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# Selective signalling of sialic acid in solution by circularly polarised luminescence spectroscopy using a dynamically racemic europium(III) complex

Emily R. Neil and David Parker\*

A europium complex bearing a phenylboronic acid group has been created that binds reversibly in methanol to sialic acid and lactic acid, as signalled by changes in the Eu total emission spectrum and the induction of strong circularly polarised luminescence. An analogue lacking the boronate moiety displays no spectral response towards sialic acid. The CPL signature is distinctive for sialic acid, and differs from that observed with methyl sialate and *N*-acetyl glucosamine. A hypothetical binding model is promulgated, where binding of the amide carbonyl to the Eu centre occurs at the same time as the terminal diol group of the glycerol moiety chelates to the boron atom.

## Introduction

Sialic acid (Neu-5-Ac) is an important biomolecule belonging to the family of 9-carbon amino sugars (neuraminic acids). It is present in serum as a mixture of  $\alpha$ - (5–8%) and  $\beta$ -pyranose (92–95%) forms; the absence of the furanose form can be attributed to the acetamide group at the C-5 position, (Fig. 1). Sialic acid can also be found occupying the terminal positions of carbohydrate chains of glycoproteins and glycolipids of biological membranes. It is over-expressed on the surface of tumour cells<sup>1,2</sup> and the variation in serum levels may serve as a useful biological marker, indicating the existence of a cancerous state.<sup>3</sup> Typical concentrations in healthy adults of free sialic acid in serum lie in the range 140–200  $\mu\text{M}$ .<sup>4</sup>

Several lanthanide probes have been reported that target sialic acid and signal the detection using MRI<sup>5–7</sup> and emission based techniques.<sup>8,9</sup> Of particular importance is the use of a boronic acid moiety in the putative selective recognition mechanism of its diol functionality. The reversible formation of five- and six-membered cyclic boronate esters often results

from the interaction with 1,2- and 1,3-diols, and has been extensively exploited in the design of various saccharide sensors.<sup>10</sup> The incorporation of a phenylboronic acid (pba) moiety into the ligand framework of a lanthanide complex has also demonstrated the utility of this moiety, in the putative dynamic covalent binding to the 1,2-diol group of sialic acid.<sup>5,6</sup>

The complexes  $[\text{Eu}\cdot\text{L}^1]^+$  and  $[\text{Eu}\cdot\text{L}^2]^+$  have been shown to exhibit high quantum yield values, anion binding capability and strong induced CPL following the addition of a chiral analyte.<sup>11,12</sup> Recently, renewed interest has been emerging in the use of CPL to allow the creation of chiral probes,<sup>13</sup> and to permit CPL imaging and microscopy.<sup>14</sup>

Here, a heptadentate Eu(III) complex based on the bis-carboxylate ligand structure containing a phenylboronic acid moiety,  $[\text{Eu}\cdot\text{L}^3]^+$ , (Fig. 2) has been designed to target sialic acid selectively. Exploiting the inherent chirality of the molecule, binding at europium was hypothesised to result in a change in emission spectral form and generation of an induced circularly polarised luminescence signal. The response of the complex is compared to the bis-carboxylate complex,  $[\text{Eu}\cdot\text{L}^1]^+$ , and the *N*-alkylated derivative,  $[\text{Eu}\cdot\text{L}^2]^+$ , which serve as controls lacking the boronate moiety.

## Synthesis of $[\text{Eu}\cdot\text{L}^3]^+$

A phenylboronic acid moiety was appended to the desired ligand framework,  $\text{L}^1$ , via a ring *N*-alkylation reaction. Initial protection of the boronic acid group as a cyclic ester was used to assist subsequent purification. The simple cyclic ester, based on ethylene glycol, was selected as the protecting group. Although rather sensitive to hydrolysis, the boronic ester was considered to be sufficiently stable towards purification using column chromatography, under carefully

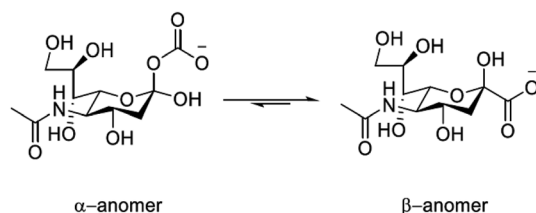


Fig. 1 Structure of sialic acid (Neu-5-Ac) showing the  $\alpha$ - and  $\beta$ -pyranose forms;  $\text{p}K_{\text{a}} = 2.6$  ( $\text{H}_2\text{O}$ ,  $I = 0.1$ , 298 K).

Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, UK.  
E-mail: david.parker@dur.ac.uk



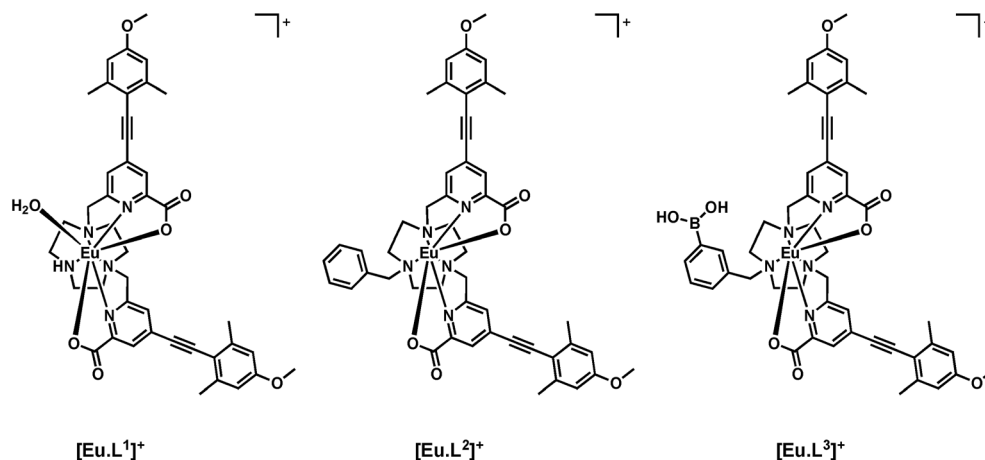


Fig. 2 Structures of the Eu(III) complexes of  $L^{1-3}$ .

controlled conditions. Subsequent removal of the ester group can be achieved using mild base hydrolysis. The use of more stable hindered cyclic esters, such as a pinacol derivative was avoided, as its conversion back to the boronic acid is particularly difficult and is not compatible with the presence of other groups in the ligand framework, such as the acid-sensitive alkyne.

Reaction of commercially available 3-(bromomethyl)phenylboronic acid with ethylene glycol in anhydrous pentane over 4 Å molecular sieves, yielded the protected phenylboronate in good yield, (Scheme 1). Subsequent alkylation with  $L^1$  gave the protected ligand,  $L^3$ , which was purified using flash column chromatography on silica gel. The presence of the boronic ester moiety was confirmed by a single resonance at 31.3 ppm in the  $^{11}\text{B}$  NMR spectrum.

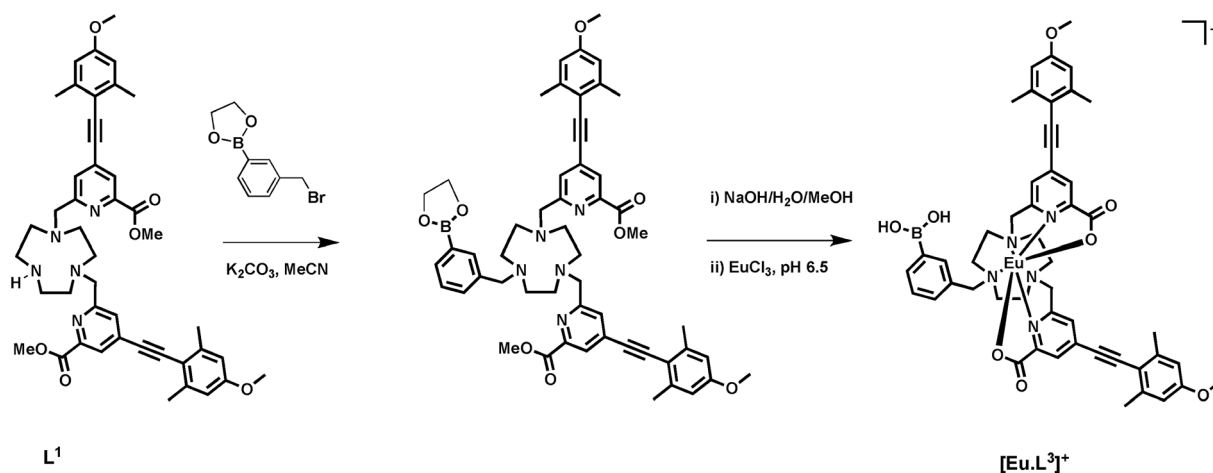
Base hydrolysis of both the methyl ester and the cyclic boronic ester groups was carried out using NaOH (aq.) in a MeOH–H<sub>2</sub>O mixture (1 : 1, v/v) at pH 12. After the completion of reaction was confirmed by LC-MS, the pH of the solution was lowered to 6.5 and  $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$  was added. Formation of the complex was confirmed by ESI-MS, in 18 h.

### A chiral probe for sialic acid

The photophysical properties of the boronic acid derivative  $[\text{Eu} \cdot L^3]^+$  were assessed in comparison to the *N*-benzyl complex  $[\text{Eu} \cdot L^2]^+$ . The capability of the complex to bind anions was tested using lactate in a model study, and the induced CPL behaviour was correlated to the configuration of the complex, using methods recently described.<sup>11–14</sup> The binding affinity of sialic acid was estimated and the emission spectral changes and induced CPL signature were compared to  $[\text{Eu} \cdot L^1]^+$  and  $[\text{Eu} \cdot L^2]^+$ . Investigation into the mode of binding of sialic acid was attempted, by comparison of the response with that observed following addition of the methyl-ester derivative of Neu-5-Ac.

### Photophysical properties of $[\text{Eu} \cdot L^3]^+$

The key photophysical parameters for  $[\text{Eu} \cdot L^3]^+$  were determined and compared to those of the related complex,  $[\text{Eu} \cdot L^2]^+$  (Table 1). The hydration state in water of the title complex,  $[\text{Eu} \cdot L^3]^+$ , was estimated to be  $q = 0.2$ .<sup>15</sup> The absence of an inner sphere metal-bound water molecule is consistent with the reported behaviour of the *N*-benzyl complex,  $[\text{Eu} \cdot L^2]^+$ .<sup>11,12</sup> This observation provides



Scheme 1 Synthesis of the Eu(III) complex of  $L^3$  containing a phenylboronic acid moiety.

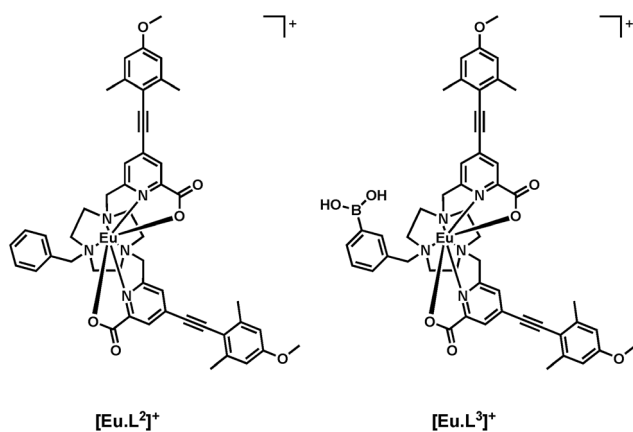


**Table 1** Photophysical properties of the Eu(III) complexes of  $L^{2,3}$  (295 K, MeOH, selected emission decay rate constants given in  $H_2O$  and  $D_2O$ ). Values of  $\phi$  are ( $\pm 15\%$ ); errors for  $k$  are ( $\pm 10\%$ )

	$[Eu \cdot L^2]^+$	$[Eu \cdot L^3]^+$
$\lambda/nm$	348	356
$\epsilon/mM^{-1} cm^{-1}$	36.0	35.0
$\phi$	0.18	0.05
$k/ms^{-1}$	1.20	2.04
$k(H_2O)/ms^{-1}$	2.00	2.63
$k(D_2O)/ms^{-1}$	1.89	2.17
$q$	0	0.2

further evidence that the steric bulk of the *N*-substituent inhibits coordination of a water molecule to the lanthanide centre.

The europium emission quantum yield of the boronic acid complex,  $[Eu \cdot L^3]^+$ , was measured to be 5%, which is significantly lower than that of the *N*-benzyl complex,  $[Eu \cdot L^2]^+$  (Table 1). Secondly, the radiative rate of decay of  $Eu^{3+}$  emission in methanol was nearly twice as fast for  $[Eu \cdot L^3]^+$  compared to  $[Eu \cdot L^2]^+$ . Such behaviour suggests that the europium(III) excited state lifetime for  $[Eu \cdot L^3]^+$  is being quenched, in a manner that is not possible for  $[Eu \cdot L^2]^+$ .



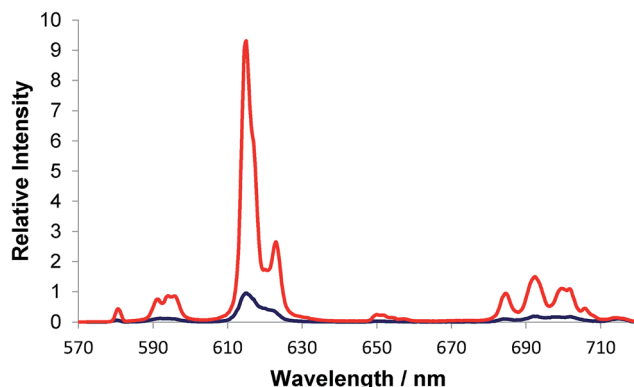
A likely explanation for the increase of the measured excited state decay rate (Table 1, where the measured value of  $k$  is given by  $k_{obs} = k_0 + k_{nr}$ , in which  $k_0$  is the radiative rate constant for the Eu excited state and  $\sum k_{nr}$  represent competing non-radiative processes) is that the presence of the boronic acid group simply enhances the rate of non-radiative decay in the monomer. Alternatively, it may be related to the ability of  $[Eu \cdot L^3]^+$  to undergo intermolecular association, e.g. dimer formation as shown in the X-ray crystal structure of phenylboronic acid,<sup>16</sup> where hydrogen bonding occurs between boronic acid groups. To test this hypothesis further, a dilution study was carried out in MeOH- $H_2O$  (1 : 1, v/v) at pH 7.4. The luminescent lifetime of the excited state was monitored as the concentration was successively lowered from 5  $\mu M$  to 0.63  $\mu M$ , at which point the sample was too dilute to obtain reliable results. No significant change in the luminescent lifetime was observed, suggesting either that the intermolecular interactions are relatively strong and not disrupted over this concentration range, or that the monomer or dimer undergo additional non-

radiative deactivation processes, in the latter case, for example, *via* intra- or intermolecular charge transfer.

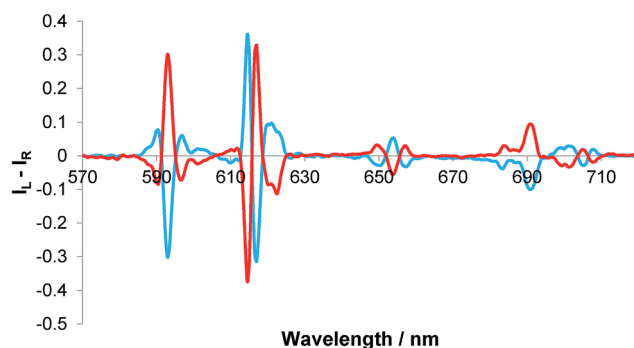
### Preliminary investigations into interactions with anions

The form and relative intensity of the electric-dipole allowed transitions in the europium emission spectrum is particularly sensitive to the metal coordination environment.<sup>13,14</sup> The addition of lactate to a series of structurally related Eu(III) complexes ( $[Eu \cdot L^{1,2}]$ ) was seen to cause significant changes in the total emission spectra, consistent with anion binding at the metal centre. With this behaviour in mind, the total emission spectral response of  $[Eu \cdot L^3]^+$  was monitored following addition of *S*-lactate (Fig. 3). A ten-fold enhancement in the total luminescence intensity was observed, following addition of 4000-fold excess of *S*-lactate. The increase in emission intensity suggested that anion binding to the metal centre results in disruption of the quenching mechanism taking place. Lactate can bind to the boronate through the carboxylate and hydroxyl group to form a 5-membered chelate,<sup>17</sup> as well as to the lanthanide centre, consistent with the idea that the strong intermolecular association that may be occurring in the monomer, is not possible when lactate binds.

The addition of *R*- and *S*-lactate to the europium complex,  $[Eu \cdot L^3]^+$ , resulted in an induced CPL response, identical in



**Fig. 3** Total emission spectra of  $[Eu \cdot L^3]^+$  (blue) and following addition of 2 mM *S*-lactate (red) (5  $\mu M$  complex,  $\lambda_{exc} = 356$  nm, MeOH, 295 K).



**Fig. 4** Induced CPL spectra of  $[Eu \cdot L^3]^+$  following the addition of 2 mM *R*- (red) and *S*-lactate (blue);  $g_{em}(593 \text{ nm}) = \pm 0.05$ ,  $g_{em}(654 \text{ nm}) = \pm 0.07$  (5  $\mu M$  complex,  $\lambda_{exc} = 356$  nm, MeOH, 295 K).



Table 2 Binding constants,  $\log K$ , for complexation of lactate and sialic acid (295 K)

Complex	<i>R</i> -Lactate <sup>a</sup>	Sialic acid <sup>b</sup>
[Eu·L <sup>1+</sup> ]	4.01(04)	5.24
[Eu·L <sup>2+</sup> ]	3.15(04)	n.r.
[Eu·L <sup>3+</sup> ]	3.20(06)	>5.3

<sup>a</sup> In 50/50 aqueous methanol. <sup>b</sup> In pure methanol; n.r. = no spectral response observed; the response with [Eu·L<sup>3+</sup>] only gave a limit, as a complex spectral response was observed.

nature to that of [Eu·L<sup>2+</sup>], (Fig. 4).<sup>11</sup> The sign and form of each transition was the same and the measured emission dissymmetry values,  $g_{em}$ , were comparable. The binding affinity was similar to that observed earlier with [Eu·L<sup>2+</sup>], (Table 2)<sup>11</sup> Empirical analysis of the  $\Delta J = 4$  transition allowed assignment of absolute configuration of the complex,<sup>11,12</sup> as addition of *R*-lactate has been shown to result in  $\Delta$  helicity while *S*-lactate gives  $\Lambda$  helicity in the ternary adduct.

The emission and CPL spectral response of [Eu·L<sup>3+</sup>] following addition of lactate, demonstrates that binding at the metal centre can occur, in principle, in a similar manner to that observed for [Eu·L<sup>1,2</sup>].

### Investigations into the binding of sialic acid

The interaction of sialic acid with the parent bis-carboxylate europium(III) complex, [Eu·L<sup>1+</sup>] was investigated first. An emission titration was carried out in methanol and spectral changes were monitored as a function of added concentration of sialic acid. A binding affinity in methanol of  $\log K = 5.24$  was estimated, (Table 2) which demonstrates that there is a significant interaction between the complex and the Neu-5-Ac anion in methanol, (Fig. 5). No significant interaction was observed in pure water at ambient pH; presumably the hydration energies of the complex and the sialic acid are much higher under these conditions.

The addition of sialic acid to [Eu·L<sup>1+</sup>] also induced significant CPL. It was interesting to note that the form of the CPL spectrum, particularly in the  $\Delta J = 1$  and  $\Delta J = 2$  manifolds, was remarkably different to that following addition of simple carboxylates such as *R*-phenylpropionate, and also  $\alpha$ -hydroxy acids, such as lactate and mandelate<sup>11</sup> (Fig. 6). The 'signature' CPL response may allow simple differentiation between the detection of sialic acid and other chiral carboxylate anions in a competitive environment. It is notable, that the  $\Delta J = 4$  region again resembles that of the *R*-lactate adduct of [Eu·L<sup>1+</sup>], allowing assignment of the complex adduct configuration as  $\Delta$ -[Eu·L<sup>1</sup>·Neu-5-Ac].

This proof of concept study demonstrated the capability of lanthanide complexes based on a heptadentate bis-carboxylate ligand, to signal the binding of sialic acid. Further development of this probe examined the behaviour of the complex, [Eu·L<sup>3+</sup>] with incorporation of a phenylboronic acid moiety into the ligand framework.

Phenylboronic acid is a weak acid ( $pK_a = 8.72$ ) that is able to bind covalently and reversibly to 1,2-diols,<sup>18</sup> such as the glycerol side-chain of sialic acid, and to  $\alpha$ -hydroxy acids, in each case forming 5-ring chelate structures.<sup>17</sup> The stability of esters formed by the tetragonal boronate anion is an order of magnitude greater than for the trigonal boronic acid.<sup>19</sup> Therefore, enhanced affinity is observed under basic conditions. It was hoped that the addition of this secondary stabilising interaction would enhance the affinity of the complex for sialic acid, and permit detection in polar media.

An emission titration of [Eu·L<sup>3+</sup>] and sialic acid in methanol was carried out to allow comparison with [Eu·L<sup>1+</sup>]. Plotting the change in  $\Delta J = 2$  and  $\Delta J = 1$  intensities *versus* concentration of anion generated a curve that did not fit to a 1 : 1 binding model, (Fig. 7). The plot revealed that another process perturbed the spectral signal variation process occurs before 1 : 1 binding between the anion and the lanthanide ion. Such behaviour indirectly supports the suggestion that there is an intermolecular association in solution between the boronate groups in two

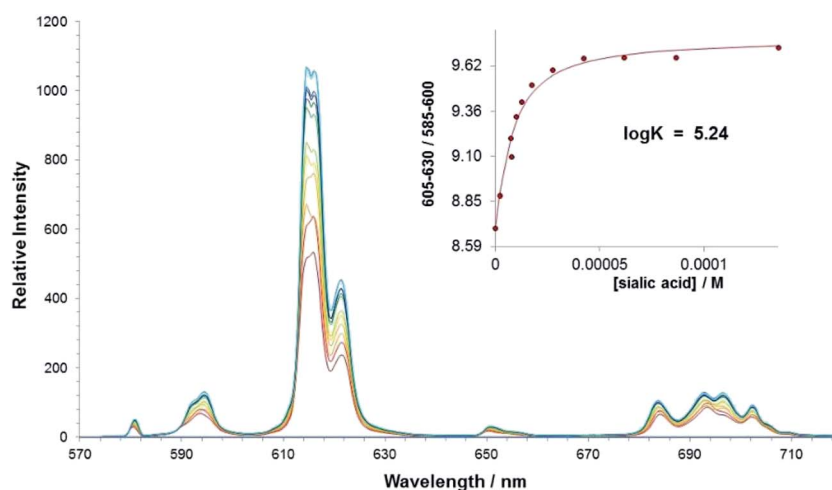


Fig. 5 Variation of the europium(III) emission profile of [Eu·L<sup>1+</sup>] as a function of added sialic acid. (5  $\mu$ M,  $\lambda_{exc} = 352$  nm, MeOH). Inset shows fit to experimental data, following iterative least squares fitting to a 1 : 1 binding model.



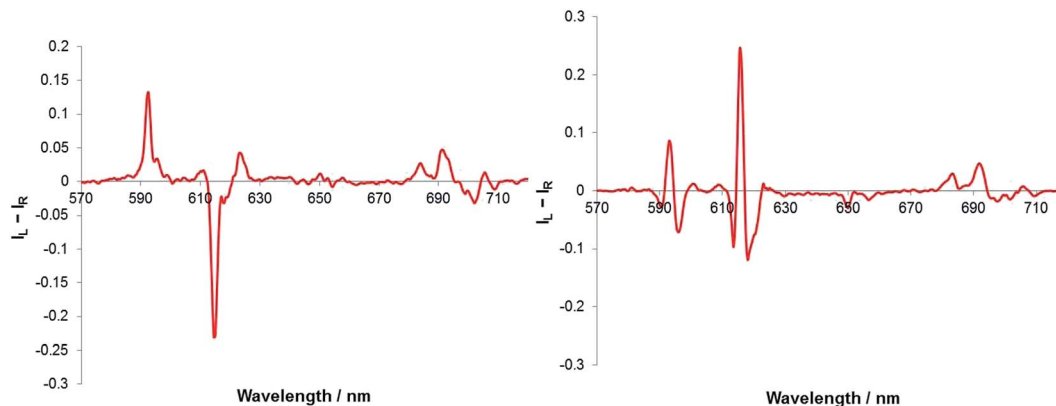


Fig. 6 CPL spectra of  $[\text{Eu}\cdot\text{L}^1]^+$  following addition of *R*-lactate (left) and sialic acid (right). ( $5\ \mu\text{M}$  complex,  $50\ \mu\text{M}$  anion,  $\lambda_{\text{exc}} = 352\ \text{nm}$ , MeOH).

or three molecules (*e.g.* hydrogen bonded dimer or trimer formation has been noted before for such boronates<sup>10c</sup>). Alternatively, the interaction may occur between the boronate group acting as a ligand to the Eu centre in a second Eu complex. In each case these interactions are disrupted following addition of an anion. It is worth noting that significant changes in the emission spectrum were observed with  $[\text{Eu}\cdot\text{L}^3]^+$  at a 3-fold lower concentration of sialic acid, compared to the response observed for  $[\text{Eu}\cdot\text{L}^1]^+$ . Such behaviour suggests that the addition of the phenylboronic acid moiety, significantly increases the affinity of the europium(III) complex for sialic acid. The complexity of the binding curve obtained (Fig. 7) precluded quantitative assessment of binding affinity. It is particularly interesting to note that there was no direct spectroscopic evidence of sialic acid binding to the control *N*-benzyl complex,  $[\text{Eu}\cdot\text{L}^2]^+$ , indicating the need for the stabilising interaction provided by the boronic acid moiety.

The europium(III) total emission studies showed that there is a well-defined interaction between  $[\text{Eu}\cdot\text{L}^1]^+$ , and sialic acid, (Fig. 5 above). However, no evidence for binding was observed for the *N*-benzyl complex,  $[\text{Eu}\cdot\text{L}^2]^+$ . Such behaviour is most likely to occur as a result of the increased steric demand around

the metal centre created by the *N*-benzyl group. Interestingly, the addition of a boronic acid group to the aromatic ring of the *N*-benzyl complex,  $[\text{Eu}\cdot\text{L}^3]^+$ , provided an additional stabilising interaction that evidently allowed sialic acid to bind to the europium(III) complex, notwithstanding the steric hindrance imposed by the benzyl substituent.

The sign of the induced CPL in the  $\Delta J = 1$  manifold was comparable to that for  $[\text{Eu}\cdot\text{L}^1]^+$ , following addition of sialic acid. However, very little optical activity was observed in the  $\Delta J = 3$  and  $\Delta J = 4$  transitions, (Fig. 8). The difference in the CPL spectral form, suggests that a different coordination environment is present in these complexes. The emission dissymmetry values,  $g_{\text{em}}$ , were larger for  $[\text{Eu}\cdot\text{L}^3\cdot\text{Neu-5-Ac}]^+$  ( $g_{\text{em}}(593\ \text{nm}) = +0.03$ , *c.f.*  $+0.015$  for  $[\text{Eu}\cdot\text{L}^1\cdot\text{Neu-5-Ac}]$ ), suggesting that enhanced binding affinity and a greater degree of conformational rigidity is present when sialic acid binds to the metal centre in the boronate complex,  $[\text{Eu}\cdot\text{L}^3]^+$ . Such behaviour may be due to an additional interaction with the boronic acid group in the adduct. The CPL spectra for sialic acid and lactic acid were also very different in overall form, suggesting that the europium was not binding in the same manner.

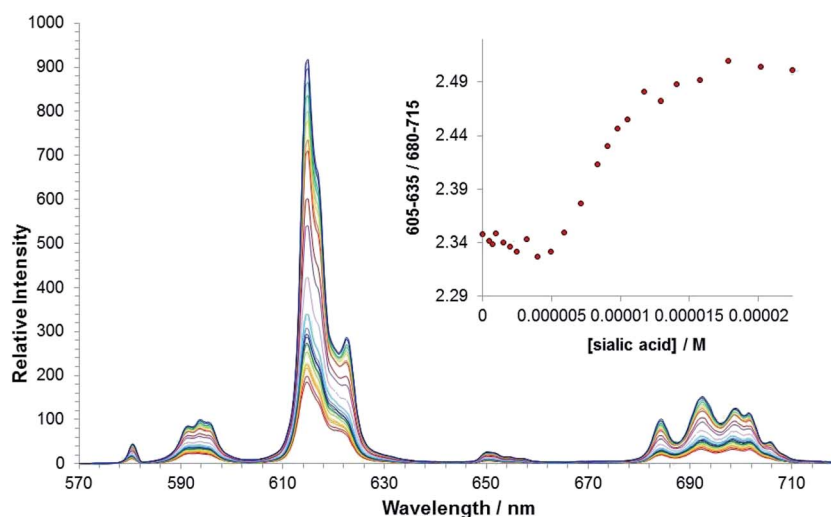


Fig. 7 Variation of the europium(III) emission profile of  $[\text{Eu}\cdot\text{L}^3]^+$  as a function of added sialic acid. ( $5\ \mu\text{M}$ ,  $\lambda_{\text{exc}} = 356\ \text{nm}$ , MeOH).



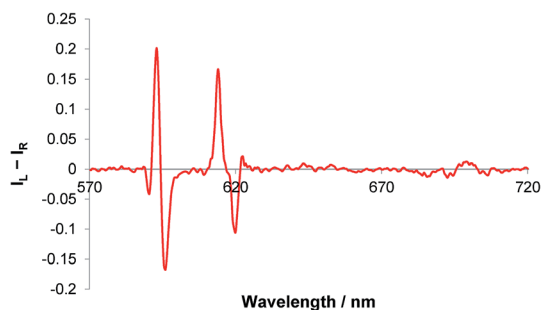


Fig. 8 CPL spectrum of  $[\text{Eu} \cdot \text{L}^3]^+$  following addition of sialic acid ( $5 \mu\text{M}$  complex,  $50 \mu\text{M}$  anion,  $\lambda_{\text{exc}} = 356 \text{ nm}$ , MeOH).

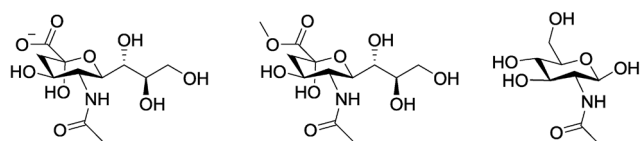


Fig. 9 Structures of sialic acid (left), the corresponding methyl ester (centre) and *N*-acetyl-*D*-glucosamine (right).

No evidence for binding was observed in pure water in the pH range 4–10 for  $[\text{Eu} \cdot \text{L}^3]^+$ . This behaviour could be related to the fact that the conformation adopted by sialic acid in solution has been reported to be solvent dependent.  $^1\text{H}$  NMR spectra of sialic acid in MeOD and  $\text{D}_2\text{O}$  were analysed at a pD of 5.5. However, no significant differences were observed in the spectra in each solvent, suggesting a minimal difference in the conformation of sialic acid.

It is likely that the difference in binding affinity of sialic acid in the two solvent systems is a direct result of the difference in

solvation energy. There are five hydroxyl groups present in the molecule, so hydration of the anion in water is likely to be very high and may explain the low affinity it has for the positively charged lanthanide complex in aqueous solution. Systematic variation of the solvent composition and the effect on the emission spectral output was monitored. It was found that significant change in form and intensity was observed when 2 mM sialic acid was added to 1 mL 4 : 1 MeOH :  $\text{H}_2\text{O}$  sample of  $[\text{Eu} \cdot \text{L}^3]^+$ . However, increasing the water mole fraction any higher, resulted in no evidence for anion binding to the metal centre. The poor aqueous solubility of the complex is a key factor in the decreased affinity for sialic acid in such aqueous systems.

### Investigating the mode of binding of sialic acid

The mode of binding of sialic acid to the europium(III) complexes,  $[\text{Eu} \cdot \text{L}^{1,3}]^+$ , was investigated further by a comparative study of induced CPL following addition of sialic acid, its methyl ester and the related molecule *N*-acetyl-*D*-glucosamine, (Fig. 9).

The form and sign of the CPL spectra of  $[\text{Eu} \cdot \text{L}^1]^+$  following addition of sialic acid and its methyl ester derivative was similar, strongly suggesting that the negatively charged carboxylate group of Neu-5-Ac is not involved in the primary binding interaction at the metal centre (Fig. 10A vs. B). Therefore, an alternative mechanism for the recognition of sialic acid must be considered, based on binding of the amide carbonyl oxygen to the lanthanide centre. Such a binding mode was hypothesised recently in the work of Ouchi, regarding sialic acid binding to a  $[\text{Ln} \cdot (\text{ABNOTA})]$  complex (Fig. 11).<sup>9</sup> However, addition of *N*-acetyl-*D*-glucosamine, a monosaccharide containing the same acetamide moiety but lacking the glycerol moiety and a carboxylate group, resulted in

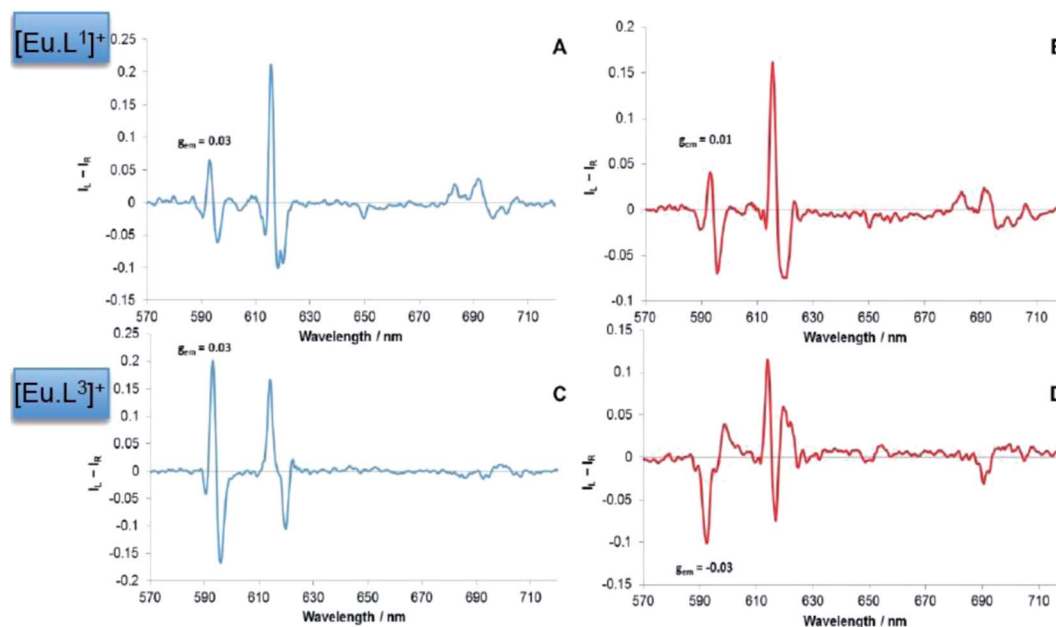


Fig. 10 Induced CPL spectra for  $[\text{Eu} \cdot \text{L}^1]^+$  following the addition of (A) 2 mM sialic acid; (B) 2 mM sialic acid methyl ester (MeOH,  $\lambda_{\text{exc}} = 352 \text{ nm}$ , 295 K). Induced CPL spectra for  $[\text{Eu} \cdot \text{L}^3]^+$  following the addition of (C) 2 mM sialic acid; (D) 2 mM sialic acid methyl ester (MeOH,  $\lambda_{\text{exc}} = 356 \text{ nm}$ , 295 K). The  $g_{\text{em}}$  values are given for the same  $\Delta J = 1$  transition at 593 nm.



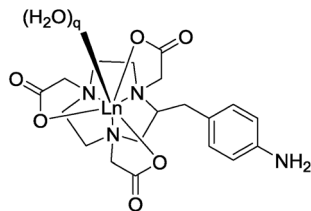


Fig. 11 Partial structure of a  $[\text{Ln}\cdot\text{ABNOTA}]$  complex;  $q = 2$  or  $3$ .<sup>9</sup>

no change to the total emission spectrum. Neither was any induced CPL observed, indicating that other groups in the Neu-5-Ac molecule must be cooperatively involved in the binding interaction.

In contrast to the behaviour of  $[\text{Eu}\cdot\text{L}^1]^+$ , the induced CPL spectra of  $[\text{Eu}\cdot\text{L}^3]^+$  were rather different, following addition of sialic acid *versus* its methyl ester (Fig. 10C *vs.* D). Subtle differences in the form of the total emission spectra, particularly in the  $\Delta J = 1$  and  $\Delta J = 4$  transitions, were also observed. Such behaviour suggests that the carboxylate group may yet be involved in some manner, even if just *via* some hydrogen bonding interaction.

In work on a Gd-based MRI contrast agent for sialic acid detection, lanthanide complexes based on diethylenetriamine pentaacetic acid (DTPA) ligands incorporating a phenylboronic acid moiety have also been used, *e.g.*  $\text{L}^4$  and  $\text{L}^5$  (Fig. 12).<sup>5</sup> The authors suggested that the interaction between the phenylboronic acid group and sialic acid occurred either with the glycerine moiety at C-6, or with the carboxylate and hydroxyl group at C-2, to form a five-membered cyclic ester. They did not consider any role for the amide carbonyl group in binding to the  $\text{Gd}^{3+}$  centre.

## Summary

This study has presented the first example of induced CPL to signal sialic acid binding. Evidence gathered from the induced CPL response of the phenylboronate–europium(III) complex

$[\text{Eu}\cdot\text{L}^3]^+$ , allows us to hypothesise that there is a synergistic interaction involved in the binding mechanism of the europium complex and sialic acid. It is possible that this interaction involves the 2,3-hydroxyl group of the glycerol moiety, forming a cyclic ester with the boronic acid, together with an additional stabilising interaction, involving the amide carbonyl oxygen. There is indirect support for this hypothesis, based on crystal structure data for the sialic acid trisaccharide derivative, Neu-5-Ac-Gal-Glc, when bound in the active site of the serum protein complement factor H. The structure reveals a potentially favourable orientation for metal binding, involving the amide carbonyl oxygen and, in that case, the first glycerol hydroxyl group (Fig. 13).<sup>20</sup>

With the methyl ester of sialic acid, the carbonyl oxygen atom is a much weaker lone pair donor and cannot reasonably be expected to engage in such a binding interaction. Therefore, the coordination geometry around the metal centre will be different, as observed by the emission behaviour. Indeed, the induced CPL response is distinctively different in form and sign, comparing the two chiral analytes.

Taken together, these results allow the development of tentative hypotheses for the binding mode of the sialic acid group to  $[\text{Eu}\cdot\text{L}^1]^+$  and  $[\text{Eu}\cdot\text{L}^3]^+$ . Accordingly, DFT modelling studies were undertaken to assess the feasibility of putative cooperative binding models, using  $[\text{Eu}\cdot\text{L}^3]^+$ . The binding to boron of the terminal diol group of the glycerol moiety, allowed the cooperative ligation of the amide carbonyl oxygen to europium, (Fig. 14). Alternative structures that were explored included one involving chelation of the alpha-hydroxyl carboxylate to boron. However, in that case no simultaneous binding to europium was geometrically feasible, and the earlier CPL work had anyway suggested very different coordination environments at Eu, in the complexation with sialic acid *vs.* lactic acid (*vide supra*).

## Conclusions and future work

The utility of dynamically racemic lanthanide probes in the sensing of important chiral biomolecules, employing CPL

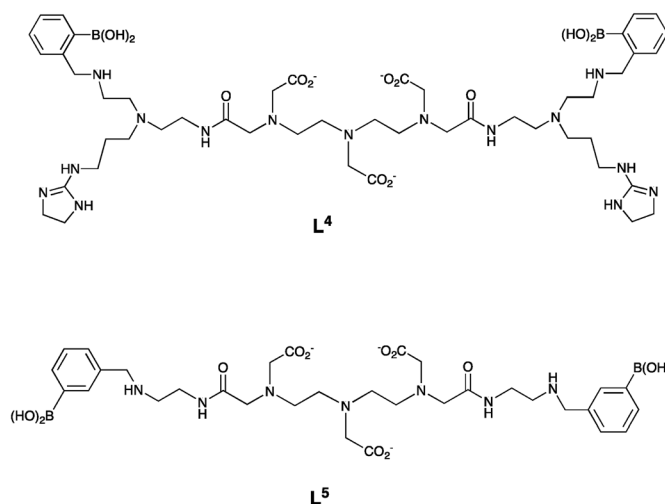


Fig. 12 DTPA-bis amide (DTPA – diethylenetriamine pentaacetic acid) ligands,  $\text{L}^4$  and  $\text{L}^5$  used in Gd-based MRI contrast agents for sialic acid detection.<sup>5</sup>



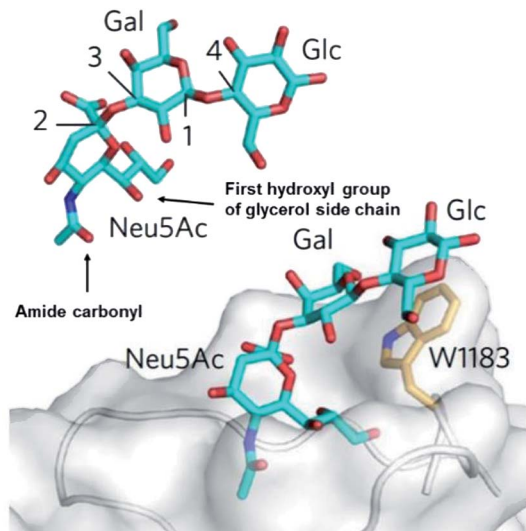


Fig. 13 The sialic acid derivative binding site of the serum protein complement factor H, showing the orientation of the amide carbonyl oxygen and the first glycerol hydroxyl group to allow a cooperative binding interaction.<sup>21</sup>

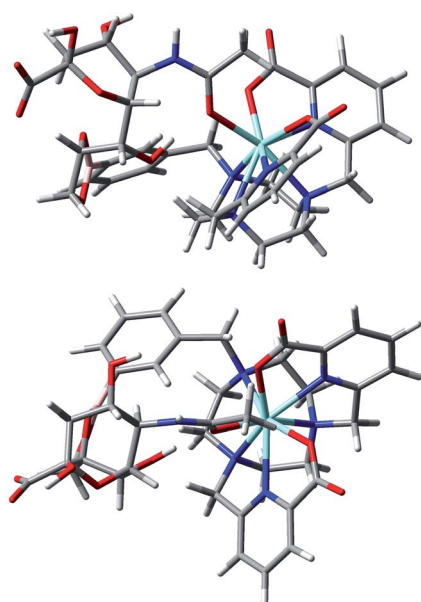


Fig. 14 Views of the hypothetical binding of the glycerol diol group to boron and cooperative ligation of the amide carbonyl oxygen to the metal ion in an optimised model geometry for the complex  $[\text{Eu} \cdot \text{L}^3 \cdot \text{sialic acid}]$ .<sup>11,12</sup>

spectroscopy as the detection technique, is a research area of growing interest. Here, the sensitivity and selectivity of this type of chirality probe has been demonstrated.

The reversible binding of sialic acid by the bis-carboxylate complex,  $[\text{Eu} \cdot \text{L}^1]^+$ , and the boronic acid complex  $[\text{Eu} \cdot \text{L}^3]^+$  was signalled *via* the induction of a 'fingerprint' CPL response accompanying changes in the total emission spectrum. This result demonstrates the first example of the use of CPL spectroscopy in conjunction with a lanthanide chirality probe to

detect sialic acid. Other workers have examined the response of selected carbohydrates to europium, but at far higher concentrations and without any real rationale for binding selectivity.<sup>22</sup> Further work investigated the sign and form of the CPL spectra to elucidate key structural information regarding the ternary adduct, by comparison with the methyl-ester derivative of sialic acid. A proposed binding mode was hypothesised, highlighting the role of the glycerol side chain with the boronic acid group.

It may also be possible to increase the binding affinity, and thus selectivity, of  $[\text{Eu} \cdot \text{L}^3]^+$  for sialic acid through the inclusion of an aminomethyl group in the *ortho*-position of the benzyl substituent. In this way, Wulff has shown that conversion of the trigonal boronic acid to the tetrahedral boronate occurs at lower pH, in the presence of this additional amino group, where the stability of the resulting ester is an order of magnitude greater.<sup>10</sup> The presence of the tetragonal species over a greater pH range can be attributed to the B–N interaction in the phenylboronate and may aid the selective recognition of sialic acid in aqueous solution at physiological pH levels.

## Experimental

Details of general experimental methods, instrumentation and purifications methods have been given elsewhere.<sup>11,12</sup> The synthesis of  $\text{L}^1$  and  $\text{L}^2$  and their europium (iii) complexes was reported in ref. 11 and 12. The methods of acquiring spectra, spectral titrations and the analysis of spectral data to estimate binding affinities are given in ref. 11.

### HPLC

Reverse-phase preparative HPLC was performed at 295 K using a Shimadzu system consisting of a Degassing Unit (DGU-20A5R), a Prominence Preparative Liquid Chromatograph (LC-20AP), a Prominence UV/Vis Detector (SPD-20A) and a Communications Bus Module (CBM-20A). An XBridge C18 OBD 19 × 100 mm, i.d. 5 μm column was used with a flow rate of 2 mL min<sup>-1</sup> (analytical) or 17 mL min<sup>-1</sup> (prep). The solvent system was H<sub>2</sub>O + 0.1% formic acid/MeOH + 0.1% formic acid (gradient elution, Table 3).

### DFT modelling and geometry optimisation

Several starting model geometries of  $[\text{Eu} \cdot \text{L}^3 \cdot \text{sialic acid}]$  with Eu replaced by Y and  $\text{C} \equiv \text{CC}_6\text{H}_3\text{Me}_2(\text{OMe})$  groups replaced by hydrogen atoms were optimised at B3LYP/3-21G\* to examine the structural feasibility of both metal-binding and boronic

Table 3 HPLC conditions for analysis

Step	Time/min	Flow (analytical/prep)/ mL min <sup>-1</sup>	% H <sub>2</sub> O (0.1% FA)	% MeOH (0.1% FA)
0	0.0	2.0/17.0	90.0	10.0
1	10.0	2.0/17.0	5.0	95.0
2	13.0	2.0/17.0	5.0	95.0
3	13.5	2.0/17.0	90.0	10.0
4	16.5	2.0/17.0	90.0	10.0

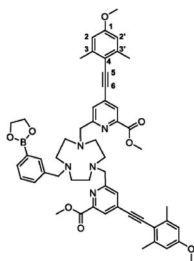


cycle formation, present in  $[\text{Eu} \cdot \text{L}^3 \cdot \text{sialic acid}]$ . The optimised geometry in Fig. 14 was confirmed as a true minimum by a frequency calculation. More details of the DFT modelling methodology used are given in ref. 11 and 12.

### 3-(Bromomethyl)phenylethylene boronate CAS: 1225388-28-2

3-(Bromomethyl)phenylboronic acid (100 mg, 0.465 mmol) was dissolved in anhydrous pentane (2 mL) over 4 Å molecular sieves. Ethylene glycol (30 µL, 0.465 mmol) was added and the white suspension was allowed to stir at room temperature for 1 h under an atmosphere of argon. Upon completion,  $\text{CH}_2\text{Cl}_2$  (15 mL) was added to the reaction mixture and the solution was decanted from the molecular sieves. The solvent was removed under reduced pressure to give 3-(bromomethyl)phenylethylene boronate as a colourless oil which gave spectral data in accord with the literature, and was used directly without further purification (80 mg, 72%). TLC analysis  $R_f$  0.28 (silica, 5%  $\text{CH}_3\text{OH}$  in  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H NMR}$  (295 K, 400 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  7.85 (1H, s,  $\text{H}_2$ ), 7.75 (1H, dt,  $^3J$  7.5,  $^4J$  1.5,  $\text{H}_6$ ), 7.51 (1H, dt,  $^3J$  7.5,  $^4J$  1.5,  $\text{H}_4$ ), 7.37 (1H, t,  $^3J$  7.5,  $\text{H}_5$ ), 4.50 (s, 2H,  $\text{CH}_2\text{Br}$ ), 4.37 (s, 4H,  $\text{BO}(\text{CH}_2)_2\text{O}$ );  $^{13}\text{C NMR}$  (295 K, 175 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  137.4 (Ar-C), 135.5 (Ar-C), 134.9 (Ar-C), 132.2 (Ar-C), 128.5 (Ar-C), 66.2 ( $\text{BO}(\text{CH}_2)_2\text{O}$ ), 33.5 ( $\text{CH}_2\text{Br}$ );  $^{11}\text{B NMR}$  (295 K, 128 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{B}}$  31.41;  $m/z$  GC-EI  $t_{\text{R}} = 4.68$  min, 240.0 ( $\text{M}^+$ ), 161.1 ( $\text{M}^+ - \text{Br}$ ).

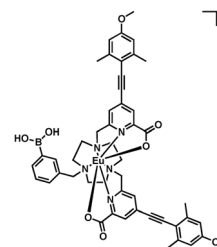
### 3-((4,7-Bis((4-((4-methoxy-2,6-dimethylphenyl)ethynyl)-6-(methoxycarbonyl)pyridin-2-yl)methyl)-1,4,7-triazonan-1-yl)methyl)phenyl)boronic acid, $\text{L}^3$



The bis-alkylated ligand,  $\text{L}^1$  (15 mg, 0.020 mmol) and  $\text{K}_2\text{CO}_3$  (3 mg, 0.020 mmol) were dissolved in anhydrous  $\text{CH}_3\text{CN}$  (2 mL) and bubbled with argon (20 minutes). 3-(Bromomethyl)phenylethylene boronate (5 mg, 0.020 mmol) was added and the mixture was stirred under argon at 55 °C and monitored by LC-MS. After 24 h the reaction was cooled and filtered to remove excess potassium salts. The solvent was removed under reduced pressure and the crude material was purified by flash column chromatography (silica, 0–5%  $\text{CH}_3\text{OH}$  in  $\text{CH}_2\text{Cl}_2$ ) to give  $\text{L}^3$  as a glassy solid (20 mg, 85%).  $^1\text{H NMR}$  (295 K, 600 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  8.02 (2H, s, py- $\text{H}_3$ ), 7.78–7.72 (3H, m, Ph-H), 7.53 (2H, s, py- $\text{H}_5$ ), 7.39–7.34 (1H, m, Ph-H), 6.62 (4H, s,  $\text{H}_{2,2'}$ ), 4.31 (2H, s, Ph- $\text{CH}_2$ ), 4.02 (4H, s, py- $\text{CH}_2$ ), 3.93 (6H, s,  $\text{CO}_2\text{CH}_3$ ), 3.80 (6H, s,  $\text{OCH}_3$ ), 3.53–2.95 (12H, br m, ring Hs), 2.46 (12H, s,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (295 K, 150 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  165.2 ( $\text{CO}_2\text{CH}_3$ ), 160.3 (C1), 157.6 (py- $\text{C}_6$ ), 148.1 ( $\text{C}_4$ ), 143.1 (Ph-C), 136.6 (Ph-C), 135.7 (Ph-C), 134.6 ( $\text{C}_{3/3'}$ ), 133.86 (Ph-C), 127.8 (py- $\text{C}_5$ ), 126.0 (py- $\text{C}_3$ ), 113.7 (py- $\text{C}_4$ ), 112.8

( $\text{C}_{2/2'}$ ), 94.8 ( $\text{C}_5$ ), 92.9 ( $\text{C}_6$ ), 66.2 (Ph- $\text{CH}_2$ ), 61.0 (py- $\text{CH}_2$ ), 55.3 ( $\text{OCH}_3$ ), 53.6 (ring Cs), 53.1 ( $\text{CO}_2\text{CH}_3$ ), 51.8 (ring Cs), 46.3 (ring Cs), 21.5 ( $\text{CH}_3$ );  $^{11}\text{B NMR}$  (295 K, 128 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{B}}$  31.3;  $m/z$  (HRMS+) 877.4350 [ $\text{M} + \text{H}$ ] $^+$  ( $\text{C}_{51}\text{H}_{57}^{10}\text{BN}_5\text{O}_8$  requires 877.4337);  $t_{\text{R}} = 8.8$  min.

### $[\text{Eu} \cdot \text{L}^3]\text{Cl}$



An aqueous solution of sodium hydroxide 0.1 M (0.5 mL) was added to a solution ligand  $\text{L}^3$  (5 mg, 6 µmol) in methanol (0.5 mL). The mixture was stirred at 65 °C for 4 h. The reaction was monitored by LC-MS. Upon completion, aqueous hydrochloric acid (0.1 M) was added until pH 6.5 was achieved. Europium chloride hexahydrate (3 mg, 8 µmol) was added and the pH was readjusted to 6.5 by addition of aqueous sodium hydroxide (0.1 M). The reaction was stirred at 65 °C for 24 h. The solvent was removed under reduced pressure to give the europium complex as a white solid (7 mg, 80%).  $m/z$  ESI ( $\text{NH}_4\text{HCO}_3/\text{MeCN}$ ) 998.2887 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{47}\text{H}_{49}^{11}\text{BN}_5\text{O}_8^{155}\text{EuNa}$  requires 998.2818;  $t_{\text{R}} = 6.2$  min;  $\lambda_{\text{exc}}$  (MeOH) = 356 nm;  $\phi_{\text{em}}$  (MeOH) 0.05,  $\epsilon$  (MeOH) 35 000  $\text{M}^{-1} \text{cm}^{-1}$ ,  $\tau$  ( $\text{H}_2\text{O}$ ) = 0.38 ms,  $\tau$  ( $\text{D}_2\text{O}$ ) = 0.48 ms,  $q = 0.2$ .

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## References

- G. Yogeewaran, B. S. Stein and H. Sebastian, *Cancer Res.*, 1978, **38**, 1336–1344.
- J. Roth, C. Zuber, P. Wagner, D. J. Taatjes, C. Weisgerber, P. U. Heitz, C. Goridis and D. Bitter-Suermann, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**, 2999–3003.
- F. J. Krolkowski, K. Reuter, T. P. Waalkes, S. M. Sieber and R. H. Adamson, *Pharmacology*, 1976, **14**, 47–51.
- E. Gruszewska, B. Cylwik, A. Panasiuk, M. Szmikowski, R. Flisiak and L. Chrostek, *BioMed Res. Int.*, 2014, **2014**, 876096.
- L. Frullano, J. Rohovec, S. Aime, T. Maschmeyer, M. I. Prata, J. J. P. de Lima, C. F. G. C. Geraldes and J. A. Peters, *Chem.–Eur. J.*, 2004, **10**, 5205–5217.
- G. A. Lemieux, K. J. Yarema, C. L. Jacobs and C. R. Bertozzi, *J. Am. Chem. Soc.*, 1999, **121**, 4278–4279.



- 7 S. Geninatti Crich, D. Alberti, I. Szabo, S. Aime and K. Djanashvili, *Angew. Chem., Int. Ed.*, 2013, **52**, 1161–1164.
- 8 M. Regueiro-Figueroa, K. Djanashvili, D. Esteban-Gómez, T. Chauvin, É. Tóth, A. de Blas, T. Rodríguez-Blas and C. Platas-Iglesias, *Inorg. Chem.*, 2010, **49**, 4212–4223.
- 9 K. Ouchi, S. Saito and M. Shibukawa, *Inorg. Chem.*, 2013, **52**, 6239–6241.
- 10 (a) T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1910–1922; (b) G. Wulff, *Pure Appl. Chem.*, 1982, **54**, 2093–2102; (c) R. Nishiyabu, Y. Kubo, T. D. James and J. S. Fossey, *Chem. Commun.*, 2011, **47**, 1124.
- 11 E. R. Neil, M. A. Fox, R. Pal, L.-O. Palsson, B. A. O'Sullivan and D. Parker, *Dalton Trans.*, 2015, **44**, 14937–14951.
- 12 E. R. Neil, M. A. Fox, R. Pal and D. Parker, *Dalton Trans.*, 2016, **45**, 8355.
- 13 For reviews addressing the scope of CPL probes: (a) F. Zinna and L. Di Bari, *Chirality*, 2015, **27**, 1; (b) J. P. Riehl and F. S. Richardson, *Chem. Rev.*, 1986, **86**, 1; (c) R. Carr, N. H. Evans and D. Parker, *Chem. Soc. Rev.*, 2012, **41**, 7673; (d) G. Muller, *Dalton Trans.*, 2009, 9692; (e) T. Wu, X.-Z. You and P. Bour, *Coord. Chem. Rev.*, 2015, **284**, 1.
- 14 A. T. Frawley, R. Pal and D. Parker, *Chem. Commun.*, 2016, **52**, 13349.
- 15 A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans. 2*, 1999, 493.
- 16 K.-H. Chen, J.-S. Yang, C.-Y. Hwang and J.-M. Fang, *Org. Lett.*, 2008, **10**, 4401–4404.
- 17 O. Alptürk, O. Rusin, S. O. Fakayode, W. Wang, J. O. Escobedo, I. M. Warner, W. E. Crowe, V. Král, J. M. Pruet and R. M. Strongin, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 9756–9760.
- 18 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769–774.
- 19 S. Friedman, B. Pace and R. Pizer, *J. Am. Chem. Soc.*, 1974, **96**, 5381–5384.
- 20 B. S. Blaum, J. P. Hannan, A. P. Herbert, D. Kavanagh, D. Uhrin and T. Stehle, *Nat. Chem. Biol.*, 2015, **11**, 77–82.
- 21 R. S. Alexander, Z. F. Kanyo, L. E. Chirlian and D. W. Christianson, *J. Am. Chem. Soc.*, 1990, **112**, 933–937.
- 22 T. Wu, J. Průša, J. Kessler, M. Dračinský, J. Valenta and P. Bouř, *Anal. Chem.*, 2016, **88**, 8878.

