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A benzimidazole functionalised DO3A chelator showing pH switchable coordination modes with lanthanide ions

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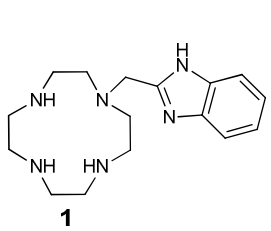
The synthesis of a new macrocyclic chelator incorporating a benzimidazole heterocycle is reported. Lanthanide complexes with macrocyclic chelators based on 1,4,7,10-tetra(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA) and 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A), are of interest in luminescent, radiopharmaceutical and magnetic resonance (MR) biomedical imaging applications. The benzimidazole DO3A chelator allows for sensitisation of europium(III), terbium(III) and ytterbium(III) luminescence by the heterocycle and also shows a pH dependent coordination change due to protonation of the chelator (pKa = 4.1 for the europium(III) complex). The thermodynamic stability of the complexes has been investigated by potentiometric titration with the gadolinium(III) complex showing significantly higher stability than the zinc(II) complex, where $\log\beta_{ZnLH} = 28.1$ and $\log\beta_{GdLH} = 32.1$.

Introduction

There is high interest in the use of metal complexes for multimodal imaging with a requirement for chelators incorporating functional groups for binding, sensitisation and conjugation. The imidazole heterocycle is a bio-relevant metal binding unit with two nitrogen donors offering multiple coordination modes and protonation states.[1] Functionalisation allows it to be incorporated into multidentate chelating constructs linking via either a nitrogen or carbon.[2] In this work we have combined an imidazole coordinating group, in the form of a benzimidazole, with an azamacrocycle to produce an octadentate ligand for the coordination of lanthanide ions. We have studied the effects of pH on the coordination mode of the chelator and investigated the photophysical properties of its lanthanide complexes. The use of a heterocycle with an N-H group for functionalisation opens up wider biomedical applications for this chelator as a multipurpose construct for use in a series of imaging techniques by varying the metal centre (e.g. ^{111}In for SPECT imaging, ^{177}Lu or ^{90}Y for radioimmunotherapy, ^{86}Y for PET imaging, Yb^{III} for NIR luminescent imaging and Gd^{III} for MRI contrast.). In this work the aim is to characterise the interactions of the chelator with lanthanide ions.[3, 4]

Benzimidazole has been used as component of a chelating ligands combined with the cyclen macrocycle but no 1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A)-benzimidazole constructs were found in database searches. Tripodal benzimidazole ligands have previously been used to form supramolecular networks with lanthanide ions[5] and it has been demonstrated that alkylation of the N-1 position can be used to influence the photophysical properties.[6] Bünzli and co-workers have synthesised chelators for coordination to lanthanide ions with a central pyridyl group and benzimidazole arms mixed with carboxylates for coordination to lanthanide ions.[7]



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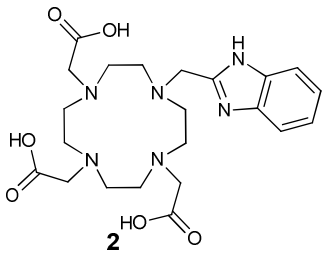

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Stoppionni and co-workers have synthesised a series of macrocyclic chelating ligands incorporating imidazole units attached at either the 2 or 4/5 position.[8-11] A cyclen derivative with two acetate and two *N*-methylimidazole pendant arms (attached via the imidazole 2-position) was synthesised and coordination chemistry investigated with a number of first row transition metals including zinc(II) and nickel(II), and cadmium(II), mercury(II) and lead(II) which were investigated for dynamic exchange processes by NMR.[12, 13] A triaza macrocycle was used to produce a tetradentate chelator with one pendant imidazole unit attached by either the 2- or 4-position on the heterocyclic ring which can form chloride bridged transition metal dimers.[14]

Other heterocycles have been combined with cyclen,

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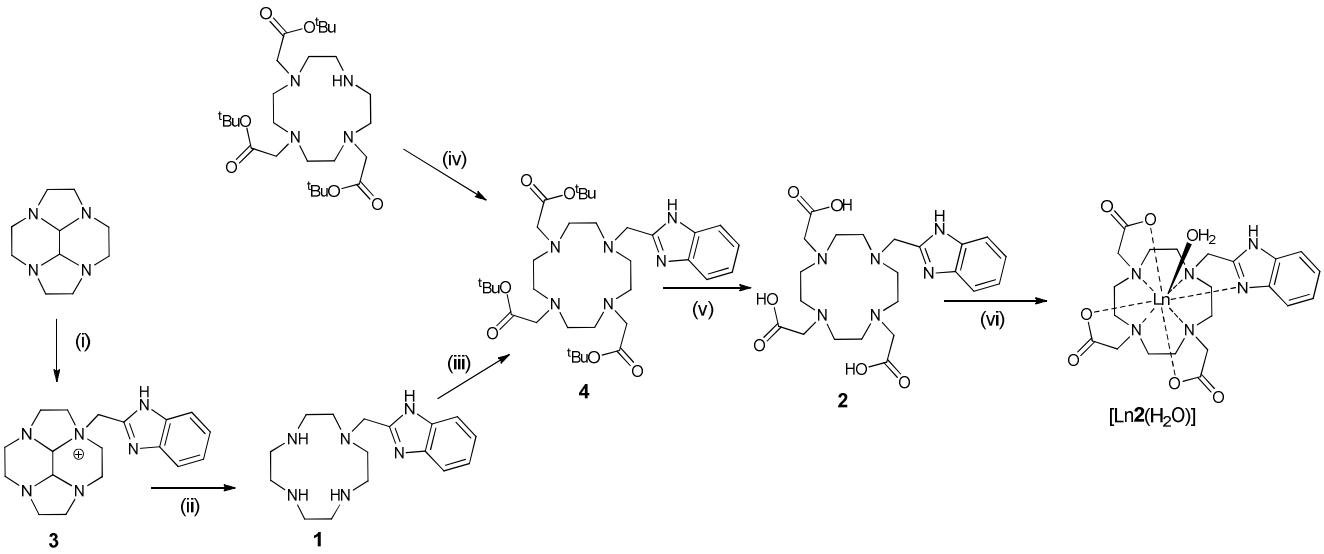
† Electronic Supplementary Information (ESI) available: Spectroscopic data (UV/vis., NMR and luminescence spectra including exponential fitted decay curves). See http://dx.doi.org /



Scheme 1 Synthesis of benzimidazole-DO3A chelators by two different routes. The chelator can either be produced by substituting 2-chloromethylbenzimidazole with protected DO3A or using the selective monosubstitution reaction with glyoxal bridged cyclen. Experimental conditions: (i) 2-chloromethyl benzimidazole, MeCN, RT (ii) hydrazine monohydrate, reflux (iii) *tert*-butylbromoacetate, K_2CO_3, EtOH, RT (iv) 2-chloromethyl benzimidazole, Cs_2CO_3, MeCN (v) 6M HCl, reflux (vi) $LnX_3.xH_2O$, K_2CO_3, H_2O, reflux.

Faulkner and co-workers utilised a pyridyl DO3A gadolinium(III) complex to link to a luminescent rhenium complex for applications in bimodal imaging.[15] Cyclen ligands with pendant benzimidazole arms have been synthesised but complex formation with lanthanide ions has not been reported. Chuburu and co-workers have developed benzimidazolylmethylcyclen **1** as a sensor for zinc(II) taking advantage of a chelation enhanced fluorescence process on binding to the metal ion.[16]

The lanthanides have relatively long lived emission lifetimes from microseconds for ytterbium(III) and neodymium(III), to milliseconds for europium(III) and terbium(III) and they are useful in time-resolved imaging where the lanthanide luminescence can be distinguished from that of shorter lived biological chromophores.[17] Thus, Parker, Faulkner, Gunnlaugsson and many others have extensively studied lanthanide complexes with applications in luminescent cellular imaging and as reactive probes. Since the dipole strengths of lanthanide(III) f-f transitions are very small, direct excitation into the 4f energy levels is inefficient. To take advantage of the remarkable lanthanide(III) luminescent properties it is necessary to embed the lanthanide(III) ion in a chelating ligand that behaves as a light harvester. Energy is then transferred from the excited states of the ligand onto the metal ion which subsequently luminesces (antenna effect). In this context, Parker has focussed on tetraazatriphenylene as the lanthanide emission sensitising chromophore.[18] Quici and co-workers reported lanthanide complexes with a DO3A 1,10-phenanthroline ligand system, where inner sphere water molecule coordination is blocked by steric hindrance.[19, 20] The benzimidazole group in our novel chelator acts as the sensitising chromophore that is required for energy efficient transfer to the metal centre via an intramolecular process.[2, 21]

Results and Discussion

Synthesis

Two synthetic routes to produce the methylbenzimidazole DO3A chelator **2** were investigated; utilising the reaction with the tris-*tert*-butylacetate cyclen derivative or via initial introduction of a single methylbenzimidazole substituent to produce **1**, see scheme 1. 1,4,7-tris(*tert*-Butoxycarboxymethyl)-1,4,7,10-tetraazacyclododecane was synthesised by following a literature procedure,[22, 23] and the cyclen secondary amine position was then reacted with 2-chloromethylbenzimidazole to give **4** as a light brown solid. Deprotection of the ester groups was carried out using 5 M HCl to give **2** as an off white solid.[24, 25] Positions for attaching imidazole coordinating groups onto tetraazamacrocycles are not limited to substitution at the macrocyclic ring nitrogens. Kimura and co-workers produced an early example where the imidazole is attached at one of the ring carbon positions of the cyclam ring.[26]

Previous preparations for N-substitution of imidazole groups onto cyclen macrocycles have used reduced temperature to control the substitution pattern. For example, Di Vaira and co-workers produce 1,7-bis(1-methylimidazol-2-ylmethyl) cyclen by carrying out the reaction in an ice-cooled DMF-acetonitrile solution over three days. This product was later functionalised with pendant carboxymethyl pendant arms using sodium bromoacetate. This chelator could form two different linkage isomers on complexation with nickel(II), binding either through the pendant imidazoles or the pendant carboxylate arms dependent on the pH of the reaction mixture.

An alternative procedure for selective functionalisation of cyclen macrocycles utilises a bis-aminal cyclen derivative, and has been used previously to produce **1**.[16] A condensation reaction between tetraazamacrocycles such as cyclen, cyclam and homocyclam with glyoxal leads to the formation of azamacrocyclic bis-aminals.[27] The glyoxal bridged bis-aminal cyclen compound (cis-13-1,4,7,10-tetraazatetracyclo[5.5.2.$0^{4,14}0^{10,13}$]tetradecane) was isolated in 88% yield.[28-30] The bis-aminal cyclen derivative was

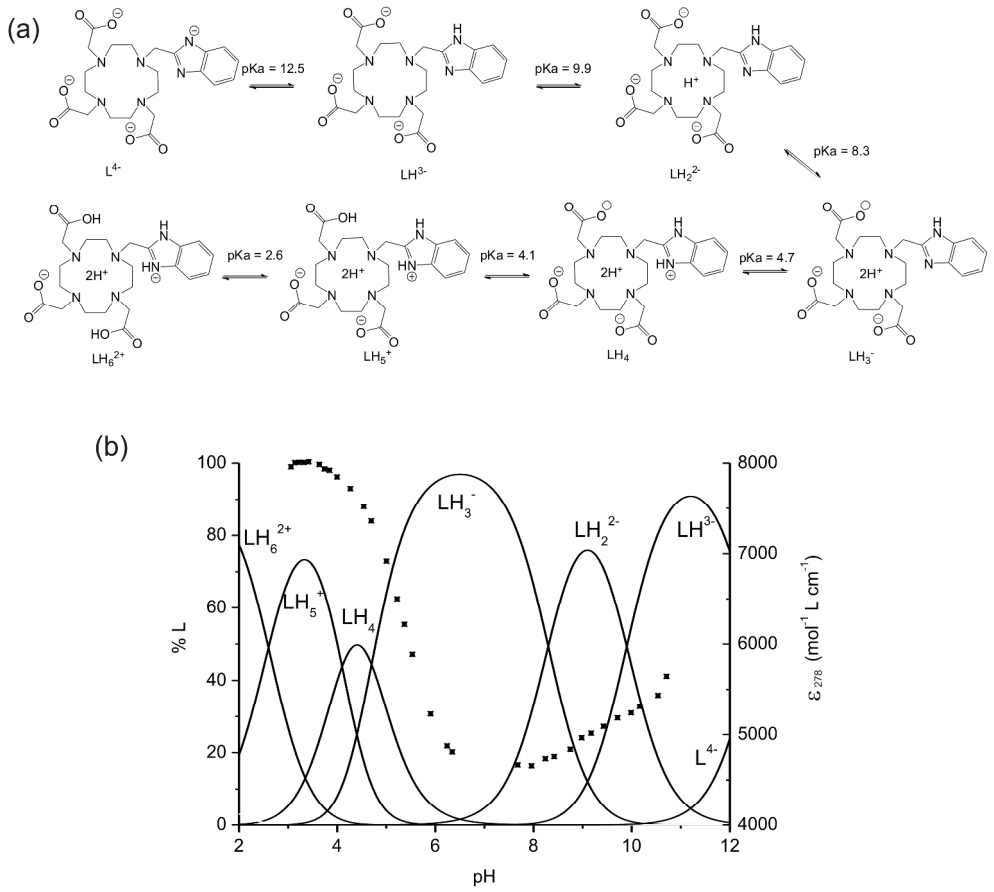


Fig. 1 (a) Protonation pattern of **2** and (b) related speciation diagram with the ε(λ= 278 nm) superimposed. In its neutral form **2** is named LH_4.

employed in a reaction with 2-chloromethyl benzimidazole to form the quaternized product **3**.[31, 32] The reaction was carried out in acetonitrile with stirring at RT for 8 days and then evaporated to dryness to yield **3** as a solid. The bisaminal ethyl bridge was successfully removed from **3** using hydrazine monohydrate to give **1**,[28] which was purified by recrystallisation from a mixture of toluene, CH_2Cl_2 and DMF. Chuburu and co-workers have published a preparation of **1** by a similar method.[16] Three acetate pendant 'arms' were then attached by reaction with *tert*-butylbromoacetate in ethanol to form **4** and deprotection to give **2** was carried out using 5M HCl. Substitution reactions were performed at room temperature to avoid side reactions, as evidence of benzimidazole N-substitution was observed at elevated temperatures.

The lanthanide complexes were formed by refluxing **2** for 3 h in water with either trifluoromethanesulfonate or nitrate lanthanide salts. [Ln**2**] complexes were purified from trace amounts of lanthanide salts and base using Amberlite^{TM} XAD 1600 resin.[20] Complex formation was confirmed by ^{1}H NMR and/or mass spectrometry. The most relevant comparison to the complexes formed here in terms of metal ions used and the similarity of donor atoms are the benzimidazole-5,6-dicarboxylate lanthanide complexes for which coordination is observed through both carboxylate groups and benzimidazole N-donors, and hence it is expected that this would also occur for chelator **2**.[33]

Potentiometric, UV and ^{1}H NMR studies: 2

The chelator **2**, which in its neutral form is represented as LH_4, was titrated with tetramethylammonium. Six protonation constants ($\log K_{01h}$) were calculated (Table 1) to give the speciation diagram of the protonated species for chelator **2** across the pH range 2-12. (Fig.1).

By comparison with the protonation constants of **1**,[16] the two LH_4 protonation constants 9.9 ($\log K_{012}$) and 8.3 ($\log K_{013}$) can be associated to the acid-base equilibria on the macrocyclic nitrogen atoms. By analogy with acetate protonation constants of DOTA[34] the two LH_4 protonation constants 4.1 ($\log K_{015}$) and 2.6 ($\log K_{016}$) can be associated to the acid-base equilibria on the acetate functions. The two remaining LH_4 protonation constants 12.5 ($\log K_{011}$) and 4.7 ($\log K_{014}$) can therefore be ascribed to the benzimidazole moiety. These values are similar to those determined for ligand **1** at 11.1 and 4.6 respectively[16] and for benzimidazole at 12.8[35] and 5.6 respectively.[36] These two latter acid-base steps can be investigated more precisely in the whole pH

Table 1 Protonation constants for **2** and literature data of equivalent protonation events for **1**[16] and benzimidazole (BIM).[35, 36]

	2	**1**	BIM
$L^{4-} + H^+ \rightleftharpoons LH^{3-}$	12.5(1)	11.1	12.8
$LH^{3-} + H^+ \rightleftharpoons LH_2^{2-}$	9.92(3)	10.2	-
$LH_2^{2-} + H^+ \rightleftharpoons LH_3^-$	8.32(2)	9.0	-
$LH_3^- + H^+ \rightleftharpoons LH_4$	4.67(3)	4.6	5.6
$LH_4 + H^+ \rightleftharpoons LH_5^+$	4.10(3)	-	-
$LH_5^+ + H^+ \rightleftharpoons LH_6^{2+}$	2.6(1)	-	-

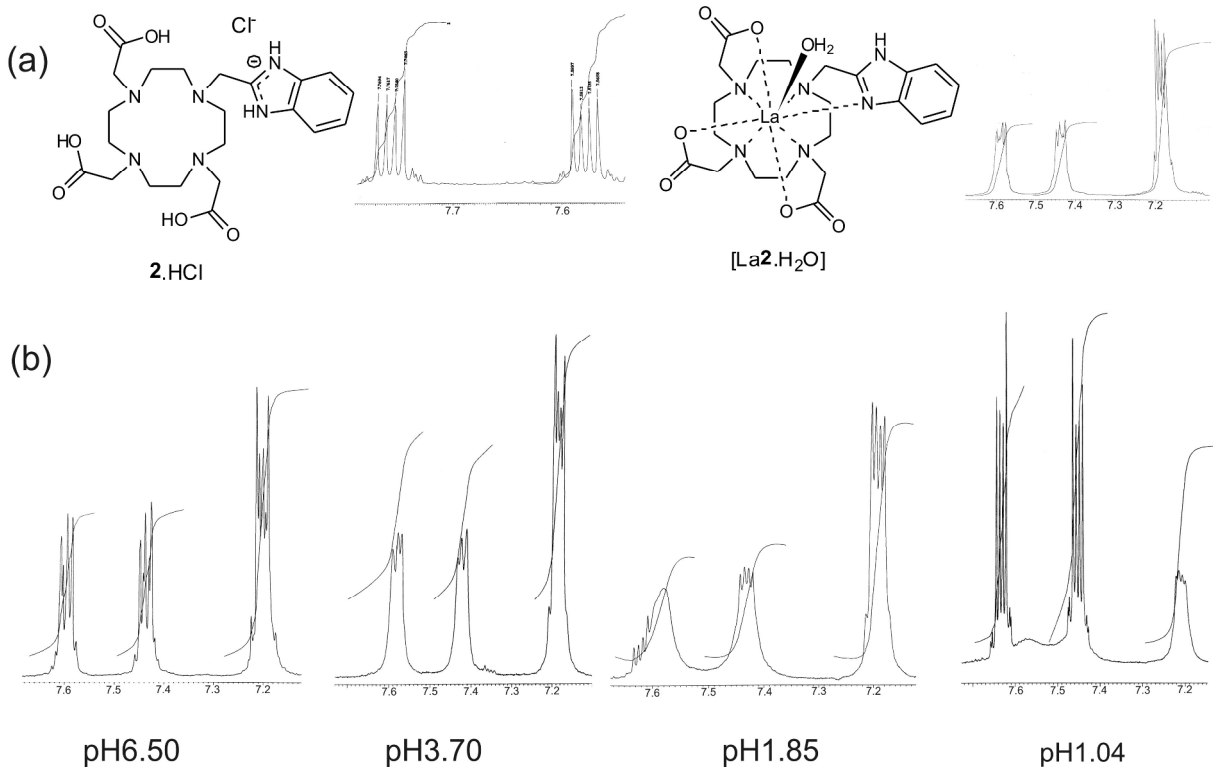


Fig. 2 (a) Aryl region of the ^{1}H NMR spectra for the symmetric protonated benzimidzole chelator **2** and unsymmetric coordinated benzimidazole unit in [La**2**.H_2O] respectively; (b) ^{1}H NMR spectral changes for the aryl region across a pH (pD) range for the complex [La**2**.H_2O].

range by following the UV absorption spectrum of the benzimidazole moiety in the range 250–290 nm. A prior study on ligand **1** has effectively shown that benzimidazole absorption is very sensitive to the protonation degree of the aromatic ring.[16] At pH 6, the UV benzimidazole spectrum shows two peaks at 272 and 278 nm that can be assigned as $\pi^*\leftarrow\pi$ singlet−singlet transitions. Acidification of the medium down to pH = 6 induces a slight blue shift (λ = 2 nm) of these bands accompanied by a strong hyperchromic effect, which results in an approximately 40% increase in the parent extinction coefficient (Fig. 1 and Fig. S2). This effect is characteristic of the formation of a benzimidazolium cation.[16] This result implies that the log*K* value of 4.7 can be attributed to the protonation of the benzimidazole group. Finally, above pH 8, the increase in pH is accompanied by an hyperchromic shift in the range 250–290 nm of the UV spectrum together with the appearance of a low energy shoulder at 300 nm (Fig. 1 and Fig. S2). These data are in agreement with the formation of a benzimidazolate species[16] and indicate that the log*K* value of 12.5 can be attributed to the deprotonation of the benzimidazole group. On the basis of these results, the acid–base behaviour of **2** (i.e. LH_4) is summarized in Fig.1. The resulting speciation diagram shows that the predominant species (LH_3^-) in pH 7.0 has all three carboxylic acids deprotonated, two cyclen ring amines protonated and a neutral benzimidazole.

NMR and Potentiometric studies: lanthanide complexes of 2

The aryl region of the ^{1}H NMR **2**.5HCl spectrum contains two peaks representing the four aryl benzimidazole protons as protonation renders the benzimidazole unit symmetrical. On complexation the data indicates that the lanthanide ion is bound to the *N*3-position of the benzimidazole, hence the ^{1}H

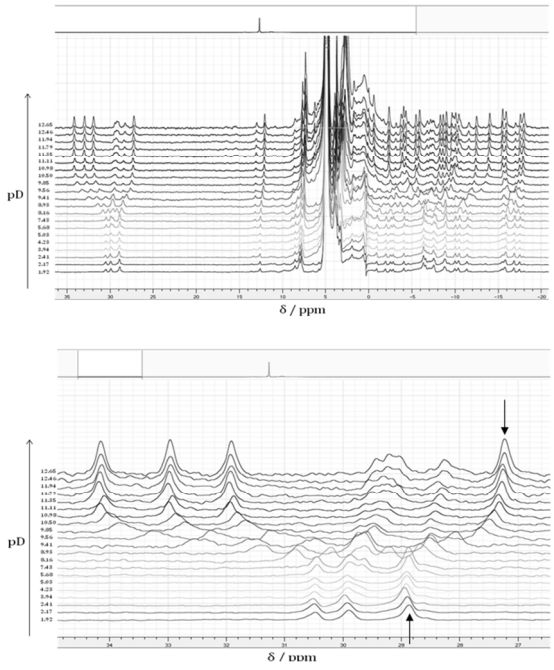


Fig. 3 (a) The complete ^{1}H spectrum over the pD range 1.92 to 12.65 at 300 MHz (b) Enlargement of the 35 –26 ppm region of the spectra of [Eu**2**]

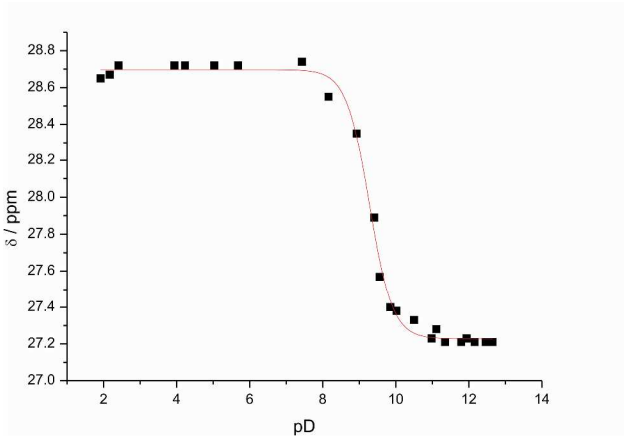


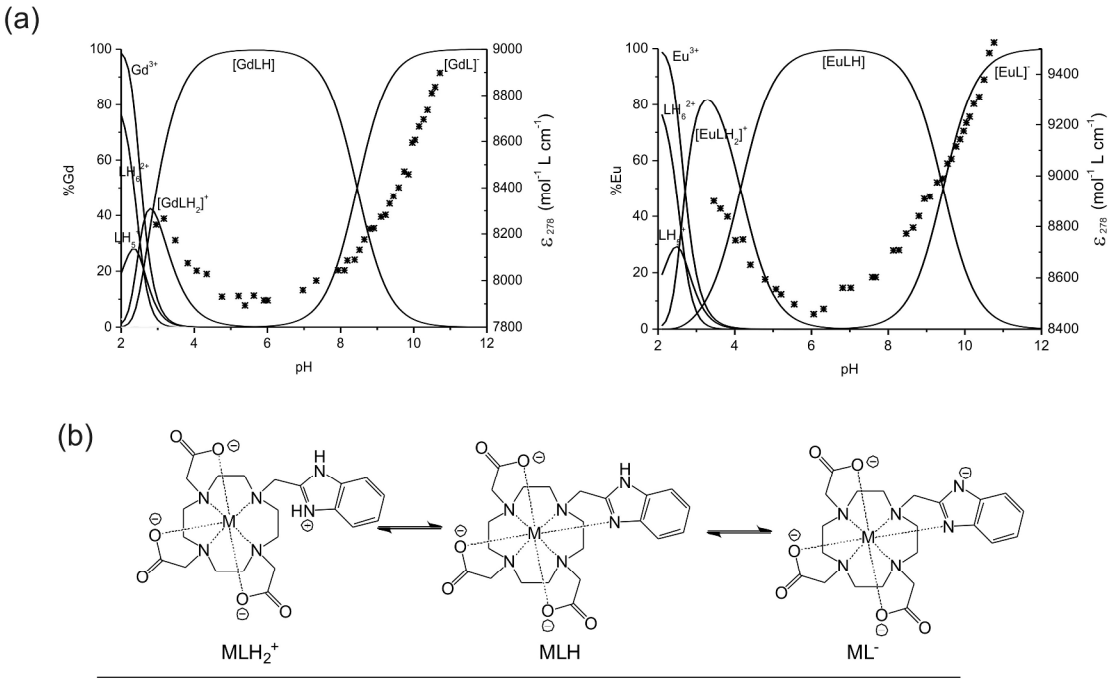
Fig. 4 Plot of δ against pD for [Eu**2**] following the δ = 27.22 ppm peak (at pD 12.68) as a function of pD (indicated by the arrows in Fig. 3).

NMR spectrum of the complex contains peaks for four different proton environments in the aryl region at due to the loss of symmetry, see Fig. 2.

We investigated the characteristics of the ^{1}H NMR spectrum of the lanthanum(III) complex over a pH/ pD range, see Fig. 2. At higher pD (6.50) the benzimidazole is coordinated and as the pH/pD decreases the benzimidazole is protonated and dissociates from the metal centre making the aryl ring proton environment symmetrical to give two peaks. At pD 1.04 only a small amount of the imidazole bound complex remains, in accordance with [Gd**2**] and [Eu**2**] speciation curves *vide infra* Fig.5. It is predicted that the complexes of chelator **2** would be nine coordinate species for the larger lanthanides such as lanthanum(III) (with a bound water molecule) but may form eight coordinate species for the smaller lanthanides. The bound water molecule (if present) could potentially deprotonate at high pH rather than the benzimidazole. However this assignment gave a poor fit to the data and is not consistent with the observed UV spectral changes.

The yttrium(III) complex of **2** was synthesised to probe any structural differences in solution that may occur with the smaller lanthanides. The solution structure of yttrium(III) DO3A complexes with a fourth pendant arm has been studied previously by NMR.[37, 38] The ^{1}H NMR spectrum of [Y**2**] shows a that it is rigid at room temperature and the cyclen ring inversion fluxionality only occurs at elevated temperature (ca. 45°C and above). At room temperature an AB quartet is observed for the benzimidazole CH_2 protons 4.3 – 3.8 ppm. The inequivalency of these protons indicates coordination of the benzimidazole the metal centre.

In the ^{1}H NMR spectra of DOTA type europium(III) complexes, the chemical shifts of the axial cyclen ring protons are characteristic of the coordination mode of the 1,4,7,10-substituted 1,4,7,10-tetrazacyclododecane macrocycle to the lanthanide. The coordination of the eight donors from a macrocyclic chelator in similar systems has been assigned as twisted square antiprism or square antiprism geometry (with the formation of diastereomers observed with chiral ligand systems) on the basis of the NMR data.[39-41] To gain insight into the coordination geometry of [Eu**2**], the paramagnetically shifted ^{1}H NMR spectrum was recorded at 300 and 500 MHz (see Figs. 3 and S1) showing peaks in the region +35 to −25 ppm. For [Eu**2**] the four axial protons are observed as a single a single set of four peaks between 32 to 28 ppm, showing that



pKa ($GdLH_n$)	3.0 (1)	8.4 (0.2)
pKa ($EuLH_n$)	4.1 (0.2)	9.3 (0.2)

Fig. 5 (a) Speciation curves, variation ε(λ=278 nm) with pH for [Gd**2**] and [Eu**2**] and (b) relevant pKa values for the protonation steps of the complexes [Gd**2**] and [Eu**2**].

a single geometric isomer predominates in solution. Assignment by analogy to the published studies indicates a square antiprismatic geometry for [Eu**2**] with traces of a minor isomer observed in the pD titration.

pH dependence of the ^{1}H NMR for [Eu2]

An NMR pH/pD dependence study was carried out with the europium(III) complex. Titration of the complex was performed with a larger number of data points (> 20) than the study with the lanthanum(III) complex to examine the equilibrium between protonated, neutral and deprotonated benzimidazole in the complex. As discussed, the axial ring protons can be identified by their large downfield shift and used to monitor structural change. pD was adjusted a using NaOD and DCl to obtain spectra over the pD range 1.92-12.65, see Fig. 3. The variation in chemical shift of the peak indicated by the arrows in Fig. 3 was plotted to give the titration curve, see Fig. 4. The following equation was used to determine pKa values, in which A1 represents the lower limit of δ, and A2 the higher; $\log x_0$ is the middle of the slope, which is equivalent to the pKa of the complex, and p is a constant that converts the normalised pKa scale to δ scale.

$$\mathbf{y = A1 + \frac{(A2 - A1)}{1 + 10^{(\log x_0 - x)p}}}$$

The results show that there are multiple species present, one at lower pH in the range pH 1.92 to 7.43 and one at higher pH in the approximate range 10.98 to 12.65. The pKa of the acid form was calculated to be 9.28, showing the increased acidity of the N–H proton due to coordination to the europium(III) ion. The results are consistent with those published by Lowe and co-workers on the pH dependence of triazole lanthanide complexes of europium(III)[42] for which the pKa of the N-H of the triazole appended EuDO3A is 7.5, with the lower pKa expected for the more acidic triazole N–H proton.

Potentiometric studies: overall complexation constants for [Gd2] and [Eu2]

Potentiometric titrations were used to investigate the Gd^{III} and Eu^{III} complexation pathways with **2** (*i.e.* LH_4). The overall stability constants calculated for the 1:1 lanthanide complexes are reported in Table 2 and the best-fit lanthanide speciation diagrams are reported Fig. 5.

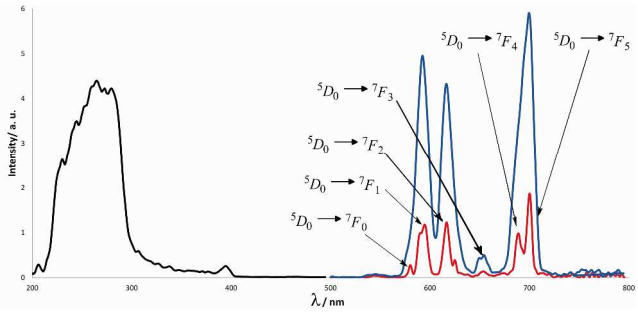


Fig. 6 Plot of the luminescence spectra of [Eu**2**] at pH 11.86 in H_2O, the dark blue line is the excitation spectrum at 616 nm emission, the black line is the emission spectrum at 265 nm excitation with a 10 nm slit width. Similarly the red line represents the emission spectrum at 265 nm excitation with a 5 nm slit width.

Table 2 $\log\beta_{MLHn}$ values for LH_4 / M systems with M = Gd^{III}, Eu^{III}, Zn^{II}, Cu^{II} and relative pK_{MLH} deprotonation constant

$\log\beta_{MLHn}$	*Gd*	*Eu*	*Zn*	*Cu*
M + L + 4H = MLH_4	-	-	41.1(1)	-
M + L + 3H = MLH_3	-	-	37.8(1)	39.8(4)
M + L + 2H = MLH_2	35.0(5)	35.0(4)	33.6(1)	35.5(5)
M + L + H = MLH	32.1(3)	30.9(2)	28.5(1)	31.0(4)
M + L = ML	23.7(4)	21.62)	18.8(2)	21.8(4)
pK_{MLH}				
MLH = ML + H	8.4(3)	9.3(2)	9.6(2)	9.2(4)

These data allow evaluation of the thermodynamic stability of lanthanide complexes and comparison with the stability of zinc(II) and copper(II) complexes. This comparison is motivated by the fact that these ions (particularly zinc(II)) could potentially compete for coordination to the chelator in a biological system. Analysis of β_{MLH} values (line 4 Table 2) indicates that both the gadolinium(III) and europium(III) complexes show significantly higher stability than the zinc(II) complex. It means that under competitive complexation conditions and from a thermodynamic point of view, it is unlikely that transmetallation will occur between lanthanide complexes and zinc(II). The potentiometric measurements also indicate that different [Gd**2**] and [Eu**2**] species of various protonation states exist in solution. Since ligand potentiometric experiments have shown that benzimidazole moiety could act as an additional acid-base centre, UV/vis titration were performed for each system between pH 2 and 12 (Fig. S3). For ligand **2**, it was previously shown that benzimidazole absorptions in the 250-290 nm range evolve according to benzimidazole protonation states. Therefore, these bands and their respective intensities could give insight into the benzimidazole protonation pattern of the complexes. For gadolinium(III) and europium(III) complexes, the evolution of the extinction coefficient at 278 nm (ε_{278}) was followed across the pH range 2-12 (Fig.5 - superimposed curve with black stars). From pH 2 to 6, ε_{278} decreases, following the formation of the formation of the [M**LH**] species. On the basis of what was observed for the ligand, this evolution could be attributed to the deprotonation of the

Table 3 Luminescence data for complexes Ln**4**.[a] Rate constants (ms^{-1}) for depopulation of the excited states of the [Tb**2**][b], [Yb**2**][c] and [Eu**2**][d, e] complexes in H_2O and D_2O at 293 k.

Complex	Lifetime (τ)	k_{H_2O}	k_{D_2O}	Δk	Δk_{corr}	q^1_{corr}
[Eu**2**]	0.63 ms	1.59	0.42	1.17	0.92[d]	1.10[h]
[Tb**2**]	1.92 ms	0.52	0.31	0.21	0.15[b]	0.75[f]
[Yb**2**]	1.23 µs	0.81[i]	0.14[j]	0.67	0.57[c]	0.57[g]

[a]Lifetimes are quoted with errors of ± 10%. [b]A correction to Δk of − 0.06 ms^{-1} has been applied, to allow for the effect of closely diffusing OH oscillators. [c]A correction to Δk of − 0.10 μs^{-1} has been applied, to allow for the effect of closely diffusing OH oscillators. [d]A correction to Δk of − 0.25 ms^{-1} has been applied, to allow for the effect of closely diffusing OH oscillators. [e]Each NH oscillator has been assumed to contribute 0.075 ms^{-1} to the quenching of the Eu $^5D_0$ excited state. [f]The value of q^1_{corr} has been obtained, by multiplying Δk_{corr} by 5.0. [g]The value of q^1_{corr} has been obtained, by multiplying Δk_{corr} by 1.0. [h]The value of q^1_{corr} has been obtained, by multiplying Δk_{corr} by 1.20. [i, j]Rate constants are in μs^{-1}.

benzimidazolium moiety (MLH_2^+ → MLH). On increasing the pH up to 12, the UV bands of the benzimidazole unit are perturbed and undergo a slight red shift of about 5 nm (Fig. S3b). Moreover, a new band appears at 284 nm from pH 8 up to pH 12, and the extinction coefficient of this band increases by about a factor of four. As indicated for the ligand, this is characteristic of deprotonation at the imidazole ring (MLH → ML^-).[43] From the log β_{MLH} values, one can calculate the deprotonation constant of [Gd**2**] and [Eu**2**] complexes (last line Table 2) which, as suggested by the UV titration, corresponds to the ionization of the pyrrole hydrogen of the benzimidazole moiety. These values are significantly lower than that observed for the analogous equilibrium in **2**, which means that in the presence of the lanthanide(III) ion, the benzimidazole pyrrole hydrogen is 10^3 (with europium(III)) and 10^4 (with gadolinium(III)) times more acidic. Similar results are obtained for Zn(II) and Cu(II) complexes (last line Table 2, Figs. S4 and S5). By analogy with [M**1**] complexes,[16] where M is a zinc(II) or copper(II) ion, this increased acidity can be correlated with the coordination of the benzimidazole moiety to the metal, which strongly influences the benzimidazole acidity.[44]

To sum up, the pKa values and species for the lanthanide complexes are identified in Fig. 5b. The data matches with the NMR titration values for the europium(III) complex with both methods giving a pKa value of 9.3 for deprotonation of the coordinated benzimidazole.

Photophysical properties of [Eu2], [Tb2] and [Yb2]

Emission spectra of the complexes were recorded in aqueous solutions with excitation at 273 nm via the benzimidazole chromophore. Europium(III) has a $^5D_0$ excited state and the complex showed a typical emission spectrum (λ_{em} = 575, 592, 616, 655 and 699 nm), see Fig. 6. The terbium(III) complex shows a characteristic four band emission in the visible region. (480 nm $^5D_4 \rightarrow {}^7F_6$; 540 nm $^5D_4 \rightarrow {}^7F_5$; 575 nm $^5D_4 \rightarrow {}^7F_4$; 624 nm $^5D_4 \rightarrow {}^7F_3$), see Fig S10. Yb has a $^2F_{5/2}$ excited state which emits in the near-IR. The ytterbium(III) complex was excited at 337 nm, using a pulsed nitrogen laser, and gave rise to an ytterbium based emission in the near-IR region at around 980 nm.

Luminescence lifetimes

The luminescence lifetimes (τ) of the complexes in D_2O and H_2O, are given in Table 3 and the fitted decay profiles are included in the supplementary data, see Figs. S7 and S8. Emissive energy peaks were recorded at the following levels; Eu λ_{em} = 615 nm, Tb λ_{em} = 545 nm, Yb λ_{em} = 977 nm.[45-47] The number of bound water molecules (*q* value, with an uncertainty of ± 0.5) was calculated by measuring the rate constant for depopulation of the excited state in H_2O and D_2O, with a correction made for the quenching effect of unbound (closely diffusing) H_2O molecules, and for other exchangeable NH/OH oscillators (for equations see supplementary data). The complexes have an NH oscillator present on the benzimidazole unit which can contribute to quenching (corrections values used for benzimidazole N-H quenching contribution: Eu -0.075 ms^{-1} and Yb 0.06 μs^{-1}, no correction applied for Tb).[48, 49] The luminescence lifetime of [Eu**2**] is 0.63 ms in an aqueous environment, which is typical for a europium(III) complex with a *q* value of 1.1 indicating that the species is nonacoordinate. For [Yb**2**] the lifetime values were obtained by iterative reconvolution of the detector response with a single exponential decay component, see Fig. 7.[50] Excitation gave rise to an ytterbium centred time resolved emission spectrum. The *q* value for [Yb**2**] is 0.6. It is lower than for the other lanthanide complexes, as would be predicted due to the reduced ionic radii of the ytterbium(III) ion compared to the europium(III) and terbium(III) ions and hence higher preference for an octacoordinate geometry.

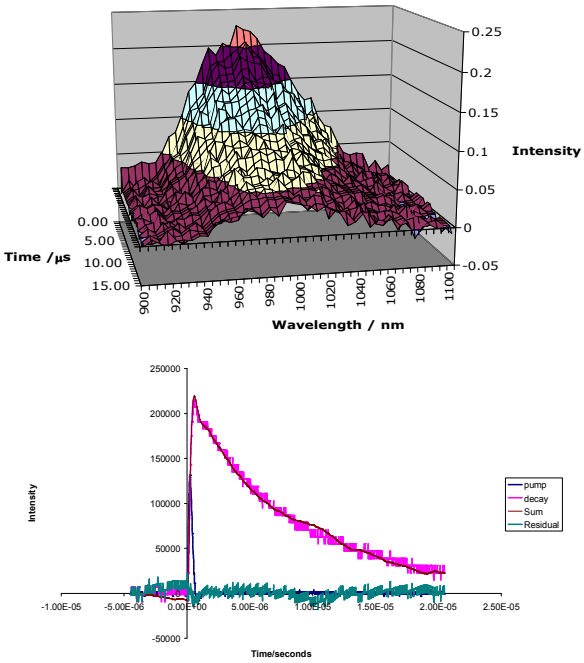


Fig. 7 Fitted decay for the emission from [Yb**2**] in D_2O (λ_{exc} = 272 nm, λ_{em} = 977 nm). The fit was obtained by iterative reconvolution with the detector response by minimisation of residuals squared. The fitted curve and the observed decay are almost exactly superimposed. Time resolved emission spectrum of [Yb**2**] (D_2O solution) is also shown.

pH dependence of luminescence lifetimes

There has been interest in modulating the lanthanide luminescence with pH to produce luminescent pH sensors.[51] One strategy to achieve this is to have a functional group that will protonate, freeing up a coordination site at the metal centre where a water molecule can bind. This will partially quench the luminescence, reducing its intensity and thereby reporting on the surrounding media.[52, 53,54] The benzimidazole in our system can perform this role and hence an the efficiency of the energy transfer step can be reduced either because the energy transfer distance is longer or due to the change in properties of the protonated chromophore. Additional luminescence quenching may also occur due to binding of additional water molecule(s) to the metal centre.

The pH/pD dependency of the luminescence lifetimes was investigated for [Eu**2**] and the *q* values calculated at each pH point, see Table 4. It would appear that changing the pH/pD has had no significant effect on the number of bound water molecules. This was an unexpected result, as correlation with the potentiometric titration results indicates that at lower pH/pD the benzimidazole group becomes protonated and is no longer bound, hence an increase in the *q* value would be expected as a water molecule filled the vacant site in the coordination sphere. This could be explained by a structural rearrangement in which the europium(III) centre adopts an octacoordinate geometry with seven donors from chelator **2** and a single water molecule. Luminescence spectra were recorded for [Tb**2**] at pH values of 4.2 and 6.2 showing quenching of the luminescence at lower pH, see Fig. S10. As the complex becomes protonated the luminescence is quenched fitting the model of less efficient transfer of energy to the metal centre by the dissociated benzimidazole chromophore.

The UV/vis spectra of [Eu**2**] recorded above and below the pKa value of 9.3, see Fig. S6, clearly show a structural change relating to the benzimidazole chromophore. This indicates that the deprotonation event is occuring at the coordinated heterocycle rather than at a bound water molecule. The luminescence studies indicate no change in the q value and little impact of the removal of an exchangeable N-H oscillator.

Irreversible pH dependent changes observed for [Yb2]

[Yb**2**] exhibits different pH dependent behaviour than [Eu**2**] although pD dependence is still observed in the ^{1}H NMR spectrum. If the pD is raised to 10.97, then lowered again down to 1.76 irreversible change is observed for the cyclen axial ring protons in the 140 – 100 ppm region of the spectrum, see Figs. S11 and S12. This indicates a reaction has occurred. Due to the strong Lewis acidity of ytterbium(III) the benzimidazole may be activated for nucleophilic attack by hydroxide at high pH. Ytterbium(III) is commonly used as Lewis acid catalyst in ring opening and acylation reactions and so a reaction of the benzimidazole would be most likely to be observed for this particular complex of those studied.[55, 56]

Table 4 Rate constants (ms^{-1}) for depopulation of the excited states of the [Eu**2**] complex at varying pH in H_2O and D_2O at 293 k[a, b].

pH	k_{H_2O}	k_{D_2O}	Δk	Δk_{corr}[a]	q^1_{corr}[c]
11.86	1.79	0.51	1.28	1.03	1.2
7.34	1.61	0.46	1.15	0.90	1.1
6.09	1.61	0.44	1.17	0.92	1.1
5.76	1.59	0.43	1.16	0.91	1.1
4.12	1.61	0.43	1.18	0.93	1.1
3.69	1.60	0.44	1.16	0.91	1.1
3.26	1.61	0.43	1.18	0.93	1.1
2.76	1.59	0.43	1.16	0.91	1.1
2.19	1.59	0.43	1.16	0.91	1.1
1.88	1.60	0.42	1.18	0.93	1.1
1.51	1.61	0.47	1.14	0.89	1.1
1.05	1.65	0.52	1.13	0.88	1.1

[a]A correction to Δk of − 0.25 ms^{-1} has been applied to allow for the effect of closely diffusing OH oscillators. [b]Each amide N-H oscillator has been assumed to contribute 0.075 ms^{-1} to the quenching of the Eu $^5D_0$ excited state. [c]The value of q^1_{corr} has been obtained, by multiplying Δk_{corr} by 1.20.

Conclusion

We have synthesised a novel chelator based on the DO3A macrocycle **2** that incorporates a benzimidazole chromophore and characterised the stability, speciation and pH dependent behaviour of its lanthanide complexes. Investigation of the luminescence behaviour of the europium(III), terbium(III) and ytterbium(III) complexes demonstrates efficient sensitisation at 272 nm. The lanthanide complexes have high thermodynamic stability in aqueous media and show pH dependent luminescence and coordination changes as the benzimidazole heterocycle is protonated. The data indicates that a coordinated benzimidazole 'arm' becomes dissociated from the metal centre.

The pKa values determined for the benzimidazole in the complex [Gd**2**] show protonation to form the benzimidazolium species at pKa = 3.0 and deprotonation to form the benzimidazolate at pKa = 8.4, with the comparable values 4.1 and 9.3 respectively for [Eu**2**]. The number of bound water molecules (q) does not show significant variation on protonation of the benzimidazole in [Eu**2**] suggesting that a rearrangement from a 9-coordinate to an 8-coordinate complex, each with one bound water molecule, occurs on deprotonation. At high pH values irreversible changes to the [Yb**2**] complex are tentatively attributed to nucleophilic attack at the benzimidazole.

This chelator offers exciting potential as a framework for the development of optical, MRI and radiopharmaceutical medical imaging constructs. A key feature that can be developed with **2** is functionalisation at the NH position of the benzimidazole group. Archibald and co-workers have investigated N-functionalisation of benzimidazole derivatives that will allow access to a series of chelators with N1 position substitution on the benzimidazole pendant arm.[57] This will allow modulation of the sensitisation properties by removal of the N-H oscillator, variation of photophysical properties and the addition of steric bulk. **2** can also be modified to form a bifunctional chelators (BFCs) useful for radiopharmaceuticals by introducing functional groups for conjugation to proteins or other biological targeting groups through attachment of pendant groups onto the benzimidazole.[58]

Experimental

All chemicals and solvents were used as supplied unless stated otherwise. The lanthanide salts were purchased from Strem Chemicals and the cyclen from Strem Chemicals or from Chematech. All other chemicals were supplied by Sigma-Aldrich or Lancaster. 2-(Chloromethyl)-benzimidazole was synthesised using a standard Phillips synthesis or purchased from Sigma Aldrich.

All ^{1}H and ^{13}C NMR experiments were carried out on a JEOL JNM-LA400 or a Bruker Avance II 500 MHz NMR spectrometer. Spectra are referenced to TMS or partially deuterated solvent. The ^{1}H NMR study of complex [La**2**] over

a pD range used solutions of the same concentration made up with calculated amounts of D_2O and DCl to give the required pD values (8 different pDs in the range 6.50 to 1.04). The pD of each sample was measured using Metrohm AG 9101 pH meter with Herisau electrodes. A standard correction factor of pD = pH meter reading + 0.4 was used to calculate pD.

Electrospray mass spectra were recorded using a Thermo Finnigan LCQ Classic Electrospray LC-MS. Electron impact mass spectra were recorded using a Shimadzu QP55A GCMS, samples were analysed using a solid probe at 70 eV. Flash chromatography was carried out using silica gel supplied by Fluorochem and Brockmann. Amberlite™ XAD 1600 was used for purification of the metal complexes.

Photophysical studies: UV/vis and luminescence measurements were carried out using an Agilent 8453E UV-visible spectroscopy system, an Aminco Bowman Series 2 Luminescence Spectrometer SLM (Aminco Spectronic Instruments) and a Perkin-Elmer LS55 fluorimeter (University of Manchester). In the case of the ytterbium complexes, the samples were excited using a pulsed nitrogen laser (PTI-3301, 337 nm) or a nitrogen pumped dye laser (PTI-330, 520 nm), operating at 10 Hz. Light emitted at right angles to the excitation beam was focused onto the slits of a monochromator (PTI120), which was used to select the appropriate wavelength. The growth and decay of the luminescence at selected wavelengths was detected using a germanium photodiode (Edinburgh Instruments, EI-P) and recorded using a digital oscilloscope (Tektronix TDS220) before transfer to a PC for analysis. Luminescence lifetimes were obtained by iterative reconvolution of the detector response (obtained by using a scatterer) with exponential components for growth and decay of the metal centred luminescence, using a spreadsheet running in Microsoft Excel.[59]

Potentiometric Measurements: Potentiometric titrations were carried out with an automatic titrator composed of a microprocessor burette Metrohm Dosimat 665 and a Metrohm 713 pHmeter, connected to a computer. All measurements were performed within a thermo-regulated cell at 25.0 ± 0.1°C under an argon stream to prevent dissolution of the carbon dioxide. The ionic strength was adjusted to 0.1 with NMe_4Cl. The combined Type "U" glass Metrohm electrode used, had a very low alkaline error. The procedures and apparatus used for protometric measurements have been previously described.[16] The ionic product of water was determined by titration of acetic acid with a CO_2-free NMe_4OH solution (pK_w=13.78(1) at 25.0 ± 0.1°C in 0.1 M of NMe_4Cl). The determination of the complexation ability of **2** towards metal salts (M = Gd^{3+}, Eu^{3+}, Zn^{2+}, Cu^{2+}) was performed according to the 'batch method'. The mother solutions of **2** (1.5×10^{-3} M) and M (5×10^{-3} M) were mixed according to different [M] / [**2**] ratios (I = 0.1). For each ratio, a series of 24 stopped flasks was prepared. Each flask corresponded to a pH value, obtained by micro-addition of NMe_4OH (5×10^{-2} M, I = 0.1). All the flasks were stored under argon and put for six weeks in a thermo-regulated enclosure at 37°C. Before pH measurements, these solutions were finally allowed to reach equilibrium temperature (20°C) for 48 h. The protometric data were processed using PROTAF[60] and Hyperquad 2008 softwares,[61] in order to obtain the best chemical model and the refined overall constants β_{MLH}.

$$m\,M + l\,L + h\,H^+ \rightleftharpoons M_mL_lH_h \qquad \beta_{MLH} = \frac{[M_mL_lH_h]}{[M]^m[L]^l[H^+]^h}$$

The speciation diagrams were obtained using HYSS.[62]

Synthesis of N-(2-methylbenzimidazolyl) cis-13-1,4,7,10-tetraazatetracyclo[5.5.2.$0^{4,14}0^{10,13}$**]tetradecane chloride 3**

2-(Chloromethyl)-benzimidazole (0.41 g, 2.46 mmol) was dissolved in acetonitrile (20 cm^3) and stirred. cis-13-1,4,7,10-Tetraazatetracyclo[5.5.2.$0^{4,14}0^{10,13}$]tetradecane (0.43 g, 2.24 mmol) was dissolved in acetonitrile (20 cm^3) and added dropwise. The reaction was stirred at RT for 8 d. The reaction mixture was then evaporated to dryness to yield a pink/red solid (0.68 g, 84%). ^{1}H NMR (400MHz, CD_3OD) δ 2.46 - 4.17 (m), 4.39 - 5.45 (m), 7.13 - 7.28 (m, 2H, Ar*H*), 7.48 - 7.59 (m, 2H, Ar*H*). MS (ESI) m/z 325 (100 $[M + H]^+$).

Synthesis of 1-(2-methylbenzimidazolyl)-1,4,7,10-tetraazacyclododecane 1 *N*-(2-methylbenzimidazolyl) *cis*-13-1,4,7,10-tetraazatetracyclo[5.5.2.$0^{4,14}0^{10,13}$]tetradecane chloride (0.44 g, 1.21 mmol) in hydrazine monohydrate (20 cm^3) was heated to reflux for 17 h. After cooling the solution was evaporated to yield a mixture of red and white solids. Recrystallisation was carried out from toluene (40 cm^3), CH_2Cl_2 (20 cm^3) and DMF (5 cm^3). On cooling in an ice bath, a white precipitate formed which was collected via filtration and dried (0.14 g, 38%): ^{1}H NMR (400MHz, CD_3OD) δ 2.58 – 2.63 (m, 4H, CH_2), 2.69- 2.77 (m, 8H, C*H*$_2$), 2.79- 2.85 (m, 4H, C*H*$_2$), 3.97 (s, 2H, C*H*$_2$-benzimid.), 7.12-717 (m, 1H, Ar*H*), 7.18-7.22 (m, 1H, Ar*H*), 7.40- 7.46 (m, 1H, Ar*H*), 7.49-7.53 (m, 1H, Ar*H*). MS (ESI) *m/z* 302 (100 $[M + H]^+$).

Synthesis of 1,4,7-tris(tert-butoxycarboxymethyl)-10-(2-methylbenzimidazolyl) tetraazacyclododecane 4

METHOD 1: *tert*-Butylbromoacetate (0.30 g, 1.55 mmol) in EtOH (60 cm^3), was added dropwise over 4 h to a stirred solution of 1-(2-methylbenzimidazolyl)-1,4,7,10-tetraazacyclododecane (0.14 g, 0.46 mmol) in EtOH (30 cm^3) in the presence of K_2CO_3 (0.06 g, 0.46 mmol). After addition was complete the solution was stirred at RT for 72 h. The inorganic salts were then filtered off, and the filtrate was evaporated to dryness to produce a cream coloured solid (0.29 g, ca. 100%).

METHOD 2: 1,4,7-tris(*tert*-butoxycarboxymethyl)-1,4,7,10-tetraazacyclododecane (3.0g, 5.83mmol) was dissolved in dry MeCN (1.2 L), 2-(chloromethyl)-benzimidazole (0.971 g, 5.83 mmol) and Cs_2CO_3 (4.5 g, 13.8 mmol) was added and the reaction was stirred for 18 hours at RT. The reaction was filtered, concentrated *in vacuo* and purified using a neutral alumina plug, eluting with ethyl acetate (100%) then MeOH (100%) to yield a crude solid which was dissolved in DCM (50ml) filtered and concentrated *in vacuo* to yield a light brown solid (3.7g, 98%).

^{1}H NMR (400 MHz, CD_3OD) δ 1.20-1.78 (m, 27H, C(C*H*$_3$)$_3$),

2.73 (s, 8H, CH_2), 2.85 (s, 4H, CH_2), 2.98 (s, 4H, CH_2), 7.06-7.20 (m, 2H, Ar*H*), 7.40-7.52 (m, 2H, Ar*H*). ^{13}C{^{1} NMR (100 MHz, CD_3OD) δ 29.2 (CH_3), 49.2 (CH_2), 49.6 (CH_2), 50.0 (CH_2), 50.4 (CH_2), 53.5 (CH_2), 58.4 (CH_2), 83.7 (C-Ar), 116.7 (CH-Ar), 124.1 (CH-Ar), 144.5 (C-Ar), 153.8 (C-Ar), 171.2 (C=O), 174.6 (C=O). MS (ESI) m/z 645.4 (100 $[M+H]^+$).

Synthesis of 1,4,7-tris(carboxymethyl)-10-(2-methylbenzimidazolyl)tetraazacyclododecane 2

1,4,7-tris(*tert*-butoxycarboxymethyl)-10-(2-methylbenzimidazolyl) tetraazacyclododecane (1.0 g, 1.55 mmol) was dissolved in 6M HCl (50 cm^3) and heated under reflux for 18 hours. The solvent was removed under reduced pressure to give the crude product which was purified by dissolving impurities into diethyl ether and decantation (3 x 20 cm^3) to leave a light brown solid (1.0g, 88%). Analysis of the chelator sample by CHN, TGA and chloride titration showed that **2** is obtained as an hydrochloride salt (the composition of the material is **2**.5HCl.4H_2O).

^{1}H NMR (D_2O) δ 2.95-2.98 (m, 4H, CH_2), 3.12-3.14 (m, 2H, CH_2), 3.21-3.30 (m, 2H, CH_2), 3.37-3.46 (m, 4H, CH_2), 3.51-3.69 (m, 4H, CH_2), 3.72-3.83 (m, 2H, CH_2), 3.94-4.02 (m, 2H, CH_2CO$_2$), 4.09-4.23 (m, 2H, CH_2CO$_2$), 4.37 (s, 2H, CH_2-benzimidazole), 7.49-7.55 (m, 2H, C*H*-Ar), 7.66-7.72 (m, 2H, C*H*-Ar). ^{13}C NMR (D_2O) δ 48.8 (CH_2), 48.9 (CH_2), 49.3 (CH_2), 51.5 (CH_2), 53.1 (CH_2), 56.2 (CH_2), 114.8 (CH-Ar), 127.5 (CH-Ar), 131.2 (C-Ar), 148.8 (C-Ar), 169.5 (C-Ar), 176.9 (C=O); MS (ES, *m/z*) 657 (100 [M + (5HCl)]$^+$), 477 (37 $[M + H]^+$). Anal. Calcd for $C_{22}H_{32}N_6O_6$.5HCl.4H_2O: C, 36.15; H, 6.21; N, 11.50; Cl 24.25%. Found: C, 36.30; H, 5.77; N, 11.52; Cl 24.30%. TGA weight loss (H_2O) Calcd for $C_{22}H_{32}N_6O_6$.5HCl.4H_2O: 9.86% Found : 10.26%.

General method for synthesis of lanthanide complexes

1,4,7-tris(carboxymethyl)-10-(2-methylbenzimidazolyl) tetraazacyclododecane (1.0 g, 1.52 mmol) was dissolved in deionised water (20 cm^3) and the lanthanide salt (1.67 mmol) was then added as solid. K_2CO_3 (1.05 g, 7.59 mmol) dissolved in H_2O (20 cm^3) was then added dropwise, then the reaction mixture was heated to reflux for 18 h. The solution was then evaporated to dryness and the residue extracted with MeOH (20 cm^3). The methanolic solution was filtered and evaporated to dryness. The crude material was passed down an Amberlite XAD 1600 column eluted first with H_2O (to remove inorganic salts) followed by (H_2O/CH_3CN 9:1); which on combination and evaporation of the fractions gave the desired product as a white solid in each case.

Synthesis of terbium(III) 1,4,7-tris(carboxymethyl)-10-(2-methylbenzimidazolyl) tetraazacyclododecane [Tb2]

$Tb(CF_3SO_3)_3$ (0.5 g, 52%). MS (ES, *m/z*) 633 $[M+1]^+$. HR $ESMS^+$ m/z calc. for $C_{22}H_{29}N_6O_6Tb$ 633.1475 $[(M+H)^+]$; found 633.1477. Anal. Calcd for $C_{22}H_{29}N_6O_6Tb.5H_2O$: C, 36.57; H, 5.44; N, 11.63%. Found: C, 36.67; H, 5.25; N, 11.32%.

Synthesis of ytterbium(III) 1,4,7-tris(carboxymethyl)-10-(2- methylbenzimidazolyl) tetraazacyclododecane [Yb2]

$Yb(CF_3SO_3)_3$. (0.51 g, 52%). ^{1}H NMR (D_2O) δ −77.9, −74.3, −66.6, −60.7, −53.2, −48.3, −44.6, −23.5, −20.0, −10.9, 8.2, 11.8, 15.2, 24.5, 34.2, 108.5, 113.8, 116.4, 120.9; HR $ESMS^+$ m/z calc. for $C_{22}H_{29}N_6O_6Yb$ 648.1610 $[(M+H)^+]$; found 648.1616. MS (ES, *m/z*) 648 $[M+1]^+$. Anal. Calcd for $C_{22}H_{29}N_6O_6Yb.4½H_2O$: C, 36.32; H, 5.26; N, 11.55%. Found: C, 36.69; H, 4.89; N, 11.16%.

Synthesis of europium(III) 1,4,7-tris(carboxymethyl)-10-(2- methylbenzimidazolyl) tetraazacyclododecane [Eu2]

$Eu(NO_3)_3.5H_2O$ (0.31 g, 33%). ^{1}H NMR (D_2O) δ −17.84, −17.30, −16.70, −13.62, −11.68, −9.18, −8.68, −6.88, −4.13, −2.81, −1.45, 1.81, 5.25, 7.39, 12.42, 28.99, 29.53, 30.59, 31.24; HR $ESMS^+$ m/z calc. for $C_{22}H_{29}N_6O_6Eu$ 627.1434 $[(M+H)^+]$; found 627.1437. MS (ES, *m/z*) 627 $[M+1]^+$, Anal. Calcd for $C_{22}H_{29}N_6O_6Eu.5½H_20$: C, 36.47; H, 5.56; N, 11.59%. Found: C, 36.46; H, 5.29; N, 11.21%.

Synthesis of lanthanum(III) 1,4,7-tris(carboxymethyl)-10-(2- methylbenzimidazolyl) tetraazacyclododecane [La2]

$La(CF_3SO_3)_3.2H_2O$. (0.23 g, 50%). ^{1}H NMR (D_2O) δ 2.44 (m, 8H, CH_2), 3.03 (m, 4H, CH_2), 3.45 (m, 4H, CH_2), 3.60 (s, 2H, CH_2), 3.72-3.85 (m, 4H, CH_2), 4.51 (s, 2H, cyclen-CH_2-benzimid), 7.26 (m, 2H, Ar*H*b,c), 7.53 (m, 1H, Ar*H*a), 7.68 (m, 1H, Ar*H*d); ^{13}C NMR (D_2O) δ 48.2 (CH_2), 49.5 (CH_2), 50.8 (CH_2), 52.2 (CH_2), 56.6 (CH_2), 114.6 (CH-Ar), 126.8 (CH-Ar), 130.9 (C-Ar), 154.4 (C-Ar), 169.5 (C-Ar), 179.8 (C=O); HR $ESMS^+$ m/z calc. for $C_{22}H_{29}N_6O_6La$ 613.1285 $[(M+H)^+]$; found 613.1285. MS (ES, *m/z*) 613 $[M+1]^+$. Anal. Calcd for $C_{22}H_{29}N_6O_6La.5H_2O$: C, 37.61; H, 5.60; N, 11.96%. Found: C, 37.89; H, 5.26; N, 11.67%.

Synthesis of yttrium(III) 1,4,7-tris(carboxymethyl)-10-(2-methylbenzimidazolyl) tetraazacyclododecane [Y2]

1,4,7-tris(carboxymethyl)-10-(2-methylbenzimidazolyl)tetraazacyclododecane (**2**) (100 mg, 0.25 mmol) was dissolved in ammonium acetate buffer (0.2M, pH 5, 5 ml). To this, yttrium(III) chloride hexahydrate (76 mg, 0.25 mmol) in ammonium acetate buffer (0.2M, pH 5, 5 ml) was added. The reaction was heated under reflux for 18 hours then concentrated *in vacuo*. The crude solid was redissolved in water (1 ml) and purified using an Amberlite XAD16N column, eluting with water (100 ml) then water:acetonitrile (9:1, 100 ml) to yield a white solid (32 mg, 37%). ^{1}H-NMR (D_2O): δ 1.63-1.70 (m, 1H, CH_2), 2.09-2.85 (m, 15H, CH_2), 3.08-3.13 (m, 2H, CH_2), 3.19-3.37 (m, 4H, CH_2), 3.47-3.53 (m, 2H, CH_2), 3.94 (d, 2H, CH_2, J = 16.2 Hz), 4.19 (d, 2H, CH_2, J = 16.2 Hz), 7.08-7.15 (m, 2H, CH-Ar), 7.41 (d, 1H, CH-Ar, J = 7.0 Hz), 7.52 (d, 1H, CH-Ar, J = 7.0 Hz). ^{13}C-NMR (D_2O) : δ 55.1 (CH_2), 55.3 (CH_2), 55.6 (CH_2), 56.1 (CH_2), 56.4 (CH_2), 56.5 (CH_2), 59.4 (CH_2), 65.8 (CH_2), 66.0 (CH_2), 66.4 (CH_2), 113.1 (CH-Ar), 117.6 (C-Ar), 123.1 (CH-Ar), 123.5 (CH-Ar), 135.1 (C-Ar) 140.5 (CH-Ar), 156.1 (C-Ar), 180.7 (C=O), 180.8 (C=O). HRMS calc. 563.1280 found 563.1288 $[M+H^+]$. Anal. Calcd for $C_{22}H_{29}N_6O_6Y.3H_2O$: C, 42.86; H, 5.72; N, 13.63%. Found: C, 43.01; H, 6.00; N, 13.70%.

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Graphical Abstract

A benzimidazole functionalised DO3A chelator showing pH switchable coordination modes with lanthanide ions

A benzimidazole coordinating unit has been incorporated into a DO3A chelator backbone and potentiometric titrations along with NMR and photophysical studies show the influence of pH on protonation of the chelator and its coordination to lanthanide(III) ions. This compound has potential applications in multimodal imaging and as a bifunctional chelator.