Technische Universität München

Fakultät für Chemie, Fachgebiet Molekulare Katalyse

Supramolecular metallocages as potential delivery systems for anticancer drugs

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Vollständiger Abdruck der von der Fakultät für Chemie der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitzender:	Prof. Dr. Lukas Hintermann
Prüfer der Dissertation:	1. Prof. Dr. Fritz E. Kühn
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Die Dissertation wurde am 19.08.2016 bei der Technischen Universität München eingereicht und durch die Fakultät für Chemie am 21.09.2016 angenommen.

„So eine Arbeit wird eigentlich nie fertig,
man muss sie für fertig erklären,
wenn man nach Zeit und Umständen
das Möglichste getan hat.“

Johann Wolfgang von Goethe,
Italienische Reise, 1787

Die vorliegende Arbeit wurde im Fachgebiet Molekulare Katalyse an der Technischen Universität München unter der Anleitung von Prof. Dr. Fritz E. Kühn in Kooperation mit der Arbeitsgruppe von Prof. Dr. Angela Casini an der Cardiff University im Zeitraum von Juni 2013 bis August 2016 angefertigt.

Mein ganz besonderer Dank gilt meinem Doktorvater

Herrn Prof. Dr. Fritz E. Kühn

für die freundliche Aufnahme in seinen Arbeitskreis, für die Schaffung eines positiven Betriebsklimas, für die unzähligen Möglichkeiten und Freiheiten sowohl bei der Gestaltung meines Promotionsprojektes als auch bei der Bearbeitung, für die stetige Unterstützung und für das entgegengebrachte Vertrauen in meine Arbeit.

Des Weiteren gilt mein besonderer Dank meiner Zweitbetreuerin

Frau Prof. Dr. Angela Casini

für die Ermöglichung der mehrmonatigen Forschungsaufenthalte in ihrer Arbeitsgruppe in Goningen und Cardiff, für die Einführung in die spannende Welt der Toxikologie und Pharmazie und für die vielfältigen Möglichkeiten.
Thank you very much for your trust and support!

Danksagung

Diese Arbeit wäre ohne den Beitrag und die Unterstützung einiger Personen in dieser Form nicht möglich gewesen. Hiermit möchte ich mich bei allen bedanken, die mich während meiner Promotion begleitet und unterstützt haben. Mein herzlicher Dank geht an:

Dr. Markus Dress für die unermüdliche Bereitschaft DFT-Berechnungen an den Käfigen durchzuführen und mir die Ergebnisse ausführlich zu erklären.

Dr. Gabriele Raudaschl-Sieber für die hervorragende Organisation der Saalpraktika und der Klausurkorrekturen, für die netten Gespräche und hilfreichen Tipps.

Dr. Alexander Pöthig für die Unterstützung bei Belangen in Röntgendiffraktometrie und Emissionsspektroskopie.

Manuela Hollering für das beharrliche Kristall-Picken und Lösen der Käfigstrukturen, für die hilfreichen Diskussionen auch außerhalb der Wissenschaft und für die konstante Wegbegleitung.

meine Masterstudenten **Felix Kaiser** und **Viviana Molano** für die tolle Zusammenarbeit auch bei den gemeinsamen Publikationen.

Jiaying Han für die ESI-MS Messungen und für den aufschlussreichen E-Mail Austausch.

Eva Hahn, **Sophie Jürgens** und **Özden Karaca** für die gute Zusammenarbeit in der MedChem Gruppe, für die gegenseitigen Hilfestellungen und für die schöne Zeit bei Forschungsaufenthalten.

meine Laborkollegen **Julia Rieb** und **Christian Jandl** für die gute Atmosphäre, die hilfreichen Tipps und Unterstützung bei Messungen. Ebenso danke ich meinen Kollegen aus dem alten Labor **Robert Reich**, **Teresa Zimmermann** und co.

alle weiteren Kollegen für eine super Arbeitsatmosphäre, hilfreiche Diskussionen und spaßige Zeiten.

den AK in Groningen, besonders an **Andreia de Almeida**, **Benoit Bertrand**, **Sarah Spreckelmeyer** und **Natalia Estrada**, für die nette Aufnahme in den Arbeitskreis und die Einführung in das Arbeiten mit Zellkulturen. Ebenso danke ich dem AK in Cardiff, besonders **Valeria Ugone**, **Margot Wenzel** und **Fabio Cocco** für die gute Zusammenarbeit.

meine **Forschungspraktikanten** und **Bacheloranden** für die tatkräftige Unterstützung im Labor.

Jürgen Kudermann und **Maria Weindl** für den herausragenden Einsatz bei NMR-technischen Fragstellungen.

Ulrike Ammari, **Petra Ankenbauer**, **Bircan Dilki** und **Rodica Dumitrescu** für die Charakterisierung zahlreicher Verbindungen.

die Sekretärinnen des Lehrstuhls **Ulla Hifinger** und **Irmgard Grötsch** für die hervorragende Arbeit in organisatorischen Belangen, sowie an **Martin Schellerer** für die Bestellung von Chemikalien.

Weiterer Dank geht auch an meine **Studienkollegen und Freunde**, die beim Mittagessen und Kaffeetrinken für die nötige Abwechslung im Laboralltag sorgten.

Von Herzen danke ich meiner **Familie** und besonders meinen **Eltern**, die mir das Studium ermöglichten, mir konstant Rückhalt geben und mit Begeisterung den Weg mit mir gegangen sind.

Zu guter Letzt danke ich meinem Freund **Patrick**, der mich immer liebevoll unterstützt, mir den nötigen Halt gibt und für ein abwechslungsreiches Leben außerhalb der Chemie sorgt.

Abstract

The anticancer drug cisplatin is widely applied as treatment for testicular and ovarian cancer among others. Tumor research is currently focusing on targeting methods to avoid the drawbacks resulting from cisplatin therapy. One targeting method is the use of supramolecular coordination complexes (SCCs) as carrier systems for anticancer drugs.

In this work, *exo*-functionalized Pd_2L_4 (L = ligand) cages were evaluated as drug delivery systems for cisplatin regarding their encapsulation properties as well as their toxicity in cancer cells and healthy tissue. Based on X-ray diffraction (XRD) and NMR studies, cisplatin can be encapsulated within the cavity of these palladium cages. The hydroxymethyl-functionalized cage encapsulating cisplatin shows an improved cytotoxic effect in cancer cells compared to cisplatin, while being less toxic in healthy rat liver tissue. These results reveal the potential of Pd_2L_4 cages functioning as delivery system for cisplatin.

The uptake of palladium cages in cells was evidenced by fluorescence microscopy using the amine-based cage with a fluorescence quantum yield of 17%. However, the emission is too low to determine the target in cancer cells. To follow the distribution of palladium cages in cells by fluorescence microscopy, highly luminescent coordination cages need to be developed.

The coupling of fluorophores, such as naphthalene or anthracene, to the Pd_2L_4 cages *via* an amide bond resulted in almost non-emissive compounds. These unexpected results were explained by density functional theory (DFT) calculations showing a disruption of the chromophoric system in the excited state by bending the amide bond, thus no emission occurs. These cages are further highly cytotoxic against cancer cells making them suitable candidates as anticancer agents.

In order to obtain highly luminescent cages, a linker was inserted between the emissive bis(pyridyl) ligand and the luminescent tag, namely a Ru(II) bipyridine complex. In contrast to previously reported Pd_2L_4 cages, this cage demonstrates a remarkable high quantum yield of 66%.

Zusammenfassung

Das Krebsmedikament Cisplatin findet eine breite Anwendung unter anderem zur Behandlung von Hoden- und Eierstockkrebs. Die Tumorforschung fokussiert sich derzeit auf gezielte Verfahren zur Vermeidung der Nachteile, welche durch die Cisplatintherapie entstehen. Eine gezielte Methode ist die Verwendung von supramolekularen Koordinationskomplexen als Transportsysteme für Krebsmedikamente.

In dieser Arbeit wurden *exo*-funktionalisierte Pd_2L_4-Käfige als Wirkstofftransportsysteme für Cisplatin bezogen auf deren Einschlusseigenschaften sowie deren Toxizität in Krebszellen und im gesunden Gewebe beurteilt. Mittels Röntgendiffraktometrie und NMR-Studien wurde gezeigt, dass Cisplatin innerhalb des Hohlraums der Palladiumkäfige eingeschlossen werden kann. Der Hydroxymethyl-funktionalisierte Käfig mit eingeschlossenem Cisplatin weist im Vergleich zu Cisplatin einen verbesserten zytotoxischen Effekt in Krebszellen auf, während dieser weniger giftig in gesundem Rattenlebergewebe ist. Diese Ergebnisse belegen, dass Pd_2L_4-Käfige als potentielle Transportsysteme für Cisplatin eingesetzt werden zu können.

Die Aufnahme der Palladiumkäfige in Zellen wurde mittels Fluoreszenzmikroskopie anhand eines Amine-basierten Käfigs, der eine Fluoreszenzquantenausbeute von 17% hat, bewiesen. Die Emission ist jedoch zu gering, um das Zielmolekül in den Krebszellen zu bestimmen. Hoch lumineszierende Koordinationskäfige müssen entwickelt werden, um die Verteilung der Palladiumkäfige in Zellen mittels Fluoreszenzmikroskopie verfolgen zu können.

Die Kupplung von Fluorophoren, wie Naphthalen oder Anthrazen, über eine Amidbindung an die Pd_2L_4-Käfige führte zu emissionsarmen Verbindungen. Diese unerwarteten Ergebnisse wurden mittels Dichtefunktionaltheorieberechnungen erklärt, wobei das chromophore System im angeregten Zustand durch das Abknicken der Amidbindung unterbrochen ist. Diese Käfige sind zudem sehr zytotoxisch gegenüber Krebszellen, weshalb sie sich als Krebswirkstoff eignen.

Um stark lumineszierende Käfige zu erhalten, wurde ein Linker zwischen den emissiven Bis(pyriydin)liganden und den lumineszierenden Marker, einen Ru(II)-Bipyridinkomplex, eingefügt. Im Gegensatz zu literaturbekannten Pd_2L_4-Käfigen, weist dieser Käfig eine erstaunlich hohe Quantenausbeute von 66% auf.

List of Abbreviations

A	adenine
CNT	carbon nanotubes
CTR	copper transporters
DACH	diaminocyclohexane
DFT	density functional theory
DNA	deoxyribonucleic acid
DOSY	diffusion-ordered spectroscopy
EPR	enhanced permeability and retention
ESI	electrospray ionization
FDA	food and drug administration in USA
G	guanine
GSH	glutathione
HOMO	highest occupied molecular orbital
HAS	human serum albumin
L	ligand
LUMO	lowest occupied molecular orbital
M	metal
MMR	mismatch repair
MOF	metal organic framework
MS	mass spectrometry
MT	metallothionein
NER	nucleotide excision repair
NMR	nuclear magnetic resonance
OCT	organic cation transporters
PEG	poly(ethylene glycol)
SCC	supramolecular coordination complexes

TD	time-dependent
TrxR	thioredoxin reductase
XRD	X-ray diffraction
2D	two-dimensional
3D	three-dimensional

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1. Introduction

1.1 Cancer – A widespread disease

Cancer is the uncontrolled growth of abnormal cells leading to malignant tumors and metastasis. The greek physician Hippokrates (460-370 BC) introduced the term “cancer” and gave the first scientific explanation attempt for the development of cancer. In his view, diseases were caused by an imbalance of the four body fluids blood, mucus, yellow and black bile, especially the cause of cancer was attributed to an excess of black bile in specific parts of the body.[1] Although the disease is known since ancient times, cancer is still a leading cause of death worldwide. The world health organization (WHO) reported a cancer incidence of approximately 14.1 million cases and a mortality of 8.2 million in the year 2012.[2] After circulatory diseases (39%), cancer is the second most common cause of death with 26% in Germany in 2014.[3] Nearly 480,000 incident cancer cases arise each year in Germany, while 220,000 people die because of cancer (Data from 2010). The percentage of all new cancer cases and cancer deaths based on the tumor localization of men and women in Germany in 2010 are depicted in Figure 1. The most frequent tumor site among men diagnosed with cancer is the prostate and among women the breast, followed by lung and colorectum. In contrast, lung cancer is the most common cause of death for men followed by colorectum and prostate, while for women it is also breast cancer followed by lung and colorectum.[4]

In all types of cancer, the genetic information is damaged resulting in deoxyribonucleic acid (DNA) mutations. Transformation of normal human cells into malignant neoplasm is a multistep process described as tumorigenesis.[5] The main phases of the development of cancer are initiation, promotion and progression. The DNA damages are induced during the initiation by physical, chemical or biological carcinogens, such as sunlight, components of tobacco smoke and viruses. Three genes are involved in tumorigenesis, DNA repair genes, tumor suppressor genes and

oncogenes.[6] DNA alterations can be either corrected by repair proteins during the replication or tumor suppressor proteins initiate the apoptosis of mutated cells. The promotion is induced by an activation of the oncogenes and an inhibition of tumor suppressor genes leading to an increased proliferation of cancer cells. The growth of the neoplastic transformation of cancer cells to a malignant tumor is called progression.[7] To further promote the tumor growth and metastasis, new blood vessels (angiogenesis) are formed for oxygen and nutrient supply.

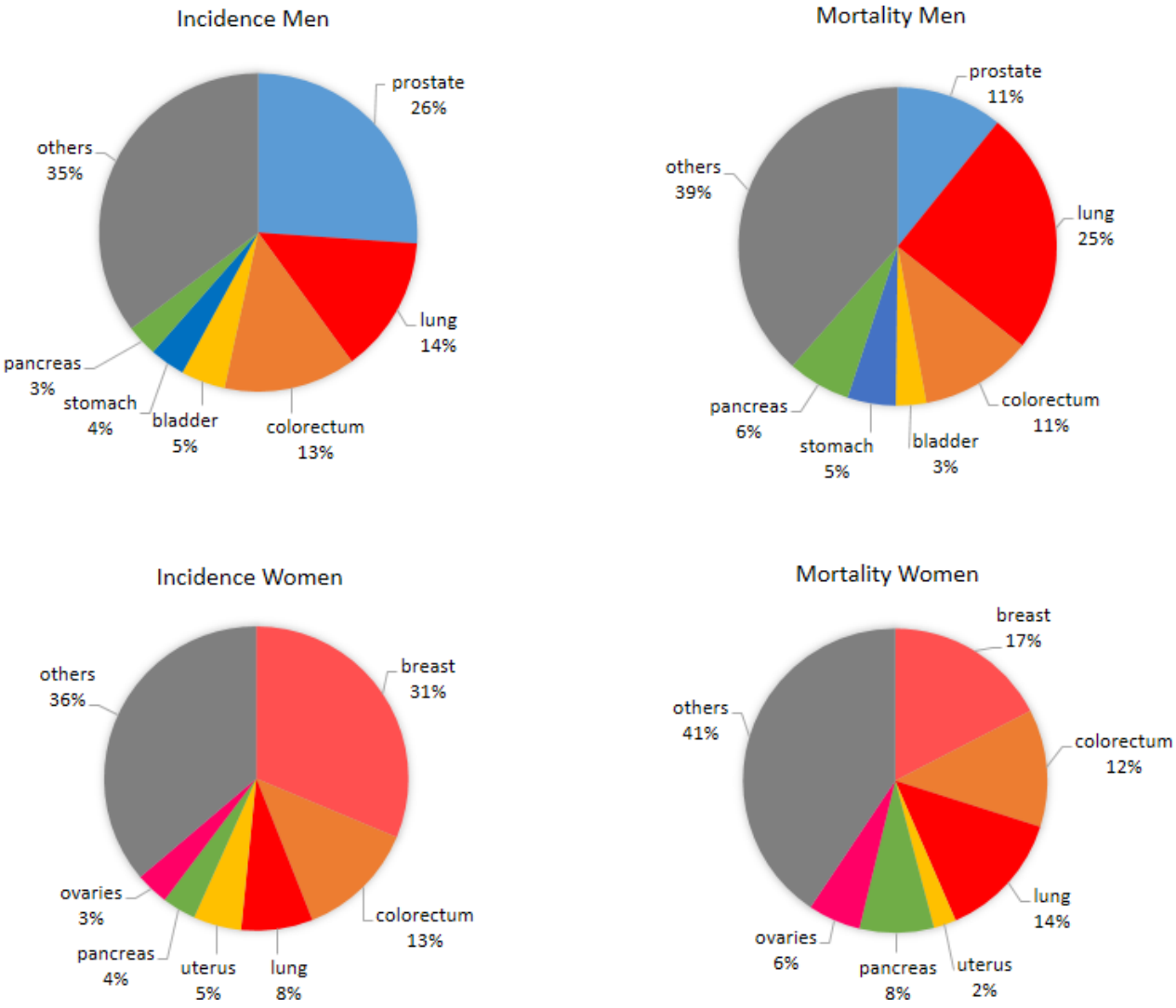


Figure 1. Most frequent tumor localization as a percentage of cancer incidence and mortality of men and women in Germany in 2010.[4]

In most cases, cancer arises due to environmental and lifestyle influences, while only 5 to 10% are attributed to the genetic predisposition.[8] Risk factors are tobacco, nutrition (*e.g.* red meat), infections (*e.g.* viruses), alcohol, sunlight exposure, environmental pollutants, obesity and

physical inactivity.[9] Three major methods are used for the treatment of cancer, surgery, radiation therapy and chemotherapy. The latter one is a method using chemical substances to treat diseases. At the beginning of the 20th century, Paul Ehrlich (1854-1915) introduced this type of treatment using the chemical substance arsphenamin (salvarsan) to cure syphilis. Among the first anticancer drugs are the antifolate methotrexate, the taxane paclitaxel (trademark: taxol) and the metal complex cisplatin (trademark: platinol).[10]

1.2 Platinum complexes in tumor therapy

Since the discovery of the first metal complex, namely cisplatin, as chemotherapeutics in cancer therapy, a vast number of organometallic and coordination complexes have been developed. However, only a few complexes have entered clinical trials and even less are approved for the clinical use. Up to date, three Pt(II)-based drugs (cisplatin, carboplatin and oxaliplatin) obtained worldwide approval and further three platinum complexes are approved for regional clinical use (Figure 2).[11] Other metal-based compounds, such as the ruthenium complex NAMI-A or the gold(I) compound auranofin, are still in clinical testing.[12] Even the first drug delivery systems of platinum complexes, *e.g*. Lipoplatin and ProLindac, entered clinical trials.[13] The development of platinum anticancer agents as well as the use of drug delivery systems is discussed in the following sections.

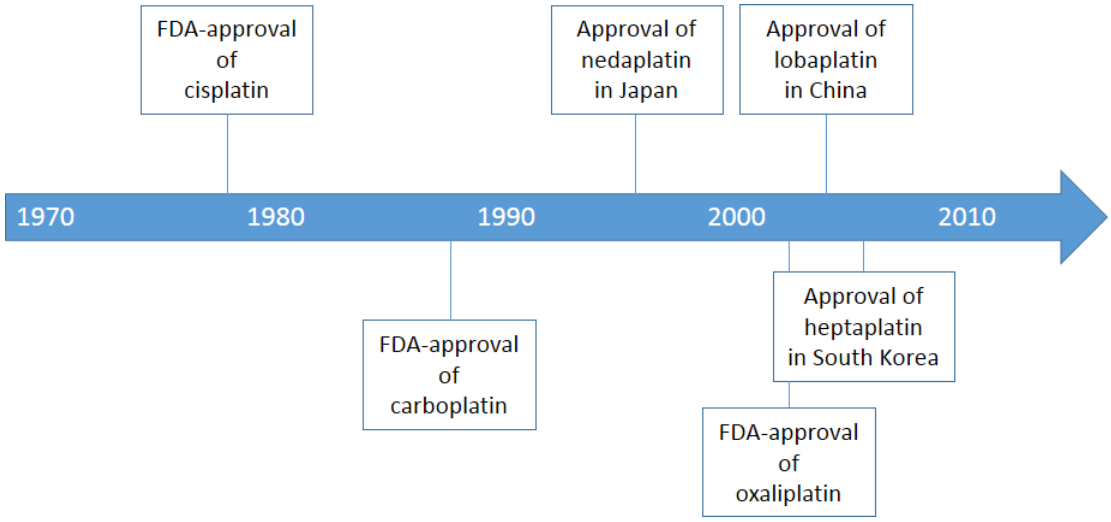


Figure 2. Milestones of platinum complexes as anticancer drugs for the clinical use.[11]

1.2.1 The anticancer drug cisplatin

In the late 1960's, Barnett Rosenberg and his coworkers discovered accidently the cytotoxic effects of the complex *cis*-diamminedichloridoplatinum(II) (cisplatin) and proved its antitumor activity in mice.[14] Cisplatin is worldwide successfully applied as anticancer drug since the FDA approval in 1978. Cisplatin is mainly used to treat cancers of the testicles, ovaries and bladder.[15]

In some cases, however, it is also applied for the treatment of head and neck cancer, lung cancer and brain tumors among others. In chemotherapy, cisplatin is administered intravenously as a saline solution. After the approval of cisplatin as chemotherapeutics, the mechanism of action has been investigated in detail and elucidated involving four main processes: cellular uptake and accumulation (1), activation by aquation (2), DNA platination (3) and cellular recognition of the DNA damage leading to apoptosis (4) (Figure 3).[15,16]

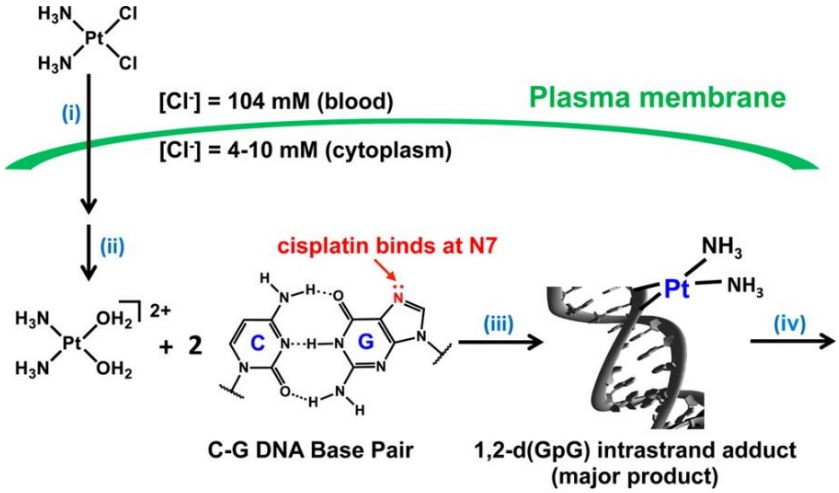


Figure 3. Mechanism of action of the anticancer drug cisplatin. (i) Cellular uptake, (ii) aquation inside the cell, (iii) DNA binding, and (iv) cellular recognition leading to apoptosis.[15] Reprinted with permission from *Chem. Rev*. **2016**, *116*, 3436-3486, copyright 2016 American Chemical Society.

The uptake of cisplatin by cells takes place *via* two different pathways (see also Figure 4), either by passive diffusion through the plasma membrane or by active transport using membrane proteins, such as copper transporters (CTRs) and organic cation transporters (OCTs). Moreover, the accumulation inside the cell is also regulated by efflux transporters (*e.g*. ATP7B). Because of the reduced chloride concentration inside the cell (4 – 10 mM) compared with that of the bloodstream (104 mM), the ligand substitution of cisplatin is facilitated, in which chloride ligands are replaced by water molecules. In the next step, aquated cisplatin enters the nucleus and interacts with its target DNA by binding to N7 atoms of guanine (G) and adenine (A) residues. The aqua ligands are substituted by purine residues forming different types of DNA cross-links, such as 1,2-GG, 1,2-AG and 1,3-GG intrastrand adducts but also GG interstrand cross-links. The cellular

recognition of the resulting platinum DNA damage initiates the inhibition of the transcription, hence the programmed cell death (apoptosis).[17]

Despite its successful use in cancer therapy with a cure rate of over 90% for testicular cancer, the treatment with cisplatin has also several drawbacks. [16] Cancer patients experience strong side effects, since cisplatin is not only toxic against malignant cells but also cytotoxic for rapidly dividing normal cells. The dose-limiting toxicities of cisplatin are nephrotoxicity, neurotoxicity and ototoxicity.[18] Further limitations for the patient's quality of life are a loss of hair, nausea and vomiting as well as disorders of the immune system. Another major problem is the development of drug resistance, which is ascribed to deactivation mechanisms (Figure 4).

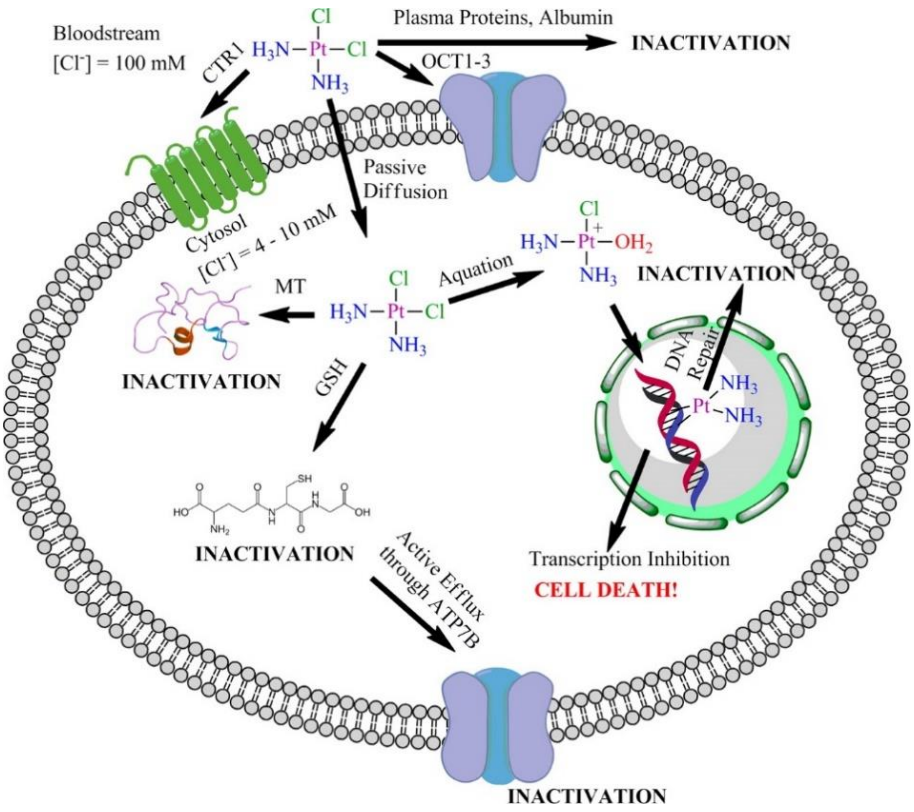


Figure 4. Extracellular and intracellular inactivation processes influencing cisplatin resistances.[19] Reprinted with permission from *Inorg. Chem*. **2013**, *52*, 12234-12249, copyright 2013 American Chemical Society.

Since cisplatin is administered intravenously, it can already be deactivated in the bloodstream by interaction with proteins, such as human serum albumins (HSA).[20] Drug resistance can be reasoned to the reduced accumulation of cisplatin inside the cell due to an altered regulation of

influx and efflux transporters.[21] Once inside the cell, aquated cisplatin can interact with sulfur-rich components by binding to glutathione (GSH) and metallothioneins (MT) leading to an increased detoxification.[22] Another reason of cisplatin resistance is an enhanced DNA repair mechanism of platinum-DNA adducts. DNA damages can be effectively removed by the nucleotide excision repair (NER) or the mismatch repair (MMR) mechanism.[23]

1.2.2 Further developed platinum anticancer agents

In order to reduce the cisplatin-associated toxicities and resistances, various platinum(II) complexes have been developed. The Pt compounds with the formula *cis*-[PtL_2X_2] are uncharged, square-planar complexes featuring *N*-based ligands (L) and leaving groups (X). The platinum complexes in clinical use are presented in Figure 5. The modification of the anionic ligands X_2 in carboplatin and nedaplatin influences the rate of aquation, thus the toxicity profile. The modification of the *N*-based ligands L_2 in oxaliplatin, lobaplatin and heptaplatin results in the formation of structurally different DNA adducts, thereby the resistance profile is affected.[18,24]

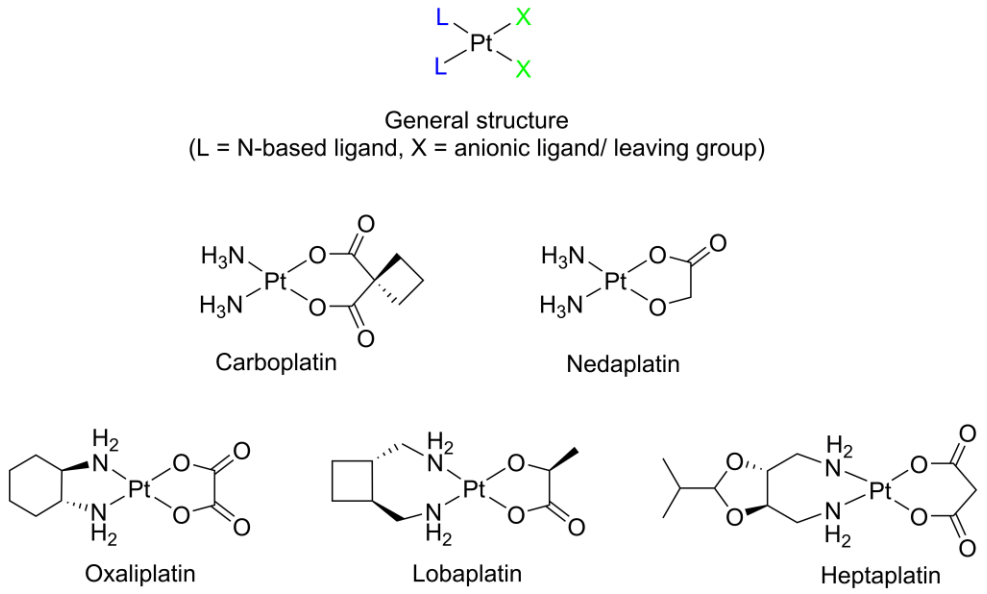


Figure 5. Platinum-based anticancer drugs approved worldwide or in specific countries.[13,24]

Carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II), trademark: paraplatin) is worldwide applied to treat ovarian cancer since 1989. The ligand substitution of chloride ligands

with a bulky bidentate ligand resulted in a reduced toxicity compared to cisplatin. The lower reactivity, thus higher stability, decreases the side effects, namely ototoxicity and nephrotoxicity, however, carboplatin is also less effective against testicular and bladder cancer.[25] Another cisplatin derivative is nedaplatin (*cis*-diammineglycolatoplatinum(II)), which is used for the treatment of small cell lung cancer, non-small cell lung cancer as well as head and neck cancers. Although the nephrotoxicity is significantly reduced using nedaplatin, it is only approved in Japan under the trademark aqupla since 1995, while still in clinical trials in the USA.[24,26]

Since the FDA approval in 2002, oxaliplatin has become a blockbuster for the clinical use against colorectal cancer. The replacement of the ammine ligands by a bulkier bidentate ligand results in a lack of cross-resistance with cisplatin. Moreover, oxaliplatin is less toxic than cisplatin.[27] The platinum complexes lobaplatin and heptaplatin obtained regional approval in China and in South Korea, respectively, for various types of cancer.[18]

The discussed Pt(II) complexes have a similar mechanism of action as the one of cisplatin with DNA as target. Since platinum(II) chemotherapeutics still exhibit several limitations, platinum(IV) prodrugs have been developed due to their favored physicochemical properties.[15,28] Platinum(IV) compounds are octahedral, six-coordinated complexes having compared to Pt(II) compounds additional ligands in axial positions. The extra ligands have an impact on the stability and fine-tuning of biological properties, lipophilicity and solubility. As platinum(IV) agents are considered as prodrugs, the reduction from Pt(IV) to Pt(II) and the loss of the axial ligands is necessary for their anticancer activity. Three examples of Pt(IV) prodrugs, which entered clinical trials, are depicted in Figure 6. Iproplatin and ormaplatin entered clinical trials in the 1980s and 1990s, respectively, but no marketing approval was given, while satraplatin is still under clinical investigation.[18]

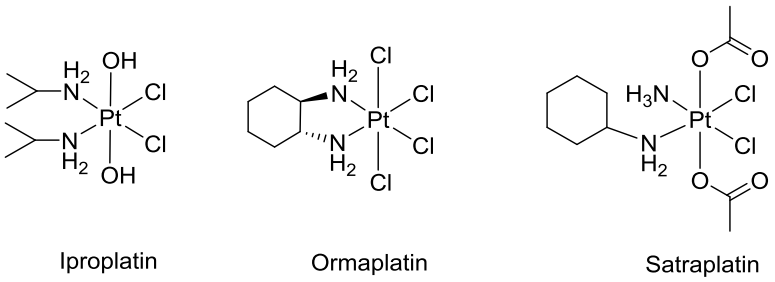


Figure 6. Examples of platinum(IV) prodrugs tested for their anticancer activity in clinical trials.[15,28]

One of the first platinum prodrugs entering clinical trials is iproplatin (JM9). The mechanism of action involves reduction to platinum(II) followed by aquation and DNA binding.[29] The advantages of iproplatin are the slow reduction rate and the high water solubility. Several Phase I, II and III clinical trials have been conducted making it the most studied platinum anticancer drug. However, marketing approval failed due to the low anticancer activity compared to cisplatin and carboplatin.[30]

Another platinum prodrug, which is structurally similar to iproplatin, is ormaplatin (tetraplatin).[24] The platinum(IV) complex is rapidly reduced due to the chloride ligands in axial positions. Although ormaplatin has been tested in Phase I clinical studies, it never entered Phase II because of severe neurotoxicity.

A promising Pt(IV) prodrug is satraplatin being the first orally administered platinum drug.[31] Once in the bloodstream, the prodrug is reduced to six different platinum(II) species. The major metabolite ammine(cyclohexylamine)dichloroplatinum(II) (JM118) is formed by the loss of two axial ligands showing also the most potent anticancer activity. The drug is effective in prostate, lung and ovarian cancer having advantages such as the type of administration, milder toxicity profile and lack of cross-resistance with cisplatin. Although satraplatin entered clinical trials in 1992, it is still under clinical investigation and did not yet obtain marketing approval.

1.2.3 Drug delivery systems

The platinum(II) complexes and platinum(IV) prodrugs interact with the classic target DNA, while other metal-based drugs (*e.g*. Au and Ru) target overexpressed enzymes and proteins in cancer cells.[32] In recent years, a contrary approach to the active targeting, namely the use of drug delivery systems, has received much attention. Passive targeting has great potential to enhance the efficacy of anticancer drugs, to reduce severe side effects and to overcome drug resistance.[18] The advantages of large carrier-based systems are the protection of the anticancer agent against early activation during the transport and the selective delivery and accumulation of the drug into the tumor tissue. The latter processes are explained by the enhanced permeability and retention (EPR) effect (Figure 7). This effect is caused by the leaky vasculature and the poor lymphatic clearance of the tumor tissue.[33] In general, small molecules can pass through the tight endothelial cell layer of blood vessels in healthy tissue as well as through the leaky vasculature in tumor tissue. However, large molecular weight molecules can only diffuse through the capillary wall of tumor tissue. While the lymphatic drainage is effective in normal tissue, macromolecules are retained in tumor tissue, thus accumulate selectively.

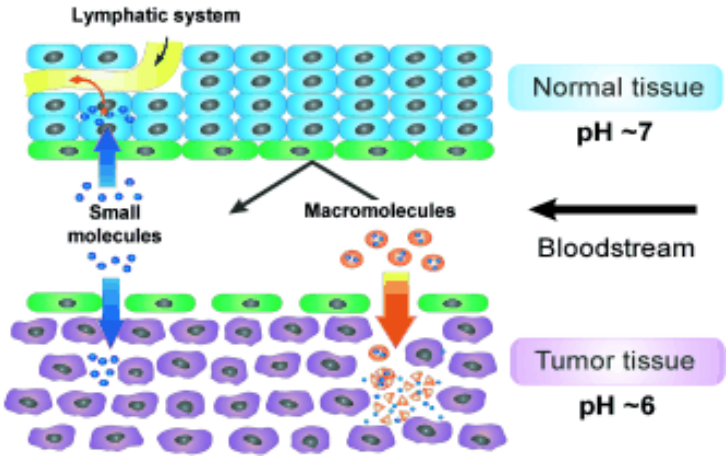


Figure 7. Schematic representation of the permeability and retention of small molecules and macromolecules in normal and tumor tissue.[34] Reprinted with permission from *Angew. Chem. Int. Ed*. **2006**, *45*, 1198 – 1215, copyright 2006 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

To provide more effective and less toxic anticancer formulations, a variety of drug delivery systems has been developed and investigated in the last years including carbon nanotubes, liposomes, nanoparticles, polymers and micelles (Figure 8). Liposomes are prepared by

amphiphilic phospholipids resulting in lipid bilayer vesicles with an aqueous cavity.[35] These systems are between 50 and 1000 nm in size and are capable to encapsulate hydrophobic and hydrophilic drugs. The major advantages of liposomes are the low toxicity, the biodegradability and the immunogenicity. A problem is the rapid elimination from the systemic circulation, which affects the biodistribution, thus reduces the biological activity. A promising liposomal drug delivery system is Lipoplatin consisting of 91% lipids and 9% cisplatin.[36] The liposomal drug formulation is currently in Phase III clinical trials showing superiority to cisplatin in non-small cell lung cancer due to the reduction of side effects. Lipoplatin can potentially replace cisplatin and be applied for various types of cancer.

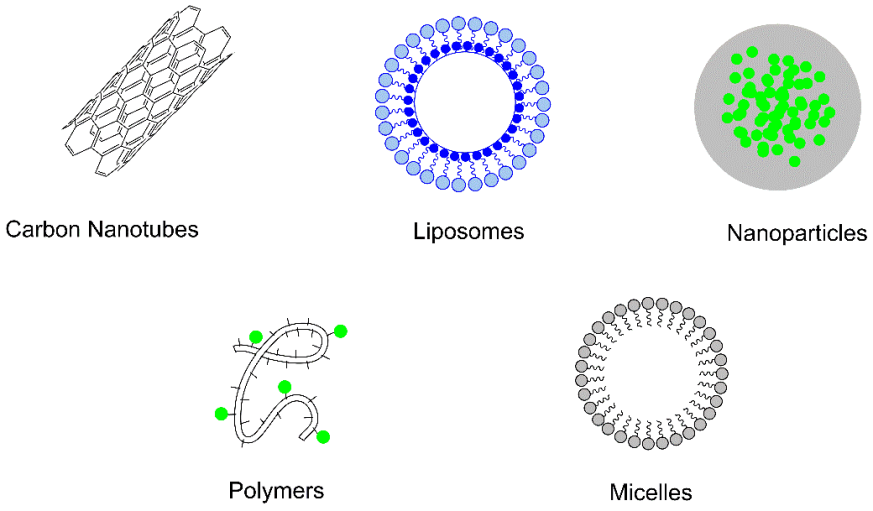


Figure 8. Various types of drug delivery systems being currently under investigation.[18]

Polymers can also function as inert carrier systems. Hereby, the therapeutic drug is covalently attached to a polymer forming polymer-drug conjugates.[37] The drug is conjugated *via* ligating groups such as nitrogen or oxygen donors, *e.g*. amine, carboxylate and hydroxyl moieties. The most common polymer for anticancer drugs is poly(*N*-(2-hydroxypropyl)-methacrylamide) (PHPMA). An example for a HPMA-platinum drug conjugate is ProLindac (AP5346), which is under clinical investigations.[38] As oxaliplatin, the polymeric delivery system contains diaminocyclohexane platinum (DACHPt). The low pH environment in tumors triggers the release of DACHPt, since the amidomalonate-platinum linker is pH-sensitive. ProLindac exhibits a higher efficacy and a better safety profile compared to oxaliplatin.

Another class of drug delivery systems are gold nanoparticles. The ease of synthesis and functionalization, the high drug load as well as the biocompatibility are advantageous, while retention and accumulation of metal particles inside the body after drug administration can have undesired toxic effects.[39] For instance, platinum-tethered PEGylated (poly(ethylene glycol) gold nanoparticles showed improved cytotoxicity in cancer cells compared to oxaliplatin *per se*.[40]

One of the most studied carrier systems are carbon nanotubes (CNTs) due to their interesting properties, such as high drug load, large surface area and the various conjugation possibilities at the inner and outer surface to functional groups and drug molecules.[41] For instance, water soluble amine-functionalized single-walled CNTs are conjugated to a Pt(IV) prodrug *via* peptide linkers.[42] A decrease in pH inside testicular cancer cells results in a reductive release of the Pt(II) complex. The platinum(IV)-conjugated CNTs exhibit a >100-fold higher cytotoxicity compared to the free Pt(IV) prodrug.

Further types of delivery systems for metal-based anticancer drugs are dendrimers,[43] polymeric micelles[44] and metal-organic frameworks (MOFs).[45] In the last years, an increasing interest of supramolecular coordination cages (SCCs) as drug delivery systems arose. In addition to the promising biological features, SCCs possess also remarkable physicochemical properties. In the next chapter, the development of supramolecular systems is discussed, particularly with regard to the application as carrier systems for anticancer drugs or as anticancer agent *per se*.

1.3 Supramolecular coordination complexes

Metallosupramolecular complexes have attracted much attention in the past decades because of their potential applications in molecular recognition, catalysis and biological processes, among others.[46] SCCs are, in contrast to MOFs, well-defined discrete two- (2D) and three-dimensional (3D) molecular entities.[47] The preparation of SCCs is based on coordination-driven self-assembly of suitable metal ions or dinuclear complexes with polydentate ligands, resulting in complexes of the type M_xL_y (M = metal, L = ligand) and [x+y] (x = number of dimetallic building blocks, y = number of ligands) assemblies, respectively. Schematic representation of the self-assembly approaches is exemplarily depicted in Figure 9. The shape and size of the metal-based entities is defined by M:L ratio, by the coordination geometry of the metal and by the ligand structure. In addition to 2D metallacycles, 3D metallocages with precise geometries and cavities are also of current interest for biological applications due to their guest binding abilities.[48] The guest molecules are bound inside the cavity by various interactions, such as hydrogen bonding, Coulomb, van der Waals, and steric interactions. The advantages of SCCs are the ease of synthesis and the possibility to modify the compound's properties by functionalization of the ligand, thus interesting chemical-physical properties, such as solubility, luminescence and guest encapsulation, can be obtained.[49]

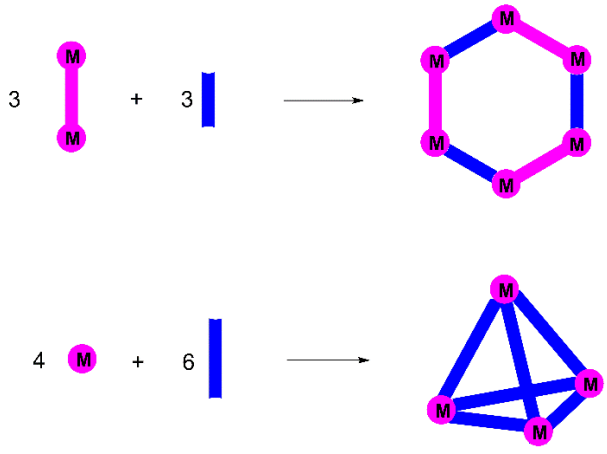
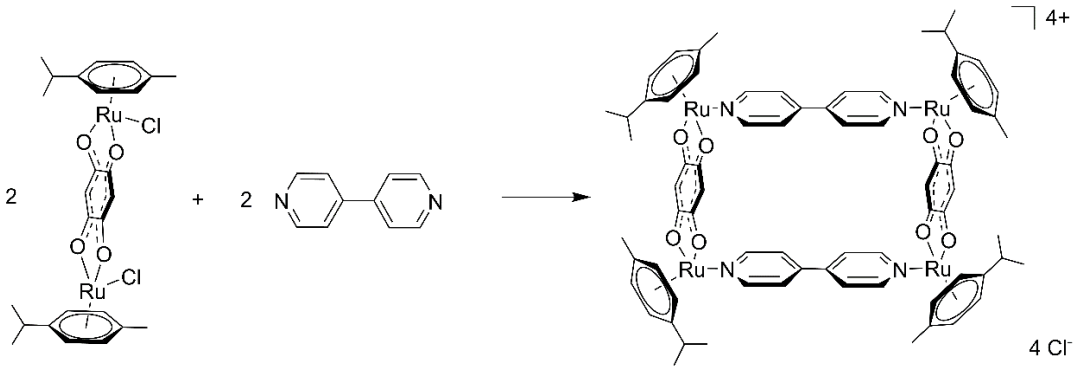


Figure 9. Schematic representation of the self-assembly of [3+3] metallacycles and M_4L_6 metallocages.

1.3.1 Metallacycles

In the last years, cyclic self-assemblies with different metal ions, such as Ru, Pd, Pt, and Ir, have been investigated for the cytotoxicity in cancer cells, the localization by fluorescence microscopy and the interaction with DNA. A series of arene ruthenium(II) metallacycles as anticancer agents was studied by the research groups of Chi and Therrien.[50,51,52,53,54] The Ru complexes are prepared using a [2+2] self-assembly approach, which is exemplarily depicted in Scheme 1. Several of these metallacycles showed similar or even improved cytotoxicity to different cancer cell lines compared to cisplatin. Some arene Ru cycles are effective against multidrug resistant cancer cells.[50,53] In addition, the uptake of these metallacycles by cancer cells and their localization within the cells was investigated by fluorescence microscopy showing an accumulation of the fluorescent compounds in the nuclei.[54]



Scheme 1. Self-assembly of an arene ruthenium(II) [2+2] cycle exhibiting anticancer activity.[51]

Another arene Ru(II) metallacycle containing a BODIPY-based linker showed selective toxicities against MCF-7 and HeLa cancer cell lines, while the respective pentamethylcyclopentadienyl Ir(III) metallarectangle is selectively toxic against U87 cancer cells.[55] Both complexes were visualized in cells by fluorescence microscopy revealing an accumulation of the compounds in the cytoplasm. In addition, the antiproliferative effects of pentamethylcyclopentadienyl iridium(III) and rhodium(III) metallacycles with lipophilic side chains have been investigated.[56] The Rh complexes are more effective than the Ir analogs. However, both complexes have a higher selectivity for cancer than for non-cancer cells.

For biological imaging, fluorescent compounds are used to follow the uptake and localization in cells by fluorescence microscopy. Therefore, highly luminescent compounds attracted attention also in the field of metallacycles. So far, only a few examples of fluorescent metallacycles, such as Pt_4L_4,[57] M_2L_2 (M = Zn, Ag)[58] and [3+3] platinum assemblies,[59] have been reported. Some of these feature fluorescence quantum yields up to 16%.

Apart from the cytotoxic tests of Ru(II), Ir(III) and Rh(III) rectangles, palladium(II) and platinum(II) cycles were studied for their toxic effects in cancer cells as well as for their DNA binding abilities.[60–63] The first *in vivo* toxicity studies of SCCs were conducted in mice using [2+2] platinum(II) metallacycles.[60] Although the Pt complexes have less cytotoxic effects towards cancer cells, efficacy in reducing the tumor growth rate in breast cancer mouse xenograft model was observed. In addition, the localization of Pt(II) cycles with fluorescence quantum yields below 10% was monitored by confocal microscopy suggesting a lysosomal accumulation of the metallacycles in cells.

In contrast, ferrocene-based M_2L_2 (M = Pd, Pt) assemblies showed higher antiproliferative effects in several cancer cell lines compared to cisplatin.[61] Interestingly, these type of metallacycles have the ability to bind and unwind supercoiled DNA.[63] Another type of metallacycles interacting with DNA is a Pt_4L_4 square.[62] An attractive target in cancer therapy, apart from the classical target DNA, is the stabilization of the G-quadruplex, thus the inhibition of the enzyme telomerase. This enzyme, being responsible for the elongation of telomeric DNA to avoid apoptosis, is overexpressed in cancer cells, while inactive in somatic cells. The platinum assembly can function as telomerase inhibitor, as it displays a high binding affinity to the G-quadruplex. The G-quadruplex structure and stabilizing Pt(II) metallacycle are depicted in Figure 10.

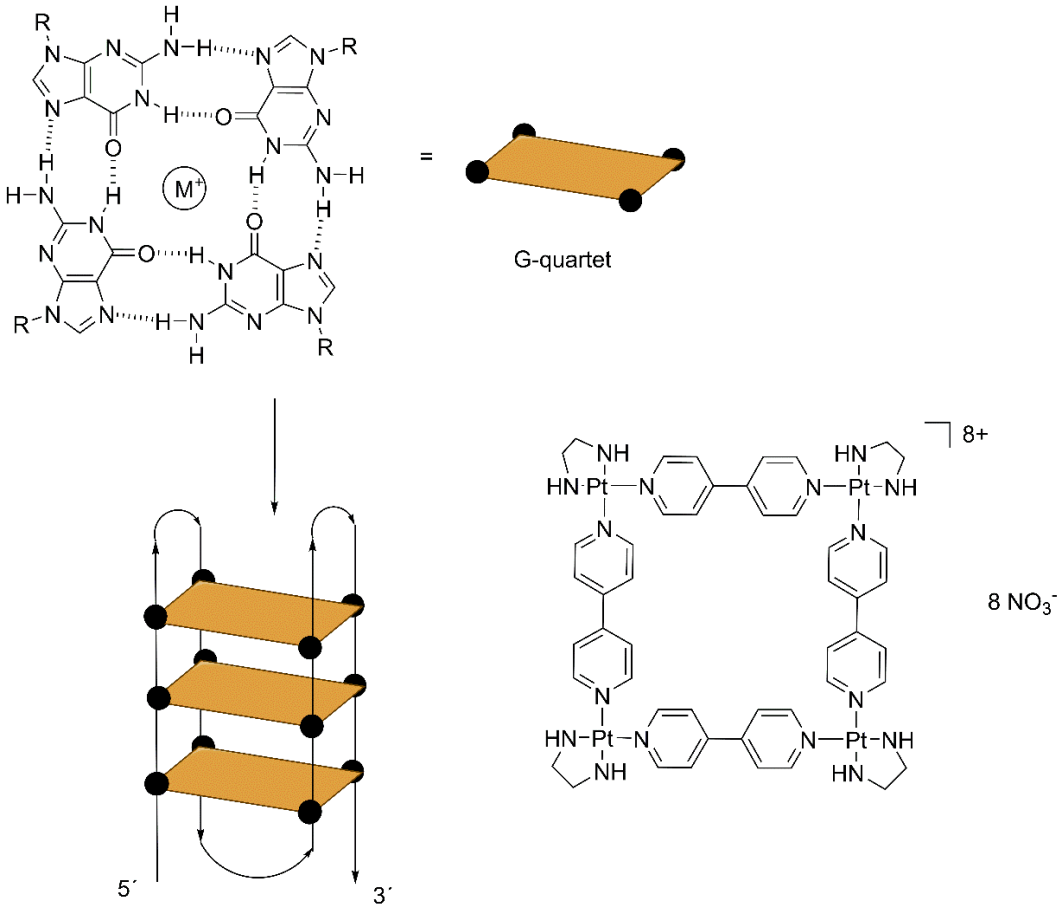


Figure 10. G-quadruplex structure consisting of 2 or more G-quartets. The G-quartets (4 guanine bases) are stabilized by sodium or potassium ions. Pt_4L_4 metallacycle interacting with telomeric DNA.[62,64]

1.3.2 Metallocages

Not only metallocycles can interact with DNA, but also metallocages have been reported to bind to DNA. Octacationic [4+2] arene ruthenium(II) cubes have been studied for their DNA binding abilities showing strong interactions with quadruplex DNA, especially with telomeric DNA.[65] One of the first supramolecular cages binding to DNA is described by Hannon *et al*.[66] The iron(II) cylinder $[Fe_2L_3]Cl_4$ targets Y-shaped DNA junctions and coils DNA. However, the cylindric assembly shows only moderate cytotoxicity in different cancer cell lines compared to cisplatin. The conjugation of arginine residues to the ligand framework of the iron cylinder enhances the cytotoxicity against cancer cells as well as the binding ability to DNA junctions.[67] The use of ruthenium(II) instead of iron(II) for the self-assembly of M_2L_3 cylinders results in a highly stable

and luminescent $[Ru_2L_3](PF_6)_4$ cage, while the cytotoxicity and the DNA binding ability is similar to Fe_2L_3.[68]

In the last years, an increasing interest on highly luminescent metallocages arose for potential applications such as biological imaging. One of the most emissive supramolecular cages is a Zn_4L_6 tetrahedron with a fluorescence quantum yield of 67%.[69] Further platinum(II) metallocages with organic luminophores are reported by Stang *et al*. resulting in quantum yields up to 82%.[59,70] In addition, luminescent metallosupramolecular complexes can be obtained by incorporation of ruthenium(II) polypyridyl complexes.[71] Ru(II) polypyridines have promising photo-physical properties. In most cases, ruthenium bipyridyl complexes exhibit higher luminescence than the Ru terpyridyl compounds.[72]

Another class of arene ruthenium(II) self-assemblies, namely hexanuclear [3+2] metallaprisms, can potentially function as anticancer agent *per se* as well as drug delivery system.[73] The first example of a metallocage acting as drug delivery system has been reported by Therrien *et al*. in 2008 (Figure 11). The hexaruthenium prism encapsulating palladium or platinum acetylacetonato complexes exhibits an enhanced cytotoxic effect against human ovarian A2780 cancer cells in comparison to the empty cage and the inactive guest molecules.[74] The ruthenium cage compound has been also studied for the guest encapsulation properties for pyrenyl derivatives and the drug release in cancer cells showing an increased anticancer activity.[75] Similar water soluble arene ruthenium metallocages were used to deliver hydrophobic pyrene and porphin guest molecules into cancer cells revealing an enhanced cytotoxicity.[76] Recently, Lippard *et al*. presented a promising strategy using a hexanuclear platinum(II) coordination cage to deliver platinum(IV) prodrugs into cancer cells (Figure 11).[77] The host-guest complex displays a high cellular uptake due to the high positive charge and a similar cytotoxicity profile as the anticancer drug cisplatin against several cancer cells. Although some reports on supramolecular coordination cages as potential drug delivery systems exist, this research field is still in its infancy.

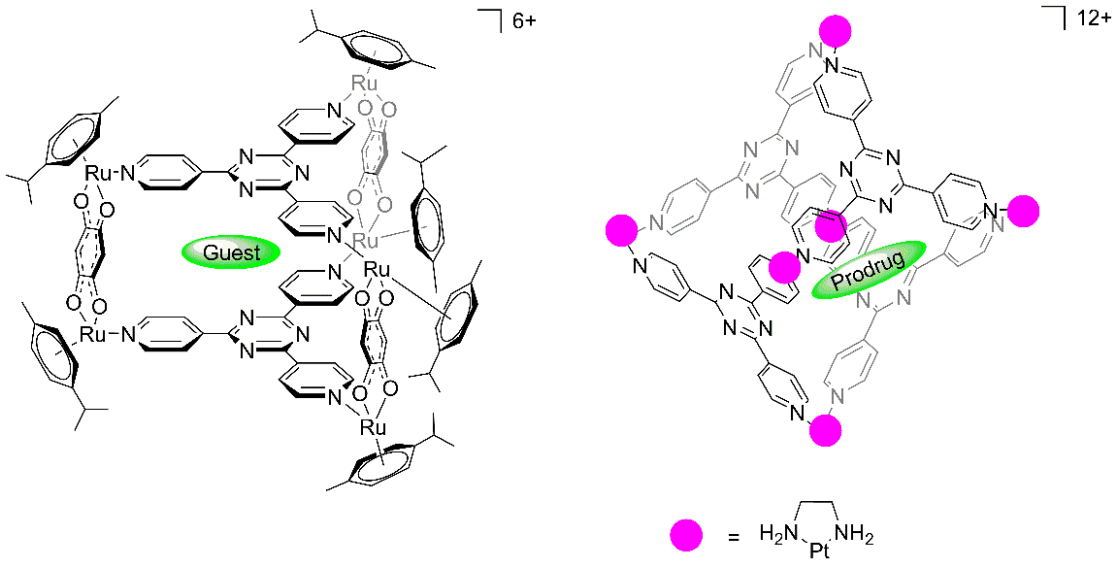


Figure 11. Examples of supramolecular coordination cages as drug delivery systems reported by the groups of Therrien and Lippard.[74,77]

1.3.3 M_2L_4 coordination cages

An attractive research field of supramolecular metallocages is the self-assembly of M_2L_4 cages. The advantages of these type of metallocages are the high symmetry, the ease of synthesis as well as the alteration of the cages' properties by variation of the metal ions and functionalization of the ligand framework. In addition, the cage can be functionalized in *exo*- and *endo*-position (Figure 12). *Endo*-functionalization has mainly effects on the formation of discrete metallocages and on the guest binding abilities within the cavity, while *exo*-functionalization affects the cages' properties, *e.g*. solubility and photo-physical behavior.[78,79]

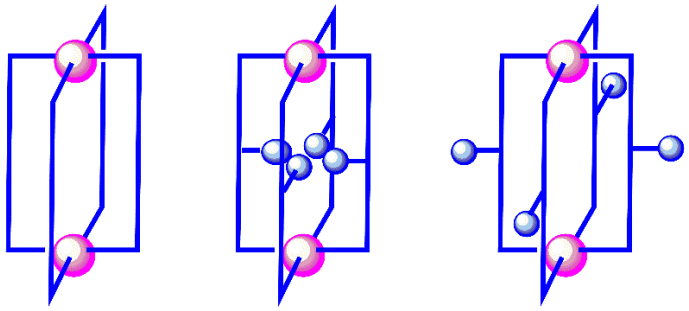


Figure 12. Graphical representation of functionalized M_2L_4 metallocages: unfunctionalized, *endo* and *exo*.

Most M_2L_4 metallocages reported to date are based on palladium(II) ions showing less or non-emissive properties due to the quenching by heavy transition metal ions. In order to obtain highly luminescent metallocages, two approaches have been exploited; the use of metal ions with photo-physical properties or the incorporation of emissive building blocks into the ligand framework. Yoshizawa *et al*. prepared several metal(II) capsules with anthracene shells (Figure 13), exhibiting strong blue fluorescence (Φ = 80%) for the zinc(II) cage, weak emission for Ni and Mn capsules and no emission for platinum, palladium and cobalt cages.[80,81] Furthermore, some luminescent alkyne-based palladium(II) coordination cages were obtained by the introduction of an amine group, of naphthalimide or of ruthenium(II) bipyridyl complexes into the ligand structure (Figure 13).[82–84] A water soluble palladium cage was obtained by conjugation of a glucose moiety.[83] The solubility of the metallocages can be also influenced by the choice of counterions.[82]

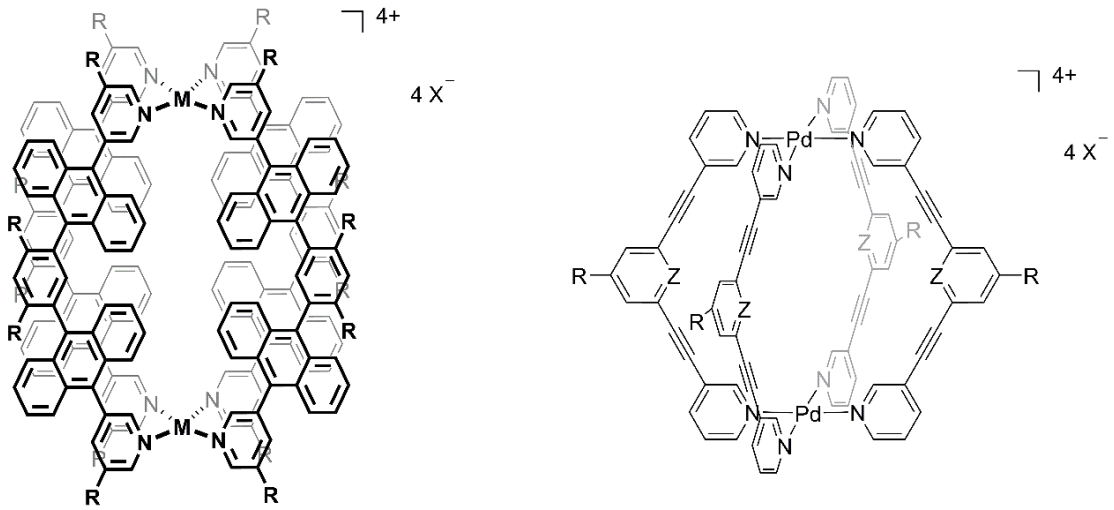


Figure 13. Anthracene-functionalized metallocages prepared by Yoshizawa *et al*. and alkyne-based palladium(II) cages with *endo*- and *exo*-functionalization reported by Hooley and Crowley.[81–83]

Another interesting feature of M_2L_4 cages is the encapsulation of guest molecules, thus the guest binding abilities. Most of these metallocages are capable to encapsulate anions, for instance nitrate, perrhenate and sulfonates,[85] but also neutral organic molecules, such as spherical, planar and large C_{60} guests.[86] Interestingly, the non-emissive anthracene-based Pt(II) capsule by Yoshizawa *et al*. exhibits blue-green, green and red fluorescence upon encapsulation of coumarin,

BODIPY and Nile red guest molecules, respectively, hence highly fluorescent host-guest complexes with quantum yields up to 50% were obtained.[87] Further groups reported the encapsulation of platinum complexes, including the anticancer drug cisplatin, into different M_2L_4 coordination cages.[79,88]

In order to use supramolecular metallocages as drug delivery systems, not only the uptake of the drug is important, but also its release. In the last years, different approaches for the guest release have been investigated. Clever *et al*. studied the light-triggered guest uptake and release using a photochromic palladium(II) cage.[89] In addition, the guest release can also be achieved by disassembling the M_2L_4 cage either by the addition of competing ligands, e.g. DMAP or chloride ions,[90] or by the addition of further equivalents of metal ions to transform the capsule into a M_2L_2 tube.[91]

Recently, the groups of Crowley and Yoshizawa studied the anticancer activity of some palladium(II) and platinum(II) coordination cages. Quadruply-stranded helicates and alkyne-based palladium cages exhibit moderate cytotoxicity against several cancer cell lines,[92] while the anthracene-based Pd and Pt cages are highly cytotoxic.[93] Notably, the anticancer activity is decreased upon encapsulation of organic molecules in the metallocages with anthracene shells.[94]

2. Objective

The drug cisplatin is widely applied in chemotherapy for various cancer types, *e.g*. testicular and ovarian carcinoma. However, cancer patients treated with cisplatin suffer from severe side effects and experience drug resistance. One targeting method to circumvent these drawbacks is the use of drug delivery systems. Supramolecular coordination complexes are attractive candidates as carrier compounds mainly because of their ease of synthesis and encapsulation properties. Nevertheless, biological investigations of SCCs, especially as drug delivery systems, are rarely reported up to date.

The prime objective of this PhD project is the evaluation of *exo*-functionalized Pd_2L_4 coordination cages as potential delivery systems for the anticancer drug cisplatin. Thus, Pd_2L_4 cage compounds bearing amino, carboxy and hydroxy groups in *exo*-position (Figure 14) are synthesized and studied for their cisplatin encapsulation abilities. For fluorescence microscopy, the photo-physical properties of the metallocages are investigated. The antiproliferative activity of the compounds is examined in cancer cells and healthy tissues. To monitor the uptake and distribution of palladium cages in cancer cells, fluorescence microscopy is performed.

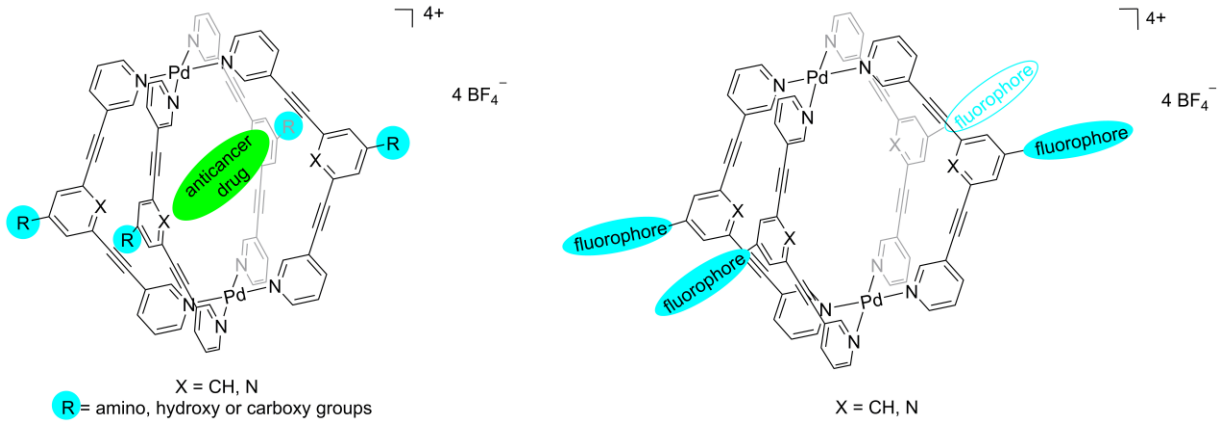


Figure 14. Structures of *exo*-functionalized Pd_2L_4 cages as potential drug delivery systems (left) and with fluorophore groups (right).

A further objective of this work is the synthesis of highly luminescent Pd_2L_4 cages for the localization in cancer cells by fluorescence microsopy. *Exo*-functionalized palladium coordination cages featuring fluorophores (Figure 14), such as anthracene, naphthalene and ruthenium(II) pyridine complexes, are synthesized and studied for their spectroscopic properties.

3. Results – Publication Summaries

In this chapter, summaries of the publications prepared during the course of this PhD project are presented. Reprint permissions and detailed bibliographic data of the articles are shown in chapter 5 and 6, respectively. The published articles as well as supporting information can be found in the appendix.

3.1 Metal complexes as anticancer drugs - Approaches in tumor research and mechanisms of action

Original title: Metallkomplexe als Antikrebsmittel - Konzepte in der Tumorforschung und Wirkmechanismen

This review article is directed at a broad circle of readers, *e.g.* teachers, students and non-professionals, interested in the development of metal complexes in cancer research. Cancer is a leading cause of death worldwide being responsible for *ca.* 14 million new cases and 8.2 million deaths in the year 2012. Since its FDA approval in 1978, the anticancer drug cisplatin is used as treatment for various cancer types, such as testicular and ovarian carcinoma. However, cancer patients treated with the platinum complex suffer from severe side effects and experience drug resistance.

The development of new anticancer drugs having a higher efficacy and a lower toxicity is imperative and currently ongoing. Platinum complexes of the second generation show already less severe side effects. The modification of the ligands influences either the anticancer activity of the complex or the biodistribution, and thus the side effects. Some platinum drugs are approved by the FDA, while others are still under clinical investigation.

In order to circumvent the platinum drug resistance, to reduce the toxic side effects and to broaden the range of application, various organometallic and coordination complexes are

investigated as anticancer agents. The targeted therapy with transition metal complexes takes advantage of the overexpression of receptors and enzymes in cancer cells. The gold complex auranofin, for instance, inhibits selectively the enzyme thioredoxin reductase (TrxR). The inhibition of TrxR induces the production of reactive oxygen species leading to apoptosis. Auranofin is currently in clinical trials for the treatment of ovarian cancer. Another complex under clinical investigations is the ruthenium complex NAMI-A, which is used to treat metastatic cancer.

Another approach to increase the efficacy and reduce the side effects is the use of delivery systems for anticancer drugs. Based on the EPR effect, the large carrier compound is selectively transported and accumulated in the tumor tissue. The liposome Lipoplatin and the polymer-based delivery system ProLindac are investigated in clinical trials. In this review article, the current strategies and enhancements of metal complexes in tumor research are discussed.

My individual contribution was literature research, conception and writing of the article.

3.2 Self-assembled M_2L_4 coordination cages: Synthesis and potential applications

This review article is directed at experts in the field of supramolecular coordination complexes. Metal-mediated self-assembly is used for the preparation of SCCs, which are promising structures, not only because of their interesting properties, but also because of their applicability in host-guest chemistry, catalysis and biomedicine. A relatively new and fast-developing research field of SCCs is the self-assembly of M_2L_4 coordination cages. This review describes the synthesis and characteristics of these metallocages bearing square-planar (Pd, Pt) as well as square-pyramidal and octahedral (Co, Cu, Ni, Zn) coordinated metal(II) ions. The discrete entities exhibit helical and interpenetrated structures and can be functionalized in *exo*- as well as in *endo*-position. A simplified example of an *exo*-functionalized M_2L_4 cage is shown in Figure 15. The solubility behavior and the photo-physical properties can be optimized by the choice of the metal and by functionalization of the ligand structure.

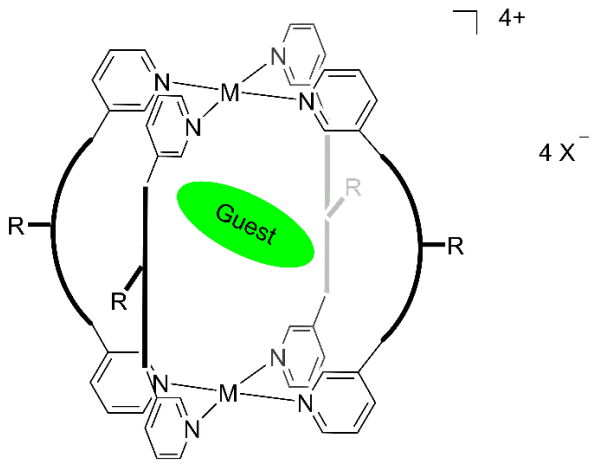


Figure 15. Sketch of an *exo*-functionalized M_2L_4 coordination cage encapsulating a guest molecule.

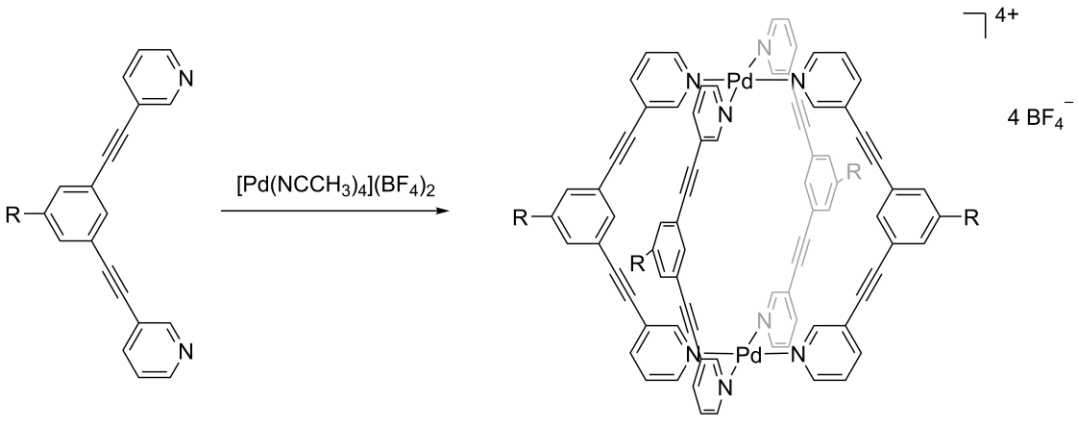
An important feature of coordination cages is the molecular recognition and encapsulation of guest molecules within the well-defined inner cavity. The self-assembled cages are capable to encapsulate anions, cations and neutral molecules mainly by hydrogen bonding and electrostatic interactions. Some of these host-guest complexes might have potential to be applied as chemosensors or drug delivery systems among others. However, the focus in this research field

is still on the structural aspects rather than on the applicability of the metallocages. This article is intended to give a detailed overview of the development of M_2L_4 coordination cages and highlights the potential applications in material science and biological systems.

My individual contribution was literature research, conception and writing of the article.

3.3 Evaluation of new palladium cages as potential delivery systems for the anticancer drug cisplatin

Supramolecular coordination cages are promising for biological applications because of their chemical-physical features and encapsulation properties. Although some metallocages have been studied for the potential use as drug delivery systems, this research field is still less explored. In this article, a series of *exo*-functionalized Pd_2L_4 cage compounds has been synthesized by self-assembly and characterized by various methods. The self-assembly reaction is exemplarily depicted in Scheme 2 for cage **1** and **2**. The successful cage formation was mainly evidenced by NMR spectroscopy, ESI mass spectrometry and X-ray diffraction. The encapsulation properties of the palladium cages were investigated in solution by NMR as well as in solid state by single-crystal XRD showing the inclusion of two cisplatin molecules in the cavity.



Scheme 2. Self-assembly of the palladium cage using the precursor $[Pd(NCCH_3)_4](BF_4)_2$ and the bidentate ligand **L**.

The antiproliferative effects of the metallocages were investigated in cancer cell lines *in vitro* being less or similar cytotoxic than the anticancer drug cisplatin. The IC_{50} values of cage **1** as well as of its respective host-guest complex (cisplatin)$_2$⊂**1** against human ovarian cancer cell line SKOV-3 are shown in Figure 16 with respect to cisplatin. Notably, cage **1** encapsulating cisplatin has an enhanced cytotoxic effect compared to free cisplatin, while it is less toxic *ex vivo* in healthy rat liver tissues. For the first time, M_2L_4 cages have been examined as drug delivery systems in

cells and tissues. The obtained results emphasize the potential of palladium cages for medicinal applications.

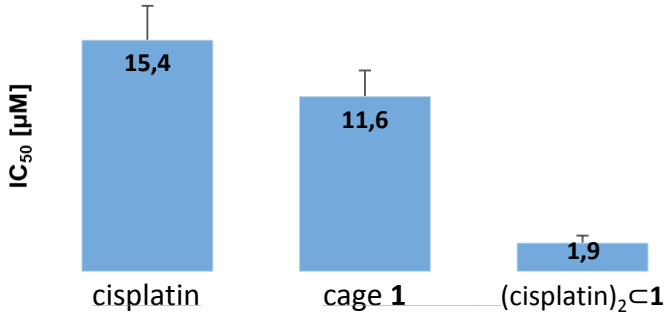


Figure 16. Cytotoxic effects of compounds (IC_{50} values in µM) against human ovarian cell line SKOV-3 after 72 h incubation.

Furthermore, the uptake of the Pd(II) cage **2** in cancer cells was monitored by fluorescence microscopy. Despite the proof of uptake, a precise localization of compound **2** inside the cell was not possible due to the low fluorescence quantum yield of 17%.

My individual contribution was the conception, experimental work and writing of the article.

3.4 Supramolecular *exo*-functionalized palladium cages: fluorescent properties and biological activity

For biological imaging in cells by fluorescence microscopy, the design and synthesis of highly fluorescent metallocages is indispensable. However, the preparation of palladium cages with enhanced luminescence is challenging due to the quenching effect of heavy transition metals. In order to obtain metallocages with improved fluorescent features, carboxy-functionalized bis(pyridyl) ligands were conjugated to fluorophore moieties *via* an amide bond and further self-assembled with Pd(II) ions to form Pd_2L_4 cages.

The photophysical properties of the cages coupled to fluorophores were compared with those of the carboxy-functionalized palladium cage. Examples of the ligands structures are shown in Figure 17. Both ligands and cages exhibit blue emission, while the fluorescence quantum yield Φ of all compounds is below 8%. As expected, the carboxy-functionalized ligand **L4** has a higher quantum yield with 8% than its respective palladium cage **4** displaying a quantum yield of 1%. However, the ligands conjugated to fluorophores show only low emission with $\Phi < 1\%$ resulting in an even lower emission for the corresponding cages.

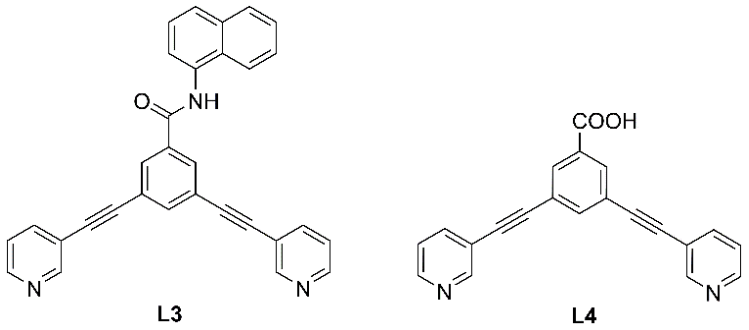


Figure 17. Structure of the naphthalene-conjugated ligand **L3** and of the carboxy-based ligand **L4.**

The unexpected low fluorescence of the ligands was investigated by time-dependent density functional theory (TD-DFT) calculations to determine the probability of a HOMO-LUMO transition and the emission wavelength. A HOMO-LUMO excitation is for ligand **L3** with 2% probability less likely than for ligand **L4** (24% probability). The calculated emission wavelength for **L4** is in the visible region at 420 nm, while **L3** is calculated to emit in the IR region at 2000 nm. Thus, the low

fluorescence of **L3** can be attributed to these points. In addition, the emission properties of **L3** can be explained using the geometry of the ground state *vs.* the geometry of the optimized first excited state. The amide bond is nearly planar in the ground state and almost orthogonal in the excited state, thus the bending of the amide bond results in a disruption of the chromophoric system.

Furthermore, the cage compounds were studied for their antiproliferative effects in cancer cell lines. The fluorophore-based cages are more cytotoxic against the tested cells than the anticancer drug cisplatin, whereas the carboxy-based cage has a very low anticancer activity. According to preliminary results, the carboxy-based cage is a suitable candidate as drug delivery system for cisplatin. In contrast, the cages attached to fluorophores might have potential to function as anticancer agents *per se*.

My individual contribution was the conception, experimental work and writing of the article.

3.5 Self-assembly of highly luminescent heteronuclear coordination cages

As previously mentioned, the development of luminescent metallocages is gaining increasing importance also for the potential application as biological markers. However, reports about highly emissive palladium cage compounds are still rare. Inspired by previous investigations, a promising approach to enhance the luminescence of palladium cages is reported.

Two Pd_2L_4 coordination cages with conjugated ruthenium(II) pyridine complexes (Figure 18) were prepared and studied for their photo-physical properties. The alteration of the ligand structure has significant effects on the emission of the ligands and also of the cages. In cage **5**, the Ru complex is directly coupled to the bis(pyridyl) ligand *via* an amide bond. In cage **6**, however, the luminescent Ru complex is separated from the emissive ligand by an alkyl bridge as spacer/ linker.

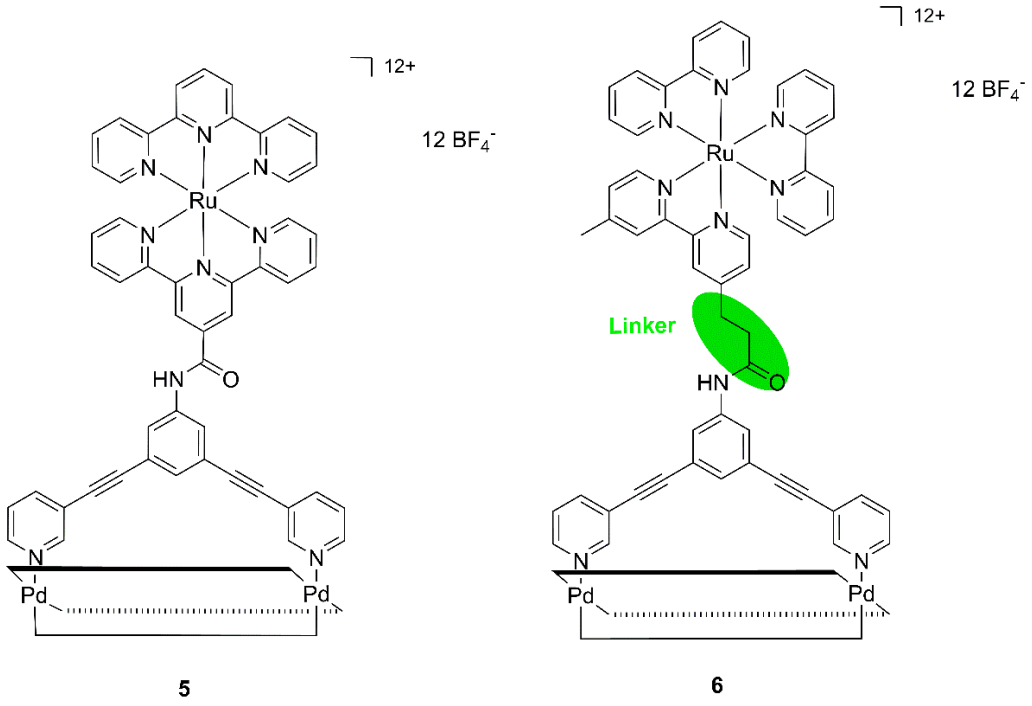


Figure 18. Pd_2L_4 coordination cage with attached Ru(II) terpyridine **5** and Ru(II) bipyridine **6.**

As expected, cage **5** and its corresponding ligand **L5** are not emissive, while the amine-based ligand features highly emissive properties by itself with a quantum yield of 52%. Notably, cage **6** and its ligand **L6** show strong orange luminescence exhibiting quantum yields of 66% and 88%,

respectively. The insertion of the alkyl bridge has a positive influence on the luminescence of **L6** since the bending of the amide bond can be avoided, thus the predicted disruption of the chromophoric system during the excitation process can be prevented. The palladium cage **6** with Φ = 66% is one of the highest luminescent SCCs known to date. The presented approach has potential for the targeted design of highly luminescent coordination complexes.

My individual contribution was the conception, experimental work and writing of the article.

4. Conclusion and Outlook

In this work, *exo*-functionalized Pd_2L_4 coordination cages were evaluated as drug delivery systems for the anticancer drug cisplatin. The applied functional groups in *exo*-position are hydoxy, amino and carboxy moieties for further coupling, anthracene and naphthalen as fluorophores as well as ruthenium(II) pyridine complexes. The metallocages were synthesized by self-assembly using alkyne-based bis(pyridyl) ligands and a palladium precursor. The successful cage formation was proven mainly by ^{1}H and DOSY NMR spectroscopy, ESI mass spectrometry and X-ray diffraction.

For the evaluation as drug delivery systems, the palladium cages were first studied for their ability to encapsulate cisplatin. As evidenced by NMR in solution and by XRD in the solid state, two cisplatin molecules are encapsulated within the cavity. The anticancer activity of the metallocages were investigated *in vitro* in different cancer cell lines. The hydroxy-, amino- and carboxy-functionalized cages exhibit a broad range of cytotoxicity (IC_{50} 8.2 - 94.4 µM) being similar cytotoxic as cisplatin to almost non-toxic. Due to the encapsulation properties and the suitable cytotoxicity profile, these cages were investigated in combination with cisplatin in human ovarian cancer cells SKOV-3 resulting in an enhanced cytotoxicity for the hydroxymethyl-based cage $(cisplatin)_2 \subset$ **1** (Figure 19). In order to assess the toxicity in healthy tissue, the cages were examined *ex vivo* in rat liver tissue slices showing a significantly lower toxicity for cage **1** than cisplatin. These results highlight the potential of cage **1** to function as drug delivery system for cisplatin. In contrast, the cages with anthracene and naphthalene moieties (Figure 19) are highly cytotoxic compared to cisplatin with IC_{50} values between 1.1 and 8.0 µM, thus these cages can potentially act as anticancer agents *per se*.

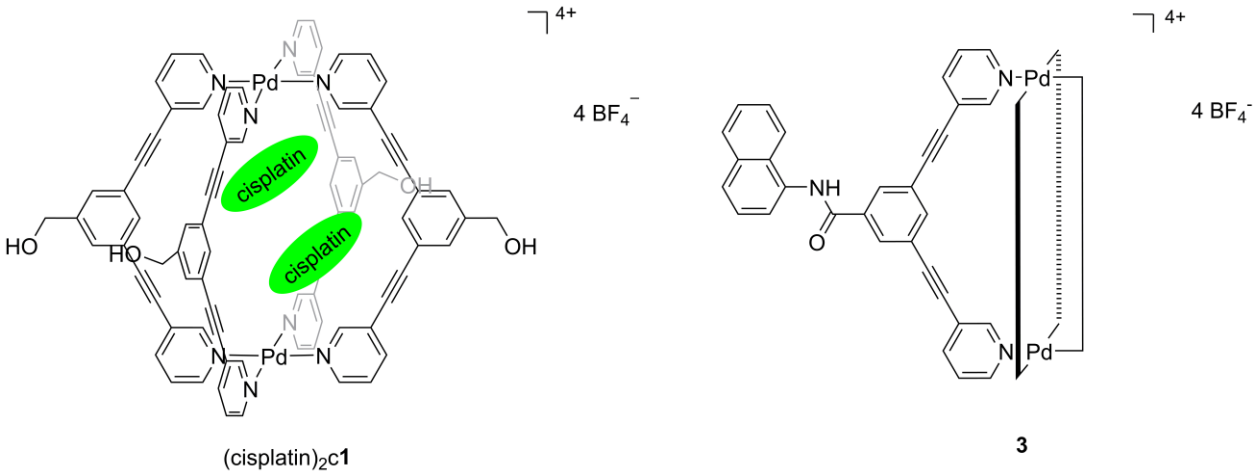
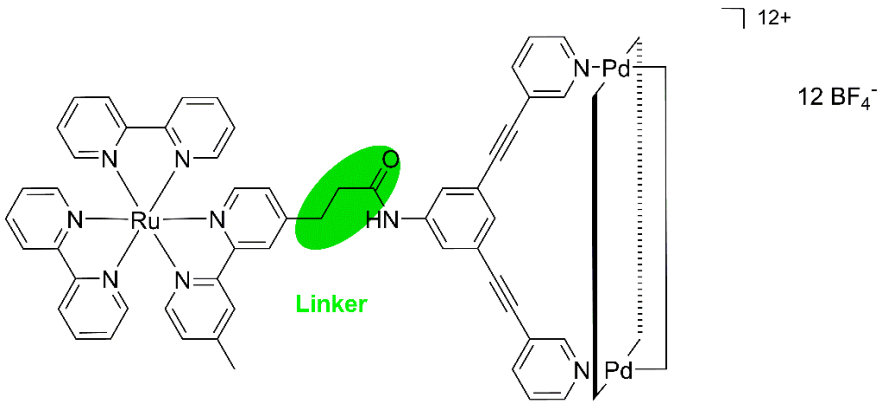


Figure 19. The hydroxymethyl-based cage (cisplatin)$_2$⊂**1** and the naphthalene-based cage **3** being attractive candidates as drug delivery system and as anticancer drug alone**.**

In order to monitor the uptake and distribution of the metallocages in cancer cells, fluorescent microscopy of cage **2** was performed. Due to the low fluorescence (Φ = 17%), only the uptake of **2** in cancer cells was proven. The determination of the distribution is necessary to examine the target and the mechanism of action. Consequently, the design of highly fluorescent metallocages gained in importance during the course of this work. Surprisingly, even the ligands and cages coupled to fluorophores (*e.g.* cage **3**) are non-emissive. An explanation was given by TD-DFT calculations revealing a bending of the amide bond in the excited state, hence the chromophoric system is disrupted and no emission occurs.

A linker, namely an alkyl bridge, was inserted between the emissive bis(pyridyl) ligand and the luminescent tag (here: Ru(II) bipyridines) to avoid the predicted torsion of the amide bond in the excited state. The resulting cage **6**, shown in Figure 20, exhibits an exceptionally high quantum yield Φ = 66%, which is one of the highest reported for SCCs so far. The introduction of a linker between two luminescent groups is a promising tool to further design highly emissive palladium cages.



6

Figure 20. Highly luminescent Pd_2L_4 cage **6** with attached Ru(II) bipyridine complexes**.**

Future studies may include the biological examination of cage **6** including cytotoxicity assays and uptake studies by fluorescence microscopy to reveal the target of these Pd_2L_4 cages in cancer cells. In addition, carboxy-functionalized cages are currently under investigation for coupling experiments to peptides in order to increase the selectivity to cancer cells by active targeting. The reported bis(pyridyl) ligands may be further exploited for self-assembly of M_2L_4 metallocages using cheaper transition metal ions, such as copper, nickel and zink.

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Andrea Schmidt and Fritz E. Kühn

Chemie in unserer Zeit **2016**, *50*.

DOI: 10.1002/ciuz.201600756

Self-assembled M_2L_4 coordination cages: Synthesis and potential applications

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DOI: 10.1016/j.ccr.2014.03.037

Evaluation of new palladium cages as potential delivery systems for the anticancer drug cisplatin

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DOI: 10.1002/chem.201504930

Supramolecular *exo*-functionalized palladium cages: fluorescent properties and biological activity

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DOI: 10.1039/c6dt00654j

Self-assembly of highly luminescent heteronuclear coordination cages

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DOI: 10.1039/C6DT02708C

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8. List of Publications and Conference Contributions

Journal articles

[1] **Andrea Schmidt**, Nidhi Grover, Teresa K. Zimmermann, Lilian Graser, Mirza Cokoja, Alexander Pöthig, Fritz E. Kühn, "Synthesis and characterization of novel cyclopentadienyl molybdenum imidazo[1,5-a]pyridine-3-ylidene complexes and their application in olefin epoxidation catalysis", *J. Catal.* **2014**, *319*, 119-126.

[2] **Andrea Schmidt**, Angela Casini, Fritz E. Kühn, "Self-assembled M_2L_4 coordination cages: Synthesis and potential applications", *Coord. Chem. Rev.* **2014**, *275*, 19-36.

[3] **Andrea Schmidt**, Viviana Molano, Manuela Hollering, Alexander Pöthig, Angela Casini, Fritz E. Kühn, "Evaluation of new palladium cages as potential delivery systems for the anticancer drug cisplatin", *Chem. Eur. J.* **2016**, *22*, 2253-2256.

[4] **Andrea Schmidt**, Manuela Hollering, Markus Drees, Angela Casini, Fritz E. Kühn, "Supramolecular *exo*-functionalized palladium cages: fluorescent properties and biological activity", *Dalton Trans.* **2016**, *45*, 8556-8565.

[5] **Andrea Schmidt**, Manuela Hollering, Jiaying Han, Angela Casini, Fritz E. Kühn, "Self-assembly of highly luminescent heteronuclear coordination cages", *Dalton Trans.* **2016**, *45*, 12297-12300.

[6] **Andrea Schmidt**, Fritz E. Kühn, "Metallkomplexe als Antikrebsmittel: Konzepte in der Tumorforschung und Wirkmechanismen", *Chem. Unserer Zeit* **2016**, *50*, DOI: 10.1002/ciuz.201600756.

[7] Felix Kaiser, **Andrea Schmidt**, Wolfgang Heydenreuter, Philipp J. Altmann, Angela Casini, Stephan A. Sieber, Fritz E. Kühn, "Self-assembled palladium and platinum coordination cages: Photophysical studies and anticancer activity", *Eur. J. Inorg. Chem.* **2016**, DOI: 10.1002/ejic.201600811.

[8] Jiaying Han, **Andrea Schmidt**, Hjalmar P. Permentier, Rainer P. H. Bischoff, Fritz E. Kühn, Peter L. Horvatovich, Angela Casini, "Bioconjugation strategies to couple supramolecular *exo*-functionalized palladium cages to peptides for biomedical applications", *manuscript in preparation*.

Talks and posters

12/2015 Pacifichem 2015, Honolulu, Hawaii, USA
Talk and poster as part of the student poster competition
"M_2L_4 coordination cages as potential drug delivery systems for cisplatin"

12/2015 Clinical and Experimental Metallodrugs in Medicine (CEMM), Honolulu, Hawaii, USA
Poster
"Biological studies of Pd_2L_4 metallocages as drug delivery systems"

07/2015 17th International Conference on Biological Inorganic Chemistry (ICBIC17), Beijing, China
Poster and talk as part of the student competition
"Pd_2L_4 metallocages as potential drug delivery systems"
Award: RSC Books Prize

08/2014 2nd International Symposium on Functional Metal Complexes that Bind to Biomolecules, Zürich, Switzerland
Poster and talk
"Self-assembled Pd_2L_4 metallocages as potential hosts for anticancer drugs"

9. Appendix