrogen carbonate concentration in physiological solution. The best sensitivity of the optical response towards HCO<sub>3</sub><sup>–</sup> in the concentration range related to metabolic acidosis has been observed for Eu(bpcd)<sup>+</sup> and Eu(QC3A) complexes. It has been possible to obtain an unprecedented affinity towards hydrogen carbonate ions by simply playing with several features of the investigated complexes such as: (i) the relatively low coordination number of the ligands, which have 6 donating atoms; (ii) the positive or neutral charge and (iii) the steric hindrance at the metal ion. The positively charged Eu(bpcd)<sup>+</sup> and Tb(bpcd)<sup>+</sup> complexes, which possess a small steric hindrance at the metal ion, reveal the highest log *K* values (5.76 and 5.94, respectively) reported up to now in the literature, for the formation of an adduct with HCO<sub>3</sub><sup>–</sup> characterized by an uncommon 1:2 stoichiometry. With regard to the state-of-the-art of the optical sensing of hydrogen carbonate ions in extracellular fluid and *in cellulo* experiments, Ln(bpcd)<sup>+</sup> (Ln = Tb and Eu) can be considered as very promising optical probes with an enhanced selectivity.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

AM acknowledges the University of Udine ("Piano Strategico d'Ateneo") for the grant to acquire the HPC hardware employed in this study.

## References

- 1 J. C. G. Bünzli, *Chem. Rev.*, 2010, **110**, 2729–2755.
- 2 H. Tsukube and S. Shinoda, *Chem. Rev.*, 2002, **102**, 2389–2404.
- 3 S. J. Butler and D. Parker, *Chem. Soc. Rev.*, 2013, **42**, 1652–1666.
- 4 J. C. G. Bünzli and S. V. Eliseeva, *Chem. Sci.*, 2013, **4**, 1939–1949.
- 5 M. C. Heffern, L. M. Matosziuk and T. J. Meade, *Chem. Rev.*, 2014, **114**, 4496–4539.
- 6 J. W. Walton, A. Bourdolle, S. J. Butler, M. Soulie, M. Delbianco, B. K. McMahon, R. Pal, H. Puschmann, J. M. Zwier, L. Lamarque, O. Maury, C. Andraud and D. Parker, *Chem. Commun.*, 2013, **49**, 1600–1602.
- 7 D. G. Smith, B. K. McMahon, R. Pal and D. Parker, *Chem. Commun.*, 2012, **48**, 8520–8522.
- 8 E. A. Weitz, J. Y. Chang, A. H. Rosenfield and V. C. Pierre, *J. Am. Chem. Soc.*, 2012, **134**, 16099–16102.
- 9 A. Brandner, T. Kitahara, N. Beare, C. Lin, M. T. Berry and P. S. May, *Inorg. Chem.*, 2011, **50**, 6509–6520.
- 10 J. C. G. Bünzli and S. V. Eliseeva, in *Comprehensive Inorganic Chemistry II (Second Edition): From Elements to Applications*, Elsevier, 2013, vol. 8, pp. 339–398.
- 11 W. T. Carnall, H. Crosswhite and H. M. Crosswhite, *Energy level structure and transition probabilities in the spectra of the trivalent lanthanides in LaF<sub>3</sub>*, Argonne, IL, United States, 1978.
- 12 K. L. Peterson, J. V. Dang, E. A. Weitz, C. Lewandowski and V. C. Pierre, *Inorg. Chem.*, 2014, **53**, 6013–6021.
- 13 A. G. Weidmann, A. C. Komor and J. K. Barton, *Philos. Trans. R. Soc. London, Ser. A*, 2013, **371**, 20120117.
- 14 C. A. Puckett and J. K. Barton, *J. Am. Chem. Soc.*, 2007, **129**, 46–47.
- 15 A. S. Chauvin, F. Thomas, B. Song, C. D. B. Vandevyver and J. C. G. Bünzli, *Philos. Trans. R. Soc. London, Ser. A*, 2013, **371**, 20120295.
- 16 E. J. New, A. Congreve and D. Parker, *Chem. Sci.*, 2010, **1**, 111–118.
- 17 B. S. Murray, E. J. New, R. Pal and D. Parker, *Org. Biomol. Chem.*, 2008, **6**, 2085–2094.
- 18 R. Carr, L. Di Bari, S. Lo Piano, D. Parker, R. D. Peacock and J. M. Sanderson, *Dalton Trans.*, 2012, **41**, 13154.
- 19 C. P. Montgomery, E. J. New, D. Parker and R. D. Peacock, *Chem. Commun.*, 2008, 4261.
- 20 E. A. Weitz and V. C. Pierre, *Chem. Commun.*, 2011, **47**, 541–543.
- 21 D. G. Smith, R. Pal and D. Parker, *Chem. – Eur. J.*, 2012, **18**, 11604–11613.
- 22 F. Piccinelli, A. Melchior, A. Speghini, M. Monari, M. Tolazzi and M. Bettinelli, *Polyhedron*, 2013, **57**, 30–38.
- 23 F. Piccinelli, M. Bettinelli, A. Melchior, C. Grazioli and M. Tolazzi, *Dalton Trans.*, 2015, **44**, 182–192.
- 24 M. Mihorianu, M. Leonzio, M. Monari, L. Ravotto, P. Ceroni, M. Bettinelli and F. Piccinelli, *ChemistrySelect*, 2016, **1**, 1996–2003.

- 25 F. Piccinelli, M. Leonzio, M. Bettinelli, M. Monari, C. Grazioli, A. Melchior and M. Tolazzi, *Dalton Trans.*, 2016, **45**, 3310–3318.
- 26 W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375–2434.
- 27 C. A. Wagner, J. Kovacicova, P. A. Stehberger, C. Winter, C. Benabbas and N. Mohebbi, *Nephron Physiol.*, 2006, **103**, p1–p6.
- 28 J. D. Kopple, K. Kalantar-Zadeh and R. Mehrotra, *Kidney Int. Suppl.*, 2005, **67**, S21–S27.
- 29 G. Gran, *Analyst*, 1952, **77**, 661.
- 30 P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739–1753.
- 31 L. Toso, G. Crisponi, V. M. Nurchi, M. Crespo-Alonso, J. I. Lachowicz, D. Mansoori, M. Arca, M. A. Santos, S. M. Marques, L. Gano, J. Niclós-Gutierrez, J. M. González-Pérez, A. Domínguez-Martín, D. Choquesillo-Lazarte and Z. Szewczuk, *J. Inorg. Biochem.*, 2014, **130**, 112–121.
- 32 G. H. Jeffrey, J. Bassett, J. Mendham and R. C. Denney, *Vogel's textbook of quantitative chemical analysis*, John Wiley & Sons, 5th edn, 1990, vol. 14.
- 33 M. Leonzio, A. Melchior, G. Faura, M. Tolazzi, F. Zinna, L. Di Bari and F. Piccinelli, *Inorg. Chem.*, 2017, **56**, 4413–4422.
- 34 M. Leonzio, A. Melchior, G. Faura, M. Tolazzi, M. Bettinelli, F. Zinna, L. Arrico, L. Di Bari and F. Piccinelli, *New J. Chem.*, 2018, **42**, 7931–7939.
- 35 P. Di Bernardo, P. L. L. Zanonato, A. Bismondo, A. Melchior and M. Tolazzi, *Dalton Trans.*, 2009, 4236–4244.
- 36 A. C. Mendonça, A. F. Martins, A. Melchior, S. M. Marques, S. Chaves, S. Villette, S. Petoud, P. L. Zanonato, M. Tolazzi, C. S. Bonnet, É. Tóth, P. Di Bernardo, C. F. G. C. Gerales and M. A. Santos, *Dalton Trans.*, 2013, **42**, 6046–6057.
- 37 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 1372–1377.
- 38 C. T. Lee, W. T. Yang and R. G. Parr, *Phys. Rev. B: Condens. Matter*, 1988, **37**, 785–789.
- 39 X. Cao and M. Dolg, *J. Chem. Phys.*, 2001, **115**, 7348–7355.
- 40 J. Tomasi, B. Mennucci and R. Cammi, *Chem. Rev.*, 2005, **105**, 2999–3093.
- 41 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox, *Gaussian 16 Rev. A03*, Wallingford, CT, 2016.
- 42 D. F. Eaton, *Pure Appl. Chem.*, 1988, **60**, 1107–1114.
- 43 H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703–2707.
- 44 P. Polese, M. Tolazzi and A. Melchior, *J. Therm. Anal. Calorim.*, 2018, **134**, 1317–1326.
- 45 D. W. Lee, H. J. Ha and W. K. Lee, *Synth. Commun.*, 2007, **37**, 737–742.
- 46 E. M. Gale, S. Mukherjee, C. Liu, G. S. Loving and P. Caravan, *Inorg. Chem.*, 2014, **53**, 10748–10761.
- 47 A. V. Rayer, K. Z. Sumon, L. Jaffari and A. Henni, *J. Chem. Eng. Data*, 2014, **59**, 3805–3813.
- 48 R. Ferreirós-Martínez, D. Esteban-Gómez, C. Platas-Iglesias, A. De Blas and T. Rodríguez-Blas, *Dalton Trans.*, 2008, 5754–5765.
- 49 G. Anderegg, *Helv. Chim. Acta*, 1974, **57**, 1340–1346.
- 50 K. Kahmann, H. Sigel and H. Erlenmeyer, *Helv. Chim. Acta*, 1964, **47**, 1754–1763.
- 51 H. Kitano, Y. Onishi, A. Kirishima, N. Sato and O. Tochiyama, *Radiochim. Acta*, 2006, **94**, 541–547.
- 52 P. Caravan, P. Mehrkhodavandi and C. Orvig, *Inorg. Chem.*, 1997, **36**, 1316–1321.
- 53 M. Wagner, R. Ruloff, E. Hoyer and W. Gründer, *Z. Naturforsch., C: Biosci.*, 1997, **52**, 508–515.
- 54 R. M. Smith and A. E. Martell, *Sci. Total Environ.*, 1987, **64**, 125–147.
- 55 P. Di Bernardo, A. Melchior, M. Tolazzi and P. L. Zanonato, *Coord. Chem. Rev.*, 2012, **256**, 328–351.
- 56 L. Tei, Z. Baranyai, L. Gaino, A. Forgács, A. Vágner and M. Botta, *Dalton Trans.*, 2015, **44**, 5467–5478.
- 57 K. Binnemans, *Coord. Chem. Rev.*, 2015, **295**, 1–45.
- 58 R. M. Supkowski and W. D. Horrocks, *Inorg. Chim. Acta*, 2002, **340**, 44–48.
- 59 W. D. Horrocks and D. R. Sudnick, *J. Am. Chem. Soc.*, 1979, **101**, 334–340.
- 60 W. D. Horrocks, J. P. Bolender, W. D. Smith and R. M. Supkowski, *J. Am. Chem. Soc.*, 1997, **119**, 5972–5973.
- 61 M. H. V. Werts, R. T. F. Jukes and J. W. Verhoeven, *Phys. Chem. Chem. Phys.*, 2002, **4**, 1542–1548.
- 62 D. L. Dexter, *J. Chem. Phys.*, 1953, **21**, 836–850.
- 63 D. Parker, P. K. Senanayake and J. A. G. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2129–2140.
- 64 C. Li, Y. Liu, Y. Wu, Y. Sun and F. Li, *Biomaterials*, 2013, **34**, 1223–1234.
- 65 Y. Bretonniere, M. J. Cann, D. Parker and R. Slater, *Org. Biomol. Chem.*, 2004, **2**, 1624–1632.
- 66 S. J. Butler, B. K. McMahon, R. Pal, D. Parker and J. W. Walton, *Chem. – Eur. J.*, 2013, **19**, 9511–9517.
- 67 D. Imperio, G. B. Giovenzana, G. L. Law, D. Parker and J. W. Walton, *Dalton Trans.*, 2010, **39**, 9897–9903.