universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Synthese und Charakterisierung neuartiger tumorhemmender Platin(IV)-Verbindungen

Verfasserin

Verena Pichler

angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag. rer. nat.)

Wien, 2009

Studienkennzahl lt. Studienblatt:	A 419
Matrikelnummer	0400151
Studienrichtung lt. Studienblatt:	Chemie
Betreuerin / Betreuer:	o. Univ.-Prof. Dr. Dr. Bernhard Keppler Ao. Univ.-Prof. Mag. Dr. Markus Galanski

universität wien

DIPLOMA THESIS

Title

Synthesis and Characterisation of Novel Antitumour Active Platinum(IV) Compounds

Author

Verena Pichler

Supervisor

o. Univ.-Prof. Dr. Dr. Bernhard Keppler

ao. Univ.-Prof. Dr. Markus Galanski

Vienna, 2009

To my parents

Acknowledgement

I would like to express my deepest gratitude to all who supported me during the time I spent working on this diploma thesis:

First of all, I would like to thank o. Univ.-Prof. Dr. Dr. Bernhard Keppler for giving me the opportunity to work in this research field and for his generous support.

My supervisor ao. Univ.-Prof. Dr. Markus Galanski for always having an open ear and being a constant source of encouragement, advice and support, for discussion about new ideas and for giving me the possibility to try them as well as for measuring numerous 2D-NMR spectra.

The NMR service team (Mag. Sergey Abramkin, Dr. Wolfgang Kandioller und DI Amitava Kundu) for measuring a plenitude of spectra and for introducing me into measurements.

Mag. Anatolie Dobrov for measuring all the mass spectra, Mag. Theiner and the team of the microanalytical laboratory for elemental analysis.

Dr. Michael Reithofer for providing the substrate for click chemistry.

Alexander Roller for all of his support: general and technical one in the lab and for always trying to find a crystal.

Mag. Elfriede Limberger for her kindness and support in all administrative affairs and Mihai Odoleanu for his support with chemicals and solvents.

My colleagues in Lab 7 and 8 (Sergey, Conny, Yulia, Hristo and Björn) for the good atmosphere and especially Sergey for chemical and off-topic discussion and for all the lunch and coffee breaks.

The whole working group for assistance whenever it was needed, in particular my study colleagues Michael Primik for the hours we spent on the tram and Lukas Filak for the fantastic time at the coffee table. Furthermore Christian Kowol for talks, whenever necessary and for one or two very important cigarettes.

Markus and Simone for accompaning me during my whole studies, without you the years of my studies could not have been that much fun and especially Markus for proof reading and bearing all my ups and downs.

All of my “non-chemistry” friends (sorry, not to name you all) for taking my mind off chemistry and recharging my batteries.

Last but not least my family, my two brothers and especially my parents for always supporting me in every possible way during my whole life.

Abstract

The advancement of cancer treatment is a major aim of modern science, according to the fact that this disease is responsible for 13% of all deaths worldwide (25% in Austria). Chemotherapy based on platinum complexes is a major part in the field of treatment of cancer. Its advent was in the 1960s when Barnett Rosenberg discovered the antitumour activity of cisplatin. Nowadays there are, besides cisplatin, two other platinum-based drugs in worldwide clinical usage: carboplatin and oxaliplatin. Because of the insufficient selectivity of these platinum(II) compounds to attack only cancer cells, patients suffer from a variety of severe side effects.

As a result, research turned its focus to the synthesis of platinum(IV) compounds, due to their chemical inertness. These characteristics are expected to raise the possibility for an oral application and increased selectivity. Platinum(IV) compounds have to be activated via reduction to develop their activity against tumours and therefore act as prodrugs. During reduction, the axial ligands are released. Hence, these ligands are of particular interest for target-oriented synthesis, since the features of a drug, like uptake into the tissue, can be improved without abating anti-tumour activity. Another great opportunity is the coupling to bioactive molecules to extend the spectrum of efficacy.

The synthesis of platinum(IV) compounds experienced a crucial advance through carboxylation with cyclic anhydrides. The resulting free carboxylic acid besides the coordinated carboxylate allows further derivatisation to yield amides and esters. It has to be mentioned that there are two functional groups available which can be of disadvantage for further reactions with carrier molecules. This diploma thesis presents the successful synthesis of asymmetric platinum(IV) complexes: carboxylation with succinic anhydride of (OC-6-43)-dichlorido(N,N-dimethylethane-1,2-diamine)dihydroxidoplatinum(IV) had led to a monocarboxylation, whereas the second hydroxido group was sterically inhibited. Derivatisation of the carboxylic acid with methanol yielded the corresponding ester. Furthermore, platinum(IV) complex was transformed with a secondary amine to the corresponding amide for the first time. Currently, cytotoxicity studies in human tumour cell lines are being performed with three of the synthesised compounds.

Zusammenfassung

Die Verbesserung der Behandlungsmethoden von Krebs ist ein wichtiges Ziel der heutigen Forschung, vor allem wenn man bedenkt, dass diese Krankheit für 13% aller Todesfälle weltweit verantwortlich ist (25% in Österreich). Eine der wichtigsten Behandlungsmethoden ist die Chemotherapie mit Platinverbindungen. Ihre Anfänge liegen in den 1960er Jahren begründet, als Barnett Rosenberg die Antitumoraktivität von Cisplatin entdeckte. Heutzutage sind, neben Cisplatin zwei weitere platinhältige Chemotherapeutika weltweit für die klinische Anwendung zugelassen: Carbo- und Oxaliplatin. Jedoch führt die unzureichende Selektivität dieser Platin(II) Verbindungen dazu, dass nicht nur Krebszellen angegriffen werden. In weiterer Folge führt dies beim Patienten zum Teil schweren Nebenwirkungen.

Dieser Umstand führte zu einer Verlagerung der Forschung hin zu Platin(IV)-Verbindungen, da diese eine erhöhte Inertheit aufweisen. Diese Eigenschaften könnten zu einer möglichen oralen Applizierbarkeit und gesteigerten Selektivität führen. Platin(IV)-Verbindungen werden durch Reduktion in die aktive Platin(II) Spezies überführt und agieren daher als Prodrugs. Die axialen Liganden werden während der Reduktion freigesetzt und sind daher für eine zielgerichtete Synthese von Wirkstoffen von besonderem Interesse. Durch Variation dieser Liganden können gewisse Eigenschaften, wie z.B. die Aufnahme in das Gewebe, verbessert werden, ohne die Aktivität der Substanz zu verändern. Darüber hinaus eröffnet sich die Möglichkeit, das Wirkspektrum durch eine Kopplung mit bioaktiven Molekülen zu erweitern.

Das Repertoire zur Synthese von Platin(IV)-Verbindungen wurde durch die Umsetzung mit zyklischen Anhydriden enorm erweitert. Bei dieser Reaktion wird eine freie Carboxylgruppe erzeugt, die weitere Derivatisierung mit Aminen oder Alkoholen ermöglicht. Es muss angemerkt werden, dass immer zwei funktionelle Gruppen erhalten werden. Dieser Umstand kann, vor allem beim Umsatz mit Trägermolekülen, zu einigen Nachteilen führen. Diese Diplomarbeit enthält die erfolgreiche Synthese asymmetrischer Platin(IV)-Komplexe. Die Asymmetrisierung erfolgt bei der Carboxylierung von (OC-6-43)-Dichlorido(N,N-dimethylethan-1,2-diamin)dihydroxidoplatinum(IV) mit Bernsteinsäureanhydrid durch sterische Hinderung der zweiten Hydroxylgruppe. Die erhaltene freie Carboxylgruppe wird

weiter umgesetzt zum Methylester. Außerdem wurde erstmalig ein Platin(IV) Komplex mit einem sekundären Amin zum entsprechenden Amid umgesetzt. Derzeit werden in vitro Untersuchungen in humanen Tumorzelllinien durchgeführt an drei der synthetisierten Verbindungen.

Table of Contents

1 Introduction

1.1 A Short History of Cancer

Cancer is a long-known disease and is not restricted to human beings. The first description of this disease was already found in ancient Egypt in a manuscript dates back as far as 1600 B.C. Additionally, fossilised malignant bone tumours (osteosarcoma) have been found in ancient human mummies.

Hippocrates (460-370 B.C.) formed the term *carcinos* and *carcinoma*, the Greek word for crab, because the appearance of tumours, finger-like spreading veins, reminded him of crabs. The Greek term was then translated into the Latin word *cancer* by Celsus (28-50 B.C.).

When scientists in the 18th century began to perform autopsies to identify the reason for a patient's death, John Hunter (1728-1793) had born the idea of removing the tumour by surgery. Down to the present day surgery is often used in cancer treatment, furthermore other methods of treatment are deployed like radiation, immunotherapy or chemotherapy. [1]

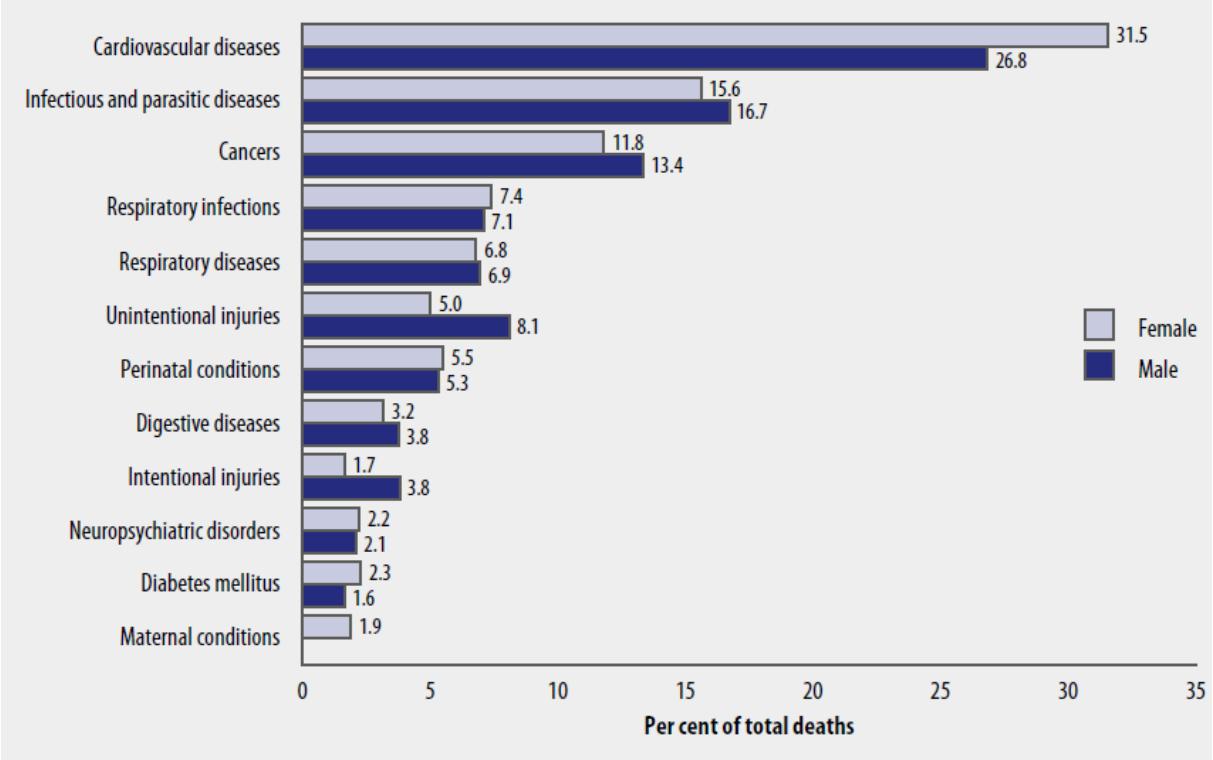
1.2 Facts about Cancer

In 2004 about 13% of all deaths worldwide were accounted for cancer diseases. In low-income countries, poor health care provokes, besides cardiovascular diseases and cancer, an especially high mortality because of infectious and parasitic diseases. In the industrialised countries, cancer is the main cause of death after cardiovascular diseases and its number is rising continuously. The increasing number is a result of the changes in living conditions of modern society (higher life expectancy, decreased mortality for example). The leading causes for cancer to emerge are, aside from the personal genetic factors, several external factors like ultraviolet and ionising radiation, infections of certain viruses (e.g. hepatitis B or human immunodeficiency virus (HIV)) and different chemical compounds people ingest in the course of a lifetime, such as tobacco smoke, aflatoxine or alcohol. By avoiding major risk factors like

[1] www.cancer.org

tobacco and alcohol abuse, overweight and stress an estimated 30% of deaths caused by cancer could be prevented.

Table 1: Worldwide death causes by sex, 2004



Which kind of cancer appears more frequently depends, apart from the aforementioned factors, on the genders. Men are more often affected by lung, stomach and liver cancer, whereas women primarily suffer from breast cancer, stomach and lung cancer.

Hence, there is a major interest to raise public awareness for preventing risk factors, to reduce deaths by early detection of malignant tumours and to improve the methods of treatment. [2]

1.3 Formation of Cancer

Cancer formation is every possible appearance of malignant tumours that can grow in every part of a human body.

[2] http://www.who.int

By outside influences cells may experience DNA damage, but in the majority of cases the body repair system can inhibit genetic harm. When cell division takes place and the DNA damage is replicated, permanent alteration in the nucleotide sequence (= *mutation*) is the consequence. Typically, a cascade of mutations is required to produce a malignant cell and in further steps a tumour, generated by uncontrolled division and proliferation of the mutated cell. Tumour growth requires formation of new blood vessels to get nutrient supply, so-called *angionesis*, otherwise the size of a tumour is limited to approximately 10^6 cells. The most dangerous development is *metastasis*: the spread of invasive cancer cells through the blood stream or lymphatic system to other parts of the body to form new tumours, which makes it difficult to eliminate the cancer.

Classification of different types of cancer results from the tissue of origin the cancer cell emanates. Due to the fact that cell division has to proceed, especially high proliferating tissue is endangered to form neoplasm, like lung and gastrointestinal tract tissue respectively. [3, 4]

1.4 Treatment of Cancer

The method of choice to tread cancer depends on several different factors, like nature of cancer, progression of the illness and its localisation. Furthermore, the general condition of the patient like nutrition stage, age and mental condition is crucial.

Nowadays, there are five types of treatment available:

- Surgical oncology
- Radiation therapy
- Hormone therapy
- Immunotherapy
- Chemotherapy

These methods are often combined, at which surgery is the main step in removing tumours.

---

[3] B. Alberts; A. Johnson; J. Lewis; M. Raff; K. Roberts and P. Walter; *Molecular biology of the cell* **2003**, 4th Edition

[4] H. Lodish; A. Berk; C. A. Kaiser; M. Krieger; M. P. Scott; A. Bretscher; *Molecular cell biology* **2003**, 5th Edition

Surgical incision of tumours is the longest-established treatment and still plays a big role to handle primary as well as secondary tumours. Surgery is also used for palliation by advanced cancer. Good results can be attained for breast and bowel cancer, but a complete removal of the tumour or the target organ has to be assured. On the contrary, survival rates for pancreatic and gastric cancer are lower than 10% for patients treated in Europe.

The success of r*adiation therapy* significantly depends on the irradiated tissue, because the cells lose reproductive capacity and infrequently they die of apoptosis. For this reason, cells with fast cellular turnover like skin or nervous system tissue respond more rapidly to radiation. The challenge is to accurately detect the total dose and continuance to harm the tumour at the most with minimum effect on the healthy surrounding tissue.

Hormones naturally produced in the body can interfere with the development of malignant tumours. *Hormone therapy* correspondingly stands for the inhibition of hormones of the afflicted target organ, which maintain the growth of cancer. However, the appliance is limited to few types, breast and prostate cancer for example, which are closely associated to the hormone balance. Often tumours are intrinsically resistant or will develop resistance during treatment, so it is considered to combine this strategy with chemotherapy.

Immunotherapy is a new method to stimulate the immune system of the patient to affect an anti-tumour response. There are two types of immunotherapy: Immunisation of the patient with agents that directly affect the tumour growth (active immunotherapy) or medication with materials to stimulate the body's own defences to fight malignant cells (passive immunotherapy). Immunotherapy is not yet fully developed.

Chemotherapy is a treatment, where cytotoxic chemical compounds are deployed. Chemotherapy is the method of choice for unlocalised tumours with high tendency towards metastasis. Furthermore it is used pre or postsurgical to minimise tumour volume or for treatment of residual tumour cells. Additionally, it is useful to combine different chemotherapeutics, which attack at different points of the cell cycle to gain overall tumour response. However, the lack of selectivity often leads to multiple severe side effects.

Nowadays there is a large amount of chemotherapeutics in clinical usage that can be classified by their mode of action:

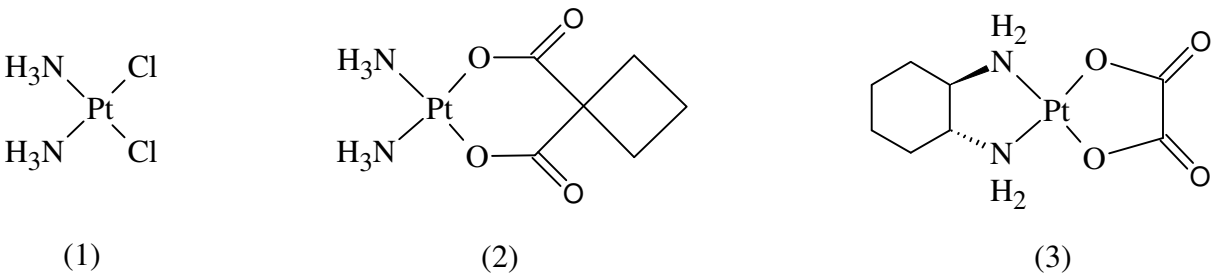
- alkylating agents (e.g. melphalan, chlorambucil)
- anti-tumour antibiotics (e.g. daunorubicin, mitomycin C)
- anti-metabolites (e.g. methotrexate, 5-flurouracil)
- topoisomerase inhibitors (e.g. topotecan, etoposide)
- signal transduction inhibitors (e.g. resveratrol)
- anti-microtubule agents (e.g. vincristine, paclitaxel)
- hormone and hormone antagonists (e.g. tamoxifen)
- anti-tumour metal complexes [5]

The last group includes cisplatin, carboplatin and oxaliplatin; the only three platinum-containing chemotherapeutics in worldwide clinical use.

[5] J. Cassidy; D. Bissett; R. AJ Spence; *Oxford Handbook of Oncology*, **2002**, 1st Edition

2 Antitumour Platinum Compounds

The history of cytotoxic platinum compounds starts in the 1960's, when Barnett Rosenberg accidentally discovered the anti-tumour activity of certain platinum containing coordination compounds. Originally, he wanted to examine the effect of an electric field on the growth of *Escherichia coli*, and found that cell division stopped, whereas the bacteria still had a filamentous growth. This effect later was ascribed to platinum complexes, which were formed in the presence of ammonium chloride, sunlight and the supposedly inert platinum electrode. [6] Based upon these results he tested various group 10 transition-metal compounds with respect to their activity against cancer. [7] The most powerful compounds were *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin, DDP) (1) and *cis*-$[PtCl_4(NH_3)_2]$, whereas the analogous *trans*-compounds showed no effect. [8-10]



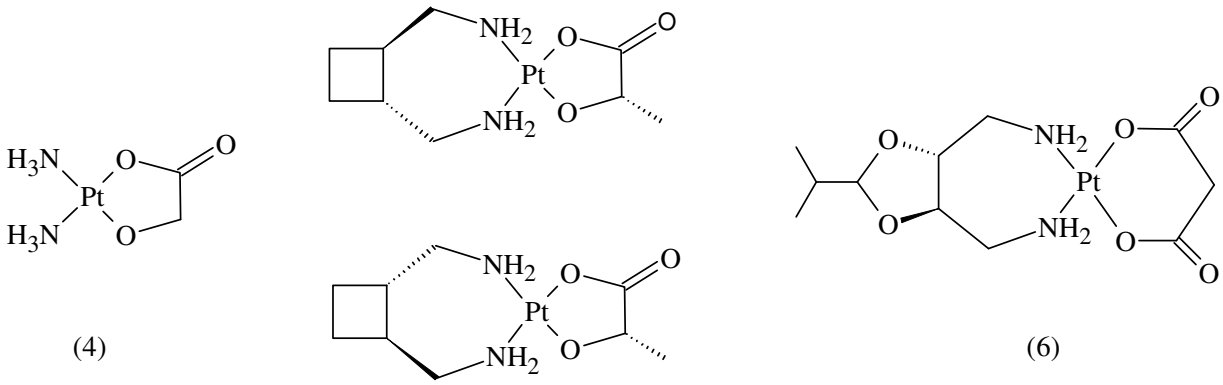
(1) (2) (3)

Figure 1: The only three platinum complexes in worldwide clinical usage: (1) cisplatin; (2) carboplatin and (3) oxaliplatin

Soon, cisplatin was established in clinical routine and its success as anticancer drug is unbroken up to date. Ever since that time an enormous amount of platinum complexes have been synthesised and about 35 compounds have entered clinical trials, but the majority of

[6] B. Rosenberg; L. Van Camp; T. Krigas; *Nature*, **1965**, 698-699
[7] B. Rosenberg; E. Renshaw; L. Van Camp; J. Hartwick; J. Drobnik*; J. Bacteriol*. **1967**, 93, 716-721
[8] B: Rosenberg; L. Van Camp; J. E. Trosko; V. H. Mansour; *Nature* **1969**, 222, 385-387
[9] B. Rosenberg; L. Van Camp; *Cancer Res.* **1970**, 30, 1799-1802
[10] B. Rosenberg; L. Van Camp; *J. Biol. Chem.* **1967**, 242, 1347-1352

these compounds were abandoned, because of their inactivity and/or toxicity. [11] Today, only three platinum(II) complexes are approved for worldwide application (cisplatin (1), carboplatin (2), oxaliplatin (3) (Figure 1)); another three complexes are only regionally approved (nedaplatin (4), lobaplatin (5), and heptaplatin (6) (Figure 2)) and currently a few are in clinical trials.



(4) (5) (6)

Figure 2: Platinum complexes in regionally limited clinical usage: (4) nedaplatin (Japan), (5) lopaplatin (China), and (6) heptaplatin (South Korea)

The small number of compounds used in chemotherapy results from enormously strict requirements in trials for clinical approval. The drug has to have at least one advantage over currently applied chemotherapeutics, like advanced or more selective activity against cancer, reduced side effects or the possibility of easier application. [12]

Despite original assumption, the annual volume of platinum compounds in chemotherapy currently amounts about 3 bn € worldwide. [13]

2.1 Structure-Activity Relationships

Approximately one in every 10 000 compounds gets approved, so the motivation to find a way to design drugs more efficiently was and still is very high. However, before starting to design a certain drug, it is important to understand the interaction between the drug and the

[11] M. Galanski; M. A. Jakupec; B. K. Keppler; *Curr. Med. Chem.* **2005**; 12, 2075-2094
[12] R. A. Alderden; M. D. Hall and T. W. Hambley; *J. Chem. Ed.* **2006**; Vol. 83; 728-734
[13] M. Galanski; B.K. Keppler; *Pharm. Unserer Zeit*, **2006**, 35; 118-122

targeted cancer cell. Based upon the analysis by Cleare and Hoeschele the following "structure-activity relationships" were first indicated: [14, 15]

The complex should have…

- *cis* geometry, whereas compounds with *trans* geometry are inactive.
- necessarily a pair of *cis* leaving groups, which is not sufficient.
- no charge.
- moderately bound leaving groups, given that labile leaving groups are toxic and firmly bound ones are inactive.
- two amine groups, with at least one proton.

The validation of these rules remained untouched for long, because all of the compounds that have entered clinical trials were consistent with them. Today, all of these theses that were thought to be essential for generating an active drug have been disproved. [16] Furthermore, the acceptation of these structure-activity criteria has shortened the focus on cisplatin analogues. This may cause the fact, that fewer Pt(IV)-complexes have been synthesised, although their anti-cancer effect has been known since Rosenberg. [17]

Concluding, although structure-activity relationships are not sufficient to find active drugs, they are still important for understanding the mechanism of action and especially for categorising complexes into groups with analogous modes of action.

2.2 Platinum(II) Compounds

2.2.1 Cisplatin

Cisplatin (1) is a long known compound and over the time, synthesis has experienced several improvements. The structure was first published as "Peyrone's salt" in 1844 by Michele Peyrone. [18] The most common and successful synthesis was reported in 1970 by *Dhara et al*, which is based on the trans-effect of ligands. [19]

[14] J. M. Cleare; J. D. Hoeschele; *Plat. Met. Rev.* **1973**, 17, 2-13
[15] J. M. Cleare; J. D. Hoeschele; *Bioinorg. Chem.* **1973**, 2, 187-210
[16] T. W. Hambley; *Coord. Chem. Rev.* **1997**; 166; 181-223
[17] M. D. Hall; T. W. Hambley; *Coord. Chem. Rev.* **2002**, 232, 49-67
[18] M. Peyrone; *Anal. Chem. Pharm.* **1844**, LI, 1-29
[19] S. J. Dhara; *Indian J. Chem.* **1970**, 8, 193-194

Cisplatin was one out of four complexes, which entered clinical trials after the discovery of Rosenberg and was found active. In April 1971, the complex was first tested in a patient in the USA and 7 years later it was approved as anticancer drug. [20] Intense clinical research showed a curative effect of cisplatin in small cell and non-small cell bronchial carcinoma, ovarian and testicular tumours, cervix carcinoma, advanced bladder carcinoma, melanoma, soft tissue sarcomas and carcinomas of the head and neck; especially testicular cancer can now be cured in over 90% of cases.

Cisplatin is applied intravenously in a chloride-containing solution over a period of 0.5 to 2 hours. Prehydration with a salt-containing fluid to minimise nephrotoxicity is fundamental. The nephrotoxicity's aggressiveness nearly led to abandonment of the clinical trials. Usually, a maximum amount of up to 200 mg/m^2 is administered for high-dose cisplatin. Besides nephrotoxicity, there are other severe side effects associated to it, like ototoxicity, neuropathy, nausea and myelosuppression. [21, 22]

There is a variety of brand names for cisplatin-containing drugs, like Platinol® (Swiss, Austria), Cis-GRY® and Platinex®.

2.2.1.1 Mode of Action

For developing new and more effective platinum drugs, it is fundamental to understand how cisplatin and analogues interact with cells and for this reason a huge amount of publications exists that analyse and discuss this mode of action, although it is not fully understood yet.

Cisplatin enters the bloodstream by intravenous administration and an immediate binding to proteins in the blood plasma up to 90% of the administered platinum drug takes place; specifically to thiol groups found in human serum albumin for example. [23, 24]

The cellular uptake can occur mainly via two pathways, primarily by passive diffusion or actively by copper transporter protein Ctr1P. [25] Aquation of cisplatin leads to the activated

---

[20] D. Lebwohl; R. Canetta; *Eur. J. Cancer*, (34), 10, **1998**, 1522-1534
[21] W. Voigt; A. Dietrich; H.J. Schmoll; *Pharm. Unserer Zeit* **2006**; 35, 134-143
[22] B. Lippert; Cisplatin; *Wiley VCH*, **1999**
[23] R. C. DeConti; B. R. Toftness; R. C. Lange; W. A. Creasey; *Cancer Res.* **1973**, 33, 1310-1315
[24] A. I. Ivanov; J. Christodoulou; J. A. Parkinson; K. J. Barnham; A. Tucker; J. Woodrow; P. J. Sadler; *J. Biol. Chem.* **1998**, 24, 14721-14730
[25] S. Ishida; J. Lee; D. J. Thiele; I. Herskowitz; *Proc. Natl. Acad. Sci. U.S.A* **2002**, 99, 14298-14302

species (Figure 3); this process is enabled by the much lower intracellular chloride concentration (~ 4 mmol/L), compared to the extracellular chloride concentration of about 100 mM. The result is a mixture of species; the distribution of these depends on the pH and chloride concentration. [26, 27]

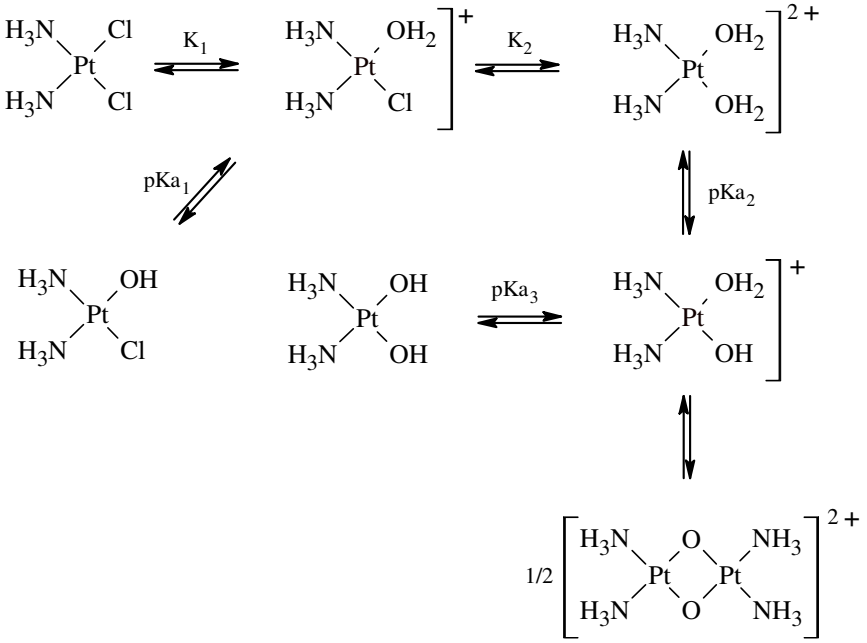


Figure 3: Behavior of cisplatin in aqueous environment

It was found that the monoaquated platinum species is the major contributor of binding to genomic DNA (gDNA) and therefore is the main initiator of anticancer activity. [28] Only 1% of the total cellular platinum reach DNA, the primary target, because of the enormous binding to other cellular components like membrane phospholipids, RNA and especially sulphur containing biomolecules (glutathione and metallothionein). The preferred DNA binding area of cisplatin is located at nucleophilic nitrogen atoms of the nucleobases, usually the N7 atom of guanine in the major groove. There is a variety of known DNA adducts, such as 60-65% 1,2-d(GpG), 20-25% 1,2-d(ApG) and 5-10% 1,3-d(GpNpG) intrastrand cross-links that can be considered the main products. Of little importance are the interstrand cross-links and monoadducts. [29-31]

---

[26] T. G. Appleton; J. R. Hall; S. F. Ralph; C. S. M. Thompson; *Inorg. Chem.* **1989**, 28; 1989-1993
[27] S. J. Berners-Price; T. A. Frenkiel; U. Frey; J. D. Ranford; P. J. Sadler; *J. Chem. Soc., Chem. Commun.* **1992**; 789-791
[28] M. S. Davies; S. J. Berners-Price; T. W. Hambley; *Inorg. Chem.* **2000**, 39, 5603-5613
[29] E. R. Jamieson; S. J. Lippard; *Chem. Rev.* **1999**, 99, 2467-2498

The consequence of the formation of these adducts is a change and distortion of the DNA conformation, like bending of the axis and unwinding of the double helix, which eliminates the possibility of replication and transcription and leads to apoptosis and necrosis. [32-36]

2.2.1.2 Mechanism of Cisplatin Resistance

It is generally accepted that on the way to the DNA the administered cisplatin in large parts is lost because of binding to a variety of proteins and cellular compounds. In addition, there are different mechanisms that lead to resistance, intrinsic (e.g. colon cancer) or acquired ones (e.g. ovarian cancer). Due to the multifactorial impacts details of the pathway are not yet entirely resolved despite intense research.

There are two possible ways to anticipate apoptosis: (i) preventing the platinum compounds to build DNA adducts by deactivation and/or (ii) removing them by different repair mechanisms of the cell. The multitude of possibilities to deactivate cisplatin before interfering with DNA was described in brief before (see Chapter 2.2.1.1), so here focus will be the cellular repair systems.

Nucleotide excision repair (NER) is thought to be the most important way of removing DNA adducts. The best described NER system is mediated by UvrABC endonuclease in *Escherichia coli*, moreover it is more sensitive to platinum adducts than others. After the damage recognition step an incision in DNA is made at a range of approximately 25-30 nucleotides in length, this sector is removed and a polymerase reconstructs up the new DNA fragment. [37-39]

Mismatch repair (MMR) is a system to correct different defects of DNA-synthesis, like mispaired nucleotides. Repeated ineffective repair triggers a process that ends in programmed

[30] M. A. Fuertes; C. Alonso; J. M. Pérez; *Chem. Rev.* **2003**, 103, 646-662
[31] Y. Jung; S. J. Lippard; *Chem. Rev.* **2007**; 107, 1387-1407
[32] J.-M. Malinge; M.-J. Giraud-Panis; M. Leng; *J. Inorg. Biochem.* 77 **1999**, 23-29
[33] Z. H. Siddik; *Oncogene* **2003**, 22, 7265-7279
[34] Y.-P. Ho; S. C. F. Au-Yeung; K. K. W. To; *Med. Res. Rev.* **2003**, 23, 633-655
[35] C. M. Sorenson; A. Eastmann; *Cancer Res.* 48, **1988**; 4484-4488
[36] D. Wang; S. J. Lippard; *Nat. Rev. Drug Discov.* **2005**, 307-320
[37] B. VanHouten; A. McCullough; *Ann. N.Y. Acad. Sci. U.S.A* **1994**, 236-251
[38] R. Visse; M. de Ruijter; G. G. Moolenaar, P. Van de Putte; *J. Biol. Chem.* **1992**; 6736-6742
[39] R. Visse; M. De Ruijter; J. Brouwer; J. A. Brandsma; P. Van de Putte; *J. Biol. Chem.* **1991**, 7609-7617

cell death (apoptosis). Therefore an enhanced cytotoxicity of DNA adducts is observed in MMR proficient cells in the case of cisplatin and opposed loss of MMR leads to greater tolerance of or resistance against platinum species, as monitored in ovarian cancer. This resistance mechanism applies only to platinum drugs that form similar DNA adducts as cisplatin. [40-43]

HMG Proteins (high mobility group proteins) are a group of small proteins, which recognise distortion of the DNA-helix and specifically bind to these sites. They play a role in damage recognition for example and initialise systems like NER and MMR. HMG proteins detect platinum DNA adducts effectively and mask them so they will not be repaired. This eventually leads to apoptosis. The ambitions to identify such proteins that bind specifically to platinum adducts and understand their biochemical processes are naturally high. [44]

Deepening the knowledge of acquired tolerances and resistances is essential for designing more effective drugs. The comprehensiveness of the topic is particularly challenging, so only some important mechanisms could be described at this point. [29-31, 33-34, 45-50]

2.2.2 Carboplatin

Carboplatin (2) is a cisplatin derivative with two chloride ligands being replaced with 1,1-cyclobutane dicarboxylate. The chelating ligand has the effect of stabilisation and in further succession reduction of nephrotoxicity without abating anti-tumour activity.

---

[40] D. A. Anthony; A. J. McIlwrath; W. G. Gallagher; A. R. M. Edlin; R. Brown; *Cancer Res.* **1996**, 1374-1381
[41] D. Fink; S. Nebel; S. Aebi; H. Zheng; B. Cenni; A. Nehmé; R. D. Christen; S. T. Howell; *Cancer Res.* **1996**; 4881-4886
[42] S. Aebi; B. Kurdi-Haidar; R. Gordon; B. Cenni; H. Zheng; D. Fink; R. D. Christen; C. R. Boland; M. Koi; R. Fishel; S. B. Howell; *Cancer Res.* **1996**, 3087-3090
[43] J. T. Drummond; A. Anthony; R. Brown; P. Modrich; *J. Biol. Chem.* **1996**, 19645-19648
[44] E. Hughes; B. N. Engelsberg; P. C. Billings; *J. Biol. Chem.* **1992**; 13520-13527
[45] J. Odenburg; A. C. Begg; M. J. H. van Vugt; M. Ruevekamp; J. H. Schornagel, H. M. Pinedo; G. Los; *Cancer Res.* **1994**, 487-493
[46] M. Bungo; Y. Fujiwara; K. Kasahara; K. Nakagawa; Y. Ohe; Y. Sasaki, S. Irino; N. Saijo; *Cancer Res.* **1990**, 2549-2553
[47] A. Eastman; N. Schulte; *Biochem.* **1988**, 27, 4730-4734
[48] R. P. Perez; *Eur. J. Cancer* **1998**, 1535-1542
[49] V. Brabec; J. Kasparkova; *Drug Res. Updates* **2002**, 147-161
[50] M. Kartalou; J. M. Essigmann; *Mut. Res.* **2001**, 23-43

Carboplatin was the result of intense research after the raise of cisplatin and had entered phase I clinical trials in the UK in 1980 and finally was approved for clinical usage in 1992. Compared to cisplatin, it is not deactivated by thiol groups in the blood stream, thereby a decrease of some side effects is achieved, only myelosuppression is higher and is hence the dose-limiting factor.

The mode of action is analogous to cisplatin, so it responds to the same tumour species, although the needed therapeutic dose of carboplatin is approximately four times higher (200-400 mg/m^2) to achieve the same effect. The clinical usage requires absence of chloride-ions in solution to avoid exchange of the ligands. The administration is intravenous without prehydration, due to the reduced effect on the kidneys. A recovery of about 90% of the injected dose is found in the urine. [20-22, 51]

Carboplatin is available as Carbosol® (Austria), Neocarbo® or Onkocarbo® (Germany); the first commercially used brand name was Paraplatin®.

2.2.3 Oxaliplatin

Synthesised by *Kidani et al*., oxaliplatin (3) is a third-generation derivative, with activity against some cisplatin-resistant tumours.[52] Oxaliplatin has an oxalato leaving group, and a non-leaving DACH ligand (= R,R-cyclohexane-1,2-diamine ligand).

The first clinical phase I study took place in France, and currently oxaliplatin combined with 5-fluorouracil is approved worldwide for colon cancer treatment. The reason for the different fields of application is caused by the fact that loss of the mismatch repair system (see Chapter 2.2.1.2) do not lead to resistance against oxaliplatin, because of the formation of different DNA adducts as opposed to cisplatin. The limiting factor of dosage is the neurotoxicity, other side effects are myelosurpression, diarrhoea and mucosa inflammation, whereas often the toxicities are reversible after completion of treatment.

There is no prehydratation necessary because of the absence of nephrotoxicity. The intravenous administration of 50 mg/m^2 is carried out in a 5% solution of glucose, and similar to cisplatin, oxaliplatin is affine towards glutathione and other plasma compounds. [20-22, 53, 54]

---

[51] J. Lokich; N. Anderson; *Ann. Onc.* **1998**, 13-21
[52] Y. Kidani; M. Noji; T. Tashiro; *Gann* **1980**, 71, 637-643
[53] B. Stordal; N. Pavlakis; R. Davey; *Cancer Treatm. Rev.* **2007**, 33, 347-357

An observable structure-activity relationship could be identified between the three DACH-isomeres, in which the activity sequentially decreases: [55]

trans-R,R-DACH > *trans*-S,S-DACH > *cis*-R,S-DACH

Oxaliplatin is commercially available as Eloxatin® (Austria, Germany) or Croloxat® (Germany).

2.2.4 Regionally Limited Platinum(II) Complexes

In the course of time it became difficult to obtain new platinum(II) compounds with better performance against neoplasms, on account of this only three more complexes were regionally approved as anticancer agents: nedaplatin (4), lobaplatin (5), and heptaplatin (6) (Figure 2). [11, 56]

In 1995 *nedaplatin* (4) was approved in Japan. Its characteristics are very similar to those of carboplatin, it shows the same side effects (e.g. myelosuppression) and a small affinity to plasma proteins. Nedaplatin is amongst others effective against small and non-small cell lung cancer, testicular tumours and cancer of the neck and head. [21]

Although *lobaplatin* (5) was developed in Frankfurt (Germany), it is only clinically used in China. Lobaplatin, which exists as a mixture of two diastereomeres, is effective against ovarian, esophageal, head and neck or small cell lung cancer. [57]

Heptaplatin (6) shows impact on some cisplatin-resistant cell lines and is approved in South Korea for treatment of gastric cancer. The major side effects at intravenous administration are hepatotoxiticy and myelosupression. [58, 59]

---

[54] S. Fu; J. J. Kavanagh; W. Hu; R. C. Bast Jr.; *Int. J. Gynecol. Cancer* **2006**; 16; 1717-1732
[55] M. Noji; K. Okamoto; Y. Kidani; T. Tashiro; *J. Med. Chem.* **1981**, 24, 508-515
[56] M. A. Jakupec; M. Galanski; B. K. Keppler; *Rev. Physiol Biochem Pharmacol* **2003**, 146; 1-53
[57] J. Welink; E. Boven; J. B. Vermorken; H. E. Gall; W. J. F. Vijgh; *Clin. Cancer Res.* **1999**, 5, 2349-2358
[58] W. Yu; H. Y. Chung; S. H. Park; Y. B. Cho; Y. S. Lim; *Cancer Res. and Treatm.* **2003**, 35, 25-29
[59] C.-H. Choi; Y.-J. Cha; C.-S. An; K.-J. Kim; K.-C. Kim; S.-P. Moon; Z. H. Lee; Y.-D. Min; *Cancer Cell Int.* **2004**, 4:6

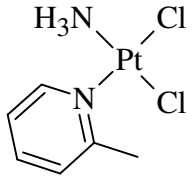
2.2.5 Other Representatives of Platinum(II) Compounds

The intense research in the field of platinum(II) complexes produced an amount of derivatives that did not correspond with the structure-activity relationships, a few of these entered even clinical trials (Figure 4 and Figure 5):

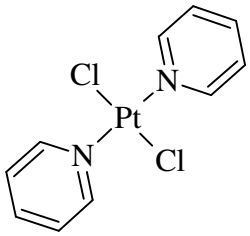
One of the intentions was to develop complexes, which are protected against the reaction with thiol groups and human cell repair mechanism through a sterically demanding ligand. *Picoplatin (NX473)* (7) provided the best results, especially in small lung cancer and currently is in clinical trials. [60 ,61]

By now, also *trans*-platinum-compounds were found active in vivo and in vitro when a bulky planar ligand replaces the ammine groups, e.g. *trans-[PtCl$_2$(py)$_2$]* (8).

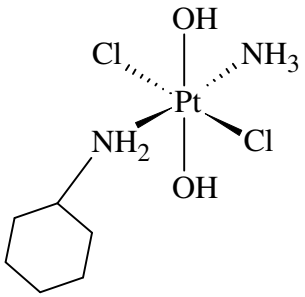
JM 335 (9), a trans-platinum(IV)-compound, has even achieved better results than the *cis*-analogue. [62, 63]



(7)



(8)



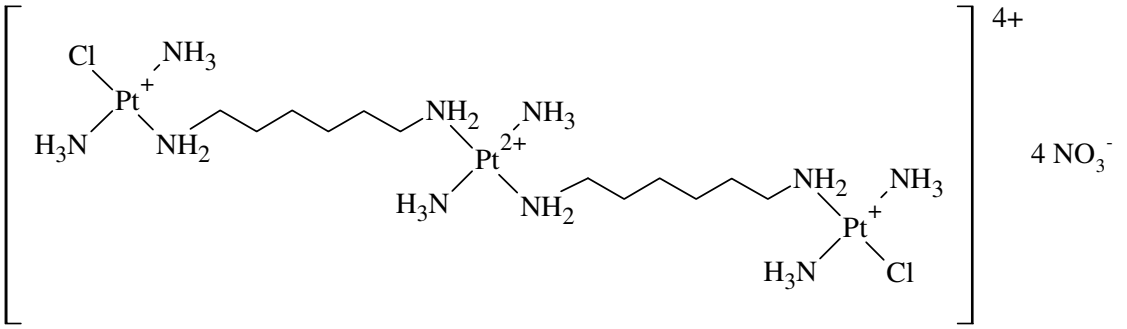
(9)

Figure 4: Structure of (7) Picoplatin, (8) trans-[PtCl$_2$(py)$_2$] and (9) JM 335

Another group of innovative anti-cancer drugs are multinuclear platinum compounds, with *BBR 3464* (10) e.g. entering phase II studies. As they build "long distance" products with DNA, several cisplatin-resistant tumours showed response. [64, 65]

---

[60] L. Kelland; *Nature Rev.* **2007**, 7, 573-584
[61] B. A. Teicher; *Clin. Cancer Res.* **2008**, 14, 1610-1617
[62] M. Van Beusichem; N. Farrell; *Inorg. Chem.* **1992**, 31, 634-639
[63] C. F. O'Neill; L. Hunakova; L. R. Kelland; *Chem. Biol. Interact. 123*, **1999**, 11-29



(10)

Figure 5: Structure of (10) BBR 3464

[64] C. Manzotti; G. Pratesi; E. Menta; R. Di Domenico; E. Cavalletti; H. H. Fiebig; L. R. Kelland; N. Farrell; D. Polizzi; R. Subino; G. Pezzoni, F. Zunino; *Clin, Cancer Res.* **2000**, 2626-2634

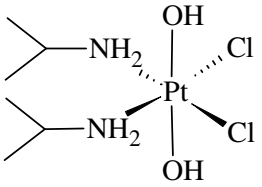
[65] D. I. Jodrell; T. R. J. Evans; W. Steward; D. Cameron; J. Prendiville; C. Aschele; C. Noberasco; M. Lind; J. Carmichael; N. Dobbs; G. Camboni; B. Gatti; F. De Braud; *Eur. J. Cancer* 40, **2004**, 1872-1877

2.3 Platinum(IV) Compounds

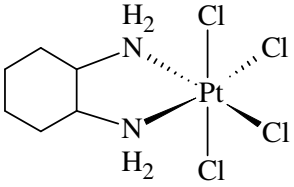
Although Rosenberg simultaneously found that $[PtCl_4(NH_3)_2]$ (= platinum(IV) compound) and cisplatin (= the platinum(II) analogue) have an antiproliferating potential on malignant tumours, platinum(IV) compounds have suffered from little attention. However, platinum(IV) compounds offer a variety of characteristics to take advantage of, especially their increased inertness and consequently reduced toxicity and possibility of oral administration. Moreover, their octahedral coordination enables more synthetic ways for designing drugs on purpose.

2.3.1 Platinum(IV) Anticancer Complexes Entering Clinical Trials

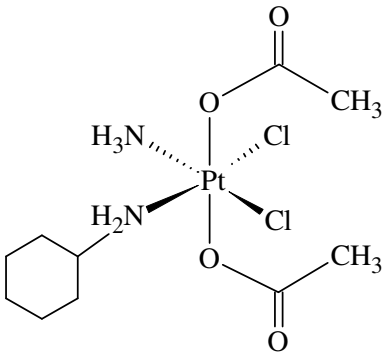
Up to date, four platinum(IV) compounds entered clinical trials (Figure 6).



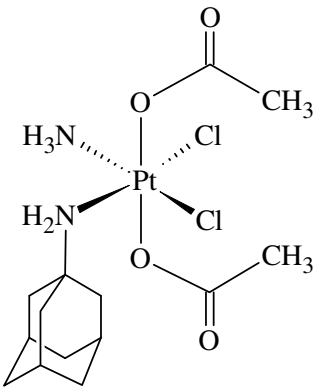
(11)



(12)



(13)



(14)

Figure 6: Platinum(IV) compounds: (11) iproplatin, (12) tetraplatin, (13) satraplatin and (14) LA-12

Iproplatin (CHIP, JM9) (11) is highly soluble and showed decreased toxicity, even so it was abandoned in phase III clinical trials, because of its decreased activity compared to

carboplatin. Recently, it was shown that iproplatin is not reduced, as expected, to *cis*-dichloro-platinum(II) and therefore interacts differently with the target DNA than cisplatin. [17, 66]

Tetraplatin (Ormaplatin) (12) was declined in phase I, although it affects a variety of tumour cells, especially several cisplatin-resistant ones. The reason for the abortion of the trials was the significant neurotoxicity. [17, 67]

Satraplatin (JM216) (13), currently in clinical investigation, is the first platinum compound, that could be administered orally. The resulting DNA adducts are not detected by mismatch repair proteins and therefore available for cisplatin-resistant tumours. [68, 69] Due to the fact that in phase III SPARC trial the overall survival rate was not significantly improved, GPC-Biotech decided not to deposit the application for approval by the FDA (US Food and Drug Administration). [70]

LA-12 (14), a satraplatin analogue with a very bulky adamantylamine ligand, has been found to be more active and in addition well-tolerated than cisplatin.
One reason for the high activity to induce apoptosis, as found by *Procháska et al*, is the inhibition of gap-junctional intercellular communication (GJIP). [11, 71, 72]

2.3.2 Mode of Action

It is generally believed that the kinetically inert platinum(IV) compounds have to be activated by reduction, through loss of their axial ligands, to the active platinum(II) species (Figure 7). They act therefore as prodrugs. Only the activated compound will bind to the targeted DNA and induce apoptosis. Essential is in this context at which point of metabolism reduction takes place. Extra-cellular activation leads to loss of an amount of drug by binding

[66] E. Volckova, E. Weaver; R. N. Bose; *Eur. J. Med. Chem.* **2008**, 43, 1081-1084
[67] R. J. Schilder; R. P. LaCreta; R. P. Perez, S. W. Johnson, J. M. Brennan; A. Rogatko; S. Nash; C. McAleer; T. C. Hamilton; D. Roby; R. C. Young; R. F. Ozols; P. J. O'Dwyer; *Cancer Res.* **1994**, 54; 709-717
[68] H. Choy; C. Park; M. Yao; *Clin Cancer Res.* **2008**; 14(6); 1633-1638
[69] L. K. Kelland; G. Abel; M. J. McKeage; M. Jones; P. M. Goddard; M. Valenti; B. A. Murrer; K. R. Harrap; *Cancer Res.* **1993**, 53, 25811-2586
[70] http://www.gpc-biotech.com/
[71] F. Žák, J. Turánek; A. Kroutil; P. Sova; A. Mistr; A. Poulová; P. Mikolin; Z. Žák, A. Kašná; D. Záluská; J. Neča; L. Šindlerová; A. Kozubík; *J. Med. Chem.* **2004**, 24, 761-763
[72] L. Procháska; J. Turánek; R. Tesařík; P. Knotigová; P. Polášková; Z. Andrysík; A. Kozubík; F. Žák; P. Sova; J. Neuzil; M. Machala; *Arch. Biochem. and Biophys.* **2007**, 462, 54-61

to sulphur containing proteins, as already seen for cisplatin and analogues. Therefore, it would be of advantage, if intra-cellular reduction happens so that immediate interaction with DNA can follow. Intra-cellular reduction implies a decrease of side effects and a smaller dosage of the administered drug. [11, 17, 73, 74, 75]

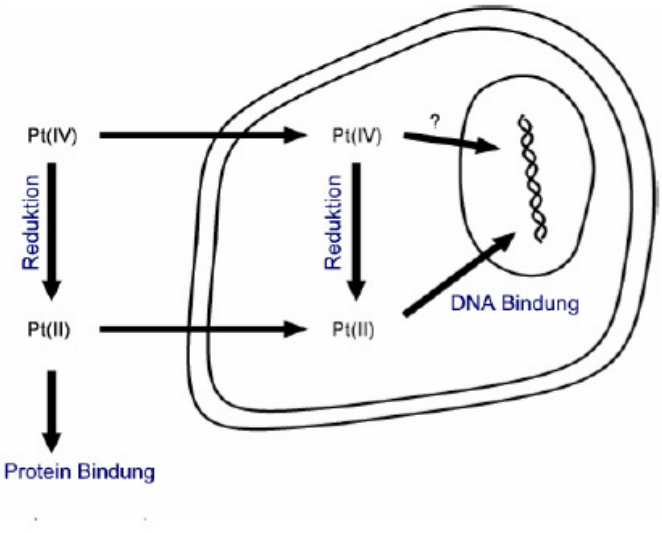


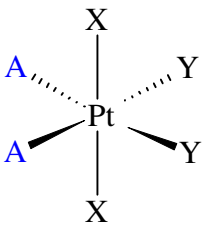
Figure 7: Mode of Action of platinum(IV) compounds[11]

However, it is not impossible that already the platinum(IV) species bind to blood-plasma proteins or even to DNA-bases. [76, 77, 78]

[73] M. D. Hall; S. Amjadi; M. Zhang; P. J. Beale; T. W. Hambley; *J. Inorg. Biochem* **2004**, 98, 1614-1624
[74] L. Pendyala; J. W. Cowens; G. B. Chheda; S. P. Dutta; P.j. Creaven; *Cancer Res.* **1988**, 44, 3533-3536
[75] M. D. Hall; R. A. Alderden; M. Zhang; P. j. Beale; Z. Cai; B. Lai; A. P. J. Stampfl; T. W. Hambley; *J. Struct. Biol.* **2006**, 155, 38-44
[76] L. T. Ellis; H. M. Er; T. W. Hambley; *Aust. J. Chem.* **1995**; 48, 793-806
[77] R. C. Dolman; G. B. Deacon; T.W. Hambley; *J. Inorg. Biochem.* **2002**, 88, 260-267
[78] M. Galanski; B. K. Keppler; *Inorg. Chim. Acta* 300-302; **2000**, 783-798

2.3.3 Structure-Activity Relationships

In general, platinum(IV) compounds consist of two axial ligands (X) that get lost upon reduction, two equatorial leaving groups (Y) for binding to DNA and furthermore two am(m)ine containing non-leaving groups (Figure 8).



(15)

Figure 8: octahedral platinum(IV) complex

Possible factors, like lipophilicity, reduction potential or cellular uptake, that influence the pharmacokinetics and activity of a drug, depend on the ligands and their position.

Especially the axial ligands alter the reduction potential, following the order Cl > OAc > OH, whereas complexes with axial chlorido ligands are reduced most easily.
Furthermore the size of the equatorial ligand influences the reduction rate, the bulkier the smaller the rate. When a drug is reduced easily the possibility of extra-cellular reduction is higher. [17, 73, 76, 79]

The lipophilic character of an anti-tumour compound is important for drug uptake, in that the ability of a lipophilic drug to enter the cell is significantly higher. This factor is mostly affected by the chain length of the axial carboxylato groups. Furthermore, it could be shown that with increasing lipophilicity cytotoxicity also increased. [17, 80]

The structure-activity relationships are a good guideline for rationally designing drugs with specific characteristics, in particular since platinum(IV) compounds have been found more active than their platinum(II) analogues. [81]

---

[79] S. Choi; C. Filotto; M. Bisanzo; S. Delaney; D. Lagasee; J. L. Withworth; A. Jusko; C. Li; N. A. Wood; J. Willingham; A. Schwenker; K. Spaulding; *Inorg. Chem.* **1998**; 37, 2500-2504
[80] L. R. Kelland; B. A. Murrer; G. Abel; C. M. Giandomenico; P. Mistry; K. R. Harrap; *Cancer Res.* **1992**, 52, 822-828
[81] G. N. Kaldujerović; D. Milijković, M. Momcilović; V. M. Djinović; M. M. Stoyković, T. J. Sabo; V. Trajković, *Int. J. Cancer* **2005**, 116, 479-486

3 Synthesis of Platinum Complexes

3.1 Synthesis of Platinum(II) Compounds

After discovering the anticancer activity of cisplatin, the synthesis was optimised by utilising the trans-effect, given that otherwise a mixture of *cis* and *trans* isomers is the result. The starting material is almost always potassium tetrachloroplatinate for all *cis* platinum(II) compounds that were synthesised since Rosenberg's discovery.

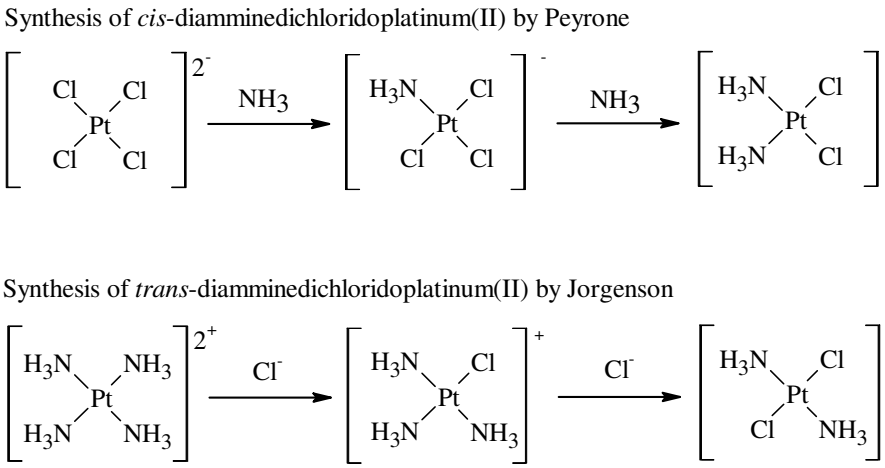


Figure 9: Preparation of cisplatin and the correspondent trans-complex

The trans-effect is a kinetic phenomenon and appears especially in square planar coordination complexes, although this effect can also be observed in octahedral ones. In general, the trans-effect is referable to an electronegative or other labilising ligand that weakens the bond in trans position to it. The trans directing ligand normally shows a very strong binding and therefore is difficult to substitute, whereas the labialised ligand can be replaced easily.[82]

[82] J. V. Quagliano; L. Schubert; *Chem. Rev.* **1952**, 50 (2), 201-260

Due to the high demands on clinically used drugs a further step in synthesis of cisplatin was established to minimise side-products and especially the formation of the trans isomer. This step includes the exchange of the chlorido to iodido ligands, taking advantage of the increased trans-effect of iodide. [83]

For synthesis of platinum(II) compounds with chelating ligands like ethane-1,2-diamine, the reaction directly takes place in aqueous solution without addition of iodido ligands. This can be explained by the single choice of binding in adjacent positions and therefore prevention of forming the trans isomer. [84]

3.2 Synthesis of Platinum(IV) Compounds

For a long time, the synthesis of platinum(IV) compounds was mainly limited to the oxidation of platinum(II) complexes. Further reactions were not performed according to the reduced reactivity and increased kinetic stability of platinum(IV) complexes.

3.2.1 Oxidation of Platinum(II) Compounds

Oxidation of platinum(II) compounds can take place basically via two ways (Figure 10):

(i) with hydrogen peroxide in aqueous solution to yield two axial hydroxido groups (A)

(ii) or with chlorine gas to obtain the tetrachloridoplatinum(IV) compound (B)

In addition, the dihydroxidoplatinum(IV) compound can be converted to the tetrachlorido analogue under extreme conditions such as high temperature and addition of concentrated hydrochloric acid (C). [85, 86, 87]

Nowadays, further derivatisation of the axial hydroxido ligands can occur because of their nucleophilic behaviour. Up to now, practicable reactions with tetrachloridoplatinum(IV) compounds are badly.

[83] R. N. Rhoda; J. N. Crosby, *U.S. Patent* **1981**
[84] H. D. K. Drew; *J. Chem. Soc.* **1932**, 2328-2331
[85] R. J. Brandon; J. C. Dabrowiak; *J. Med. Chem.* **1984**, 861-866
[86] J. F. Vollano; E. E. Blatter; J. C. Dabrowiak; *J. Am. Chem. Soc,* **1984**, 106, 2732-2733
[87] J. F. Vollano; S. Al-Baker; J. C. Dabrowiak; J. E. Schurig; *J. Med. Chem.* **1987**, 30, 716-719

3.2.2 Carboxylation Reported in Literature

Carboxylation with typical agents used in organic chemistry leads to lipophilic platinum(IV) compounds and is furthermore of particular interest for the combination with bioactive components, due to the fact that the axial ligands are released during activation of the prodrug.

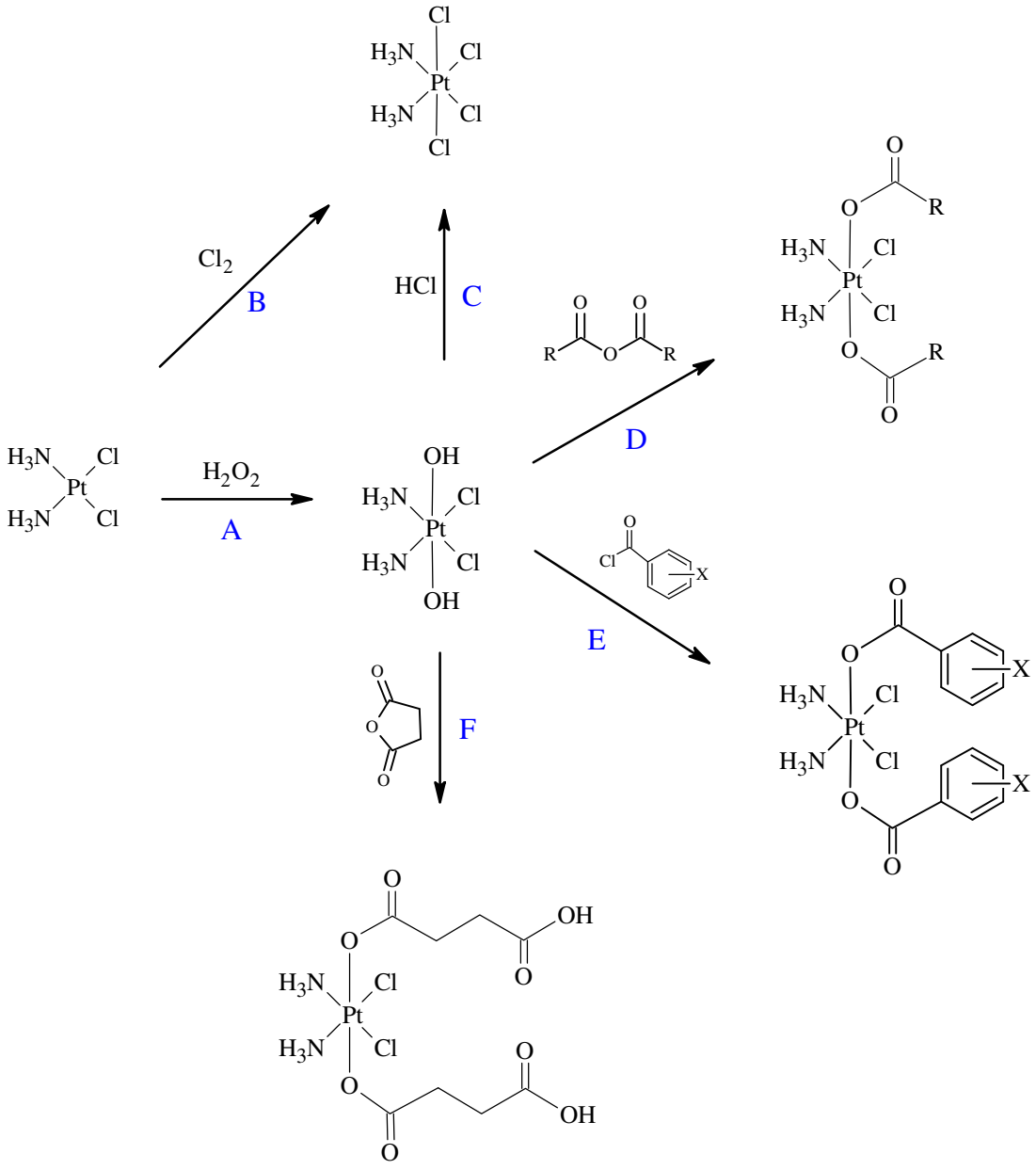
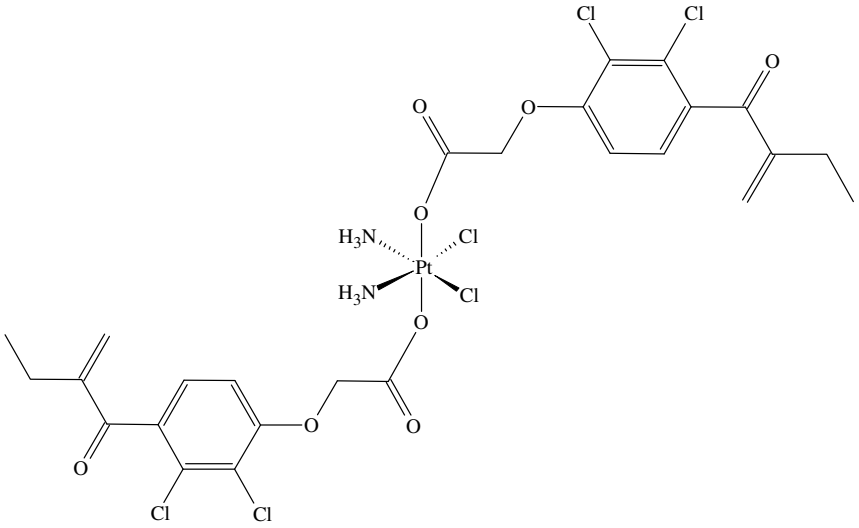


Figure 10: Synthesis of platinum(IV) compounds

Recently, a variety of publications appeared that deal with the carboxylation of axial hydroxido ligands by using various anhydrides and acyl chlorides (Figure 10). [88]

In 1995 Giandomenico *et al.* synthesised platinum(IV) carboxylates, carbonates and carbamates by carboxylation of the axial hydroxido groups with anhydrides, pyrocarbonates or isocyanates (D). This approach enables most notably the coupling with bioactive molecules. [89]

Shortly after, Galanski *et al.* published the successful derivatisation of the hydroxido ligands with fatty acyl chlorides of moderate reactivity in the presence of pyridine to trap released hydrochloric acid. [90]



(16)

Figure 11: Structure of ethacraplatin

Dyson and colleagues used this method to prepare aryl platinum(IV) complexes (E) showing improved cellular drug uptake due to the increased lipophilicity. [91] Furthermore, a platinum compound with ethacrynic acid as axial ligand (ethacraplatin (16)) was synthesised by using this synthetic procedure. Ethacrynic acid is a glutathione-S-transferase (GST) inhibitor, playing a central role in the mechanism of resistance against cisplatin. The synthesis

[88] M. D. Hall; H. R. Mellor; R. Callaghan; T. W. Hambley; *J. Med. Chem.* **2007**, 50, 1-9
[89] C. M. Giandomenico; M. J. Abrams; B. A. Murrer; J. F. Vollano; M. I. Rheinheimer; S: B. Wyer; G. E. Bossard; J. D. Higgins III; *Inorg. Chem.* **1995**, 34, 1015-1021
[90] M. Galanski; B. K. Keppler; *Inorg. Chem.* **1996**, 35, 1709-1711
[91] W. H. Ang; S. Pilet; R. Scopelliti; F. Bussy; L. Juillerat-Jeanneret; P. J. Dyson; *J. Med. Chem.* **2005**, 48, 8060-8069

of ethacraplatin was only possible without addition of an amine base. Activation of the prodrug leads to one equivalent cisplatin and two equivalents of ethacrynic acid. Ethacraplatin leads to a faster growth inhibition (after ~24 h) in different cancer cell lines compared to cisplatin (after ~72 h). [92]

Finally, the turnover with cyclic anhydrides afforded terminal uncoordinated carboxy groups (F) and therefore enables the accessebility for further reactions, which were first reported by Navarro-Ranninger *et al.*. [93] The optimisation of the reaction by changing the solvent from CH_2Cl_2 to DMF brought about increased yields and decreased reaction time, as shown by Keppler and co-workers. [94]

3.2.3 Further Derivatisation of the Terminal Carboxylic Acid

Straight forward, derivatisation of the uncoordinated free carboxylic group was first utilised by Lippard and colleagues. They coupled estrogen via an amide bond to the carboxylic acid.

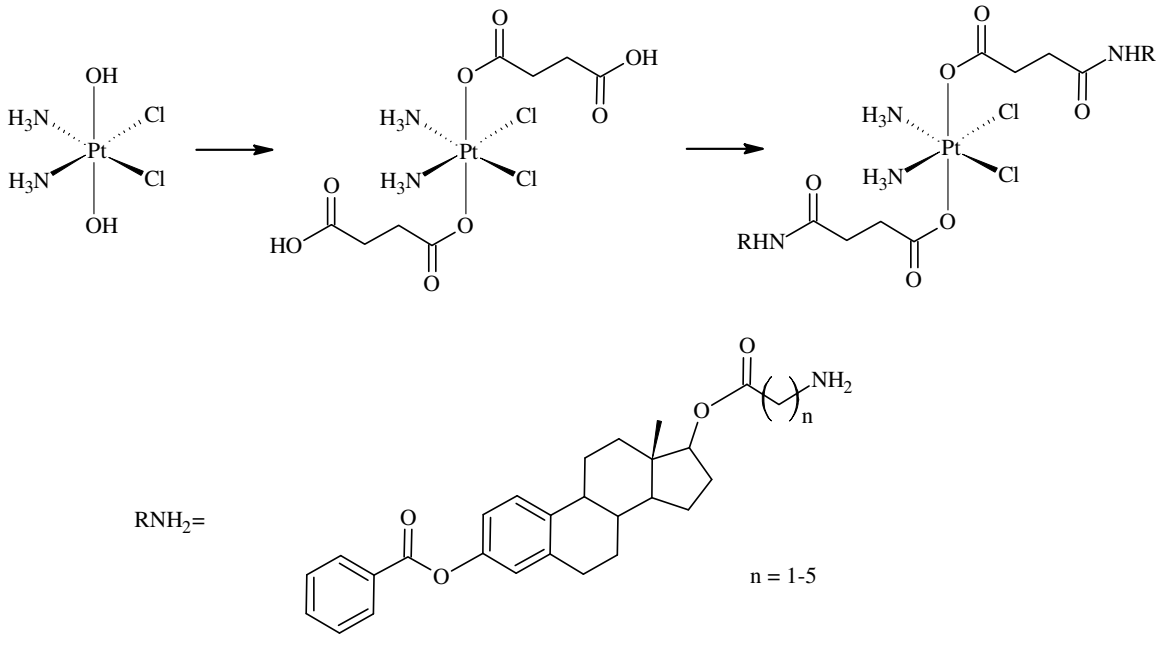


Figure 12: Scheme of synthesis: linking estrogen to platinum(IV) complexes

[92] W. H. Ang; I. Khalaila; C. S. Allardyce; L. Juillerat-Jeanneret; P. J. Dyson; *J. Am. Chem. Soc*. **2005**, 127, 1382-1383
[93] A, Alvarez-Valdés; J. M. Pérez; I. López-Solera; R. Lannegrand; J. M. Continente; P. Amo-Ochoa; M. H. Camazón; X. Solans; M. Font-Bardía; C. Navarro-Ranninger; *J. Med. Chem.* **2002**, 45, 1835-1844
[94] M. R. Reithofer; M. Galanski; A. Roller; B. K. Keppler; *Eur. J. Inorg. Chem.* **2006**, 2612-2617

The idea behind this strategy is based on the fact that estrogen sensitises ER(+) cells (estrogen receptor positive) to cisplatin. Estrogen causes overexpression of HMGB1 (high mobility group B1) and subsequently the HMBG1 protein protects cisplatin adducts from NER thereby increasing the efficiency of the drug. [95]

Keppler *et al*. then created a generic scheme for derivatisation of the free carboxylic acid with primary amines and alcohols to yield amides and esters. However, they used the formerly known peptide-coupling reagents 1,1'-carbonyldiimidazole (CDI) for activation of the carboxylic group. Additionally, the formation of esters requires a catalytic amount of a strong base, in this case the corresponding sodium alcoxide of the applied alcohol, while the amine reacts without addition of a strong base. [94, 96, 97] (for mechanism aspects see chapter 5.1.2)

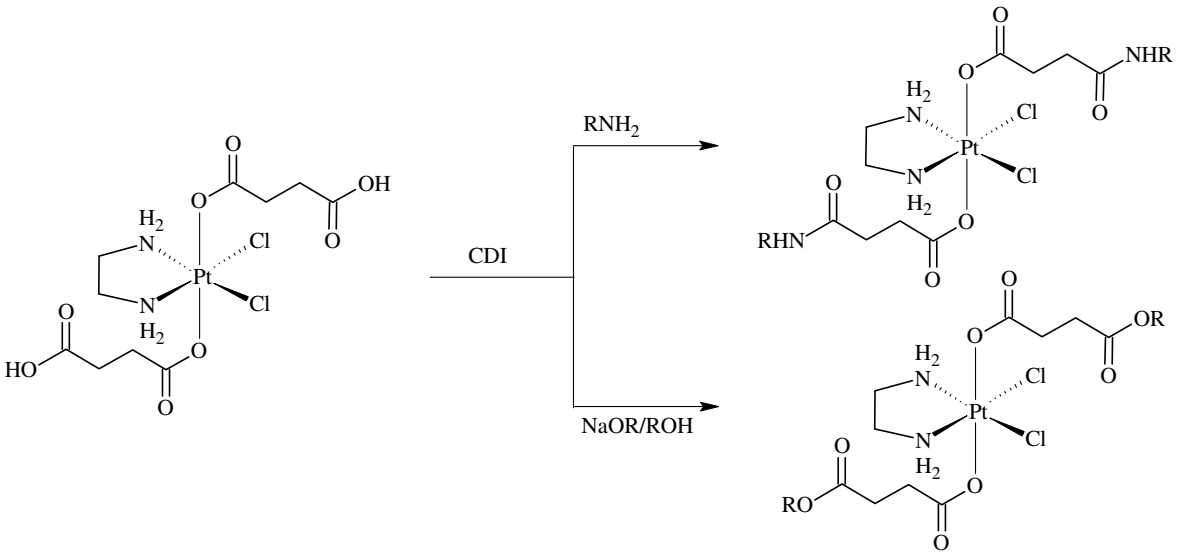


Figure 13: Derivatisation of bis(carboxylato)platinum(IV) complexes

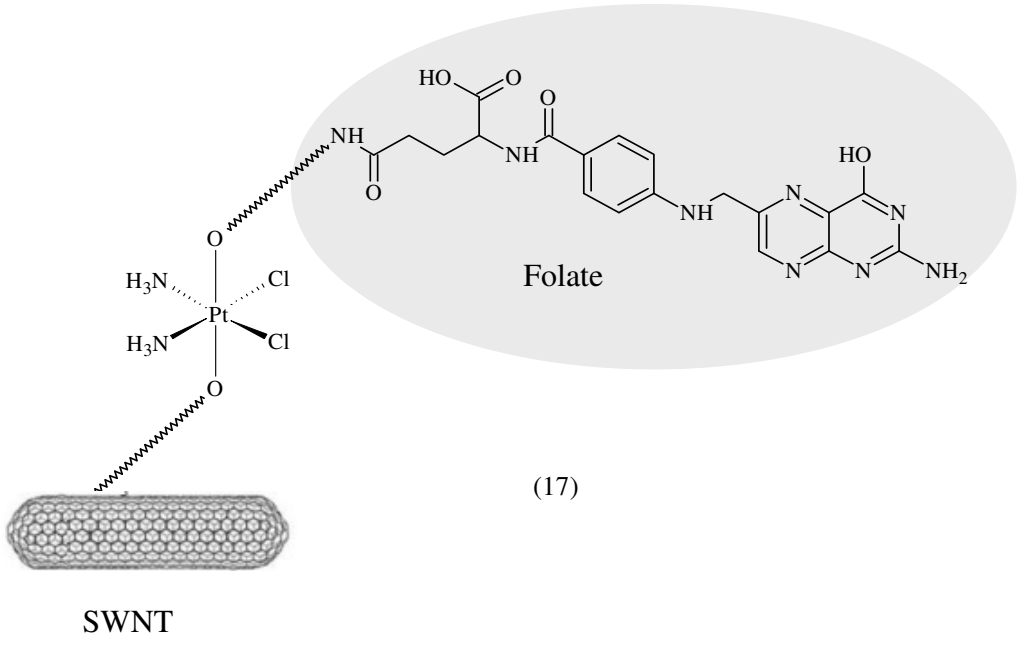
[95] K. R. Barnes. A. Kutikov; S. J. Lippard; *Chem. Biol.* **2004**, 557-564
[96] M. R. Reithofer; A. Schwarzinger; S. M. Valiahdi; M. Galanski; M. A. Jakupec; B. K. Keppler; *J. Inorg. Biochem*. 102, **2008**, 2072-2077
[97] M. R. Reithofer; S. M. Valiahdi; M. A. Jakupec; V. B. Arion; A. Egger; M. Galanski; B. K. Keppler; *J. Med. Chem.* **2007**, 50, 6692-6699

3.2.4 Recent Developments in the Synthesis of Platinum(IV) Complexes

Developments in the field of platinum(IV) compounds are numerous and therefore only a selection of interesting approaches is shown.

Minimising the loss of platinum(IV) drugs on the way to the targeted tumour is a particular aim and for this reason Lippard and co-workers coupled platinum(IV) complexes with water soluble single-walled carbon nanotubes (SWNTs). SWNTs function as effective transporting vehicles to get the drug into the cell. The synthesis was carried out by asymmetric oxidation of cisplatin with hydrogen peroxide in excess of ethanol to yield only one axial hydroxido ligand, whereas the second axial position is protected by an alkoxido ligand. Carboxylation of the free hydroxido ligand took place with succinic anhydride and further linkage by amide bonds to the SWNT surface. One SWNT incorporates an average of 65 platinum(IV) centers. [98]

In 2008, the same group expanded the system and coupled the platinum(IV) compounds with folate instead of the axial alkoxido protecting group. Folic acid acts as substrate of the folate receptor (α-FR) which was found to be overexpressed in a wide variety of tumours.



(17)

Figure 14: Targeted prodrug-delivery system

[98] R. P. Feazell, N. Nakayama-Ratchford, H. Dai; S. J. Lippard; *J. Am. Chem. Soc.* **2007**, 129, 8438-8439

So a very selective agent was produced; the delivery system increased the uptake into the cell and ensured subsequent targeting of cancer cells by the folate ligand. [99]

Furthermore, Lippard et al. synthesised some platinum(IV)-peptide complexes that show high affinity to the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$ and the membrane-spanning surface protein aminopeptidase N (APN or CD13). Integrins are involved in cell-cell and cell-matrix interactions as transmembrane-spanning receptors. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are overexpressed during tumour-induced angiogenesis of endothelial cells making them a possible target. The same applies to the aminopeptidase N. It was reported that, the tripeptide sequence RGD (Arg-Gly-Asp) and NGR (Asn-Gly-Arg) can be bound to these receptors effectively.

Accordingly, a mixture of mono- and disubstituted peptide complexes with this tripeptide sequences was prepared and subsequently separated via HPLC. As a reference the tripeptide AGR (Ala-Gly-Arg) which is known to bind unspecifically was used. [100]

3.3 Click Chemistry as Possible Source for Biologically Active Ligands

All times scientists have been occupied with the quest for effective procedures to produce highly demanded, common natural materials. Regarding nature's chemistry, an obvious preference for carbon-heteroatom bonds as opposed to carbon-carbon bonds can be observed.

In 2001, Sharpless *et al.* characterised "Click Chemistry" as a strong approach to generate molecules by composing small and easily available building blocks via carbon-heteroatom bond forming reactions. A reaction has to display a set of criteria to be suitable for Click Chemistry, as well-defined by Sharpless: the process has to be *"modular, wide in scope, high yielding, generate inoffensive by-products (removed without chromatography) and stereospecific, furthermore it should display simple reaction conditions, readily available starting materials and benign or easily removed solvents"*. [101]

There are several types of reactions which pass the "Click Chemistry Criteria", whereas the spectrum ranges from non-aldol carbonyl chemistry, carbon-carbon multiple bonds addition, nucleophilic substitution reactions, especially ring opening chemistry, to

[99] S. Dhar; Z. Liu; J. Thomale; H. Dai; S. J. Lippard; *J. Am. Chem. Soc.* **2008**, 130, 11467-11476
[100] S. Mukhopadhyay; C. M. Barnés; A. Hasekl; S. M. Short; K. R. Barnes; S. J. Lippard; *Bioconj. Chem.* **2008**, 19, 39-49
[101] H. C. Kolb; M. G. Finn; K. B. Sharpless; *Angew. Chem., Int. Ed.* **2001**, 40, 2004

cycloadditions of unsaturated molecules. The last group includes the Huisgen 1,3-dipolar cycloaddition which is the most potent Click Chemistry reaction. [100, 102, 103]

3.3.1 Huisgen 1,3-Dipolar Cycloaddition

Huisgen cycloadditions are reactions with two unsaturated molecules to yield five-membered heterocycles. The most powerful and popular of this family is the formation of 1,2,3-triazoles made of azides and alkynes.

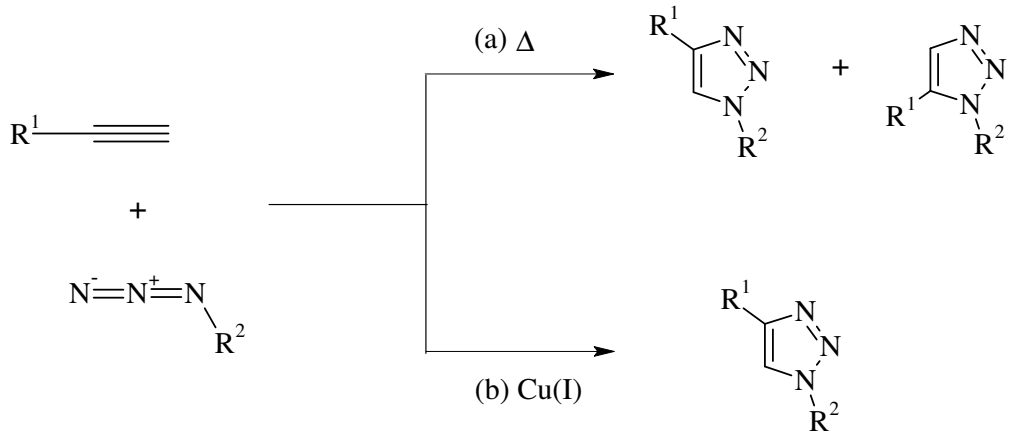


Figure 15: Huisgen-1,3-dipolar cycloadditions: (a) thermic conditions yield an approximate 1:1 ratio of anti and syn product; (b) copper(I)-catalysed Huisgen leads to the anti product.

The formation of the 1,2,3-triazole can occur either thermic, though high temperature and long reaction times are required and the result is a mixture of regioisomers, or copper(I)-catalysed, which offers increased reaction rate and regioselectivity (Figure 15). An enormous benefit is the stability of all reagents in water and in presence of atmospheric oxygen, which enables the reaction in water as a solvent.

The Cu(I) catalysed alkyne-azide coupling shows the possibility of a variety of different conditions without loss of reactivity. Copper in the oxidation state I is relatively instable in the presence of oxygen. Although the reaction takes place when directly adding Cu(I) salts (normally CuI or CuBr in presence of a base), triazole formation via reduction of Cu(II) salts ($CuSO_4$ reduced by ascorbic acid for example) or oxidation of metallic copper (nanosized Cu(0) activated by amine hydrochloride e.g.) is favoured. For reactions sensitive to redox-systems, the possibility of ligand-assisted Cu(I) 1,3-cycloaddition exists. The ligand has the

[102] J. E. Moses; A. D. Moorhouse; *Chem. Soc. Rev.* **2007**, 36, 1249-1262
[103] V. D. Bock; H. Hiemstra; J. H. van Maarseveen; *Eur. J. Org. Chem*. **2006**, 51-68

task to stabilise the oxidation state, whereas the problem is to choose a ligand which is neither too labile to lose the stabilisation potential nor too strong so that the catalytic activity is lost. [103]

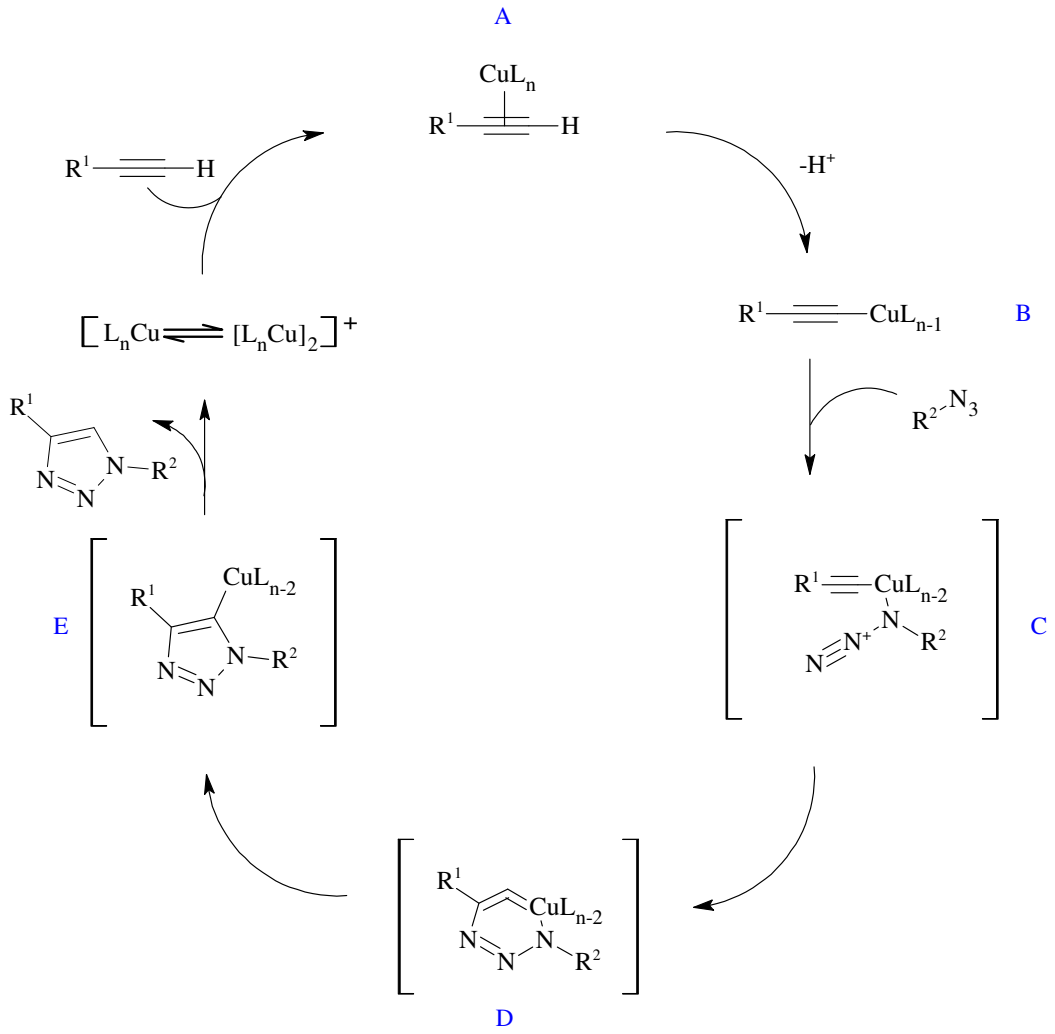


Figure 16: Catalytic cycle of Cu(I) assistant click chemistry

It exist a proposed stepwise mechanism for click chemistry supported by copper being based on kinetic studies. The first step is the formation of a copper-acetylene π complex (A) and subsequent ligand dissociation yields the acetylide (B). Then the azide displaces a further ligand (C). Benefited of the complexation a metallocycle (D) can be built and afterwards a

transformation to the triazole (E) takes place. The final step is regeneration of the catalyst. [103 -105]

The mechanism demonstrates that only terminal alkynes can react in this way, furthermore formation of the copper-acetylene π complex explains the high tolerance against a variety of functional groups. It has to be mentioned that on the contrary the thermal cycloaddition favours a concerted mechanism.

Due to the toxicity and the potential explosiveness, azide chemistry was neglected for a long time and nowadays experiences a renaissance. This may be caused by the fact that 1,2,3-triazole derivatives offer a diversity of biological activities, like HIV inhibition [106] or as antibacterial agents. [107]

[104] V. R. Rostovtsev; L. G. Green; V. V. Fokin; K. B. Sharpless; *Angew. Chem. Int. Ed.* **2002**, 41
[105] F. Himo; T. Lovell; R. Hilgraf; V. V. Rostovtsev; L. Noodleman; K. B. Sharpless; V. V. Fokin; *J. Am. Chem. Soc.* **2005**; 127, 210-216
[106] R. Alvarez; S. Velazquez; F. San; S. Aquaro; C. De; C. Perno; A. Karlsson; J. Balzarini; M. H. Camarasa; *J. Med. Chem.* **1994**, 37, 4185-4194
[107] M. J. Genin; D. A. Allwine; D. J. Anderson; M. R. Barbachyn; D. E. Emmert; S. A. Garmon; D. R. Grabner; K. C. Gregy; J. B. Hester; D. K. Hutchinson; J. Morris; R. J. Reischer; C. W. Ford; G. E. Zurenko; J. C. Hamel; R. D. Schaadt, D. Stapert; B. H. Yagi; *J. Med. Chem.* **2000**, 43, 953-970

4 Research Objective

The objective of this diploma work was the development and characterisation of novel platinum(IV) complexes. The focus was on the synthesis of near derivatives of already investigated compounds to examine consequences in small variances in structure such as methyl groups at different positions.

The synthesis included the oxidation of platinum(II) to *trans*-dihydroxido compounds, transformation of the resulting compounds with succinic anhydride to obtain uncoordinated, free carboxylic acids, which were further used for derivatisation with support of CDI to receive the corresponding amides and esters.

Furthermore, the challenge was to produce 1,2,3-triazoles as ligands by performing the Huisgen 1,3-dipolar cycloaddition of platinum(IV) alkyne derivatives. Triazoles are interesting ligands for coupling with platinum(IV) complexes because of their reported biological activity.

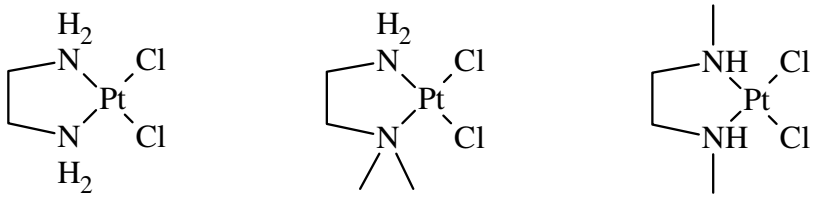
The structures of the resulting complexes could be verified by elemental analysis, ESI-MS, one- and two-dimensional multinuclear NMR spectroscopy (^{1}H, ^{13}C, ^{195}Pt) and IR spectroscopy.

5 Results and Discussion

5.1 Synthesis

The origin of this work was given by three platinum(II) compounds, all of which were synthesised via established synthetic routes described in chapter 3:

- (SP-4-2)-Dichlorido(ethane-1,2-diamine)platinum(II) (18)
- (SP-4-3)-Dichlorido(N,N-dimethylethane-1,2-diamine)platinum(II) (19)
- (SP-4-2)/(SP-4-3)-Dichlorido(N,N'-dimethylethane-1,2-diamine)platinum(II) (20)



(18) (19) (20)

Figure 17: Platinum(II) compounds used as starting complexes

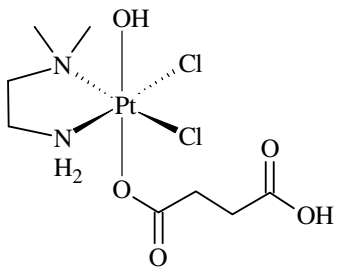
The starting material was $K_2[PtCl_4]$ that was stirred in an aqueous solution with corresponding diamines to gain the platinum(II) compounds, followed by oxidation with 30% H_2O_2. It has to be pointed out that there are great differences in the solubility of the obtained dihydroxido compounds in water. While the ethane-1,2-diamine derivative precipitated from the reaction mixture, the methylated compounds had to be isolated by removing the solvent.

5.1.1 Carboxylation

For carboxylation of the dihydroxido compounds, succinic anhydride was used as reported by Reithofer *et al.*.

Taking into consideration that methyl groups led to sterical hindrance of the hydroxido groups, various products could be achieved. Carboxylation of *enPtCl$_2$(OH)$_2$* brought about only the dicarboxylated product, whereas *N,N-dmenPtCl$_2$(OH)$_2$* exclusively became mono-carboxylated (21). This result can be attributed to the axial position of the methyl group. A particular challenge was the N,N'-dimethylethane-1,2-diamine derivative, due to the fact of the present stereoisomeres. The ratio of the diastereomeres of 1:1.4 could be deduced from the ^{1}H NMR of the dihydroxidoplatinum(IV) complex (see chapter 5.2.4). After reaction with the anhydride a mixture of di- and monocarboxylated product could be isolated, unfortunately the stereoisomeres could not yet be separated.

The whole synthesis was carried out in DMF as a polar solvent and at 70 °C. During the reaction time of about 24 h, the starting suspension turned clear to indicate the end of the reaction. After removing the solvent, the residue was dissolved in acetone, filtrated and the product could be isolated by precipitating with diethylether. No changes in reaction time or condition comparing the three dihydroxido complexes could be observed.



(21)

Figure 18: Monocarboxylated product.

5.1.2 Formation of Amides

For the formation of amides a nucleophilic attack of the carboxylic group was required. Better yields could be achieved by activating the carboxylic acid with CDI, a compound that is widely spread as a peptide coupling reagent and in organic synthesis. [108, 109] The splitting off of CO_2 and imidazole shifted the reaction equilibrium to the formation of the imidazolide intermediate. This intermediate is easily attacked by primary amines for example.

[108] H. A. Staab; *Justus Liebigs Annalen der Chemie* **1957**, 609, 75-83
[109] R. Paul; G. W. Anderson; *J. Am. Chem. Soc.* **1960**, 82, 4596-4600

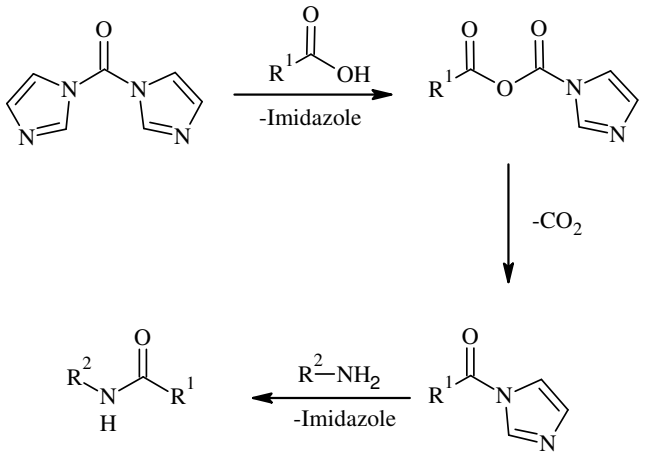


Figure 19: Formation of an amide bond with primary amines.

Due to the obviously lower reactivity of secondary amines another synthetic pathway for formation of the amide bond was chosen. Activation of the amine took place by treatment with CDI and subsequent reaction with methyliodide. The corresponding carbamoylimidazole suffered from significant inertness against nucleophilic attack, therefore a further step with methyliodide to gain the carbonylimidazolium salt was required. [110] The salt readily reacted with the platinum(IV) carboxylic acid under mild condition (stirring at 60°C) in the presence of a base.

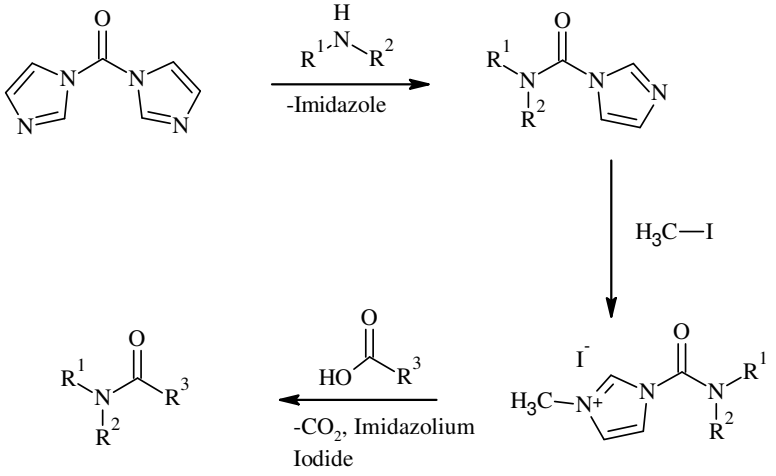


Figure 20: Formation of an amide bond with secondary amines.

This procedure was successfully applied to the synthesis of *enPtCl$_2$(C$_6$H$_{10}$NO$_3$)$_2$* with moderate yields.

[110] J. A Grzyb, M. Shen, C. Yoshina-Ishii, W. Chi, R. S. Brown, R. A. Batey; *Tetrahedron 61* **2005**, 7153-7175

5.1.3 Formation of the Ester

Transformation of the imidazolide intermediate with an alcohol was aggravated because of its minor nucleophilicity, hence the alcohol had to be deprotonated. The method of choice was the preparation of an alkoxide solution with metallic sodium, whereas only a catalytic amount was required. This can be accounted to the formation of the sodium salt of imidazole that was in equilibrium with the alkoxide

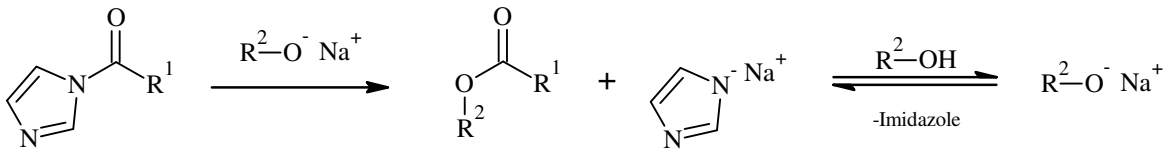


Figure 21: Catalytic process of esterification with sodium alkoxide

A disadvantage of this method of esterification is that alcohols with other functional groups could prefer side reactions, as obtained in the case of propargyl alcohol. The alkyne group of propargyl alcohol shows a tendency for polymerisation in the presence of a base or heat. Another previously published method making use of propargyl bromide and K_2CO_3 was also not successful. [111]

5.1.4 Synthesis of a 1,2,3-Triazole Derivative

The presence of platinum restricted the choise of different conditions for click chemistry. The fact that platinum(IV) could easily be reduced made it impossible to use methods that were carried out via reduction of Cu(II). Consequently the only two ways for performing this reaction with platinum(IV) complexes were on the one hand the thermal type or on the other hand based on ligand assisted Cu(I).

The starting component $enPtCl_2(C_7H_8O_3N)_2$ was prepared by Michael Reithofer and placed at the disposal. Phenyl azide was used as azide component and was prepared via standard literature procedures. [112]

---

[111] R. Berscheid; M. Nieger; F. Voegtle; *Chem Ber.* **1992**, 125(11), 2539-1552
[112] A. B. Theocharis; N. E. Alexandrou; A. Terzis, *J. Heterocyc. Chem.* **1990**, 27, 1741-1744

The thermal dipolar cycloaddition was carried out in aqueous solution at 100°C, within an hour, the solution turned black. Neither substrate nor product could be isolated, consequently the presumption was that decomposition took place. [113]

For reaction with ligand assisted copper(I) the first choice was TBTA (tris(triazolylmethyl)amine) as ligand. [114] Initially a copper-TBTA complex had to be prepared to stabilise the oxidation state I of copper. Therefore, $CuCl_2*2H_2O$ was added to a solution of TBTA in CH_2Cl_2 and the mixture was refluxed for 3 h. The result was a green solid of the complex $[Cu(Cl(TBTA))]Cl*1.5H_2O$. This complex subsequently was added to a solution of alkyne and azide in DMSO:H_2O (2:1) and was stirred at rt for 24 h. Unfortunately, the reaction did not take place and the starting substances were isolated.

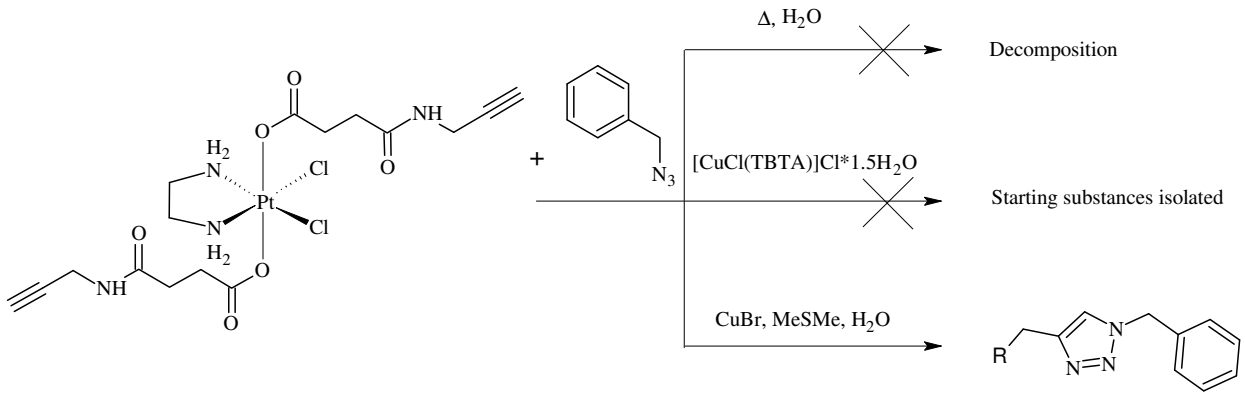


Figure 22: Conducted experiments of Click Chemistry

Another method, that combined Cu(I)Br with sulphur containing ligands in water as a solvent was evaluated. In this case, the azide and alkyne were suspended in water and 5 mol% Cu(I)Br and 10 mol% of dimethylsulfide were added. After stirring for 4 h, a small amount of triazole (less than 5% yield) could be isolated. By increasing the reaction time to about 24 h, the yield could be raised noticeable (~ 50%). Compared to published reaction rates for organic substrates, times were in the range of 5 min to 2 h, which means an enormous decrease of the reaction rate for platinum complexes. [115] Additionally, due to the insolubility of the isolated crude product (only soluble in DMSO and DMF) further purification could not be realised.

[113] Z.-X. Wang; H.-L. Qin; *Chem. Comm.* **2003**, 2450-2451
[114] P. S. Donnelly; S. D. Zanatta; S. C. Zammit; J. M. White; S. J. Williams; *Chem. Comm.* **2008**, 2459-2461
[115] F. Wang, H. Fu; Y. Jiang; Y. Zhao; *Green Chem.* **2008**, 10, 452-456

The observed broad NMR-signals of the product allowed the presumption that possibly complexation of copper by the product took place.

However, the preparation of the close analogue of $enPtCl_2(C_7H_8O_3N)_2$ with propargyl alcohol was not successful because of the problem of polymerisation (see chapter 5.1.3). Nevertheless, 1,3 cycloaddition of propargyl alcohol with phenyl azide to yield the corresponding triazole as white crystals was successful. The free alcohol group could afterwards be coupled to $enPtCl_2(C_4H_5O_4)_2$ trough an ester bond. Likewise, the obtained substance could not be purified.

5.2 Characterisation

The synthesised platinum complexes were characterised by elemental analysis, electrospray ionisation mass spectrometry (ESI MS), infrared spectroscopy (IR) and multinuclear NMR spectroscopy (^{1}H, ^{13}C, ^{195}Pt). The spectroscopic data are discussed in the following sections.

5.2.1 Elemental Analysis

All synthesised compounds were analysed for their percentage composition of the elements C, H and N.

Table 2: Elemental analysis data of selected compounds

Compound		C	H	N
$enPtCl_2$	Calculated	7.37	2.47	8.59
	Found	7.46	2.36	8.47
N,N-$dmenPtCl_2$	Calculated	13.57	3.42	7.91
	Found	13.40	3.05	7.92
N,N'-$dmenPtCl_2$	Calculated	13.57	3.42	7.91
	Found	13.61	3.18	7.84
$enPtCl_2(OH)_2$	Calculated	6.67	2.80	7.78
	Found	6.41	2.51	7.42
N,N-$dmenPtCl_2(OH)_2$	Calculated	12.38	3.64	7.22
	Found	12.36	3.49	6.95
N,N'-$dmenPtCl_2(OH)_2$	Calculated	12.38	3.64	7.22
	Found	12.27	3.46	6.91
$enPtCl_2OH(C_4H_5O_4)$	Calculated	21.44	3.24	5.00
	Found	21.25	2.97	5.11
N,N-$dmenPtCl_2OH(C_4H_5O_4)$	Calculated	19.68	3.72	5.74
	Found	19.95	3.63	5.48
$enPtCl_2(C_6H_9O_4)_2$	Calculated	27.28	4.25	4.55
	Found	26.63	4.09	4.35
$enPtCl_2(C_7H_{12}O_3N)_2$	Calculated	29.91	5.02	8.72
	Found	30.00	4.86	8.30
$enPtCl_2(C_6H_{10}O_3N)_2$	Calculated	26.59	4.78	8.86
	Found	26.47	4.64	8.61
N,N-$dmenPtCl_2OH(C_5H_7O_4)$	Calculated	21.52	4.01	5.58
	Found	21.50	3.77	5.12

5.2.2 ESI-MS spectra

ESI is a soft ionisation method and therefore allows non-destructive detection of molecules. These mass spectra show most of the mono-adducts that were formed with the matrix, like H^+, Na^+ or K^+ adducts in positive ion mode or Cl^- in negative.

Table 3: Mass spectra data of selected compounds

Compound	M/[g/mol]	$[M+H^+]^+$	$[M+Na^+]^+$	$[M+K^+]^+$
$enPtCl_2OH(C_4H_5O_4)$	560.3	561.5	582.8	598.7
N,N-dmen-$PtCl_2OH(C_4H_5O_4)$	488.2	-	511.0	-
$enPtCl_2(C_7H_{12}O_3N)_2$	642.4	-	663.9	679.1
$enPtCl_2(C_6H_9O_4)_2$	616.4	-	637.9	654.8
$enPtCl_2(C_6H_{10}O_3N)_2$	614.4	-	637.1	-
N,N-dmen-$PtCl_2OH(C_5H_7O_4)$	502.3	-	525.0	-
N,N-dmen-$PtCl_2OH(C_6H_9O_3N)$	515.3	-	537.9	553.9

5.2.3 Infrared Spectroscopy

IR spectroscopy is a useful method for quickly determining of a reaction's success, especially the purity of compounds can be ascertained. The most important advantage of this method is the independency from the solubility of the compound, by reason that measurements are possible in all aggregate states. In particular for $enPtCl_2(OH)_2$ IR was the only method for structure-determination, beside elemental analysis, due to its insolubility.

The complexation of ligands to a platinum centre has a remarkable effect on measured wavelengths. Depending on the distance to the platinum, ligands are shifted to lower wavelengths (reddish range) as observed for the C=O bond (general 1750-1735 cm^{-1}, platinum bound 1650 cm^{-1}) or the directly coordinated amines (general 3500-3300 cm^{-1}, platinum bound 3200 cm^{-1}) for example.

Oxidation with Hydrogenperoxid

The obtained hydroxido groups of the platinum(IV) compound after oxidation with hydrogen peroxide (see Figure 23) display one very characteristic peak at 3538 cm^{-1}, that is attributable to the Pt-OH stretching vibration, the shape of which is very sharp and intense.

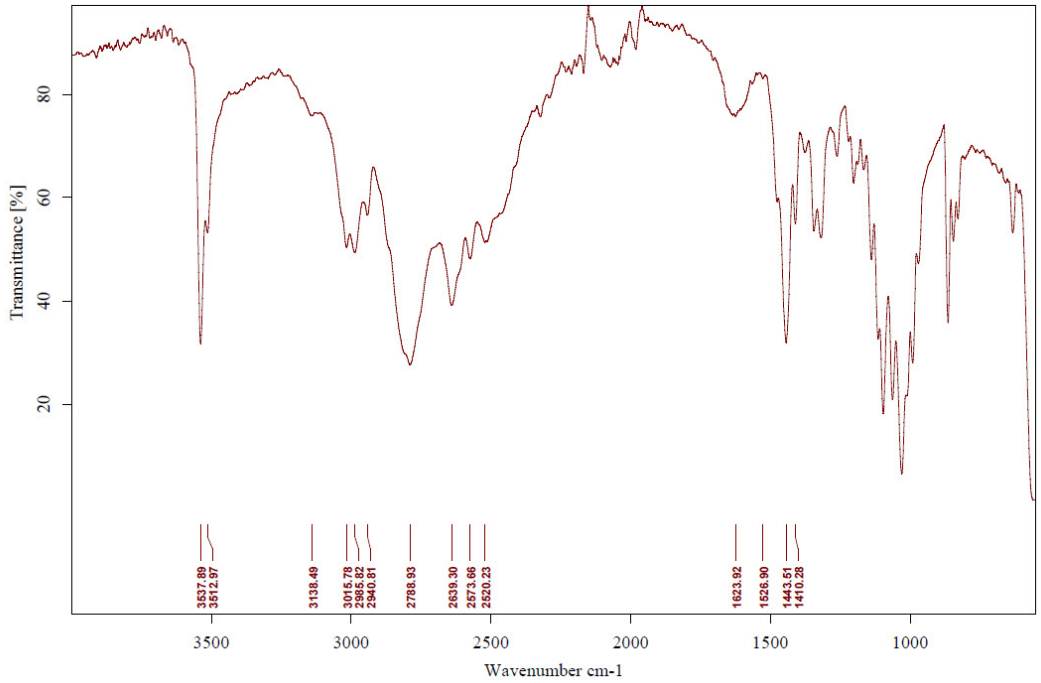


Figure 23: IR-spectrum of *N,N'-dmenPtCl₂(OH)₂*

Acylation of the Dihydroxido Compounds

IR spectroscopy is particularly useful for reaction control of the turnover of dihydroxido complexes with cyclic anhydrides. Here, the Pt-OH vibration disappears and the C=O peak between 1730-1600 cm^{-1} arises, if dicarboxylation takes place.

The spectrum of the asymmetric product (see Figure 24) shows as expected both the Pt-OH and the C=O signal. The broad absorption in the range of 3300-2700 cm^{-1} results from the amine, amide and saturated alkyl groups and is by reason of interferences not qualified for analysis.

However, it can be determined whether the product is clean, as the substrate succinic anhydride has an absorption maximum typical at 1787 cm^{-1} from C=O vibration and the succinic acid at 1693 cm^{-1}.

Furthermore, the asymmetric vibrations of the C=O groups can be well differentiated. The peak at 1726 cm^{-1} can be referred to the uncoordinated carboxylic acid, whereas the shoulder at 1662 cm^{-1} is attributable to the coordinated one.

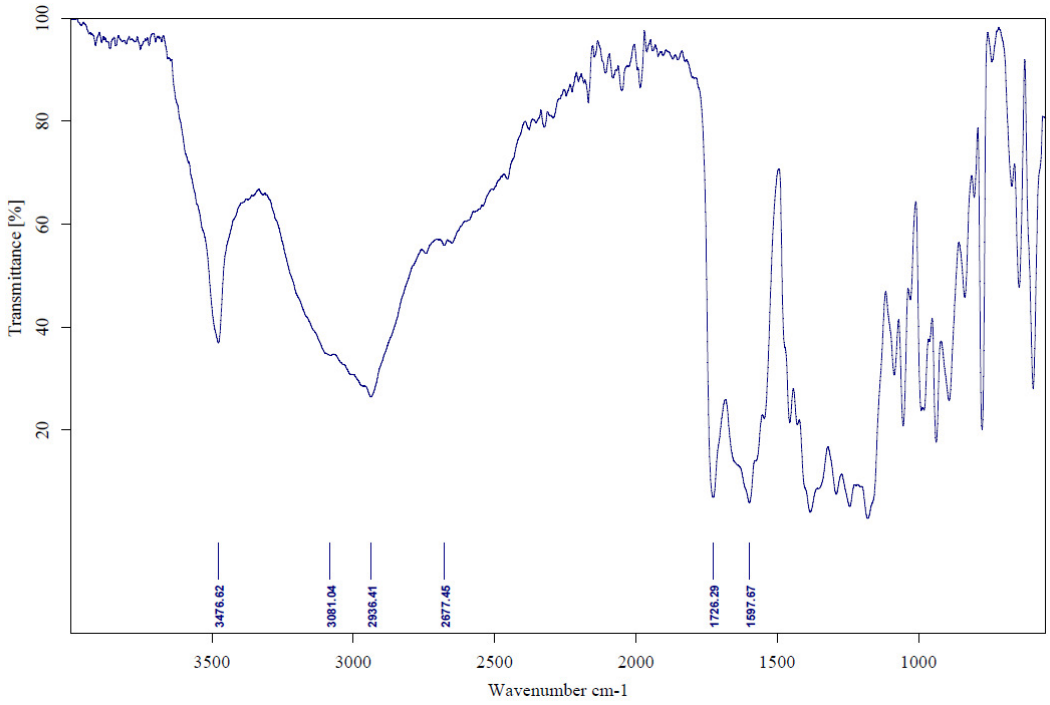


Figure 24: IR-spectrum of *N,N-dmenPtCl$_2$OH(C$_4$H$_5$O$_4$)*

Derivatisation of the Free Carboxylic Acid

Figure 25 shows the spectra of the two synthesised derivatives of $enPtCl_2(C_4H_5O_4)_2$. Both the ester ($(enPtCl_2(C_6H_9O_4)$ red graph) and the amide ($(enPtCl_2(C_7H_{12}O_3N)_2)$ blue graph) have an absorption maximum at about 3200 cm^{-1} that can be assigned to the N-H vibration. Differences can be illustrated through the varying C=O asymmetric stretches. These vibrations can be clearly differentiated by looking at the ester spectrum (ester: 1714 cm^{-1}, platinum coordinated carboxylic acid: 1643 cm^{-1}) while the two C=O peaks of the amide overlap at about 1738 cm^{-1} and only a small shoulder can be seen.

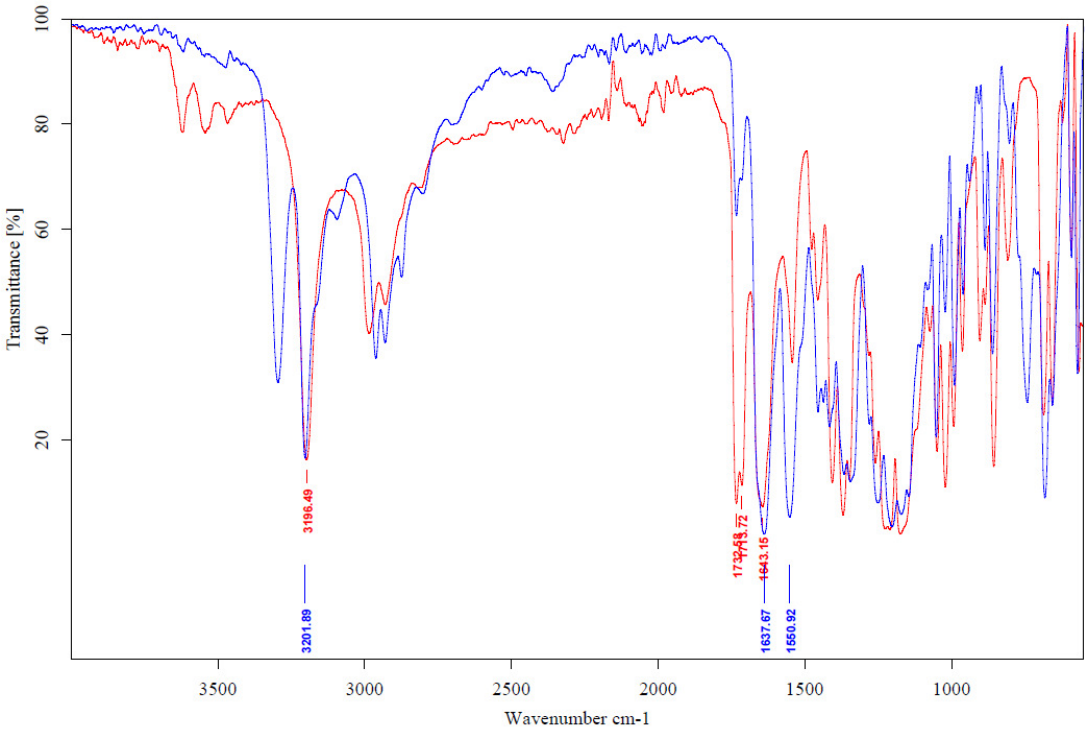


Figure 25: IR-spectra of $enPtCl_2(C_5H_7O_4)_2$ and $enPtCl_2OH(C_6O_3H_{10}N)_2$

5.2.4 NMR Spectroscopy

5.2.4.1 Asymmetric Platinum(IV) Complexes

Since there are only a few asymmetric platinum(IV) compounds published, the focus will be put on the discussion of NMR data of *N,N-dmenPtCl$_2$OH(C$_4$H$_5$O$_4$)* (21) and its methylester.

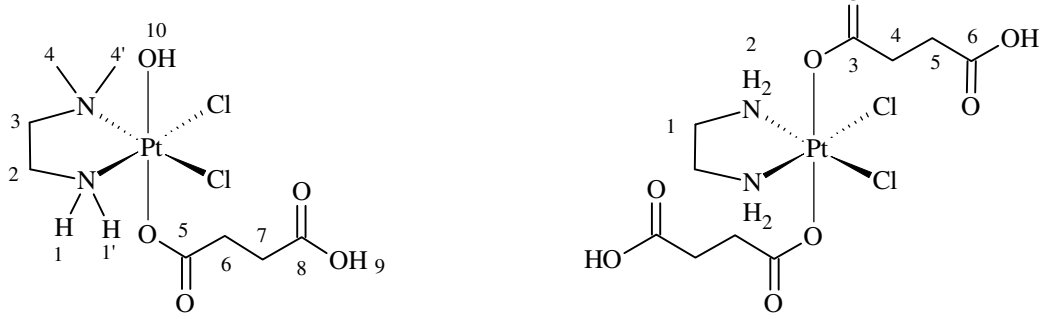
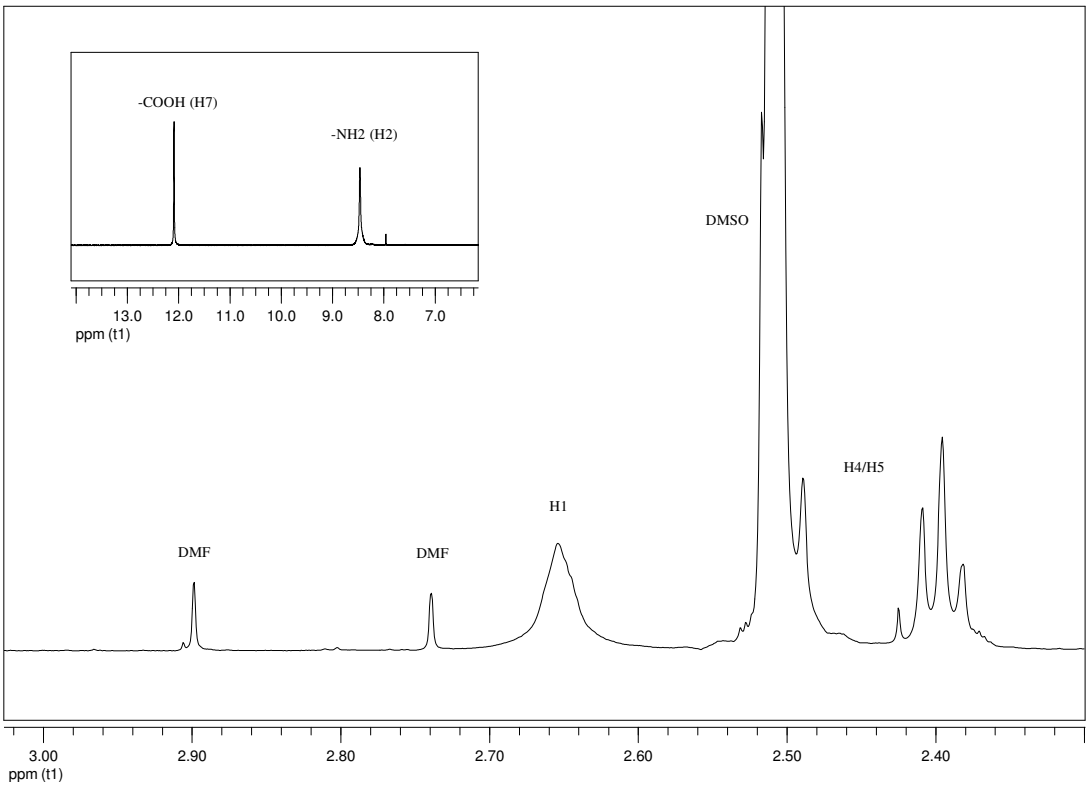
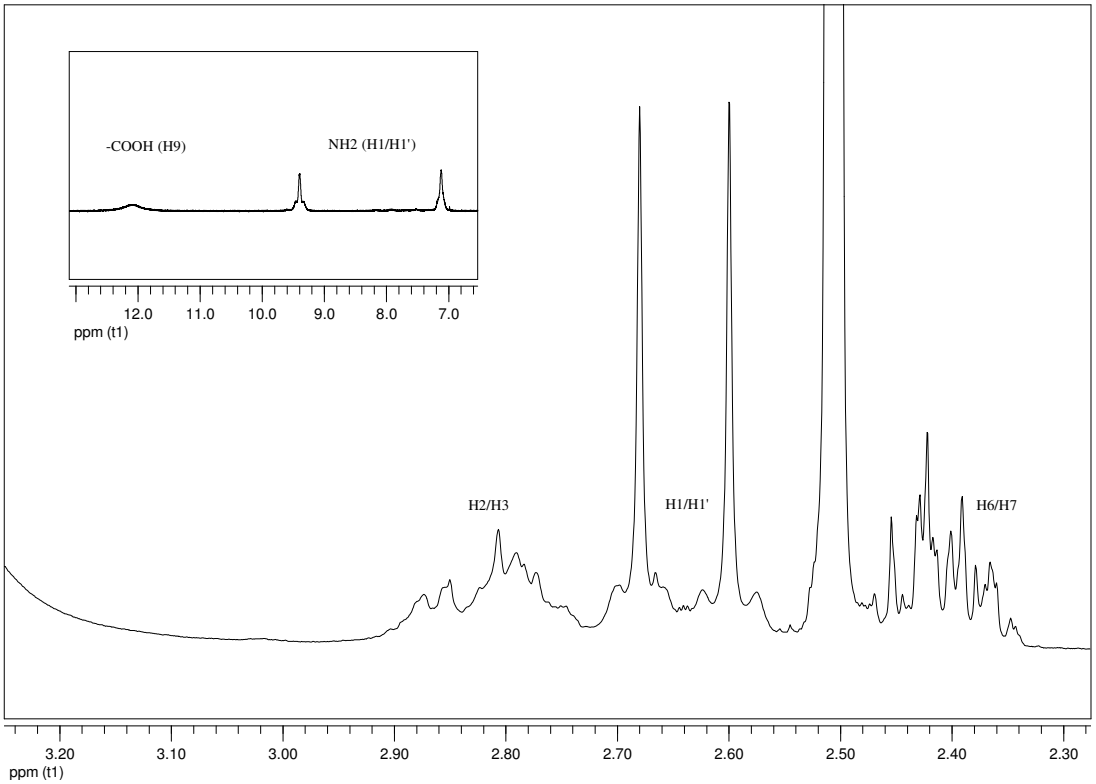


Figure 26: Comparison of symmetric and asymmetric platinum(IV) compound

In comparison to *enPtCl$_2$(C$_4$H$_5$O$_4$)$_2$* there is a break in symmetry. Consequently, the signal reduction that occurred in highly symmetric compounds is abrogated. The platinum centre functions as a chiral centre because of the presence of four different ligands. Therefore the amine protons (1 + 1') and methyl groups (4 + 4') became differentiable.



^{1}H spectrum of $enPtCl_2(C_4H_5O_4)_2$ (22)



^{1}H spectrum *N,N-dmen*$PtCl_2OH(C_4H_5O_4)$ (21)

Figure 27: Comparison of ^{1}H spectra of a symmetric and asymmetric platinum(IV) compound.

Interpretation of the ^{1}H spectra without 2D-data is ambiguous; especially the adjacent methylene groups (C2/C3 and C6/C7) revealed very broad multipletts.

Owing to the complexation of a platinum(IV) centre, platinum satellites of the methyl-signals can be observed. The broadening of the multiplet shifted to lower field is attributable to the relaxation time of amines, and therefore can be associated with C2/C3.

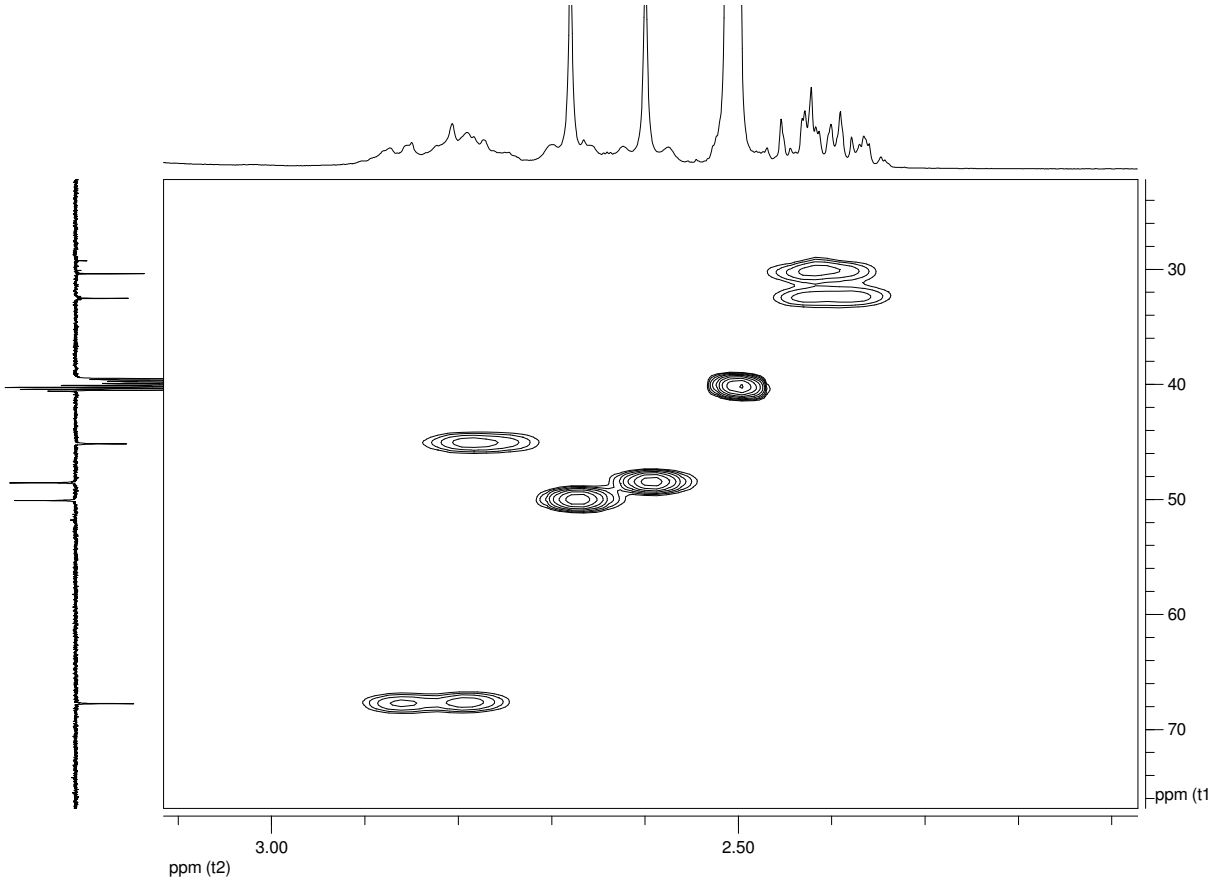


Figure 28: Detail of HSQC spectrum of *N,N-dmenPtCl$_2$OH(C$_4$H$_5$O$_4$)* (21)

By the use of HSQC and HMBC spectra the carbon signals could be assigned readily except for C6/C7. The differentiation of C2/C3 can be made considering the cross peaks of the carbon signal at 67.8 ppm (C3) to the methyl groups, whereas the signal at 45.2 ppm (C2) does not show these cross peaks.

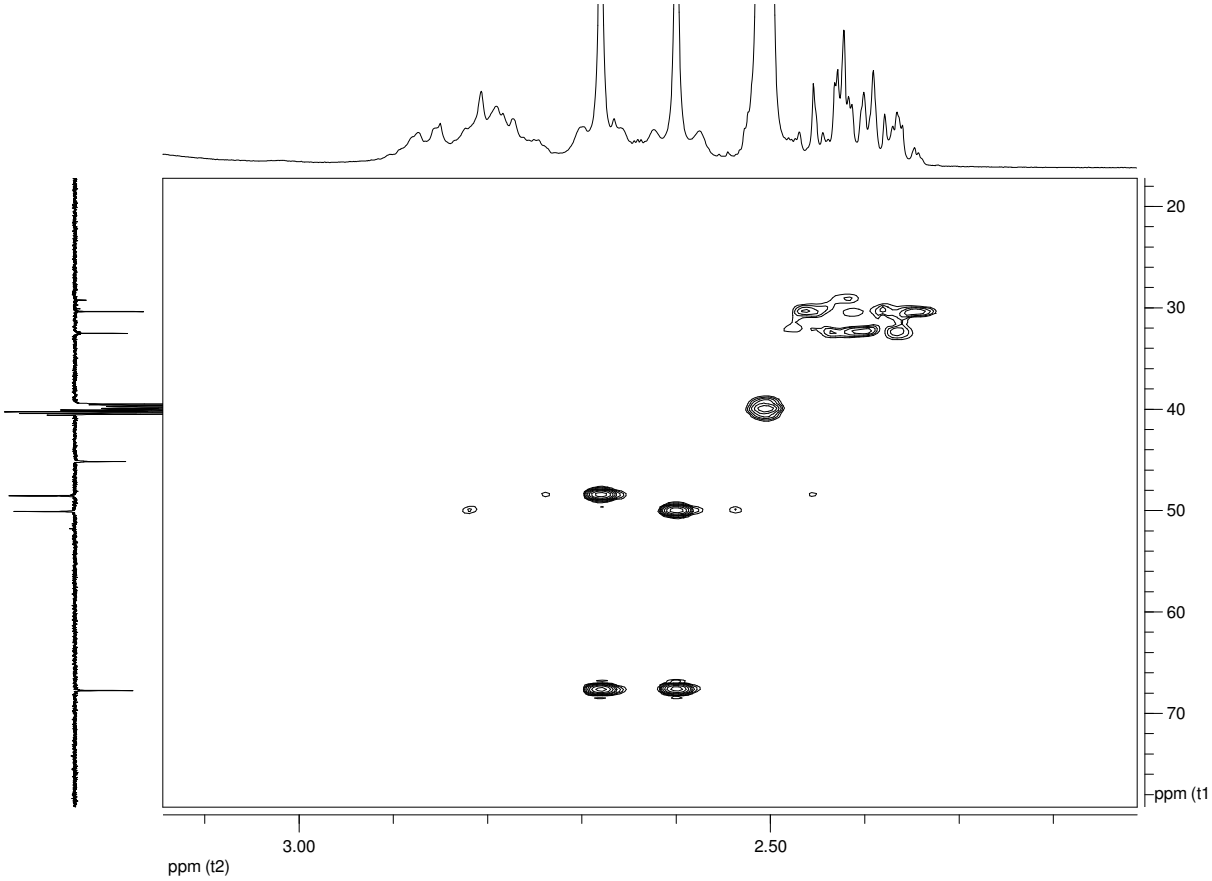
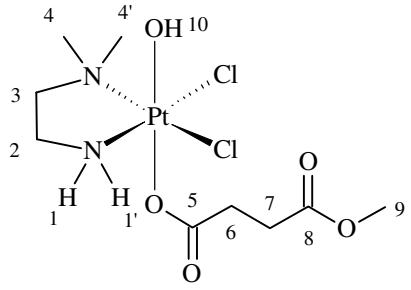


Figure 29: Detail of HMBC spectrum of *N,N-dmenPtCl$_2$OH($C_4H_5O_4$)* (21)

For assignment of the residual C6/C7 signals and the two quaternary carbons C5/C8, chemical correlation by comparison of the acid with the methyl ester derivative was used.



(23)

Figure 30: *N,N-dmenPtCl$_2$OH($C_5H_7O_4$)*

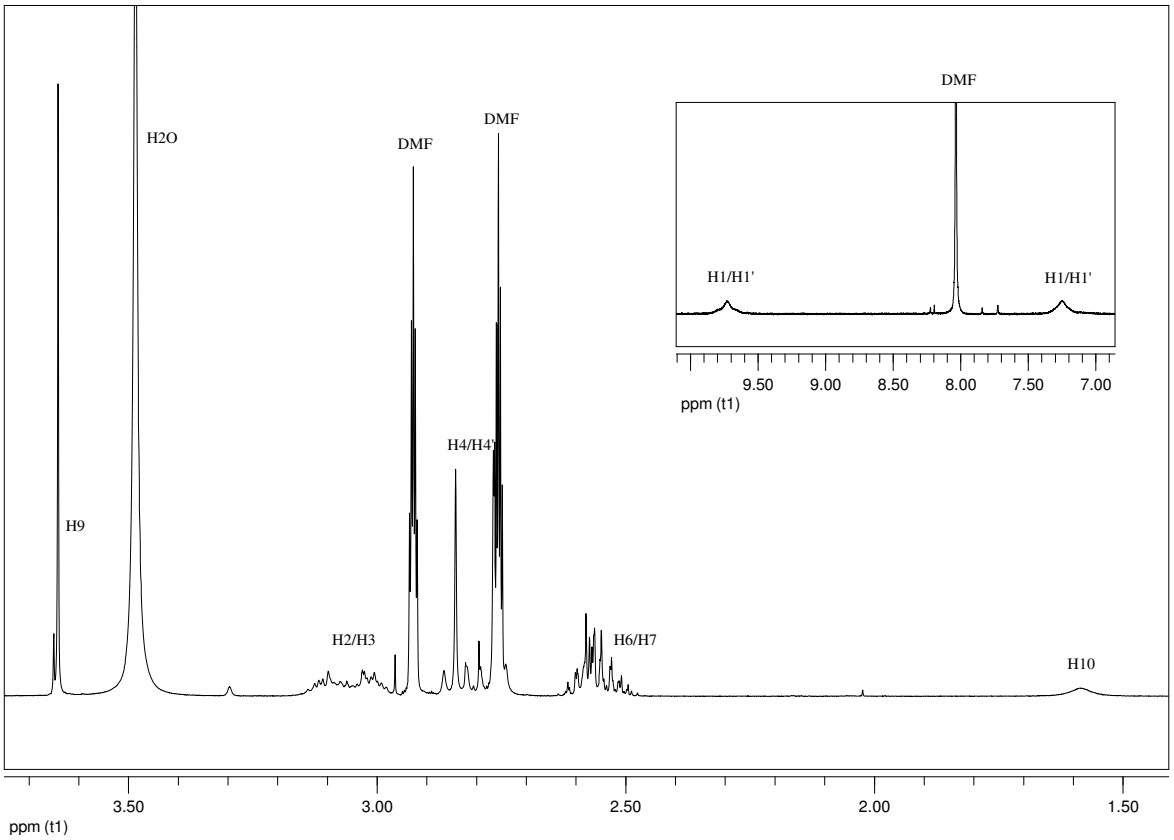


Figure 31: ^{1}H spectrum of *N,N-dmenPtCl$_2$OH(C$_5$H$_7$O$_4$)*

A closer look at the HMBC spectrum of *N,N-dmenPtCl$_2$OH(C$_5$H$_7$O$_4$)* shows a crosspeak of the shifted quarternary carbon signal (173.4 ppm) to H9 of the methyl ester, whereas the signal (C5) at 181.1 ppm did not.

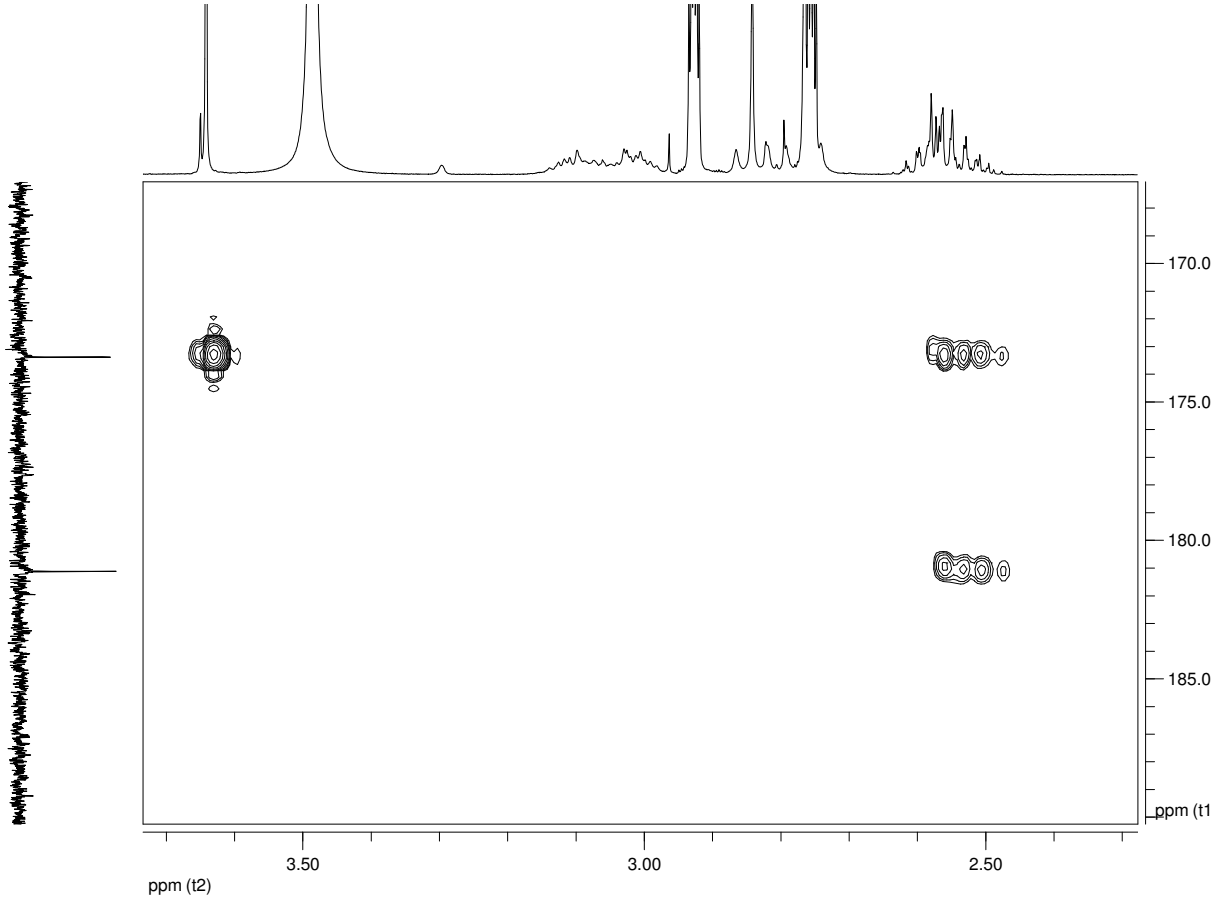
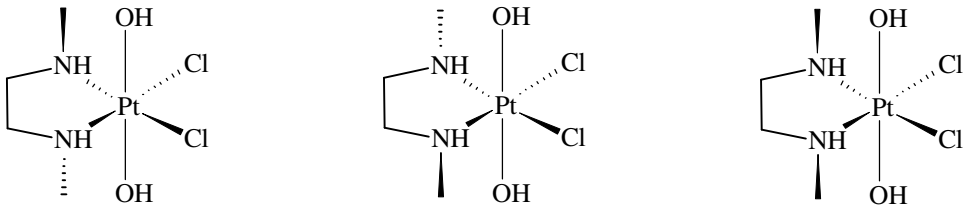


Figure 32: Detail of HMBC spectrum of *N,N-dmenPtCl$_2$OH(C$_5$H$_7$O$_4$)*

5.2.4.2 (SP-4-2)-Dichlorido(N,N'-dimethylethane-1,2-diamine)platinum(II)

As mentioned before, *N,N'-dmenPtCl$_2$(OH)$_2$* is a mixture of stereoisomers. The enantionmeres (A/B) can not be differentiated in NMR spectroscopy without a chiral environment, nevertheless the diastereomeres (A/C and B/C) induce different signals. At this point it has to be mentioned that compound C is not chiral, as it is a meso compound.



(20)

Figure 33: Stereoisomeric forms of *N,N'-dmenPtCl$_2$(OH)$_2$*

The diastereomers show different signals in all measured spectra. The resultant peaks, for example at ^{195}Pt spectrum, display different intensities. This fact permits a correlation of the signals to the isomer, after determination of the ratio.

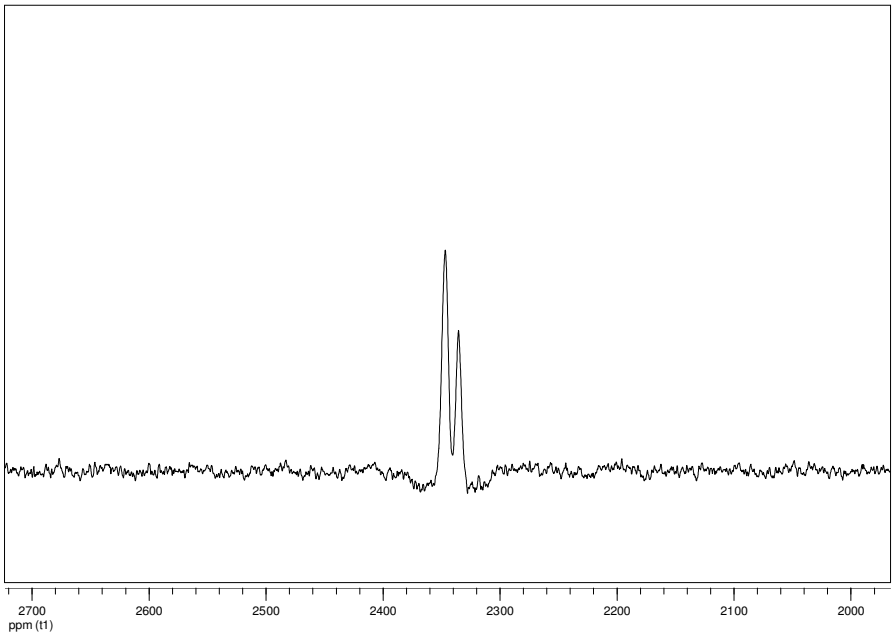


Figure 34: ^{195}Pt spectrum of *N,N'-dmenPtCl$_2$(OH)$_2$* (20)

In the ^{1}H-NMR spectrum measured in D_2O the acidic protons of the amino functions and the hydroxido groups can not be seen because of the rapid chemical exchange with D_2O. Also in ^{1}H and ^{13}C spectra, there are two sets of signals for the diastereomeres, which can be related via HMBC-spectrum.

The methylene protons of the meso compound ((20) C) are split in two, nevertheless the methyl group show only one signal. It is obvious that the reason for this is attributable to a favoured conformation wit a mirror plane.

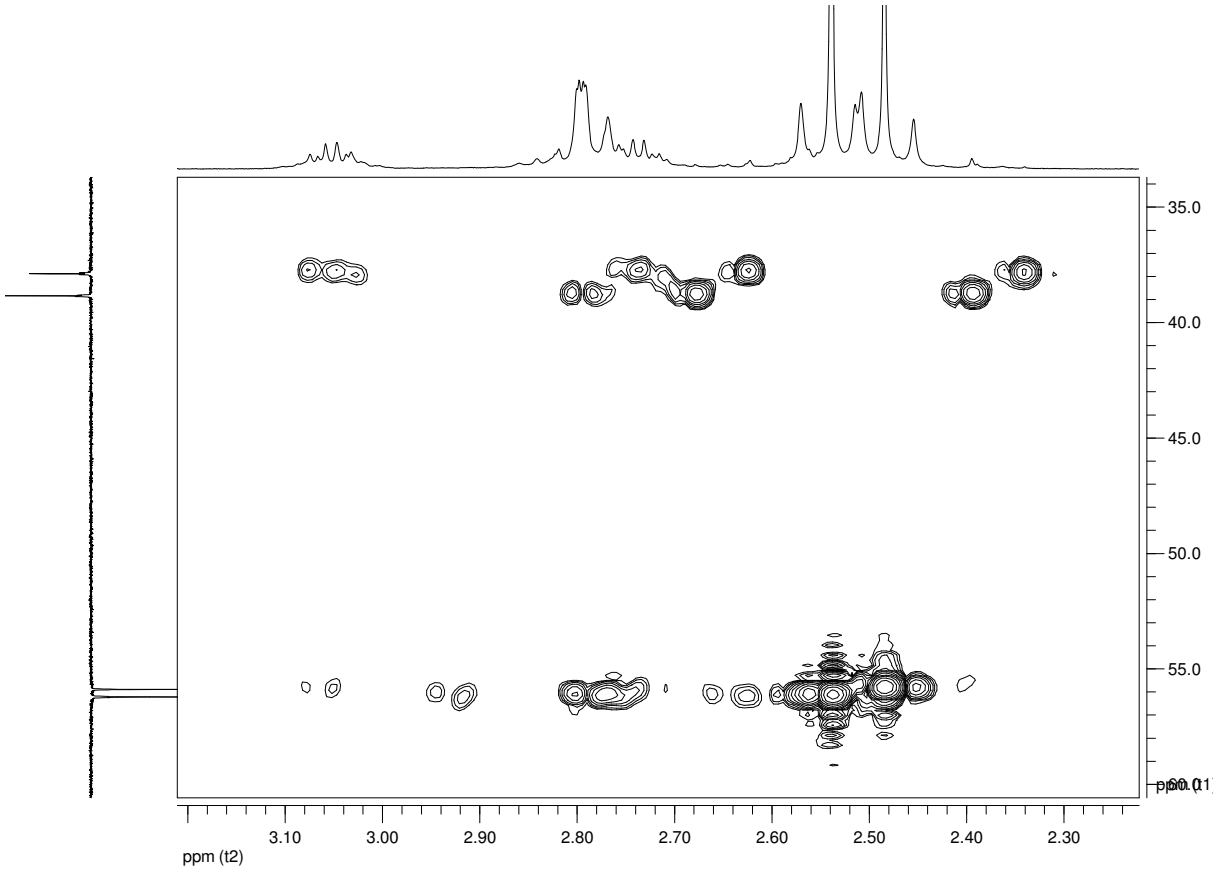


Figure 35: HMBC spectrum of *N,N'-dmenPtCl$_2$(OH)$_2$*

After correlation of the isomers and the methyl groups, computation of the diastereomeres ratio was made. To afford analysis of the overlapping peaks, deconvolution was performed by using a Lorentzian function as an apodization function. The calculated relative integrations can be set in direct proportion to each other.

A ratio of 1:1.4 of compound C to the enantiomeres A/B was determined.

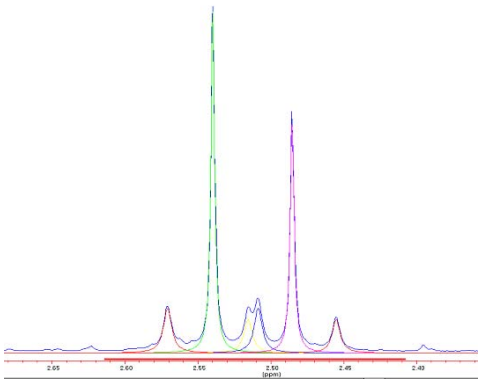


Figure 36: Deconvolution of the overlapping methyl groups of stereoisomeres.

6 Experimental Section

6.1 General Remarks

Platinum(II) compounds are prone to disproportion to metallic platinum and Platinum(IV) in presence of light, metals or metal compounds, accordingly all reactions were carried out with protection from light and with thrice distilled water, if necessary. Purification steps were also, if possible, performed with exclusion of light. Magnetic stirrers were coated with glass to prevent the possibility of reduction to platinum(II) with the ferromagnetic metal.

All glassware needed for reactions was dried in compartment drier at 110°C. For reactions that are sensitive to atmospheric oxygen or moisture, they were operated under argon atmosphere and with use of anhydrous solvents. All magnetic stirrers and suction filters were cleaned by treatment with *aqua regia* to eliminate traces of platinum.

The synthesised compounds were stored under argon atmosphere at 4°C.

6.1.1 Chemicals

All commercially available chemicals were purchased from Sigma Aldrich, Fluka, Acros or Merck and used without further purification, except for $K_2[PtCl_4]$ from Johnson Matthey.

Anhydrous DMF was bought by Sigma Aldrich and stored over molecular sieves (4 Å). Methanol and ethanol were freshly distilled over CaO and Mg and CH_2Cl_2 over phosphorus pentoxide. Water was distilled thrice for removing traces of metal.

For TLC Fluka silica gel TLC-PET foils (medium pore diameter 60 Å) and for preparative column chromatography silica gel 60 also from Fluka (particle size 35-70 µm) were used.

6.2 Instrumental Analysis

Elemental Analysis

The analyses were performed by the Microanalytical Laboratory of the University of Vienna, under the direction of Mag. Theiner. Determination of the composition (elements C, H and N) was performed on a 2400 CHN Elemental Analyser produced by Perkin Elmer.

Infrared Spectroscopy

IR-spectra were recorded on a Bruker Vertex 70 FT-IR-spectrometer with ATR-unit (attenuated total reflection unit) in the range of 4000-400 cm^{-1}.

Mass Spectrometry

ESI-MS spectra were performed in methanol in positive and negative mode on a BRUKER $Esquire_{3000}$ ion trap spectrometer.

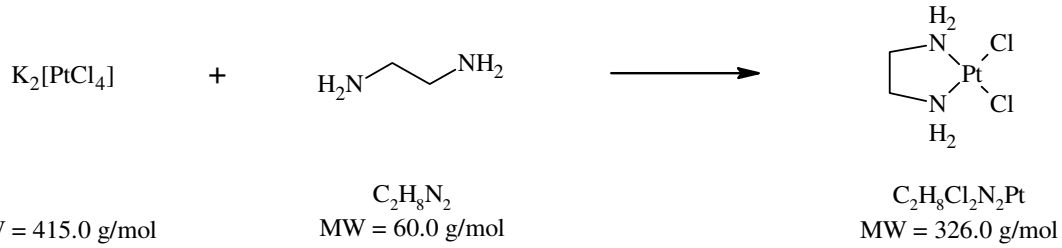
NMR Spectroscopy

NMR spectra were recorded in d_7-DMF, d_6-DMSO and D_2O, referring to the respective solubilities of the synthesised compounds, with a Bruker FT-NMR Avance III 500 MHz spectrometer at 500.32 (^{1}H), 125.81 (^{13}C), 107.55 (^{195}Pt) MHz at 298 K. Chemical shifts (ppm) were referenced internal to the solvent residual peaks or external relative to $K_2[PtCl_4]$.

For description of spin multiplicity the following abbreviations were used: s = singlet; d = duplet; m = multiplett; b = broad signal.

6.3 Platinum(II) Compounds

6.3.1 (SP-4-2)-Dichlorido(ethane-1,2-diamine)platinum(II)



$K_2[PtCl_4]$ MW = 415.0 g/mol

$C_2H_8N_2$ MW = 60.0 g/mol

$C_2H_8Cl_2N_2Pt$ MW = 326.0 g/mol

Ethane-1,2-diamine (145 mg, 2.409 mmol) in tridest. H_2O (6.5 mL) was added to a filtered solution of *$K_2[PtCl_4]$* (1 g, 2.409 mmol) in tridest. H_2O (16.5 mL) and the mixture was stirred at rt. The precipitating product was repeatedly filtered off and washed with tridest. H_2O. The filter crucible was changed when the colour of the precipitate was turning brown. The crude product was collected until the primarily red solution turned yellow and subsequently dried in vacuo. The impurified product could be recristallised from DMF to yield a yellow solid.

Yield: 0.537 g = 1.648 mmol (68%)

Analytical determination:

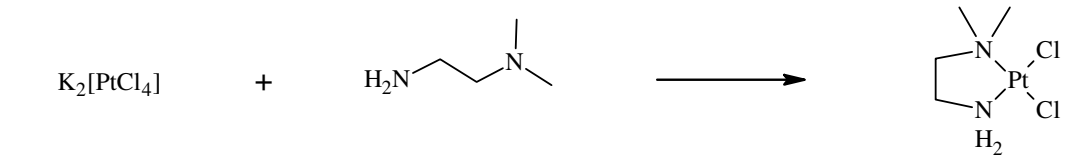
Elemental analysis: $C_2H_8Cl_2N_2Pt$

Element	C	H	Cl	N	Pt
Calculated	7.37	2.47	21.75	8.59	59.83
Found	7.46	2.36	-	8.47	-

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3279, 3249, 3205, 3127	ν (N-H)
2962, 2899	ν (C-H)
1564	δ (N-H)

6.3.2 (SP-4-3)-Dichlorido(N,N-dimethylethane-1,2-diamine)platinum(II)



$K_2[PtCl_4]$		$C_4H_{12}N_2$	$C_4H_{12}Cl_2N_2Pt$
MW = 415.0 g/mol		MW = 88.1 g/mol	MW = 354.1 g/mol

N,N-dimethylethane-1,2-diamine (212 mg, 2.409 mmol) in tridest. H_2O (17 mL) was added to a filtered solution of *$K_2[PtCl_4]$* (1000 mg, 2.409 mmol) in tridest. H_2O (6.5 mL), and the mixture was stirred at rt. The precipitating product was repeatedly filtered off and washed with tridest. H_2O. The filter crucible was changed when the colour of the precipitate was turning brown. The yellow crystalline product was collected until the primarily red solution turned yellow and subsequently dried in vacuo.

Yield: 0.497 g = 1.403 mmol (58%)

Analytical determination:

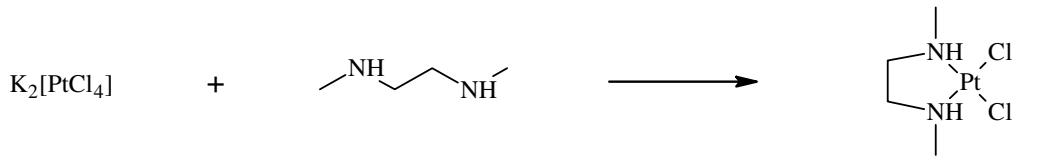
Elemental analysis: $C_4H_{12}Cl_2N_2Pt$

Element	C	H	Cl	N	Pt
Calculated	13.57	3.42	20.02	7.91	55.09
Found	13.40	3.05	-	7.92	-

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3211, 3126, 3019	ν (N-H)
2989, 2926	ν (C-H)
1569	δ (N-H)
1452, 1391, 1307	δ (C-H)

6.3.3 (SP-4-2)/(SP-4-3)-Dichlorido(N,N'-dimethylethane-1,2-diamine)platinum(II)



$K_2[PtCl_4]$ MW = 415.0 g/mol

$C_4H_{12}N_2$ MW = 88.1 g/mol

$C_4H_{12}Cl_2N_2Pt$ MW = 354.1 g/mol

N,N'-dimethylethane-1,2-diamine (212 mg, 2.409 mmol) in tridest. H_2O (17 mL) was added to a filtered solution of *$K_2[PtCl_4]$* (1000 mg, 2.409 mmol) in tridest. H_2O (6.5 mL) and the mixture was stirred at rt. The precipitating product was repeatedly filtered off and washed with tridest. H_2O. The filter crucible was changed when the colour of the precipitate was turning brown. The light yellow product was collected until the primarily red solution turned yellow and dried in vacuo.

Yield: 0.487 g = 1.375 mmol (57%)

Analytical determination:

Elemental analysis: $C_4H_{12}Cl_2N_2Pt$

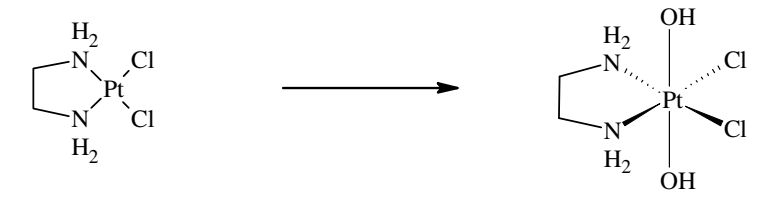
Element	C	H	Cl	N	Pt
Calculated	13.57	3.42	20.02	7.91	55.09
Found	13.61	3.18	-	7.84	-

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3123	ν (N-H)
2936, 2854	ν (C-H)
1732, 1633, 1617	δ (N-H)
1455, 1428	δ (C-H)

6.4 Oxidation to Platinum(IV)

6.4.1 (OC-6-33)-Dichlorido(ethane-1,2-diamine)dihydroxidoplatinum(IV)



$C_2H_8Cl_2N_2Pt$
MW = 326.0 g/mol

$C_2H_{10}Cl_2N_2O_2Pt$
MW = 360.1 g/mol

enPtCl₂ (537 mg, 1.648 mmol) was suspended in tridest. H_2O (14 mL) and 30% H_2O_2 (14 mL) was added. After stirring at rt for 30 min the mixture was refluxed for 10 min. The suspension was cooled down to rt and was left in the refrigerator overnight. The pale yellow precipitate was filtered off, washed with tridest. H_2O, ethanol and diethylether and dried in vacuo.

Yield: 0.406 g = 1.127 mmol (68%)

Analytical determination:

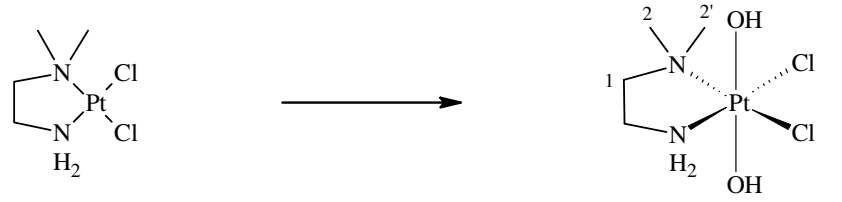
Elemental analysis: $C_2H_{10}Cl_2N_2O_2Pt$

Element	C	H	Cl	N	O	Pt
Calculated	6.67	2.80	19.69	7.78	8.89	54.17
Found	6.41	2.51	-	7.42	-	-

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3480	ν (Pt-OH)
3157	ν (N-H)
2951-2904	ν (C-H)
1586	δ (N-H)

6.4.2 (OC-6-43)-Dichlorido(N,N-dimethylethane-1,2-diamine)dihydroxidoplatinum(IV)



$C_4H_{12}Cl_2N_2Pt$
MW = 354.1 g/mol

$C_4H_{14}Cl_2N_2O_2Pt$
MW = 388.1 g/mol

N,N-dmenPtCl₂ (900 mg, 2.541 mmol) was suspended in tridest. H_2O (22 mL) and 30% H_2O_2 (22 mL) was added. After stirring at rt for about 1 h the suspension turned clear. The yellow solution was filtered and the solvent was removed under reduced pressure to gain a yellow solid. The residue was repeatedly dissolved in tridest. H_2O and carefully concentrated to remove peroxides completely (caution: peroxide). The obtained product was dried in vacuo.

Yield: 0.980 g = 2.525 mmol (99%)

Analytical determination:

Elemental analysis: $C_4H_{14}Cl_2N_2O_2Pt$

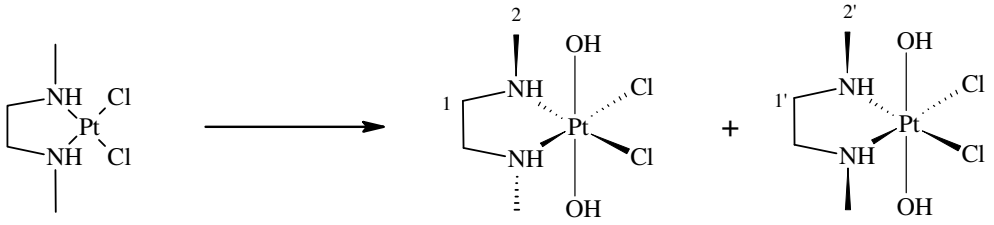
Element	C	H	Cl	N	O	Pt
Calculated	12.38	3.64	18.27	7.22	8.24	50.26
Found	12.36	3.49	-	6.95	-	-

^{1}H-NMR (D_2O): δ = 2.67 (t, 6H, H2); 2.89 (m, 4H, H1) ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3596	ν (Pt-OH)
3248, 3212	ν (N-H)
2915	ν (C-H)
1594	δ (N-H)

6.4.3 (OC-6-33)/(OC-6-43)-Dichlorido(N,N'-dimethylethane-1,2-diamine)dihydroxido platinum(IV)



$C_4H_{12}Cl_2N_2Pt$
MW = 354.1 g/mol

$C_4H_{14}Cl_2N_2O_2Pt$
MW = 388.1 g/mol

N,N'-dmenPtCl₂ (2 g, 5.648 mmol) was suspended in tridest. H_2O (50 mL) and 30 % H_2O_2 (50 mL) was added. After stirring at rt for about 2.5 h the suspension turned clear. The yellow solution was filtered and the solvent was removed under reduced pressure to gain a yellow solid. The residue was repeatedly dissolved in tridest. H_2O and concentrated to remove peroxides completely (caution: peroxide). The obtained mixture of isomeres was dried in vacuo.

Yield: 2.19 g = 5.643 mmol (100%)

Analytical determination:

Elemental analysis: $C_4H_{14}Cl_2N_2O_2Pt$

Element	C	H	Cl	N	O	Pt
Calculated	12.38	3.64	18.27	7.22	8.24	50.26
Found	12.27	3.46	-	6.91	-	-

^{1}H-NMR (D_2O): δ = 2.49 (s+d, H2'); 2.54 (s+d, H2); 2.78 (m, H1'), 3.05 (m, H1-H1') ppm

^{13}C-NMR (D_2O): δ = 37.9 (C2') ; 38.8 (C2); 55.9 (C1'); 56.2 (C1) ppm

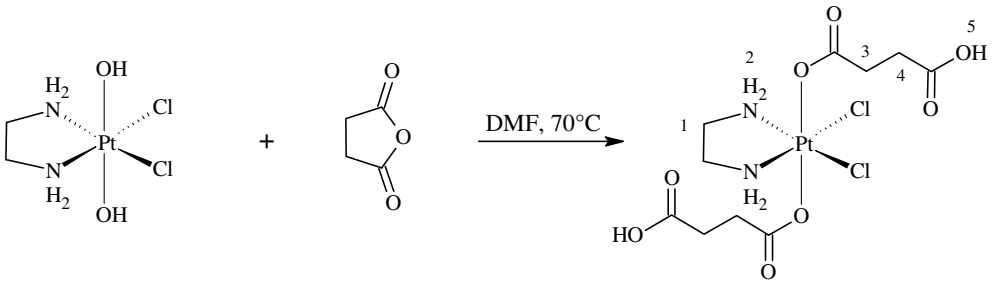
^{195}Pt NMR (D_2O): δ = 2335; 2346 ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3538	ν (Pt-OH)
3016, 2986, 2941	ν (N-H)
2789	ν (C-H)
1633	δ (N-H)

6.5 Carboxylation with Succinic Anhydride

6.5.1 (OC-6-33)-Bis(3-carboxypropanoato)dichlorido(ethane-1,2-diamine) platinum(IV)



$C_2H_{10}Cl_2N_2O_2Pt$
MW = 360.1 g/mol

$C_4H_4O_3$
MW = 100.1 g/mol

$C_{10}H_{18}Cl_2N_2O_8Pt$
MW = 560.3 g/mol

enPtCl$_2$(OH)$_2$ (400 mg, 1.111 mmol) and *succinic anhydride* (456 mg, 4.555 mmol) were suspended in DMF (8 mL). The suspension was stirred at 70 °C until the solution turned clear. The solvent was removed under reduced pressure and the brown residue was dissolved in acetone. The mixture was filtered, concentrated under reduced pressure and the pale yellow solid was precipitated by slow addition of diethyl ether.

Yield: 490 mg = 0.875 mmol (79%)

Analytical determination:

Elemental analysis: $C_{10}H_8Cl_2N_2O_8Pt$

Element	C	H	Cl	N	O	Pt
Calculated	21.44	3.24	12.66	5.00	22.85	34.82
Found	21.25	2.97	-	5.11	-	-

^{1}H-NMR (d$_6$-DMSO): δ = 2.39 (t, 4H, H3 or H4, $^3J_{H\text{-}H}$ = 7.0 Hz); 2.50 (t, 4H, H3 or H4, $^3J_{H\text{-}H}$ = 6.7 Hz); 2.65 (bs, 4H, H1); 8.46 (bs, 4H, H2); 12.08 (bs, 2H, H5) ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3465	ν (-COOH)
3198	ν (N-H)
3040-2929	ν (C-H)
1711, 1652	ν_{as} (C=O)
1592	δ (N-H)

ESI-MS: M_r = 560.3 g/mol

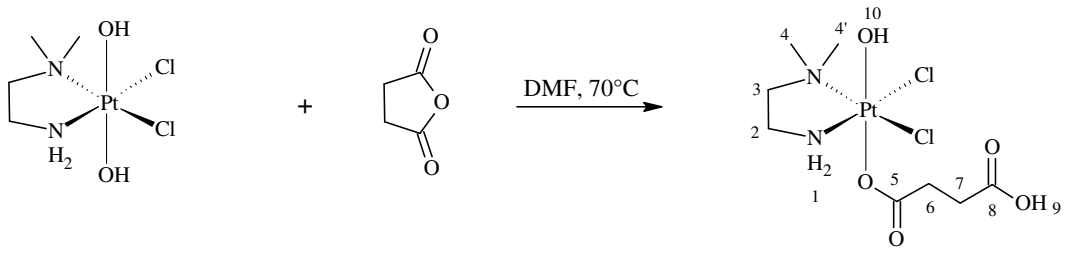
MS^+ in MeOH:

$[m/z]^+$	molecular formula	Ion
1140.9	$C_{20}H_{36}Cl_4N_4O_{16}Pt_2Na$	$[2M+Na]^+$
598.7	$C_{10}H_{18}Cl_2N_2O_8PtK$	$[M+K]^+$
582.8	$C_{10}H_{18}Cl_2N_2O_8PtNa$	$[M+Na]^+$
561.5	$C_{10}H_{19}Cl_2N_2O_8Pt$	$[M+H]^+$
347.0	$C_2H_8Cl_2N_2Pt$	$[M+Na]^+$ - axial ligands

MS^- in MeOH

$[m/z]^+$	molecular formula	Ion
1117.7	$C_{20}H_{37}Cl_4N_4O_{16}Pt_2$	$[2M-H]^-$
558.9	$C_{10}H_{17}Cl_2N_2O_8Pt$	$[M-H]^-$

6.5.2 (OC-6-54)-(3-Carboxypropanoato)dichlorido(N,N-dimethylethane-1,2-diamine) hydroxideplatinum(IV)



$C_4H_{14}Cl_2N_2O_2Pt$
MW = 388.1 g/mol

$C_4H_4O_3$
MW = 100.1 g/mol

$C_8H_{18}Cl_2N_2O_5Pt$
MW = 488.2 g/mol

N,N-dmenPtCl$_2$(OH)$_2$ (800 mg, 2.061 mmol) and *succinic anhydride* (845 mg, 8.450 mmol) were suspended in DMF (16 mL). The suspension was stirred at 70 °C for 18h, after about 30 min the solution turned clear. The solvent was removed under reduced pressure and the yellow residue was dissolved in acetone. The mixture was filtered, concentrated under reduced pressure and the pale yellow solid was precipitated by slow addition of diethyl ether.

Yield: 738 mg = 1.511 mmol (73%)

Analytical determination:

Elemental analysis: $C_8H_{18}Cl_2N_2O_5Pt$

Element	C	H	Cl	N	O	Pt
Calculated	19.68	3.72	14.52	5.74	16.39	39.96
Found	19.95	3.63	-	5.48	-	-

^{1}H-NMR (d$_6$-DMSO): δ = 2.41 (m, 4H, H6/H7); 2.60 (bs, 3H, H4 or H4'); 2.68 (bs, 3H, H4 or H4'); 2.81 (m, 4H, H2/H3); 7.12 (bs, 1H, H1); 9.39 (bs, 1H, H1); 12.09 (bs, 2H, H9) ppm

^{13}C-NMR (d_6-DMSO): δ = 30.4 (C6 or C7); 32.5 (C6 or C7); 45.2 (C2); 48.6 (C4 or C4'); 50.1 (C4 or C4'); 67.8 (C3); 174.6 (C10); 181.3 (C7) ppm

^{195}Pt NMR (d_6-DMSO): δ = 2497 ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

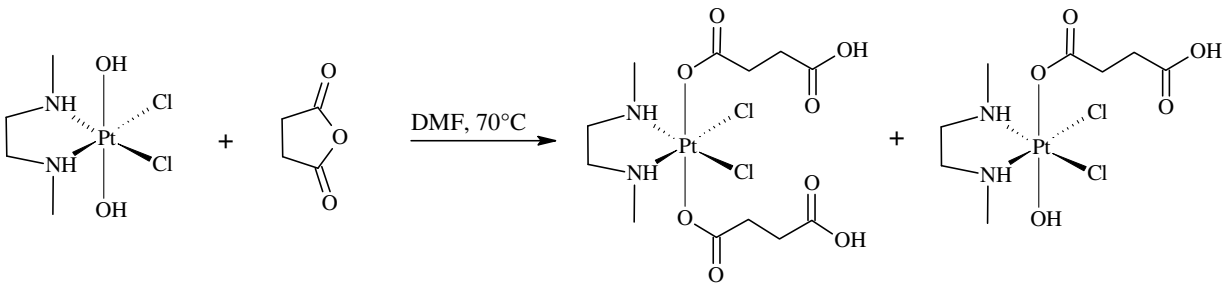
3477	ν (Pt-OH)
3081	ν (N-H)
2936	ν (C-H)
1726	ν_{as} (C=O)
1598	δ (N-H)

ESI-MS: M_r = 488.2 g/mol

MS^+ in MeOH:

$[m/z]^+$	molecular formula	ion
511.0	$C_8H_{18}Cl_2N_2O_5PtNa$	$[M+Na]^+$
493.1	$C_8H_{16}Cl_2N_2O_4PtNa$	$[M+Na]^+ - H_2O$
375.2	$C_4H_{12}Cl_2N_2Pt$	$[M+Na]^+$ - 2*axial ligands

6.5.3 (OC-6-33)/(OC-6-43)-Bis(3-carboxypropanoato)dichlorido(N,N'-dimethylethane-1,2-diamine)platinum(IV)



$C_4H_{14}Cl_2N_2O_2Pt$
MW = 388.1 g/mol

$C_4H_4O_3$
MW = 100.1 g/mol

$C_{12}H_{22}Cl_2N_2O_8Pt$
MW = 588.3 g/mol

$C_8H_{18}Cl_2N_2O_5Pt$
MW = 488.2 g/mol

N,N'dmen-PtCl$_2$(OH)$_2$ (1.60 g, 4.123 mmol) and *succinic anhydride* (1.69 mg, 16.492 mmol) were suspended in DMF (32 mL). The yellow suspension was stirred at 70 °C for 18 h, after about 1 h the solution turned clear and the colour turned to reddish brown. The solvent was removed under reduced pressure and the brown residue was dissolved in acetone. The mixture was filtered, concentrated under reduced pressure and the brown solid was precipitated by slow addition of diethyl ether. A mixture of mono- and dicarboxylated product was obtained.

Analytical determination:

Elemental analysis: $C_{12}H_{22}Cl_2N_2O_8Pt$

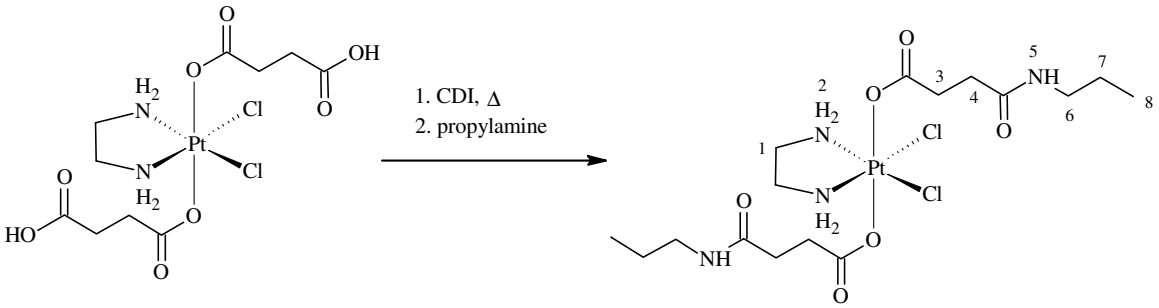
Element	C	H	Cl	N	O	Pt
Dicarboxylated	24.50	3.77	12.05	4.76	21.76	33.16
monocarboxylyted	19.68	3.72	14.52	5.74	16.39	39.96
Found	20.21	3.39	-	5.51	-	-

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3454	ν (-COOH)
3127	ν (N-H)
2941-2872	ν (C-H)
1721, 1621	ν_{as} (C=O)

6.6 Derivatisation of the Carboxyl Group

6.6.1 (OC-6-33)-Dichlorido(ethane-1,2-diamine)-bis[4-(propylamino)-4-oxobutanoato]platinum(IV)



$C_{10}H_{18}Cl_2N_2O_8Pt$
MW = 560.3 g/mol

$C_{16}H_{32}Cl_2N_4O_6Pt$
MW = 642.4 g/mol

enPtCl$_2$(C$_4$H$_5$O$_4$)$_2$ (200 mg, 0.357 mmol) was dissolved in 4 mL DMF under argon atmosphere. The yellow solution was heated to 70 °C and *1,1-carbonyldiimidazole* (118.6 mg, 0.732 mmol) in 8 mL DMF was added. After stirring for 10 min the solution was allowed to cool to rt when simultaneously flushing with argon to remove emerging CO_2. Then *propylamine* (60.4 µL, 0.732 mmol) in 12 mL DMF was added, after stirring for 24 h the solvent was removed under reduced pressure to get a brown oil. The residue was dissolved in MeOH and purified by column chromatography (silica, MeOH:EE = 1:4).

Yield: 0.143 g = 0.223 mmol (62%)

Analytical determination:

Elemental analysis: $C_{16}H_{32}Cl_2N_4O_6Pt$

Element	C	H	Cl	N	O	Pt
Calculated	29.91	5.02	11.04	8.72	14.94	30.37
Found	30.00	4.86	-	8.30	-	-

^{1}H-NMR (d_6-DMSO): δ = 0.83 (t, 6H, H8, $^3J_{H, H}$ = 7.4 Hz); 1.38 (m, 4H, H7); 2.28 (t, 4H, H3 or H4, $^3J_{H, H}$ = 7.3 Hz); 2.46 (t, 4H, H3 or H4, $^3J_{H, H}$ = 7.3 Hz); 2.67 (bs, 4H, H1); 2.98 (m, 4H, H6); 7.77 (t, 2H, H5, $^3J_{H, H}$ = 5.5 Hz); 8.44 (bs, 4H, H2) ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3294, 3202	ν (N-H)
3040-2929	ν (C-H)
1711, 1652	ν_{as} (C=O)
1551	δ (N-H)

ESI-MS: M_r = 642.4 g/mol

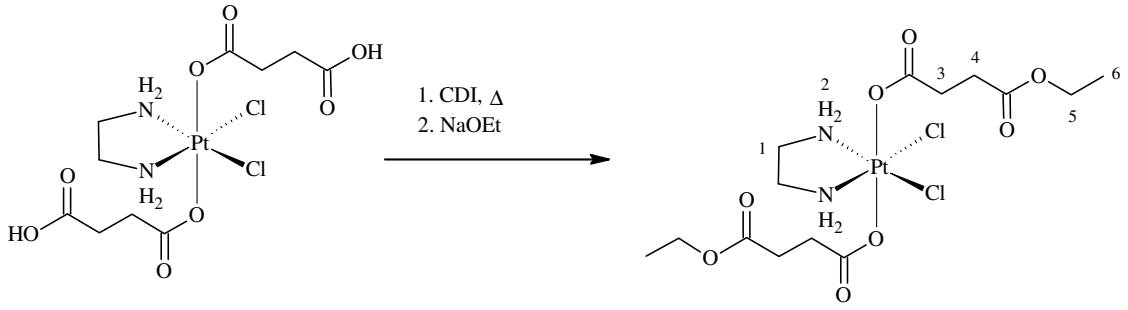
MS^+ in MeOH:

$[m/z]^+$	molecular formula	ion
679.1	$C_{16}H_{32}Cl_2N_4O_6PtK$	$[M+K]^+$
663.9	$C_{16}H_{32}Cl_2N_4O_6PtNa$	$[M+Na]^+$
505.8	$C_9H_{20}Cl_2N_3O_3PtNa$	$[M+Na]^+$ - 1*axial ligand
346.9	$C_2H_8Cl_2N_2Pt$	$[M+Na]^+$ - 2*axial ligands

MS^- in MeOH

$[m/z]^+$	molecular formula	ion
641.3	$C_{16}H_{33}Cl_2N_4O_6Pt$	$[M+H]^-$
676.8	$C_{16}H_{32}Cl_2N_4O_6PtCl$	$[M+Cl]^-$

6.6.2 (OC-6-33)-Dichlorido(ethane-1,2-diamine)bis[(4-ethoxy)-4-oxo-butanoato]platinum(IV)



$C_{10}H_{18}Cl_2N_2O_8Pt$
MW = 560.3 g/mol

$C_{14}H_{26}Cl_2N_2O_8Pt$
MW = 616.4 g/mol

enPtCl$_2$(C$_4$H$_5$O$_4$)$_2$ (200 mg; 0.357 mmol) was dissolved in 4 mL DMF under an argon atmosphere. The yellow solution was heated to 70 °C and *1,1-carbonyldiimidazole* (118.6 mg, 0.732 mmol) in 8 mL DMF was added. After stirring for 10 min the solution was allowed to cool to rt when simultaneously flushing with argon to remove emerging CO_2. Dissolving s*odium* (about 6 mg) in anhydrous EtOH (10 mL) gave a mixture of *ethanolate/ethanol* which was added to the reaction mixture. The reaction mixture was stirred for 24 h and subsequently the solvent was removed under reduced pressure to get a brown oil. The residue was dissolved in MeOH and purified by column chromatography (silica, MeOH:EE = 1:7).

Yield: 0.052 g = 0.084 mmol (24%)

Analytical determination:

Elemental analysis: $C_{14}H_{26}Cl_2N_2O_8Pt$

Element	C	H	Cl	N	O	Pt
Calculated	27.28	4.25	11.50	4.55	20.77	31.65
Found	26.63	4.09	-	4.35	-	-

^{1}H-NMR (d$_6$-DMSO): δ = 1.19 (m, 6H, H6); 2.46 (t, 4H, H3 or H4, $^3J_{H,H}$ = 6.3 Hz); 2.54 (t, 4H, H3 or H4, $^3J_{H,H}$ = 6.4 Hz); 2.65 (bs, 4H, H1); 4.06 (m, 4H, H5); 8.43 (bs, 4H, H2) ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3196	ν (N-H)
2984-2929	ν (C-H)
1714, 1643	ν_{as} (C=O)
1543	δ (N-H)

ESI-MS: M_r = 616.4 g/mol

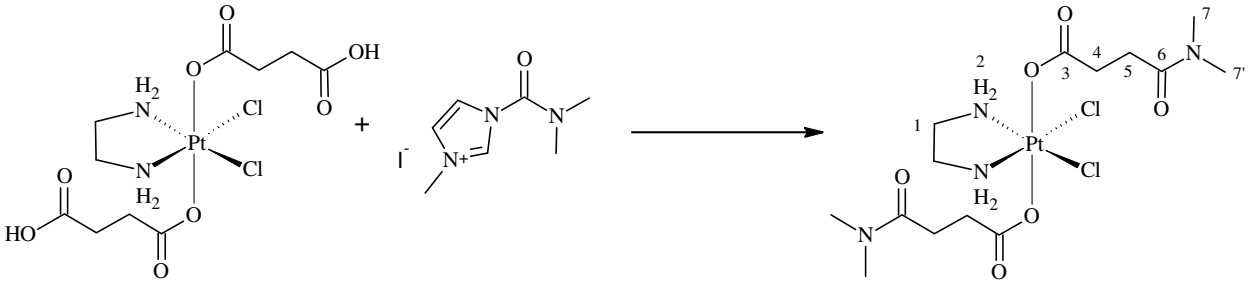
MS^+ in MeOH:

$[m/z]^+$	Molecular formula	ion
637.9	$C_{14}H_{26}Cl_2N_2O_8PtK$	$[M+K]^+$
654.8	$C_{14}H_{26}Cl_2N_2O_8PtNa$	$[M+Na]^+$
492.8	$C_8H_{17}Cl_2N_2O_4PtNa$	$[M+Na]^+$ - 1*axial ligand
346.9	$C_2H_8Cl_2N_2Pt$	$[M+Na]^+$ - 2*axial ligands

MS^- in MeOH

$[m/z]^+$	Molecular formula	ion
641.3	$C_{14}H_{27}Cl_2N_2O_8Pt$	$[M+H]^-$

6.6.3 (OC-6-33)-Dichlorido(ethan-1,2-diamine)bis[(4-dimethylamine)-4-oxobutanoato] platinum(IV)



$C_{10}H_{18}Cl_2N_2O_8Pt$
MW = 560.3 g/mol

$C_7H_{12}IN_3O$
MW = 281.1 g/mol

$C_{14}H_{28}Cl_2N_4O_6Pt$
MW = 614.4 g/mol

N,N-dmenPtCl$_2$(C$_4$H$_5$O$_4$)$_2$ (300 mg, 0.535 mmol) and *1-(dimethylcarbamoyl)-3-methyl-1H-imidazol-3-ium iodide* (309 mg, 1.098 mmol) were dissolved in 2 mL DMF under argon atmosphere and *triethylamine* (111 mg, 1.098 mmol) was added. The suspension was heated to 60 °C and after 1 h a clear, reddish brown solution was obtained. The reaction mixture was stirred for 24 h and subsequently the solvent was removed under reduced pressure to get a brown oil. The residue was dissolved in MeOH and purified by column chromatography (silica, MeOH:EE = 1:1).

Yield: 0.125 g = 0.203 mmol (38%)

Analytical determination:

Elemental analysis: $C_{14}H_{28}N_4Cl_2O_6Pt*1H_2O$

Element	C	H	Cl	N	O	Pt
Calculated	26.59	4.78	11.21	8.86	17.71	30.85
Found	26.47	4.64	-	8.61	-	-

^{1}H-NMR (d$_6$-DMSO): δ = 2.47 (bs, 8H, H4-H5); 2.69 (bs, 4H, H1); 2.80 (s, 6H, H7 or H7'); 2.96 (s, 6H, H7 or H7'); 8.45 (bs, 4H, H2) ppm

^{13}C-NMR (d_6-DMSO): δ = 28.9 (C5); 31.7 (C4); 35.4 (C7 or C7'); 37.0 (C7 or C7'); 49.0 (C1); 171.6 (C6); 182 (C3) ppm

^{195}Pt-NMR (d_6-DMSO): δ = 2659 ppm

ESI-MS: M_r = 614.4 g/mol

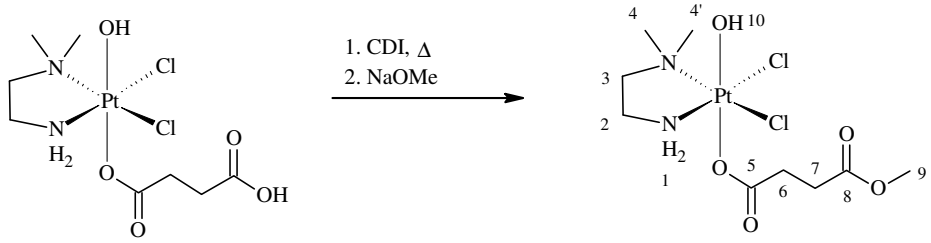
MS^+ in MeOH:

$[m/z]^+$	Molecular formula	ion
637.1	$C_{14}H_{28}N_4Cl_2O_6PtNa$	$[M+Na]^+$
491.0	$C_8H_{18}N_3O_3Cl_2PtNa$	$[M+Na]^+$ - 1*axial ligand
347.0	$C_2H_8N_2Cl_2PtNa$	$[M+Na]^+$ - 2*axial ligands

MS^- in MeOH

$[m/z]^+$	Molecular formula	ion
613.0	$C_{14}H_{27}N_4Cl_2O_6Pt$	$[M+H]^-$

6.6.4 (OC-6-54)-Dichlorido(N,N-dimethylethane-1,2-diamine)hydroxido[(4-methoxy)-4-oxo-butanoato]platinum(IV)



$C_8H_{18}Cl_2N_2O_5Pt$
MW = 488.2 g/mol

$C_9H_{20}Cl_2N_2O_5Pt$
MW = 502.3 g/mol

N,N-dmenPtCl$_2$OH(C$_4$H$_5$O$_4$) (200 mg, 0.409 mmol) was dissolved in 4 mL DMF under argon atmosphere. The yellow solution was heated to 70 °C and *CDI* (136 mg, 0.839 mmol) in 8 mL DMF was added. After stirring for 10 min the solution was allowed to cool to rt when simultaneously flushing with argon to remove emerging CO_2. Dissolving s*odium* (about 6 mg) in anhydrous MeOH gave a mixture of *methanolate/methanol* which was added to the reaction mixture. The reaction mixture was stirred at rt for 24 h and subsequently the solvent was removed under reduced pressure to get a brown oil. The residue was resolved in MeOH and purified by column chromatography (silica, MeOH:EE = 1:1).

Yield: 0.045 g = 0.089 mmol (22%)

Analytical determination:

Elemental analysis: $C_9H_{20}Cl_2N_2O_5Pt$

Element	C	H	Cl	N	O	Pt
Calculated	21.52	4.01	14.12	5.58	15.93	38.84
Found	21.50	3.77	-	5.12	-	-

^{1}H-NMR (d$_7$-DMF): δ = 1.59 (bs, 1H, H10); 2.56 (m, 4H, H6/H7); 2.77 (t, 3H, H4 or H4'); 2.84 (t, 3H, H4 or H4'); 3.06 (m, 4H, H2/H3); 3.64 (s, 3H, H9); 7.25 (bs, 1H, H1); 9.73 (bs, 2H, H1) ppm

^{13}C-NMR (d_7-DMF): δ = 29.8 (C6 or C7); 32.2 (C6 or C7); 45.1 (C2); 48.1 (C4 or C4'); 49.7 (C4 or C4'); 51.1 (C9); 67.8 (C3); 173.4 (C8); 181.1 (C5) ppm

^{195}Pt-NMR (d_7-DMF): δ = 2457 ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3480	ν (Pt-OH)
2923-2852	ν (C-H)
1723, 1626	ν_{as} (C=O)
1559	δ (N-H)

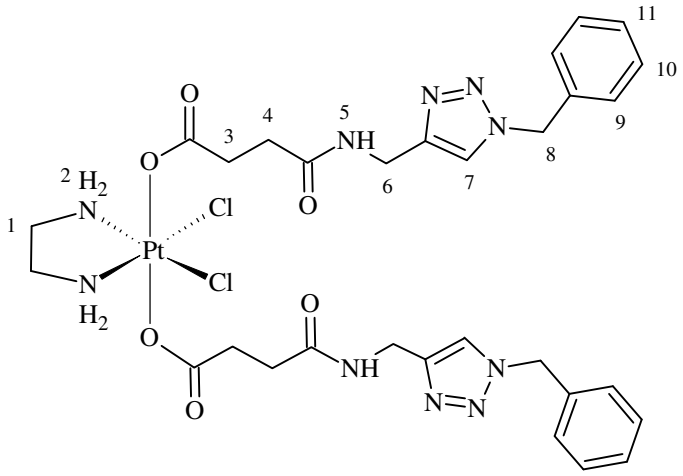
ESI-MS: M_r = 502.3 g/mol

MS^+ in MeOH:

$[m/z]^+$	molecular formula	ion
525.0	$C_9H_{20}Cl_2N_2O_5PtNa$	$[M+Na]^+$
375.2	$C_4H_{12}Cl_2N_2PtNa$	$[M+Na]^+$ - axial ligands
339.3	$C_4H_{11}ClN_2PtNa$	$[M+Na]^+$ - axial ligands + HCl

6.7 Click Chemistry

6.7.1 (OC-6-33)-Bis{4-{[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amino}-4-oxo-butanoato}dichlorido(ethane-1,2-diamine)platinum(IV)



$C_{30}H_{38}Cl_2N_{10}O_6Pt$
MW = 900.7 g/mol

enPtCl$_2$(C$_7$H$_8$O$_3$N)$_2$ (100 mg, 0.158 mmol) and *benzylazide* (41.9 mg, 0.315 mmol) were suspended in 3 mL of tridest. H_2O and a catalytic amount of *CuBr* (2 mg, 0.016 mmol) and *Me$_2$S* (5.9 mg, 0.095 mmol) were added. The reaction mixture was stirred at rt for 24 h. The solid was filtered off and washed with water, ethanole and diethylether to yield a green product. Due to the bad solubility further purification was impossible.

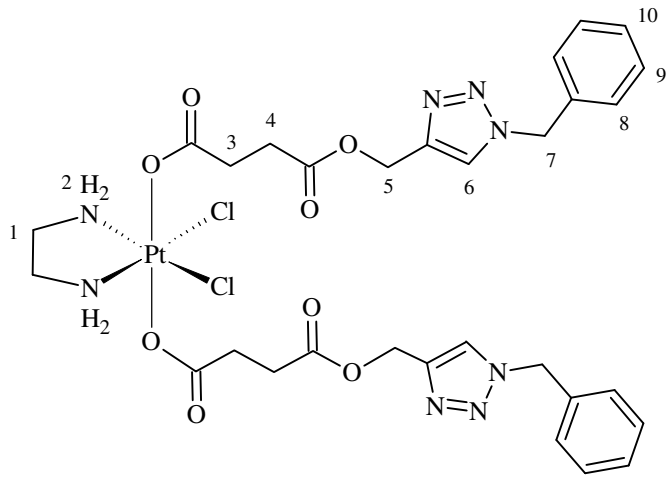
Analytical determination:

^{1}H-NMR (d$_6$-DMSO): δ = 2.50 (m, 8H, H3/H4); 2.64 (bs, 4H, H1); 4.27 (bs, 4H, H6); 5.56 (s, 4H, H8); 7.36 (m, 10H, H9-H11); 7.99 (s, 2H, H7); 8.31 (bs, 2H, H5); 8.42 (bs, 4H, H2) ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3293,56	ν (N-H)
2988-2928	ν (C-H)
1641	ν_{as} (C=O)
1564	δ (N-H)

6.7.2 (OC-6-33)-Bis{4-[(1-benzyl-1H-1,2,3-triazol-4-yl)methoxy]-4-oxo-butanoato}dichlorido(ethane-1,2-diamine)platinum(IV)



$C_{30}H_{36}Cl_2N_8O_8Pt$
MW = 902.6 g/mol

enPtCl$_2$(C$_2$H$_5$O$_4$)$_2$ (100 mg, 0.178 mmol) was dissolved in 1.5 mL DMF under argon atmosphere. The yellow solution was heated to 70 °C and *CDI* (59 mg, 0.366 mmol) in 3 mL DMF was added. After stirring for 10 min, the solution was allowed to cool to rt when simultaneously flushing with argon to remove emerging CO_2. Dissolving s*odium* (about 6 mg) in a solution of *(1-benzyl-1H-1,2,3-triazole-4-yl)methanol* (100 mg, 0.529 mmol) in 3 mL anhydrous THF gave the corresponding alkoxide. THF was removed under reduced pressure and to the exclusion of air and water and the alkoxide was resolved in 3 mL DMF. This solution was added to the reaction mixture. After stirring for 24 h the solvent was removed under reduced pressure to get a yellow oil. The residue was dissolved in MeOH and a brown residue was obtained. The residue was filtered off and washed with water, methanol and diethylether. Due to the bad solubility further purification was impossible.

Analytical determination:

^{1}H-NMR (d_6-DMSO): δ = 2.52 (m, 8H, H3/H4); 2.62 (bs, 4H, H1); 5.12 (s, 4H, H5); 5.60 (s, 4H, H7); 7.36 (m, 10H, H8-H10); 8.19 (s, 2H, H6); 8.40 (bs, 4H, H2) ppm

IR (4000 – 400 cm^{-1}) selected bands in cm^{-1}:

3196	ν (N-H)
2980-2850	ν (C-H)
1730, 1643	ν_{as} (C=O)

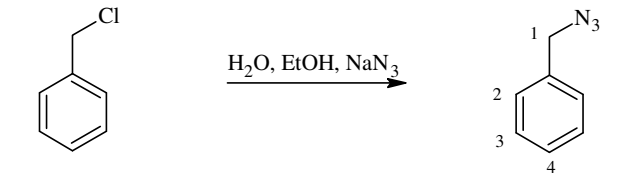
ESI-MS: M_r = 902.6 g/mol

MS^+ in MeOH:

$[m/z]^+$	Molecular formula	ion
942.1	$C_{30}H_{36}Cl_2N_8O_8PtK$	$[M+K]^+$
925.6	$C_{30}H_{36}Cl_2N_8O_8PtNa$	$[M+Na]^+$
888.2	$C_{30}H_{35}ClN_8O_8PtNa$	$[M+Na]^+$-HCl
636.0	$C_{16}H_{22}Cl_2N_5O_4PtNa$	$[M+Na]^+$ - 1*ligand
346.9	$C_2H_8Cl_2PtNa$	$[M+Na]^+$ - 2*ligand
312.1	$C_{14}O_4N_3H_{15}Na$	$[ligand+Na]^+$

6.8 Synthesis of Ligands

6.8.1 Benzylazid



C_7H_7Cl
MW = 126.58

$C_7H_7N_3$
MW = 133.15

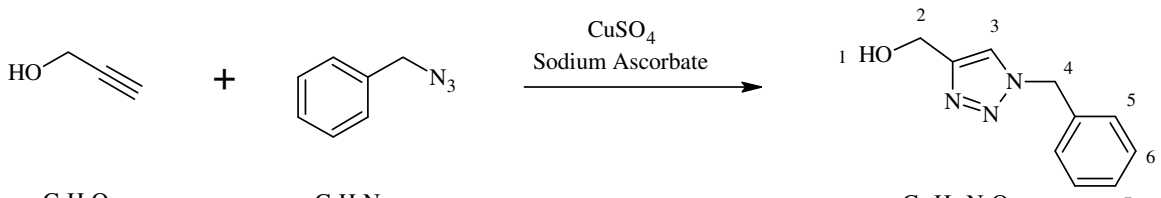
Benzylchloride (6.0 g; 47.5 mmol) and *sodiumazide* (3.4 g, 52.2 mmol) were dissolved in EtOH/H_2O (48 mL, 7:1). The reaction mixture was refluxed for 24 h. The solution was slowly cooled to rt and then poured on H_2O (220 mL). The aqueous layer was extracted with diethylether (3x80 mL). The combined organic layer was washed with water, dried with Na_2SO_4, filtered and concentrated in vacuo to yield a clear solution.

Yield = 4.98 g = 0.037 mol (78 %)

Analytical determination:

^{1}H-NMR (d_6-DMSO): δ = 4.46 (s, 2H, H1); 7.39 (m, 5H, H2-H4) ppm

6.8.2 (1-Benzyl-1H-1,2,3-triazole-4-yl)methanol



C_3H_4O
MW = 56.06

$C_7H_7N_3$
MW = 133.15

$C_{10}H_{11}N_3O$
MW = 189.21

Benzylazide (1.00 g; 7.51 mmol) and propargylalcohol (0.42 g, 7.51 mmol) were dissolved in THF (40 mL). $CuSO_4*5H_2O$ (94 mg, 0.375 mmol) and sodium ascorbate (297 mg, 1.49 mmol) in H_2O (25 mL) were added. The yellow suspension was stirred for 7 h at rt. The solvent was removed under reduced pressure and the residue was redissolved in toluene. The organic layer was washed with H_2O and brine, dried with $MgSO_4$ and concentrated under reduced pressure to yield a white solid. The product was recrystallized in toluene to receive white needles.

Yield: 0.705 g = 3.73 mmol (50 %)

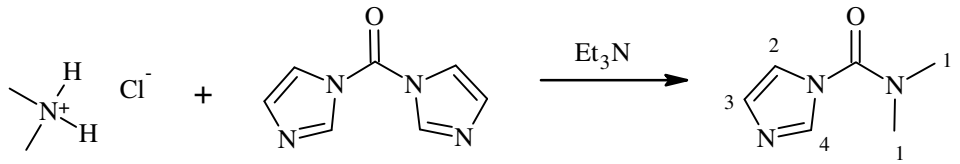
Analytical determination:

Elemental analysis: $C_{10}H_{11}N_3O$

Element	C	H	Cl	N	O	Pt
Calculated	63.48	5.86	-	22.21	8.46	-
Found	63.55	5.69	-	22.09	-	-

^{1}H-NMR (d_6-DMSO): δ = 4.51 (d, 2H, H2, $^3J_{H,H}$ = 5.7 Hz); 5.15 (t, 1H, H1, $^3J_{H,H}$ = 5.7 Hz); 5.57 (s, 2H, H4); 7.35 (m, 5H, H5-H7); 8.01 (s, 1H, H3) ppm

6.8.3 *N*,*N*-Dimethyl-1*H*-imidazole-1-carboxamide



C_2H_8ClN
MW = 81.5 g/mol

$C_7H_6N_4O$
MW = 162.1 g/mol

$C_6H_9N_3O$
MW = 139.2 g/mol

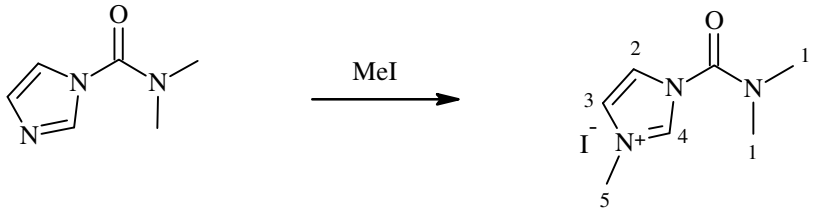
1,1-carbonyldiimidazol (1.40 g, 8.637 mmol) and *triethylamine* (850 mg, 8.400 mmol) were dissolved in 20 mL of absolute CH_2Cl_2. The solution was cooled to 0 °C and *dimethylamine hydrochloride* (500 mg, 6.135 mmol) was slowly added. After about 10 min the solution was allowed to reach rt and was stirred over night. Then the reaction mixture was quenched with water, the aqueous layer was extracted thrice with CH_2Cl_2. The organic layers were combined, dried with Na_2SO_4, filtrated and the solvent was removed under reduce pressure to yield white crystals.

Yield: 782 mg = 5.622 mmol (92%)

Analytical determination:

^{1}H-NMR (d_6-DMSO): δ = 3.01 (bs, 6H, H1); 7.02 (m, 1H, H4) 7.50 (t, 1H, H3, $^3J_{H,H}$ = 1.4 Hz); 8.05 (t, 1H, H2, $^3J_{H,H}$ = 0.9 Hz) ppm

6.8.4 1-(Dimethylcarbamoyl)-3-methyl-1*H*-imidazol-3-ium iodide



$C_6H_9N_3O$
MW = 139.2 g/mol

$C_7H_{12}IN_3O$
MW = 281.1 g/mol

N,N-dimethyl-1H-imidazole-1-carboxamide (750 mg, 5.392 mmol) was dissolved in 10 ml of *acetonitril p.a.* and *methyliodid* (3.06 g, 21.56 mmol) was added slowly. The reaction mixture was stirred over night and meanwhile the clear colourless solution turned yellow. The solvent was removed under reduced pressure to yield a yellow oil.

Yield: 1.17 g = 4.163 mmol (77%)

Analytical determination:

^{1}H-NMR (d_6-DMSO): δ = 3.06 (bs, 6H, H1); 3.92 (s, 3H, H5); 7.84 (t, 1H, H2, $^3J_{H,H}$ = 2.0 Hz); 8.06 (t, 1H, H3, $^3J_{H,H}$ = 1.8 Hz); 9.58 (s, 1H, H4) ppm

List of Abbreviations

gDNA	genomic deoxyribonucleic acid
RNA	ribonucleic acid
A	adenine
G	guanine
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
CDI	1,1-carbonyldiimidazole
HPLC	high performance liquid chromatography
TLC	thin layer chromatography
ESI MS	electrospray ionisation mass spectrometry
Arg (R)	arginine
Gly (G)	glycine
Asp (D)	aspartic acid
Asn (N)	asparagine
Ala (A)	alanine
h	hour
min	minute
rt	room temperature

Shortcuts for Platinum(IV) Complexes

$enPtCl_2$	(SP-4-2)-Dichlorido(ethane-1,2-diamine)platinum(II)
N,N-$dmenPtCl_2$	(SP-4-3)-Dichlorido(N,N-dimethylethane-1,2-diamine) platinum(II)
N,N'-$dmenPtCl_2$	(SP-4-2)/(SP-4-3)-Dichlorido(N,N'-dimethylethane-1,2-diamine)platinum(II)
$enPtCl_2(OH)_2$	(OC-6-33)-Dichlorido(ethane-1,2-diamine)dihydroxido platinum(IV)
N,N-$dmenPtCl_2(OH)_2$	(OC-6-43)-Dichlorido(N,N-dimethylethane-1,2-diamine) dihydroxidoplatinum(IV)
N,N'-$dmenPtCl_2(OH)_2$	(OC-6-33)/(OC-6-43)-Dichlorido(N,N'-dimethylethane-1,2-diamine)dihydroxidoplatinum(IV)
$enPtCl_2(C_4H_5O_4)_2$	(OC-6-33)-Bis(3-carboxypropanoato)dichlorido(ethane-1,2-diamine)platinum(IV)
N,N-$dmenPtCl_2OH(C_4H_5O_4)$	(OC-6-54)-(3-Carboxypropanoato)dichlorido(N,N-dimethyl ethane-1,2-diamine)hydroxidoplatinum(IV)
N,N'-$dmenPtCl_2(C_4H_5O_4)_2$	(OC-6-33)/(OC-6-43)-Bis(3-Carboxypropanoato)dichlorido (N,N'-dimethylethane-1,2-diamine)platinum(IV)
$enPtCl_2(C_7H_8O_3N)_2$	(OC-6-33)-Dichlorido(ethane-1,2-diamine)bis(4-oxo-4-(ethenylamino)butanoato)platinum(IV)
$enPtCl_2(C_7H_{12}O_3N)_2$	(OC-6-33)-Dichlorido(ethane-1,2-diamine)bis[4-(propyl amino)-4-oxobutanoato]platinum(IV)
$enPtCl_2(C_6H_9O_4)_2$	(OC-6-33)-Dichlorido(ethane-1,2-diamine)bis[(4-ethoxy)-4-oxobutanoato]platinum(IV)
$enPtCl_2(C_6H_{10}O_3N)_2$	(OC-6-33)-Dichlorido(ethane-1,2-diamine)bis[(4-dimethyl amine)-4-oxobutanoato]platinum(IV)
N,N-$dmenPtCl_2OH(C_5H_7O_4)$	(OC-6-54)-Dichlorido(N,N-dimethylethane-1,2-diamine) [(4-methoxy)-4-oxobutanoato)]hydroxidoplatinum(IV)

Curriculum vitae

Verena Pichler

eMail: Verena.Pichler@univie.ac.at

Date of Birth: 03.12.1984
Marital Status: Single
Nationality: Austria

Education

2005 – to date	Studies of chemistry (University of Vienna)
2004 – to date	Studies of economics (Vienna University of Economics and Business)
1999 – 2004	HLW Steyr (High School)
1995 – 1999	Dr. Rudolf-Kirschschläger Schule Steyr (Secondary School)
1991 – 1995	Elementary School, Steyr

Employment History

Mar–Jul 2009	Tutor at Undergraduate Inorganic Chemistry Labs
Jul–Aug 2008	DSM Fine Chemicals Austria – R&D
Jul 2007	Association for the Prevention of Pollution (Reinhaltungsverband Steyr) – Analytical Laboratory
Aug 2005	Municipal Administration – Public Swimming Bath
Aug 2004	Municipal Administration – Department “Conservation of the Historic City”
Sep 2003	Municipal Administration – Department “Community Services” – Administrative Office
Jul–Sep 2002	BMW Steyr - Catering Staff Traineeship
Aug 2001	Municipal Administration - Public Swimming Bath

Scholarship

2009	Performance Scholarships (Leistungsstipendium aus Stiftung und Sondervermögen) of the University of Vienna offered by private institutions
2008	Grant of the Dr.-Wilhelm-Groß foundation