

**Historical Perspective of Ruthenium Anticancer Agents**

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**Paramagnetic Lanthanide Chelates in MRI: from Extracellular Contrast Agents to Probes for Molecular Imaging**

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## Design of Novel, Innovative Metal Anticancer Drugs

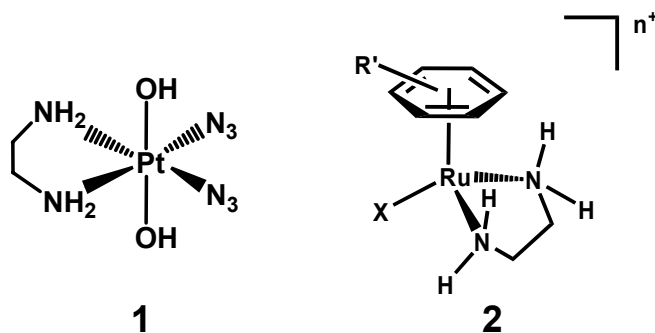
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Inorganic medicinal chemistry will have a major impact on pharmaceutical research if we can demonstrate that novel, innovative drugs can be designed. Here I will discuss recent work of my group on the design photoactivatable platinum anticancer drugs, and organometallic ruthenium arene anticancer complexes.

The success of the diammine Pt(II) complexes cisplatin and carboplatin as clinical anticancer drugs is well known, and several related complexes have entered clinical trials recently. However their use is often limited by the side-effects which accompany treatment, by the resistance which can develop after continued therapy, and the limited range cancers which is treatable.

To overcome side-effects, it would be attractive to design a complex which is non-toxic until activated at the site at which activity is required, for example using laser irradiation. Photodynamic therapy, as it is often called, is already in clinical use using porphyrin derivatives as photosensitizers. The success of photodynamic therapy in this form depends on there being available oxygen at the target site which is then activated to toxic singlet oxygen. However, tumours can be hypoxic. Our approach does not depend on oxygen. We have designed Pt(IV) azide complexes, e.g. *cis,trans*-[Pt(en)(N<sub>3</sub>)<sub>2</sub>(OH)<sub>2</sub>] (**1**), which are highly stable and inert in the dark [1], but can platinate DNA when irradiated with visible light. We have achieved a pattern of DNA platination similar to that of cisplatin [2]. Experiments are in progress to investigate the photoactivation of such complexes in cells.



We have designed organometallic Ru(II) arene complexes of the type [(η<sup>6</sup>-arene)Ru(II)(en)X] (**2**), where en is ethylenediamine, which are cytotoxic to a range of cancer cells, including cisplatin-resistant cells, and also exhibit activity *in vivo* [3,4]. The arene, leaving group X, and chelated ligand en can have a major influence on the rate of the hydrolysis and the pK<sub>a</sub> of the aqua adduct [5]. Reactions with S-containing amino acids are relatively slow [6]. The complexes incorporate a number of potential DNA recognition features. They can form monofunctional adducts with DNA bases, and with en as the chelating ligand exhibit a high preference for G N7 binding with little binding to A [7]. A strong H-bond forms between en NH and the C6 carbonyl oxygen of G. However, if en is replaced by acetylacetonate (acac) then binding to A as well as G occurs [8]. The arene ligand can have a significant influence on the rate of G binding [9], perhaps due in part to arene-purine base stacking with extended arene π systems such as those of biphenyl and anthracenes. Such a π - π interaction introduces

the possibility of intercalation of the arene ligand into DNA which contributes to the large conformational distortions induced in double helical DNA even though these complexes are only monofunctional [10]. Ru(II) arene complexes appear to have a unique profile of DNA binding and this may lead to a profile of biological activity different from metal-based anticancer drugs already in the clinic. They are therefore of interest for further development.

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## Novel Aqueous Chemistry of a *trans* Diamine Anticancer Complex

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There is increasing interest in the anticancer activity of *trans* diamine Pt(II) complexes. Motivated by the lack of knowledge about their mechanism of action, together with the assumption that hydrolysis of Pt(II) complexes is the rate-limiting step in reactions with the target site, DNA,<sup>[1-3]</sup> we have studied the aqueous chemistry of a novel *trans*-platinum complex: *trans*-[PtCl<sub>2</sub>(NH<sub>3</sub>)(2-Me-butylamine) (**1**).

The <sup>15</sup>N-labelled complex was synthesised and characterised to aid the understanding of the hydrolysis of **1** using 2D [<sup>1</sup>H, <sup>15</sup>N] NMR spectroscopy. pKa values for the mono-aqua and diaqua adducts (5.90 for the mono-aqua and 4.16 and 7.17 for the diaqua) were found to be comparable to those reported for aquation products of transplatin under similar conditions<sup>[4]</sup>. Surprisingly, the NMR spectra revealed unpredicted additional reactions, with extra products observed. These unexpected species, which we have assigned as Pt(IV) products, were not observed in the presence of NaCl, which suppressed hydrolysis or when the reaction was carried out under argon. Consequently, we can conclude that the aqua adducts of **1** are readily oxidized in the presence of air (oxygen) in aqueous solution.

This work suggests that some *trans* diamine Pt(II) complexes have different and novel chemistry in comparison to their *cis* analogues. Further investigation may help to establish structure-activity relationships for future design of *trans*-Pt drugs.

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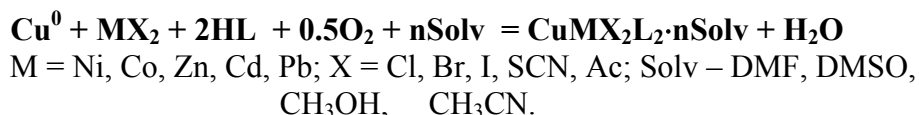
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## Direct Synthesis of Bioactive Heterobimetallic Complexes

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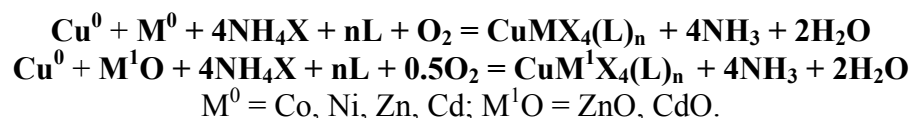
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In the report the most perspective approaches to synthesis of heterometallic complexes directly from metal powders or metal oxides ("anion-limited", "ligand-limited" and direct template synthesis) are considered. The "anion-limited" method of direct synthesis is developed for protonic ligands and it can be illustrated by the reaction scheme to obtain heterometallic Cu/M complexes with aminoalcohols (HL):



The general deficiency of anions in the system results in the deprotonation of the HL ligands that become a source of anions and also function as bridges between atoms of different metals.

For the case of aprotic ligands the "ligand-limited" method is offered:



The deficiency of ligand in the system can be created easily what forces available anions to display bridging functions and join atoms of different metals. The achievements of the latter approach are demonstrated taking the synthesis of heterometallic complexes with ethylenediamine as an example.

Synthetic reactions involving two zerovalent metals, ammonium salt and ethylenediamine in the presence of acetone result in the formation of heterometallic complexes containing a cation with an open-chain ligand formed by condensation of acetone with diamines what became the beginning of direct template synthesis of heterometallic complexes. In the report typical examples of direct template synthesis of heterometallic complexes of 3d-metals with ligands formed as a result of the condensation of aliphatic carbonyl compounds (formaldehyde, acetone) with ethylenediamine and its derivatives are given.

Preliminary investigations have shown that prepared complexes exhibit antitumour, antiviral and antimicrobial activity. For example, Cu/Zn compounds with 2-dimethylaminoethanol decrease the tumour growth in the range 14–28 (Adenocarcinoma Ca755), 27–33 (Solid Melanoma B16), 23–41 (Ascitic Sarcoma S37), 18–22 % (Krocker Sarcoma).

## Metal Ion Complexes of Nucleic Acid Constituents with Potentials in Antiviral and Anticancer Therapy

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The *Working Group* COST D20-0002-00 with the above mentioned title (put into action at January 1st, 2001) consists, next to the *Coordinator* (H.S.; Switzerland) and Bernhard Lippert (*1st Co-Applicant*; Germany), of Paolo Carloni (Italy), Antonín Holy (Czech Republic), Andrzej Okruszek (Poland) and Jan Reedijk (The Netherlands). The collaboration within the *Group* is excellent and very fruitful as is evidenced by the fact that within the first two and a half years more than 25 relevant papers have been published in international journals. Below, only a few points are highlighted.

**Complexes of Artificial Nucleotides with Antiviral Activity.** 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA) -- also called *Adefovir* -- is considered as an analogue of (2'-deoxy)adenosine 5'-monophosphate, (d)AMP<sup>2-</sup>. This compound -- first described in 1986 -- exhibits remarkable antiviral and cytostatic properties and in fact its bis(pivaloyloxymethyl)ester (*Adefovir dipivoxil*) was very recently (second half of 2002) [1] approved for use in hepatitis B therapy by the US Food and Drug Administration (FDA). This diester, after being transported into the cell, is hydrolyzed and then the released PME<sup>2-</sup> is diphosphorylated to PMEApp<sup>4-</sup>; this analogue of (d)ATP<sup>4-</sup> is recognized by nucleic acid polymerases as a substrate (for refs see [2]) and after incorporation of the corresponding "nucleotidyl" part into the growing nucleic acid chain this is terminated because of the lack of a 3'-hydroxy group.

The ether oxygen in the aliphatic chain of PME<sup>2-</sup>, Ade(N9)-CH<sub>2</sub>CH<sub>2</sub>-O-CH<sub>2</sub>-PO<sub>3</sub><sup>2-</sup>, is known to be compulsory for a biological activity and it has been suggested [2,3] that PMEApp<sup>4-</sup> is initially an excellent substrate for DNA polymerases because of the facilitated formation of the needed M(P<sub>α</sub>)-binding mode, M being a divalent metal ion (M<sup>2+</sup>); this binding type then also facilitates formation of the two-metal ion containing reactive M(P<sub>α</sub>)-M(P<sub>β</sub>,P<sub>γ</sub>) mode which is crucial for the transfer of a nucleotidyl unit in the polymerase reaction [2]. This M(P<sub>α</sub>) coordination is promoted by the formation of a 5-membered chelate of the α-phosphate-coordinated M<sup>2+</sup> with the above mentioned ether oxygen. Replacement of this ether oxygen by a sulfur atom or a CH<sub>2</sub> unit leads to a loss of the biological activity. In accord herewith are also 9-(5-phosphonopentyl)adenine (dPEEA<sup>2-</sup>) [4] and the quaternary 1-(2-phosphonomethoxy)ethyl derivative of 2,4-diaminopyrimidine (PMEDAPy<sup>-</sup>) antivirally inactive. In fact, for the latter case it could be shown [5] that the positive charge at N1 of the pyrimidinium residue prevents the M<sup>2+</sup>-ether oxygen interaction [5] due to charge repulsion [6]. In other words, we are now able to define the structural requests that an 'acyclic nucleoside phosphonate' must fulfill to be antivirally active.

**Cisplatin and Related Compounds with Anticancer Activity:** *Cisplatin* exerts its biological action by preferential binding of the *cis*-(NH<sub>3</sub>)<sub>2</sub>Pt<sup>2+</sup> unit to the N7 sites of adjacent guanine residues of DNA forming intrastrand cross-links [7,8]. The recent observation that derivatives of 5- and 6-uracilmethylphosphonate (Umpa<sup>2-</sup>), in combination with *Cisplatin*, prolong the survival time of mice with lymphoid leukemia [9], prompted a study of their acid-base properties [10] and for the Mg<sup>2+</sup> and Ca<sup>2+</sup> comple-

xes of  $5\text{Umpa}^{2-}$  and  $6\text{Umpa}^{2-}$  it was shown that the stability of these  $\text{M}(\text{Umpa})$  complexes [11] is solely determined by the basicity of the phosphonate group and this means, that these two ligands may be considered in this respect as simple analogues of uridine 5'-monophosphate ( $\text{UMP}^{2-}$ ).

In an effort to combine the antiviral with the anticancer effects, the acid-base and metal ion-binding properties of PMEAs (see above) and of its ternary complexes,  $(\text{Dien})\text{Pt}(\text{PMEA-}N1)$  and  $(\text{Dien})\text{Pt}(\text{PMEA-}N7)$  ( $\text{Dien}$  = diethylenetriamine), were studied and compared [12]. One of the most remarkable results is that the metal ion-binding properties of the phosphonate group are only little affected by the nucleobase-coordinated  $(\text{Dien})\text{Pt}^{2+}$  units. In this context it is important to understand the effect which metal ions exert upon their binding to nucleobase residues, i.e. how they affect the acid-base properties of nucleobases; this is crucial with regard to their metal ion-binding, hydrogen-bonding [13] and stacking properties. However, in this context it was also necessary to know the intrinsic proton affinities of the various basic sites present in purines [14]. Therefore, we have measured for several examples the corresponding micro acidity constants [14] and it turns out that for an adenine residue the acidity of the  $(N7)\text{H}^+$  site is not much lower than that of the  $(N1)\text{H}^+$  site; the corresponding micro acidity constants are  $\text{p}k_{(N7)\text{H}}^{\text{H}} = 3.0$  and  $\text{p}k_{(N1)\text{H}}^{\text{H}} = 4.1$ , respectively. This means, N7 can also be an excellent binding site for metal ions. Indeed, it was now proven that  $\text{Pt}(\text{II})$  coordinated to  $(N1)^{-1/0}$  sites in guanine, hypoxanthine, or adenine residues acidifies the  $(N7)\text{H}^+$  unit to the same extent as  $(N7)$ -coordinated  $\text{Pt}(\text{II})$  acidifies the  $(N1)\text{H}^{0/+}$  site; in other words, the acidifications are reciprocal and identical [15]. The strongest acidification occurs by a proton and for divalent metal ions it decreases in the series  $\text{Cu}^{2+} > \text{Ni}^{2+} > \text{Pt}^{2+} \sim \text{Pd}^{2+}$  [15].

**Complexes of Phosphorothioate Derivatives:** Nucleoside phosphorothioates are nucleotide analogues in which a terminal phosphate O is replaced by an S atom. They are often used in studies of ribozymes, as artificial substrates in enzymatic reactions, and they also have a potential in the antisense strategy; in fact, one phosphorothioate oligodeoxynucleotide is already a commercially available drug [16].

Since  $\text{Pb}^{2+}$  is essential for the so-called leadzymes and because it is known that this metal ion has a significant affinity for S donors, at first the interactions of  $\text{Pb}^{2+}$  with natural nucleotides and their constituents were investigated and summarized [17] and the following affinity order, relevant for nucleic acids, was revealed:  $\text{guanine-}N7(\text{O}6) \geq \text{cytosine-}N3(\text{O}2) \geq \text{R}'\text{OP}(\text{O})_2\text{OR} \geq \text{adenine} > \text{uracil} \sim \text{thymine}$  [17]. Studies with methyl thiophosphate and uridine 5'-*O*-thiomonophosphate ( $\text{UMPS}^{2-}$ ) showed that the uracil residue of  $\text{UMPS}^{2-}$  is not participating in  $\text{Pb}^{2+}$  binding [18]. Consequently, the large stability increase of about 2.4 log units observed for  $\text{Pb}(\text{UMPS})$ , in comparison with its parent complex  $\text{Pb}(\text{UMP})$ , has to be attributed to an interaction between  $\text{Pb}^{2+}$  and the sulfur of the thiophosphate group [18]. The corresponding result was observed for the  $\text{Cd}(\text{UMPS})$  and  $\text{Cd}(\text{UMP})$  complexes [19]. Studies with other divalent metal ions, like  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$ , were just completed [19]; e.g., in the case of  $\text{Zn}^{2+}$  the S-bonded isomer has a formation degree of  $76 \pm 6\%$  ( $3\sigma$ ), whereas for  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$  the O-bonded isomer forms to nearly 100%. At this point it may also be emphasized that the antisense strategy mentioned above is further pursued with monofunctionally *trans*-diammine  $\text{Pt}(\text{II})$ -modified peptide-nucleic acid oligomers [20] and this demonstrates how closely interlinked the apparently different studies in fact are.

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**Biochemistry, Structural and Cellular Biology of Non-Classical Antitumor Platinum Compounds**

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The COST Working Group D20/0003/00 started in 2000 and was a natural continuation of the activities within COST working group in the Chemistry Action D8/0009/97, “Metal Recognition of DNA and Drug Design”. It was motivated by the fact that despite their success, the first platinum antitumor drugs introduced in the clinic (cisplatin and carboplatin) have several disadvantages. The drawbacks coupled with cisplatin and carboplatin clinical use have stimulated development of an improved platinum drug. It is therefore of great interest to understand the details of molecular mechanisms underlying biological efficacy of the new compounds. Such an understanding may help broaden the chemotherapeutic arsenal by laying the groundwork for systematic development and description of new modes of attack on DNA. One part of this project is focused on finishing the structural analysis of major DNA adducts of several new platinum agents. In further extension of this multilateral project the more extensive work is carried out to understand how the structures of these adducts modulate recognition by specific proteins including DNA-processing enzymes and other cell components and how these adducts are removed from DNA. The overall goal is to combine the complementary strengths of the eleven laboratories from eight countries (synthesis, physical studies, molecular modeling, physical and inorganic chemistry, biochemistry, molecular and cellular biology) to produce the structures of specific DNA adducts and to understand how these adducts modulate the processes in tumor cells following the primary damage to DNA, such as specific protein recognition by DNA-processing enzymes and repair.

The principal aims of the Working Group are: (i) to develop and possibly bring into clinical phase, or advanced preclinical testing, new platinum-based antitumor drugs and (ii) to gain understanding of their mechanism of action.

In the framework of this Working Group, the participants have investigated the anticancer properties of a number of platinum compounds. Several new compounds have been synthesized, characterized structurally and spectroscopically, and their chemical behavior under physiological conditions investigated; the cytostatic activity of selected platinum agents has been investigated *in vitro* and their specific interactions with cellular components, such as nucleic acids and proteins, have been studied with the aim of understanding mechanism underlying their biological activity.

The 11 laboratories from 8 COST-member countries participating to the Working Group have shared their ideas and results on the above subjects and fruitful collaborations among them have been established, as witnessed by the joint publications. The COST Working Group was successful in bringing together the European expertise in the emerging field of platinum anticancer drugs. COST chemistry networking has been particularly helpful to young scientists, supporting their participation to Working Group meetings and symposia, where they have very often reported their results in the form of oral presentations and have had the chance of critical discussion with senior scientists in a constructive and supportive environment.

## Synthesis and Antitumor Activity of Novel *trans*-Diaminedichloroplatinum(II) Derivatives

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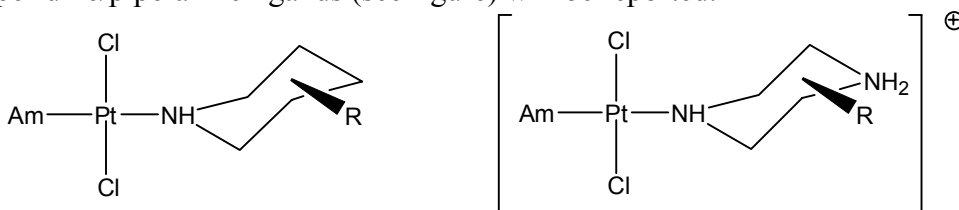
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Cisplatin [*cis*-diamminedichloroplatinum(II)] and carboplatin [*cis*-diamminecyclobutane-dicarboxylatoplatinum(II)] are two of the most widely used anticancer drugs. Their inability to overcome inherent and acquired resistance of cancer cells is a drawback that limits their clinical usefulness. Our goal is to develop new platinum(II) based cytotoxic agents that are capable of overcoming cisplatin resistance. Our working hypothesis is that we need to prepare novel platinum(II) complexes that will differ from cisplatin and carboplatin in their binding properties to biomolecules (proteins, peptides and DNA) and especially in the types of adducts that they form with double stranded DNA. The design, rational, synthesis as well as the biological evaluation of a new series of *trans* oriented platinum(II) complexes with piperidine/piperazine ligands (see figure) will be reported.



**Development of Platinum and non-Platinum Anticancer Compounds**

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Abstract not received

## Pt(II) Acidification of the N(6)H<sub>2</sub> Group of Adenines

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Although the common nucleobases (G, C, A, T) have  $pK_a$  values in the range  $4 > pK_a > 9$ , hence values well outside the physiological pH range, there is increasing evidence that nucleobase  $pK_a$  values are sometimes shifted into the physiological pH range. As a consequence, protonated and unprotonated species coexist at physiological pH, with the possibility of acid-base catalysis. For example, the Hepatitis Delta Virus ribozyme appears to use a  $H^+$  transfer mechanism for catalysis,<sup>[1]</sup> and peptide bond formation in the ribosome is related to the mode of action of an adenosine with a  $pK_a$  of ca. 7.6.<sup>[2]</sup> It is generally assumed that these shifted  $pK_a$  values refer to an increase in basicity of an endocyclic ring N atom and stabilization of the protonated nucleobase.

An alternative would be to shift the  $pK_a$  of a weakly acidic proton, e.g. of the endocyclic N(1)H of guanine or the exocyclic N(6)H<sub>2</sub> group of adenine into the physiological pH range. Here we demonstrate that twofold Pt(II) coordination can lead to a dramatic lowering in the  $pK_a$ , from 16.7 to 7.9.<sup>[3]</sup> Efficient stabilization of the deprotonated group, here the amido group, by neighboring H donors is essential.<sup>[4]</sup>

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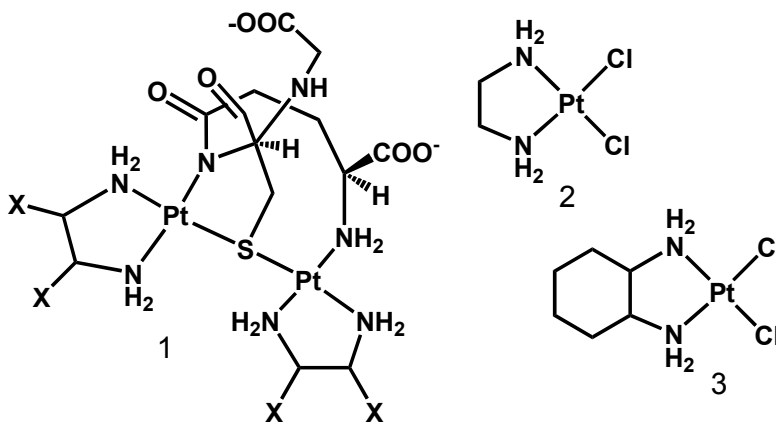
## Platinum Anticancer Complexes: Role of Redox Reactions

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Factors that influence the interaction of cytotoxic platinum anticancer drugs and their biological target, DNA, include redox reactions, co-ordination geometry and steric bulk. Here we present recent work in our group that has concentrated on gaining a better understanding of the biological implications of novel redox chemistry and its relationship to the mechanism of action of this class of drugs.

An example of such redox reactions is the formation of the macrochelate  $[(Pt(Am))_2SGH_{-1}]$  (**1**), a product from the reactions of both glutathione ( $\gamma$ -L-Glu-L-Cys-Gly, GSH), and oxidised glutathione with  $[PtCl_2(en)]$  and  $[PtCl_2(dach)]$  (en = ethylenediamine) (**2**) (dach = diaminocyclohexane) (**3**).<sup>1,2</sup> Glutathione is a sulfur-containing tripeptide present in cells at millimolar concentrations, and is believed to be involved in the deactivation of cytotoxic platinum drugs. Interestingly, these novel  $[(Pt(Am))_2SGH_{-1}]$  products are not formed in reactions with the analogous platinum(IV) complexes. Redox reactions of Pt(II) complexes that can induce disulfide bond cleavage of sulfur-containing biomolecules, including peptides and proteins, may play a significant role in the *in vivo* activity of these drugs.



We thank the BBSRC and Wellcome Trust (Edinburgh Protein Interaction Centre) for their support in this work, and COST D20 for stimulating discussions.

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## Chiral Platinum Complexes Reacting With DNA: Perturbation of the Double-Helix Studied by NMR, Molecular Modeling, and Biophysical Methods

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Enantiomeric analogs of the anticancer drug cisplatin such as [PtCl<sub>2</sub>(DAB)] (DAB = 2,3-diaminobutane) or [PtCl<sub>2</sub>(DACH)] (DACH = trans-1,2-diaminocyclohexane) show different biological activities. For example, the RR isomer of [PtCl<sub>2</sub>(DACH)] has higher antitumor activity than the SS isomer. It has been suggested that these differences could be related to different structural perturbations that both isomers cause to DNA.<sup>1</sup> These structural differences could also explain why DNA adducts of both isomers are differently recognized by the HMGB1 protein and by the nucleotide excision repair system.

As with cisplatin, [PtCl<sub>2</sub>(R,R-DACH)] binds preferentially to GG sequences of DNA.<sup>2</sup> An X-ray structure of a duplex DNA oligonucleotide bearing the GG-Pt(R,R-DACH) crosslink has been reported.<sup>3</sup> This structure features a hydrogen bond between one NH<sub>2</sub> group of DACH and the O6 atom of the 3'-guanine. In aqueous solution, such hydrogen bonding interaction could be mediated by water molecules,<sup>4</sup> which could affect the overall structural perturbation. Particularly intriguing is the difference between the adduct structures formed with the R,R- and S,S-isomers. To clarify this point, we have reacted the hairpin-stabilized, double-stranded DNA oligonucleotide containing a single, central GG sequence with either isomer of [PtCl<sub>2</sub>(DACH)], purified the duplexes containing single, intrastrand GG-adducts and determined their solution structure at pH 7 using multinuclear NMR and molecular dynamics simulations. The molecular models were correlated with conformational alterations induced in DNA including bending and unwinding determined by chemical probes of DNA conformation and electrophoretic retardation (phasing) assay.

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**Hydroxamate Complexes of Ruthenium and Platinum: Synthesis,  
Characterisation and Biological Activity**

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Hydroxamic acids (RCONHOH) represent an intriguing family of bioligands with hypotensive, anti-cancer, anti-malarial and anti-tuberculosis properties and act as potential chemotherapeutics targeting enzymes such as matrix metalloproteases, cardiovascular diseases, HIV and Alzheimer's disease.<sup>1</sup>

Whilst many of their biomedical applications are undoubtedly due to their ability to bind metal centres, we have firmly established that hydroxamic acids are also effective NO donors by virtue of the fact that they readily transfer NO to Ru(III) and activate the iron containing enzyme guanylate cyclase under physiological conditions.<sup>2</sup> Herein we describe the denitrosylation reactions of hydroxamic acids by Ru(III) and also report the first example of a unique Ru complex containing both coordinated NO in addition to a free NO donor group in the form of a hydroxamic acid.

Furthermore, in view of the fact that some hydroxamic acids can act as anticancer agents, together with the fact that Pt derivatives (e.g. cisplatin) have a now firmly established role in the treatment of certain cancers and Ru derivatives (e.g. NAMI-A) are potential anti-metastatic agents, we have synthesised and characterised an extensive library of pyridine hydroxamic acid Pt(II), Pt(IV) and Ru(III) complexes. These complexes are not only novel Pt and Ru derivatives but each have the potential to release NO. A summary of the above results will be presented

We thank Enterprise Ireland, RCSI, EU COST D20 and EU COST D21 for funding and the University of Trieste, Italy.

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**Metal Ion Complexes with Antibacterial Quinolones and Antiviral Nucleotide Analogues (MAQA)**

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The participants involved in the project are: prof. E. Sletten (Bergen), prof. J. Arpalathi (Turku), prof. J. Fiol (Mallorca), prof. N. Poklar (Ljubljana), prof. T. Varadinova (Sofia), prof. K. Varnagy (Debrecen) and prof. I. Turel (Ljubljana).

Research has been carried out in the following areas:

**Synthesis and characterization of new metal-quinolone complexes** (Ljubljana, Turel).

**The interactions of quinolones with DNA and the role of metal ions in these processes** (Bergen, Ljubljana (Poklar, Turel), Mallorca).

**Synthesis of new N<sup>6</sup>,N<sup>6'</sup>- and N<sup>9</sup>,N<sup>9'</sup>-polymethylene-bis-purines complexes** (Mallorca).

**The study of Pt(II) binding to bis-adenines** (Mallorca-Turku).

**The study of influence of different cations on the binding properties and the stability of N<sup>6</sup> platinated adenines** (Turku-Debrecen).

**Metal complexes of 5-X-uracilato derivatives (X= H, F, Cl, Br, I)** (Mallorca).

**Cytotoxicity and activity of various metal complexes of the antiviral drug acyclovir (ACV) and its acetylated form (Ac-ACV) against Herpes simplex virus (HSV) infection** (Sofia-Mallorca).

**The synthesis of Ru(II) and Ru(III) complexes of the type Na[RuCl<sub>4</sub>(DMSO)L], [RuCl<sub>3</sub>(DMSO)<sub>2</sub>(L)] or HL[RuCl<sub>4</sub>L<sub>2</sub>] with various ligands** (Mallorca, Ljubljana).

Parts of this work will be presented as posters and as a short oral presentation and this report will focus on general data and especially on the quinolone part of the project.

### New N<sup>6</sup>,N<sup>6'</sup>-Polymethylene Bisadenines and their Chelating Properties

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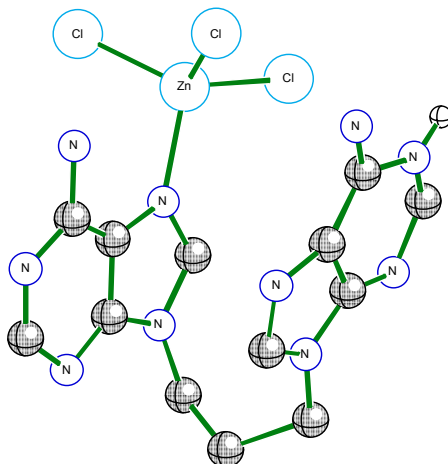
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Although the synthesis of inner sphere metal complexes of N<sup>6</sup>,N<sup>6'</sup>- and N<sup>9</sup>,N<sup>9'</sup>-polymethylene-bis-purines is difficult, our previous results indicate that inner sphere complexes can be obtained by increasing the distance between the bisprotonated ligand which occurs in the complex [N<sup>7,7'</sup>-ZnCl<sub>3</sub>, N<sup>1,1'</sup>-H<sub>2</sub>N<sup>6,6'</sup>-(CH<sub>2</sub>)<sub>3</sub>]-bisadenine [1]. In addition, with a severe pH control it is possible to obtain inner sphere complexes of Zn(II) or Cd(II) and the N<sup>9,9'</sup> monoprotinated trimethylene-bis adeninium ligand (see figure). Without this strict control on the preparation conditions the products obtained are invariably outer sphere complexes as in the case of the previously described Zn(II), Cd(II) and Hg(II) complexes with N<sup>9</sup>, N<sup>9'</sup> polymethylene-bis-adenines [2]. Moreover, previous results related to other polymethylene-bis-purines or pyrimidines will be also presented.



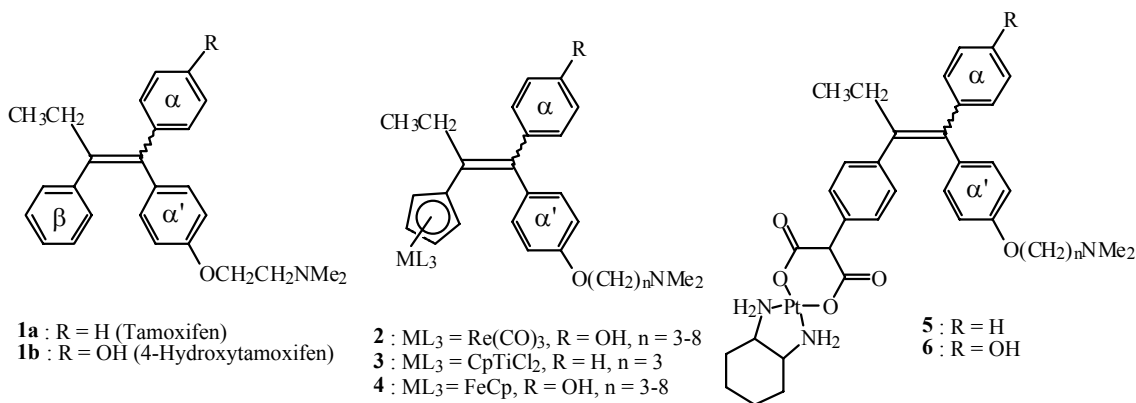
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- [3] We are grateful to DGICYT (BQU2002-02546) and COST D20, WG 0006-01 for financial support.

## Metal Complexed SERMs (Selective Estrogen Receptor Modulators). Synthesis and Antiproliferative Effects

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In the overall scheme of the future development of new drugs for the treatment of breast cancer, specially tamoxifen resistant tumors, we have explored the unprecedented use of organometallic or coordination SERMs. The initial idea is to enhance the efficacy of the current standard, i.e. tamoxifen **1a**, and its active metabolite, 4-hydroxytamoxifen **1b**, by modifying the structure through judicious incorporation of an organometallic and coordination moieties possessing novel properties. Results have been varied, justifying a systematic approach that has proved to be full of surprised.



The following differing situations were observed (a) The antiproliferative effect is due to the vector and the organometallic moiety does not improve the effects of the SERM, no matter what concentration is used. In particular, this is the case for **2**, the hydroxytamoxifen derivatives bearing a  $CpRe(CO)_3$  group, which behave almost identically to hydroxytamoxifen. These stable species have future promise for use with radionuclides of Re and Tc (b) The effect of the organometallic moiety counteracts the antiestrogenic behavior of the vector and leads to a species with proliferative activity; this is the case for **3** where the  $Cp_2TiCl_2$  entity behaves as a powerful estrogen when attached to tamoxifen. This is probably due to in situ release of Ti(IV) (c) There is also the case observed with the Platinum complexes **5** and **6** in which coupling of the antiestrogenic skeleton to a cytotoxic inorganic moiety results in a product with properties superior to those of the coordination complex alone, but here the principal antiproliferative component appears to be associated with the antiestrogenic organic skeleton (d) Finally, a synergy exists between the potential cytotoxic organometallic moiety and its organic vector, leading to unique antiproliferative effects on breast cancer cells classed ER<sup>+</sup> and ER<sup>-</sup>. This is the case with **4**, the ferrocene derivatives. This result, probably related to the in situ oxidation of ferrocene opens a new window on organometallic oncology. It is also clear that the range of possibilities is broad,

varied and currently unpredictable. A systematic study combining organometallic chemistry and biology is the only option in the search for new SERMs with novel properties.

**Novel Polynuclear Ruthenium and Platinum Complexes and their Interaction with DNA-Model Bases**

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Polynuclear platinum complexes represent a novel class of anticancer agents.<sup>1,2</sup> A challenging extension of this concept is to utilize inequivalent coordination spheres to achieve selective reactivity at each metal center.

Inspired by the mononuclear antitumor-active complex<sup>3</sup> [Ru(tpy)Cl<sub>3</sub>] and the cytotoxic complex [Pt(tpy)Cl]Cl·2H<sub>2</sub>O (tpy = 2,2':6',2''-terpyridine), a new class of polynuclear ruthenium and platinum complexes has been designed and synthesized, using the dinucleating ligand, bis[4'-(2,2':6',2''-terpyridyl)] diethyleneglycolether (dtdeg).

The complexes have been fully characterized. Their binding to the DNA-model base 9-ethylguanine has been studied and the adducts are characterized by <sup>1</sup>H NMR spectroscopy. The cytotoxicity of the complexes is being studied in order to obtain structure-activity relationships.

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**Title to be announced**

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Abstract not received

## The Interplay of Metallodrugs and Plasma Proteins

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Plasma proteins play a central role in the metabolism of drugs including several metallodrugs. Drug binding to plasma proteins has a strong influence on their biodistribution and pharmacokinetics. The most important protein in this respect is albumin, by far the most abundant protein in the plasma. Another protein, which may be crucial for inorganic drug transport is serum transferrin. Studies of the interactions of the new and currently used anticancer metal compounds (based on platinum, ruthenium, gold, tin and gallium) with albumin and transferrin, and of their transport into the cells is the major aim of this D20 WG. Particular attention is directed to drug-protein adducts that may be prepared for drug targeting purposes. The state of the art will be illustrated as well as recent results obtained within the coordinated group. Specifically, recent results obtained on the interactions of novel gold(III) compounds with serum albumin will be described. Attention will be paid to the binding of classical antimetastatic ruthenium(III) complexes to plasma proteins.



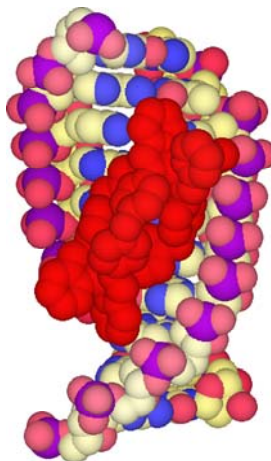
## **Non-Covalent DNA Recognition and Remarkable DNA Structural Effects Induced by Supramolecular Cylinders**

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The design of complex molecular architectures is an important goal as it offers a route to encode the properties of materials at the molecular level. We have shown that supramolecular architectures can be prepared quickly in one-pot reactions by mixing suitable metals with pyridylimine ligand, prepared in-situ from commercial amines and aldehydes. The architecture adopted is controlled by the selection of the metal and the design of the ligand.

The molecular shape or architecture may be used to encode the molecular function. We have designed cylindrical agents that are the right size and shape to recognize the major groove of DNA. These agents not only target the DNA major groove but induce dramatic intramolecular DNA coiling that is unprecedented with synthetic DNA binders.



## Chemical Structure-Activity Correlation in Photodynamic Therapy of Cancer

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Photodynamic therapy (PDT) is a cancer treating modality which involves a combined action using photosensitizers, laser light and tissue oxygen to achieve photochemical destruction of biological tissue. It has been successfully applied to treat a variety of cancers experimentally and clinically. Porphyrins, free-base and metallo-complexes, are promising photosensitizers for the photodynamic therapy, which attacks tumor cells by the combined action of oxygen, light and drug. Crucial requirement for a photosensitizer (PS) to be effective is its lower toxicity for normal cells compared to tumor tissue.

The aim of this study is to evaluate the cytotoxic effect of different porphyrins - free bases and metallic complexes with Zn (symmetrical and unsymmetrical substituted) on tumor and non-tumor cell lines.

The combination of analytical methods including UV/VIS, fluorescence, NMR and ESR spectrometry, laser-induced optoacoustic spectroscopy (LIOAS), scanning and atomic force microscopy, have been applied for evaluation of photophysics, photochemistry and cytological effects of these metallo-porphyrins with applications in PDT.

The light dose-response curves (relative cell viability as a function of the light dose) are obtained for each cell line at low (10 µg/ml) and high (200 µg/ml) concentrations of the photosensitizers. The cytotoxic effect of metallo-porphyrins are most distinguished for the cell lines than the free bases, indicating that the metallic photosensitizers are more appropriate for photodynamic application.

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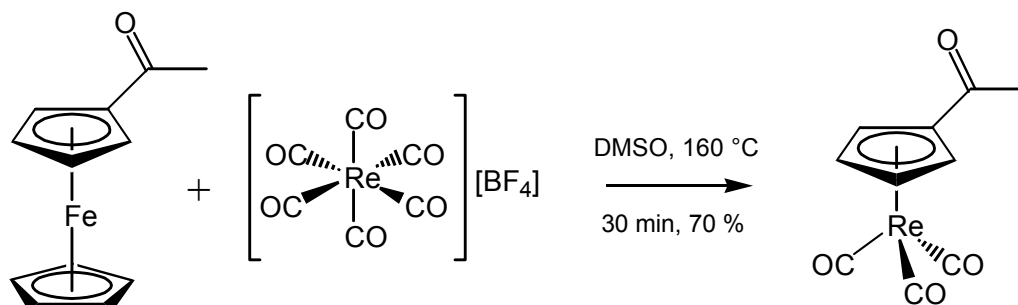
## New Methods for the Synthesis of CpRe(CO)<sub>3</sub> and CpTc(CO)<sub>3</sub> Units from Ferrocene Unit

Stéphane Masi,<sup>a</sup> Siden Top,<sup>a</sup> Gérard Jaouen,<sup>a</sup> Stefan Mundwiler,<sup>b</sup> Bernhard Spingler,<sup>b</sup> and Roger Alberto<sup>b</sup>

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Preliminary results describing the reactivity of [Re(CO)<sub>6</sub>][BF<sub>4</sub>], or the aqua ion *fac*-[<sup>99m</sup>Tc(CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup>, with ferrocene derivatives are presented. The reaction of ketoferrocene with [Re(CO)<sub>6</sub>][BF<sub>4</sub>] leads to the transfer of a cyclopentadienyl ligand from the monosubstituted ferrocene to the *fac*-[Re(CO)<sub>3</sub>]<sup>+</sup> moiety of the hexacarbonylrhenium complex. This cyclopentadienyl transfer is activated by the presence of the ketone group on the cyclopentadienyl ligand (Scheme 1). Reactivity was solvent dependent with higher yields obtained in DMSO > DMF > HMPA.<sup>[1]</sup>



**Scheme 1**

This research was extended to the synthesis of cyclopentadienyltricarbonyltechnetium derivatives in collaboration with Prof. R. Alberto at the University of Zurich. The organometallic aqua complex *fac*-[<sup>99m</sup>Tc(CO)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]<sup>+</sup> reacts with ketoferrocenes to give almost quantitative yield of the expected radiolabeled compound. The cyclopentadienyl-transfer reactions described above were applied to the syntheses of two novel 17 $\alpha$ -ethynyl estradiol derivatives containing one atom of Re or one atom of <sup>99m</sup>Tc. The results will be presented and potential radiopharmaceutical applications of these processes will be discussed.

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## Interaction of Metal Complexes with Biomolecules

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The interactions of metal complexes with biomolecules present in the bloodstream as well as in the cell are of high importance for the anticancer chemotherapeutic success in the clinic. In this communication the applicability of four different analysis methods for studying the binding of biomolecules on metal complexes influencing the mode of action will be discussed.

It is widely accepted that DNA represents the target molecule for platinum complexes in the cell. A simple assay system to determine antitumor activity could help to reduce the high costs and long duration of cell culture tests. Such a test system could be based on capillary electrophoresis which offers a number of prominent advantages like the possibility to simulate physiological conditions, low sample consumption, short analysis time, separation of differently charged species, etc.

Another method suitable to characterize biomolecule-complex binding is single crystal X-ray diffraction analysis. The crystal structure of a newly developed ruthenium complex with an derivative of the DNA building block adenine will be presented.

In 2002 the development of electrospray ionization mass spectrometry (ESI-MS), an MS technique suitable to determine the molecular weight of proteins, was awarded with the Nobel Prize for chemistry. The suitability of applying this analytical tool to investigate the binding of the ruthenium complex KP1019 (FCC14a) which entered clinical trials phase 1 lately to transferrin was recently evaluated.

Hyphenated techniques gained by combining the separation power of chromatographic/electrophoretic methods and the structural information of mass spectrometry are of special interest in pharmaceutical research. In this context the time-dependent binding of Pt-complexes toward albumin was determined by CE-ICP-MS.

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## Platinum-Modified DNA and PNA Oligomers in Antisense and Antigene Strategies

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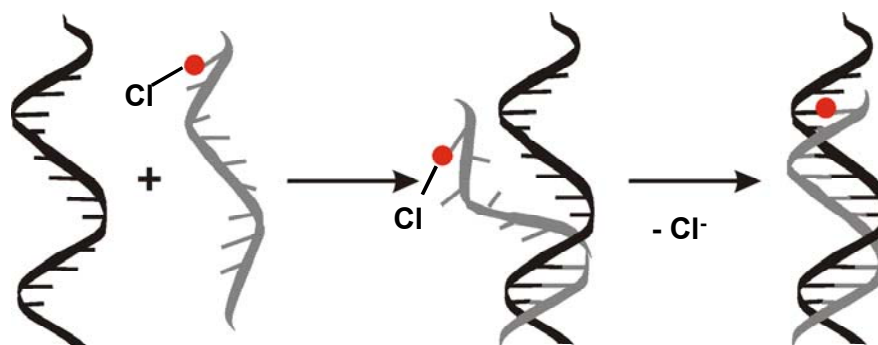
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The antisense and antigene strategies are promising approaches in the field of gene silencing. One frequent challenge is the relatively low affinity of the oligonucleotide (analogue) to its target sequence. To increase this affinity, we employed *trans*-coordinating platinum complexes to covalently cross-link the two complementary strands.

Whereas both the direct reaction of the oligonucleotide with *trans*-DDP as well as the use of platinated monomers during solid-phase synthesis of the targeting oligonucleotide still leave several problems unsolved such as limited applicability within poly-pyrimidine sequences only, newly developed monofunctionally *trans*-DDP modified peptide nucleic acid (PNA) oligomers display promising features.

In this presentation, the results of a feasibility study of a platinated triple helix will be presented together with an overview of how monofunctionally platinated monomers for the solid-phase syntheses of the targeting DNA and PNA oligomers can be synthesized.



## Use of Mass Spectrometry to Help Identify Drug-Protein Interactions

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One of the most difficult tasks in drug development is the identification of the molecular mechanism of drug activity, which is integral for the entry of the candidate drug into clinical trials. The difficulty lies in the complexity of living things and the number of biomolecules that the drug could target either to induce the therapeutic effect or to cause unwanted side-effects.

Mass spectroscopy (MS) has already been shown to be extremely useful in probing disease mechanisms in whole cell systems through its central role in proteomics methods, which can handle complex protein mixtures. However, proteomics has limited applications to probing drug mechanism as drug modified proteins often do not have significantly different mobility in polyacrylamide gels used in proteomics, compared to their unmodified equivalents, to allow their detection. In contrast, ICP-MS can be used to specifically detect metal-drug modified protein species in protein gels. Subsequently the protein can be extracted and identified using standard proteomics methods. Further, with covalent-modification, the MS-MS sequencing methods can be used to identify the drug-modified amino acid residues due to the change in molecular weight of the residue and the characteristic isotope pattern of the bound metal.