Synthesis of novel silver(I) based complexes with 3-(1-pyrazolyl)-L-alanine

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by **Paul Thomashausen**

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Aufgabenstellung für die Bachelorarbeit

Thema: Synthesis of novel silver(I) based complexes with 3-(1-pyrazolyl)-L-alanine

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This bachelor's thesis focuses on the synthesis of new complexes coordinated with the ligand system 3-(1-Pyrazolyl)-L-alanine. The study is conducted during an exchange stay in Novi Sad, with the primary goal of synthesizing and characterizing new metallorganic complexes.

A special emphasis is placed on silver as the central atom, as previous studies have already shown an interesting coordination between silver and similar ligand systems. After the synthesis of the complexes, the characterization of those compounds is carried out using various analytical methods, such as single-crystal X-ray diffraction, as well as the investigation of the antioxidant activity of the newly synthesized compounds.

In dieser Bachelorarbeit liegt der Schwerpunkt auf der Synthese neuer Komplexe, die mit dem Ligandensystem 3-(1-Pyrazolyl)-L-alanin koordiniert sind. Der Auslandsaufenthalt in Novi Sad bildet dabei den Rahmen für die Durchführung dieser Arbeiten, wobei es primär darum geht, neue metallorganische Komplexe zu synthetisieren und diese zu charakterisieren.

Ein besonderer Fokus wurde auf das Metall Silber als Zentralatom gelegt, da frühere Studien bereits eine interessante Koordination zwischen Silber und ähnlichen Ligandensystemen gezeigt hatten. Nach der Synthese der Komplexe erfolgt eine umfassende Charakterisierung durch verschiedene analytische Methoden, wie der Einkristallstrukturanalyse, oder aber auch der Untersuchung der antioxidativen Wirkung der neu synthetisierten Verbindungen.

Acknowledgement

Firstly, I would like to thank Prof. Dr. Goran Kaluđerović, as my thesis advisor, for making it possible to work on this topic, for reading and revising my thesis and his leading hands throughout this work.

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Finally, I want to thank my family for giving me immense support and inspiration throughout my years of study. Without you I probably would not even study chemistry.

Statement of Authorship

I hereby declare that I wrote this bachelor thesis by myself without unauthorized external assistance, unless otherwise acknowledged in the text. All verbatim and referenced passages and all sources of information have been quoted and indicated.

This thesis has not been submitted for any other degree.

Rothenschirmbach, 04.06.2025

Signed in, date

Signature

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List of Abbreviations

DAP – 2,6-diacetylpyridine

DPPH – 2,2-diphenyl-1-picrylhydrazyl

DMSO – dimethyl sulfoxide

EA – elemental analysis

FBN – 4-formylbenzonitrile

IR – infrared spectroscopy

LED – light emitting diode

MeOH – methanol

oop – out-of-plane bending vibrations

pz – pyrazole

pzAla – 3-(1-pyrazolyl)- L-alanine

rt – room temperature

SC-XRD – single crystal X-ray diffraction

TMGa – trimethylgalllium

TMIn – trimethylindium

Trolox – 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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1. Introduction

1.1. General Introduction

In the 19th century, the coordination chemistry started to take off as a main new branch of chemistry. Nowadays, it plays a vital role in fields like catalysis (e.g., Ziegler-Natta catalyst [1]), pharmaceutical applications (anticancer drugs, e.g. cis-platin and derivatives) or in the process of manifacturing LEDs (e.g. TMGa, TMIn). Since then, many breakthroughs in every field were achieved.

Pyrazole derivatives are an important type of heterocyclic compounds for the organometallic chemistry, that are able to coordinate to a big variety of metal centers. They are also capable of engaging in hydrogen bonding with suitable counterparts. In the behavior, and therefore the usage of pyrazole compounds, plenty of differences can be found. Some can be used as a drug to help against an inflammation (Antipyrine) or cancer (Ga(III)-(*N*,*N*,*O*)-tridentate pyrazole-imine-phenol [2]).

Silver(I) shows, because of its d^{10} configuration, perfectly suited characteristics to be used as a central atom in coordination chemistry. It can adapt to a variety of different geometries (e.g., linear, trigonal, square-planar, trigonal pyramidal, octahedral) and it is generally known that silver(I), either in the form of its salts or in coordination compounds, can be used in pharmaceutical ways to treat microbial infections [3].

All those characteristics and properties lead to this work, within which two novel 3-(1-pyrazolyl)-L-alanine-silver(I) compounds **1** $[Ag_2(pzAla)_2](NO_3)_2$ and **2** $[Ag(pzAla)_2]ClO_4$ were successfully synthesized and characterized by IR and SC-XRD. The attempt was made to obtain another mode of crystals from those compounds by the usage of the moderator 4-formylbenzonitrile, which did not yield any of the desired newly oriented crystal structures. Only single crystals of the moderator were isolated. The structure of the two newly gained compounds was solved throughout the SC-XRD, IR measurements were conducted and the purity was confirmed by elemental analysis. The antioxidative behavior of both of the compounds was tested.

1.2. Silver as the metal backbone

Silver, known as one of the coinage metals, can be found in its native form, like gold, platinum or copper, in the nature, but only in lower quantities compared to those other native metals. The usage of silver grew around 3000 BC as soon as the method of cuppellation was known to mankind. Through this method silver could be gained from its ores. One of the most found silver ores is argentite (silver glance, AgS_2) but also other sulfide ores can contain silver. Horn silver (AgCl) counts to one of the more commonly found silver ores. Silver nowadays is produced as a byproduct in the smelting process of metals like copper or zinc. It is further purified through electrolysis to yield silver with a purity of 99.9 % or greater. This silver can then be used in the electronic industry for LED or in the field of photography [4]. Some important properties of silver are given in Table 1.

Table 1: Properties of silver [4]

Atomic number	47
Naturally occurring isotopes	2
Atomic weight	107.87
Electronic configuration	[Kr] $4d^{10}\,5s^{1}$
Electronegativity	1.9
Metal radius [pm]	144
Density (20 °C) [$g\cdot cm^{-3}$]	8.95
Melting point [°C]	1083
Boiling point [°C]	2570

Like already mentioned, silver may be used in various applications. Silver(I) compounds are known as antimicrobial and anti-inflammatory agents [5]. For this work, the metal complexes of silver are the particularly interesting ones. The silver(I) ion can form coordination polymers that show photocatalytic and photoluminescent properties. It is often found bicoordinated in complexes, but in coordination polymers it forms three or four coordinative bonds to the ligand system [6]. Such a system with Ag(I) that is coordinated to four nitrogen atoms of pyrazol rings is [Ag(TPEA)](PF_6) (TPEA = *tris*(1-pyrazolylethyl)amine, CSD refcode: RAJDIR) [7]. Other examples are, [AgL_2]ClO_4 (L = ethyl-5-amino-1-methyl-1*H*-pyrazole-4-carboxylate, CSD refcode: LOFWIQ) and, {[AgL_2]ClO_4}$_n$ (L = ethyl-5-amino-1-methyl-1*H*-pyrazole-4-carboxylate, CSD refcode: LOFWOW) [6]. [AgL_2]ClO_4 shows just a bicoordinated Ag(I) central atom between two pyrazole rings, while {[AgL_2]ClO_4}$_n$ is a monoperiodic polymer that is connected by the perchlorate anion.

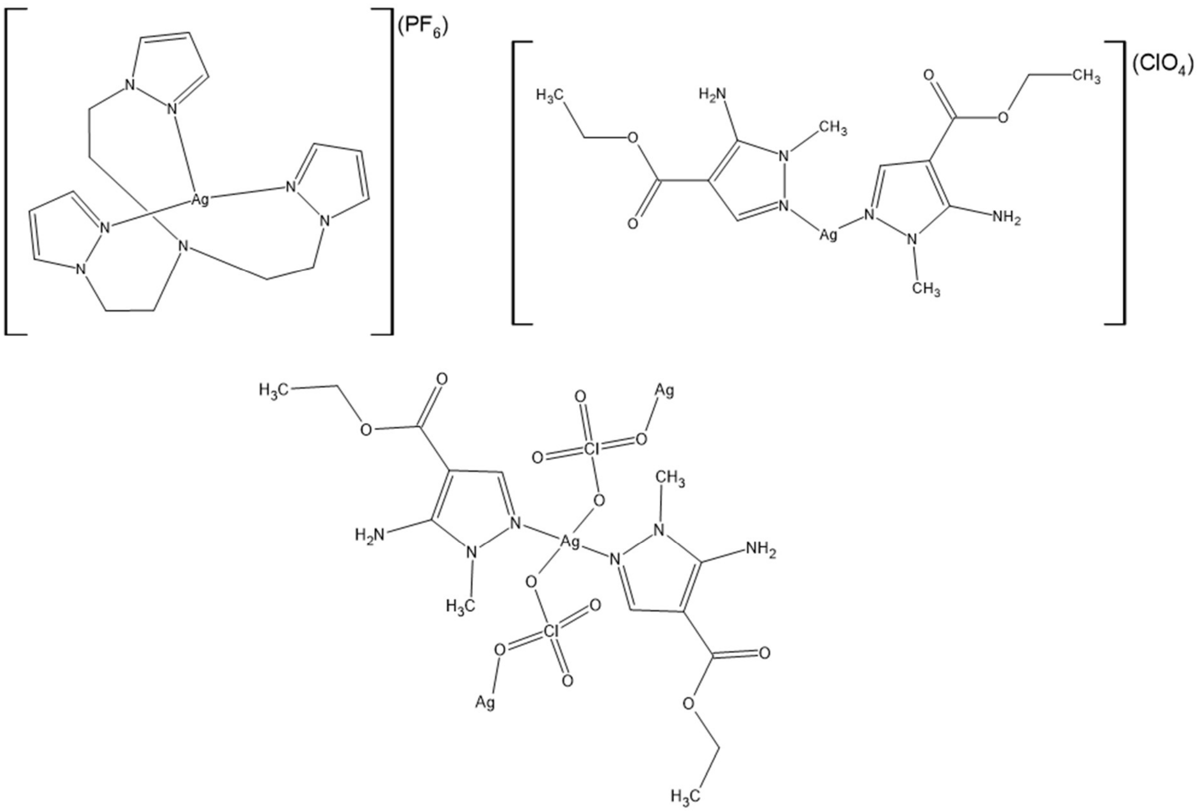


Figure 1: Structural formulas of the three complexes containing silver top left to bottom: [Ag(TPEA)](PF₆), [AgL₂]ClO₄ and {[AgL₂]ClO₄}ₙ

1.3. The ligand system

Pyrazole (1-*H*-pyrazole, 1,2-diazacyclopenta-2,4-diene) is an aromatic heterocycle derived from cyclopentane, with three carbon atoms and two adjacent nitrogen atoms in ortho substitution. It belongs to the azole class (Fig. 2), nitrogen atom one (N^1) is bound to a hydrogen atom and its unshared electrons are conjugated with the aromatic system, which yields a “pyrrole-like” behavior. The second nitrogen atom (N^2) is “pyridine-like” since its free electrons are not compromised with resonance. The difference in these two nitrogen atoms leads to the fact that pyrazole can react with both acids and bases (Fig. 3) [8].

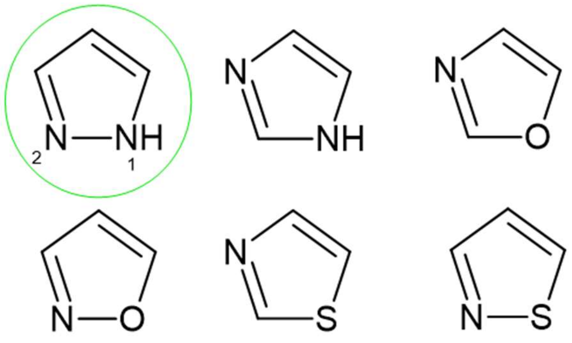


Figure 2: Some azoles, from top left to bottom right: pyrazole (marked green), imidazole, oxazole, isoxazole, thiazole and isothiazole

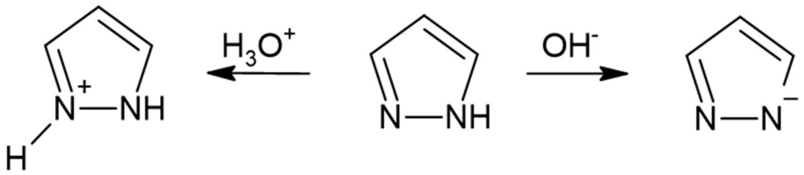


Figure 3: Cations and anions produced from pyrazole [9]

Pyrazole also shows the ability to form tautomers. Unsubstituted pyrazole is able to form three tautomers while a monosubstituted pyrazole can yield five different tautomers (Fig. 4). The green marked tautomers preserve the aromaticity of the ring system and are therefore the most relevant ones [8].

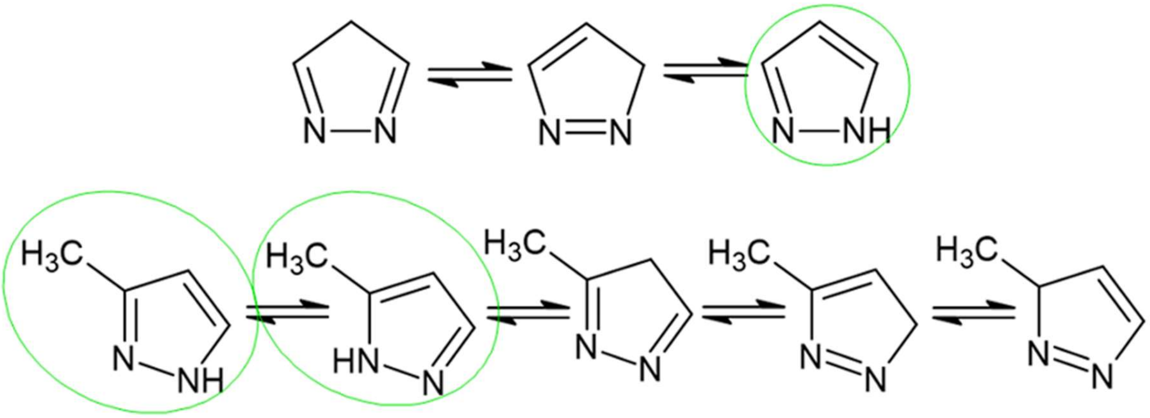


Figure 4: Tautomers of unsubstituted (upper) and substituted (exemplarly 3-methylpyrazole) pyrazoles) (lower)

Pyrazoles can be synthesized in different ways. One of such ways that yields pyrazoles is the condensation of hydrazine with an 1,3-dicarbonyl compound (Knorr pyrrole synthesis). Another communly used synthesis is the Pechmann synthesis [8]. In Table 2 some of the main properties of pyrazole are given.

Table 2: Properties of pyrazole [9]

IUPAC Name	1H-pyrazole
Molecular Formula	$C_3H_4N_2$
Molecular Weight [g mol^{-1}]	68.08
Melting point [°C]	68
Boiling point [°C]	187
Solubility in water (25 °C) [g· **L**$^{-1}$]	19.4
Dissociation Constant (25 °C)	2.48
Vapor Pressure [mmHg]	1.19

Pyrazole shows a wide range of usages as shown in Figure 5. There are plenty of different drugs that contain pyrazole as one of the main components in their structure. Some of these drugs are known for their antimicrobial, anti-inflammatory, antipyretic, or antitumor activity. Pyrazole derivatives also show a wide range of biological activity and are excellent candidates that can coordinate to an active proteine center and can therefore be used for enzymatic or other biochemical reactions. But not only these biological activities make pyrazole compounds interesting, they are also relatively stable at high temperatures and easily form chemical bonds. Pyrazole can yield porous metal-organic frameworks, coordination polymers, or others, which then get used in fields like chemical vapor deposition or in the reserarch for new energetic materials [10].

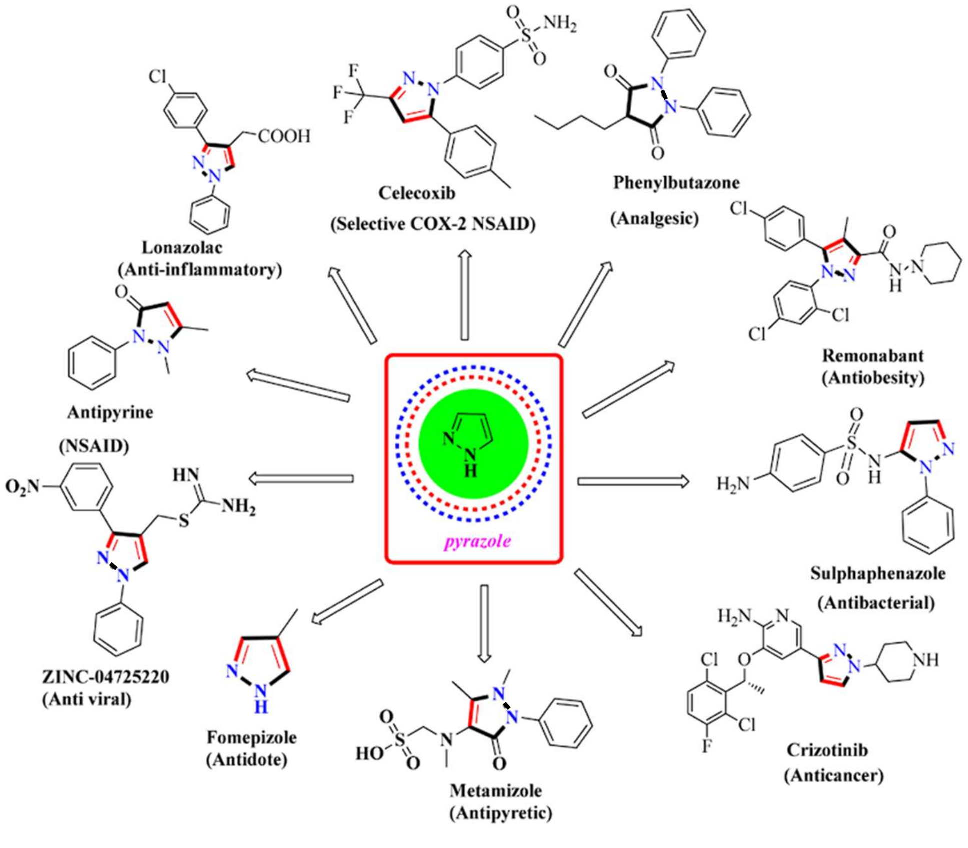


Figure 5: Application field of pyrazole in the pharmacology [11]

PzAla as a ligand is expected to shows similar characteristics like the other pyrazole derivatives. Research showed that pzAla is capable of form complexes with metal ions like copper(II) or zinc(II). Moreover, pyrazole derivatives yielded complexes of silver(I) [6]. The silver complexes are formed with ethyl-5-amino-1-methyl-1*H*-pyrazole-4-carboxylate as the ligand and showed a linear and square-planar coordination. Those characteristics of pyrazole compounds, and the fact that an amino acid forms a part of the molecule, made PzAla interesting as a new ligand for metal complexes.

2. Results and discussion

2.1. Synthesis of the different silver(I) complexes

2.1.1. Synthesis of [$Ag_2(pzAla)_2](NO_3)_2$

The [$Ag_2(pzAla)_2](NO_3)_2$ complex **1** was obtained through the reaction of pzAla with silver(I) nitrate (Fig. 6). The reaction was carried out in water. Both reactants were used in equimolar ratio . The reaction flask was covered in aluminum foil to keep the reaction solution in dark, due to the photosensitivity of Ag(I) cation. After four weeks when all of the used solvent evaporated, whitish crystals with brown spots and small brown particles on them had formed on the walls and at the bottom of the used flask. The brown particles started to form after about a week, floating in the solution. The complex was washed with some water and filtered to get rid of some of the particles, which are most likely degradation products of the silver salt (Fig. 7).

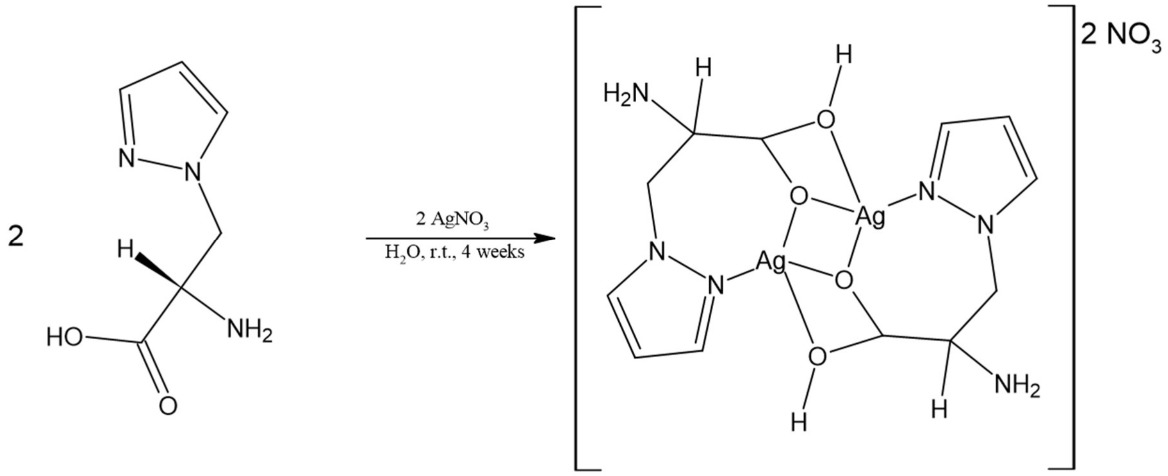


*Figure 6: Synthesis of [$Ag_2(pzAla)_2](NO_3)_2$ (**1**)*

*Figure 7: Obtained crystals from compound **1** under the microscope*

2.1.2. Synthesis of [Ag(pzAla)$_2$]ClO$_4$

For the synthesis of the [Ag(pzAla)$_2$]ClO$_4$ complex **2** the synthetic procedure of **1** was applied. However, in this reaction instead of silver(I) nitrate the perchlorate salt was used (Fig. 8). After five weeks, some clear appearing yellow/brown colored crystals, with the same brown chunks on them, were removed from the solution by tweezers. After seven more days newly formed crystals were obtained from the solution. These two crystal fractions show two types of different crystals. Specifically, the second fraction constist of the same crystals as the first one, however some small could be also identified (Fig. 9).

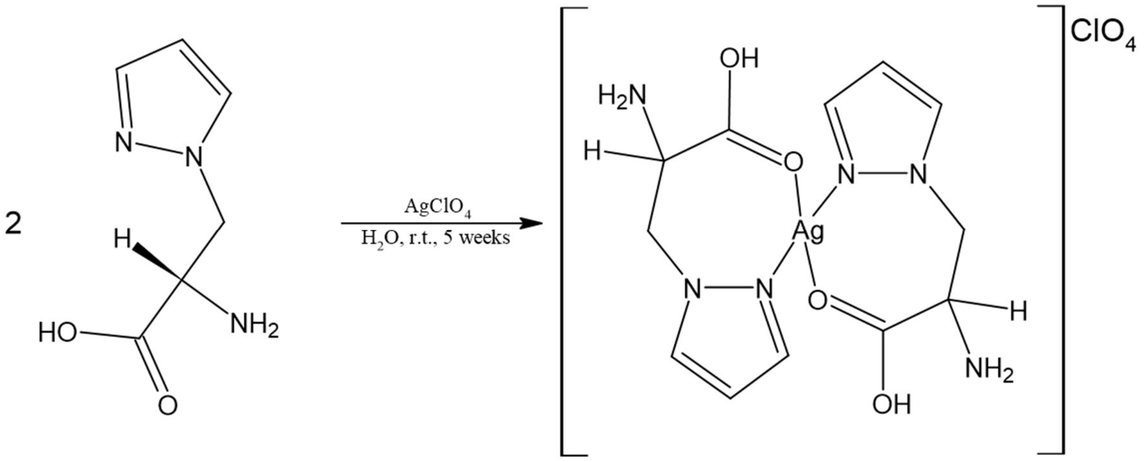


*Figure 8: Synthesis of [Ag(pzAla)$_2$]ClO$_4$ (**2**)*

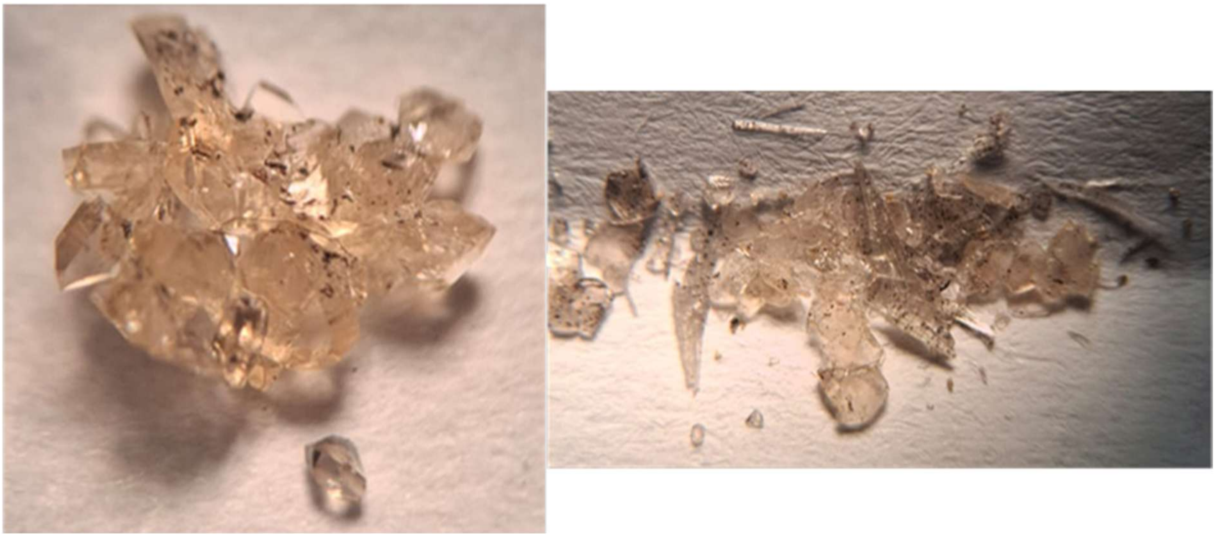


*Figure 9: Crystals obtained from the solution of **2** (left) and from the filtrate (right) under the microscope*

2.1.3. Attempt to modulate the crystal structure of **1** in the presence of 4-FBN

Under similar reaction condidtions it was attempted to obtained the desired complex **3** as for **1**, just with the additon of 4-FBN to yield an other orientation of the crystal lattice. Firstly the ligand pzAla and the 4-FBN were refluxed in water at equimolarmolar ratio. To the cooled solution of pzAla and 4-FBN the silver(I) nitrate salt was added (Fig. 10). From the added 4-FBN a new orientation mode of the crystal structure was expected since 4-FBN showed such behavior in similar cases [6]. The used amount of silver(I) nitrate was lowered. The molarity of it was halved to yield a decrease in the activity of silver(I) ions in the reaction, to see if this would yield any crystals with the 4-FBN. After three weeks, clear crystals were obtained from the solution (Fig. 11).

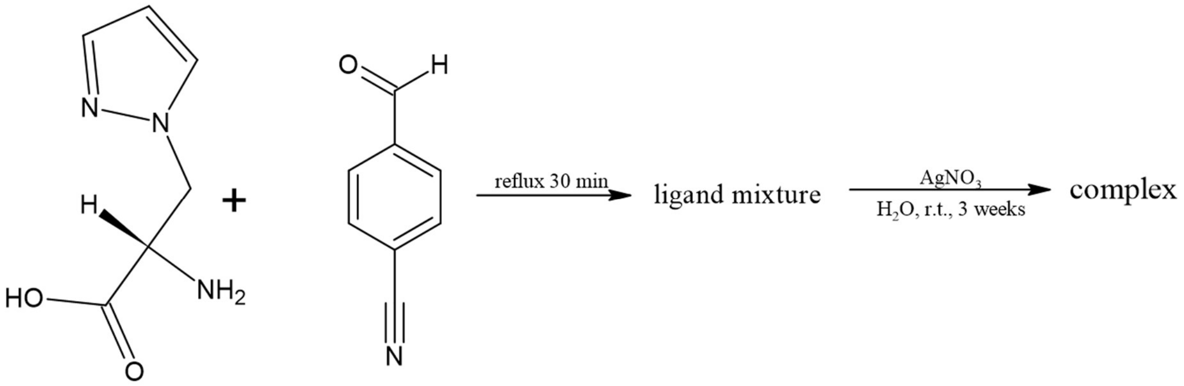


*Figure 10: Attempted synthesis of the modulation of the crystal structure of **1** with 4-FBN*

Figure 11: Crystals obtained in the presence of 4-FBN under the microscope

2.1.4. Attempt to modulate the crystal structure of **2** in the presence of 4-FBN

The desired complex **4** was also attempted to synthesized according to the procedure for complex **3** just with the perchlorate salt (Fig. 12). After three weeks, crystals were obtained from the solution. These show two different appearances, longer clear needles and some smaller clear crystals in between them (Fig. 13).

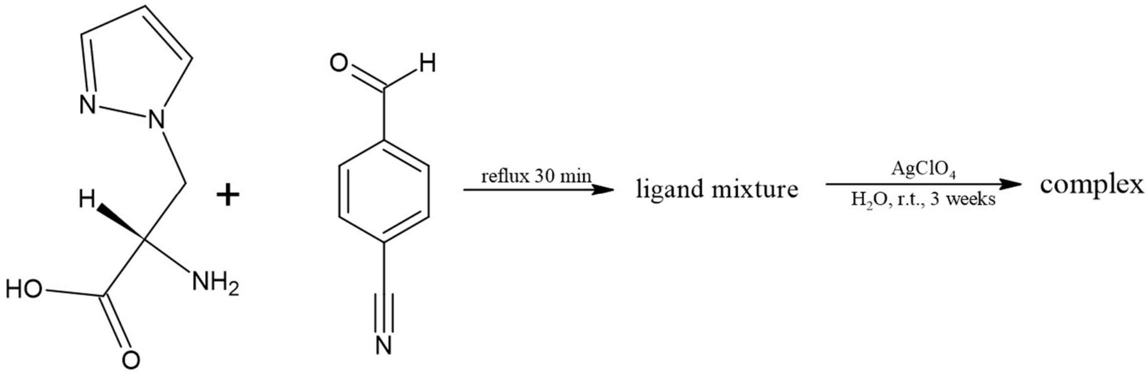


*Figure 12: Attempted synthesis of the modulation of the crystal structure of **2** with 4-FBN*

Figure 13: Crystals obtained in the presence of 4-FBN under the microscope

2.2. Comparison and discussion of the gained products

2.2.1. Synthesis and characterization of the compounds

Before comparing the prepared complexes, it has to be stated, that the syntheses in the presence of 4-FBN (compounds **3** and **4**) did not yield any silver(I) complexes. The obtained crystals looked promising, but the SC-XRD and IR analysis confirmed that the obtained material was in both cases just the 4-FBN that crystallized out from the reaction mixture. Those clear crystals appeared similar to the complexes. The desired effect of the 4-FBN in which it acts as a moderator to form new orientation modes of the crystal lattice, could not be achived since no complexes where isolated.

Complex **1** appears as flaky white crystals with brown spots on them, mixed in between are needles with the same color. The flakes are about 0.5 to 1 mm large, while the needles can reach up to 2 mm in legnth.

Complex **2** appears as clear crystals with a few small brown dots on the surface, about 0.5 mm in size, that formed an aggregate (Fig. 9 left), where several different crystals sticked together. Like in **1** smaller needles can be seen in between the clear crystals, where more are present in the crystals obtained from the filtrate.

2.2.2. Crystal structure

The molecular structure of **1** (Fig. 14) in the asymmetric unit consists of two Ag^+, two pzAla molecules in their zwitter-ionic form, and two nitrate anions. Each silver atom is coordinated by four atoms, one nitrogen and three oxygen atoms. The free nitrogen atom (N2/N2') of the pyrazole ring binds to one of the two silver ions (Ag1), while one oxygen of the carboxyl group (O1') binds with the same Ag^+ ion (Ag1) but also coordinates to the other Ag^+ ion (Ag2) of the asymmetric unit. The remaining oxygen of the carboxyl group (O2') is able to coordinate with an adjacent Ag^+ ion from another complex molecule, from which its own oxygen atom of the neighbouring carboxyl group coordinates to the Ag^+ ion (Ag1). The fourth bond is formed by the carboxyl oxygen atom of the second ligand molecule (O1). For the other Ag^+ (Ag2) the coordination environment is similar, besides the fact, that just one carboxyl oxygen from the amino acid coordinates with it (O2). This structual formation yields a six-membered bimetallocycle with its metal centers in a distorted tetrahedral environment ($\tau_4 = 0.673$) in a somehow angled seesaw shape. The axial ligand pair for Ag1 consists of N2' and O1 and shows a angle of 161.8 ° which is smaller than the ideal angle of 180 °. This smaller bond angle leads to the used term of the angled seesaw shape. Compound **1** shows polymer strands of the oxygen-silver(I) linkage network. If both Ag^+ ions are compared to each other a similar behaviour in bond lengths and angles can be observed (Tab. 3). The silver-nitrogen bonds Ag1—N2' and

Ag2—N2 show a value of 2.204(15) Å and 2.193(17) Å, compared to other compounds with similar structure fragments of Ag—N(pz) like $Ag_4(tbz)_2(H_2bdpm)_2(HPMo_{12}O_{40})]\cdot 2H_2O$ (Htbz = thiabendazole, H_2bdpm = 1,1′-*bis*(3,5-dimethyl-1*H*-pyrazolate)methane, CSD refcode: ZULJUN [12]), $[Ag(3{,}5dmpz)_2]SbF_6$ (3,5dmpz = 3,5-dimethyl-1*H*-pyrazole, CSD refcode: UNONER [13], $[Ag(H_4thinz)_2]NO_3$ (4,5,6,7thinz = 4,5,6,7-tetrahydro-1*H*-indazole, CSD refcode: JAXKOL [14], $[Ag(dphypz)_2]NO_3$ (dphypz = 3,5-diphenylpyrazole, CSD refcode: ABOREQ [15]] they are slightly higher than those found in other similar compounds (up to 4 %; up to 0.1 Å). Others like $[Ag(TPEA)](PF_6)$ [7] show a similar value in the bond length. All geometric parameters can be seen in Table 3.

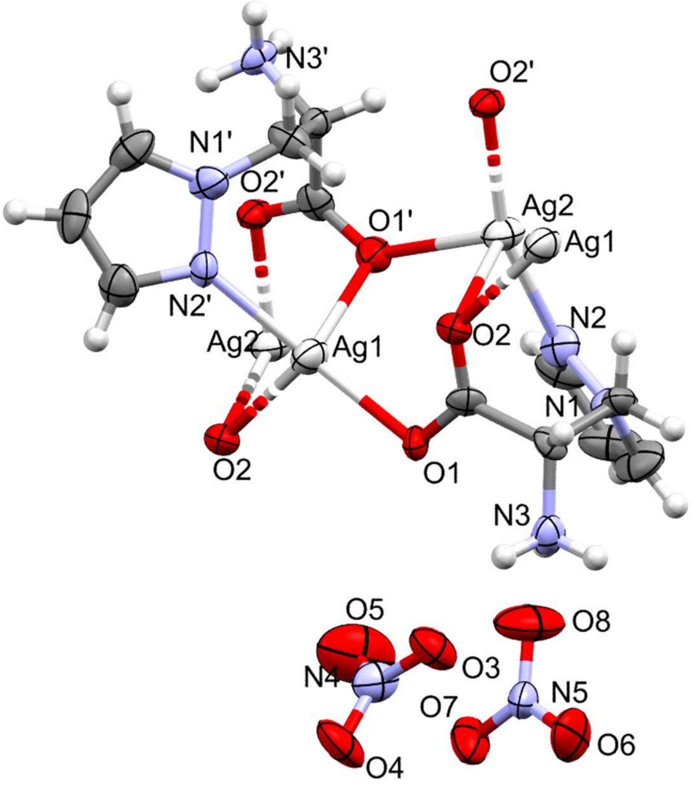


*Figure 14: Crystal structure of complex **1***

Compound **1** consists of complex polymer strands that follow the 0-a axis (a-axis), in which the silver-nitrogen-oxygen network is centered. On the left and right sides of the strand are the remaining ligand molecules situated. Each of those strands is connected by hydrogen bonds (Tab. 4) of the NH_3^+ group to the adjacent strands through the nitrate anions (Fig. 15). The nitrate anions assamble closely to the positive charged NH_3^+ group and form a linkage between different strands of the polymer. The distance between the hydrogen of the NH_3^+ and the oxygen of the nitrate anion (N3—H3A···O3) is around 2.08 Å. The opposing charges of those two yield a electrostatic bonding of the strands which additionally holds them together. In the face of the whole structure of the crystal lattice two different orientaions of the strands can be observed. Both of them are tilted at roughly 130° and are alternating between horizontal orientation and tilted orientation. The lattice can be seperated in diagonal layers of either horizontal (green) or tilted (orange) oriented polymer strands (Fig. 16).

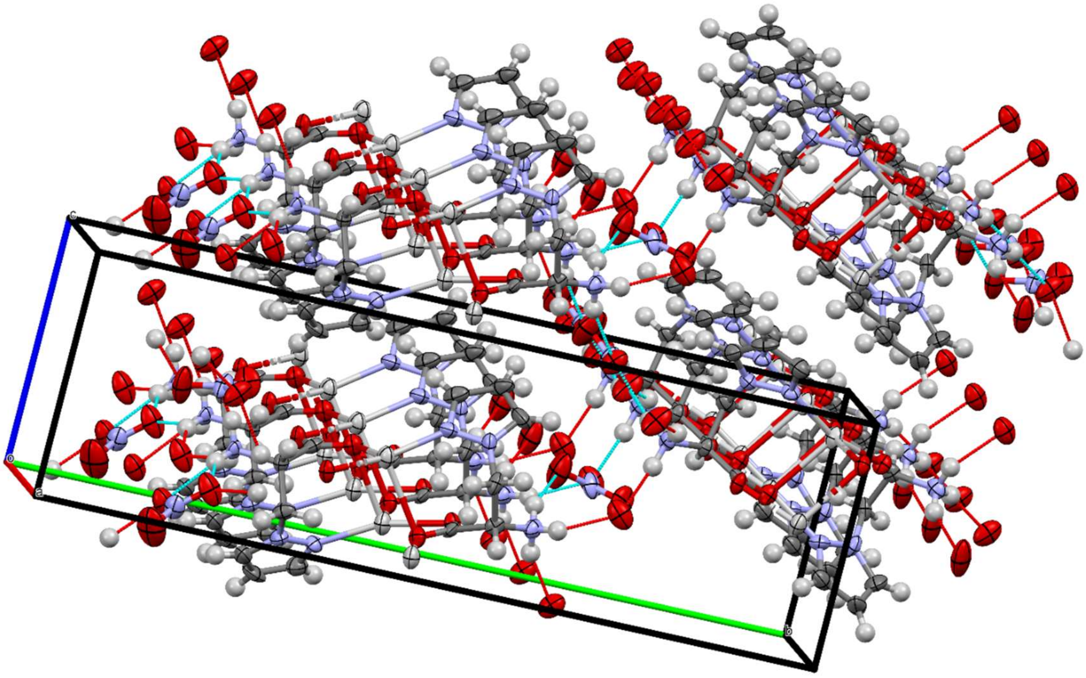


*Figure 15: Crystal packing of complex **1** in the lattice*

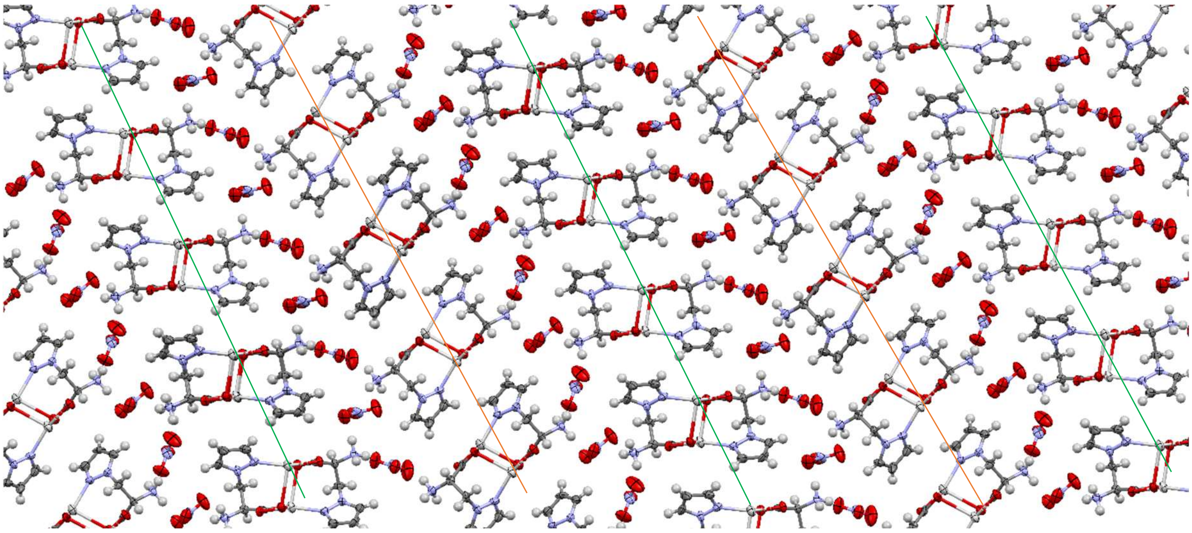


*Figure 16: Orientation of the different strands in the lattice of compound **1** (shown through the 0-a axis, green horizontal oriented layer, orange tilted layer)*

*Table 3: Geometric parameters of complex **1***

Bonds	*Length [Å]*	*Bonds*	*Angle [°]*
Ag1—N2'	2.204 (15)	N2'—Ag1—O1	161.8 (5)
Ag1—O1	2.223 (12)	N2'—Ag1—O1'	88.98
Ag1—O1'	2.662	O1—Ag1—O1'	103.19
Ag1—O2'^{i}	2.507 (12)	N2'—Ag1—O2'^{i}	88.9 (5)
Ag2—N2	2.193 (17)	N2—Ag2—O1'	90.0 (5)

Ag2—O1'	2.519 (12)	N2—Ag2—O2'$^{\mathrm{i}}$	161.38
Ag2—O2	2.649	N2—Ag2—O2	88.53
Ag2—O2'$^{\mathrm{i}}$	2.244 (12)	O1'—Ag2—O2	97.10
		O2'$^{\mathrm{i}}$—Ag2—O1'	103.6 (4)

Symmetry code: (i) x+1, y, z

The existing hydrogen bonds between the NH_3^+ groups and oxygen atoms of the perchlorate anions are induced through the fact that both of those components are attracted by each other since their opposing charges. The formed hydrogen bonds, of roughly 2 Å in distance between hydrogen atom and the acceptor atom, which is similar to the ones shown in [6], support the positioning of the polymer strands to its neighbouring ones in the lattice.

Table 4: Hydrogen-bond geometry of compound ***1***

D—H···A	*D—H [Å]*	*H···A [Å]*	*D···A [Å]*	*D—H···A [°]*
N3—H3A···O3	0.89	2.08	2.923 (19)	157
N3—H3B···O7$^{\mathrm{i}}$	0.89	2.05	2.89 (2)	157
N3—H3C···O8	0.89	2.01	2.82 (2)	152
N3′—H3′A···O3$^{\mathrm{iii}}$	0.89	2.07	2.91 (2)	157
N3′—H3′A···O4$^{\mathrm{iii}}$	0.89	2.30	3.06 (2)	142
N3′—H3′B···O4$^{\mathrm{iv}}$	0.89	2.08	2.84 (2)	142
N3′—H3′C···O6$^{\mathrm{v}}$	0.89	2.46	3.00 (2)	119
N3′—H3′C···O7$^{\mathrm{vi}}$	0.89	2.12	2.93 (2)	151

Symmetry codes: (i) x+1, y, z; (iii) −x+1, y−1/2, −z; (iv) −x, y−1/2, −z; (v) −x+1, y−1/2, −z+1; (vi) −x, y−1/2, −z+1.

Complex **2** shows a molecular structure, which consists of one Ag^+ central atom, two ligand molecules and one perchlorate anion (Fig. 17). The Ag^+ cation (Ag1) is chelated by both ligand molecules through each of their free nitrogen atoms of the pyrazole ring (N2 and N5). One carbonyl oxygen of the amino acid end of two adjacent complex molecules (O1 and O4) in the crystal lattice can coordinate to the silver central atom to form a four-membered metallocycle ($\tau_4 = 0.693$) that shows like complex **1** an angled seesaw geometry. The ligand molecules both exist in their zwitter-ionic form and each exhibits a *NO* coordination mode. The geometric parameters of complex **2** are shown in Table 5.

Since the oxygen atoms bind to the adjecent silver atoms the neighbouring complex molecule has to rotate 90° to make those bonds possible. Figure 18 shows that the complex molecules alternate in their rotation the middle one shows a horizontal nitrogen-silver-nitrogen bond but both its neighbours show a vertical nitrogen-silver-nitrogen bond. On those two again a horizontal complex molecule coordinates and then again a vertical one, continuing to alternate between those two rotations. This pattern extends from each silver atom in all four directions of the binding atoms, towards the nitrogen atoms to the left and right but also through the oxygen atoms in and out of plane.

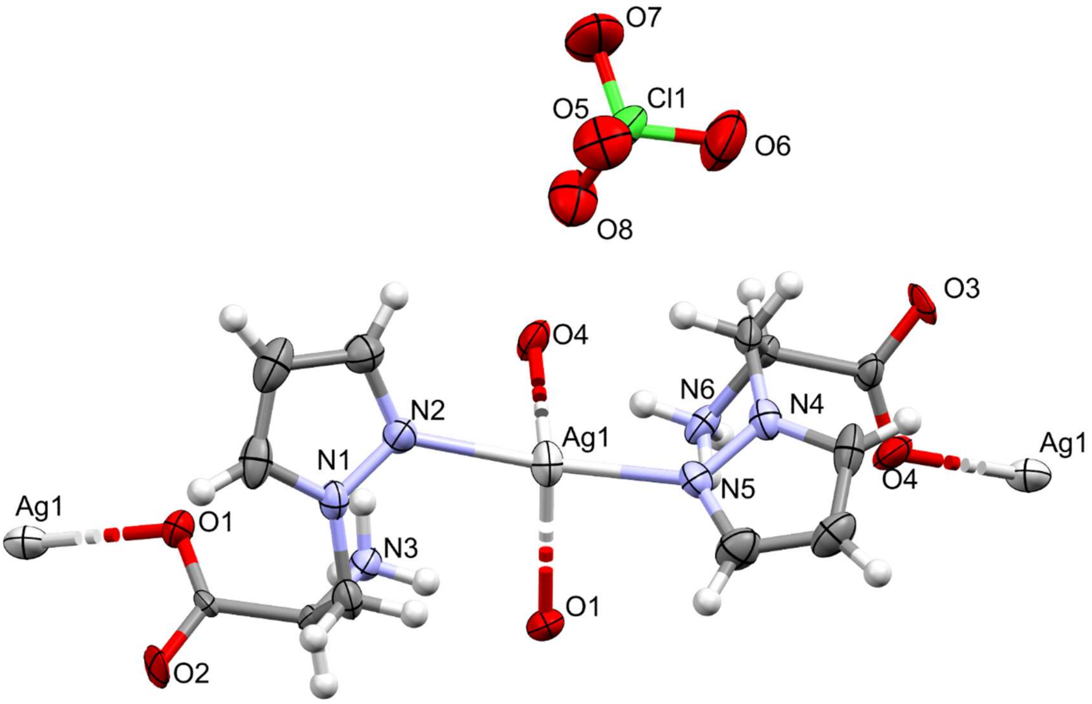


*Figure 17: Crystal structure of complex **2***

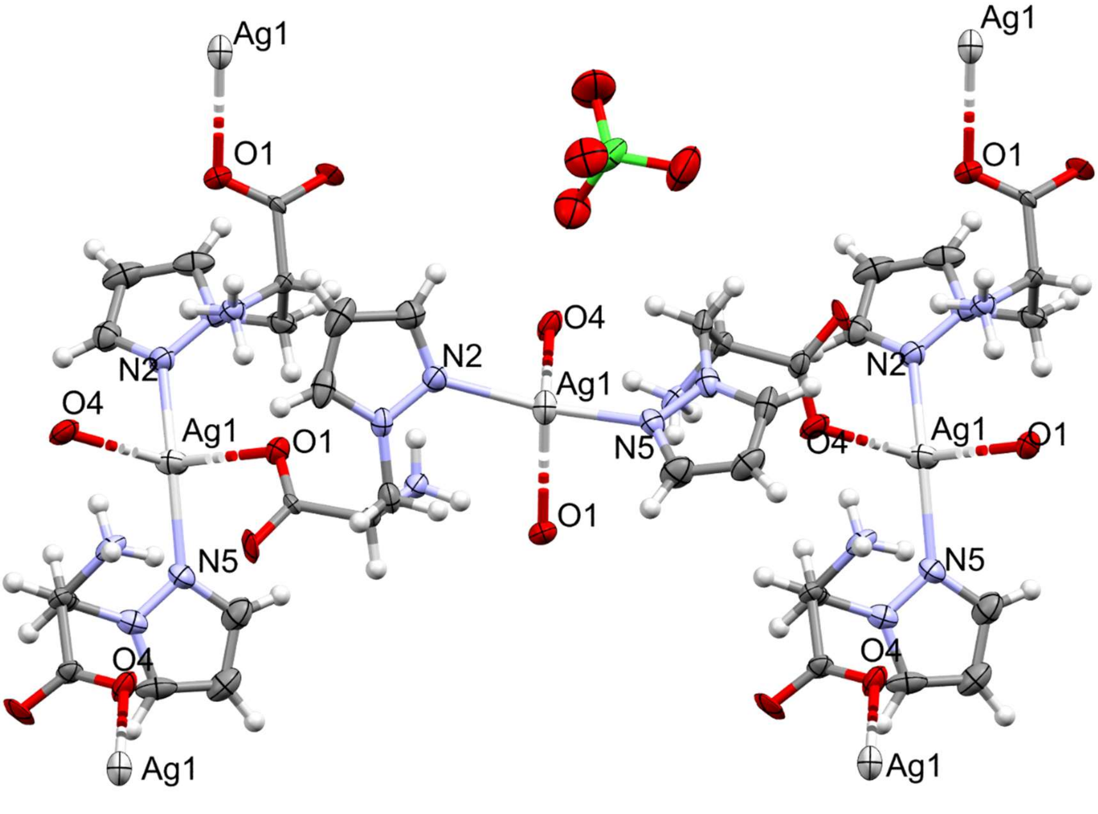


*Figure 18: Crystal structure with adjacent molecules of complex **2***

In the crystal lattice the chains of the alternating complex molecules form layers that are connected like in compound **1** but this time through the perchlorate anion. The electrostatic forces pull both ions close together so that hydrogen bonds (Tab. 6) with the amine group (N3—H3B···O5^{iii}) of 2.24 Å length can be acchieved. The amine group also forms hydrogen bonds to the oxygens of the amino acid (N3—H3A···O3^{ii} and N3—H3C···O1^{iv}) in a length of 1.95 Å and 1.97 Å. Figure 19 shows the layers in the lattice.

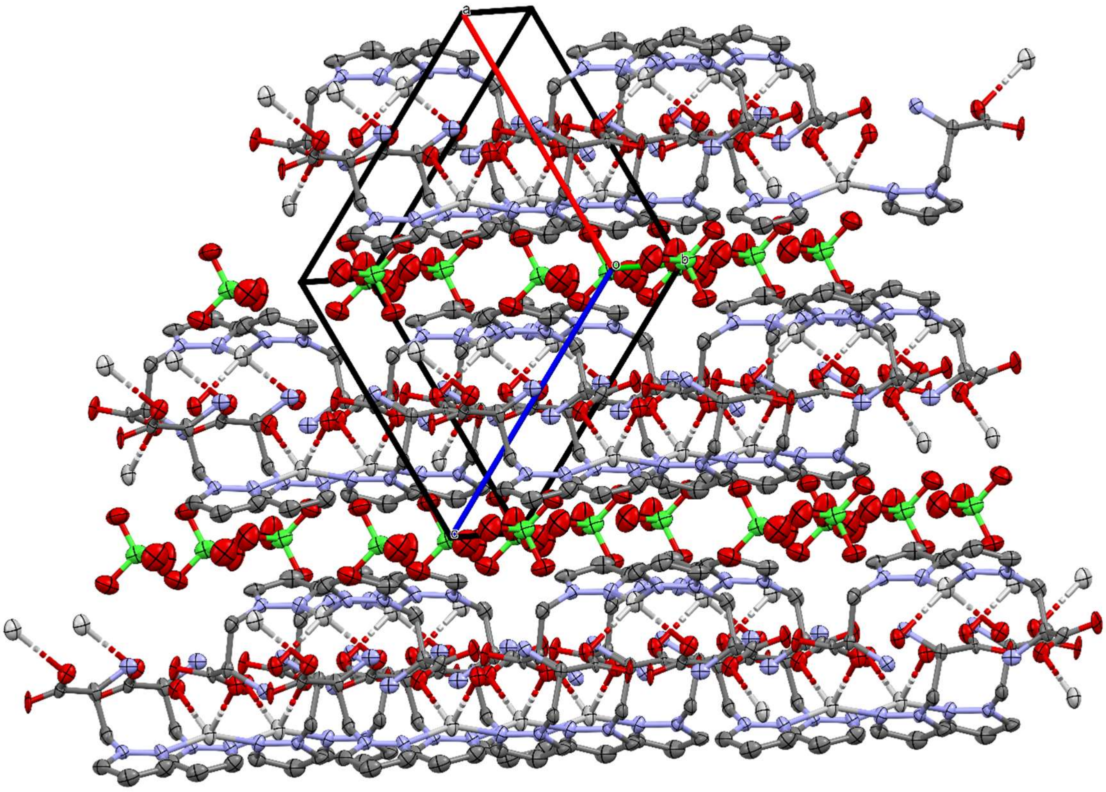


*Figure 19: Packing of complex **2** in the lattice (hydrogen atoms are omitted for clarity)*

*Table 5: Geometric parameters of complex **2***

Bonds	*Length [Å]*	*Bonds*	*Angle [°]*
Ag1—N2	2.178 (10)	N2—Ag1—N5	156.0 (4)
Ag1—N5	2.168 (11)	N2—Ag1—O1	104.82
Ag1—O1	2.600	N2—Ag1—O4	88.0 (3)
Ag1—O4	2.585	N5—Ag1—O1	90.49
		N5—Ag1—O4	106.3 (3)
		O1—Ag1—O4	104.06

The hydrogen bonds act just like in **1** as a stabilizer and form the structure of the complex molecules in the crystal lattice. But comparing the NH_3^+—ClO_4^- to the NH_3^+—NO_3^- bond shows that the bond in **1** is around 0.2 Å shorter than the one formed in **2**. This can be explained through the different sizes of the anions.

*Table 6: Hydrogen-bond geometry of **2***

D—H···A	*D—H [Å]*	*H···A [Å]*	*D···A [Å]*	*D—H···A [°]*
N3—H3A···N2	0.89	2.58	3.150 (14)	123
N3—H3A···O3^{ii}	0.89	1.95	2.718 (14)	144
N3—H3B···O5^{iii}	0.89	2.24	3.083 (15)	158
N3—H3C···O1^{iv}	0.89	1.97	2.811 (13)	156
N6—H6A···O8^{i}	0.89	2.24	3.104 (15)	162
N6—H6B···O4^{ii}	0.89	1.99	2.814 (13)	153
N6—H6C···N5	0.89	2.68	3.256 (15)	124
N6—H6C···O2^{iv}	0.89	1.86	2.660 (14)	148

Symmetry codes: (i) −x, y−1/2, −z+1; (ii) −x, y+1/2, −z+1; (iii) x, y, z+1; (iv) −x+1, y−1/2, −z+2.

2.2.3. IR measurements

If both IR spectras of compounds **1** and **2** are compared to the ligand pzAla (Fig. 20), three main differences can be observed. One in the region of 3300 to 2800 cm^{-1} where the O–H, N–H and C–H vibrations are found, the second between 1500 and 1250 cm^{-1} and the third between 1150 and 950 cm^{-1}. Since pzAla coordinates for both complexes through the free nitrogen atom, but diffrenetly through the oxygens atoms for each of the complexes, differences in the spectra are unavoidable. For **1** it binds through both O atoms from the alanine part and also through neighbouring oxygen atmos, while for **2** pzAla just binds through one O atom of the adjecent ligand molecules. The occuring differences between the spectra are smaller, besides the already meantioned ones, and mostly result from different interactions in the crystal latice of the new compounds. Compound **1** and **2** both show in the range of 3300 to 2800 cm^{-1} bands of lower intensity than the ligand, **1** shows a similar but weaker band as the ligand, while **2** shows two strong bands, one at 3211 cm^{-1} and the other at 3115 cm^{-1}. The difference of those absorptions is influenced by the capability of forming hydrogen bonds either intramolecular or with other close complex molecules in the lattice. The strong band of compound **1** at apr. 1300 cm^{-1} can be assigned to the band of the nitrate anion in the structure of the complex. Compound **2** shows a strong band at around 1100 cm^{-1} which can be assigned to the perchlorate anion which should appear at apr. 1100 cm^{-1}. The C=O stretching vibration from the ligand (1576 cm^{-1}) differs from the one of the compounds. Complex **1** shows a narrow band at the same wavelenght, while **2** shows a shift up to 1624 cm^{-1}. This is caused by the hydrogen bonds to either of the anions in the lattice. Through the coordination of the silver to the nitrogen of the pyrazole ring the normaly appearing vibrations of C=C, C=N (1505 cm^{-1}), N—N (1084 cm^{-1}), C—N (1181 cm^{-1}) and the pyrazole ring deformation (620 cm^{-1}) get shifted away from these values. For both compounds the C=C, C=N vibration shifts downwards to around 1495 cm^{-1} for **1** and for **2** to around 1497 cm^{-1}. The N—N vibration shifts for **1** to 1065 cm^{-1} and for **2** it is not possible to be observed since the strong band of the perchlorate anion overlaps with this vibration band. The C—N vibration just slightly shifts higer to 1183 cm^{-1} for **1** and 1186 cm^{-1} for **2**. The ring deformation of the pyrazole ring also stays at around 620 cm^{-1}, **1** shows it at 618 cm^{-1} and **2** at 624 cm^{-1}.

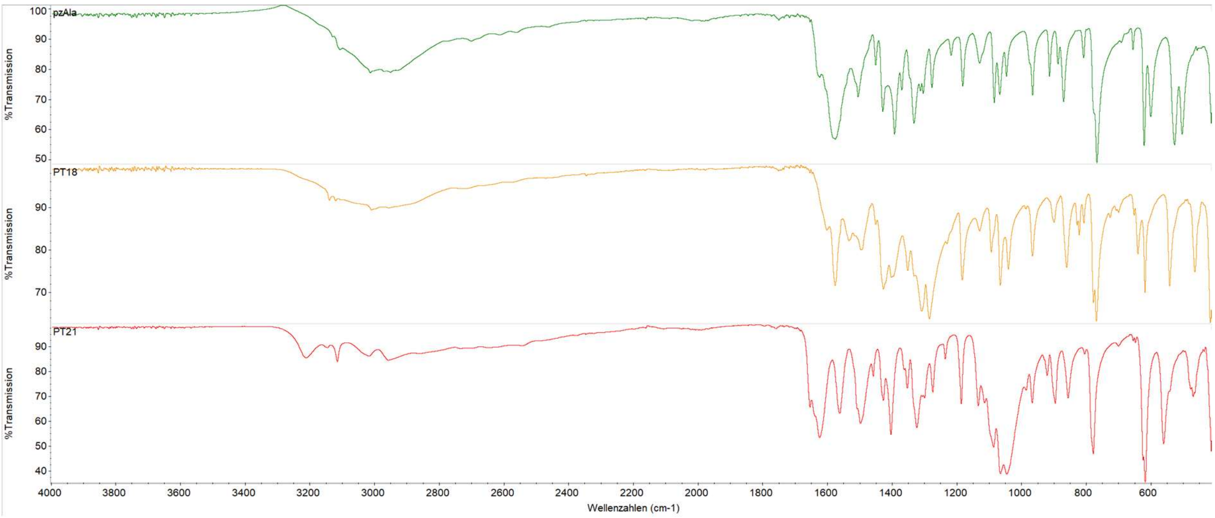


*Figure 20: IR-spectra of compounds **1** (middle, yellow), **2** (lower, red) and pzAla (upper, green)*

The 4-FBN which was used in the synthesis of the crystals was measured as a reference for the IR. In the trial for the synthesis of compounds **3** and **4** the correspoding IR spectra are compared to this reference spectra of 4-FBN (Fig. 21). It become cleary that all three spectra are identical. Just a slightly increase of band intensity at around 3380 cm^{-1} in both samples can be seen. This can be caused by some remaining water of the synthesis.

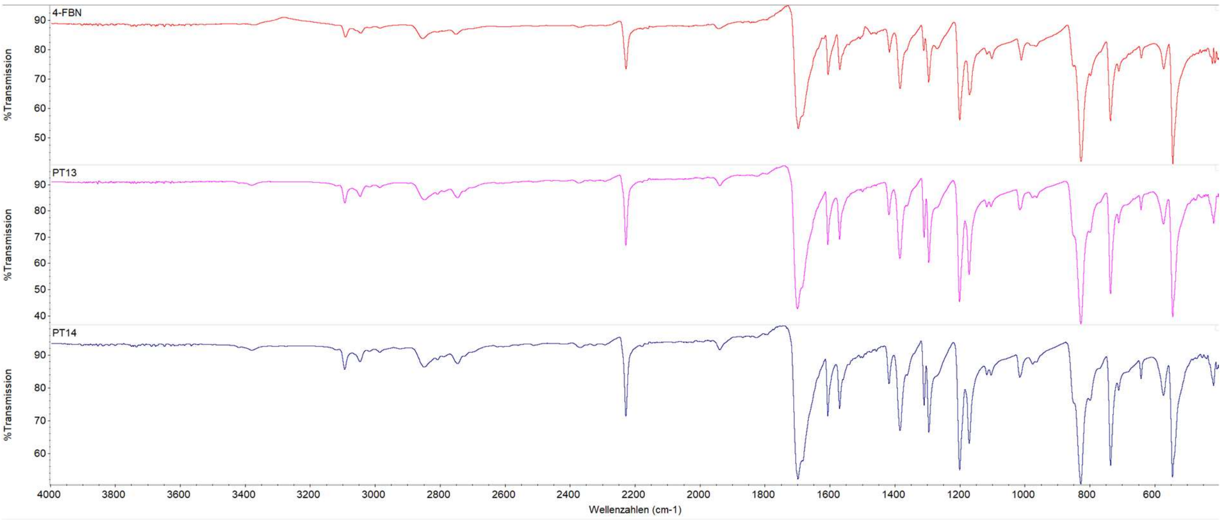


*Figure 21: IR-spectra of compounds **3** (middle, pink), **4** (lower, blue) and 4-FBN (upper, red)*

2.2.4. Radical scavenging activity

As previously stated silver(I) on its own and its compounds shows antimicrobial and anti-inflammatory capabilities. Silver(I) complexes with ethyl-5-amino-1-methyl-1H-pyrazole-4-carboxylate as its ligand showed promising antioxidative results, also zinc(II) complexes containing pyrazole derivatives showed a significant antioxidative capacity [6] . Those results lead to the screening of the antioxidative activity of the newly synthesized compounds **1** and **2**. The ligand shows, as presented in Figure 22, an antioxidative capacity of 176.51 µg TE/g dsm (Trollox equivalent per gram of dry sample mass). Both synthesized compounds show a higher antioxidative activity than the ligand on its own. **1** has with 272.30 µg TE/g dsm the highest antioxidative activity of both compounds, **2** shows a slightly higher activity as the ligand of 195.53 µg TE/g dsm. The forming of the ligand-silver bond seems to increases the antioxidative capability of the ligand system, like in [6] mentioned, it is most likely that the NH_3^+ group of the alanine substituent of the pyrazole ring neutralizes the DPPH• radical. The formed silver nitrogen bond supports this by stabalizing the missing hydrogen atom throughout the molecule. The polymer strands of **1** either seem to increase this effect, or just the fact that, in compound **1**, the ammount of present silver is doubled in comparison to compound **2** and there for yields a higher antioxidative activity. Also the effect of the existing anion could lead to these differences in the activity of **1** and **2**. To clearify this further test have to be conducted.

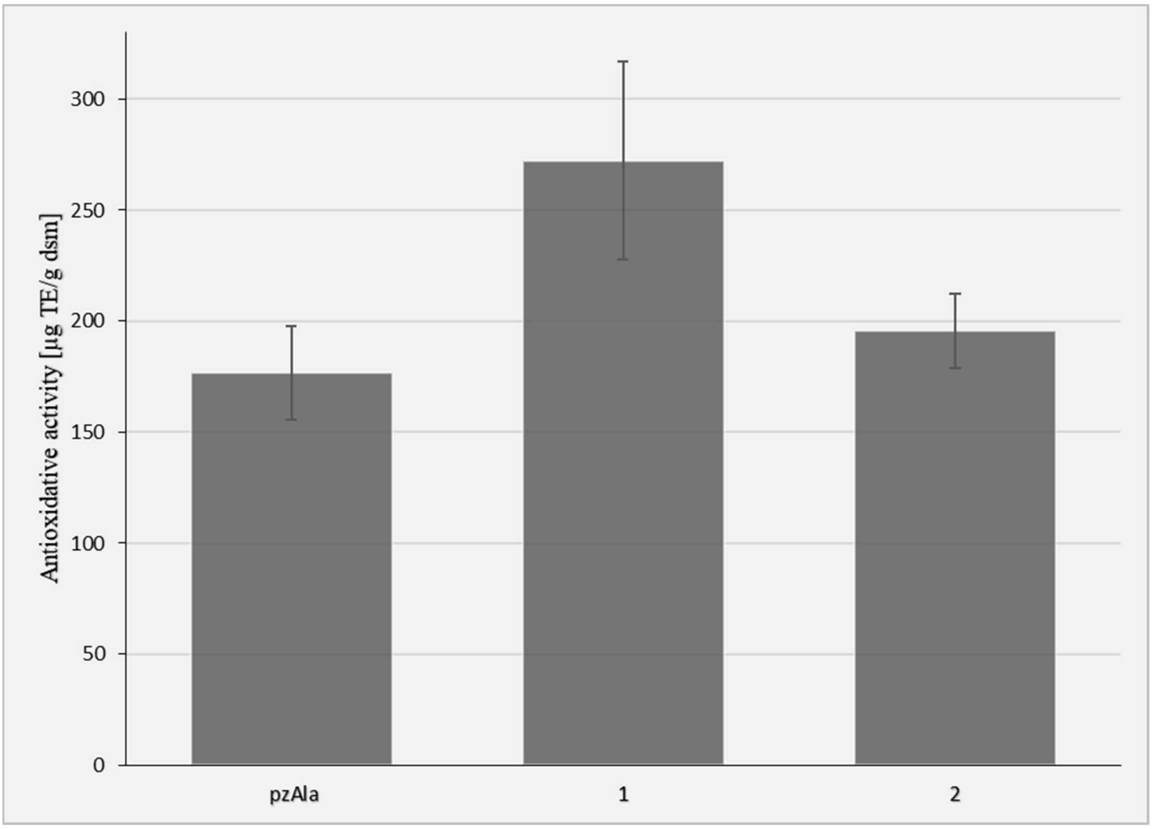


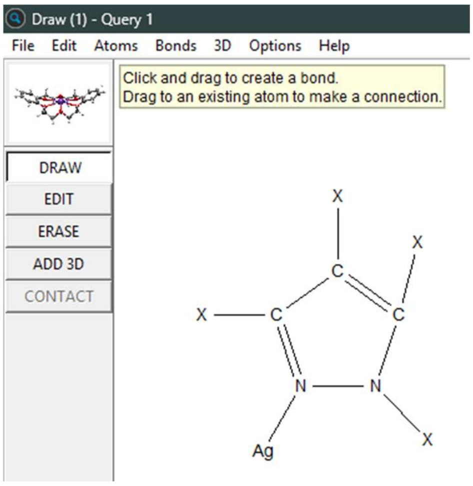
Figure 22: Trolox equivalent antioxidant capacity of the ligand and both compounds

2.2.5. ConQuest search

To compare and validate the characteristics of newly obtained complexes the Cambridge Structural Database by the Cambridge Crystallographic Data Centre can be used. It is a database that was establish 1965 and contains over 1.25 million 3D structures of compounds yielded by XRD and neutron diffraction analyses. Those structures make things like predictions about the structure of newly compounds, or a classifications of those in comparison to similar structures possible [16]. It was searched through the program ConQuest [17].

Different queries where used to yield structures similar to the predicted and then obtained one. Before the structure of the compounds was known through the SC-XRD prediction searches were done to see if those structures are already known. It was searched for silver(I) compounds, either bound by just the pyrazole ring nitrogen, or also through the oxygen atoms and/or the nitrogen atom of the amine group. These searches did not yield any results. Also searches for compounds **3** and **4** were done. Since both compounds **1** and **2** just differentiate, besides their 3D orientation and their number of atoms per asymmetric unit, by the anions, the search was conducted for both together. Not all conducted searches are shown.

The first search was used to gain an overview of the pyrazole containing silver compounds. No specifications were made for the structure of the query as shown in Figure 23. This yielded a total of 739 results of structures containing that query as a part of their structure (Fig. 24). Some showed similarities to **1** and **2** in things like bond length or orientation of the ligands.



Click and drag to create a bond.
Drag to an existing atom to make a connection.

Figure 23: First query

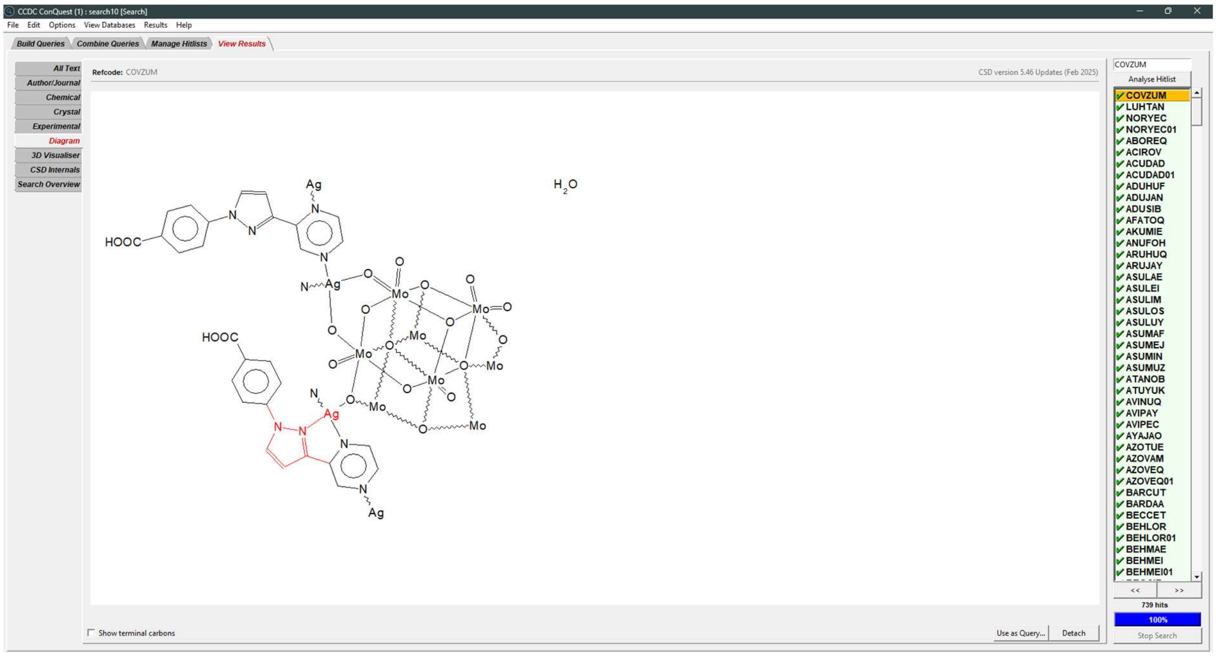
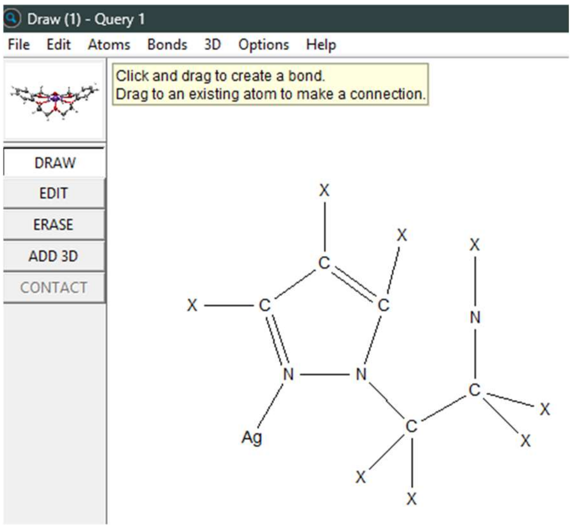


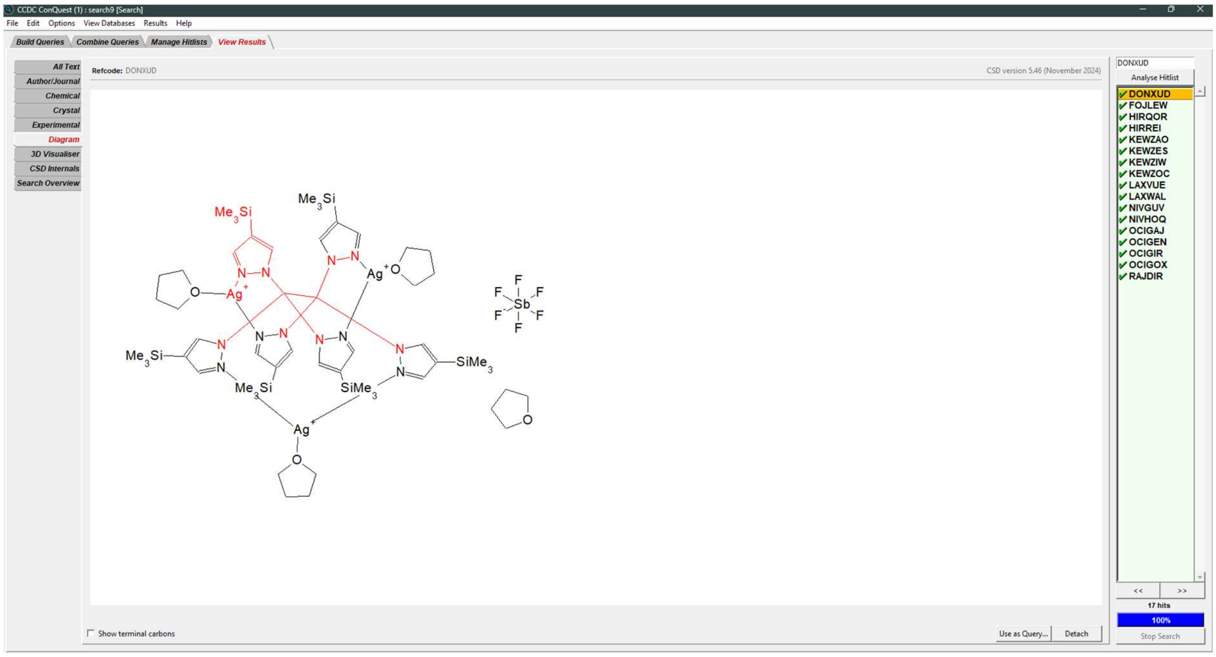
Figure 24: Hit list for query one

The second search tried to yield structures that are more similar to the one of pzAla. On the free nitrogen of the pyrazole ring a two carbon long chain was added with an amine group on the second carbon. Through this addition to the structure of the query (Fig. 25) the resulting structures got shrunk down to 17, all of them are more accurate and closer to both new compounds **1** and **2**. The complex with the refcode RAJDIR for example was used as a comparison and is also mentioned in the introduction part.



Click and drag to create a bond.
Drag to an existing atom to make a connection.

Figure 25: Second query



Refcode: DONXUD

CSD version 5.46 (November 2024)

DONXUD

- ✓ DONXUD
- ✓ FOJLEW
- ✓ HIRQOR
- ✓ HIRREI
- ✓ KEWZAO
- ✓ KEWZES
- ✓ KEWZIW
- ✓ KEWZOC
- ✓ LAXVUE
- ✓ LAXWAL
- ✓ NIVGUV
- ✓ NIVHOQ
- ✓ OCIGAJ
- ✓ OCIGEN
- ✓ OCIGIR
- ✓ OCIGOX
- ✓ RAJDIR

17 hits

☐ Show terminal carbons

Figure 26: Hit list for query two

3. Conclusion

Silver(I) compounds have gained significant interest as potential candidates for futher research in fields like pharmaceuticals, as novel antimicrobial and anti-inflammatory agents, or in material science for an application in photovoltaics or biotechnology. Hence the well known and documented linkage between a (chronic) inflammation and the growth and progession of cancer, which is one of mankinds biggest challenges, the demand for newly anti-inflammatory drugs is growing. Due to their promising redox and biological activity silver based agents are particular promising in this regard.

This research contributes to the ongoing efforts and discussion in this field by synthesizing and characterizing two novel silver(I) complexes featuring a fused ligand system consisting of a pyrazole ring and the amino acid L-alanine, forming 3-(1-pyrazolyl)-L-alanine. The ligand was successfully coordinated to the silver(I) starting from appropriate nitrate and perchlorate salts. Two silver(I) compounds were obtained: $[Ag_2(pzAla)_2](NO_3)_2$ (**1**) and $[Ag(pzAla)_2]ClO_4$ (**2**). Both complexes were characterized using spectroscopic and analytical techniques, and their absolute structures were determined via single crystal X-ray diffraction. The antioxidative behavior of both compounds showed promising results which could indicate a potential usage as bioactive agents. However further research is necessary to fully gain insight in their behavior, so things like the influence of the anion on the antioxidative behaviour and stability, have to be studied.

In summary the main objective of this study, namely, the synthesis of novel silver(I) complexes and their structural characterization, was successfully achieved. However further research is required to explore their full range of biological and physicochemical properties, to lead the way for potential applications in medicinal and materials chemistry.

4. Experimental part

Caution! Perchlorate compounds are potentially explosive, thus, they have to be handled with great care.

4.1. Materials and methods

Every reaction was done in normal ambient atmosphere, either in an Erlenmeyer flask or if refluxed in a round-bottom flask. All used reagents were comercially available and used as purchased:

3-(1-pyrazol)-L-alanine (Fluka Analytical), 4-formylbenzonitrile (Sigma Aldrich, 95 %), silver(I) nitrate (Alkaloid Skopje), silver(I) perchlorate-monohydrate (Fluka, <12 % water).

4.2. Characterisation of the silver(I) compounds

IR measurements were recorded on a Thermo Nicolet iS20 FTIR spectrophotometer (Thermo Fisher SCIENTIFIC) with Smart iTR™ ATR Sampling accessories, in a range of 4000 to 400 cm^{-1}.

The elemental analysis was done after standard micro-methods on an Elementar vario EL III.

Synthesised compounds were characterised through X-ray diffrraction analysis. Those experiments were performed on a four-circle single crystal X-ray diffractometer Gemini S (Oxford Diffraction), equipped with a position-sensitive CCD detector Sapphire3 and with a Mo Kα X-radiation (λ = 0.71073 Å) source. Measurements were made at room temperature. Instrument control and raw data reduction were performed with the CrysAlisPro [18]. Absorption corrections were performed by analytical integration of absorption equations with the help of the multifaceted crystal model [19].

The structure was solved by using ShelXle [20], shown and analysed with the programms Mercury [21] and PublCIF [22]. Chemical drawings were done with ChemSketch [23]. Crystallographic and refinement details are listed in Table 7.

4.3. Evaluation of radical scavenging activity

The synthesized complexes were subjected to evaluation of their DPPH radical scavenging activity using a slightly modified protocol based on the method described in [24]. This approach involves spectrophotometric measurement of the reduction of the violet-colored, nitrogen-centred, stable DPPH• radical to its reduced, yellow-colored form, DPPH-H. The DPPH radical is a stable molecule that exhibits a characteristic purple color in methanol, with a maximum absorption peak at λ_{max} = 515 nm. In the presence of electron or hydrogen atom donors, the DPPH• radical undergoes neutralization, resulting in a transition to its neutral form, accompanied by a decrease in absorbance and loss of the purple coloration. The analyzed samples were dissolved in a mixture of DMSO/MeOH, specifically in a 15% DMSO/MeOH solution. All experiments were performed using 96-well microplates. A volume of 50 μL of each sample solution was combined with 160 μL of a DPPH solution prepared at an initial concentration of 0.4 mmol/L in 96 % ethanol, followed by fourfold dilution in methanol to achieve an absorbance of 0.9 at 515 nm. Additionally, 90 μL of methanol was added to reach a final reaction volume of 300 μL. Blank controls (where the corresponding solvent replaced the sample) and matrix blanks (containing the solvent and sample without DPPH) were also analyzed. In parallel, a series of Trolox standard solutions (1–100 mg/mL) were prepared to construct a calibration curve. Trolox solutions were treated identically to the test samples, with DPPH and methanol added in the same volumes. All sample measurements were done in quadruplicate, while Trolox samples were analyzed in triplicate. After incubation for 60 minutes, absorbance was measured at 515 nm.

UV-Vis absorbance measurements were conducted using an Evolution™ Pro UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA), at room temperature, equipped with a xenon flash lamp, mercury lamp, and a dual-matched silicon photodiode detector. The spectrophotometer was operated over a wavelength range of 205 to 800 nm. Data analysis was conducted using the Thermo Scientific™ Insight™ Pro Software [26].

4.4. Synthesis of the complexes **1** and **2**

In an Erlenmeyer flask 0.25 mmol (40 mg) of pzAla were dissolved in 5 mL of water. The compound dissolved after some mixing to form a clear lightly yellow solution. In another flask 0.25 mmol of the silver(I) salt (52 mg for sliver(I) nitrate (**1**) and 51 mg for silver(I) perchlorate (**2**)) were also dissolved in another 2 mL of water. Both solutions were mixed in the Erlenmeyer flask, which was then covered in some aluminum foil to prevent the exposure to light and was put aside to crystalize out. After four weeks for the silver nitrate and five weeks for the silver perchlorate the crystals were obtained.

1 yield: 50 mg (30.7 %) calculated (%) for $C_6H_9N_5O_4Ag$ (M_r = 324.07): C 22.24, H 2.78, N 17.29; found C 22.45, H 2.53, N 17.03

IR-measurment of **1**: 3140 cm^{-1} (m) and 3009 cm^{-1} (m) N—H and O—H, 1576 cm^{-1} (s) C=O, 1496 cm^{-1} (m) C=C, C=N, 1285 cm^{-1} (s) N—O sym., 1183 cm^{-1} (m) C—N, 1065 cm^{-1} (m) N—N, 778 cm^{-1} (m) C—H oop, 620 cm^{-1} (m) ring deformation of pyrazole

2 yield: 24 mg (18.5 %) calculated (%) for $C_{12}H_{18}N_6O_8ClAg$ (M_r = 517.64): C 27.84, H 3.51, N 16.24; found C 27.53, H 3.37, N 15.96

IR-measurment of **2**: 3212 cm^{-1} (m) and 3115 cm^{-1} (m) N—H and O—H, 2958 cm^{-1} (w) C—H, 1624 cm^{-1} (s) C=O, 1497 cm^{-1} (m) C=C, C=N, 1186 cm^{-1} (m) C—N, 1087 cm^{-1} (s) ClO_4^-, 778 cm^{-1} (m) C—H oop, 624 cm^{-1} (m) ring deformation of pyrazole

4.5. Synthesis of the complexes in the presence of 4-FBN

In a round-bottom flask 0.25 mmol of pzAla (40 mg) and 4-FBN (32 mg) were dissolved in 12 mL of water to yield a clear slightly yellow colored solution which was then refluxed for 30 min. After refluxing and cooling off to room temperature 0.125 mmol of the silver salt was added (21 mg for silver(I) nitrate and 25 mg for silver(I) perchlorate). The mixture was then transfered to an Erlenmeyer flask which was covered with aluminum foil and let to crystalize. After three weeks the crystals were obtained.

4.6. Crystallographic data

Table 7: Crystallographic details of both complexes

	1	**2**
Chemical formula	$C_{12}H_{18}Ag_2N_8O_{10}$	$C_{12}H_{18}AgClN_6O_8$
M_r	650.08	517.64
Crystal system	Monoclinic	Monoclinic
Space group	*P*2$_1$	*P*2$_1$
a [Å]	5.2092 (3)	10.0057 (9)
b [Å]	24.7366 (19)	9.3727 (5)
c [Å]	7.7314 (6)	10.6564 (9)
β [°]	90.241 (6)	117.076 (11)
V [Å^3]	996.24 (12)	889.83 (14)
Z	2	2
R factor	0.0645	0.0677
Radiation type	Mo Kα (0.71073 *Å)*	Mo Kα (0.71073 *Å)*
μ [mm^{-1}]	2.04	1.34
Absorption correction	Analytical	Analytical
Measured reflections	10732	15804
Independent reflections	4586	4325
Observed reflections [I>2σ(I)]	3373	3202

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6. Appendix

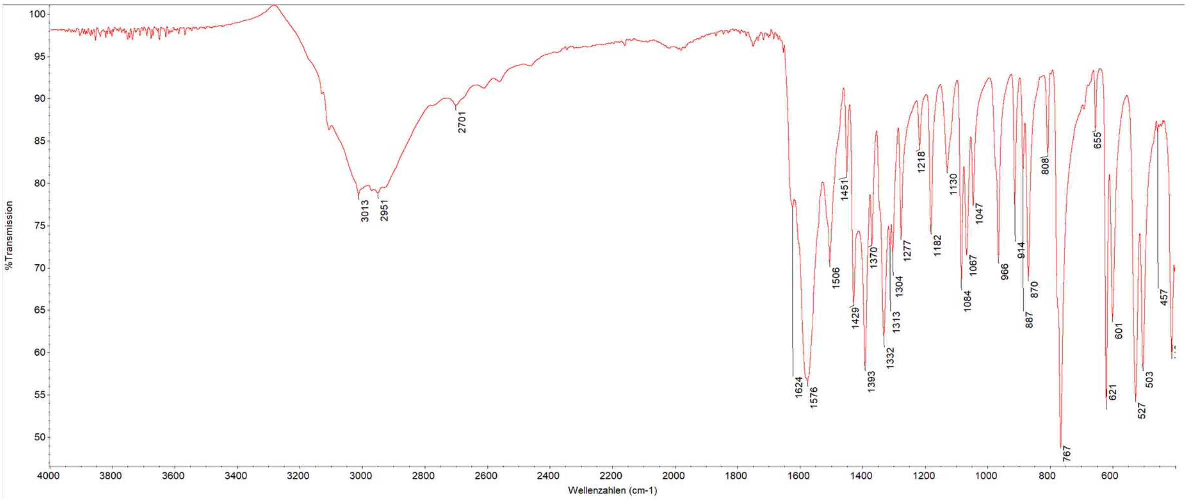

Figure 27A: IR-spectra of pzAla

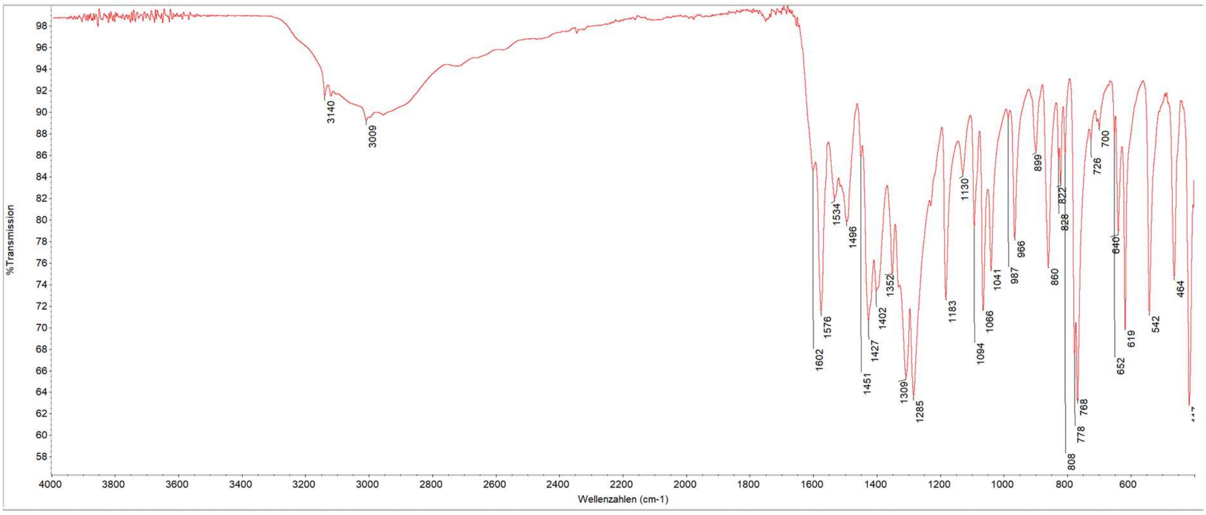

Figure 28A: IR-spectra of 1

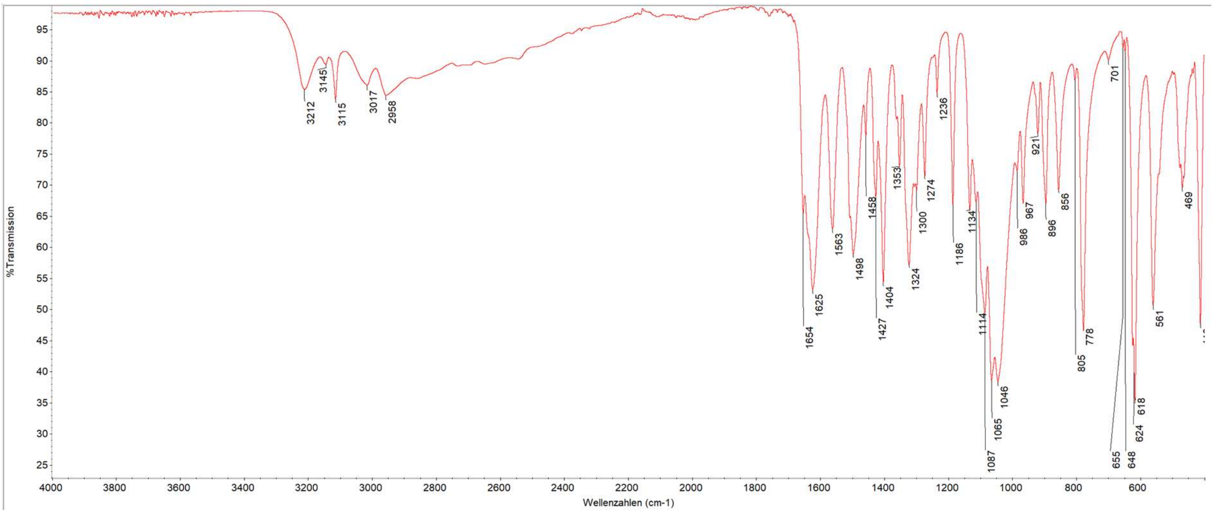

Figure 29A: IR-spectra of 2

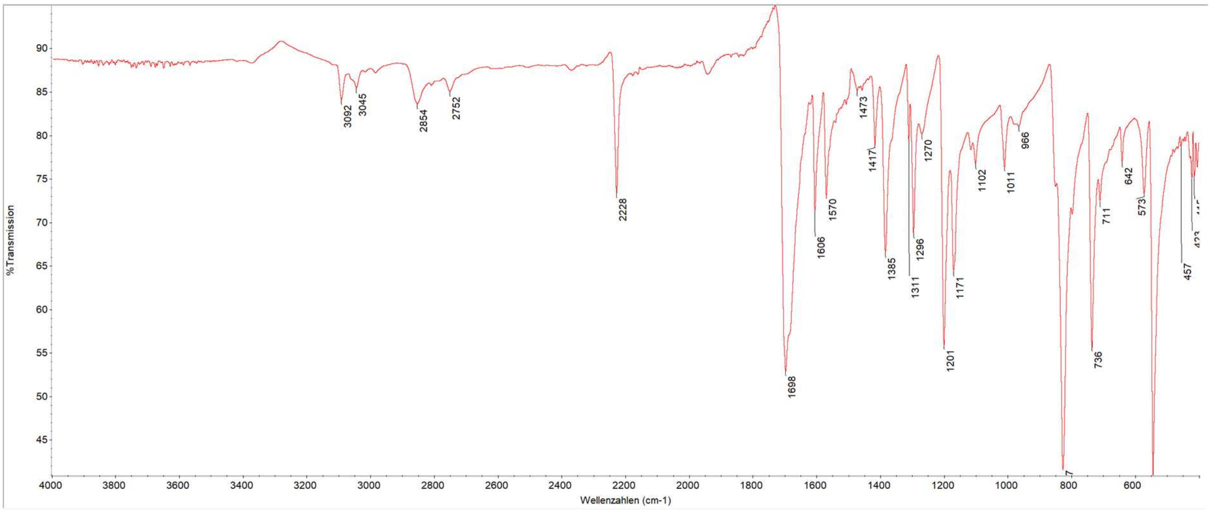

Figure 30A: IR-spectra of 4-FBN

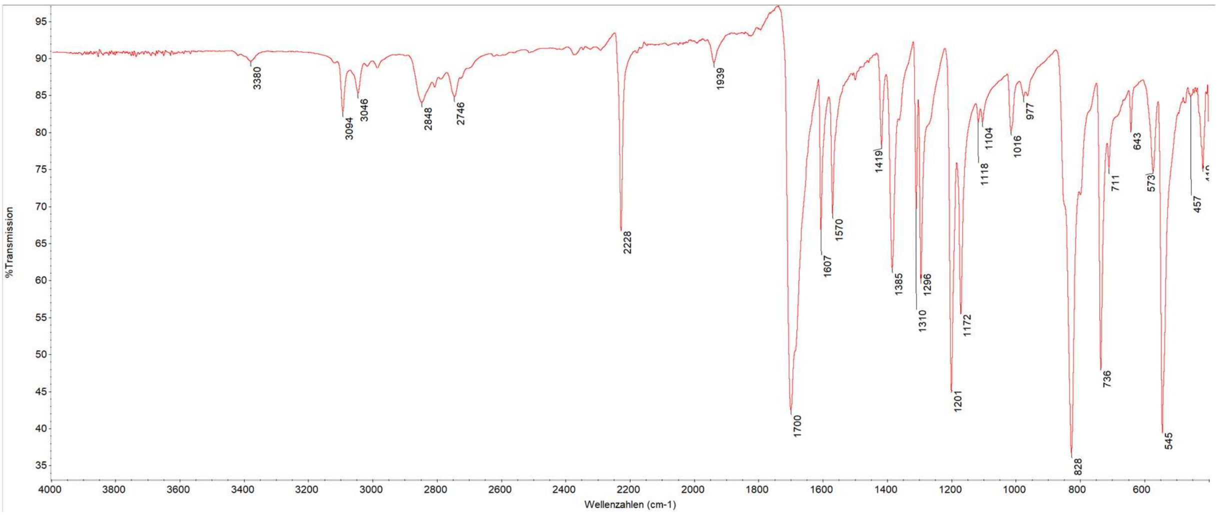

Figure 31A: IR-spectra of 3

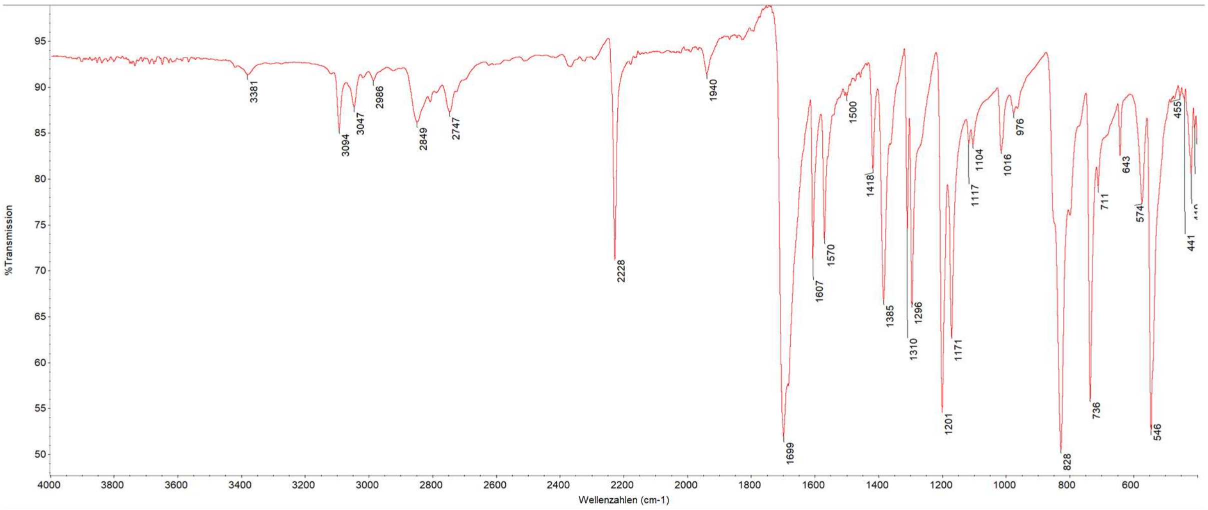

Figure 32A: IR-spectra of 4