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DIPLOMARBEIT

„Organometallische Ru(II)- und Os(II)-Komplexe
von 2- und 4-Pyridonen als potentielle
tumorstimmende Wirkstoffe“

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DIPLOMA THESIS

„2-Pyridone vs 4-Pyridone organometallic Ru(II)- and
Os(II)-complexes as potential anticancer agents“

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Submitted in part fulfillment of the requirements for the degree
Magistra rerum naturalium (Mag. rer. nat.)

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Dedicated to my Oma

*„Denn was man schwarz auf weiß besitzt, kann man getrost
nach Hause tragen.“ -Faust I, Vers 1966 f. / Schüler*

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¹ William Shakespeare, *Julius Caesar*, Act III, Scene 1

Abstract

Since the introduction of cisplatin and other platinum-based drugs in cancer chemotherapy, metal complexes and organometallic compounds have been gaining growing importance in oncology. Recently, organometallic Ru(II) compounds bearing 1,3,5-triaza-7-phosphaadamantane (pta), ethylenediamine, sugar- and maltol-derived ligands have shown promising anticancer properties with cytotoxicity comparable or in some case superior to cisplatin. Some of the compounds are even active in cisplatin resistance cell lines, probably due to different mode of actions. The content of this diploma project involves the synthesis of a series of novel M(II)-arene half-sandwich complexes (M=Ru, Os) with derivatives of 3-hydroxy-2-pyridone ligands, characterization by standard methods such as NMR spectroscopy, ESI-MS, elemental analysis and determination of solid state structures by X-ray diffraction analysis. In vitro anticancer assays in human cancer cell lines will demonstrate the potential of the new compounds for further development.

Zusammenfassung

Seit Cisplatin und andere auf Platin basierende Medikamente in die Chemotherapie zur Behandlung von Krebs eingeführt wurden, haben Metallkomplexe und organometallische Verbindungen wachsende Wichtigkeit im Bereich der Onkologie erhalten. In letzter Zeit haben Ru(II) Verbindungen, die Derivate von 1,3,5-triaza-7-phosphaadamantan (pta), Ethylendiamin, Zuckern und Maltol als Liganden besitzen, vielversprechende tumorhemmende Eigenschaften gezeigt. Die Zytotoxizität dieser Verbindungen ist vergleichbar mit und in manchen Fällen sogar höher als die von Cisplatin. Manche dieser Verbindungen sind sogar in Zelllinien aktiv, die gegen Cisplatin resistent sind. Dies ist möglicherweise auf eine andere Wirkungsweise der Substanzen zurückzuführen. Der Inhalt dieser Diplomarbeit beinhaltet die Synthese einer Serie von neuen M(II)-Aren Halb-sandwich Komplexen ($M = \text{Ru}, \text{Os}$) mit 3-Hydroxy-2-pyridon Derivaten als Liganden. Die Charakterisierung dieser Verbindungen erfolgte mit Standardmethoden, wie NMR Spektroskopie, ESI-MS, Elementalanalyse, und Analyse der Festkörperstrukturen durch Röntgen Diffraktionsanalyse. In vitro Antikrebsassays in humanen Krebszelllinien werden das Potential dieser neuen Verbindungen zeigen, woraus sich wiederum neue Konzepte für zukünftige Entwicklungen ableiten lassen.

Table of Contents

1	INTRODUCTION	13
1.1	CANCER	13
1.2	CANCER TREATMENT	19
1.2.1	Surgery	19
1.2.2	Radiation Therapy	19
1.2.3	Immunotherapy and monoclonal antibody therapy	20
1.2.4	Hormonal therapy	21
1.2.5	Chemotherapy	21
1.3	METAL-BASED ANTICANCER DRUGS	24
1.3.1	Introduction	24
1.3.2	Platinum Compounds	24
1.3.2.1	Cisplatin	25
1.3.2.2	Carboplatin	26
1.3.2.3	Oxaliplatin	26
1.3.3	Non-platinum Compounds	27
1.3.3.1	General Overview	27
1.3.3.2	Ruthenium Compounds	27
1.3.3.3	Ruthenium(II)-arene compounds	29
1.3.3.4	Maltol-derived compounds	32
1.4	HYDROXYPYR(ID)ONES (HP)	34
1.4.1	Introduction	34
1.4.2	Selected Applications in Medicinal Inorganic Chemistry	35
1.4.2.1	Treatment of iron deficiency and overload	35
1.4.2.2	Treatment of Alzheimer's disease (AD)	36
1.4.2.3	Treatment of Diabetes	36
1.4.2.4	Diagnostic use	37
1.4.2.5	Tris(maltolato)gallium(III) as potential anticancer drug	38
1.4.3	Coordination Chemistry	39
1.4.4	Synthetic considerations	39
1.4.4.1	(Thio)hydroxypyr(id)ones	39
2	RESULTS AND DISCUSSION	40
2.1	SYNTHESIS AND CHARACTERIZATION OF THE LIGANDS	40
2.1.1	Synthesis	40
2.1.2	Characterization	43
	¹ H-NMR spectroscopy	43
	¹³ C{ ¹ H}-NMR spectroscopy	44
	2D-NMR spectroscopy	44

2.2 SYNTHESIS AND CHARACTERIZATION OF THE COMPLEXES	45
2.2.1 Synthesis	45
2.2.2 Characterization	46
¹ H-NMR spectroscopy	47
¹³ C{ ¹ H}-NMR spectroscopy	47
2D-NMR Spectroscopy	48
3 CONCLUSIONS AND OUTLOOK	50
4 EXPERIMENTAL	52
4.1 MATERIALS AND EQUIPMENT	52
4.2 SYNTHESIS	53
4.2.1 Precursors	53
4.2.2 General Procedures	53
4.2.3 Amides	55
[Halido(η^6 - <i>p</i> -cymene){ <i>N</i> -[(1-propylamide)carbonylmethyl]-3-oxo- κ O-2-(1 <i>H</i>)-pyridonato- κ O}ruthenium(II)] – 1	55
[Halido(η^6 - <i>p</i> -cymene){ <i>N</i> -[(4-morpholinoaniline)carbonylmethyl]-3-oxo- κ O-2-(1 <i>H</i>)-pyridonato- κ O}ruthenium(II)] – 2	58
[Chlorido(η^6 - <i>p</i> -cymene){ <i>N</i> -[(4-phenylbenzylamide)carbonylmethyl]-3-oxo- κ O-2-(1 <i>H</i>)-pyridonato- κ O}ruthenium(II)] – 3f	62
[Chlorido(η^6 - <i>p</i> -cymene){ <i>N</i> -[(4-(3-aminopropyl)morpholine)carbonylmethyl]-3-oxo- κ O-2-(1 <i>H</i>)-pyridonato- κ O}ruthenium(II)] – 4f	65
4.2.4 Variation of the Metal-arene moiety of pyridone complexes	67
❖ Ruthenium complexes	67
❖ Osmium complex	70
5 SUPPLEMENTARY DATA	71
5.1 ¹H- AND ¹³C{¹H}- NMR SPECTRA	71
5.1.1 Amides	71
5.1.2 Variation of the Metal-arene moiety of pyridone complexes	79
Curriculum Vitae	85

Abbreviations

acac	Acetylacetonate
Ar	Argon
BEOV	bis(ethylmaltolato)oxovanadium(IV)
BMOV	bis(maltolato)oxovanadium(IV)
CDCl ₃	deuterated chloroform
d	doublet (NMR)
d ₄ -MeOH	deuterated methanol
d ₆ -DMSO	deuterated DMSO
DACH	1,2-diaminocyclohexane
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
Deferiprone	1,2-dimethyl-3-hydroxypyrid-4-one
DMF	dimethylformamid
DMSO	dimethyl sulfoxide
DNA	2' - deoxyribonucleic acid
en	ethylenediamine
FDA	US Food and Drug Administration
h, hrs	hour(s)
HBV	Human Hepatitis B Virus
HCV	Human Hepatitis C Virus
Him	imidazole
HPV	Human papilloma virus
Hz	Hertz
J	coupling constant (NMR)
KP 1019	indazolium <i>trans</i> -[tetrachloridobis(1 <i>H</i> -indazole)ruthenate(III)]
KP46	tris(quinolinolato)gallium(III)
Lawesson's Reagent	2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-dithione
m	multiplet (NMR)
MRI	Magnetic Resonance Imaging
NAMI-A	(H ₂ im)[<i>trans</i> -RuCl ₄ (Him)(dmso)]
NaOMe	sodium methoxide
NHS	<i>N</i> -hydroxysuccinimide
PAH	polycyclic aromatic hydrocarbons
RAPTA	1,3,5-triaza-7-phosphatricyclo[3.3.1.]decane

ROS	reactive oxygen species
RT	room temperature
Ru-dimer	$[(\eta^6\text{-arene})\text{RuX}(\mu\text{-X})]_2$ (X=Cl, Br, I)
s	singlet (NMR)
TBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
WHO	World Health Organization
δ	chemical shift (NMR)

1 Introduction

1.1 Cancer

Cancer – by definition a class of diseases – is one of the major causes of deaths worldwide. There might be the need to explain the difference between what is commonly known as cancer and for example a tumor. One speaks of a benign tumor when there is abnormal and uncontrolled growth of cells. It is called a malignant tumor under the following circumstances: The symptoms are coupled with invasion of the surrounding tissue by cancerous cells. Additionally the formation of metastasis occurs, where abnormal cells travel to different parts of the body – sometimes located far away from the original tumor – via the bloodstream and the lymphatic system. Only with these conditions the term cancer applies².

The starting point is a mutation in one single cell from which cancer can arise. This mutation comes either from genetics (5-10%) or is ascribed to external factors including lifestyle and environmental causes (90-95%)³ (Fig. 1).

The latter can be categorized as follows:

- Physical carcinogens, e.g. UV radiation
- Chemical carcinogens, e.g. tobacco smoke
- Biological carcinogens, e.g. infections from viruses like human papilloma virus (HPV) and its relation to cervical cancer, hepatitis B and liver cancer, and bacteria like *heliobacter pylori* and their relation to stomach cancer⁴

As we are ageing our exposure to risk factors accumulates and our cells gradually lose their ability to repair themselves. This leads to the fact that the chance to develop cancer grows greatly with age.

² US National Institute of Health, <http://cancer.gov/cancertopics/understandingcancer>, (4/2/2011)

³ Anand P, *et al.* Pharm Res. 2008 September; 25(9): 2097–2116.

⁴ WHO, Fact sheet N°297, February 2009

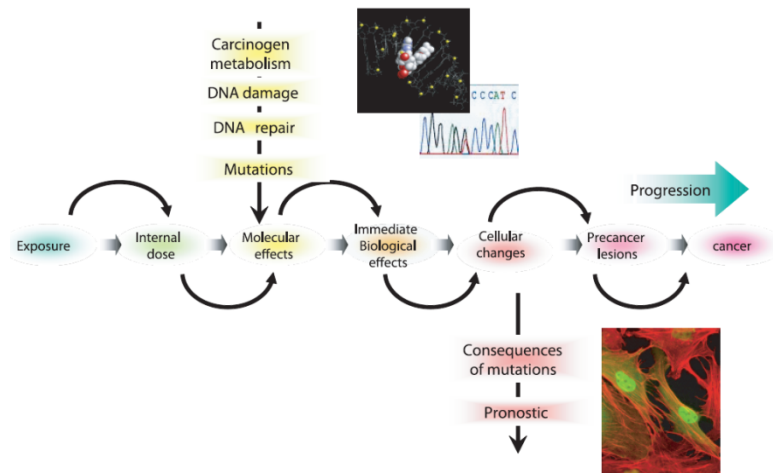


Fig. 1: The progression of cancer⁵

Chemical Carcinogens

Tobacco

13 types of cancer can be attributed to smoking. Each additional cigarette smoked amplifies the risk proportionally. Carcinogenic substances in tobacco smoke include PAH and nitrosamines. Passive smoking, due to the chemical composition of the smoke, causes 10-15% of lung cancers in never-smokers. Smokeless tobacco is also linked to cancers, i.e. oral cancers. Here the plausible cause for cancer is derived from the elevated level of nitrosamines (Fig. 2).

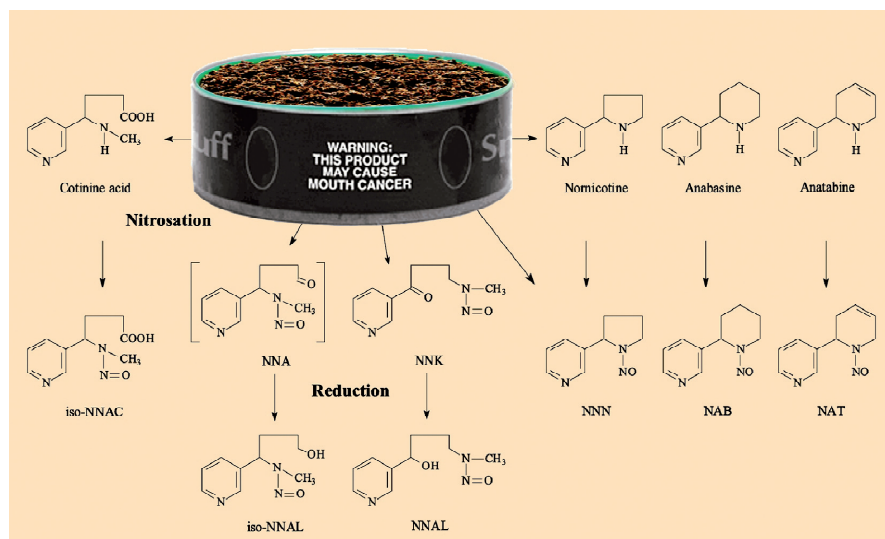


Fig. 2: Tobacco can cause cancer not only when smoked but also when chewed or snuffed⁵

Alcohol

In recent times a correlation between the consumption of alcohol and colorectal and breast cancer was derived additionally to the already confirmed links to liver cancer and cancers of the gastro-intestinal tract. This might be related to its ability to act as a solvent for carcinogenic compounds and a rise of the estrogen level.

Biological Carcinogens

Chronic infections and vaccinations

Chronic infections differ in the way they disrupt the body which can lead to cancer. HPV interrupts biochemical pathways while Human Hepatitis B Virus (HBV), Human Hepatitis C Virus (HCV) and *H. pylori* are responsible for constant tissue inflammation (Fig. 3). HIV weakens the immune system leaving it vulnerable to carcinogenic infections.

HBV and HPV vaccines have shown to be great tools in the prevention of cancer. The problem is to reach people who need it most, which is at least partially because of the high costs of the drugs.

For infections like HCV, HIV and *H. pylori*, it is more likely to develop a vaccine against the formation of symptoms, e.g. chronic infection, than a prophylaxis against the disease itself.⁵

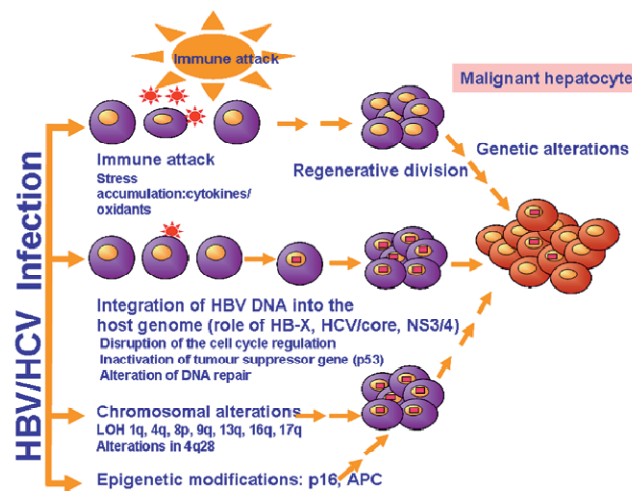


Fig. 3: Carcinogenesis induced by HBV and HCV⁵

Formation

The relation between cell growth and cell death in healthy tissue is balanced, but in cancer it is in disarray. This can be traced back to either uncontrolled growth or a cell is no longer able to undergo apoptosis, the programmed cell death. Now cell division exceeds cell death and unlike in healthy tissue the newly generated cells do not lose their ability for further cell

⁵ World Cancer Report 2008

division. This results in augmentation of the overall number of cells with the capacity to divide still intact. The tissue generated from this continuous cell production is called tumor or neoplasm. As this process moves along, the tissue loses its normal arrangement (Fig. 4).²

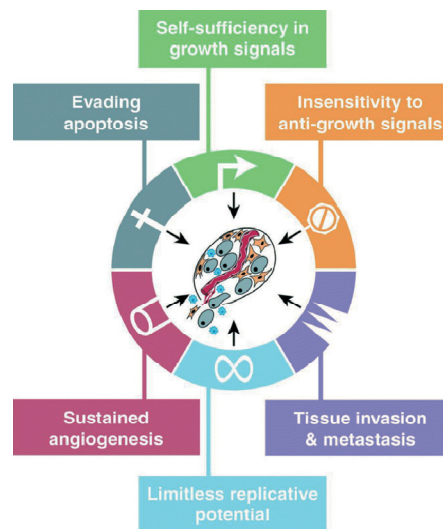


Fig. 4: Devices of Cancer⁵

Classification

The classification of cancer types derived from the origin of the abnormal cells are done as follows:

Carcinoma, the type of cancer where the tumorous cells arise from epithelial cells, is the most common form of cancer. This category comprises lung, prostate, breast and colon cancer.

Sarcoma is the name given to cancer developed from connective tissue, e.g. muscle, fat, cartilage and bone.

Special types of cancer are *lymphoma* and *leukemia* because they do not form solid tumors. These cancers arise in the lymphatic system and the bone marrow, respectively. The abnormal growth of white blood cells in leukemia is subsequently found in the blood stream. To differentiate between the different forms of cancer, Latin prefixes are added to the name. Each prefix is derived from the location where the tumorous growth arised, e.g., myosarcoma is the term for cancer of the muscle.²

Statistics

The types of cancer that are the most common causes of death are breast, lung, stomach, liver and colon cancer (Fig. 5). When the data is separated by gender, prostate cancer is the most common cancer in men and breast cancer the most common in women, the numbers of the other types of cancer mentioned before vary between men and women (Fig. 6). When looking at the data worldwide one can see that tobacco and alcohol use is a problem everywhere. In high-income countries additional cancer occurrences come from overweight and obesity, while in low- and middle-income countries they derive from low fruit and vegetable intake and chronic infections from Hepatitis B, C and Human Papilloma Virus (HPV), the latter leading to many cases of cervical cancer. Low- and middle-income countries have more than 70% of the world's cancer fatalities.⁴

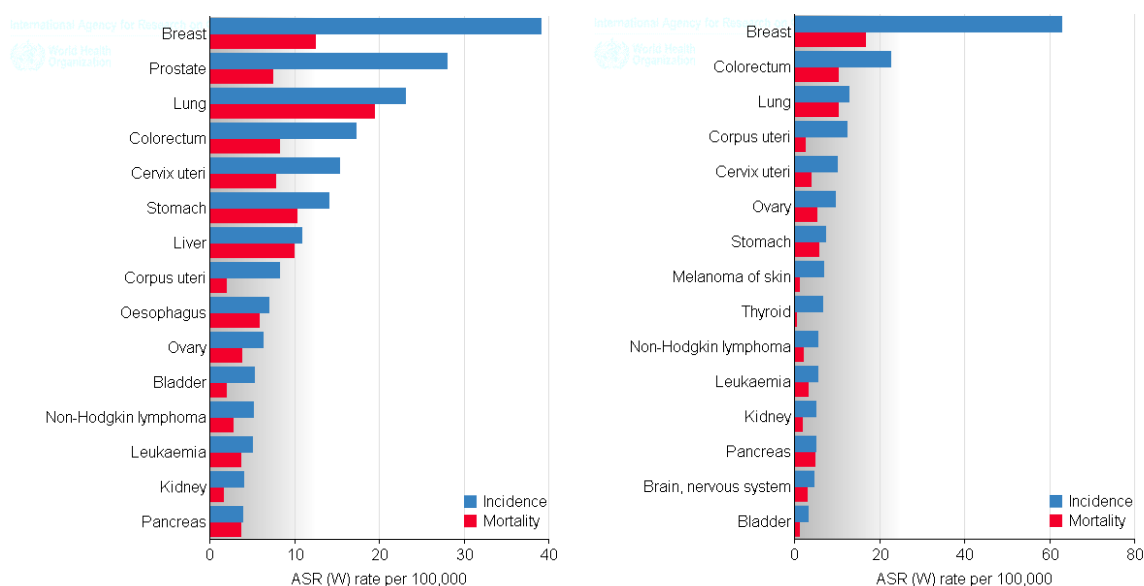


Fig. 5: Most frequent cancers for both genders in Europe (left) and the world (right)⁶

⁶ GLOBOCAN 2008 (IARC) *Section of Cancer Information* (4/2/2011), <http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900>

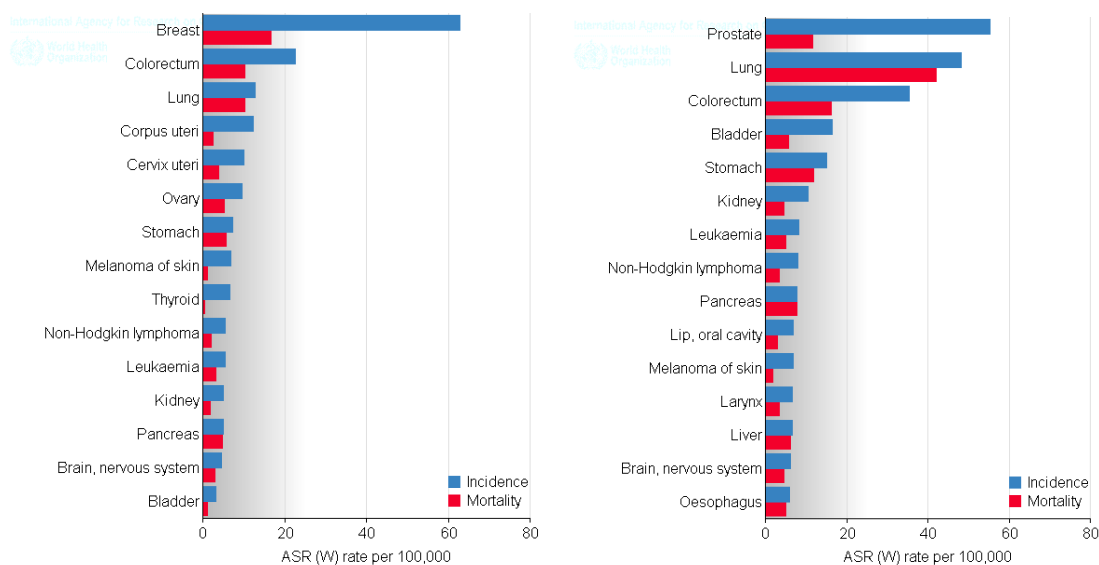


Fig. 6: Most frequent cancers in Europe: women (left) and men (right)⁷

Prevention

The development of cancer in more than 30% of cases could be prevented. Strategies for this include avoiding risk factors like the aforementioned tobacco and alcohol use, overweight and obesity, low fruit and vegetable intake, but also physical inactivity, urban air pollution, indoor smoke from household use of solid fuels, and sexually transmitted HPV infections. Vaccination against HPV and Hepatitis B, control of occupational hazards and the reduction of sunlight exposure help to lower the occurrence of cancer.⁴

Outlook

The projections for cancer to become the major cause of death worldwide are continuously increasing and it is estimated to reach 12 million deaths in 2030.⁴

⁷ GLOBOCAN 2008 (IARC) *Section of Cancer Information* (4/2/2011), <http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=994>

1.2 Cancer Treatment

The treatment of cancer can be divided into several categories, the main three being surgery, radiation therapy and chemotherapy. Other options include hormonal therapy, monoclonal antibody therapy and immunotherapy. The ideal first treatment option is the physical excision of the cancerous tissue. Sometimes this is impossible because of the location of the tumor, its type or stage of development. On the other hand the patient's overall state of health is an important criterion, which influences the type of therapy chosen. Chemotherapy – as well as radiation therapy – can be used to reduce the tumor size to facilitate surgical removal or to reduce number and size of the metastases. The aim to reach is remission.⁸ This is the fact when both symptoms and signs of the disease have disappeared or decreased.⁹

1.2.1 Surgery

The practice of surgery as cancer treatment is known since the ancient Egyptians. Later it was disapproved by Hippocrates and was also condemned by the Christian church during the Middle Ages. Surgery is an important part of modern cancer management. On the one hand, diseases can be treated with surgery, sometimes before they turn into malignancies. On the other hand, healthy tissue can be excised prophylactically when the genetic risk factors are heightened. Furthermore, it is used as a diagnostic tool when tissue samples need to be collected for pathologists. Surgery is also a valuable part of the staging of cancer. Surgery as cancer treatment is the best option when the entire cancerous tissue can be excised. When the cancer is not easily accessible sometimes only the accessible part is cut out. Surgery can also be done in a palliative way. It is then used to improve the living quality of the patients by procedures like blocking of nerves, parenteral nutrition and the removal of obstructions.⁵

1.2.2 Radiation Therapy

X-rays, i.e. photons, are used in conventional radiotherapy to damage the DNA which leads to apoptosis.¹⁰ Radiation therapy can be chosen either as primary treatment option or in an adjuvant fashion, where it can work in a palliative or therapeutic way.⁵ At the moment three methods of radiation therapy are in use: External beam therapy, proton therapy and brachytherapy. The first two involve external treatment whereas the latter is also called internal radiotherapy.

⁸ C. M. Haskell, *Cancer Treatment*, Saunders, Philadelphia 2000

⁹ US National Institute of Health, <http://www.cancer.gov/dictionary?Cdrid=45867> (11/3/2011)

¹⁰ W.P. Levin, et al., *British Journal of Cancer*, 2005, 93, 849 – 854

External beam therapy uses high-energy X-rays to destroy the cancerous tissue and is commonly used for breast, colorectal, head and neck, lung, prostate and brain tumors.¹¹

In proton therapy the energy and dose can be applied extremely accurately due to the physical properties of the protons.¹⁰ Therefore the side effects are less severe because the protons release almost all of their energy when they get in contact with the cancerous tissue thus not releasing any exit dose to the surrounding healthy tissue. Subsequently a higher dose of radiation can be administered. This method finds its most useful application in the treatment of children. Leaving the surrounding tissue intact is of utmost importance because the body is still in the developing phase. The cancers treated this way include lung, prostate, brain, spinal or vertebral body tumors, skull base sarcomas, head and neck cancer, pediatric brain tumors and eye melanomas.¹²

Brachytherapy applies ionizing radiation for the partial or total destruction of cancerous tissue.¹³ With this method the radioactive material is placed either directly inside, i.e. interstitial brachytherapy, or adjacent to the abnormal growth, i.e. contact brachytherapy. The advantages over external radiation treatment lie with the possibility of a higher administered dose of radiation applied to a smaller area during a shorter amount of time. Both the area to be treated and the time of the treatment can be reduced. It also offers the possibility of either permanent implantation of radioactive material or a temporary therapy with both high- and low-dose rates.¹⁴ Brachytherapy finds its application in the following areas: prostate, cervix, head and neck, skin, breast, gallbladder, uterus, vagina, lung, rectum and eye cancer.¹³

1.2.3 Immunotherapy and monoclonal antibody therapy

In immunotherapy the aim is to activate the immune system of the patient to destroy the tumor. Different strategies are in use at the moment including the injection of interferons, tumor antigens or dead cancer cells and the activation of the immune system through cytokines.

If monoclonal antibody therapy is chosen as the treatment option, the patient's immune system will be stimulated by an antibody with the ability to attach itself to the surface of

¹¹ Radiological Society of North America, 2010, <http://www.radiologyinfo.org/en/info.cfm?pg=ebt> (20/1/2011), <http://www.radiologyinfo.org/en/info.cfm?pg=protonthera> (20/1/2011)

¹² Radiological Society of North America, 2010, <http://www.radiologyinfo.org/en/info.cfm?pg=protonthera> (20/1/2011)

¹³ Radiological Society of North America, 2010, <http://www.radiologyinfo.org/en/info.cfm?pg=brachy> (20/1/2011)

¹⁴ A. Gerbaulet (ed.) et al., *The GEC ESTRO Handbook of Brachytherapy*, 2002

cancerous cells by binding specifically to protein-receptors localized there.⁸ To take this measure even further, the antibodies can be combined with toxins or radionuclides.¹⁵

1.2.4 Hormonal therapy

The aim of hormonal therapy is to block the hormones that are involved in the expansion of tumors by inhibiting the binding of the hormones to the receptors (in case of prostate and breast cancer this comprises testosterone and estrogen, respectively). Tamoxifen is the most important estrogen receptor antagonist. The drug arimedex limits the estrogen production.⁵

1.2.5 Chemotherapy

Frequently cancers cannot be treated by surgery or radiotherapy. Then chemotherapy comes in as the best treatment option⁵ and it is chosen either as primary treatment or in an adjuvant way post-surgery, where it is applied either palliative or therapeutic. When it is necessary to use chemotherapy before the cancerous tissue can be removed surgically we speak of neoadjuvant chemotherapy. Chemotherapy will be applied against metastases to shrink the tumor and to reduce the possibility of the recurrence of the cancer. It involves the application of drugs especially developed to interfere with the tumor cells' ability to replicate and to ideally leave the healthy cells intact.⁸

Toxicity of chemotherapeutics

The major disadvantage of the "anticancer drugs" available at the moment is their inability to differentiate between cancerous cells and other fast replicating cells, like epithelia of both hair and oral mucosa, bone marrow and gastrointestinal crypt cells. This results in various side effects which are correlated to the type of the administered drug.

The fact that mutations are much faster accumulated in cancer cells compared to healthy cells seems to be responsible for the development of both cancer and drug resistance, which is the main cause of failure of chemotherapy.⁵

One drug can also be applied to a spectrum of tumors and shows no differentiation between them.⁸

Chemotherapeutics can be categorized as follows (not including all classes):

DNA adductors bind covalently to the metal center of the nucleic acids. This leads to an obstruction of the synthesis of DNA or causes changes in the DNA structure. This category includes *alkylating agents*, *cytotoxic metal complexes* and *mitomycin C*.⁵ **Alkylating agents**,

¹⁵ T.A.Waldmann, *Nat. Med.*, **2003**, 9(3)

like nitrosoureas, attach an alkyl group to DNA via the N7 atom of guanine. This damage of the DNA leads to disruption of the replication.¹⁶ **Cytotoxic metal complexes**, like platinum(II) complexes, e.g. *cisplatin*, *carboplatin* and *oxaliplatin* bind covalently to the purine of the DNA and thus cause structural changes.¹⁷ **Mitomycin C** acts as a cross-linker of the complementary DNA strands, which leads to inhibition of the DNA replication. It can also work as a monofunctional alkylating agent.¹⁸

Topoisomerase I and II inhibitors and antibiotics are used to **prevent DNA from unwinding**. This is achieved by binding and inhibition of the responsible enzymes, which leads to apoptosis.

Mitotic inhibitors inhibit mitosis for which cancer cells are more perceptive than healthy cells. They are often derivatives of plant alkaloids and terpenoids and interfere with the movement and separation of chromosomes as they hinder the formation of microtubuli. They either prevent the formation of the tubulin scaffold or hinder its disintegration. Main representatives of this type of drugs are *vinca alkaloids*, *taxanes*, and *epothilones*.

The similar structures of **antimetabolites** and of nucleobases of DNA help in chemotherapy as they disturb the cell's metabolism leading to cell death. *Methotrexate* was one of the first developed antimetabolites. It inhibits dihydrofolate reductase and therefore the synthesis of thymidine (Fig. 7). Another example is *5-fluorouracil*, which is converted into 5-fluorodeoxyuridine monophosphate. This leads to inhibition of the synthetic pathway of the DNA.

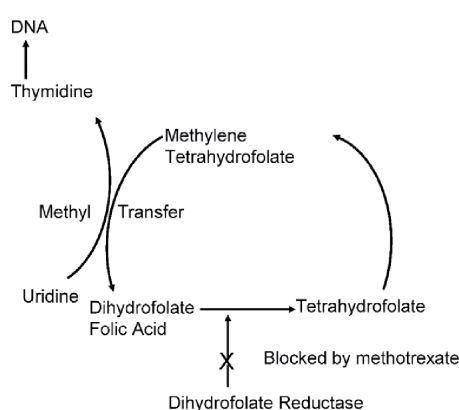


Fig. 7: Mode of action of methotrexate⁵

¹⁶ D. Jensen, et al., *Biochemistry*, 1978, 17, 5098–5107

¹⁷ L. Kelland, *Nat. Rev. Cancer*, 2007, 7, 573–584.

¹⁸ M. Tomasz, *Pharmacol. Ther.*, 1997, 76, 73–87

Combination therapy is the term for treating the patients with more than one chemotherapeutic agent at once to tackle cancer cells by hitting several targets. Most of the time two or three drugs are combined to yield an effect.⁵

A very common application of combination therapy is FOLFOX, where oxaliplatin is combined with 5-fluorouracil and leucovorin. This combination shows a higher activity against metastatic colon cancer than 5-fluorouracil and leucovorin without the platinum complex. Oxaliplatin on its own has no activity against this type of cancer.¹⁷

Summarizing, according to the world cancer report 2008, 20-30 cytotoxic drugs are in use. They are frequently combined to yield better results. Unfortunately the drugs often possess severe side effects including damages to both kidney and nerves and tumors are becoming resistant against treatment. These drugs are the reason the survival rate of some types of cancer, for example testicular, breast and colorectal cancer, could be enhanced considerably. Due to getting more and more insight into the mode of action, new drugs can be developed.⁵

1.3 Metal-based Anticancer Drugs

1.3.1 Introduction

Metal ions, in particular transition metal ions, play an invaluable part in biological systems. Although organic compounds take the leading role in medicinal chemistry, metal-based drugs have picked up momentum in this field and find application in both diagnostics and curative treatment.¹⁹

1.3.2 Platinum Compounds

Modern chemotherapy is unthinkable without the three Pt complexes which have gained worldwide approval for clinical practice: cisplatin, carboplatin and oxaliplatin.¹⁹ (Fig. 8)

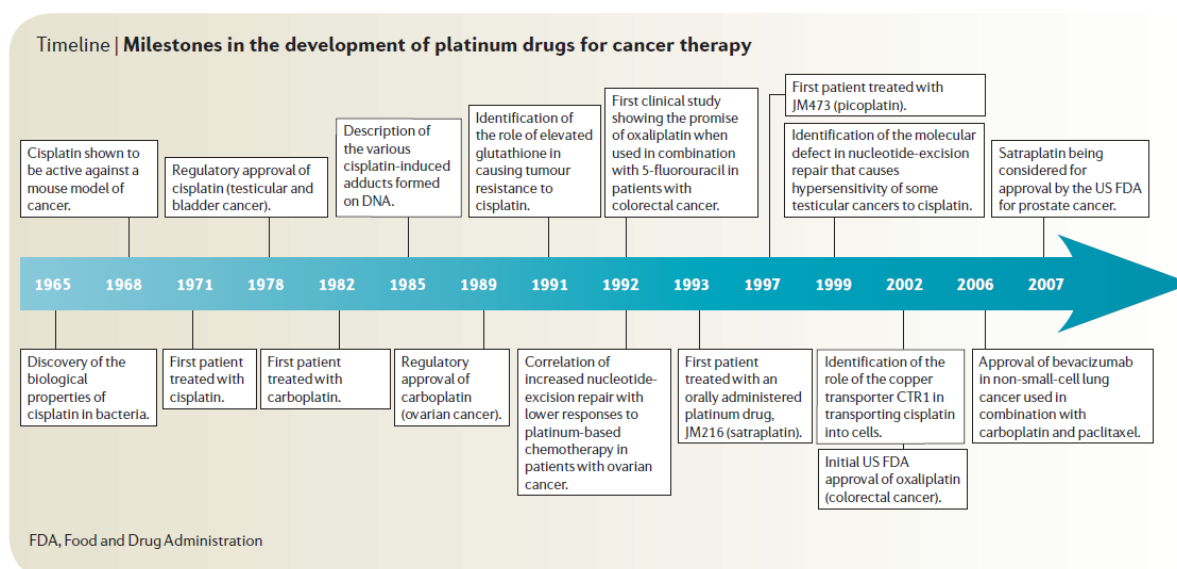


Fig. 8: Timeline: Platinum compounds as anticancer drugs¹⁷

Cisplatin is still one of the best drugs for treating ovarian and testicular cancers despite its nephrotoxicity. The severe side effects of cisplatin were the reason to search for a drug with the same effectiveness but considerably lower side effects. This led to the discovery of carboplatin. The mode of action of both drugs relies on forming bonds between the drug and DNA resulting in apoptosis. The development of oxaliplatin can be lead back to the need for a drug to treat cancers that are resistant to cisplatin.¹⁷

¹⁹ C.G. Hartinger, et al., *Journal of Inorganic Biochemistry*, 2006,100, 891–904

1.3.2.1 Cisplatin

The development of cisplatin occurred serendipitously in the 1960s. Barnett Rosenberg, a physicist working at Michigan State University, was eager to investigate if bacterial growth can be stimulated by magnetic dipole or electric fields. The setup for the experiment included *Escherichia coli* and platinum electrodes and the bacteria started a filamentous growth. This could be traced back not to the effects of the electric field but to the experimental conditions and to the platinum electrodes, where accidentally *cis*-diamminedichloridoplatinum(II) was being synthesized. This byproduct, better known as cisplatin (Fig. 9), has already been prepared by Michele Peyrone in 1844. After his discovery Rosenberg tested the cytotoxicity of cisplatin first on various bacteria and after that on mice and proved its healing properties against solid tumors. In the early 1970s clinical trials were started by the US National Cancer Institute. At the end of this decade the FDA had already approved cisplatin under the name of Platinol®.¹⁷ The main field of application for cisplatin is ovarian and testicular cancer, but it is also used in the treatment of cervical, bladder and bronchogenic carcinoma, gastric and head and neck cancer as well as osteosarcoma.²⁰

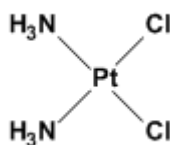


Fig. 9: Structure of cisplatin

The process of the cellular uptake of cisplatin is not thoroughly understood, though passive distribution and active transport by copper transporters are considered important; the following mode of action is proposed: Activation of cisplatin occurs in the cell and results from the exchange of one of the two chloride group with water. Afterwards it is able to form covalent DNA adducts. It does this by forming a bond with the *N7* of guanine and also to the other purine based nucleobase, i.e. adenine, but not to same extent. This results in monofunctional or bifunctional adducts, most of the time with neighboring nucleotides forming crosslinks. The platinum-DNA adducts can have different effects on the DNA, e.g. bending and unwinding and this can also hinder mechanisms like transcription and replication.¹⁷

²⁰ M. Galanski, et al., *Current Medicinal Chemistry*, 2005, 12(18), 2075-2094(20).

1.3.2.2 Carboplatin

The discovery of cisplatin and its tumor-inhibiting abilities led to the development of a great number of platinum compounds. Approximately 30 of them, including carboplatin, i.e. *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) (Fig. 10), and oxaliplatin, were tested in clinical trials. Studies demonstrated that the change in leaving group from the chloride ligand in cisplatin to 1,1-cyclobutanedicarboxylate in carboplatin causes the compound to be more stable, exhibiting lower side effects, while obtaining the same activity. The FDA approved carboplatin under the name Paraplatin® for palliative application in 1989 and for first line treatment of ovarian cancer in 1991.¹⁷

The coordination of carboplatin to the *N7* of the DNA was proven by experiments involving the model compound 5'-GMP. These experiments, though in cell free environment, demonstrated differences to cisplatin like the lack of monoadduct formation and hydrolysis of products, which lead to the conclusion that carboplatin does not need to be activated by hydrolysis before application.²¹

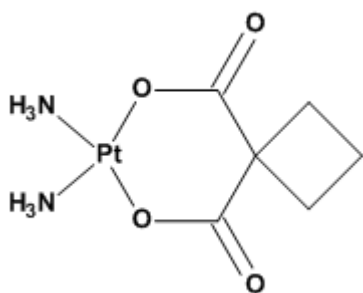


Fig. 10: Structure of carboplatin

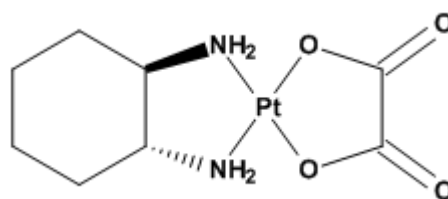


Fig. 11: Structure of oxaliplatin

1.3.2.3 Oxaliplatin

In the late 1970s, a platinum compound was synthesized incorporating a 1,2-diaminocyclohexane (DACH) ligand, namely [(1*R*,2*R*)-diaminocyclohexane](oxalato)platinum(II), better known as oxaliplatin (Fig. 11). It is active against cisplatin resistant tumors and was FDA approved by the end of the millennium. It is available under the trade name Eloxatin®. Oxaliplatin is one of the components of FOLFOX, used very commonly in combination therapy to treat metastatic colorectal cancer.¹⁷ It causes other types of DNA damages than cisplatin, which can be related to the size and structure of the ligand.²²

²¹ A. Küng, et al., *J. Inorg. Biochem.* 2001, 83, 181-186.

²² M.A. Jakupec, et al., *Rev. Physiolog. Biochem. Pharmacol.*, 2003, 146, 1-53

1.3.3 Non-platinum Compounds

1.3.3.1 General Overview

Due to the severe side effects of platinum compounds and their use limited to special forms of tumors, the search for other metal compounds with tumor-inhibiting properties is crucial.¹⁹ New drugs need to have improved selectivity and activity, their toxicity should be lower and a new mode of action would be preferable.²³ At the moment two non-platinum tumor-inhibiting compounds show great promise in clinical trials: tris(quinolinolato)gallium(III) (KP46) (Fig. 12) and indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (KP1019).²⁴

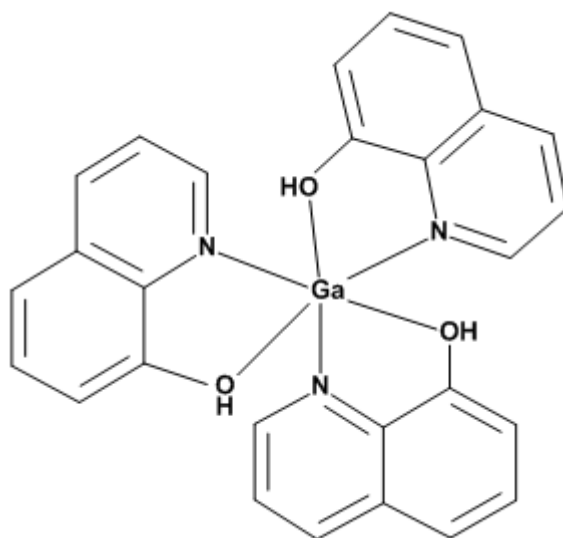


Fig. 12: Structure of KP46

1.3.3.2 Ruthenium Compounds

An encouraging alternative to platinum is provided by ruthenium complexes due to their well understood chemistry and the advancing comprehension of their biological activities.¹⁹ The main advantages of ruthenium complexes as drug candidates are believed to be related to their ability to mimic iron, their ligand exchange rate and the fact that several oxidation states are available under physiological conditions.²⁵

NAMI-A and KP1019 (Fig. 13) differ from most of the tumor-inhibiting pharmaceuticals that are based on platinum in the following points: ruthenium complexes are octahedral whereas platinum(II) compounds are square-planar and the Ru(II)/Ru(III) redox couple is reversible

²³ W. Kandioller et al., *J. Organomet. Chem.*, 2011, 696, 999-1010

²⁴ M. Hanif, et al., *Inorg. Chem.* 2010, 49, 7953–7963

²⁵ C.S. Allardyce, et al. *Platinum Metals Rev.*, 2001, 45(2), 62-69.

whereas when Pt(IV) complexes are reduced to Pt(II) the interatomic bond distances as well as the coordination number change. As a result differences in the mode of action regarding these drugs are proposed. This could account for the capability of KP1019 to be effective against tumors resistant to cisplatin. Another advantage for ruthenium chemotherapeutics is their lower toxicity which could be explained by the ability of ruthenium(III) complexes to work as prodrugs, which means that they are converted into their active species *in vitro* or *in vivo*.

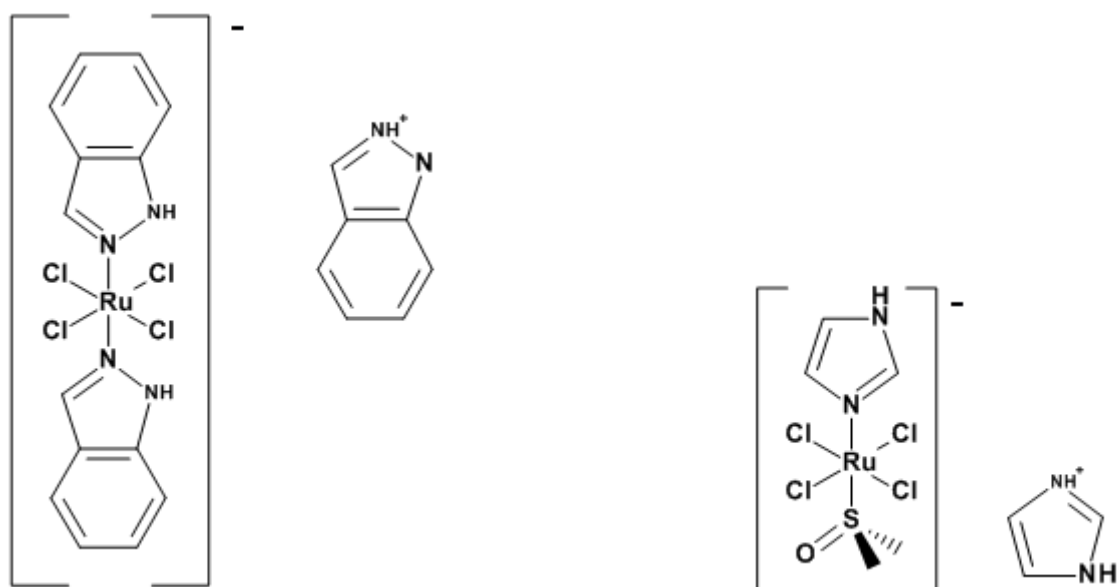


Fig. 13: Structures of KP1019 (left) and NAMI-A (right), two Ru(III) complexes in clinical trials

After learning of the antitumor effects of ruthenium red in the 1970s and the examination of *fac*-[RuCl₃(NH₃)₃] and *cis*-[RuCl₂(NH₃)₄]Cl as the first ruthenium antitumor agents in the early 1980s, KP1019 and (H₂im)[*trans*-RuCl₄(Him)(dmsO)] (NAMI-A, Him=imidazole) are the first tumor-inhibiting drugs with ruthenium as their metal center that are in clinical trials (Fig. 14). The results of preclinical experiments show very different points of application for these two anticancer drugs. KP1019 is active against different primary explanted human tumors and colon carcinomas while NAMI-A displayed effective results in the inhibition of metastasis.

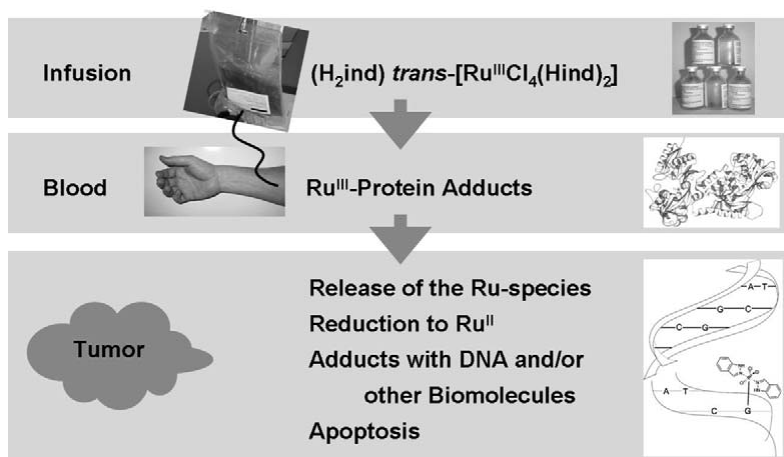


Fig. 14: Proposed mode of action for KP1019 ¹⁹

Transferrin and albumin are the most important potential binding partners of Ru(III) compounds when given intravenously because drugs that contain transition metals show a high affinity to their soft sulfur and nitrogen donors.²⁶ However, the initial binding event is related to hydrophobic interactions.²⁷

It is speculated that the large accumulation of Ru(III) complexes in tumors might be ascribed to transferrin. This protein is responsible for the transport of iron, for which the need is much higher in tumor cells than in healthy cells and therefore more iron receptors are expressed. This leads to the notion of transferrin as a “Trojan Horse” accountable for the transport of the ruthenium complex into the cell whether in the place of or additionally to the much needed iron. The current notion contains the exchange of the ligand with the histidine of the iron-binding pocket and so forming a covalent bond between complex and transferrin.²⁶ Albumin on the other hand is believed to be used as storage for the medication as the greater part of ruthenium complexes (80-90%) is bound to this protein while in the blood stream.¹⁹

1.3.3.3 Ruthenium(II)-arene compounds

Ru(II)-arene compounds along with their corresponding Os(II)-arene analogues can be designed to tune important factors like chemical reactivity and pharmacological characteristics, including oral bioavailability, pharmacokinetic behavior, water solubility and stability in plasma. This can be achieved by choosing different ligands.²⁴

²⁶ M. Grössl, et. al., *Electrophoresis* 2009, 30, 2720–2727

²⁷ N. Cetinbas, et. al., *J Biol Inorg Chem* 2010, 15, 131–145

Due to the stabilizing properties of the η^6 -coordination of the aromatic ligand, Ru(II)-arene complexes are stable enough to come into consideration for appliance as a drug. Additionally the Ru(II) complex is positively affected by the arene so that both biological and physicochemical attributes are changed.²³ These Ru(II)-arene complexes can be written as $[\text{Ru}(\eta^6\text{-arene})(\text{X})(\text{Y})(\text{Z})]$ (Fig. 15). X and Y are non-leaving ligands whereas Z is a monofunctional leaving group.²⁸ The most commonly used leaving group is a halide, which promotes aquation. This helps the compound to react to biological targets.²³ The geometry of these complexes is described as “piano stool” configuration. This derives from its appearance, where the arene serves as the seat of the piano stool and the legs are made up by the ligands.²⁸

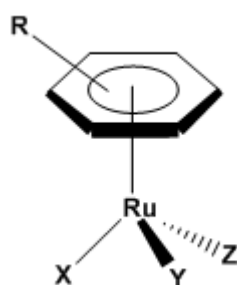


Fig. 15: General structure of Ru(II)-arene complexes

The idea behind the first organometallic ruthenium chemotherapeutics was the incorporation of bioactive ligands into the metal-arene complex. The first tests were conducted with $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)\text{Cl}_2(\text{metronidazole})]$ given the fact that metronidazole is a well known anti-infective agent.²⁴ Another early investigated compound was $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)\text{Cl}_2(\text{dmsO})]$, which possesses topoisomerase II inhibiting properties, but only in high concentrations.²³

These initial results led to the development of compounds with various enzyme inhibitors as ligands like ethacrynic acid, paullones and staurosporine. On the other hand the use of non-cytotoxic ligands like 1,3,5-triaza-7-phosphatricyclo[3.3.1.]decane (pta) and ethylenediamine resulted in compelling anticancer activity.²⁴ The pta complexes are known as the RAPTA family. The pta ligand amplifies the aqueous solubility and the activity towards metastases.²³ When carbo- and oxaliplatin analogous are formed by exchanging the chlorido ligands with cyclobutane-1,1-dicarboxylate or oxalate, the resulting complexes show similar activity as the parent compounds, yet they are of minor *in vitro* anticancer activity.

²⁸ R.E. Morris, et.al., *J. Med. Chem.* 2001, 44, 3616-3621

Complexes bearing bidentate ligands with O,O-, N,N-, N,O-, and S,O- donor systems in the form of a $[M(\eta^6\text{-arene})(XY)Cl]^{n+}$ metal-arene complex ($n = 0, 1$) were evaluated on their cytotoxic properties. The use of compounds like ethylenediamine as N,N-ligands coordinated to Ru(II)-arene gave high cytotoxic values even in cell lines resistant to cisplatin.²⁴ The studies demonstrated highest activity in cancer cell lines of complexes bearing the tetrahydroanthracene ligand and lowest activity with benzene derivatives. This shows that the chelating ligand has a high significance and impact with regard to biological, physical and chemical attributes of Ru(II) complexes.²³ The discussed mode of action involves a hydrogen bond and monofunctional covalent binding to DNA nucleobases, but without any clear comprehension of the ultimate targets of bioorganometallics the topic remains discussed with much controversy.²⁴ Neutral complexes can be formed by substituting ethylenediamine with acetylacetonate. The Ru(II) center of these compounds has a higher electron density, which promotes hydrolysis and the exchange of ligands, Ethylenediamine complexes bind preferably to guanine while on the other hand acetylacetonate complexes favor adenine.²³ Using paullone as N,N-chelating ligands coordinated to Ru or Os reveals high activity *in vitro* independent of the metal center. The use of O,O- and N,O-chelating ligands such as amino acids and derivatives in similar metal complexes yielded only moderate cytotoxicity, but picolinate and picolinamide ligands show great promise regarding their *in vitro* activities even in cells resistant to cisplatin.²⁴

1.3.3.4 Maltol-derived compounds

An important group of ligands are derivatives of maltol (Fig. 16) which are bound to the metal center of both di- and trivalent metal ions via an O,O-chelating moiety. The resulting complexes are more stable than with acetylacetonato ligands and exhibit minor cytotoxicity.²⁹ This can be ascribed to the fact that a ruthenium-hydroxo dimer, which is formed in aqueous solution, is a very stable but inactive species. This disadvantage can be overcome by replacing maltol with 3-hydroxypyrrone derivatives, which include groups having both lipophilic and aromatic properties. Similar to platinum compounds, the interactions of these ruthenium-arene complexes with DNA were examined with 5'-GMP and demonstrated reactivity to the N7 of guanine.³⁰

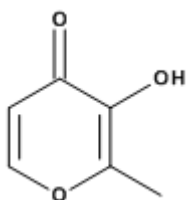


Fig. 16: Structure of maltol

3-Hydroxy-2-methyl-4-pyridones have been investigated as ligands for both mononuclear and dinuclear ruthenium complexes. Dinuclear complexes in the form of PyrRu_2^n , with n as the chain length of the alkyl spacer, displayed encouraging cytotoxicity. The cytotoxicity correlates with the length of the spacer, which is related to the hydrophobicity of the compound and probably facilitates the cellular uptake. The dinuclear ruthenium(II)-arene complex showing the best results was PyrRu_2^{12} (Fig. 17) with activities higher than cisplatin and in the same range as oxaliplatin.³¹ The two chloride ligands are easily exchanged with water. As with the other compounds before, guanine is the binding site of choice and results in inter-duplex cross-linked DNA adducts with good stability.³²

²⁹ A. Peacock, et al., *Chem. Eur. J.* 2007, 13 (9), 2601-2613.

³⁰ W. Kandioller, et al. *J. Organomet. Chem.* 2009, 694, 922-929.

³¹ M. Mendoza-Ferri, et al., *Organometallics* 2008, 27, 2405-2407.

³² O. Nováková, et al., *Biochem. Pharmacol.* 2009, 77, 364-374.

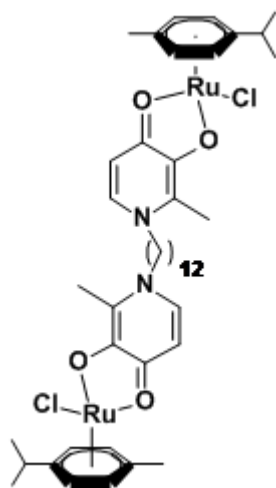


Fig. 17: Structure of PyrRu₂¹²

For mononuclear complexes the substitution of O,O- with S,O-chelating ligands results in higher *in vitro* anticancer activity which can be deduced from their increase in stability when biomolecules are present.³³ The anticancer activity of pyridones used instead of pyrones is high and shows promising results for overcoming drug resistance.²⁴

³³ W. Kandoll, et al., *Chem. Eur. J.* 2009, 15, 12283-12291.

1.4 Hydroxypyr(id)ones (HP)

1.4.1 Introduction

Hydroxypyridones have increasingly become of interest in medicinal inorganic chemistry. Their anions are able to build stable metal complexes with various metals thus showing great versatility as chelating ligands. During the last years there have been miscellaneous studies regarding hydroxypyridones and hydroxypyridone complexes. These involved using HP as anticancer drugs, matrix metalloprotein inhibitors, magnetic resonance imaging (MRI) contrast agents and anthrax lethal factor inhibitors. Also their use as antidiabetic, antibacterial, antiviral agents and as Na^+ - and Li^+ -receptors, as well as a treatment for manic depression was studied.³⁴ Furthermore, hydroxypyridones, especially 1,2-dimethyl-3-hydroxypyrid-4-one, i.e. deferiprone, are currently in use as therapeutics against iron-overload diseases.²³ They are also under discussion for treating misplaced iron and aluminium in neurodegenerative disorders such as Parkinson's (PD) and Alzheimer's (AD) diseases.³⁵

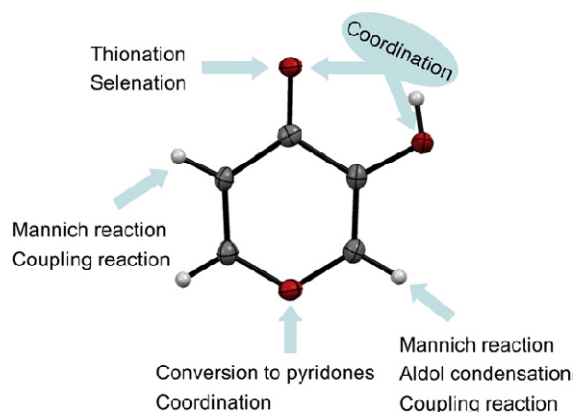


Fig. 18: Possible sites for derivatization of pyrones²³

Hydroxypyridones display favorable bioavailability and toxicity profiles. Additionally their lipophilic properties and the fact that their metal ion complexes are notably stable make hydroxypyridones an interesting compound class.

There are three classes of hydroxypyridones known, i.e., 3-hydroxy-2-pyridone, 3-hydroxy-4-pyridone, and 1-hydroxy-2-pyridone. The structure of hydroxypyridones contains the following elements: a secondary or tertiary amino group and a hydroxyl group ortho to a ketone. The amino group is easily functionalized and therefore a likely place to alter

³⁴ M. Hanif, et al., *Inorg. Chem.*, 2010, 49 (17), 7953–7963

³⁵ M. Amélia Santos, et al., *Coord. Chem. Rev.*, 2008, 252, 1213–1224.

biological and physicochemical properties, like lipophilicity, oral bioavailability and chelating ability (Fig. 18).³⁶

In recent times thiopyridones became of interest as S,O-metal chelating agents because their affinity to softer metal ions is higher than the one of the O,O-counterparts as is the stability of the complexes.²³

1.4.2 Selected Applications in Medicinal Inorganic Chemistry

1.4.2.1 Treatment of iron deficiency and overload

The control of iron concentration in cells, called “iron homeostasis”, is very important due to the fact that deficiency as well as overload of this essential trace element can create complications in the body.³⁷ The affinity of 3-hydroxypyridones towards iron is high³⁸ thus being suitable candidates for the treatment of iron overload diseases by substituting the moiety at the pyridone's nitrogen while keeping the chelating properties.³⁹ The aforementioned deferiprone was synthesized at the beginning of the 1980s⁴⁰ and its clinical application started at the end of the millennium.⁴¹ One major advantage is that it can be taken orally.⁴² It was discovered that 85% of deferiprone was inactivated by O-glucuronation during the metabolism and excreted through the urine. Due to these findings it became a challenge to find new pyridone-derivatives lacking this particular inactivation.⁴³

People affected by iron deficiency have the need to enhance their diet with iron supplements. This can be problematic due to the potential toxicity of Fe(III), the oxidation state of iron when bound to transferrin. Said toxicity occurs because of a much higher production of reactive oxygen species (ROS).⁴⁴ The difference of Fe(III) and Fe(II) is that under physiological conditions Fe(III) is hydrolyzed and precipitates.⁴⁵ On the other hand Fe(II) has to be dosed very highly in order to reach the desired effects.⁴⁶ When complexes of Fe(III) and maltol derivatives are formed, these drug compounds have favorable properties as compared to administration of iron salts. These include non-toxic ligands, neutral complexes and a more

³⁶ K. H. Thompson, et al., *Chem. Soc. Rev.*, 2006, 35, 545–556

³⁷ G. Papanikolaou, et al., *Toxicol. Appl. Pharmacol.*, 2005, 202, 199-211.

³⁸ W.C. Tsai, et al., *Biochem. Soc.*, 1973, 2, 70.

³⁹ W. Schlindwein, et al., *Dalton Trans.*, (2006), 1313-1321.

⁴⁰ G.J. Kontoghiorghe, et al., *Inorg. Chim. Acta*, 1987, 136, L11-L12.

⁴¹ A.V. Hoffbrand, et al., *Blood*, 2003, 102, 17-24

⁴² S. Singh, et al., *Drug Metab. Dispos.*, 1992, 20, 256-261.

⁴³ Z.D. Liu et al., *J. Med. Chem.*, 1999, 42, 4814-4823.

⁴⁴ M.A. Barrand, et al., *J. Pharm. Pharmacol.*, 1990, 42, 279-282

⁴⁵ T. Hedlund, et al., *Acta Chem. Scand. Ser. 1988 A A42*, 702.

⁴⁶ B. Morgan, et al., *Chemosphere*, 2007, 68, 2080-2084.

stable intermediate, hindering hydrolysis and the formation of iron precipitates.⁴⁵ The main advantage of these complexes is their bioavailability *in vivo*, which allows to administer lower doses to patients in comparison to treatment with FeSO₄.⁴⁷

1.4.2.2 Treatment of Alzheimer's disease (AD)

The goal of the application of pyr(id)ones in the treatment of AD is to chelate Al(III) to reduce its influences on the brain and thus the progression of Alzheimer's disease.⁴⁸ Al(III) is particularly dangerous because of its similarity to Fe(III) with regard to their coordination chemistry. Since investigations into [tris(maltolato)aluminum(III)] (Fig. 19) revealed neurotoxicity, the current focus lies on 3-hydroxypyridones because of their stronger complexation properties.⁴⁹

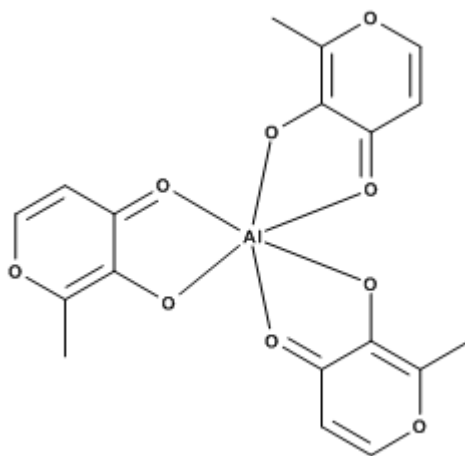


Fig. 19: Al(III) maltolato complex

1.4.2.3 Treatment of Diabetes

Two maltol-based compounds, namely [bis(maltolato)oxovanadium(IV)] (BMOV) (Fig. 20) and [bis(ethylmaltolato)oxovanadium(IV)] (BEOV) have reached clinical phase II trials as potential treatment of type-2 diabetes.⁵⁰ The insulin mimicking property of vanadium has been known since the 1980s but its use has been limited by the low bioavailability of its inorganic salts.⁵¹ Other maltol-based ligands have been coordinated to metals like Mo(VI),⁵²

⁴⁷ D.G. Maxton, et al., *Br. J. Nutr.*, 1994, 71, 203-207

⁴⁸ D.R. Crapper, et al., *Brain*, 1976, 99, 67-80

⁴⁹ M.M. Finnegan, et al., *Inorg. Chem.*, 1987, 26, 2171-2176

⁵⁰ A. Sheela, et al., *Eur. J. Med. Chem.*, 2008, 43, 2206-2210

⁵¹ K.H. Thompson, et al., *J. Inorg. Biochem.*, 2009, 103, 554-558

⁵² S. Chaves, et al., *Metallomics*, 2010, 2, 220-227

Cu(II), Co(II)⁵³ and Zn(II). The first three showed a lower activity than BMOV and BEOV, but the last showed promising results with (thio)pyr(id)onate as ligand both *in vitro*⁵² and *in vivo*.⁵⁴

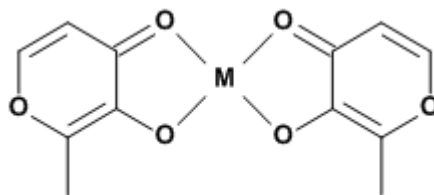


Fig. 20: BMOV, M=VO

1.4.2.4 Diagnostic use

Today nearly a third of all MRI examinations require the use of paramagnetic metal ions, like Gd(III), as contrast agents to get clearer images. A good way to reduce the toxic effects of this metal is to bind it to multidentate ligand systems, but to leave one coordination site minimum accessible for an aqua species. The availability of coordination sites for aqua ligands is of the utmost importance to yield the desired contrast improvement. The higher the number of available coordination sites is, the better the contrast will be.⁵⁵ When employing such compounds the resulting complexes will gain in relaxivity and do not lose their stability. Following this assumption, MRI contrast agents, involving Gd(III) with trisbidentate hydroxypyridonate ligands, have been developed,⁵⁶ where six coordination sites of Gd(III) are taken by these ligands leaving two sites free to bind water molecules.⁵⁷ One of the first pyridone-based ligands assessed when coordinated to Gd was tris[(3-hydroxy-1-methyl-2(1H)-pyridone-4-carboxamido)ethyl]amine. The Gd(III) complex showed improved relaxivity as well as stability in comparison to the contrast agents that were already in use.⁵⁸ Different linkers were used to determine the optimal parameters.⁵⁷ The complexes were also tested *in vivo* yielding the highest success rate when two⁵⁹ or three⁵⁷ water molecules were present in the inner coordination sphere.⁶⁰

⁵³ K.H. Thompson, et al., *J. Inorg. Biochem.*, 2004, 98, 683-690

⁵⁴ A. Katoh, et al., *J. Inorg. Biochem.*, 2009, 103, 567-574

⁵⁵ S. Aime, et al., *Adv. Inorg. Chem.*, 2005, 57, 173-237

⁵⁶ A. Datta, et al., *Acc. Chem. Res.*, 2009, 42, 938-947

⁵⁷ E.J. Werner, et al., *J. Am. Chem. Soc.*, 2007, 129, 1870-1871

⁵⁸ J. Xu, et al., *J. Am. Chem. Soc.*, 1995, 117, 7245-7246

⁵⁹ K.N. Raymond, et al., *Bioconjug. Chem.*, 2005, 16, 3-8

⁶⁰ M.K. Thompson, et al., *J. Med. Chem.*, 2005, 48, 3874-3877

1.4.2.5 Tris(maltolato)gallium(III) as potential anticancer drug

Ga(III) compounds were on the one hand already in use for the imaging of soft tumor tissue and bear tumor inhibiting characteristics.⁶¹ On the other hand they possess an analogous coordination chemistry to Fe(III) and hence could use the same biochemical transport pathways.²⁶ Therefore it was not farfetched to examine them as potential anticancer drugs. The biggest difference between Ga(III) and Fe(III) is the redox inertness of Ga(III) thus destabilizing biological functions when replacing Fe(III) in redox-active enzymes. The FDA approved gallium nitrate under the name GaniteTM for treating cancer related hypocalcaemia. During its clinical trials it proved to be active against bladder cancer and lymphoma. Due to the grave side effects, which include the drug being both nephrotoxic and neurotoxic, and its reduced bioavailability no further investigation were performed.⁶² The new focus was put on other ligands with the ability to increase the bioavailability of the gallium complex and to prevent the compound from hydrolyzing. This search concluded in clinical trials of gallium maltolate, i.e. [tris(maltolato)gallium(III)]. The advantages of gallium maltolate lie in its ability of oral administration and better bioavailability as compared to gallium salts. It completed phase I⁶³ but was terminated in phase I/II.⁶⁴

⁶¹ R.E. Coleman, *Cancer*, 1991, 67, 1261-1270

⁶² C.R. Chitambar, *Curr. Opin. Oncol.*, 2004, 16, 547-552

⁶³ L.R. Bernstein, et al., *Met. Based Drugs*, 2000, 7, 33-47

⁶⁴ <http://clinicaltrials.gov/show/NCT00050687> (26/6/2011)

1.4.3 Coordination Chemistry

The structure of a hydroxypyridone contains a 6-membered N-heterocycle and has zwitterionic “aromatic” character (Fig. 21).

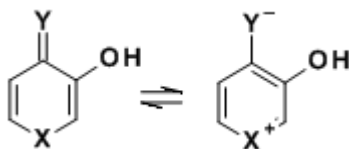


Fig. 21: Mesomeric forms, X=O, N-R; Y=S,O

3-Hydroxy-2-pyridones and 3-hydroxy-4-pyridones have O,O-chelating properties. Therefore and because of their strong affinity to metal ions they serve as likely ligands to form stable complexes. The formed 5-membered ring is thermodynamically stable at physiological pH values.⁶⁵ The complex stability decreases in the following order: 3-hydroxy-4-pyridone, 3-hydroxy-2-pyridone, and 1-hydroxy-2-pyridone and the complexes formed by pyridones are more stable than their pyrone analogues.⁶⁶

1.4.4 Synthetic considerations

1.4.4.1 (Thio)hydroxypyr(id)ones

The most general approach to convert hydroxypyrones into hydroxypyridones involves the protection of the hydroxyl group. This is done via benzylation and assists the progress of conversion. To obtain the product in high yield, it is very important to maintain the pH value at approximately 12 during the reaction with the primary amine. The last step includes the cleavage of the protection group via hydrogenation (Fig. 22).⁶⁶

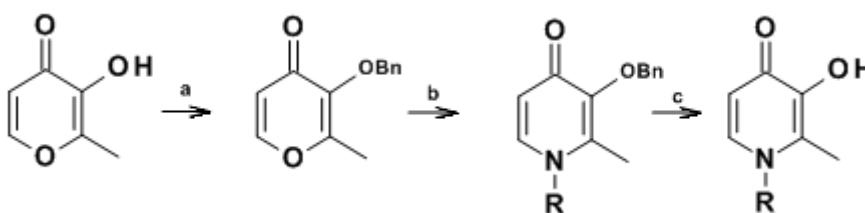


Fig. 22: Reaction conditions: a) BnBr, b) H₂N-R, c) H₂, Pd/C

To prepare the thionated analogues of 3-hydroxy-4-pyr(id)ones, approaches include (a) adding 1 eq of Lawesson's reagent⁶⁷ or (b) reacting them with P₄S₁₀ in excess.⁶⁸

⁶⁵ R.D. Hancock, et al., *Chem. Rev.*, 1989, 89, 1875

⁶⁶ P.S. Dobbin, et al., *J. Med. Chem.*, 1993, 36, 2448-2458

⁶⁷ S. Chaves, et al., *Dalton Trans.*, 2008, 1773-1782

⁶⁸ V. Monga, et al., *Inorg. Chem.*, 2005, 44, 2666-2677

2 Results and Discussion

The research during my diploma thesis was aimed at the synthesis of 3-hydroxypyridone-derived compounds and their Ru(II)(η^6 -arene) complexes with tumor-inhibiting properties.

2.1 Synthesis and Characterization of the Ligands

2.1.1 Synthesis

The ligands were prepared in several steps. First, compound (c) has to be prepared from which (d) and (e) can be derived, which includes a protection step to yield (c). Afterwards ester (c) was converted into amides (d). Subsequently the protection group was removed by hydrogenation resulting in ligand (e) which then can be coordinated to different metal ions.

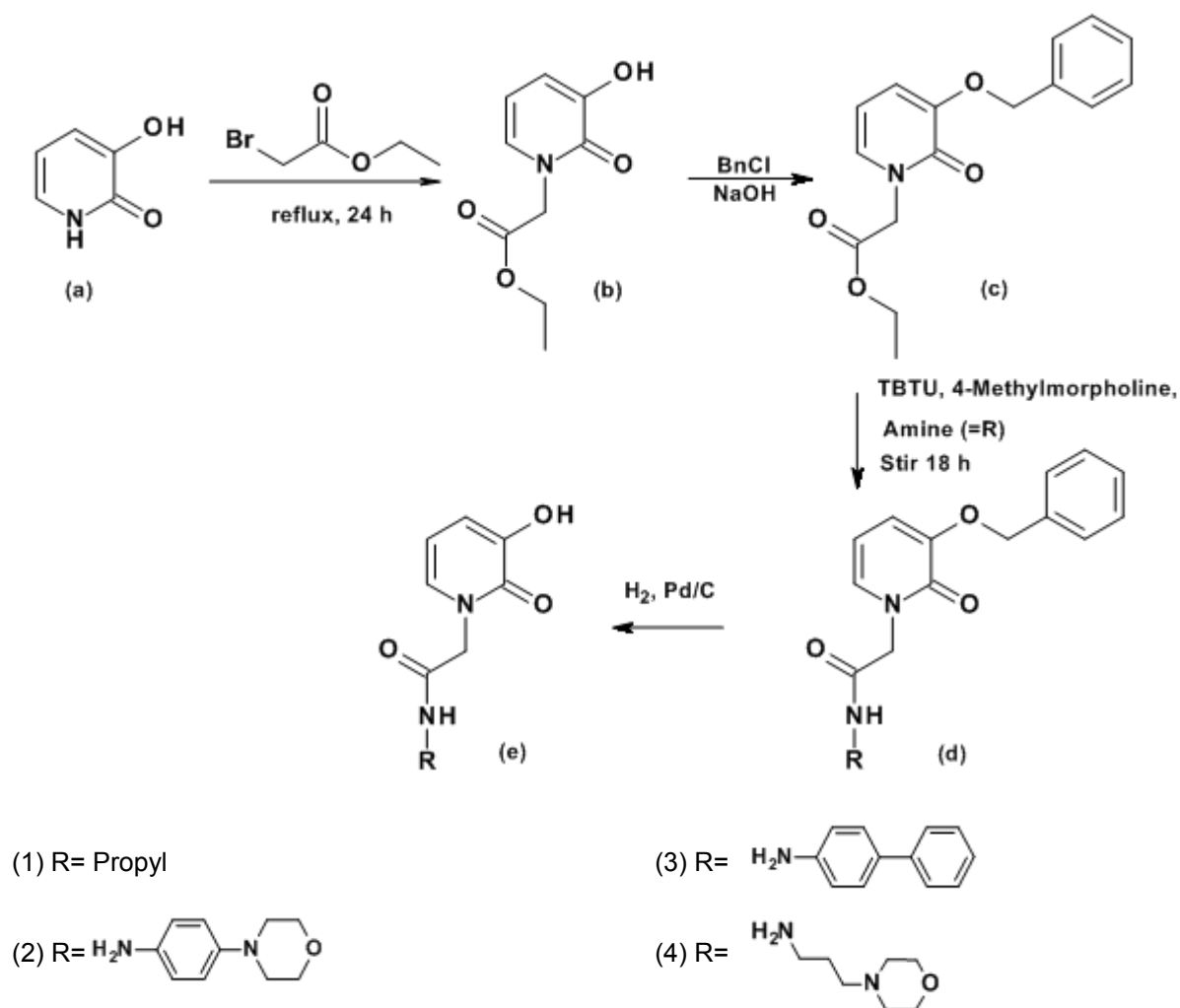


Fig. 23: General procedure for the synthesis of the ligands

The synthesis of the ligand 1-[(ethoxycarbonyl)methyl]-3-hydroxy-2-(1H)-pyridone (b) was done by N-alkylation of 3-hydroxy-2-pyridone with an alkyl halide, e.g. ethyl bromoacetate, which also acts as a solvent in this reaction.⁶⁹ This results in the formation of the ethyl ester. The next step is the protection of the hydroxyl group yielding 3-benzyloxy-1-carboxymethyl-2-pyridone (c) in a S_N2 reaction (Fig. 24). To get to the ether, we used an alkyl halide, e.g. benzyl chloride and an alkoxide anion, e.g. the pyridone anion. The pyridone serves as a good nucleophile and chloride as the leaving group. In this case NaOH is strong enough to be used as a base.⁶⁹

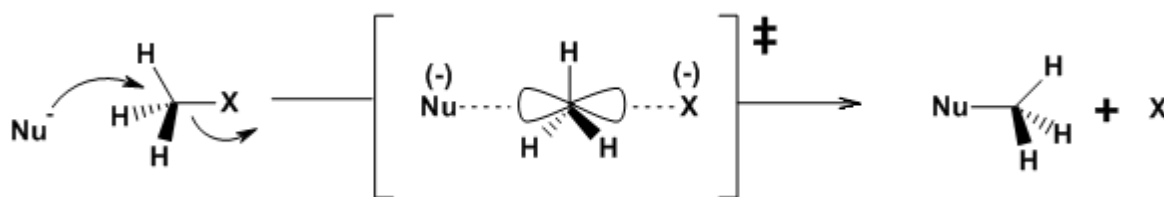


Fig. 24: S_N2 reaction – scheme⁶⁹

The most important step in the preparation of the ligands was the exchange of the ester moiety with an amine forming the respective amides (d). The mechanism is shown in Fig. 25. The ester (A) is hydrolyzed under alkaline conditions (B) and reacts with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) under formation of the reactive O-acyl-N,N,N',N'-tetramethyluronium intermediate (C). The formed active ester (D) reacts with the respective amines (E) yielding amides (F).⁷⁰ To make the ligand ready for complexation, the protecting group has to be removed (Fig. 23-(e)). This deprotection step uses catalytic hydrogenation to cleave off the benzyl protecting group using hydrogen gas and a palladium catalyst. The removal of benzyl ethers can also be done by acids, where the conjugate base is nucleophilic.⁶⁹

Results:

The goal of this part of the synthesis was to obtain straightforward procedures, which do not need harsh conditions and result in high yields.

❖ Amide formation

The yields ranged from 66 - 87%. All coupling steps were done following this synthetic strategy. After successful preparation of the respective ligands, another amide formation procedure was evaluated in a very small scale yielding ligand 1d'. This included the use of

⁶⁹ J. Clayden, et al.; *Organic Chemistry*, Oxford University Press, 2001 (8)

⁷⁰ S. Fuchs; *Oberflächenfunktionalisierte Poly(amidoamin)-Dendrimere als potentielle Trägermoleküle für Cytostatika*, 2004

N,N'-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide instead of TBTU and of diisopropylethylamine as base. The interesting part of this alternative synthesis route is not the reduction of the reaction time by two hours, but the lack of need for the purification by solvent extraction.

❖ Deprotection

This step yielded approximately 70% of the desired product. The preferred procedure involved the use of an autoclave apparatus, only not applied when the respective ligand was not soluble in the highest usable amount (250 ml) of solvent, e.g. methanol and acetic acid, for the autoclave apparatus, as in case of compound 3e. The insolubility of ligand 3e can be attributed to the phenylbenzylamido moiety and its aromaticity.

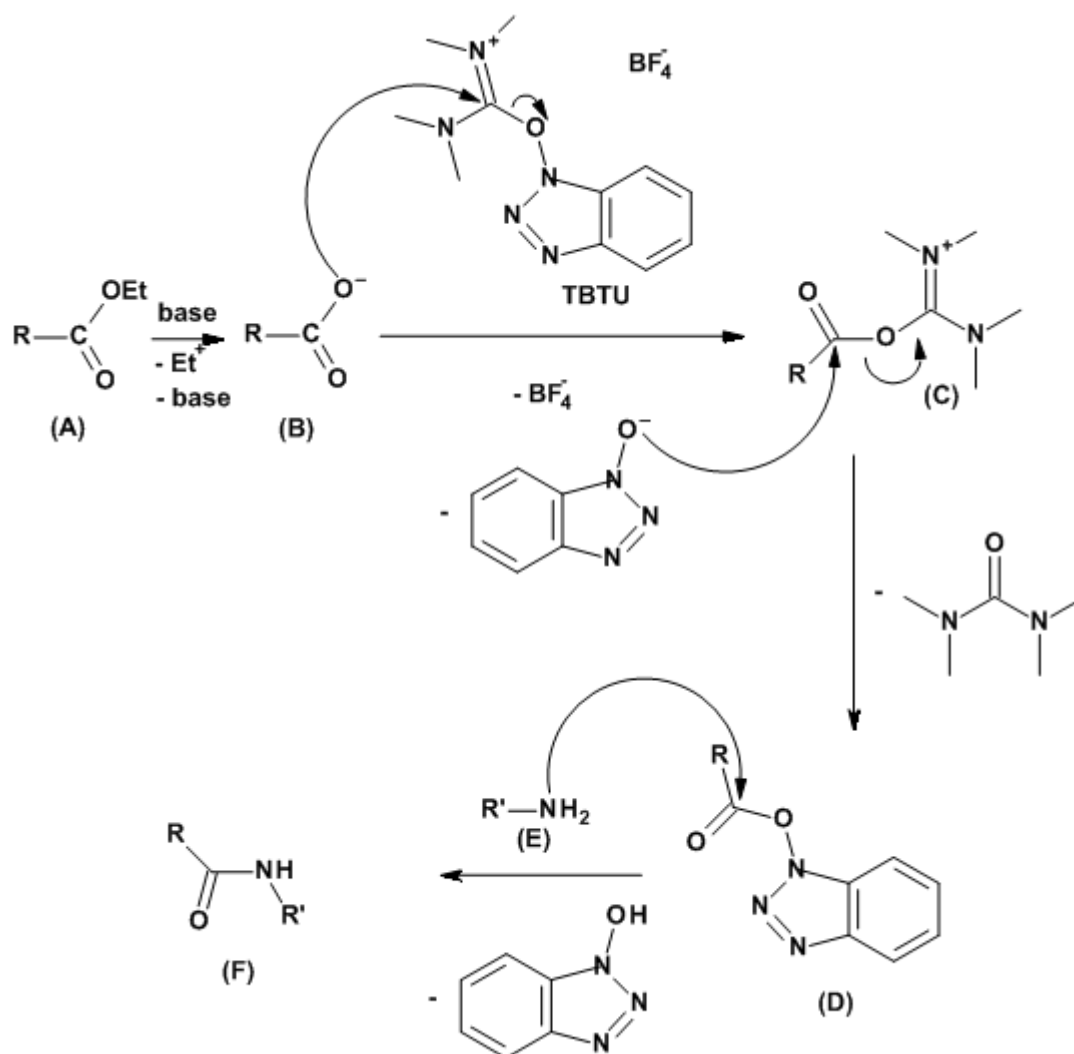


Fig. 25: Reaction mechanism for the preparation of the amide (Fig. 23-d) from the ester (Fig. 23-c)⁷⁰

2.1.2 Characterization

The characterization of the prepared compound was carried out by ^1H - and $^{13}\text{C}\{^1\text{H}\}$ -NMR spectroscopy in d_6 -DMSO, d_4 -MeOH and CDCl_3 .

^1H -NMR spectroscopy

The -NH- of the amides were found around 10 ppm (singlet). Signals with chemical shift of 9 ppm were assigned to the -OH group of the pyridone. The appearance of this peak in NMR spectra recorded in d_6 -DMSO is a clear indication that the deprotection was successful. The signals of the aromatic protons of the benzyl protection group can be found at approximately 6.9 and 7.5 ppm. In some cases they overlapped with aromatic protons of the respective amide, e.g. 2d (Fig. 26). The protons of the pyridone ring can be found at approximately 7.3, 7.2 and 7.0 ppm, with the H in meta position showing up as a dd. The two protons of the O- CH_2 - group of the protection group are detected at around 5 ppm. Lack of these peaks is another indicator for a successful deprotection. The $\text{N}_{\text{pyr}}\text{-CH}_2$ - group is found at 4.7 ppm. The signals of the aliphatic components of the amide moiety appear high field < 4 ppm.

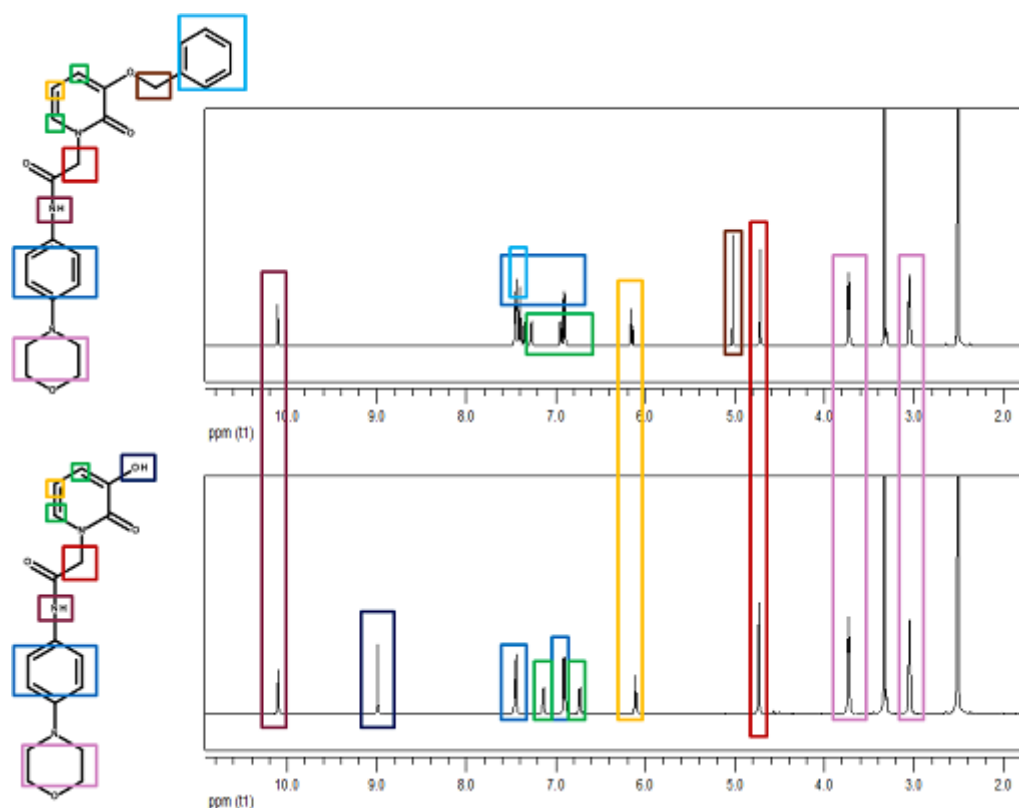


Fig. 26: Comparison of ^1H NMR spectra: 2d (protected) and 2e (unprotected)

$^{13}\text{C}\{^1\text{H}\}$ -NMR spectroscopy

The resonances of the two C=O groups appear at around 170 and 160 ppm, followed by the quaternary groups at approximately 129 ppm and at higher field the $-\text{CH}_2-$ groups. The signals assignable to the aromatic carbon atoms are found between 131 and 75 ppm.

2D-NMR spectroscopy

In order to assign the peaks unambiguously 2D NMR spectroscopy studies were conducted. Different 2D methods are in use, e.g. $^1\text{H}, ^1\text{H}$ -COSY, HSQC and HMBC. With $^1\text{H}, ^1\text{H}$ -COSY, i.e. Correlation Spectroscopy, we are able to identify the proton-coupling and see if they are neighboured or spin coupled. In order to assign protons bound to carbon atoms at larger structural features, the coupling of carbon atoms and protons was recorded by HMBC (Fig. 31) and HSQC. HSQC, i.e. Heteronuclear Single Quantum Coherence provides information about the coupling of carbon atoms and protons over a single bond. To collect the information on the coupling of carbon atoms and protons separated by two to four bonds HMBC, i.e. Heteronuclear Multiple Bond Coherence, can be applied.

2.2 Synthesis and Characterization of the Complexes

2.2.1 Synthesis

For the preparation of complexes 1f-11 (Fig. 27 and 28), the ligands were activated by sodium methoxide in methanol. Subsequently the dimer $[(\eta^6\text{-arene})MX(\mu\text{-X})_2]_2$ (M = Ru, Os, X = Cl, Br, I) in dichloromethane was added and the reaction mixture was stirred for a minimum of 3 h at r.t. Purification was achieved by recrystallization from diethyl ether.²⁹

Results

The yield of the chlorido complexes was around 60%. The complexation of the ligands with *p*-cymene was straight-forward while using biphenyl as arene proved to be more difficult and often did not work. When substituting the chlorido leaving group with either bromide (1f', 2f') or iodide (1f'', 2f'') the yield of the complexes was much lower.

The following ligands were used in the complexation

- 3-hydroxy-2-pyridone derived amides to yield their respective Ru(II)-arene complexes (1-4) (Fig. 27)

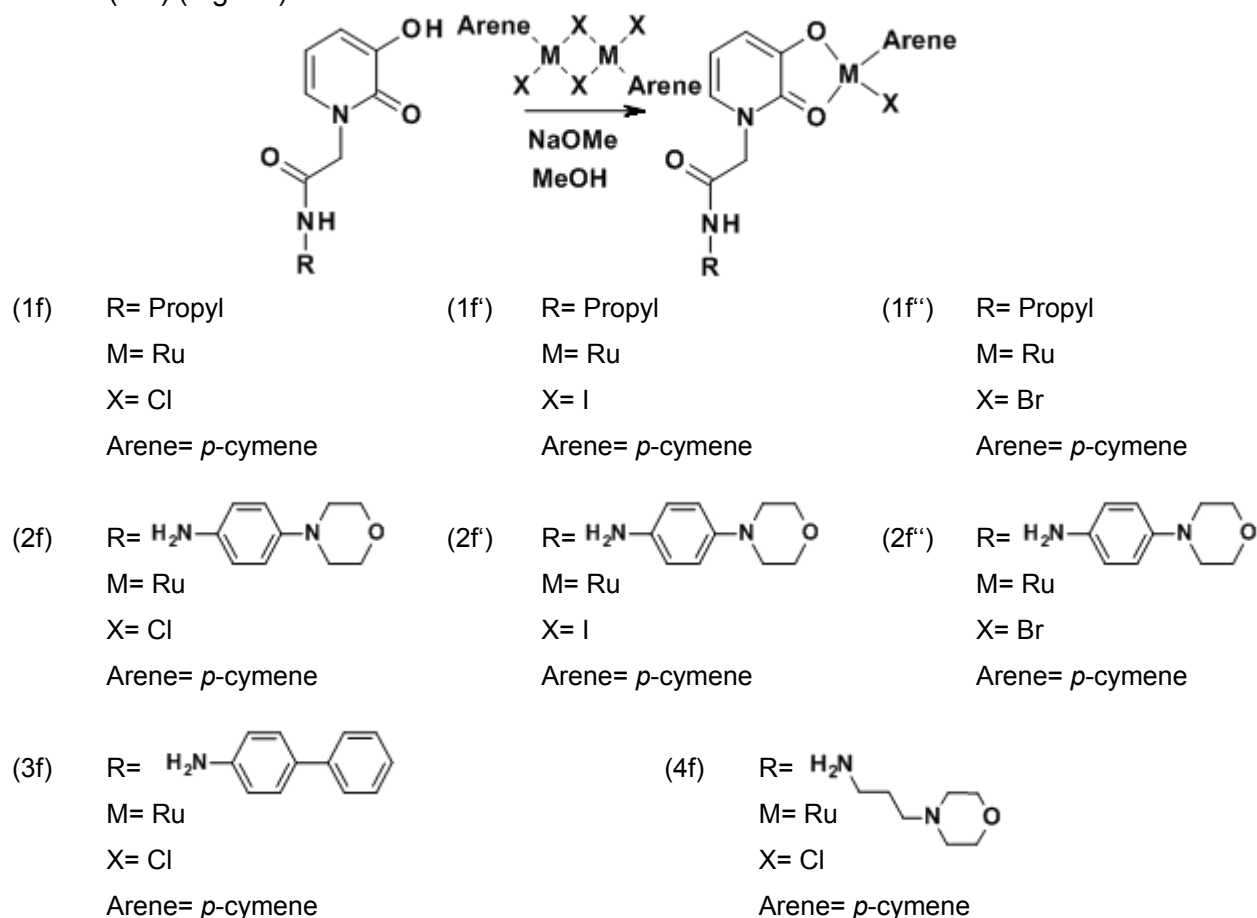


Fig. 27: Complexation of amides

- 1,2-dimethyl-3-hydroxy-4(1*H*)-(thio)pyridones, which were kindly provided by Muhammad Hanif, to yield Ru(II)- and Os(II)-arene complexes (5-11) (Fig. 28)

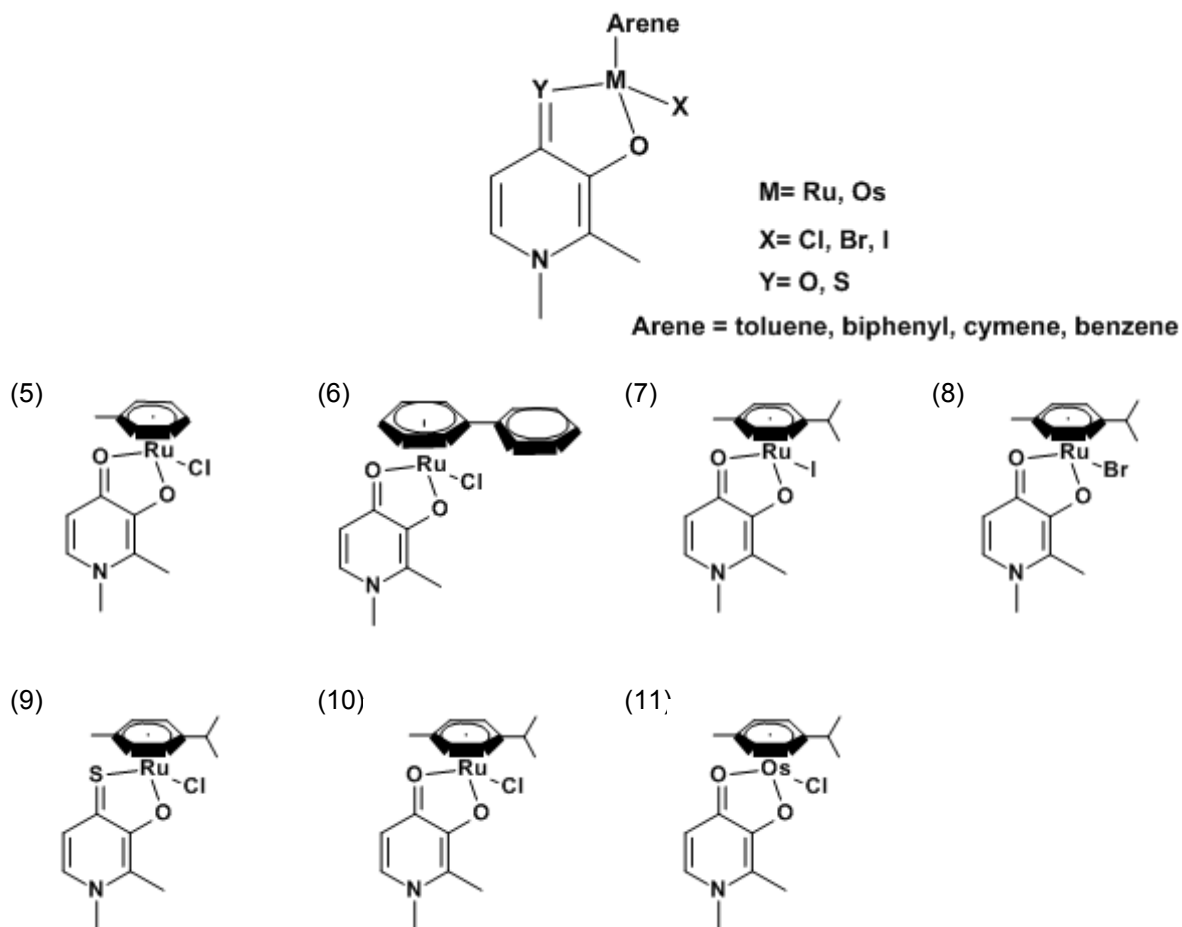


Fig. 28: 1,2-Dimethyl-3-hydroxy-4(1*H*)-(thio)pyridone-M(II)arene complexes

2.2.2 Characterization

The characterization was carried out by ^1H - and $^{13}\text{C}\{^1\text{H}\}$ -NMR spectroscopy, melting point and elemental analysis. $\text{d}_4\text{-MeOH}$ and CDCl_3 were used as solvents because the ruthenium complexes decomposed slower than in $\text{d}_6\text{-DMSO}$.

When measuring the melting points most of the complexes decomposed at temperatures $>200^\circ\text{C}$. The elemental analysis data collected for the complexes were in general in good agreement for the theoretical values. The only exception was a deviating oxygen value in 2f'. The NMR spectra of 2f and 2f' showed that the protons in the $\text{N-CH}_2\text{-CO}$ group were non-equivalent and were found at ca 5.22 and 4.35 ppm. For the complexes with the iodide

leaving group the NMR showed hindered rotation of the *p*-cymene probably due to the size of the iodide.

The spectra of 1f, 1f', 2f, 2f', 3f and 8 showed impurities due to traces of ligand still being present.

The complexes with the 1,2-dimethyl-3-hydroxy-4(1*H*)-(thio)pyridone ligands were difficult to purify. While using the same recrystallization step as with all the other complexes resulted in the precipitation of the desired product, the complexes were not sufficiently soluble in solvents such as CDCl₃ and d₄-MeOH. Efficient characterization by NMR spectroscopy was not achievable, even though the elemental analysis confirmed the expected composition of the compound. For 7 the values found for C, N and H in elemental analysis were out of the acceptable range.

¹H-NMR spectroscopy

The spectra of the ligands and the respective complexes are difficult to compare in terms of chemical shift because they were not recorded in the same solvent.

The aromatic protons of the cymene are found in the range of 5.3-5.6 ppm. The only exception was the iodide complex with all 4 protons non-equivalent due to the steric hindrance of the rotation. The -CH-CH₃ proton is found as a multiplet at approximately 3 ppm. The -CH-CH₃ methyl groups appear in the lower ppm range.

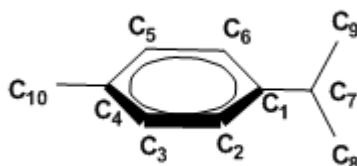


Fig. 29: Structure of the *p*-cymene moiety

¹³C{¹H}-NMR spectroscopy

Chemical shifts of the coordinated ligands are similar to those found in the spectra for the free ligands. The signals of 2f, which are representative of the cymene signals, are detected as follows: the -CH- groups are found at 80 (C₂/C₆), 78 (C₃/C₅) and 31 ppm (C₇). The -CH₃ groups are found at 22 ppm (C₈/C₉) and 19 ppm (C₁₀). The two quaternary carbon atoms are detected at 99 (C₁) and 96 ppm (C₄) (Fig. 29).

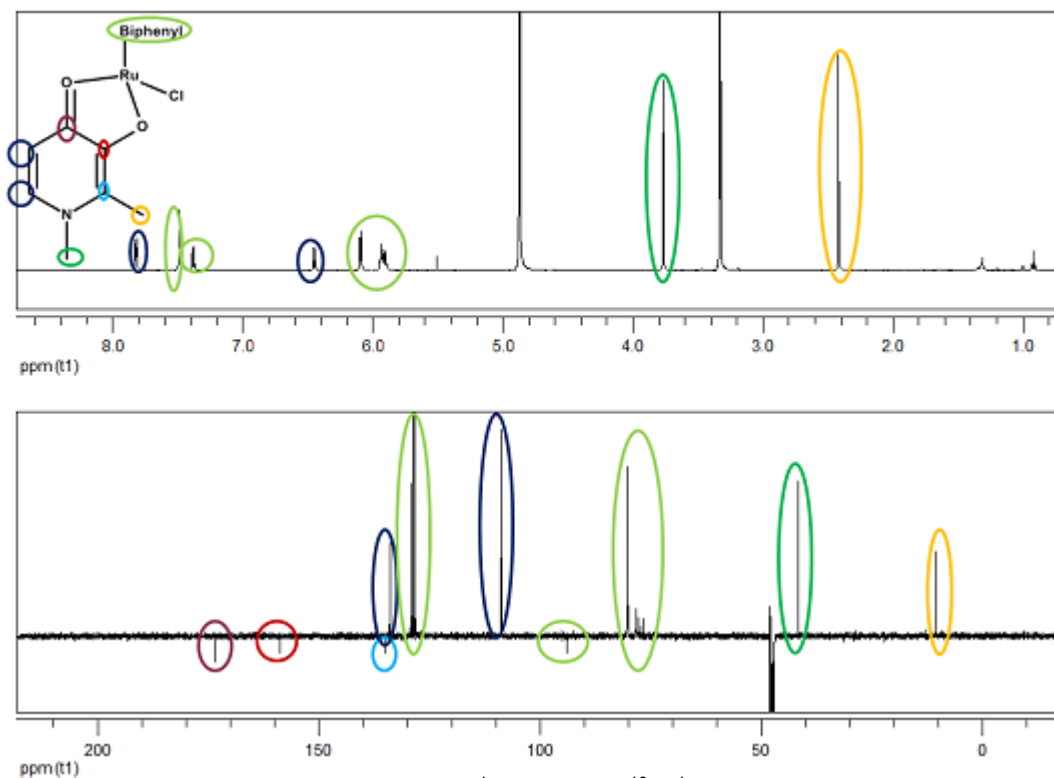


Fig. 30: Spectra of 6: ^1H (top) and $^{13}\text{C}\{^1\text{H}\}$ (bottom)

2D-NMR Spectroscopy

The same methods as with the 2D NMR of the ligands were used to perform the measurements.

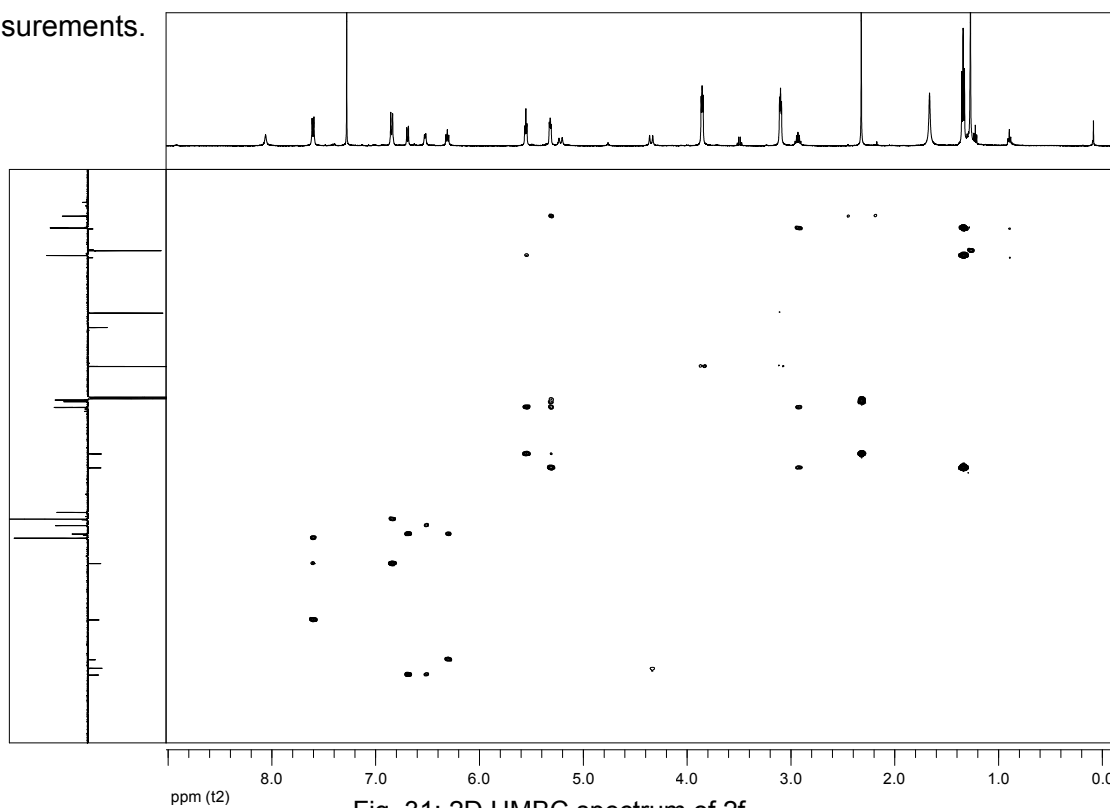


Fig. 31: 2D HMBC spectrum of 2f

2.3 Characterization overview

compound	¹ H-NMR	¹³ C{ ¹ H}-NMR	Elemental Analysis	Melting point [°C]	Yield [%]
1d	✓				87
1d'					
1e	✓			209-211	71
1f	impurities (ligand)		✓	137-142	60
1f'	✓		✓		9
1f''	impurities (ligand)		✓		10
2d	✓	✓		211-212	66
2e	✓	✓		246-247	72
2f	✓	✓	✓	decomp	64
2f'	impurities (ligand)		✓		41
2f''	impurities (ligand)		✓		35
3d	✓	✓		203-204	73
3e	✓	✓		236-237	72
3c	impurities (ligand)	noise	✓	decomp	65
4d	✓				70
4e	✓				69
4f					67
5	✓	✓			
6	✓	✓			
7	✓	✓			
8	impurities (ligand)	✓	✓		
9	✓	✓			
10	✓	✓	✓		
11	✓		✓		

3 Conclusions and Outlook

Developing new tumor inhibiting drugs is very important, due to the fact that the number of cancer patients and deaths is extremely high and steadily increasing. Compounds with different modes of action are tested in the hope to find a successful way to cure cancer. A relatively new class of compounds involves the use of hydroxypyridone ligands coordinated to metal centers, which are of great interest for the medical community as pyridones are already applied in various areas of medicine.

During the course of this diploma thesis new Ru(II)-arene complexes substituted with hydroxypyridone ligands have been synthesized and different approaches to the synthesis of this type of complexes were tested.

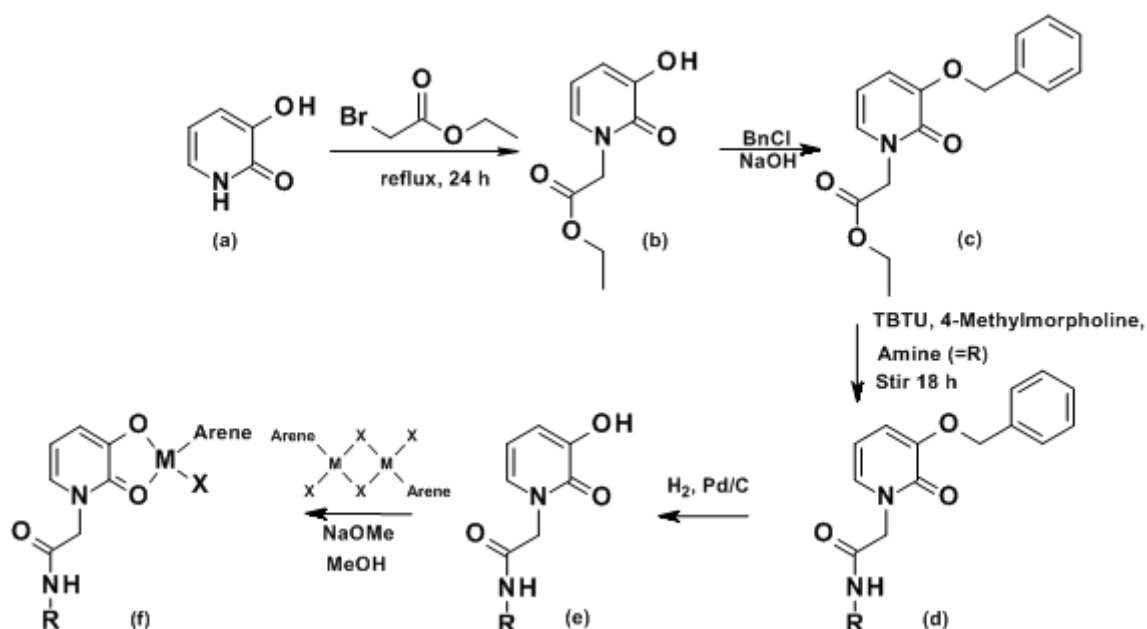


Fig. 32: General procedure for the synthesis of the complexes

First, two precursors (b) and (c) had to be synthesized in large quantities. The main part of the synthesis (Fig. 32) comprised the amide formation (d), where the amine was coupled to the hydroxypyridone backbone, followed by a deprotection step from the benzyl group (e), and the complexation of the activated ligand to ruthenium with η^6 -*p*-cymene as arene and a chlorido leaving group (f). In addition, complexes with both iodido and bromido leaving groups were prepared.

Another part of this thesis was the synthesis of 1,2-dimethyl-3-hydroxy-4(1*H*)-(thio)pyridone-M(II)arene complexes (Fig. 33). Here the arene was not only *p*-cymene and biphenyl, but also toluene and benzene. Again complexes with chlorido as well as bromido and iodo leaving groups were prepared. Additionally the oxygen of the pyridone was exchanged with sulfur resulting in thiopyridone and ruthenium was also replaced with osmium.

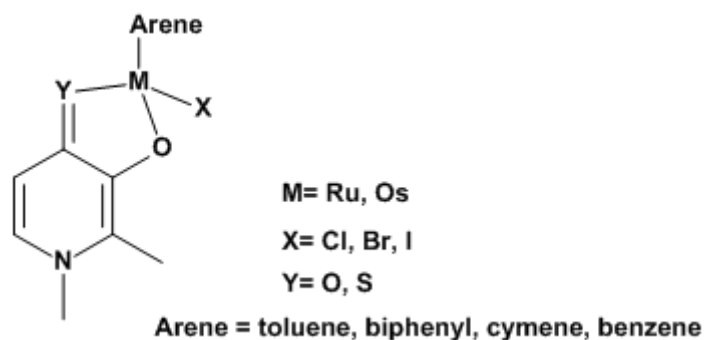


Fig 33: 1,2-Dimethyl-3-hydroxy-4(1*H*)-(thio)pyridone-M(II)arene complexes

This lead to a number of complexes with only small differences, which could be interesting for pinpointing the influences of various parts of the complexes and thus gaining more knowledge on the tumor-inhibiting effects of these compounds.

It would be of great interest to see if these types of complexes exhibit cytotoxic properties. On the synthetic level it would be good to improve isolation and purification.

4 Experimental

4.1 Materials and equipment

The chemicals were purchased at analytical grade from commercial providers. Dry solvents were produced following standard procedures. Bis[dichlorido(η^6 -*p*-cymene)ruthenium(II)], bis[diiodido(η^6 -*p*-cymene)ruthenium(II)], bis[dibromido(η^6 -*p*-cymene)ruthenium(II)], bis[dichlorido(η^6 -biphenyl)ruthenium(II)], bis[dichlorido(η^6 -toluene)ruthenium(II)], bis[dichlorido(η^6 -benzene)ruthenium(II)], 1,4-dihydrobiphenyl,⁷¹ and thiomaltol⁷² were synthesized according to literature procedures.

A Bruker FT-NMR Avance III 500 MHz spectrometer was used to record the ^1H (500.10 MHz) and $^{13}\text{C}\{^1\text{H}\}$ (125.75 MHz) as well as the 2D NMR spectra at 25 °C.

A Büchi B-540 apparatus was used to determine the melting points.

Samples for elemental analysis were submitted to the Microanalytical Laboratory, University of Vienna. These samples were measured on a Perkin–Elmer 2400 CHN Elemental Analyzer. A Bruker esquire₃₀₀₀ was used to record the electrospray ionization mass spectra.

⁷¹ M.G. Mendoza-Ferri, et al., *Organometallics* 2009, 28, 6260–6265

⁷² J.A. Lewis, et.al., *Inorg. Chem.* **2003**, 42, 7455–7459

4.2 Synthesis

4.2.1 Precursors

1-[(Ethoxycarbonyl)methyl]-3-hydroxy-2-(1H)-pyridone

A mixture of ethyl bromoacetate (83.5 g, 0.5 mol) and 3-hydroxy-2-(1H)-pyridone (11.1 g, 0.1 mol) was purged with Ar and heated until refluxation under Ar for 24 hours. Precipitation occurred when the solution was kept at 4 °C. The crude product was isolated by filtration and recrystallization from ethanol, which yielded yellow crystals. Yield: 15.2 g (77%)⁷³

3-Benzoyloxy-1-carboxymethyl-2-pyridone

Benzyl chloride (25 g, 0.2 mol) was added to a solution of 1-[(ethoxycarbonyl)methyl]-3-hydroxy-2-(1H)-pyridone (10 g, 0.05) in a mixture of methanol:water (9:1 v/v). The adjustment to pH 12 was done with a sodium hydroxide solution, which was further added during the course of the reaction to maintain a pH > 12. Refluxation over night yielded a clear orange solution, which was cooled at room temperature. Methanol was removed on the rotary evaporator. Afterwards, the product was extracted with dichloromethane to remove benzyl chloride. Addition of water to the aqueous phase and acidification with concentrated hydrochloric acid to pH 2 resulted in the precipitation of the product. The solution was cooled by further addition of water. The product was separated by filtration and recrystallization from ethanol. Yield: 10.8 g (83 %)⁷³

4.2.2 General Procedures

❖ Amide formation

Dry 4-methylmorpholine was added to a solution of 3-benzyloxy-1-carboxymethyl-2-pyridone and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in dry DMF under Ar and stirred at RT for 20 min. Subsequently the respective amine was added and the reaction occurred while stirring for another 18 hours at RT. DMF was removed under vacuum. The resulting product was dissolved in dichloromethane and purified by extraction with 5% aqueous HCl (2x), 5% aqueous NaOH (3x) and water (2x). The solvent was removed on the rotary evaporator and the crude product was recrystallized from 96% ethanol.⁷⁴

⁷³ M. Streater, et al.; *J. Med. Chem.*, 1990, 33, 1749-1755

⁷⁴ B.L. Rai, et al.; *Tetrahedron*, 1999, 55, 1129-1142

❖ Deprotection

1) The respective ligand was dissolved in an adequate solvent and 10% Pd/C was added. The solution was put into the autoclave and the apparatus was flushed with Ar and evacuated (3x). H₂ gas at a pressure of 10-15 bar was applied and the solution was stirred at RT over night. Subsequently the solution was filtered through Celite to remove Pd/C and the solvent was removed by rotary evaporation. Recrystallization from 96% ethanol gave the pure product.

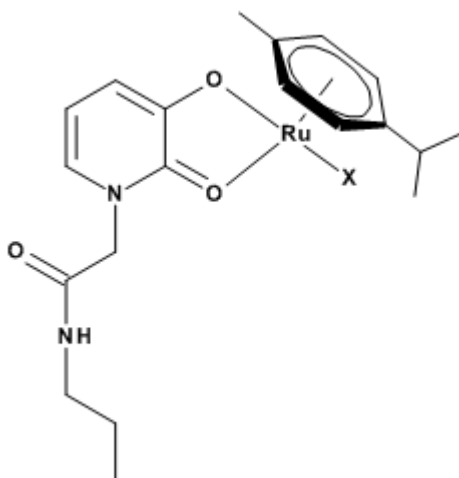
2) The respective ligand was dissolved in HCl : CH₃COOH = 1:1 and stirred at r.t. for 3-4 days. The mixture of HCl and acetic acid was removed by rotary evaporation and the residue was washed with methanol and put on the rotary evaporator to remove acetic acid (3x). Recrystallisation from ethanol gave the pure product.

❖ Complexation

The ligands were activated by sodium methoxide in methanol. Subsequently the dimer $[(\eta^6\text{-arene})MX(\mu\text{-X})_2]$ (M = Ru, Os, X = Cl, Br, I) was dissolved in dichloromethane and added in one portion. The reaction mixture was stirred at RT for a minimum of 3 hours. In some cases the solution had to be stirred at 60 °C for a minimum of 4 h. Afterwards the solvent was removed by rotary evaporation and put on high vacuum for further drying. Then the product was dissolved in dichloromethane, filtered and washed with dichloromethane to remove NaOMe. The solution was concentrated on the rotary evaporator. Finally diethyl ether was added until precipitation occurred.²⁹

4.2.3 Amides

[Halido(η^6 -*p*-cymene){*N*-[(1-propylamide)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 1



❖ Amide formation

N-[(1-Propylamide)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone – 1d

The reaction was performed according to the general procedure, using 3-benzyloxy-1-carboxymethylpyrid-2-one (4.00 g, 15.4 mmol), TBTU (5.28 g, 16.4 mmol), 4-methylmorpholine (3.12 g, 30.8 mmol) and propylamine (0.89 g, 6.0 mmol).

Yield: 1.10 g (87%);

^1H NMR (500.10 MHz, d_4 -MeOH): δ = 7.48 (d, 2H, $^3J(\text{H,H})=7.3\text{Hz}$, $\text{H}_{\text{Benzene}}$), 7.38 (dd, 1H, $^3J(\text{H,H})=7.4\text{Hz}$, $\text{H}_{\text{Benzene}}$), 7.32 (dd, 1H, $^3J(\text{H,H})=7.3\text{Hz}$, $\text{H}_{\text{Benzene}}$), 7.18 (dd, 1H, $^3J(\text{H,H})=1.6\text{Hz}$, $^3J(\text{H,H})=6.9\text{Hz}$, $\text{H}_{\text{Pyridone}}$), 6.99 (dd, 1H, $^3J(\text{H,H})=1.5\text{Hz}$, $^3J(\text{H,H})=7.6\text{Hz}$, $\text{H}_{\text{Pyridone,meta}}$), 6.28 (m, 1H, $\text{H}_{\text{Pyridone}}$), 5.12 (s, 1H, O-CH₂), 4.66 (s, 2H, N-CH₂), 3.19 (t, 2H, $^3J(\text{H,H})=7.1\text{Hz}$, CH₂, amide moiety), 1.54-1.59 (m, 2H, CH₂, amide moiety), 0.96 (t, 3H, $^3J(\text{H,H})=7.4\text{Hz}$, CH₃) ppm;

1d'

The reaction mixture of 3-benzyloxy-1-carboxymethyl-2-pyridone (200 mg, 0.75 mmol), propylamine (74 mg, 0.91 mmol), *N,N'*-dicyclohexylcarbodiimide (200 mg, 0.96 mmol), *N*-hydroxysuccinimide (102 mg, 0.88 mmol) and 11 ml of DMF was stirred under Ar. Diisopropylethylamine (258.5 mg, 2 mmol) was added and the solution was refluxed at 80 °C for 16 hours. The solvent was removed on the rotary evaporator and the product was recrystallized from isopropanol.

❖ Deprotection

***N*[(1-Propylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 1e**

The reaction was performed according to the general procedure – deprotection 1), using methanol, *N*[(1-propylamide)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone (1.1 g, 3.7 mmol) and 10% Pd/C (0.2 g).

Yield: 0.5 g (71%); Mp: 209 – 211 °C

¹H NMR (500.10 MHz, d₆-DMSO): δ = 8.93 (s, 1H, OH), 8.11 (t, 1H, J=5.4Hz, NH), 7.06 (d, 1H, ³J(H,H)=6.5Hz, H_{Pyridone}), 6.70 (d, 1H, ³J(H,H)=7.2Hz, H_{Pyridone}), 6.07 (t, 1H, ³J(H,H)=6.7Hz, H_{Pyridone,meta}), 4.53 (s, 2H, N-CH₂-CO), 3.04 (q, 2H, ³J(H,H)=6.5Hz, CH₂, amide moiety), 1.43 (m, 2H, CH₂, amide moiety), 0.86 (t, 3H, ³J(H,H)=7.2Hz, CH₃) ppm;

❖ Complexation

[Chlorido(η⁶-*p*-cymene){*N*[(1-propylamide)carbonylmethyl]-3-oxo-κO-2-(1*H*)-pyridonato-κO}ruthenium(II)] – 1f

The reaction was performed according to the general procedure, using *N*[(1-propylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (101 mg, 0.48 mmol), NaOMe (32 mg, 0.6 mmol) and [(η⁶-cymene)RuCl(μ-Cl)]₂ (123 mg, 0.2 mmol).

Yield: 115 mg (60%); Mp: 137 – 142 °C

¹H NMR (500.10 MHz, d₄-MeOH): δ = 8.18 (s, 1H, NH), 6.83 (m, 1H, H_{Pyridone}), 6.71 (m, 1H, H_{Pyridone}), 6.39 (m, 1H, H_{Pyridone}), 5.67 (m, 2H, H_{cymene}), 5.45 (m, 2H, H_{cymene}), 4.66 (s, 1H, N-CH₂-CO), 3.20 (m, 2H, CH₂, amide moiety), 2.89 (m, 1H, CH-CH₃, cymene), 2.27 (s, 3H, CH₃, cymene), 1.54-1.64 (m, 6H, CH-CH₃, cymene), 1.34 (m, 2H, CH₂, amide moiety), 0.97 (m, 3H, CH₃, amide moiety) ppm;

Elemental analysis, Anal.Calc. for C₂₀H₂₇O₃N₂RuCl·0.25CH₂Cl₂: C 48.53, H 5.53, N 5.59. Found: C 48.31, H 5.22, N 5.94 %.

[Iodido(η^6 -*p*-cymene){*N*-[(1-propylamide)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 1f'

The reaction was performed according to the general procedure, using *N*-[(1-propylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (70 mg, 0.33 mmol), NaOMe (24 mg, 0.45 mmol) and [$(\eta^6$ -cymene)RuI(μ -I)]₂ (120 mg, 0.15 mmol).

Yield: 15 mg (9%)

¹H NMR (500.10 MHz, d₄-MeOH): δ = 6.81 (brs, 1H, H_{Pyridone}), 6.65 (brs, 1H, H_{Pyridone}), 6.35 (brs, 1H, H_{Pyridone}), 5.69 (brs, 2H, H_{cymene}), 5.47 (brs, 2H, H_{cymene}), 4.66 (s, 1H, NH-CH₂-CO), 3.15 (s, 2H, NH-CH₂-CO), 2.92 (brs, 1H, CH-CH₃, cymene), 2.27 (s, 3H, CH₃, cymene), 1.58 (m, 2H, CH₂-CH₃), 1.34 (m, 6H, CH(CH₃)₂), 0.97 (t, J=7.0 Hz, CH₃) ppm;

Elemental analysis, Anal.Calc. for C₂₀H₂₇O₃N₂RuI*0.25CH₂Cl₂: C 41.03, H 4.68, N 4.73. Found: C 41.08, H 4.47, N 4.46 %.

[Bromido(η^6 -*p*-cymene){*N*-[(1-propylamide)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 1f''

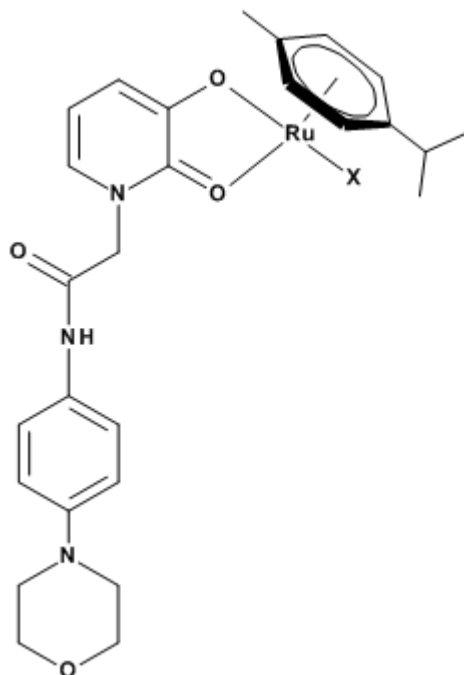
The reaction was performed according to the general procedure, using *N*-[(1-propylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (70 mg, 0.33 mmol), NaOMe (24 mg, 0.45 mmol) and [$(\eta^6$ -cymene)RuBr(μ -Br)]₂ (106 mg, 0.15 mmol).

Yield: 15 mg (10%)

¹H NMR (500.10 MHz, d₄-MeOH): δ = 6.79-6.83 (m, 1H, H_{Pyridone}), 6.60-6.70 (m, 1H, H_{Pyridone}), 6.30-6.40 (m, 1H, H_{Pyridone}), 5.62 (d, 2H, ³J(H,H)=5.4Hz, H_{cymene}), 5.41-5.42 (m, 2H, H_{cymene}), 4.66 (s, 1H, NH-CH₂-CO), 3.21-3.24 (m, 2H, CH₂, amide moiety), 2.81-2.90 (m, 1H, CH-CH₃, cymene), 2.27 (s, 3H, CH₃, cymene), 1.54-1.64 (m, 6H, -CH-CH₃, cymene), 1.33-1.37 (m, 2H, CH₂, amide moiety), 0.91-1.00 (m, 3H, CH₃, amide moiety) ppm;

Elemental analysis, Anal.Calc. for C₂₀H₂₇O₃N₂RuCl*0.25CH₂Cl₂: C 48.53, H 5.53, N 5.59. Found: C 48.31, H 5.22, N 5.94 %.

[Halido(η^6 -*p*-cymene){*N*-[(4-morpholinoaniline)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 2



❖ **Amide formation**

***N*-[(4-Morpholinoaniline)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone - 2d**

The reaction was performed according to the general procedure, using 3-benzyloxy-1-carboxymethylpyrid-2-one (2.0 g, 7.5 mmol), TBTU (2.6 g, 8 mmol), 4-methylmorpholine (1.5 g, 15 mmol) and 4-morpholinoaniline (1.4 g, 7.5 mmol).

Yield: 2.1 g (66%); Mp: 211 - 212 °C

¹H-NMR (500.10 MHz, d₆-DMSO) δ = 10.11 (s, 1H, NH), 7.39-7.46 (m, 6H, H_{benzene}), 7.27 (dd, 1H, J=1.5Hz, J=6.9Hz, H_{Pyridone}), 6.95 (dd, 1H, J=1.5Hz, J=7.5Hz, H_{Pyridone}), 6.91 (d, 2H, J=9.1Hz, H_{Benzene}), 6.15 (t, 1H, J=7.1Hz, H_{Pyridone}), 5.03 (m, 2H, O-CH₂-), 4.72 (m, 2H, N-CH₂-CO) 3.73 (m, 4H, CH₂-O), 3.05 (m, 4H, CH₂) ppm;

¹³C{¹H}-NMR (125.75 MHz, CDCl₃): δ = 165.4 (-C=O), 157.6 (-C=O), 148.3 (-CO-Bz), 147.7 (-C-N), 137.1 (-C-O-Bz), 132.0 (CH_{arom}), 131.6 (-C-N), 128.9 (CH_{arom}), 128.43 (CH_{arom}), 128.39 (CH_{arom}), 120.5 (CH_{arom}), 116.3 (CH_{arom}), 115.9 (CH_{arom}), 103.9 (CH_{arom}), 70.3 (O-CH₂-Bn), 66.6 (-CH₂O_{morph}), 52.3 (N-CH₂-CO), 49.3 (CH₂-N_{morph}) ppm;

❖ Deprotection

***N*-[(4-Morpholinoaniline)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 2e**

The reaction was performed according to the general procedure – deprotection 1), using acetic acid, *N*-[(4-morpholinoaniline)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone (2.0 g, 4.78 mmol) and 10% Pd/C (0.5 g).

Yield: 1.1 g (72%); Mp: 246 – 247 °C

¹H-NMR (500.10 MHz, d₆-DMSO), δ = 10.10 (s, 1H, NH), 8.99 (s, 1H, OH), 7.45 (d, 1H, ³J(H,H)=9.0Hz, H_{benzene}), 7.14 (dd, 1H, ³J(H,H)=1.4Hz, ³J(H,H)=6.8Hz, H_{Pyridone}), 6.91 (d, 1H, ³J(H,H)=9.0Hz, H_{benzene}), 6.74 (dd, 1H, ³J(H,H)=1.5Hz, ³J(H,H)=7.2Hz, H_{Pyridone}), 6.11 (t, 1H, ³J(H,H)=7.0 Hz, H_{Pyridone}), 4.73 (s, 2H, N-CH₂-CO), 3.73 (m, 2H, CH₂-O), 3.05 (m, 2H, CH₂-N) ppm;

¹³C{¹H}-NMR (125.75 MHz, CDCl₃): δ = 165.4 (-C=O), 158.4 (-C=O), 147.7 (-C-OH), 147.1 (-C-N), 131.5 (-C-N), 130.1 (CH_{arom}), 120.5 (CH_{arom}), 120.5 (CH_{arom}), 115.9 (CH_{arom}), 115.6 (CH_{arom}), 105.1 (CH_{arom}), 66.6 (-CH₂O_{morph}), 52.2 (N-CH₂-CO), 49.3 (-CH₂N), ppm;

❖ Complexation

[Chlorido(*η*⁶-*p*-cymene){*N*-[(4-morpholinoaniline)carbonylmethyl]-3-oxo-κO-2-(1*H*)-pyridonato-κO}ruthenium(II)] – 2f

The reaction was performed according to the general procedure, using *N*-[(4-morpholineamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (145 mg, 0.44 mmol), NaOMe (32 mg, 0.60 mmol) and [(*η*⁶-cymene)RuCl(μ-Cl)]₂ (123 mg, 0.20 mmol).

Yield: 155 mg (64%); Mp: Decomposition at 241 - 244 °C

¹H-NMR (500.10 MHz, CDCl₃): δ = 8.06 (s, 1H, NH), 7.60 (d, 1H, J=8.9Hz, H_{benzene}), 6.85 (d, 1H, ³J(H,H)=8.9Hz, H_{benzene}), 6.69 (d, 1H, ³J(H,H)=7.7Hz, H_{Pyridone}), 6.52 (d, 1H, ³J(H,H)=6.1 Hz, H_{Pyridone}), 6.31 (m, 1H, H_{Pyridone}), 5.55 (t, 2H, ³J(H,H)=5.2Hz, H_{cymene}), 5.32 (m, 2H, H_{cymene}), 5.22 (d, 1H, ³J(H,H)=15.1Hz, N-CH₂-CO), 4.35 (d, 1H, ³J(H,H)=15.1Hz, N-CH₂-CO), 3.86 (t,

4H, $^3J(\text{H,H})=7.0\text{Hz}$, O-CH₂), 3.10 (t, 4H, $^3J(\text{H,H})=7.0\text{Hz}$, N-CH₂), 2.93 (m, 1H, -CH-CH₃, cymene), 2.32 (s, 3H, CH₃, cymene), 1.34 (m, 6H, -CH-CH₃, cymene) ppm;

$^{13}\text{C}\{^1\text{H}\}$ -NMR (125.75 MHz, CDCl₃): δ = 166.1 (-C=O), 163.9 (-C=O), 161.2 (-C-O), 148.4 (-C-N), 130.3 (-C-N), 122.1 (CH_{arom}), 120.8 (CH_{arom}), 118.0 (CH_{arom}), 115.9 (CH_{arom}), 113.8 (CH_{arom}), 99.5 (-C-CH₃, cymene), 95.0 (-C-CH_{cymene}), 80.1 (-CH-_{cymene}), 80.0 (-CH-_{cymene}), 78.2 (-CH_{cymene}), 77.7 (-CH-_{cymene}), 66.9 (-CH₂-O), 54.4 (N-CH₂-CO), 49.7 (-CH₂-N), 31.2 (-CH-CH₃, cymene), 22.5 (-CH-CH₃, cymene), 22.3 (-CH-CH₃, cymene), 18.5 (-C-CH₃, cymene) ppm;

Elemental analysis, Anal.Calc. for C₂₇H₃₂O₄N₃RuCl: C 54.13, H 5.38, N 7.01. Found: C 54.18, H 5.24, N 7.04 %.

[Iodido(η^6 -*p*-cymene){*N*-[(4-morpholinoaniline)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 2f'

The reaction was performed according to the general procedure, using *N*-[(4-morpholineamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (109 mg, 0.33 mmol), NaOMe (24 mg, 0.45 mmol) and [η^6 -cymene)Ru(μ -I)]₂ (120 mg, 0.15 mmol).

Yield: 86 mg (41%)

^1H -NMR (500.10 MHz, CDCl₃): δ = 8.07 (s, 1H, NH), 7.60 (m, 1H, H_{benzene}), 6.86 (d, 1H, $J=8.5\text{Hz}$, H_{benzene}), 6.70 (t, 1H, $J=7.7\text{Hz}$, H_{Pyridone}), 6.59 (d, 1H, $J=6.2\text{Hz}$, H_{Pyridone}), 6.29-6.36 (m, 1H, H_{Pyridone}), 5.59 (m, 1H, H_{cymene}), 5.38 (d, 1H, H_{cymene}), 5.31-5.33 (m, 1H, H_{cymene}), 4.74 (s, 1H, N-CH₂-CO), 2.94 - 2.99 (m, 1H, -CH-CH₃, cymene), 2.36 (m, 3H, CH₃, cymene), 1.36 (m, 6H, -CH-CH₃, cymene) 1.28 (m, 3H, CH₃, cymene) ppm;

Elemental analysis, Anal.Calc. for C₂₇H₃₂O₄N₃Ru*0.5H₂O: C 46.36, H 4.75, N 6.01. Found: C 46.33, H 4.38, N 6.24 %.

[Bromido(η^6 -*p*-cymene){*N*-[(4-morpholinoaniline)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 2f''

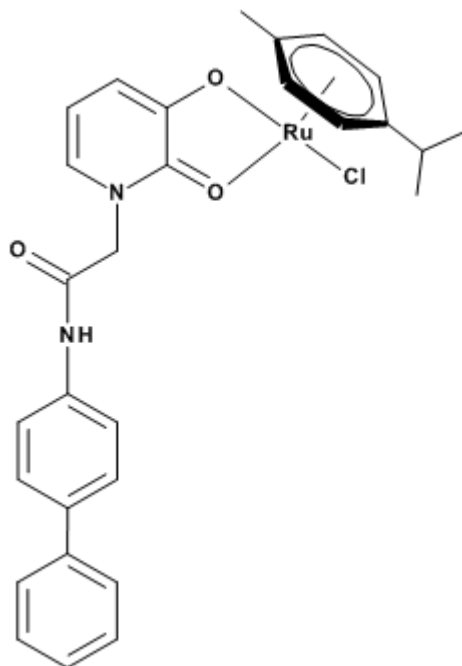
The reaction was performed according to the general procedure, using *N*-[(morpholineamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (109 mg, 0.33 mmol), NaOMe (24 mg, 0.45 mmol) and [η^6 -cymene)RuBr(μ -Br)]₂ (106 mg, 0.15 mmol).

Yield: 67 mg (35%)

¹H-NMR (500.10 MHz, CDCl₃): δ = 7.99 (s, 1H, NH), 7.59 (d, 1H, *J*=8.9Hz, H_{benzene}), 6.85 (d, 1H, ³*J*(H,H)=8.9Hz, H_{benzene}), 6.69 (d, 1H, ³*J*(H,H)=7.7Hz, H_{Pyridone}), 6.52 (d, 1H, ³*J*(H,H)=6.1Hz, H_{Pyridone}), 6.31 (m, 1H, H_{Pyridone}), 5.55 (t, 2H, ³*J*(H,H)=5.2Hz, H_{cymene}) 5.32 (dd, 2H, ³*J*(H,H)=3.6Hz, ³*J*(H,H)=5.3Hz, H_{cymene}), 5.21-5.24 (m, 1H, N-CH₂-CO), 4.29-4.32 (m, 1H, N-CH₂-CO), 2.92 - 2.95 (m, 1H, -CH-CH₃, cymene), 2.32 (s, 3H, CH₃, cymene), 1.34 (m, 6H, *J*=6.4Hz, -CH-CH₃, cymene) ppm;

Elemental analysis, Anal.Calc. for C₂₇H₃₂O₄N₃Ru*0.05 CH₂Cl₂: C 50.15, H 4.99, N 6.49. Found: C 49.86, H 4.78, N 6.81%.

[Chlorido(η^6 -*p*-cymene){*N*-[(4-phenylbenzylamide)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 3f



❖ **Amide formation**

***N*-[(4-phenylbenzylamide)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone – 3d**

The reaction was performed according to the general procedure, using 3-benzyloxy-1-carboxymethylpyrid-2-one (1.4g, 5.3 mmol), TBTU (1.8 g, 5.6 mmol), 4-methylmorpholine (1.1 g, 10.6 mmol) and 4-phenylbenzylamine (1.0 g, 5.3 mmol).

Yield: 1.6 g (73%); Mp: 203 - 204°C

$^1\text{H-NMR}$ (500.10 MHz, d_6 -DMSO): δ = 8.69 (t, 1H, $J=5.5\text{Hz}$, NH), 7.64 (m, 4H, $\text{H}_{\text{Benzene}}$), 7.3-7.5 (m, 10H, $\text{H}_{\text{Benzene}}$), 7.25 (d, 1H, $J=6.6\text{Hz}$, $\text{H}_{\text{Pyridone}}$), 6.94 (d, 1H, $J=7.5\text{Hz}$, $\text{H}_{\text{Pyridone}}$), 6.14 (t, 1H, $J=7.0\text{Hz}$, $\text{H}_{\text{Pyridone}}$), 5.02 (s, 2H, O-CH₂), 4.63 (s, 2H, N-CH₂-CO) ppm;

$^{13}\text{C}\{^1\text{H}\}$ -NMR (125.75 MHz, CDCl₃): δ = 167.4 (-C=O), 157.6 (-C=O), 148.4 (-CO-Bz), 140.4 (-C-), 139.3 (-C-), 138.9 (-C-), 137.1 (-C-), 132.0 (CH_{arom}), 129.4 (CH_{arom}), 128.9 (CH_{arom}), 128.45 (CH_{arom}), 128.36 (CH_{arom}), 127.8 (CH_{arom}), 127.1 (CH_{arom}), 116.2 (CH_{arom}), 103.9 (CH_{arom}), 70.3 (O-CH₂-), 51.8 (N-CH₂-CO) ppm;

❖ Deprotection

***N*-[(4-phenylbenzylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 3e**

The reaction was performed according to the general procedure – deprotection 2), using *N*-[(4-phenylbenzylamide)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone (1.6 g, 3.83 mmol) in HCl : CH₃COOH.

Yield: 0.2 g (72%); Mp: 236 – 237 °C

¹H-NMR (500.10 MHz, *d*₆-DMSO), δ = 9.00 (s, 1H, OH) , 8.69 (t, 1H, ³J(H,H)=5.8Hz, NH), 7.64 (m, 4H, H_{Benzene}) , 7.48 (t, 2H, J=7.7Hz, H_{Benzene}) , 7.37 (m, 3H, H_{Benzene}), 7.12 (dd, 1H, J=1.7Hz, J=6.9Hz, H_{Pyridone}) , 6.72 (dd, 1H, J=1.7Hz, J=7.2Hz, H_{Pyridone}), 6.10 (t, 1H, J=7.0Hz, H_{Pyridone}), 4.64 (s, 2H, N-CH₂-CO) ppm

¹³C{¹H}-NMR (125.75 MHz, CDCl₃): δ = 167.3 (-C=O), 158.4 (-C=O), 147.1 (-C-O), 140.4 (-C-), 139.3 (-C-), 138.9 (-C-), 130.0 (CH_{arom}), 129.4 (CH_{arom}), 128.4 (CH_{arom}), 127.8 (CH_{arom}), 127.1 (CH_{arom}), 115.4 (CH_{arom}), 105.2 (CH_{arom}), 51.8 (N-CH₂-CO) ppm

❖ Complexation

[Chlorido(η⁶-*p*-cymene){*N*-[(4-phenylbenzylamide)carbonylmethyl]-3-oxo-κO-2-(1*H*)-pyridonato-κO}ruthenium(II)] – 3f

The reaction was performed according to the general procedure, using *N*-[(4-phenylbenzylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (130 mg, 0.44 mmol), NaOMe (32 mg, 0.6 mmol) and [(η⁶-cymene)RuCl(μ-Cl)]₂ (123 mg, 0.2 mmol).

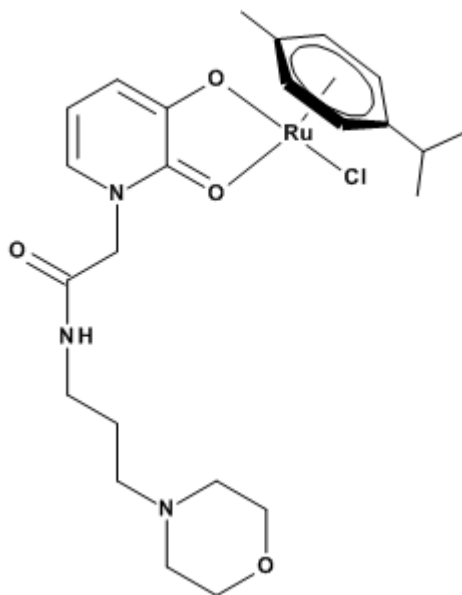
Yield: 153 mg (65%); Mp: Decomposition at 122 - 126 °C

¹H-NMR (500.10 MHz, CDCl₃): δ = 7.64 (m, 4H, J=7.8Hz, J=14.5Hz, H_{Benzene}), 7.41-7.59 (m, 9H, H_{Benzene}), 6.69 (d, 1H, J=7.4Hz, H_{Pyridone}), 6.48 (d, 1H, J=6.1Hz, H_{Pyridone}), 6.29 (dd, 1H, J=7.0Hz, J=14.4Hz, H_{Pyridone}), 5.55 (t, 2H, ³J(H,H)=5.2Hz, H_{cymene}) 5.32 (dd, 2H, ³J(H,H)=3.6Hz, ³J(H,H)=5.3Hz, H_{cymene}), 4.76 (s, 1H, N-CH₂-CO), 2.87-2.96 (m, 1H, -CH-CH₃), 2.32 (s, 3H, CH₃, cymene), 1.34 (m, 6H, -CH-CH₃, cymene) ppm;

The $^{13}\text{C}\{^1\text{H}\}$ -NMR (125.75 MHz, CDCl_3) was very noisy, because the amount of substance was very small: δ = 166.6 (-C=O), 165.8 (-C=O), 140.4 (-C-O), 136.5 (-C-), 128.7 (CH_{arom}), 127.6 (CH_{arom}), 127.1 (CH_{arom}), 120.8 (CH_{arom}), 118.0 (CH_{arom}), 114.7 (CH_{arom}), 113.8 (CH_{arom}), 107.7 (CH_{arom}), 101.3 (-C- CH_3 , *cymene*), 95.1 (-C-CH- CH_3 , *cymene*), 81.3 (-C- *cymene*), 80.5 (-C- *cymene*), 54.4 (N- CH_2 -CO), 30.5 (-CH- CH_3 , *cymene*), 22.5 (-CH- CH_3 , *cymene*), 22.3 (-CH- CH_3 , *cymene*), 18.5 (-C- CH_3 , *cymene*) ppm.

Elemental analysis, Anal.Calc. for $\text{C}_{29}\text{H}_{29}\text{O}_3\text{N}_2\text{ClRu}$: C 59.03, H 4.95, N 4.75. Found: C 58.63, H 5.04, N 4.55 %.

[Chlorido(η^6 -*p*-cymene){*N*-[(4-(3-aminopropyl)morpholine)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 4f



❖ **Amide formation**

***N*-[(4-(3-aminopropyl)morpholine)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone – 4d**

The reaction was performed according to the general procedure, using 3-benzyloxy-1-carboxymethylpyrid-2-one (2.0 g, 7.5 mmol), TBTU (2.6 g, 8 mmol), 4-methylmorpholine (1.5 g, 15 mmol) and 4-(3-aminopropyl)morpholine (1.1 g, 7.5 mmol).

Yield: 2.0 g (70%)

$^1\text{H-NMR}$ (500.10 MHz, $\text{d}_6\text{-DMSO}$): δ = 8.12 (t, 1H, $J=5.5\text{Hz}$) 1H, NH), 7.19-7.45 (m, 5H, $\text{H}_{\text{benzene}}$), 7.20 (dd, 1H, $J=1.6\text{Hz}$, $J=6.9\text{Hz}$, $\text{H}_{\text{Pyridone}}$) 6.92 (dd, 1H, $J=1.6\text{Hz}$, $J=7.5\text{Hz}$, $\text{H}_{\text{Pyridone}}$), 6.10-6.13 (m, 1H, $\text{H}_{\text{Pyridone}}$), 5.00 (s, 2H, $\text{O-CH}_2\text{-Ph}$), 4.51 (s, 2H, $\text{N-CH}_2\text{-CO}$), 3.55-3.57 (m, 4H, $\text{CH}_2\text{-O}_{\text{morph}}$), 3.10 (m, 2H, CH_2), 2.35 (brs, 4H, N-CH_2), 2.27-2.30 (m, 2H, CH_2), 1.54-1.69 (m, 2H, CH_2) ppm.

❖ Deprotection

***N*-[(4-(3-aminopropyl)morpholine)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 4e**

The reaction was performed according to the general procedure – deprotection 1), using methanol, *N*-[(4-(3-aminopropyl)morpholine)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone (2 g, 5.2 mmol) and 10% Pd/C (400 mg).

Yield: 1.070 g (69%)

¹H-NMR (500.10 MHz, d₆-DMSO): δ = 8.94 (s, 1H, OH), 8.11 (t, 1H, J=5.5Hz, NH), 7.06 (dd, 1H, J=1.7Hz, J=6.9Hz, H_{Pyridone}), 6.70 (dd, 1H, J=1.7Hz, J=7.2Hz, H_{Pyridone}), 6.07 (t, 1H, J=7.0Hz, H_{Pyridone}), 4.53 (s, 2H, N-CH₂-CO), 3.56-3.58 (m, 4H, CH₂-O_{morpholine}), 3.11 (m, 2H, CH₂), 2.29-2.30 (m, 2H, CH₂), 2.35 (brs, 4H, CH₂N_{morph}) 1.54-1.60 (m, 2H, CH₂) ppm

❖ Complexation

[Chlorido(η^6 -*p*-cymene){*N*-[(4-(3-aminopropyl)morpholine)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 4f

The reaction was performed according to the general procedure, using *N*-[(4-(3-aminopropyl)morpholine)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone (130 mg, 0.44 mmol), NaOMe (32 mg, 0.60 mmol) and [(η^6 -cymene)RuCl(μ -Cl)]₂ (123 mg, 0.20 mmol).

Yield: 151 mg (67%)

The spectra showed that the compound is not pure.

4.2.4 Variation of the Metal-arene moiety of pyridone complexes

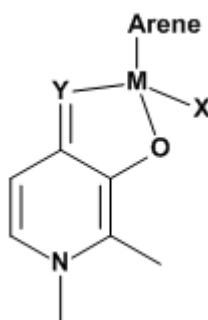


Fig. 34: General scheme for metal-arene complexes bearing 1,2-dimethyl-3-hydroxy-4-(thio)pyridone ligands

❖ Ruthenium complexes

[Ru(η^6 -toluene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 5

The reaction was performed according to the general procedure, using 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone (92 mg, 0.66 mmol), NaOMe (39 mg, 0.72 mmol) and [(η^6 -toluene)RuCl(μ -Cl)]₂ (158 mg, 0.30 mmol).

¹H-NMR (500.10 MHz, d₄-D₂O): δ = 7.40 (d, 1H, J=6.8Hz, H_{Pyridone}), 6.49 (d, 1H, J=6.8Hz, H_{Pyridone}), 5.82 (t, 2H, J=5.7Hz, H_{Toluene}), 5.60 (t, 1H, J=5.5Hz H_{Toluene}, tip), 5.42 (d, 2H, J=5.9Hz H_{Toluene}), 3.79 (s, 3H, N-CH₃), 2.48 (s, 3H, CH₃), 2.27 (s, 3H, CH₃, Toluene) ppm

¹³C{¹H}-NMR (125.75 MHz, CDCl₃): δ = 173.7 (-C=O), 158.8 (-C-O-), 134.9 (-C-CH₃, Pyridone), 134.0 (CH_{Pyridone}), 108.8 (CH_{Pyridone}), 83.6 (CH_{Toluene}), 76.1 (CH_{Toluene}), 75.4 (CH_{Toluene}), 41.7 (N-CH₃), 17.3 (CH₃, Toluene), 10.7 (CH₃, Pyridone) ppm;

[Ru(η^6 -biphenyl){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 6

The reaction was performed according to the general procedure, using 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone (92 mg, 0.66 mmol), NaOMe (39 mg, 0.72 mmol) and [(η^6 -biphenyl)RuCl(μ -Cl)]₂ (196 mg, 0.30 mmol).

¹H-NMR (500.10 MHz, d₄-MeOH): δ = 7.82 (m, 2H, H_{biphenyl}, not coordinated), 7.47-7.50 (m, 3H, H_{biphenyl}, not coordinated), 7.38 (d, 1H, J=6.8Hz, H_{Pyridone}), 6.45 (d, 1H, J=6.8Hz, H_{Pyridone}), 6.09 (d,

2H, $J=5.8\text{Hz}$ $H_{\text{biphenyl, coordinated}}$), 5.89-5.94 (m, 3H, $H_{\text{biphenyl, coordinated}}$) 3.77 (s, 3H, N-CH₃), 2.42 (s, 3H, CH₃) ppm;

$^{13}\text{C}\{^1\text{H}\}$ -NMR (125.75 MHz, CDCl₃): δ = 173.5 (-C=O), 158.7 (-C-O-), 135.1 (-C-CH₃, Pyridone), 133.9 (CH_{Pyridone}), 129.2 (CH_{Biphenyl}), 128.49 (CH_{Biphenyl}), 128.46 (CH_{Biphenyl}), 108.8 (CH_{Biphenyl}), 94.9 (CH_{Biphenyl}), 93.9 (C_{Biphenyl}), 80.3 (CH_{Biphenyl}) 78.3 (CH_{Biphenyl}) 41.6 (N-CH₃), 10.6 (CH₃, Pyridone) ppm;

[Ru(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}] – 7

The reaction was performed according to the general procedure, using 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone (92 mg, 0.66 mmol), NaOMe (39 mg, 0.72 mmol) and [(η^6 -*p*-cymene)Ru(μ -I)]₂ (293 mg, 0.30 mmol).

^1H -NMR (500.10 MHz, d₄-MeOH): δ = 7.35 (d, 1H, $J=6.8\text{Hz}$, H_{Pyridone}), 6.43 (d, 1H, $J=6.8\text{Hz}$, H_{Pyridone}), 5.61 (d, 2H, $J=6.1\text{Hz}$, H_{Cymene}), 5.42 (d, 2H, $J=6.0\text{Hz}$, H_{Cymene}), 3.77 (s, 3H, N-CH₃), 2.92 (m, 1H, CH-(CH₃)₂), 2.44 (s, 3H, CH₃), 2.34 (s, 3H, CH₃), 1.33 (s, 6H, CH₃, Cymene) ppm;

$^{13}\text{C}\{^1\text{H}\}$ -NMR (125.75 MHz, CDCl₃): δ = 174.0 (-C=O), 159.1 (-C-O-), 134.5 (-C-CH₃, Pyridone), 133.6 (CH_{Pyridone}), 106.6 (CH_{Pyridone}) 100.0 (C_{Cymene}), 94.9 (C_{Cymene}), 79.1 (CH_{Cymene}), 78.3 (CH_{Cymene}), 41.6 (N-CH₃), 31.2 (CH-CH₃, Cymene), 21.2 (-CH₃, Cymene), 18.0 (CH-(CH₃)₂, Cymene) 10.7 (C-CH₃, Pyridone) ppm;

[Ru(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Br] – 8

The reaction was performed according to the general procedure, using 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone (92 mg, 0.66 mmol), NaOMe (39 mg, 0.72 mmol) and [(η^6 -*p*-cymene)RuBr(μ -Br)]₂ (237 mg, 0.30 mmol).

^1H -NMR (500.10 MHz, CDCl₃): δ = 6.92 (d, 1H, $J=6.8\text{Hz}$, H_{Pyridone}), 6.41 (d, 1H, $J=6.8\text{Hz}$, H_{Pyridone}), 5.39-5.52 (m, 4H, H_{Cymene}), 3.62 (s, 3H, N-CH₃), 2.8(m, 1H, CH_{Cymene}) 2.44 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 1.28-1.33 (m, 6H, CH₃, Cymene) ppm;

$^{13}\text{C}\{^1\text{H}\}$ -NMR (125.75 MHz, CDCl₃): δ = 173.7 (-C=O), 159.0 (-C-O-), 134.7 (-C-CH₃, Pyridone), 133.8 (CH_{Pyridone}), 108.7 (CH_{Pyridone}), 98.8 (C_{Cymene}), 95.4 (C_{Cymene}), 79.1 (CH_{Cymene}), 77.7

(CH_{Cymene}), 41.6 (N-CH₃), 31.0 (CH-CH₃, Cymene), 21.2 (CH-CH₃, Cymene), 17.3 (CH_{Cymene}) 10.6 (C-CH₃, Pyridone) ppm;

Elemental analysis, Anal.Calc. for C₁₂H₁₁O₃ClRu: C 45.04, H 4.89, N 3.09. Found: C 44.80, H 4.67, N 3.07 %.

[Ru(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-thiopyridone}Cl] – 9

The reaction was performed according to the general procedure, using 1,2-dimethyl-3-hydroxy-4(1*H*)-thiopyridone (69 mg, 0.44 mmol), NaOMe (32 mg, 0.6 mmol) and [(η^6 -*p*-cymene)RuCl(μ -Cl)]₂ (123 mg, 0.6 mmol).

¹H-NMR (500.10 MHz, d₄-MeOH): δ = 7.51 (d, 1H, J=6.3Hz, H_{Pyridone}), 7.38 (d, 1H, J=6.2Hz, H_{Pyridone}), 5.82 (d, 1H, J=5.8Hz, H_{Cymene}), 5.72 (d, 1H, J=5.9Hz, H_{Cymene}), 5.50-5.56 (m, 2H, H_{Cymene}), 4.01 (s, 3H, N-CH₃), 2.77 (m, 1H, CH-CH₃, Cymene), 2.37 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 1.27 (d, 3H, J=6.9Hz, CH₃, Cymene), 1.15 (d, 3H, J=6.9Hz, CH₃, Cymene) ppm;

¹³C{¹H}-NMR (125.75 MHz, CDCl₃): δ = 172.4 (-C=S), 146.2 (-C-O-), 141.4 (-C-CH₃, Pyridone), 128.5 (CH_{Pyridone}), 128.3 (CH_{Pyridone}), 107.2 (C_{Cymene}), 101.8 (C_{Cymene}), 86.3 (C_{Cymene}), 84.2 (C_{Cymene}), 83.9 (CH_{Cymene}), 82.1 (CH_{Cymene}), 43.9 (N-CH₃), 30.6 (CH-CH₃, Cymene), 21.4 (CH-CH₃, Cymene), 20.9 (CH-CH₃, Cymene), 17.2 (C-CH₃, Cymene) 11.1 (C-CH₃, Pyridone) ppm;

[Ru(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 10

The reaction was performed according to the general procedure, using 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone (62 mg, 0.44 mmol), NaOMe (32 mg, 0.6 mmol) and [(η^6 -*p*-cymene)RuCl(μ -Cl)]₂ (123 mg, 0.6 mmol).

¹H-NMR (500.10 MHz, CDCl₃): δ = 7.39 (d, 1H, J=6.8Hz, H_{Pyridone}), 6.48 (d, 1H, J=6.8Hz, H_{Pyridone}), 5.66 (d, 2H, J=6.1Hz, H_{Cymene}), 5.44 (d, 2H, J=6.0Hz, H_{Cymene}), 3.78 (s, 3H, N-CH₃), 2.86 (m, 1H, CH-CH₃, Cymene), 2.47 (s, 3H, CH₃), 2.27 (s, 3H, CH₃), 1.33 (d, 6H, J=6.9Hz, CH₃, Cymene) ppm

¹³C{¹H}-NMR (125.75 MHz, CDCl₃): δ = 173.7 (-C=O), 158.9 (-C-O-), 134.8 (-C-CH₃, Pyridone), 133.8 (CH_{Pyridone}), 108.7 (CH_{Pyridone}), 98.5 (C_{Cymene}), 95.6 (C_{Cymene}), 79.2 (CH_{Cymene}), 77.5

(CH_{Cymene}), 41.6 (N-CH₃), 31.0 (CH-CH₃, Cymene), 21.1 (CH-CH₃, Cymene), 17.1 (-C-CH₃, Cymene) 10.6 (C-CH₃, Pyridone) ppm

Elemental analysis, Anal.Calc. for C₁₂H₁₁O₃ClRu: C 49.94, H 5.42, N 3.43. Found: C 49.70, H 5.19, N 3.37 %.

❖ Osmium complex

[Os(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 11

The reaction was performed according to the general procedure, using 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone (61 mg, 0.40 mmol), NaOMe (27 mg, 0.50 mmol) and [(η^6 -*p*-cymene)OsCl(μ -Cl)]₂ (158 mg, 0.20 mmol).

¹H-NMR (500.10 MHz, CDCl₃): δ = 6.99 (d, 1H, J=6.8Hz, H_{Pyridone}), 6.51 (d, 1H, J=6.8Hz, H_{Pyridone}), 6.00 (d, 1H, J=5.2Hz, H_{Cymene}), 5.93 (d, 1H, J=5.1Hz, H_{Cymene}), 5.78 (d, 1H, J=5.2Hz, H_{Cymene}), 5.73 (d, 1H, J=5.2Hz, H_{Cymene}), 3.67 (s, 3H, N-CH₃), 2.74 (m, 1H, CH-CH₃, Cymene), 2.45 (s, 3H, CH₃), 2.37 (s, 3H, CH₃), 1.24-1.27 (m, 6H, CH₃, Cymene) ppm

Elemental analysis, Anal.Calc. for C₁₇H₂₂NO₂OsCl: C 41.00, H 4.45, N: 2.81. Found: C 40.64, H 4.18, N 2.94 %.

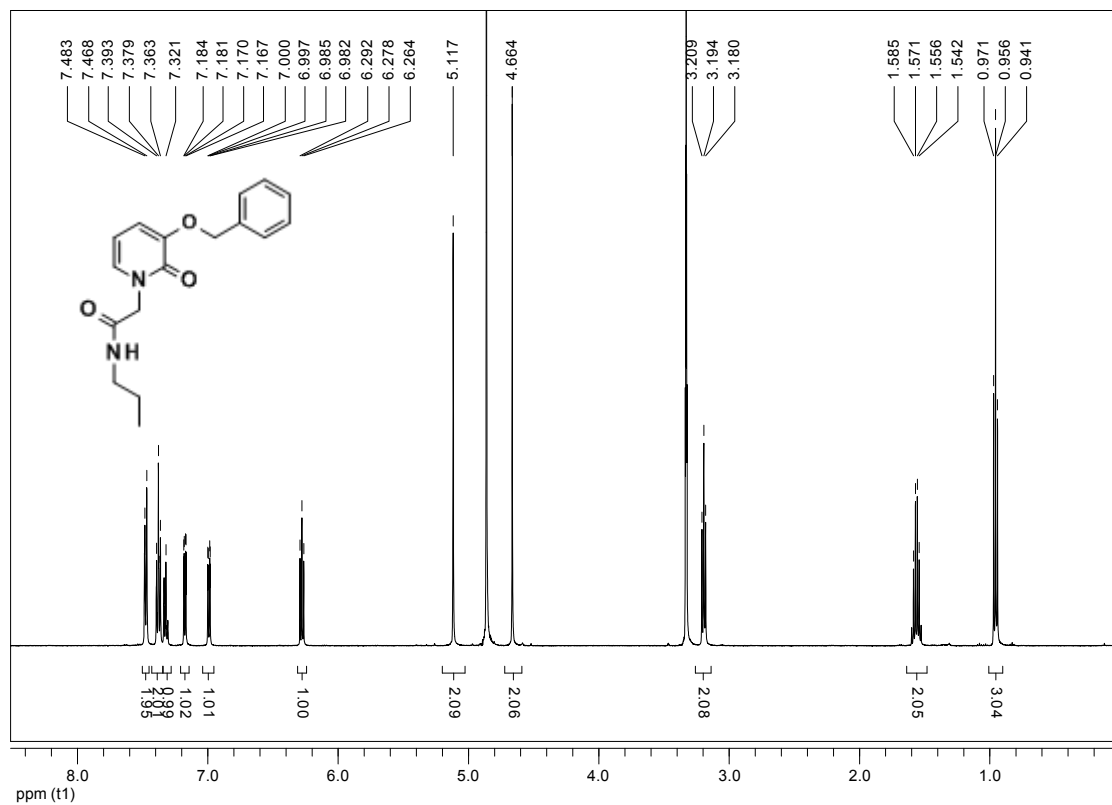
5 Supplementary Data

5.1 ^1H - and $^{13}\text{C}\{^1\text{H}\}$ - NMR spectra

5.1.1 Amides

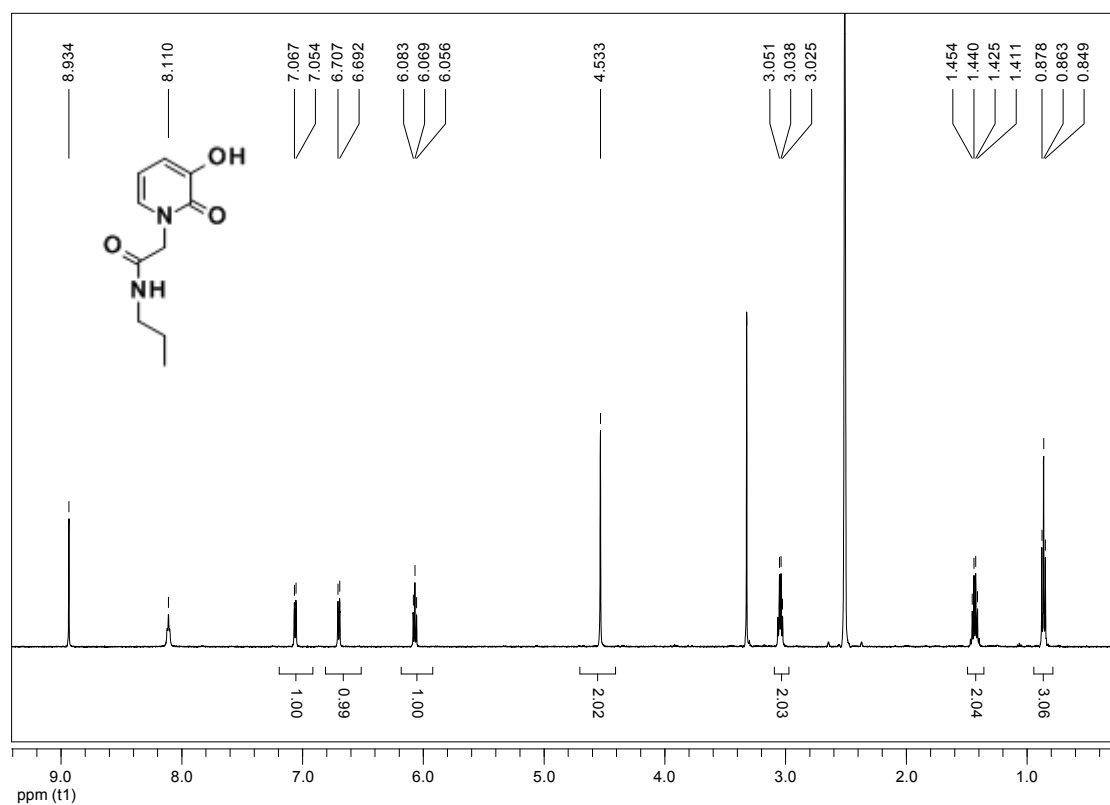
N-[(1-propylamide)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone – 1d

^1H -NMR



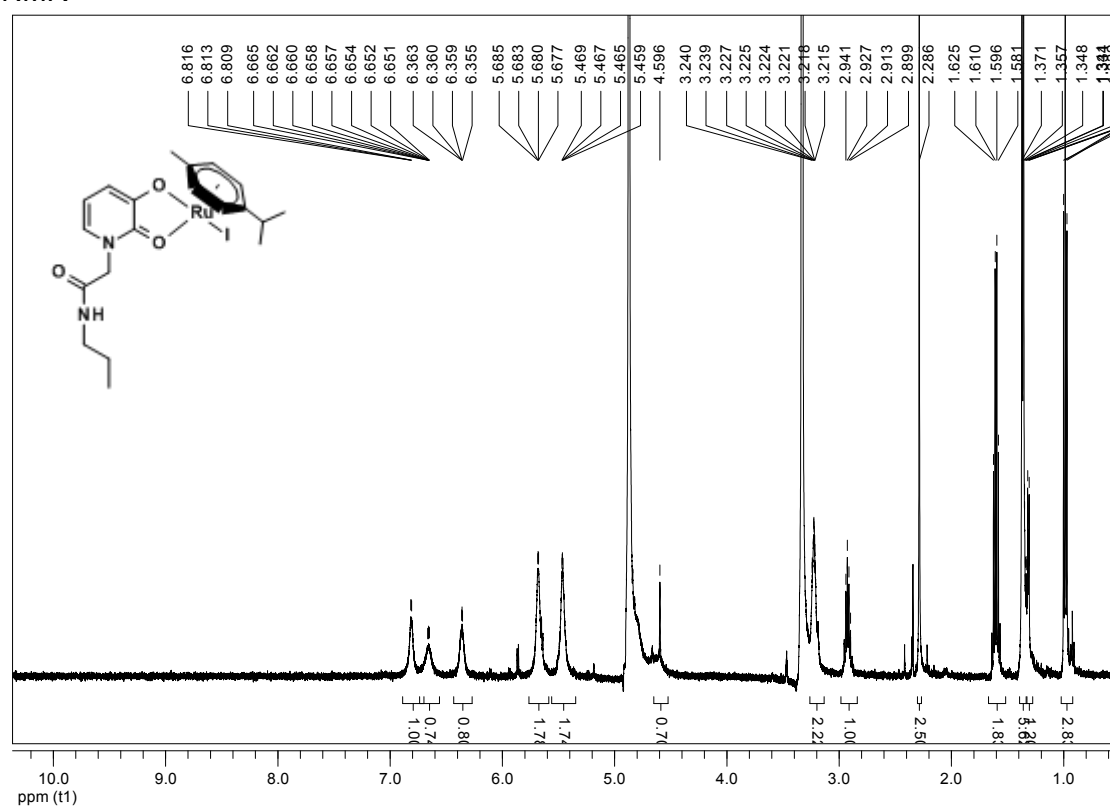
***N*-[(1-propylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 1e**

¹H-NMR



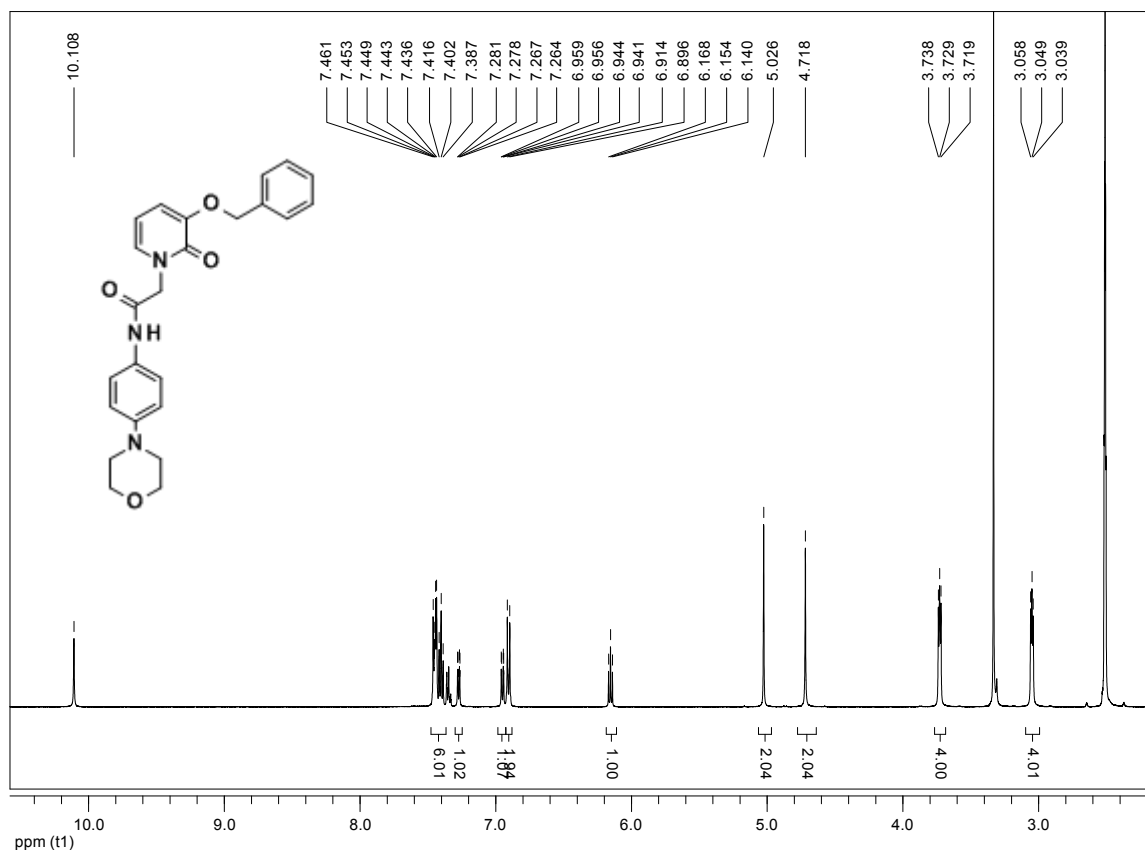
[Iodido(η^6 -*p*-cymene){*N*-[(1-propylamine)carbonylmethyl]-3-oxo- κ O-2-(1*H*)-pyridonato- κ O}ruthenium(II)] – 1f'

¹H-NMR

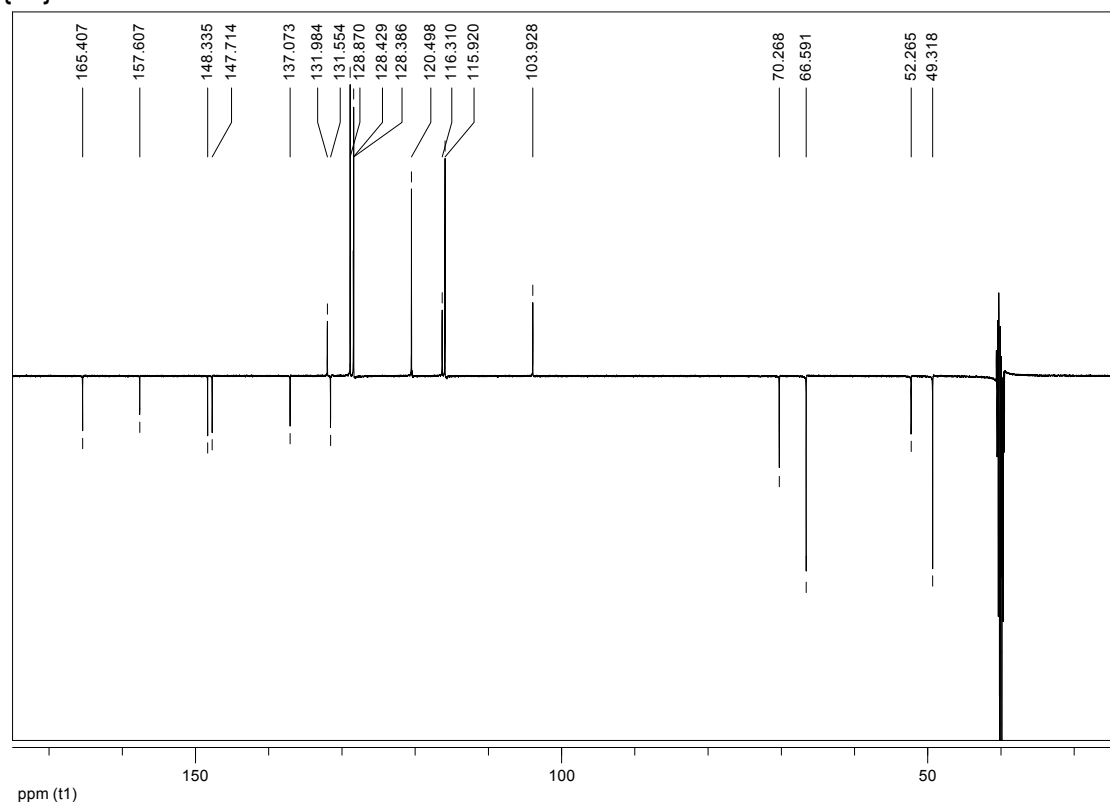


***N*-[(morpholinoaniline)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridon - 2d**

¹H-NMR

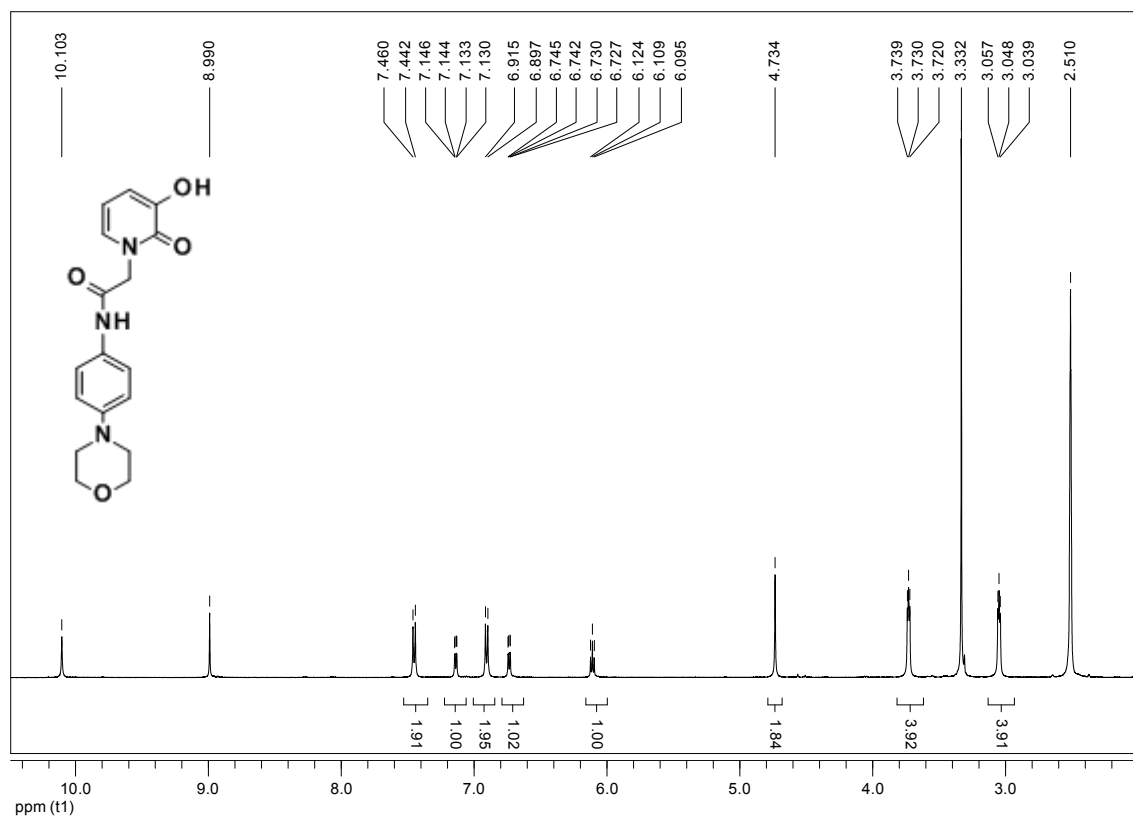


¹³C{¹H}-NMR

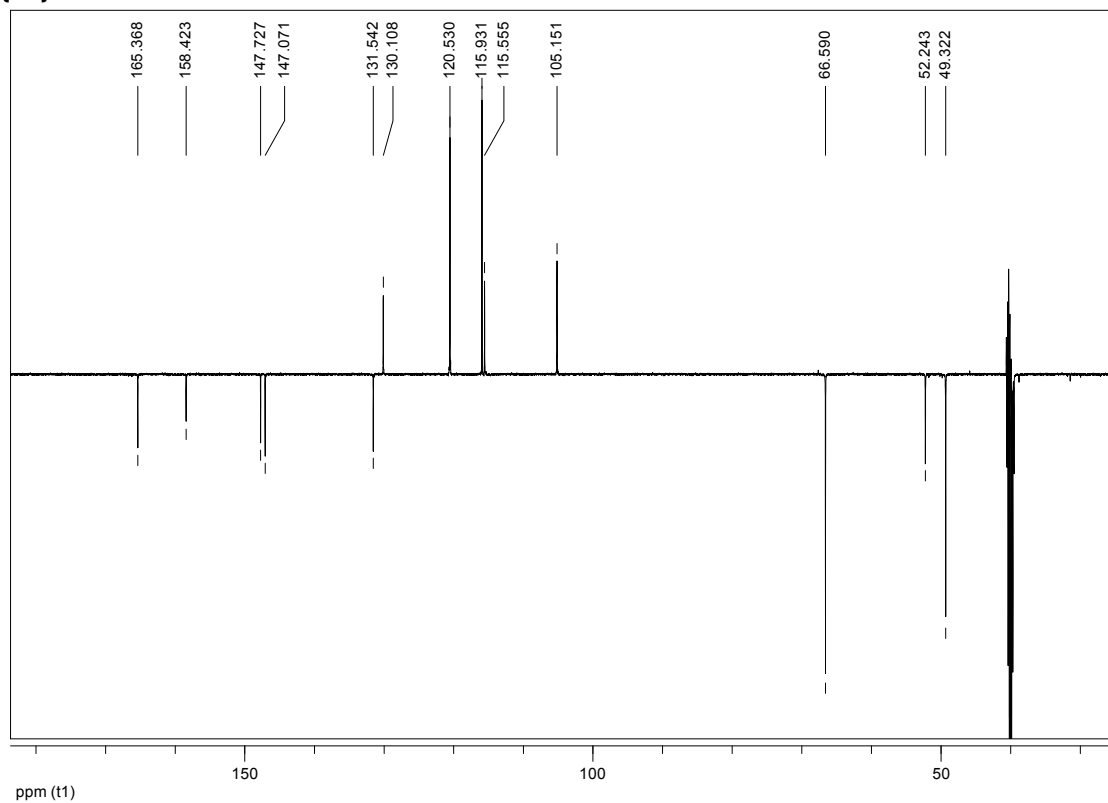


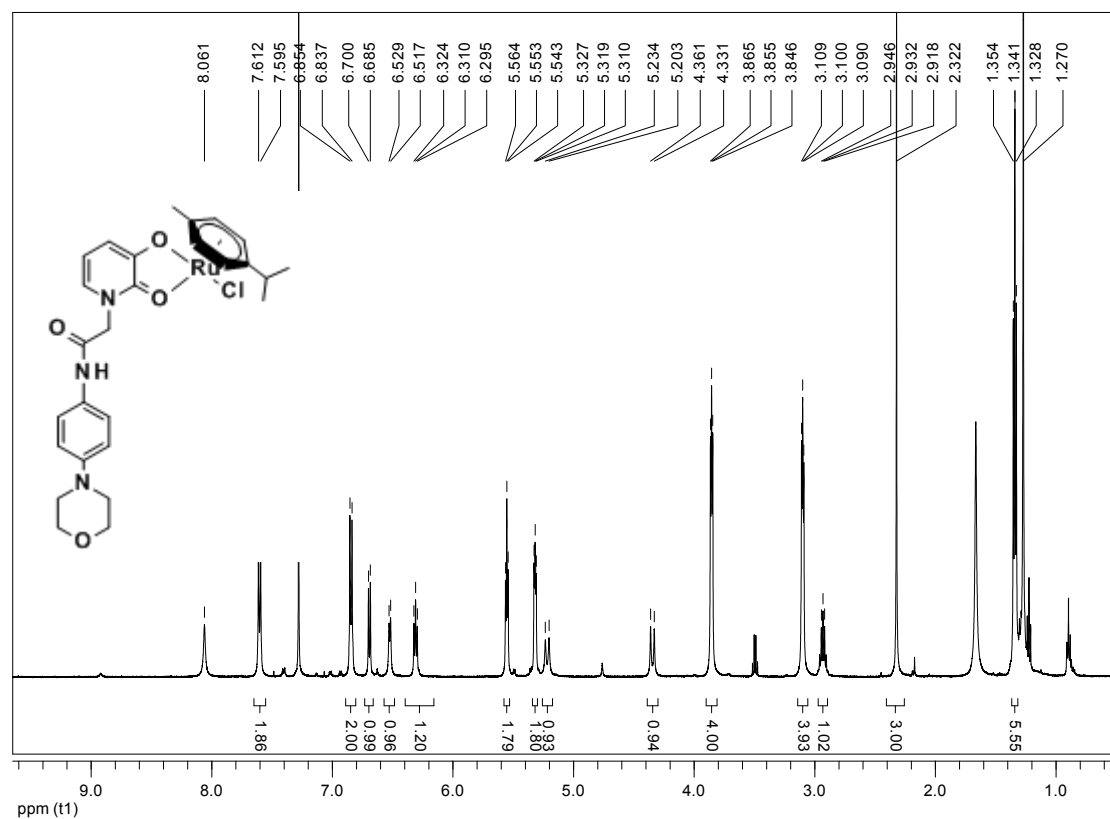
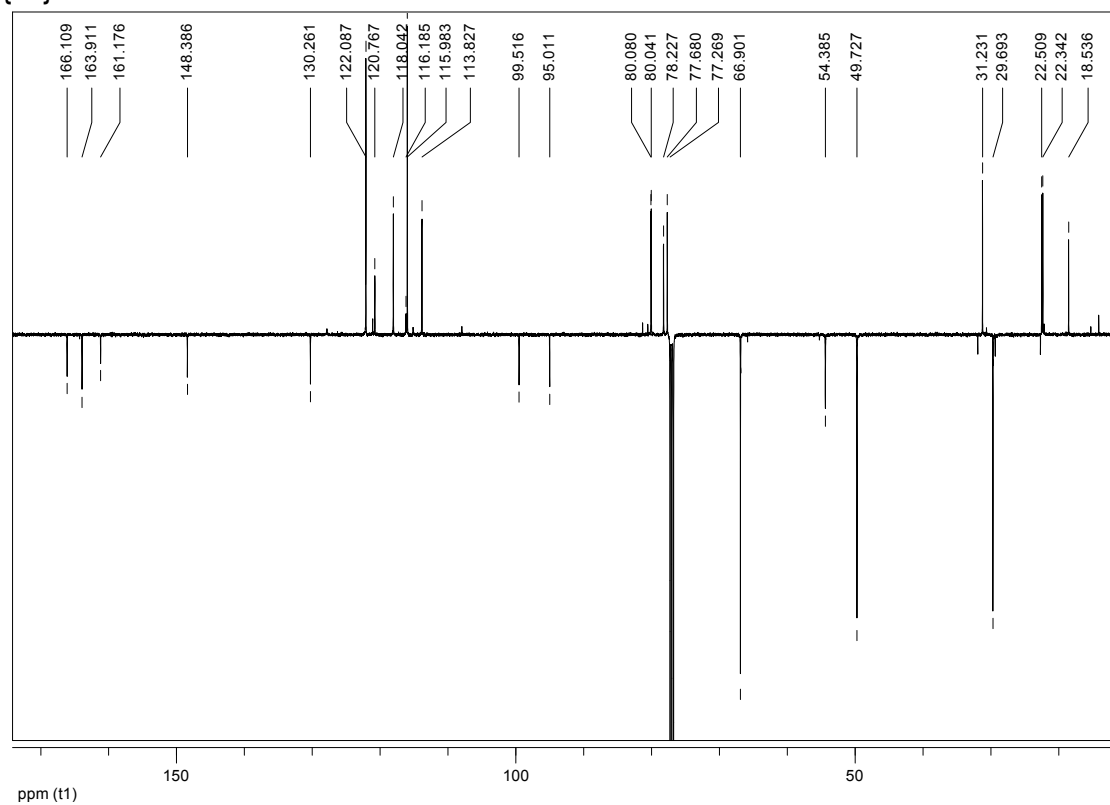
***N*-[(morpholinoaniline)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 2e**

¹H-NMR



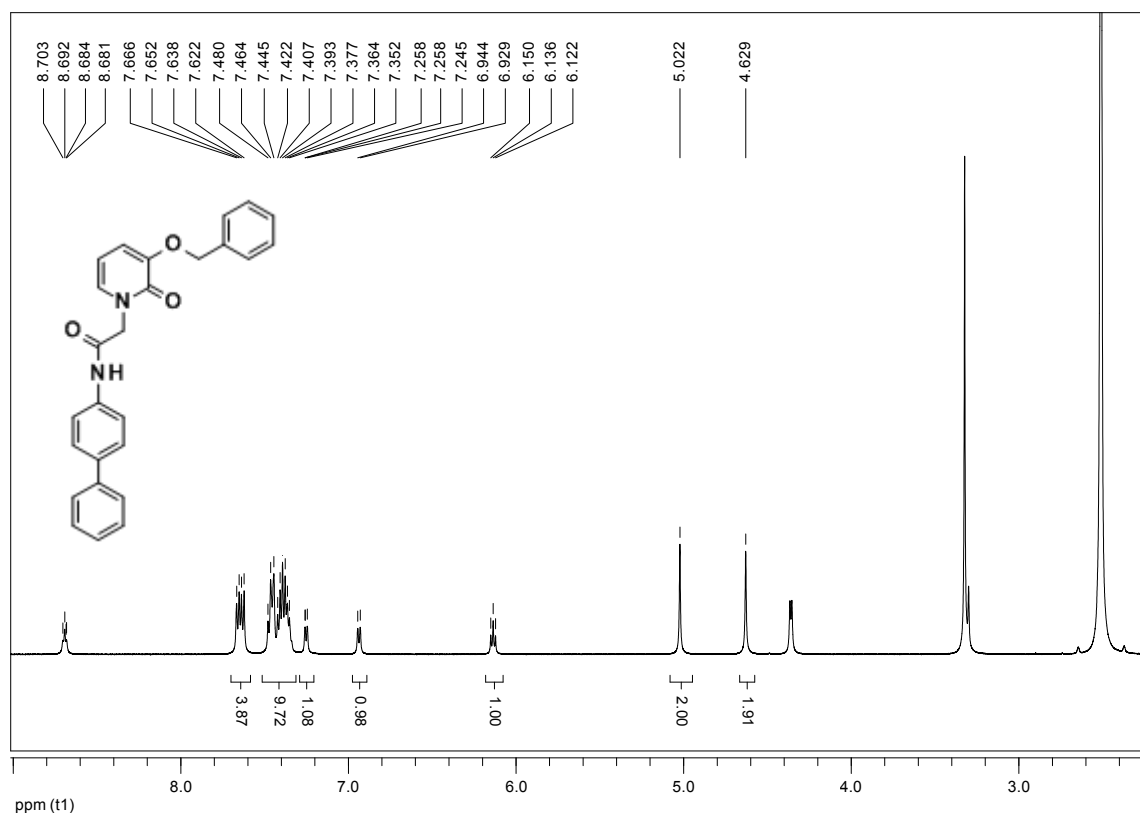
¹³C{¹H}-NMR



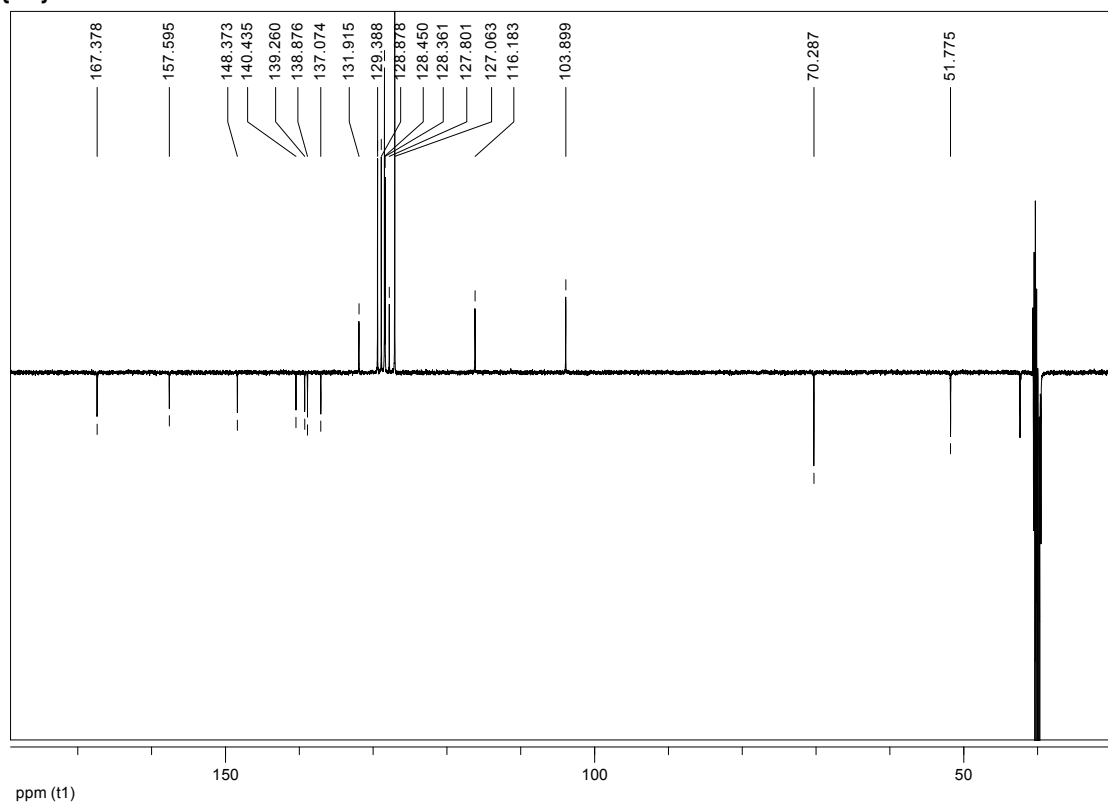
¹H-NMR $^{13}\text{C}\{^1\text{H}\}\text{-NMR}$ 

***N*-[(4-phenylbenzylamide)carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone – 3d**

¹H-NMR

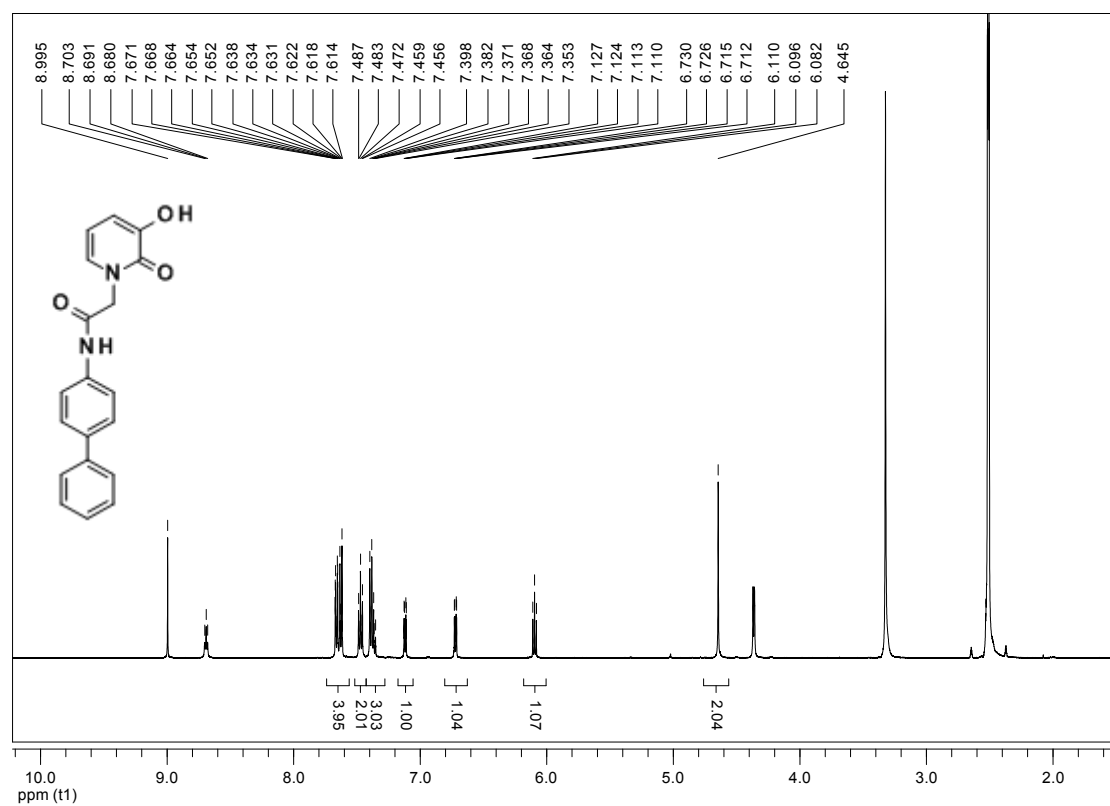


¹³C{¹H}-NMR

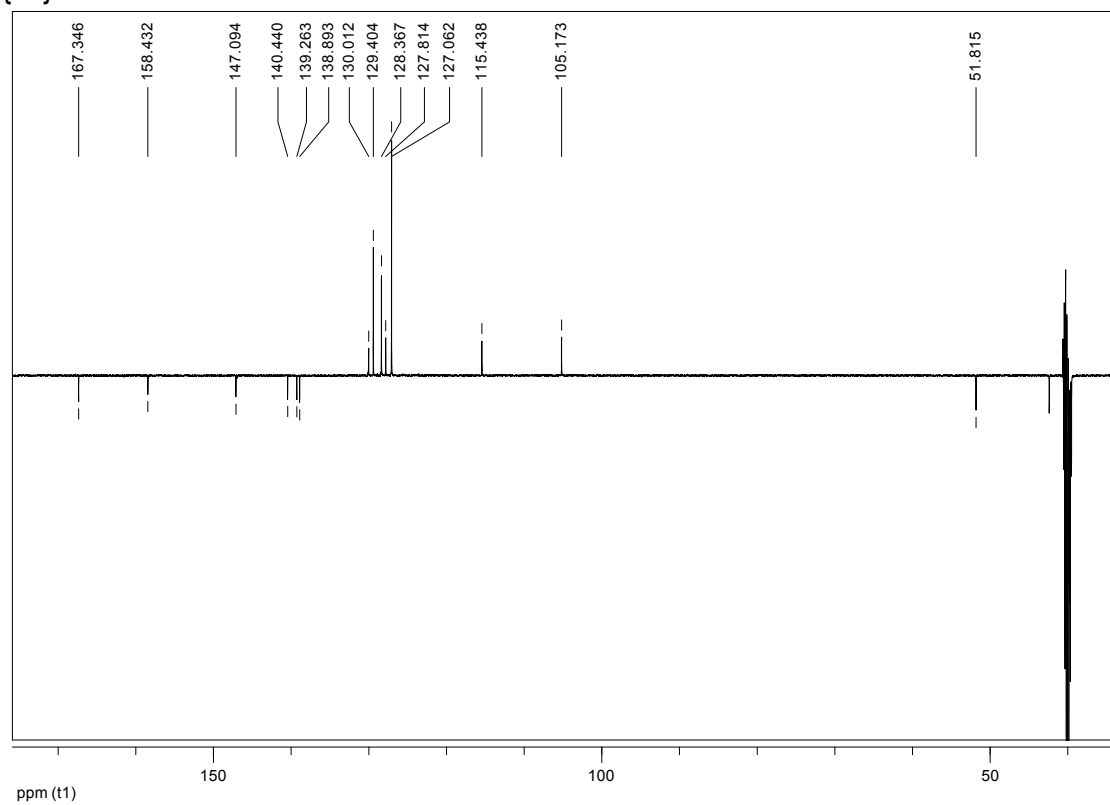


***N*-[(4-phenylbenzylamide)carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 3e**

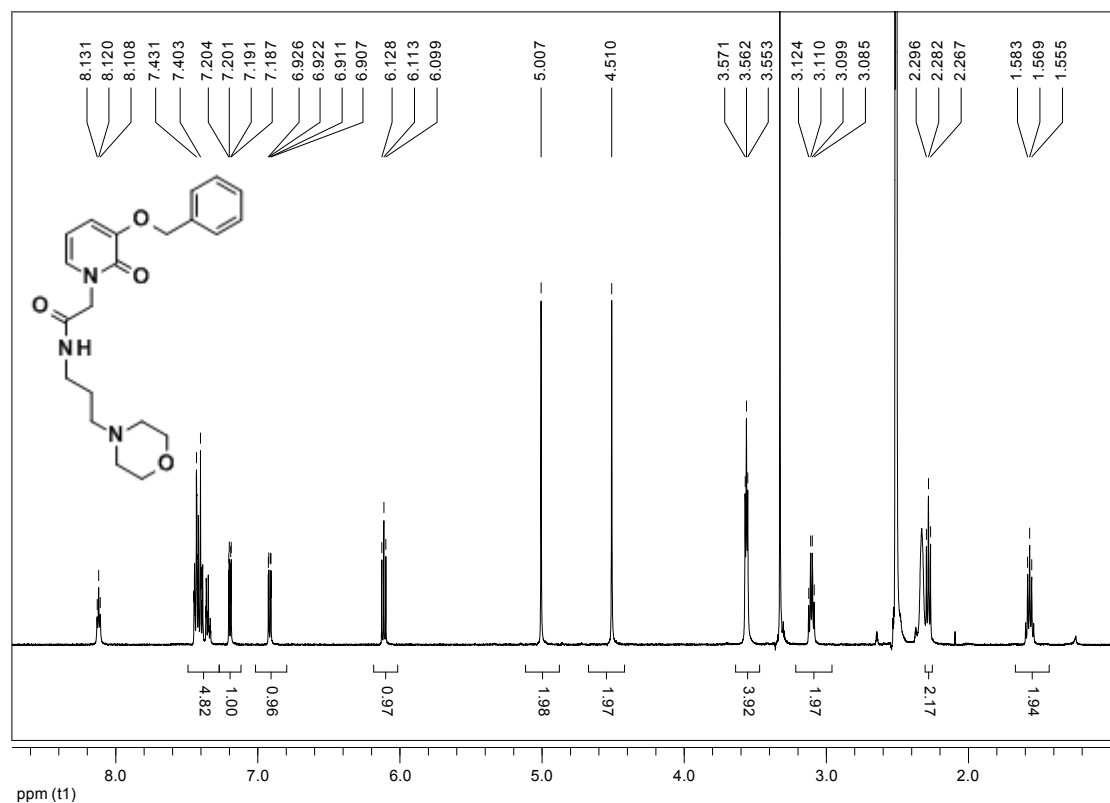
¹H-NMR



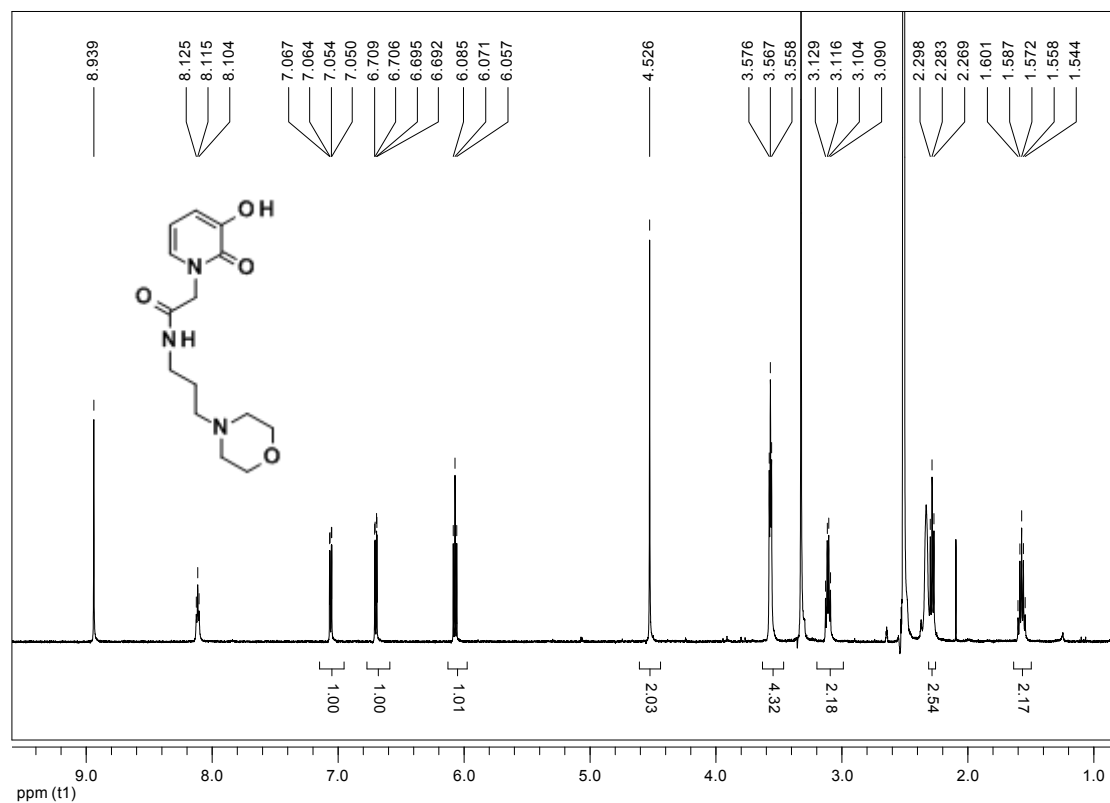
¹³C{¹H}-NMR



***N*-[4-(3-aminopropyl)morpholine]carbonylmethyl]-3-benzyloxy-2-(1*H*)-pyridone – 4d**
¹H-NMR



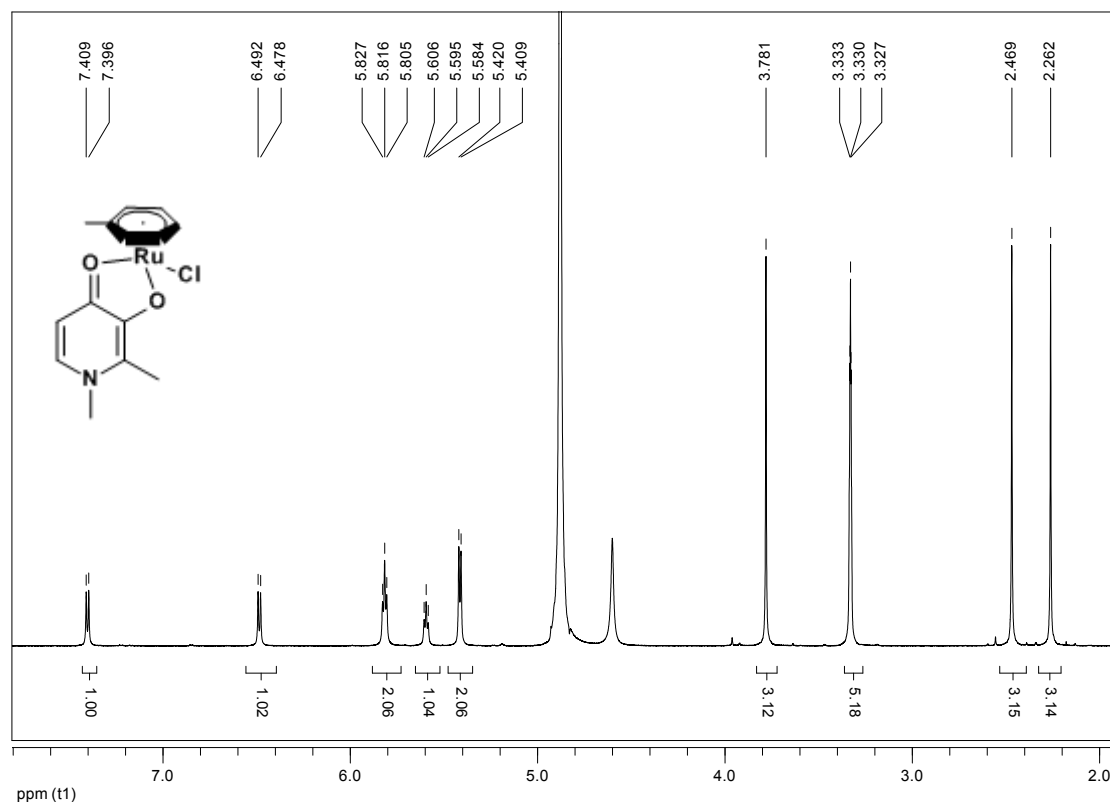
***N*-[4-(3-aminopropyl)morpholine]carbonylmethyl]-3-hydroxy-2-(1*H*)-pyridone – 4e**
¹H-NMR



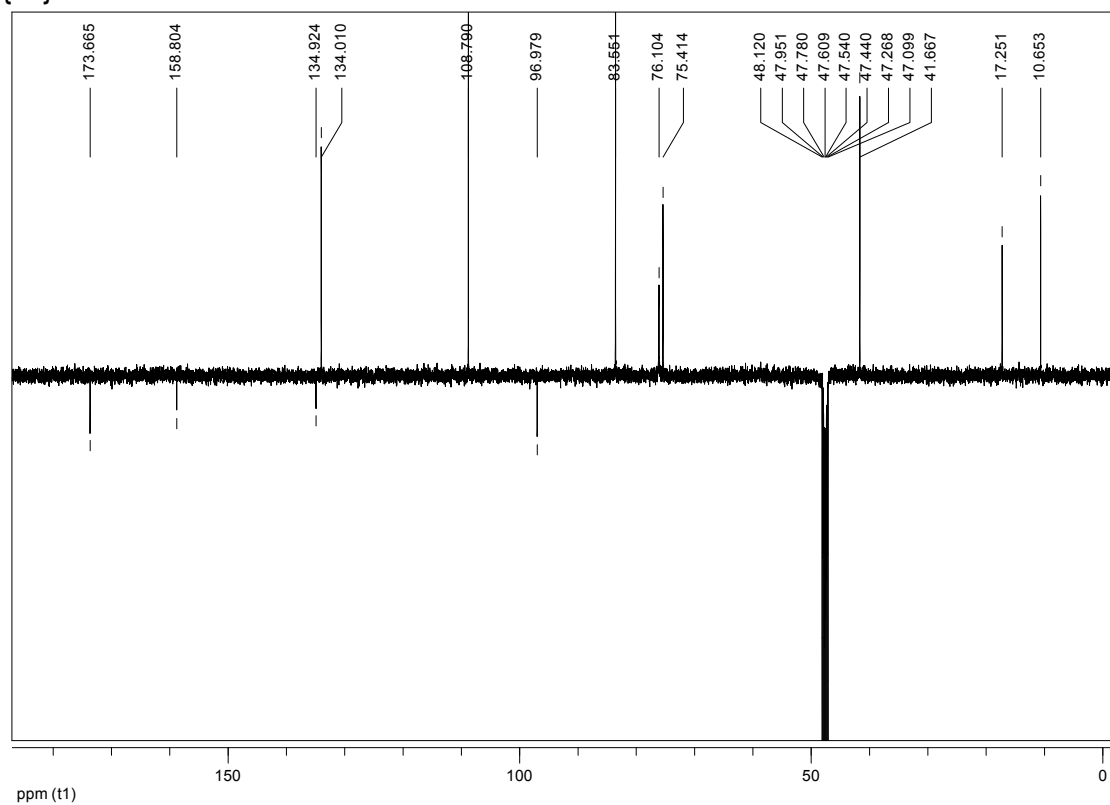
5.1.2 Variation of the Metal-arene moiety of pyridone complexes

[Ru(η^6 -toluene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 5

^1H -NMR

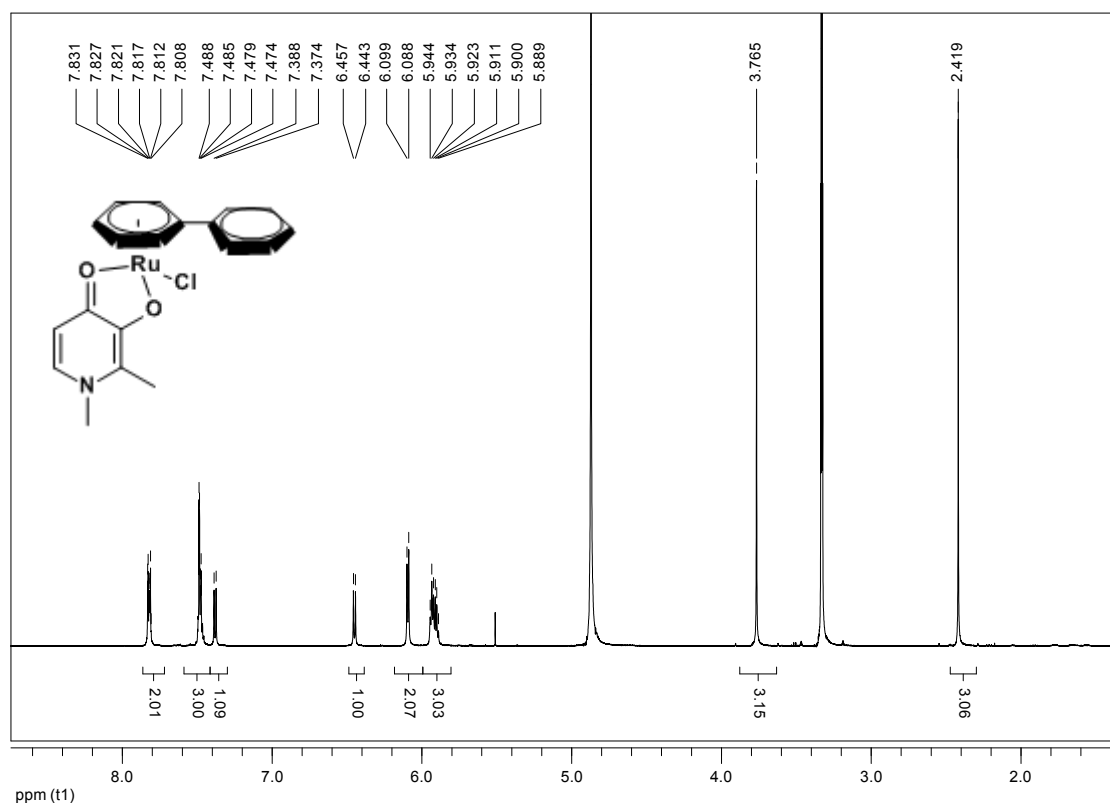


$^{13}\text{C}\{^1\text{H}\}$ -NMR

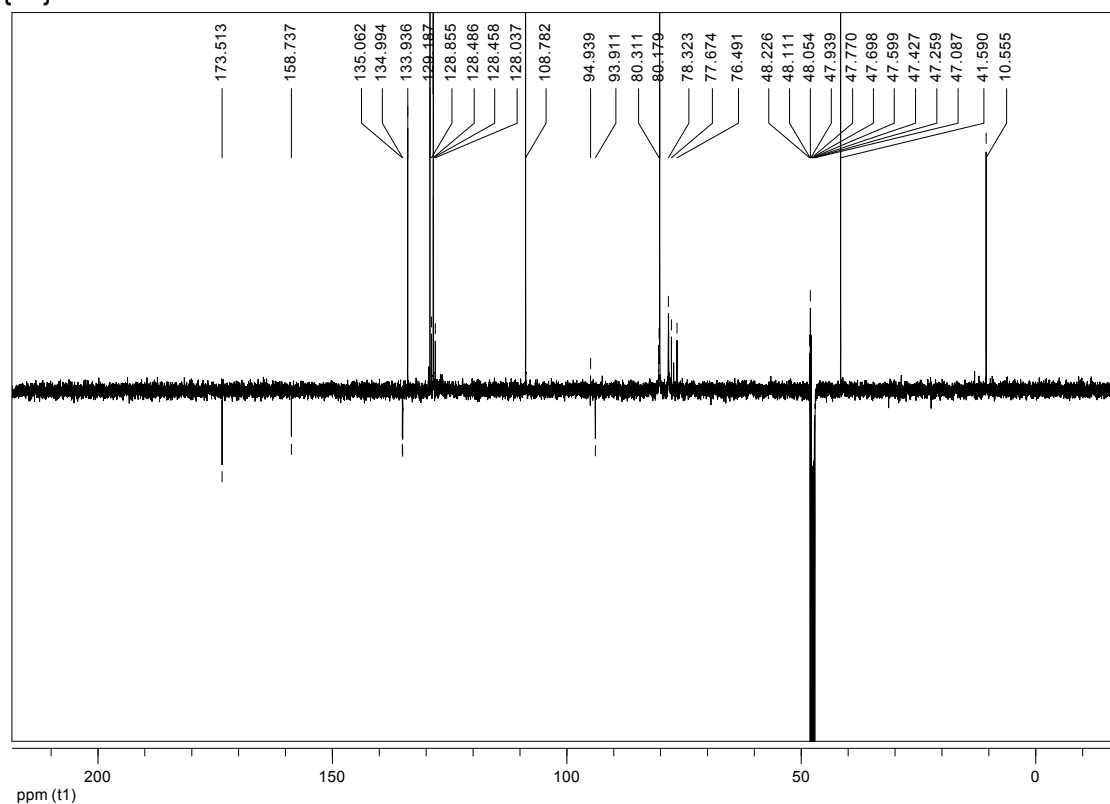


[Ru(η^6 -biphenyl){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 6

¹H-NMR

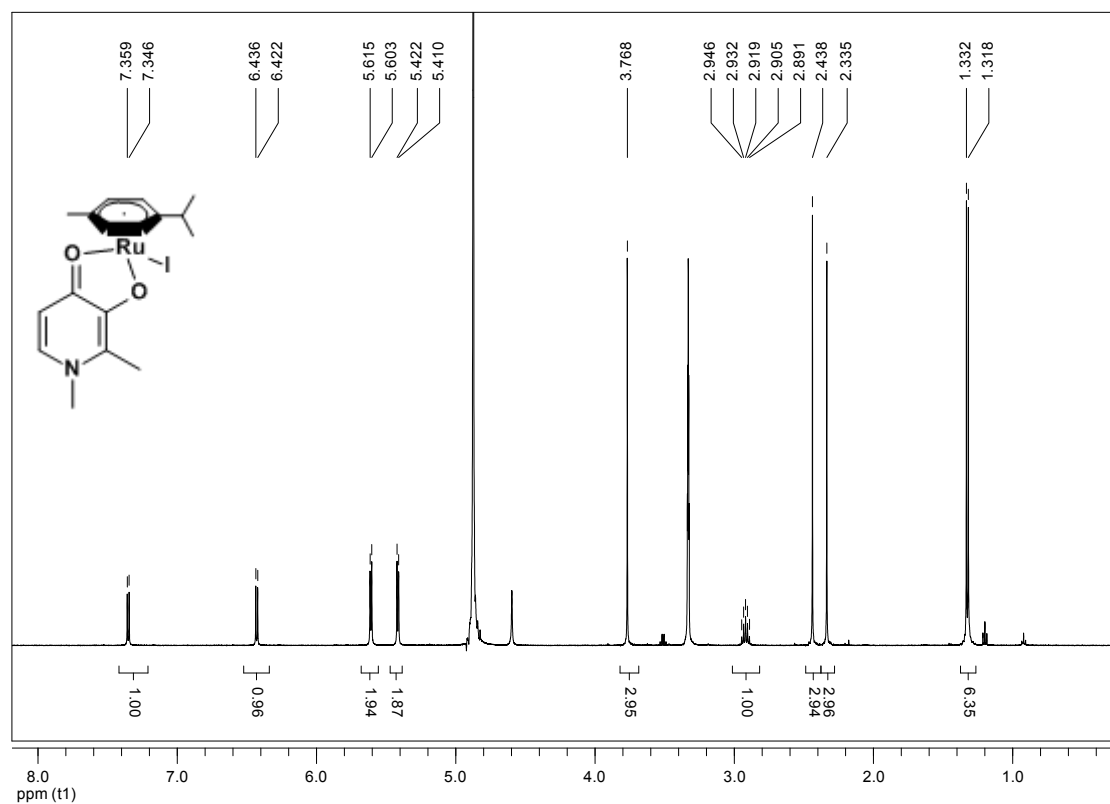


¹³C{¹H}-NMR

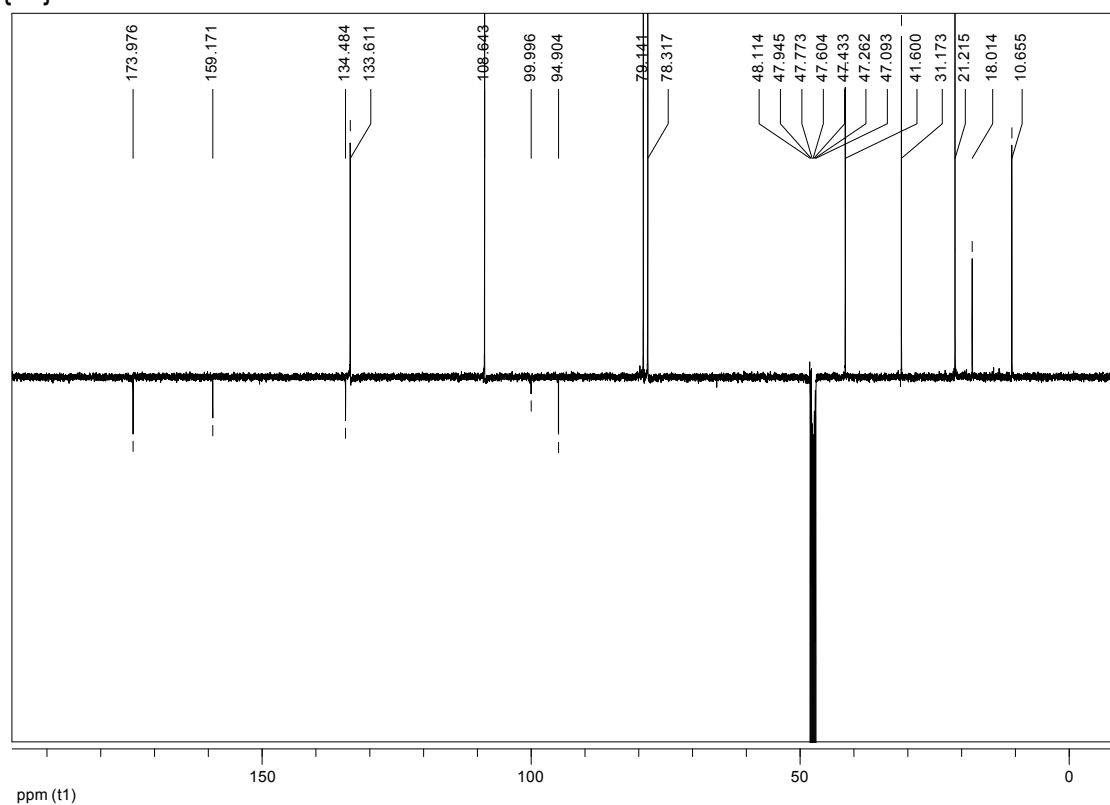


[Ru(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}] – 7

¹H-NMR

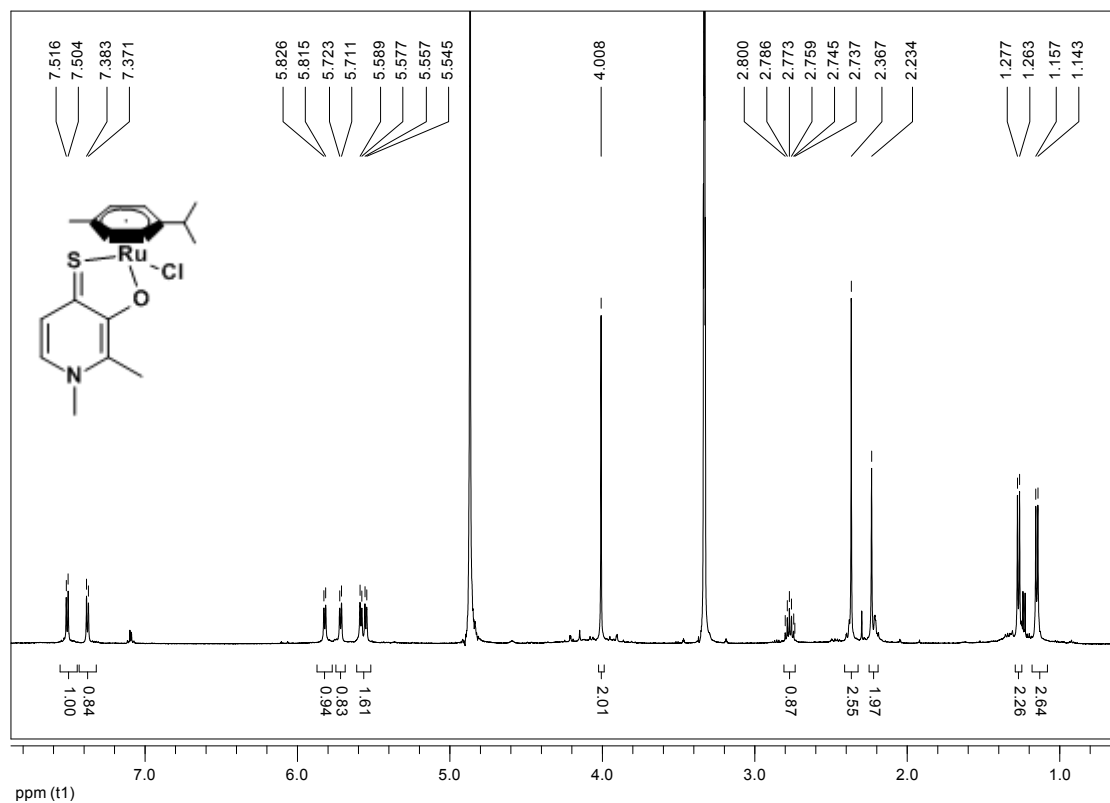


¹³C{¹H}-NMR

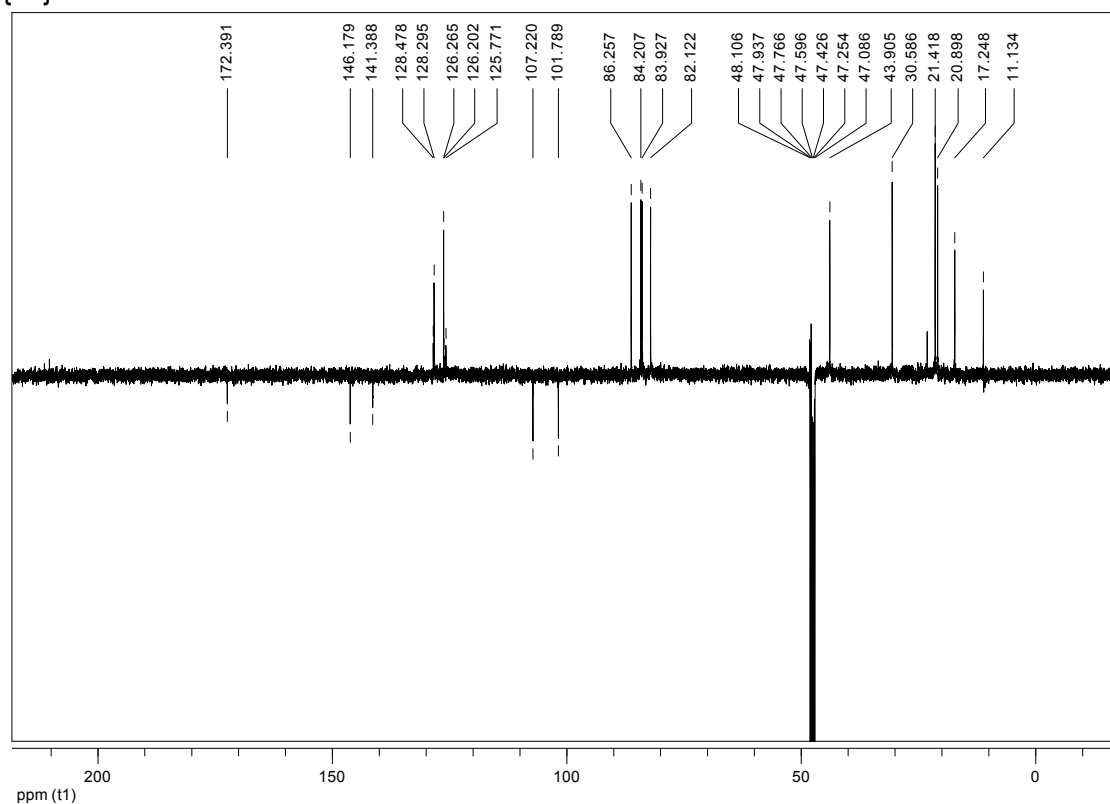


[Ru(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-thiopyridone}Cl] – 9

^1H -NMR

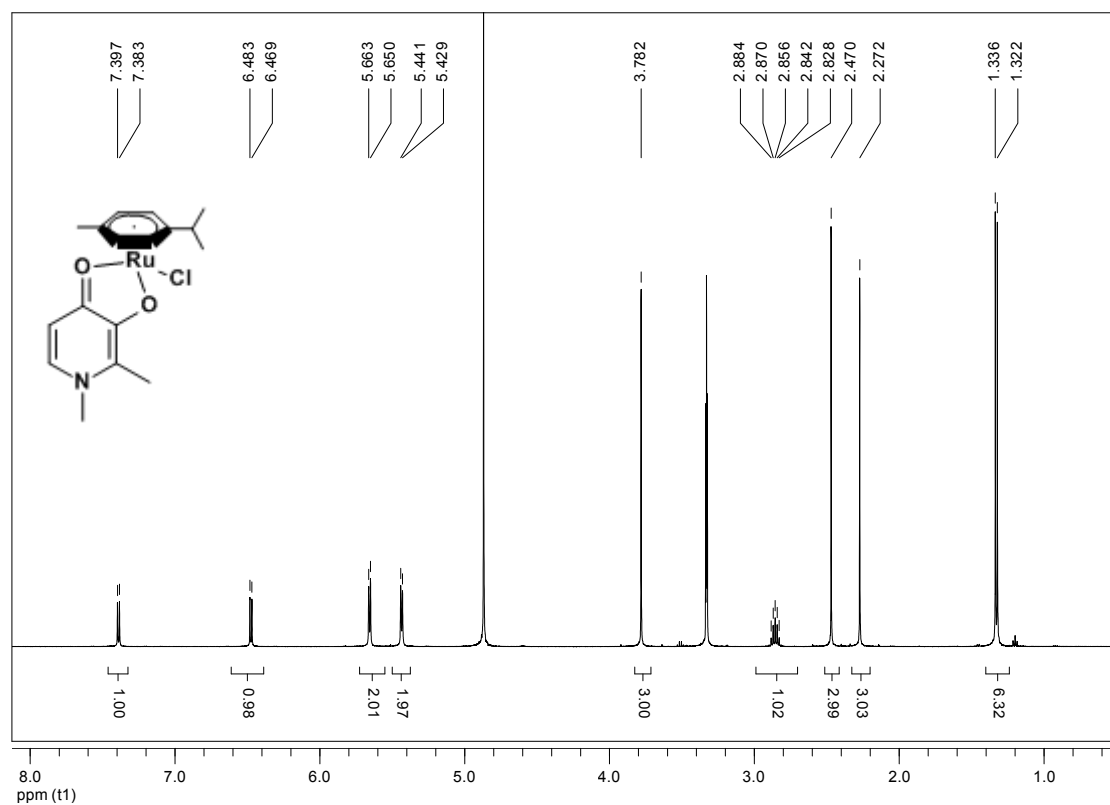


$^{13}\text{C}\{^1\text{H}\}$ -NMR

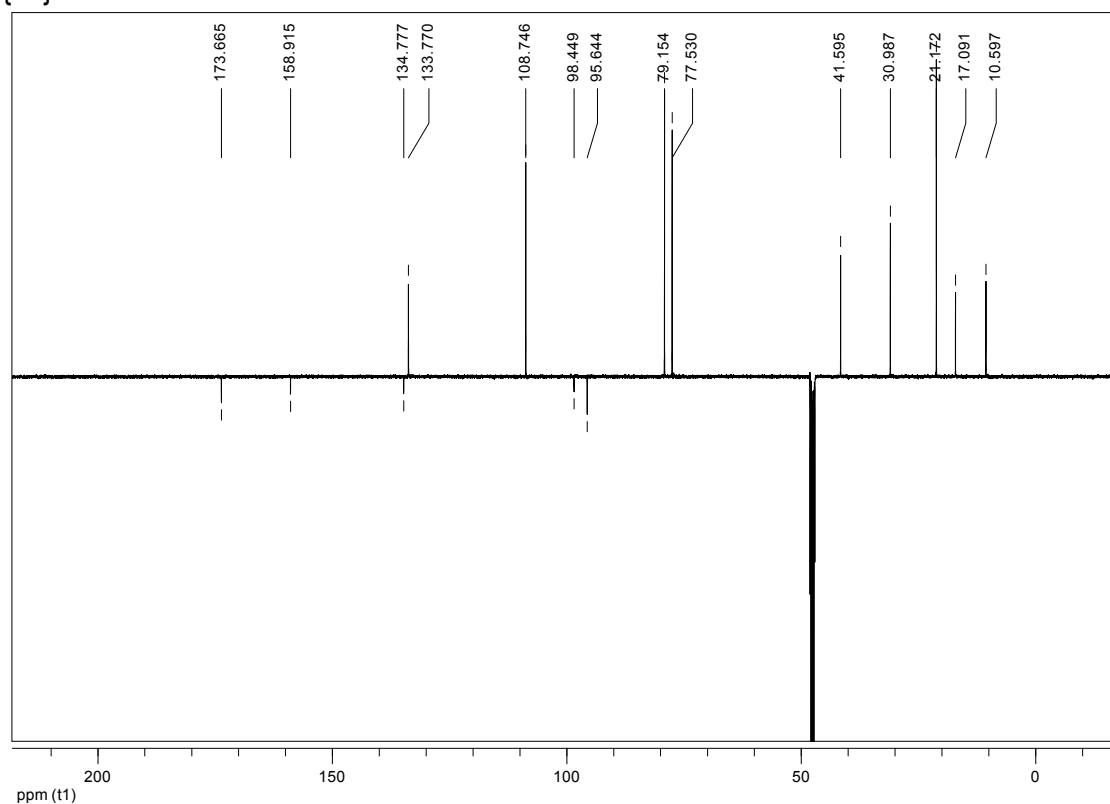


[Ru(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 10

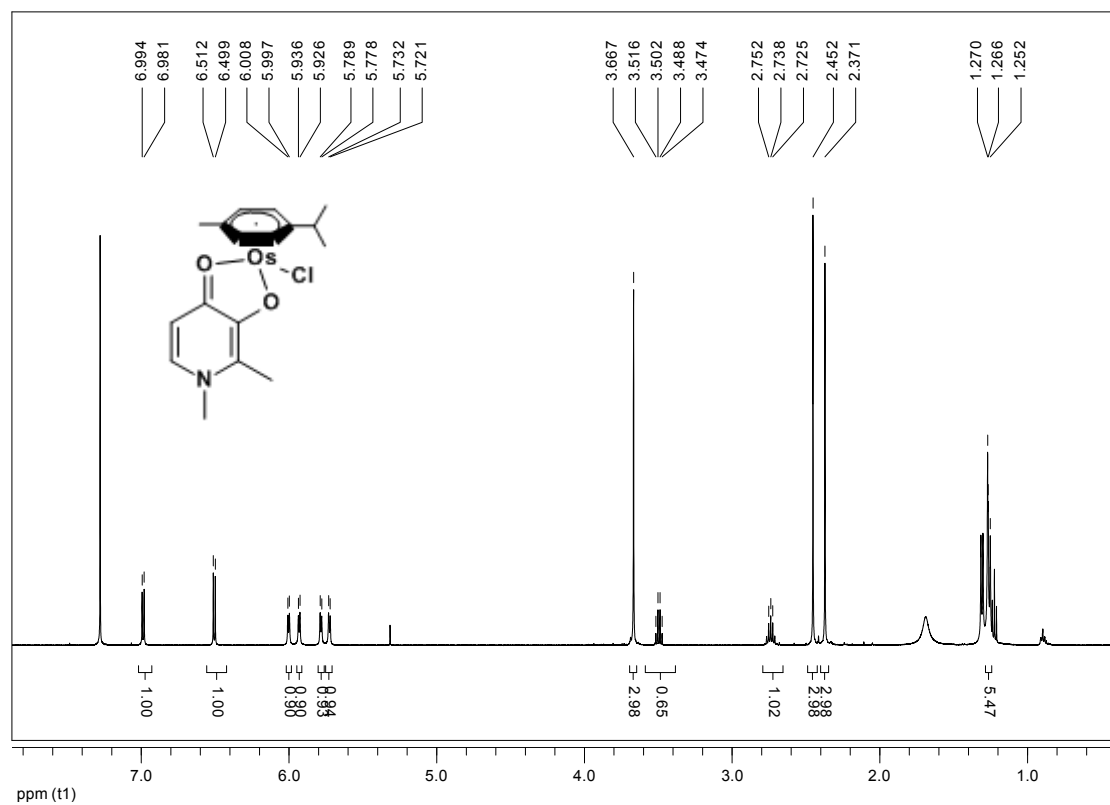
^1H -NMR



$^{13}\text{C}\{^1\text{H}\}$ -NMR



[Os(η^6 -*p*-cymene){1,2-dimethyl-3-hydroxy-4(1*H*)-pyridone}Cl] – 11
¹H-NMR



Curriculum vitae

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EDUCATION

2009 to date	Member of the working group of Prof. Dr. Dr. Keppler, Department of Inorganic Chemistry, University of Vienna
12.2009 to date	Diploma Thesis , Bioinorganic Chemistry
2001-2011	Diploma Student of Chemistry at the University of Vienna, Austria
June 2001	High School degree (Matura)
1993-2001	High School at BG & BRG Perchtoldsdorf
1989-1993	Elementary School, VS Auhofstraße 49, Vienna

PUBLICATIONS

- 1) M. Hanif, **H. Henke**, S. M. Meier, S. Martic, M. Labib, W. Kandioller, M. A. Jakupec, V. B. Arion, H. B. Kraatz, B. K. Keppler, C. G. Hartinger; *Is the Reactivity of M(II)–Arene Complexes of 3-Hydroxy-2(1H)-pyridones to Biomolecules the Anticancer Activity Determining Parameter?* **Inorganic Chemistry**, **2010**, 49 (17), 7953–7963.
- 2) W. Kandioller, A. Kurzwehnart, M. Hanif, S. M. Meier, **H. Henke**, B. K. Keppler, C. G. Hartinger, *Pyrone derivatives and metals: From natural products to metal-based drugs*, **Journal of Organometallic Chemistry**, **696** (2011) 999-1010.

CONTRIBUTIONS TO CONFERENCES/SYMPOSIUMS/WORKSHOPS

- 1) M. Hanif, **H. Henke**, S. M. Meier, M. A. Jakupec, B. K. Keppler, C. G. Hartinger; *Metal(II)-Arene Anticancer Complexes of 3-Hydroxy-2-pyridones: Synthesis, Cytotoxicity and Reactivity to Biomolecules*; **15th International Conference on Biological Inorganic Chemistry (ICBIC 15)**, August 07-12, 2011, Vancouver, Canada.
- 2) C. G. Hartinger, W. Kandioller, M. Hanif, A. Kurzwehnart, **H. Henke**, R. Trondl, C. Bartel, G. Mühlgassner, M. A. Jakupec, M. G. Mendoza-Ferri, A. A. Nazarov, B. K. Keppler; *Organometallic Pyrone and Pyridone Complexes as Anticancer Agents*, **5th International Symposium on Bioorganometallic Chemistry (ISBOMC10)**, July 5-9, 2010, Bochum, Germany.
- 3) W. Kandioller, **H. Henke**, M. Hanif, P. Schaaf, C. Bartel, M. A. Jakupec, A. A. Nazarov, C. G. Hartinger, B. K. Keppler; *Pyrone vs. Pyridinone as Ligands for Ru-arene Anticancer Agents*; **10th European Biological Inorganic Chemistry Conference (EUROBIC10)**, June 22-26, 2010, Thessaloniki, Greece.
- 4) **H. Henke**, M. Hanif, C. G. Hartinger, W. Kandioller, M. A. Jakupec, V. B. Arion, B. K. Keppler; *Synthesis and Cytotoxicity of Metal-Arene Complexes of 3-Hydroxy-2-pyridinone-Derived Ligands*, **6th Workshop on Inorganic Chemistry (6.WACÖ)**, March 29-30, 2010, Linz, Austria.