ChemComm

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/c7cc06753d

Received 28th August 2017,
Accepted 9th October 2017

DOI: 10.1039/c7cc06753d

rsc.li/chemcomm

Selective cytotoxicity and luminescence imaging of cancer cells with a dipicolinato-based Eu^{III} complex†

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Four new species $[Ln(dipicNH_2)_3]^{3-}$ (Ln = La^{III}, Eu^{III}, Gd^{III}, Tb^{III}), with the ligand $dipicNH_2^{2-}$ (dipic = dipicolinato), were synthesized. Incubation of the Eu^{III} complex with glioma NG97 and pancreatic cancer PANC1 cells showed that it penetrates the cell membrane and can be used to image the cells, while also being moderately cytotoxic.

Cell luminescence imaging is of interest to aid in the characterization, *in situ* and *in vivo*, of cellular processes,[1,2] or in disease diagnosis.[3,4] Luminescent labels, such as organic dyes,[3–5] transition metal complexes[6] and nanoparticles,[7–11] are known, yet photobleaching in the case of the organic dyes, as well as short emission lifetimes, and narrow Stokes shifts, limit their application. In the case of some types of cancer, such as glioblastoma and pancreatic cancer, both common and aggressive, early detection and subsequent treatment are challenging, as many luminescent labels and drugs cannot cross the blood–brain barrier (BBB) and location of the tumour impedes surgical removal or there is a lack of effective drugs.[12–15] Thus, the search for new cancer-specific imaging agents, which can, for example, aid in cell identification pre- and post-surgery and/or drugs capable of crossing the BBB, is ongoing.[16,17]

Lanthanide(III) [Ln^{III}] ions are very attractive for application as photoluminescent labels[18] due to long emission lifetimes, which enable time-gated detection and thus increased signal-to-noise ratio, and narrow emission bands. As the emission is due to parity-forbidden f–f transitions, a chromophore bound to the metal ion is used as sensitizer; it absorbs energy and transfers it to the Ln^{III} ion, which then emits light.[19–21] For use as labels the compounds should be water-soluble, and should show high emission efficiencies.[22] A wide variety of ligands[23–30] has been shown to efficiently sensitize Ln^{III} emission. Among these are ligands based on dipicolinato; they are well known to form water-soluble Ln^{III} complexes with high emission efficiencies along with long emission lifetimes.[31,32] In addition, they are very easy to functionalize at the *para* position of the pyridine ring, for example with groups that allow interaction with biomolecules (such as –NCS or $–NH_2$, as used in this study).[18,33,34] At low concentrations most Ln^{III} complexes show no or low cytotoxicity, but, in some cases, at high concentrations cytotoxicity has been observed,[35] and has enabled their use as cytotoxic agents against OE33 (esophageal carcinoma),[36] HeLa (cervical cancer)[37] and B16 (melanoma).[38,39] Ln^{III} complexes which can be used as photoluminescent labels, but also display cytotoxicity, are not widely known[5,10,40] and thus of interest for both therapy and diagnostics, and their development contributes to the growing field of theranostics.

With the goal of increasing our knowledge of luminescent complexes for use as imaging agents, as well as potential therapeutics for glioblastomas should they display cytotoxicity, we isolated Ln^{III} (Ln^{III} = La^{III}, Eu^{III}, Gd^{III}, Tb^{III}) complexes of an NH_2-functionalized dipicolinato, $dipicNH_2^{2-}$. $H_2dipicNH_2$ was synthesized from chelidamic acid in 33% yield,[41,42] (Scheme S1, ESI†) and characterized using standard techniques (Fig. S4, S6(c) and S7, ESI†). Its 3 : 1 (L : Ln) metal complexes were obtained by mixing the Ln^{III} (Ln = La^{III}, Eu^{III}, Gd^{III}, Tb^{III}) oxide with $H_2dipicNH_2$ in water, adjusting the pH with M_2CO_3 (M = Na^+, K^+ or Cs^+), and purifying by re-crystallization.[43] The complexes were characterized by standard techniques (Fig. S5 and S8–S14, ESI†).

X-ray quality crystals of $[CsK_2(H_2O)_5(MeOH)_2][Gd(dipicNH_2)_3]$ (A) and $[CsNa_2(H_2O)_5(MeOH)][Eu(dipicNH_2)_3]$ (B) were obtained through vapour diffusion. Both complexes crystallize in the monoclinic space group $P2_1/n$ with cell parameters a = 9.5125(2) Å, b = 24.0522(6) Å, c = 14.1809(3) Å, β = 104.6492(15)°, V = 3139.07(12) Å^3 and a = 9.4903(1) Å, b = 24.0900(3) Å, c = 14.2494(2) Å, β = 104.2850(7)°, and V = 3156.99(7) Å^3, respectively. Except for

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† Electronic supplementary information (ESI) available: Experimental procedures, NMR and FT-IR spectroscopy, mass spectrometry of ligand and complexes, cell viability studies, confocal microscopy images and emission spectra of cell. CCDC 1534740 and 1534741. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7cc06753d

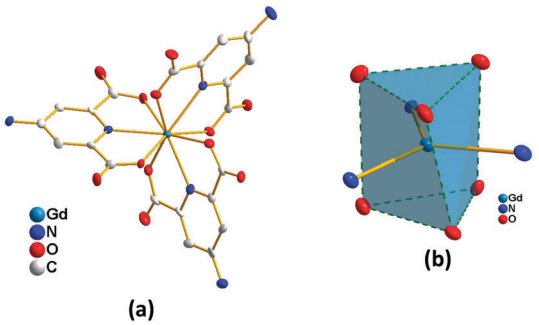

Fig. 1 (a) Plot of $[Gd(dipicNH_2)_3]^{3-}$ with thermal ellipsoids at 50% probability. Hydrogen atoms, solvated counter-cations and solvent molecules of crystallization were omitted for clarity. (b) Tricapped trigonal prism coordination polyhedron around the Gd^{III} ion.

solvent molecules of crystallization and solvated counter-cations, the complexes are isostructural with similar coordination environments around the Ln^{III}. A will be discussed here representatively (plots of B are shown in Fig. S19, ESI†). As shown in Fig. 1a, the metal ion is bound to six oxygen and three nitrogen atoms of three $dipicNH_2^{2-}$ ligands for a coordination number of 9 with a tricapped trigonal prism geometry with pseudo-D_3 symmetry (Fig. 1b), similar to analogous dipicolinato-based complexes. Eu–O distances, in the range 2.437–2.462 Å, Eu–N distances, in the range 2.482–2.523 Å, and Gd–O and Gd–N distances, in the ranges 2.423–2.451 and 2.473–2.516 Å, respectively, are similar to analogous reported complexes.[43,44]

Aqueous solutions of the potassium salts of the Eu^{III} and Tb^{III} complexes display the characteristic metal-centred emission spectra (Fig. 2 right) with transitions $^5D_0 \rightarrow {}^7F_J$ (J = 0–4) for Eu^{III} (Fig. 2(a)) and $^5D_4 \rightarrow {}^7F_J$ (J = 6–0) for Tb^{III} (Fig. 2(b)). The excitation spectra (Fig. 2, left) closely resemble the absorption spectra (Fig. S16, ESI†), indicating that the metal-centred emission is sensitized by the ligand. The stability constants (log β) determined for the 1 : 1, 1 : 2 and 1 : 3 species (Eu : $dipicNH_2$) are 7.2 ± 0.1, 15.0 ± 0.1, and 22.4 ± 0.3, respectively (Fig. S18, ESI†). The value for the 3 : 1 species is comparable with

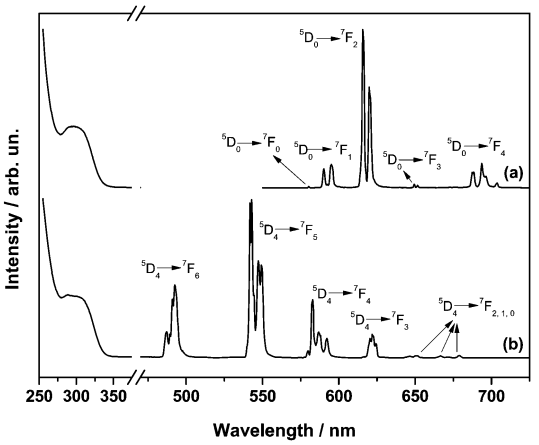

Fig. 2 Excitation (left) and emission spectra (right) in aqueous TRIS/HCl buffer (pH ~ 7.4) of $[Ln(dipicNH_2)_3]^{3-}$. (a) Ln = Eu^{III}, (b) Ln = Tb^{III}. (λ_{exc} = 300 nm and λ_{em} = 615 nm, for Eu^{III}, or 524 nm, for Tb^{III}).

log β_3 = 22.4 ± 0.3 determined for $[Eu(dpa)_3]^{3-}$ (dpa = dipicolinato).[45] The latter species is known to dissociate upon dilution under physiological conditions (water and pH ~ 7.4).[45] The concentration chosen by us to obtain the spectroscopy data and cell imaging was, at 1×10^{-4} M and 12.5–200 μg mL^{-1} (ESI†), respectively, high enough to ensure the presence of almost exclusively the 1 : 3 (Ln : L) species, as seen by determining the speciation from complex emission lifetimes as a function of the concentration (Fig. S17, ESI†).

The emission efficiencies ϕ and excited state lifetimes τ of the complexes in aqueous TRIS/HCl buffered solutions are summarized in Table 1 and Table S3 (ESI†). Efficiencies of ~33% for Eu^{III} and 24% for Tb^{III}, respectively, were observed. These data are similar to related dipicolinato-based complexes[46] and compare favourably with Eu^{III} and Tb^{III} emission in other aqueous systems.[46] The lifetimes in buffered water and D_2O (Table 1) confirm the absence of water molecules (q) coordinated to the metal ion and therefore that the ligand effectively prevents vibrational quenching of the luminescence through the high energy O–H oscillators,[47] resulting in high intrinsic quantum yields (ϕ_{Ln}^{Ln}) and emission lifetimes of 1.2 and 1.5 ms, respectively. The emission lifetimes of ~1.48 ms of the Tb^{III} complexes are slightly lower than the ~1.58 ms reported by Lamture and co-workers,[48] yet these authors used a different experimental method for the measurement and did not report the experimental error. The favourable position of the singlet and triplet states of the ligand and short donor–acceptor distance (R_L, Table S3, ESI†),[44] results in high sensitization efficiencies η_{sens} for the Eu^{III} complexes (Table 1 and Table S3, ESI†).

Cancerous cells and fibroblasts were incubated for 48 h hours with aqueous solutions of $H_2dipicNH_2$ and $K_3[Ln(dipicNH_2)_3]$ (Ln = La^{III}, Eu^{III}, Tb^{III}) (ESI†). *In vitro* cytotoxicity studies show that $H_2dipicNH_2$, and the Ln^{III} triflate and Ln^{III} chloride salts do not affect the viability of NG97 (brain) and PANC-1 (pancreatic) cancer cells. However, the Ln^{III} complexes greatly reduce their viability from 100 to ~50% (Fig. S20–S23, ESI†). As a proof of concept, NIH/3T3 fibroblasts were used to represent a generic non-cancerous cell.[49–51] For these cells, none of the species, metal salts, ligands or the metal complexes, was cytotoxic.

The cancer cells incubated with $K_3[Eu(dipicNH_2)_3]$ showed red emission (Fig. 3 and Fig. S24 for NG97 and Fig. S25 (ESI†) for PANC-1), while NIH/3T3 fibroblasts do not show red luminescence (Fig. S26, ESI†), which we attribute to lack of penetration of the complexes into the latter, non-cancerous cells. The analogous Tb^{III} complex was not used for imaging due to overlap of the emission with cell auto-fluorescence.

High resolution emission spectra of the cells incubated with the complexes could only be obtained by confocal Raman spectroscopy, due to the small amount of cells available, and show the characteristic peaks of the f–f transitions (Fig. 4 for NG97 and Fig. S28 (ESI†) for PANC-1), confirming that the emission is from Eu^{III}. For the fibroblasts only very low intensity metal-centred emission peaks are seen, confirming that the complex does not penetrate the cell membrane (Fig. S27, ESI†). The difference in peak splitting between the emission spectra of the complex in the cells and in solution (Fig. 2 and 4) is

Table 1 Singlet (^{1}S) and triplet (^{3}T) state energies of the ligand in the Gd^{III} complex, quantum yield (ϕ), intrinsic emission efficiency (ϕ_{Ln}^{Ln}), sensitization efficiency (η_{sens}), lifetime (τ) indicated with standard deviations and water molecules (q) in the first coordination sphere obtained for the complexes in aqueous TRIS/HCl or TRIS/DCl buffered solutions (pH $\sim$ 7.4)

Complexes	^{1}S [cm^{-1}]	^{3}T [cm^{-1}]	ϕ [%]	ϕ_{Ln}^{Ln} [%]	η_{sens} [%]	τ_{H_2O} [ms]	τ_{D_2O} [ms]	q
$K_3[Eu(dipicNH_2)_3]$	26 200 $\pm$ 150	22 080 $\pm$ 130	33 $\pm$ 2	55	60	1.210 $\pm$ 0.001	2.282 $\pm$ 0.002	0.1
$K_3[Tb(dipicNH_2)_3]$			24 $\pm$ 1	—	—	1.477 $\pm$ 0.001	1.771 $\pm$ 0.005	0.5

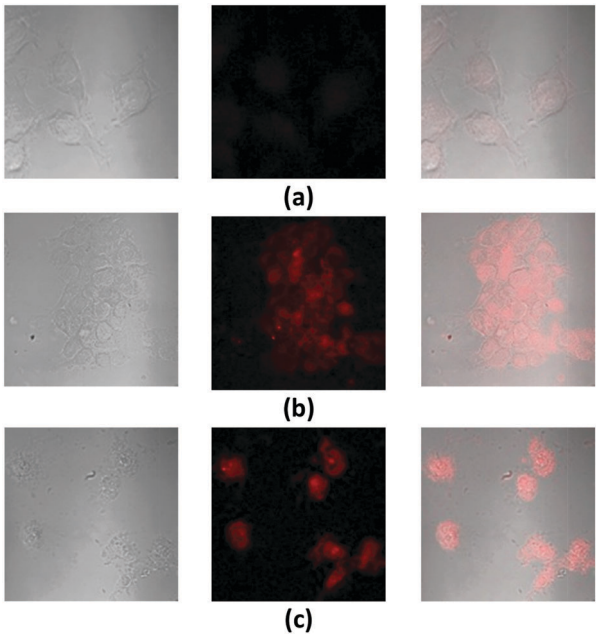

Fig. 3 Confocal imaging of the NG97 cells after incubation with $K_3[Eu(dipicNH_2)_3]$ in (a) absence of complex and after an incubation time of (b) 12 and (c) 24 h. The first column is the bright field image, the second is the luminescence and the third is the overlay between first and second columns ($K_3[Eu(dipicNH_2)_3]$ = 200 μg mL^{-1}, λ_{exc} = 405 $\pm$ 10 nm).

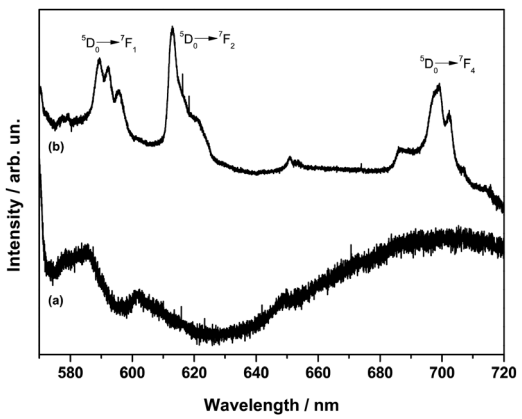

Fig. 4 Emission spectra of the NG97 cells in (a) absence of complex and (b) after incubation for 24 h. ($K_3[Eu(dipicNH_2)_3]$ = 200 μg mL^{-1}, λ_{exc} = 488 nm).

attributed to changes in the Eu^{III} symmetry, due to possible interaction with intracellular species.[52] While we cannot fully exclude decomplexation within the cell environment as a reason for changes in peak splitting, we can reasonably assume that complex is present, since we observe that its solutions are cytotoxic, while solutions of the Ln^{III} salts and of the ligand are not. Further, as evidenced by unchanged emission lifetimes and mono-exponential decays, the 3 : 1 species do not undergo decomplexation in the presence of the biologically relevant cations Ca^{II}, Zn^{II} and Fe^{III} (Table S2, ESI†).[53]

To evaluate the $K_3[Eu(dipicNH_2)_3]$ complex as possible therapeutic and luminescent dye for glioblastomas, we tested its ability to cross the BBB.[54] The barrier was simulated according to a previously described procedure.[54,55] After 24 hours of simulation (Fig. S29, ESI†), the NG97 cells exhibit Eu^{III}-centred luminescence, indicating that the complex crosses the simulated barrier.

In summary, an easily accessible ligand derived from dipicolinic acid with the functional group –NH_2, H_2dipicNH_2, was synthesized and its 3 : 1 (L : Ln) Ln^{III} ion complexes isolated and characterized. The Eu^{III} and Tb^{III} complexes showed efficient metal-centred luminescence in aqueous solution at physiological pH and stability of the 3 : 1 species at the concentrations used. The complexes showed elevated cytotoxicity against NG97 and PANC-1 cancer cells, but not the healthy NIH/3T3 cells. Owing to its high emission efficiency in the easily accessible red region of the spectrum and ability to transfect the cell membrane of the cancerous cells chosen, $K_3[Eu(dipicNH_2)_3]$ was used as luminescent dye for the NG97 and PANC-1 cells. Finally, this complex was able to cross a simulated BBB. The results shown here indicate the promise of this type of Ln^{III} complexes for use as theranostic agents; they show the characteristic metal-centred luminescence within cancer cells, and, albeit moderate, selective cytotoxic towards those cells.

Financial support from NSF-CHE 1363325 (AdBD), FAPESP 08/53868-0 (FAS), CNPq 303433/2014-0 (FAS and AdBD), FAPESP 2012/15064-3 (ML) is gratefully acknowledged. The authors thank Prof. Carlos Lenz Cesar, for access to equipment and assistance provided by INFABIC at the University of Campinas.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 X. He, Y. Hu, W. Shi, X. Li and H. Ma, *Chem. Commun.*, 2017, **53**, 9438–9441.
2 D. Kim, R. R. Jetson and C. J. Krusemark, *Chem. Commun.*, 2017, **53**, 9474–9477.
3 Z. Liu, P. Koczera, D. Doleschel, F. Kiessling and J. Gatjens, *Chem. Commun.*, 2012, **48**, 5142–5144.
4 N. K. Devaraj, R. Upadhyay, J. B. Hatin, S. A. Hilderbrand and R. Weissleder, *Angew. Chem., Int. Ed.*, 2009, **48**, 7013–7016.
5 I. A. Karpenko, M. Collot, L. Richert, C. Valencia, P. Villa, Y. Mely, M. Hibert, D. Bonnet and A. S. Klymchenko, *J. Am. Chem. Soc.*, 2015, **137**, 405–412.

6 H. L. Steel, S. L. Allinson, J. Andre, M. P. Coogan and J. A. Platts, *Chem. Commun.*, 2015, **51**, 11441–11444.
7 S. C. Wu, Y. L. Zhong, Y. F. Zhou, B. Song, B. B. Chu, X. Y. Ji, Y. Y. Wu, Y. Y. Su and Y. He, *J. Am. Chem. Soc.*, 2015, **137**, 14726–14732.
8 X. D. Zhang, X. Xie, H. Wang, J. J. Zhang, B. C. Pan and Y. Xie, *J. Am. Chem. Soc.*, 2013, **135**, 18–21.
9 Z. L. Zhao, H. H. Fan, G. F. Zhou, H. R. Bai, H. Liang, R. W. Wang, X. B. Zhang and W. H. Tan, *J. Am. Chem. Soc.*, 2014, **136**, 11220–11223.
10 I. Schick, S. Lorenz, D. Gehrig, A. M. Schilmann, H. Bauer, M. Panthofer, K. Fischer, D. Strand, F. Laquai and W. Tremel, *J. Am. Chem. Soc.*, 2014, **136**, 2473–2483.
11 Q. Liu, Y. Sun, T. S. Yang, W. Feng, C. G. Li and F. Y. Li, *J. Am. Chem. Soc.*, 2011, **133**, 17122–17125.
12 R. Qiao, Q. Jia, S. Huewel, R. Xia, T. Liu, F. Gao, H.-J. Galla and M. Gao, *ACS Nano*, 2012, **6**, 3304–3310.
13 H. Yan, L. Wang, J. Wang, X. Weng, H. Lei, X. Wang, L. Jiang, J. Zhu, W. Lu, X. Wei and C. Li, *ACS Nano*, 2012, **6**, 410–420.
14 J. V. Georgieva, R. P. Brinkhuis, K. Stojanov, C. A. G. M. Weijers, H. Zuilhof, F. P. J. T. Rutjes, D. Hoekstra, J. C. M. van Hest and I. S. Zuhorn, *Angew. Chem., Int. Ed.*, 2012, **51**, 8339–8342.
15 Y. S. Yim, J.-S. Choi, G. T. Kim, C. H. Kim, T.-H. Shin, D. G. Kim and J. Cheon, *Chem. Commun.*, 2012, **48**, 61–63.
16 H. Jing, C. Weidensteiner, W. Reichardt, S. Gaedicke, X. K. Zhu, A. L. Grosu, H. Kobayashi and G. Niedermann, *Theranostics*, 2016, **6**, 862–874.
17 W. Yuan, D. P. Yang, Q. Q. Su, X. J. Zhu, T. Y. Cao, Y. Sun, Y. Dai, W. Feng and F. Y. Li, *Adv. Funct. Mater.*, 2016, **26**, 8631–8642.
18 J.-C. G. Bünzli, *Chem. Rev.*, 2010, **110**, 2729–2755.
19 J.-C. G. Bünzli, *Coord. Chem. Rev.*, 2015, **293-294**, 19–47.
20 A. de Bettencourt-Dias, in *Luminescence of Lanthanide Ions in Coordination Compounds and Nanomaterials*, ed. A. de Bettencourt-Dias, Wiley, 2014.
21 J.-C. G. Bünzli and C. Piguet, *Chem. Soc. Rev.*, 2005, **34**, 1048–1077.
22 M. Rajendran, E. Yapici and L. W. Miller, *Inorg. Chem.*, 2014, **53**, 1839–1853.
23 C. P. Montgomery, B. S. Murray, E. J. New, R. Pal and D. Parker, *Acc. Chem. Res.*, 2009, **42**, 925–937.
24 S. Mizukami, T. Yamamoto, A. Yoshimura, S. Watanabe and K. Kikuchi, *Angew. Chem., Int. Ed.*, 2011, **50**, 8750–8752.
25 A. de Bettencourt-Dias, P. S. Barber and S. Bauer, *J. Am. Chem. Soc.*, 2012, **134**, 6987–6994.
26 A. de Bettencourt-Dias, P. S. Barber, S. Viswanathan, D. T. de Lill, A. Rollett, G. Ling and S. Altun, *Inorg. Chem.*, 2010, **49**, 8848–8861.
27 S. V. Eliseeva, G. Auboeck, F. van Mourik, A. Cannizzo, B. Song, E. Deiters, A.-S. Chauvin, M. Chergui and J.-C. G. Bünzli, *J. Phys. Chem. B*, 2010, **114**, 2932–2937.
28 J. Andres and A.-S. Chauvin, *Phys. Chem. Chem. Phys.*, 2013, **15**, 15981–15994.
29 A. Bourdolle, M. Allali, J. C. Mulatier, B. Le Guennic, J. M. Zwier, P. L. Baldeck, J.-C. G. Bünzli, C. Andraud, L. Lamarque and O. Maury, *Inorg. Chem.*, 2011, **50**, 4987–4999.
30 A.-S. Chauvin, S. Comby, M. Baud, C. De Piano, C. Duhot and J.-C. G. Bünzli, *Inorg. Chem.*, 2009, **48**, 10687–10696.
31 A. D'Aleo, A. Picot, A. Beeby, J. A. G. Williams, B. Le Guennic, C. Andraud and O. Maury, *Inorg. Chem.*, 2008, **47**, 10258–10268.
32 J. Andres and A.-S. Chauvin, *Inorg. Chem.*, 2011, **50**, 10082–10090.
33 Q. Ju, D. Tu, Y. Liu, R. Li, H. Zhu, J. Chen, Z. Chen, M. Huang and X. Chen, *J. Am. Chem. Soc.*, 2012, **134**, 1323–1330.
34 Z. Tang, Z. Lin, G. Li and Y. Hu, *Anal. Chem.*, 2017, **89**, 4238–4245.
35 I. Kostova, *Curr. Med. Chem.*, 2005, 591–602.
36 D. M. Miller-Shakesby, B. P. Burke, S. Nigam, G. J. Stasiuk, T. J. Prior, S. J. Archibald and C. Redshaw, *CrystEngComm*, 2016, **18**, 4977–4987.
37 T. F. A. F. Reji, A. J. Pearl and B. A. Rosy, *J. Rare Earths*, 2013, **31**, 1009–1016.
38 I. Kostova and T. Stefanova, *J. Rare Earths*, 2010, **28**, 40–46.
39 I. Kostova and T. Stefanova, *J. Trace Elem. Med. Biol.*, 2010, **24**, 7–13.
40 B. Liu, C. Li, P. Yang, Z. Hou and J. Lin, *Adv. Mater.*, 2017, **29**, 1605434.
41 Z. E. A. Chamas, X. Guo, J. L. Canet, A. Gautier, D. Boyer and R. Mahiou, *Dalton Trans.*, 2010, **39**, 7091–7097.
42 B. H. Huang, M. A. Prantil, T. L. Gustafson and J. R. Parquette, *J. Am. Chem. Soc.*, 2003, **125**, 14518–14530.
43 P. A. Brayshaw, J.-C. G. Bünzli, P. Froidevaux, J. M. Harrowfield, Y. Kim and A. N. Sobolev, *Inorg. Chem.*, 1995, **34**, 2068–2076.
44 J. H. S. K. Monteiro, A. de Bettencourt-Dias and F. A. Sigoli, *Inorg. Chem.*, 2017, **56**, 709–712.
45 A. S. Chauvin, F. Gumy, D. Imbert and J. C. G. Bünzli, *Spectrosc. Lett.*, 2004, **37**, 517–532.
46 A.-L. Gassner, C. Duhot, J.-C. G. Bünzli and A.-S. Chauvin, *Inorg. Chem.*, 2008, **47**, 7802–7812.
47 R. M. Supkowski and W. D. Horrocks, *Inorg. Chim. Acta*, 2002, **340**, 44–48.
48 J. B. Lamture, Z. H. Zhou, A. S. Kumar and T. G. Wensel, *Inorg. Chem.*, 1995, **34**, 864–869.
49 M. Danihelová, M. Veverka, E. Šturdík and S. Jantová, *Interdiscip. Toxicol.*, 2013, **6**, 209.
50 L. Y. Wang, Y. C. Chen, H. Y. Lin, Y. T. Hou, L. C. Yang, A. L. Y. Sun, J. Y. Liu, C. W. Chang and D. H. Wan, *ACS Appl. Mater. Interfaces*, 2017, **9**, 3873–3884.
51 N. Tyagi, N. F. Attia and K. E. Geckeler, *J. Colloid Interface Sci.*, 2017, **498**, 364–377.
52 P. Atkinson, B. S. Murray and D. Parker, *Org. Biomol. Chem.*, 2006, **4**, 3166–3171.
53 U. Carpentieri, J. Myers, C. W. Daeschner and M. E. Haggard, *Biol. Trace Elem. Res.*, 1988, **16**, 165–176.
54 J. Banerjee, Y. J. Shi and H. S. Azevedo, *Drug Discovery Today*, 2016, **21**, 1367–1386.
55 R. F. C. Pereira and M. Lancellotti, *Br. Pat.*, 2011003016A2, 2014.

