Iron-Manganese complexes as mimics of the cofactor in Class I Ribonucleotide Reductases

MICHELE BEDIN
A group of enzymes has recently been reported to have a manganese-iron carboxylate cofactor containing one Mn ion and one Fe ion in the active site. This cofactor is closely related to, and has chemical properties similar to, the well-known diiron-carboxylate cofactor in proteins like the ribonucleotide reductase (RNR) present in pathogens like *Mycobacterium* T or *Chlamydia* T.

In order to provide a model to the study these mixed metal cofactors three different ligands were synthesized. The first ligand was the very well-known BPMP, the second ligand called DPCPMP had one carboxylate instead of a pyridine and the third ligand, which was a modification of the second one, was called BPCPMP and had two carboxylates in opposite position. From the first and the second ligands it was possible to obtain six complexes: two low-valent homometallic Mn/Mn or Fe/Fe complexes and a heterometallic complex per each ligand. The homometallic complexes were synthesized by addition of the respective metal ions to the ligands. In the case of the heterometallic Mn/Fe complex, instead, the two metal ions were added separately and consecutively to the ligands. For what concerns the third ligand, only the Fe/Fe complex was synthesized.

All the seven complexes were characterized by electrochemistry, UV-vis spectroscopy, Mössbauer, and EPR spectroscopy. Our results revealed that the presence of carboxylate in the ligand shifted the redox potential towards negative values, particularly in the case of the homometallic Fe/Fe complex. Next, the behaviour of the complexes in presence of molecular oxygen was assessed and the analysis showed that, even though the metals changed the oxidation state, particularly the iron, no oxygen molecule was trapped inside the complex.

In addition, the relative stability of the homometallic dimers versus the heterometallic dimer and the possibility to transform a homometallic complex into a heterometallic complex were investigated. By titrating one metal into a solution containing the other homometallic dimer it was possible to observe that Fe$^{2+}$ into a Mn/Mn solution led to the replacement of one Mn ion in the complex. In contrast, the titration of the Fe/Fe complex with Mn$^{2+}$ did not lead to any replacement of iron ions in the complex. In the case of the second ligand, it was also possible to observe that the metal selection of the pocket was not dictated by the asymmetry, and that some complexes showed a disproportionate reaction with oxidants like the hydrogen peroxide.

**Keywords:** Iron - Manganese complexes, Ribonucleotide Reductase, biomimetic

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urn:nbn:se:uu:diva-395608 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-395608)
To my wife
And my family

... Cosiderate la vostra semenza:
Fatti non foste a viver come bruti,
ma per seguir virtute e canoscenza.

[Eng] ...consider well the seed that gave you birth:
You were not made to live as brutes,
But to follow virtue and knowledge.

Dante Alighieri (1265-1321) - Divina Commedia
Canto XXVI.
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Contribution to the papers

**Paper I**  Performed the synthesis of the ligand, and the metal complexes, performed the characterization by mass spectrometry, electrochemistry and UV-Vis, NMR, and IR spectroscopy and did the analysis and discussion of the data. Prepared the sample for the Mössbauer analysis. Designed and performed the oxygen activation and metal exchange experiments and did the analysis and discussion of the data. Wrote the first draft of the manuscript.

**Paper II**  Designed the project and developed the synthesis of the ligand and complexes. Performed the synthesis of the ligand and the complexes, the characterization by mass spectrometry, electrochemistry and UV-Vis, NMR, and IR spectroscopy and did the analysis and discussion of the data. Performed the oxidation experiments and did the analysis and discussion of the data. Prepared the sample for Mössbauer analysis and was involved in the analysis of the data. Wrote the first draft of the manuscript.

**Paper III**  Designed the synthesis of the ligand and complex. Supervised the synthesis of the ligand and all the characterization steps. Took part in the synthesis of the iron complex, performed the characterization by mass spectrometry, and IR spectroscopy and did the analysis and discussion of the data. Prepared the sample for Mössbauer analysis and was involved in the analysis of the data. Wrote the first draft of the manuscript.

**Paper IV**  Was involved in the oxygen evolution experiment and supervised the mass spectrometry analysis. Performed the NMR analysis of the complex after water oxidation.
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### Abbreviations

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<tr>
<td>OEC</td>
<td>Oxygen evolving complex</td>
</tr>
<tr>
<td>RNR</td>
<td>ribonucleotide reductase</td>
</tr>
<tr>
<td>MMOs</td>
<td>methane monooxygenase</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>CV</td>
<td>cyclic voltammogram</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
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<tr>
<td>TOF</td>
<td>time of flight</td>
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<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>HR-MS</td>
<td>high resolution mass spectrometry</td>
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<tr>
<td>ICP-MS</td>
<td>inductively coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>δ</td>
<td>isomer shift</td>
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<tr>
<td>ΔE_Q</td>
<td>quadrupole splitting</td>
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<tr>
<td>HS</td>
<td>high-spin</td>
</tr>
<tr>
<td>MLCT</td>
<td>metal to ligand charge transfer</td>
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<tr>
<td>EPR (ESR)</td>
<td>electronic paramagnetic resonance (electron spin resonance)</td>
</tr>
<tr>
<td>hTH</td>
<td>human tyrosine hydroxylase</td>
</tr>
<tr>
<td>LiqN₂</td>
<td>liquid nitrogen</td>
</tr>
<tr>
<td>TBAP</td>
<td>tetrabutylammonium perchlorate</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>DPA</td>
<td>di-(2-picoyl)amine</td>
</tr>
<tr>
<td>TON</td>
<td>turnover number</td>
</tr>
<tr>
<td>TOF</td>
<td>turnover frequencies</td>
</tr>
<tr>
<td>WOC</td>
<td>water oxidation catalyst</td>
</tr>
<tr>
<td>Bpy</td>
<td>2,2’-Bipyridine</td>
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HBPMP (L1)  2,6-bis[(bis(-2-pyridylmethyl)amino)methyl]-4-methylphenol
H₂DPCPMP (L2)  2-(N-(3-((bis((pyridin-2-yl)methyl)amino)methyl)-2-hydroxy-5-methylbenzyl)-N-((pyridin-2-yl)methyl)amino acetic acid
H₃BPCPMP (L3)  2,6-bis[N-[N-(carboxymethyl)-N-pyridylmethyl]amino]methyl]-4-methylphenolate
NHC  N-heterocyclic carbene
Fe₂L₁  [Fe₂(BPMP)(OAc)₂](PF₆)
Mn₂L₁  [Mn₂(BPMP)(OAc)₂](PF₆)
FeMnL₁  [FeMn(BPMP)(OAc)₂](PF₆)
Fe₂L₂  [Fe₂(DPCPMP)(OAc)₂]
Mn₂L₂  [Mn₂(DPCPMP)(OAc)₂]
FeMnL₂  [FeMn(DPCPMP)(OAc)₂]
Fe₂L₃  [Fe₂(BPCPMP)(OAc)₂](PF₆)
Introduction

Introduction to Bioinorganic Chemistry

A third of the chemical reactions happens in nature thanks to enzymes that contain metal cofactors\(^1\) thus linking together two different fields: biochemistry and inorganic chemistry. The presence of metals adds to the topic an inorganic aspect hence defining this subject as biological inorganic chemistry or bioinorganic chemistry.

One of the earlier milestones in this now well-established branch of chemistry was the first resolved X-ray structure of hemoglobin\(^2,3\) where one for the first time could see how a metal ion interacts with a protein. Since then, the field has been growing exponentially, increasing in focus in the past few decades. The main goal of this field is to understand the role of metals and non-metals in biological systems like enzymes, which are involved in numerous processes such as respiration, nitrogen fixation, photosynthesis, metabolic processing and, as particular focus for this thesis, the reparation and production of DNA\(^4\).

Metals in Biology

Elements are organized in the period table depending on their atomic number, electronic configuration and chemical properties. Based on that, elements can thus be divided into three main categories: non-metals and metals, respectively at the right and left of the table, and metalloids, which are elements that share properties with both metals and non-metals.

The non-metals category is quite small and includes the six most abundant elements that are fundamental for life, namely C, O, N, P, S and H\(^5\). The second category, metals, includes elements that are good conductors of heat and electricity, and are generally hard, resilient, malleable and denser than non-metals. It is possible to divide this group of elements into other subcategories such as alkali, alkaline earth and transition metals. Only a few metals are however involved in biochemical processes, and among them Na, K, Mg, Ca, Mo
and the first row of the transition metals subgroup play a fundamental role in living cells\textsuperscript{6}.

One can also classify the metals depending on their role in fundamental biological processes. For example, Fe and Cu are involved in electron storage and transfer, and in dioxygen binding, storage and activation. Other metals like Mg, Mn, Co, Zn and Mo are predominantly involved in substrate activation and catalysis\textsuperscript{7}. All these metals are present as cofactor in enzymes, either as metallic ions or as part of the non-protein groups e.g. heme groups. In many cases the proteins coordinates directly to the metal ions via the side-chains of amino acids\textsuperscript{8}.

\textbf{Classification of enzymes}

In biological systems there are many types of enzymes that are classified in six groups based on their function\textsuperscript{9,10}. The \textit{transferases} catalyze the transfer of some group (methyl-, acyl-, amino- or phosphate groups) from one substrate to another. There are other enzymes involved in the breaking or formation of bonds, like the \textit{hydrolases}, which hydrolyze any kind of bond, and the \textit{lyases}, responsible for non-hydrolytic addition or removal of groups from the substrate. Enzymes that re-arrange intramolecular bonds are called \textit{isomerases}. The \textit{ligases} are instead enzymes that catalyze the formation of specific bonds like C-O, C-S, C-N or C-C with the investment of energy from ATP. The last and most relevant group of enzymes for this thesis is the \textit{oxidoreductases} that are responsible for redox reactions by transferring electrons or transferring H or O\textsuperscript{11}.

\textbf{Iron and Manganese in Biological systems}

Among all the transition metals, iron and manganese are of particular interest in this thesis. Iron is one of the most abundant elements in the earth’s crust. In biological systems, most of the time it is coordinated with a porphyrin ligand that transports and stores oxygen (like in myoglobin and hemoglobin) or catalyzes oxidations (like in cytochrome P-450)\textsuperscript{12}. Iron is also present in iron-sulfur proteins that transfer electrons and for example participate in nitrogen fixation via electron transfer catalysis\textsuperscript{13}. Of fundamental importance for this work is a third class of iron-binding proteins, the transferrins and catechol dioxygenases\textsuperscript{14}. These enzymes contain one
or more iron ions and have oxygen atoms as bridging oxo-, hydroxo- or alkoxo-ligands. Some examples of proteins belonging to this group are the Hemerythrin proteins, which are present in marine invertebrates and transport O$_2$, the ferritin-like superfamily, which has the function to store and transport iron inside the cells, the Flavo-diiron proteins, which provide protection against reactive oxygen or nitrogen species, and the methane monooxygenases (MMOs), which are present in methanotrophic bacteria that use methane as energy and carbon source. However, for this work the most relevant proteins in this third class of iron-binding proteins are the ribonucleotide reductases (RNRs) that produce DNA from RNA.

Manganese is a constituent of the oxygen evolving complex (OEC), present in Photosystem II in the organisms that perform photosynthesis. The OEC is a catalytic site designated to oxidize water into molecular oxygen. Manganese is also present in dinuclear metallo-enzymes, like the lectins-family present in animals, plants, and bacteria, and it was first discovered inside the Concanavalin A. Other biological systems where it is possible to find Mn include the Mn catalases, which disproportionate hydrogen peroxide into water and molecular oxygen, and the arginases, dinuclear manganoenzymes that catalyze the final step to convert ammonia to urea.
Ribonucleotide reductase (RNR) is a fundamental protein for life. Its main function is to produce the building blocks of the genetic material. Specifically, it converts ribonucleotides to deoxyribonucleotides by removing the hydroxyl group on the 2’ carbon in the pentose ring and substituting it with a hydrogen (Figure 1).

RNR was discovered in Sweden almost 60 years ago in *Escherichia coli* even though it is present in all the beings of the living kingdom. RNR is defined as a radical enzyme because the reaction that it catalyzes go through the formation of a radical. The majority of these radicals are protected inside the protein from potential reductants. Most of the radical enzymes like the RNR have, intimately close to the radical site, a metal active site that is fundamental for the stabilization and the formation of radicals.

The classification of RNR includes three main groups named Class I, Class II and Class III depending on the following aspects: the structural differences, the mechanism by which radicals are generated and whether the presence of oxygen is necessary or dangerous for the protein. The first class is further divided into three subclasses: Class Ia (or R2), Class Ib (or R2F) and Class Ic (or R2c).

The Class III of RNR is very sensitive to oxygen that has the ability to destroy the protein. Due to the need of complete anaerobic conditions, this class of
proteins is present only in obligate anaerobes and sometime in facultative ones. These enzymes are formed by two dimeric proteins, namely the NrdD and the NrdG. The cofactor has a \([4\text{Fe}-4\text{S}]^+\) center that reacts with the S-adenosylmethionine to generate a glycyl radical for the activation of the substrate that NrdD uses to catalyze the reaction.

Class II was found instead in archaea and it is completely indifferent to the presence of oxygen, meaning that the protein can work in presence and in absence of oxygen. The enzymes in this class are formed by a single subunit (NrdJ), present as monomer or dimer, and the presence of adenosylcobalamin (AdoCbl) is required to start the radical chain reaction. Additionally, the crystal structure of RNRs in Class II shows three essential redox-active cysteine residues inside the protein (an arrangement that is similar to Class I) even though only one of them is eventually converted in a cysteine radical to start the nucleotide reduction.

Class I is divided in three subclasses, as mentioned above. Due to the importance of Class I for this thesis, each one of the three subclasses will be described separately.

**Class Ia**

This was the first subclass discovered and the most extensively studied due to its similarity to the ferritin-like superfamily\(^2^8\). The protein was found in living cells that require oxygen to perform the radical reaction\(^1^0\). RNRs in this class contain two iron ions in the metals site, close to a tyrosine residue that can form a stable radical\(^3^0\). It is composed by two different subunits called R1 and R2, or \(\alpha\) and \(\beta\), depending on whether the European or American notation is used\(^2^8, 2^9, 3^1\). For consistency throughout this thesis we will always use the European version. R1 has the active side for ribonucleotide reduction but R2 houses the metal active site fundamental to start the nucleotide reaction\(^2^9, 3^2\).
**Class Ib**

This class does not need to react with $\text{O}_2$ or $\text{H}_2\text{O}_2$ in order to be activated. The composition of the protein is mostly similar to Class Ia as it has two homodimer subunits that contain, however, two manganese ions inside the cofactor. This difference seems to be evolutionary, and iron deficiency has led some mammalian pathogens like *Mycobacterium tuberculosis* to adapt by producing Class Ib enzymes as a response.

**Class Ic**

The last subclass, that is also the most important for this thesis, is the Class Ic. It was first discovered in *Chlamydia trachomatis* and it differs from the
other two subclasses due to the presence of a heterobimetallic metal site containing one iron in one pocket and one manganese in another pocket in the active site. In addition this subclass shows some structural differences like the presence of one molecule of water in the reduced active site resembling the sMMO.\textsuperscript{17} Interestingly, Class Ic can react either with oxygen or with H\textsubscript{2}O\textsubscript{2} to produce the same active form of Mn\textsuperscript{IV}/Fe\textsuperscript{III}\textsuperscript{29, 37}.\textsuperscript{}

![Figure 4: Structure of reduced form of Class Ic RNRs. The blue dot represents a coordinated water molecule.](image)

**Structure of the dimetal sites**

To have a better idea about how the Class I RNRs work it is necessary to have a closer look at the structure of the dimetal site of three different subgroups. In the reduced form of the Class Ia, the iron in position one is coordinated with one oxygen of an aspartate and the nitrogen from a histidine. The other iron is coordinated with the nitrogen from another histidine and an oxygen from a glutamate residue. The two metals are bridged by the carboxylate groups from two glutamate residues\textsuperscript{29} (Figure 2). In the oxidized and active form the two iron ions are hexacoordinate and have additional water ligands and a µ-oxo bridge (Figure 5, lower left structure).

When it comes to the Class Ib, the structure is identical to Class Ia in the oxidized form, but instead of two iron ions it contains two manganese ions. However, in the reduced form there are a few differences (Figure 3). The manganese ions in Class Ib maintain the hexacoordinate structure, that the iron ions in Class Ia lose, the manganese ions coordinate one solvent molecule each and one histidine residue each. Three glutamate residues are bridging the two manganese ions and another aspartate coordinate the metal in site 1\textsuperscript{29}.\textsuperscript{}}
In case of Class Ic the most obvious distinction from the previous subclasses is the presence of two different metal ions: one iron and one manganese. It has been discovered that the position of the two metals is not random and that the manganese preferentially coordinates in position one, while position two is filled by the iron ion\textsuperscript{38}. The amino acid residues coordinating the metals in the reduced form are, in each side, a histidine, namely His105 for the manganese and His205 for the iron, and a glutamate, namely Glu69 for the manganese and Glu167 for the iron. Two glutamate residues bridges the two metals\textsuperscript{39} (Figure 4). In the oxidized form the two metal ions also have a peroxo bridge.

![Diagram of metal coordination and peroxo bridge](image)

*Figure 5: Mechanism for the formation of the tyrosyl radical in Class Ia RNRs* \textsuperscript{40}.

**Mechanism of radical formation**

As previously described, the conversion of ribonucleotides into deoxyribonucleotides is performed by a radical reaction. The unpaired electrons in the rad-
icals make the system very unstable and highly reactive. Several ionic reactions are possible, to remove an oxygen from a simple alcohol, but the sterically hindered position of the OH in the ribonucleotide poses a limitation to the reaction. An S_{n}2 reaction involving the substitution of a –OH group with a –H would be inhibited both electronically and sterically. The formation of a carbocation in a S_{n}1 reaction is instead prevented by bonding to the adjacent carbon, thus promoting the elimination of the base at C1’. This leaves the radical approach as the only possible reaction pathway.

The Class I RNR proteins share common features for the radical reaction. The cysteine residue that forms the radical and three different radical precursors are indeed present in the three types of RNRs. In addition, in all three cases the dimetal center is required to generate the cysteine radical. In Class Ia and Class Ib a tyrosyl radical (TyrO·) is part of this process. The Class Ia RNRs are the most studied and proposed mechanism for the formation of a tyrosyl radical in these enzymes is shown in Figure 5. The two reduced metals ions in the active site react with a molecule of O_{2} and a molecule of water that lead to an unstable intermediate peroxo species. From the dimetal center two electron are transferred to the oxygen creating an iron-bound peroxide. An additional one-electron reduction helps the cleavage of the O-O bond allowing the generation of a further intermediate named compound X. Spectroscopical analyses show that these metals are in the oxidation state Fe^{III}/Fe^{IV} where the one with lower oxidation binds with water and the other metal coordinates with a glutamate residue and one oxo ion. Ultimately, the last intermediate converts the tyrosine into a tyrosyl radical and the two Fe^{III} ions are bridged by one glutamate and one oxo bridge in the activate form^{41,42,44}. In Class Ib the mechanism is similar and the active forms is Mn^{III}/Mn^{IV} (TyrO·) species. However in Class Ic no tyrosine is present and the active form is instead proposed to be an oxo-hydroxo bridged Fe^{III}/Mn^{IV} species.

**Synthetic model complexes of Class I RNRs**

One role of synthetic chemistry in the bioinorganic field is to create models able to reproduce interesting structural and/or functional aspects of the metalloenzymes, and to provide mechanistic, structural and spectroscopic data comparable to the macromolecule in order to gain detailed information about the biological system^{45}. This characterization can potentially help to explain the
biological mechanism and possibly isolate an intermediate and can lead eventually to the development of drugs to treat diseases.

In the past years many attempts meant at mimicking class I RNRs have been reported and reviewed. The first part of this study, for instance, was based on HBMP (see Figure 15, on the left below), a ligand that can coordinates two metal ions creating two different pockets. Latour et al. prepared an iron-manganese complex with an asymmetric version of this ligand, a pyridine arm has been replaced with a non-coordinating benzyl group in one of the pockets, creating a potential open coordination site on one of the metals. In the crystal structure a molecule of water completed the octahedral coordination sphere around the manganese ion, similar to the case in the proposed active form of Class Ic RNRs. Tolman et al. used a ligand that coordinates only one metal to make an iron complex that, in the presence of dioxygen, forms a µ-oxo bridged FeIII dimer that models intermediates found in Class Ia RNRs. In order to study a system similar to a porphyrin, Dutta and colleagues prepared a macrocyclic ligand containing four nitrogen and thus creating two pockets linked with two p-cresol groups to close the cycle. All the above-mentioned studies contain some common features that make them mimics for the Class I RNRs. The metal ions are hexacoordinate, the ligands are containing nitrogen donors and the complexes have additional carboxylate groups bridging the two metal sites.

In a quite different approach Friedle et al. used a series of bulky carboxylate building blocks to create a complex that was sterically controlled to form a diiron species, creating a structure very similar to Class I RNRs with bridging and monodentate carboxylates.
Figure 6: Examples of synthetic model complexes for Class I RNRs reported by, from top left to bottom right, Tolman et al.\textsuperscript{54}, Latour et al.\textsuperscript{57}, Dutta et al.\textsuperscript{56}, and Friedle et al.\textsuperscript{49}
As mentioned above, the most common and the well-known biological system that contains Mn is the OEC present in Photosystem II. This metal-oxygen cluster has a cubane like structure with a $\text{Mn}_4\text{CaO}_5$ composition. Inspired by nature, a first series of Mn-based complexes was reported in the middle of the 90’s from Naruta et al.\textsuperscript{58}. This series of complexes consisted of a dimeric porphyrin complex able to perform O-O bond formation. A few years later, however, the very first manganese dimer able to produce oxygen from water was reported by Crabtree and Brudvig\textsuperscript{59, 60}.

![Scheme of dimanganese complexes used as WOCs reported by, from left to right, Naruta et al.\textsuperscript{58}, Crabtree and Brudvig et al.\textsuperscript{59, 60}.](image)

In the past few years iron-based complexes have become popular to investigate as water oxidation catalysts (WOCs). The possibility to use metals that are cheap and very abundant in nature, like iron, opens the possibility to use this reaction on a global scale in the future. In 2010 Collins et al.\textsuperscript{61} reported the first iron-based WOC. The authors reported a notable TOF of $> 1.3$ s$^{-1}$ even though the complex was not stable on a longer time-scale. Inspired by this work, Fillol and Costas et al.\textsuperscript{62} presented several other iron-based WOCs with a very high TON.
Unfortunately, in order to have high TON and very good stability in WOCs it is still necessary to employ expensive and rare metals like iridium or ruthenium. Ten years ago, Bernhard’s group reported the first series of molecular iridium-based WOC (Figure 9, on the left), with 2,2'-bipyridine as a ligands, which did not have a very high TOF but a TON of 2490. Crabtree and Brudvig developed the system substituting the bipyridine with Cp* to reach very high TOF but decreasing the stability. Combining the two developments, Bernard and Albrecht used the Cp* and a carbene ligand increasing the stability and preserving the high TON. This development was based on the idea that iridium-oxo intermediates could be stabilized by the electron rich Cp*, especially at the high valent states that the complex reaches during the water oxidation. After Bernhard proposed the involvement of iridium in high oxidation states as Ir^{IV} and Ir^{V} in WOCs, several other groups has started to use the IrCp* moiety as part of WOCs, driven by ceric ammonium nitrate (CAN) or sodium periodate (NaIO_{4}) as sacrificial oxidants.

N-heterocyclic carbenes (NHCs) have been used as ligands for various types of catalysts in particular WOCs. The strength of this type of structure is the relative high σ-donor capacity that results in a tight bond to the metal and thus helps in stabilizing high-valent metal oxidation states. Hetterscheid et al. reported in 2011 a WOC with a NHC ligand suggesting that the presence of chloride hinder the catalysis.

An aspect of using CAN as the oxidant is the highly acidic conditions that is necessary for the work. These conditions can be detrimental for the IrCp* moiety and some of the catalysts can degrade and form nanoparticles of IrO_{x}. This would not be a big problem if the nanoparticles were inert, but it was...
observed that also the nanoparticles can be utilized as catalyst for water oxidation. 

![Figure 9: Scheme of Ir complexes as WOCs, from the first Bernhard et al. (left), then Crabtree and Brudvig (center) and Bernhard with the improvement by the Cp* (right).]
The principal aim of this thesis was to use bioinorganic model complexes to gain more understanding of why there are two different metals present in Class Ic RNRS, and how that can affect the reactivity of the di-metal site. The starting point was to synthesize three different dinuclear complexes from a known symmetric ligand (L1). Two homometallic complexes, containing two iron and two manganese ions were prepared, in addition to the heterobimetallic complex. These three complexes were then characterized to highlight the differences and similarities in their chemistry (Paper I).

Since the studies on Class Ic RNRS show a unique behavior of the manganese ion, it seems that the metals prefer one site over the other. A further development of the ligand system was made by using an asymmetric ligand (L2) in order to investigate whether the asymmetry would direct the different metals to different pockets in the ligand (Paper II).

In order to have a complex with more carboxylate donors to the metals, better resembling the active site of the Class I RNRS, a third symmetric ligand (L3) was prepared and its diiron complex was characterized (Paper III).

In paper IV, many of the techniques used to study metal complexes and their oxidative chemistry in Paper I-III were employed in a different context, investigating an Ir-based WOC and characterizing high-valent intermediates. In particular, the importance of pendant groups such as hydroxyl or amino groups to stabilize higher oxidation state was demonstrated. The Ir complexes are more stable than first row transition metal complexes, but the obtained results can probably be transferred also to Fe- or Mn-based complexes.
Techniques

Mass spectrometry

Mass spectrometry (MS) is one of the pillar techniques used to identify compounds, either known or not, and to detect side products even at very low concentrations. The first published study where MS was performed on a metal complex was in the 90’s and involved the tris(bipyridine)ruthenium (II) complex. For this work, MS was of fundamental importance to identify the new compounds and to characterize already known ones. Since the mass spectrometer detects only charged species, the unit used in this technique is mass-to-charge ratio, represented by $m/z$. This unit is dimensionless and indicates the atomic or molecular mass divided by the elementary charge.

The peak represented in the graph is correlated with the abundance of the ion. When it comes to metal complexes it is common to identify several peaks due to the natural presence of several isotopes of the metal atom, hence the name isotope pattern. The isotope pattern makes the identification simpler thanks to the unicity of the pattern, and in the case of this thesis, a clear example is represented by the isotope pattern of iron-manganese complexes compared to iron-iron or manganese-manganese complexes.

There are several methods to ionize the sample but the most common for this type of analysis is the electrospray ionization (ESI) thanks to the gentle solution-to-gas phase transition and its high sensitivity and selectivity.

The mass analyzer has the function to divide the different fragments according to their $m/z$. Among the several types of mass analyzers, two types were used in this work: the RF octopole and time-of-flight (TOF). The RF octopole separates the different fragments by polarity. The main advantage is that this system offers a higher transmission of the ions. In addition, it allows high scan speed that makes this type of mass analyzer perfect for coupling with liquid chromatography. A limitation of the RF octopole is however the relatively small range of $m/z$ (80 to 2000) thus making impossible to analyze a whole...
range of compounds. On the contrary, the TOF has a completely different set-up giving unlimited detection range, high sensitivity that allows the separation of very small fragments (thanks to the high ion transmission) and a complete ionization created during a short ionizing event.

Analytical challenges are common features in MS. In fact, the poor stability of metal complexes during the analysis causes the complex to break creating fragments that are detected by the MS thus constraining the identification of the correct metal complex.\textsuperscript{78} In addition, other problematic phenomena can happen during the electrospray ionization. For example, the reduction of metals like Fe\textsuperscript{III}, Cu\textsuperscript{II}, with chelating agents during the analysis, or some fragments can react forming new species\textsuperscript{74, 79}.

**High performance liquid chromatography**

High performance liquid chromatography (HPLC) is a separation technique. The use of the HPLC was necessary to check the different steps of the synthesis and verify the purity of the compound. The HPLC used was coupled with the MS and was an analytical size, which allowed to have a good separation with crude product up to 500 mg. The instrument eluent was a mixture of acetonitrile-water with 0.05% of formic acid. In addition, there were two columns (15 cm length and 3.4 mm internal diameter, one with pore size of 5\(\mu\)m and the other 10\(\mu\)m) inside a thermostatic oven at 25 °C to separate the material. The analyte was detected by a photodiode array (PDA) detector from 200 nm to 800 nm wavelength.

**IR spectroscopy**

Vibrational infrared (IR) spectroscopy uses the interaction of electromagnetic radiation of energies between 500 cm\(^{-1}\) to 4000 cm\(^{-1}\) with a sample and can be used for example to obtain information about the structure of organic molecules.

The absorbed infrared radiation leads to molecular vibrations that cause a change in the dipole moment of the molecule\textsuperscript{80}. The absorption frequencies depend on the mass of atoms, geometrical arrangement and type of vibration and altogether provides a “fingerprint” of particular molecules and functional groups (Figure 10).
The types of vibrations that can occur can be summarized in six main groups: Symmetric and Asymmetric stretching, Wagging, Twisting, Scissoring, and Rocking. The characteristic movements of the atoms define each group. For instance, a vibration in the planar space can fall in the stretching, scissoring or rocking groups, while a vibration outside of the plane defines the wagging or twisting groups.

We used IR spectroscopy to determine the nature of the coordination between carboxylate groups in the complexes and the metals in order to understand the structure of the complex and the coordination of the metals with the ligand. Hence, it is fundamental to know the “fingerprint” of the different types of interactions between carboxylate groups and metals ions.

We can summarize the type of coordination of metals to carboxylates in four categories as delineated in Figure 11. It has been shown that the difference (Δ) between the symmetric ($v_s$) and asymmetric ($v_{as}$) vibration of the coordinated carboxylate in the IR spectrum can be used to distinguish the different coordination modes.

Deacon and Philips studied carboxylates coordinated to metals ions comparing the stretching frequencies combined with the crystal structure led to three main conclusions:

1) Carboxylates with unidentate coordination show much greater Δ values compared to ionic carboxylates.

2) Chelating carboxylates complexes show smaller Δ values than ionic carboxylates.

3) The Δ value of bridging bidentate carboxylates is greater than the Δ value of chelating carboxylates, and closer to ionic values.
Figure 11: Categories of coordination between carboxylates and metal (M) ions

1. Unidentate

2. Chelating Ligand

3. Bridging Bidentate

4. Monoatomic Bridging Ligand

For instance, in almost all acetate complexes with $\Delta < 150\ \text{cm}^{-1}$ the carboxylates are recognized as bridging between metals ions. However, in some rare cases, when there is a metal on one side and a hydrogen on the other, this rule is not applicable. In these cases, the complexes are called “pseudo-bridging” and the values obtained are similar to those of the bridging bidentate carboxylate.

Other information about how metal ions coordinate to ligands is also possible to obtain from IR spectroscopy. For example, for two metal ions bridged by an oxo ligand (M-O-M) IR spectroscopy can be used determine if oxo bridge is linear or bent. In a bent system the $\nu_{\text{as}}$ and $\nu_s$ are visible in IR and Raman.
spectroscopy but when the system is approaching linearity one of the two vibration become only IR active ($\nu_{as}$). This band has been observed particularly in diiron model complexes for metazidohemerythrin. Where each iron ion was fac-coordinated by a tridentate nitrogen donating ligand and two metal ions were bridged by one $\mu$-oxobridge and two symmetrical bridging bidentate $\mu$-carboxylate ligands.

UV-Vis spectroscopy

The ultraviolet-visible (UV-Vis) spectroscopy is a well-known technique used for example to obtain information about the electronic properties of the studied molecule.

The absorption of the light happens when the energy of incident radiation is the same as the energy of an electronic transition of the compound studied. This absorption, called electronic excitation, is associated with a moving electron from a low energy level (ground state) to a high energy level (excited state).

The main types of electronic transition that can be observed in a UV-Vis spectrum ones to be considered are:

1. Transitions involving $\sigma$ and $\pi$ electrons
2. Transitions involving charge-transfer electrons
3. Transitions involving $d$ and $f$ electrons.

The first group can be divided in four subclasses. The $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions are more common in organic compounds and ligands and are visible between 200 nm and 700 nm. The other electronic transitions are $n \rightarrow \sigma^*$ and $\sigma \rightarrow \sigma^*$ and are visible in the region below 190 nm.

When a metal is coordinated to a ligand, transitions from the second group are also possible, namely metal-to-ligand and ligand to metal charge-transfer (MLCT and LMCT respectively).

The third type of transitions include the $d-d$ transitions that are common in the elements of the d-block and are associated with weak-colored metal complex solutions.
Electrochemistry

Electrochemistry can be used to investigate the redox behavior of metal centers within in different complexes. There are many different electrochemical techniques that can be used but for the purpose of this thesis cyclic voltammetry (CV) also called linear potential sweep chronoamperometry (or linear sweep voltammetry) was mostly used.

![Figure 12: A scheme of three-electrode cell for electrochemistry](image)

The equipment used in these studies was a three-electrode cell which is composed of the reference electrode, the counter electrode, and the working electrode (Figure 12). In order to make electrons move freely in the cell, a dry polar solvent with added electrolyte was used. The compound studied was maintained in the central solution of the cell and in contact with the working electrode, a glassy carbon rod with an area of 0.0707 cm². The reference electrode consisted of a silver wire immersed in a solution of silver nitrate in a small salt bridge, which was immersed in an electrolyte solution in a bigger salt bridge (not shown in Figure 12). The counter electrode was a glassy carbon rod immersed in an electrolyte solution inside a second salt bridge.

The entire cell was a closed environment in order to avoid oxygen contamination which can create background noise or signals in the scan. For this purpose, an inert dry gas such as argon was bubbled through a vessel with dry molecular sieves into the solvent used for the electrochemical solution (Figure 12). This bubbling prevents the solvent to dry or the concentration to change during the various steps of the electrochemical analysis. In the cases that the
CV is recorded in a controlled environment like the glove box where there is not presence of oxygen, the bubbler is not necessary.

In CV (Figure 13) the current is measured at the working electrode from a starting potential to a vortex potential and back. Normally the experiment begins with a few seconds of equilibration of the solution next to the electrode (often 0.0 V). In a typical CV each reversible redox couple gives rise to two peaks, one in the anodic scan (E_{pa}) and one in the cathodic scan (E_{pc}). The potential of the redox couple (E^0) is then the average of the two.

Another technique used in this thesis was chronoamperometry uses a working electrode that has a larger surface area. The instrument is set to a specific potential and it records the current in a stirred solution for the desired amount of time.

Because of the mechanical movement of the liquid, the whole complex in the solution is oxidized or reduced in comparison with the CV where instead only
a small amount (on the surface of the working electrode) is oxidized or reduced. The measurement normally finishes when the current reaches a plateau, meaning indeed that the whole solution has changed state. This technique can be useful to convert a complex in solution to a desired oxidation state for example to prepare a sample for EPR analysis.

Mössbauer spectroscopy

The physicist R. Mössbauer discovered the nuclear resonance absorption of γ-rays – or Mössbauer effect – in 1957. This technique is based on the recoilless emission and the recoilless resonant absorption of γ-rays by the nucleus and it is so powerful that a Mössbauer spectrometer was installed in the Mars rovers Opportunity and Spirit. In detail, two nuclei of the same isotope of an element are necessary to observe the Mössbauer effect, and 57Fe is far the most studied isotope in Mössbauer spectroscopy. The first iron nucleus is the source that emits γ photons and 57Co embedded in Rh metal the most commonly used for this purpose. This unstable isotope decays by electron capture to the 57Fe excited state and in a few ns the 57Fe goes to the ground state by the emission of a γ-photon at 14.4 keV radiation. This radiation is recorded by the Mössbauer spectrometer. Due to the necessity of an exact amount of energy a synchrotron can also be used as a source instead of radioactive sources. The nuclear resonance absorption of γ-photons is observable when the metal ion is coordinated by other atoms, but it is not possible for free metal atoms because the energy is lost by the recoil. Not all the elements are suitable for Mössbauer spectroscopy, and the second metal used more frequently in this thesis, manganese, is one of these.

From a Mössbauer spectrum it is possible to extract three parameters. The electric monopole interaction is the first parameter, a hyperfine interaction that in the Mössbauer analysis is called the isomer shift δ. This interaction is between the nucleus and the electrons inside the nuclear field. Hence, the information that is possible to obtain include the oxidation state, spin state and bonding properties. As shown in Figure 14 the isomer shift falls in different ranges making it possible to identify the oxidation state of the iron and its spin state based on the value of δ. This made it the most useful parameter for the work in this thesis. For high-spin systems, the isomer shift is higher the lower
the oxidation state is. However, for low-spin systems the trend is not so clear. The second parameter of the Mössbauer spectrum is the electric quadrupole interaction $\Delta E_Q$. This interaction is between the nuclear quadrupole moment and the inhomogeneous electric field at the nucleus. From this hyperfine it is possible to obtain information about the symmetry around the iron ion. The third and last parameter is the magnetic dipole interaction that is between the nuclear magnetic dipole moment and the magnetic field at the nucleus. This gives information about the magnetic properties of the material under study, but it is not deeply evaluated in this thesis.  

Due to the possibility to investigate the oxidation state and coordination environment of iron ions, this technique has been used to study iron-containing proteins, for example RNR and MMO$^{89}$. In biological systems, most of the proteins have to be enriched with nonradioactive $^{57}$Fe because, due to the low concentration of iron, the signal is not otherwise visible.$^{94}$ Through the isomer shift it is possible to identify high-spin Fe$^{II}$ center and also evaluate if the iron ion is coordinated with nitrogen and oxygen donors inside the cofactor. High quadrupole splitting have been shown to correspond to a highly distorted octahedral geometry around the two irons in RNR, which was also confirmed by X-ray crystallography.$^{95, 96}$ The Mössbauer spectrum of MMO has shown the asymmetry at the active site, which indicates that the two iron ions are in two different pockets.$^{95, 97}$
EPR

Electron paramagnetic resonance (EPR) spectroscopy can be used to study paramagnetic centers in compounds or materials. EPR detects the absorption of a paramagnetic species from a continuous microwave source in a strong magnetic field (B)\(^9\). There is a wide range of microwave frequencies that could be applied but the most common one is the X-band (9 GHz)\(^9\). The microwaves generated outside the magnetic field move through a waveguide to the cavity that it is immersed inside the magnet\(^1\). The sample is placed in the cavity, for the detection of transition metals species it is most often cooled down to the temperature of liquid helium or liquid nitrogen in order to perform the measurements\(^1\). An electron has a magnetic moment (m\(_s\)) and a spin quantum number. If it is immersed in a magnetic field, like in the case of EPR, it tends to align in par-
allel \((s = +\frac{1}{2})\) or antiparallel \((s = -\frac{1}{2})\) to the magnetic field. Due to the Zee-
man effect\(^{102}\) each alignment of the magnetic moment has a specific energy\(^{101}\).
The specificity of these two states makes the unpaired electron able to jump, at
different magnetic field strength, from one level to the other absorbing or
emitting \(h \nu = g \mu_b B\) amount of energy, where \(g\) is the so called \(g\) value and
\(\mu_b\) is the Bohr magneton. The nuclei can also have spin \((I)\) and interact with
the field, this type of interaction is called nuclear Zeeman interaction \((I \leftrightarrow B)\).
One more interaction that is necessary to take into consideration is the electron
spin or hyperfine interaction \((S \leftrightarrow I)\), which is the interaction between the elec-
tron and the nuclei\(^{101}, 103\). The hyperfine interaction can often be observed in
the EPR spectrum, particularly in the case of Mn\(^{II}\) \((I = 5/2)\) species. Because
the nucleus and the electron spins are coupled weakly, the microwave radia-
tion changes slightly (in a constant value called \textit{hyperfine splitting constant})
the spin of the electron, resulting in 6 lines centered on the field corresponding
to the \(g\) value\(^{104}\).
Because all these interaction are depending each other, changing the field
could influence each one of the different interactions.
The transition metal complexes, prepared in this work can have different oxida-
tion states with one or more unpaired electrons, and therefore EPR was of
great interest for this thesis work.\(^{105}\)
The core of this thesis work was to synthesize and characterize biomimetic models of the di-metal sites in Class I RNRs, with special focus on the recently discovered iron-manganese cofactor in the RNR from *Mycobacterium tuberculosis*. In the X-ray crystallographic structure of that protein the two metals have an octahedral coordination environment. The coordination is orchestrated by five oxygens and one nitrogen for both the metal ions even they are differently distributed between the two ions. The two metals share one oxygen from a carboxylate, one bidentate carboxylate bridge from a glutamate and an additional bidentate carboxylate from a fatty acid molecule. The iron ion coordinates with two carboxylates from two glutamate residues while the manganese ion coordinates with only one carboxylate from a glutamate residue and one molecule of water. To complete the octahedral geometry one nitrogen donor from a histidine residue coordinates to each metal.

The coordination environment in the dimetal sites of Class I RNRs, with nitrogen and oxygen donors, inspired the choice of the three different dinucleating ligands used in this thesis (Figure 15). We started by using a well-known ligand, the HBPMP (H\(^1\)L\(^1\), Figure 15, left). The dimetal complexes with this ligand have two additional acetate bridges and the metals ions are coordinated by three nitrogen and three oxygens in each side of the ligand. In the study of the heterobimetallic protein in the *C. Trachomatis* Anderson and Högbom,\(^{38}\) found that in the construction of the dimetal site the manganese ion prefers one position over the other, suggesting that the asymmetry in the pocket could be directing the metals to different sites in the enzyme. Therefore we also decided to study complexes made with an asymmetric ligand and chose a scaffold with one carboxylate donor coordinating to only one of the metal ions in the dinucleating ligand, H\(_2\)DPCPMP (H\(_2\)L\(_2\), Figure 15, center).

The third ligand, the H\(_3\)BPCPMP (H\(_3\)L\(_3\), Figure 15, right), is instead symmetric and it has a higher number of oxygen donors and the coordination environment is thus more similar to the situation in the dimetal sites of Class I RNRs if compared to the HBPMP ligand.
Figure 15: Exposition of the ligands HBMP (HL1) H2DPCPMP (H2L2) H3BPCPMP (H3L3), in their protonated forms. The two ligands with carboxylate groups (L2 and L3) were stored protected as ethyl esters, and deprotected the day before use.

General preparation

In the synthesis of the three ligands all reagents and solvents were purchased from commercial sources and all the used solvents were distilled under \( \text{N}_2 \). The solvents were dried in different ways: acetonitrile and methanol were distilled over CaH\(_2\) while THF was distilled over sodium-benzophenone. Once dried, all the solvents were collected and transferred to dry flasks using a cannula before every reaction. Since methanol is very hygroscopic, it was collected and stored under argon in a Schlenk flask with 4 Å molecular sieves.

2,6-bis(hydroxymethyl)-4-methylphenol

The 2,6-bis- (hydroxymethyl)-4-methylphenol (I) that was used as starting material for all the three ligands was purchased from commercial sources, but the first attempt of synthesis of the L1 ligand was not satisfactory, and the starting material was further purified by recrystallization in acetone before use. The solid was partially dissolved in acetone, the solution was warmed up until the solid residue was completely dissolved. The mixture was left to cool down overnight, and the pure material was collected the day after by filtration.

Di-(2-picolyl)amine (DPA)

DPA was synthetized using slightly modified procedure from the literature.\(^{107}\) The reaction can be considered as a condensation between a carboxylic acid and an amine and, even if it was not strictly necessary, it was performed in dry conditions to increase the yield and the purity. The 2-aminomethylpyridine was dissolved in dry methanol. The other reagent, 2-pyridinecarboxaldehyde,
was first transferred in a round bottom flask and then placed under high vacuum to remove solvent residues. During this process the oily compound turned into a foamy solid thus suggesting that all the water had left the flask. The reagent was then dissolved in dry methanol and transferred to a dropping funnel connected with the amine solution. The system was cooled down twice, first when the aldehyde was added and then for the addition of sodium borohydride. The resulting reaction mixture was poured into ice, the pH was adjusted to 3-4 and the final product was extracted with dichloromethane until the water phase became colorless. In order to increase the yield, the water phase was neutralized with potassium carbonate and extracted again with dichloromethane. All the organic phases were collected and dried with magnesium sulfate, and the solvent was then removed under reduced pressure. The residue was further purified on an alumina column and the eluent used was a mixture of dichloromethane, methanol and small amount of triethylamine.

![Figure 16: Synthesis of DPA.](image)

**BPMP (L1)**

As mentioned before, the first ligand that was synthetized was the symmetric 2,6-bis[(bis(-2-pyridylmethyl)amine)methyl]-4-methylphenol or HBMP. The synthesis followed a modified literature procedure.\(^{108, 109}\)

![Figure 17: Synthesis of HBMP (HL1). The first step gave II in 82% yield and second step gave HL1, as a yellow oil, in 96.5% yield.](image)

The first step was to substitute the two alcohol groups in the 2 and 6 positions on I with chlorides. This passage is needed because the chloride is a better leaving group for the second substitution with DPA. The reaction started with
dissolving I in dichloromethane and hydrochloric acid, and the mixture was left to stir for 24 hours. The compound was extracted with water in order to remove the excess of hydrochloric acid and the organic phase was dried with magnesium sulfate. The solvent was removed under reduced pressure leaving yellow crystals of 2,6-bis(chloromethyl)-4-methyl phenol (II). In the next step two equivalents of DPA were added to obtain the HL ligand. The procedure started with the DPA dissolved in THF and triethylamine. The amine is necessary to make an ammonium salt together with the chloride released. Compound II was added dropwise to DPA at 0 °C. After the complete addition the solution was left to stir at room temperature for four days. The triethylammonium salt was removed by filtration and washed with THF in order to recover as much product as possible, and the solvent was removed under vacuum. The residue contained some impurities so an extraction with dichloromethane was performed. The final yellow oil did not need any further purification and it was used directly in the synthesis of the three different complexes.

DPCPMP (L2)

In order to make an asymmetric ligand, we substituted one pyridine with one carboxylate in the scaffold of the BPMP-ligand. The synthesis of the 2-(N-(3-((bis((pyridin-2-yl)methyl)amino)methyl)-2-hydroxy-5-methylbenzyl)-N-((pyridin-2-yl)methyl)amino)acetic acid], or DPCPMP, was quite different compared to the synthesis of BPMP. The synthesis is based on a method explored by Jarenmark et al.110 for another ligand. Instead of simply substituting the alcohol group with the chloride, the alcohol was oxidized to an aldehyde before adding the DPA.

The first step was to create the monoaldehyde of I. Two approaches were attempted. In the first, I was dissolved in chloroform and manganese oxide (freshly bought) was used as the oxidant and added to the mixture that was refluxed overnight. Once the mixture was filtered and washed with chloroform three times, silica gel column chromatography was performed. The eluent used was a gradient 0 to 40% of ethyl acetate in dichloromethane. The starting material and the two products, namely the monoaldehyde (IV) and the double aldehyde, are at this point separated. These two products were always present in every synthesis, but the yield of IV could be increased by reducing the equivalents of manganese oxide compared to the starting material.
Figure 18: Synthesis of IV. Compound IV was obtained in 43% yield with MnO₂ and 43% yield with BaMnO₄. In the next step the yield of V was 97%. Compound VI was obtained in 60.65% yield.

A second more reliable method was used because the manganese oxide lost efficiency and caused the yield to decrease over time. In this second method a suspension of barium manganate in dry dichloromethane was used and the mixture was left to stir overnight at room temperature. The next day the barium manganate was removed by filtration and the mixture washed with methanol. In this case a column chromatography step was also performed in order to purify the final product. Since the absence of the double aldehyde, which run very close to the monoaldehyde, reduces any chance of contamination between the products this procedure is easier. Even if it is possible to purify the final product and the starting material separately, the final yield was not higher than in the first method (Figure 18).

After the monoaldehyde was obtained, the DPA “arm” was attached. The reaction was straightforward. The DPA was dissolved in dichloromethane with molecular sieves (4 Å) in order to remove the water that forms during the reaction. Compound IV was dissolved in dry dichloromethane in a separate flask under inert condition and it was added to the flask with DPA via a dropping funnel under argon atmosphere. After a couple of hours of stirring the solution was cooled down to 0 °C and sodium borohydride was added. The
solution was allowed to warm up overnight and was then quenched with sodium carbonate very slowly due to the high reactivity of the borohydride. The final product V was extracted with dichloromethane and dried under vacuum. The purity of compound IV is fundamental because the presence of two aldehydes in the p-cresol at this stage can create the L1. As the difference of HL1 and the H2L2 is only one pyridine any further step from column chromatography to crystallization of the final complex becomes very difficult if both are present.

The N-(2-pyridylmethyl)glycine ethyl ester arm

Several oxidation methods were first tested in order to attach the second “arm”. These methods were applied in order to avoid the use of chlorine but it did not work properly. In the first try the Swern oxidation was applied. This oxidation is used to produce aldehyde and ketones avoiding the use of toxic metals. One of the strategies to get good results in the reaction is to keep the temperature as close as possible to −78 °C. However, in our case this did not work. In fact, after adding the base in order to obtain the final product, the crude material was analyzed with HPLC-MS and the m/z matched the thioacetal’s mass. The presence of two pyridines in one side of the ligand makes the alkoxy sulphonium ion unstable as it quenches before adding the base.

In the second attempt the Dess-Martin oxidation was applied. This method involves a mild oxidation that can be performed at room temperature. However, also in this case the reaction did not work and the HPLC MS only showed a peak matching the mass of the starting material.

The last and final attempt involved the use of manganese oxide as a strong oxidant in the oxidation of the p-cresol. Also in this case the HPLC-MS did not show a peak matching with the expected product since the reaction was too strong and all the product was oxidized.

Since none of the oxidations worked as expected the decision to following the original procedure from the paper111 using thionyl chloride was inevitable. First the thionyl chloride was purified, since it is subject to degradation and sulphur accumulation. The reported method uses linseed oil112 but in our case extra virgin olive oil was used. The thionyl chloride was mixed with 10 percent of extra virgin olive oil, stirred for an hour and distilled and collected in three fractions, the first and the third fraction was discarded and the second was stored for a couple of minutes under argon. The amount collected was sufficient to use immediately. In a round bottom flask the compound V was dissolved in dichloromethane under inert conditions and twelve equivalents of
fresh thionyl chloride was added using a dropping funnel. After one night of stirring the solution was removed under high vacuum overnight to give the intermediate with the chloride. The yellow solid was cooled down with acetone and dry ice in dry condition, and in a separate flask the N-(2-pyridylmethyl)glycine ethyl ester was dissolved in ethanol and cooled to −78 °C. The second solution was added slowly dropwise at low temperature. After 30 min of stirring the solution was allowed to warm to room temperature, refluxed for 2h and stirred overnight at room temperature. Finally, the solvent was removed under vacuum and the remaining solid was re-dissolved in phosphate buffer (pH 7.4, 0.1 M) and extracted with dichloromethane. The final product VI was purified on a neutral alumina column using heptane, 2-propanol, and 1% of trimethylamine as eluent. The deprotection step was performed before the synthesis of the different complexes, and is described in the beginning of the complex synthesis.

**N-(2-pyridylmethyl) glycine as the second arm in L2**

An alternative synthesis to get H2L2 more directly was also tested. N-(2-pyridylmethyl) glycine was prepared and used for the synthesis of the unprotected ligand. The synthesis of N-(2-pyridylmethyl) glycine was a modification of the DPA synthesis, where the 2-pyridinecarboxaldehyde was condensed with a solution of glycine (Figure 19). The reaction worked properly, and the desired product was obtained in reasonable yield and purity. However, when the N-(2-pyridylmethyl) glycine was attached to make the complete H2L2 ligand, the product started to polymerize over several days. This was detected in the HPLC-MS, where peaks of a high mass started to dominate the spectrum over time. Hence, the decision to use an ester protection on the glycine was necessary to maintain the ligand available for the complexation.

![Figure 19: Synthesis of N-(2-pyridylmethyl) glycine.](image)

**BPCPMP (L3)**

The synthesis of the third ligand is shown in Figure 20. The oxidation to get VIII was done similarly to the first step in the L2 ligand synthesis. In this
particular case ten equivalents of manganese oxide were necessary to promote the double oxidation of \( \text{I} \). After 16 h the mixture was filtered through celite and washed several times with chloroform. The bright yellow solution was dried under high vacuum to give orange crystals of \( \text{VIII} \). The double aldehyde was purified on a silica column using a mixture of heptane and ethyl-acetate with 1 percent of acetic acid as eluent.

For the preparation of the complete ligand the same method as for \( \text{L1} \) was used\(^{110} \). The N-(2-pyridinemethyl)glycine ester was dissolved in dry dichloromethane and cooled in an ice-bath with calcium chloride to get the solution below 0 °C. Compound \( \text{VIII} \) was dissolved in dichloromethane, transferred to a dropping funnel, and slowly added to the N-(2-pyridinemethyl)glycine ester solution. The solution was left to stir for a couple of hours at low temperature before adding the sodium borohydride and it was then allowed to warm to room temperature. The solution was quenched with sodium hydrogen carbonate and the ligand was extracted with dichloromethane. The product \( \text{IX} \) was purified by a column chromatography on silica with heptane ethyl acetate and two percent of trimethylamine as eluent. As in the \( \text{L2} \) synthesis, the deprotection step was performed directly before the synthesis of the metal complex.

![Diagram](image_url)

\( \text{Figure 20: Synthesis of \text{IX}, precursor of \text{L3}. \text{VIII} \) was obtained in 5\% yield and \text{IX} \) was obtained in 59.2\% yield.\)

**Synthesis of the metal complexes**

The complexes from the \( \text{L1} \) and \( \text{L2} \) ligands were synthesized under an inert atmosphere in the glovebox.

For the synthesis of the iron manganese complex with \( \text{L1} \), Fe[CH\(_3\)CN]\(_6\)(PF\(_6\))\(_2\) was used as source of iron ions. It was synthetized following a literature procedure\(^{113} \) and the complex salt was then stored inside the glovebox.
**Deprotection of L2 and L3**

The ethyl esters in L2 and L3 was removed in a deprotection step before use in complexation reactions. As shown in Figure 21 a solution of methanol with the compound VI or IX was left to stir at room temperature for 24 h with lithium hydroxide. The amount of lithium should not be more than 1.5 equivalent for L2 and 2.5 equivalent for L3 because it also can form a phenolate salt which is difficult to remove.

\[
\begin{align*}
\text{VI} & \quad \text{1.5 equiv. LiOH} \quad \text{MeOH} \quad \text{LiHL2} \\
\text{IX} & \quad \text{2.5 equiv. LiOH} \quad \text{MeOH} \quad \text{Li2HL3}
\end{align*}
\]

*Figure 21 Deprotection of L2 and L3.*

**Synthesis of Mn2L1 and Fe2L1**

The synthesis of the two homometallic complexes [Fe2L1(OAc)2(PF6)] and [Mn2L1(OAc)2(PF6)], hereafter called Fe2L1 and Mn2L1, was performed applying some modifications to a procedure from the literature.51

The L1 ligand was yellow and oily so it was weighed in a flask and put under vacuum, to remove any residue of water and solvent, and became a solid spongy mass. The flask was transferred into the glovebox where, thanks to the inert environment, the metal complex could be kept at a low oxidation state. The ligand was dissolved in dry acetonitrile and, after Fe(CH3COO)2 was added, the mixture was left to stir for a few minutes in order to let the metal incorporate into the ligand. The salt was used not only as iron source but also as a source for the acetate bridges. Since the formed complex had a charge of
+1, one equivalent of ammonium hexafluorophosphate was added using PF$_6^-$ as the counterion source. The ammonium ion also functions as positive counterion to the excess acetate.

Until this point the complex was in solution and a large amount of dichloromethane was added, to form a red-brown precipitate in the bottom of the flask with a yield of 95%.

The synthesis of Mn$_2$L$_1$ was performed following almost the same procedure. The spongy solid mass of L$_1$ was dissolved in dry acetonitrile in the glove box and Mn$_2$(CH$_3$COO)$_2$ was added. The cloudy mix was left to stir for a couple of minutes until the solution turned clear. As for the Fe$_2$L$_2$, the same steps of addition of ammonium hexafluorophosphate and precipitation of the complex with dichloromethane were performed. The solid formed in the bottom of the flask as a pale-yellow dust (yield 73%).

Synthesis of MnFeL$_1$

The difficult part in the synthesis of [FeMnL$_1$(CH$_3$COO)$_2$](PF$_6$) (FeMnL$_1$) was to place two different metals ions with almost the same ionic radius in two different pockets. As mentioned above L$_1$ was treated under vacuum in order to remove residues of solvent and water. The compounds used for the synthesis of the FeMnL$_1$, were Mn(CH$_3$COO)$_2$ as source of Mn$^{2+}$ and acetate bridges, and [Fe(CH$_3$CN)$_6$](PF$_6$)$_2$ as source of Fe$^{2+}$ and PF$_6^-$. For the synthesis the Irving-Williams order$^{114}$ was followed for the introduction of the metals. After dissolving the ligand in dry acetonitrile under inert conditions, Mn(CH$_3$COO)$_2$ was added to the mixture that was left to stir for a couple of minutes before the [Fe(CH$_3$CN)$_6$](PF$_6$)$_2$ was added. The solution turned from clear and yellow to dark with blue shadows. The mixture was left to stir for 1 h and the solvent was removed under reduced pressure. The complex was precipitated twice in acetonitrile/diethyl ether mixture (yield 82%). The complex was obtained as a dark blue solid and the ICP-MS analysis confirmed the presence of manganese and iron in a ratio of 1.2:1.

Even if the complex was prepared in Fe$^{II}$/Mn$^{II}$ oxidation state, the difference between the theoretical elemental analysis and the obtained shows that there is a second PF$_6^-$ entrapped in the lattice of the precipitate, even after several recrystallizations. In paper I we formulate the complex as [FeMnL$_1$(CH$_3$COO)$_2$](PF$_6$)(HPF$_6$).

All metal complexes were stored in an inert atmosphere (glove box) until further characterization.
Synthesis of \(\text{Mn}_2\text{L}_2\) and \(\text{Fe}_2\text{L}_2\)

The syntheses of the complexes with the \(\text{L}_2\) ligand did not follow any published procedure but it was adjusted from the \(\text{L}_1\) complexation. Both the syntheses of \(\text{Fe}_2(\text{L}_2)(\text{CH}_3\text{COO})_2\) \((\text{Fe}_2\text{L}_2)\) and \(\text{Mn}_2(\text{L}_2)(\text{CH}_3\text{COO})_2\) \((\text{Mn}_2\text{L}_2)\) were performed in the glove box in order to avoid oxidation of the metal ions. After deprotection the ligand \(\text{L}_2\) was isolated as an oil on the bottom of the flask. For the synthesis of \(\text{Mn}_2\text{L}_2\) the ligand was dissolved in acetonitrile and \(\text{Mn}(\text{CH}_3\text{COO})_2\) was added forming a foggy solution. In this situation the total charge of the ligand is -2 and, adding the two negative acetate bridges and the two metal ions (\(\text{Mn}^{2+}\)) the final charge is neutral, thus there is no need to add any counterion. After one hour a very small amount of methanol was added to the foggy solution that became yellow and clear. The mixture was left to stir overnight. The day after a white precipitate, presumably the \(\text{Li}(\text{CH}_3\text{COO})\), appeared in the bottom of the flask. The reaction mixture was filtered with a filter cannula into another flask, the solvent was removed and the metal complex precipitated (35% yield).

For the synthesis of \(\text{Fe}_2\text{L}_2\), the deprotected ligand was dissolved in acetonitrile and \(\text{Fe}(\text{CH}_3\text{COO})_2\) was added. The solution color changed from red, due to the dissolution of the iron salt, to yellow/green when the iron was complexed. As in the case of \(\text{Mn}_2\text{L}_2\), the overall charge of the complex was zero thus no counterion was needed. After one night of stirring the liquid was filtered to remove the \(\text{Li}(\text{CH}_3\text{COO})\) precipitate. The liquid was transferred in another flask and the solvent was removed under high vacuum leaving a solid precipitate on the bottom of the flask (58% yield).

Synthesis \(\text{FeMnL}_2\)

The synthesis of the \(\text{FeMn}(\text{L}_2)(\text{CH}_3\text{COO})_2\) \((\text{FeMnL}_2)\) was a little more complicated, due to the use of two different metals. The deprotected ligand was dissolved in \(\text{CH}_3\text{CN}:\text{CH}_3\text{OH}\) (5:1) mixture. Following the experience of the synthesis of \(\text{FeMnL}_1\), one equivalent of \(\text{Mn}(\text{CH}_3\text{COO})_2\) was added to the ligand and stirred for about 1 h due to the difficulty in dissolving the manganese salt. When the solution turned clear, one equivalent of \(\text{Fe}(\text{CH}_3\text{COO})_2\) was added and the flask was left to stir overnight. The day after the solvent was reduced to half of the volume and a precipitate developed. The precipitate was removed by filtration, and after diethyl ether was added a yellow precipitate appeared (21% yield).
**Synthesis Fe₂L₃**

The amount of IX obtained was not enough for deprotection and for the synthesis of all three metal complexes. The diiron complex [FeIII₂(L3)(CH₃COO)₂](PF₆) (Fe₂L₃) was prepared since the corresponding complexes had been easily obtained for the previous two ligands. This complexation was made outside the glove box under ambient conditions. The deprotected Li₂HL₃ was dissolved in methanol and two equivalents of Fe(CH₃COO)₂ were added. The color of the mixture changed from amber to dark brownish-red. In this case the L₃ has charge of −3, and with the two carboxylate bridges the total charge of the ligands are −5. Thus, NOPF₆ was added to oxidize all the iron but also to provide a source of counter ion, since the total charge of the complex was +1. After a few minutes the mixture was overlaid with diethyl ether and a lilac precipitate appeared. This solid was purified via recrystallization by dissolving the complex in a mixture of methanol and acetonitrile and precipitating it with diethyl ether. After three times the liquid was removed and the solid was dried overnight under high vacuum (40% yield).
Characterization of the complexes

Mass spectrometry

As mentioned above, two types of instruments with different mass analyzers were used to identify and confirm the presence of the metal complexes. The instrument used to analyze the compounds was a Finningan LCQ system coupled with Dionex HPLC. The ionization system was an electrospray ionization source with two RF only octopoles in tandem as mass analyzer. In all the analyses performed in direct injection mode the complex was dissolved in dry and O<sub>2</sub> free acetonitrile. The solution in a syringe was injected in the instrument by a syringe pump.

In addition, a second instrument used for HR-MS was the Bruker MicroTOF that uses the TOF as mass analyzer. In addition, HR-MS of the complexes with L<sub>1</sub> was also performed by Organisch-Chemisches Institut der Westfälischen Wilhems-Universität, Münster, Germany.

In order to discuss the complexes, they are presented organized by their metal content. Firstly, the three complexes containing two iron ions, where the isotope pattern is always visible and unique, then the two complexes containing two manganese ions and at the end the two complexes containing one iron and one manganese ion.

Before going into a detailed analysis of the complexes, a small consideration should be done. In the complexes with L<sub>2</sub> the ligand was deprotected by hydrolysis of the ester group using LiOH before being used for complexation. After the synthesis of the three complexes no lithium contamination was detected in any of the three spectra. This was also confirmed with ICP-MS were no lithium was observed.
Diiron complexes

In the MS of each of the complexes (Fe₂L₁, Fe₂L₂, Fe₂L₃) it is possible to observe the presence of 6 peaks with different abundances, due to the natural isotope abundance of iron.

Starting from the Fe₂L₁, in the total spectrum from HR-MS (Figure A1 in the appendix) a main peak at m/z =379.584 with z = 2 is visible. All the other peaks are below 15% indicating a high purity of the compound. The fragment could be identified as [Fe₂L₁(CH₃COO)₂]^2+ (calc. m/z = 379.5835). According with the total charge +2, the metals are in the Fe²⁺/Fe³⁺ oxidation state and the complex is not attached to the counter ions. The presence of Fe³⁺ could be caused by the conditions during the analysis since because the synthesis was performed in inert and controlled conditions.

The detailed spectrum (Figure 22) shows the typical isotope pattern for a complex with two iron ions. The different peaks are comparable with the calculated m/z values up to the third digit so the complex is quite pure and clean.

![Figure 22: Isotope pattern of Fe₂L₁, z = 2. Above the spectrum obtained from the HR-MS analysis, below the theoretical spectrum.](image-url)
In the full MS of the Fe\textsubscript{2}L\textsubscript{2} (Figure A2) there is also only one major species at \( m/z = 725.1335 \) present. Some other small peaks are visible but with intensity lower than 10% suggesting also in this case that the solution injected is rather pure. The main peak has \( z = 1 \) and could be associated to the complex [Fe\textsubscript{2}L\textsubscript{2}(CH\textsubscript{3}COO)\textsubscript{2}]\textsuperscript{+} (calc. \( m/z = 725.1230 \)). This suggests that even here the metals are in Fe\textsuperscript{II}/Fe\textsuperscript{III} probably due to the oxidation of one of the two metals during the ionization\textsuperscript{70}. Also in this case the complex “fly alone” without the counter ion.

Looking more carefully at the detailed MS (Figure 23) it is possible to observe the isotope pattern of the two iron ions demonstrating the incorporation of the metals in the ligand. Also in this complex observed and calculated \( m/z \) values are in excellent agreement.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{isotope_pattern.png}
\caption{Isotope pattern of Fe\textsubscript{2}L\textsubscript{2}.}
\end{figure}

In the full spectrum of Fe\textsubscript{2}L\textsubscript{3} (Figure A6) it is possible to observe there is one big peak at \( m/z = 691.06682 \) with \( z = 1 \). This fragment is attributable to [Fe\textsubscript{2}L\textsubscript{3}(CH\textsubscript{3}COO)\textsubscript{2}]\textsuperscript{+} (calc. \( m/z = 691.0784 \)). Analyzing the isotope pattern of the Fe\textsubscript{2}L\textsubscript{3} (Figure 24) is it possible to observe the fingerprint of the iron incorporate in the ligand. The presence of many other peaks in the spectrum suggests that the mixture injected is not completely clean. In fact, the signal with \( m/z = 706.0869 \) is slightly above the mass calculated and it matches properly with the mass of Fe\textsubscript{2}L\textsubscript{3} and two lithium ions, meaning that part of the total complex could be [Fe\textsubscript{2}L\textsubscript{3}Li\textsubscript{2}(CH\textsubscript{3}COO)\textsubscript{2}]\textsuperscript{+} (calc. \( m/z = 706.1183 \)). The lithium was thus not completely removed and can still associate with the complex.
Dimanganese complexes

Manganese has only one stable natural isotope $^{55}\text{Mn}$ which makes the identification of the manganese complexes less straightforward due to the lack of a unique isotope pattern.

In the MS of $\text{Mn}_2\text{L}_1$ (Figure A5) there are two main peaks present, a sign of purity of the compound obtained. The MS shows a main peak at $m/z = 757.17330$ with $z = 1$ that can be attributed to $[\text{Mn}_2\text{L}_1(\text{CH}_3\text{COO})_2]^+$ (calc. $m/z = 757.1732$). Unlike $\text{Fe}_2\text{L}_1$ the charge is +1 and the two manganese ions are both in the +2 oxidation state. The stability of the manganese ions in a low oxidation state also observed in the UV-Vis chapter below.
The complex \( \text{Mn}_2 \text{L}_2 \) was difficult to identify since the total charge of the ligand, Mn\(^{II}\) ions and acetate bridge is 0. To overcome this problem the complex \( \text{Mn}_2 \text{L}_2 \) was dissolved in a solution of acetonitrile and 0.05% of formic acid so that the protons from the formic acid can protonate the complex to give a positively charged ion. Thanks to these protons it is possible to see the complex with one additional mass unit in the MS (Figure 26) without changing the oxidation state of the metals, the ion \([\text{Mn}_2 \text{L}_2(\text{CH}_3\text{COO})_2 + \text{H}]^+\) was thus identified\(^{70, 73}\) (observed \(m/z = 724.0907\), calculated \(m/z = 724.1276\)). However, the formate ions react with the complex, as was the case observed in Figure 26 where one acetate bridge was substituted with a formate obtaining \([\text{Mn}_2 \text{L}_2(\text{CH}_3\text{COO})(\text{HCOO}) + \text{H}^+]^+\) with mass \(m/z = 710.2244\) (calc. \(m/z = 710.1213\)).
Figure 26: Mass spectrum of Mn₂L₂. On the right of the spectrum is the peak associated with [Mn₂L₂(CH₃COO)₂⁺H⁺]+ with two acetate bridges (m/z = 724.091) and with the related isotope pattern. On the center-left the peak of the complex with one acetate bridge substituted with a formate ion [Mn₂L₂(CH₃COO)(HCOO)+H⁺]+ (m/z = 710.078) and the related isotope pattern.

Iron-manganese complexes

In FeMnL₁ the presence of two types of metals and the difference of just one mass unit between them make the identification an interesting challenge. In the MS of FeMnL₁ (Figure A3) it is possible to observe the main peak at m/z = 379.0852 with z = 2 that is the exact mass of the complex formulated as [FeMnL₁(CH₃COO)₂]²⁺ (calc. m/z = 379.0851). This suggests that one of the two metals, probably the Fe, is oxidized from the +2 to the +3 oxidation state thus doubling the charge of the complex. The challenge of this type of heterobimetallic complex is that the calculated mass is exactly one unit less than Fe₂L₁ and one unit more than Mn₂L₁, two possible side products. However, the isotope pattern of the complex is sufficiently unique to identify FeMnL₁. Observing the isotope pattern of FeMnL₁ (Figure 27) in particular focusing on the peak at m/z = 379.58652 it is possible to detect that the difference of the relative abundance of the signal between the calculated and the obtained is about the 20%. The peak selected is close
with the main peak of Fe$_2$L$_1$ ($m/z = 379.58374$), so it is possible that the excess is attributable to the presence of Fe$_2$L$_1$.

To quantify the presence of manganese and iron in the sample, ICP-MS was used and confirmed the ratio of 1.2:1 of Mn:Fe.

Figure 27: Isotope pattern of FeMnL$_1$. Total charge of the complex is +2. Above the spectrum obtained from the HR-MS analysis, below the theoretical spectrum.

As the overall charge of FeMnL$_2$ all complexes in the M$^{II}$/M$^{II}$ state is neutral, thus undetectable by MS, a few drops of acetonitrile mixed with 0.05% of formic acid were used as solvent prior to the injection into the instrument. Like in the heterometallic complex with L$_1$, the FeMnL$_2$ should exhibit an intermediate mass between the Fe$_2$L$_2$ and Mn$_2$L$_2$, two likely side products. A peak with $m/z = 725.135$ is observed in the MS of FeMnL$_2$ and could be associate with [FeMnL$_2$(CH$_3$COO)$_2$ + H$^+$]$^+$ (calc. $m/z = 725.1339$) but the peak does not show a perfect isotope pattern (Figure 28), a symptom of presence of impurities as the above-mentioned side products.

The higher abundance of the peak at $m/z = 724.1372$ in the measured isotope pattern compared with the calculated isotope pattern could be associated with the presence of the Mn$_2$L$_2$ but also potentially the presence of the oxidized FeMnL$_2$ ([FeMnL$_2$(CH$_3$COO)$_2$]$^+$ with the metals in a M$^{III}$/M$^{II}$ state, (calc. $m/z = 724.1261$).
IR spectroscopy

The IR-spectra of all the seven complexes Fe$_2$L$_1$, Mn$_2$L$_1$, FeMnL$_1$, Fe$_2$L$_2$, Mn$_2$L$_2$, FeMnL$_2$ and Fe$_2$L$_3$ were recorded on an ATR-IR system. From the IR spectra of the complexes with L$_1$ in Figure 29, we can find the two characteristic signals from the bridging acetate ligands that coordinate the two metal ions, the asymmetric ($\nu_{as}$) and symmetric ($\nu_s$) vibrations. The $\nu_{as}$ was observed at 1590 cm$^{-1}$, 1568 cm$^{-1}$, and 1589 cm$^{-1}$, and the $\nu_s$ 1416 cm$^{-1}$, 1420 cm$^{-1}$, and 1436 cm$^{-1}$ in Fe$_2$L$_1$, Mn$_2$L$_1$, and FeMnL$_1$ respectively. The difference in wavenumbers between these two types of vibrations in a complex, $\Delta$, is associated with the coordination mode of the acetate ligand. This parameter is $\Delta = 173$ cm$^{-1}$, 168 cm$^{-1}$ and 153 cm$^{-1}$ for Fe$_2$L$_1$, Mn$_2$L$_1$ and FeMnL$_1$ respectively. This matches the expected type of bidentate coordination in syn-syn.$^{82,116}$

Many of the features in the IR spectra of the complexes with L$_1$ can also be seen in and compared to the IR spectra of the complexes with L$_2$ (Figure 30). For example, the $\Delta = 172$ cm$^{-1}$, 160 cm$^{-1}$ and 154 cm$^{-1}$ for Fe$_2$L$_2$, Mn$_2$L$_2$, and FeMnL$_2$, respectively.
Another structural feature that can expected in this type of complex is a uni-
dentate coordination from the carboxylate in the L2 ligand itself.

Comparison of IR spectra between complexes with different ligands

In our studies, we were able to synthesize three complexes with different metal composition using the same ligands but also complexes with the same metal composition in three different ligands. The comparison between the diiron complexes with the three ligands is shown in Figure 31.
Figure 30: IR spectra of Fe\textsubscript{2}L\textsubscript{2}, Mn\textsubscript{2}L\textsubscript{2}, and FeMnL\textsubscript{2}.

Comparing the IR spectra of the three iron complexes, the carboxylate region (ca 1700-1350 cm\textsuperscript{-1}) is particularly interesting and gives information about the coordination between the metals and the acetate ligands plus the metal and the dinucleating ligand. In Fe\textsubscript{2}L\textsubscript{1}, the peak of the asymmetric carboxylate stretching (\(v_{as}\)) at 1590 cm\textsuperscript{-1} is very sharp and the \(v_{s}\) at 1416 cm\textsuperscript{-1} has equal intensity. When one pyridine is substituted with one carboxylate in Fe\textsubscript{2}L\textsubscript{2}, there is a change both the peaks are broader but the \(v_{as}\) peak at 1583 cm\textsuperscript{-1} now has higher intensity than the \(v_{s}\) at 1411 cm\textsuperscript{-1}. When instead two carboxylic groups are part of the dinucleating ligand as in Fe\textsubscript{2}L\textsubscript{3}, the change is even more visible the \(v_{as}\) is very broad and the \(v_{s}\) is much lower in intensity and split in two peaks that are not found in the other two complexes.

These differences are probably due to the presence of two type of carboxylates with different coordination modes in Fe\textsubscript{2}L\textsubscript{2} and Fe\textsubscript{2}L\textsubscript{3}. The carboxylate arms that are incorporated in the dinucleating ligand can coordinate to the metal in the unidentate fashion. These carboxylates should have a significantly larger \(\Delta\) than the bridging carboxylate, and possibly the \(v_{as}\) is present as broad shoulder at \(\sim 1650\) cm\textsuperscript{-1} on the \(v_{as}\) bridging peak in the spectra of Fe\textsubscript{2}L\textsubscript{2} and Fe\textsubscript{2}L\textsubscript{3} while the \(v_{s}\) peak should cause the broadening in the \(\sim 1400\) cm\textsuperscript{-1} region for Fe\textsubscript{2}L\textsubscript{2} and the split in two peaks in Fe\textsubscript{2}L\textsubscript{3}.
Some other peaks in the three spectra can be identified as originating from the ligand backbone and can be compared with the already existing literature. For example, previous studies have suggested that the peaks at 1476 cm⁻¹ [Fe₂L₁], 1479 cm⁻¹ [Fe₂L₂], and 1475 cm⁻¹ [Fe₂L₃] are due to the semi-circle stretching of pyridine rings. Other small peaks at 761 cm⁻¹ [Fe₂L₁], 766 cm⁻¹ [Fe₂L₂], 766 cm⁻¹ [Fe₂L₃] are associated to a pyridine ring substituted in the 2 position, and are defined as the wagging vibration (vibration in couples out of plane) of the four adjacent protons. This signal unfortunately appear in the same region as a possible peak from the νₓs from M-O-M group. In the region from 1160 to 1000 cm⁻¹, it is possible to distinguish five peaks for Fe₂L₁ and four peaks for Fe₂L₂ and Fe₂L₃ at similar wavenumbers. These five peaks can also be identified with pyridine vibrations.
Interestingly, there is a difference in the peaks between 1320 and 1270 cm$^{-1}$, where in Fe$_2$L$_1$ there are three distinct signals but only weak shoulders in Fe$_2$L$_2$. The peaks at 838 cm$^{-1}$ and 557 cm$^{-1}$ in the spectra of Fe$_2$L$_1$ and Fe$_2$L$_3$ are associated with the PF$_6^-$ counterion in these two complexes.

**IR spectra of DPA and IX**

In Figure 32, it is possible to compare the IR spectra of IX, the deprotected form of L$_2$, where there are three pyridine groups and a carboxylic ester, with the DPA fragment which involves only two pyridine groups connected by an amine and further identify some important structural parts of the ligands. In the spectrum of IX there is a sharp signal at 1737 cm$^{-1}$ and a signal at 1188 cm$^{-1}$ both associated with the carboxylic ester$^{80}$. The disappearance of the later signal can be used as marker for the deprotection of IX before complexation with metals.

The quadrant stretching of the pyridines are seen both for DPA (1593 cm$^{-1}$ and 1569 cm$^{-1}$) and for IX (1591 cm$^{-1}$ and 1571 cm$^{-1}$). The semicircle stretching in the pyridine rings is also visible in the IX spectrum at 1475 cm$^{-1}$ and 1434 cm$^{-1}$, and in the DPA spectrum at 1480 cm$^{-1}$, 1538 cm$^{-1}$ and 1415 cm$^{-1}$ $^{80}$.

![Figure 32: IR spectra of the DPA and IX.](image)
Mössbauer spectroscopy

The Mössbauer technique can be used to analyze the coordination environment of iron ions in different samples. The analysis of the iron containing complexes $\text{Fe}_2\text{L}_2$, $\text{FeMnL}_2$ and $\text{Fe}_2\text{L}_3$ were performed at room temperature (295 K) using a $^{57}\text{CoRh}$ source. The compounds were dried and mixed with boron nitrate in the glove box in order to avoid any oxidation and moisture contamination. The concentration of iron in the sample was 5 mg/cm$^2$ in order to have a good signal. The holder of the sample was held in a chamber flushed with nitrogen gas. For $\text{Fe}_2\text{L}_1$ and $\text{FeMnL}_1$ the analysis was performed at 78 K in collaboration with Prof. Volker Schünemann at Techische Universität Kaiserslautern, Germany.

$\text{Fe}_2\text{L}_1$ and $\text{FeMnL}_1$

The samples of $\text{Fe}_2\text{L}_1$ and $\text{FeMnL}_1$ were prepared in a glove box, frozen at N$_2$(l) temperature in a holder and measured at low temperature. The analysis of the $\text{L}_1$ complexes at 78 K (Table 1) shows the presence of two types of iron species in $\text{Fe}_2\text{L}_1$ (Figure 33). The most abundant species (62%) is form a Fe$^{\text{II}}$ species. The high isomer shift $\delta = 1.19$ and $\Delta E_Q = 2.80$ are matching with the typical features of a high-spin (HS) Fe$^{\text{II}}$ species. The second species (39%) is a Fe$^{\text{III}}$ ion. Also in this case the isomer shift is high for Fe$^{\text{III}}$ so it is in a HS state. The high quadrupole splitting is sign of a distorted an octahedral environment around the iron ion$^{118}$.

<table>
<thead>
<tr>
<th>Metals</th>
<th>$\delta$</th>
<th>$\Delta E_Q$</th>
<th>Area (%)</th>
<th>$\Gamma$</th>
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</thead>
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<td>0.43</td>
<td>0.45</td>
<td>20</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Table 1 Mössbauer data for $\text{Fe}_2\text{L}_1$ and $\text{FeMnL}_1$ at 78 K.

Comparing with the Mössbauer data from Borovik,$^{51,119}$ where they also were able to crystallize diiron complexes with the $\text{L}_1$ ligand, it possible to see that their value of $\delta =1.21$ mm/s and $\Delta E_Q = 2.86$ mm/s for Fe$^{\text{II}}$ at 55 K and 93 K,
are comparable with our data. This is confirming that the Fe\textsuperscript{II}-HS, in an octahedral coordination, and surrounded by nitrogen and/or oxygen donor, is present in Fe\textsubscript{2}L\textsubscript{1}\textsuperscript{119,120}.

![Mössbauer spectra of Fe\textsubscript{2}L\textsubscript{1} at 77K](image.png)

Figure 33: Mössbauer spectra of Fe\textsubscript{2}L\textsubscript{1} at 77K, the red line shows the resonance of the Fe\textsuperscript{III}, the green line shows the resonance of the Fe\textsuperscript{II}.

The Mössbauer spectrum of FeMnL\textsubscript{1} also has two components, Fe\textsuperscript{II}-HS and Fe\textsuperscript{III}-HS. In FeMnL\textsubscript{1} the most abundant form is also Fe\textsuperscript{II} (80%) while Fe\textsuperscript{III} is present in a much lower amount (20%). Considering the isomer shift of 1.19 and the high quadrupole splitting it is possible to suggest a coordination with nitrogen and oxygen in a distorted octahedral geometry also for the iron in FeMnL\textsubscript{1}\textsuperscript{118,120}.

The Mössbauer analysis could also confirm the hypothesis that FeMnL\textsubscript{1} is mainly in the Fe\textsuperscript{II}/Mn\textsuperscript{II} oxidation state with a small part in the Fe\textsuperscript{III}/Mn\textsuperscript{II} oxidation state.

\textbf{Fe\textsubscript{2}L\textsubscript{2} and FeMnL\textsubscript{2}}

The Mössbauer spectrum of Fe\textsubscript{2}L\textsubscript{2} also have contributions from more than one iron species and the high difference in δ reveals the presence of both Fe\textsuperscript{II} and Fe\textsuperscript{III}. In this complex the two metal ions are in an asymmetric ligand, and the surrounding should be different based on the presence of different amount of nitrogen and oxygen donors. In one pocket there are 4 oxygen donors and 2 nitrogen donors, and the second pocket has 3 nitrogen donors and 3 oxygen donors. The spectrum of Fe\textsubscript{2}L\textsubscript{2} presents four signals, two signals are from Fe\textsuperscript{II}-HS and two signals are from Fe\textsuperscript{III}-HS, presumably one Fe\textsuperscript{II}-HS signal and
one Fe$^{III}$-HS signal from each pocket. Overall the Fe$^{II}$ species (65%) are more abundant than the Fe$^{III}$-HS species (35%) indicating a mixture of oxidation states in the product. The spectra of Fe$_2$L2 have lower δ than for Fe$_2$L1$^{120}$. According with Schünemann, the δ for Fe$^{II}$-HS decreases with decreasing of the coordination number$^{120}$. However, also the temperature has an effect on the δ. In fact the study from Borovik et al.$^{51,119}$ shows a shifting of δ towards lower values with increasing temperature. Thus we can assume that our low δ value could be due to that the analysis was performed at room temperature. This is supported with the Fe$^{III}$ signal, in the same complex, that presents δ values very close to what is expected for octahedral coordination around a Fe$^{III}$-HS ion.

<table>
<thead>
<tr>
<th>Metals</th>
<th>δ</th>
<th>ΔE$_Q$</th>
<th>Area (%)</th>
<th>Γ</th>
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<tbody>
<tr>
<td>A</td>
<td>Fe$^{2+}$- HS</td>
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<tr>
<td>B</td>
<td>Fe$^{2+}$- HS</td>
<td>1.10</td>
<td>2.51</td>
<td>39</td>
</tr>
<tr>
<td>C</td>
<td>Fe$^{3+}$- HS</td>
<td>0.39</td>
<td>1.47</td>
<td>19</td>
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<tr>
<td>D</td>
<td>Fe$^{3+}$- HS</td>
<td>0.33</td>
<td>0.53</td>
<td>16</td>
</tr>
<tr>
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<td>Fe$^{2+}$- HS</td>
<td>1.08</td>
<td>1.92</td>
<td>27</td>
</tr>
<tr>
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<td>2.61</td>
<td>39</td>
</tr>
<tr>
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<td>1.23</td>
<td>14</td>
</tr>
<tr>
<td>D'</td>
<td>Fe$^{3+}$- HS</td>
<td>0.27</td>
<td>0.30</td>
<td>20</td>
</tr>
<tr>
<td>Fe$_2$L3</td>
<td>Fe$^{3+}$- HS</td>
<td>0.39</td>
<td>1.62</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Fe$^{3+}$- HS</td>
<td>0.39</td>
<td>0.80</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 2: Mössbauer data at room temperature for Fe$_2$L2, and FeMnL2, and Fe$_2$L3.

If the sum of all the four signals of Fe$_2$L2 is considered 100% it is possible to proposed the pair of signals that come from each pocket, regardless of the oxidation state of the iron ion, as these pairs should be close to 50% of the area together. The most balanced aggregate is then that A+C (45%) is associate with one pocket and B+D (55%) with the other.

For the FeMnL2 complex, there is also two Fe$^{II}$-HS signals and two Fe$^{III}$-HS signals in the Mössbauer spectrum. Also in this case the isomer shift is lower compared to the FeMnL1 probably due to the measurement being performed at room temperature. Interestingly, the area of the four different signals are not very different compared to the Fe$_2$L2 so it appears that the iron ion has no clear preference for a specific pocket in FeMnL2.
**Fe₂L₃**

Fe₂L₃ was the only complex synthetized with the L₃ ligand, and both the metal positions should have equal occupancy and most importantly the same atomic surrounding with 3 nitrogen donors and 3 oxygen donors. The Mössbauer spectrum (Figure 34) confirmed the presence of only Fe³⁺-HS in the sample, as both the species found have the same δ (Table 2). A Fe³⁺-HS octahedrally coordinated by nitrogen and/or oxygen donors has a δ from 0.4 mm/s to 0.6 mm/s, so this coordination can be expected for Fe₂L₃. The presence of two different Fe³⁺-HS species could come from two different isomers of Fe₂L₃, and a X-ray crystal structure of the complex could give more information if this is the case.

![Figure 34](image)

*Figure 34: Room temperature Mössbauer spectra for, from top to bottom, Fe₂L₂ (a), FeMnL₂ (b), and Fe₂L₃ (c). The red and blue sub spectra shows the Fe²⁺ and Fe³⁺ resonance signals, respectively.*

**Electrochemistry**

The electrochemical analysis mostly performed in this project was cyclic voltammetry. The procedure was the same for all the complexes. All analyses were performed with 0.1 M tetrabutylammonium perchlorate (TBAP) as electrolyte. The reference electrode used was the Ag/AgNO₃ (10 mM) in acetonitrile. Between scans, the working electrode was polished with a pad and alumina (grade 0.005 µm), then soaked in acetonitrile to clean the electrode. All
the potentials are given vs Ag/AgNO₃. The scan speed was 100 mV/s unless otherwise specified.

**Diiron complexes**

The series of the diiron complexes gave the opportunity to analyze the influence of carboxylate groups in the ligand on the redox behavior of the metal ions. In Fe₂L₁ (Figure 35), there are two distinct and reversible redox couples for the changes in oxidation state of the metal ions in the complex. E₁₁₁₁₁₁ at -0.34 V corresponds to the Fe³⁺-Fe²⁺ / Fe²⁺-Fe²⁺ couple and E₁₁₁₁₁₂ at 0.37 V correspond to the Fe³⁺-Fe³⁺ / Fe³⁺-Fe²⁺ couple. The difference between the two redox couples is 740 mV.

In the CV of Fe₂L₂, the two redox couples are less reversible and broader. The Fe³⁺-Fe²⁺ / Fe²⁺-Fe²⁺ couple is at a much more negative potential (E₂₂₂₂ = -0.74 V) while the Fe³⁺-Fe³⁺ / Fe³⁺-Fe²⁺ is almost not affected (E₂₂₂₂ = 0.30 V) this gives a difference between the redox couples of 1040 mV. Since one the redox couples is very similar between the two complexes (E₁₁₁₁₁₂ = 0.37 V and E₂₂₂₂ = 0.30 V), this couple is likely associated with the iron ion in the pocket with the two pyridines in Fe₂L₂. The iron in the other pocket should feel the presence of the carboxylate group making the oxidation easier and shifting the redox couple to more negative values. In the CV of Fe₂L₃, both redox couples are shifted to much more negative values, with E₃₃₃₃₃₃ = -1.67 V and E₃₃₃₃₃₂ = -1.16 V with a difference between the couples of 510 mV. Comparing the three spectra CVs it is possible to see that the presence of one carboxylate arm in Fe₂L₃ lowers the oxidation potential for the metal in one pocket much more than for the other and increases the distance between the two redox couples. When the complex is symmetric with one carboxylate in each pocket as in Fe₂L₃, the difference between the redox couples are smaller and at more negative values.
Figure 35: From top to bottom, CVs of Fe$_2$L$_1$, Fe$_2$L$_2$ and Fe$_2$L$_3$. Working electrode and counter electrode were glassy carbon. The CVs of Fe$_2$L$_1$ and Fe$_2$L$_2$ were recorded in a glove box, and Fe$_2$L$_3$ under Ar in a set up similar to the one shown in Figure 12.
**Dimanganese complexes**

The CV of \(\text{Mn}_2L_1\) (Figure 36) shows two reversible redox couples, for the \(\text{Mn}^{III}-\text{Mn}^{II}/\text{Mn}^{II}-\text{Mn}^{II}\) couple \(E_{L1b1} = 0.17\) V and for the \(\text{Mn}^{III}-\text{Mn}^{II}/\text{Mn}^{II}-\text{Mn}^{II}\) couple \(E_{L1b2} = 0.73\) V. These potentials are similar to values from literature for this complex\textsuperscript{121-124}. The difference between the redox couples is 560 mV similar to the literature value of 550 mV\textsuperscript{122}. In the CV of \(\text{Mn}_2L_2\) (Figure 36) the redox couples are again less reversible and broader, and the first is shifted to more negative values (\(E_{L2b1} = -0.08\) V) and the second almost unaffected (\(E_{L2b2} = 0.61\) V). The difference between the couples increases in \(\text{Mn}_2L_2\) to 690 mV.

![Figure 36: Comparison of CVs for the dimanganese complexes with \(\text{Mn}_2L_1\)(above) and \(\text{Mn}_2L_2\)(below). The electrochemistry was performed in a glove box. Working electrode and counter electrode were glassy carbon.](image)

**Iron-manganese complexes**

The CV of \(\text{FeMnL1}\) was first reported in Paper I, but similar complexes exist in the literature\textsuperscript{52}. The CV of the heterobimetallic complex (Figure 37) shows more peaks than the homometallic analogues. There is a non-reversible reduction at \(-0.35\) V and a non-reversible oxidation at \(0.16\) V together with two more reversible redox couples (\(E_{L1c3} = 0.37\) V and \(E_{L1c4} = 0.77\) V). As all
these redox events are at values close to redox couples in Fe$_2$L$_1$ and Mn$_2$L$_1$, there was the possibility that the two homometallic complexes are coexisting in solution rather than the heterobimetallic FeMn complex. Hence, in order to remove any doubt about the formation of FeMnL$_1$ and gather additional information, a solution with Fe$_2$L$_1$ and Mn$_2$L$_1$ was mixed in an electrochemical cell. The obtained CV (dashed line, figure 3 in Paper I) did not match with the CV of FeMnL$_1$, especially in the more negative region, and excludes the presence of homometallic complexes in any appreciable amount. The non-reversible oxidation at 0.07 V and reduction at -0.44 V are indications of a chemical charge in the complex upon the first oxidation. This could be associated to a change in coordination of the acetate bridges but more experiments would be needed to confirm this.

The CV of FeMnL$_2$ resembles the CV of FeMnL$_1$ even though the current is lower in comparison. Overall, the carboxylate arm in FeMnL$_2$ appears to shift the potential of the redox couples to more negative values. Comparing with figure 3 in Paper II, it is possible to associate the redox couples with the homometallic complexes, so the $E_{L2c^4} = 0.59$ V can be associated with $E_{L2b^2} = 0.63$ V and $E_{L2c^1} = -0.62$ V with $E_{L2a^1} = -0.74$ V. The two central couples $E_{L2c^2} = -0.075$ V and $E_{L2c^3} = 0.18$ V do not have a corresponding redox couple in the respective homometallic complexes. There are two possible explanations that could justify this behavior. The first hypothesis is that a mix of homometallic Fe$_2$L$_2$ and Mn$_2$L$_2$ – rather than FeMnL$_2$ – formed in the synthesis. Hence, what is measured corresponds to the result of the sum of the two single CVs. The second hypothesis is that two forms of FeMnL$_2$ are present. This matches with the nature of the two pockets gives two different environments for the metals ions. As we know from the Mössbauer analysis, it was not possible to control the position of the metal ions in the different pockets of FeMnL$_2$ and the metals find the position randomly. Some complexes will have the Fe ion in the pocket with three nitrogen donor and the Mn ion in the pocket with two nitrogen donors and one oxygen donors. At the same time other complexes with have the position of the two metals in the pocket reversed. This condition created four different redox couples, which are independent to each other. To understand better this concept please see table in Paper II.
Metal exchange

The presence of iron-manganese cofactors in nature in addition to the diiron and dimanganese cofactors raises questions on how assembly of a heterometallic cofactor can be made in the presence of both metals. The relative stability of homometallic complexes with the ligand L1 and L2 ligands was investigated with this in mind. The experiment consisted in a simple titration of the homometallic complexes with the opposite metal, followed by CV.
When Mn$_2$L$_1$ was titrated with [Fe(CH$_3$CN)$_6$(PF$_6$)$_2$] (Figure 38) the peaks associated with the Mn redox couples gradually diminished. A new peak at -0.25 V appeared and increased in intensity with increasing iron concentration, indicating that a new species was formed. In fact, the CV after addition of one equivalent of Fe$^{2+}$ showed large similarities with the CV of FeMnL$_1$ (Figure 37), suggesting that a Fe-Mn complex was formed during the titration of Mn$_2$L$_1$ with Fe$^{2+}$. When Fe$_2$L$_1$ was titrated with Mn(ClO$_4$)$_2$ no changes were observed in the CV after the first equivalent of Mn$^{2+}$, indicating that the two iron centers stayed coordinated to the ligand and that the complex is stable towards metal exchange.

The same experiment was performed starting from Mn$_3$L$_2$ (Figure 39) titrating with [Fe(CH$_3$CN)$_6$](PF$_6$)$_2$. Similar to the first titration some changes was observed. The redox wave $E_{L2b1} = -0.065$ V almost disappear after the first addition of Fe$^{2+}$. Interestingly, two redox wave formed, on oxidative at -0.49 V and one reductive at -0.64 V both signals can be associated with the insertion of the Fe in the ligand, comparing with $E_{L2c1} = -0.615$ V in the CV of FeMnL$_2$ in Figure 37.
As previously mentioned, the function of RNR is to remove one oxygen from the ribonucleotides in order to obtain deoxyribonucleotides. In the enzyme a cysteine radical plays a crucial role in this reaction. In Class I RNRs this radical is formed with the help of a tyrosyl radical and/or the oxidized dimetal site.

In this thesis UV-Vis spectroscopy was used to assess the reactivity of the different complexes and the ability to form higher valent complexes in the presence of different oxidants. The analysis was performed for all the complexes with \( \text{L1} \) and \( \text{L2} \). The solutions of the complexes were prepared in the glove box under inert conditions and analyzed in a gas-tight cuvette. Molecular oxygen was added using a gas-tight syringe. For the other oxidants, a separate solution was prepared in dry and degassed solvent and injected with a syringe. In the UV-Vis spectra of the \( \text{L1} \) complexes in acetonitrile (Figure 40)
Fe$_2$L1 has strong band at 433 nm, FeMnL1 has broad shoulder at 394 nm and a weak band at 570 nm, and Mn$_2$L1 has almost no absorption in the visible region.

Comparing the spectral changes on addition of O$_2$ to the complexes with L1 it was observed that Mn$_2$L1 is not reacting with oxygen, but both Fe$_2$L1 and FeMnL1 did react with O$_2$. The diiron complex reacted slowly with the added oxygen over the course of an hour, the band at 430 nm decreased in intensity and a new shoulder ~600 nm increased in the UV-Vis spectrum (Figure 41).
Figure 41: Fe$_2$L$_1$ reacted with O$_2$.

The spectral changes showed two isosbestic points suggesting that the complex converted to a single product. This product was proposed to be singly oxidized complex Fe$^{	ext{III}}$Fe$^{	ext{II}}$L$_1$, based on comparison with literature data as described in Paper I. Interestingly, for FeMnL$_1$ the oxygen reacted much faster with the complex and the band at 570 nm increased in intensity (Figure 6 in paper I), the reaction was over within a minute.

Figure 42: Comparison of UV-Vis spectra of Fe$_2$L$_2$, Mn$_2$L$_2$, and FeMnL$_2$ in methanol.

The UV-Vis spectra of the L$_2$ complexes in methanol are less intense and with fewer distinct features than the corresponding L$_1$ complexes. Fe$_2$L$_2$ has a
band at ~378 nm, FeMn\(\text{L2}\) only has a very broad tail that stretches out in the visible region, and Mn\(\text{2L2}\) has almost no absorption in the visible region.

For the \(\text{L2}\) complexes the reactivity of a broader range of oxidants were tested. Molecular oxygen was used also in this case together with H\(_2\)O\(_2\) (30 wt% in water), tert-Butyl hydroperoxide (TBHP) (70 wt% in water), and 3-chloroperbenzoic acid (mCPBA). When H\(_2\)O\(_2\) and TBHP were added they were in aqueous solution so mCPBA dissolved in dry methanol was used as an oxidant were the effect of water could be removed in the analysis. The addition of oxygen to Fe\(\text{2L2}\) caused only small spectral changes below 400 nm. In contrast, addition of the other oxidants caused a large increase in the absorption between 300 and 400 nm (Figure 6 in Paper II). The addition of oxidant to Mn\(\text{2L2}\) caused less dramatic increases, but the addition of H\(_2\)O\(_2\) caused the appearance of a peak at 430nm that was best visible after one equivalent (Figure A 10).

The addition of 1 equivalent of oxygen to FeMn\(\text{L2}\) caused a decrease in the intensity of the UV-vis spectrum. However, the addition of one equivalent of mCPBA caused an increase at wavelengths below 400 nm and the appearance of a weak band at ~500 nm.

\[\text{FeMnL2} \quad \text{+mCPBA} \quad \text{+O}_2\]

\[\begin{array}{cccc}
300 & 350 & 400 & 450 & 500 & 550 & 600 & 650 & 700 & 750 & 800 \\
0.0 & 0.2 & 0.4 & 0.6 & 0.8 & 1.0 \\
\end{array}\]

\[\text{Absorbance}\]

\[\text{wavelength (nm)}\]

\[304\]

\[\sim 500\]

\[\text{Figure 43: UV-vis spectra of FeMnL2 before (black) and after addition of O}_2\text{ (red) or mCPBA (green). All spectra are in methanol.}\]
EPR spectroscopy

Samples for EPR spectroscopy were prepared in the glove box. The complexes were dissolved in acetonitrile transferred to EPR tubes. After the tubes were sealed inside the glove box, the samples were taken out and immediately frozen in liquid nitrogen.

Diiron complexes

The EPR spectrum of Fe$_2$L$_2$ in acetonitrile (Figure A8) is not showing any EPR-signals since Fe$^{II}$ have an even number of unpaired electrons, making the system EPR silent in the normal perpendicular detection mode. In addition, the two Fe$^{II}$ ions in Fe$_2$L$_2$ are likely antiferromagnetically coupled, giving a total spin system with $S = 0$. However, it is possible to see a very small signal at $g \sim 4.3$ due to the presence of small amount of Fe$^{III}$ in the sample. Fe$^{III}$ has an odd number of unpaired electrons that makes the complex visible in the EPR. For high-spin Fe$^{III}$ species the EPR-signal is normally found in the $g \sim 4$ region.
The oxidation experiments with \( \text{Fe}_2\text{L}_2 \) described in the UV-vis chapter in this thesis were combined with the EPR analysis. A small volume (0.3 mL) of each of the oxidized samples was transferred from the gas-tight cuvette to an EPR tube and frozen in liquid nitrogen. In Figure 44 the EPR spectra of \( \text{Fe}_2\text{L}_2 \) in the \( g = 4 \) region, before and after addition of the different oxidants, are shown. In all the EPR spectra, it is possible to observe the formation of Fe\(^{III}\). An EPR signal at \( g \approx 4.3 \) is often associated with mononuclear Fe species, especially octahedrally solvated iron ions. In this case, the EPR signals are different for different oxidants suggesting that some of the oxidants can interact or coordinate with the metal ion (e.g. \( \text{H}_2\text{O}_2 \) where there is a shoulder in the EPR signal with a \( g \)-value > 4.3). However, it is difficult from this experiment to determine if the complexes are oxidized but still intact, of if the oxidation has led to degradation where some of the iron is still coordinated to a ligand but not as a dimeric complex.

The EPR-spectrum of \( \text{Fe}_2\text{L}_3 \) (Figure 45) has a signal with \( g \approx 4.22 \) associated with Fe\(^{III}\)-HS. In line with the proposed structure of the complex, the two metal ions should probably be antiferromagnetically coupled, making the complex
EPR-silent. However, the low intensity of the spectrum could mean that the concentration of the species giving rise to the signal is very low compared to the total amount of the complex. Since the signal also has a g-value at \( \sim 9.5 \) it could originate from a Fe\(^{III}\)-HS in a distorted octahedral coordination environment and perhaps originate from some of the impurities observed in MS.

![EPR spectrum of Fe\(_2\)L\(_3\) in acetonitrile at 7K recorded with a microwave power of 20mW.](image)

**Figure 45:** EPR spectrum of Fe\(_2\)L\(_3\) in acetonitrile at 7K recorded with a microwave power of 20mW.

**Dimanganese complexes**

Mononuclear Mn\(^{II}\) species generally have a characteristic 6-line EPR signal at \( g \sim 2 \) due to the interaction between the nuclei \( (I = 5/2) \) and the electron. This signal can act as a Mn\(^{II}\) fingerprint.

Mn\(_2\)L\(_1\) has been studied using EPR spectroscopy previously and shows an EPR-signal in the Mn\(^{II}\)-Mn\(^{II}\) oxidation state\(^{124,125}\). This signal is not from the antiferromagnetically coupled ground state but has been proposed to originate from a low-lying excited state.

The EPR-spectrum of Mn\(_2\)L\(_2\) is very broad (Figure 46, bottom), with features over the whole range from 500-5000 G. The spectrum changes shape when the temperature is increased (Figure A9), and a feature at \( g \sim 2.5 \) is increasing in intensity. This feature was also observed in the EPR-spectrum of Mn\(_2\)L\(_1\), which indicates that the EPR signal from Mn\(_2\)L\(_2\) also could originate from a low-lying excited state.

Interestingly, when Mn\(_2\)L\(_2\) was reacted with H\(_2\)O\(_2\) a small 16-line EPR signal centered on \( g \sim 2 \) appeared (Figure 46, top). This signal is a fingerprint of a
Mn^{III}-Mn^{IV} system with two \( \mu \)-oxo bridges and is showing that Mn\(_2\)L\(_2\) can be oxidized to a high-valent state.

**Figure 46**: EPR spectra of Mn\(_2\)L\(_2\) before (bottom) and after addition of H\(_2\)O\(_2\). Both spectra were recorded at 7 K with a microwave power of 2 mW. The asterisk (*) at \( g \approx 2 \) is marking a signal coming from Cu\(^{2+}\) in the cavity.

**Iron Manganese complexes**

In the EPR-spectrum of FeMnL\(_1\) there is a signal centered at \( g = 2.12 \) with six lines indicating the presence of Mn\(^{II}\) in the complex (Figure 1 in Paper I). In the EPR spectrum of FeMnL\(_2\) at 5 K there is instead a broad signal at \( g \approx 2 \) and at higher temperatures (20 K) a small feature at \( g \approx 2.5 \), similar to the features observed for the dimanganese complexes above (Figure 47).
Figure 47: EPR spectra of FeMn$\text{L}_2$ at 5K (red) and 20 K (black). The asterisk (*) indicates the feature at $g \sim 2.5$ visible at 20 K. The spectra were recorded with a microwave power of 200 $\mu W$.

The EPR spectrum of FeMn$\text{L}_1$ was also recorded after a bulk electrolysis at $-0.1$ V. For this experiment the complex was dissolved in 5 mL of acetonitrile with TBAClO$_4$ as electrolyte. The working electrode and counter electrode were glassy carbon and the analysis was performed inside the glove box. The potential was maintained constant at $-0.1$ V in continuing stirring of the solution in order to allow the complex to get completely reduced. After 6 h, 0.3 mL of sample was collected and frozen in liquid nitrogen inside an EPR tube. The resulting EPR spectra are shown in Figure 48 and Figure 49.
Figure 48: EPR spectrum of FeMnL1 in acetonitrile after a bulk electrolysis at -0.1 V for 6 h. The spectrum was recorded at 12 K with a microwave power of 2 mW.

The six lines associated with MnII at g-value ~ 2.0 are still visible, but there is also another signal at g ~4. Figure 49 is showing the magnification of the area around g ~4 and it is possible to observe three overlapping series of six lines. We propose that this signal originates from the presence of the two metal ions magnetically coupled inside the ligand. To understand this signal better further analysis has to be made.

Figure 49: Magnification of the g ~4 region in the EPR spectrum of FeMnL1 after bulk electrolysis at -0.1 V. Recorded in acetonitrile at 12 K with a microwave power of 2 mW.
Oxygen evolution reactions

The main idea of this analysis was to evaluate whether the complexes could produce O$_2$ by disproportionation of oxidants. The oxygen produced were detected by a Clark-type electrode$^{126}$, which is commonly used to detect dissolved oxygen$^{127,128}$. The chamber of the Clark electrode is separated from the cathode by a Teflon permeable membrane that allows the passage of oxygen to the surface of the platinum cathode. The oxygen that reaches the surfaces of the platinum electrode is reduced to water.

The analysis (Figure 50) was performed on Mn$_2$L$_2$ and Fe$_2$L$_2$ and the complexes were dissolved in oxygen free methanol. Before the experiment started, the atmospheric oxygen present in the solvent was removed by bubbling the solvent solution with argon.

When the system showed no trace of oxygen, the oxidant was added in large excess. The oxidants used were H$_2$O$_2$, mCPBA and TBHP i.e. the three oxidants that were used for the oxidation experiments followed by UV-vis spectroscopy. As mentioned before H$_2$O$_2$ and TBHP were in water solution, and

![Figure 50: Oxygen evolution from Fe$_2$L$_2$ and Mn$_2$L$_2$. The oxidants were added at the 250 s mark. H$_2$O$_2$ was 30 wt% in water, TBHP was 70 wt% in water and mCPBA was dissolved in methanol. All the analysis were performed in methanol.](image)
mCPBA was dissolved in dry and oxygen free methanol. Fe$_2$L$_2$ can produce oxygen by disproportionation of H$_2$O$_2$ and mCPBA, but not from TBHP. Mn$_2$L$_2$ showed only oxygen evolution when H$_2$O$_2$ was used as an oxidant. The complex therefore mimics Mn-catalase, an enzyme that contains two Mn-ions and that disproportionate H$_2$O$_2$.$^{129}$
Water oxidation with an Ir-based catalyst (paper IV)

Nowadays, one of the most important challenges in society is to supply energy for the increasing global demand without producing greenhouse gases, radioactive waste or other forms of waste that damages the environment. One alternative could be to develop artificial photosynthesis as a method to convert solar energy to a chemical fuel. Water splitting is then the reaction of interest and it is fundamental to develop robust and cheap catalyst, especially for the water oxidation reaction.

Molecular catalysts for this reaction are at the moment mostly based on metal complexes that include rare metals like ruthenium. In the study in Paper IV, four Iridium complexes with heterocyclic carbene (NHCs) ligands that contain pendent coordinating groups are investigated as catalyst (Figure 51).

Figure 51: complexes 1-3 and 5 studied in this work as catalysts for water oxidation.
The water oxidation was performed by dissolving the catalysts in a methanolic solution at pH = 1 (0.1 M HNO₃) and adding a large amount of ceric ammonium nitrate (CAN) as chemical oxidant⁶⁴. The oxygen produced by the reaction was detected by a Clark electrode.

After the addition of CAN, each complex presented a different lag time before the oxygen evolution started, the shortest was 5-10 s for 1, for 2 it was about 30 s, and for 3 it was instead 3-5 min. This type of lag time has also been reported by other groups¹³⁰,¹³¹ and it is associated with the time it takes for the initial complex to transform into the active catalytic species. A possible explanation of the long lag time of 3 could be that the pendant amine coordinating the Ir³⁺ has to disconnect to create an open coordination site for the water to bind. If the first three complexes present different lag times due to the release of chloride to create the site for the water than a more interesting result was obtained with complex 5 that lacks chloride ligands. In this latter complex, the lag time was similar to complex 1 but the TON and TOF were much lower than 1, suggesting that the chloride is important in the catalytic cycle.

To investigate the durability of the catalysts, 1 and 2 were utilized to perform a series of multiple additions of the oxidant. As reported in paper IV, a series of aliquots of 400 equivalent of CAN was injected in sequence, after purging with argon prior the addition. After the first three additions, the amount of evolved oxygen was the same and it started decreasing from the fourth addition. Interestingly, the two catalysts were active even after the seventh additions. Complex 1 was also utilized to perform a long time scale experiment in which the oxygen was monitored by gas-chromatography. With 16.67 µM of complex 1 and 0.182 M of CAN at pH of 1.0 the complex generates a TON of 2520 molO₂ molcat⁻¹ that consists in a yield for the oxygen evolution of 92%. Also, 1 has one pendant hydroxyl group that is lacking in an analogue catalyst previously reported by Hetterscheid et al.⁶⁸ but the catalytic activity in our case is higher suggesting that the hydroxyl group plays a role for the catalytic activity.

Photo-induced water oxidation activity was another analysis performed on complexes 1-3 with the employment of [Ru⁰(bpy)₃]Cl₂ as photosensitizer and Na₂S₂O₈ as sacrificial electron acceptor in a borate buffer (pH 7.6). However, under these conditions the complexes produced significantly low levels of oxygen.
Along with the water oxidation reaction, UV-vis spectroscopy and mass spectrometry were used to identify active species and intermediates in the reaction. Following the UV-vis spectra of 1, a peak at 455nm appeared soon after the addition of CAN. This peak then converted into a peak at 555 nm within a few minutes, accompanied by a change of color of the solution from transparent to purple. The peaks at 455nm and 555 nm are manifestations of in situ generated high-valent molecular iridium species. \(^{69}\) The formation of IrO\(_x\) nanoparticles\(^{132}\) have been reported to cause a broad peak at 580 nm, but in the case of 1, no indications of nanoparticle formation was observed in e.g. dynamic light scattering experiments.

A similar picture merge in case of 2, for which two peaks at 475 nm and 640 nm appeared after the addition of CAN, with a following rise of a peak at 555 nm indicating that the same type of species forms in this complex.

Mass spectrometry is useful to identify and compare the different species that are formed under similar conditions but with different catalysts. Catalysts 1 and 2 were initially dissolved in an aqueous solution at pH = 7 and then injected in the mass spectrometer giving peaks at \(m/z = 523.21\) and \(m/z = 539.17\) that are associated with \([\text{IrCp}^*(\text{NHC}) - 2\ \text{Cl}^-]\) and \([\text{IrCp}^*(\text{NHC}) - \text{H}^+]\). These two forms of the complexes are stable enough to be present also after the addition of CAN. The addition of CAN, also causes the appearance of new peaks at \(m/z = 539.14\) and \(m/z = 555.19\), which are assigned to new species that are derived from 1 and 2 that carry an additional oxygen atoms, forming species like \([\text{IrCp}^*(\text{NHC}) + \text{O} - 2\text{Cl}^-]\) and \([\text{IrCp}^*(\text{NHC}) + \text{O} - \text{H}^+]\). A similar species with an oxygen atom inserted has also observed in the work of Macchioni and co-workers\(^{67}\).
Conclusions

This aim of the thesis project was to gain further insight in the different metal cofactors of enzymes like the Class I RNRs. During these years of work, three different ligands and several iron and manganese complexes with the ligands were synthetized. The purpose of these ligands is to give a structure that mimic interesting features of the real cofactors in order to mimic aspects of their structure and function.

For the iron complexes, a first interesting observation was that Fe₂\(L₁\) and Fe₂\(L₂\) are more stable in the Fe\(\text{II}-\text{FeIII}\) mixed oxidation state than the Fe\(\text{II}-\text{FeII}\) state. This was seen in the Mössbauer analysis, which confirmed the presence of signals for Fe\(\text{II}\) and Fe\(\text{III}\), and the mass spectrometry, which showed a species with a charge of +2 for Fe₂\(L₁\) and +1 for Fe₂\(L₂\), indicating a mixed valence state in both cases. Analyzing all the CV of the three diiron complexes with the different ligands it was observed that the presence of one carboxylate donor instead of one pyridine donor shifts the redox potential into more negative values, as expected. This trend was especially clear in Fe₂\(L₃\) where two carboxylates donors, one in each pocket moved the potential of both redox couples to much more negative value.

The two manganese complexes, Mn₂\(L₁\) and Mn₂\(L₂\) are much more stable at low oxidation states. Indeed, mass spectrometry showed the presence of a species with charge +1 in Mn₂\(L₁\) and in the case of Mn₂\(L₂\) the complex had to be protonated to be detectable with MS.

In the case of FeMn\(L₁\) and FeMn\(L₂\) it was observed in a titration experiment followed by CV, that the heterobimetallic complexes are more stable than the manganese homometallic ones. In fact it was observed that, in the presence of free iron ions in the solution, only one metal was substituted to form a heterobimetallic complex.

It was also shown that the presence of two different metals in FeMn\(L₁\) makes the reaction with oxygen faster than the homometallic complexes containing only iron or only manganese. This reactivity difference with oxygen could be
one of the advantages of a FeMn cofactor in the Class Ic RNRs compared to the FeFe or MnMn forms.

The results in paper IV suggest that, among the four Ir complexes investigated as water oxidation catalysts, 1 is the most active with a TON >2500 and an oxygen yield of more than 90% relative to the amount of oxidant used. A possible explanation for this high activity could be the chloride ligand coordinating to the iridium center, which presence enhances the activity about 5 times over 5 that lacks a chloride. The presence of pendant groups on the NHC, particularly the hydroxy group, was also proposed to stabilize the structural integrity of the complex for high-valent iridium species. In general, with the hydroxy-containing substituent on the NHC ligand a robust molecular catalyst for water oxidation with high TON was obtained.

Future outlook.

In conclusion of this work, three ligands were made and seven metal complexes where synthesized and characterized. In order to continue these studies, further analysis should be made starting with the natural development to synthetize and characterize the Mn homometallic complex as well as the heterobimetallic Fe-Mn complex with the L3 ligand. It would also be interesting to characterize with IR-spectroscopy all the complexes with L2 and L3 using 13C-labelled acetate bridges. This should change the vibration frequency of the bridging acetate and allow the identification of the peaks from all the carboxylate groups in the complex, also the ones in the dinucleating ligand. Possible further synthesis includes developing further ligands. Two examples are shown here, the first present two carboxylate donors in one side and DPA on the other side (Figure 52, on the left). This type of structure could be interesting to make in order to more strongly try to select the pocket for the different metals in a heterobimetallic complex. Complexes with this ligand would also be very interesting to compare with the L3 complexes as the ligands have the same composition but different symmetry. Another interesting system will be the ligand with 4 carboxylate donors (Figure 52, on the right). In order to the position of the metals could be to create an asymmetric ligand where is possible to place DPA or the two carboxylate groups on one side of the ligand.
and in the opposite side a bulky non-coordination system in order to prevent of the formation of a bimetallic complex. In this way is possible to generate mononuclear complexes and characterize and identify characteristic features of the coordination of the metals with the pyridine groups or carboxylate groups. Last but not least, one could consider to add different R groups to the pyridine donors in this type of ligands. For example, some studies have been reported\textsuperscript{133-135} where a bulky R group in the 5 position on the pyridine allowed the coordination of only one carboxylate bridge. An alternative idea could be instead of using 5 position, the R group should be on the 4 position in order to influence the electronic of the ring and consequently the placement and the stability of the two metal ions.

\textit{Figure 52: Proposed structure of new ligands}
Livet som vi känner till det kräver endast sex grundämnen från den periodiska tabellen, de så kallade CHNOPS-grundämnen, som är förkortningen för elementen Kol (C), väte (H), kväve (N), syre (O), fosfor (P) och Svavel (S). Dessa sex grundämnen är byggnestenarna biologiska molekyler så som proteiner, nukleinsyror, lipider och polysackarider. Andragrundämnen som är nödvändiga för ett antal processer inom signalering, transport och katalys finns även i levande celler men i små mängder. Dessa grundämnen är metaller som natrium (Na), kalium (K), magnesium (Mg), klor (Cl), kalcium (Ca), kisel (Si) och vissa övergångsmetaller som mangan (Mn), järn (Fe), zink (Zn), nickell (Ni), kobolt (Co), koppar (Cu), molybdén (Mo), vanadium (V), volfram (W) och kadmium (Cd). Biologisk-oorganisk (eller biooorganisk) kemi är den kemi-disciplin där metaller och deras roll i biologiska system studeras.


Huvudfunktionen för oxidoreduktas-enzyme är att, beroende på substrat, katalysera oxidations- / reduktionsreaktioner på cellerna. Enzymet som har nyckelrollen i denna avhandling är ribonukleotidreduktas (RNR) och det omvandlar ribonukleotiden i deoxyribonukleotidan genom att ersätta en hydroxyl i position 2 'med ett väte. RNR finns i alla levande celler och det finns tre huvudtyper av RNR: klass I, klass II och klass III. Den senare klassen är mycket känslig för syre och innehåller järn-svavelkluster som metallkofaktor. Klass II-enzyme fullständigt likgiltiga med syre och använder en kobaltkofaktor, så kallad adenosylocobalamine, som är ett derivat från vitamin B12. Tvärtom, i klass I är syret grundläggande för att starta reaktionen på metallstationen i kofaktorn. Denna klass av enzym är indelad i tre underklasser, nämligen

Målet med denna avhandling var att syntetisera biomimetiska komplex för att bättre förstå de två metalljonernas roll i klass Ic RNR.

Utgångspunkten var att syntetisera en ligand (L1) med fyra pyridiner som skapade två fickor där de arrangerade metalljonerna. De första två metallerna arrangerades, sedan testades liganden med två manganjoner, och så småningom med båda i det heterobimetalliska komplexet. Dessa tre komplex karakteriserades sedan för att förstå hur deras likheter och olikheter (artikel I). Denna studie visade ett unikt beteende hos Mn-jonen eftersom det verkar som att denna metall föredrar en position i komplexet framför den andra. En vidareutveckling av detta system inkluderade således syntesen av en asymmetrisk ligand (L2) genom att ersätta en pyridin med en karboxylgrupp för att undersöka om asymmetri skulle rikta de olika metallerna till olika fickor (artikel II). Till skillnad från våra förväntningar observerade vi inte en preferens av plats, men det verkar snarare som att en serie komplex som innehåller båda metalljoner i slumpmässiga platser bildas. Den tredje och sista försöket involverade en annan symmetrisk ligand (L3), där de två pyridinerna ersattes med två karboxylgrupper, och som var mer syredonerande än L1 för att mer specifikt likna metallbindingssättet i RNR (artikel III). I detta sammanhang observerade vi att redoxparet skiftade mot mer negativa värden, vilket innebär att närvaron av syre gör metallen lättare att oxidera.

Sammantaget indikerar dessa data att det två metallerna inte har någon positionspreferens och att metallerna oxiderar snabbare i närvaro av syre, men ytterligare studier behövs dock för att bättre belysa rollen för två olika metaller i Ic-kofaktorn.
The life as we know it is primarily built up from only six elements from the periodic table, called CHNOPS, which are the abbreviation of the elements Carbon (C), Hydrogen (H), Nitrogen (N), Oxygen (O), Phosphorous (P) and Sulphur (S). These six elements are the building blocks of major cellular components like proteins, nucleic acids, lipids, polysaccharides. Other elements are also present in living cells but in small traces that are necessary to catalyse particular reactions like signalling, transportation, and catalysis. These elements are metals like Sodium (Na), Potassium (K), Magnesium (Mg), Chlorine (Cl), Calcium (Ca), Silicon (Si), and some transition metals like Manganese (Mn), Iron (Fe), Zinc (Zn), Nickel (Ni), Cobalt (Co), Copper (Cu), Molybdenum (Mo), Vanadium (V), Tungsten (W) and Cadmium (Cd). Understanding the role of how the metals interact with each other and their structure inside biological systems is the objective of a branch of chemistry called Biological-Inorganic (or bioinorganic) Chemistry.

The fundamental biological structures in this study are the enzymes, particular protein that catalyse the chemical reactions inside the cells. Enzymes are divided by the international Union of Biochemistry and Molecular Biology in six big categories based on their function: oxidoreductases (which are the focus of this thesis), transferases, hydrolases, lyases, isomerases, ligases and translocases. Some enzymes need also an additional component - called cofactor - to do the catalysis. The cofactor can be an organic or inorganic structure and in this thesis we focused on the latter one, particularly on metals ions. The main function of the oxidoreductase enzymes is to catalyse oxidation/reduction reactions inside the cells, depending on the type of substrate. The enzyme that has the key role in this thesis is the ribonucleotide reductase (RNR) and it converts the ribonucleotide in deoxyribonucleotide by substituting a hydroxyl in position 2’ with a hydrogen. RNRs are present in all living cells and there are three main types of RNR: class I, class II and class III. The latter are very sensitive to oxygen and contain an iron – sulphur cluster as metal cofactor. Class II enzymes instead are completely indifferent to the presence of oxygen and they have a cobaltous cofactor, called adenosylcobalamine, which is
a derivative from vitamin B$_{12}$. On the contrary, in Class I the oxygen is fundamental to start the reaction in the metal site of the cofactor. This class of enzymes is subdivided in three subclasses, namely class Ia, Ib, and Ic, depending on the cofactor they have. The first group, class Ia, is the most extensively studied and it has a Fe-Fe metal cofactor close to a tyrosine residue. The class Ib is similar to class Ia by having a tyrosine residue but at the same time it differs by having a Mn-Mn cofactor. The last and most important class for this thesis, the class Ic, has a Fe-Mn heterobimetallic cofactor and it has a phenyl residue instead of a tyrosine one.

The aim of this thesis work was then to synthetize complexes that mimic the function of these cofactors to better understand the role of the two different metals in class Ic RNRs.

The starting point was to synthetize a ligand (L$_1$) with four pyridines that created two pockets where arrange the metal ions. The first two metals arranged were Fe, then the ligand was tested with two Mn ions, and eventually with both ions in the heterobimetallic complex. These three complexes were then characterized to highlight differences and similarities in their chemistry (Paper I). This study showed a unique behaviour of the Mn ion as it seems that this metal prefers one site over the other. Thus, a further development of this system included the synthesis of an asymmetric ligand (L$_2$), by substituting one pyridine with one carboxylic group, in order to investigate whether the asymmetry would direct the different metals to different pockets (Paper II). Contrarily to our expectations we did not observe a preference of site, but it rather appears that a series of complexes containing both metal ions in random sites forms. The third and last trial involved another symmetric ligand (L$_3$), where the two pyridines were substituted with two carboxylic groups, and that had more oxygen donors than L$_1$ to resemble more specifically the metal binding site of the RNRs (Paper III). In this context, we observed that the redox couple shifted toward more negative values, meaning that the presence of oxygen makes the metal easier to oxidize.

Taken together these data indicate that there is no preference of arrangement site and the metals oxidize faster in presence of oxygen, but further studies are however needed to better elucidate the role of two different metals in the Ic cofactor.
La vita come la conosciamo richiede solo sei elementi della tavola periodica, identificati come CHNOPS, che consiste nell'abbreviazione degli elementi carbonio (C), idrogeno (H), azoto (N), ossigeno (O), fosforo (P) e zolfo (S). Questi sei elementi sono i mattoncini dei principali componenti cellulari come le proteine, gli acidi nucleici, i lipidi ed i polysaccaridi. Ci sono anche altri elementi nelle cellule viventi, ma questi si ritrovano in piccole trace, e sono necessari per catalizzare reazioni particolari come la comunicazione, il trasporto e la catalisi. Questi elementi sono metalli come il sodio (Na), potassio (K), magnesio (Mg), cloro (Cl), calcio (Ca), silicio (Si) e alcuni metalli di transizione come il manganese (Mn), ferro (Fe), Zinco (Zn), Nichel (Ni), Cobalto (Co), Rame (Cu), Molibdeno (Mo), Vanadio (V), Tungsteno (O), Cadmio (Cd), Cromo (Cr). Comprendere il ruolo di come i metalli interagiscono tra loro e la loro struttura all'interno dei sistemi biologici è l'obiettivo di un ramo della chimica chiamato Chimica Biologico-Inorganica (o bioinorganica).

Le strutture biologiche di fondamentale importanza per questo studio sono gli enzimi, delle proteine particolari che catalizzano le reazioni chimiche all'interno delle cellule. Gli enzimi sono divisi dall'Unione Internazionale di Biochimica e Biologia Molecolare in sei categorie principali in base alla loro funzione: ossidoriduttasi, che sono i protagonisti questa tesi, e poi le transferasi, idrolasi, liasi, isomerasi, ed infine le ligasi e traslocasi. Alcuni enzimi necessitano anche di un componente aggiuntivo, chiamato cofattore, per eseguire la catalisi. Il cofattore può essere una struttura organica o inorganica e in questa tesi ci siamo concentrati su questi’ultimi, ed in particolare sugli ioni di metalli.

La funzione principale delle ossidoreduttasi è di catalizzare le reazioni di ossidazione / riduzione all'interno delle cellule a seconda del tipo di substrato disponibile. L'enzima che ha il ruolo chiave in questa tesi è la ribonucleotide reduttasi (RNR) che converte il ribonucleotide in desossiribonucleotide sostituendo un idrossile in posizione 2' con un idrogeno. Gli RNR sono presenti in tutte le cellule viventi e ci sono tre tipi principali di RNR: classe I, classe II e
classe III. Questi ultimi sono molto sensibili all'ossigeno e contengono un cluster ferro-zolfo come cofattore metallico. Gli enzimi di classe II invece sono completamente indifferenti alla presenza di ossigeno e hanno un cofattore con cobalto, chiamato adenosilcobalamina, che è un derivato della vitamina B12. Al contrario, nella classe I l'ossigeno è fondamentale per iniziare la reazione nel sito del cofattore. Quest'ultima classe di enzimi è suddivisa in tre sottoclassi, vale a dire classe Ia, Ib, e Ic a seconda del cofattore che hanno. Il primo gruppo, la classe Ia, è il più ampiamente studiato e ha un cofattore in metallo Fe-Fe vicino ad un residuo di tirosina. La classe Ib è simile alla classe Ia in quanto ha un residuo di tirosina ma è al tempo diversa poiché il cofattore è composto da ioni Mn-Mn. L'ultima e più importante classe per questa tesi, la classe Ic, ha un cofattore eterobimetallico Fe-Mn e ha un residuo fenilico invece di uno tirosina.

Lo scopo di questa tesi era quindi quello di sintetizzare complessi che mimano la funzione di questi cofattori per comprendere meglio il ruolo dei due diversi metalli nella classe RNR Ic.

Il punto di partenza è stato sintetizzare un ligando (L1) con quattro piridine per creare due tasche in cui posizionare gli ioni metallici. I primi due metalli disposti sono stati ioni Fe, poi il ligando è stato testato con due ioni Mn, ed infine con entrambi gli ioni per creare il complesso eterobimetallico. Questi tre complessi sono stati quindi caratterizzati per evidenziare differenze e somiglianze nella loro chimica (Studio I). Tale studio ha mostrato un comportamento unico dello ione Mn poiché sembra che questo metallo preferisca un sito rispetto all'altro. Pertanto, un ulteriore sviluppo di questo sistema includeva la sintesi di un ligando asimmetrico (L2), sostituendo una piridina con un gruppo carbossilico, al fine di studiare se l'asimetria avrebbe diretto i diversi metalli verso tasche diverse (Studio II). Contrariamente alle nostre aspettative, non abbiamo osservato una preferenza per il sito, ma sembra piuttosto che si formi una serie di complessi contenenti entrambi gli ioni metallici in siti casuali. Il terzo ed ultimo approccio ha coinvolto un altro ligando simmetrico (L3), in cui le due piridine sono state sostituite con due gruppi carbossilici, e con più donatori di ossigeno rispetto a L1 per assomigliare più specificamente al sito attivo delle RNR (Studio III). In questo contesto, abbiamo potuto osservare che la coppia redox si è spostata verso valori più negativi, il che significa che la presenza di ossigeno rende il metallo più facile da ossidare.
Nel loro insieme queste dati indicano che non vi è alcuna preferenza per il sito di disposizione e che i metalli si ossidano più rapidamente in presenza di ossigeno, ma sono tuttavia necessari ulteriori studi per chiarire meglio il ruolo di due diversi metalli nel cofattore Ic.
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Appendix – additional spectra

Figure A1: Full MS of Fe\textsubscript{2}L\textsubscript{1}. The complex was dissolved in acetonitrile.

Figure A2: Full MS of Mn\textsubscript{2}L\textsubscript{1}. The complex was dissolved in acetonitrile.
Figure A3: Full MS of FeMnL1. The complex was dissolved in acetonitrile.

Figure A4: Full MS of Fe2L2. The complex was dissolved in acetonitrile.
Figure A5: Full MS of Mn$_2$L$_2$. The complex was dissolved in acetonitrile. The main peak (m/z = 710.078) is related to protonated Mn$_2$L$_2$ with one acetate bridge substituted with a formate ion [Mn$_2$L$_2$(CH$_3$COO)(HCOO) + H$^+$]$^+$. The second peak (m/z = 724.09) is the protonated Mn$_2$L$_2$ [Mn$_2$L$_2$(CH$_3$COO)$_2$ + H]$^+$.  

Figure A6: Full MS of the complex Fe$_2$L$_3$. The complex was dissolved in acetonitrile.
IR spectroscopy

$\Delta = 193$

Figure A7: IR spectrum of Fe$_2$L$_3$. 
EPR spectroscopy

Figure A8: EPR spectrum of Fe$_2$L$_2$ (above) compared with the cavity (below). The cavity signal is due to the release of Cu$^{2+}$ from the heating coil. The spectra were recorded at 5K, with a microwave power of 20 µW.

Figure A9: EPR spectra of Mn$_2$L$_2$ at different temperatures. The feature indicated at g $\sim$2.5 (*) is more intense at higher temperature. All spectra were recorded with a microwave power of 2 mW.
UV-vis spectroscopy

Figure A 10: UV-vis spectra of $\text{Mn}_2\text{L}_2$ with the addition of $\text{H}_2\text{O}_2$

Figure A 11: Comparison of the UV-vis spectra of $\text{Fe}_2\text{L}_1$ (in acetonitrile), $\text{Fe}_2\text{L}_2$ (in methanol) and $\text{Fe}_2\text{L}_3$ (in acetonitrile).
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