

Synthesis, characterization, and biological evaluation of platinum IV complexes exhibiting anticancer properties
by Wayne Ross Edwards

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University
© Copyright by Wayne Ross Edwards (1989)

Abstract:
The chemotherapeutic agent cisplatin is widely used in the treatment of head, neck, ovarian, testicular and bladder cancers. The major disadvantages in using cisplatin are its low solubility and high toxicity, in particular nephrotoxicity. These two limitations prevent cisplatin from being administrated at higher concentrations than 1.0 mg/Kg.

Fifteen platinum IV compounds were synthesized and characterized by NMR. Nine of the 15 were soluble enough in Fetal Bovine Serum to be evaluated in a tissue culture assay using murine B-16 melanoma and Lewis Lung carcinoma cells. Toxicity and chemotherapeutic activity in tumor bearing mice was used to evaluate those compounds showing high solubility and chemotherapeutic activity in tissue culture. Only one of the nine compounds showed high solubility and chemotherapeutic activity in the assay. The toxicity of . this compound,11, using C57B1 mice was determined to be LD50 >41 mg/Kg, compared to cisplatins' LD50 of 13-14 mg/Kg. The compound was evaluated for its chemotherapeutic effects in tumor bearing mice and it was found to . give a dose response against the B-16 melanoma tumor.

Of the other compounds tested in the tissue culture assay one in particular showed significant activity, 20. The LD100 was 29 µM while cisplatin and dichloro-(ethylenediamine)Pt(II) were 15 µM and 75 µM respectively.

It was discovered that dicarboxylates substituents increased the solubility of platina(IV)cylcobutanes more so than alcohols, ethers and aldehydes. Amine platinum ligands influenced the chemotherapeutic activity, with ethylenediamine complexes showing more activity than either pyridine or 2,2'-dipyridine complexes.

SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF PLATINUM IV COMPLEXES EXHIBITING ANTICANCER PROPERTIES

by

Wayne Ross Edwards

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Chemistry

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 1989

N378
Ed985

APPROVAL

of a thesis submitted by

Wayne Ross Edwards

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

July 25, 1989 — Date

Dr. P. W. Jennings — Chairperson, Graduate Committee

Approved for the Major Department

July 25, 1989 — Date

Edwin H. Abbott — Head, Major Department

Approved for the College of Graduate Studies

September 15, 1989 — Date

Henry L. Parsons — Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgement of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his/her absence, by the Dean of Libraries when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my permission.

Signature Waynel Edwards

Date 9/12/89

ACKNOWLEDGEMENTS

During the last 35 months numerous people have shaped and changed a block of clay into a form that is more alive and need they to be mentioned.

Dr. Wyn Jennings for believing, Dr. Jean Starkey for patience, Tana Wilson for assisting, Jeanne Nash for helping, Dr. Steve Guggenheim for advising, Gayle Callis for histological work, the Jennings' Group for smoothing the bumps, and Edie for being there.

TABLE OF CONTENTS

APPROVAL ii

STATEMENT iii

VITA iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vi

LIST OF TABLES ix

LIST OF FIGURES xi

ABSTRACT xvii

INTRODUCTION 1

Organometallic Chemistry. 1
Preparation of Pt(IV) metallacyclobutanes . . 2
Preparation of Platina(IV)cyclopentanes. . . . 8
Characterization of Platinum Compounds 13
Model for Platinum Interacting with Cyclopropanes 15
Mechanism of Platinum Insertion. 16
Puddephatt Rearrangement 17

Cis-Platin History and Usage. 22
Areas of Investigation 24
Clinical 24
Pharmokinetics and Distribution 25
Mechanism of Action. 27
Interactions between Cisplatin and DNA 30
Evaluation of Potential Chemotherapeutic Agents 41

STATEMENT OF PROBLEM 45

CHEMISTRY RESULTS AND DISCUSSION 47

Dicarboxylate Platinacyclobutanes 47
Syn Hydroxymethyl Platinacyclobutanes 62
Syn Methyl Ether and Diol Ether 70
Hydroxy Platinacyclobutanes and Pentanes. 72

Oxidation of Syn Hydroxymethyl to Carbonyl Derivatives 81

BIOLOGICAL RESULTS AND DISCUSSION. 89

Tissue Culture Results. 89
Solubility of Compounds Synthesized 119
Ultraviolet Irradiation Experiments 120
Generating Dichloro(ethylenediamine)Pt(II) from 11. 120
Murine Toxicity Study 127
Black and White Photos 1 and 2 of Liver and Kidney Tissue from Group I, Toxicity Experiment. 135
Black and White Photos 3 and 4 of Liver and Kidney Tissue from Group V, Toxicity. 136
Black and White Photos 5 and 6 of Liver and Kidney Tissue from Group VI, Toxicity. 137
Compound 11 Evaluation in vivo 138
Summary 147

EXPERIMENTAL METHODS FOR CHEMICAL SYNTHESIS. 148

Synthesis of 4 148
Synthesis of 5. 148
Synthesis of 7. 149
Synthesis of 9 and 10 149
Synthesis of 11 and 12. 150
Synthesis of 13 151
Synthesis of 15 151
Synthesis of 16 152
Synthesis of 17 and 18. 152
Synthesis of 19 153
Synthesis of 20 153
Synthesis of 21 153
Synthesis of 23 and 25. 154
Synthesis of 24 and 26. 154
Synthesis of 28 155
Synthesis of 29 155
Synthesis of 29, 30, and 31 156
Synthesis of 33 156
Synthesis of 34 157
Synthesis of 36 157
Synthesis of 37 157

Experimental for Biological Experiments 158

Tissue Culture Assay Experimental. 158
Adding Cells to Dishes 158
Addition of Test Compounds to the Dishes . . .160

Removing Solution Containing Compounds from Dishes 160
Staining and Counting Colonies 161
Subculturing of Cells. 161
Dissolving Test Compounds in FBS 162
Toxicity Test Experimental Methods. 162
In Vivo Anticancer Experimental Study Methodologies. 165

SUMMARY. 169

REFERENCES CITED 171

APPENDICES 179

Appendix A--Structures of Methotrexate, Fluorouracil, Etoposide Vinblastine Sulfate, Bleomycin and Adriamycin. 180

Appendix B--Standard Error for Tissue Culture Survival Curves. 184

Appendix C--Defined Fetal Bovine Serum Analysis for First and Second Batches. 189

Appendix D--Biological Solutions and Supplies . . .192

Appendix E--List of All Compounds Used in Thesis. .196

LIST OF TABLES

Table — Page

1. Tetrameric Platinacyclobutanes, [$PtCl_2$ (cyclopropane)$_4$. 5

2. Structural Data for Monomer Platinacyclobutanes. 6

3. Infrared Data for cyclopropane, a Platina(IV)cyclopropane, and the Tetramer. 7

4. Examples of Platina(IV)cyclopentanes. 13

5. Organ Distribution of Cisplatin in Walker-256-Carcinosarcoma-Bearing Rats (3 hr After Pulse Injection) 28

6. Evidence Supporting DNA-Cisplatin Interaction. 29

7. Activity of Amine Complexes with Chelated Dicarboxylate Ligands X in [$Pt(NH_3)_2X$].. . 35

8. Various Amine Complexes Containing Oxalate and Malonate Ligands [PtA_2X] or [PtAX].. . 36

9. Changes in Activity on Varying A in cis-[PtA_2Cl_2]. 38

10. Straight Chain Derivatives of Cis Pt II.. . . . 39

11. Branched Chain Derivatives of Pt II- Pt IV. . . 39

12. Using ADJ/PC6 Plasma Cell Tumor to Test Cyclic Amines. 40

13. Solubility data for Pt(II) and Pt(IV) Complexes. 41

14. Antitumor Activity of Pt(II) and Pt(IV).. . . . 42

15. Major Clinical Toxicities of Cisplatin in Various Animals Species. 43

16. LD_{50} and LD_{100} of Compounds Tested. 115

17. Concentrations Used For Groups I-IX. 128

18. Serum Creatinine Values for Toxicity Study. . .130

19. Histological Summary of Liver Toxicity Day 5 Testing Compound 11. 132

20. Histological Summary of Kidney Toxicity Day 5 Testing Compound 11 133

21. Dosages Used for in vivo Study. 139

22. Liver Histological Evaluations of Compound 11 in Tumor Bearing Mice. 146

23. Kidney Histological Evaluations of Compound 11 in Tumor Bearing Mice. 146

24. Cisplatin, 11 and 20 Evaluation Data Compared 147

25. Standard Error for Tissue Culture Survival Curves. 185

26. First Lot of Defined Fetal Bovine Serum Analysis 190

27. Second Lot of Defined Fetal Bovine Serum Analysis 191

28. Biological Solutions and Supplies 193

29. List of All Compounds Used in Thesis. 197

LIST OF FIGURES

Figure		Page
1.	Tipper's Reaction Scheme.	2
2.	Reaction Scheme Using Zeise's Dimer	3
3.	Tetrameric Structure.	4
4.	Monomer Structure..	4
5.	Cis Substituted Platinacyclobutanes..	8
6.	Three Possible Pathways for Platinacyclobutanes.	8
7.	First Method to Synthesize Platina(IV) cyclopentanes.	9
8.	Second Method to Synthesize Platina (IV) cyclopentanes.	9
9.	Puddephatt's Method to Synthesize Ring Expanded Products.	10
10.	Third Method by Puddaphatt to Synthesize Platina(IV)cyclopentanes	11
11.	Puddephatt's Ring Expansion..	11
12.	Alcoholysis and Acid Catalysis Reactions. . .	12
13.	Neilsen's Ring Expansion.	12
14.	Pt(IV)cyclopentane synthesized by Waddington and Jennings..	13
15.	Walsh Molecular Orbital Interactions.	16
16.	Formation of an Edge-bound Platinum Complex.	17
17.	Platinacyclobutane Rearrangement.	18
18.	First Mechanism of Puddephatt Rearrangement.	19

19. Second Puddephatt Rearrangement Mechanism. 19

20. Concerted Mechanism for Rearrangement.. . . . 20

21. Isolobal Analogy to Platina(IV) cyclobutanes 20

22. Proposed Mechanism for the Platina(IV) cyclobutane Complex Using the Isolobal Analogy.. 21

23. Cisplatin and Dichloro(ethylenediamine) Pt(II) 23

24. Seven Compartmental Model.. 27

25. Aquation Reactions of Cisplatin 29

26. Cisplatin DNA Interactions: a) N_7-(G)-O_6(G), b) intrastrand, c) DNA-Protein.. 30

27. Structure of Cisplatin-GpG Adduct 32

28. Cis and Trans DichlorodiaminePt(II) Survival Curves Using HeLa Cells 43

29. Target Molecule 10. 47

30. 1,2-Dimethylester-1,4-norbornadiene, 4. . . . 48

31. Proton NMR Spectrum of Compound 10. 49

32. Carbon NMR of Compound 10 50

33. COSY Spectrum of Compound 10. 51

34. HETCOR Spectrum of Compound 10. 52

35. Mono and Di cyclopropanated methyl esters, 5 and 6.. 53

36. Hydrolysis Dicarboxylate Products 7 and 8.. . 53

37. Dicarboxylate Platinacylcobutane with ethylenediamine, (11) 54

38. Proton NMR Spectrum of Compound 11 55

39. Dicarboxylate platinacyclobutane with 2,2'-bipyridine 12.. 56

40. Carbon NMR Spectrum of Compound 12. 57

41. Dicarboxylate Compound with L = 4-Pyridyl Carbinol, 13.. 58

42. Carbon NMR Spectrum of Compound 13. 59

43. Summary of Reactions Involving Dicarboxylates.. 61

44. LAH Reduction Scheme. 62

45. Syn Hydroxymethyl Platinacyclobutane I.P.C. 17. 63

46. Carbon NMR Spectrum of Compound 18. 64

47. Carbon NMR Spectrum of Compound 21. 65

48. DEPT NMR Spectrum of Compound 21. 66

49. I.P.C. 17 to products 20 and 21.. 67

50. DEPT NMR Spectrum of Compound 20. 68

51. Summary of Reactions of Syn Hydroxymethyl Platinacyclobutanes. 69

52. Synthetic Scheme for Compounds 35, 36 and 37 71

53. Compounds Synthesized by Burton.. 72

54. Reaction Scheme for 25. 73

55. Burton's Synthetic Scheme for 24. 74

56. Synthetic Scheme for 24 Involving Ether.. . . 74

57. Hydroxy Platinacyclobutane and Pentane 24 and 25. 75

58. Addition of Acid to Synthesize 24 and 25.. 76

59. Hydroxy Platinacyclopentane with 2,2'-bipyridine, 28.. 77

60. Carbon NMR Spectrum of Compound 28. 78

61. Reaction Scheme for Compounds 24 and 25 using THF.. 79

62. Reaction Scheme for Compounds 24 and 25 using Diethyl Ether.. 80

63. Reaction Scheme to Synthesis 30.. 81

64. Oxidation Products of 20 with PDC 82

65. Carbon NMR Spectrum of Compound 20. 83

66. Neilsen's Synthesis of 31 84

67. Neilsen's Ring Expansion Mechanism. 84

68. Twenty Four Hour and 7 days Oxidations of 20. 86

69. Compound 20 with 50:50 Pyridine-chloroform.. 86

70. Oxidation of Compound 21. 87

71. Reaction Scheme for Compounds 29, 30 , 31. 88

72. Cisplatin and $Cl_2(H_2NCH_2CH_2NH_2)Pt(II)$.. . . . 90

73. Survival Curves for Cisplatin and Dichloro(ethylenediamine)Pt(II). 91

74. Reductive-Elimination Products from Platinacylcobutanes. 92

75. Compounds Synthesized for Tissue Culture Testing. 93

76. Compounds Not Tested in the Tissue Culture Assay Due to Insolubility in Fetal Bovine Serum. 95

77. Survival Graph of Cisplatin with B-16.. . . . 96

78. Survival Graph of Cisplatin with Lewis Lung.. 97

79. Survival Graph of Dichloro(ethylene-diamine)Pt(II) with B-16 98

80. Survival Graph of Dichloro(ethylene-diamine)Pt(II) with Lewis Lung 99

81. Survival Graph of Dicarboxylate with Pyridine using B-16. First Experiment Done. 100

82. Survival Graph of Dicarboxylate with Pyridine using B-16. Last Experiment Done.. 101

83. Survival Graph of Dicarboxylate with Pyridine using Lewis Lung. 102

84. Survival Graph of Dicarboxylate with ethylenediamine using B-16.. 103

85. Survival Graph of Dicarboxylate with ethylenediamine using Lewis Lung.. . . .104

86. Survival Graph of Syn Hydroxymethyl with Pyridine using B-16.. 105

87. Survival Graph of Syn Hydroxymethyl with Pyridine using Lewis Lung.. 106

88. Survival Graph of Syn Hydroxymethyl with Ethylenediamine using B-16.. 107

89. Survival Graph of Syn Aldehyde with Pyridine using B-16. 108

90. Survival Graph of Syn Aldehyde with Pyridine using Lewis Lung. 109

91. Survival Graph of Syn Diol Ether using Lewis Lung.. 110

92. Survival Graph of Oxygen Bridge with Pyridine using B-16. 111

93. Survival Graph of Oxygen Bridge with Pyridine using Lewis Lung. 112

94. Survival Graph of Dicarboxylate Cyclopropane with Lewis Lung.. 113

95. Survival Graph of Epimer Alcohols/ Cyclopropane using Lewis Lung. 114

96. B-16 Cells Survival Curve Using 254 nm Light. 121

97. Serum Containing Medium with 11 Irradiated Without Cells Present. 122

98. Five Hours of UV in the Absence of B-16 cells with 11 123

99. Five Hours of UV With Dichloro-(ethylenediamine)Pt(II). 123

100. Five Hours UV Irradiation in the Presence of 10. 125

101 Black and White Photos of Liver and Kidney Tissue from Group I, Toxicity Experiment 135

102. Black and White Photos of Liver and Kidney Tissue from Group V, Toxicity Experiment 136

103. Black and White Photos of Liver and Kidney Tissue from Group VI, Toxicity Experiment. 137

104. Percent Growth and Survival Curves for Females. 143

105. Percent Growth and Survival Curves for Males. 144

106. Percent Growth and Survival Curves for All Mice. 145

107. Chemical Structures of Methotrexate and Fluorouracil 181

108. Chemical Structures of Etoposide and Vinblastine Sulfate. 182

109. Chemical Structures of Bleomycin and Adriamycin 183

ABSTRACT

The chemotherapeutic agent cisplatin is widely used in the treatment of head, neck, ovarian, testicular and bladder cancers. The major disadvantages in using cisplatin are its low solubility and high toxicity, in particular nephrotoxicity. These two limitations prevent cisplatin from being administrated at higher concentrations than 1.0 mg/Kg.

Fifteen platinum IV compounds were synthesized and characterized by NMR. Nine of the 15 were soluble enough in Fetal Bovine Serum to be evaluated in a tissue culture assay using murine B-16 melanoma and Lewis Lung carcinoma cells. Toxicity and chemotherapeutic activity in tumor bearing mice was used to evaluate those compounds showing high solubility and chemotherapeutic activity in tissue culture. Only one of the nine compounds showed high solubility and chemotherapeutic activity in the assay. The toxicity of this compound, 11, using C57Bl mice was determined to be LD_{50} >41 mg/Kg, compared to cisplatins' LD_{50} of 13-14 mg/Kg. The compound was evaluated for its chemotherapeutic effects in tumor bearing mice and it was found to give a dose response against the B-16 melanoma tumor.

Of the other compounds tested in the tissue culture assay one in particular showed significant activity, 20. The LD_{100} was 29 μM while cisplatin and dichloro-(ethylenediamine)Pt(II) were 15 μM and 75 μM respectively.

It was discovered that dicarboxylates substituents increased the solubility of platina(IV)cylcobutanes more so than alcohols, ethers and aldehydes. Amine platinum ligands influenced the chemotherapeutic activity, with ethylenediamine complexes showing more activity than either pyridine or 2,2'-dipyridine complexes.

INTRODUCTION

Organometallic Chemistry

The growth of organometallic chemistry over the last three decades has been remarkable. What started out with several compounds and applications has grown into a multitude of industries. The applications of this chemistry to synthesis, catalysis, and transformations are well documented. Synthesis, catalysis, regioselectivity and stereoselectivity are areas where platinum group metals (Pt,Pd,Ir,Rh,Os,Ru) have been used with great success.

The first organo-transition metal complex synthesized was one containing platinum and ethylene, ($K[(C_2H_4)PtCl_3]$. This compound was prepared by William C. Zeise in Copenhagen, 1827.[1] Since Zeise's discovery, and in particular in the last three decades the area of organometallic chemistry involving platinum has had tremendous growth. Most of the complexes formed are stable and this lends to easily isolated and characterized complexes.[2]

An area of interest in recent years is that of metallacyclobutanes. These complexes are of interest because of their catalyzed reactions, such as, olefin metathesis, and polymerization.[3] [4] [5] Puddephatt[6], in 1980 reviewed

platinacyclobutanes and their chemistry. Even though these complexes are stable, they will perhaps help in elucidating mechanism(s), involving metallacyclobutanes with transition metals.[7 8 9]

Preparation of Pt(IV) metallacyclobutanes

There are two main synthetic routes used in the preparation of platinum (IV) metallacyclobutanes. The original method was developed by Tripper in 1955 and is shown below in Figure 1.[10] The reference to platinum IV complexes or platinum IV's in this thesis will be restricted to organometallic complexes. The monomer from Tipper's synthesis is stable after addition of pyridine. Zeise's dimer is used as the platinum source in the second method, Figure 2.

Figure 1. Tipper's Reaction Scheme.

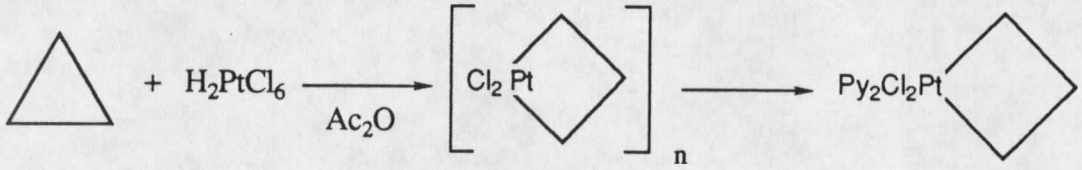
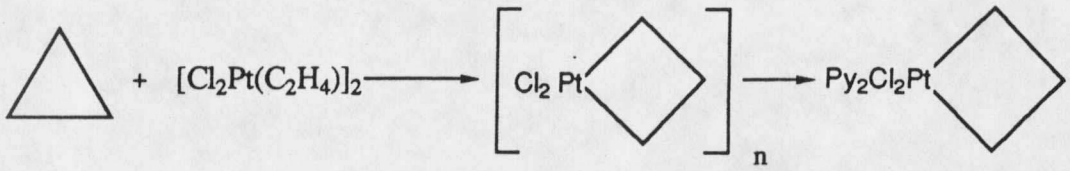


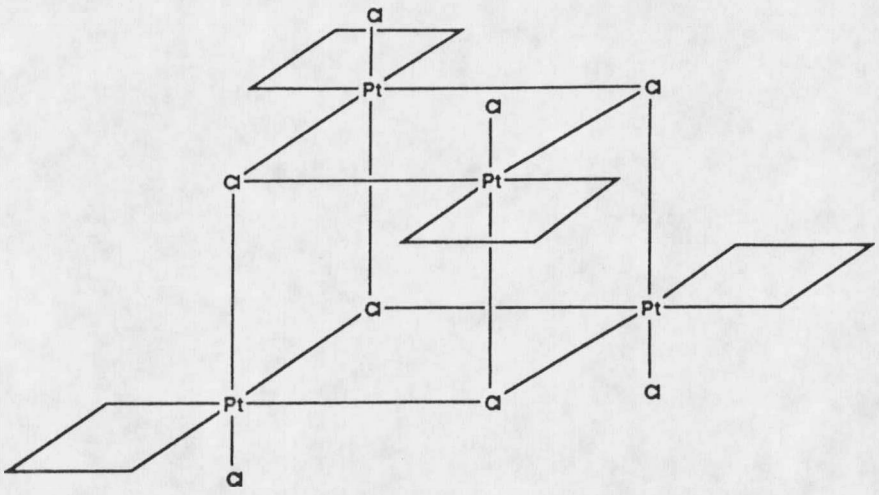
Figure 2. Reaction Scheme Using Zeise's Dimer.



Using Zeise's dimer as the platinum source instead of hexachloroplatinic(IV) acid makes it possible for substituted derivatives to be synthesized. McQuillin originally reported the use of Zeise's dimer in 1976.[11] The dimer is an orange solid that is very soluble in tetrahydrofuran (THF) and is slightly soluble in diethyl ether. Upon addition of the cyclopropane starting material, a yellow precipitate will form, indicating oxidative addition. This initially precipitated complex (I.P.C.) is believed to be a tetrameric species, Figure 3. Owing to the nature of the tetrameric complex's solubility, full characterization has not been achieved to date. Table 1 lists data on known tetrameric platinacyclobutanes.

The tetrameric species can be converted to monomers by adding a good donor ligand, such as pyridine. Monomers are octahedral in geometry at the metal center and have a general structure as shown in Figure 4.

Figure 3. Tetrameric Structure.



The monomeric species is well characterized due to its enhanced solubility. Once the tetramer dissociates to form monomers, the donor ligands are cis and the chlorines trans.[12 13 14] Examples of platina(IV)cyclobutane monomers are listed in Table 2.

Figure 4. Monomer Structure.

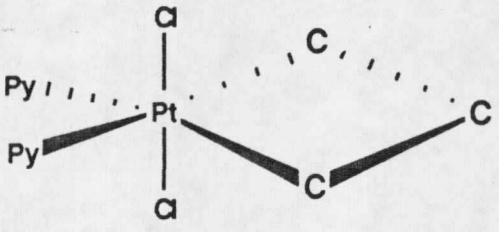
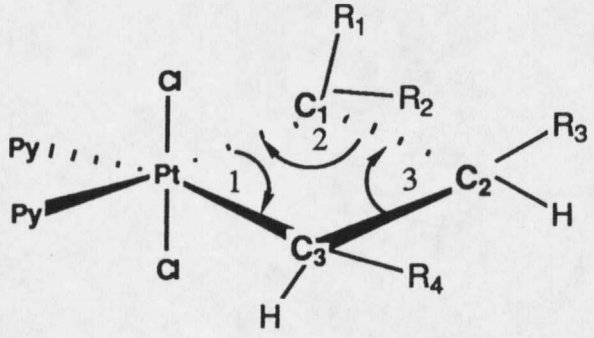


Table 1. Tetrameric Platinacyclobutanes, $[PtCl_2(cyclopropane)_4]$.

R $[(RC_3H_5)PtCl_2]_4$	Solvent	mp(°)	Ref.
H	CH_2Cl_2	148 d	40,41
Me	THF		42
Et	Et_2O		43
i Pr	Et_2O		43
Bu	THF		42
nC_6H_{13}	Et_2O	120 d	29,40,44
$PhCH_2$	Et_2O	123 d	29,40,44
Ph	Et_2O	135 d	29,34,40,44
4-MeC_6H_4	Et_2O	125 d	29,34,40,44
2-Me_6H_4	Et_2O		34
2-$NO_2C_6H_4$	Et_2O	148 d	29,40,44
4-$EtOC_6H_4$	THF		34
4-$EtOC_6H_4$	THF		34
R,R' $[(RR'C_3H_4)PtCl_2)_4$			
1,1-Me_2	Et_2O, THF		42,43
trans-1,2-Me_2	Et_2O, THF		42,43
trans-1-Me-2-Bu	Et_2O		29,40,44
trans-1-Me-2-Ph	THF		40
trans-1,2-Ph_2	Et_2O		29,40,44
cis-1.2-Ph_2	Et_2O		37
trans-1,2-(4-$MeC_6H_4)_2$	Et_2O		40

Table 2. Structural Data for Monomer Platinacyclobutanes.



	Alkyl Groups				Bond Distances A					
No.	R_1	R_2	R_3	L_2	Pt-C_1	Pt-C_2	Pt-C_3	C_1-C_2	C_2-C_3	C_1-C_3
1	H	H	H	Py_2	2.04	2.69	2.19	1.48	1.82	2.55
2	H	Ph	H	Py_2	2.06	2.60	2.11	1.59	1.48	2.39
3	H	H	H	Py_2	2.05	2.62	2.17	1.59	1.71	2.60
4	H	H	H	bipy	2.07	2.76	2.07	1.63	1.63	-

No.	Dihedral Angle	1	2	3	Ref.
1	12°	74°	101°	99°	40,41
2	28°	70°	102°	90°	29,40,44
3	22°	76°	104°	91°	29,40,44
4	0°	72°	96°	96°	45

It is worth noting that all of the platinacyclobutanes in Table 2 show puckering at the cyclobutane ring. The degree of puckering is measured in the dihedral angle between the $C_1C_2C_3$ plane and the C_1PtC_3 plane. These dihedral angles can vary between 0 and 30 degrees, Table 2. Table 3 lists infrared data characteristic of cyclopropane and platina(IV)cyclobutanes.

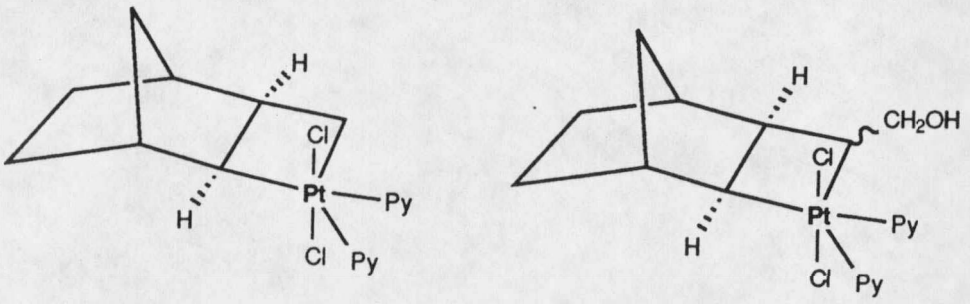
Table 3. Infrared Data* for Cyclopropane, a Platina(IV)-cyclopropane, and the Tetramer.

Bands due to the C_3H_6 unit	C_3H_6	$Pt(C_3H_6)Cl_2Py_2$	$[Pt(C_2H_4)Cl_2]_2$
C-H	3103	2992	3025
		2938	2954
		2917	2948
C-H_2 deformation	1442	1437	1414
CH_2		1238	1255
			1237
CH_2 twist		1217	1165
ring deformation		1134	1149
		1025	1087
CH_2 rock	1029	1038	1087
			1022
ring deformation		980	981
CH_2 rock	869	976	948
		892	890
			663
ring deformation			563

(*) = cm^{-1}

Novel platinum complexes of the form, $PtCl_2(C_3H_4R'R'')L_2$, and $PtCl_2(C_3H_3R'R''R''')L_2$ have been synthesized and characterized by Jennings and coworkers, forming the first cis 2,3-disubstituted[15] and 1,2,3-trisubstituted[16] platinacyclobutanes, Figure 5.

Figure 5. Cis Substituted Platinacyclobutanes.

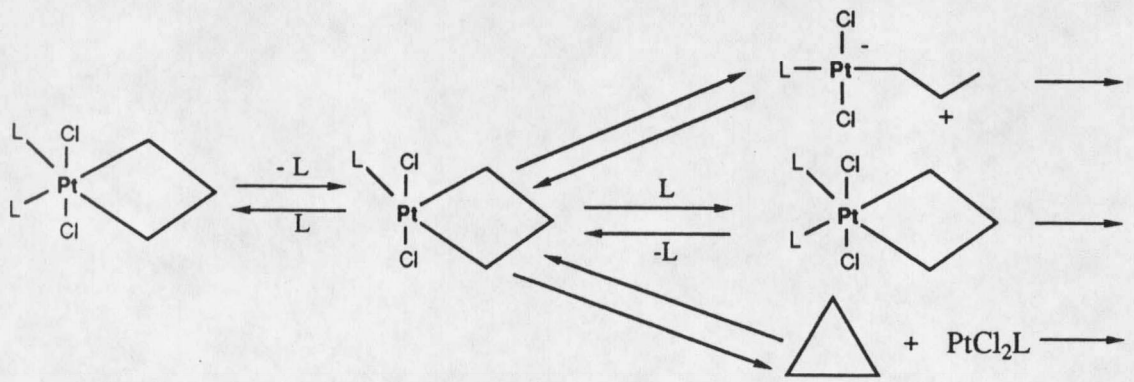


Reactions of platina(IV)cyclobutanes in the monomer form generally proceed by neutral ligand dissociation followed by three possible pathways, Figure 6.

Preparation of
Platina(IV)cyclopentanes

There are three basic methods to synthesize platina-(IV)cyclopentanes. The first two methods, Figures 7 and 8, involve synthesis of a platinum(II) species prior to the platinum(IV) complex.

Figure 6. Three Possible Pathways for Platinacyclobutanes.



This first method, Figure 7, is more synthetically useful, since it is capable of synthesizing substituted platina(IV)cyclopentanes.[17 18]

Figure 7. First Method to Synthesize Platina(IV)cyclopentanes.

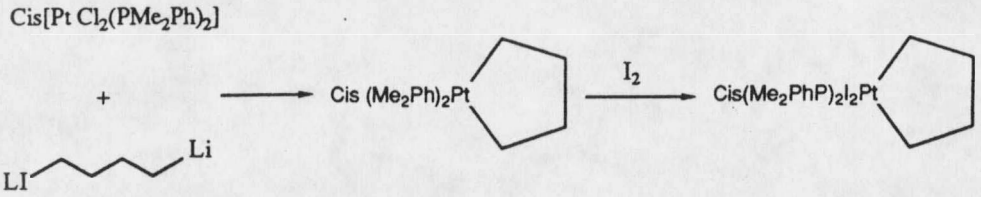
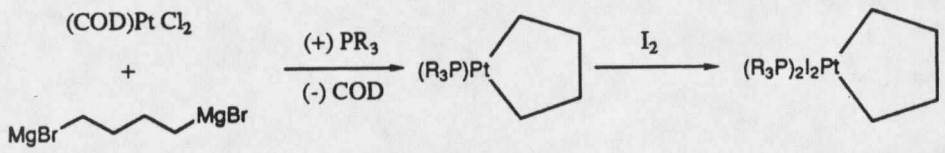


Figure 8. Second Method to Synthesize Platina(IV)cyclopentanes.

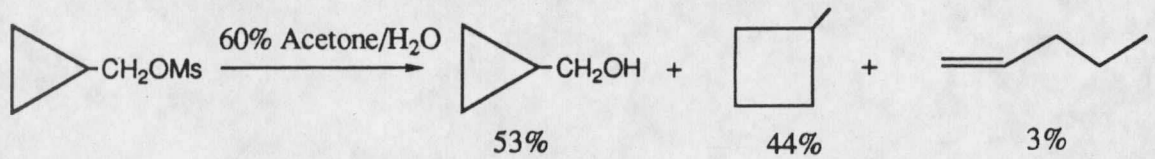


The last method was reported by Puddephatt and Burton[19] using cyclopropyl and cyclobutylcarbinyl esters in a solvolysis reaction which will ring expand by one carbon[20 21], Figure 9. Puddephatt and Burton capitalized on the chemistry in Figure 9 by having the starting

material a platina(IV)cyclobutane moiety instead of a cyclobutane. Substituents on the ring are good leaving groups during subsequent solvolysis reactions to produce the desired ring expanded product. Figure 10 outlines these synthetic steps.

Puddephatt and Burton proposed a mechanism, Figure 11, to explain these results, but also add that the products could be achieved by alcoholysis and acid catalysis, Figure 12.[22 23 24]

Figure 9. Puddaphatt's Method to Synthesize Ring Expanded Products.



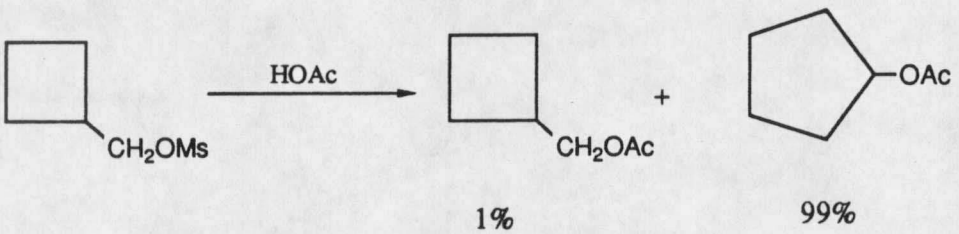


Figure 10. Third Method by Puddaphatt to Synthesize Platina(IV)cyclopentanes.

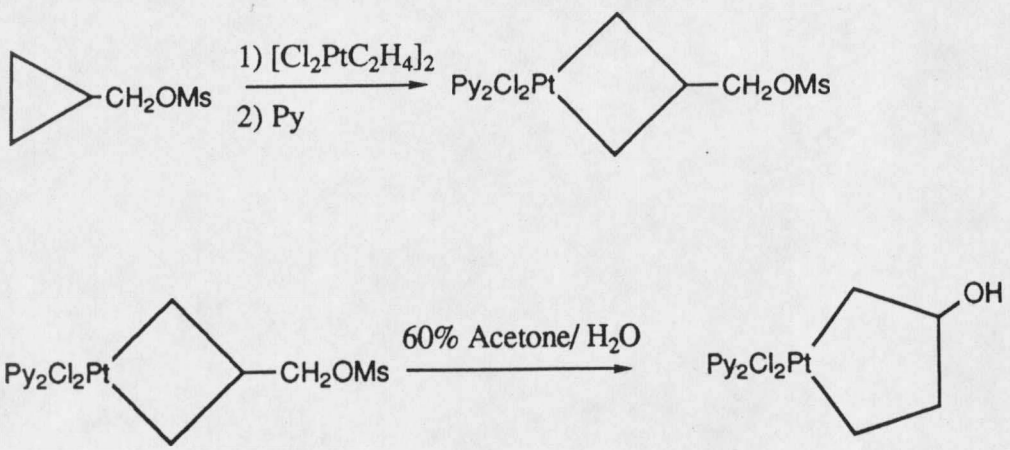


Figure 11. Puddephatt's Ring Expansion.

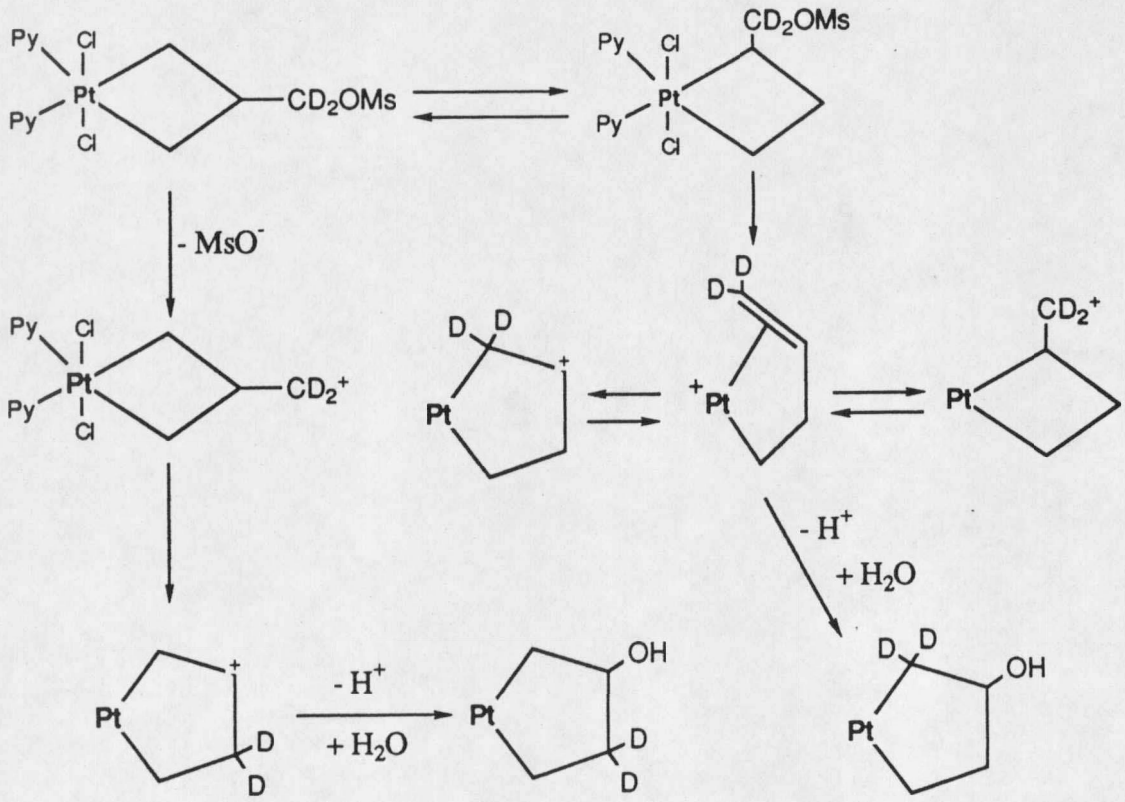
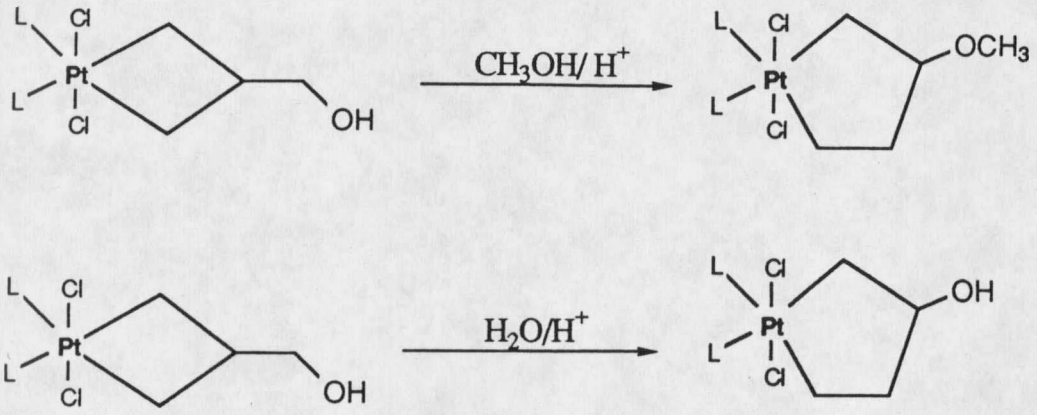
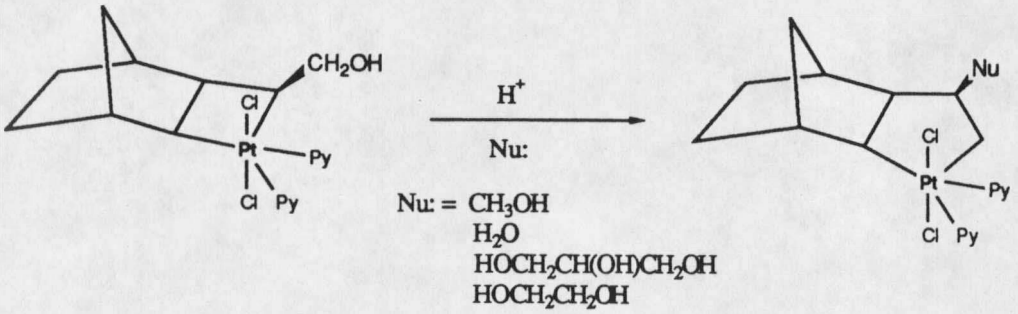


Figure 12. Alcoholysis and Acid Catalysis Reactions.



Reactions outlined in Figure 12 were found not to provide the ring expanded product. Neilsen[25] showed that acid catalysis could be used to obtain a Pt(IV)cyclopentane, Figure 13.

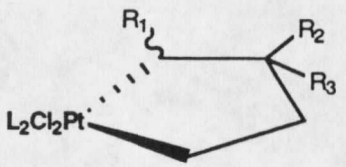
Figure 13. Neilsen's Ring Expansion.



Examples of Pt(IV)cyclopentanes synthesized by Puddephatt and Burton are presented in Table 4 and Figure

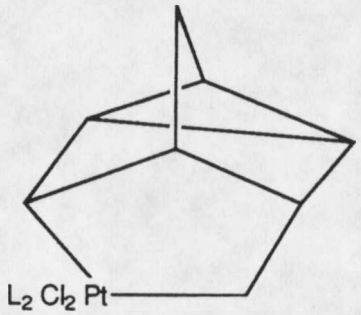
Table 4. Examples of Platina(IV)cyclopentanes.

R_1	OH	OH	OH	OH
R_2	H	Me	Ph	H
R_3	H	H	H	Me
L	Py or Bipy	Py	Bipy	Py



14 shows an example of a platina(IV)cyclopentane synthesized by Waddington and Jennings. [26]

Figure 14. Pt(IV)cyclopentane Synthesized by Waddington and Jennings.



Characterization of Platinum Compounds

Characterization of platinum-carbon complexes is enhanced by platinum being NMR active. Platinum-195 has a

spin of 1/2 for its NMR active isotope, in 33.7% natural abundance. Coupling of commonly used nuclei (^{1}H, ^{13}C, ^{15}N, ^{31}P) to platinum can be readily observed. The combination of spin 1/2 and the 33.7% natural abundance creates a well recognized triplet in the ^{13}C or ^{1}H NMR spectra of significant proportion. Intensity of the central resonance is 66.3% and the satellites resonances are each 16.8%. The frequency difference between the two satellites represents the platinum-coupling constant. Platinum coupling constants are used as a measure of distance between a particular carbon and the platinum, along with the ring size.

When ^{1}H NMR is used for characterization of platinacyclobutanes or pentanes the platinum-hydrogen couplings can provide conclusive evidence that platinum is present. It also provides information about the location of the proton relative to the platinum. Platinum-hydrogen satellites can be overlapping with other resonances and therefore obscured. The coupling constants for ^{2}J range from 70-100 Hz, ^{3}J range from 0-50 Hz, while ^{4}J range from 0-10 Hz.

Carbon-13 NMR is generally more useful in determining platinum-carbon structures.[27] Typical ^{195}Pt-C coupling constants for platinacyclobutanes are between 335-460 Hz for ^{1}J and 80-130 Hz for ^{2}J. Coupling constants for

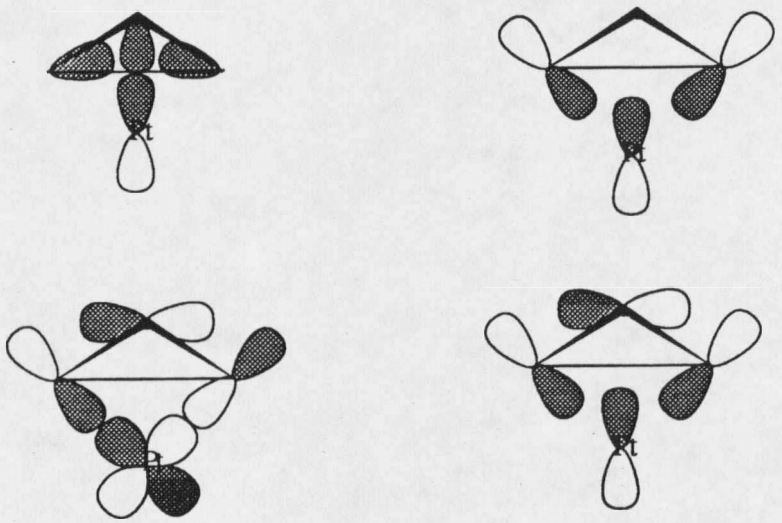
platinacyclopentanes are between 450-550 Hz for ^{1}J, 50-100 Hz for ^{2}J and 20-40 Hz for ^{3}J.

Platinum-195 NMR can be used to distinguish between Pt(IV) and Pt(II). The platinum resonances are found over a wide range, encompassing 13000 ppm.[28] Platinum II resonances will be observed at higher fields than Pt(IV) resonances.

Model for Platinum Interacting with Cyclopropanes

The interaction between the platinum II and cyclopropane was first proposed using overlapping Walsh orbitals.[29] [30] McQuillin's model has the vacant p-orbitals of the platinum being filled by the cyclopropane orbitals. The anti-bonding molecular orbitals of the cyclopropane are filled from the filled d-orbitals on the platinum, in a backbonding fashion. Figure 15 shows these orbital interactions. This model can account for the formation of either a platinacyclobutane or a Pt-cyclopropane complex. If the backbonding orbitals from the platinum to the cyclopropane dominate, a platinacyclobutane results, and if the cyclopropane orbitals filling the p-orbitals on platinum dominate, then a Pt-cyclopropane complex will result.

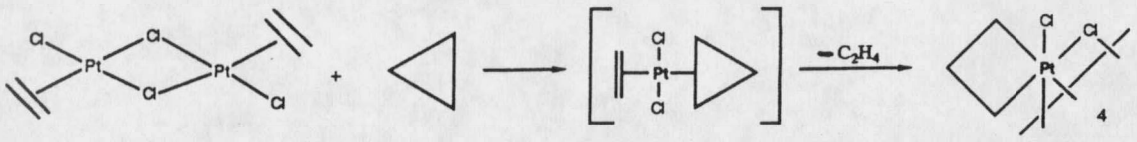
Figure 15. Walsh Molecular Orbital Interactions.



Mechanism of Platinum Insertion

The mechanism of platinum II insertion into a cyclopropane ring is postulated to proceed by edge attack of the platinum into the cyclopropane. An intermediate in this mechanism is an edge-bound complex, which has yet to be observed, Figure 16 .[31] Substituents on the cyclopropane can either enhance or retard insertion: electron-donating groups will enhance while electron-withdrawing groups will retard.[32] Formation of the platinacyclobutane species results in retention of the stereochemistry on the cyclopropane ring.[33] This would then presumably eliminate any ionic or freely tethered intermediate species.

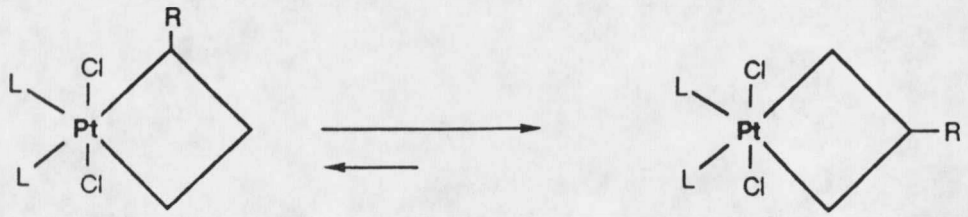
Figure 16. Formation of an Edge-Bound Platinum Complex.



Puddephatt Rearrangement

The observation that platinum will insert into the most substituted carbon-carbon bond and then rapidly rearrange is shown in Figure 17. This has lead to four possible rearrangement mechanisms. Puddephatt first observed this rearrangement upon reacting Zeise's dimer with phenylcyclopropane.[34] [35] Deuterium labeling experiments ruled out any possibility of phenyl migration. External chlorine is not exchanged for the chlorines originally on platinum and it is not a reductive elimination step followed by reinsertion to produce the beta-phenyl isomer. Another observation was that excess pyridine inhibits the isomerization, while a bidentate ligand completely suppresses the process. A criterion that is associated with all four proposed mechanisms is that the platinum has to be coordinatively unsaturated, resulting from loss of a single pyridine ligand.

Figure 17. Platinacyclobutane Rearrangement.



The first mechanism is shown in Figure 18. A loss of stereochemistry results in this product and was not observed with the labeled cis-2-phenyl-4-deuterioplatinacyclobutane.[36] The second mechanism is one where the platinum forms an edge complex followed by isomerization and then reinsertion, Figure 19.

Al-Essa[37] states that the edge complex is thermodynamically unstable which is supported by the fact that no edge type complexes have ever been observed. Thus, this mechanism is discounted as an unlikely possibility.

The last two mechanisms are very similar and possibly the same, since they cannot be distinguished experimentally. Puddephatt proposed that the platinum is bound to all three carbons equally. The platinum-carbon, and carbon-carbon bond formation and cleavage occur concertedly, Figure 20. Hoffman[38] [39] proposed the last mechanism, it is a system involving a transition state where all three carbons are attached to platinum, thus preventing rotation. Hoffman used Extended Huckel calculations with the assumption that platinum becomes

Figure 18. First Mechanism of Puddephatt Rearrangement.

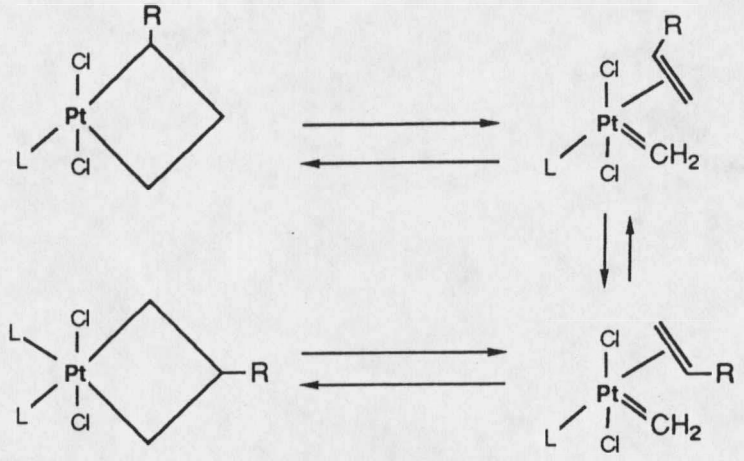
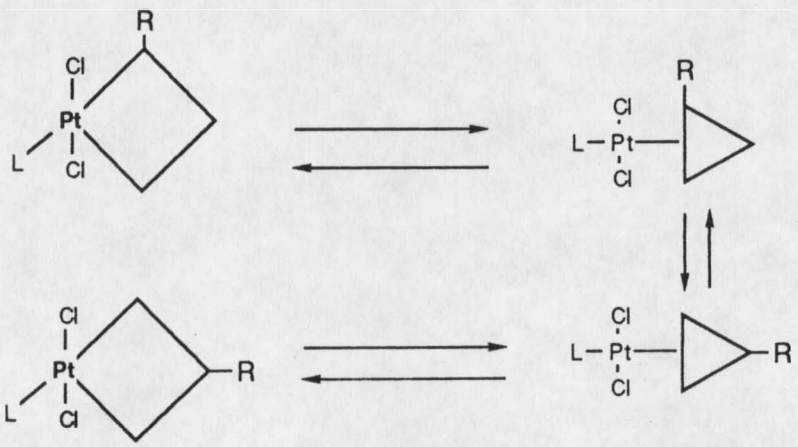


Figure 19. Second Puddephatt Rearrangement Mechanism.



5-coordinate square pyramidal after loss of a ligand. This complex is then isolobal to cyclobutyl cation and used as an analogy to platinum-cyclobutane, Figure 21. Figure 22 shows the proposed mechanisms using the analogy to cyclobutyl cation.

Figure 20. Concerted Mechanism for Rearrangement.

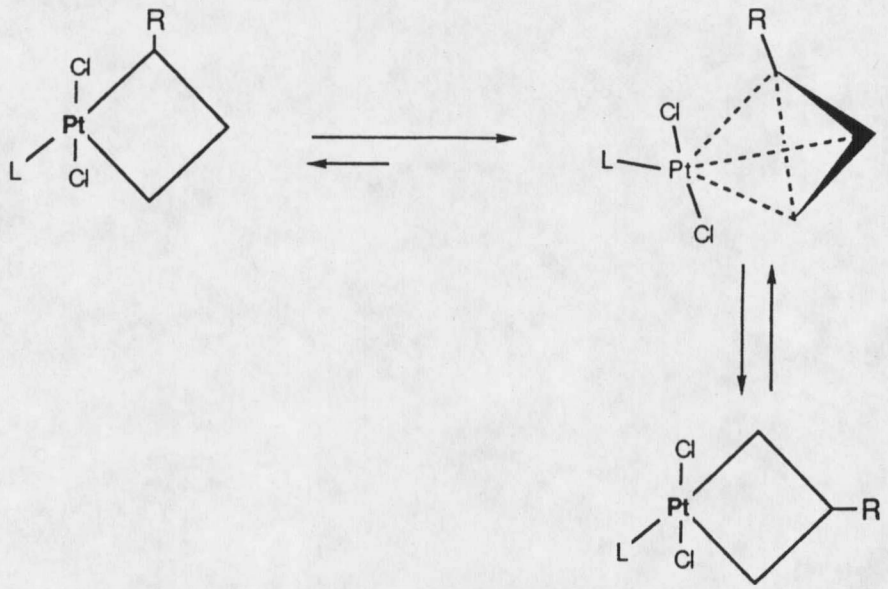


Figure 21. Isolobal Analogy to Platinacylcobutane.

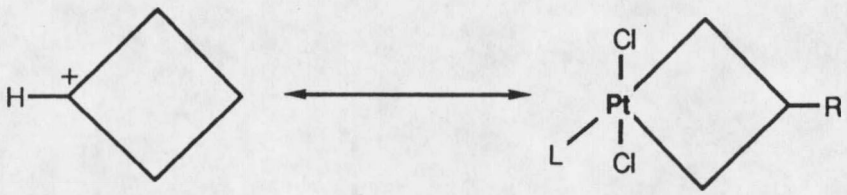
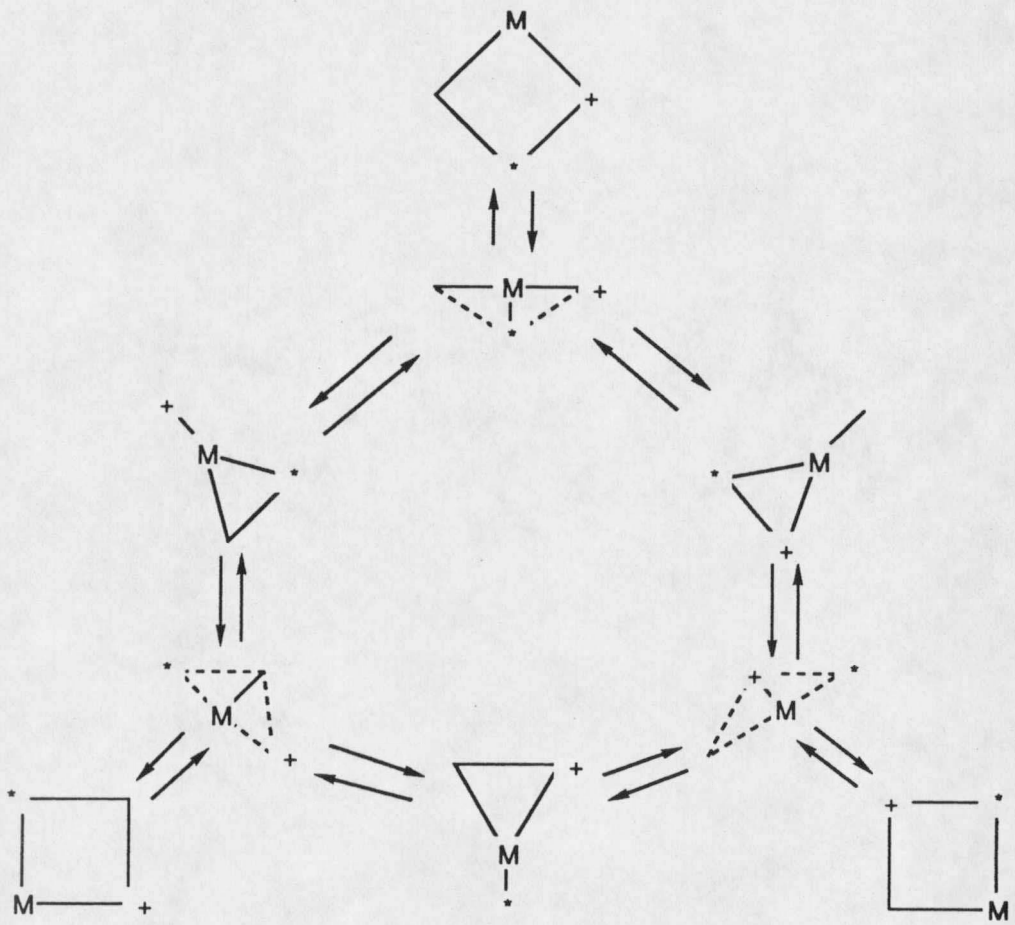


Figure 22. Proposed Mechanism for the Platinacyclobutane Complex Using the Isolobal Analogy.

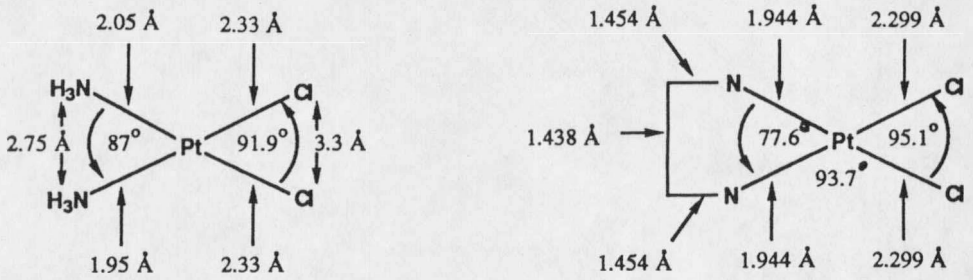


Cis-Platin History and Usage

In 1965 Barnett Rosenburg serendipitously discovered that an electric field applied to Escherichia coli caused filamentous growth. The cellular changes were due to platinum compounds being generated from platinum electrodes.[46] Further investigations showed that platinum compounds, such as, (1) cis-Pt(IV)$(NH_3)_2Cl_4$; (2) cis-Pt-(II)$(NH_3)_2Cl_2$; (3) Pt(II)$(NH_2CH_2CH_2NH_2)Cl_2$; and (4) Pt-(IV)$(NH_2CH_2CH_2NH_2)Cl_4$ could in fact inhibit carcinoma 180 and leukemia L1210 growth in mice.[47] These studies determined that cis-Pt$(NH_3)_2(Cl_2)$ was the most effective compound in inhibiting tumor growth. Figure 23 shows some structural features for cis-dichlorodiaminePt(II) and dichloro(ethylenediamine)Pt(II). There are many recognized synonyms for cis-dichlorodiamineplatinum(II), eg. cis-platin, cis-platinum, CDDP, DDP, cis-DDP, and Platinol^{TM}.

Cis-dichlorodiaminePt(II) is commonly referred to as cisplatin in the United States, but prior to 1965 it was known to chemists as Peyrone's chloride. It was first synthesized in 1845 and structurally characterized in 1893.[48] Interest in cisplatin markedly accelerated after Rosenburg's discovery and has continued to the present.

Figure 23. Cisplatin and Dichloro(ethylenediamine)Pt(II).



Cisplatin went into clinical trials in 1971 and obtained approval from the Food and Drug Administration (FDA) in 1979.[49] It has since become a leading chemotherapeutic agent for a variety of cancers. Response rates are higher for solid tumors than with lymphomas. Head, neck, ovarian, testicular, and bladder cancers show a positive response when cisplatin is used as a single agent. Therapeutic synergism with methotrexate, 5-fluorouracil, etoposide, vinblastine, bleomycin, and adriamycin have been shown to occur either singly or in multiple combinations (see Appendix A).[50 51 52 53 54 55] Synergism has resulted in new areas of research, which include combination therapy and varying schedule regimes. A successful synergistic combination for treating testicular cancer is cisplatin, vinblastine and bleomycin. The chemotherapeutic agents mentioned above can be categorized according to their mechanism of action. Adriamycin and bleomycin are antibiotics produced by microbial fermentation. Adriamycin

is an anthracycline which acts as an intercalating agent and bleomycin intercalates and causes DNA single strand breakage from generation of free radicals. Methotrexate is a folic acid analogue which competitively inhibits mammalian dihydrofolate reductase. This leads to DNA synthesis inhibition by inhibiting the synthesis of TMP. Five-fluorouracil is a pyrimidine analogue and prevents DNA synthesis while etoposide and vinblastine are antimetabolites which arrest cells in the S or G_2 phase. Cisplatin is an metallating agent inhibiting cell division and DNA synthesis by forming DNA cross-links.[56]

Areas of Investigation

Numerous investigators have undertaken the challenge to determined clinical applications, pharmacokinetics, cellular and body distribution, mechanism of action and most significantly, structural modifications for cisplatin. This last point is in an effort to synthesize analogs that have greater tumor specificity along with reduced toxicity.

Clinical

Clinical research involving cisplatin encompasses a broad spectrum. Attempts to find synergistic combinations with cisplatin predominates the clinical literature. Synergism with cisplatin can occur with other chemotherapeutic drugs, radiation, or hyperthermia. These combinations can be varied according to dosages and order.

Optimal effectiveness of the drug can be either on the primary tumor, metastases, or as a salvage therapy. Sex and age have also been investigated with an advantage in selectivity for males over females in reproductive cancers. Females had a response rate of 13-26 percent in one study and 40 percent in another study when cisplatin was used as a single agent. [57] [58] Response rates in males have been as high as 80 percent when cisplatin was used as a single agent.[59] It was also discovered that cisplatin is more toxic to the auditory systems in children than in adults.[60]

Pharmokinetics and Distribution

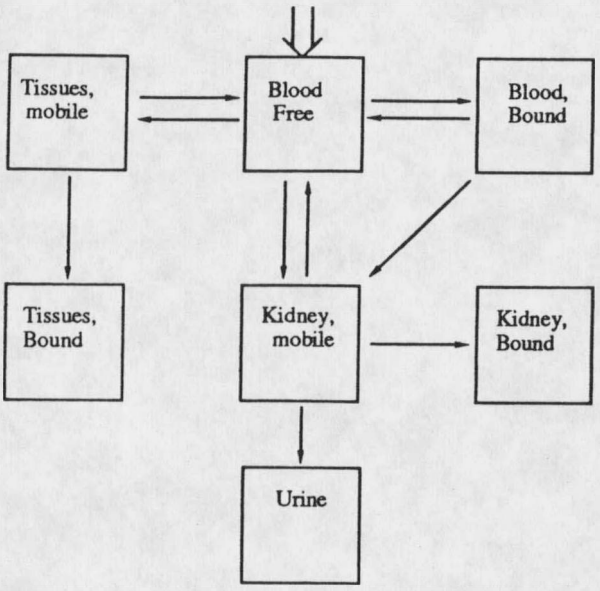
Pharmokinetics and distribution of cisplatin has been investigated extensively in order to understand the drug's mechanism of action. Pharmokinetic data indicates that cisplatin is rapidly eliminated from the body, both in laboratory animals and in humans. Elimination from a single dose is biphasic. The first phase is due to cisplatin being rapidly eliminated with a half-life on the order of minutes. The second phase has a half-life of days and is not cisplatin in its original state.[61] [62] Lange et. al.[63] showed that 79 percent was excreted during the first 24 hours in mice.

Cisplatin's effectiveness is determined by the quantity of bound versus unbound complexes. Several models have been proposed to account for the distribution and bound versus unbound cisplatin. A seven compartment model

is shown in Figure 24. All mammalian species tested show a plasma clearance of between 70-90 percent of the injected cisplatin within the first 15 minutes. Plasma bound cisplatin has been shown to be present up through the 6th day following a single injection.[64] Other studies suggested that the uptake of cisplatin by the tissues takes place over approximately 0.6 minutes and then the drug is released at a significant rate within 3.0 minutes following injection. At the same time, but at a much slower rate, cisplatin is protein-bound in blood and in tissues. Protein binding is estimated to have a half-life around 1.0 hour.[65] In yet another study, the reversibility of the protein-binding reaction was estimated conservatively at 71 hours.[66] Perhaps this estimate of 71 hours is more realistic than 1.0 hour knowing that 40-50% of a platinum dose was excreted in the urine in the first 72 hours.[67] Proteins, peptides, and amino acids in the plasma which contain sulfhydryl groups would be expected to react with cisplatin most rapidly due to their high nucleophilicity[68] and millimolar concentrations.[69] These reactions are important in the loss of free-cisplatin.

No single body tissue actively accumulates platinum preferentially. Table 5 lists the distribution of platinum in various tissues. This table illustrates what has been determined from many similar studies in that the kidneys, liver and lungs have a greater concentration of platinum

Figure 24. Seven Compartmental Model*.



(*) see reference 60

than the tumor. Platinum preferential accumulation in a tissue would be expected to show a greater difference then that observed in Table 5 and would not be due to circulation or excretion factors. This suggests that tumor cells do not have a propensity for concentrating platinum.

Mechanism of Action

The mechanism of action begins at the moment cisplatin is injected into the blood stream. Even though the plasma chloride ion concentration is around 103 mM[70] one or both of the chlorides on platinum can be substituted by water forming monoaquo and diaquo complexes. Figure 25 illustrates the aquation of cisplatin.[71]

Once the aquo products have formed, reactions with sulfur containing plasma molecules can occur rapidly

leading to bound platinum species. It was discovered that when cisplatin is administrated with NaCl the percentage of protein bound platinum is much lower, thereby increasing

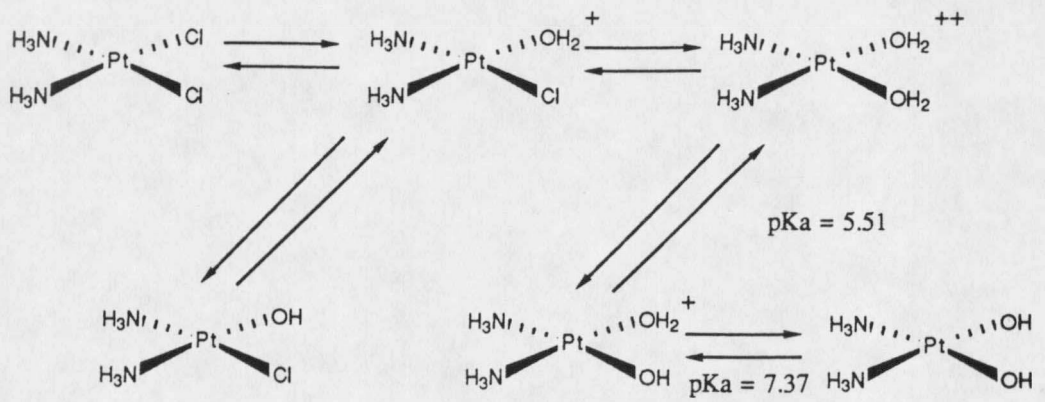
Table 5. Organ Distribution of Cisplatin in Walker-256-Carcinosarcoma-Bearing Rats (3 hr After Pulse Injection)*

Organ	% of injection dose per gram of tissue
Kidneys	1.747
Liver	0.755
Tendon	0.675
Adrenals	0.614
Bladder	0.520
Lung	0.569
Bone	0.584
Skin	0.495
Small Intestine	0.411
Blood	0.378
Large Intestine	0.351
Spleen	0.334
Pancreas	0.306
Marrow	0.234
Tumor	0.226
Heart	0.166
Thyroid	0.119
Stomach	0.110
Brain	0.035
Muscle	0.002

(*) see reference 72

the percentage of unbound active platinum. In fact, Bristol-Myers Co. packages cisplatin with NaCl and mannitol added to each dosage vial. Chloride ion concentration in the cell drops to a value of 4 mM, thereby increasing the exchange of chloride ion.[73] These aquo complexes are proposed to react with the target molecule, presumably

Figure 25. Aquation Reactions of Cisplatin.



DNA. Evidence supporting DNA as the primary target has been accumulated by Zwelling et. al. (1979) and is listed in Table 6.

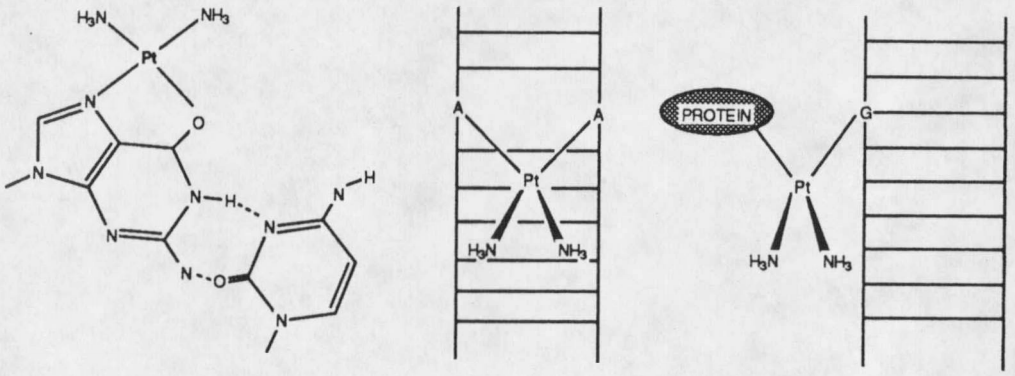
Table 6. Evidence Supporting DNA-Cisplatin Interaction.

1) Inhibited bacterial cell division
2) Reduced viability of bacteria deficient in DNA repair as compared to wild-type organisms
3) Induced prophage from lysogenic bacteria
4) Induced mutations in bacteria
5) Inactivated transforming DNA
6) Inactivated DNA-containing bacteriophage
7) Selectively inhibited DNA synthesis in several mammalian cell systems including human cells
8) Induced mutations in mammalian cells
9) Reduced viability of human cells deficient in DNA repair(xeroderma pigmentosum cells) compared to normal fetal lung cells
10) Preferentially bound to intracellular DNA
11) Toxic doses of cis-platinum in vitro and tumoricidal doses of cis-platinum in vivo produced comparable DNA-platinum binding
12) Inactivated DNA template for DNA polymerase

Interactions between Cisplatin and DNA

Interactions between DNA and cisplatin can occur four ways. These interactions are reactions between the purine and/or pyrimidine bases and not the sugar-phosphate moieties of DNA. Interaction between cisplatin species and the purine and/or pyrimidine bases are important for antitumor activity by inhibiting replication.[74] [75] These interactions are shown in Figures 26 and 27.

Figure 26. Cisplatin DNA Interactions: a) N_7-(G)-O_6(G), b) intrastrand, c) DNA-Protein.

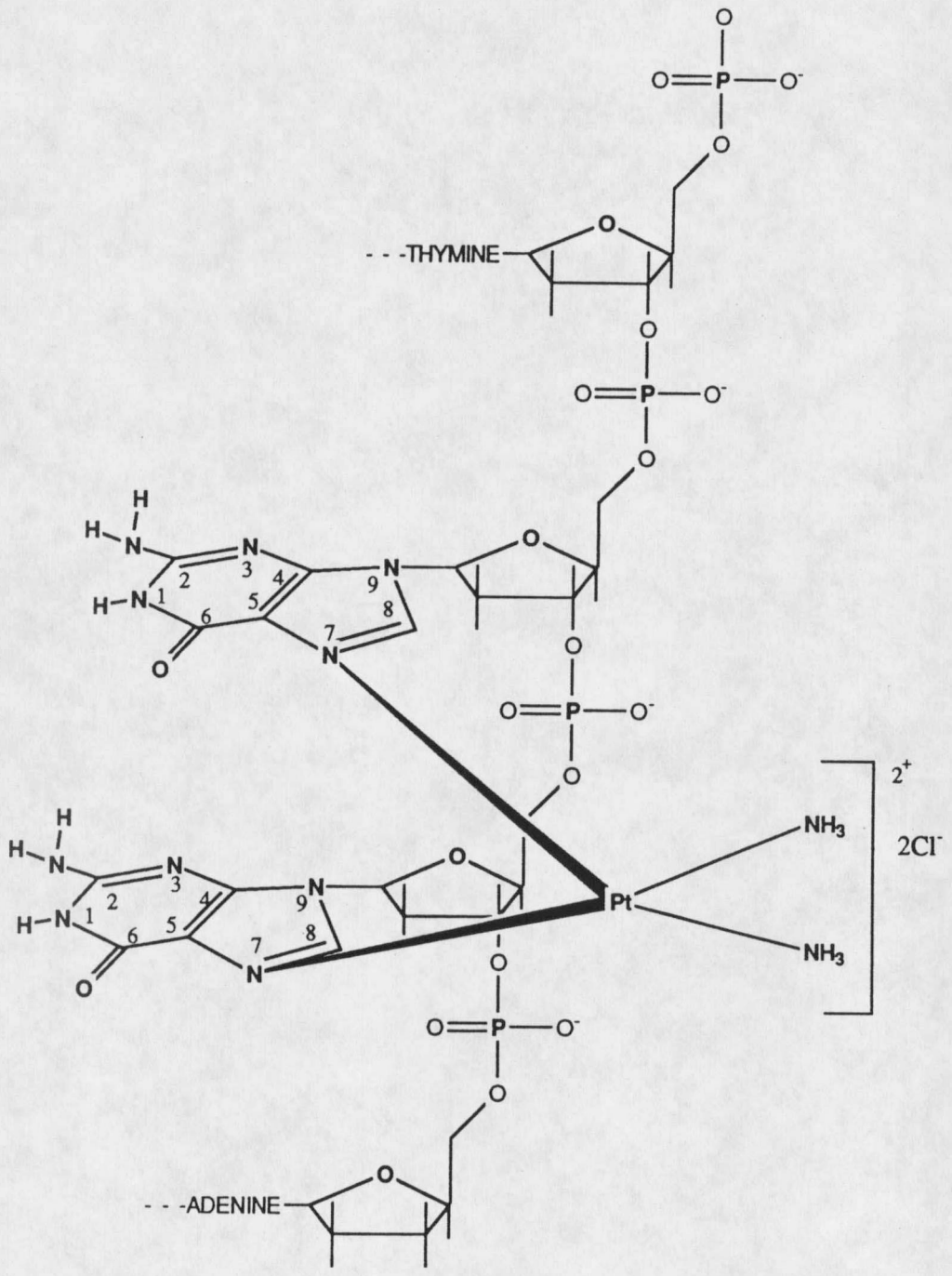


a b c

Even though all the bases have the potential to bind with cisplatin, guanosine has been shown to form a majority of the complexes.[76] There are several ways cisplatin can bind to guanosine as described below. The N-7 position of guanosine dominates the DNA-platinum interaction. This

position is the most nucleophilic and hence the site of electrophilic attack by aquated cisplatin. The O-6 position is also capable of binding to cisplatin but is not a major site of attack.[77] Current evidence indicates that the intrastrand cross-linking complex is the predominating one, determined by DNA analysis. Numerous investigators have further demonstrated that the intrastrand cross-link is between adjacent guanine residues.[78] [79] [80] [81] The approximate intrastrand adduct composition is: GpG, 50-65%; GpNpG, 5-10%; and ApG, 25%.[82] Interstrand cross-links account for <1% of platinum-DNA adducts.[83] [84] Intrastrand adducts are thermodynamically stable at 37°C and irreversible at the N-7 positions. When the Pt-DNA adducts are present, DNA replication is inhibited and the cell dies.

Figure 27. Structure of Cisplatin-GpG Adduct.



This mechanism is certainly not specific for only cancer cells, since normal cells are destroyed as well. The distinct difference, though, is the rate of replication of cancer cells versus normal cells making cancer cell populations more susceptible.

Following the discovery of cisplatin as an important chemotherapeutic agent, research efforts were directed towards synthesizing analogs superior to cisplatin. It was quickly determined that the structural geometry of the platinum had to be cis and not trans. There generally had to be amine ligands to have anticancer activity. Initial structural activity studies started by varying anionic ligands (X) in species similar to $Pt(A_2)(X)_2$,[85] (A_2 = two monodentate or one bidentate amine ligand; X_2 = two monodentate or one bidentate anionic ligand). These studies indicated that substitution of the chloride ions with labile groups such as NO_3^- and H_2O produced highly toxic compounds while strongly bound ligands such as SCN^- and NO_2^- formed inactive complexes. Much of the toxicity of the aquo complexes is due to polymerization in forming hydroxo bridge complexes. There was activity in complexes where bromine was substituted for chlorine, thus changing the monodentate ligand to intermediate lability.

A major advance stemming from this early work was the activity found for chelating dicarboxylate ligands, oxalate, malonate and substituted malonates, Table 7.

These compounds were more water soluble in comparison to other complexes synthesized.[86]

Additional testing with a variety of amine ligands has shown that these malonate and oxalate complexes have comparable or superior activity to the analogs containing chloride groups, Table 8.

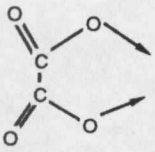
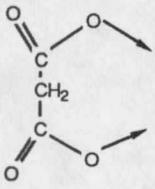
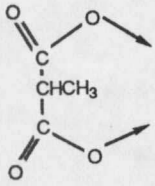
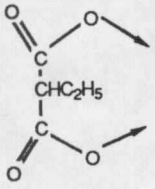
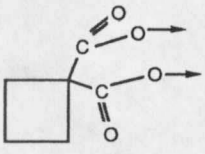
Changes in activity for varying A in cis-$[PtA_2Cl_2]$, where the amines are heterocyclic and alicyclic, are given in Table 9. Straight and branched chain alkylamines complexes are given in Tables 10 and 11. In Tables 9, 10, and 11 the complexes with a higher activity were the ones with low aqueous solubility. Chelating amines such as 1,2-diaminocyclohexane and o-phenylenediamine were also effective, Table 12.

Amine ligand substitutions were less sensitive to variations than substitution of the other platinum ligands, although primary alkylamines did show a higher chemotherapeutic activity than secondary amines.

Attempts to increase the solubility by adding trans-OH ligands to platinum were successful, Table 13. In these new Pt (IV) complexes, the anticancer activity was maintained also, Table 14.

The most promising Pt(IV) complex at this time is cis-dichloro-trans-dihydroxy-bisisopropylamine. This compound has an aqueous solubility of 44 mM and is less nephrotoxic than cis-platin.

Table 7. Activity of Amine Complexes with Chelated Dicarboxylate Ligands X in $[Pt(NH_3)_2X]$.*

Sarcoma 180 X	Solvent	Dose Range mg / Kg	Response	Toxic Level mg / Kg	T / C	Dose mg / Kg
	DMSO (slurry)	5-20	+	16-20	9	15
	WS	12-18	+	17	24	14-16
	W	10-20	+	10	0	10
	W	0.5-6	+	3-4	25	2.5
oxalate						
	WS	10-60	+	35	7	30
	W	5-24	+	24	28	20-24
	W	1-7	+	8	12	7
malonate						
	W	10-80	+	65	7	60
methyl malonate						
	W	30-80	+	>80	17	70-80
ethyl malonate						
	W	20-160	+	150	18	120
1,1-cyclobutane dicarboxylate						

(*) = see reference 90

WS = water slurry

W = water

T/C = tumor vulome of test group/ tumor volume of control group X 100

Table 8. Various Amine Complexes Containing Oxalate and Malonate Ligands [PtA_2X] or [PtAX].*

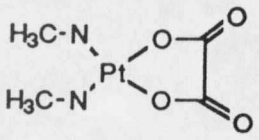
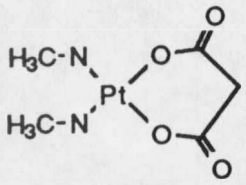
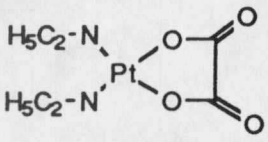
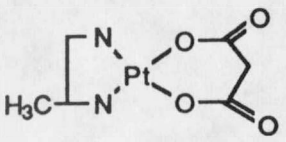
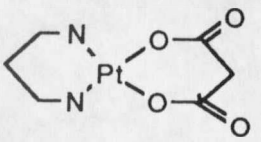
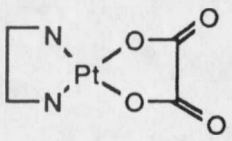
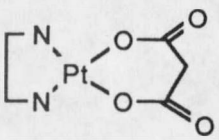
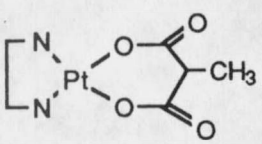
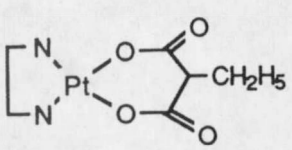
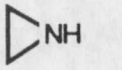
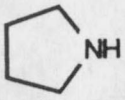
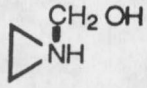
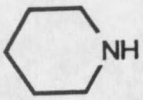
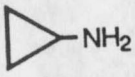
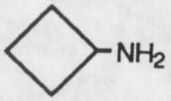
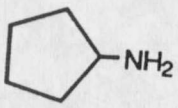
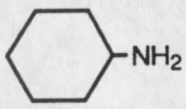
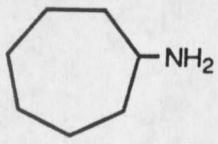
Sarcoma 180 X	Solvent	Dose Range mg / Kg	Response	Toxic Level mg / Kg	T / C	Dose mg / Kg
	W	5-80	+	20-30a	21	30
oxalatobis(methylamine) platinum (II)						
	W	80-180	+	>180	20	120-180
malonatobis(methylamine) platinum (II)						
	W	10-80	+ -	20-40	21	10
oxalatobis(ethyleneamine) platinum (II)						
	W	45-90	+	65	9	60
malonato-1,2-proplyenedi-amineplatinum (II)						
	W	20-80	+	90	28	60-80
malonato-1,3-propylene diamineplatinum (II)						

Table 8 Continued.

Sarcoma 180 X	Solvent	Dose Range mg / Kg	Response	Toxic Level mg / Kg	T / C	Dose mg / Kg
 oxalatoethylene diamineplatinum (II)	W	0.25-16	-	3 b	75	0.25-2
 malonatoethylene diamineplatinum (II)	W	5-80	+	45-60	18	40
 methylmalonatoethylene diamineplatinum (II)	W	30-90	+	>90	4	90
 ethylmalonatoethylene diaminoplatinum (II)	WS	40-120	+ -	>120	51	90-120

a = only 50% survivors
b = highly toxic-convulsions
WS = water slurry
W = water
T / C = tumor volume of test group/tumor volume of control group X 100
(*) = see reference 85

Table 9. Changes in Activity on Varying A in cis-$[PtA_2Cl_2]$.*

ADJ / PC6 plasma cell tumor A	Solvent	Dose Range mg / Kg	Dose Response	LD_{50}	ID_{90}	TI
NH_3	Ar	0.1-40	+	13.0	1.6	8.1
CH_6NH_2	Ar		-	18.5	18.5	1.0
$ClC_2H_4NH_2$	Ar		+	45.0	17.5	2.6
	Ar	2.5-160	+	56.5	2.6	21.7
	Ar	3-200	+	141	10.8	13.1
	Ar		-	90	>90	<1.0
	Ar		-	18	>18	<1.0
	Ar	1-80	+	56.5	2.3	24.6
	Ar	6-750	+	67	<6	>11.1
	Ar	1-3200	+	480	2.4	200
	Ar	1-3200	+	>3200	12	>267
	Ar	5-625	+	>625	18	>35

LD_{50} = lethal dose for 50% kill

ID_{90} = inhibiting dose for 90% tumor growth inhibition

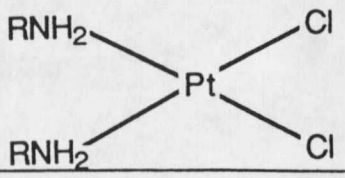
TI = therapeutic index, LD_{50} / ID_{90}

Ar = arachis oil

(*) = see reference 87 and 89

Table 10. Straight Chain Derivatives of Cis Pt II.*

R	LD_{50}	ID_{90}	TI
H	13	1.6	8
CH_3	18.5	18.5	1
CH_3CH_2	26.5	<12	>2.2
$CH_3(CH_2)_2$	26.5	<12	>2.2
$CH_3(CH_2)_3$	110	<10	>1.1
$CH_3(CH_2)_4$	1000	>1500	-



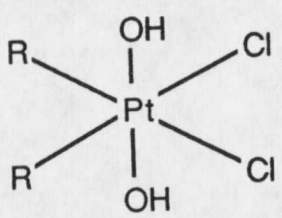
LD_{50} = lethal dose for 50% kill

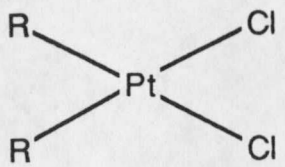
ID_{90} = inhibiting dose for 90% tumor growth inhibition

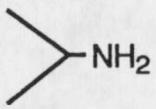
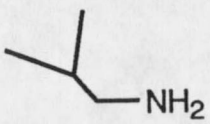
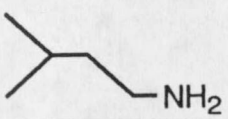
TI = therapeutic index, LD_{50} / ID_{90}

(*) = see reference 88

Table 11. Branched Chain Derivatives of Pt II - Pt IV.*





Ligands R	LD_{50}*	ID_{90}*	TI	LD_{50}*	ID_{90}*	TI
	33.5	0.9	37.5	90	7.5	12
	83	6.2	13.4	410	19.5	21
	1150	5.8	198	720	19.0	38

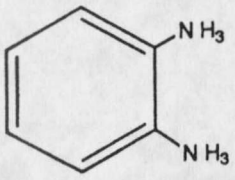
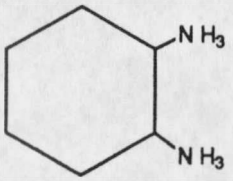
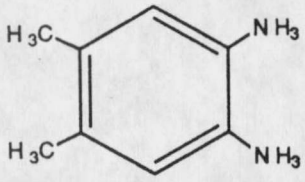
LD_{50} = lethal dose for 50% kill

ID_{90} = inhibiting dose for 90% tumor growth inhibition

TI = therapeutic index, LD_{50} / ID_{90}

(*) = see reference 88

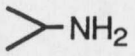
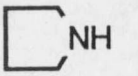
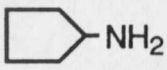
Table 12. Using ADJ/PC6 Plasma Cell Tumor to Test Cyclic Amines.*

	Solvent	Dose Range (mg/ Kg)	Dose Response	LD_{50}	ID_{90}	TI
	H_2O	0.3-40	+	141	2.1	6.9
	H_2O	0.6-80	+	48	2.43	20.4
	H_2O	12-1500	+	680	<12	>56.7

(*) = see reference 89

Toxicities associated with platinum based complexes are similar to other heavy metals and presents a major limitation with cisplatin and other analogs. Toxicity, primarily nephrotoxicity, associated with cisplatin was discovered during animal testing and clinical trials. Mannitol was subsequently discovered to reduce nephrotoxicity when administrated with cisplatin. Table 15 lists some other toxicities associated with cisplatin.[90]

Table 13. Solubility Data for Pt(II) and Pt(IV) Complexes.*

Amine	H_2O (mM)	Cl_2 $CHCl_3$ (mM)	CH_3OH H_2O	H_2O (mM)	Cl_4 $CHCl_3$ (mM)	CH_3Cl H_2O	H_2O (mM)	$Cl(OH)_2$ $CHCl_3$ (mM)	CH_3Cl H_2O
NH_3	8.9	<0.0712	0.008	4.58	ND	0	44.1	0.053	0.0012
	0.22	0.0038	0.0173	0.015	0.015	1.0	44.1	0.053	0.0012
	0.058	-	-	0.2	-	-	1.23	-	-
$n\text{-}C_4H_9NH_2$	0.053	0.0872	1.7	0.656	0.453	0.66	0.451	-	-
	0.013	0.0112	0.86	0.133	0.1995	1.5	7.95	0.0119	0.0015

(*) = see reference 89

N D = not determined

Cl_2 = cis-Pt am_2 Cl_2

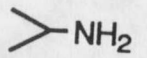
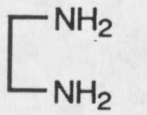
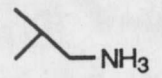
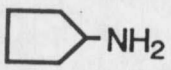
Cl_4 = cis-Pt am_2 Cl_4

$Cl_2(OH)$ = $Pt(am_2)Cl_2(OH)_2$-cis-$(am)_2$trans-$(OH)_2$

Evaluation of Potential Chemotherapeutic Agents

The evaluation of chemotherapeutic drugs proceeds through various test phases before FDA approval. At the time Rosenburg discovered cisplatin, evaluation procedures started with bacterial tests, then animals (mice, dogs, and monkeys) before human testing. With the need to have a

Table 14. Antitumor Activity of Pt(II) and Pt(IV).

Amine	$Pt(IV)Cl_2$ LD_{50}	$Pt(IV)Cl_2$ ID_{90}	$Pt(IV)Cl_2$ TI	$Pt(IV)Cl_4$ LD_{50}	$Pt(IV)Cl_4$ ID_{90}	$Pt(IV)Cl_4$ TI	$Pt(IV)Cl_4OH$ LD_{50}	$Pt(IV)Cl_4OH$ ID_{90}	$Pt(IV)Cl_4OH$ TI
NH_3	13.0	1.6	8.1	-	a	-	135	4.8	28.1
	33.5	0.9	37	132	>60	<2.0	90	7.5	12.0
	22.5	10	2.25	-	-	-	135	60	2.2
	83	6.2	13.4	111	<10	>11.1	410	19.5	21
	556	2.4	236	141	3.0	48	83	b	-

LD_{50} = lethal dose for 50% kill (mg/ Kg)
ID_{90} = inhibiting dose for 90% tumor growth inhibition
TI = therapeutic index, LD_{50}/ ID_{90}

a = active against other systems
b = 89 percent inhibition obtained
(*) = see reference 89

cost efficient method of screening hundreds of compounds, tissue culture assays were developed. These assays replaced the use of animals for routine testing.

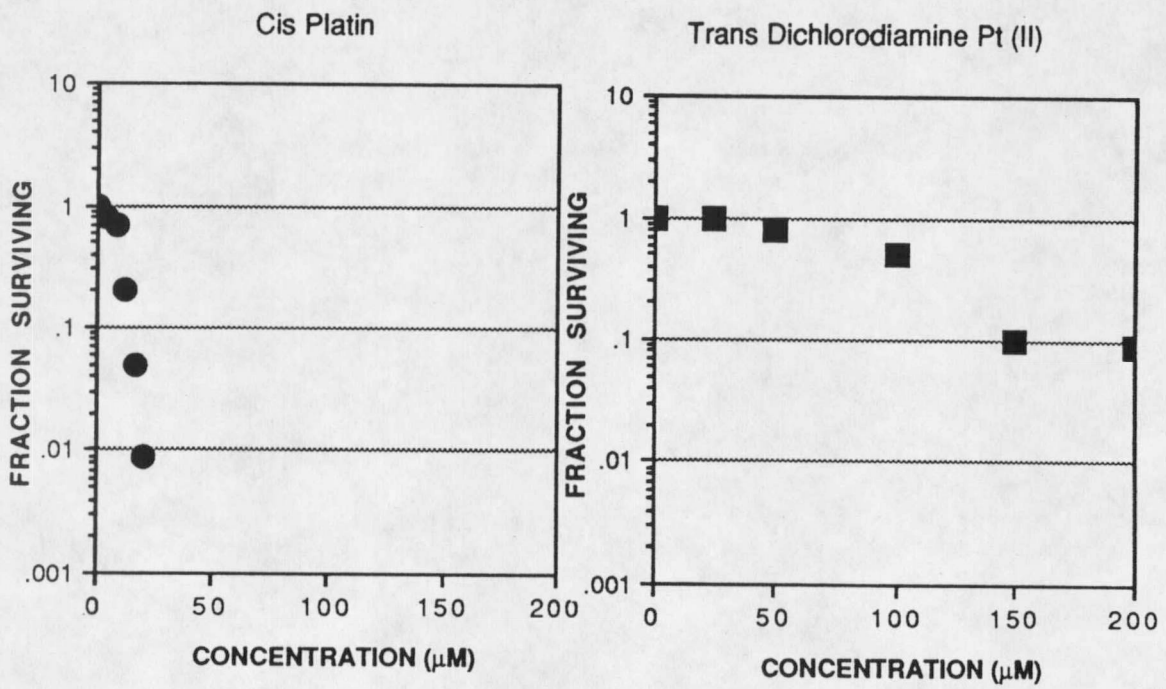
Tissue culture assays were conducted with a variety of cultured mammalian cancer cells. These tests determined whether or not the compounds were cytotoxic and/or cytostatic. The experimental results from tissue culture assays are expressed in percent survival curves. The percent survival curves for cisplatin and trans dichlorodiamine Pt(II) using HeLa (human ovarian cancer cells) cells is shown in Figure 28.[91] Note differences between cis and trans isomers.

Table 15. Major Clinical Toxicities of Cisplatin in Various Animals Species.

Type of toxicity	Species			
	Mouse	Rat	Dog	Monkey
GASTROINTESTINAL				
Emesis	-	-	+	+
Anorexia	+	+	+	+
RENAL				
BUN increase	+	+	+	+
Creatinine increase	ND	+	+	+
Polyurea	ND	+	+	+
Tubular necrosis	+	+	+	+
HEMATOLOGIC				
WBC decrease	ND	+	+	+
Platelet decrease	ND	+	+	-
Bone marrow hypocellularity	+	+	+	+
Otologic	ND	+	ND	+

\+ = positive
\- = negative
ND = not determined

Figure 28. Cis and Trans DichlorodiaminePt(II) Survival Curves Using HeLa Cells.



Bacterial testing has become almost obsolete in determining anticancer activity due to the inconsistencies in predicting anticancer activity in mammalian systems. Using bacteria to investigate the possible cellular mechanism(s) associated with new and possible active anticancer compounds is routinely done. Those compounds found to be active in tissue culture tests are then advanced to toxicity studies involving animals, usually mice. If results indicate minimal toxicity at high dosages then further testing is done in vivo. The in vivo experiments involve tumor bearing animals. If the compound is shown to be highly active in vivo, compared to cisplatin, it can proceed to clinical trials in humans. Clinical trials consist of three phases: 1) determine human tolerance; 2) preliminary efficacy studies and 3) broad clinical evaluations.

STATEMENT OF PROBLEM

Tissue toxicities associated with cis-platin limit the utility of this anticancer drug. A large number of analogs have been synthesized and tested in an attempt to decrease the toxicity. To date, no platinum compound has been synthesized that is lower in toxicity and still capable of killing cancer cells compared to cisplatin. Few platinum IV complexes have been fully investigated and even fewer organometallic compounds.

The goals of this project are as follows: 1) to synthesize a variety of organometallic platinum IV complexes, 2) increase the aqueous solubility of these complexes over cisplatin in an attempt to reduce nephrotoxicity, and 3) determine what structural modifications on the organic fragment and what amine ligands will enhance the anticancer activity.

Functional groups (eg. alcohols, aldehydes, carboxylates, ethers) and amine ligands (eg. pyridine, ethylenediamine, 2,2'-bipyridine) will be used to vary the compounds synthesized. Each compound will be evaluated using an <u>in vitro</u> assay with two murine cell lines, B-16 melanoma and Lewis Lung carcinoma, to determine effectiveness as an anticancer agent. Solubility will be determined using fetal bovine serum as the "aqueous"

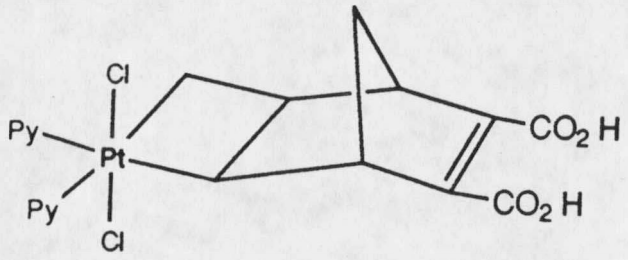
solvent. Compounds showing significant anticancer activity in the tissue culture assay and excellent solubility will be evaluated in an *in vivo* study, using C57Bl/6 mice. The first *in vivo* study will be to determine the general toxicity, while the second study will be to evaluate the effectiveness of the compound(s) in tumor bearing mice.

CHEMISTRY RESULTS AND DISCUSSION

Dicarboxylate Platinacyclobutanes

The target molecule 10, Figure 29, was originally synthesized by R. A. Moats.[92] Synthesis proceeded by using a fractional distillation apparatus to "crack"

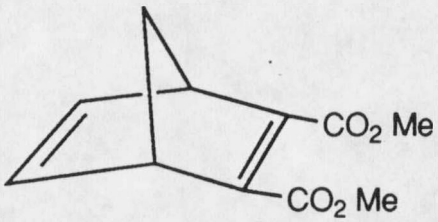
Figure 29. Target Molecule 10.



dicyclopentadiene (1) to cyclopentadiene (2) which was condensed with dimethylacetylenedicarboxylate (3) in an ice bath. The Diels-Alder product 1,2-dimethylcarboxylate-1,4-norbornadiene (4) was obtained as the only product, Figure 30. Figures 31-34 show ^{1}H, ^{13}C, COSY and HETCOR NMR spectra of 10.

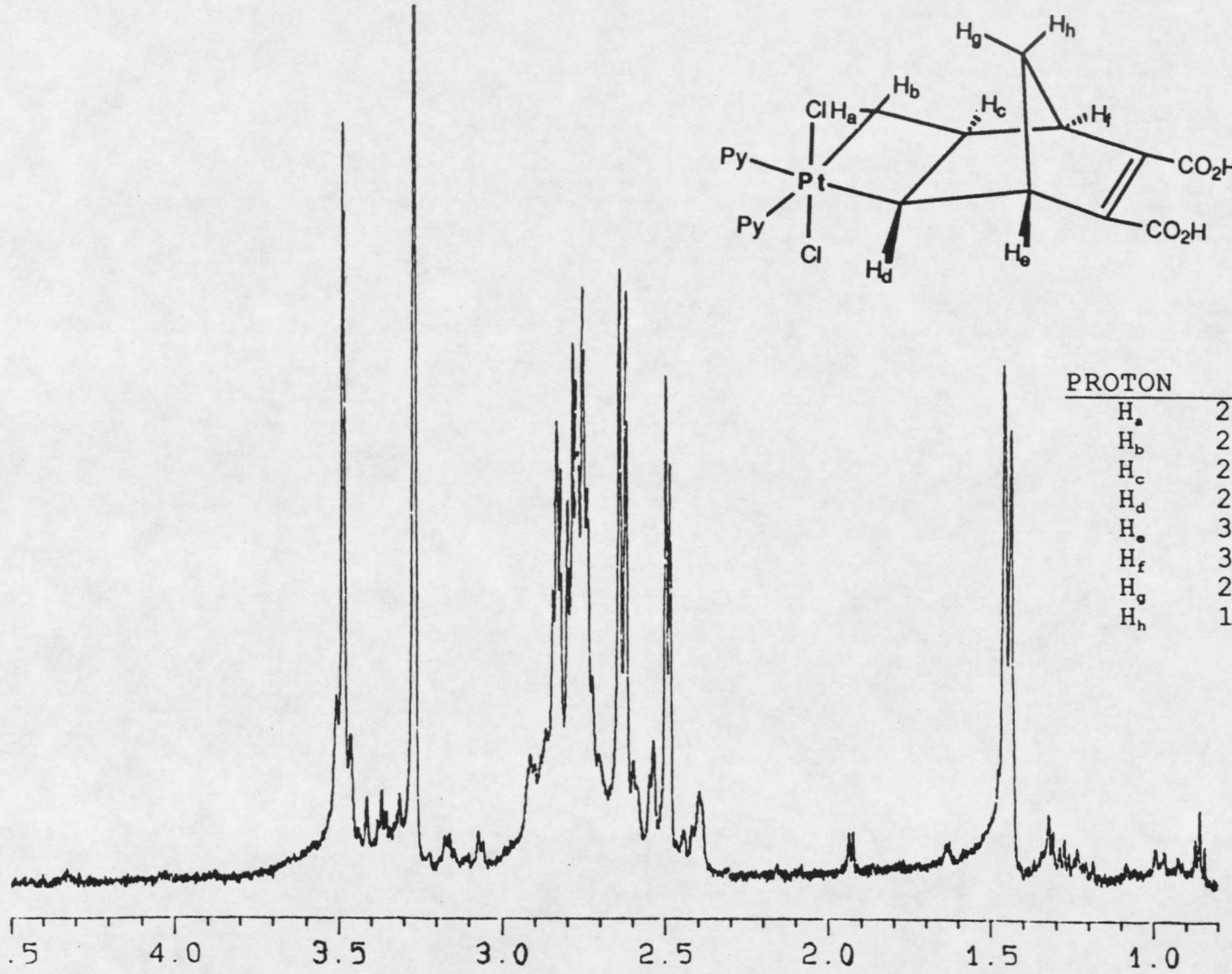
Compound 5 was prepared by reacting diazomethane with 4 in the presence of a catalytic amount of palladium (II) acetate. Proton NMR was used to monitor the reaction

Figure 30. 1,2-Dimethylester-1,4-norbornadiene, 4.



mixture in an effort to maximize the monocyclopropanated product 5, over the dicyclopropanated product 6, Figure 35. Compound 6 showed distinctive ^{1}H NMR resonances, at the high field signals, further upfield by ca. 0.8 ppm than those of 5. The reaction was terminated after 4-6 hours and the product mixture was chromatographed. A dark clear amber solution was left after rotoevaporating the eluent. Binding of the palladium catalyst to the oxygen atoms was evident by Pd(O) solid precipitating out over a period of days even after chromatographing with silica gel.

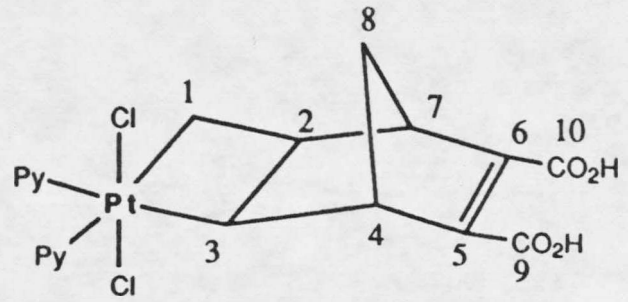
Hydrolysis of 5 produced the monocyclopropanated diacid (7), Figure 36. It was discovered that the monocyclopropanated product 7 is slightly less soluble in diethyl ether than the hydrolyzed product 8, Figure 36. This difference was advantageous in obtaining pure 7 (>90 %). The difficulty with using a mixture of the hydrolysis products of 4 and 5 is that if the diolefin is >20% of the mixture, yields are markedly reduced (<10%)



PROTON	ppm	
H_a	2.8	m
H_b	2.8	m
H_c	2.7	dd
H_d	2.5	d
H_e	3.5	s
H_f	3.2	s
H_g	2.6	d
H_h	1.4	d

Figure 31. Proton NMR Spectrum of Compound 10.

CARBON #	ppm	$J_{(Pt-C)}$ Hz
1	-3.6	(366.2)
2	51.8	(81.4)
3	5.3	(423.9)
4	49.7	
5	147.6	
6	147.6	
7	51.2	(20.3)
8	41.8	
9	168.0	
10	166.9	



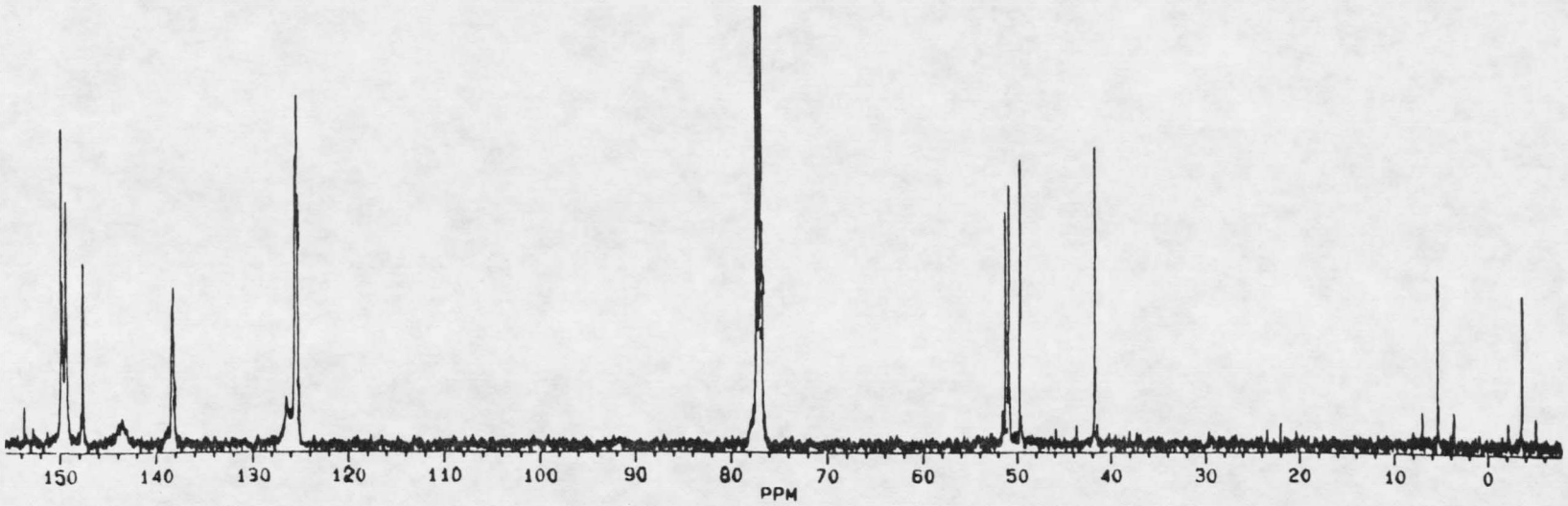


Figure 32. Carbon NMR Spectrum of Compound 10.

Figure 33. COSY NMR Spectrum of Compound 10.

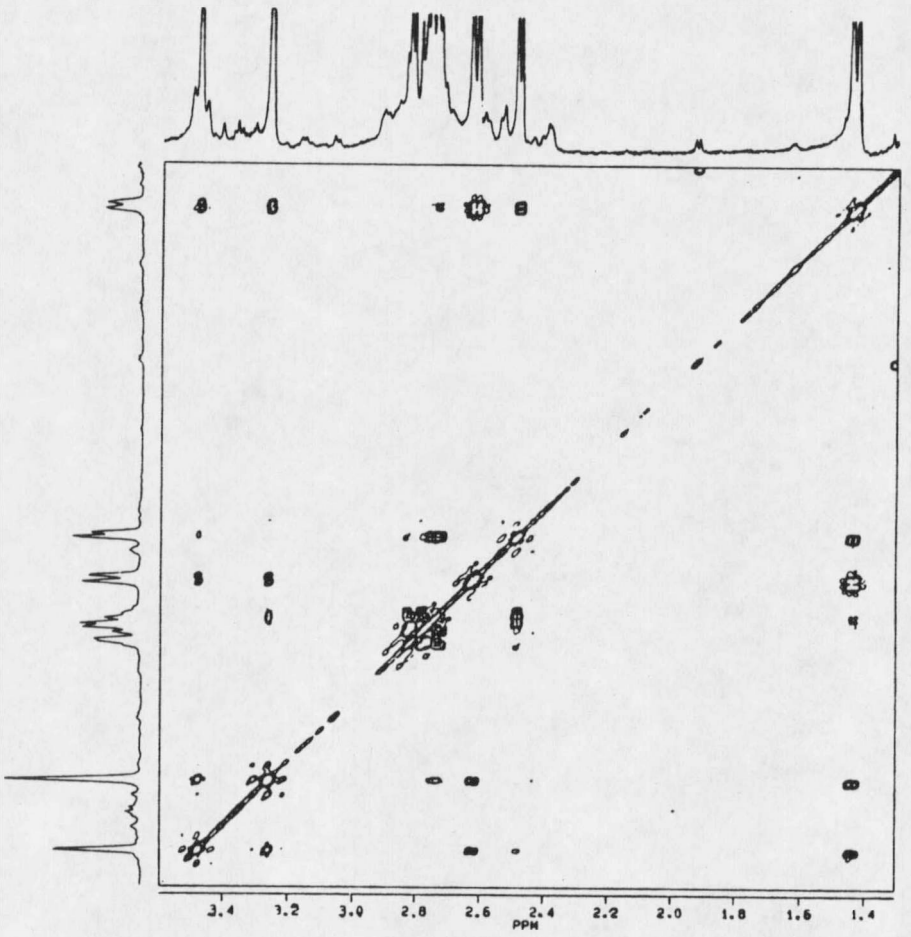


Figure 34. HETCOR NMR Spectrum of Compound 10.

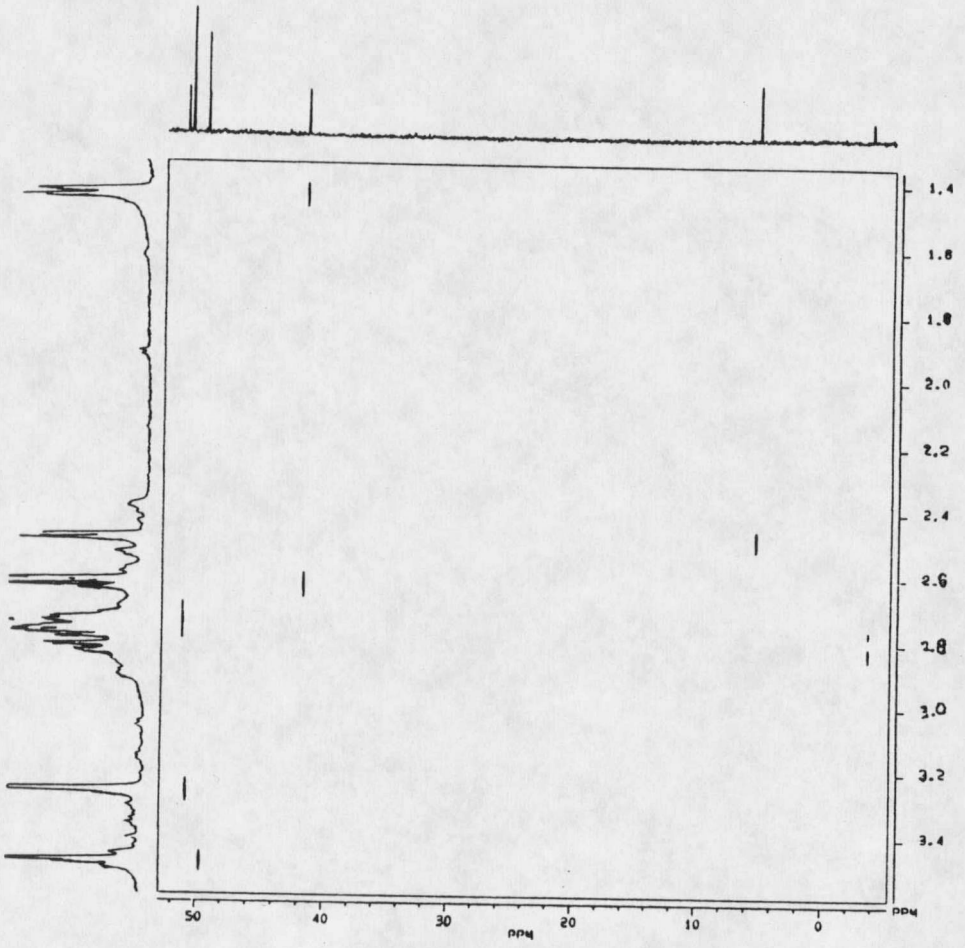
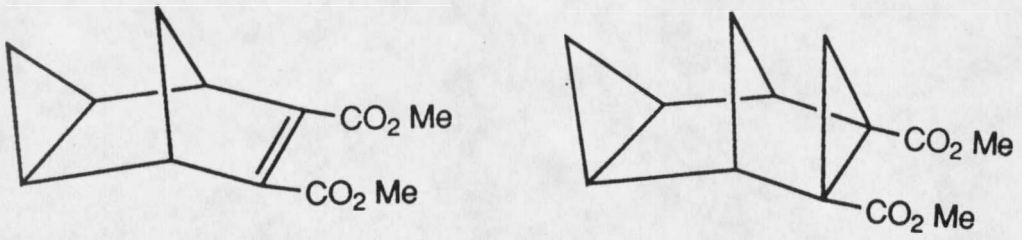
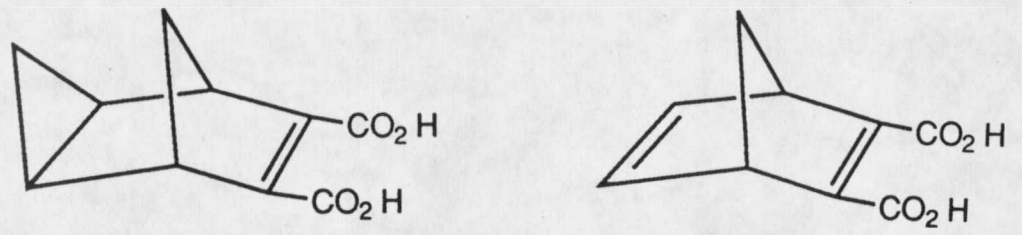


Figure 35. Mono and Di Cyclopropanation Methyl Esters, 5 and 6.



when reacted with Zeise's Dimer. Obtaining 7 in the highest percent yield was critical if the products were to be obtained in high yields. It seemed that either the additional olefin or the additional carboxylic groups of the hydrolysis product 8 had an adverse effect on Zeise's dimer inserting into 7.

Figure 36. Hydrolysis Dicarboxylate Products 7 and 8.



Zeise's dimer ($5.1X10^{-4}$ mol) was added to a 5 dram vial containing 7 and diethyl ether. A fluffy yellow precipitate, (I.P.C.) 9, formed after 6 hours following the addition of Zeise's dimer. The I.P.C. was washed with pentane 3 times before adding $CHCl_3$ and 2 equivalents of

pyridine to form 10. Complex 10 was soluble in chloroform and upon adding 1 equivalent of ethylenediamine (en), the pyridines were displaced to produce 11, Figure 37.[93]

Figure 37. Dicarboxylate Platinacyclobutane with Ethylenediamine, (11).

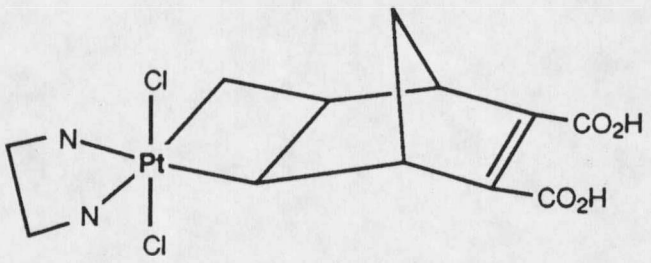
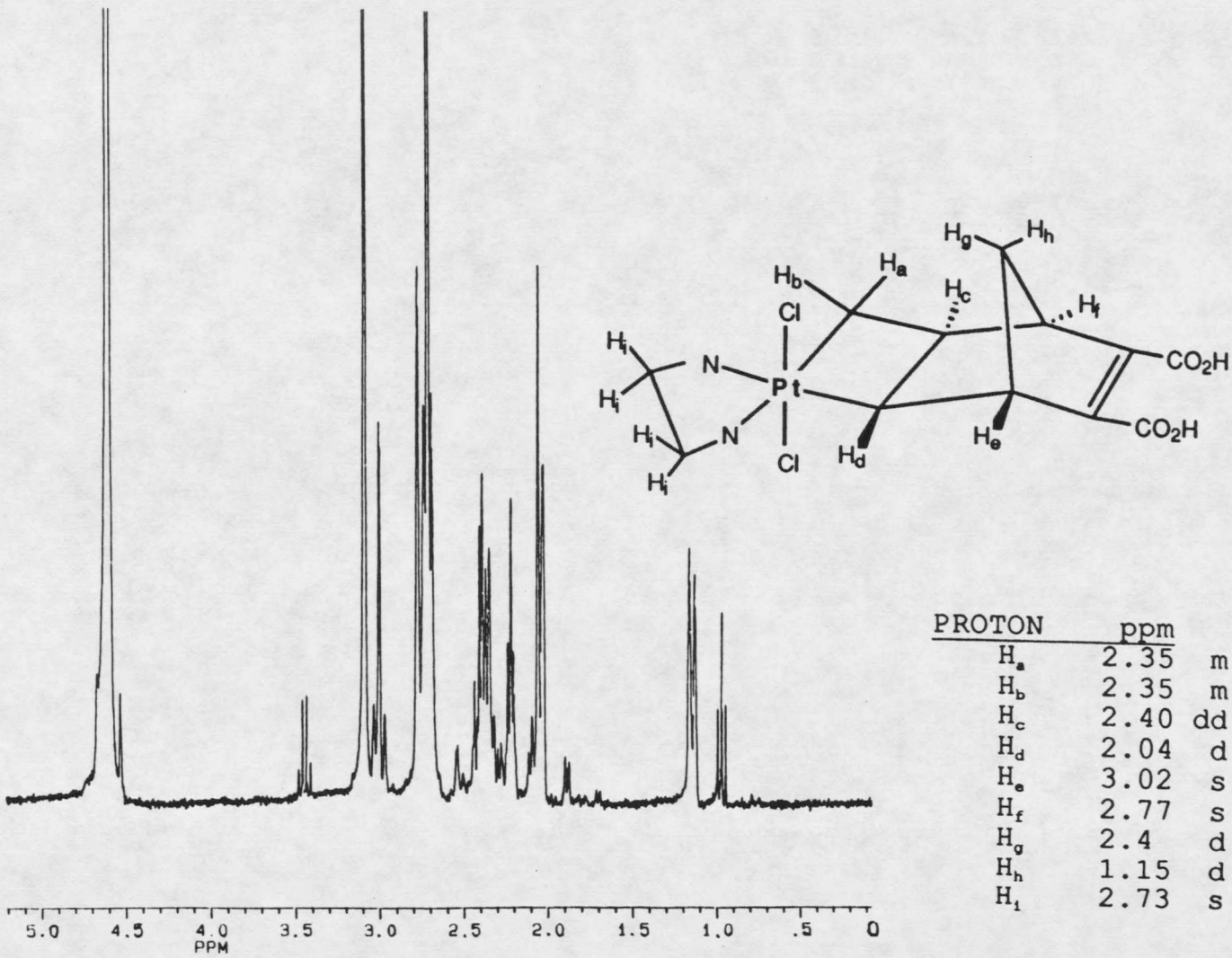


Figure 38 shows the ^{1}H NMR of 11. Due to the low solubility of 11 a ^{13}C NMR was not done. Displacement of pyridine by ethylenediamine was very rapid for a total reaction time of 60 seconds. Complex 11 is a chloroform insoluble white precipitate. The precipitate was washed three times using chloroform and then evaporated to dryness using N_2, a white-tanish solid remained. The only solvent that 11 was found to be soluble in was basic H_2O. Therefore, proton NMR samples were prepared by dissolving 20-30 mg of the platinacyclobutane in basic (NaOH) D_2O at pH 8-9.

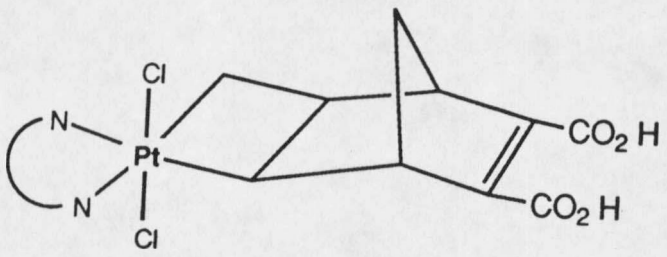
A slight deviation on 11 was synthesized by reacting one equivalent of 2,2'-bipyridine with 10 in chloroform to produce 12, which did not form a precipitate, Figure 39. Figure 40 shows a ^{13}C of compound 12. The chelating effect



PROTON	ppm	
H_a	2.35	m
H_b	2.35	m
H_c	2.40	dd
H_d	2.04	d
H_e	3.02	s
H_f	2.77	s
H_g	2.4	d
H_h	1.15	d
H_i	2.73	s

Figure 38. Proton NMR Spectrum of Compound 11.

Figure 39. Dicarboxylate Platinacyclo with 2,2'-Bipyridine, 12.



of a bidentate ligand lends stability to platinacyclobutanes over pyridine. This point will be discussed later when addressing solubility.

The chloroform volume with 12 was reduced and pentane added to initiate formation of a white precipitate. A white-tanish solid was left after evaporating to dryness using N_2. In order to obtain a ^{13}C NMR spectrum the compound was dissolved in d_6-DMSO.

The dicarboxylate ethylenediamine platinacyclobutane compound 11 was synthesized with the hopes that 1) the dicarboxylate moieties would enhance solubility, and 2) the product from reductive-elimination would be dichloro-(ethylenediamine)Platinum(II). This product is a recognized anticancer drug and will be discussed later. Synthesis of 11 confirmed that functionality could be attached to the norbornyl fragment, opposite the

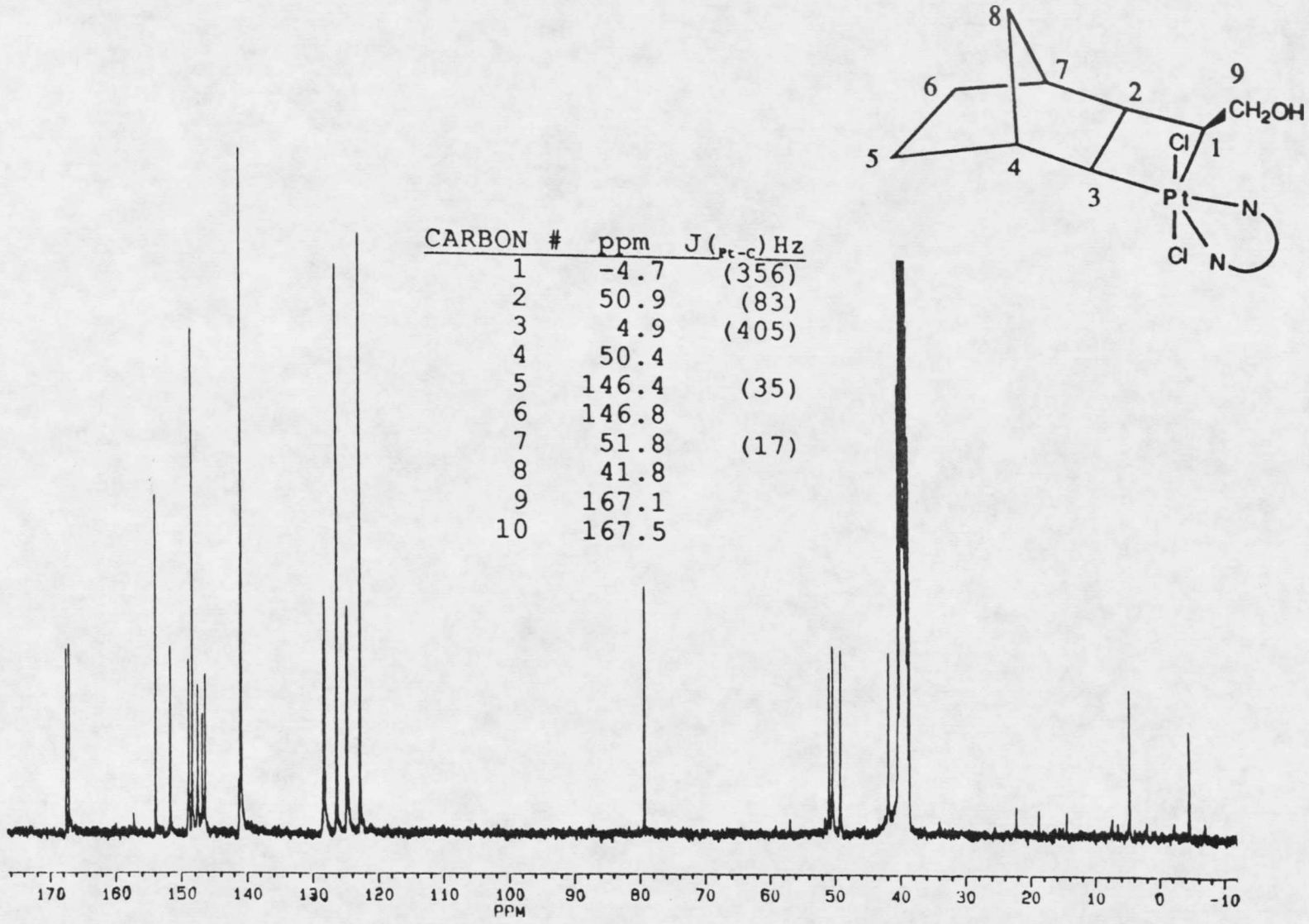
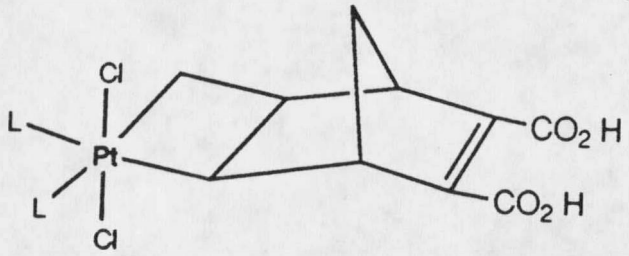


Figure 40. Carbon NMR Spectrum of Compound 12.

platinacyclobutane, without destroying the platinacyclobutane's integrity.

Compound 13, Figure 41, was synthesized with the possibility that solubility could be increased by using amine ligands with hydrophilic functionality. Four-pyridyl carbinol was chosen for its minimal steric hindrance when approaching the platinum center. Figure 42 shows the ^{13}C NMR spectrum of compound 13. Presence of the dicarboxylate

Figure 41. Dicarboxylate Compound with L= 4-Pyridyl Carbinol, 13.



functionality made it difficult to determine any difference in solubility between 10 and 13 using fetal bovine serum, FBS.

Compounds 10, 11, and 12 differ only in the type of amine ligand(s). Several experiments were carried out using these three compounds to determine what differences pyridine, ethylenediamine and 2,2'-dipyridine had on solubility and chemotherapeutic activity in tissue culture. These results will be discussed throughout this thesis.

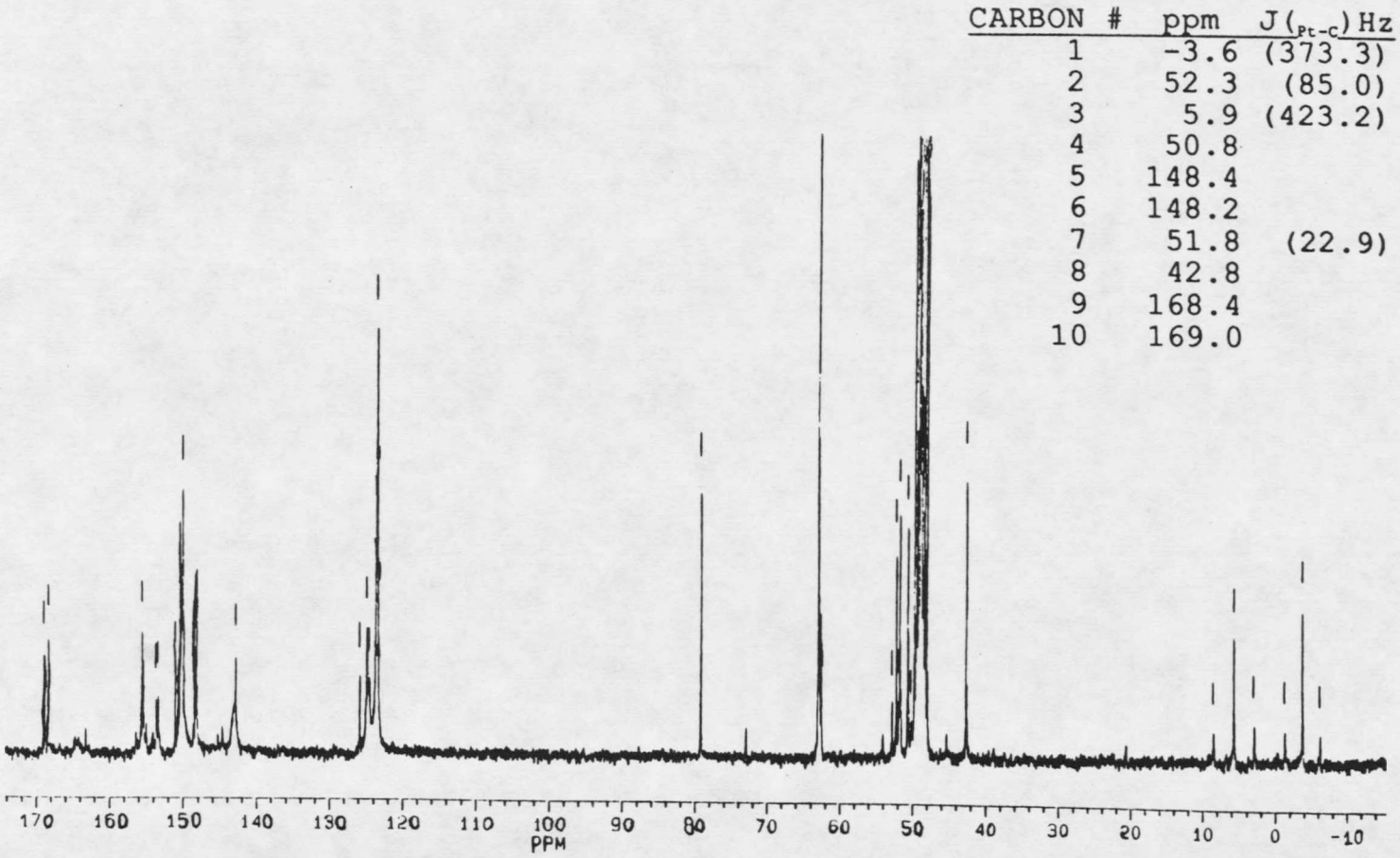
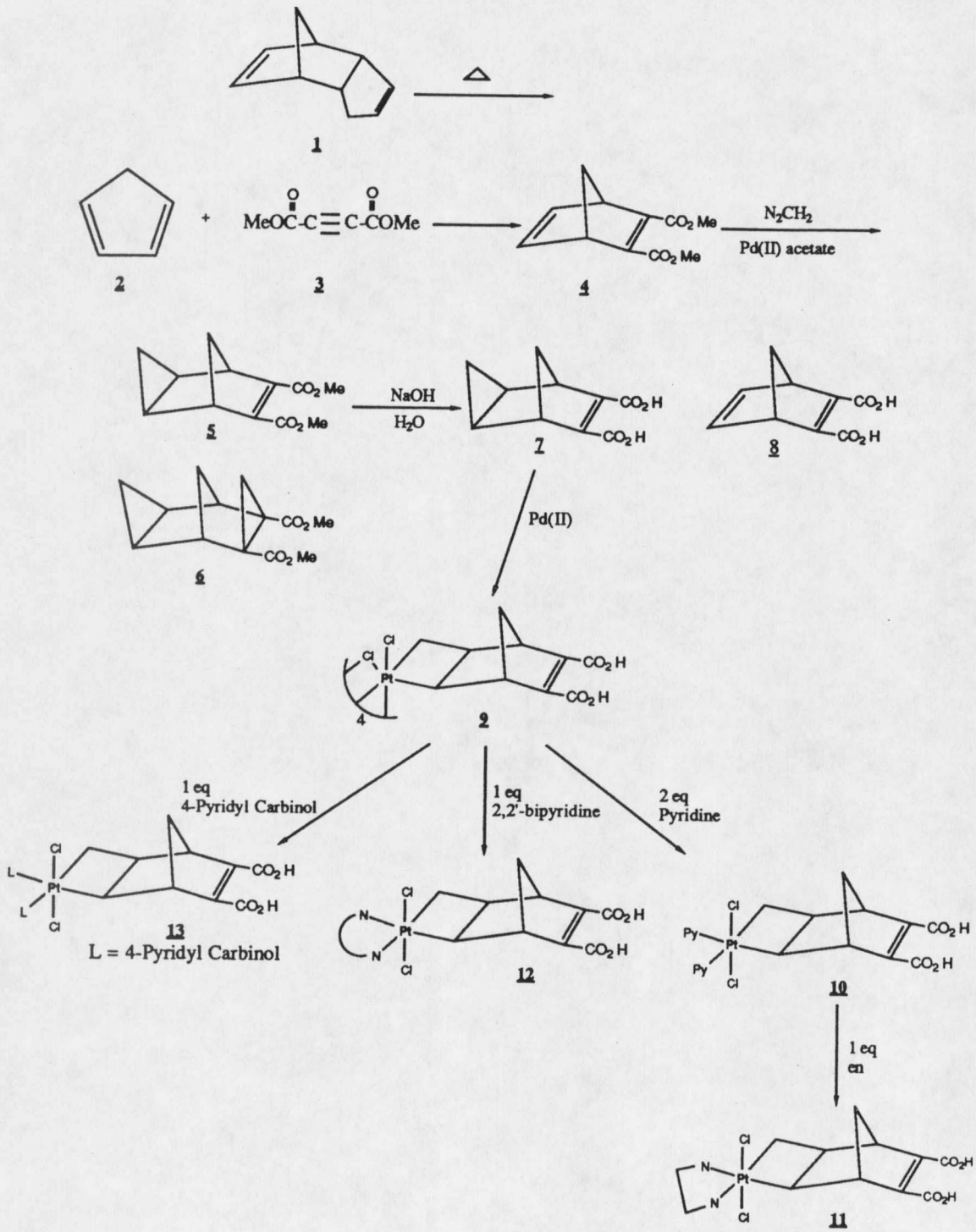


Figure 42. Carbon NMR Spectrum of Compound 13.

The first comparison was solubility in various solvents. Compounds 11 and 12 were insoluble in $CHCl_3$, CH_2Cl_2, and CH_3OH. These two compounds with bidentate ligands were also insoluble in H_2O, while 10 was slightly soluble (5-10 mg/ 1 ml). When NaOH was dissolved in H_2O all three were very soluble (50-60 mg/ 1 ml), pH 8-9. The three compounds were also added separately to FBS during experiments involving tissue cultures. Compound 12 was slightly soluble at 20-30 mg/ 10 mls, while 10 and 11 dissolved immediately at 50-60 mg/10 mls.

Further comparative studies were done involving anticancer activity and will be discussed in the tissue culture section. Figure 43 shows a synthetic scheme for products 10 through 13.

Figure 43. Summary of Synthetic Reactions Involving Dicarboxylates.



Syn Hydroxymethyl Platinacyclobutanes

In order to obtain more comparative data on the platinacyclobutane system, a new series of compounds was synthesized. These compounds have the norbornyl platinacyclobutane as the base structure with various amine ligands. Norbornene (14) reacts with ethyldiazoacetate and a catalytic amount of Rh (II) acetate dimer to form the epimeric ester isomers 15, Figure 44. Lithium aluminum hydride was used to reduce 15 to the epimeric alcohols 16 which were obtained in a ratio of 2.5:1.0, anti to syn, respectively. It was discovered by Neilsen[94] that Zeise's dimer reacted preferentially with the syn isomer of 16 to form the desired syn hydroxymethyl platina(IV)cyclobutane I.P.C. (17), Figure 45.

Figure 44. LAH Reduction Scheme.

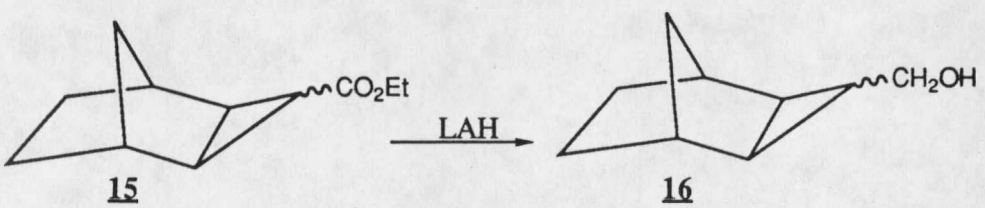
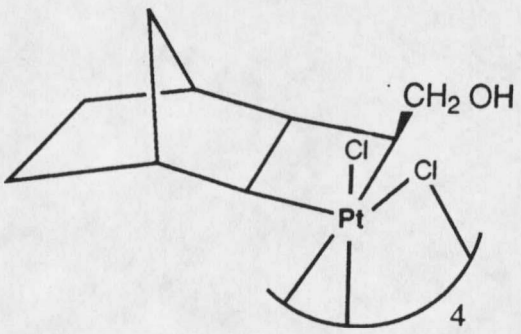


Figure 45. Syn Hydroxymethyl Platinacyclobutane I.P.C. 17.

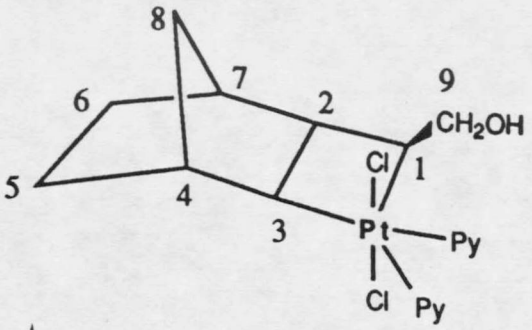


Anti alcohol 16 remained in the chloroform solution and was removed completely by washing the I.P.C. with pentane. Two equivalents of pyridine were added to the I.P.C. resulting in only one product,(18). The anti alcohol I.P.C. was synthesized by reacting Zeise's dimer with the anti alcohol followed by two equivalents of pyridine to produce compound 19,(see Figure 52). Synthesis of 18 and 19 was first done by Neilsen.[95] Figure 46 shows a ^{13}C NMR spectrum of compound 18, while Figures 47 and 48 show a ^{13}C and DEPT NMR spectrum of compound 21, respectively.

Compounds 20 and 21 were synthesized by adding the appropriate amine ligand, ethylenediamine or 2,2'-bi-pyridine, with 17, Figure 49.

In the syn hydroxymethyl series functional moieties were only two carbons from the platinum in contrast to the dicarboxylate compounds. The hydroxymethyl moiety was used

CARBON #	ppm	$J_{(Pt-C)}$ Hz
1	6.3	(372)
2	56.8	(97)
3	13.1	(395)
4	40.3	(10)
5	28.7	(42)
6	29.0	
7	38.0	(9)
8	37.5	
9	63.8	(24)



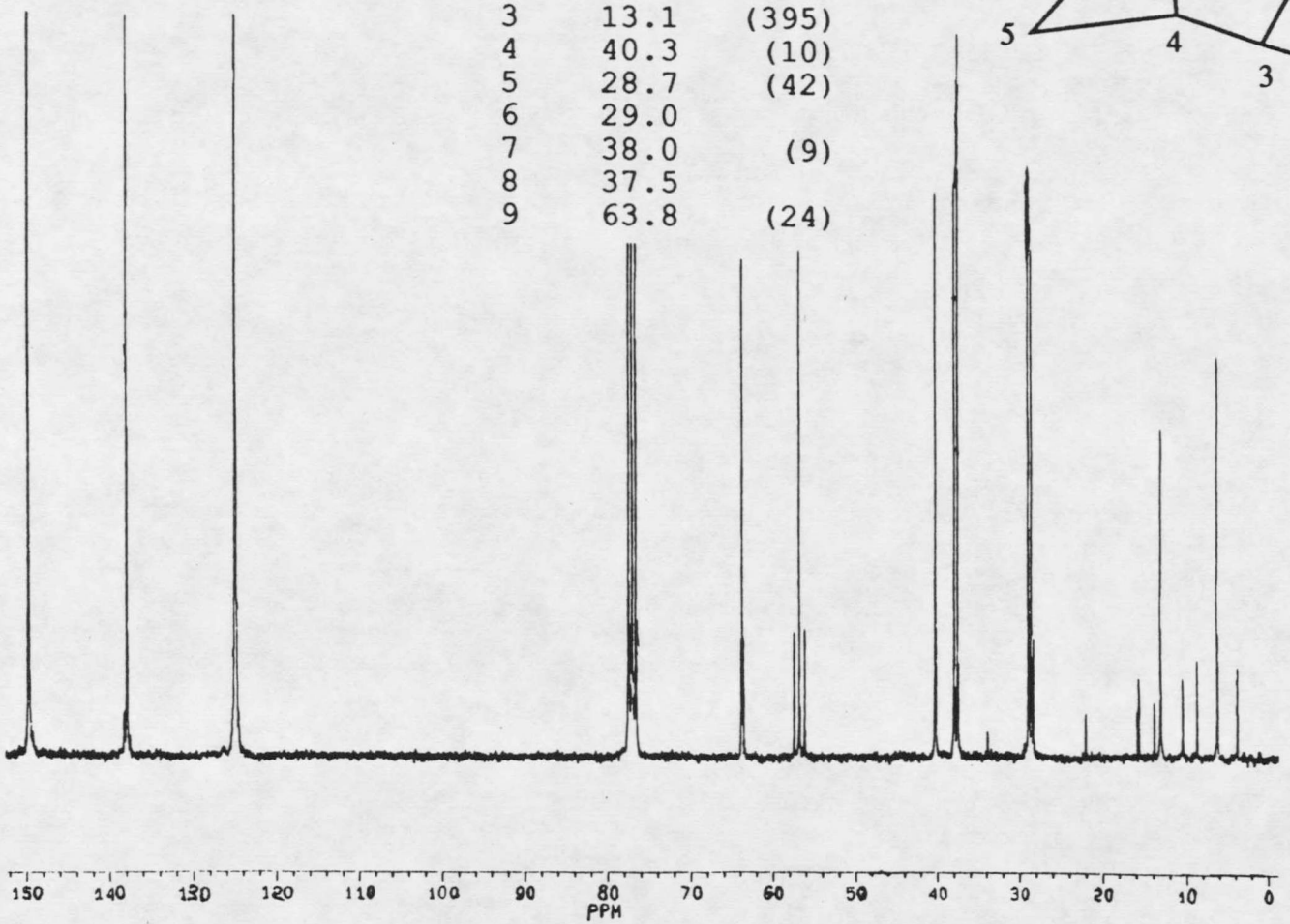


Figure 46. Carbon NMR Spectrum of Compound 18.

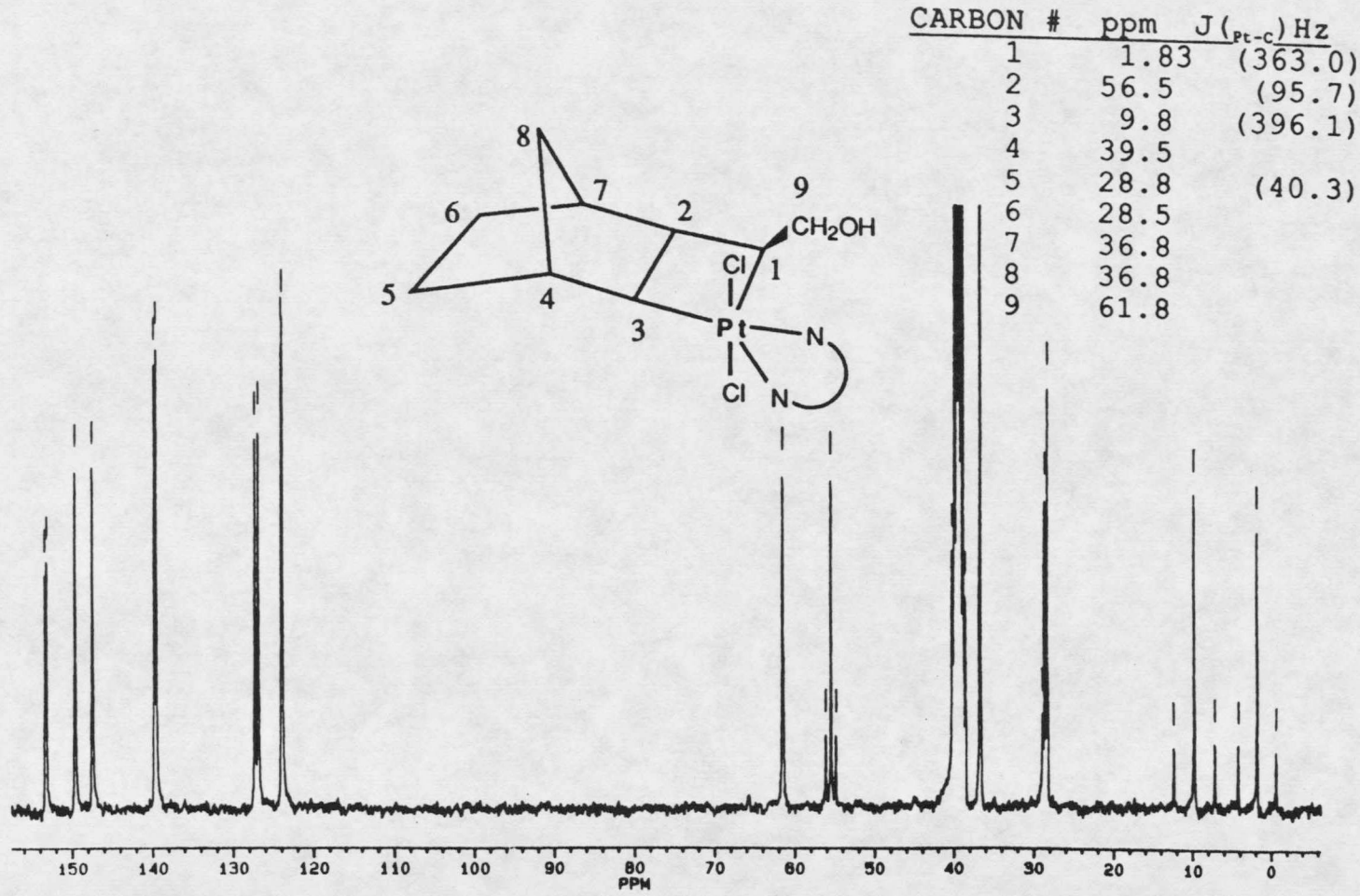


Figure 47. Carbon NMR Spectrum of Compound 21.

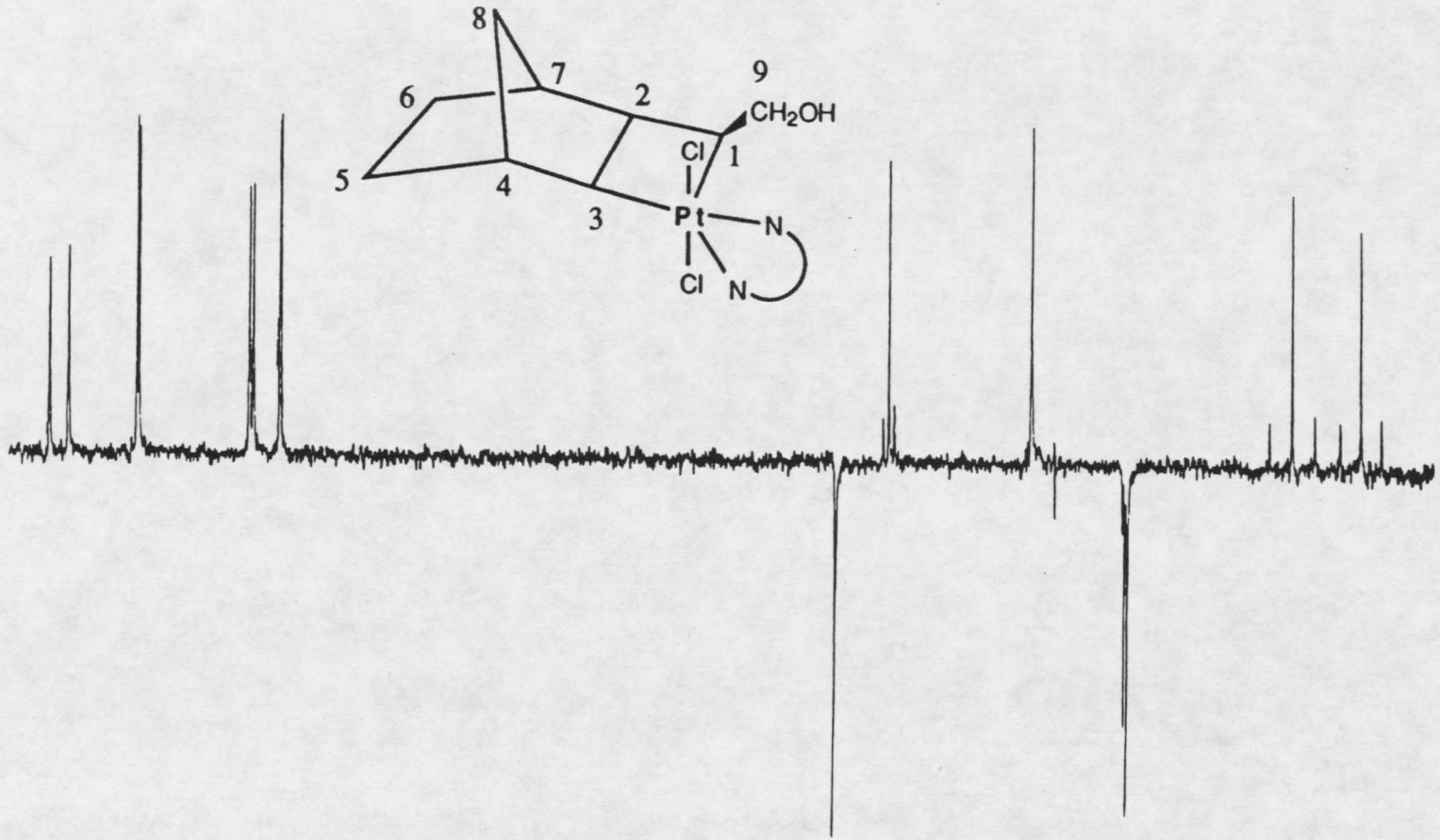
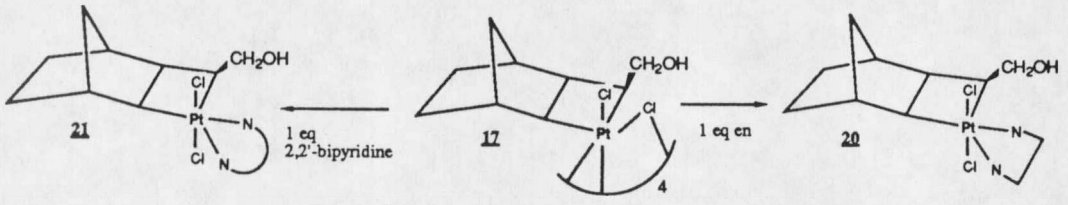


Figure 48. DEPT NMR Spectrum of Compound 21.

to, once again, influence the solubility of the complexes. Platinum ligands were altered in this series to determine the extent different ligands played in solubility and anticancer activity. Solubility of 20 in $CHCl_3$ was markedly reduced compared to 18 and 19 while compound 21 was only slightly soluble in $CHCl_3$. Dimethylsulfoxide (d_6) was used to solubilize 20 and 21 for NMR characterization.

When testing their solubility in FBS, compounds 19 and 21 were very insoluble while 18 was soluble at 15 mg/ 10 mls. Compound 20 was only slightly soluble (5 mg/ 10 mls). Due to the insoluble nature of the antihydroxymethyl-platinacyclobutane (19) no analogs were synthesized with 2,2'-bipyridine or ethylenediamine ligands. Figure 50 shows a DEPT NMR spectrum of compound 20. Figure 51 shows a synthetic scheme for products 18 through 21.

Figure 49. I.P.C. 17 to Products 20 and 21.



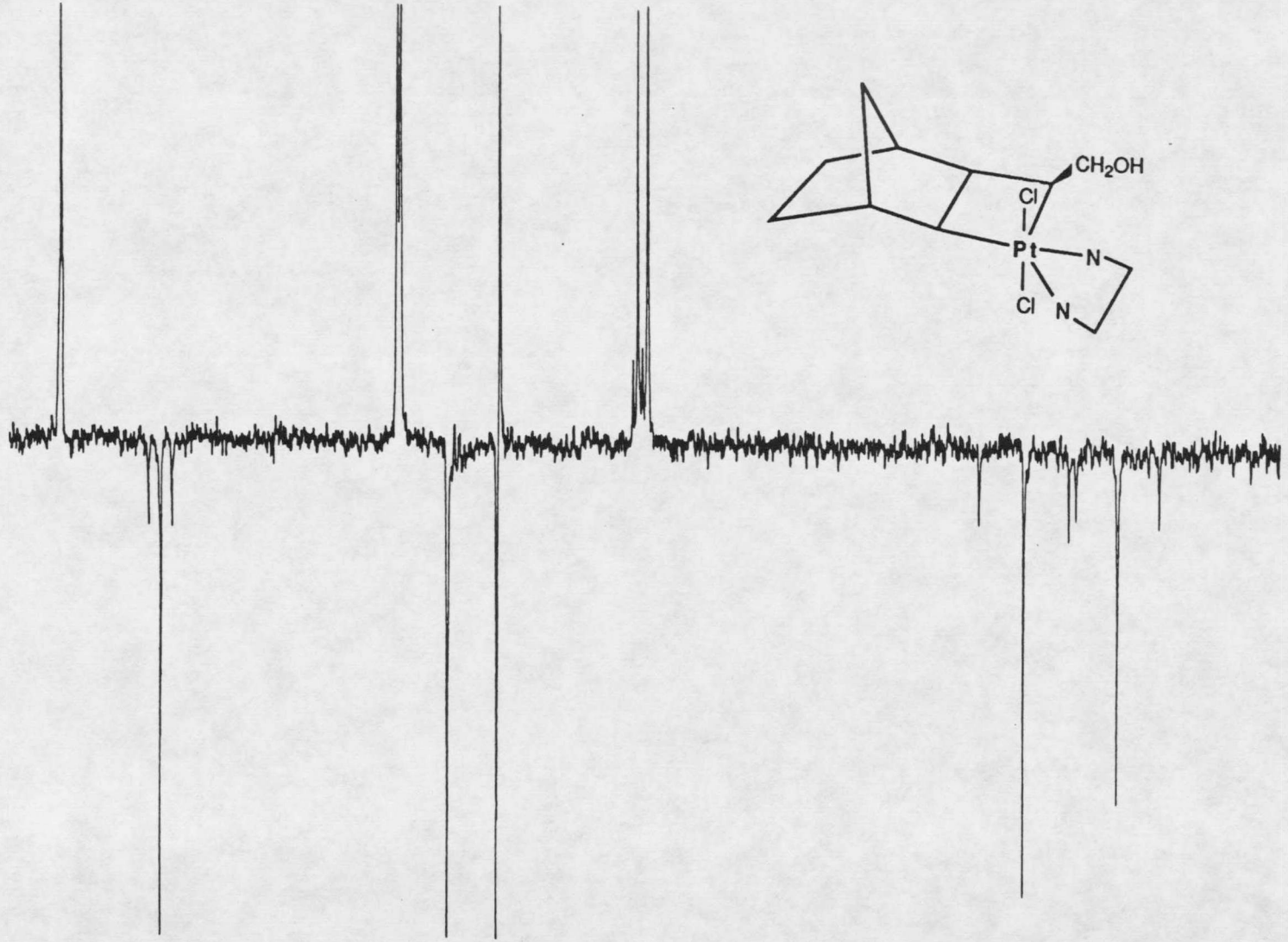
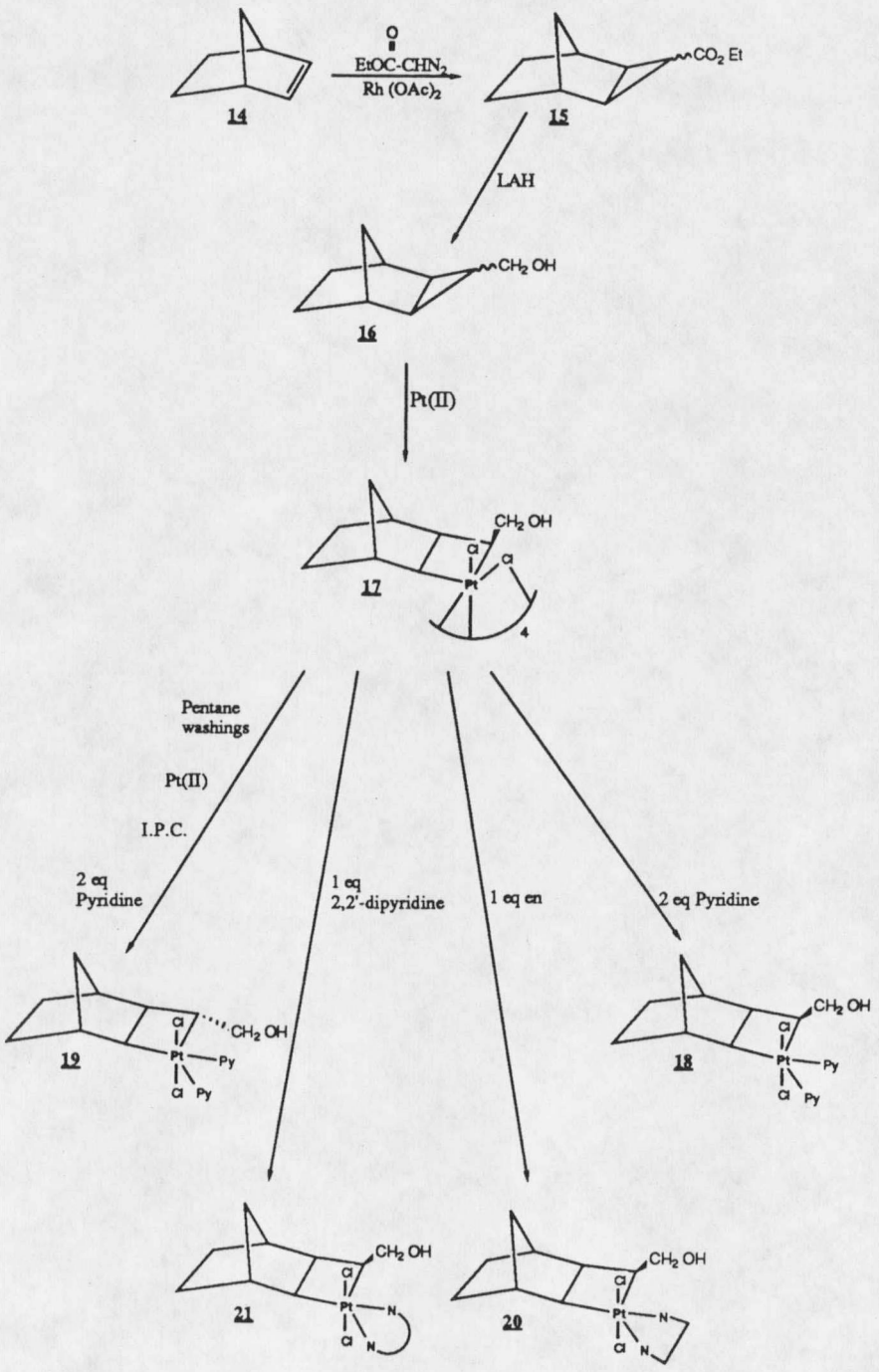


Figure 50. DEPT NMR Spectrum of Compound 20.

Figure 51. Summary of Synthetic Reactions of Syn Hydroxymethyl Platinacyclobutanes.

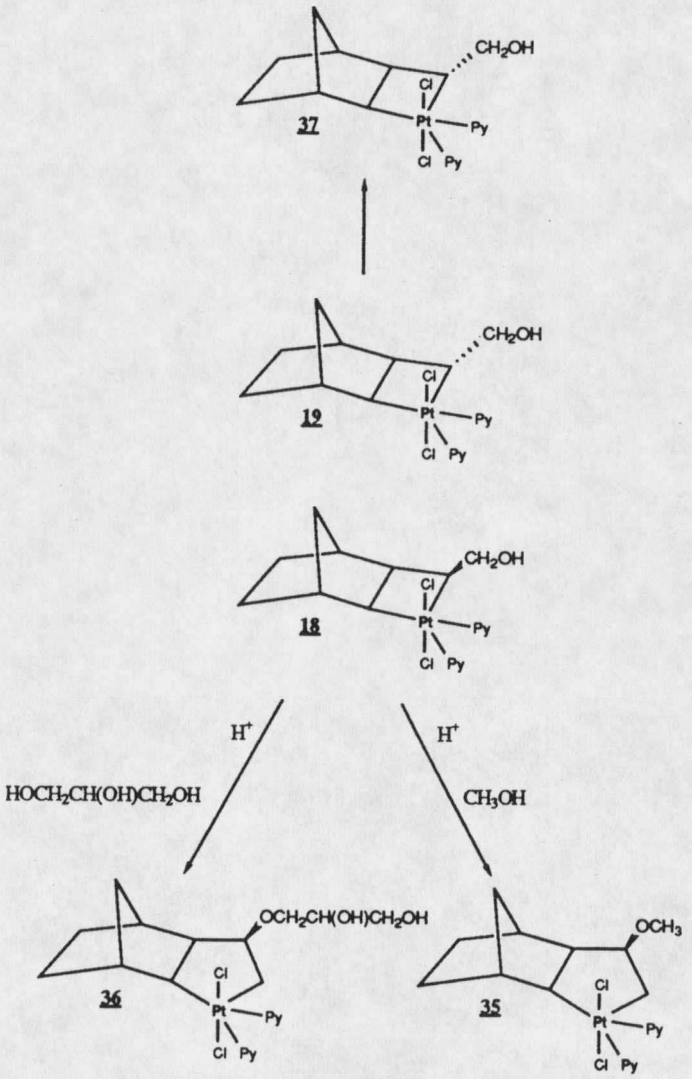


Syn Methyl Ether and Diol Ether

Neilsen[96] synthesized 36 and 35, by adding glycerin and methanol respectively, to 18 followed by sulfuric acid, Figure 52. The ring expanded products were confirmed by ^{13}C NMR Pt-C coupling constants, ~540 Hz for ^{1}Js. A x-ray crystal structure of an analogous complex was done to confirm the platinacyclopentane structure.[97] The differences between these two compounds and the substituted platinacyclobutanes are that the ring substituents are one carbon farther away from the platinum and are platinacyclopentanes. This could affect the solubility and/or chemistry of the compound if platinum is capable of interacting with the tethered substituents. Also, substituents on the platinacyclopentane ring, supposedly affecting solubility, have changed from a primary alcohol to a methyl or diol ether.

Compound 36 was more soluble in $CHCl_3$ than 35. Compound 36 was very insoluble in FBS whereas 35 was only slightly soluble. The additional oxygen functionality, 36 produced a more soluble compound in comparison to 35. Compound 37 was synthesized with the same procedure used for compound 35.[98]

Figure 52. Synthetic Scheme for Compounds 35, 36 and 37.

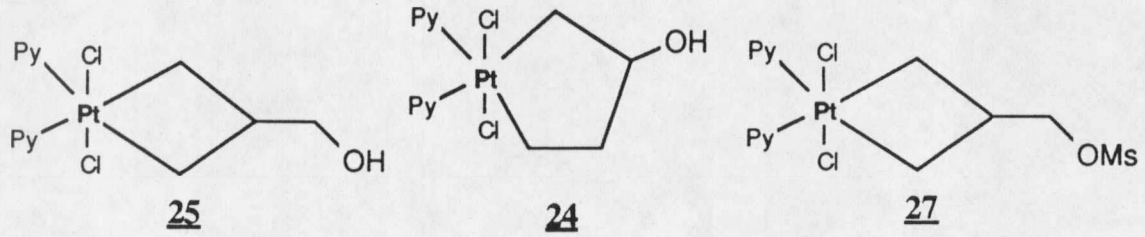


Hydroxy Platinacyclobutanes and Pentanes

In 1983, Burton and Puddephatt, synthesized complexes 24, 25 and 27, Figure 53. Their method of synthesis for 25 involved adding Zeise's dimer to cyclopropyl carbinol (22) in tetrahydrofuran (THF) and stirring for twelve hours. The reaction mixture was first filtered and dried using N_2. Pyridine was then added to the precipitate at zero degrees celsius in methylene chloride. The resulting precipitate was dried using N_2, washed with ether, dried again, and re-crystallized to yield a pale yellow solid.

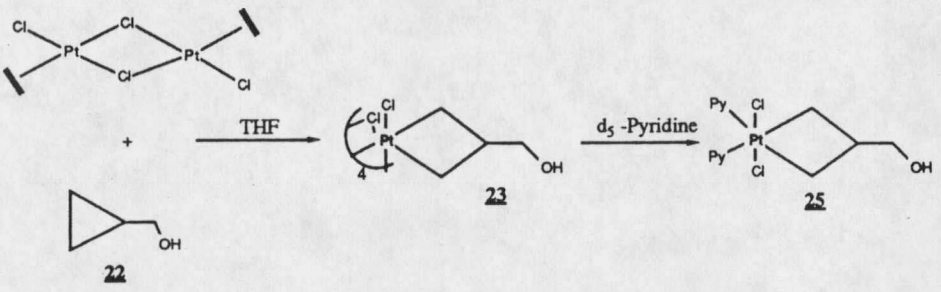
A simpler method in synthesizing 25 was discovered by this author. Cyclopropyl carbinol (22) was mixed with Zeise's dimer in freshly distilled THF for 24 hrs, at which time the solution was filtered, Figure 54. Figure 55 shows the ^{13}C NMR Spectrum for 25. The volume was reduced to 0.5 mls by evaporating over N_2 followed by the addition of

Figure 53. Compounds Synthesized by Burton.



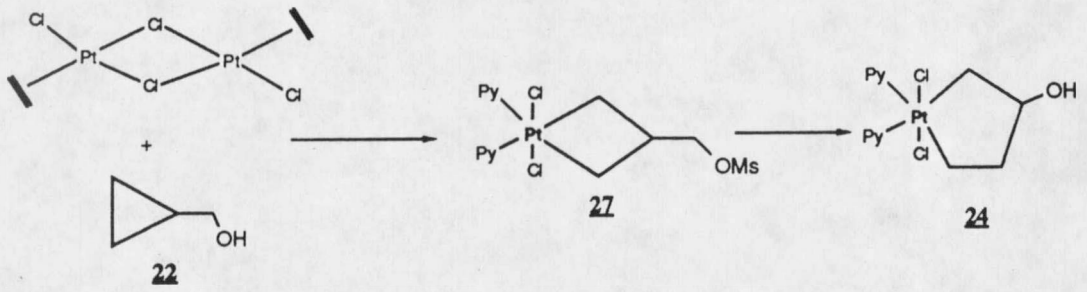
pentane to initiate precipitation of a white fluffy solid. Pentane was used to wash the precipitate three times yielding a yellow solid, 23. Due to the instability of 25 in $CDCl_3$, d_5-pyridine was used as the NMR solvent. The ^{13}C NMR spectrum showed conclusively that the platinacyclobutane was present when compared to Burton's ^{13}C NMR data. There were resonances in the same spectrum at the correct chemical shifts, just above base line, to indicate 24 had also been synthesized. If the reaction was left to react for 4 days and worked up as previously described there was only one product, platinacyclobutane 25.

Figure 54. Reaction Scheme for 25.



In making 24, Burton's method was even more lengthy. Cyclopropylcarbinyl methanesulphonate was used with Zeise's dimer to initially prepare 27. This was then the precursor to 24 which underwent solvolysis in 60% acetone-d_6/D_2O for 100 hours, followed by a cleanup procedure. This yielded a pale yellow solid 24, Figure 55.

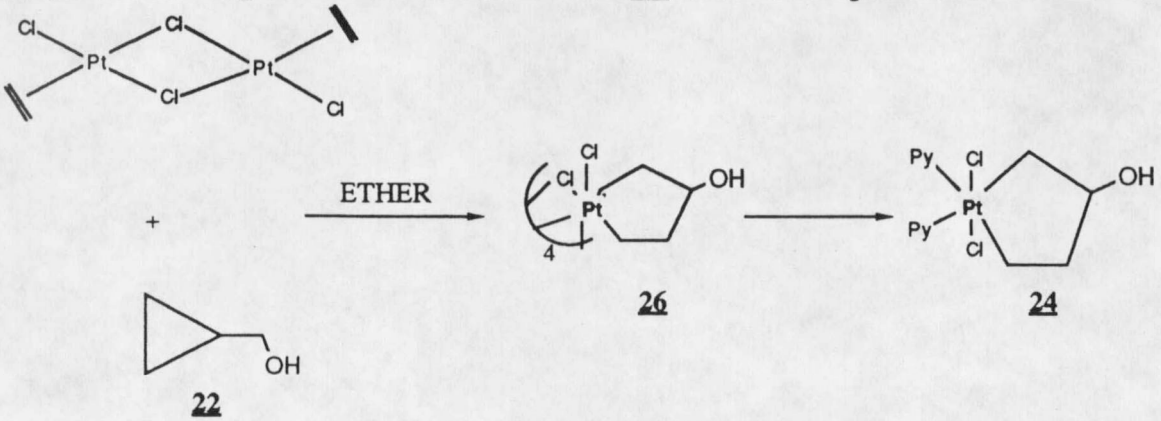
Figure 55. Burton's synthetic scheme for 24.



An alternative method from this laboratory for synthesizing 24 was set up exactly like the procedure outlined above for 25, except that the reaction solvent was freshly distilled diethyl ether. The carbon NMR spectrum of the resulting fine white solid showed only one product, the platinacyclopentane 24, Figure 56.

A reaction was set up to run for 24 hours, but by accident the reaction vial cap came off sometime during the first 12 hours. The solvent had evaporated leaving a dried yellow solid. A ^{13}C NMR spectrum revealed both the

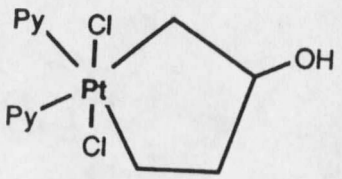
Figure 56. Synthetic Scheme for 24 Involving Ether.



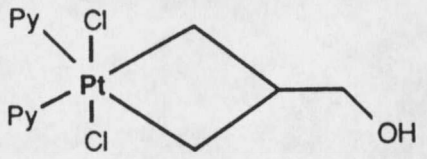
platinacyclobutane and the platinacyclopentane in roughly a 1:1 ratio, Figure 57. Another reaction was set up to stir for 3.5 days and the ^{13}C NMR spectrum showed that the major product was the platinacyclopentane 24 with a possible minor product, platinacyclobutane 25. Carbon 13 resonances for the platinacyclobutane were at the appropriate chemical shifts and just above base line.

To address the question as to whether the platinacyclopentane was resulting from adventitious acid catalysis as had been shown by Neilsen,[99] two reactions

Figure 57. Hydroxy Platinacyclobutane and Pentane 24 and 25.



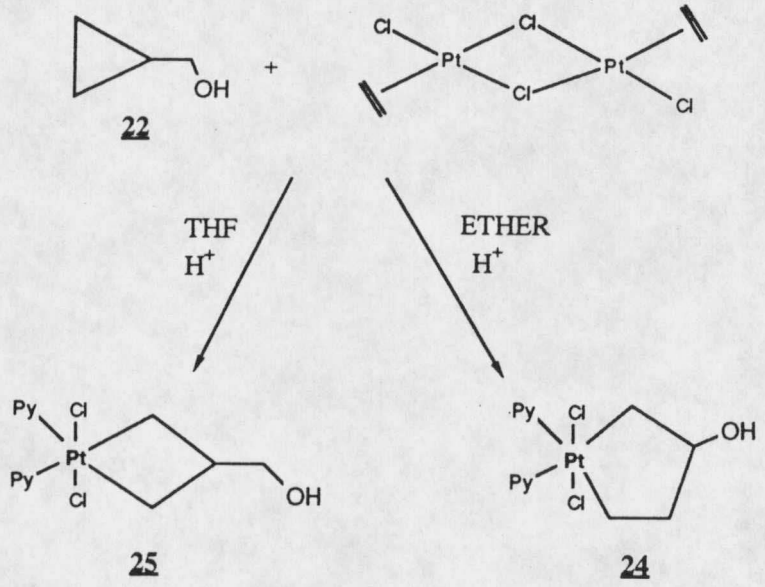
24



25

were set up One was in diethyl ether and the other in THF as previously described. The only difference was that 2 drops of concentrated sulfuric acid were added to both reaction vials. Carbon 13 NMR results showed that running the reaction in ether produced the platinacyclo-pentane exclusively and the THF reaction had the platinacyclobutane as the sole product, Figure 58.

Figure 58. Addition of Acid to Synthesize 24 and 25.



These results indicate the following: 1) the ring expansion process is not mineral acid catalyzed, and 2) the formation of the platinacyclobutane or pentane is solvent dependent. Perhaps the coordination of THF to platinum prevents an open coordination site from being vacant, which is needed for Lewis acid catalyzed ring expansion. In the case of diethyl ether, either a long reaction time or evaporating to dryness will produce the platinacyclobutane. Whereas a 24 hour reaction in a capped vial with or without H^+ will produce only the platinacyclopentane.

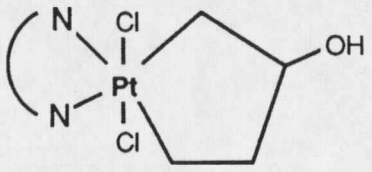
Complex 28, Figure 59, was synthesized by adding 1 equivalent of 2,2'-bipyridine to the I.P.C. 26 from the diethyl ether reaction described above. The only product in the ^{13}C NMR spectrum observed was the platinacyclopentane 28. The ^{13}C resonances for 28 do not correlate

with those from Burton. Figure 60 shows the carbon NMR spectrum for compound 28.

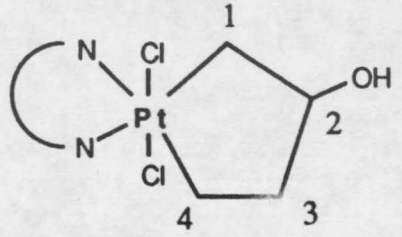
In contrast to 24 and 25, 28 was insoluble in $CHCl_3$ and CH_2Cl_2. This difference must be attributed to the bidentate ligand. Compounds 24 and 25 were not added to FBS to determine solubility, because of their instability. The platinacyclopentane with 2,2'-bipyridine was added to FBS and was completely insoluble at 5 mg/ 10 mls.

Figures 61 and 62 show the reaction scheme for synthesizing compounds 24 and 25.

Figure 59. Hydroxy Platinacyclopentane with 2,2'-Bipyridine, 28.



28



CARBON #	ppm	J($_{Pt-C}$) Hz
1	13.8	(479.6)
2	81.6	(38.2)
3	36.0	
4	27.7	(540.6)

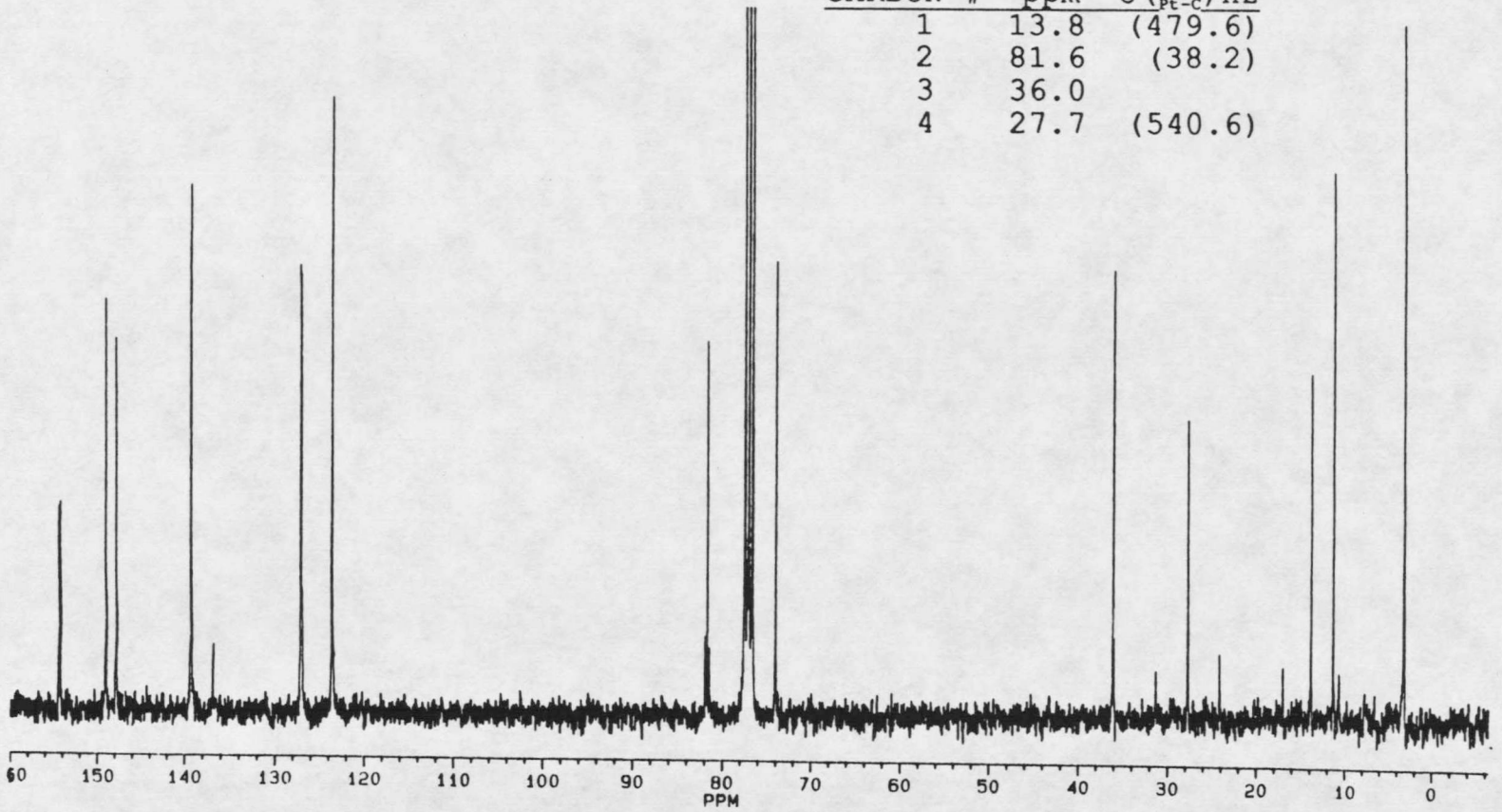


Figure 60. Carbon NMR Spectrum of Compound 28.

Figure 61. Reaction Scheme for Compounds 24 and 25 using THF.

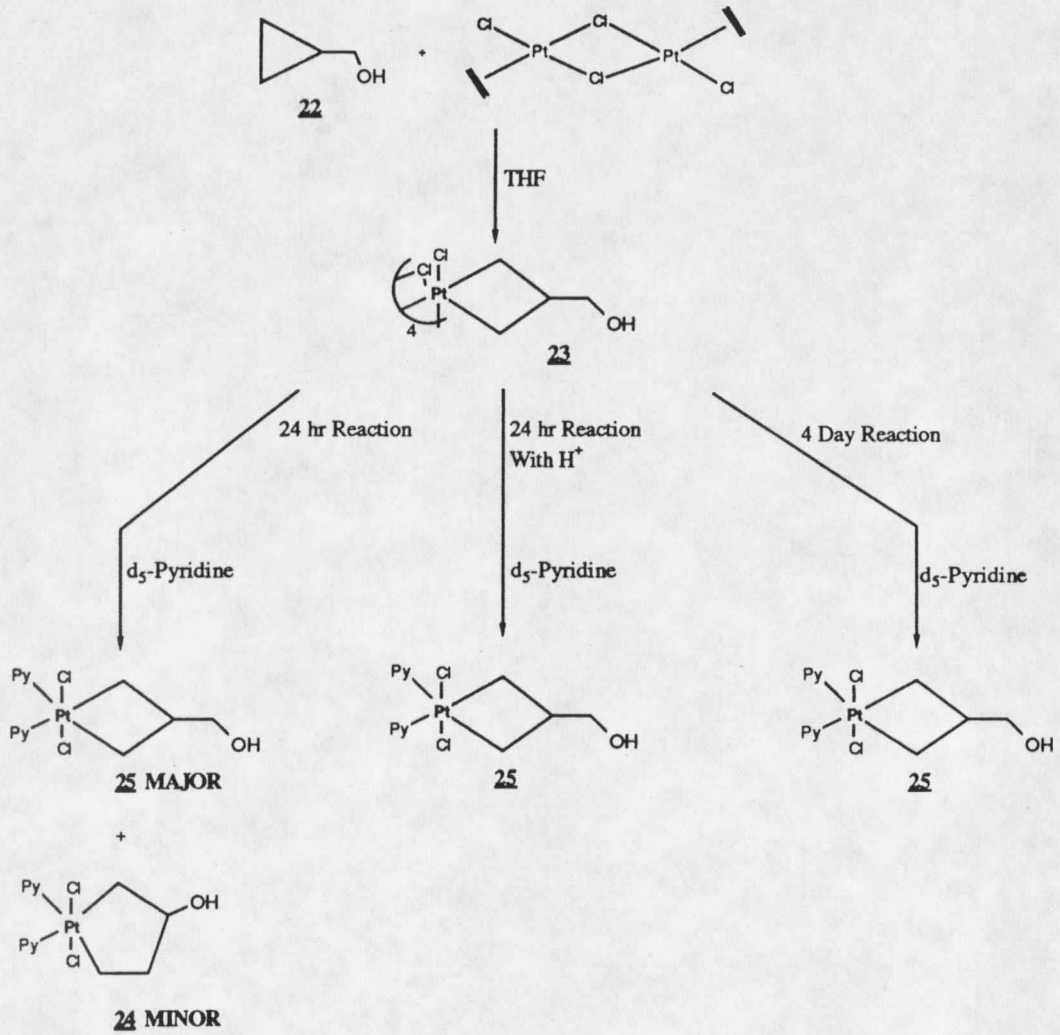
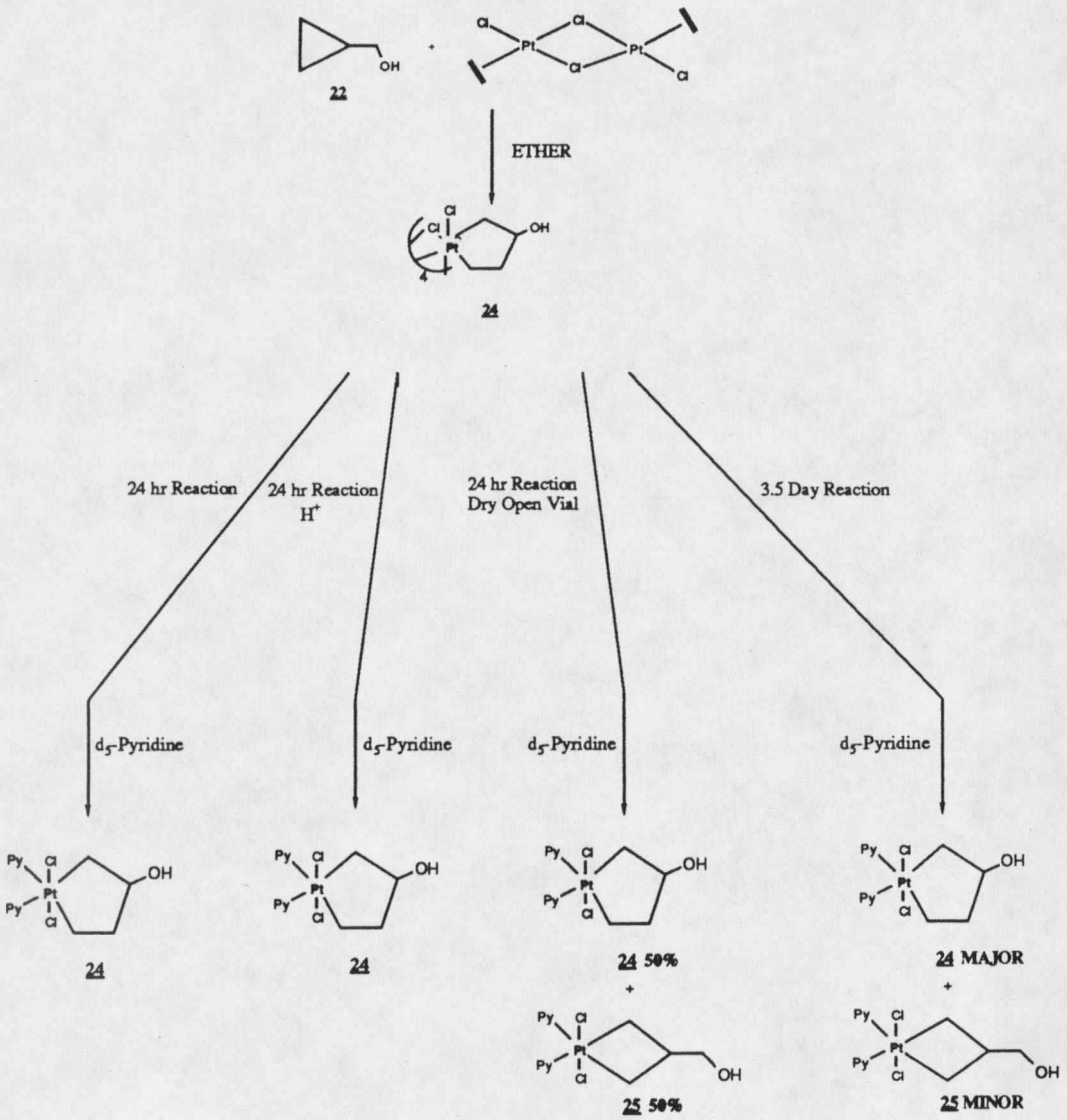


Figure 62. Reaction Scheme for Compounds 24 and 25 using Ether.



Oxidation of Syn Hydroxymethyl to Carbonyl Derivatives

Oxidation of 18 with PDC to yield 29 was carried out earlier by Neilsen[100]. In an attempt to react 29 with ethylenediamine to yield 30, it was determined that the amine ligands did not exchange as expected, but ethylenediamine did react with the aldehyde to form what was believed to be the Schiff base, Figure 63. An alternative synthetic route was to first add ethylenediamine to 18 to make 20 and then oxidized to 30. Instead of synthesizing 30, 31 and 29 were the resulting products, Figure 64. It is of interest to note that in both cases ethylenediamine was replaced with pyridine. Free pyridine was not added at any step in the synthesis, therefore the pyridine had to come from the oxidizing agent, pyridinium dichromate. Figure 65 shows a carbon NMR spectrum of 20.

Figure 63. Reaction Scheme to Synthesis 30.

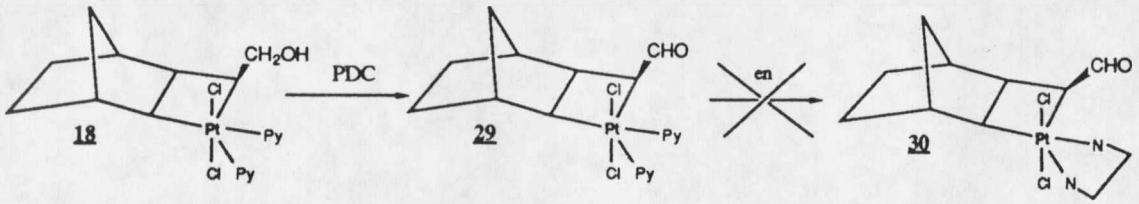
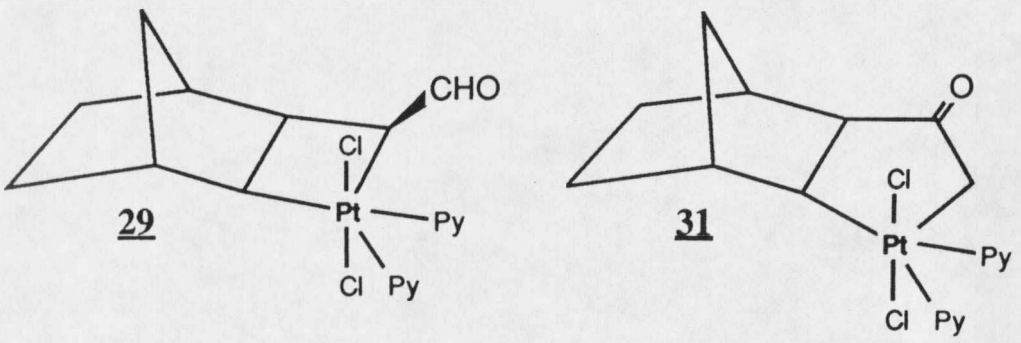
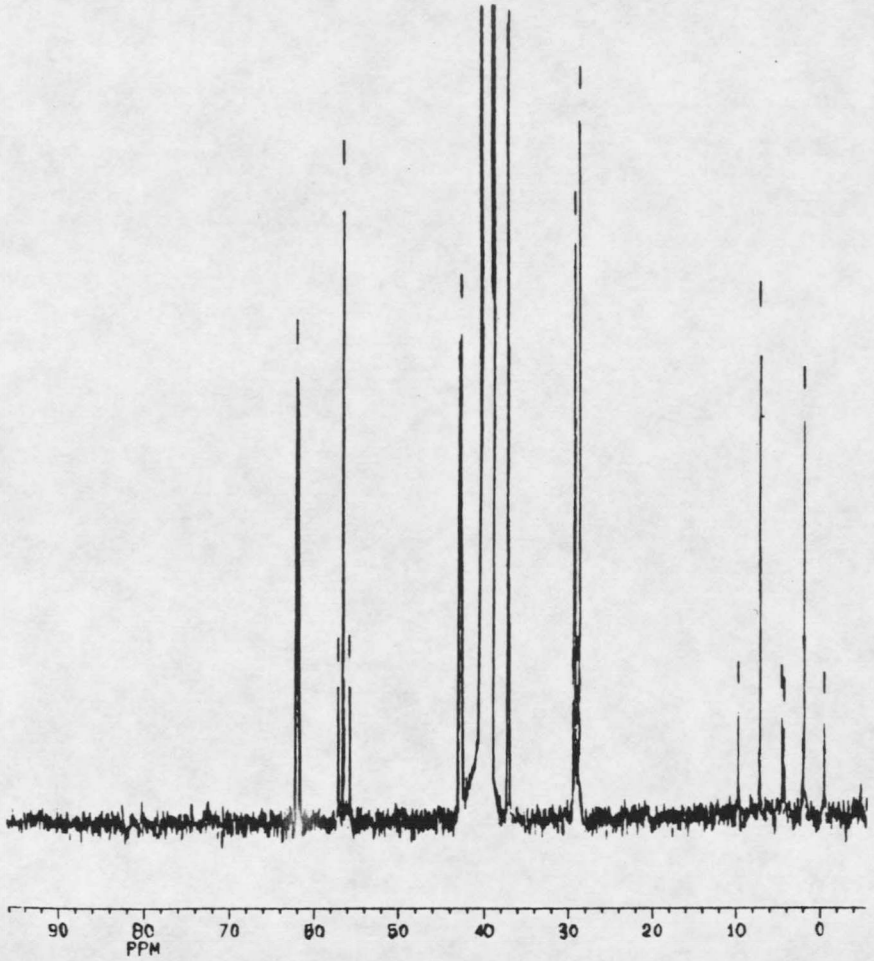


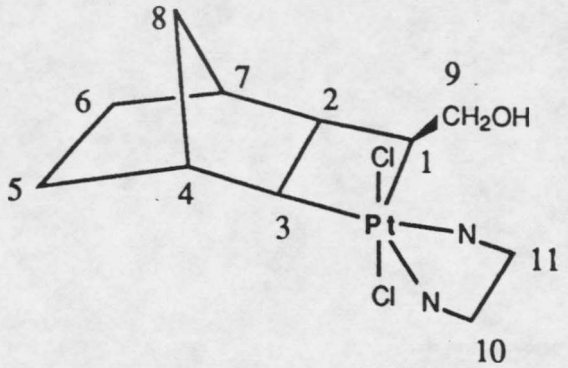
Figure 64. Oxidation Products of 20 with PDC.



The platina(IV)cyclopentanone was unexpected and had not been observed before in this oxidation reaction. Compound 31 was first synthesized by Neilsen[101] by a somewhat different method. Neilsen took 18 in a 60% acetone/H_2O mixture and added 4 drops of concentrated sulfuric acid to yield 32. This complex was subjected to PDC for 24 hours to yield 31, Figure 66. Neilsen had then shown that the oxidation was acid catalyzed. The reaction discussed above where no sulfuric acid was added could still have been acid catalyzed, since PDC is somewhat acidic, $[(C_5H_5NH^+)_2\ Cr_2O_7]$. Neilsen proposed a ring expansion mechanism to explain his results as shown in, Figure 67.

A point of interest in this mechanism is that the hydroxy oxygen originally on the platinacyclobutane leaves as H_2O and the oxygen on the final product comes from the attacking nucleophile. In the case presented above there was no nucleophilic source added, thus the





CARBON #	ppm	J(Pt-C) Hz
1	1.9	(366.2)
2	56.3	(99.2)
3	7.1	(391.3)
4	39.5	
5	29.1	(39.2)
6	28.6	
7	37.2	
8	37.0	
9	61.9	
10	42.8	
11	42.6	

Figure 65. Carbon NMR Spectrum of Compound 20.

Figure 66. Neilsen's synthesis of 31.

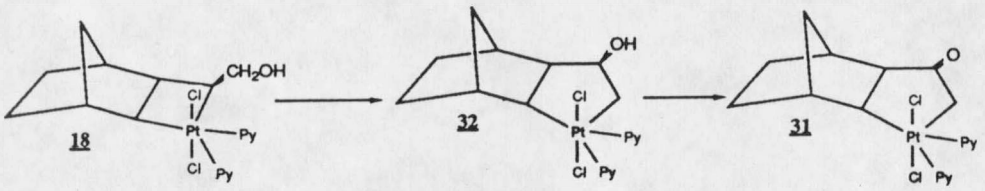
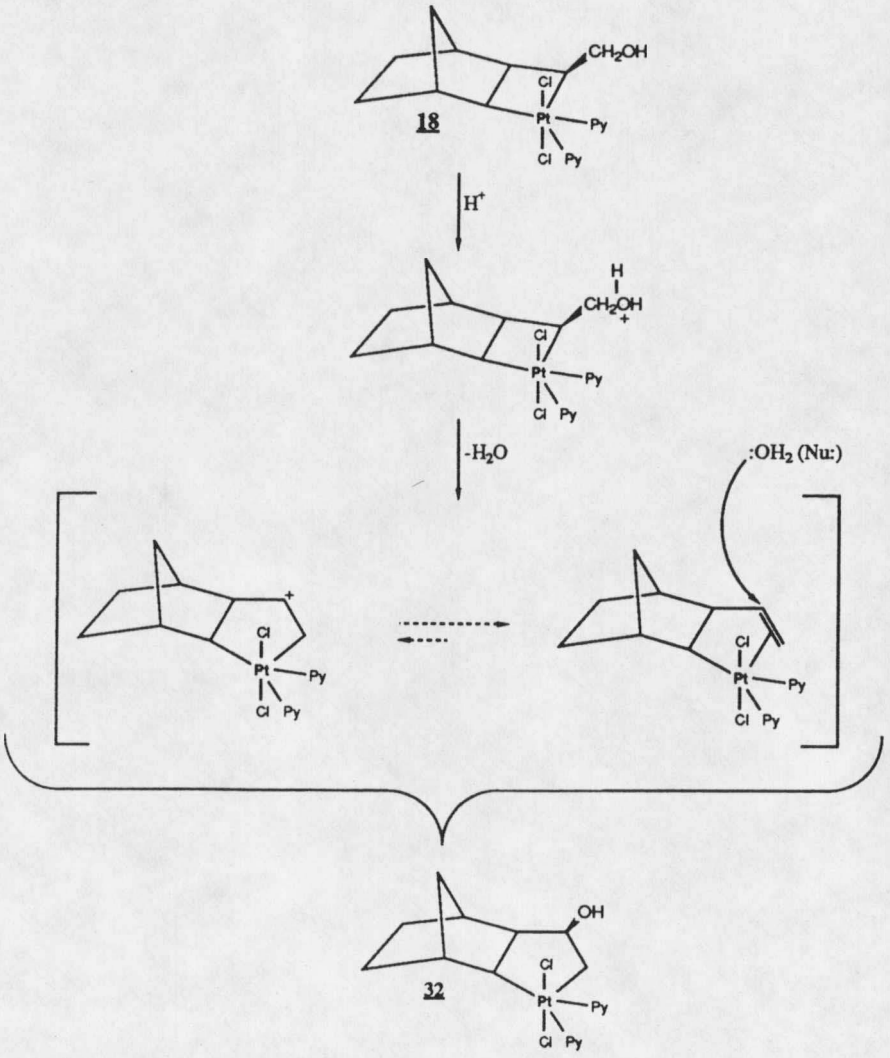


Figure 67. Neilsen's Ring Expansion Mechanism.



oxygen must have come from the alcohol or chromate ester. Perhaps, the oxygen does leave as H_2O and then also acts as the nucleophile. If so, this would explain the long reaction time needed to synthesize 31. The weaker acidic source would also cause a longer reaction time. This would mean that 32 must be present and then goes on to the platinacyclopentanone. Unfortunately, the ^{13}C NMR resonances for 32 were not seen in spectra of 31 and 29.

There is evidence to indicate that 29 is converted to complex 31. If the oxidation of 20 is left to continue for 24 hours, 29 is the predominate product with 31 a minor product. A seven day reaction will produce 31 as the major product and 29 in now the minor product, Figure 68.

The exchange of amine ligands is very interesting considering that ethylenediamine is a stronger ligand then pyridine by virtue of its chelate effect. In an earlier experiment where 20 was mixed in a 50:50 solution of chloroform/pyridine. The reaction was run for 36 hours and resulted in no reaction with only starting material present, Figure 69. Since the ethylenediamine had not been replaced by pyridine, PDC must be directly involved in the exchange of ethylenediamine with pyridine in the reaction.

To further investigate oxidation reactions with PDC, 21 was oxidized for 7 days with PDC, Figure 70. The reaction mixture was worked up as previously described and

Figure 68. Twenty Four Hour and 7 Days Oxidations of 20.

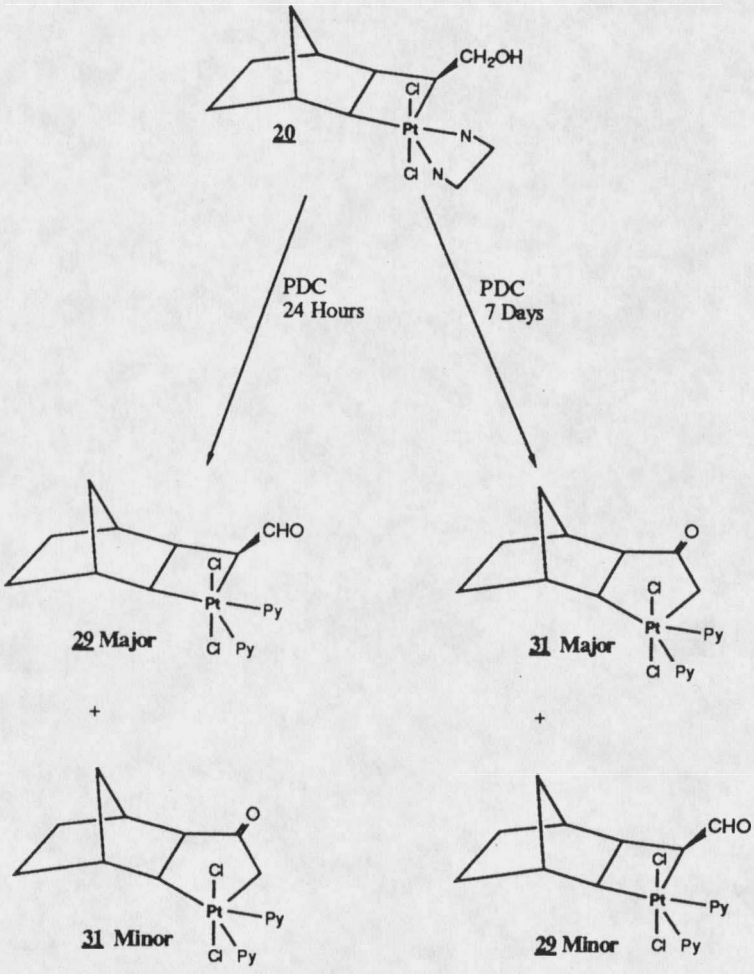
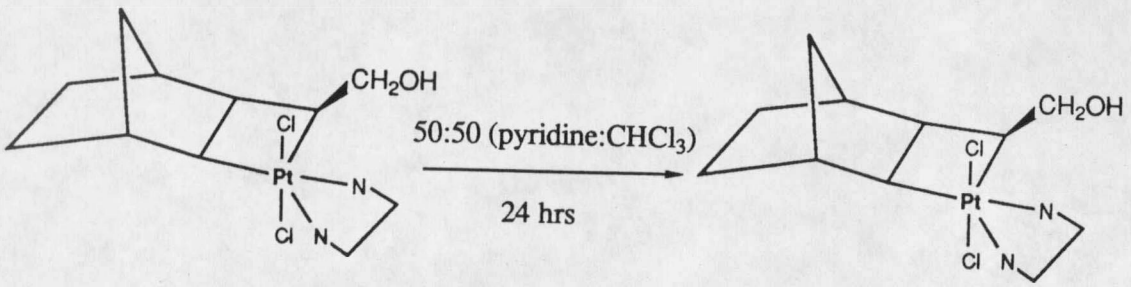


Figure 69. Compound 20 with 50:50 Pyridine-Chloroform.



the ^{13}C NMR spectrum showed only product, 33. This is in contrast to when ethylenediamine was used, 2,2'-bipyridine seemed to prevent ligand exchange and rearrangement to the platinacyclopentanone. Figure 71 outlines the reactions used to synthesize compounds 29, 30, 31.

Figure 70. Oxidation of Compound 21.

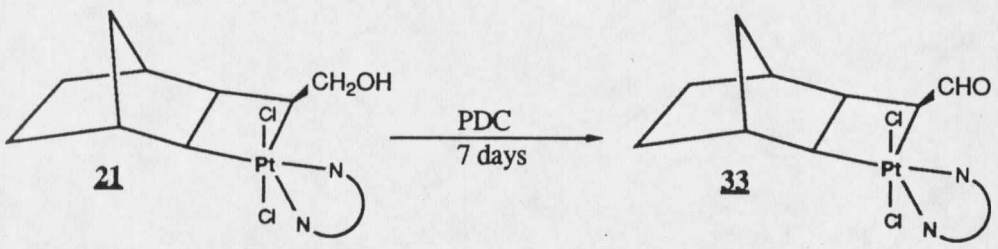
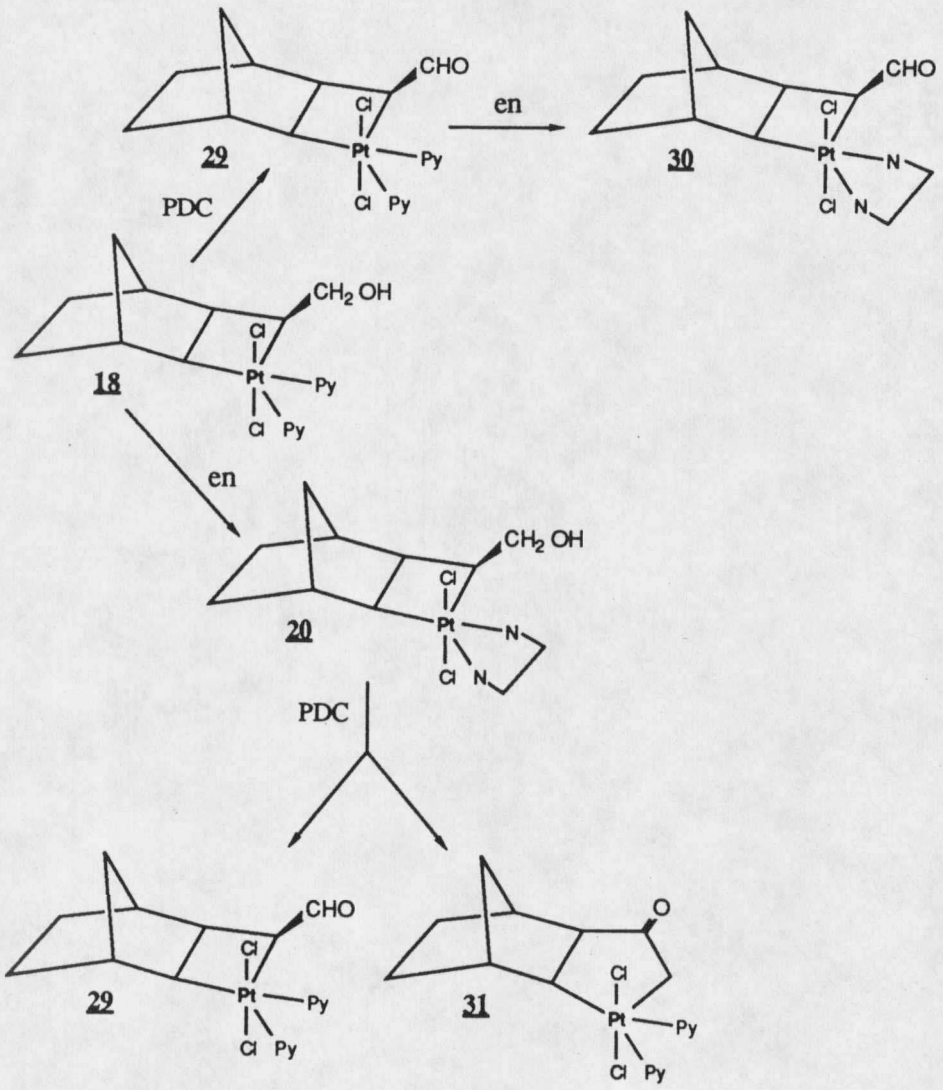


Figure 71. Reaction Scheme for Compounds 29, 30, 31.



BIOLOGICAL RESULTS AND DISCUSSION

Tissue Culture Results

An assay was developed to evaluate the anticancer activity of the platinum IV's synthesized using two murine cell lines; B-16 melanoma and Lewis Lung carcinoma. The cells were grown in tissue culture dishes (60 x 15mm). An appropriate clonal density was determined prior to testing by plating 100, 500, and 1000 monodispersed cells per dish. There was an insufficient number of colonies at 100 and 500, while 1000 cells per dish yielded sufficient colonies with some coalescing. The clonal plating density was therefore set at 900 cells.

The assay was designed to determine percent of surviving cells at each concentration following exposure to the platinum compounds. The colonies resulting from individual cells were counted and not individual cells, therefore the colonies were indicative of the number of cells surviving exposure to the platinum compounds. Surviving colonies were counted on each dish. The highest and lowest values were not used in calculating group averages. Percent survival was calculated for each group based upon the control group having 100% survival. A percent survival curve was established for each compound

tested. Concentrations causing 100% mortality and initial mortality were used to construct each curve.

Cisplatin and dichloro(ethylenediamine)Pt(II) were used as standards to determine relative effectiveness of the platinum IV's synthesized, Figure 72. Survival curves

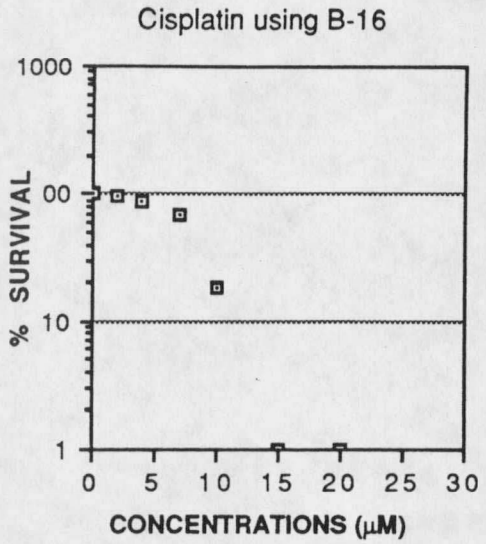
Figure 72. Cisplatin and $Cl_2(H_2NCH_2CH_2NH_2)Pt(II)$.

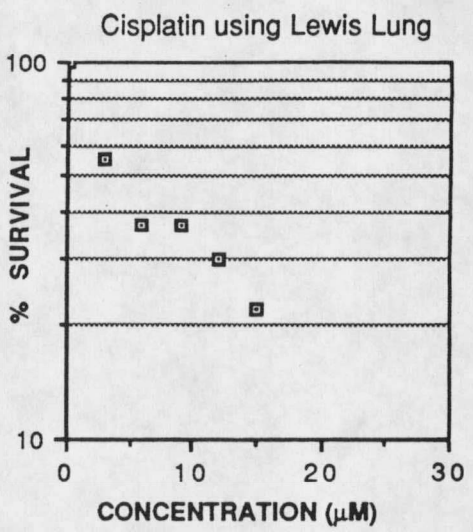


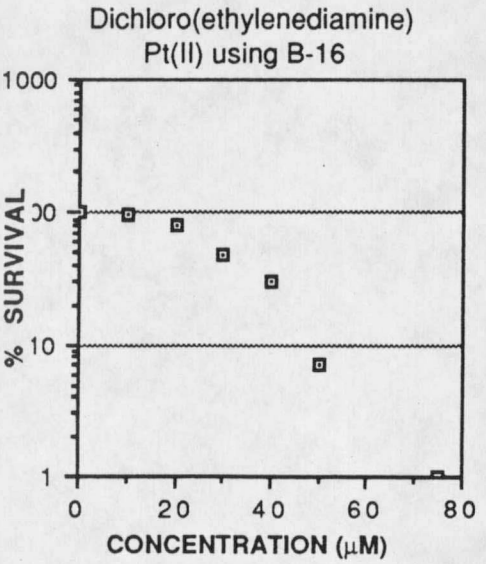
are shown in Figure 73 for both drugs using B-16 melanoma and Lewis Lung carcinoma cells. Cisplatin was included as a standard due to its effectiveness and wide use. It is therefore the primary standard in these tissue culture experiments. Dichloro(ethylenediamine)Platinum(II) was chosen for its similarity to possible reductive-elimination products from the synthesized Pt(IV)'s, Figure 74.

The goal of the tissue culture assay work was to test all platinum IV's synthesized, Figure 75. However, 6 compounds were not tested due to their low solubility in fetal bovine serum, Figure 76.

Figure 73. Survival Curves for Cisplatin and Dichloro (ethylenediamine) Pt(II).







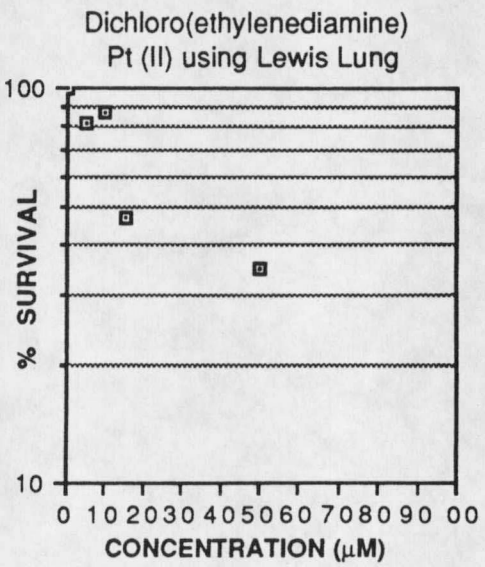
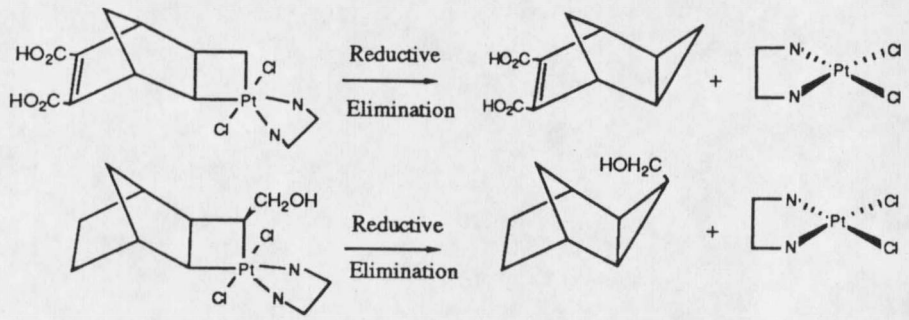
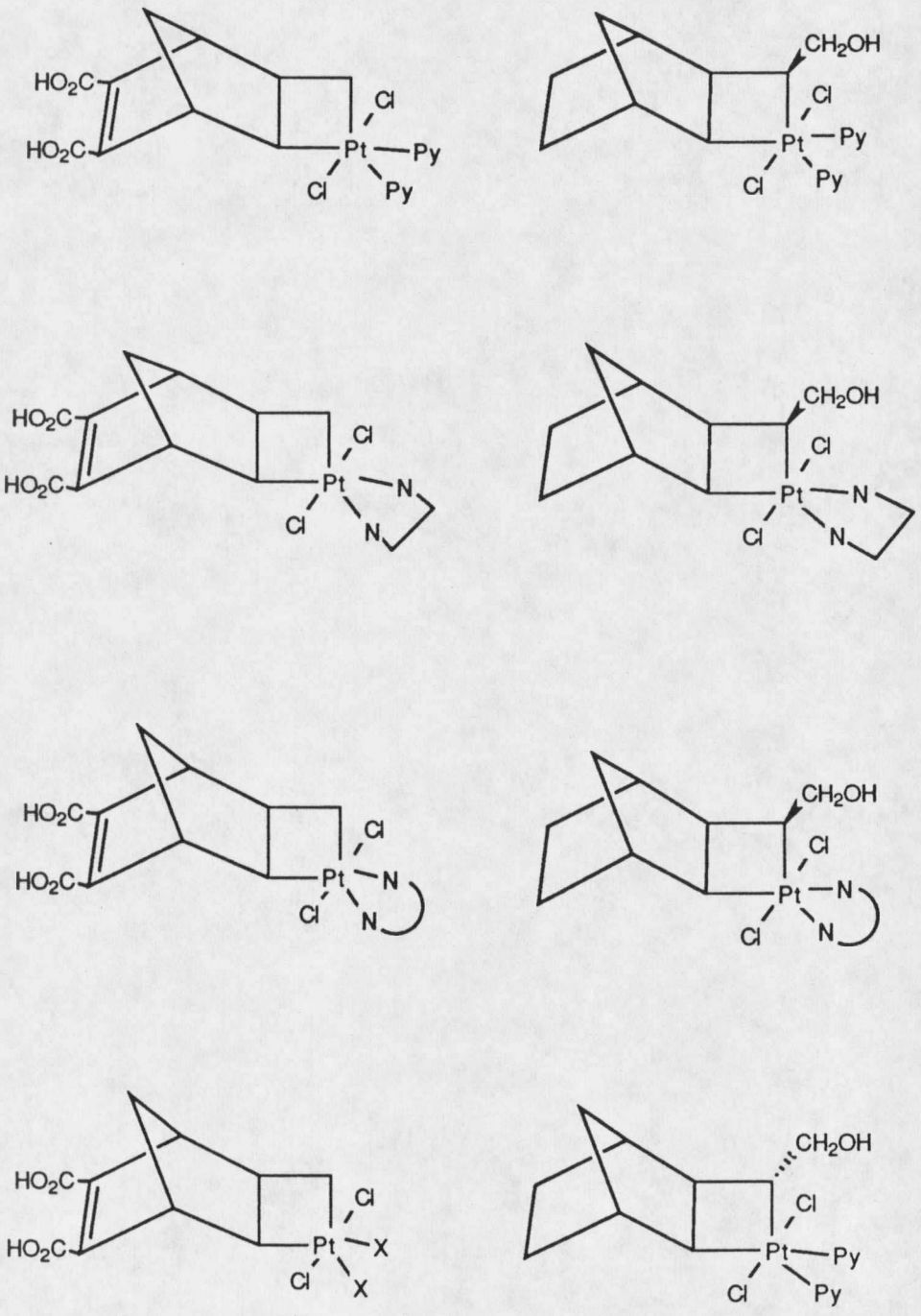


Figure 74. Possible Reductive-Elimination Products from Platinacyclobutanes.



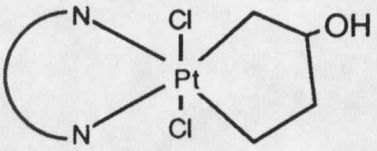
In most cases the platinum compounds were tested using both cell lines. The compounds were tested using these two cell lines to determine if the compounds were more sensitive to one cell line or the other. Lewis Lung cells formed fewer colonies and spread out easier causing coalescing of colonies. The standard errors for each experiment involving both cell lines are in Appendix B. The standard error was calculated for each group as follows: standard deviation/number of plates counted. The survival curves for each compound are shown in Figures 77-95 using both B-16 melanoma and Lewis Lung carcinoma cell lines.

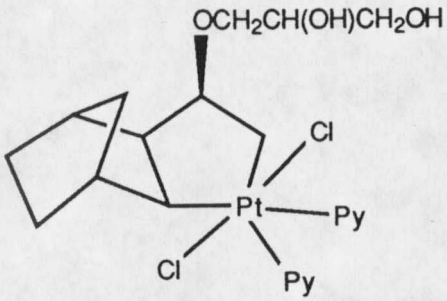
Figure 75. Compounds Synthesized for Tissue Culture Testing.

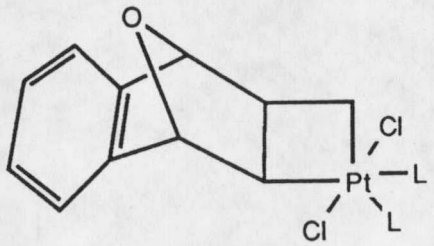


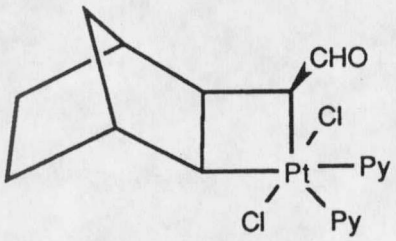
x = 4-Pyridylcarbinol

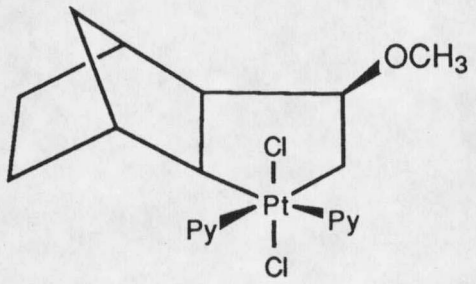
Figure 75 Continued.

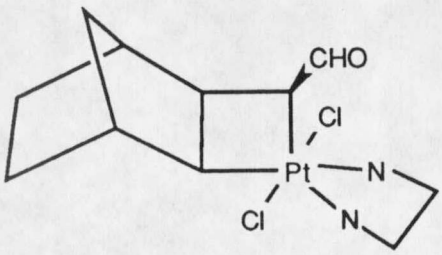












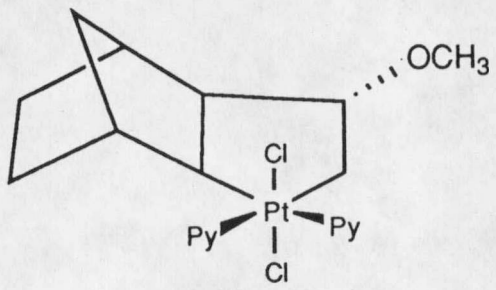
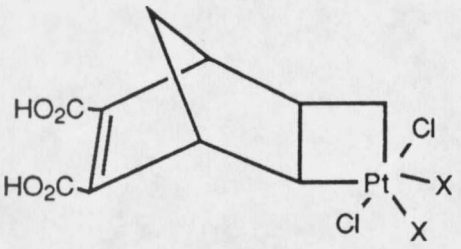
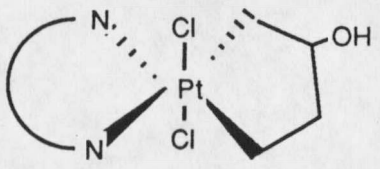
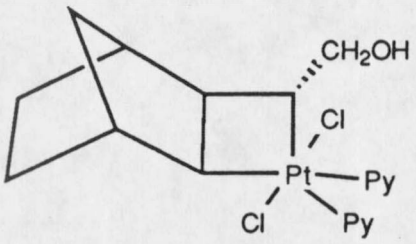


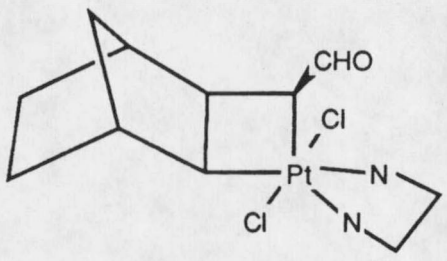
Figure 76. Compounds Not Tested in the Tissue Culture Assay Due to Insolubility in Fetal Bovine Serum.

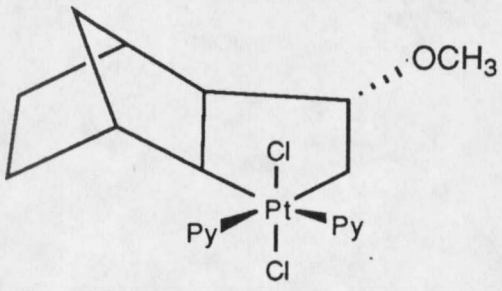


x = 4-Pyridylcarbinol









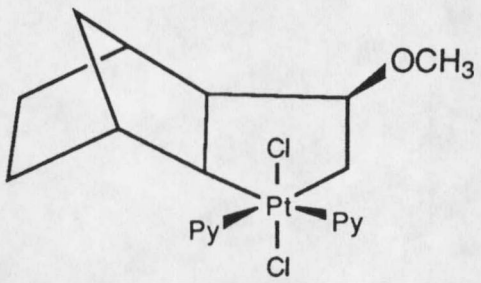
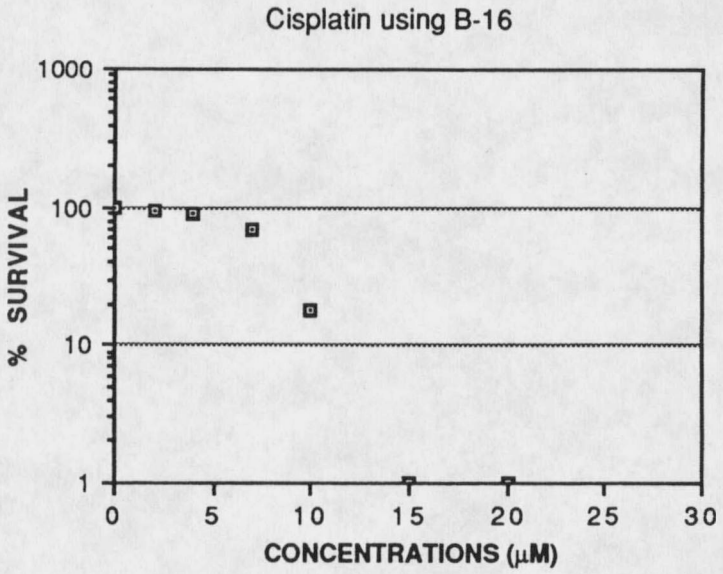


Figure 77. Survival Graph of Cisplatin with B-16.



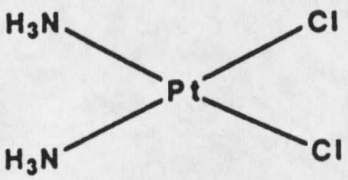
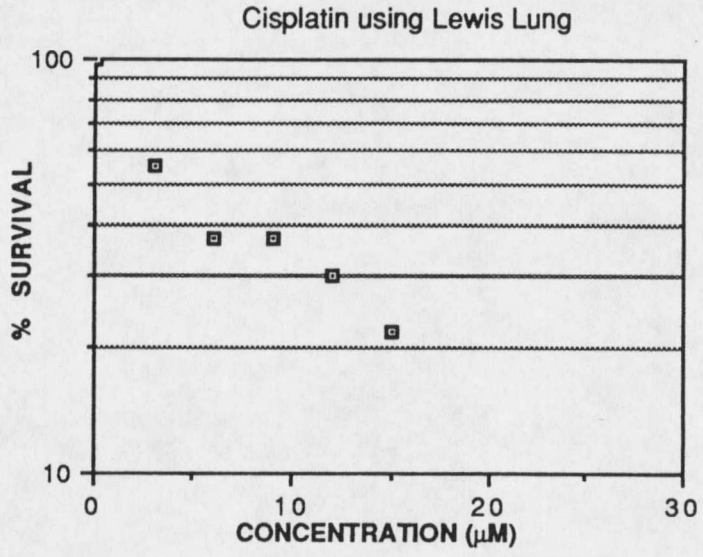


Figure 78. Survival Graph of Cisplatin with Lewis Lung.



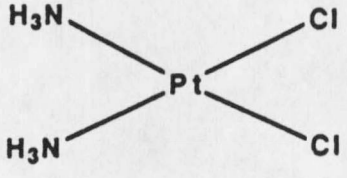
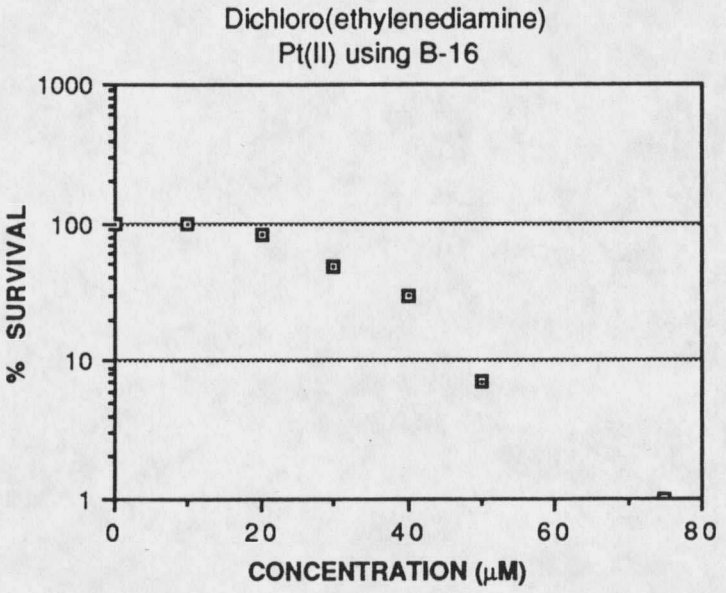


Figure 79. Survival Graph of Dichloro(ethylenediamine) Pt(II) with B-16.



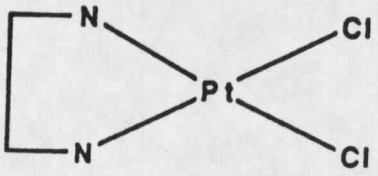
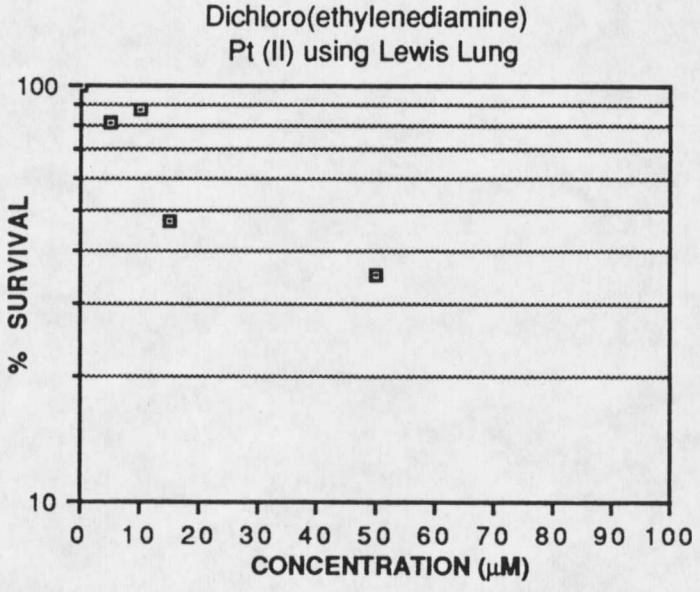


Figure 80. Survival Graph of Dichloro(ethylenediamine) Pt(II) with Lewis Lung.



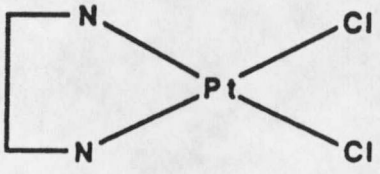
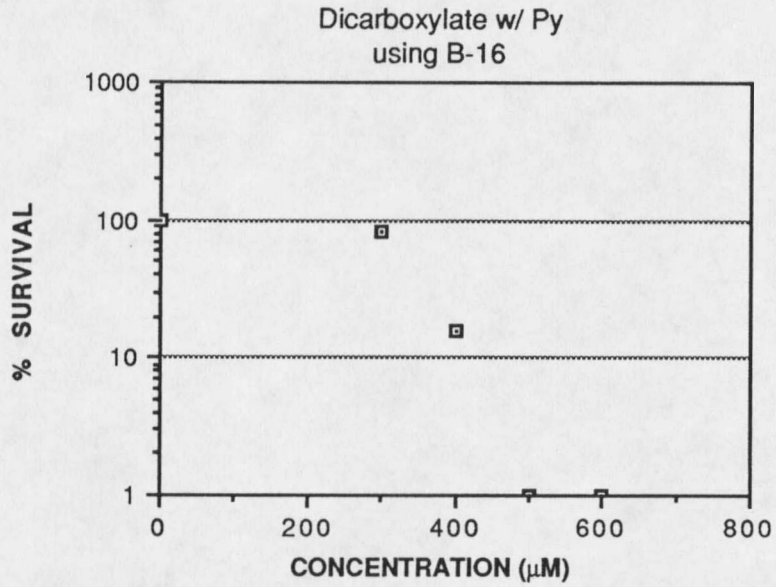


Figure 81. Survival Graph of Dicarboxylate with Pyridine using B-16.
First Experiment Done.



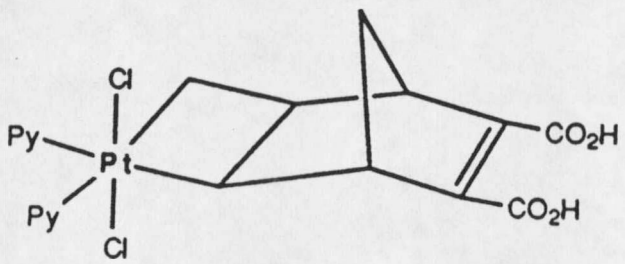
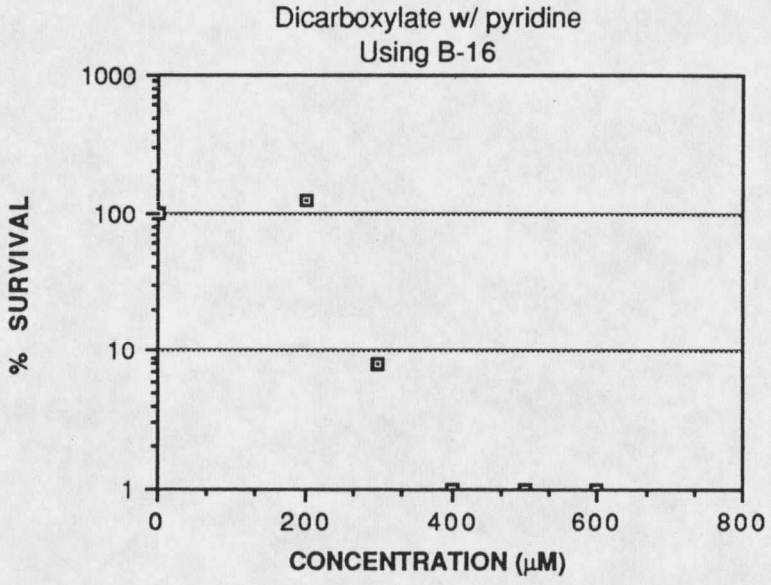


Figure 82. Survival Graph of Dicarboxylate with Pyridine using B-16.
Last Experiment Done.



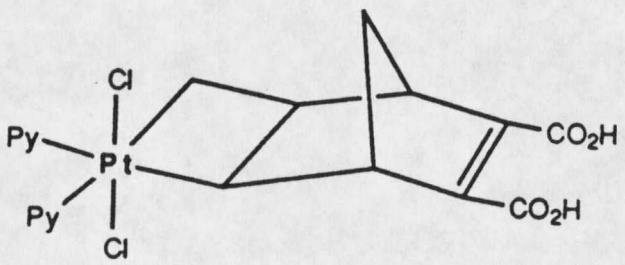
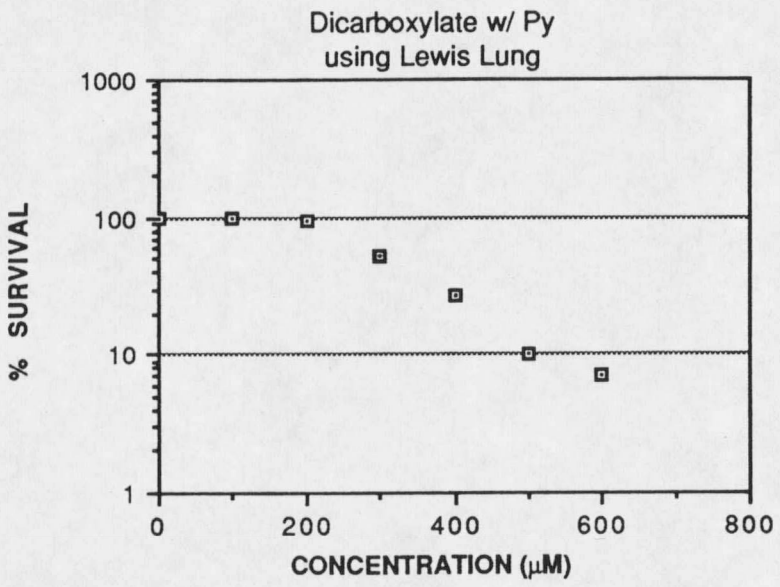


Figure 83. Survival Graph of Dicarboxylate with Pyridine using Lewis Lung.



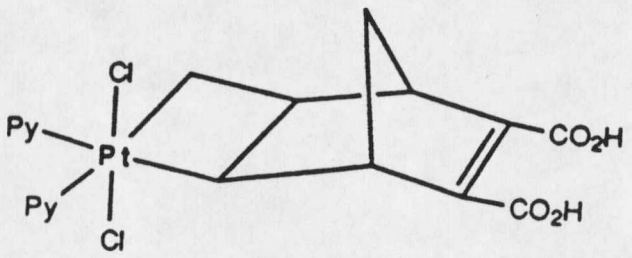
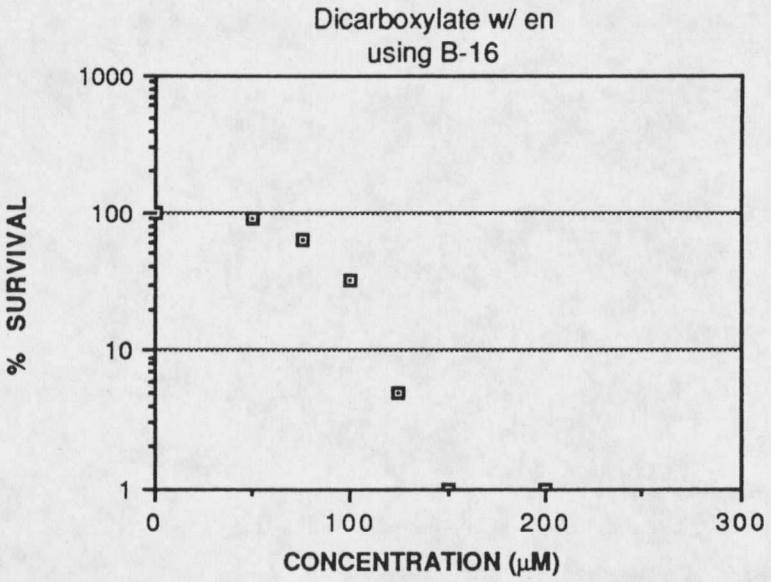


Figure 84. Survival Graph of Dicarboxylate with Ethylenediamine using B-16.



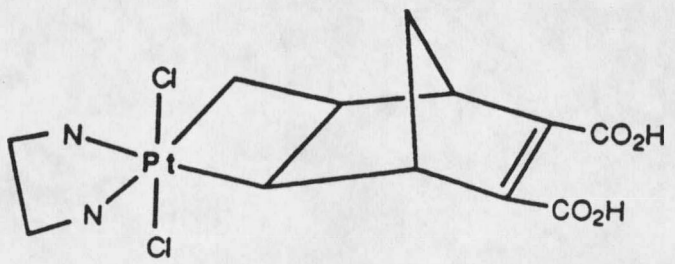
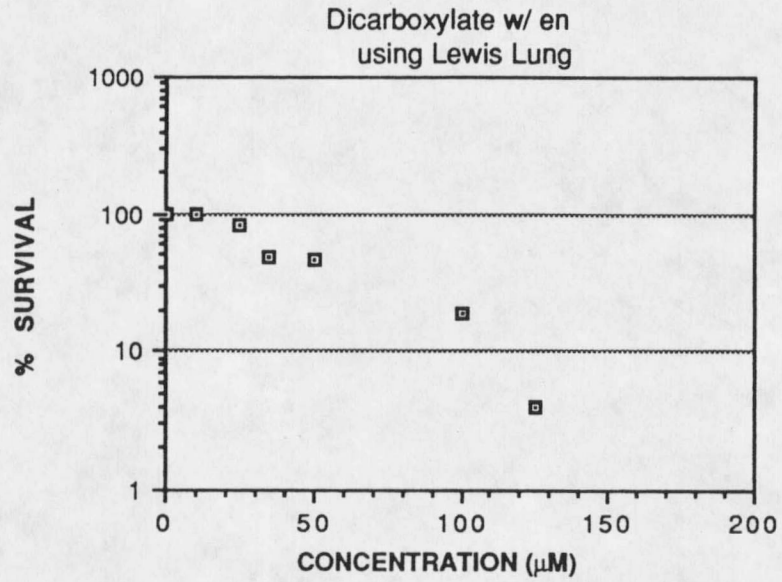


Figure 85. Survival Graph of Dicarboxylate with Ethylenediamine using Lewis Lung.



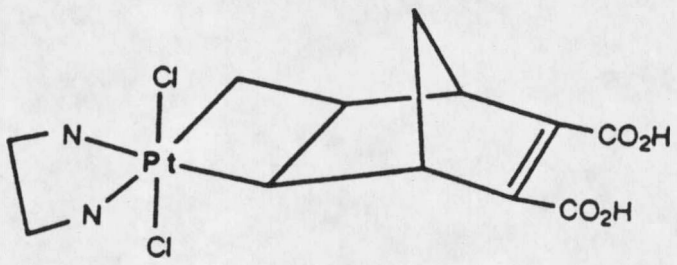
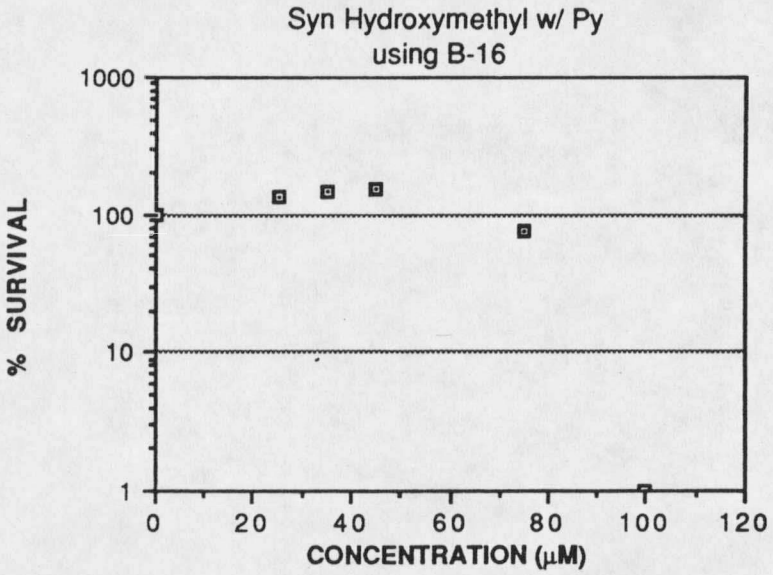


Figure 86. Survival Graph of Syn Hydroxymethyl with Pyridine using B-16.



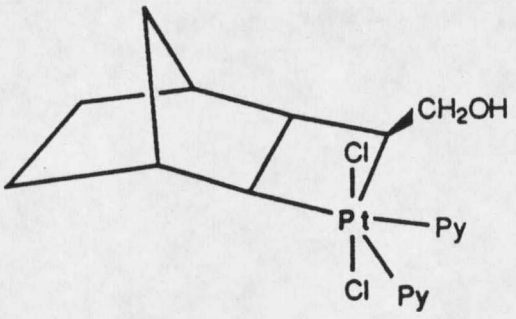
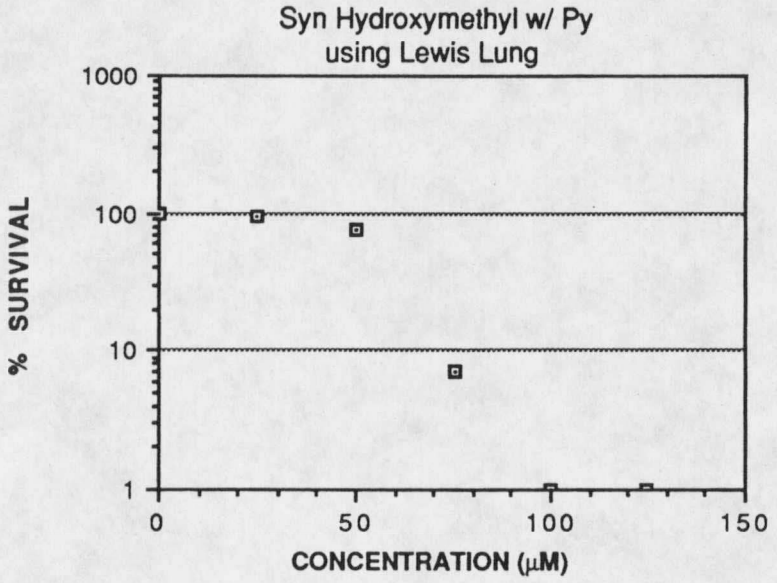


Figure 87. Survival Graph of Syn Hydroxymethyl with Pyridine using Lewis Lung.



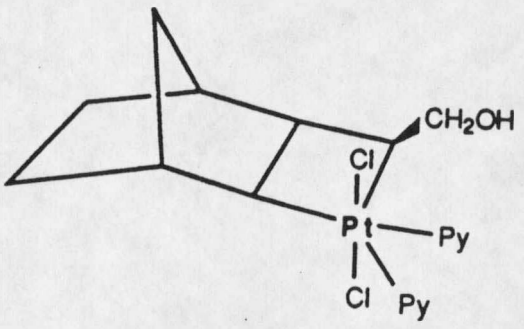
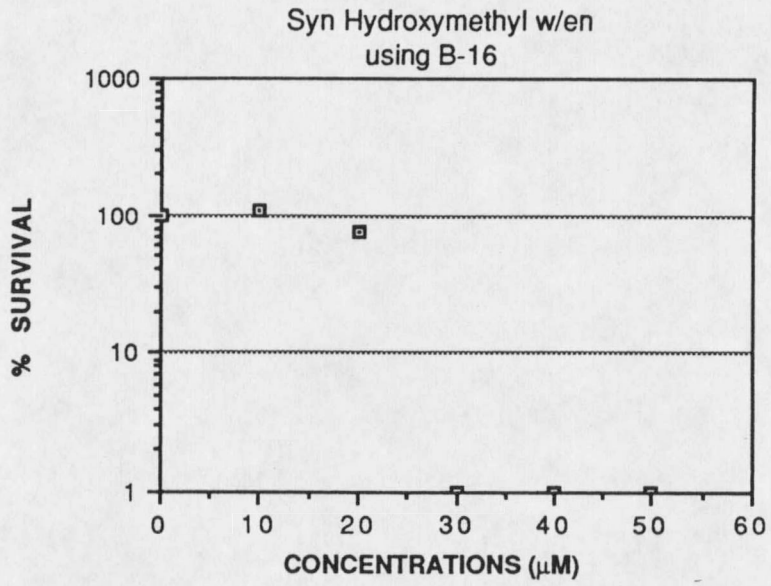


Figure 88. Survival Graph of Syn Hydroxymethyl with Ethylenediamine using B-16.



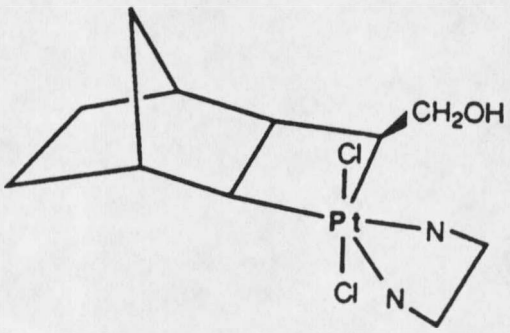
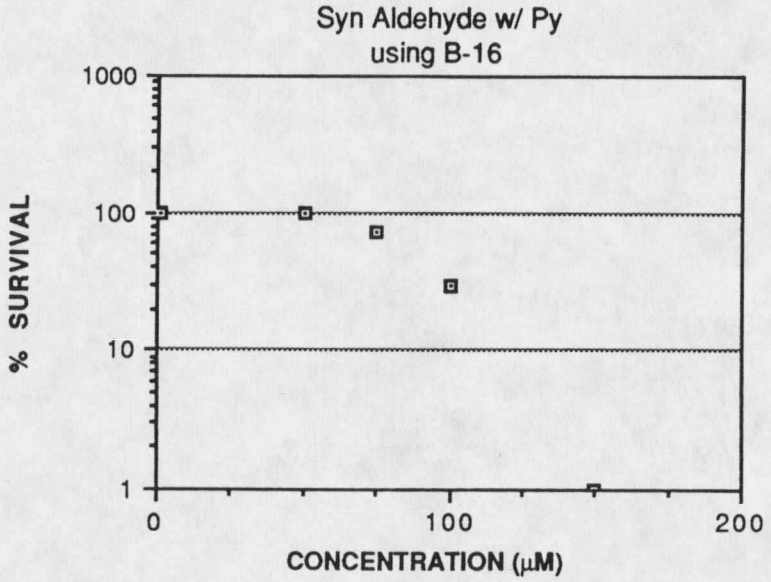


Figure 89. Survival Graph of Syn Aldehyde with Pyridine using B-16.



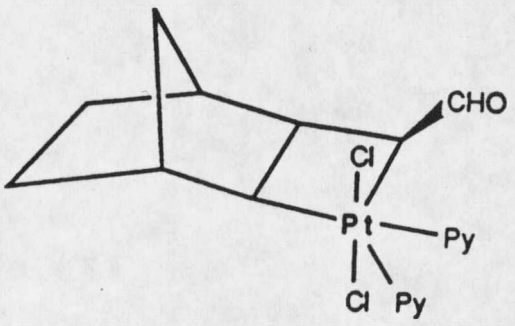
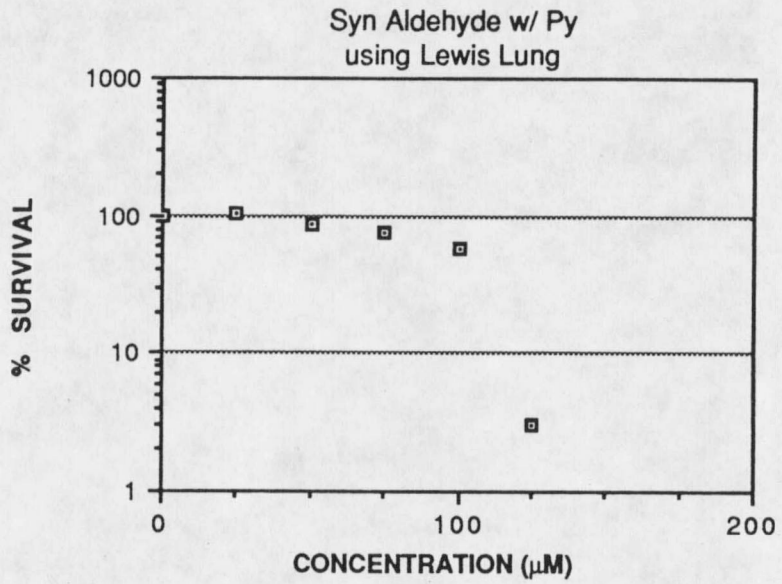


Figure 90. Survival Graph of Syn Aldehyde with Pyridine using Lewis Lung.



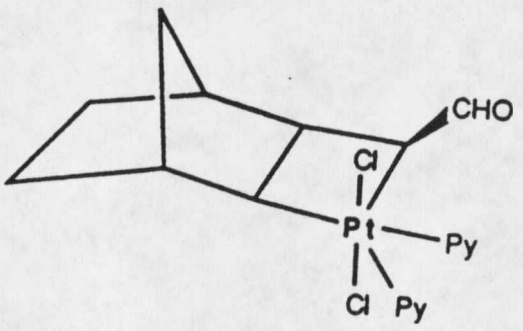
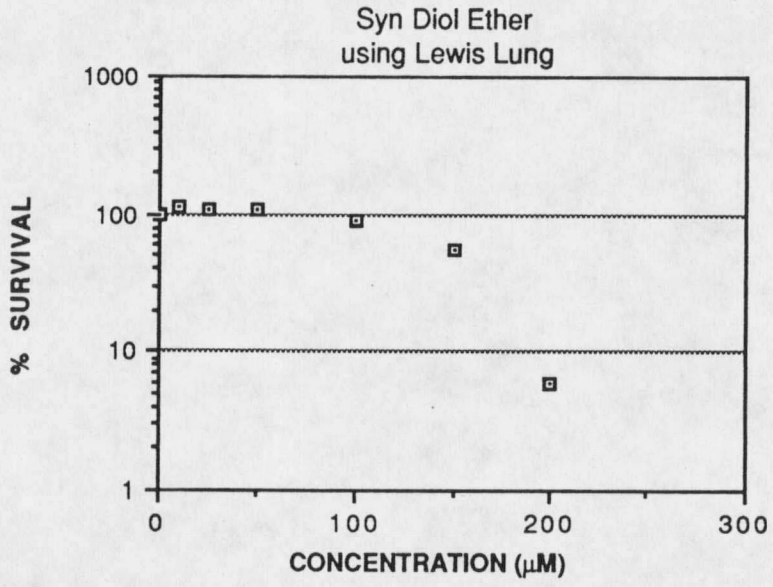


Figure 91. Survival Graph of Syn Diol Ether using Lewis Lung.



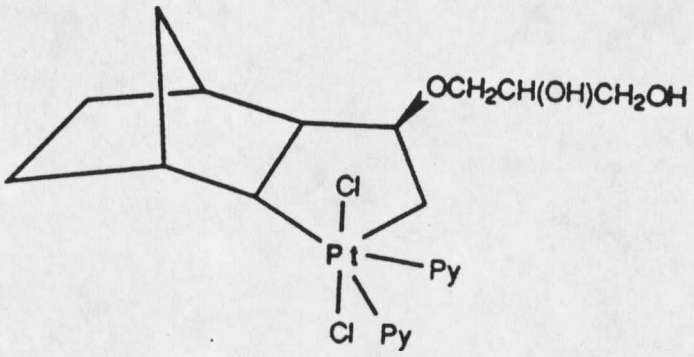
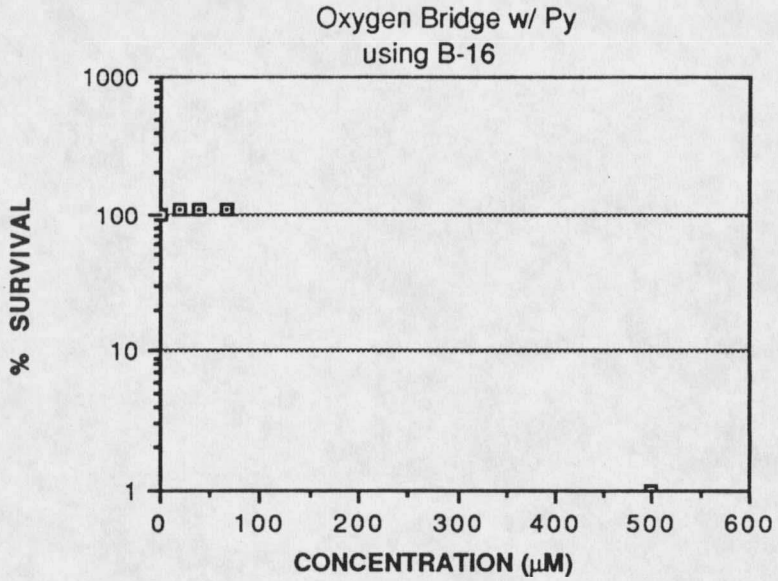


Figure 92. Survival Graph of Oxygen Bridge with Pyridine using B-16.



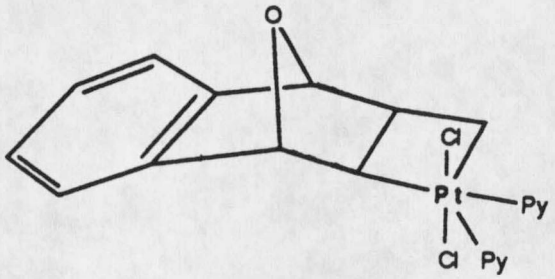
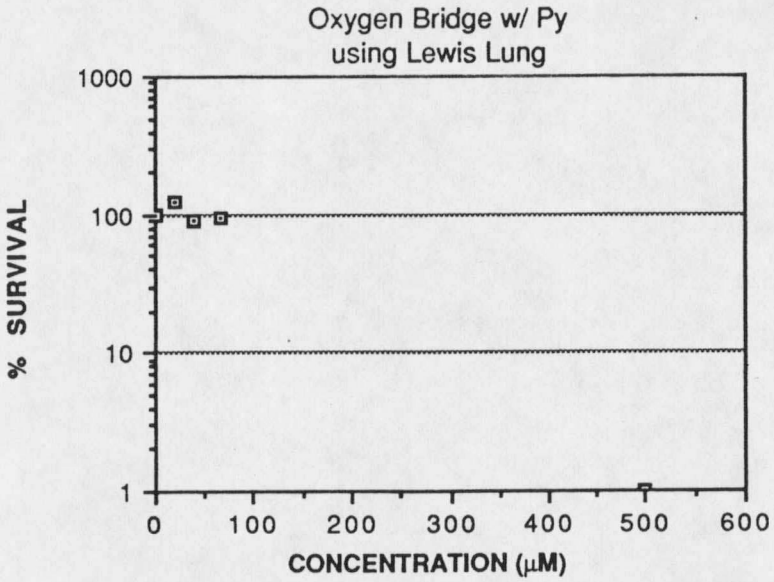


Figure 93. Survival Graph of Oxygen Bridge with Pyridine using Lewis Lung.



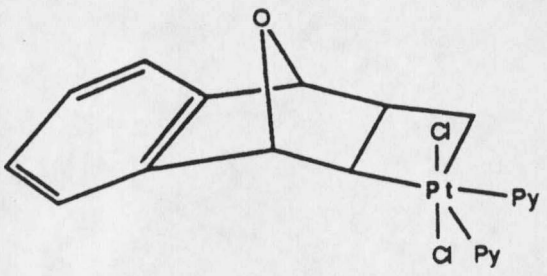
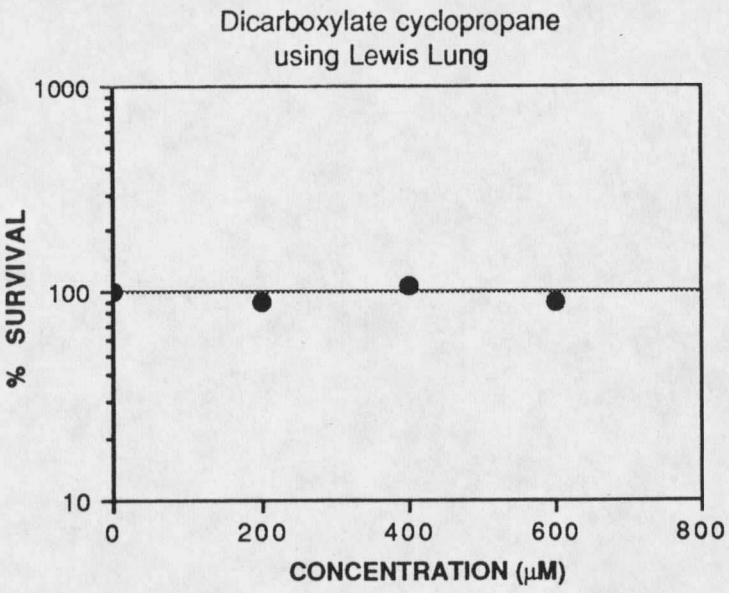


Figure 94. Survival Graph of Dicarboxylate cyclopropane using Lewis Lung.



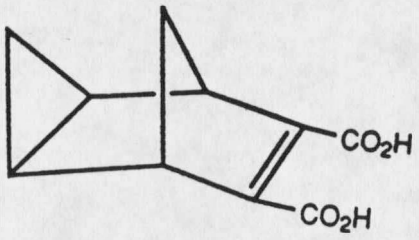
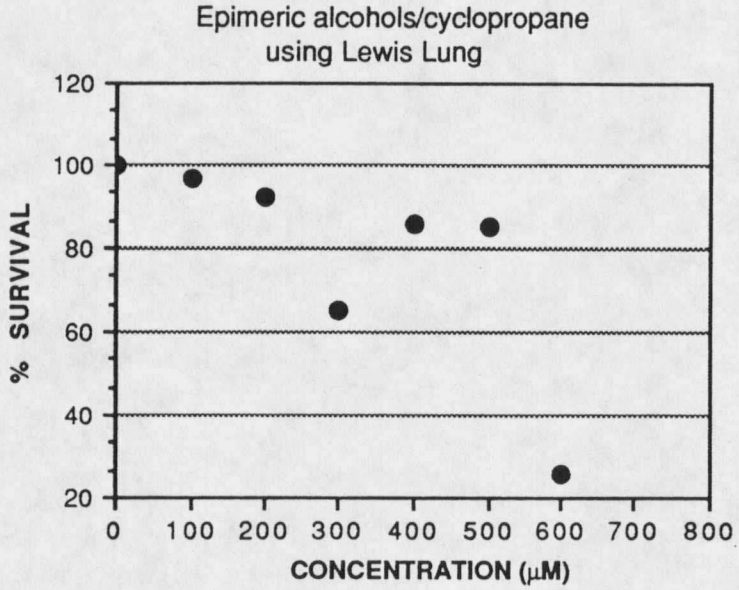


Figure 95. Survival Graph of Epimer Alcohols/ Cyclopropane using Lewis Lung.



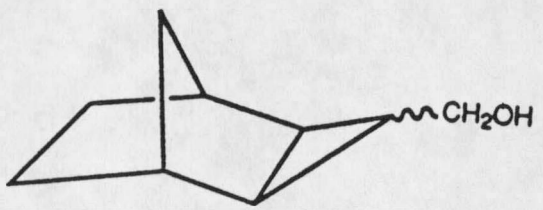


Table 16 summarizes the tissue culture results by listing each compound along with the LD_{50} and LD_{100} values, in μM.

Table 16. LD_{50} and LD_{100} of Compounds Tested.

Compounds	Cell Line	LD 50's	LD 100's
Cisplatin:			
	B-16	7	14
	Lewis Lung	6	20
Dichloro(ethylenediamine)Pt(II):			
	B-16	26	67
	Lewis Lung	22	70
Dicarboxylates:			
10			
First Experiment*			
	B-16	350	450
	Lewis Lung	340	750
Last Experiment*			
	B-16	250	370
11			
	B-16	90	145
	Lewis Lung	50	175
Syn Hydroxymethyl:			
18			
	B-16	80	95
	Lewis Lung	55	95
20			
	B-16	22	28
Syn Aldehyde:			
29			
	B-16	90	140
	Lewis Lung	100	160

Table 16 Continued.

Oxygen-Bridge: 34		B-16	190	400
		Lewis Lung	190	400
Syn Diol Ether: 37		B-16	ND	ND
		Lewis Lung	155	220
Dicarboxylate Cyclopropane: 7		Lewis Lung	?	>700
Epimer Alcohols Cyclopropane: 16		Lewis Lung	?	>600

ND = not determined
(*) = see page 118

Results from the tissue culture experiments were encouraging. Differences in LD_{100} values between the two cell lines were not significant enough to speculate on any difference in specificity of the platinum compounds towards either cell line. Significant differences did result from substitution of platinum ligands and structural changes on the norbornyl moiety or platinacyclobutane.

The dicarboxylate series produced the most varying results. Compound 11 had the lowest LD_{100} in the series with ethylenediamine, 175 μM, and compound 10 had the highest value of 750 μM with Lewis Lung cells. Lability

of the amine ligands is substantially greater for pyridine than for either bidentate ligand. This would effectively increase the chance of interaction between platinum and biomolecules in the serum. Bond strength between pyridine and platinum may be too weak to survive for an extend period of time in serum. If this is the case then it would seem possible for the platinum to bind nitrogen or sulfur containing molecules and be converted to a bound inactive species. This would then prevent any interaction of the platinum species with the target molecule. Using the analogy of cisplatin the target molecule would be DNA, but this would not necessarily need to be the case. Other possible target sites in cellular metabolism could cause cell death, as well.

The syn-hydroxymethyl series was the most promising compounds tested. The differences in platinum ligands were once again observed in this series. Compound 20 with ethylenediamine was more active than 18 with pyridines. Lability of the ligands must play a role in affecting anticancer activity. In comparing the activity between the dicarboxylate series and this series, two points are worth noting. Either the substituents on the platinacyclobutane ring increases the activity or the carboxylate groups reduce activity. Reactivity of the carboxylate groups might well be a disadvantage if they interact with non-target

biomolecules. These bound complexes can either be totally inactive and/or slower in diffusing into the cell.

The results from compounds 29, 34 and 37 can only be analyzed separately as to their degree of activity, since they were not part of a series .

The activity of 29 lends support to the idea that it might be substitution of the platinacyclobutane ring that increases or at least positively influences anticancer activity. Syn diol ether 37 was the only platinacyclopentane tested. A LD_{100} of 220 μM places its activity in the middle range of all compounds tested. Due to the low activity and the very low solubility, further testing was not carried out with variations of this molecule. This compound had too many different features to speculate on why it was not soluble and relatively nonactive.

Compound 34 was also not pursued further after the first two tissue culture tests. Judging from its survival curve the LD_{100}, at best, would be 200 μM. The oxygen bridge and aromatic moiety clearly did not substantially increase the anticancer activity.

The dicarboxylate compounds were the first ones tested with compound 20 being tested last. Each new compound tested in the tissue culture assay showed higher activity then the previously tested compounds. To discount the possibility that this trend was solely due to improved techniques, compound 10 was retested. Results from the second test

showed a LD_{100} value slightly above 370 μM compared to the earlier value of 450 μM. This difference is likely attributable to experimental variation. One major factor resulting in this variation, in this particular case, is the use of two different lots of FBS. The analysis of those two lots numbers are listed in Appendix C. Thus, the observed increasing activities over the course of the experiment was real and not due to improved techniques or changes in serum.

Solubility of Compounds Synthesized

An important consequence of the tissue culture testing was generation of solubility data for comparison between compounds. Fetal bovine serum (FBS) was the "aqueous" solvent used with all compounds. The carboxylates and compound 35 with the diol ether were compounds with a high oxygen content, thus suggesting increased solubility. This proved to be the case with the carboxylates, which showed excellent solubility. However, the diol ether compound was very insoluble and took 4 days to dissolve in FBS. Compounds 20 and 34 showed equivalent solubility characteristics, each taking 4 days to dissolve.

Syn hydroxymethyl and syn aldehyde compounds with pyridines were comparable in solubility taking 2-3 days to dissolve. In contrast, the syn hydroxymethyl with ethylenediamine took ca. 9-10 days. There was a distinct

difference in solubility depending on whether pyridine or a bidentate ligand was used. In general, as the lability of the amine ligands increased so did the solubility.

A surprising result was that both anti substituted compounds tested, 19 and 38, were extremely insoluble compared to the syn analogs.

Compounds synthesized with 2,2'-bipyridine were again extremely insoluble, with the exception of the dicarboxylate compounds. These other compounds with 2,2'-bipyridine showed no signs of being soluble, even over a period of 14 days with stirring. This would support the idea that a loss of ligand is needed to facilitate solubility. Bidentate ligands are less freely able to leave platinum than pyridine, thus decreasing the chance of solvent molecular interactions.

In the platinacyclobutane system, solubility is still a major limiting factor. However, this work has shown that the solubility can be influenced at the platinum center with the appropriate ligand or with hydrophilic functionality placed away from the platinacyclobutane.

Ultraviolet Irradiation Experiments

Generating Dichloro(ethylene-diamine) Pt(II) from 11

One of the products from platinacyclobutane decomposition is the original starting cyclopropane, Figure 6. If compound 11 undergoes decomposition it is possible that the platinum species generated will be dichloro(ethylene-

diamine)Pt(II), see Figure 74. Several tissue culture experiments were conducted to test this hypothesis. The first experiment determined the length of time B-16 cells could tolerate 254 nm light. Figure 96 shows cell survival vs exposure to 254 nm light.

Reductive-elimination of 11 was believed to take a longer exposure time then could be tolerated by the cells. To compensate for this, the platinum compound in serum medium was irradiated at 254 nm void of cells. Following irradiation, the platinum-serum medium was added to the cells. Results are shown in Figure 97 with the graph illustrating that irradiation is not enhancing activity of 11. Thus dichloro(ethylenediamine)Pt(II) is possibly not being generated in this case.

Figure 96. B-16 Cells Survival Curve Using 254 nm Light.

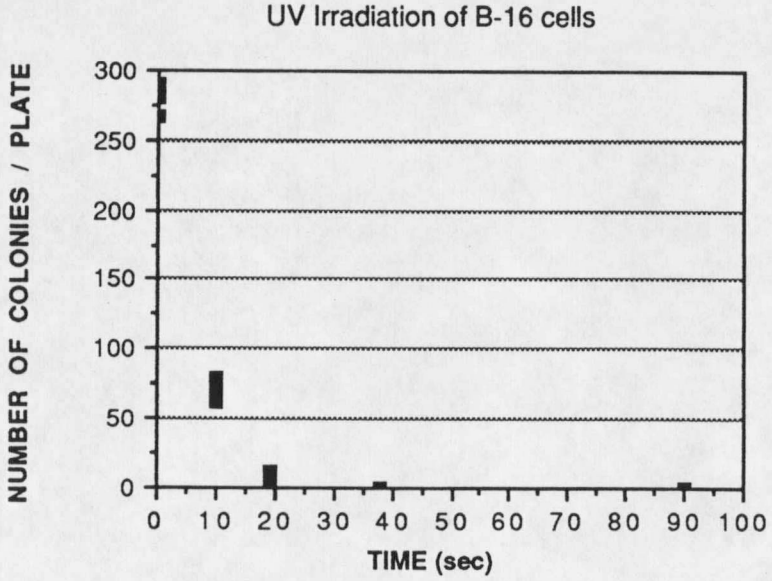
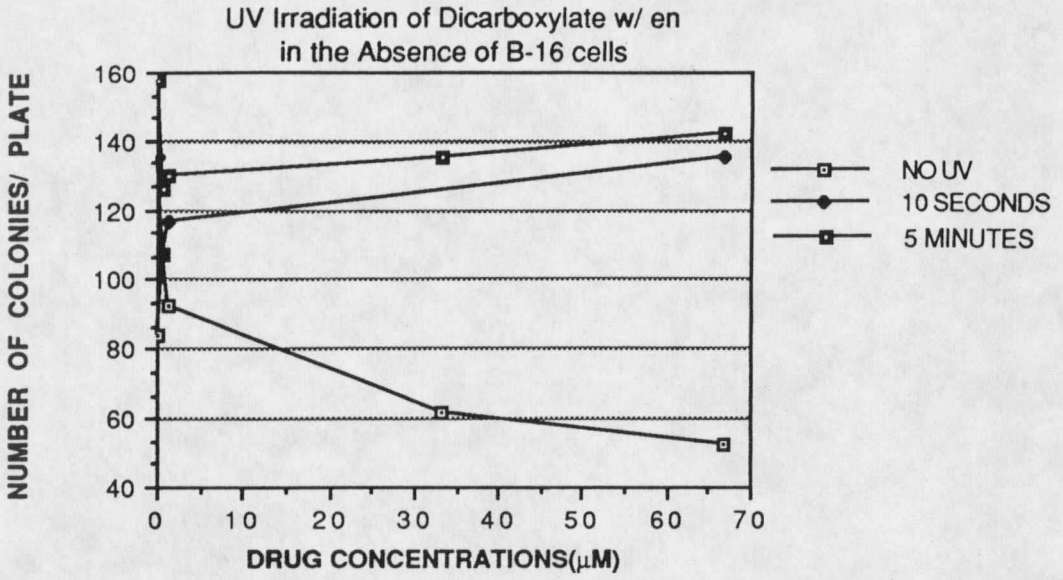
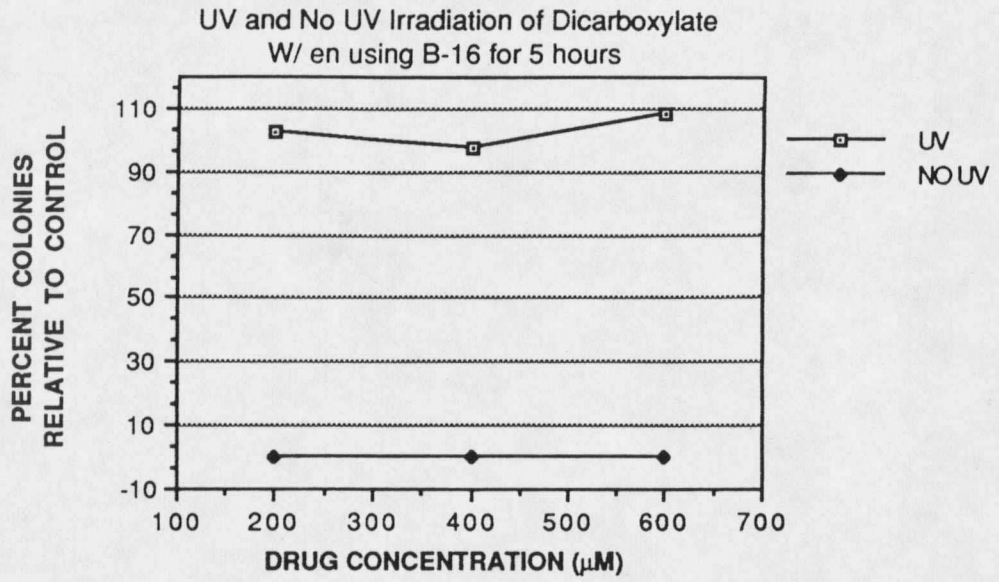


Figure 97. Serum Containing Medium with 11 Irradiated in the Absence of B-16 cells.



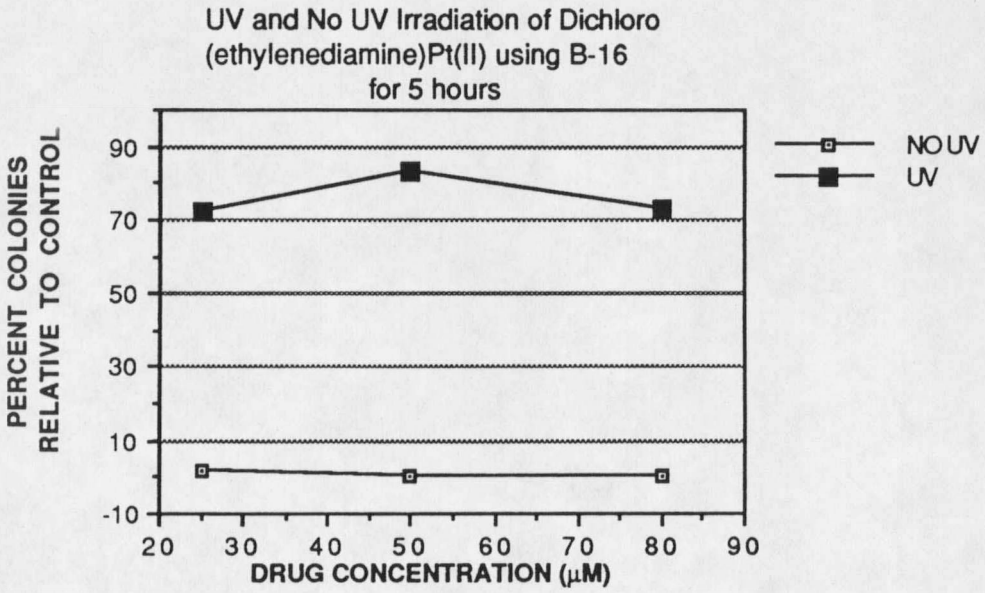
A second more powerful UV-VIS Hanovia 450 watts lamp (Engelhard Hanovia Inc. Newark, N.J.), was chosen to emit a broader spectrum of light. Platinum compounds were dissolved in fetal bovine serum and irradiated in a 125 ml sodium glass bottle. Three separate experiments were done using this UV lamp. The platinum compounds were exposed to the UV source for 5 hours in all three experiments. The first experiment was conducted using compound 11. The objective was to cause reductive-elimination and generate dichloro(ethylene-diamine)Pt(II). If this were to occur, an increase in anticancer activity would be observed. The experimental results are illustrated in Figure 98 and an increased in activity was not observed. The same results were observed at 5 hours or 5 minutes of UV exposure.

Figure 98. Five Hours of UV With 11.



Dichloro(ethylenediamine)Platinum (II) was the second compound tested. Figure 99 shows that 5 hrs of irradiation also decreases the activity of this platinum drug.

Figure 99. Five Hours of UV With Dichloro(ethylene-diamine)Pt(II).

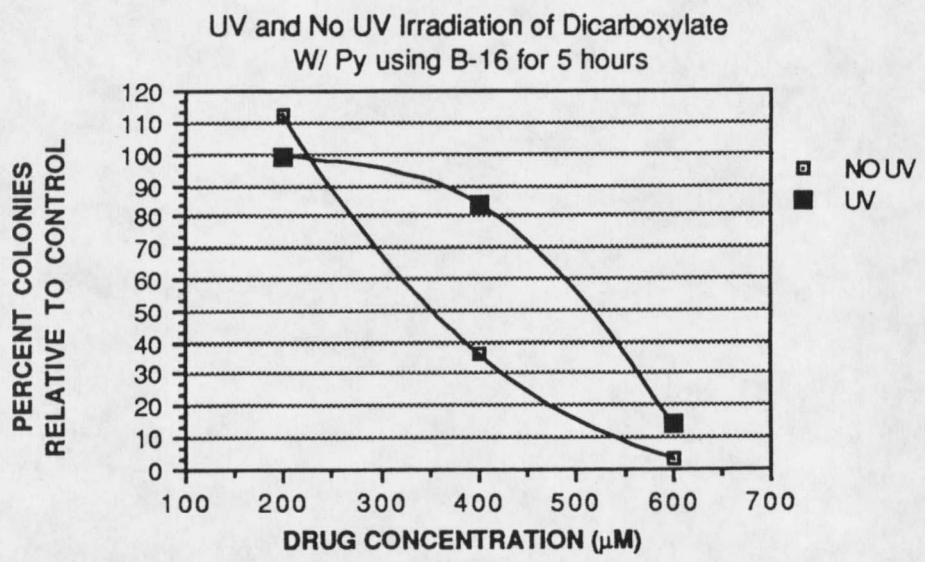


The ultraviolet irradiation experiments involving 11 and dichloro(ethylenediamine)Pt(II) seemed to indicate that the active platinum species are being destroyed. The third experiment was done to test the effect of UV irradiation on a molecule without similarity to dichloro(ethylenediamine)Pt(II). The molecule chosen was 10. Results in Figure 100 show that UV irradiation decreased the activity, but not to the extent found in the two previous experiments. In this experiment the irradiation seemed to delay the anticancer activity of the platinum compound without destroying the active species.

In this series of experiments, if UV irradiation of 11 did cause reductive-elimination to generate dichloro-(ethylenediamine)Pt(II) an increase in activity would not have been observed. Irradiation not only changed the activity of 11, but also dichloro(ethylenediamine)Pt(II). Thus the ethylenediamine compound could have been generated but then subsequently destroyed.

A 500 MHz spectrometer set up for platinum-195 NMR was used in an attempt to determine the platinum species being generated by UV irradiation. UV exposure was reduced from 5 hrs to three 20 minute periods. Following each 20 minute period, the sample was removed and placed in the NMR spectrometer. Each compound was dissolved in D_2O and irradiated in a 10 mm NMR tube.

Figure 100. Five Hours of UV Irradiation in the Presences of 10.



Compound 11 was irradiated while dichloro(ethylenediamine)Pt(II) was used as the platinum reference. The same UV lamp was used as in the 5 hour experiments previously mentioned. A platinum resonance at 2259 ppm was observed for the standard, referenced to $Na_2Pt(CN)_4$ 1.0 M. Prior to irradiation compound 11 produced a platinum singlet at 2982 ppm. This signal was observed to decrease in intensity, after each 20 minute period until it became undistinguishable in the base line noise. There were several new resonances observed, but upon repeating the experiment none of the new resonances consistently showed up at the same chemical shift. The new resonances did not coincide with the chemical shift of the nonirradiated standard sample. Thus, no identification of any new platinum species were determined. It was concluded that compound 11

does decompose to at least one and possibly several species over a period of 20 minutes. In addition the new product, or perhaps its more than one, did not show any chemotherapeutic activity.

Murine Toxicity Study

Compound 11 was chosen to be evaluated in a murine toxicity study, based on three criteria: 1) high aqueous solubility, 2) a low LD_{100} value in tissue culture assay, and 3) the possibility of generating dichloro(ethylenediamine)Pt(II) from *in vivo* degradation.

The LD_{10}, LD_{50}, and LD_{100} for 11 values would determine if this compound was more or less toxic than cisplatin. A compound of higher toxicity would be removed from further testing while one showing less toxicity would be advanced to an *in vivo* antitumor study. Platinum toxicity of various tissues were also determined by histological analysis. Sites of tissue toxicity and severity were important considering compound 11's high solubility compared to cisplatin. The higher solubility should be reflected in minimal damage in the liver and kidneys. These two organs are major sites of platinum toxicity, as evident in toxicity associated with cisplatin.

The protocol was set up as follows. One hundred and nineteen C57Bl/J6 mice were divided into 9 groups. All groups had 14 animals, seven females and seven males, except group VIII which had only seven males. There were seven test groups and two controls. Concentrations of compounds tested are given in Table 17.

Table 17. Concentrations Used For Groups I-IX.

Control Group I	Females: 2.0 mls of PBS Males : 2.0 mls of PBS		
Compound 11			
Group II	Females:	4.78×10^{-8} mol	1.3 mg/Kg
	Males :	6.30×10^{-8} mol	1.3 mg/Kg
Group III	Females:	4.78×10^{-7} mol	13 mg/Kg
	Males :	6.30×10^{-7} mol	13 mg/Kg
Group IV	Females:	8.24×10^{-7} mol	23 mg/Kg
	Males :	1.08×10^{-6} mol	23 mg/Kg
Group V	Females:	1.51×10^{-6} mol	41 mg/Kg
	Males :	1.98×10^{-6} mol	41 mg/Kg
Group VI	Females:	4.78×10^{-6} mol	130 mg/Kg
	Males :	6.30×10^{-6} mol	130 mg/Kg
Group VII	Females:	4.78×10^{-5} mol	1310 mg/Kg
	Males :	6.30×10^{-5} mol	1310 mg/Kg
Compound Dichloro(ethylenediamine)Pt(II)			
Control Group VIII	Females: 2 mls "Vehicle" Males : 2 mls "Vehicle"		
Group IX	Females:	8.24×10^{-7} mol	14 mg/Kg
	Males :	1.08×10^{-6} mol	14 mg/Kg

Groups I through VII compounds were solubilized in phosphate buffered saline (PBS) prior to intraperitoneal injection. Dichloro(ethylenediamine)Pt(II) was not soluble in PBS, so a commercial drug "vehicle" had to be used. A second control group was established to test the effects of this "vehicle", Group VIII. Animals went into rapid shock when injected with the vehicle, but within 12 hours had

stabilized and were as mobile as the control group injected with PBS. All dosages were referenced to the LD_{50} value of cisplatin, 13 mg/Kg. Values for cisplatin are given below for comparison. All groups had 2.0 mls of solution injected i.p.

Females: 8.23×10^{-7} mol (13 mg/Kg)
Males : 1.08×10^{-6} mol (13 mg/Kg)

Animals from Group VII never recovered from the initial shock following injection and died within twelve hours. Group VI animals recovered from the shock, but later died within 24 hours. No mortality, due to toxicity, was observed in any other group. Thus the LD_{10} value is greater than 41 mg/Kg for compound 11. A subsequent toxicity study was not undertaken to determined the actually LD_{10}, LD_{50}, and LD_{100} values.

Nephrotoxicity is the major toxicity seen with cisplatin in mice, rats and humans.[102] [103] [104] [105] [106] [107] The creatinine serum levels were measured in an effort to determine renal function over the course of the experiment. Cardiac puncture was preformed on three mice from each group. A total of 0.3 mls of blood was withdrawn from each mouse. The mice were anesthetized using Metaphane™. Whole blood was collected on the first day and every third day of experiment thereafter. Red blood cells were removed by centrifugation and creatinine values determined on the resulting blood serum from each group. Creatinine levels were measured for each group, except group VII

because all were found already exhibiting rigor mortis. The results indicated that creatinine values were normal at each measuring, with the exception of Groups VI and VIII. Only one serum sample was obtained from group VI, resulting in only one creatinine measurement at 24 hours. Group VIII showed elevated creatinine values 24 hours after injection, but these returned to normal by day six. The published average creatinine value is 0.44 (0.43-0.46) mg/dl[108] for normal mice, while values obtained from this experiment are listed in Table 18. The discrepancy of 0.1 was attributed to groups IV, VIII and IX serum samples being tested at a later time than the other groups.

Table 18. Serum Creatinine Values for Toxicity Study*

		Bleeding Intervals in Days		
		1^{st}	4^{th}	7^{th}
Control	I	0.4	0.4	0.4
Group	II	0.4	0.4	0.4
Group	III	0.4	0.4	0.4
Group	IV	0.5	0.5	0.5
Group	V	1.3	0.7	0.6
Group	VI	1.9	---	---
Group	VII	---	---	---
Control	VIII	0.5	0.5	0.5
Group	IX	0.5	0.6	0.5

* = Values are mg/dl

To learn what effects the platinum compound had on various tissues, 50% of each group was sacrificed using carbon dioxide on day five of the experiment. The remaining animals were terminated on the 10^{th} day, except for group

V. Group V was continued beyond the 10^{th} day for evaluation of possible long term effects. This group was terminated on the 91^{st} day. All animals were terminated using either diethyl ether or CO_2. A postmortem evaluation was conducted and various tissues were removed for histological examination. The following tissues were collected and placed in neutral buffered formalin (NBF): brain, lung, liver, spleen, intestines, and kidney.

During the postmortem examination it was discovered that the livers and kidneys of the animals in groups V and VI were pink instead of the normal dark red color. Blood pooling under the nails and in the nose was observed in both groups. No other gross abnormalities were detected.

A histological examination of tissues from each animal was conducted and the results tabulated. Two animals were included in the histological evaluation that served as normal controls. No morphological changes could be seen in the brain, spleen, intestinal, or lung tissue. Liver and kidneys showed significant changes indicating these two tissues were the primary sites of toxic damage. Liver sections were evaluated both on severity and nature of the microscopic damage. Three specific structural alternations were looked for and each judged on a scale of 0 to 3+, 0 representing normal tissue, while 3+ was very severely damaged. The three alternations were: 1) hepatocellular necrosis, 2) fatty deposits, and 3) central lobular congestion, Table 19.

Table 19. Histological Summary of Liver Toxicity on Day 5 Testing Compound 11.

Groups	I	II	III	IV	V	VI	VII	VIII	IX
hepatic necrosis	0	0	0	0	0	2	0	0	0
fatty deposits	0	0	0	1	0	3	2	1-	1
central congestion	0	0	0	0	0	3	1	0	0

Necrosis indicated cell death due to the test compound, while fatty deposits indicated cell stress. Central congestion referred to blood pooling in the hepatic lobules, due to necrosis and cell stress.

Kidney sections were evaluated using the same scale, but five different alterations were looked for. The five categories were: 1) cytoplasmic necrosis, 2) sloughed cytoplasm in renal tubule lumens, 3) tubular dilation in cortical areas, 4) interstitial inflammation, and 5) tubular regeneration, Table 20. Sloughed cytoplasm indicated cell necrosis in the cortical region. Tubular dilation represented the lack of cells lining the tubules, thus increasing the lumen diameter. Interstitial inflammation indicated stress and irritation, while tubular regeneration indicated the degree of cellular regrowth in the tubules.

Table 20. Histological Summary of Kidney Toxicity Day 5 Testing Compound 11.

Groups	I	II	III	IV	V	VI	VII	VIII	IX
tubule necrosis	1	0	0	1	2	2	1-	1	1+
sloughing cytoplasm	1+	1	1+	1+	3	3	1+	1+	1+
tubular dilation	1+	1	1+	2-	3	3	1-	1+	1+
interstial inflammation	0	0	0	1-	2	2	1-	0	0
tubular regeneration	0	0	0	0	1	0	0	0	0

The values in Tables 19 and 20 reflect the histological condition observed five days after injection of the test compound. On the 10th day, remaining animals in groups IV, VIII, and IX showed no microscopic signs of cellular damage. The damage identified on day 5 was completely repaired by the 10th day. As mentioned above the remaining animals in group V were not terminated until the 91st day. The histological results from this group indicated that renal and liver structural morphology was normal. Previously observed renal damage seen on day 5 had been repaired. The only observable long term consequence was small surface scars on the kidneys. Body weights and sizes of these seven animals were normal. Figures 101-103 show black and white photos of liver and kidney sections for groups I, V and VI, (magnification 37X).

There was no evidence from the toxicity study to indicate a gender difference to compound 11.

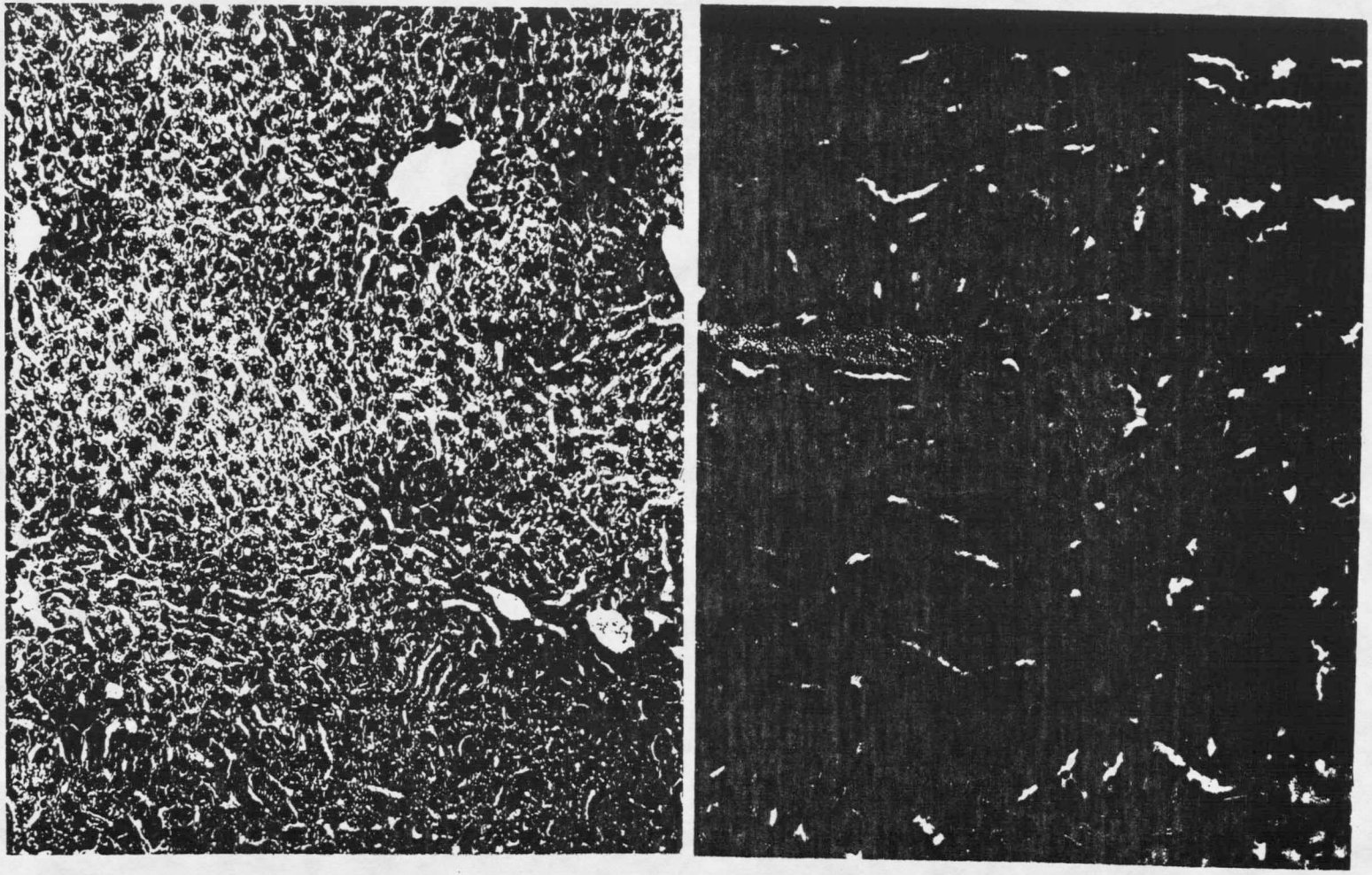


Figure 101. Black and White Photos of Liver and Kidney Tissue from Group I.

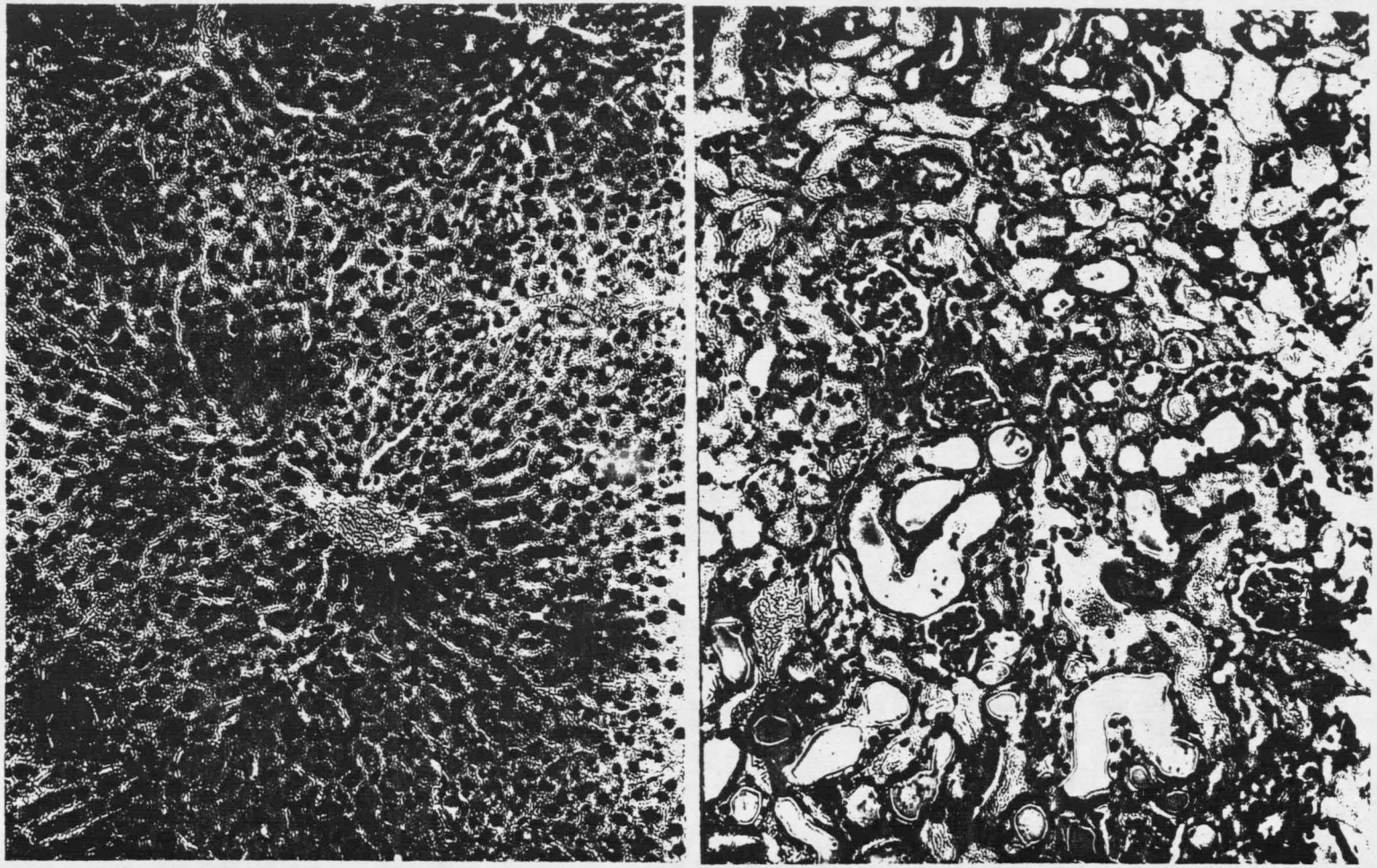


Figure 102. Black and White Photos of Liver and Kidney Tissue from Group V.

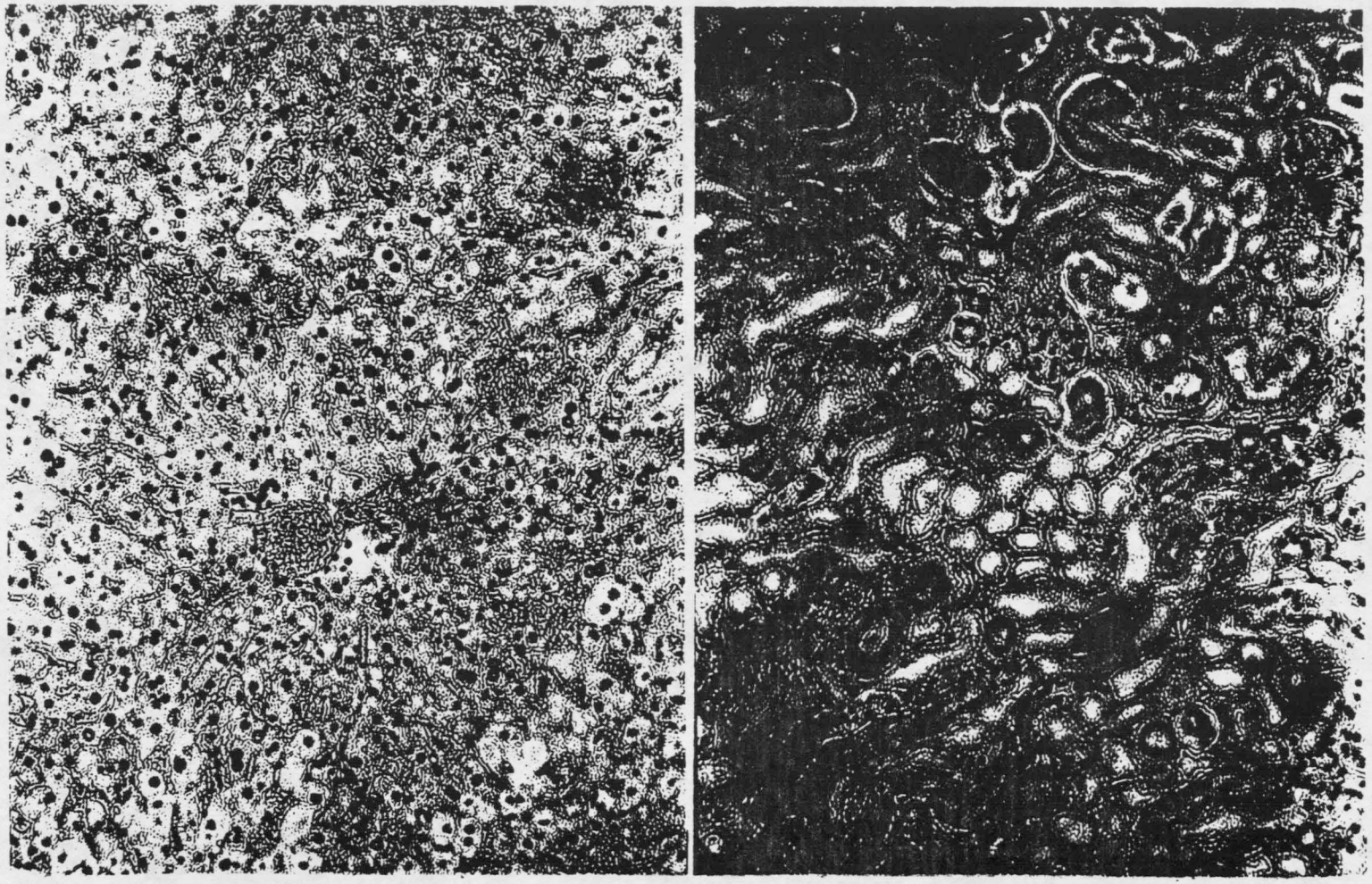


Figure 103. Black and White Photos of Liver and Kidney Tissue from Group VI.

Compound 11 Evaluation in vivo

Once the solubility, relative toxicity, and anticancer activity *in vivo* for compound 11 had been determined, the compound was advanced to an *in vivo* study. This study would establish whether 11 was active *in vivo*. Fifty C57Bl/6 mice were injected sc (subcutaneous) with 9 x 10^5 B-16 melanoma cells in 0.2 mls of serum-free medium. Injections were done on day one of the experiment and by day 12 the tumors were easily palpated. Cisplatin was tested in Groups I and II at the same concentration while compound 11 was tested at two different concentrations in Groups III and IV. Each group consisted of 10 mice: five males and five females. Cisplatin from Bristol-Myers Co. and Aldrich Chemical Co. were used in groups I and II, respectively. Bristol-Myers Co. provided a 50 mg dosage vial that had an expiration date of 6-87. When the vial was reconstituted with 50 mls of dH_2O (distilled H_2O), each ml contained 1 mg cisplatin, 10 mg mannitol, and 9 mg NaCl and represents Platinol™. The cisplatinum from Aldrich Chemicals did not contain additives. Compound 11 was tested at two different concentrations below 41 mg/Kg. In doing so, it was hoped that one of the concentrations would be close to compound 11's therapeutic dose. The therapeutic dose for cisplatin is 7 mg/Kg, which is below the LD_{10} value. Assuming 41 mg/Kg is close to the

LD_{10} of compound 11, then the therapeutic dose should be around 15 mg/Kg. The concentrations of 10 and 18 mg/Kg were used for groups III and IV, respectively. Table 21 outlines the numbers of moles and mg/Kg used for each group.

Table 21. Dosages Used for in vivo Study

Control	0.5 mls	dH_2O	
Group I*	0.5 mls	5.83×10^{-7} mol/mouse	7 mg/Kg
Group II*	0.5 mls	5.83×10^{-7} mol/mouse	7 mg/Kg
Group III**	0.5 mls	4.81×10^{-7} mol/mouse	10 mg/Kg
Group IV**	0.5 mls	8.65×10^{-7} mol/mouse	18 mg/Kg

* = Cisplatin Groups
** = Compound 11 Groups

Both platinum compounds were solubilized in sterilized dH_2O and injected i.p. Total volume injected for each animal was 0.5 mls. The control group was injected with 0.5 mls of sterilized dH_2O. Tumors were measured with a 25mm caliper or metric ruler at the time the compounds were injected and every 2 days thereafter. Six tumor volume measurements were taken over the twelve day period. Each tumor was measured in three dimensions in order to calculate volume. Tumor volumes were calculated and recorded for each group according to sex.

There was an excessive number of deaths following injection of test compounds in Group I, see percent survival curves (Figures 104-106), with only 30% of the animals

surviving when the experiment was terminated. In an effort to try and explain the high mortality, a tissue culture experiment was done using the same drug mixture. LD_{50} and LD_{100} concentrations had been previously determined for cisplatin in constructing the percent survival, Figure 77. Mortality of 100% was observed at every test concentration. This result demonstrated that the drug mixture from Bristol-Myers Co. was more toxic than could be attributed to just the platinum drug. The vial containing the drug was used past the expiration date as mentioned above. This group was removed from further analysis because of the high mortality. On the 25^{th} day of the experiment all remaining animals were euthanized. A postmortem evaluation was conducted on each animal. The tumor, both kidneys, two sections of liver, two sections of lung, and one section of intestines were removed and placed in NBF. Tumor volume data was plotted to show percent tumor growth from when the test compounds were injection until the animals were terminated for each group. Percent tumor growth and survival data was categorized to show females, males, and both sexes and is presented in Figures 104-106. These results indicate that compound 11 is active against B-16 melanoma *in vivo*. This is well illustrated by the dose response seen between the two dosage groups. The males showed a more pronounced response than the females to compound 11. Groups III and IV females rarely showed reduction in tumor volumes over

the control group. In fact, compound 11 stimulated tumor growth. This could be explained if the dosage given to females was too high. In fact, females were injected with a higher concentration than males since dosages were not mixed up to account for female/male weight differences. The overall effect of this higher dosage would be to depress the immune system, resulting in tumor growth. Platinum compounds have been shown to be immunosuppressive.[109] dosage group showed a larger percent tumor growth than the low group supports this point.

An immune and/or hormonal response in the females is most likely the cause of the absolute differences in control tumor growth between females and males.

In contrast to the in vivo results, the concentrations of compound 11 in tissue culture were low enough at times to stimulate cell growth. The increase in growth rate would be the result of cells responding to injury in an attempt to repair the damage. At higher concentrations though, the cells would be incapable of repairing all alterations and succumb to the compound. This stimulation of cancer cell growth at low concentration was seen in some of the tissue culture tests with data points over 100% survival.

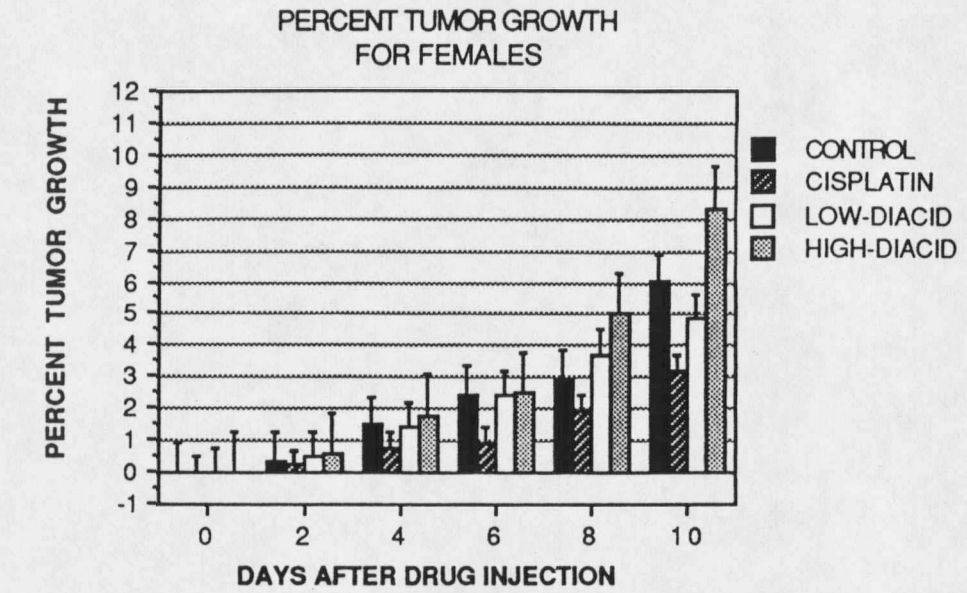
Tumor growth was significantly inhibited in the cisplatin group. The percent tumor inhibition in groups III and IV were not close to the cisplatin group, but perhaps this is due only to the fact that dosages of 10 and

18 mg/Kg were too low. A higher dose could theoretically show inhibition of tumor growth compared to cisplatin without surpassing the compound's LD_{10} value.

Histological analysis were conducted on three specimens taken randomly from each group. The tissues were processed as previously described in the toxicity section and evaluated using the same criteria and scale. Results from the histological evaluations are listed in Table 22.

These histological data substantiates the fact that the dosage vial containing cisplatin, obtained from Bristol-Myers Co., was in fact highly toxic. The other results were expected considering dosages were below the LD_{10} values for both compounds.

Figure 104. Percent Growth and Survival Curves for Females.



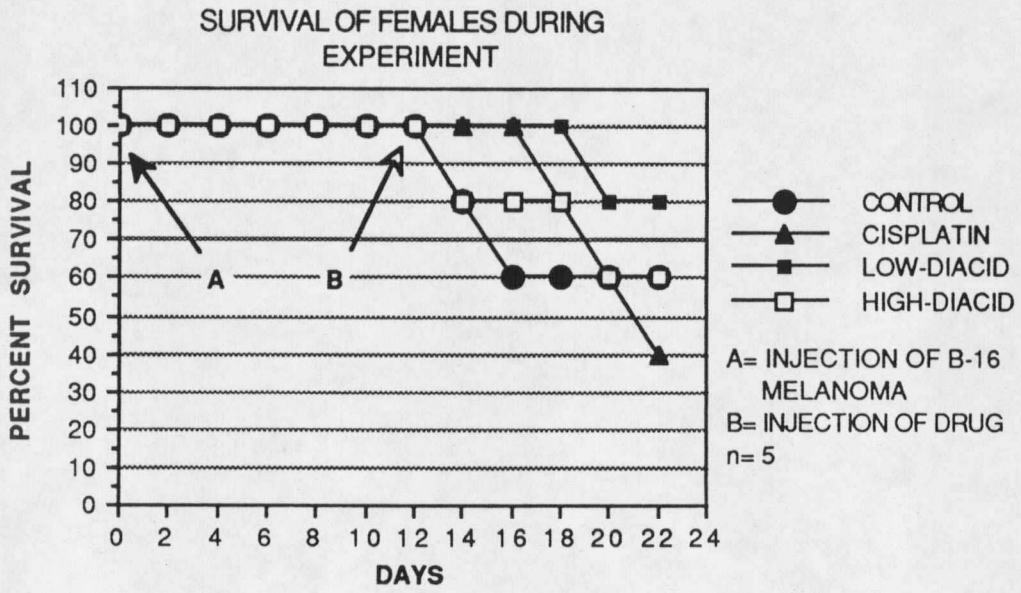
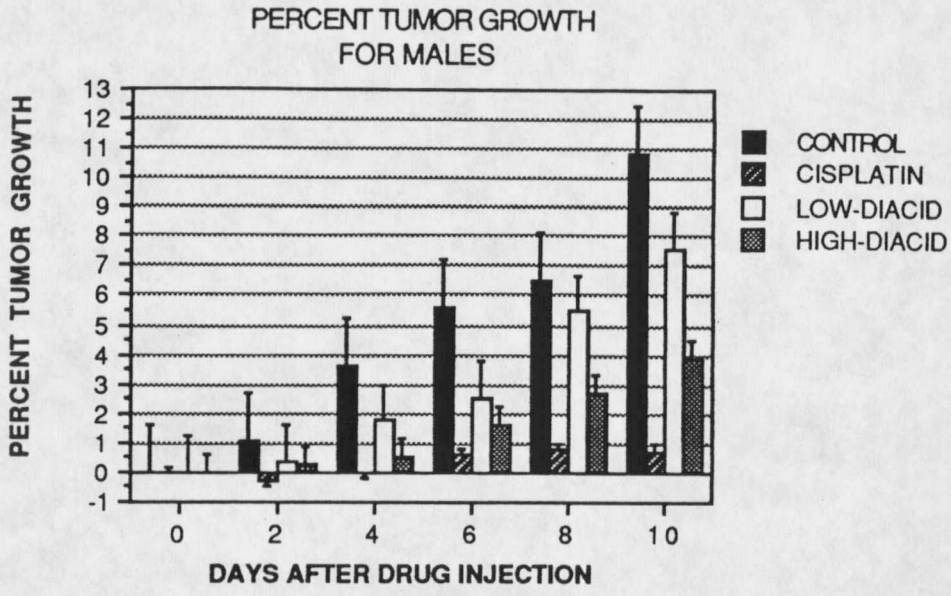


Figure 105. Percent Growth and Survival Curves for Males.



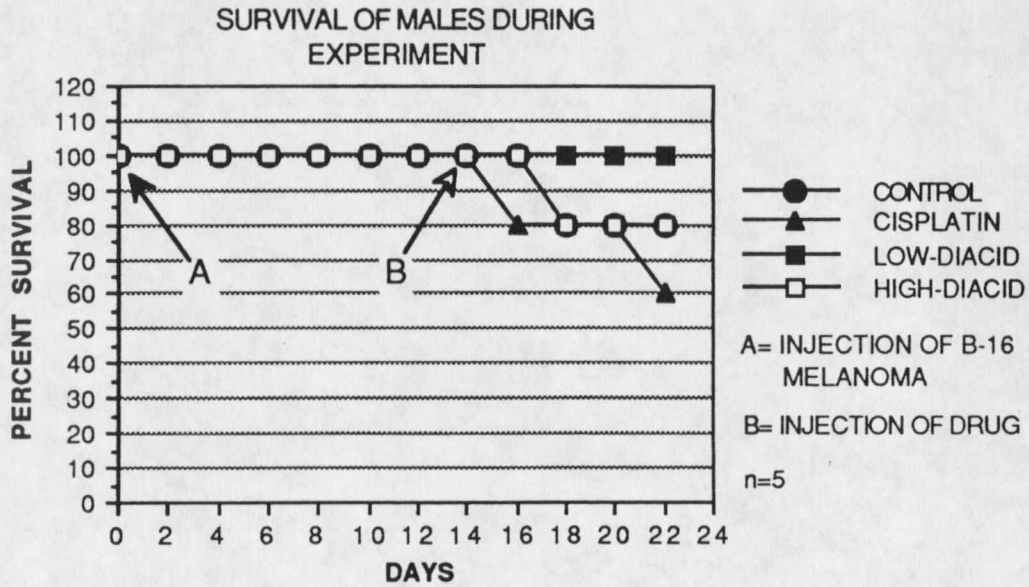
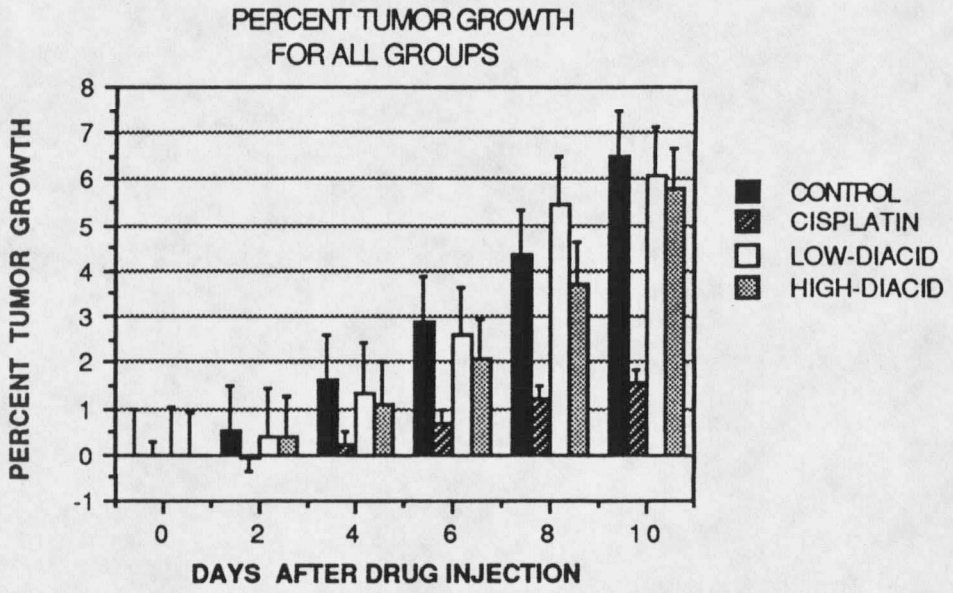


Figure 106. Percent Growth and Survival Curves for All Mice.



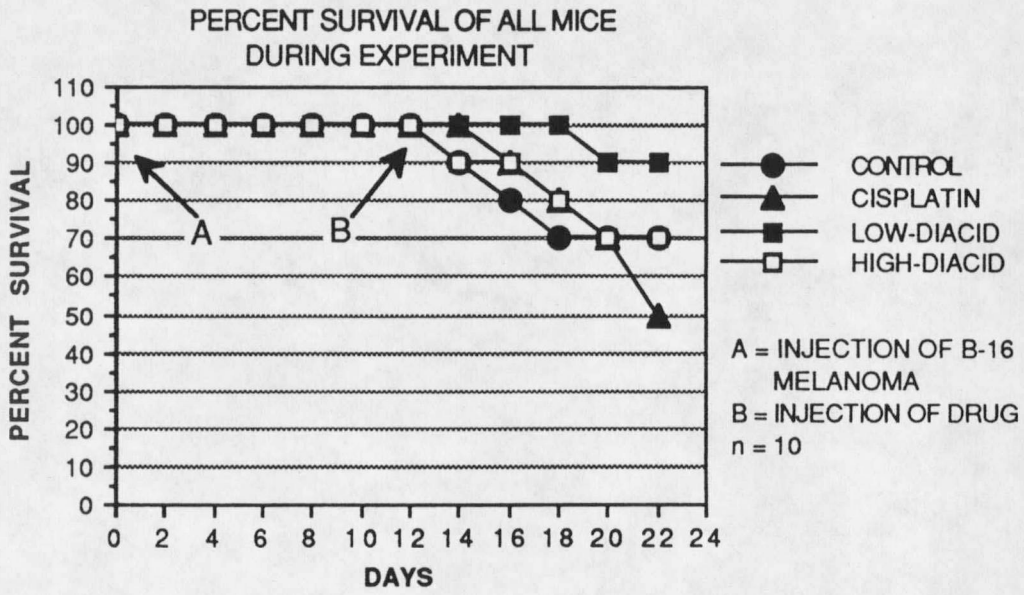


Table 22. Liver Histological Evaluations of Compound 11 in Tumor Bearing Mice.

Liver Histological Evaluation

GROUPS	CONTROL	I	II	III	IV
hepatic necrosis	0	1	0	0	0
fatty deposits	0	1+	0	0	0
central congestion	0	0	0	0	0

Table 23. Kidney Histological Evaluations of Compound 11 in Tumor Bearing Mice.

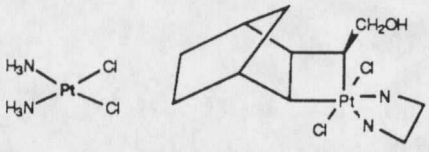
Kidney Histological Evaluations

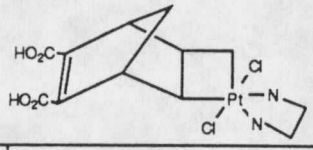
GROUPS	CONTROL	I	II	III	IV
tubule necrosis	0	1	0	0	0
slough cytoplasm	0	1+	0	0	0
tubular dilation	0	0	0	0	0
interstitial inflammation	0	0	0	0	0
tubular regeneration	0	0	0	0	0

Summary

The experimental data obtained on compounds 11 and 20 are listed in Table 24 along with values for cisplatin.

Table 24. Cisplatin, 11 and 20 Evaluation Data Compared.





Solubility	1.0 mg/ ml	1.0 mg/ ml	10 mg/ ml
Toxictiy	13 mg/ Kg	NA	> 41 mg/ Kg
Nephrotoxicity	severe	NA	moderate
Tissue Culture LD_{50}	15 μM	28 μM	145 μM

These results indicate that 11 could be more effective as a chemotherapeutic agent than cisplatin. Even though 11 did not show comparable activity in tissue culture, the higher solubility and lower toxicity could give 11 the advantage as a chemotherapeutic agent. Compound 11's full therapeutic effect has yet to be determined. Compound 20 shows significant activity, but needs to be tested *in vivo* before any valid comparisons can be made.

EXPERIMENTAL METHODS FOR CHEMICAL SYNTHESIS

Synthesis of 4

Excess dicyclopentadiene (1) was placed in a round bottom of a fractional distillation apparatus to produce cyclopentadiene, 2. Fifty mls of dimethylacetylene-dicarboxylate, 3, was placed in a receiving round bottom, with a condenser, in an ice/H_2O bath. Both 1 and 3 were purchased from Aldrich Chemical Co. Rotoevaporation was used to remove any excess cyclopentadiene once the reaction mixture volume had doubled. 1,2-diethylcarboxylate-1,4-norbornadiene (4), was obtained in 99% yield.

Synthesis of 5

Cyclopropanation of 4 was carried out by generating diazomethane. Seventy six grams of Diazald^{TM} and 300 mls of ether were added dropwise to 125 mls of 2-(2-ethoxy-ethoxy)ethanol, 35 mls ether, 21.2 g KOH, and 30 mls H_2O. The receiving flask contained 10 grams of 4, 3-4 mls of ether, a catalytic amount of palladium (II) acetate, and a stir bar. Proton NMR was used to monitor the reaction mixture in order to maximum the product (5) and not the dicyclopropanated product (6). The reaction was terminated after 4 to 6 hrs and allowed to stir for additional 12 hours to remove any excess diazo species. Solid Pd(0) was

removed from the solution by gravity filtration, #5 paper, and chromatographed on a silica gel column using diethyl ether. A dark clear amber mixture resulted after removal of ether by rotoevaporation. Additional solid Pd(0) continued to precipitate out over a period of days.

Synthesis of 7

Ten grams of 5 was placed in a 250 ml round bottom along with 20 grams of Sodium Hydroxide and 200 mls of distilled H_2O. The mixture was mildly refluxed for 48 hours. Hydrochloric acid was added to acidify (pH 2) the solution, at which time a white precipitate formed in a clear yellow solution. Three diethyl ether extractions were used to remove the protonated diacid from the acidic solution. A tanish solid resulted when all three were combined and rotoevaporated. The tanish solid was purified by redissolving in a minimal amount of diethyl ether. A white solid remained and was collected. Proton NMR showed a 70-90% yield for 7 with the remainder being the hydrolysis product of the diene 4.

Synthesis of 9 and 10

Compound 7 (100 mg, 0.515 mmol) and Zeise's dimer (150 mg, 0.255 mmol) were added to a 5 dram vial containing diethyl ether and a stir bar. The vial was capped and allowed to stir for ca. 5 hours or until the orange solid (Zeise's dimer) was dissolved and a light fluffy yellow

precipitate (I.P.C.) had formed. The I.P.C. (9) was washed three times using diethyl ether, then evaporated to dryness with N_2. Chloroform (10 mls) and a stir bar were added to the remaining yellow pale solid along with 81 l of pyridine to form the monomer platinacyclobutane 10. The platina(IV)cyclobutane formed was soluble in $CHCl_3$, where as the I.P.C. was not. Filtering the clear brownish solution using #5 paper resulted in a clear yellow solution, which was reduced in volume to 0.5 mls by evaporating with N_2. Pentane was added to initiate precipitation of a thick white precipitate. The precipitate was washed three times using pentane and nitrogen gas was used to evaporate the precipitate to dryness, yielding a light tanish powder 10. Carbon 13, ^1H, COSY and HETCOR NMR spectra were obtained on this compound.

Synthesis of 11 and 12

Platinacyclobutane 10 was dissolved in 10 mls of chloroform. One equivalent of ethylenediamine (en) was added dropwise. A fine white precipitate was immediately observed. The precipitate was washed with chloroform three times, with centrifugation to expedite the washing process. Evaporating the precipitate to dryness with N_2 yielded a light brownish powder. Proton NMR analysis showed that the platinacyclobutane 11 was 97% pure.

Tetramer 9 was added to 10 mls of chloroform with one equivalent of 2,2'-bipyridine. A clear yellow solution

resulted. The platinacyclobutane was precipitated out of chloroform by first reducing the volume to 0.5 mls and then adding pentane. A small quantity of a light yellow powder remained after three washings with pentane and evaporated to dryness with N_2. The ^{13}C NMR spectrum using d_6-DMSO indicated the platinacyclobutane 12.

Synthesis of 13

A 150 mg of Zeise's Dimer was added to a 5 dram vial containing 10 mls of chloroform and a stir bar. Four-pyridyl carbinol (111 mg) was added to the stirring chloroform solution. The solution remained clear yellow after 60 minutes after which time the reaction was stopped. The reaction solution was worked up as previously described in the synthesis of 10. A yellowish solid resulted from the work up.

Synthesis of 15

Norbornylene (14), from Alrich Chemical Co., (10 g, 0.106 mol) was cyclopropanated using ethyl diazoacetate (1 eq.). The hydrocarbon was dissolved in diethyl ether with a catalytic amount of Rhodium (II) acetate. Ethyl diazoacetate was dissolved in diethyl ether and added to the hydrocarbon mixture over a 2 hour period. A green color was maintained in the reaction mixture by addition of catalyst. The final mixture was allowed to stir an additional 2 hours to complete the diazo reaction.

Rotoevaporation was used to remove excess 14 and diethyl ether, resulting in a viscous yellow liquid. This liquid was chromatographed using a silica gel column with diethyl ether. All ether extractions were combined and rotoevaporated to yield a clear yellow liquid. Proton and ^{13}C NMR as used to identified the liquid as being both acetate isomers, 15, along with fumarate and maleate esters.

Synthesis of 16

The product mixture resulting from the synthesis of 15 was reduced to the corresponding alcohols. Diethyl ether (80 mls) was used to dissolve the product mixture which was added to a diethyl ether / Lithium Aluminum Hydride slurry by an addition funnel. Water was used to destroy any unreacted LAH after 24 hours. The resulting mixture was extracted with diethyl ether. Ether washings were extracted with H_2O. The ether extractions were rotoevaporated yielding a yellow liquid, 15.

Synthesis of 17 and 18

A proton integratable NMR spectrum was required of mixture 16 to determine a ratio of anti to syn isomers. Protons on the methylene carbon next to both hydroxyl groups, at 2.25 and 2.38 ppm, were integrated. Neilsen discovered that Zeise's dimer reacted preferentially with the syn isomer. The anti isomer could be obtain if one equivalent of Zeise's dimer was allowed to react with 2 equivalent of

syn isomer, yielding 19. The reaction was done in a 5 dram vial using diethyl ether and a stir bar. The anti isomer was collected by washing the I.P.C. with pentane three times. Chloroform (10 mls) and a stir bar were added to the I.P.C., which had been evaporated to dryness with N_2. Two equivalents of pyridine resulted in a clear yellow solution. The chloroform solution was reduced in volume to 0.5 mls and pentane added to initiate precipitation of the platinacyclobutane. Three washing with pentane produced pure 18.

Synthesis of 19

Compound 19 was synthesized by W. D. Neilsen, according to Neilsen, 1989.

Synthesis of 20

I.P.C. 17 was added to 10 mls of chloroform in a 5 dram vial along with a stir bar. One equivalent of ethylenediamine was added dropwise. After 20 minutes the newly formed precipitate was washed with chloroform three times. A yellow solid, 20, resulted after evaporating to dryness with N_2.

Synthesis of 21

I.P.C. 17 was added to 10 mls of chloroform in a 5 dram vial along with a stir bar. Addition of 2,2'-bipyridine produced a yellow precipitate in a clear yellow solution. The solution was reduced in volume to 0.5 mls and pentane added to initiate precipitation of any additional soluble

platinacyclobutane. Pentane was used three times to wash the resulting yellow solid, pure 21.

Synthesis of 23 and 25

Procedure of Burton, 1983, was followed with some modifications. Zeise's dimer (100 mg, 0.170 mmol), and a stir bar were placed in a 5 dram vial along with 5 mls of THF. Cyclopropyl carbinol (125 mg, 1.72 mmol) was added dropwise. The vial was capped and left to stir for 4 days. The product mixture was filtered prior to the volume being reduced to 0.5 mls. Pentane was added to initiate precipitation of a tanish solid. Additional pentane was used to wash the precipitate three times. A tanish solid resulted after evaporating to dryness using N_2, I.P.C. 23. Neat d_5-pyridine dissolved the I.P.C., thus forming the monomer. Carbon-13 NMR identified the solid as the platinacyclobutane 25.

Synthesis of 24 and 26

The same conditions were used to synthesize 26 and 24 as in synthesizing 25, except that diethyl ether was used and the reaction proceeded for 24 hrs. The I.P.C. (26) was washed 2 times with ether and evaporated to dryness with N_2 resulting in a tanish powder. Neat d_5 pyridine was added to the I.P.C. forming the monomer. A carbon 13 NMR spectrum showed resonances for the platinacyclopentane 24.

Synthesis of 28

I.P.C. (26) was added to 10 mls of chloroform in a 5 dram vial along with a stir bar. A clear yellow solution resulted following the addition of 1 equivalent of 2,2'-bipyridine. Nitrogen gas was used to reduce the volume to 1.0 ml. The 1.0 ml solution was added to 100 mls of pentane forming a cloudy white solution. A yellow-whitish precipitate formed after several hours. A very fine white precipitate (28) was left once the yellow-whitish precipitate was evaporated to dryness using N_2. The ^{13}C NMR spectrum showed only (28) when dissolved in $CDCl_3$.

Synthesis of 29

Pyridinium dichromate (PDC) oxidize 18 to 29. A 150 mg (0.267 mmol) of 18 , 400 mg of PDC, and 25 mls of methylene chloride were added along with a stir bar to a 100 mls round bottom. The reaction was terminated after 24 hours and then chromatographed using a silica gel column with methylene chloride. A small yellow band appeared slightly below the PDC layer in the column and descended very slowly. Acetone was added to the methylene chloride (40:60) to facilitate separation. Extractions containing the yellow solution were reduced in volume by rotoevaporation to 1.0 ml and transferred to a 150 ml beaker. Pentane was added to initiate the formation of a fluffy white precipitate which

became a heavy yellow powder after a few minutes. The yellow powder was washed with pentane yielding pure 29.

Synthesis of 29, 30, and, 31

Synthesis of 29 (in this scheme) proceeded by making 20. I.P.C. 17, 10 mls of chloroform, and a stir bar were added to a 5 dram vial. One equivalent of ethylenediamine was added and caused the precipitation of a yellow solid. Washing the precipitate three times with pentane yielded pure 20. The oxidation reaction of 20 was set up identical to the oxidation of 18. A yellow band was observed during chromatographing with methylene chloride. Carbon 13 NMR characterization of the yellow precipitate showed predominantly compound 29 with a trace of 31. However, if the identical oxidation reaction and workup were conducted and left for 7 days, 31 became the dominate product with a trace of 29.

Synthesis of 33

Synthesis of 33 proceeded by making 21. Pyridinium dichromate (0.40 g) was dissolved in 25 mls of methylene chloride and added to a 125 ml round bottom. Compound 21 (150 mg, 0.269 mmol) was added to the mixture along with a stir bar. A condenser was placed on the round bottom and the reaction stirred for 7 days. The reaction was worked up according to the procedure for 29. A yellow solid was left after evaporating to dryness with N_2.

Synthesis of 34

Compound 34 was synthesized by R. A. Moats, according to Moats, unpublished.

Synthesis of 36

Compound 36 was synthesized by W. D. Neilsen, according to Neilsen, 1989.

Synthesis of 37

Compound 37 was synthesized by W. D. Neilsen, according to Neilsen, 1989.

Experimental for Biological Experiments

Tissue Culture Assay Experimental

B-16 melanoma and Lewis Lung cell lines were obtained from Dr. Jean Starkey, Montana State University. New cells were initiated from frozen stock after 25 passages. Cells were cultured in 25 cm^3 flasks between experiments (Costar; Cambridge, MA) and incubated (Queue Systems, Cell Culture Incubator; Parkersburg, WA) at 37°C in a humidified atmosphere of 7% CO_2 in air. Both cell lines were grown in 10% fetal bovine serum containing medium.

A Sterile Guard Hood (The Baker Co. Inc., Sanford, ME) equipped with germicidal light and blowers was used. The hood was also equipped with a bunsen burner. Glassware and pipets were autoclaved prior to use. Individual 10 ml and pasteur pipets were flamed prior to use.

Adding Cells to Dishes

Cells were removed from the 25 cm^3 flask by removing the growth medium with a pasteur pipet attached to a vacuum pump. Five mls of trypsin/EDTA was added to the flask and left on the cells for 1.0 minute. This was removed and approximately 0.3 mls were left in the flask to be washed over the cells by tilting flask from side to side. Three mls of serum containing medium was added after ca. 3

minutes. A pasteur pipet with a rubber bulb was used to rinse the cells from the surface of the flask. The resulting cell suspension was transferred to a sterile 10 ml plastic test tube which was centrifuged for 10 minutes at 1000 rpm. The medium was removed leaving a cell pellet. Three mls of Tyrodes Ca^{++} and Mg^{++}-free saline medium was added to the test tube and the cells resuspended, 100 μl of the suspension was transferred to a test tube containing 900 μl of trypan blue stain. The remaining cell suspension was placed on ice. The trypan blue/cell solution was thoroughly mixed and one drop placed on a hemocytometer. Cells were counted on the four corners of one grid on the hemocytometer. This cell count was divided by four and multiplied by 1.0×10^5 to give the number of cells per ml in the original suspension. A series of dilutions were done in serum containing medium to achieve the correct cell concentration of 900 cell/ml. One ml of the cell suspension was added to each tissue culture dish, followed by 9 mls of serum containing medium. The dishes were swirled prior to being placed in the incubator to prevent cell aggregation in center of dishes. Incubation for 24 hours was adequate time to allow all cells to adhere. Five dishes were used for each group.

Addition of Test Compounds to the Dishes

Test compounds were dissolved in fetal bovine serum in 125 ml bottles. Mixtures were filter sterilized 30 minutes before adding to cells through 0.22μm filter attached to a 10 ml sterile syringe. The filtrate was kept in a 125 ml bottle for ease of dispensing. Individual dosages were prepared separately in sterile 50 mls beakers. The concentration of FBS was maintained at 10% in all dosage groups, including the control group. Test compound/serum mixtures were dispensed into the beakers using 200μl and/or 1000μl pipetman(s). A final volume of 50 mls was achieved by adding serum-free medium to each beaker. The growth medium was removed from the cultures, 24 hours after plating, by vacuum suction. Ten mls of test compound-serum solution was immediately added to each dish following removal of growth medium. Each beaker was thoroughly mixed before adding solutions to dishes. To prevent contamination the test compound/serum solution was added to one group at a time. The cultures were incubated in the test mediums for 24 hours.

Removing Solution Containing Compounds from Dishes

The test compound solution was removed and collected in a beaker for proper disposal. Ten mls of serum-free medium

was added to each dish and then removed by vacuum suction. One group was washed at a time to avoid contamination. Ten mls of serum containing medium was added to each dish following three serum-free washes. The dishes were incubated for 4-5 days to allow colony formation.

Staining and Counting Colonies

The experiments were terminated when colonies were observed without the aid of a microscope. The growth medium was removed and each dish was rinsed three times in phosphate buffered saline (PBS). Five mls of methanol was immediately added to each dish. Methanol was removed after 10 minutes and 5 mls of hematoxylin stain was added to each dish. The stain was left on the dishes for 10 minutes, followed by 3 rinses with dH_2O, a single rinse with 50:50 dH_2O:ethanol mixture, and 95% ethanol. Colonies were counted using a Biotran II Automatic Colony Counter (New Brunswick Scientific Co. Inc., Edison, N.J.).

Subculturing of Cells

Cells were subcultured between experiments to propagate the cell lines. Serum containing medium was removed from flasks by vacuum suction and 5.0 mls of trypsin/EDTA added. After one minute all but ca. 0.3 mls were removed. The remaining solution was washed over the cells by tilting flask from side to side. Four mls of serum medium was added after ca. 3.0 minutes. A cell suspension was formed

by using a pasteur pipet with a rubber bulb to loosen cells from flask. One drop of the cell suspension was placed in a sterile 25 cm^3 flask along with 10 mls of serum medium. Cells were incubated until needed.

Dissolving Test Compounds in FBS

Compounds were added to 125 ml bottles along with a 8mm x 1.5mm spin bar. The spin bar was washed in ethanol and flamed prior to placing in bottle. Ten mls of FBS was also added to bottle. See Appendix C for FBS analysis.

A stir motor was used to gently stir the contents of each bottle. The bottle was removed when all visible solids were dissolved and the serum regained its clear amber color. Periodically the bottle was removed to gently swirl the solution. All bottles were wrapped in aluminum foil to avoid direct exposure to natural and artificial light. The length of time the mixtures were left to dissolve depended on solubility of compounds, which varied from 30 minutes with the dicarboxylates to 7 days with syn hydroxymethylplatinacyclobutane with ethylenediamine. Once the compounds were dissolved the mixture was added to the cells within 12 hours.

Toxicity Test Experimental Methods

C57Bl/6 mice were obtained from The Jackson Laboratory, Bar Harbor, ME. The mice were put on water containing

tetramycin and quarantined for 2 weeks at the Animal Resource Center, Montana State University. The mice were initially housed 3-4 per cage, but after injection of test compounds were housed 2 per cage. They were 6-7 weeks old and averaged 19 g/female and 25 g/male when the experiment started.

Concentrations for groups II, III, VI, and VIII were prepared by doing serial dilutions, as follows. Group VIII female vial contained 25 mls dH_2O and 311 mg of 10. Three serial dilutions (10:1) of this mixture produced the appropriate concentrations for females groups VI, III, and II. Group VIII male vial contained 25 mls dH_2O and 409 mg of 10. Three serial dilutions (10:1) of this mixture produced the correct concentrations for male groups VI, III, and II. For both males and females the first dilution was for group VI, the second dilution for group III, and third dilution for group II.

The vial for group IV females contained 19.655 mls dH_2O and 0.345 mls from the group VII female solution vial, while group IV male vial contained 19.657 mls dH_2O and 0.343 mls from the group VII male solution vial. Group V female vial had 19.368 mls dH_2O and 0.632 mls from the group VII female solution vial, while group V males had 19.371 mls dH_2O plus 0.629 mls from the group VII male solution vial.

Twenty five milligrams dichloro(ethylenediamine)Pt(II) in 25 mls of "vehicle" was prepared for group IX. The

concentration for females was 17.5 mls of vehicle plus 2.5 mls of the 1 mg/1 ml drug solution. Concentration for males was 16.75 mls of vehicle plus 3.25 mls of the 1 mg/1 ml drug solution.

The platinum compounds were initially dissolved in 125 ml bottles and the final concentrations were put into 50 ml dosage vials. All groups except IX and VIII had H_2O as the solvent, while a "drug vehicle" was used for IX and VIII. The "drug vehicle" was prepared 3 days in advance, see Appendix D.

Each mouse was anesthetized with ether and injected i.p. with a total volume of 2.0 mls using a 10 ml syringe with a 27G gauge 0.5 inch needle. The mice were warmed in an incubator at 37°C for approximately 15-20 minutes after injections to reduce the effects of shock.

Each cage was checked every 6.0 hrs for the first 48 hrs and then every 12 hrs thereafter. Necropsies were done on all mice. Carbon dioxide was used to euthanize animals during the experiment. Removed organs were placed in neutral buffered formalin (NBF). Tissue paper was placed on top of lungs to keep them from desiccating in the NBF solution.

Tissue samples were left in NBF for ca. 72 hours prior to placing cut sections in plastic cassettes for histological processing. Tissues were sectioned as follows: brain, medial section; lung, cross section of two lobes; liver,

cross sections of two lobes; kidneys, cross section; intestine, one short segment; and spleen, whole specimen. The histological preparations were done at the Veterinary Research Laboratory at Montana State University by Gayle Callis. All specimens were processed using a Miles Scientific (Elkhar,IN), VIP-1000 automatic tissue processor. One change of the following was completed by the processor: ethanol 70%, 80%, and three changes of 95% and 100%; two changes of xylene; and four changes of Surgipath (Grayslake, IL) infiltration media (m.p. 56°C) using 5.0 hour changes. Specimens were embedded with Surgipath embedding media and sectioned at 5.0 μm with an AO 820 rotary microtome and AO Reichert disposable blades (Cambridge Reichert, Buffalo, N.Y.). Each slide was stained with Gill II (hematoxylin and eosin) following the procedure in Sheehan, 1980.[110]

Creatinine determinations were carried out at the Veterinary Research Laboratory, Montana State University, by Lezann Bennet.

In Vivo Anti-cancer Experimental Study Methodologies

Fifty C57Bl/6 mice were obtained from Dr. Jean Starkey, Montana State University, and divided into 5.0 groups of 10 at ca. 4.0 weeks of age. Each group contained five females and five males and no group had a high proportion of older or younger animals. At six weeks of age 900 x 10^5 B-16 cells were inoculated into the left flank sc, using a 1.0

ml syringe with a 27G 0.5 inch needle. Harvesting and determining concentration of cells were done as previously described in "Adding Cells to Dishes". Cells were injected in a solution of serum-free medium. Metaphane^{TM} (Pitman-Moore, Co. Inc., Washington Crossing,N.J.) was used to anesthetize the animals prior to injection.

The platinum compounds were injected when the tumors were palpated in all fifty animals. Compounds were injected i.p. using a 1.0 ml syringe with a 27G 0.5 inch needle. Total volume of solutions injected was 0.5 mls in all groups. The dH_2O used for dissolving compounds was filter sterilized using a 0.22 μm filter. Once the compounds were completely dissolved, they were again filter sterilized. Solutions for the control and four test groups were prepared in a 50 ml beaker and then transferred to a 50 ml dosage vial.

Group I was injected with cisplatin obtained from Bristol-Myers Co. This drug was packaged in a dosage vial containing 50 mg cisplatin, 500 mg mannitol, and 450 mg NaCl. Fifty mls of dH_2O was used to reconstitute the mixture. This was done within 6.0 hours of injection. The vial was kept at room temperature and 8.75 mls was extracted and placed in a 50 ml beaker. Sixteen and a quarter mls of distilled water, filter sterilized, was also added to the beaker for a final concentration of 0.35 mg/ml. Group II received the same concentration of

cisplatin as group I less the mannitol and NaCl. This source of cisplatin was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI).

Group III received a concentration of 0.5 mg/ml which was prepared by adding 12.5 mg of 10 in 25 mls of dH_2O. Group IV concentration was prepared by dissolving 22.5 mg of 10 in 25 mls of dH_2O for a final concentration of 0.9 mg/ml. The final concentrations for groups II, III, and IV were prepared 2.0 hours prior to injection.

Tumors were first measured at the time the compounds were injected and every two days thereafter. A 25 mm caliber and small metric ruler were used to measure tumor dimensions. Tumor depth was measured by abducting the whole tumor.

Necropsies of animals that had died prior to the sixth measuring were conducted within 5.0 hours of death. Tumors, kidneys, 2.0 liver lobes, lungs and a section of intestines were removed and placed in NBF. Remaining mice were euthanized using CO_2 and the same tissues removed and placed in NBF, when the experiment was terminated. Tissues were sectioned and placed in histological cassettes after a minimum of 2 days in NBF. The same histological procedures were followed as outlined above in the toxicity experimental methodologies.

All mice were housed and maintained in the Animal Resource Center, Montana State University. The mice were in

a controlled environment with ample water and WAYNE, MRH 22/5 Rodent Blox pellets.

Both experiments involving mice were preapproved by the Animals Rights Committee, Montana State University. The committee was chaired by Dr. Jack Frost, Animal Resource Center.

SUMMARY

Fifteen platinum IV compounds were synthesized for testing in a tissue culture assay. Only 9 of the 15 compounds were soluble in fetal bovine serum. The dicarboxylate moieties increased the solubility more so than syn hydroxymethyl, syn aldehyde, methyl ether, and syn diol ether. The anti analogs of hydroxymethyl, diol ether were highly insoluble. Platinum amine ligands affected solubility to a greater extent than syn substituents on the platinacyclobutane or pentane. Complexes were found to be more soluble when pyridine was used as a ligand than either ethylenediamine or 2,2'-bipyridine.

Tissue culture results indicated that slight structural changes could in fact influence the anticancer activity. Complexes with pyridine showed substantially less activity than complexes with ethylenediamine. No compounds were found to be more active than cisplatin, but several did approached the 75 μM value of dichloro (ethylenediamine) platinum (II). The most active compound synthesized was 20 with a LD_{100} value of ca. 28 μM using B-16 melanoma. Cisplatin showed a LD_{100} value of ca. 12 μM. Compound 11 was chosen for further testing based on its solubility, tissue culture activity, and possible generation of dichloro(ethylenediamine)Pt II. Toxicity of compound 11

was shown to be less than that observed for cisplatin and dichloro(ethylenediamine) Pt(II). Cisplatin has a LD_{50} of 13 mg/Kg and compound 11's LD_{10} is > than 41 mg/kg. The LD_{50} for dichloro(ethylenediamine) Pt(II) is 20 mg/Kg with a ID_{90} of 8.2 mg/Kg.[111] This was reflected in the histological evaluation with 41 mg/Kg causing short term liver and kidney damage. Irreparable tissue damage was not observed in animals surviving 91 days.

In an *in vivo* study using tumor bearing mice, compound 11 showed a dose response against B-16 melanoma. Concentrations used were 10 mg/Kg and 18 mg/Kg, suggesting that a higher dose could inhibit tumor growth in a manner comparable to that of cisplatin without surpassing the LD_{10} value.

Also, organometallics complexes in the form of platinacylcobutanes were shown to act as chemotherapeutic agents.

REFERENCES CITED

References Cited

1. Zeise, W.C. Ann. Phys. 1827, 9, 932.

2. Collman, J.P.; Hegedus, L.S.; Norton, J.R.; Finke, R.G. Principles and Application of Organotransition Metal Chemistry, University Science Books, Mill Valley, California, 1987.

3. Ivin, K.J.; Rooney, J.J.; Stewart, C.D.; Green, M.L.H.; Mahtab, R. J. Chem Soc., Chem. Commun. 1978, 604.

4. Herisson, J.L. and Chauvin, Y. Makromol. Chem. 1970, 141, 61.

5. Bishop, K.C. III Chem Rev. 1976, 76, 161.

6. Puddephatt, R.J. Coord. Chem Rev. 1980, 33, 149.

7. Groves, J.T.; Avaria-Neisser, G.E.,; Fish, K.M.; Imachi, M. and Kuczkowshi, T. J. Am. Chem. Soc. 1986, 108, 3837-38.

8. Groves, J.T. and McClusky, G.A. J. Am. Chem. Soc. Commun., 1976, 98, 3, 859-861.

9. Groves, J.T., and Nemo, T.E. J. Am. Chem. Soc. 1983, 105, 5786-5791.

10. Tipper, C.F.H. J. Chem. Soc. 1955, 2045.

11. Irwin, W.J.; McQuillin, F.J.; Powell, K.G. Inorg. Synth. 1976, 16, 113.

12. Adams, D.M.; Chatt, J.; Guy, R.G.; Sheppard, N. J. Chem. Soc. 1961, 738.

13. McGinnery, J.A. J. Organomet. Chem. 1973, 59, 429.

14. Gillard, R.D.; Keeton, M.; Mason, R.; Pillgrow, M.F.; Russel, D.R. J. Organomet. Chem. 1971, 33, 247.

15. Waddington, M.D. and Jennings, P.W. Organomet. 1982, 1, 1370.

16. Neilsen, W.D.; Jennings, P.W., J. Am. Chem. Soc. 1988, 110, 8657.

17. Cheetham, A.K.; Puddephatt, R.J.; Zalkin, A.; Templeton, D.H.; Templeton, L.K. Inorg. Chem. 1976, 15, 2997.

18. Young, G.B. and Whitesides, G.M. J. Am. Chem. Soc. 1978, 100, 5808.

19. Burton, J.T.; Puddephatt, R.J. J. Am. Chem. Soc. 1982, 104, 4242.

20. Wilberg, K.B. and Hess, B.A. J. Am. Chem. Soc. 1966, 88, 4433.

21. Gajewski, J.J.; Lyle, R.L.; Gagewski, R.P. Tetrahedron Lett. 1970, 14, 1189.

22. Burton, J.T. and Puddephatt, R.J. J. Am. Chem. Soc. 1982, 104, 4242.

23. Burton, J.T. and Puddephatt, R.J. Organometallics 1986, 5, 1312.

24. Burton, J.T.; Ph.D. Thesis; University of Western Ontario, 1983.

25. Neilsen, W.D., Ph.D. Thesis, Montana State University, 1989.

26. Waddington, M.D.; Campbell, J.A.; Jennings, P.W. Organometallics 1983 , 2, 1269.

27. Waddington, M.D. and Jennings, P.W. Organomet. Chem. 1982, 1, 385.

28. Pregosin, P.S. Coord. Chem. Rev. 1982, 44, 247.

29. Powell, K.G. and McQuillin, F.J. Tetrahedron Lett. 1971, 3313.

30. Al-Essa, R.J.; Puddephatt, R.J.; Quyser, M.A.; Tipper, C.F.H. Inorg. Chim. Acta 1979, 34, L187.

31. Al-Essa, R.J.; Puddephatt, R.J.;Quyser, M.A.; Tipper, C.F.H. J. Organomet. Chem. 1978, 150, 295.

32. Moats, R.A. and Jennings, P.W., Unpublished results.

33. Puddephatt, R.J. Coord. Chem. Rev. 1980, 33, 149.

34. Al-Essa, R.J.; Puddephatt, R.J.; Quyser, M.A.; Tipper, C.F.H. J. Am. Chem. Soc. 1979, 101, 364.

35. Puddephatt, R.J.; Quyser, M.A.; Tipper, C.F.H. J. Chem. Soc., Chem. Commun. 1976, 626.

36. Casey, C.P.; Scheck, D.M.; Shusterman, A.J. J. Am. Chem. Soc. 1979, 101, 4233.

37. Al-Essa, R.J.; Puddephatt, R.J.; Quyser, M.A.; Tipper, C.F.H. Inorg. Chim. Acta 1979, 34, L187.

38. Wilker, C.N. and Hoffman, R.J. J. Am. Chem. Soc. 1983, 105, 5285.

39. Hoffman, R.J. Angew. Chem. Int. Ed. Engl. 1982, 21, 711.

40. Littlecott, G.W.; McQuillin, F.J.; Powell, K.G. Inorg. Synth. 1976, 16, 113.

41. Brown, D.B. J. Organomet. Chem. 1970, 24, 787.

42. Al-Essa, R.J.; Puddephatt, R.J.; Tipper, C.F.H.; Thompson, P.J. J. Organomet. Chem. 1978, 157, C40.

43. Cushman, B.M.; Earnest, S.E.; Brown, D.B. J. Organomet. Chem. 1978, 159, 431.

44. McQuillin, F.J.; Powell, D.G. J. Chem. Soc. Dalton Trans. 1972, 2123.

45. Klinger, R.J.; Hoffman, J.C.; Kochi, J.K. J. Am. Chem. Soc. 1982, 104, 2147.

46. Rosenburg, B.; Van Camp, L.; and Krigas, T. Nature, 1965, 698-9.

47. Rosenburg, B. et. al. Nature, 1969, 222, 385-6.

48. Powis/Prough (ed.) Metabolism and Action of Anticancer Drugs Taylor and Francis, 1987.

49. Biochim. Biophys. Acta 1984, 780, 167-180.

50. Powis/Prough (ed.) Metabolism and Action of Anticancer Drugs Taylor and Francis, 1987.

51. Klastersky, J. and Sculier, J.P., Seminars in Oncology 1985 12 (Suppl. 6), 38-48.

52. Ozols, T.F. and Young, R.C., Seminars in Oncology 1984, 11, 251-263.

53. Ervin, T.J.; Clark, J.R. and Wechselbaum, R.R., Seminars in Oncology 1985 12 (Suppl. 6), 71-78.

54. Rooney, M., Kish, J., Jacobs, J., Kinzie, J., Weaver, A., Crissman, J. and Al-Sarrof, M. Cancer, 1985, 55 1223-1128.

55. Guthrie, D. Clin. Obst. Gyn. 1985, 29-246.

56. AMA Drug Evaluations 5th Ed., W.B. Sanders Co., 1983, 1481-1561.

57. Henney, J.E. and et. al. Biochemie, 1978, 60, 931-934.

58. Hayat, M. et. al. Biochemie, 1978, 60, 935-40.

59. ibid.

60. Prestayko, Archie W., Crooke, Stanley T., and Carter, Stephen K. (ed.) Cisplatin Academic Press, 1980.

61. DeConti, R.C.; Toftness, B.R.; Lange, R.C. and Creasey, W.A. Cancer Res. 1973, 33, 1310-1315.

62 Taylor, David M. Biochemie 1978, 60, 949-956.

63. Lange, R.C., Spencer, R.P., and Harder, H.C. J. Nucl. Med. 1973, 14, 191-195.

64. ibid.

65. Manaka, R.C. and Wolf, Walter Cisplatin Academic Press, 1980.

66. Repta, A.J. and Long, R. Cisplatin Academic Press, 1980.

67. Daley-Yates, P.T. and McBrian, D.C.H. Biochem. Pharmacol. 1985, 34, 1423-1428.

68. Basolo, F. and Pearson, R. G. Mechanisms of Inorganic Reactions 2nd. ed. Wiley, New York, 1967, 351-453.

69. Geigy, Basel. Geigy Scientific Tables 6th ed. 546-593, 1962.

70. Zwelling, L.A. and Kohn, K.W. Cancer Treat. Rep. 1979, 63, 9-10, 1439-1444.

71. Rosenburg, B. Cancer Treat. Rep. 1979, 63, 9-10, 1433-1438.

72. Wolf, W. and Manaka, R.C. J. Clin. Hematol. Oncol. 1979, 7, 1979.

73. Zwelling, L.A., 1979.

74. Stone, P.J.; Kelman, A.D. and Sinex, F.M. Nature, 1974, 251, 736-737.

75. Horacek, P. and Drobnik, J. Biochim Biophys Acta. 1971, 254, 341-347.

76. Fichtinger-Schepman, A.M.J.; vanderVeer, J.L.; denHartog, J.H.; Lohman, P.H.M. and Reedijk, J. Biochemistry 1985, 24, 707-713. Eastman, A. Biochemistry 1986, 25, 3912-3915.

77. Rosenburg, B. Cisplatin Academic Press, Chap. 2, 1980.

78. Eastman, A. Biochemistry 1983, 22, 3927-3933.

79. Eastman, A.,1986.

80. Rahn, R.O. J. Inorg. Biochem. 1984, 21, 311-321.

81. Fichtinger-Schepman, A.M.J., 1985.

82. Fichtinger-Schepman et. al., 1985: Eastman, A., 1986.

83. Roberts, J.J., and Feidelos, F. Chem. Biol. Interactions 1982, 39, 181-189.

84. Eastman, A. Biochemistry 1985, 24, 5027-5032.

85. Cleare, M.J. and Hoeschele, J.D. Platinum Metals Review 1973, 17, 2.

86. Wilkinson, R.; Cox, P.J.; Jones, M. and Harrap, K.R. Biochimie 1978, 851-857.

87. Connors, T.A.; Jones, M.; Ross, W.C.J.; Braddlock, P.D.; Khokhar, A.R. and Tobe, M.L. Chem. Biol. Interactions 1972, 5, 415.

88. Braddlock, P.D., Connors, T.A., Jones, M., Khokhar, A.R., Melzack, D.H., and Tobe, M.L. Chem. Bio. Interactions 1975, 11, 145-161.

89. Tobe, M.L. and Khokhar, A.R. J. Clin. Hem. Oncol. 1977, 7,(1), 114.

90. Guarino, A.M.; Miller, D.S.; Arnold, S.T.; Pritchard, J.B.; Davis, R.D.; Urbanek, M.A.; Miller, T.J. and Litterst, C.L. Cancer Treat. Rep. 1979, 63, 1475.

91. Connors, T.A., and Roberts, J.J. Recent Results in Cancer Research Springer-Verlag, New York, 1974.

92. Moats, R.A. unpublished results.

93. ibid.

94. Neilsen, W.D., Larsen, R.D., and Jennings, P.W., 1988, J Am. Chem. Soc., 110, 3307.

95. Neilsen, W.D., Ph.D. Thesis Montana State University, 1989.

96. ibid.

97. ibid.

98. ibid.

99. ibid.

100. ibid.

101. ibid.

102. Mizushima, Yutaka; Nagahyama, Hirotoku; Tokoyama, Akihito; Morikage, Toshihiko; and Yano, Saburo. Tohoku J. Exp. Med. 1987, 151, 129-135.

103. Hayes, D.M.; Cvitkivoc, E.; Gobey, B.R.; Scheiner, E.; Helson, L.; and Krokoff, I. Cancer. 39:1374-1381, 1977.

104. Kociba, R. and Sleight, S.D. Cancer Treat. Rep. 55, No.1, 1971.

105. Schaeppi, U.; Heyman, I. A.; Fleischman, R.W.; Rosenkrantz, H.; Ilievski, V.; Phelfan, R.; Cooney, D.; and Davis, R. Toxicology and Applied Pharmacology 1973, 25, 230-241.

106. Jones, T. W.; Chopra, S.; Kaufman, J. S.; Flamenbaum, W. and Trump, B.F., Laboratory Investigation, 52, No.4, 363-374.

107. Chopra, Shreekant; Kaufman, J.S.; Jones, T. W.; Hong, W. K.; Gehr, M. K.; Hamberger, R. J.; Flamenbaum, W.; Trump, B. F.; and Chase, D.H. Kidney International 1982, 21, 54-64.

108. Foster, Henry; Small, J. D. and Fox, J.G. The mouse in Biomedical Research vol III, Academic Press, 1983.

109. Khan, A. and Hill, J.M. Advances in Antimicrobial and Antineoplastic Chemotherapy Vol II, University Park Press, Baltimore, 1972, 259-60.

110. Sheehan, D.C. and Hrapchak, B.B. Theory and Practice of Histotechnology 2nd ed. C.V. Mosby, 1980.

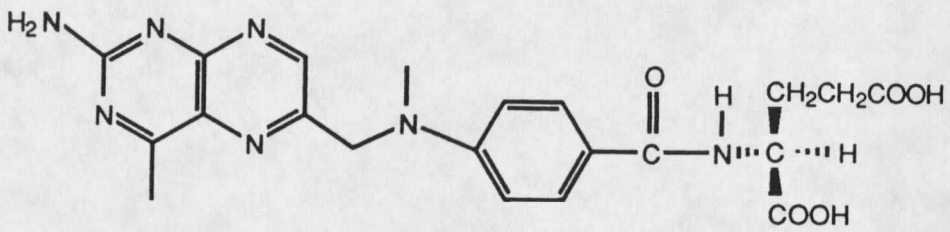
111. Connors, T.A. Advances in Antimicrobial and Antineoplastic Chemotherapy Vol II, University Park Press, Baltimore, 1972, 237-8.

APPENDICES

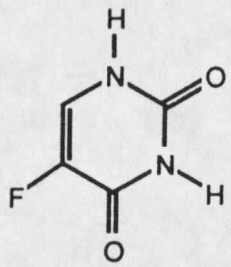
Appendix A

Structures of Methotrexate, Fluorouracil, Etoposide Vinblastine Sulfate, Bleomycin and Adriamycin

Figure 107. Chemical Structures of Methotrexate and Fluorouracil.

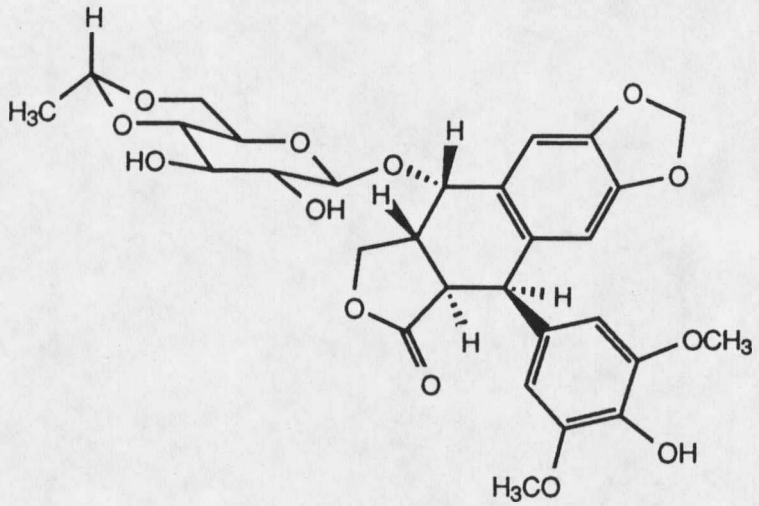


METHOTREXATE

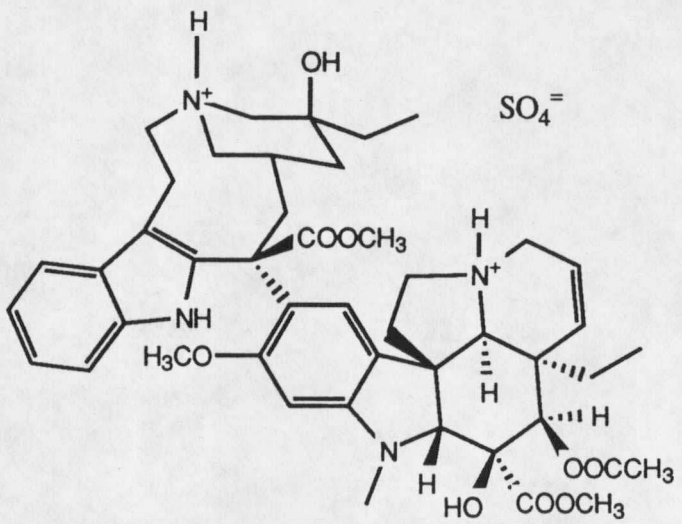


FLUOROURACIL

Figure 108. Chemical Structures of Etoposide and Vinblastine Sulfate.

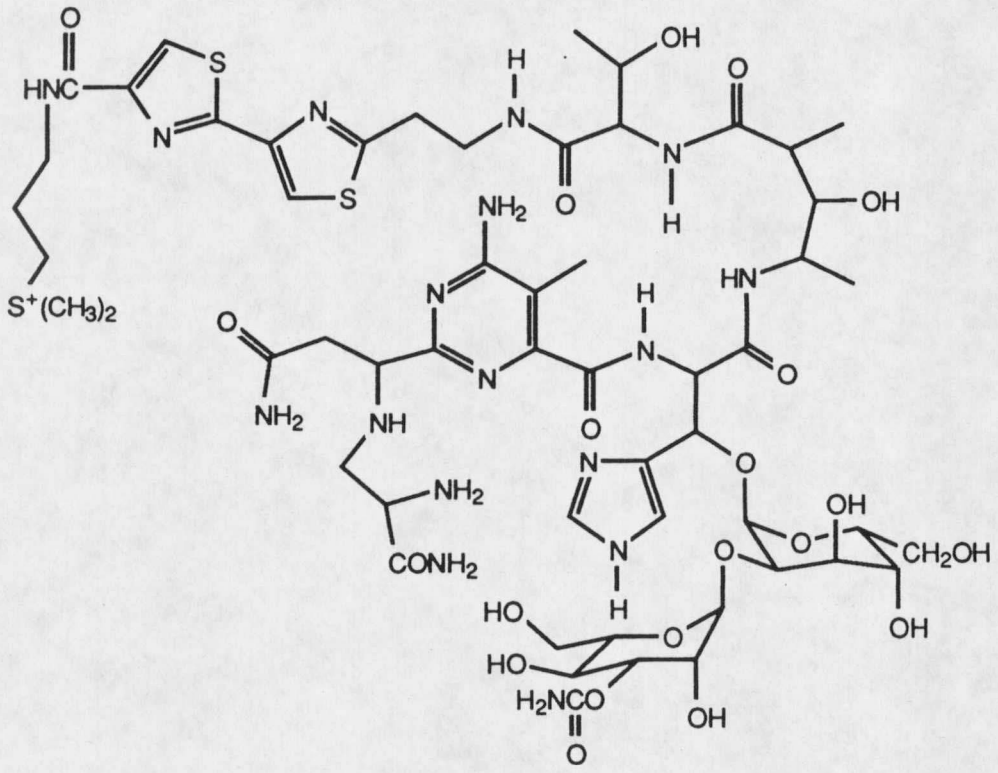


ETOPOSIDE

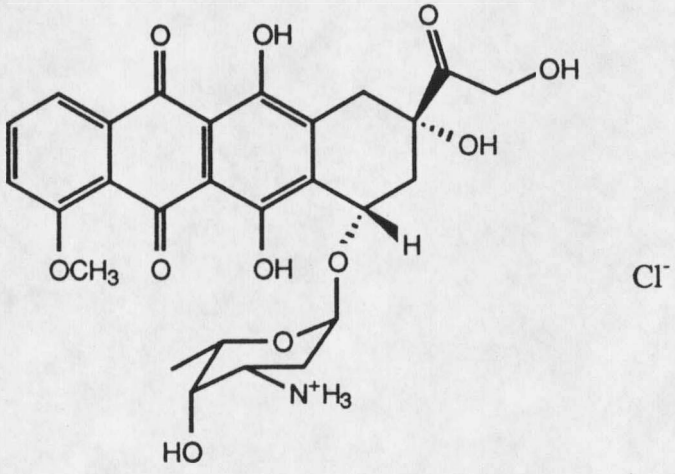


VINBLASTINE SULFATE

Figure 109. Chemical Structures of Bleomycin and Adriamycin.



BLEOMYCIN A_2



ADRIAMYCIN

Appendix B

Standard Error for Tissue Culture Survival Curves

Table 25. Standard Error for Tissue Culture Survival Curves.

Dichlorodiamine Pt(II)

With B-16

[μM]	E
Control	10.0
2	20.1
4	9.7
7	6.2
10	3.2
15	4.0
20	-

With Lewis Lung

[μM]	E
Control	0.2
5	0.4
10	1.8
15	2.4
25	3.2
50	1.2

Dichloro(ethylenediamine) Pt(II)

With B-16

[μM]	E
Control	0.8
10	5.4
20	1.6
30	8.1
40	3.9
50	4.5
75	2.0

With Lewis Lung

[μM]	E
Control	4.2
3	5.5
6	0.9
9	1.8
12	2.1
15	1.2

Compound 10

First Experiment B-16

[μM]	E
Control	1.6
200	1.7
300	1.0
400	-
500	-
600	-

Last Experiment B-16

[μM]	E
Control	2.2
300	6.9
400	4.8
500	-
600	-

Table 25 Continued.

Last Experiment Lewis Lung

[μM]	E
Control	5.1
100	1.4
200	0.8
300	2.9
400	0.8
500	0.2
600	3.1

Compound 11

With B-16

[μM]	E
Control	5.0
50	8.0
75	1.7
100	1.5
125	0.3
150	0.2
200	-

With Lewis Lung

[μM]	E
Control	0.2
10	3.5
25	5.8
35	5.0
50	5.4
100	6.5
125	0.3

Compound 29

With B-16

[μM]	E
Control	1.5
50	9.1
75	4.7
100	6.0
150	0.3

With Lewis Lung

[μM]	E
Control	1.1
25	1.8
50	0.5
75	1.4
100	2.7
125	0.2
150	-

Table 25 Continued.

Compound 36

With B-16

[μM]	E
Control	3.2
10	1.4
25	1.7
50	1.0
100	1.7
150	3.7

Compound 35

With B-16

[μM]	E
Control	0.7
19	4.8
39	6.6
67	8.0
500	-

With Lewis Lung

[μM]	E
Control	0.9
19	1.5
39	1.0
67	0.9
500	-

Compound 18

With B-16

[μM]	E
Control	13.7
25	3.0
50	4.0
75	3.3
100	-
125	-

With Lewis Lung

[μM]	E
Control	2.4
25	1.9
35	3.0
45	6.1
75	3.0
100	-

Table 25 Continued.

Compound 20

With B-16

[μM]	E
Control	2.3
10	1.3
20	6.3
30	-
40	-
50	-

Epimer Alcohols-Cyclopropane

With Lewis Lung

[μM]	E
Control	0.7
100	3.6
200	1.8
300	3.3
400	1.6
500	0.3
600	1.1

Dicarboxylate Cyclopropane

With B-16

[μM]	E
Control	2.2
200	11.0
400	3.6
600	3.9

With Lewis Lung

[μM]	E
Control	5.3
200	9.2
400	14.7
600	5.8

Appendix C

Defined Fetal Bovine Serum Analysis for First and Second Batches

Table 26. First Lot of Defined Fetal Bovine Serum Analysis.

TEST	VALUE
Hemoglobin (mg/dl)	7.00
Endotoxin (ng/dl)	0.200
Bacteria & Fungi	Negative
Mycoplasma	Negative
Bacteriophage VS C-3000 K-12	Negative
Virus Screening	
BVD CPE IF	Negative
IBR CPE	Negative
PI3 CPE	Negative
OTHER CPE	Negative

Supplementary Information

TEST	VALUE
Sodium (meq/l)	135
Potassium (meq/l)	10.0
Chloride (meq/l)	100.0
Glucose (mg%)	88
Blood Urea Nitrogen (mg%)	17
Creatinine (mg%)	2.9
Calcium (mg%)	13
Phosphorus (mg%)	9.2
Total Protein (gm%)	3.3
Albumin (gm%)	2.4
Uric Acid (mg%)	3.5
pH	7.34
Osmolality (mOsm/kg)	297
Iron (ug/dl)	114
TIBC (ug/dl)	154
Percent Saturation (%)	74

Table 27. Second Lot of Defined Fetal Bovine Serum Analysis.

TEST	VALUE
Hemoglobin (mg/dl)	6.00
Endotoxin (ng/dl)	0.200
Bacteria & Fungi	Negative
Mycoplasma	Negative
Bacteriophage VS C-3000 K-12	Negative
Virus Screening	
BVD CPE IF	Negative
IBR CPE	Negative
PI3 CPE	Negative
OTHER CPE	Negative

Supplementary Information

TEST	VALUE
Sodium (meq/l)	141
Potassium (meq/l)	10.0
Chloride (meq/l)	104.0
Glucose (mg%)	101
Blood Urea Nitrogen (mg%)	14
Creatinine (mg%)	3.3
Calcium (mg%)	13
Phosphorus (mg%)	9.4
Total Protein (gm%)	3.6
Albumin (gm%)	2.6
Uric Acid (mg%)	2.9
pH	7.33
Osmolality (mOsm/kg)	306
Iron (ug/dl)	170
TIBC (ug/dl)	205
Percent Saturation (%)	83

Appendix D

Biological Solutions and Supplies

Table 28. Biological Solutions and Supplies.

Serum Medium

Fetal Bovine Serum	100 mls (16.4 g)
RPMI 1640	1 bottle
Insulin	5 mls
Pen-Strep	10 mls
Sodium Bicarbonate	2 g
H_2O	900 mls

Serum-Free Medium

RPMI 1640	1 bottle (16.4 g)
Insulin	5 mls
Pen-Strep	10 mls
Sodium Bicarbonate	2 g
H_2O	1 L

Trypsin/EDTA

EDTA	0.02%
Trypsin	0.05%
Tyrodes Ca^{++}/Mg^{++} Free	

Dolbecco's Phosphate Buffered Saline (PBS)

NaCl	8.0 g
KCl	0.2 g
CaCl2	0.1 g
$MgCl_2.6H_2O$	0.1 g
$Na_2HPO_4.2H_2O$	1.15 g
KH_2PO_4	0.2 g
H_2O	1.0 L

TYRODES Ca^{++}/Mg^{++} Free (CMF)

NaCl	8.0 g/l
KCl	0.2 g/l
$NaH_2PO_4.H_2O$	0.21 g/l
$Na_2HPO_4.12H_2O$	0.48 g/l
$NaHCO_3$	0.5 g/l
Glucose	1.0 g/l
Phenol Red	2.0 ml/l

Table 28 Continued.

Drug Vehicle

Carboxymethylcellulose*	5mg/ml
Polysorbate 80*	4mg/ml
Sodium Chloride*	9mg/ml
Benzyl Alcohol*	9mg/ml

(*) Purchased from Sigma Chemical Co.

RPMI 1640 Medium

Sigma Hybrimax w/ L-Glutamine
4500mg glucose/l
15 mM HEPES

Hematoxylin

Eastman Practical

Trypan Blue Stain

0.4% in CMF

Formaldehyde

37% from Baker Chemical Co.

Neutral Buffered Formalin (NBF)

10% formaldehyde in PBS

Pen-Strep

GIBCO Labs, Grand Island, NY. 20ml
Penicillin-streptomycin lyophilized

Insulin

Boehringer-Mannheim, Crystalline, mycoplasma free, from Bovine pancreas

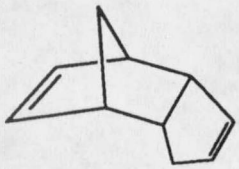
Table 28 Continued.

Defined Fetal Bovine Serum (FBS)	
500 ml bottles	
Hyclone Laboratories. Inc., Logan, Utah	

Appendix E

List of All Compounds Used in Thesis

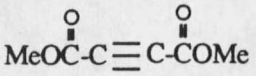
Table 29. List of all Compounds Used in Thesis.



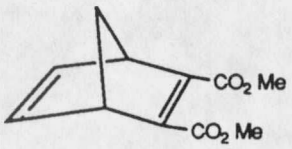
1



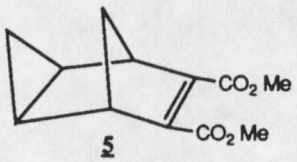
2



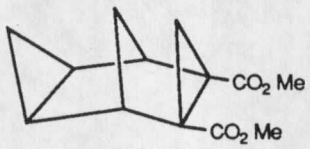
3



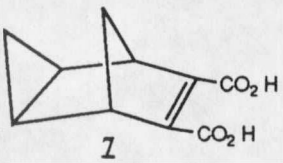
4



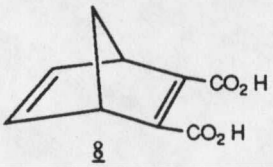
5



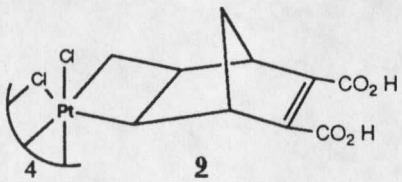
6



7

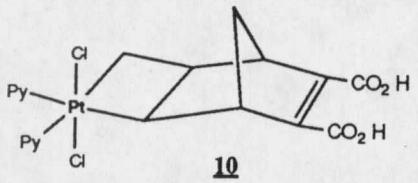


8

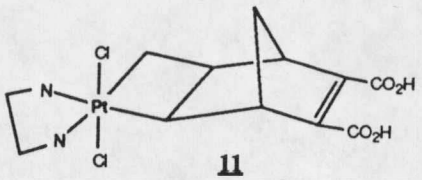


9

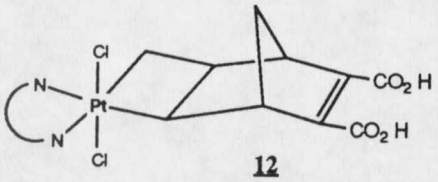
Table 29 Continued.



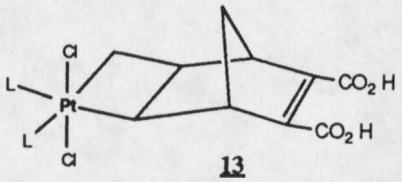
10



11

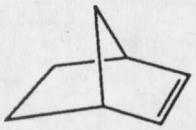


12

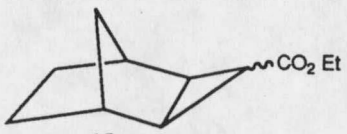


13

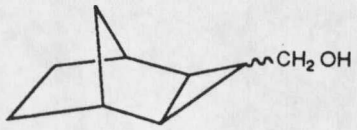
L = 4-Pyridyl Carbinol



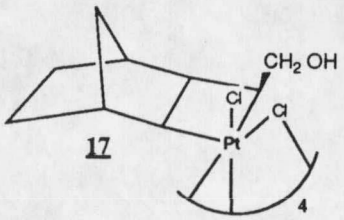
14



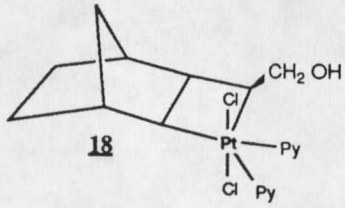
15



16

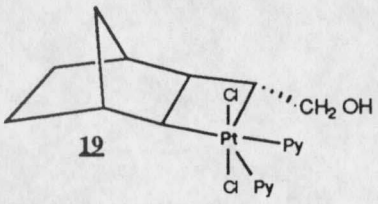


17

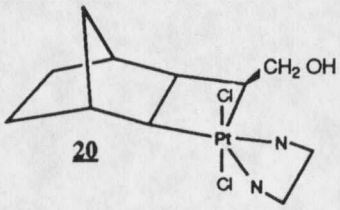


18

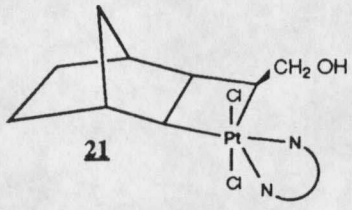
Table 29 Continued.



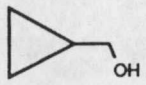
19



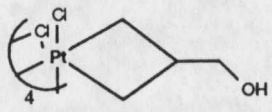
20



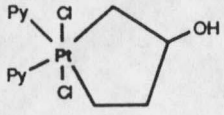
21



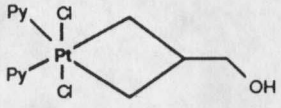
22



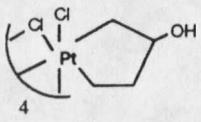
23



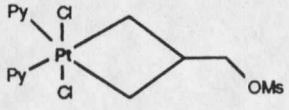
24



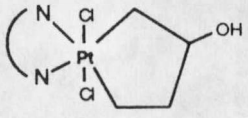
25



26

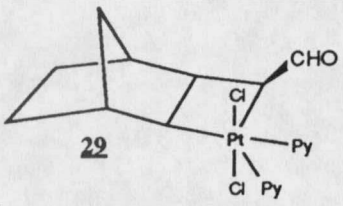


27

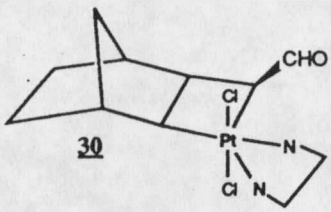


28

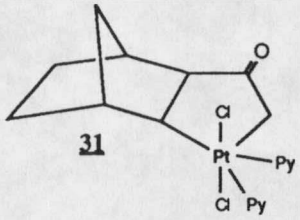
Table 29 Continued.



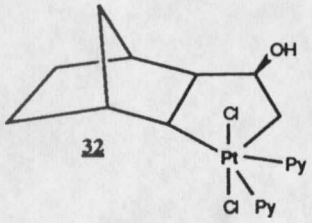
29



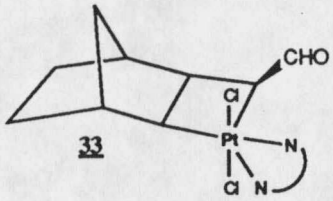
30



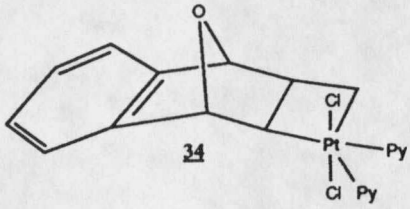
31



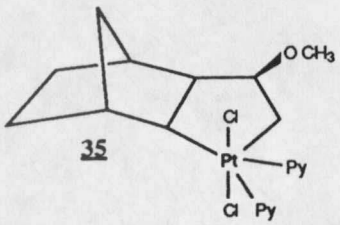
32



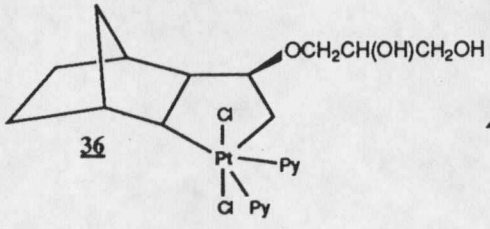
33



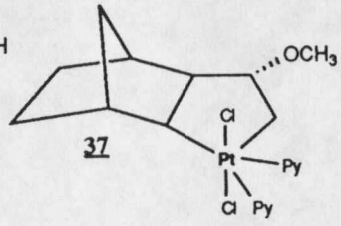
34



35



36



37

MONTANA STATE UNIVERSITY LIBRARIES
3 1762 10062098 6