

Subject Heading

Lanthanide complexes of azidophenacyl-DO3A as new synthons for click chemistry and the synthesis of heterometallic lanthanide arrays

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Dedication ((optional))

Abstract: Lanthanide complexes of azidophenacyl DO3A are effective substrates for click reaction with ethyne derivatives, giving rise to aryl triazole appended lanthanide complexes, in which the aryl triazole acts as an effective sensitising chromophore for lanthanide luminescence. They also undergo click chemistry with propargylDO3A derivatives, giving rise to heterometallic complexes.

Lanthanide complexes have proved very useful as luminescent probes in bioassay and imaging,^[1] since their long lived luminescence facilitates time-gated separation of the desired signal from noise arising from scatter or autofluorescence.^[2] Such systems are particularly effective when combined with sensitizing chromophores that circumvent the problem of weak absorption by lanthanide ions.^[3] In such systems, energy transfer from the excited chromophore to the lanthanide is usually mediated by the chromophore triplet state,^[4] except in the case of ytterbium (III) complexes, where a sequential charge transfer mechanism is observed if it is thermodynamically feasible.^{[5],[6]}

While gadolinium complexes are rarely luminescent, they have become of remarkable importance as contrast agents in MRI imaging.^[7] Clinically useful agents must have high kinetic stability, combined with exchange of bound water with bulk to be effective in generating image contrast. MRI can be combined with other techniques in multimodal imaging,^[8] and heterometallic d-f hybrid complexes have been shown to have potential as bimodal imaging agents.^[8b] While gadolinium is optimal in generating MRI image contrast, a dual imaging probe could

immensely benefit from the unique properties of luminescence of other lanthanide ions, possessing sharp and characteristic emissions with long lifetimes. Furthermore, the use of two different lanthanide ions could potentially facilitate other dual-imaging modalities.

Almost ten years ago it occurred to us that such kinetically robust complexes could be linked together to form heterometallic lanthanide complexes containing different lanthanides in different binding pockets.^[9] Subsequently, we and others have extended this approach to a range of covalently linked and self-assembled systems.^[10]

Click chemistry has also proved to be an effective methodology for generating bioconjugates and lanthanide containing architectures,^[11] and we recently showed that alkyne appended DO3A derivatives are effective substrates for click chemistry.^[11b, 12] Furthermore, extensive work has been carried out recently on microwave enhanced click chemistry reactions based on lanthanide DO3A derivatives.^[13] We recently reported the synthesis of an azide-bearing lanthanide complex that incorporates a sensitizing chromophore (Ln.1) and its use in detection of H₂S.^[14] In this manuscript, we describe how Ln.1 complexes can be used as kinetically stable building blocks for "click" chemistry in the preparation of heterometallic complexes such as Ln.2.Ln'.

The complexes Ln.1 (Ln =Eu,Yb) were prepared as shown in Scheme 1. Reaction between 4-azidophenacylbromide (**3**) and the well-known triester^[15] (**4**) yielded the protected proligand (**5**). Subsequent deprotection of **5** by treatment with trifluoroacetic acid yielded H₃.1, which was used to form the lanthanide complexes by reaction with the appropriate trifluoromethanesulfonate salts.

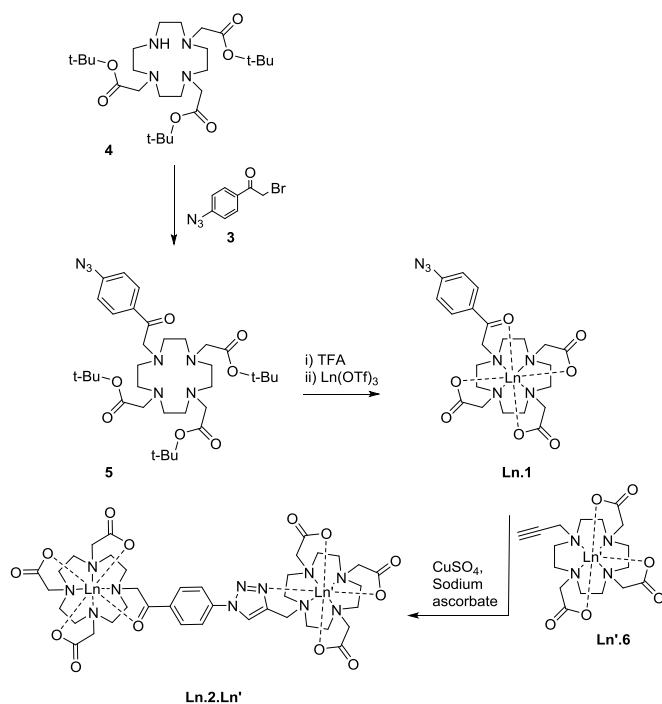
The structures of the Ln.1 complexes were studied in solution by NMR and luminescence spectroscopy. ¹H NMR on the paramagnetic europium and ytterbium complexes revealed the

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presence of two diastereoisomeric forms of the complex in slow exchange on the NMR timescale (Figure S1), with the dominant isomer corresponding to a square antiprismatic arrangement of atoms around the metal centre.^[16] The presence of two isomers in slow exchange confirms the coordination of the ketone to the the lanthanide; a more open structure in which the ligand was heptadentate would be expected to give rise to fast exchange between isomers, as is commonly observed with DO3A derivatives.^[11b]



Scheme 1. Synthesis of Ln.1 and Ln.2.Ln'

Luminescence measurements confirmed the coordination of the ketone to the metal centre. Table 1 shows photophysical properties for the complexes discussed in this paper. The inner sphere solvation, calculated from the observed lifetimes in D₂O and H₂O using established procedures^[17] revealed that the europium and ytterbium complexes had, on average, 1.1 and 1.4 inner sphere water molecules respectively.

We then investigated the suitability of the Ln.1 complexes as substrates in click reactions with lanthanide complexes of P-DO3A (Ln'.6). Coupling yielded the heterometallic complexes (Ln.2.Ln'). Aside from being an extremely straightforward way to elaborate Ln.1 complexes into more complicated structures, this method has the additional advantage of extending the absorption of the new chromophoric system further into the visible part of the spectrum (Figures S17-18 show the absorption spectra).

The heterometallic complexes Ln.2.Ln' display extremely interesting properties. As might be expected, their NMR properties differ depending upon which lanthanide is in which binding site (Figure 1 shows the ¹H NMR spectra of Eu.2.Yb and Yb.2.Eu). It is clear from these spectra that both centres are in slow exchange between isomers on the timescale of the NMR experiment (i.e. that both lanthanides are in octadentate binding

pockets). Furthermore, there are more diastereoisomers present in this case than in a simple monometallic complex (cf. the spectra in Figure S1). If we consider the signals associated with the axial ring protons on the macrocycle backbone of the SAP form of the Yb binding site there are clear differences in observed chemical shift that can be accounted for on the basis of differences in the local ligand donor set. Furthermore, there are eight resonances corresponding to these four protons, implying that the chirality on the second binding site creates a pair of diastereoisomers. The remoteness of the binding domains from one another means that neither isomer is particularly favoured, with NMR suggesting roughly equal populations of both.

The luminescence spectra of Yb.2.Eu and Eu.2.Yb are totally different, as can be seen from Figure 2. In Yb.2.Eu the europium emission is much less intense than the europium emission in Eu.2.Yb. Similarly, the emission from ytterbium (980 nm) is much more intense in Yb.2.Eu than in Eu.2.Yb. It can also be seen that the local environment at ytterbium differs between the two complexes - the fine structure of the peak at 980 nm differs considerably. Ligand to Metal Charge Transfer (LMCT) processes are known to lead to low quantum yields in europium containing systems, while they can mediate formation of ytterbium containing excited states. In this case, the formation of an ICT state within the chromophore would appear to favour energy transfer to europium only when the europium is bound in one pocket of the complex. This may suggest that the direction of charge transfer within the chromophore favours LMCT to europium only when the lanthanide is in a specific binding pocket. Though further study is required before this hypothesis can be

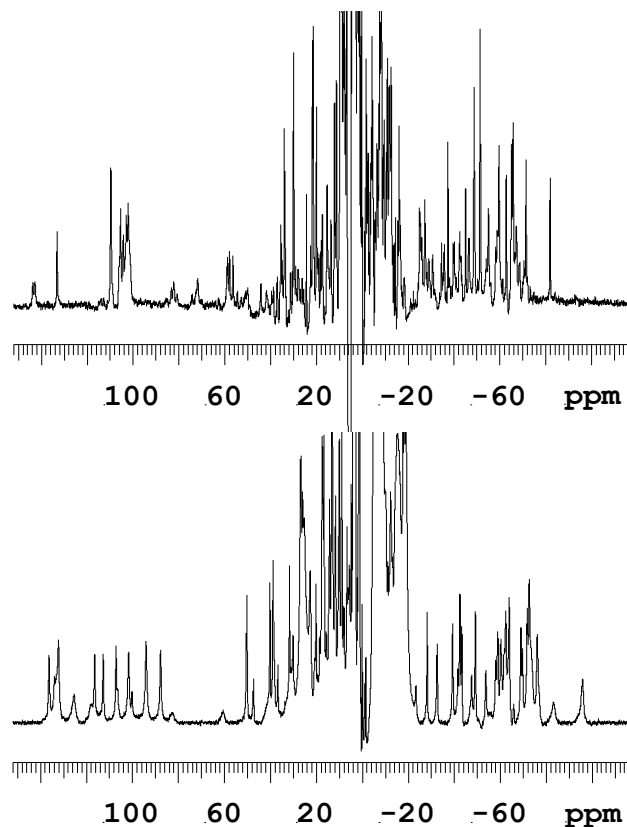


Figure 1. ¹H NMR spectra (700 MHz, D₂O) of Eu.2.Yb (top) and Yb.2.Eu (bottom).

confirmed, it is clear that the effect itself is real. It is worth noting that excitation of Eu.2.Yb at 300 nm (outside the region of absorption by the ICT state) does give rise to europium centred emission as well as ytterbium centred emission, suggesting that energy transfer from states other than the ICT state is more straightforward. The fine structure of the band at 980 nm remains the same regardless of the wavelength used to excite the chromophore in Eu.2.Yb, confirming that emission in both cases arises from the same entity.

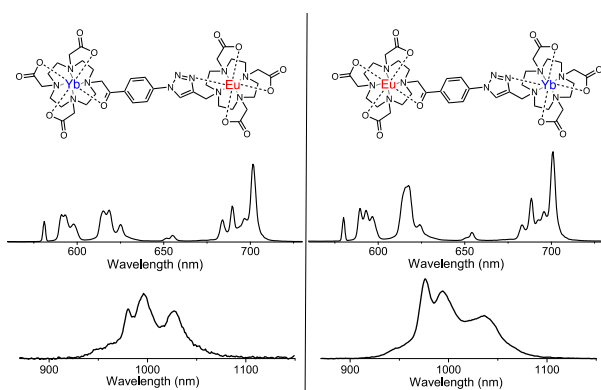


Figure 2. Luminescence spectra of Eu.2.Yb and Yb.2.Eu (left and right respectively), expanded to show the europium emission (upper set of spectra) and ytterbium emission (lower set of spectra) regions.

From these results it is clear that complexes of the form Ln.1 are effective substrates for Click chemistry. As such, they have considerable potential as tags for use in luminescence imaging, but also in MRI applications (for Gd.1 derivatives) and radioisotope tomography (where ^{177}Lu derivatives may have potential). Perhaps the key observation from this work is that it is important to consider the directionality of energy transfer processes that can yield excited states. This is clearly of critical importance in tuning the intensities of emission from two (or more) different lanthanides, but is also of general relevance when designing effective and brightly emissive mononuclear lanthanide complexes. Furthermore, the 4-triazolophenacyl chromophore clearly has potential as a useful general purpose sensitizer.

Acknowledgements

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Keywords: keyword 1 • keyword 2 • keyword 3 • keyword 4 • keyword 5

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Table 1. Photophysical properties of Ln.1 and Ln.2.Ln' recorded in H ₂ O and D ₂ O and derived hydration numbers. (λ_{ex} as specified, 295 K).					
Compound	$\lambda_{\text{ex}}/\text{nm}$	$\lambda_{\text{em}}/\text{nm}$	$\tau_{\text{H}_2\text{O}}/\mu\text{s}$	$\tau_{\text{D}_2\text{O}}/\mu\text{s}$	q
Eu.1	300	615	600	2050	1.1
Yb.1	337	980	0.61	6.4	1.4
Eu.2.Yb	300	615	640	1880	0.9
	337	980	1.29	5.67	0.5
Yb.2.Eu	300	615	590	1810	1.1
	337	980	0.73	6.51	1.1

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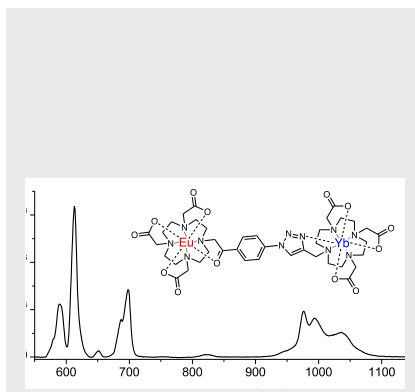
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Entry for the Table of Contents (Please choose one layout only)

Layout 1:

COMMUNICATION

Lanthanide complexes with high kinetic stabilities have been used to form heterobimetallic compounds containing two lanthanide ions in distinct binding pockets. The bimetallic species are characterised by distinct emission profile in the red and near-infrared regions resulting from Eu(III) and Yb(III) respectively.



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Layout 2:

COMMUNICATION

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