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14. ABSTRACT
Controlled hydrolysis of lanthanide element or yttrium salts in the presence of amino acids yields a series of polynuclear clusters with two, four, twelve, fourteen, and fifteen lanthanide or yttrium centers bridged by hydroxides and possessing chelating amino acids. The MRI relaxivity of the gadolinium (Gd) clusters has been studied in vitro, with an unprecedented, large, pH-dependent value of 165 mM-1 s-1 for the Gd14 cluster. We have crystallized dysprosium (Dy) analogs of the dinuclear and tetranuclear clusters for neutron diffraction analysis in order to determine the dysprosium…hydrogen (hydroxide) distance. Yttrium analogs have been prepared, structurally characterized, and studied by yttrium-89 NMR spectroscopy in order to determine solution structures. The first attempt at preparing a Y15 cluster gave, in the presence of residual carbonate, an unprecedented Y60 polyhedral cluster that suggest routes to larger Gd clusters. Europium (Eu) cluster analogs have been prepared and their reduction explored in order to exploit the Eu(II)-Eu(III) redox couple and develop a redox-sensitive MRI reporter for reactive oxygen species in tumors. A potentially multinuclear chelating ligand with amino acid serine moieties at each end has been prepared by multi-step organic synthesis for ditopic coordination of the Gd14 cluster.

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INTRODUCTION

This research involves the development of polynuclear gadolinium cluster chemistry, with the purpose of developing high-sensitivity magnetic resonance imaging (MRI) contrast agents that could be bioconjugated to PSMA inhibitors for targeted imaging of prostate cancer and its metastases. The chemistry scope of the project involved (1) synthesis, solid-state, and solution characterization of polynuclear gadolinium clusters with amino acid ligands, in order to determine the reaction conditions and amino acid ligands needed for clusters with different gadolinium numbers, (2) the development of the corresponding chemistry for related lanthanide elements (dysprosium, europium, yttrium) that would provide clearer insight into the physicochemical properties of the gadolinium clusters, and (3) the design and organic synthesis of ditopic ligands, based on amino acids, that could span adjacent gadolinium centers in the clusters. The ditopic ligands were designed to encapsulate the clusters in order to improve biocompatibility and provide reactive chemical sites for future bioconjugation to small-molecule inhibitors of PSMA, prostate-specific membrane antigen.

BODY

MRI is at present a minor imaging modality in diagnosis of prostate cancer, and has not played a role in detection of metastases such as skeletal metastases. A major limitation of current MRI contrast agents for prostate cancer diagnosis and metastatic imaging is their low sensitivity, relative to nuclear medicine imaging agents, and lack of specificity. This precludes the ready use of contrast-enhanced MRI in noninvasive imaging, which is unfortunate because of the far higher resolution inherent in MRI over nuclear medicine imaging. Current MRI contrast agents are based on a single Gd(III) ion (the most paramagnetic metal ion, with 7 unpaired electrons) within an encapsulating acyclic or macrocyclic ligand that binds the toxic Gd(III) ion while providing inner sphere coordination of one water molecule. We postulated that polyhedral polygadolinium complexes, particularly nonspherical ones, would represent a paradigm shift in MRI contrast agent design by multiplying the relaxivity, and thus the imaging signal, on a molecular level.

We have been investigating the application of inorganic cluster chemistry to biomedical imaging for several years, with an emphasis on high atomic number elements (tantalum, tungsten, bismuth) for X-ray imaging and gadolinium for MRI. We have also developed several metal clusters, particularly of tantalum, as phasing compounds for protein and macromolecular crystallography. We discovered, prior to PCRP proposal preparation, that simple hydrolysis of $Gd(ClO_4)_3$ (ClO_4^- = perchlorate) with aqueous sodium hydroxide in the presence of the amino acid L-serine gave in high (>90%) yield the novel, water-soluble cluster $[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(OH_2)_8(ser)_{20}](ClO_4)_4$ (= Gd_{14}). It can be described as two Gd_8 square antiprisms sharing a common four-fold face, with a Gd(III) in the center of each antiprism and each face having a bridging hydroxo. Its solid-state molecular structure is shown below. Initial *in vitro* measurements (without pH control via buffers) gave a remakably high value for relaxivity, 145 $mM^{-1}\ s^{-1}$, approximately 30 times that of clinical MRI contrast agents based on mononuclear Gd chelates. Based on this key discovery of a multiplied-relaxivity molecule, we proposed several specific tasks.

The specific tasks in the Statement of Work in the proposal were

Task #1: Prepare bioconjugate of polygadolinium cluster tethered to PSMA inhibitor

a. Perform molecular modelling of tether for bis(serine) ligand
b. Prepare bis(serine) ligand and examine ligand exchange reactions with Gd_{14} cluster
c. Prepare PSMA inhibitor with functionalizable group on para position of aryl group
d. Couple aryl group to a PEG-like tether

e. Couple tether to bis(serine) ligand
f. Perform ligand exchange with Gd_{14} cluster
g. Characterize Gd_{14}-tether-PSMA inhibitor bioconjugate agent

Task #2: Determine *in vitro* relaxivity of Gd_{14} bioconjugate agent on MR scanner

Task #3: Determine binding and relaxivity of bound bioconjugate
a. Add bioconjugate agent to cell cultures containing PSMA-positive and PSMA-negative (as control) prostate cancer cells, wash away unbound bioconjugate agent, and assay cells for Gd content
b. Determine relaxivity of bioconjugate bound to PSMA-positive prostate cancer cells

After initial chelation attempts failed with simple mononuclear chelating agents (all led to disassembly and precipitation of the cluster), and recent reports that clinical Gd MRI contrast agents were of sufficient toxicity to lead to a fatal dermatologic disease for kidney-compromised patients, we decided that it was crucial to determine the solution structure of Gd_{14}, to prepare other polygadolinium clusters, each of which could provide insight into the molecular bases for the high relaxivity of Gd_{14}, and to design new ditopic ligands (ones that could coordinate to two or more different Gd centers in a cluster. The relaxivity per Gd for various Gd nuclearity clusters would provide insight into the role of the molecular shape (and thus molecular correlation time) on relaxivity. The strong dependence of relaxivity on Gd···H distance, as noted for mononuclear Gd MR contrast agents, meant that future molecular design would be influenced by the distance between three Gd's and the μ_3-OH hydrogen and any inner sphere waters. Furthermore, the presence of triply-bridging hydroxides on each polyhedral face might provide a novel, new mechanism for relaxation via hydrogen-bonding/exchange of the hydroxide proton with that of a water proton through a four-center transition state.

The research proceeded in three parallel chemistry directions: (1) preparation of known and new polygadolinium clusters, in order to study relaxivity as a function of Gd content; (2) preparation of dysprosium, europium, and yttrium analogs for neutron diffraction and solution NMR structural studies; (3) design and synthesis of ditopic ligands for Gd_{14} based on serine.

Preparation of known and new polygadolinium and polylanthanide clusters, in order to study relaxivity as a function of Gd content and obtain physicochemical preperties

In order to examine relaxivity as a function of cluster size and also the presence or absence of μ_3-OH ligands, we prepared several known polygadolinium clusters based on literature reports and discovered new clusters by varying the amino acid. The following reactions were used to prepare these clusters, with yields listed.

Ln_2:

$$Ln(ClO_4)_3\ (Ln = Gd, Dy) + 2\ L\text{-alanine} \rightarrow [Ln_2(ala)_4(OH_2)_8](ClO_4)_6$$

$$Ln(ClO_4)_3\ (Ln = Gd, Y) + 2\ L\text{-valine} \rightarrow [Ln_2(val)_4(OH_2)_8](ClO_4)_6$$

Reaction conditions: no NaOH used; yield: 25 - 30%

Figure 1 shows the solid-state structure of $[Gd_2(H\text{-ala})_4(OH_2)_8](ClO_4)_6$. Figure 2 shows the isostructural view of $[Y_2(H\text{-val})_4(OH_2)_8]\ (ClO_4)_6$.

Figure 1: Gd_2 structure

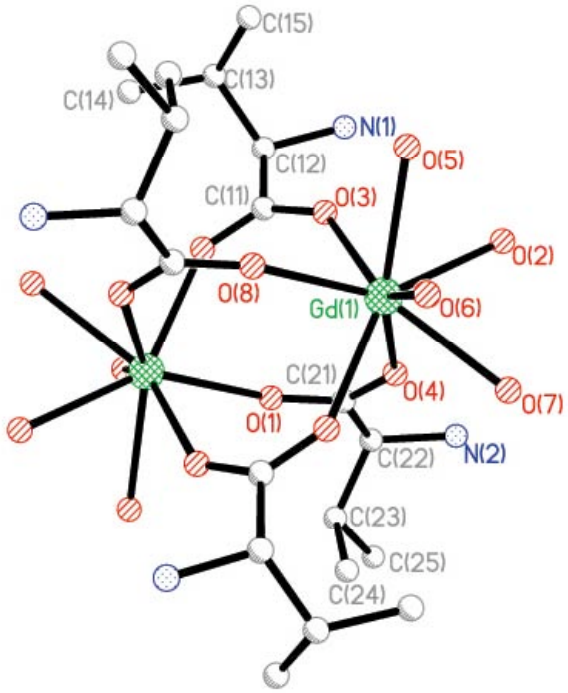
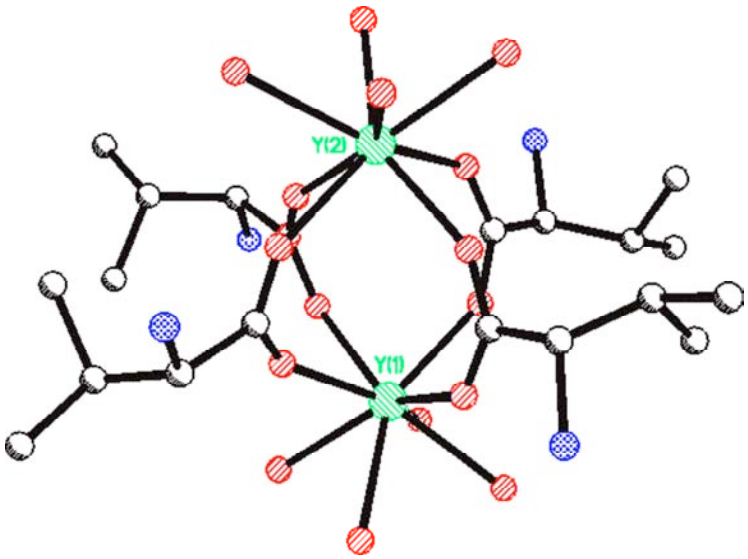


Figure 2: Y_2 structure



<u>Ln_4</u>:

$2Ln(ClO_4)_3$ (Ln = Gd, Dy) + L-proline + NaOH $\rightarrow$ $[Ln_4(\mu_3\text{-}OH)_4(pro)_6(OH_2)_8](ClO_4)_8$

$2Ln(ClO_4)_3$ (Ln = Gd, Dy) + L-Valine + NaOH $\rightarrow$
$[Ln_4(\mu_3\text{-}OH)_4(val)_6(OH_2)_8]$ + $[Ln_4(\mu_3\text{-}OH)_4(val)_6(OH_2)_7(ClO_4)](ClO_4)_{15}$
Reaction conditions: pH adjusted to ~ 6.5 with 0.3 M NaOH, yield: ~ 20%

Figure 3 shows the solid-state molecular structure of $[Gd_4(\mu_3\text{-}OH)_4(proline)_6(OH_2)_8](ClO_4)_8$. Four Gd(III) form a fairly regular tetrahedron with distances of Gd···Gd: 3.811(5), 3.903(4) Å. Each of the four triangular faces is capped by a μ_3-OH.

Figure 3: Gd_4 structure

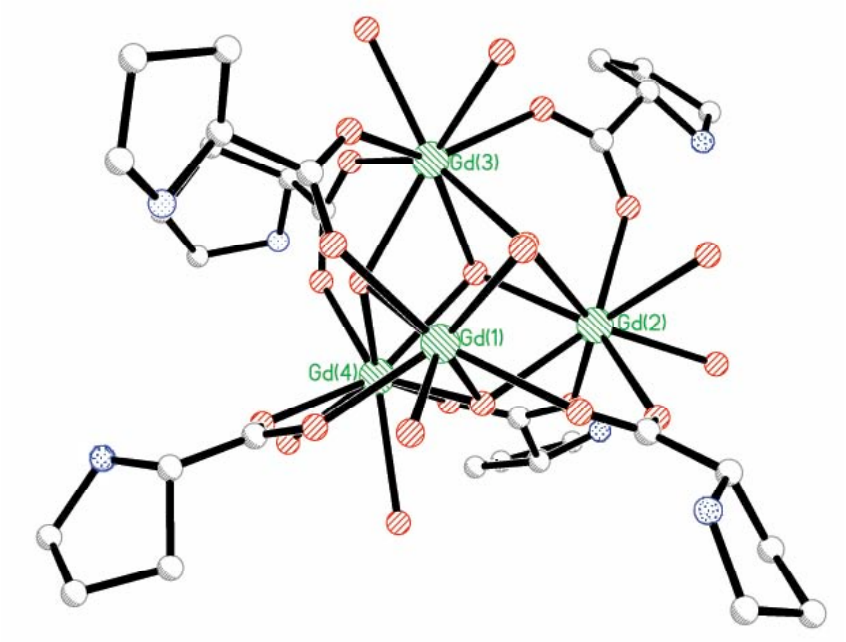
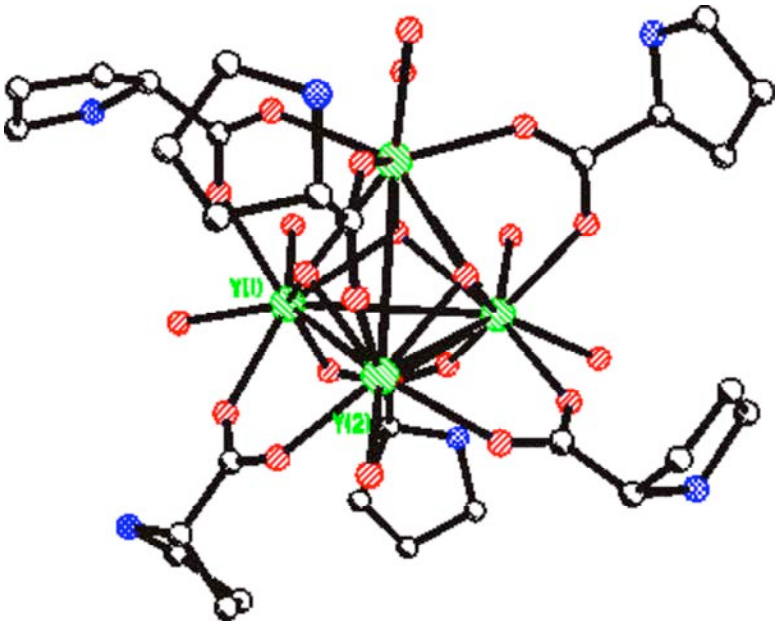


Figure 4 shows the corresponding structure of the yttrium analog, $Y_4(\mu_3\text{-}OH)_4(proline)_6(OH_2)_8]$ $(ClO_4)_8$. It is isostructural to the Gd_4 compound above.

Figure 4: Y_4 structure



<u>Ln_{14}</u>:

$$Gd(ClO_4)_3 + NaOH + \text{L-serine} \rightarrow [Gd_{14}(\mu_3\text{-}OH)_{16}(\mu_4\text{-}OH)_2(ser)_{20}(OH_2)_8]\,(OH)(ClO_4)_3$$

92% yield

Figures 5, 6, and 7 show the molecular structure, basic coordination geometries, and the binding modes (three types) of the twenty serine ligands. The serines coordinate

coordinated by the carboxylates, amino groups, and in one case the hydroxyl also; the latter is an unprecedented coordination mode for serine as a ligand. The central portion of six Gd's does not contain a μ_6-O. A novel feature of the structure are the two end-capping μ_4-OH groups, which provide hints as to the mechansim of formation as well as being potential sites for lengthening the rod-like cluster.

Figure 5: Gd_{14} structure

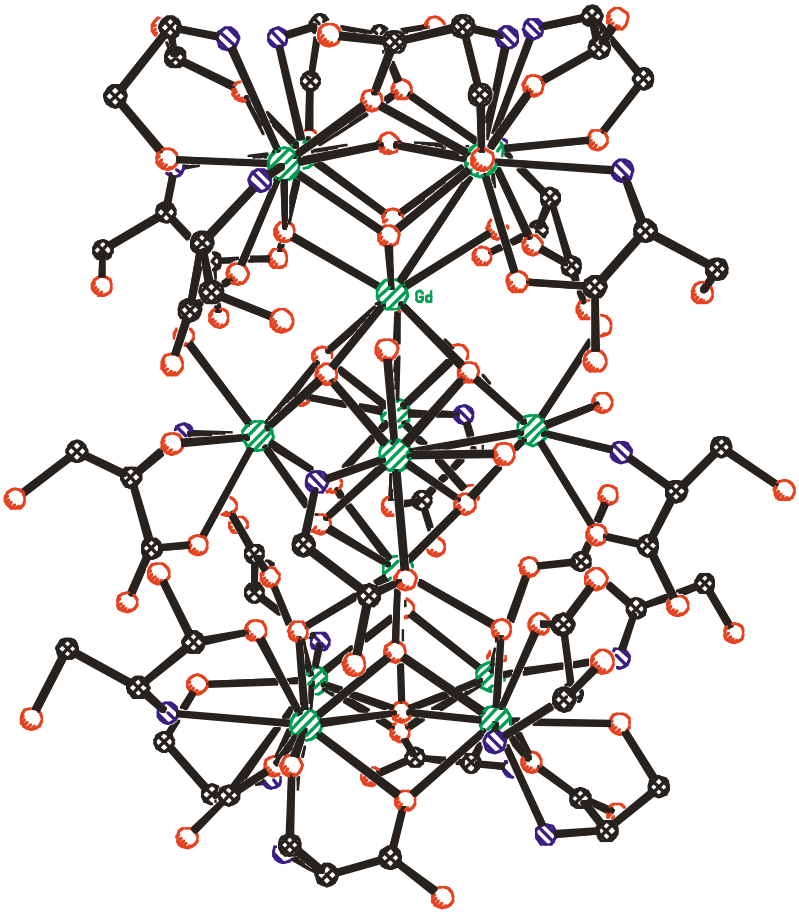


Figure 6: Gd_{14} ligand coordination

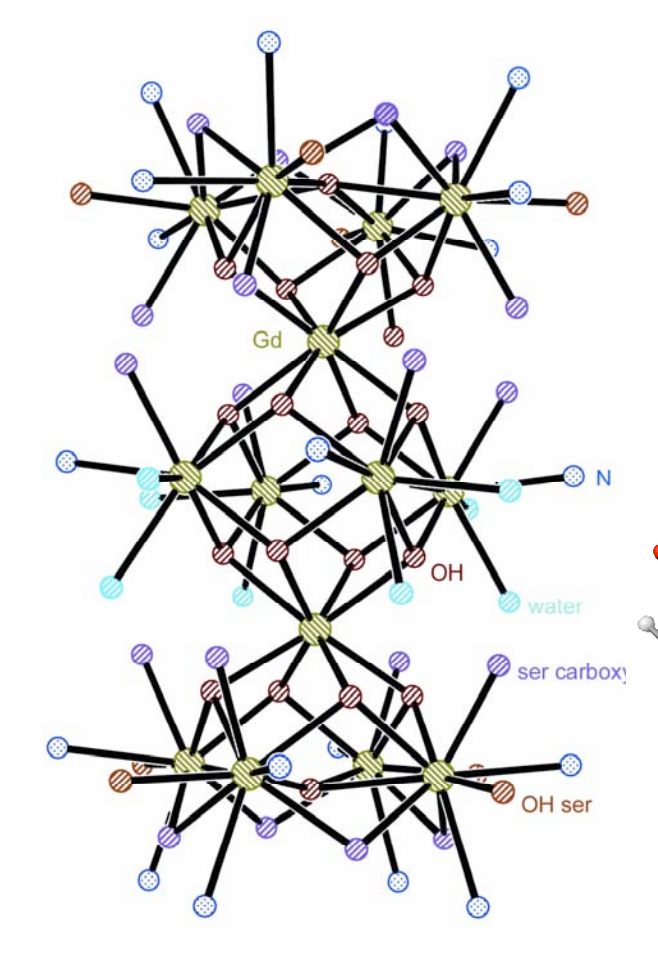
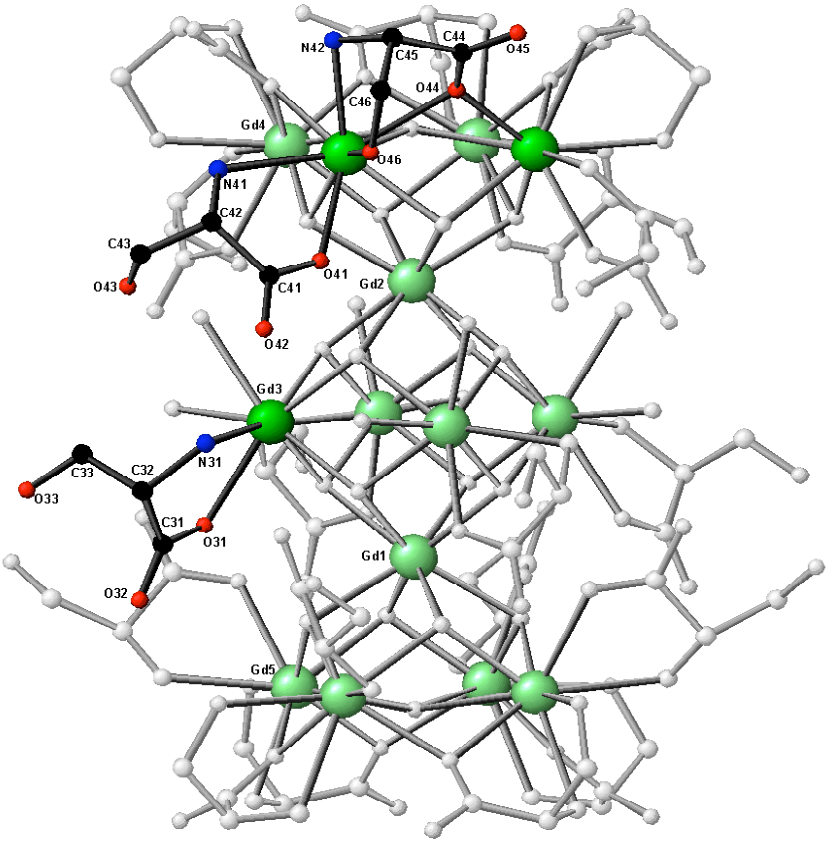


Figure 7: Gd_{14} view depicting three different serine coordination modes



The yttrium analog has also been prepared in ~50% yield. It is isostructural to the Gd_{14} compound, but less soluble in water, which has limited ^{89}Y NMR studies.

Table 1 summarizes the key molecular structure data for several new Ln_{14} complexes as determined by single-crystal X-ray diffractometry:

Table 1:

Structural parameter	Y_{14}	Dy_{14}	Gd_{14}
μ_4-OH above plane	0.410	0.406	0.375
Ln-O (μ_4-OH)	2.638	2.649	2.676
Ln-O (μ_3-OH)	2.36	2.37	2.39
M-M (apical)	5.496	5.542	5.562
M-M (apex-plane)	3.722	3.738	3.917
M-M (adjacent plane)	3.724	3.744	3.901
M-M (opposite plane)	5.404	5.496	5.517

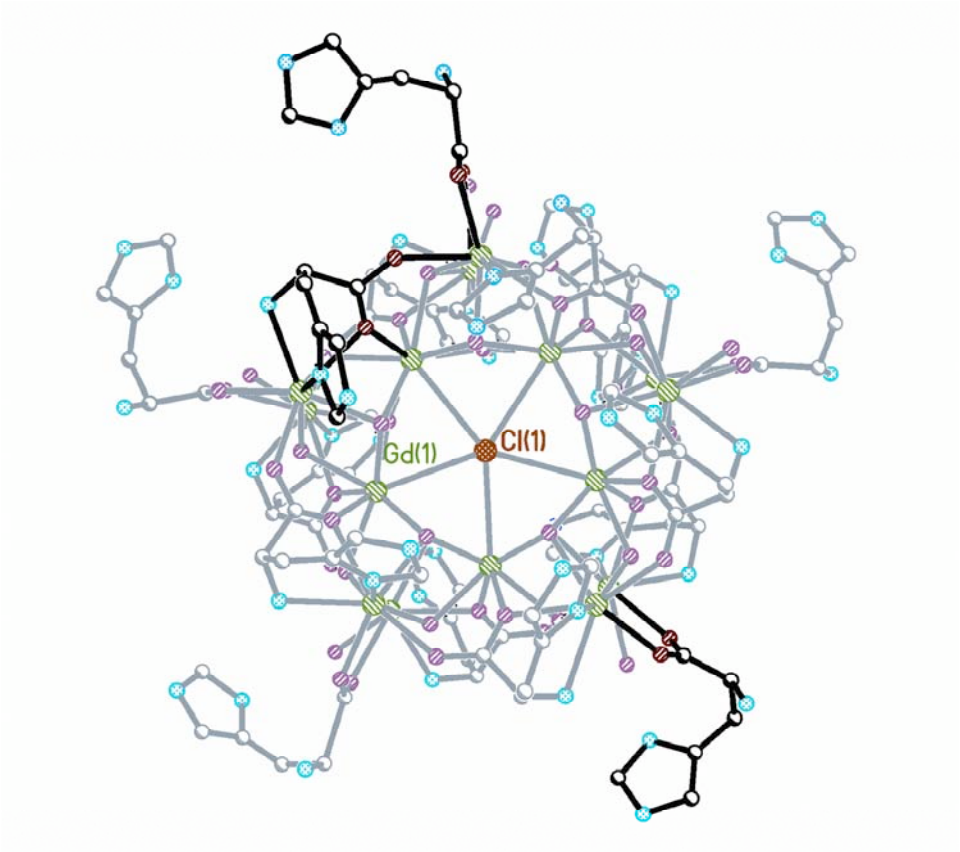
$\underline{Ln_{15}}$:

$$Gd(ClO_4)_3 + 2\ \text{L-histidine} + KX + NaOH \rightarrow [Gd_{15}(\mu_5\text{-}X)(\mu_3\text{-}OH)_{20}(\text{histidinate})_{15}(OH_2)_8](ClO_4)_9$$

Reaction conditions: 0.3 M NaOH added dropwise, pH 7; yield: 63% (Cl), 70% (Br)

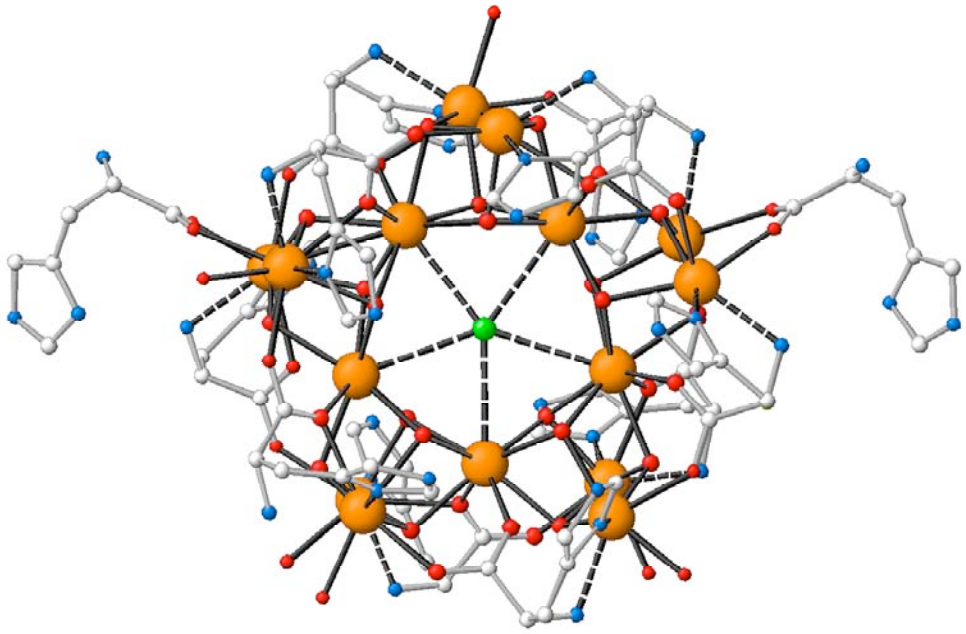
The structure of $Gd_{15}(\mu\text{-Cl})$ is shown in Figure 8. This is the first instance of the use of histidine to make a polygadolinium cluster. There are five vertex-sharing cubanes in three parallel layers of five Gd(III) ions, with a Gd···Gd separation of 6.028Å between outer Gd's and 3.828 Å between inner Gd's. The average Gd-Cl distance is 3.236 Å.

Figure 8: Gd_{15} structure



The corresponding Y_{15} has been prepared straightforwardly in good yield, and was found to be isostructural to the Gd_{15} compound. Figure 9 depicts the molecular structure.

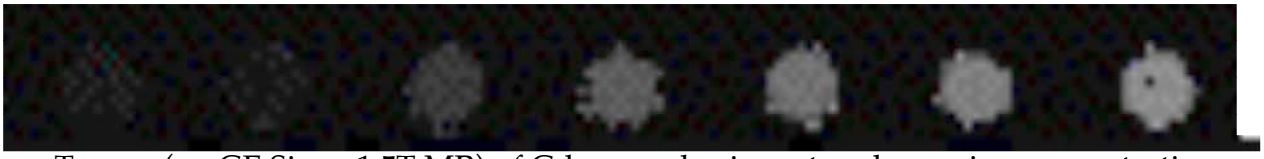
Figure 9: Y_{15} structure



Relaxivity Studies:

Aqueous solutions of varying concentrations of Gd_{14} and $Gd_{15}Cl$ were placed in vials within a vial file and positioned in a 1.5 T MR scanner, and T1 measurements were made by inversion recovery with differing delay times compared to various controls. The resulting T_1 maps are shown in Figure 10 and Figure 11. Figure 12 shows the first plot of the T1 data vs coincentration, with the slope indicating a high relaxivity of $mM^{-1}\ s^{-1}$. Table 2 shows a comparison of the molecular relaxivity compared to clinical mononuclear (single Gd) MR contrast agents.

Figure 10: Gd_{14} relaxivity images



T_1 map (on GE Signa 1.5T MR) of Gd_{14} complex in water, decreasing concentrations (left to right) of

0.1	0.05	0.025	0.01	0.005	0.0025	0.001 mM
with T_1's of						
0.07	0.13	0.63	1.25	1.69	1.96	2.26 s

Figure 11: $Gd_{15}(\mu\text{-Cl})$ relaxivity: T_1 map

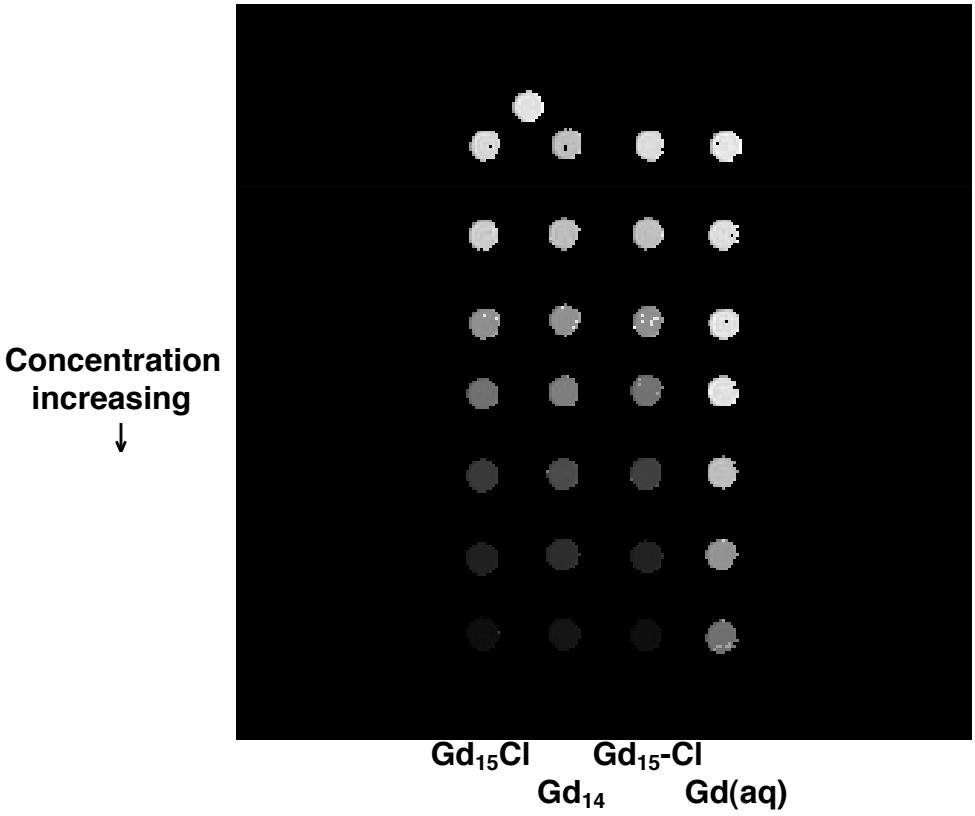


Figure 12: Plot of $1/T_1$ vs. Gd_{14} concentration

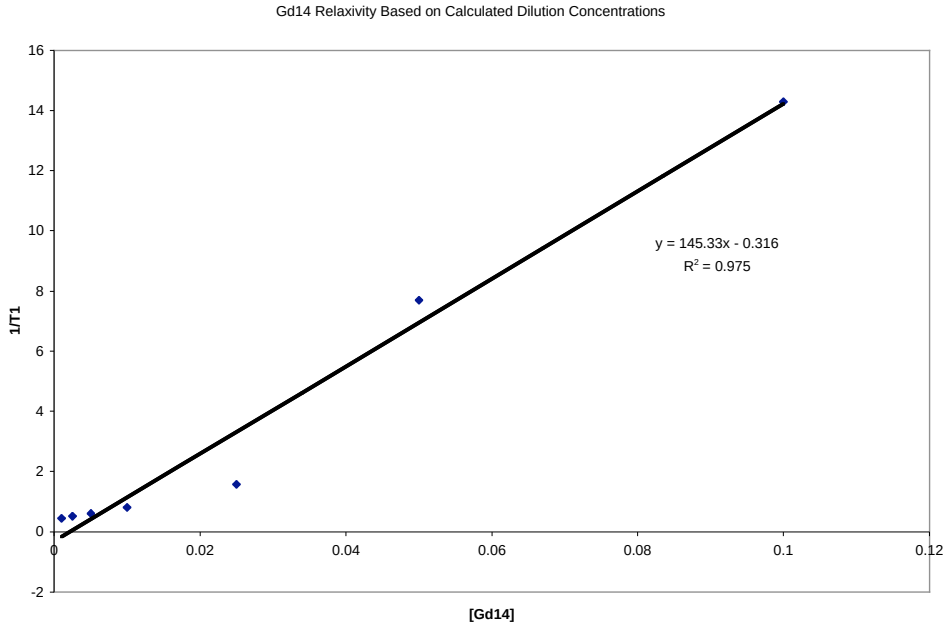


Table 2: Relaxivities of mononuclear Gd vs. Gd_{14}

Complex	Gd-OH_2 (Å)	r_1 ($mM^{-1}s^{-1}$)
ProHance™ [Gd(HP-DO3A)(H_2O)]	2.50	3.7
Magnevist™ $[Gd(DTPA)(H_2O)]^{2-}$	2.49	4.3
Omniscan™ [Gd(DTPA-BMA)(H_2O)]	2.46	4.58
$Gd_6(\mu_6\text{-}O)(\mu_3\text{-}OH)_8(H_2O)_{20}$	2.411-2.597	46
$Gd_{14}(ser)_{20}(OH_2)_8$	2.44(3)-2.52(2)	145

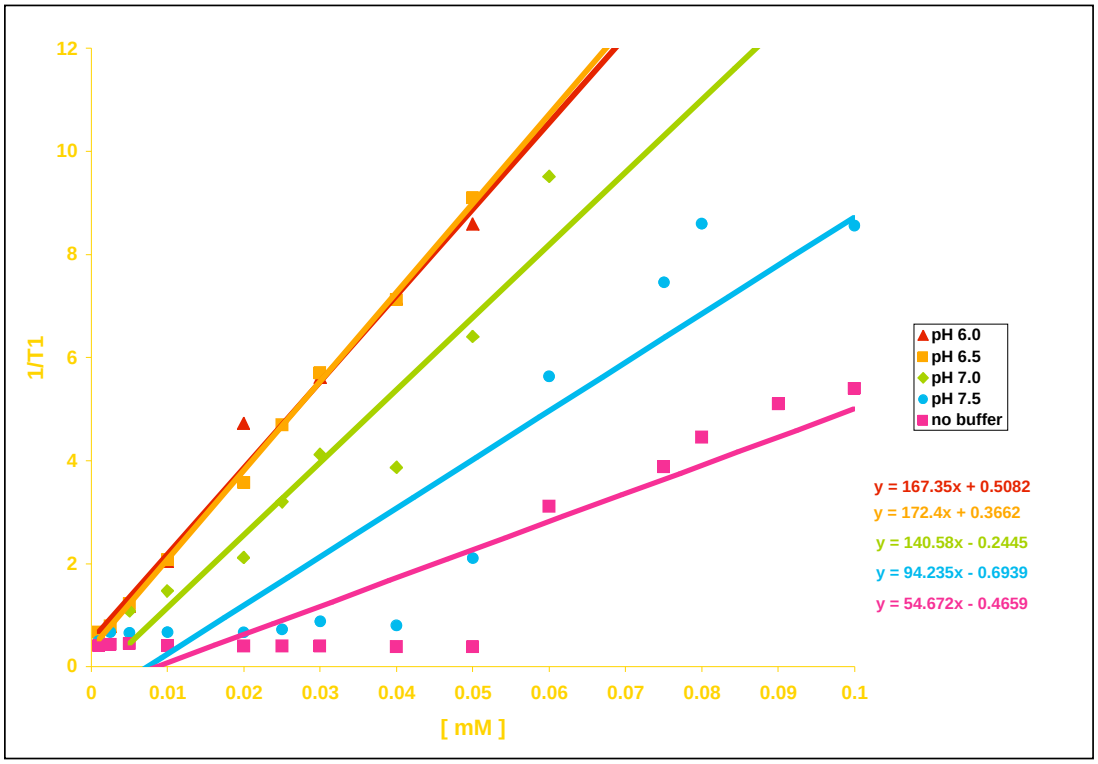
In later studies we observed variations in the relaxivity of Gd_{14}, and decided to probe the pH dependence of the relaxivity by using buffered solutions (using 3-(N-morpholino)propane sulfonate, MOPS, as buffering agent). Table 3 lists the relaxivity data as a function of pH, showing a clear pH dependence. These observations suggest that a prototopic relaxation mechanism is operative, with higher relaxivities at lower pH's. Figure 13 shows a plot of $1/T_1$ vs Gd_{14} concentration at different pH's. The nonlinearity at low concentrations is not understood at present.

Table 3: Gd_{14} relaxivity dependence on pH

Complex	r_1 ($mM^{-1}s^{-1}$)
$[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8]$ (MOPS buffer – pH 6.0)	167.35
$[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8]$ (MOPS buffer – pH 6.5)	172.40
$[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8]$ (MOPS buffer – pH 7.0)	140.58
$[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8]$ (MOPS buffer – pH 7.5)	94.24
$[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8]$ (NEM buffer – pH 7.0)	104.55
$[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8]$ (water – pH ~ 8.5)	54.67

(0.1 – 0.001 mM Gd_{14}, 300 mM MOPS buffer, pH 6.0, 6.5, 7.0, & 7.5)

Figure 13: Plot of Gd_{14} $1/T_1$ vs concentration as a function of pH



We then examined the relaxivity of various Gd_n at a buffered pH = 7.0. Table 4 shows the comparative relaxivity at 1.5 T per Gd as a function of Gd_n cluster size. Assuming that the cluster structures are retained in solution, then the less spherical, rod-shaped Gd_{14} has the highest relaxivity per Gd. This points to a rotational correlation time component to the relaxivity.

Table 4: Gd_n relaxivity in buffered solution vs n

Complex	r_1	r_1 / Gd
$[Gd_2(H\text{-}ala)_4(OH_2)_8]^{6+}$	14.13(14)	7
$[Gd_4(\mu_3\text{-}OH)_4(H\text{-}pro)_6(OH_2)_8]^{8+}$	20.47(70)	5.1
$[Gd_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8]^{4+}$	132(14)	9.4
$[Gd_{15}(\mu_5\text{-}Br)(\mu_3\text{-}OH)_{20}(his)_{15}(OH_2)_8]^{9+}$	110.67(29)	7.4

(r_1 in units of mM^{-1} s^{-1}; number in parentheses represents standard deviation; MOPS buffer, pH 7.0)

Ongoing synthetic studies are involving non-perchlorate starting materials (tosylate, triflate) and anion metathesis in order to move to less toxic anions.

<u>Preparation of dysprosium, yttrium, and europium analogs for neutron diffraction and solution NMR structural studies, and redox sensing</u>

Neutron diffraction is the most accurate technique for determining element-H bond distances, since unlike X-ray diffraction it is not dependent on the number of core and valence electrons, but rather the neutron crossection, and hydrogen positions can be determined easily. Gadolinium has the highest neutron cross section of nearly all elements, so neutron diffraction is not possible on gadolinium compounds. The close lanthanide relative dysprosiumhas a low neutron crosssection, so neutron diffraction on dysprosium

analogs can provide the needed data to model structural paramters for gadolinium compounds. The only requirement for neutron diffraction, besides access to a neutron source, is very large crystals. We were fortunate in being able to grow large single crystals of Dy_2 (to provide Dy···H distances for inner sphere water molecules) and Dy_4 (to provide Dy···H distances for μ_3-OH groups on Dy_3 faces of the Dy_4 tetrahedron) complexes. We were successful in obtaining neutron source time at Argonne National Laboratory via competitive proposals to DOE, and both data sets have now been collected. The final refinement of the Dy_2 structure shows Dy···H distances of 2.91-3.11 Å. Structure refinement via merging with single-crystal X-ray diffraction data is now complete for Dy_4. We are preparing a manuscript that details the first single-crystal neutron diffraction study of lanthanide···H distances to bridging hydroxides and to terminal water ligands. Previous studies have determined Ln···H water distances only in solution for the aquo ions.

The key question in our studies of polynuclear gadolinium complexes is the *solution* structure of the cluster compounds. Single-crystal X-ray diffraction is a powerful tool for determining molecular structure, but is only relevant to the solid-state structure. Solution structures, particularly for aqueous solutions, can be very different – in fact, it is possible that a polynuclear complex can dissociate in solution to mononuclear aquated/solvated fragments. The high paramagnetism of gadolinium precludes the use of the most powerful routine spectroscopic method for determining solution structures, nuclear magnetic resonance (NMR) spectroscopy. We decided to tackle this solution structure problem by the use of yttrium-89 NMR spectroscopy on polyyttrium analogs of our new polygadolinium clusters. Yttrium is chemically very similar to the lanthanides, with only a slightly smaller ionic radius. Yttrium-89 is the only naturally-occurring isotope of yttrium, being 100% abundant. It has a spin of 1/2, highly desirable for an NMR-active nucleus, and has a resonant frequency within the range of most NMR instrument probes. The only drawback is the very long relaxation times of several hundred seconds. Thus, yttrium-89 NMR spectroscopy can take several days to perform for reasonably soluble compounds. We have obtained several yttrium-89 NMR spectra on polyyttrium complexes (Y_2, Y_4, and Y_{15}) on a 400 MHz NMR spectrometer with a pulsed-field gradient (PFG) probe design and digital electronics, though significant and inexplicable experimental artifacts in the spectra have hindered data collection and analysis (we hope to solve this problem by use of an older spectrometer that uses different RF electronics). However, we have obtained the following yttrium-89 chemical shifts (referenced to aqueous YCl_3) with an acceptable degree of uncertainty:

$[Y_2(\text{valine})_4(OH_2)_8](ClO_4)_6$ δ 24.7

$[Y_4(\mu_3\text{-}OH)_4(\text{proline})_6(OH_2)_8]\ (ClO_4)_8$ δ 32.1

$[Y_{15}(\mu_5\text{-}Cl)(\mu_3\text{-}OH)_{20}(\text{histidinate})_{15}(OH_2)_8](ClO_4)_9$ δ 75.4 (integration = 2), δ 64.9 (1)

The Y-89 spectrum of Y_{15} is particularly intriguing in that two resonances, in a 2:1 ratio, are obtained, consistent with the solid-state structure showing two chemically and magnetically inequivalent yttrium centers in a 2 (outer-edge):1 (inner pentagon) ratio. This suggests that Y_{15} doesn't dissociate in solution, the first proof that at least some Gd_n clusters remain intact in water.

All attempts to date to obtain the Y-89 NMR spectrum of Y_{14} have failed because of the limited solubility of this compound in water; we are still exploring alternate solvent systems. The PI attempted to use an identical 400 MHz PFG NMR instrument while on a three-month sabbatical last spring at Los Alamos National Laboratory during three weekend runs when the instrument was not being utilized; all attempts failed because of spurious signals/artifacts that did not shift appropriately with changes in spectrometer frequency parameters. We hope to refurbish the magnet of an NMR spectrometer with older, non-pulsed-field gradient probe technology since this instrument previously demonstrated a capability to obtain ^{89}Y NMR spectra without artifacts in the early stages of

our research into polylanthanides.

We encountered a very surprising, serendipitous result during our initial attempts to prepare $Y_{15}(\mu_5\text{-Br})$ with histidine. A solution sample that had been set up for long-term crystallization was inadvertently exposed to the air during the time period. Adventitious amounts of carbonate formed from atmospheric carbon dioxide, leading to small cubic crystals that were unlike any seen previously. The resulting X-ray diffraction result was astonishing – instead of preparing the expected Y_{15} analog of Gd_{15}, we obtained instead a self-assembled Y_{60} cluster, shown in Figure 14. The keys to its formation were the presence of adventitious carbonate as well as chloride from contamination of the perchloric acid used to prepare $Y(ClO_4)_3$. The Y_{60} compound can be described as a stellated doubly-truncated octahedron, with this geometric dissection shown in Figure 15. The histidinate ligands are bound by their carboxylate groups near the four-fold axes of the octahedron, with the histidine imidazole side chains σ-π–stacking with those of adjacent Y_{60} clusters to give a supramolecular solid with cubic symmetry (Figure 16). In essence, this is to our knowledge the first example in supramolecular chemistry of self-assembly to give a cluster tecton (building unit) that then self-assembles by a separate mechanism into a supramolecular solid. While chemically exciting and unprecedented, the result is also important to future high sensitivity prostate MRI contrast agents because it suggests that larger Gd clusters such as Gd_{60} may be accessible by controlled addition of other anion templates (notably, carbonate has not been studied in polylanthanide cluster chemistry because of the well-known tendency of anions such as carbonate and phosphate to quickly precipitate lanthanides). In other words, this research has shown that strong-binding anion templates could be used to give discrete molecular species.

Figure 14: Molecular structure of Y_{60}

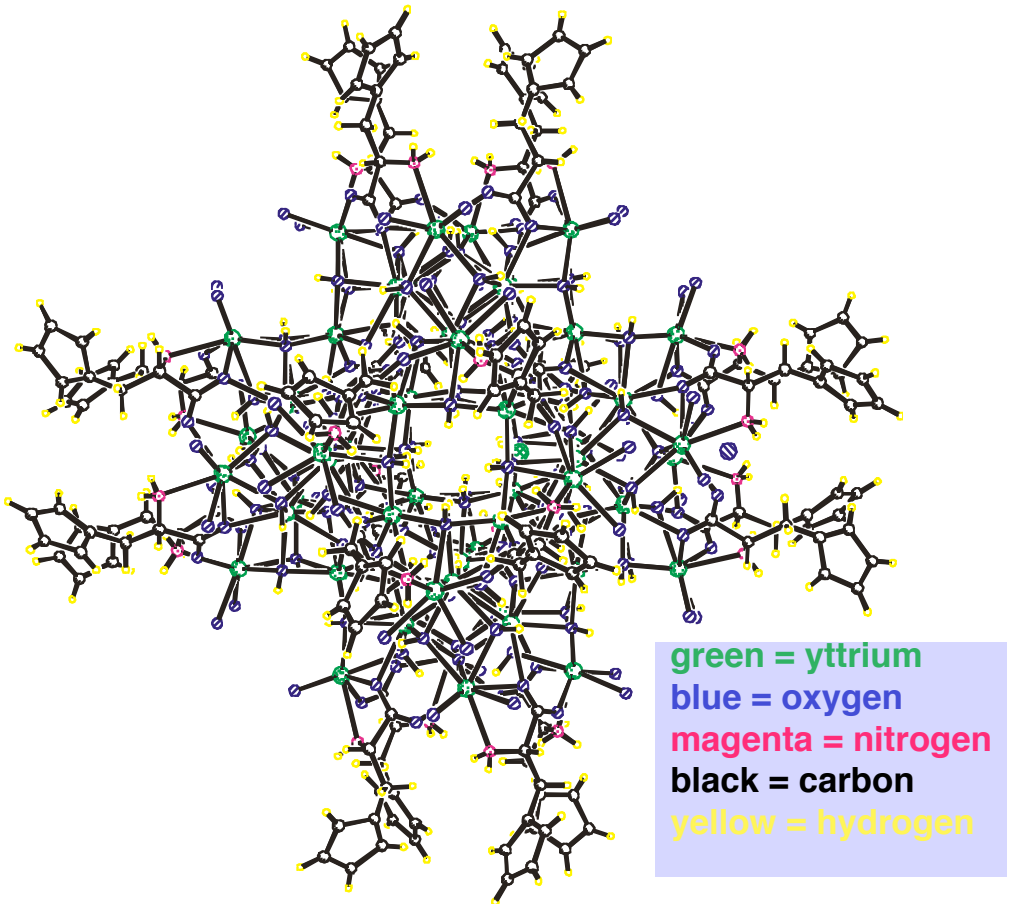
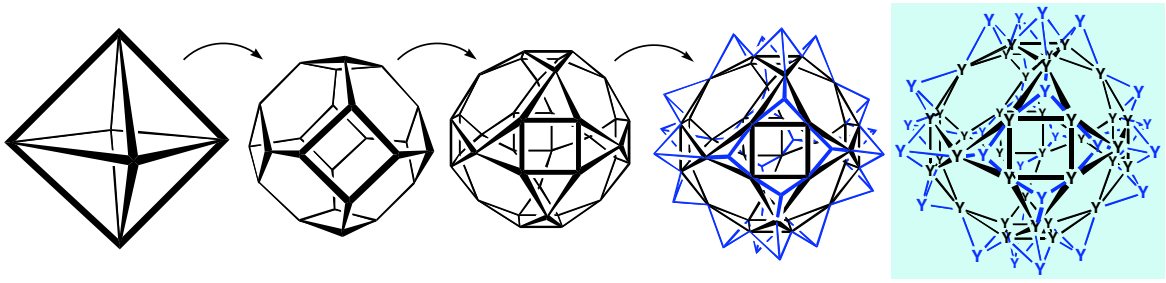
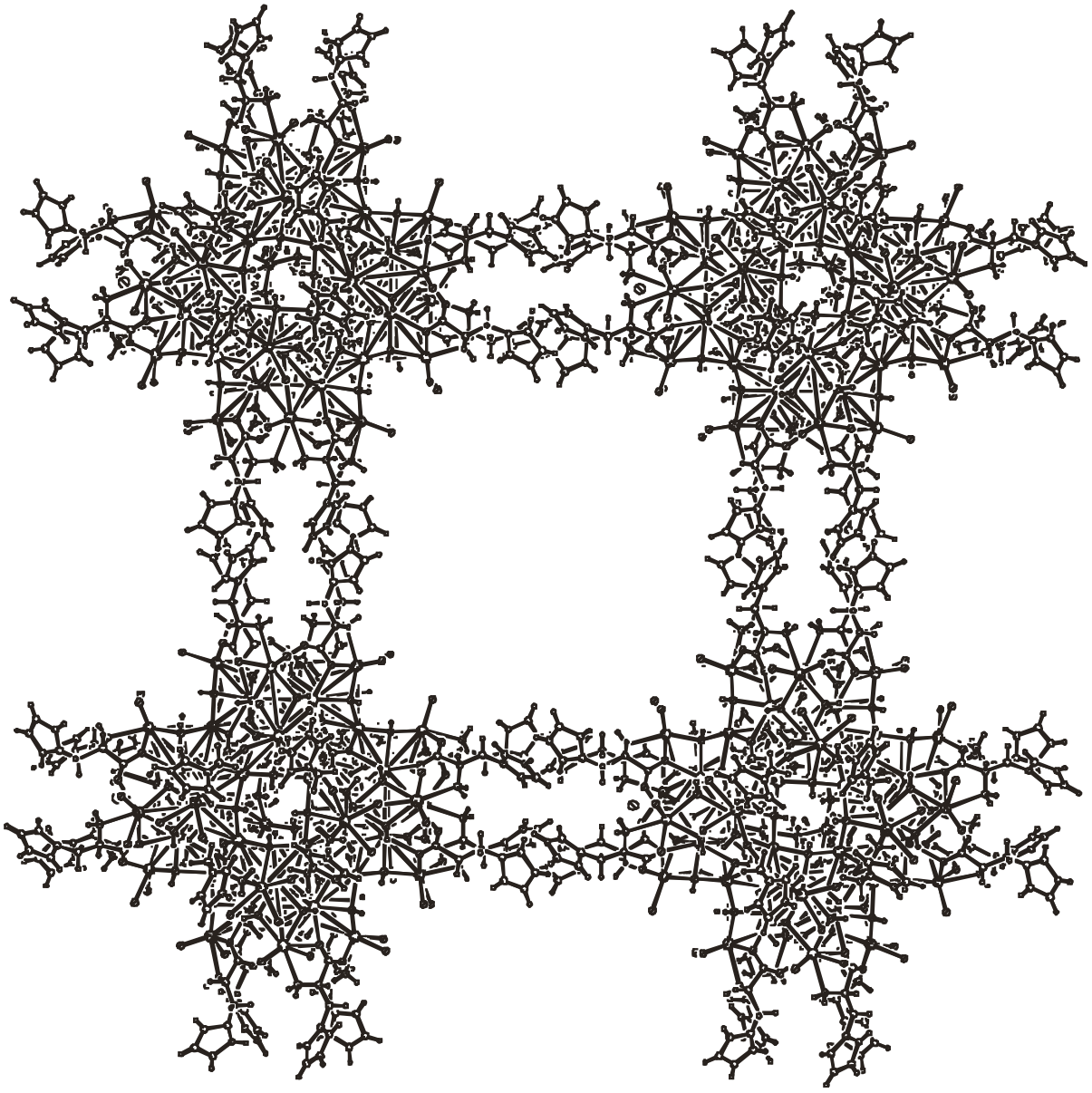


Figure 15: Geometric analysis of Y_{60} cluster



Conversion of Octahedron to Truncated Octahedron to Doubly-Truncated Octahedron to Stellated Doubly-Truncated Octahedron to Y_{60} (Y = yttrium)

Figure 16: Supramolecular self-assembly of Y_{60} tectons



We have successfully prepared europium(III) analogs of a number of the above polygadolinium complexes because of the potential use of the Eu(III)-Eu(II) redox couple to act as non-invasive physiological parameter reporters. These investigations include structural characterization and studying the electrochemical properties of these compounds. We have successfully synthesized $[Eu_{15}(\mu_3\text{-}OH)_{20}(\mu_5\text{-}Cl)(his)_{15}(OH_2)_8](ClO_4)_9$ and $[Eu_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8](ClO_4)_3(OH)$ as characterized by XRD. The crystal structures show that these europium compounds are isostructural to the gadolinium and yttrium analogues previously synthesized.

The crystals structures of the pentadecanuclear europium complex demonstrate an extensive network of σ-π interactions by the histidine ligands, which result in very large unit cell dimensions. X-Ray Diffraction data has been collected and solved for the following compounds: Eu_{14} and Eu_{15}; the following compounds are currently being refined: Eu_{15} synthesized with equivalent molar ratios of NaBr and NaCl; Eu_{15} synthesized with exact molar ratios (Eu:His:X^- ; 1:1:1/15); and Eu_{15} synthesized with NaI.

The electrochemical properties of the pentadecanuclear and tetradecanuclear compounds have been investigated using a CH Instruments Potentiostat. It is thought that because lanthanides are non-electrically communicating sites, that it may be possible to reduced europium in equivalent sites while not reducing europium in other non-equivalent sites. For example, the pentadecanuclear complex contains two in-equivalent sites with five europium ions occupying one site (all equivalent to one another) and ten europium ions occupying the other site (all equivalent to one another). We believe that we can reduce the five europium ions in one site before reducing the ten europium ions in the other site (or visa-versa). We believe we can similarly reduce the sites in the tetradecanuclear complex as it contains three in-equivalent sites with eight europium ions occupying one site (all equivalent to one another), two in another site (all equivalent to one another) and four in the final site (all equivalent to one another).

Electrochemistry brings an interesting new insight to the stability of these molecules in solution. It is because these complexes contain sites of varying chemical nature that we should be able to observe reduction potentials for the different sites and be able to determine how many electrons are being pushed into these sites. In view of the fact that lanathanides are non-electrically communicating centers, we can directly correlate the number of electrons being pushed into the system with the number of europium ions in the system. Since the pentadecanuclear complex contains five equivalent europium ions and ten other in-equivalent europium ions we should be able to determine a ratio of 1:2 for the two different reduction potentials, if the molecule is initially stable in solution in the unreduced state. The same logic can be used for the tetradecanuclear europium complex to determine a ratio of 3:8:2.

The results from the electrochemical studies thus far have been very promising with our initial hypothesis. As seen in Figure 17 there are two reduction potentials for the two in-equivalent sites of the pentadecanuclear europium complex and in Figure 18 there are three reduction potentials for the three in-equivalent sites of the tetradecanuclear europium complex prior to solvent reduction.

The CV of the pentadecanuclear europium complex demonstrates two reductive peaks but only one corresponding oxidative peak, which suggests that the compound is initially stable in solution but upon the second reduction falls apart in solution. This could also begin to explain why the peaks integrate to a 7:10 instead of a 5:10 ratio. As the molecule obtains more and more electrons, the overall charge of the molecule increases and may destabilize it. We may have reached the reduction threshold prior to complete reduction of the molecule and thus obtained a ratio of 7:10 instead of the 5:10. Likewise, in CV of the tetradecanuclear complex, we observe three reduction peaks as we expected for the three in-equivalent sites. However, these peaks were not integratable and no statistical data could be obtained. As in the pentadecanuclear complex the tetradecanuclear complex does not

demonstrate the corresponding oxidative peaks and thus is probably unstable following reduction.

From the XRD data, we can conclude that the pentadecanuclear complex preferentially forms with chloride anion over the bromide anion. The pentadecanuclear may be formed with the use of a chloride, bromide, or iodide as a templating agent. This complex may also be readily formed with exact ratios of ligand and templating agent while the tatradecanuclear complex is stubborn to form with exact ratios, indicating another role of the amino acid in the tetradecanuclear complex formation. The electrochemical data suggests that these compounds are stable in solution in the unreduced form and begin to destabilize upon reaching a reduction threshold.

Figure 17: CV of $[Eu_{15}(\mu_3\text{-}OH)_{20}(\mu_5\text{-}Cl)(his)_{15}(OH_2)_8](ClO_4)_9$

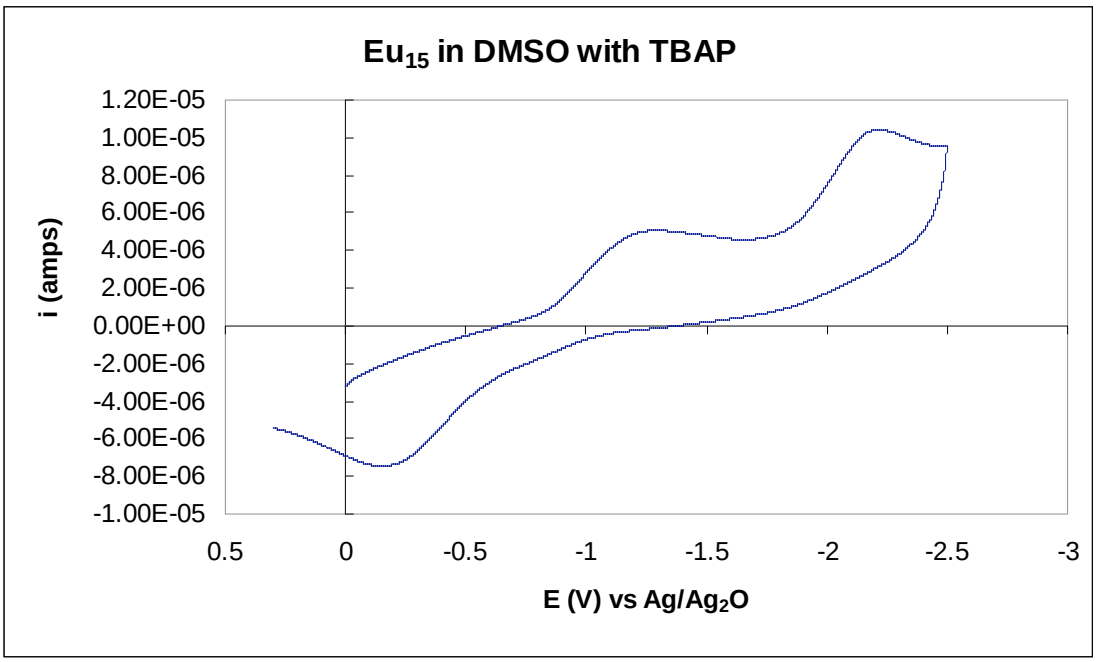
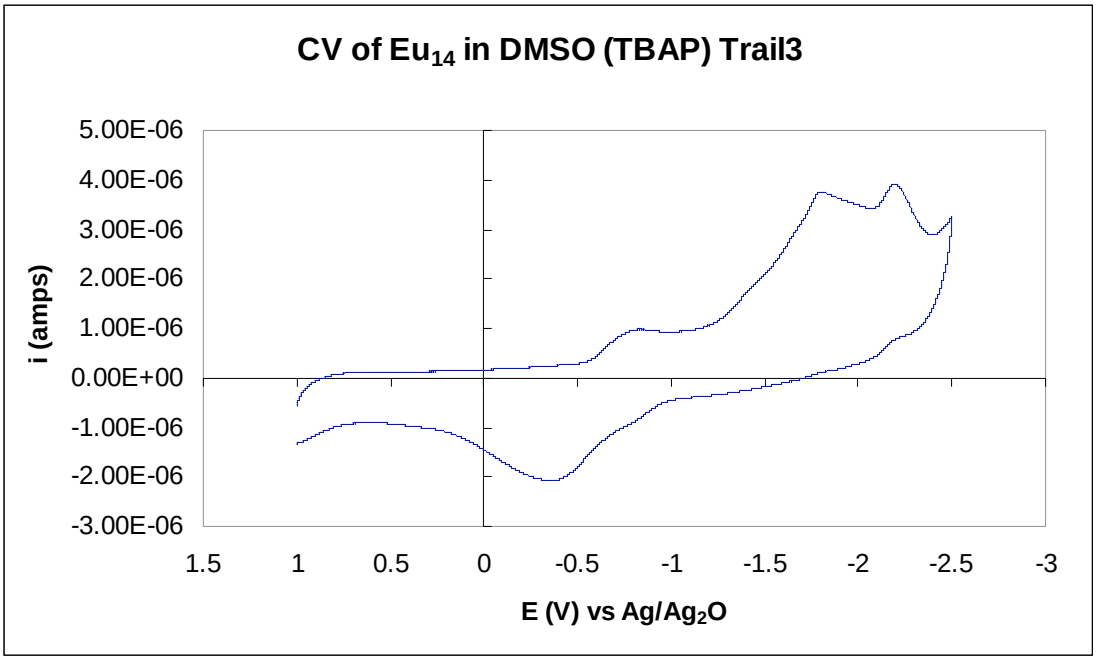


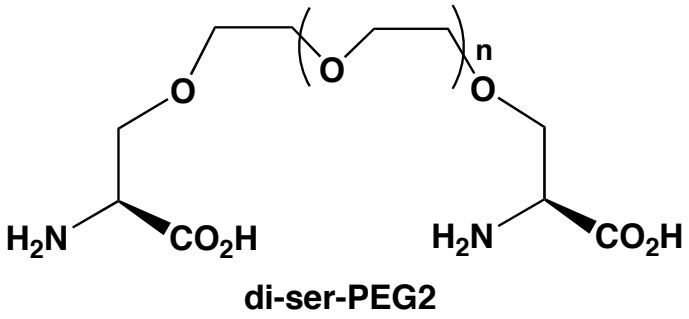
Figure 18: CV of $[Eu_{14}(\mu_4\text{-}OH)_2(\mu_3\text{-}OH)_{16}(ser)_{20}(OH_2)_8](ClO_4)_3(OH)$



Design and synthesis of ditopic ligands for Gd_{14} based on serine

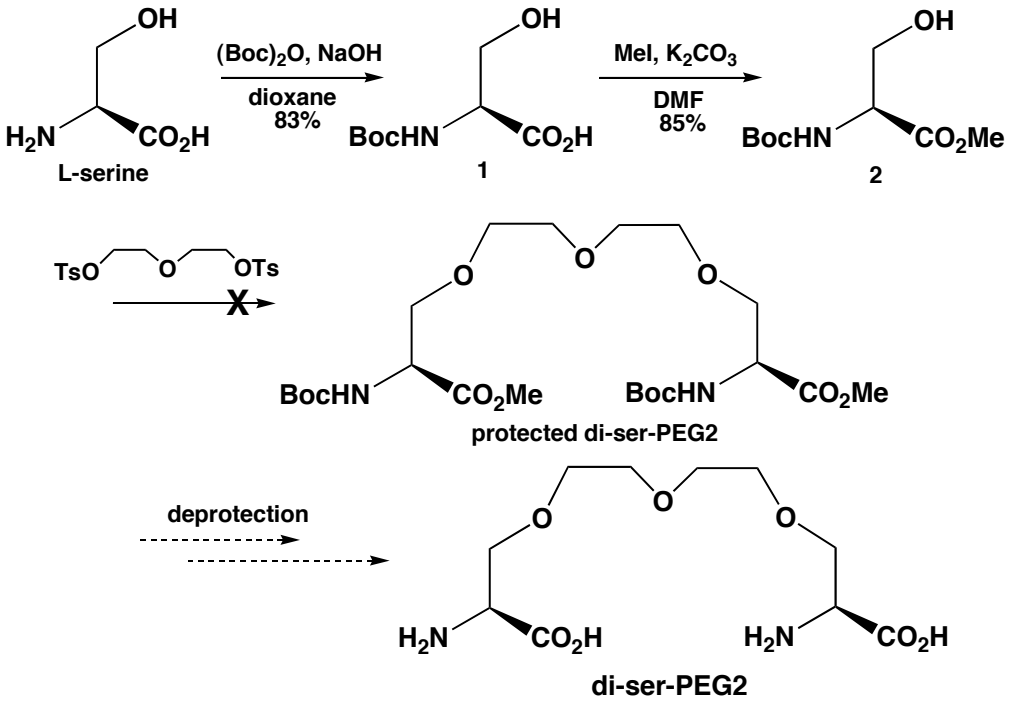
Since the nuclearity of polygadolinium complexes seems to be strongly dependent on the amino acid and the presence or absence of templating inorganic anions, we decided that the coordination in a ditopic manner (i.e., coordinating to two different lanthanides such as gadolinium within a cluster) to Gd_{14} should be based on ligands that most closely resembled L-serine. A serine dipeptide such as ser-serH would not suffice since the two ends are different – one is the free amino end while the other possesses only the carboxy terminus. This suggested that we needed to bridge two serines via a linkage between their a-carbons, so that each end would possess both carboxy and amino groups. In order to enhance aqueous solubility, we decided to use a PEG-like (PEG = poly(ethylene glycol)) spacer as the linker. Two other advantages of a PEG-like linker are the ability to tune the linker length, depending on the number of ethylene glycol spacers in the link, and the known tendency of PEG to mask small molecules from the immune system. Therefore, our general target is shown in Figure 19.

Figure 19: Ditopic ligand target, di-ser-PEG2



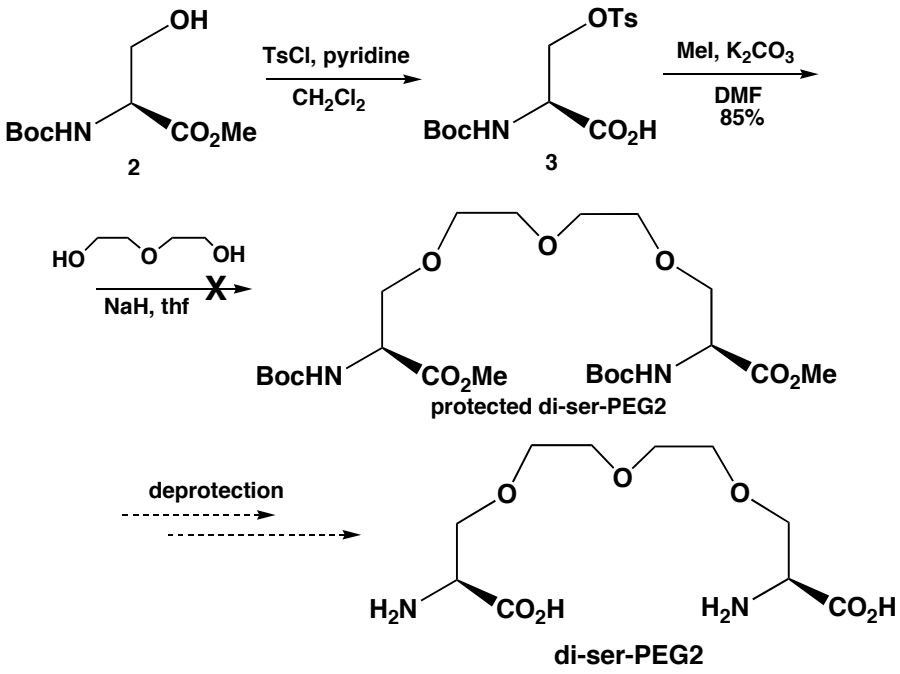
We devised and tested three different strategies for the synthesis of our first target, di-ser-PEG2, with n = 1 in the above general formula. The first strategy is shown in Scheme 1.

SCHEME 1

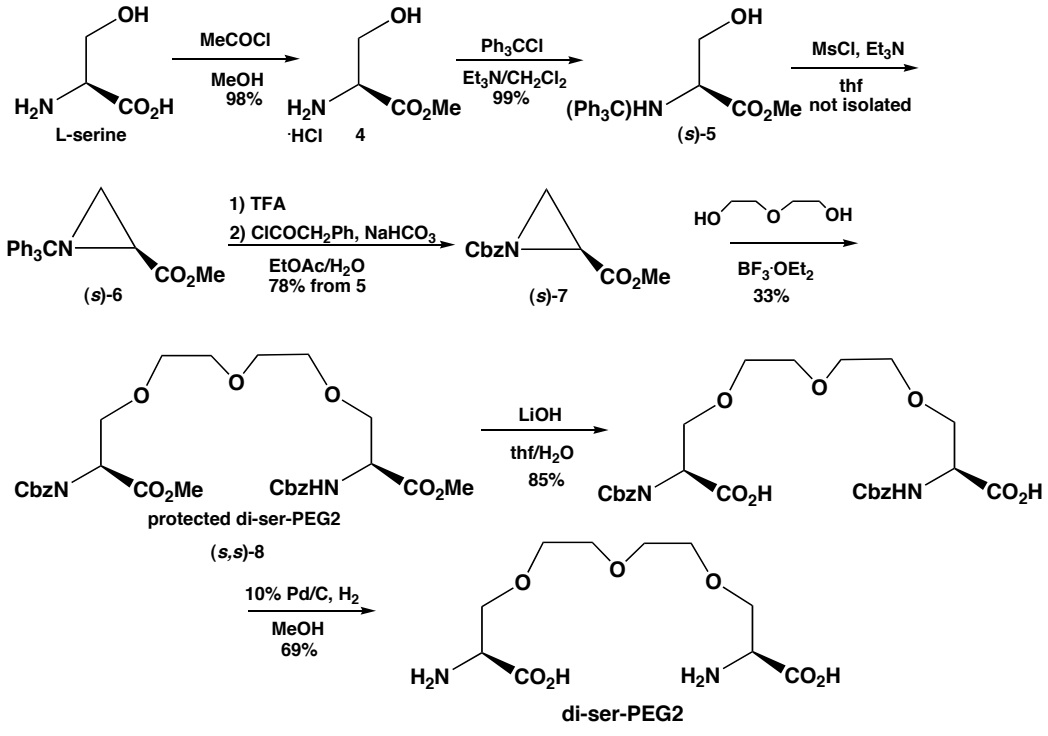


Our failure to affect step 3 (compound **2** as reactant) in Scheme 1 above led us to evaluate the synthestic strategy in Scheme 2.

SCHEME 2



Step 2 (starting with reactant **3**) in Scheme 2 proved to be a roadblock. Therefore, we designed and tested Scheme 3. The utility of Scheme 3 is that different PEGs can be used in step 5 to give diffferent linker lengths, for coordination to different sites in the cluster.



This approach was successful in giving us the desired di-ser-PEG2 target in an overall yield of 15%. We now have 450 mg of di-ser-PEG2 to test in coordination studies on Ln_{14} complexes. We have held off on its use (since obtaining that quantity involved a substantial effort) until we have successfully obtained ^{89}Y NMR spectra on Y_{14}, so we can ascertain that the solution structure is similar to or matches that of the solid-state structure. Instrumental problems and the lower solubility of Y_{14} have limited our ability to obtain this needed information to show that coordination-induced ^{89}Y chemical shifts can be observed.

KEY RESEARCH ACCOMPLISHMENTS

- Preparation of new Gd_4, Gd_{14}, and Gd_{15} clusters and Y, Eu, and Dy analogs
- Determination of high relaxivity of Gd_{14} and Gd_{15}, pH dependence, and physicochemical probes of contributions to relaxivity
- Development of Y-89 NMR spectroscopy as a probe of solution structure of polylanthanides
- X-ray and neutron diffraction structure determination on a group of new polylanthanides of MR relevance
- Design and synthesis of a novel ditopic ligand for coordination studies on Gd_{14}
- Serendipitous discovery of a novel, high nuclearity Y_{60} cluster, a precedent for obtaining higher nuclearity Gd clusters
- Initial electrochemical study of reduction of polyeuropium(III) clusters

REPORTABLE OUTCOMES: Provide a list of reportable outcomes

Manuscripts

No manuscripts have been submitted, but six manuscript drafts (on Y_{60}, planned submission to *Nature*; on Gd_{14}, to *Inorganic Chemistry*; on Y-89 NMR of polyyttrium complexes, to *Inorganic Chemistry*; on di-SER-PEG synthesis, to *Organic Letters*; $Gd_{12}I_2$ (histidine) and $Gd_{15}Cl$, to *Dalton Transactions*); neutron diffractometry on polydysprosium clusters, to *Journal of the American Chemical Society*) are near completion and await some final data and analysis.

Presentations:

Invited Lectures and Invited Conference Presentations

a. International

3. Bayer Schering Pharma, Berlin, Germany, 3/28/08
   "Beyond Iodine and Monogadolinium: "Anorganische Gruppe" Paradigms For Multiplied Detectabilty, Multi-Modality Contrast Agents"
   L. Messerle
2. Contrast Media Research Conference, CMR 2003, San Diego, CA, 10/28/03
   "Transition Metal Cluster and Polygadolinium Compounds as a New Paradigm for High Attenuation and/or Relaxivity in Contrast Media Design: Crashing the Molecular $r_1 = 100$ Barrier"
   L. Messerle, D. Nolting, L. Bolinger, A. H. Stolpen, B. F. Mullan, D. Swenson, and M. Madsen

1. Contrast Media Research Conference, CMR 2001, Capri, Italy, 10/14/01
"Hexanuclear Tantalum, Tungsten, and Gadolinium Cluster Compounds: A New Paradigm for Multiplied Attenuation and Relaxivity in Contrast Media Design"
travel not completed and talk not presented because of State Department travel advisory, issued preceding week before conference, for Italy in aftermath of September 11 World Trade Center/Pentagon attacks

b. National

64. American Chemical Society, 231st National Meeting, Atlanta, GA, March 26, 2006, Symposium on "Dinuclear, Polynuclear, and Cluster Chemistry: Frontiers in the New Millenium"
"Organopolymetallic, Cluster, and Polynuclear Supramolecular Chemistries of the Early Transition Metals, Lanthanides, and Bismuth with Nuclearities n = 2–60"
63. Chemistry Division, Los Alamos National Laboratory, Los Alamos, NM, February 23, 2006
"A Smorgasbord of Half-Sandwiches and Meatballs of the Early Transition Metals, Lanthanides, and Bismuth"
62. Mark Twain Section Meeting, American Chemical Society, April 1, 2005, Culver-Stockton College, MO.
"New Vistas in Half-Sandwich and Meatball Complexes Seasoned with Electrons"
61. International Society for Optical Imaging (SPIE) Conference on Medical Imaging, San Diego, CA, February 12-17, 2005; Conference 5746, Physiology, Function, and Structure from Medical Images: Workshop on CT Contrast
"A New Paradigm for Multiplied Attenuation and/or Relaxivity in X-Ray, MRI, and Combined X-Ray/MRI Contrast Agent Design: An Inorganic Chemist's Perspective on Novel Targeted Contrast Agents"

Contributed Presentations (presenter underlined; oral unless denoted by poster)

77. Pacifichem 2005 Conference, American Chemical Society, Honolulu, Hawaii, December 16, 2005
"Polylanthanide and Polyyttrium Amino Acid Complexes with μ_3-OH Ligands: Synthesis, ^{89}Y NMR and Spectroscopic Characterization, Neutron Diffractometry and MRI Relaxivities"
L. Messerle, D. D. Nolting, C.-T. Yang, J. H. Thurston, A. H. Stolpen, L. Bolinger, and D. C. Swenson
Session Title: Chemistry of Molecular Imaging
76. 229th ACS National Meeting, San Diego, CA, March 13-17, 2005 "Polynuclear Lanthanide(III) Complexes with α-Amino Acid and μ_3-Hydroxo Ligands"
C.-T. Yang and L. Messerle (poster; also selected by organizers for Sci-Mix poster presentation)

Degrees obtained that were supported by this award

Ph.D., 2005, Donald Nolting. Thesis title: "Polynuclear Lanthanide and Yttrium Complex and Tungsten Bromide Cluster Chemistries Relevant to Diagnostic Imaging and Protein Crystallographic Phasing"
Current position: Postdoctoral, Department of Radiology, Vanderbilt University School of Medicine

Funding applied for based on work supported by this award

Two proposals to NIH on Gd_n in MR imaging were not funded; revisions in preparation to be submitted after manuscripts are accepted for publication.

Employment or research opportunities received based on experience/training

The following postdoctoral researchers received partial funding for the work described in this report:

Dr. John Thurston (Y-89 NMR studies). Current position: Assistant Professor, Chemistry, Albertson College, Caldwell, Idaho

Dr. Yibo Zhou (organic ligand synthesis). Current position: Postdoctoral, Chemistry, Iowa State University

Dr. Chang-Tong Yang (Gdn synthesis). Current position: Postdoctoral, Chemistry, Purdue University

CONCLUSION

The key results have been the chemical development of a new family of polylanthanide complexes, particularly polygadolinium complexes, as potential high sensitivity/detectability MR contrast agents. A number of new clusters have been prepared and characterized, and the relaxivities of the gadolinium variants have been determined. The pH dependence of the relaxivity has been demonstrated, and nonspherical molecular shape seems to contribute to relaxivity enhancement. Key inorganic chemistry aspects have been ascertained, along with tools developed for further physicochemical study. The NMR studies have shown that at least one of the clusters is stable in water, with a solution structure consistent with the solid-state structure. The first generation of encapsulating ligands have been synthesized by organic methods. This Exploration-Hypothesis project has succeeded in proving that the chemistry has promise for giving multiplied relaxivity MR contrast agent for cancer and metastatic imaging.

As a medical product, more research is needed; it took many years to develop the basic chelate chemistry and toxicology of Gd for current clinical MR contrast agents. The short timespan of the project has not led as far as needed for multitopic coordination and bioconjugation to PSMA small molecule inhibitors, because of the unexpected chemical complexity, so *in vivo* cell studies were not initiated. Future funding and effort will be needed to push the concept forward to cell biology/*in vitro* bioimaging studies.

REFERENCES none