TRANSITION METAL COMPLEXES OF NUCLEOSIDES FOR
CANCER CHEMOTHERAPY

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TRANSITION METAL COMPLEXES OF NUCLEOSIDES FOR
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ABSTRACT

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Platinum(II) complexes of the type PtCl₂L₂, such as cisplatin---PtCl₂(NH₃)₂, have been widely studied and shown to have anti-tumor activity. On the other hand, ruthenium(II) complexes are known for their intense photophysical properties, biodiversity, and tumor targeting capabilities, which make them suitable as photosensitizers in photodynamic therapy. The efficacy of these complexes as anti-tumor agents is controlled by many variables such as cell permeability, availability of DNA repair processes, etc. A platinum(II)-DMSO complex and a ruthenium(II)-bypyridine complex have been incorporated into a thymidine based nucleoside through an N-3 pendant pyridyl nitrogen. Nucleosides are transported into the cell by particular trans-membrane proteins. This could increase the cellular uptake and reduce toxic side effects. Incubation of these two complexes with pUC18 circular plasmid DNA has been
performed and followed by gel electrophoresis. The results show the formation of linearized forms of DNA. The structures of all intermediates and final compounds were confirmed by 1D-NMR (\textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR), 2D-NMR (H-C correlation, HMQC and HMBC), TOF-MS and \textsuperscript{195}Pt-NMR methodologies.
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LIST OF SYMBOLS AND ABBREVIATIONS

δ: Chemical Shifts (ppm, downfield from TMS)

δΔ: Coordination Shift

ADDP: 1,1’-(Azodicarbonyl)-dipiperidine

bp: Base Pair

BPY: Bipyridine

$^{13}$C: Carbon-13

CBDCA: Cyclobutane Dicarboxylicacid

CDI: Carbonyl Diimidazole

CH$_2$Cl$_2$: Methylene chloride

CNT: Concentrative Nucleoside Transporters

COSY: Correlation Spectroscopy

DDP: Diammineplatinum

DEPT: Distortionless Enhancement by Polarization Transfer

DMF: Dimethylformamide

DMSO: Dimethyl Sulfoxide

DNA: Deoxyribonucleic acid

Dppz: Dipyrido[3,2-a:2’,3’-c]phenazine

ENT: Equilibrative Nucleoside Transporter
Eq: Equivalent
EthBr: Ethidium Bromide
EtOAc: Ethyl acetate
EtOH: Ethanol
h: Hour (s)
$^{1}$H: Proton
hCNT: Human Concentrative Nucleoside Transporters
HCOSY: Proton Correlation Spectroscopy
HETCOR: Heteronuclear Correlation Spectroscopy
HMBC: Heteronuclear Multiple Bond Correlation Spectroscopy
HPD: Hematoporphyrin Derivative
Hz: Hertz
$J$: Coupling Constant
$\mu$: Micro
MHz: Mega Hertz
MLCT: Metal to Ligand Charge Transfer
MS: Mass Spectrum
$N_{2}$: Nitrogen Gas
NMR: Nuclear Magnetic Resonance
Phen: Phenanthroline
PDT: Photodynamic Therapy
ppm: Parts per million
PPY: Ruthenium-Polypyridyl
Pt: Platinum

pUC 18: Plasmid University of California 18

Pyr: Pyridine

R_f: Retention Factor

ROS: Reactive Oxygen Species

r.t.: Room Temperature

Ru: Ruthenium

SOD: Superoxide Dismutase

TBA: Tetrabutylammonium cation

TBAF: Tetrabutylammonium Fluoride

TBDMSiCl: Tert-Butyldimethylsilyl Chloride

TBP: Tributyl Phosphate

THF: Tetrahydrofuran

TLC: Thin Layer Chromatography

TMG: 1,1,3,3-Tetramethylguanidine

TMS: Tetramethylsilane, also Trimethylsilyl

TOF-MS: Time-Of-Flight Mass Spectrometry

UV-Vis: Ultraviolet-Visible Spectroscopy

V: Volt
CHAPTER 1
INTRODUCTION

Cancer is now everywhere in our world and kills about 7.6 million people every year. Cancer is a disease that is caused by dysfunctional and uncontrollably dividing cells, this means that if a compound can inhibit cell division, it can treat cancer. Medicine is developing so fast nowadays, however, our knowledge of cancer and our abilities to combat cancer also increases significantly. Current treatments, such as cisplatin \(^{[1,2]}\), carboplatin \(^{[1,3]}\) and Photodynamic Therapy (PDT) have been successfully used to treat some types of cancer such as skin cancer, but we still have to confront a large number of other types of cancer. This is why research must continue in the field of platinum complexes and Photodynamic Therapy.

**Anti-cancer platinum(II) complexes**

As we all know, DNA plays a key role in the cell. It is a genetic material needed for cell division and replication. If the structure of DNA is destroyed and cannot be repaired, then the cell will stop dividing and replicating, in turn, the cell will kill itself in a process called apoptosis. The mechanism of those anticancer drugs to treat cancer is to attack the DNA of a cancer cell. If we can alter the DNA of a cancer cell permanently, then we may kill the cell. Platinum(II) complexes are a key pharmaceutical anti-cancer drug. They have been shown to have abilities to cross-link DNA \(^{[4,5]}\). The cross-link is a process that the platinum complex can covalently bind to the bases of single DNA strand such that
this single DNA strand cannot combine with another DNA strand any more. The position of attachment is usually N-7 of guanine base. Figure 1 shows the picture of the product results from the reaction of cisplatin with DNA \([6, 7]\). Figure 2 shows some examples of platinum (II) complexes that have been shown to have crosslink activity \([1, 8]\).

![Figure 1: Platinum complex combines with guanine bases](image1)

**Figure 1** Platinum complex combines with guanine bases

![Figure 2: Examples of platinum (II) complexes](image2)

**Figure 2** Examples of platinum (II) complexes

After the complex combines with one of the DNA single strands, a kink is made such that the DNA becomes unwound. Figure 3 shows the three-dimensional kinked structure of the DNA.
Cisplatin is currently one of the leading drugs used against cancer (Figure 4a). As we have mentioned above, one way the platinum drugs can interfere with DNA strands is to induce structural changes in the DNA. By now the most effective drugs that can achieve this goal is the anticancer drug cisplatin. Although it has been identified that several binding sites in the DNA can be utilized for the cisplatin\cite{9, 10}, the dominate one is N7, N7-bidentate cross-links between adjacent purine (usually Guanine) residues within one DNA strand which is also known as intra-strand cross-links\cite{4, 10} (Figure 4b).

Another very important feature of cisplatin that makes it become one of the most effective anticancer drugs is that the damage caused by it can be poorly repaired\cite{11}. On the other hand, its isomer---transplatin (Figure 5a), is barely used because it usually forms interstrand crosslinks\cite{12, 13, 14} (Figure 5b) which can be repaired more efficiently\cite{14}.
Despite its success, cisplatin still has toxic side effects caused by the chloride ligands and inability to differentiate normal vs. cancer cells.\cite{15} These effects, such as nephrotoxicity, neurotoxicity, and emetogenesis\cite{16}, limit the dose of cisplatin that can be given to patients everyday. The general mechanism of cisplatin is to displace the two chloride ligands by water to form two active species, cis-[PtCl (NH\textsubscript{3})\textsubscript{2}(OH\textsubscript{2})]\textsuperscript{+} and cis-[Pt (NH\textsubscript{3})\textsubscript{2}(OH\textsubscript{2})\textsubscript{2}]\textsuperscript{2+}, that covalently bind to DNA\cite{17,18}.

\[
\text{Figure 5a} \quad \text{Figure 5b}
\]

Carboplatin, [Pt (NH\textsubscript{3})\textsubscript{2}(CBDCA-O, O')] (Figure 6), where CBDCA is CycloButane DiCarboxylic Acid, is a widely used second-generation Pt(II) anticancer drug\cite{19,20}. This drug is less oto- and nephrotoxic than cisplatin\cite{20,21} but is effective against about the same tumor types as cisplatin.

\[
\text{Figure 6 Carboplatin}
\]

Compared to the cisplatin, carboplatin has a dicarboxylate chelate ring instead of two chloride ligands. Carboplatin is much less reactive than cisplatin because it is difficult to displace the bidentate chelate ring in substitution reactions\cite{22}. The lower reactivity of carboplatin with nucleophile makes it less toxic than cisplatin as well as less effective than cisplatin as a chemotherapy drug. The overall binding kinetics of carboplatin to DNA have been determined experimentally\cite{21}. Once bound to DNA, the
actions of cisplatin and carboplatin are the same. Figure 7\textsuperscript{[23]} shows a brief mechanism of carboplatin with DNA to form monoadducts and diadducts. A represents unreacted carboplatin, B is the carboplatin-DNA monoadduct, and C is the free CBDCA resulting from Pt-DNA diadduct formation and other types of nucleophilic displacement.

![Figure 7 Mechanism of carboplatin with DNA](image)

Nedaplatin(Figure 8) is a second-generation platinum derivative. It is shown to be effective against various solid tumors, including lung cancer, head and neck cancer and testicular cancer\textsuperscript{[24-26]}.

![Figure 8 Nedaplatin](image)

Nedaplatin contains a ring structure in which glycolate is bound to platinum by a bidentate ligand. It makes the platinum complex reactive to nucleophilic groups in DNA which results in the inter- and intra-strand DNA crosslinks\textsuperscript{[27]}. Nedaplatin has shown a higher antitumor activity to head, neck, lung and cervical cancer compared to cisplatin\textsuperscript{[24-26]}. On the other hand, it also shows less toxic activity than cisplatin due to the less toxic leaving group and lower reactivity with nucleophile\textsuperscript{[24-26]}. 
Oxaliplatin (Figure 9) is a novel third-generation platinum coordination complex that is effective to treat metastatic colorectal carcinoma. Oxaliplatin is capable of overcoming cisplatin resistance because of the different binding mechanism with DNA.

![Figure 9 Oxaliplatin](image)

Oxaliplatin exhibits activity against metastatic colorectal cancer when it is used together with 5-fluorouracil and leucovorin.

One of the most important problems that need to be considered is transport of the platinum compound into the cell. Most platinum(II) complexes are polar and not transported well into the cell. One strategy is to change the structures of the platinum complexes to something that resembles a nucleoside. Then these nucleoside-derived drugs have affinity to the membrane transporter protein. For nucleoside-derived drugs to exert their action, they must enter the cell via nucleoside transporters from two gene families, SLC28 and SLC29 (CNT and ENT, respectively). The human concentrative nucleoside transporter 3 (hCNT3) is a broad-selectivity, high-affinity protein implicated in the uptake of most nucleoside-derived anticancer and antiviral drugs. Regulated trafficking of hCNT3 has been recently postulated as a suitable way to improve nucleoside-based therapies. Once inside the cell, these drugs must be phosphorylated to their active forms. We propose the study of a Pt(II) DMSO-pyridine complex (Figure 10) which is based on thymidine to improve the cell permeability and uses a pyridyl
group as the platinum attachment point. DNA binding assays will be performed using pUC18 plasmid DNA.

Figure 10 Target nucleoside structure

Photodynamic Therapy (PDT)

Photodynamic Therapy (PDT) is an experimental treatment for tumors utilizing a special drug, called a photosensitizer or photosensitizing agent, along with particular type of light. When a photosensitizer is irradiated by light at a specific wavelength, it produces a cytotoxic oxygen singlet ($^1$O$_2$) which can kill nearby cells.$^{[30]}$

In the 1960s, a hematoporphyrin derivative was first discovered by R. L. Lipson and S. Schwartz who observed fluorescence of neoplastic lesions visualized during surgery when they injected crude hematoporphyrin into the cell. Hematoporphyrin was modified with acetic acid and sulfuric acid to obtain hematoporphyrin derivative (HPD) which was termed by S. Schwartz.$^{[31]}$ The HPD was purified by removing less-active porphyrins’ monomers to get photofrin which is the most widely used photosensitizing agent in clinical PDT. Recently, some new photosensitizers have been developed.$^{[32]}$

The first step of PDT treatment for cancer is to inject the photosensitizer into the bloodstream or to put it on skin. Although the drug is absorbed by all cells throughout the
body, it stays in cancer cells longer than it does in normal, healthy cells \cite{33, 34}. Approximately 2-3 days after injection, most of photosensitizer drug has gone from healthy cells but still remains in cancer cells. Then the tumor or diseased area is exposed to light with specific wavelength. This light might be applied inside the body with a thin tube or on the outside from a light source \cite{35}. The photosensitizer in the tumor absorbs the specific wavelength light and produces an activated-form oxygen—\(^1\text{O}_2\) radical.

There are three main steps to form the oxygen singlet. Figure 11 shows a typical energy level diagram for photosensitizing agent activation.

![Energy level diagram for photosensitizing agent activation](image)

**Figure 11 Energy level diagram for photosensitizing agent activation**

A photon with specific wavelength is absorbed by the photosensitizing agent molecule, raising it from the ground state to the excited state (singlet). Since the singlet state has a short lifetime the molecule undergoes inter system crossing to a long-lived triplet state. Then there are two types of reaction that can occur here. In the type I
reaction, the excited-triplet can react directly with a substrate to transfer an electron to form radicals which can further react with $O_2$ to form the reactive oxygen species (ROS). Alternatively, in type II reaction, the excited-triplet state photosensitizer molecule exchanges energy with the oxygen molecule and return to the ground state, the oxygen molecule is then activated to its excited state to produce the highly active singlet oxygen($^{1}O_2$) (Figure 11). These two types of reactions are summarized in Figure 12.

Both reactions occur simultaneously, and the ratio depends on the type of photosensitizer, the concentration of oxygen and substrate.

There are three main mechanisms that are already known for PDT’s effects on tumors. In the first case, the reactive oxygen radical can directly kill the tumor cells. The photosensitizer can also damage blood vessels which supply oxygen to the tumor, leading to severe tissue hypoxia and anoxia$^{[33,36]}$. Finally, an immune response can be activated by PDT to against the tumor$^{[33,37]}$. All these three mechanisms can combine with each other to make a better result for treating cancer.
Ruthenium complex used for PDT

Platinum complexes are one of the most successful anti-cancer drugs in the clinic. However, their clinical use is limited to their toxic side effects\textsuperscript{[15,16]} and a narrow range of cancer types, which promotes the development of other transition metal complexes. Among these novel complexes, ruthenium complexes have attracted much interest.

Ruthenium has three main properties that make it suitable for biological applications. The first one is that Ru(II) and Ru(III) compounds have very good ligand exchange kinetics which are very similar with those of platinum complexes\textsuperscript{[38]}. Ligand exchange plays a key role in biological activity. The second one is that Ruthenium has a wide range of oxidation states; Ru(II), Ru(III) and Ru(IV) are all accessible. The redox potential of Ru complexes can be modified by attaching to different ligands and it can also be utilized to improve the effectiveness of the Ru complex drugs. For example, by reducing Ru(III) to Ru(II) one can activate a ruthenium complex from its inert form to excited form when it is in diseased tissues\textsuperscript{[39]}. The last one is that ruthenium has the ability to mimic iron in binding with many biomolecules, which lowers toxicity of ruthenium drugs \textsuperscript{[40, 41]}. Many types of ruthenium complexes have been developed, including dimethyl sulfoxide ruthenium complexes, heterocyclic ruthenium complexes, polypyridyl compounds, ruthenium(II) arene complexes etc. Figure 13 shows some examples of ruthenium complexes.
Among these complexes, Porphyrin-Ruthenium complexes have been shown to have intense photophysical properties, biodiversity, and tumor targeting capabilities, which make them suitable as photosensitizers. One of the examples of this kind of complex was μ-{meso-5, 10, 15, 20 –tetra (4-pyridyl) porphyrin}- tetrakis – { bis- (bipyridine) chlororuthenium(II) [TRP] complex[^43] (Figure 14). When TRP and pBR322 were irradiated with light in aqueous solution, the supercoiled DNA was converted to the nicked form by breaking a single strand of DNA. All these observations were determined by gel electrophoresis[^44]. One of the most well studied ruthenium complexes is Ru (bpy)\(_3\)\(^{2+}\) (bpy, bipyridine) (Figure 15)[^44].
The ruthenium-polypyridyl (PPY) complexes were first developed as DNA intercalating agents in the late 1990’s [45]. The luminescence of the complex is quenched by forming H-bonds between the complex and water molecules in the aqueous solution, however, when double-stranded DNA was added into the solution the luminescence was observed. This can be explained by the shielding of the complex from water deactivation through intercalation into the DNA matrix. The studies of electron transfer through DNA base pairs showed that the DNA-inserted ruthenium(III) complex ([Ru(phen)$_2$dppz]$^{2+}$)
had the ability to quench the luminescence of the ruthenium(II) complex which had the same DNA intercalated \cite{44, 45}. This study developed the research of ruthenium complexes as potential photosensitizer for PDT.

Ruthenium complexes also have the same transport problem as we have mentioned for platinum complexes above. We also propose to synthesize and evaluate a ruthenium(II)-PPY nucleoside-complex (Figure 16) to increase its cell permeability.

![Figure 16 Ru (II)-PPY nucleoside](image-url)
Overview of proposed synthesis

According to the structure of our target compounds, the synthesis of compound (5) and (6) is outlined in Scheme 1. The 3’ and 5’ alcohols of Thymidine 1 will be protected by silylation with TBDMSiCl to give intermediate (2). Then a pyridyl methyl group will be introduced at the N3 succinimide position of the protected thymidine (2) by the Mistsunobu coupling reaction to give compound (3). The intermediate (3) is then treated with tetrabutylammonium fluoride in THF and subsequent hydrolysis to give the deprotected nucleoside (4). Treatment of the nucleoside ligand (4) with PtCl₂(DMSO)₂ could give the target compound (5), or treatment of (4) with Ru(bpy)₂Cl₂ could give the other target compound (6).
Scheme 1 Synthetic pathway to Cis- \{PtCl_2 [DMSO]\}[N4(N-3(4-Pyridylmethyl) Thymidine]} and Ru (II)-PPY nucleoside.
CHAPTER 2
RESULTS AND DISCUSSION

Part 1: Platinum- nucleoside Complex

We began our work by protecting the 3’ and 5’ alcohols of thymidine with TBDMSiCl. The reaction is shown in Scheme 2.

\[
\begin{align*}
\text{(1)} & \quad \text{HO} & \quad \text{HO} & \quad \text{N} & \quad \text{N} & \quad \text{CH}_3 & \quad \text{O} & \quad \text{O} & \quad \text{H} \\
\text{(2)} & \quad \text{O} & \quad \text{TBDMSO} & \quad \text{TBDMSO} & \quad \text{N} & \quad \text{N} & \quad \text{CH}_3 & \quad \text{O} & \quad \text{O} & \quad \text{H} \\
\text{Yield:56%}
\end{align*}
\]

Scheme 2 Protection of 3’ and 5’ alcohols of Thymidine.
Reagents and conditions: DMF, TBDMSiCl, imidazole, reflux, 24h.

We first attempted to use 2.2 molar equivalents of TBDMSiCl and 2.2 molar equivalents of imidazole to react with thymidine, but the reaction gave poor yields. We then increased the molar equivalents of TBDMSiCl and imidazole to 8.0 and 10.0, respectively. Finally we isolated compound (2) in 56% yield. The TBDMSi protecting group has the added advantage of decreasing the polarity of the nucleoside making it more soluble for the next step.

The next step is to introduce a platinum attachment point at the N3 succinimide position. Pt(II) DMSO-pyridine complexes have been shown to be reactive in
organic solution and tend to lose the heteroaromatic ligand in favor of other added ligands \[^{[46]}\]. The alcohol protected nucleoside (2) was reacted with 4-pyridinemethanol through the Mitsunobu coupling reaction to obtain nucleoside (3) in 65% yield (As shown in Scheme (3). We used 1.1 molar equivalents of 4-pyridinemethanol, 1.5 equivalents of tributylphosphine and 1.5 equivalents of 1,1’-(azodicarbonyl)dipiperidine (ADDP) \[^{[47]}\]. The Mitsunobu reaction requires an acidic proton which is available at the succinimide position.

![Scheme 3 Synthesis of 3',5'-Di-O-t-butyldimethylsilyl-N-3-(4-pyridylmethyl)-thymidine (3). Reagents and conditions: Benzene, 4-Pyridinemethanol, tributylphosphine, ADDP, r.t. 24h, N\(_2\).](image)

The structure of compound (3) was fully characterized by NMR\(^{\text{1H}}\)NMR, \(^{13}\)CNR, COSY, HMBC, and HMQC. A full discussion of absorbance assignment techniques will be shown for compound (5). The structure and numbering system is portrayed in the appendix.

Since the polarity of compound (2) and compound (3) were similar and they showed very similar R\(_t\) values by TLC analysis, we didn’t realize we successfully made compound (3) after quite a long time. During this period of time, we also attempted to couple other possible heteroaromatic ligands to compound (2) as the platinum attachment point. First, we tried 5-methyl-8-quinoline (As shown in Scheme 4).
Scheme 4  Synthesis of compound (7)
Reagents and conditions: DMF, 5-(chloromethyl)-8-quinolin hydrochloride, TMG, r.t., 24h.

We treated compound (2) with 1.3 molar equivalents of 5-(chloromethyl)-8-quinolinol hydrochloride and 3 molar equivalents of the strong base 1, 1, 3, 3-Tetramethylguanidine (TMG) at room temperature for 24 hours. Then we analyzed by TLC for product (7) and compound (2). We found most of the material was still compound (2) and the different components had very similar polarities so it was very hard to purify through flash chromatography. The reaction was deemed unsuccessful. Then we tried to couple 9-Acridine Carboxamide (10) to compound (2) via Mistunobu coupling as before. The acridine nucleus could also act as a Pt (II) or Ru (II) attachment ligand. The reaction is shown in Scheme 5.
Scheme 5  Synthesis of compound (11)
Reagents and conditions: (a) CH$_3$CN, CDI, ethanolamine (b) Thionyl chloride, reflux, 3h; (c) CH$_3$CN, ethanolamine, triethylamine, r.t., 24h, N$_2$. (d) Benzene, TBP, ADDP, r.t., 24h, N2.

Since 9-Acridine Carboxamide (10) is not commercially available we had to synthesize it. We started with compound (8), 9-Acridinecarboxylic acid, and tried two different pathways. The first was to utilize carbonyldiimidazole (CDI) (path a) to directly synthesize compound (10) based on the literature procedures$^{[48]}$, but the reaction did not work. TLC analysis showed no product formation. The second path was to use thionyl chloride to convert the starting material to acid chloride (9) (path b) first. We then treated compound (9) with ethanolamine and triethylamine to give compound (10), 9-Acridine Carboxamide (47% yield), which is the reagent required for the next Mistunobu reaction. After 24h, we filtered the solid from the solution and the residue was purified by flash chromatograph on silica gel, eluting with acetone/CH$_2$Cl$_2$ (1:1). Then the crude product was dissolved in water to get rid of some ionic impurities and finally we obtained pure
compound (10) which was identified by $^1$H NMR $^{[49]}$. The next step was to perform the Mistunobu reaction to add compound (10) to compound (2) by using the same reagents and conditions as in Scheme 4. Unfortunately this step failed to give the desired product (11) as shown by NMR. We researched the reagents and conditions for Mistunobu reaction but could not ascertain why the reaction did not work. We assumed that it was because the benzene was not polar enough to dissolve all the starting materials completely so we replaced benzene with 1-methyl-2-pyrrolidinone as the solvent and did this reaction again, but still failed.

Continuing our work with the pyridyl methyl nucleoside ligands. Scheme 6 shows the deprotection of nucleoside (3). According to the literature $^{[50]}$, we treated compound (3) with 1.1 molar equivalent of TBAF solution in THF and subsequent hydrolysis gave the deprotected nucleoside (4) in 62% yield.
Scheme 6  Synthesis of N-3-(4-pyridylmethyl)-thymidine (4)
Reagents and conditions: (a) THF, TBAF in THF, r.t., 24h, N$_2$; (b) H$_2$O, r.t., 30mins.

Nucleoside ligand (4) was also fully analyzed by $^1$H, $^{13}$C NMR, and 2-D NMR. Peak assignments were made using 2D-HCOSY, HETCOR and HMBC experiments. Figure 17 shows the $^1$H NMR of compound (4).
The key step in the project is metal coordination to the pyridyl group of nucleoside (4) since it is platinum that gives the final product its anti-tumor activity. Cis-[PtCl$_2$(DMSO)$_2$] was one of the reactants in this reaction and was synthesized according to the literature.$^{[51]}$ We then reacted compound (4) with cis-[PtCl$_2$(DMSO)$_2$] as shown in Scheme 7.
Scheme 7  Synthesis of PtCl₂(DMSO)₂ {N₄[N-3(4-Pyridylmethyl)]}Thymidine (5)
Reagents and conditions: ethanol, reflux, 18h.

The first problem we encountered was how to choose the appropriate solvent for compound (4) due to its high polarity. Neither DMSO or DMF could be utilized since DMSO could displace the pyridyl group of nucleoside (4) from the product. DMF is very hard to get rid of and could make purification difficult. The first attempt was to use 1.0 molar equivalent of Cis-[PtCl₂(DMSO)₂] to react with compound (4) in 100ml refluxing ethanol for 18 hours to give compound (5) as a white solid in 60.0% yield. Then we attempted to reduced the volume of the solvent to 20ml and increased molar equivalents of cis-[PtCl₂(DMSO)₂] to 2.0, but the reaction gave 44.3% yield which was even less than the yield of the first attempt. Reaction with 4.0 mole equivalents of cis-[PtCl₂(DMSO)₂] led to a gray solid and a metal coating on the reaction flask, which was probably caused by the decomposition of cis-[PtCl₂(DMSO)₂]. So reaction of nucleoside (4) with higher mole equivalents of cis-[PtCl₂(DMSO)₂] did not lead to increased yields of compound (5). Coordination of platinum(II) at the pyridine nitrogen of compound (5) was confirmed by several spectroscopic lines of evidence. ¹H NMR and COSY spectroscopy are depicted below (Figure 18 & 19).
Figure 18 $^1$H-NMR of the target compound (5)

A singlet at 8.04 ppm ($H_6$) represents the vinylic proton of the thymine moiety. $H_6$ is confirmed in the COSY spectrum (Figure 19) which indicates a long range coupling of the proton with the adjacent methyl group $H_7$ of the thymine moiety. The doublets at 8.70 ppm and 7.64 ppm correspond to $H_{11}$ and $H_{10}$ of the pyridine group respectively. $H_{10}$ and $H_{11}$ are also confirmed from the COSY spectrum (Figure 19) which indicates the coupling of $H_{11}$ with the adjacent proton $H_{10}$. A triplet at 6.33 ppm and a multiplet at 2.26 ppm account for the aliphatic proton $H_1'$ and $H_2'$ of the pentose sugar moiety which can be conformed by the COSY spectrum (Figure 19), indicating proton $H_1'$ coupling with $H_2'$. A multiplet at 4.44 ppm which is coupled to $H_2'$ corresponds to $H_3'$ of the sugar moiety. The proton at 4' position (multiplet at 3.93 ppm) which is coupled to $H_3'$ can be easily
identified from the COSY spectrum as only H$_{2}'$ and H$_{4}'$ can be coupled to H$_{3}'$. A multiplet at 3.76 ppm which is coupled to H$_{4}'$ can be accounted for H$_{5}'$. A multiplet at 5.24 ppm and a doublet at 5.37 ppm correspond to 3’- OH and 5’-OH. The 3’-OH and 5’-OH should have very similar chemical shifts and OH protons have wider chemical shift range than other protons so it is hard to tell which peak listing is 3’-OH and which is 5’-OH. However it should be noted that there is a very weak coupling of the proton at 5.24 ppm with the proton H$_{5}'$ and 3’-OH should be doublet since there is only one adjacent proton, so the peak listing at 5.24 ppm can be confirmed as 5’-OH and the doublet at 5.37 ppm can be validated as 3’-OH. The reason why 3’-OH is more intense than 5’-OH is because the overlap of H8 and 3’-OH. The two multiplets at about 2.73 and 2.91 ppm are the DMF$_{d7}$ solvent peaks.
Figure 19 $^1$H-COSY spectrum of the target compound (5)

$\delta \Delta$ complex represents the coordination shift due to platinum complexation.

$\delta \Delta$ complex = $\delta$(5)-$\delta$(4). Table 1 shows the Coordination shift of compound (5) in DMF$_{d7}$.

Table 1 Effect of pyridyl platination on $^1$H and $^{13}$C NMR chemical shifts of (5) in DMF$_{d7}$

<table>
<thead>
<tr>
<th>Proton</th>
<th>$\delta$ (4)</th>
<th>$\delta$ (5)</th>
<th>$\delta \Delta$ complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_{11}$</td>
<td>8.533</td>
<td>8.691</td>
<td>0.158</td>
</tr>
<tr>
<td>H$_{10}$</td>
<td>7.317</td>
<td>7.646</td>
<td>0.329</td>
</tr>
<tr>
<td>H$_8$</td>
<td>5.118</td>
<td>5.243</td>
<td>0.125</td>
</tr>
<tr>
<td>H$_6$</td>
<td>8.033</td>
<td>8.037</td>
<td>0.004</td>
</tr>
<tr>
<td>H$_7$</td>
<td>1.887</td>
<td>1.882</td>
<td>-0.005</td>
</tr>
<tr>
<td>Carbon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C$_{11}$</td>
<td>150.10</td>
<td>151.87</td>
<td>1.77</td>
</tr>
<tr>
<td>C$_{10}$</td>
<td>122.68</td>
<td>124.66</td>
<td>1.98</td>
</tr>
<tr>
<td>C$_9$</td>
<td>146.75</td>
<td>162.48</td>
<td>15.73</td>
</tr>
<tr>
<td>C$_8$</td>
<td>43.35</td>
<td>43.10</td>
<td>-0.25</td>
</tr>
</tbody>
</table>
Protons H₁₁, H₁₀ and H₈ of compound (5) are all deshielded relative to compound (4) and this effect tails off as the distance from platinum center increases. The $^{13}$C data also gives a similar trend. There are no significant differences in chemical shifts in the sugar protons and carbons. The C₉ gives a relatively large downfield coordination shift comparing with known Pt (II) complexes, which requires further discussion.

According to the literature, deshielding of heteroaromatic carbons in platinum(II) complexes have maximum coordination shifts of about +10 ppm $^{[52-53]}$. We could not find any reference examples of $^{13}$C NMR chemical shifts in mixed- pyridine complexes similar to compound (5). The strong deshielding of C₉ could be explained by a resonance effect that places positive charge on a tertiary carbon. Scheme 8 shows the resonance structures in the pyridyl group of compound (5).

Another spectral technique we used for analysis was $^{195}$Pt NMR which has been shown to be a valuable tool to study the environment around platinum in platinum complexes $^{[54]}$. The literature work on PtCl₂(DMSO)Py complexes, where Py are various substituted pyridines, gave $^{195}$Pt NMR shifts of about -2850 ppm for cis-isomers and -3320 ppm for trans-isomers, relative to K₂PtCl₆ $^{[54]}$. All these complexes are bound to platinum via the sulfur atom of the sulfoxide. The chemical shift of compound (5) is
-3124 ppm relative to K$_2$PtCl$_6$ dissolved in 50:50 DMF/D$_2$O solution. This result matches the NMR data of trans-isomers but we do not possess both isomers for comparison. Another study also showed that an initial trans-isomer was formed and followed by isomerization to the cis geometric isomer$^{[5]}$. Figure 20 shows the parent ion region of electrospray TOF Mass Spectrometry of compound (5). The presence of two chlorine isotopes and five major platinum isotopes make this region very complicated. The experimental data agree well with the calculated spectrum inserted for comparison.

![Figure 20 Parent ion region of TOF-MS spectrum of (5) with calculated spectrum insert](image)

Early in the structure determination of compound (5) we found that decomposition occurred when we dissolved it in DMSO$_{d6}$ solution. It was stable in many other solvents such as THF$_{d8}$, DMF$_{d7}$ and CD$_3$CN. In consideration of the solubility issues and
instability in DMSO, we decided to use DMF\textsubscript{d7} solution as the solvent for NMR. The aromatic region of the \textsuperscript{1}H-NMR spectrum of compound (5) in DMSO\textsubscript{d6} is shown in Figure 21.

A significant portion of compound (5) was converted to compound (4) just 10 minutes after dissolution. It has been shown that PtCl\textsubscript{2}(Pyridine)\textsubscript{2} complexes undergo a similar solvolysis in DMSO solution to give an equilibrium mixture of cis- PtCl\textsubscript{2}(DMSO)(Pyridine) and trans- PtCl\textsubscript{2}(DMSO)(Pyridine)\textsuperscript{[46]}.

![Figure 21 Aromatic region of a solution of compound (5) in DMSO\textsubscript{d6}](image)

A proposed pathway of decomposition is outlined in Scheme 9. We prefer the bimolecular associative process since the intermediate of the dissociative process would be unstable. Further experimentation and discussion will be required for a more detailed mechanism.
We then began studies of the DNA binding activity of compound (5). Compound (5) was incubated with pUC18 plasmid DNA for 4 hours and subsequently we performed gel electrophoresis to separate the different DNA forms. Ethidium bromide was utilized for staining DNA fragments. It is known that relaxed forms (i.e. linearized and nicked) of DNA are retarded in the migration through the gel relative to fully supercoiled DNA if they are generated by reacting with chemical reagents. As we have mentioned in the introduction part the general mechanism of cisplatin is the hydrolysis of cisplatin followed by coordination to adjacent guanine groups at the N7 position.\textsuperscript{[17-18]} This coordination will impede the migration of the DNA through the gel, as shown in Figure 22A \& 22B lane 5. We used 20\% DMF buffered aqueous solutions due to the low solubility of compound (5) in buffered aqueous solutions and it’s decomposition in DMSO solutions. Since we had no idea if DMF would affect the migration of plasmid DNA; incubation of pUC18 with 20\% DMF aqueous buffer at 37\textdegree C for 4h was performed as a reference. Lane 1, Fig. 22A \& 22B shows the migration of DNA without DMF, while Lane 2 shows the migration of DNA in the presence of DMF. There is no
significant difference between Lane 1 and Lane 2 indicating DMF has no effect on the conformation of the DNA. Fig. 22A lane 4 represents the incubation of the DNA with nucleoside (4) at a 1:1 DNA-base pair to compound ratio, while Fig. 22B lane 4 shows a 5:1 DNA-base pair to compound ratio, both of which do not have noticeable observed interaction. This is expected and when the DNA is incubated with compound (5) at a 5:1 bp-compound ratio, a significant fragmentation is observed as shown in Fig. 22B lane 3. Compound (5) gives mainly linearized DNA as a result. Increasing the concentration of compound (5) to 1:1 DNA-bp to compound ratio (Fig. 22A, lane 3) led to a complete loss of DNA signal. This could be interpreted that most of the DNA double strands have been cleaved or denatured and ethidium can no longer bind to the DNA and fluoresce. This result shows high reactivity of compound (5) towards DNA, which indicates that compound (5) has the Cis geometry about platinum center since trans-Pt (II) configuration is less reactive and causes different types of DNA damage.

![Figure 22 Effect of (5) on the migration of pUC18 circular plasmid DNA](image)

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Part 2: Ruthenium-nucleoside Complex

We did not have trouble in choosing a solvent for this step since we already knew ethanol was successful in platinum-nucleoside formation. The reaction is shown in Scheme 10.

![Scheme 10](image)

Scheme 10  Synthesis of Ruthenium-PPY (6)
Reagents and conditions: Ethanol, Ru(bpy)$_2$Cl$_2$, reflux, 2h.

We added 1.2 eq. Ru (bpy)$_2$Cl$_2$ to compound (4) in refluxing ethanol. The reaction mixture was added to a saturated aqueous solution of NH$_4$PF$_6$ to precipitate the complex as the PF$_6$ salt. Flash precipitation from diethyl ether was performed to purify the crude product, giving the proposed Ruthenium-PPY nucleoside (6) as a brown powder (60% yield). Figure 23 shows the $^1$H NMR of compound (6). From the $^1$H NMR spectrum we can see all nucleoside protons as in compound (4), then we can also see addition of 16 bipyridyl protons in the aromatic region from ppy complexation.
Table 2 shows the coordination shifts of (6) in acetonitrile-d3. These results provide further proof of ruthenium coordination to the pyridyl group.

**Table 2 Coordination shifts of Compound (6) in Acetonitrile-d3**

<table>
<thead>
<tr>
<th>Proton</th>
<th>$\delta$ (4)</th>
<th>$\delta$ (6)</th>
<th>$\delta$ complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_{11}$</td>
<td>8.48</td>
<td>9.93</td>
<td>1.45</td>
</tr>
<tr>
<td>$H_{10}$</td>
<td>7.22</td>
<td>7.80</td>
<td>0.58</td>
</tr>
<tr>
<td>$H_8$</td>
<td>5.05</td>
<td>5.00</td>
<td>-0.05</td>
</tr>
<tr>
<td>$H_7$</td>
<td>1.88</td>
<td>1.84</td>
<td>-0.04</td>
</tr>
<tr>
<td>$H_6$</td>
<td>7.68</td>
<td>7.67</td>
<td>-0.01</td>
</tr>
<tr>
<td>Carbon</td>
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<td></td>
</tr>
<tr>
<td>$C_{11}$</td>
<td>149.45</td>
<td>152.52</td>
<td>3.21</td>
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<td>$C_{10}$</td>
<td>122.13</td>
<td>152.66</td>
<td>30.39</td>
</tr>
<tr>
<td>$C_9$</td>
<td>146.17</td>
<td>147.51</td>
<td>1.34</td>
</tr>
<tr>
<td>$C_8$</td>
<td>42.91</td>
<td>42.43</td>
<td>-0.48</td>
</tr>
</tbody>
</table>
Figure 24 shows the parent ion region of the electrospray TOF Mass Spectrum of compound (6). The presence of two chlorine isotopes and five major ruthenium isotopes gives a similar result as the platinum complex. The experimental data agree well with the calculated spectrum inserted.

Since Ruthenium (II) and (III) are easily discerned by electrochemistry, the next experiment we performed was cyclic voltammetry. The experiment was run under a nitrogen atmosphere in a three compartment cell equipped with a platinum auxiliary electrode, a glassy carbon working electrode and referenced to the Ag/AgCl reference.
electrode. Figure 25 shows the cyclic voltammograms of nucleoside ligand (4) and ruthenium complex (6). A broad irreversible oxidation wave at about 0.8V vs. Ag/AgCl and a irreversible reduction wave with Epc= -1.41V vs. Ag/AgCl are observed in electrochemical oxidation of compound (4) (Figure 25A). Figure 25B shows the electrochemical oxidation of compound (6). A quasireversible redox couple with an E1/2=0.82V (△E= 90mV) vs. Ag/AgCl indicates the presence of a Ru(III/II) couple. The oxidation current is larger than its coupled reduction current, which could be explained by the overlapping oxidation of both Ru (II) and nucleoside (4). Two irreversible redox waves were observed in the cathodic direction at about -1.49 V and -1.71 V vs. Ag/AgCl due to one electron reduction of compound (4) and the bipyridyl groups in compound (6) respectively.

![Figure 25 Cyclic voltammograms of the nucleoside (A) and the Ru(II)-PPY nucleoside (B)](image)

The electronic absorption spectroscopy of compounds (4) and (6) were then run in 1cm quartz cuvettes in acetonitrile. Figure 26 shows the results. From the figure we can see compound (4) (blue line) has an intense π-π* transition (λmax = 257nm, Ε= 12,267 M⁻¹cm⁻¹). However, in compound (6) (red line) the π-π* transition associated with nucleoside ligand (4) shifts to a higher absorptivity and lower wavelength (about 245nm), which indicates higher energy. Both of these two changes reveal a destabilization of the
π* orbitals of the nucleoside in compound (6). There is also a very intense absorption at about 295 nm with a molar absorptivity around 30,000 M⁻¹cm⁻¹, which can be assigned to the π-π* transition of the bipyridyl groups coordinated to the Ru metal. Another two weak peaks at about 353 nm and 502 nm are most likely metal to ligand charge transfer (MLCT) transitions with the shorter wavelength associated with a Ru(dπ)-bpy(π*) transition and the longer wavelength associated with a Ru(dπ)-pyridyl(π*). These studies in addition to the cyclic voltammetry are all good evidence for the assigned structure of complex (6).

![Electronic absorption spectroscopy of nucleoside (blue) and the Ru(II)-PPY nucleoside complex (red)](image)

Figure 26 Electronic absorption spectroscopy of nucleoside (blue) and the Ru(II)-PPY nucleoside complex (red)

The ability of compound (6) to photo-react with DNA was evaluated by combining an aqueous solution of compound (6) with pUC18 plasmid DNA at a 10:1 DNA-base pairs to complex (6) ratio. The solutions were irradiated with a 300W mercury arc lamp equipped with a long band pass 420 nm filter. Samples were irradiated at 10 minute intervals and the extent of photoinduced DNA damage was determined by the subsequent gel electrophoresis. The results are shown in Figure 27. The intact supercoiled-DNA travels the farthest in the gel and the nicked-DNA which has one single strand broken...
travels slower through the gel. When both the strands break the circular plasmid will become linear and generally migrates between the nicked circular and the supercoiled circular.

![Figure 27 Gel electrophoresis of circular plasmid DNA (pUC 18) in the presence and absence of Ru(II)-PPY nucleoside](image)

From the Figure 27 we can see that Lane C shows the movement of the supercoiled-DNA without complex (6) and irradiation. The reason why there is a faint band behind the supercoiled-DNA band is probably because the pUC18 plasmid DNA sample contains relaxed or nicked-DNA. Lane 1 is a combination of complex (6) and DNA sample at a 10:1 DNA-base pairs ratio but without any irradiation. We can observe that Lane 1 has a slightly slower migration compared to Lane C, which represents the DNA sample is slightly damaged. Lane 2-9 represent the combination of complex (6) and DNA is irradiated at 10 minute intervals. It can be seen that the bands of nicked-DNA are more intense and the bands of supercoiled-DNA become less as the irradiation time increases. These changes indicate that the complex (6) with irradiation is causing significant DNA damage.

As mentioned in the introduction part, reactive oxygen species (ROS) is one of the most important intermediates in the PDT since it kills the cancer cells directly. Examples of ROS include superoxide, hydroxyl radical and singlet oxygen. Our next goal was to determine the mechanism of this photo-reaction, in other words, which reactive oxygen
species was generated in this photo reaction. We made a solution that contained complex (6) and DNA sample at a 5:1 bp to complex ratio. The solution was irradiated as we did above but in the presence of specific ROS inhibitors. Figure 28 is the results of gel electrophoresis analysis.

![Figure 28 Photoinduced cleavage of pUC18 in the presence of Ru(II)-PPY nucleoside and different inhibitors](image)

The control Lane 1 shows the movement of double strand DNA on the gel. In Lane 2 it can be seen that the band of double strand DNA disappears which means all of the double strand DNA is gone. It should be noted that there is a light band between the double strand DNA and relaxed DNA, this band indicates both of the strands are broken in the DNA sample and it becomes lineared. This linearized DNA was not observed in 10:1 bp to complex experiment. In Lane 3 the experiment was run in the presence of 200 mM DMSO solution which is hydroxyl radical scavenger. The result shows little difference compared to Lane 2, which indicates that almost no hydroxyl radicals are generated in this reaction. Then the reaction was performed in the presence of D$_2$O (can extend the lifetime of singlet oxygen), the result (Lane 4) is almost the same as Lane 3. The Lane 5 represents the effect of the inhibitor NaN$_3$ which is known as singlet oxygen quencher indicating that singlet oxygen is involved in this photoreaction. As we discussed
in the introduction, the photosensitizer would undergo type II mechanism---energy transfer from triplet state and ground state will generate singlet oxygen. We can now draw a conclusion that a type II mechanism is involved in this photoreaction. Lane 6 also has a large effect as in Lane 5 when superoxide dismutase (SOD, an enzyme, a known superoxide radical quencher) is present, this means superoxide radicals also exist in the reaction indicating a type I mechanism is also taking place. From Figure 28 now we know that both type I and type II mechanisms, in other words---electron and energy transfer between the excited state and ground state, are taking place in this photo reaction.
CHAPTER 3

EXPERIMENTAL

General

All reagents chemicals were purchased from Sigma-Aldrich and used without further purification. PtCl₂ (DMSO)₂ and Ru (bpy)₂Cl₂ was prepared according to literature methods [56, 57]. NMR analysis was performed on a Bruker AVANCE 300 system operating at 300.13 MHZ for ¹H, 75.47 MHz for ¹³C and 64.30 MHz for ¹⁹⁵Pt. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane for ¹H and ¹³C and K₂PtCl₆ for ¹⁹⁵Pt NMR. All coupling constants are given in Hertz. Electrospray Ionization Quadrapole TOF-Mass spectral analysis was performed on a Bruker maXis instrument at the Ohio State University CCIC. Mass spectrum modeling was performed with an Isotope Distribution Calculator available online at Scientific Instrument Services. Thin Layer chromatographic analysis was performed with Merck DC Kieselgel 60 analytical plates and flash chromatography was carried out using 60A flash silica from Dynamic Adsorbents Inc. Tris Base, ethidium bromide (EthBr), and agarose low EEO were purchased from Fisher. The plasmid DNA, pUC18 (1ug/µL), was from Aldrich, and superoxide dismutase (SOD) from bovine erythrocytes was obtained from Sigma. All aqueous solutions were prepared using doubly distilled water.
Synthetic Procedures and Spectroscopic Data

3’, 5’-Di-O-tbutyldimethylsilylthymidine (2): To a mixture of 1 (1.0g, 4.13mmol) and imidazole(2.81g, 41.3mmol) in DMF (40ml) TBDMSiCl (4.98g, 32mmol) was added dropwise. The solution was heated to reflux under nitrogen atmosphere. After being stirred for 24h, the reaction solution was poured into brine (100ml) followed by dichloromethane (140ml). The mixture was then filtered by vacuum through celite-pad and the solution was collected into a separatory funnel. The organic layer was separated and the aqueous layer was further extracted with dichloromethane (100ml) three more times. The drying agent MgSO₄ was added to the combined organic layer. After filtration the drying agent, the solution was evaporated to dryness. The residue was purified by flash chromatography on silica gel, eluting with CH₃OH/ CHCl₃ (1:49), to give 1.08g (yield 55.7%) of product 2 as white powder. 1H and 13C NMR data are consistent with a literature reference [58]. TOF-MS; C₂₂H₄₂N₂Si₂O₅ M+Na calc. for 493.2525, observed 493.2528.

3’,5’-Di-O-t-butyldimethylsilyl-N-3-(4-pyridylmethyl)-thymidine (3): Compound 2 (0.60g, 1.28mmol) was dissolved in benzene (6 ml) at 0°C and treated with 4-Pyridinemethanol (0.25g, 1.28mmol) and Tributylphosphine (0.47ml, 1.92mmol). After being stirred at 0°C for 10 mins, the whole solution was taken to room temperature and treated with ADDP (0.48g, 1.92mmol). The reaction mixture was stirred under nitrogen environment overnight, then added to EtOAc (100ml) and washed with H₂O (100ml). The organic layer was dried by MgSO₄ and evaporated to dryness. The residue was chromatographed on a column of silica gel, eluting with chloroform/acetone (9:1) to give product 3 0.47g (yield 65.3%) as clear yellow liquid. ¹H-NMR(CDCl₃) δ 0.011, 0.07,
0.077, 0.1140 (4 s, 12H, Si(CH₃)₂C(CH₃)₃), 0.89 (s, 9H, Si(CH₃)₂C(CH₃)₃), 0.93 (s, 9H, Si(CH₃)₂C(CH₃)₃), 1.61 (s, 3H, H₇), 1.97-2.04 (m, 1H, H₂⁻), 2.23-2.29 (m, 1H, H₂⁻), 3.73-3.95 (m, 3H, H₄⁻ and H₅⁻), 4.40 (m, 1H, H₃⁻), 5.11 (dd, 2H, J=14.4 and 17.3, H₈), 6.36 (dd, 1H, J=5.7 and 8.00, H₁⁻), 7.30 (d, 2H, J=5.97, H₁₀), 7.52 (s, 1H, H₆), 8.53 (d, 2H, J=5.9, H₁₁); ¹³C-NMR (CDCl₃) δ 163.24 (C₂), 150.80 (C₄), 149.92 (C₁₁), 145.54 (C₉), 133.97 (C₆), 123.36 (C₁₀), 110.07 (C₅), 87.91 (C₁⁻), 85.61 (C₃⁻), 72.34 (C₄'), 63.01 (C₈), 43.50 (C₅'), 41.46 (C₂⁻), 25.91, 25.71 (Si(CH₃)₂C(CH₃)₃), 18.38, 17.91 (Si(CH₃)₂C(CH₃)₃), 13.26 (C₇), -5.47, -4.67 (Si(CH₃)₂C(CH₃)₃). TOF-MS C₂₈H₄₇N₃Si₂O₅, M+Na; calcd. for 584.2946, observed 584.2963.

**N-3-(4-pyridylmethyl)-thymidine (4):** A solution of compound 3 (0.65g, 1.15mmol) in THF (10ml) was cooled at 0°C and 0.73ml of TBAF (1.0M solution in THF 2.53mmol) was added dropwise under N₂ environment. The solution was taken to room temperature and stirred for 20hrs. Then H₂O (2ml) was added to it and stirred for another 30 mins. After evaporating to dryness, the residue was purified by flash chromatography on silica gel, eluting with acetone/chloroform (7:3) to give 0.24g (yield 62.3%) of product 4 as a white powder. ¹H-NMR(DMF) δ 1.89 (s, 3H, H₇), 2.20-2.34 (m, 2H, H₂⁻), 3.75 (brd-m, 2H, H₅⁻), 3.93 (q, 1H, J=3.06, H₄⁻), 4.46 (m,1H, H₃⁻), 5.11 (dd, 2H, J=15.9 and 15.8, H₈), 5.27, 5.41 (brd-s, brd-s, 2H, OH₅⁻ and OH₃⁻), 6.35 (t, 1H, J=6.00, H₁⁻), 7.32 (d, 2H, J=5.25, H₁₀), 8.03 (s, 1H, H₆), 8.53 (d, 2H, J=5.31, H₁₁). ¹³C-NMR (DMF) δ 163.17 (C₄), 151.14 (C₂), 149.50 (C₁₁), 146.75 (C₉), 135.70 (C₆), 122.68 (C₁₀), 109.10 (C₅), 88.37 (C₄'), 85.74 (C₁⁻), 71.16 (C₃'), 62.04 (C₅'), 43.35 (C₈), 40.39 (C₂⁻), 12.67 (C₇). TOF-MS; C₁₆H₁₉N₃O₅, M+Na calc. for 356.1217 observed 356.1230.
PtCl₂·{DMSO}{N₄[N-3(4-Pyridylmethyl)]}Thymidine (5): Compound 4 (0.10g, 0.30mmol) was dissolved in ethanol (100ml) and Pt(DMSO)₂Cl₂ (0.13g, 0.30mmol) was added. The solution was heated to reflux under N₂ atmosphere for 20hrs. After cooling to room temperature the reaction mixture was stored at -30°C for 2 days. The precipitate was collected by vacuum filtration and dried under reduced pressure to give 0.12g (yield 60.0%) of product 5 as a light yellow powder. ¹H-NMR(DMF d₇) δ 1.88 (s, 3H, H₇), 2.25-2.28 (m, 2H, H₂'), 3.51 (s, 6H, SO(CH₃)₂), 3.76 (m, 2H, H₅'), 3.93 (m, 1H, H₄'), 4.44 (m, 1H, H₃'), 5.24 (m, 3H, H₈ and OH₅'), 5.37 (d, 1H, J=4.16, OH₃'), 6.33 (t, 1H, J=6.87, H₁'), 7.64 (d, 2H, J=6.66, H₁₀'), 8.04 (s, 1H, H₆), 8.69 (d, 2H, J=6.8, H₁₁). ¹³C-NMR (DMF d₇) δ 163.14 (C₄), 162.48 (C₉), 151.87 (C₁₁), 151.10 (C₂), 135.87 (C₆), 124.66 (C₁₀), 109.10 (C₅), 88.37 (C₄'), 85.83 (C₁'), 71.10 (C₃'), 62.02 (C₅'), 43.28 (C₆), 43.03 (SO(CH₃)₂), 40.60 (C₂'), 12.62 (C₇). TOF-MS; C₁₈H₂₅N₃SO₆PtCl₂, M+Na calc. for 700.0366 observed 700.0368.

Nucleoside-PPY (6): Compound 4 (17.6mg, 0.053mmol) was dissolved in ethanol (10ml) and Ru(bpy)₂Cl₂ (30.6mg, 0.0636mmol) was added. The solution was heated to reflux for 3h. The reaction mixture was cooled to room temperature and added to H₂O (100ml) containing NH₄PF₆. The precipitate was filtered and washed with H₂O and then air dried for 20h. The crude product was dissolved in Acetonitrile and the solution was poured into ethyl ether (100ml). The precipitate was collected by vacuum filtration and dried under reduced pressure overnight to give product 6 (yield 60.1%) as brown powder. ¹H NMR(CD₃CN) δ 1.84 (d, 3H, J=0.93 H₇), 2.11-2.16 (m, 2H, H₂'), 3.11 (t, 1H, J=5.1, 5'-OH), 3.31 (d, 1H, J=4.3, 3'-OH), 3.68 (m, 2H, H₅'), 3.82 (m, 1H, H₄'), 4.33 (m, 1H, H₃'), 5.00 (s, 1H, H₆), 6.16 (t, 1H, J=6.3, H₁'), 7.13-7.20 (m, 4H, bipy-H), 7.57-7.62 (m,
2H, bipy-H), 7.67 (s, 1H, H₆), 7.73-7.84 (m, 3H, bipy-H), 7.80 (d, 2H, H₁₀), 8.02-8.09 (m, 2H, bipy-H), 8.24 (d, 1H, J=7.9, bipy-H), 8.34 (d, 2H, J=8.2, bipy-H), 8.46-8.54 (m, 3H, bipy-H), 9.93 (d, 2H, J=5.4, H₁₁). ¹³C NMR (CD₃CN) δ 162.74 (C₄), 152.66 (C₁₀), 152.52 (C₁₁), 151.68 (bipy-C), 150.53 (C₂), 147.51 (C₉), 136.01, 135.80, 135.64, 134.99 (bipy-C), 134.77 (C₆), 126.67, 126.52, 126.15, 125.52, 123.55, 123.50, 123.30, 123.05, 122.86, 122.53 (bipy-C), 108.92 (C₅), 86.95 (C₄'), 85.22 (C₁'), 70.30 (C₃'), 61.14 (C₅'), 42.43 (C₈), 39.56 (C₂'), 11.90 (C₇). TOF-MS; C₃₆H₃₅ClN₇O₅Ru, M-PF₆+H calc. for 782.1432 observed: 782.1428.

**DNA Gel Binding Assay:** The reactions of compound 4, 5 and cis-platin with DNA were conducted using pUC18 circular plasmid DNA (1µg/µl) and analyzed by gel electrophoresis. All reactions contain 1µl of DNA in a total volume of 100µl. To the first sample 99µl H₂O was added giving control lane 1. Lane 2 contains 20µl DMF and 79µl H₂O. Lanes 3, 4 and 5 contain, respectively, 20µl 15µmol/L compound 4, 5 and cis-platin in DMF solution with 79µl H₂O in each sample to give a final concentration of 3µmol/L for gel test. Then all samples were incubated at 37.8°C for 4h and analyzed by electrophoresis in 1% agarose gel by applying 230mA for 40 mins in approximately 500ml of a Tris buffer solution. Gels were then stained in Ethidium bromide and photographed with UV illumination.

**Electronic spectroscopy:** An HP8453 photodiode array spectrophotometer with 2 nm resolution was utilized for electronic absorption spectra at room temperature. The nucleosides and the complexes were run in acetonitrile. All spectra were recorded at 298K.
**Electrochemistry:** A one-compartment, three electrode cell, CH-instruments analyzer, equipped with a platinum wire auxiliary electrode were used for cyclic voltammograms. The working electrode was a 2.0 mm diameter glassy carbon disk from CH- Instruments. Before using this electrode, it was polished first by using 0.30 um followed by 0.50 um alumina polish (Buehler) and then sonicated for 10 seconds. Potentials were referenced to an Ag/AgCl electrode, CH-Instruments. Tetrabutylammonium hexafluorophosphate ($\text{Bu}_4\text{NPF}_6$) (0.1M) was the supporting electrolyte and the measurements were taken in acetonitrile.

**Plasmid Photocleavage:** Buffered solutions of pUC18 DNA and pUC18/ruthenium complexes at a ratio of 10:1 bp to complex were placed side by side in quartz cuvettes and irradiated with a 300W mercury arc lamp (Oriel) equipped with a colored glass filter (Newport FSR-GG420) blocking wavelengths shorter than 420 nm. Samples were run in 1% agarose gel using 150 V for 1 hour in 300 mL of Tris buffer solution. Samples were analyzed in 10 minute intervals. Gels were then stained in Ethidium bromide and photographed with UV illumination.
CHAPTER 4
CONCLUSIONS

We synthesized and characterized several synthetic intermediates in the process of preparation of the target compound, 5 and 6, as listed in the Table 3. All compounds were characterized through $^1$H and $^{13}$C NMR, 2-D NMR techniques, TOF-MS spectroscopy and for (5) $^{195}$Pt-NMR. We also attempted to synthesize other intermediates for metal complexation but failed to obtain the product. Incubation of compound 5 and 6 with pUC18 circular plasmid DNA has been performed and followed by gel electrophoresis. All results show the formation of linearized forms of DNA. Electronic spectroscopy and electrochemistry indicate correct structure of Nucleoside-PPY (6). DNA plasmid photocleavage indicates the high reactivity towards DNA. This result bodes well for their possible anticancer activity.
<table>
<thead>
<tr>
<th>Compounds synthesized</th>
<th>% yield</th>
</tr>
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<tr>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>55.7%</td>
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<tr>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>65.3%</td>
</tr>
<tr>
<td><img src="image3" alt="Chemical Structure" /></td>
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</tr>
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<td><img src="image4" alt="Chemical Structure" /></td>
<td>60.0%</td>
</tr>
<tr>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>60.1%</td>
</tr>
</tbody>
</table>

Data: \(^1\text{H NMR, }^{13}\text{C NMR, TOF-MS}\)
The large downfield coordination shift of the C9 carbon of the target compound (5) by +15.7 ppm and the C10 carbon of the target compound (6) by +30.39 ppm require further discussion. There may be conformational effects on NMR shifts induced by complexation to a chiral nucleoside. Also further experimentation will be required to confirm the decomposition mechanism of the target compound (5) in DMSO$_{6}$ solution. The compounds should be tested on mammalian cancer cells grown in culture to determine their utility as antineoplastic agents.
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   Carboplatin-DNA Binding in Genomic DNA and Bladder Cancer Cells As 
   622-626


APPENDIX

STRUCTURE NUMBERING

\textbf{N-3-(4-pyridylmethyl)-thymidine (4)}

\begin{center}
\includegraphics[width=0.7\textwidth]{structure.png}
\end{center}