#### PHOTOCHEMICAL RELEASE OF METAL IONS: A MODIFIED CAGING

## DESIGN OF A PHOTOCLEAVABLE CHELATOR FOR

#### THE LIGHT DIRECTED RELEASE OF METAL IONS

by

MATTHEW ALAN MCKINLEY

(Under the Direction of Vladimir Popik)

#### ABSTRACT

In our search for a modified photosensitive metal chelator we have designed a new platform for the photochemical release of metal ions. The tetradentate ligand, having a high binding affinity for metal ions, contains a photolabile ether linkage. Upon irradiation one of the bidentate side chains is cleaved off resulting in a dramatic reduction of the ligand to metal affinity. The cleavage occurs within 12  $\mu$ s after the laser pulse permitting spacial and temporal control of the metal ion release.

**Keywords:** Photosensitive, Tetradentate, Caging compound, Metal chelator, Spacial control, Temporal control

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# DEDICATION

To the best wife in the world, Molly.

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## LIST OF ABBREVIATIONS

ATPadenosine	triphosphate
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BAPTA.....(1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*<sup>*II*</sup>,*N*<sup>*II*</sup>-tetraacetic acid)

acid)-3-hydroxynaphthalene

BBr<sub>3</sub>.....boron tribromide

Br<sub>2</sub>.....bromine

cAMP.....cyclic adenosine monophosphate

CDCl<sub>3</sub>.....deuterated chloroform

DCM.....dichloromethane

DIBAL.....diisobutylaluminum hydride

DMF.....dimethylformamide

EDTA.....ethylenediaminetetraacetic acid

EGTA.....ethylene glycol tetraacetic acid

ESI.....electrospray ionizaiton

ESIPT.....excited state intramolecular proton transter

GFP.....green fluorescent protein

HOMO.....highest occupied molecular orbital

HRMS.....high resolution mass spectrometry

IC.....internal conversion

ISC.....intersystem crossing

IUPAC.....International Union of Pure and Applied Chemistry

LUMO.....lowest unoccupied molecular orbital

mCPBA.....meta-chloroperoxybenzoic acid

MgSO<sub>4</sub>.....magnesium sulfate

MOMCI.....chloromethyl methyl ether

NaH.....sodium hydride

NaOAc.....sodium acetate

NBS.....N-bromosuccinimide

oNQM.....o-naphthoquinone methide

PCC.....pyridinium chlorochromate

Pd/C.....palladium on carbon

PPG.....photoremovable protecting group

SnCl<sub>2</sub>.....stannous chloride

TBDMS.....tert-butyldimethylsilyl ether

THF.....tetrahydrofuran

ZnCl<sub>2</sub>.....zinc chloride

#### CHAPTER 1

#### Introduction to "Caging" compounds

"Caged" compounds are biologically useful molecules whose functionality is controlled by light. The ability to achieve a chemical transformation from an inactive to an active state under photochemical conditions is a useful concept in nearly all branches of biological and physical sciences. Caging molecular hosts have been used to encapsulate everything from adenosine triphosphate (ATP) used in muscle activity studies<sup>(1-2)</sup> to organometallics used in drug delivery.<sup>(3-4)</sup>

The word "caged" is based off of an older concept that smaller active compounds or species can be encapsulated or trapped inside of a larger inactive host. Upon photolysis, the host would be dismantled or severed exposing the active species and therefore deeming it "uncaged." Only within the past few decades has this total imprisonment from the outside environment been successfully accomplished.<sup>(5)</sup>

In 1992, Donald J. Cram defined specific parameters and proposed the common mechanics involved between hosts and their caged species. These interactions included hydrogen bonding, ion pairing,  $\pi$ – $\pi$  binding dispersion interactions, van der Waals attractions, metal ion ligation, and solvophobic phenomenon.<sup>(5)</sup> Simple covalent bond formations (the caging of the APT phosphate group using *o*-nitrobenzene) are also used as "caging" interactions

and are more commonly known as photoremovable protecting groups (PPG's). However, because the majority of these types of compounds do not directly involve the chelation with metal ions, they will not be discussed further.

In order for the binding energy ( $E_d$ ) between the host and guest to be strong enough it is often necessary for multiple interactions between the two to occur concurrently. (**Figure 1.1 (a) and (b)**) In most cases, the binding energy needed for the host to hold in a guest species is 50 – 100 kcal mol<sup>-1</sup>. In return, an accumulation of these weak 2 – 20 kcal mol<sup>-1</sup> interactions are needed in order to bind the guest inside the host cavity, making it a polydentate ligand.

Even though the traditional definition of a cage is a full enclosure having some openwork for confining, the goal of a caging molecule is not to fully encapsulate its guest. Rather, it simply has to mask the active species from its surroundings. As we can see with EGTA and 2,2,2-Cryptand buffers, binding selectivity is achieved as a result of the host framework incorporating electron donating heteroatoms arranged in a unique way creating a specifically sized pocket. Thus, organic chemists now have a myriad of options when selecting or synthesizing caging compounds that chelate (the presence of two or more separate coordinate bonds between a polydentate ligand and a single central atom) to electron deficient guests of their choice.<sup>(6)</sup> Starting from simple derivatives of one of the simplest naturally occurring biological ligands, citrate (**Fig 1.1 (c)**), chemists have synthesized hundreds of chelating hosts that can be used as tools specific to research studies and the cations needed to be worked on.





Typically, when dealing with electron donating atoms oriented around a binding pocket, there is a strong affinity for alkali and alkaline earth metals. The reason for this is their tendency to solubilize ionic substances and chelate with the metal cation. As a result, the metal cation is masked from its surroundings.

One of the main factors contributing to binding selectivity is the cavity size. Looking again at EGTA, there are four oxygen atoms and two nitrogen atoms contributing to the size and binding selectivity of the pocket. The binding pocket is the right size for Ca<sup>2+</sup> with an ionic radius of 99 pm and cannot constrict any further to securely complex Mg<sup>2+</sup>, which is a smaller ion with an ionic radius of 65 pm. (**Table 1.1**) The same goes for smaller ions such as Li<sup>+</sup> and Be<sup>2+</sup>. On the other hand, metal cations that are too big, simply will not fit inside of the bind pocket.

Another way to manipulate binding selectivity is the number and type of donor atoms used in the ligand. Over the past decade, hosts containing different

quantities of heteroatoms (nitrogen, oxygen, and sulfur) in differing arrangements have shown an increasing amount of sophistication and specificity with bound metal ions and uses.<sup>(6)</sup> Common examples of these are tetradentate ligands(Figure 1.1 (a)), crown ethers, cryptands (Figure 1.1 (b)), and aza-crown ethers. These macrocyclic and polydentate molecules are modified with different types and quantities of heteroatoms not only manipulating the size of the binding pocket but also modifying the number and arrangement of coordination bonds. Coordination bonds, or dipole-dipole interactions, lock the metal ion in its place within the binding pocket. Thus, if you have 6 electron-donating atoms, you will form 6 coordination bonds with the metal (permitted the metal can coordinate with that many or that few). Metals that are more prone to bind are those with a coordination number of 6. With both Mg<sup>2+</sup> and Ca<sup>2+</sup> having a coordination number of 6, it then becomes binding affinity and pocket size that contribute to the selectivity between the two. As a result, a collection of parameters contribute to the overall selectivity of the ligand.

A chemical ligand is any ion or molecule with the capability of donating or withdrawing one or more electron pairs that contributes to the formation of coordinate bonds leading to the encapsulation of the cation or anion.<sup>(7)</sup> Between the valence lone pairs of heteroatoms and cations with empty 3d, 4s, and 4p orbitals, these coordinate bonds influence selectivity and establish rigid binding pockets that will drastically reduce which cations will hold inside the polydentate ligand.

Alkali Metals	Ionic Radius (pm)	Alkaline Earth	Ionic Radius (pm)
		Metals	
Li <sup>+</sup>	68	Be <sup>2+</sup>	31
Na⁺	95	Mg <sup>2+</sup>	65
K <sup>+</sup>	135	Ca <sup>2+</sup>	99
Rb⁺	148	Sr <sup>2+</sup>	113
Cs⁺	169	Ba <sup>2+</sup>	135

Table 1.1: List of ionic radii for alkali and alkaline earth metals.<sup>(7)</sup>

Ideally, other metal ions would compete for the binding pocket. However, because nitrogen atoms bind protons the binding constant becomes very sensitive to pH levels. Hence lower pH levels can greatly reduce the rate of metal uptake in result from the binding competition of protons to the nitrogen atoms. For instance, it is reported that the rate constant for the reaction of Ca<sup>2+</sup> with EGTA is 10<sup>6.3</sup> M<sup>-1</sup>s<sup>-1</sup> at a pH of 7.0, whereas at physiological pH (7.365) with EGTA being fully ionized the rate constant is 2 to 3 times faster.<sup>(7)</sup>

There has been substantial progress in the design and synthesis of caging compounds that are utilizing all of the traits discussed and overcoming binding and selectivity problems. The "caging" compound encompasses a vast field and is rapidly expanding the ligands being used as well as incorporating novel chromophores providing diverse functionality. It is becoming very clear the potential caging ligands have in the medical field as well as throughout chemical laboratories.

#### The Photochemical backbone of Caging hosts

Having defined what "caging" ligands are, it is important to explain the functionality behind these photosensitive molecules. It is one thing to synthesize

a compound that chelates a specific metal ion and another to synthesize a compound that can bind a specific metal ion and release it in response to an outside signal. Thanks to Kaplan,<sup>(1)</sup> Schlaeger,<sup>(8)</sup> and Tsein<sup>(9)</sup> for their work on the photochemical release of ATP and cAMP via the *o*-nitrobenzyl protecting group and binding affinity reduction by photochemical transformations in ligand backbones, chemists know how to prepare polydentate chelators by synthesizing ligands consisting of or protected by a chromophore.

As defined by IUPAC, a chromophore is the part of a molecule capable of selective light absorption leading to the coloration of certain atoms in the molecule. Localized electronic transitions occur in result from absorption of a photon at a certain wavelength of light that matches the energy gap between the ground state and an excited state. This in turn can lead to a photophysical or photochemical process. These transitions are more commonly taught as the excitation of an electron from a lower-lying quantized energy state (ground state) to a higher-lying energy state (excited state).

There are five electronic transitions that are involved in electron excitation including  $\sigma - \sigma^*$ ,  $\sigma - \pi^*$ ,  $\pi - \pi^*$ ,  $n - \sigma^*$ , and  $n - \pi^*$  with  $\pi - \pi^*$  and  $n - \pi^*$  being the two main transitions that are readily involved in photochemistry. The reason for this is  $\pi - \pi^*$  and  $n - \pi^*$  transitions occur at a lower energies on the UV region while it is necessary for energy in the far-UV to obtain  $\sigma$  electronic transitions.

Alexander Jablonski, a Polish physicist, proposed the diagram (**Figure 1.2**) relating the different photophysical processes upon excitation. Upon absorption of a photon, an electron moves from the ground state (S<sub>0</sub>) to a higherlying quantized state ( $S_n$ ). Once an electron is in an excited state ( $S_n$ ), several processes may occur depending on the molecular structure and the matrix surrounding the compound. These photophysical processes include the loss of a photon by fluorescence, the interconversion from a singlet state to a triplet state by intersystem crossing (ISC), the release of heat to its surrounds ( $S_n$  to  $S_1$ ) by internal conversion (IC), non-radioactive decay, photolysis, or the release of a triplet state photon by phosphorescence (after ISC).

Typically, organic chromophores absorb in the UV/vis range via the low energy  $\pi - \pi^*$  and  $n - \pi^*$  transitions. With an absorption range expanding from 180 nm to well above 650 nm,<sup>(11)</sup> organic molecules can be modified in order to absorb within a specific energy range. There are several characteristics that chemists modify when designing chromophores to help achieve maximal absorbance ( $\lambda_{max}$ ) at certain wavelengths. One of these is the extended  $\pi$ system. The more conjugation a molecule has, the more red shifted (increase in wavelength) its  $\pi - \pi^*$  transition ( $\lambda_{max}$ ) will be. This is due to the fact that the more electrons are delocalization, the greater the wavelength it will absorb. Secondly, n- $\pi^*$  transitions occur at longer wavelengths than  $\pi$ - $\pi^*$  transitions meaning its transition will also occur at a lower energy. On the other hand, with an extinction coefficient (how strongly a molecule absorbs light at a specific wavelength,  $\varepsilon_{max}$ ) greater than 10<sup>3</sup>,  $\pi$ - $\pi$ <sup>\*</sup> transitions tend to have a greater intensity than n- $\pi$ <sup>\*</sup> transitions. Using these molecular patterns, chemists have a good framework to predict the type of chromophore to use or develop based on its photophysical and photochemical properties.



Figure 1.2: The Jablonski diagram.<sup>(10)</sup>

After a chromophore has been excited by the absorption of a photon, one of its chemical bonds may cleave resulting in photolysis or photodissociation. These intermediates produced may include free atoms, radicals, or electronically excited species which are highly reactive with extremely short lifetimes. These intermediates may themselves carry excess energy and perform secondary reactions.

One of the fundamental characteristics which has been observed is the rate of absorption and photolysis for several chromophores. While it could take several minutes after absorption for photolysis and secondary reactions to complete, there are a few chromophores which photolyze in microseconds. Absorption, being one of the fastest chemical processes, can take place in just

 $10^{-16} - 10^{-14}$  s because only electron movement is involved. This concept lead to the Franck-Condon principle which states that the most favorable electronic transitions are ones in which the geometries of the initial and final states are similar.<sup>(11)</sup>

Another key concept in photochemistry that not all molecules absorb 100 percent of the photons emitted. With a maximum of 1.0 (except for chain reactions), the number molecules decomposed divided by the quantity of photons absorbed is called the quantum yield of a decomposition reaction. (**Eq 1.1**) The closer your quantum yield is to 1, the more efficient your photochemical reaction. Several factors may contribute to a poor quantum yield including compound structure, the matrix surrounding the molecule, and quenching.

$$\phi = \frac{\begin{array}{c} \text{Number of molecules} \\ \text{decomposed} \\ \hline \\ \text{Number of photons} \\ \text{absorbed} \end{array}}$$

Eq. 1.1

Now that a brief overview of the chemical processes behind photosensitive chelators has been discussed, how exactly have photochemists utilized chromophores as caging and protecting compounds? Several reviews have been published during the past decade covering novel modifications and applications of photosensitive protecting groups and ligands releasing metal cations, organic molecules,<sup>(12, 13)</sup> and even primary messengers including neurotransmitters.<sup>(14)</sup> However, only Ca<sup>2+</sup> binding photosensitive ligands will be discussed in depth in this report. One of the pioneer chemists contributing to the research and understanding of photosensitive Ca<sup>2+</sup> chelators was Roger Tsien, a Nobel Prize winner in chemistry for his discovery and research on the green fluorescent protein (GFP). Dr. Tsien took the readily available Ca<sup>2+</sup> EGTA (**Figure 1.1 (a)**) and manipulated the ligand structure to incorporate a chromophore with the intention of creating a molecule having a significant difference in UV absorption between the bound Ca<sup>2+</sup> and the free Ca<sup>2+</sup> states. Still maintaining EGTA's selectivity for Ca<sup>2+</sup> over Mg<sup>2+</sup> (K<sub>a</sub><sup>Ca2+</sup> = 10.97, K<sub>a</sub><sup>Mg2+</sup> = 5.2), Tsien et al tethered on aromatic rings (commonly known as BAPTA) (**Figure 1.3**) not only providing a chromophore for the ligand but also contributing to the rigidity of the cavity size.<sup>(15)</sup> The absorption maximum at 254 nm for the Ca<sup>2+</sup> free BAPTA ligand at a pH of 7.29 shifts to 203 nm for the bound Ca<sup>2+</sup> ligand resulting in a hypsochromic



Figure 1.3: BAPTA<sup>(15)</sup>

shift (blue shift) in the absorption spectrum. The trend continues as the pH is increased to around 6.5. Below 6.5 protonation of the nitrogen atoms will reduce

the conjugation between themselves and the aromatic rings leading to the decrease of binding affinity with Ca<sup>2+</sup>. Even though BAPTA was observed to have fast kinetics of binding and release of Ca<sup>2+</sup> ions, the quantum yield observed was low ( $\phi = 0.029$ ) and the fact that it absorbed well below 300 nm means that it will not suitable in biological environments due to the fact that the range below 300 nm will damage nucleic acids.

Less than a decade later, Dr. Tsien proposed another BAPTA (**Figure 1.4**) derivative this time incorporating a backbone chromophore that undergoes structural change lowering the binding affinity upon irradiation.<sup>(9)</sup> Tethering on an *o*-nitrobenzhydryl ether located in the para position to one of the amine groups, Tsein et al proposed the idea of using a chromophore that undergoes a photochemical change to influence the binding affinity of the metal cation with the





tetradentate ligand pocket. Upon irradiation, a hydrogen atom is transferred from the ortho-alkyl group to one of the oxygen atoms on the nitro group. After ISC to the triplet excited state, the (E) *aci*-nitro intermediate then cyclizes followed by the release of the alcohol upon ring opening, creating an oxidized electron-withdrawing substituent and in result reduce the affinity for Ca<sup>2+</sup>. The mechanism of this photochemical reaction closely resembles that of the release of the *o*-nitrobenzene protecting group via the ground state *aci*-nitro intermediate (**Figure 1.5**).

With an absorbance above the 300 nm, these ligands commonly known as the nitr-(1-7) series, have proven to meet nearly all of the requirements photochemists are looking for use in biological environments. By tagging on alkoxy substituents to the nitrobenzyl ring the absorption wavelengths are red shifted well above 300 nm thereby making them eligible for cell studies.



Figure 1.5: Accepted photochemical mechanism for the cleavage of the benzyl

12

ether. (16)

With constant breakthroughs with chromophore functionality over the past decade, the problem of quantum yield and speed can be addressed using photoelimination of alcohols from 3-(alkoxymethyl)-2-naphthol as the tetradentate ligand backbone chromophore. With spacial and temporal control, the generation of the *o*-naphthoquinone methide intermediate can be produced photochemically in microseconds first by the excitation of the hydroxyl group. Because phenols are much more acidic at S<sub>1</sub> (pka = 4) than at S<sub>0</sub> (pka = 10), an adiabatic deprotonation occurs within nanoseconds. The negative charge on the phenolate is strongly delocalized by the aromatic system, providing the driving force for heterolytic cleavage of the benzylic ether. Being very reactive to nucleophiles, the methylene then hydrolyzes in water regenerating the o-hydroxynapthalene chromophore and.<sup>(17)</sup>

Just as Dr. Tsien was able to predict how chromophores would affect the binding pockets of ligands,<sup>(9)</sup> we can say that based on the photochemical process via the *o*-naphthoquinone methide (*o*NQMs) intermediate, not only should there be a rapid release of Ca<sup>2+</sup> by the dramatic change in binding affinity due to the loss of half of the binding pocket but also the quantum yields for oNQM are very reasonable ( $\phi \sim 0.20$ ). <sup>(17)</sup>

With the overall goal for these ligands for be used in biological environments it is crucial that these caging molecules are biologically inert, start with a high affinity for  $Ca^{2+}$  and rapidly fall under photolysis, bind with a dissociation constant equal to  $10^7$  M or lower, have a selectivity for  $Ca^{2+}$  over

other biological metal cations, have an ability to release the ion in less than 10<sup>-3</sup>s, and results in non-toxic by products.

## The Biological implications of caged Ca<sup>2+</sup>

Secondary messengers play a managing role in relaying signals from receptors located on the outside surface of cells to specific target molecules within the cytoplasm. They act as linkers between primary stimulus and cell activity. There is a wide variety of these messengers including cAMP (hydrophilic cyclic nucleotides) involved in the regulation of glycogen and sugars, Ca<sup>2+</sup> involved in muscle contraction and the release of neurotransmitters, and hydrophobic phosphatidylinositol's involved in membrane signaling and lipid communication.

Since the majority of the cells within the body require Ca<sup>2+</sup> to function, the ability to gain spacial and temporal control of calcium fluctuations are greatly needed. The biological roles calcium plays are diverse including cell structure, intracellular regulator, and electrical signaling. However, nearly all of these biological activities are regulated by fluctuations and ionic gradient changes in and out of the cell.

The primary stimulus can initiate a change in calcium either by bringing it in from the outside of the cell or by release from Ca<sup>2+</sup> storage site within, such as the endoplasmic reticulum and the mitochondria. In muscle contraction for example, calcium is used as a physiological regulator by being concentrated within the cytoplasm of muscle cells and in return becoming a signaling vessel between the primary stimulus and the proteins involved with muscle contraction. With the calcium concentration of a resting cell being around 0.1  $\mu$ m, there is a large concentration gradient existing across the cell membrane of over 10000 fold.<sup>(7)</sup> As a result to only needing a very small amount of calcium fluctuation, the time from the primary stimulus to the activation of the cell is in milliseconds.<sup>(15)</sup>

Just as with muscle contraction, neurosynaptic transmissions and other biological activities occur by a similar process at similar rates. It would be advantageous for chemists to be able to generate localized Ca<sup>2+</sup> fluctuations for the advancement in the understanding of the biochemistry behind intact cell functions.

This methodology can be expanded upon to be used in many different biological situations including the caging of gallium (Ga) ions with an azo-crown ether ligand. Intracellular Ga is known to inhibit cell growth and can be lethal in excess. <sup>(18-20)</sup> Just as with Ca<sup>2+</sup>, caged Ga can be administered and selectively released in cancerous tissues upon irradiation with spatial and temporal control.

The advantages of using a caging compound modified with the o-NQMP chromophore on the BAPTA ligand is that the selectivity for  $Ca^{2+}$  over Mg<sup>+</sup> is conserved, the chromophore absorbs above 325 nm making the wavelength harmless to cells, because of the all or nothing cleavage of the benzylic ether there will be a dramatic reduction in  $Ca^{2+}$  affinity in microseconds, and the side products produced are not toxic.

#### CHAPTER 2

#### **Discussion of NQMP-BAPTA**

Our investigation in the synthesis and testing of a modified Ca<sup>2+</sup> caging chelator began by first designing a light-sensitive ligand predicted to have a high probability of meeting the requirements to be a biologically useful caging compound: 1. Having a dissociation constant equal to or lower than the free Ca<sup>2+</sup> concentration of a resting cell ( $\leq 10^{-7}$ M);<sup>(7)</sup> 2. Selectivity for Ca<sup>2+</sup> over Mg<sup>2+</sup> ions by having a dissociation constant  $\geq 10^{-3}$ ;<sup>(15)</sup> 3. Using a chromophore that absorbs at 300 nm or greater, 4. After photolysis; there should be a dramatic reduction in the binding affinity with Ca<sup>2+</sup>; 5. The photochemical release of Ca<sup>2+</sup> should occur at rates comparable with biological and enzymatic processes (nanoseconds); and 6. The chelator should be biologically inert before being irradiated and not produce toxic by-products upon photolysis.



Figure 2.1: 2-(1,2-bis(o-aminophenoxy)ethane-N,N,N<sup>2</sup>,N<sup>2</sup>-tetraacetic acid)-3-

hydroxynaphthalene (NQMP-BAPTA)

Starting with the commonly used BAPTA Ca<sup>2+</sup> buffer, we modified the rigid tetradentate ligand (**Figure 2.1**) to include an ortho-hydroxynaphthalene chromophore which upon irradiation will result in ESIPT from the phenol proton to the oxygen atom in the benzylic position. It is believed that the C-O bond cleavage occurs after proton transfer in the ground state resulting in an elimination of one of the bidentate groups. Rapid hydration then follows to regenerate the *o*-hydroxynapthalene chromophore (**Figure 2.2**).



Figure 2.2: Photochemical mechanism of oNQM

Our synthesis of NMQP-BAPTA began by protection of the readily available methyl 3-hydroxy-2-naphthoate using MOMCI under standard conditions by treating the phenol with sodium hydride (NaH) followed by the substitution of the primary chloride on the methoxy ether with the benzyloxy (**Figure 2.3**). It was decided to protect the alcohol with a MOM group after having problems with both TBDMS and methyl protecting groups later in the reaction scheme. The TBDMS group fell off during the LAH reduction of the ester and also in later reactions. Starting with the cheap and readily available methoxy 3-methoxy-2-naphthoate, it was believed that a protection step could be avoided using a reagent already containing the methyl protected alcohol. However, the methyl group proved to be very difficult to remove without destroying the compound.

With a protected alcohol, the ester was reduced using LAH in THF (**2.3.2**). Careful attention was placed on the quenching of the reaction using ethyl acetate (EtOAc) and water as well as controlling the massive amounts of aluminum emulsions formed. It was initially thought that Rochelle's salt (sodium potassium tartrate) would have to be used to break up the aluminum emulsions for a higher product yield but by performing extra extractions with EtOAc and purification through recrystallization with diethyl ether and hexanes resulted in a good yield.



Figure 2.3: Formation of 3-(methoxymethoxy)-2-naphthaldehyde

Next, the protected benzylic alcohol ((3-(methoxymethoxy)naphthalen-2yl)methanol) (**2.3.2**) was oxidized using PCC in DCM. Purification was done through recrystallization using ether and hexanes gave nice yellow crystals (**2.3.3**). It needs to be explained as to why it was decided to reduce the ester to an alcohol and then oxidize it back to an aldehyde. There were several attempts to use DIBAL as a mild reducing agent to produce the aldehyde directly from the ester. However, because DIBAL does not react with aromatic esters, no reaction occurred.

Once 3-(methoxymethoxy)-2-naphthaldehyde (**2.3.3**) was formed, there were two different routes taken to form the epoxide. At first, a Wittig reaction was done using methyltriphenylphosphonium bromide to form the styrene derivative (**2.4.1**), followed by *m*CPBA to generate the epoxide (**Figure 2.4**). With only mediocre yields, it was decided to transform the aldehyde directly into the



Figure 2.4: Formation of 2-(3-(methoxymethoxy)naphthalen-2-yl)oxirane

epoxide using the Corey-Chaykovski epoxidation reaction with trimethylsulfonium iodide (**2.4.2**).

When trimethylsulfonium iodide is treated with strong base (**Figure 2.5**, **2.5.1**), dimethylsulfonium methylide (sulfur ylide) is formed. When the sulfur ylide is mixed with an aldehyde the carbonyl carbon is attacked by the nucleophilic

methylide anion (**2.5.2**). Being a good leaving group, the dimethyl sulfide cleaves off upon attack from the hydroxyl anion, thus forming the epoxide. With a standard water workup and easy purification using column chromatography this reaction not only produces high yields but also helped reduce total steps in the synthesis.



Figure 2.5: Corey-Chaykovski epoxidation mechanism

Standard epoxide opening using 2-nitrophenol under basic conditions yielded 1-(3-(methoxymethoxy)naphthalen-2-yl)-2-(2-nitrophenoxy)ethanol (2.4.1). Treatment of 2-nitrophenol with NaH deprotonated the hydroxy group which in turn attacked the least substituted side of the epoxide, opening the ring and leaving a secondary alcohol. The solution was neutralized making sure the secondary alcohol remained protonated in the organic solution. A standard workup and purification by column chromatography to remove any leftover 2nitrophenol provided the pure oil.

To deprotonate the secondary alcohol, 1-(3-(methoxymethoxy)naphthalen-2-yl)-2-(2-nitrophenoxy)ethanol (**Figure 2.6, 2.6.1**) was treated with sodium hydride in DCM. 1-fluoro-2-nitrobenzene was added leading to the substitution of the fluoride halide with the hydroxy anion. 2-(1,2-*bis*(2-nitrophenoxy)ethyl)-3- (methoxymethoxy)naphthalene (**2.6.2**) was produced in decent yield with a standard water workup and purification by column chromatography.



**Figure 2.6:** Formation of 2-(1,2-*bis*(2-nitrophenoxy)ethyl)-3-(methoxymethoxy)naphthalene

Several different reaction methods were attempted to synthesize this intermediate (**2.6.2**). One route was the bromination of 2-(methoxymethoxy)-3-vinylnaphthalene. However, there were issues with brominating the naphthalene ring as well. Several bromine sources including Br<sub>2</sub>, NBS, and BBr<sub>3</sub> were studied but all led to aromatic bromination. It was then thought that maybe by epoxide opening with water both alcohols could simultaneously substitute out the fluoride

on the 1-fluoro-2-nitrobenzene. This too led to a dead end with elimination of the secondary alcohol.

The selective reduction of the nitro groups was carefully tested so as to not cleave the benzylic ether. Initially, there were several attempts to reduce via hydrogenation using 3%, 5% and 10% Pd/C at 15, 30, 40, and 60 psi of H<sub>2</sub>. The problem with using 3% and 5% Pd/C as the catalyst was that it required too much pressure to get the nitro groups to reduce. Again, this resulted in a messy mixture of partially reduced nitro groups and elimination at the benzylic ether position. Eventually, mediocre yields were achieved using 10% Pd/C at 40 psi. But partial cleavage of the benzylic ether was also observed. Thus we decided to use a different reduction process to avoid this problem.

After attempting the reduction with SnCl<sub>2</sub> and ZnCl<sub>2</sub>, there still remained a problem with selective reduction. Searching through the literature finally paid off with the possibility of selective reduction using 8 equivalents of zinc dust and acetic acid in methanol. By chelating with the nitro group, the nitrogen then becomes protonated forming a hydroxylamine intermediate. Upon longer reaction times, the hydroxylamine gets reduced to the amine releasing zinc oxide as a by-product. With high reaction yields, this reaction proved to be a clean reaction avoiding the problem of cleavage of the benzylic ether (**Figure 2.7**).

In synthesizing the tetradentate arms the amines were alkylated using methylbromoacetate and sodium iodide in DMF (a Finkelstein reaction). Halide substitution between bromine and iodine yielded methyl iodioacetate. With iodine being a much better leaving group than bromine, the amine groups

22



Figure 2.7: Selective reduction of aromatic nitro groups

undergo an  $S_N^2$  reaction binding the methyl acetate and displacing iodine. A second equivalent of methyl iodoacetate underwent another Sn2 reaction with





Figure 2.8: Formation of NQMP-BAPTA

the now secondary amine. As a result, four methyl acetates where successfully introduced onto the two aminophenoxy groups (**Figure 2.8, 2.8.1**). In order to

avoid partial or *bis*- and *tris*- amino alkylation the reaction performed under heat 90°C.

To hydrolyze the methyl acetate groups, different methods were tested. In hopes of hydrolyzing the esters and simultaneously removing the MOM protecting group, tetraacetate (**2.6.2**) was treated with conc. HCl in water and acetonitrile (co-solvent). While the MOM group was easily removed at 60 °C, there was a problem with fully hydrolyzing all of the acetate groups. Based on <sup>1</sup>H NMR, a mixture of *bis*- and *tris*- acetates remained after reacting 72 hours. A saponification reaction was then performed to generate the tetraacetic acid ligand (**2.8.2**). Even though NQMP-BAPTA was synthesized and characterized, in hindsight it would be wise to perform a saponification reaction, making sure all four acids are reduced and then removing the MOM protecting group with a mild acid.

#### **Future Studies**

Several additional photochemical investigations need to be made with the binding affinity, rate of Ca<sup>2+</sup> release, extinction coefficient, and quantum yield in order to complete this study of the modified caging ligand.

The UV/Vis absorption spectra of BAPTA-OHN will first confirm where the absorption maximum is and at what wavelength the photochemical transformation will occur at. After irradiation, the photolytic conversion in the absence and presence of Ca<sup>2+</sup> will be followed by HPLC and <sup>1</sup>H NMR. The affinity for NQMP-BAPTA and Ca<sup>2+</sup> will be obtained by titrating in the ligand with

incremental additions of Ca<sup>2+</sup>.

The work done in this report has laid the foundation for future research and exploration of caging metal ions involving crown-ethers. (**Figure 2.9**) Utilizing NQMP-BAPTA as a platform to photochemically cleave a benzylic ether, we can broaden the scope and based on which ion needed for caging we can tag on different sized crown ethers and cryptands.



Figure 2.9: NQMP-15-crown-5

#### EXPERIMENTAL SECTION

Synthesis: Methyl 3-(methoxymethoxy)-2-naphthoate, (2.3.1).<sup>(21)</sup> Sodium hydride (3.86 g, 96.59 mmol; 60% in oil) was suspended in dry DMF (150 mL) at 0 °C, followed by the dropwise addition of methyl 3-hydroxy-2-naphthoate (15.0 g, 74.3 mmol) in dry DMF (10 mL) under N<sub>2</sub>. The reaction mixture stirred for 30 minutes and was then treated with chloromethyl methyl ether (6.77 mL, 89.16 mmol). The mixture was then allowed to warm up to rt and stir for 24 hours. The resulting homogeneous solution was cooled back down to 0 °C and guenched with water (100 mL). The aqueous solution was extracted with ether (3 x 75 mL). The combined organic extracts were washed with brine (75 mL), dried (MgSO<sub>4</sub>), and concentrated to provide methyl-3-(methoxymethoxy)-2-naphthoate as a pale orange oil (18.15 g, 99 %): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.31 (s, 1H), 7.83 (d, J = 8 Hz, 1H), 7.75 (d, J = 8 Hz, 1H), 7.52 (m, 2H), 7.40 (t, J = 8 Hz, 1H), 5.37 (s, 2H), 3.97 (s, 3H), 3.57 (s, 3H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 166.83, 153.17, 136.06, 132.68, 128.77, 128.50, 126.99, 125.05, 122.60, 111.64, 95.33, 56.51, 52.42, 31.09; HRMS (ESI) calcd for  $C_{14}H_{14}O_4$  (M<sup>+</sup> + H) m/z 247.0926, found 247.0962.

**(3-(Methoxymethoxy)naphthalen-2-yl)methanol, (2.3.2).** <sup>(22)</sup> Lithium aluminum hydride (5.12 g, 135 mmol) was suspended in dry THF (100 mL) at 0 °C, followed by the dropwise addition of the methyl-3-(methoxymethoxy)-2-naphthoate (11.11

g, 45 mmol) in dry THF (5 mL) under N<sub>2</sub>. The reaction mixture was allowed to warm to rt and stir for 4 hours. The resulting homogeneous mixture was cooled back down to 0 °C and quenched with ethyl acetate (100 mL) and water (100 mL). The solution was extracted with ethyl acetate (3 x 75 mL). The combined organic extracts were washed with brine (75 mL), dried (MgSO<sub>4</sub>), and concentrated. The crude was washed with haxanes leaving white crystals (8.67 g, 88 %, M.P. 46 – 48 °C). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.77 (m, 3H), 7.42 (m, 2H), 7.38 (t, J = 8 Hz, 1H), 5.38 (s, 2H), 4.875 (d, J = 4 Hz, 2H), 3.54 (s, 3H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  153.50, 134.18, 130.99, 129.42, 127.82, 127.80, 127.00, 126.53, 124.57, 109.27, 94.80, 62.49, 56.52; HRMS (ESI) calcd for C<sub>13</sub>H<sub>13</sub>O<sub>3</sub> (M<sup>+</sup> + Na) *m/z* 241.0841, found 241.0833.

**3-(Methoxymethoxy)-2-naphthaldehyde, (2.3.3).** <sup>(23)</sup> Pyridinium chlorochromate (28.8 g, 133.6 mmol) and sodium acetate (10.9 g, 133.6 mmol) were suspended in dry DCM (150 mL) at 0 °C, followed by the dropwise addition of (3-(methoxymethoxy)naphthalene-2-yl)methanol (14.56 g, 66.8 mmol) in dry DCM (10 mL) under N<sub>2</sub>. The reaction mixture stirred at 0 °C for 1 hour and was then allowed to warm to rt and continued to stir for 4 hours. The resulting reaction mixture was diluted with ether (75 mL) and concentrated. The oil was taken back up in ether (100 mL), washed with water (3 x 75 mL), brine (75 mL), dried (MgSO<sub>4</sub>), and concentrated. The crude was washed with hexanes leaving yellow crystals (10.41 g, 72 %, M. P. 66 – 67 °C). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.40 (s, 1H), 7.9 (d, J = 8 Hz, 1H), 7.76 (d, J = 12 Hz, 1H), 7.56 (t, J = 8 Hz, 1H), 7.51 (s, 1H), 7.42 (t, J = 8 Hz, 1H), 5.43 (s, 2H), 3.58 (s, 3H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  190.43, 155.35, 137.65, 131.00, 130..11, 129.44, 128.56, 127.22, 126.06, 125.32, 110.42, 95.07, 56.73; HRMS (ESI) calcd for C<sub>13</sub>H<sub>12</sub>O<sub>3</sub> (M<sup>+</sup> + H) *m/z* 217.0820, found 217.0857, (M<sup>+</sup> + Na) *m/z* 239.0684, found 239.0673.

**2-(Methoxymethoxy)-3-vinyInaphthalene, (2.4.1).** <sup>(24)</sup> *n*-BuLi (1.01 mL, 2.5 M) was added dropwise to a stirring solution of methyltriphenylphosphonium bromide (0.9 g, 2.53 mmol) in THF (30 mL) under N<sub>2</sub> at rt. After stirring for 20 minutes, 3-(methoxymethoxy)-2-naphthaldehyde (0.43 g, 2.0 mmol in 2 mL of THF) was added dropwise over 5 minutes. The reaction mixture refluxed at 70°C for 4 hours and was cooled to rt and washed with ammonium chloride (3 x 15 mL), brine (15 mL), dried (MgSO<sub>4</sub>), and concentrated. Purification by flash chromatography using silica gel (10 % EtOAc/Hexanes) provided a yellow oil (0.37 g, 86 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.94 (s, 1H), 7.78 (d, J = 8 Hz, 1H), 7.72 (d, J = 8 Hz, 1H), 7.41 (m, 2H), 7.36 (t, J = 8 Hz, 1H), 7.20 (dd, J = 8, 4 Hz, 1H), 5.93 (d, J = 20 Hz, 1H), 5.39 (d, J = 12 Hz, 1H), 5.36 (s, 2H), 3.55 (s, 3H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  153.13, 134.23, 132.28, 129.49, 129.01, 127.89, 126.91, 126.43, 126.06, 124.48, 115.92, 109.49, 94.91, 56.41.

#### 2-(3-(Methoxymethoxy)naphthalen-2-yl)oxirane, (2.4.2). (25)

Trimethylsulfonium iodide (9.45 g, 46.3 mmol) and KOH (6.5 g, 115.8 mmol) were added to a solution of 3-(methoxymethoxy)-2-naphthaldehyde (8.34 g, 38.6 mmol) in DMSO (100 mL) at rt. The reaction mixture was heated to 80 °C and stirred for 4 hours. The homogeneous reaction mixture was diluted with water (100 mL), extracted with ether (3 x 75 mL), washed with brine (75 mL), dried (MgSO<sub>4</sub>), and concentrated leaving an orange oil (8.35 g, 94%). <sup>1</sup>H NMR (400

MHz, CDCl<sub>3</sub>)  $\delta$  7.76 (m, 3H), 7.69 (s, 1H), 7.43 (m, 2H), 7.37 (t, J = 8 Hz, 1H), 5.39 (s, 2H), 4.34 (s, 1H), 3.57 (s, 3H), 3.24 (t, J = 4 Hz, 1H), 2.79 (m, 1H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  153.86, 134.11, 129.33, 128.45, 127.95, 127.02, 126.62, 124.72, 124.59, 108.96, 94.84, 56.50, 51.51, 49.01; HRMS (ESI) calcd for C<sub>14</sub>H<sub>14</sub>O<sub>3</sub> (M<sup>+</sup> + H) *m*/*z* 231.0976, found 231.1013, (M<sup>+</sup> + Na) *m*/*z* 253.0841, found 253.0832.

1-(3-(Methoxymethoxy)naphthalen-2-yl)-2-(2-nitrophenoxy)ethanol, (2.6.1). <sup>(9)</sup> Sodium hydride (1.45 g, 36.3 mmol; 60% in oil) was added to a stirring solution of 2-nitrophenol (6.07 g, 43.6 mmol) in dry DMF (25 mL) at rt under N<sub>2</sub>. The reaction mixture stirred for 30 minutes, followed by the dropwise addition of 2-(3-(methoxymethoxy)naphthalene-2-yl)oxirane (8.35 g, 36.3 mmol) in dry DMF (5 mL). The mixture refluxed for 48 hours and was then cooled to rt, diluted with water (20 mL), and extracted with toluene (3 x 30 mL). The combined organic extracts were washed with brine (25 mL), dried  $(MqSO_4)$ , and concentrated. Purification by flash chromatography in silica gel (10% EtOAc/Hexanes) provided an orange oil (5.07 g; 38 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 (s, 1H), 7.91 (d, J = 8 Hz, 1H), 7.84 (d, J = 8 Hz, 1H), 7.76 (m, 2H), 7.53 (t, J = 8 Hz, 1H), 7.44 (m, 4H), 7.09 (m, 2H), 5.64 (d, J = 8 Hz, 1H), 5.38 (s, 2H), 4.57 (d, J = 8 Hz, 1H), 4.12 (m, 1H), 3.54 (s, 3H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 152.42, 152.17, 134.59, 129.43, 128.13, 127.09, 126.90, 126.75, 126.15, 124.65, 121.18, 115.49, 109.04, 94.81, 74.43, 67.80, 56.63, 31.14; HRMS (ESI) calcd for  $C_{20}H_{19}NO_6$  (M<sup>+</sup> + Na) *m*/*z* 329.111, found 329.10976.

2-(1,2-*bis*(2-Nitrophenoxy)ethyl)-3-(methoxymethoxy)naphthalene, (2.6.2). <sup>(9)</sup> 1-Fluoro-2-nitrobenzene (2.17 mL, 20.55 mmol) was added dropwise to a stirring solution of 1-(3-(methoxymethoxy)naphthalen-2-yl)-2-(2-nitrophenoxy)ethanol (5.07 g, 13.7 mmol) in dry DCM (100 mL) at 0 °C under N<sub>2</sub>. The reaction mixture was treated with NaH (0.71 g, 60% in oil, 17.81 mmol) and stirred for 10 minutes before warmed to rt and stirred overnight. The mixture was diluted with water (75 mL), and extracted with chloroform (3 x 75 mL). The combined organic extracts were washed with brine, dried (MgSO<sub>4</sub>), and concentrated. Purification by flash chromatography in silica gel (10% EtOAc/Hexanes) provided an orange oil (3.94 g, 59 %). 1H NMR (400 MHz, CDCl3) δ 8.06 (s, 1H), 7.79 (m, 4H), 7.52 (m, 2H), 7.46 (t, J = 8 Hz, 1H), 7.38 (t, J = 8 Hz, 2H), 7.26 (t, J = 8 Hz, 1H), 7.08 (m, 2H), 6.98 (t, J = 8 Hz, 1H), 6.33 (dd, J = 4, 4 Hz, 1H), 5.47 (q, J = 4 Hz, 2H),4.60 (m, 1H), 4.51 (dd, J = 4, 8 Hz, 1H), 3.60 (s, 3H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 152.28, 151.95, 151.29, 140.64, 140.55, 134.51, 134.39, 134.19, 128.30, 127.73, 126.98, 125.79, 125.73, 125.58, 121.50, 121.08, 116.44, 116.31, 109.56, 95.00, 75.67, 73.46, 68.00, 56.75; HRMS (ESI) calcd for  $C_{26}H_{22}N_2O_6$  (M<sup>+</sup> + Na) *m*/*z* 513.1247, found 513.1262.

# **2-(1,2-***bis*(**2-**Aminophenoxy)ethyl)-**3-(methoxymethoxy)naphthalene, (2.7.1).** Zinc dust (3.98 g, 60.8 mmol) was added to a stirring solution of 2-(1,2-*bis*(2nitrophenoxy)ethyl)-**3-(methoxymethoxy)naphthalene (3.69 g, 7.6 mmol) in** absolute methanol (150 mL) at r.t. The solution was treated with glacial acetic acid (3.48 mL, 60.8 mmol) and stirred at r.t. overnight. The reaction mixture was diluted with EtOAc (100 mL) and basicified with sodium bicarbonate to a pH of 8.

The solution was extracted with EtOAc (3 x 75 mL), washed with brine, dried (MgSO<sub>4</sub>), and concentrated. The crude was recrystallized with EtOAc/Hexanes forming a pale white crystal (2.90 g, 89 %, M.P. 134 – 135 °C). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.03 (s, 1H), 7.77 (d, J = 8 Hz, 1H), 7.50 (s, 1H), 7.46 (t, J = 8 Hz, 1H), 7.37 (t, J = 8 Hz, 1H), 6.88 (d, J = 8 Hz, 1H), 6.82 (m, 1H), 6.75 (m, 4H), 6.62 (d, J = 8 Hz, 1H), 6.50 (t, J = 8 Hz, 1H), 6.015 (d, J = 4 Hz, 1H), 5.44 (s, 2H), 4.41 (m, 2H), 4.0 (s, broad 4H), 3.55 (s, 3H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  152.07, 146.55, 146.20, 137.51, 137.02, 134.32, 129.39, 128.15, 127.84, 127.36, 127.02, 126.89, 124.70, 122.28, 121.97, 118.52, 118.47, 115.64, 115.57, 114.86, 112.56, 109.29, 94.73, 75.95, 71.89, 56.69; HRMS (ESI) calcd for C<sub>26</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub> (M<sup>+</sup>+H) *m/z* 431.1926 found 431.1965.

## 2-(1,2-bis(o-Aminophenoxy)ethane- $N,N,N^2,N^2$ -tetramethylacetate)-3-

(methoxymethoxy)naphthalene, (2.8.1). <sup>(9)</sup> Sodium iodide (4.2 g, 28.0 mmol) was added to s stirring solution of methyl bromoacetate (2.83 mL, 28.0 mmol) in dry DMF (30 mL) at r.t. under N<sub>2</sub>. The reaction mixture stirred for 1 hour followed by the addition of 2-(1,2-bis(2-aminophenoxy)) ethyl)-3-

(methoxymethoxy)naphthalene (0.3 g, 0.7 mmol) in dry DMF (2 mL). Potassium carbonate (3.87 g, 28.0 mmol) was added and the reaction mixture stirred at 90 °C for 48 hours. The mixture was cooled to r.t. and diluted with 30 mL of toluene. It was then washed with water (35 mL), brine, dried (MgSO<sub>4</sub>), and concentrated. Purification by flash chromatography in silica gel (30% EtOAc/Hexanes) provided a colorless oil (0.1 g, 20 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.06 (s, 1H), 7.79 (d, J = 8 Hz, 1H), 7.72 (d, J = 8 Hz, 1H), 7.46 (s, 1H), 7.41 (t, J = 4 Hz, 1h), 7.33 (t, J =

8 Hz, 1H), 6.88 (m, 3H), 6.83 (m, 2H), 6. 73 (m, 2H), 6.68 (m, 1H), 6.25 (t, J = 8 Hz, 1H), 5.42 (s, 2H), 4.30 (s, 4H), 4.25 (s, 4H), 3.60 (s, 6H), 3.55 (s, 3H), 3.45 (s, 6H); <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  172.35, 171.87, 152.37, 150.61, 149.47, 140.02, 139.06, 134.25, 128.29, 127.83, 127.00, 126.80, 124.38, 122.51, 122.26, 121.33, 121.25, 119.47, 114.25, 94.57, 73.38, 71.34, 56.49, 53.82, 53.12, 51.71, 51.57; HRMS (ESI) calcd for C<sub>38</sub>H<sub>42</sub>N<sub>2</sub>O<sub>12</sub> (M<sup>+</sup>+H) *m/z* 719.2771 found 719.2753.

## 2-(1,2-bis(o-Aminophenoxy)ethane-N,N,N<sup>2</sup>,N<sup>2</sup>-tetraacetic acid)-3-

hydroxynaphthalene (2.8.2). A stirring solution of (2.6.1) (2.43 g, 3.38 mmol) in 1:1 CH<sub>3</sub>CN/H<sub>2</sub>O (25 mL) was treated with concentrated HCI (0.05 mL). The solution stirred at 60 °C for 16 hours and was then cooled to rt. The solution was neutralized to a pH of 7 using 1M sodium bicarbonate, extracted with ether (3 x 30 mL), washed with brine, dried (MgSO<sub>4</sub>), and concentrated. Purification using silica gel (10% MeOH/DCM) followed by an acid base extraction yielded 1.36 g of a mixture of the fully hydrolyzed and partially hydrolyzed ligand. The intermediate was taken up in methanol (30 mL) and treated with KOH (0.59 g, 10.5 mmol). The mixture stirred overnight at 70 °C and was then cooled to rt. The solution was acidified using 5% HCl and extracted using ether (3 x 30 mL), washed with brine, dried (MgSO<sub>4</sub>), and concentrated. Purification using silica gel (10% MeOH/DCM) followed by recrystallization using ether and hexanes provided a light ten crystal (0.12 g, 6%, M.P. 93-94 °C). <sup>1</sup>H NMR (400 MHz,  $CD_3OD$ )  $\delta$  7.73 (s, 1H), 7.65 (d, J = 8 Hz, 1H), 7.52 (d, J = 8 Hz, 1H), 7.26 (t, J = 8 Hz, 1H), 7.16 (t, J = 8 Hz, 1H), 7.05 (s, 1H), 6.75 (m, 8H), 5.07 (t, J = 8 Hz, 1H), 4.12 (d, J = 12 Hz, 1h), 4.07 (m, 1H), 4.04 (m, 2H), 3.61 (s, 2H), 3.21 (s,

4H); <sup>13</sup>C NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  175.65, 175.21, 174.06, 173.09, 154.86, 152.15, 140.33, 136.08, 129.88, 128.95, 128.15, 127.97, 127.34, 126.92, 124.30, 123.70, 122.31, 120.71, 114.98, 110.27, 79.06, 73.07, 61.66, 57.83, 55.10, 54.91, 52.38, 33.19, 30.88, 23.86, 21.01, 14.61; HRMS calcd for C<sub>32</sub>H<sub>30</sub>N<sub>2</sub>O<sub>11</sub> (M<sup>+</sup>+H) *m*/*z* 619.1993 found 619.2567.

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APPENDIX

<sup>1</sup>H NMR and <sup>13</sup>C NMR Relevant to the Synthesis of NQMP-BAPTA























2.3.3

EIDOD 75.7-

TS'2 #5'2~ 95'2~ 85'2—



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55'8-













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24.2



δ

HO-









3.60











CO<sub>2</sub>Me













2.8.2

	I.	0
		0
	T9.41	1
	10°12	5
	61.85	- M
-48.51 cd3od	J	40
- 49'69 69'64 - 49'19 69'94 - 75'38	Ť	- 22
01 '55' 88 '25 00 '79		- 9
	۲۵.٤۲	70
	90.97	- 80
		- 06
	12011 86'0'11 12'021-	100
	-15'21 0/'EZI- 05'#210	110 (ppm
		120 f3
	56'8ZI	130
	EE.04.1	140
	ST/2ST	150
	98751	160
	60°£/1 90°¢/1	170
со <sub>2</sub> н со	12'SLT 7 59'SLT 7	180
		190
		200
		210
	1	220
		230
		-