Primary Amine-Functionalized Ligand Substitution and Biotinylation of Co(III) for Ligand-Anchored Artificial Metalloenzymes

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Abstract

Group IX metals in the +3-oxidation state can be incorporated into $[Cp^*MCl_2]_2$ complexes for the design and synthesis of new artificial metalloenzymes for biological functions. While previous studies have applied the more costly and less abundant rhodium- or iridium-(III) metals, use of a cobalt(III) metal center provides a low-cost route to preparing artificial metalloenzymes. Herein, a new metal cofactor is acquired through synthesis of a primary amine-functionalized Cp* ligand and subsequent complexation with a Co(III) metal center. Key signals corresponding to the Cp* and NH_2 groups are visible by Fourier Transform Infrared spectroscopy where the aromatic signal of the Cp* and the stretching mode of the primary amine are present in their characteristic regions, supporting the formation of a tentatively assigned bis[dichloro-η^5(1-ethylamine-2,3,4,5-tetramethylcylopentadienyl)-cobalt(III)]. Biotinylation of this complex would allow for pairing with engineered proteins to foster catalytic C—H activation, improving enzymatic processes in biological and biochemical applications.

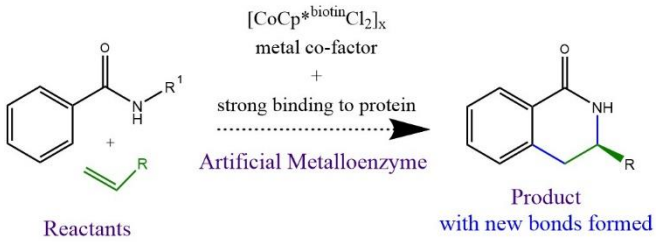


Figure 1A. Benzannulation reaction, common to essential metabolic processes, catalyzed by an artificial metalloenzyme formed through strong binding of a biotinylated Co(III) cofactor and native protein.

I. Introduction

Metalloenzymes, or proteins which incorporate a firmly-bound metal ion with a labile coordination site, play a range of important biological roles including electron transfer and oxygen delivery and are known to incorporate several types of metal complexes.[1-3] Most importantly, these enzymes catalyze organic reactions that are difficult to achieve due to unfavorable thermodynamics such as endergonic processes (in which more energy is consumed than is produced) or those with high activation energies that must be overcome in converting reactants to products.[4-6] For example, deoxyribozymes, also called DNAzymes or catalytic DNA, are metalloenzymes that contribute to the catalysis of RNA/DNA cleavage and ligation, amino acid phosphorylation and dephosphorylation, and carbon-carbon bond formation.[7,8] Many of these metalloenzymes have been shown to be quite metal-specific, meaning only by incorporating certain metal ions are they capable of fulfilling these roles in biological systems.[7] However, known native metalloenzymes utilize a limited range of 14 metals to complete their catalytic processes, and most known metalloenzymes rely on magnesium, iron, and/or zinc for catalysis.[9,10]

Artificial metalloenzymes (ArMs), on the other hand, have the ability to promote high activity and selectivity in these catalytic processes using a wide range of metals.[11] ArMs have therefore been employed since the 1970s to overcome limitations for the catalysis of biochemical transformations in laboratory experiments.[4,12] Design and optimization of active and selective ArMs is desirable for both practical applications in converting reactants to products and in fundamental studies to understand and potentially improve enzymatic mechanisms of catalysis.

ArM synthesis can be achieved by several methods aiming to incorporate the non-native metallo-organic cofactor into a protein scaffold.[13,14] While earlier studies focused on introducing artificial cofactors into these protein scaffolds via metal exchange, a newer approach which has received attention in more recent studies is the binding of catalytic metal complexes through ligand anchoring.[15] The former approach involves the fine-tuning of cofactor-protein interactions within the active metal site, partially controlled by the selection of the transition metal center.[10,16] Several studies have utilized group IX metals rhodium(III) and iridium(III) to generate $[Cp^*MCl_2]_2$ complexes to serve as non-natural protein cofactors for ligand anchoring. The η^5-pentamethylcyclopentadienyl (Cp*) ligands are methylated, aromatic 5-membered rings bound to the metal center through each of the five carbon atoms within the ring providing stabilization of the metal center and have been demonstrated to be effective catalysts.[17,18] The later transition metals have more recently been incorporated into side-chain-functionalized half-sandwich complexes using Cp* ligands demonstrating improved suitability and increased reaction rates via bifunctional mechanisms.[19,20] Cp*-functionalization by primary amine derivatives would replace a single methyl group on the 5-membered ring with an amine tether which is particularly rare, yet would be of great interest as the new amine functional group is quite common in bioactive compounds and appropriate for biological applications.[19,21]

Combining this common approach of metal exchange with the newer method of ligand anchoring, as reported in 2012 by Hyster, et al., a biotinylated rhodium(III) metallo-organic cofactor can be successfully paired with amino acid-engineered streptavidin proteins to produce an ArM that fosters catalytic asymmetric carbon-hydrogen (C—H) activation with reaction rate accelation increasing by nearly 100-fold in the coupling of benzamides and alkene reactants.[17] However, *in vivo* performance of these ArMs has not been studied, and many non-native metal incorporations have failed to produce the same results in cells as they do in solution.[22] Due to the low cost and high abundance of cobalt relative to rhodium, accomplishing the above procedure using a Co(III) metal center as opposed to Rh(III) would make for a more economically feasible and widely applicable ArM synthesis.[23,24] As d^6 metals in the 3+-oxidation state, cobalt and rhodium can be expected to exhibit similar electron configurations, molecular geometries, and d-orbital splitting patterns.[25,26]

Further, some native metalloenzymes already utilize cobalt as an active metal ion for their catalytic processes, such as cobalamin – better known as vitamin B_{12} – which serves as an essential cofactor for two metalloenzymes: methionine synthase and methylmalonyl-CoA mutase. These two enzymes catalyze metabolic reactions responsible for production of methionine and tetrahydrofolate, and succinyl-CoA, respectively.[27] Methionine is a well-documented essential amino acid, while tetrahydrofolate is utilized in the synthesis of numerous amino and nucleic acids.[28,29] Succinyl-CoA serves in the metabolism of fatty and amino acids, another essential process in biological systems.[30]

Given that cobalt is naturally incorporated into the catalytic production of numerous essential molecules, reasonable deduction would lead one to hypothesize that it may be successfully incorporated elsewhere via introduction of artificial metalloenzymes. Synthetic amino-pyridine cobalt complexes have had reported success in electro- and photocatalytic H_2 evolution, and Co(III) complexes have previously been applied to synthesis of ArMs for catalyzed phosphate

hydrolysis.[31,32] Further, biotin is another B vitamin utilized as a metalloenzyme cofactor in processes related to metabolism of carbohydrates, fats, and amino acids.[33] The catalytic efficiency of these processes has been linked to low rates of biosynthesis-related deficiencies and high immune response capability.[34,35] The similarity of a cobalt-biotin ligand to coenzymes produced naturally makes the incorporation of such an ArM seem promising as it relates to mimicking and improving upon catalysis by native metalloenzymes. Here we report the synthesis of a primary amine-functionalized Cp* ligand and subsequent new complexation with a Co(III) metal center. The potential for biotinylating this complex to produce a bifunctional ArM is also explored.

II. General Methods

Materials and Methods. All proton-Nuclear Magnetic Resonance (^{1}H-NMR) and carbon-13-Nuclear Magnetic Resonance (^{13}C-NMR) spectra were obtained using a JEOL ECX-400 spectrometer, operating at 400 MHz and 100 MHz, respectively; NMR samples were dissolved in deuterated chloroform (Acros, >99.75% D enrichment) or dimethyl sulfoxide (DMSO-d_6, Sigma-Aldrich. 99.9 atom % D enrichment), and spectra were referenced to the residual solvent peak. Fourier Transform Infrared (FTIR) spectra were taken on the Thermo Scientific Nicolet iS10 with Smart iTR from 600-4000 cm^{-1}, dissolved in the same solvents used for NMR. Solvent removal was completed using the Buchi Rotavapor R-114 connected to the Buchi water bath B-480, and air-free procedures were performed under argon in a Vacuum Atmospheres Company Controlled Atmosphere Systems apparatus or under nitrogen using standard Schlenk line techniques.

Chemicals and Reagents. Tetrahydrofuran (THF, ≥99.9%, anhydrous), n-butyllithium solution (2.5M in hexanes), 2,3,4,5-tetramethyl-2-cyclopentenone (mixture of cis and trans, 95%), ether (≥99.0%, anhydrous, ACS reagent), lithium aluminum hydride (1M in diethyl ether), diethyl ether (≥99.7%, anhydrous), N,N-Dimethylformamide (DMF, anhydrous, 99.8%) and triethylamine (>99.5%) were purchased from Sigma-Aldrich and used as received. Concentrated hydrochloric acid (ACS Plus), sodium sulfate (granular, anhydrous, 99.4%), n-hexane (ACS grade), and dichloromethane (ACS grade) were purchased from Fisher Scientific and used as received. Acetonitrile (ACS grade) was obtained from Eastman Kodak Company, and ethyl alcohol (200 proof, anhydrous) was obtained from Pharmco-Aaper; both were used without modification. Perfluorophenyl-5((3aS,4S,6aR)-2-oxohexa-hydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (95%) was purchased from Ambeed and used as received. The $[Co(pyridine)_4Cl_2]Cl$ used was synthesized previously, as reported by Hart, et al.;[36] this product was used without modification.

Synthesis of the Organic Ligand

(2,3,4,5-Tetramethylcyclopentadienyl)acetonitrile (1). Synthesis Steps A and B were achieved following a modified version of a previous literature procedure, which was developed following the field precedent established by Okuda and Zimmerman, as well as Bensley, Mintz, and

Sussangkarn in 1988.[18,37,38] Under an inert nitrogen atmosphere, 1.2 mL of acetonitrile in THF was cooled in a dry ice-acetone bath to -75 °C before 8.8 mL (22.0 mmol) of a 2.5M n-butyllithium solution was added. After 15 minutes, 3.0 mL (22 mmol) of 2,3,4,5-tetramethyl-2-cyclopentenone was added to the solution dropwise over 45 minutes, then the reaction was gradually brought up to 0 °C over three hours. Following the addition of 5.0 mL of water, the solution was washed and treated with 2.0 mL (4.00 mmol) of 2M HCl, then the solution was shaken vigorously and separated over a period of 48 hours. The mixture was washed with brine, dried with sodium sulfate and concentrated. Distillation of this product at 120 °C under 0.4 mmHg produced 1.061 g of a thick, yellow oil (32.9% yield – **Table 1**, entry 1). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): δ_H (ppm)= 4.82 d, 2.38 t, 2.03 br, 1.76 br, 1.56 d, 1.19 br, 1.09 br, 0.97 d. FTIR (cm^{-1}): 3460 s, 2960 s, 2870 m, 2250 m, 2210 s, 1600 s,1450 m, 1380 m, 1090 w.

(2-(2,3,4,5-Tetramethylcyclopentadienyl)ethylamine) ***(2)***. Under an inert atmosphere, 0.25 g (0.372 mmol) of (2,3,4,5-tetramethylcyclopentadienyl)acetonitrile prepared in step A was added with 3.2 mL of diethyl ether to a solution of 2.4 mL (2.4 mmol) of lithium aluminum hydride. The solution was refluxed for 3 hours at 35 °C before quenching with water to produce a foamy white solid. After being left to sit for 19 hours at room temperature, the product was dissolved in diethyl ether and dried with sodium sulfate before filtering off the salts and washing with five 10 mL portions of ether. Filtrates were then combined and concentrated to yield 0.0951 g of product as a thick yellow-orange oil (37.11% yield – **Table 1**, entry 2). ^{1}H-NMR (400 MHz, $CDCl_3$, δ): δ_H (ppm)= 5.13 t, 3.36 d, 2.63 m, 2.43 d, 2.33 q, 2.02 m, 1.70 s, 1.61 s, 1.56 m, 1.27 br, 1.16 d, 1.01 t, 0.96 m; ^{12}C-NMR (100-1 MHz, $CDCl_3$, δ): δ_C (ppm)= 40.3 m. FTIR (cm^{-1}): 2953 s, 2870 s, 1640 m, 1610 m, 1450 m, 1870 w, 1300 w.

Complexation with Co(III)

[(η^5-Me$_4$Cp(CH$_2$)$_2$NH$_3$)CoCl$_2$]$_2$Cl$_2$ ***(3)***. Step C was completed according to a modified literature procedure, originally adapted from the widely used method of Reiner et al., published in 2009.[17,39] After dissolving 0.496 g (0.1514 mmol) of (2-(2,3,4,5-tetramethylcyclopentadienyl)-ethylamine), synthesized in Step B, in 10 mL of diethyl ether, it was treated with 0.147 mL (0.588 mmol) of 4M hydrochloric acid and 2 mL (15.3 mmol) of hexane. The aqueous solution was filtered and washed twice with hexane, and to it 5 mL (85.6 mol) of ethyl alcohol and 0.084 g (0.0353 mmol) of *trans*-dichlorotetrakis(pyridine)cobalt(III) chloride were added. After refluxing for 5 days at 85 °C and storing for 48 hours in the refrigerator, the dark green solution was once again filtered and washed with hexane and diethyl ether to produce 28 mg of extremely fine green crystals (45.17% yield – **Table 1**, entry 3). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): δ_H (ppm)= 8.25 br; ^{13}C-NMR (100 MHz, DMSO-d6, δ): δ_C (ppm)= 3.30 s, 2.47 s. FTIR (cm^{-1}): 3140 s, 2890 m, 2800 m, 2670 w, 2250 w, 2130 w, 1880 w, 1680 s, 1660 s, 1610 s, 1550 s, 1510 s, 1440 s, 1370 s, 1260 s, 1110 m, 1020 s, 1000 s, 835 s, 760 m.

Coordination of Biotin

[CoCp*^{biotin}Cl$_2$]$_2$ (4). Synthesis Step D was performed according to a known procedure, with modifications made to scale.[17] In 1.6 mL of DMF, 16.053 mg (0.0122 mmol) of [η^5-($C_5Me_4(CH_2NH_3)CoCl_2]_2Cl_2$ synthesized in Step C was combined with 3.693 mg (0.0232 mmol) of perfluorophenyl-5-((3aS,4S,6aR)-2-oxohexahydro1H-thieno[3,4-d]imidazol-4-yl)pentanoate and 0.0343 mL (0.246 mmol) of triethylamine. The blue-green solution was stirred overnight at room temperature, then filtered and washed with three 1.5 mL portions of hexane and three 1.5 mL portions of dichloromethane to yield 8 mg of white solid product (57.97% yield - **Table 1**, entry 4). No spectroscopic data was collected.

Table 1. Expected and observed product and intermediate species yields for synthesis of bis[dichloro(1-biotin-2,3,4,5-pentamethylcyclopentadienyl)cobalt(III)] from 2,3,4,5-tetramethylcyclopentenone.

Synthesis Step	Product		Reported Yield (%)	Actual Yield (%)
A	(2,3,4,5-Tetramethylcyclopentadienyl)acetonitrile	**1**	98 [18]	32.9
B	(2-(2,3,4,5-tetramethylcyclopentadienyl)ethylamine)	**2**	70 [18]	37.1
C	bis[dichloro-η^5(1-ethylamine-2,3,4,5-tetramethylcylopentadienyl)cobalt(III)]	**3**	-*	24.2
D	bis[dichloro-η5(1-biotin-2,3,4,5-pentamethylcyclopentadienyl)cobalt(III)]	**4**	-*	-

*No previous yields have been reported for products **3** or **4**; however, previous literature does describe the synthesis of equivalent complexes wherein the cobalt(III) metal center has been substituted for rhodium(III), giving yields of 72% and 85% for products **3** or **4**, respectively.[17]

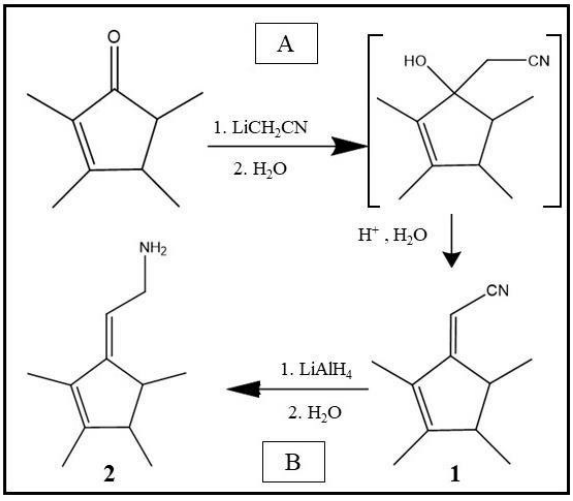
III. Results and Discussion

Synthesis of Organic Ligand

The reaction sequence is illustrated in **Scheme 1** for the synthesis of the organic aminoethyl-functionalized Cp* ligand via the addition of lithiated acetonitrile to 2,3,4,5-tetramethylcyclo-2-pentenone, followed by hydrolysis and finally the reduction of (2,3,4,5-tetramethylcyclopentadienyl)acetonitrile (**1**) with lithium aluminum hydride to yield (2-(2,3,4,5-tetramethylcyclopentadienyl)ethylamine) (**2**).

The synthesis of (2,3,4,5-tetramethylcyclopentadienyl)acetonitrile (**1**) has been reported previously by Van Leusen, et al. and can be achieved

Scheme 1. Synthetic pathway for preparation of organic ligand **2**.



by nucleophilic addition to 2,3,4,5-tetramethylcyclo-2-pentenone of n-butyllithium, first generating the intermediate identified in **Scheme 1.**[18] An acidic workup of this intermediate followed by short path vacuum distillation results in condensation and formation of the exocyclic double bond present in **1.** Isomerization of this bond creates a favorable electronic configuration within a Cp* system, necessary for coordination to a transition metal center.[40]

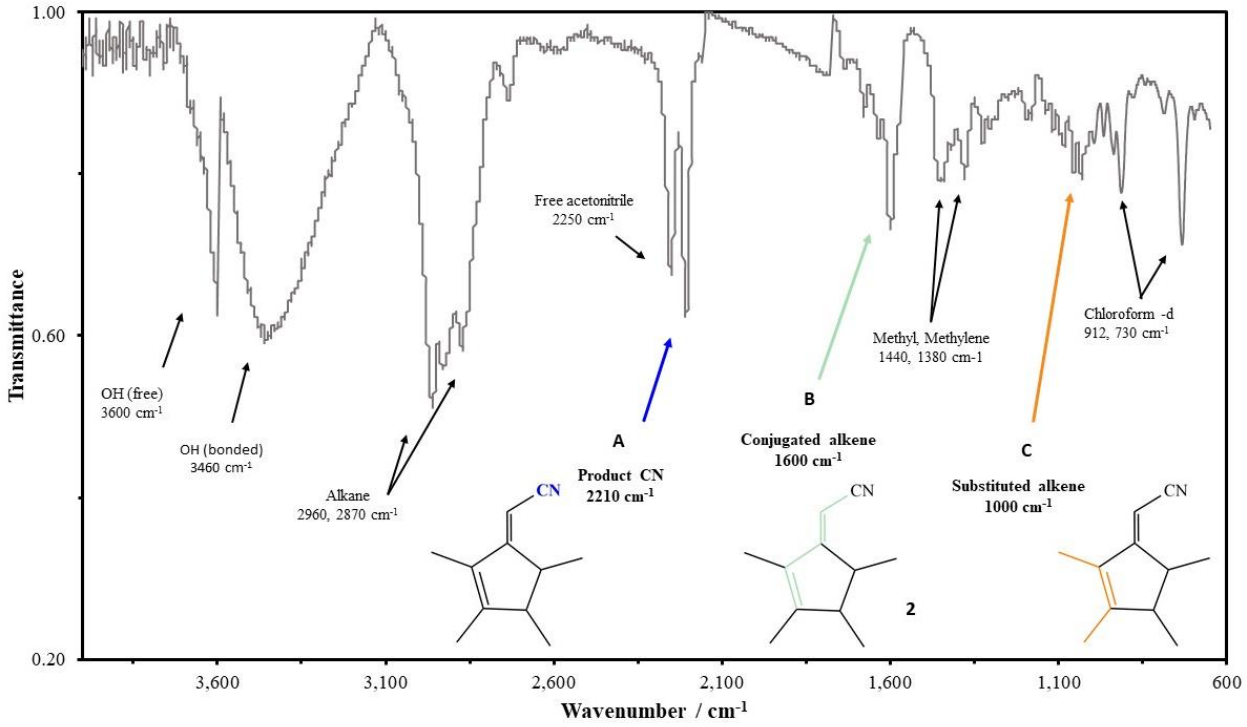


Figure 1. Three significant characteristics of (2,3,4,5-tetramethylcyclopentadienyl)acetonitrile (**1**) are observed in the IR spectrum of the product formed and distilled in Synthesis A (diluted with deuterated chloroform). A strong peak at 2210 cm^{-1} corresponds to the added nitrile group (A) and a signal at 1600 cm^{-1} indicates a successful synthesis of a conjugated alkene product (B). Along with the signal at 1000 cm^{-1} from a substituted alkene, these results indicate a successful synthesis of the product. The presence of acetonitrile and OH groups suggests either incomplete conversion of the intermediate species or the generation of various side products not removed by distillation.

Spectroscopic analysis by FTIR and ^{1}H-NMR support the successful formation of **1** as well as isomers and possible side products arising from acid-base enolate and Michael Addition reactions.[41] A key singlet signal identified in previous literature is present at δ_H = 4.82 ppm in the ^{1}H-NMR spectrum as expected, corresponding to the cyanomethyl hydrogen. Significant FTIR signals are identified in **Figure 1**, including a strong peak at 2210 cm^{-1} from the added nitrile group and a peak of medium intensity at 1600 cm^{-1}, confirming the presence of the conjugated alkene system that characterizes this molecule. Van Leusen et al. have described four isomeric species of **1** which, in addition to the above-mentioned side products, may account for many of the unidentified signals within the C—C region of the FTIR spectrum.[18] The favored of

the four species in this reaction environment is that with the exocyclic double bond, which can be most likely attributed to the stability of the conjugated system. Higher relative intensity of C=C signals supports favorable formation of the conjugated isomer in this reaction. While the above referenced literature reported a yield of 98%, a series of four repeated experiments (with slight modifications – see Methods) in this case produced a maximum yield of 32.9% (**Table 1**, entry 1), likely due to formation of the above-mentioned side products.

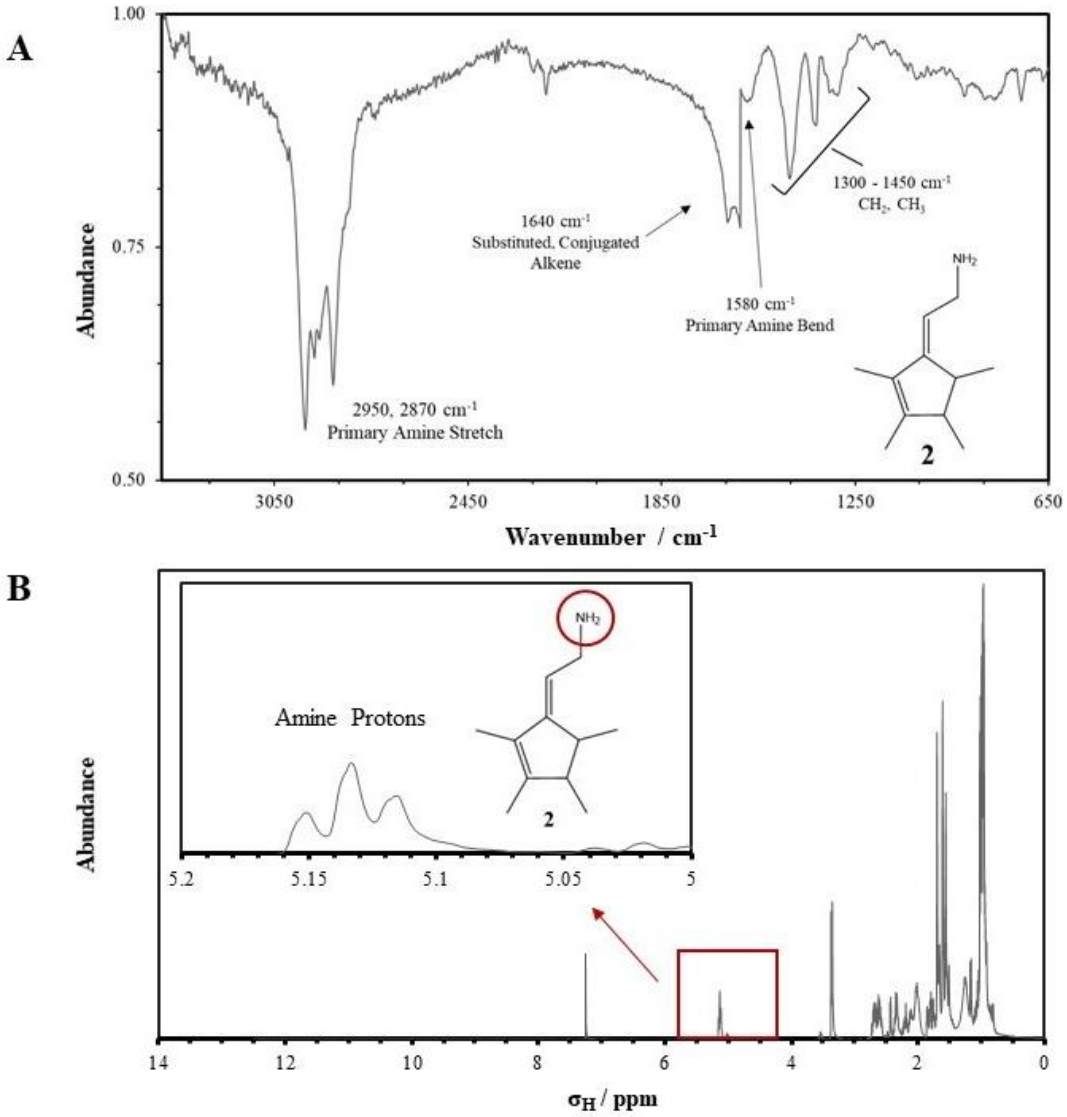


Figure 2. A) Formation of organic ligand (2-(2,3,4,5-tetramethylcyclopentadienyl)ethylamine) (**2**) is supported by FTIR spectra (dissolved in deuterated chloroform solution). Successful nitrile reduction and formation of product **2** is evidenced by the presence of a primary amine group (2950 & 2870 cm^{-1}) and the much lower relative intensity of the nitrile functionality (2210 cm^{-1}) which was replaced in Reaction B. B) ^{1}H-NMR for **2** indicates successful conversion of the nitrile group in **1** to a primary amine functionality. The singlet previously observed at 4.8 ppm in the spectrum of **1** now appears as a triplet at 5.1 ppm.

Nitrile reduction of **1** with lithium aluminum hydride results in activation of the Cp-ring and the formation of an amino-ethyl functional group to produce (2-(2,3,4,5-tetramethylcyclopenta-dienyl)ethylamine), **2**.[19,34] Synthesis of **2** is also shown in **Scheme 1.** The undesirable attraction of the nitrile group to Lewis-acidic centers such as Co(III) makes reduction of the nitrile to an aminoethyl functionality favorable for coordination to the transition metal center.[42] Formation of **2** is evident by FTIR and by ^{1}H-NMR (**Figure 2**). FTIR signals corresponding to the amino group can be identified in the expected range, and the signals corresponding to the nitrile group identified in **1** are present at low relative intensities. As with the synthesis of **1**, originally reported in the same publication, the actual yield of 37.1% is considerably lower than expected based on the reported yield of 70% (**Table 1**, entry 2).[18] This could be due to the formation of unidentified side products, or product instability and degradation resulting from heat and oxidation.[43,44]

Complexation with Co(III)

Scheme 2. Synthetic pathway for attachment of the aminated organic ligand to the Co(III) transition metal center.

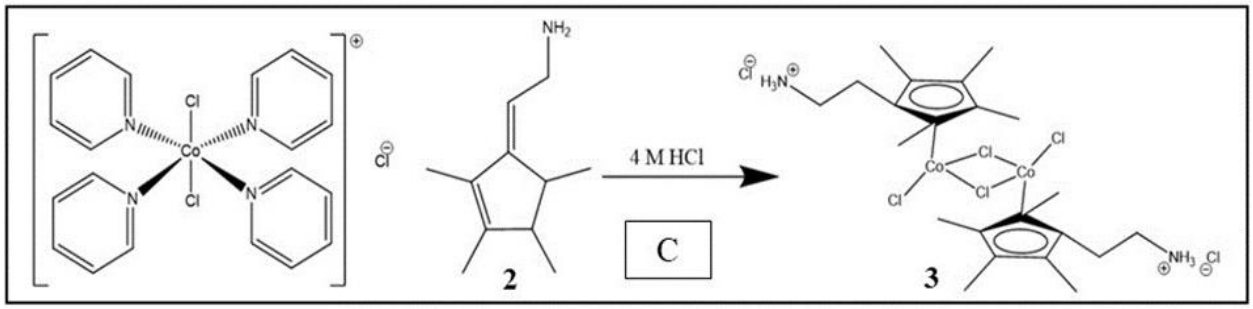


Figure 3. Product **3** crystals dissolved in DMSO-d possess a brilliant emerald green color characteristic of Co(III), evidence for complexation of the organic ligand to the Co(III) center while avoiding undesired reduction to Co(II).

Generation of the dark green crystalline metallo-organic complex tentatively assigned as bis[dichloro-η^5(1-ethylamine-2,3,4,5-tetramethyl-cylopentadienyl)cobalt(III)] (**Figure 3**) was attempted through substitution of the organic ligand described above for pyridine around the Co(III) center of the starting material, illustrated in **Scheme 2**. Successful syntheses of equivalent complexes using Ir(III) and Rh(III) metal centers have been reported previously by Reiner, et al.[19] These complexes have assumed dimeric structure based on their large molecular weight, though group IX metals have not been often noted to form μ-chloro-bridged η^5-cyclopentadienyl equivalents under similar reaction conditions.[2,45]

Crystals dissolved in DMSO-d_6 were analyzed by ^{1}H- and ^{13}C-NMR and by FTIR; overlain FTIR spectra for **2** and **3** (**Figure 4**) show high relative intensity of CH_2 and CH_3 groups in **3**, as well as C=C double bond signals in the 1600 cm^{-1} region indicative of a conjugated Cp* system.

A broad singlet in the ^{1}H-NMR at 8.25 ppm integrating to 3 and corresponding to the NH_2 protons further confirms the ligand is not coordinated through the nucleophilic nitrogen atom (**Figure 5**). As with the synthesis of the organic ligand, the 24.2% product yield was considerably lower than expected, based on the 72% yield reported for Rh(III) and Ir(III) equivalents (**Table 1**, entry 3).

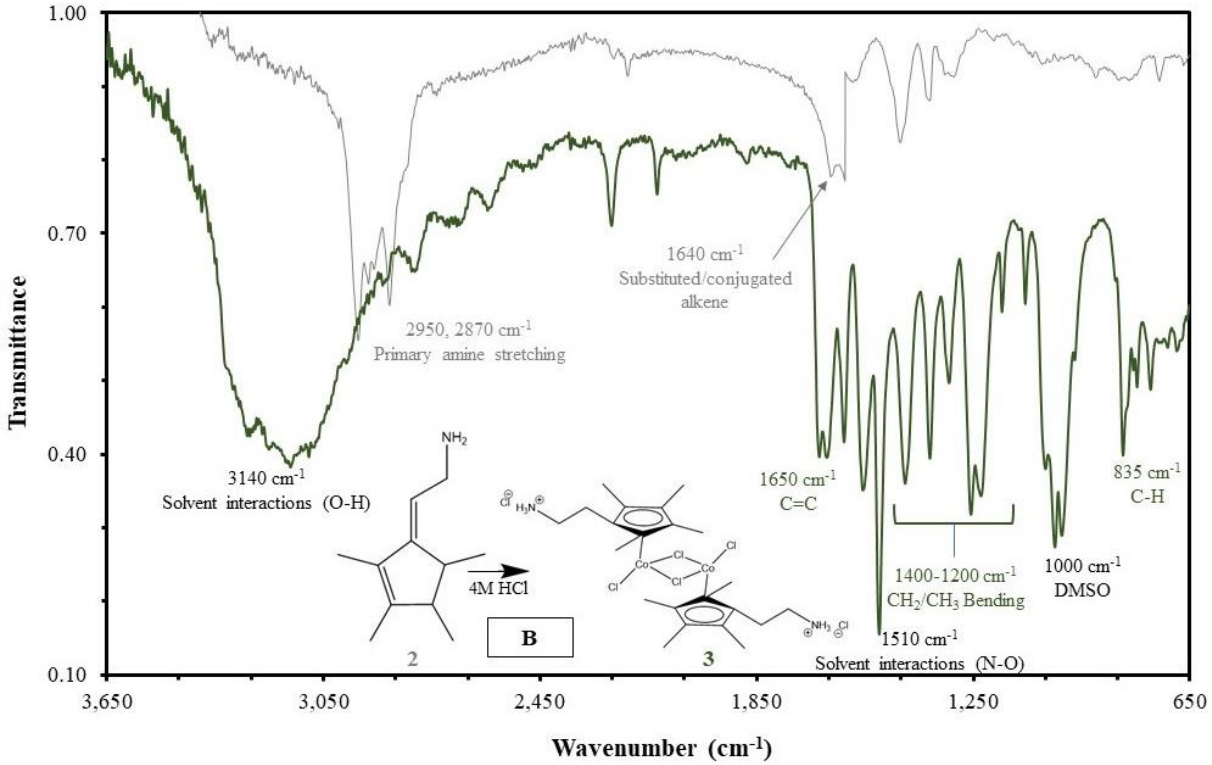
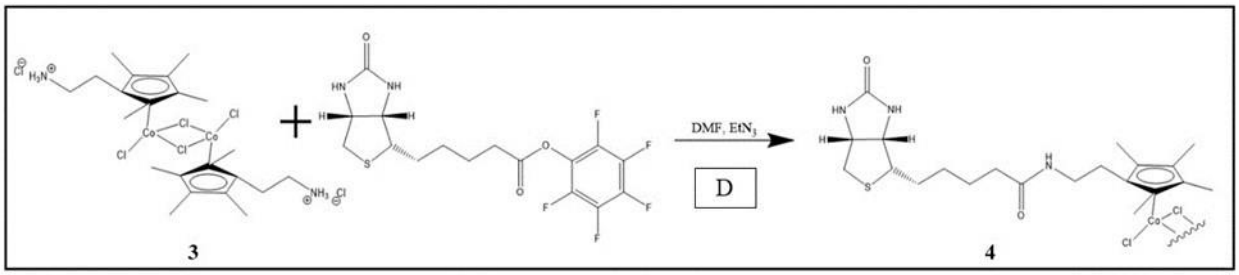


Figure 4. Overlay of FTIR spectra for **2** in chloroform-d (grey) and **3** in DMSO-d_6 (green) indicates successful conversion of $[CoCl_2(pyridine)_4]Cl$ to $[(\eta^5\text{-}Me_4Cp(CH_2)_2NH_3)CoCl_2]_2$. Methyl and aromatic C—H signals from **3** match closely with those reported in the literature[1], and the presence of N—O interactions with the solvent (1510 cm^{-1}) is consistent with the protonation of the primary amine. RNH_3^+, the key evidence of product formation, typically also exhibits signals in the region of the product's CH_3 and CH_2 bending modes; these signals would not be distinguishable from those of the alkyl and aromatic groups (which would be expected to show up at higher intensities than RNH_3^+ for **3**).

Coordination of Biotin

Scheme 3. Biotinylation of the Co(III) metallo-organic complex (**3**) to produce bis[dichloro(1-biotin-2,3,4,5-pentamethylcyclopentadienyl)cobalt(III)] (**4**).



Biotin has successfully been coordinated to similar compounds with Ir(III) and Rh(III) metal centers thanks to the pseudo-octahedral molecular geometries formed by these group IX metals.[17] The addition of perfluorophenyl-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (PFP) to **3** to form bis[dichloro(1-biotin-2,3,4,5-pentamethylcyclo-pentadienyl)cobalt(III)] (**4**) was performed under room-temperature atmospheric conditions and is described in **Scheme 3**. Following procedures developed from Hyster, et al., a granular solid white product was obtained, but no mass or spectral data was recorded. However, based on appearance and similarity to the biotin starting material, it was predicted that **4** was not successfully generated, but the PFP had precipitated back out of solution. This prediction was further supported by the color of the product; Hyster, et al. described the Rh(III) compound as a dark orange powder.[17] The Co(III) equivalent was therefore expected to retain the emerald color characterized in **3** (**Figure 5**).

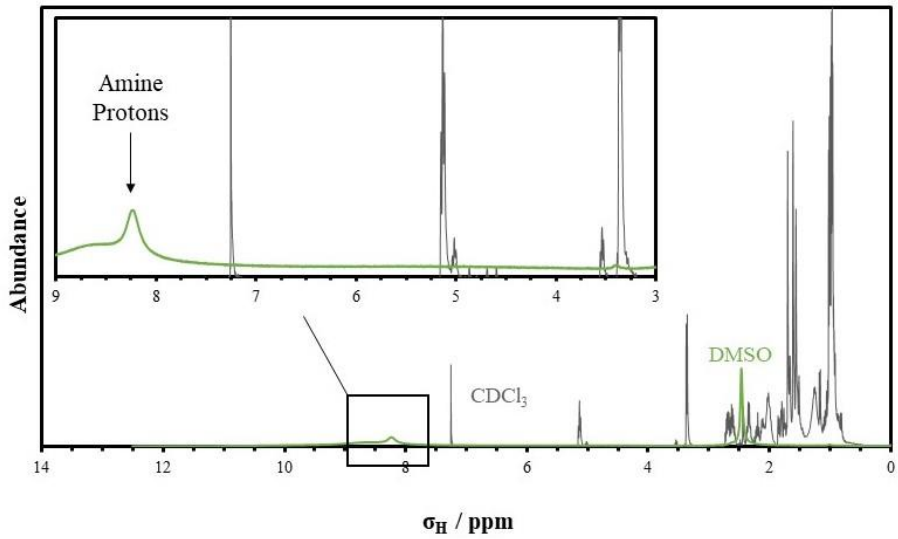


Figure 5. Overlain ^{1}H-NMR spectra of **2** (grey) and **3** (green) supports formation of **3** with a broad singlet at 8.25 ppm integrating to 3 and corresponding to the NH_2 protons. This signal is not visible in the spectrum of **2**, suggesting this group is in a new chemical environment.

Additional analytical methods will be required to fully confirm formation of **3** based on the procedure described herein, and procedural adaptations to maximize yield would improve viability of this reaction. Further experimental trials following confirmation of **3** will be necessary to determine the feasibility of the reported methods for the coordination of **3** to biotin, and the general use of Co(III) in place of Rh(III) and Ir(III) equivalents for enzymatic catalysis.

Successful biotinylation of this Co(III) catalyst will yield a cofactor to coordinate to biotin-binding proteins and produce an ArM capable of accelerating organic reactions within essential metabolic processes which rely on asymmetric C—H bond activation. Known to participate in a variety of such reactions, biotin and cobalt together hold strong potential to mimic activities of native metalloenzymes. It is therefore of great interest to apply this cofactor to produce highly efficient ArMs for these essential processes.

IV. Conclusion

Preparation of a new Co(III) metallo-organic catalyst was achieved in a two-step process. Synthesis of the organic amine functionalized Cp* ligand was confirmed upon characterization by FTIR, ^{1}H-NMR, and ^{13}C-NMR. Analyses suggest the generation of numerous undesired organic byproducts, making maximization of synthetic yields of particular interest going forward. Complexation was subsequently achieved through substitution of the Cp* ligand around a Co(III) metal center and verified by the same spectroscopic techniques previously mentioned although further studies are needed to fully assign the structure.

Further work is needed to achieve esterification of the Cp* ligand to generate a biotinylated Co(III) complex for the exploration of the molecule and its ability to aid with cofactor localization in medicinal and biochemical applications. Biotinylation of a Co(III) metal center will have a range of implications in the field, due primarily to the low cost and high abundance of cobalt relative to iridium and rhodium, which have been applied for similar acceleration of enzymatic catalysis in the past.

Given the incorporation of a cobalt active ion in known native metalloenzymes, a Co(III) catalytic complex may perform with higher success in biological systems than the aforementioned iridium- and rhodium-(III) analogs. More specifically, native metalloenzymes which utilize cobalt in Vitamin B cofactors have been shown to play significant roles in catalyzing numerous essential metabolic processes in humans and other mammals. This ability to mimic native proteins offers high potential for *in vivo* applications where previous ArMs have failed, allowing for fine-tuned improvements in metabolism of nucleic and amino acids, as well as carbohydrates. Such applications hold relevance in the treatment of diseases and defects, from biosynthesis-related deficiencies to immune response.

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