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Vienna University of Technology

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İYTE

DIPLOMARBEIT

Synthesis and Applications of Oxomolybdenum Compounds

ausgeführt am

Izmir Institute of Technology

in Kooperation mit dem

Institut für Angewandte Synthesechemie
der **Technischen Universität Wien**

unter der Anleitung von

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Das, wobei unsere Berechnung versagt, nennen wir Zufall.

Albert Einstein

Danksagung

Zu aller erst möchte ich mich bei **Univ.Prof. Dr. Işil Sözüer** und **Univ.Prof. Dr. Wolfgang Linert** bedanken, die meine Arbeit durch ihre excellente internationale Zusammenarbeit ermöglichten.
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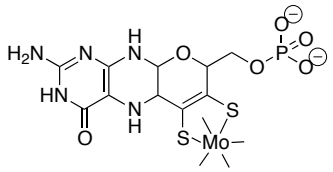
Schließlich möchte ich meiner Chemieprofessorin **Mag. Elisabeth Dietrich**, die bereits während meiner Zeit im Gymnasium mein Interesse an der Chemie geweckt hat, außerordentlich danken.

Abstract

Molybdenum enzymes, which carry a pterine-based molybdenum cofactor in their center, are enzymes, which catalyze plenty of reactions in the human body and play therefore a very important role in its metabolism.



Pterine-based molybdenum cofactor (Moco)

Redox reactions and oxygen transfer reactions, which are omnipresent in the human's metabolism, are performed with the aid of these enzymes.

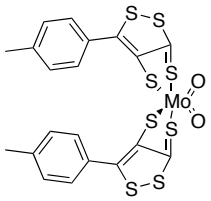
The absence of enzymes of this kind can cause various health problems, which can lead to several disorders or even death in the early childhood.

Since research is very important in this area, model systems of Moco are formed, which hold the possibility to examine the reactivity, the redox potential and also the geometry of different pterine-based molybdenum cofactors.

In model systems of Moco bidentate sulfur donor ligands should be coordinated to the molybdenum metal center.

bidentate sulfur donor ligands

In this thesis the synthesis of a respective ligand was performed and the coordination to molybdenum succeeded, yielding the compound shown in the figure below.

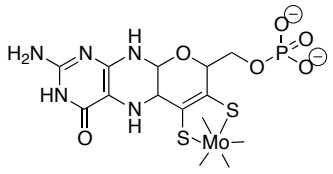


Synthesized model compound

Parts of this thesis were performed at the Institute of Technology in Izmir (IZTECH) in course of my ERASMUS-year in Turkey.

Kurzfassung

Enzyme, die den Molybdäncofactor (Moco) in ihrem Zentrum enthalten, sind von imenser Bedeutung für zahlreiche biochemische Zyklen im menschlichen Körper und spielen daher eine sehr wichtige Rolle im Metabolismus.



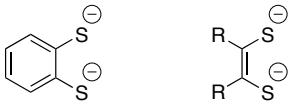
Molybdän-Cofactor (Moco)

Mit Hilfe dieser Enzyme werden sowohl Redoxreaktionen, als auch der Transport von Sauerstoff katalysiert.

Ohne diese Enzyme könnten im menschlichen Körper eine Vielzahl von Problemen auftreten, die zu Bedhinderungen oder sogar dem Tod in der frühen Kindheit führen könnten.

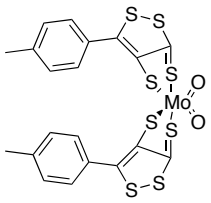
Da die Forschung auf diesem Gebiet von sehr großer Bedeutung ist, wurden Modellverbindungen von Moco hergestellt, durch die es möglich ist, die Reaktivität, das Redoxpotential und auch die Geometrie von Cofaktoren zu untersuchen, die auf dem Molybdäncofaktor basieren.

In Modellsystemen des Molybdäncofaktors wird Molybdän mit einem zweizähnigen Schwefelliganden koordiniert und analysiert.



zweizähnige Schwefelliganden

Im Zuge dieser Diplomarbeit wurde erfolgreich ein derartiger zweizähniger Schwefelligand synthetisiert und die Koordination mit Molybdän durchgeführt, wobei die folgende Verbindung gebildet wurde:



synthetisierte Modellverbindung

Teile dieser Arbeit wurden in Izmir Izmir Institute of Technology (IZTECH) im Zuge meines ERASMUS-Aufenthaltes durchgeführt.

Abbreviations

Besides common abbreviations of the English language and chemical symbols the short forms below are used:

A	absorption
acac	acetylacetonato
AMP	adenosine monophosphate
ATP	adenosine triphosphate
Bp	boiling point
BuLi	butyllithium
C	concentration (mol/l)
cPMP	cyclic monopterin monophosphate
CN	Cyanide
cys	cysteine
cyt c	cytochrome c
DCM	dichloromethane
DMF	N,N-Dimethylformamid
DMSO	dimethylsulfoxide
ε	extinction coefficient (l/mol*cm)
E_a	activtion energy
Et_2O	diethylether
EtOH	ethanol
ΔG	Gibbs free energy
GTP	guanosine triphosphate
HPLC	high pressure liquid chromatography
IR	Infrared
kDa	kilodalton
K_{eq}	equilibrium constant
λ	wavelength (nm)
m	medium
MCP	Moco carrier protein

Me	methyl
MeOH	methanol
Moco	molybdenum cofactor
Mp	melting point
MPT	molybdopterin
MS	mass spectrometry
NMR	Nuclear Magnetic Resonance
PE	petrolether
Ph	Phenyle
R	gas constant
RT	room temperature
SAM	S-adenosylmethionine
st	strong
T	temperature
THF	tetrahydrofuran
TLC	thin layer chromatography
v_0	velocity at the beginning
w	weak

General remarks

Description of Compounds in the text

Compounds, which were mentioned before in the chemical literature are provided with Arabic numbers. In this work synthesized compounds, which were not mentioned in the chemical literature before, are provided with Roman numbers.

References to the literature

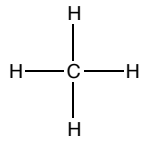
Within the text, references are marked with Arabic numbers in angular brackets.

Nomenclature

The nomenclature of compounds, which were not mentioned yet in the chemical literature, is done by using the IUPAC based rules of Chemical Abstracts. Well known chemicals and reagents are partly designated by their trivial names.

In the experimental part the empirical formula and the molar mass in [g/mol] are written below the structures:

Example:



chemical structure

CH_4 sum formula

16.04 molar mass [g/mol]

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A) Formula scheme

A.1 Synthesis of 4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione

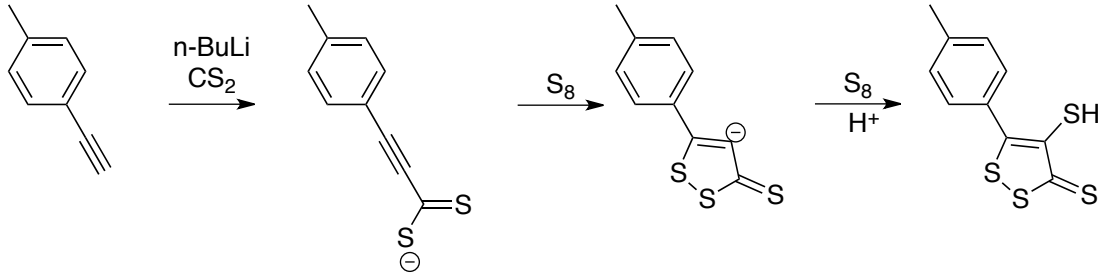

Figure 1: Synthesis of 1,3-Dithiole-2-thiones

A.2 Synthesis of (Bis-(4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione)-dioxomolybdenum)

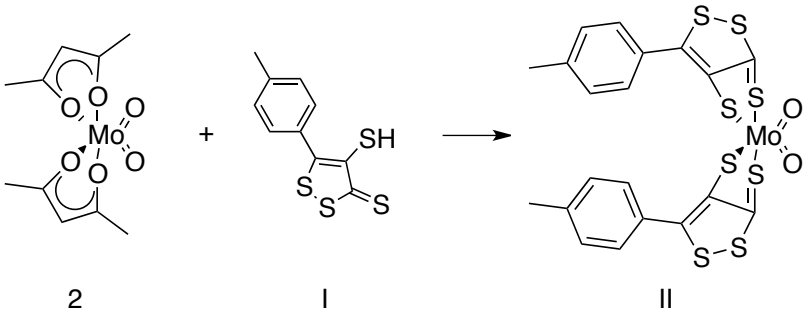

Figure 2: Synthesis of the Mo-Complex

B) General aspects

B.1 What is a metalloenzyme?

Enzymes are catalysts in biological and biochemical systems. The majority of the reactions in the organism, which are called metabolism, would not work without enzymes. In simple terms, enzymes speed up chemical or biochemical reactions. [1]

B.1.1 Specificity and catalysis

In contrast to chemical catalysts, enzymes act very specific. The active site of an enzyme is very sensitive to one certain intermediate of the metabolism. Thus, it reacts with one specific substrate only, while molecules, which are structurally quite similar to the actual substrate, are not able to interact with the enzyme. [1]

B.1.2 Chemical equilibria and energy

All chemical reactions end up in a chemical equilibrium at a certain point. This means that the concentrations of reactants and products are constant and will not change in time. In general, for equilibria the law of mass and action is applied. K_{eq} is the equilibrium constant in the following formulas. A, B, C and D are the concentrations of reactants and products. [2]

$$A + B \leftrightarrow C + D \tag{1}$$

$$K_{eq} = \frac{C * D}{A * B} \tag{2}$$

The chemical equilibrium is connected with the Gibbs free energy by the following equation. The energy gap between reactants and products result in the Gibbs free energy, which can be seen in Figure 3. In the equation below ΔG is the Gibbs free energy, R is the gas constant and T the temperature of the system. [2]

$$\Delta G = \Delta G^0 + R * T * lnK_{eq} \tag{3}$$

Reactions with $K_{eq} > 1$ or $\Delta G < 0$ take place voluntarily, which means that energy is emitted to the environment (exergonic reaction). An exergonic reaction can take place, as long as $\Delta G < 0$. When the reaction reaches the equilibrium, $\Delta G = 0$, which means that the energy of

the system is used up and the reaction stops. In the reversed case the reaction is called endergonic reaction, which means that energy is required for its progress.[2]

In biochemical systems redox reactions play a very important role, when it comes to obtaining energy. Energy can be obtained via the oxidation of nutrients in this systems. [1]

B.1.3 Activation energy and enzymes

In order to be able to start a chemical or biochemical reaction, a certain amount of energy is needed. This energy is called activation energy and is shown in detail in the diagram in Figure 3. The relation between the activation energy and the reaction's velocity at the beginning is given by the Arrhenius equation below. [2]

$$v_0 = v_0^{max} * e^{\frac{E_a}{RT}} \tag{4}$$

In the equation above v_0 is the reaction's velocity at the beginning and E_a is the activation energy. Energy can be provided to the system by increasing the temperature, which means that more collisions between reactants are produced, which result in an activated transition state. Thus, the velocity of a reaction is depending on the number of molecules in this activated transition state. If the activation energy is very high, the reactants will react very slow with each other. [2]

B.1.4 Decreasing of the activation energy by adding enzymes

Catalysts and enzymes decrease the activation energy as can be seen in Figure 3 below. The blue curve shows the energy usage of a biochemical reaction without the use of an enzyme, while the red curve shows the same reaction, but with the use of an enzyme. The net amount of the energy of the system ΔG remains the same in both cases. With or without catalyst the system always reaches the equilibrium state.

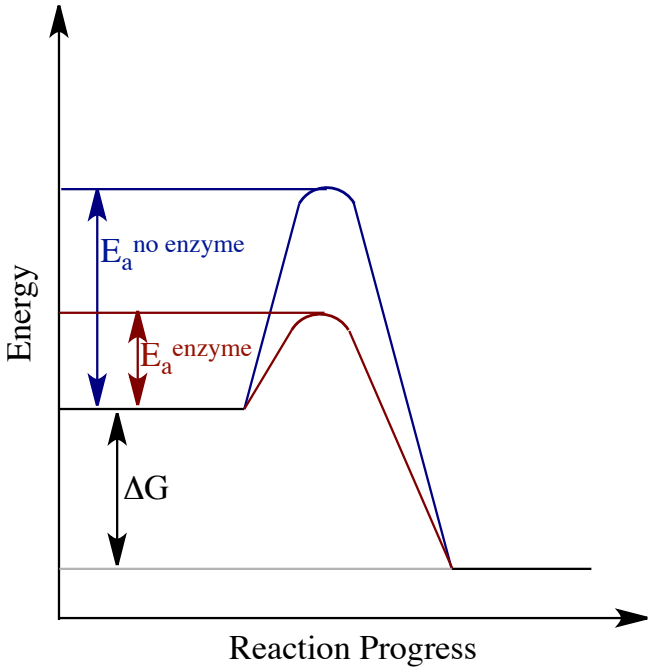

Figure 3: Enegy diagram of a reaction with enzyme (red) and without enzyme (blue)

In order to be able to influence a reaction's velocity, an enzyme or catalyst has to participate in the reaction itself. Thus, enzymes have a great influence on a reaction's mechanism, but are not used up within its progress. In general, enzymes are recycled after each reaction step. Thus, an enzyme does not occur in the overall reaction equation. [3]

B.1.5 The structure of enzymes – the active center

In general, enzymes are globular proteins with a molecular mass of about 10 to 100 kDa. Mostly, they consist of a couple of subunits, so their molecular mass can be even greater. On top of that, different enzyme-proteins can be combined to multi-enzyme-complexes, bearing an active center stabilized by the huge polypeptide skeleton. Here, in the active center, substrates are bound selectively and thus side chains of the enzyme's aminoacids can interact with it. The active center itself is just a very little part of the overall enzyme-structure and is positioned in most enzymes in a cavern. The enzyme's structure itself is quite flexible due to conformational changes, which are of great importance for the substrate binding process as well as the catalytic mechanism. Due to the fact that intermediates are stabilized by binding to the enzyme's active site, only very certain reactions can be catalyzed and thus increased in their velocity. [1]

In some cases the binding process of the enzyme to the substrate occurs via ionic interactions, sometimes even in cooperation with a metal cation as the enzyme's cofactor,

which makes a metalloenzyme out of an enzyme. Since many metals can exist in different oxidation states, their presence can also enable electron transfer reactions (redox reactions). Due to the reason that the conformation of an enzyme is correlated with its activity, the presence of a metal ion can have a big influence. By forming hydrogen bonds, ionic bonds or Van der Waals interactions the final structure of an enzyme – its conformation – can change and thus controls the catalytic activity. [1]

B.2 Molybdenum

Molybdenum is a 4d transition metal, which can easily change between the oxidation states +III, +IV, +V and +VI, which especially faciliates the catalysis of redox-rections. In the nature molybdenum occurs in the mineral Wulfenite $Pb[MoO_4]$, which occurs with a tetragonal crystal structure. The crystal has a very intensive red colour, as shown in the figure below. [1]

Figure 4: Wulfenite Pb[MoO4], found in the Red Cloud Mine (Yuma country, Arizona, USA) [3a]

In fact, among the second-row transition metals, molybdenum is the only one, which is needed by most organisms and living cells. Because molybdenum and tungsten, which is located just below molybdenum in the periodic table, are so similar, organisms can also use tungsten instead. Due to their manifold chemical applications and high bioavailability, these metals are essential, when it comes to enzyme's cofactors. [4]

Because the high-valent oxides of molybdenum are well soluble in water, it takes part in many biochemical circles in biochemical systems. [5]

B.3 The molybdenum cofactor

B.3.1 Uptake of molybdenum into the cell

In fact, molybdenum is essential for plants, animals and microorganisms. Molybdenum itself is transported into the cell as a molybdate oxoanion (MoO_4^{2-}). In presence of other ions there are competing forces with them concerning the transport through the membrane into the cell. Molybdenum itself is not able to show any enzymatic activity without any coordination to a special cofactor. It is bound immediately after the transport to a certain cofactor, in order to gain enzymatic activity. [6]

For the transport through the membrane a couple of enzymes are needed. These "transporting enzymes" have a high affinity to ATP, which provides the necessary energy by releasing a phosphate group through hydrolysis. This enables a conformational change of the transmembrane protein forming a transmembrane channel for the molybdate oxoanion. [7]

In general we can differentiate two different kind of molybdenum consisting enzymes:

- Nitrogenases (which contain the iron-molybdenum cofactor)
- Molybdoenzymes (which contain the pterin-based molybdenum cofactor (Moco))

B.3.1.1 Iron-molybdenum cofactor (nitrogenases)

Although nearly 80 % of the earth's atmosphere consist of nitrogen, it can not be used easily as a nitrogen source due to its inert character. Human beings and animals can absorb nitrogen, which is very important for several cycles in the metabolism, only via food. In the degradation process of organic compounds in the metabolism ammonia is formed, but it is immediately oxidized by bacteria to nitrate. That's the reason why soil contains nitrate. Plants and microorganisms now have to absorb nitrate from soil and water and thus build nitrogen consisting compounds therefrom. [1]

Figure 5 below shows the FeMo-cofactor. In order to be able to differentiate the atoms, they have different colors: Carbon is gray, nitrogen is blue, oxygen is red, sulfur is yellow, iron is green and molybdenum pink.

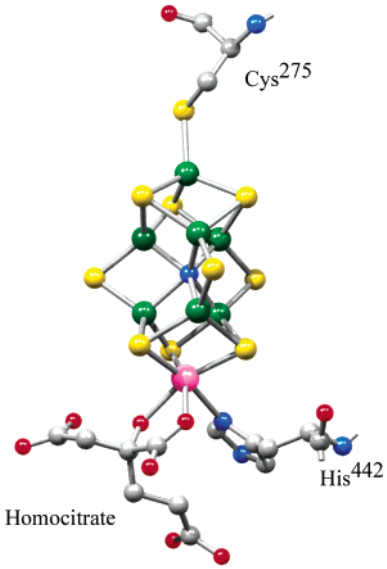

Figure 5: FeMo-cofactor [8]

The active site of the nitrogenase enzyme is the FeMo-cofactor, which is shown above. It consists of two units, the $MoFe_3S_3$ and the Fe_4S_4, which are connected by three sulfur atoms. The molybdenum itself in this compound is coordinated octahedrally. [8]

B.3.1.2 Pterin-based molybdenum cofactor (Moco)

Molybdoenzymes are enzymes, which catalyze plenty of chemical reactions including the transfer of oxygen in biological systems in the nitrogen, sulfur and carbon cycles. Relating to their function, we have to differentiate between two different classes:

- **Hydroxylases**:

  These use water as a source of oxygen. Oxygen is inserted into a C-H bond:

$$RH + H_2O \leftrightarrow ROH + 2H^+ + 2e^- \quad (5)$$

- **Oxotransferases**:

  They simply transfer oxygen from donor- to acceptor molecules:

$$R + H_2O \leftrightarrow RO + 2H^+ + 2e^- \quad (6)$$

The active site of the pterin-based molybdenum cofactor (Moco) is, in contrast to nitrogenase, a mononuclear molybdenum compound, which is coordinated to a bidentate sulfur donor ligand. The simplest form of the molybdoenzyme cofactor is shown in Figure 6.

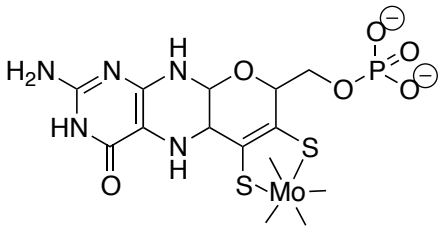

Figure 6: MOCO – Molybdenum Cofactor

Moco is based on a pterin derivative, which is bound in its 6-position to a phosphorylated pyran-ring, which itself is bound to the pterin-moiety with two sulfur atoms in cis-position. The coordination to the molybdenum metal center occurs via these sulfur atoms in a ratio 1:1.

In fact, the purpose of the pterin moiety is not completely understood yet. First of all, it makes a coordination to the molybdenum metal center, which builds the cofactor and causes the catalytic activity. It can act as a regeneration tool for the metal center by allowing an electron transfer between molybdenum and the pterin moiety. [6, 9]

B.3.1.2.1 Biosynthesis of Moco

The biosynthesis of Moco can be subdivided in four partial steps, as shown below:

1. Circularization
2. Formation of the dithiolene group
3. Adenylation
4. Mo-insertion

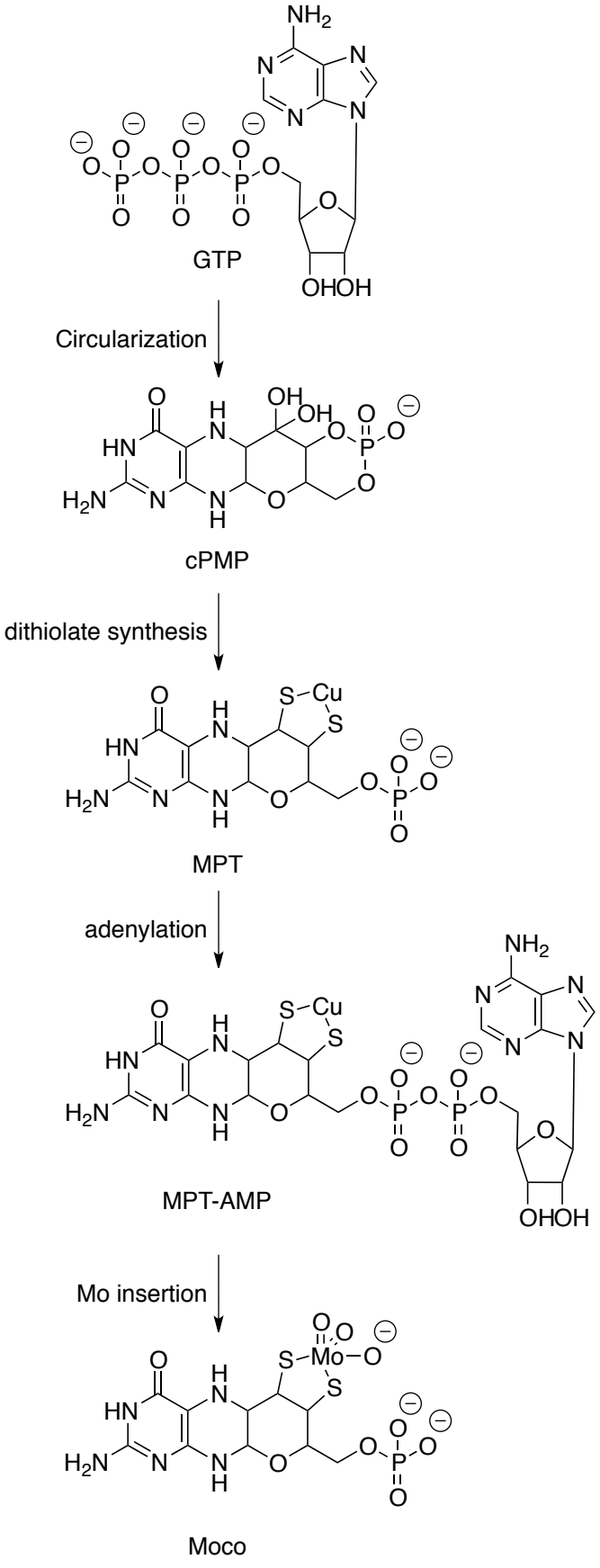

Figure 7: Biosynthesis pathway of Moco

Each reaction is catalyzed by certain enzymes. Depending on the organism (human, plants, *Escherichia coli*), the enzymes can differ a lot. [6] [10]

B.3.1.2.1.1 Step 1: Circularization

The biosynthesis of Moco starts with guanosine 5'-triphosphate (5'-GTP), which forms the cyclic monopterin monophosphate (cPMP) also known as precursor Z. The intermediate cPMP seems to be the first stable compound, which is very sensitive to oxygen, whereas in low pH it can resist at least a few hours. This first step of the biosynthesis takes place in the mitochondria. [6, 11]

Figure 8 below shows the mechanism of the first step of the Moco-biosynthesis in detail.

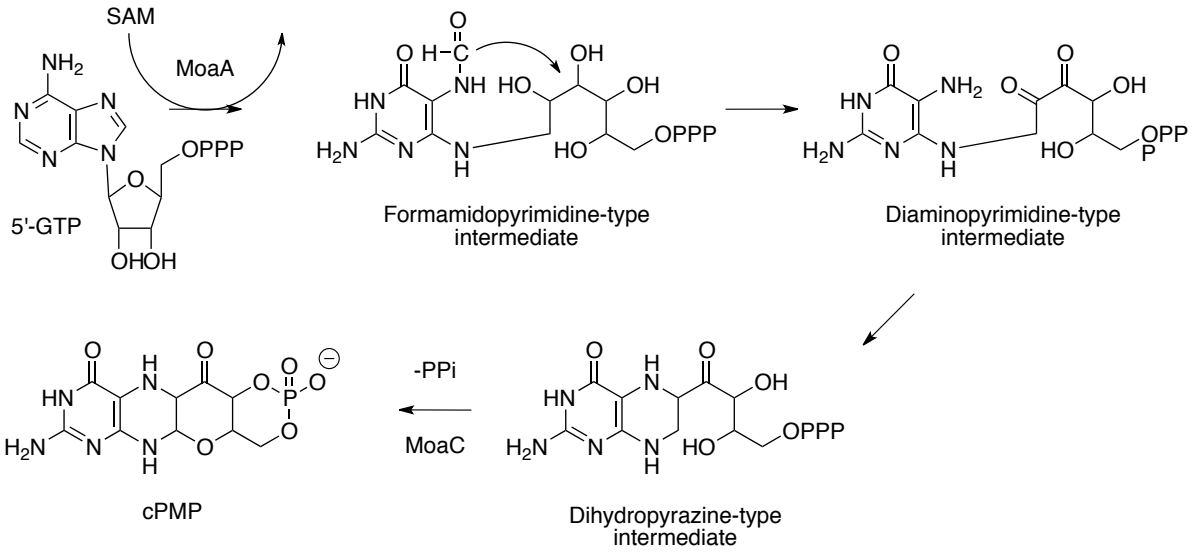

Figure 8: Mechanism of the first step of the biosynthesis of Moco. [12]

As shown above the enzyme MoaA, which works in combination with SAM, first catalyzes the opening of the pterin-moiety by forming the formamidopyrimidine-type intermediate. The final formation of the cPMP intermediate is catalyzed by the enzyme MoaC (used in *E. Coli*) by closing the furan ring under secession of the pyrophoaphate group. [12]

B.3.1.2.1.2 Step 2: Formation of dithiolene group

In the next step dithiolene groups are inserted to cPMP, forming the molybdopterin dithiolate (MPT dithiolate). This step is catalyzed by the enzyme MPT synthase, which consists of four subunits. Two of these four subunits carry thiocarboxylate groups, enabling

the enzyme to transfer sulfur atoms. Thus, these groups are the active sites of this enzyme. [6]

The Figure 9 below shows the detailed mechanism of the formation of the dithiolene groups.

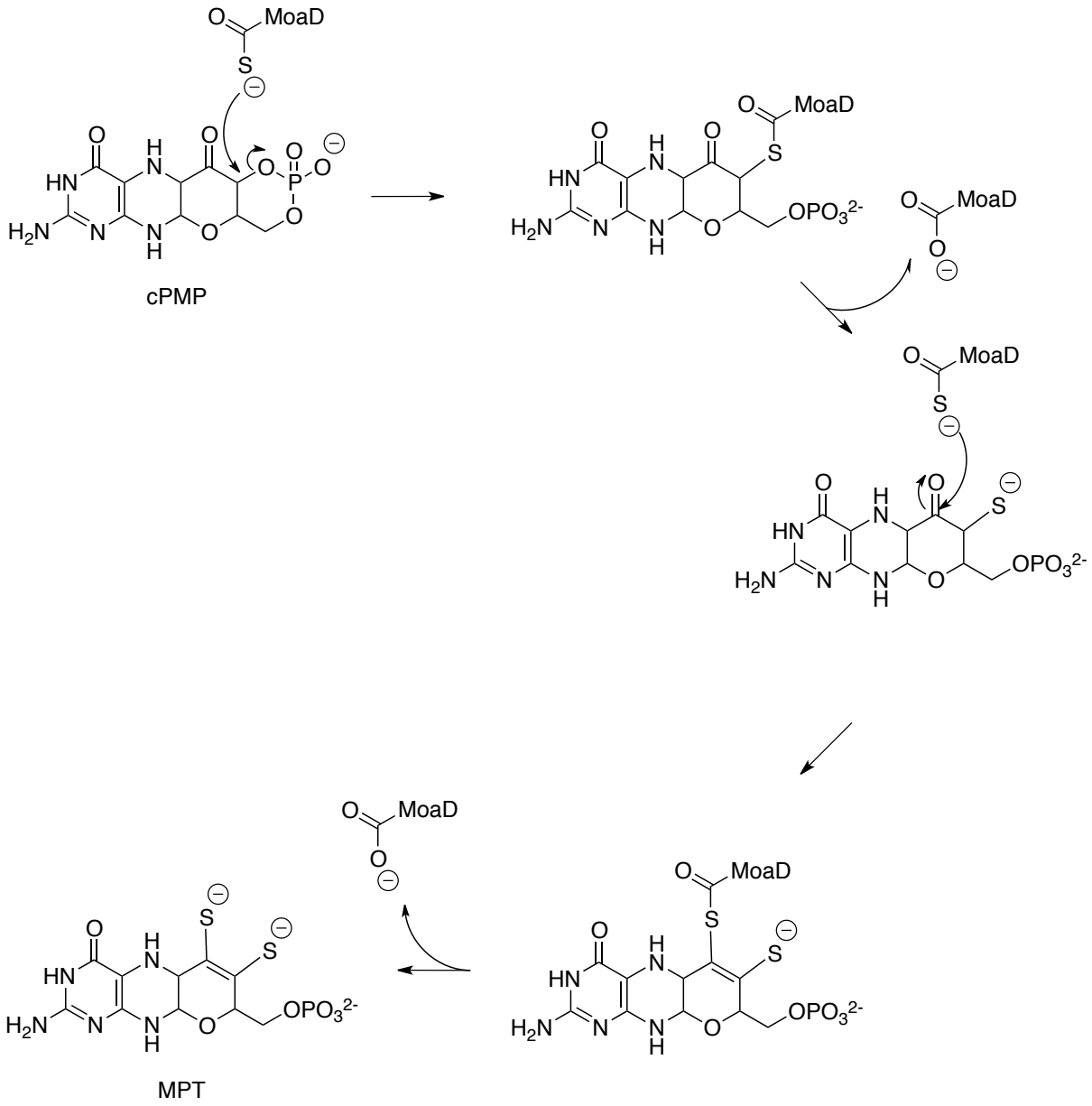

Figure 9: Mechanism of the formation of the dithiolene groups (2nd step) [12]

As seen above, the sulfur atoms are inserted one after another. When the first sulfur is inserted from the enzyme to the C2' carbon of the furan moiety, the cleavage of the bond of the cyclic phosphate group occurs. The second sulfur atom is inserted at the C1' atom yielding MPT. [12]

B.3.1.2.1.3 Step 3: Adenylation

The third step is decisive for the adenylation of the MPT moiety forming the adenylated molybdopterin (MPT-AMP). In fact, this intermediate has been found, when the crystal structure of the enzyme Cnx1G (used in plants), which binds with high affinity to MPT and MPT-AMP and catalyzes the Mo-insertion in plants, was determined.[13] It was found that the final coordination of the sulfur atoms to the molybdenum atom is associated with the cleavage of the MPT adenylate. [14]

Figure 10 shows the final two steps of the biosynthesis of Moco.

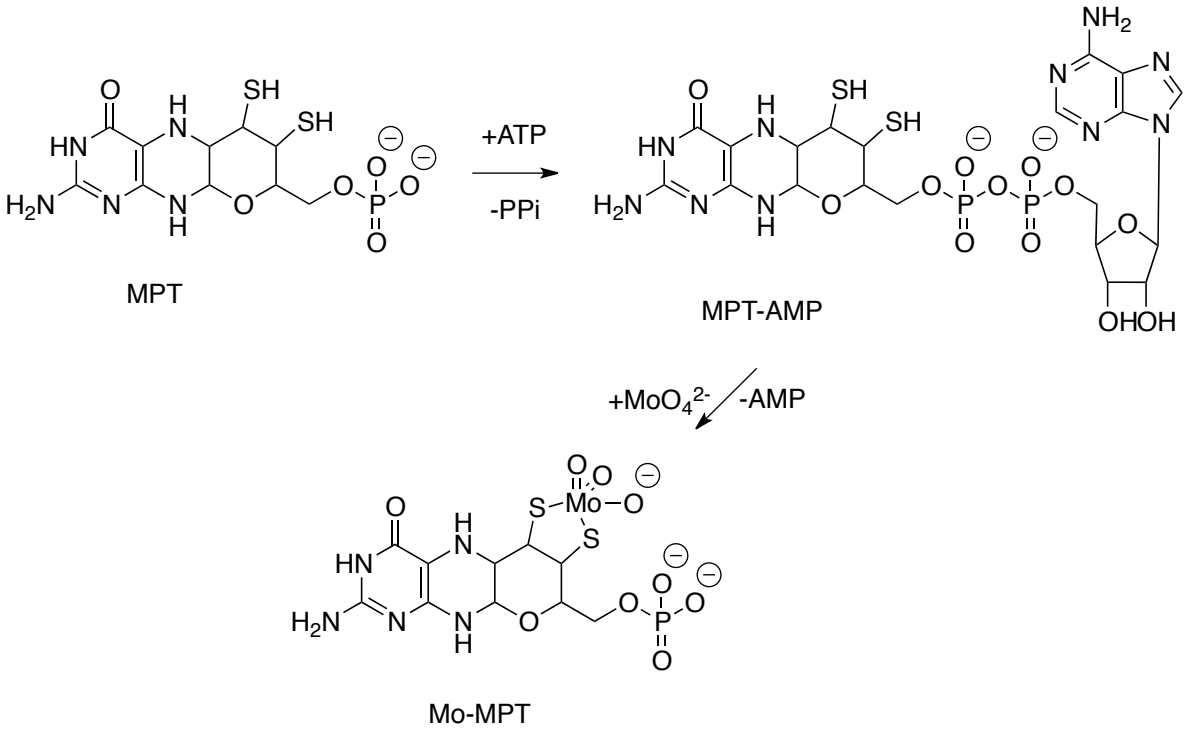

Figure 10: Adenylation and insertion of molybdenum – the final two steps in the biosynthesis of Moco. [12]

The adenylation is catalyzed by the enzyme MogA (used in *E. Coli*) using ATP as a source for adenosine monophosphate. In this reaction step a pyrophosphate group is removed. [12]

B.3.1.2.1.4 Step 4: Mo-insertion

In fact, the determination of the crystal structure of the enzyme Cnx1G (used in plants) revealed another structural feature of the intermediate synthesized in step 2. It turned out that the sulfur atoms, which were inserted in the second step, were coordinated to a copper atom immediately after sulfur was bound to the intermediate (Figure 7). In this compound copper is coordinated tetragonally. The coordination of copper might happen for two

reasons: It can act as a protection group, thus preventing the sulfur atoms from oxidation after their transfer. Also, copper is a suitable leaving group for molybdenum. [6, 14-15]

The enzyme MoaA (used in *E. Coli*), which cleaves the bond to AMP, also causes the release of copper (Figure 10). Thus, there is the opportunity for molybdenum, which was transported into the cell as a molybdate oxoanion (MoO_4^{2-}) prior, to make a coordination to the sulfur atoms. With the last step of the insertion of the molybdenum atom the active form of the molybdenum cofactor is formed and ready for further modification. [6, 12, 15]

In procaryotes a further modification of the active molybdenum cofactor occurs, which is shown in Figure 11 below.

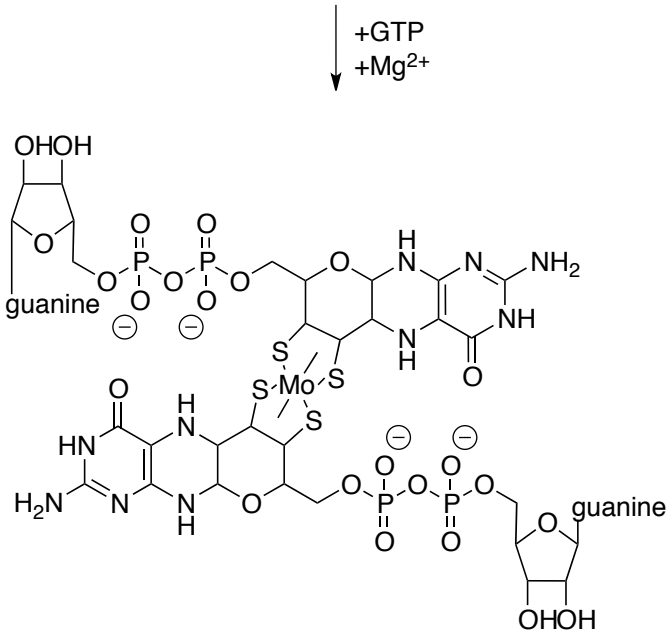

Figure 11: Modification of Moco in procaryotes [7]

For organisms as for example *E. Coli* it occurs that a second MPT guanine dinucleothide group is coordinated to molybdenum, building up an enzyme belonging to the DMSO reductase family. [7, 12]

B.3.1.2.2 Storage and transfer of Moco

Due to the fact that Moco itself in its free condition (without any connection to the enzyme) is very unstable, there has to be a "transportation system", whereby it can reach to its final destination immediately after biosynthesis. Proteins can act as such tools, helping to protect Moco from external influences as for example oxidations. Such a "transportation system"

should bind to the molybdenum enzyme immediately after biosynthesis. Right after it forms a connection to the cofactor it should enable the transport to its final destination, the enzyme. Depending on the organism, this "transportation system" can be different. [6-7]

In the green alga *Chalmydomonas reinhardtii* the first Moco carrier protein (MCP) was found, which is able to bind to Moco immediately after biosynthesis in a quite soft way and thus has the potential to protect the cofactor from oxidational influences. [16]

B.3.1.2.3 Insertion of Moco into molybdoenzymes

When the transport of the cofactor to the enzyme was successful, the next step is the insertion into the enzyme, whereby the exact mechanism is not completely understood yet. In fact, the molybdenum cofactor is not placed on the surface of the enzyme, as it is shown below. The active site is located in the middle of the enzyme in its center. Contact between the cofactor and the substrate is provided by a system, which is similar to a tunnel, whereby the interaction between substrate and cofactor is ensured. [17]

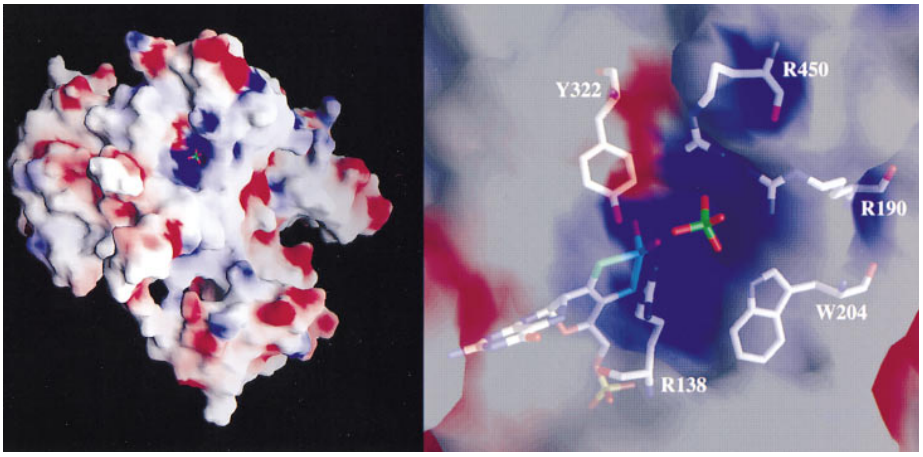

Figure 12: Placement of Moco within the enzyme (for Sulfite Oxidase)[17]

When it comes to the insertion of the cofactor into the enzyme, there seems to be a certain order, according to which the parts of Moco are introduced. First of all, the molybdenum center is inserted. Then the pyranopterin moiety enters. Subsequently, the phosphate side chain is introduced into the enzyme having an important function. Due to its negative charge there is the possibility to interact with positively charged side chains of amino acids of the enzyme. Thus, the tunnel, by which the entry of the cofactor was possible, may be closed again, leaving the cofactor deep inside the enzyme's center. [9]

B.3.1.2.4 Moco-dependent enzymes

Regarding to reactivity and structure there are three different subgroups of molybdoenzymes:

- Xanthine oxidase Family
- Sulfite oxidase Family
- DMSO reductase Family

The figure below shows the general structures of each subgroup.

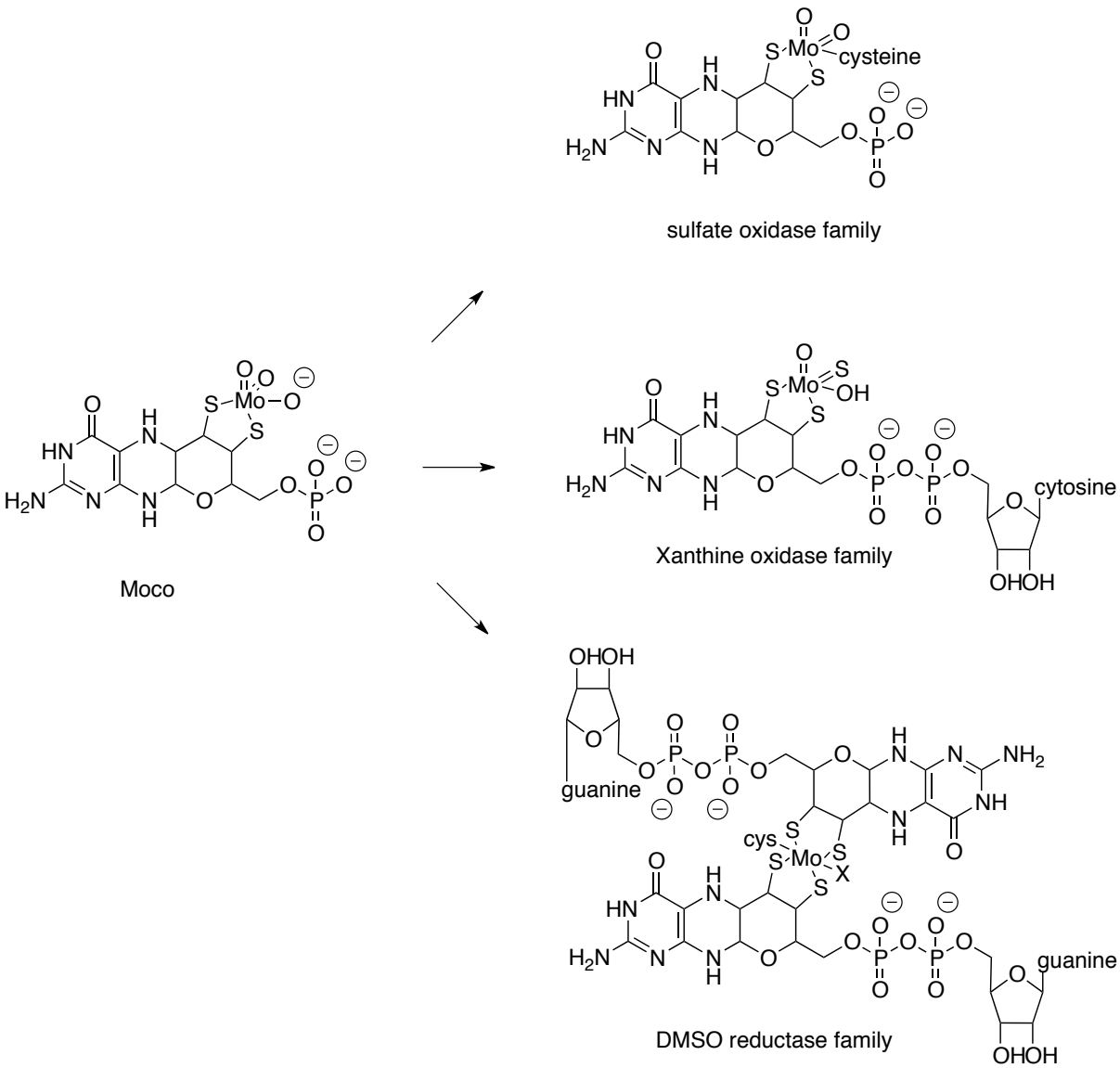

Figure 13 shows the three subgroups of the Mo-cofactor [12]

As it is shown in Figure 13 all of these Moco-cofactor families have one structural property in common. In each of them molybdenum is coordinated to a bidentate sulfur ligand, specifically the pterin moiety.

B.3.1.2.4.1 Xanthine oxidase family

First of all, xanthine oxidase is an enzyme, which enables the insertion of an oxygen atom to xanthine or similar substrates as for example aromatic heterocycles and/or substrates, which have an oxy-group. Xanthine itself occurs in the nucleotide metabolism and is an intermediate in the degradation of purine. The structure of xanthine is shown in the figure below. [18]

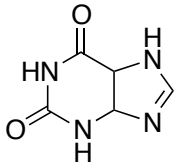

Figure 14: Structure of xanthine

Due to the fact that the bond between the molybdenum central atom and the oxygen atom in the cofactor is not very strong, it entails the possibility of the transfer of this atom to a substrate. In order to close the catalytic cycle and to regain catalytic activity, a source of oxygen is needed in order to regenerate the enzyme. In fact, this oxygen source is found in water. [5, 19]

Figure 16 shows the mechanism of the catalytic cycle of xanthine oxidase. At the beginning of the reductive catalytic half-reaction the molybdenum central atom has an oxidation state of +VI. For initializing the reaction xanthine is deprotonated at the C-8 carbon atom causing a negative charge, which enables a nucleophilic attack, which is shown in detail in Figure 15.

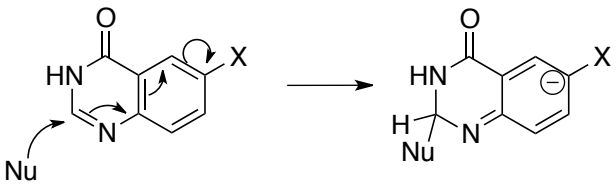

Figure 15: Nucleophilic attack at the C-8 atom[5]

Due to the aromatic character of xanthine, the negative charge is delocalized in the whole molecule. Subsequently, a MoOR group is formed. At this point the oxidation state of molybdenum is +V. The regeneration of the cofactor in the oxidative half-reaction is performed by using water as a source of oxygen, raising up the oxidation state of molybdenum again up to +VI. [5, 19c, 20]

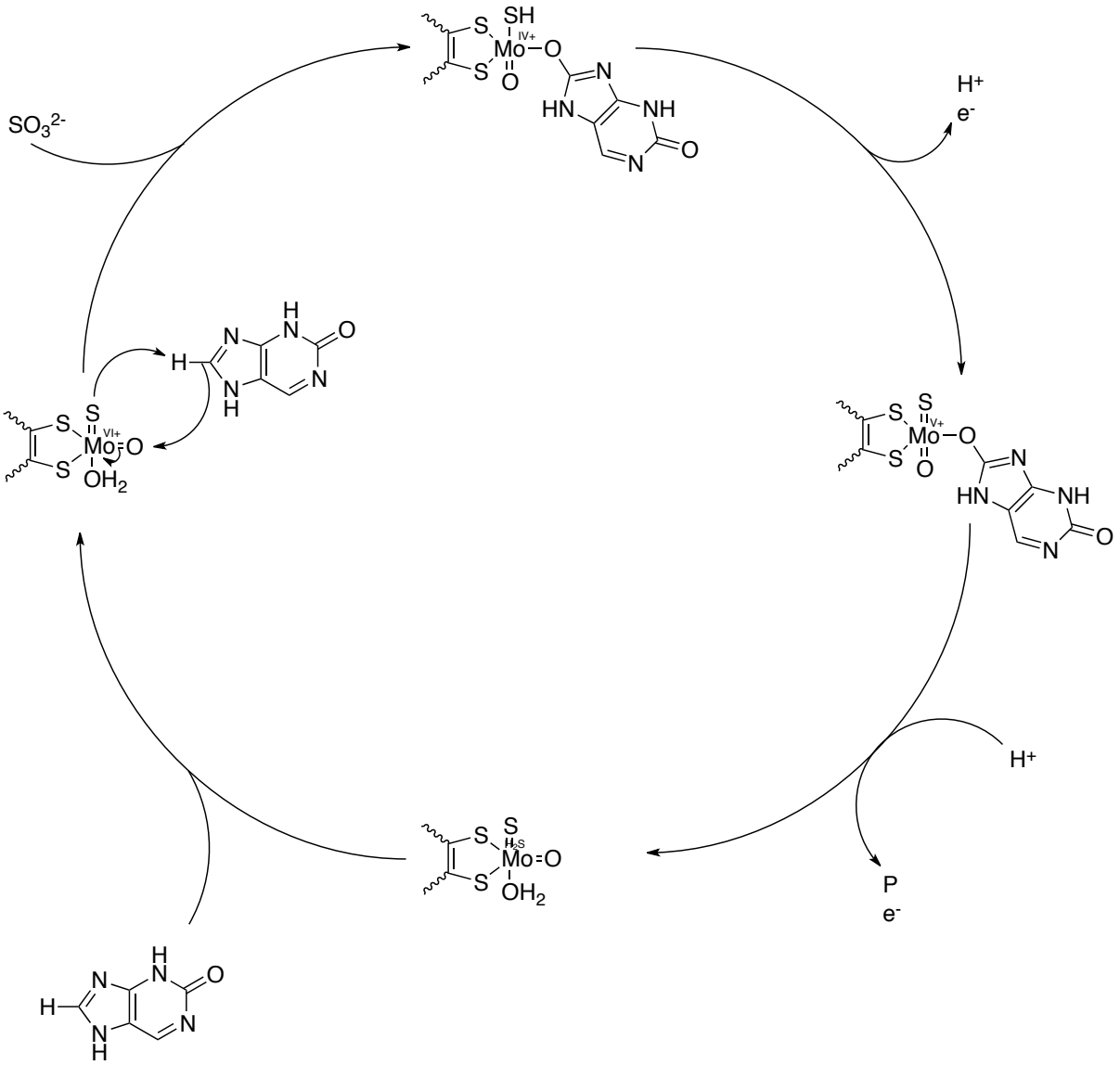

Figure 16: The mechanism of Xanthine Oxidase [5, 19c, 20]

The oxidation state of the oxidized form of the cofactor of xanthine oxidase bearing a thione group is +VI. In the reduced form of the enzyme the oxidation state is reduced by two. In this state the thione group is converted to a thiole group by the uptake of a hydrogen atom. [5]

Figure 17 shows the oxidized and reduced form of the xanthine oxidase cofactor.

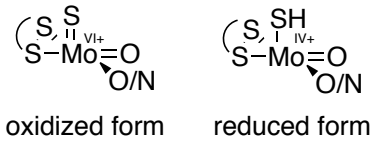

Figure 17: Oxidized and reduced form of the cofactor of xanthine oxidase

B.3.1.2.4.2 Sulfite oxidase family

Sulfite oxidase is an enzyme, which catalyzes the oxidation of sulfite to sulfate, using cytochrome c as an electron carrier. In fact, this reaction is very important in the overall environmental cycle of sulfur. [5]

Figure 18 shows the catalytic cycle of sulfite oxidase.

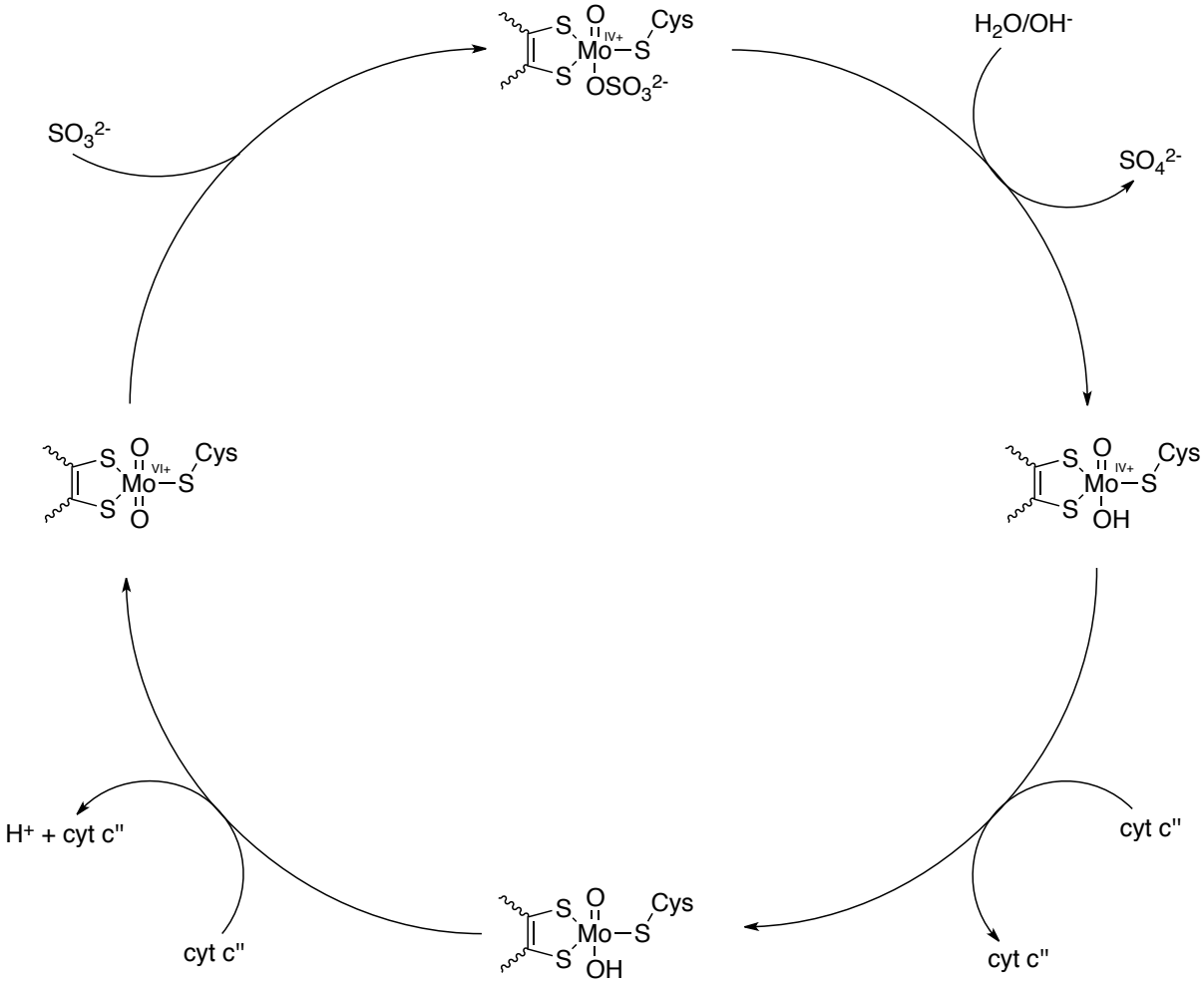

Figure 18: Catalytic cycle of sulfite oxidase[21]

In the oxidative half reaction, molybdenum is first reduced from the +VI to the +IV oxidation state. Alos in this half-reaction the oxidation of sulfite to sulfate takes place. The regeneration of the molybdenum center, which is characteristic for the oxidative half-reaction, is provided by electron exchange between the molybdenum center and cytochrome c. [5]

One suggested mechanism for this catalytic cycle is the nucleophilic attack of a sulfite lone pair on one of the oxygen atoms of the oxidized molybdenum cofactor. [21]

B.3.1.2.4.3 DMSO reductase family

In fact, the DMSO reductase family enzymes are very different from the other two groups mentioned before. While in the others are mostly catalyzing oxygen atom transfer reactions, in this group the majority of reactions are simple oxidation/reduction reactions. There isn't only a difference concerning the catalytic behavior. Also structural properties differ a lot. As mentioned before, the molybdenum center is bound to two oxygen atoms in the oxidized form for the first two groups, while the reduced form carrys only one oxygen atom after the transfer reaction. Relating to the DMSO-reductase family there is a change between a monooxo molybdenum(VI+) (oxidized form) and desoxo molybdenum (IV+) (reduced form) as it is seen in Figure 20 below.[18]

Figure 19 shows the reductive half reaction of DMSO.

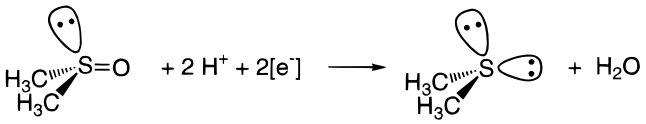

Figure 19: Reduction of DMSO [5]

The following Figure 20 shows the catalytic cycle of the reduction of DMSO using DMSO reductase. After the reduction of DMSO there is the oxidized form of the molybdenum cofactor. Thus, there has to be a reduction agent. It was found that the phosphine, shown in Figure 20, is a very efficient regeneration agent for the enzyme. [22]

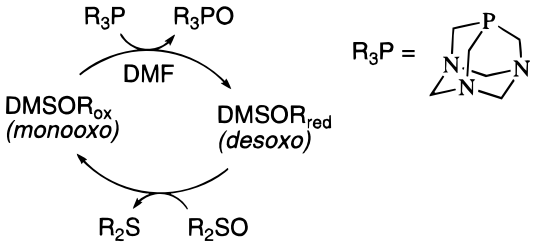

Figure 20: Catalytic cycle of the DMSO reductase family[5]

By making an experiment with labelled oxygen (^{18}O) there is a proof that the oxygen is completely transferred from the molybdenum atom to the phosphine. This is another proof that the bond between molybdenum and oxygen is quite wak as discussed before in section B.3.1.2.4.1. It is easy to transfer an oxygen between the molybdenum cofactor and a substrate. [22]

B.4 Molybdenum cofactor deficiency

In fact, when the molybdenum cofactor is missing, there is a loss of all molybdenum based enzymes (xanthine oxidase, sulfite oxidase, DMSO reductase) discussed in the chapter B.3.1.2.4, which causes a serious disease. The classification of the disease is done in consideration of the synthetic pathway of the pterine based molybdenum cofactor as it is shown in Figure 21. Each biosynthetic step of the cofactor requires certain enzymes in the human organism. Thus, the disease is classified by the affected biosynthetic step. [7]

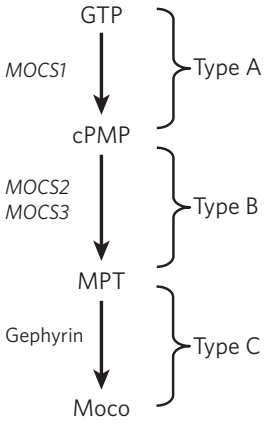

Figure 21: Subdivision of molybdenum deficiency in 3 types [7]

The disease is very serious. In fact, the molybdenum cofactor deficiency is an inborn neurological disease with seizures, which affect the patients metabolism causing several

symptoms. Patients born with this disease are often mentally retarded and have seizures. [23]

There is another kind of disease, causing the deficiency of sulfite oxidase. In this case, there is no problem with the biosynthesis of the molybdoenzyme, but due to the mutation of a gene, which encodes the sulfite oxidase protein itself, the enzyme is not active. The result of both diseases is the absence of the sulfite oxidase protein. [23a]

The role of the sulfite oxidase is quite important. In fact, it is positioned in the intermembrane space as it is shown in Figure 22. It oxidizes sulfite to sulfate in the final step of the degradation of amino acids and lipids containing sulfur. Due to the high toxicity of sulfite, organs, especially the brain, are damaged in the absence of sulfite oxidase. [7]

The symptoms of sulfite oxidase deficiency, which are quite similar to those of molybdenum cofactor deficiency, can be caused by a too high level of sulfite, but also a too low level of sulfate, which is required for the formation of sphingolipids. Thus, a too low concentration of sulfate can cause serious problems too. [24]

Figure 22 shows the degradation of amino acids in the human organism in detail.

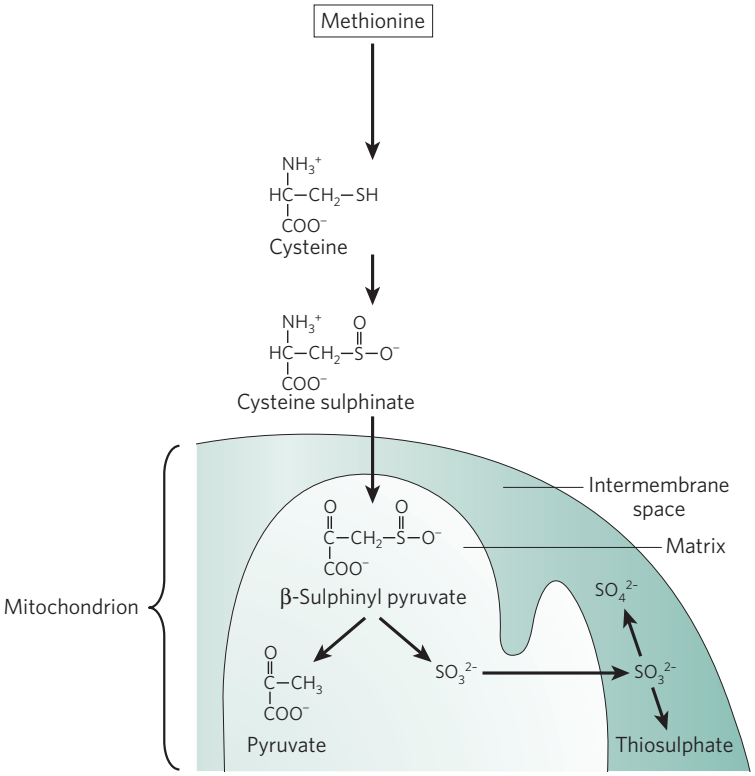

Figure 22: Sulfite Oxidase Deficiency[7]

At first the amino acid is oxidized at the SH-group, building up a sulfinate, which enters the matrix. There a degradation into pyruvate and sulfite takes place, which in healthy organisms is oxidized in the intermembrane space to sulfate, which is not toxic and harmful anymore.[7]

C) Synthesis and characterization of new species

C.1 Goal of the thesis

First of all a ligand had to be found, whose coordination to molybdenum we wish to investigate. Due to the facts that sulfur has a big attraction to molybdenum and that the molybdenum cofactor is coordinated to a bidentate sulfur donor ligand, these group of ligand are of big interest. Subsequently, the goal of this thesis is the coordination of a bidentate sulfur donor ligand to molybdenum, thus forming a mononuclear molybdenum complex.

C.2 Objective – modelling of enzymes

Although molybdoenzymes have been studied very well in recent years, it is very important to form model systems for them for further investigation. The best approach for modeling enzymes is as follows. First of all, model systems have to be built. By studying them, it might be easier to understand the mechanism of potential enzymes. Model compounds with similar ligands might give some information about the enzyme's geometry. Each ligand has an influence on the metal's redox potential. By investigating many different ligand systems, it can be possible to predict, how each ligand influences the molybdenum center, and thus substrate selectivity can be understood. Subsequently, the behavior of found model compounds can be investigated in biochemical systems. [25]

C.3 Sulfur ligands

Generally, there are two different types of bidentate sulfur ligand systems as shown in Figure 23. The residue R in the structure can be for example H, Me, Ph or CN. [21]

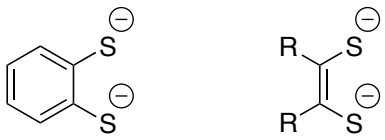

Figure 23: General types of bidentate sulfur ligands (ene-1,2-dithiolates)

In fact, the ligand itself acts as a Lewis base, donating an electron pair to the molybdenum center atom. Molybdenum on the other side functions as a Lewis acid. By changing the R-group in Figure 23, the electron distribution of the final molybdenum complex can vary and thus the final redox potential changes. [21]

C.3.1 4-mercapto-1,2-dithiole-3-thiones

In fact, this group of compounds, which show cancer chemo preventive activity, are well known. The general structure is shown below in Figure 24. In the last decades, many compounds of this kind have been synthesized, in which a few were successful in animal trials and thus were applicated in medical trials and finally gained the permission for therapeutical use. [26]

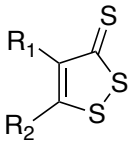

Figure 24: General structure of dithiolethiones

The R-groups R_1 and R_2 in the structure above can differ a lot. They can be just simple aliphatic carbon chains, but may also be aromatic systems with hetero atoms as for example nitrogen, oxygen or sulfur. [26]

In order to be able to use this compound as a bidentate sulfur ligand, R_1 has to be a SH-group, which bears the possibility to coordinate to a molybdenum atom. The R_2-group is a toluyl-group. The final structure of the ligand, which was synthesized succesfully in this thesis, is shown in Figure 25.

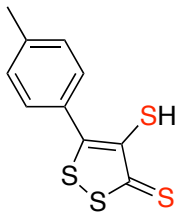

Figure 25: bidentate sulfur ligand (coordinating sulfur atoms in red)

The ligand coordinates only with the two sulfur atoms, which are colored red in the figure above. The other two sulfur atoms within the five-membered ring do not play any role concerning the coordination to the metal.

C.3.1.1 Synthesis

The mechanism of the synthesis of the ligand, which is shown in detail in Figure 26, is quite simple. The first step – the deprotonation of the acetylene-derivative – was carried out at -80 °C. This kinetically controlled reaction with n-BuLi results in a negative charge on the carbon atom at the end of the triple bond. Thus, a nucleophilic attack, which was also carried out at low temperature, on the positively polarized carbon atom of CS_2 can be performed. By the addition of sulfur (S_8) a further sulfur atom was integrated into the structure. In the following step, the closing of the ring occurs, which results in a five-membered ring. This ring now carries a negative charge at the 4th position. On this position a thiole-group was formed by the addition of sulfur and HCl.

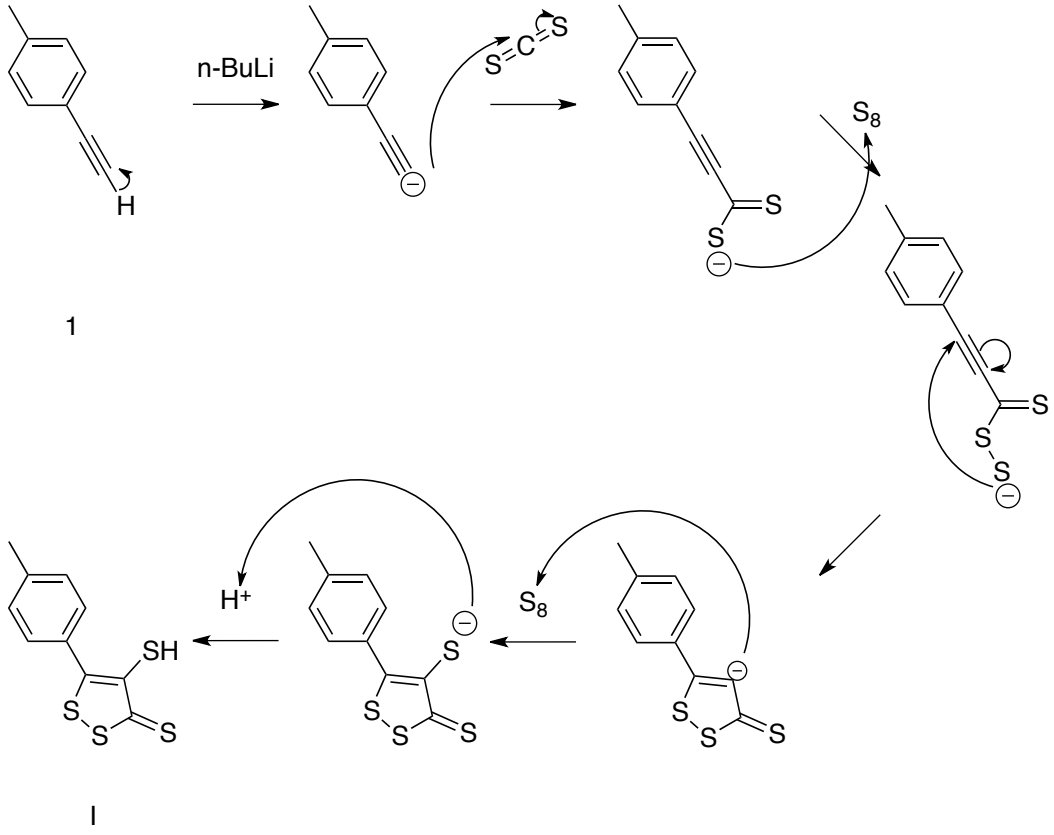

Figure 26: Mechanism of the synthesis of I

This reaction was carried out with an overall yield of 23 %. IR- and NMR-spectra were recorded for characterization reasons (see section F.1 Spectra of the ligand, page 43).

C.4 The coordination of the ligand to molybdenum

C.4.1 Synthesis

The coordination of the bidentate sulfur donor ligand to molybdenum was carried out at room temperature. The starting material was bis(acetylacetonato)dioxomolybdenum(VI). With a mild acid treatment, the acetylacetonato-ligands could be removed from the molybdenum atom. Therefore, $Mo(acac)_2O_2$ was dissolved in MeOH prior to the coordination.

When both, the solution of $Mo(acac)_2O_2$ and the solution of the ligand, were combined at room temperature, the red powder of the product precipitated immediately. Molybdenum is octahedral coordinated in the complex.

For analytical reasons IR- and NMR-spectra and and UV/VIS spectrum were recorded (see section F.2 Spectra of the complex, page 46)

C.4.2 UV-VIS-spectroscopy

The absorption spectrum of the complex was recorded on a Perkin Elmer Lambda 900 (UV/VIS/NIR spectrometer) in a solution of DCM (HPLC grade) with a concentration of 0.0227 mmol/l at room temperature using quartz glass cuvettes with a diameter of 1 cm.

Figure 27 shows the UV-VIS-spectrum of the complex.

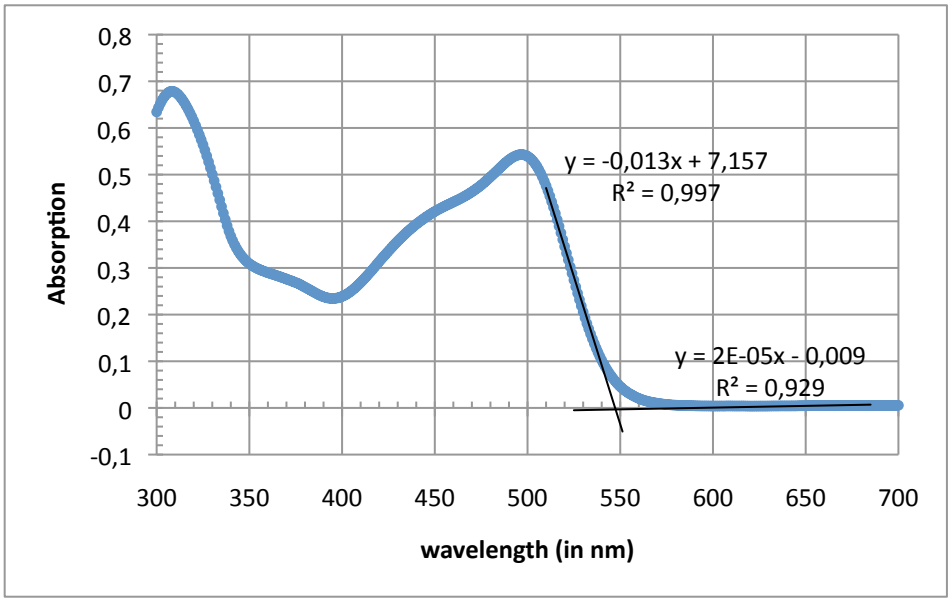

Figure 27: UV-VIS spectrum of

C.4.2.1 Determination of the onset-point

The onset-point is a point in the spectrum, at which the compound starts to absorb light. For the determination of this point two straight lines are needed as it is shown in Figure 27. The point, in which these two lines intersect with each other, is called the onset-point.
The formulas of the two straight lines are given below:

$$y = -0,013x + 7,157 \tag{7}$$

$$y = 2 * 10^{-5}x - 0,009 \tag{8}$$

The solution of the equation system with two unknown variables provides the onset point, which was found out to be at a wavelength of **λ = 551 nm**.

C.4.2.2 Determination of the extinction coefficient, maxima and minima

Maxima and minima are given in Table 1 below.

	A	λ (nm)	ε (l/mol*cm)
Maxima	0.68	309.18	29912
	0.54	496.84	23921
Minimum	0.27	372.88	11982

Table 1: Results of the UV/VIS measurement of compound II

Also, the extinction coefficient was determined in the maxima and minima using Formula 9 below.

$$A = \epsilon * c * d \tag{9}$$

C.4.3 IR-spectroscopy

In Figure 28 the IR-spectra of the ligand (I) (in red) and of the molybdenum complex (II) (in blue) are plotted for an easy comparison. It is possible to observe, that some vibrational bands of the ligand are shifted, after the coordination to molybdenum.

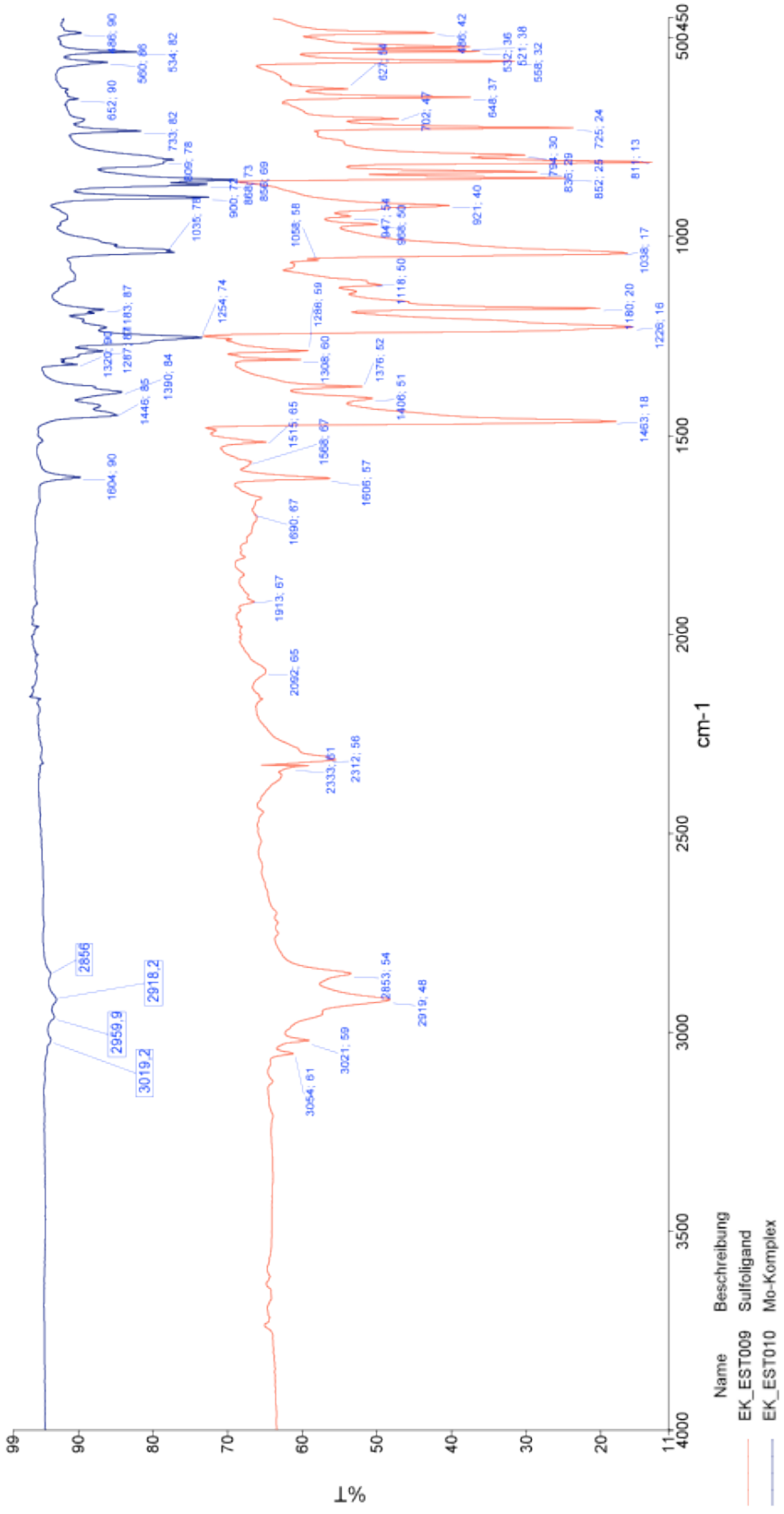

Figure 28: Comparison of the IR-measurement of the compounds I (red) and II (blue)

In Table 2 the characteristic IR-bands of the compounds I and II are compared. It is apparent that vibrations between atoms, which are close to the molybdenum metal center in the complex, have a shift to a bigger value, while those, which are far away from molybdenum nearly do not show any change. The C=S-bond for example, which is just next to the molybdenum metal center undergoes a shift of about 74 cm^{-1}, while the other values stay nearly the same.

Compound	ν (C-H arom.)	ν (C-H) → CH_3)	ν (C=C arom.)	ν (C=S)	ν (Mo=O)
I (ligand)	2919.48 (w)	2853.54 (w)	1607.56 (m)	1180.20 (st)	-
II (complex)	2917.30 (w)	2852.60 (w)	1604.90 (m)	1254.74 (st)	868.73 (st), 900.72 (st)

Table 2: Comparison of the characteristic IR-bands of the compounds I and II (s = strong, m = medium, w = weak) (values in cm^{-1})

D) Experimental

D.1 General remarks

All reagents, which were purchased from commercial suppliers, were used without prior purification.

All solvents were distilled before use. Anhydrous solvents like methanol, diethylether, dichloromethane and tetrahydrofurane were purified using the PURESOLV-system from it-innovative-technology-inc.

The commercially available lithiation reagent n-BuLi was used without additional quantitative analysis, using the declared value.

D.2 Chromatographic methods

D.2.1 Thin layer chromatography

TLC was performed by using TLC aluminum foil (Merck, Silica gel 60 F_{254}).

D.2.2 Column chromatography

The glass columns were packed with silica gel (Merck, 40-60 μm). Solvents, which were used for column chromatography, were distilled before use.

D.3 Analysis methods

D.3.1 NMR-spectroscopy

NMR spectra were recorded at 200 MHz for ^{1}H on a Bruker Avance 200 and at 400 MHz for ^{13}C. Data for ^{1}H NMR are reported as follows: chemical shift in parts per million from TMS (tetramethylsilane) with the residual solvent signal as an internal reference [27], multiplicity (s = singlet, d = doublet, t = triplet and m = multiplet), coupling constant in Hz and integration.

^{13}C NMR spectra are reported in ppm from TMS using the central peak of the solvent as reference[27], multiplicity with respect to proton (deduced from APT experiments, s = quaternary C, d = CH, t = CH_2, q = CH_3).

D.3.2 IR-spectroscopy

IR-spectra were recorded using a Perkin Elmer UATR Two spectrometer in the ATR mode. Spectra were recorded of solid samples at room temperature.

D.3.3 UV-VIS-spectroscopy

The UV-VIS spectrum was recorded on a Perkin Elmer Lambda 900 (UV/VIS/NIR spectrometer). The spectrum was measured from 300 to 700 nm at room temperature using quartz glass cuvettes with a diameter of 1 cm. For the measurement of the spectrum, solutions were prepared in DCM.

D.4 Synthesis and characterization

D.4.1 Synthesis of 4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione

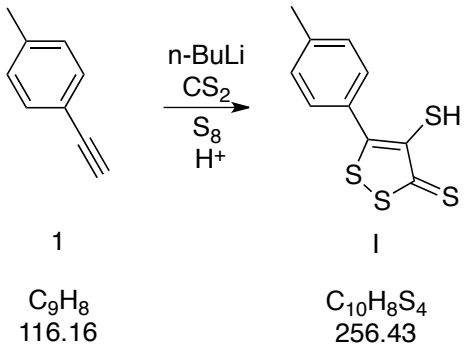

Figure 29: Synthesis of 4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione

Synthesis according to [28]:

Figure 30: Color of the product 4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione

First **1** (2 ml, 15.8 mmol, 1.0 eq) was dissolved in 110 ml abs. THF under argon atmosphere. After cooling to -80 °C, n-BuLi (2.5 M in Hexane, 6.8 ml, 17.0 mmol, 1.1 eq) was added drop wise, while keeping the temperature constant, causing a color change to pale yellow. The reaction mixture was then allowed to warm up to RT. After stirring for 30 min at RT the reaction mixture was again cooled to -80 °C. Then CS_2 (1 ml, 15.8 mmol, 1.0 eq) was added drop wise causing a further color change to deep red. The reaction mixture was then stirred at RT for one hour. After S_8 (1.52 g, 47.4 mmol, 3.0 eq) was added at RT, the color of the reaction mixture immediately changed to purple. After stirring for 2 hours, TLC analysis (PE : DCM = 1 : 1) indicated completion of the reaction. The reaction mixture was then extracted with 2 M HCl. The water layer was extracted three times with DCM. The combined organic layers were dried over anhydrous sodium sulfate. Evaporation of the solvent yielded 4.7 g of a red-brown solid. The residue was purified by column chromatography (PE : DCM = 15 : 1) yielding 0.91 g (22.5 g) of a orange solid.

Yield 0.91 g (22.5 % d. Th.)

D.4.1.1 Analysis

D.4.1.1.1 ^{1}H NMR

^{1}H NMR	200 MHz, $CDCl_3$	δ = 7.582 (d, J = 8.23 Hz, 2H), 7.351 (d, J = 8.00 Hz, 2H), 5.981 (s, 1H), 2.436 (s, 3H)

D.4.1.1.2 ^{13}C DEPT NMR

^{13}C NMR	200 MHz, $CDCl_3$	δ = 130.390 (s), 129.789 (s), 128.983 (s), 128.409 (s), 21.755 (s)

D.4.1.1.3 IR-spectroscopy

IR-ATR	ν (C-H arom.)	2919.48 cm^{-1} (w)
	ν (C-H --> CH_3)	2853.54 cm^{-1} (w)
	ν (C=C arom.)	1607.56 cm^{-1} (m)
	ν (C=S)	1180.20 cm^{-1} (st)

D.4.2 Synthesis of the molybdenum complex (II)

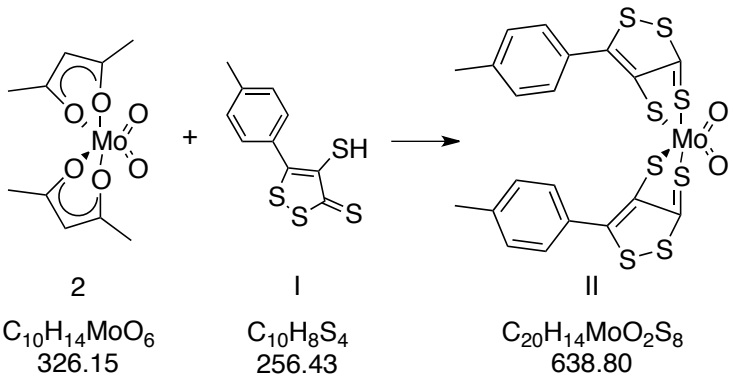

Figure 31: Synthesis of

Synthesis according to [29]:

The whole reaction was carried out under argon atmosphere.

At first two solutions were prepared:

- **Solution 1:**

  2 (77.1 mg, 0.24 mmol, 1.0 eq) was dissolved in 13 ml abs. MeOH (yellow solution).

- **Solution 2:**

  I (121.1 mg, 0.48 mmol, 2.0 eq) was dissolved in 2.6 ml abs. THF (orange solution).

Figure 32: Color of the product

When solution 2 was added drop wise to solution 1 at RT, a red solid was formed immediately. After stirring at RT for 22 h the red solid was obtained by filtration and washed with cold abs. MeOH. The solid was then dried in vacuo.

Yield 44.13 mg (35 % d. Th.)

D.4.2.1 Analysis

D.4.2.1.1 ^{1}H NMR

^{1}H NMR 200 MHz, DMSO (d_6) δ = 7.979-7.311 (m, 8H), 2.395 (s, 6H)

D.4.2.1.2 ^{13}C NMR

^{13}C NMR	400 MHz, DMSO (d_6)	δ = 213.413 (s), 177.604 (s), 141.709 (s), 129.637 (s), 129.469 (s), 129.156 (s), 128.681 (s), 21.075 (s)

D.4.2.1.3 IR-spectroscopy

IR-ATR	ν (C-H arom.)	2917.30 cm^{-1} (w)
	ν (C-H --> CH_3)	2852.60 cm^{-1} (w)
	ν (C=C arom.)	1604.90 cm^{-1} (m)
	ν (C=S)	1254.74 cm^{-1} (st)
	ν (Mo=O)	868.73 cm^{-1} (st), 900.72 cm^{-1} (st)

D.4.2.1.4 UV-VIS-spectroscopy

Maxima	A(309.18 nm) = 0.68
	A(496.84 nm) = 0.54
Minimum	A(372.88 nm) = 0.27

E) Synopsis

In this section a summary of the results will be given.

E.1 General aspects

E.2 Synthesis of the ligand

The synthesis of the ligand was successful and resulted in a sufficient yield for analysis and further coordination experiments. For analytical reasons NMR- and IR-measurements were done.

E.3 Coordination to molybdenum

The coordination of the prior synthesized ligand to molybdenum succeeded. Of the obtained complex NMR- IR- and UV-VIS-data was acquired and confirmed the given structure of the complex.

F) Appendix

Spectra, which are new in literature, are given here:

F.1 Spectra of the ligand

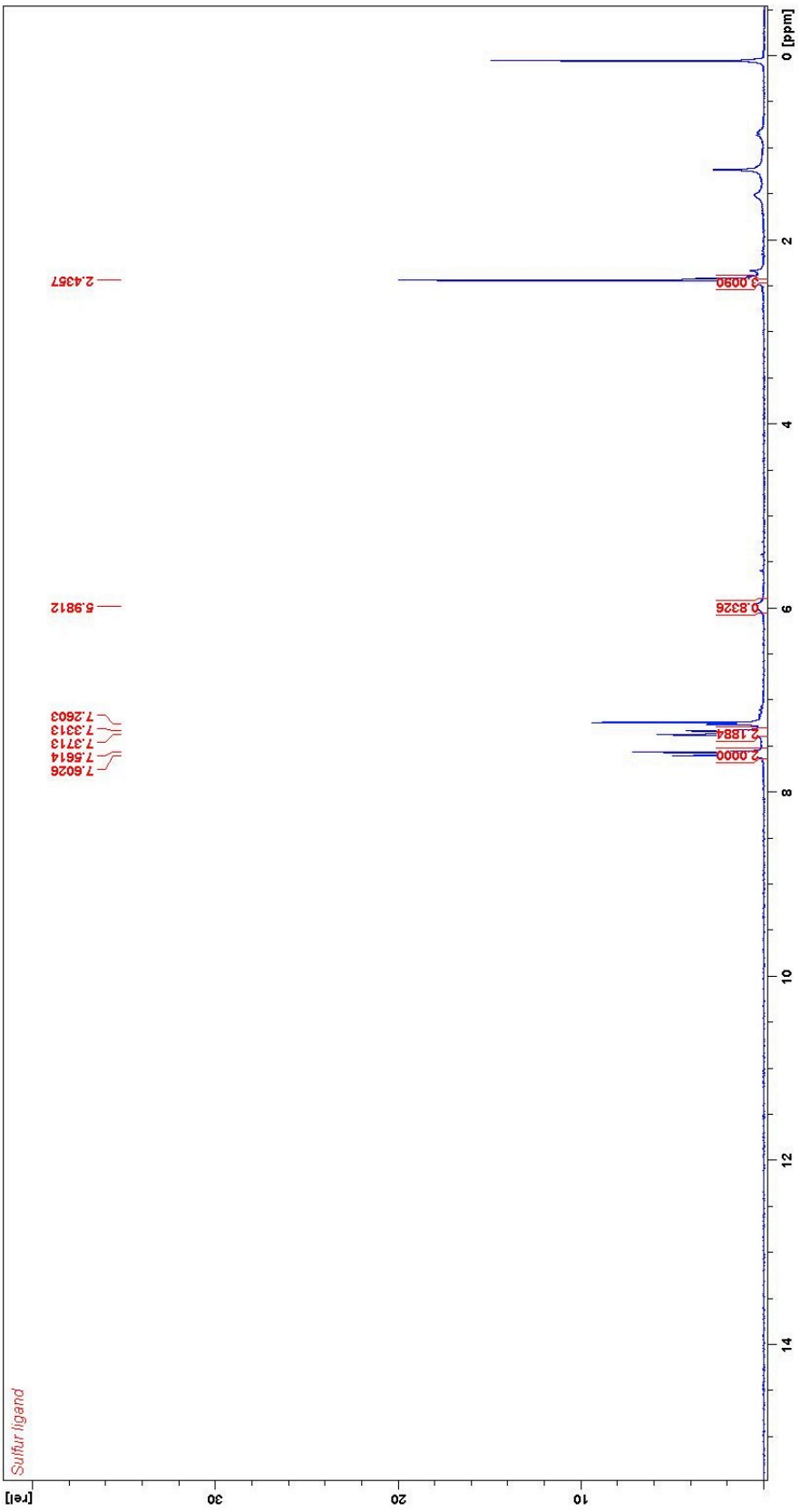

Figure 33: ^{1}H NMR of 4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione (in $CDCl_3$)

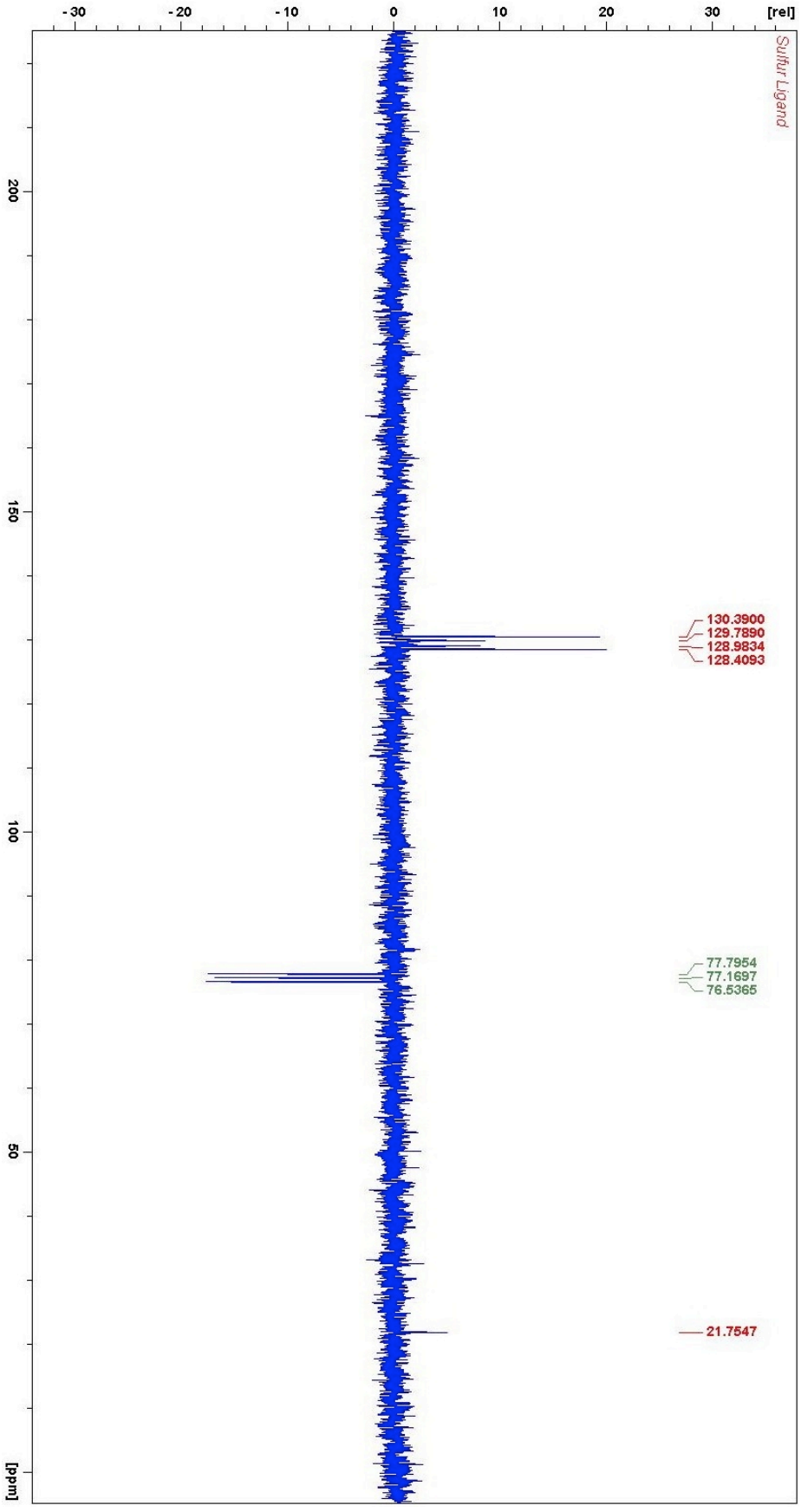

Figure 34: ^{13}C NMR of 4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione (in $CDCl_3$)

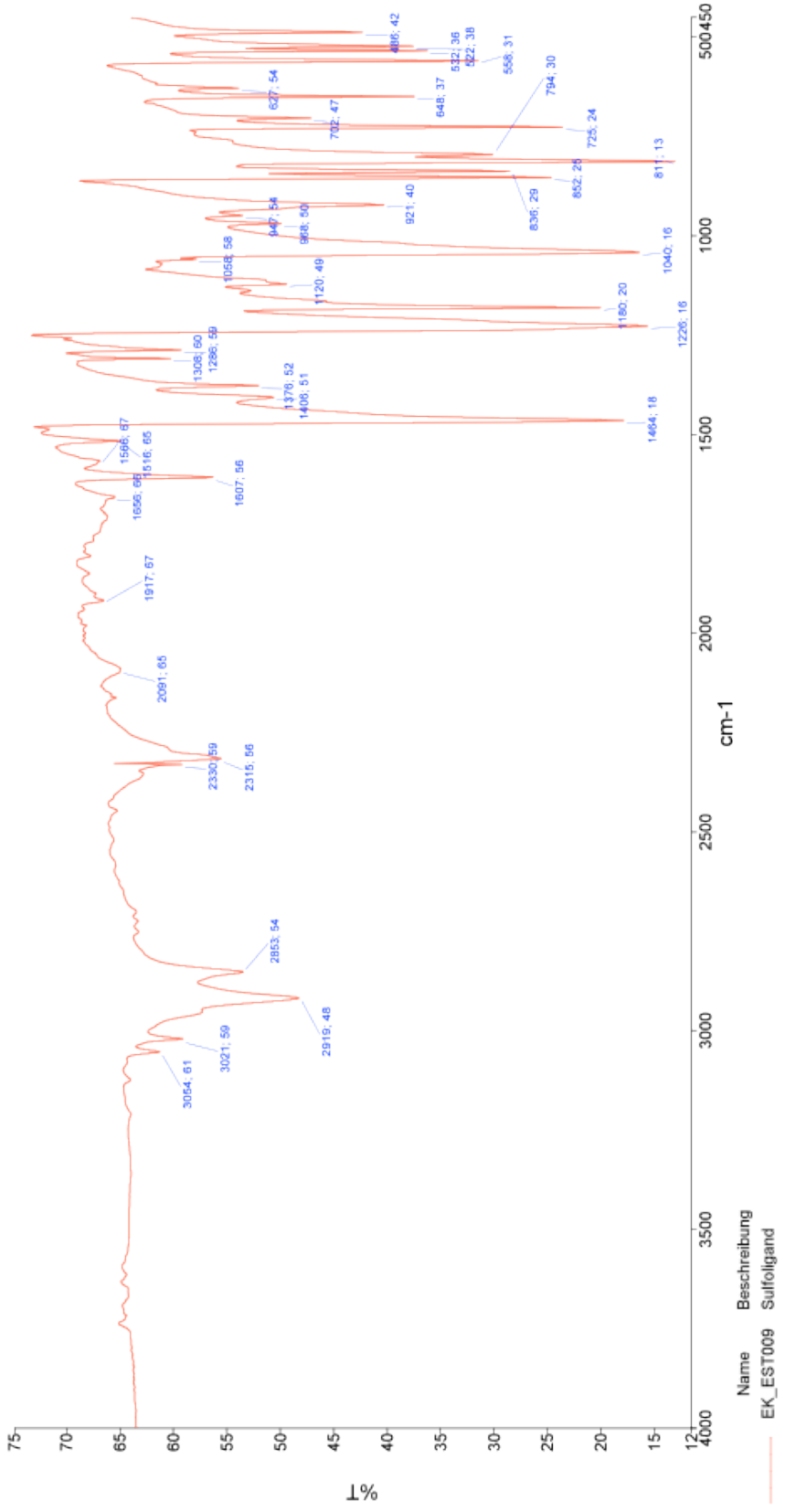

Figure 35: IR-spectrum of 4-mercapto-5-(*p*-tolyl)-3*H*-1,2-dithiole-3-thione

F.2 Spectra of the complex

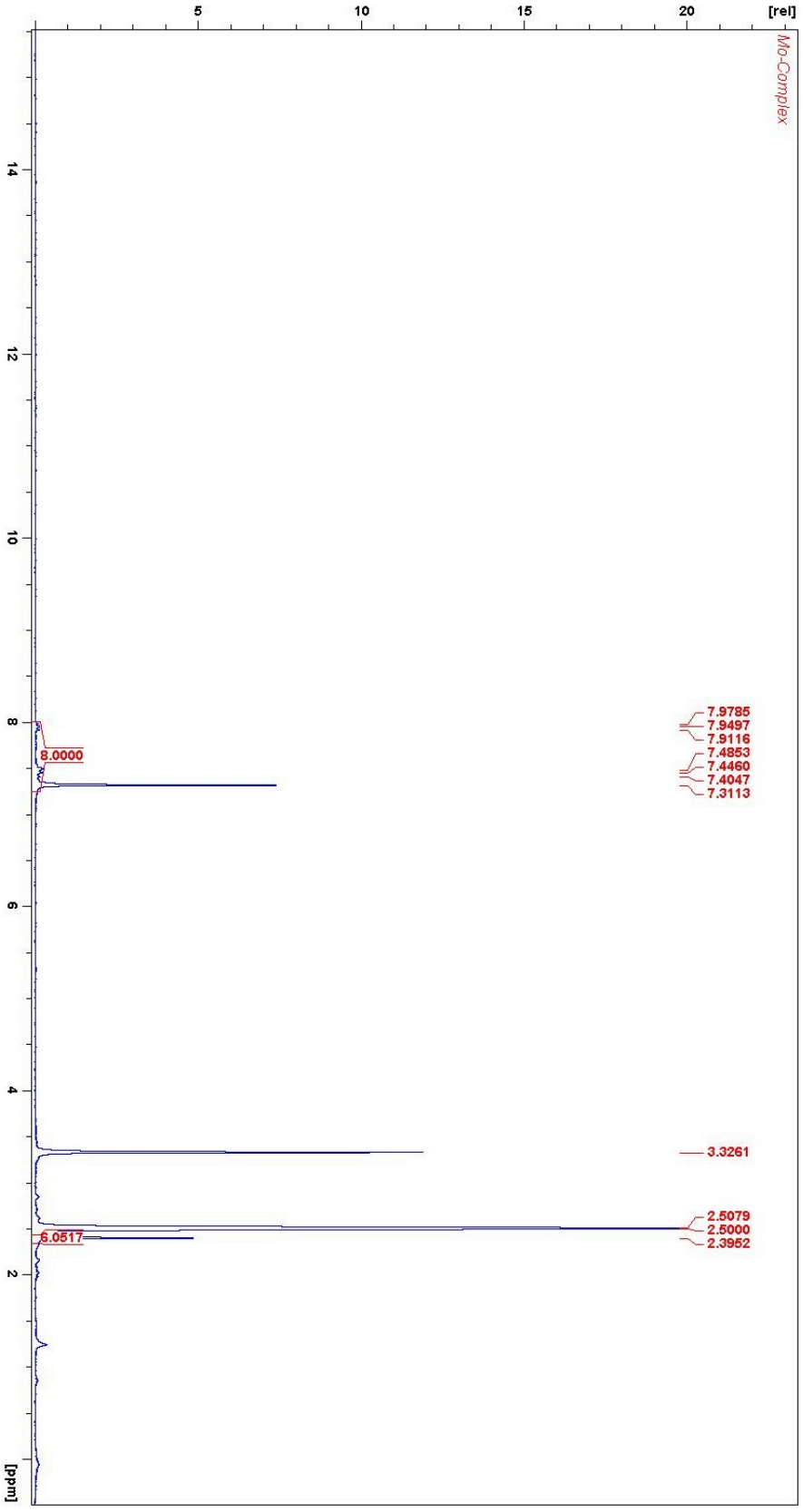

Figure 36: ^{1}H NMR of the molybdenum complex (II) (in DMSO d_6)

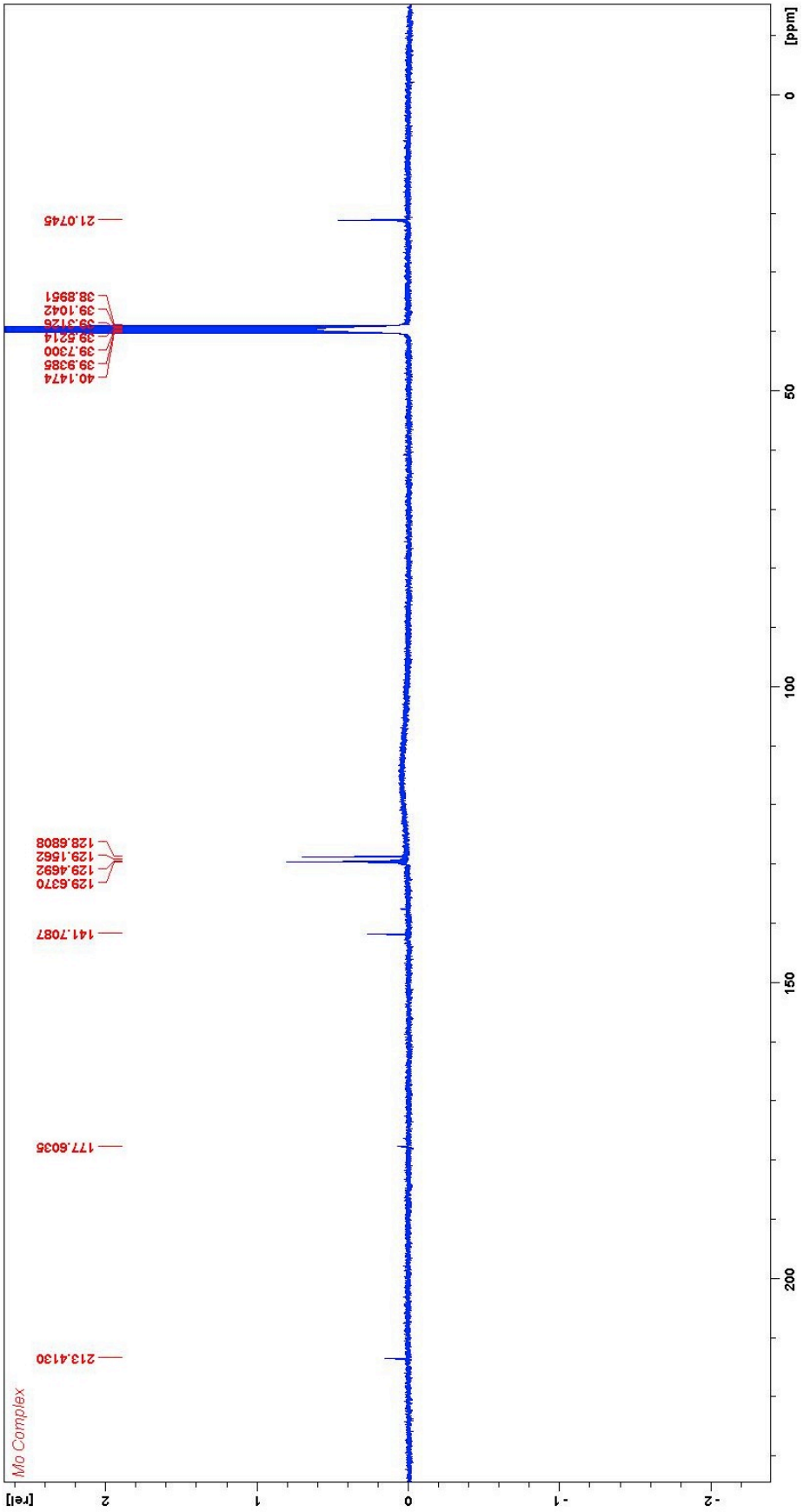

Figure 37: ^{13}C DEPT NMR of the molybdenum complex (II) (in DMSO d_6)

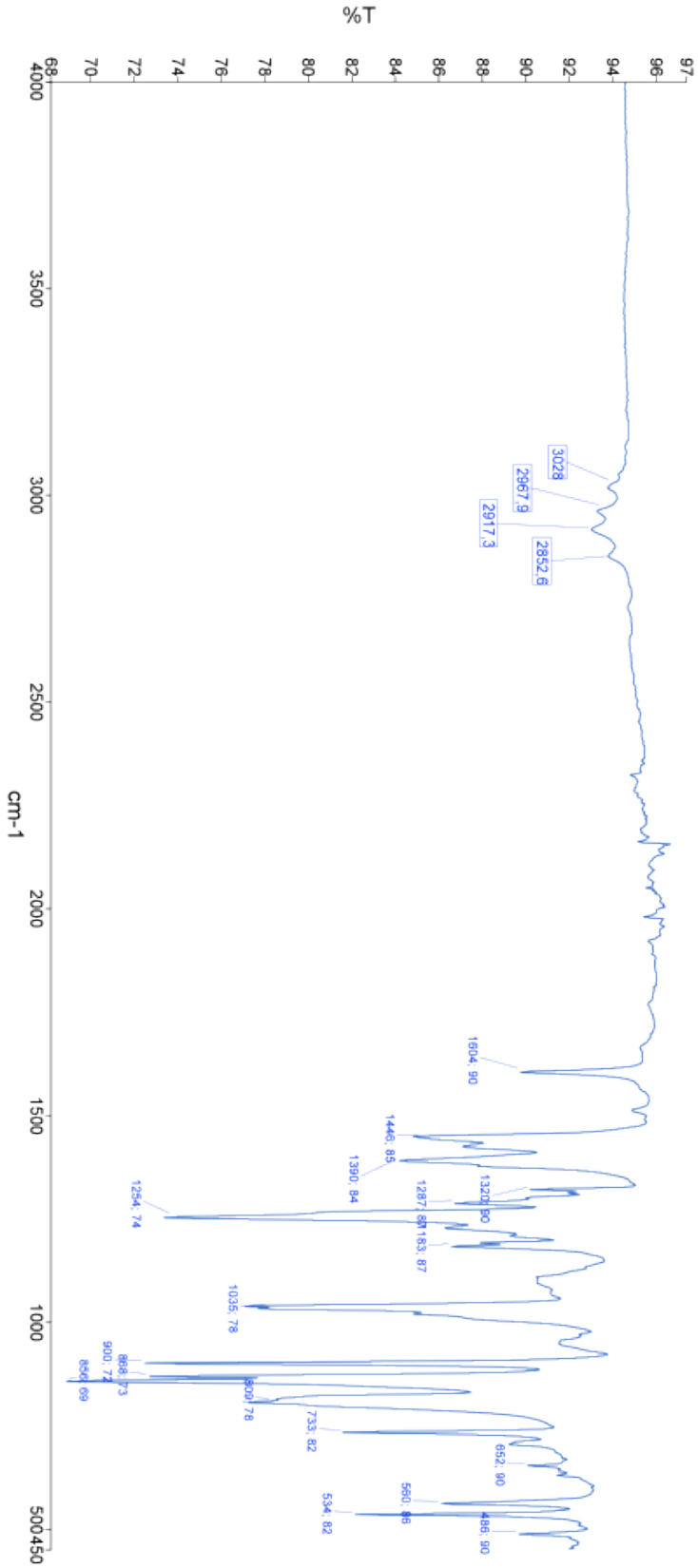

Figure 38: IR-spectrum of the molybdenum complex (II)

G) Table of illustrations

H) References

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