Characterization of Co (Am$_2$Bcyclam) PF$_6$

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ABSTRACT

The complex ([CoL]$^{3+}$) of ligand 4, 11 – bisacetamido – 1, 4, 8, 11 – tetraazabicyclo [6.6.2] hexadecane (L) and Co^{3+} has been characterized. Characterization of the complex was achieved by mass spectrometry, infrared absorption (IR), elemental analysis, proton and ^{13}C nuclear magnetic resonance spectroscopy (NMR), electronic spectroscopy (UV/Vis), conductance measurements, and cyclic voltammetry. Characterization through testing and comparison with similar Co complexes support the purposed complex structure (Figure 5 – [CoL]$^{3+}$).

Keywords: *cross-bridged ligands, tetraazamacrocycle, cobalt, macrobicycle*

INTRODUCTION

The occurrence of tetraazamacrocyclic ligands in key biological complexes, such as porphyrin of the heme proteins and corrin of vitamin B-12 (Figure 1), suggests the advantages of the ligands' structure (Hung, *et al*, 1976). The most applicable advantage, from the chemistry perspective, is the stability the ligand provides it's complex. With this advantage as a motivating force, the tetraazamacrocycle ligand has become the prototypical motif for much of coordination chemistry (Hung, *et al*, 1976).
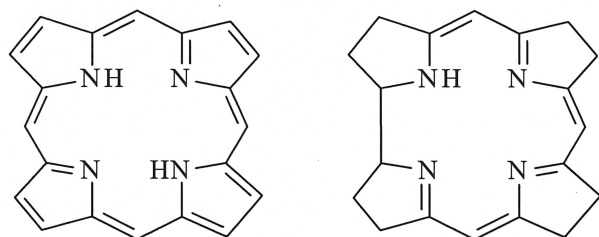

Figure 1 Porphyrin and Corrin, two naturally occurring tetraazamacrocyclic ligands.

Weisman and Ciampolini rigidified simple monocyclic tetraazamacrocyclic ligands (Figure 1 – L$_3$) by adding an ethylene cross-bridge (Figure 2 – L$_2$) (Bencini, *et al*, 1994; Weisman, *et al*, 1990). Cross-bridged tetraazamacrocyclic ligand complexes were shown to be kinetically stable after prolonged exposure to hostile environments failed to decompose the complexes (Hubin, *et al*, 1998; Hubin, *et al*, 2000).
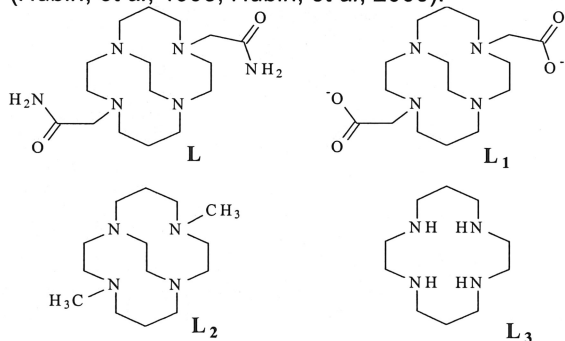
The unfavorable interaction of cross-bridged tetraazamacrocyclic ligands with the protons in reaction media hindered the binding of transition metals used in coordination chemistry studies (Hubin, *et al*, 1998). This undesirable characteristic earned these ligands the label "proton sponge" (Weisman, *et al*, 1990; Bencini, *et al*, 1994). Competition between metal and protons was eliminated when protons were purged from the reaction media (Hubin, *et al*, 1998). Without proton interference, metal complexes were produced in high yields (Hubin, *et al*, 1998).

Construction of a Magnetic Resonance Imaging (MRI) contrast agent is one goal of research involving these ligands (Hubin, *et al*, 1998). The ultimate MRI contrast agent will utilize Gd^{3+} because the metal's high magnetic moment (Aime, *et al*, 1998) will favorably change the local magnetic field of the tissue water the MRI is probing (Tweedle and Kumark, 1999). Some form of a tetraazamacrocyclic ligand will be utilized to prevent the release of the free toxic metal ion, which is harmful to the human subject. Experimentation is being done to find a ligand that will aid in achieving the goal of constructing a viable MRI contrast agent (Grillo, 2002; Hubin, *et al*, 1998; Jonas, 2001). Figure 3 illustrates how the amide pendant arms used in this study may secure the Gd^{3+} while allowing the metal to interact with water. The figure also shows how amide pendant arms may aid in alteration of local tissue water through it's own H-bonding with water.
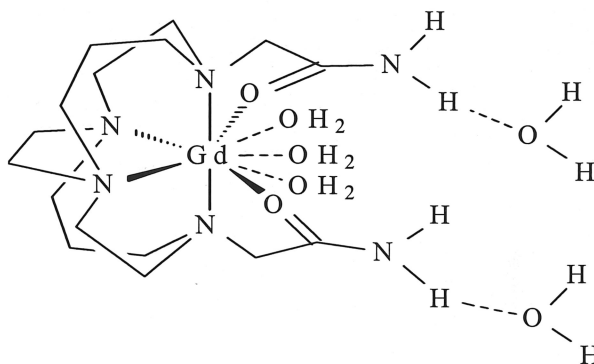

Figure 2 Structures of ligands discussed in this paper.

Figure 3 Potential H-bonding interactions of L.

This study will employ Co^{3+} instead Gd^{3+} because previous research involving Co^{3+} allows comparison of the new complex with an immense catalog of Co^{3+} complexes (Cotton and Wilkinson, 1988). Comparison of similar complexes allows for more accurate characterization of the tetraazamacrocyclic ligand in question. Once the synthesis and characterization of the Co complex is completed, similar methods should allow efficient synthesis of the more difficult to synthesize and characterize Gd^{3+} complex.

This study will look at the properties produced when amide pendant arms are added to a cross-bridged tetraazamacrocyclic ligand. The expected structure and purity of the complex was determined using mass spectrometry, infrared spectroscopy, elemental analysis, and proton and ^{13}C NMR spectroscopy. UV/Vis spectroscopy provided a glimpse at the ligand field strength. Conductance provided proof for positioning of the pendant arms. Reversibility of reduction and oxidation of the metal in the complex was examined using cyclic voltammetry. Comparison of the properties of the amide-armed complex to previously characterized complexes will be utilized where appropriate.

MATERIALS AND METHODS

Ferrocene and tetrabutylammonium hexafluoro-phosphate (TBAPF₆) were purchased from Aldrich. Acetonitrile (CH_3CN) (HPLC grade), methanol (HPLC grade), and KBr were purchased from Fischer Scientific. Adedamola Grillo provided the complex [Co(L)][PF₆]₃. Jonas Lichty provided the complex [Co(L₁)][PF₆].

FAB⁺ mass spectrometry of [Co(L)][PF₆]₃ was carried out by the Analytical Service of the University of Kansas on a VG ZAB HS spectrometer outfitted with a xenon gun and making use of acetonitrile and the matrices nitrobenzyl alcohol (NBA). An infrared spectrum of [Co(L)][PF₆]₃ was collected at McPherson College on a Nicolet 320 Avatar FT-IR spectrometer on a KBr pellet. Elemental analysis of [Co(L)][PF₆]₃ was performed by Quantitative Technologies, Inc. The UV-Vis absorption of [Co(L)][PF₆]₃ was measured at McPherson College on a Varian Cary 50 Bio spectrophotometer from 200-900nm using a 1mM solution. The molar conductance of the complex was determined at McPherson College using 1mM solutions on a Chemtrix 700 conductivity meter. Cyclic voltammetry was performed at McPherson College on an Obbligato Objectives Faraday 1 potentiostat equipped with a platinum working electrode, a platinum auxiliary electrode and a silver pseudoreference electrode at a sweep rate of 200mV/sec. Solutions used in the cyclic voltammetry contained complexes at 1 mM, 0.1M TBAPF₆ and ferrocene as an internal standard. NMR spectra were taken at the University of Kansas NMR lab on a Bruker DRX 500 spectrometer.

RESULTS AND DISCUSSION

The initial test that complexation of Co^{3+} with the ligand was successful was a FAB⁺ mass spectrum. This spectrum exhibited peaks at m/z = 398.2 (CoL⁺) and m/z = 544.2 (CoLPF₆⁺). These peaks are typical for metal complexes undergoing the FAB ionization process (Hubin, *et al*, 1998). The mass spectrum confirms that the cobalt has successfully complexed with the ligand.

Additional evidence that the desired complex had formed was obtained by infrared spectroscopy. The infrared spectrum of the complex in a KBr pellet produced assignable peaks at 3469 cm⁻¹, 3384 cm⁻¹, 3338 cm⁻¹ (all three peaks indicate the presence of NH₂), 1680 cm⁻¹ (amide carbonyl C=O), 843 cm⁻¹ (PF₆) (Silverstein, Bassler, and Morrill, 1963). The infrared spectrum confirms the presence of the amide pendant arms and that PF₆ is the anion.
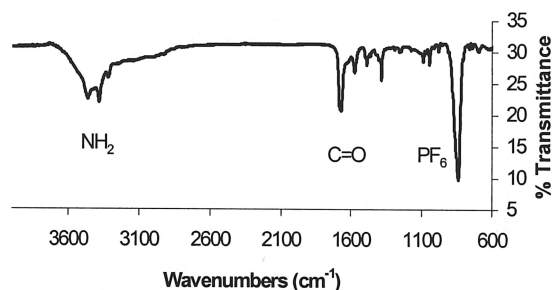

Figure 4. IR Spectrum of [Co(L)][PF₆]₃.

Once these initial tests suggested that the desired complex had been synthesized, its purity needed to be investigated. An elemental analysis of the sample should match the theoretical elemental composition if the complex is pure. If the complex contains other impurities, the experimentally determined composition will not match what is calculated for the pure complex. In this case, the analytical calculations predicted: C 23.03%, H 3.87%, N 10.07%. The experimentally determined elemental analysis was found as: C 23.00 %, H 3.60%, N 9.96%. Elemental analysis confirms the identity of the complex and that the complex is pure.

Proton NMR spectroscopy gave a spectrum that integrated for the correct number of protons (32) for the proposed complex structure. The two peaks at 2.2 ppm and 2.4 were assigned to the protons at the 4 position because of their distance from any N. These same protons were found at 1.5 ppm in L without Co (Grillo, 2002). The four peaks between 4.2 – 4.6 ppm assigned to the protons at the 7 position because of the protons proximity to nitrogen and oxygen. These protons were found at 3.0 ppm in L (Grillo, 2002). The peak at 8.2 ppm was assigned to the protons at the 8 position because this range is characteristic for amide groups. These protons were found at 7.0 ppm in L (Grillo, 2002). The numerous peaks between 2.8 – 3.8 ppm could not be assigned due to the nearly identical chemical environment of the protons at positions

1,2,3,5, and 6. Splitting of some protons at same positions shows that the complex is asymmetric. Asymmetry is due to one of the protons being in closer to the Co^{3+} than the other proton at the same position.
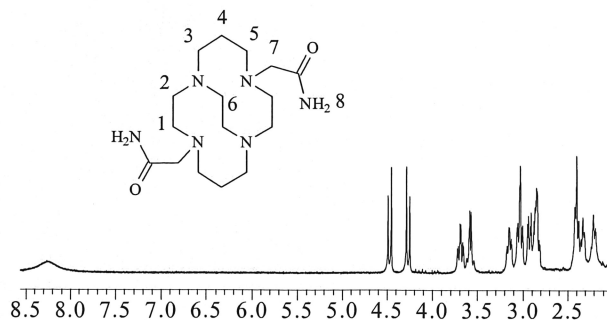

Figure 9. Proton NMR of $[Co(L)][PF_6]_3$ and labeled L to help with identification of hydrogens.

^{13}Carbon NMR spectroscopy is shown in Figure 10. The peak at 22.737 ppm was assigned for the carbon at position 4 due to the distance of carbon 4 from the nitrogen. This carbon was found at ~ 26 ppm in the L (Grillo, 2002). The peak at 69.510 ppm assigned for the carbon at position 7 because of its position between the nitrogen and oxygen. This carbon was found at shifts between 50 – 60 ppm in L (Grillo, 2002). The peak at 182.159 ppm assigned for the carbon at position 8 because of its close proximity with oxygen. This carbon was found at a position between 170 – 180 ppm in L (Grillo, 2002). The five peaks between 57.122 – 65.74 ppm could not be assigned due to the nearly identical chemical environment of the carbons at positions 1,2,3,5, and 6. ^{13}Carbon NMR confirms the purity of the complex because no "stray" peaks were observed. The fact that we even got a NMR spectrum confirms that the Co is Co^{3+} because the metal must be diamagnetic in order to get spectra, a property unique to Co^{3+} in the Co family.
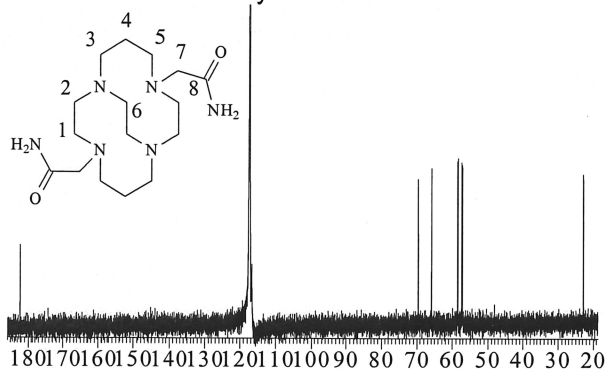

Figure 10 ^{13}C NMR of $[Co(L)][PF_6]_3$ and labeled L to help with identification of carbons.

An interesting feature of this class of complexes is that UV/Vis, or electronic, spectrum provides an estimate of ligand field strength (Wentworth and Piper, 1965). Ligand field strengths are equivalent to the average of the strengths of the cobalt-nitrogen and cobalt-oxygen bond strengths in these complexes (Cotton and Wilkinson, 1988). The ultraviolet spectrum showed only two absorbance bands, the most intense absorption at 353 nm and the lowest intensity absorption at 489 nm. The value for the lowest absorption band (489 nm) is most useful and was converted to units of wave numbers (20,450 cm^{-1}) and that value was added to the Racah parameter for Co^{3+} (3800 cm^{-1}) for a ligand field strength value (Δ_0) (Hung, *et al*, 1977). The determined value for $[CoL][PF_6]_3$ was $\Delta_0 = 24,250$ cm^{-1}. The value for $[CoL_1][PF_6]$ was $\Delta_0 = 24,040$ cm^{-1} (Lichty, 2000). Comparison shows that the field strengths of these two complexes are very similar considering experimental error. Higher field strength was expected for $[CoL_1][PF_6]$ because the complex has negatively charged carboxyl arms with which to bind to the Co^{3+} (Figure 5). A negatively charged ligand might be expected to bind a 3+ charged metal ion more strongly than a neutral ligand. The value for *cis* – $[CoL_2(CO_3)]^+$ was $\Delta_0 = 23,030$ cm^{-1} (Hung, *et al*, 1977). It follows from comparison of the three acquired field strengths that the ethylene cross-bridged molecules have only slightly stronger metal – donor bonds. Earlier work shows that this family of cross-bridged complexes is incredibly kinetically stable (Hubin, *et al*, 1998; Hubin and Meade, 2002). This additional stability could be a result of increased ligand field strength or increased rigidity. Since ligand field strength is about the same for unbridged and bridged ligands the source of stability must come from increased rigidity (Hubin, *et al*, 2002).
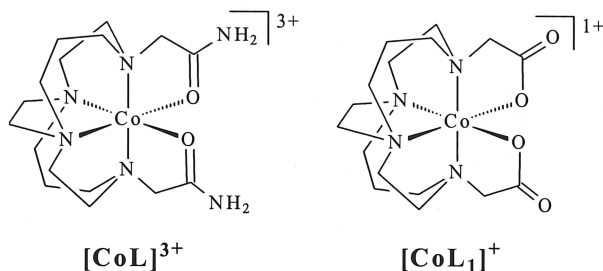

$$[CoL]^{3+} \qquad\qquad [CoL_1]^+$$

Figure 5 The amide arms of $[CoL]^{3+}$ provide less negative charge than the carboxyl arms of $[CoL_1]^+$.
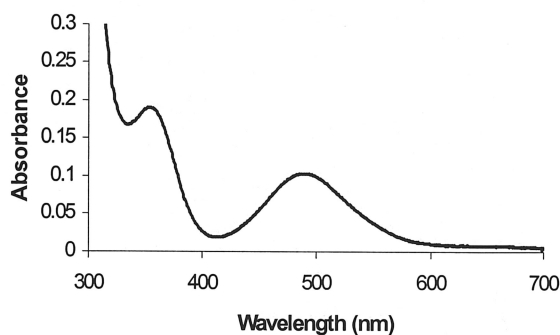
Figure 6 UV/Vis of $[Co(L)][PF_6]_3$.

The molar conductance of $[CoL][PF_6]_3$ in water was

Λ_O = 310 ohm^{-1} cm^2 mol^{-1}, in methanol was Λ_O = 170 ohm^{-1} cm^2 mol^{-1}, and in acetonitrile was Λ_O = 425 ohm^{-1} cm^2 mol^{-1}. The water and methanol values suggest a 2:1 dissociation while the acetonitrile value supports the expected 3:1 dissociation (Angelici, 1986; Sneed and Maynard, 1942). The molar conductance of [CoL₁][PF₆] in water was Λ_O = 79.5 ohm^{-1} cm^2 mol^{-1}, and in acetonitrile was Λ_O = 92.4 ohm^{-1} cm^2 mol^{-1} (Lichty, 2000). The dissociation values for this complex were slightly low but clearly represented the expected 1:1 ratio (Angelici, 1986; Sneed and Maynard, 1942). Dissociation of [CoL][PF₆]₃ may have been hindered by H-bonding between [Co(L)][PF₆]₃'s NH₂ and the PF₆⁻ (Figure 7); a phenomenon that would not occur between [CoL₁][PF₆]'s oxygen and PF₆⁻. The experimental values also confirm the charge of the complexes by showing that the expected number of ions are produced in solution.

Cyclic voltammetry shows the voltage at which the complexes are reduced or oxidized. Cyclic voltammetry performed using the internal reference ferrocene ($E_{1/2}$ = +0.40 V relative to the Standard Hydrogen Electrode, SHE) provides a value from which the voltage scale can be standardized. The placement of the internal standard's peak in solution with [CoL][PF₆]₃ presented a
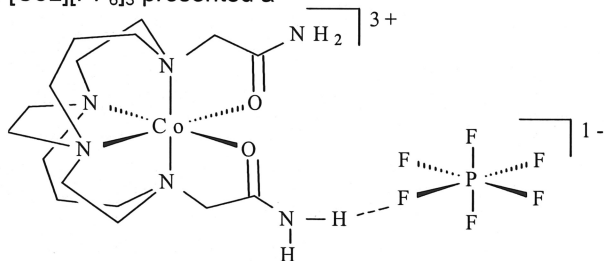

Figure 7. Proposed H-bonding between [Co(L)][PF₆]₃'s NH₂ and the PF₆⁻.

correction factor = 0.065 V with a peak separation ☐ = 92 mV. The peak separation shows the reversibility of a compound's redox process. For example, if a compound has a reversible reduction, the reduced and oxidized forms differ only in the charge on the metal ion and not in any structural way. Reversibility is a measure of structural rearrangement following a redox process. A peak separation of 59 mV is considered ideal for ferrocene, which is known to be completely reversible (Gagne, Koval and Lisensky, 1980).

Cyclic voltammetry performed on [CoL][PF₆]₃ revealed a reversible reduction to Co²⁺ that corrected for a potential value $E_{1/2}$ = 0.013 V. Peak separation of 103 mV was observed. A second, unaccompanied reduction peak (Co²⁺/Co¹⁺) was corrected to provide a potential value $E_{1/2}$ = -1.721 V. A similar experiment performed with ferrocene in solution with [CoL₁][PF₆] presented a correction factor = 0.040 V and a peak separation ☐ = 76 mV. [CoL₁][PF₆] exhibited a reversible reduction to Co²⁺ which corrected for a potential value $E_{1/2}$ = -0.565 V. Peak separation of 75 mV was observed. [CoL₁][PF₆] required half a volt more

than [CoL][PF₆]₃ to be reduced. This occurrence can be explained because the ligand of [CoL][PF₆] is -2 charged, making the complex more difficult to reduce. The more negative ligand of [CoL₁][PF₆] works to prevent the metal from reducing from Co²⁺ to Co¹⁺. Comparison of reversibility via peak separation is possible; but it should be noted that the peak separation of the identical internal standards differed by 16 mV. An experimental difference of 28 mV was observed between the two complexes, with [CoL₁][PF₆] being the most reversible.
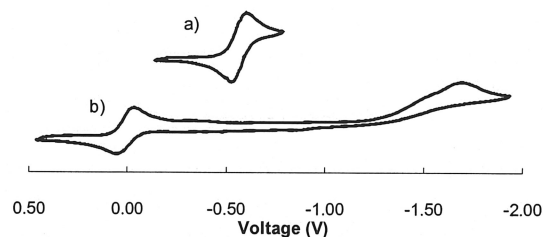

Figure 8 Cyclic voltammetry of a) [CoL₁][PF₆] and b) [CoL][PF₆]₃.

In conclusion, the successful synthesis and characterization of an amide pendant-armed cross-bridged tetraazamacrocycle complex has been achieved. The relevant data points to coordination of Co³⁺ by the amide oxygens as well as the tetraazamacrocyclic nitrogens. UV/Vis spectroscopy confirms that the ligand field is typical for Co³⁺ complexes. Electrochemical experiments correlate reduction potentials with ligand charge for two similar Co³⁺ complexes. Finally, NMR and elemental analysis confirms the purity of the complexes synthesized by this route (Lichty, 2001; Grillo, 2002). The next step for research along these lines is to insert Gd³⁺ into these ligands using similar conditions and evaluate the complexes as MRI contrast agents.

ACKNOWLEDGEMENTS

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