

Fluorescence-based diagnostics for understanding platinum-based therapy



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Abstract

This thesis explores the design of a series of platinum(IV) fluorescent probes as imaging tools to study the intracellular reduction of Pt(IV) prodrugs in real-time *via* fluorescence microscopy.

Chapter 1 outlines the fundamental properties of platinum. The mode of action of Pt(II) drugs within cells, the development and reduction mechanisms of Pt(IV) prodrugs is discussed. The final sections review prior work on Pt fluorescent sensors, Pt-tagged complexes, and Pt theranostics.

Chapter 2 details the synthetic procedures and characterisation of two novel fluorescent diazido platinum(IV) conjugates for potential PACT-PDT application. The photophysical properties and stability of the complexes are examined.

Chapter 3 presents a set of fluorescence responsive oxaliplatin(IV) carbamate complexes, of which the photophysical and photochemical characteristics in the presence of reductants are studied in detail. The reduction and cytotoxic properties of the OxPt(IV) prodrugs in cancer cells are also investigated.

Chapter 4 extends the work presented in Chapter 3 to prepare the cisplatin-based and carboplatin-based platinum(IV) analogues. The stability, reduction, and cytotoxic profiles of the fluorescent CisPt(IV) and CarboPt(IV) compounds are compared to the OxPt(IV) analogues.

Chapter 5 evaluates the combinatory effect of copper metal ions and ascorbate in an attempt to promote the reduction of less reactive platinum compounds.

Chapter 6 describes the development and characterisation of fluorescent platinum therapeutics as potential dual-action anticancer agents.

Chapter 7 provides an overview of the conclusions met in this work and the consecutive research strands that could be investigated in the future.

Chapter 8 contains the experimental procedures and synthetic methods.

Publications

1. M. H. C. Boulet, L. K. Marsh, A. Howarth, A. Woolman and N. J. Farrer, *Dalton Trans.*, 2020, **49**, 5703-5710, DOI:10.1039/c9dt04862f.
2. M. H. C. Boulet, H. R. Bolland, E. M. Hammond and A. C. Sedgwick, *J. Am. Chem. Soc.*, 2023, **145**, 12998-13002, DOI:10.1021/jacs.3c03320.

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Abbreviations

BODIPY	boron dipyrromethene
BSA	bovine serum albumin
DCM	dichloromethane
dH ₂ O	deionised water
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenyl phosphoryl azide
DSC	<i>N,N'</i> -Disuccinimidyl carbonate
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
em	emission
en	ethylenediamine
ex	excitation
GF-AAS	graphite furnace atomic absorption spectroscopy
5'-GMP	guanosine 5'-monophosphate
GSH	glutathione
H ₂ O	water
H ₂ O ₂	hydrogen peroxide
HBTU	<i>O</i> -(Benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrometry
HSA	human serum albumin
ICP-MS	inductively coupled plasma mass spectrometry
IR	infrared
L	ligand
LA-ICP-MS	laser ablation inductively coupled plasma mass spectrometry
LC-MS	liquid chromatography mass spectrometry
L-Cys	L-cysteine
LMCT	ligand-to-metal charge transfer
λ	wavelength
λ_{max}	wavelength of maximum intensity
λ_{irr}	wavelength of irradiation

MeCN	acetonitrile
MeOH	methanol
NaAsc	sodium ascorbate
NADH	reduced nicotinamide adenine dinucleotide
NanoSIMS	nano-scale secondary ion mass spectrometry
NHS	<i>N</i> -hydroxysuccinimide
NMR	nuclear magnetic resonance
PACT	photoactivated therapy
PBS	phosphate buffered saline
PDT	photodynamic therapy
ppm	parts per million
PS	photosensitiser
py	pyridine
UV-vis	ultraviolet-visible
UVA	ultraviolet A
ROS	reactive oxygen species
RP-HPLC	reverse phase high performance liquid chromatography
RT	room temperature
TBTU	O-(Benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium tetrafluoroborate
TEA	triethylamine
TEM	transmission electron microscopy
TFA	trifluoroacetic acid
TLC	thin layer chromatography
XANES	X-ray absorption near-edge spectroscopy

Chapter 1 Introduction

The term 'cancer' is a broad designation used to describe more than 200 diseases distinguished by the unregulated proliferation of abnormal cells.¹ Cancer is an extremely critical global health issue, standing as the second leading cause of death worldwide and responsible for approximately 10 million fatalities (accounting for one-six of all deaths). According to the World Health Organization (WHO), it is anticipated that the annual number of newly diagnosed cancer cases will rise to about 30 million by the year 2040. In the next twenty years, the approximate 60 % increase in cancer incidence will exert additional pressure on both health systems and populations.²

Current conventional treatments typically involve three main approaches applied alone or in combination: surgery (removal of localised solid tumours), radiotherapy (radiation using X-rays), and chemotherapy (use of antineoplastic drugs). Amongst the various anticancer agents administered, platinum-based chemotherapeutics (e.g., cisplatin, carboplatin and oxaliplatin) are extensively employed, providing treatment for nearly half of all cancer patients. Although platinum drugs have proven highly effective against a wide spectrum of malignancies, chemoresistance of cancer cells and the occurrence of undesired and debilitating side-effects are significant problems encountered with their continuous use.^{3,4} Hence, present challenge lies in the development of new and more precise platinum-based therapies to efficiently treat and improve outcomes of cancer patients.

Platinum(IV) prodrugs remain one of the leading strategies investigated to reduce the systemic side-effects experienced with clinically approved platinum agents. However, there is still a lack of complete understanding of their mechanisms of action and the intracellular components involved in their activation. This thesis explores the design of a range of platinum(IV) fluorescent complexes with the aim to examine the reduction process of platinum(IV) prodrugs in cancer cells. The fluorescent probes could aid in identifying novel approaches to develop the next generations of Pt-based anticancer agents. Thus, this chapter

initially introduces the properties of platinum, the mode of action of Pt(II) drugs such as cisplatin and the development of Pt(IV) prodrugs. In the final sections, an overview of several platinum fluorescent sensors and platinum theranostics devised for dual chemotherapy and fluorescence imaging applications reported in the literature is provided.

1.1. Platinum chemistry

Natural platinum consists of six isotopes: ^{190}Pt , ^{192}Pt , ^{194}Pt , ^{195}Pt , ^{196}Pt and ^{198}Pt . Of these, ^{195}Pt is the most abundant, accounting for 33.83 % of the total Pt abundance and is the only one with magnetic properties due to its spin quantum number $I = \frac{1}{2}$, making the ^{195}Pt nucleus NMR active. Platinum chemical shifts ($\delta^{195}\text{Pt}$) have an extensive range of values, from +8000 to -7000 ppm (usually referenced to a standard sample $[\text{PtCl}_6]^{2-}$ in D_2O) and are sensitive to several parameters which include the oxidation state, type of ligands in the coordination sphere, stereochemistry, solvent, and temperature. As a result, $\delta^{195}\text{Pt}$ responds to small variations in the chemical environment and ^{195}Pt NMR spectroscopy can be used to study the speciation and biomolecular interactions of platinum complexes in solution.⁵⁻⁸ Figure 1.1 illustrates the approximate $\delta^{195}\text{Pt}$ range for Pt(0), Pt(II) and Pt(IV) compounds.

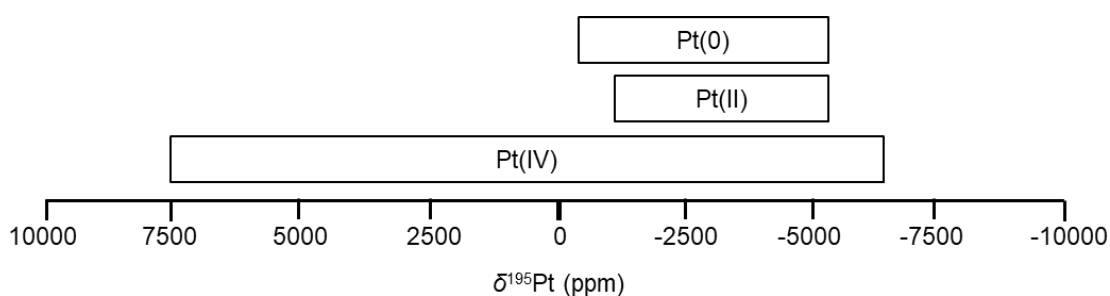


Figure 1.1. Approximate range of chemical shifts ($\delta^{195}\text{Pt}$) according to the different oxidation states of platinum. The chemical shifts are relative to $[\text{PtCl}_6]^{2-}$ in D_2O . (Scheme adapted from Still et al.⁵)

Coupling of the ^{195}Pt isotope to other nuclei can be observed in the NMR spectra of the coupled nucleus (such as ^1H , ^{13}C , ^{15}N , ^{19}F and ^{31}P) as small resonances surrounding the central chemical shift. Referred to as “ ^{195}Pt satellites”, the NMR resonances appear as a multiplet with a relative intensity of 1 : 4 : 1 (Figure 1.2).^{5,9}

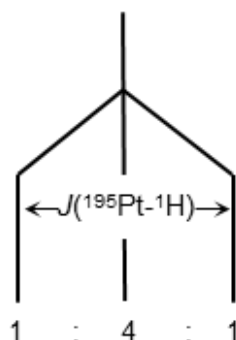


Figure 1.2. Illustration of $^{195}\text{Pt}-^1\text{H}$ satellites.

Platinum (Pt) is a transition metal with the electronic configuration $[\text{Xe}] 4f^{14} 5d^9 6s^1$. The d-block element can have a range of oxidation states from 0 to +6, but the most prevalent are +2 and +4.¹⁰ Because platinum(II) has a $5d^8$ electronic configuration, it generally adopts a square-planar geometry in the +2-oxidation state and has a coordination number of four. The square-planar geometry is preferred over the alternative tetrahedral geometry due to the favourable splitting of the d-orbitals. The lower orbitals are completely filled whilst the highest energy orbital ($d_{x^2-y^2}$) is left empty. Platinum can also be found in the +4-oxidation state with a d^6 electronic configuration. In this oxidation state, platinum has 6 substituents and forms octahedral complexes. The low-spin d^6 configuration exhibit fully occupied t_{2g} orbitals (d_{xy} , d_{xz} and d_{yz}) (Figure 1.3).¹¹

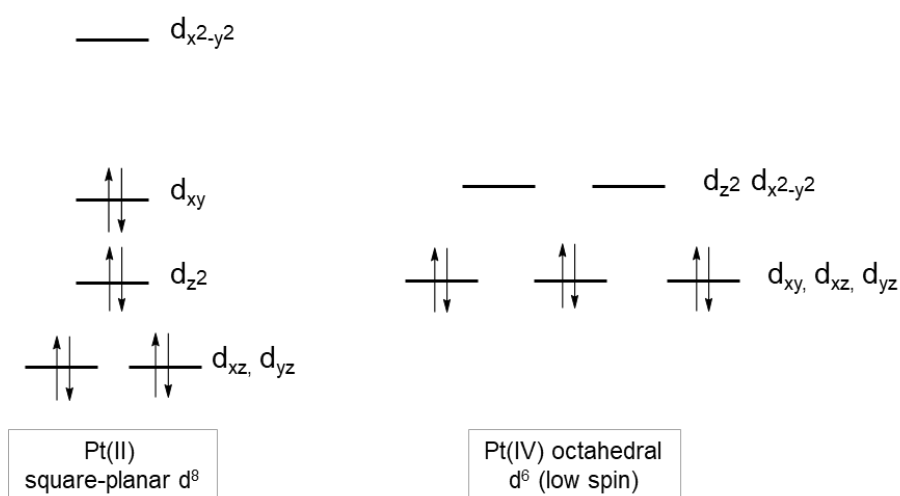


Figure 1.3. Crystal field splitting of the d-orbitals in square-planar Pt(II) complexes (d^8) and octahedral Pt(IV) complexes (d^6) configurations.

As per Pearson's "hard and soft acids and bases" (HSAB) principle, Pt(II) is classified as a soft acid which indicate a preference to interact with soft bases such as S-containing ligands. Halides, S- and P-donor ligands are therefore often found to form stable complexes with Pt(II). Additionally, Pt(II) demonstrates a strong preference for certain O-donor ligands like carboxylates and acetates groups while typically displaying lower affinity for hard bases (such as F and O ligands). Although Pt(IV) is categorised as a soft acid within the HSAB theory, it is comparatively harder than Pt(II).^{12,13}

1.2. Platinum chemotherapy

1.2.1. Cisplatin and mechanism of action

The cytotoxic property of cisplatin (*cis*-diamminedichloroplatinum(II), also referred to as cDDP) was initially identified by Barnett Rosenberg back in the 1960s.^{14,15} The complex was rapidly introduced as a drug candidate for clinical testing and approved by the FDA (Food and Drug Administration) for the treatment of testicular and ovarian cancer in the late 1970s, becoming the first metal-based anticancer agent used in the clinic.¹⁶ Cisplatin is now commonly administered worldwide to treat other kind of solid malignancies such as lung, bladder as well as head and neck cancers.¹⁷

The Pt-based drug is delivered intravenously by saline injection. The high concentration of chloride ions within the bloodstream (~100 mM) is believed to prevent premature hydrolysis of its chlorido ligands. However, interaction of the drug with biological nucleophiles such as plasma proteins and in particular human serum albumin (HSA) has been reported to considerably decrease the concentration of cisplatin able to reach DNA, thus reducing its efficacy.¹⁸ The mechanism of action of cisplatin consists of four strategic steps and is illustrated in Figure 1.4. The first stage involves the cellular uptake of the drug (**1**) which can be done *via* two pathways: passive diffusion through the cell membrane and active transport *via* copper transporter protein (CTR1).^{19–21} After entering the cell, cisplatin undergoes aquation (**2**) which corresponds to the substitution of one or two chlorido ligands with water molecules and results

in the production of positively charged mono- or bis-substituted adducts. This hydrolysis event is made possible by the lower concentration of chloride ion (~4-20 mM) in the intracellular environment.^{22,23} These active aquated species are subsequently able to cross into the nucleus (3) and bind to the DNA via the formation of bifunctional adducts which damage the double helix (4).²⁴

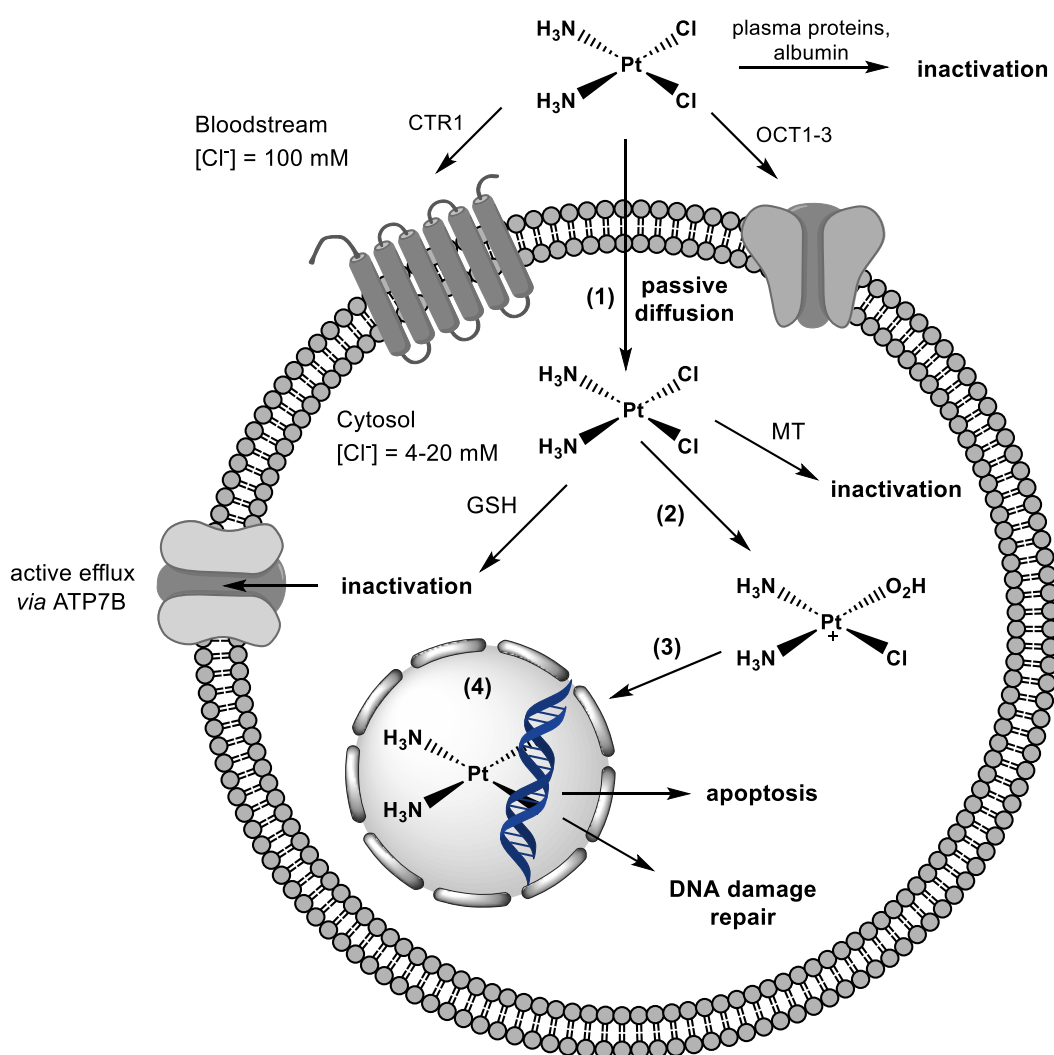


Figure 1.4. Various pathways of cisplatin: cellular uptake, aquation and deactivation pathways (Scheme adapted from Johnstone et al.²¹).

Although cisplatin can react with various other molecules within cells, its primary target is nuclear DNA. The active drug mainly binds to the nucleophilic N₇ position of purine residues, preferentially with guanine and to a lesser extent with adenine (Figure 1.5). This reaction leads to the deformation of the DNA helix and triggers several cell-signalling pathways able to initiate apoptosis.^{25,26}

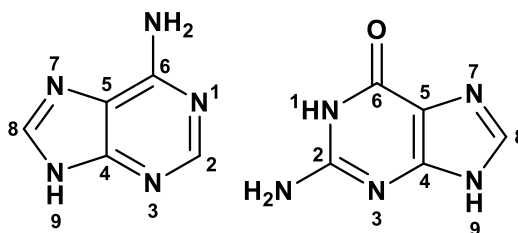


Figure 1.5. Chemical structures of the nucleotide bases adenine (left) and guanine (right).

¹⁹⁵Pt NMR studies demonstrated that aquated species initially react with DNA to form monofunctional adducts and subsequently bind to another nucleotide to create bifunctional crosslinks.^{27,28} Cisplatin can generate intrastrand DNA crosslinks, by binding two adjacent nucleobases, or interstrand DNA crosslinks (Figure 1.6). It predominantly forms intrastrand crosslinks 1,2-d(GpG) (65%) and 1,2-d(ApG) (25%). Other possible crosslinks encountered are 1,3-d(GpTpG) intrastrand (5-10%), which involve two guanines separated by another base, monofunctional adducts, G-G interstrand crosslinks and adducts between nucleotides and proteins. The formation of 1,2-intrastrand crosslinks distorts the DNA structure and result in the DNA replication and transcription processes inhibition leading to the death of the cancer cell.²⁹ High Mobility Group-domain (HMG) proteins were also found to be involved in the mechanism of cisplatin cytotoxicity by preventing the repair of these Pt-DNA adducts.³⁰

In addition to cisplatin's ability to act as a DNA alkylating agent, other apoptosis signalling pathways contribute to its toxicity. Cisplatin accumulation can increase the generation of intracellular reactive oxygen species (ROS) *via* different mechanisms. As mentioned previously, the drug can interact with other components within the cellular environment, including sulphur-containing biomolecules such as glutathione (GSH) and

thioredoxin reductase (TrxR), which are responsible for regulating the cellular homeostasis. The formation of cisplatin-GSH conjugate and the inhibition of the TrxR enzyme can interrupt normal cellular processes and cause ROS overproduction.^{31,32}

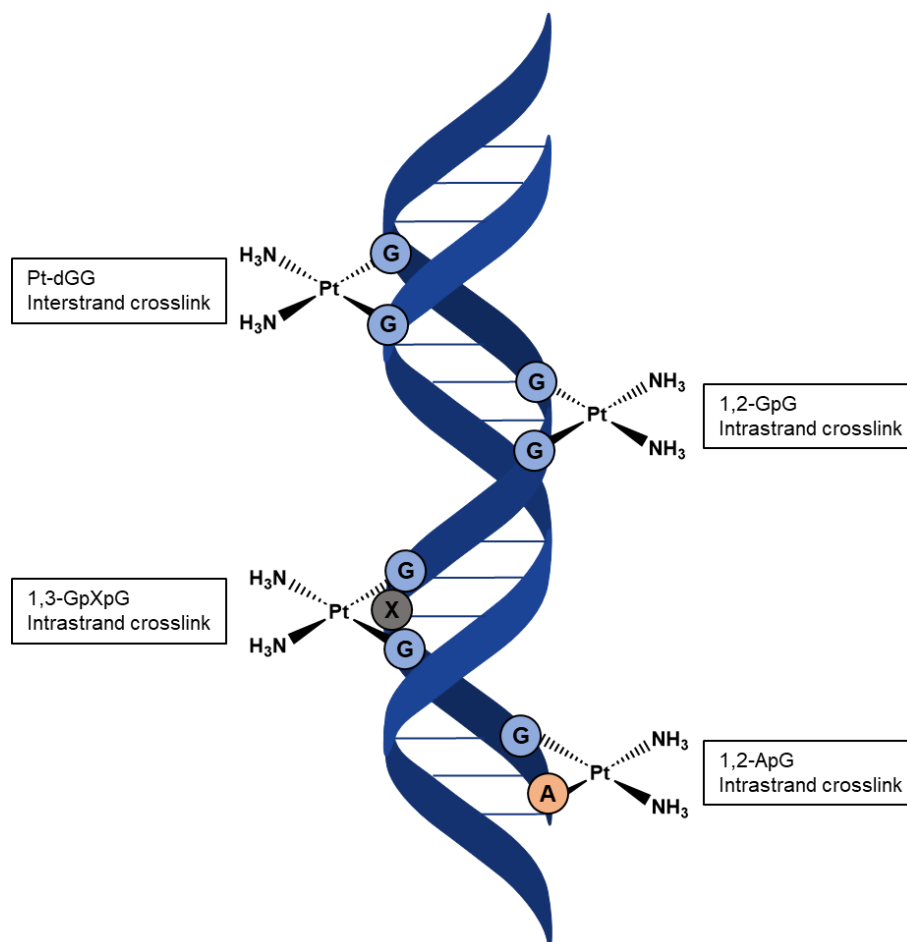


Figure 1.6. Illustration of DNA with the most common cisplatin-DNA adducts.

1.2.2. Next-generations Pt(II) anticancer agents

Despite being effectively used in cancer therapy, treatment with the chemotherapeutic agent faces many limitations. Severe side-effects such as ototoxicity, neurotoxicity, nephrotoxicity, nausea, vomiting and fatigue which can be dose-limiting have been induced by the repeated use of cisplatin.³³ Moreover, the therapeutic effectiveness of the drug is further limited by the development of chemoresistance, either innate or acquired, and has been linked to several factors including the enhanced DNA repair, increased cisplatin inactivation, and the

decreased cellular accumulation of the drug.³⁴

Due to the high toxicity profile of cisplatin and the resistance displayed by cancerous cells, alternative platinum-based chemotherapeutics have been investigated in the hope to diminish the systemic toxicities experienced with the initial Pt(II) anticancer drug. Numerous cisplatin derivatives have been designed including the second- and third-generation Pt-based complexes, carboplatin and oxaliplatin, clinically approved worldwide. Three additional platinum(II) chemotherapeutics have been developed, namely nedaplatin, heptaplatin and lobaplatin, and are used in some parts of Asia (Figure 1.7).³⁵

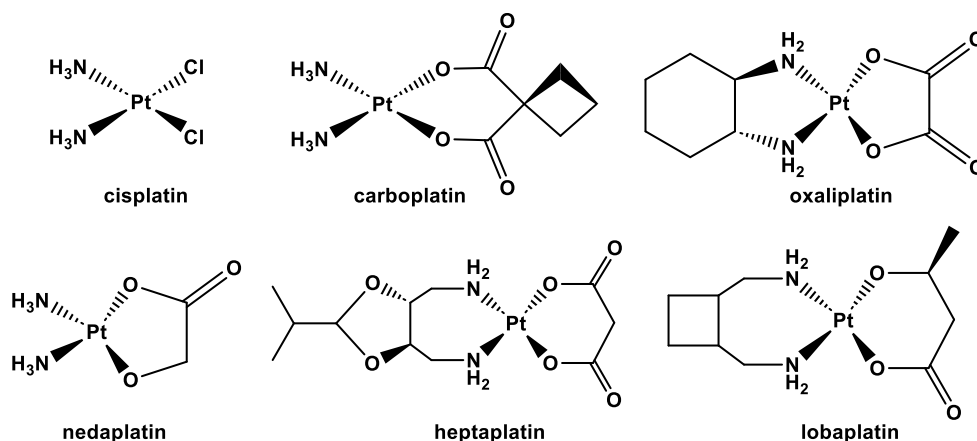


Figure 1.7. Chemical structures of approved Pt(II) chemotherapeutic drugs used in the clinic.

Carboplatin has a similar mechanism of action to cisplatin, but its structure differs by the introduction of a bidentate cyclobutanedicarboxylate ligand instead of two chlorido ligands. As a result, the complex is characterised by a slower aquation rate, around 10-fold slower than cisplatin, making carboplatin less reactive towards thiol-containing molecules and thus less toxic. Treatment with carboplatin leads to less side-effects which allow for higher doses to be administered. Carboplatin is primarily used to treat ovarian and small cell lung cancers.¹⁶ Oxaliplatin features a bidentate oxalate ligand and a bidentate 1,2-diaminocyclohexane ligand which enhance its stability. The drug presents less cytotoxic adverse effects than cisplatin and is used in the treatment of metastatic colorectal cancer. Both drugs are however still capable

of causing severe dose-limiting side-effects such as myelosuppression (carboplatin) and neurotoxicity (oxaliplatin).³⁶

Replacing the chlorido and ammine ligands significantly modified the activity and toxic profile of next-generation anticancer agents. Nevertheless, current Pt(II)-based therapies still pose significant problems, mainly extensive systemic toxicities, narrow spectrum of activities and the development of tumour resistance. Several strategies have been employed to enhance the therapeutic efficacy and selectivity whilst reducing the adverse effects of these Pt(II) chemotherapeutics such as combination therapy, the use of drug delivery systems to improve cancer-cell selectivity and retain drug effectiveness, and the design of safer and more effective Pt drugs.¹⁶

1.3. Platinum(IV) prodrugs

1.3.1. Overview

Extensive research efforts have been dedicated to developing platinum alternatives which are more stable and exhibit lower toxicity. Amongst these, platinum(IV) prodrugs represent a promising avenue due to their higher kinetic stability compared to Pt(II) complexes. They are therefore less likely to interact with other biological nucleophiles before reaching cancer cells, which in turn would reduce undesirable side-effects (Figure 1.8). They are regarded as prodrugs because they need to be reduced to produce the active Pt(II) drug able to display anticancer effect.³⁷ Moreover, the introduction of two axial ligands can contribute to the optimisation of their chemical properties such as the reduction potential, lipophilicity, and the drug solubility. These axial positions can also be used to incorporate various functionalities for targeting tumour or specific organelles, combination therapy, bioimaging and sensing, and to control the activation of platinum(IV) prodrugs, thus improving the delivery and treatment efficacy of platinum-based chemotherapeutics.^{21,38–44}

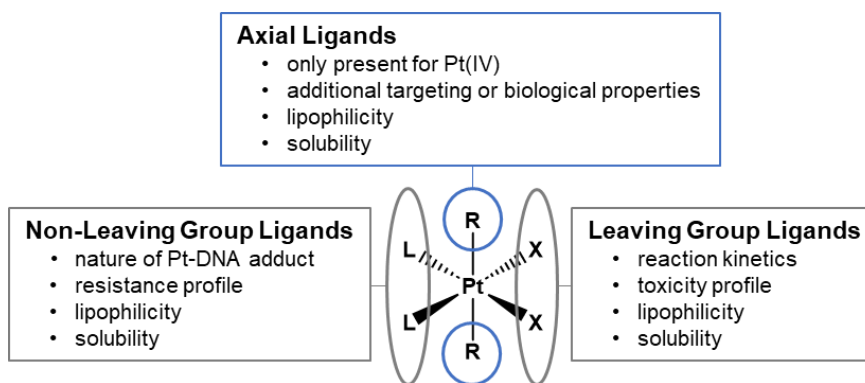


Figure 1.8. General structure of platinum(IV) prodrugs (Scheme adapted from Johnstone et al.²¹).

Several platinum(IV) prodrugs have been developed including tetraplatin, iproplatin, satraplatin and LA-12 which have reached clinical trials (Figure 1.9). However, none have yet received approval. Tetraplatin, also known as ormaplatin, and iproplatin were the first to be assessed. Tetraplatin was discontinued due to severe neurotoxicity which was attributed to its rapid reduction whilst iproplatin presented a lower cytotoxic profile than cisplatin and carboplatin.^{17,45} Satraplatin is the first Pt(IV) prodrug able to be administered orally and is currently under investigations in combination with radiotherapy.⁴⁶ The satraplatin derivative LA-12 is in phase I clinical trials.⁴⁷

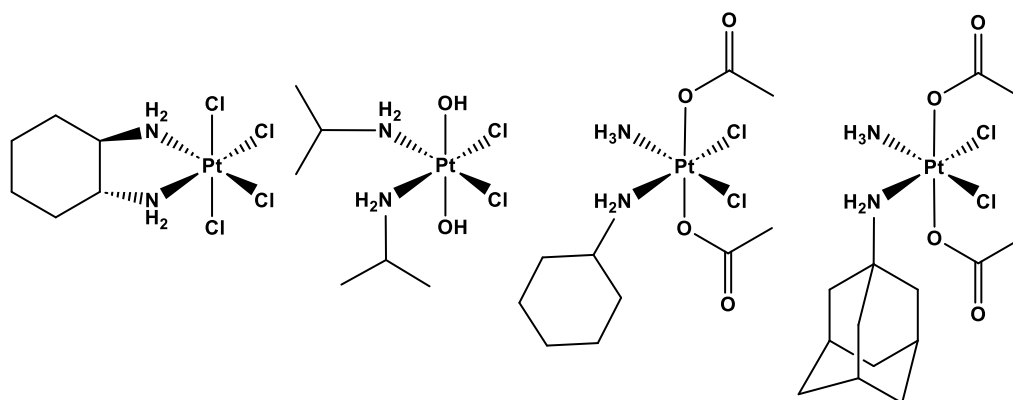


Figure 1.9. Chemical structures of platinum(IV) prodrugs that have entered clinical trials (L-R): tetraplatin, iproplatin, satraplatin and LA-12.

1.3.2. Reduction of platinum(IV) complexes

Platinum(IV) prodrugs are believed to be reduced *in vivo* by biological reducing agents to release the corresponding toxic Pt(II) drug and its axial ligands (Figure 1.10).⁴⁸ Previous studies have also shown that equatorial ligands can be released instead upon activation.^{49,50} The reduction of Pt(IV) prodrugs can be influenced by various factors which need to be investigated to develop improved Pt-based therapies. The prodrug should be stable enough to avoid deactivation before reaching its target but not completely inert to reduction as to remain non-toxic within cells. The redox potential is a crucial element to examine in order to understand the reduction behaviour of Pt(IV) complexes. Based on the structure of the complexes, reduction studies have been conducted in an attempt to decipher the impacts of the axial and equatorial ligands on the rate of Pt(IV) reduction. For instance, prior reports have revealed that Pt(IV) complexes bearing axial hydroxido ligands were more slowly reduced than their Pt(IV) analogues containing axial carboxylato or chlorido ligands due to the lower reduction potential of the former.⁵¹ This was found to be accurate for Pt(IV) complexes characterised by *cis* chlorido and *cis* diamine ligands at the equatorial sites but not necessarily applicable to all Pt(IV) prodrugs. In fact, Gibson *et al.* reported that oxaliplatin-based Pt(IV) complexes carrying axial hydroxido ligands were more quickly reduced than their diacetate derivative.⁵²

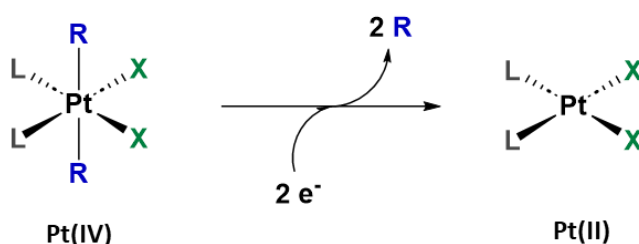


Figure 1.10. Schematic, showing reduction of platinum(IV) prodrug to generate the cytotoxic Pt(II) agent and release both axial ligands.

The reduction process of Pt(IV) prodrugs is thought to occur in the presence of cellular reducing agents with ascorbate and glutathione (GSH) generally assumed to be the ones involved in this event. They are therefore often used in solution and cell studies to examine the reduction of Pt(IV) prodrugs.⁵³ Ascorbic acid is a two-electron reducing agent whilst GSH is a one-electron reductant (Figure 1.11). Both are present within cells in high concentrations, approximately 1 mM and 2 mM, respectively.^{54–56} The reduction reaction of platinum(IV) prodrugs is believed to involve electron transfer from the bioreductant to the platinum centre followed by the elimination of two of its ligands. Different mechanisms have been studied to explain Pt(IV) reduction such as inner-sphere and outer-sphere two-electron transfer processes which are dependent on the type of ligands of the Pt(IV) complex and the nature of the bioreductant.^{57,58}

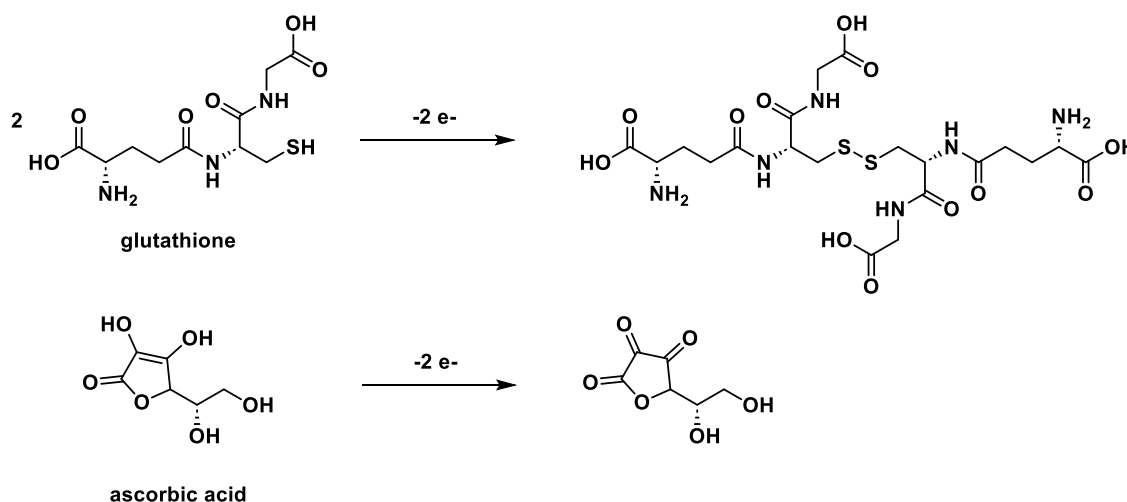


Figure 1.11. Possible cellular reducing agents responsible for Pt(IV) prodrugs reduction: glutathione and ascorbic acid (Scheme adapted from Wexselblatt *et al.*⁵⁷).

Moreover, several other biological reductants were found to be involved in the reduction of Pt(IV) prodrugs, including sulphur-containing biomolecules like L-cysteine and L-methionine as well as nicotinamide adenine dinucleotide (NADH), small cellular proteins, and metal-containing redox proteins (Figure 1.12).^{53,57} For example, iproplatin was shown to be reduced by metallothionein, a small metal-binding protein, which contains 20 cysteine residues and one acetylated methionine.⁵⁹ Carr *et al.* reported that metal-containing redox proteins can reduce

Pt(IV) complexes when in the presence of NADH. In fact, satraplatin was found to be stable in the presence of cytochrome c but rapidly reduced when NADH was added.⁶⁰ Salassa and co-workers also showed that the reduction of Pt(IV) prodrugs could be catalysed by the flavoenzyme NADH oxidase (NOX) when combined with NADH.⁶¹ Overall, there is clear evidence indicating that various biomolecules present in the physiological environment of cancer cells are able to activate Pt(IV) complexes, either alone or in combination with other cellular components.

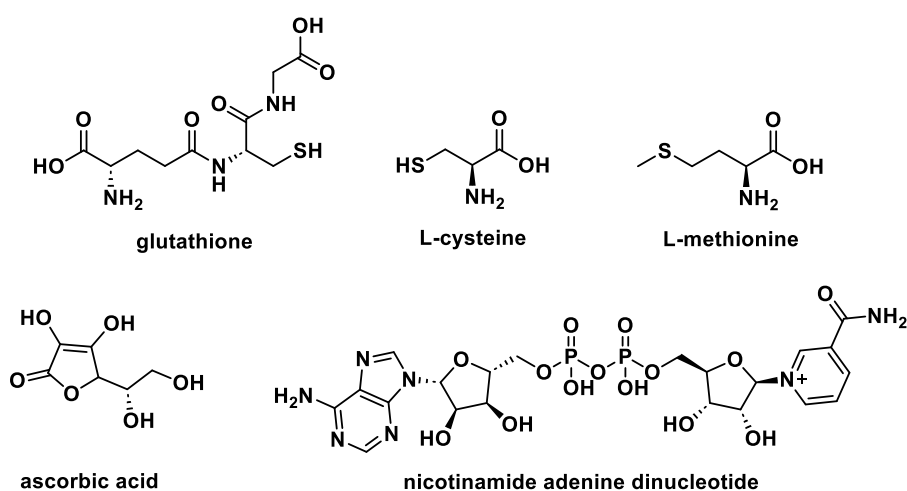


Figure 1.12. Cellular reducing agents involved in the activation of platinum(IV) prodrugs.⁵⁸

As previously mentioned, multiple parameters can impact the rates of reduction of Pt(IV) prodrugs including the chemical structure of the complex and the nature of the biological reducing agent used in the activation of the compound. Hence, to understand the *in vivo* behaviour and mechanisms of action of platinum(IV) prodrugs, the different factors that regulate the reduction process of these complexes need to be thoroughly studied to facilitate the design and optimisation of platinum-based chemotherapeutics.

1.4. Fluorescence imaging to monitor platinum-based drugs

1.4.1. Analytical techniques to study platinum complexes

Considerable research has been dedicated to the understanding of platinum metabolism, distribution, and interactions with biomolecules within biological systems. A range of analytical techniques have been employed and have offered valuable insights into the cellular mechanisms and pathways by which these compounds exert their therapeutic effects.

Elemental spectroscopy and spectrometry techniques such as graphite furnace atomic absorption spectroscopy (GF-AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are sensitive methods of analysis which allow to assess platinum content at very low concentrations in biological fluids, cancer cells and tissue samples. The combination of ICP-MS with laser ablation facilitated the design of a new technique known as LA-ICP-MS, enabling the measurement of Pt levels in specific tissues regions.⁶²⁻⁶⁶

Nano-scale secondary ion mass spectrometry (NanoSIMS) is an imaging technique which has been used to visualise the localisation of isotopically labelled platinum-based chemotherapeutics (with ^{195}Pt and ^{15}N isotopes) within cells by mapping their cellular uptake and distribution with high sensitivity.^{67,68} NanoSIMS is often utilised in combination with other analytical techniques. For instance, Keppler and colleagues have used LA-ICP-MS, NanoSIMS and transmission electron microscopy (TEM) to study platinum distribution in tumour tissues previously exposed to Pt(IV) complexes. LA-ICP-MS was initially applied to evaluate platinum concentration in different tissues regions and identify regions of interest.⁶⁹ Whilst NanoSIMS can effectively monitor platinum biodistribution, it does not provide information on the oxidation states of platinum complexes.

X-ray absorption near-edge spectroscopy (XANES) has been extensively employed to examine the oxidation states and distribution of platinum complexes within tumour cells.^{49,70-74} This technique can differentiate between platinum(II) and platinum(IV) species as their oxidation states are characterised by distinct absorption profiles in XANES spectra, resulting

in different peak heights which can be measured to determine the proportion of platinum(II) and platinum(IV) complexes present in a sample. Hambley and co-workers have also applied XANES to study the reduction behaviour of platinum(IV) prodrugs in biological fluids and in cells and were able to quantify the relative amount of platinum(IV) and platinum(II) species overtime, giving information into the stability and rate of reduction of these complexes.^{73,74}

Although these methods aid in getting a deeper understanding into the cellular processes of platinum compounds, they involve sample destruction and are therefore not compatible with live-cell imaging.

1.4.2. Fluorescence microscopy

In comparison to the analytical techniques outlined previously, fluorescence imaging is a powerful and non-invasive method used for the real-time observation of biomolecules or targeted structures within living biological samples. This technique can be utilised to analyse complex biological processes with high sensitivity and spatial resolution, by giving information on the localisation, distribution, and function of specific molecules or cellular components.⁷⁵

Fluorescence refers to the emission of light by molecules which are able to absorb light at a specific wavelength, usually within the ultraviolet or visible range. This process is often illustrated using a Jablonski diagram, as shown in Figure 1.13. It initiates with the absorption of a photon by a molecule, causing it to become excited and to transition to a higher energy state (S_1 or S_2). The electrons in the excited state subsequently undergo rapid relaxation to the lowest energy level (S_1) through internal conversion (IC). The molecule in a singlet excited state then returns to its ground state (S_0), resulting in photon emission. The entire process is rapid, occurring within nanoseconds to microseconds, following the molecule excitation. Furthermore, due to the loss of energy during the relaxation event, the emitted photon exhibit a longer wavelength compared to the photon that was absorbed.⁷⁶

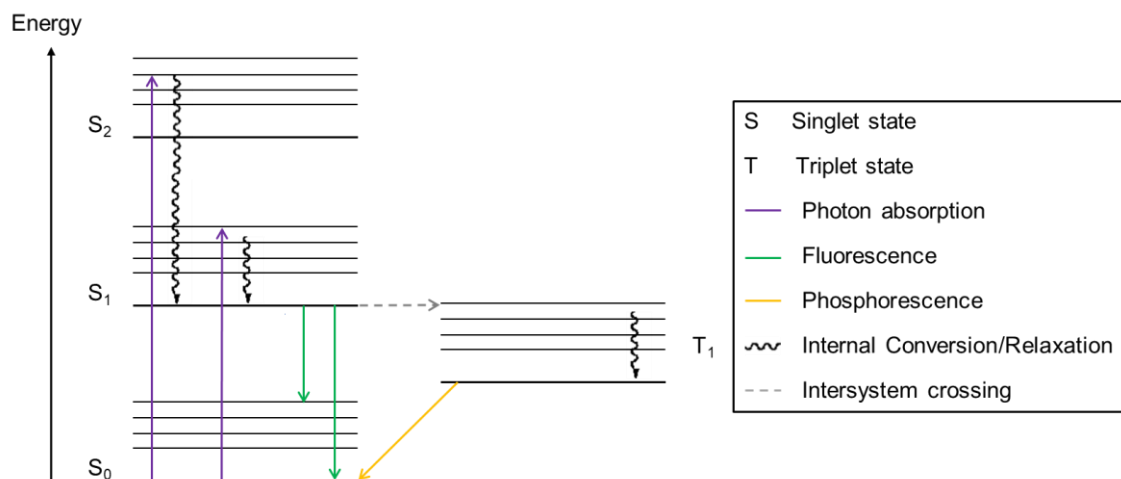


Figure 1.13. A Jablonski diagram displaying possible deactivation pathways of a molecule in an excited state (Scheme adapted from J. Lakowicz⁷⁶).

Fluorescent molecules, also called fluorophores, are widely employed as probes due to their versatile properties (i.e., high sensitivity, specificity, tunable excitation and emission spectra, high spatial and temporal resolution) to examine biological systems and processes in various field of applications such as bioimaging, sensing, drug discovery, and diagnostics. Figure 1.14 displays some of the most extensively utilised fluorescent frameworks for probes construction, which have also been used to analyse platinum compounds in biological settings.^{77–80}

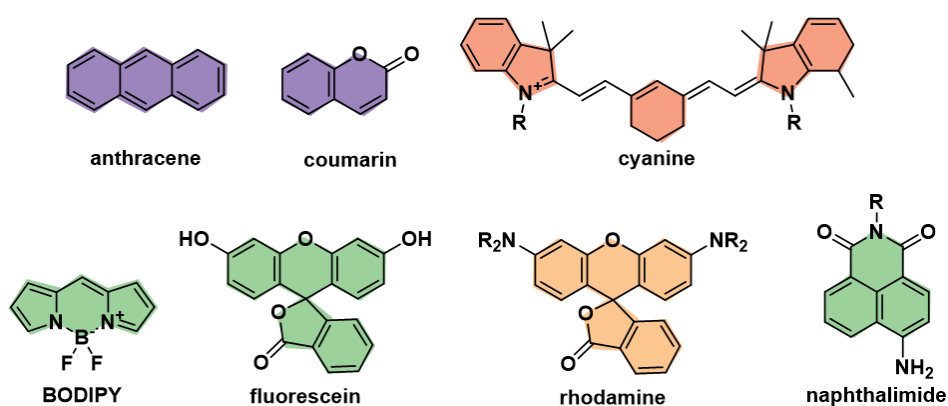


Figure 1.14. Common fluorescent scaffolds used for bioimaging applications.⁷⁷

Fluorescence microscopy can therefore aid in the comprehensive understanding of platinum complexes mode of action by mapping the biodistribution, interactions, and cellular uptake of these compounds *in vivo*. Two different approaches have been primarily studied and are discussed in the next sections: the use of exogenous fluorescent chemosensors and the tagging of platinum complexes with fluorophores to enable the direct visualisation of their behaviour within living cells.

1.4.3. Fluorescent sensors for platinum complexes

Fluorescence-sensing strategies have been exploited by several research groups to monitor the cellular uptake and activity of platinum complexes in biological systems. These fluorescent sensors are generally based on fluorescein or rhodamine dyes and functionalised with a sensing unit able to probe platinum both in solution and within cells. This section provides an overview of the imaging probes used to detect different types of platinum complexes reported in the literature.

The first fluorescence-sensing system for platinum was reported by Garner and Koide and involved a fluorescein-based probe which can detect platinum *via* a Tsuji-Trost allylic oxidative insertion mechanism (Figure 1.15, **1.1**).⁸¹ The reduction of Pt(II) and Pt(IV) species to Pt(0) is, however, initially required to initiate the reaction with the chemosensor. This is achieved by treatment with a reducing agent such as triphenylphosphine or in the presence of phosphine ligands on the complex, leading to the formation of Pt(0) species able to catalyse the deprotection of the allylic group of the probe and generate the fluorescent fluorescein product. As a result, the sensor is not applicable for real-time monitoring of platinum compounds. However, this method can be applied to assess the amount of Pt present in complex samples (even at low nanomolar levels) and has been used by Ahn *et al.* to quantify the concentration of platinum in a cisplatin drug delivery system.⁸²

In 2010, Tae and co-workers developed a rhodamine-triazole conjugate as a fluorescent chemosensor to monitor Pt^{2+} ions in aqueous solutions (Figure 1.15, **1.2**).⁸³ In its close-ring form the platinum probe is non-fluorescent, but addition of Pt^{2+} ions can induce a ring-opening of the spirolactam moiety leading to a fluorescence “turn-on” response. The sensor was selective to platinum ions over 22 other metal ions and the detection of platinum complexes (K_2PtCl_4 , $\text{Pt}(\text{COD})\text{Cl}_2$, PtCl_2 , and cisplatin) was also investigated. Whilst the sensor exhibited a noticeable fluorescence response with cisplatin, the signal was comparatively diminished in contrast to the ones observed with platinum salts. Moreover, the system was not applied under conditions other than water.

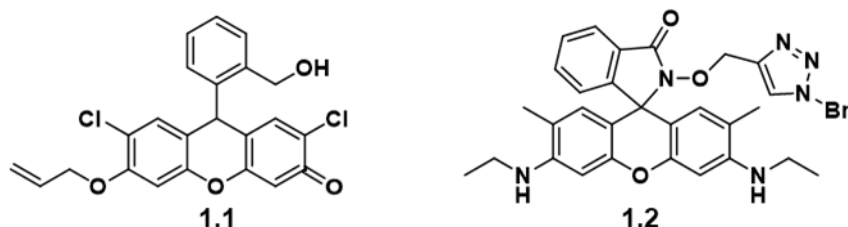


Figure 1.15. Structures of fluorescent chemosensors for platinum developed by Garner and Koide (**1.1**) and Tae et al. (**1.2**).^{81,83}

The Ang group has reported an extensive array of fluorescent probes for platinum recognition which was based on the **Rho-DDTC** sensor scaffold (Figure 1.16).^{84–88} The first designed rhodamine-based sensor was tethered to a diethyldithiocarbamate sensing group to yield **Rho-DDTC**.⁸⁴ The motif can interact with platinum *via* the dithiocarbonyl bond which trigger an opening of the spirolactam ring with a resulting fluorescence “turn-on” signal. The probe response in the presence of various $\text{Pt}(\text{II})$ and $\text{Pt}(\text{IV})$ species such as K_2PtCl_4 , cisplatin, carboplatin and satraplatin was studied in aqueous ethanol solutions. **Rho-DDTC** was revealed to be selective for $\text{Pt}(\text{II})$ complexes as no fluorescence enhancement was monitored with $\text{Pt}(\text{IV})$ compounds, making the sensor a valuable imaging tool to study the reduction of $\text{Pt}(\text{IV})$ complexes inside cells. The activation of platinum(IV) prodrugs was successfully monitored in HeLa cells and an accumulation of $\text{Pt}(\text{II})$ species was visualised in the cytoplasm

following reduction.

Later work expanded on the **Rho-DDTC** probe to explore the activation mechanism of the sensor through structure-activity investigations.⁸⁵ A series of structural analogues was designed, altering the linker chain lengths between the dithiocarbamate unit and the spirolactam motif or by modifying the spirolactam ring with amine and sulphur functional groups. The fluorescent probe **Rho(S)-OC2** demonstrated a 4-fold improvement in fluorescence response with cisplatin compared to **Rho-DDTC**. In addition, the sensor was found to respond more rapidly (reduced from 120 to 20 minutes) and maintained similar sensitivity to Pt(II) complexes as its precursor **Rho-DDTC**.

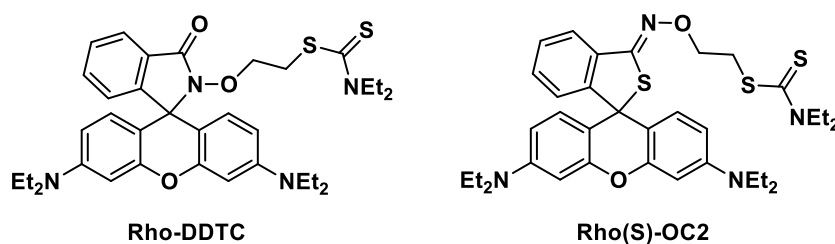


Figure 1.16. Structures of Pt-selective “turn-on” fluorescent probes **Rho-DDTC** and **Rho(S)-OC2** designed by Ang and co-workers.^{84,85}

Subsequent fluorescent systems involved the synthesis of two ratiometric rhodamine-based fluorescent derivatives of **Rho(S)-OC2** functionalised with a coumarin unit (**RDC1**) and a naphthalimide dye (**NPR1**) (Figure 1.17).^{86,87} The chemosensors are based on the Förster Resonance Energy Transfer (FRET) and Through-Bond Energy Transfer (TBET) mechanisms, respectively. FRET and TBET are energy transfer processes which involve two molecules referred to as donor and acceptor. In FRET, energy is transferred from the excited donor fluorophore to the ground-state acceptor fluorophore via dipole-dipole interactions, making it a distance-dependent process. In contrast, TBET involves energy transfer through a conjugated system and is independent of the spatial distance between both molecules. **RDC1** and **NPR1** exhibited similar characteristics to the original sensor and demonstrated selectivity towards Pt(II) species like cisplatin. They were thus used for ratiometric sensing of Pt(II) complexes

and for tracking the reduction of Pt(IV) prodrugs in cells. Recent efforts focused on designing a mitochondria-targeted analogue by incorporating a triphenylphosphonium unit on the probe to monitor the accumulation of cisplatin in real-time within mitochondria (**Rho-Mito**).⁸⁸ By targeting specific cellular components or organelles such as mitochondria, which is essential for cellular function, information can be obtained on the effect of platinum complexes on the metabolism and processes of cancer cells.

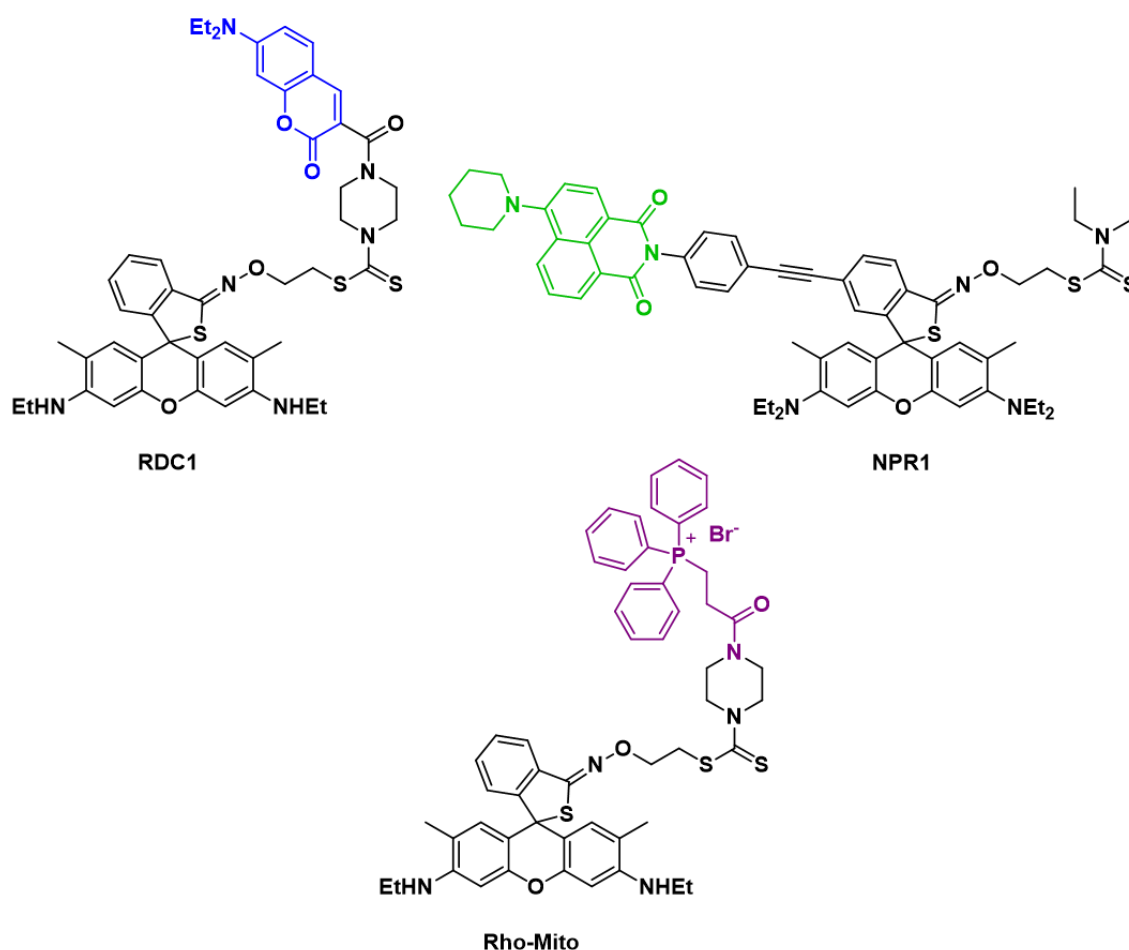


Figure 1.17. Structures of the ratiometric fluorescent probes **RDC1** and **NPR1**, and the mitochondria-targeting sensor **Rho-Mito** for Pt(II) recognition developed by Ang and co-workers.^{86–88}

Several Pt-selective fluorescent probes were also developed by the New group.^{89,90,91} Two of the initial fluorescent chemosensors designed, **FDCPt1** and **RPt1**, are shown in Figure 1.18. In the first system, a dithiocarbamic acid binding motif was introduced onto the spirolactam ring of a fluorescein-based sensor to design **FDCPt1**.⁸⁹ The chemosensor was

screened against a range of platinum compounds with various ligands but could only detect monofunctional Pt(II) species. The absence of response to other platinum complexes was believed to be caused by the resulting fluorescence quenching of π - π stacking interactions between two fluorophores when binding to bifunctional Pt(II) compounds. In addition, the probe was selective to Pt species over other metal ions (Cu^+ , Cu^{2+} , Ni^{2+} , Zn^{2+} , Fe^{3+} and Co^{2+}). **FDCPt1** was applied in biological settings to examine the cellular metabolism of Pt(II) drugs. The sensor was able to provide a fluorescence response in colorectal cancer cells treated with oxaliplatin and was subsequently used to track the metabolism of cisplatin and carboplatin in different cell lines using fluorescence microscopy. Thus, **FDCPt1** could be an effective probe to visualise the transformation of Pt(II) complexes within live cells. A rhodamine-based sensor, namely **RPt1**, conjugated to a phenyl isothiocyanate motif *via* the spirolactam ring was later synthesised.⁹⁰ The fluorescent probe was specifically created to track *trans*-configured platinum complexes containing aryl ligands and explore their metabolic stability and interactions with biological reductants like glutathione (GSH). More recently, the group expended their work by developing a library of sensors capable of detecting platinum complexes in clinical samples using an array-based sensing approach.⁹¹

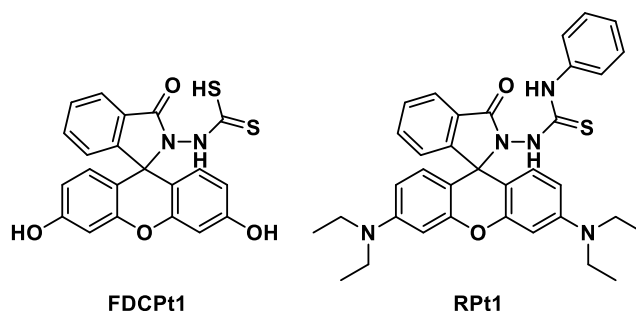
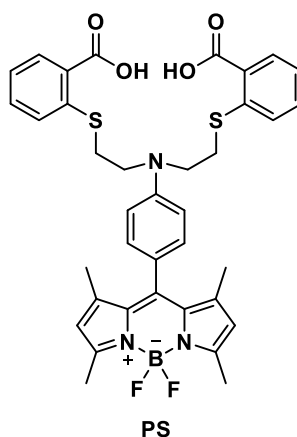


Figure 1.18. Structures of the chemosensors **FDCPt1** and **RPt1** developed by the New group.^{89,90}

One of the most recent examples of Pt-chemosensors was presented by Leung and colleagues (Figure 1.19).⁹² The BODIPY-based probe (**PS**) was functionalised with a NO_4S_2 podand ligand to study the cellular localisation of cisplatin and Pt(II) derivatives. The thioethers moieties on the ligand function as the sensing group for Pt(II) species. The sensor can



1.4.4. Fluorophore-labelled platinum complexes

Fluorescent tagging is another fluorescence-based imaging strategy employed to monitor platinum compounds within biological systems using fluorescence microscopy. By introducing fluorescent tags onto platinum complexes, researchers are able to directly visualise the localisation, cellular behaviour and uptake of these compounds in living cells. This approach can help in gaining insights into the mechanisms of action and potential applications of platinum-based chemotherapeutics for cancer therapy.

Clinically relevant platinum(II) complexes have been labelled with fluorescent dyes to track their localisation and pathways within malignant cells, facilitating the investigation of their cellular processing.^{78–80,93–97} For instance, Kalayda *et al.* developed a fluorescein-tagged oxaliplatin(II) derivative to examine the localisation and cellular mechanisms associated with tumour resistance against the platinum(II) chemotherapeutic agent.⁹⁷ Howell and colleagues devised a cisplatin derivative labelled with a fluorescein dye to study the accumulation and the intracellular transport pathways involved in the compound distribution in cancer cells.⁹³ Weissleder and co-workers synthesised several fluorescent platinum complexes, which combine a carboplatin or cisplatin scaffold and a bodipy fluorophore, to explore the biological interactions and distribution of these complexes in tumours.⁹⁵ Another method extensively employed to investigate the cellular targets of platinum(II) complexes is the use of a post-treatment fluorescent labelling approach.^{98–104} Using this strategy, DeRose and co-workers designed several oxaliplatin- and cisplatin-based platinum(II) derivatives functionalised with azide moieties to study the cellular components involved in the nucleolar stress response generated by the Pt(II) drug and leading to the death of cancer cells.¹⁰⁵

Based on the same approach used for monitoring Pt(II) species in cells, fluorophores have been conjugated to platinum(IV) complexes to examine the intracellular activation mechanisms of platinum(IV) prodrugs. When coordinated to the platinum centre, the fluorescence of the probe is suppressed, resulting in non-fluorescent compounds. The reduction of these Pt(IV) species to Pt(II) can restore fluorescence emission, enabling the

visualization of the activation process in both solution and biological investigations. Several examples of fluorescent Pt(IV) conjugates are illustrated in Figure 1.20 and reviewed in the following paragraphs.^{106–109}

Hambley and co-workers designed a fluorescent cisplatin-based Pt(IV) compound by replacing one of the equatorial amines with a coumarin derivative to study the reduction of the platinum(IV) complex in cells.¹⁰⁶ The fluorescence emission of the Pt(II)-coumarin complex was quenched following oxidation to the Pt(IV)-coumarin derivative (**1.3**), facilitating the examination of its reduction within cancer cells. However, despite observing a significant fluorescence intensity in treated cells, the distribution pattern of the platinum(IV) compound appeared to resemble the one monitored for the free coumarin dye. Hence, coordination of the fluorophore to the equatorial sites may not be the most appropriate approach to probe the activation of Pt(IV) complexes in biological systems.

Introducing a fluorescent dye at the axial sites, Lippard and colleagues reported a fluorescein-based Pt(IV) conjugate to monitor the fate of the cisplatin derivative in cell studies (**1.4**).¹⁰⁷ Solution-based investigations were conducted in the presence of the biological reductants glutathione or ascorbate and led to a fluorescence “turn-on” response due to the reduction to Pt(II) and the dissociation of its axial ligands. Fluorescence microscopy was subsequently used to track the cellular uptake and activation of the complex in HeLa cancer cells. Due to the poor cellular permeability of the fluorescein dye, its fluorescence emission was not readily observed within cells. However, an increase in fluorescence intensity was detected in cells exposed to the Pt(IV) conjugate, indicative of the complex uptake into the cell and its subsequent reduction to the active Pt(II) form. Cytotoxic studies revealed that **1.4** induced comparable DNA damage and cell cycle arrests to those of cisplatin, suggesting its potential as an effective prodrug of the Pt(II) complex and to investigate the intracellular reduction of cisplatin-based Pt(IV) prodrugs.

The use of self-immolative linkers to improve dual-drug delivery of platinum complexes within cancer cells was later explored by the Ang group. Self-immolative systems are molecules which can undergo spontaneous decomposition in response to a specific stimulus, resulting in the release of a functional moiety. These types of linkers were tethered to platinum(IV) prodrugs to enable the controlled release of both the active platinum(II) complex and an additional active agent or co-drug in cells upon reduction. Several cisplatin(IV) conjugates were designed to validate this approach, including a platinum(IV) complex bearing a coumarin derivative at the axial position (**1.5**) to examine the reduction process and elimination kinetic of the ligand through fluorescence-based studies.¹⁰⁸ In the presence of sodium ascorbate in phosphate buffered solution, a steady increase in fluorescence intensity was observed with time for the complex, which was attributed to the loss of both axial ligands following reduction of the Pt(IV) prodrug. *In vitro* experiments were subsequently conducted in HeLa cancer cells lines to evaluate the cytotoxicity and cellular uptake of the complex. However, fluorescence imaging was not applied to examine the reduction behaviour and monitor the release of the co-drug within cells in a real-time mode or to establish a correlation between these parameters and the increased cytotoxicity and platinum accumulation observed.

Recent work by Zhu and co-workers focused on the synthesis of a carboplatin(IV) prodrug functionalised with an axial bodipy ligand (**1.6**). The compound was developed to track, in real-time, the reduction process of the carboplatin derivative within cancer cells and to quantify its activation by measuring changes in fluorescence intensity.¹⁰⁹ In solution-based experiments, the sensor was exposed to sodium ascorbate and the fluorescence intensity was monitored over a 24-hour period. Overtime, a significant increase in fluorescence emission was detected, confirming that **1.6** can be used to effectively monitor and measure the activation of the carboplatin(IV) prodrug. Subsequently, the reduction of the complex was investigated in the presence of different cell extracts obtained from various cancer cell lines. These extracts contained proteins of varying molecular weights, from low (<3 kDa) to high (>3, <10, <50 and

<100 kDa) molecular weights, to examine the reduction process of the complex in different cellular environments. Whilst the reduction of platinum(IV) complexes is commonly attributed to the bioreductants ascorbate and glutathione, cellular proteins have also demonstrated the ability to activate Pt(IV) prodrugs. The results indicated that proteins with higher molecular weights played a predominant role in the activation of the complex, as opposed to proteins with lower molecular weights. The reduction of the carboplatin(IV) derivative was then studied across multiple cancer cell lines in real-time, allowing for the quantification of the complex reduction rate. In addition, the cellular accumulation and cytotoxicity of **1.6** were evaluated in the same cancer cell lines. These three parameters were analysed simultaneously to reveal a correlation between the rate of reduction and accumulation of the sensor with its cytotoxicity.

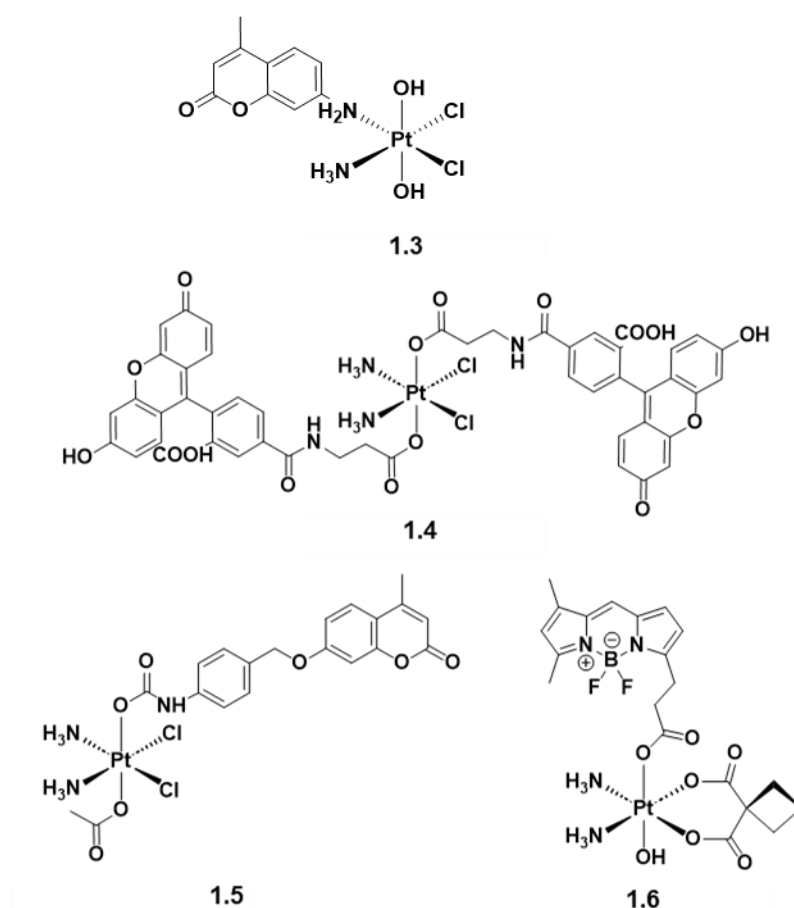


Figure 1.20. Structures of reported fluorescent platinum(IV) conjugates for studying Pt(IV) prodrug reduction.^{106–109}

Overall, platinum-based fluorescent sensors provide a method for tracking the activation of platinum(IV) prodrugs in a real-time manner within cancer cells, offering information on their mode of action and behaviour in response to cellular components present in the physiological environment. However, as mentioned previously, structural changes of platinum complexes can considerably affect the activity and properties of these compounds (i.e., cellular uptake of the drug, interactions with biomolecules, pharmacokinetics...). The integration of fluorophores will therefore result in platinum complexes that differ from the original platinum-based chemotherapeutics.

1.5. Platinum theranostics

The development of theranostic systems is a rapidly evolving strategy in personalised medicine for cancer treatment. These platforms incorporate both therapeutic and diagnostic functionalities within the same molecule which enables drug monitoring using different imaging modalities. This approach aims to provide real-time insights into the localisation, delivery, and efficacy of the anticancer agent, with the hope to develop more effective and precise cancer treatments strategies whilst reducing systemic side-effects. Ultimately, the information acquired could help improve patient outcomes by designing therapies specific to patients.

Several examples of platinum theranostics which combine a platinum-based chemotherapeutic component with a diagnostic moiety for fluorescence imaging or magnetic resonance imaging (MRI) applications have already been reported.^{109–116} In this section, the focus will be directed towards four targeted platinum(IV) theranostic agents developed by the Liu group.^{117–120} These conjugates were designed for distinct applications such as photodynamic therapy, assessing the therapeutic effects of a specific bioactive agent, and dual-drug monitoring, including the direct visualisation of Pt(IV) reduction in living cells using confocal microscopy. Each system is composed of four parts: a cisplatin(IV) prodrug modified at the axial sites with a targeting unit (cRGD), an aggregation-induced emission (AIE) fluorophore, and a hydrophilic component or additional anticancer drug. A cyclic arginine–

glycine–aspartic acid (cRGD) tripeptide was chosen to achieve selective delivery of the chemotherapeutic by targeting specific integrins ($\alpha_v\beta_3$) on the surface of cancer cells. On the other hand, a fluorophore featuring AIE properties was incorporated to offer real-time imaging of the Pt(IV) activation or co-drug release following reduction of the Pt(IV) complex. These fluorescent molecules are characterised by an enhanced fluorescence emission intensity in the aggregated state due to the restriction of their intramolecular rotations, making them attractive as fluorescent probes to study the intracellular reduction of platinum(IV) prodrugs in biological investigations.

The first of these platinum theranostics (**1.7**) is illustrated in Figure 1.21 and was designed to study the targeted delivery and reduction process of the cisplatin(IV) complex within cancer cells.¹¹⁷ Reduction studies in solution-based experiments were performed in the presence of bio-reductants such as ascorbic acid and glutathione, along with cellular proteins and other bio-acids. The complex demonstrated selectivity for both reducing agents, exhibiting significant fluorescence increases upon platinum(IV) activation but remained stable in the presence of other analytes. Cellular uptake investigations were carried out using confocal microscopy in two breast cancer cell lines: MDA-MB-231 cells, which present a high level of integrin receptors, and MCF-7 cells, serving as the negative control. Selective cellular uptake and high cytotoxic effects were reported in MDA-MB-231 cells whilst negligible fluorescence intensity and low toxicity were detected in MCF-7 cells due to the theranostic poor cellular uptake. In contrast, cisplatin demonstrated comparable cytotoxicity in both cancer cell types. The theranostic agent was thus shown to achieve selective drug delivery to specific cancer cells, enhancing the therapeutic efficacy, and may be further modified by substituting the cRGD targeting moiety with other tumour-targeting groups. In addition, real-time tracking of cisplatin(IV) prodrug activation within tumour cells could be visualised and quantified *via* fluorescence imaging using this system.

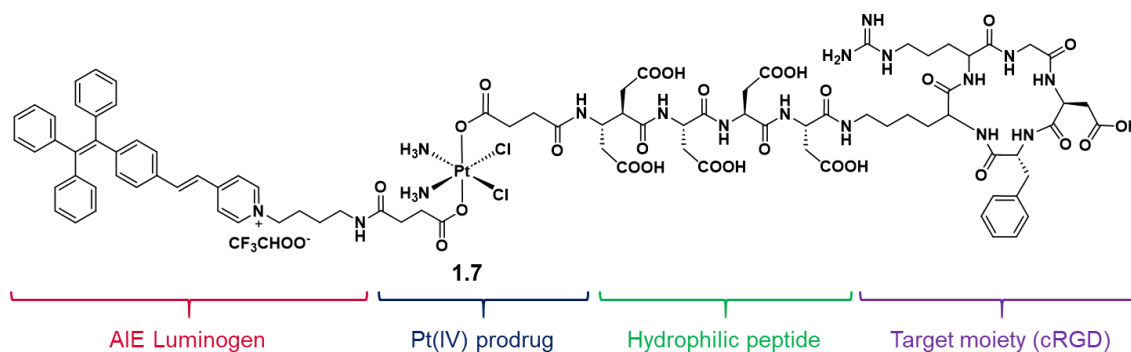


Figure 1.21. Structure of the water-soluble Pt(IV) theranostic PyTPE-Pt-D5-cRGD (**1.7**) (Scheme adapted from Yuan et al.¹¹⁷).

Additional work by Liu and co-workers expanded on the same structural model to develop three novel platinum(IV) theranostic prodrugs shown in Figure 1.22 and Figure 1.23. The original system was modified to integrate different functionalities for combinatorial applications. An AIE-active photosensitiser (PS) (**1.8**) was added for dual chemotherapy and photodynamic therapy (PDT) purposes.¹¹⁸ The platinum(IV) prodrug **1.8** was shown to induce comparable anticancer efficacy as cisplatin ($IC_{50} = 37.1 \mu M$) in the absence of light in MDA-MD-231 cells, a cisplatin-resistant cell line. Under light irradiation, however, **1.8** demonstrated increased cytotoxicity with an IC_{50} value of $4.2 \mu M$, indicative of the synergetic anticancer effect of the theranostic agent when PDT and chemotherapy are used in combination.

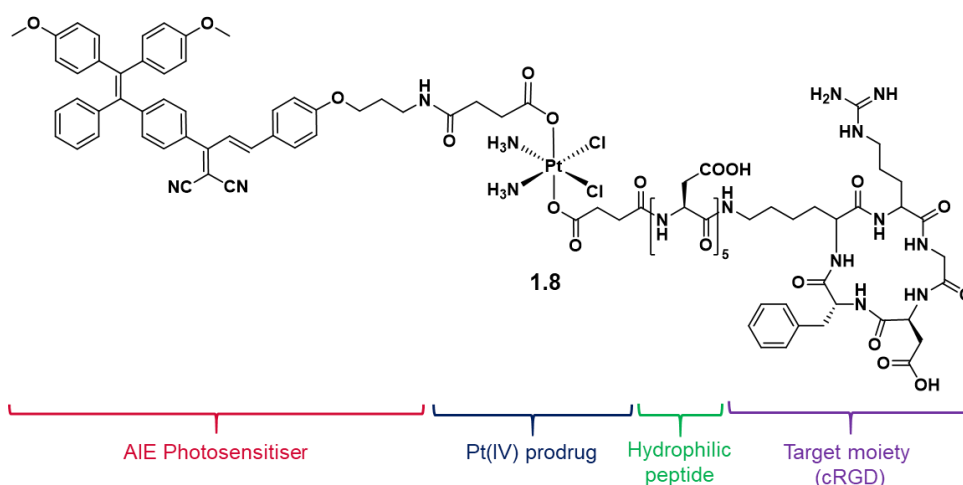


Figure 1.22. Structure of the targeted theranostic Pt(IV) prodrug TPECB-Pt-D5-cRGD (**1.8**).¹¹⁸

In the subsequent system (**1.9**), an apoptosis sensor was incorporated to image the cellular apoptosis induced by the reduced Pt(II) complexes in real-time and assess the effectiveness of the active agent within living cells.¹¹⁹ The sensor consists of an AIE fluorophore coupled to the peptide sequence (DEVD) specific to the enzyme caspase-3 which is involved in the apoptosis pathway. Upon intracellular reduction of the Pt(IV) prodrug, both cytotoxic Pt(II) species and the apoptosis indicator are released. The imaging sensor can interact with the caspase-3 enzyme activated during apoptosis, leading to the cleavage of the fluorophore and peptide units. The free AIE fluorophore is then able to aggregate in cells and generate an intense fluorescent signal. Cellular imaging studies revealed that the platinum theranostic **1.9** is capable of directly imaging apoptosis in cells and evaluating the therapeutic response induced by the apoptotic agent. The last platinum theranostic developed integrated the fluorescent anticancer agent doxorubicin (**1.10**) to monitor the Pt(IV) and co-drug delivery, cellular uptake, and activation in cancer cells.¹²⁰ The cellular uptake and cytotoxicity studies indicated that the co-drug was ultimately localised in the nuclei (within 6 hours) after Pt(IV) reduction. Additionally, the theranostic demonstrated a synergistic cytotoxic effect in MDA-MB-231 cells with an IC_{50} of 0.69 μ M, compared to 1.6 μ M for DOX and 18.1 μ M for cisplatin. Taken together, these fluorophore-based systems enable the monitoring of various functionalities and applications, providing great insights for the optimisation of platinum-based theranostic agents for cancer therapy.

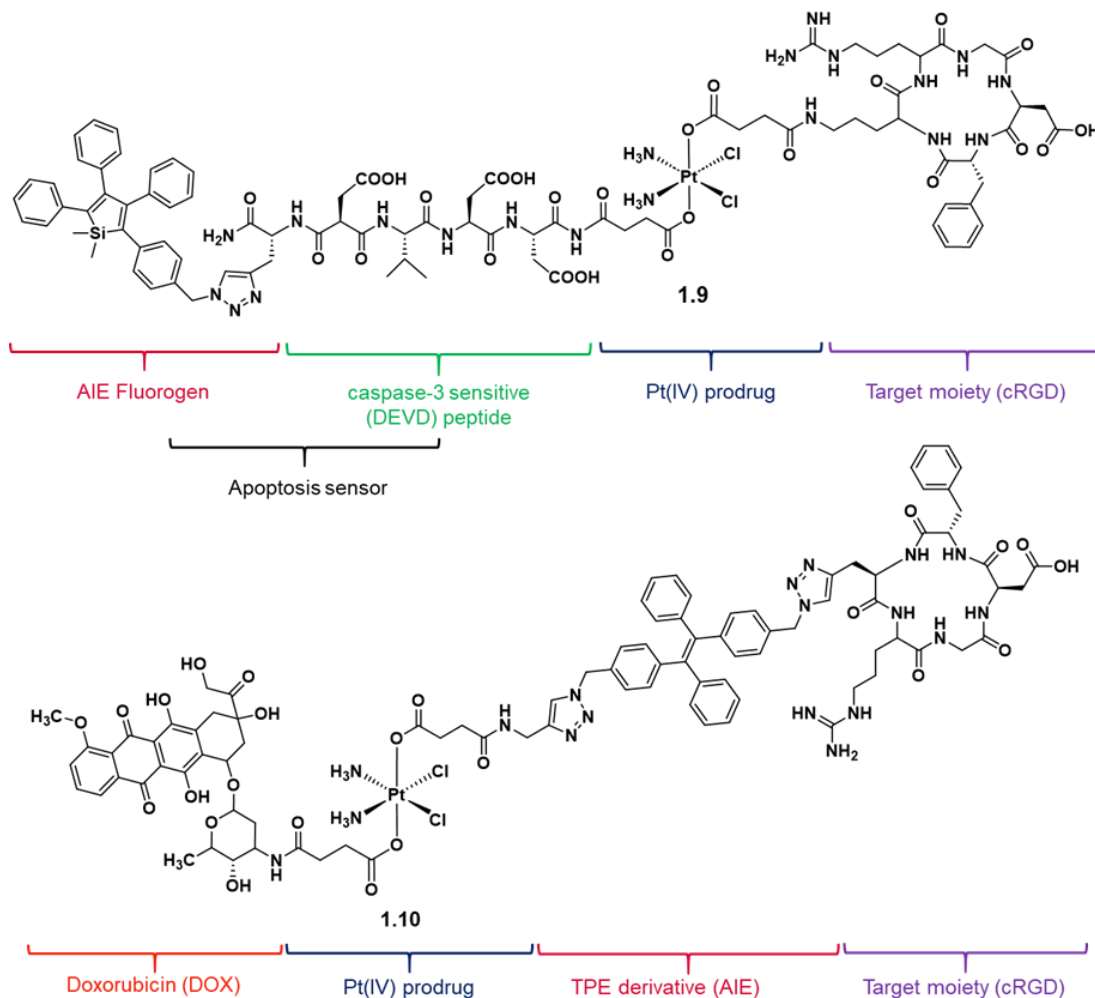


Figure 1.23. Structures of the targeted theranostic Pt(IV) prodrugs TPS-DEVD-Pt-cRGD (**1.9**) and Dox-Pt-TPE-cRGD (**1.10**).^{119,120}

1.6. Project aims

The aim of this research is to design and synthesise a set of fluorophore-labelled platinum(IV) prodrugs as fluorescent sensors to investigate the intracellular activation process of platinum(IV) complexes, attempting to better understand their mechanisms of action within cancer cells. The study will explore how different axial and equatorial ligands affect the stability, reduction behaviour, as well as the cytotoxic properties of these compounds. In addition, this project will examine the impact of copper metal ions, when combined with ascorbate, on the reduction of several fluorescent platinum(IV) probes. Finally, the development of novel platinum(IV) theranostic agents for dual-drug tracking applications will be investigated.

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Chapter 2 A photoactivatable platinum(IV) diazido anticancer complex functionalised with fluorescent dyes

2.1. Introduction

Photoactivatable d-block metal complexes have been extensively studied for application in photodynamic therapy (PDT) and more recently in photoactivated therapy (PACT) for cancer treatment. Anticancer phototherapy uses chemotherapeutic drugs which in response to light exposure can damage the DNA and promote cell death. To be effective, the photosensitizing agent should be non-toxic to cells in the dark, stable under biological conditions in the presence of reductants and be activated in a controlled manner upon photo-irradiation. While PDT involves a photosensitizer, light, and molecular oxygen to kill cancer cells, PACT is an emerging form of phototherapy that works *via* oxygen-independent pathways.^{1,2} This specific feature is of great interest for the treatment of malignant solid tumours characterised by hypoxic microenvironments (i.e., low oxygen contents) which are particularly hard to treat and prone to resist conventional treatments.

Metal-based photosensitizers containing Ru(II)³⁻⁷, Re(I)^{8,9,10}, Ir(III)^{11,12}, Pt(II)^{13,14} and Pt(IV)^{15,16} have shown great potential as photoactive anticancer drug candidates for PACT application. Ru(II) polypyridyl complexes have also been designed for dual PACT-PDT activity and have been implicated in the formation of reactive oxygen species, mostly singlet oxygen (¹O₂), and release of anticancer agents (active ligands or/and Ru(II) photoproducts) upon visible light activation¹⁷⁻²⁰, with some ruthenium compounds even able to retain photocytotoxic efficacy under hypoxic conditions in selected cancer cell lines.^{6,21}

Photosensitized Pt(II) derivatives of the clinically approved platinum(II) anticancer drugs have been explored to enhance the cytotoxic activity and reduce the side-effects of the chemotherapeutic agents.²² Luminescent cyclometalated platinum(II) complexes have also been reported for cellular imaging and have demonstrated selective phototoxicity towards

cancer cells.^{23,24,25} Unlike square-planar Pt(II) complexes, octahedral platinum(IV) prodrugs are kinetically less reactive which minimises off-target reactions with other biomolecules. They can be controllably reduced using different approaches such as ultrasound, the tumour microenvironment or light to release the active Pt(II) drugs at the tumour site.^{26,27} The first-generation of photoactive Pt(IV)-based drugs was developed by Bednarski and colleagues with the design of the diiodo-Pt(IV)-ethylenediamines series of the form *trans, cis*-[Pt(X)₂(en)I₂] (Figure 2.1, complexes **2.1-2.4**).^{15,28} Each platinum(IV) diiodo complex synthesised was based on the same Pt(II) scaffold, bearing two *cis* iodide groups and the ethylenediamine ‘en’ bidentate ligand. The chelating group helped maintain the *cis* configuration of the complexes since the *trans* analogue of cisplatin (namely transplatin) was shown to be less potent.²⁹ The prodrugs were only modified at the axial sites with various functional groups (chloride, acetate, hydroxide or methylsulfonate) to improve their dark stabilities against biological reductants (e.g., ascorbic acid and glutathione (GSH)). They depicted similar intense ligand-to-metal charge-transfer (LMCT) absorption bands centred around 400 nm and can be reduced upon exposure to light ($\lambda_{\text{irr}} > 375$ nm). Despite demonstrating good photocytotoxicity *via* DNA platination in cancer cells, no significant differences were found with their dark toxicity profiles.^{15,30} NMR experiments showed that the presence of biological reductants, especially thiols-containing molecules like GSH found in large concentrations in the cellular environment, promoted the rapid reduction from Pt(IV) to the toxic Pt(II) species, preventing their application as photochemotherapeutic agents.^{31,32}

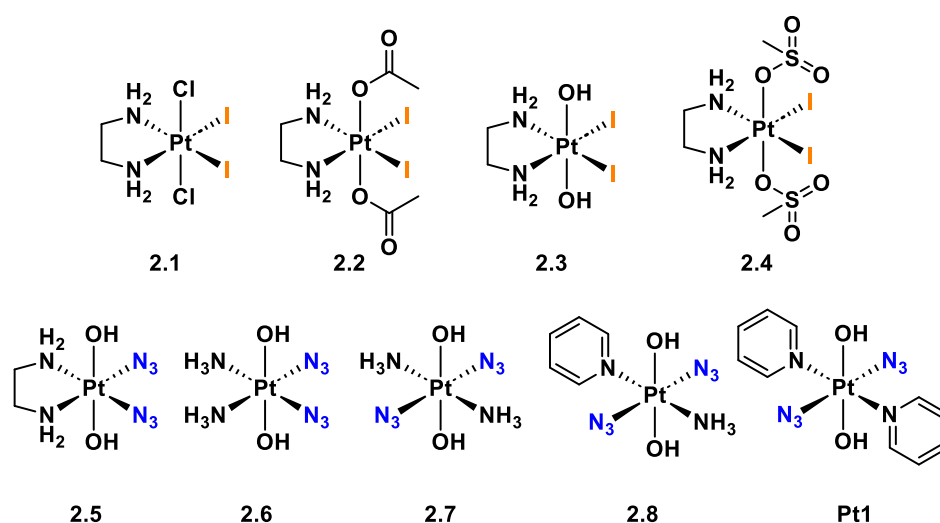


Figure 2.1. Photoactivatable diiodo and diazido platinum(IV) complexes.

The second-generation of photoactive platinum(IV) complexes was subsequently developed, changing the iodide groups with photolabile azido ligands. Sadler, Bednarski and co-workers designed two azido-based platinum(IV) anticancer complexes as derivatives of the diiodo Pt(IV) complex (**2.1**) and cisplatin: *cis, trans*-[Pt(en)(N₃)₂(OH)₂] (**2.5**) and *cis, trans, cis*-[Pt(N₃)₂(OH)₂(NH₃)₂] (**2.6**) (Figure 2.1).³³ The absorption profiles of complexes **2.5** and **2.6** were characterised by a strong ligand-to-metal charge-transfer (LMCT) band in the UV region (256 nm and 257 nm, respectively) associated to the azide to platinum (N₃ → Pt) transition with an absorption tail reaching to the visible region at approximately 460 nm. Upon irradiation in tumour cells with UVA light (365 nm), both complexes displayed good photocytotoxicity but failed to surpass cisplatin efficacy.³³ Biological studies on a series of *cis* and *trans* diazido Pt(IV) complexes revealed the higher cytotoxicity profile of the *trans* isomers.³⁴ Furthermore, the LMCT bands of *trans* platinum(IV) complexes were shifted to longer wavelengths (285 to 294 nm) in comparison to the *cis* isomers.¹⁵ Complex **2.7** exhibited no dark toxicity but demonstrated similar potency as cisplatin upon UVA light activation.³⁵ Attempting to increase the cytotoxicity of the *trans* diazido complexes, the ammine groups were replaced by pyridine ligands. Complexes **2.8** and **Pt1** displayed superior efficacy to that of cisplatin *via* a different mechanism of action involving *trans* platinum-guanine adducts. This was observed by NMR spectroscopy experiments using the mononucleotide 5'-GMP (guanosine 5'-monophosphate)

as a model for DNA binding studies. Upon irradiation with UVA light or visible blue light (420 nm), loss of the azido ligands as radicals and release of Pt(II) species able to coordinate to 5'-GMP were demonstrated.^{36,37} In addition, **Pt1** displayed high stability in aqueous solution over weeks and for days in the presence of reducing agents such as GSH.³⁷

The aim of this project was to develop two diazido-platinum(IV) carboxylate and carbamate fluorescent conjugates and investigate their photophysical, photochemical and biological properties as potential dual-action PACT/PDT chemotherapeutic agents. The photoactive *trans, trans, trans*-[Pt(py)₂(N₃)₂(OH)₂] complex (**Pt1**) presented above (Figure 2.1), and two green-emitting fluorescent probes were conjugated to red-shift the excitation wavelength of the precursor **Pt1**, optimising its clinical applications and enabling the visualisation of the platinum(IV) prodrugs reduction upon photo-irradiation. The resulting fluorescence “switch-on” response would also provide information on the cellular localisation of the designed Pt(IV) complexes when reduced. BODIPY and naphthalimide-based derivatives were chosen in this study due to the considerable number of synthetic methods available in the literature, which could later be applied to modify their emission properties to the near-infrared region, ideal for *in vivo* studies (Figure 2.2). They are also well-known photosensitisers able to generate reactive oxygen species which could enhance the photocytotoxicity efficacy of the diazido photochemotherapeutic agents.^{38,39} Lastly, naphthalimide derivatives have also been studied as anticancer agents as they can target and intercalate with the DNA.⁴⁰

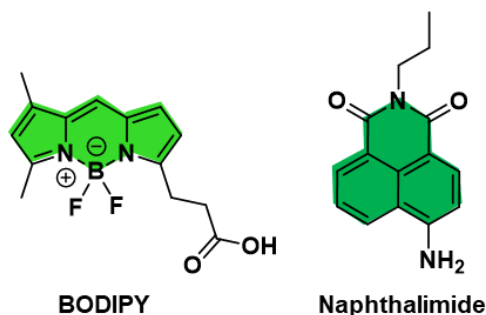


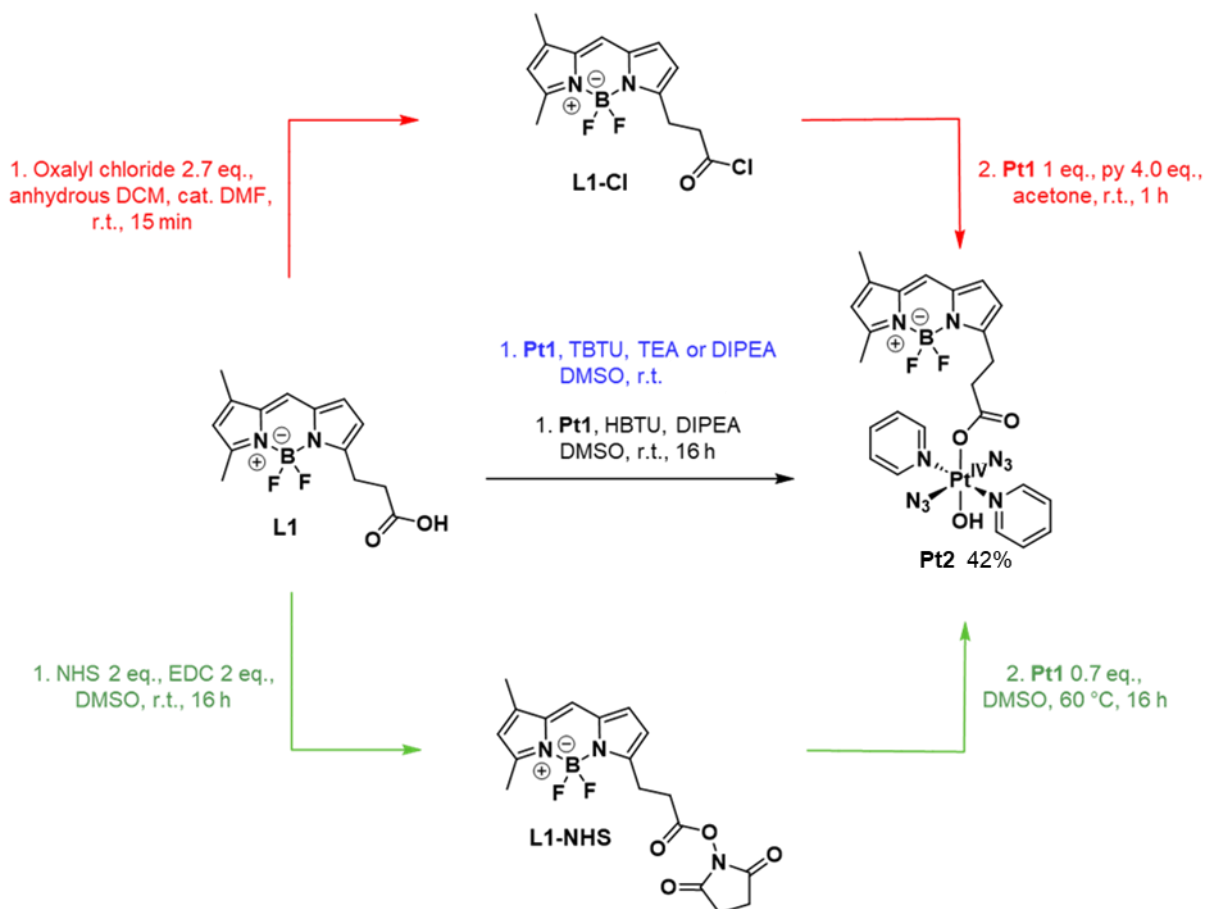
Figure 2.2. Fluorophores used in this study.^{41,42}

2.2. Diazido platinum(IV)-bodipy carboxylate conjugate

2.2.1. Synthetic approaches of Azido-BDP

Coupling strategies for the axial coordination of carboxylato ligands onto platinum(IV) complexes have been widely studied and reported in the literature.⁴³⁻⁴⁷ Depending on the synthetic approach, symmetric or asymmetric *mono*- and *bis*-functionalised compounds can be obtained. Several coupling procedures for the synthesis of the *mono*-substituted complex **Azido-BDP** (Complex **Pt2**) were initially investigated to maximise the reaction yield.

The reaction pathway between *trans, trans, trans*-[Pt(N₃)₂(OH)₂(py)₂] (Complex **Pt1**) and BODIPY-FL propionic acid (**L1**) to afford **Azido-BDP** is illustrated in Scheme 2.1. The BDP-FL ligand (**L1**) and **Pt1** were easily prepared following known literature procedures.^{37,41} Lee *et al.* reported a two-step synthesis for the axial functionalisation of a cisplatin-based platinum(IV) complex, which involved conversion of the carboxyl group into an acid chloride using oxalyl chloride.⁴⁸ Adapting this procedure (Scheme 2.1, red synthetic route), **L1** was treated with 2.7 equivalents of oxalyl chloride in anhydrous DCM and a catalytic amount of dimethylformamide to produce the corresponding acyl chloride. The reaction was completed under 15 minutes and the solvent completely dried. Toluene was used to wash the crude product and removed excess of oxalyl chloride. The activated carboxylate **L1-Cl** was immediately added dropwise to a stirring solution of **Pt1** mixed with pyridine in acetone to generate **Pt2** by rapid esterification. Unfortunately, this reaction resulted in the formation of a mixture of *mono*- and *bis*-substituted complexes among other byproducts that were difficult to separate and multiple purification steps by column chromatography were needed to isolate **Azido-BDP**.



Scheme 2.1. Synthetic strategies explored to obtain **Azido-BDP (Pt2)**. Adapted synthetic methods: red procedure reported by Lee et al.⁴⁸, green procedure reported by Wang et al.⁴⁹, blue procedure reported by Śmiłowicz and Metzler-Nolte⁵⁰, and black procedure reported by Ravera et al.⁵¹

Due to the challenging purification and extremely poor yield (<15 %) of this first method, an alternative procedure using *N*-hydroxysuccinimide (NHS) to activate **L1** was explored (Scheme 2.1, green pathway).⁴⁹ The succinimidyl BDP FL ester derivative of **L1** was prepared using NHS and carbodiimide hydrochloride (EDC) in DMSO at room temperature for 16 hours. Due to the relative stability of the formed active ester towards hydrolysis, purification and characterisation of the intermediate were this time possible. In a second step, BDP-FL NHS ester (**L1-NHS**) was reacted with **Pt1** in DMSO at 60 °C overnight. The rate of reaction was slower than when using the acid chloride method, due to the use of a weaker electrophile, and only formation of the *mono*-functionalised complex was observed with very few undesired side products. **Azido-BDP** was obtained in reasonable yield (51 %) after simple purification by column chromatography.

Because these procedures required two distinct steps, coupling reagents which can directly react and activate carboxylic acid groups in a one-pot synthesis were also investigated (Scheme 2.1, blue and black pathways).^{50,51} The benzotriazole-based uronium reagents *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) and *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) are known for their fast reaction rates, mild reaction conditions and few byproducts formation.⁴³ In each cases, various reaction conditions and equivalents of the activating agents and base (triethylamine (TEA) or *N,N*-diisopropylethylamine (DIPEA)) were studied and the reaction monitored by TLC and LC-MS. *In situ* activation of **L1** using TBTU and TEA was the first method attempted.⁵⁰ The desired complex **Pt2** was usually observed after 15-30 minutes. This procedure, however, seemed to produce an adduct as the major product and prevented the reaction to proceed. Formation of the same adduct was observed when DIPEA was reacted with TBTU but as a minor product of the reaction. Due to the substantial number of byproducts created using these conditions, isolation of **Azido-BDP** turned out to be quite arduous with low yield (< 25 %). The HBTU-based coupling reaction in combination with DIPEA was the last strategy explored.⁵¹ However, no adduct were detected with this method. The optimised reaction conditions used 2.5 equivalents of HBTU and 3.0 equivalents of DIPEA at room temperature, giving the final product in up to 62 % yield after purification by silica gel column chromatography.

2.2.2. Characterisation

Azido-BDP was fully characterised by ¹H, ¹¹B, ¹⁹F, ¹³C and ¹⁹⁵Pt NMR spectroscopy as well as high-resolution mass spectrometry. Crystals of **Pt2** were grown from a concentrated solution in methanol as dark-green crystals and analysed by Dr Kirsten E. Christensen of the University of Oxford. The crystal structure displayed in Figure 2.3 confirmed that **Pt2** was obtained as the *mono*-substituted complex. Single crystal X-ray crystallography of the precursor complex **Pt1** has been previously reported.³⁷ The angle and bond distances of the

azido ligands ($\text{Pt-N}_\alpha\text{-N}_\beta\text{-N}_\gamma$) were found to be alike between **Pt1** and **Pt2** with $175.8(4)/175.4(4)^\circ$ and $1.228(5)/1.223(6)$ Å ($\text{N}_\alpha\text{-N}_\beta$), $1.139(6)/1.142(5)$ Å ($\text{N}_\beta\text{-N}_\gamma$) for **Pt2** vs $175.7(4)/175.9(4)^\circ$ and $1.215(6)/1.218(5)$ Å ($\text{N}_\alpha\text{-N}_\beta$), $1.140(6)/1.146(5)$ Å ($\text{N}_\beta\text{-N}_\gamma$) for the precursor **Pt1**. A slightly shorter bond length is observed for the hydroxido ligand (Pt-OH) ($1.962(3)$ vs $1.990(3)$ Å) whilst the bond length value of the carboxylato ligand (Pt-OC) appears to be slightly longer ($2.045(3)$ vs $2.027(3)$ Å). The Pt-O-C and O-Pt-O angles values are noticeably more acute compared to the previous Pt-O-H ($125.3(3)^\circ$ vs 144.2°) and O-Pt-O ($174.98(10)^\circ$ vs 180°) angles and are similar with those previously reported for diazido-Pt(IV) monocarboxylato complexes.⁵² (See Appendix A.1 for further data).

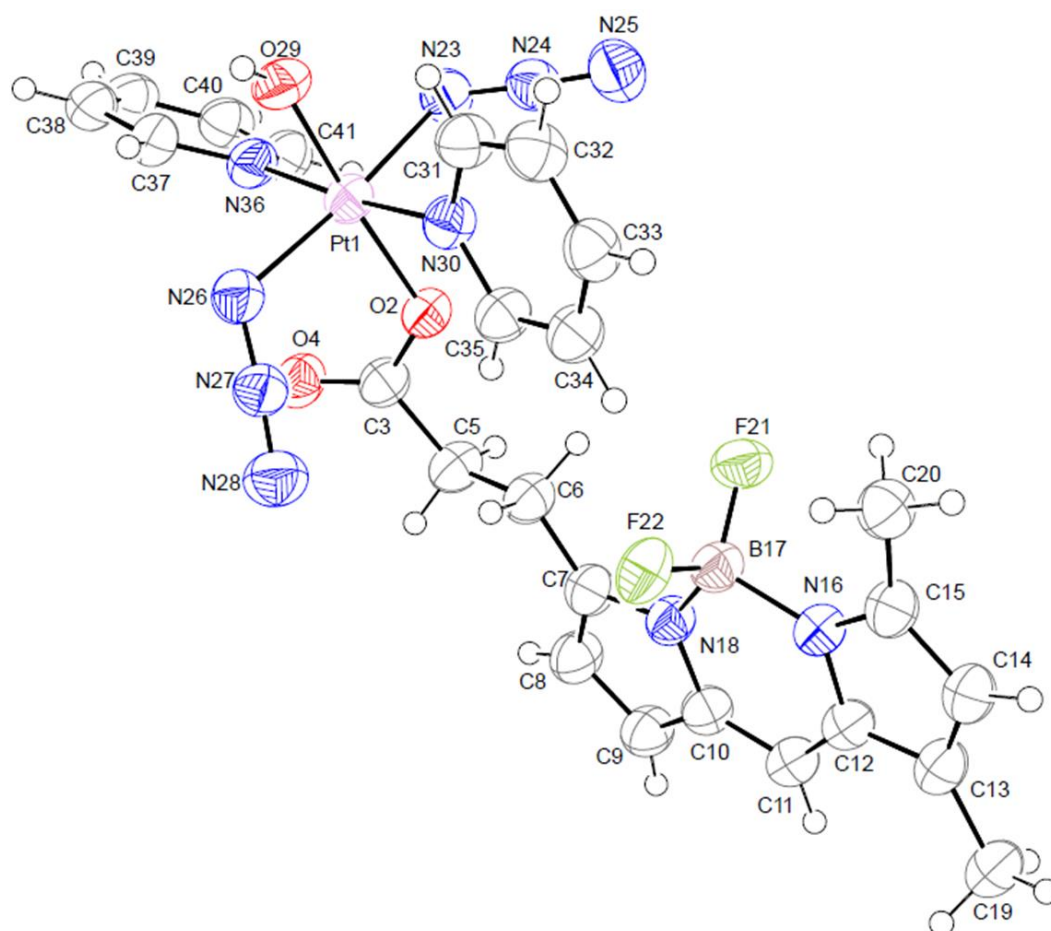


Figure 2.3. Molecular structure of **Azido-BDP (Pt2)** determined by X-ray diffraction showing thermal ellipsoids at 50% probability. Pt-azido group naming convention: $\text{N}_{23/26} = \text{N}_\alpha$, $\text{N}_{24/27} = \text{N}_\beta$ and $\text{N}_{25/28} = \text{N}_\gamma$.

Figure 2.4 and Figure 2.5 illustrate the assigned ^1H NMR and ^{195}Pt NMR spectra of **Pt2** in deuterated chloroform. All resonances in the ^1H NMR spectrum of **Azido-BDP** are relatively similar to the chemical shifts observed for **Pt1** and **L1**. The signal at 8.93 ppm corresponds to the four protons in the *ortho* positions of the pyridine ligands and is easily recognizable due to the characteristic sharp ^{195}Pt satellites. A chemical shift at 961 ppm is observed in the ^{195}Pt NMR spectrum of **Pt2**, downfield compared to the **Pt1** precursor (857 ppm in deuterated methanol for **Pt1**) due to the coordination of **L1**, resulting in the decrease in electron density around the platinum centre.

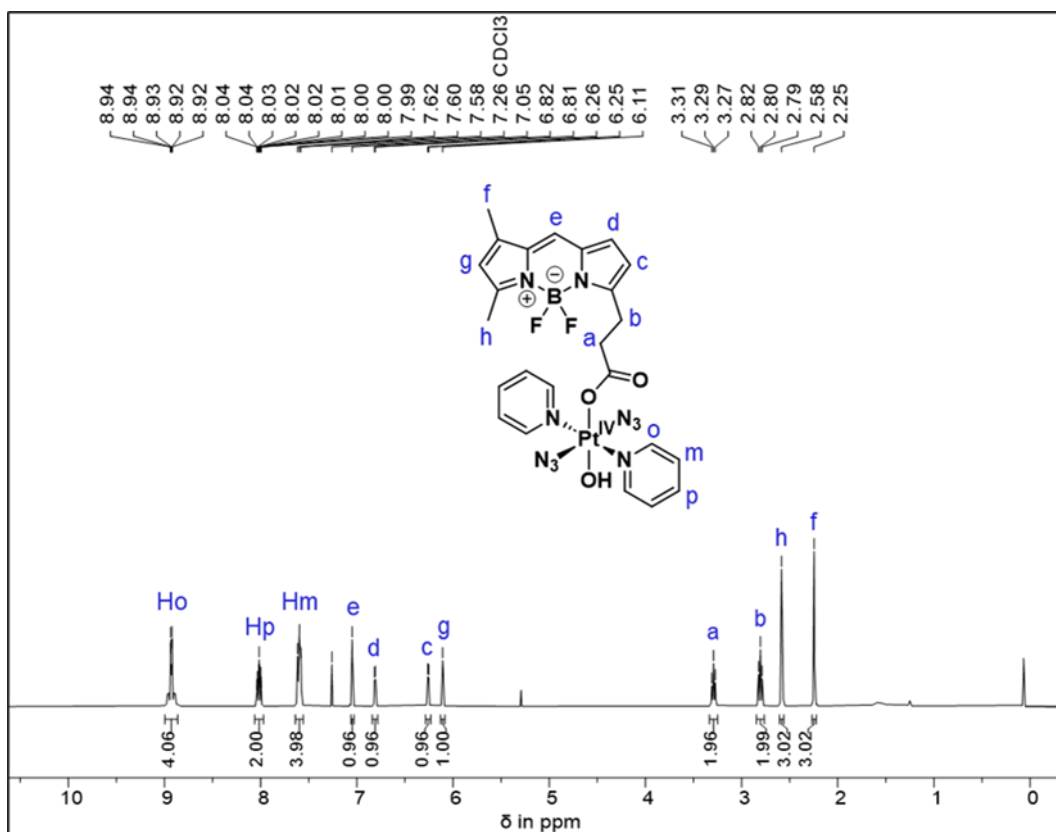


Figure 2.4. ^1H NMR (400 MHz, CDCl_3) of **Azido-BDP (Pt2)**.

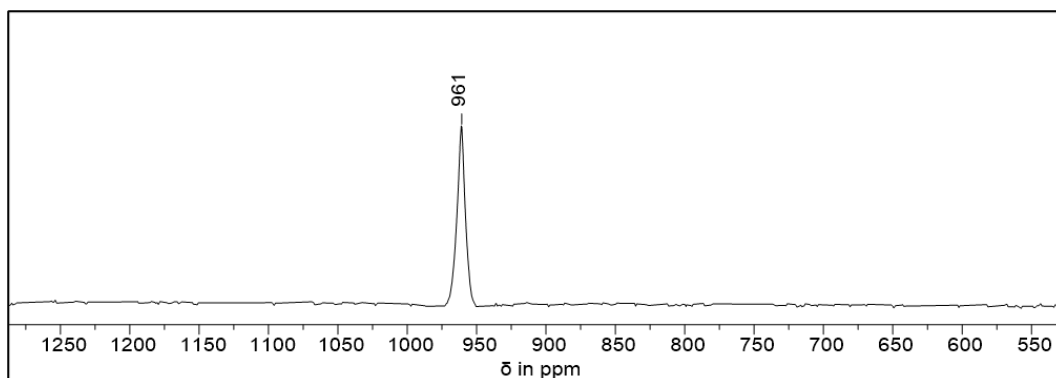


Figure 2.5. ^{195}Pt NMR (86 MHz, CDCl_3) of **Azido-BDP (Pt2)**.

The absorption spectrum of **L1** displays a shoulder in the region of 300–400 nm and a main absorption band at 504 nm which corresponds to the low-energy $S_0 \rightarrow S_1$ transition, characteristic of the BODIPY dye (Figure 2.6a). A similar absorption maximum at 505 nm is observed for **Pt2**, noticeably diminished compared to the intense absorption of the free ligand. The absorbance of the ligand-to-metal charge-transfer (LMCT) transition band $\text{N}_3 \rightarrow \text{Pt}$ is present in the UV region (around 300 nm) following a high-energy band at 220 nm. The fluorescence intensity of **Pt2** appears to be considerably lower compared to **L1**, likely to be quenched due to the heavy-metal effect, favouring intersystem crossing (Figure 2.6b).

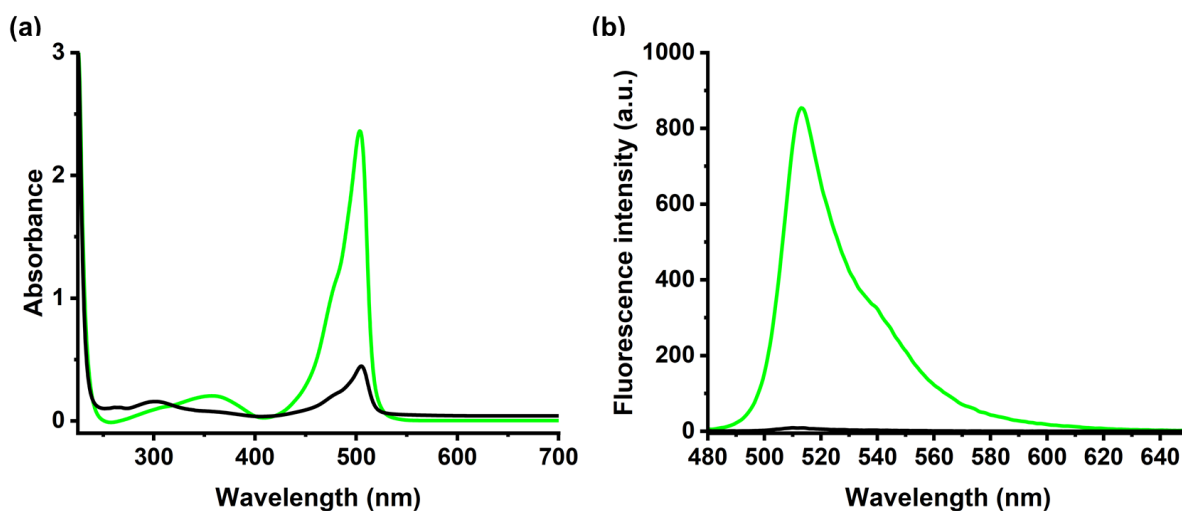


Figure 2.6. (a) Absorbance spectra of BDP-FL (green, 15 μM) and **Azido-BDP** (black, 15 μM) in PBS buffer (pH = 7.40). (b) Fluorescence intensity of BDP-FL (green, 0.2 μM) and **Azido-BDP** (black, 0.2 μM) in PBS buffer (pH = 7.40), λ_{ex} = 400 nm, slit widths: 10 nm and 2.5 nm.

2.2.3 Analytical studies

2.2.3.1. Photophysical properties and stability

A carboplatin-based platinum(IV) bodipy derivative of complex **1.6** presented in Chapter 1 and designed by Yao *et al.* was shown to be stable in the dark and in the presence of sodium ascorbate (NaAsc, 1 mM) for 24 hours but could be rapidly activated by green light ($\lambda_{\text{max}} = 495$ nm). A cisplatin analogue was also reported and proved to be unstable in the dark and completely reduced under 6 hours after treatment with NaAsc.⁵³ Based on these reports, we initially studied the stability and photophysical properties of our diazido-Pt(IV) bodipy complex in the dark with and without the reducing agent. **Azido-BDP** was found to be less soluble in organic solvents (methanol and acetonitrile) compared to the precursor **Pt1**. Therefore, the conjugate was first diluted in a small amount of DMSO before being added to a solution of PBS buffer or deionised H₂O (<1 % DMSO) in the following experiments. As displayed in Figure 2.7, no differences in the absorption **(a)** and minimal changes in the fluorescence emission intensity of **Pt2 (b)** were recorded after two hours in the absence of light.

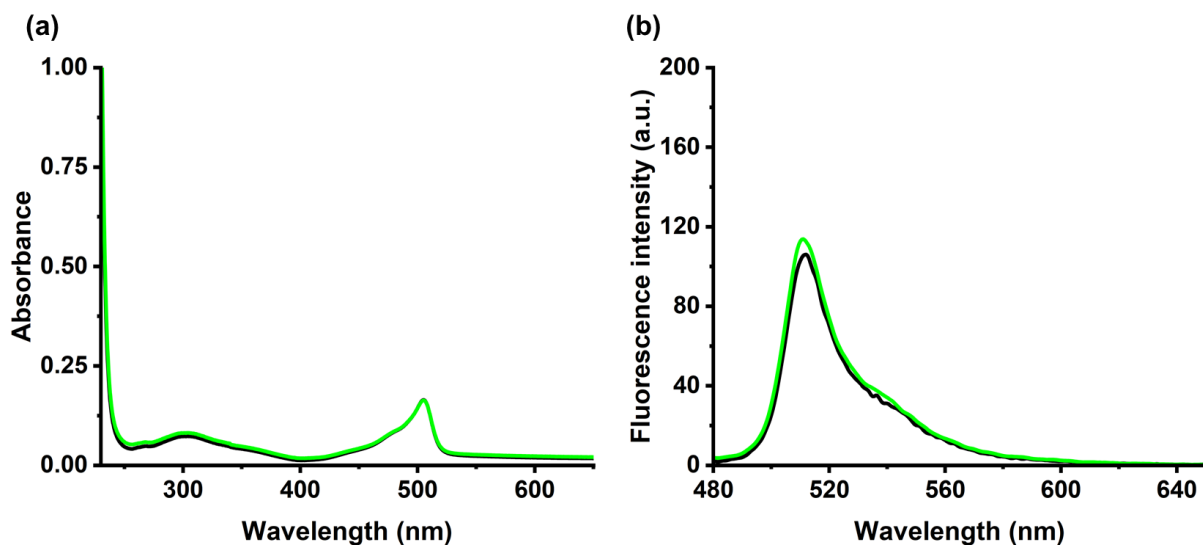


Figure 2.7. **(a)** Absorbance spectra of **Azido-BDP** (15 μM) in PBS buffer (pH = 7.40) in the dark (2 h incubation). **(b)** Fluorescence intensity of **Azido-BDP** (5 μM) in PBS buffer (pH = 7.40) (2 h incubation), $\lambda_{\text{ex}} = 400$ nm, slit widths: ex = 10 nm, em = 2.5 nm.

Incubation of **Azido-BDP** with 4 millimolar of sodium ascorbate was monitored by UV-vis spectroscopy (Figure 2.8). After one hour, formation of bodipy was observed as the spectrum depicted a new and clear absorption wavelength between 300-400 nm corresponding to the free ligand **L1**, as seen previously in Figure 2.6. Similarly, an increase in fluorescence emission intensity at 511 nm was witnessed after treatment with 1 mM of NaAsc (Figure 2.9). In contrast to the BODI-Pt complex reported by Zhu's group, our **Azido-BDP** complex appears to be reduced and releasing the fluorophore rapidly in the presence of NaAsc, in a similar manner to their cisplatin-bodipy conjugate.⁵³

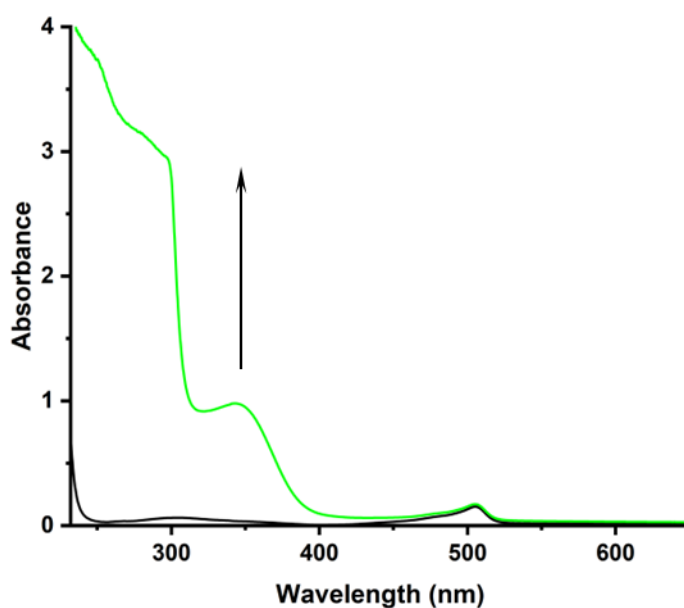


Figure 2.8. Absorbance spectra of **Azido-BDP** (15 μ M) in PBS buffer (pH = 7.40) before (**black**) and after (**green**) addition of NaAsc (4 mM). The second measurement was recorded after 60 minutes incubation.

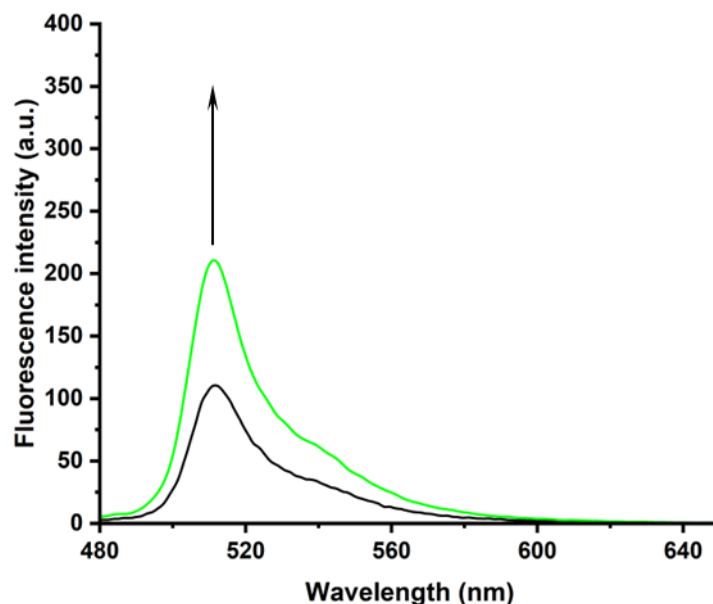


Figure 2.9. Fluorescence spectra of **Azido-BDP** (5 μ M) with 1 mM of NaAsc (1 h incubation) in PBS buffer (pH = 7.40), λ_{ex} = 450 nm, slit widths: ex = 10 nm, em = 2.5 nm.

Longer incubation times were used to evaluate the stability of the complex in the dark and monitored by fluorescence emission spectroscopy. As illustrated in Figure 2.10, a distinct fluorescence response (3.5-fold) was observed for **Azido-BDP** upon treatment with NaAsc after 8 hours. However, in the absence of the reducing agent, a slow but constant increase in fluorescence intensity was also recorded, suggesting release of the axial ligand **L2** overtime and therefore instability of **Pt2** in aqueous solution.

Azido-BDP was incubated in deionised H₂O and analysed by LC-MS which confirmed our previous observations. The free fluorophore, complex **Pt1** and the Pt(II) diazido precursor *trans, trans*-[Pt(N₃)₂(py)₂] were detected upon reinjection of **Azido-BDP** after 3 hours (Figure 2.11). The presence of **L1** and Pt(II) and (IV) species are indicative of the poor stability of the complex towards hydrolysis with time.^{54,55} Thus, due to the low aqueous stability of **Azido-BDP** in the dark and its fast reduction in the presence of NaAsc, the complex was judged unsuitable for *in vitro* imaging studies and as a selective photoactivatable prodrug.

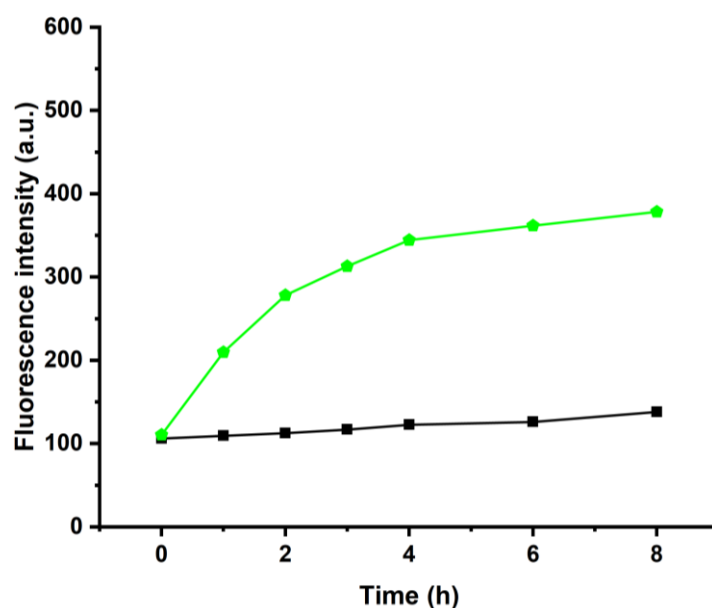


Figure 2.10. Changes in the fluorescence intensity of **Azido-BDP** ($5\ \mu\text{M}$) at $512\ \text{nm}$ with (**green**) and without (**black**) the addition of NaAsc ($1\ \text{mM}$) in PBS buffer ($\text{pH} = 7.40$), $\lambda_{\text{ex}} = 450\ \text{nm}$, slit widths: $\text{ex} = 10\ \text{nm}$, $\text{em} = 2.5\ \text{nm}$.

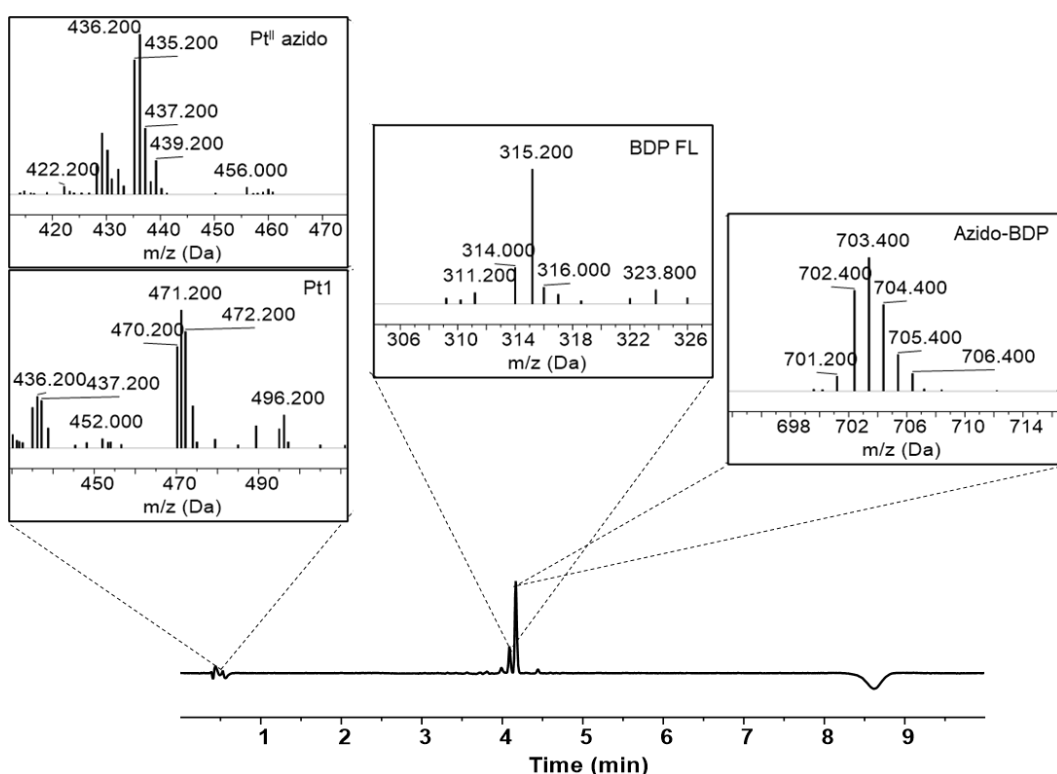


Figure 2.11. Reinjection of **Azido-BDP** (**Pt2**, $50\ \mu\text{M}$) incubated in deionised H_2O after 3 hours recorded by LC-MS (Absorption at $450\ \text{nm}$).

2.3. Diazido platinum(IV)-naphthalimide carbamate conjugate

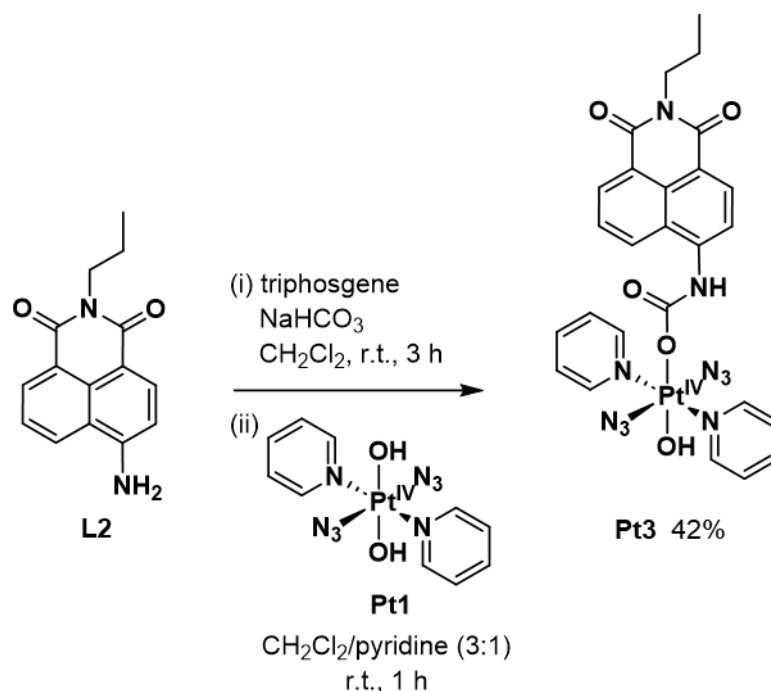
2.3.1. Synthesis of AzidoNap

In contrast to the vast number of synthetic methods described for the functionalisation of platinum(IV) complexes with carboxylato, hydroxido and chlorido axial ligands, few procedures for the preparation of Pt(IV) carbamate complexes have been reported. The first examples found in the literature used purchased alkyl or aryl isocyanates to produce symmetric *bis*-substituted cisplatin(IV) carbamates.^{56,57} In 2020, Gibson and co-workers presented two new strategies for the direct coordination of amine-containing ligands which involved the coupling reagent *N,N'*-Disuccinimidyl carbonate (DSC) to generate mono- and dicarbamate complexes.⁵⁸ The first method required activation of the axial hydroxyl group of the cisplatin-based platinum(IV) complex by DSC to create an electrophilic intermediate able to react with the amino group of the ligand. In the second procedure, the amine was initially treated with the coupling reagent before reacting with the Pt(IV) complex *via* the axial OH ligand to obtain the carbamates. Several complexes were synthesised using both protocols, however, the first approach enabled the synthesis of the desired carbamates in the highest yields and easiest reaction conditions with few side-products formation.⁵⁸

In this section, we explored the synthesis of a monocarbamate diazido-Pt(IV) complex carrying a naphthalimide-based fluorophore (**L2**) (Scheme 2.2). Known literature methods were used to synthesise **L2**.⁴² We first attempted to prepare the complex **AzidoNap** (Complex **Pt3**) based on the first synthetic approach developed by Babu *et al.*⁵⁸ To this end, **Pt1** was treated with DSC in acetonitrile and stirred at room temperature. After 3 hours, the activated carbonate complex was purified by centrifugation and washed twice with diethyl ether to obtain a bright yellow solid. Characterisation of the intermediate by mass spectrometry and NMR spectroscopy confirmed the activation of the platinum(IV) complex with a new chemical shift at 1034 ppm observed in the ¹⁹⁵Pt NMR spectrum. Unfortunately, the second step did not yield the desired product, even when attempting to optimise the reaction conditions. This may

be due to the low nucleophilicity of the amino group and the higher steric hindrance of the naphthalimide ligand. The method reported involved coordination of primary, secondary aliphatic and anilinic amines. In general, the use of aromatic amines gave rise to the lowest yields and longer reaction times were needed to yield the carbamates.⁵⁸

A wide number of literature protocols exist in organic chemistry for the activation of various functional groups including alcohols, carboxylic acids, and amines. For instance, triphosgene has been studied to transform amino groups into more reactive species, such as carbamoyl chloride and isocyanates.⁵⁹ Following a slightly adapted procedure by Sabatino *et al.*, **L2** was reacted with triphosgene and sodium bicarbonate in dichloromethane at room temperature and monitored by TLC.⁶⁰ Conversion of the amine moiety to a carbamoyl chloride intermediate was achieved after 3 hours. The reaction was concentrated to eliminate the volatiles and subsequently redissolved in dichloromethane. Due to the low solubility of **Pt1** in chlorinated solvents, multiple solvents systems and reactions conditions were investigated in the second step. The use of acetonitrile, methanol and dimethylformamide with triethylamine and 4-(dimethylamino)pyridine (DMAP) were explored. However, the presence of DCM was required to avoid transformation of the reactive intermediate back to the amino group. Combination of dichloromethane with MeCN, MeOH and DMF in the following step did not generate the targeted product. Addition of a small amount of pyridine, however, not only helped solubilise **Pt1** but also permitted the reaction between the intermediate and the platinum(IV) complex to go forward. The carbamoyl chloride species in DCM were slowly added dropwise to a mixture of **Pt1** in dichloromethane and pyridine in a 3:1 ratio at 0°C and the reaction was completed under 60 minutes. **AzidoNap** was isolated by column chromatography in moderate yield (42 %).



Scheme 2.2. Synthesis of **AzidoNap** (**Pt3**).

2.3.2. Characterisation

Characterisation of **AzidoNap** was achieved by high-resolution mass spectrometry and ¹H NMR, ¹³C NMR and ¹⁹⁵Pt NMR spectroscopy in DMSO-d₆. For comparison, the ¹H NMR spectra of the synthesised fluorophore **L2** and **AzidoNap** are shown in Figure 2.12 and Figure 2.13, respectively. The resonance at 7.41 ppm assigned to the free amine of **L2** is absent in **AzidoNap** spectrum whilst a new chemical shift is observed at 9.50 ppm for the proton NH of the carbamate bond. Both observations are indicative of the formation of the carbamate linker. All protons of the pyridine ligands are accounted for, but the platinum satellites surrounding the doublet of the *ortho* protons are not exhibiting the sharp pattern expected for a Pt(IV)-diazido complex and observed previously for **Azido-BDP**. This might be due to the choice of deuterated solvent (DMSO-d₆ vs CDCl₃) used to record the spectrum. Interestingly, the resonance of proton 'a' is now downfield to the resonance of proton 'd', reversed in the free ligand ¹H NMR spectrum. Proton 'a' appears to be less shielded in **AzidoNap**, due to its proximity to the carbamate linkage.

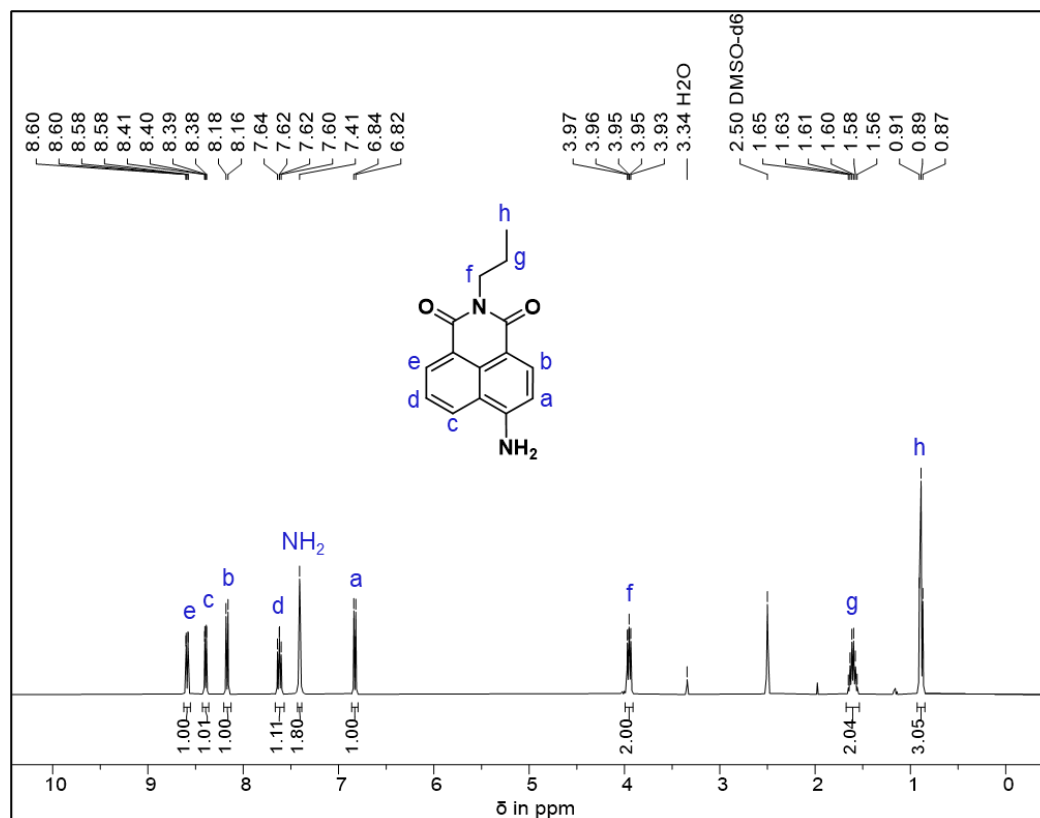


Figure 2.12. ¹H NMR (400 MHz, DMSO-d₆) of 6-amino-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (4-NH₂-Nap, L2).

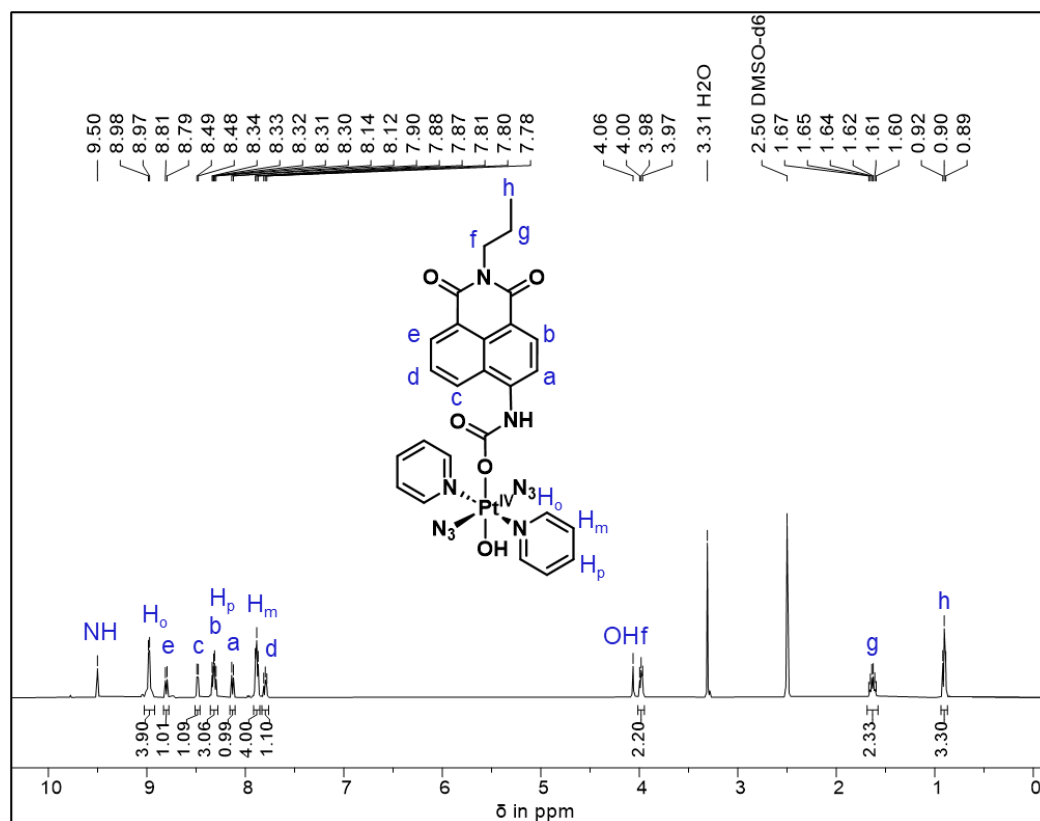


Figure 2.13. ¹H NMR (500 MHz, DMSO-d₆) of AzidoNap (Pt3).

The ^{195}Pt NMR spectrum of **AzidoNap** is presented in Figure 2.14 and displays a single chemical shift at 1011 ppm, in the same Pt(IV) region as **Azido-BDP**.

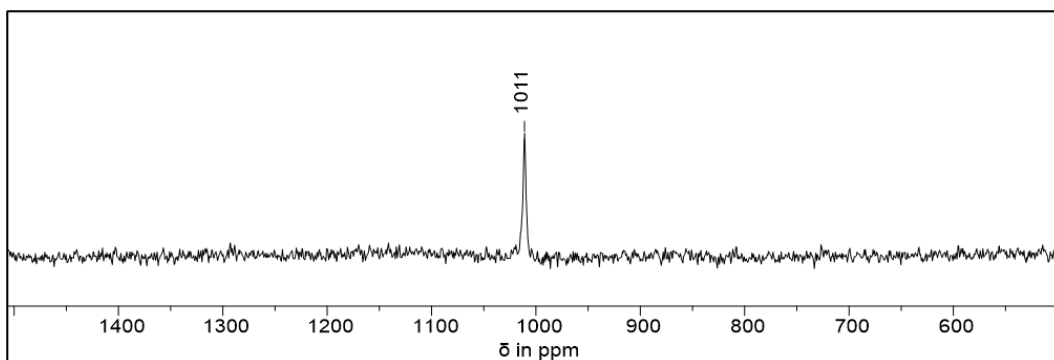


Figure 2.14. ^{195}Pt NMR (107 MHz, DMSO-d_6) of **AzidoNap** (**Pt3**).

UV-vis spectroscopy of **L2** exhibits an intense absorption band at 256 nm with a maximum wavelength absorption (λ_{max}) at 433 nm whilst the main absorption of **Pt3** was blue shifted to 372 nm (Figure 2.15a). **AzidoNap** spectrum also displays a high energy band at 215 nm followed by a shoulder at 300 nm corresponding to the $\text{N}_3 \rightarrow \text{Pt}$ LMCT transition band. These observations are in agreement with previously reported absorption profiles of diazido-Pt(IV) carboxylate complexes containing naphthalimide-based ligands.⁵² The fluorescence intensity of **AzidoNap** is significantly reduced compared to the free ligand **L2**, due to the heavy-metal effect (same as **Azido-BDP**) (Figure 2.15b).

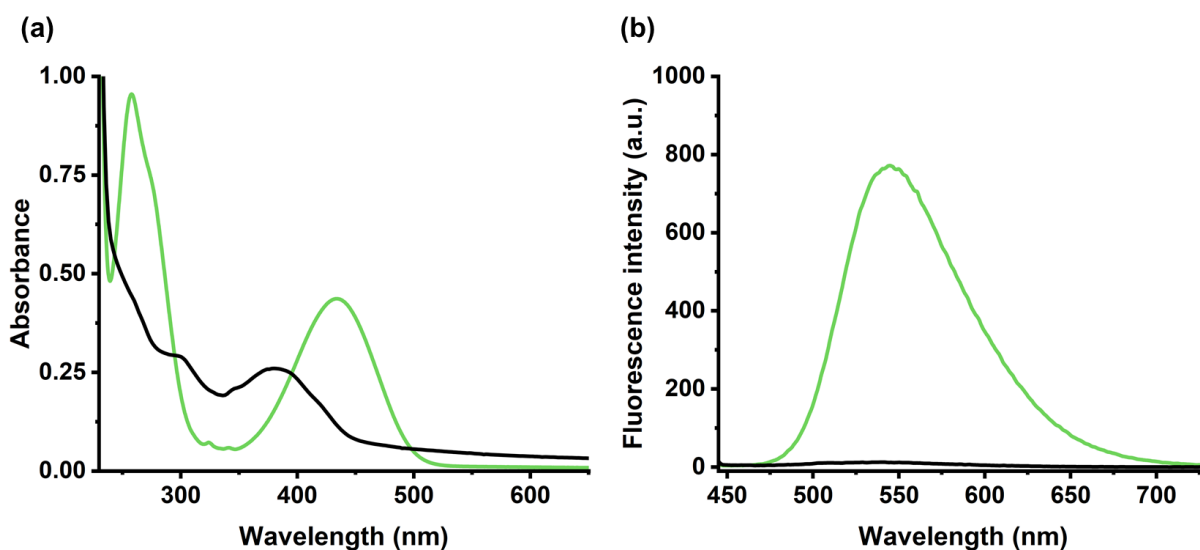


Figure 2.15. (a) Absorbance spectra of **Nap-NH₂** (green, 5 μ M) and **AzidoNap** (black, 5 μ M) in PBS buffer (pH = 7.40). (b) Fluorescence intensity of **Nap-NH₂** (green, 0.6 μ M) and **AzidoNap** (black, 0.6 μ M) in PBS buffer (pH = 7.40), λ_{ex} = 430 nm, slit widths: 10 nm and 5 nm.

2.3.3. Analytical studies

2.3.3.1. Photophysical properties and stability

A stability study of **AzidoNap** by UV-vis and fluorescence spectroscopy was undertaken and compared to the results obtained for the platinum(IV) monocarboxylato complex **Azido-BDP**. Surprisingly, a significant decrease was observed in the absorption emission of **AzidoNap** after 2 hours (in the dark), suggesting that the complex is not stable in aqueous solution and undergoes rapid hydrolysis (Figure 2.16).⁶¹ Furthermore, the fluorescence emission spectra displayed a small increase in fluorescence intensity at 545 nm over the same period of time, which points to the loss of the axial ligand **L2**. These experiments were repeated three times with freshly prepared samples to ensure consistency of the results.

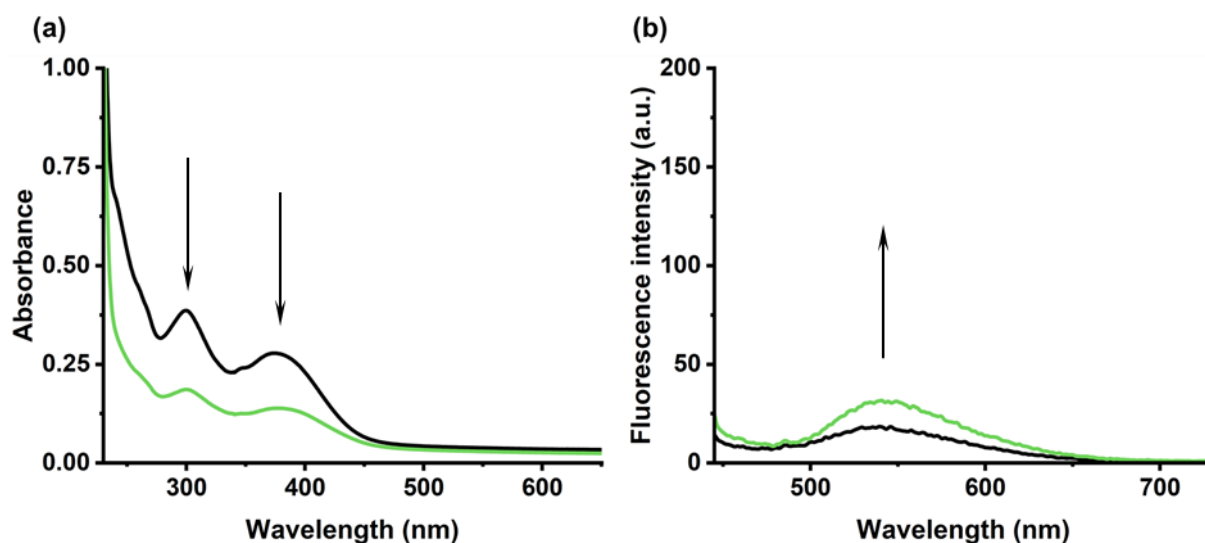


Figure 2.16. **(a)** Absorbance spectra of **AzidoNap** (15 μM) in PBS buffer (pH = 7.40) in the dark (2 h incubation). **(b)** Fluorescence intensity of **AzidoNap** (5 μM) in PBS buffer (pH = 7.40) (2 h incubation), $\lambda_{\text{ex}} = 430 \text{ nm}$, slit widths: slit widths: ex = 10 nm, em = 3.5 nm.

Based on these observations, the stability of the complex was studied by fluorescence spectroscopy in aqueous solution over a period of 8 hours (Figure 2.17). In a comparable way to **Azido-BDP**, a small fluorescence increase was observed when **AzidoNap** was left standing in the dark. In addition, incubation of **Pt3** with 1 mM of sodium ascorbate in the absence of light led to a fast increase in fluorescence intensity. **AzidoNap** appears to be reduced more rapidly than **Azido-BDP** in the presence of the reducing agent. In fact, previous work by Zhu, and others, have showed that carbamate complexes tend to be reduced more quickly than their carboxylate counterparts.^{55,57,58} LC-MS analysis of the sample after 2 hours of incubation in dH₂O showed slow decomposition of the complex with presence of the naphthalimide fluorophore **L2** and the *trans*-diazido Pt(IV) precursor **Pt1** (Figure 2.18). The complex exhibits similar aqueous hydrolysis as **Azido-BDP**, but to a slightly more rapid extent. One strategy to improve the overall stability of both complexes may involve replacing the axial OH ligand with a carboxylate substituent.^{58,62}

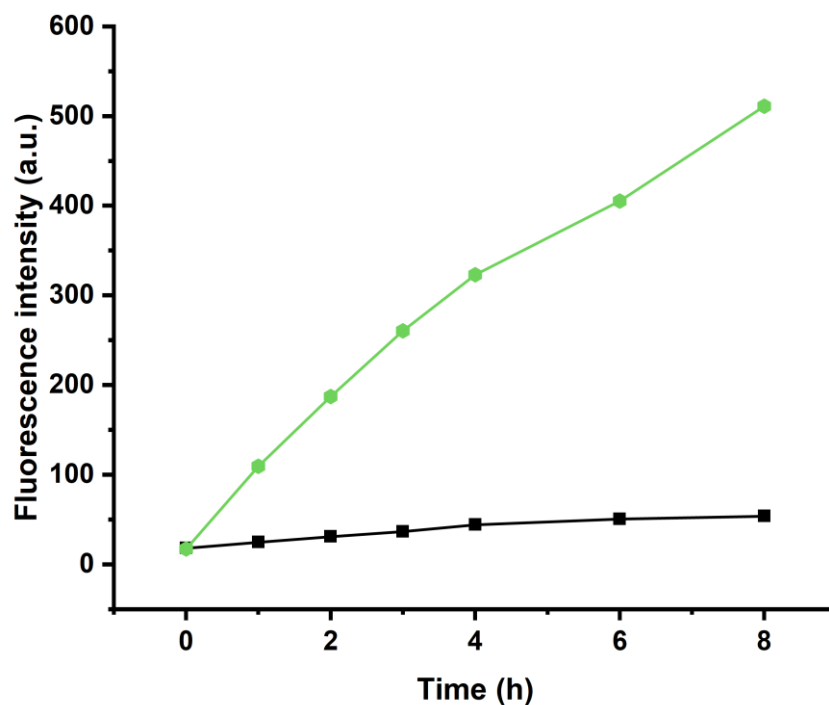


Figure 2.17. Changes in the fluorescence intensity of **AzidoNap** (5 μ M) at 430 nm with (**green**) and without (**black**) the addition of NaAsc (1 mM) in PBS buffer (pH = 7.40), λ_{ex} = 430 nm, slit widths: ex = 10 nm, em = 3.5 nm.

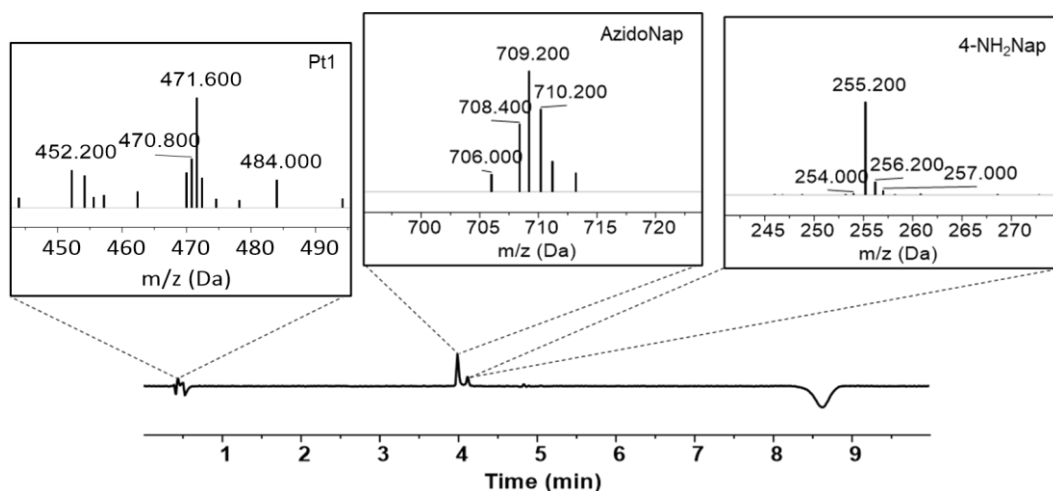
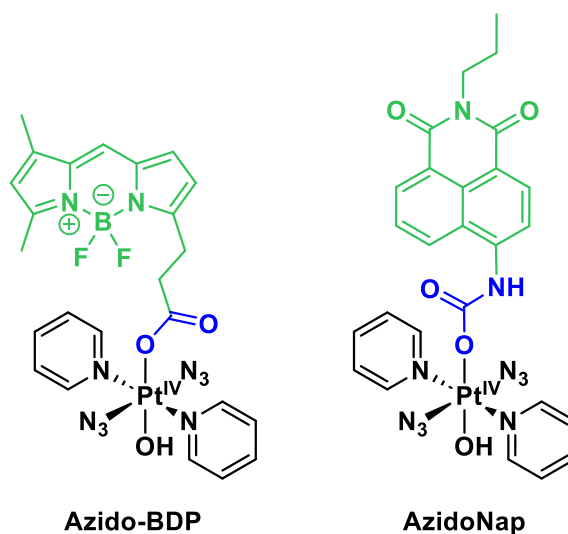


Figure 2.18. Reinjection of **AzidoNap** (**Pt3**, 50 μ M) incubated in deionised H_2O for 2 hours recorded by LC-MS (Absorption at 450 nm).

2.4. Conclusion

In this chapter, several methods for the axial functionalisation of the photoactive *trans*, *trans*, *trans*-[Pt(N₃)₂(OH)₂(py)₂] (**Pt1**) anticancer complex with two fluorophores: a boron dipyrromethene (BODIPY) dye and a naphthalimide-based derivative were investigated. Two novel diazido-Pt(IV) complexes bearing carboxylate and carbamate ligands, **Azido-BDP** (**Pt2**) and **AzidoNap** (**Pt3**), were prepared and fully characterised by NMR spectroscopy, mass spectrometry, UV-vis and fluorescence spectroscopies. The platinum(IV)-bodipy conjugate (**Pt2**) was structurally characterised using X-ray crystallography which confirmed synthesis of the desired monocarboxylato complex. A new synthetic strategy for conjugating amine-containing ligands to Pt(IV) complexes *via* a carbamate linkage was developed. Preliminary studies on the photophysical properties and stability of **Azido-BDP** and **AzidoNap** in phosphate buffered solution were conducted using fluorescence and UV-vis absorption spectroscopies. Unfortunately, the complexes demonstrated poor hydrolytic stability in the dark with time and further experiments on their photophysical and photoactivation properties could not be undertaken. Future work presented in this thesis will focus on the FDA-approved Pt(II) drugs and use the diazido-Pt(IV) complex as a synthetic model to investigate the coordination of different amine-containing molecules onto Pt(IV) complexes.



2.5. References

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Chapter 3 The study of platinum(IV) reduction: Oxali(IV)Fluors

3.1. Introduction

Platinum-based antineoplastic agents, cisplatin, carboplatin and oxaliplatin, are routinely applied in chemotherapy for the treatment of several types of malignancies. The clinical application of these platinum(II) drugs is however restricted due to high systemic toxicities, intrinsic or acquired tumour resistance and devastating side-effects caused by the lack of selectivity to cancerous cells of the chemotherapeutic agents.^{1,2} The development of platinum(IV) prodrugs has become an attractive strategy to overcome the dose-limiting toxicities and minimise the side-effects associated with the well-known platinum(II) anticancer drugs. A wide variety of Pt(IV) complexes have already been developed and have displayed promising results (overcome resistance, high tumour selectivity, low off-target toxicity...) but have failed to demonstrate superior therapeutic efficacy.^{3,4,5} To help explain the poor therapeutic outcome observed, gaining further insights into the mechanisms of action of Pt(IV) prodrugs is therefore crucial and may facilitate the design of efficient platinum(IV)-based therapy.

Platinum(IV) prodrugs are described as relatively inert complexes in comparison to their Pt(II) forms which prevents their unexpected reaction with other components of the bloodstream once administered. They often require the presence of bioreductants such as sodium ascorbate, cysteine or glutathione to promote their reduction to the corresponding toxic Pt(II) agent and release the two axial ligands in cancer cells.⁶⁻⁹ The choice of axial ligands is important as they play major roles in the rate of reduction, lipophilicity, and solubility of the platinum(IV) complexes and can also be used to enhance their cytotoxic and selectivity profiles (addition of antitumour drugs and/or targeting units).^{9,10,11} Different methods of analysis are currently exploited to obtain a deeper understanding of the mode of action of platinum(IV) prodrugs. Analytical techniques (e.g., XANES, NanoSIMS and LA-ICP-MS) have provided

valuable information on the spatial distribution and accumulation of Pt species in cell culture and tumour samples but cannot be used to visualise the fate of platinum drugs in a real-time manner.^{12,13,14} High performance liquid chromatography (HPLC) is the main solution-based analytical technique used to monitor the reduction process of Pt(IV) complexes when exposed to reducing agents but cannot be applied to live-cell imaging.^{15,16} One strategy to bypass these limitations, which restricts our comprehension of Pt(IV) prodrugs activation, is to use fluorescence microscopy to directly observe changes in biological systems.

As discussed in *Chapter 1*, fluorescence-based sensors have been designed to study the activation of platinum(IV) prodrugs within a cellular environment. The platinum probes were able to differentiate between Pt(II) and Pt(IV) species as no fluorescence was observed in the presence of Pt(IV) complexes. The probes could, however, detect Pt(II) compounds such as cisplatin in aqueous solution and in cells as an “off-on” fluorescence response was produced following reduction.¹⁷⁻²⁰ Platinum(IV) complexes bearing fluorescent dyes have also been reported. Once coordinated to the platinum centre at the equatorial or axial site, the fluorescence emission of the fluorophore is quenched to generate non-fluorescent conjugates. Reduction of the Pt(IV) to Pt(II) products leads to a fluorescence “turn-on” response, making it possible to track the intracellular activation process of these compounds.²¹⁻²⁴ Despite being extensively investigated, the reduction of Pt(IV) prodrugs in biological environments is still not well understood and additional information on the factors involved in their activation are needed to develop more effective treatments.^{6,9}

In this chapter, we focused our attention on oxaliplatin-based platinum(IV) (OxPt(IV)) complexes and examined the reduction mechanism of these OxPt(IV) prodrugs in cancer cells using fluorescence imaging. To this end, we aimed to develop two fluorescent OxPt(IV) conjugates, also referred to as Oxali(IV)Fluors, *via* distinct sensing strategies. In the first system, oxaliplatin(IV) was functionalised with the red fluorophore resorufin using a self-immolative linker whilst the second approach involved the direct attachment of a naphthalimide dye to the platinum(IV) centre (Figure 3.1). Upon intracellular reduction, a fluorescence

“switch-on” response would be observed following the release of the fluorescent probes allowing us to directly visualise the activation of both Pt(IV) complexes in cancer cells.²⁵

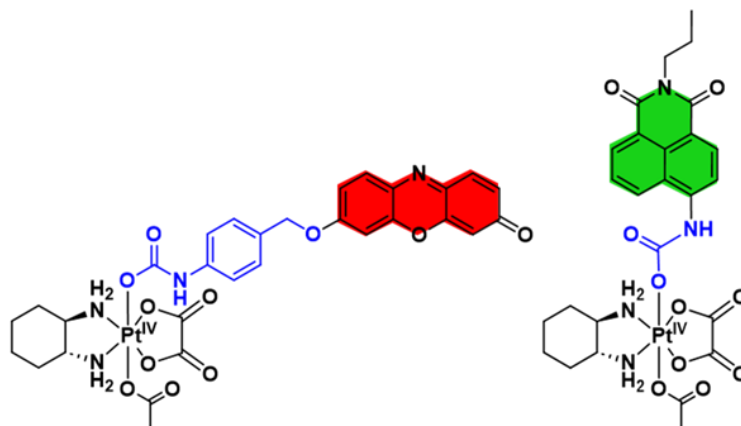


Figure 3.1. Self-immolative system (left) and direct coordination (right) strategies for OxPt(IV) prodrugs designed in this project.

3.2. Synthesis of Oxali(IV)Fluors

3.2.1. Synthesis and characterisation of OxaliRes

Carbamate linkers were selected based on recent studies by Gibson and Zhu which demonstrated that Pt(IV) complexes containing carbamate ligands displayed good stability under aqueous conditions and can be rapidly reduced in the presence of reducing agents.^{15,16} Moreover, a wider range of molecules can be conjugated to platinum(IV) complexes using this type of linkers. Furthermore, carbamate self-immolative systems for the controlled delivery of active compounds have been extensively studied by a number of research groups since the initial prodrug model reported by Katzenellenbogen and co-workers.²⁶⁻²⁹ Figure 3.2 illustrates the general release mechanism of this model system (**3.1**) with 4-aminobenzyl alcohol acting as self-immolative linker (**blue**) to connect the reporter 4-nitroaniline (**green**) and a protecting group (**black**). The latter is used as a trigger moiety and can be cleaved *via* hydrolysis upon addition of trypsin to free the deprotected intermediate **3.2**. The self-immolative spacer is then able to undergo a *para*-azaquinone-methide 1,6-elimination process and release the linker **3.3** and the drug **3.4**.^{29,30}

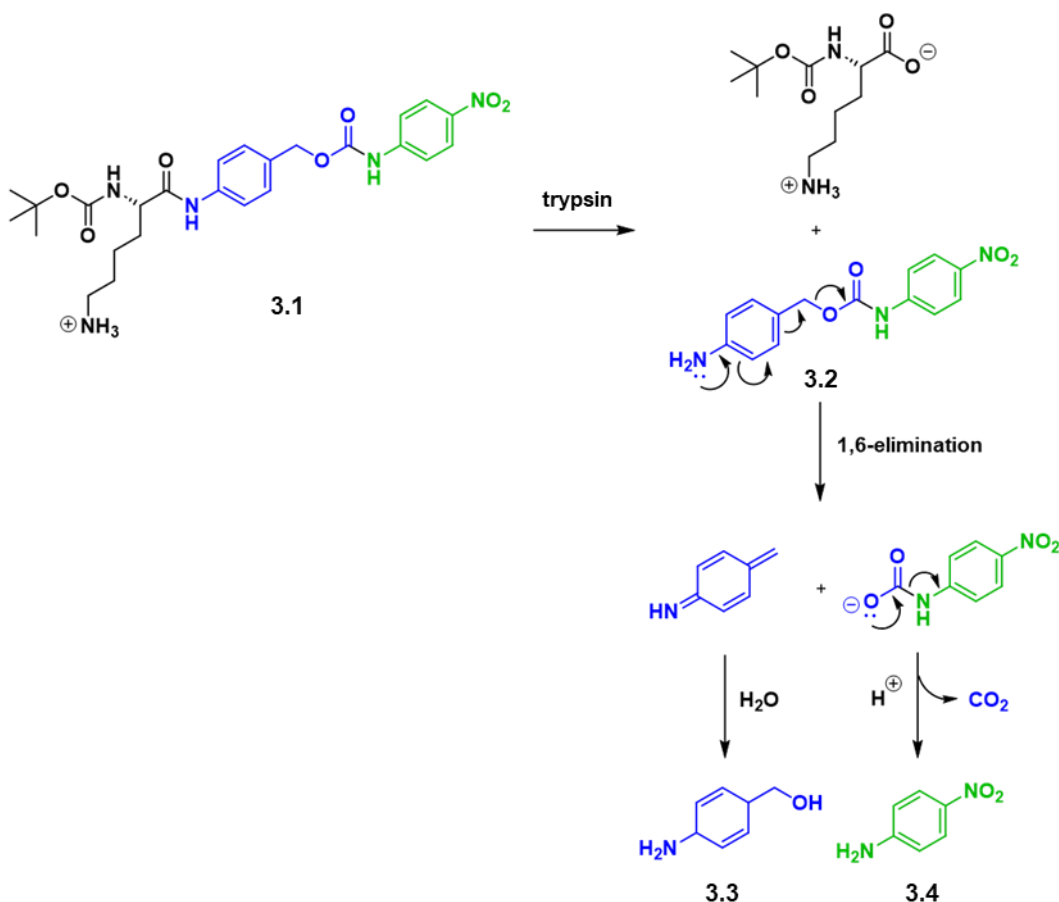


Figure 3.2. Release of the 4-nitroaniline group (**3.4**) upon enzymatic cleavage by trypsin via a self-immolative elimination of the 4-aminobenzyl alcohol linker (**3.3**).²⁹ (Scheme adapted from a review by Gavriel et al.²⁷).

The system presented above was applied in this work to develop an oxaliplatin(IV) carbamate complex attached to the fluorescent dye resorufin via the self-immolative linker 4-aminobenzyl alcohol. Reduction of the platinum(IV) centre would result in the loss of the axial ligands which could trigger the decarboxylation of the self-immolative resorufin unit and release the fluorophore via a 1,6-elimination reaction (Figure 3.3). Thus, this activation would be accompanied by an increase in fluorescence emission, indicative of the reduction of the Pt(IV) complex to Pt(II) in cancer cells. In 2021, Ang and co-workers used the same approach to design a dual-drug delivery system for combination therapy application using cisplatin(IV) prodrugs with other bioactive agents. HPLC studies on the activation of these compounds showed controlled release of the co-drug upon reduction of the cisplatin(IV) conjugate with sodium ascorbate.³¹

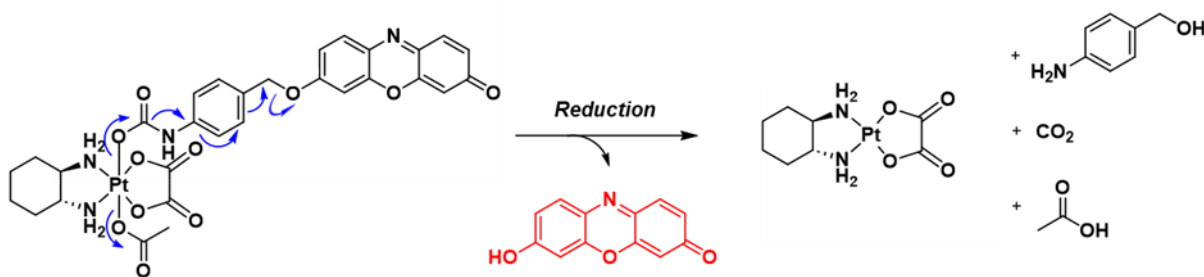
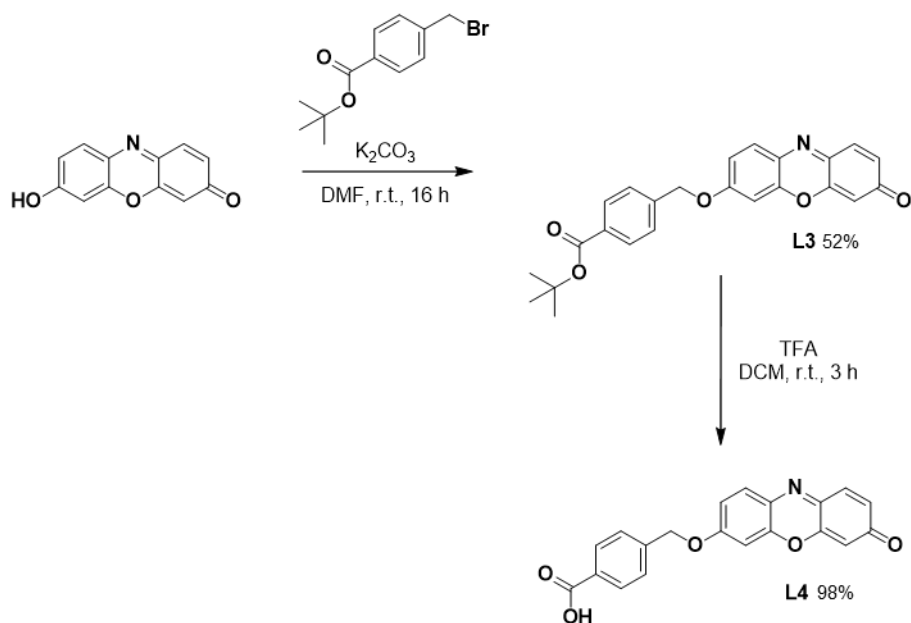


Figure 3.3. Self-immolative system used in this work for the release of the fluorescent dye resorufin upon reduction.

The initial step involved the synthesis of the self-immolative resorufin component **L4**, outlined in Scheme 3.1. *Tert*-butyl 4-(bromomethyl)benzoate was first attached to resorufin via a nucleophilic substitution ($\text{S}_{\text{N}}2$) reaction using potassium carbonate as base in dimethylformamide. After overnight stirring, the reaction was diluted with dichloromethane and extracted with brine to get the red compound *tert*-butyl 4-(((3-oxo-3*H*-phenoxazin-7-yl)oxy)methyl)benzoate **L3** in reasonable yield (52 %) and high purity. The product was characterised using high-resolution mass spectrometry, ^1H NMR and ^{13}C NMR spectroscopy.



Scheme 3.1. Synthesis of 4-(((3-Oxo-3*H*-phenoxazin-7-yl)oxy)methyl)benzoic acid (**L4**).

Crystals suitable for X-ray diffraction were obtained from slow evaporation of deuterated chloroform and analysed by Dr Kirsten E. Christensen (University of Oxford). The crystal structure of **L3** is shown in Figure 3.4 and revealed coordination of the protected self-immolative linker via the hydroxyl group of resorufin, as expected (further data available in Appendix A.2).

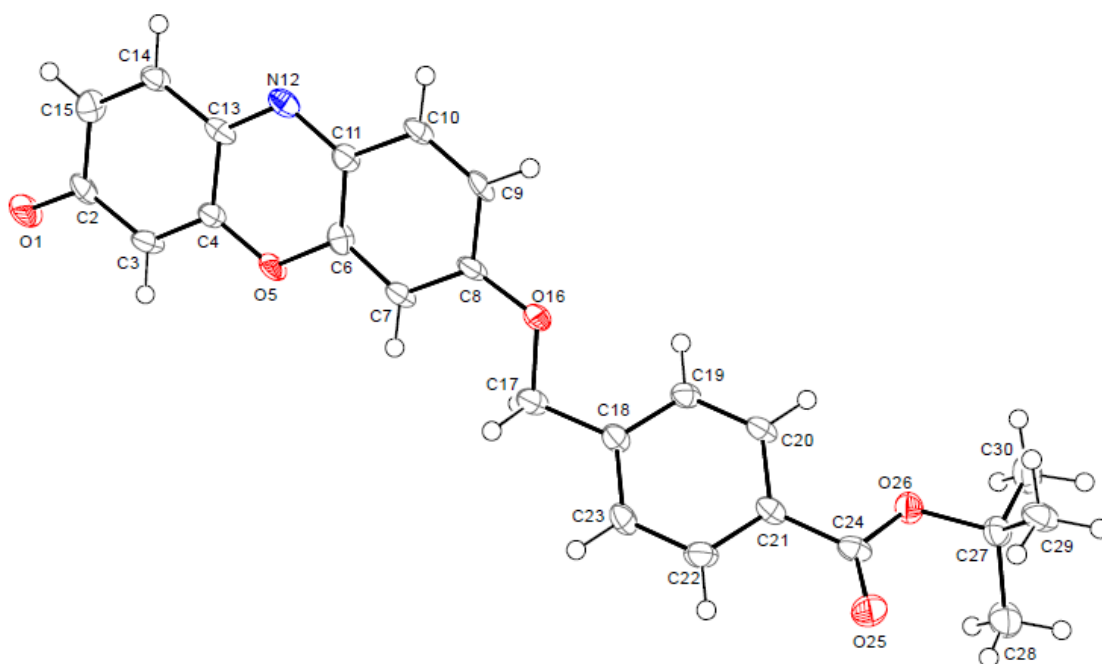


Figure 3.4. Molecular structure of *tert*-butyl 4-(((3-oxo-3*H*-phenoxazin-7-yl)oxy)methyl)benzoate (**L3**) determined by X-ray diffraction with thermal ellipsoids displayed at 50% probability.

Deprotection of the *tert*-butyl ester was carried out using TFA in DCM at room temperature for 3 hours. The crude product was completely dried and washed twice with methanol to remove residual trifluoroacetic acid. **L4** was obtained in high yield (98 %) and characterised by ^1H and ^{13}C NMR spectroscopy in DMSO-d_6 and high-resolution mass spectrometry.

Figure 3.5 displays the ^1H NMR spectra of the synthesised ligands **L3** and **L4**. The resonance of the *tert*-butyl groups observed at 1.59 ppm in spectrum (a) has disappeared in

the deprotected product **(b)**. A broad singlet is now present at 13.02 ppm and assigned to the proton of the carboxylic acid group of **L4**.

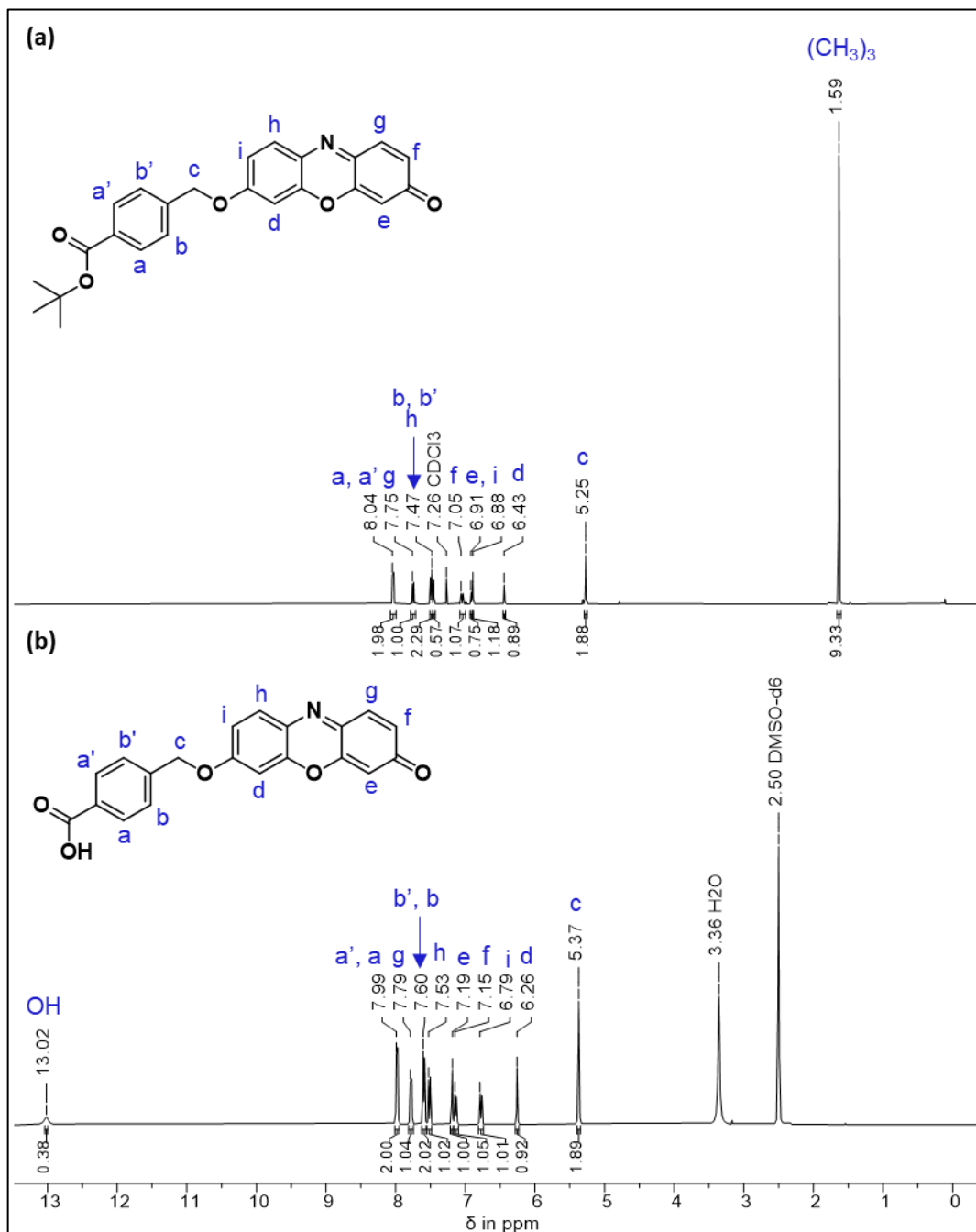
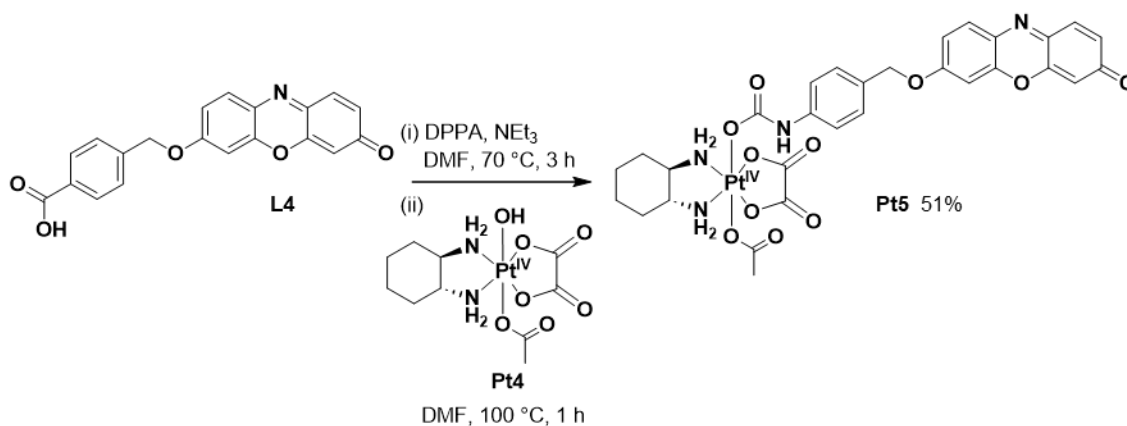


Figure 3.5. (a) ^1H NMR (400 MHz, CDCl_3) of *tert*-butyl 4-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)benzoate (**L3**), (b) ^1H NMR (400 MHz, DMSO-d_6) of 4-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)benzoic acid (**L4**).

OxPt(OH)(OAc) (Complex **Pt4**) was easily prepared by treating oxaliplatin with hydrogen peroxide and acetic acid according to literature method.³² Scheme 3.2 presents the synthetic pathway of **OxaliRes** (Complex **Pt5**), which was synthesised following a modified literature procedure by Yee Lee *et al.*³¹ Excess of diphenyl phosphoryl azide (DPPA) and triethylamine (TEA) (3.0 equiv.) were used to achieve transformation of the carboxyl group of **L4** to the corresponding isocyanate *via* a Curtius rearrangement in a one-pot procedure. **L4** and DPPA were mixed in the presence of TEA to first form an intermediate acyl azide rearranging into an isocyanate moiety through thermal decomposition and the release of N₂ gas.³³ The reaction was performed in dimethylformamide and heated at 70 °C while monitored by TLC and subsequently cooled to room temperature. Dropwise addition of **Pt4** in DMF to the isocyanate-containing solution and heating at 100 °C afforded the formation of **OxaliRes** by acylation in the second step. **Pt5** was isolated by column chromatography on silica gel in moderate yield (51 %).



Scheme 3.2. Synthesis of **OxaliRes** (**Pt5**).

Characterisation of **OxaliRes** was performed by ¹H, ¹³C and ¹⁹⁵Pt NMR spectroscopy, and high-resolution mass spectrometry. Figure 3.6 illustrates the ¹H NMR spectrum of **Pt5** in DMSO-d₆. A slight upfield shift for the resonances of the self-immolative resorufin ligand and the absence of the COOH proton signal are noticed. A small resonance is detected at 9.21 ppm for the NH proton of the carbamate. The chemical shifts of the 1,2-diaminocyclohexane

(DACH) ring of oxaliplatin are observed between 1.16-2.75 ppm with the presence of a large singlet at 1.97 ppm, corresponding to the methyl group of the acetato ligand.

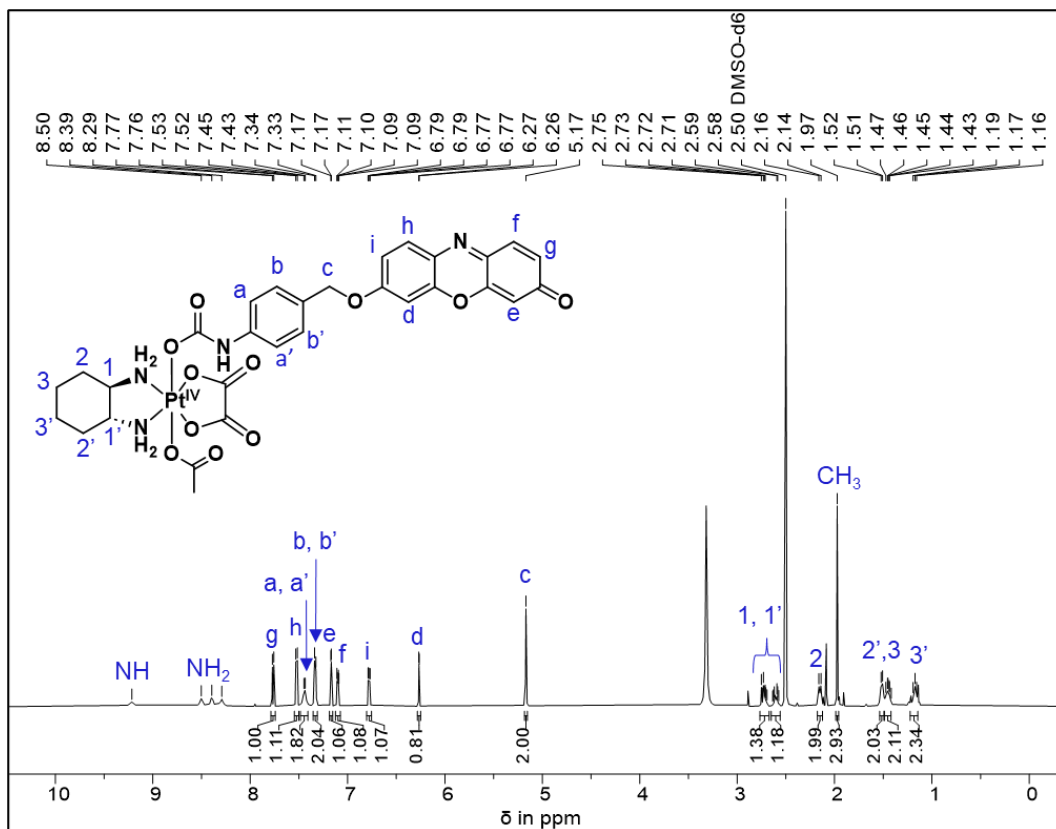


Figure 3.6. ^1H NMR (600 MHz, DMSO-d_6) of **OxaliRes (Pt5)**.

The ^{195}Pt NMR spectrum of **OxaliRes** presented in Figure 3.7 gave a single resonance at 1626 ppm, exhibiting a strong downfield shift in comparison to OxPt(OH)(OAc) (**Pt4**) (1315 ppm in D_2O), due to the coordination of **L4** at the axial site.

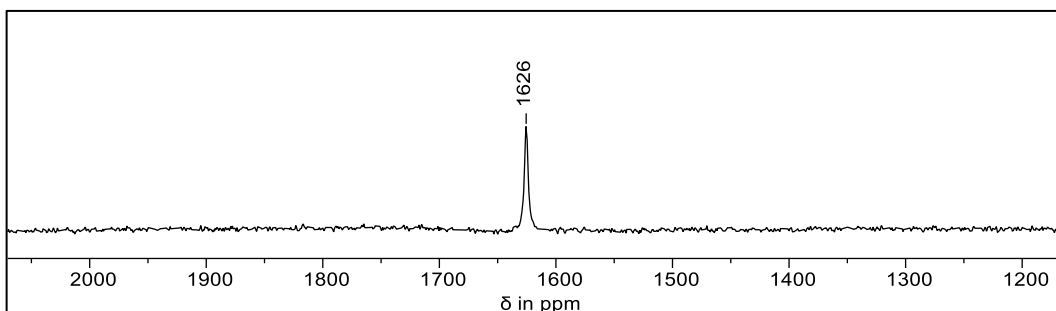
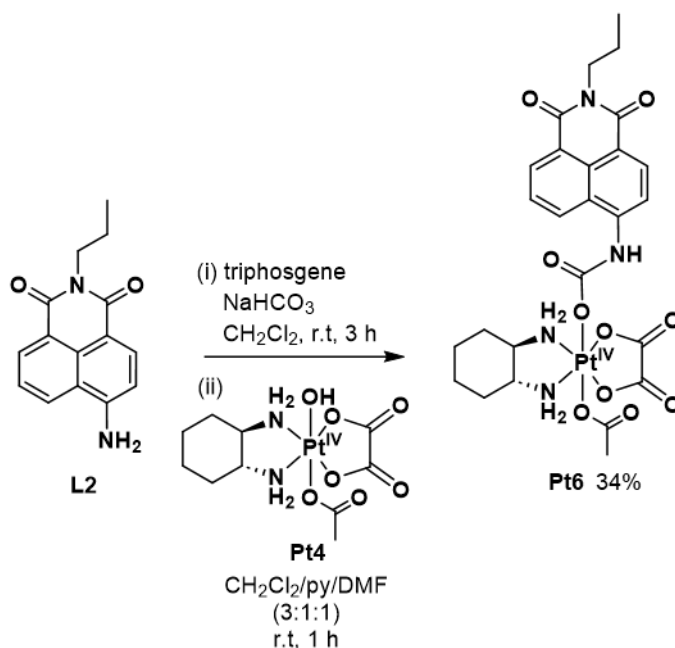


Figure 3.7. ^{195}Pt NMR (107 MHz, DMSO-d_6) of **OxaliRes (Pt5)**.

3.2.2. Synthesis and characterisation of OxaliNap

The synthesis of the second Oxali(IV)Fluors involved direct coordination of the fluorophore 4-aminonaphthalimide (4-NH₂Nap, **L2**) onto **Pt4**, as displayed on Scheme 3.3. Following the same procedure described in *Chapter 2* for the preparation of **AzidoNap (Pt3)**, 4-NapNH₂ was first treated with triphosgene and added dropwise in a second step to a solution of OxPt(OH)(OAc) in dichloromethane, pyridine and dimethylformamide in a 3:1:1 ratio. DMF was added to the solvent mixture to improve the solubility of **Pt4** whereas pyridine was required for the reaction to proceed and appeared to function as a catalyst. **OxaliNap (Complex Pt6)** was obtained in average yield (34 %) after purification by column chromatography and characterised by high-resolution mass spectrometry and ¹H, ¹³C and ¹⁹⁵Pt NMR spectroscopy.



Scheme 3.3. Synthesis of **OxaliNap (Pt6)**.

The ¹H NMR spectrum of **OxaliNap** in DMSO-d₆ is shown in Figure 3.8. The chemical shifts of **OxaliNap** are similar to the resonances observed for the starting materials **Pt4** and **L2**, with a broad resonance between 8.2-8.6 ppm corresponding to the two NH₂ groups of oxaliplatin DACH ligand. The chemical shift associated to the proton NH of the carbamate bond

is seen at 9.71 ppm. The ^{195}Pt NMR spectrum of **Pt6** presents an analogous downfield shift as **OxaliRes** with a sharp resonance at 1634 ppm (Figure 3.9).

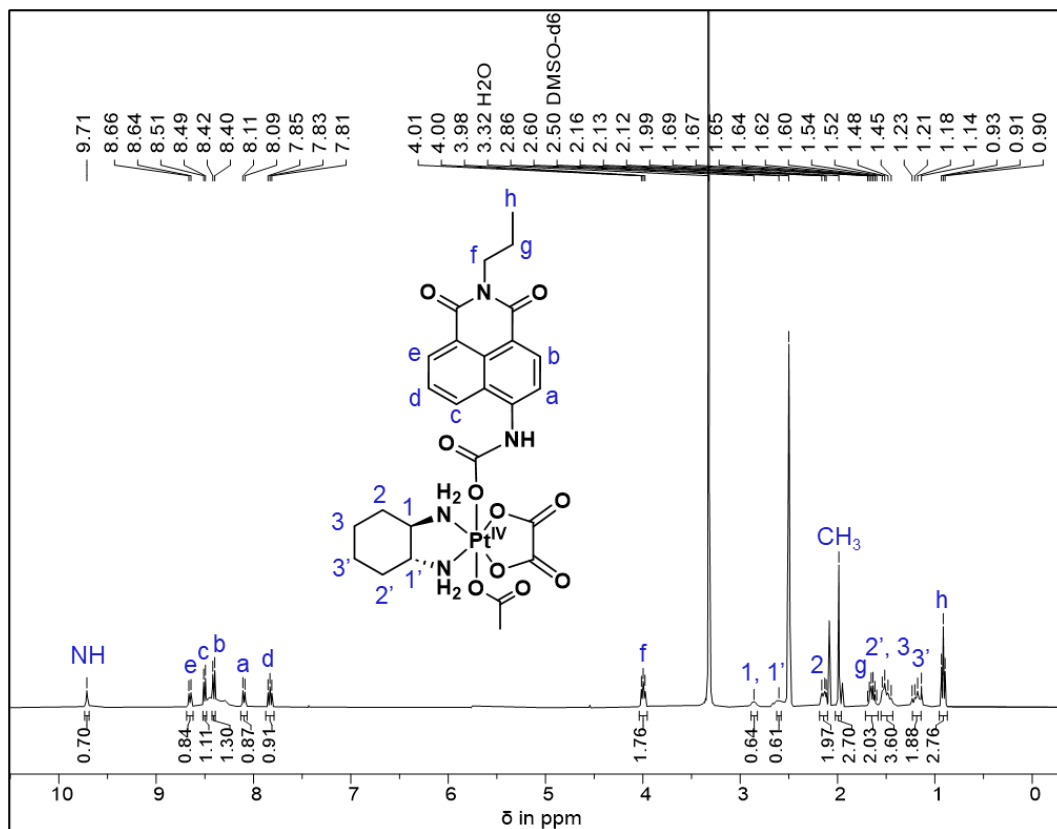


Figure 3.8. ^1H NMR (400 MHz, DMSO-d_6) of **OxaliNap (Pt6)**.

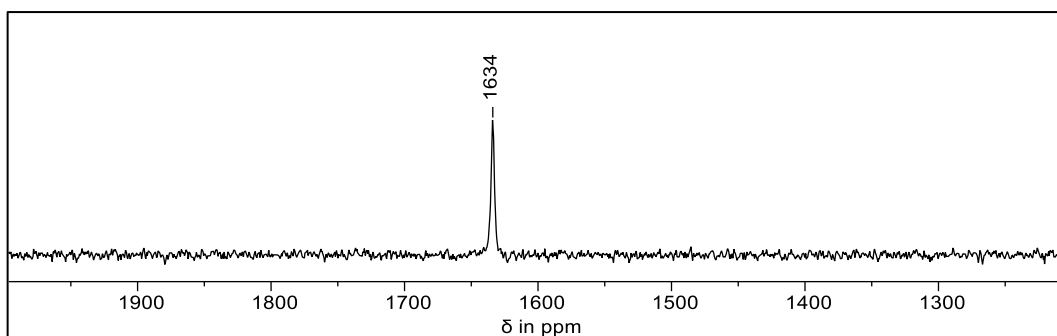


Figure 3.9. ^{195}Pt NMR (107 MHz, DMSO-d_6) of **OxaliNap (Pt6)**.

3.3. Analytical studies on Oxali(IV)Fluors

With **OxaliRes (Pt5)** and **OxaliNap (Pt6)** in hand, a comparative study of their photophysical and chemical properties was carried out in aqueous solution.²⁵ Fluorescence titration experiments in PBS buffer (pH = 7.40) were first undertaken to investigate the reduction properties of the complexes in the presence of sodium ascorbate (NaAsc). As shown in Figure 3.10 and Figure 3.11, increases in fluorescence emission intensities at 585 nm and 545 nm were recorded for **Pt5** and **Pt6**, respectively. The fluorescence response appears to be concentration-dependent of NaAsc. These results suggest reduction of the Pt(IV) complexes to their Pt(II) counterparts in the presence of NaAsc, noticeable through the release of free resorufin (585 nm) and 4-NapNH₂ (545 nm). A higher concentration of NaAsc (up to 10 mM) was, however, required for **OxaliNap** to produce a fluorescence response (4-fold), as displayed in Figure 3.12. In comparison, a 9-fold “turn on” response was observed for **OxaliRes** when using up to 4 millimolar of NaAsc, showing a clear difference in sensitivity between the two Oxali(IV)Fluors.

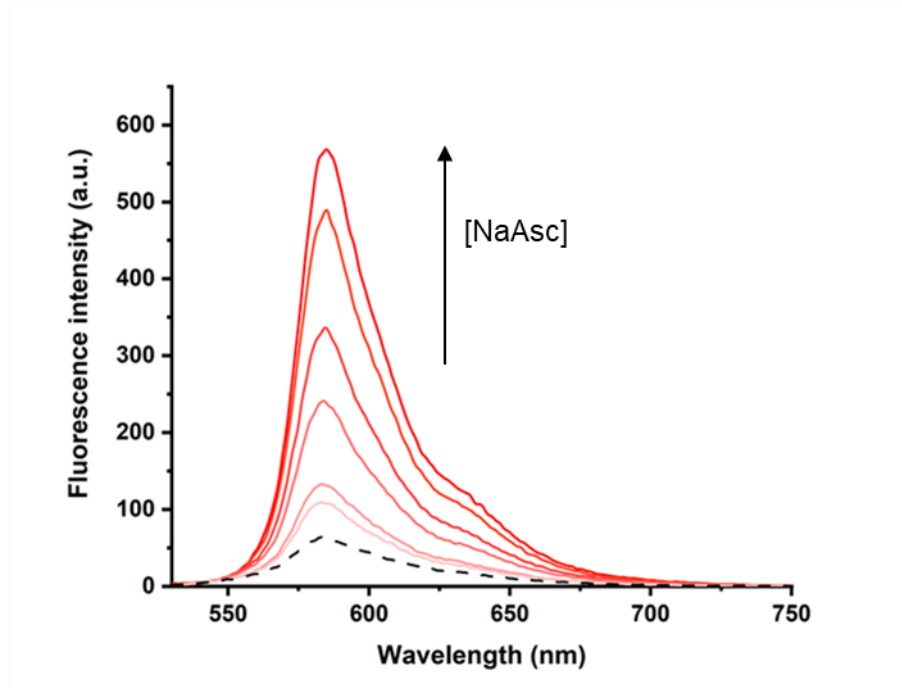


Figure 3.10. Fluorescence spectra of **OxaliRes (Pt5, 5 μ M)** with increasing concentrations of NaAsc (1 h incubation) in PBS buffer (pH = 7.40). Fluorescence intensities were recorded with $\lambda_{\text{ex}} = 500$ nm, slit widths: ex = 10 nm, em = 2.5 nm.

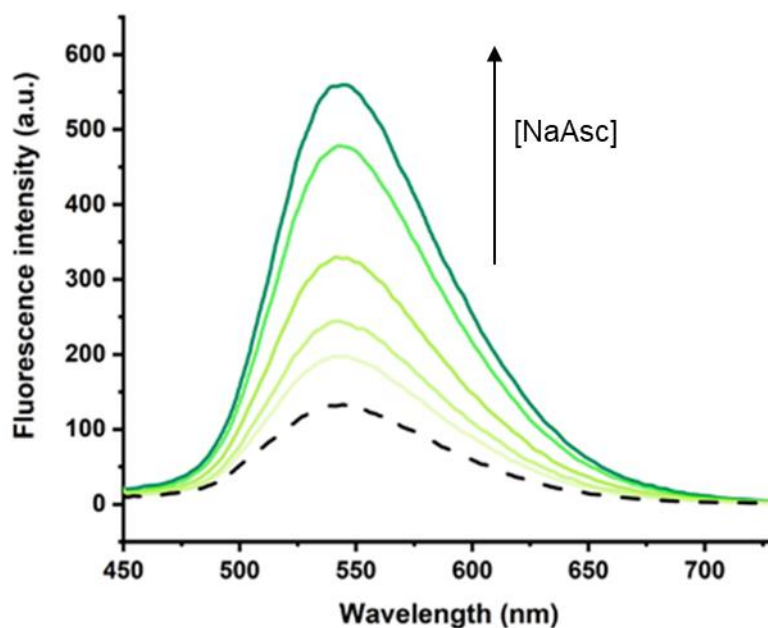


Figure 3.11. Fluorescence spectra of **OxaliNap (Pt6, 5 μ M)** with increasing concentrations of NaAsc (1 h incubation) in PBS buffer (pH = 7.40). Fluorescence intensities were recorded with $\lambda_{\text{ex}} = 430$ nm, slit widths: ex = 10 nm, em = 10 nm.

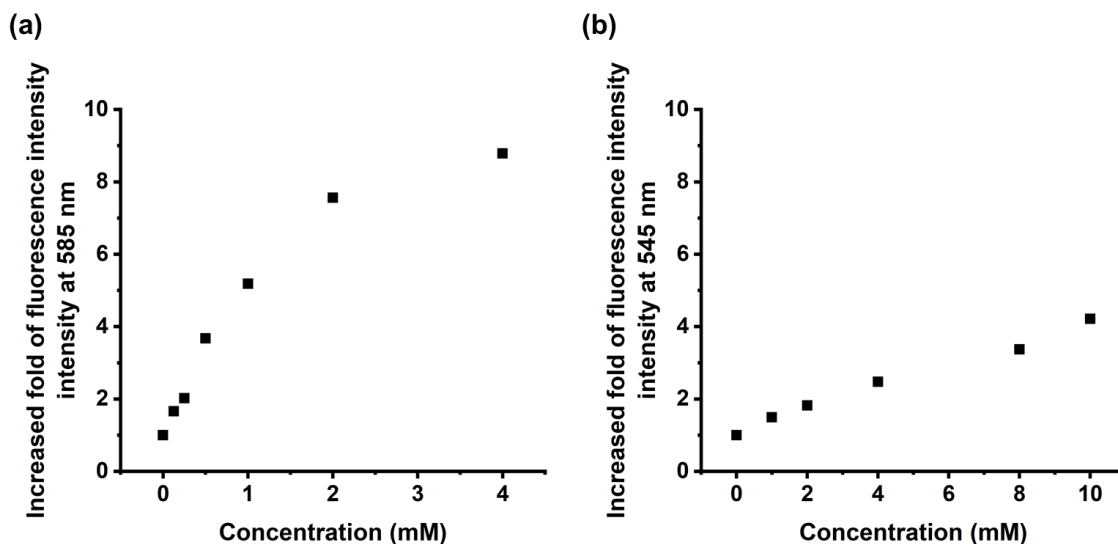


Figure 3.12. **(a)** Fluorescence intensity changes (I/I_0) for **OxaliRes (Pt5, 15 μ M)** at 585 nm as a function of NaAsc concentration (0–4 mM, 1 h incubation) in PBS buffer (pH = 7.40). Fluorescence intensities were recorded with $\lambda_{\text{ex}} = 500$ nm, slit widths: ex = 10 nm, em = 2.5 nm. **(b)** Fluorescence intensity changes (I/I_0) for **OxaliNap (Pt6, 15 μ M)** at 545 nm as a function of NaAsc concentration (0–10 mM, 1 h incubation) in PBS buffer (pH = 7.40). Fluorescence intensities were recorded with $\lambda_{\text{ex}} = 430$ nm, slit widths: ex = 10 nm, em = 10 nm.

The absorption properties of **OxaliRes** and **OxaliNap** were studied with NaAsc (4 mM) over 60 minutes (Figure 3.13 and Figure 3.14). In the absence of NaAsc, a broad absorption band around 480 nm was observed for **OxaliRes**. After incubation, the spectrum displayed a distinct bathochromic shift in the maximum absorption band from 480 nm to 570 nm, with a strong absorption emission characteristic of resorufin. A clear colour change was also observed in the samples before and after treatment, changing from clear to a bright pink solution. Similarly, the UV-vis spectrum of **OxaliNap** exhibited a weak and broad peak at 380 nm before treatment. Addition of NaAsc produced a small spectral shift in the absorption maxima from 380 to 340 nm, corresponding to 4-NapNH₂. Once again, **OxaliNap** response was not as significant as the one observed for **OxaliRes** under the same conditions, displaying the low sensitivity of **Pt6** towards NaAsc. Nevertheless, formation of resorufin and 4-NapNH₂ were observed for **OxaliRes** and **OxaliNap**, respectively, further supporting the reduction of Pt(IV) to Pt(II).

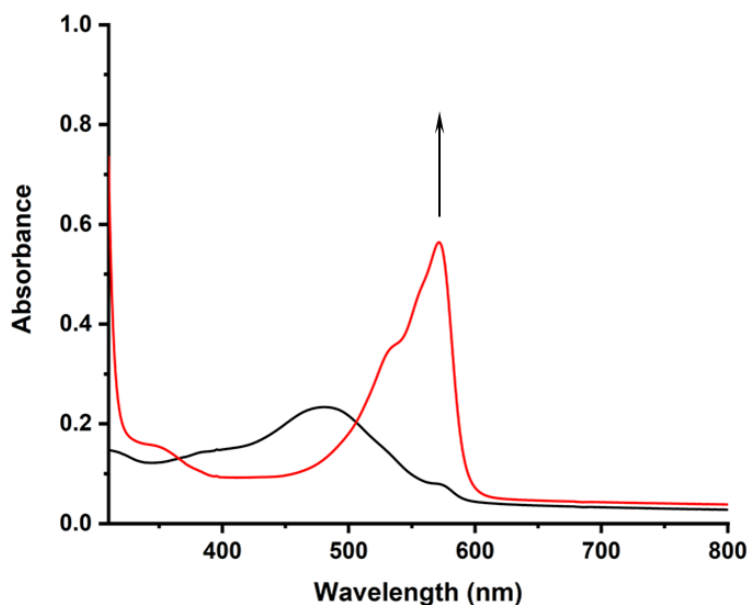


Figure 3.13. Absorbance spectra of **OxaliRes** (**Pt5**, 15 μ M) in PBS buffer (pH = 7.40) before (**black**) and after (**red**) addition of NaAsc (4 mM). The second measurement was recorded after 60 minutes incubation.

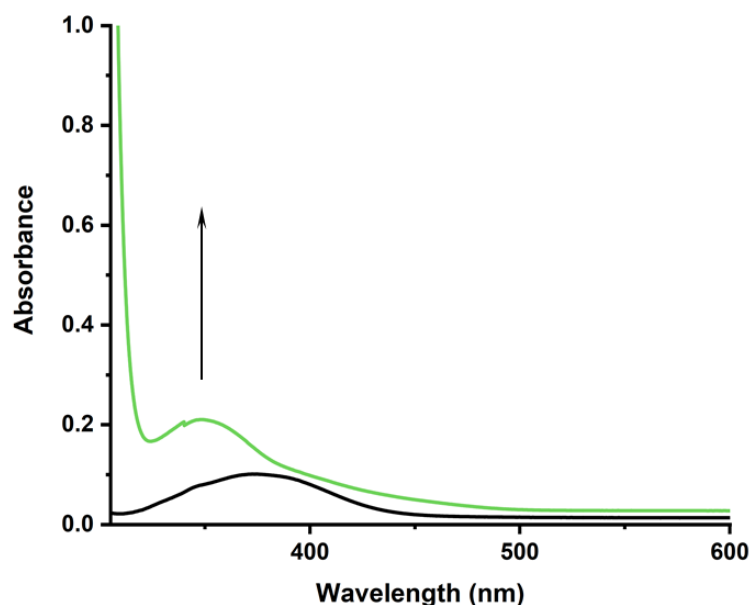


Figure 3.14. Absorption spectra of **OxaliNap** (**Pt6**, 15 μM) in PBS buffer (pH = 7.40) before (**black**) and after (**green**) addition of NaAsc (4 mM). The second measurement was recorded after 60 minutes incubation.

Changes in the fluorescence emission intensities were also monitored over the course of 60 minutes in the presence of a range of NaAsc concentrations, as displayed in Figure 3.15 and Figure 3.16. Due to the less sensitive nature of **OxaliNap** against sodium ascorbate, higher concentrations of the reducing agent were again utilized (ranging from 1 to 10 mM). Steady increases of fluorescence emissions were observed for both complexes. However, a one-hour period was required to see a clear fluorescence response.

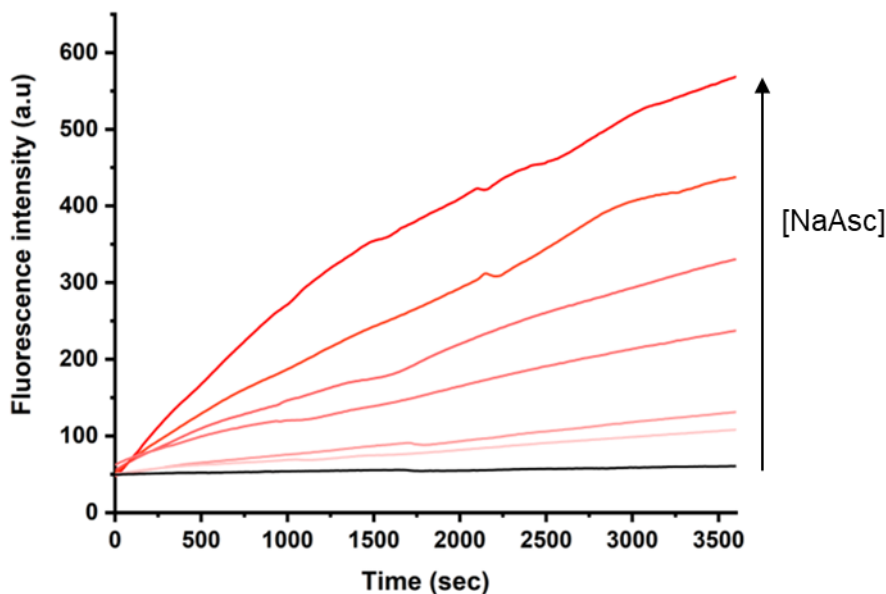


Figure 3.15. Changes in fluorescence emission intensity of **OxaliRes (Pt5, 5 μ M)** at 585 nm with different concentrations of NaAsc (125 μ M, 250 μ M, 500 μ M, 1 mM, 2 mM, 4 mM) over one hour. All measurements were performed in PBS buffer (pH = 7.40), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

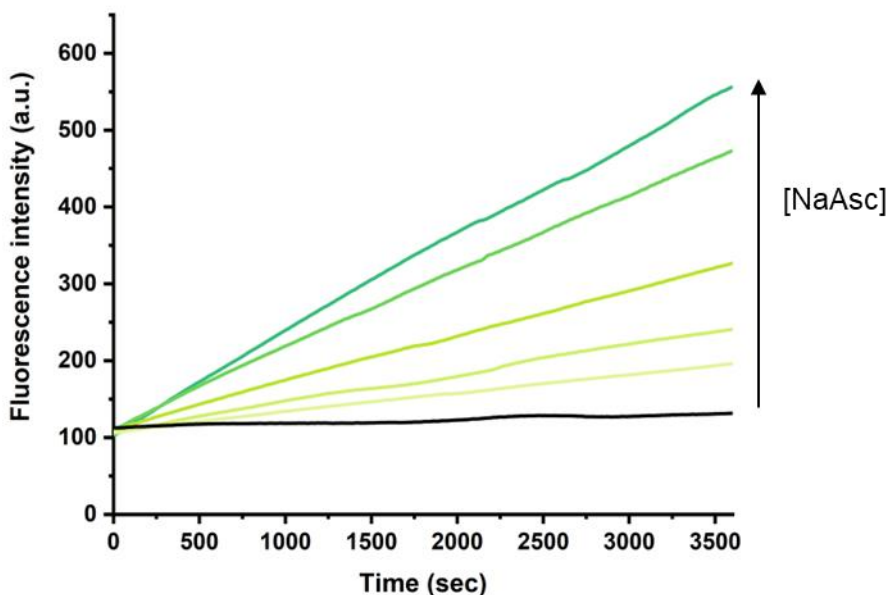


Figure 3.16. Changes in fluorescence emission intensity of **OxaliNap (Pt6, 5 μ M)** at 545 nm with different concentrations of NaAsc (1 mM, 2 mM, 4 mM, 8 mM, 10 mM) over one hour. All measurements were performed in PBS buffer (pH = 7.40), λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 10 nm.

Attempting to explain the discrepancies observed in the rate of reduction between our two Oxali(IV)Fluors, cyclic voltammetry experiments were conducted by Aaron J. King and Eric S. Yang of the University of Oxford. Due to the poor aqueous solubility of **OxaliRes** and **OxaliNap**, the measurements were conducted in DMF with the OxPt(OH)(OAc) precursor (**Pt4**) used for comparison (Figure 3.17, Figure 3.18, and Figure 3.19). An irreversible reduction process was observed for all three complexes due to the reduction from Pt(IV) to Pt(II) and the loss of the two axial ligands. Overall, the reduction potential (E_p) of the resorufin ligand ($E_p = -1.20$ V vs $\text{Fc}^{0/+}$) was slightly higher (more positive) than the reduction peak of the naphthalimide ligand (**L2**) ($E_p = -2.03$ V vs $\text{Fc}^{0/+}$) and the one of **Pt4** ($E_p = -1.88$ V vs $\text{Fc}^{0/+}$). These results might explain the faster reduction seen for **OxaliRes** in comparison to **OxaliNap**, as the axial ligands (**L2** and **L4**) may influence the rate of reduction of the two Oxali(IV)Fluors.

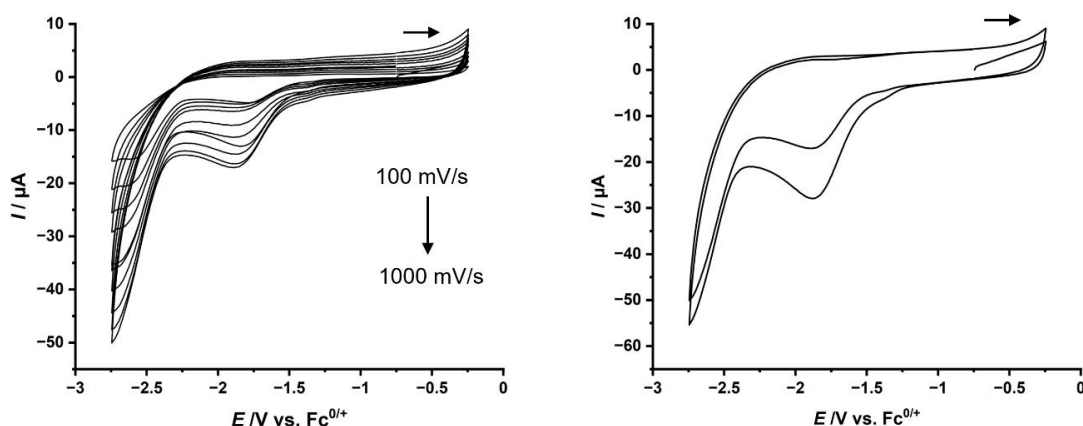


Figure 3.17. Left: Cyclic voltammogram (DMF, 0.1 M $[\text{Bu}_4\text{N}][\text{PF}_6]$) of a 1 mM solution of OxPt(OH)(OAc) (**Pt4**). Potentials are referenced to $[\text{Cp}_2\text{Fe}]^{+/0}$. Right: Cyclic voltammogram (DMF, 0.1 M $[\text{Bu}_4\text{N}][\text{PF}_6]$) of a 1 mM solution of OxPt(OH)(OAc) (**Pt4**). Potentials are referenced to $[\text{Cp}_2\text{Fe}]^{+/0}$. The scan rate was 1000 mV/S.

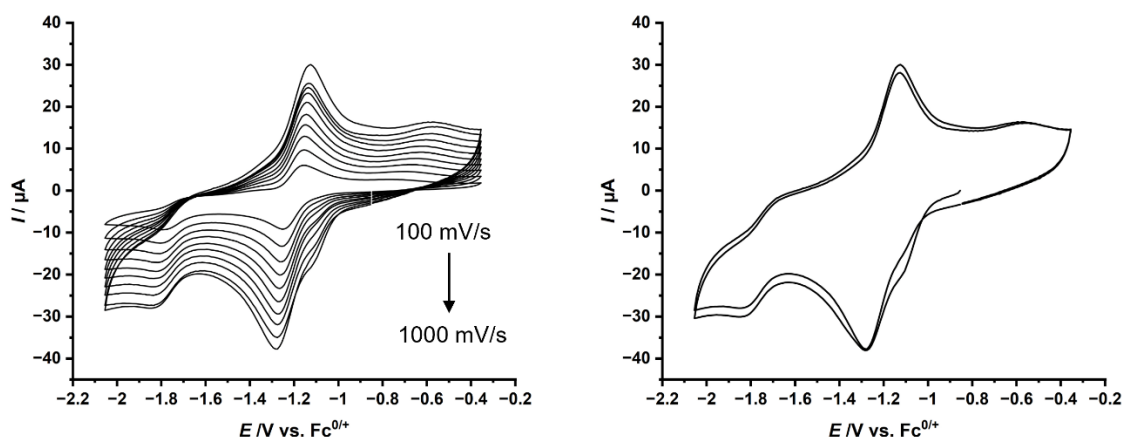


Figure 3.18. Left: Cyclic voltammogram (DMF, 0.1 M $[Bu_4N][PF_6]$) of a 1 mM solution of **OxaliRes (Pt5)**. Potentials are referenced to $[Cp_2Fe]^{+/0}$. Right: Cyclic voltammogram (DMF, 0.1 M $[Bu_4N][PF_6]$) of a 1 mM solution of **OxaliRes (Pt5)**. Potentials are referenced to $[Cp_2Fe]^{+/0}$. The scan rate was 1000 mV/S.

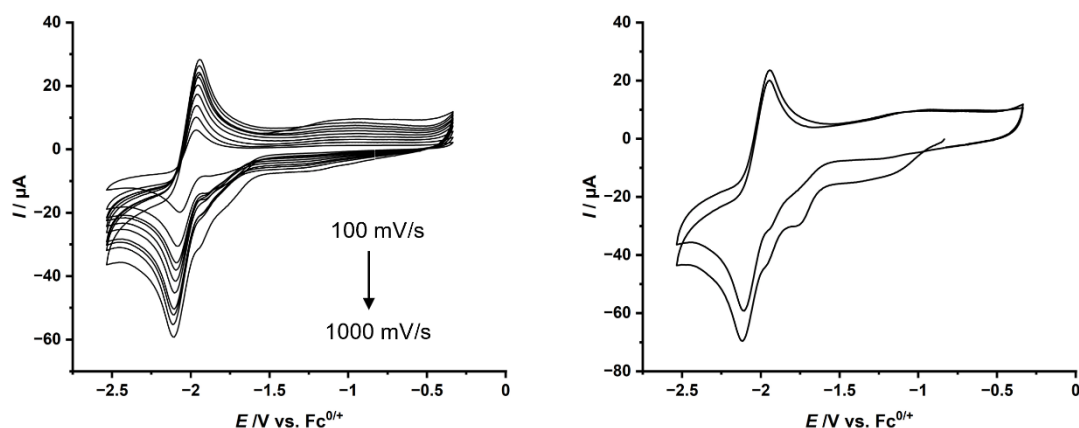


Figure 3.19. Left: Cyclic voltammogram (DMF, 0.1 M $[Bu_4N][PF_6]$) of a 1 mM solution of **OxaliNap (Pt6)**. Potentials are referenced to $[Cp_2Fe]^{+/0}$. Right: Cyclic voltammogram (DMF, 0.1 M $[Bu_4N][PF_6]$) of a 1 mM solution of **OxaliNap (Pt6)**. Potentials are referenced to $[Cp_2Fe]^{+/0}$. The scan rate was 1000 mV/S.

To monitor the reduction of **OxaliRes** and **OxaliNap** in cancer cells, a stability study was first conducted by fluorescence spectroscopy and HPLC analysis. The emission properties of the Oxali(IV)Fluors were recorded with and without sodium ascorbate (1 mM) over 8 hours (Figure 3.20 and Figure 3.21). Treatment with sodium ascorbate resulted in a time-dependent increase in red and green fluorescence intensities but no significant changes were detected in the fluorescence emission of **OxaliRes** and **OxaliNap** alone. Figure 3.22 and Figure 3.23 display the HPLC traces of **OxaliRes** and **OxaliNap** at 254 nm and 360 nm,

respectively. Good stability for both Pt(IV) complexes was demonstrated over the course of 12 hours, making them suitable for cell imaging studies.

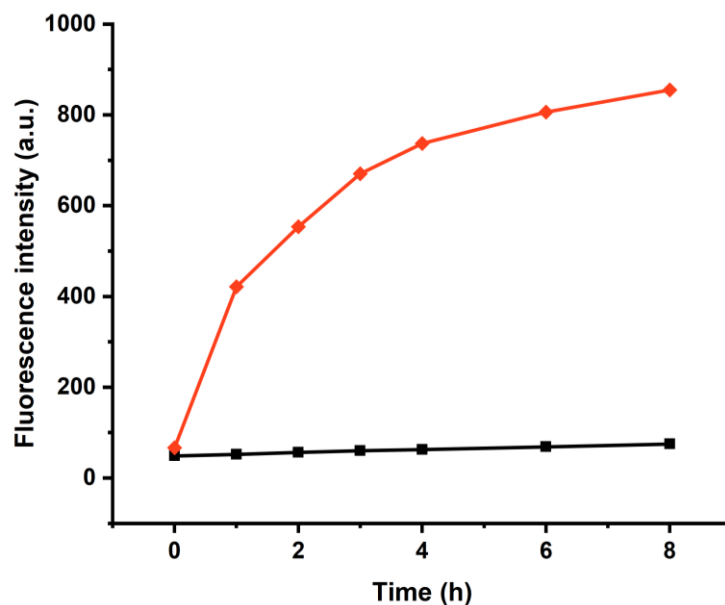


Figure 3.20. Changes in the fluorescence intensity of **OxaliRes (Pt5, 5 μ M)** at 585 nm with (**red**) and without (**black**) the addition of NaAsc (1 mM). All measurement were performed in PBS (pH = 7.40), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

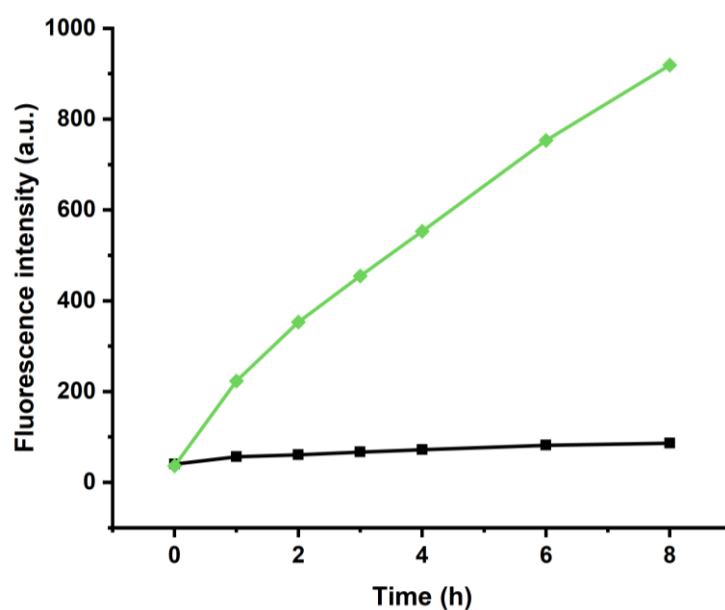


Figure 3.21. Changes in the fluorescence intensity of **OxaliNap (Pt6, 5 μ M)** at 545 nm with (**green**) and without (**black**) the addition of NaAsc (1 mM). All measurement were performed in PBS (pH = 7.40), λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 10 nm.

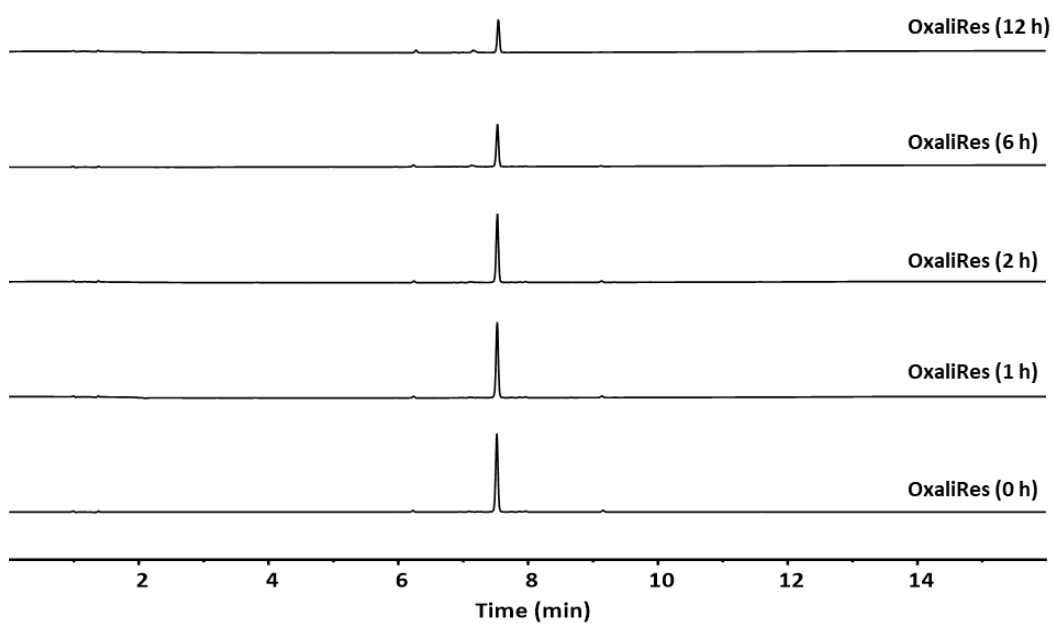


Figure 3.22. The stability of **OxaliRes** (**Pt5**, 50 μ M) in PBS buffer (pH 7.40) recorded by HPLC (Absorption at 254 nm).

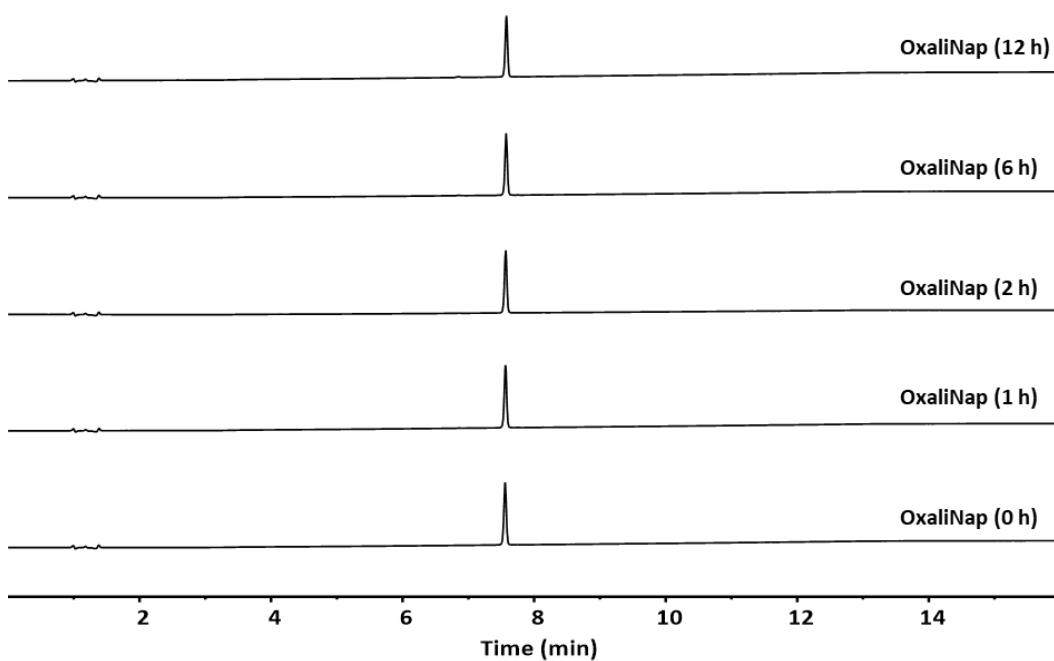


Figure 3.23. The stability of **OxaliNap** (**Pt6**, 50 μ M) in PBS buffer (pH 7.40) recorded by HPLC (Absorption at 360 nm).

Due to the abundant presence of reducing agents in malignant cells, the fluorescence response of **OxaliRes** and **OxaliNap** towards various reductants was studied. Minimal changes in the emission profile of **OxaliRes** were detected in the presence of L-glutathione (GSH, 4 mM), L-Cysteine (L-Cys, 100 μ M), hydrogen peroxide (H_2O_2 , 100 μ M) and bovine serum albumin (BSA, 1 equiv.), as seen in Figure 3.24. Upon addition of 0.5 mM NADH, however, a time-dependent increase in fluorescence intensity was observed (Figure 3.25).

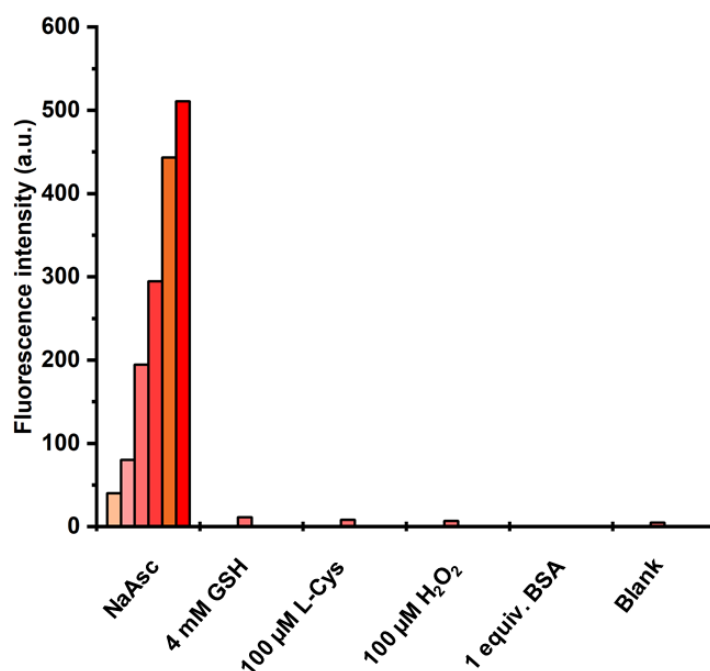


Figure 3.24. Relative fluorescence intensity of **OxaliRes** (**Pt5**, 5 μ M) in the presence of various analytes: NaAsc (125 μ M, 250 μ M, 500 μ M, 1 mM, 2 mM, 4 mM); GSH (4 mM); L-Cysteine (100 μ M); H_2O_2 (100 μ M) and BSA (1 equiv.) in PBS buffer (pH = 7.40), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

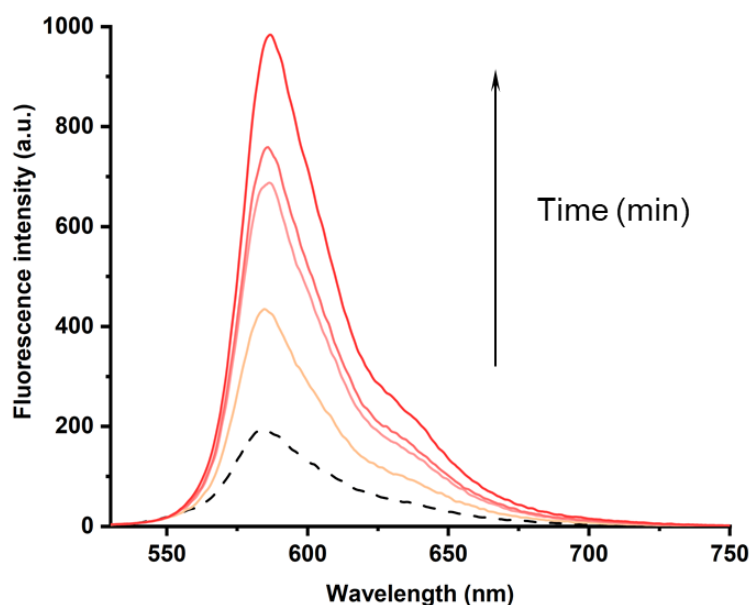


Figure 3.25. Changes in fluorescence emission intensity of **OxaliRes (Pt5, 5 μ M)** in the presence of NADH (0.5 mM) at different time points (0 min, 5 min, 10 min, 15 min, 20 min). All measurements were performed in PBS buffer (pH = 7.40), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

Comparable results were recorded for **OxaliNap** after incubation with GSH (4 mM), L-Cys (100 μ M) and H_2O_2 (100 μ M), as displayed in Figure 3.26. Interestingly, in the presence of BSA a small blue shift in the emission wavelength from 545 to 520 nm was observed (Figure 3.27). Previous studies demonstrated the possible interaction between naphthalimide-based probes and human serum albumin (HSA), observed by fluorescence spectroscopy, isothermal titration calorimetry (ITC) and small-angle X-ray scattering (SAXS) experiments.^{34,35,36} HSA has also been extensively investigated as a delivery drug carrier due to its ability to transport hydrophobic molecules.³⁷ Based on these reports, our results might involve similar binding between **OxaliNap** and BSA via the 4-NH₂Nap unit. Furthermore, treatment of **OxaliNap** with NADH led to a 1.5-fold “turn-on” response, which indicate that both Oxali(IV)Fluors are selective for sodium ascorbate and NADH.

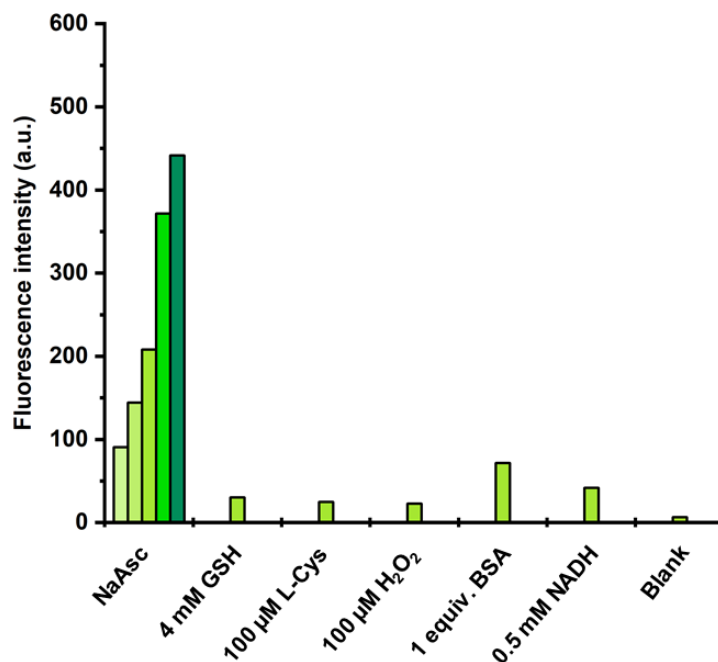


Figure 3.26. Relative fluorescence intensity of **OxaliNap (Pt6, 5 μ M)** in the presence of various analytes: NaAsc (1 mM, 2 mM, 4 mM, 8 mM, 10 mM); GSH (4 mM); L-Cysteine (100 μ M); H₂O₂ (100 μ M); BSA (1 equiv.) and NADH (0.5 mM) in PBS (pH = 7.40), λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 10 nm.

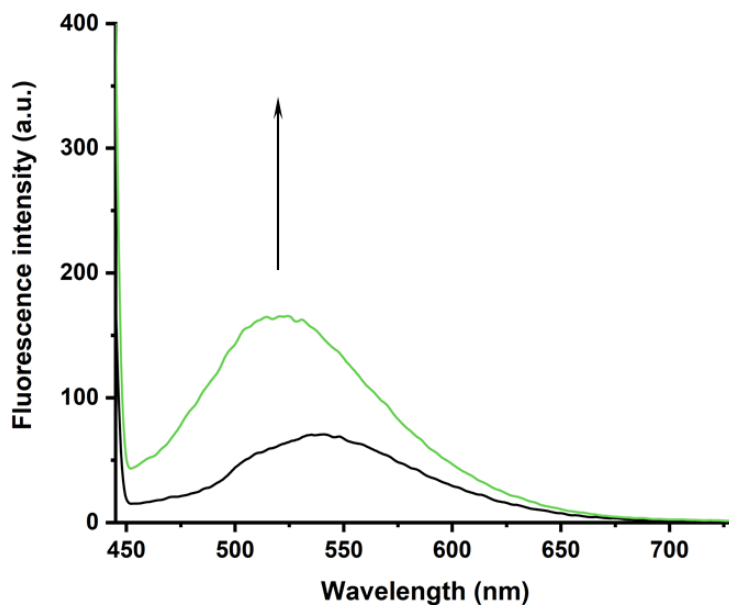


Figure 3.27. Relative fluorescence intensity of **OxaliNap (Pt6, 5 μ M)** in PBS buffer (pH = 7.40) before (**black**) and after (**green**) addition of BSA (1 equiv.). The second measurement was recorded after 60 minutes incubation, λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 10 nm.

To confirm our previous hypothesis on the reduction mechanism of **OxaliRes** and **OxaliNap**, HPLC and LC-MS analyses in the presence of sodium ascorbate were conducted. As shown in Figure 3.28 and Figure 3.29, **OxaliRes** was reduced to its Pt(II) form by NaAsc (1 mM) overtime, as observed via the release of free resorufin and oxaliplatin (OxPt(II)). The complex appeared to be completely activated after 12 hours.

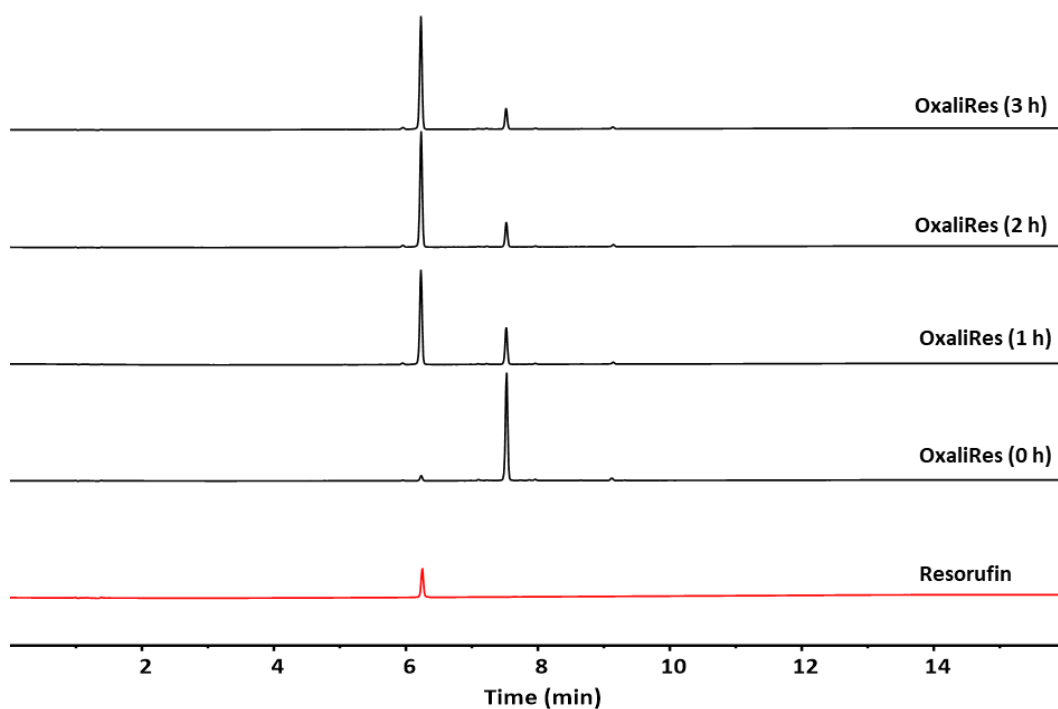


Figure 3.28. The reduction of **OxaliRes** (Pt5, 50 μ M) in PBS buffer (pH 7.40) with 1 mM sodium ascorbate recorded by HPLC (Monitoring absorption at 480 nm).

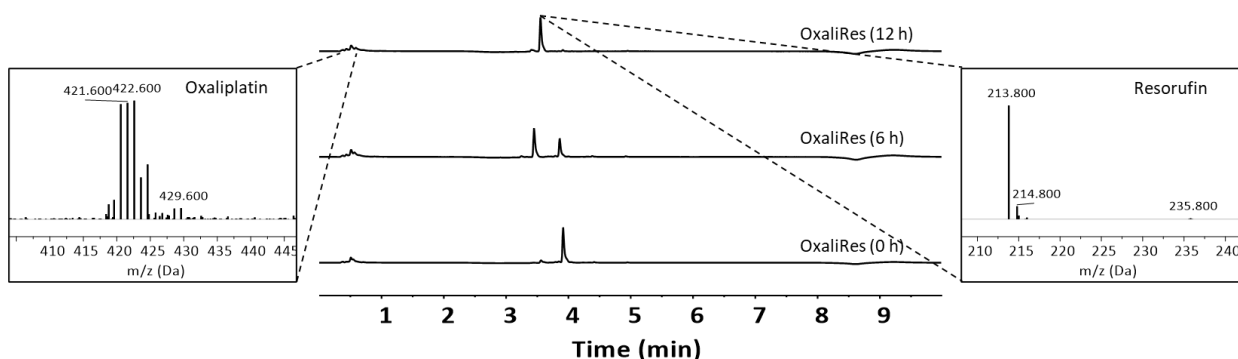


Figure 3.29. The reduction of **OxaliRes** (Pt5, 50 μ M) in deionised H_2O containing 1 mM sodium ascorbate over 12 h. The reduction process and products were recorded by LC-MS.

Upon treatment with 8 mM of NaAsc, the reduction of **OxaliNap** was barely observed after 3 hours with 12 % of the free fluorophore **L2** detected (Figure 3.30).³⁸ After 12 hours, 47 % of **L2** was recorded, showing that although NaAsc is able to reduce **OxaliNap**, larger concentrations of the reducing agent are necessary to efficiently activate the naphthalimide-Pt(IV) conjugate. In the presence of 4 mM NaAsc, formation of free 4-NH₂Nap and OxPt(II) were detected after 24 hours of incubation (Figure 3.31). These results confirm our previous observations and demonstrate the lack of sensitivity of **OxaliNap** towards sodium ascorbate in comparison to **OxaliRes**.

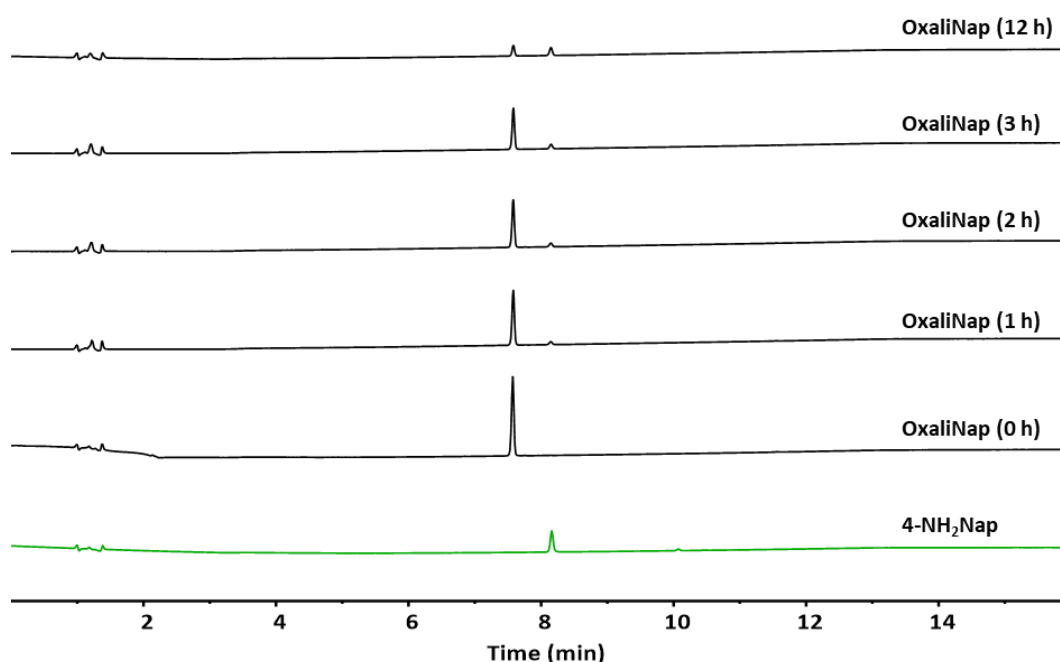


Figure 3.30. The reduction of **OxaliNap** (**Pt6**, 50 μ M) in PBS buffer (pH 7.40) with 8 mM sodium ascorbate recorded by HPLC (Monitoring absorption at 360 nm).

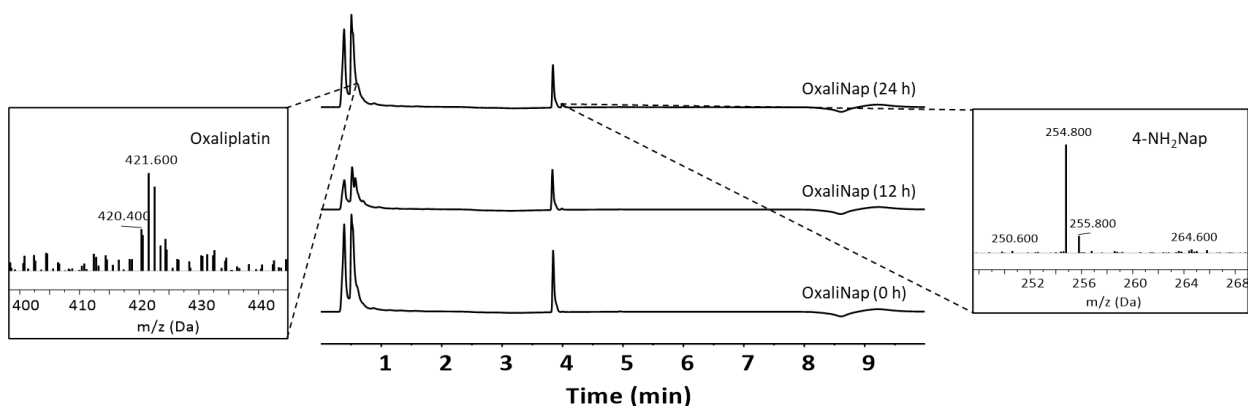


Figure 3.31. The reduction of **OxaliNap** (**Pt6**, 50 μM) in deionised H_2O containing 4 mM sodium ascorbate over 24 h. The reduction process and products were recorded by LC-MS.

3.4. Biological studies of Oxali(IV)Fluors

Based on the results described above, cellular imaging and cytotoxicity studies of **OxaliRes** and **OxaliNap** in cancerous cells were performed by Dr. Hannah Bolland in collaboration with Professor Ester Hammond at the University of Oxford.²⁵ Due to the routine administration of oxaliplatin (OxPt(II)) in the clinic for the treatment of colon cancer, two colorectal cancer cell lines HCT116 and RKO were used for biological testing.³⁹ HCT116 cells were incubated with **OxaliRes** or **OxaliNap** in the presence of increasing concentrations of NaAsc and observed under confocal microscopy (Figure 3.32). Negligible changes were recorded without sodium ascorbate. However, a clear concentration-dependent response was monitored in the presence of the reducing agent for the two Oxali(IV)Fluors. These results indicate that the presence of reducing agent in these cells is necessary to reduce the OxPt(IV) complex to the active OxPt(II). In addition, higher concentration of NaAsc and longer incubation times (120 min vs 80 min) were required to induce a “switch-on” response for **OxaliNap**, which corroborate what was previously seen in solution-based studies (Section 3.3).

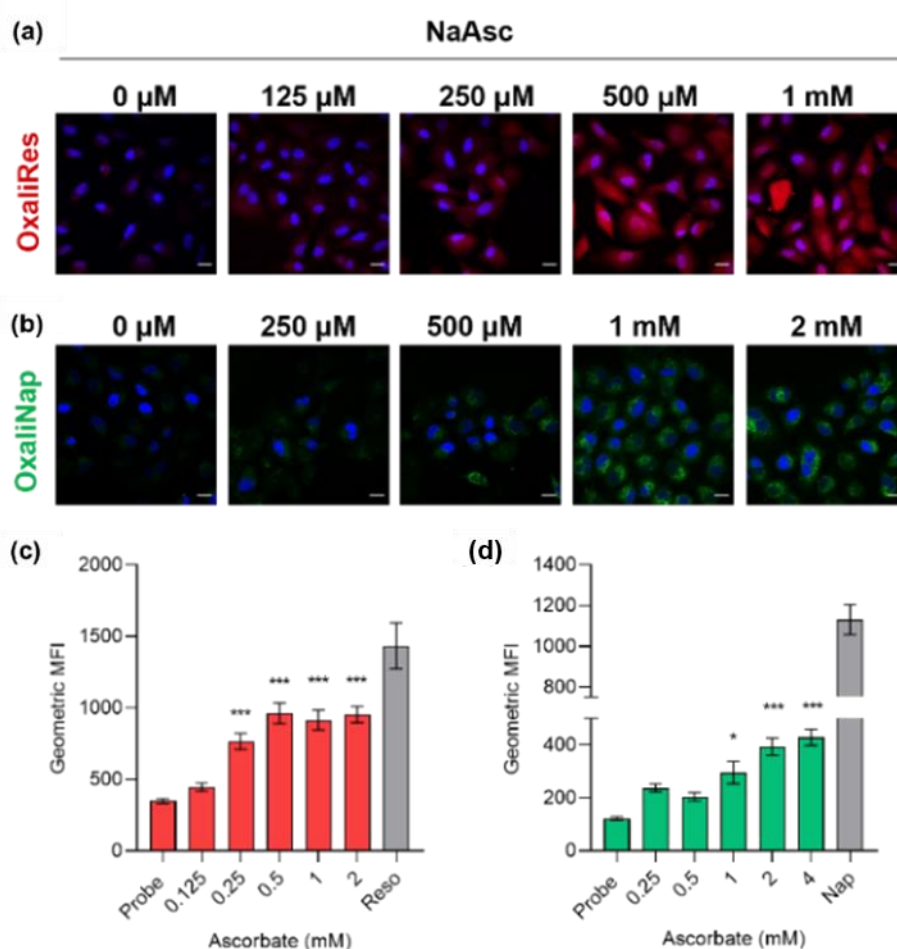


Figure 3.32. **(a)** Representative fluorescence images of HCT116 cells treated with **OxaliRes** (10 μ M) with NaAsc (0-1 mM). Cells were fixed after 80 minutes. **(b)** Representative fluorescence images of HCT116 cells treated with **OxaliNap** (10 μ M) with NaAsc (0-2 mM). Cells were fixed after 120 minutes. Scale bar in **(a)** and **(b)** represents 20 μ m and blue colour represents DAPI stain. **(c, d)** Flow cytometry analysis of HCT116 cells treated with **OxaliRes** (10 μ M) or **OxaliNap** (10 μ M) with the indicated concentrations of NaAsc. Relative fluorescence is displayed as geometric mean intensity (MFI). Error bars represent SD. Significance compared to probe alone condition. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n=3$.

The poor tissue vascularisation and abnormal rapid proliferation of cancer cells often lead to the development of regions of low oxygen content (1 %–2 % O_2 and below) in solid tumours. Malignant cells characterised by this hypoxic microenvironment are difficult to destroy as they are more resistant to the current clinical treatments (chemo-, radio- and immunotherapy) and usually result in the treatment failure and/or recurrence of the cancerous cells.^{40,41} Due to the therapeutic challenge presented by tumour hypoxia, different diagnostic and therapeutic strategies have been investigated based on the reducing microenvironment of hypoxic cancer cells.⁴² Fluorescent indicators able to detect hypoxic conditions and generate

distinct fluorescence response depending on the oxygen concentrations found in living cells have been reported.^{43,44,45} Moreover, a range of hypoxia-targeted chemotherapeutic agents have shown enhanced cytotoxic efficacy upon selective reduction under low oxygen content via the production of active species.^{46,47} Using the same approach, several Pt(IV) prodrugs were developed and demonstrated increased toxicity in hypoxic cancer cells. Although these studies are suggestive of the hypoxic-mediated reduction of Pt(IV) complexes to their Pt(II) precursors under these low oxygen conditions, the direct visualisation of the Pt(IV) prodrug activation and how it correlates to the cytotoxicity observed has not been reported.⁴⁸⁻⁵¹

With this in mind, the fluorescence response of the Oxali(IV)Fluors under normoxia (>21 % O₂) and hypoxia (<0.1 % O₂) was investigated by confocal microscopy and quantified by flow cytometry analyses. HCT116 cells were treated with **OxaliRes** or **OxaliNap** and monitored over a period of 6 hours while being exposed to severe oxygen levels (<0.1 % O₂). As displayed in Figure 3.33, fluorescence emission intensity increases were observed with time for both OxPt(IV) conjugates which might indicate reduction of the Pt(IV) complexes in hypoxic conditions. Since cancer cells with hypoxic microenvironments can possess varying levels of oxygen concentrations (from mild (≤ 2 %) to severe (<0.1 %)), **OxaliRes** and **OxaliNap** were exposed to a range of O₂ levels to study the dependency of the OxPt(IV) complexes to oxygen (Figure 3.34). Fluorescence imaging after 6 hours of incubated HCT116 cells displayed an oxygen-dependent response in green and red emissions with increases in fluorescence intensity recorded under 4 % O₂ for **OxaliRes** and under 1.5 % O₂ for **OxaliNap**. These observations revealed a potential correlation between the sensitivity to NaAsc and the oxygen dependency of the oxaliplatin(IV) carbamates. Furthermore, the results obtained suggest that **OxaliRes** and **OxaliNap** are reduced to the OxPt(II) complex under hypoxic conditions in the selected cancer cell line.

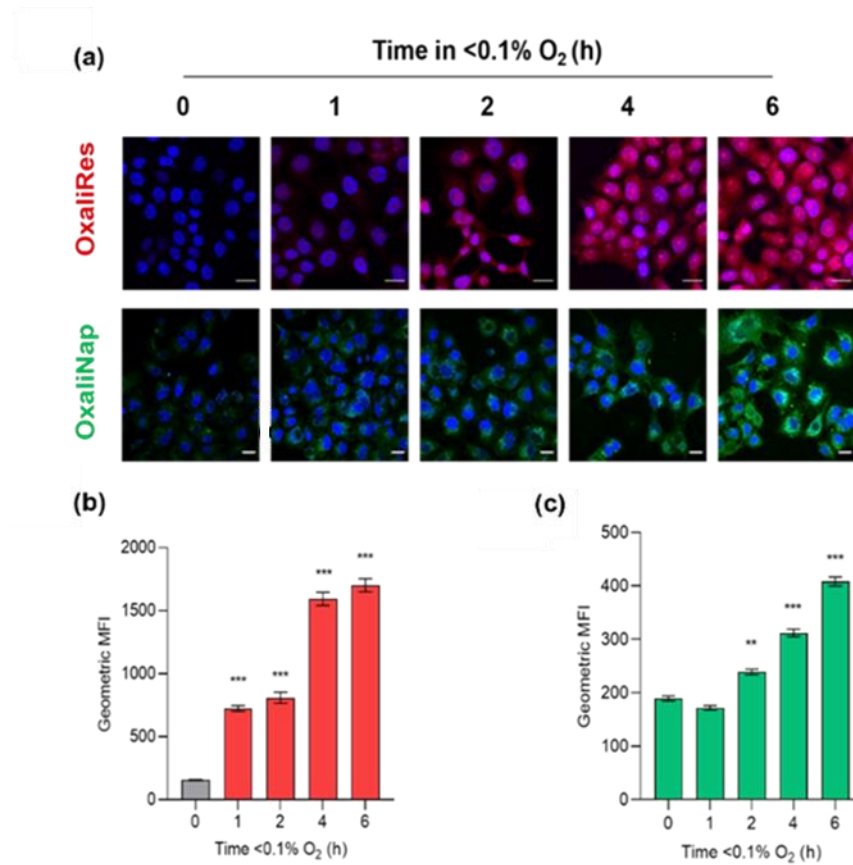


Figure 3.33. HCT116 cells were treated with **OxaliRes** or **OxaliNap** (10 μ M) for the times indicated (0-6 h) in <0.1 % O₂. **(a)**. Representative images are shown. Images were taken at 63x magnification. Scale bar represents 20 μ m. HCT116 cells were treated with **OxaliRes** (10 μ M) for the times indicated in hypoxia followed by flow cytometry. HCT116 cells were treated with **OxaliNap** (10 μ M) for the times indicated in hypoxia followed by flow cytometry. In **(b)** and **(c)** relative fluorescence is displayed as geometric mean intensity (MFI). Error bars represent SD. Significance compared to normoxic control. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n=3$.

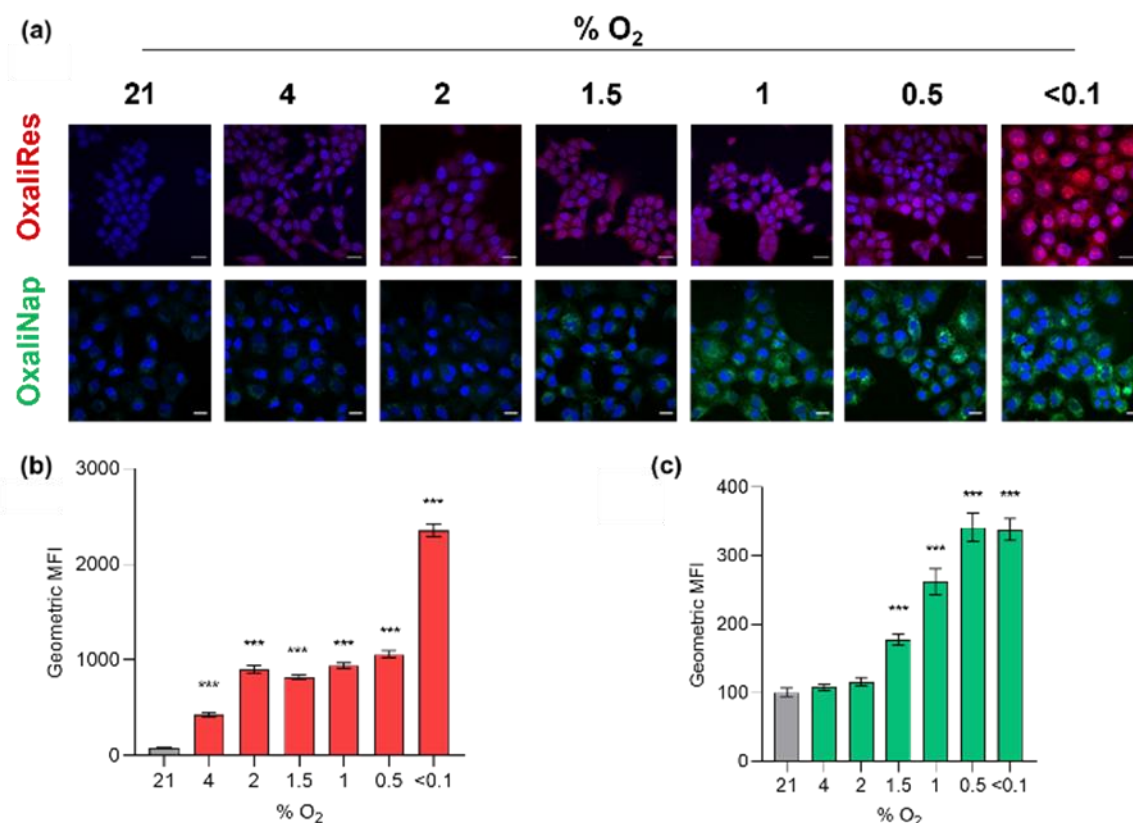


Figure 3.34. **(a)** Representative fluorescence images of HCT116 cells treated with **OxaliNap** or **OxaliRes** (10 μ M) for 6 hours in the oxygen conditions indicated. Scale bar represents 20 μ m and blue colour represents DAPI stain. **(b, c)** Flow cytometry of HCT116 cells treated with **OxaliRes** (10 μ M) or **OxaliNap** (10 μ M) for 6 hours at the oxygen level indicated. Relative fluorescence is displayed as geometric mean intensity (MFI). Error bars represent SD. Significance compared to normoxic control. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n=3$.

Next, clonogenic cells survival and MTT assays were conducted to evaluate the cytotoxicity profiles of our Oxali(IV)Fluors against two colorectal cancer cell lines, HCT116 and RKO cells, under normoxic (21 % O₂) and severe hypoxic conditions (<0.1 % O₂). HCT116 cells were initially treated with increasing concentrations of the fluorophores over 3 days (Figure 3.35). Results demonstrated that resorufin and the naphthalimide-based derivative displayed no cytotoxic activity in HCT116 cells in both conditions.

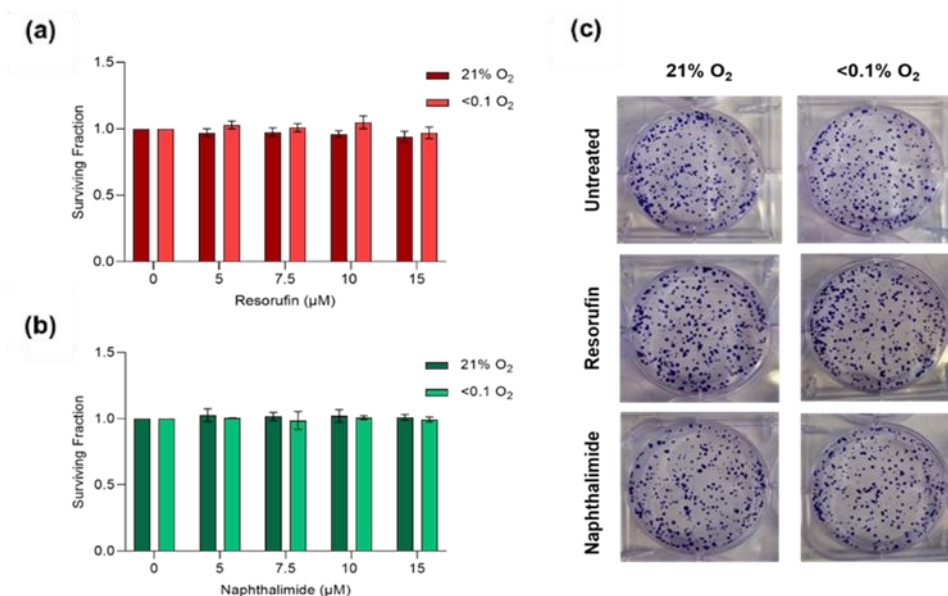


Figure 3.35. HCT116 cells were treated with the indicated concentrations of **Resorufin** or **Naphthalimide** (4-NH₂Nap) for 3 days. Hypoxic cells were exposed to <0.1 % O₂ for 16 hours with addition of either Resorufin or Naphthalimide. Cell survival was measured via clonogenic assay. **(a) Resorufin** surviving fraction. **(b) Naphthalimide** surviving fraction. **(c)** Representative images of clonogenic assay. Error bars represent SD.

The cytotoxicity study of the fluorescence responsive oxaliplatin(IV) complexes in HCT116 and RKO cell lines is shown in Figure 3.36 and Figure 3.37, respectively. Cells were treated with the Oxali(IV)Fluors for 3 days and exposed to low oxygen content (<0.1% O₂) over 16 hours. **OxaliRes** displayed higher cytotoxicity under hypoxia compared to normoxia against HCT116 and RKO cells **(a)**. Similar results were recorded for **OxaliNap** **(b)**, however, the Pt(IV) complex also exhibited cytotoxic effect in normoxic conditions. The cell imaging and toxicity studies revealed that even though **OxaliNap** is less sensitive to reduction than **OxaliRes**, the naphthalimide-OxPt(IV) conjugate might be toxic to cancer cells prior to its reduction to the Pt(II) precursor.

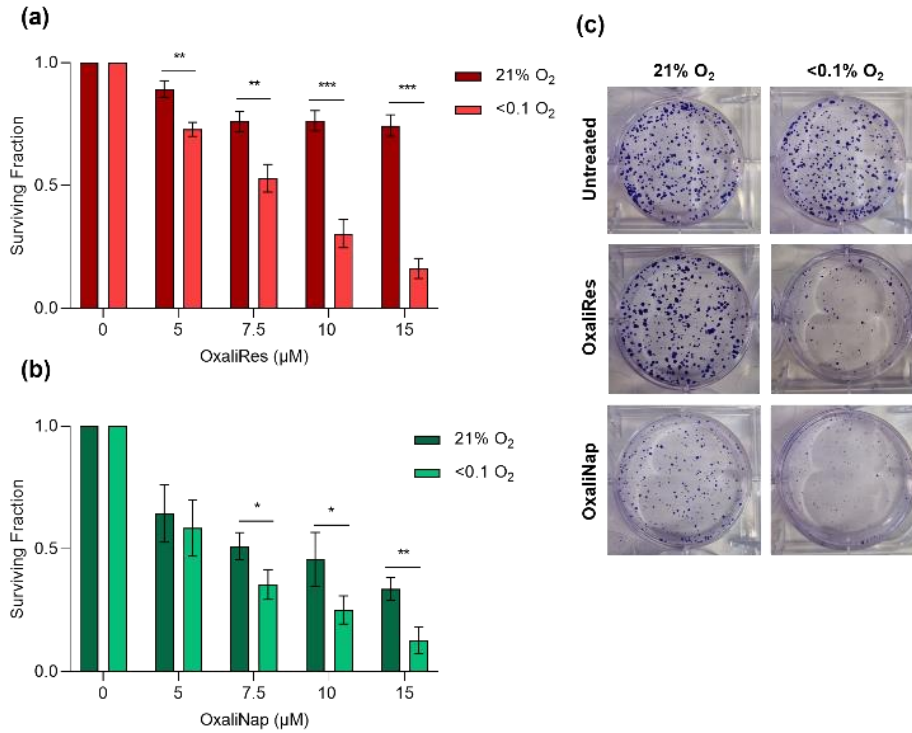


Figure 3.36. HCT116 cells were treated with the indicated concentrations of **OxaliRes** (a) or **OxaliNap** (b) for 3 days. Hypoxic cells were exposed to <0.1 % O₂ for 16 hours. Cell survival was measured via clonogenic assay. (c) Representative images of clonogenic assay. Error bars represent SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n=3$.

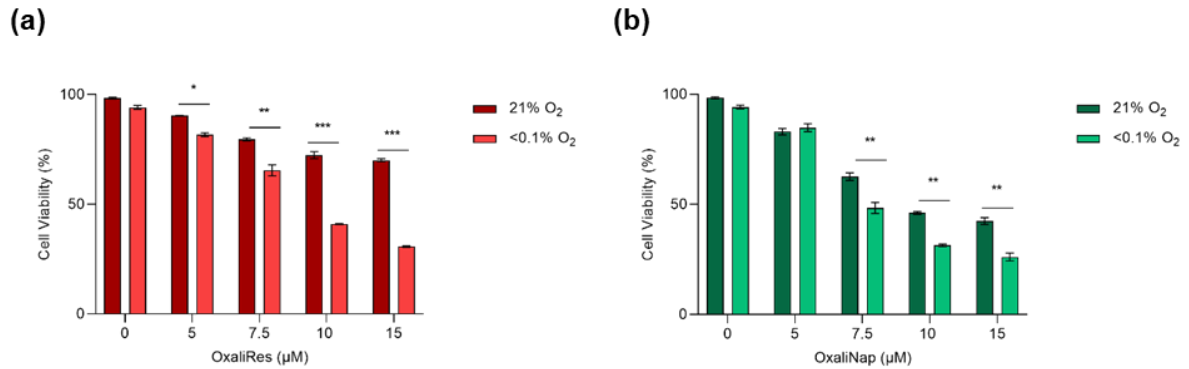


Figure 3.37. RKO cells were treated with the indicated concentrations of **OxaliRes** or **OxaliNap** for 3 days. Hypoxic cells were exposed to <0.1 % O₂ for 16 hours with addition of either **OxaliRes** or **OxaliNap**. Cell viability was measured via MTT assay. (a) **OxaliRes** cell viability. (b) **OxaliNap** cell viability. Data presented are percentage cell viability relative to untreated control. Error bars represent SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n=3$.

Finally, the cytotoxicity of oxaliplatin (OxPt(II)) and its oxidised form OxPt(IV) in HCT116 and RKO cells were investigated (Figure 3.38 and Figure 3.39). As expected, OxPt(II) **(a)** was significantly more potent than any other compounds and displayed similar cytotoxicity profiles under both normoxic and hypoxic conditions. In comparison, minor differences in cytotoxic efficacy were observed for OxPt(IV) between normoxia and hypoxia **(b)**. Altogether, the results obtained in this study demonstrated that the carbamate ligands greatly influence the cytotoxic profile of the oxaliplatin(IV) complexes before reduction in malignant cells. Thus, to differentiate between the potency of the complexes in hypoxia compared to non-hypoxic conditions, looking into the differences in sensitivity to reduction of functionalised Pt(IV) complexes is essential.

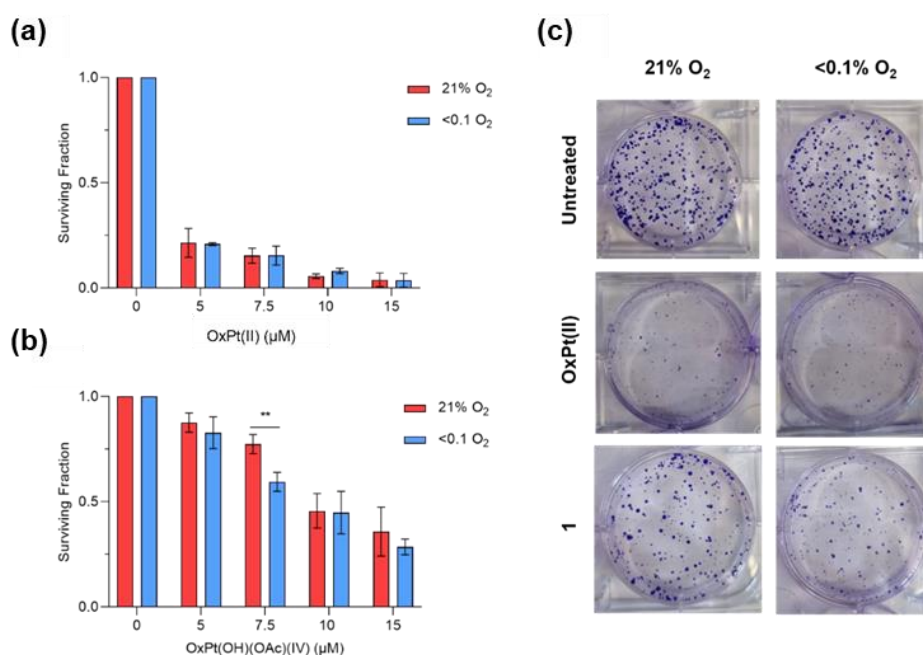


Figure 3.38. HCT116 cells were treated with the indicated concentrations of **Oxaliplatin (OxPt(II))** or **(Pt4)** for 3 days. Hypoxic cells were exposed to <0.1 % O₂ for 16 hours with addition of either **OxPt(II)** or **(Pt4)**. Cell survival was measured via clonogenic assay. **(a)** **OxPt(II)** surviving fraction. **(b)** **(Pt4)** surviving fraction. **(c)** Representative images of clonogenic assay. Error bars represent SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n=3$.

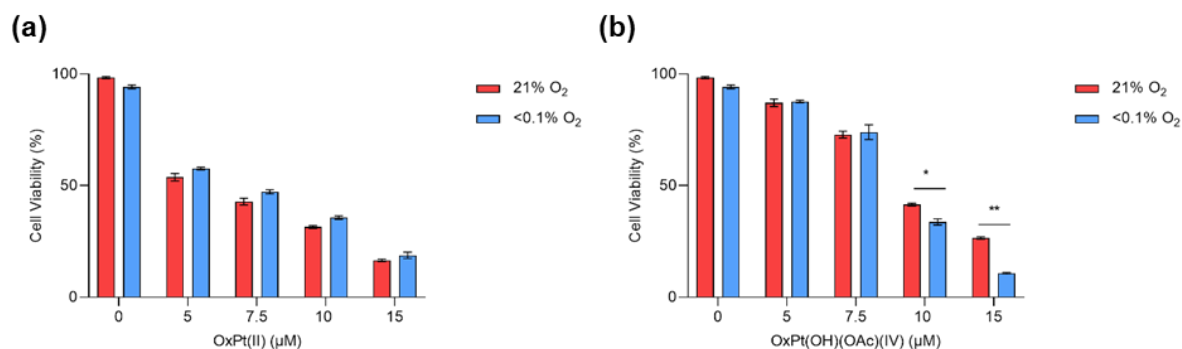
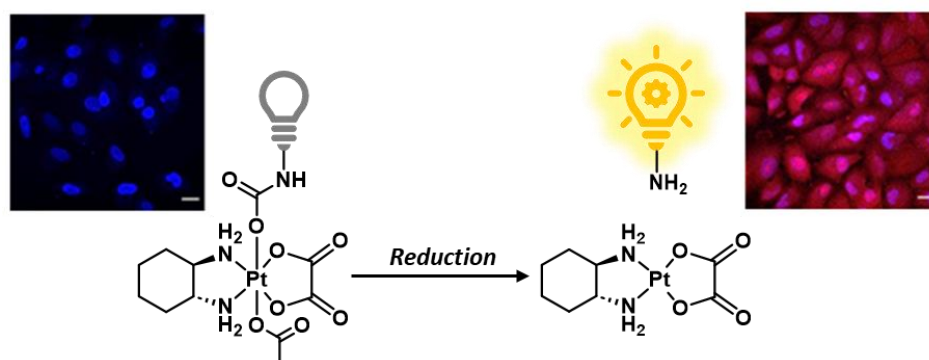


Figure 3.39. RKO cells were treated with the indicated concentrations of **OxPt(II)** or (**Pt4**) for 3 days. Hypoxic cells were exposed to <0.1 % O_2 for 16 hours with addition of either **OxPt(II)** or (**Pt4**). Cell viability was measured via MTT assay. **(a) OxPt(II)** cell viability. **(b) (Pt4)** cell viability. Data presented are percentage cell viability relative to untreated control. Error bars represent SD. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n=3$.

3.5. Conclusion

This chapter presented the synthesis and characterisation of two fluorescence responsive oxaliplatin-based platinum(IV) carbamate complexes: **OxaliRes (Pt5)** and **OxaliNap (Pt6)**. The OxPt(IV) carbamates were designed according to two different sensing strategies: a self-immolative linker approach involving the red fluorophore resorufin (**OxaliRes**) and the direct coordination of a naphthalimide dye (**OxaliNap**). A comparative study of the photophysical properties, stability, and reduction profiles of the Oxali(IV)Fluors in solution-based studies was conducted. A fluorescence “turn-on” response was observed for the two complexes in the presence of sodium ascorbate, accompanied by a significant difference in sensitivity towards the reducing agent between the two strategies. Similar results were monitored upon treatment with NaAsc in colorectal cancer cell lines using fluorescence imaging. In general, **OxaliNap** required greater concentrations of NaAsc to trigger a proper fluorescence response in solution and in malignant cells. Cell imaging experiments revealed the ability of tumour hypoxia to reduce OxPt(IV) carbamate complexes to the active oxaliplatin(II) drug. The greatest fluorescence emission intensity was observed under severe hypoxic conditions (<0.1 % O₂) for **OxaliRes** and **OxaliNap**, with a distinct difference in cytotoxicity efficacy compared to normoxia (21 % O₂). Overall, the use of fluorescence imaging enabled the direct visualisation of the Oxali(IV)Fluors activation under hypoxia in selected cancer cell lines and helped identify tumour hypoxia as a potential therapeutic target.²⁵



3.6. References

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Chapter 4 The study of platinum(IV) reduction: *Cis(IV)Fluors* and *Carbo(IV)Fluors*

4.1. Introduction

Despite the adverse effects caused by the repeated administration of cisplatin, the platinum anticancer drug remains one of the leading chemotherapeutic agents prescribed in the clinic due to its high potency and efficacy to treat a wide spectrum of cancers. The square-planar Pt(II) complex is characterised by two labile *cis*-chlorido ligands opposing non-leaving diammine groups.^{1,2} The compound is highly susceptible to aquation due to the presence of the equatorial chlorido ligands, resulting in their substitution with water molecules at a fast exchange rate upon cisplatin uptake in cells. The activated complex is subsequently able to form intrastrand crosslink cisplatin-DNA adducts promoting cell death mechanisms.^{3,4} The activation of the complex can, however, take place in both malignant and healthy tissues as the drug is defined by a low selectivity for cancer cells which results in chemotherapy-induced toxic effects (e.g., nephrotoxicity, neurotoxicity, and emetogenesis). Another major problem related to the use of cisplatin is the acquired chemoresistance developed overtime by tumours.^{5,6,7} The search for platinum-based chemotherapeutics with less side-effects was motivated by the great cytotoxic efficacy demonstrated by cisplatin and led to the development of novel Pt(II) complexes including the second FDA-approved anticancer drug carboplatin.⁸ Instead of two chlorido ligands, the complex was functionalised with a chelating cyclobutane-1,1-dicarboxylate (CBDCA) group which significantly altered the activity profile of the drug in comparison to cisplatin. The stability of the complex was increased whilst its potency diminished mainly due to the delayed aquation of the bidentate leaving ligand once inside the cells.^{4,9} Despite exhibiting fewer side-effects, severe systemic toxicities are still experienced with the clinical administration of carboplatin and limit its therapeutic application.

In an attempt to improve the therapeutic efficacy and avoid undesired toxicities, several approaches have been explored including the design of kinetically inert Pt(IV) derivatives able to be reduced in a controlled manner in cancer cells. A wide array of axial ligands with versatile

functions have been combined to Pt(IV) prodrugs to customize their pharmacological properties, targeting abilities and kinetic profiles.¹⁰⁻¹³ Fluorescent systems have been developed to monitor the reduction process and examine the cellular localisation and accumulation of cisplatin-, carboplatin- and oxaliplatin-based platinum(IV) complexes to provide a better understanding of their intracellular mechanisms.¹⁴⁻¹⁷ Photoactive Pt(IV) prodrugs have also been extensively investigated to achieve precise activation at the tumour site upon irradiation using external triggers such as visible to near-IR light sources¹⁸⁻²² and more recently with ultrasound.²³ The dark stability, photo-activity, and cytotoxic properties of these compounds were, however, found to be highly dependent on the ligand surrounding the platinum centre. A great number of studies on the effects of the axial, leaving and non-leaving ligands on these parameters have been conducted and might contribute to the development of more selective Pt-based drugs for cancer therapy.^{24,25,26}

As presented in *Chapter 3*, two fluorescence responsive oxaliplatin(IV) prodrugs were designed to study the intracellular activation of OxPt(IV) complexes and a hypoxic-mediated reduction was identified in colorectal cancer cells.²⁷ In this chapter, the same strategy was extended to develop the fluorescent cisplatin- and carboplatin-based platinum(IV) analogues. The aim of this project was to compare the reduction properties, stability, and cytotoxic profiles of these novel complexes containing distinct Pt(II) scaffolds (Figure 4.1).

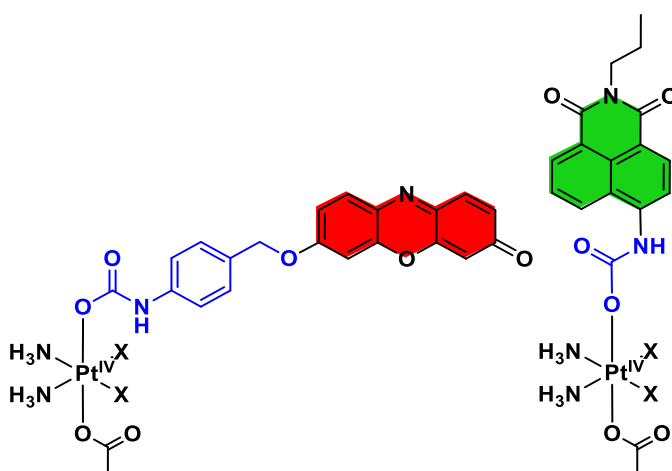
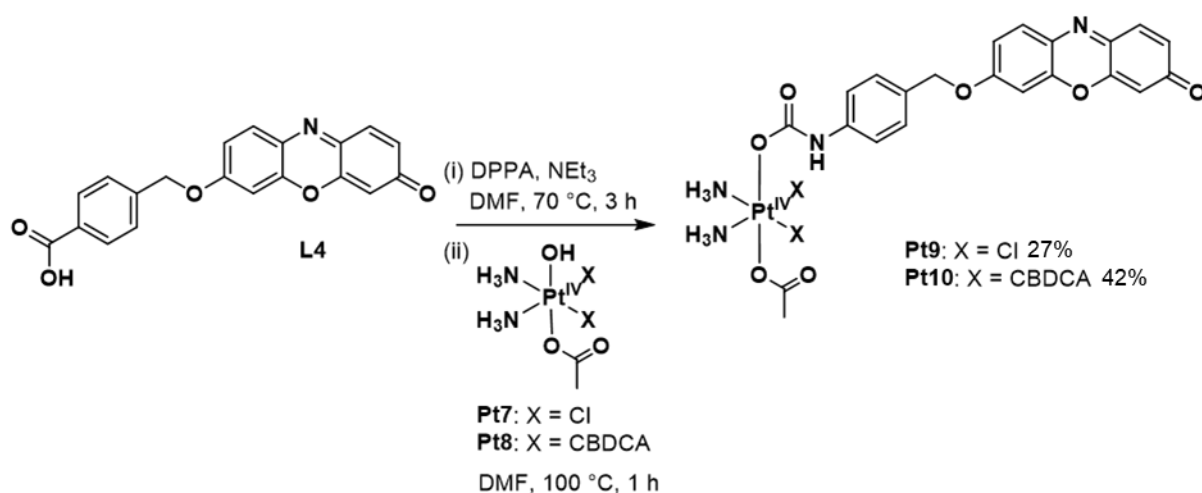


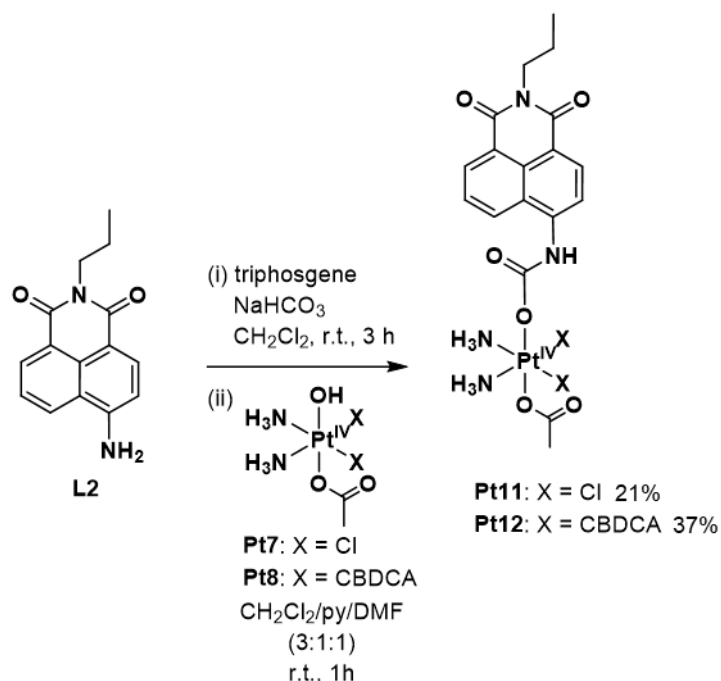
Figure 4.1. Self-immolative system (left) and direct coordination (right) strategies for CisPt(IV) (with X = Cl) and CarboPt(IV) (with X = CBDCA) prodrugs applied in this project.

4.2. Synthesis and characterisation of Cis(IV)Fluors and Carbo(IV)Fluors

Scheme 4.1 and Scheme 4.2 illustrate the synthetic routes of the cisplatin- and carboplatin-based platinum(IV) derivatives of **OxaliRes** and **OxaliNap**. Following previously reported methods, oxidation reactions of the Pt(II) drugs cisplatin and carboplatin were performed using hydrogen peroxide and acetic acid to yield CisPt(OH)(OAc) (Complex **Pt7**) and CarboPt(OH)(OAc) (Complex **Pt8**).^{28,29} The two procedures presented in *Chapter 3* for the synthesis of the oxaliplatin(IV) carbamates were applied to prepare **CisRes (Pt9)**, **CarboRes (Pt10)**, **CisNap (Pt11)** and **CarboNap (Pt12)**.²⁷ Trituration of the complexes with ethyl acetate, methanol or chloroform was required after purification by column chromatography to remove the remaining impurities. The Cis(IV)Fluors were more difficult to synthesise compared to the Carbo(IV)Fluors and Oxali(IV)Fluors and were collected in lower yields: 27 % for **CisRes** and 21 % for **CisNap**. **CarboRes** and **CarboNap** were obtained in decent yields 42 % and 37 %, respectively. The Pt(IV) complexes were characterised by high-resolution mass spectrometry and ¹H, ¹³C and ¹⁹⁵Pt NMR spectroscopy.



Scheme 4.1. Synthesis of Pt(IV)-resorufin derivatives: **CisRes (Pt9)** and **CarboRes (Pt10)**.



Scheme 4.2. Synthesis of the Pt(IV)-naphthalimide derivatives: **CisNap** (**Pt11**) and **CarboNap** (**Pt12**).

Figure 4.2 and Figure 4.3 display the assigned ¹H NMR spectra of **CisRes** and **CarboRes** whilst the ¹H NMR spectra of **CisNap** and **CarboNap** are illustrated in Figure 4.4 and Figure 4.5, respectively. All resonances corresponding to the fluorophores resemble the ones observed in the ¹H NMR spectra of **OxaliRes** and **OxaliNap**. The chemical shift of the equatorial NH₃ protons is present between 6.3-6.8 ppm for all four complexes and the resonance of the CONH proton is detected around 9 ppm. The signals of the NH proton of the carbamates are slightly downfield for the two naphthalimide-platinum(IV) conjugates compared to the two resorufin-platinum(IV) complexes (9.75 ppm vs 9.11/9.16 ppm). As expected, the chemical shifts of the bidentate dicarboxylate (CBDCA) ligand of carboplatin are situated in the upfield region of the spectrum between 1.80-2.59 ppm. A sharp singlet around 1.9 ppm is present in the spectra and assigned to the CH₃ protons of the axial acetate group.

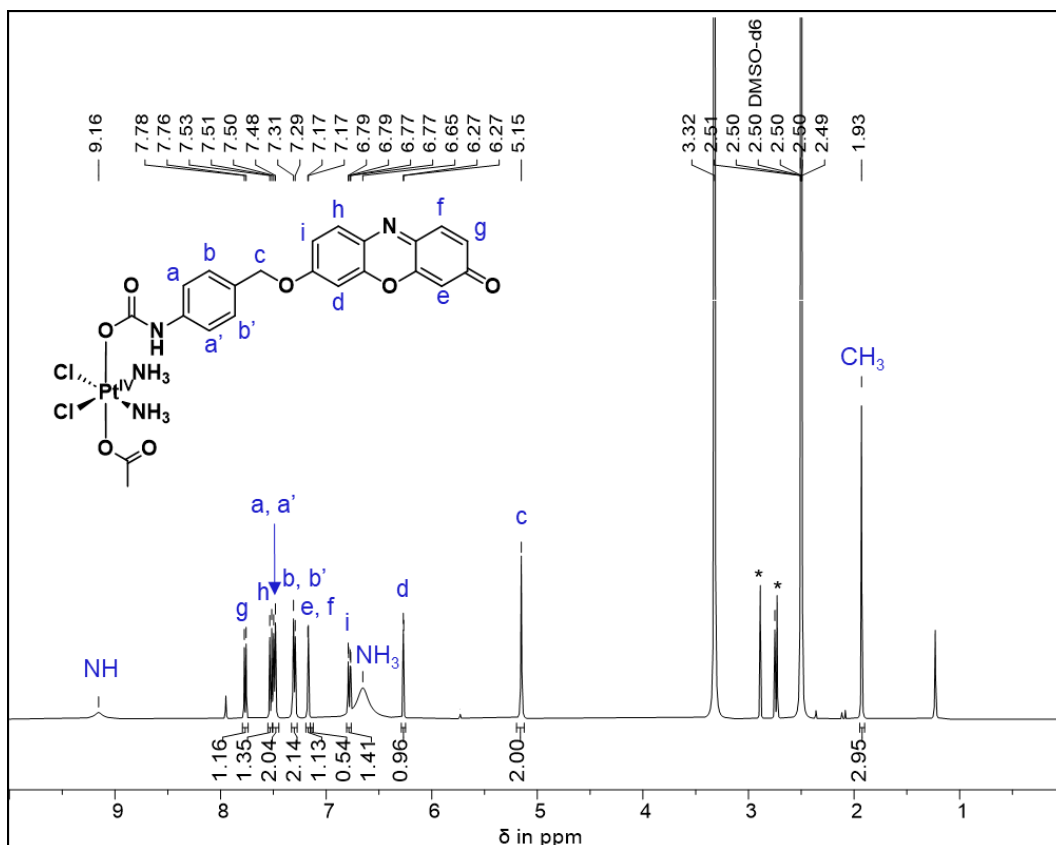


Figure 4.2. ^1H NMR (400 MHz, DMSO-d_6) of *CisRes* (**Pt9**) (*DMF).

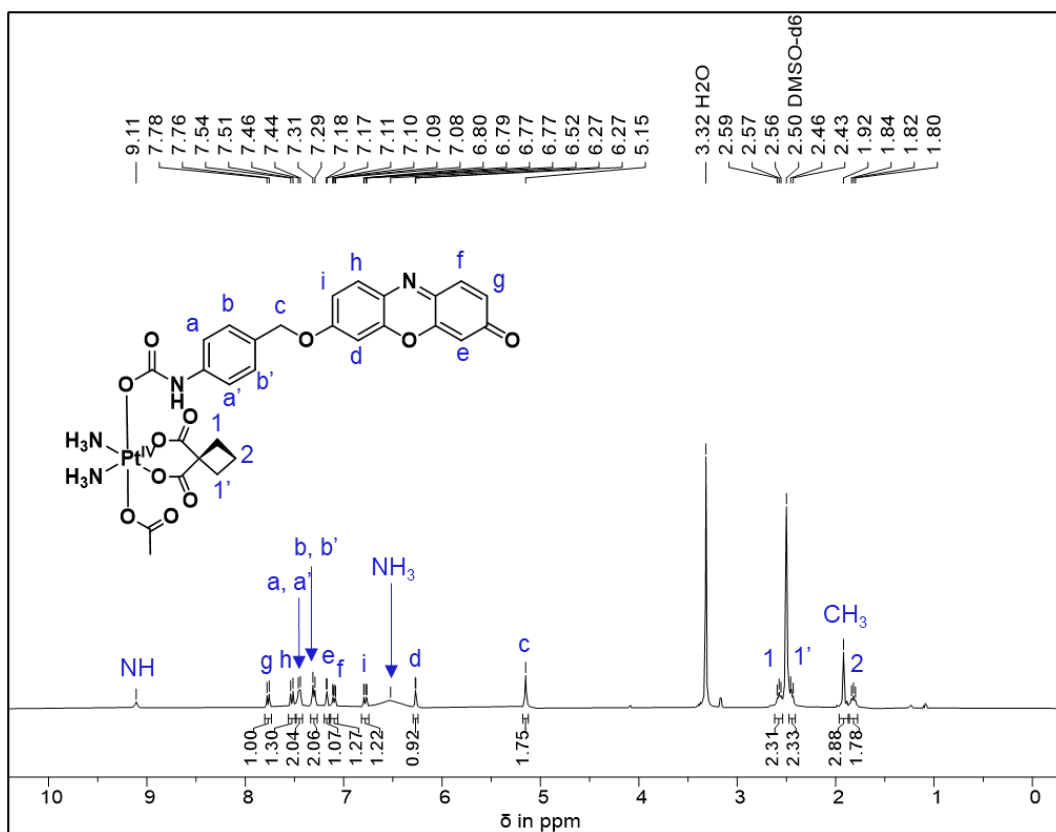


Figure 4.3. ^1H NMR (400 MHz, DMSO-d_6) of *CarboRes* (**Pt10**).

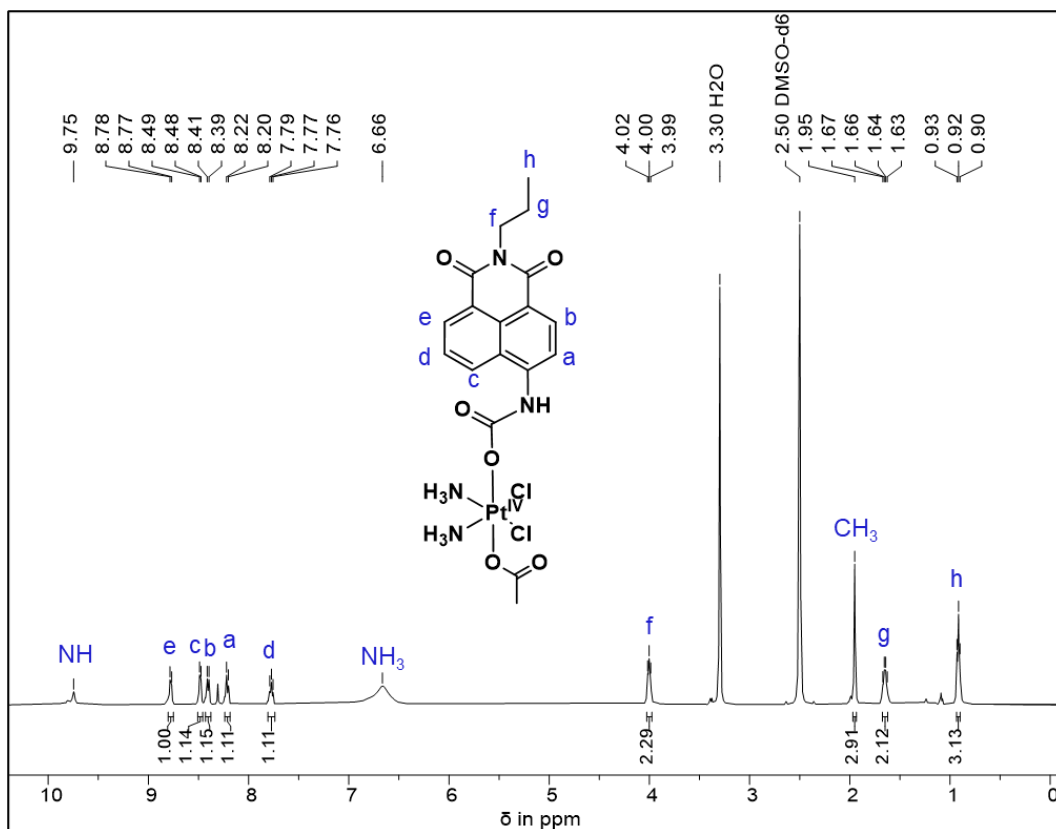


Figure 4.4. ¹H NMR (500 MHz, DMSO-d₆) of *CisNap* (Pt11).

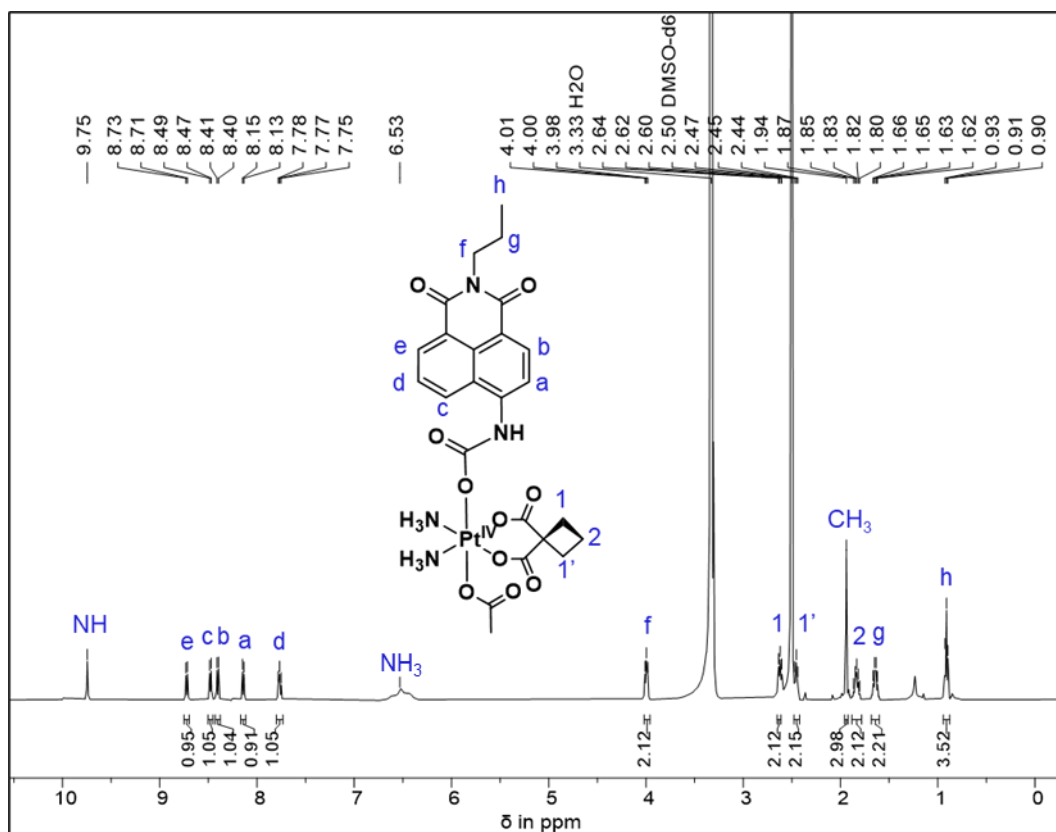


Figure 4.5. ¹H NMR (500 MHz, DMSO-d₆) of *CarboNap* (Pt12).

The ^{195}Pt NMR spectra of the fluorescent Pt(IV) conjugates are shown in Figure 4.6. Coordination of the fluorophores resulted in a similar downfield shift of approximately 200 ppm in the platinum resonances of **CisRes** (1257 ppm), **CarboRes** (1970 ppm), **CisNap** (1256 ppm) and **CarboNap** (1972 ppm). Due to differences in solubility, the ^{195}Pt NMR spectra of **OxaliRes** and **OxaliNap** were recorded in DMSO- d_6 and in D_2O for the precursor $\text{OxPt}(\text{OH})(\text{OAc})$, causing a slightly larger downfield shift to be observed (300 ppm). Overall, the chemical shifts listed are in the expected range for cisplatin(IV) and carboplatin(IV) complexes.^{30,31}

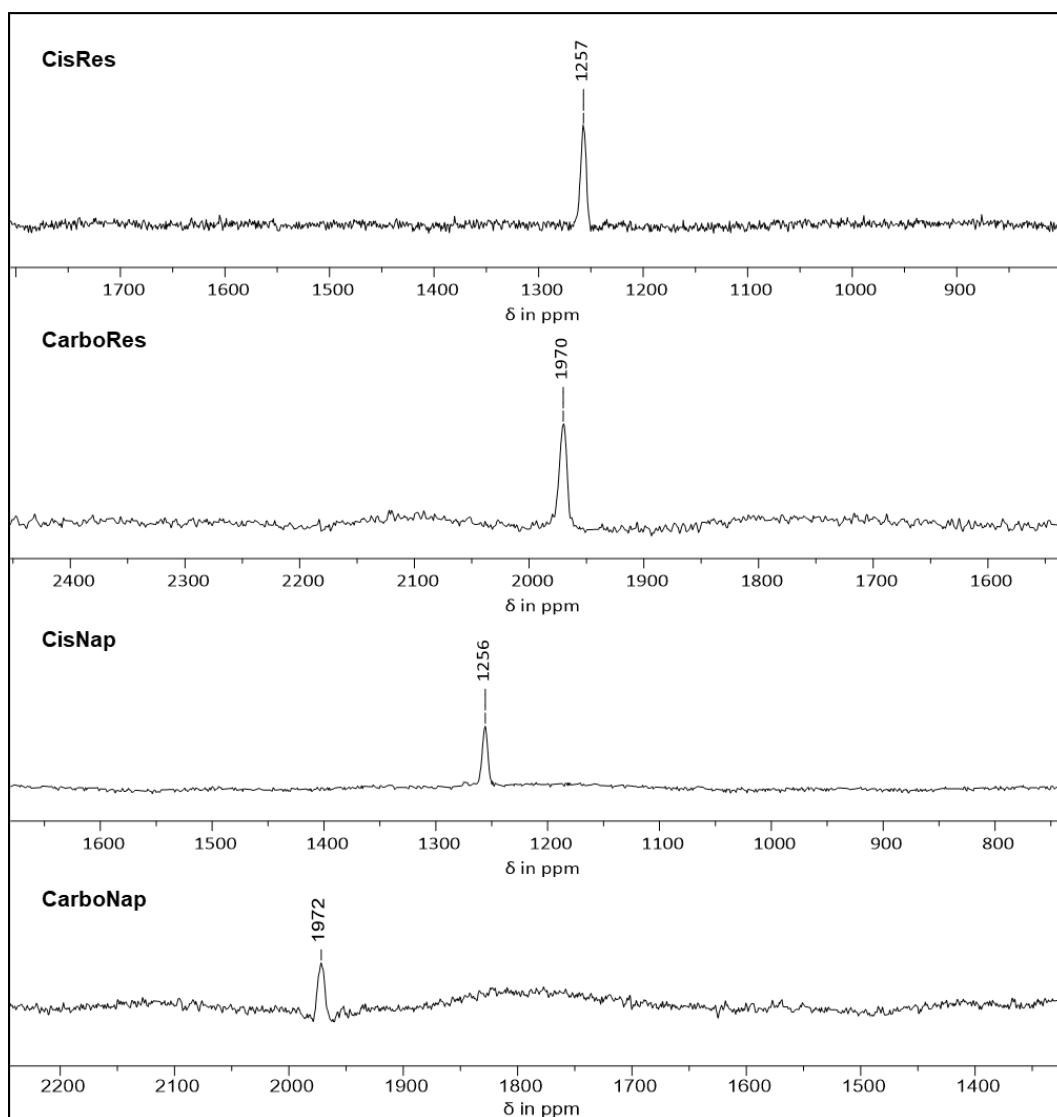


Figure 4.6. ^{195}Pt NMR (107 MHz, $\text{DMSO}-d_6$) of **CisRes** (Pt9), **CarboRes** (Pt10), **CisNap** (Pt11) and **CarboNap** (Pt12).

4.3. Analytical studies on *Cis(IV)Fluors* and *Carbo(IV)Fluors*

Based on the results described in *Chapter 3* on the *Oxali(IV)Fluors*, comparable experiments investigating the reduction properties, sensitivity, and stability of the *Cis(IV)Fluors* and *Carbo(IV)Fluors* analogues were conducted in aqueous buffered solution. We initially studied the sensitivity to reduction of each Pt(IV) complexes with sodium ascorbate (NaAsc) over a period of 8 hours. As displayed in Figure 4.7, treatment of **CisRes** with 500 μ M of NaAsc led to a rapid “turn-on” response with a 9-fold change recorded after only two hours. Under the same conditions, **CarboRes** was significantly less sensitive towards the reducing agent and a small fluorescence increase was observed at 585 nm over 8 hours (3-fold). A higher concentration of sodium ascorbate (1 mM) and longer incubation times were required to generate a distinct fluorescence response for **CisNap** (Figure 4.8). In comparison, **CarboNap** was almost unresponsive to the reducing agent over the course of 8 hours. The fluorescence response of the *Oxali(IV)Fluors* to sodium ascorbate was added to compare the sensitivity profiles of the three platinum(IV) derivatives between the two sensing strategies.

Depending on the functionalisation of the axial ligands (self-immolative linker vs direct attachment) and the Pt(II) scaffolds used, we observe distinct differences in the reduction profiles of these platinum(IV) prodrugs. The resorufin-Pt(IV) conjugates seem to be reduced more quickly and therefore display higher sensitivity towards NaAsc than their naphthalimide-Pt(IV) counterparts. This is supported by our previous findings on the influence played by the axial carbamate ligands on the rate of reduction and sensitivity of the *Oxali(IV)Fluors* in solution and in cancer cells.²⁷ In general, cisplatin(IV) is more prone to reduction (most sensitive) than its oxaliplatin(IV) analogue whereas the carboplatin(IV) derivative appears to be the hardest to reduce (least sensitive). These observations are consistent with an in-depth study conducted by Zhu and colleagues on the relationship between the reduction properties of cisplatin-, carboplatin- and oxaliplatin-based Pt(IV) carbamate analogues and the coordinated ligands to the Pt centre. A similar trend in the reduction rates of these Pt(IV) complexes in the presence of sodium ascorbate or glutathione was demonstrated by HPLC analysis and NMR

spectroscopy experiments.³¹ Altogether, the results presented so far suggest that the axial and equatorial ligands play an important role in the reduction profiles of Pt(IV) prodrugs.

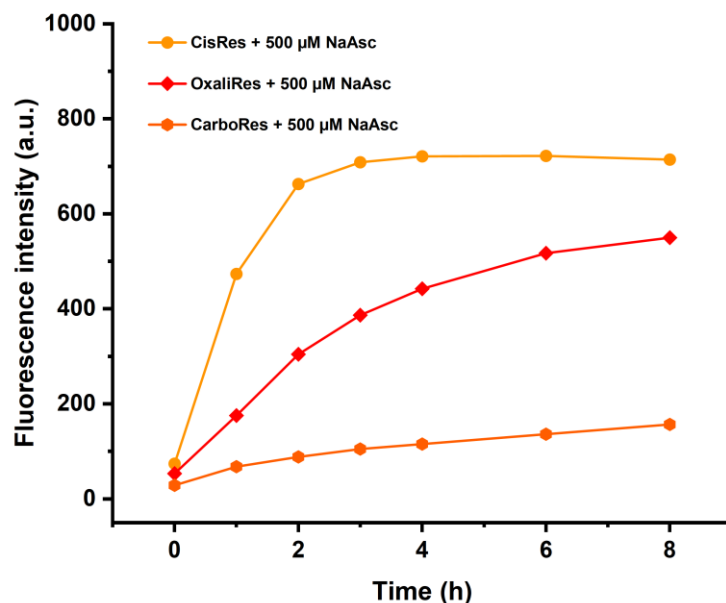


Figure 4.7. Changes in the fluorescence intensity of **CisRes**, **OxaliRes** and **CarboRes** (5 μM) at 585 nm with the addition of NaAsc (500 μM) in PBS buffer (pH = 7.40), λ_{ex} = 500 nm, slit widths: λ_{ex} = 10 nm and λ_{em} = 2.5 nm, ($n=1$).

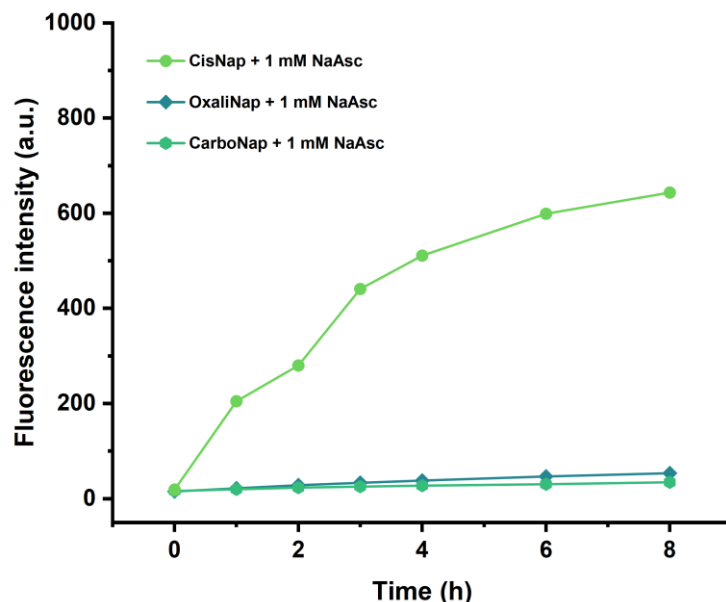


Figure 4.8. Changes in the fluorescence intensity of **CisNap**, **OxaliNap** and **CarboNap** (5 μM) at 545 nm with the addition of NaAsc (1 mM) in PBS buffer (pH = 7.40), λ_{ex} = 430 nm, slit widths: λ_{ex} = 10 nm and λ_{em} = 3.5 nm, ($n = 1$).

Due to the lack of sensitivity observed for **CarboRes** and **CarboNap**, fluorescence titration experiments were conducted with sodium ascorbate and collected by another PhD student from our group Jevon W. Marsh (University of Oxford). To generate a significant fluorescence response, higher concentrations of the reductant were needed for **CarboRes** (500 μ M to 10 mM) and to a greater extent for **CarboNap** (5 mM to 50 mM). As shown in Figure 4.9 and Figure 4.10, **CarboRes** exhibited a clear “turn-on” response upon incubation with 10 mM NaAsc (7.5-fold change). On the other hand, addition of 50 mM of reducing agent to **CarboNap** resulted in minimal increases in fluorescence emission intensity, thus highlighting the extremely low sensitivity of the naphthalimide-carboplatin(IV) conjugate towards sodium ascorbate (Figure 4.11 and Figure 4.12).

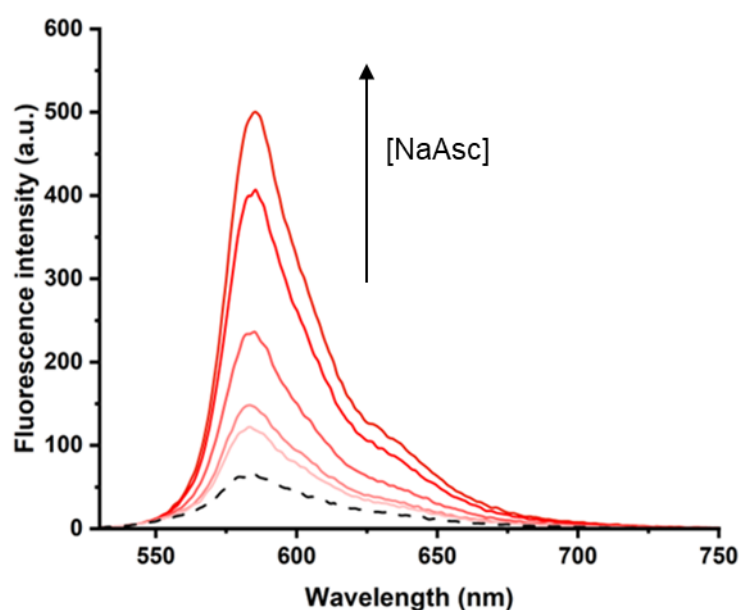


Figure 4.9. Fluorescence spectra of **CarboRes** (**Pt10**, 5 μ M) in PBS buffer (pH = 7.40) with increasing concentrations of NaAsc (500 μ M, 1 mM, 2 mM, 6 mM, 10 mM) (1 h incubation), λ_{ex} = 500 nm, slit widths: λ_{ex} = 10 nm, λ_{em} = 2.5 nm.

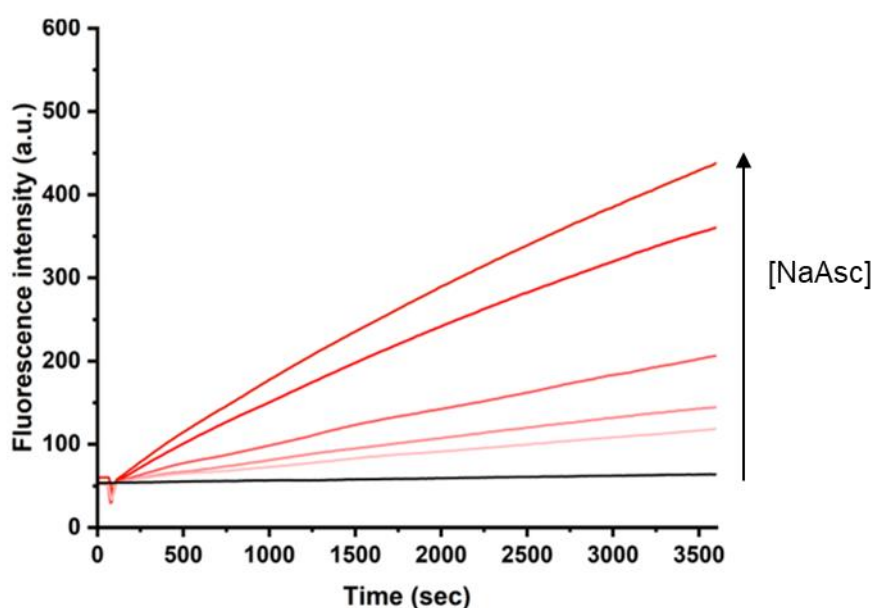


Figure 4.10. Changes in fluorescence emission intensity of **CarboRes (Pt10, 5 μ M)** at 585 nm with different concentrations of NaAsc (500 μ M, 1 mM, 2 mM, 6 mM, 10 mM) over one hour. All measurements were performed in PBS Buffer (pH = 7.4), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

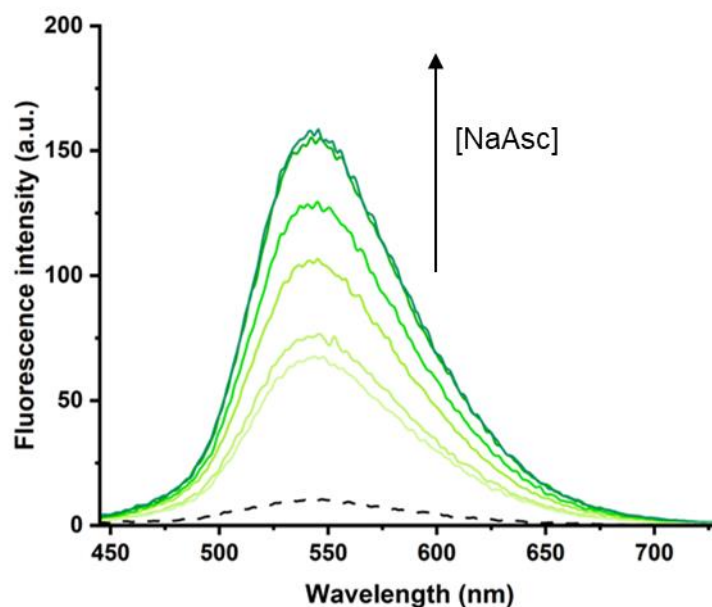


Figure 4.11. Fluorescence spectra of **CarboNap (Pt12, 5 μ M)** in PBS buffer (pH = 7.40) with increasing concentrations of NaAsc (5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM) (1 h incubation), λ_{ex} = 430 nm, slit widths: ex = 10 nm, em = 3.5 nm.

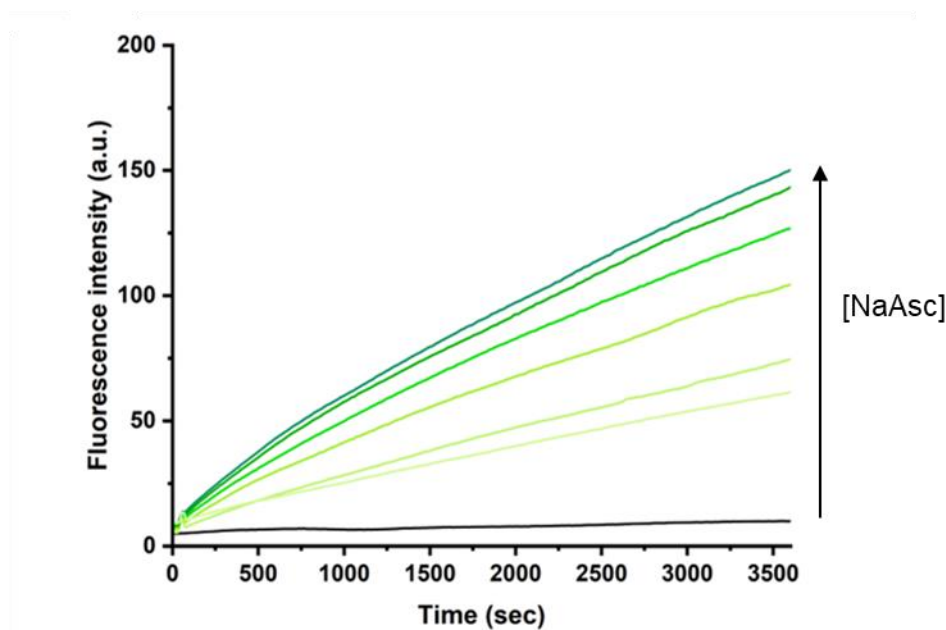


Figure 4.12. Changes in fluorescence emission intensity of **CarboNap** (**Pt12**, 5 μ M) at 545 nm with different concentrations of NaAsc (5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM) over one hour. All measurements were performed in PBS Buffer (pH = 7.4), λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 3.5 nm.

When monitoring the stability of the cisplatin(IV) complexes by fluorescence spectroscopy, gradual increases in fluorescence emission for **CisRes** (Figure 4.13), and at a slower rate for **CisNap** (Figure 4.14), were seen over time. These observations indicate that the Cis(IV)Fluors are likely undergoing slow decomposition *via* hydrolysis and releasing their respective fluorophores in aqueous solution. In comparison, no change in fluorescence intensity was observed for **CarboRes** and **CarboNap**, which suggest that the Carbo(IV)Fluors are stable towards hydrolysis during that timeframe (Figure 4.15 and Figure 4.16). Although a faster rate of reduction was witnessed for cisplatin(IV) carbamates, the complexes appear to be relatively unstable under aqueous conditions. The carboplatin(IV) derivatives, however, remained unchanged over 8 hours but are more difficult to reduce due to their low sensitivity against sodium ascorbate. These results seem to point towards a correlation between the reduction profile and the hydrolytic stability of platinum(IV) complexes.

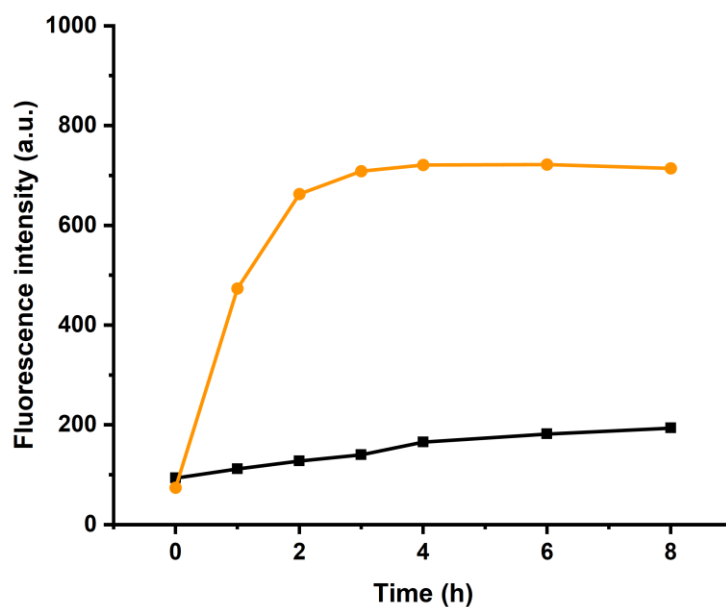


Figure 4.13. Changes in the fluorescence intensity of **CisRes (Pt9, 5 μ M)** at 585 nm with (**orange**) and without (**black**) the addition of NaAsc (500 μ M) in PBS buffer (pH = 7.40), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

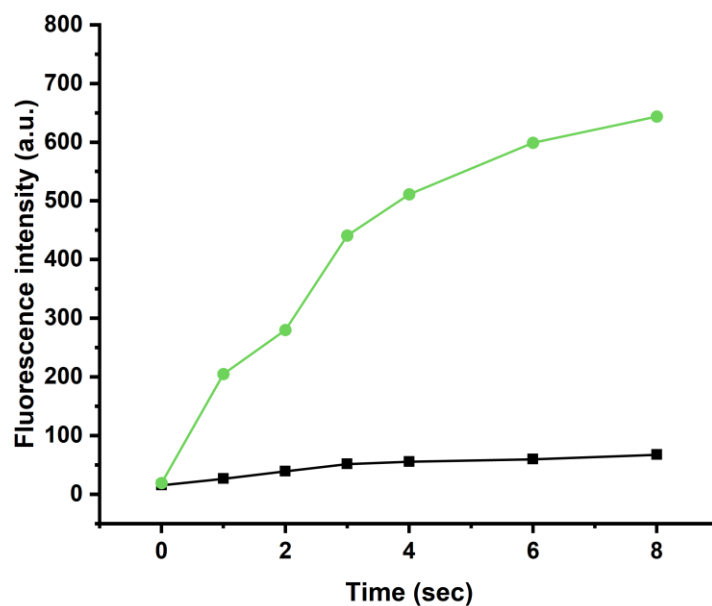


Figure 4.14. Changes in the fluorescence intensity of **CisNap (Pt11, 5 μ M)** at 545 nm with (**green**) and without (**black**) the addition of NaAsc (1 mM) in PBS buffer (pH = 7.40), λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 3.5 nm.

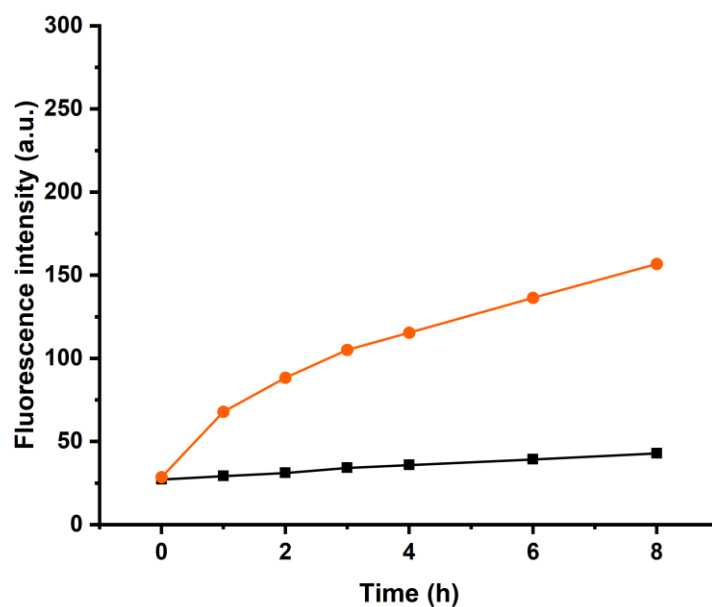


Figure 4.15. Changes in the fluorescence intensity of **CarboRes (Pt10, 5 μ M)** at 585 nm with (**orange**) and without (**black**) the addition of NaAsc (500 μ M) in PBS buffer (pH = 7.40), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

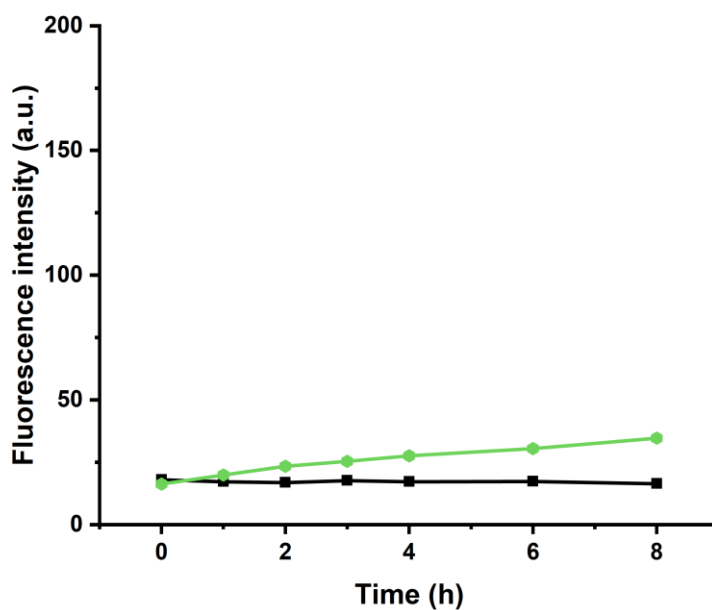


Figure 4.16. Changes in the fluorescence intensity of **CarboNap (Pt12, 5 μ M)** at 545 nm with (**green**) and without (**black**) the addition of NaAsc (1 mM) in PBS buffer (pH = 7.40), λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 3.5 nm.

The poor aqueous stability of the *Cis(IV)Fluors* was also observed by UV-vis spectroscopy experiments. Spontaneous release of the fluorophores was recorded over a period of two hours in the absence of reducing agent for **CisRes** and **CisNap** (Figure 4.17 and Figure 4.18). In addition, a slight colour change was observed in the samples containing **CisRes**, going from a clear to bright pink solution.

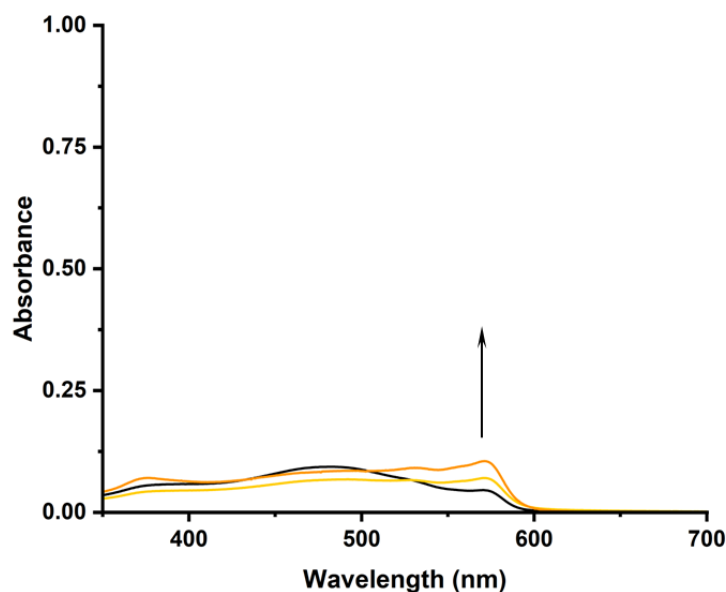


Figure 4.17. Absorbance spectra of **CisRes** (Pt9, 15 μ M) in PBS buffer (pH = 7.40). The second and third measurements were recorded after 60- and 120-minutes.

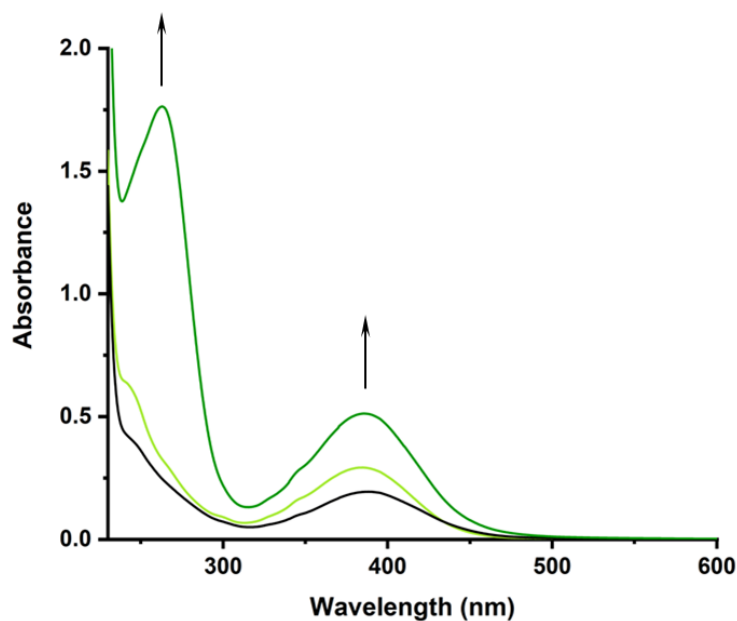


Figure 4.18. Absorbance spectra of **CisNap** (Pt11, 15 μ M) in PBS buffer (pH = 7.40). The second and third measurements were recorded after 60- and 120-minutes.

LC-MS analysis confirmed these observations (Figure 4.19 and Figure 4.20). Free resorufin and 4-NapNH₂ were detected alongside Pt-species of cisplatin after reinjection of the complexes upon 60 minutes of incubation in deionised water, which indicate that the cisplatin(IV) carbamates hydrolysed within a short period of time in aqueous solution.^{31,32} The self-immolative linker was previously applied by the Ang group to functionalised cisplatin(IV) complexes with diverse active ligands and the stability of these CisPt(IV) prodrugs was demonstrated by RP-HPLC in PBS over the course of 24 hours.³³ Since the reported self-immolative co-drug systems displayed great stability, the resorufin unit used in this strategy might be affecting the overall stability of the axial ligand inducing rapid elimination *via* hydrolysis. Different approaches can be investigated to improve the hydrolytic stability of the cisplatin(IV) conjugates like adding an electron-withdrawing group on the self-immolative spacer³⁴ or replacing the axial acetato ligand with a hydroxyl group or another carboxylate substituent.^{30,35} Due to the low stability of **CisRes** and **CisNap**, the following studies will solely focus on the *Carbo(IV)Fluors*.

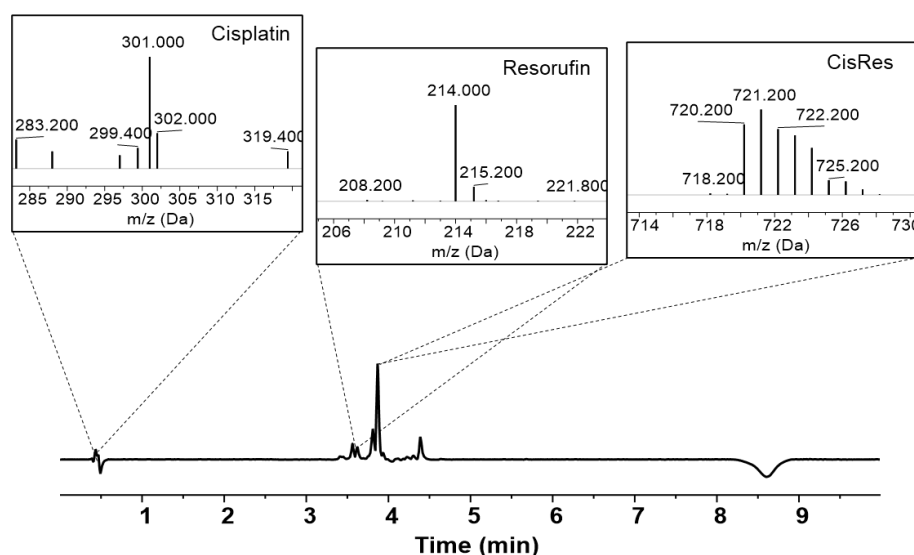


Figure 4.19. LC-MS analysis of an injection of **CisRes** (**Pt9**, 50 μ M) incubated for 60 minutes in deionized H₂O (Absorption at 365 nm).

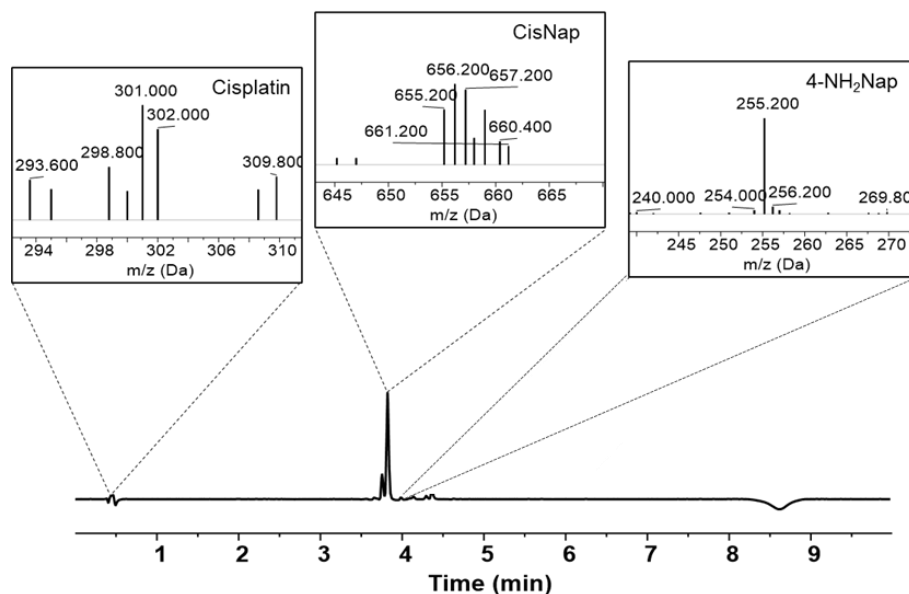


Figure 4.20. LC-MS analysis of an injection of **CisNap** (**Pt11**, 50 μ M) incubated for 60 minutes in deionized H₂O (Absorption at 365 nm).

Next, we monitored the stability of the carboplatin-based platinum(IV) complexes by LC-MS over a period of 24 hours. As illustrated in Figure 4.21, **CarboRes** displayed good stability before exhibiting a slight decomposition with 26 % of free resorufin detected beyond 24 hours. In contrast, **CarboNap** demonstrated excellent stability in deionised water (Figure 4.22). Over time, a clear difference in stability can be observed between the two carboplatin-based platinum(IV) complexes bearing distinct axial ligands. Nevertheless, the carboplatin(IV) prodrugs were found to be suitable for imaging and cytotoxicity studies in malignant cells.

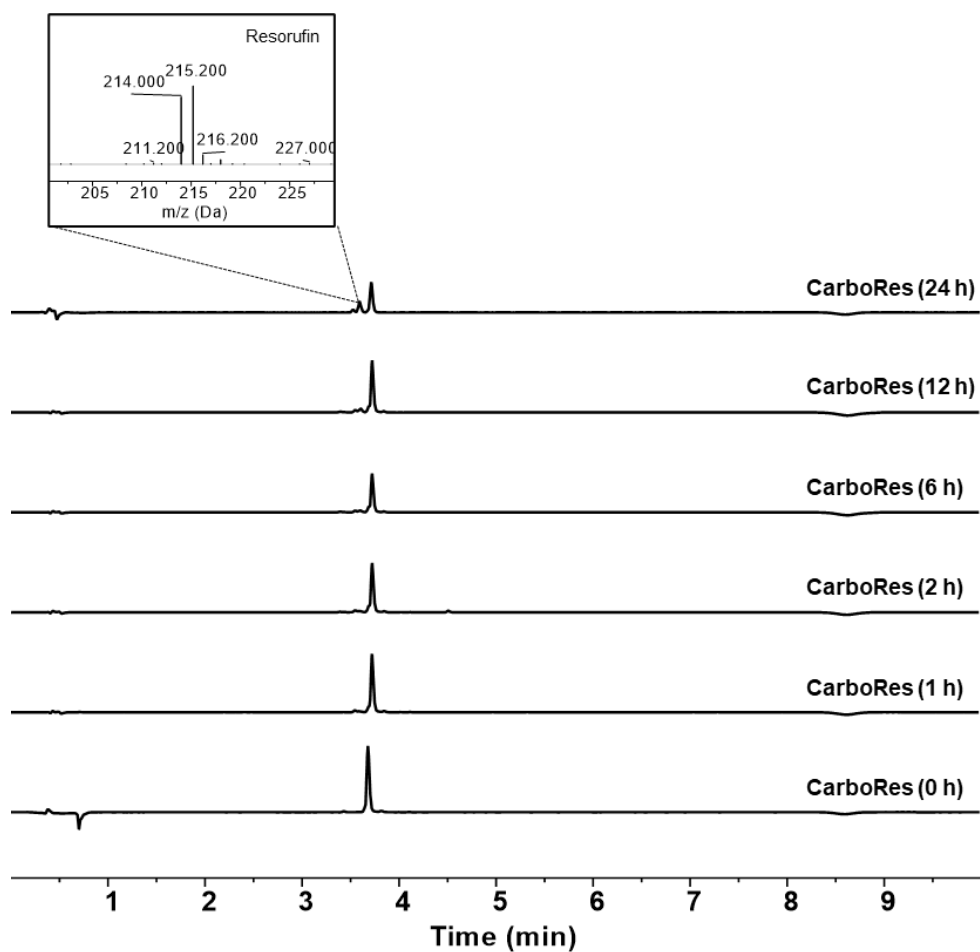


Figure 4.21. Stability study of **CarboRes** (**Pt10**, 50 μM) in deionised H_2O recorded by LC-MS (Absorption at 365 nm).

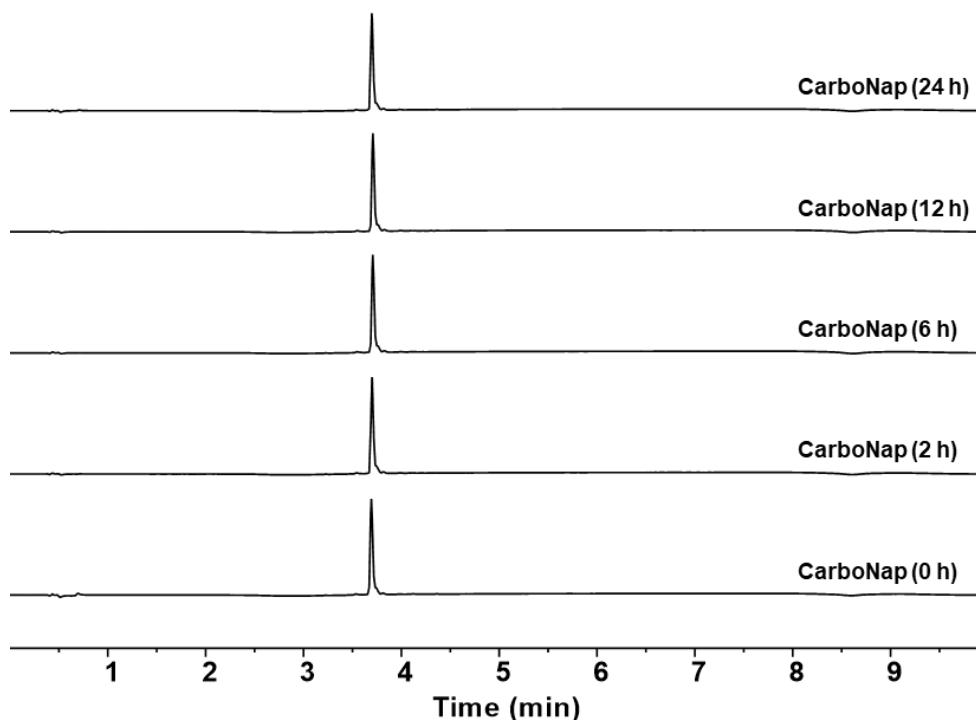


Figure 4.22. Stability study of **CarboNap** (**Pt12**, 50 μ M) in deionised H_2O recorded by LC-MS (Absorption at 365 nm).

Lastly, the Carbo(IV)Fluors were screened against several biological reductants. The results were studied by fluorescence spectroscopy and collected by Jevon W. Marsh (University of Oxford). As displayed in Figure 4.23, treatment of **CarboRes** with GSH (4 mM), L-Cysteine (100 μ M), H_2O_2 (100 μ M), and BSA (1 equiv.) resulted in negligible fluorescence changes. However, incubation with 0.5 mM NADH provided a large “turn-on” response (6-fold change). As was the case with **OxaliRes**, the carboplatin(IV) derivative is selective for the two bio-reductants sodium ascorbate and NADH.

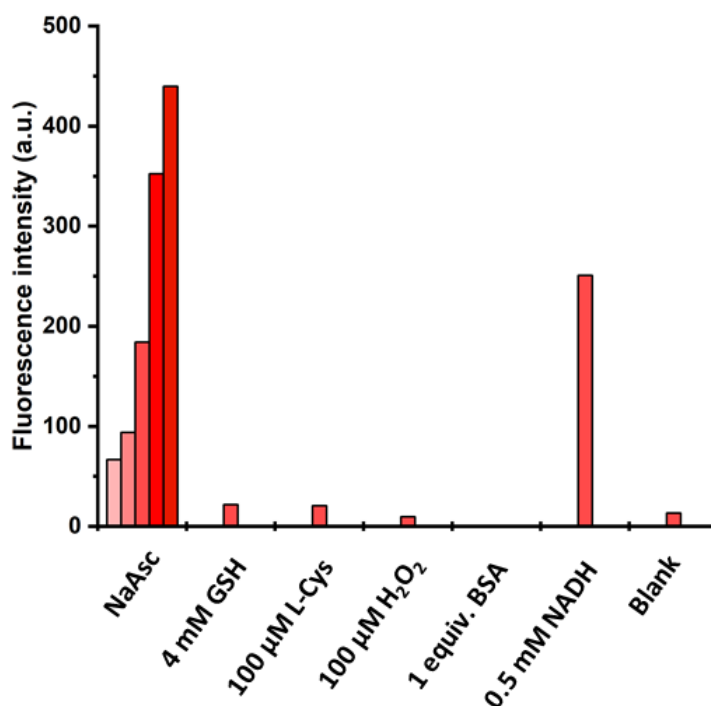


Figure 4.23. Changes in fluorescence emission intensity of **CarboRes** (**Pt10**, 5 μ M) at 585 nm in the presence of various analytes over one hour: NaAsc (500 μ M, 1 mM, 2 mM, 6 mM, 10 mM); NADH (0.5 mM); GSH (4 mM); L-Cysteine (100 μ M); BSA (1 equiv.) and H₂O₂ (100 μ M). All measurements were performed in PBS Buffer (pH = 7.4), λ_{ex} = 500 nm, slit widths: ex = 10 nm and em = 2.5 nm.

Little to no changes were monitored for **CarboNap** upon addition of GSH (4 mM), L-Cysteine (100 μ M) and H₂O₂ (100 μ M), as shown in Figure 4.24. Upon incubation with BSA (1 equiv.), however, a similar blue shift (from 545 to 522 nm) as **OxaliNap** was observed for **CarboNap** which is suggestive of a potential binding between the coordinated fluorophore **L2** and BSA (Figure 4.25).^{36,37,38} Unlike **OxaliNap**, however, **CarboNap** appeared to only respond to sodium ascorbate as no obvious changes were recorded with NADH (0.5 mM). Due to the lack of sensitivity of **CarboNap**, higher concentrations of the reductant might be needed to induce reduction of the Pt(IV) to Pt(II) and generate a noticeable fluorescence response.

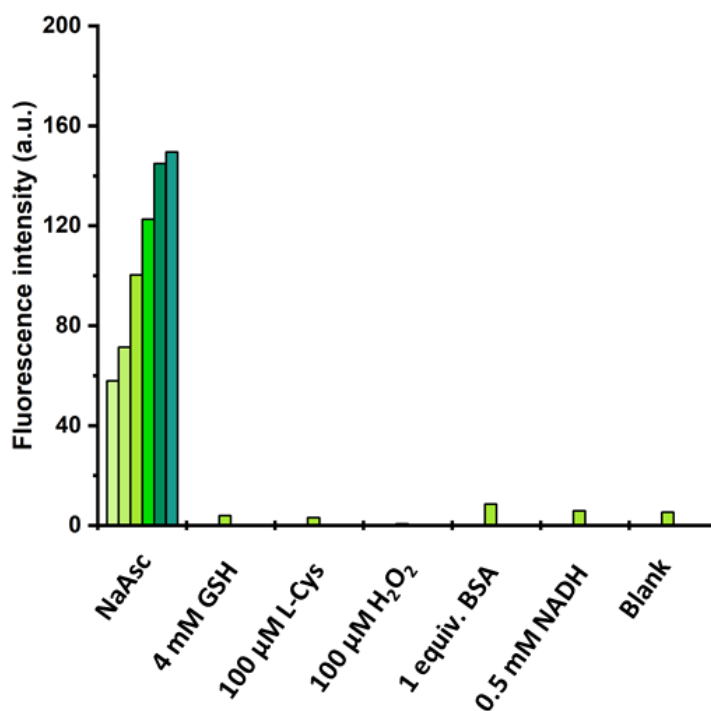


Figure 4.24. Changes in fluorescence emission intensity of **CarboNap (Pt12, 5 μ M)** at 545 nm in the presence of various analytes over one hour: NaAsc (5 mM, 10 mM, 20 mM, 30 mM, 40 mM); NADH (0.5 mM); GSH (4 mM); L-Cysteine (100 μ M); BSA (1 equiv.) and H₂O₂ (100 μ M). All measurements were performed in PBS Buffer (pH = 7.4), λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 3.5 nm.

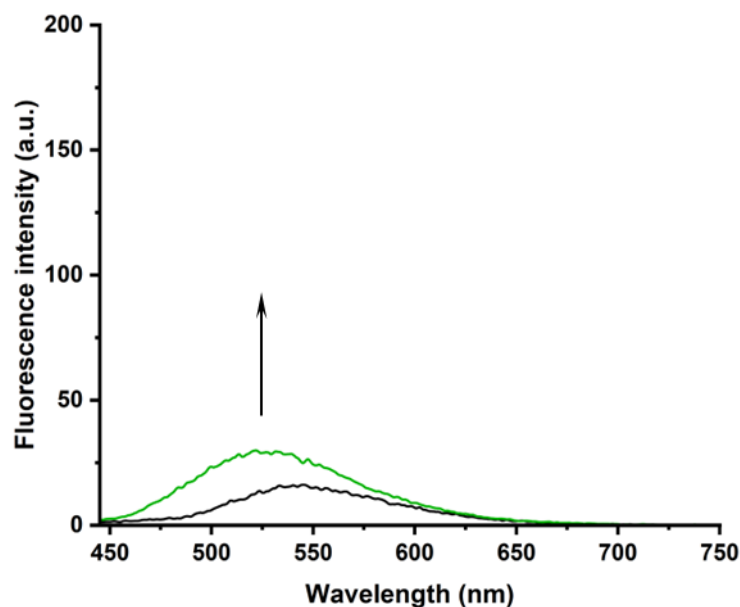


Figure 4.25. Relative fluorescence intensity of **CarboNap (Pt12, 5 μ M)** in PBS buffer (pH = 7.40) before (**black**) and after (**green**) addition of BSA (1 equiv.). The second measurement was recorded after 60 minutes incubation, λ_{ex} = 430 nm, slit widths: ex = 10 nm and em = 3.5 nm.

4.4. Biological studies of Carbo(IV)Fluors

Following our investigations on the Carbo(IV)Fluors in solution-based experiments, the imaging properties and biological activities of **CarboRes** and **CarboNap** were examined against A549 lung adenocarcinoma cell line since carboplatin (CarboPt(II)) is commonly used in the clinic to treat small-cell lung cancer.³⁹ The study was conducted by Dr. Lina Hacker in collaboration with Prof. Ester Hammond (University of Oxford). Confocal microscopy and flow cytometry analyses were first carried out in A549 cells treated with the respective carboplatin(IV) (CarboPt(IV)) prodrugs and increasing concentrations of sodium ascorbate (ranging from 0 to 4 mM) over 6 hours (Figure 4.26). In the absence of reducing agent, no changes in fluorescence emission were observed. However, incubation with sodium ascorbate led to a concentration-dependent response for both Carbo(IV)Fluors, indicative of the reduction from CarboPt(IV) to CarboPt(II). Under the same conditions, **CarboNap** was visibly less sensitive to the reductant considering the fluorescence response observed in selected cancer cell line. These results are consistent with our previous observations in aqueous solution discussed in *Section 4.3*.

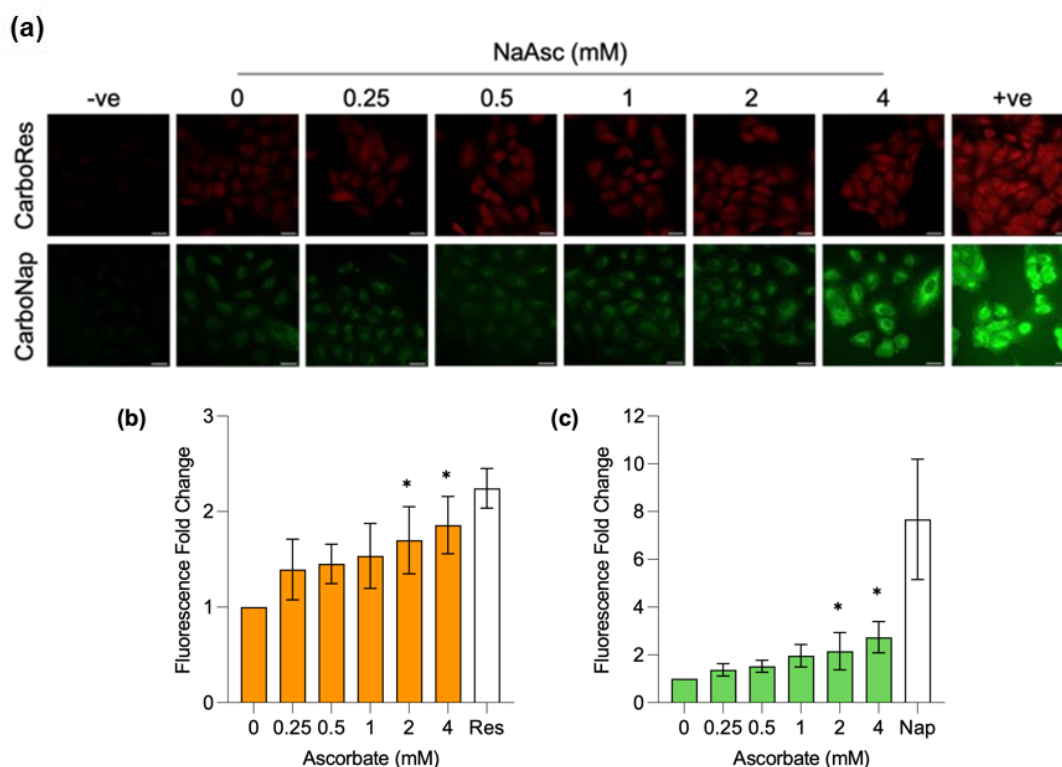


Figure 4.26. **(a)** Representative fluorescence images of A549 cells treated with **CarboRes** (top, 10 μ M) or **CarboNap** (bottom, 10 μ M) with NaAsc (0–4 mM, 6 hours). Scalebar represents 12 μ m. Positive controls (+ve) are Resorufin and Naphthalimide, respectively. The negative (-ve) control is untreated cells. **(b, c)** Flow cytometry analysis of A549 cells treated with **CarboRes** (10 μ M, **a**) or **CarboNap** (10 μ M, **b**) with the indicated concentrations of NaAsc. Fluorescence fold change of the geometric mean intensity is displayed. Error bars represent SD. Significance compared to probe alone condition. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n = 3$.

Since we are able to visualise the intracellular reduction of **CarboRes** and **CarboNap** in A549 cells, the fluorescence response of both CarboPt(IV) complexes was investigated in normoxic (21 % O_2), mild (2 % O_2) and severe (<0.1 % O_2) hypoxic conditions. A549 cells were treated with the fluorescent CarboPt(IV) conjugates and exposed to three distinct oxygen levels for a period of 6 hours. As displayed in Figure 4.27, negligible changes were monitored with **CarboNap** in cells exposed to 2 % oxygen content whereas a fluorescence “turn-on” response was seen under severe oxygen concentrations (<0.1 % O_2). Increases in fluorescence emission intensities were observed for **CarboRes** under mild hypoxic condition (2 % O_2) and to a greater extent in oxygen levels inferior to 0.1 %. Just like the Oxali(IV)Fluors, the sodium ascorbate sensitivity observed and the dependency to oxygen of the CarboPt(IV)Fluors appear to be related. Moreover, the results of the cell imaging study

suggest that both Carbo(IV) carbamates are being reduced to the CarboPt(II) precursor under severe hypoxic conditions in A549 cells.⁴⁰

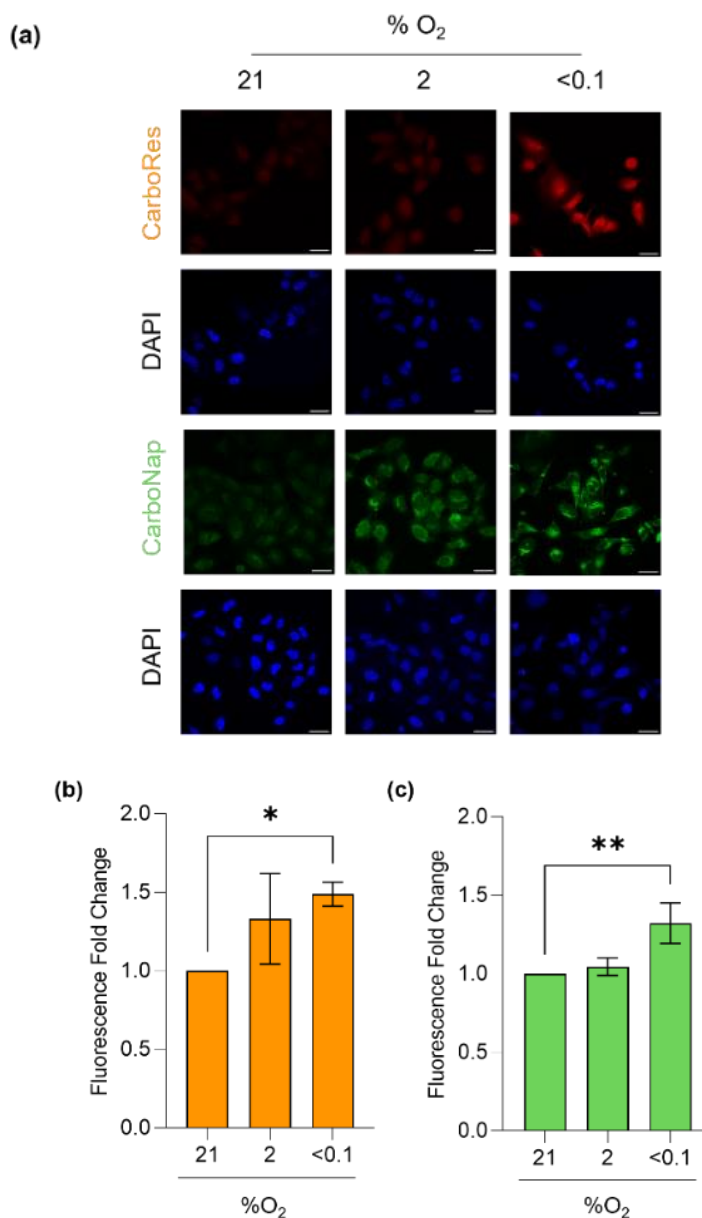


Figure 4.27. (a) Representative fluorescence images of A549 cells treated with **CarboRes** (10 μ M) or **CarboNap** (10 μ M) for 6 hours in the oxygen conditions indicated. Scale bar represents 12 μ m; blue colour represents DAPI stain. (b, c) Flow cytometry of A549 cells treated with **CarboRes** (10 μ M) or **CarboNap** (10 μ M) for 6 hours at the oxygen level indicated. Fluorescence fold change of the geometric mean intensity is displayed. Error bars represent SD. Significance compared to normoxic control. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n = 3$.

Subsequently, the cytotoxic activities of **CarboRes** and **CarboNap** under hypoxic and normoxic conditions were studied and compared to the fluorescence imaging results obtained. The antitumor activity of the Carbo(IV)Fluors was evaluated in A549 cell lines under normoxia (21 % O₂) and hypoxia (<0.1 % O₂) using clonogenic cell survival assays, as illustrated in Figure 4.28. Cells were incubated with different concentrations of **CarboRes** or **CarboNap** over an extended period (10 days) and exposed to hypoxic conditions for 16 hours upon treatment **(a)**. A greater cytotoxic potency was observed for **CarboRes (b)** under hypoxia compared to non-hypoxic conditions. However, cells treated with larger concentrations of the CarboPt(IV) prodrug demonstrated cell proliferation inhibition in both conditions. On the other hand, **CarboNap (b)** exhibited higher cytotoxic effect under hypoxia in cells treated with 10 µM or more or the naphthalimide-Pt(IV) conjugate.

Lastly, the cytotoxic study of CarboPt(II) and CarboPt(IV) in A549 cells was performed in hypoxic (<0.1 % O₂) and normoxic (21 % O₂) conditions. As seen in Figure 4.29, the complexes demonstrated high potency in cancer cells with minor differences under both oxygen contents. Taken together, the biological studies of the CarboPt(IV) complexes revealed distinct differences in cytotoxic efficacy under hypoxia and non-hypoxic conditions. Based on these results, carboplatin(IV) carbamates may be suitable as hypoxia-activated prodrugs in the treatment of solid hypoxic tumours.

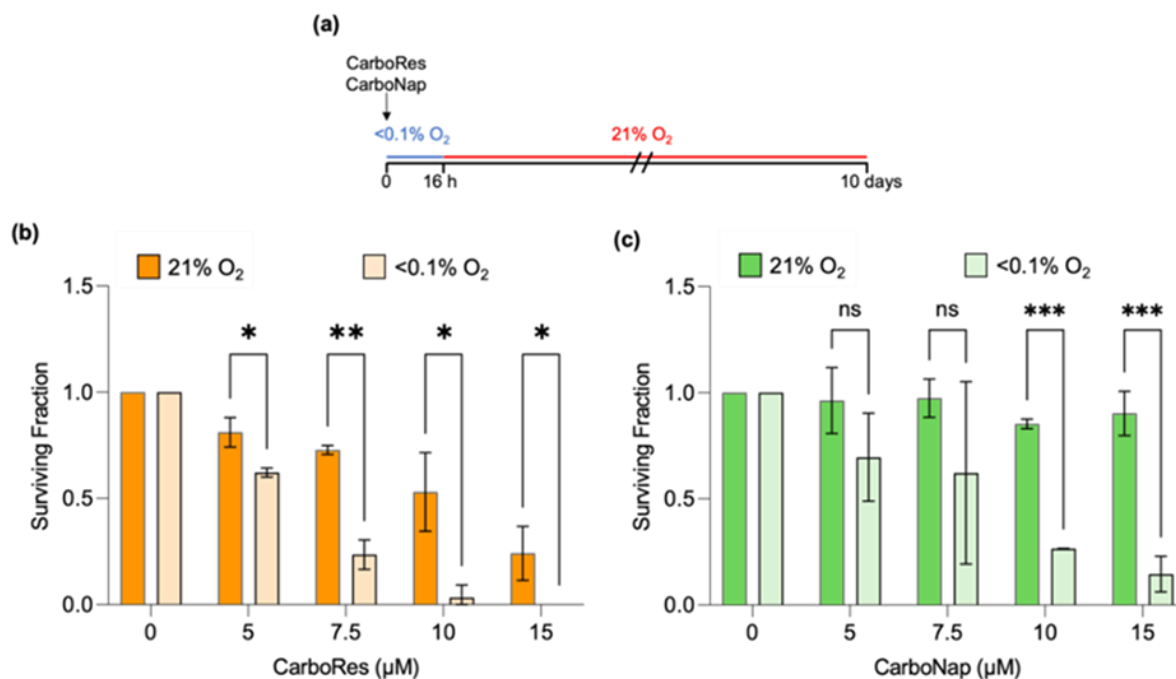


Figure 4.28. (a) A549 cells were treated with the indicated concentrations of **CarboRes** or **CarboNap** for 10 days as indicated in the schematic. Cell survival was measured via clonogenic assay after exposure to (b) **CarboRes** and (c) **CarboNap**. Error bars represent SD * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n = 3$.

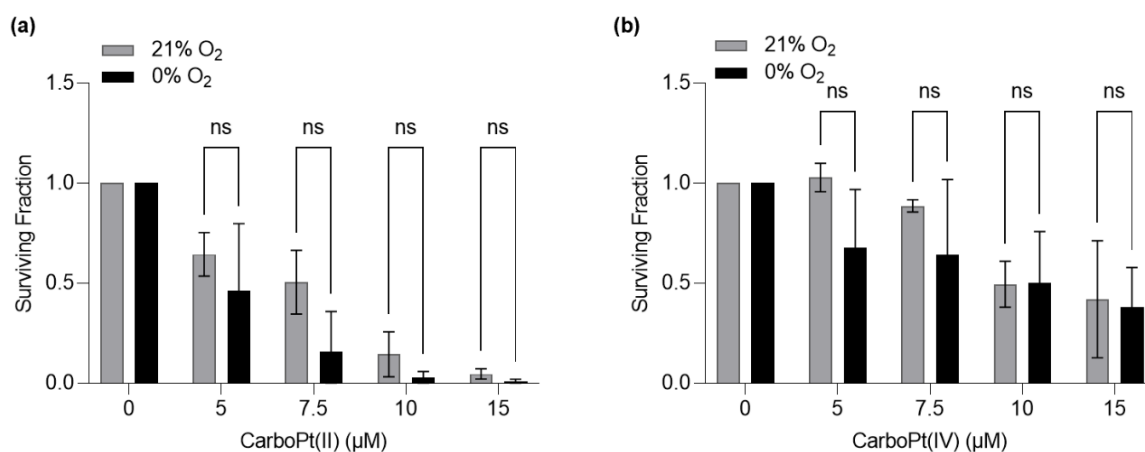
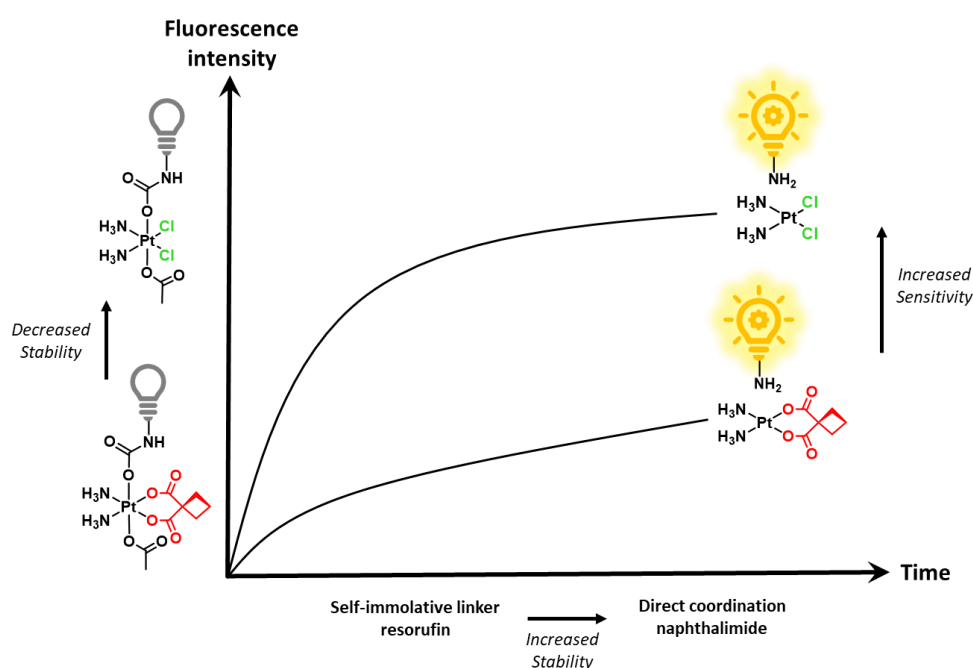


Figure 4.29. A549 cells were treated with the indicated concentrations of **CarboPt(II)** (a) or **CarboPt(IV)** or **(Pt8)** (b) and exposed to <0.1 % O₂ for 16 hrs. After 10 days, cell survival was measured via clonogenic assay. Error bars represent SD * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. $n = 3$.

4.5. Conclusion

This chapter described the synthesis and characterisation of a series of fluorescence responsive cisplatin(IV) and carboplatin(IV) carbamate complexes, **CisRes (Pt9)**, **CisNap (Pt11)**, **CarboRes (Pt10)** and **CarboNap (Pt12)**, functionalised with resorufin *via* a self-immolative spacer and a naphthalimide fluorophore directly coordinated to the platinum centre. The reduction profiles, sensitivity, and stability of the Cis(IV)Fluors and Carbo(IV)Fluors were investigated in aqueous solution. The fluorescent CisPt(IV) conjugates demonstrated poor hydrolytic stability and were therefore precluded from the remaining studies. In contrast, the CarboPt(IV) carbamates showed good stability under aqueous conditions and displayed increases in fluorescence emission upon treatment with sodium ascorbate in both solution and cancer cells with significant differences in sensitivity to reduction. A fluorescence “switch-on” response was seen for both Carbo(IV)Fluors under severe hypoxia (<0.1 % O₂) in small-cell lung cancer cells using confocal microscopy, characteristic of the reduction from Pt(IV) to Pt(II) and accompanied by clear disparities in potency under hypoxic (<0.1 % O₂) and normoxic conditions (21 % O₂). Overall, the data presented in this chapter revealed that the reduction properties, aqueous stability, and cytotoxic efficacy of platinum(IV) prodrugs are strongly influenced by the choice of equatorial and axial ligands.



4.6. References

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Chapter 5 Improving the sensitivity to reduction of platinum(IV) complexes

Chapter 6 Fluorescent therapeutics

6.1. Introduction

Although cisplatin, carboplatin and oxaliplatin are widely applied in the clinic to treat many malignancies, the continuous use of these anticancer agents often results in systemic toxicities and acquired resistance of cancer cells.^{1,2} Platinum-based chemotherapy is therefore frequently co-administered with other antineoplastic drugs able to exert cytotoxic activity *via* distinct mechanisms of action to maximise the therapeutic efficacy.^{3,4} Few examples of FDA-approved agents used in combination therapy in the clinic include gemcitabine, doxorubicin, taxol, and 5-fluorouracil.^{5,6,7} However, despite the overall improvement in therapeutic outcome, severe side-effects are still being experienced with the current chemotherapy regimens and finding alternative treatments remain challenging.

To provide an optimal anticancer efficacy and minimise adverse effects, platinum(IV) prodrugs functionalised with potent chemotherapeutics are of great interest. The Pt(II) drug and the targeted agent could be delivered together in a controlled manner and work synergistically upon activation to destroy malignant cells.^{8,9} A wide number of platinum(IV) complexes in which the axial ligands have been modified with antitumour drugs have already been reported.^{9,10,11} For instance, cisplatin-based Pt(IV) prodrugs conjugated to known anticancer drugs gemcitabine (**6.1**), taxol (**6.2**), estramustine (**6.3**) *via* carbonate linkers were developed by Yempala *et al.* (Figure 6.1). Analogues of these complexes (**6.1** and **6.3**) containing succinate spacers were also prepared. Comparable reduction rates of the Pt(IV) complexes in the presence of reducing agents but different rates of the co-drug release between the carbonate and succinate derivatives were observed by HPLC and NMR spectroscopy experiments. Moreover, cytotoxic studies revealed discrepancies in the potency of the complexes depending on the choice of linker.¹² These reports and others highlight the difficulty in understanding the mode of action of these platinum(IV) complexes and the

bioactive ligand efficient release upon cell internalisation and how it correlates to the overall cytotoxicity measured using standard analytical methods.

In 2014, Ang and colleagues designed a cisplatin(IV) derivative tethered to the drug doxorubicin (Dox), which possesses fluorescence properties, with a succinate linker (**6.4**). They used a carbon nanotubes system functionalised with tumour-targeting units to selectively deliver the complex in cancer cells and achieve a similar distribution profile between the two anticancer components. Even though, increased cytotoxic efficacy was demonstrated, confocal microscopy studies showed different sub-localisation of the free drug and the ligand, potentially released as the Dox-linker moiety.¹³ Furthermore, as explored in *Chapter 1*, several platinum(IV) theranostic agents have also been developed for dual diagnostic and therapeutic purposes in order to study the prodrug activation and monitor the release of the bioactive ligand in real-time mode using fluorescence imaging.^{14,15,16}

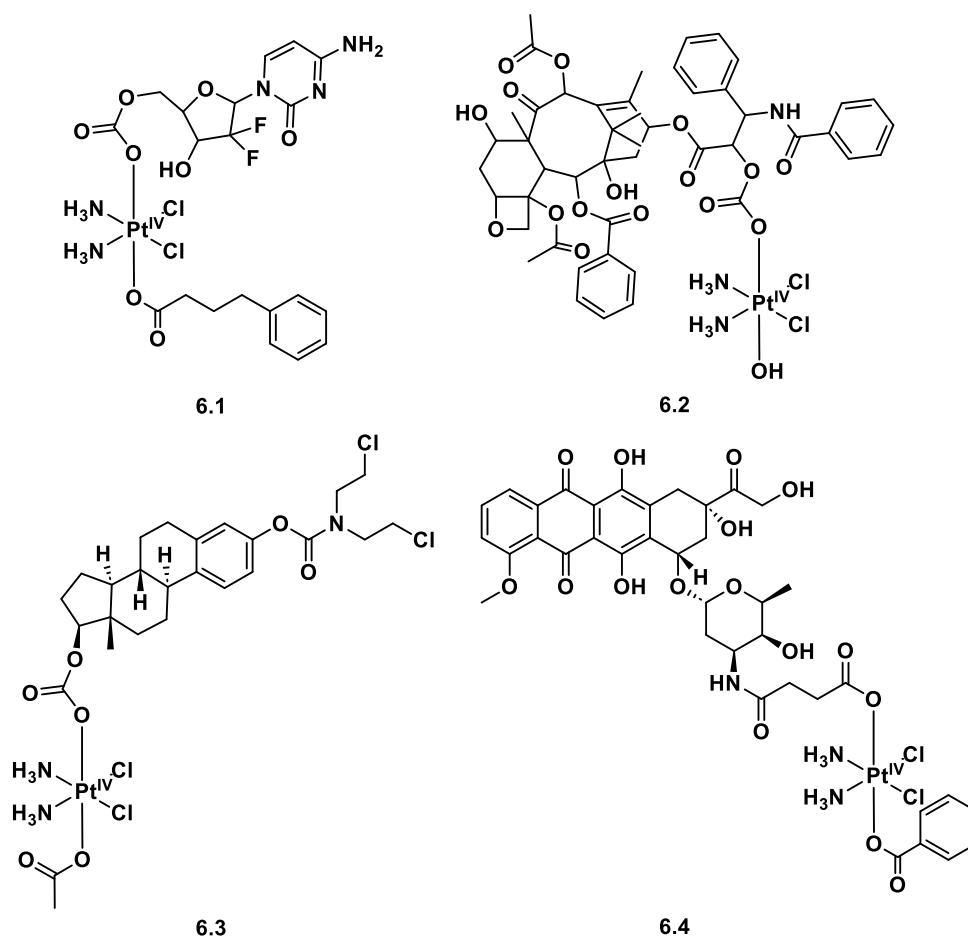


Figure 6.1. Four cisplatin-based Pt(IV) multi-action prodrugs functionalised with gemcitabine (**6.1**), taxol (**6.2**), estramustine (**6.3**) and doxorubicin (**6.4**) using carbonate and succinate linkers.^{12,13}

The aim of this project was to design several fluorescent platinum(IV) prodrugs carrying antineoplastic drugs and explore their photophysical and cytotoxic characteristics as potential dual-action therapeutic agents. Two antitumour drugs with fluorescence properties were conjugated to the photoactivatable diazido-Pt(IV) and oxaliplatin-based Pt(IV) complexes which would allow real-time visualisation of the co-drug release upon intracellular reduction to Pt(II) species. The anticancer agents studied in this chapter involved the fluorinated benzothiazole derivative 5F203 and the naphthalimide-based DNA intercalator Amonafide (Figure 6.2).^{17,18} The blue-emitting drug 5F203 (2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole) is a potent anticancer agent which has shown great efficacy in *in vivo* and *in vitro* models of ovarian and breast cancers.^{19,20} Poor aqueous solubility has, however, limited its clinical application.¹⁷ To address this issue, a water-soluble derivative has been developed by combining a lysyl unit to the pharmacophore to generate the prodrug Phortress. Upon cellular uptake, the drug is hydrolysed to release 5F203 resulting in the formation of DNA adducts and inducing apoptosis. To date, the chemotherapeutic has reached phase I clinical trials for the treatment of solid tumours.²¹ Amonafide is a DNA intercalating agent and topoisomerase II inhibitor and has been approved by the FDA for the treatment of secondary acute myeloid leukaemia.^{18,22} The antitumour drug has, however, failed to demonstrate great therapeutic efficacy against other type of cancers and has been shown to cause toxic side-effects (e.g., myelosuppression, nausea and vomiting, seizures).²³ The coordination of 5F203 and Amonafide to platinum(IV) prodrugs might improve the aqueous solubility and enhance the cytotoxic efficacy of these anticancer agents, respectively. Due to the presence of amino groups on both bioactive moieties, a direct coordination approach using the method previously developed was investigated. The platinum(IV) carbamates and antineoplastic agents could work in a synergistic manner upon reduction to provide an optimal therapeutic effect in malignant cells.

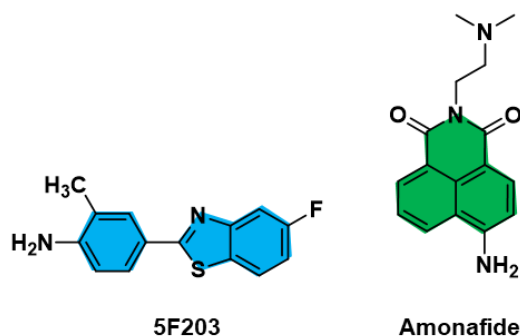
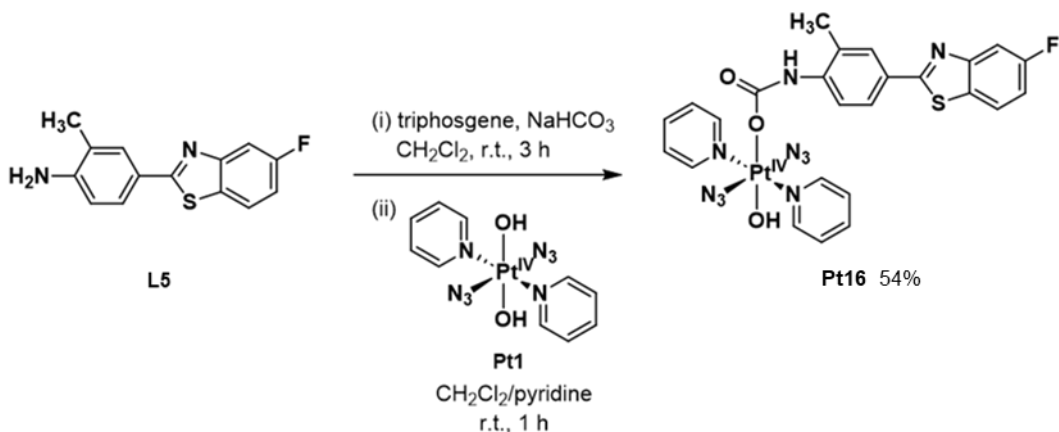


Figure 6.2. Antineoplastic agents used in this project.^{17,18}

6.2. Platinum(IV)-5F203 conjugates

6.2.1. Synthesis and characterisation of Azido-5F203

The antitumor agent **5F203** (**L5**) was synthesised over five-steps according to literature protocols.¹⁷ The synthetic method reported in *Chapter 2* enabled the preparation of the diazido-Pt(IV) 5F203 conjugate (Complex **Pt16**), as outlined in Scheme 6.1. **Azido-5F203** was obtained in good yield (54 %) and characterised by ¹H, ¹³C and ¹⁹⁵Pt NMR spectroscopy and high-resolution mass spectrometry.



Scheme 6.1. Synthesis of **Azido-5F203** (**Pt16**).

Yellow crystals of **Azido-5F203** were obtained by slow evaporation of a concentrated solution of the product in a mixture of dichloromethane and methanol and analysed by Kirsten E. Christensen (University of Oxford). The molecular structure is shown in Figure 6.3

and verified that the desired monocarbamate Pt(IV) 5F203 complex was obtained. In terms of angle and bond length values, **Azido-5F203** presents few differences to the precursor **Pt1** and the complex **Azido-BDP** presented in Chapter 2.²⁴ The Pt-O-C angle is noted to be slightly more acute (121.02 (3) vs 125.3(3)°) whereas the O-Pt-O angle appears to be more obtuse (177.85(6) vs 174.98(10)°) than the angles observed for the monocarboxylato diazido-Pt(IV) bodipy conjugate (**Pt2**) (further data available in Appendix A.3).

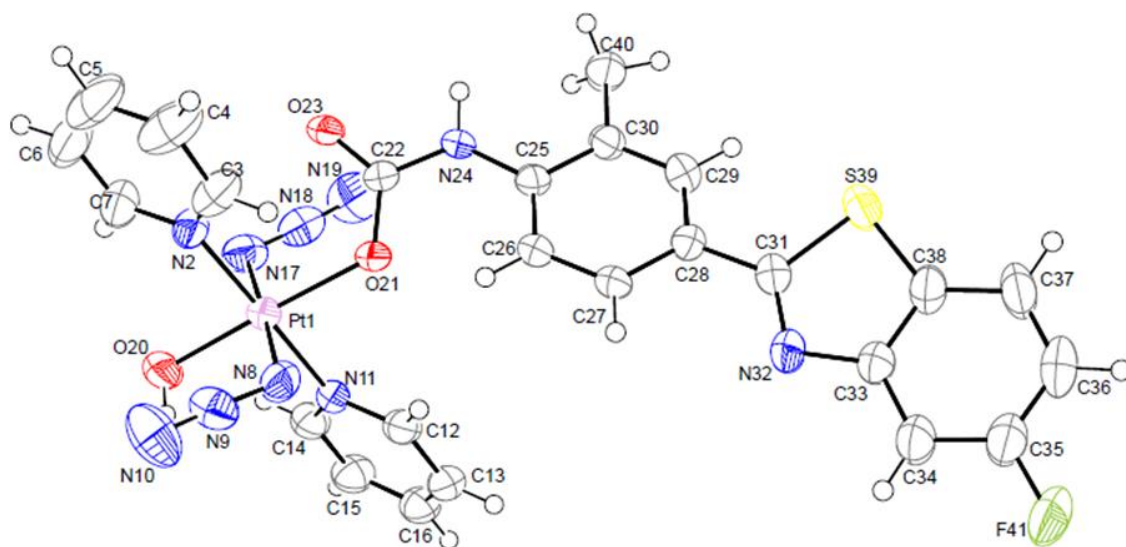


Figure 6.3. Molecular structure of **Azido-5F203 (Pt16)** determined by X-ray diffraction with thermal ellipsoids display at 50% probability. Pt-azido group naming convention; $N_{8/17} = N\alpha$, $N_{9/18} = N\beta$ and $N_{10/19} = N\gamma$.

The assigned ^1H NMR spectra of **Azido-5F203** and the synthesised ligand **5F203** in DMSO- d_6 are illustrated in Figure 6.4. The ^1H NMR spectrum of **Pt16 (b)** denotes a small downfield shift for the resonances of **L5** and a new chemical shift at 3.95 ppm assigned to the axial OH ligand of the Pt(IV) complex. Furthermore, the resonance of the free NH_2 group of **5F203 (a)** is absent in **Azido-5F203** spectrum (**b**), indicative of the coordination between the ligand and **Pt1** via the amine. Compared to the ^1H NMR spectrum of **L5**, the chemical shift of proton 'a' is seen downfield to the signal of proton 'e' in the complex **Pt16** spectrum, due to the new carbamate linker. The platinum satellites of the pyridine *ortho* protons exhibit the same broad pattern as the one seen for **AzidoNap** in deuterated DMSO. Although the resonance of the CONH proton is not observed, the ^{195}Pt NMR spectrum of **Azido-5F203** shown in

Figure 6.5, displays a new downfield chemical shift at 1010 ppm, which supports the formation of the conjugate.

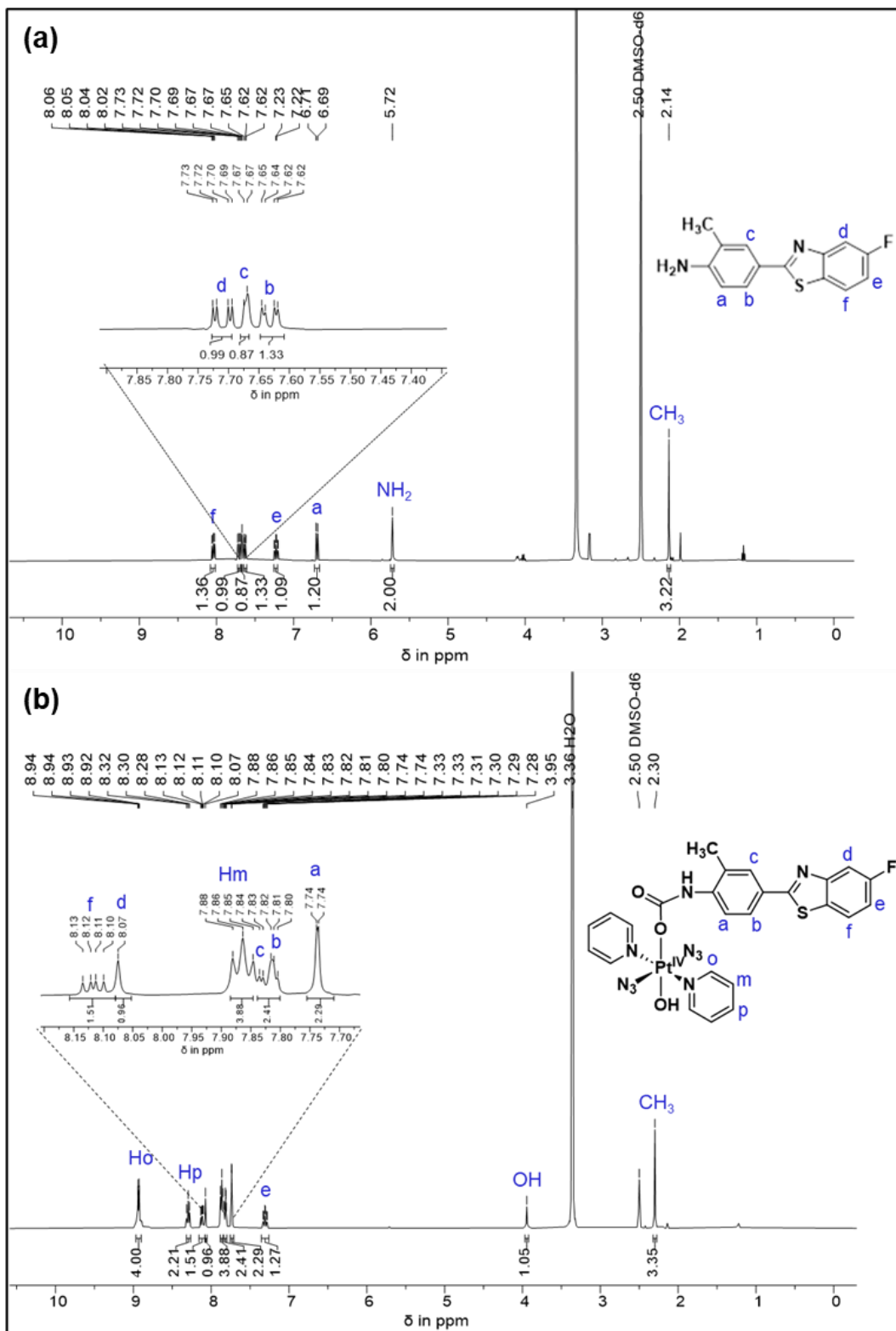


Figure 6.4. (a) ^1H NMR (400 MHz, DMSO-d_6) of 5F203 (L5). (b) ^1H NMR (400 MHz, DMSO-d_6) of Azido-5F203 (Pt16).

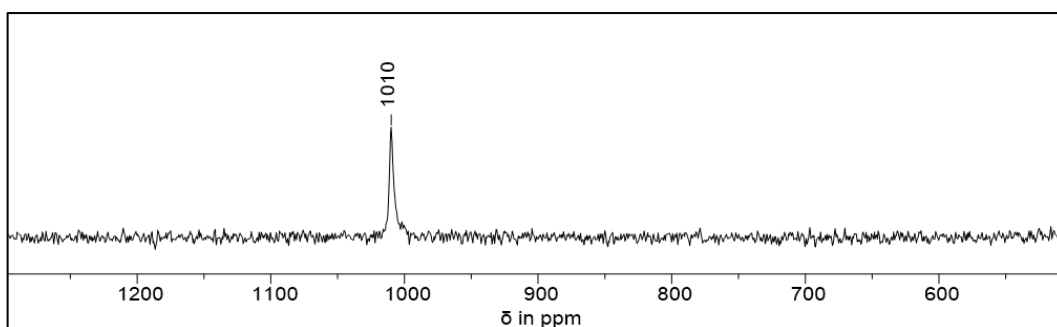
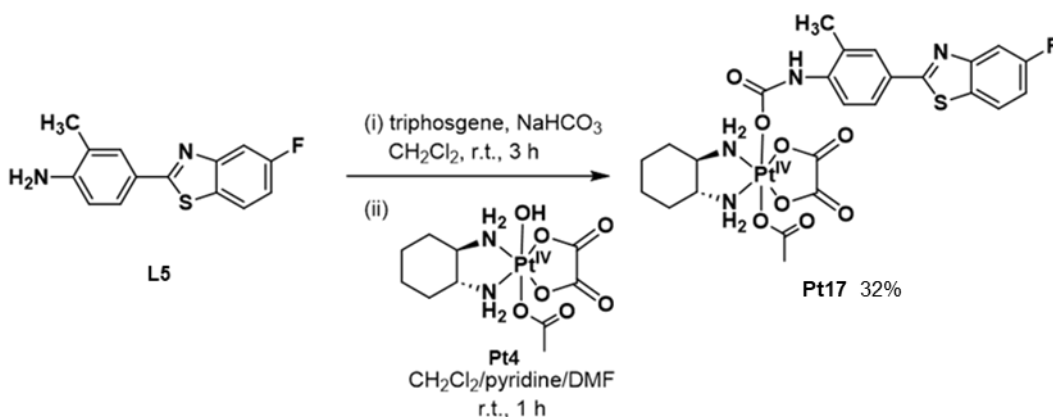


Figure 6.5. ^{195}Pt NMR (107 MHz, $\text{DMSO}-d_6$) of **Azido-5F203** (**Pt16**).

6.2.2. Synthesis and characterisation of Oxali-5F203

Based on the same procedure used to synthesize **OxaliNap** in Chapter 3, the oxaliplatin(IV) derivative **Oxali-5F203** (Complex **Pt17**) was easily prepared and collected in moderate yield (32 %). The synthetic route of **Oxali-5F203** is displayed in Scheme 6.2.



Scheme 6.2. Synthesis of **Oxali-5F203** (**Pt17**).

Characterisation of **Oxali-5F203** was performed by high-resolution mass spectrometry and ^1H , ^{13}C , ^{195}Pt NMR spectroscopy in CD_3OD . In contrast to the anticancer drug **5F203** which displayed poor solubility in aqueous solution, the complex **Pt17** was found to be highly soluble in methanol and sparingly soluble in water. The ^1H NMR spectrum shown in Figure 6.6 is rather similar to the one of **Azido-5F203** for the resonances of **L5** with the signal of proton 'a' less shielded when **5F203** is axially coordinated to the platinum(IV) complex. As expected, the resonances of the equatorial DACH ligand are detected between 1.29-2.94 ppm. Two large

singlets are present at 2.08 ppm and 2.30 ppm and correspond to the axial acetato ligand of oxaliplatin(IV) and the methyl group of **5F203**, respectively. The ^{195}Pt NMR spectrum of **Oxali-5F203** is presented in Figure 6.7 and displays a major resonance at 1590 ppm.

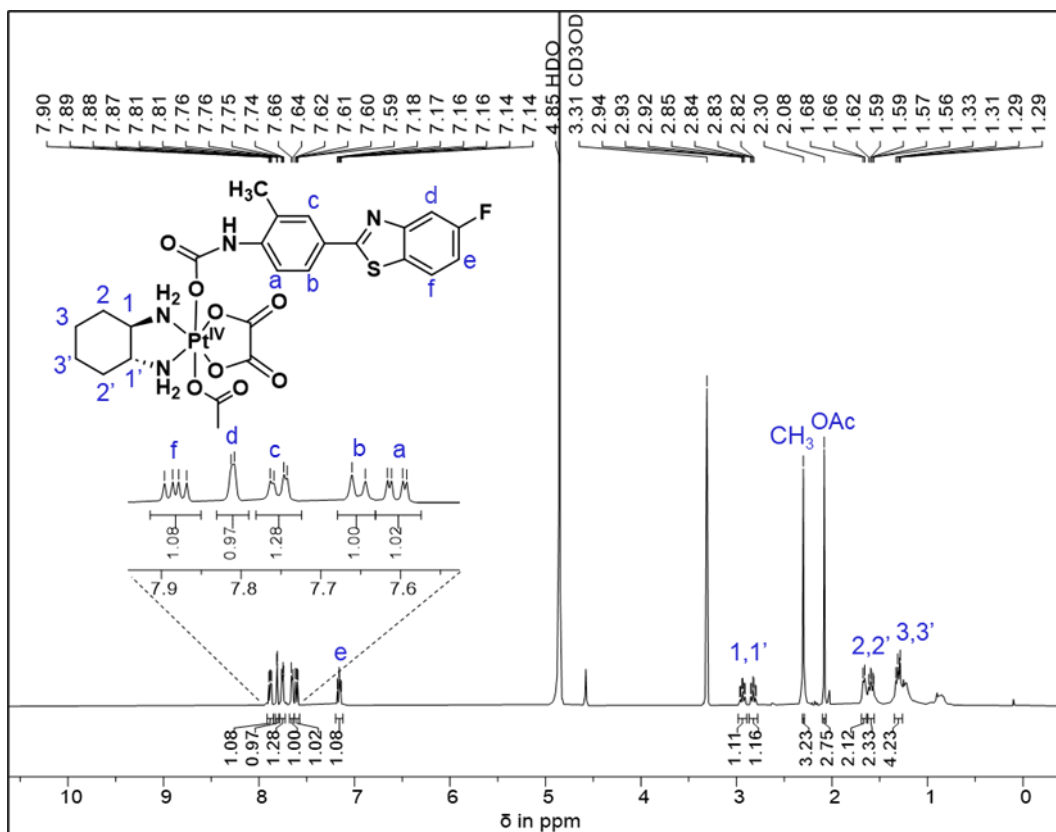


Figure 6.6. ^1H NMR (500 MHz, CD_3OD) of **Oxali-5F203 (Pt17)**.

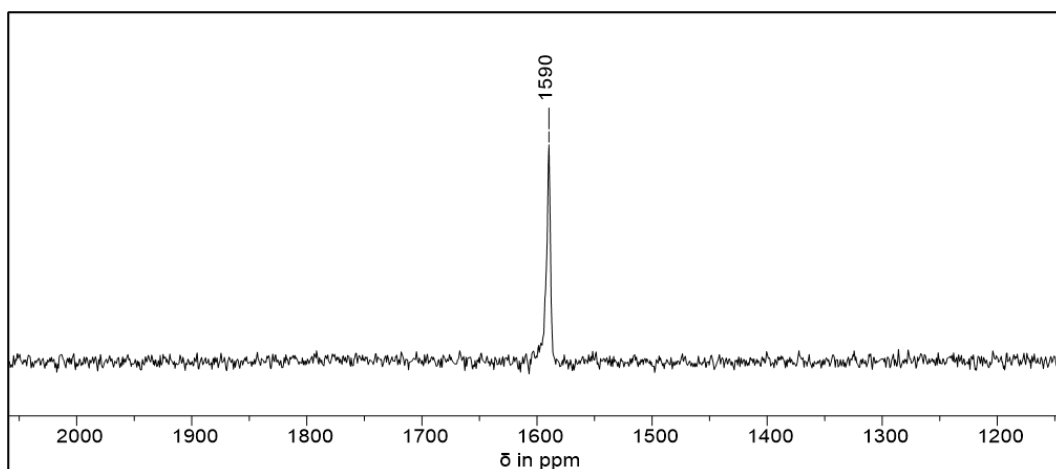


Figure 6.7. ^{195}Pt NMR (107 MHz, CD_3OD) of **Oxali-5F203 (Pt17)**.

The absorption and fluorescence emission profiles of **5F203 (L5)** and the platinum(IV) conjugates **Azido-5F203** and **Oxali-5F203** in PBS buffer (pH = 7.40) are shown in Figure 6.8. The ligand **L5** displays an intense absorption band at 341 nm, whilst the absorption maximum for **Oxali-5F203** and **Azido-5F203** are blue shifted to 324 nm and 307 nm, respectively. The fluorescence emission peak of **5F203** is observed at 433 nm. As expected, the fluorescence intensity of both Pt(IV)-5F203 complexes is quenched following the coordination of the ligand **L5** to the platinum centre.

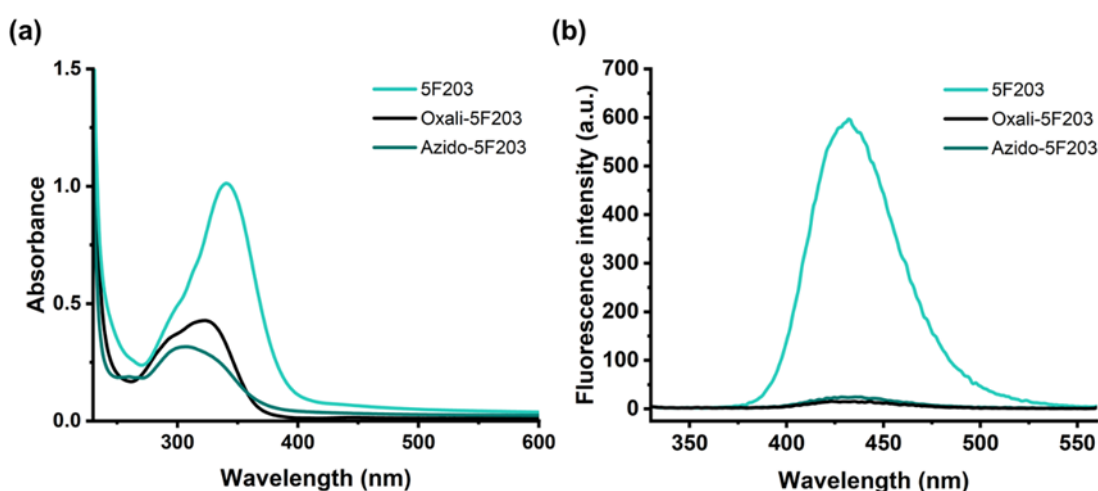


Figure 6.8. (a) UV-vis spectrum of **5F203 (L5, 15 μ M)**, **Azido-5F203 (Pt16, 15 μ M)** and **Oxali-5F203 (Pt17, 15 μ M)** in PBS buffer (pH = 7.40). (b) Fluorescence intensity of **5F203 (L5, 0.6 μ M)** compared to **Azido-5F203 (Pt16, 0.6 μ M)** and **Oxali-5F203 (Pt17, 0.6 μ M)** in PBS buffer (pH = 7.40), with $\lambda_{\text{ex}} = 290$ nm, slit widths: ex = 10 nm, em = 2.5 nm.

6.2.3. Photophysical and stability studies of the platinum(IV)-5F203 conjugates

This section presents the preliminary experiments conducted on the Pt(IV)-5F203 complexes in aqueous solution. In a first instance, we studied the stability and sensitivity of the Pt(IV) conjugates to sodium ascorbate (NaAsc) over the course of 8 hours by fluorescence spectroscopy. The results of the stability study are regrouped in Figure 6.9 for comparison. **Oxali-5F203** appeared to be stable for the duration of the experiment as no significant changes in fluorescence emission were observed. However, **Azido-5F203** exhibited gradual increases in fluorescence intensity with time, which indicate potential hydrolysis of the complex. These results seem to be consistent with our previous observations for the stability of **Azido-BDP** and **AzidoNap** in aqueous solution (*Chapter 2*). Surprisingly, upon treatment with sodium ascorbate, an instantaneous decrease in fluorescence intensity was observed for **Azido-5F203** and **Oxali-5F203** (Figure 6.10 and Figure 6.11). The fluorescence emission of the platinum(IV) conjugates appeared to be completely quenched after addition of the reducing agent.

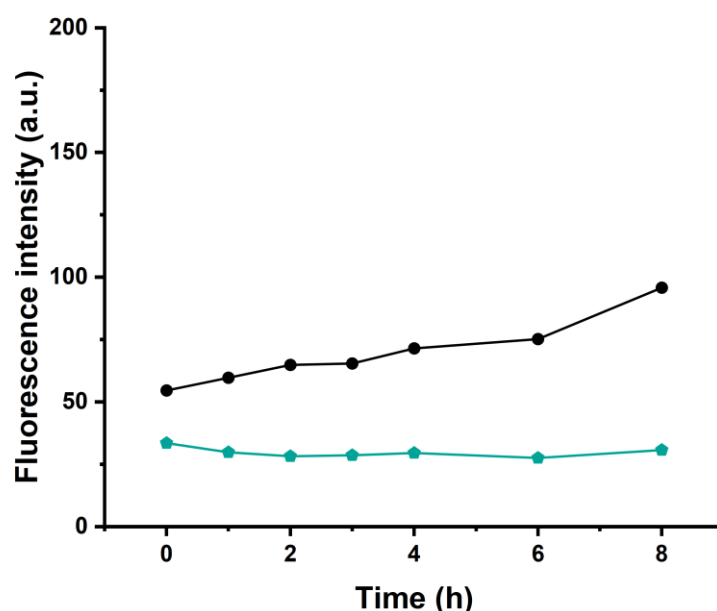


Figure 6.9. Changes in the fluorescence intensity of **Oxali-5F203** (Pt17, 5 μ M, blue) and **Azido-5F203** (Pt16, 5 μ M, black) at 430 nm in PBS buffer (pH = 7.40). Fluorescence intensities were recorded with $\lambda_{\text{ex}} = 290$ nm, slit widths: ex = 10 nm, em = 2.5 nm.

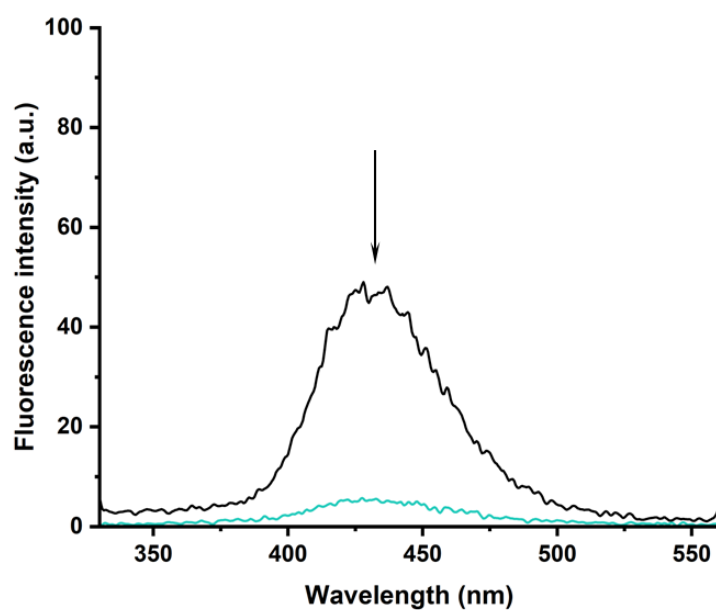


Figure 6.10. Fluorescence intensity changes of **Azido-5F203 (Pt16)**, 5 μM before (**black**) and after (**blue**) addition of 1 mM NaAsc in PBS buffer (pH = 7.40), λ_{ex} = 290 nm, slit widths: ex = 10 nm, em = 2.5 nm.

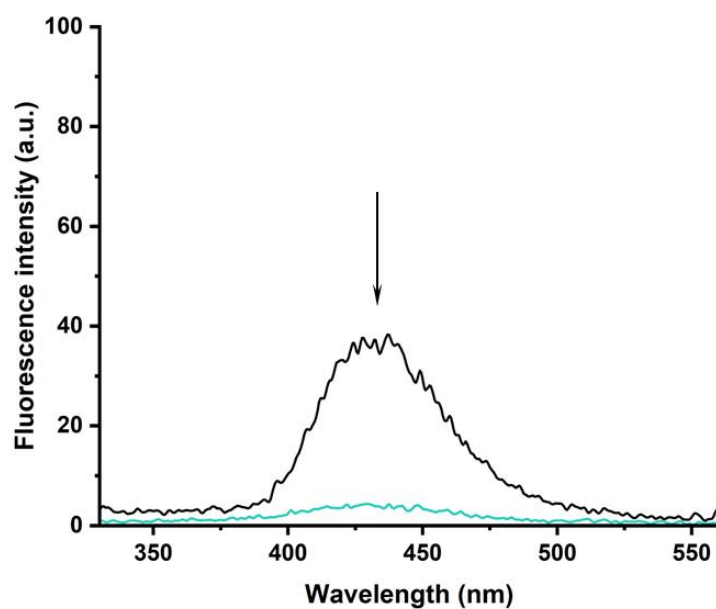


Figure 6.11. Fluorescence intensity changes of **Oxali-5F203 (Pt17)**, 5 μM before (**black**) and after (**blue**) addition of 1 mM NaAsc in PBS buffer (pH = 7.40), λ_{ex} = 290 nm, slit widths: ex = 10 nm, em = 2.5 nm.

Attempting to understand these results, the fluorescence response of the ligand **5F203** in the presence of sodium ascorbate was recorded before and after 60 minutes of incubation and a similar loss of fluorescence emission was witnessed (Figure 6.12). LC-MS analysis of the ligand **L5** with 1 mM NaAsc over the same period did not show any differences or formation of a new signal (Figure 6.13). Treatment of the anticancer drug with the reductant may result in the protonation of the benzothiazole amino group leading to fluorescence quenching of the fluorophore. Additional analyses are required to determine the type of species produced from this reaction and are currently being conducted. Nonetheless, due to the loss of fluorescence emission when using sodium ascorbate, the biological reductant NADH may be utilised instead to monitor the reduction of the OxPt(IV)-5F203 complex in solution- and cell-based studies.

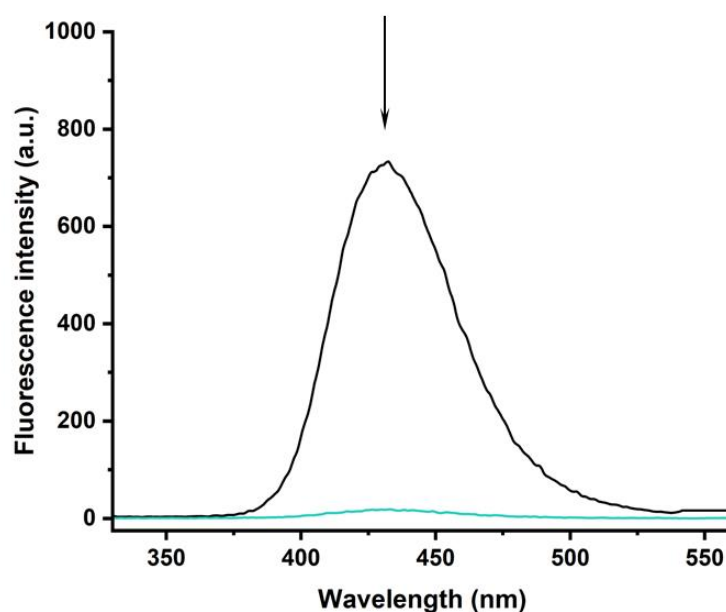


Figure 6.12. Fluorescence intensity changes of **5F203** (**L5**, 0.5 μ M) before (**black**) and after (**blue**) addition of 1 mM NaAsc in PBS buffer (pH = 7.40), λ_{ex} = 290 nm, slit widths: ex = 10 nm, em = 2.5 nm.

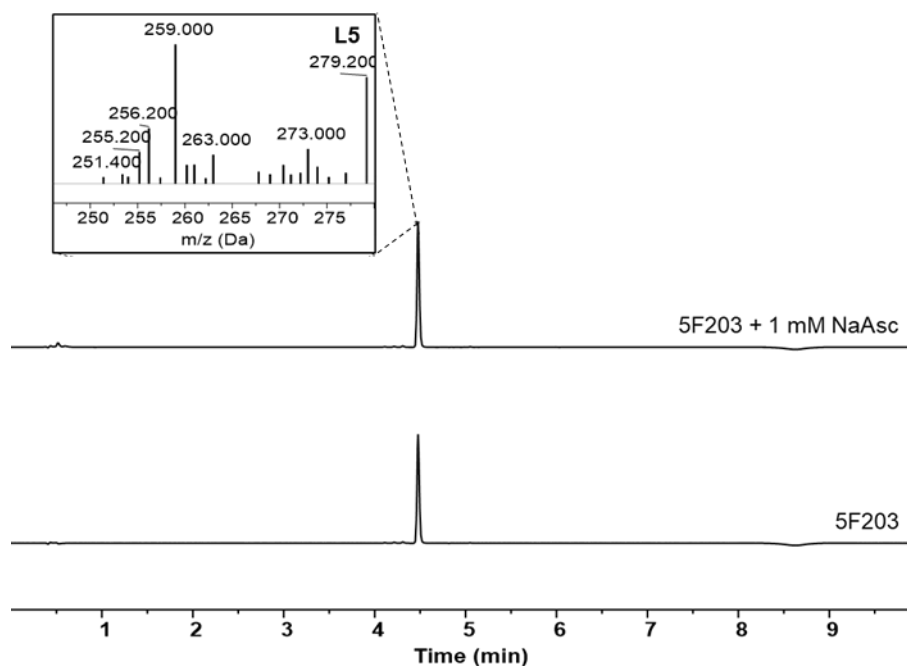
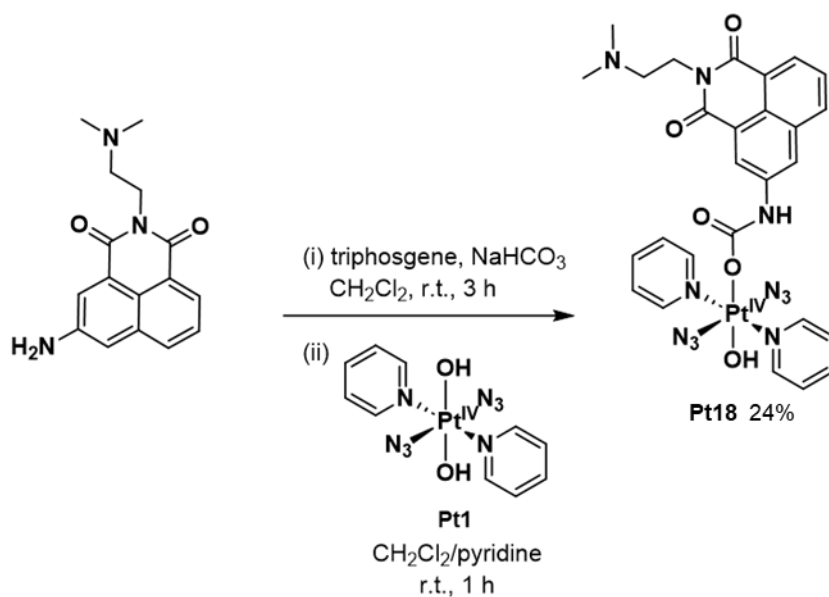


Figure 6.13. Injection of 5F203 before (**bottom**) and after (**top**) incubation with 1 mM NaAsc for 60 minutes recorded by LC-MS (Absorbance at 365 nm).

6.3. Platinum(IV)-Amonafide conjugate

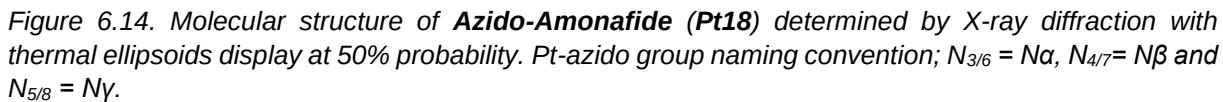
6.3.1. Synthesis and characterisation of Azido-Amonafide

The synthetic pathway of **Azido-Amonafide** (Complex **Pt18**) is illustrated in Scheme 6.3. The **Pt18** conjugate was notably more difficult to prepare and purify compared to the previous platinum(IV) complexes synthesised using the triphosgene activation method and was collected in lower yield (24 %). Upon completion of the first step, the solution was not evaporated as the intermediate could not be successfully redissolved in dichloromethane afterwards. This might be due to the protonation of the terminal nitrogen in the substituted group of the ligand which prevents its dissolution in the chlorinated solvent. The mixture was therefore centrifugated and the solution directly added dropwise to a mixture of DCM and pyridine containing **Pt1** at 0 °C in the following step. Isolation of the complex **Pt18** by silica gel column chromatography was distinctly more challenging due to the presence of the tertiary amino group of Amonafide. Thus, trituration of the recovered product with methanol and ethyl acetate was required to remove the residual impurities. Characterisation of the complex was achieved by ^1H , ^{13}C and ^{195}Pt NMR spectroscopy and high-resolution mass spectrometry.



Scheme 6.3. Synthesis of **Azido-Amonafide (Pt18)**.

Crystals of **Azido-Amonafide** suitable for X-ray diffraction were obtained from slow evaporation of a mixture of dichloromethane and methanol and analysed by Dr Kirsten E. Christensen (University of Oxford). The crystal structure of **Azido-Amonafide** is presented in Figure 6.14 and revealed conjugation of the antineoplastic drug to the platinum (IV) complex *via* carbamate linkage. Both diazido-Pt(IV) carbamate conjugates, **Azido-5F203** and **Azido-Amonafide**, share similar molecular structures around the platinum centre (further data available in Appendix A.4).



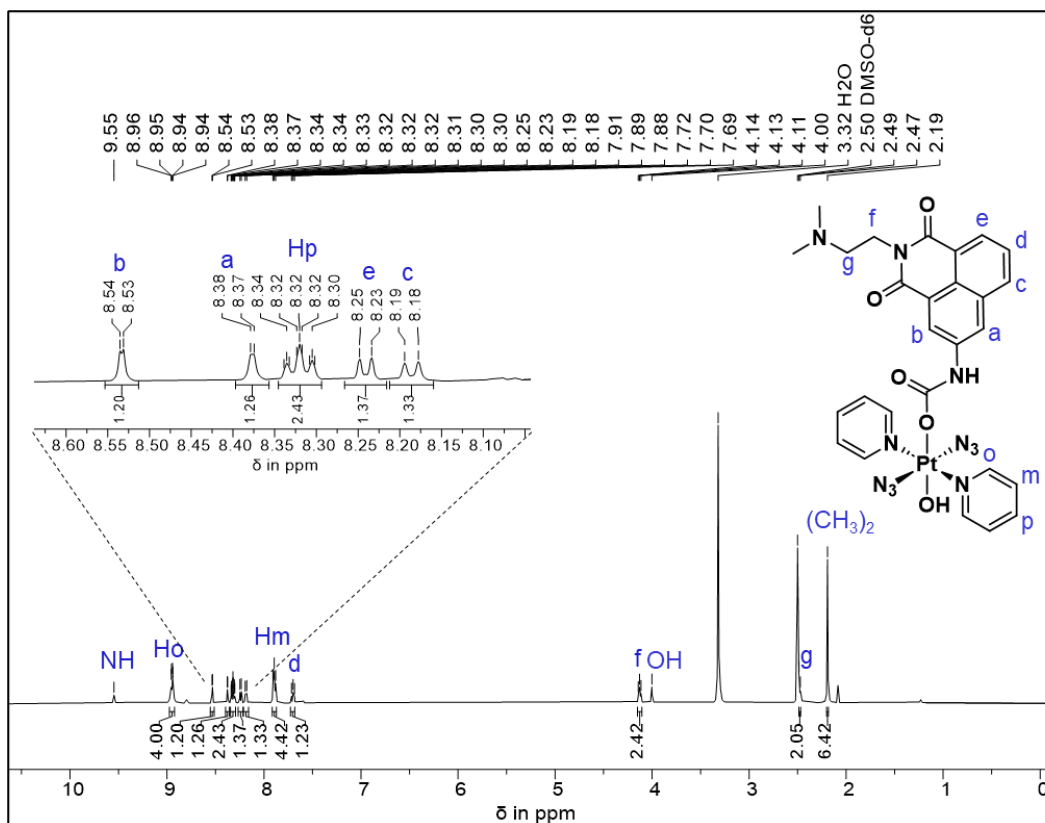


Figure 6.15. ¹H NMR (500 MHz, DMSO-d₆) of **Azido-Amonafide (Pt18)**.

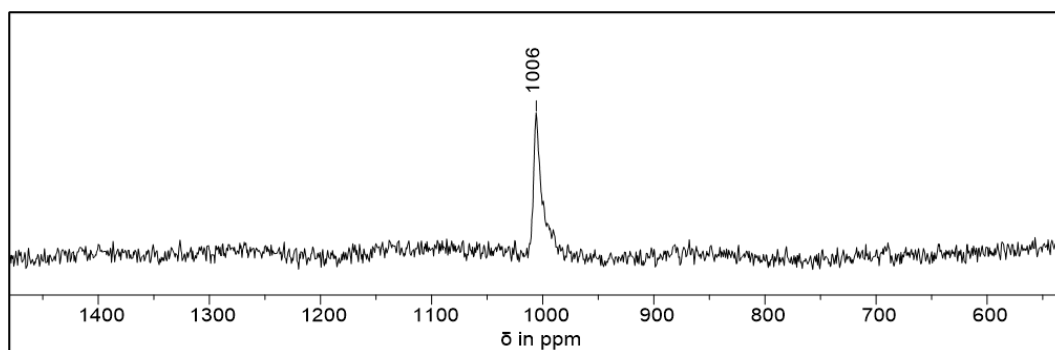
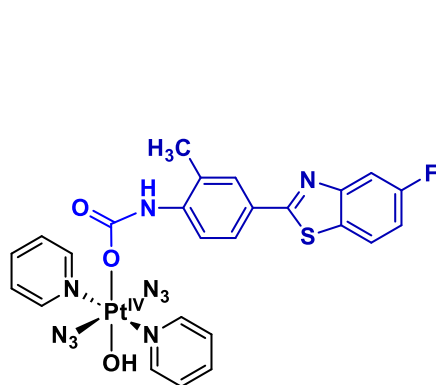


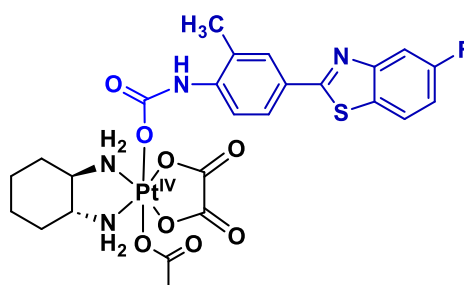
Figure 6.16. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆) of **Azido-Amonafide (Pt18)**.

6.4. Conclusion

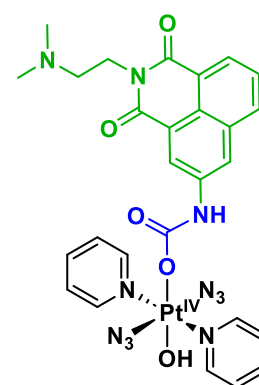
In this chapter, three platinum-based fluorescent therapeutics have been prepared following the synthetic procedure previously described for the synthesis of the naphthalimide-Pt(IV) carbamates. The coordination of two antineoplastic agents, 5F203 and Amonafide, to *trans, trans, trans*-[Pt(N₃)₂(OH)₂(py)₂] (**Pt1**) was initially explored. The diazido-platinum(IV) complex was used as a synthetic model as it can be obtained in high yield and purity following a straightforward three-steps synthesis. **Azido-5F203** and the OxPt(IV) analogue **Oxali-5F203** were synthesised in decent yields, whereas **Azido-Amonafide** was collected in poor yield. All three conjugates were characterised by NMR spectroscopy and mass spectrometry. Crystal structures of the diazido-platinum(IV) carbamates were acquired and confirmed coordination of both antitumor drugs to the axial hydroxido ligand of the Pt(IV) complex *via* a carbamate linker. Synthesis of the oxaliplatin(IV)-Amonafide complex was attempted and gave rise to the conjugate in even poorer yield than its diazido-Pt(IV) derivative. ¹H and ¹⁹⁵Pt NMR were indicative of the formation of the targeted complex, however, residual impurities could be observed in the spectra. Therefore, an alternative synthetic approach might be better suited for the preparation of platinum(IV)-based Amonafide carbamates. Preliminary studies on the stability and photophysical properties of the Pt(IV)-5F203 conjugates were undertaken. Unexpectedly, sodium ascorbate triggered a fluorescence quenching of the complexes' emission, preventing the use of the reductant to study platinum(IV) reduction in solution and biological studies.



Azido-5F203



Oxali-5F203



Azido-Amonafide

6.5. References

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Chapter 7 Conclusions and future work

The main aim of this work was to develop a range of fluorescence responsive platinum(IV) prodrugs as imaging agents to examine the intracellular activation of Pt(IV) complexes in response to stimuli in cancer cells. The reduction profiles, stability and cytotoxic properties of the novel compounds were investigated to study the influence of the equatorial and axial ligands on the relationship between these parameters. The information provided from this research could help identify new strategies for the design of novel Pt-based anticancer agents.

The project initially explored the conjugation of two fluorophores to the photoactive diazido-Pt(IV) complex (**Pt1**) to generate fluorescent platinum(IV) carboxylate and carbamate conjugates. Various coupling strategies were thoroughly investigated and led to the design of a new procedure for the incorporation of amine-containing compounds. Experimental analyses were conducted to assess the photophysical properties and stability of the complexes in aqueous solution. In the absence of light, both compounds exhibited low hydrolytic stability over a short period of time which precluded additional investigations. Prior work by Zhu, Gibson and co-workers showed that several asymmetric platinum(IV) complexes functionalised with a carboxylate or carbamate substituent were more stable when bearing a carboxylate (acetate or 4-phenyl butyrate, respectively) in *trans* compared to their hydroxido derivatives.^{1,2} Future work could therefore involve the introduction of an acetato ligand or another carboxylate group at the opposite axial position of the fluorophore which might have the potential of improving the aqueous stability of the BODIPY- and naphthalimide-Pt(IV) conjugates.

A set of platinum(IV) fluorescent probes based on the oxaliplatin, cisplatin and carboplatin scaffolds were subsequently synthesised and characterised. A self-immolative linkage system and a direct attachment strategy were used to compare the photophysical, photochemical and biological properties of these complexes. The results obtained in solution studies highlighted the considerable differences in terms of stability and sensitivity to reduction

between each compound according to the six ligands coordinated to the Pt centre. The OxPt(IV) and CarboPt(IV) carbamates were found to be stable in aqueous conditions and thus suitable for *in vitro* imaging studies. The activation of these complexes to the parent Pt(II) drug was visualised under severe hypoxia in colorectal and lung cancer cells, respectively. Increased cytotoxic activity was also measured for each complexes in hypoxic conditions compared to normoxia.³ Following these results, several directions can be investigated to design the next-generations of fluorescence responsive Pt(IV) prodrugs. The first approach should focus on enhancing the aqueous stability of the resorufin Pt(IV) complexes and the naphthalimide CisPt(IV) conjugate (Figure 7.1). A series of Pt(IV) carbamate derivatives in which the counter axial acetato ligand is substituted with various functional groups (e.g., hydroxyl, carboxylates, halogens) could be prepared to investigate the impact of these substituents on the hydrolytic stability of the resulting complexes.^{2,4} This might lead to the development of more stable Pt-based chemotherapeutics and allow for the visualisation of CisPt(IV) complexes reduction in biological studies. Moreover, previous reports have extensively examined the influence of electron-withdrawing and -donating substituents on the stability and reaction kinetics of *ortho*-quinone methide species. It was found that the presence of an electron-withdrawing group could significantly destabilise the quinone methide precursor and delay the elimination event.⁵ Hence, the addition of an electron-withdrawing group (such as a methyl ester, cyanide, or nitro substituent) on the linker might enhance the overall stability of the Pt(IV) complex and prevent the rapid hydrolysis of the self-immolative system. Furthermore, the two sensing strategies presented in these chapters could be extended to other well-known Pt(IV) prodrugs such as iproplatin and satraplatin to monitor their reduction to Pt(II) species in cancer cells.

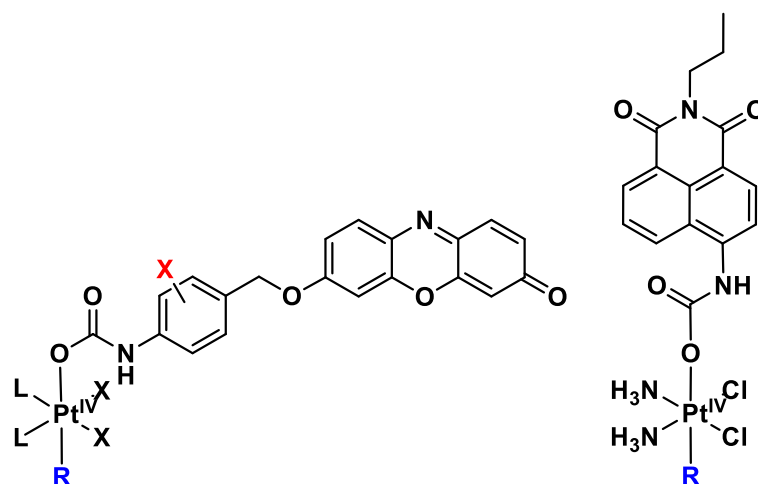


Figure 7.1. Proposed strategies to increase the hydrolytic stability of fluorescent Pt(IV) conjugates via the modification of the axial ligand (R) or/and the addition of EWG on the self-immolative linker.

In addition, the hypoxia-mediated activation of oxaliplatin- and carboplatin-based Pt(IV) carbamates generated new insights into the next therapeutic strategies to explore. The design of Pt(IV) prodrugs able to target specific hypoxic markers or cell organelles is highly attractive as it could improve the cancer-cell selectivity and therefore favour the accumulation of these complexes in tumours over normal cells. This would in return minimise toxic side-effects whilst enhancing anticancer efficacy. Carbonic anhydrase IX (CAIX) is an enzyme involved in the pH-regulation of the intra- and extracellular environments. CAIX has been investigated as a potential antitumour target due to its overexpression in solid hypoxic tumours.⁶ The combination of CAIX inhibitors to hypoxia-activatable OxPt(IV) and CarboPt(IV) prodrugs could be studied as dual-action chemotherapeutics for selectively targeting hypoxic cancer cells and achieve localise toxicity (Figure 7.2).^{7,8} Moreover, naphthalimide-based derivatives can be further functionalised at the imide position with organelle-targeting units. These molecules could be coordinated to OxPt(IV) and CarboPt(IV) compounds using the same synthetic method previously used to target certain sub-cellular components (lysosomes, mitochondria, and endoplasmic reticulum).^{9,10,11} The selective accumulation of these complexes upon cellular uptake might trigger several cell death-related pathways following organelle damage and increase the therapeutic effect (Figure 7.3).¹²

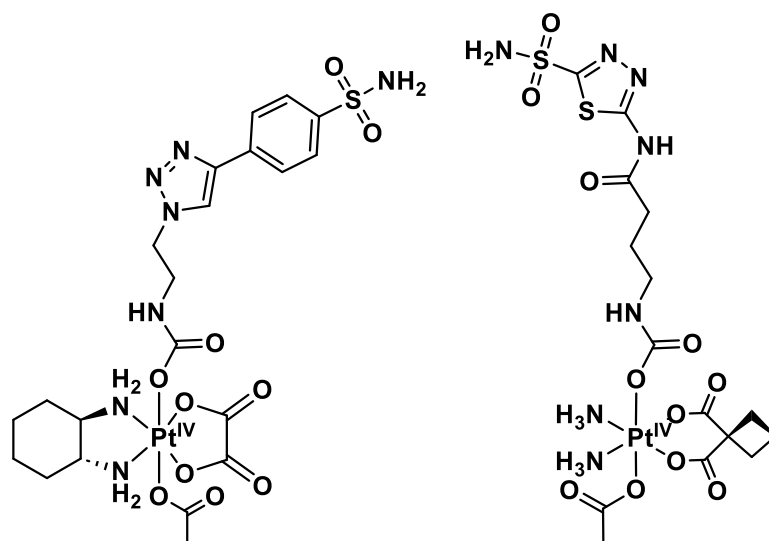


Figure 7.2. Examples of OxPt(IV) and CarboPt(IV) complexes containing hypoxia-targeting ligands.

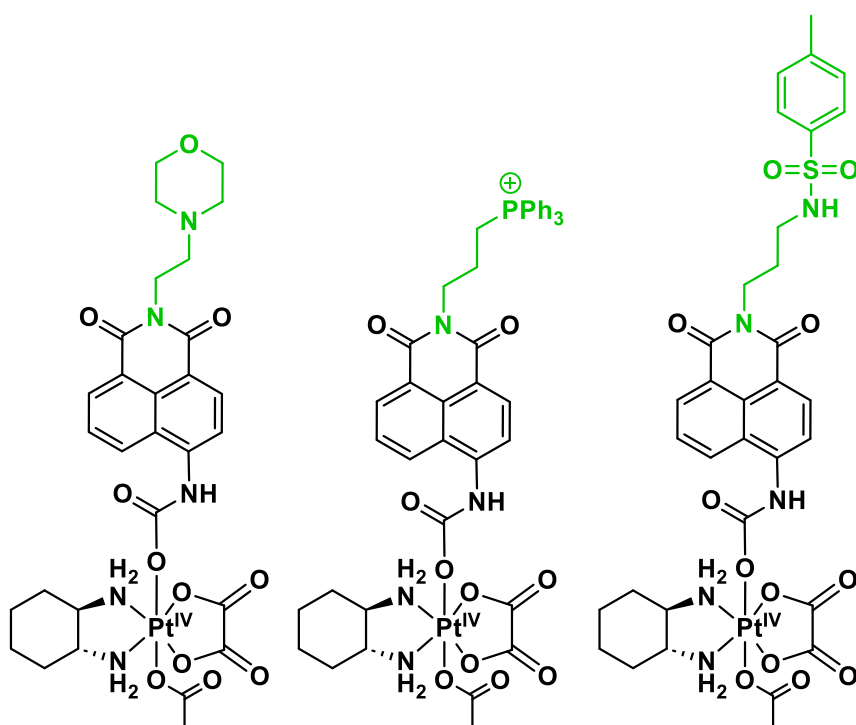


Figure 7.3. Examples of OxPt(IV) prodrugs containing organelle-targeting moieties: lysosome (left), mitochondria (middle), and ER (right). The same strategy could be extended to CarboPt(IV) prodrugs.

The development of platinum(IV) fluorescent therapeutics was initiated in the final project with the synthesis and characterisation of the diazido-Pt(IV) and OxPt(IV)-5F203 conjugates. Stability of each compound was monitored by fluorescence spectroscopy over an extended period. The OxPt(IV) complex was shown to be stable for the duration of the experiment whilst rapid hydrolysis of its diazido derivative was recorded. LC-MS analysis should be conducted to verify these results. Preliminary work investigating the reduction properties of these complexes revealed a fluorescence quenching effect caused by the addition of sodium ascorbate. Other reductants such as NADH might be considered to examine the reduction profile of the OxPt(IV) conjugate. Development of its carboplatin(IV) and cisplatin(IV) analogues could be undertaken following the same procedure and their photophysical properties and aqueous stability compared in solution-based studies. Depending on the hydrolytic stability demonstrated by the prodrugs, biological studies to observe their activation in cancer cells could be attempted. The synthesis and characterisation of the diazido-Pt(IV) Amonafide conjugate were also reported but alternative methods to couple Amonafide to oxaliplatin(IV), carboplatin(IV) and cisplatin(IV) complexes may be searched to produce the compounds in higher yields.

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Chapter 8 Experimental section

8.1. General procedures

Commercially available solvents and reagents were used without further purification unless otherwise stated. Reagents were laboratory grade and obtained from standard commercial sources. Anhydrous solvents were degassed with nitrogen and then used immediately. Water was deionised and microfiltered using an Elix® Essential water purification system. TLC Silica gel 60 plates and Geduran® Si 60 Silica gel for column chromatography were purchased from Merck (pore size 60 Å, 230-400 mesh, 40-63 µm). (IM) indicates use of a nylon syringe filter (pore size 0.2 µm).

NMR Spectroscopy: NMR spectra were acquired at 298 K. 499.9 MHz ^1H NMR, 125.7 MHz ^{13}C NMR, 107.57 MHz ^{195}Pt NMR, 160.4 MHz ^{11}B NMR, and 470.4 MHz ^{19}F NMR spectra were recorded on a Bruker Avance III NMR equipped with a 11.75 T magnet. 400.2 MHz ^1H NMR, 100.6 MHz ^{13}C NMR, 86.11 MHz ^{195}Pt NMR, 128.4 MHz ^{11}B NMR and 376.5 MHz ^{19}F NMR spectra were recorded on a Bruker Avance III HD nanobay NMR spectrometer equipped with a 9.4 T magnet. 600.4 MHz ^1H NMR and 151.0 MHz ^{13}C NMR spectra were recorded on a Bruker NEO600 with broadband helium cryoprobe equipped with a 14 T magnet. 699.9 MHz ^1H NMR and 176.0 MHz ^{13}C NMR spectra were recorded on a Bruker Avance III 700 with a $^1\text{H}(^{13}\text{C}/^{15}\text{N})$ inverse TCI cryoprobe equipped with a 16.44 T magnet. All spectra are referenced to the residual solvent peak. The chemical shifts (δ) are reported in part per million (ppm) and coupling constants (J) in Hertz (Hz). NMR abbreviations used are as follows: singlet (s), doublet (d), triplet (t), quintet (qu), pentet (p), sextet (sext), and multiplet (m). NMR data were processed using MestreLab MestreNova or Bruker TopSpin™.

Mass Spectrometry: Low-resolution mass spectra were acquired on an Agilent Technology 1260 Infinity or a Waters LCT Premier XE spectrometer. LC-MS were obtained on a Waters LCT Premier bench-top orthogonal acceleration time-of-flight LC-MS system. High-resolution accurate mass spectra were performed by the staff of the Chemistry Research

Laboratory at the University of Oxford using Bruker μ TOF or Micromass® GCT systems and recorded to 4 decimal places.

HPLC analysis: Agilent 1260 Infinity II® Poroshell 120 EC-C18 column [4 μ m, 4.6 $\times$ 100 mm]; [95:5 H₂O:MeCN $\rightarrow$ 5:95 H₂O:MeCN with 0.1 % TFA modifier, 10 min; 5 min hold; 1 mL.min⁻¹]. Ascentis® C18 column [5 μ m, 15 cm $\times$ 4.6 mm]; [98:2 H₂O:MeCN $\rightarrow$ 0:100 H₂O:MeCN with 0.05 % TFA modifier, 27 min; 8 min hold; 1.5 mL.min⁻¹].

LC-MS Analysis: Agilent 1260 Infinity II® ZORBAX SB C18 column [1.8 μ m, 2.1 $\times$ 50 mm]; [95:5 H₂O:MeCN $\rightarrow$ 5:95 H₂O:MeCN with 0.1 % FA modifier, 10 min; 5 min hold; 0.4 mL.min⁻¹].

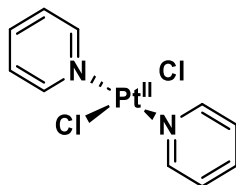
Infrared Spectroscopy: Solid-state IR spectra were recorded using a Bruker Tensor 27 FT-IR spectrometer with an IR Diamond Attenuated Total Reflectance module. The data was processed using Bruker OPUS software.

UV-Vis Spectroscopy: UV-vis spectra were recorded on a Jasco V-770 UV-visible/NIR spectrophotometer. The data was recorded using Spectra Manager™ Suite and the spectra were processed using OriginLab Origin®.

Luminescence Spectroscopy: Luminescence spectra were measured on a PerkinElmer LS-55 Fluorescence Spectrometer (equipped with a Pulsed Xenon lamp) using quartz cuvettes of 1 cm path length and operated on FL WinLab™ software. Spectra were processed using OriginLab Origin®.

8.2. Synthetic methods

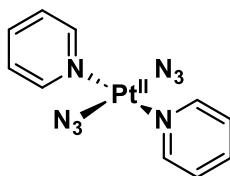
Trans-[Pt(py)₂Cl₂]¹



Pyridine (5.8 mL, 74.6 mmol, 20x excess) was added to a solution of K₂PtCl₄ (1.5 g, 3.6 mmol) in distilled water (38 mL). The reaction mixture was heated at 85 °C for 1 hour until colourless and filtered with IM. The solvent was removed, and the resulting white salt was washed with diethyl ether to remove the excess of pyridine. HCl (2 M, 37.5 mL) was added, and the solution was stirred at 75 °C for 24 hours. The yellow suspension was cooled on ice, filtered by Büchner funnel, and washed with cold H₂O, ethanol, and diethyl ether to give a pale-yellow solid (1.40 g, 3.30 mmol, 91 %).

¹H NMR (400 MHz, d₆-acetone) δ_H: 8.94 (dd, ³J_{HPt} = 31.0 Hz, ³J_{HH} = 8.0 Hz, 4H, Ho), 8.03 (t, ³J_{HH} = 7.0 Hz, 2H, Hp), 7.54 (t, ³J_{HH} = 7.0 Hz, 4H, Hm) ppm.

Trans-[Pt(py)₂(N₃)₂]¹

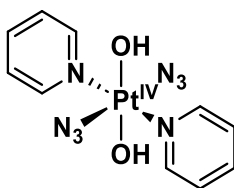


Silver nitrate (0.802 g, 4.73 mmol, 2 equiv.) was added to a solution of *trans*-[Pt(py)₂(Cl)₂] (1 g, 2.36 mmol) in distilled water (170 mL). The reaction was stirred at 60 °C overnight. The grey precipitate of AgCl was filtered with celite and IM. NaN₃ (0.77 g, 23.64 mmol, 5 equiv.) was added to the colourless solution which was stirred at room temperature for 4 hours. Another 5 mol equiv. of NaN₃ was added and the reaction mixture was stirred for an additional 4 hours. The solution was cooled on ice, filtered by Büchner funnel, and washed with cold H₂O, ethanol, and diethyl ether. The yellow crude product was recrystallized from pyridine, followed by

filtration with IM and cooling to -4 °C. The yellow product was filtered by Büchner funnel and washed with cold H₂O, ethanol, and diethyl ether and dried under vacuum (0.73 g, 1.67 mmol, 71 %).

¹H NMR (400 MHz, d₆-acetone) δ_{H} : 8.86 (dd, ³J_{HPt} = 36.0 Hz, ³J_{HH} = 8.0 Hz, 4H, Ho), 8.12 (t, ³J_{HH} = 7.0 Hz, 2H, Hp), 7.67 (t, ³J_{HH} = 7.0 Hz, 4H, Hm) ppm.

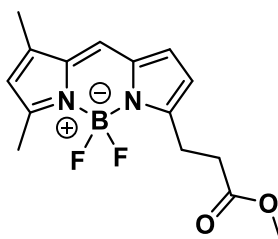
***Trans, trans, trans*-[Pt(py)₂(OH)₂(N₃)₂]¹ (Pt1)**



Trans-[Pt(py)₂(N₃)₂] (0.550 g, 1.258 mmol) was suspended in hydrogen peroxide – 34.5-36.5 % in water (43 mL). The solution was stirred at 45 °C for 3 hours. The solvent was reduced to 5 mL by rotary evaporator at 50 °C. Ethanol (10 mL) was added to the yellow solution which was filtered with IM. Diethyl ether (30 mL) was added, and the solution briefly mixed and left in the freezer overnight. The yellow crystals were filtered by Büchner funnel and washed with cold ethanol and diethyl ether (0.48 g, 1.02 mmol, 81 %).

¹H NMR (400 MHz, CD₃OD) δ_{H} : 9.01 (d, ³J_{HPt} = 32.0 Hz, ³J_{HH} = 8.0 Hz, 4H, Ho), 8.23 (t, ³J_{HH} = 8.0 Hz, 2H, Hp), 7.79 (t, ³J_{HH} = 7.0 Hz, 4H, Hm) ppm. ¹³C NMR (100 MHz, CD₃OD) δ_{C} : 150.6 (Co), 143.1 (Cp), 127.5 (Cm, ³J_{Cpt} = 27.9 Hz) ppm. ¹⁹⁵Pt NMR (86 MHz, CD₃OD): 857 ppm. ESI-MS (MeOH): *m/z* calcd. for C₁₀H₁₂N₈O₂Pt [2M+H]⁺ 943.68, found 943.01, [3M+H]⁺ 1414.99, found 1415.02. IR ν (cm⁻¹): 2044.77 (N₃).

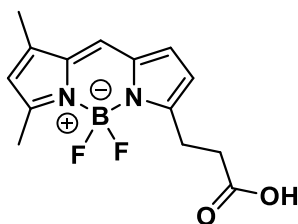
4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-methyl propanoate - BDP FL methyl propanoate²



POCl₃ (1.34 mL, 14.4 mmol, 1.1 equiv.) was added dropwise to a solution of methyl 3-(1*H*-pyrrol-2-yl)propanoate (2 g, 13.08 mmol) and 3,5-dimethylpyrrole-2-carboxaldehyde (1.772 g, 14.4 mmol, 1.1 equiv.) in anhydrous CH₂Cl₂ (120 mL) at 0 °C. The solution was slowly warm to room temperature and stirred for 3 hours. The reaction mixture was cooled to 0 °C and *N,N*-diisopropylethylamine (DIPEA, 10.2 mL, 58.86 mmol, 4.5 equiv.) was added dropwise and the solution was stirred at 0 °C for 20 minutes. Boron trifluoride diethyl etherate (6.4 mL, 52.32 mmol, 4 equiv.) was added and the reaction was stirred at room temperature overnight. The solution was diluted with CH₂Cl₂ (60 mL) and brine (140 mL), filtered through celite and diluted with brine (140 mL) and extracted with CH₂Cl₂ (5 x 150 mL). The organic layers were dried over MgSO₄, filtered, and evaporated by rotary evaporator. The crude product was purified by flash chromatography (100 % CH₂Cl₂) to yield a dark-green crystalline solid (2.26 g, 7.28 mmol, 56 %).

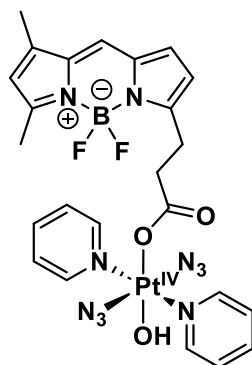
¹H NMR (400 MHz, CDCl₃) δ_H: 7.07 (s, 1H, C=CH-C), 6.88 (d, *J* = 4.0 Hz, 1H, C=CH-CH), 6.26 (d, *J* = 4.0 Hz, 1H, CH-CH=C), 6.11 (s, 1H, CH₃-C=CH), 3.69 (s, 3H, C-O-CH₃), 3.29 (t, *J* = 8.0 Hz, 2H, CH₂-CH₂-COOCH₃), 2.77 (t, *J* = 8.0 Hz, 2H, CH₂-CH₂-COOCH₃), 2.56 (s, 3H, CH-C-CH₃), 2.24 (s, 3H, C-C-CH₃) ppm.

4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid - BDP FL propionic acid² (L1)



BDP FL methyl propanoate (1.82 g, 5.97 mmol) was stirred in THF (45 mL) at 0 °C and deionised water (30 mL) and conc. HCL (15 mL) were added. The reaction was stirred at room temperature for 52 hours. The solution was diluted with water (100 mL) and extracted with CH₂Cl₂ (3 x 100 mL), and the organic layers were combined, dried with MgSO₄, and evaporated on rotary evaporator. The crude product was purified by flash chromatography (CH₂Cl₂/AcOH 100:1) to give a bright red solid (0.79 g, 2.70 mmol, 41 %).

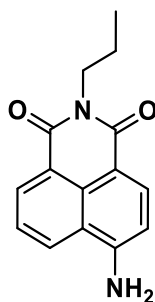
¹H NMR (400 MHz, CDCl₃) δ_H: 7.09 (s, 1H, C=CH-C), 6.89 (d, *J* = 4.0 Hz, 1H, C=CH-CH), 6.28 (d, *J* = 4.0 Hz, 1H, CH-CH=C), 6.12 (s, 1H, CH₃-C=CH), 3.30 (t, *J* = 8.0 Hz, 2H, CH₂-CH₂-COOH), 2.83 (t, *J* = 8.0 Hz, 2H, CH₂-CH₂-COOH), 2.57 (s, 3H, CH-C-CH₃), 2.25 (s, 3H, C-C-CH₃) ppm. ¹¹B NMR (160 MHz, CDCl₃) δ: 0.92 (t, 1B) ppm. ¹⁹F NMR (470 MHz, CDCl₃) δ: -145.28 (d, *J* = 33.3 Hz, 1F), -145.42 (d, *J* = 33.3 Hz, 1F) ppm. ESI-MS (MeOH): *m/z* calcd. for C₁₄H₁₅BF₂N₂O₂ [M+Na]⁺ 315.09, found 315.09, [2M+Na]⁺ 607.18, found 607.18.

***Trans, trans, trans*-[Pt(py)₂(OH)(O-CO-BDP)(N₃)₂]³ (Azido-BDP, Pt2)**

Trans, trans, trans-[Pt(py)₂(OH)₂(N₃)₂] (0.050 g, 0.106 mmol) was dissolved in dimethylformamide (3 mL). BDP FL propionic acid (0.031 g, 0.106 mmol, 1 equiv.), HBTU (0.080 g, 0.211 mmol, 2.5 equiv.) and DIPEA (0.04 mL, 0.211 mmol, 3 equiv.) were added and the reaction mixture was stirred at room temperature overnight. The crude product was purified by column chromatography on silica gel (100 % Hexane to 100 % EtOAc to CHCl₃/MeOH 100:3) to give a red solid (0.048 g, 0.066 mmol, 62 %).

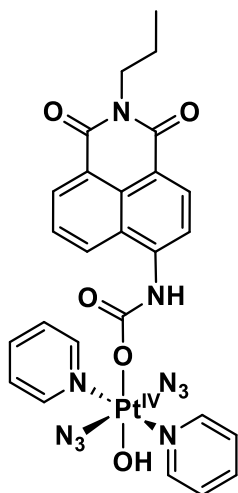
¹H NMR (400 MHz, CDCl₃) δ_H: 8.94 (d, ³J_{HPt} = 33.0 Hz, ³J_{HH} = 5.0 Hz, 4H, Ho), 8.02 (tt, ³J_{HH} = 8.0 Hz, 2H, Hp), 7.60 (t, ³J_{HH} = 7.0 Hz, 4H, Hm), 7.05 (s, 1H, C=CH-C), 6.82 (d, *J* = 4.0 Hz, 1H, C=CH-CH), 6.26 (d, *J* = 4.0 Hz, 1H, CH-CH=C), 6.11 (s, 1H, CH₃-C=CH), 3.29 (t, *J* = 8.0 Hz, 2H, CH₂-CH₂-COOPt), 2.80 (t, *J* = 8.0 Hz, 2H, CH₂-CH₂-COOPt), 2.58 (s, 3H, CH-C-CH₃), 2.25 (s, 3H, C-C-CH₃) ppm. ¹³C NMR (100 MHz, CDCl₃) δ_C: 176.1 (COOPt), 159.6 (CH-C-CH₃), 159.2 (CH-C-CH₂), 149.6 (Co), 143.4 (C-C-CH₃), 141.2 (Cp), 134.9 (C-CH-C-CH), 135.5 (C-CH-C-CH), 128.4 (C-CH-CH), 126.0 (Cm, ³J_{Cpt} = 27.6 Hz), 123.7 (C-CH-C-CH), 120.2 (CH₃-C-CH-C-CH₃), 117.1 (CH-CH-C), 35.4 (C-CH₂-CH₂), 25.5 (C-CH₂-CH₂), 15.1 (CH-C-CH₃), 11.4 (C-C-CH₃) ppm. ¹⁹F NMR (376 MHz, CDCl₃) δ: -145.57 (d, *J* = 33.3 Hz, 1F), -145.74 (d, *J* = 33.3 Hz, 1F) ppm. ¹¹B NMR (128 MHz, CDCl₃) δ: 0.95 (t, 1B) ppm. ¹⁹⁵Pt NMR (86 MHz, CDCl₃): 961 ppm. HRMS (*m/z*) calcd. for C₂₄H₂₅BF₂N₁₀O₃Pt [M+Na]⁺ 768.1712, found 768.1715.

Crystals suitable for single crystal X-ray diffraction were obtained from a concentrated solution of the product in MeOH. Please see Appendix A.1 for X-ray diffraction data.

6-amino-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (4-NH₂Nap, L2)⁴

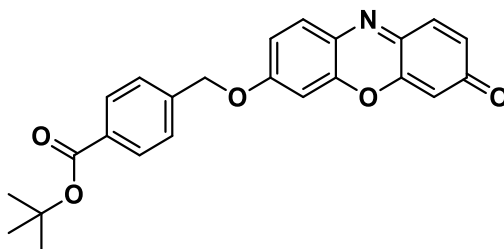
SnCl₂ (6.00 g, 32.00 mmol) was added to a solution of 6-Nitro-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (1.50 g, 5.28 mmol) in MeOH (50 mL) and concentrated HCl (10 mL). The reaction mixture was stirred at room temperature and monitored by TLC. Upon completion, the solvent was removed under reduced pressure, and the aqueous layer was adjusted to a basic solution by aqueous NaOH (2M). The title compound was extracted with DCM (3 x 50 mL), dried over MgSO₄, and purified by column chromatography on silica gel.

¹H NMR (400 MHz, DMSO-d₆) δ_H: 8.60 (dd, *J* = 1.4, 8.5 Hz, 1H, CH=CH-CH), 8.41 (dd, *J* = 1.4, 7.4 Hz, 1H, CH=CH-CH), 8.18 (d, *J* = 8.5 Hz, 1H, NH₂-C-CH=CH), 7.62 (t, *J* = 7.4 Hz, 1H, CH=CH-CH), 7.41 (s, 2H, NH₂), 6.84 (d, *J* = 8.3 Hz, 1H, NH₂-C-CH=CH), 3.95 (t, *J* = 7.5 Hz, 2H, N-CH₂-CH₂-CH₃), 1.60 (sext, *J* = 7.4 Hz, 2H, N-CH₂-CH₂-CH₃), 0.89 (t, *J* = 7.4 Hz, 3H, N-CH₂-CH₂-CH₃) ppm. ¹³C NMR (151 MHz, DMSO-d₆) δ_C: 163.7, 162.9, 152.6, 133.9, 130.9, 129.6, 129.2, 123.9, 121.8, 119.3, 108.1, 107.6, 40.7, 21.0, 11.4 ppm. HRMS (*m/z*) calcd. for C₁₅H₁₄N₂O₂ [M+H]⁺ 255.1128, found 255.1123.

***Trans, trans, trans*-[Pt(py)₂(OH)(O-CONH-Nap)(N₃)₂] (AzidoNap, Pt3)**

To a solution of 6-amino-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (0.040 g, 0.157 mmol, 1.0 equiv.) in CH₂Cl₂ (3 mL) was added triphosgene (0.093 g, 0.314 mmol, 2.0 equiv.) and NaHCO₃ (0.040 g, 0.471 mmol, 3.0 equiv.). The mixture was stirred at room temperature for 3 hours, followed by evaporation of the volatiles. The crude product was redissolved in CH₂Cl₂ and was added dropwise to a stirring solution of *trans, trans, trans*-[Pt(py)₂(OH)₂(N₃)₂] (0.037 g, 0.079 mmol, 0.5 equiv.) in CH₂Cl₂/pyridine (3:1) at 0 °C. After 5 minutes, the reaction mixture was warmed to room temperature and the solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting yellow solid was purified by column chromatography on silica gel (gradient method CH₂Cl₂/MeOH 1-3%) to give a yellow solid (0.025 g, 0.033 mmol, 42 %).

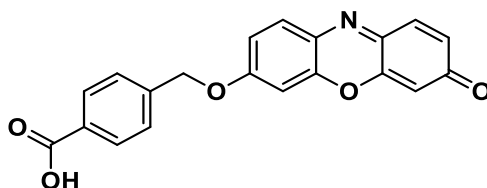
¹H NMR (500 MHz, DMSO-*d*₆) δ_H: 9.50 (s, 1H, NH), 8.98 (d, *J* = 6.7 Hz, 4H, Ho), 8.81 (d, *J* = 8.0 Hz, 1H, CH=CH-CH), 8.49 (d, *J* = 6.7 Hz, 1H, CH=CH-CH), 8.32 (m, 3H, Hp & CH=CH), 8.14 (d, *J* = 8.9 Hz, 1H, CH=CH), 7.88 (t, *J* = 6.2 Hz, 4H, Hm), 7.80 (t, *J* = 6.7 Hz, 1H, CH=CH-CH), 4.06 (s, OH), 3.98 (t, *J* = 7.5 Hz, 2H, N-CH₂-CH₂-CH₃), 1.64 (sext, *J* = 7.7 Hz, 2H, N-CH₂-CH₂-CH₃), 0.90 (t, *J* = 7.3 Hz, 3H, N-CH₂-CH₂-CH₃) ppm. ¹³C NMR (151 MHz, DMSO-*d*₆) δ_C: 163.6, 163.0, 156.5, 149.3, 143.5, 142.1, 132.1, 130.7, 129.4, 128.5, 126.3, 125.5, 122.5, 122.0, 115.7, 114.4, 41.0, 20.9, 11.4 ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-*d*₆): 1011 ppm. HRMS (*m/z*) calcd. for C₂₆H₂₄N₁₀O₅Pt [M+Na]⁺ 774.1471, found 774.1442.

Tert-butyl 4-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)benzoate (L3)

Resorufin (0.500 g, 2.35 mmol) was dissolved in dimethylformamide (3 mL) and *tert*-butyl 4-(bromomethyl)benzoate (0.637 g, 2.35 mmol) and K_2CO_3 (0.487 g, 3.525 mmol) were added to the solution. The reaction mixture was stirred at room temperature overnight. The solution was diluted with CH_2Cl_2 (30 mL) and extracted with brine (5 x 50 mL). The organic layers were dried over $MgSO_4$, filtered, and dried under reduced pressure to give a bright red solid (0.535 g, 1.33 mmol, 56 %).

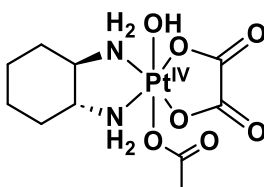
^1H NMR (400 MHz, $CDCl_3$) δ_H : 8.04 (d, J = 8.4 Hz, 2H, COOH-C=CH, COOH-C-CH), 7.75 (d, J = 8.8 Hz, 1H, N=C-CH), 7.49 (d, J = 9.4 Hz, 2H, COOH-C=CH-CH, COOH-C-CH=CH), 7.44 (s, 1H, N-C=CH), 7.05 (dd, J = 2.6, 9.0 Hz, 1H, N-C=CH-CH), 6.91 (d, J = 2.0 Hz, 1H, N=C-CH=CH), 6.88 (d, J = 2.3 Hz, 1H, N-C=CH-CH), 6.43 (d, J = 2.0 Hz, 1H, O-C-CH), 5.25 (s, 2H, O-CH₂), 1.59 (s, 9H, (CH₃)₃) ppm. ^{13}C NMR (151 MHz, DMSO- d_6) δ_C : 186.1, 165.3, 162.7, 149.9, 145.8, 145.7, 139.9, 134.9, 134.1, 132.3, 131.9, 130.1, 128.9, 127.0, 114.6, 106.8, 101.3, 81.4, 70.4, 28.3 ppm. HRMS (m/z) calcd. for $C_{24}H_{21}NO_5$ $[M+Na]^+$ 426.1312, found 426.1308.

Crystals suitable for single crystal X-ray diffraction were obtained by slow evaporation of a concentrated solution of the product in $CDCl_3$. Please see Appendix A.2 for X-ray diffraction data.

4-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)benzoic acid (L4)

Tert-butyl 4-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)benzoate (0.515 g, 1.28 mmol) was dissolved in CH₂Cl₂ (5 mL). Trifluoroacetic acid (5 mL) was added, and the reaction mixture was stirred at room temperature for 3 hours. The solvent was removed under reduced pressure, washed with MeOH twice and dried to give a bright red solid (0.435 g, 1.25 mmol, 98 %).

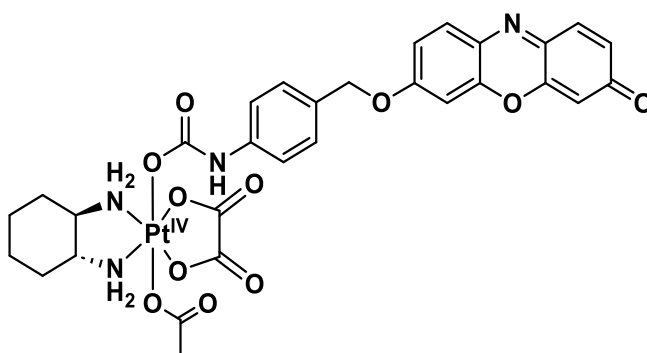
¹H NMR (400 MHz, DMSO-d₆) δ_H: 13.02 (s, 1H, OH), 7.99 (d, *J* = 8.0 Hz, 2H, COOH-C=CH & COOH-C-CH), 7.79 (d, *J* = 8.8 Hz, 1H, N=C-CH), 7.60 (d, *J* = 8.0 Hz, 2H, COOH-C=CH-CH & COOH-C-CH=CH), 7.53 (d, *J* = 9.5 Hz, 1H, N-C=CH), 7.19 (d, *J* = 2.4 Hz, 1H, O-C=CH), 7.15 (dd, *J* = 2.4, 8.5 Hz, 1H, N=C-CH=CH), 6.79 (dd, *J* = 2.0, 9.6 Hz, 1H, N-C=CH-CH), 6.25 (d, *J* = 2.0 Hz, 1H, O-C-CH), 5.37 (s, 2H, O-CH₂) ppm. ¹³C NMR (151 MHz, DMSO-d₆) δ_C: 185.3, 167.0, 162.1, 157.8, 157.6, 149.7, 145.4, 145.2, 141.0, 134.9, 133.8, 131.4, 130.6, 129.6, 128.1, 127.6, 118.4, 116.4, 114.3, 105.7, 101.3, 69.7 ppm. HRMS (*m/z*) calcd. for C₂₀H₁₃NO₅ [M+H]⁺ 348.0867, found 348.0862.

OxPt(OH)(OAc)⁵ (Pt4)

Hydrogen peroxide – 34.5-36.5 % in water (2 mL) was added to oxaliplatin (0.200 g, 0.503 mmol) in acetic acid (80 mL) and the reaction was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residual solid was washed with diethyl ether to obtain a white solid (Quant. Yield).

^1H NMR (500 MHz, D_2O) δ_{H} : 2.87 (m, 2H), 2.30 (m, 2H), 2.06 (s, 3H), 1.65-1.55 (m, 4H), 1.25 (m, 2H) ppm. ^{195}Pt NMR (107 MHz, D_2O): 1315 ppm. ESI-MS (H_2O): m/z calcd. for $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_7\text{Pt}$ $[\text{M}+\text{H}]^+$ 474.08, found 474.05, $[\text{2M}+\text{H}]^+$ 947.16, found 947.09, $[\text{3M}+\text{H}]^+$ 1419.24, found 1419.93.

OxPt(O-CONH-L4)(OAc) (OxaliRes, Pt5)



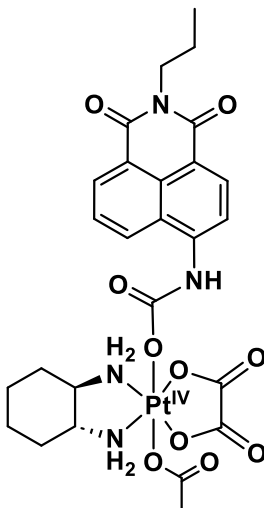
4-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)benzoic acid (0.040 g, 0.107 mmol, 3.0 equiv.) was suspended in dimethylformamide (3 mL). Triethylamine (Et_3N , 22 μL , 0.107 mmol, 3.0 equiv.) and diphenyl phosphoryl azide (DPPA, 34 μL , 0.107 mmol, 3.0 equiv.) were added and the reaction mixture was stirred for 30 minutes at room temperature followed by heating at 70 $^\circ\text{C}$ for 3 hours. The reaction mixture was cooled to room temperature. OxPt(OH)(OAc) (0.024 g, 0.052 mmol) was added and the reaction was stirred at 100 $^\circ\text{C}$. Upon completion, the crude mixture was purified via column chromatography (100 % Hexane to 100 % EtOAc to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 100:5) to afford the title compound as a red solid (0.022 g, 0.27 mmol, 51 %).

^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ_{H} : 9.21 (s, 1H, NH), 8.50 (m, 4H, NH_2), 7.77 (d, $J = 8.8$ Hz, 1H, $\text{N}=\text{C}-\text{CH}$), 7.53 (d, $J = 9.7$ Hz, 1H, $\text{N}-\text{C}=\text{CH}$), 7.45 (d, $J = 7.8$ Hz, 2H, $\text{NH}-\text{C}=\text{CH}$ & $\text{NH}-\text{C}-\text{CH}$), 7.31 (d, $J = 8.3$ Hz, 2H, $\text{NH}-\text{C}=\text{CH}-\text{CH}$ & $\text{NH}-\text{C}-\text{CH}=\text{CH}$), 7.17 (d, $J = 2.7$ Hz, 1H, $\text{CO}-\text{CH}$), 7.11 (dd, $J = 2.6, 8.9$ Hz, 1H, $\text{N}=\text{C}-\text{CH}=\text{CH}$), 6.79 (dd, $J = 2.1, 9.8$ Hz, 1H, $\text{O}-\text{C}=\text{CH}$), 6.27 (d, $J = 2.1$ Hz, 1H, $\text{O}-\text{C}=\text{CH}$), 5.17 (s, 2H, $\text{O}-\text{CH}_2$), 2.75-2.64 (m, 2H, DACH), 2.16 (m, 2H, DACH), 1.97 (s, 3H, CH_3), 1.52 (m, 2H, DACH), 1.47 (m, 2H, DACH), 1.19 (m, 2H, DACH) ppm. ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ_{C} : 185.3, 178.2, 163.4, 163.4, 162.4, 161.0, 149.7, 145.2, 145.2, 140.1, 134.9, 133.7, 131.3, 128.9, 128.7, 127.9, 118.3, 114.4, 105.6, 101.2, 70.3, 61.1,

61.0, 30.9, 30.9, 30.8, 29.6, 23.5, 23.5, 22.9 ppm. ^{195}Pt NMR (107 MHz, DMSO- d_6): 1626 ppm.

HRMS (m/z) calcd. for $\text{C}_{30}\text{H}_{30}\text{N}_4\text{O}_{11}\text{Pt}$ $[\text{M}+\text{Na}]^+$ 840.1451, found 840.1449.

OxPt(O-CONH-Nap)(OAc) (OxaliNap, Pt6)



To a solution of 6-amino-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (0.040 g, 0.157 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL) was added triphosgene (0.093 g, 0.314 mmol, 2.0 equiv.) and NaHCO_3 (0.040 g, 0.471 mmol, 3.0 equiv.). The mixture was stirred at room temperature for 3 hours, followed by evaporation of the volatiles. The crude product was redissolved in CH_2Cl_2 and was added dropwise to a stirring solution of OxPt(OH)(OAc) (0.037 g, 0.079 mmol, 0.5 equiv.) in CH_2Cl_2 /pyridine/DMF (3:1:1) at 0 °C. After 5 minutes, the reaction mixture was warmed to room temperature and the solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting yellow solid was redissolved in DMF and purified by column chromatography on silica gel (100 % Hexane to 100 % EtOAc to gradient method CH_2Cl_2 /MeOH 2-4%) to give a yellow solid. The recovered solid was triturated with EtOAc and CHCl_3 to yield a yellow solid (0.020 g, 0.027 mmol, 34 %). ^1H NMR (600 MHz, DMSO- d_6) δ_{H} : 9.71 (s, 1H, NH), 8.66 (dd, J = 1.2, 8.4 Hz, 1H, CH=CH-CH), 8.51 (dd, J = 1.2, 7.2 Hz, 1H, CH=CH-CH), 8.44-8.30 (m, 4H, NH_2), 8.42 (d, J = 8.3 Hz, 1H, NH-C-CH=CH), 8.11 (d, J = 8.3 Hz, 1H, NH-C-CH=CH), 7.83 (t, J = 7.34 Hz, 1H, CH=CH-CH), 4.00 (t, J = 7.4 Hz, 2H, N- CH_2 - CH_2 - CH_3), 2.86 (m, 1H, DACH), 2.60 (m, 1H, DACH), 2.16-

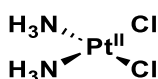
2.12 (m, 2H, DACH), 1.99 (s, 3H, OCO-CH₃), 1.65 (sext, $J = 8.4$ Hz, 2H, N-CH₂-CH₂-CH₃), 1.54-1.45 (m, 4H, DACH), 1.23-1.14 (m, 2H, DACH), 0.91 (t, $J = 7.8$ Hz, 3H, N-CH₂-CH₂-CH₃) ppm. ¹³C NMR (151 MHz, DMSO-d₆) δ_C: 178.3, 163.6, 163.0, 159.9, 142.0, 131.7, 130.8, 129.5, 128.4, 125.9, 123.4, 122.2, 117.9, 115.9, 61.2, 61.0, 41.0, 30.9, 30.8, 30.7, 23.6, 23.5, 22.8, 20.9, 11.4 ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1634 ppm. HRMS (m/z) calcd. for C₂₆H₃₀N₄O₁₀Pt [M+Na]⁺ 776.1502, found 776.1492.

***Cis*-[Pt(II)(NH₃)₂]⁶**



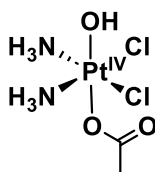
K₂PtCl₄ (1.5 g, 3.61 mmol) was dissolved in 25 mL of deionised H₂O with KI (6 g, 36.1 mmol, 10 equiv.). The reaction was stirred at room temperature for 2 hours. NH₄Cl (0.39 g, 7.29 mmol) was then added to the mixture and the pH adjusted to 10 using a 2M KOH solution. The solution was stirred at room temperature for 1 hour and the yellow precipitate was isolated by filtration, washed with cold water, ethanol, and ether, and dried under suction (1.21 g, 2.50 mmol, 69 %).

Cisplatin⁶



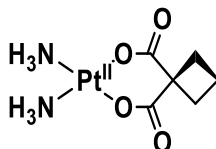
Cis-[Pt(II)(NH₃)₂] (0.400 g, 0.828 mmol) was suspended in 30 mL of H₂O. AgNO₃ (0.280 g, 1.65 mmol, 2 equiv.) was added and the reaction was heated at 55 °C overnight. The precipitate of silver iodide was filtered, and the filtrate was concentrated until ~5 mL remained. KCl (0.248 g, 3.33 mmol, 4 equiv.) was then added and a yellow precipitate immediately formed. The mixture was stirred for 2 hours at room temperature and cooled to 0 °C. The yellow solid was isolated by filtration, washed with minimal cold water, ethanol, and ether, and dried under suction (0.206 g, 0.69 mmol, 83 %).

¹H NMR (400 MHz, DMSO-d₆) δ_H: 3.95 (s, 6H) ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): -2091 ppm.

CisPt(OH)(OAc)⁷ (Pt7)

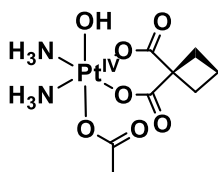
Cis-[Pt(Cl)₂(NH₃)₂] (0.100 g, 0.33 mmol) was suspended in 40 mL of acetic acid to which hydrogen peroxide 34.5-36.5 % in water (2 mL) was added. The mixture was stirred at room temperature for 3 hours. The colourless solution was concentrated under reduced pressure and ethanol, and diethyl ether were added to the residual solution. A pale-yellow precipitate was recovered by centrifugation and washed twice with diethyl ether (0.113 g, 0.30 mmol, 91 %).

¹H NMR (500 MHz, DMSO-d₆) δ_H: 5.97 (t with ¹⁹⁵Pt satellites peaks, ¹J_{H-N} = 52.6 Hz, ²J_{H-Pt} = 53.1 Hz, 6H, NH₃), 1.87 (s, 3H, CH₃) ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1054 ppm. ESI-MS (MeOH): *m/z* calcd. for C₂H₁₀Cl₂N₂O₃Pt [2M+H]⁺ 753.20, found 753.81.

Carboplatin⁸

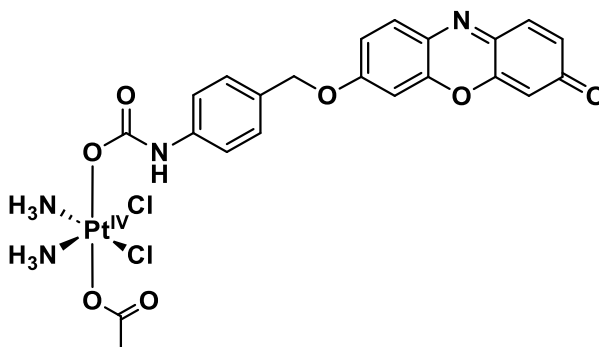
Cis-[Pt(II)₂(NH₃)₂] (0.240 g, 0.497 mmol) was suspended in 20 mL of H₂O. AgSO₄ (0.153 g, 0.491 mmol, 2 equiv.) was added and the reaction was stirred at room temperature for 4 hours. The precipitate of silver iodide was filtered and a solution of 1,1-cyclobutanedicarboxylic acid (0.082 g, 0.569 mmol) and Ba(OH)₂·8H₂O (0.154 g, 0.488 mmol) in 5 mL H₂O was added to the filtrate. The reaction mixture was stirred at room temperature overnight, filtered and concentrated under reduced pressure to give a white solid (0.120 g, 0.32 mmol, 66 %).

¹H NMR (400 MHz, DMSO-d₆) δ_H: 4.10 (s, 6H), 2.66 (t, *J* = 7.85 Hz, 4H), 1.65 (qu, *J* = 7.86 Hz, 2H) ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): -1710 ppm.

CarboPt(OH)(OAc)⁹ (Pt8)

Carboplatin (0.050 g, 0.135 mmol) was suspended in 20 mL of acetic acid and 2 mL of hydrogen peroxide – 34.5-36.5 % in water was added. The reaction mixture was stirred at room temperature for 2 hours. The colourless solution was concentrated under reduced pressure and diethyl ether was added to the residual solution. The white precipitate was recovered by centrifugation and washed twice with diethyl ether (0.054 g, 0.12 mmol, 90 %).

¹H NMR (500 MHz, DMSO-d₆) δ_H: 5.98-5.72 (m, 6H, NH₃), 2.54 (d, *J* = 8.0 Hz, 2H), 2.47 (d, *J* = 8.0 Hz, 2H), 1.86 (s, 3H), 1.78 (dt, *J* = 7.7, 15.7 Hz, 2H) ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1758 ppm. ESI-MS (MeOH): *m/z* calcd. for C₈H₁₆N₂O₇Pt [M+H]⁺ 448.06, found 448.02, [2M+H]⁺ 895.12, found 895.18.

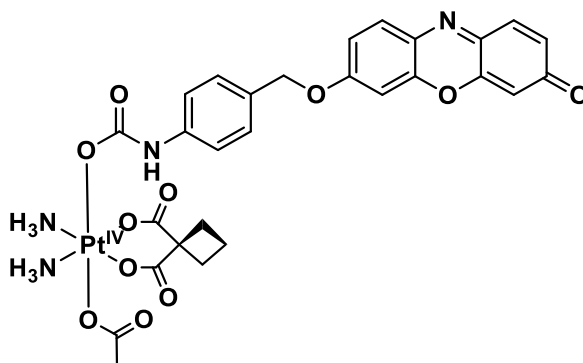
CisPt(O-CONH-L4)(OAc) (CisRes, Pt9)

4-(((3-oxo-3*H*-phenoxazin-7-yl)oxy)methyl)benzoic acid (0.040 g, 0.107 mmol, 3.0 equiv.) was suspended in dimethylformamide (3 mL). Triethylamine (Et₃N, 22 μL, 0.107 mmol, 3.0 equiv.) and diphenyl phosphoryl azide (DPPA, 34 μL, 0.107 mmol, 3.0 equiv.) were added and the reaction mixture was stirred for 30 minutes at room temperature followed by heating at 70 °C for 3 hours. The reaction mixture was cooled to room temperature. CisPt(OH)(OAc) (0.019 g, 0.052 mmol, 2 equiv.) was added and the reaction was stirred at 100 °C. Upon completion, the crude mixture was purified via column chromatography (100 % Hexane to 100 % EtOAc to

gradient method CH₂Cl₂/MeOH 6-8 %). The recovered solid was triturated with EtOAc and MeOH to afford the title compound as a dark red solid (0.010 g, 0.0139 mmol, 27 %).

¹H NMR (400 MHz, DMSO-d₆) δ_H: 9.16 (s, NH), 7.78 (d, *J* = 8.8 Hz, 1H, O=C-CH=CH), 7.53 (d, *J* = 9.4 Hz, 1H, O-C=CH-CH), 7.48 (d, *J* = 8.2 Hz, 2H, NH-C=CH & NH-C-CH), 7.31 (d, *J* = 8.9 Hz, 2H, NH-C=CH-CH & NH-C-CH=CH), 7.17 (d, *J* = 2.6 Hz, 2H, O=C-CH & O=C-CH=CH), 6.79 (dd, *J* = 2.0, 9.5 Hz, 1H, O-C=CH-CH), 6.65 (m, NH₃), 6.27 (d, *J* = 3.0 Hz, 1H, O-C=CH), 5.15 (s, 2H, O-CH₂), 1.93 (s, 3H, CH₃) ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1257 ppm. HRMS (*m/z*) calcd. for C₂₂H₂₂Cl₂N₄O₇Pt [M+Na]⁺ 742.0406, found 742.0403. *a suitable ¹³C NMR spectrum could not be recorded due to stability issues.

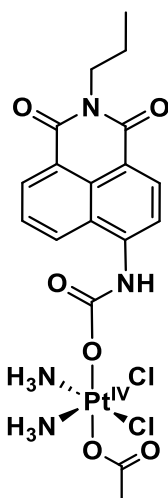
CarboPt(O-CONH-L4)(OAc) (CarboRes, Pt10)



4-(((3-oxo-3*H*-phenoxazin-7-yl)oxy)methyl)benzoic acid (0.040 g, 0.107 mmol, 3.0 equiv.) was suspended in dimethylformamide (3 mL). Triethylamine (Et₃N, 22 μL, 0.107 mmol, 3.0 equiv.) and diphenyl phosphoryl azide (DPPA, 34 μL, 0.107 mmol, 3.0 equiv.) were added and the reaction mixture was stirred for 30 minutes at room temperature followed by heating at 70 °C for 3 hours. The reaction mixture was cooled to room temperature. CarboPt(OH)(OAc) (0.016 g, 0.036 mmol, 1.0 equiv.) was added and the reaction was stirred at 100 °C. Upon completion, the crude mixture was purified via column chromatography (100 % Hexane to 100 % EtOAc to CH₂Cl₂/MeOH 9:1). The recovered solid was triturated with EtOAc and MeOH to afford the title compound as a dark red solid (0.012 g, 0.015 mmol, 42 %).

^1H NMR (400 MHz, DMSO-d_6) δ_{H} : 9.11 (s, NH), 7.78 (d, $J = 8.9$ Hz, 1H, $\text{O}=\text{C}-\text{CH}=\text{CH}$), 7.54 (d, $J = 9.5$ Hz, 1H, $\text{O}-\text{C}=\text{CH}-\text{CH}$), 7.46 (d, $J = 8.2$ Hz, 2H, $\text{NH}-\text{C}=\text{CH}-\text{CH}$ & $\text{NH}-\text{C}-\text{CH}=\text{CH}$), 7.31 (d, $J = 8.9$ Hz, 2H, $\text{NH}-\text{C}=\text{CH}-\text{CH}$ & $\text{NH}-\text{C}-\text{CH}=\text{CH}$), 7.18 (d, $J = 2.6$ Hz, 1H, $\text{O}=\text{C}-\text{CH}$), 7.11 (dd, $J = 2.6, 8.9$ Hz, 1H, $\text{O}=\text{C}-\text{CH}=\text{CH}$), 6.80 (dd, $J = 2.0, 9.5$ Hz, 1H, $\text{O}-\text{C}=\text{CH}-\text{CH}$), 6.52 (m, NH_3), 6.27 (d, $J = 3.0$ Hz, 1H, $\text{O}-\text{C}=\text{CH}$), 5.15 (s, 2H, $\text{O}-\text{CH}_2$), 2.57 (t, $J = 7.0$ Hz, 2H, $\text{CH}_2-\text{CH}_2-\text{CH}_2$), 2.46 (m, 2H, $\text{CH}_2-\text{CH}_2-\text{CH}_2$), 1.92 (s, 3H, CH_3), 1.82 (t, $J = 7.6$ Hz, 2H, $\text{CH}_2-\text{CH}_2-\text{CH}_2$) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ_{C} : 185.3, 177.5, 176.1, 162.5, 160.0, 149.7, 145.2, 145.1, 140.7, 134.9, 133.7, 131.1, 128.7, 128.3, 127.9, 117.9, 114.4, 105.6, 101.1, 70.4, 55.4, 32.8, 29.5, 22.4, 15.7 ppm. ^{195}Pt NMR (107 MHz, DMSO-d_6): 1970 ppm. HRMS (m/z) calcd. for $\text{C}_{28}\text{H}_{28}\text{N}_4\text{O}_{11}\text{Pt}$ $[\text{M}+\text{Na}]^+$ 814.1295, found 814.1302.

CisPt(O-CONH-Nap)(OAc) (CisNap, Pt11)

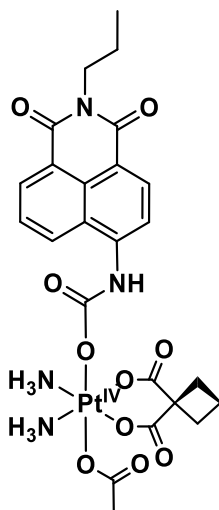


To a solution of 6-amino-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (0.040 g, 0.157 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL) was added triphosgene (0.093 g, 0.314 mmol, 2.0 equiv.) and NaHCO_3 (0.040 g, 0.471 mmol, 3.0 equiv.). The mixture was stirred at room temperature for 3 hours, followed by evaporation of the volatiles. The crude product was redissolved in CH_2Cl_2 and was added dropwise to a stirring solution of CisPt(OH)(OAc) (0.030 g, 0.079 mmol, 0.5 equiv.) in CH_2Cl_2 /pyridine/DMF (3:1:1) at 0 °C. After 5 minutes, the reaction mixture was warmed to room temperature and the solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting yellow

solid was redissolved in DMF and purified by column chromatography on silica gel (100 % Hexane to 100 % EtOAc to CH₂Cl₂/MeOH 100:8) to give a yellow solid. The recovered solid was triturated with EtOAc and CHCl₃ to yield a yellow solid (0.010 g, 0.0152 mmol, 21 %).

¹H NMR (500 MHz, DMSO-d₆) δ_H: 9.75 (s, NH), 8.78 (d, *J* = 8.3 Hz, 1H, CH=CH-CH), 8.49 (d, *J* = 7.0 Hz, 1H, CH=CH-CH), 8.41 (d, *J* = 8.6 Hz, 1H, CH=CH), 8.22 (d, *J* = 8.6 Hz, 1H, CH=CH), 7.77 (t, *J* = 8.0 Hz, 1H, CH=CH-CH), 6.66 (m, NH₃), 4.00 (t, *J* = 7.3 Hz, 2H, N-CH₂-CH₂-CH₃), 1.95 (s, 3H, CH₃), 1.66 (q, *J* = 7.3 Hz, 2H, N-CH₂-CH₂-CH₃), 0.92 (t, *J* = 7.3 Hz, 3H, N-CH₂-CH₂-CH₃) ppm. ¹³C NMR (151 MHz, DMSO-d₆) δ_C: 178.3, 163.6, 163.1, 160.1, 142.9, 131.7, 130.7, 129.9, 128.4, 125.7, 123.5, 123.4, 122.0, 118.1, 115.3, 41.0, 22.8, 20.9, 11.4 ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1256 ppm. HRMS (*m/z*) calcd. for C₁₈H₂₂Cl₂N₄O₆Pt [M+Na]⁺ 678.0457, found 678.0435.

CarboPt(O-CONH-Nap)(OAc) (CarboNap, Pt12)

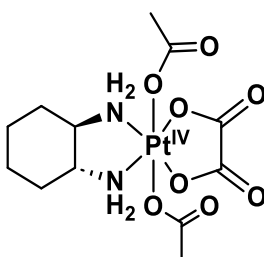


To a solution of 6-amino-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (0.040 g, 0.157 mmol, 1.0 equiv.) in CH₂Cl₂ (3 mL) was added triphosgene (0.093 g, 0.314 mmol, 2.0 equiv.) and NaHCO₃ (0.040 g, 0.471 mmol, 3.0 equiv.). The mixture was stirred at room temperature for 3 hours, followed by evaporation of the volatiles. The crude product was redissolved in CH₂Cl₂ and was added dropwise to a stirring solution of CarboPt(OH)(OAc) (0.035 g, 0.079 mmol, 0.5 equiv.) in CH₂Cl₂/pyridine/DMF (3:1:1) at 0 °C.

After 5 minutes, the reaction mixture was warmed to room temperature and the solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting yellow solid was redissolved in DMF and purified by column chromatography on silica gel (100 % Hexane to 100 % EtOAc to CH₂Cl₂/MeOH 9:1) to give a yellow solid. The recovered solid was triturated with EtOAc and CHCl₃ to yield a yellow solid (0.021 g, 0.029 mmol, 37 %).

¹H NMR (600 MHz, DMSO-d₆) δ_{H} : 9.75 (s, 1H, NH), 8.73 (dd, $J = 1.0, 8.4$ Hz, 1H, CH=CH-CH), 8.49 (dd, $J = 1.1, 7.7$ Hz, 1H, CH=CH-CH), 8.41 (d, $J = 8.4$ Hz, 1H, NH-C-CH=CH), 8.15 (d, $J = 8.8$ Hz, 1H, NH-C-CH=CH), 7.83 (t, $J = 7.3$ Hz, 1H, CH=CH-CH), 6.53 (m, NH₃), 4.00 (t, $J = 7.3$ Hz, 2H, N-CH₂-CH₂-CH₃), 2.62 (t, $J = 7.7$ Hz, 2H), 2.45 (t, $J = 7.3$ Hz, 2H), 1.94 (s, 3H, OCO-CH₃), 1.83 (quintet, $J = 7.3$ Hz, 2H), 1.65 (sext, $J = 7.7$ Hz, 2H, N-CH₂-CH₂-CH₃), 0.91 (t, $J = 7.7$ Hz, 3H, N-CH₂-CH₂-CH₃) ppm. ¹³C NMR (151 MHz, DMSO-d₆) δ_{C} : 178.0, 176.7, 164.1, 163.5, 160.0, 143.2, 132.2, 132.1, 130.0, 128.9, 126.3, 123.9, 122.5, 118.3, 115.9, 55.9, 41.5, 33.8, 29.5, 22.9, 21.4, 16.2, 11.8 ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1972 ppm. HRMS (m/z) calcd. for C₂₄H₂₈N₄O₁₀Pt [M+Na]⁺ 750.1346, found 750.1330.

OxPt(OAc)₂¹⁰ (Pt13)

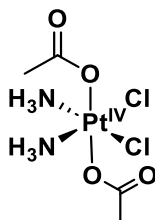


OxPt(OH)(OAc) (0.100 g, 0.211 mmol) was dissolved in dimethylformamide (1 mL) and acetic anhydride (1 mL) was added. The reaction was stirred at room temperature for 3 hours. The solvent was removed, and the white solid washed with diethyl ether to yield the desired product (Quant. Yield.).

¹H NMR (500 MHz, DMSO-d₆) δ_{H} : 2.57 (d, $J = 8.9$ Hz, 2H, DACH), 2.12 (d, $J = 12.2$ Hz, 2H, DACH), 1.95 (s, 6H, (CH₃)₂), 1.51 (d, $J = 9.6$ Hz, 2H, DACH), 1.43-1.40 (m, 2H, DACH), 1.15 (t,

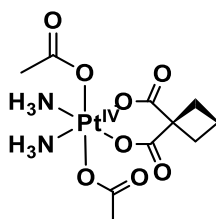
$J = 10.1$ Hz, 2H, DACH) ppm. ^{195}Pt NMR (107 MHz, DMSO- d_6): 1618 ppm. ESI-MS (H_2O): m/z calcd. for $\text{C}_{12}\text{H}_{20}\text{N}_2\text{O}_8\text{Pt}$ $[\text{M}+\text{Na}]^+$ 538.38, found 538.96, $[\text{2M}+\text{Na}]^+$ 1053.76, found 1053.89.

CisPt(OAc) $_2$ ¹¹ (Pt14)



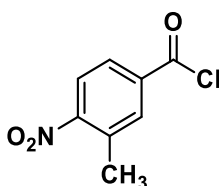
Cisplatin (0.100 g, 0.33 mmol) was dissolved in deionised water (3.5 mL) and hydrogen peroxide – 34.5-36.5 % in water (6 mL) was added. The solution was stirred at room temperature for 26 hours. The solvent was reduced, and the product was precipitated using methanol and diethyl ether and recovered by centrifugation. The pale-yellow solid was suspended in acetic anhydride (6 mL) and the reaction was stirred at room temperature for 3 days. The solution was concentrated under reduced pressure and diethyl ether was added to the residual solution. The pale-yellow precipitate was recovered by centrifugation and washed twice with diethyl ether (0.095 g, 0.23 mmol, 68 %).

^1H NMR (500 MHz, DMSO- d_6) δ_{H} : 6.51 (t, $^1J_{\text{H-N}} = 50.1$ Hz, $^2J_{\text{H-Pt}} = 51.8$ Hz, 6H, NH_3), 1.90 (s, 6H, $(\text{CH}_3)_2$) ppm. ^{195}Pt NMR (107 MHz, DMSO- d_6): 1234 ppm. ESI-MS (MeOH): m/z calcd. for $\text{C}_4\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_4\text{Pt}$ $[\text{M}+\text{H}]^+$ 419.13, found 419.89, $[\text{M}+\text{Na}]^+$ 441.13, found 441.87.

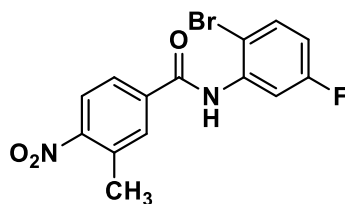
CarboPt(OAc)₂¹¹ (Pt15)

Carboplatin (0.100 g, 0.27 mmol) was dissolved in deionised water (2.7 mL) and hydrogen peroxide – 34.5-36.5 % in water (5 mL) was added. The solution was stirred at room temperature for 26 hours. The solvent was reduced, and the product was precipitated using methanol and diethyl ether and recovered by centrifugation. The pale-yellow solid was suspended in acetic anhydride (5 mL) and the reaction was stirred at room temperature for 3 days. The solution was concentrated under reduced pressure and diethyl ether was added to the residual solution. The white precipitate was recovered by centrifugation and washed twice with diethyl ether (0.098 g, 0.20 mmol, 74 %).

¹H NMR (500 MHz, DMSO-d₆) δ_H: 6.47-6.26 (m, 6H, NH₃), 2.52 (m, 2H, CBDCA), 2.48 (m, 2H, CBDCA), 1.90 (s, 6H, (CH₃)₂), 1.81 (m, 2H, DACH) ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1953 ppm. ESI-MS (MeOH): *m/z* calcd. for C₄H₁₂Cl₂N₂O₄Pt [M+H]⁺ 490.34, found 490.96, [M+Na]⁺ 512.34, found 512.92.

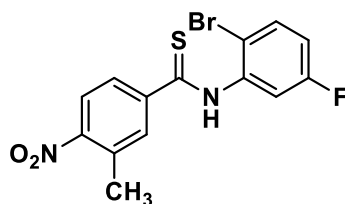
3-Methyl-4-nitrobenzoyl chloride¹²

3-Methyl-4-nitrobenzoic acid (5.0 g, 27.62 mmol) in dry CH₂Cl₂ (40 mL) was stirred at 0 °C. (COCl)₂ (4.8 mL, 56.06 mmol) and DMF (250 μL) were added to the solution which was stirred at 0 °C for 10 minutes and then warmed to room temperature for 4 hours. The yellow solution was concentrated under reduced pressure to obtain a yellow solid used immediately in the following reaction.

***N*-(2-bromo-5-fluorophenyl)-3-methyl-4-nitrobenzamide¹²**

3-Methyl-4-nitrobenzoyl chloride (5.682 g, 28.55 mmol) in 6 mL of pyridine was added to a stirring solution of 2-bromo-5-fluoroaniline (5.426 g, 28.55 mmol) in pyridine (10 mL). The dark brown solution was heated under reflux for 2 hours. Water (40 mL) was then added to the reaction, forming a white precipitate that was filtered, washed with water and methanol, and dried under suction to afford a white solid (6.546 g, 0.019 mmol, 68 %).

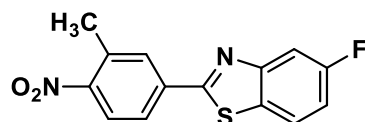
¹H NMR (400 MHz, DMSO-*d*₆) δ_{H} : 10.38 (s, 1H, *NH*-CO), 8.13 (d, *J* = 8.4 Hz, 1H, NO₂-C=CH), 8.07 (bs, 1H, CH₃-C=CH), 8.00 (dd, *J* = 2.0, 8.4 Hz, 1H, NO₂-C=CH-CH), 7.78 (dd, *J* = 5.9, 8.9 Hz, 1H, Br-C=CH), 7.53 (dd, *J* = 3.1, 9.8 Hz, 1H, F-C=CH), 7.18 (td, *J* = 3.1, 8.8 Hz, 1H, F-C=CH), 2.59 (s, 3H, CH₃) ppm. ¹³C NMR (101 MHz, DMSO-*d*₆) δ_{C} : 164.0, 162.3, 159.9, 150.8, 137.6, 134.0, 133.9, 132.9, 126.6, 124.6, 123.9, 115.9, 115.6, 19.3 ppm.

***N*-(2-bromo-5-fluorophenyl)-3-methyl-4-nitrobenzothioamide¹²**

To a solution of *N*-(2-bromo-5-fluorophenyl)-3-methyl-4-nitrobenzamide (6.546 g, 18.53 mmol) in HMPA (10 mL) was added Lawesson's reagent (5.278 g, 13.05 mmol) and the reaction mixture was heated at 100 °C for 16 hours. Water (70 mL) was added to the solution and the product was extracted with diethyl ether (3 x 70 mL). The organic layers were combined and dried over Na₂SO₄. The product was recrystallised from methanol as orange needles (5.426 g, 79 %).

^1H NMR (400 MHz, DMSO- d_6) δ_{H} : 12.04 (s, 1H, NH-CS), 8.10 (d, $J = 8.4$ Hz, 1H, $\text{NO}_2\text{-C=CH}$), 7.93 (s, 1H, $\text{CH}_3\text{-C=CH}$), 7.88 (d, $J = 8.7$ Hz, 1H, $\text{NO}_2\text{-C=CH-CH}$), 7.83 (dd, $J = 5.6, 8.9$ Hz, 1H, Br-C=CH), 7.51 (dd, $J = 3.1, 9.3$ Hz, 1H, F-C-CH), 7.27 (td, $J = 3.1, 8.6$ Hz, 1H, F-C=CH), 2.59 (s, 3H, CH_3) ppm.

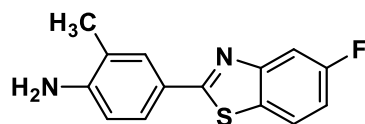
5-Fluoro-2-(3-methyl-4-nitrophenyl) benzothiazole¹²



To a stirring solution of *N*-(2-bromo-5-fluorophenyl)-3-methyl-4-nitrobenzothioamide (1.00 g, 2.71 mmol) in dry *N*-methyl-2-pyrrolidinone (2.6 mL) was slowly added sodium hydride 60% dispersion in mineral oil (0.130 g, 3.25 mmol). The reaction mixture was slowly warmed to 100 °C for 1 hour, followed by 120 °C for 1 hour and 140 °C for 4 hours. After reaction completion, water (50 mL) was added and the brown precipitate was filtered and dried under vacuum (0.712 g, 0.015 mmol, 91 %).

^1H NMR (400 MHz, DMSO- d_6) δ_{H} : 8.28 (dd, $J = 5.3, 8.9$ Hz, 1H, $\text{NO}_2\text{-C=CH}$), 8.23 (bs, 1H, $\text{CH}_3\text{-C=CH}$), 8.18-8.14 (m, 2H, $\text{NO}_2\text{-C=CH-CH}$ & F-C=CH-CH), 7.98 (dd, $J = 2.6, 9.8$ Hz, 1H, F-C-CH), 7.47 (td, $J = 2.7, 9.3$ Hz, 1H, F-C=CH), 2.63 (s, 3H, CH_3) ppm.

4-(5-Fluorobenzothiazol-2-yl)-2-methylaniline (5F203, L5)¹²

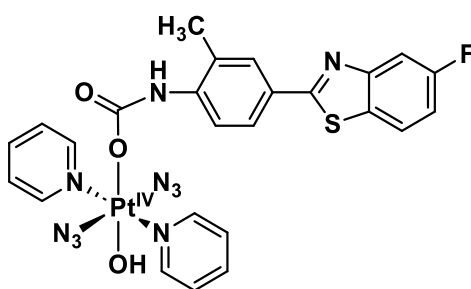


5-Fluoro-2-(3-methyl-4-nitrophenyl) benzothiazole (0.712 g, 2.47 mmol) was suspended in ethanol (10 mL) and tin(II) chloride dihydrate (2.79 g, 10.9 mmol) was added. The reaction mixture was reflux for 3 hours. The solvent was removed under reduced pressure to give a dark brown oil. EtOAc (100 mL) was added to the oil and washed with 2M NaOH (1 x 70 mL), H_2O (20 mL) and brine (10 mL). The organic layer was dried over MgSO_4 , and the solvent was

removed under reduced pressure. The brown solid was washed with MeOH, filtered, and dried under vacuum to yield a yellow solid (0.480 g, 1.86 mmol, 75 %).

^1H NMR (400 MHz, DMSO-d_6) δ_{H} : 8.06 (dd, $J = 5.4, 8.7$ Hz, 1H, F-C=CH-CH), 7.73 (dd, $J = 2.5, 10.0$ Hz, 1H, F-C-CH), 7.67 (d, $J = 2.2$ Hz, 1H, $\text{CH}_3\text{-C=CH}$), 7.62 (dd, $J = 2.3, 8.4$ Hz, 1H, $\text{NH}_2\text{-C=CH-CH}$), 7.25 (td, $J = 2.6, 9.0$ Hz, 1H, F-C=CH), 6.71 (d, $J = 8.2$ Hz, 1H, $\text{NH}_2\text{-C=CH}$), 5.72 (bs, 2H, NH_2), 2.14 (s, 3H, CH_3) ppm.

***Trans, trans, trans*-[Pt(py)₂(OH)(O-CONH-5F203)(N₃)₂] (Azido-5F203, Pt16)**



To a solution of 4-(5-Fluorobenzothiazol-2-yl)-2-methylaniline (5F203, 0.045 g, 0.175 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) was added triphosgene (0.104 g, 0.350 mmol, 2.0 equiv.) and NaHCO_3 (0.044 g, 0.525 mmol, 3.0 equiv.). The mixture was stirred at room temperature for 3 hours, followed by evaporation of the volatiles. The crude product was redissolved in CH_2Cl_2 and was added dropwise to a stirring solution of *trans, trans, trans*-[Pt(py)₂(OH)₂(N₃)₂] (0.041 g, 0.088 mmol, 0.5 equiv.) in CH_2Cl_2 /pyridine (3:1) at 0 °C. After 5 minutes, the reaction mixture was warmed to room temperature and the orange solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting yellow solid was purified by column chromatography on silica gel (gradient method $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 0.2-1%) to give a yellow solid (0.036 g, 0.048 mmol, 54 %).

^1H NMR (500 MHz, DMSO-d_6) δ_{H} : 8.94 (d, $J = 5.3$ Hz, 4H, Ho), 8.30 (t, $J = 7.6$ Hz, 2H, Hp), 8.12 (dd, $J = 5.4, 8.8$ Hz, 1H, F-C=CH-CH), 8.07 (bs, 1H, F-C-CH), 7.85 (t, $J = 6.7$ Hz, 4H, Hm), 7.83 (d, 1H, $J = 2.3$ Hz, $\text{CH}_3\text{-C=CH}$), 7.81 (m, 1H, NH-C=CH-CH), 7.74 (bs, 1H, NH-C=CH), 7.31 (td, 1H, $J = 2.6, 9.0$ Hz, F-C=CH), 3.96 (s, 1H, OH), 2.30 (s, 3H, CH_3) ppm.

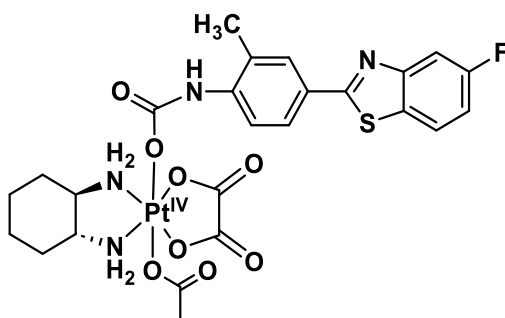
^{13}C NMR (126 MHz, DMSO-d_6) δ_{C} : 170.2, 160.4, 156.8, 154.5, 149.3, 142.5, 142.1, 130.0,

128.8, 128.2, 128.1, 126.2, 126.0, 125.2, 123.4, 121.3, 113.4, 108.5, 108.3, 18.0 ppm.

^{195}Pt NMR (107 MHz, DMSO- d_6): 1010 ppm. ^{19}F NMR (470 MHz, DMSO- d_6) δ : -115.91 (sext, 1F) ppm. HRMS (m/z) calcd. for $\text{C}_{25}\text{H}_{21}\text{FN}_{10}\text{O}_3\text{PtS}$ $[\text{M}+\text{H}]^+$ 756.1224, found 756.1216.

Crystals suitable for single crystal X-ray diffraction were obtained by slow evaporation of a concentrated solution of the product in a mixture of $\text{CH}_2\text{Cl}_2/\text{MeOH}$. Please see Appendix A.3 for X-ray diffraction data.

OxPt(O-CONH-5F203)(OAc) (Oxali-5F203, Pt17)

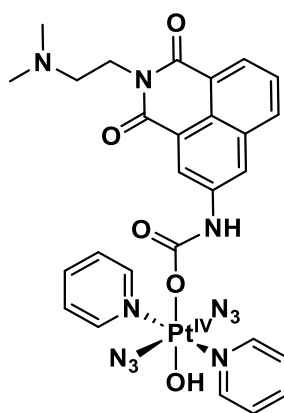


To a solution of 4-(5-Fluorobenzothiazol-2-yl)-2-methylaniline (5F203) (0.045 g, 0.175 mmol, 1.0 equiv.) in CH_2Cl_2 (4 mL) was added triphosgene (0.104 g, 0.350 mmol, 2.0 equiv.) and NaHCO_3 (0.044 g, 0.525 mmol, 3.0 equiv.). The mixture was stirred at room temperature for 3 hours, followed by evaporation of the volatiles. The crude product was redissolved in dry CH_2Cl_2 and was added dropwise to a stirring solution of OxPt(OH)(OAc) (0.050 g, 0.106 mmol) in $\text{CH}_2\text{Cl}_2/\text{DMF}/\text{pyridine}$ (3:1:1) at 0 °C. After 5 minutes, the reaction mixture was warmed to room temperature and the yellow solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting yellow solid was purified by column chromatography on silica gel (100 % Hexane to 100 % EtOAc to gradient method $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 6-8%) to give a yellow solid (0.026 g, 0.034 mmol, 32 %).

^1H NMR (500 MHz, CD_3OD) δ_{H} : 7.90 (dd, J = 3.7, 5.2 Hz, 1H, S-C=CH-CH), 7.81 (d, J = 2.0 Hz, 1H, F-C-CH), 7.76 (dd, J = 2.2, 8.4 Hz, 1H, CH-CH), 7.66 (d, J = 8.4 Hz, 1H, CH_3 -C=CH), 7.62 (dd, J = 2.5, 9.5 Hz, 1H, S-C=CH-CH), 7.16 (tt, J = 2.5, 8.9 Hz, 1H, CH-CH), 2.94 (tt, J = 4.1, 11.8 Hz, 1H, DACH), 2.83 (tt, J = 3.5, 11.2 Hz, 1H, DACH), 2.30 (s, 3H, CH_3),

2.08 (s, 3H, OCO-CH₃), 1.62 (d, *J* = 9.6 Hz, 2H, DACH), 1.59 (tt, *J* = 3.0, 11.8 Hz, 2H, DACH), 1.33-1.22 (m, 4H, DACH) ppm. ¹³C NMR (126 MHz, CD₃OD) δ_C: 181.7, 172.1, 167.0, 164.2, 163.0, 162.3, 155.9, 155.8, 141.6, 131.7, 131.3, 130.1, 129.4, 126.5, 124.4, 123.7, 114.5, 114.3, 109.3, 109.1, 63.0, 56.0, 32.5, 29.5, 25.1, 22.9, 18.3 ppm. ¹⁹⁵Pt NMR (107 MHz, CD₃OD): 1590 ppm. HRMS (*m/z*) calcd. for C₂₅H₂₇FN₄O₈PtS [M+H]⁺ 758.1254, found 758.1251.

***Trans, trans, trans*-[Pt(py)₂(OH)(O-CONH-Amonafide)(N₃)₂] (Azido-Amonafide, Pt18)**



To a solution of amonafide (0.045 g, 0.150 mmol, 1.0 equiv.) in CH₂Cl₂ (3 mL) was added triphosgene (0.094 g, 0.317 mmol, 2.0 equiv.) and NaHCO₃ (0.040 g, 0.476 mmol, 3.0 equiv.). The mixture was stirred at room temperature for 3 hours. The mixture was centrifuged, and the solution was added dropwise to a stirring solution of *trans, trans, trans*-[Pt(py)₂(OH)₂(N₃)₂] (0.037 g, 0.080 mmol, 0.5 equiv.) in CH₂Cl₂/pyridine (3:1) at 0 °C. After 5 minutes, the reaction mixture was warmed to room temperature and the orange solution was stirred for 1 hour. The solvent was removed under reduced pressure and the resulting yellow solid was purified by column chromatography on silica gel (gradient method CH₂Cl₂/MeOH 5-7%) to give a yellow solid. The solid was triturated with MeOH and EtOAc to obtain an orange solid (0.015 g, 0.019 mmol, 24 %).

¹H NMR (500 MHz, DMSO-*d*₆) δ_H: 9.55 (s, NH), 8.96 (dd, *J* = 1.6, 7.0 Hz, 4H, Ho), 8.54 (d, *J* = 2.2 Hz, 1H, CH=CH-CH), 8.38 (d, *J* = 2.2 Hz, 1H, CH=CH-CH), 8.32 (t, *J* = 7.7 Hz, 2H, Hp), 8.25 (d, *J* = 7.1 Hz, 1H, NH-C=CH), 8.19 (d, *J* = 8.4 Hz, 1H, NH-C-CH), 7.89 (t, *J* = 7.0 Hz, 4H,

Hm), 7.70 (t, $J = 7.7$ Hz, 1H, CH=CH-CH), 4.13 (t, $J = 5.5$ Hz, 2H, N-CH₂-CH₂), 4.00 (s, OH), 2.49 (m, 2H, N-CH₂-CH₂), 2.19 (s, N-(CH₃)₂, 6H) ppm. ¹³C NMR (126 MHz, DMSO-d₆) δ_c : 163.6, 163.0, 156.5, 149.3, 143.5, 142.1, 132.1, 130.7, 129.4, 128.5, 126.3, 125.5, 122.5, 122.0, 115.7, 114.4, 41.0, 20.9, 11.4 ppm. ¹⁹⁵Pt NMR (107 MHz, DMSO-d₆): 1006 ppm. HRMS (m/z) calcd. for C₂₇H₂₇N₁₁O₅Pt [M+H]⁺, 781.1917 found 781.1884.

Crystals suitable for single crystal X-ray diffraction were obtained by slow evaporation of a concentrated solution of the product in a mixture of CH₂Cl₂/MeOH. Please see Appendix A.4 for X-ray diffraction data.

8.3. References

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Appendix A X-ray crystallographic data

A.1. Azido-BPD (Complex Pt2)

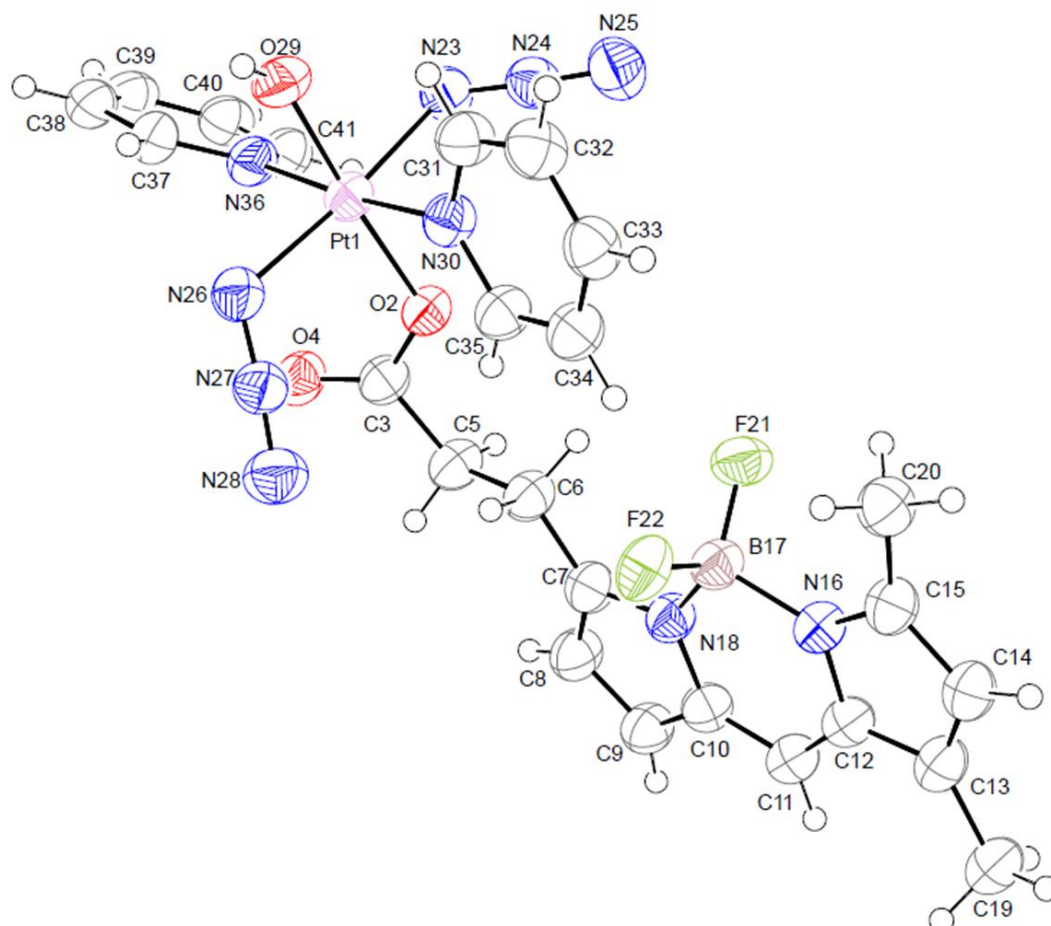


Figure A.1. Molecular structure of **Pt2** determined by X-ray diffraction showing thermal ellipsoids at 50% probability.

Table A.1. Crystal data and structure refinement for **Pt2**.

Identification code	Pt2
Empirical formula	C ₂₅ H ₂₉ B F ₂ N ₁₀ O ₄ Pt
Formula weight	777.47 g.mol ⁻¹
Temperature	150 K
Wavelength	1.54184 Å
Crystal system	Triclinic

Space group	P -1	
Unit cell dimension	a = 9.8829(2) Å	$\alpha = 102.5337(18)^\circ$.
	b = 10.5362(2) Å	$\beta = 93.0927(18)^\circ$.
	c = 14.6135(3) Å	$\gamma = 107.173(2)^\circ$.
Volume	1407.78(5) Å ³	
Z	2	
Density (calculated)	1.834 Mg/m ³	
Absorption coefficient	9.895 mm ⁻¹	
F(000)	764	
Crystal size	0.20 x 0.10 x 0.07 mm ³	
Theta range for data collection	3.123 to 76.160°.	
Index ranges	-11<=h<=12, -13<=k<=13, -18<=l<=18	
Reflections collected	29174	
Independent reflections	5826 [R(int) = 0.040]	
Completeness to theta = 73.875°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.50 and 0.07	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	5824 / 0 / 388	
Goodness-of-fit on F ²	0.9999	
Final R indices [I>2sigma(I)]	R1 = 0.0432, wR2 = 0.1055	
R indices (all data)	R1 = 0.0432, wR2 = 0.1057	
Largest diff. peak and hole	3.28 and -0.41 e.Å ⁻³	

A.2. *Tert*-butyl 4-(((3-oxo-3*H*-phenoxazin-7-yl)oxy)methyl)benzoate (**L3**)

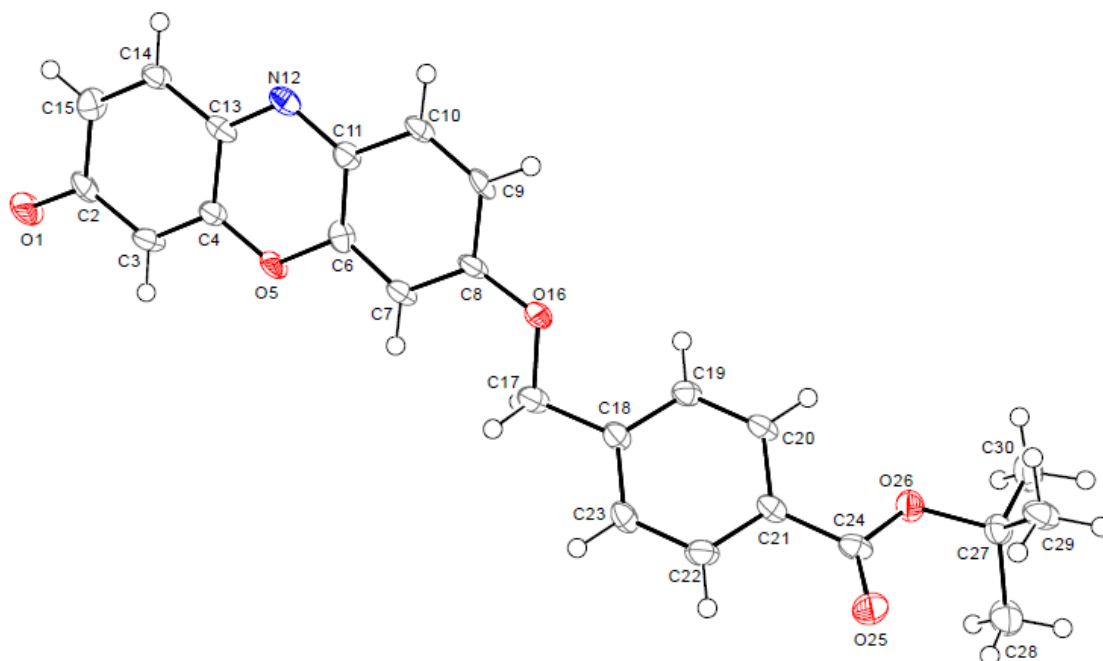
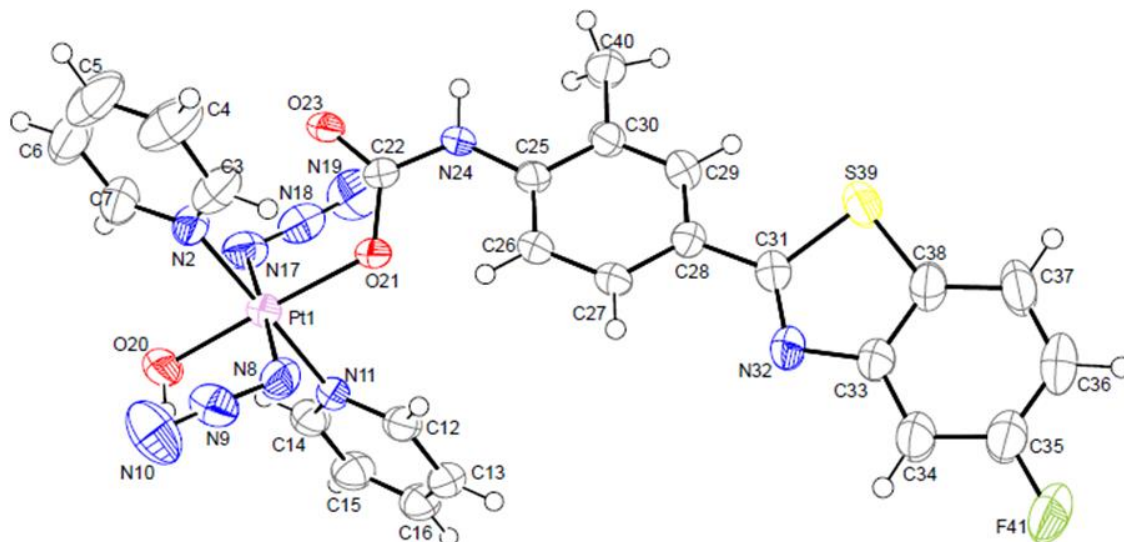


Figure A.2. Molecular structure of **L3** determined by X-ray diffraction showing thermal ellipsoids at 50% probability.

Table A.2. Crystal data and structure refinement for **L3**.

Identification code	L3	
Empirical formula	C ₂₄ H ₂₁ N O ₅	
Formula weight	403.43 g.mol ⁻¹	
Temperature	100 K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimension	a = 6.1844(2) Å	α = 101.008(4)°.
	b = 11.4092(4) Å	β = 99.313(4)°.
	c = 14.9583(9) Å	γ = 101.210(3)°.
Volume	994.33(8) Å ³	

Z	2
Density (calculated)	1.347 Mg/m ³
Absorption coefficient	0.095 mm ⁻¹
F(000)	424
Crystal size	0.20 x 0.09 x 0.02 mm ³
Theta range for data collection	3.432 to 23.706°.
Index ranges	-8<=h<=8, -14<=k<=15, -19<=l<=19
Reflections collected	20243
Independent reflections	4930 [R(int) = 0.047]
Completeness to theta = 73.875°	99.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1.00 and 0.65
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2986 / 0 / 273
Goodness-of-fit on F ²	1.0464
Final R indices [I>2sigma(I)]	R1 = 0.1042, wR2 = 0.3013
R indices (all data)	R1 = 0.1105, wR2 = 0.3036
Extinction coefficient	140(30)
Largest diff. peak and hole	0.60 and -0.55 e.Å ⁻³

Table A.3. Crystal data and structure refinement for **Pt16**.

Identification code	Pt16		
Empirical formula	C25 H21 F N10 O3 Pt S		
Formula weight	755.66 g.mol ⁻¹		
Temperature	150 K		
Wavelength	1.54184 Å		
Crystal system	Monoclinic		
Space group	C 2/c		
Unit cell dimension	a = 31.4441(5) Å	α = 90°.	
	b = 8.02160(10) Å	β = 96.5427(13)°.	
	c = 24.5474(3) Å	γ = 90°.	
Volume	6151.32(15) Å ³		
Z	8		

Density (calculated)	1.632 Mg/m ³
Absorption coefficient	9.594 mm ⁻¹
F(000)	2944
Crystal size	0.08 x 0.04 x 0.01 mm ³
Theta range for data collection	3.625 to 75.897°.
Index ranges	-39<=h<=39, -9<=k<=10, -26<=l<=30
Reflections collected	74828
Independent reflections	6308 [R(int) = 0.095]
Completeness to theta = 73.875°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.91 and 0.84
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	6304 / 0 / 370
Goodness-of-fit on F ²	1.0357
Final R indices [I>2sigma(I)]	R1 = 0.0373, wR2 = 0.0776
R indices (all data)	R1 = 0.0546, wR2 = 0.0835
Largest diff. peak and hole	0.99 and -1.43 e.Å ⁻³

A.4. Azido-Amonafide (Complex Pt18)

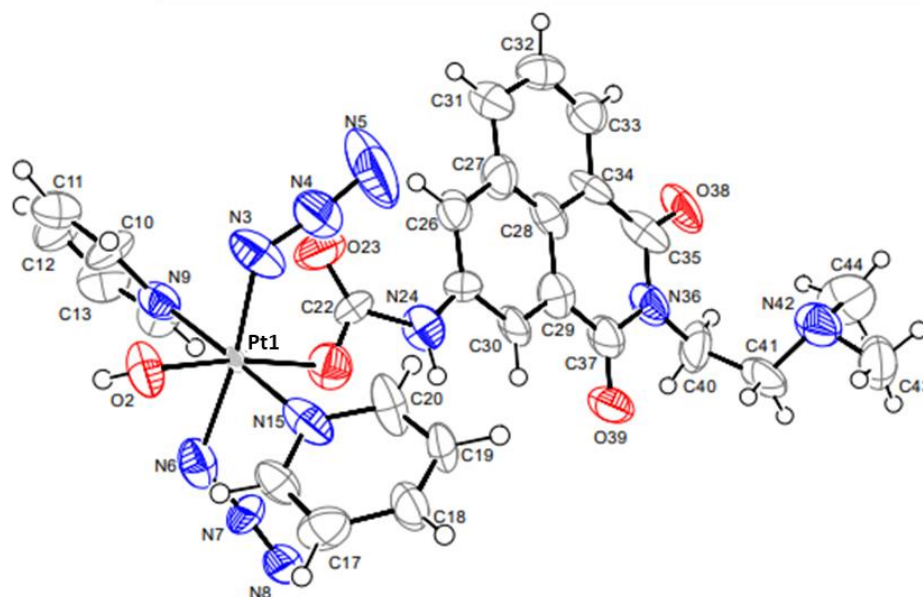


Figure A.4. Molecular structure of **Pt18** determined by X-ray diffraction with thermal ellipsoids displayed at 50% probability.

Table A.4. Crystal data and structure refinement for **Pt18**.

Identification code	Pt18	
Empirical formula	C ₂₇ H ₂₇ N ₁₁ O ₅ Pt	
Formula weight	780.67 g.mol ⁻¹	
Temperature	100 K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	F d d 2	
Unit cell dimension	a = 77.4882(18) Å	α = 90°.
	b = 37.7584(11) Å	β = 90°.
	c = 7.7316(3) Å	γ = 90°.
Volume	22621.4(12) Å ³	
Z	32	
Density (calculated)	1.834 Mg/m ³	

Absorption coefficient	5.023 mm ⁻¹
F(000)	12288
Crystal size	0.19 x 0.03 x 0.02 mm ³
Theta range for data collection	3.191 to 23.257°.
Index ranges	-86<=h<=86, -41<=k<=41, -8<=l<=8
Reflections collected	160131
Independent reflections	8129 [R(int) = 0.235]
Completeness to theta = 73.875°	99.6 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.90 and 0.62
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	8129 / 814 / 782
Goodness-of-fit on F ²	0.9852
Final R indices [I>2sigma(I)]	R1 = 0.0459, wR2 = 0.0511
R indices (all data)	R1 = 0.0666, wR2 = 0.0523
Absolute structure parameter	-0.012(5)
Largest diff. peak and hole	1.37 and -1.35 e.Å ⁻³

Appendix B HPLC and LC-MS data

B.1. Chapter 3 The study of platinum(IV) reduction: Oxali(IV)Fluors

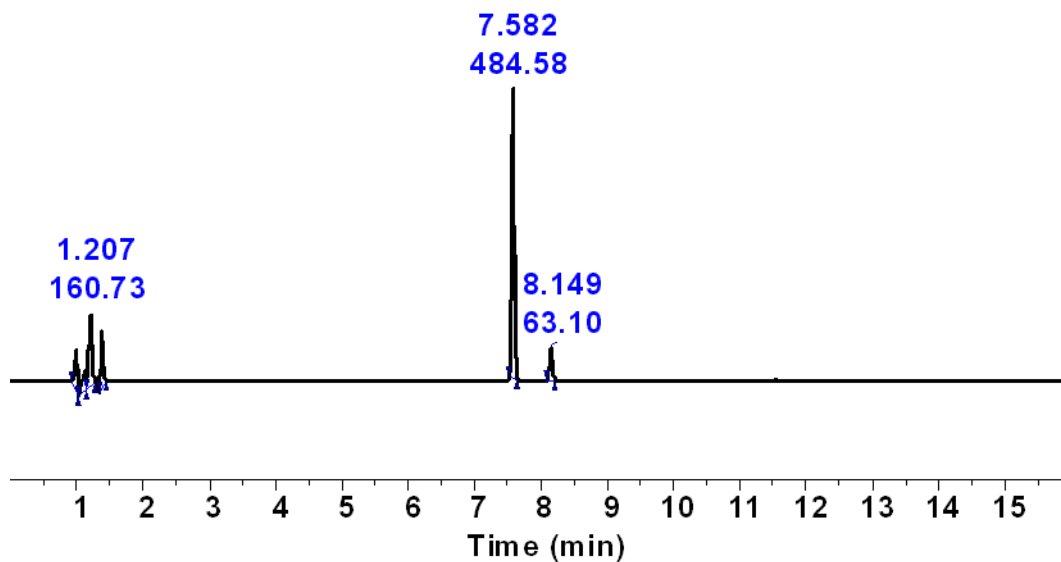


Figure B.1A. The reduction of **OxaliNap** (**Pt6**, 50 μM) in PBS buffer (pH 7.40) with 8 mM sodium ascorbate recorded by HPLC after 3 hours of incubation (Monitoring absorption at 360 nm).

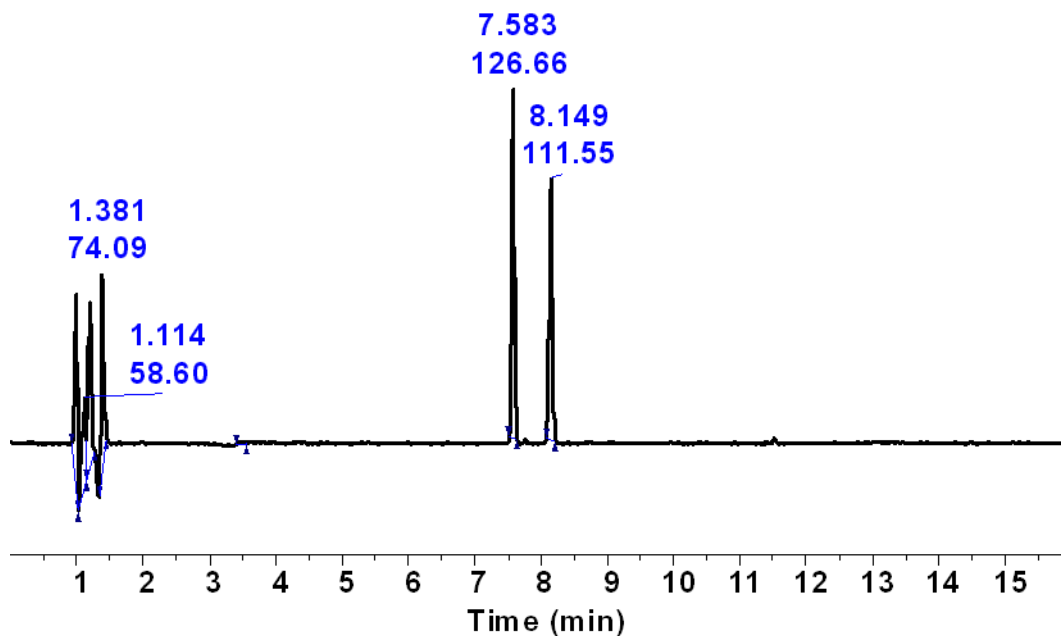


Figure B.1B. The reduction of **OxaliNap** (**Pt6**, 50 μM) in PBS buffer (pH 7.40) with 8 mM sodium ascorbate recorded by HPLC after 12 hours of incubation (Monitoring absorption at 360 nm).

The percentage of free ligand was calculated based on the equation adapted from: Z. Deng, C. Li, S. Chen, Q. Zhou, Z. Xu, Z. Wang, H. Yao, H. Hirao and G. Zhu, *Chem.Sci.*, 2021, **12**, 6536-6542.

Percentage of free 4-NapNH₂ = $A_{L2}/(A_{L2}+A_{OxaliNap}) \times 100\%$, with A_{L2} corresponding to the peak area of the fluorophore **L2** and $A_{OxaliNap}$ the peak area of **OxaliNap** recorded by HPLC with the absorption set at 360 nm.

After 3 hours, percentage of free 4-NapNH₂ = $63.10/(63.10+484.58) \times 100\% = 12\%$ (Figure B.1A).

After 12 hours, percentage of free 4-NapNH₂ = $111.55/(111.55+126.66) \times 100\% = 47\%$ (Figure B.1B).

B.2. Chapter 4 The study of platinum(IV) reduction: Cis(IV)Fluors and Carbo(IV)Fluors

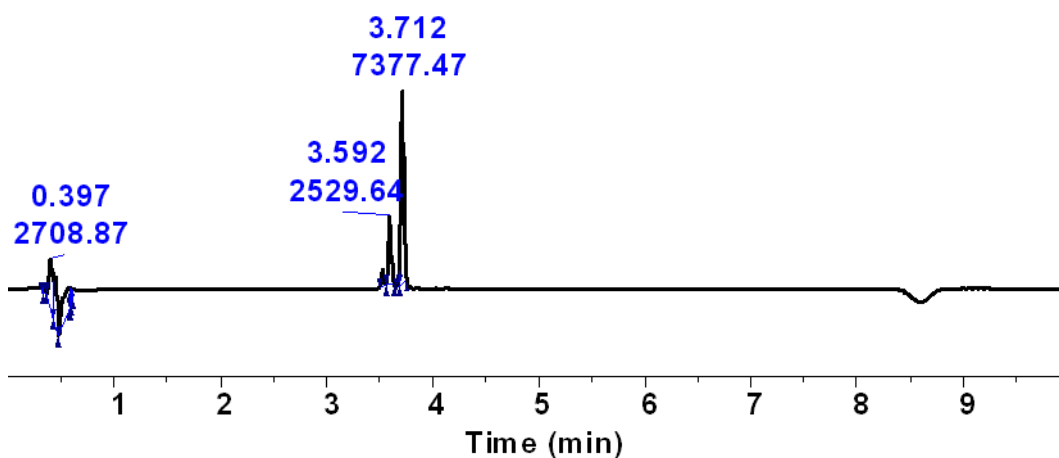


Figure B.2A. Stability of **CarboRes** (**Pt10**, 50 μ M) after 24 hours in deionised H_2O recorded by LC-MS (Absorption at 365 nm).

Percentage of free resorufin = $A_{\text{resorufin}} / (A_{\text{resorufin}} + A_{\text{CarboRes}}) \times 100\%$, with $A_{\text{resorufin}}$ corresponding to the peak area of the resorufin fluorophore and A_{CarboRes} the peak area of **CarboRes** recorded by LC-MS (absorption at 365 nm).

After 24 hours, percentage of free resorufin = $2529.64 / (2529.64 + 7377.47) \times 100\% = 26\%$ (Figure B.2A).

B.3. Chapter 5 Improving the sensitivity to reduction of platinum(IV) complexes

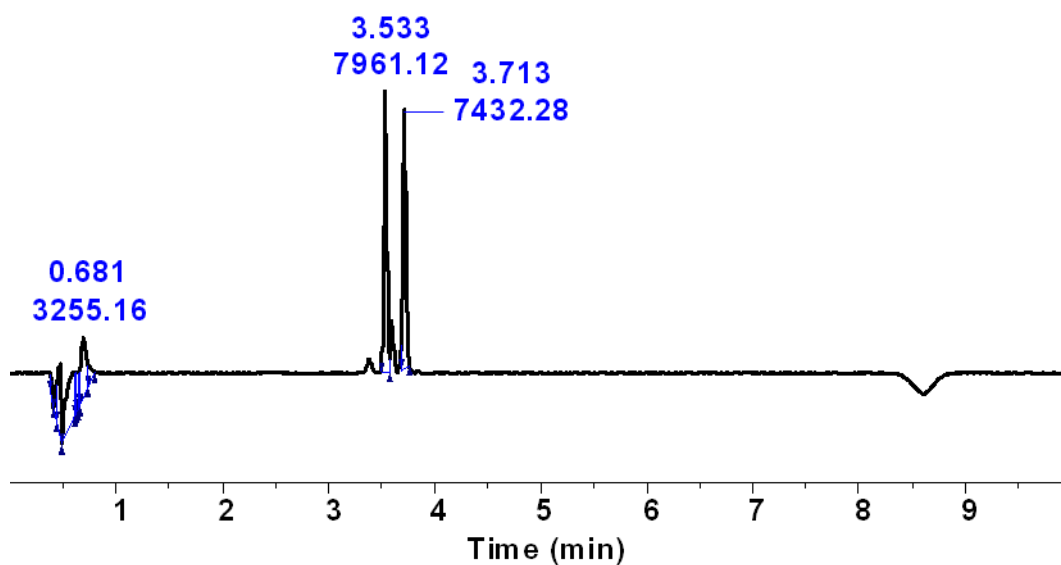


Figure B.3A. The reduction of **CarboRes (Pt10)** in deionised H_2O containing 1 mM sodium ascorbate after 24 hours. The reduction process and products were recorded by LC-MS (monitoring absorption at 365 nm).

Percentage of free resorufin = $A_{\text{resorufin}} / (A_{\text{resorufin}} + A_{\text{CarboRes}}) \times 100\%$, with $A_{\text{resorufin}}$ corresponding to the peak area of the resorufin fluorophore and A_{CarboRes} the peak area of **CarboRes** recorded by LC-MS (absorption at 365 nm).

After 24 hours, percentage of free resorufin = $7961.12 / (7961.12 + 7432.28) \times 100\% = 52\%$ (Figure B.3A).

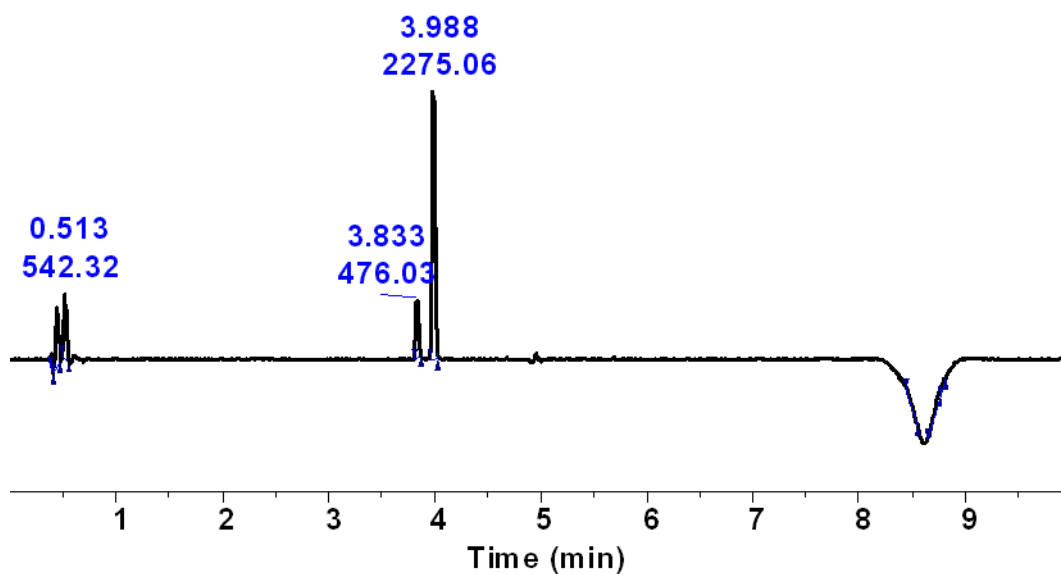


Figure B.3B. The reduction of **OxaliNap (Pt6)** in deionised H₂O containing 1 mM sodium ascorbate with 50 μ M Cu(II) acetate after 3 hours recorded by LC-MS (monitoring absorption at 365 nm).

Percentage of free 4-NapNH₂ = $A_{L2}/(A_{L2}+A_{\text{OxaliNap}}) \times 100\%$, with A_{L2} corresponding to the peak area of the fluorophore **L2** and A_{OxaliNap} the peak area of **OxaliNap** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free 4-NapNH₂ = $2275.06/(2275.06+476.03) \times 100\% = 83\%$ (Figure B3.B).

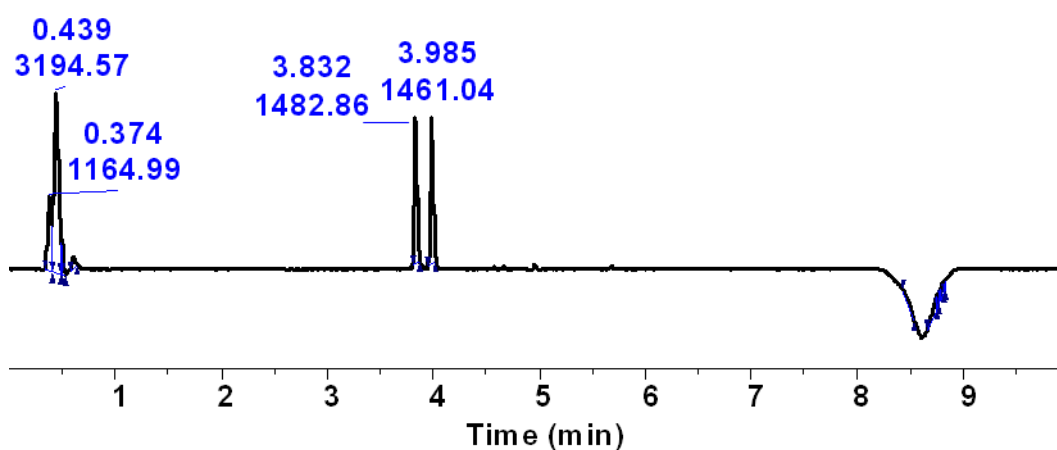


Figure B.3C. The reduction of **OxaliNap (Pt6)** in deionised H_2O containing 1 mM sodium ascorbate with 50 μM Fe(III) chloride after 3 hours recorded by LC-MS (monitoring absorption at 365 nm).

Percentage of free 4-NapNH₂ = $A_{L2}/(A_{L2}+A_{OxaliNap}) \times 100\%$, with A_{L2} corresponding to the peak area of the fluorophore **L2** and $A_{OxaliNap}$ the peak area of **OxaliNap** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free 4-NapNH₂ = $1461.04/(1461.04+1482.86) \times 100\% = 50\%$ (Figure B.3C).

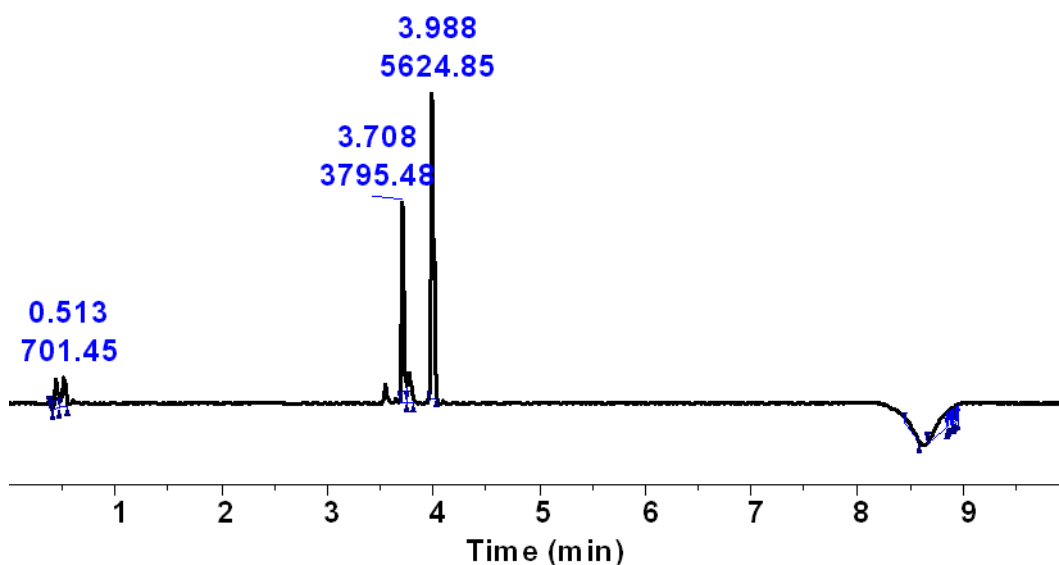


Figure B.3D. The reduction of **CarboNap (Pt12)** in deionised H_2O containing 1 mM sodium ascorbate with $50 \mu M$ $Cu(II)$ acetate after 3 hours recorded by LC-MS (monitoring absorption at 365 nm).

Percentage of free 4-NapNH₂ = $A_{L2}/(A_{L2}+A_{CarboNap}) \times 100\%$, with A_{L2} corresponding to the peak area of the fluorophore **L2** and $A_{CarboNap}$ the peak area of **CarboNap** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free 4-NapNH₂ = $5624.85/(5624.85+3795.48) \times 100\% = 60\%$ (Figure B.3D).

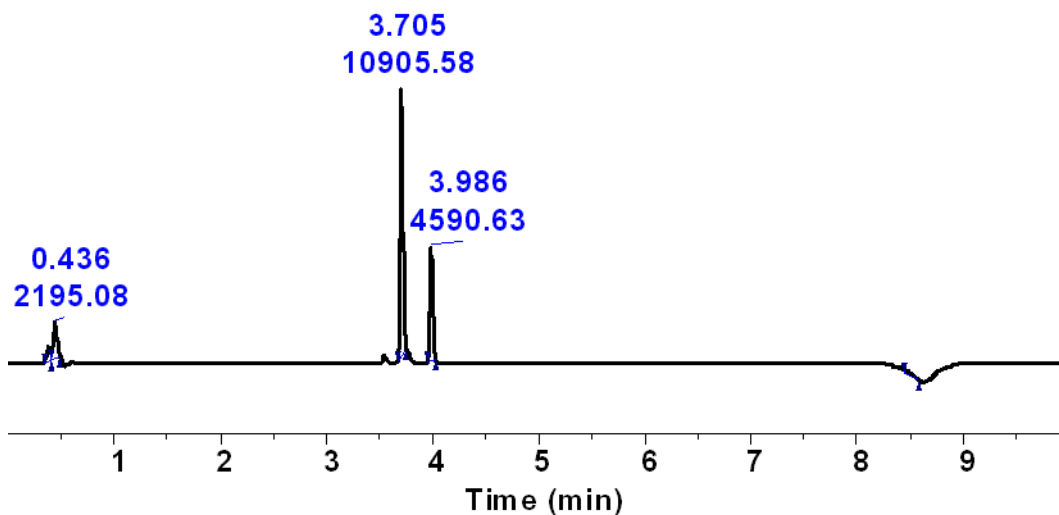


Figure B.3E. The reduction of **CarboNap** (**Pt12**) in deionised H_2O containing 1 mM sodium ascorbate with 50 μM Fe(III) chloride after 3 hours recorded by LC-MS (monitoring absorption at 365 nm).

Percentage of free 4-NapNH₂ = $A_{L2}/(A_{L2}+A_{\text{CarboNap}}) \times 100\%$, with A_{L2} corresponding to the peak area of the fluorophore **L2** and A_{CarboNap} the peak area of **CarboNap** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free 4-NapNH₂ = $4590.63/(4590.63+10905.58) \times 100\% = 30\%$ (Figure B.3E).

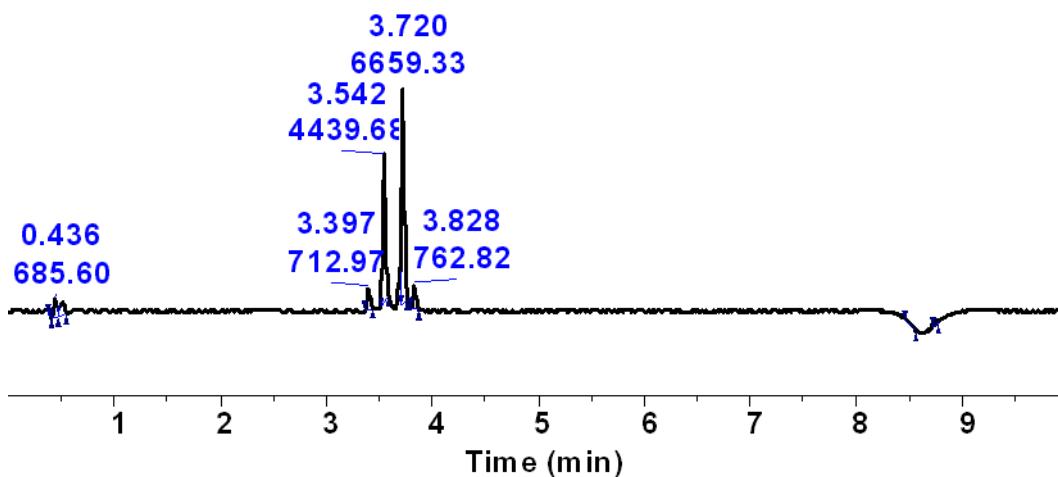


Figure B.3F. The reduction of **CarboRes (Pt10)** in deionised H_2O containing 1 mM sodium ascorbate with 50 μM Cu(II) acetate after 3 hours recorded by LC-MS (monitoring absorption at 365 nm).

Percentage of free resorufin = $A_{\text{resorufin}} / (A_{\text{resorufin}} + A_{\text{CarboRes}}) \times 100\%$, with $A_{\text{resorufin}}$ corresponding to the peak area of the resorufin fluorophore and A_{CarboRes} the peak area of **CarboRes** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free resorufin = $4439.68 / (4439.68 + 6659.33) \times 100\% = 40\%$ (Figure B.3F).

Percentage of free dihydroresorufin = $A_{\text{dihydroresorufin}} / (A_{\text{dihydroresorufin}} + A_{\text{CarboRes}}) \times 100\%$, with $A_{\text{resorufin}}$ corresponding to the peak area of the dihydroresorufin and A_{CarboRes} the peak area of **CarboRes** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free dihydroresorufin = $712.97 / (712.97 + 6659.33) \times 100\% = 10\%$ (Figure B.3F).

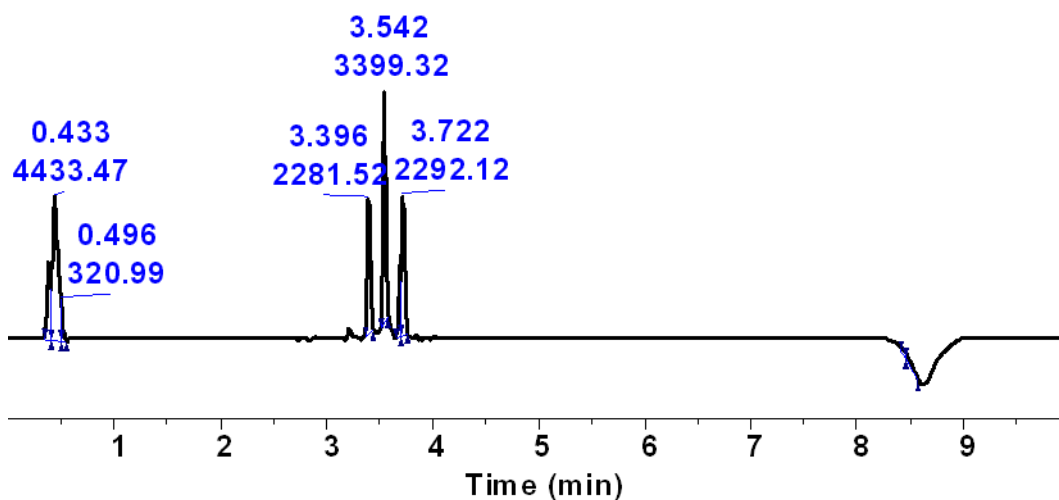


Figure B.3G. The reduction of **CarboRes (Pt10)** in deionised H_2O containing 1 mM sodium ascorbate with $50 \mu M$ Fe(III) chloride after 3 hours recorded by LC-MS (monitoring absorption at 365 nm).

Percentage of free resorufin = $A_{\text{resorufin}} / (A_{\text{resorufin}} + A_{\text{CarboRes}}) \times 100\%$, with $A_{\text{resorufin}}$ corresponding to the peak area of the resorufin fluorophore and A_{CarboRes} the peak area of **CarboRes** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free resorufin = $3399.32 / (3399.32 + 2292.12) \times 100\% = 60\%$ (Figure B.3G).

Percentage of free dihydroresorufin = $A_{\text{dihydroresorufin}} / (A_{\text{dihydroresorufin}} + A_{\text{CarboRes}}) \times 100\%$, with $A_{\text{resorufin}}$ corresponding to the peak area of the dihydroresorufin and A_{CarboRes} the peak area of **CarboRes** recorded by LC-MS (absorption at 365 nm).

After 3 hours, percentage of free dihydroresorufin = $2281.52 / (2281.52 + 2292.12) \times 100\% = 50\%$ (Figure B.3G).