SANDIA REPORT
SAND2013-7827
Unlimited Release
September 17, 2013

Luminescent Lanthanide Reporters for High-Sensitivity Novel Bioassays

Mitchell R. Anstey
Julia A. Fruetel
Michael E. Foster
Carl C. Hayden

Prepared by
Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation,
a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's
National Nuclear Security Administration under contract DE-AC04-94AL85000.

Issued by Sandia National Laboratories, operated for the United States Department of Energy by Sandia Corporation.

Available to DOE and DOE contractors from
U.S. Department of Energy
Office of Scientific and Technical Information
P.O. Box 62
Oak Ridge, TN 37831

Telephone: (865) 576-8401
Facsimile: (865) 576-5728
E-Mail: reports@adonis.osti.gov
Online ordering: http://www.osti.gov/bridge

Available to the public from
U.S. Department of Commerce
National Technical Information Service
5285 Port Royal Rd.
Springfield, VA 22161

Telephone: (800) 553-6847
Facsimile: (703) 605-6900
E-Mail: orders@ntis.fedworld.gov
Online order: http://www.ntis.gov/help/ordermethods.asp?loc=7-4-0#online

SAND2013-7827
Unlimited Release
September 17, 2013

Luminescent Lanthanide Reporters for High-Sensitivity Novel Bioassays

Mitchell R. Anstey, Julia A. Fruetel, Michael E. Foster, Carl C. Hayden
8125, 8114, 8223, 8353
Sandia National Laboratories
7011 East Avenue
Livermore, California 94550

Abstract

Biological imaging and assay technologies rely on fluorescent organic dyes as reporters for a number of interesting targets and processes. However, limitations of organic dyes such as small Stokes shifts, spectral overlap of emission signals with native biological fluorescence background, and photobleaching have all inhibited the development of highly sensitive assays. To overcome the limitations of organic dyes for bioassays, we propose to develop lanthanide-based luminescent dyes and demonstrate them for molecular reporting applications. This relatively new family of dyes was selected for their attractive spectral and chemical properties. Luminescence is imparted by the lanthanide atom and allows for relatively simple chemical structures that can be tailored to the application. The photophysical properties offer unique features such as narrow and non-overlapping emission bands, long luminescent lifetimes, and long wavelength emission, which enable significant sensitivity improvements over organic dyes through spectral and temporal gating of the luminescent signal.

Growth in this field has been hindered due to the necessary advanced synthetic chemistry techniques and access to experts in biological assay development. Our strategy for the development of a new lanthanide-based fluorescent reporter system is based on chelation of the lanthanide metal center using absorbing chromophores. Our first strategy involves “Click” chemistry to develop 3-fold symmetric chelators and the other involves use of a new class of tetrapyrrole ligands called corroles. This two-pronged approach is geared towards the optimization of chromophores to enhance light output.

ACKNOWLEDGMENTS

This work was funded under LDRD Project Number 157690 and Title "Luminescent Lanthanide Reporters for High-Sensitivity Novel Bioassays".

CONTENTS

NOMENCLATURE

HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
DOE	Department of Energy
SNL	Sandia National Laboratories
PCR	Polymerase Chain Reaction
DFT	Density Functional Theory
DNA	Deoxyribonuclease
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid

1. PROPOSAL

1.1. Introduction

Biological imaging and assay technologies rely on fluorescent organic dyes as reporters for a number of interesting targets and processes. However, limitations of organic dyes such as small Stokes shifts, spectral overlap of emission signals with native biological fluorescence background, and photobleaching have all inhibited the development of highly sensitive assays. The Polymerase Chain Reaction, PCR, is a current work-around for increasing sensitivity, but PCR can introduce bias into the sample and complicate data interpretation. In addition, a need is arising for identifying multiple targets for biomarker discovery or pathogen detection from a bioterrorist attack. These multiplex assays are currently expensive and complex, utilizing microarray plates or multi-wavelength excitation/emission detection systems, which limit their application. What is needed is a new type of fluorescent moiety that offers improved properties over organic dyes, enables cost-effective multiplexed analysis, and provides opportunities for new and novel high sensitivity bioassays.

To overcome the limitations of organic dyes for bioassays, we propose to develop lanthanide-based luminescent dyes and demonstrate them for molecular reporting applications. This relatively new family of dyes was selected for their attractive spectral and chemical properties. Luminescence is imparted by the lanthanide atom and allows for relatively simple chemical structures that can be tailored to the application. The photophysical properties offer unique features such as narrow and non-overlapping emission bands, long luminescent lifetimes, and long wavelength emission, which enable significant sensitivity improvements over organic dyes through spectral and temporal gating of the luminescent signal.

Growth in this field has been hindered due to the necessary advanced synthetic chemistry techniques and access to experts in biological assay development. At Sandia, a multidisciplinary partnership between chemistry, biology, and engineering is easily formed and will allow this project to advance the state of the art.

1.2. Research Approach

Our approach is to develop a lanthanide-based luminescent reporter system capable of increased sensitivity over current organic dyes that incorporates a multiplexing component and a modular molecular scaffold. The final products are anticipated to yield superior fluorescent assays to current methods, with the potential to introduce a novel assay developed through exploitation of a unique “proximity” effect. For these applications, development of our dyes will necessitate several synthetic and analytical strategies that explore using lanthanides in various sensing modes.

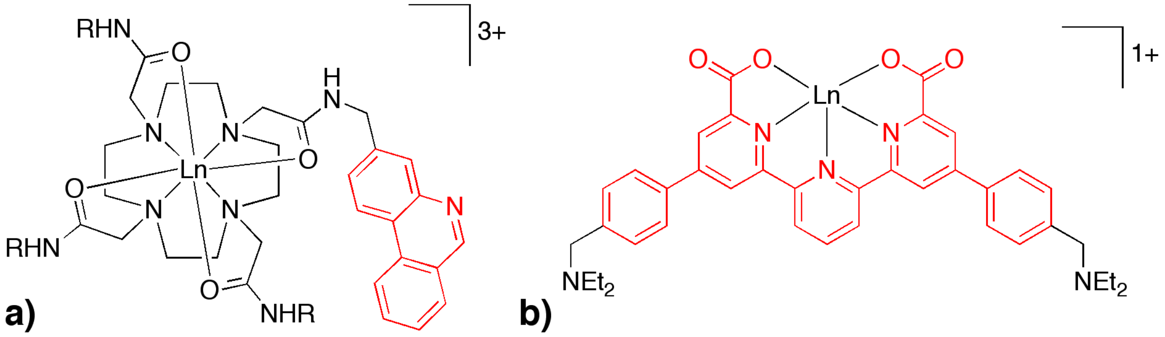


Figure 1. a) tethered antenna to lanthanide complex, and b) chromophore-as-ligand complex (antenna in red)

Fluorescence is a well-known property of lanthanides and was initially explored in the fields of materials science[1], crystal display engineering[2], and laser physics[3]. Through these studies, it was discovered that lanthanides were not easily excited by direct illumination. However, a separate chromophore, or antenna, could be excited and transfer its energy to the lanthanide. This antenna-lanthanide energy transfer is affected by the excited energy states of these two moieties and also by the length of separation. This proximity effect will be discussed later on as it relates to potential applications in novel bioassays.

The first generation of luminescent lanthanide probes utilized small molecule chromophores tethered to simple ligand structures (Figure 1a). As this area matured, more sophisticated probes turned the chromophores into the ligand (Figure 1b). This chromophore-as-ligand strategy brought the chromophores into direct contact with the lanthanide as a means to increase quantum yield and reduce leeching of lanthanides into solution. Some notable examples have shown that nitrogen-containing heterocycles used in this way have achieved quantum yields as high as 62%[4].

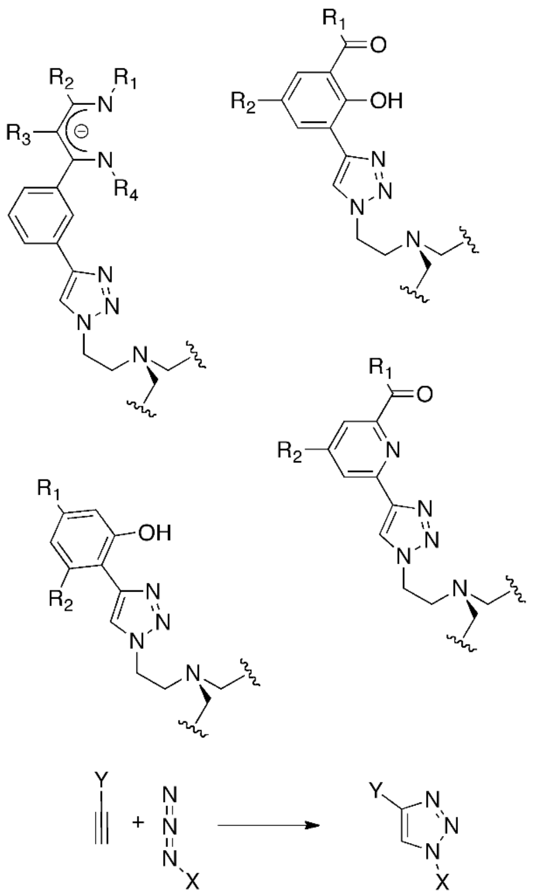


Figure 2. "Click Chemistry" methodology and diverse preliminary candidates

Both the chromophore-as-ligand and tethered antenna approaches have virtues that merit our attention. We see chromophore-as-ligand reporter molecules as the best candidate for improving the sensitivity of fluorescent assays such as PCR or immunoassay, where the fluorescent moiety is to attached directly to the DNA probe or antibody. Multiplexing these assays may be possible by using different lanthanide ions (Tb, Eu, etc.) in the complexes, or varying the R groups attached to the chormophores, or both, providing a unique reporter for each analyte of interest. The tethered antenna systems are highly versatile, as the antenna can be composed not only of synthetic chromophores, but also of amino acid side chains or oligonucleotides from biomolecules, which may be useful for protein or nucleic acid binding studies. Within these two general classes, there are multiple avenues for assay applications.

1.2.1. Chromophore-as-ligand approach

Recent reports have targeted lanthanide complexes with ligands composed of anionic oxygen donors with appended nitrogen heterocycles. While these have been promising, they have suffered from laborious synthetic strategies and poor structural characteristics. For example, Ziessel and coworkers reported their studies on a tripodal pyrazole-based ligand framework,[5] which gives excellent quantum yields in aprotic solvents but is quenched in aqueous solution. The chromophore functions properly but their ligand structure is difficult to make (seven synthetic steps) and harder to derivatize. It also allows solvent into the coordination sphere of the lanthanide reducing its efficacy.

Acknowledging these shortcomings, our chromophore-as-ligand strategy will take the current state-of-the-art in chromophores and marry them to a simple synthetic chemistry method called "Click Chemistry"[6] (Figure 2). This method forms the core of our target chromophore (three-Nitrogen triazole ring) while simultaneously linking the larger ligand framework. Libraries of nitrogen-containing heterocycles with various functionalities can be prepared from cheap and abundant starting materials where available, but a significant amount of laboratory work will be devoted to the derivatization of the most promising candidates. Our method allows us the ability to introduce a diverse number of functionalities to tune the excitation wavelength, quantum yield, and binding affinity while excluding water from the coordination sphere. In general, this strategy will enable a survey of the chemical landscape to find the optimal reporter system that shows improved sensitivity over organic dyes.

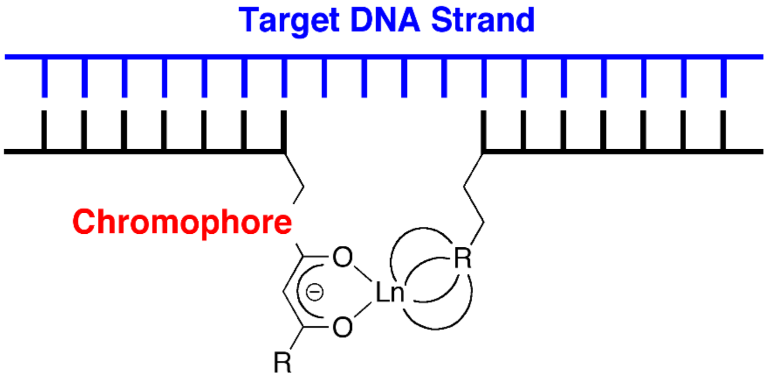


Figure 3. Target DNA strand binds chromophore and lanthanide complex closely as a "turn-on" mechanism

Beyond ligand and complex construction, a significant amount of effort will be spent on determining the photophysical properties of the complexes and using computational methods such as Density Functional Theory (DFT) to understand the excited-state energies of the ligands and metals. Currently, very little is known about the excited states of these complexes, but it is generally agreed upon that the energy match of the ligand and metal plays a key role in efficient energy transfer. Comparing experimental and computational data will aid the development of more sensitive lanthanide-based dyes for our purposes and also in the larger research community.

1.2.2. Tethered and untethered antennae approach

As mentioned above, the antenna does not need to be intimately bound to the lanthanide (Figure 1a) to enable luminescence; it only needs to be closely associated. This feature allows for scenarios where the two moieties can be attached to separate molecules as long as they are brought closely together by a third target binding unit (Figure 3). Several reports have shown how this antenna effect can be exploited as an on-off mechanism for detection, but further work has not been performed to optimize the energy transfer of the antenna to the metal, which will enable improved detection sensitivity. This optimization is where our (un)tethered antenna approach will begin. Lessons learned in our chromophore-as-ligand strategy will be applied to a simpler "tethered antenna" complex (Figure 1a). The triazole-based chromophores can be made singly and separate from a ligand and then linked to a more classical multidentate lanthanide binder such as a porphyrin or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). Synthetic moieties and relevant biomolecule functional groups such as tyrosine, tryptophan, and various nucleosides will be examined for their ability to act as antennae. If it becomes necessary to attach these antennae directly to a lanthanide, multidentate binders such as porphyrins, Cyclen, Cyclam, and DOTA are commercially available, bind lanthanides strongly, and can be easily augmented to attach a chromophore.

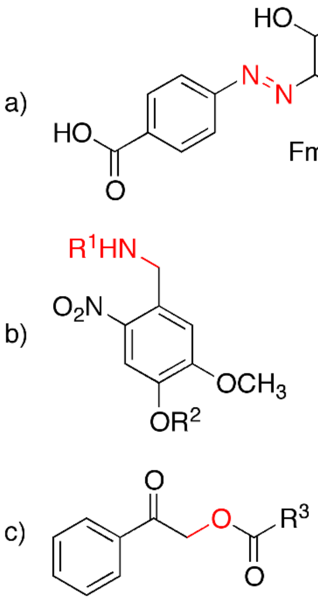


Figure 4. Cleavable linkers: a) diazo, b) 2-nitrophenyl, c) phenacyl

Once the most promising candidates are known, linkers will be made to attach these complexes to biomolecules. Photo- or chemically-cleavable linkers may be desired to allow for analysis of the dyes apart from the remaining sample; for example, multiplexing may be achieved using capillary electrophoresis to separate and detect the various unique reporter dyes. Three such cleavable moieties are the diazo compounds,[7] 2-nitrophenyl groups,[8] and phenacyl esters[9] (Figure 4).

From the lanthanide dye chemistry and characterization work we envision several publications: two dealing with lanthanide complex structure and photophysical properties, and two outlining the computational modeling of the two types of complexes.

1.2.3. Proof-of-principle bioassays

During the latter part of this project, we will assess the reporters' feasibility in several proof-of-principle bioassays. Because of the uncertainty in the structure, light yield, and solubility, it is too early to commit to several assays before knowing what might be the most fruitful assay. It is important to note here that there are experts in various fields here at Sandia that are interested in highly sensitive luminescent dyes, and collaborations will be made whenever possible to explore applications of newly developed dyes. However, we have outlined some specific applications for each of the reporter types.

The chromophore-as-ligand reporters can be applied to a multiplexed bead-based immunoassay and/or PCR. Our fluorescent lanthanide reporter can be attached to an antibody with an azoarene cleavable moiety. Another complementary antibody is attached to a bead. The target antigen binds simultaneously to the two antibodies and can then be washed to remove non-specifically bound lanthanide reporters. For PCR, the lanthanide reporter is attached to the DNA probe and is cleaved by the polymerase enzyme during amplification. The cleaved reporters can be detected using capillary electrophoresis. Additionally, there are several researchers at Sandia exploring time-resolved and hyperspectral fluorescence imaging, and we will leverage that expertise to explore potential applications.

For the untethered antenna, a homogeneous DNA hybridization assay is a potential application to be explored during the second year. In this scheme, the antenna is not tethered to the lanthanide but instead to the terminus of a DNA probe. By attaching the lanthanide complex to a complementary DNA probe that binds to the target DNA in a position immediately adjacent to the first probe, fluorescence would only occur when the two are bound to the target DNA strand.

1.3. Conclusion

In summary, our strategies will develop successful lanthanide-based luminescent reporter systems that are highly sensitive, modular, cost-effective, multiplexed, applicable to several assays, and refined by computational studies. We see this combination of approaches as the most successful tactic to improve upon the current state-of-the-art. Assays outlined above for each class of reporter are examples, and as the unique properties of our lanthanide reporters are understood, collaborations can be made to explore additional proof-of-principle options.

2. LANTHANIDE CORROLES: A NEW CLASS OF MACROCYCLIC LANTHANIDE COMPLEXES

2.1. Introduction

Lanthanide complexes and nanomaterials are used extensively in biological imaging and analyses due to the confluence in these metals of low toxicity and interesting photophysical properties. Chelating organic ligands often serve as vehicles for lanthanides in imaging agents because they can serve as the chromophore while maintaining solution stability and preventing coordination of quenching moieties (e.g., water). [1-5] For these reasons, strongly absorbing ligand scaffolds such as conjugated macrocycles are of great interest for lanthanide complex construction. [2,6,7]

Extensive work has been done in the preparation and characterization of lanthanide porphyrin complexes, [8,9] which were first synthesized in the 1970s for use as NMR shift reagents. [6] Single porphyrin, double-decker porphyrin, and supramolecular complexes are known. [8,10-12]

In contrast to lanthanide porphyrins, there have been no examples of lanthanide corroles reported prior to this work. This is unsurprising given that good methods for the preparation of free-base corroles have existed for just over a decade; [13,14] this breakthrough has led to a flurry of activity in which late transition metal, main group, [15] and, recently, alkali metal as well as early transition metal corroles have been isolated and characterized. [16,17] The relatively small size of the corrole cavity forces larger metals to sit quite far (up to 1.144 Å) out of the N_4 plane; this is exemplified in recent examples of iridium, gold, lead and bismuth complexes.

A collaboration exists between Sandia and Prof. John Arnold at the University of California, Berkeley to develop new lanthanide complexes using corrole molecules; a cousin of the porphyrin. The corrole molecule is interesting because of its unique spectroscopic and electronic properties, and, paired with a lanthanide atom, new corrole compounds have the potential to push lanthanide luminescence into new research areas. The current collaboration has laid the groundwork, and we are now interested in extending this research to the applications noted above. Specifically, we are interested in Europium corrole compounds because of the bright fluorescence and phosphorescence that these atoms exhibit, respectively. The corrole may play a significant role in modulating the electronics of these atoms leading to new luminescent properties not seen in other known lanthanide compounds. Additionally, we are interested in Cerium corrole compounds because of their interesting redox properties and the relatively modest potentials needed to get to the Ce(III)/(IV) couple.

2.2 Research and Discussion

Here we present two methods for the preparation of lanthanide corroles. The first is from free-base corrole and lanthanide silyl amides, and the second is by metathesis of the recently reported lithium corrole[17] and the metal chloride.

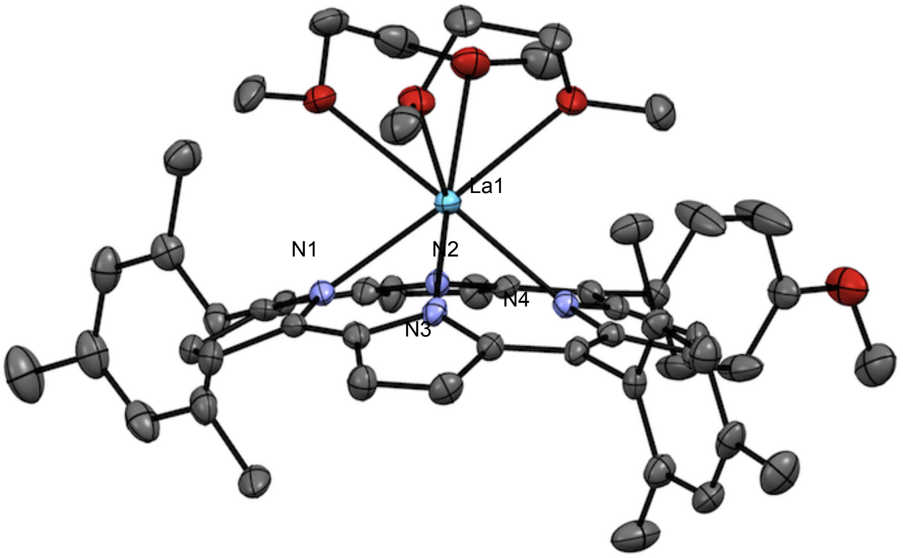
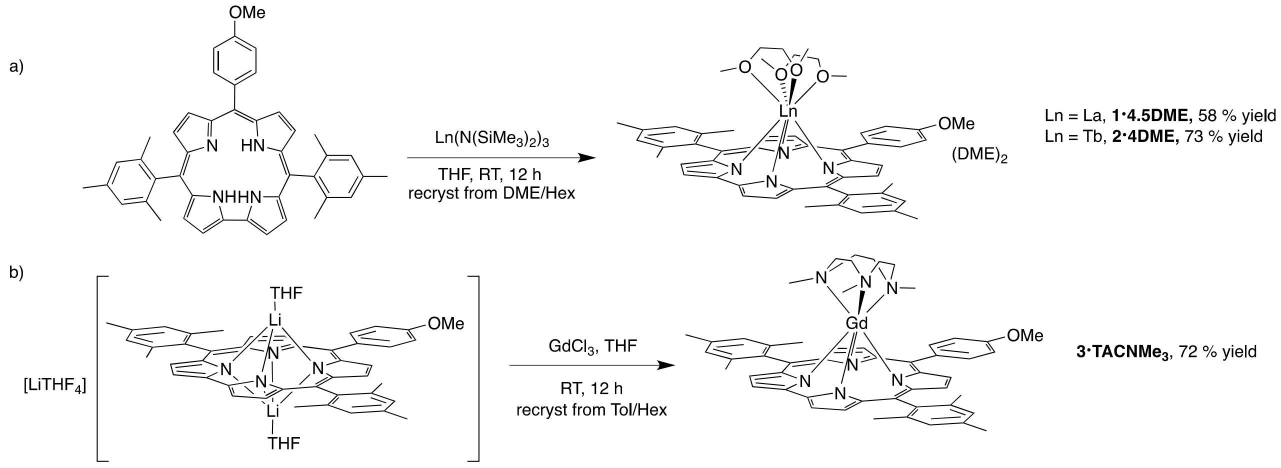


Figure 5. Molecular structure of lanthanum corrole 1·4.5DME, determined by single-crystal X-ray diffraction. H atoms and non-coordinated solvent omitted for clarity; thermal ellipsoids at 50 % probability level.

Combination of the free base 10-(4-methoxyphenyl)-5,15-dimesitylcorrole ((Mes$_2$(*p*-OMePh)corrole)H$_3$) with 1.1 equivalents of La(N(SiMe$_3$)$_2$)$_3$ at room temperature in THF leads to an immediate colour change from deep purple to a deep green solution. Stirring of this solution overnight, removal of the solvent under vacuum and recrystallization of the solid by vapour diffusion at room temperature (1,2-dimethoxyethane (DME)/hexanes) provided dark purple plates from a dark green solution that were suitable for characterization by X-ray crystallography (58 % yield). Complex (**1·4.5DME**) (Scheme 1a) has been characterized by ^{1}H NMR and UV-visible spectroscopies and ESI mass spectrometry.

The crystal structure of lanthanum corrole **1·4.5DME** is shown in Figure 5. The lanthanum atom is located 1.469 Å outside of the N$_4$ plane of the corrole with an average N-La bond distance of 2.438(6) Å. This is the largest metal-N$_4$ plane separation recorded for a corrole complex to-date, with the previous record held by a recently reported bismuth (III) corrole structure at 1.144 Å. This separation is also consistent with the κ^2-coordination of the La atom by two molecules of DME; excepting cases with η^5-Cp$^{(*)}$ as a capping ligand, there are no examples of metallocorrole complexes where the coordination number at the metal exceeds six.



Scheme 1. Synthesis of a) lanthanum (III) corrole 1·4.5DME and terbium (III) corrole 2·4DME b) gadolinium (III) corrole 3·TACNMe$_3$

The terbium (III) corrole complex (Mes$_2$(*p*-OMePh)corrole)Tb·4DME (**2·4DME**) was prepared in a manner analogous to **1·4.5DME** in 72 % yield. The complex has been characterized by UV-visible spectroscopy, ESI mass spectrometry, and X-ray crystallography. ESI MS shows peaks consistent with the incorporation of two atoms of oxygen (corroleTbO$_2$H$^+$, calculated 829.2192, observed 829.2198) as well as the coordination one and two molecules of THF to this species, consistent with the availability of adventitious oxygen in the spectrometer and the use of THF to prepare solutions for ESI MS. The crystal structure of **2·4DME** shows a Tb-N$_4$ plane distance of 1.272 Å, consistent with the smaller ionic radius of Tb (III) as compared to La (III).

The gadolinium (III) corrole complex (Mes$_2$(*p*-OMePh)corrole)Gd·TACNMe$_3$ (**3·TACNMe$_3$**) was prepared by the combination in THF at room temperature of (Mes$_2$(*p*-OMePh)corrole)Li$_3$·6THF with 1.1 equivalents of GdCl$_3$ and 1.0 equivalents of 1,4,7-trimethyl-1,4,7-triazacyclononane (TACNMe$_3$) (Scheme 1b). Stirring overnight results in a dark green solution. Removal of the solvent and recrystallization of the solid by vapour diffusion at room temperature (toluene/hexane) yields crystals suitable for X-ray diffraction (73 % yield). This species has been characterized by UV-visible spectroscopy and ESI MS. The crystal structure of **3·TACNMe$_3$** is shown in Figure 6 and shows a Gd-N$_4$ plane distance of 1.262 Å. The structure shows seven-fold coordination of the Gd, which like the La complex is a higher coordination number than previously observed for corroles, but consistent with other lanthanide complexes and with the distance of the metal centre from the N$_4$ plane.

In addition to the three isolated lanthanide (III) corrole complexes, the three analogues with complementary ligands were prepared *in situ* for comparison by UV-visible spectroscopy. Thus, UV-visible spectra of **1·4.5DME**, **1·TACNMe$_3$**, **2·4DME**, **2·TACNMe$_3$**, **3·nTHF**, and **3·TACNMe$_3$** were obtained in THF (THF likely undergoes rapid exchange with DME, thus the spectra are of THF rather than DME adducts). The spectra for **2·4DME** and **2·TACNMe$_3$** are unremarkable; both Tb(III) complexes display identical spectra with a single Soret peak at 433 nm and three Q-peaks at 541, 580, and 611 nm. A single Soret band is consistent with the spectra of other metallocorroles with the metal sitting outside of the plane, including Al, [18] Ga, and Zr. [17]

However, the spectra for the La and Gd complexes are more interesting; both display a single Soret peak in the presence of only DME/THF as ancilliary ligands, but display two Soret peaks in the presence of $TACNMe_3$ and a spectrum essentially identical to that of (Mes_2(*p*-OMePh)corrole)Li_3·6THF. The latter situation is also consistent with sterically hindered (i.e. mesityl-susbsituted) free-base corroles that have retained planarity in solution; this data suggests that the corrole rings in **1·TACNMe$_3$** and **3·TACNMe$_3$** may be roughly planar in solution.

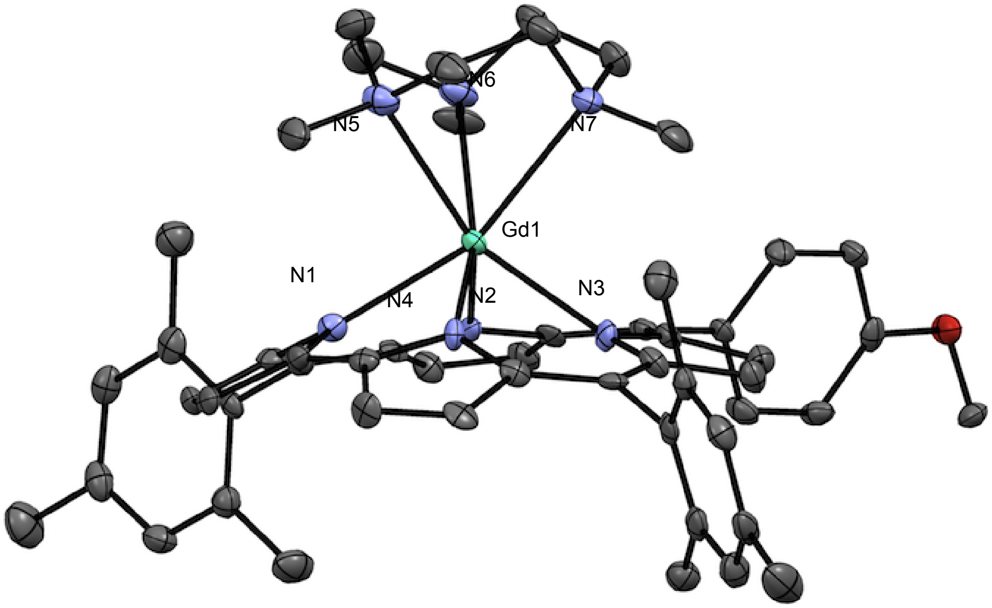


Figure 6. Molecular structure of gadolinium corrole 3·TACNMe$_3$, determined by single-crystal X-ray diffraction. H atoms and non-coordinated solvent omitted for clarity; thermal ellipsoids at 50 % probability level.

The ^{1}H NMR spectrum of **1·4.5DME** was obtained in *d$_8$*-toluene at room temperature, and it was found that all corrole peaks at this temperature are broad and the number of peaks exceeds the number anticipated. This is attributed largely to rapid exchange of the DME ligands. Variable temperature (VT) NMR was used to determine that the broadening is exacerbated in the case of the aromatic protons of the *p*-methoxyphenyl ring at the 10-position of the corrole; the barrier to rotation of this ring relative to the corrole is low but significant at room temperature. At lower temperatures (263 K) some sharpening of the peaks is observed and it is possible to see doublets partially resolved for the β-Hs, as well as sharpening of some of the aromatic *p*-methoxyphenyl peaks as non-equivalent peaks with an integration of 1 H each. The symmetric inequivalence of these peaks is consistent with the structure observed by X-ray crystallography, where the aryl rings clearly sit out of the plane of the corrole. At high temperatures, all peaks become too broad to provide any useful data.

To complement the variable temperature NMR spectroscopy performed on **1·4.5DME** and to confirm that a single species has in fact been isolated, a slight excess of $TACNMe_3$ was added to an NMR tube containing **1·4.5DME**. At room temperature, the main result of the addition was a considerable sharpening of the peaks in the aromatic region and the clear emergence of only four β-*H* doublets and two aromatic singlets from the mesityl groups at room temperature. At 323 K, both the ortho- and meta- protons of the *p*-methoxyphenyl ring were observed to coalesce into doublet, confirming that their apparent absence at room temperature was indeed due to the moderate barrier to rotation of that ring.

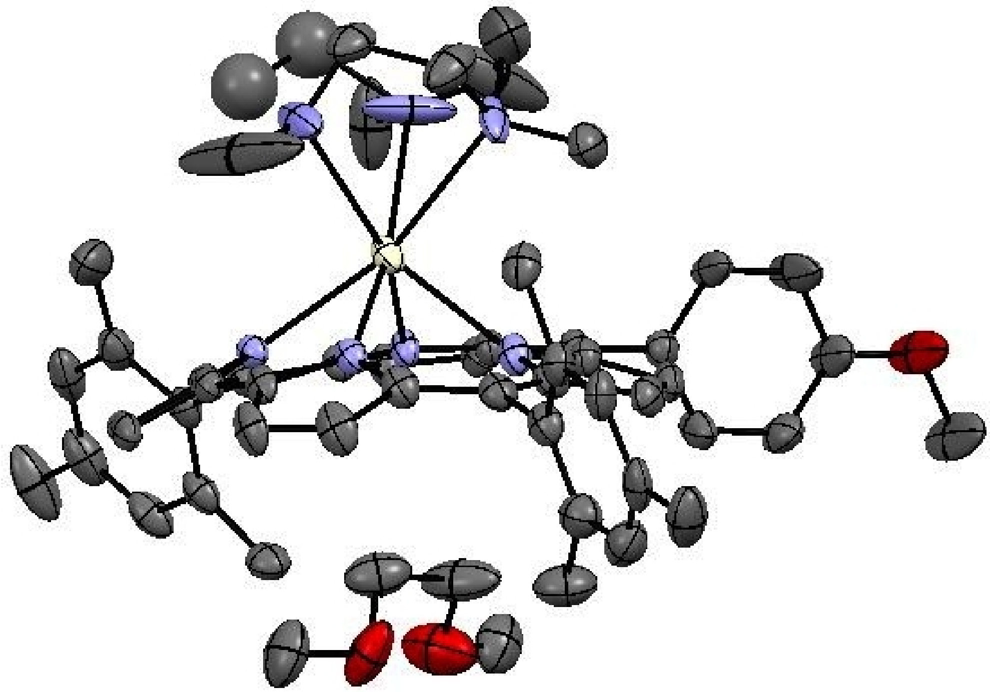


Figure 7. Molecular structure of cerium corrole 4·TACNMe$_3$, determined by single-crystal X-ray diffraction. H atoms and non-coordinated solvent omitted for clarity; thermal ellipsoids at 50 % probability level.

Beyond our demonstration of the synthesis of metallocorrole complexes of redox-inactive metals, we have conducted preliminary research into the preparation and interconversion of Ce(III) and (IV) complexes. The Ce(III) corrole complex (Mes$_2$(*p*-OMePh)corrole)Ce·TACNMe$_3$ (**4·TACNMe$_3$**) has been isolated and structurally characterized. Preliminary ^{1}H NMR spectroscopy studies of the oxidation of this species by ferrocenium salts have indicated formation of a diamagnetic species consistent with a Ce(IV). The isolation of solid crystalline material was achieved and single-crystal X-ray diffraction determined the structure of the compound pictured in Figure 7. Preliminary electrochemistry was inconclusive. This led to further computational studies to determine whether the cerium atom or the corrole ligand itself was redox active.

2.3. Computational Studies on Cerium Corrole

Due to uncertainty related to the electrochemistry of the cerium corrole complex, a brief computational investigation of the lanthanide corrole structure was performed. The basis set for this study is DFT-B3LYP/TZVP and using relativistic effects (ZORA) to more accurately model the lanthanide electronic structure. The cerium and lanthanum compounds were chosen for this study. In its 3+ oxidation state, the cerium has one unpaired valence electron, which is the target of our electrochemical studies. The lanthanum exists in the 3+ oxidation state with no electrons in its outer f-shell. This comparison here is whether or not the highest occupied molecular orbital (HOMO) in the cerium and lanthanum structures is the same and if so, is it a corrole- or metal-based orbital.

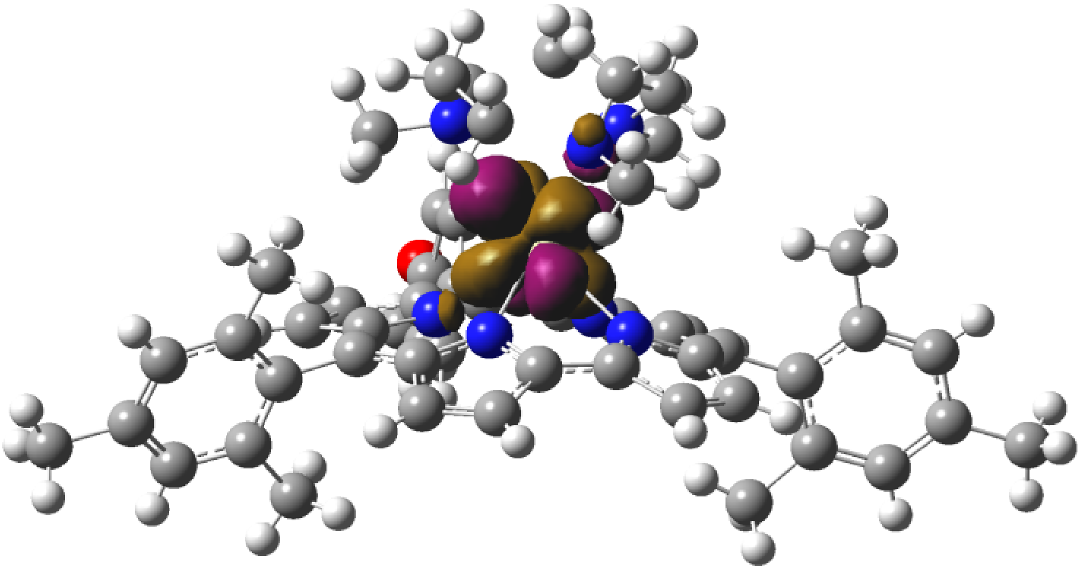


Figure 8. HOMO of neutral model cerium corrole complex.

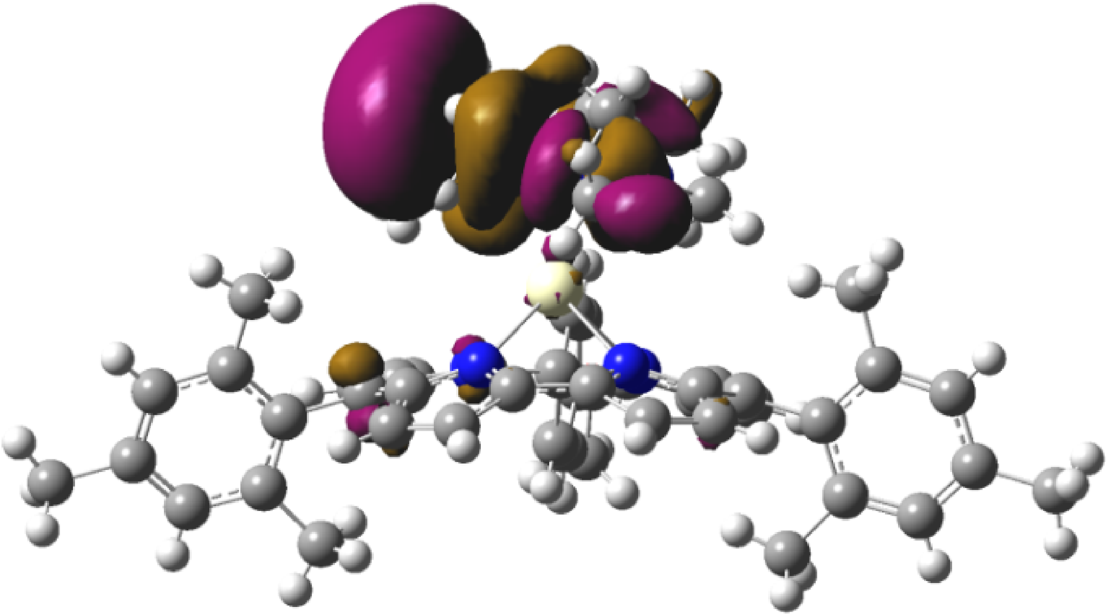


Figure 9. LUMO of neutral model cerium corrole complex.

As can be seen in Figure 8, the HOMO is a metal-based orbital with very little to no contribution from the ligand framework. Curiously, the lowest unoccupied molecular orbital (LUMO) is an orbital on the capping ligand (Figure 9). This should not affect the oxidation of the neutral compound in this case, only the reduction. Once the oxidation of the compound is performed, the compound is now in the 1+ oxidation state, and presumably the electron in the HOMO removed. In Figure 10, the LUMO of the oxidized cerium corrole is shown, and that orbital is predominantly metal-based. The HOMO of the oxidized cerium corrole is a ligand-based corrole orbital (Figure 11). This is as we expected. The highest energy electron in the system resides on the cerium atom, allowing us to achieve oxidation and reduction of the molecule and accessing the Ce(III)/(IV) redox couple.

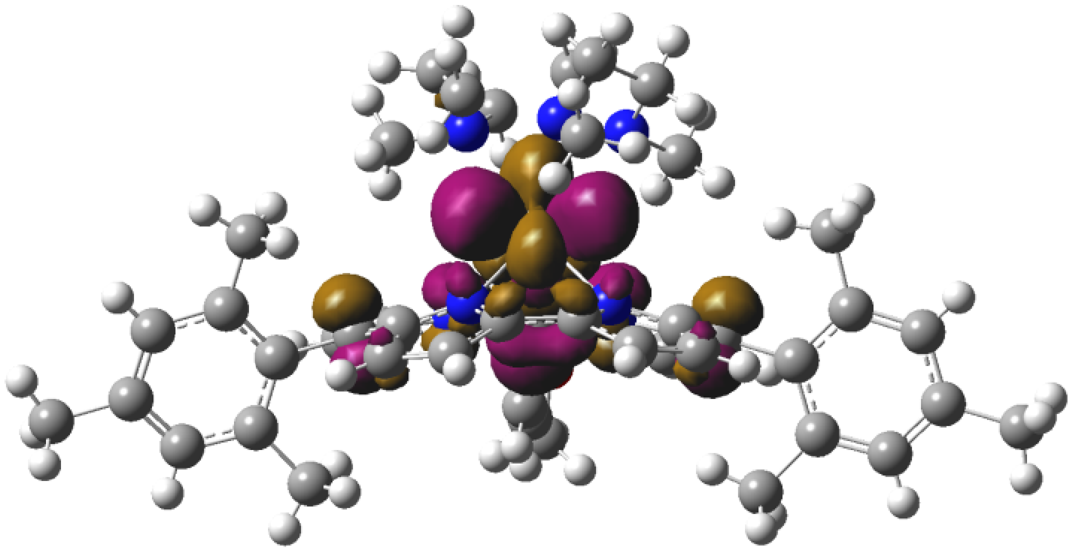


Figure 10. LUMO of cationic model cerium corrole complex.

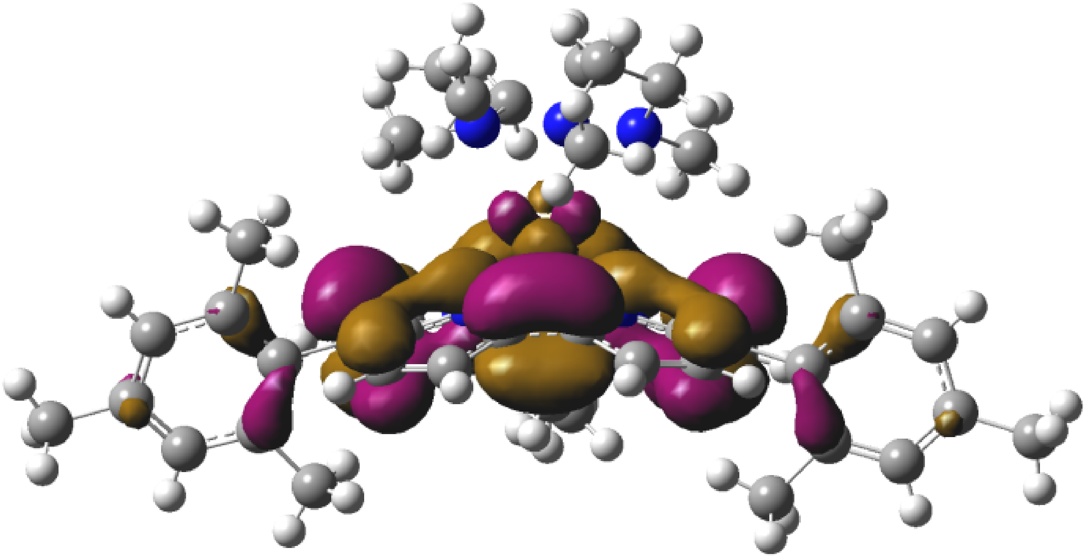


Figure 11. HOMO of cationic model cerium corrole complex.

In the case of the lanthanum corrole, we expect to see a similar electronic structure (HOMO and LUMO) to the cationic cerium corrole complex. This seems to be the case in that the HOMO of the neutral lanthanum corrole complex is a ligand-based orbital (Figure 12). The LUMO picture in Figure 13 is still predominantly on the capping ligand and not the metal, but considering the electron count and the difficulty in reducing lanthanum, this is not unexpected. Additionally, the oxidized lanthanum corrole has both the LUMO and HOMO depicted as corrole-based orbitals (Figure 14, 15).

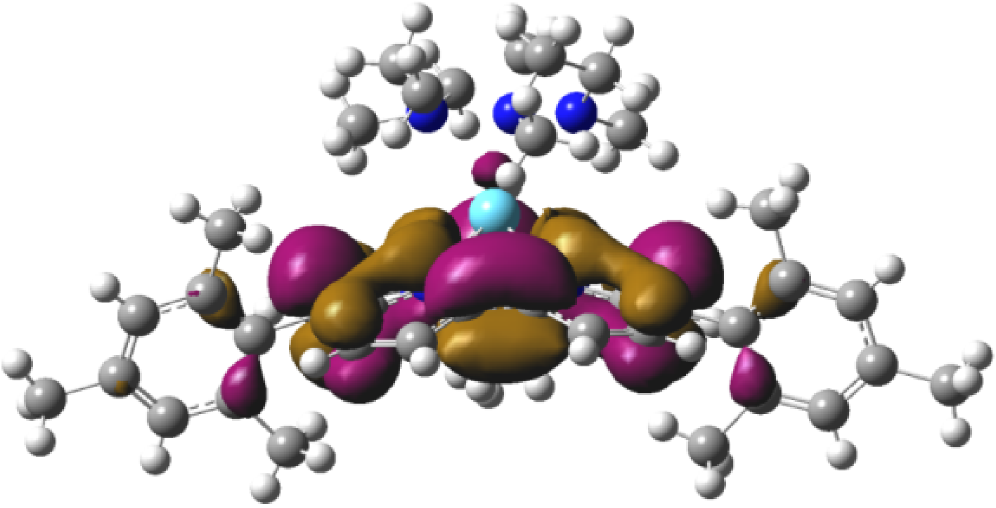


Figure 12. HOMO of neutral model lanthanum corrole complex.

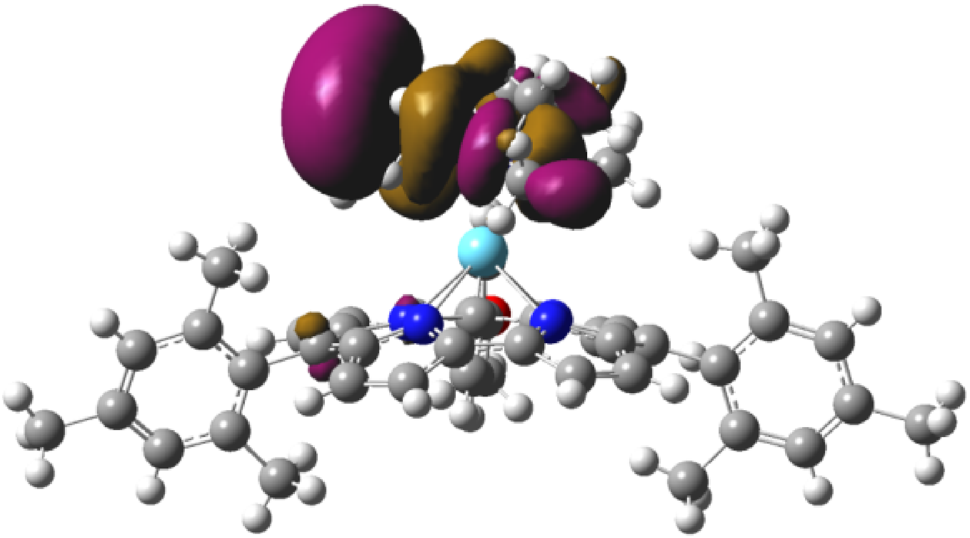


Figure 13. LUMO of neutral model lanthanum corrole complex.

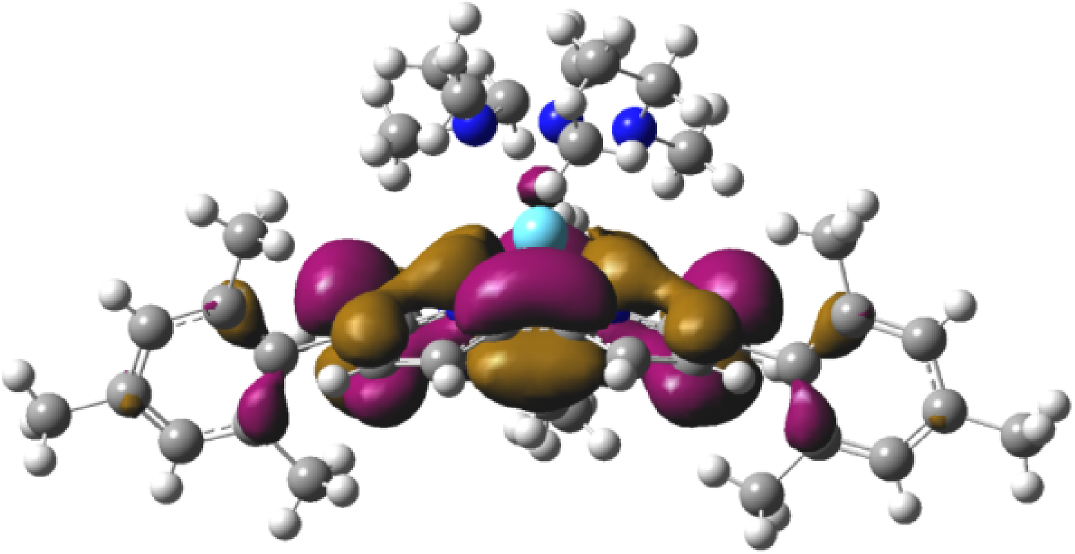


Figure 14. LUMO of cationic model lanthanum corrole complex.

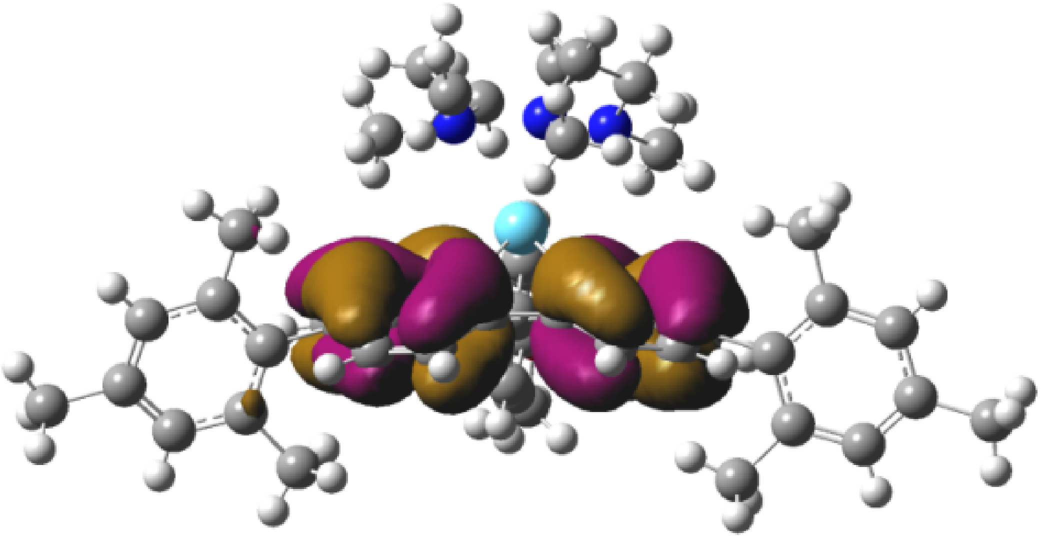


Figure 15. HOMO of cationic model lanthanum corrole complex.

This study suggests that the oxidation of the neutral cerium corrole compounds should yield a Cerium 4+ complex. Further experimental studies are needed to find the appropriate conditions for this reaction.

2.4. Conclusions

In summary, the first examples of lanthanide corrole complexes (of La, Gd, Tb, and Ce) have been prepared by two routes and have been characterized spectroscopically and structurally. In addition to salt-metathesis chemistry, the amine-elimination route shown here provides a further attractive avenue to prepare new metallocorrole complexes. Given the large molar absorption coefficient of corrole ligand and the possibility to use various excitation wavelengths, this strategy has the potential to be used for sensitization of NIR-emitting lanthanide cations. Efforts are underway to extend this methodology across the lanthanide series and to explore the use of these materials as fluorescence imaging agents.

[Blank page following section.]

3. CLICK-DERIVED LANTHANIDE CHELATORS

3.1. Introduction

As was stated in section 1.1 and 1.2, our aim has been to develop chelators that can bind strongly to a lanthanide metal center while also effectively transfer energy through the well-known antennae effect. As is necessary with all chelators, several points of contact need to be made between the ligand and metal center, and the more that are present, the better the overall binding should be. Lanthanides are known to be 9-coordinate and higher due to their large size and also the dominance of electrostatics in the overall binding.

The quest for the perfect binding unit is inhibited in the case of lanthanide-based luminescent reporters by the need for the exclusion of water and other solvents from the coordination sphere. These molecules usually lead to non-radiative decay of the excited state of the lanthanide. This means that the ligand in question must occupy the entire coordination sphere and crowd out all other competitors. For a small molecule such as water, this can be a difficult proposition. The chelator needs to be sterically bulky. Additionally, it would be beneficial to make the molecule easy to synthesize in large quantities and done cheaply.

All of these limitations and demands lead our team to the use of “Click” chemistry as the methodology for ligand synthesis. This method allows us to quickly and selectively link together small pieces to create a larger scaffold. Using a 3-fold symmetric core, we can create several binding points on a single linked scaffold. That coupling reaction creates not only the entire backbone of the ligand but also create triazole moieties that operate as antennae for the lanthanide energy transfer process. What follows are the results of that investigation, and the potentially promising avenues for further study.

3.2. Yearly Research Reports

3.2.1. FY11 Year-End Report

The first course of action on this project was to construct the organic architectures that would simultaneously encapsulate and electronically excite the lanthanide atom in question. Because our synthetic methodology is based around the “Click” reaction developed by Prof. Barry K. Sharpless, several azide and alkyne substrates were made. Some examples include 2-ethynylphenol, methyl 2-ethynylbenzoate, methyl 6-ethynylpicolinate, methyl 6-azidopicolinate, tris(2-azidoethyl)ammonium chloride, methyl 2-azidobenzoate, methyl 3-azidobenzoate, and 1-azido-2-methoxybenzene. Along with the synthesis of these molecular building blocks, a proof of concept experiment was performed to verify that the “Click” chemistry will work in this application. A full ligand, 3,3',3''-(4,4',4''-(nitrilotris(methylene))tris(1H-1,2,3-triazole-4,1-diyl))tribenzoic acid, was made and is now ready for lanthanide encapsulation experiments.

During this time, a computational collaborator at Sandia, CA was identified and investigations into the excited state electronics of these complexes are underway. The program GaussView 5.0 is being used as a molecular modeling aid, and the eventual computational work will be performed on Sandia's supercomputer RedSky using a proprietary molecular orbital energy minimization algorithm. Three additional synthetic chemistry collaborators have been identified who are interested in aspects of lanthanide luminescence (inorganic extended solids, hybrid gold-lanthanide materials, and organic chromophore architectures). Project development discussions are underway and in one case, the ligand 3,3',3''-(4,4',4''-(nitrilotris(methylene))tris(1H-1,2,3-triazole-4,1-diyl))tribenzoic acid is being shared to investigate its radiation-induced scintillation properties with iridium metal centers.

3.2.2. FY12 Year-End Report

The previous year was spent developing lanthanide-based dyes that would be amenable to the various types of biological assays that are performed here at Sandia. Bright dyes with many different luminescent lanthanides were made and then up/down-selected based on performance, solubility, ease of synthesis, and other structural characteristics. With these dyes in hand, the remainder of this fiscal year will be devoted to preparing them for use in assays. These tasks include development of cleavable linkers to allow for further conjugation to receptor molecules.

These dyes and this work in general have generated a great deal of interest among Sandians and academics. Currently, we now have a collaboration with Prof. John Arnold in the UC Berkeley Chemistry Department exploring lanthanides bound in ligand/chromophore frameworks. Additionally, two new research proposals for LDRD and NNSA (NA-22) were submitted based on luminescent Cerium compounds similar to the structures proposed in this work but excited by radiation in place of incident light. New directions are also being explored with these lanthanide compounds that include multiphoton absorption and multiphoton upconversion technologies.

3.2.3. FY13 Year-End Report

The final year of the project demonstrated a viable but limited methodology for using these ligands as chelators for lanthanides. It was determined that despite the large scaffolds being synthesized, the binding pockets they created were not sufficient to encapsulate a lanthanide atom with ease. This led to a host of other unrelated products such as interconnected linked structures, dimers, and so forth.

In an effort to position this work for continuation funding, attention was devoted completely to the lanthanide corrole work and to the completion of the complexation studies with the ligands that were already in hand. Additionally, we determined that these compounds are capable of multi-photon upconversion through a collaboration with Carl Hayden. Attempts to incorporate cleavable linkers into the scaffolds and to use these dyes in proof-of-principle assays were abandoned in favor of finishing the basic science needed to determine viability.

Finally, this work was successful in starting a new LDRD project based on cerium-based emission for radiation detection. There was initial interest in a lanthanide-based quantum magnet project where light is used for the read/write mechanism. After getting to the second round of the LDRD review, the project was not chosen.

3.3. “Click” Ligands for Lanthanides

As mentioned above, the “Click” strategy involved using a 3-fold symmetric core and coupling together several pieces to create a scaffold that also transfers energy using arene moieties. The amine core works well for the 3-fold symmetry in these systems, and commercially available tripropargylamine and the easily synthesized tri(2-azidoethyl)amine were chosen as the cores to be derivatized (Figure 16).

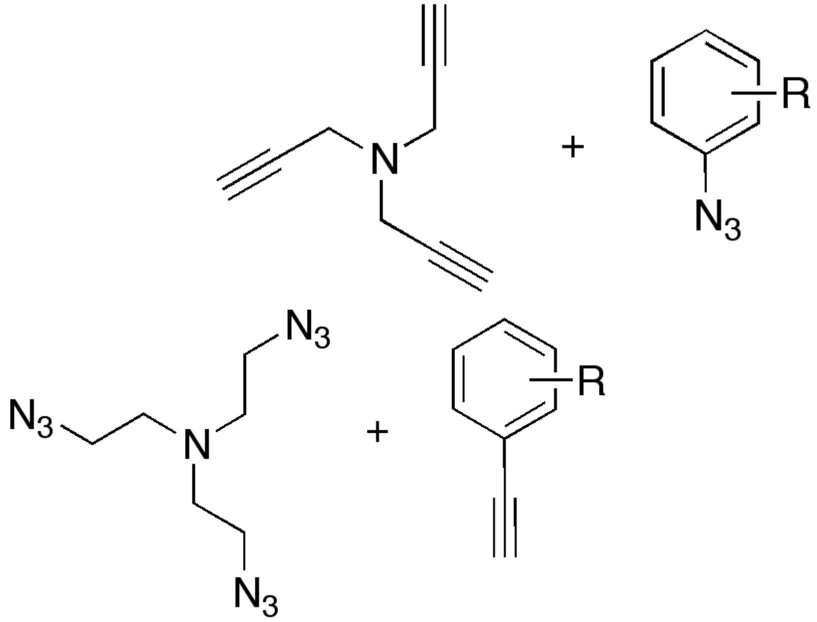
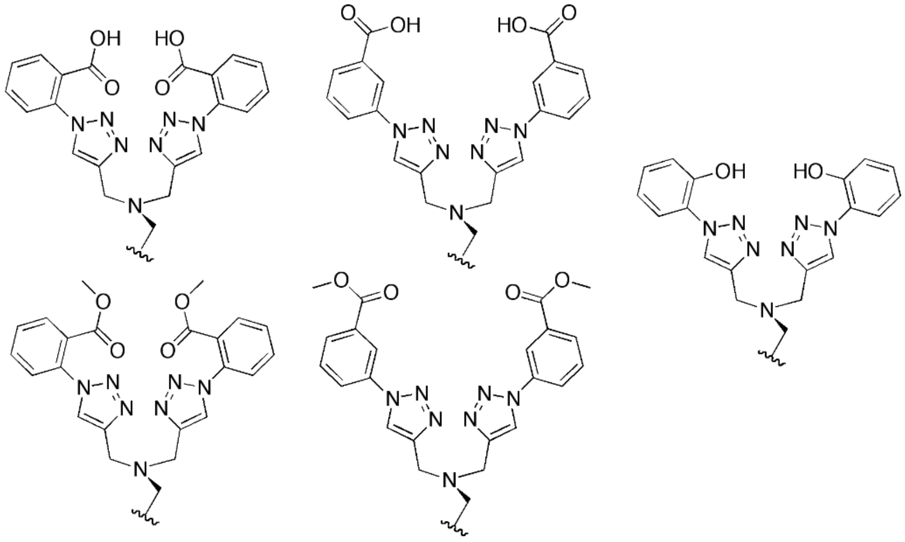


Figure 16. Two avenues for derivatization of ligands.

Using these two strategies, our team synthesized a small library of chelators. Predominantly, they incorporate arene carboxylates as the binding functional group, but pyridines, alcohols, pyridine N-oxides, and esters were also investigated (Figure 17).



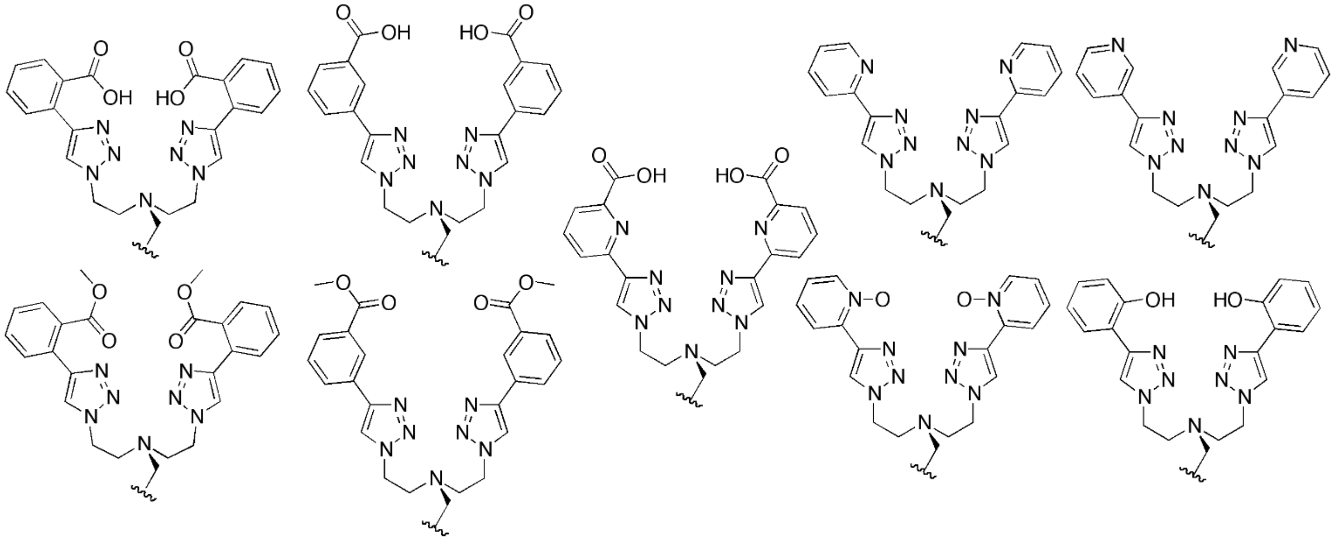


Figure 17. Library of synthesized scorpionate-type chelators.

It was determined that anionic functional groups performed well in binding to the lanthanide metal center. Specifically, carboxylates worked very well in this case. Lanthanides tend to be oxophilic, and while the alcohols and N-oxides fall into this group, they did not yield tractable products upon exposure to lanthanide salts. Esters also did not yield products, but this could be attributed to poor binding due to the lack of an anionic moiety for electrostatic attraction.

The best performing ligand in our library was 2,2',2''-(4,4',4''-(nitrilotris(methylene))tris(1H-1,2,3-triazole-4,1-diyl))tribenzoic acid. However, this ligand did not encapsulate a single lanthanide atom. Instead, two of these ligands link two lanthanide atoms to form a dimer of sorts. The crystal structure is pictured in Figure 18.

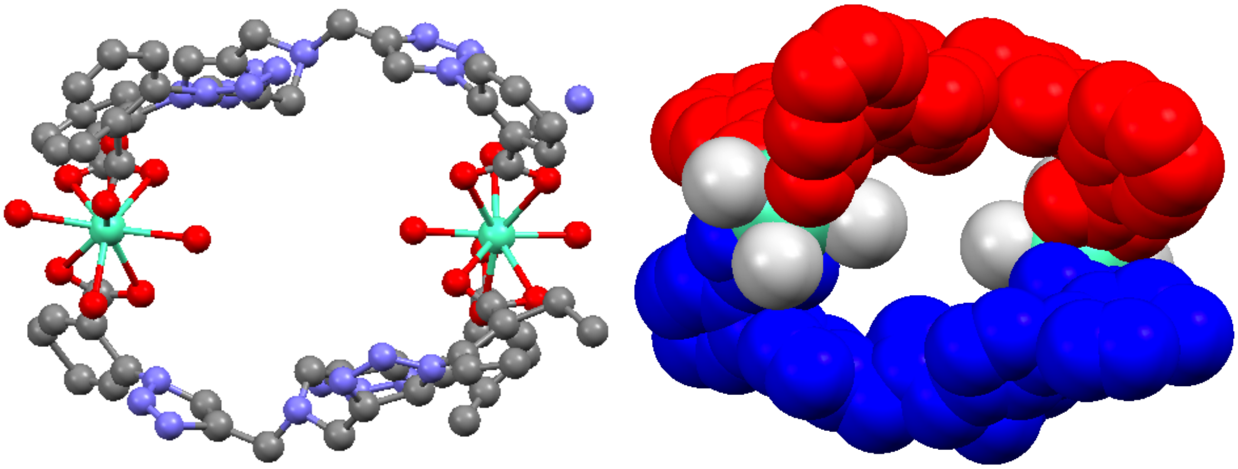


Figure 18. Crystal structure (ball-and-stick on left, space-filling on right) of L_2Eu_2.

3.4. Luminescence Studies on L_2Eu_2

3.4.1. Photoluminescence

The compound luminesces strongly under UV irradiation with a maximum absorption at 260 nm (Figure 19). The emission spectrum shows a distinctive pattern that is associated with the emission of a Europium atom (Figure 20). This results in a Stokes' shift of roughly 450 nm from the respective maxima.

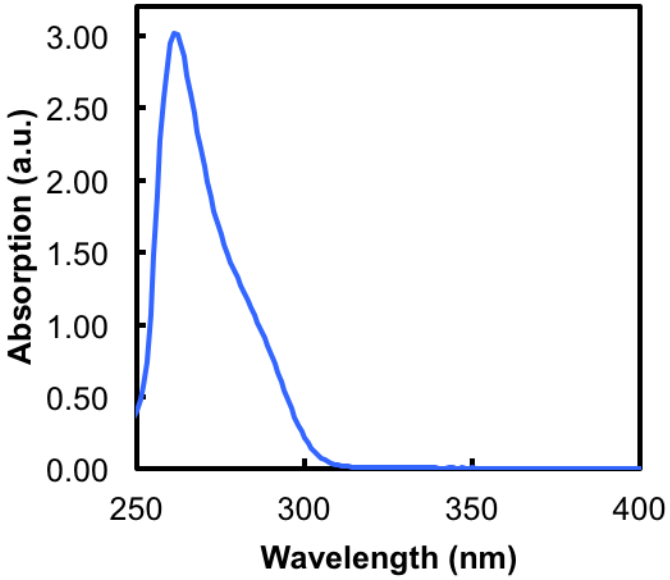


Figure 19. Excitation spectrum for L_2Eu_2.

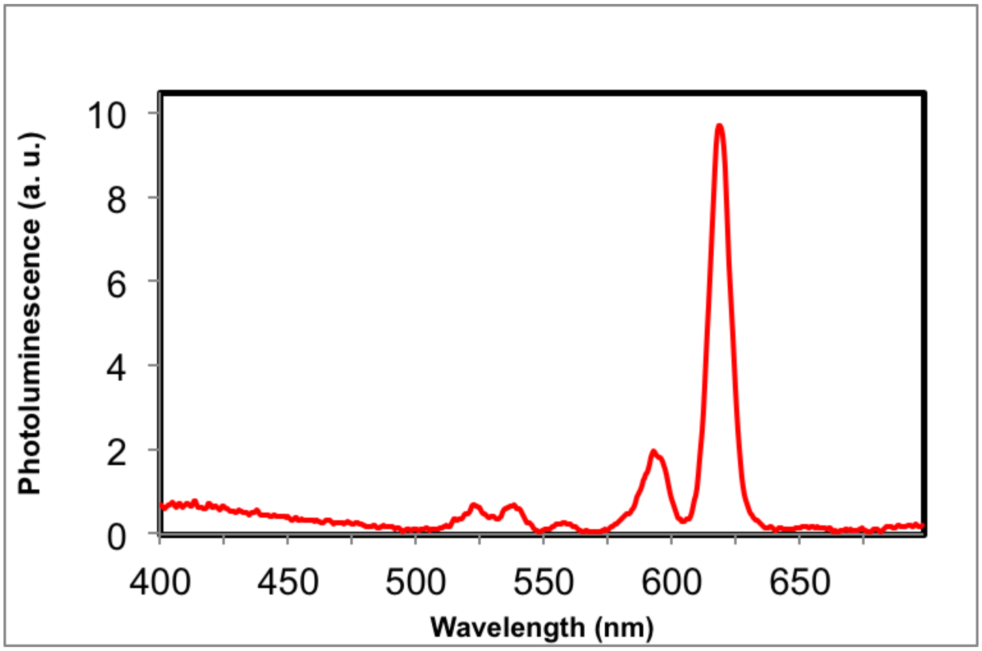


Figure 20. Emission spectrum for L_2Eu_2.

Figure 21 shows the clear solutions of L_2La_2, L_2Eu_2, L_2Tb_2, L_2Dy_2, and L_2Sm_2 in dimethyl sulfoxide. Figure 22 shows the emission of the compounds using a 256 nm UV light.

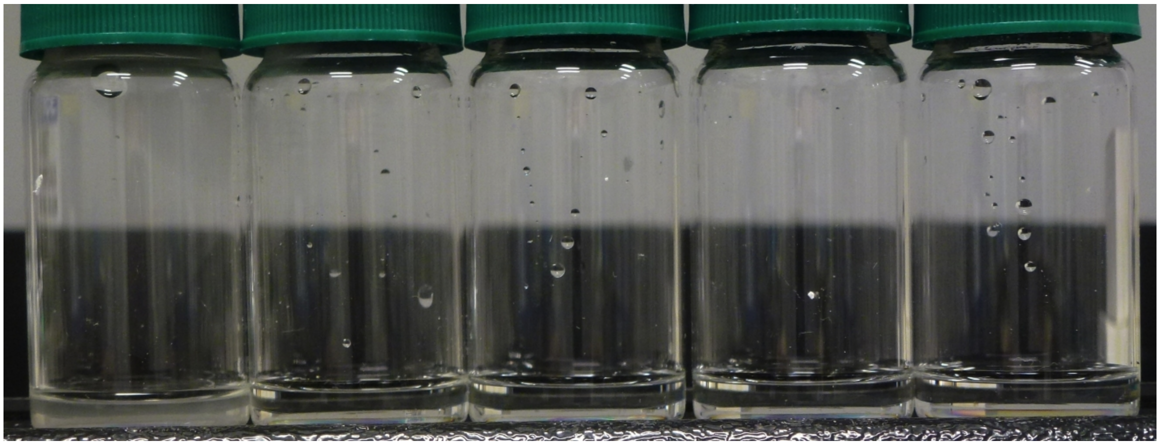


Figure 21. Solutions of L_2La_2, L_2Eu_2, L_2Tb_2, L_2Dy_2, and L_2Sm_2 in dimethyl sulfoxide.

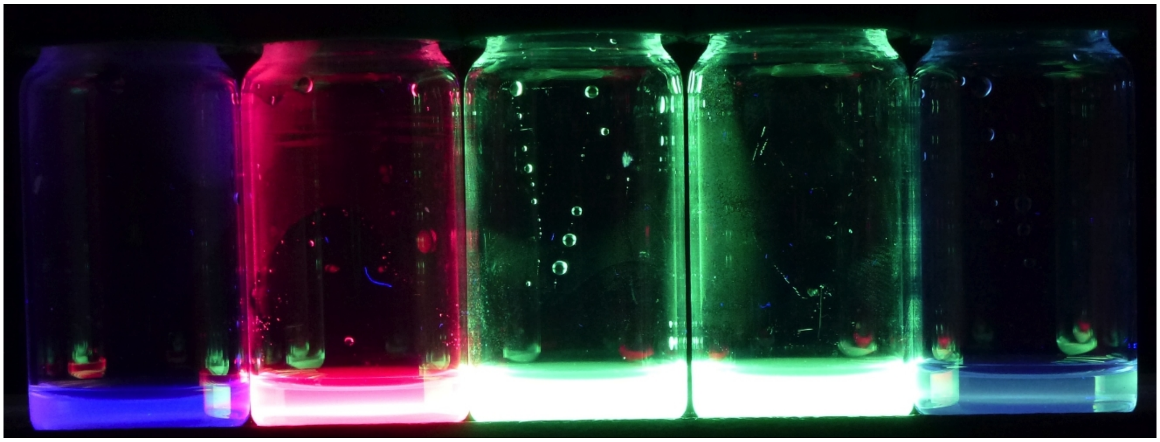


Figure 22. Solutions of L_2La_2, L_2Eu_2, L_2Tb_2, L_2Dy_2, and L_2Sm_2 in dimethyl sulfoxide emitting under UV irradiation (256 nm)

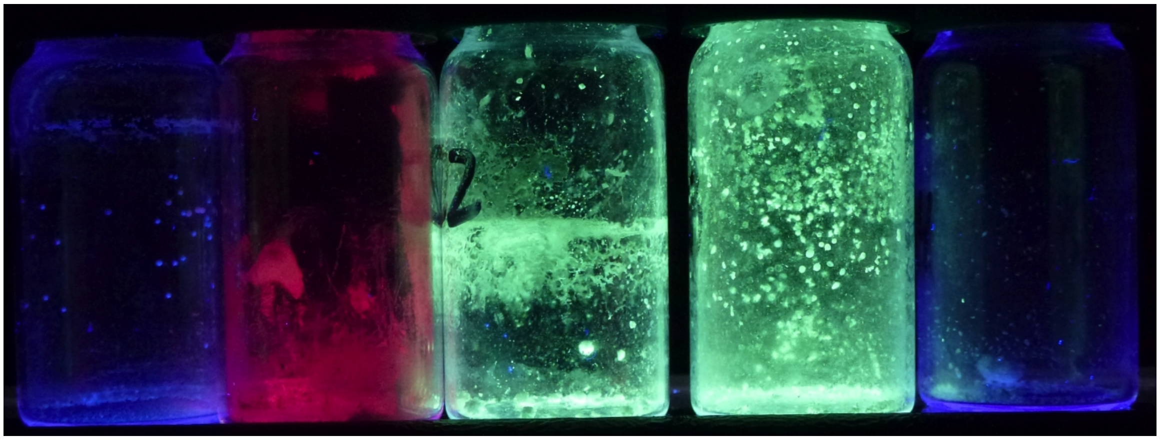


Figure 23. Solids L_2La_2, L_2Eu_2, L_2Tb_2, L_2Dy_2, and L_2Sm_2 emitting under UV irradiation (305 nm).

A qualitative measure of the intensity of emission of a material is whether that emission can be seen with the naked eye especially in the solid state. In both the solution phase and solid state, these compounds are intense emitters showing the viability of this strategy.

3.4.2. Two-Photon Upconversion

In collaboration with Carl Hayden, we were able to observe the L2Eu2 compound absorb two photons upon excitation with a laser at 532 nm. This is not a common feature of lanthanide compounds, at least at the intensities that we are performing our excitation. This might be of use in the detection community, especially if we can excite using wavelengths further into the infrared region.

3.5. Final Overall Conclusions

This project has been objectively successful from the standpoint of the LDRD Early Career program. The intention to integrate a new scientist into the Sandia R&D community was achieved. Publications were written and accepted, collaborations both external and internal were identified and created, and new proposals were successfully funded.

Professor John Arnold and Heather Buckley at UC Berkeley were key contributors to this project. It is also likely that our collaboration will continue beyond the period of performance because the work was so successful. The LDRD proposal based on lanthanide tetrapyrroles developed in our project went to the second round, which I consider an achievement.

Another lanthanide LDRD based on the luminescence of cerium for the purposes of radiation detection was successfully funded for three years starting in FY13.

Integration of my work with the biological sciences here at Sandia has been limited, because the project had not achieved a sufficient level of sophistication to be incorporated into biological assays. This was unfortunate, but with continued work done at Berkeley and peripherally on these systems, a collaboration is still a possibility.

[Blank page following section.]

4. REFERENCES

(1) Bünzli, J.-C. G. *Acc. Chem. Res.* **2006**, *39*, 53–61.
(2) Parker, D.; Dickins, R.; Puschmann, H. *Chemical* … **2002**.
(3) Bünzli, J.-C. G. *Chem. Rev.* **2010**, *110*, 2729–2755.
(4) Richardson, F. S. *Chem. Rev.* **1982**, *82*, 541–552.
(5) Moore, E. G.; Samuel, A. P. S.; Raymond, K. N. *Acc. Chem. Res.* **2009**, *42*, 542–552.
(6) Horrocks, W. D., Jr; Wong, C. P. *J. Am. Chem. Soc.* **1976**, *98*, 7157–7162.
(7) Khalil, G. E.; Thompson, E. K.; Gouterman, M.; Callis, J. B.; Dalton, L. R.; Turro, N. J.; Jockusch, S. *Chemical Physics Letters* **2007**, *435*, 45–49.
(8) Jiang, J.; Ng, D. K. P. *Acc. Chem. Res.* **2009**, *42*, 79–88.
(9) Zhu, X.; Wong, W.-K.; Wong, W.-Y.; Yang, X. *Eur. J. Inorg. Chem.* **2011**, *2011*, 4651–4674.
(10) Moussavi, M.; De Cian, A.; Fischer, J.; Weiss, R. *Inorg Chem* **1986**, *25*, 2107–2108.
(11) George, S.; Lipstman, S.; Goldberg, I. *Crystal Growth & Design* **2006**, *6*, 2651–2654.
(12) Ishikawa, N.; Kaizu, Y. *Coordination Chemistry Reviews* **2002**, *226*, 93–101.
(13) Semenishyn, N.; Gross, Z. *Dalton Trans.* **2013**.
(14) Koszarna, B.; Gryko, D. T. *J. Org. Chem.* **2006**, *71*, 3707–3717.
(15) Thomas, K. E.; Alemayehu, A. B.; Conradie, J.; Beavers, C.; Ghosh, A. *Inorg Chem* **2011**, *50*, 12844–12851.
(16) Thomas, K. E.; Alemayehu, A. B.; Conradie, J.; Beavers, C. M.; Ghosh, A. *Acc. Chem. Res.* **2012**, 120323124845004.
(17) Buckley, H. L.; Chomitz, W. A.; Koszarna, B.; Tasior, M.; Gryko, D. T.; Brothers, P. J.; Arnold, J. *Chem. Commun.* **2012**, *48*, 10766.
(18) Vestfrid, J.; Botoshansky, M.; Palmer, J. H.; Durrell, A. C.; Gray, H. B.; Gross, Z. *J. Am. Chem. Soc.* **2011**, *133*, 12899–12901.

[Blank page following section.]

DISTRIBUTION

1	MS9055	Carl Hayden	8353 (electronic copy)
1	MS9291	Blake Simmons	8630 (electronic copy)
1	MS9292	Benjamin Wu	8634 (electronic copy)
1	MS9292	Mitchell Anstey	8125 (electronic copy)
1	MS9292	Kamlesh Patel	8125 (electronic copy)
1	MS9402	Julie Fruetel	8114 (electronic copy)
1	MS9403	Michael Foster	8223 (electronic copy)
1	MS0899	Technical Library	9536 (electronic copy)
1	MS0359	D. Chavez, LDRD Office	1911

Sandia National Laboratories