Emissive and Cell-Permeable 3-Pyridyl- and 3-Pyrazolyl-4-azaxanthone Lanthanide Complexes and Their Behaviour *in cellulo*

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Dedicated to Professor *Jean-Claude Bünzli* on the occasion of his 65th birthday

A series of seven emissive europium(III) and terbium(III) complexes was prepared, incorporating a 3-pyridyl-4-azaxanthone or 3-pyrazolyl-4-azaxanthone sensitising moiety within a polydentate macrocyclic ligand. High overall emission quantum yields in aqueous media are attenuated in the presence of protein or certain oxy anions due to displacement of the *N,N′*-chelated sensitiser. Nevertheless, these complexes are taken into cells and tend to localise over the first few hours in mitochondria before being trafficked to endosomal compartments. Cell uptake studies, in the presence of competitive inhibitors or promoters of well-defined uptake pathways, reveal a common uptake mechanism involving macropinocytosis.

Introduction. – H_2O-soluble complexes of lanthanide(III) ions (Ln^{3+}) that are amenable to sensitised emission have been studied intensively over the past few decades for three main reasons, each related to a particular technological application. First, they may serve as components in various time-resolved assays, most commonly serving as a donor to a proximate acceptor lumophore, usually involving an intermolecular energy-transfer process [1–4]. Second, they can function as responsive probes, in which the energy lifetime or polarisation of the emitted light is monitored. For example, ratiometric sensors have been devised, in which the relative intensity of a pair of emission bands in a single complex (usually Eu^{III}) reports on the change in the composition of the local environment, on a millisecond timescale. These include systems designed to report changes in pH [5] or the concentration of anions, such as hydrogencarbonate [6] or citrate and lactate [7][8]. Alternative strategies have been devised in which Eu^{III} and Tb^{III} complexes of a common ligand are used. In these cases, it is the intensity ratio of red (Eu) to green (Tb) light that is measured. By careful consideration of the nature of the sensitising moiety and the constitution of the ligand, systems able to report on changes in pO_2 or p[urate] have been developed [9][10]. Third, emissive probes suitable for application in living cells are being evaluated [11–15]. Probes for this purpose should possess the following characteristics: cell permeability and minimal toxicity over the relevant range of concentrations for the duration of the observation period; resistance to photofading and photobleaching; kinetic stability with respect to chemical degradation, such as loss of the metal ion from the ligand or rapid oxidative metabolism of the sensitiser or ligand; immunity, or at

least relative insensitivity, to quenching of the metal-based excited state and those intermediate excited states associated with excitation of the integral sensitising moiety [11][16–18].

A plethora of hepta-, octa-, and nonadentate ligands designed to form robust complexes with lanthanide(III) ions has been reported [1–4][16][19–21], including some elegant dinucleating systems [14]. The ligand should be designed to suppress vibrational deactivation of the excited Ln^{III} ion by proximate NH, OH, or CH oscillators [22]. It also should include an aromatic or heterocyclic sensitising moiety, possessing a small singlet/triplet energy gap, with a rapid rate of intersystem crossing that facilitates efficient population of the triplet state. The 4-azaxanthone sensitisers **1**[1]) have recently been introduced for this purpose [23] (4-azaxanthone[1]) = 5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one) and have proved to be effective sensitisers for Eu and Tb emission in aqueous media [12][17][18]. In seeking to extend their scope, we resolved to prepare bidentate analogues with an increased conjugation length, allowing excitation at longer wavelengths [24]. Both 2,2′-bipyridines **2** and 2-pyrazolyl pyridines **3** are well-established chelating ligands in Ln coordination chemistry [20][23][25] and possess desirable photophysical characteristics allowing their introduction into a sensitising moiety. Accordingly, we set out to prepare these compounds and incorporate them into well-defined, macrocyclic ligand frameworks, *e.g.*, **L[1]**–**L[6]**, to define a new sub-set of nonadentate ligands. Here, we describe their synthesis and characterisation and report the outcomes of various studies to assess their utility as time-resolved assay components and as selective cellular probes. A preliminary account of part of this work has been published [24].

Ligand and Complex Synthesis and Characterisation. – The synthetic intermediate **4** was identified as a key primary target for the preparation of chromophores **2** and **3** (*Scheme 1*). It included a ^{t}Bu group at the 7-position to aid solubility, without unduly perturbing the energy of the S_1/T_1 excited states. Nucleophilic attack of 4-(*tert*-butyl)phenoxide on 2-chloronicotinic acid, followed by electrophilic cyclisation mediated by polyphosphoric acid (PPA), afforded **4a** in 77% overall yield. *N*-Methylation with methyl triflate and subsequent anion exchange yielded the H_2O-soluble chloride salt. Oxidative hydrolysis, following a literature protocol [26] using basic ferricyanide, gave the *N*-methylpyridinone in 74% yield. Conversion to the 2-chloro-derivative **4b** was undertaken with $POCl_3$ in 49% yield after chromatographic purification. A *Stille* coupling of **4b** with 6-methyl-2-(tributylstannyl)pyridine ($[Pd(PPh_3)_4]$/PhMe; 61% yield) afforded the bipyridine derivative **2a**, whilst reaction of **4b** with 3-methyl-1*H*-pyrazole (NaH/THF; 90% yield) yielded the pyrazolylpyridine intermediate **3a**. Selective benzylic bromination of both **2a** and **3a** (NBS/CCl_4) gave the bromomethyl derivatives **2b** and **3b**, respectively. These reactions also led to significant quantities of the corresponding dibrominated products **2c** or **3c**, typically in *ca.* 30% isolated yield, following separation by column chromatography. Each dibrominated compound could subsequently be converted into the desired monobrominated compound by reaction with diethyl phosphite in the presence of *Hünig*'s

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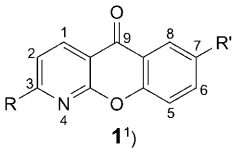
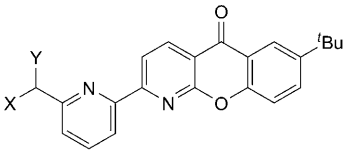
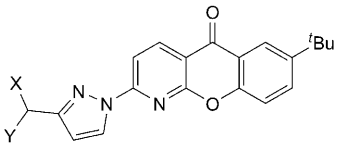
[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part*. Notice that compounds of type **1** have also been designated as 1-azaxanthones.



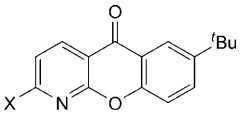
1[1])



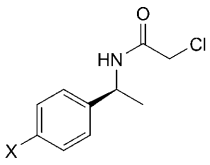
2a X = Y = H
2b X = Br, Y = H
2c X = Y = Br



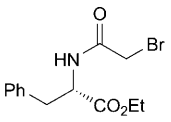
3a X = Y = H
3b X = Br, Y = H
3c X = Y = Br



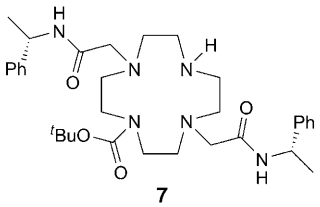
4a X = H
4b X = Cl



(*S*)-**5a** X = H
(*S*)-**5b** X = CO_2Me



(*S*)-**6**



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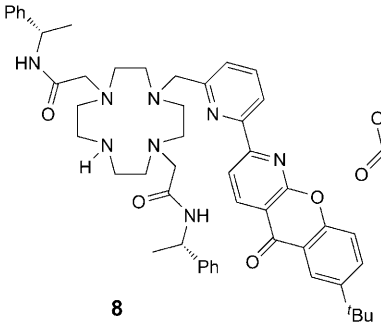

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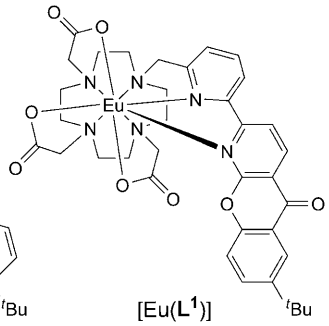
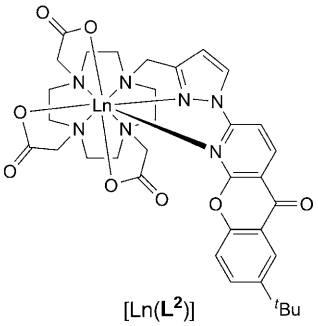
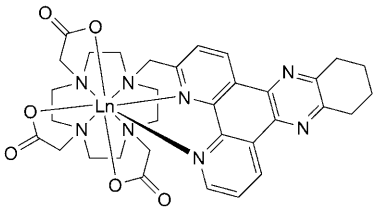


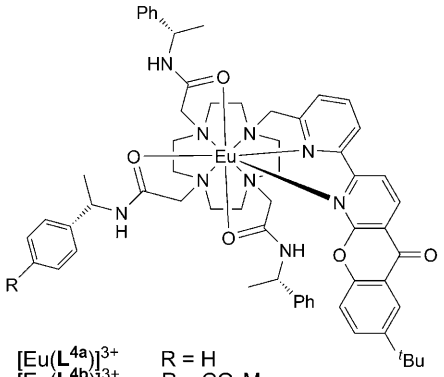
[Eu(**L**[1])]



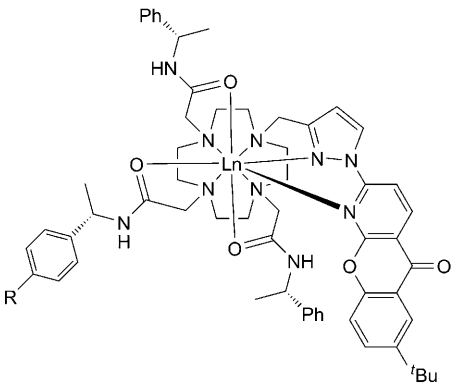
[Ln(**L**[2])]



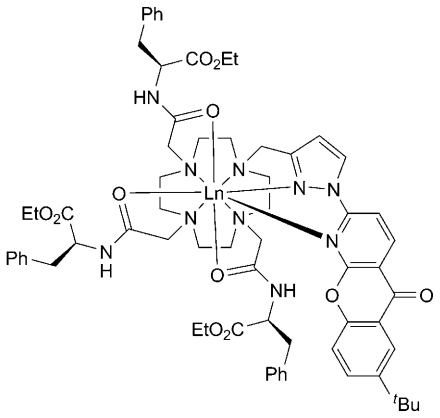
[Ln(**L**[3])]



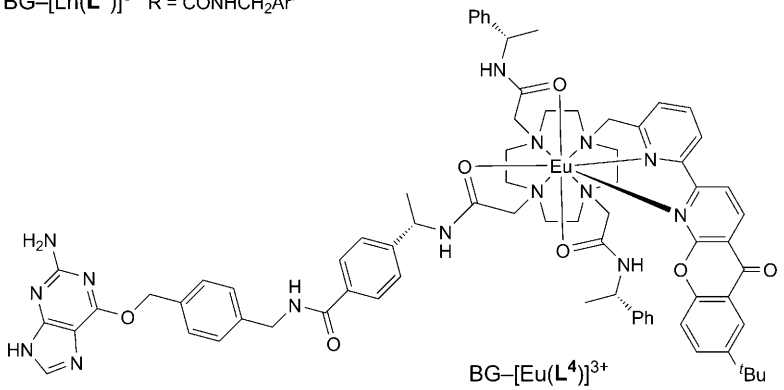
[Eu(**L**[4a])]$^{3+}$ R = H
[Eu(**L**[4b])]$^{3+}$ R = CO_2Me
BG–[Eu(**L**[4])]$^{3+}$ R = $CONHCH_2Ar'$



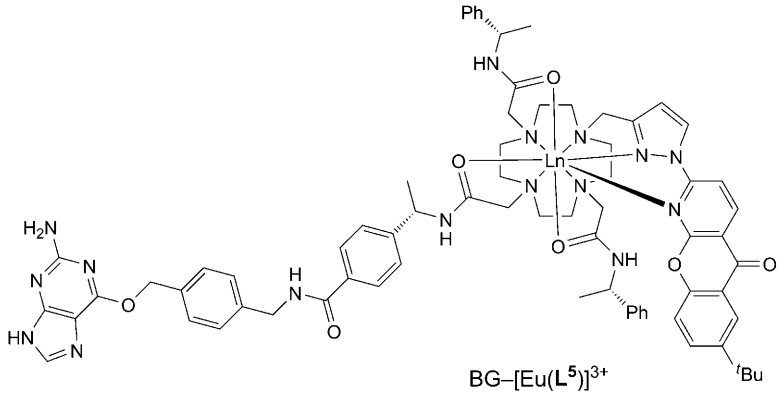
$[Ln(\mathbf{L^{5a}})]^{3+}$ R = H
$[Ln(\mathbf{L^{5b}})]^{3+}$ R = CO_2Me
$BG–[Ln(\mathbf{L^{5}})]^{3+}$ R = $CONHCH_2Ar'$



$[Ln(\mathbf{L^{6}})]^{3+}$

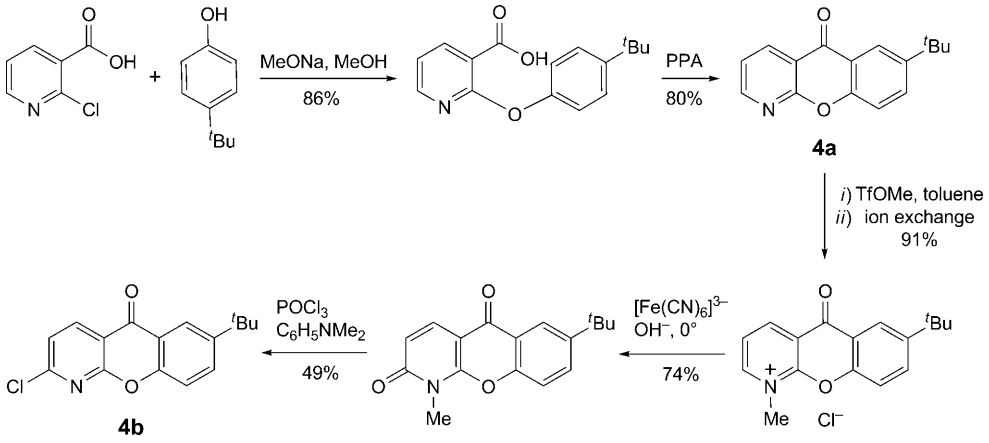


$BG–[Eu(\mathbf{L^{4}})]^{3+}$



$BG–[Eu(\mathbf{L^{5}})]^{3+}$

Scheme 1. *Synthesis of the Key Intermediates* **4a** *and* **4b**



base in THF. The constitution of compounds **2a** and **3b** was confirmed by single-crystal X-ray-analysis (*Fig. 1*)[2]).

The absorption and emission spectral properties of **2a** and **3a** were examined in MeOH. The absorption spectrum of the pyridyl system **2a** (λ_{max} 348 nm, ε 10100 M^{-1} cm^{-1}) was slightly broader in form than that of the pyrazolyl analogue **3a** (λ_{max} 346 nm, ε 15400 M^{-1} cm^{-1}) and extended further to the red, absorbing 5 times more strongly at 365 nm, for example. Each gave rise at room temperature to a very weak and unstructured fluorescence emission band in the range 390–430 nm. Phosphorescence emission spectra (77 K, Et_2O/isopentane/EtOH glass) revealed a lowest-energy T_1-S_0 transition at 20960 and 23470 cm^{-1} for **2a** and **3a**, respectively. Fine structure was observed in each phosphorescence spectrum (*Fig. 2*), with three distinct bands observed at higher energy than the zero–zero transition, separated by 1650 cm^{-1}. Such a spectral form is characteristic of C=O vibrational fine structure, consistent with dominant $n\pi^*$ character for the triplet state [27][28]. Therefore, given that the excited-state energies of the emissive states of Tb ($^5D_4$) and Eu ($^5D_0$) are at 20400 and 17200 cm^{-1}, respectively, only **3a** is suitable as a sensitiser for both ions, without the prospect of thermally activated back energy transfer.

The lanthanide(III) complexes of ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ were prepared by using similar methods to those defined for the tetraazatriphenylene analogue $\mathbf{L^3}$ [12]. For example, stepwise reaction of **2b** with 1,4,7-tri(*tert*-butyl) 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (MeCN, Cs_2CO_3; 90% yield), followed by treatment with CF_3COOH (CH_2Cl_2, 20°; 50% yield) afforded $\mathbf{L^1}$ as its protonated trifluoroacetate salt. Complexation of the ligand with $Eu(OAc)_3$ (pH 5.5, 50% aq. MeOH, 15 h, 80°) gave the neutral complex, [Eu($\mathbf{L^1}$)], which was purified by chromatography (neutral Al_2O_3). The other series of complexes that was prepared was based on the neutral ligands $\mathbf{L^4}$–$\mathbf{L^6}$. Each chiral ligand was prepared in enantiomerically pure form. Similar series of ligands

[2]) CCDC-724804 and -724805 contain the supplementary crystallographic data for **2a** and **3b**. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif.

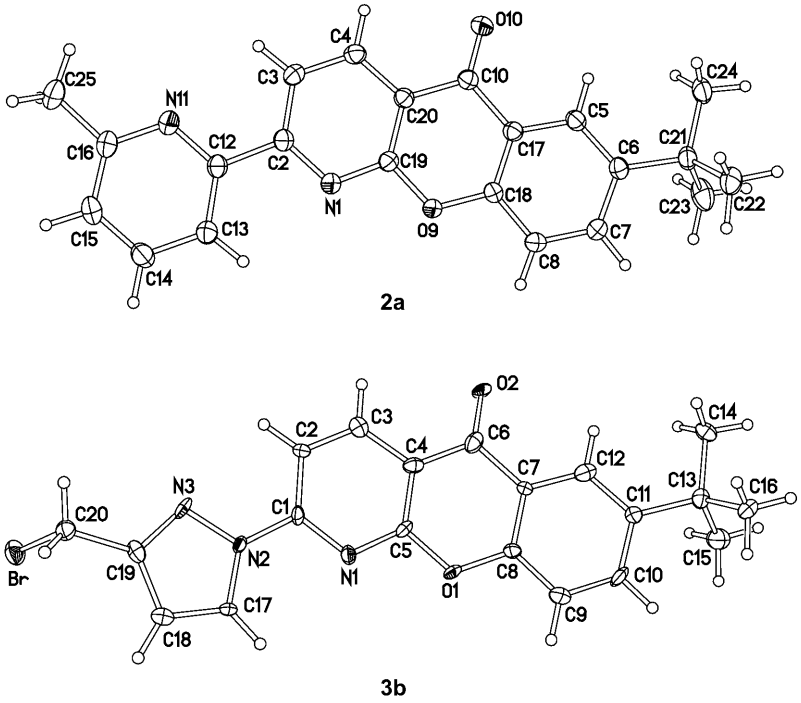


Fig. 1. *X-Ray crystal structure of* **2a** *and* **3b**, *showing atomic displacement ellipsoids at the 50% probability level.* Arbitrary atom numbering.

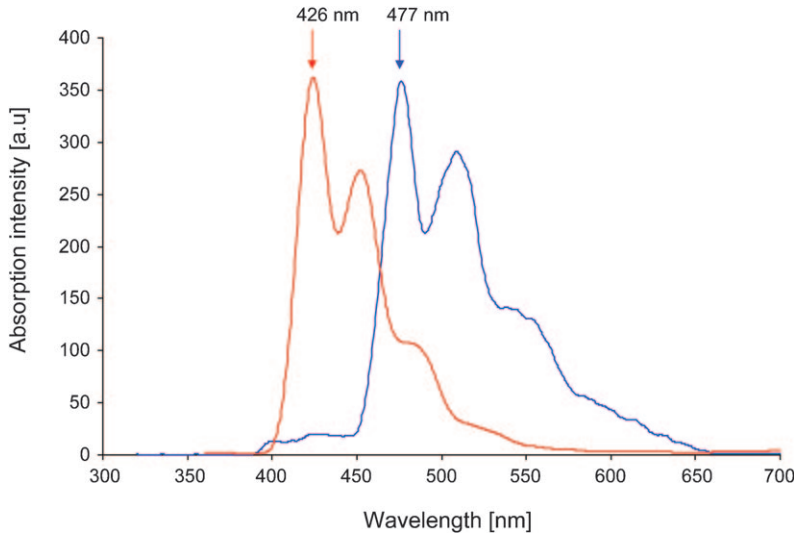
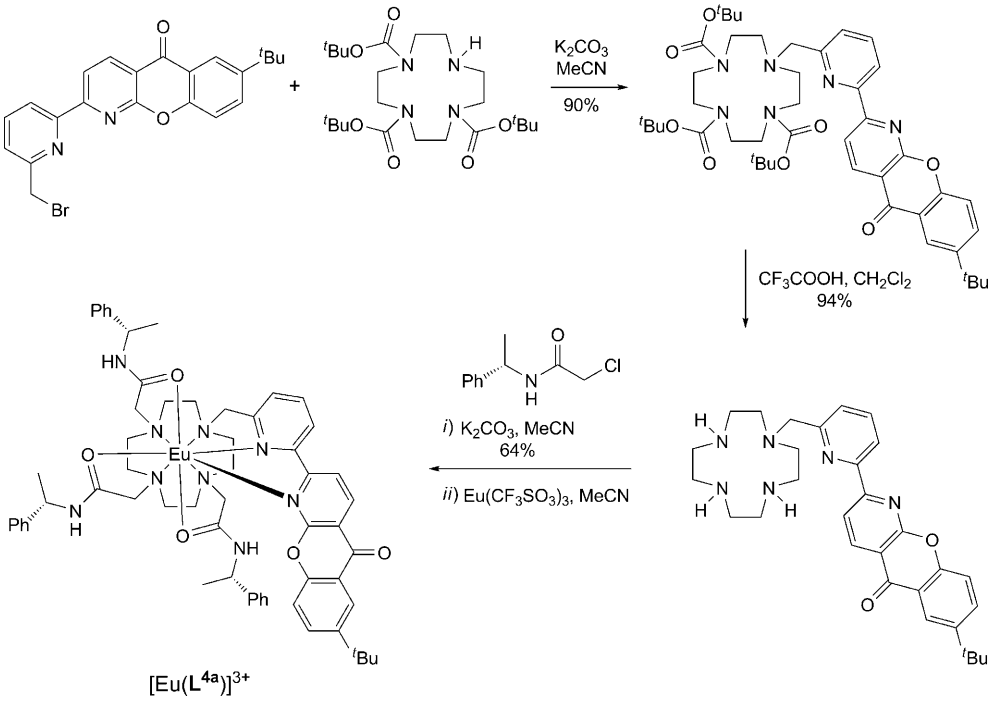


Fig. 2. *Phosphorescence emission spectra for* **2a** (right) *and* **3a** (77 K, Et_2O/isopentane/EtOH frozen glass)

incorporating such chiral amide substituents have been studied quite intensively [29][30] as they give rise to formation of preferred isomeric species in their Ln^{III} complexes, and these relatively hydrophobic complexes tend to resist excited-state quenching processes well [17][23]. Reaction of 1,4,7-tri(*tert*-butyl) 1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate with **2b**, followed by (*tert*-butoxy)carbonyl(Boc)-deprotection and *N*-alkylation with three equiv. of (*S*)-**5a** (MeCN, K_2CO_3) afforded $\mathbf{L^{4a}}$ (*Scheme 2*). Complexation of $\mathbf{L^{4a}}$ (MeCN, 40°) with an anhydrous lanthanide(III) triflate salt, followed by anion-exchange chromatography, led to isolation of the H_2O-soluble cationic complex $[Ln(\mathbf{L^{4a}})]Cl_3$. Similar methods were used to prepare Ln^{III} complexes of ligands $\mathbf{L^{5a}}$ and $\mathbf{L^{6}}$.

Scheme 2. *Synthesis of the Cationic Europium Complex [Eu(*$\mathbf{L^{4a}}$*)]*$^{3+}$

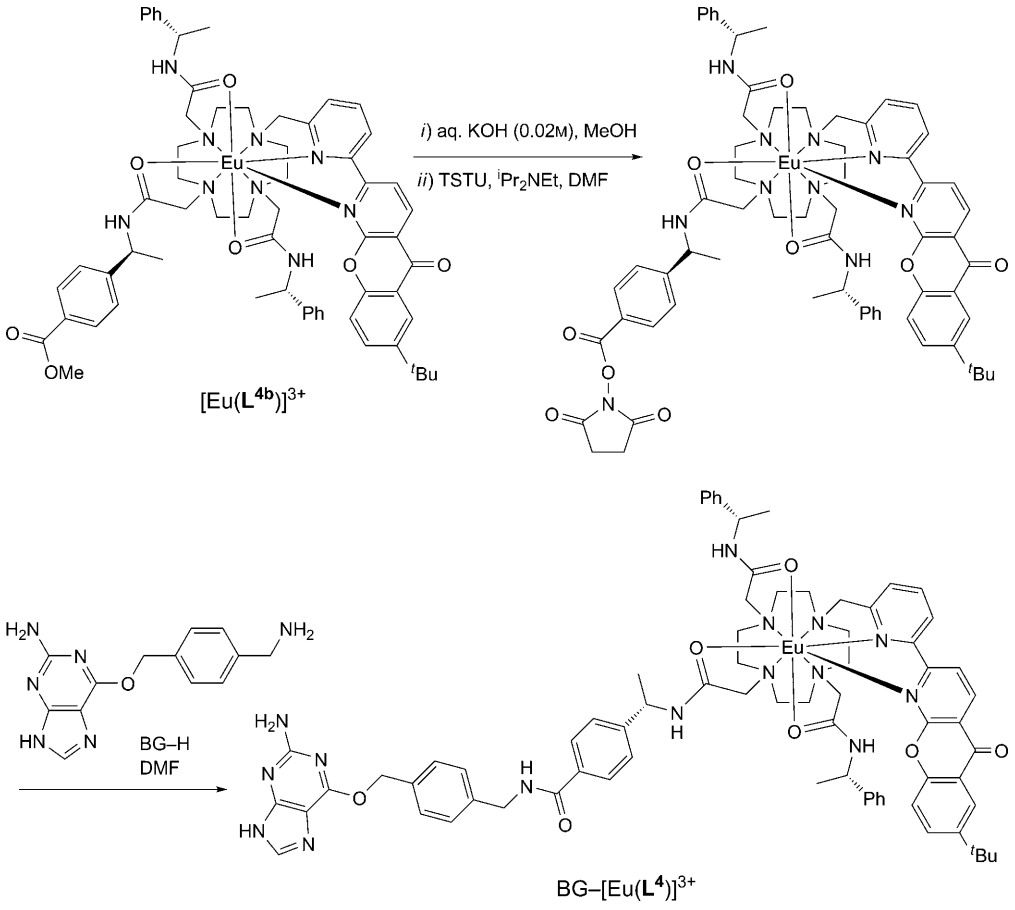


A different strategy was required for the synthesis of the complexes of $\mathbf{L^{4b}}$ and $\mathbf{L^{5b}}$ bearing a *p*-(methoxycarbonyl)phenyl group at the amide substituent. This *p*-(methoxycarbonyl) group is introduced to allow subsequent conjugation of the Ln complex to different linkers or biomolecules. Reaction of the mono-Boc derivative **7** with **2b** [31] (K_2CO_3, MeCN; 74% yield), followed by Boc deprotection afforded the diamide **8** with a secondary amino group. *N*-Alkylation of the latter with (*S*)-**5b** under basic conditions (MeCN, K_2CO_3; yield 64%) yielded the ligand $\mathbf{L^{4b}}$. The pyrazolyl analogue $\mathbf{L^{5b}}$ was prepared by a similar strategy, and the Eu^{III} or Tb^{III} complexes were isolated, as described for $[Ln(\mathbf{L^{4a}})]X_3$. Various strategies are available for linking these emissive probes to a particular protein or antibody [32], allowing selective FRET experiments to be undertaken. An alternative approach has recently been devised by

Covalys ('SNAP-tag'™ technology), that simplifies the procedure and enables the covalent labelling of proteins (*e.g.*, for use as acceptors in a FRET system), with compatible donor lumophores, labelled with an *O*-benzylguanine moiety. A mutant DNA repair enzyme, human O^6-alkylguanine-DNA alkyltransferase (LAGT) [33], irreversibly and specifically reacts with O^6-alkylguanine derivatives (*e.g.*, BG–H, a benzylguanine derivative) to form a thioether bond and release free guanine. Thus, the BG conjugate of an emissive donor is transferred to a reactive cysteine residue on the protein [34][35].

Hydrolysis of the methyl ester in $[Eu(\mathbf{L^{4b}})]^{3+}$ and $[Tb(\mathbf{L^{5b}})]^{3+}$ was undertaken in basic aqueous MeOH (pH 10, MeOH/H_2O 1:1, 2 h) (*Scheme 3*). After neutralisation, the acid salt was converted into a succinimido ester (TSTU = *N*,*N*,*N*′,*N*′-tetramethyl-*O*-succinimidouronium tetrafluoroborate), $^{i}Pr_2NEt$, DMF), monitoring conversion by anal. HPLC. Direct reaction with excess *O*-[4-(aminomethyl)benzyl]guanine (= BG–H) afforded the desired coupled amide which was purified by prep. reversed-phase

Scheme 3. *Synthesis of the* O-*Benzylguanine Conjugate BG–[Eu(*$\mathbf{L^4}$*)]*$^{3+}$



HPLC. These BG conjugates, BG–[Eu(**L**^{4})]$^{3+}$ and BG–[Tb(**L**^{5})]$^{3+}$, possessed virtually identical absorption and emission spectral properties to the precursor methyl esters.

Photochemical Characterisation and Solution Stability. – Salient photophysical data, defining the absorption and emission spectral characteristics of Eu^{III} and Tb^{III} complexes are summarised in the *Table*. Measurements of radiative rate constants in H_2O and D_2O allowed hydration states for each complex to be estimated. Only [Eu(**L**^{2})] possesses a coordinated H_2O molecule, *i.e.*, [Eu(**L**^{2})(H_2O)]. Its differing coordination environment is also apparent in the relative form of the Eu^{III} emission spectrum, in comparison to those of [Eu(**L**^{1})] and [Eu(**L**^{4})]$^{3+}$ (*Fig. 3*). With [Eu(**L**^{2})(H_2O)], the observed ligand fluorescence at 410 nm is quite strong ($\phi^f_{em} \approx$ 25%) and is barely shifted from that of the free ligand. This behaviour contrasts with that observed with [Eu(**L**^{1})] and [Eu(**L**^{4a})]$^{3+}$. In each of these cases, the fluorescence emission centred at *ca.* 400 nm is much weaker, and the form of the Eu spectral fingerprint is very similar. This behaviour is consistent with the adoption of a common coordination environment for the latter pair of complexes, very similar to that observed for [Eu(**L**^{3})]. In this case, the 680 nm band is postulated to characterise axial coordination of an N-atom donor in the 9-coordinate complex [12][36]. With [Eu(**L**^{2})(H_2O)], the pyrazolyl and pyridine N-atoms presumably do not chelate, and **L**^{2} serves as a heptadentate ligand with one coordinated H_2O molecule completing the coordination sphere. The reduced quantum yield and the differing Eu emission spectral form – resembling that of analogous (*N*-alkyl-DO3A) europium(III) complexes [37] – and unshifted ligand fluorescence emission accords with this hypothesis (DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetato).

Table. *Photophysical Data for Selected Lanthanide(III) Complexes* (295 K, pH 5.5)

Complex	λ_{max} [nm]	ε [M^{-1} cm^{-1}]	k_{H_2O}[a])[b]) [ms^{-1}]	k_{D_2O}[a])[b]) [ms^{-1}]	$\phi_{em}(H_2O)$[c]) [%]	E_T^{Gd} [cm^{-1}] (77 K)[d])
[Eu(**L**^{1})]	356	10100	1.00	0.74	14	20950
[Eu(**L**^{2})(H_2O)]	345	10100	1.43	0.57	1.2	23450
[Tb(**L**^{2})]	348	10050	0.44	0.37	15	23450
[Eu(**L**^{3})]	347	8300	0.95	0.63	18	23800
[Tb(**L**^{3})]	347	8300	0.64	0.58	40	23800
[Eu(**L**^{4a})]$^{3+}$	356	10050	1.00	0.74	24	20950
[Eu(**L**^{4b})]$^{3+}$	356	10050	0.98	0.73	25	20950
[Tb(**L**^{5a})]$^{3+}$	348	15050	0.50	0.42	46	23470
[Tb(**L**^{5b})]$^{3+}$	349	15050	0.45	0.36	61	23470
[Tb(**L**^{6})]$^{3+}$	348	15050	0.44	0.40	54	23450

[a]) Hydration numbers q are zero for each complex examined, except for [Eu(**L**^{2})(H_2O)], for which q = 0.73 [22]. [b]) Errors in lifetimes are estimated to be ±10%, and ±15% for quantum yields. [c]) Note that lifetime data and ϕ_{em} values may differ considerably as a function of pH in the presence of different anions and/or protein in the aq. solution. For example, with [Eu(**L**^{4a})]Cl_3, values were reduced to 3% in the presence of 0.4 mM human-serum albumin and to 2% in the cellular growth medium. [d]) Triplet energies were determined with Gd analogues in a frozen glass of Et_2O/isopentane/EtOH or EtOH/MeOH 2 : 1.

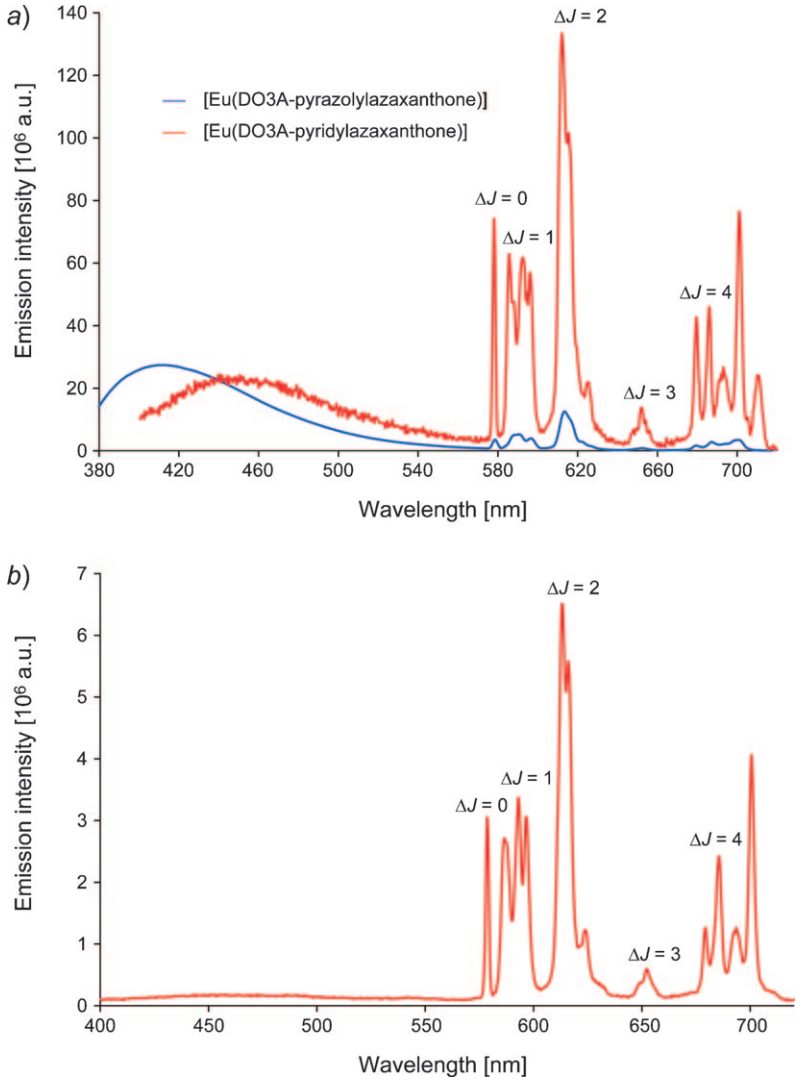


Fig. 3. *Total emission spectra for* a) *[Eu(***L**^{1}*)]* (red) *and [Eu(***L**^{2}*)(H$_2$O)]* (blue) *compared to* b) *[Eu(***L**^{4a}*)]*$^{3+}$ (295 K, aerated H_2O, λ_{exc} 355 nm). **L**^{1} = DO3A-pyridylazaxanthone, **L**^{2} = DO3A-pyrazolyl-azaxanthone.

The inability of the pyrazolyl-azaxanthone moiety to chelate to the Eu^{III} centre in [Eu(**L**^{2})] prompted an examination of the pH sensitivity of the Ln^{III} complexes and their ability to resist chelation by common oxy anions, such as HCO_3^-, HPO_4^{2-}, lactate, and citrate. Such anions are well known to form ternary adducts with Ln^{III} complexes of related heptadentate ligands, several of which have been characterised by X-ray crystallography [38]. The emission spectra of [Eu(**L**^{1})] and [Eu(**L**^{4a})]$^{3+}$ did not vary over the pH range 3 – 9. With [Tb(**L**^{5a})]$^{3+}$, the intensity of emission at 542 nm decreased

five-fold over the pH range 5 to 8, with an apparent pK_a of 6.50 (±0.05) (*Fig. 4*). Such behaviour may tentatively be assigned to partial dissociation of the chelating sensitising moiety (*Scheme 4*). The measured q values at pH 5 and 8 were both zero. Similar behaviour was exhibited by [Tb(**L²**)], and in each case, the spectral changes were reversible.

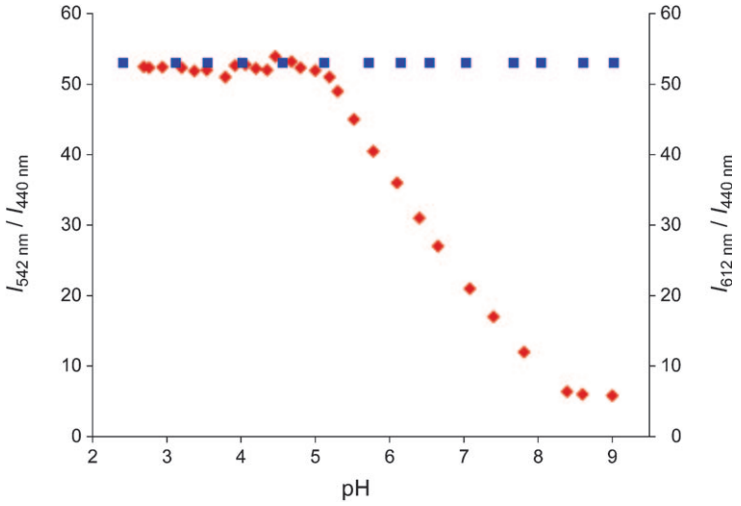


Fig. 4. *Variation of the ratio of Ln^{III} emission intensity* vs. *residual ligand fluorescence with pH for [Tb(**L**^{5a})]$^{3+}$* (◆) *and [Eu(**L**^{4a})]$^{3+}$* (■) (295 K, 0.1M NaCl)

Scheme 4. *Dissociative Equilibrium Occurring with [Tb(**L**^{5})]$^{3+}$*

Addition of a ten-fold excess of sodium lactate, phosphate, or citrate (pH 5.5) to [Tb(**L**^{5a})]Cl_3 resulted in a marked decrease in Tb emission intensity at 542 nm, consistent with the observed pH instability. With [Eu(**L**^{4a})]Cl_3, incremental addition of aqueous solutions of lactate or citrate (pH 7.4), in up to a 10-fold excess, caused a change in the form and a reduction in the intensity of the Eu emission (*Fig. 5*). Addition of a 0.4 mM solution of human serum albumin (HSA) also changed the spectral form, but in each of these cases less than a 10% reduction of the measured Eu emission lifetime occurred. The changes to the form and relative intensity of the hypersensitive $\Delta J = 2$ (around 620 nm) and $\Delta J = 4$ (680–710 nm) transitions were most

distinctive, and the limiting spectra (*e.g.*, strong $\Delta J = 2$ transition) observed were strongly reminiscent of ternary adducts observed in related triamide complexes [10][36][38] based on an N_4O_3 (ligand) plus O_2 (with one or more polarisable O-atoms) donor array. The loss of the transition at 680 nm is also consistent with cleavage of a Ln – N interaction. In addition, absorption spectra of solutions of [Eu(**L**^{4a})]Cl_3, in the presence of added citrate or HSA, exhibited a distinct shift ($\approx$5 nm) to the blue of the low-energy $\pi - \pi^*$ transitions at 356 and 325 nm, consistent with dissociation of the chelating N-atoms of the sensitising moiety.

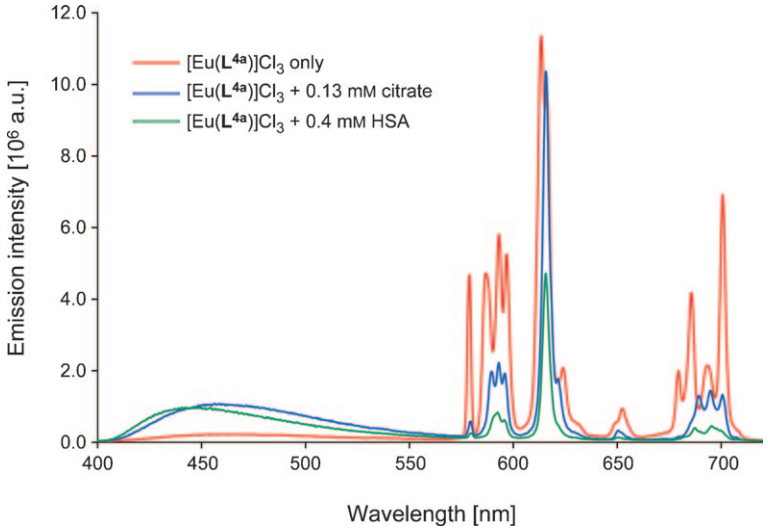


Fig. 5. *Emission spectra for [Eu(***L**^{4a}*)]Cl$_3$* (295 K, λ_{exc} 355 nm, 10 μM complex) (red) *and in the presence of 0.13*M *citrate* (blue) *and 0.4* mM *human-serum albumin* (HSA; green)

In summary, these competition experiments strongly indicate that for the Ln^{III} complexes examined here, the sensitising chromophore is likely to dissociate *in cellulo*, lowering the overall emission quantum yield and modulating the emission spectral form, notably for Eu^{III} complexes. This tendency – not observed for complexes of [Ln(**L**^{3})] and its congeners – will tend to restrict the range of applications of such complexes as cellular probes but does not preclude their usage *in cellulo*, as they remain emissive. This is particularly pertinent for the complexes of **L**^{4a} and **L**^{1}.

Behaviour as Cellular Probes. – In striving to develop Ln^{III} complexes as cellular probes, and not simply cellular stains or labels, it is important to understand their mechanism of cell entry. In addition, the relationship between probe structure and its cytotoxicity and compartmentalisation profile needs to be established [11][39]. Initial work in Durham has examined over 70 emissive Eu and Tb complexes. This study has revealed that uptake and compartmentalisation profiles are dependent upon the nature and mode of linkage of the heterocyclic sensistising moiety, rather than upon complex charge, lipophilicity, or relative protein affinity [11][12][18][40][41]. Three main classes of behaviour have been observed so far. The majority of complexes are

noncytotoxic and are taken up quite quickly and exhibit localisation to the perinuclear endosomes/lysosomes [11]. A second set localises in protein-dense regions, such as ribosomes or nucleoli and exhibit rather slow uptake/egress. Recently, it has been suggested that the presence of these probes in the growth medium during incubation may enhance membrane permeability [40]. A third series of complexes localises quickly to mitochondria with subsequent shuttling to endosomal/lysosomal compartments [41].

Cytotoxicity studies [39–41] were undertaken by using the 'MTT' assay in mouse-skin fibroblasts (NIH-3T3 cells), monitoring, over a 24 h period, the perturbation of mitochondrial dehydrogenase enzymes in viable cells. Cytotoxicity values (IC_{50}; with the standard deviation in parenthesis) for [Eu(**L**^{4a})]Cl_3, [Tb(**L**^{5a})]Cl_3, and [Tb(**L**^{6})]Cl_3 were 73(9), 42(9), and 101(13) μM, respectively. This is in the same range as other cell-permeable complexes that exhibit similar cellular behaviour [18]. Cellular uptake and localisation profiles of [Eu(**L**^{4a})]Cl_3 and [Tb(**L**^{5a})]Cl_3 were examined in Chinese-hamster ovarian (CHO) cells and mouse-skin fibroblasts (NIH-3T3) by means of fluorescence microscopy. Cultured cells were exposed to a growth medium with complex concentrations of between 10 and 50 μM, and living cells were examined at time intervals from 20 min to 24 h, post-incubation. Optical sections throughout the observed cells were undertaken to ensure the probe was internalised, rather than simply associated with the cell membrane. With [Eu(**L**^{4a})]Cl_3, after a 20 min incubation with a 50 μM solution, red Eu emission was observed clearly. Co-localisation with *Mitotracker Green*™ after 2 h confirmed this as a predominant mitochondrial distribution, with merged images showing good correspondence (*Fig. 6*). Other luminescence was observed, which was attributed to the lysosomes. For incubation times of greater than 4 h, such merged images showed inconsistencies, and after more than 16 h, the complex appeared to have migrated from the mitochondria to the perinuclear endosomes/lysosomes. This hypothesis was indicated by a co-localisation study with *Lysotracker Green*™ (*Fig. 7*).

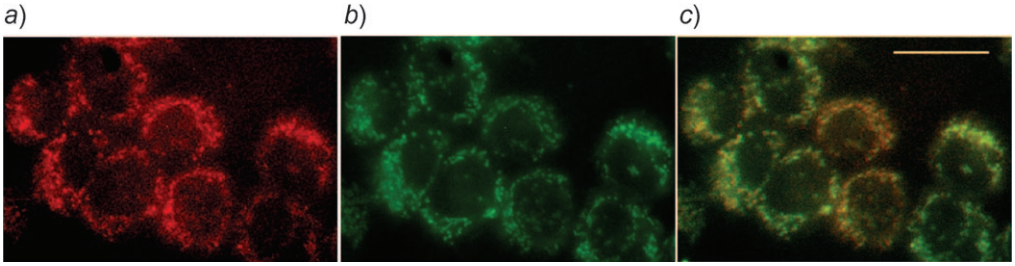


Fig. 6. *Fluorescence microscopy co-localisation study with [Eu(***L**^{4a}*)]Cl_3* (50 μM, 2 h): a) *red Eu emission* ($\lambda_{obs} > 600$ nm), b) Mitotracker Green™ *emission* (λ_{obs} 450–500 nm), *and* c) *merged image showing good correspondence* (scale bar 20 μm)

Like [Eu(**L**^{4a})]Cl_3, the pyrazolyl (*S*,*S*,*S*)-complex [Tb(**L**^{5a})]Cl_3 exhibited time-dependent intracellular trafficking between mitochondrial and endosomal compartments. No difference in localisation behaviour was observed with the enantiomeric complex (*R*,*R*,*R*)-[Tb(**L**^{5a})]Cl_3. Furthermore, [Tb(**L**^{5a})]Cl_3 and [Tb(**L**^{6})]Cl_3 (the latter with three amide substituents derived from L-phenylalanine replacing the (1-phenyl-

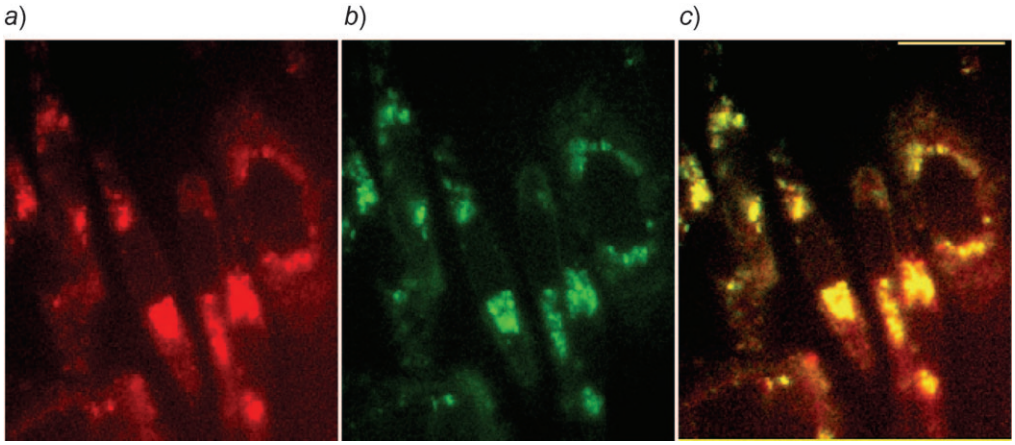


Fig. 7. *Fluorescence microscopy co-localisation study with [Eu(**L**^{4a})]Cl$_3$* (50 μM, 24 h): a) *Eu emission,* b) Lysotracker Green™, *and* c) *merged image* (scale bar 20 μm)

ethyl)amino moiety) also behaved more or less identically in their uptake and localisation behaviour. Such behaviour lends further support to the hypothesis that the nature of the pendant arms in homologous series of macrocyclic Ln^{III} complexes does not determine uptake/egress or intracellular localisation behaviour. Similar results were obtained recently for a set of six complexes related to [Ln(**L**^{3})] [40]. The observations reported here lend further support to the theory that it is the constitution of the sensitising moiety that primarily determines uptake and trafficking. The condensed aromatic moiety is the structural element that is most likely to be recognised in protein association, which must be a key step in intramolecular trafficking involving recycling vesicles.

Information on the speciation of Eu^{III} complexes can be gleaned by analysis of the form and relative intensity of the emission bands. With [Eu(**L**^{4a})]Cl$_3$, following a 6-h incubation in Chinese-hamster ovarian cells (50 μM, complex concentration in growth medium), the cells were inspected by microscopy to confirm the mixed endosomal/mitochondrial localisation profile. A set of cells (*ca.* 10^6) was then washed and immediately suspended in an optical cell and the emission spectrum measured (*Fig. 8*). The spectrum is consistent with a protein-bound ternary adduct, given its correspondence to that measured in the presence of human-serum albumin *in vitro* (*Fig. 5*).

Measurement of Cell Uptake. – The cellular behaviour of [Eu(**L**^{4a})]Cl$_3$ and [Tb(**L**^{5a})]Cl$_3$ was further examined by studying the effect of adding to the growth medium various established inhibitors and promoters of defined cell uptake pathways [40]. This was addressed by examining their effect the on observed image intensity under standardised conditions by means of microscopy, as well as by measuring the percentage of the complex in sorted and counted cell populations by means of flow cytometry and ICP-MS to determine the Ln-ion concentration. Suppressed uptake (30% (±10)) was observed at 4°, consistent with an energy-dependent process such as endocytosis [42]. The influence of the following was examined; wortmannin (blocking

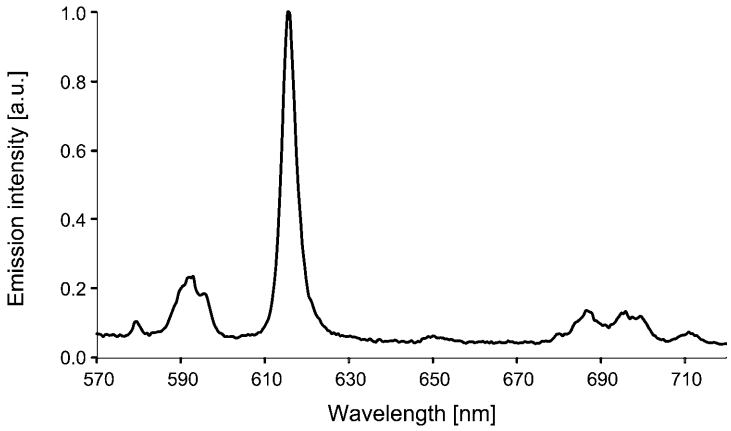


Fig. 8. *Europium emission spectrum of [Eu(***L**^{4a}*)]Cl$_3$ localised in Chinese-hamster ovarian cells* (50 μM, 6 h incubation) *revealing a spectral fingerprint consistent with formation of a ternary adduct with protein* (295 K, λ_{exc} 365 nm)

P1-3 kinase and hence macropinocytosis) [43], amiloride (blocking the Na^+/H^+ pump and hence macropinocytosis) [44], sucrose (inhibiting clathrin-dependent endocytosis) [45], chlorpromazine (interacting with clathrin), filipin (sequestering cholesterol, thereby perturbing caveolae), chloroquine (increasing endosomal pH), phorbol esters (activating protein kinase C and stimulating macropinocyotosis) [46], and long-chain di-*O*-acylglycerols (stimulating macropinocytosis) [47]. Both wortmannin and amiloride suppressed cell uptake of each complex (by up to 40%), whilst phorbol esters and fatty-acid-derived di-*O*-acylglycerol enhanced uptake (by up to 100%). The other treatments did not give rise to significant deviations from the control. Variations in cell-image intensity in CHO, HeLa, and NIH-3T3 cells were consistent with this trend (*Fig. 9*).

Taken together, these results reinforce the mechanistic conclusions derived very recently [40] that pinpoint macropinocytosis as the common cell uptake pathway for this class of complexes. Neither evidence for clathrin-mediated uptake, nor processes involving caveolae was found.

Conclusions. – The Eu^{III} and Tb^{III} complexes of macrocyclic complexes incorporating a 3-pyridyl-4-azaxanthone or an analogous 3-pyrazolyl-4-azaxanthane sensitising moiety function as effective emissive probes in aqueous media. Each complex displays a tendency to undergo dissociation of the metal-coordinated sensitiser at elevated pH ($pH > 7$) or in the presence of chelating anions or endogenous protein. Whilst this tendency lowers the measured quantum yield in such media, it has not inhibited their application as cellular probes. The complexes appear to enter the cell *via* macropinocytosis. This is an attractive mechanism for cell labelling, as it is not receptor-mediated, and the probe is not necessarily confined to endosomal or lysosomal compartments [48]. Indeed, macropinosomes are generally regarded as rather leaky compartments that tend to discharge their contents into the cell [48].

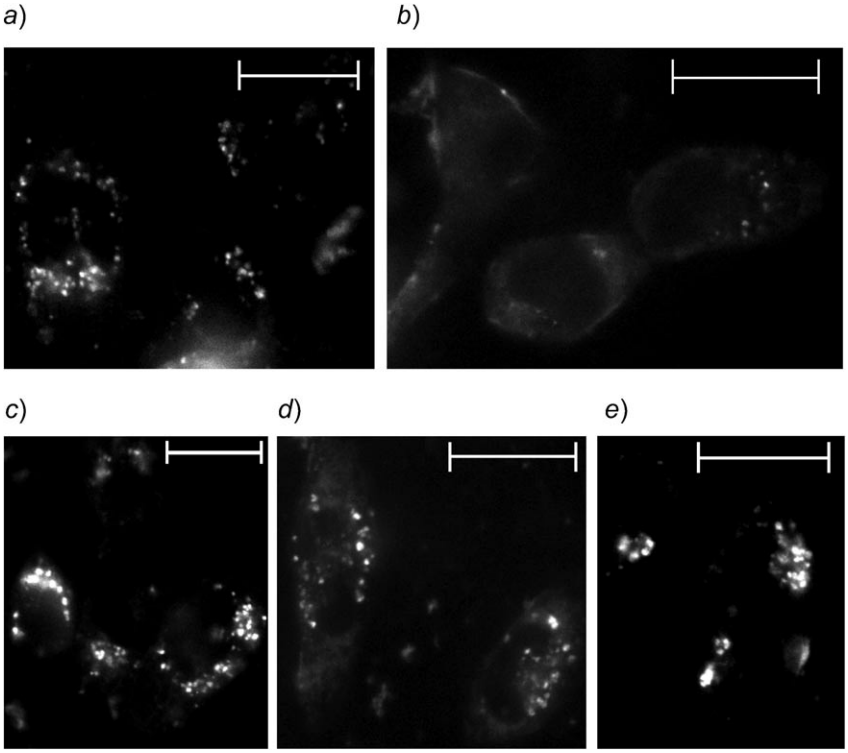


Fig. 9. *Fluorescence microscopy images of Chinese-hamster ovarian cells in the presence of [Tb(***L**^{5a}*)]Cl*$_3$ (50 μM, 4 h), *following a 30 min pretreatment with activators or inhibitors of endocytosis:* a*) control,* b*) 300 nM wortmannin,* c*) 500 ng/ml of 1,2-dipalmitoyl-derived* rac-*di*-O-*acylglycerol,* d*) 50* μM *chlorpromazine, and* e*) 1 μg/ml of filipin*

The lower affinity of these chelating sensitisers for the Ln ion compared to those based on a bipyridine subunit, may be related to the increased steric demand imposed by the substituted azaxanthone moiety and the reduced basicity of the pyridine N-atom donor atoms. This is particularly true of the azaxanthone 'pyridine' N-atom that is subject to the inductive effect of the adjacent endocyclic O-atom. Future probe design should take heed of this behaviour.

We thank *CISbio* (*C. P. M.*), the *Association of Commonwealth Universities* for a scholarship (*E. J. N.*), *COST-Action D38*, and the EC-network DIMI for support. It is a pleasure to acknowledge the inspiration of the work of Prof. *Jean-Claude Bünzli*, particularly for his indomitable enthusiasm and passion for lanthanide chemistry, and his buoyant attitude to scholarship and education.

Experimental Part

1. *General.* Details of instrumentation, spectral acquisition and abbreviations, separation methods, solvent purification, photochemical analyses, and flow cytometry have been reported earlier [39–41]. Cell culture methods, inhibition/complex uptake studies, and cytotoxicity measurements are detailed below.

2. *Cell Culture.* Three cell lines were selected for cellular studies: CHO (Chinese-hamster ovary), NIH 3T3 (mouse-skin fibroblast), and HeLa (human endothelial carcinoma cells). Cells were maintained in exponential growth as monolayers in F-12 (Ham) medium, DMEM (*Dulbecco*'s modified eagle medium), and RPMI 1640 medium, resp. For each cell line, the medium was supplemented with 10% foetal bovine serum (FBS) and 1% penicillin and streptomycin. Cells were incubated at 37°, 20% average humidity, and 5% (*v*/*v*) CO_2/air.

3. *Microscopy.* Cells were seeded in 12-well plates on glass cover slips and allowed to grow to 40–60% confluence at 37° in 5% CO_2. At this stage, the medium was replaced, and cells were treated with drugs and complexes as described below. For NIH 3T3 and HeLa cells, the non-phenol-red-containing DMEM was used. Following incubation, the cover slips were washed with phosphate-buffered saline (PBS; pH 7.5), mounted on slides and sealed with nail varnish. Epifluorescence images were taken on a *Zeiss-Axiovert-200M* epifluorescence microscope with a digital camera, and were processed with the *Zeiss* Axiovision software. G365 filters (*Zeiss*) were employed for excitation of the complexes, with 546/12 and 575–625 filters (*Comar*) used for emission of Tb and Eu, resp.

4. *Uptake Studies.* Cells were seeded in 6-well plates and allowed to grow to 80–100% confluence at 37° in 5% CO_2/air. At this stage, the medium was replaced, and cells were treated with drugs and complexes as described below. Following incubation, the medium was removed, and the cells were washed three times with PBS. Lysis buffer (500 µl; 10 mM *Tris*, pH 7.5, 100 mM NaCl, 1 mM EDTA, 1% *Triton X-100*, 0.1% protease inhibitor cocktail) was then added to each well, and the cells were incubated at 5° for 15 min. Aliquots (3×25 µl) of the supernatant were taken from each well for the BCA (bicinchoninic acid) assay, as described below, and a sample (400 µl) was submitted for ICP-MS analysis.

5. *BCA Assay.* Total protein content was determined in lysed cells by the bicinchoninic acid protein assay (*Pierce* BCA protein assay kit). A BSA (bovine-serum albumin) standard curve was constructed in the range 0.2 to 1 mg/ml. Standards (25 µl) and samples (25 µl) were transferred into triplicate wells of a 96-well plate, and the BCA reagent mix (200 µl) was added to each well. Absorbance at 540 nm was measured after 10 h incubation at r.t. with a microplate reader. Protein concentration for each sample was determined against the standard curve.

6. *ICP-Mass Spectrometry.* Inductively coupled plasma mass spectrometry (ICP-MS) determinations of Eu or Tb concentrations were made by Dr. *C. Ottley* in the Department of Earth Sciences at Durham University.

7. *Drug Treatments.* For the 5° treatment, cells were incubated in a refrigerator set at 5°. Concentrations of inhibiting or activating drugs used were 10 µM chloroquine, 50 µM chlorpromazine, 1 µg/ml of filipin, 2 µM monensin, 0.01% poly-L-lysine, 50 mM sucrose, 300 nM wortmannin, 3 mM amiloride, 50 ng/ml of phorbol 12-myristate 13-acetate, and 500 ng/ml of *rac*-1,2-di-*O*-palmitoylglycerol. Cells were subjected to appropriate treatments for 30 min prior to treatment with 50 µM complex for 4 h.

8. *Cytotoxicity.* IC_{50} Values were determined with the MTT assay, which makes use of the conversion of MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) to a purple formazan product by the mitochondrial dehydrogenase of viable cells. This insoluble formazan was quantified spectrophotometrically upon dissolution in DMSO. About $1 \cdot 10^4$ NIH-3T3 cells in DMEM (100 µl) were seeded into each well of flat-bottomed 96-well plates and allowed to attach overnight. Complex solns. were added to triplicate wells to give final concentrations over a 2-log range. Following 24 h incubation, MTT (1.0 mM) was added to each well, and the plates were incubated for a further 4 h. The culture medium was removed, and DMSO (150 µl) was added. The plates were shaken for 20 s and the absorbance measured immediately at 540 nm in a microplate reader. IC_{50} Values were determined as the drug concentration required to reduce the absorbance to 50% of that in the untreated control wells, and represent the mean for data from at least three independent experiments.

9. *HPLC Analysis.* Retention time t_R in min. Anal. reversed-phase HPLC: at 298 K; *Perkin-Elmer-200* pump/autosampler/diode array detector with a fluorescence detector; *Chromolith-RP18e* column (100×4.6 mm); flow rate 1 ml/min; *Method A:* running over 20 min, from H_2O/MeCN 95:5 (each with 0.1% HCOOH) to 100% MeCN at 15 min; *Method B:* running over 13 min, from H_2O/MeCN 95:5 to H_2O/MeCN 35:65 at 8 min, and to 100% MeCN at 10 min; *Method C:* running over 13 min, from H_2O/MeCN 85:15 to 100% MeCN at 10 min; *Method D:* running over 17 min, from H_2O/MeCN 90:10 to H_2O/MeCN 30:70 at 11 min, and to 100% MeCN at 12 min.

Semi-prep. HPLC (*Method E*): *Chromolith* performance *RP18e* column (100 × 10 mm); flow rate 14 ml/min; running over 13 min, from H_2O/MeCN 80:20 to H_2O/MeCN 45:55 at 8 min, and to 100% MeCN at 10 min.

10. *Chromophore Synthesis. 2-[4-(*tert*-Butyl)phenoxy]nicotinic Acid* (*= 2-[4-(1,1-Dimethylethyl)-phenoxy]pyridine-3-carboxylic Acid*). To a stirred soln. of Na metal (1.02 g, 44.4 mmol) in anh. MeOH (25 ml), 4-(*tert*-butyl)phenol (15.2 g, 101 mmol) was added to form a thick cream-coloured soln. The solvent was evaporated to afford a cream-coloured residue. Then 2-chloronicotinic acid (3.31 g, 21.0 mmol) was added to the residue and the resulting mixture heated at 190° with stirring for 20 h. The mixture was allowed to cool to 100° and poured into H_2O (200 ml). The aq. soln. was extracted with Et_2O (2 × 150 ml) and then acidified to pH 5 by the addition of AcOH to afford a fine precipitate. The precipitate was filtered, washed with H_2O (100 ml), and dried under vacuum: colourless crystalline solid (4.89 g, 86%). M.p. 180–181°. ^{1}H-NMR ($CDCl_3$, 500 MHz): 1.37 (*s*, ^{t}Bu); 7.14 (*d*, *J* = 8.5, H–C(2′,6′)); 7.20 (*dd*, *J* = 7.5, 5.0, H–C(5)); 7.49 (*d*, *J* = 9.0, H–C(3′,5′)); 8.35 (*dd*, *J* = 4.5, 2.0, H–C(6)); 8.55 (*dd*, *J* = 8.0, 2.0, H–C(4)). ^{13}C-NMR ($CDCl_3$, 125 MHz): 31.7 (*Me*$_3$C); 34.8 (Me$_3$*C*); 113.5 (C(3)); 119.7 (C(5)); 121.4 (C(2′)); 127.1 (C(3′)); 143.8 (C(4)); 149.3 (C(4′)); 149.8 (C(1′)); 152.4 (C(6)); 161.5 (C(2)); 164.9 (C=O). HR-ESI-MS (neg.): 270.1135 ([*M* – H]$^-$, $C_{16}H_{16}NO_3^-$; calc. 270.1130). Anal. calc. for $C_{16}H_{17}NO_3$ (271.31): 70.83, H 6.32, N 5.16; found: C 70.54, H 6.20, N 4.91.

*6-(*tert*-Butyl)-10*H*-9-oxa-1-azaanthracen-10-one* (*= 7-(1,1-Dimethylethyl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **4a**). A stirred mixture of polyphosphoric acid (70 g) and 2-[4-(*tert*-butyl)phenoxy]nicotinic acid (2.15 g, 7.93 mmol) was heated at 120° for 16 h. The brown mixture was allowed to cool to r.t., before being poured onto iced water (400 ml) to afford a pale yellow soln. The pH of the aq. soln. was adjusted to pH 7 by the addition of conc. aq. NaOH soln. The aq. soln. was extracted with Et_2O (3 × 300 ml) and the combined extract dried ($MgSO_4$) and concentrated: **4a** (1.79 g, 89%). Yellow solid. M.p. 97–98°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.42 (*s*, ^{t}Bu); 7.45 (*dd*, *J* = 7.5, 4.5, H–C(2)); 7.58 (*d*, *J* = 8.5, H–C(10)); 7.86 (*dd*, *J* = 9.0, 3.0, H–C(9)); 8.30 (*d*, *J* = 2.5, H–C(7)); 8.73 (*d*, *J* = 7.5, H–C(1)); 8.76 (*d*, *J* = 7.5, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 31.6 (*Me*$_3$C); 35.1 (Me$_3$*C*); 117.0 (C(4)); 118.4 (C(10)); 121.1 (C(2)); 121.1 (C(1)); 122.7 (C(7)); 133.9 (C(9)); 137.6 (C(3)); 148.2 (C(6)); 154.1 (C(8)); 154.3 (C(12)); 160.6 (C(11)); 178.1 (C(5)). HR-ESI-MS (pos.): 254.1177 ([*M* + H]$^+$, $C_{16}H_{16}NO_2^+$; calc. 254.1181). Anal. calc. for $C_{16}H_{15}NO_2$ (253.30): 75.87, H 5.97, N 5.53; found: C 75.80, H 5.91, N 5.61.

*6-(*tert*-Butyl)-1-methyl-10*H*-9-oxa-1-azoniaanthracen-10-one Trifluoromethanesulfonate* (*= 7-(1,1-Dimethylethyl)-1-methyl-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridinium 1,1,1-Trifluoromethanesulfonate*). To a soln. of **4a** (1.00 g, 3.95 mmol) in anh. toluene (20 ml) at 0°, methyl trifluoromethanesulfonate (6 ml, 8.70 g, 53.0 mmol) was added over 10 min. The soln. was stirred at 0° for 1 h, to afford a fine precipitate that was filtered and dried under vacuum: colourless solid (1.49 g, 91%). ^{1}H-NMR (CD_3OD, 400 MHz)[3]): 1.43 (*s*, ^{t}Bu); 4.51 (*s*, MeN); 7.84 (*d*, *J* = 9.0, H–C(10)); 7.99 (*dd*, *J* = 8.0, 6.0, H–C(2)); 8.15 (*dd*, *J* = 9.0, 2.0, H–C(9)); 8.33 (*d*, *J* = 2.5, H–C(7)); 9.14 (*dd*, *J* = 6.0, 2.0, H–C(1)); 9.30 (*dd*, *J* = 8.0, 2.0, H–C(3)). ^{13}C-NMR (CD_3OD, 100 MHz)[3]): 30.3 (*Me*$_3$C); 34.8 (Me$_3$*C*); 41.7 (Me); 118.2 (C(10)); 120.4 (C(4)); 120.8 (C(6)); 121.2 (C(2)); 122.6 (C(7)); 135.4 (C(9)); 145.9 (C(3)); 149.1 (C(1)); 151.1 (C(8)); 152.4 (C(11)); 156.3 (C(12)); 173.8 (C(5)). ^{19}F-NMR (CD_3OD, 188 MHz, ^{1}H-decoupled 400 MHz): 80.5 (*s*, CF_3). HR-ESI-MS: 268.1332 (*M*$^+$, $C_{17}H_{18}NO_2^+$; calc. 268.1338).

*6-(*tert*-Butyl)-1-methyl-10*H*-9-oxa-1-azoniaanthracen-10-one Chloride.* Ion exchange of 6-(*tert*-butyl)-1-methyl-10*H*-9-oxa-1-azoniaanthracen-10-one trifluoromethanesulfonate to the corresponding chloride salt was undertaken to enhance H_2O solubility. The trifluoromethanesulfonate (1.00 g, 2.40 mmol) was dissolved in H_2O/MeOH 1:1 (20 ml) and added to an excess of a *Dowex* anion-

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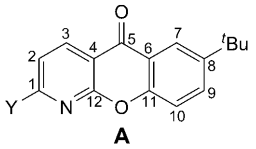
[3]) The atom numbering in the NMR spectra of 10*H*-9-oxa-1-azaanthracen-10-one (=4-azaxanthone) derivatives is arbitrary, see **A**.



A

exchange resin (0.5 g, 200–400 mesh, Cl^- form). The mixture was stirred for 2 h, filtered, and the solvent evaporated to yield the corresponding chloride salt. ^{1}H-NMR (CD_3OD, 500 MHz)[3]): 1.46 (*s*, ^{t}Bu); 4.55 (*s*, MeN); 7.88 (*d*, *J* = 9.0, H–C(10)); 8.03 (*t*, *J* = 6.5, H–C(2)); 8.18 (*dd*, *J* = 9.0, 2.0, H–C(9)); 8.36 (*d*, *J* = 2.0, H–C(7)); 9.22 (*d*, *J* = 6.5, H–C(1)); 9.33 (*d*, *J* = 7.5, H–C(3)). ^{13}C-NMR (CD_3OD, 125 MHz)[3]): 30.4 (*Me*$_3$C); 34.8 (Me$_3$*C*); 41.8 (Me); 118.2 (C(10)); 120.5 (C(4)); 120.8 (C(6)); 121.2 (C(2)); 122.6 (C(7)); 135.4 (C(9)); 145.9 (C(3)); 149.1 (C(1)); 151.1 (C(8)); 152.4 (C(11)); 156.3 (C(12)); 173.8 (C(5)).

*6-(*tert*-Butyl)-1-methyl-1*H*-9-oxa-1-azaanthracene-2,10-dione* (*= 7-(1,1-Dimethylethyl)-1-methyl-2*H*-[1]benzopyrano[2,3-*b*]pyridine-2,5(1*H*)-dione)*. A soln. of 6-(*tert*-butyl)-1-methyl-10*H*-9-oxa-1-azoniaanthracen-10-one chloride (0.361 g, 1.18 mmol) in H_2O (10 ml) was added over 30 min to a stirred soln. of $K_3[Fe(CN)_6]$ (1.16 g, 3.54 mmol) in H_2O (6 ml) at 0°. This was followed by the dropwise addition of an aq. soln. of NaOH (0.850 g, 21.2 mmol) in H_2O (10 ml) to the stirred soln. over 30 min. The soln. was allowed to warm to r.t. and stirred for 8 h. The soln. was acidified to pH 3 by the addition of conc. H_2SO_4 soln. to afford a green precipitate. The precipitate was filtered and dissolved in $CHCl_3$ (50 ml) and the soln. washed with H_2O (2 × 50 ml), dried ($MgSO_4$), and concentrated: red solid (0.251 g, 74%). M.p. 243–244°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.41 (*s*, ^{t}Bu); 3.76 (*s*, MeN); 6.54 (*d*, *J* = 9.5, H–C(2)); 7.47 (*d*, *J* = 8.5, H–C(10)); 7.79 (*dd*, *J* = 9.0, 2.0, H–C(9)); 8.21 (*d*, *J* = 9.5, H–C(3)); 8.29 (*d*, *J* = 2.0, H–C(7)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 28.5 (Me); 31.6 (*Me*$_3$C); 35.2 (Me$_3$*C*); 102.8 (C(4)); 116.0 (C(2)); 117.3 (C(10)); 121.6 (C(6)); 122.9 (C(7)); 132.3 (C(9)); 135.7 (C(3)); 149.7 (C(8)); 152.0 (C(11)); 156.5 (C(12)); 162.3 (C(1)); 174.2 (C(5)). HR-ESI-MS: 284.1281 ($[M+H]^+$, $C_{17}H_{18}NO_3^+$; calc. 284.1287). Anal. calc. for $C_{17}H_{17}NO_3$ (283.32): C 72.07, H 6.05, N 4.94; found C 72.02, H 5.99, N 4.90.

*6-(*tert*-Butyl)-2-chloro-10*H*-9-oxa-1-azaanthracen-10-one* (*= 2-Chloro-7-(1,1-dimethylethyl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **4b**). A stirred soln. of 6-(*tert*-butyl)-1-methyl-1*H*-9-oxa-1-azaanthracene-2,10-dione (0.180 g, 0.636 mmol) in $POCl_3$ (10 ml) and *N*,*N*-dimethylaniline (0.30 ml, 0.313 g, 2.59 mmol) was heated under reflux for 18 h. The mixture was allowed to cool to r.t. and slowly added to iced water (300 ml). The aq. soln. was extracted with CH_2Cl_2 (2 × 200 ml), the combined org. phase washed with aq. dil. K_2CO_3 soln. (100 ml), dried (K_2CO_3), and concentrated, and the residue dried over silica gel (SiO_2) and purified by column chromatography (CC) (SiO_2, hexane → 10% AcOEt/hexane by 1% AcOEt increments): **4b** (0.090 g, 49%). Pink solid. R_f 0.33 (SiO_2, hexane/AcOEt 9 : 1). M.p. 132–133°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.41 (*s*, ^{t}Bu); 7.43 (*d*, *J* = 8.0, H–C(2)); 7.54 (*d*, *J* = 9.0, H–C(10)); 7.86 (*dd*, *J* = 9.0, 2.5, H–C(9)); 8.27 (*d*, *J* = 2.5, H–C(7)); 8.65 (*d*, *J* = 8.0, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 31.5 (*Me*$_3$C); 35.1 (Me$_3$*C*); 115.6 (C(4)); 118.4 (C(10)); 121.1 (C(6)); 121.9 (C(2)); 122.7 (C(7)); 134.1 (C(9)); 139.9 (C(3)); 148.8 (C(8)); 153.8 (C(11)); 155.6 (C(12)); 159.7 (C(1)); 177.2 (C(5)). HR-ESI-MS: 288.0785 ($[M+H]^+$, $C_{16}H_{15}ClNO_2^+$; calc. 288.0791).

*6-(*tert*-Butyl)-2-(6-methylpyridin-2-yl)-10*H*-9-oxa-1-azaanthracen-10-one* (*= 7-(1,1-Dimethylethyl)-2-(6-methylpyridin-2-yl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **2a**). Under Ar, **4b** (0.201 g, 0.696 mmol), 6-methyl-2-(tributylstannyl)pyridine (0.293 g, 0.766 mmol), and degassed toluene (5 ml) were added to tetrakis(triphenylphosphine)palladium(0) (0.040 g, 0.034 mmol). The mixture was stirred and heated under reflux under Ar for 16 h. After cooling to r.t. and filtering, solvent was evaporated to afford a pale brown oil. The crude material was triturated with Et_2O (10 ml) to yield a fine precipitate. The solvent was decanted and the solid dried under vacuum: **2a** (0.145 g, 61%). Colourless solid. M.p. 191–192°. ^{1}H-NMR ($CDCl_3$, 700 MHz)[3]): 1.37 (*s*, ^{t}Bu); 2.60 (*s*, Me); 7.18 (*d*, *J* = 8.0, H–C(5′)); 7.53 (*d*, *J* = 8.0, H–C(10)); 7.69 (*t*, *J* = 8.0, H–C(4′)); 7.79 (*dd*, *J* = 8.0, 2.0, H–C(9)); 8.26 (*d*, *J* = 2.0, H–C(7)); 8.29 (*d*, *J* = 8.0, H–C(3′)); 8.54 (*d*, *J* = 8.5, H–C(2)); 8.74 (*d*, *J* = 8.5, H–C(3)). ^{13}C-NMR ($CDCl_3$, 176 MHz)[3]): 24.8 (Me); 31.5 (*Me*$_3$C); 35.0 (Me$_3$*C*); 116.4 (C(4)); 118.3 (C(10)); 118.5 (C(2)); 119.7 (C(5′)); 121.3 (C(6)); 122.7 (C(7)); 124.9 (C(2′)); 133.6 (C(9)); 137.4 (C(4′)); 138.2 (C(3)); 148.0 (C(8)); 153.6 (C(1′)); 154.2 (C(11)); 158.6 (C(6′)); 160.2 (C(1)); 160.6 (C(12)); 177.8 (C(5)). HR-ESI-MS: 345.1596 ($[M+H]^+$, $C_{22}H_{21}N_2O_2^+$; calc. 345.1603).

The structure of **2a** was confirmed by single-crystal X-ray diffraction: $C_{22}H_{20}N_2O_2$, M_r 344.40, monoclinic ($P2_1/c$); $a = 13.472(1)$ Å, $b = 6.7227(7)$ Å, $c = 19.449(2)$ Å, $V = 1760.7(3)$ Å^3, $\beta = 91.702(6)°$, $Z = 4$; $\mu = 0.08$ mm^{-1}, $D_{calc.} = 1.299$ g cm^{-3}, T 120(2) K; 4671 independent reflections ($R_{int} = 0.028$), $R_1 = 0.042$, $wR_2 = 0.118$ ($I > 2\sigma(I)$); CCDC 724804.

*2-[6-(Bromomethyl)pyridin-2-yl]-6-(*tert*-butyl)-10*H*-9-oxa-1-azaanthracen-10-one* (*= 2-[6-(Bromomethyl)pyridin-2-yl)-7-(1,1-dimethylethyl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **2b**). A stirred mix-

ture of **2a** (0.200 g, 0.581 mmol), *N*-bromosuccinimide (NBS; 0.129 g, 0.725 mmol), and benzoyl peroxide (0.010 g, 0.041 mmol) in CCl_4 (5 ml) was heated under reflux for 16 h (^{1}H-NMR monitoring). After 8 h, NBS (0.100 g, 0.562 mmol) and dibenzoyl peroxide (0.010 g, 0.041 mmol) were added, and the mixture was heated under reflux for a further 16 h. The mixture was then allowed to cool to r.t. and filtered, the filtrate concentrated, and the yellow residue purified by CC (SiO_2; CH_2Cl_2): **2b** (0.101 g, 41%). Colourless solid. R_f 0.35 (SiO_2, CH_2Cl_2). M.p. 186–187°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.42 (*s*, ^{t}Bu); 4.66 (*s*, CH_2Br); 7.56 (J = 7.5, H–C(5′)); 7.60 (*d*, J = 9.0, H–C(10)); 7.85 (*dd*, J = 8.5, 2.5, H–C(9)); 7.90 (*t*, J = 8.0, H–C(4′)); 8.32 (*d*, J = 2.5, H–C(7)); 8.48 (*d*, J = 8.0, H–C(3′)); 8.63 (*d*, J = 8.0, H–C(2)); 8.82 (*d*, J = 8.0, H–C(3)). ^{13}C-NMR ($CDCl_3$, 126 MHz)[3]): 31.6 (*Me*$_3$C); 34.0 (CH_2Br); 35.1 (Me$_3$*C*); 116.8 (C(4)); 118.3 (C(10)); 118.7 (C(2)); 121.3 (C(6)); 121.8 (C(5′)); 122.8 (C(7)); 125.1 (C(3′)); 133.8 (C(9)); 138.4 ((C(4′)); 138.6 (C(3)); 148.2 (C(8)); 154.0 (C(1′)); 154.2 (C(11)); 157.0 (C(6′)); 160.0 (C(1)); 160.2 (C(12)); 177.9 (C(5)). HR-ESI-MS: 423.0700 ($[M+H]^+$, $C_{22}H_{20}{}^{79}BrN_2O_2^+$; calc. 423.0708).

*6-(*tert*-Butyl)-2-[6-(dibromomethyl)pyridin-2-yl]-10*H*-9-oxa-1-azaanthracen-10-one* (=*2-[6-Dibromomethyl)pyridin-2-yl]-7-(1,1-dimethylethyl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **2c**). As described for **2b**: **2c** (0.061 g, 33%). Colourless solid. R_f 0.71 (SiO_2, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.41 (*s*, ^{t}Bu); 6.78 (*s*, $CHBr_2$); 7.58 (*d*, J = 8.5, H–C(10)); 7.84 (*dd*, J = 9.0, 2.5, H–C(9)); 7.92 (*d*, J = 8.0, H–C(5)); 7.98 (*t*, J = 8.0, H–C(4′)); 8.30 (*d*, J = 2.5, H–C(7)); 8.50 (*d*, J = 7.5, H–C(3′)); 8.59 (*d*, J = 8.0, H–C(2)); 8.81 (*d*, J = 8.0, H–C(3)). ^{13}C-NMR ($CDCl_3$, 126 MHz)[3]): 31.6 (*Me*$_3$C); 35.1 (Me$_3$*C*); 41.6 ($CHBr_2$); 116.9 (C(4)); 118.3 (C(10)); 118.7 (C(2)); 121.3 (C(6)); 122.8 (C(7)); 122.9 (C(3′)); 123.6 (C(5′)); 133.9 (C(9)); 138.7 (C(3)); 139.1 (C(4′)); 148.3 (C(8)); 152.7 (C(2′)); 154.2 (C(11)); 159.0 (C(6′)); 159.1 (C(1)); 160.2 (C(12)); 177.8 (C(5)). HR-ESI-MS: 500.9807 ($[M+H]^+$, $C_{22}H_{19}{}^{79}Br_2N_2O_2^+$; calc. 500.9813).

*6-(*tert*-Butyl)-2-(3-methyl-1*H*-pyrazol-1-yl)-10*H*-9-oxa-1-azaanthracen-10-one* (=*7-(1,1-Dimethylethyl)-2-(3-methyl-1*H*-pyrazol-1-yl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **3a**). NaH (0.030 g, 1.25 mmol) was added to a stirred soln. of 3-methyl-1*H*-pyrazole (0.880 g, 1.07 mmol) in anh. THF (5 ml) under Ar. A soln. of **4b** (0.281 g, 0.97 mmol) in anh. THF (5 ml) was added, and the mixture was heated at 65° for 16 h. The mixture was allowed to cool to r.t., and H_2O (10 ml) was added to induce precipitation. The precipitate was collected by centrifugation and the resultant solid triturated with Et_2O (10 ml). The solvent was decanted and the solid dried under vacuum: **3a** (0.294 g, 90%). Colourless solid. M.p. 128–130°. ^{1}H-NMR ($CDCl_3$, 400 MHz)[3]): 1.39 (*s*, ^{t}Bu); 2.37 (*s*, Me); 6.31 (*d*, J = 3.0, H–C(2′)); 7.51 (*d*, J = 9.0, H–C(10)); 7.80 (*dd*, J = 9.0, 3.0, H–C(9)); 7.99 (*d*, J = 8.5, H–C(2)); 8.27 (*d*, J = 3.0, H–C(7)); 8.51 (*d*, J = 3.0, H–C(1′)); 8.72 (*d*, J = 8.5, H–C(3)). ^{13}C-NMR ($CDCl_3$, 100 MHz)[3]): 14.2 (Me); 31.6 (*Me*$_3$C); 35.1 (Me$_3$*C*); 109.7 (C(2)); 110.1 (C(2′)); 113.9 (C(4)); 118.1 (C(10)); 121.4 (C(6)); 122.7 (C(7)); 129.0 (C(1′)); 133.4 (C(9)); 140.1 (C(3)); 148.3 (C(8)); 153.7 (C(11)); 153.9 (C(12)); 154.0 (C(3′)); 160.0 (C(1)); 177.0 (C(5)). HR-ESI-MS: 334.1551 ($[M+H]^+$, $C_{20}H_{20}N_3O_2^+$; calc. 334.1556).

*2-[3-(Bromomethyl)-1*H*-pyrazol-1-yl]-6-(*tert*-butyl)-10*H*-9-oxa-1-azaanthracen-10-one* (=*2-[3-(Bromomethyl)-1*H*-pyrazol-1-yl]-7-(1,1-dimethylethyl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **3b**). *Procedure A*. A stirred mixture of **3a** (0.212 g, 0.636 mmol), NBS (0.113 g, 0.635 mmol), and benzoyl peroxide (0.010 g, 0.041 mmol) in CCl_4 (15 ml) was heated under reflux for 16 h. The mixture was allowed to cool to r.t. and filtered, the filtrate concentrated, and the pale yellow oil purified by CC (SiO_2, CH_2Cl_2): **3b** (0.148 g, 56%). Colourless solid. R_f 0.28 (SiO_2, CH_2Cl_2). M.p. 184–185°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.42 (*s*, ^{t}Bu); 4.56 (*s*, CH_2Br); 6.62 (*d*, J = 2.5, H–C(2′)); 7.56 (*d*, J = 9.0, H–C(10)); 7.85 (*dd*, J = 9.0, 2.5, H–C(9)); 8.06 (*d*, J = 8.0, H–C(2)); 8.31 (*d*, J = 2.5, H–C(7)); 8.63 (*d*, J = 2.5, H–C(1′)); 8.80 (*d*, J = 8.5, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 24.6 (CH_2Br); 31.6 (*Me*$_3$C); 35.1 (Me$_3$*C*); 109.7 (C(2′)); 110.0 (C(2)); 114.6 (C(4)); 118.1 (C(10)); 121.4 (C(6)); 122.8 (C(7)); 129.8 (C(1′)); 133.6 (C(9)); 140.5 (C(3)); 148.6 (C(8)); 153.3 (C(11)); 153.7 (C(3′)); 153.9 (C(12)); 159.9 (C(1)); 177.0 (C(5)). HR-ESI-MS: 434.0475 ($[M+Na]^+$, $C_{20}H_{18}{}^{79}BrN_3NaO_2^+$; calc. 434.0480).

The structure was confirmed by a single crystal X-ray analysis. $C_{20}H_{18}BrN_3O_2$, M_r 412.28, monoclinic ($P2_1/c$); a = 17.044(3) Å, b = 13.303(2) Å, c = 8.079(1) Å, β = 97.124(15)°, V = 1817.6(3) Å^3, Z = 4; μ = 2.28 mm^{-1}, $D_{calc.}$ = 1.507 g/cm^3, T 120(2) K; 3201 independent reflections (R_{int} = 0.104), R_1 = 0.073, wR_2 = 0.125 ($I > 2\sigma(I)$); CCDC-724805.

Procedure B. A soln. of **3c** (0.150 g, 0.307 mmol), ^{i}Pr$_2$NEt (0.157 g, 0.211 ml, 1.22 mmol) and diethyl phosphite (0.168 g, 0.157 ml, 1.22 mmol) in anh. THF (10 ml) was stirred at r.t. for 16 h. The solvent was

evaporated and the residue partitioned between $CHCl_3$ (10 ml) and H_2O (10 ml). The org. phase was concentrated and the crude material purified by CC (SiO_2, CH_2Cl_2): **3b** (0.085 g, 67%). Colourless solid.

*6-(*tert*-Butyl)-2-[3-(dibromomethyl)-1*H*-pyrazol-1-yl]-10*H*-9-oxa-1-azaanthracen-10-one* (=*2-[3-(Dibromomethyl)-1*H*-pyrazol-1-yl]-7-(1,1-dimethylethyl)-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*; **3c**). As described for **3b**: **3c** (0.970 g, 31%). Colourless solid. R_f 0.33 (SiO_2, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.42 (*s*, ^{t}Bu); 6.79 (*s*, $CHBr_2$); 6.89 (*d*, $J = 2.5$, H–C(2′)); 7.56 (*d*, $J = 8.5$, H–C(10)); 7.85 (*dd*, $J = 8.5$, 2.5, H–C(9)); 8.03 (*d*, $J = 8.5$, H–C(2)); 8.31 (*d*, $J = 2.5$, H–C(7)); 8.67 (*d*, $J = 2.5$, H–C(3′)); 8.81 (*d*, $J = 8.5$, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 31.0 ($CHBr_2$); 31.6 (Me_3C); 35.1 (Me_3C); 108.8 (C(2′)); 110.0 (C(2)); 114.9 (C(4)); 118.1 (C(10)); 121.3 (C(6)); 122.8 (C(7)); 130.1 (C(3′)); 133.8 (C(9)); 140.6 (C(3)); 148.6 (C(8)); 153.1 (C(12)); 153.9 (C(11)); 157.0 (C(1′)); 159.8 (C(1)); 177.0 (C(5)). ESI-MS: 486.3 (100, $[M+H]^+$).

11. *Eu^{III} Complex of Ligand* $\mathbf{L^1}$. *Ligand* $\mathbf{L^1}$ *Precursor 1,4,7-Tri(*tert*-butyl) 10-{[6-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]pyridin-2-yl}methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate* (=*1,4,7-Tris(1,1-dimethylethyl) 10-{[6-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]pyridin-2-yl]methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate*). A stirred mixture of 1,4,7-tri(*tert*-butyl) 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (0.032 g, 0.062 mmol), **2b** (0.024 g, 0.057 mmol), and Cs_2CO_3 (0.026 g, 0.080 mmol) in anh. MeCN (5 ml) was heated under reflux for 16 h. The mixture was allowed to cool to r.t. and syringe filtered, the filtrate concentrated, and the yellow oil purified by CC (Al_2O_3, $CH_2Cl_2 \rightarrow 2\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): triester precursor of $\mathbf{L^1}$ (0.035 g, 74%). Pale yellow solid. R_f 0.22 (Al_2O_3, CH_2Cl_2/MeOH 19:1). ^{1}H-NMR ($CDCl_3$, 700 MHz)[3]): 1.29 (*s*, ^{t}Bu); 1.32 (br. *s*, Boc); 1.35 (br. *s*, Boc); 2.71 (br. *s*, 8 H, cyclen CH_2); 3.24 (br. *s*, 8 H, cyclen CH_2); 3.50 (br. *s*, 2 $CH_2CO_2{}^t$Bu); 3.55 (br. *s*, 1 $CH_2CO_2{}^t$Bu); 3.69 (*s*, pyCH_2); 7.25 (*d*, $J = 8.0$, H–C(5′)); 7.45 (*d*, $J = 8.5$, H–C(10)); 7.69 (*t*, $J = 8.0$, H–C(4′)); 7.71 (*dd*, $J = 9.0$, 3.0, H–C(9)); 8.17 (*d*, $J = 3.0$, H–C(7)); 8.32 (*d*, $J = 8.0$, H–C(3′)); 8.36 (*d*, $J = 7.0$, H–C(2)); 8.64 (*d*, $J = 8.0$, H–C(3)). ^{13}C-NMR ($CDCl_3$, 176 MHz)[3]): 28.3 (Me_3C); 31.5 (2 Me_3CO); 35.1 (1 Me_3CO); 50.6–56.7 (cyclen CH_2 and NCH_2CO); 57.1 (pyCH_2); 82.4 (4 Me_3C); 116.4 (C(4)); 118.2 (C(10)); 118.3 (C(2)); 121.0 (C(3′)); 121.2 (C(6)); 122.6 (C(7)); 126.2 (C(5′)); 133.6 (C(9)); 137.4 (C(4′)); 138.2 (C(3)); 148.0 (C(8)); 153.6 (C(1′)); 154.2 (C(11)); 158.6 (C(6′)); 160.2 (C(1)); 160.6 (C(12)); 177.8 (C(5)). ESI-MS (pos.): 857.5 (100, $[M+H]^+$). HR-ESI-MS: 857.5176 ($[M+H]^+$, $C_{48}H_{69}N_6O_8^+$; calc. 857.5177).

[Eu($\mathbf{L^1}$*)]*. A soln. of the ligand $\mathbf{L^1}$ precursor (see above; 0.035 g, 0.0419 mmol) in CH_2Cl_2/CF_3COOH 1:1 (2 ml) was stirred at r.t. for 16 h to afford an orange soln. The solvent was evaporated to yield a glassy solid. The crude material was repeatedly dissolved 3× in CH_2Cl_2 (5 ml) and the solvent evaporated (removal of excess acid and ^{t}BuOH). The desired ligand, $\mathbf{L^1} \cdot 3\,H^+$, as its trifluoroacetate salt, was examined by ^{1}H-NMR to ensure complete ester hydrolysis, and the material was used immediately for complexation. The ligand was dissolved in MeOH/H_2O 1:1 (4 ml), and $Eu(OAc)_3 \cdot 6\,H_2O$ (0.017 g, 0.039 mmol) was added to the mixture. The pH of the soln. was raised to 5.5 by the addition of 1M aq. KOH, then stirred and heated at 80° for 15 h. The mixture was allowed to cool to r.t. before raising the pH to 10.0 with dil. aq. KOH soln. The mixture was stirred for 1 h to allow precipitation of excess Eu metal as its hydroxide salt $Eu(OH)_3$. The solid precipitate was removed by syringe filtration and the pH of the colourless aq. filtrate lowered to pH 5.5. The solvent was removed by lyophilization to yield a colourless solid. The material was purified by CC (neutral Al_2O_3, CH_2Cl_2/MeOH 8:2): [Eu($\mathbf{L^1}$)]. Colourless solid (0.024 g, 68%). R_f 0.41 (Al_2O_3, CH_2Cl_2/MeOH 7:3). λ_{max} (H_2O) 356 nm; τ (H_2O) 1.00 ms; τ (D_2O) 1.34 ms; ϕ_{Eu} (H_2O, pH 7.4, λ_{exc} 365 nm) 14%.

12. *Complexes of Ligand* $\mathbf{L^2}$. *Ligand* $\mathbf{L^2}$ *Precursor 1,4,7-Tri(*tert*-butyl) 10-{[1-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate* (=*1,4,7-Tris(1,1-dimethylethyl) 10-{[1-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]-1*H*-pyrazol-3-yl}methyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate*). As described for the ligand $\mathbf{L^1}$ precursor, with 1,4,7-tri(*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (0.075 g, 0.146 mmol), **3b** (0.060 g, 0.146 mmol), Cs_2CO_3 (0.050 g, 0.153 mmol), and MeCN (5 ml). CC (SiO_2, $CH_2Cl_2 \rightarrow 8\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): triester precursor of $\mathbf{L^2}$ (0.090 g, 0.106 mmol, 73%). Pale yellow solid. R_f 0.36 (SiO_2, CH_2Cl_2/MeOH 19:1). ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.40 (*s*, ^{t}Bu); 1.51 (br. *s*, 3 Boc); 2.67 (br. *s*, 8 H, cyclen CH_2); 3.27 (br. *s*, 8 H, cyclen CH_2); 3.44 (br. *s*, 2 $CH_2CO_2{}^t$Bu); 3.51 (br. *s*, 1 $CH_2CO_2{}^t$Bu); 3.55 (*s*, pzCH_2); 6.57 (*d*, $J = 2.5$, H–C(2′));

7.56 (*d*, *J* = 8.0, H–C(10)); 7.86 (*dd*, *J* = 9.0, 2.5, H–C(9)); 8.23 (*d*, *J* = 8.0, H–C(2)); 8.27 (*d*, *J* = 2.5, H–C(7)); 8.57 (*d*, *J* = 2.5, H–C(1′)); 8.62 (*d*, *J* = 8.5, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 28.3 (*Me*$_3$C); 31.5 (2 *Me*$_3$CO); 35.1 (1 *Me*$_3$CO); 50.6–56.1 (cyclen CH_2, br.); 56.7 (2 cyclen CH_2, pz*C*H_2); 82.4 (4 Me$_3$*C*); 110.8 (C(2)); 110.9 (C(2′)); 114.3 (C(4)); 118.2 (C(10)); 121.1(C(6)); 122.7 (C(7)); 129.6 (C(1′)); 133.9 (C(9)); 140.1 (C(3)); 148.7 (C(8)); 153.6 (C(11)); 153.9 (C(12)); 155.3 (C(3)); 159.8 (C(1)); 172.9 (3 C=O of ester); 176.9 (C(5)). ESI-MS: 846.5 (100, $[M+H]^+$).

*[Tb(***L**^{2}*)]*. As described for [Eu(**L**^{1})] (above), with the ligand **L**^{2} precursor (see above; 0.045 g, 0.053 mmol) in CH_2Cl_2/CF_3COOH 2:1 (3 ml) at r.t. in a sealed flask for 16 h: [Tb(**L**^{2})] (0.026 g, 61%). Colourless solid. R_f 0.39 (Al_2O_3, CH_2Cl_2/MeOH 7:3). λ_{max} (H_2O) 348 nm; τ (H_2O) 2.24 ms; τ (D_2O) 2.65 ms; ϕ_{Tb} (H_2O, pH 6.0, λ_{exc} 355 nm) 15%.

*[Eu(***L**^{2}*)H*$_2$*O]*. As described for [Tb(**L**^{2})]: [Eu(**L**^{2}) (H_2O)] (0.015 g, 52%). Colourless solid. λ_{max} (H_2O) 346 nm; τ (H_2O) 0.70 ms; τ (D_2O) 1.76 ms.

13. *Eu*^{III} *Complex of Ligand* **L**^{4a}. *Tri(*tert*-butyl) 10-{[6-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]pyridin-2-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate* (= *Tris(1,1-dimethylethyl) 10-{[6-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]pyridin-2-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate*). A stirred mixture of tri(*tert*-butyl) 1,4,7-tetraazacyclododecane-1,4,7-tricarboxylate (0.117 g, 0.248 mmol), **2b** (0.100 g, 0.236 mmol), and K_2CO_3 (0.049 g, 0.354 mmol) in anh. MeCN (4 ml) was heated under reflux and Ar for 16 h. The mixture was allowed to cool to r.t. and syringe filtered, the filtrate concentrated, and the residual yellow oil purified by CC (SiO_2, $CH_2Cl_2 \rightarrow 3\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): pale yellow solid (0.173 g, 90%). R_f 0.70 (SiO_2, CH_2Cl_2/MeOH 49:1). ^{1}H-NMR ($CDCl_3$, 700 MHz)[3]): 1.29 (*s*, ^{t}Bu); 1.32 (*s*, 1 Boc); 1.35 (br. *s*, 2 Boc); 2.71 (br. *s*, 4 H, cyclen CH_2); 3.24 (br. *s*, 8 H, cyclen CH_2); 3.50 (br. *s*, 4 H, cyclen CH_2); 3.87 (*s*, py*C*H_2); 7.25 (*d*, *J* = 8.0, H–C(5′)); 7.45 (*d*, *J* = 8.5, H–C(10)); 7.69 (*t*, *J* = 8.0, H–C(4′)); 7.71 (*dd*, *J* = 9.0, 3.0, H–C(9)); 8.17 (*d*, *J* = 3.0, H–C(7)); 8.32 (*d*, *J* = 8.0, H–C(3′)); 8.36 (*d*, *J* = 7.0, H–C(2)); 8.64 (*d*, *J* = 8.0, H–C(3)). ^{13}C-NMR ($CDCl_3$, 176 MHz)[3]): 28.6 (3 *Me*$_3$CO); 31.5 (*Me*$_3$C); 35.0 (Me$_3$*C*); 47.3 (cyclen CH_2); 47.7 (cyclen CH_2); 48.0 (cyclen CH_2); 48.4 (cyclen CH_2); 50.1 (cyclen CH_2); 51.1 (cyclen CH_2); 54.4 (cyclen CH_2); 55.2 (cyclen CH_2); 57.1 (py*C*H_2); 79.5 (3 Me$_3$*C*); 116.4 (C(4)); 118.2 (C(10)); 118.3 (C(2)); 121.0 (C(3′)); 121.2 (C(6)); 122.6 (C(7)); 126.2 (C(5′)); 133.6 (C(9)); 137.4 (C(4′)); 138.2 (C(3)); 148.0 (C(8)); 153.6 (C(1′)); 154.2 (C(11)); 155.9 (3 C=O of Boc); 158.6 (C(6′)); 160.2 (C(1)); 160.6 (C(12)); 177.8 (C(5)). HR-ESI-MS: 815.4710 ($[M+H]^+$, $C_{45}H_{63}N_6O_8^+$; calc. 815.4707).

*6-(*tert*-Butyl)-2-[6-(1,4,7,10-tetraazacyclododec-1-ylmethyl)pyridin-2-yl]-10*H*-9-oxa-1-azaanthracen-10-one* (= *7-(1,1-Dimethylethyl)-2-[6-(1,4,7,10-tetraazocyclododec-1-ylmethyl)pyridin-2-yl]-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]-5-one*). A soln. of tri(*tert*-butyl) 10-{[6-[6-(*tert*-butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]pyridin-2-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (0.173 g, 0.212 mmol) in CH_2Cl_2/CF_3COOH 2:1 (3 ml) was stirred at r.t. for 6 h (→ orange soln.). The solvent was evaporated to yield a glassy orange solid. The crude material was dissolved 3 × in CH_2Cl_2 (5 ml) and the solvent evaporated (removal of excess acid and ^{t}BuOH). The residue was finally taken into 1M aq. KOH (10 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined org. extract was dried (K_2CO_3) and concentrated: orange solid (0.065 g, 94%). ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.36 (*s*, ^{t}Bu); 2.54 (*s*, 4 H, cyclen CH_2); 2.68 (*s*, 8 H, cyclen CH_2); 2.77 (*s*, 4 H, cyclen CH_2); 3.85 (*s*, py*C*H_2); 7.44 (*d*, *J* = 7.5, H–C(5′)); 7.54 (*d*, *J* = 9.0, H–C(10)); 7.79 (*dd*, *J* = 8.5, 2.5, H–C(4′)); 7.81 (*t*, *J* = 7.5, H–C(9)); 8.26 (*d*, *J* = 2.5, H–C(7)); 8.38 (*d*, *J* = 7.5, H–C(3′)); 8.62 (*d*, *J* = 8.0, H–C(2)); 8.73 (*d*, *J* = 8.0, H–C(3)). ^{13}C-NMR ($CDCl_3$, 126 MHz)[3]): 31.5 (*Me*$_3$C); 35.0 (Me$_3$*C*); 45.3 (cyclen CH_2); 46.6 (cyclen CH_2); 47.4 (cyclen CH_2); 51.8 (cyclen CH_2); 60.8 (py*C*H_2); 116.4 (C(4)); 118.2 (C(10)); 118.6 (C(2)); 121.0 (C(5′)); 121.3 (C(6)); 122.6 (C(7)); 124.6 (C(2′)); 133.7 (C(9)); 137.8 (C(4′)); 138.1 (C(3)); 148.1 (C(8)); 153.5 (C(1′)); 154.2 (C(11)); 159.7 (C(6′)); 160.2 (C(1)); 160.6 (C(12)); 178.0 (C(5)). ESI-MS (pos.): 289.4 (100, $[M+Zn]^{2+}$). HR-ESI-MS: 515.3130 ($[M+H]^+$; $C_{30}H_{39}N_6O_2^+$; calc. 515.3134).

*10-{[6-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]pyridin-2-yl}methyl}-*N*^{1},*N*^{4},*N*^{7}-tris-[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetamide* (= *10-{[6-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]pyridin-2-yl}methyl}-*N*^{1},*N*^{4},*N*^{7}-tris[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetamide*; **L**^{4a}). A stirred mixture of 6-(*tert*-butyl)-2-[6-(1,4,7,10-tetraazacyclododec-1-ylmethyl)pyridin-2-yl]-10*H*-9-oxa-1-azaanthracen-10-one (0.060 g, 0.116 mmol), 2-chloro-*N*-[(1*S*)-1-phenylethyl]acetamide ((*S*)-**5a**; 0.080 g, 0.408 mmol), and K_2CO_3 (0.064 g,

0.466 mmol) in anh. MeCN (5 ml) was heated under reflux for 16 h. The resultant mixture was allowed to cool to r.t. and syringe filtered, the filtrate concentrated, and the residual yellow oil purified by CC (neutral Al_2O_3, $CH_2Cl_2 \rightarrow 2\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): **L^{4a}** (0.074 g, 64%). Pale yellow solid. R_f 0.21 (Al_2O_3, CH_2Cl_2/MeOH 19:1). ^{1}H-NMR ($CDCl_3$, 700 MHz)[3]): 1.38 (*s*, ^{t}Bu); 1.45 (br. *s*, 3 Me); 2.60 (br. *s*, 8 H, cyclen CH_2); 2.92 (br. *s*, 8 H, cyclen CH_2); 3.06 (br. *s*, CH_2CO); 3.54 (br. *s*, CH_2CO); 3.67 (br. *s*, CH_2CO); 3.96 (br. *s*, pyCH_2); 4.96 (*q*, 1 CH); 5.11 (*q*, 2 CH); 7.11–7.30 (*m*, 3 Ph); 7.45 (br. *s*, H–C(5′)); 7.58 (*d*, *J* = 9.0, H–C(10)); 7.70 (br. *s*, H–C(4′)); 7.82 (*dd*, *J* = 9.0, 2.0, H–C(9)); 8.28 (*d*, *J* = 3.0, H–C(7)); 8.43 (br. *s*, H–C(3′)); 8.62 (*d*, *J* = 8.5, H–C(2)); 8.77 (*d*, *J* = 8.5, H–C(3)). HR-ESI-MS: 998.5661 ($[M+H]^+$, $C_{60}H_{72}N_9O_5^+$; calc. 998.5656).

*[Eu(***L^{4a}***)]Cl$_3$*. A soln. of **L^{4a}** (0.040 g, 0.040 mmol) and $Eu(OTf)_3 \cdot 6\,H_2O$ (0.034 g, 0.041 mmol) in anh. MeCN (1 ml) was heated under reflux and Ar for 18 h. The resultant soln. was allowed to cool to r.t. followed by evaporation of the solvent to afford a glassy orange solid. CH_2Cl_2 (10 ml) was added to the solid and the mixture sonicated for 10 min. The solvent was then decanted and the solid material dissolved in a minimum volume of MeCN (0.5 ml). The soln. was added slowly onto Et_2O (25 ml) to induce precipitation. The solid material was isolated by centrifugation, and the process of induced precipitation repeated twice more to yield [Eu(**L^{4a}**)](TfO)$_3$. The off-white solid was rendered H_2O-soluble by the exchange of triflate anions for Cl anions with *Dowex* (1×8, 200–400 mesh, Cl^-) resin. The solid material was dissolved in H_2O/MeOH 1:1 (16 ml) and prepared resin (0.8 g) was added to the soln., which was stirred at r.t. for 3 h. The resin was removed by filtration and the filtrate concentrated: [Eu(**L^{4a}**)]Cl_3 (0.034 g, 68%). HPLC (*Method A*): t_R 10.9. λ_{max} (H_2O) 356 nm; τ (H_2O) 1.00 ms; τ (D_2O) 1.34 ms; ϕ (H_2O, pH 7.4, λ_{exc} 365 nm) 24%.

14. *Complexes of Ligand* **L^{5a}**. *Tri(*tert*-butyl) 10-{{1-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate* (= *Tris(1,1-dimethylethyl) 10-{{1-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]-1*H*-pyrazol-3-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate*). A stirred mixture of 1,4,7-tri(*tert*-butyl) 1,4,7-tetraazacyclododecane-1,4,7-tricarboxylate (0.045 g, 0.109 mmol), **3b** (0.057 g, 0.119 mmol), K_2CO_3 (0.019 g, 0.131 mmol), and a cat. amount of KI (2 mg) in anh. MeCN/CH_2Cl_2 1:1 (5 ml) was heated for 16 h. The mixture was allowed to cool and syringe filtered, the filtrate concentrated, and the residual oil purified by CC (SiO_2, $CH_2Cl_2 \rightarrow 3\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): pale yellow solid (0.075 g, 85%). R_f 0.41 (SiO_2, CH_2Cl_2/MeOH 24:1). ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.44 (br. *s*, 3 Boc, ^{t}Bu); 2.78 (br. *s*, 4 H, cyclen CH_2); 3.39 (br., 8 H, cyclen CH_2); 3.59 (*s*, 4 H, cyclen CH_2); 3.89 (*s*, pzCH_2); 6.44 (*d*, *J* = 3.0, H–C(2′)); 7.56 (*d*, *J* = 8.5, H–C(10)); 7.86 (*dd*, *J* = 9.0, 2.5, H–C(9)); 8.04 (*d*, *J* = 8.5, H–C(2)); 8.30 (*d*, *J* = 2.5, H–C(7)); 8.60 (*d*, *J* = 2.0, H–C(1′)); 8.78 (*d*, *J* = 8.5, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 28.7 (3 *Me*$_3$CO); 31.5 (*Me*$_3$C); 35.5 (Me$_3$*C*); 47.5 (cyclen CH_2); 47.9 (cyclen CH_2); 48.4 (2 cyclen CH_2); 50.1 (2 cyclen CH_2); 53.6 (cyclen CH_2); 54.0 (cyclen CH_2); 55.3 (pz*C*H$_2$); 79.5 (3 Me$_3$*C*O); 109.9 (C(2)); 110.8 (C(2′)); 114.2 (C(4)); 118.1 (C(10)); 121.4 (C(6)); 122.8 (C(7)); 129.0 (C(1′)); 133.6 (C(9)); 140.3 (C(3)); 148.5 (C(8)); 153.6 (C(11)); 153.9 (C(12)); 155.6 (C(3)); 155.9 (3 C=O of Boc); 159.9 (C(1)); 177.0 (C(5)). ESI-MS: 804.3 (100, $[M+H]^+$).

*6-(*tert*-Butyl)-2-[3-(1,4,7,10-tetraazacyclododec-1-ylmethyl)-1*H*-pyrazol-1-yl]-10*H*-9-oxa-1-azaanthracen-10-one* (= *7-(1,1-Dimethylethyl)-2-[3-(1,4,7,10-tetraazacyclododec-1-ylmethyl)-1*H*-pyrazol-1-yl]-5*H*-[1]benzopyrano[2,3-*b*]pyridin-5-one*). A soln. of tri(*tert*-butyl) 10-{{1-[6-(*tert*-butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate (0.110 g, 0.137 mmol) in CH_2Cl_2/CF_3COOH 2:1 (3 ml) was stirred at r.t. for 16 h, generating a red soln. The solvent was evaporated to yield an orange glassy solid. The crude material was repeatedly ($3\times$) dissolved in CH_2Cl_2 (5 ml) and the solvent evaporated. The residue was taken into 1M aq. KOH (15 ml) and extracted with CH_2Cl_2 (3×15 ml). The combined org. extract was dried (K_2CO_3) and concentrated: pale orange solid that was used directly in the next step without further analysis (0.065 g, 94%). ^{1}H-NMR ($CDCl_3$, 200 MHz)[3]): 1.39 (*s*, ^{t}Bu); 2.70 (br. *s*, 4 H, cyclen CH_2); 2.80 (br. *s*, 8 H, cyclen CH_2); 2.85 (br. *s*, 4 H, cyclen CH_2); 3.83 (*s*, CH_2); 6.51 (*d*, *J* = 3.0, H–C(2′)); 7.54 (*d*, *J* = 8.5, H–C(10)); 7.82 (*dd*, *J* = 9.0, 2.0, H–C(9)); 8.04 (*d*, *J* = 8.5, H–C(2)); 8.28 (*d*, *J* = 2.0, H–C(7)); 8.58 (*d*, *J* = 2.0, H–C(1′)); 8.74 (*d*, *J* = 8.5, H–C(3)).

*10-{{1-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl}-*N^1,N^4,N^7*-tris[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetamide* (= *10-{{1-[7-(1,1-Dimethyleth-*

*yl)-5-oxo-5*H-*[1]benzopyrano[2,3-*b*]pyridin-2-yl]-1*H-*pyrazol-3-yl}methyl}-*N^{1},N^{4},N^{7}-*tris[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triacetamide*; **L^{5a}**). A stirred mixture of 6-(*tert*-butyl)-2-[3-(1,4,7,10-tetraazacyclododec-1-ylmethyl)-1*H*-pyrazol-1-yl]-10*H*-9-oxa-1-azaanthracen-10-one (0.039 g, 0.078 mmol), 2-chloro-*N*-[(1*S*)-1-phenylethyl]acetamide ((*S*)-**5a**) (0.050 g, 0.250 mmol), and Cs_2CO_3 (0.081 g, 0.251 mmol) in anh. MeCN (5 ml) was heated for 16 h. The mixture was allowed to cool and filtered, the filtrate concentrated, and the orange glassy solid sonicated in Et_2O (15 ml) to yield a fine, pale yellow precipitate that was isolated *via* centrifugation. The material was sonicated in Et_2O and centrifuged twice more to yield **L^{5a}** (0.062 g, 81%). Pale yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.41 (*s*, ^{t}Bu); 1.52 (br., 3 Me); 2.48–3.12 (br. *m*, 20 H, cyclen CH_2, CH_2CONH); 3.77 (*s*, CH_2); 4.05 (*s*, pzCH_2); 5.01 (*m*, 3 CH); 6.38 (br. *s*, H–C(2′)); 7.03–7.19 (br. *m*, 3 Ph); 7.54 (*d*, *J* = 8.0, H–C(10)); 7.84 (*dd*, *J* = 8.0, 2.0, H–C(9)); 7.96 (*d*, *J* = 8.0, H–C(2)); 8.30 (*d*, *J* = 2.5, H–C(7)); 8.60 (*d*, *J* = 2.0, H–C(1′)); 8.81 (*d*, *J* = 8.0, H–C(3)). ESI-MS: 998.4 (100, $[M+H]^+$).

*[Tb(***L^{5a}***)]Cl$_3$*. A stirred mixture of **L^{5a}** (0.044 g, 0.044 mmol) and $Tb(OTf)_3 \cdot 6\,H_2O$ (0.030 g, 0.049 mmol) in anh. MeCN (1 ml) was heated under reflux for 16 h. The soln. was allowed to cool and the solvent evaporated to afford a glassy orange solid. CH_2Cl_2 (5 ml) was added to the solid, and the mixture was sonicated for 10 min. The solvent was decanted and the solid dissolved in a minimum volume of MeCN (0.4 ml). The soln. was added slowly onto Et_2O (15 ml) to induce precipitation. The solid material was isolated by centrifugation, and the process of induced precipitation repeated twice more to yield [Tb(**L^{5a}**)]$(TfO)_3$. The off-white solid was made H_2O-soluble by the exchange of triflate anions for Cl anions with *Dowex* (1 × 8, 200–400 mesh, Cl^-) resin: [Tb(**L^5**)]Cl_3 (0.040 g, 69%). Colourless solid. HPLC (*Method A*): t_R 11.0. λ_{max} (H_2O) 348 nm; τ (H_2O) 2.00 ms; τ (D_2O) 2.38 ms; ϕ_{Tb} (H_2O, pH 6.0, λ_{exc} 355 nm) 46%.

*[Eu(***L^{5a}***)]Cl$_3$*. An analogous procedure with **L^{5a}** (0.042 g, 0.042 mmol) and $Eu(OTf)_3 \cdot 6\,H_2O$ (0.033 g, 0.046 mmol) in anh. MeCN (1 ml), following anion exchange, yielded [Eu(**L^{5a}**)]Cl_3 (0.027 g, 54%). Colourless solid. HPLC (*Method A*): t_R 11.1. λ_{max} (H_2O) 348 nm.

15. *Complexes of Ligand* **L^6**. N^2,N$^{2'}$,N$^{2''}$-*{{10-{{1-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl}tris(1-oxoethane-2,1-diyl)}tris[*L*-phenylalanine] Triethyl Ester* (= N^2,N$^{2'}$,N$^{2''}$-*{{10-{{1-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]-1*H*-pyrazol-3-yl}methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl}tris(1-oxoethane-2,1-diyl)}tris[*L*-phenylalanine] Triethyl Ester*; **L^6**). A stirred mixture of 6-(*tert*-butyl)-2-[3-(1,4,7,10-tetraazacyclododec-1-ylmethyl)-1*H*-pyrazol-1-yl]-10*H*-9-oxa-1-azaanthracen-10-one (0.033 g, 0.065 mmol), *N*-(2-bromo-1-oxoethyl)-L-phenylalanine ethyl ester ((*S*)-**6**) (0.064 g, 0.206 mmol), and Cs_2CO_3 (0.073 g, 0.231 mmol) in anh. MeCN (7 ml) was heated under reflux for 16 h. The mixture was allowed to cool to r.t. and filtered, the filtrate concentrated, and the orange glassy solid sonicated in Et_2O (15 ml) to yield a fine pale yellow precipitate that was isolated by centrifugation. The material was sonicated in Et_2O and centrifuged twice more to yield **L^6** (0.028 g, 36%). Pale yellow solid. ^{1}H-NMR ($CDCl_3$, 400 MHz)[3]): 1.13 (*t*, *J* = 6.0, 3 *Me*CH_2O); 1.35 (*s*, ^{t}Bu); 2.41–2.60 (br. *m*, 12 H, cyclen CH_2); 2.68–3.08 (br. *m*, 14 H, cyclen CH_2, CH_2CO; PhCH_2); 4.02 (*s*, pzCH_2); 4.06 (*t*, *J* = 6.0, 3 MeCH_2O); 4.76 (*q*, 3 CH); 6.38 (br. *s*, H–C(2′)); 7.03–7.19 (br. *m*, 3 Ph); 7.50 (*d*, *J* = 8.0, H–C(10)); 7.77 (*dd*, *J* = 8.0, 2.0, H–C(9)); 7.94 (*d*, *J* = 8.0, H–C(2)); 8.24 (*d*, *J* = 2.5, H–C(7)); 8.52 (*d*, *J* = 2.0, H–C(1′)); 8.92 (*d*, *J* = 8.0, H–C(3)). HR-ESI-MS: 1203.6241 ($[M+H]^+$, $C_{67}H_{83}N_{10}O_{11}^+$; calc. 1203.6243).

*[Tb(***L^6***)]Cl$_3$*. A stirred soln. of **L^6** (0.013 g, 0.011 mmol) and $Tb(OTf)_3 \cdot 6\,H_2O$ (0.007 g, 0.012 mmol) in anh. MeCN (1 ml) was heated under reflux for 10 h. The complex was prepared as described for [Eu(**L^{4a}**)]Cl_3: [Tb(**L^6**)]Cl_3 (0.008 g, 56%). Colourless solid. HPLC (*Method A*): t_R 11.2. λ_{max} (H_2O) 348 nm, τ (H_2O) 2.25 ms; ϕ_{Tb} (H_2O, pH 6.0, λ_{exc} 355 nm) 54%.

*[Eu(***L^6***)]Cl$_3$*. Similarly, [Eu(**L^6**)]Cl_3 was isolated as a colourless solid. HPLC (*Method A*): t_R 11.2.

16. *Eu^{III} Complex of Ligand* **L^{4b}**. tert-*Butyl 7-{{6-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-pyridin-2-yl}methyl}-4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl}-1,4,7,10-tetraazacyclododecane-1-carboxylate* (= tert-*Butyl 7-{{6-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]pyridin-2-yl}methyl}-4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl}-1,4,7,10-tetraazacyclododecane-1-carboxylate*). A stirred mixture of *tert*-butyl 4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl}-1,4,7,10-tetraazacyclododecane-1-carboxylate (**7**; 0.325 g, 0.547 mmol), **2b** (0.254 g, 0.601 mmol), and K_2CO_3 (0.113 g, 0.821 mmol) in anh. MeCN (10 ml) was heated under reflux for

16 h. The mixture was allowed to cool and filtered, the solvent evaporated, and the yellow oil purified by CC (neutral Al_2O_3, $CH_2Cl_2 \rightarrow 0.5\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): orange glassy solid (0.379 g, 74%). R_f 0.74 (Al_2O_3, CH_2Cl_2/MeOH 49:1). M.p. 87–89°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.38 (br. *s*, ^{t}Bu, Boc); 1.46 (*d*, *J* = 4.0, 2 Me); 2.61 (br. *s*, 8 H, cyclen CH_2); 2.82 (br. *s*, 4 H, cyclen CH_2); 3.07 (br. *s*, 4 H, cyclen CH_2); 3.33 (br. *s*, 1 CH_2CO); 3.40 (br. *s*, 1 CH_2CO); 3.61 (br. *s*, pyCH_2); 5.12 (br. *s*, 2 CH); 7.19 (*d*, *J* = 8.0, H–C(5′)); 7.26 (br. *s*, 2 Ph); 7.46 (br. *s*, NH); 7.58 (*d*, *J* = 8.5, H–C(10)); 7.64 (*t*, *J* = 8.0, H–C(4′)); 7.74 (br. *s*, NH); 7.82 (*dd*, *J* = 9.0, 2.5, H–C(9)); 8.29 (*d*, *J* = 2.5, H–C(7)); 8.41 (*d*, *J* = 8.0, H–C(3′)); 8.46 (*d*, *J* = 8.0, H–C(2)); 8.78 (*d*, *J* = 8.0, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 21.3 (Me); 22.0 (Me); 28.7 (Me_3CO); 31.5 (Me_3C); 35.1 (Me_3C); 48.0 (CH); 48.4 (CH); 52.5 (2 cyclen CH_2); 53.2 (2 cyclen CH_2); 53.8 (2 cyclen CH_2); 54.6 (2 cyclen CH_2); 59.4 (pyCH_2); 59.8 (CH_2CO); 60.6 (CH_2CO); 80.2 (Me_3CO); 116.6 (C(4)); 118.3 (C(10)); 121.1 (C(3′)); 121.3 (C(6)); 122.7 (C(7)); 124.8 (C(5′)); 126.5 ($C_{o/m}$); 126.8 ($C_{o/m}$); 127.3 (2 $C_{o/m}$); 127.8 (2 $C_{o/m}$); 128.7 ($C_{o/m}$); 128.9 ($C_{o/m}$); 133.8 (C(9)); 137.6 (C(4′)); 138.5 (C(3)); 143.0 (C_{ipso}); 143.8 (C_{ipso}); 148.2 (C(8)); 153.8 (C(1′)); 154.2 (C(11)); 156.1 (C=O of Boc); 158.0 (C(6′)); 160.1 (C(1)); 160.3 (C(12)); 170.1 (2 amide C=O); 177.8 (C(5)). HR-ESI-MS: 937.5331 ($[M+H]^+$, $C_{55}H_{69}N_8O_6^+$; calc. 937.5340).

*4-{{6-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]pyridin-2-yl}methyl}-*N^1,N^7*-bis[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,7-diacetamide* (= *4-{{6-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]pyridin-2-yl}methyl}-*N^1,N^7*-bis[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,7-diacetamide*; **8**). A soln. of *tert*-butyl 7-{{6-[6-(*tert*-butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]pyridin-2-yl}methyl}-4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl}-1,4,7,10-tetraazacyclododecane-1-carboxylate (0.320 g, 0.341 mmol) in CH_2Cl_2/CF_3COOH 2:1 (6 ml) was stirred at r.t. for 12 h. The solvent was evaporated to yield a glassy solid. The crude material was repeatedly (3×) dissolved in CH_2Cl_2 (5 ml) and the solvent evaporated. The residue was finally taken into 0.02M aq. KOH (10 ml) and extracted with CH_2Cl_2 (3 × 15 ml). The combined org. layer was dried (K_2CO_3) and concentrated: **8** (0.254 g, 89%). Pale yellow solid. M.p. 92–94°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.38 (*s*, ^{t}Bu); 1.47 (*d*, *J* = 7.0, 2 Me); 2.58 (br. *s*, 8 H, cyclen CH_2); 2.64 (br. *s*, 8 H, cyclen CH_2); 3.09 (*q*, *J* = 17.0, 2 CH_2CO); 3.60 (*d*, *J* = 15.0, 1 H, pyCH_2); 3.68 (*d*, *J* = 15.0, 1 H, pyCH_2); 5.14 (*q*, *J* = 7.5, 2 CH); 7.16 (*q*, *J* = 8.0, H–C(5′), 2 H_p); 7.24 (*t*, *J* = 7.5, 4 H_m); 7.30 (*d*, *J* = 7.5, 4 H_o); 7.56 (*d*, *J* = 9.0, H–C(10)); 7.62 (*t*, *J* = 7.5, H–C(4′)); 7.82 (*dd*, *J* = 8.5, 2.0, H–C(9)); 8.06 (*d*, *J* = 8.5, NH); 8.28 (*d*, *J* = 2.5, H–C(7)); 8.37 (*d*, *J* = 7.5, H–C(3′)); 8.44 (*d*, *J* = 8.5, H–C(2)); 8.77 (*d*, *J* = 8.5, H–C(3)). ^{13}C-NMR ($CDCl_3$)[3]): 21.7 (2 Me); 31.5 (Me_3C); 35.0 (Me_3C); 47.4 (2 cyclen CH_2); 48.6 (2 CH); 52.5 (2 cyclen CH_2); 53.4 (2 cyclen CH_2); 53.7 (cyclen CH_2); 54.8 (cyclen CH_2); 58.7 (pyCH_2); 60.2 (2 CH_2CO); 116.6 (C(4)); 118.3 (C(2); C(10)); 121.0 (C(3′)); 121.3 (C(6)); 122.7 (C(7)); 125.0 (C(5′)); 126.7 (4 C_o); 127.5 (2 C_p); 128.8 (4 C_m); 133.8 (C(9)); 137.6 (C(4′)); 138.5 (C(3)); 143.5 (2 C_{ipso}); 148.2 (C(8)); 153.6 (C(1′)); 154.2 (C(11)); 158.0 (C(6′)); 160.2 (C(1)); 160.3 (C(12)); 170.8 (2 amide C=O); 177.8 (C(5)). HR-ESI-MS: 837.4817 ($[M+H]^+$, $C_{50}H_{61}N_8O_4^+$; calc. 837.4816).

*Methyl 4-{(1*S*)-1-{{2-{7-{{6-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]pyridin-2-yl}-methyl}-4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl}-1,4,7,10-tetraazacyclododec-1-yl}acetyl}amino}ethyl}benzoate* (= *Methyl 4-{(1*S*)-1-{{2-{7-{{6-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]pyridin-2-yl}methyl}-4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl}-1,4,7,10-tetraazacyclododec-1-yl}acetyl}amino}ethyl}benzoate*; **L^{4b}**). A mixture of **8** (0.131 g, 0.157 mmol), methyl 4-{(1*S*)-1-[(2-chloroacetyl)amino]ethyl}benzoate ((*S*)-**5b**; 0.050 g, 0.196 mmol), and K_2CO_3 (0.043 g, 0.313 mmol) in anh. MeCN (7 ml) was heated under reflux for 18 h. After cooling and filtering, the solvent was evaporated and the orange oil purified by CC (neutral Al_2O_3, $CH_2Cl_2 \rightarrow 1.0\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): **L^{4b}** (0.105 g, 64%). Orange solid. R_f 0.27 (Al_2O_3, CH_2Cl_2/MeOH 49:1). M.p. 87–89°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.40 (*s*, ^{t}Bu); 1.44 (*d*, *J* = 7.0, 2 Me); 2.71 (br. *s*, 16 H, cyclen CH_2); 3.25 (br. *s*, 3 CH_2CO); 3.75 (*s*, pyCH_2); 3.84 (*s*, COOMe); 5.05 (*q*, 3 CH); 7.16 (*d*, *J* = 8.0, 2 H, H–C(5′)), H_p); 7.28 (*m*, 2 Ph); 7.33 (*d*, *J* = 8.0, 2 H, $H_{o/m}$); 7.60 (*d*, *J* = 8.5, H–C(10)); 7.73 (*t*, *J* = 8.0, H–C(4′)); 7.85 (*dd*, *J* = 8.5, 2.5, H–C(9)); 7.89 (*d*, *J* = 8.0, 2 H, $H_{o/m}$); 8.30 (*d*, *J* = 2.5, H–C(7)); 8.43 (*d*, *J* = 8.0, H–C(2), H–C(3′)); 8.77 (*d*, *J* = 8.0, H–C(3)). ^{13}C-NMR ($CDCl_3$, 125 MHz)[3]): 21.9 (3 Me); 31.5 (Me_3C); 35.0 (Me_3C); 48.9 (3 CH); 51.5 (2 cyclen CH_2); 52.3 (COO*Me*); 52.5 (2 cyclen CH_2); 53.0 (2 cyclen CH_2); 53.7 (2 cyclen CH_2); 58.5 (3 CH_2CO); 60.2 (pyCH_2); 116.6 (C(4)); 118.3 (C(2); C(10)); 121.3 (C(3′)); 121.4 (C(6)); 122.7 (C(7)); 125.4 (2 C, Ph); 126.2 (2 C, Ph); 126.5 (4 C, Ph); 127.5 (C(5′));

128.8 (4 C, Ph); 129.2 (Ph); 130.1 (2 C, Ph); 133.8 (C(9)); 137.8 (C(4′)); 138.6 (C(3)); 143.5 (2 C_{ipso}); 147.7 (1 C_{ipso}); 148.2 (C(8)); 153.6 (C(1′)); 154.2 (C(11)); 159.9 (C(6′)); 160.2 (C(1)); 160.3 (C(12)); 166.9 (*C*OOMe); 170.8 (3 amide C=O); 177.8 (C(5)). HR-ESI-MS: 1056.5703 ($[M+H]^+$, $C_{62}H_{74}N_9O_7^+$; calc. 1056.5711).

*[Eu(***L**^{4b}*)]Cl*$_3$. The complex was prepared as described for [Eu(**L**^{4a})]Cl_3: [Eu(**L**^{4b})]Cl_3 (0.044 g, 64%). Pale yellow solid. HPLC (*Method A*): t_R 10.9. λ_{max} (H_2O) 356 nm; τ (H_2O) 1.02 ms; τ (D_2O) 1.34 ms.

17. *Complex of Ligand* **L**^{5b}. *4-{[1-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl]-*N^1,N^7*-bis[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,7-diacetamide* (= *4-{[1-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]-1*H*-pyrazol-3-yl]methyl}-*N^1,N^7*-bis[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,7-diacetamide*). A stirred mixture of *N*^{1},*N*^{7}-bis[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,7-diacetamide [41] (0.104 g, 0.211 mmol), **3b** (0.087 g, 0.211 mmol), and $NaHCO_3$ (0.020 g, 0.231 mmol) in anh. MeCN (5 ml) was heated at 60° for 18 h. The mixture was cooled and filtered, the solvent evaporated, and the yellow residue purified by CC (neutral Al_2O_3, $CH_2Cl_2 \rightarrow 0.5\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): pale yellow solid (0.124 g, 71%). M.p. 152–154°. ^{1}H-NMR ($CDCl_3$, 500 MHz)[3]): 1.40 (*s*, *t*Bu); 1.47 (*d*, *J* = 7.0, 2 Me); 2.58 (br. *s*, 4 H, cyclen CH_2); 2.79 (br. *s*, 4 H, cyclen CH_2); 2.91 (br. *s*, 8 H, cyclen CH_2); 3.36 (*s*, 2 CH_2CO); 3.71 (*s*, pzCH_2); 5.07 (*q*, *J* = 14.0, 7.5, 2 CH); 6.31 (*d*, *J* = 2.5 H–C(2′)); 7.13 (*t*, *J* = 7.5, 2 H, Ph); 7.21 (*t*, *J* = 7.5, 4 H, Ph); 7.35 (*d*, *J* = 7.5, 4 H, Ph); 7.54 (*d*, *J* = 9.0, H–C(10)); 7.84 (*dd*, *J* = 8.5, 2.5, H–C(9)); 7.91 (br. *s*, 2 NH); 7.98 (*d*, *J* = 8.5, H–C(2)); 8.29 (*d*, *J* = 2.5, H–C(7)); 8.55 (*d*, *J* = 2.5, H–C(1′)); 8.76 (*d*, *J* = 8.5, H–C(3)). HR-ESI-MS: 826.4769 ($[M+H]^+$, $C_{48}H_{60}N_9O_4^+$; calc. 826.4768).

*Methyl 4-{(1*S*)-1-{{2-{7-{{1-[6-(*tert*-Butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl}-4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}methyl}-1,4,7,10-tetraazacyclododec-1-yl}acetyl}-amino}ethyl}benzoate* (= *Methyl 4-{(1*S*)-1-{{2-{7-{{1-[7-(1,1-Dimethylethyl)-5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridin-2-yl]-1*H*-pyrazol-3-yl}methyl}-4,10-bis{2-oxo-2-{[(1*S*)-1-phenylethyl]amino}ethyl}-1,4,7,10-tetraazacyclododec-1-yl}acetyl}amino}ethyl}benzoate*; **L**^{5b}). A mixture of 4-{{1-[6-(*tert*-butyl)-10-oxo-10*H*-9-oxa-1-azaanthracen-2-yl]-1*H*-pyrazol-3-yl}methyl}-*N*^{1},*N*^{7}-bis[(1*S*)-1-phenylethyl]-1,4,7,10-tetraazacyclododecane-1,7-diacetamide (0.060 g, 0.073 mmol), (*S*)-**5b** (0.021 g, 0.084 mmol), and Cs_2CO_3 (0.031 g, 0.095 mmol) in anh. MeCN (5 ml) was heated under reflux for 16 h. The mixture was cooled and filtered, the solvent evaporated, and the yellow residue purified by CC (neutral Al_2O_3, $CH_2Cl_2 \rightarrow 0.5\%$ MeOH/CH_2Cl_2, by 0.1% MeOH increments): **L**^{5b} (0.060 g, 79%). Pale yellow solid. R_f 0.68 (Al_2O_3, CH_2Cl_2/MeOH 49:1). M.p. 187–189°. ^{1}H-NMR ($CDCl_3$, 400 MHz)[3]): 1.41 (br. *s*, *t*Bu, 1 Me); 1.45 (*d*, *J* = 7.0, 2 Me); 2.57 (br., 16 H, cyclen CH_2); 2.86 (br. *s*, 1 CH_2CO); 2.99 (br. *s*, 2 CH_2CO); 3.62–3.84 (br., pzCH_2, CO_2Me); 5.14 (*q*, *J* = 7.0, 3 CH); 6.30 (*d*, *J* = 2.5, H–C(2′)); 6.21–7.32 (br., 12 arom. H); 7.47 (*d*, *J* = 8.0, 2 arom. H); 7.55 (*d*, *J* = 9.0, H–C(10)); 7.82 (*dd*, *J* = 8.0, 2.5, H–C(9)); 7.98 (*d*, *J* = 8.0, H–C(2)); 8.31 (*d*, *J* = 2.5, H–C(7)); 8.78 (*d*, *J* = 8.0, H–C(3)). HR-ESI-MS: 1045.5706 ($[M+H]^+$, $C_{60}H_{73}N_{10}O_7^+$; calc. 1045.5664).

*[Tb(***L**^{5b}*)]Cl*$_3$. The complex was prepared as described for [Tb(**L**^{5a})]Cl_3: [Tb(**L**^{5b})]Cl_3 (0.037 g, 55%). Pale yellow solid. HPLC (*Method A*): t_R 10.9. λ_{max} (H_2O) 349 nm; τ (H_2O) 2.27 ms; ϕ_{Tb} (H_2O, pH 6.0, λ_{exc} 355 nm) 63%.

18. *Conjugate Formation. Ester Hydrolysis.* A soln. of [Tb(**L**^{5b})]Cl_3 (1.3 μmol) in MeOH/0.02M aq. KOH 1:1 (2 ml) was stirred at 20° and the progress of hydrolysis monitored by reversed-phase HPLC (*Chromolith* performance *RP18e* column (100 × 4.6 mm), *Method C*): t_R (ester) 6.07, t_R (acid) 5.78. The reaction ran to completion in *ca.* 2 h, and the mixture was neutralised with a dil. aq. HCl soln. The solvent was evaporated and the crude residue used directly in the next step without further purification. ESI-MS: 594 ($[M-H]^{2+}$), 1187 ($[M-2H]^+$).

*BG-[Tb(***L**^{5}*)]Cl*$_3$. To a stirred soln. of the above acid in anh. DMF (2 ml) was added TSTU and ^{i}Pr_2NEt (50 μl of a 100 mM soln. in DMF in each case; 5 μmol). The progress of the reaction was monitored by reversed-phase HPLC (*Method C*): t_R (acid) 6.07, t_R (NHS = succinimide) 6.28. BG–H was added to the stirred soln. of the active ester (1 μmol) derived from [Tb(**L**^{5b})]Cl_3 in anh. DMF (400 μl). The mixture was stirred and monitored by reversed-phase HPLC (*Method C*): t_R (succinimido ester) 6.28, t_R (BG–[Tb(**L**^{5b})]Cl_3) 5.97. The reaction ran to completion in *ca.* 15 min. The product was purified by

reversed-phase semi-prep. HPLC (*Method E*; t_R (conjugate) 5.23): BG–[Tb(**L**^{5b})]Cl_3 (380 nmol, 38%). Colourless solid. ESI-MS: 721 ($[M-H]^{2+}$), 1440 ($[M-2H]^{+}$).

*BG–[Eu(**L**^{4})]Cl_3*. This conjugate was prepared similarly to BG–[Tb(**L**^{5b})]Cl_3. HPLC (*Method E*); BG–Eu(**L**^{4})]Cl_3 at t_R 6.67. HR-ESI-MS: 697.7724 ($[M-H]^{2+}$, $C_{68}H_{78}{}^{151}EuN_{17}O_7^{2+}$; calc. 697.7729.

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