

# Table of contents

<b>Acknowledgments</b> .....	I
<b>Abstract</b> .....	III
<b>Abbreviations</b> .....	V
<b>1. Introduction</b> .....	9
1.1 Metals in medicine .....	9
1.1.1 Platinum complexes in cancer therapy .....	10
1.1.2 Ruthenium in cancer therapy .....	11
1.2 Analytical methods to characterize metallodrug – biomolecule interactions .....	16
1.2.1 Capillary electrophoresis .....	16
1.2.2 Raman and UV-Vis spectroscopy.....	17
1.2.3 X-Ray spectroscopy.....	18
1.2.4 Chromatography .....	19
1.2.5 Circular dichroism spectroscopy .....	19
1.2.6 Mass spectrometry .....	20
1.2.7 NMR spectroscopy .....	22
1.2.7.1 Theoretical aspects.....	22
1.2.7.2 Application in organometallic chemistry .....	23
1.2.7.3 HR-MAS NMR spectroscopy .....	24
1.2.7.4 DOSY NMR spectroscopy.....	25
1.3 Aim of the thesis .....	26
1.4 References .....	27
<b>2. Reaction of [(<i>p</i>-cym)<sub>6</sub>Ru<sub>6</sub>dhbq<sub>3</sub>tpt<sub>2</sub>]<sup>6+</sup> with amino acids and glutathione.....</b>	<b>39</b>
2.1 Introduction .....	39
2.2 Experimental section .....	41
2.2.1 Material and methods .....	41
2.2.2 Stability in aqueous solution.....	43
2.2.3 Reactions with biological ligands monitored by NMR spectroscopy .....	43
2.2.4 Mass spectrometry studies on the binding to selected biological ligands .....	44
2.3 Results and discussion.....	45
2.3.1 Synthesis and characterization.....	45
2.3.2 Stability in aqueous solution.....	45

2.3.3 Reactions with biological ligands.....	47
2.3.3.1 Reactions with ascorbic acid and glucose.....	47
2.3.4 Reactions with amino acids and GSH .....	49
2.3.4.1 Reactions with Met .....	49
2.3.4.2 Reactions with Cys .....	50
2.3.4.3 Reactions with GSH.....	52
2.3.4.4 Reactions with Arg .....	54
2.3.4.5 Reactions with Lys.....	57
2.3.4.6 Reactions with His .....	59
2.3.5 Competition experiments.....	61
2.4 Conclusions .....	63
2.5 References .....	65
<b>3. Reaction of [(<i>p</i>-cym)<sub>6</sub>Ru<sub>6</sub>dhnq<sub>3</sub>tpt<sub>2</sub>]<sup>6+</sup> with amino acids and glutathione.....</b>	<b>69</b>
3.1 Introduction .....	69
3.2 Materials and methods .....	70
3.2.1 NMR spectroscopy .....	71
3.2.2 Electrospray ionisation mass spectrometry .....	72
3.3 Results and discussion.....	73
3.3.1 Synthesis and characterisation of the metallaprism.....	73
3.3.2 Stability in aqueous solution.....	74
3.3.3 Reactions with ascorbic acid and lactic acid .....	75
3.3.4 Reactions with Arg, Lys and His.....	78
3.3.5 Reactions with Cys and GSH .....	81
3.4 Conclusions .....	84
3.5 References .....	85
<b>4. Reaction of [(<i>p</i>-cym)<sub>6</sub>Ru<sub>6</sub>oxa<sub>3</sub>tpt<sub>2</sub>]<sup>6+</sup> with amino acids and glutathione .....</b>	<b>87</b>
4.1. Introduction .....	87
4.2. Results and discussion.....	88
4.2.1 Synthesis and characterisation of the hexacationic arene ruthenium assembly .....	88
4.2.2 Stability in aqueous solution.....	89
4.2.3 Correlation between cytotoxicity and reactivity.....	91
4.2.4 Reactions with biological ligands.....	92
4.2.4.1 Reactions with the basic amino acids arginine, histidine and lysine .....	92

4.2.4.2 Reactions with glutamine.....	93
4.2.4.3 Reactions with tryptophan .....	95
4.2.4.4 Reactions with sulphur-containing biomolecules cysteine, methionine and glutathione .....	95
4.2.4.5 Reactions with glutamic acid, proline, serine, tyrosine and alanine .....	99
4.2.4.6 Competition experiments .....	101
4.3 Conclusions .....	103
4.4 Experimental .....	104
4.4.1 General remarks.....	104
4.4.2 NMR spectroscopy .....	104
4.4.3 Electrospray ionisation mass spectrometry (ESI-MS).....	105
4.4.4 Cell culture and inhibition of cell growth.....	106
4.5 References .....	107
<b>5. Interactions of the unfilled Ruthenium metallaprisms with proteins .....</b>	<b>111</b>
5.1 Introduction .....	111
5.2 Results and discussion.....	113
5.2.1 NMR and MS investigations of the interaction between metallaprisms and proteins .....	113
5.2.2 CD investigation of the interaction between the metallaprisms and proteins .	116
5.3 Conclusions .....	121
5.4 Experimental .....	122
5.4.1 General remarks.....	122
5.4.2 NMR spectroscopy .....	122
5.4.3 Mass spectrometry studies.....	123
5.4.4 Circular dichroism studies .....	123
5.5 References .....	124
<b>6. Interactions of the unfilled Ruthenium metallaprisms with nucleotides and     DNA .....</b>	<b>127</b>
6.1 Introduction .....	127
6.2 Material and methods .....	129
6.2.1 Chemicals .....	129
6.2.2 NMR spectroscopy .....	129
6.2.3 Mass spectrometry studies.....	130
6.2.4 Circular dichroism studies .....	131

9.2 Experimental section .....	207
9.2.1 Synthesis of the ruthenium metallaprism .....	207
9.2.2 Cell cultures.....	207
9.2.2.1 Cell material and cytotoxicity test.....	207
9.2.2.2 Cell growth and exposure to the Ru-complex.....	208
9.2.2.3 Trypan blue exclusion assay .....	209
9.2.2.4 Sample preparation for NMR measurements.....	209
9.2.3 <sup>1</sup> H high resolution magic angle spinning (HR-MAS) NMR spectroscopy .....	209
9.2.3.1 Acquisition .....	209
9.2.3.2 Spectral processing.....	210
9.2.4 Data analysis.....	210
9.2.4.1 Multivariate analysis of 1D NOESY spectra .....	211
9.2.4.2 Univariate analysis of 1D cpmg spectra.....	211
9.3 Results and discussion.....	212
9.3.1 <sup>1</sup> H HR-MAS NMR spectra of cells.....	212
9.3.1.1 The metabolic profile of untreated cells - PCA of control cells .....	217
9.3.1.2 Toxicity of the metallaprism [1] <sup>6+</sup> against cells.....	220
9.3.1.3 Cellular response to drug treatment – multivariate analysis for the spectral region 0 – 6.4 ppm .....	220
9.3.1.4 Changes in lipids and choline containing compounds.....	224
9.3.1.5 Changes in non-lipid small metabolites.....	227
9.3.1.6 Cellular response to drug treatment – analysis of single components in the spectral region 5 – 8.4 ppm .....	229
9.3.1.7 Potential processes underlying the different metabolic responses to Ru- complex treatment.....	231
9.3.1.8 Lipids and choline containing compounds .....	232
9.3.1.9 Nucleotides and nucleotide sugars.....	234
9.3.1.10 Glutamate and glutathione .....	235
9.4 Conclusions .....	236
9.5 References .....	237
<b>10. Summary and Conclusions .....</b>	<b>241</b>
<b>Communicated Results.....</b>	<b>243</b>
<b>Curriculum Vitae.....</b>	<b>245</b>