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# Using remote substituents to control solution structure and anion binding in lanthanide complexes

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**Abstract:** A study of the anion-binding properties of three structurally related lanthanide complexes which all contain chemically identical anion-binding motifs has revealed dramatic differences in their anion affinity. These arise as a consequence of changes in the substitution pattern on the periphery of the molecule, at a substantial distance from the binding pocket. In this paper, we explore these remote substituent effects, and explain the observed behaviour through discussion of the way in which remote substituents can influence and control the global structure of a molecule through their demands upon conformational space. Peripheral modifications to a binuclear lanthanide motif derived from α,α'-bis(DO3A)-*m*-xylene are shown to result in dramatic changes to the binding constant for isophthalate. In this system, the parent compound displays considerable conformational flexibility, yet can be assumed to bind to isophthalate through a well-defined conformer. Addition of steric bulk remote from the binding site restricts conformational mobility, giving rise to an increase in binding constant on entropic grounds as long as the ideal binding conformation is not excluded from the available range of conformers.

**Keywords:** anion binding • luminescence • remote effects • lanthanides • conformational space

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## Introduction

In this paper, we show that by controlling the available conformational space through using remote substituents, it is possible to influence the affinity of lanthanide containing hosts for anionic guests. Such ideas of remote influence on behaviour already have some currency in other fields. For instance, Reetz has shown that directed evolution can be mediated through changes in protein sequence far from an active site,[1] while Clayden has observed stereochemical induction transmitted over considerable distances in large organic molecules.[2]

Here, we use simple lanthanide containing building blocks to prepare a series of related multinuclear lanthanide complexes, all of which feature the same lanthanide-containing isophthalate binding motif, to explore the role of remote substituent effects in coordination chemistry. We find that the association constant can be influenced by remote substituents to a degree where an increase of two orders of magnitude is observed.

Lanthanide ions have been widely used in optical applications and magnetic resonance imaging for a generation.[3] Gadolinium complexes have been, and are, widely used as contrast agents in clinical magnetic resonance imaging, exploiting the high paramagnetism and rapid water exchange associated with gadolinium centres. In a biological context, the long-lived emission from *f-f* transitions from lanthanide ions such as $Eu^{3+}$ and $Tb^{3+}$ can

be exploited in luminescence imaging and assay, offering very low limits of detection when combined with time-gating methods that separate the lanthanide centred emission from the short lived autofluorescence and scattering arising from biological samples [4][5].

Application of lanthanides *in vivo* requires the use of kinetically stable lanthanide complexes, since lanthanide aqua ions are toxic.[6] Their large size and high ionic character means that kinetically stable lanthanide complexes must be derived from rigid ligands, typically azamacrocycles, bearing hard donor atoms such as oxygen and nitrogen. In such systems, the arrangement of donor atoms around the lanthanide is defined in large part by the structure of the ligand. For instance, the lanthanide complexes of DOTA and its derivatives (e.g. the DOTA tetraamides) have been widely studied owing to the importance of $[Gd.DOTA]^-$ (DOTAREM®) as an MRI contrast medium.[3d] These complexes exhibit exceptional kinetic robustness owing to the rigidity of the cyclen backbone from which four donor acetate groups are appended.

DOTA and DOTTA are octadentate ligands, and exhibit isomerism when binding to lanthanides: Chart 1 shows how both square antiprismatic (SAP) and twisted square antiprismatic (TSAP) geometries can be adopted.[7] Both types of complex are robust and can interconvert through arm rotation, which is relatively facile but slow on the NMR timescale, or ring inversion, which is slower still. For $[Eu.DOTA]^-$ at 298 K, the rates for arm rotation and ring inversion are 78 $s^{-1}$ and 35 $s^{-1}$ respectively.[8] The heptadentate ligand DO3A displays similar behaviour,[9] forming stable complexes in which exchange between SAP and TSAP isomers is much more facile, with fast exchange on the NMR timescale as a consequence of arm rotation, though the stability of DO3A analogues can vary with the nature of the appended donor groups.[10]

The robustness of these complexes means that they are stable to a wide variety of organic reactions/transformations; hence they serve as a versatile building block for a diverse range of syntheses.[11] We and others have used derivatives of DOTA and DO3A complexes with lanthanides to access more complicated architectures such as heterometallic lanthanide complexes, *d-f* hybrids and multi-modal imaging agents.[12] Such architectures can be achieved only where complexes are kinetically stable—otherwise lanthanide exchange results in intractable mixtures of products under thermodynamic control.

There are many circumstances in which thermodynamic control can be exploited to advantage.[13] In supramolecular self-assembly and dynamic combinatorial chemistry, reversible interactions between species have been widely exploited in the construction of very large molecular assemblies. In the field of lanthanide chemistry, the assembly of lanthanide helicates is probably the classic example, and the formation of triple helicate assemblies has been explored extensively.[14] We have taken a different approach, and shown that kinetically robust binuclear lanthanide complexes can form stable self-assemblies with 1,3 dicarboxylate dianions, even in phosphate buffered saline solutions.[15] While helicates dissociate and associate reversibly, leaving the potential for kinetic trapping of released lanthanide, the use of robust hepta-dentate binding pockets ensures that the lanthanide remains complexed throughout an experiment. These kinetically stable complexes can be considered as a molecular entity, rather than a typical metal complex under thermodynamic control.

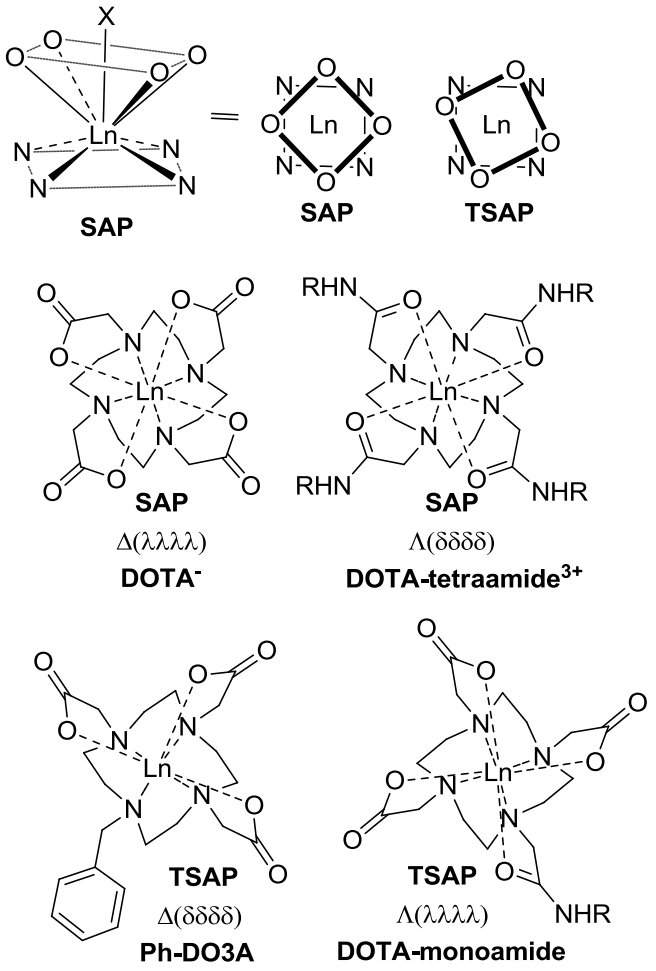
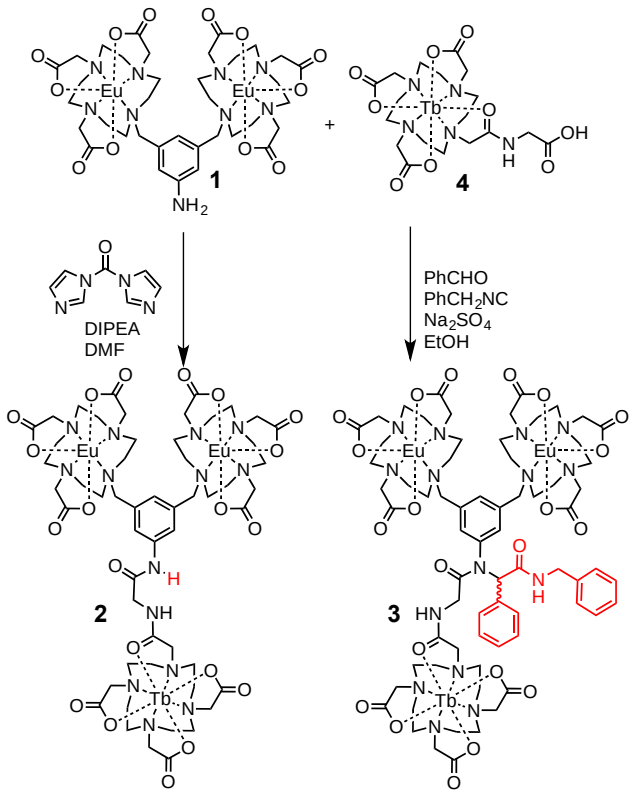



Chart 1. 3-dimensional representation of the coordination sphere of lanthanides in DOTA ligands in solution, X is the ninth, capping, ligand position. Uncapped structures of hepta- and octa-dentate lanthanide complex of cyclen-based ligands with carboxylate arms. The isomers of DOTA are illustrated.

## Results

Scheme 1 shows how the lanthanide complex **1,** α,α'-bis(Eu.DO3A)-*m*-xylene (*m*-bis(europium 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate-10-methylene)-benzene), can be derivatized to prepare the related structures **2** and **3**, which are accessed by peptide and Ugi coupling methodologies respectively. The versatility of particularly the multicomponent Ugi reaction gives rise to considerable potential for derivatisation.[16] All three of these molecules contain the same *m*-xylyl bridged binuclear europium complex.[17] In the case of **1**, this fragment has already been shown to bind to isophthalate in aqueous buffers and methanol/water solvent mixtures.[15a, 15b] The approach used to achieve the synthesis of these compounds relies upon the kinetic stability of the complexes **1** and **4**, which has already been amply demonstrated through their use in preparing lanthanide arrays.[11b, 18] It should also be noted that **2** and **3** contain a terbium ion, bound in a stable domain in the lower part of the structure as drawn. We envisaged that this different metal centre would provide a reporter group that is not itself involved in anion binding—as the octa-dentate binding pocket in which the terbium is bound leaves insufficient space for coordination of an anion (Further information, spectroscopic and synthetic details can be found in the SI).




Scheme 1 Synthesis of complexes **2** and **3** by peptide and Ugi coupling.

Having prepared the complexes, we studied their isophthalate binding properties in methanolic solution. A $10^{-5}$ or $10^{-6}$ M solution of the lanthanide complex in methanol was made. This stock was used to prepare a $10^{-3}$ M solution of isophthalic acid. By doing so, the concentration of the lanthanide complex remains constant during the titration. 2 ml of the stock was placed in a cuvette, and the luminescence spectrum recorded. An aliquot, ranging from 10 µL to 100 µL of the titrant, was added and the luminescence spectrum recorded anew. The titration was continued until the binding isotherm reached a plateau. A representative binding isotherm is shown in figure 1, arising from a titration of isophthalic acid to complex **2**. The isotherms from thetitrations of isophthalate with complexes **1**, **2** and **3** can be found as SI, the determined association constants ($K_a$) are compiled in table 1. For titrations with isophthalate, equimolar concentrations of isophthalic acid and lithium hydroxide were dissolved in the titrant solution. The association constant of isophthalic acid was found to be 39,400 $M^{-1}$, several orders of magnitude weaker than the binding of isophthalate (see table 1).

Figure 1 shows the changes in the emission spectra of complexes **2** and **3** upon addition of isophthalate. In these complexes emission is observed from both the terbium center ($^5D_4$-$^7F_n$) and the europium centers ($^5D_0$-$^7F_n$). Both complexes clearly respond to isophthalate, but the observed properties of the two complexes are very different. In **2**, there are dramatic changes to the intensities of both terbium and europium emission bands. However, in **3** the changes observed to the terbium centered emission are very small, while the europium emission changes dramatically with increasing isophthalate concentration.

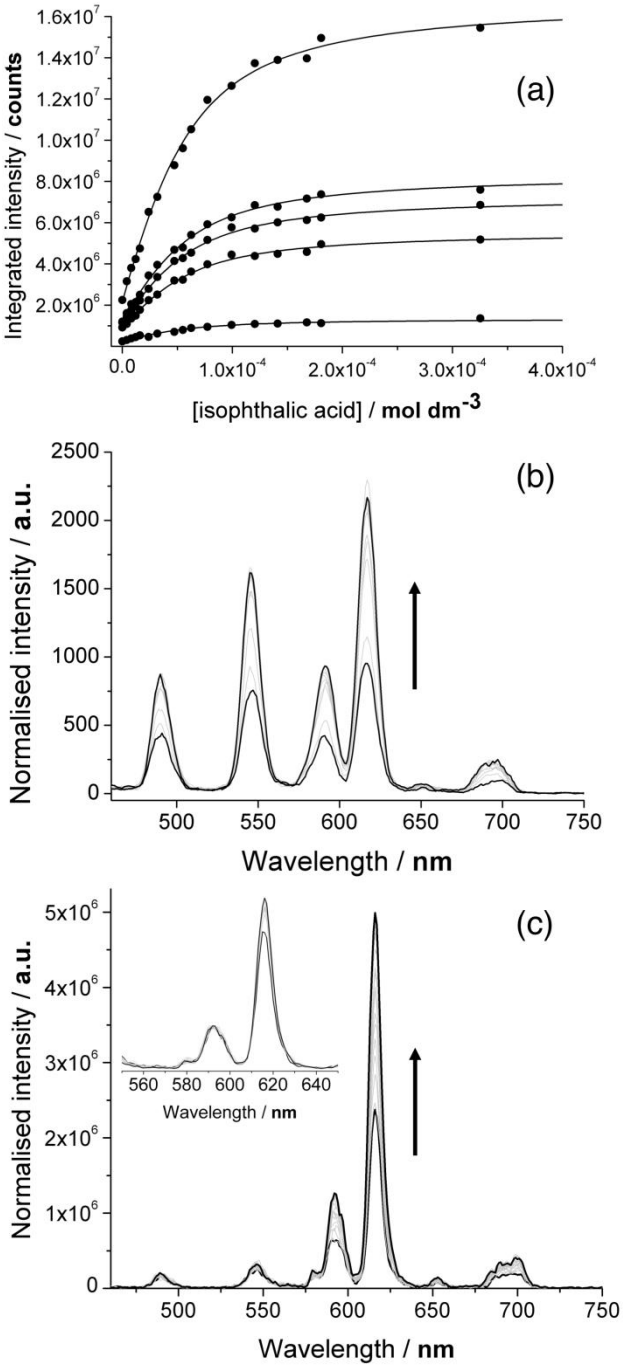
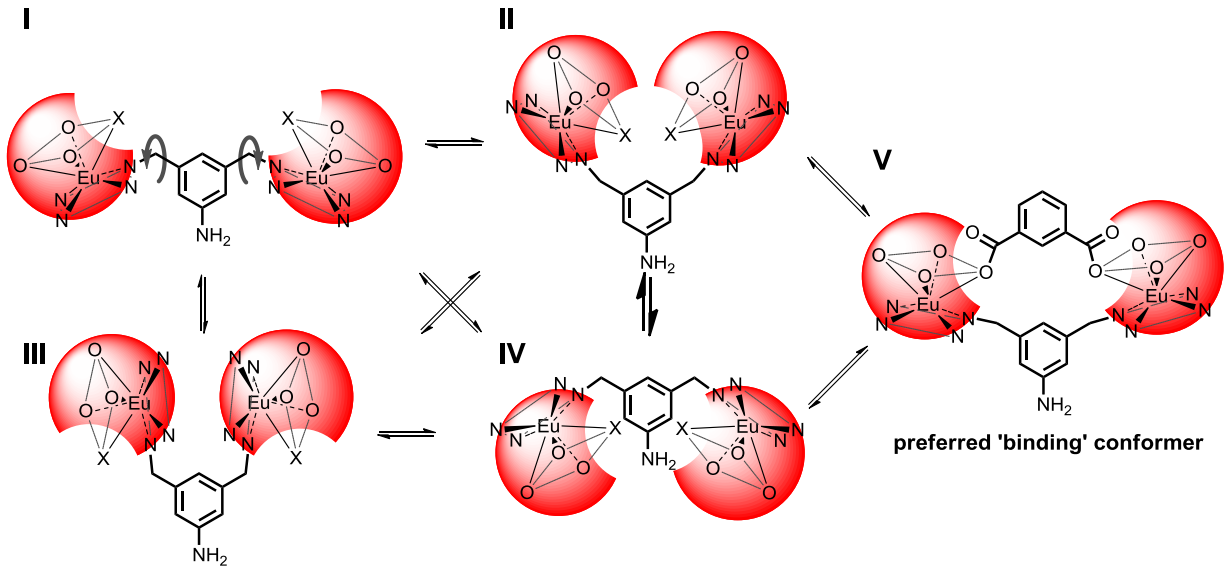



Figure 1.(a) Titration of a 3 ×$10^{-6}$ mol $dm^{-3}$ solution of **2** with isophthalic acid in methanol. The curves correspond to each of the well resolved bands with excitation at 275 nm. (b) Titration of a 3 × $10^{-6}$ mol $dm^{-3}$ solution of **2** with isophthalate in methanol exciting at 275 nm. (c) Titration of a 5 × $10^{-5}$ mol $dm^{-3}$ solution of **3** isophthalate in methanol exciting at 275 nm. The inset shows the **Δ**J =1 and the **Δ**J =2 peaks with intensity normalised to the **Δ**J =1 peak.




**Scheme 2**. Illustrating the wide variety of conformations available to *m*-xylyl bridged bis-DO3A compounds, and showing that only a small range of conformers can bind effectively to an isophthalate guest.

The binding constants were obtained by fitting the observed luminescence data using Dynafit®.[19] The association constants for binding of isophthalate by each of the systems **1**-**3** are shown in Table 1. Further discussion of the fitting methodology and the Dynafit software used can be found in the SI and reference 33. From the values shown in Table 1, it is clear that the affinity for isophthalate in methanol changes by two orders of magnitude between **1** and **3**.

Table 1. Association constants ($K_a$) of complexes **1**-**3** with isophthalate in methanol, the concentration of the complex is $10^{-5}$ M. See SI for details regarding the determination of $K_a$. The asymmetric 99%-confidence intervals is given in brackets.

| Complex | $K_a$ (isophthalate)/$M^{-1}$ |
|---|---|
| **1** | $1.18 \cdot 10^5$ [$1.13 \cdot 10^5$-$1.24 \cdot 10^5$] |
| **2** | $2.0 \cdot 10^6$ [$1.0 \cdot 10^6$ - $5.5 \cdot 10^6$] |
| **3** | $1.0 \cdot 10^7$ [$9.7 \cdot 10^6$ - $1.1 \cdot 10^7$] |

## Discussion

We have synthesised two complex lanthanide containing architectures by coupling of DO3A-monoamide building blocks, demonstrating the versatility and stability of kinetically stable lanthanide complexes. Three of the lanthanide complexes **1**-**3** contain an identical binuclear binding pocket, which can bind isophthalate strongly. We found association constants ranging over two orders of magnitude from $1 \cdot 10^5$ $M^{-1}$ for **1** over $2 \cdot 10^6$ $M^{-1}$ for **2** to $1 \cdot 10^7$ $M^{-1}$ for **3** by using a robust luminescence approach to determine binding isotherms.

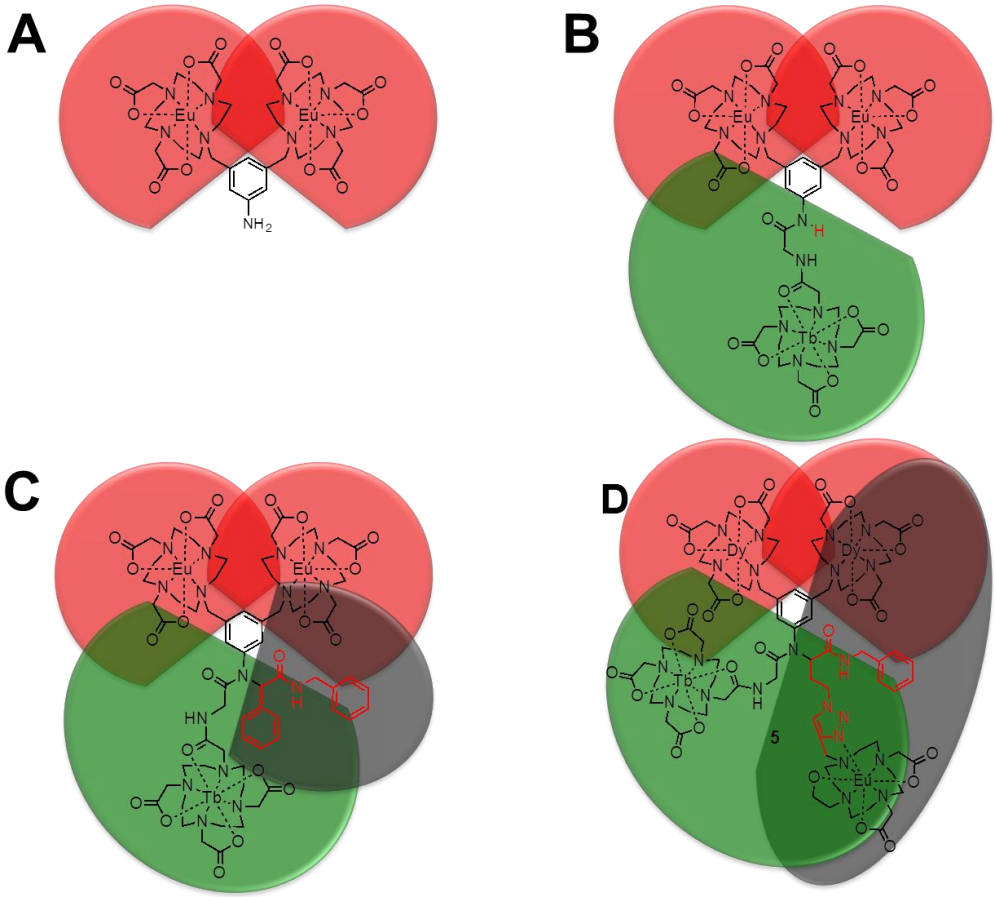
It is necessary first of all to rationalise the observed trends in binding for the three complexes studied. To do so, we must begin by considering the nature of the isophthalate binding domain, which is identical for complexes **1**-**3.** Scheme 2 illustrates a simplified view of the conformational possibilities in **1** that arise as a consequence of possible rotation about the C-C and C-N single bonds that link the two macrocycles to the aryl ring. In fact many more possible conformers exist: a multitude will arise as a consequence of the rotation just mentioned, while it should also be noted that both square antiprismatic and twisted square antiprismatic geometries are to be expected for each of the europium complexes.[7]

However, from all these possible conformers, only a few will bind effectively to isophthalate. In scheme 2 illustrated with conformers I-IV, where bidentate binding of isophthalate is impossible. While conformer V is pre-organised for binding of isophthalate. Thus the interaction between host complex and anionic guest will result in a perturbation to the multitude of equilibria that govern the structure in solution, and will result in a consequent loss of entropy.

Although the 16-membered ring, formed by the binding of isophthalate to **1** (scheme 2) seems improbable, when considered from the standpoint of traditional supramolecular chemistry. We have amply demonstrated that the binding occurs and that it involves both europium centres and both carboxylic acid residues.[15] We feel that this point can be used to highlight that *f*-block chemistry is—in many ways—set apart from our general understanding of the rest of the periodic table.

In more detail, if we consider these possible conformational equilibria as allowing each macrocycle to occupy a multitude of positions, it is possible to define a volume of space that each might occupy close to the aryl ring. This is represented in 2D cartoon form in Scheme 3A for **1**. It can be seen from the deeply shaded red area in this figure that there is a space that both europium domains might occupy. Since this is clearly impossible, it follows that the position of one metal centre will influence that of the other.

Addition of remote substituents will further restrict the available conformational space. Thus in the peptide coupled system **2** the terbium domain also impinges upon the conformational freedom of the europium domains (and thus dictates the structure of the binding pocket to some degree). It is clear that this restriction increases the




**Scheme 3:** A simplified 2D representation, showing how the steric demand from remote substituents can restrict the available conformational space in A) complex **1**, B) complex **2**, C) complex **3** and D) complex **5**. DO3A domains are shown in red, dotamonoamide domains in green and the peptide side-chain in **3** and **5** in grey. Areas of heavy shading show where two groups will compete to occupy the same space, restricting conformational mobility and coupling their motion.

affinity of the complex for isophthalate. As such, we may infer that the conformation that binds effectively to anions is unaffected by this bulky group, and that the increased (pre-)organisation of the structure results in a reduced loss of entropy upon binding.

Furthermore, in complex **3** the tertiary amide is now substituted with an additional bulky fragment, which will restrict rotation further, giving rise to the largest observed binding constant (while still leaving the preferred binding conformation unaffected).

It is perhaps worth noting that such structural variations can have unpredictable consequences. The tetranuclear trimetallic complex **5**, which we reported recently, is closely related to the other structures described here.[18] However, it has no detectable affinity for isophthalate. From this, we may deduce that it is possible to go too far, and that restriction of conformational freedom can actually exclude the preferred binding conformation. Furthermore we must note that the strength of the binding of isophthalate to the europium-binding pocket must be large to overcome the lost entropy, when an increase in binding of two orders of magnitude can be readily gained by reducing the entropy loss by remote substituents. We are currently working towards other systems, where the loss of entropy is reduced by other means

## Conclusion

These results clearly show the importance of considering the whole structure of the molecule when designing a binding cavity. By doing so it is possible to both enhance and inhibit the binding of a guest. Our results should inform the design of hosts for a wide variety of anions, and we are currently working on the use of alternative binuclear domains that will permit us to extend our approach to ions of biological interest. In this we have been aided by both the conformational and rotational properties of organic ligand frameworks, and by the absence of directionality in the bonds between lanthanide and associated donor atoms. Both these features are essential in controlling the properties of the host species. In particular, the lanthanide binding domains of an organic ligand must bind irreversibly to a lanthanide while still retaining flexibility within the ligand structure if this approach is to pay dividends.

In this work, we have shown that a delicate balance exists between excluding unwanted degrees of conformational freedom while not excluding the desired binding conformer. Such pre-organisation can be achieved by linking building blocks together, and potentially allows a small variety of lanthanide containing motifs for anion recognition to be optimised for a wide range of guests.

These results have shown what can be achieved by tuning the binding for one guest. It is, however, clear that a binding site that contains a flexible binding motif is likely to bind to different guests by favouring different conformers. It should therefore be possible to tune the selectivity of a single flexible host to a range of guests. Indeed, this is already observed in biology and in the directed evolution of enzyme hosts. It remains to be seen whether it is possible to do so with simpler coordination domains.

## Experimental Section

The synthetic procedures and characterisation of the lanthanide complexes presented above is reported in the supporting information. The data from titrations and the fitting methodology employed to determine association constants is found in the SI.

## Acknowledgements

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**Entry for the Table of Contents** (Please choose one layout only)

Layout 1:

**Remote substituent effects**

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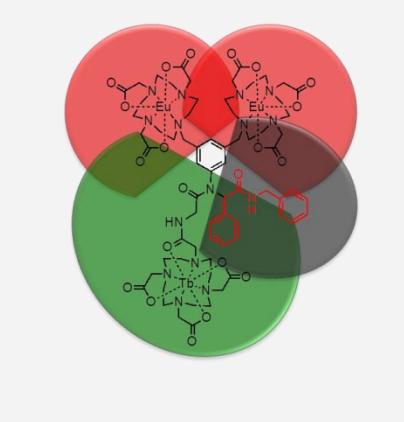
**Using remote substituents to control solution structure and anion binding in lanthanide complexes**




In this paper the influence of remote substituents on the conformational space of a lanthanide containing binding domain is investigated, the effect on the binding is found to be several orders of magnitude.