

## The Synthesis and Characterization of Co(Am<sub>2</sub>Bcyclam)PF<sub>6</sub> A model for new MRI Contrast Agents

Adedamola Grillo

### ABSTRACT

The ligand 4, 11 – Bisacetamido – 1, 4, 8, 11 – tetraazabicyclo[6.6.2]hexadecane (**L**) has been synthesized. Characterization of this product has been achieved by mass spectrometry, elemental analysis, and nuclear magnetic resonance spectroscopy. Assignment of the proton NMR spectrum in CDCl<sub>3</sub> has been carried out and confirms the structure of the ligand. A preliminary study of the ligand's binding ability has been performed through complexation to Co(III) in the complex [Co(**L**)]PF<sub>6</sub><sub>3</sub>

Keywords: *cross – bridged ligands, tetraazamacrocyclic, Cobalt, macrobicyclic*

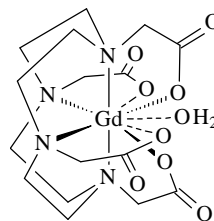
### INTRODUCTION

A long-term goal of the study of cross-bridged ligands in our lab is the development of improved MRI contrast agents (Hubin and Meade, 2000). Magnetic resonance imaging (MRI) provides an unparalleled view of human tissue. The amount of information that can be obtained through MRI is higher than in most other diagnostic methods (Aime et al., 1999; Lauffer, 1987). MRI images display sharp contrast differences across tissue boundaries as a result of their different water contents and water environments. Contrast agents help improve the quality of the image. MRI contrast agents work by altering the local magnetic field in the tissue being examined (Tweedle and Kumark, 1999). Normal and abnormal tissue will respond differently to this slight alteration, resulting to differing signals. This gives better visualization of several different types of tissue abnormalities and disease development compared to ordinary MRI.

MRI contrast agents generally utilize Gd<sup>3+</sup> because it has a high magnetic moment due to its seven unpaired electrons (Aime, et al, 1998). As a result, it modulates the magnetic properties of water in tissues (Aime, et al, 1998) and this modification results in improved contrast in the resulting MRI images. Theoretically, the more open coordination sites in the Gd complex there are to interact with water, the better the resolution of the MRI image (Koenig and Brown, 1990). However, at the same time, the Gd<sup>3+</sup> must be held in place firmly enough by the ligand so that it does not come off under physiological conditions because Gd<sup>3+</sup> is toxic to humans (Koenig and Brown, 1990).

Presently, the ligands used for MRI contrast agents have only one open coordination site on Gd<sup>3+</sup> to interact with water (Koenig and Brown, 1990). Most ligands leaving more open sites on Gd do not form strong enough complexes with the ion to avert release of the toxic metal. Cross – bridged tetraazamacrocyclics such as ligand 4, 11 – Bisacetamido–1, 4, 8, 11 tetraazabicyclo[6.6.2] hexadecane **L** have been proposed to leave more (3)

open coordination sites on the Gd<sup>3+</sup>, and at the same time provide exceptional kinetic stability (Hubin, et al., 1998). Both of these aspects make them potentially ideal candidates for MRI contrast agents (Hubin and Meade, 2000).

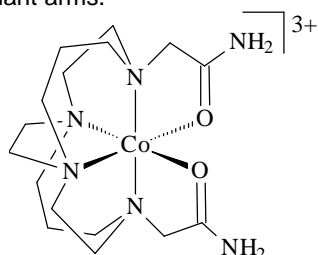


**Figure 1.** The Gd(III) complex of 1,4,7,10-tetraacetato-1,4,7,10-tetraazacyclododecane(DOTA), a commonly used MRI Contrast agent. Note there is no bridge between any of the nitrogens.

This particular study will look at how adding amide pendant arms to cross – bridged tetraazamacrocyclics affects the physical and chemical properties of this type of ligand. The purpose of this experiment is to open two more sites on the metal and yet have the complex stable in order not to expose the gadolinium to the tissue. The ligand synthesized will have a cross-bridge between two nitrogens, hopefully providing sufficient complex stability, and amide pendant arms that could further modulate the metal's interaction with water through hydrogen bonding. The ethylene cross-bridge has two purposes: (1) to increase the binding ability of the ligand allowing the now hexadentate ligand to hold Gd<sup>3+</sup> strongly, and (2) to open up two more coordination sites for water (Hubin and Meade, 2000). The ultimate goal is synthesis of a Gd(III) complex as in Figure 2. The first step toward this goal, and the subject of this paper, is the synthesis and characterization of the ligand 4, 11 – Bisacetamido – 1, 4, 8, 11 – tetraazabicyclo [6.6.2] hexadecane (**L**) (Weisman, et al., 1990; Weisman, et

al., 1996; Weisman, et al., 2000). Finally, we will provide evidence of ligand complexation to a transition metal as the next step toward the more difficult final goal of Gd(III) complexation (Hubin, et al., 1999).

Co<sup>II</sup> and Co<sup>III</sup> complexes are the standard for modern coordination chemistry (Cotton and Wilkinson, 1988). Complexes made with new ligands and these metals can be compared to the properties of the large database of known Co<sup>II</sup> and Co<sup>III</sup> complexes. In this case, the new ligand will be an ethylene cross-bridged tetraazamacrocyle having amide pendant arms.



**Figure 2.** The proposed Gd(III) complex of 4, 11 – Bisacetamido – 1, 4, 8, 11 – tetraazabicyclo[6.6.2] hexadecane. Note the bridge between the two nitrogens absent in Fig. 1

## MATERIALS AND METHODS

Dr. T.J. Hubin supplied solutes and solvents. The Natural Science Department provided hydrogen and nitrogen gas, glassware and other equipment. Reactions done with hydrogen and nitrogen gas were done in the hood with proper supervision. Overnight reactions were checked every three to five hours.

### Cyclam (1)

NiCl<sub>2</sub>·H<sub>2</sub>O (35.66g, 0.15mol) was dissolved in 100ml distilled water. 1,5,8,12 tetraazadodecane (26g, 0.15mol) was added. The resulting purple solution was cooled to 0°C in an ice bath. A solution of 40% glyoxal (22.5ml) was added and stirred for 4 hours. The solution was then cooled again to 0°C. NaBH<sub>4</sub> (11g) was added over a period of 30 minutes. The resulting solution was heated at 90°C for 20 minutes on a hot plate. The solution was filtered through celite on a medium frit. The solution was stored in the fridge until the next day. The solution was transferred to 1liter round-bottom flask. KCN (39g, 0.6mol) was added and stirred. The resulting solution was refluxed with condenser for 2 hours and cooled to room temperature and NaOH (15g) was added and stirred. Evaporation to semisolid was achieved using the rotovap. CHCl<sub>3</sub> (100ml) was then added. This was filtered through medium size frit and the solid washed twice with 100ml CHCl<sub>3</sub>. Using a separatory funnel, the product was extracted five 5 times with 50 ml CHCl<sub>3</sub>. The organic layer was dried overnight over Na<sub>2</sub>SO<sub>4</sub>, which was filter on fast filter

paper. (Barefield, et al., 1976). The solvent was removed yielding pure Cyclam (1) (16g).

***cis-Decahydro-1H,6H-3a,5a,8a,10a-tetraazapyrene (2)***

A portion of **(1)** (11.30g) was flushed with N<sub>2</sub> gas for 15 minutes and dry CH<sub>2</sub>CN (40ml) was added. 40% Glyoxal (13ml) was then added. The resulting solution was stirred at 50-65°C for 3 hours under N<sub>2</sub> gas. The solution was cooled to room temperature. Solvent was then evaporated. The residue was extracted with CHCl<sub>3</sub> (6x40ml). The solution was then filtered to remove CH<sub>3</sub>CN layer. The organic layer was then dried over Na<sub>2</sub>SO<sub>4</sub> and then dried to a crude oily product. Column chromatography using basic alumina as the stationary phase and 1% methanol in methylene chloride as the eluent purified the product, a waxy, colorless solid **(2)** (9.94g).

***-Decahydro-3a-8a-bis(9phenylmethyl)-1H,6H-3a,5a-8a,10a-tetraazapyrenium-Dibromide Monohydrate (3)***

A small amount of **(2)** (9.94g) was dissolved in CH<sub>3</sub>CN (350ml) and added to a 3 neck round bottom flask and flushed with N<sub>2</sub> gas for 15 min. BnBr (92.51ml) was added and stirred at room temperature under N<sub>2</sub> gas for 4 days. The resulting off-white precipitate **(3)** (20.00g) was filtered under vacuum and washed with ethyl acetate to remove excess BnBr.

***4,11-Dibenzyl-1,4,8,11-tetraazabicyclo[6,6,2]hexadecane (4)***

A portion of **(3)** (20.00g) was stirred with 95% ethanol (1.5liters) under N<sub>2</sub> gas. NaBH<sub>4</sub> (40g) was added slowly. This was stirred under N<sub>2</sub> gas for 5 days. Excess NaBH<sub>4</sub> was decomposed with 10% HCl (3molar) to a pH of 1. Ethanol was then rotovapped off. The residue was dissolved in H<sub>2</sub>O (150ml) and then made strongly basic with 30% KOH (100ml) followed by KOH pellet to a pH of 14. The product was then extracted in benzene and dried overnight over Na<sub>2</sub>SO<sub>4</sub>. Benzene was evaporated to give **(4)** (9.00g).

***1,4,8,11-Tetraazabicyclo[6,6,2]hexadecane(5)-***

A huge fraction **(4)** (8.61g) was stirred with 85% acetic acid (50ml) and degassed by bubbling N<sub>2</sub> for 30 minutes. 10% Pd on Carbon (2g) was added to the reaction slowly while stirring. N<sub>2</sub> was bubbled through the solution for 10 more minutes. H<sub>2</sub> was bubbled through the mixture from a balloon four times to remove N<sub>2</sub> and O<sub>2</sub>. The reaction was stirred under H<sub>2</sub> via a balloon (which was refilled when necessary) for four days. Solvent was evaporated and the residue dissolved in pH = 14 KOH solution. Product was extracted in benzene. Benzene was evaporated to give **(5)** (3.86g) and stored in the fridge.

***4,11-Bisacetamido-1,4,8,11-tetraazabicyclo[6,6,2]hexadecane(6)***

A small amount of **(5)** (2g) was dissolved in MeCN

(200ml) and treated with anhydrous K<sub>2</sub>CO<sub>3</sub> and KI (2g) and α-chloroacetamide (3.4g). The resulting solution was heated at 60°C under N<sub>2</sub> for 24 hours. The solvent was then rotovapped off and the residue was dissolved in H<sub>2</sub>O (120ml). The solution was brought to a pH of 14 with KOH pellet. The product was extracted in CHCl<sub>3</sub> (4x50ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> overnight. The Na<sub>2</sub>SO<sub>4</sub> was filtered and CHCl<sub>3</sub> was rotovapped off. The residue was then dissolved in ethanol (200ml) (Weisman, et al., 1990; Weisman, et al., 1996; Weisman, et al., 2000). The solution was rotovapped off to give **(6)** (2.34g). (Anal. Calcd for C<sub>16</sub>H<sub>32</sub>N<sub>6</sub>O<sub>2</sub>: C, 56.44; H, 9.47; N, 24.68. Found: C, 50.43; H, 8.65; N, 20.23.)

**Co (III) COMPLEX (7)-AIM VIII**

**(6)** (0.25g) was dissolved in 4:1 acetonitrile methanol (40ml) in a 100ml round bottom flask. N<sub>2</sub> gas was bubbled through the solution for 15 minutes. CoCl<sub>2</sub> (0.095g) was added and left stirring for 24 hours under N<sub>2</sub> gas. The blue color solution was then refluxed under N<sub>2</sub> gas for 2 days. The solution was then rotovapped to a blue brown solid. The resulting solution was dissolved in distilled H<sub>2</sub>O (15ml) and 6 M HCl (5ml). Compressed air was then bubbled through the solution. H<sub>2</sub>O was then rotovapped off. A solution of NH<sub>4</sub>PF<sub>6</sub> (0.603g) dissolved in methanol (10ml) was made and filtered. Both solutions were then mixed and left to precipitate in the fridge. The solution was filtered off and the purple solid product kept **(8a)** (Anal. Calcd for CoC<sub>16</sub>H<sub>32</sub>N<sub>6</sub>O<sub>2</sub>P<sub>3</sub>F<sub>18</sub>: C 23.03; H 3.87; N 10.07. Found: C 23.26; H 3.60; N 9.96.). A second crop of product was obtained by adding ether to the filtrate **(8b)**

**RESULTS AND DISCUSSION**

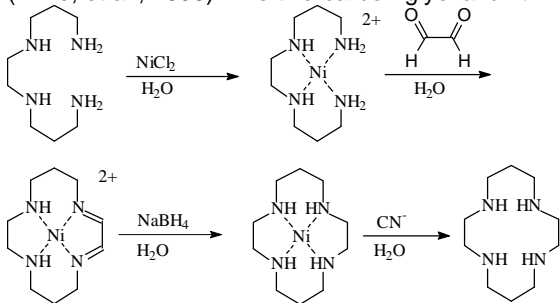
The mass spectrometry, <sup>1</sup>H and <sup>13</sup>C NMR was achieved at Kansas University. Quantitative analysis obtained from Quantitative Analysis Inc. New Jersey.

***Synthesis. 1 (Cyclam (1))***

Cyclam, or 1,4,8,11-tetraazacyclotetradecane, is a tetraazamacrocyclic compound commonly used for complexation of transition and other metal ions. It is rather expensive to buy (\$50/gram) but can be synthesized from a linear tetraamine and glyoxal (Fig. 3). The ring closure is facilitated by the metal ion organizing the flexible tetraamine into the correct geometry for reaction with glyoxal (Barfield, et al., 1976). There was only about 50% yield for this product, which is about average for this synthesis. No superior route to cyclam has been achieved. Characterization of this compound using melting point and Infrared Spectrometry (IR) has already been achieved in chemistry literature (Barfield, et al., 1976).

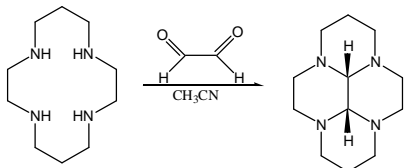
***Synthesis 2 (cis-Decahydro-1H,6H-3a,5a,8a,10a-tetraazapyrene (2))***

The synthesis of the cross-bridged ligand begins with the condensation of Glyoxal and Cyclam to give a 78% yield of tetracyclic bisaminal (Fig. 3) (Aime, et al., 1998). The two-carbon glyoxal unit will



**Figure 3** Synthesis of Cyclam

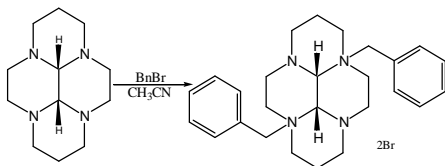
eventually become the two-carbon cross-bridge. This condensation reaction places each of these carbons in the center of the cyclam ring bound to two adjacent nitrogens each resulting from a modified Schiff-base condensation.



**Figure 4.** Condensation of glyoxal into the cyclam ring.

**Synthesis 3 (-Decahydro-3a-8a-bis(9phenylmethyl)-1H,6H-3a,5a-8a,10a-tetraapyrenium-Dibromide Monohydrate (3))**

The tetracyclic tetraamine resulting was dialkylated with excess benzyl bromide to give an 85% yield of bis-quaternary ammonium halide. Other alkyl groups, such as methyl iodide can be used for this type of quaternization, but the benzyl groups offer the ability to be removed at a further step in order to replace them with other pendant arms. The reaction proceeds slowly, but with good yield. The product is easily collected (by filtration) and purified (by washing with ethyl acetate).

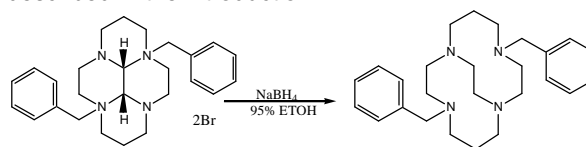


**Figure 5** Bis-alkylation of the tetracyclic tetraamine.

**Synthesis 4 (4,11-Dibenzyl-1,4,8,11-tetraazabicyclo [6,6,2] hexadecane (4))**

The bis-quaternary ammonium halide product was now much more reactive than the neutral tetraamine. Specifically, reductive ring cleavage with

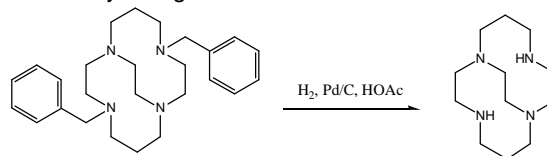
sodium borohydride gives a 63.6% of the double ring expanded cross-bridge ligand (Weisman, et al., 1990; Weisman, et al., 1996; Weisman, et al., 2000). This is the crucial step in producing the cross-bridged architecture having the desirable properties described in the introduction.



**Figure 6.** Reductive ring cleavage of the alkylated salt.

**Synthesis 5 (1,4,8,11-Tetraazabicyclo [6,6,2]hexadecane(5))**

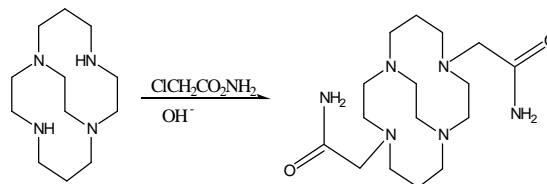
This neutral, bis-alkylated ligand was cleanly debenzylated by atmospheric pressure hydrogenolysis to give an 80.58% yield of the parent cross bridge ligand. This ligand was then ready to have various pendant arms attached at the secondary nitrogen sites.



**Figure 7** Debenzylation by hydrogenolysis.

**Synthesis 6 (4,11-Bisacetamido-1,4,8,11 tetraazabicyclo [6,6,2]hexadecane(6))**

The (CONH<sub>2</sub>) pendant arms were then be added according to a literature procedure (Hubin, et al., 1999). This reaction had a 76.1% yield.



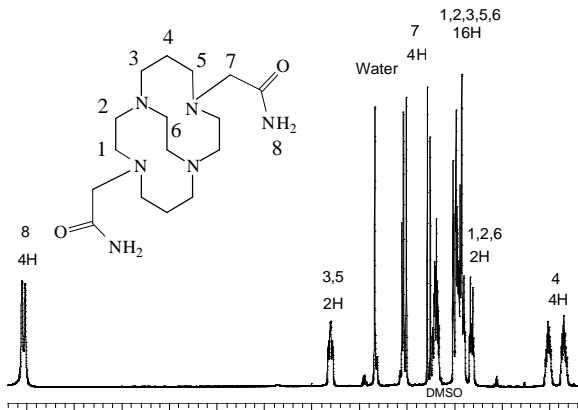
**Figure 8** Attachment of amide pendant arms.

*Characterization of the Ligand.* Mass spectrometry provided the first evidence of successful ligand synthesis. A very clean FAB<sup>+</sup> spectrum was obtained of the ligand exhibiting a large peak at  $m/z = 341$ , corresponding to the protonated ligand. This is characteristic of this ligand family (Weisman, et al, 1990; Weisman, et al, 1996; Weisman, et al, 2000).

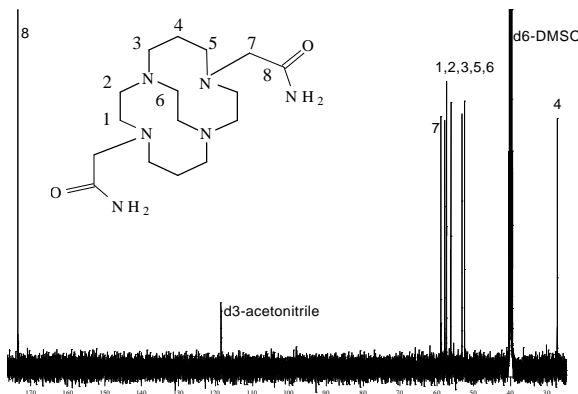
The elemental analysis performed on the ligand showed there are some impurities present. The likely contaminant was water, as amines are often hygroscopic. The actual analysis of the ligand was closer to a calculated formula including two water molecules. The amount of ligand on hand was small enough that purification would be difficult to achieve.

Therefore no further purification was made.

NMR spectroscopy showed that whatever impurities are present, the majority of the compound is the desired ligand. The proton spectrum is shown in figure 9. Integration revealed the correct number and ratio of protons for the theoretical structure. The peak at 1.5 ppm is easily assigned as the four hydrogens of the secondary alkyl carbon at position 4. The peak at position 3.0 ppm integrated for 4 protons, which can be assigned to the methylene group next to the amide carbonyl at position 7. The 4 amine protons (position 8) are located at 7.0 ppm. The signal at 3.4 ppm is water contamination of the ligand, which also showed up in the elemental analysis. Other assignments are less straightforward. The multiplet around 2.3-2.5 ppm accounts for 18 protons located at nearly indistinguishable positions 1,2,3,5, and 6. A two-proton peak at about 3.8-3.9 ppm most likely come from protons in positions 3 or 5, according to 2D studies (not shown). Finally, the multiplet at around 2.7 ppm is due to the d7-DMSO solvent.



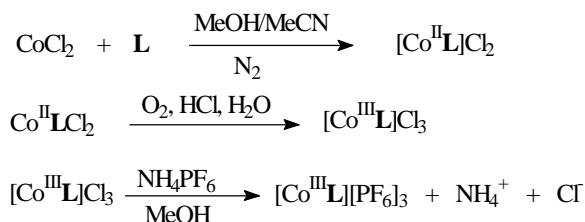
**Figure 9.**  $^1\text{H}$  NMR spectrum of the Ligand (4,11-Bisacetamido-1,4,8,11-tetraazabicyclo[6,6,2]hexadecane).



**Figure 10.**  $^{13}\text{C}$  NMR spectrum of our Ligand (4,11-Bisacetamido-1,4,8,11-tetraazabicyclo[6,6,2]hexadecane)

The  $^{13}\text{C}$  NMR spectrum in d6-DMSO is Figure 10. Again, only some of the signals can definitively be assigned. First is the central secondary alkyl carbon at  $\sim 26$  ppm, clearly the central methylene group of the propylene chain at position 4. Two peaks in this spectrum come from solvents, d7-DMSO at 40 ppm and d3-acetonitrile (residue remaining in the sample from a failed attempt to dissolve the compound) at 120 ppm. The nearly identical methylene carbons adjacent to nitrogen all had shifts between 50 and 60 ppm. Finally, the carbonyl carbon of the amide group was found at its characteristic position between 170 and 180 ppm. Although neither NMR spectrum could be completely assigned, they both indicate a relatively pure ligand consistent with the proposed structure. The theoretical Analytical Calculation for  $\text{C}_{16}\text{H}_{32}\text{N}_6\text{O}_2$  is C, 56.44%; H, 9.47%; N, 24.68%. The experimental Analytical Calculation found was C, 50.43%; H, 8.65%; N, 20.23%.

**Complexation of Ligand.** The ligand was reacted with  $\text{CoCl}_2$  to generate  $[\text{Co}^{\text{II}}\text{L}]$ . The divalent cobalt ion is labile enough to expect rapid complexation, but the resulting product is air sensitive. To produce a more easily manipulated, air-stable species, this complex was converted to the Co(III) chloride complex by simply bubbling air through an aqueous acid solution. Oxygen acted as the oxidant. The resulting chloride salt is difficult to purify, so it was converted to the hexafluorophosphate salt with  $\text{NH}_4\text{PF}_6$  to give  $[\text{Co}^{\text{III}}\text{L}][\text{PF}_6]_3$ . This compound is easier to purify due to its solubility in acetonitrile. Recrystallization of the  $\text{PF}_6$  salt from acetonitrile gave a pure complex, as confirmed by elemental analysis. Further research will be carried out on the characterization of the complex (Lichty, 2000; Allen 2002).



**Figure 11.** Synthetic scheme for  $[\text{CoL}][\text{PF}_6]_3$ .

## ACKNOWLEDGEMENTS

I would like to thank Dr. Hubin for his ideas and contribution in this project. Without his timeless support this project could not have been possible. He supplied some of the material used during this reaction.

## LITERATURE CITED

Aime, S., M. Botta, M. Fasano, and E. Terreno. 1998. Lanthanide(III) chelates for NMR biomedical

- applications. *Chemical Society Reviews* 27: 19-29
- Aime, S., M. Botta, M. Fasano, E. Terreno. 1999. Prototropic and Water-Exchange Processes In Aqueous Solutions of Gd(III) Chelates. *Accounts of Chemical Research* 32: 941-949.
- Allen, S. 2002. In Preparation
- Barefield, E.K., F. Wagner, and A.W. Herlinger. 1976. (1,4,8,11-tetraajacyclotetradecane) Nickel(II) perchlorate and 1,4,8,11-tetraajacyclotetradecane *Inorganic Synthesis* 16: 220.
- Cotton, F.A., and G. Wilkinson, 1988. *Advanced Inorganic Chemistry*, 5<sup>th</sup> edition.
- Hubin, T.J., J.M. McCormick, N.W. Alcock, D.H. Busch, and N.J. Clase. 1999. Crystallographic Characterization of Stepwise Changes in Ligand Conformations as Their Internal Topology Changes and Two Novel Cross-Bridged Tetraazamacrocyclic Copper(II) Complexes *Inorganic Chemistry* 38: 4435-4446.
- Hubin, T.J., J.M. McCormick, N.W. Alcock, H.J. Clase, and D.H. Busch. 1998. Ultra-rigid gross-bridged tetraajamacrocycles and Ligands—the challenge and the solution. *Journal of the Chemical Society, Chemical Communication* 16: 1609
- Hubin, T.J., and T.J. Meade. 2000. In Preparation
- Koenig, S.H., and R.D. Brown VI. 1990. Field-cycling relaxation of protein solutions and tissue-Implications for MRI *Progress in NMR Spectroscopy* 22: 487.
- Lauffer, R.B. 1987. Paramagnetic metal complexes as water proton relaxation agents for NMR Imaging- theory and design. *Chemical Reviews* 87: 901.
- Lichty, J. 2001. The synthesis and characterization of Co(AcBcyclam)PF<sub>6</sub>. *Centaurus* 8:
- Tweedle, M.F., and K. Kumark. 1999. In *Magnetic Resonance Imaging (MRI) Contrast Agents*; Clarhe, M.I., Sadler, P.J., Eds.; Springer; Berlin, Germany, 2: 1
- Weisman G.R., M.E Rogers, E.H. Wong, J.P. Jasinski, and E.S. Paight. 1990. Cross-Bridged Cyclam. Protonation and Li<sup>+</sup> Complexation in a Diamond-Lattice Cleft. *Journal of the American Chemical Society* 112: 8604-8605.
- Weisman G.R., E.H. Wong, D.C. Hill, M.E. Rogers, D.P. Reed, and J.C. Calabrese. 1996. Synthesis and Transition Metal Complexes of New Cross-Bridged Tetraamine Ligands. *Journal of the Chemical Society, Chemical Communication*. 947- 948.
- Weisman G.R. E.H. Wong, D.C Hill, D.P. Reed, M.E. Rogers, J.S. Condon, M.A. Fagan, J.C. Calabrese, K. Lam, I.A. Guzei, and A.L. Rheingold, 2000. Synthesis and Characterization of Cross-Bridged Cyclams and Pendant-Armed Derivatives and Structural Studies of their Copper(II) Complexes. *Journal of the American Chemical Society* 122: 10561-10572