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Cisplatin analogues containing 4,4'-bis[alkyl]-2,2'-bipyridine derivatives

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CISPLATIN ANALOGUES CONTAINING 4,4′-BIS[ALKYL]-2,2′-BIPYRIDINE DERIVATIVES

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ABSTRACT

Cisplatin Analogues Containing 4,4′-Bis[alkyl]-2,2′-bipyridine Derivatives

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Cisplatin is used clinically to treat various types of cancer. While effective in treating a variety of cancers it exhibits a wide range of side effects. Synthesis of novel cisplatin analogs, which exhibit increased cytotoxicity at lower doses, may alleviate some of the side effects.

These analogs utilize 4,4′-bis(alkyl)-2,2′-bipyridine, a diimine, in place of the ammonia ligands present in cisplatin and the addition of substituents to the 4,4′-position as well as coordination to $PtCl_2$ to yield a series of (4,4′-bis[alkyl]-2,2′-bipyridine) $PtCl_2$ compounds. Modifications at the 4,4′-position occur by generating a benzylic dianion by way of lithium diisopropyl amide (LDA) and then substituting with the desired alkyl halide in a S_N2 reaction. The different substitutions made in the 4,4′ position are as follows: ethyl, propyl, *n*-butyl, methoxy and 3-methoxy-*n*-propyl. All of the coordination reactions have been done by ligand exchange where by $PtCl_2$ exchanges from 1,5-cyclooctadiene (COD) to the bipyridine ligand.

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ABBREVIATIONS

Abbreviation	Definition
AAG	3-methyladenine DNA glycosylase
bipy	2,2′-bipyridine
n-BuLi	*n*-butyllithium
Cisplatin	*cis*-diamminedichloroplatinum (II)
$CDCl_3$	deuterated chloroform
COD	1,5-cyclooctadiene
COD-$PtCl_2$	1,5-cyclooctadiene platinum (II) dichloride
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNA-PK	deoxyribonucleic acid protein kinase
EC_{50}	effective concentration (50%)
G	guanine
G_2	gap 2 phase of cell cycle
GG	guanine-guanine
GC/MS	gas chromatography/ mass spectroscopy
HBV	hepatitis B virus
HeLa	human cervix carcinoma

HMG	high mobility group proteins
His	histidine
HMG	high mobility group proteins
HPV	human papiloma virus
LDA	lithium diisopropyl amide
Met	methionine
MMR	mismatch repair
NER	nucleotide excision repair
NMR	nuclear magnetic resosnance
$K_2Cr_2O_7$	potassium dichromate
K_2PtCl_4	potassium tetrachloroplatinate (II)
Pd/C	palladium over carbon
ppm	parts per million
ppt	precipitate
PPTS	pyridinium *p*-toluene sulfonate
RBF	round-bottomed flask
RNA	ribonucleic acid
SSRP1	structure-specific recognition protein 1
TBP	TATA-binding protein
THF	tetrahydrofuran

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CHAPTER 1

INTRODUCTION

Cisplatin is used clinically to treat various types of cancer. While effective in treating testicular, ovarian, head and neck tumors, it exhibits side effects including "nephrotoxicity, neurotoxicity and ototoxicity" [1]. The objective of the project was to synthesize novel cisplatin analogs that may exhibit increased cytotoxicity at lower doses and alleviate some of the side effects. These analogs utilized 2,2′-bipyridine, a diimine, in place of the ammonia ligands present in cisplatin. Further modifications included the addition of substituents to the 4,4′-position. Over the course of the investigation, we have generated a few novel 4,4′-disubstituted-2,2′-bipyridine compounds as part of a homologous series of cisplatin analogues as well as made improvements to synthetic procedures of previously synthesized compounds.

According to the American Cancer society it is expected that in this year alone 1,444, 920 new cases of cancer will be diagnosed [2]. Cancer is one of the most complicated and idiosyncratic group of diseases fought by modern medicine today. Cancer is often characterized by uncontrolled growth and motility of abnormal cells, which cause problems in vital organs, circulatory and other systems. Various factors are causative agents of these diseases and these

factors act either singly or synergistically. However, it is not known specifically what the actual causative agents of cancer are from patient to patient. External or environmental factors include exposure to carcinogenic and mutagenic chemicals such as tobacco smoke, solvents etc., radiation exposure, such as UV rays and exposure to infectious organisms such as Hepatitis B Virus (HBV) and Human Papiloma Virus (HPV) [2]. Internal factors are often inherited mutations, which can exhibit problems in hormonal, immune and in general metabolic systems [2]. Various methods exist for treating cancer such as chemotherapy, radiation and surgery. These therapies can be used individually or concomitantly [2]. Cisplatin is a commonly used chemotherapy drug.

1.1 A Brief History of Cisplatin

In 1845, Michael Peyrone synthesiszed cis-diamminedichloroplatinum (II), then called Peyrone´s chloride, currently known as cisplatin. The structure of this compound was not elucidated until 1893 by Alfred Werner [3]. (See Figure 1) In 1965, Rosenberg and his team discovered, quite by accident, when using a platinum electrode, there was inhibition of cell division in *Escherichia coli* grown in ammonium chloride media. Later the compound causing this inhibition was found to be cisplatin [4]. Afterward, Rosenberg and his team isolated and tested this compound on sarcoma 180 and leukaemia L1210 in mice and found the compound to be a potential new class of anti-tumour agents [5]. In 1971, cisplatin entered clinical trials, and in 1977 was marketed exclusively by Bristol-Myers Squibb, under the brand name Platinol [3.] By 1985 approximately 2000 analogs

of cisplatin and cisplatin-related compounds had been investigated [6] in an effort to ameliorate the high toxicity exhibited by cisplatin. Nephrotoxicity in cisplatin is dose limiting, [7], however hydration therapy diminishes dramatically the toxicity allowing the increase of the dose up to 3 times [8].

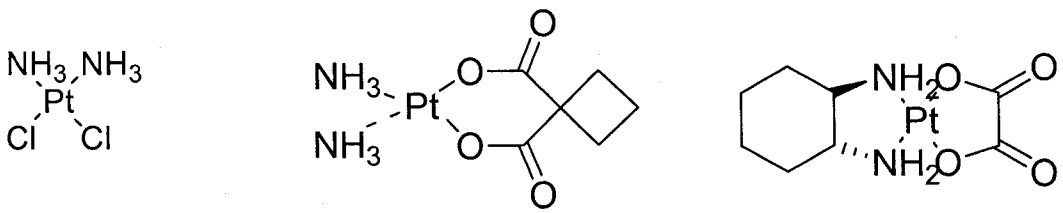


Figure 1. Cisplatin, Carboplatin and Oxaliplatin, respectively.

1.2 Mechanism of Action of Cisplatin

The exact mechanism by which cisplatin is able to execute its cytotoxicity is not well known. High levels of chlorides are found in extracellular and extranuclear fluids allowing cisplatin to remain in its stable, nonreactive dichloro form [9]. Within the cellular membranes the concentration of chloride drops dramatically from approx 1.5*M* to approx 4m*M*, thus kinetic activation of cisplatin is rendered once it crosses the cell membrane and it exchanges its chlorides for water yielding a more reactive monoaqua and diaqua species [10].

cis-$Pt(NH_3)_2Cl_2$ $(H_20)\rightarrow$*cis*$[Pt(NH_3)_2Cl(H_20)]^+ + Cl^-\rightarrow$*cis*$[Pt(NH_3)_2Cl(H_20)_2]^{++} + Cl^-$

Cisplatin binds in a 1,2-G,G-intrastrand and G,C-interstrand adduct crosslinks with DNA (see Figure 2), specifically binding to N7 atoms of guanine residues in the same or opposite strands, correspondingly [11,12]. With the formation of the 1,2-G,G-intrastrand adducts important modifications are made to the topological structure of DNA which becomes bent or kinked [13, 14]. It is believed at this

point the HMG (High Mobility Group) proteins recognize the adduct formed [15,16] and DNA repair mechanisms are triggered. The nucleotide excision repair (NER) system seems to be the main system for repair of damage caused by cisplatin [17,18,19,20]. Mismatch Repair (MMR) system recognizes 1,2-G,G-intrastrand adduct, via hMutS-α, but does not remove the adduct or is incapable of doing so, thus inducing signaling to an apoptotic pathway [21,22]. It has been reported that the cisplatin-DNA adduct has the capability to inhibit the kinase activity of DNA-PK protein, a repair protein, by binding to the Ku subunit of DNA-PK [23]. The HMG DNA-binding moiety is found in structure-specific recognition protein 1 (SSRP1) has a high specificity recognition for 1,2-G,G-intrastrand adducts [24] reinforcing the unknown but highly speculated hypothesis that the 1,2-G,G-intrastrand adduct cross-links are the major adducts formed in the action mechanism of cisplatin [25].

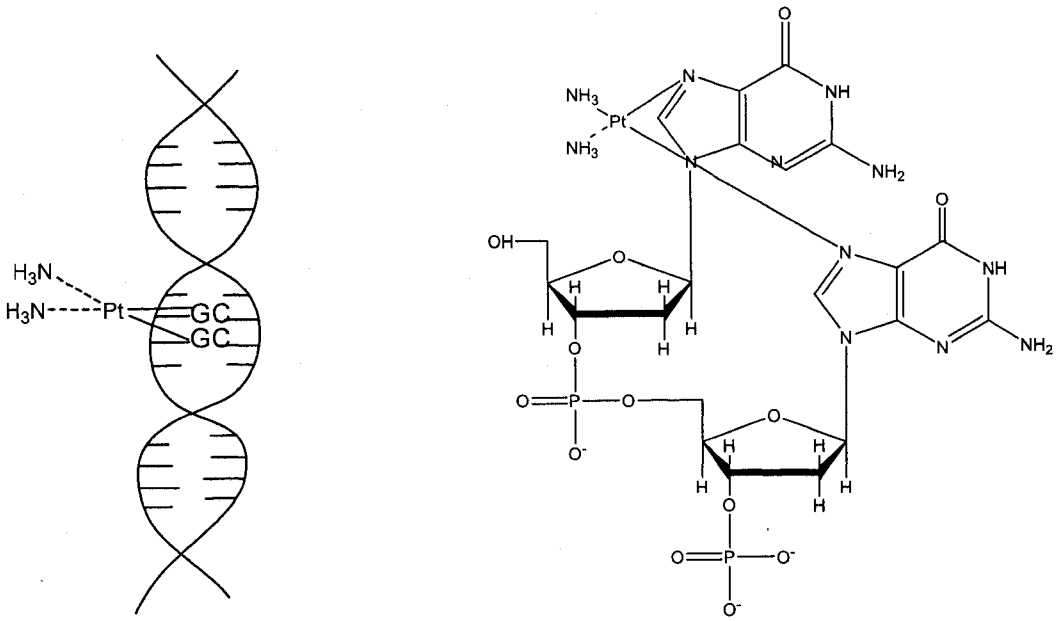


Figure 2. 1,2-G,G-Intrastrand Adduct.

Two mechanisms of action via HMG proteins have been postulated: The "repair shielding model" proposes that the protection of adducts by HMG proteins could be affecting recognition, by DNA repairing enzymes [26] and the "hijacking model" implies triggering of cell cycle events leading to cell death by SSRP1 and/or other HMG proteins [27]. Besides the HMG-domain family of proteins, other proteins such as TATA-Binding Protein (TBP) and 3-methyladenine DNA glycosylase (AAG) seem to recognize the cisplatin-DNA adduct [28,29]. Cisplatin degrades and shortens telomeres suggesting yet another mechanism of cell death in HeLa (human cervix carcinoma) cells [30]. G_2 phase cell cycle arrest induced by cisplatin-adducts apparently indicates inhibition of transcription by RNA Polymerase II and its consequent degradation by ubiquitination [31]. A more direct interaction with ubiquitin may engender a different cytotoxic response, cisplatin binds to ubiquitin protein in two sites: Met1 and His68, and 4 types of adducts are known to be formed [32]. A direct relationship has not yet been established between p53-mediated response to cisplatin induced cell damage, for each cell type and cell environment there seems to be an idiosyncratic response [33]. All the previously mentioned mechanisms could work individually or concomitantly, or could be part of the same cascade of events or related to yet unknown events, it is not known how they relate to each other based on cisplatin adduct formation and induced damage. [31]

1.3 A Brief History of 2,2′-Bipyridine

2,2′-bipyridine was first synthesized by Fritz Blau in 1888 via dry-distillation of picolinic acid copper salt [32]. The first synthesis of substituted 2,2′-bipyridines was carried out by Case in 1946 utilizing an Ullman reaction with a copper metal catalyst [33]. From that point on the popularity of bipyridines as a building block has increased dramatically, many preparation improvements have been made and its use in various subfields of chemistry, predominantly as a metal chelating agent in coordinate systems, has found numerous applications in: analytical chemistry [34], electrochemistry [35], polymer chemistry [36], photonics and optoelectronics [37] and more recently although less thoroughly investigated in biochemistry (see section 1.4 for more detail and references).

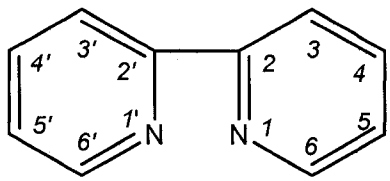


Figure 3. 2,2′-Bipyridine with Ring Numbering Scheme.

Symmetric substitution synthesis in the 3,3′, 4,4′, 5,5′, 6,6′ positions of the 2,2′-bipyridine has been thoroughly researched [38], however there seems to be a need for more efficient synthetic systems to be designed as will be discussed in the results section. Our primary research focus is on the 4,4′ position substitution of 2,2′-bipyridine system. Many 4,4′-disubstituted-2,2′-bipyridines where synthesized by utilizing similar classic organic reactions, such as nitration, for the introduction of substituents into the conjugated rings [33, 39]. Consideration must be given to the fact that the 2,2′-bipyridyl moiety has a

tendency to form *N,N′*-dioxide (or 1,1′-dioxide) occurring after an oxidation reaction, and must be reduced [39]. 4,4′-Bis(methyl)-2,2′-bipyridine was synthesized utilizing 2-bromo-4-methylpyridine heated with copper powder to 220 °C [33]. 4,4′-Bis(ethyl)-2,2′-bipyridine was first synthesized by utilizing 4-ethylpyridine by refluxing with degassed Raney Ni as the catalyst, to generate a heterocyclic biaryl [40]. 4,4′-Bis(*n*-butyl)-2,2′-bipyridine was first synthesized utilizing 4-*n*-butylpyridine catalyzed by Pd/C [41]. 4,4′-Bis(methoxy)-2,2′-bipyridine was fist synthesized from 4,4′-Bis(nitro)-2,2′-bipyridine and sodium methoxide yielding 4,4′-bis(methoxy)-2,2′-bipyridine-1,1′-dioxide and reduced by phosphorous trichloride in chloroform [39].

1.4 Generation of Novel Compounds as Cisplatin Analogues

Various reasons generate a demand for novel pharmaceutical compounds, specially in cancer pharmacology, for example: side-effects that are extremely taxing to patients, inactivity or drug-resistance from one cancer-type to the next and differences in response to drug, from patient to patient. Despite the large number of compounds related to cisplatin that have been synthesized over the years, only two related anti-cancer compounds, besides cisplatin, have been FDA approved, carboplatin and oxaliplatin [42]. (see Figure 1)

Before considering what to synthesize as an analogous compound, one must look at the structure and properties that incite such activity. General structure-activity relationships for cisplatin-related compounds have the following properties: 1) complexes should be electrically neutral, 2) one bidentate or two

monodentate structure with *cis* conformation are the requirement for the non-exchanging ligand as well as the exchanging or leaving groups, 3) leaving groups should not be too stable or too strongly reactive, 4) the non-exchanging ligand should be firmly bonded and be a strongly donating group such as a primary or secondary amine [43].

The 2,2′-bipyridine structure is considered a bidentate ligand in which its nitrogen atoms bind, in a coordinate manner, to metals, in this case specifically platinum. By binding the bipyridine ligand to Pt (II), a structural moiety similar to that of cisplatin is achieved. Both cisplatin and a Pt (II) coordinated 2,2′-bipyridine molecules are square planar [44], hypothetically rendering both similar functionality and binding.

It has been demonstrated that the 2,2′-bipyridyl Pt (II) complex can intercalate DNA [45]. Whether this intercalation is similar to the binding of cisplatin to DNA is not known and has not yet been explored. Garelli et al. have synthesized a series of 4,4′-perfluoroalkyl-2,2′-bipyridine ligands complexed to Pt (II) incorporated into liposomes and tested these compounds for cytotoxic activity in CAL 27 cell line. [46,47,48]. These compounds show cell growth inhibition but in a lesser magnitude than cisplatin [48]. In another study, 3,3′-disubstituted-2,2′-bipyridines ligands complexed to Pt (II) were synthesized and tested for their activity in leukemia L1210 cell line, and showed no anti-tumor activity[49]. It must be considered that the 3,3′ substituted position has a non-coplanar conformation due to steric hindrance [38] and this may be the reason for its lack of cytotoxic activity. Another study dealing with a similar structure of a

2,2′-bipyridine ligated to Pt(II) and 1,1-cyclobutanedicarboxylic acid as the anion demonstrated lesser cytotoxic activity than cisplatin in P_{388} lymphocytic leukemia cells [50]. Elwell et al. demonstrated that [4,4′-Bis(4,4,4-trifluorobutyl)-2,2′-bipyridine] $PtCl_2$ and [4,4′-Bis(methyl)-2,2′-bipyridine] $PtCl_2$ exhibited a higher cytotoxicity than that of cisplatin, from 14 to 125 fold depending on the compound and the cell line [51, 52]. Based on these results, it was decided to synthesize a homologous series of [4,4′-Bis(alkyl)-2,2′-bipyridine] $PtCl_2$, of increasing chain size in the 4,4′ position for the specific purpose of future testing in various cell lines and in order to produce some type of structure-activity relationship specific to these compounds and how these particular substituents in the 4,4′ position affect biochemical activity and perhaps determine the mechanism of action within the cell.

In the following investigation, 4,4′-bis(methyl)-2,2′-bipyridine was used as the starting material for the synthesis of the 4,4′-bis(alkyl)-2,2′-bipyridines. A typical procedure involved generating a dianion species at the benzyl position, utilizing lithium diisopropylamide (LDA) as the strong base to remove the methyl protons, and a consequent S_N^2 reaction with an alkyl halide afforded the modifications to the ligand [46, 51, 53]. Then a ligand exchange reaction between 1,5-cyclooctadiene-$PtCl_2$ (COD-$PtCl_2$) and the 4,4′-Bis(substituted)-2,2′-bipyridine ligand was executed, yielding the final Pt (II) coordinated bipyridine [51,54].

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

The following chemicals were purchased from Alfa Aesar: 2,2′-bipyridine, 4,4′-dimethyl-2,2′-bipyridine, 2.5 and 2.9 M *n*-BuLi (in hexanes), diisopropyl amine, iodomethane, iodoethane, 2-bromoethyl methyl ether, 2-(2-bromoethoxy) tetrahydro-2H-pyran, 2-(2-Bromoepropoxy) tetrahydro-2H-pyran, PPTS and acetone-d_6 with 1% TMS. 4,4′-dimethoxy-2,2′-bipyridine, 4,4′-dialdehyde-2,2′-bipyridine, 1,5-cyclooctadiene $SnCl_2$ and DMSO-d_6 with 0.1% TMS were purchased from Aldrich. K_2PtCl_4, $CDCl_3$ with 0.03% TMS and $K_2Cr_2O_7$, were purchased from Acros. *n*-Propyl iodide was obtained from TCI America. . 4,4'-diamine-2,2'-bipyridine was purchased from Carbosynth Limited, UK. All materials purchased were above 95% purity and were used without further purification unless noted in the procedure.

2.2. Spectroscopy and Analytical Methods.

NMR spectra were obtained utilizing Varian 400 MHz VnmrJ 2.1A (Palo Alto, CA.) ^1H, 400 MHz and ^{13}C, 100 MHz spectra were obtained utilizing the following deuterated solvents:$CDCl_3$, DMSO-d_6 and acetone-d_6, all of which

contain TMS and were used for reference peaks. GC/MS spectra were obtained utilizing Varian 4000 GC/MS/MS (Palo Alto, CA.) Elemental Analysis was provided by Desert Analytics, Tucson, AZ, USA and by Dr. S. Steinberg in the UNLV Chemistry department utilizing an Exeter CE 440 (UK)

2.3 Synthetic Methods

General note for synthetic methods: all reactions substituting the 4,4´ position in the bipyridine that utilized the strong base lithium diisopropylamide (LDA) were carried out under inert atmosphere, N_2 or Ar. This atmosphere was exchanged utilizing a Schlenk line gas and vacuum set up. All transfers were done via purged syringes and canulas. The solvent utilized in these reactions was anhydrous THF and it was directly used without further drying. All glassware was cleaned and dried in an oven and purged before use.

2.3.1. Preparation of COD-$PtCl_2$, **1**

A 500 mL round-bottomed flask (RBF) was charged with 2.5 g (6.0 mmol) of K_2PtCl_4, exchanged atmosphere to N_2 using a vacuum Schlenk line, and 40 mL of distilled H_2O was added to the flask. Then on a 50 mL RBF, 27.5 mL of *n*-propanol, 5 mL of 1,5-cyclooctadiene (COD) and 37.5 mg of $SnCl_2$ were combined and allowed to dissolve. After dissolving, the solution was added to the 500 mL RBF and stirred at room temperature under N_2 for 94 hrs. The crude product was filtered through a coarse fritted funnel and washed with ethanol and distilled H_2O and afterward allowed to air dry. The washed solid was weighed

yielding 2.107g (5.63 mmol) (94% yield) in the form of a white powder, which was used without further purification [54].

2.3.2. Preparation of (2,2′-bipyridine) $PtCl_2$, **2**

A 100 ml RBF was charged with 0.150 g (0.960 mmol) of 2,2′-bipyridine and 0.359 g (0.960 mmol) of COD-$PtCl_2$ along with 20 mL of acetonitrile. The RBF was set-up with a condenser and in an oil bath to reflux at 95 °C for 24 hrs. The RBF was cooled, all solvent was evaporated to dryness, then additional acetonitrile was added to the crude product and filtered onto a fritted filter. The solid was washed with copious amounts of water, and allowed to air dry. The dry solid weighed 0.3528g (0.836 mmol) (87% yield) and was in the form of a bright yellow powder, m.p. >380 °C (decomposition).

2.3.3.A. Preparation of 4,4′-bis[ethyl]-2,2′-bipyridine, **3**

A 3-neck 250 ml RBF, with a drip funnel attached, was charged with a stir bar and 1.54 mL (11 mmol) of diisopropyl amine and 20 mL of THF was added via syringe under an argon atmosphere at 0 °C in a brine bath. Then 4.4 mL (11 mmol) of *n*-BuLi (2.5 M) was added, via syringe, drip wise to the same flask. A 50 ml RBF was charged with 0.920 g (5 mmol) of 4,4′-dimethyl-2,2′-bipyridine, degassed with a vacuum and dissolved in 30 mL of anhydrous THF and transferred to the drip funnel. The dissolved bipyridyl was dripped slowly onto the lithium diisopropylamide (LDA) to produce a dark red colored anion. After 10 min, 0.68 mL (11 mmol) of iodomethane was added to the 3-necked RBF via syringe. The solution was stirred for 24 hrs and allowed to warm to room temperature. The reaction was under an argon atmosphere at all times. After 24

hrs, the product had a yellow oily appearance. This crude product was extracted with water and diethyl ether, and dried with Na_2SO_4. The final product was isolated 0.9137g (4.309 mmol) (86% yield) in the form of a yellow oily liquid.

2.3.3.B. Preparation of (4,4′-bis[ethyl]-2,2′-bipyridine) $PtCl_2$, **4**

Coupling of compound **3** to COD-$PtCl_2$, **1**, was carried out in the following manner. A 100 mL RBF was charged with 0.212 g (1.0 mmol) of compound **3** and 0.374 g (1.0 mmol) of COD-$PtCl_2$ and 10 mL of acetonitrile, set up with a condenser and allowed to reflux for 24 hrs in an oil bath at 95 °C. The RBF containing the mixture was cooled to room temperature. The solvent was removed by rotary evaporation. The resulting precipitate was washed in acetonitrile and filtered through a fritted funnel. The final product was weighed 0.3483g (0.729 mmol) (73% yield) in the form of a bright yellow powder, m.p. 368-370 °C.

2.3.4.A. Preparation of 4,4′-bis[propyl]-2,2′-bipyridine, **5**

A 3-necked 250 ml RBF, with a drip funnel attached, was charged with stir bar and 1.54 mL (11 mmol) of diisopropyl amine and 20 mL of THF under an argon atmosphere at 0 °C in a brine bath. Then 3.79 mL (11 mmol) of *n*-BuLi (2.9 M) was added drop wise to the same flask. A 50 ml RBF was charged with 0.920 g (5 mmol) of 4,4′-dimethyl-2,2′-bipyridine, degassed under a vacuum and dissolved in 30 mL of anhydrous THF and transferred to the drip funnel. The dissolved bipyridyl was dripped slowly onto the LDA to produce a dark red colored anion. After 10 min, 0.88 mL (11 mmol) of iodoethane was added to the 3-neck RBF via syringe. The solution was stirred for 24 hrs and allowed to warm

to room temperature. The reaction was under an argon atmosphere at all times. After 24hrs, the product has yellow oily appearance. This crude product was extracted with water and diethyl ether, and dried with Na_2SO_4. The crude product gave a mixture of two compounds monosubstituted (1/3 of total product) and disubstituted (2/3 of total product). This is discussed in more depth in the results and discussion section. Due to this mixture, silica gel column chromatography was used for separation of products, with ethyl acetate as the eluent. The final product was isolated 0.800 g (3.3mmol) (66% yield) in the form of a yellow oily liquid.

2.3.4.B. Preparation of (4,4′-bis[propyl]-2,2′-bipyridine) $PtCl_2$, **6**

Coupling of compound **5** to COD-$PtCl_2$, **1**, was carried out in the following manner. A 100 mL RBF was charged with 0.127 g (0.529 mmol) of compound **5** and 0.1979 g (0.529 mmol) of COD-$PtCl_2$ and 10 mL of acetonitrile, set up with a condenser and allowed to reflux for 24 hrs in an oil bath at 95 °C. The RBF containing the mixture was cooled to room temperature. The solvent was removed by rotary evaporation. The resulting precipitate was washed in acetonitrile, filtered through a fritted funnel and allowed to air dry. The final product was isolated 0.1869 (0.369 mmol) (70% yield) in the form of a bright yellow powder, m.p. 214-216 °C.

2.3.5.A. Preparation of 4,4′-bis[n-butyl]-2,2′-bipyridine, **7**

A 250 ml 3-neck RBF, with a drip funnel attached, was charged with stir bar and 1.75 mL (12.5 mmol) of diisopropyl amine and 20 mL of THF under a N_2 atmosphere at 0 °C in a brine bath. Then 4.8 mL (12.5 mmol) of *n*-BuLi (2.6 M)

was added drip wise to the same flask. A 50 ml RBF was charged with 0.920 g (5mmol) of 4,4′-dimethyl-2,2′-bipyridine, degassed under a vacuum and dissolved in 30 mL of anhydrous THF and transferred to the drip funnel. The dissolved bipyridyl was dripped slowly onto the LDA to produce a dark red colored anion. After 10 min, 1.21 mL of *n*-propyl iodide, that was passed through a mini pipette silica column, was added to the 3-neck RBF. The solution was stirred for 24 hrs and allowed to warm to room temperature. The reaction was under a N_2 atmosphere at all times. After 24hrs, the product had a yellow oily appearance. This crude product was extracted with water and diethyl ether, and separated through a silica column using ethyl acetate. The final product was isolated 0.9751g (3.638 mmol) (73% yield) in the form of a yellow oil [55].

2.3.5.B. Preparation of (4,4′-Bis[*n*-butyl]-2,2′-bipyridine) $PtCl_2$, **8**

Coupling of compound **7** to COD-$PtCl_2$, **1**, was carried out in the following manner. A 100 mL RBF was charged with 0.1151 g (0.429 mmol) of 1 and 0.1607 g (0.429 mmol) of COD-$PtCl_2$ and 10 mL of acetonitrile, set up with a condenser and allowed to reflux for 24 hrs in an oil bath at 95 °C. The RBF containing the mixture was cooled to room temperature. The solvent was removed by rotary evaporation. The resulting precipitate was washed in acetonitrile and filtered through a fritted funnel. The final product was weighed 0.1743g (0.3262 mmol) (76% yield) in the form of a bright yellow powder, m.p. 215-218 °C.

2.3.6. Preparation of (4,4′-bis[methoxy]-2,2′-bipyridine) $PtCl_2$, **9**

In a 100 ml RBF was charged with 0.216 g (1.0 mmol) of 4,4′-dimethoxy-2,2′-bipyridine and 0.374 g (1.0 mmol) of COD-PtCl2 along with 20 mL of acetonitrile. The RBF was set-up with a condenser and in an oil bath to reflux at 95 °C for 24 hrs. The RBF was cooled, all solvent was evaporated to dryness, then acetonitrile was added to the crude product and filtered in to a fritted filter. The solid was washed with copious amounts of water and allowed to air dry. The final solid was weighed 0.3075g (0.6377 mmol) (64% yield) in the form of a bright yellow powder, m.p. 318-320 °C.

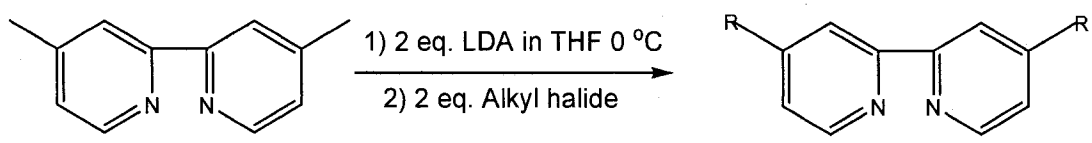
2.3.7.A. Preparation of 4,4′-bis[3-methoxy-*n*-propyl]-2,2′-bipyridine, **10**

The same method for preparing compound **3** applies. The only difference was 4 equivalence of 2-Bromo-ethyl methyl ether was added. If not used in excess, this substituents gives part monosubstituted (10 % of total yield) and part disubstituted (90 % of total yield) products based on possible side reactions and elimination in a strong base. The product was extracted and purified same as compound **3**. The final product was isolated 0.5229g (1.743 mmol) (35 % yield) in the form of a white crystal, m.p. 38-40 °C.

2.3.7.B. Preparation of (4,4′-bis[3-methoxy-*n*-propyl]-2,2′-bipyridine) $PtCl_2$, **11**

Compound **11** was synthesized in the same manner as was compound **2** utilizing 0.1059 g (0.353 mmol) of 4,4′-bis[n-butyl-4-methoxy]-2,2′-bipyridine and 0.1321g (0.353 mmol) of COD-$PtCl_2$. The final product was weighed 0.1738g (0.307 mmol) (87% yield) in the form of a bright yellow powder, m.p.177-180 °C.

2.4 Structures and Synthetic Schemes

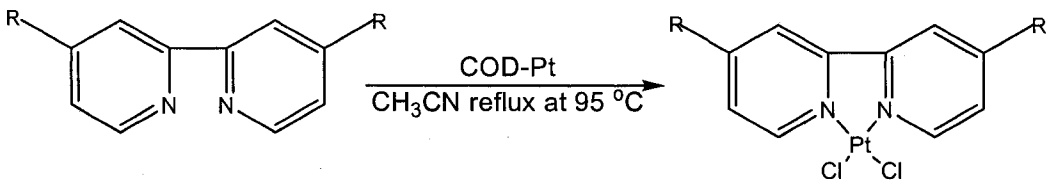


Alkyl Halides Used

CH_3I
CH_3CH_2I
$CH_3CH_2CH_2I$
$CH_3OCH_2CH_2Br$

3 R=-CH_2CH_3
5 R=-$CH_2CH_2CH_3$
7 R=-$CH_2CH2CH_2CH_3$
10 R=-$CH_2CH_2CH_2OCH_3$

Figure 4. Synthetic scheme for 4,4′-Bis(substituted)-2,2′-bipyridine ligands.



3 R=-CH_2CH_3
5 R=-$CH_2CH_2CH_3$
7 R=-$CH_2CH2CH_2CH_3$
10 R=-$CH_2CH_2CH_2OCH_3$

2 R=-H
4 R=-CH_2CH_3
6 R=-$CH_2CH_2CH_3$
8 R=-$CH_2CH2CH_2CH_3$
9 R=-OCH_3
11 R=-$CH_2CH_2CH_2OCH_3$

Figure 5. Synthetic scheme for [4,4′-Bis(substituted)-2,2′-bipyridine] $PtCl_2$.

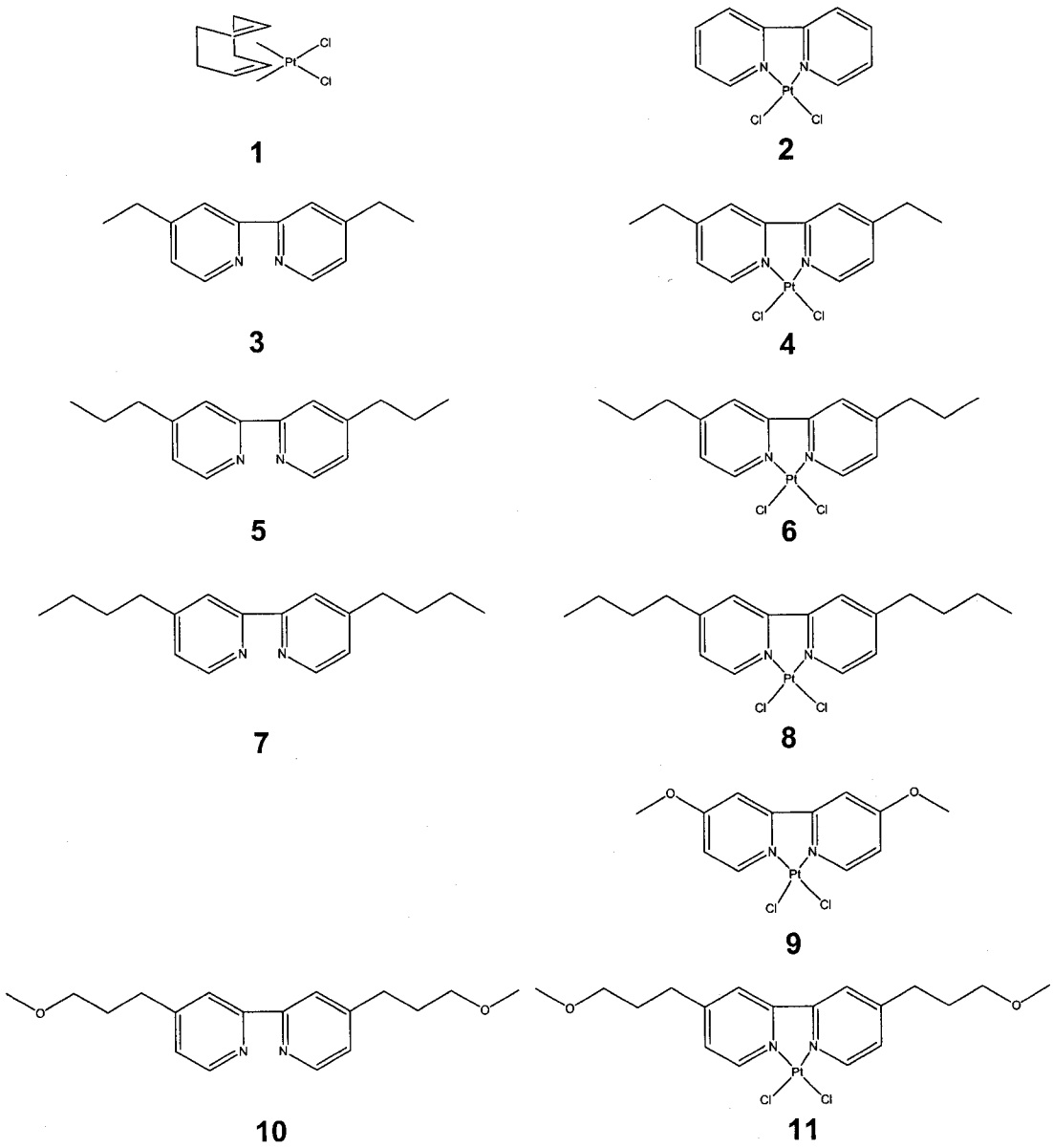


Figure 6. Structures of Compounds.

2.5 Attempted Synthesis of 7 Compounds.

2.5.1 Attempted synthesis of 4,4´-bis[hydroxy]-2,2'-bipyridine, **I**

The synthesis of compound **I** from 4,4´-bis[methoxy]-2,2'-bipyridine was executed via ether cleavage reaction utilizing HBr and glacial acetic acid [56]. However this reaction did not yield a single product and was difficult to isolate.

2.5.2 Attempted synthesis of 4,4´-bis[hydroxymethyll]-2,2'-bipyridine, **II**

The synthesis of compound **II** from 4,4´-bis[methyl]-2,2´-bipyridine was carried out by first oxidizing with $K_2Cr_2O_7$, this product was isolated yielding 4,4´-bis[carboxy]-2,2´-bipyridine. The acid was reacted with H_2SO_4 and CH_3OH to give 4,4´-bis[methoxycarbonyl]-2,2´-bipyridine which was also isolated, then it was reduced with $LiAlH_4$ and did not yield product. It was also consequently reduced with $NaBH_4$ and did not yield the desired product either, regardless of following the literature step by step [57,58].

2.5.3 Attempted synthesis of 4,4´-bis[hydroxypropyl]-2,2'-bipyridine, **III**

The synthesis of **III** from 4,4´-bis[methyl]-2,2´-bipyridine was carried out as in reaction of compound **3** utilizing 2-(2-bromoethoxy) tetrahydro-2*H*-pyran yielding a mono and disubstituted product, which was difficult to isolate via column chromatography [59,60].

2.5.4 Attempted synthesis of 4,4´-bis[hydroxy-*n*-butyl]-2,2'-bipyridine, **IV**

The synthesis of **IV** from 4,4´-bis[methyl]-2,2´-bipyridine was carried out as in reaction of compound **3** utilizing 2-(2-bromopropoxy) tetrahydro-2*H*-pyran yielding a mono and disubstituted product, which was difficult to isolate via column chromatography. [59,60,61]

2.5.5 Attempted synthesis of (4,4′-bis[formyl]-2,2′-bipyridine) $PtCl_2$, **V**

The synthesis of **V** from 4,4′-bis[formyl]-2,2′-bipyridine was carried out with similar methodology as in compound **2**. In this reaction there were two products that were not isolable one was yellow and another green. This is the first instance of synthesis of this compound. The yellow product is believed to be the desired product, the green one seems to have a similar arrangement such as a Magnus' salt [62], however this has not been concretely established.

2.5.6 Attempted synthesis of (4,4′-bis[carboxy]-2,2′-bipyridine) $PtCl_2$, **VI**

The synthesis of **VI** from 4,4′-bis[carboxy]-2,2′-bipyridine was carried out with similar methodology as in compound **2** but did not yield the desired product. This is the first instance of synthesizing this compound via this methodology.

2.5.7 Attempted synthesis of (4,4′-bis[amine]-2,2′-bipyridine) $PtCl_2$, **VII**

The synthesis of **VII** from 4,4′-bis[amine]-2,2′-bipyridine was carried out with similar methodology as in compound 2. This reaction gave the desired product confirmed by ^1H NMR, however the elemental analysis shows a higher percentage for C and H. Analytical Calculated: C 26.56 %, H 2.23 %, N 12.39 %. Experimental: C 32.40 %, H 3.16 %, N 11.32 %. The yellow solid seems to be the correct product but it is contaminated with COD.

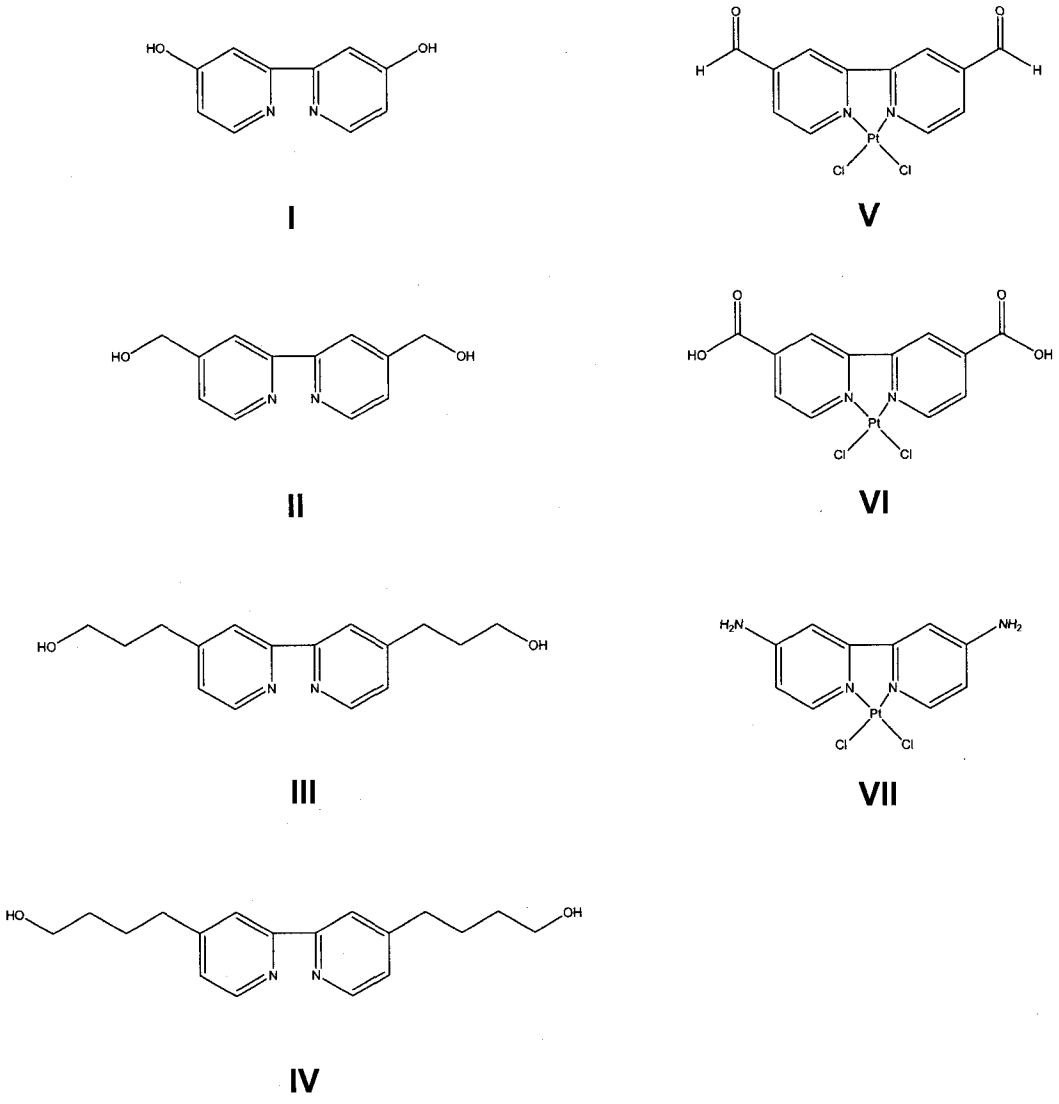


Figure 7. Attempted Synthesis of 7 Compounds.

CHAPTER 3

RESULTS

The following contains the analytical and spectroscopic characterization results of the products synthesized by the reactions described in the Materials and Methods section. (See Appendix for ^{1}H, ^{13}C NMR and GC/MS spectra.)

3.1. (2,2´-Bipyridine) $PtCl_2$, **2**

Analytical Calculated for $C_{10}H_8Cl_2N_2Pt$: C 28.45 %, H 1.91 %, N 6.64 %. Experimental: C 28.49 %, H 1.77 %, N 6.42 %.

NMR: ^{1}H (400 MHz, 298.1 K, DMSO-d_6) δ 9.514, (d, *J*=4.00 Hz, 2H, Ar-H 6,6´), 8.599, (d, *J*=7.60 Hz, Ar-H 3,3´) 8.440, (m, 2H, Ar-H 4,4´), 7.864 (m, 2H, Ar-H 5,5´).

NMR: ^{13}C {^{1}H} (100 MHz, 298.1 K, DMSO-d_6) δ 157.505 (2C, 2,2´) 149.074 (2C,6/6´) 141.216 (2C, 3/3´) 128.395 (2C, 4/4´) 124.868 (2C, 5/5´)

The synthesis for compound **2** has been previously executed [63] by K_2PtCl_4 in HCl and refluxing, however this is the first instance of synthesis utilizing COD-$PtCl_2$ and the methodology mentioned in the previous synthesis section.

3.2.A. 4,4´-Bis[ethyl]-2,2´-bipyridine, **3**

MS (EI: 70 eV, *m/z*) 211 (M^{-1}, 100%), 196 (M^+ -CH_3, 15.2%) 184 (M^+ -CH_3, 21.25%) 169 (M^+ -CH_2, 12.5%)

Compound **3** has previously been synthesized [40]. However, this is the first synthesis of this compound utilizing 4,4′-Bis(methyl)-2,2′-bipyridine as the starting material.

3.2.B. (4,4′-Bis[ethyl]-2,2′-bipyridine) $PtCl_2$, **4**

Analytical Calculated for $C_{14}H_{16}Cl_2N_2Pt$: C 35.16 %, H 3.37 %, N 5.86 %. Experimental: C 34.81 %, H3.41 %, N 5.86 %.

NMR: ^1H (400 MHz, 298.1 K, DMSO-d_6) δ 9.279, (d, $^3J_{HH}$=6.00 Hz, 2H, Ar-H 6,6′), 8.494, (d, $^4J_{HH}$=1.60 Hz 2H, Ar-H 3,3′), 7.697, (dd, $^3J_{HH}$=6.00 Hz, $^4J_{HH}$=1.60 Hz, 2H, Ar-H 5,5′), 2.827, (q, *J*=7.60 Hz, 4H Ar-C$H_2$$CH_3$), 1.337, (t, *J*=7.60 Hz, 4H, Ar-CH_2CH_3);

NMR: ^{13}C {^1H} (100 MHz, 298.1 K, DMSO-d_6) δ 157.140 (2C, 2,2′) 157.110 (2C, 4/4′) 148.322 (2C, 6/6′) 127.986 (2C, 3/3′) 124.719 (2C, 5/5′) 37.338 (2C, Ar-$CH_2$$CH_3$) 23.147 (2C, Ar-$CH_2CH_3$)

Compound **4** is novel.

3.3.A. 4,4′-Bis[propyl]-2,2′-bipyridine, **5**

MS (EI: 70 eV, *m/z*) 241 (M^{+1}, 72.5%), 212 (M^+ -CH_3, 100%) 184 (M^+ -CH_2CH_3, 14.75%)

Compound **5** has previously been synthesized [64].

3.3.B. (4,4′-Bis[propyl]-2,2′-bipyridine) $PtCl_2$, **6**

Analytical Calculated for $C_{16}H_{20}Cl_2N_2Pt$: C37.95 %, H 3.98 %, N 5.53 %. Experimental: C 37.53 %, H3.98 %, N 5.53 %.

NMR: ^1H (400 MHz, 298.1 K, DMSO-d_6) δ 9.286, (d, $^3J_{HH}$=6.00 Hz, 2H, Ar-H 6,6′), 8.491, (d, $^4J_{HH}$=1.602H, Ar-H 3,3′), 7.682, (dd, $^3J_{HH}$=6.00 Hz, $^4J_{HH}$=1.60 Hz,

2H, Ar-H 5,5′), 2.764 (t, *J*=7.60 Hz, 4H, Ar-C*H*$_2$CH$_2$CH$_3$), 1.776, (m, 4H, Ar-CH$_2$C*H*$_2$CH$_3$), 0.972, (t, *J*=7.20 Hz, 6H, Ar-CH$_2$CH$_2$C*H*$_3$)

NMR: ^{13}C {^{1}H} (100 MHz, 298.1 K, DMSO-d$_6$) δ 157.140 (2C, 2,2′) 157.110 (2C, 4/4′) 148.322 (2C, 6/6′) 127.986 (2C, 3/3′) 124.719 (2C, 5/5′) 37.338 (2C, Ar-*C*H$_2$CH$_2$CH$_3$) 23.147 (2C, Ar-CH$_2$*C*H$_2$CH$_3$) 14.151 (2C, Ar-CH$_2$CH$_2$*C*H$_3$)

Compound **6** is novel.

3.4.A. 4,4′-Bis[n-butyl]-2,2′-bipyridine, **7**

NMR: ^{1}H (400 MHz, 298.1 K, CDCL$_3$) δ 8.55, (d, $^3J_{HH}$=4.80 Hz, 2H, Ar-H 6,6′), 8.23, (s, 2H, Ar-H 3,3′), 7.14, (d, $^3J_{HH}$=5.20 Hz, 2H, Ar-H 5,5′), 2.66, (t, *J*=7.60 Hz, 4H, Ar-C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.66, (m, 4H, Ar-CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.36 (m, 4H, Ar-CH$_2$CH$_2$C*H*$_2$CH$_3$), 0.94 (t, *J*=7.20 Hz, 6H, Ar-CH$_2$CH$_2$CH$_2$C*H*$_3$);

MS (EI: 70eV, *m/z*) 269 (M^{+1}, 60%), 226 (M^+-CH$_2$CH$_2$CH$_3$, 100%) 183 (M^+-CH$_2$CH$_2$CH$_3$, 21%)

3.4.B. (4,4′-Bis[butyl]-2,2′-bipyridine) PtCl$_2$, **8**

NMR: ^{1}H (400 MHz, 298.1 K, DMSO-d$_6$) δ 9.266 (d, $^3J_{HH}$=6.00 Hz, 2H, Ar-H 6,6′), 8.486 (d, $^4J_{HH}$=1.20, 2H, Ar-H 3,3′), 7.678 (dd, $^3J_{HH}$=6.40 Hz, $^4J_{HH}$=2.00, 2H, Ar-H 5,5′),), 2.786 (t, *J*=7.60 Hz, 4H, Ar-C*H*$_2$CH$_2$CH$_2$CH$_3$) 1.720 (m, 4H, Ar-CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.398 (m, 4H, Ar-CH$_2$CH$_2$C*H*$_2$CH$_3$), 0.957 (t, *J*=7.20 Hz, 6H, Ar-CH$_2$CH$_2$CH$_2$C*H*$_3$);

NMR: ^{13}C {^{1}H} (100 MHz, 298.1 K, DMSO-d$_6$) δ 157.378 (2C, 2/2′) 157.073.1 (2C, 4/4′) 148.308 (2C, 6/6′) 127.904 (2C, 3/3′) 124.704 (2C, 5/5′) 35.142 (2C, Ar-*C*H$_2$CH$_2$CH$_2$CH$_3$) 31.980 (2C, Ar-CH$_2$*C*H$_2$CH$_2$CH$_3$) 22.455 (2C, Ar-CH$_2$CH$_2$*C*H$_2$CH$_3$) 14.352 (2C, Ar-CH$_2$CH$_2$CH$_2$CH$_3$);

Compound **8** has been previously been synthesized [55]. However, this is the first synthesis of this compound utilizing COD-$PtCl_2$.

3.5. (4,4′-Bis[methoxy]-2,2′-bipyridine) $PtCl_2$, **9**

Analytical Calculated for $C_{12}H_{12}Cl_2N_2O_2Pt$: C 29.89% , H 2.51 %, N 5.81 %. Experimental: C 29.68 %, H 2.51 %, N 5.66 % .

NMR: ^1H (400 MHz, 298.1 K, DMSO-d_6) δ 9.068, (d, $^3J_{HH}$=6.80 Hz, 2H, Ar-H 6,6′), 8.129, (d, $^4J_{HH}$=2.80 2H, Ar-H 3,3′), 7.360, (dd, $^3J_{HH}$=6.80 Hz, $^4J_{HH}$=2.80 Hz, 2H, Ar-H 5,5′), 4.021, (s, 6H, Ar-OCH_3);

NMR: ^{13}C {^1H} (100 MHz, 298.1 K, DMSO-d_6) δ 168.443 (2C, 2,2′) 158.584 (2C, 4/4′) 149.692 (2C, 6/6′) 113.468 (2C, 3/3′) 111.340 (2C, 5/5′) 57.875 (2C, Ar-OCH_3)

Compound **9** is novel.

3.6.A. 4,4′-Bis[n-butyl-4-methoxy]-2,2′-bipyridine, **10**

Analytical Calculated for $C_{18}H_{24}N_2O_2$: C 71.97 %, H 8.05 %, N 9.33 %. Experimental: C 71.82 %, H 7.75 %, N 9.41 %.

NMR: ^1H (400 MHz, 298.1 K, acetone-d_6) δ 8.52, (dd, $^3J_{HH}$=4.80 Hz, $^5J_{HH}$=0.80 Hz, 2H, Ar-H 6,6′), 8.33, (s, 2H, Ar-H 3,3′), 7.21, (dd, $^3J_{HH}$=4.80 Hz, $^4J_{HH}$=2.00 Hz, 2H, Ar-H 5,5′), 3.35, (t, *J*=6.40 Hz, 4H, Ar-$CH_2CH_2CH_2OCH_3$), 3.27, (s, 6H, Ar-$CH_2CH_2CH_2OCH_3$), 2.73 (t *J*=8.00 Hz, 4H, $ArCH_2$), 1.86 (m, 4H, Ar-$CH_2CH_2CH_2OCH_3$);

NMR: ^{13}C {^1H} (100 MHz, 298.1 K, acetone-d_6) δ 156.3 (2C, 2/2′) 152.1 (2C, 4/4′) 149.3 (2C, 6/6′) 124.2 (2C, 3/3′) 120.9 (2C, 5/5′) 71.4 (2C, Ar-

$CH_2CH_2CH_2OCH_3$) 57.9 (2C, Ar-$CH_2CH_2CH_2OCH_3$) 31.8 (2C, Ar-$CH_2CH_2CH_2OCH_3$) 30.5 (2C, Ar-$CH_2CH_2CH_2OCH_3$);

MS (EI: 70eV, *m/z*) 301 (M^{+1}, 34%), 255 (M^+-CH_2OCH_3, 64%), 242 (M^+-$CH_2CH_2OCH_3$, 100%), 184 (M^+- $CH_2CH_2OCH_3$, 32.5%).

Compound **10** is novel.

3.6.B. (4,4´-Bis[n-butyl-4-methoxy]-2,2´-bipyridine) $PtCl_2$, **11**

Analytical Calculated for $C_{18}H_{24}Cl_2N_2O_2Pt$: C 38.17 %, H 4.27 %, N 4.95 %. Experimental: C 38.24 %, H 4.05 %, N 5.12 %.

NMR: ^1H (400 MHz, 298.1 K, DMSO- d_6)δ 9.307, (d $^3J_{HH}$=6.40 Hz, 2H, Ar-H 6,6´), 8.523, (d, $^4J_{HH}$=1.60Hz, 2H, Ar-H 3,3´), 7.703, (dd, $^3J_{HH}$=6.00 Hz, $^4J_{HH}$=1.60 Hz, 2H, Ar-H 5,5´), 3.400, (t, *J*=6.40 Hz, 4H, Ar-$CH_2CH_2CH_2OCH_3$), 3.37, (s, 6H, Ar-$CH_2CH_2CH_2OCH_3$), 2.828 (t *J*=7.60 Hz, 4H, Ar-CH_2), 1.976 (m, 4H, Ar-$CH_2CH_2CH_2OCH_3$)

NMR: ^{13}C {^1H} (100 MHz, 298.1 K, DMSO-d_6) δ 157.378 (2C, 2/2´) 157.073.1 (2C, 4/4´) 148.308 (2C, 6/6´) 127.904 (2C, 3/3´) 124.704 (2C, 5/5´) 71.4 (2C, Ar-$CH_2CH_2CH_2OCH_3$) 57.9 (2C, Ar-$CH_2CH_2CH_2OCH_3$) 31.8 (2C, Ar-$CH_2CH_2CH_2OCH_3$) 30.5 (2C, Ar-$CH_2CH_2CH_2OCH_3$);

Compound **11** is novel.

Note: Peaks appearing in ^1H NMR spectra at 2.50 ppm belong to DMSO-d_6 and at 3.33 ppm belong to H_2O. Peaks appearing in ^{13}C NMR spectra at 40 ppm belong to DMSO-d_6. For GC/MS most of the parent peaks have a M^{+1}, this is due to sample overloading in the column, these compounds correspond correctly in their breakage pattern to the compound characterized.

CHAPTER 4

DISCUSSION

In this investigation six distinct compounds of the type [4,4′-bis(substituted)-2,2′-bipyridine] $PtCl_2$ were successfully synthesized, isolated, purified and characterized. Four of these are novel compounds: **4**, **6**, **9** and **11**, these have not been previously reported in the literature. Compounds **2**, **4**, **6**, **8** are part of a homologous series of increasing number of methylene groups in an alkyl chain at the 4,4′ position. In this series [4,4′-bis(methyl)-2,2′-bipyridine] $PtCl_2$ was omitted due to the fact that it had previously been synthesized as well as biologically tested [51]. Compounds **9** and **11** are part of an incomplete homologous series of increasing number of methylene groups in an alkyl-methoxy chain at the 4,4′ position.

The purpose of this investigation was to synthesize compounds for the purpose of cytotoxic assays, carried out by another team. Some preliminary cytotoxic assay results will be discussed briefly, and they demonstrate the therapeutic potential of the compounds synthesized by this project.

Table 1 presents a comparative analysis of ^1H NMR chemical shifts (400 MHz, 298.1 K, DMSO-d_6) and how these chemical shifts are affected by the

different substituents in the 4,4′ position as well as their effect by the increasing chain length size.

Compound	NMR ^{1}H: (ppm) Ar-6,6′	NMR ^{1}H: (ppm) Ar-5,5′	NMR ^{1}H: (ppm) Ar-4,4′	NMR ^{1}H: (ppm) Ar-3,3′
(2,2′-bipyridine) $PtCl_2$, **2** CAS # 13965-31-6	9.515	7.865	8.441	8.601
(4,4′-bis[ethyl]-2,2′-bipyridine) $PtCl_2$, **4** **Novel Compound**	9.284	7.70	N/A	8.497
(4,4′-bis[propyl]-2,2′-bipyridine) $PtCl_2$, **6** **Novel Compound**	9.286	7.682	N/A	8.491
(4,4′-bis[butyl]-2,2′-bipyridine) $PtCl_2$, **8** CAS # 728910-99-4	9.266	7.678	N/A	8.486
(4,4′-bis[methoxy]-2,2′-bipyridine) $PtCl_2$, **9** **Novel Compound**	9.068	7.360	N/A	8.129
(4,4′-bis[n-butyl-4-methoxy]-2,2′-bipyridine) $PtCl_2$, **11** **Novel Compound**	9.307	7.703	N/A	8.523

TABLE 1. Comparison of H^1 NMR peak shifts in the aromatic region for 6 coordinated compounds.

NMR Comparison between ligand and coordinated compounds show the electron withdrawing effect of the Pt (II) on the bipyridine ring. The effect spans approximately from 0.1 to 1.00 ppm of values shifted downfield. The most affected positions in decreasing order are as follows: 6,6′ with a difference of 0.928 ppm, 4,4′ with a difference of 0.783, 5,5′ with a difference of 0.741 and 3,3′ with a difference of 0.105 ppm. See Table 2 below for actual values.

Compound	NMR ^{1}H: (ppm) Ar-6,6′	NMR ^{1}H: (ppm) Ar-5,5′	NMR ^{1}H: (ppm) Ar-4,4′	NMR ^{1}H: (ppm) Ar-3,3′
(2,2′-bipyridine) CAS # 366-18-7	8.587	7.124	7.658	8.496
(2,2′-bipyridine) $PtCl_2$, **2** CAS # 13965-31-6	9.515	7.865	8.441	8.601

TABLE 2. Comparison of the aromatic region of ^{1}H NMR values for the ligand and its corresponding coordinated compound.

Problems encountered in the synthesis of the ligand:

Having to substitute two identical positions in a molecule presented one of the main obstacles in synthesis and purification of the ligand species. Generating a dianion in the 4,4′ (benzylic) position of a 4,4′-bis(methyl)-2,2′-bipyridine via LDA is generally easy to achieve, as well as substituting that position with an alkyl halide in an S_N2 reaction. The problem arrives with not knowing exactly how

much excess of base needs to be added considering the high reactivity of the base toward trace amounts moisture present in the purging gas. Typically *n*-BuLi reacts with water to form LiOH and release butane, in an acid-base reaction. The reaction for the substitution of the ligand was carried under and inert gas, N_2 or Ar. Even with precautions, there is always an amount of moisture in the system causing the base to be consumed. Optimizations of a specific system must be explored, until the excess has been optimized to yield substitutions at two positions yielding one single disubstituted product. Very often these reactions run even at 2.5 excess of base will yield two products a monosubstituted and disubstituted (the desired product).

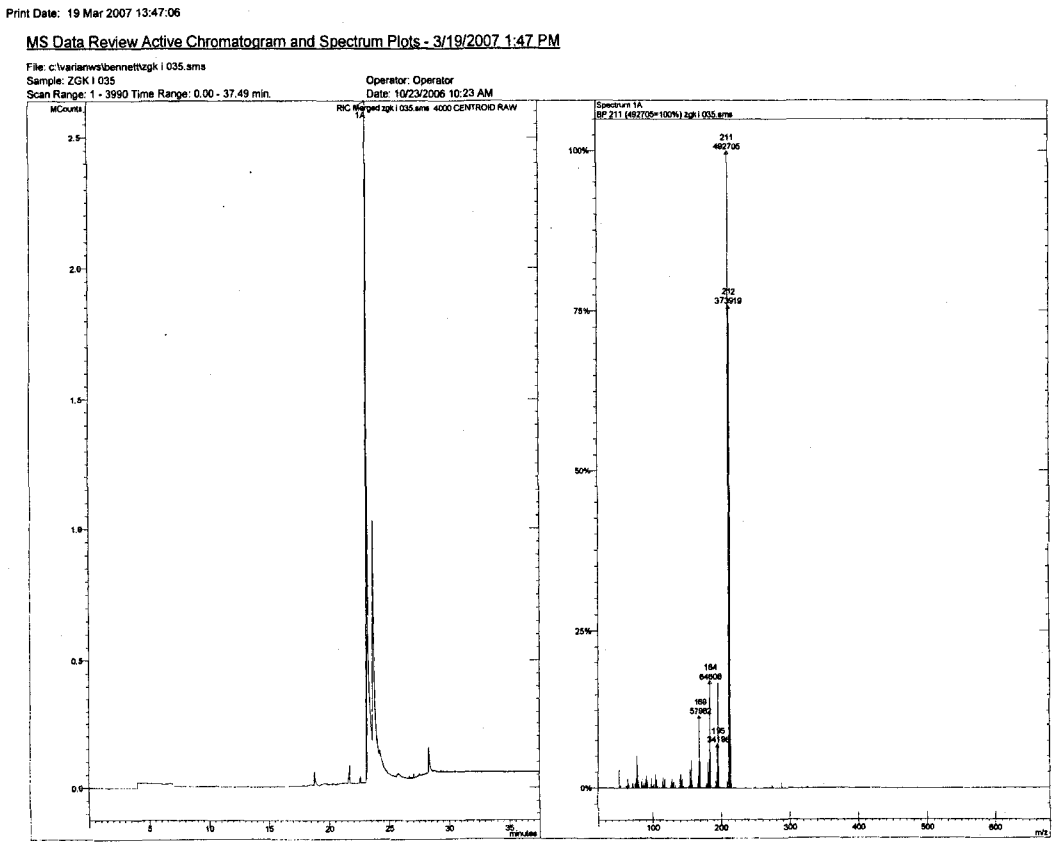


Figure 8a.

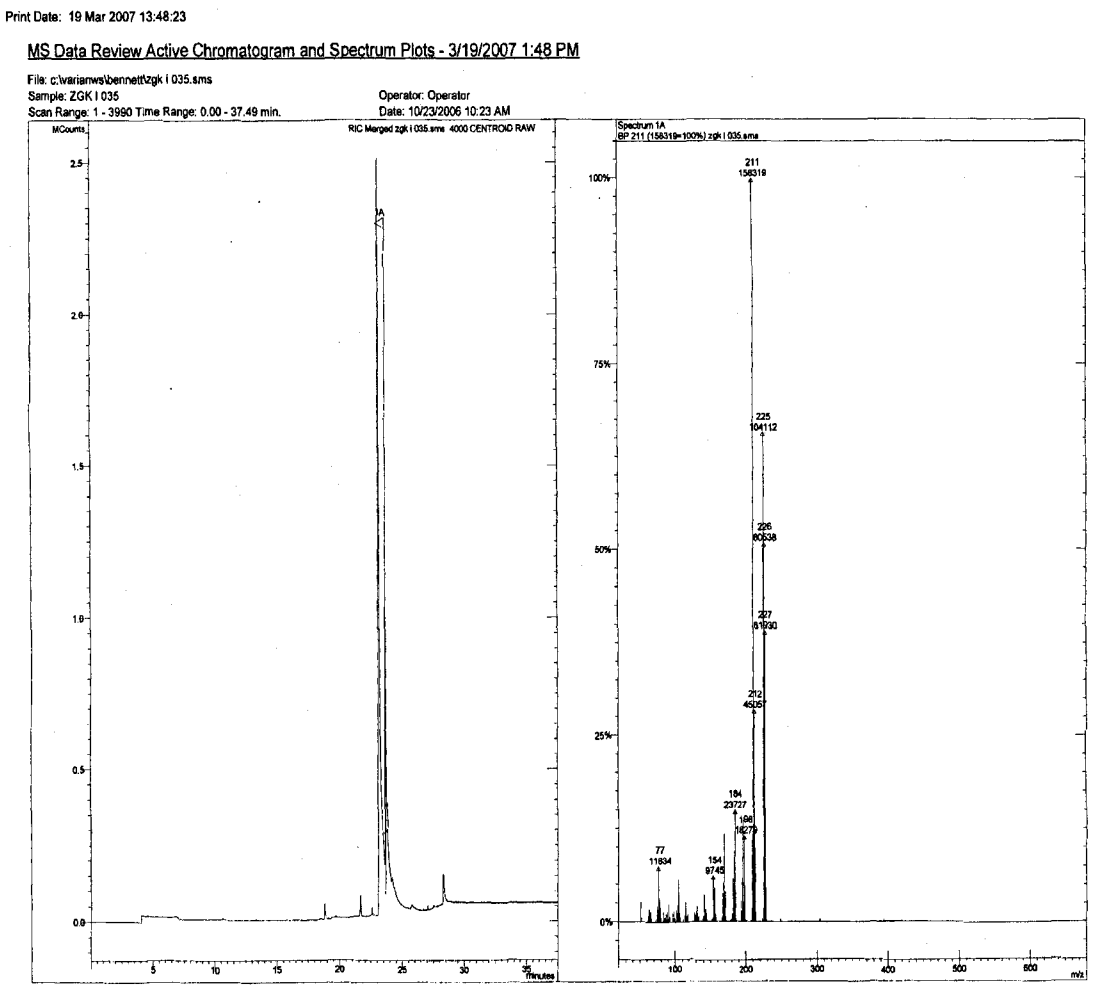


Figure 8b.

Figure 8a-b. GC/MS for a synthesis of compound **3** utilizing 2.5 eq. of base.

On a few occasions, the system is all too efficient yielding two products as well, a disubstituted and a trisubstituted product. This is demonstrated in a synthesis of compound **3** where 2.5 equivalence of base was used. Figure 8a. Illustrates the disubstituted product and Figure 6b shows the trisubstituted product with the GC distribution as well as the MS break down. This can be compared to the final synthesis of compound **3** where 2.2 eq. of base was used,

thus yielding one product, the desired disubstituted. (See page 34 of Appendix for GC/MS spectra of final compound **3**).

Preliminary cytotoxicity:

S. Carper et al. (personal communication) have assessed preliminary results of the cytotoxic activity of compounds **8** and **11** in various cancer cell lines utilizing a clonogenic survival assay.

Cancer Cell Line	Cancer Type	Cisplatin (μM)	Compound **8** (μM)	Compound **11** (μM)
MDA-MB-435	Breast	320	1.4	4.8
MDA-MB-231	Breast	350	1.5	16
MCF-7	Breast	305	6.4	33.2
DC-4	Breast	5.01	1.9	1.9
DB-46	Breast	4.29	1.5	10.2
DU-145	Prostate	490	2.5	22.2
A-549	Lung	900	9	24.6

Table 3. EC_{50} comparison of cisplatin, compound **8** and **11** in breast cancer.

These results are promising considering the range of activity is approx. 2.6 to 233.3 fold of cisplatin cytotoxicity, depending on the compound and cell line.

CHAPTER 5

CONCLUSION

In this investigation successful synthesis of substituted ligands of the type 4,4′-bis(alkyl)-2,2′-bipyridines and their consequent coordination to Pt (II) was achieved. Syntheses of six cisplatin-analogs were generated and four of these compounds are novel. Two of these compounds, (4,4′-Bis[butyl]-2,2′-bipyridine) $PtCl_2$, **8** and (4,4′-Bis[n-butyl-4-methoxy]-2,2′-bipyridine) $PtCl_2$, **11,** have been preliminarily tested for their biological properties and their cytotoxic properties are higher then cisplatin by 2.6 to 233.3 fold, depending on the compound and the cell line. These numbers demonstrate the potential for treatment, however the mechanism of these compounds is not know and assessments about their behavior inside a body can not be properly made at this time.

Future prospects related to this investigation would yield a broader range of coordinated compounds to Pt (II) with different functional group substituents in the 4,4′ position, such as hydroxyl, a series of increasing alkyl chains with hydroxyl substituents, amine, aldehyde, carboxylic acid and various other compounds.

REFERENCES

[1] J.C. Dabrowiak, W.T. Bradner. In: G.P. Ellis, W.T. West. (Eds.), Progress in Medicinal Chemistry, Elsevier, Amsterdam 24 (1987) 129-58.

[2] Cancer Facts & Figures 2007, American Chemical Society

[3] S. Trzaska, Chem. & Eng. News, 83 (2005) 3.

[4] B. Rosenberg, L. Van Camp, T. Krigas, Nature 205 (1965) 698-9.

[5] B. Rosenberg, L. Van Camp. J.E. Trosko, V.H. Mansour, Nature 222 (1969) 385-6.

[6] C.C. Riley, L.A. Sternson, In: K. Florey (Eds.), Cisplatin: Analytical profiles of drug substances, New York : Academic press, 14 (1985) 77-105.

[7] A.H. Rossof, S.E Slayton, C.P Perlia, Cancer 30 (1972) 1451-6.

[8] B. Rosenberg, *Cancer* 55 (1985) 2303-16

[9] J.W. Reishus, D.S. Martin, Jr. J. Amer. Chem. Soc. 83 (1961) 2457.

[10] N.P. Johnson, J.D. Hoeschele, R.O. Rahn Chem. Biol. Interact. 30 (1987) 151-169.

[11] A.M.J Fichtinger-Schepman, Biochemistry 24 (1985) 707-13.

[12] A. Eastman, Pharmacol. Ther. 34 (1987) 155-166.

[13] A. Gelasco, S. J. Lippard, Biochemistry 37 (1998) 9230–9239.

[14] P.M. Takahara, A.C. Rosenzweig, C. A. Frederick, S. J. Lippard, Nature 377 (1995) 649–652.

[15] P.M. Pil, S.J. Lippard, Science 256 (1992) 234.

[16] D. Wang, S.J. Lippard, Nat. Rev. Drug Discov. 4 (2005) 307-320.

[17] T. Lindahl, P. Karran, R. D. Wood Curr. Opin. Genet. Dev. 7 (1997) 158–169.

[18] D.B. Zample S.J. Lippard, Trends Biochem. Sci. 20 (1995) 435–39.

[19] D.B Zamble, D. Mu , J.T. Reardon, A. Sancar, S. J. Lippard, Biochemistry 35 (1996) 10004–13.

[20] J.G. Moggs, D.E. Szymkowski, M. Karran, P. Yamada, R. D Wood. Nucleic Acids Res. 25 (1997) 480–91.

[21] M. Yamada, E. O´Regan, R. Brown, P. Karrap, Nucleic Acids Res. 25 (1997) 491.

[22] E.R. Jamieson, S.J. Lippard, Chem. Rev. 99 (1999) 2467.

[23] K.M. Henkels, J.J. Turchi, Cancer Res. 57 (1997) 4488.

[24] A.T. Yarnell, S. Oh, D. Reinberg, S.J. Lippard, J. Biol. Chem. 276 (2001) 25736.

[25] E.R. Jamieson, S.J. Lippard, Chem. Rev. 99 (1999) 2467.

[26] S.J. Brown, P.J. Kellet, S.J. Lippard, Science, 261(1993) 603.

[27] G.Orphanides, W.H. Wu, W.S. Lane, M. Hampsey, D. Reinberg, Nature 400 (1999) 284.

[28] Z.S.Juo, T.K. Chiu, P.M. Leiberman, I. Baikalov, A.J. Berk, R.E. Dickerson, J. Mol. Biol., 261, (1996) 239.

[29] M. Kartalou, L.D. Samson, J.M. Essigman, Biochemistry 39 (2000) 8032.

[30] T. Ishibasi, S.J. Lippard, Proc. Natl. Acad. Sci. USA 95 (1998) 4219.

[31] M.A. Fuertesa, J. Castillab, C. Alonsoa ,J.M. Pérez, Curr. Med. Chem. 10 (2003) 257-266.

[32] T. Peleg-Shulman, D. Gibson, J. Am. Chem. Soc. 123 (2001) 3171.

[33] M. Weller, Cell Tissue Res. 292 (1998) 435.

[32] F. Blau, Ber. Dtsch. Chem. Ges. 21 (1988) 1077-8.

[33] F.H. Case, J. Amer. Chem. Soc. 68 (1946) 2574-7.

[34] A. M. Knight, G.M. Greenway, The Analyst 119 (1994) 879.

[35] M. Venturi, A. Credi, V. Balzani, Coord. Chem. Rev. 186 (1999) 233-56.

[36] K. Matyjaszewski, T. E. Patten, J. Xia, J. Am. Chem. Soc. 119 (1997) 674-80.

[37] K. Kaylanasundanaram, M. Grätzel, Coord. Chem. Rev. 177 (1998) 347-414.

[38] G.R. Newkome, A.K. Patri, E. Holder, U.S. Schubert, Eur. J. Org. Chem. (2004) 235-54.

[39] G. Maerker, F. Case, J. Amer. Chem. Soc. 80 (1958) 2745-8.

[40] G.M. Badger, W.H.F. Sasse J Chem. Soc. (1956) 616-20.
[41] P.E. Rosevear, W.H.F. Sasse J Heterocyclic Chem. 8(3) (1971) 483-5.
[42] http://www.accessdata.fda.gov/scripts/cder/onctools/druglist.cfm
[43] T.J.M. Hodes, W.J.M. Underberg, G. Los, J.H. Beinjnene, Pharmaceutisch Weekblad Scientific Edition, 14(3) (1992) 61-77.
[44] V.H. Houlding, V.M. Miskowski, Coord. Chem. Rev. 111 (1991) 145-52.
[45] M. Cusumano, M.L. Di Pietro, A. Giannetto, P.A. Vainiglia, J. Inorg. BioChem. 99 (2005) 560-5.
[46] N. Garelli, P. Vierling, J. Org. Chem. 57 (1992) 3046-51.
[47] N. Garelli, P. Vierling, Inorg. Chim. Acta 194 (1992) 247-53.
[48] N. Garelli, P. Vierling, J.L. Fischel, G. Milano, Eur. J. Med. Chem. 28 (1993) 235-42.
[49] J. Yoo, J. Kim, Y.S. Sohn, Y. Do. Inorg. Chim. Acta 263 (1997) 53-60.
[50] H. Mansuri-Torshizi, S. Ghadimy, N. Akbarzadeh, Chem. Pharm. Bull. 49(12) (2001) 1517-20.
[51] K.E. Elwell, C. Hall, S. Tharkar, Y. Giraud, B. Bennett, C. Bae, S.W. Carper, Bioorg. & Med. Chem. 14 (2006) 8692-8700.
[52] K.E. Elwell, S.W. Carper (2005) Novel cisplating analogs: Synthesis, characterization and cytotoxicity in breast cells, Thesis (M.S.) University of Nevada, Las Vegas, 2005.
[53] C.G. Griggs, D.J.H. Smith, J. Chem. Soc., Perkin Trans. 1 (1982) 3041-3.
[54] H.C. Clark, L.E. Manser, J. OrganoMetallic Chem, 59 (1973) 411-28.
[55] T.M. Cocker, R.E. Backman, Molecular Crystals and Liquid Crystals, 408 (2004) 1-19.
[56] Y.R. Hong, C.B. Gorman, J. Org. Chem. 68 (2003) 9019-25.
[57] Z .SU, L. Chen, L..Wu, H. Xu, D. Zhu, R. Wang, Chem. Res. Chinese U., 18(3) (2001) 345-7
[58] A.R. Oki, R.J. Morgan, Synth. Comm. 25(24) (1995) 4093-7.
[59] J.C. Chambron, J.P. Savage, Tetrahedron 43(5) 1987 895-904
[60] J.C. Chambron, J.P. Savage, Tetrahedron Lett. 27(7) 1986 865-8

[61]C. Kaes, M.W. Hosseini, A. DeCian, J. Fischer, Tetrahedron Letters, 38(22) (1997) 3901-4

[62] E. Bielli, P.M. Gidney, R.D. Gillard, B.T. Heaton, J. Chem. Soc.:Dalton Trans. (1974) 2133-9.

[63] G.T.Morgan, F.H. Burstall, J. Chem. Soc. (1934) 965.

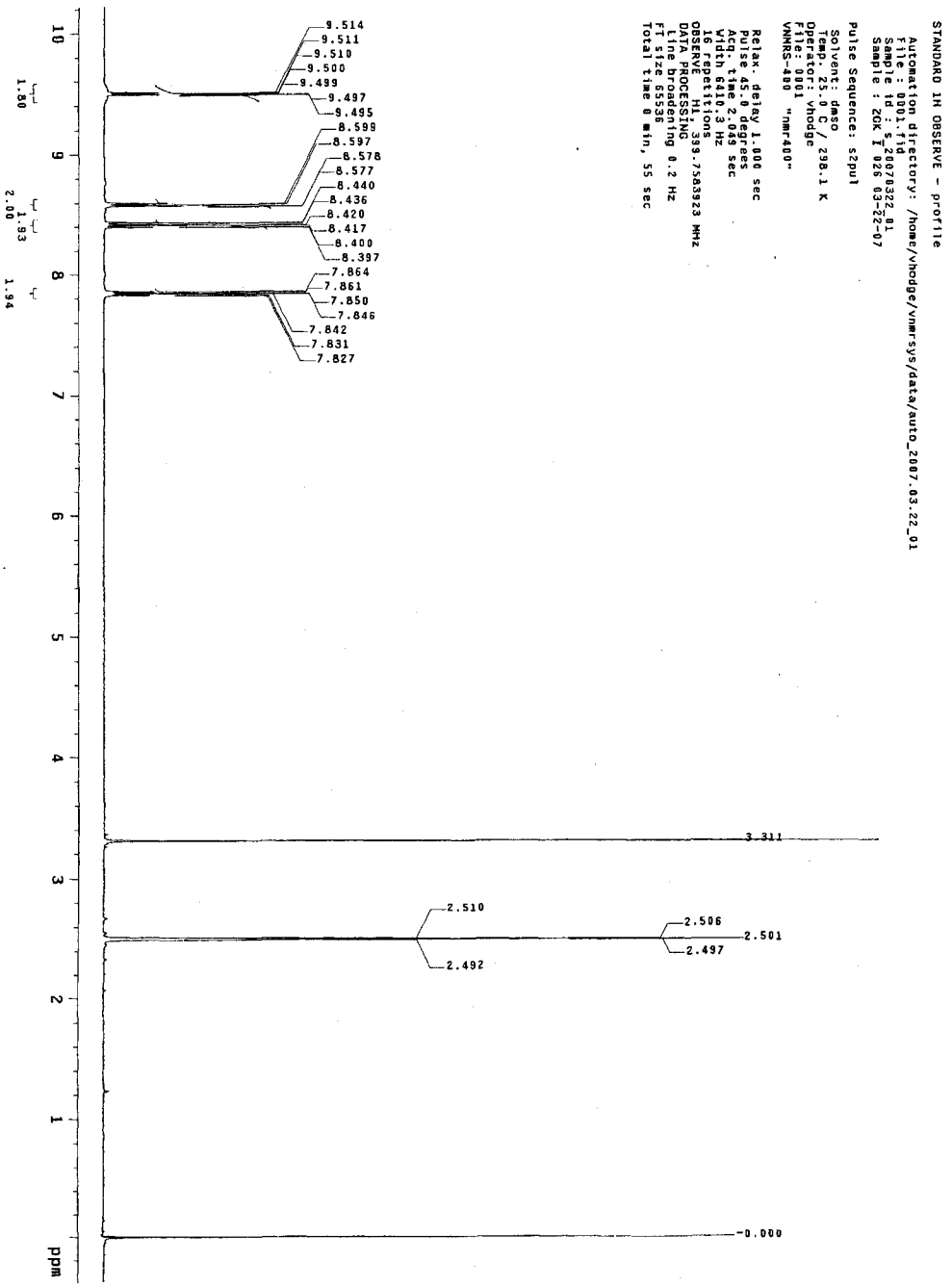
[64] S.H. Yoo, J.H. Lee, J. Lee, J.Y. Jho, Macromolecules 35(4) (2002) 1146-48.

APPENDIX I

^{1}H, ^{13}C NMR AND GC/MS SPECTRA

^{1}H NMR of (2,2′-bipyridine) $PtCl_2$, **2**

(400 MHz, 298.1 K, DMSO-d_6)



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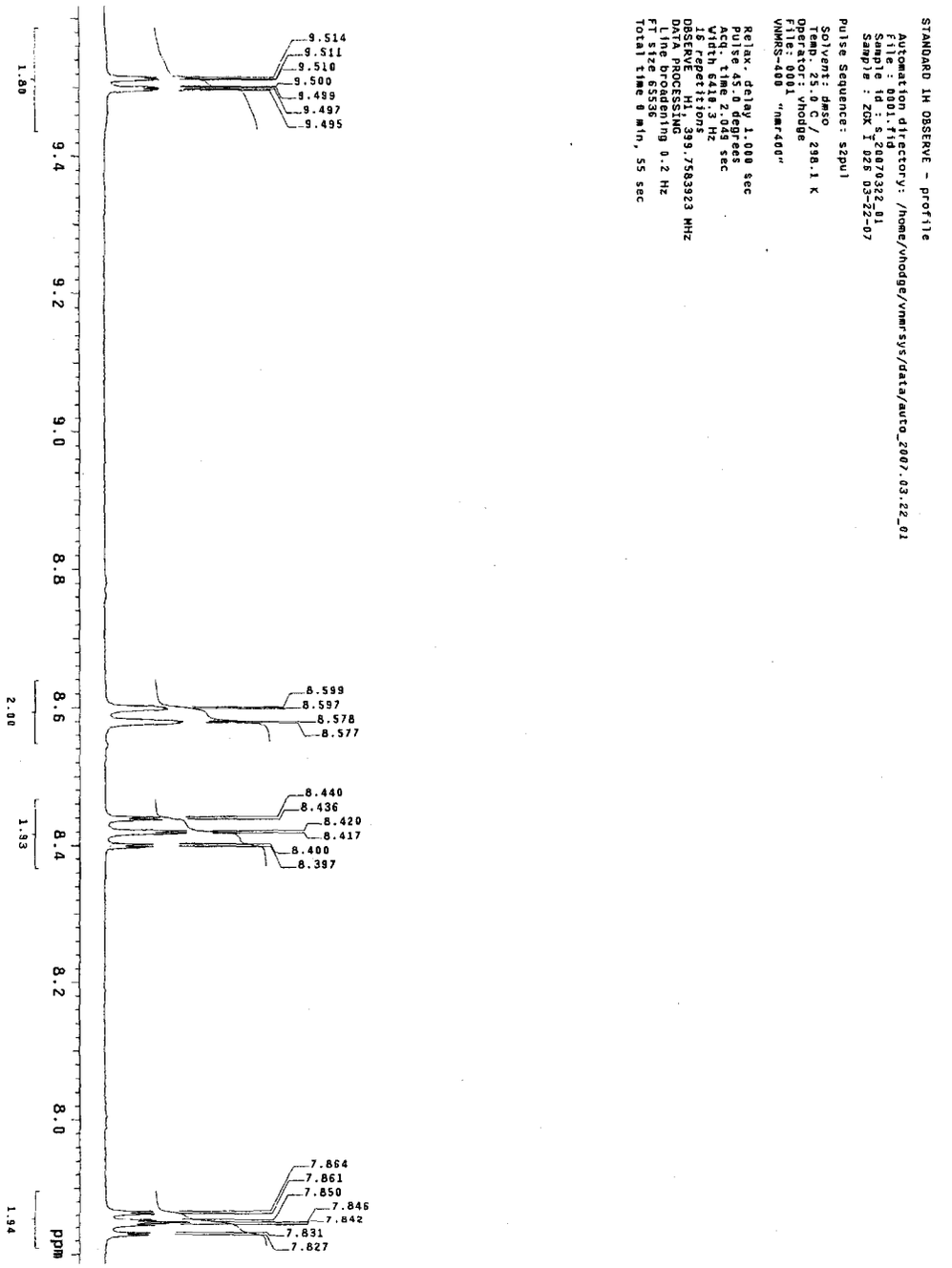
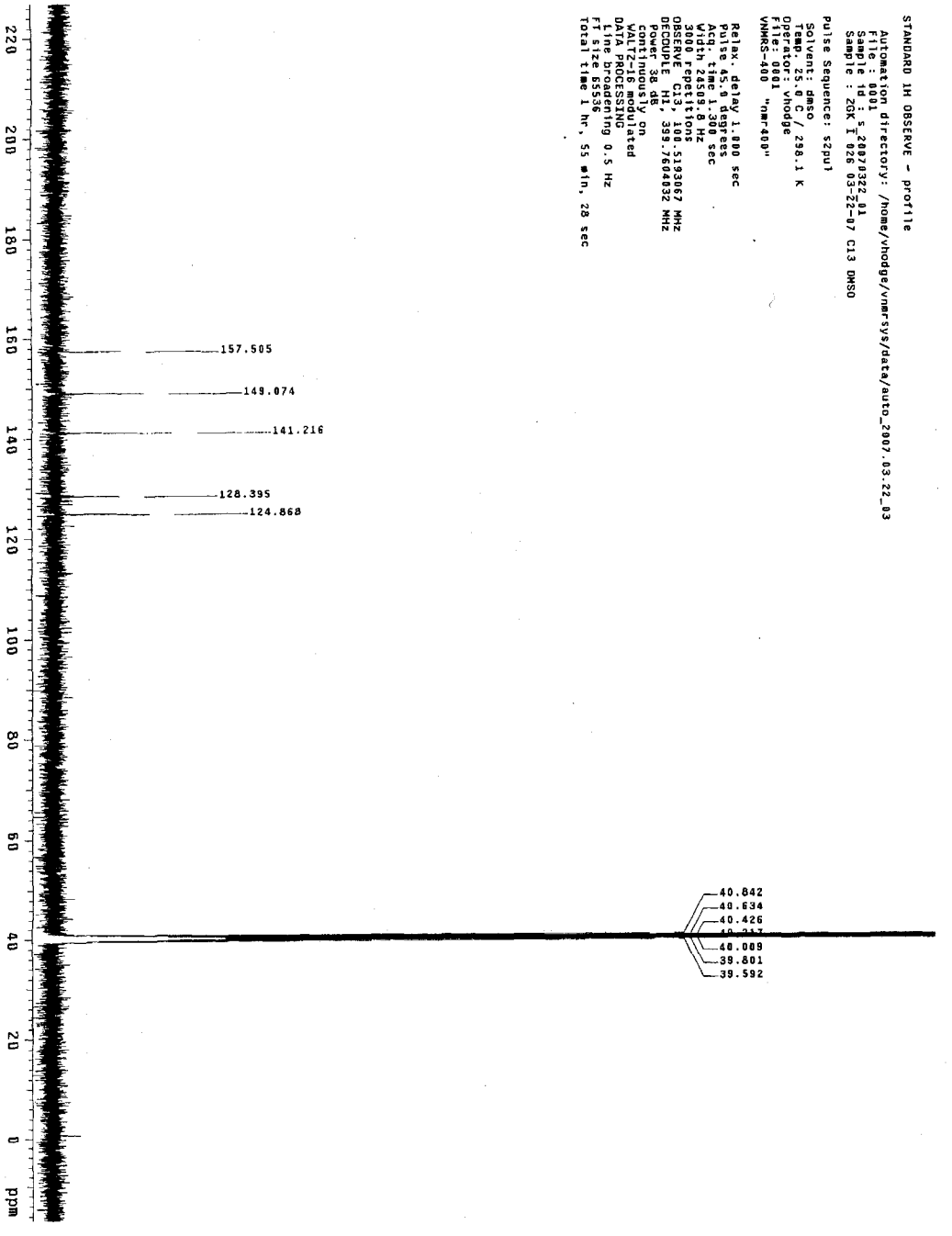


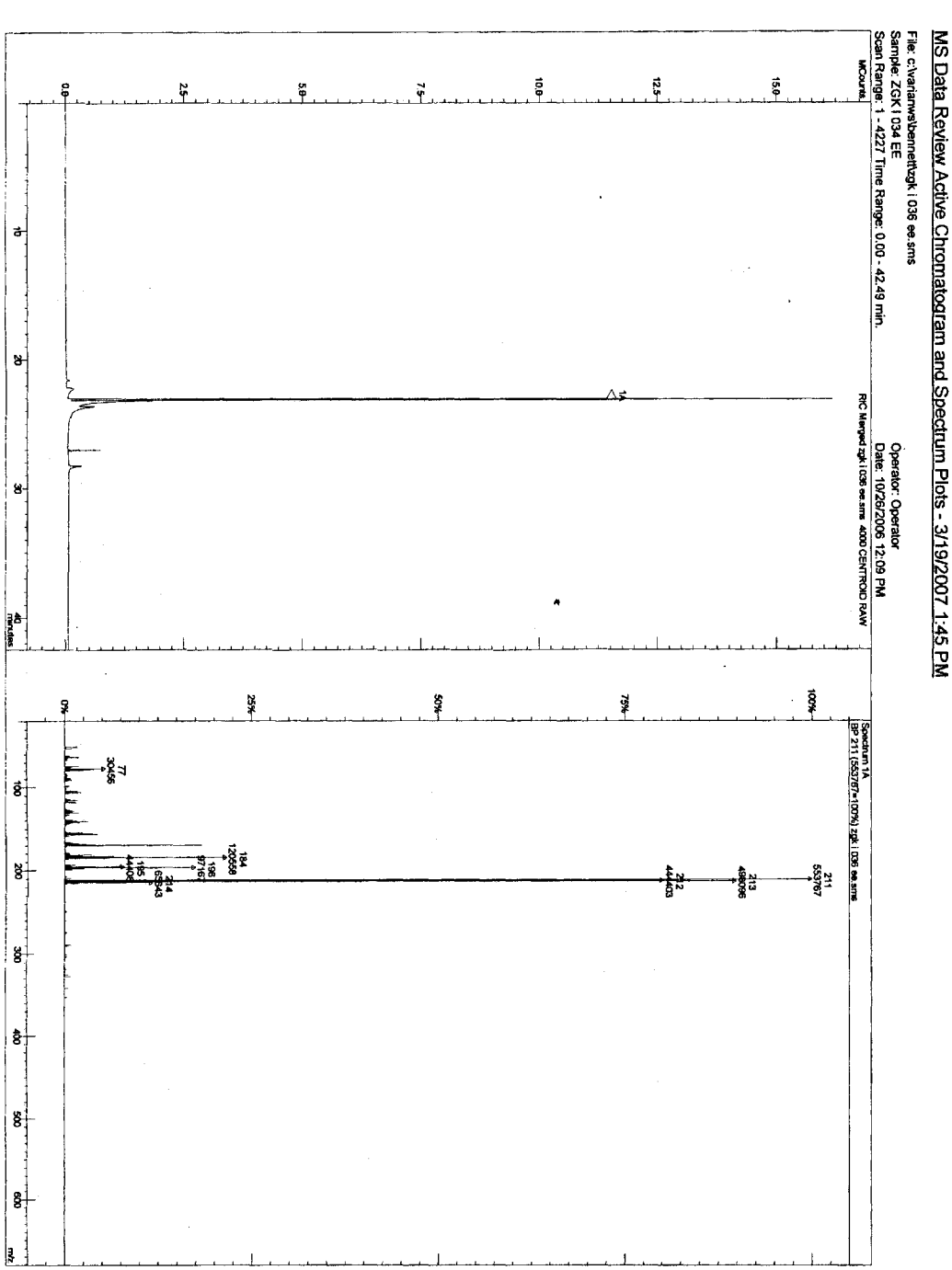
^{13}C NMR of (2,2′-bipyridine) $PtCl_2$, **2**

(100 MHz, 298.1 K, DMSO-d_6)



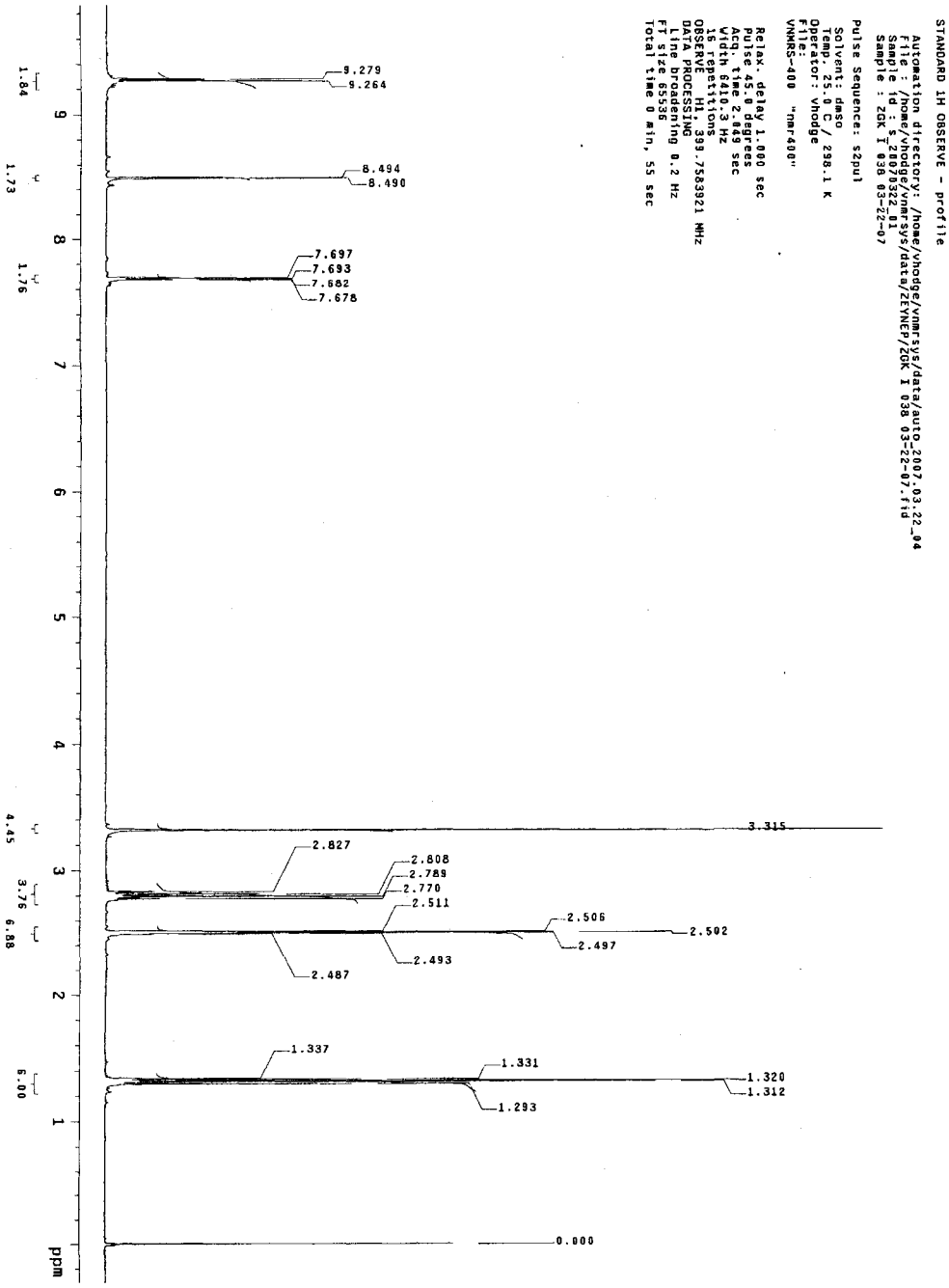
GC/MS of 4,4´-Bis[ethyl]-2,2´-bipyridine, **3**

(CH_2Cl_2)



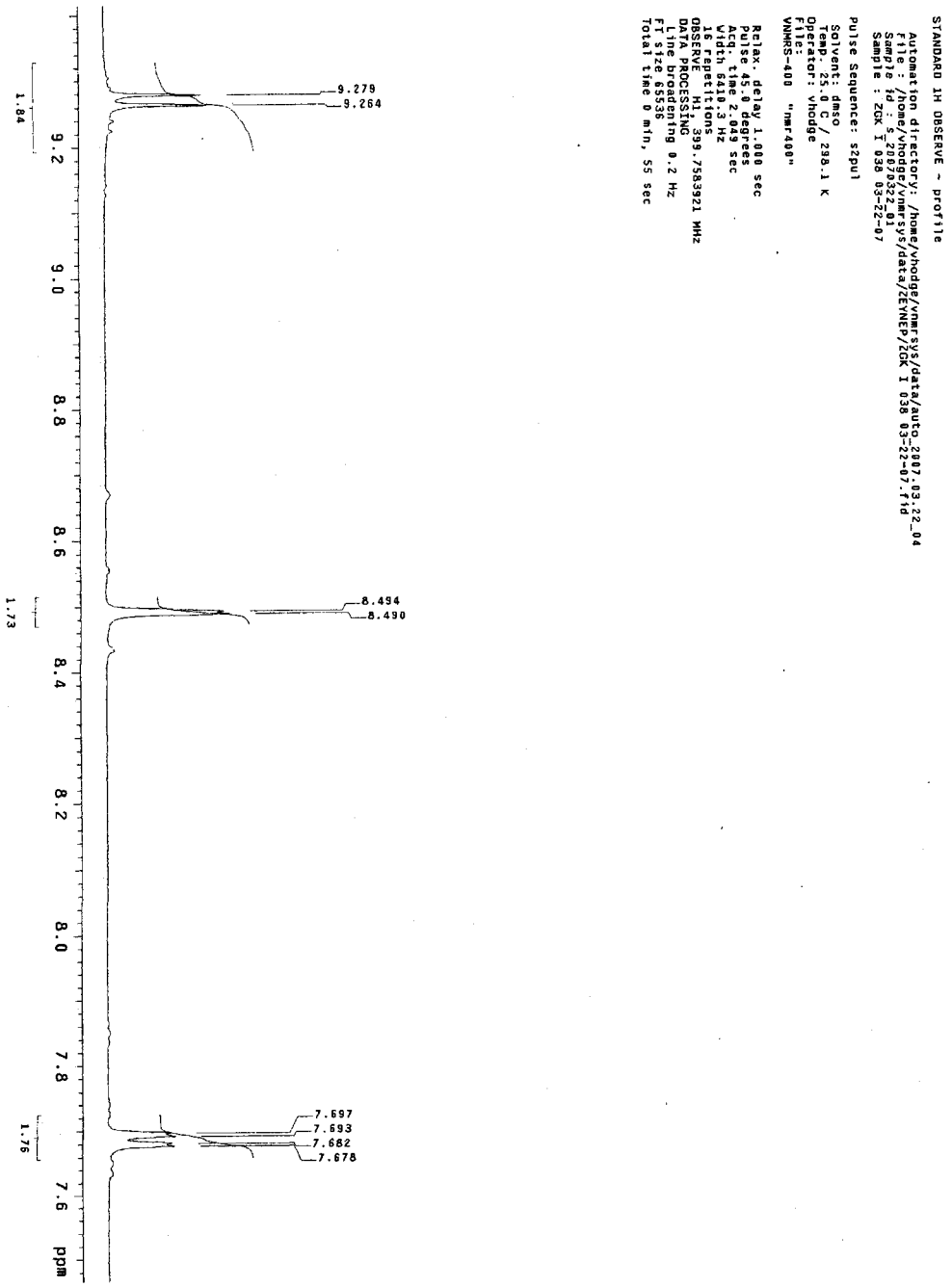
^{1}H NMR of (4,4´-Bis[ethyl]-2,2´-bipyridine) $PtCl_2$, **4**

(400 MHz, 298.1 K, DMSO-d_6)



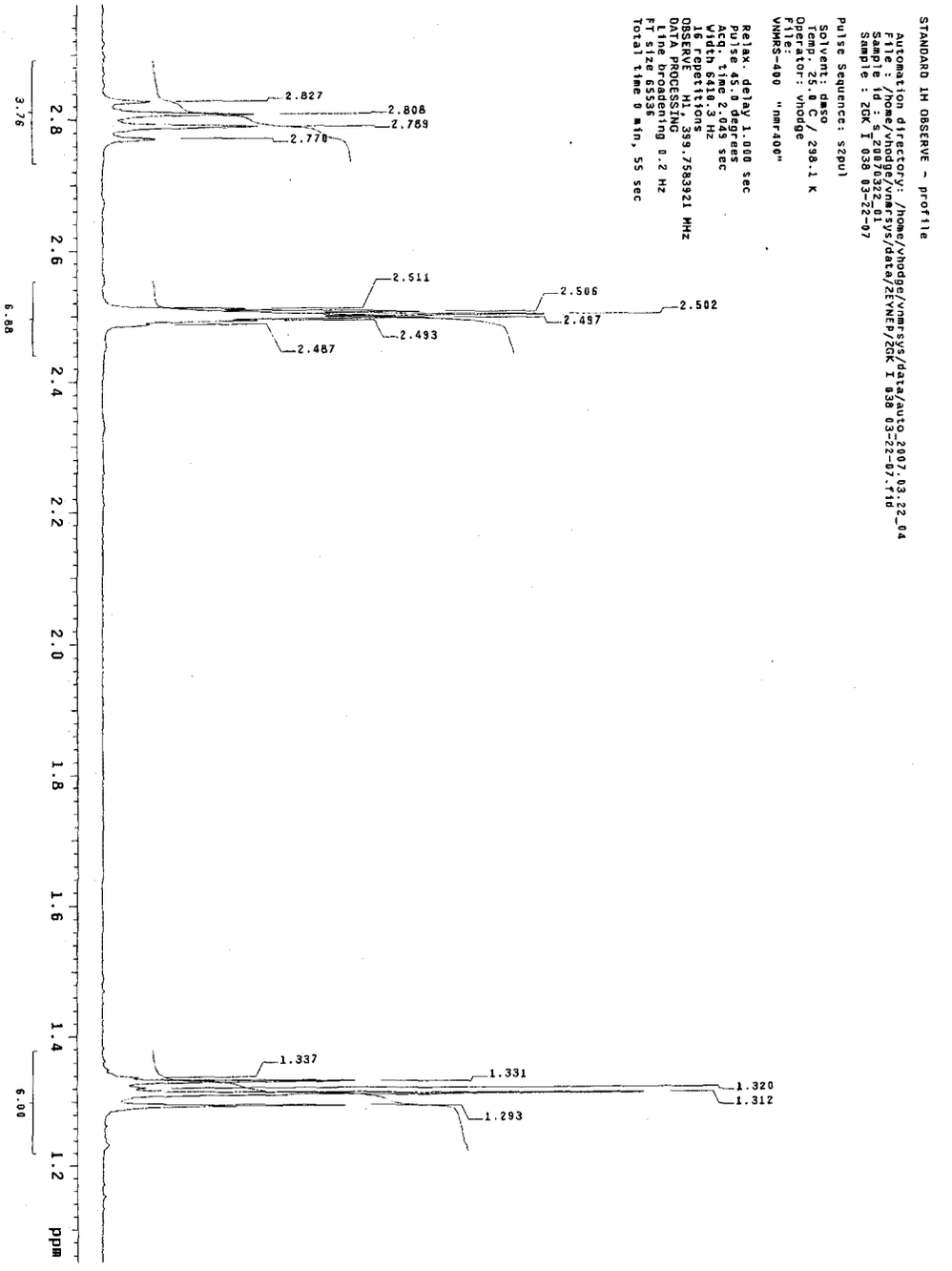
^{1}H NMR of (4,4′-Bis[ethyl]-2,2′-bipyridine) $PtCl_2$, **4**

(400 MHz, 298.1 K, DMSO-d_6)



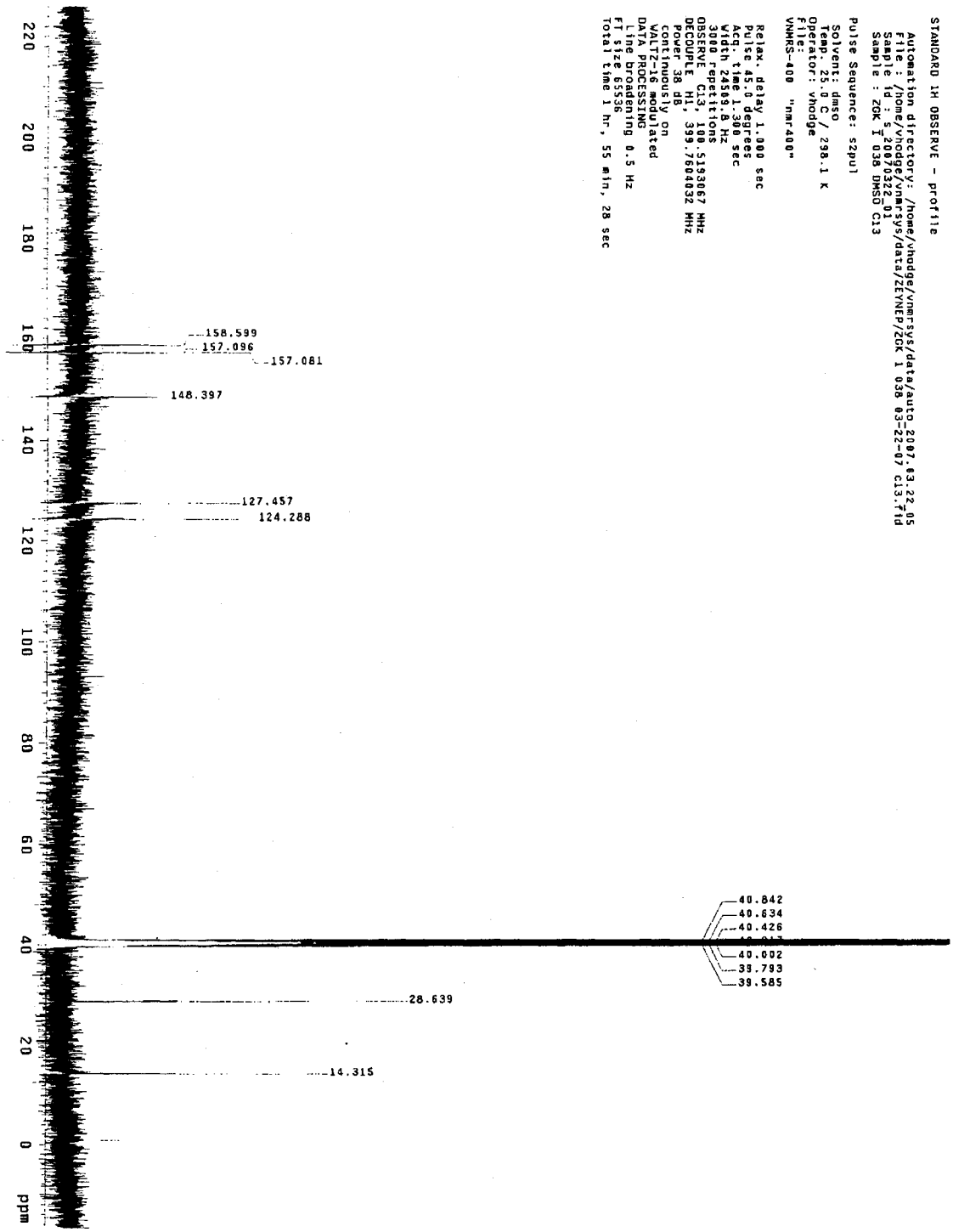
^{1}H NMR of (4,4´-Bis[ethyl]-2,2´-bipyridine) $PtCl_2$, **4**

(400 MHz, 298.1 K, DMSO-d_6)



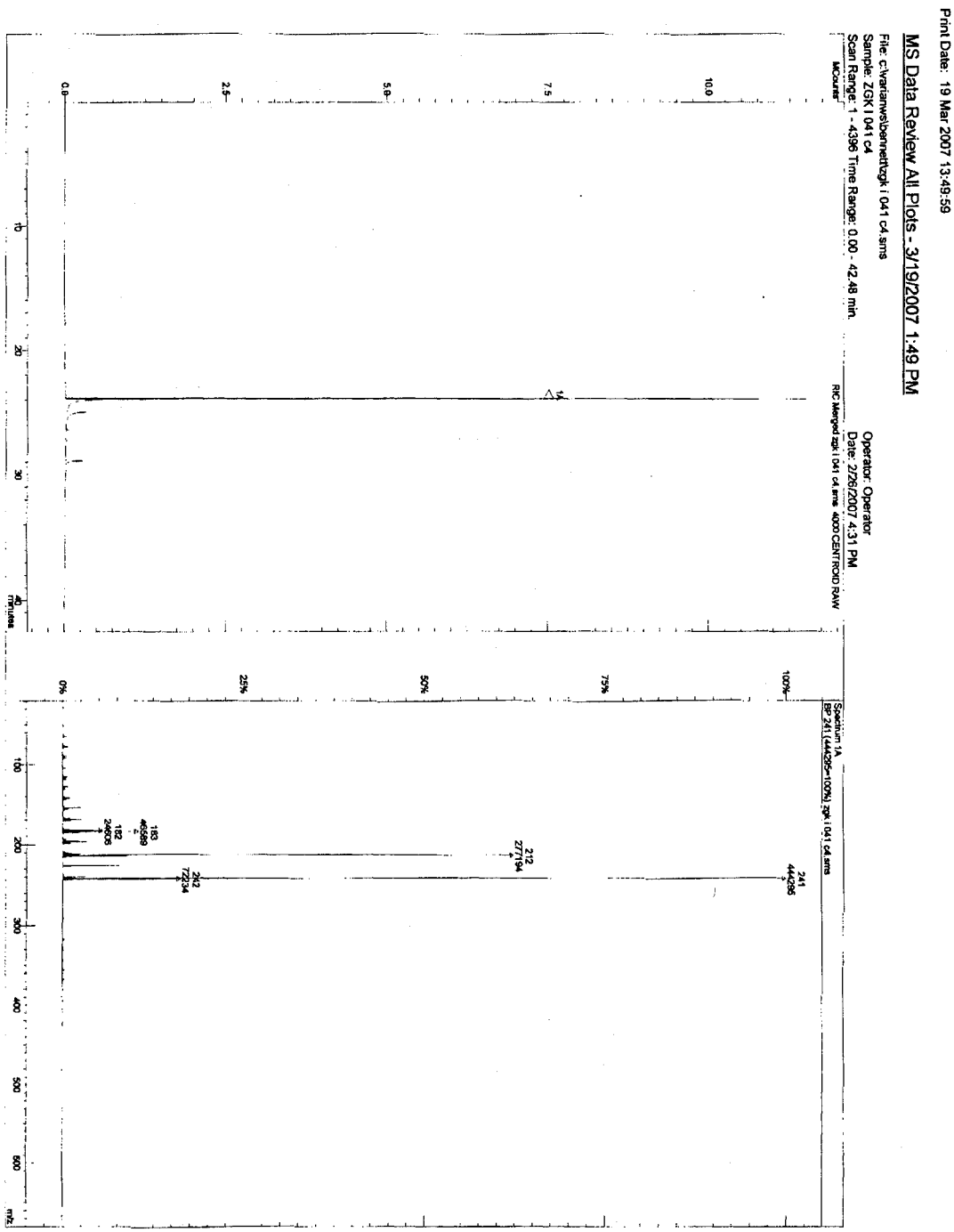
^{13}C NMR of (4,4′-Bis[ethyl]-2,2′-bipyridine) $PtCl_2$, **4**

(100 MHz, 298.1 K, DMSO-d_6)



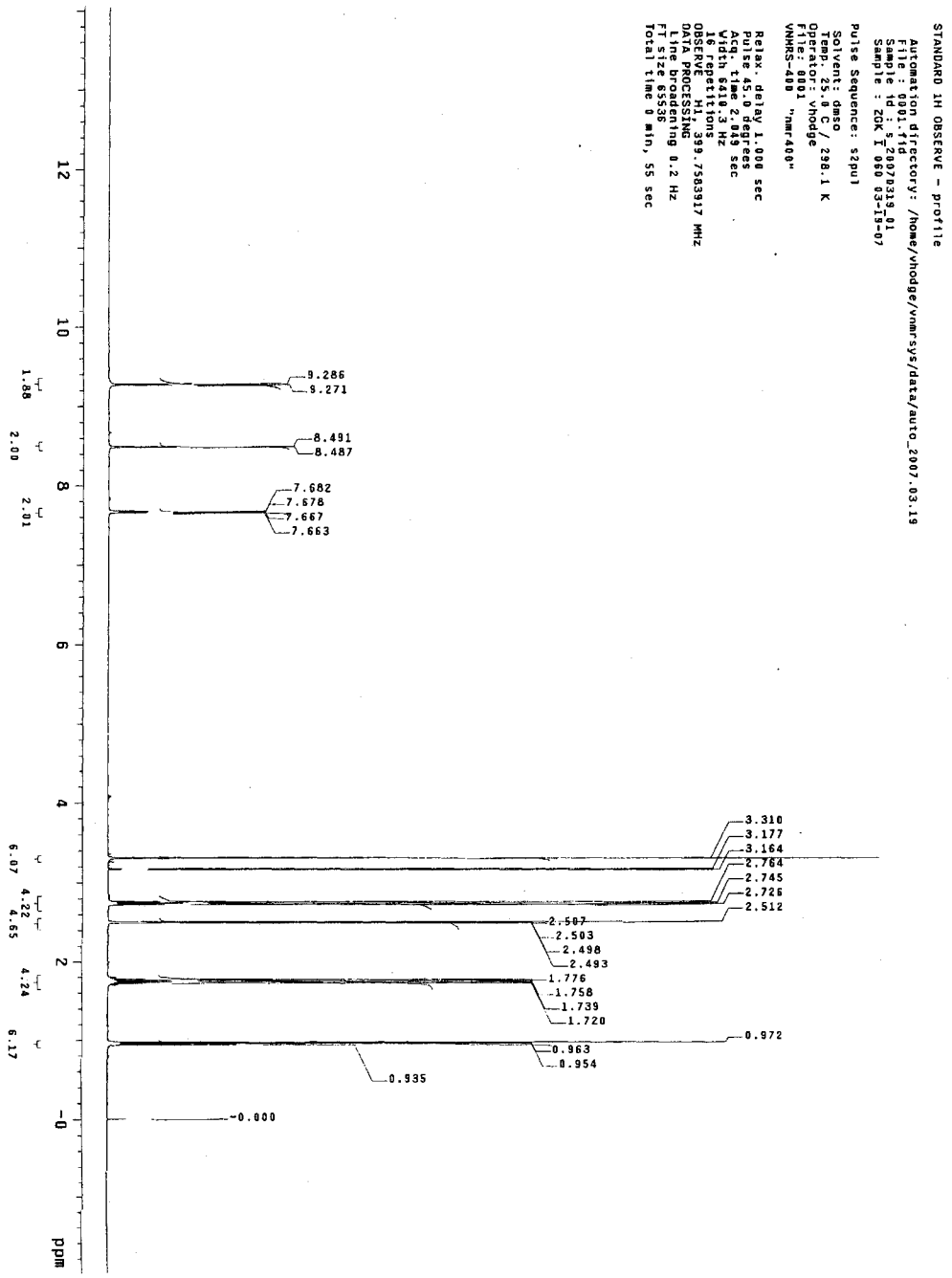
GC/MS of 4,4´-Bis[propyl]-2,2´-bipyridine, **5**

(CH_2Cl_2)



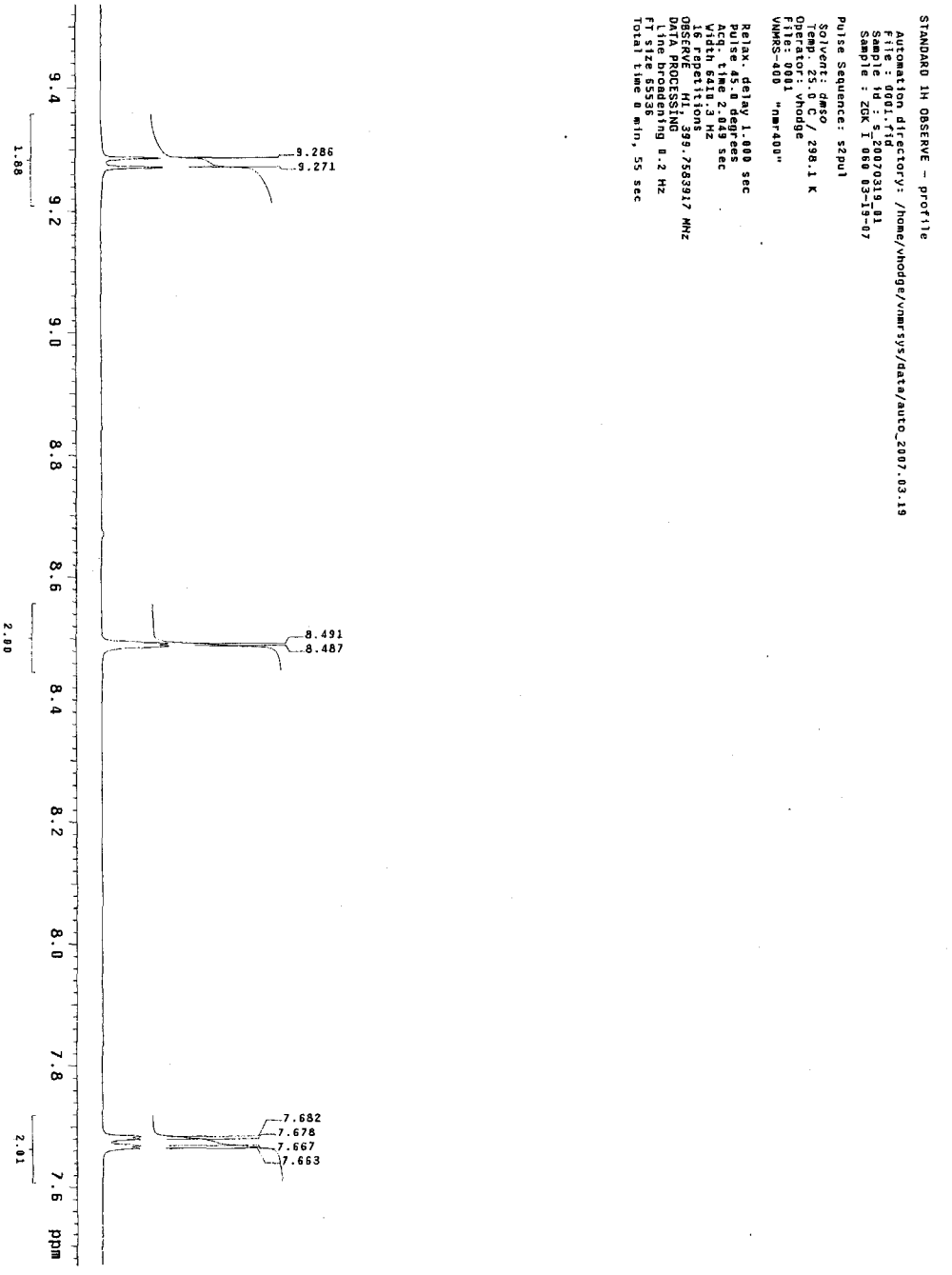
^{1}H NMR of (4,4´-Bis[propyl]-2,2´-bipyridine) $PtCl_2$, **6**

(400 MHz, 298.1 K, DMSO-d_6)



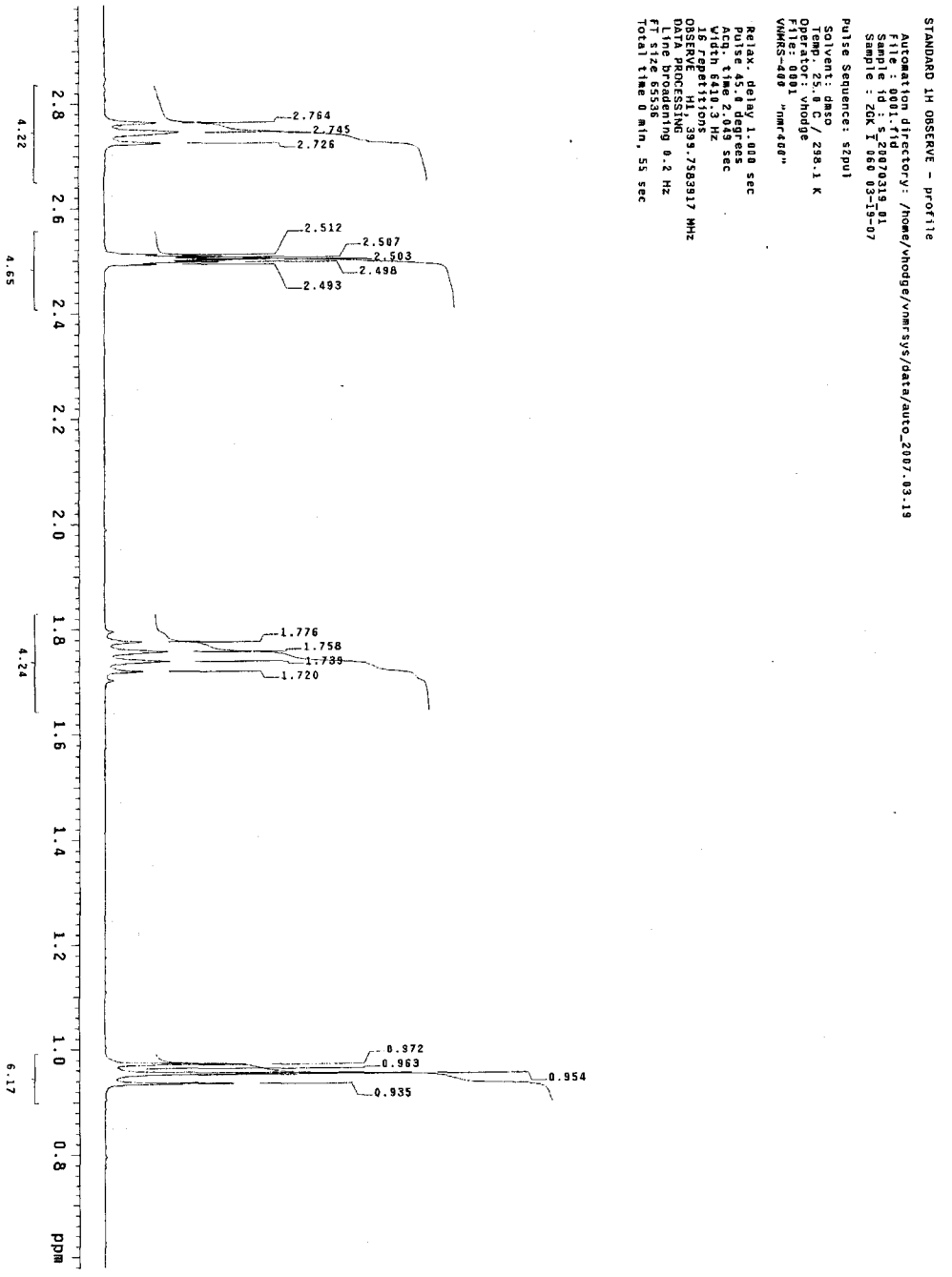
^{1}H NMR of (4,4′-Bis[propyl]-2,2′-bipyridine) $PtCl_2$, **6**

(400 MHz, 298.1 K, DMSO-d_6)



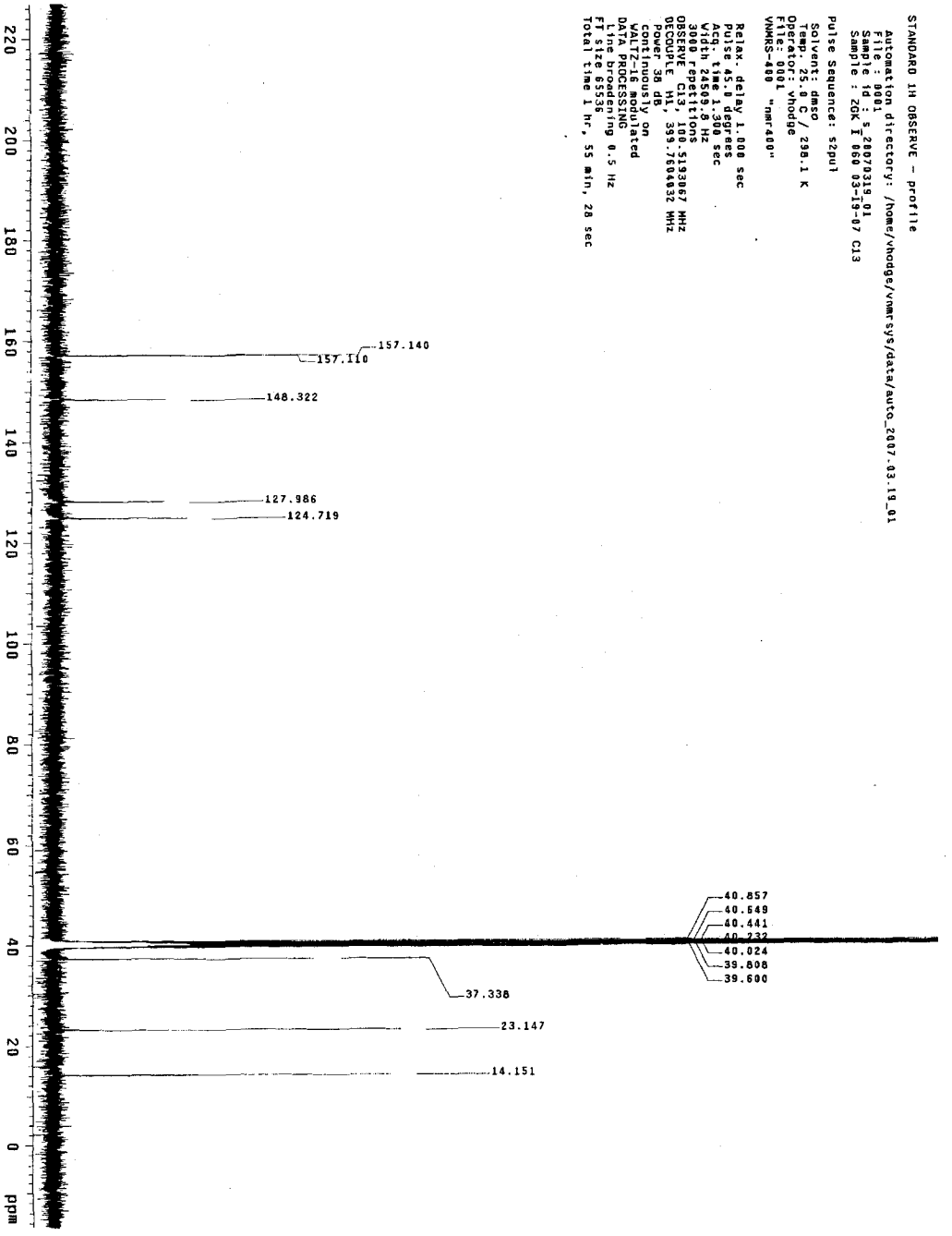
^{1}H NMR of (4,4´-Bis[propyl]-2,2´-bipyridine) $PtCl_2$, **6**

(400 MHz, 298.1 K, DMSO-d_6)



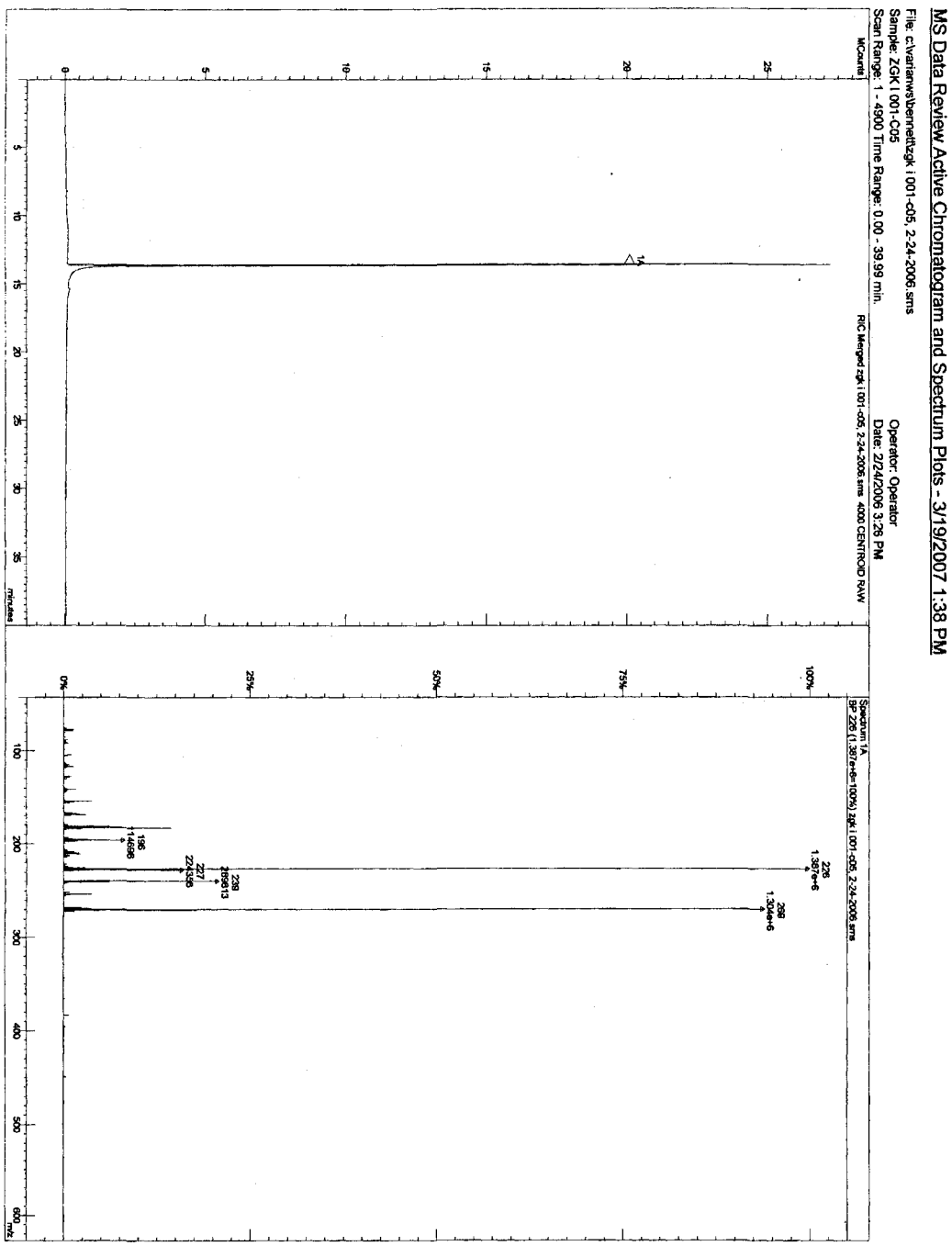
^{13}C NMR of (4,4´-Bis[propyl]-2,2´-bipyridine) $PtCl_2$, **6**

(100 MHz, 298.1 K, DMSO-d_6)



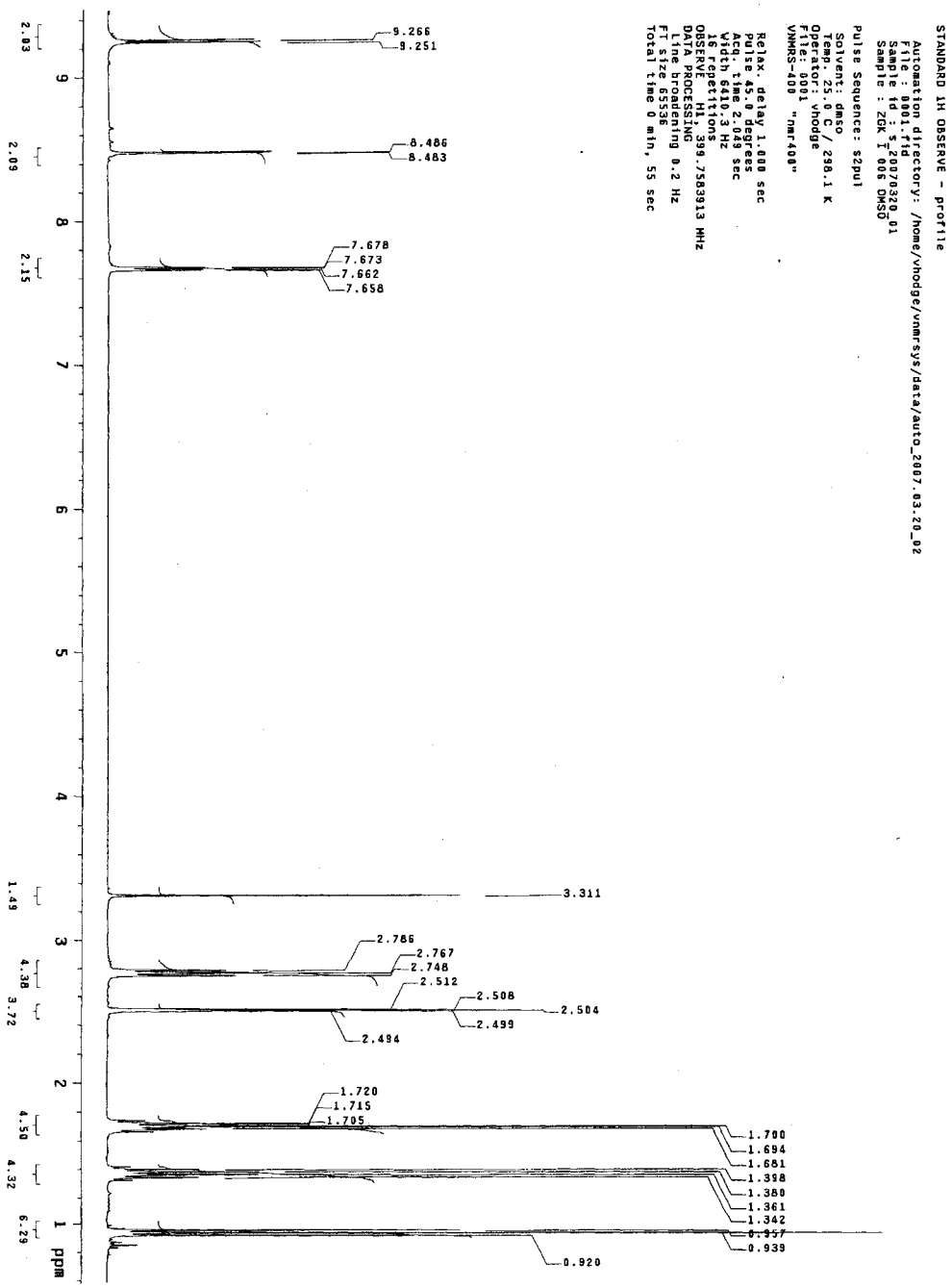
GC/MS of 4,4´-Bis[n-butyl]-2,2´-bipyridine, **7**

(CH_2Cl_2)



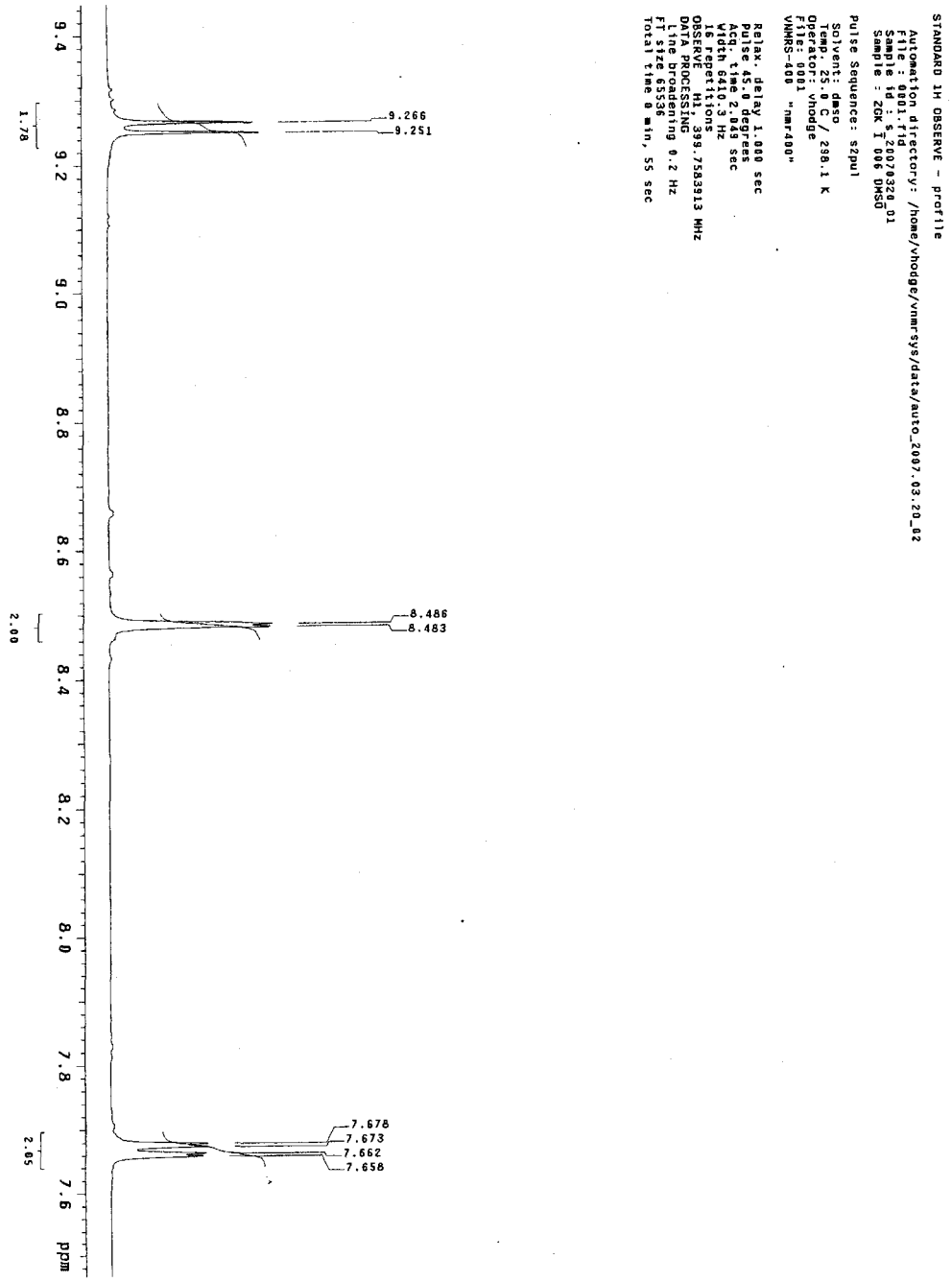
^{1}H NMR of (4,4´-Bis[butyl]-2,2´-bipyridine) $PtCl_2$, **8**

(400 MHz, 298.1 K, DMSO-d_6)



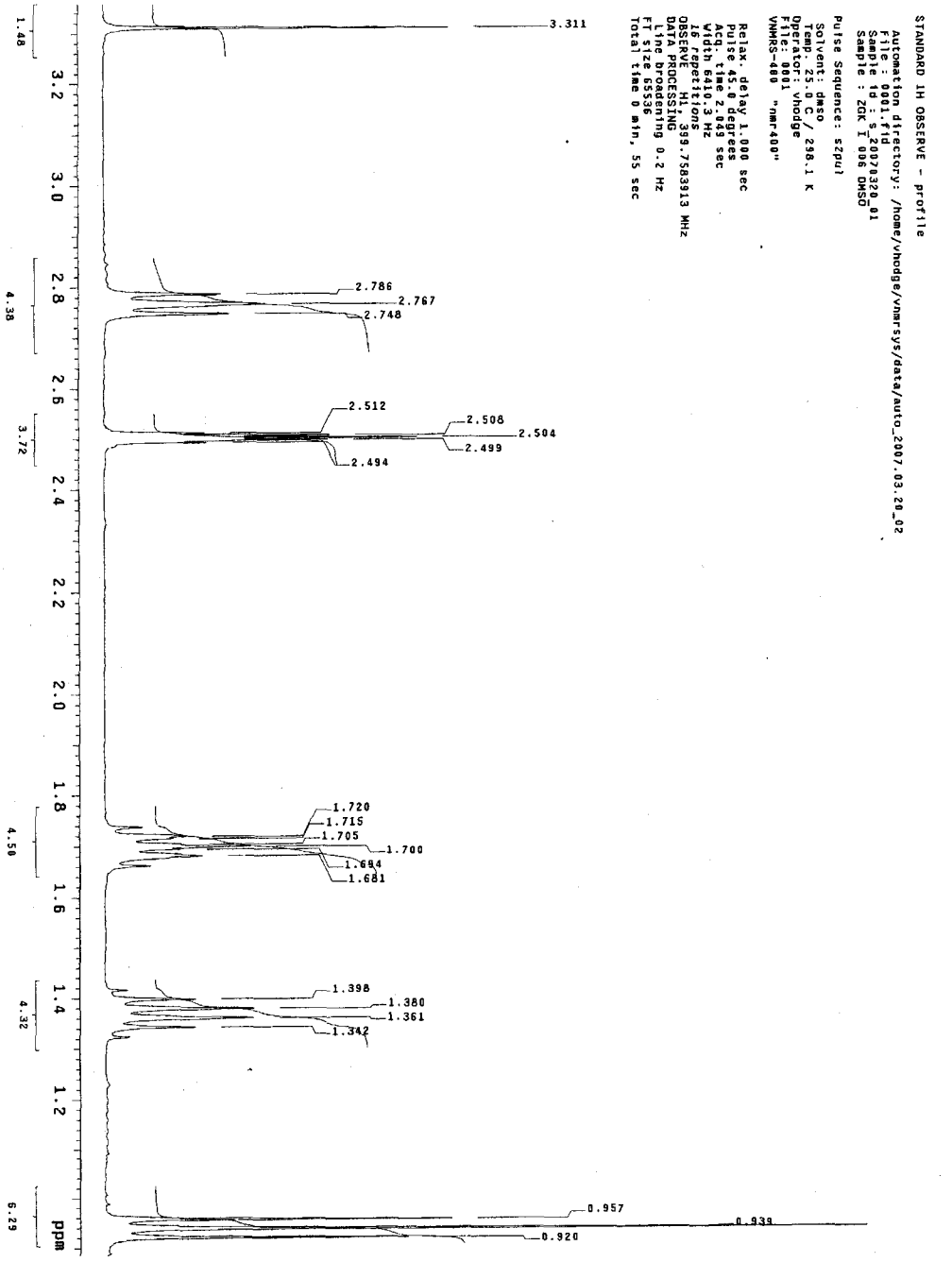
^{1}H NMR of (4,4´-Bis[butyl]-2,2´-bipyridine) $PtCl_2$, **8**

(400 MHz, 298.1 K, DMSO-d_6)



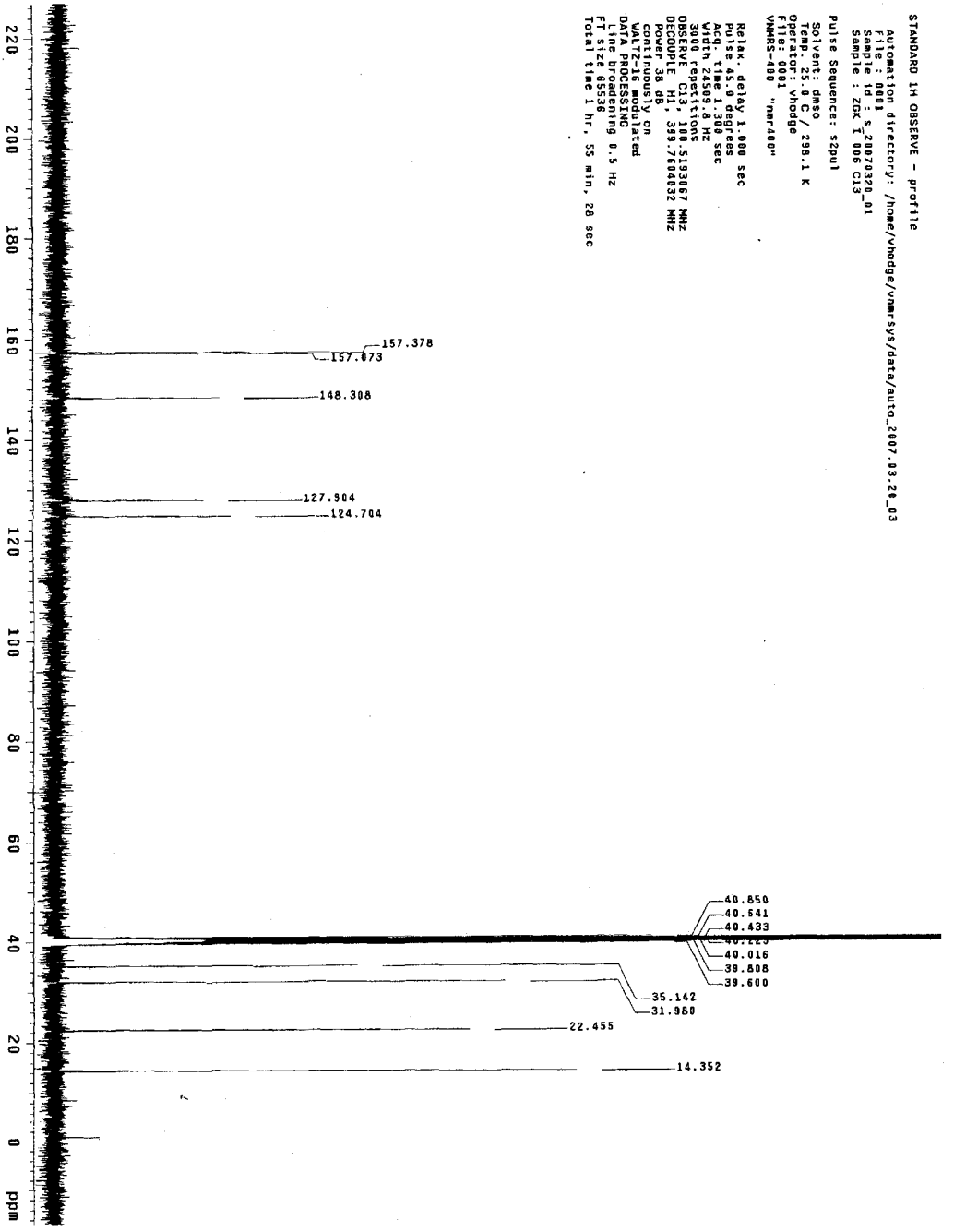
^{1}H NMR of (4,4′-Bis[butyl]-2,2′-bipyridine) $PtCl_2$, **8**

(400 MHz, 298.1 K, DMSO-d_6)



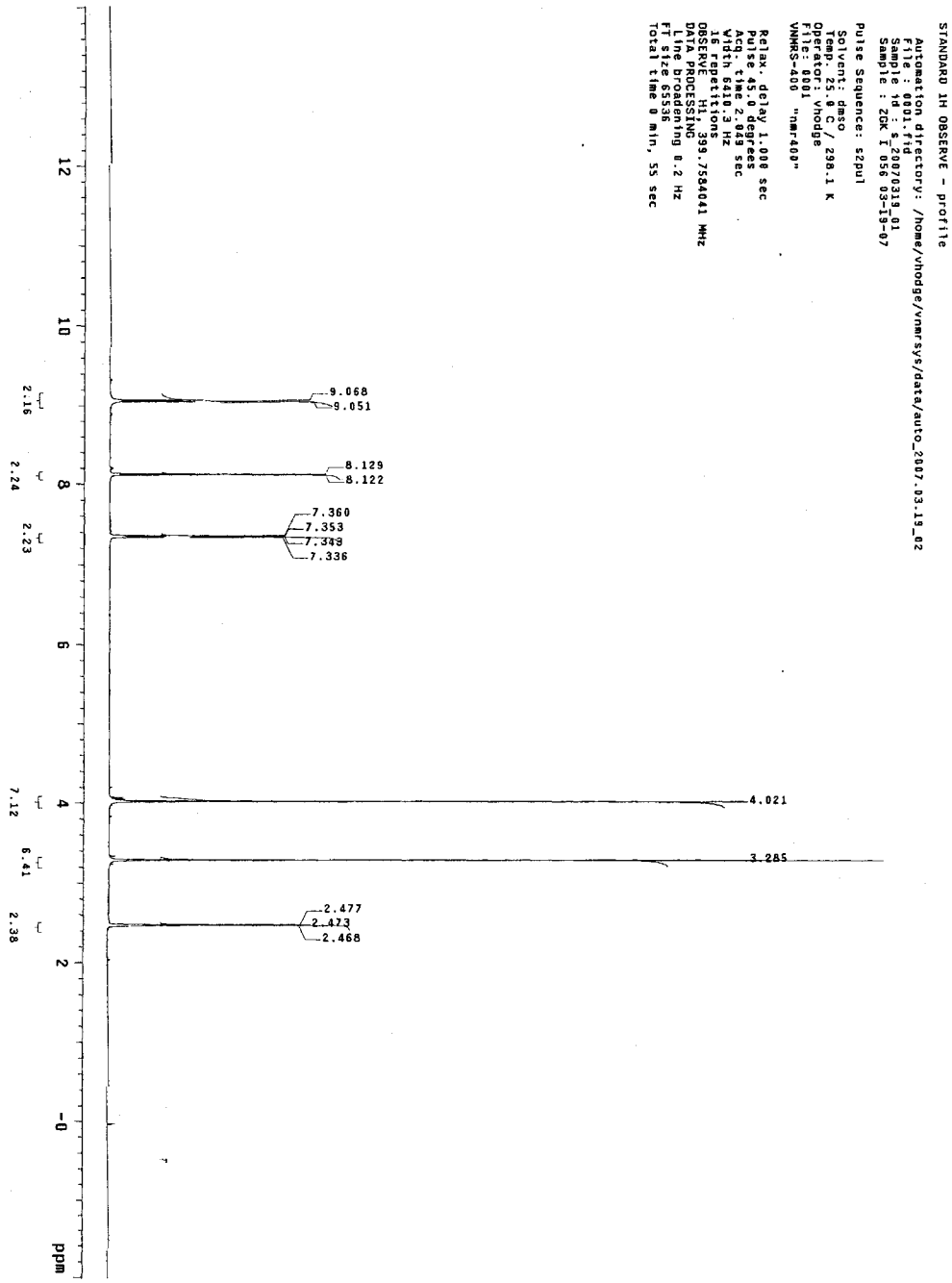
^{13}C NMR of (4,4´-Bis[butyl]-2,2´-bipyridine) $PtCl_2$, **8**

(100 MHz, 298.1 K, DMSO-d_6)



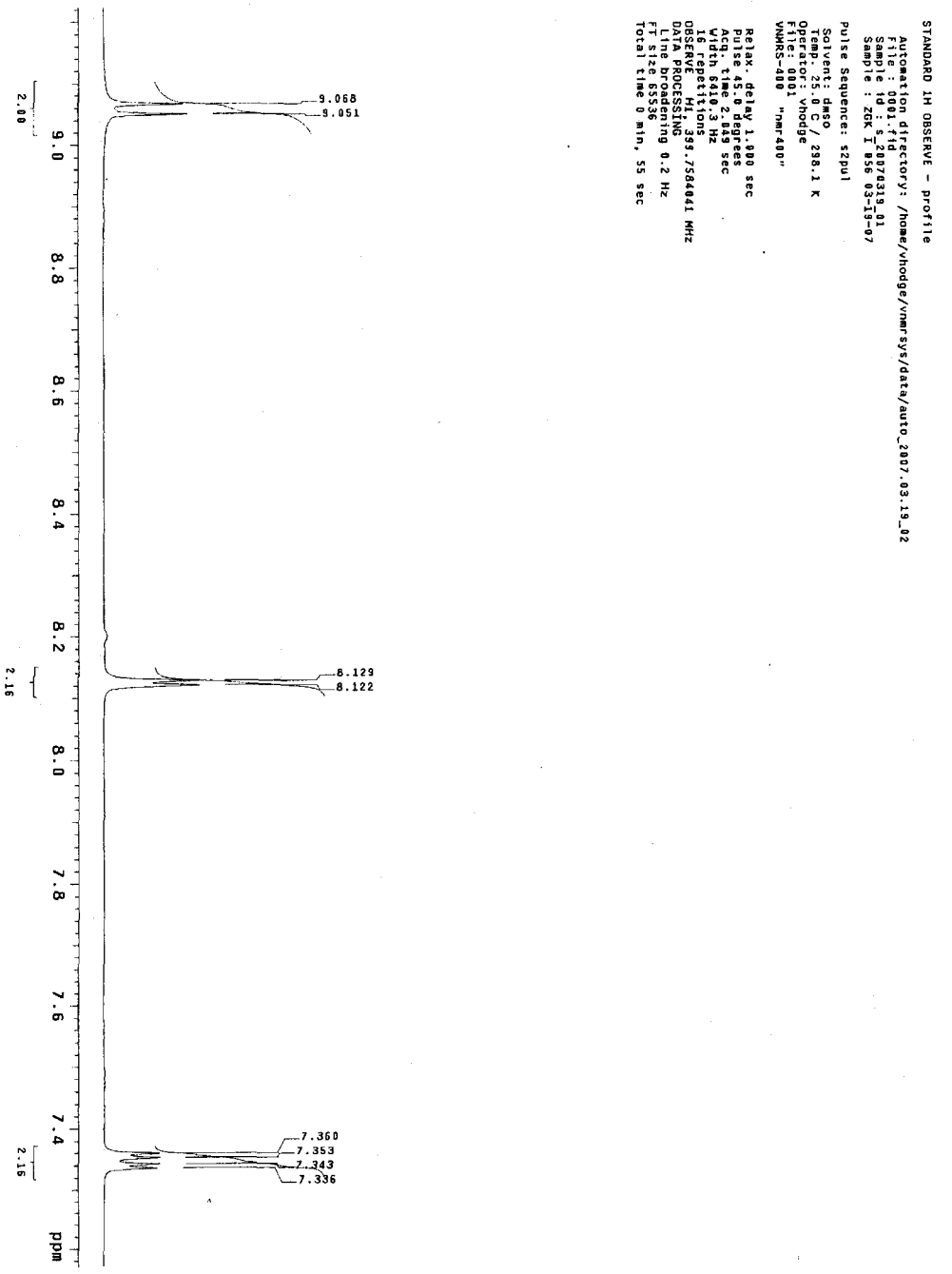
^{1}H NMR of (4,4´-Bis[methoxy]-2,2´-bipyridine) $PtCl_2$, **9**

(400 MHz, 298.1 K, DMSO-d_6)



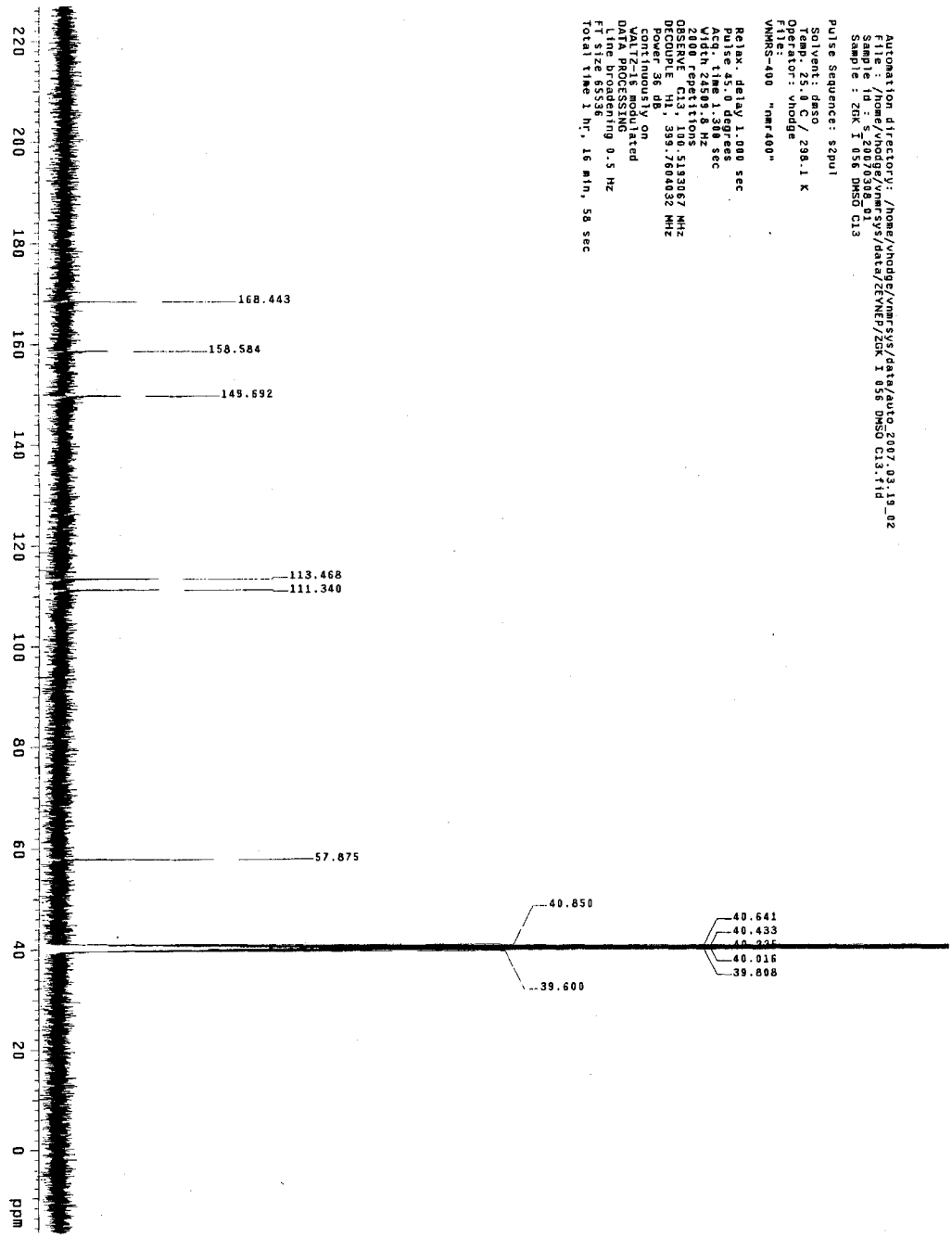
^{1}H NMR of (4,4´-Bis[methoxy]-2,2´-bipyridine) $PtCl_2$, **9**

(400 MHz, 298.1 K, DMSO-d_6)



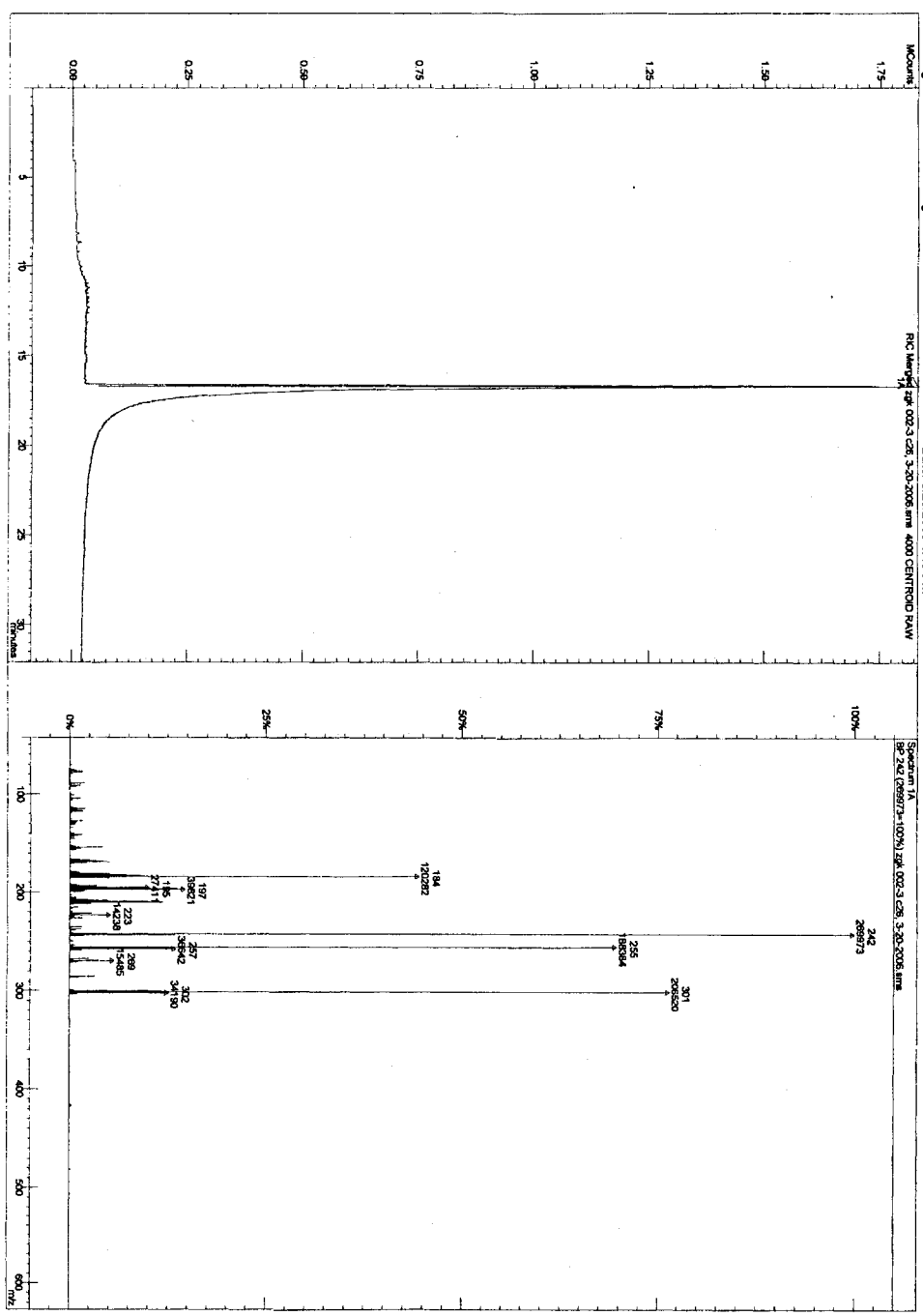
^{13}C NMR of (4,4´-Bis[methoxy]-2,2´-bipyridine) $PtCl_2$, **9**

(100 MHz, 298.1 K, DMSO-d_6)



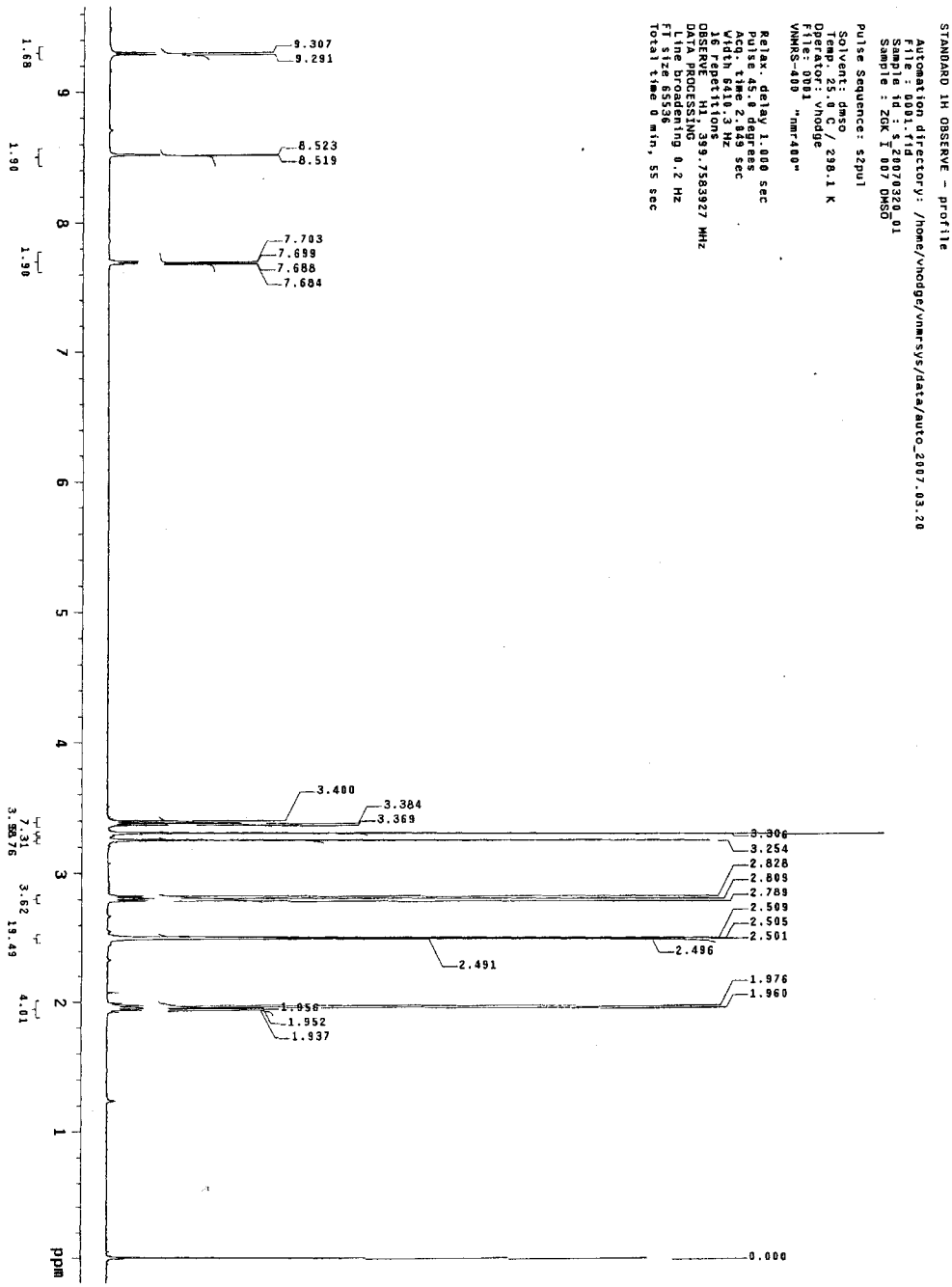
GC/MS of 4,4´-Bis[n-butyl-4-methoxy]-2,2´-bipyridine, **10**

(CH_2Cl_2)



^{1}H NMR of (4,4´-Bis[n-butyl-4-methoxy]-2,2´-bipyridine) $PtCl_2$, **11**

(400 MHz, 298.1 K, DMSO-d_6)



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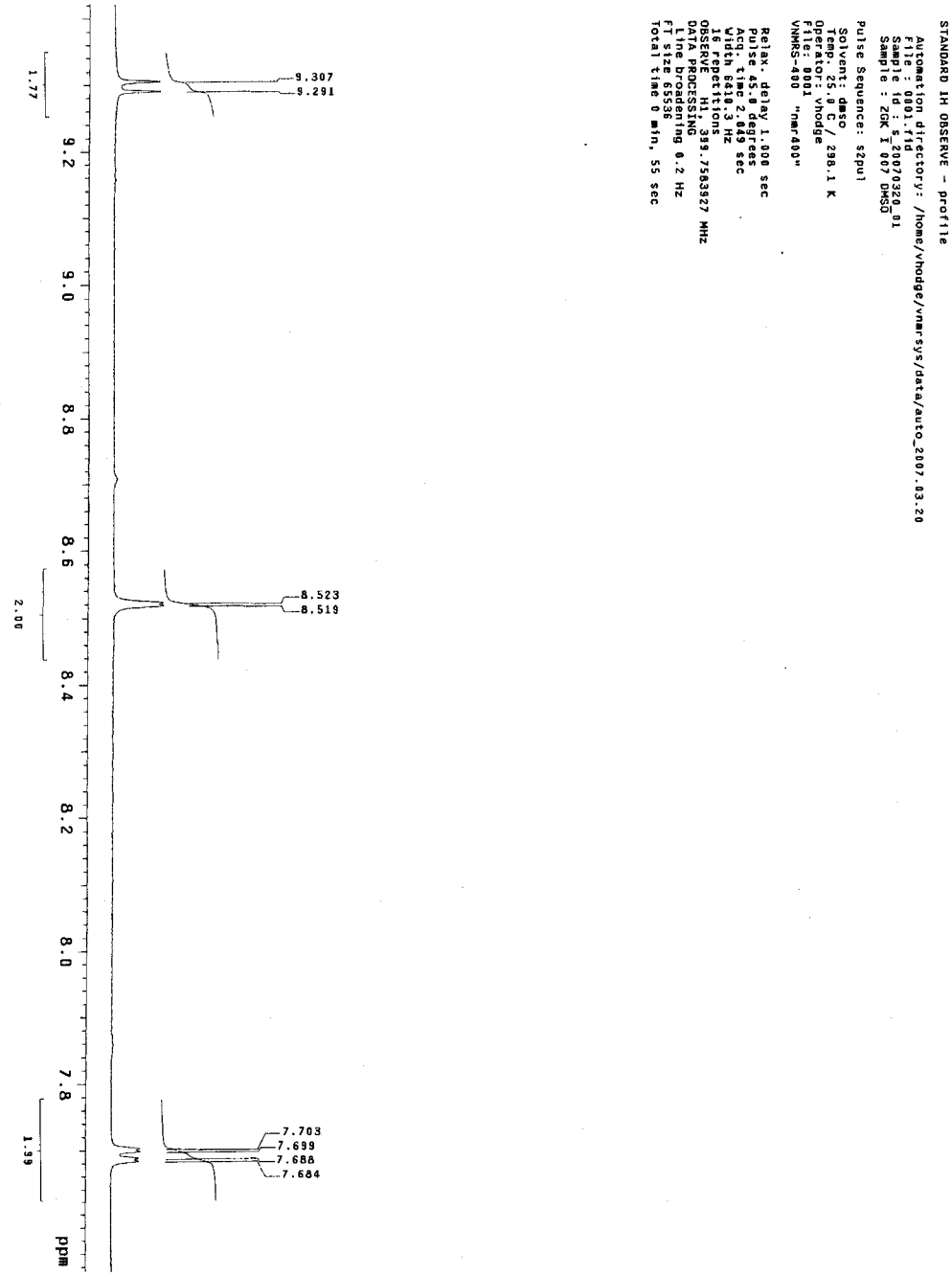
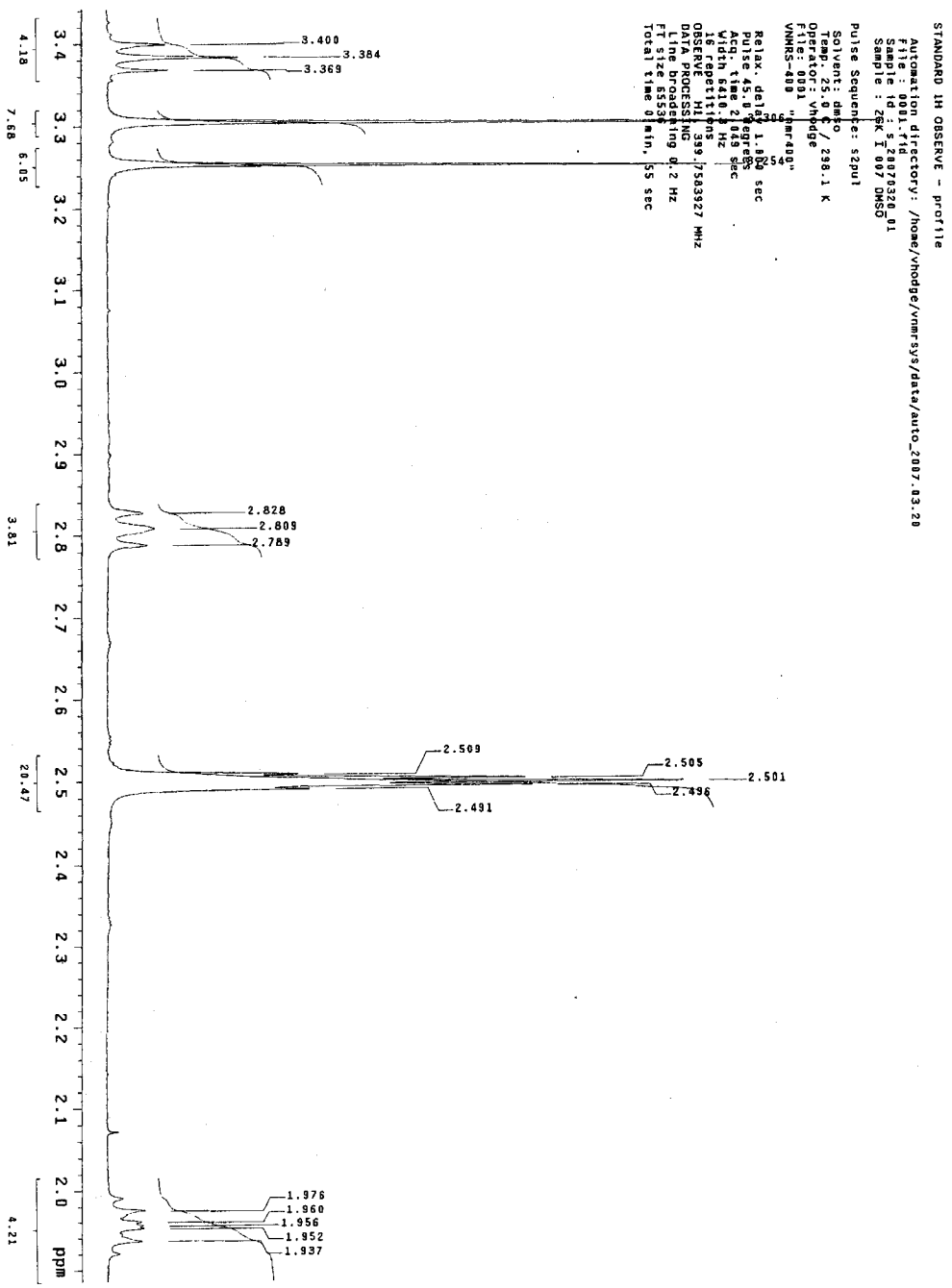
(400 MHz, 298.1 K, DMSO-d_6)



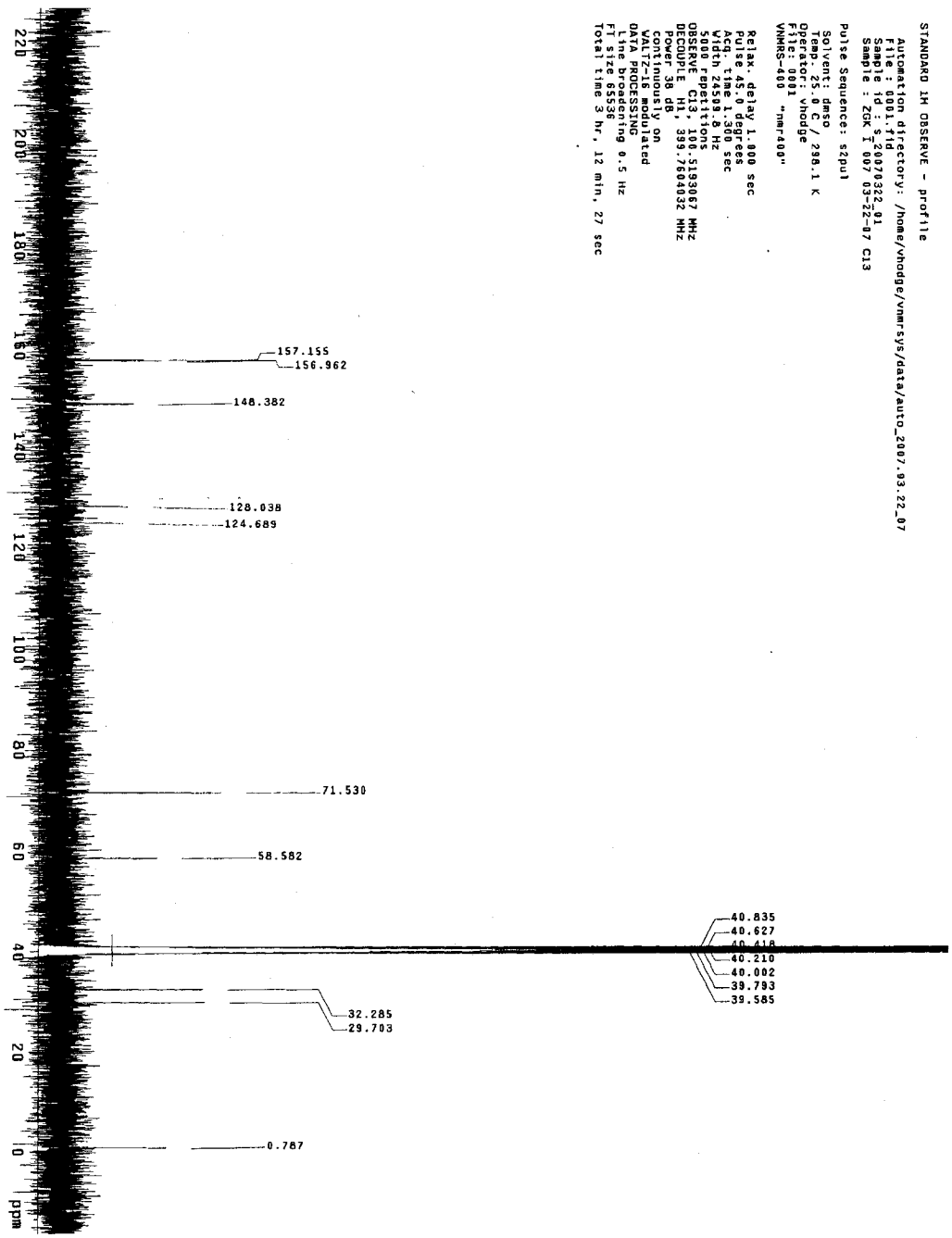
^{1}H NMR of (4,4´-Bis[n-butyl-4-methoxy]-2,2´-bipyridine) $PtCl_2$, **11**

(400 MHz, 298.1 K, DMSO-d_6)



^{13}C NMR of (4,4´-Bis[n-butyl-4-methoxy]-2,2´-bipyridine) $PtCl_2$, **11**

(100 MHz, 298.1 K, DMSO-d_6)



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