SYNTHESIS AND KINETICS STUDY OF DIIRON-HYDROGENASE ACTIVE SITE
MIMICS

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BY
KATHERINE MACRI
ADVISOR: JESSE W. TYE

BALL STATE UNIVERSITY
MUNCIE, INDIANA
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The hydrogenase enzyme is an effective replacement for the expensive platinum catalysts used in hydrogen fuel cells today. However, many enzymes themselves are found in extreme environments and are inactive under standard conditions, but current active site models have a much larger over-potential for H⁺ reduction than the actual enzyme. Most research today involves the improvement of these synthetic models in an attempt to lower reduction potential, increase reaction kinetics, or improve catalytic activity.

Research focuses on the synthesis of active site models with a carbon chain bridgehead linker of varying length. Synthesis of these molecules is achieved by the reaction of a dithiol with triiron dodecacarbonyl under an inert atmosphere to avoid the formation of by-products. Dithiols with four or more carbon atoms must first be converted to cyclic disulfides before the reaction with the iron dodecacarbonyl. This prevents the formation of an unwanted side product. Both butyl- and
pentyldithiolatohexacarbonyldiiron model complexes have been characterized by IR, NMR, and X-ray spectroscopy.

Active site models can also feature two unlinked sulfur atoms. These models have two conformational isomers that depend on the spatial location of the R-group bonded to each sulfur atom. This research also focuses on the synthesis of unlinked active site models with a variety of R-groups, and a temperature controlled NMR study of the isomeration reaction to determine the reaction rate.
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Chapter 1

Review of Literature

I. Discovery of Hydrogenase Enzymes

II. Use as a Fuel Cell Catalyst

III. Types of Hydrogenase Enzymes
   i. [NiFe]-Hydrogenases
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IV. Synthetic Models
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I. DISCOVERY OF HYDROGENASE ENZYMES

Many microorganisms use the hydrogenase enzyme to catalyze the oxidation-reduction equilibrium of dihydrogen, as shown in the following reaction, $2H^+ + 2e^- \leftrightarrow H_2$. Since its discovery in 1931, the hydrogenase enzyme has been divided into three different classes based on the metal ions present at its active site. All are believed to be unrelated and are classified as [NiFe]-hydrogenases, [FeFe]-hydrogenases, and [Fe]-hydrogenases. Most hydrogenases found in nature belong to the [NiFe]-H$_2$ase or [FeFe]-H$_2$ase class. [Fe]-hydrogenases are different because they contain a mononuclear iron active site and no iron-sulfur bonds characteristic of the other hydrogenases. The production of dihydrogen has many biotechnological applications, including an alternative to carbon based fossil fuels as an energy source.

The hydrogenase enzyme was discovered after Stephenson and Stickland demonstrated that colon bacteria evolved H$_2$ during growth and could use H$_2$, but not N$_2$, to reduce other substrates. A hydrogenase was thought to be the enzyme responsible. Over the years, the hydrogenase enzyme was isolated from prokaryotes...
found in both Bacteria and Archaea domains. These species have hydrogenases instead of mitochondria. In 2007, Vignais and Billoud reported that over 450 hydrogenases have been sequenced. Many of these enzymes are found in the same organism, and often individual species will contain hydrogenases with different metal centers.

Until 1984, it was believed that all hydrogenases contained nickel as a cofactor in the active site. The absence of a strong electron paramagnetic resonance (EPR) signal from nickel in the anaerobe Megasphaera elsdenii led researchers to conclude the metal was not found at the active site. A variety of other [FeFe]-hydrogenases were soon isolated, the majority of them found in anaerobic bacteria. Extensive EPR spectroscopy of the enzyme lead to the discovery of multiple [4Fe-4S] clusters thought to be involved in the H₂ catalysis. A variety of different clusters were synthesized and compared to the known data. The findings suggest a binuclear active site structure, but this was not proven until 1999. In recent years, the discovery of the mononuclear [Fe]-hydrogenase has led to a third class of hydrogenase enzymes.

II. USE AS A FUEL CELL CATALYST

The hydrogenase enzymes found in nature have many parallels to the dihydrogen fuel cell. A fuel cell generates electricity through an electrochemical reaction. Hydrogen is the most commonly used fuel, but hydrocarbons or alcohols will
also work as reducing agents.\(^3\) All fuel cells contain an anode, cathode, and electrolyte.

In the hydrogen fuel cell, $\text{H}_2$ enters the cell at the anode where it is oxidized into protons and electrons by a catalyst. The positively charged ions travel through the electrolyte to the cathode. Simultaneously, the electrons are channeled through a wire producing an electric current. Both protons and electrons are reunited at the cathode with oxygen and react with the help of another catalyst to form water. The only by-product is heat which could also be used outside the fuel cell.\(^4\)

The fuel cell is similar to a battery in that it converts the potential energy of a chemical reaction into electrical energy. However, a battery has a finite supply of energy and must be replaced or recharged once that fuel is depleted.\(^3\) On the other hand, a fuel cell uses an external source of chemical energy, and in theory can produce electricity as long as it is supplied with hydrogen.\(^3\) The output of power generated by a fuel cell can easily be increased by combining individual cells in series to form a stack.\(^4\) This allows manufacturers to customize the voltage to power anything from laptops to a central power system.\(^4\)

In practice, the fuel cell is far perfect. Intensive of research is being conducted to increase efficiency, lower cost, and improve the durability of current models. The fuel cell must be cost effective and perform as well or better than current technologies to be used as an alternative energy source.\(^4\) If fuel cells can be optimized and made commercially available they will have numerous applications. They could be used to power almost any portable device that currently uses batteries.\(^4\) Fuels cells also have
the potential to replace the petroleum used in transportation vehicles with hydrogen, and stationary cells could be used for household power.\textsuperscript{3,4}

Fuel cells can vary based on their electrodes and composition of the electrolyte. Two cells that use hydrogen as a fuel are the proton exchange membrane (PEM) fuel cell (also known as polymer electrolyte membrane) and the phosphoric acid fuel cell (PAFC). The PEM fuel cell uses a water-based, acidic polymer as the electrolyte.\textsuperscript{3} The cell won’t leak or crack and operates at relatively low temperatures (less than 100 °C).\textsuperscript{3} This makes it ideal for use in light duty vehicles and some stationary applications.\textsuperscript{3} However, both electrodes employ a platinum catalyst and must operate on pure hydrogen.\textsuperscript{5} The PAFC also uses platinum at both electrodes but operates at higher temperatures around 180 °C.\textsuperscript{3} Other fuels besides hydrogen can be used in a phosphoric acid fuel cell.\textsuperscript{5} It can still have an overall efficiency over 80% and is often used in stationary power generators around the world.\textsuperscript{3} Figure 1.1 shows the basic mechanism of both a PEM and PAFC using hydrogen as the fuel source.\textsuperscript{5}

One big problem with each of these fuel cells is the use of platinum at both the cathode and anode. Today platinum can cost up to $214.50 per gram and accounts for only 0.0000037% of the elements in the earth’s crust.\textsuperscript{6,7} Trace impurities in the hydrogen source like hydrogen sulfide and carbon monoxide can also irreversibly erode the platinum electrodes.\textsuperscript{8} The biological counterpart to the dihydrogen fuel cell is the hydrogenase enzyme. These enzymes were found to use less expensive base metals such as iron and nickel to catalyze the redox reactions of H\textsubscript{2}. Unlike their platinum
counterparts, hydrogenase enzymes are able to regain catalytic activity after exposure to carbon monoxide. However, the majority of isolated hydrogenase enzymes come from anaerobic bacteria and are extremely sensitive to oxygen. Air-stable models of enzyme active sites are currently being synthesized to provide an alternative to expensive platinum and anaerobic hydrogenase catalysts currently used in fuel cells.

**Figure 1.1:** Graphic representation of a hydrogen fuel cell.

### III. CLASSES OF HYDROGENASE ENZYMES

Three different types of hydrogenase enzymes have been discovered and classified based on the metal atoms found at their active sites. Both [NiFe]-hydrogenases and [FeFe]-hydrogenases have a binuclear active site while [Fe]-hydrogenases contain a single Fe atom. In every enzyme, each Fe atom is coordinated to at least one carbonyl
ligand. Most metal centers also coordinate to one or more cyanide ligands as well. The hydrogenases with a binuclear center also have Fe-S clusters that transport electrons to and from the active site.

i. [Ni-Fe]-HYDROGENASES

Of all classes, [NiFe]-hydrogenases found in bacteria have been the most studied. Eleven different redox states of the [NiFe]-hydrogenases have been discovered. Some of these have a physiological importance, and the reactivity of the species drives the research of this class of hydrogenases. The first IR spectroscopic studies showed that biologically unusual CO and CN ligands were present at the center. These initial studies also proposed a mononuclear nickel center as the active site. Iron was only considered to be part of the electron transport relay system.

Volbeda and co-workers provided the first X-ray crystal structure of a heterodimeric hydrogenase isolated from Desulfovibrio gigas at a resolution of 2.85 Å. The active site was found to contain a nickel atom as well as a second unidentified metal ion. The enzyme itself forms a globular heterodimer, where the larger α-subunit contains the active site and the smaller β-subunit holds the iron-sulfur clusters. The active site is coordinated to the protein by four cysteine residues. Crystallography has shown that three Fe-S clusters (a [3Fe-4S] cluster
between two [4Fe-4S] clusters) form a tunnel to the center of the enzyme where the active site is buried.\textsuperscript{11}

![Figure 1.2: Active site of [NiFe]-hydrogenase.\textsuperscript{11}](image)

Soon after, Volbeda confirmed the second metal atom to be iron and provided a more detailed description of the active site.\textsuperscript{12} A metal content analysis suggested the presence of 12 iron atoms in the enzyme.\textsuperscript{12} The group confirmed no other significant peaks besides the Fe-S clusters (which contain 11 iron atoms) and the active site, which establishes the second active site metal ion to be iron.\textsuperscript{12} This study also confirmed that the iron atom is coordinated to three buried non-protein ligands.\textsuperscript{12} The higher resolution experiment revealed these must be diatomic molecules, as neither smaller nor larger ligands could be correctly fitted to the electron density map.\textsuperscript{12} Furthermore, a bridging ligand was identified between the metal centers of the inactive form and tentatively assigned to be oxygen, Figure 1.3.\textsuperscript{12}
Figure 1.3: Inactive form of the [NiFe]-hydrogenase. (X=HOO\(^-\) or HO\(^-\)).

At least three redox states are believed to be actively involved in catalysis.\(^9\) Exposure to \(O_2\) dramatically reduces catalytic activity in all hydrogenases but tends to be more reversible for [NiFe]-hydrogenases.\(^9\) Therefore, these have been studied in the most detail, but the location of the \(H_2\) binding site is still uncertain.\(^9,10\) The nickel atom is believed to be the primary dihydrogen binding site because of its proximity to the electron transport channel.\(^10\) However, some authors favor the iron atom as a binding site based on density functional theory calculations and the affinity low spin \(d^6\) metals have for \(H_2\).\(^10\)

ii. [FeFe]-HYDROGENASES

[FeFe]-Hydrogenases are similar to [NiFe]-hydrogenases in that they both have a binuclear active site. The [FeFe]-hydrogenase plays an important role in bacterial energy metabolism by reversibly converting protons and electrons to dihydrogen, but the majority of these enzymes are committed only to proton
Molecular masses of the enzymes can vary from 40 – 130 kDa, and unlike their [NiFe]-hydrogenase counterparts, [FeFe]-hydrogenases are mainly monomeric in the cytoplasm, but are known to be dimeric, trimeric, and tetrameric in the periplasm.

The enzymes X-ray crystal structure was determined independently by two different groups using different organisms in 1998. \textit{Clostridium pasteurianum} (CpI) is an enzyme that catalyzes proton reduction in the cytoplasm. The periplasmic \textit{Desulfovibrio desulfuricans} catalyzes the oxidation of H\textsubscript{2}. For both enzymes the active site is buried within the protein and connected to the surface by a continuous hydrophobic channel. The active site, also called the H-cluster, was found to contain 6 iron atoms arranged in a [4Fe4S] cubane core covalently linked by a cysteiny1 residue to a 2Fe subcluster. The [4Fe4S] core is linked to the protein backbone by three cysteiny1 residues.

![Figure 1.4: Structural representation of the active site of a [FeFe]-hydrogenase enzyme. (X=CH\textsubscript{2}, NH, or O).]
The binuclear iron cluster is set up in a butterfly arrangement with an undetermined dithiolate linker bridging the iron atoms (Figure 1.4).\textsuperscript{10} The metal center is also coordinated to biologically unusual carbonyl and cyanide ligands.\textsuperscript{10} Like [NiFe]-hydrogenases there are also many redox states of [FeFe]-hydrogenases. High concentrations of CO can lead to a completely inhibited oxidized state of the enzyme (H$_{\text{ox}}$-CO state).\textsuperscript{10} The structure of Cpl in the H$_{\text{ox}}$-CO state shows the terminal binding of CO on the distal iron.\textsuperscript{13} This evidence supports the theory that dihydrogen oxidation and reduction occurs at the distal site.\textsuperscript{10,13} One major unknown is the nature of the dithiolate ligand. Currently it remains undetermined if the unknown group is CH$_2$, NH, or O.\textsuperscript{10} High resolution X-ray crystallographic data and density functional theory calculations favor the X=O.\textsuperscript{10}

iii. [Fe]-HYDROGENASES

The third class of hydrogenase enzymes contains only one iron atom at the active site and are found in some methanogenic archaea.\textsuperscript{10} The H$_2$ forming methylene-tetrahydromethanopterin (Hmd) does not contain nickel or iron-sulfur clusters and induced under limited nickel growth conditions.\textsuperscript{10,15} This enzyme performs as an intermediary step in the reduction of carbon dioxide to methane. Hmd is limited to methanogenic archaea and is still not universal in those organisms.\textsuperscript{10} Many other methanogens have both a [NiFe]-hydrogenase (Frh) and a methylene-H$_4$MPT dehydrogenase (Mtd) instead.\textsuperscript{10}
The Hmd enzyme is composed of two identical subunits. During purification of the protein a small amount of iron was detected but was not considered functional since the iron was not redox active. It was first suggested in 1992, by Thauer and co-workers that Hmd was not “metal-free” as initially believed. It was hard to confirm this hypothesis because large quantities of purified Hmd did not become available until recently. Hmd will also lose its catalytic activity in the presence of $O_2$, $Cu^{2+}$, and UV-A (blue) light. Low quantities of the protein and its light sensitivity can help explain why [Fe]-hydrogenases were considered metal free until recently.

A model for the active site was first reported in 2003, and no crystal structure of the native enzyme has been obtained. The composition and structure has been determined using a wide variety of spectroscopic and crystallographic methods. There is still some uncertainty in structure, but the current model suggests that the iron atom is coordinated to two CO ligands, a cysteine sulfur atom, a bidentate pyridine molecule, and a still unidentified ligand (Figure 1.5).

![Figure 1.5: Proposed active site of the [Fe]-hydrogenase.](image)
IV. SYNTHETIC MODELS

The number of active site models has grown exponentially since x-ray crystal structures of hydrogenases were first published. While the [NiFe]-hydrogenase enzymes are more heat and air stable, synthetic models of the [FeFe]-hydrogenases have proven easier to synthesize.\(^\text{18}\) Due to its recent discovery, only a few synthetic models of the [Fe]-hydrogenase currently exist.\(^\text{17}\) That number will continue to grow as more is discovered about the enzyme.

i. SYNTHETIC [NiFe]-HYDROGENASE MODELS

Biochemical studies of the [NiFe]-hydrogenase enzymes exceed the study of [FeFe]-hydrogenases, but chemical models of [NiFe]-hydrogenases are much fewer than their di-iron counterparts.\(^\text{10}\) A large part of the challenge is assembling a molecule with both nickel and iron centers in a single molecule with the proper ligands.\(^\text{10}\) The first synthetic models coordinated nitrogen and sulfur ligands to the nickel atom, which could then allow for sulfur bridging to the iron center, as long as the iron atom was cationically charged or possessed electron withdrawing ligands.\(^\text{19}\) Figure 1.6 is an example of the first synthetic Ni-Fe complexes.
Figure 1.6: [Ni(bme-daco)Fe(CO)₄]; first synthesized by Darensbourg and co-workers.¹⁰,¹⁹ The Ni●●●Fe bond length is longer than the natural system, but the model does have CO ligation around the iron.¹⁰,¹⁹

In parallel with the N,S models, many groups began work on the synthesis of heterometallic complexes using phosphine ligands.¹⁰ The majority of these complexes had a Ni-Fe bond length greater than 3 Å, signifying no metal – metal bonding.¹⁰ However, a few (Figure 1.7) possess a Ni – (μ–S₂) – Fe dihedral angle and Ni-Fe bond length comparable to the enzyme in its reduced form.²⁰

Figure 1.7: {P₂Ni(μ-S)₂Fe}-motifs found to be most similar to the natural enzyme.²⁰
More recent work has focused on assembling complexes with the \( \{S_2\text{Ni(\(\mu\)-S)}_2\text{Fe}\}\)-core. This lead to the synthesis of bridging-dithiolate \(\{\text{NiFe}\}\)-complexes with both CO and CN ligands coordinated to the iron atom.\(^{21}\) Although there is still some controversy over the location of the “extra” carbonyl ligand in the CO-inhibited state, these complexes remain some of the closest analogs for the active site of [NiFe]-hydrogenases in terms of structure.\(^{10}\)

Scientists have also synthesized around 20 \(\{\text{Ni}_x(\mu\text{-S})_y\text{Fe}_z\}\)-complexes that feature three or four metal atoms held together by sulfur ligands.\(^{10}\) Of all [NiFe]-model systems that have been synthesized, only two show any electrocatalytic activity.\(^{10}\) Both of these models are trinuclear species (Figure 1.8), and both catalyze the reduction of protons.\(^{10}\) However, Rakouski and co-workers have reported the synthesis of mononuclear nickel diphosphine systems that will oxidize dihydrogen, and Rauchfuss has developed related \(\{\text{NiRu}\}\)-systems that have been reported to catalyze proton reduction.\(^{10}\)

**Figure 1.8**: Trinuclear \(\{\text{Ni}_x(\mu\text{-S})_y\text{Fe}_z\}\)-complexes. These are the only two synthetic [NiFe]-models reported to show electrocatalytic activity.\(^{10}\)
ii. SYNTHETIC [FE-FE]-HYDROGENASE MODELS

As mentioned above, the x-ray crystal structure of the [FeFe]-hydrogenase enzyme was discovered independently by two different groups using different enzymes in 1998. In the following years the number of active site mimics has increased exponentially due to limited involvement by the protein at the active site and its similarity to a known organometallic complex (Figure 1.9). There has been a notable advance in the synthesis of molecules with a mixed-valent iron center, as well as a better understanding of the hydride chemistry of the active site mimics.

![Figure 1.9](image)

**Figure 1.9:** µ-(1,3-Propanedithiolato)-hexacarbonyldiiron is similar in structure to the [FeFe]-hydrogenase active site.

The synthesis of [Fe₂(CO)₆(μ-SEt)₂] was first published by Reihlen and coworkers in 1929. When the crystal structure of the active site became known in 1999, its resemblance to the molecule synthesized by Reihlen (Figure 1.10a) was readily apparent. In late 1999, it was determined that two carbonyl ligands of µ-(1,3-Propanedithiolato)-hexacarbonyldiiron could be replaced by cyanide to
better model the active site ([Figure 1.10b]). Work by Rauchfuss and Song soon showed that nitrogen and oxygen bridged models were also accessible ([Figure 1.10 c,d]). A wide variety of analogs have since been developed containing a \(\text{\{2Fe2S\}}\)-butterfly core similar to the enzyme.

![Figure 1.10: [FeFe]-hydrogenase active site models.](image)

However, on the functional side, electrocatalytic systems rarely match that of the enzyme subsite. For example, there are very few working models that contain dicyanide ligation. Also, many synthetic models cycle through Fe(I) – Fe(0) oxidation states rather than the Fe(II) – Fe(I) levels like the enzyme, and these systems operate at a considerably more negative potentials than the \(\text{H}^+/\text{H}_2\) pair. There has also been no di-iron systems designed thus far that will oxidize dihydrogen to protons. Today the majority of research involves improving a general active site model, [Figure 1.9], by lowering reduction potentials,
increasing reaction rates, and improving the overall stability of the molecule as a catalyst.

Following the initial discovery and synthesis of the propane dithiolate system, a large fraction of hydrogenase research focused on modifying the bridgehead group or replacing the carbonyl ligands with biological phosphine, isocyanide, nitrosyl, or N-heterocyclic carbene ligands. The thiolates have also been replaced with selenide, phosphide, amide, and peptide groups in a number of syntheses. The goals of this research are to confer additional functionality on the system and provide methods to anchor the system to electrode surfaces and polymers. The immense number of new derivatives has lead to a modification of the redox and catalytic properties of these materials. This work has inadvertently led to the synthesis of stable models with mixed-valent systems and structurally and spectroscopically characterizable hydride intermediates.

As with [NiFe]-hydrogenases, there is more than one redox state of the [FeFe]-enzyme. Most researchers agree on the redox models in Figure 1.11, which have been determined by X-ray crystallography and computational calculations.
Figure 1.11: Select redox states of the [FeFe]-hydrogenase enzyme. Systems were determined by x-ray crystallography and confirmed with FTIR spectra and computational studies.¹⁰, 2⁶, 2⁷

When the catalytically active $H_{ox}$ form is exposed to CO gas, a new inhibited form of the active site results named $H_{ox}$–CO.²⁶ Both of these systems are unique in that they contain a mixed-valent di-iron center. These mixed-valent species are unstable, and a lot of research has gone into the synthesis of a stable di-iron unit with a bridging CO ligand.¹⁰ This synthesis was first achieve in diferrous, iron(II), systems with electron donating isocyanide, phosphine, or cyanide ligands.¹⁰ Some examples are shown in Figure 1.12. Soon after, the synthesis of mixed-valent Fe(I) – Fe(II) systems was reported by a number of different groups using monophosphine, diphosphine, and carbene ligands, Figure 1.13.¹⁰ These models exhibit a vacant coordination sphere on the distal iron that is also observed in the $H_{ox}$ form of the enzyme.¹⁰ The bulky carbene and phosphine ligands allow for the isolation and crystallization of these synthetic complexes.¹⁰
Other groups have focused on modeling a hydrogenase active site that includes the \{4Fe4S\}-cubic cluster. In 2004, Li-Cheng Song and co-workers were the first to report the synthesis of a \[3Fe3S\]-structure, where an iron atom of the \(\text{Fe}_2\text{S}_2\) butterfly cluster is ligated to the sulfur atom of a metallo-thioester.\(^{28}\) This paved the way for the synthesis of models with a \(\{4\text{Fe}4\text{s}\}\)-cluster that act as an electrocatalyst for proton reduction.\(^{29}\) These analogs, developed by Chris Pickett and co-workers, have a reduction potential between \(-0.59\) V and \(-1.09\) V vs a saturated calomel electrode (SCE) that depends on the substitution of the cubane cluster.\(^\text{10, 29}\)
The formation of hydride intermediates is inherent to the catalytic cycle of the [FeFe]-hydrogenase enzyme. The vacant coordination site in the $H_{ox}$ state is generally accepted to be the place where a proton or $H_2$ interacts with the active site; however, there are other binding sites that could be considered.\textsuperscript{10} The first hydride complexes of the di-iron dithiolate systems were characterized in 1976, long before their role in hydrogenase chemistry was recognized.\textsuperscript{30} All of these initial studies contained a bridging hydride, (Figure 1.14a).\textsuperscript{10,30} It was not until 2007, that the first terminal hydride was reported and confirmed using $^1H$ and $^{13}C$ NMR experiments (Figure 1.14b).\textsuperscript{31}

Barton and Rauchfuss expanded on the study of terminal hydrides to include azadithiolate diiron complexes (Figure 1.14c).\textsuperscript{32} They discovered that the stability of the terminal hydride depends on the basicity of the Fe-Fe center, as well as the steric size of the dithiolate model.\textsuperscript{32} Cyclic voltammetric (CV) studies showed that terminal hydrides reduce at 200 mV milder than the isometric bridging hydride that was also considered as a possible mechanism.\textsuperscript{32} The terminal hydrides slowly isomerize to the more thermodynamically state bridging hydride.\textsuperscript{32,33} The discovery that diiron dithiolates initially protonate to give terminal hydrides opens the door to a new phase determining the role of the dithiolate cofactor in the catalysis.\textsuperscript{32}
Figure 1.14: Synthetic hydride models. a) bridging hydride.\textsuperscript{30} b and c) terminal hydrides.\textsuperscript{31,32}

Of the large number of synthetic di-iron bridging hydride systems known to date, many of them are capable of electrocatalytically reducing protons to form dihydrogen.\textsuperscript{10} Terminal hydride systems are rare, but significant advances have been made in the last few years.\textsuperscript{10} Rauchfuss and co-workers recently reported the synthesis of ruthenium analogs of the di-iron system.\textsuperscript{34} Their work on Ru$_2$(S$_2$C$_3$H$_6$)(CO)$_4$(PCy$_3$)$_2$ (Figure 1.15) and its analogs has produced a system that will reduce protons through a terminal hydride.\textsuperscript{34} The same system will oxidatively add H$_2$ to form a rare dihydrido complex.\textsuperscript{34} The chemistry of active site models with terminal and bridging hydrides will continue to expand.

Figure 1.15: Ruthenium based active site model that gives a bridging/terminal hydride product.\textsuperscript{34}
While it is important to create a more accurate model of the active site, understanding the electrochemistry of the system is crucial to design better systems for catalysis. Several detailed electrochemical studies have been conducted on di-iron dithiolate models to determine the electron transfer chemistry of the system. Tard and Pickett summarized the reduction potentials for a wide variety of active site models in a 2009 review. Replacing the CO ligands on the µ-(1,3-Propanedithiolato)-hexacarbonyldiiron base unit (Figure 1.9) leads to a more negative reduction potential. However, increasing the electron donating ability of the ligands can shift the reduction potential to more positive values because protonation at the iron – iron bond increases. All models synthesized to date require much harsher condition than their enzyme counterparts. These synthetic molecules either require a strong acid and a moderate overpotential or a weak acid and an even more negative reduction potential to produce H₂. A large part of research today focuses finding the most stable synthetic model that also has a low reduction potential.

Another large area of research uses density functional theory (DFT) calculations to learn more about enzyme structure. Computational chemistry has been used to verify X-ray crystal structures of enzymes, determine oxidation states the active site, and explore the electron transfer mechanism in the catalytic cycle. There are also many groups that use DFT calculations to
determine the best molecular structure for active site models. It is much more efficient to first discover what ligands, oxidation states, or modifications to the dithiolate linker will provide the most stable molecules before attempting a total synthesis.

During the last decade, a lot of progresses have been made on the synthetic models of the [FeFe]-hydrogenase active sites. A large number of structural analogs have been characterized, and advances have been made on mixed-valent systems and hydride chemistry of the catalyst. There is still an incomplete picture of how then enzyme works, and scientists disagree about the molecular composition of the bridging dithiolate unit and the location of the hydride ligand. Currently, no active site models exist that reduce H⁺ to dihydrogen at low overpotentials, and synthetic models that oxidize H₂ are notably absent.

iii. SYNTHETIC [Fe]-HYDROGENASE MODELS

The chemistry of synthetic [Fe]-hydrogenases is just beginning to grow. The enzyme was not discovered until 2004, and a model of the active site did not become available until 2008. This hydrogenase is unique because the metal center is not redox active, and it will only catalyze the oxidation of H₂ in the presence of its substrate, N⁵,N¹⁰-methenyltetrahydromethanopterin, under nickel limiting conditions. The active site is bound to the backbone through one
guanyl nucleotide, featuring limited protein involvement and making it easier to model.36

Synthesis of active site models began before the crystal structure was reported, using spectroscopic data to recreate parts of the molecule.35 Rauchfuss and co-workers synthesized the first model using a pyridinol group, replacing the iron center with a ruthenium atom (Figure 1.16a).36 A study done by Rauchfuss also showed that donor-functionalized thioesters were oxidatively added to Fe(0), making functional models of the [Fe]-hydrogenase a realistic future goal.37

Soon after the discovery of the enzyme crystal structure only a few iron pyridone complexes had been reported and none featured one pyridone ligand coordinated to one iron atom.38 The following year the first mononuclear iron bis(carbonyl) pyridonate complex was reported (Figure 1.16b).38 This model suggested a 2+ oxidation state for the iron atom and is often used as a precursor to current models.38

Figure 1.16: Some of the first synthetic active site models featuring a pyridinol ligand.36,38
The number of [Fe]-hydrogenase models continues to grow as more new compounds and structural analogs are rapidly synthesized. Some questions still remain concerning the structure of the enzyme, but an increase in model complexes will help provide answers about enzyme structure and function. Research groups are just beginning to explore the reaction mechanism of the enzyme. Recently, Stiebritz and Reihner have even suggested a link between the reaction mechanisms of the [Fe]- and [FeFe]-hydrogenases.
Chapter 2

Synthesis of [FeFe]-Hydrogenase Active Site Mimics with Bridged Sulfur Atoms

I. Introduction
II. General Experimental
III. Synthesis of Cyclic Disulfides
IV. Synthesis of Methanedithiol Precursors
V. Synthesis of Diiron-dithiolato Complexes
VI. Results and Conclusions
VII. Data
I. INTRODUCTION

Of all the classes, [FeFe]-hydrogenases have proven to be the easiest to model. The active site contains two metal atoms of the same species, and is very similar in structure to a previously known organometallic complex. This simple molecule, \( \mu-(1,3\text{-propane dithiolato)hexasacarbonyl} \text{diiron} \), can be modified a number of ways with the hope of creating a better catalyst. Using this model as a starting point it is possible to: substitute one or many carbonyl ligands with different species, change the group bridging the sulfur atoms by modifying the length of the linker, inserting heteroatoms, or exploring a linked vs. non-linked motif, or change the oxidation states of the metal centers.

The research presented here explores the synthesis of active site models with a varying linker length \([\text{Fe}_2\text{SC}_x\text{H}_{2x}\text{S(CO)}_6]\), where the number of bridging carbon atoms, \(x\) =1, 2, 3, 4, 5. Models with a bridge having two and three carbons have been synthesized in relatively high yields using the representative dithiol and triirondodecacarbonyl. Repeating the procedure using a larger dithiol resulted in a thick, brown oil that was insoluble in most organic solvents, including hexanes. To solve this problem, the dithol
was first oxidized to a cyclic disulfide and then refluxed with triirondodecacarbonyl to afford the desired product.

II. GENERAL EXPERIMENTAL

MATERIALS

All reagents were obtained from commercial sources and used as received. The benzene and toluene used as solvents in an inert atmosphere were dried by distillation. All products were chromatographed on a 40 cm x 2 cm column using Merck grade 9385, 230 – 400 mesh silica gel, 60 Å.

INSTRUMENTATION

All NMR spectra were collected using either a JEOL NMR 300 MHz or 400 MHz Multinuclear NMR spectrometer. $^1$H chemical shifts in CDCl$_3$ are reported in ppm downfield from trimethylsilane. $^1$H chemical shifts in benzene are reported relative to the solvent C$_6$D$_6$ (7.16ppm), and $^{13}$C chemical shifts are reported relative to CDCl$_3$ (77.00 ppm) or benzene (128.3 ppm). Infrared spectra were obtained with a Perkin Elmer Spectrum 100 FT-IR spectrometer using a NaCl sample cell. Gas Chromatogram – Mass Spectroscopy (GC-MS) data was analyzed using an Agilent Technologies 7890A gas chromatography system with Agilent Technologies 5975C inert XL EI/CI MSD detector. The gas chromatography column used was an Agilent 190915-433 column, 30 m x 0.25 mm, 0.25-μm film thickness. 200 °C injection block, 0.949 mL/min carrier gas flow rate.
The oven temperature profile was as follows: start at 50 °C, hold for 2 min, ramp to 260 °C at 20 °C/min.

III. SYNTHESIS OF CYCLIC DISULFIDES

Disulfide bonds play an important part in biochemistry, thus many studies have been done concerning the thiol-disulfide interchange. The reaction mechanism is relatively simple, involving three reversible steps (Scheme 2.1). The nucleophilic attack in the second step follows a normal $S_N2$ pathway that occurs along the sulfur-sulfur bond axis. However, oxidations of $\alpha,\omega$-dithiols can lead to a mixture of products including cyclic monomeric disulfides, cyclic dimeric bis(disulfides), and oligomeric disulfides. Cyclic monomeric disulfides are the major product when the thiol groups are separated by three to six atoms. Larger rings are unfavorable, as the angle and torsional strain on the molecule increases. An increase in ring strain also makes small cyclic disulfides unfavorable.

The most stable cyclic disulfides should contain a strain-free six-membered ring. A kinetics study by Houk and Whitesides supports this hypothesis, but even some of these compounds are only marginally stable depending on their substituents. Larger rings can still be synthesized in dilute solutions (<0.01 M), but they are only slightly more stable than their corresponding polymers. A competing intermolecular reaction leads to polymerization in larger chains.
Scheme 2.1: Reversible thiol-disulfide interchange reaction. An oxidation of dithiols to disulfides using molecular bromine and silica gel was used to synthesize the desired products (Scheme 2.2). This procedure was employed to avoid any side reactions from hydrogen bromide created in solution. It is proposed that the silica gel removes any hydrogen bromide formed in the reaction, making it unavailable for side reactions. It is also postulated that the large surface area created by the silica gel acts to disperse the dithiol reactant and prevent any intermolecular polymerization reactions. A few small modifications were made to the procedure to increase overall yields and prevent polymerization. Before the addition of bromine, the dithiol was allowed to stir and sonicate in solution, giving the molecules time to adhere to the silica gel. The product was also extracted with a sodium thiosulfate solution to scavenge any excess Br$_2$, and a sodium hydroxide solution as a precaution against hydrogen bromide.
Based on GCMS data, this reaction affords the desired cyclic disulfide in very high yield. However, evaporation of the solvent always polymerized the product. Many attempts were made to prevent the polymerization reaction and achieve a solid product. In the end, the cyclic disulfide must be left in solution. The solvent can easily be exchanged for another with a higher vapor pressure to prepare for the next step.

**PREPARATION OF 1,2-DITHIANE \([C_4H_8S_2] \) (1)**\(^{42,43}\)

The following procedure was modified from work Ali and co-workers and Ananikov and co-workers.\(^{42,43}\) Water (5.0 mL) was added dropwise to 10.0 g of flash chromatography grade silica gel while stirring in a 100 mL round-bottomed flask. When no clumps remained, 50 mL dichloromethane was added to the flask followed by a solution of 0.465 mL 1,4-butanedithiol (0.489 g, 4.0 mmol) in 5 mL dichloromethane. This solution was allowed to stir for 1 hour and sonicate for 30 minutes. Bromine (0.703 g, 4.4 mmol) was mixed with 4 mL of dichloromethane. This solution was added slowly through a septum with a pipet. When the solution turned a light yellow color that persisted for more than 10 minutes, addition of the bromine ceased and the reaction
mixture was vacuum filtered through a fritted funnel. The solid residue was washed with dichloromethane (30 mL). The reaction mixture was transferred to a 250 mL separatory funnel and extracted with an aqueous solution of sodium thiosulfate (1.5 g in 25 mL H$_2$O, rinsed with 2x50 mL H$_2$O) to remove any excess bromine. The organic layer was also extracted with an aqueous NaOH solution (1.2 g in 24 mL H$_2$O, rinsed with 2x50 mL H$_2$O) to neutralize the HBr in solution and dried with anhydrous Na$_2$SO$_4$. The solvent was evaporated using a rotary evaporator. Before all of the dichloromethane could be removed, toluene (11.8 mL) was added to prevent polymerization of the cyclic disulfide. The remaining dichloromethane was removed leaving the 1,2-dithiane in toluene. Argon was bubbled through the solution for 1 hour to remove the oxygen.

**PREPARATION OF 1,2-DITHIEPANE [C$_4$H$_8$S$_2$] (2)\textsuperscript{42,43}**

The synthesis of 1,2-dithiane was repeated using 0.537 mL of 1,5-pentanedi(thiol (0.545 g, 4.0 mmol) as the starting material. Again, to prevent polymerization of the disulfide, the product was left in solution. Dichloromethane was exchanged with toluene and degassed using argon for 1 hour to remove the oxygen.

**IV. SYNTHESIS OF METHANEDITHIOL PRECURSORS**

While a cyclic disulfide can be used as an intermediate step in the synthesis of hydrogenase mimics with a bridgehead group of four or five carbons, a single carbon linker presents a greater challenge. There is too much ring strain to create a cyclic
molecule with only three members, and methanedithiol is not commercially available. This research focuses on synthesizing methanedithiol and its precursors.

The synthesis of methylene bis(thiocyanate) \((3)\) from dibromomethane and potassium thiocyanate was modified from the original procedure. The solution was heated to reflux instead of undergoing a microwave-assisted reaction\(^{44}\) This product was used in the synthesis of diiron-dithiolato complexes discussed in the next section. The remaining complexes discussed below were used as precursors to the target molecule, methanedithiol.

**PREPARATION OF METHYLENE BIS(THIOCYANATE) \([\text{CH}_2\{\text{SCN}\}_2]\) (3)\(^{44}\)**

Dibromomethane (6.953 g, 40 mmol) and potassium thiocyanate (5.053 g, 52 mmol) were added to a 250 mL round-bottomed flask with 100 mL water. The solution was heated at reflux for 26 hours then cooled to room temperature. Any yellow solid was removed and discarded through gravity filtration. The reaction mixture was extracted with dichloromethane (3x25 mL) and dried with \(\text{Na}_2\text{SO}_4\). After rotary evaporation of the solvent, 1.028 g white crystals remained (20%).

**PREPARATION OF DITHIOACETYL METHANE (4)\(^{45}\)**

A solution of 3.552 g of potassium hydroxide (58.90 mmol) in 50 mL of absolute ethanol was refluxed under argon for 1 hour in a 100 mL three-neck round bottom flask. The solution was cooled to room temperature and 4.4 mL of thioacetic acid (62.79 mmol) was added through the septum and allowed to stir at room temperature for 20
minutes. The dark yellow solution was cooled in an ice bath and 2.09 mL of dibromomethane (5.22 g, 30 mmol) was added slowly. After the addition was completed, the reaction mixture was heated to reflux for 1 hour to form the red-brown dithioacetylmethane. The flask is opened to the vacuum line to remove the solvent, followed by the addition of dichloromethane (50-60 mL). The solid potassium bromide was filtered out and washed with dichloromethane until the wash was clear. The solvent was removed with a rotary evaporator yielding 4.589 g of crude product. A mixture of 10% ethyl acetate and 90% hexanes was used to separate the crude sample on a silica column. The second fraction was found to contain the pure product, as checked by $^1$H NMR spectroscopy and GCMS. The solvent was removed to yield 2.6805 g dithioacetylmethane (54% yield).

**PREPARATION OF METHYLENE DIISOTHIURONIUM BROMIDE (5)**

Thiourea (6.091 g, 80 mmol) and 27.5 mL of 95% ethanol were placed in a 50 mL round-bottomed flask and heated to reflux temperature. The heat was turned off, and dibromomethane (7.515 g, 40 mmol) was added to the flask. No reaction was observed, but the solution was allowed to reflux for 30 minutes after the addition of dibromomethane. The solution was cooled to room temperature to yield white crystals that were gravity filtered. The filtrate was placed in an ice bath for 15 minutes that produced more crystals. The second set of crystals were filtered and combined with the first to yield the final product (3.162 g, 48% yield).
PREPARATION OF SODIUM S-METHYLENEDIITHIOSULFATE (BUNTE SALT) (6)\textsuperscript{47}

Dibromomethane (3.476 g, 20 mmol) was added to a 100 mL round-bottomed flask containing 24 mL methanol. Approximately 20 mL of water was added dropwise to the reaction mixture until a slight turbidity developed. The reaction flask was fitted with a condenser and brought to reflux. Sodium thiosulfate pentahydrate (12.409 g, 50 mmol) was dissolved in 10 mL of water and the resulting solution was slowly added to the reaction flask through the reflux condenser. This light yellow solution continued to heat at reflux for 3.5 hours. After cooling to room temperature, the methanol was evaporated using a rotary evaporator leaving a bright yellow precipitate in an aqueous solution. The remaining solution was diluted to 25 mL and extracted with 2 x 25 mL aliquots hexanes. The presence of a bunte salt (5) was confirmed by GCMS, and stored in the aqueous layer in the freezer.

V. SYNTHESIS OF DIIRON-DITHIOLATO COMPLEXES

The synthesis of dinuclear hexacarbonyls had been known many years prior to the discovery of the hydrogenase enzyme. A number of different methods exist to create these compounds. The models with a four or five carbon linker were synthesized by minor variations of literature methods.\textsuperscript{48} An equimolar amount of triirondodecacarbonyl and the appropriate cyclic disulfide were heated in toluene below reflux temperature. The reaction was stopped after it stopped bubbling and a color
change was observed. A similar procedure was used for the attempted preparation of (methanedithiolato)hexacarbonyldiiron using a thiocyanate starting product. However, this reaction was carried out at reflux temperature for a shorter period of time.

**PREPARATION OF (1,4-BUTANEDITHIOLATO)HEXACARBONYLDIIRON (7)**

Under an argon atmosphere, triirondodecacarbonyl (2.017 g, 4.0 mmol) was added to an oven-dried Schlenk flask equipped with a magnetic stir bar and sealed with a rubber septum. The flask was connected to a Schlenk line, and the 1,2-dithiane solution was transferred through a cannula to the reaction flask. This solution was heated in an oil bath for 11 hours at 90 °C. During this time, the solution changed to a dark red color and stopped bubbling. After allowing the reaction mixture to cool to room temperature, it was vacuum filtered through Celite and the toluene was removed using a rotary evaporator. The resulting product was an air-stable red-brown oil. A small amount of silica gel was added to the sample along with minimal dichloromethane. After evaporation of the solvent the dry sample was column chromatographed using hexanes. The first red-orange fraction was collected and the solvent evaporated to yield 0.268 g of dark red crystals. These were recrystallized in hexanes to obtain the final product (0.181 g, 11%).
PREPARATION OF (1,5-PENTANEDITHIOLATO)HEXACARBONYLDIIRON (8) \(^{48}\)

Under argon, triirondodecacarbonyl (2.017 g, 4.0 mmol) was added to a oven-dried Schlenk flask containing a magnetic stir bar and sealed with a rubber septum. The flask was connected to a Schlenk line fitted with a reflux condenser, and the 1,2-dithiepane solution was transferred to the reaction flask through a cannula. The dark green solution was heated in an oil bath at 80 °C for 34 hours. The flask was cooled to room temperature once the color changed to a dark red and stopped bubbling. The resulting solution was vacuum filtered through Celite, and the toluene was removed with a rotary evaporator. The resulting crude product was column chromatographed using hexanes. The first red fraction was collected, and the solvent was evaporated to yield 0.274 g of dark red crystals. These were recrystallized in pentane to yield 0.162 g (10%).

ATTEMPTED PREPARATION OF (METHANEDITHIOLATO)HEXACARBONYLDIIRON (9) \(^{49}\)

Triirondodecacarbonyl (0.141 g, 0.28 mmol) and methylene bis(thiocyanate) (0.037 g, 0.28 mmol) were added to 25 mL dry toluene in a Schlenk flask under an argon atmosphere. The solution was heated to reflux for 1 hour and cooled to room temperature. Vacuum filtration through Celite and removal of the solvent using a rotary evaporator affords 0.0349 g of a dark brown oil. The crude product was column chromatographed using hexanes. Two bands were collected. IR analysis of both showed a mixture of different products.
VI. RESULTS AND CONCLUSIONS

This research presents two synthetic models for the [FeFe]-hydrogenase active site, and the attempted synthesis of a third. As mentioned, models that have a dithiolate bridge linker with two or three carbons are relatively easy to synthesize. Modifying the procedure to include a cyclic disulfide (1, 2) as the starting material has afforded the synthesis of bridging linkers with four (7) and five (8) carbon atoms.

Progress was also made towards a bridging dithiolate linker with one carbon atom. Products 3-6 were successfully synthesized with the goal to remove the end groups and protonate the remaining thiolate ion, resulting in methanedithiol. A number of different methods50-54 were used to attempt this final step, but none were successful. The thiocyanate compound (3) was also used as a reactant in the synthesis of a diiron-dithiolate complex with the hope of creating a one carbon bridging link (9). There is a chance this synthesis was successful, but the method of chromatography used was unable to separate the different products for analysis.
In the synthesis of both 7 and 8, a second orange fraction was collected during chromatography. IR analysis of the second fraction reveals a different complex which is not the desired product. After evaporating the solvent, the second fraction is a red oil. Many attempts have been made to crystallize that fraction to determine its x-ray crystal
structure, but none have yet to be successful. In 2006, Song and co-workers presented a study of metallocrown ethers with a structure similar to the models presented here.\textsuperscript{54} They discovered that the reaction of the ether-containing dithiol, HSCH\textsubscript{2}CH\textsubscript{2}OCH\textsubscript{2}CH\textsubscript{2}SH with Fe\textsubscript{3}(CO)\textsubscript{12} proceeded through a coordinatively unsaturated diiron intermediate that will either undergo an \textit{intra}molecular cyclization or an \textit{inter}molecular dimerization to yield two different products (\textbf{Scheme 2.3}).\textsuperscript{54} It is hypothesized that a similar mechanism occurs in this study, and that the second fraction is actually a dimer containing two Fe\textsubscript{2}S\textsubscript{2} butterfly cluster cores. A detailed structure of this dimer is shown below in \textbf{Figure 2.3}. 

\textbf{Figure 2.2}: Structure of synthesized active site mimics.
Scheme 2.3: Possible pathway for the formation of diiron-dithiolate complexes.\textsuperscript{54}
This study explores the synthesis of [FeFe]-hydrogenase active site mimics with a varying carbon chain linker. The synthesis of models with four and five carbon linkers (7, 8) were successfully characterized by IR, NMR, and x-ray crystallography. The synthesis of (methanedithiolato)hexacarbonyldiiron and a methanedithiol precursor was also studied. However, in order to act as a replacement catalyst for platinum, these molecules must exhibit a low over-potential for proton reduction. Future electrochemical studies must be conducted to determine the catalytic activity of these models.
VII. DATA

**1,2-dithiane (1)**

The presence of 1,2-dithiane was confirmed by GC-MS. An exact yield was not determined since removal of the solvent led to polymerization of the product. GC-MS: 6.2 min, m/z = 120.0.

**1,2-dithiepane (2)**

The presence of 1,2-dithepane was confirmed by GC-MS. An exact yield was not determined to prevent polymerization of the product. GC-MS: 5.9 min, m/z = 134.0.

**Methylene bis(thiocyanate) (3)**

The representative procedure followed to scale yielded 1.028 g (20%) of white crystals. $^1$H NMR (CDCl$_3$ – 400 MHz) δ 4.40 (s, 2H). $^{13}$C NMR (CDCl$_3$ – 100 MHz) δ 108.6, 38.0. GC-MS: 7.1 min, m/z = 130.0.
Dithioacetylmethane (4)

The representative procedure followed to scale using thioacetic acid (4.4 mL, 62.79 mmol) and dibromomethane (5.22 g, 30 mmol) yielded 2.6805 g dark brown solid (54%). $^1$H NMR (CDCl$_3$ – 400 MHz) δ 4.13 (s, 2H), 2.24 (s, 6H). GC-MS (50 °C): 7.17 min, m/z = 164.0

Sodium S-methylidithiosulfate (Bunte Salt) (6)

The presence of the bunte salt (6) was confirmed by GC-MS, along with other products. This molecule was never isolated, but kept in solution to attempt the removal of the thiosulfate group to form methanedithiol. GC-MS: 9.9 min, m/z = 187.9.

(1,4-butanedithiolato)hexacarbonyldiiron (7)

The representative procedure followed to scale using triirondodecacarbonyl (2.017 g, 4.0 mmol) and 1,2-dithiane yielded a red solid (0.181 g, 11%); mp 104-106 °C. IR (ν CO – Hexanes): 2075, 2036, 2007, 1992, 1982 cm$^{-1}$. $^1$H NMR (C$_6$D$_6$ – 400 MHz) δ 1.83 – 1.80 (m, 4H), 0.91 – 0.89 (m, 4H). $^{13}$C NMR (C$_6$D$_6$ – 100 MHz) δ 207.9, 32.9, 25.3.
(1,5-pentanedithiolato)hexacarbonyldiiron (8)

The representative procedure followed to scale using triirondodecacarbonyl (2.017 g, 4.0 mmol) and 1,2-dithiepane yielded a dark red solid (0.162 g, 10%); mp 98 – 101 °C. IR (ν CO – Hexanes): 2075, 2036, 2007, 1991, 1982 cm\(^{-1}\). \(^1\)H NMR (\(\text{C}_6\text{D}_6\) – 400 MHz) δ 2.02 – 1.99 (m, 4H), 1.19 – 1.13 (m, 4H), 0.90 – 0.84 (m, 2H). \(^{13}\)C NMR (\(\text{C}_6\text{D}_6\) – 100 MHz) δ 207.8, 29.6, 28.8, 27.8

(Methanedithiolato)hexacarbonyldiiron (9)

The representative procedure followed to scale using triirondodecacarbonyl (0.141 g, 0.28 mmol) and methylene bis(thiocyanate) (8) (0.037 g, 0.28 mmol) yielded a red-brown oil. IR (ν CO – Hexanes): 2079, 2062, 2044, 2040, 2024, 2008, 1999.
Chapter 3

Preliminary Kinetics Study of [FeFe]-Hydrogenase Active Site Mimics

I. Introduction
II. General Experimental
III. Synthesis of Unlinked Models
IV. Solvent Study
V. Results and Conclusions
VI. Data
I. INTRODUCTION

The chemistry of stable organosulfur derivatives of metal carbonyls dates back to the synthesis of [Fe(CO)\(_3\)SEt]\(_2\) by Reihlen and co-workers in 1929.\(^{23}\) Since its discovery, compounds of the general formula [Fe(CO)\(_3\)SR]\(_2\) have been isolated from the reaction of triirondodecabarbonyl with dialkyl sulfides, alkyl mercaptans, and dialkyl disulfides by many different groups.\(^{55}-57\) These compounds differ from the models in the previous chapter by their bridgehead group. The sulfur atoms in both 7 and 8 are connected by an alkyl chain, while the complexes discussed here feature an unlinked motif. These unlinked R-groups are free to rotate, which can produce stereoisomers that depend on the relative orientation of their R-groups.\(^{58}\)

In 1964, R. B. King isolated and characterized the two isomer products of the reaction of triirondodecaborbonyl with dimethyl disulfide.\(^{57}\) Based on \(^1\)H NMR data, he determined that the two methyl groups in isomer A were in different positions, while isomer B had two identical methyl groups (see Figure 3.1).\(^{57}\) However, he was unable to distinguish the exact location of the R-groups.\(^{57}\) Future computational studies would show isomer A has the lowest energy conformation with the R-groups in an “anti” position.\(^{59}\) Isomer B is most likely “syn-down”, which is lower in energy than the “syn-
up” conformation. Figure 3.1 shows isomers A and B in the anti and syn-down conformation, respectively.

![Figure 3.1: Isomer A of [Fe(CO)_3SCH_3]_2 in the anti-conformation and isomer B in the syn-down conformation.](image)

While a lot of research has gone into the synthesis of these unlinked compounds, the kinetics of the isomerization reaction remains largely unexplored. Presented here is the synthesis of a variety of [Fe(CO)_3SR]_2 complexes with different alkyl groups as well as a preliminary look into the kinetics of isomerization.

II. GENERAL EXPERIMENTAL

MATERIALS

All reagents were obtained from commercial sources and used as received. Benzene used in anaerobic reactions was dried by distillation and stored in a dry box. All products were chromatographed on a 40 cm x 2 cm column using Merck grade 9385,
230 – 400 mesh silica gel or \( \text{Al}_2\text{O}_3 \) as the stationary phase. Reagent grade pentane or hexanes was used for the mobile phase.

**INSTRUMENTATION**

All NMR spectra were collected using either a JEOL NMR 300 MHZ or JEOL NMR 400 MHz Multinuclear NMR spectrometer. All \(^1\text{H}\) and \(^{13}\text{C}\) chemical shifts are reported relative to \( \text{C}_6\text{D}_6 \) (7.16 ppm for \(^1\text{H}\) and 128.3 ppm for \(^{13}\text{C}\)). Infrared spectra were obtained with a Perkin Elmer Spectrum 100 FT-IR spectrometer using a NaCl liquid sample cell.

**III. SYNTHESIS OF UNLINKED MODELS**

Compounds 10 – 13 were synthesized from the appropriate disulfide and triirondodecacarboxylic. Model 14 used two equivalents of cyclohexyl mercaptan and triirondodecacarbonyl. Each reaction produced red-orange crystals that were column chromatographed to remove an excess ligand and collected in one large fraction. All products were analyzed using \(^1\text{H}\) NMR and infrared spectroscopies.

**PREPARATION OF \([\text{Fe(CO)}_3\text{SCH}_3]_2\) (10)**

An oven-dried Schlenk flask was charged with triirondodecacarbonyl (3.2 g, 6.36 mmol) and 20 mL benzene under an argon atmosphere. The flask was connected to the Schlenk line and 0.705 mL dimethyl disulfide (0.748 g, 7.95 mmol) was injected through the septum with a syringe. A reflux condenser was connected to the reaction flask and
the solution heated to reflux in an oil bath for 10 hours. After allowing the reaction mixture to cool to room temperature, it was filtered through Celite and the solvent was evaporated using a rotary evaporator. The crude product yielded 2.418 g of dark red solid. This product was dissolved in minimal hexanes and columned on a Al₂O₃ column. Three distinct bands were collected and found to contain both isomers. These were recombined to yield 2.164 g red-orange crystals (91% yield) of compound 10.

**PREPARATION OF [Fe(CO)₃SCH₂CH₃]₂ (11)**

Triirondodecacarbonyl (3.2 g, 6.36 mmol) and 20 mL of dry benzene were added under an argon atmosphere to an oven-dried Schlenk flask with a magnetic stir bar. Diethyl disulfide (0.987 mL, 0.972 g, 7.95 mmol) was added through the septum with a syringe. The solution was heated at reflux for 10 hours until the color changed to a dark red. Once the solution cooled to room temperature, it was filtered through Celite and the solvent evaporated with a rotary evaporator. The crude product (2.637 g) was dissolved in minimal pentane and columned chromatographed with silica gel. One large red band was collected and the solvent evaporated with a rotary evaporator. To ensure the product was dry, it was connected to the vacuum line for 6 hours to remove any excess pentane. Dark red crystals (2.432 g, 95%) were collected and both isomers were confirmed by ¹H NMR data analysis.

**PREPARATION OF Fe(CO)₃SCH(CH₃)₂]₂ (12)**
Under an argon atmosphere, 3.2 g triirondodecacarbonyl (6.36 mmol) was added to a Schlenk flask charged with 20 mL benzene. This was connected to a Schlenk line, and 1.27 mL isopropyldisulfide (1.12 g, 7.95 mmol) was added through the septum with a syringe. A reflux condenser was connected and the reaction mixture was heated at reflux for 10 hours. The solution was cooled to room temperature, vacuum filtered through Celite, and the solvent was evaporated with a rotary evaporator. The crude product (3.774 g) was dissolved in minimum pentane and column chromatographed in silica gel. One large red band was collected and the solvent was evaporated to yield 2.609 g (95%) of red-orange crystals. The sample was connected to the vacuum line to remove any excess solvent. The presence of both isomers was confirmed by $^1$H NMR data analysis.

PREPARATION OF Fe(CO)$_3$SC(CH$_3$)$_3$ (13) $^{40}$

Triirondodecacarbonyl (3.2 g, 6.36 mmol) and 20 mL dry benzene were added to a Schlenk flask under an argon atmosphere. Tert-butyldisulfide (1.418 g, 7.95 mmol) was added to the Schlenk flask through the septum with a syringe. A reflux condenser was attached, and the reaction mixture was heated at reflux for 10 hours. After cooling to room temperature, the red solution was vacuum filtered through Celite and the solvent was evaporated using a rotary evaporator leaving a dark red oil that crystallized to yield 2.971 g of crude product. The solid was dissolved in minimal hexanes and column chromatographed using silica gel. One band was collected and afforded 2.426 g (83%) of red crystals after the solvent was evaporated.
**PREPARATION OF $[\text{Fe(CO)}_3\text{SC}_6\text{H}_{11}]_2$ (14)**\(^{40}\)

Triirondodecacarbonyl (3.2 g, 6.36 mmol) and 20 mL benzene were added to a Schlenk flask under an argon atmosphere. The flask was connected to the Schlenk line, and 1.949 mL cyclohexylmercaptan (1.848 g, 15.9 mmol) was added to the flask through a septum with a syringe. A reflux condenser was attached and the solution heated at reflux for 4 hours. After cooling to room temperature, the reaction mixture was vacuumed filtered through Celite and the solvent was evaporated using the rotary evaporator yielding 3.765 g of a thick red oil. The resulting product was dissolved in minimal hexanes and columned chromatographed using silica gel. One large red band was collected, and the solvent was evaporated to yield 2.370 g (73\%) of red crystals.

**IV: SOLVENT STUDY**

In order to study the kinetics of the isomerization reaction using \(^1\)H NMR a suitable solvent must be determined. A number of different solvents were tested including CDCl\(_3\), CD\(_2\)Cl\(_2\), C\(_6\)D\(_6\), D\(_2\)O, DMSO-d\(_6\), and toluene-d\(_8\). Deuterated chloroform, benzene and toluene were determined to have the highest solubility, as well as the best separation of chemical shifts. However, CDCl\(_3\) exhibits a water peak that overlaps sample peaks.\(^{60}\) A residual solvent peak from toluene-d\(_8\) also appears far enough upfield to infer with the sample.\(^{61}\) Therefore, it was determined that C\(_6\)D\(_6\) was the best solvent to use in this analysis.
V: RESULTS AND CONCLUSIONS

The synthesis of organosulfur derivatives of iron carbonyls has been attempted by many different groups. The procedure published by Rauchfuss and Mack in 2010, was employed for the kinetics study presented here. It afforded high yields as well as a pure sample product for derivatives 10 – 14. An initial column was done for each crude product to remove any unreacted iron and excess ligand. Subsequent columns ran to exclusively separate the isomers all proved unsuccessful. Both Al₂O₃ and silica gel were used as a stationary phase, and hexanes, pentane, and a variety of hexanes/ethyl acetate mixtures were attempted for the mobile phase. Pentane provided the best separation of isomers, but the column was still not long enough to allow a total separation of the two bands.

While it would be helpful to have completely separated the two isomers by chromatography, it is not necessary to study the kinetics reaction. Proton NMR provides enough separation of chemical shifts, and the relative intensities of the peaks allow for the characterization of each isomer. Products 10 – 12 are easily identified and labeled. Complexes 13 and 14 characterizable as the correct product, but could not be isometrically identified using the specified parameters and solvent. Therefore, only 10 – 12 are included in this preliminary kinetics experiment.
Experimentation began by collecting an initial $^1$H NMR of the sample spiked with 1,3,5-trimethoxybenzene as an internal standard. Samples were heated in an oil bath at 40, 50, 60, and 70 °C to determine the temperature with the best rate of reaction. 70 °C proved to be the best temperature as a noticeable change could be seen within 1-2 hours. Lowering the temperature to 60 °C did not result in any observable isomerization, even after heating for 48 hours. Once the temperature was determined, sample of 10 – 12 were spiked with the internal standard and heated overnight in an oil bath at 70 °C. $^1$H NMR spectra were collected before heating, at 2, 4, and 6 hours, and
after 25 hours. **Figure 3.3 (a-c)** is an example of an observable increase in the concentration of isomer B over time. The data collected in Table 3.1 reports the relative concentrations of each isomer at time, t, in relation to the concentration of 1,3,5-trimethoxybenzene.

**Figure 3.3 (a)**: Initial $^1$H NMR of sample 10.
**Figure 3.3 (b):** $^1$H NMR of sample 10 after 2 hours at 70°C.

**Figure 3.3 (c):** $^1$H NMR of sample 10 after 25 hours at 70°C
Table 3.1: Relative Concentrations and Equilibrium Constants for [Fe(CO)$_3$SR]$_2$ Isomers at 70 °C

<table>
<thead>
<tr>
<th>Sample # - Time</th>
<th>Relative Concentration*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isomer A</td>
<td>Isomer B</td>
</tr>
<tr>
<td>10 - 0 hr</td>
<td>0.49</td>
<td>0.03</td>
</tr>
<tr>
<td>10 - 2 hr</td>
<td>0.36</td>
<td>0.15</td>
</tr>
<tr>
<td>10 - 4 hr</td>
<td>0.36</td>
<td>0.15</td>
</tr>
<tr>
<td>10 - 6 hr</td>
<td>0.36</td>
<td>0.15</td>
</tr>
<tr>
<td>10 - 25 hr</td>
<td>0.34</td>
<td>0.14</td>
</tr>
<tr>
<td>11 - 0 hr</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>11 - 2 hr</td>
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</tr>
<tr>
<td>11 - 4 hr</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>11 - 6 hr</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>11 - 25 hr</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>12 - 0 hr</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>12 - 2 hr</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>12 - 4 hr</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>12 - 6 hr</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>12 - 25 hr</td>
<td>0.10</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Concentration of each isomer relative to the internal standard, 1,3,5-trimethoxybenzene

The equilibrium constant for each sample appears to stabilize after 3 – 4 hours.

Heating the sample overnight produced a small amount of solid in the NMR tube.

Additional small peaks (C, in Figure 3.3c) also began to appear in the NMR spectra as the heating time increased past 6 hours. The chemical shifts of these new peaks suggest they are the corresponding sulfide, disulfide, or thiol of the sample. The appearance of these peaks, along with the formation of a solid suggests the sample is decomposing.
over time. This also explains the slight increase in the equilibrium constant when \( t = 25 \) hours, as the decomposition of the sample changes the absolute concentration (see Table 3.1).

However, the research presented here shows that it is possible to measure the rate of isomerization using \(^1H\) NMR spectroscopy. The preliminary work finds benzene to be a suitable solvent for the majority of \([\text{Fe(CO)}_3\text{SR}]_2\) complexes. Heating at 70 °C provides a measurable change in the concentration of each isomer over a relatively short time period. The future direction of this research is to automate the isomerization reaction in the NMR to provide more accurate results. It is also important to separate the isomers and study their electrochemical properties. These samples must still have a low reduction potential in order to be considered good catalysts. Future research could also include the modification of the ligands on these models, and a study of the effect it would have on the electrocatalysis and rate of isomerization.

VI: DATA

\([\text{Fe(CO)}_3\text{SCH}_3]_2\) (10)

The representative procedure followed to scale using triirondodecacarbonyl (3.2 g, 6.36 mmol) and dimethylisulfide (0.748 g, 7.95 mmol) yielded a red solid (2.164 g,

¹H NMR (C₆D₆ – 400 MHz) δ 1.49 (s, 3H), 1.44 (s, 6H), 1.12 (s, 3H).

[Fe(CO)₃SCH₂CH₃]₂ (11)

The representative procedure followed to scale using triirondodecacarbonyl (3.2 g, 6.36 mmol) and diethyldisulfide (0.972 g, 7.95 mmol) produced a red solid (2.432 g, 95%). IR (v CO – Hexanes): 2072, 2069, 2036, 2001, 1995, 1955. ¹H NMR (C₆D₆ – 400 MHz) δ 1.95 (q, J=7.4 Hz, 6H), 1.77 (q, J=7.4 Hz, 2H), 0.91, (t, J=7.4, 2H), 0.85, (t, J=7.4 Hz, 4H), 0.76, (t, J=7.4 Hz, 2H).

[Fe(CO)₃SCH(CH₃)₂]₂ (12)

The representative procedure followed to scale using triirondodecacarbonyl (3.2 g, 6.36 mmol) and isopropyldisulfide (1.12 g, 7.95 mmol) afforded a red solid (2.609 g, 95%). IR (v CO – Hexanes): 2071, 2067, 2034, 2000, 1994, 1989, 1953. ¹H NMR (C₆D₆ – 400 MHz) δ 2.62 (sep, J=6.6 Hz, 1H), 2.43 (sep, J=6.6 Hz, 2 H), 2.33(sep, J=6.6 Hz, 1 H), 1.01 (d, J=6.6 Hz, 6H), 0.95 (d, J=6.6 Hz, 12H), 0.89 (d, J=6.6 Hz, 6H). ¹³C NMR (C₆D₆ – 100 MHz)
\([\text{Fe(CO)}_3\text{SC(CH}_3\text{)}_2]\) (13)

The representative procedure followed to scale using triirondodecacarbonyl (3.2 g, 6.36 mmol) and tert-butyldisulfide (1.418 g, 7.95 mmol) yielded a red solid (2.426 g, 83\%). IR (v CO – Hexanes): 2070, 2063, 2055, 2042, 2034, 1999, 1987 cm\(^{-1}\). \(^1\)H NMR (C\(_6\)D\(_6\) – 400 MHz) \(\delta\) 1.15 (s, 9H), 1.14 (s, 18H), 1.01 (s, 9H).

\([\text{Fe(CO)}_3\text{SC}_6\text{H}_{11}]_2\) (14)

The representative procedure followed to scale using triirondodecacarbonyl (3.2 g, 6.36 mmol) and cyclohexylmercaptan (1.848 g, 15.9 mmol) produced 2.37 g of a red-orange solid (73\%). IR (v CO – Hexanes): 2070, 2066, 2056, 2034, 1999, 1993, 1986, 1965.
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59. Unpublished data, Dr. Jesse Tye.


62. Dr. Phillip Fanwick, Purdue University.
APPENDIX
X-Ray Crystal Structure of (1,4-butanedithiolato)hexacarbonyldiiron $^{62}$ (7)

Fe – Fe = 2.51 Å
Fe – S (avg) = 2.25 Å

R-factor: 0.048