University of Texas at El Paso
DigitalCommons@UTEP

Open Access Theses & Dissertations

2010-01-01

Synthesis and Self-Assembly of Novel Amphiphilic Cisplatin Analogues

Gabriel Angel Gonzalez
University of Texas at El Paso, gagonzalez3@miners.utep.edu

Follow this and additional works at: https://digitalcommons.utep.edu/open_etd

Recommended Citation
Gonzalez, Gabriel Angel, "Synthesis and Self-Assembly of Novel Amphiphilic Cisplatin Analogues" (2010). *Open Access Theses & Dissertations*. 2690.
https://digitalcommons.utep.edu/open_etd/2690

This is brought to you for free and open access by DigitalCommons@UTEP. It has been accepted for inclusion in Open Access Theses & Dissertations by an authorized administrator of DigitalCommons@UTEP. For more information, please contact lweber@utep.edu.

www.manaraa.com

SYNTHESIS and SELF-ASSEMBLY of NOVEL AMPHIPHILIC CISPLATIN ANALOGUES

GABRIEL ANGEL GONZALEZ ESPARZA

Department of Chemistry

APPROVED:

_____
Juan C. Noveron, Ph.D., Chair.

_____
Jose Nunez, Ph.D.

_____
German Rosas-Acosta, Ph.D.

_____
Patricia D. Whiterspoon, Ph.D.
Dean of the Graduate School

www.manaraa.com

Copyright

by

Gabriel Angel Gonzalez Esparza

2010

www.manaraa.com

Dedication

To all those persons that I have encountered in my life whose dreams have been part of my dreams and whose advice, respect, love and appreciation have been an important motivation in my career.

www.manaraa.com

SYNTHESIS and SELF-ASSEMBLY of CHEMOTHERAPEUTIC CISPLATIN ANALOGUES

by

GABRIEL ANGEL GONZALEZ ESPARZA, B. S.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER of SCIENCE

Department of Chemistry

THE UNIVERSITY OF TEXAS AT EL PASO

May 2010

www.manaraa.com

Acknowledgements

Through the process of learning and acquiring new skills in chemistry, it has been necessary and fundamental the knowledge and advice from my professors, faculty, staff and colleagues at the chemistry department. Especially the advice and comments from my professor and mentor Dr. Juan Carlos Noveron were really important in my career in a way to improve my techniques in chemical reactions as well as using different techniques to characterize chemical compounds. Dr. Noveron's enthusiasm has been very contagious in the laboratory, his creativeness has been influential in my way of approaching chemistry to the point where I could work enthusiastically, carefully and correctly.

The strength, dedication and discipline have been values that I have found and observed in different persons from whom I also have received very important advice and I thank them all and appreciate their encouragement and support in my career.

The family is the nucleus of every society and strong framework in every individual and in my case I thank my family since all of them have played an important role in my personal life and career, particularly my mother Maria Genoveva Esparza, my aunt Maria Rafaela Esparza and my brothers and sisters.

www.manaraa.com

Abstract

The discovery of cisplatin (cis-diamminedichloroplatinum(II)) is regarded as a medical revolution in cancer therapy. Following studies have shown activity against cancers of the head, lung, ovarian, testicular, and cervical. The reaction mechanism of cisplatin is established on the intrastrand cross linking by the formation of covalent bonds with the N7 of the purine bases which causes an irregular effect impeding the normal transcription and DNA replication mechanisms of the cell. Only cisplatin and carboplatin (cis-diamimine-1,1'-cyclobutane dicarboxylate platinum) are clinically used as antitumor agents today. Carboplatin is an analogue of cisplatin and is used more often because it has a reduced amount of side effects. Analogues of cisplatin having heterocyclic compounds with aromatic N-containing ligands have shown very promising antitumor properties *in vitro* and *in vivo* in cisplatin resistant model systems. Heterocyclic compounds function as DNA intercalating agents which insert between the base pairs of the double helix unwinding it disrupting the normal function of DNA and leads to interference with gene transcriptions, gene expression, carcinogenesis, mutagenesis and cell death.

This research focuses on the design and synthesis of amphiphilic molecules having a lipid hydrophobic chain containing ester functional groups and a hydrophilic head which has a platinum coordinated bond with aromatic N-containing heterocyclic compounds such as bipyridine and biquinoline to form cisplatin analogues. Amphiphilic cisplatin analogues emulsify to make micelles that project the Pt-Cl groups on the surface of the micelle. These platinum micelles may be used as alkylating-like agents, intercalating agents and as drug delivery systems that encapsulate anticancer drugs. The action of over expressed esterases will cleave the ester group found in the lipid chain and micelles. The micelles of platinum biquinoline and platinum

www.manaraa.com

bipyridine will disassemble by the action of the esterases releasing the anticancer drugs, the Pt-Cl will bind to the DNA and the heterocyclic rings will intercalate in the DNA disrupting the DNA structure leading to cell death.

www.manaraa.com

Table of Contents

www.manaraa.com

www.manaraa.com

List of Figures

www.manaraa.com

www.manaraa.com

List of Schemes

www.manaraa.com

Chapter 1: Introduction

Metal based drugs are extensively under research in the studies of different cancer therapies. Metal compounds having positive charges at the metal center have better binding to negatively charged molecules such as proteins and nucleic acids.[1] Cis-[diamminedichloroplatinum(II)] known as cisplatin is a square planar complex of platinum(II) which was accidentally discovered by Barnet Rosenberg in the 1960s (Figure 1.1)[2].

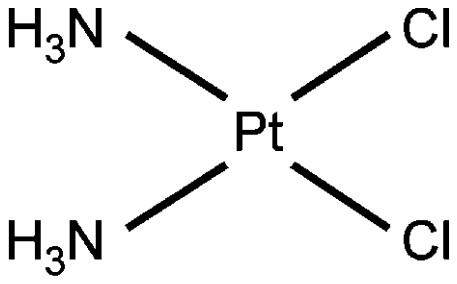


Figure 1.1 Cisplatin[2]

Cisplatin is an important chemotherapeutic agent effective against head, lung, ovarian, testicular and cervical cancers.[2,3,4,5,6,7] The reaction mechanism of cisplatin is established on the intrastrand cross linking DNA lesion. Cisplatin, subsequent to hydrolysis of the chloride ligands, (Figure 1.2), forms adducts with DNA by coordination bonds with purine bases preferably with guanine.[8,9,10]

www.manaraa.com

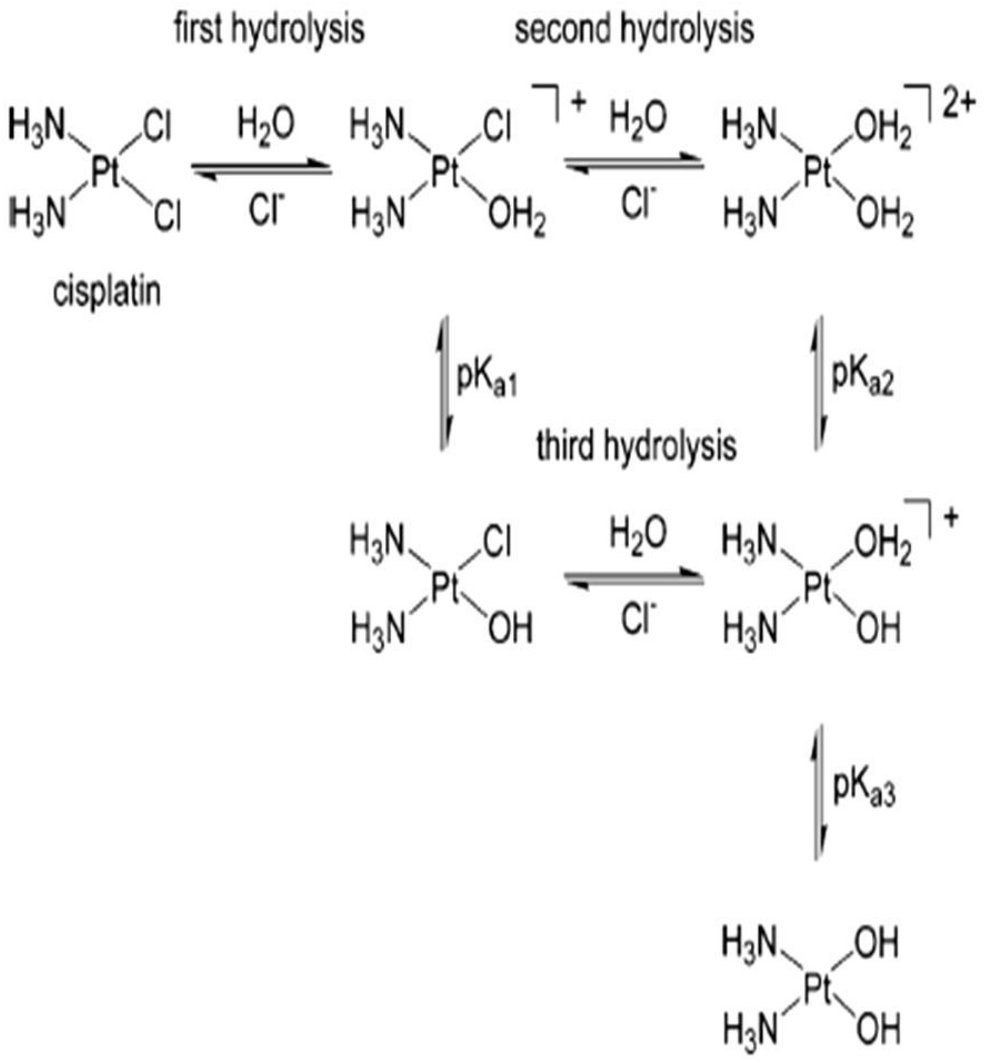


Figure 1.2 Cisplatin Hydrolysis[11]

Platinum compounds have been used in cancer therapy since the introduction of the parent compound cisplatin. Platinum based compounds are still under research since certain tumors are limited by inherent or acquired resistance to the clinically used anticancer drugs cisplatin and carboplatin.[11,12,13] In addition cisplatin has several side effects such as nephrotoxicity, nausea, vomiting, myelosuppression, ototoxicity, neurotoxicity, gastrointestinal toxicity, and is active to only a limited number of tumor types.[14,15] Since the discovery of cisplatin about 3000 platinum compounds have been synthesized only 30 compounds have been under clinical trials and more

www.manaraa.com

than half of them have been discarded. Currently four platinum compounds similar to cisplatin such as carboplatin, oxaliplatin and nedaplatin, and lobaplatin are used in the treatment of cancer (Figure 1.3).[16]

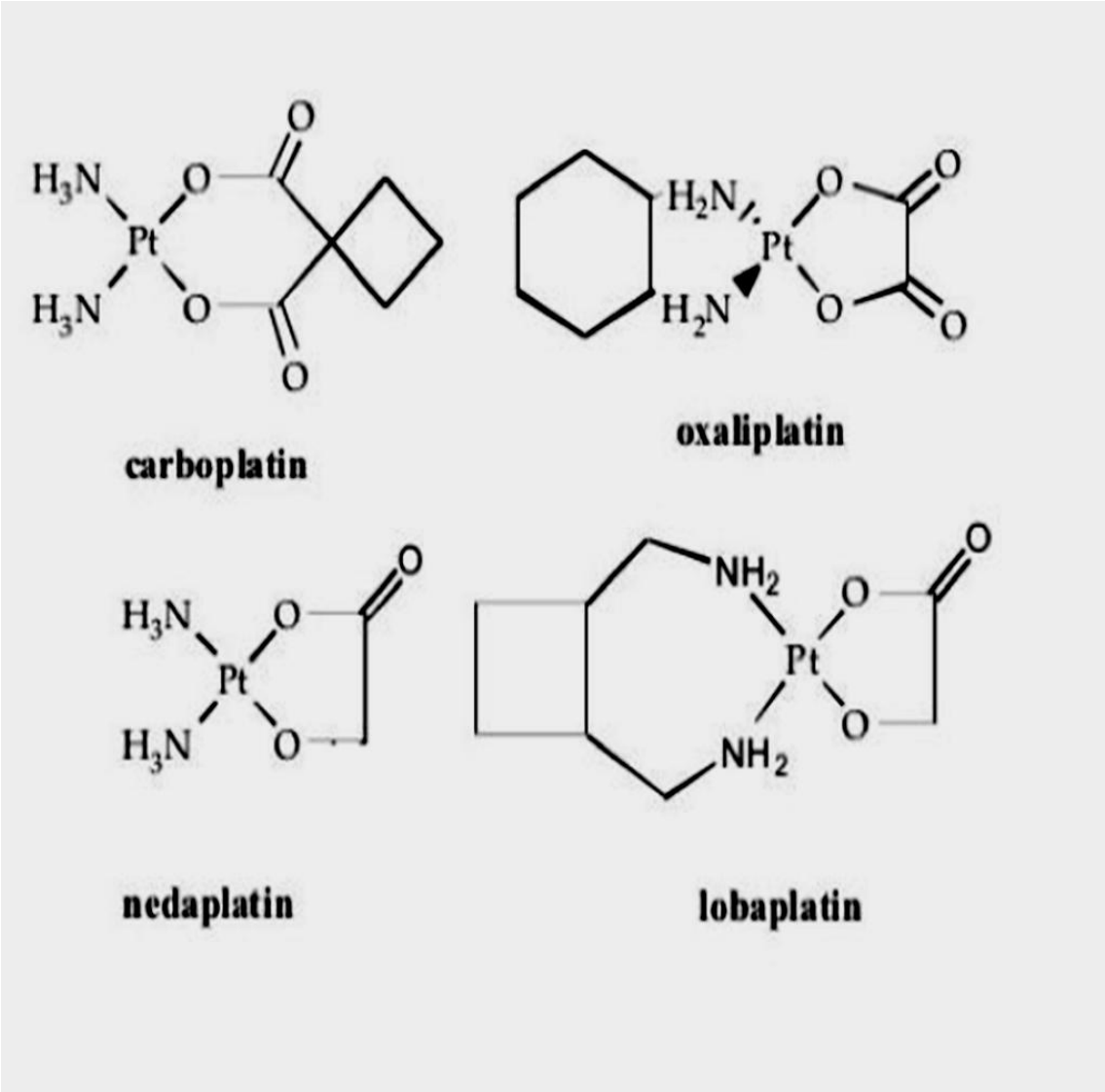


Figure 1.3 Carboplatin, oxaliplatin, nedaplatin, lobaplatin[17]

Research on analogues of cisplatin having N-heterocyclic compounds have shown very promising antitumor properties *in vitro* and *in vivo* in cisplatin resistant model systems.

N-heterocyclic cisplatin analogues function as DNA intercalating agents which insert between the base pairs of the double helix, unwinding it and disrupting the normal function of DNA

www.manaraa.com

leading to interference with gene transcriptions, gene expression, carcinogenesis, mutagenesis and cell death.[17,18]

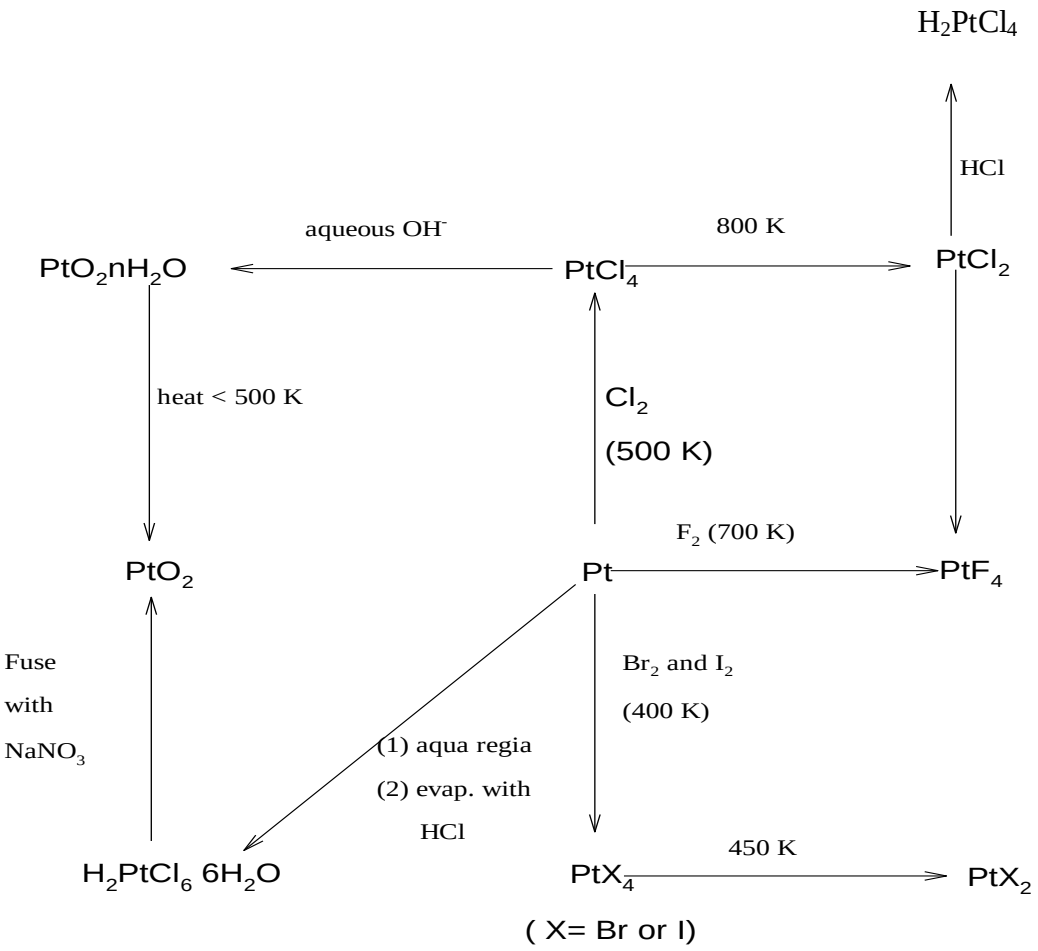
1.1 Chemistry of Cisplatin

Cisplatin was first discovered by Italian chemist Michel Peyrone in 1845, and called for a long time Peyrone's salt. Cisplatin can be prepared in different ways, such as reacting aqueous ammonia with potassium tetrachloroplatinate(II), heating trans-diamminedichloroplatinum(II) or tetraammineplatinum(II) chloride with aqueous ammonia, and by mixing potassium tetrachloplatinate(II) with potassium iodide and aqueous ammonia to give as product diamminediiodoplatinum(II) which can be reacted with silver nitrate followed by potassium chloride giving cisplatin.[19]

1.2 General Chemistry of Elemental Platinum and its Precursor Chloroplatinic Acid $H_2PtCl_66H_2O$ used in the Production of Cisplatin.

Chloroplatinic acid $H_2PtCl_6H_2O$ (Scheme 1)[20] is used as a precursor for the synthesis of potassium tetrachloroplatinate. Potassium tetrachloroplatinate is used as the starting material in the synthesis of cisplatin.

www.manaraa.com



Scheme 1. Chemistry of elemental platinum for the production of platinum precursors.[20]

1.2.1 Synthesis of Chloroplatinic Acid $H_2(PtCl)_6$. [21,22,23]

$Pt + 4HNO_3 + 6HCl \rightarrow H_2PtCl_6 + 4NO_2 + 4H_2O$

As shown on the above chemical equation the chloroplatinic acid can be obtained by treating elemental platinum with aqua regia which is a mixture of nitric acid and hydrochloric acid.

1.2.2 Synthesis of Potassium Tetrachloroplatinate(II) (K_2PtCl_4).

Potassium tetrachloroplatinate(II) (K_2PtCl_4) [22] is obtained by using chloroplatinic acid (H_2PtCl_6) as described on chemical equations below.

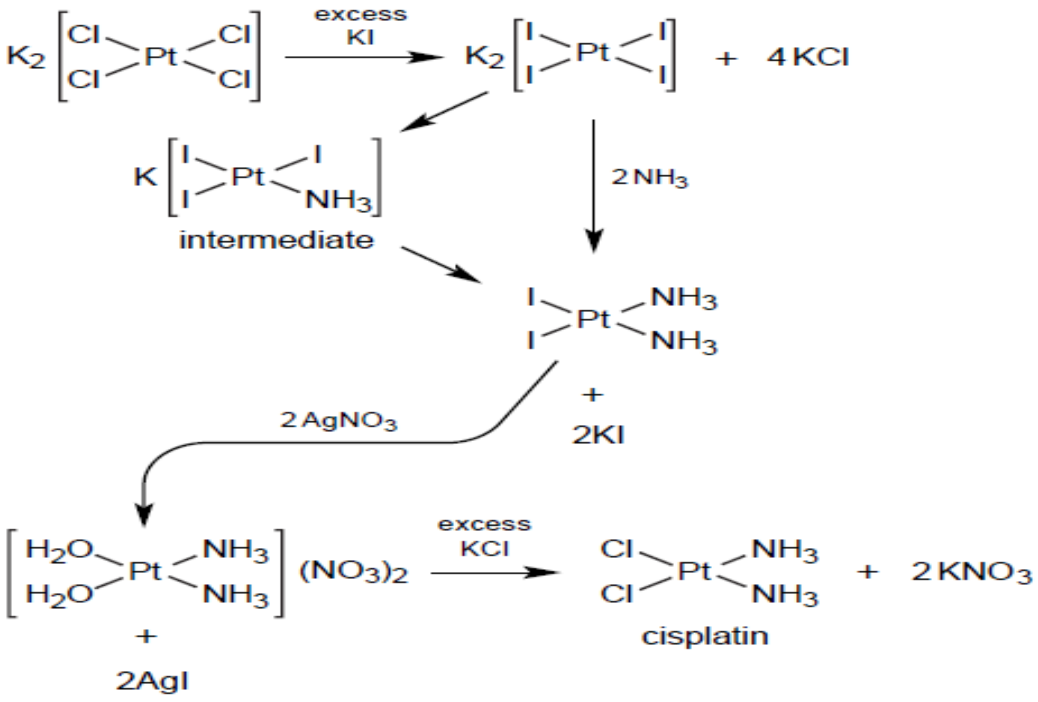
www.manaraa.com

$H_2PtCl_6 + 2KCl \rightarrow K_2PtCl_6 + 2HCl$

$K_2PtCl_6, + SO_2 + H_2O \rightarrow K_2PtC1_4 + 2HC1 + H_2SO_4$

1.2.3 Synthesis of Cisplatin.

Potassium tetrachloroplatinate(II) is the starting material in the synthesis of cisplatin[23] (Scheme 2).



Scheme 2. Synthesis of cisplatin.[23]

1.3 Mechanism of Action

Anticancer platinum compounds exert their cytotoxic effect when binding to DNA[24] via chloride ligand exchange.[25] Different mechanisms have been described for cisplatin mechanism of action such as intracellular accumulation of cisplatin, impaired DNA repair processes, and decreased levels of cisplatin inactivating factors such as metallothioneins and glutathione.[26] Although cisplatin forms covalent adducts with different biological molecules, it binds principally to

www.manaraa.com

DNA.[27] Phosphate groups, sugar oxygen atoms, and heterocyclic nucleobases form part of DNA and have lone pairs of electrons where metal ions may bind, however studies have shown that cisplatin binds preferentially at the nitrogen atoms of the nucleobases.[28] Cisplatin's mechanism of action is by binding to DNA forming different types of adducts.[29] Cisplatin chlorides are substituted by positively charged aqua groups or neutral hydroxyl groups[30] that can react with nucleophilic sites intracellularly to form protein, RNA, DNA intrastrands and interstrand adducts crosslinks[31] causing damage to the cell mitochondria, inhibiting ATPase activity, changing the cellular transport system, causing inflammation, apoptosis and death in cells.[32]

1.4 Effects of Platinum Compounds on the DNA

DNA is the main target of platinum compounds being that DNA adduct formation is key for cisplatin cytotoxicity.[33] Cisplatin interacts with different biomolecules and its anticancer activity originates from its facility to form bifunctional DNA crosslinks. Cisplatin binding to DNA activates a distortion or bending which is considered a serious lesion the DNA causing a chain of events on intracellular macromolecules to form DNA, RNA, and protein adducts that causes damage to the cells. Cisplatin binding to N7 guanines is due to the electron density of the N7 atoms and because their accessibility sites in the DNA electrophilic attack by platinum[34,35]. The higher affinity of cisplatin to sulfur donors, such as methionines and cystines, than to nitrogen donors on DNA has as consequence the inactivation of cisplatin by the plethora of sulfur containing cytoplasmatic ligands[36]. Approximatelly 90% of the cisplatin is bound to plasma proteins in the blood[37] and about 1% of intracellular cisplatin that reacts with DNA results in intrastrand and interstrand crosslinks, in which the intrastand cross link between adjacent guanines is the most common adduct. The DNA adducts are thought to be the key toxic lesions caused by cisplatin[38]. Research in vitro have shown that cisplatin in DNA forms approximately

www.manaraa.com

65% 1, 2-(GpG), 1,2-d(GpG), 25% 1,2-d(ApG), and 5-10% 1,3-d(Gp-NpG) intrastrand cross links (Figure 1.4) [39].

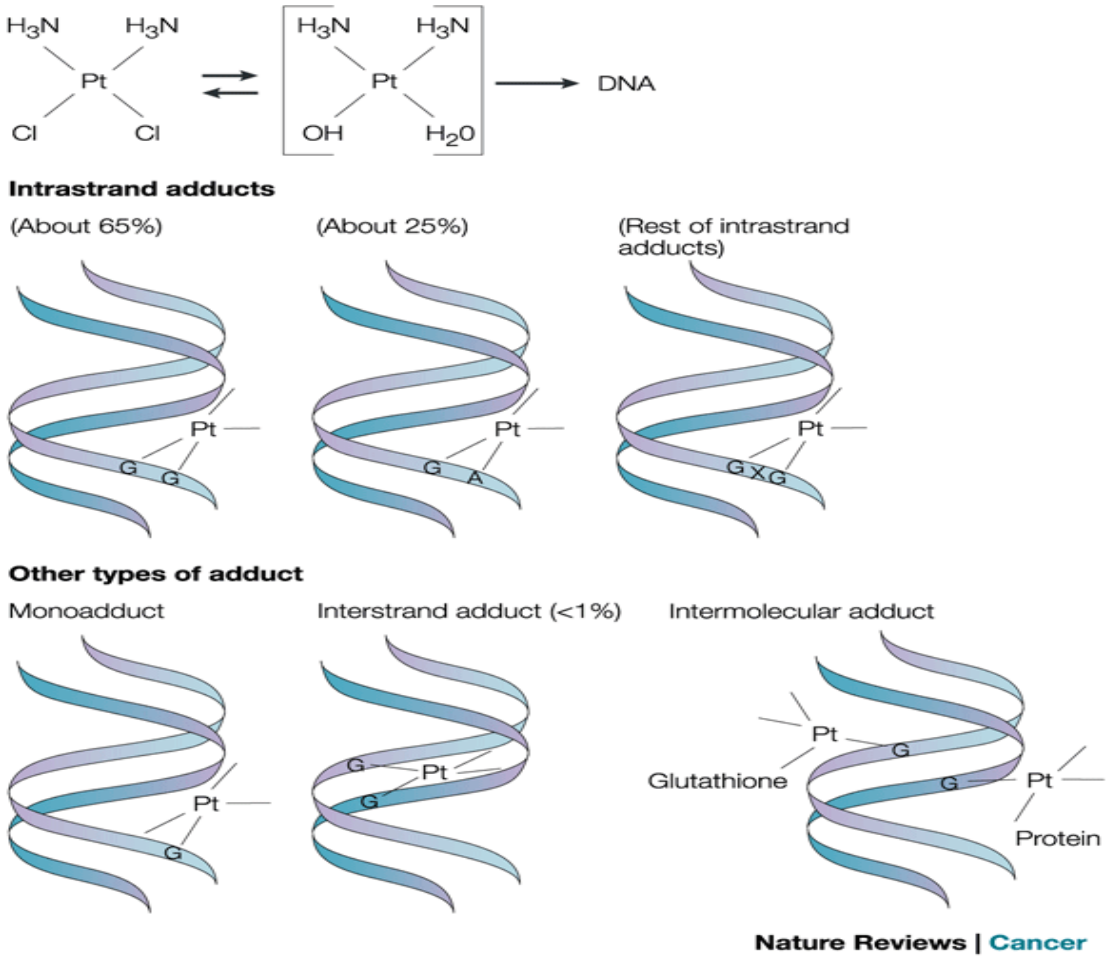


Figure 1.4 DNA adducts.[40]

1.5 Development of Resistance

The development of cellular resistance to cisplatin and its side effect such as nephrotoxicity, nausea, vomiting, myelosuppression, ototoxicity, neurotoxicity, and gastrointestinal toxicity are the limitations in cancer therapy which inhibits its clinical trials.[41] The mechanism that limits the damage includes cellular defense, change in drug accumulation, cellular thiol levels, and DNA repair.[42] Increased efflux and decreased uptake is one of the reasons for which intracellular accumulation of cisplatin is reduced and is very often observed in cisplatin resistant cell lines.

www.manaraa.com

Intracellular proteins such as metallothioneins and sulfur containing molecules also induce resistance to cisplatin. Variations in expression of oncogenes and tumor suppressor genes have also been related to cellular resistance to cisplatin.[43] The primary mechanism of platinum compounds is the formation of platinum DNA adducts which causes cell cycle arrest and apoptosis. The reparation of these DNA adducts involves polymorphisms in genes such as Mismatch Repair (MMR), Nucleotide Excision Repair (NER), and Base Excision Repair (BER).[44] The DNA mismatch repair (MMR) pathway is very important and vital for conserving the genomic integrity.[45] MMR is the system that corrects mistakes in DNA polymerases, these errors can be detected in DNA because they do not form the Watson-Crick base pairs,[46] the MMR repairs the mismatched nucleotides resulting from replication errors.[47] The nucleotide excision repair pathway eliminates bulky DNA adducts induced by a wide variety of chemotherapeutic drugs as cisplatin and electrophilic chemicals. Oxidative lesions and other types of endogenous DNA damage can be processed by the NER pathway considered as an important biological cellular defensive system.[48] The base excision repair is the major pathway that corrects most common forms of DNA damage[49]. The BER pathway first recognize damaged DNA and excision is carried out by glycosylases aimed to differentiate base lesions and in second place a damaged general stage.[50]

Chapter 2: Polymeric Drug Systems as a Therapeutic Strategy against Cisplatin Resistance in Cancer Cells

Polymeric drug delivery systems are high molecular compounds used as delivery systems of medicines in the treatment of cancer. Polymeric drugs accumulate selectively in tumor due to the enhanced permeability retention effect and high molecular weight. These properties of polymeric drugs give a high period of circulation in the blood and retention time in the solid cancer tumors.

www.manaraa.com

Polymeric drug delivery systems include polymer drug conjugates, polymer protein conjugates, polymer DNA complexes, dendrimers and polymeric micelles (Figure 2.1)[54]. Polymeric drugs do not need to have a special design for special receptors to improve the release of the medications since divinding cancerous cells have more active endocytosis than normal cells. Polymeric drug delivery systems have been designed on the basis of the enhanced permeability retention (EPR) effect.[51,52, 53]

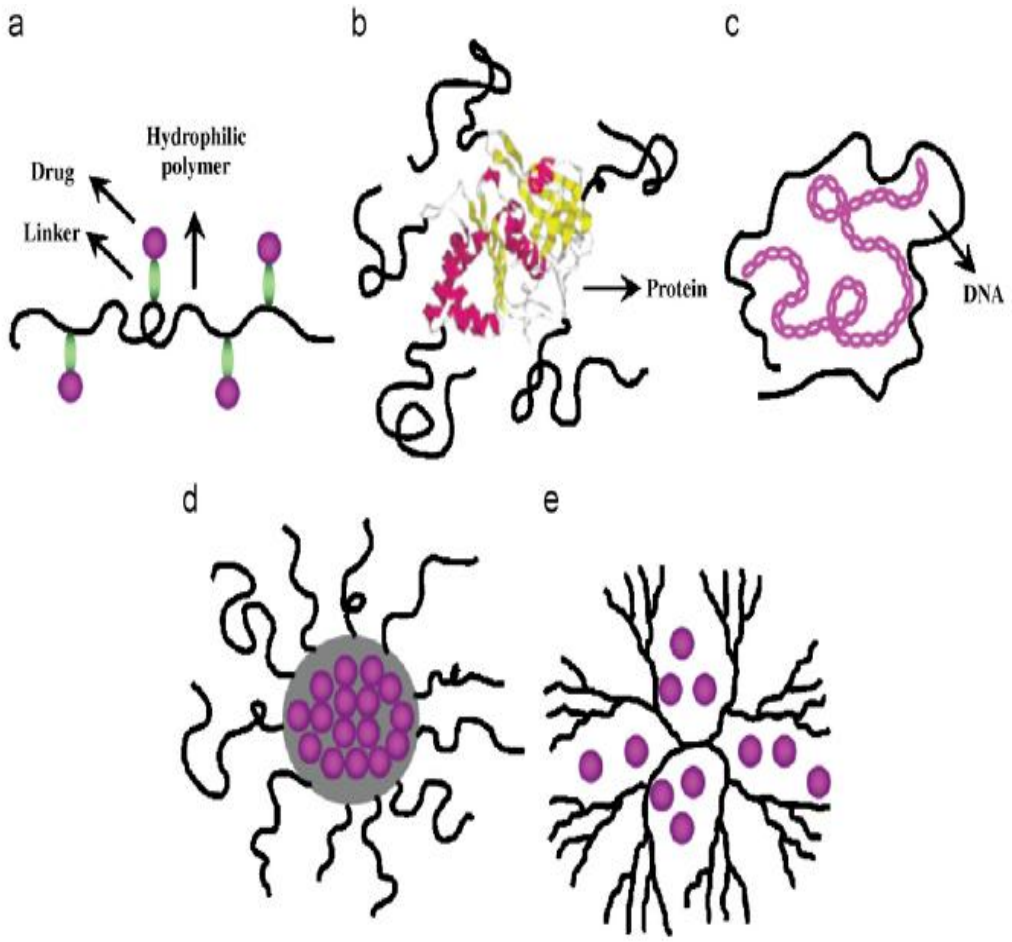


Figure 2.1 Schematic illustration of representative polymeric delivery systems : (a) polymer drug conjugates; (b) polymer protein conjugates; (c) polymer DNA complexes; (d) polymeric micelles; (e) dendrimers.[54]

www.manaraa.com

2.1 The Enhanced Permeability Retention Effect

The enhanced permeability retention effect, discovered by Dr. Hiroshi Maeda is a mechanism based on the efficiency of polymeric drugs (Figure 2.2).[55]

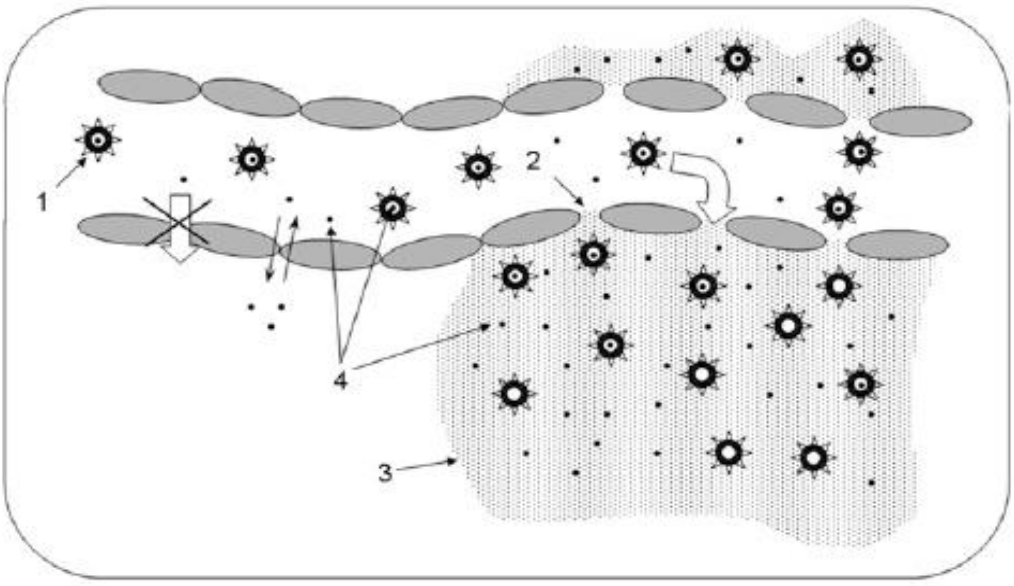


Figure 2.2 Enhanced permeability and retention (EPR). Long-circulating drug carriers (1) penetrate through the leaky pathological vasculature (2) into the tumor interstitium (3) and degrade there, releasing a free drug (4) and creating its high local concentration.[55]

The EPR effect is achieved because of the tumor blood vessels are found defective with malformations in the cancer zone. Blood tumor vessels have a quick increase of the endothelial cells. The tumor blood vessels are crooked, have deficient mural cells and have abnormal basement membrane formation as well as defective vascular structure which are the results of the rapid vascularization fundamental to provide oxygen and nutrients for the growing of cancer cells. The deficiencies of the cancer vasculature system decrease the lymphatic drainage, because of the high molecular weight polymeric drug systems can be retained in the tumor for a longer time (Figure 2.3).

www.manaraa.com

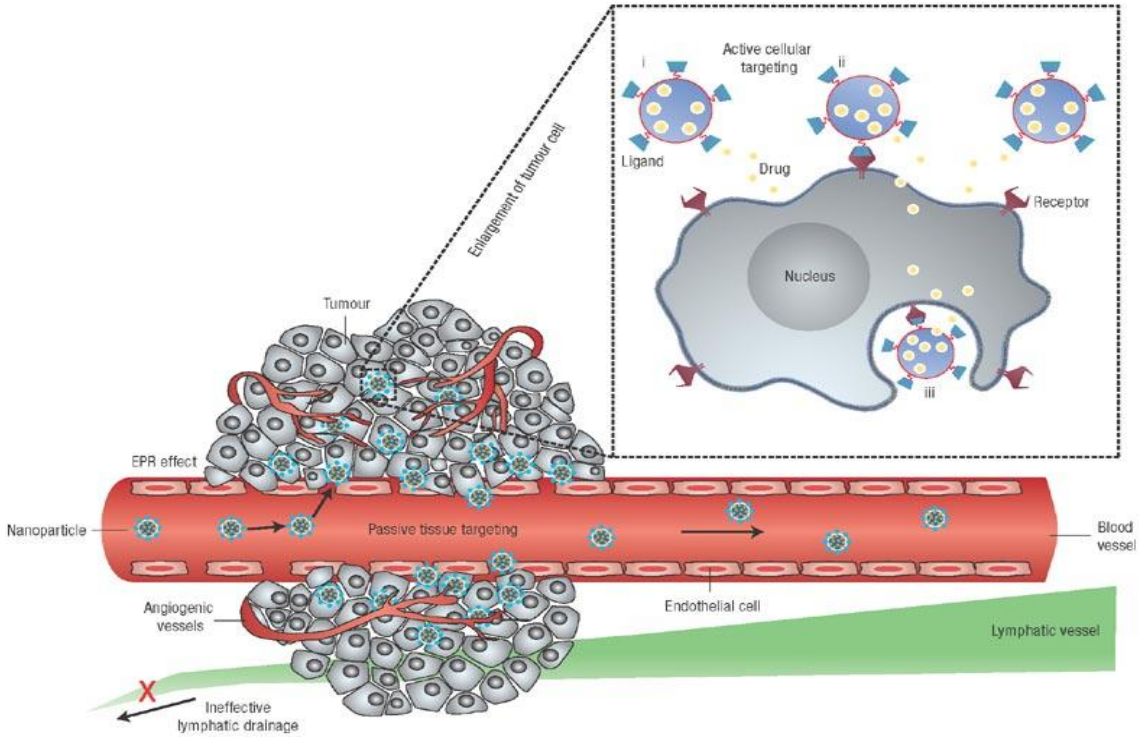


Figure 2.3 Schematic representation of different mechanisms by which nanocarriers can deliver drugs to tumours. Passive tissue targeting is achieved by extravasation of nanoparticles through increased permeability of the tumor vasculature and ineffective lymphatic drainage (EPR effect). Active cellular targeting (inset) can be achieved by functionalizing the surface of nanoparticles with ligands that promote cell-specific recognition and binding. The nanoparticles can (i) release their contents in close proximity to the target cells; (ii) attach to the membrane of the cell and act as an extracellular sustained-release drug depot or (iii) internalize into the cell.[56]

Many studies have proven that the EPR effect is a passive accumulation of macromolecules in solid tumor tissues, elevating the therapeutic efficiency for the treatment of cancers and decreasing the side effects.[57,58,59,60]

2.2 Polymeric Drug Conjugates as Anticancer Delivery Systems

In the 1970s Dr. Helmut Ringdorf visualized the idea of attaching anticancer agents to a water soluble polymer, which could improve the pharmacokinetics and the active targeting of chemotherapy drugs. Clinical trials using polymeric drug conjugates have shown some advantages over the parent drugs, exhibiting fewer side effects, ease of drug administration, enhanced therapeutic efficacy, and improved patient compliance (Figure 2.4).[61] Polymeric drug

www.manaraa.com

conjugates are delivery systems in which a drug is covalently attached to a polymer carrier via biodegradable linker.[62] The majority of the anticancer drugs in clinical use such as cisplatin, doxorubicin, and paclitaxel lack tumor selectivity and have short circulation time in the blood stream. Cisplatin and platinum based compounds with a low molecular weight have an unfavorable pharmacokinetic pathway and short half life in the blood stream. Polymeric conjugate drugs are thought to overcome the limitations of low molecular platinum based drugs with tailored selective tumor targeting and controlled releasing properties.[63]

www.manaraa.com

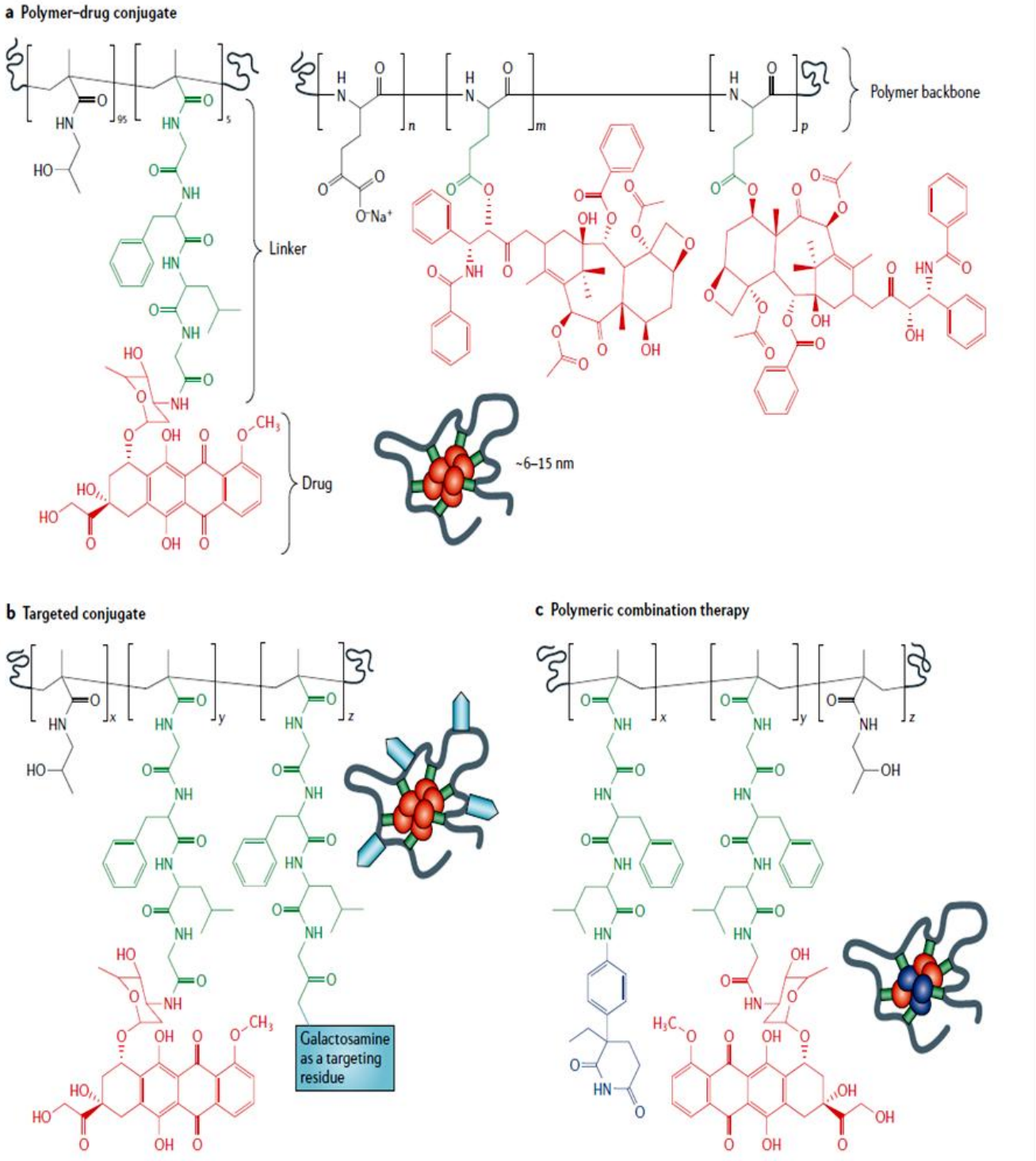


Figure 2.4 Polymer-anticancer drug conjugates. Each panel shows both the detailed chemical structure and a cartoon of the general structure. The polymer backbone is shown in black, linker region in green, drug in red and additional components (for example, a targeting residue) in blue. (a) Two examples of more simple polymer–drug conjugates containing doxorubicin (left) and paclitaxel (right) that have progressed to clinical trial. (b) A multivalent receptor targeted conjugate containing galactosamine (light blue) to promote liver targeting. (c) Polymer combination therapy containing the aromatase inhibitor amino gluthethimide (red) and doxorubicin (blue)[64].

www.manaraa.com

2.3 Polymeric Protein Drugs as Anticancer Delivery Systems

Biotechnology revolution has provided a growing number of peptide, protein, and antibody based medications. Studies in the 1970s anticipated the possible conjugation of polyethylene glycol to proteins. This method is now well recognized and is called PEGylation and is designed to enhance protein solubility and stability, as well as to decrease protein immunogenecity. Furthermore, by avoiding renal clearance of small proteins and receptor mediated protein uptake by cells of the reticulum endothelial system, PEGylation can be used to prolong plasma half life. Lower doses of the medication are of good benefit to the patient motivating conformity. Polyethylene glycol (PEG) is a very important polymer for conjugation. Polyethylene glycol has been in the pharmaceutical industry since it is a versatile compound, with high water solubility, giving hydrodynamic radius that is five to ten times greater than that of a globular protein of equivalent molecular weight. As an example of the efficiency of the PEGylation method a medication called ADAGEN^{R} was obtained from this method and ADAGEN^{R} was the first PEGylated protein to enter the market, in 1990 (Figure 2.5).

Figure 2.5 Adagen.[65]

www.manaraa.com

ADAGEN[R] is used to treat severe combined immunogenicity syndrome, as a substitute to bone marrow transplantation and enzyme substitute by gene therapy. The introduction of ADAGEN[R] was a cornerstone for a great number of PEGylated protein and peptide pharmaceuticals have continued the search for more polymeric drugs. A polymeric drug synthesized by the PEGylation method called PEG-L-asparaginase known in the market as ONCASPAR[R] is used for treatment of acute lymphoblastic leukemia (Figure 2.6).

Figure 2.6 Oncaspar.[68]

Compared to other enzyme based medications PEG-L-asparaginase has the advantage of decreased hypersensitivity, a longer plasma half life and slower total clearance. Therefore PEG-L-asparaginase can be given every two weeks, instead of the two to three times per week required by most enzyme based medications. Moreover PEGylation of L-asparaginase has showed the decrease of hypersensitivity reactions and the polymer conjugated drugs can be used to treat patients that are hypersensitive to the based enzyme medications.

Another PEGylated medication made of methinyl human granulocyte colony stimulating factor (G-CSF) was formulated to prevent severe cancer chemotherapy induced neutropaenia which is the low level of white blood cells. This medication is known in the market as NEULASTA[R], and has the benefit of less repeated administration, being given by a single subcutaneous injection on

www.manaraa.com

day 2 of each chemotherapy cycle (Figure 2.7). The regular G-CSF medication without the PEGylation must be given daily for two weeks to have the same effect as the PEGylated.[66, 67]

Figure 2.7 Neulasta[68]

2.4 Polymer DNA Complexes as Anticancer Delivery Systems

Lipoplexes are composed of nucleic acids and cationic lipids. Polyplexes are composed of cationic polymers and nucleic acid. These complexes are designed to be immunologically inert, and safer than viral vectors.[69] However lipoplexes and polyplexes have some disadvantages they lack specificity, low biodegradability, stability, and cell toxicity. Studies are focusing in the improvement of these non-viral gene delivery systems.[70] Gene therapy studies are important in the treatment of genetic and acquired diseases. Cationic lipids and cationic polymers which are expected to have an important role in gene therapy are highly under research (Figures 2.8 and 2.9).[71,72]

www.manaraa.com

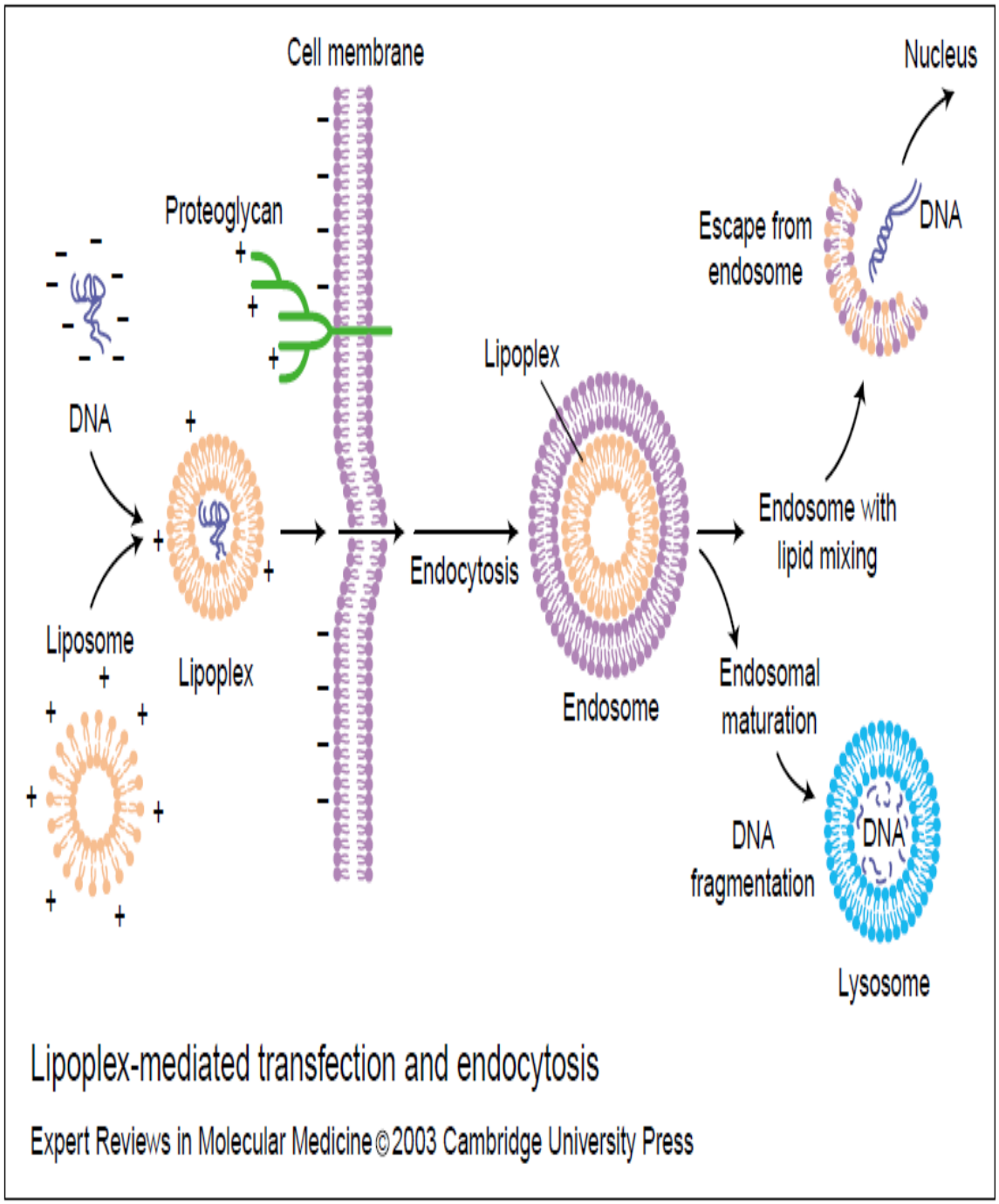


Figure 2.8 Lipoplex mediated transfection and endocytosis. Cationic lipids forming micellar structures called liposomes are complexed with DNA to create lipoplexes. The structures fuse with the cell membrane, at least sometimes after interactions with surface proteoglycans. The complexes are internalized by endocytosis, resulting in the formation of a double layer inverted micellar vesicle. During the maturation of the endosome into a lysosome, the endosomal wall might rupture, releasing the contained DNA into the cytoplasm and potentially towards the nucleus. DNA imported into the nucleus might result in gene expression. Alternatively, DNA might be degraded within the lysosome.[73]

www.manaraa.com

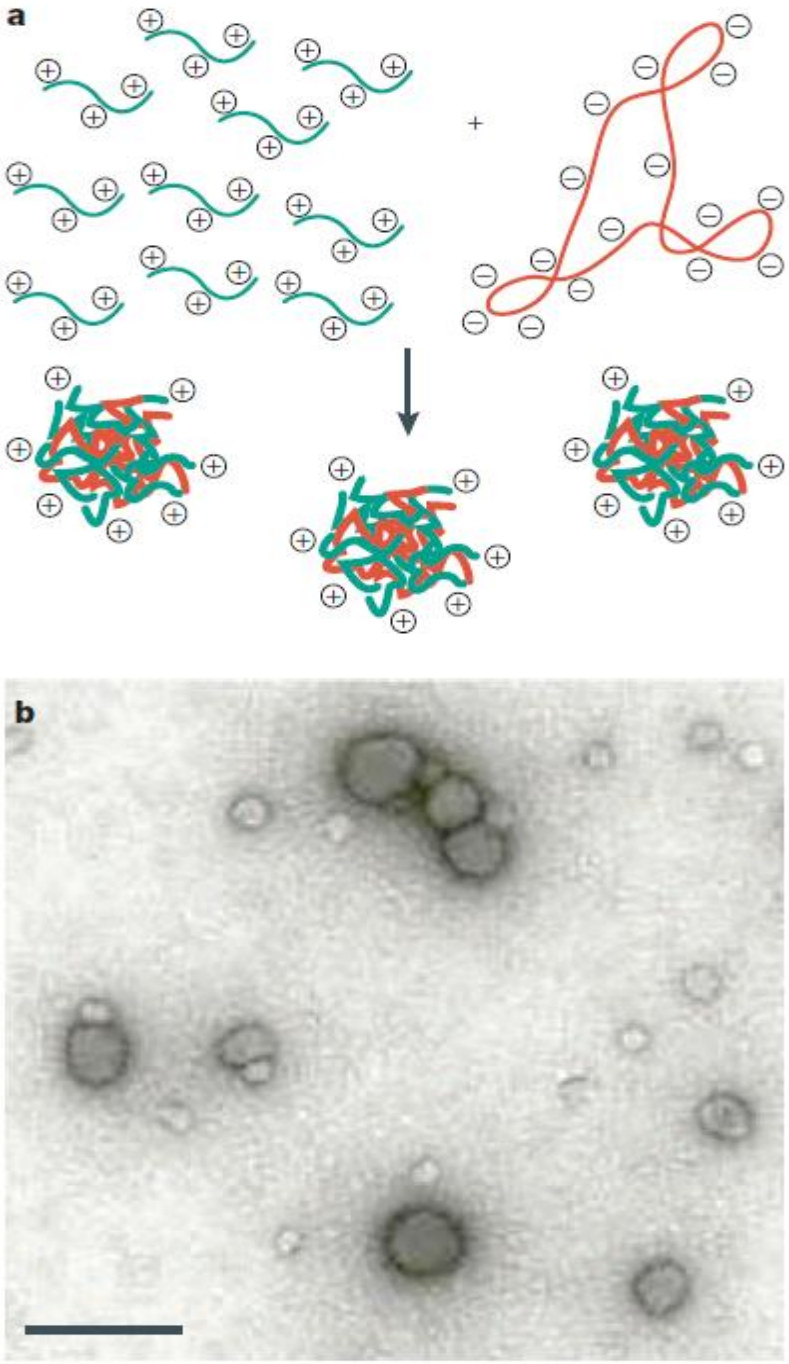
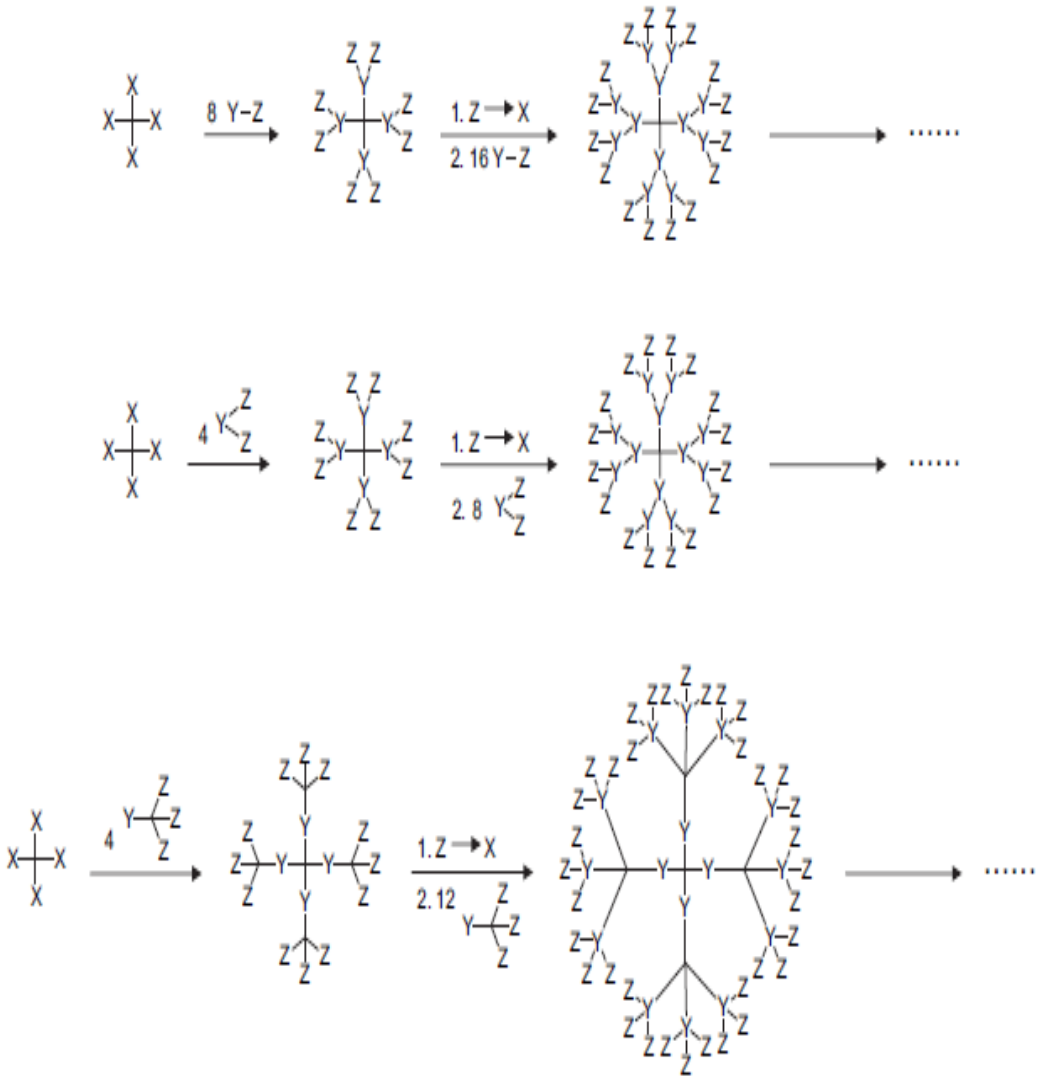


Figure 2.9 Polyplex formation. Polyplexes are formed by electrostatic interactions between polycations and DNA. (a) When aqueous solutions of a polycation and DNA are mixed, polyplexes form spontaneously. The interaction is entropically driven. For gene delivery, an excess of polycation is typically used, which generates particles with a positive surface charge. Each particle consists of several plasmid DNA molecules and hundreds of polymer chains and is 100–200 nm in diameter. (b) Transmission electron micrograph of polyplexes comprising plasmid DNA and a polycation, in this case cyclodextrin modified, branched polyethylenimine (PEI) 165. Scale bar = 200 nm.[74]

www.manaraa.com

2.5 Dendrimers as Anticancer Delivery Systems

Dendrimers (Scheme 3) are macromolecules that can be used as delivery systems, they have attractive properties in biomedical applications such as increasing bioavailability, cellular uptake, improvement of biodistribution, reduction of the systemic toxicity, clearance, and degradation rate.[75]



Scheme 1. Divergent procedures for macromolecular construction of dendrimers.[76]

The anticancer properties of cisplatin are limited because of its low water solubility, low lipophilicity and drug resistance. Dendrimers have been used to encapsulate cisplatin, which

www.manaraa.com

giving complexes with higher accumulation in solid tumors, slower release, and lower toxicity than cisplatin by itself.[77] Most of the dendrimer studies have been performed on modified polyamidoamine (PAMAM) dendrimer because PAMAM compounds are commercially available, possess a wide number of peripheral groups, and group functionality[78] (Figure 2.10).

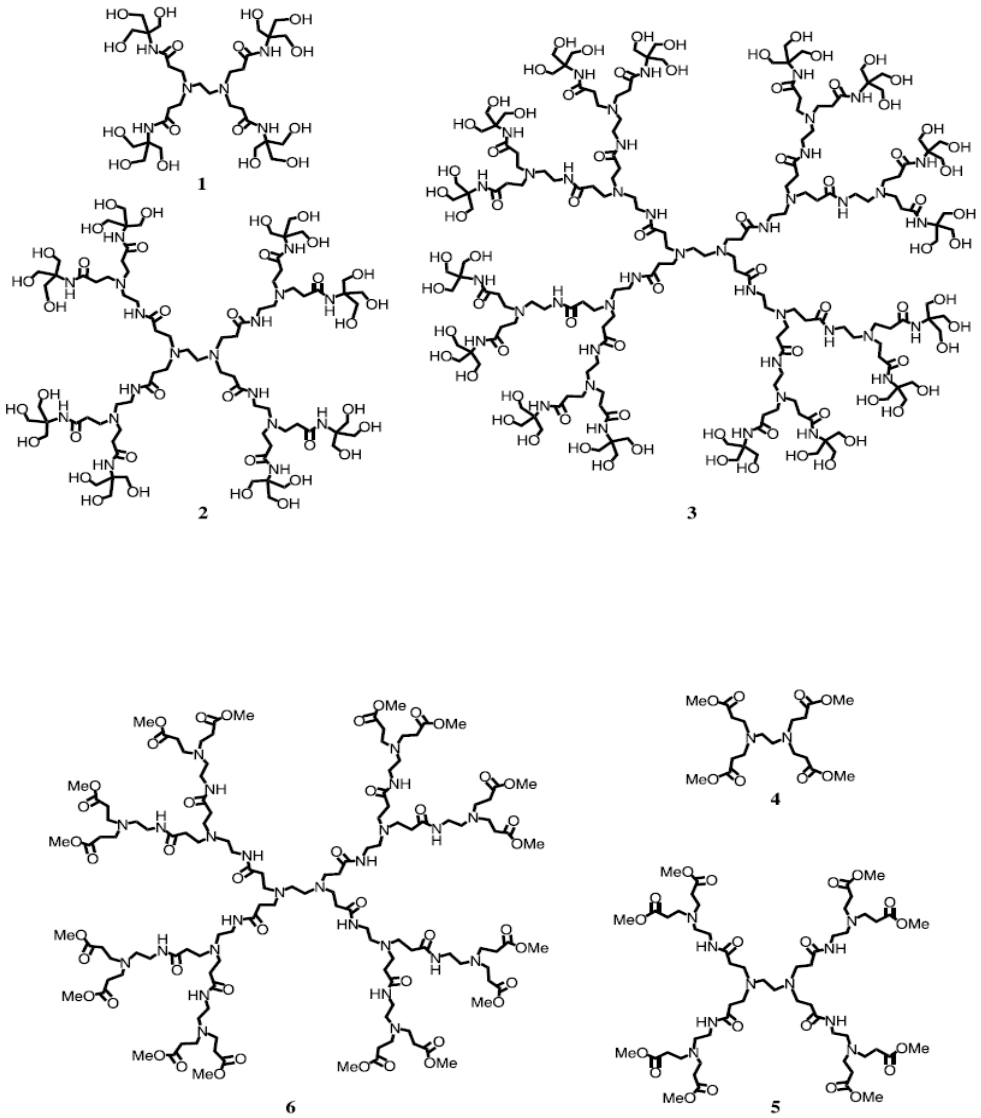


Figure 2.10 Hydroxyl terminated PAMAM dendrimers and their ester terminated precursors.[79]

2.6 Polymeric Micelles as Anticancer Delivery Systems

Polymeric micelles (Figure 2.11) are nanoscopic core shell structures made of amphiphilic copolymers, which give a cotrolled and selective way to deliver encapsulated anticancer drugs.

www.manaraa.com

Polymeric micelles consisting of amphiphilic copolymers with hydrophilic and hydrophobic blocks for solubilization of insoluble drugs have been researched intensively as an example the preparation and physical characterization of multifunctional micelles for lung cancer, imaging therapy, biocirculation.

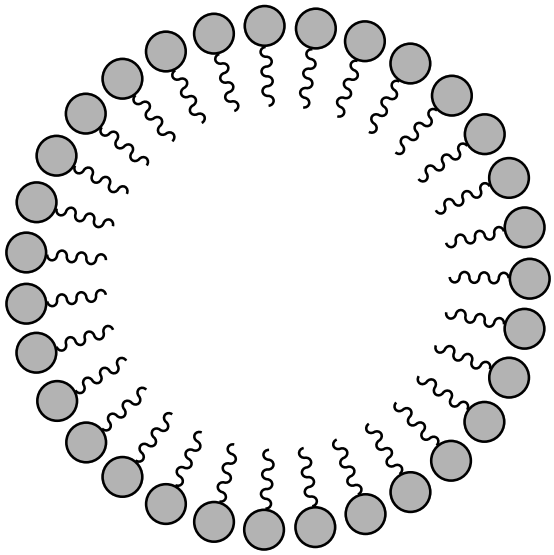


Figure 2.11 Diagram of a micelle. A sphere with hydrophilic heads (gray) at the surface, and hydrophobic tails (black) sequestered inside.[80]

Polymeric drugs are intended to decrease drug degradation and loss as well as to prevent side effects and to increase bioavailability.[81] The framework of polymeric micelles consists of an inner core surrounded by an outer shell of hydrophilic polymers. The inner core serves as a container of hydrophobic drugs and these types of delivery systems have shown longer retention time in the bloodstream and effective tumor accumulation after their admistration.[82] In our research we focused on the design and synthesis of amphiphilic molecules having a lipid hydrophobic chain containing ester functional groups and a hydrophilic head which has a platinum coordinated bond with aromatic N-containing heterocyclic compounds such as bipyridine and biquinoline to form cisplatin analogues. Amphiphilic cisplatin analogues emulsify

www.manaraa.com

to make micelles that project the Pt-Cl groups on the surface of the micelle. These platinum micelles may be used as alkylating-like agents, intercalating agents and as drug delivery systems that encapsulate anticancer drugs. The intracellular activation of esterases in cancerous cells will cleave the ester group found in the lipid chain of the micelles. The micelles of platinum biquinoline and platinum bipyridine will disassemble by the action of the esterases on the ester releasing the anticancer drugs, the Pt-Cl part of the molecule will bind to the DNA and the heterocyclic rings of the molecule will intercalate in the DNA disrupting the DNA structure leading to cell death.

Chapter 3: Objetives in the Synthesis of the Therapeutic Cisplatin Analogues.

Chemotherapy is the most important medical method for the treatment of most cancers. Some of the most used chemotherapeutic drugs are used for destroying or controlling cancerous cells, but also chemotherapeutic drugs tend to be toxic to healthy cells. In our laboratory we synthesized platinum micelles which are amphiphilic molecules that have a hydrophilic polar head and a hydrophobic tail, which self-assemble by forming platinum micelles. These platinum micelles compounds may have applications on cancers of the brain, breast, lung, testicular, ovarian, cervical and bladder which require hydrophobic molecules to permeate the surrounding tissue of such organs.

Chapter 4: Synthesis and Self-Assembly of Platinum Bipyridine Complex

4.1 Materials

The materials for the sections 4.1 and 5.1 were the same in both sections. Triethylene Glycol was purchased from TCI Tokio Kasei. The 2,2'-bipyridine-5,5'-dicarboxylic acid and 2,2'-biquinoline-4,4'-dicarboxylic acid, decanoyl chloride and benzonitrile were purchased from Sigma Aldrich. The thionyl chloride was purchased from Alfa Aesar. The potassium

www.manaraa.com

chloroplatinite crystal was purchased from Johnson Matthey Co. The solvents used were obtained from Sigma Adrich.

4.2 Instrumental Methods

The instrumental methods for the sections 4.2 and 5.2 were the same for both sections. NMR spectra was recorded on a JOEL 600 MHz spectrometer at room temperature, the solvent used was chloroform-d. Mass Spectrometry data was obtained from JOEL USA AccuTOF^{TM} DART at 200 ^{o}C and 1500 volts. Flourescence microscopy was obtained from a Nikon AZ100 with confocal C1 at a magnification 5X objective, 8X zoom and 0.6 dimagnifier and a Carl Zeiss axioskop microscope at 20X objective. The hydrodynamic diameter of the micelles was determined by dynamic light scattering using PDDLS/CoolBatch 90T and PD2000DLS^{Plus} Dynamic Light Scattering (Precision Detectors). Infrared spectroscopy information was obtained from an IR Bruker Tensor 27. The TEM images were obtained from a Carl Zeiss EM-10 instrument.

4.3 Analysis Data

4.3.1. 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate Analysis Data.

^{1}H NMR (600 MHz, $CDCl_3$) : δ 4.05, 3.88, 3.50, 3.49, 3.37, 1.98, 1.2952, 1.2837, 0.99.

^{13}C NMR (150 MHz, $CDCl_3$): δ 172.7204, 71.4193, 70.72, 70.39, 68.74, 63.05, 61.42, 33.63, 31.73, 29.33, 29.21, 22.47, 13.78. Appendix Section ^{1}H NMR and ^{13}C NMR spectrums (Figure A.1 and A.2 respectively).

4.3.2. Bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-bipyridine-5,5'-dicarboxylate Analysis Data.

^{1}H NMR (600 MHz, $CDCl_3$): 9.1789, 8.4838, 8.4700, 4.44, 4.1331, 3.8846, 3.6086, 3.5524, 2.2228, 1.4979, 1.1532, 0.7696. ^{13}C NMR (150 MHz, $CDCl_3$): 174.0704, 165.0988, 158.1571, 150.3250, 137.9065, 126.3210, 122.0315, 70.4014, 70.2608, 69.0161, 64.1712, 63.2999,

www.manaraa.com

34.1447, 31.8085, 30.2478, 29.3765, 29.2137, 24.8476, 22.6072, 14.2292. Appendix Section ^{1}H NMR and ^{13}C NMR spectrums (Figure A.3 and A.4 respectively).

4.3.3. Cis(benzonitrile)dichloropatinum(II) Analysis Data.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.7980, 7.7852, 7.4199. ^{13}C NMR (150 MHz, $CDCl_3$) δ 135.2926, 133.8564, 133.5979, 116.8707, 109.5460. Appendix Section ^{1}H NMR and ^{13}C NMR spectrums (Figure A.5 and A.6 respectively).

4.3.4. Platinum Bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2, 2'-bipyridine-5,5'-dicarboxylate Analysis Data.

^{1}H NMR (600 MHz, $CDCl_3$): δ 10.2543, 9.4939, 8.8422, 4.5728, 4.1938, 3.8857, 3.6051, 3.4723, 3.2993, 2.3385, 1.5586, 1.2391, 0.8474. ^{13}C NMR (150 MHz, $CDCl_3$): δ 173.8885, 165.1371, 159.6888, 138.2416, 127.5562, 121.1698, 72.1, 70.8, 70.5, 66.8043, 65.5027, 64.9659, 34.00, 31.0042, 30.9659, 29.7404, 22.70, 15.26. Appendix Section ^{1}HNMR and ^{13}CNMR spectrums (Figure A.7 and A.8 respectively).

4.4 Chemical Synthesis

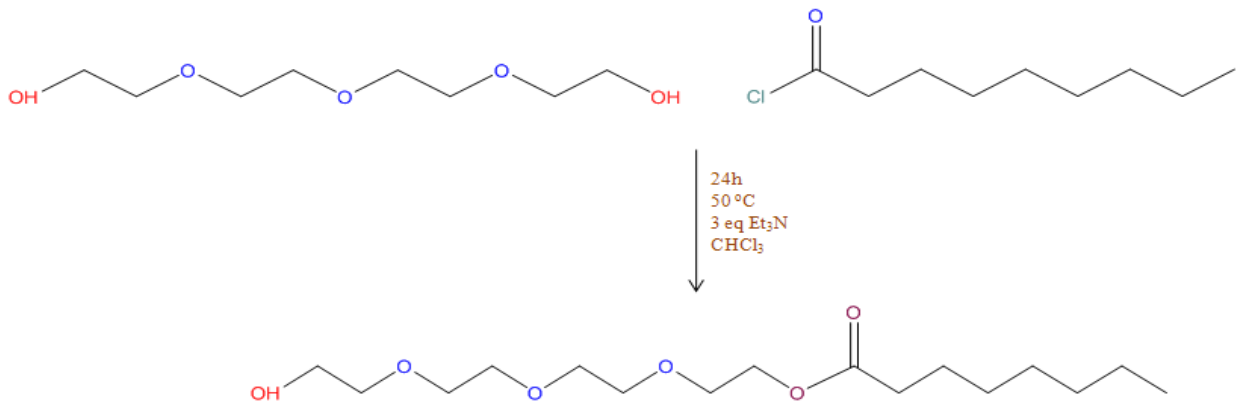
4.4.1 Synthesis of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate.

The 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate was prepared by adding 2 equivalents of tetraethylene glycol 10g (51.5 mmol) in anhydrous chloroform and 1 equivalent of decanoyl chloride 4.90g (25.7 mmol) in chloroform mixed in a round flask at room temperature, the mixture was left stirring for 2 h. The crude was purified by preparative plate using 9:1 v/v chloroform/ methanol.

^{1}H NMR (600 MHz, $CDCl_3$): δ 4.05, 3.88, 3.50, 3.49, 3.37, 1.98, 1.2952, 1.2837, 0.99.

^{13}C NMR (150 MHz, $CDCl_3$): δ 172.7204, 71.4193, 70.72, 70.39, 68.74, 63.05, 61.42, 33.63, 31.73, 29.33, 29.21, 22.47, 13.78 (Scheme 4.1).

www.manaraa.com

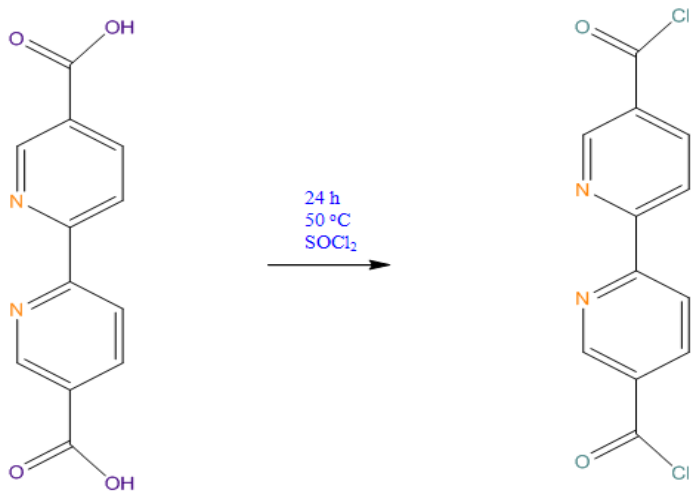


Scheme 4. 1 Synthesis of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate

4.4.2. Synthesis of 2, 2'-biquinoline-4,4'dicarboxylic dichloride

A treatment of 2,2'-bipyridine -5,5'dicarboxylic acid .58g (2.4 mmol) with thionyl chloride 10ml was refluxed for 24 h at 50 ^{o}C to obtain the compound 2, 2'-biquinoline-4,4'dicarboxylic dichloride .68g (2.3 mmol). The thionyl chloride excess was removed by vacuum obtaining a white powder (Scheme 4.2).

www.manaraa.com

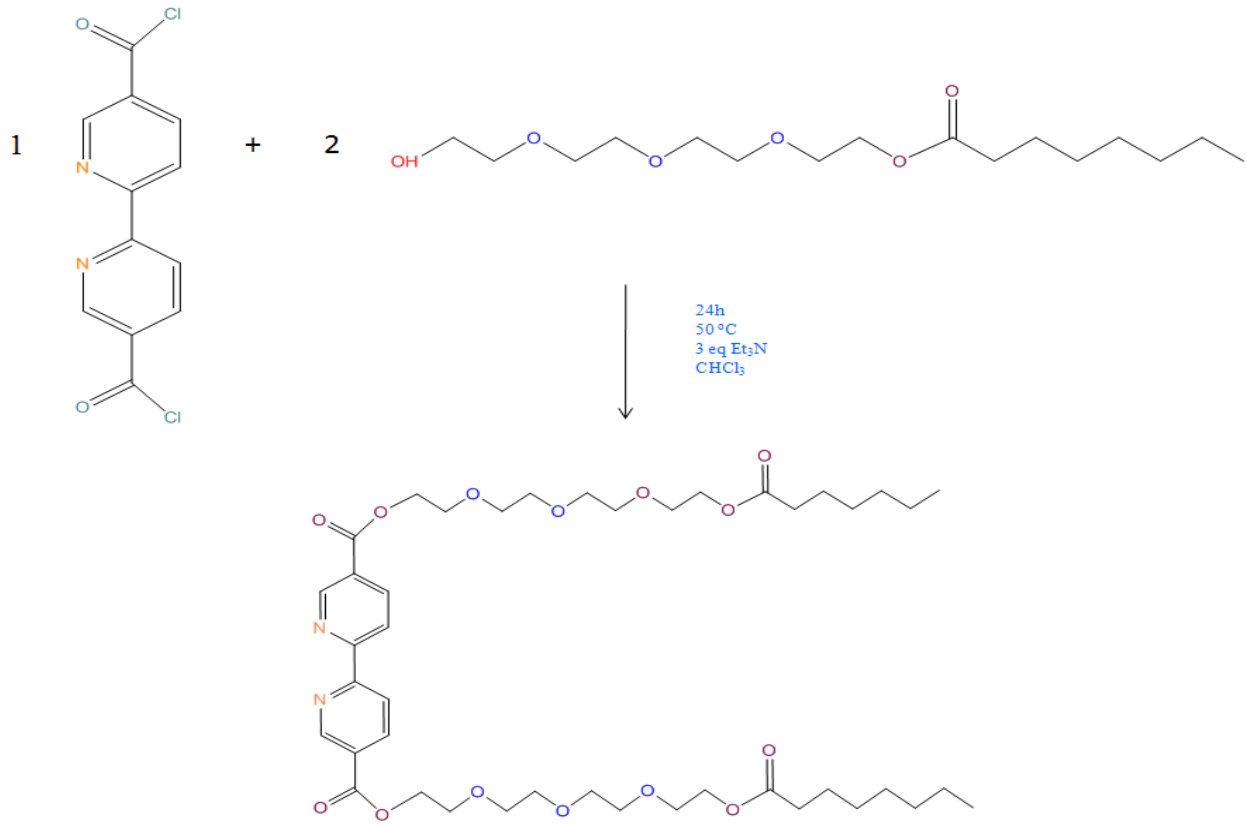


Scheme 4. 2 Synthesis 2, 2'-biquinoline-4,4'dicarboxylic dichloride

4.4.3. Bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-bipyridine-5,5'-dicarboxylate

The (2,2'-biquinoline-4,4'dicarboxylic dichloride .68g (2.3 mmol) was dissolved in chloroform and two equivalents of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate 1.6g (4.6 mmol) compound were added as well as 2 equivalents of triethylamine 0.47g (4.67 mmol). The mixture was left refluxing for 24 h at 50 ^{o}C to obtain 1.80g (1.5mmol) of bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-bipyridine-5,5'-dicarboxylate. ^{1}H NMR(500 MHz, $CDCl_3$): δ 9.1789, 8.4838, 8.4700, 4.44, 4.1331, 3.8846, 3.6086, 3.5524, 2.2228, 1.4979, 1.1532, 0.7696. ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.0704, 165.0988, 158.1571, 150.3250, 137.9065, 126.3210, 122.0315, 70.4014, 70.2608, 69.0161, 64.1712, 63.2999, 34.1447, 31.8085, 30.2478, 29.3765, 29.2137, 24.8476, 22.6072, 14.2292 (Scheme 4.3).

www.manaraa.com



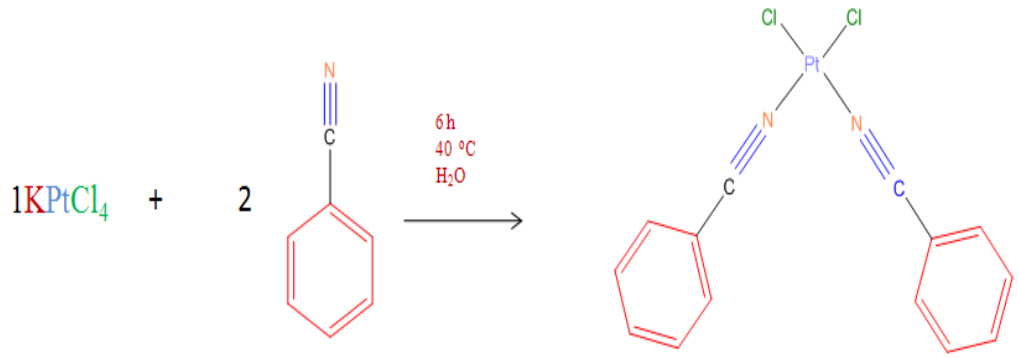
Scheme 4.3 Synthesis of bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-bipyridine-5,5'-dicarboxylate

4.4.4. Synthesis of Cis(benzonitrile)dichloropatinum(II)

The synthesis of the cis(benzonitrile)dichloropatinum(II) was carried out by adding 1 equivalent of potassium tetrachloroplatinate(II) 3.35g (8 mmol) and 1 equivalent of benzonitrile 1.66g (16.1 mmol) in 50 ml of water mixed in a round flask at 60 ^{o}C for 6 h. Cis(benzonitrile)dichloroplatinum(II) green compound 1.9 g (3.8 mmol) was obtained then filtered and dried at vacuum for 3 h. The dried compound was washed 3 times with diethyl ether and 1.9g (3.8 mmol) were obtained (scheme 4.4).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.7980, 7.7852, 7.4199. ^{13}C NMR (150 MHz, $CDCl_3$) δ 135.2926, 133.8564, 133.5979, 116.8707, 109.5460. Mass spectrum cis(benzonitrile)dichloplatinum(II) (M.W= 472.2326) (Figure 4.1).

www.manaraa.com



Scheme 4.4 Synthesis of cis(benzonitrile)dichloroplatinum(II)

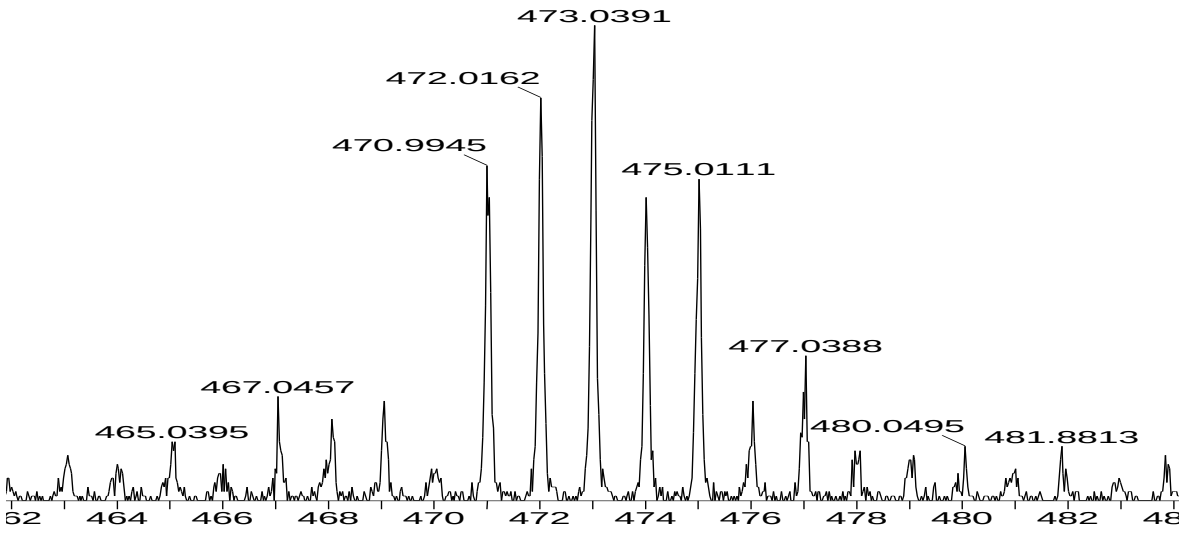
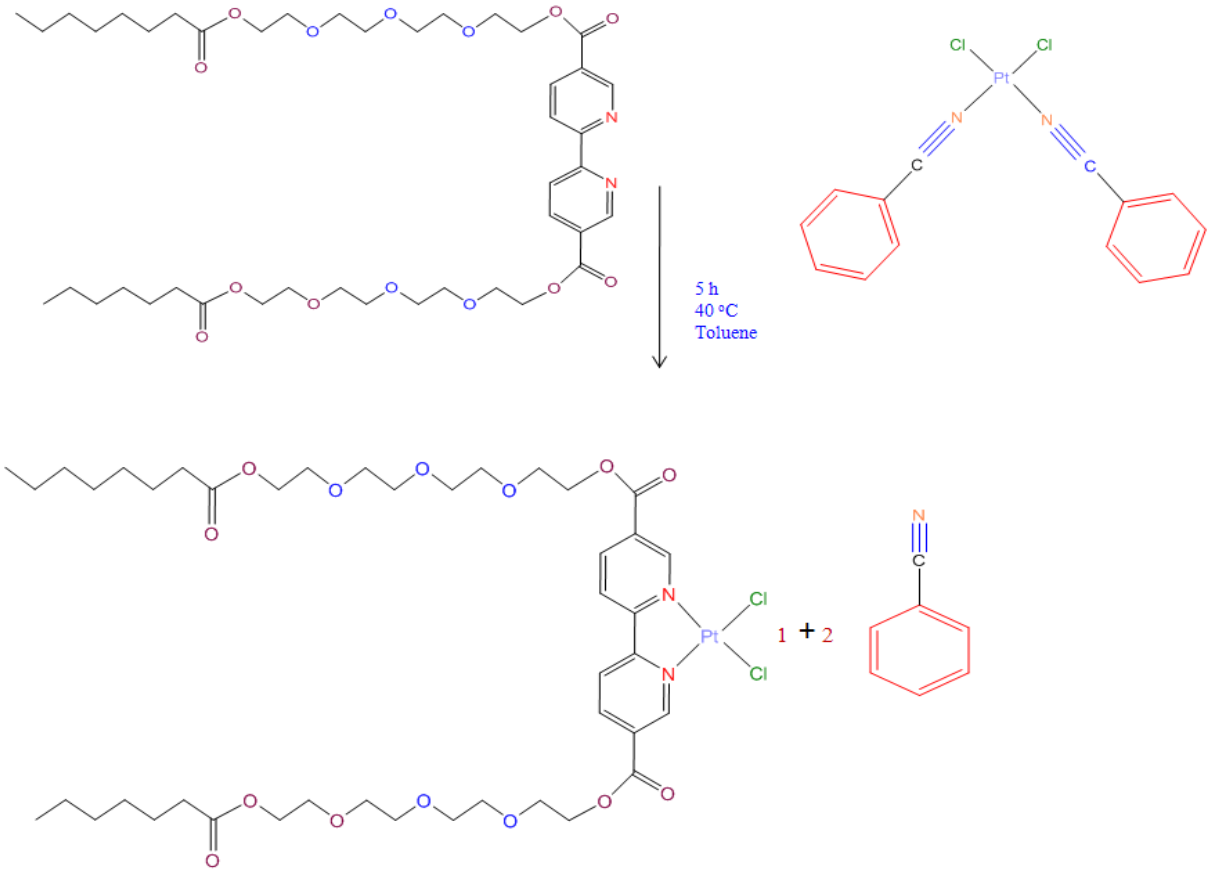


Figure 4.1 Mass Spectrum of cis(benzonitrile)dichloroplatinum(II) (M.W= 472.2326)

4.4.5. Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate.

An equivalent of bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate 1.80g (1.53 mmol) was added to an equivalent of cis(benzonitrile)dichloropatinum(II) 0.76g (1.53 mmol) in 30 ml of toluene and refluxed at 40 ^{o}C for 24 h. The solvent was removed at vacuum and the solid obtained was dissolved in chloroform and removed with the rotovap then the solid was washed with diethyl ether and centrifuged to obtain a platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate compound 2.2 g (1.86 mmol) (scheme 4.5).

www.manaraa.com



Scheme 4.5 Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate

^{1}H NMR (600 MHz, $CDCl_3$): δ 10.2543, 9.4939, 8.8422, 4.5728, 4.1938, 3.8857, 3.6051, 3.4723, 3.2993, 2.3385, 1.5586, 1.2391, 0.8474. ^{13}CNMR (150 MHz, $CDCl_3$): δ 173.8885, 165.1371, 159.6888, 138.2416, 127.5562, 121.1698, 72.1, 70.8, 70.5, 66.8043, 65.5027, 64.9659, 34.00, 31.0042, 30.9659, 29.7404, 22.70, 15.26. The IR spectrum of the platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate showed vibrational absorption frequencies at the 520 cm^{-1}, 344 cm^{-1} which were assigned to the *ν*(Pt-N) and *ν*(Pt-Cl) respectively. These assignments are consistent with the literature frequency values according to the literature.[83,84,85]

www.manaraa.com

Fluorescence microscope image, light scattering hydrodynamic diameter, HeLa cells percent availability, IR irrational frequencies, Mass Spectrum, and TEM image for pt-bipyridine complex are shown on figures 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7 respectively.

Fluorescence microscope image (Figure 4.2) indicates the formation of micelles structures of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate using 1:1 v/v chloroform/methanol with Nile red (7-diethylamino-3,4-benzophenoxazine-2-one) as fluorescence indicator.

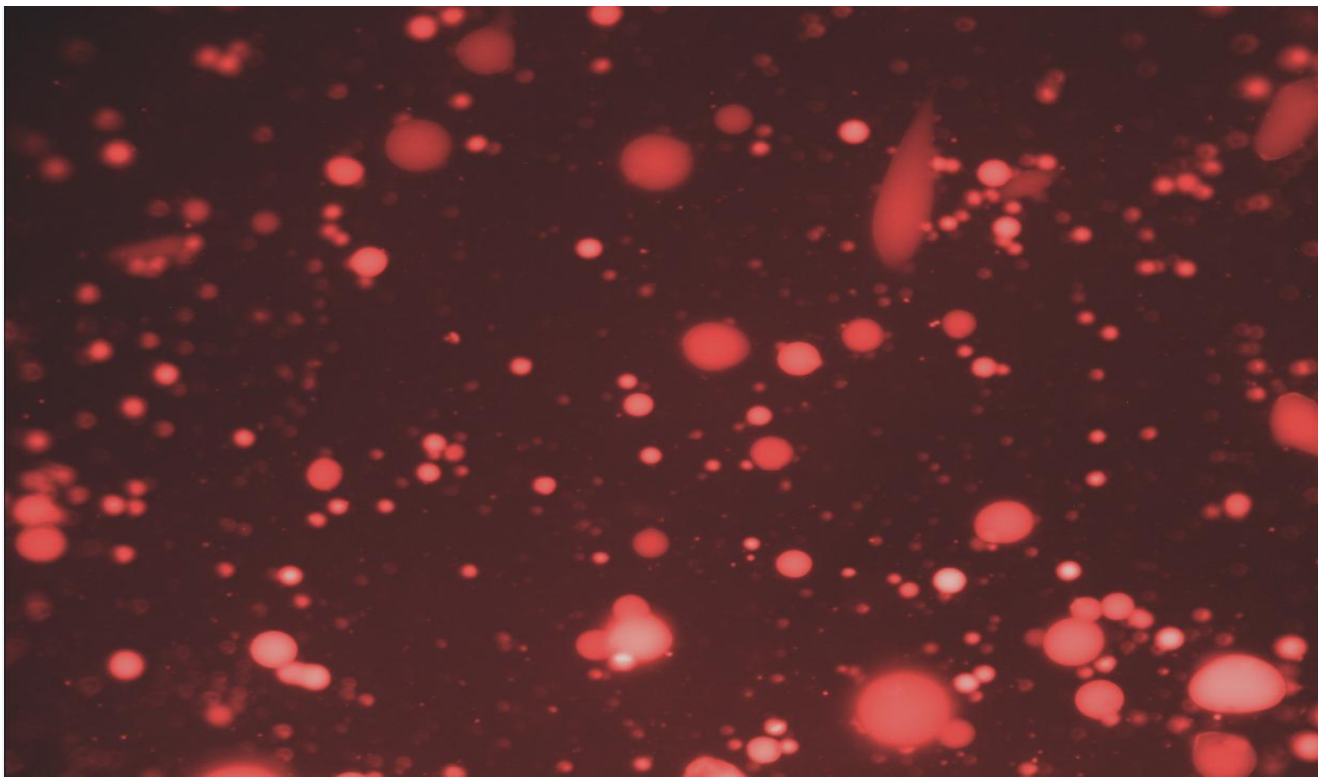


Figure 4.2 Fluorescence Microscope Image of platinum bis (13-oxo-3, 6, 9, 12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate.

www.manaraa.com

The light scattering hydrodynamic displays diameter of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2, 2'-bipyridine-5,5'-dicarboxylate (Figure 4.3) diameter population ranges from 40nm, 200-400nm and from 1100-1300 nm.

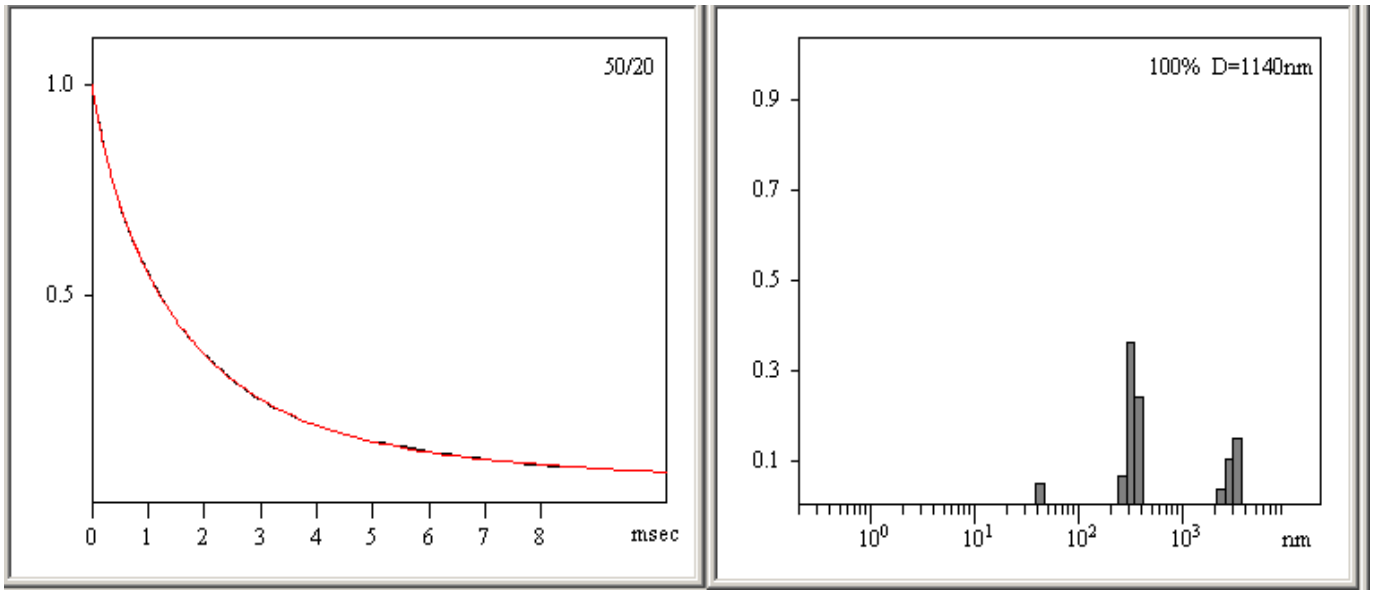


Figure 4.3 DLS of Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate

www.manaraa.com

The percent availability of HeLa cancer cells (Figure 4.4) indicates low toxicity of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate complex for the HeLa cancer cells. Each triplicate at different concentrations is shown and compared with their respective percentage difference and bar errors.

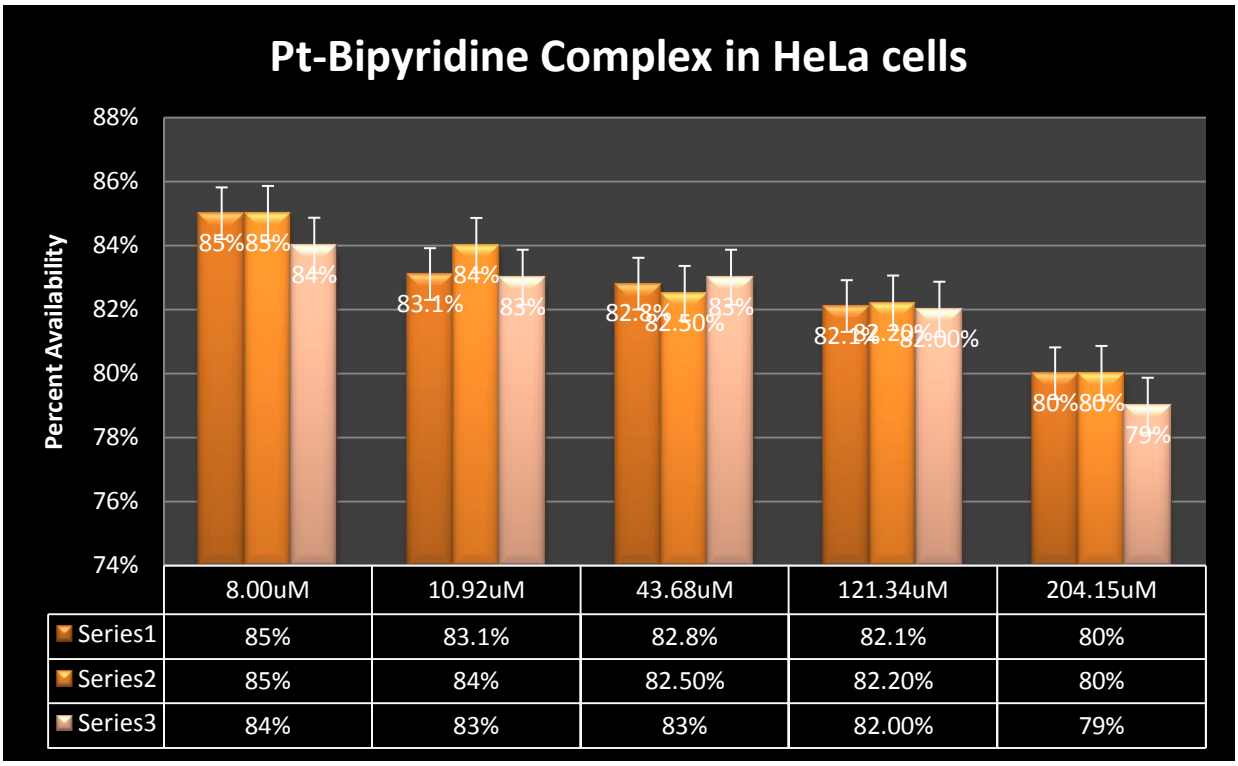


Figure 4.4 Percent availability for HeLa cells treated with platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate Complex

www.manaraa.com

The infrared spectrum Figure (4.5) displays selected vibrational frequencies at the 550 cm^{-1}, 355 cm^{-1} which were assigned to the ν(Pt-Cl) and ν(Pt-N) respectively.

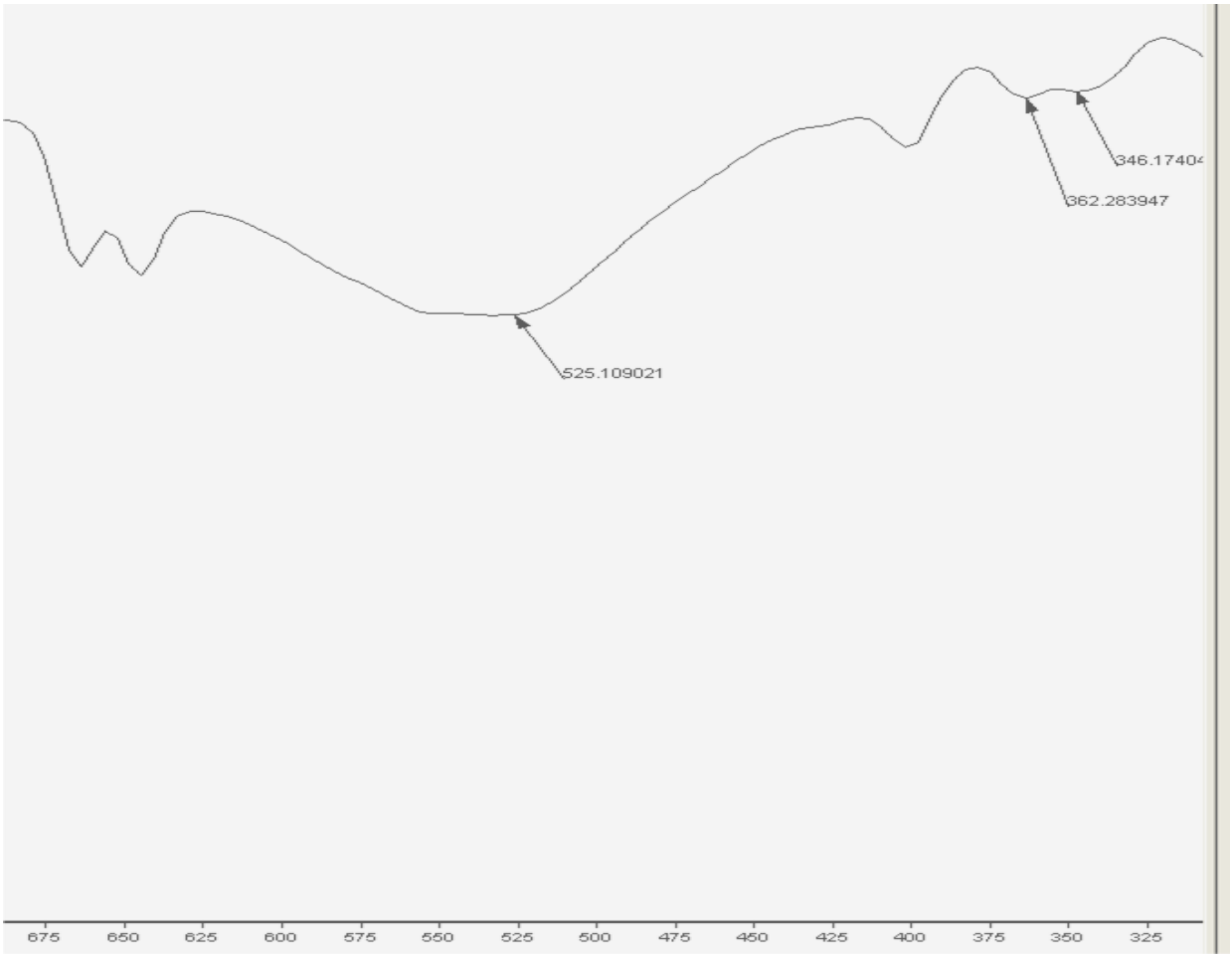


Figure 4. 5 Infrared vibrational frequencies for platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate.

The mass spectrum (Figure 4.6) for platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate (M.W = 1171.11) indicates the signals of platinum isotopes where the presence of the platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate can be observed and determined.

www.manaraa.com

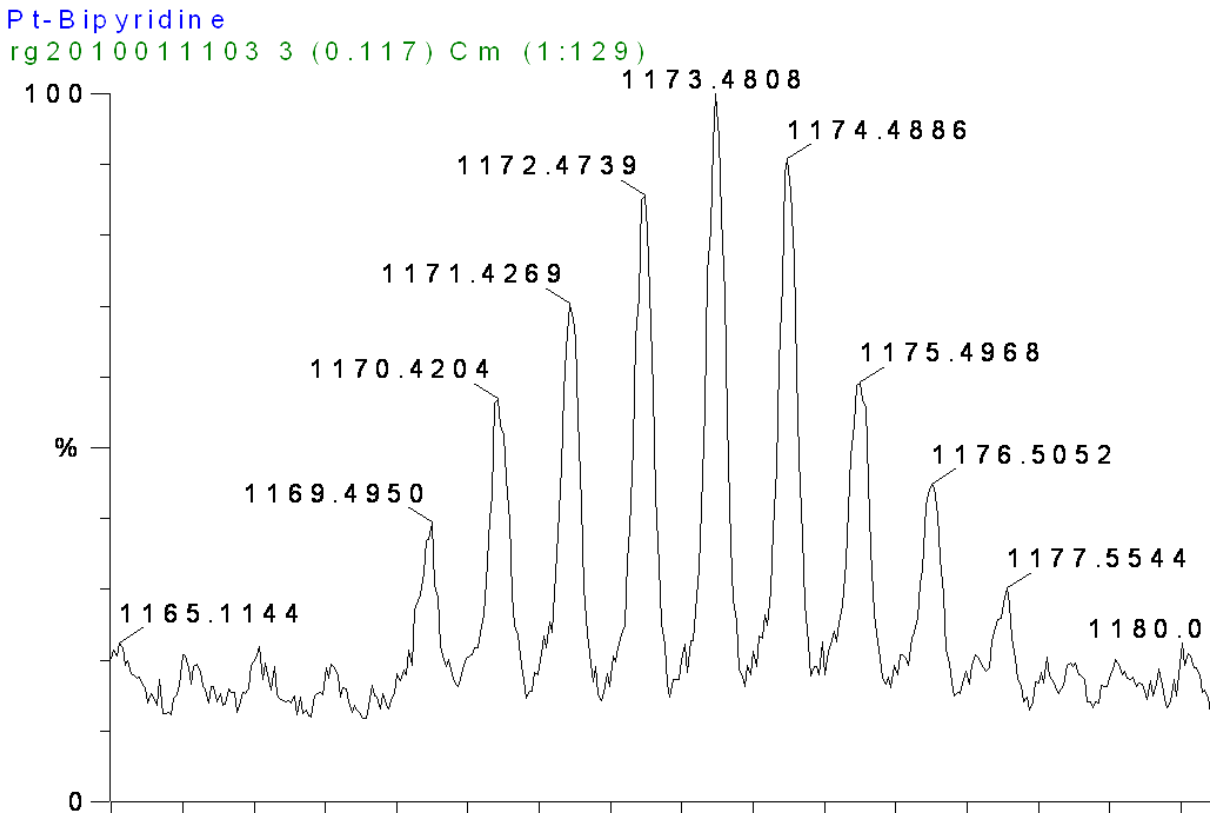


Figure 4.6 Mass Spectrum for platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate (M.W.= 1171.11).

The TEM picture (Figure 4.7) displays a spherical structure which has the form of a micelle with an empty core surrounded by amphiphilic molecules of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate.

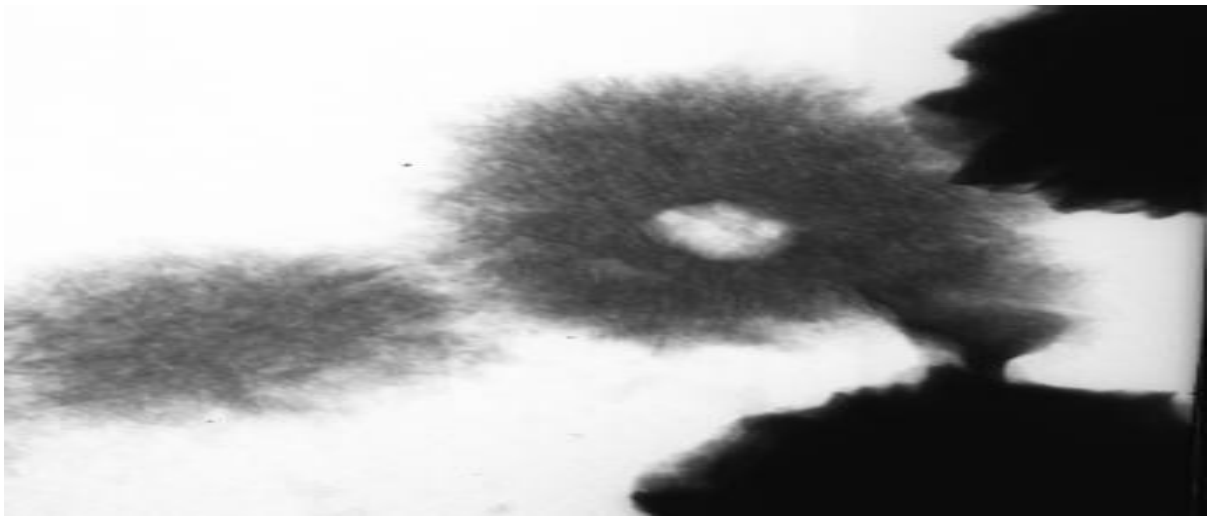


Figure 4.7 TEM for platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate.

www.manaraa.com

Chapter 5: Synthesis and Self-Assembly of Platinum Biquinoline Complex

5.1 Materials

The material for this section were the same as on section 4.1

5.2 Instrumental Methods

The instrumental method for this section were the same as on section 5.1

5.3 Analysis Data

5.3.1. 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate Analysis Data

^{1}H NMR (600 MHz, $CDCl_3$): δ 4.05, 3.88, 3.50, 3.49, 3.37, 1.98, 1.2952, 1.2837, 0.99. ^{13}C NMR (150 MHz, $CDCl_3$):172.7204, 71.4193, 70.72, 70.39, 68.74, 63.05, 61.42, 33.63, 31.73, 29.33, 29.21, 22.47, 13.78. Appendix Section ^{1}H NMR and ^{13}C NMR spectrums (Figure A.9 and A.10 respectively).

5.3.2. Bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate Analysis Data.

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.9281, 8.4219, 8.2364, 7.9123, 4.3601, 4.2722, 3.89, 3.64, 3.50, 2.0064, 1.8312, 1.28, 0.9631. ^{13}C NMR (150 MHz, $CDCl_3$): δ 173.7161, 166.3531, 155.5720, 148.5728, 136.0203, 130.5052, 130.1509, 129.3754, 128.4275, 127.9009, 127.5466, 126.8285, 120.2698, 70.3948, 70.2703, 70.2225, 68.0394, 64.2287, 63.2042, 32.0479, 31.7032, 29.5393, 29.2616, 22.5114, 14.0760. Appendix Section ^{1}H NMR and ^{13}C NMR spectrums (Figure A.11 and A.12 respectively).

5.3.3. Cis(benzonitrile)dichloropatinum(II) Analysis Data.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.7980, 7.7852, 7.4199. ^{13}C NMR (150 MHz, $CDCl_3$): δ 135.2926, 133.8564, 133.5979, 116.8707, 109.5460. Appendix Section ^{1}H NMR and ^{13}C NMR spectrums (Figure A.13 and A.14 respectively).

www.manaraa.com

5.3.4. Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate Analysis Data.

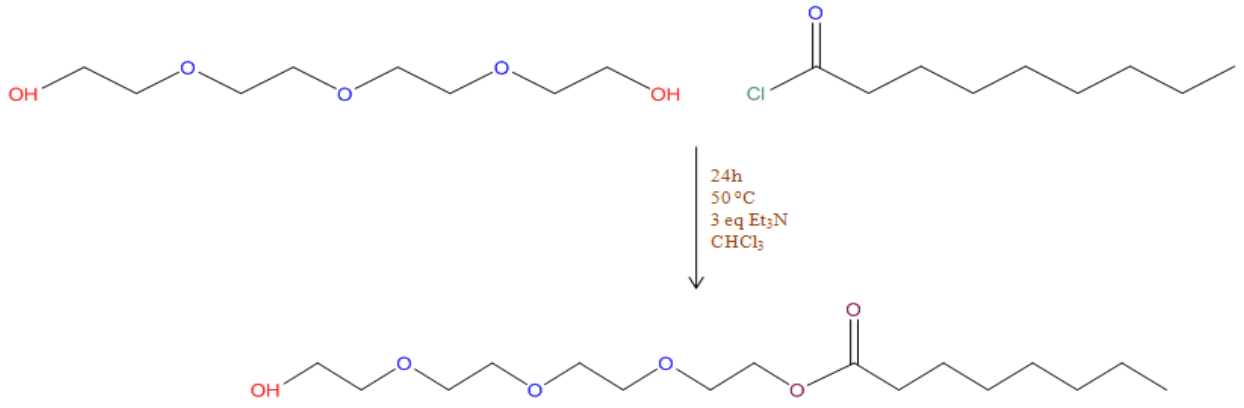
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.8273, 8.7644, 8.4906, 8.2731, 4.2121, 4.1067, 3.6429, 3.5856, 2.3293, 2.3179, 2.3053, 1.5815, 1.2654, 0.8531. ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9747, 166.6021, 148.7643, 148.7387, 136.3458, 132.2383, 130.4956, 120.5953, 70.4140, 70.0597, 68.8150, 64.1042, 63.3957, 34.2884, 31.9330, 29.3382, 29.2042, 22.7316, 14.2580. Appendix section ^{1}H NMR and ^{13}C NMR spectrums (Figure A.15 and A.16 respectively).

5.4. Chemical Synthesis

5.4.1. Synthesis of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl decanoate analysis.

The 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate was prepared by adding 2 equivalents of tetraehylene glycol 10g (51.5 mmol) in anhydrous chloroform and 1 equivalent of decanoyl chloride 4.90g (25.7 mmol) in chloroform mixed in a round flask at room temperature, the mixture was left stirring for 2 h. The crude was purified by preparative plate using 9:1 v/v chloroform/ methanol.

^{1}H NMR (600 MHz, $CDCl_3$): δ 4.05, 3.88, 3.50, 3.49, 3.37, 1.98, 1.2952, 1.2837, 0.99. ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.7204, 71.4193, 70.72, 70.39, 68.74, 63.05, 61.42, 33.63, 31.73, 29.33, 29.21, 22.47, 13.78 (Scheme 5.1).

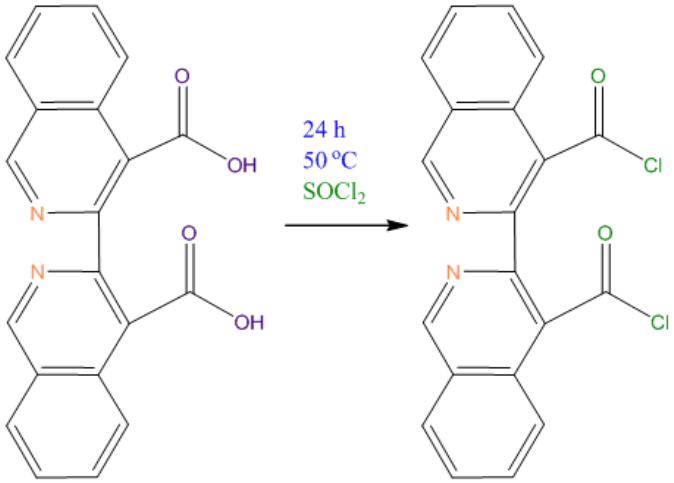


www.manaraa.com

Scheme 5.1 Synthesis of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl decanoate.

5.4.2. Synthesis of 2, 2'-biquinoline-4,4'dicarboxylic dichloride

A treatment of 2, 2'-biquinoline-4,4'dicarboxylic 1g (2.9 mmol) acid in 15 ml of thionyl chloride was refluxed for 24 h at 50 ^{o}C obtaining the compound 2, 2'-biquinoline-4,4'dicarboxylic dichloride 1.2g (3.15 mmol).[86] The thionyl chloride excess was removed by vacuum (scheme5.2).



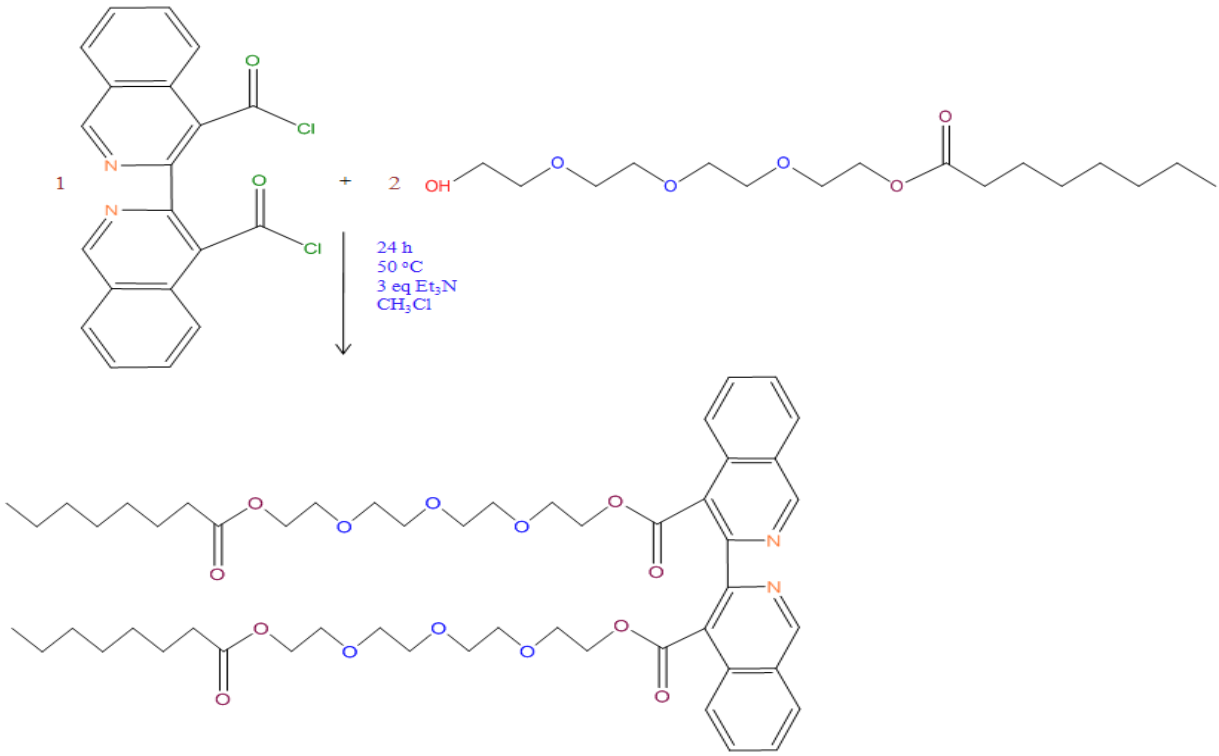
Scheme 5.2 Synthesis of 2,2'-biquinoline-4,4'dicarboxylic dichloride

5.4.3. Synthesis of bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate.

The 2,2'-biquinoline-4,4'dicarboxylic-dichlorid 0.45g (1.2 mmol) was dissolved in chloroform and 2 equivalents of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl decanoate 0.82 (2.4 mmol) was added as well as 2 equivalents of triethylamine 0.24g (2.4 mmol). The mixture was left refluxing for 24 h at 50 ^{o}C temperature to obtain bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate 1.2 g (0.78 mole). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.9281, 8.4219, 8.2364, 7.9123, 4.3601, 4.2722, 3.89, 3.64, 3.50, 2.0064, 1.8312, 1.28, 0.9631. ^{13}C NMR(150

www.manaraa.com

MHz, $CDCl_3$) : δ 173.7161, 166.3531, 155.5720, 148.5728, 136.0203, 130.5052, 130.1509, 129.3754, 128.4275, 127.9009, 127.5466, 126.8285, 120.2698, 70.3948, 70.2703, 70.2225, 68.0394, 64.2287, 63.2042, 32.0479, 31.7032, 29.5393, 29.2616, 22.5114, 14.0760 (Scheme 5.3).



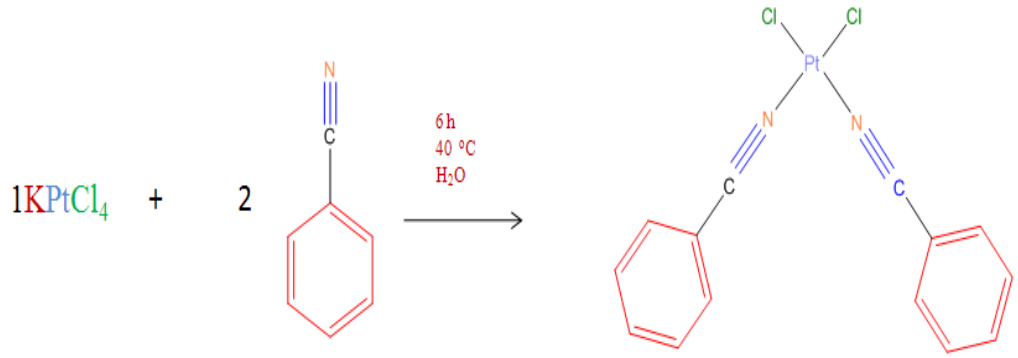
Scheme 5.3 Synthesis of bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate

5.4.4. Synthesis of Cis(benzonitrile)dichloropatinum(II).

The synthesis of the cis(benzonitrile)dichloropatinum(II) was carried out by adding 1 equivalent of potassium tetrachloroplatinate(II) 3.35g (8 mmol) and 1 equivalent of benzonitrile 1.66g (16.1 mmol) in 50 ml of water mixed in a round flask at 60 ^{o}C for 6 h. Cis(benzonitrile)dichloroplatinum(II) green compound was obtained then filtered and dried at vacuum for 3 h. The dried compound was washed 3 times with diethyl ether and 1.9g (3.8 mmol) were obtained ^{1}HNMR

www.manaraa.com

(600 MHz, $CDCl_3$): δ 7.7980, 7.7852, 7.4199. ^{13}CNMR (150 MHz, $CDCl_3$) δ 135.2926, 133.8564, 133.5979, 116.8707, 109.5460 (scheme 5.4). Mass spectrum of cis(benzonitrile)di-chloroplatinum (II) (Figure 5.1).



Scheme 5.4 Synthesis of cis(benzonitrile)dichloroplatinum(II)

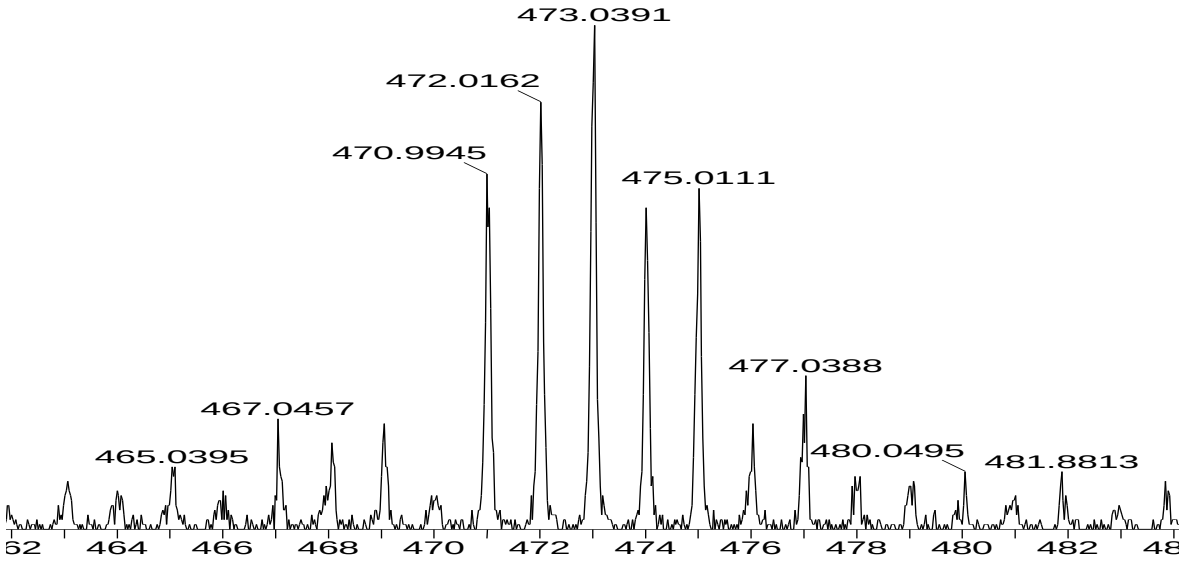
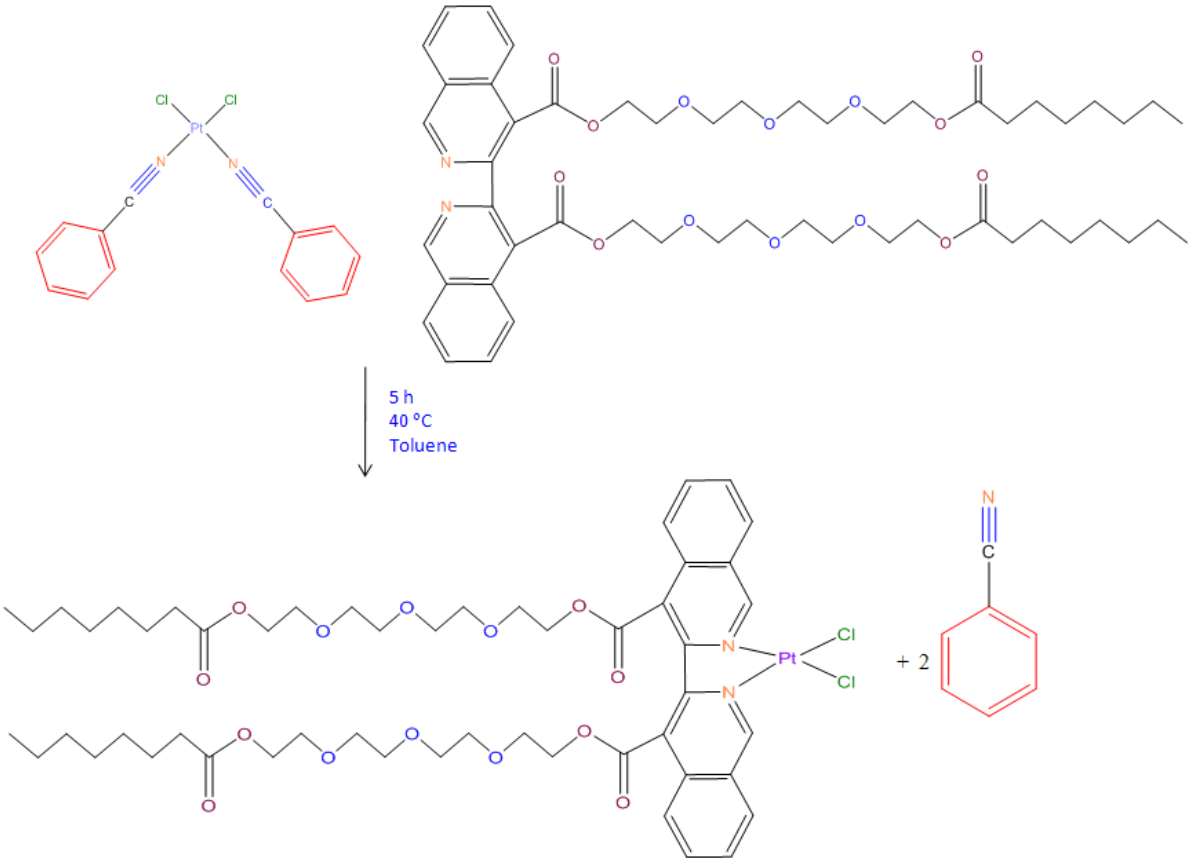


Figure 5.1 Mass Spectrum of cis(benzonitrile)dichloroplatinum(II) (M.W= 472.2326)

www.manaraa.com

5.4.5. Synthesis of Platinum Bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'-dicarboxylate.

One equivalent of bis(13-oxo-3,6,9,12-tetraoxadocosy) 2,2'-biquinoline-4,4'dicarboxylate (Ligand 2) was added to one equivalent of cis(benzonitrile)dichloropatinum(II) compound in toluene and refluxed for 24 hours the solvent was removed at vacuum and solid obtained was dissolved in chloroform and removed with the rotovap the solid was washed with diethyl ether and centrifuged to obtain a platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate (Scheme 5.5)



Scheme 5.5 Synthesis of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate

www.manaraa.com

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.8273, 8.7644, 8.4906, 8.2731, 4.2121, 4.1067, 3.6429, 3.5856, 2.3293, 2.3179, 2.3053, 1.5815, 1.2654, 0.8531. ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9747, 166.6021, 148.7643, 148.7387, 136.3458, 132.2383, 130.4956, 120.5953, 70.4140, 70.0597, 68.8150, 64.1042, 63.3957, 34.2884, 31.9330, 29.3382, 29.2042, 22.7316, 14.2580 (scheme 4).

The IR spectrum of the platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate complex showed vibrational absorption frequencies at the 550 cm^{-1}, 355 cm-1 which were assigned to the *v*(Pt-Cl) and *v*(Pt-N) respectively. These values are consistent according to the literature[87,88]. Fluorescence microscope image, light scattering hydrodynamic diameter, HeLa cells percent availability, IR vibrational frequencies, and mass spectrum for the platinum platininum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate complex are shown on figures 5.2, 5.3, 5.4, 5.5, and 5.6 respectively.

The flourescence microscope image (Figure 5.2) indicates the formation of micelle structures of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate using 1:1 v/v chloroform/methanol with Nile red (7-diethylamino-3,4-benzophenoxazine-2-one) as fluorescence indicator.

www.manaraa.com

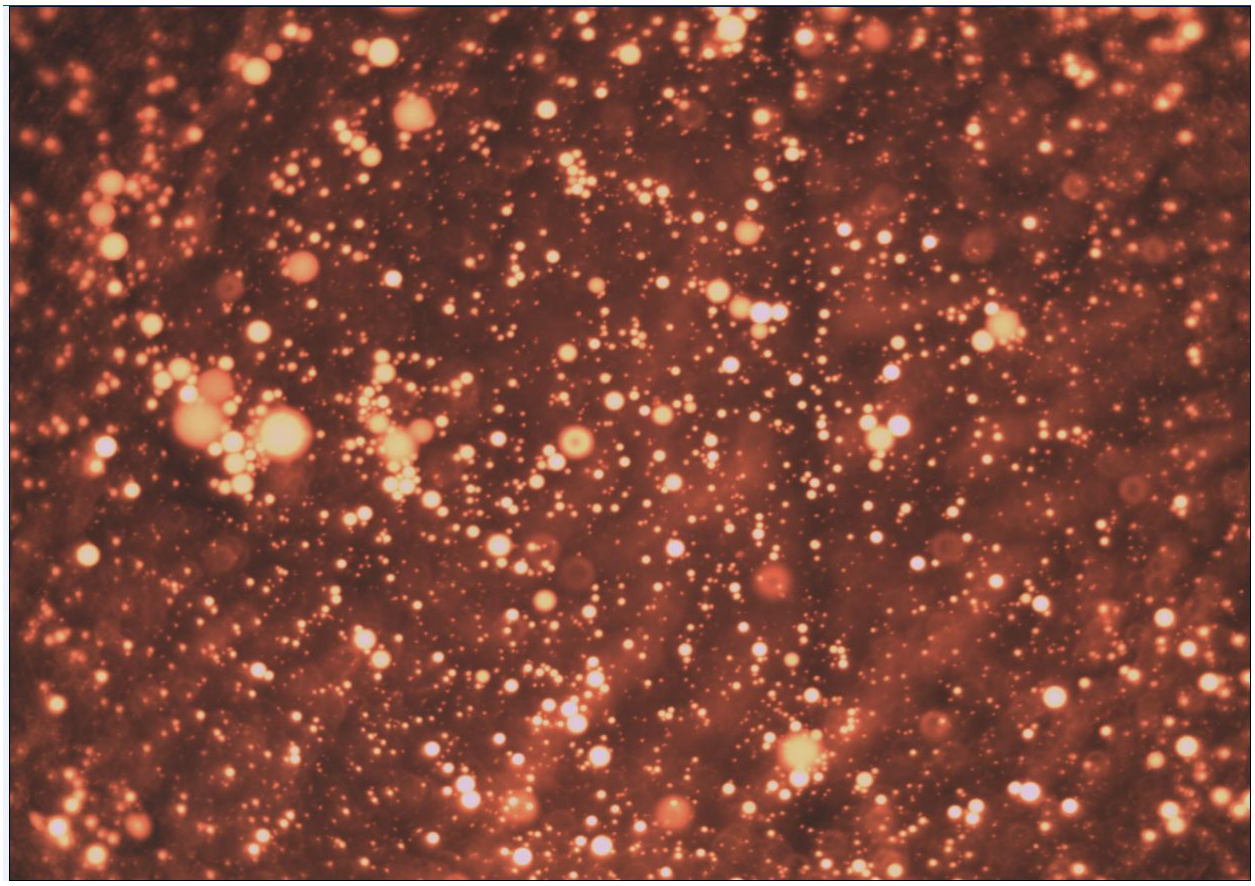


Figure 5.2 Fluorescence microscope Image of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate.

The direct light scattering image of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate (Figure 5.3) displays the diameter sizes of the micelle structures ranging from 10^1 to 10^3 nm.

www.manaraa.com

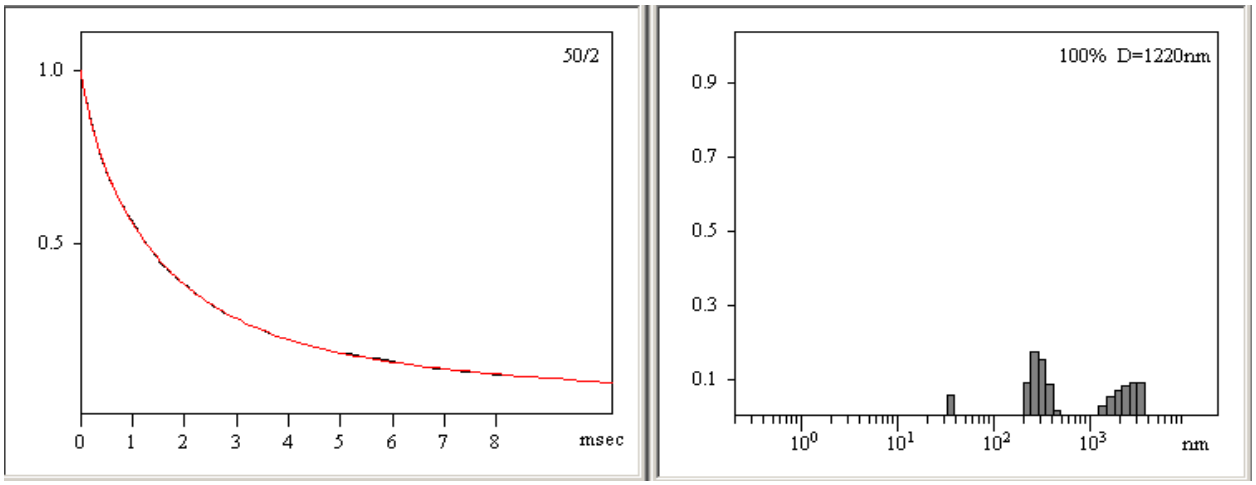


Figure 5.3 DLS of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate

The percent availability of HeLa cancer cells (Figure 5.4) indicates that at 24 uM the LD_{50} of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2, 2'-biquinoline-4,4' dicarboxilate kills about 50% of the HeLa cancer cells. Each triplicate at different concentrations is shown and compared with their respective percentage difference and bar errors.

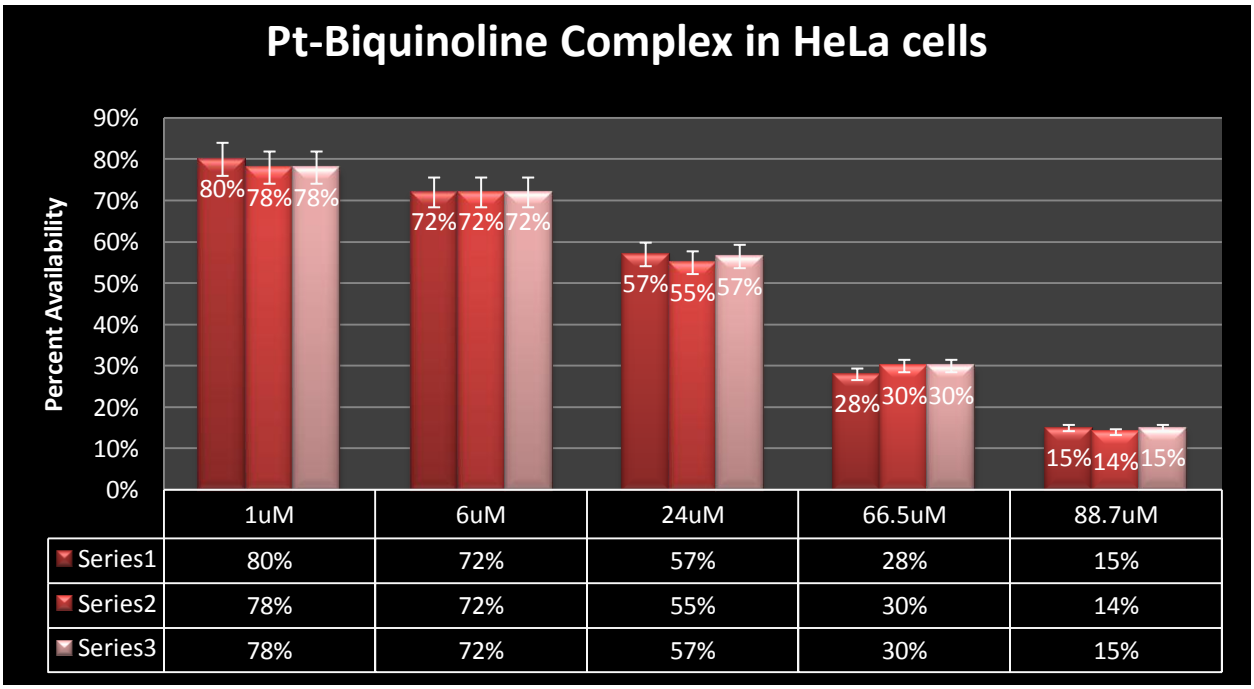


Figure 5.4 Percent availability for HeLa cells treated with platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate.

www.manaraa.com

The infrared spectrum (Figure 5.5) displays selected vibrational frequencies at the 550 cm^{-1}, 355 cm^{-1} which were assigned to the *v*(Pt-Cl) and *v*(Pt-N) respectively of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate. These values are consistent according to the literature.

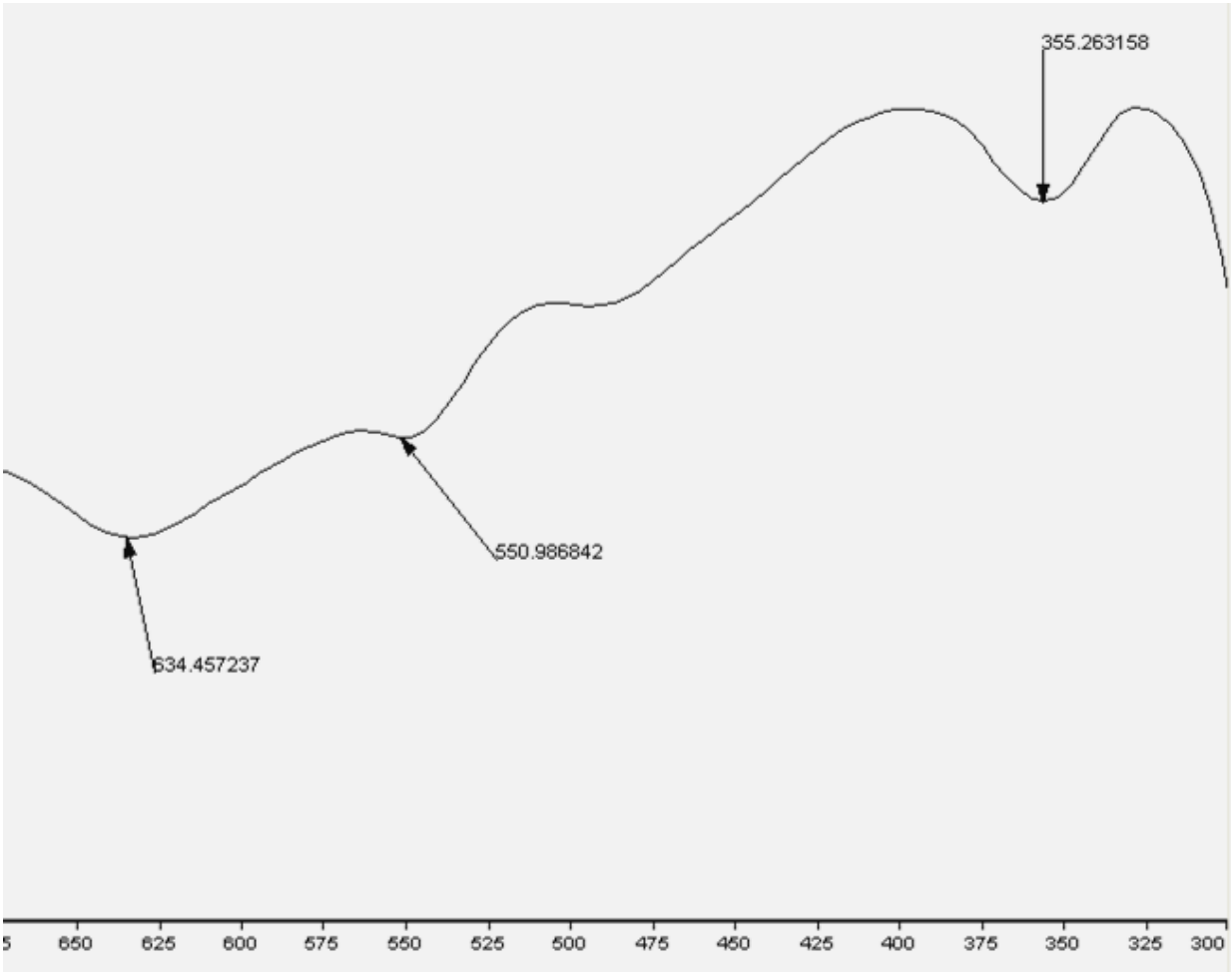


Figure 5.5 Infrared irrational frequencies for platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate.

www.manaraa.com

The mass spectrum (Figure 5.6) of platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate (M.W=1171.11) indicates the signals of platinum isotopes where the presence of the platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4'dicarboxylate can be observed and determined.

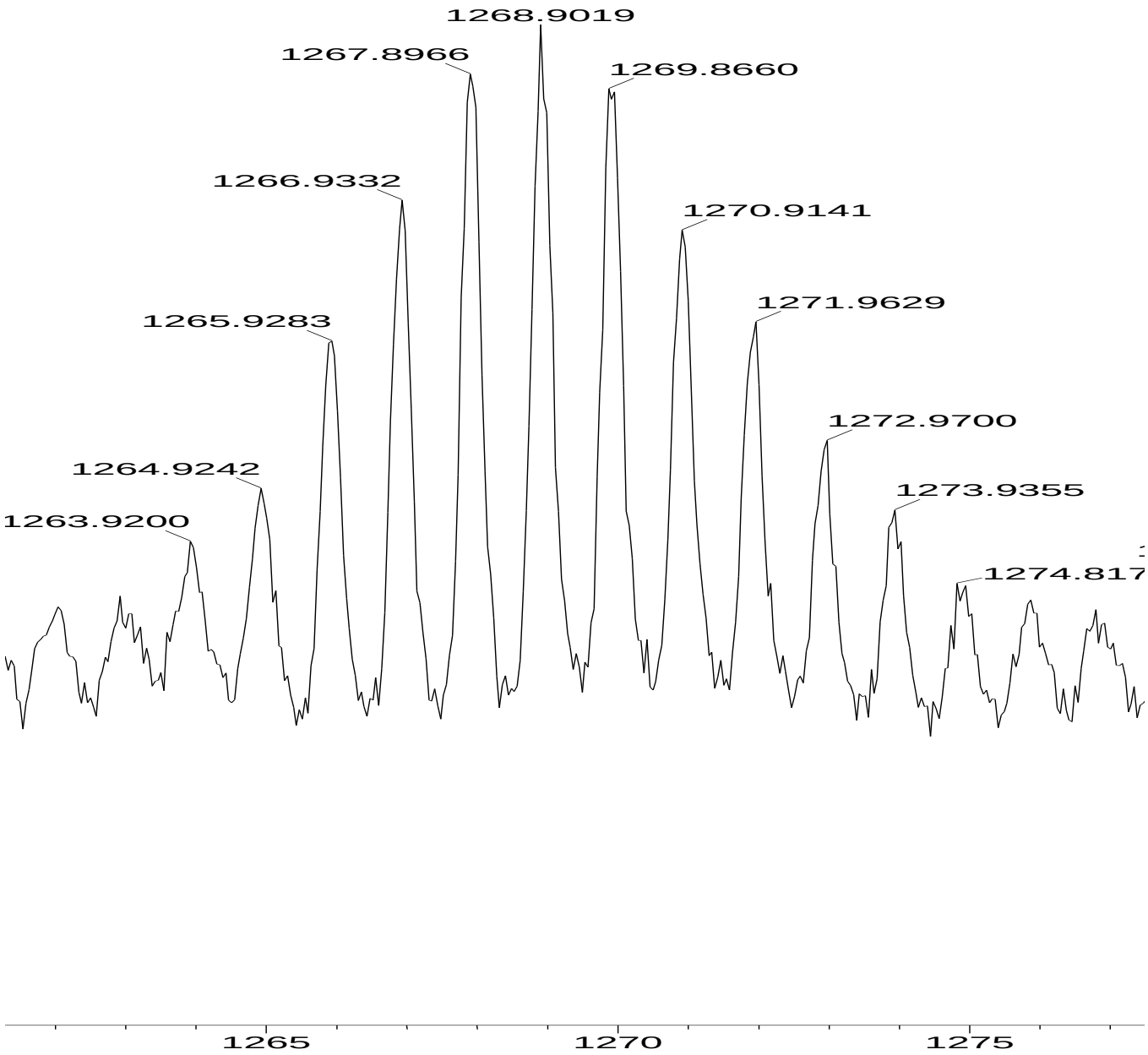


Figure 5.6 Mass Spectrum for bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate (M.W.= 1171.11)

www.manaraa.com

Conclusion

In our research we focused on the design and synthesis of amphiphilic molecules having a lipid hydrophobic chain containing ester functional groups and a hydrophilic head which has a platinum coordinated bond with aromatic N-containing heterocyclic compounds such as bipyridine and biquinoline to form cisplatin analogues. Amphiphilic cisplatin analogues emulsify to make micelles that project the Pt-Cl groups on the surface of the micelle. These platinum micelles may be used as alkylating-like agents, intercalating agents and as drug delivery systems that encapsulate anticancer drugs. The intracellular activation of esterases in cancerous cells will cleave the ester group found in the lipid chain of the micelles. The micelles of platinum biquinoline and platinum bipyridine will disassemble by the action of the esterases on the ester functional group releasing the anticancer drugs, the Pt-Cl part of the molecule will bind to the DNA bases forming DNA adducts and the heterocyclic rings of the molecule will intercalate in the DNA disrupting the DNA structure. The Pt-micelles may have applications on cancers of the brain, breast, lung, testicular, ovarian, cervical, colon and bladder which requires hydrophobic molecules to permeate the surrounding tissue of such organs.

.

www.manaraa.com

Appendix

HNMR and CNMR Spectra supporting data for Chapter 4.

2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate. ^{1}HNMR: 4.05, 3.88, 3.50, 3.49, 3.37, 1.98, 1.2952, 1.2837, 0.99. ^{13}CNMR:172.7204, 71.4193, 70.72, 70.39, 68.74, 63.05, 61.42, 33.63, 31.73, 29.33, 29.21, 22.47, 13.78 (Figure A.1 and A.2).

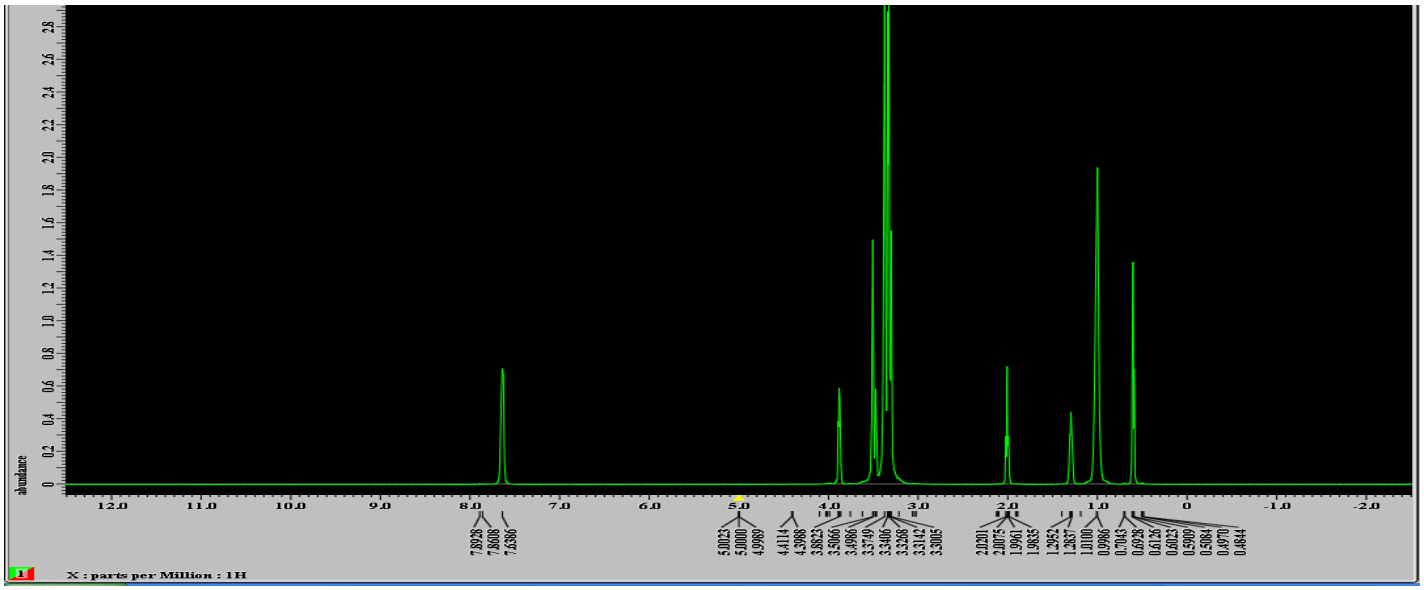


Figure A.1. ^{1}HNMR of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate.

www.manaraa.com

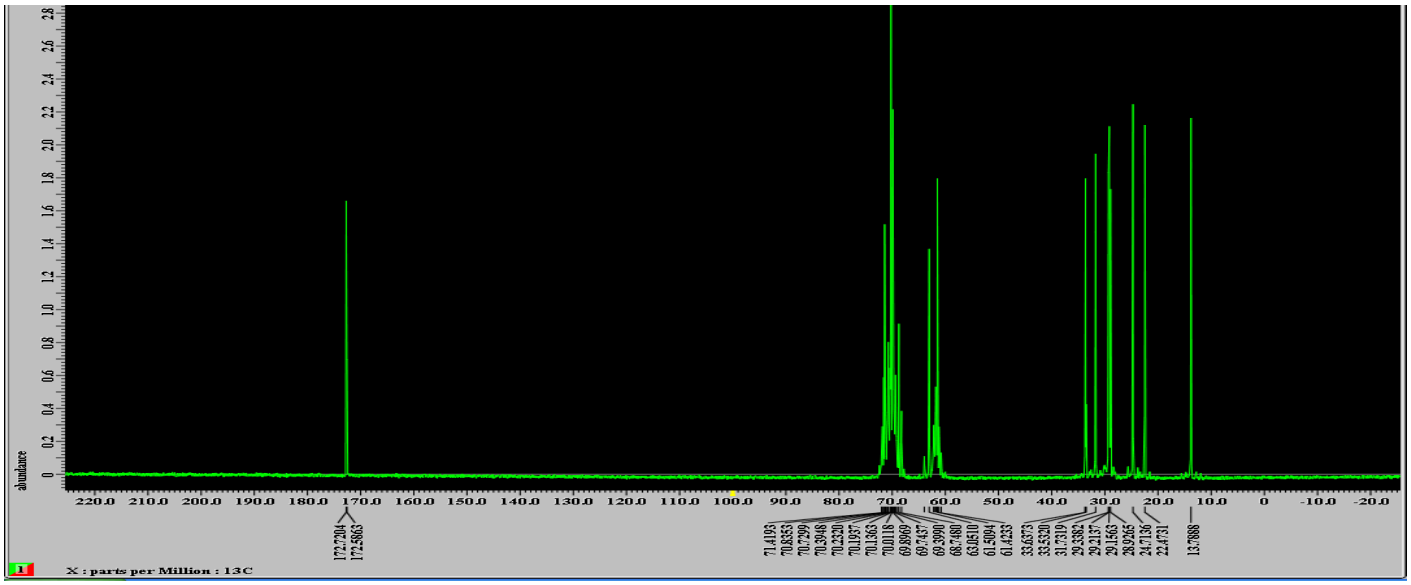


Figure A.2. ^{13}CNMR of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate.

Bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-bipyridine-5,5'-dicarboxylate. ^{1}HNMR: 9.1789, 8.4838, 8.4700, 4.44, 4.1331, 3.8846, 3.6086, 3.5524, 2.2228, 1.4979, 1.1532, 0.7696. ^{13}CNMR: 174.0704, 165.0988, 158.1571, 150.3250, 137.9065, 126.3210, 122.0315,70.4014, 70.2608, 69.0161, 64.1712, 63.2999, 34.1447, 31.8085, 30.2478, 29.3765, 29.2137, 24.8476, 22.6072, 14.2292 (Figures A3 and A4).

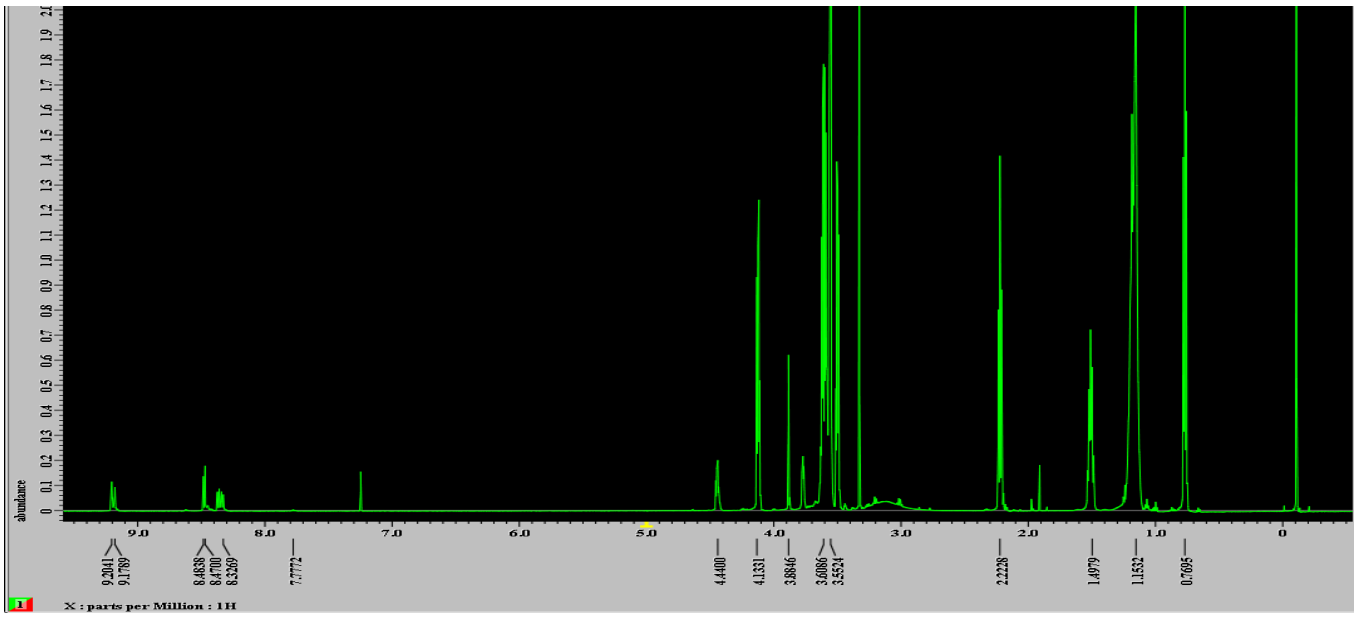


Figure A.3. ^{1}HNMR of Bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-bipyridine-5,5' dicarboxylate.

www.manaraa.com

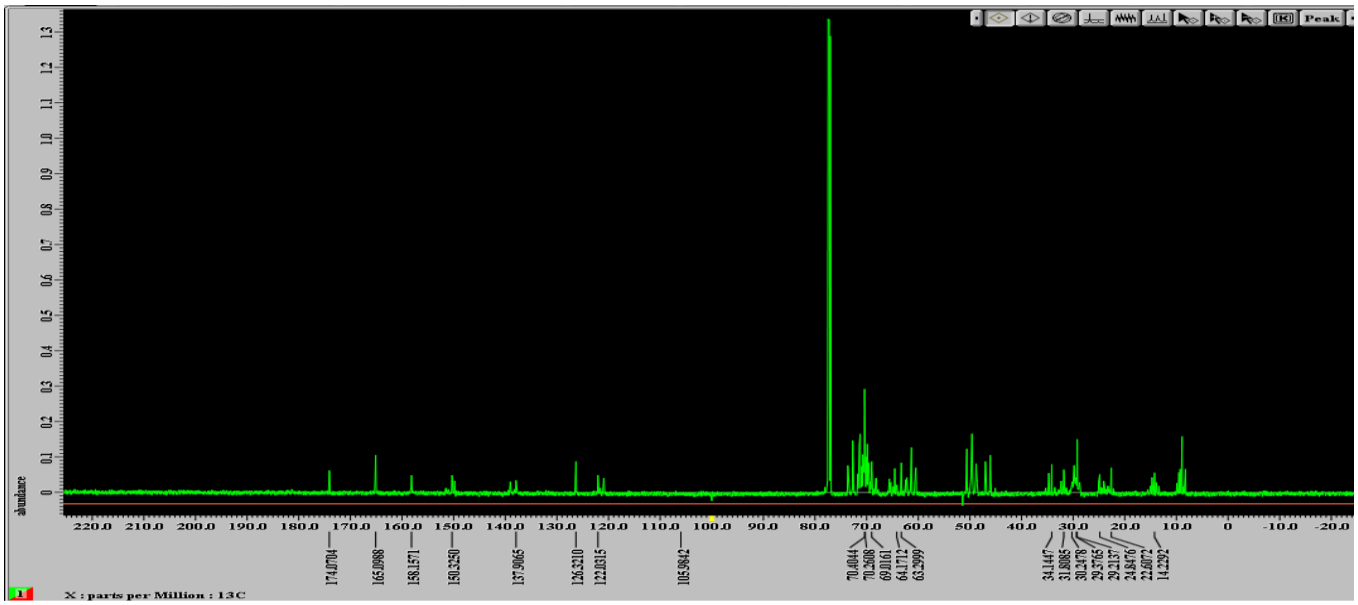


Figure A.4. [1]CNMR of Bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-bipyridine-5,5' dicarboxylate.

Cis(benzonitrile)dichloropatinum(II). [1]HNMR (600 MHz, $CHCl_3$): δ 7.7980, 7.7852, 7.4199. [13]CNMR (600 MHz, $CHCl_3$) δ 135.2926, 133.8564, 133.5979, 116.8707, 109.5460 (Figures A.5 and A.6).

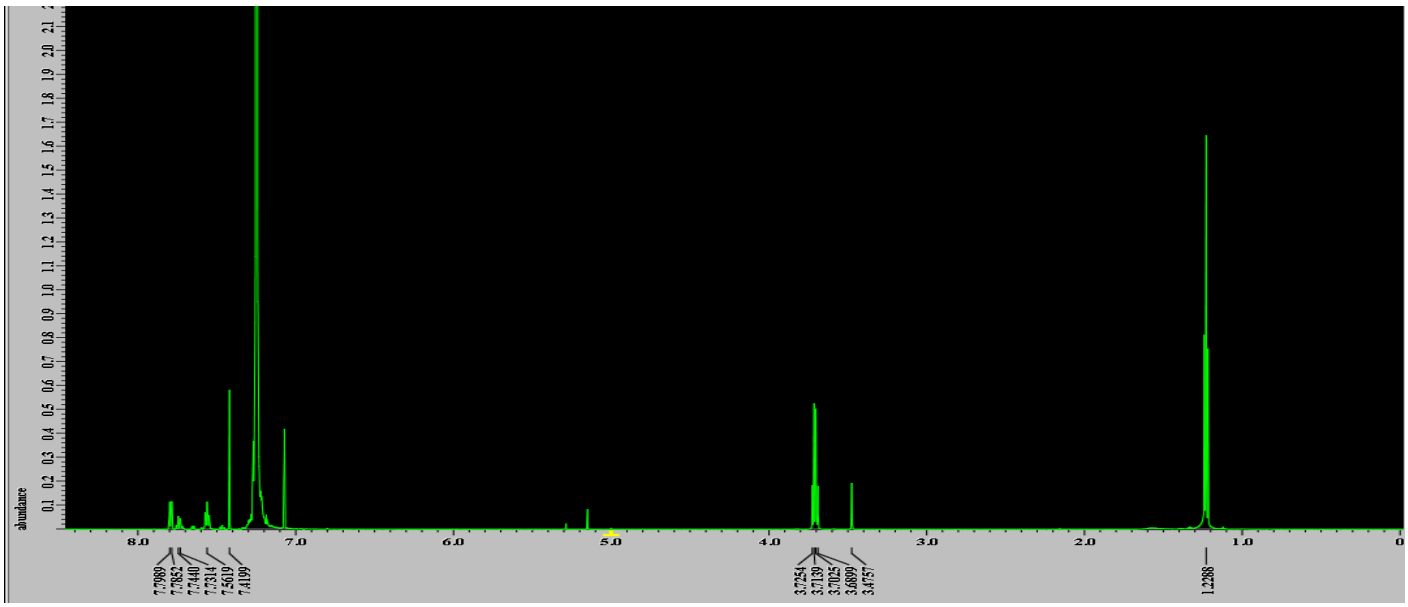


Figure A.5. [1]HNMR cis(benzonitrile)dichloropatinum(II).

www.manaraa.com

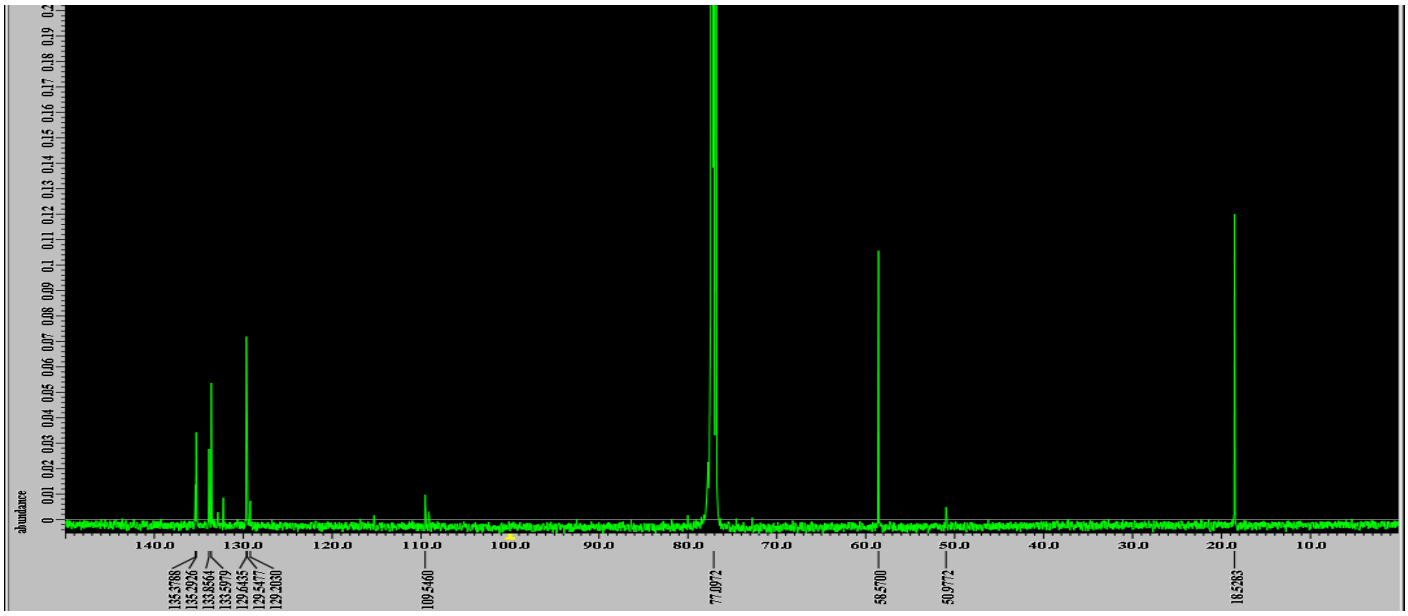


Figure A.6. ^{1}CNMR cis(benzonitrile)dichloropatinum(II).

Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate. ^{1}HNMR (600 MHz, $CHCl_3$): δ 10.2543, 9.4939, 8.8422, 4.5728, 4.1938, 3.8857, 3.6051, 3.4723, 3.2993, 2.3385, 1.5586, 1.2391, 0.8474. ^{13}CNMR (600 MHz, $CHCl_3$): δ 173.8885, 165.1371, 159.6888, 138.2416, 127.5562, 121.1698, 72.1, 70.8, 70.5, 66.8043, 65.5027, 64.9659, 34.00, 31.0042, 30.9659, 29.7404, 22.70, 15.26 (Figures A.7 and A.8).

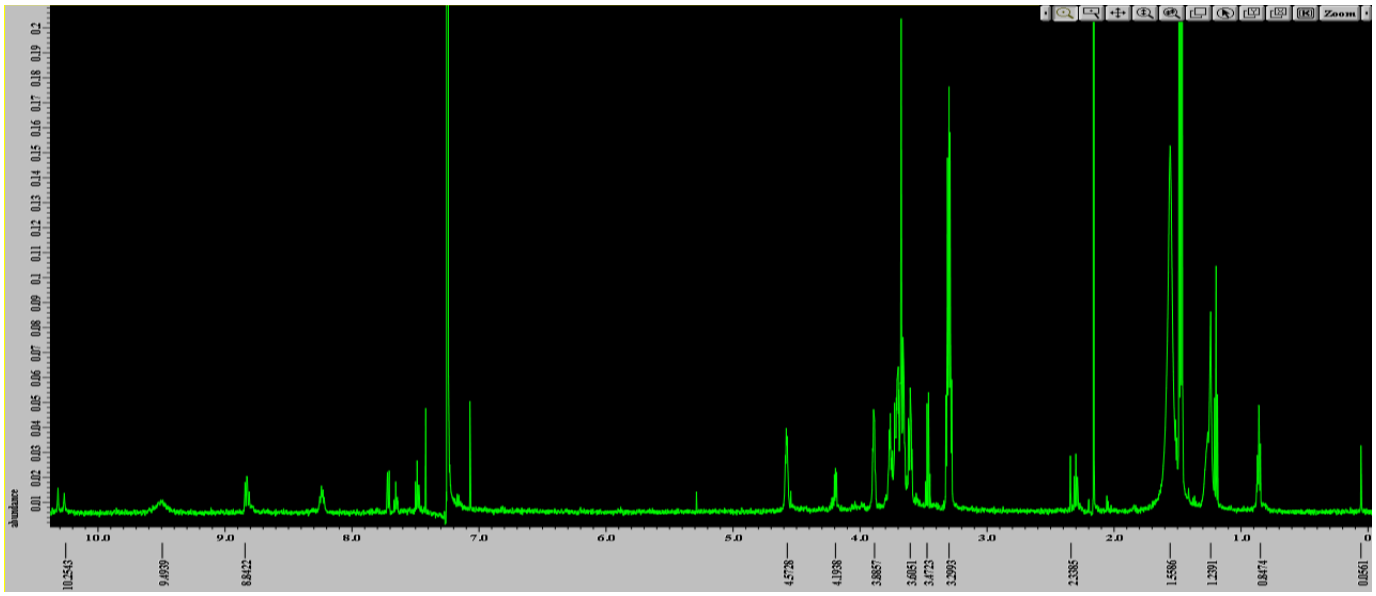


Figure A.7. ^{1}HNMR of Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate.

www.manaraa.com

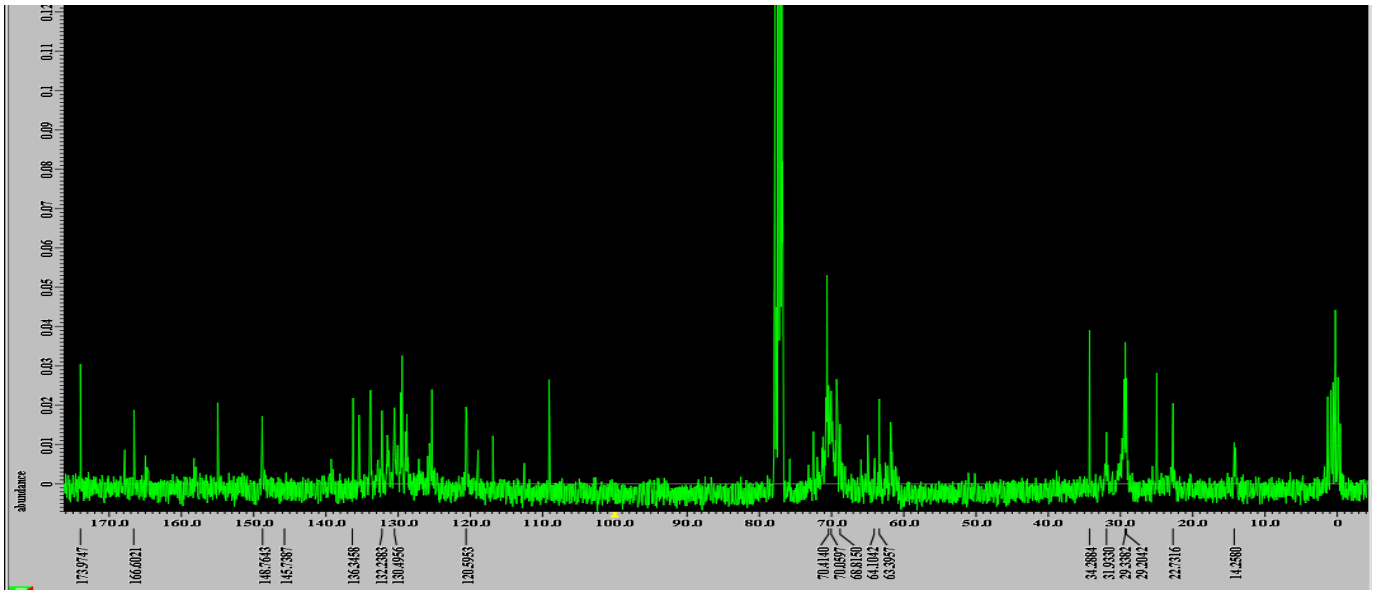


Figure A.8. [1]CNMR of Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-bipyridine-5,5'-dicarboxylate.

HNMR and CNMR Spectra supporting data for Chapter 5.

2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate. [1]HNMR: 4.05, 3.88, 3.50, 3.49, 3.37, 1.98, 1.2952, 1.2837, 0.99. [13]CNMR:172.7204, 71.4193, 70.72, 70.39, 68.74, 63.05, 61.42, 33.63, 31.73, 29.33, 29.21, 22.47, 13.78 (Figure A9 and A10).

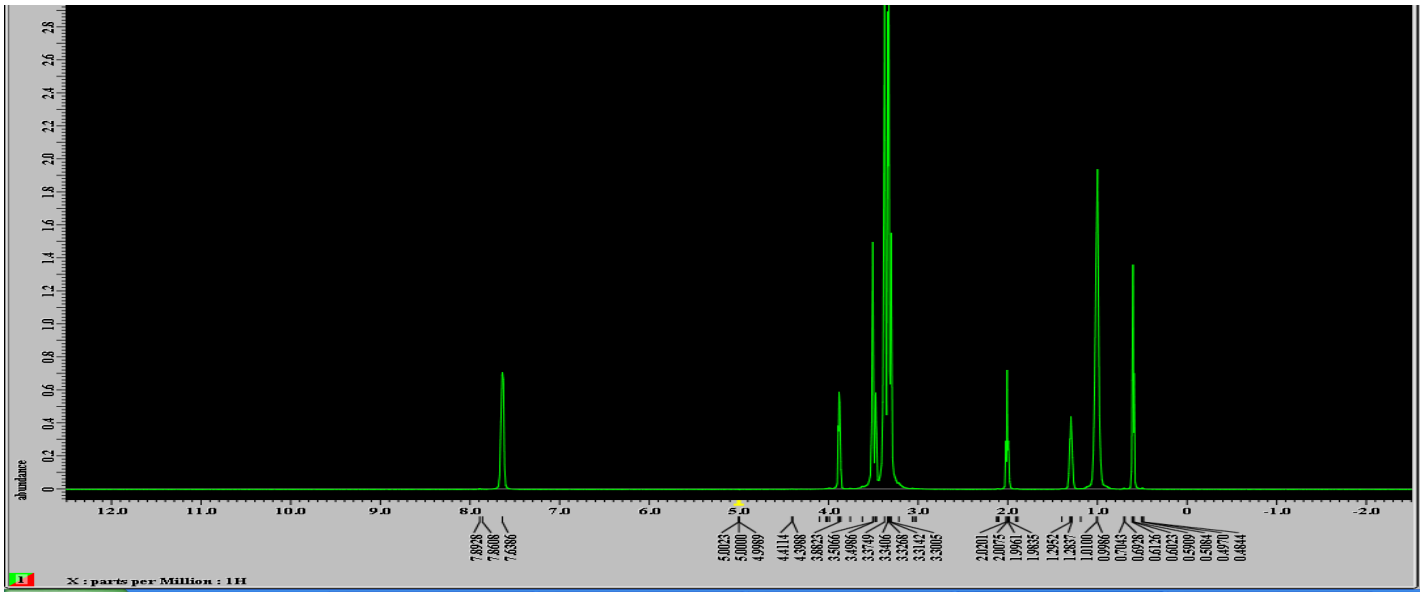


Figure A.9. [1]HNMR of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate.

www.manaraa.com

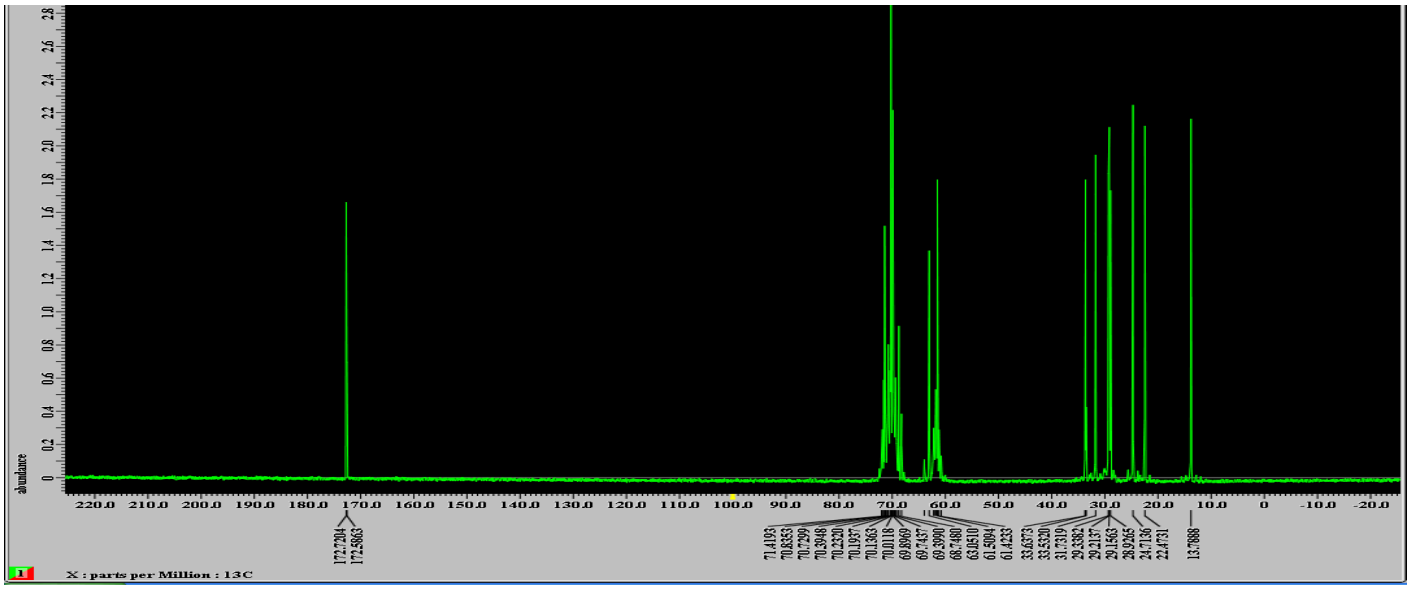


Figure A.10. ^{13}CNMR of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl-decanoate.

Bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate. ^{1}HNMR (600 MHz, $CHCl_3$): δ 8.9281, 8.4219, 8.2364, 7.9123, 4.3601, 4.2722, 3.89, 3.64, 3.50, 2.0064, 1.8312, 1.28, 0.9631. ^{13}CNMR(600 MHz, $CHCl_3$: δ 173.7161, 166.3531, 155.5720, 148.5728, 136.0203, 130.5052, 130.1509, 129.3754, 128.4275, 127.9009, 127.5466, 126.8285, 120.2698, 70.3948, 70.2703, 70.2225, 68.0394, 64.2287, 63.2042, 32.0479, 31.7032, 29.5393, 29.2616, 22.5114, 14.0760 (Figure A11 and A12).

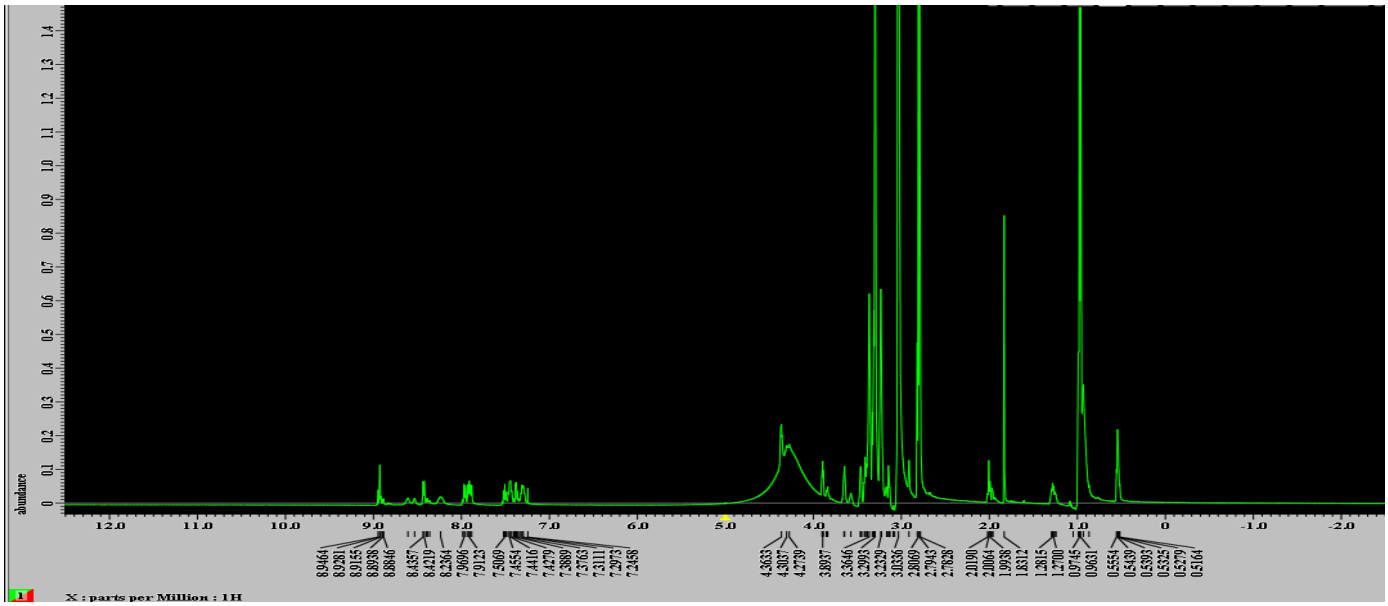


Figure A.11. ^{1}HNMR of bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate.

www.manaraa.com

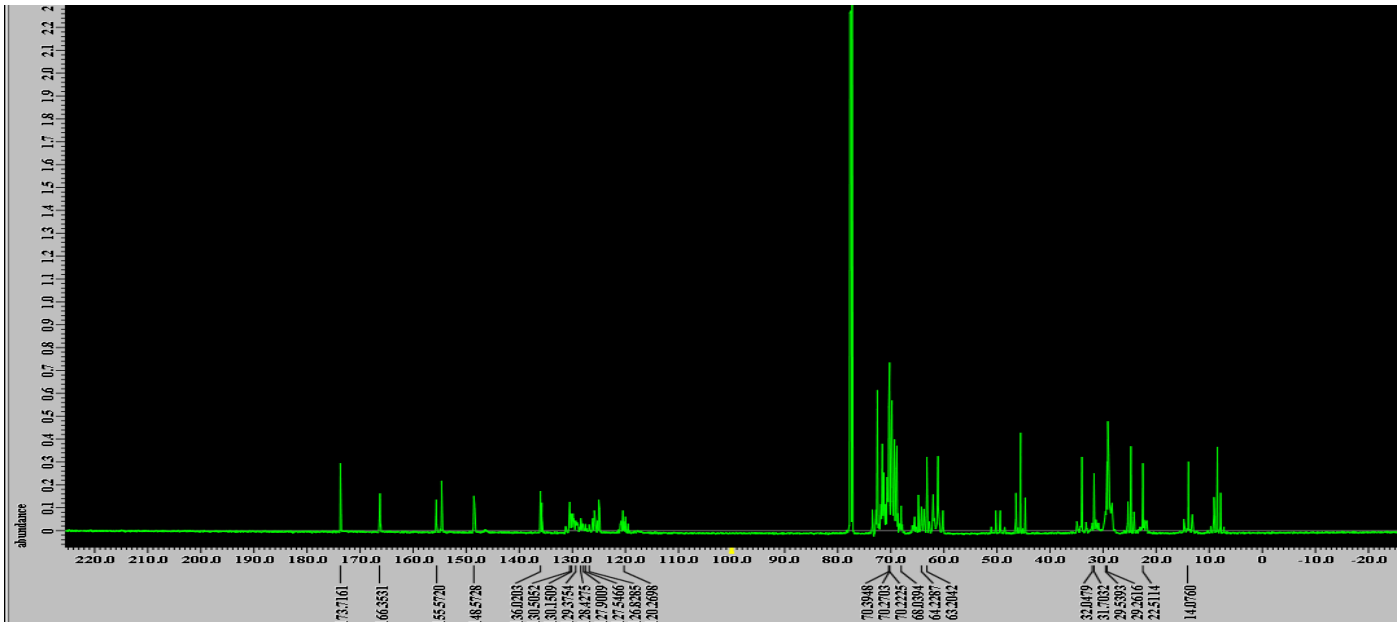


Figure A.12. ^{13}CNMR of bis(13-oxo-3,6,9,12-tetraoxadocosyl)2,2'-biquinoline-4,4'-dicarboxylate.

Cis(benzonitrile)dichloropatinum(II). ^{1}HNMR (600 MHz, $CHCl_3$): δ 7.7980, 7.7852, 7.4199. ^{13}CNMR (600 MHz, $CHCl_3$) δ 135.2926, 133.8564, 133.5979, 116.8707, 109.5460 (Figure A.13 and A.14).

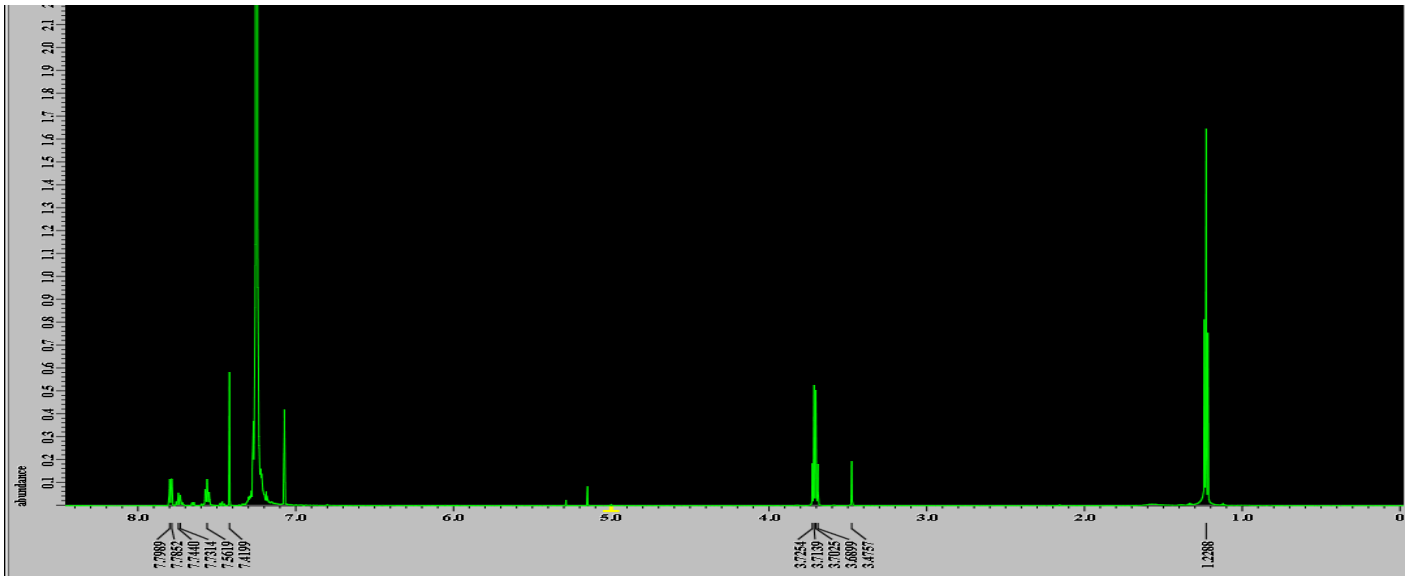


Figure A.13. ^{1}HNMR cis(benzonitrile)dichloropatinum(II).

www.manaraa.com

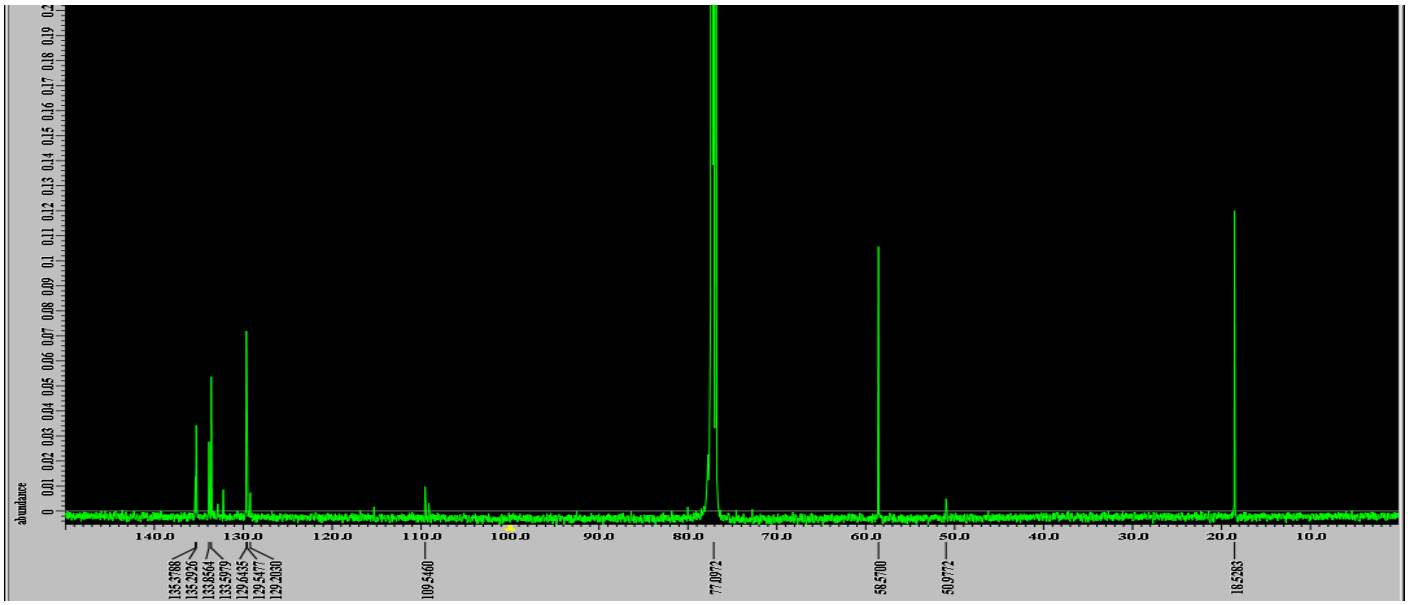
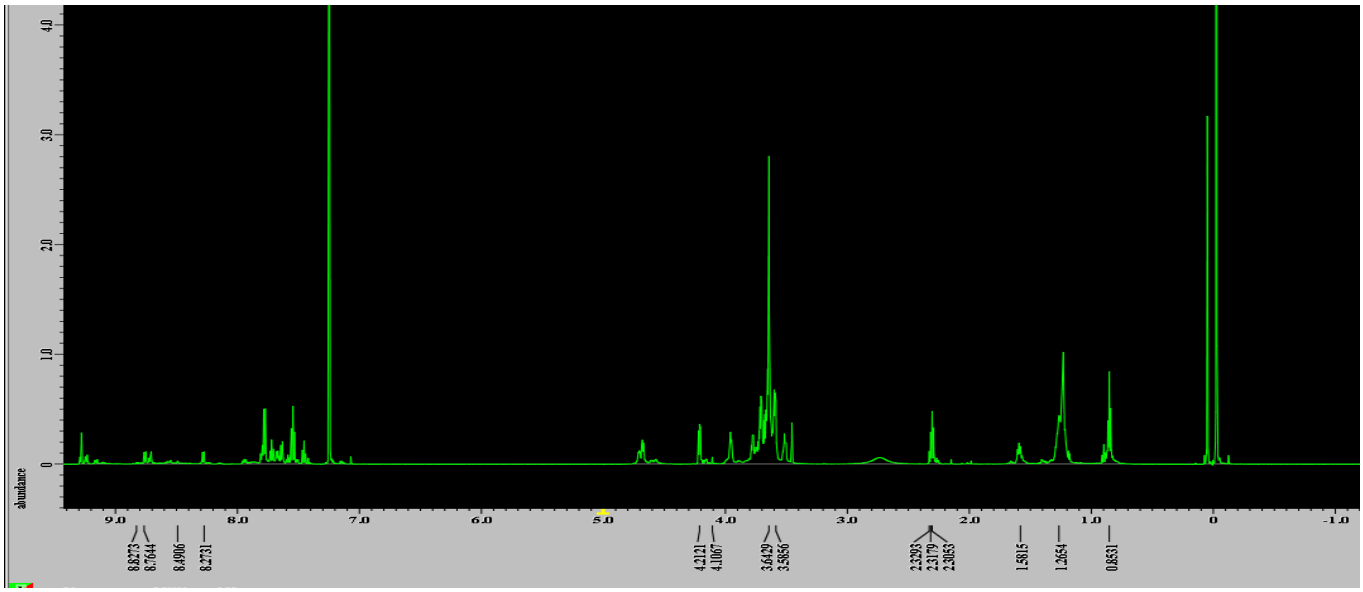


Figure A.14. ^{1}CNMR cis(benzonitrile)dichloropatinum(II).

Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate. ^{1}HNMR (600 MHz, $CHCl_3$): δ 8.8273, 8.7644, 8.4906, 8.2731, 4.2121, 4.1067, 3.6429, 3.5856, 2.3293, 2.3179, 2.3053, 1.5815, 1.2654, 0.8531. ^{13}CNMR (600 MHz, $CHCl_3$) δ 173.9747, 166.6021, 148.7643, 148.7387, 136.3458, 132.2383, 130.4956, 120.5953, 70.4140, 70.0597, 68.8150, 64.1042, 63.3957, 34.2884, 31.9330, 29.3382, 29.2042, 22.7316, 14.2580 (Figure A15 and A16).



www.manaraa.com

Figure A.15. [1]HNMR of Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate.

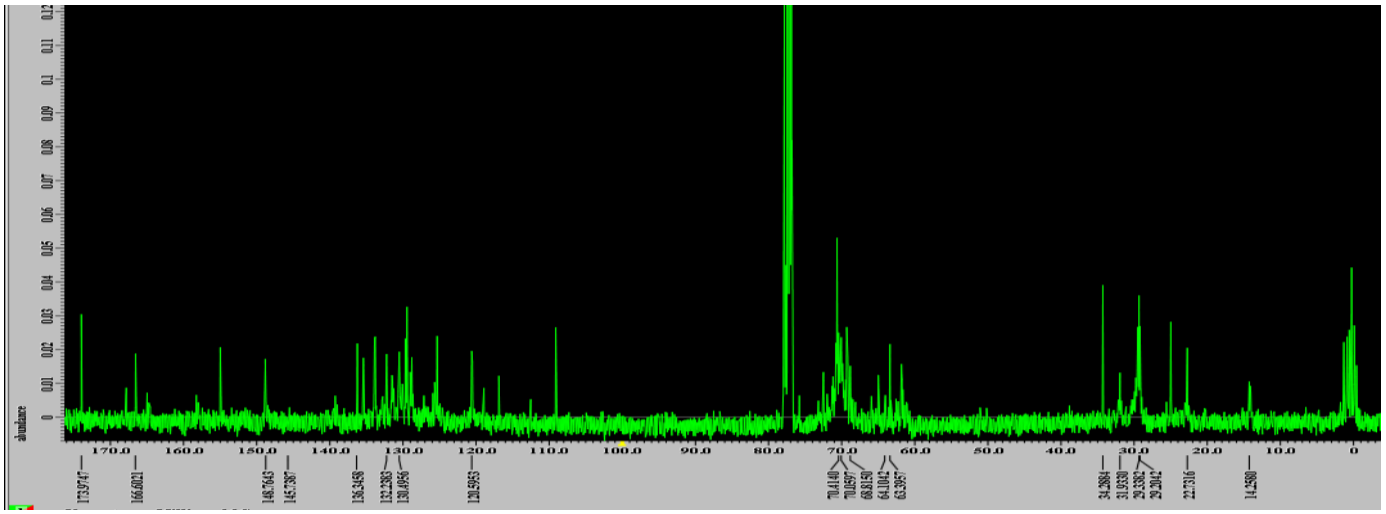


Figure A.16. [1]CNMR of Platinum bis(13-oxo-3,6,9,12-tetraoxadocosyl) 2,2'-biquinoline-4,4' dicarboxylate.

References

1. Zhang, C. X., Lippard, S. J. New metal complexes as potential therapeutics. Current Opinion in Chemical Biology 2003, 7:481–489.

2. Briere, K. M., Goel, R., Shirazi, F. H., Stewart, D. J., Smith, I. C. P. The integrity of cisplatin in aqueous and plasma ultrafiltrate mediastudied by ^{195}Pt and ^{15}N nuclear magnetic resonance. Cancer Chemother Pharmacol (1996) 37: 518-524

3. Brabec, V., Novakova, O. DNA binding mode of ruthenium complexes and relationship to tumor cell toxicity. Drug Resistance Updates 9 (2006) 111–122

4. Fojo, T., Farrell, N., Ortuzar, W., Tanimura, H., Weinstein, J., Myers, T. G. Identification of non-cross-resistant platinum compounds with novel cytotoxicity profiles using the NCI

www.manaraa.com

anticancer drug screen and clustered image map visualizations. Critical Reviews in Oncology/Hematology 53 (2005) 25-34.

5. Elwell, K. E., Hall, C., Tharkar, S., Giraud, Y., Bennet, B., Bae, C., Carper, S. W. A fluorine containing bipyridine cisplatin analog is more effective than cisplatin at inducing apoptosis in cancer cell lines. Bioorganic and Medicinal Chemistry 14 (2006) 8692-8700.

6. Corbi, P. P., Castellano E. E., Cagnin, F., Massabni, A. C. Crystal structure and infrared analysis of new trinuclear platinum (II) complex with L-cysteine. Journal of Chemical Crystallography, Vol. 37, No. 2, February 2007 (2007).

7. Sadiq, M. F., Zaghal, M. H. Mutagenic Activity of Rhodium(III) complexes with

2, 2'-Biquinoline and 2-(2'-pyridyl) Quinoline. Polyhedron Vol 16. No. 9.pp. 1483-1486,

1997.

8. Bianchi, N., Ongaro, F., Chiarabelli, C., Guadalandi, L., Mischiati, C., Bergamini, P., Gambari, R. Induction of Erythoid Differentiation of Human K562 Cells by Cisplatin Analogs. Biochemical Pharmacology, Vol 60. Pp. 31-4, 2000.

9. Roy, S., Westmaas, J. A., Buda, F., Reedijk, J. Platinum(II) compounds with chelating ligands based on pyridine and pyrimidine: Synthesis, characterizations, DFT calculations, cytotoxic assays and binding to a DNA model base. Journals of inorganic Biochemistry 103 (2009) 1278-1287.

10. Margaret, W., Solipuram, R., Gambrell, R. P., Aggarwal, S., Hammer, R. P. Peptide Targeting of Platinum Anti-Cancer Drugs. Bioconjugate Chem. 2009, 20, 1869-1878.

13. Bugarcic, Z. D., Petrovica, B., Bugarcic, Z. M., Jankovic, S. V, Lukic, G., Novakovic, M., Kostovic, V., Popovic, S., Djurdjevic, P., Baskic, D., Arsenijevic, N. Effects of cisplatin and

www.manaraa.com

other Pt(II) complexes on spontaneous motility of isolated human oviduct. Toxicology in Vitro 22 (2008) 1878–1882.

12. Wysokinski, R., Hernik, K., Szostak, R., Michalska, D. Electronic structure and vibrational spectra of cis-diammine-(orotato)platinum(II), a potential cisplatin analogue: DFT and experimental study. Chemical Physics 333 (2007) 37–48.

13.Rubino, S., Portanova, P., Girasolo, A., G. Calvaruso, S. Orecchio, G.C. Stocco. Synthetic, structural and biochemical studies of polynuclear platinum(II) complexes with heterocyclic ligands. European Journal of Medicinal Chemistry 44 (2009) 1041-1048.

14. Shamsuddin, S., Takahashi, I., Siddik, Z. H., Khokhar, A. R. Synthesis, Characterization, and Antitumor Activity of a Series of Novel Cisplatin Analogs with cis-1,4-Diaminocyclohexane as Nonleaving Amine Group. Journal of Inorganic Biochemistry, 61, 291-301 (1996).

15. Jain, A., Wang, J., Mashack, E. R, Winkel, B. S., Brewer, K. J. Multifunctional DNA Interactions of Ru-Pt Mixed Metal Supramolecular Complexes with Substituted Terpyridine Ligands. Inorg. Chem. 2009, 48, 9077–9084 9077.

16. Desoize, B., Madoulet, C. Particular aspects of platinum compounds used at present in cancer treatment. Critical Reviews in Oncology/Hematology 42 (2002) 317–325.

17. Zhao, G., Lin, H. Metal Complexes with Aromatic N-Conjugating Ligands as Potential Agents in Cancer Treatment. Curr. Med. Chem.-Anti-Cancer Agents, 2005, 5,137-147.

18. Mark B. Lyles, Ivan L. Cameron. Interactions of the DNA intercalator acridine orange, with itself, with caffeine, and with double stranded DNA. Biophysical Chemistry 96 (2002) 53–76

19 Foye. O. W. Cancer Chemotherapeutic Agents. ACS Professional Reference Book. American Chemical Society 1995. Platinum Complexes Page 121.

20.Durrant, P. J., Durrant, B. Introduction to Advanced Inorganic Chemistry. John Wiley and Sons Inc. New York. N.Y. 1962. Chapter 24 Page 1055.

www.manaraa.com

21. Durrant, P. J., Durrant, B. Introduction to Advanced Inorganic Chemistry. John Wiley and Sons Inc. New York. N.Y. 1962. Chapter 24 Page 1055

22. Keller, R. N. Potassium tetrachloroplatinate (II). Inorganic Syntheses, Volume II Edited by W. Conard Fernelius 1946 by McGraw-Hill Book Company, Inc.

23. Alderden, R. A., Hall., M. D., Hambley, T. W. The Discovery and Development of Cisplatin. Journal of Chemical Education. Vol. 83 No. 5 May 2006.

24. J. J. Roberts and M. F. Pera, JR. DNA as a Target for Anticancer Coordination Compounds. American Chemical Society 1983.

25. Boerner, L., Zaleski, J. M. Metal complex–DNA interactions: from transcription inhibition to photoactivated cleavage. Current Opinion in Chemical Biology 2005, 9:135–144.

26. Lu, Y., Cederbaum, A. The mode of cisplatin-induced cell death in CYP2E1-overexpressing HepG2 cells: Modulation by ERK, ROS, glutathione, and thioredoxin. Free Radical Biology & Medicine 43 (2007) 1061–1075.

27. Zamble, D. B., Lippard, S. J. Cisplatin and DNA repair in cancer chemotherapy.

28. Reedijk, J., Lohman P.H.M. Cisplatin: synthesis, antitumour activity and mechanism of action. Pharmaceutisch Weekblad Scientific Edition Vol. 7 - I985.

29. Crul, M., van Waardenburg, R. C. A. M., Beijnen, J. H., Schellens, J. H. M. DNA-based drug interactions of cisplatin. Cancer treatment reviews 2002; 28: 291-303.

30. Stewart, D. J. Mechanisms of resistance to cisplatin and carboplatin. Critical Reviews in Oncology/Hematology 63 (2007) 12–31.

31. Kartalou, M., Essigmann, J. M. Mechanisms of resistance to cisplatin. Mutation Research 478 (2001) 23–43.

www.manaraa.com

32. Badreldin H. A., Mansour S., Moundhri, A. Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: A review of some recent research. Food and Chemical Toxicology 44 (2006) 1173–1183.

33. Aris, S. M., Farrell, N. P. Towards Antitumor Active trans-Platinum Compounds. Eur. J. Inorg. Chem. 2009, 1293–1302.

34. Pizarro, A. M., Sadler, P. J. Unusual DNA binding modes for metal anticancer complexes. Biochimie 91 (2009) 1198–1211.

35. Hall, M. D, Mellor, H. R., Callaghan, R., Hambley, T.W. Basis for Design and Development of Platinum(IV) Anticancer Complexes. Journal of Medicinal Chem, Volume 50, Number 15.

36. Yonit Kasherman, Stefan Sturup, and Dan Gibson. Is Glutathione the Major Cellular Target of Cisplatin? A Study of the Interactions of Cisplatin with Cancer Cell Extracts. Journal of Medicinal Chemistry 2009, 52, 4319–4328.

37. Mario Casolaro, Renzo Cini, Barbara Del Bello, Marco Ferrali, and Emilia Maellaro. Cisplatin/Hydrogel Complex In Cancer Therapy. Biomacromolecules 2009, 10, 944–949.

40. Mandic, A., Hansson, J., Linder, S., Shoshan, M. C. Cisplatin Induces Endoplasmic Reticulum Stress and Nucleus-independent Apoptotic Signaling. The journal of Biological Chemistry, Vol. 278, No. 11, Issue of March 14, pp. 9100–9106, 2003.

39. Kartalou, M., Essigmann, J. M. Recognition of cisplatin adducts by cellular proteins. Mutation Research 478 (2001) 1–21.

40. Masters, J. R., Köberle, B. Curing metastatic cancer: Lessons from testicular germ cell Tumors. Nature Reviews, Cancer Volume 3, page 517, July 2003.

www.manaraa.com

41. Siddik, Z. H., Hagopian, G. S., Thai, G., Tomisaki, S., Toyomasu, T., Khokhar, A. R. Role of p53 in the ability of 1, 2-diaminocyclohexane-diacetato-dichloro-Pt(IV) to circumvent cisplatin resistance. Journal of Inorganic Biochemistry 77 *(*1999*)* 65–70.

42. Perez R. P. Cellular and Molecular Determinants of Cisplatin Resistance. European Journal of Cancer, Vol. 34, No. 10, pp. 1535-1542, 1998.

43. Kartalou, M., Essigmann, J. M. Mechanisms of resistance to cisplatin. Mutation Research 478 (2001) 23–43.

44. Kweekela, D. M., Gelderblomb, H., Guchelaara, H. J. Pharmacology of oxaliplatin and the use of pharmacogenomics to individualize therapy. Cancer Treatment Reviews (2005) 31, 90–105.

45. Mastrocola, A. S., Heinen, C. D. Nuclear reorganization of DNA mismatch repair proteins in response to DNA damage. DNA Repair 9 (2010) 120–133.

46. Stojic, L., Brun, R., Jiricny, J. Mismatch repair and DNA damage signaling. DNA Repair 3 (2004) 1091–1101.

47. Borgdorff, V., Pauw, B., Hees-Stuivenberg, S. V., de Wind, N. DNA mismatch repair mediates protection from mutagenesis induced by short-wave ultraviolet light. DNA repair 5 (2006) 1364–1372.

48. Camenisch, U., Dip, R., Vitanescu, M., Naegeli, H. Xeroderma pigmentosum complementation group A protein is driven to nucleotide excision repair sites by the electrostatic potential of distorted DNA. DNA repair 6 (2007) 1819–1828.

49.Wilson. D. M., Bohr, V. A. The mechanics of base excision repair, and its relationship to aging and disease. DNA repair 6 (2007) 544–559.

www.manaraa.com

50. Hitomia, K., Iwaia, S., Tainer, J. A. The intricate structural chemistry of base excision repair machinery: Implications for DNA damage recognition, removal, and repair. DNA repair 6 (2007) 410–428.

51. Jae, Jong-Ho, Ick Chan, Kwanmeyung, Kyeongsoon, Seulki (September 29, 2007).

52. Casolaro, M., Cini, R., Del Bello, B., Ferrali, M., Maellaro, E. Cisplatin/Hydrogel Complex In Cancer Therapy. Biomacromolecules 2009, 10, 944–949.

53. Gaspar, R., Duncan, R. Polymeric carriers: Preclinical safety and the regulatory implications for design and development of polymer therapeutics. Advanced Drug Delivery Reviews 61 (2009) 1220–1231.

54. Parka, J. H, Leeb, S., Kimb, J. H., Park, K., Kim, K., Kwon, I. C. Polymeric nanomedicine for cancer therapy. Prog. Polym. Sci. 33 (2008) 113–137.

55. Vladimir, P. T. Targeted Pharmaceutical Nanocarriers for Cancer Therapy and Imaging. The AAPS Journal 2007; 9 (2) Article 15.

56. Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R., Langer, R., Langer, R. Nanocarriers as an emerging platform for cancer therapy. Nature nanotechnology, Vol 2, Deceber 2007.

57. Maeda, H., Bharate, G. Y., Daruwalla, J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. European Journal of Pharmaceutics and Biopharmaceutics 71 (2009) 409–419.

58. Maeda, H., Wua, J., Sawa, T., Matsumura, Y., Horic, K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. Journal of Controlled Release 65 (2000) 271–284.

www.manaraa.com

59. Maeda, H., Tomohiro, S., Konno, T. Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. Journal of Controlled Release 74 (2001) 47–61.

60. Hara, T., Iriyama, S., Makino, K., Terada, H., Ohya, M. Mathematical description of drug movement into tumor with EPR effect and estimation of its configuration for DDS. Colloids and Surfaces B: Biointerfaces 75 (2010) 42–46.

61. Li, C.,Wallace, S. Polymer-drug conjugates: Recent development in clinical oncology. Advanced Drug Delivery Reviews 60 (2008) 886–898.

62. Greco, F., Vicent, M. J. Combination therapy: Opportunities and challenges for polymer drug conjugates as anticancer nanomedicines. Advanced Drug Delivery Reviews 61 (2009) 1203 1213.

63. Yu, J. Y., Jun, Y. J., Jang, S. H., Lee, H. J., Sohn, Y. S. Nanoparticulate platinum(II) anticancer drug: Synthesis and characterization of amphiphilic cyclotriphosphazene–platinum(II) conjugates. Journal of Inorganic Biochemistry 101 (2007).1931–1936.

64. Duncan, R. Polymer conjugates as anticancer nanomedicines. Nature Reviews, Cancer Volume 6, September 2006.

65 Enzon Pharmaceuticals: www.enzon.com

66. Duncan, R. The Dawning Era of Polymertherapeutics. Nature Reviews and Drug Discovery,Volume 2, May 2003.

67.Vicent, M. J., Ringsdorf, H., Duncan, R. Polymer therapeutics: Clinical applications and challenges for development. Advanced Drug Delivery Reviews 61 (2009) 1117–1120.

68 Amgen: www.neulasta.com

www.manaraa.com

69. Wasungu, L., Hoekstra, D. Cationic lipids, lipoplexes and intracellular delivery of genes. Journal of Controlled Release 116 (2006) 255–264.

70. Eliyahua, H., Josepha, A, Schillemansa, J. P., Azzamb, T., Dombb, A. J., Barenholza, Y. Characterization and in vivo performance of dextran–spermine polyplexes and DOTAP/cholesterol lipoplexes administered locally and systemically. Biomaterials 28 (2007) 2339–2349.

71. Zhang, S., Yingmei, S., Wang, B., Weihong, Q., Liu, D., Li, Z. Cationic compounds used in lipoplexes and polyplexes for gene delivery. Journal of Controlled Release 100 (2004) 165–180.

72. Cruz-Campa, I., Arzola, A., Santiago, L., Parsons, J. G, Varela-Ramirez, A., Aguilera, R. J, Noveron, J. C. A novel class of metal-directed supramolecular DNA-delivery systems. The Royal Society of Chemistry. Chem. Commun, 2007, 2944–2946.

73. Parker, A. L., Newman, C., Briggs, S., Seymour, L., Sheridan, P. J. Lipoplex-mediated transfection and endocytosis. Expert reviews in molecular medicine. Vol. 5; 3 September 2003.

74. Pack, D. W., Hoffman, A. S., Pun, S., Stayton, P. S. The design and development of polymers for gene delivery. Nature Reviews. Drug Discovery Volume 4, July 2005.

75. Yiyun, C., Tongwen, X. The effect of dendrimers on the pharmacodynamic and pharmacokinetic behaviors of non-covalently or covalently attached drugs. European Journal of Medicinal Chemistry 43 (2008) 2291-2297.

76. Newkome, G. R., Shreiner, C. D. Poly(amidoamine), polypropylenimine, and related dendrimers and dendrons possessing different 1/2 branching motifs: An overview of the divergent procedures. Polymer 49 (2008) 1-173

77. Sonke, S. Dendrimers as versatile platform in drug delivery applications. European Journal of Pharmaceutics and Biopharmaceutics 71 (2009) 445–462.

www.manaraa.com

78. Wolinsky, J. B, Grinstaff, M. W. Therapeutic and diagnostic applications of dendrimers for cancer treatment. Advanced Drug Delivery Reviews 60 (2008) 1037–1055.

79. Beezer, A. E., King, A. S. H., Martin, I. K., Mitchel, J. C., Twyman, L. J., Wain, C. F. Dendrimers as potential drug carriers; encapsulation of acidic hydrophobes within water soluble PAMAM derivatives. Tetrahedron 59 (2003) 3873–3880.

80. Kathryn E. Parent. Simple Green Uses Green Chemistry to Make Cleaning Possible. Simple Chemistry, 2002.

81. Vladimir, P. T. Structure and design of polymeric surfactant-based drug delivery systems. Journal of Controlled Release 73 (2001) 137–172.

82. Nobuhiro, N., Kazunori, K. Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. Pharmacology & Therapeutics 112 (2006) 630–648.

83. Corbi, P. P., Castellano, E. E., Cagnin, F., Massabni, A. C. Crystal structure and infrared analysis of new trinuclear platinum(II) complex with L-cysteine. Journal of Chemical Crystallography, Vol. 37, No. 2, February 2007.

84. Wysokinski, R., Hernik, K., Szostak, R., Michalska, D. Electronic structure and vibrational spectra of cis-diammine- (orotato)platinum(II), a potential cisplatin analogue: DFT and experimental study. Chemical Physics 333 (2007) 37–48.

85. Xizhu, C., Mingjin, X., Weiping, L., Qingsong, Y., Ya, Y., Shuqian, H., Wengui, G., Yang, L. Synthesis and structure of platinum(II) complexes containing an asymmetric chelating diamine 2-morpholinoethylamine as the carrier. Inorganica Chimica Acta 360 (2007) 2851–2856.

www.manaraa.com

86. Yu, L., Yun, S., Yong, C., Xue-Qing, L., Fei, D., Rui-Qin . Biquinolino-Modified b-Cyclodextrin Dimers and Their Metal Complexes as Efficient Fluorescent Sensors for the Molecular Recognition of Steroids. Chem. Eur. J. 2004, 10, 3685-3696.

87. Mohammad, S. A, Abdul, R. K. Cis-1,4-Diaminocyclohexane–Pt(II) and –(IV) adducts with DNA bases and nucleosides. Journal of Inorganic Biochemistry 96 (2003) 452–456.

88. Pantoja, E., Alvarez-Valdes, A., Perez, J. M., Navarro-Ranninger, C., Reedijk, Jan. Synthesis and characterization of new cis-[PtCl2(isopropylamine)(amine)] compounds: cytotoxic activity and reactions with 5-GMP compared with their trans-platinum isomers. Inorganic Chimica Acta 339 (2002) 525- 531.

www.manaraa.com

Curriculum Vita

Gabriel Angel Gonzalez Esparza was born in Irapuato Guanajuato where he spent his childhood years. In his adolescence years he moved to El Paso Texas where he learned English as second language at Lydia Patterson Institute and also attended Austin High School where he graduated on the fall of 1999. On the spring of 2000 he entered El Paso Community College receiving on the fall 2002 his Associates of Arts degree and on the spring of 2003 he entered the University of Texas at El Paso receiving his Bachelor of Science in Chemistry in 2007. While pursuing his Bachelor of Science degree he worked in the copy mine center and print shop at UTEP as well as undergraduate research assistant for Dr Elizabeth Garner from 2005 to 2007. After his graduation with his Bachelor of Science in Chemistry he worked as a technician for the Texas A & M research station at El Paso Texas from August 2007 to December 2007. On the spring of 2008 he entered the graduate school program in chemistry with Professor Dr. Juan Carlos Noveron and worked in the chemistry department as research assistant as well as teacher assistant from fall 2008 to spring 2010.

www.manaraa.com