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The Ligand Unwrapping/Rewrapping Pathway that Exchanges Metals in S-acetylated, Hexacoordinate N$_2$S$_2$O$_2$ Complexes

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The effect of S-acetylation in MN$_2$S$_2$ complexes on metal exchange reactivity was examined in a series of MN$_2$S$_2$O$_2$ complexes. While clean exchange processes do not occur for the MN$_2$S$_2$ derivatives where formation of S-bridged aggregates predominate, acetylation permits the metal exchange with hierarchy that follows the Irving-Williams series of stability for first row transition metals: Fe$^{2+}$ < Co$^{2+}$ < Ni$^{2+}$ < Cu$^{2+}$ > Zn$^{2+}$. The rate determining step consistent with kinetic parameters depends on both M and M’, supporting a mechanism of exchange that involves ligand unwrapping/rewrapping process as earlier defined by Margerum et al. for M(EDTA) systems. The enhanced metal exchange deriving from S-acetylation is of significance to probes and detection of cysteine-S metallo-proteins and metallo-enzyme active sites, and highlights a new role for S-acetylation.

Introduction

For the coordination chemist, there are no “free” metal ions. In solution, all synthetic procedures entail the transfer of metal ions from one binding site to another, usually involving intricate mechanisms of stepwise and cascading de- and re-ligation.1 An analogue of this process of particular interest to bioinorganic chemistry lies in the construction of catalytic sites of metalloenzymes involving nitrogen and sulfur donor atoms from protein-bound histidine imidazoles, cysteine thiolates, methionine thiioethers, and tripeptide motifs with deprotonated amido nitrogens.2-4 In the form of a protein-Cys-X-Cys-protein chain, such a motif results in a contiguous, largely square planar S-N-N-S tight binding site, displayed in the distal nickel site of acetyl-CoA synthase,5 the cobalt site of thiocyanate hydratase,6 and in the iron and cobalt forms of nitrile hydratase (NHase),7 Figure 1. The sulfurs of the Cys-Ser-Cys tripeptide binding motif found in Co- and Fe-NHase are “post-translationally modified” with oxygen, yielding metallasulfanyl (R-S=O) and metallosulfonato (R-S(=O)$_2$) units.8 Similar sulfur reactivity is characteristic of synthetic N$_2$S$_2$ complexes of nickel, where the N$_2$S$_2$ binding is maintained throughout a variety of nickel-bound S-modifications; including metallation, oxygenation, and simple alkylation, Figure 2.9-13

The reactivity of cysteiny1 sulfur in proteins with acetylating agents, such as iodoacetamide, AA, and iodoacetate, Ac, has found application for cysteine protection and as an analytical tool for protein sequencing/mass spectrometry experiments.14 The latter is used to monitor the increased mass of the cysteine residues and of the protein as a whole, and in the former, to prevent the formation of disulfide bonds in the tertiary structure of proteins.10 While thioethers are typically poorer metal-binding ligands, the carboxylate or carboxamido terminus is a potential donor, leading to the possibility of coordination number expansion.11 It is generally expected that alkylation of cysteines in an apo-active site can prevent the coordination of metal(s); alternatively, alkylation on sulfurs of metal-bound cysteines may deactivate the enzyme.15, 16 As alkyllating and acetyllating agents are known carcinogens, knowledge of potential sites of reactivity in biomolecules is of even greater significance.

The work described herein was initiated in response to a need to understand the properties of zinc in N$_2$S$_2$ binding environments and its response to extraneous metals. As the second most abundant transition metal in the human body, zinc is most commonly found as a structural element. The exchange of Zn$^{2+}$ by Pt$^{2+}$ in zinc finger sites on transcription factors is theorized to be part of the mechanism by which cisplatin can interrupt DNA replication in cells.22 Zinc is a catalytic center in enzymes such as carboxy anhydrase, and carboxypeptidase where exchange with metal ions that have...
useful spectroscopic features has proven useful to define the roles of the spectroscopically silent zinc.\textsuperscript{23-25} In metallothionein proteins its kinetic lability may be exploited in exchanges with toxic metals. It may act as a place-holder in a preformed apo-enzyme active site,\textsuperscript{26} for example, HypB in the maturation cycle of NiFe-hydrogenase.\textsuperscript{27, 28} In abiological areas, Escudero-Adan \textit{et al.} have used transmetallation of Zn-salphen complexes as a synthetic approach to a library of transition metal salphen complexes.\textsuperscript{29}

In addition to the significance of metal exchange processes described above, chelation therapy and/or toxic metal removal has inspired extensive studies of the transmetallation of Ni(EDTA)\textsubscript{2-} with Cu\textsuperscript{2+} by Margerum, \textit{et al.} where kinetic data served as basis for a proposed mechanism.\textsuperscript{1, 30} The likely similarity of the functional carboxylate exterior of the EDTA complexes and the acetylated N\textsubscript{2}S\textsubscript{2} complexes has guided interpretation of the kinetic studies described below.

![Figure 2](image2.png)

**Figure 2.** Synthetic MN\textsubscript{2}S\textsubscript{2} complexes showing modification of thiolates by oxygenation, alkylation, or metallation.

Results and Discussion

**Templated Synthesis of M-1’-Ac\textsubscript{2}**. Similar to the preparation of diacetylated Ni(bme-daco), Ni-1’-Ac\textsubscript{2},\textsuperscript{32} an excess of Na\textsuperscript{+}[ICH\textsubscript{2}CO\textsubscript{2}]\textsuperscript{-} reacts with the thiolate sulfurs of the parent [M-1’]\textsubscript{2} complexes in methanol to yield the Co-1’-Ac\textsubscript{2} and Fe-1’-Ac\textsubscript{2} complexes, isolated in 98% and 72% yields, respectively, route a), Figure 4.

![Figure 3](image3.png)

**Figure 3.** Scheme for the synthesis of Ni-1’-Ac\textsubscript{2} and Zn-1’-Ac\textsubscript{2} in MeOH, adapted from reference 30,\textsuperscript{31}

Figure 3 summarizes our earlier synthesis and reactivity studies that showed acetylation of metal-bound thiolates in zinc and nickel complexes containing the tight N\textsubscript{2}S\textsubscript{2} binding site, converting the thiolate sulfurs into thioethers, concomitant with expansion of ligand denticity through the carboxylate group, i.e., resulting in MN\textsubscript{2}S\textsubscript{2}O\textsubscript{2} formulation.\textsuperscript{31, 32} The thus derived thioethers maintained binding to the metal with notable differences in chemical properties between the unmodified M-1’ complexes and their counterparts, M-1’-Ac\textsubscript{2}, Figure 3. Herein we advance such S-acetylations, yielding a series of six-coordinate complexes that have been characterized for comparison to the products of metal exchange reactions between M-1’-Ac\textsubscript{2} complexes and exogenous metal ions. Despite their coordinative saturation within a hexadentate ligand, the M-1’-Ac\textsubscript{2} complexes readily undergo transmetallation. Kinetic studies indicate a bimolecular process, presumably involving a ligand unwrapping/wrapping mechanism similar to what was proposed for metal exchange in the EDTA complexes and their derivatives.\textsuperscript{1, 30, 33-35}

**Metallation of the Sodium Salt of the Hexadentate Ligand Na\textsubscript{2}-1’-Ac\textsubscript{2} to Form M-1’-Ac\textsubscript{2}**. The hexadentate ligand Na\textsubscript{2}-1’-Ac\textsubscript{2} was synthesized as previously reported through the reaction of a slight excess of Na\textsuperscript{+}[ICH\textsubscript{2}CO\textsubscript{2}]\textsuperscript{-} with the H\textsubscript{2}bme-dach ligand in MeOH, Figure 4.\textsuperscript{36} The products isolated from addition of Co(NO\textsubscript{3})\textsubscript{2} and Fe(NO\textsubscript{3})\textsubscript{2} route b), Figure 4, had physical properties matching the M-1’-Ac\textsubscript{2} complexes produced by route a). In addition, the copper product obtained by route b) was identical to that from reaction c), the Cu\textsuperscript{2+}/Zn\textsuperscript{2+} transmetallation, i.e., the [Cu-1’-Ac\textsubscript{2}]\textsubscript{2} product.

Zinc/Metal Transmetallation. In a previous study, nickel was shown to rapidly replace zinc in the hexadentate N\textsubscript{2}S\textsubscript{2}O\textsubscript{2} ligand as shown in Figure 3.\textsuperscript{31} Similarly, on addition of a light pink solution of Co(NO\textsubscript{3})\textsubscript{2} to a colorless methanolic solution of Zn-1’-Ac\textsubscript{2} an immediate color change to a deep magenta was observed, producing Co-1’-Ac\textsubscript{2} that was isolated in >70% yield. Likewise, Cu(NO\textsubscript{3})\textsubscript{2} mixed with Zn-1’-Ac\textsubscript{2} gave a 30% isolated yield of [Cu-1’-Ac\textsubscript{2}]\textsubscript{2} (deep blue). The characteristic properties of Co-1’-Ac\textsubscript{2} and [Cu-1’-Ac\textsubscript{2}]\textsubscript{2} matched those of the direct synthesis, route b), Figure 4, products. Thus, a facile
transmetallation reaction between the kinetically labile zinc center in Zn-1'-Ac₂ and an exogenous metal ion occurs only with the aid of the acetate arms. It is notable that no exchange was observed between ZnNi⁺⁻ in Zn-1'-Ac₂ and Fe(NO₃)₃.

Control Experiments and Qualitative Rankings of Transmetallations. Previous results along with several control experiments provided insight into the metal exchange process. The addition of Ni(NO₃)₂ in the presence and absence of NaOAc to a stirred solution of [Co-1']₂, both at 22 °C and 70 °C, resulted in the formation of metallo-aggregates rather than a quantitative exchange of metals, processes 1 and 2 of Figure 5. However, if Ni(NO₃)₂ and Na⁺[ICH₂CO₂] of NaOAc to a stirred solution of Fe(NO₃)₂ however does not. Stepwise exchanges in a single flask readily monitored by color changes and UV-Vis spectroscopy, beginning with Fe-1'-Ac₂, both at 22 °C and 70 °C, molecules that are H-bonded to the free carboxylate oxygens, vide infra.

The possibility of exchange or metal scrambling between N₆S₂ and N₆S₂O₂ binding sites was also explored. In the first case a mixture of Ni-1' and Zn-1'-Ac₂ were dissolved in a methanol solution and stirred for 7 days over which time there was, from MS and UV-Vis analysis, no indication of any reaction occurring. We conclude that nickel cannot be transferred from its tight N₆S₂ binding site; nor can the acetyl group be transferred from the zinc to the nickel thiolate sulfur. Likewise, the opposite conditions of a mixture of Ni-1'-Ac₂ and [Zn-1']₂, indicated no metal exchange occurred between the two complexes even after 7 days.

Properties of M-1'-Ac₂ Complexes. Infrared data for the M-1'-Ac₂ complexes in CH₂Cl₂ solutions are found in the experimental section. All exhibit a strong solution IR stretch at ca. 1630 cm⁻¹ corresponding to the acetate C=O group, with no apparent trend in the relative positions. Assignments of two prominent bands in the 1300's region for all are equivocal, but preserved to correspond to M-O or C-O stretches.

As described in the experimental section, Gouy balance and Evans’ method magnetic studies established that the octahedral complexes of Cu⁺², Ni⁺², Co⁺², and high-spin Fe⁺² are paramagnetic with experimental magnetic moments largely matching the expected μₛ values; the d¹⁰ Zn⁺² derivative is diamagnetic.

The cyclic voltammograms for Ni-1'-Ac₂ and Co-1'-Ac₂ are shown in Figures S1 and S3, respectively. Both complexes show an oxidation event assigned to the reversible Ni⁺²/Nil⁺³ couple at 0.69 V and a quasi-reversible Co⁺²/Co⁺³ couple at -0.24 V. An irreversible event is seen at -2.33 V and -2.31 V for Ni-1'-Ac₂ and Co-1'-Ac₂, respectively. Data relating to reversibility are as follows: for the Ni-1'-Ac₂, Epa, Ep, AE, and ip/ipp are 0.656 V, 0.716 V, 60 mV, and 0.86, respectively; and for Co-1'-Ac₂, 0.390 V, -0.090 V, 300 mV, and 0.74, respectively.

X-ray Diffraction Analyses of Molecular Structures. Crystals of the Co-1'-Ac₂, Fe-1'-Ac₂, and [Cu-1'-Ac₂]₂ complexes were obtained as racemic mixtures from layering methanol solutions with diethyl ether. The structures were refined in the P-1 (triclinic), P-1 (triclinic), and C 2/c (monoclinic) space groups, respectively. The former two co-crystallize with two MeOH molecules that are H-bonded to the free carboxylate oxygens, vide infra.
Figure 6 shows the thermal ellipsoid renderings for the Fe-1'Ac2 and Co-1'Ac2 structures. The distorted octahedral Co-1'Ac2 molecule contains a near perfect N2S2 plane with a mean atom deviation of 0.007 Å (without Co). The cobalt center is displaced from the best N2S2 plane by 0.001 Å and the Co displacement is 0.014 Å. Note that the O—M—O angles are ≠180° and are bent toward the M—S side of the molecule as observed in the previously reported Zn and Ni analogs.

The [Cu-1'Ac2]2 complex exists as a dimer resulting from detachment of one of the thioether arms which allows the carboxylate oxygens to bridge to a second copper center, Figure 7. The copper centers thus display pentacoordination in a distorted square pyramidal structure, CuN2SO2, with τ value of 0.015 and an almost ideal N2OS plane (with a mean atom deviation of 0.051 Å). The CuII metal is displaced from this N2OS best plane by 0.208 Å and the Cu—Cu distance is 7.189 Å. Also of note, the two N2OS planes present in the [Cu-1'Ac2]2 dimer intersect at an angle of 64.56°.

Table 1 lists crystallographic data and selected metric parameters for the three new complexes of this study along with Ni-1'Ac2 and Zn-1'Ac2.31 Note that the Zn-1'Ac2 is completely analogous to the Co and Fe analogues. The M-S distances from Fe to Cu are found to diminish and then increase at the end with the Zn-1'Ac2 complex, a trend which tracks their ionic radii and as noted by the Irving-Williams Series.37 The same trend can be seen with the M-N distances; however, the M-O bond distances actually increase across the row as the radii decrease. This is likely a result of the more drastic decrease in M-S distances coupled into the torsion angles through the acetate arm. Thus, the oxygen donors are prohibited from moving toward the metal center, but rather shift away as the metal ion gets smaller. Overall, the bond distances for the Zn-1'Ac2 complex fall between those of the Fe-1'Ac2 and Co-1'Ac2 complexes.

For comparison with the analogous NiN2S2 that is S-modified by acetamide, AA, the structure of [Ni-1'AA2]I2 is shown in Figure 8, along with Ni-1'Ac2. The metric parameters of [Ni-1'AA2]I2 largely track with the Ni-1'Ac2 structure in terms of the first coordination sphere angles and bond distances. All compounds in the M-1'Ac2 and Ni-1'AA2 series co-crystallize with MeOH or H2O in a H-bonded network that links the solvent molecules and the =O or –NH2 groups that are directed into the interstitial space between molecules. Figure S4 shows an example of this H-bonding network in Co-1'Ac2.

![Figure 6](image_url)

![Figure 7](image_url)

**Figure 6.** Thermal ellipsoid renderings for the Fe-1'Ac2 and Co-1'Ac2 structures. The distorted octahedral Co-1'Ac2 molecule contains a near perfect N2S2 plane with a mean atom deviation of 0.007 Å (without Co). The cobalt center is displaced from the best N2S2 plane by 0.001 Å and the Co displacement is 0.014 Å. Note that the O—M—O angles are ≠180° and are bent toward the M—S side of the molecule as observed in the previously reported Zn and Ni analogs.

**Figure 7.** Thermal ellipsoid plot of [Cu-1'Ac2]2 molecular structure showing a) the carboxylate bridged dimer and b) one unit of the dimer in ball and stick from XRD and in Chemdraw graphic, inverted and rotated by 90°.

**Figure 8.** Comparison of the metric parameters in Ni-1'Ac2 (left) and [Ni-1'AA2]I2 (right). Hydrogen atoms, counter ions, and solvent molecules have been removed for clarity. Color coding as in Figure 6; Ni in light blue.

**Equilibrium Constants.** Equilibrium constants of the metal exchange reactions were measured in aqueous solutions using UV-Vis spectroscopy. Metal salts, see Table 2, and M-1'Ac2 complexes, dissolved in water, were combined in a 1:1 ratio at 0.007-0.008 M concentrations and allowed to equilibrate for 3 h at 22 °C. The UV-Vis spectra were then recorded and, in combination with molar absorptivity values at selected wavelengths, Table S1, the concentrations of the species in solution at equilibrium were calculated.

\[
\text{M-1'Ac}_2 + \text{M'}(\text{H}_2\text{O})_x^{2+} \rightleftharpoons \text{M'-1'Ac}_2 + \text{M(H}_2\text{O)}_x^{2+}
\]  

Thus, the equilibrium constants shown in Table 2 assumed that each metal is contained in one of the two forms present in Eq. 1 and 2, see Chart S1 for equations used. Of the possible aggregates or mixed metal complexes in the likely complicated mechanism, vide infra, none are observed in the UV-Vis monitor. In the following discussion, the exchange pairs are expressed as M-1'Ac2/M'2+ → M'-1'Ac2/M2+ and shortened to M/M' and M'/M, respectively.
A chemical exchange hierarchy, which is again consistent with the Irving-Williams series Zn\(^{2+}\) < Co\(^{2+}\) < Ni\(^{2+}\).\(^{37}\) Ni/Co (Keq = 0.0002) exchange pairs show discrepancies in the values that we suggest are within error of molar absorptivity equilibrium constants using the available simple techniques.

Overall, the estimated Keq values serve to accentuate the exchange hierarchy, which is again consistent with the Irving-Williams series Zn\(^{2+}\) < Co\(^{2+}\) < Ni\(^{2+}\).\(^{37}\)

**Kinetic Studies.** Monitors of the addition of Ni(NO\(_3\))\(_2\), Co(NO\(_3\))\(_2\), or Cu(NO\(_3\))\(_2\) to a MeOH solution of Zn-1'-Ac\(_2\) found reactions completed within the time of mixing and thus too fast for rate measurements by our available techniques, Figure S5. Thus, the displacement of Co-1'-Ac\(_2\) by Ni\(^{2+}\) in MeOH was selected as a reaction sufficiently slow for kinetic monitoring. Figures 9 and S6 display full UV-Vis scans for this metal exchange reaction, with alternate pseudo first order conditions for each reactant. Figure 9 includes an inset showing the UV-Vis spectra for the pure Ni-1'-Ac\(_2\). The ten-fold excess of Co-1'-Ac\(_2\) reacting with Ni\(^{2+}\) in MeOH show the UV-Vis absorptions at 362 and 845 nm increase while a peak at 487 nm decreases in absorbance, Figure 9. If the opposite molar ratio is used (1 Co-1'-Ac\(_2\): 10 Ni\(^{2+}\)) then the peaks at 362, 580, and 845 nm increase corresponding to the formation of Ni-1'-Ac\(_2\); the peak at 487 nm decreases as Co-1'-Ac\(_2\) undergoes metal exchange, Figure S6.

![Figure 9](image-url)
change in the UV-Vis spectrum was observed, requiring approximately 20 mins at room temperature.

In a typical kinetics experiment the Co-1’-Ac2 concentration was 0.0050 M and the Ni(NO3)2 was in 12.5, 25, or 50-fold excess. The reactions were monitored at ambient temperature by appearance of the UV-Vis band at 845 nm, with data collection started as soon as the two solutions were injected into the cuvette, and followed to completion. The linearity of the natural log plot of the absorbance at 845 nm, versus time, Figure 10, indicates that the reaction is first order in the Co-1’-Ac2 complex.

The order of reaction dependence on Ni2+ was determined by monitoring the exchange at multiple concentrations of excess Ni2+. A plot of k_{obs} vs. [Ni^{2+}], Figure S7, is linear with a y-intercept of nearly zero indicating a first-order dependence for Ni^{2+}. Thus, the complete bimolecular rate law is shown below.

\[
rate = k_{obs}[\text{Co1’Ac2}]^1
\]

\[
k_{obs} = k[\text{Ni}^{2+}]^{n}
\]

\[
rate = k[\text{Co1’Ac2}]^1[\text{Ni}^{2+}]^1
\]

The temperature dependence of the rate constant, k, was measured over a 37 K range (286-313 K), Figure S8, for the conversion of Co-1’-Ac2 to Ni-1’-Ac2. During these experiments the Co-1’-Ac2 solution was equilibrated in the temperature-controlled cuvette holder before injection of the temperature-adjusted Ni(NO3)2 solution. The temperatures, k_{obs}, and k values are listed in Table 3.

Activation parameters for the formation of Ni-1’-Ac2 from Co-1’-Ac2 were determined by an Eyring analysis which found ΔH‡ of 15.7 ± 0.7 kcal/mol and a ΔS‡ of −14.3 ± 2.3 e.u., Figure 11. The ΔG‡ can be calculated at 298 K as 19.9 ± 0.7. The small ΔH‡ and negative ΔS‡ values are indicative of an associative mechanism for the Co/Ni transmetallation reaction, consistent with the bimolecular rate law above. ΔH‡ and ΔS‡ may represent composite values of both activation parameters and equilibrium constants dependent on the rate limiting step in the mechanism (vide infra).

Table 3. Kinetic parameters obtained from the natural log plots from varying temperature for the reaction of Co-1’-Ac2 with excess Ni(NO3)2 in MeOH. The concentration of Co-1’-Ac2 was 4.75 x 10^{-3} M and Ni(NO3)2 was 0.119 M i.e., 1:25 ratio.

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<th>k_{obs} (s^{-1})</th>
<th>k (M^{-1} s^{-1})</th>
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Experimental Section

General Methods and Materials. Solvents were dried and degassed using a Bruker solvent system. The products are air stable; however, as a precautionary measure, all reactions were carried out under an inert atmosphere of argon using standard Schlenk procedures unless otherwise noted. The acetylated products can be extremely hygroscopic, necessitating exclusion of moisture. Separations used silica gel chromatography both for thin layer and column purifications. Reagents were used as acquired from standard vendors. The bis(2-mercaptoethyl)-1,4-diazacycloheptane (H2bme-dach),12 N,N′-bis(2-mercaptoethyl)-1,4-diazacycloheptane zinc(II) dimer [Zn-1’]2,31 N,N′-bis(2-mercaptoethyl)-1,4-diazacycloheptane cobalt(II) dimer [Co-1’]2,40 N,N′-bis(2-mercaptopoethyl)-1,4-diazacycloheptane nickel(II) dimer [Ni-1’]2,12 1,4-diazacycloheptane-1,4-diybis(3-thiapentanoato) zinc(II) Zn-1’-Ac2,31 and 1,4-diazacycloheptane-1,4-diybis(3-thiapentanoato) nickel(II) Ni-1’-Ac2,31 were synthesized according to published procedures. Additional details for the synthesis of Co-1’-Ac2, Fe-1’-Ac2 and Cu-1’-Ac2 are available in the Supplemental Information.

Physical Measurements. Elemental analyses were performed by Atlantic Microlab, Inc. Norcross, GA, USA. Electrospray
ionization mass spectrometry (ESI-MS) was performed by the Laboratory for Biological Mass Spectrometry at Texas A&M University. Solution infrared spectra were recorded on a Bruker Tensor 37 Fourier Transform – IR spectrometer, using a CaF\(_2\) cell with 0.2 mm path length. UV-Visible spectra were obtained using a Shimadzu UV-2450 spectrophotometer with 1.0 cm path length quartz cells. Cyclic voltammograms were recorded on a BAS-100A electrochemical analyzer. All experiments were performed at room temperature under Ar in MeCN solution containing 0.1 M [n-Bu\(_4\)N][PF\(_6\)] as the electrolyte, with a 3.0 mm glassy carbon working electrode, an Ag/AgNO\(_3\) reference electrode, and a Pt coil counter electrode. All values have been internally referenced to the Fc/Fc\(^+\) couple.

**X-ray Crystallography.** The X-ray data for Ni\(^{1+}\)-AA\(_2\), Co\(^{1+}\)-Ac\(_2\), Fe\(^{1+}\)-Ac\(_2\), and [Cu\(^{1+}\)-Ac\(_2\)]\(_2\) (CCDC #1407471-1407474) were obtained on a single-crystal APEX2 CCD diffractometer (Mo Ka radiation, \(\lambda = 0.71073 \) Å) in the X-ray Diffraction Laboratory at Texas A&M University. Crystal samples were crushed to obtain a powder, affixed to a Nylon loop, and placed under streaming \(\text{N}_2\) gas. The structures were solved by direct methods. H atoms were placed at idealized positions and refined with fixed isotropic displacement parameters, and anisotropic displacement parameters were employed for all non-hydrogen atoms. The following programs were used: data collection and reduction, APEX2;\(^{41}\) absorption correction SADABS;\(^{42}\) cell refinement, SHELXTL;\(^{43}\) structure solutions, SHELXS-97;\(^{43}\) and structure refinement, SHELXL-97.\(^{43}\) The final data presentation and structure plots were generated in X-Seed Version 2.0.\(^{44}\) CIF files were prepared for publication using WinGX and its included programs.\(^{45}\) Data acquisition and refinement data are in Supporting Information.

**Synthesis and Characterization**

1,4-diazacycloheptane-1,4-diylbis(3-thiapentanoamide) nickel(II) iodide, [Ni\(^{1+}\)-AA\(_2\)]\(_2\).

Ni\(_2\)S\(_2\) Templated Synthesis. A portion of Ni\(^{1+}\) (0.25 g, 0.90 mmol) was placed in a 100 mL Schlenk flask and degassed prior to addition of 30 mL MeCN. Iodoacetamide, AA, (0.35 g, 1.90 mmol) in 20 mL MeCN was cannulated into the stirring Ni\(^{1+}\) solution. The reaction mixture was stirred at 22 \(°C\) for 48 hr yielding a blue precipitate. The mixture was filtered and the solid was washed with 3x5 mL MeCN and 3x10 mL Et\(_2\)O and dried in vacuo to yield 0.48 g (0.74 mmol, 82%) of [Ni\(^{1+}\)-AA\(_2\)]\(_2\) solid. ESI-mass spectrum in CH\(_3\)OH: \[\text{Ni}_2\text{S}_2\text{O}_2\text{C}_13\text{H}_{26}\] \(m/z = 196.0 (100 \%)\) \[\text{Ni}_2\text{S}_2\text{O}_2\text{C}_13\text{H}_{26} + \text{H}^+\] \(m/z = 391.0 (44 \%)\). UV-Vis (CH\(_3\)OH): \(\lambda_{\text{max}}\), nm (ε, M \(\text{cm}^{-1}\)) = 295 (13), 358 (56), 255 (6580) nm. IR (in MeOH, cm\(^{-1}\)): 1673 (vs, sharp). Magnetic moment, Guoy Balance: 2.93 B.M. Elem. Anal. Calc’d for Ni\(^{1+}\)-AA\(_2\)•2MeOH, Ni\(_2\)S\(_2\)O\(_2\)C\(_{15}\)H\(_{36}\)I\(_2\): C: 25.4 (25.24), H: 6.82 (7.62).

**Templated Synthesis, Route a.**

1,4-diazacycloheptane-1,4-diylbis(3-thiapentanoic) cobalt(II), Co\(^{1+}\)-Ac\(_2\). A sample of [Co\(^{1+}\)]\(_2\) (0.50 g, 0.90 mmol) within a 250 mL Schlenk flask was dissolved in 50 mL of dry MeOH, producing a green solution. Sodium iodoacetate, Na\(^{1+}\)Ac\(_2\) (0.84 g, 4.0 mmol) in 40 mL MeOH, was added via cannula to the stirring [Co\(^{1+}\)]\(_2\) solution. The reaction mixture was stirred at 22 \(°C\) for 24 hr; the solution was still red/brown and stirring was continued for 24 hr; the solution was still red/brown. The mixture was filtered to remove any NaI formed. The filtrate was then chromatographed on a silica gel column (3 x 20 cm) using a 1:1 MeOH:MeCN solvent mixture as eluent. An initial band of yellow material was discarded and the magenta product, with an R\(_f\) value of 0.45, was collected. The solvent was removed in vacuo, and precipitation of a powder forced with addition of ether. The product was collected by filtration, washed 3x with ether, and dried in vacuo yielding 0.30 g (0.92 mmol, 40%) of Co\(^{1+}\)-Ac\(_2\)•2MeOH solid. ESI-mass spectrum in CH\(_3\)OH: \[\text{Co}_2\text{S}_2\text{O}_2\text{C}_13\text{H}_{26} + \text{H}^+\] \(m/z = 416 (27%)\). UV-Vis (CH\(_3\)OH): \(\lambda_{\text{max}}\), nm (ε, M \(\text{cm}^{-1}\)) = 346 (2660), 280 (1560) nm. IR (in CH\(_3\)Cl\(_2\), cm\(^{-1}\)): 1631 (vs, sharp), 1348 (m), 1327 (m). 1,4-diazacycloheptane-1,4-diylbis(3-thiapentanolate) iron(II), Fe\(^{1+}\)-Ac\(_2\). As in the cases above, Fe\(^{1+}\)-Ac\(_2\) solid was isolated in 72% yield. ESI-mass spectrum in CH\(_3\)OH: \[\text{Fe}_2\text{S}_2\text{O}_2\text{C}_13\text{H}_{26} + \text{H}^+\] \(m/z = 391.0 (44 \%)\). UV-Vis (CH\(_3\)OH): \(\lambda_{\text{max}}\), nm (ε, M \(\text{cm}^{-1}\)) = 346 (2660), 280 (1560) nm. IR (in CH\(_3\)Cl\(_2\), cm\(^{-1}\)): 1631 (vs, sharp), 1348 (m), 1327 (m).

1,4-diazacycloheptane-1,4-diylbis(3-thiapentanolate) copper(II), [Cu\(^{1+}\)-Ac\(_2\)]\(_2\). As a discrete Cu\(^{1+}\) complex analogous to Ni\(^{1+}\) or [Co\(^{1+}\)]\(_2\), Cu\(^{1+}\) is not known, thus a templated synthesis was not attempted.

Hexadentate N\(_2\)S\(_2\)O\(_2\) Ligand Synthesis Followed by Metallation, Route b.

Co\(^{1+}\)-Ac\(_2\). The H\(_2\)bme-dach ligand (0.50 g, 2.3 mmol) was placed in a 500 mL Schlenk flask and dissolved in 50 mL MeOH. To this flask Na\(^{1+}\)Ac\(_2\) (1.0 g, 5.0 mmol) in 50 mL MeOH was added. The pale yellow solution was magnetically stirred for 16 hr before it was used in situ. To the stirring acetylated bme-dach ligand solution, Co(NO\(_3\))\(_2\) (0.66 g, 2.3 mmol) was added as a clear pink solution in 50 mL of dry MeOH whereupon a magenta color developed. The solution volume was partially reduced in vacuo before filtering to remove Na\(^+\) salts formed during the reaction. Addition of Et\(_2\)O resulted in precipitation of a magenta solid, which was isolated by filtration. This powder was redissolved in MeOH and chromatographed through a silica gel column with MeOH as the eluent yielding 0.36 g (0.92 mmol, 40%). The properties of this product matched those from the templated synthesis, route a.

Fe\(^{1+}\)-Ac\(_2\). In a similar manner, Fe\(^{1+}\)-Ac\(_2\) solid was isolated in 66% yield from the reaction of Fe(NO\(_3\))\(_2\) and the N\(_2\)S\(_2\)O\(_2\) ligand. The product had identical characteristics as the product from route a.
[Cu-1'-Ac2]. The reaction of Cu(NO3)2 and the N2S2O2 ligand was performed similar to the cobalt case above to yield 24% [Cu-1'-Ac2] solid. ESI-mass spectrum in CH2OH: [CuN2S2O2C6H4H2 + H]+ m/z = 398. UV-Vis (CH2OH): λmax nm (ε M⁻¹ cm⁻¹) = 607 (202), 348 (2660), 287 (1560) nm. IR (in CH2Cl2, cm⁻¹): 1631 (vs, sharp), 1347 (m), 1329 (m). E1/2 = -360 mV vs. Fc/Fc⁺ in CH2Cl2 for the Cu II/CuI couple. Magnetic moment, was performed similar to the cobalt case above to yield 24 % of Ni-1'-Ac2. A 100 mL Schlenk flask was charged with [Co-1']2 + Ni(NO3)2 + Na'ICH2COO⁻ with heating. The above reaction conditions were repeated with the reactants stirred at 70 °C and product purified by the same means to yield 0.043 g (0.11 mmol, 59 %) of Ni-1'-Ac2.

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Metal Exchange into Zn-1'-Ac2, Route c). Co-1'-Ac2. To a 0.20 g, 0.51 mmol, sample of Zn-1'-Ac2, 75 mL of MeOH was added, producing a clear colorless solution to which was added 0.15 g, 0.51 mmol of Co(NO3)2 as a light pink solution in 25 mL dry MeOH. The mixture was stirred for 24 h. The solid magenta product was isolated as above to yield 0.14 g (0.37 mmol, 72%); characterization matched above results.

Fe-1'-Ac2. Transmetallation between Fe2⁺ from Fe(NO3)2 and Zn-1'-Ac2 did not occur in MeOH.

[Cu-1'-Ac2]. Analogous to the cobalt reaction [Cu-1'-Ac2] could be isolated in 30% yield as a blue solid. Characterization matched that obtained by route b.

Control Reactions: Metal Exchange between N2S2 Bound and Nitrate Salts

[Co-1']2 + Ni(NO3)2. The cobalt dimer [Co-1']2 (0.050 g, 0.18 mmol) and Ni(NO3)2 (0.052 g, 0.18 mmol) were placed in a 30 mL vial followed by addition of 20 mL of MeOH. The solution was stirred overnight both at 22 °C (trial 1) and 70 °C (trial 2); no changes in UV-Vis spectra were observed. (Co-1')2 + Ni(NO3)2 + Na'CH3COO⁻. A 30 mL vial was charged with the cobalt dimer [Co-1']2 (0.050 g, 0.18 mmol), Ni(NO3)2 (0.052 g, 0.18 mmol), and 2 equiv. of NaOAc (0.299 g, 0.36 mmol); these were dissolved in 35 mL of MeOH. The resulting mixture was stirred overnight at 22 °C. No change was observed in the UV-Vis spectrum.

[Co-1']2 + Ni(NO3)2 + Na'CH3COO⁻ with heating. The above reaction conditions were repeated with the reaction stirring at 70 °C overnight. No changes were observed.

[Co-1']2 + Ni(NO3)2 + Na'ICH2COO⁻. The cobalt dimer [Co-1']2 (0.050 g, 0.18 mmol), Ni(NO3)2 (0.052 g, 0.18 mmol), and 20 equiv. of Na' ICH2COO⁻ (0.015 g, 0.072 mmol) were added to a 30 mL vial and dissolved in 20 mL of MeOH. The solution was stirred overnight at 22 °C. No change was observed when compared to control reaction without Na' ICH2COO⁻, an additional 8/5 equiv. of Na' ICH2COO⁻ (0.060 g, 0.29 mmol) in 5 mL MeOH was added and stirred overnight. Again, no change was observed by 18 equiv. of Na' ICH2COO⁻ (0.68 g, 3.25 mmol) in 10 mL MeOH was added and the solution was stirred overnight. The reaction mixture was then purified by silica gel column chromatography. The blue band was collected and reduced in vacuum. Et2O was added and the mixture left overnight at 22 °C to isolate 0.041 g (0.10 mmol, 58 %) of Ni-1'-Ac2.

Ni-1'-Ac2 + Co(NO3)2. A 100 mL Schlenk flask was charged with Ni-1'-Ac2 (0.050 g, 0.13 mmol) and Co(NO3)2 (0.037 g, 0.13 mmol) and degassed prior to the addition of 25 mL MeOH. No exchange product was observed by UV-Vis spectroscopy under these conditions so an additional 9 equiv. of Co(NO3)2 (0.33 g, 1.14 mmol) in 10 mL MeOH was added and stirred for 6 h. No product formation was observed by UV-Vis analysis so a further 90 equiv. of Co(NO3)2 (3.33 g, 11.44 mmol) in 15 mL was added. The reaction volume was reduced in vacuo and the mixture components were separated by silica gel column chromatography using MeOH as an eluent. The M(NO3)2 salts elute first. A second blue/purple band, was collected, and the volume was reduced in vacuo. UV-Vis and mass spec analysis was used to quantitate the amount of Co-1'-Ac2 (18 - 19 %) formed since the M-1'-Ac2 species could not be separated from one another.

Ni-1' + Zn-1'-Ac2. A 100 mL Schlenk flask was charged with Ni-1' (0.050 g, 0.18 mmol) and Zn-1'-Ac2 (0.072 g, 0.18 mmol) and degassed. To this 50 mL of MeOH was added yielding a brown solution. After stirring overnight at room temperature no Ni-1'-Ac2 formation was observed by UV-Vis spectroscopy. The solution was then stirred overnight at 70 °C and still no Ni-1'-Ac2 was observable by UV-Vis spectroscopy.

Conclusions

The N2S2 ligand binding site has been found to be largely inert to metal exchange, rather yielding S-based aggregation products of various structural types when MN2S2 complexes are exposed to exogeneous metals.9, 46 Such a tight binding...
characteristic of the tetradentate N₂S₂ ligands is lessened on S-alkylation. For example, S-methylation of [Zn(bme-dach)]₂ with MeI results in deligation of the newly formed thioethers and coordination of the iodide ions yielding a tetrahedral ZnN₂I₂ as shown in Figure 12.13 The zinc is readily displaced by nickel, whereupon the thioether sulfur reclaims the metal binding in square planar coordination geometry. However, if the alkylation agent creates additional donor atoms within the ligand framework as in the iodoacetamide and iodoacetate derivatives, the chelate effect might be expected and indeed does keep the poor thioether donors bound to the Zn in a hexacoordinate, octahedral ZnN₂S₂O₂ site. However, such S-modification with sodium iodoacetate can also render the metal center labile and replaceable by exogenous metal ions through interaction with the available carboxylate oxygen atoms. The proposed mechanism resulting from this interaction entails a ligand unwrapping /rewrapping process, Figure 18, leading to facile metal exchange rather than aggregation products. Modeled after the mechanistic suggestions of Margarum, et al., the rate determining step is expected to involve the M-N bond-breaking/forming step to give a bimetallic intermediate.1,30

Lending veracity to the proposed intermediates of Figure 13, are two crystal structures: first, the [Cu-1'-Ac₂]₂ dimer, Figure 7, finds carboxylate oxygens bound to copper resulting from one of the ligand arms which has become partially “unwrapped”. The second is a structure of an N₂S₂O₂ free ligand based on bis-mercaptoethane-diazacycloheptane that has been S-alkylated by paraformaldehyde (CCDC #1407475). The diazacycle of the resulting molecule is in a conformation that would allow it to bridge to two metals using the inverted nitrogens. As shown, the Bronsted acid proton of the OH group replaces the Lewis acidic metals, Figure 14.

Thus even with what would appear to be a very rigid ligand framework, and the requirement of bond breaking processes expected to have a high energy barrier, the unwrapping/rewrapping process gains feasibility by the multiple interactions resulting from the additional carboxylate functionality. Of prime significance, this work illustrates a consequence of S-acetylation that to our knowledge has not been previously expressed, i.e., a pathway for metal exchange. A wider application of such sequential binding/rebinding mechanisms involving oxygen, nitrogen, and sulfur donors is likely in many metal transport processes. For example, the methanobactin Cu⁺ chelator ultimately produces a four-coordinate N₂S₂ site for Cu⁺ with an uptake/release mechanism that involves peptide amido groups for which precise pathways are difficult to mimic.
reaction of H2bme-dach with paraformaldehyde in chloroform.

bonding to the respective pendent –OH groups. The ligand was derived from the membered diazacycle with the nitrogen lone pairs anti to each other and hydrogen bonding with the respective pendent –OH groups. The ligand was derived from the reaction of H2bme-dach with paraformaldehyde in chloroform.

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Notes and References