Ruthenium-based structural mimics of [Fe]-hydrogenase

Barik, Chandan Kr

2018

<http://hdl.handle.net/10356/73456>

<https://doi.org/10.32657/10356/73456>

RUTHENIUM-BASED STRUCTURAL MIMICS OF [Fe]-HYDROGENASE

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

Chandan Kr Barik

School of Physical and Mathematical Sciences

Nanyang Technological University

2017

RUTHENIUM-BASED STRUCTURAL MIMICS OF [Fe]-HYDROGENASE

Chandan Kr Barik

B.Sc. (Hons., WBSU, 2011); M.Sc (IITBombay, 2013)

A Thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

Division of Chemistry and Biological Chemistry,
School of Physical and Mathematical Sciences

2017

Acknowledgement

First and foremost, I would like to express my sincere gratitude to my supervisor Associate Professor Leong Weng Kee for giving me the opportunity to join his research group and his constant invaluable guidance, wisdom, patience, motivation and continuous support for the past four years of the PhD program. I am very thankful for the education he has given and also for believing in my capability to find out and solve the various scientific problems. I am also thankful for a relaxed and suitable platform to finish my PhD study. I am quite impressed by his strict and serious attitude on research and life.

I would also like to express my gratitude to Prof Michele Salmain (Sorbonne Universites, Univ. Paris 6, IPCM, UPMC) and Associate Professor Richard David Webster (CBC, SPMS, NTU) for giving me the opportunities to work in their laboratory. I am very grateful for their valuable suggestions, ideas and encouragement for the collaboration projects.

I would also like to thank Dr. Rakesh Ganguly and Dr. Li Yongxin for determining the crystal structures and all the staff in the instrument labs of NTU (Ee Ling, Derek from the NMR laboratory; Wen Wei from the mass spectrometry laboratory and Pang Yi from the elemental analysis laboratory) for their assistance in data acquisition.

I would also like to thank my past and current group members: Kumaran, Liu Yu, Jingwei, Yuqian, Yongjia, Yingzhou, Zhi Yong, Bo Yang, Marcus, Sock Li, Wei Xiang, Po Kai, Kwan Jian Ming, Julita, Aaron, Shi Ning, Eric, Poh, Shin Yiing, Hui Xian, Edward, Fan Yi, Carmen, Alvis, Yu Han and Zhi Ming for their assistance, advice, never-ending discussions and ideas etc, and maintaining a comfortable working environment in the laboratory.

I would also like to thank Nanyang Technological University (NTU) for giving me the opportunities of being a student here and providing me scholarship for the last four years.

Lastly, I would like to thank my parents, brothers and friends, especially my mom and daddy whom do not even understand what I am doing in the lab, for their care, believing in me and giving me moral support throughout my graduate study.

Abstract

In this thesis, a series of group VIII metal complexes which are structural mimics of the [Fe]-hydrogenase active site were examined. These were monodentate thiolato complexes of formulae $[\text{Ru}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_3\text{NMe})(\text{CO})_2(o,o\text{-Me}_2\text{C}_6\text{H}_3\text{S})(\text{L})]$ ($\text{L} = \text{MeCN}$ or PMe_3), and *N,S*-bidentate mercaptopyridine complexes of formulae $[\text{M}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(2\text{-SC}_5\text{H}_4\text{N})]$ ($\text{M} = \text{Fe}$, Ru and Os). Substitution at the 6 and 2,6-position of the pyridine ligand and thiol ligand, respectively, were critical to successful synthesis of the thiolato structural mimics. The mercaptopyridine complexes were found to exist as a mixture of isomers in solution, and the most thermodynamically stable isomer depended on the nature of the metal and the substituent at the 6-position of the pyridine ligand. Preliminary investigations into bioconjugation of a ruthenium-based structural mimic with papain suggested that the ruthenium centre was anchored via the cysteine and imidazole residues of the active site responsible for peptidase activity.

The catalytic potential of some of the related complexes was also evaluated. Thus for the group VIII metal complexes $[\text{M}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(2\text{-SC}_5\text{H}_4\text{N})]$ ($\text{M} = \text{Fe}$, Ru and Os), the iron complex was much more efficient than the ruthenium or osmium analogues as an electrocatalyst in proton reduction. These complexes were also efficient and regioselective catalysts for the cyclisation of α,ω -acetylenic acids to exocyclic enol lactones. The structural mimic precursors $[\text{RuBr}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_3\text{NR})(\text{CO})_2(\text{MeCN})]$ ($\text{R} = \text{H}$, Me) were also found to catalyze the very rapid hydrolysis of silanes to produce hydrogen gas ($\text{TOF} \sim 6000 \text{ h}^{-1}$ over 20 s), and showed selectivity for primary over tertiary silanes.

Finally, the idea of encapsulation of ruthenium with silica instead of a protein was attempted via hydrolysis and calcination from a molecular single-source precursor, *viz.*, $[\text{Ru}_2(\text{CO})_4(\mu\text{-O}_2\text{CCH}_2\text{OSi}(\text{OEt})_3)_2(\text{PPh}_3)_2]$. Interestingly, while the silica-encapsulated ruthenium catalyst and the molecular precursor catalysed the formation of cyclopropanes from styrene and ethyl diazoacetate, the product obtained from hydrolysis of the molecular precursor directed the reaction towards a pyrazoline via a [2+3] cycloaddition, the first metal-catalyzed example.

Table of Contents

Acknowledgement	I
Abstract	II
Table of contents	III
Abbreviations and conventions	VII
Compound numbering scheme	VIII
List of schemes	XII
List of figures	XIV
List of tables	XVI
Chapter 1: [Fe]-Hydrogenase	1
1.1 [Fe]-hydrogenase	2
1.2 The active site of [Fe]-hydrogenase (Hmd)	3
1.3 Possible mode of action of [Fe]-hydrogenase	5
1.4 Synthetic models of [Fe]-hydrogenase	7
1.4.1 Structural models	7
1.4.2 Functional models	8
1.5 Aim and objectives of this project	10
1.6 References	11
Chapter 2: Ruthenium Structural Mimics of the [Fe]-Hydrogenase	14
2.1 Ruthenium structural mimics	15
2.1.1 Reaction of 1a with 2-aminopyridine	15
2.1.2 Reaction of 4n with acetonitrile	18
2.1.3 Reaction of 5a and 5b with thiophenols	22
2.1.4 Reaction of 7d with phosphines	26
2.2 Other attempts at the synthesis of structural mimics	28
2.2.1 From the reaction of phosphine derivatives with thiols	28
2.2.2 Attempt at installing the thiolate ligand first	32
2.2.3 An attempt with a chloro derivative	34
2.3 Reaction of 5n with papain	35
2.3.1 Kinetic studies	38
2.3.2 Computational studies	42

2.4 Conclusion	44
2.5 Experimental section	45
2.5.1 General procedures	45
2.5.2 Synthesis of 3-5	45
2.5.3 Synthesis of 7 and 8	49
2.5.4 Synthesis of 9a	49
2.5.5 Synthesis of 10a-c	49
2.5.6 Synthesis of 11a	52
2.5.7 Synthesis of 12	52
2.5.8 Synthesis of 5e	52
2.5.9 Reaction of 5e with PPh ₃	53
2.5.10 Reaction of 5b with 6c	53
2.5.11 Reaction of 5b with imidazole	53
2.5.12 Reaction of 5b with 6c and imidazole in aqueous medium	54
2.5.13 Synthesis of 9b	54
2.5.14 Preparation of papain samples	54
2.5.14.1 Purification by gel filtration (GF)	54
2.5.14.2 Purification by affinity chromatography	55
2.5.15 Reaction between papain sample resulting from GF and 5n at the preparative scale	55
2.5.16 Reaction between affinity-purified papain and 5n at the preparative scale	56
2.5.17 Computation studies	56
2.5.18 X-ray crystallographic studies	57
2.6 References	62

Chapter 3: A Series of Group-VIII Structural Mimics Based on 2-Mercaptopyridine 64

3.1 Synthesis of structural mimics based on 2-Mercaptopyridine	65
3.2 Solid state structures of 15n and 16n	70
3.3 Solution structures of 15n and 16n	73
3.4 Reactivity of 15a with phosphines	76
3.5 Conclusion	81
3.6 Experimental section	82
3.6.1 General procedures	82

3.6.2 Synthesis of 15a/b and 16a/b	82
3.6.3 Synthesis of 15a'' and 16a''	84
3.6.4 Synthesis of 15a' and 16a'	84
3.6.5 Reaction of 15a with PMe_3	85
3.6.6 Reaction of 15a with PBu_3	85
3.6.7 Computational studies	87
3.6.8 X-ray crystallographic studies	87
3.7 References	91
 Chapter 4: Catalytic Studies	 92
4.1 Hydrogenase activity	92
4.1.1 Electrochemical properties of 15n	94
4.1.2 Electrocatalytic hydrogen production	96
4.2 Hydrolysis of organosilanes	98
4.3 Hydrocarboxylation of terminal alkynes	101
4.4 Conclusion	104
4.5 Experimental section	105
4.5.1 General procedures	105
4.5.2 Electrochemical and electrocatalytic experiments	105
4.5.3 Controlled potential electrolysis	106
4.5.4 CPE-GC analysis for measurement of hydrogen	106
4.5.5 General procedures for the hydrolysis of organosilane	106
4.5.6 General procedure for the lactonization of α,ω -acetylenic acid	107
4.5.7 Synthesis of 2-oxopropyl benzoate	108
4.6 References	109
 Chapter 5: Single Source Precursor for Silica Encapsulation	 111
5.1 Synthesis and characterization of a single source precursor	112
5.2 Formation of ruthenium supported on silica	115
5.3 Catalytic studies	118
5.4 Characterization of A	121
5.5 Conclusion	122

5.6 Experimental section	123
5.6.1 General procedures	123
5.6.2 Synthesis of 30	123
5.6.3 Synthesis of 31	124
5.6.4 Synthesis of 32	124
5.6.5 Hydrolysis of 31	124
5.6.6 Calcination	125
5.6.7 Synthesis of D	125
5.6.8 General procedure for catalytic reactions	125
5.6.9 X-ray crystallographic studies	127
5.7 References	129
 Chapter 6: Conclusion	 131
 Appendix (IR, NMR, Mass etc)	 CD-ROM

Abbreviations and Conventions

ACN	Acetonitrile
CV	Cyclic voltammetry
DCM	Dichloromethane
ESI	Electrospray ionization
HRMS	High resolution mass spectroscopy
IR	Infrared
LCMS	Liquid chromatography-mass spectroscopy
m/z	Mass-to-charge-ratio
NMR	Nuclear magnetic resonance
Ph	Phenyl
TEM	Transmission electron microscopy
THF	Tetrahydrofuran
TLC	Thin layer chromatography

Vibrational Spectroscopy (IR)

ν_{CO}	Stretching frequency in the carbonyl region (2200-1800 cm^{-1})
ν_{w}	Very weak
w	Weak
m	Medium
s	Strong
vs	Very strong
br	Broad
sh	Shoulder

Nuclear magnetic resonance (NMR) spectroscopy

δ	Chemical shift
ppm	Parts per million
$\{^1\text{H}\}$	Protons decoupled
s	Singlet
d	Doublet
dt	Doublet of triplet
t	Triplet
m	Multiplet
COSY	Correlation spectroscopy

Compound Numbering Scheme

Compound No	Formula	Structure
1a' M = Fe, 1a M = Ru	$M(CO)_4Br_2$	
2n R ₁ = R ₂ = H (a) R ₁ = H, R ₂ = Me (b) R ₁ = COOMe, R ₂ = H (c) R ₁ = OH, R ₂ = H (d)	2-NH ₂ C ₅ H ₂ NR ₁ R ₂	
3n' M = Fe, 3n M = Ru R ₁ = R ₂ = H (a) R ₁ = H, R ₂ = Me (b) R ₁ = COOMe, R ₂ = H (c) R ₁ = OH, R ₂ = H (d)	$[M(CO)_3(Br_3)]^-$ $[2-NH_2C_5H_2NHR_1R_2]$	
4n' M = Fe, 4n M = Ru R ₁ = R ₂ = H (a) R ₁ = H, R ₂ = Me (b) R ₁ = COOMe, R ₂ = H (c) R ₁ = OH, R ₂ = H (d)	$[M(Br_2)-(2-NHC(O)C_5H_2NR_1R_2)-(CO)_2][2-NH_2C_5H_2NHR_1R_2]$	
5n' M = Fe, 5n M = Ru R ₁ = R ₂ = H (a) R ₁ = H, R ₂ = Me (b) R ₁ = COOMe, R ₂ = H (c) R ₁ = OH, R ₂ = H (d)	$[M(Br)-(2-NHC(O)C_5H_2NR_1R_2)-(CO)_2(NCMe)]$	
6n R = <i>P</i> -Me ₂ -C ₆ H ₄ (a) R = <i>o,o</i> -Me ₂ -C ₆ H ₃ (b) R = CH ₂ CHCOOMeNHC OMe (c)	RSH	RSH
7n' M = Fe, 7n M = Ru R = <i>P</i> -Me-C ₆ H ₄ , R ₂ = H (a) R = <i>o,o</i> -Me ₂ -C ₆ H ₃ , R ₂ = H (b) R = <i>P</i> -Me-C ₆ H ₄ , R ₂ = Me (c) R = <i>o,o</i> -Me ₂ -C ₆ H ₃ , R ₂ = Me (d) R = Me, R ₂ = Me (e)	$[M(2-NHC(O)C_5H_3NR_2)-(CO)_2(CO)_2(SR)(NCMe)]$	

<p>8n R = <i>P</i>-Me-C₆H₄, R₂ = H (a) R = <i>o,o</i>-Me₂-C₆H₃, R₂ = H (b) R = <i>P</i>-Me-C₆H₄, R₂ = Me (c) R = <i>o,o</i>-Me₂-C₆H₃, R₂ = Me (d) R = CH₂CHCOOMeNHC OMe, R₂ = Me (e)</p>	<p>[Ru(2-NHC(O)C₅H₃NR₂)- (CO)₂(SR)]₂</p>
<p>9n R₂ = Me, L = PMe₃ (a) R₂ = Me, L = C₃H₄N₂ (b)</p>	<p>[Ru(2-NHC(O)C₅H₄NR₂)- (CO)₂(<i>o,o</i>-Me₂-C₆H₃S)(L)]</p>
<p>10n R₂ = H, L = PPh₃ (a) R₂ = H, L = PMe₃ (b) R₂ = Me, L = PMe₃ (c) R₂ = Me, L = C₃H₄N₂ (d)</p>	<p>[Ru(Br)- (2-NHC(O)C₅H₃NR₂)- (CO)₂(L)]</p>
<p>11n X = Br, L = PPh₃ (a) X = Cl, L = PPh₃ (b) X = SC₅H₄N, L = PMe₃ (c) X = SC₅H₄N, L = PBu₃ (d)</p>	<p>[Ru(X)(2-NHC(O)C₅H₄N)- (CO)(L)₂]</p>
<p>12</p>	<p>[Ru(CO)₃(2,6-Me₂-C₆H₃S)₂]</p>
<p>13n R₁ = R₂ = H (a) R₁ = H, R₂ = Me (b) R₁ = COOMe, R₂ = H (c) R₁ = OH, R₂ = H (d)</p>	<p>[Ru(2-NHC(O)C₅H₂NR₁R₂)- (CO)₂(papain)]</p>

15n' M = Fe, 15n M = Ru, 15n'' M = Os R ₂ = R ₃ = H (a) R ₂ = Me, R ₃ = H (b) R ₂ = Me, R ₃ = Me (c) R ₂ = OMe, R ₃ = Me (d) R ₂ = CH ₂ OCOMe, R ₃ = H (e) R ₂ = CH ₂ OCOPh, R ₃ = H (f)	[M(2- NHC(O)C ₅ H ₃ NR)(CO) ₂ - (2-SC ₅ H ₄ N)]	
16n' M = Fe, 16n M = Ru, 16n'' M = Os R ₂ = R ₃ = H (a) R ₂ = Me, R ₃ = H (b) R ₂ = Me, R ₃ = Me (c) R ₂ = OMe, R ₃ = Me (d)	[M(2- NHC(O)C ₅ H ₃ NR)(CO) ₂ - (2-SC ₅ H ₄ N)]	
17''	[Os(CF ₃ COO)(CO) ₃ - (2-SC ₅ H ₄ N)]	
18n L = PMe ₃ (a) L = PBu ₃ (b)	[Ru(2- NHC(O)C ₅ H ₄ N)(CO) ₂ - (2-SC ₅ H ₄ N)(L)]	
19n L = PMe ₃ (a) L = PBu ₃ (b)	[Ru(2-NHC(O)C ₅ H ₄ N)(CO)- (2-SC ₅ H ₄ N)(L)]	
20n 21n R = Ph, n = 3 (a) R = Ph(CH ₂) ₃ , n = 3 (b) R = n-C ₆ H ₁₃ , n = 3 (c) R = Ph, n = 2 (d) R = o-ClC ₆ H ₄ CH ₂ , n = 2 (e) R = I _{pr} , n = 1 (f) R = Et, n = 1 (g) R = PMH, n = 1 (h)	R _{4-n} SiH _n R _{4-n} Si(OH) _n	
22n n = 1 (a), 2 (b), 3 (c), 7 (d)	HCC(CH ₂) _n CH ₂ COOH	

23n n = 1 (a), 2 (b), 3 (c), 7 (d)	$\text{H}_2\text{CC}(\text{CH}_2)_n\text{CH}_2\text{COO}$
24n n = 1 (a), 2 (b), 3 (c), 7 (d)	$\text{HCCH}(\text{CH}_2)_n\text{CH}_2\text{COO}$
25	$\text{C}_6\text{H}_5\text{COOCH}_2\text{COCH}_3$
30	$[\text{M}(\text{HOCH}_2\text{COO})(\text{CO})_2-(\text{PPh}_3)]_2$
31	$[\text{Ru}(\text{EtO})_3\text{SiOCH}_2\text{CO}_2-(\text{CO})_2(\text{PPh}_3)]_2$
32	$[\text{Ru}(\text{Me})_3\text{SiOCH}_2\text{CO}_2-(\text{CO})_2(\text{PPh}_3)]_2$
33n 34 35	$\text{C}_6\text{R}_1(\text{R}_2\text{R}_3)_2\text{CHCH}_2\text{PhCHCH}_2\text{CHCO}_2\text{Et}$
36n $\text{R}_1 = \text{R}_2 = \text{R}_3 = \text{H}$ (a) $\text{R}_1 = \text{Cl}, \text{R}_2 = \text{R}_3 = \text{H}$ (b) $\text{R}_1 = \text{R}_2 = \text{R}_3 = \text{F}$ (c)	$\text{C}_6\text{R}_1(\text{R}_2\text{R}_3)_2\text{CHCH}_2\text{C}-(\text{CO}_2\text{Et})\text{NNH}$

List of Schemes

Scheme	Content	Page
1.1	Reversible oxidation of molecular hydrogen by [NiFe] and [FeFe]-hydrogenases	1
1.2	Reversible reduction of methenyl-H ₄ MPT ⁺	3
1.3	Activation of molecular hydrogen by [Fe]-hydrogenase.	5
1.4	Mechanism for the activation of molecular hydrogen via η^2 -mode	6
1.5	Mechanism for the activation of molecular hydrogen from Fe(0) centre	6
1.6	Mechanism of hydrogen evolution by model complex 14	9
2.1	Synthetic route for the preparation of structural model of the active site of [Fe]-hydrogenase	15
2.2	Synthesis of 3n and 4n .	16
2.3	Mechanism for the formation of carbamoyl complex	16
2.4	Synthesis of 5n	18
2.5	Synthesis of structural mimic 7d	22
2.6	Synthesis of 9a	26
2.7	Synthesis of 10a-c	28
2.8	Reaction of 10c with 6b	28
2.9	Reaction of 10a with 6b	30
2.10	Reaction of 1a with thiol	32
2.11	Synthesis of chloro derivative	34
2.12	Synthesis of mimic of [Fe]-hydrogenase	35
2.13	Mechanism of peptidase activity of papain	36
2.14	Reaction of 5b with cysteine and imidazole	37
2.15	Reaction of 5b with cysteine and imidazole in presence of water	38
2.16	Hydrolysis of ZGlyONP	38
2.17	Synthesis of structural mimic	44
3.1	Synthesis of structural mimic using thiol	64
3.2	Synthesis of 15a/b	65
3.3	Synthesis of osmium structural mimic	67
3.4	Proposed mechanism for the formation of osmium structural mimic	68
3.5	Synthesis of iron structural mimic	69
3.6	Isomerization under presence of uv light	74
3.7	Proposed mechanism for the isomerization	76

3.8	Reaction of 15a with phosphines	76
3.9	Disproportionation of 18	78
4.1	Reversible reduction of methenyl-H ₄ MPT ⁺	93
4.2	Simplified model of biological substrate and its reduction	93
4.3	Proposed mechanism for the electrocatalytic proton reduction	96
4.4	Hydrolysis of tertiary silane by reported complex 4a'	98
4.5	Hydrolysis of primary silane by 5a	98
4.6	Hydrocarboxylation reaction of terminal alkynes	101
4.7	Cyclization of α,ω -acetylenic acid	102
4.8	Intermolecular hydrocarboxylation of terminal alkynes	102
4.9	Catalytic cycle for the hydrocarboxylation of terminal alkyne	104
5.1	Schematic representation of the synthesis of ruthenium on silica support	112
5.2	Synthesis of single source precursor	113
5.3	Synthetic route for the preparation of ruthenium on silica support	115
5.4	Cyclopropanation of styrene with ethyl diazoacetate	118
5.5	Synthetic route for the preparation of ruthenium on silica support	122
6.1	Synthesis of structural mimic 7d	131
6.2	Catalytic reaction by ruthenium on silica support	133

List of Figures

Figure	Content	Page
1.1	Active site of the [NiFe] and [FeFe]-hydrogenases	1
1.2	Left: <i>Mathnopyrus kandleri</i> and right: <i>Methanothermobacter thermautotrophicus</i> methanogenic archaea	2
1.3	X-ray crystal structures and schematic representations of the currently acceptable structure of the active site of the [Fe]-hydrogenase	3
1.4	Ribbon diagrams of the [Fe]-hydrogenase. (A) open conformation of the structure of the holoenzyme. The FeGP cofactor is depicted as a color-coded stick model. (B) closed conformation of the structure of the apoenzyme	4
1.5	Structural models of the active site of the [Fe]-hydrogenase	8
1.6	Functional models of the active site of the [Fe]-hydrogenase	9
2.1	Current model of the active site of [Fe]-hydrogenase	14
2.2	Functional models for the activation of hydrogen	14
2.3	ORTEP plot of the anion of 4a	17
2.4	ORTEP plot of the complex 5a	19
2.5	Computed free energy of the isomers of 5a and 5b	20
2.6	The V-T ¹ H NMR spectra of 5a	21
2.7	ORTEP plot of the structural mimic 7d	23
2.8	ORTEP plots of 8b and 8c	24
2.9	ORTEP plot of the complex 9a	27
2.10	ORTEP plot of the complex 10a	29
2.11	IR spectral monitoring of the reaction of 10c with 6b	30
2.12	ORTEP plot of the complex 11a	31
2.13	³¹ P NMR spectra [in CD ₃ CN] of 10a showing its disproportionation at room temperature into 11a and 5a	32
2.14	ORTEP plot of the complex 12	33
2.15	X-ray structure of papain and a close-up structure on the active site of papain	36
2.16	Graph of ZGlyONp hydrolysis rate (V ₀) vs incubation time for 5a , 5b , 5c and 5d and the pseudo-first order rate constants (k _{obs})	39
2.17	Graph of % activity of papain vs incubation time	40
2.18	Infrared spectra of 5b (gray dotted line) and 13b (black full line)	41
2.19	Mass spectrum of 13b	41

2.20	Absorbance spectra of 5b , papain and 13b	42
2.21	Structural model for the active site of the [Fe]-hydrogenase	43
3.1	Reported structural models containing a bidentate 2-mercaptopyridine ligand	65
3.2	¹ H NMR spectra of 15a and 16a	66
3.3	¹ H NMR spectra of 15a'' and 16a''	68
3.4	ORTEP plot of 16b , 16a'' and 15a'	71
3.5	¹ H NMR spectra of 15a' and 16'	73
3.6	ORTEP plot of complex 18a	77
3.7	³¹ P{ ¹ H} NMR spectra of 18a	78
3.8	ORTEP plot of complex 19a	79
3.9	ORTEP plot of complex 11d	80
3.10	Computed relative thermodynamic stability of the isomers 15n and 16n	81
4.1	Synthesized structural mimics and precursors	92
4.2	Reported functional mimics	93
4.3	Cyclic voltammograms of 15a' , 15a and 15a''	94
4.4	Cyclic voltammograms of 15a before and after the oxidation process	95
4.5	Cyclic voltammograms of 15a before and after the reduction process	95
4.6	Cyclic voltammogram of 15a' , 15a and 15a'' in the presence of TFA	97
4.7	Recyclability of 5a (left) and 5b (right) for the hydrolysis of phenylsilane	100
4.8	Structures of parthenolide, helenalin and arglabin.	101
5.1	Metal encapsulated in a protein and in silica	111
5.2	ORTEP plots of 31	113
5.3	TGA analysis of A	116
5.4	Electron micrographs of B	116
5.5	XPS spectra of B	117
5.6	IR spectrum of B	117
5.7	Recycling of A for the cycloaddition reaction	120
5.8	Electron micrographs of A	121
5.9	XPS spectra of A	121
5.10	IR spectrum of A	122
6.1	Computed relative thermodynamic stability of the isomers 15n and 16n	132
6.2	Papain bioconjugates 13n	133

List of Tables

Table	Content	Page
2.1	Common atom labelling Scheme and selected bond parameters for 5a , 5b and 5d	19
2.2	Common atom labelling Scheme and selected bond parameters for 8a-c	25
2.3	Common atom labelling Scheme and selected bond parameters for 10a , 10b and 10c	29
2.4	Common atom labelling scheme and selected bond parameters for two independent molecules of 12	33
2.5	Common atom labelling scheme and selected bond parameters for 11a and 11b	34
2.6	ν_{CO} (cm^{-1}) for all the complexes	43
2.7	Yield, HRMS and IR data for 3n and 4n	46
2.8	Yield, MS and IR data for 5n	47
2.9	^1H NMR data for 4n	47
2.10	^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR data for 5n	48
2.11	Yield, MS and IR data for 7n and 8n	50
2.12	^1H NMR data for 7n and 8n	50
2.13	Yield, MS and IR data for 10n	51
2.14	^1H and $^{31}\text{P}\{^1\text{H}\}$ NMR data for 10n	51
2.15	Crystal and refinement data for 3a , 4a , 5a and 5b	58
2.16	Crystal and refinement data for 5d , 7d , 8a and 8b	59
2.17	Crystal and refinement data for 8c , 9a , 10a and 10b	60
2.18	Crystal and refinement data for 10c , 11a , 11b and 12	61
3.1	Common atom labelling scheme and selected bond parameters for 16a , 15b , 16b , 16a'' , 15a' and 15c'	72
3.2	Computed free energy and isomeric ratios of the complexes 15n and 16n	74
3.3	Common atom labeling ORTEP plots showing optimized structures of the two isomers for all the complexes	75
3.4	Yield, HRMS and IR data for 15a/b and 16a/b	83
3.5	^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR data for 15a/b and 16a/b	83
3.6	Yield, HRMS and IR data for 18a , 19a and 11c	86

3.7	¹ H and ³¹ P NMR data for 18a , 19a and 11c	86
3.8	Crystal and refinement data for 15a' , 16a , and 15b .	88
3.9	Crystal and refinement data for 16b , 16a'' and 18a .	89
3.10	Crystal and refinement data for 11c , 11d and 19a	90
4.1	Hydrolysis of various organosilane catalyzed by 5a and 5b	99
4.2	Optimisation of catalyst loading of 5a and 5b in the hydrolysis of phenylsilane	100
4.3	Catalytic performance of various catalysts in the hydrolysis of phenylsilane	100
4.4	Catalyzed cyclization of alkynoic acids to enol lactones	103
4.5	Optimization of catalyst loading for 15a using 5-hexynoic acid	103
5.1	Common atom labelling Scheme and selected bond parameters for 30-32	114
5.2	Catalytic efficiencies of all the catalysts	118
5.3	Optimization for the catalyzed formation of pyrazoline 36a	119
5.4	Substrate scope for the catalyzed cycloaddition reaction	120
5.5	Crystal and refinement data for 30-32	128

Chapter 1: [Fe]-Hydrogenase

The hydrogenase was first discovered in the 1930s in microorganisms,¹ and identified as part of the family of metalloproteinase.² The term “hydrogenase” was first introduced by Majory Stephenson in 1931 because they catalyse the reversible oxidation of molecular hydrogen (H₂) (Scheme 1.1).^{3,4} Until recently, only two types of hydrogenases were known and they were designated as [NiFe] (Type-I) and [FeFe] (Type-II) hydrogenases, according to the metal atoms present in the active site (Figure 1.1).^{5,6,7}

Scheme 1.1. Reversible oxidation of molecular hydrogen by [NiFe] and [FeFe]-hydrogenases.

Figure 1.1. Active site of the [NiFe] (left) and [FeFe] (right)-hydrogenases.

Although both types of hydrogenases catalyze the two reactions above, it has been found that the [NiFe]-hydrogenase (obtained from the *Ralstonia eutropha* H16 bacterium) is an efficient catalyst for the oxidation of molecular hydrogen,^{8,9} while the [FeFe]-hydrogenase is more efficient in the production of molecular hydrogen.¹⁰ The [FeFe]-hydrogenase obtained from the *Clostridium pasteurianum* bacterium showed a turnover frequency (TOF) in the order of 10,000 s⁻¹.¹⁰ This has led to research focusing on the use of [FeFe]-hydrogenase for sustainable molecular hydrogen production which has potential impact for the “hydrogen economy”.¹¹ Both types of hydrogenase were also found to be redox active as each metal could exist in various oxidation states, suggesting that they could be used to produce molecular hydrogen electrocatalytically, i.e., the conversion of protons and electrons into molecular hydrogen.^{12,13}

1.1 [Fe]-hydrogenase

In 1990, a third type of hydrogenase was discovered by Thauer and co-workers in the body of some hydrogenotrophic methanogenic archaea such as *Methanocaldococcus jannaschii*, *Methanothermobacter marburgensis*, *Methanothermobacter thermautotrophicus* and *Mathnopyrus kandleri* (Figure 1.2).¹⁴ This was termed the H₂-forming methylenetetrahydromethanopterin dehydrogenase (Hmd) (Type-III). Unlike the [NiFe]- and [FeFe]-hydrogenases which catalyze the molecular hydrogen oxidation/production reactions, Hmd catalyzes the reversible reduction of a biological carbocationic substrate, *N*⁵, *N*¹⁰-methenyltetrahydromethanopterin (methenyl-H₄MPT⁺), with H₂ to produce the neutral substrate methylenetetrahydromethanopterin (methenyl-H₄MPT) and a proton through heterolytic (H⁺ and H⁻) cleavage of dihydrogen ($\Delta G^\circ = -5.5$ kJ/mol) (Scheme 1.2).^{14,15} The reduction of methenyl-H₄MPT⁺ is an important catalytic reaction in biology as it is an intermediary step in the conversion of carbon dioxide to methane.¹ Unlike the [NiFe] and [FeFe]-hydrogenases, purified Hmd does not catalyze *para*-H₂ to *ortho*-H₂ conversion, or the isotopic exchange reaction of H₂/D₂O.^{16,17} In addition, purified Hmd requires the presence of the biological substrate.^{16,17,18,19}

Figure 1.2. Left: *Mathnopyrus kandleri* and right: *Methanothermobacter thermautotrophicus* methanogenic archaea.

Scheme 1.2. Reversible reduction of methenyl-H₄MPT⁺.

1.2 The active site of [Fe]-hydrogenase (Hmd)

Initially, Hmd was believed to be a metal-free hydrogenase but in 2004, Thauer *et al.* found that it contained an iron atom in its active site and renamed it the [Fe]-hydrogenase.²⁰ The determination of the structure of the [Fe]-hydrogenase, and especially of its active site, involved a lot of twists and turns,^{19,20,21,22} but based on all the interpreted data, including several crystallographic studies,^{23,24,25,26} the currently acceptable configuration of the active site of the [Fe]-hydrogenase is shown in Figure 1.3.

Figure 1.3. X-ray crystal structure and schematic representation of the currently accepted structure of the active site of the [Fe]-hydrogenase.

This structure is compatible with the previously obtained IR, Mossbauer and EXAFS data for wild type [Fe]-hydrogenase. The IR absorptions for the active [Fe]-hydrogenase arises at 2011 and 1944 cm⁻¹ in water, and at 1996 and 1928 cm⁻¹ in the solid state, consistent with a

cis arrangement of two CO ligands.²⁵ The iron atom exists in the +2 oxidation state and unlike the [NiFe] and [FeFe]-hydrogenases, is redox inactive.¹⁹

The high resolution crystal structure of the [Fe]-hydrogenase apoenzyme from the *Methanocaldococcus jannaschii* methanogen was reported in 2006 (Figure 1.4 B),¹⁸ and that of the [Fe]-hydrogenase with FeGP cofactor (holoenzyme) at a resolution of 1.75 Å two years later (Figure 1.4 A).²³ It is a homodimer with a molecular mass of ~76,000 Da, comprising two peripheral (green) and a central domain (blue). These three domains produce two cavities with a mononucleotide-binding site in each cavity into which the FeGP cofactor of the [Fe]-hydrogenase active site could be identified.

Figure 1.4. Ribbon diagrams of the [Fe]-hydrogenase. (A) open conformation of the structure of the holoenzyme. The FeGP cofactor is depicted as a color-coded stick model. (B) closed conformation of the structure of the apoenzyme.

1.3 Possible mode of action of [Fe]-hydrogenase

In [NiFe] and [FeFe]-hydrogenases, electrons are transferred from the oxidation of dihydrogen to the active site and then to an electron-acceptor protein through a branch of iron-sulfur clusters.^{27,28} In [Fe]-hydrogenase, electrons are not released but rather, the carbocation substrate is thought to directly accept a hydride from molecular hydrogen.²⁹ NMR and labelling experiments show that a hydride is stereoselectively transferred from molecular hydrogen onto the *pro*-R side of the sp^2 carbon atom of methenyl- H_4MPT^+ (Scheme 1.3). Griesinger and co-workers showed that there is an exchange of the *pro*-R hydrogen atom of the methylene carbon with the protons of water.³⁰

Scheme 1.3. Activation of molecular hydrogen by [Fe]-hydrogenase.

Although no intermediate for the hydrogenation of the natural substrate has been determined crystallographically, several pathways have been proposed. Shima and Thauer proposed a mechanism for the activation of molecular hydrogen which involves the coordination of molecular hydrogen to an Fe(II) center via an η^2 -mode; an adjacent base (OH group) then heterolytically cleaves the dihydrogen by accepting a proton.²⁹ This leaves a hydride on the iron center for subsequent transfer to the carbocation substrate (Scheme 1.4). The chemistry of base-catalysed heterolytic cleavage of coordinated dihydrogen to place a hydride ligand onto a metal centre is well-known.³¹

Scheme 1.4. Proposed mechanism for the activation of molecular hydrogen via η^2 -mode.

An alternative pathway, also proposed by Shima and Thauer, involves the oxidative addition of molecular hydrogen to an Fe(0) center to produce a dihydride species. Abstraction of one hydride by the carbocation substrate affords the reduced form of the natural substrate (Scheme 1.5).²⁹ This is also a well-established type of reaction.³² Although the CO ligand appears to play only an observer role in the two mechanisms above, it is probably important in modulating H-H bond cleavage as CO ligands are good π -acceptors and will exert electronic influence on the metal-bound H_2 .

Scheme 1.5. Mechanism for the activation of molecular hydrogen from Fe(0) centre.

1.4 Synthetic models of [Fe]-hydrogenase

In order to study the chemistry of metalloenzymes, it has been desirable to construct molecular models of their active sites. These models are typically small molecules which mimic the structure and/or function of the biological metalloenzymes. In the case of the [NiFe] and [FeFe] hydrogenases, these model compounds have allowed research over the last few years covering structural and functional aspects.^{33,34,35,36} In contrast, much less is known of the [Fe]-hydrogenase as its structure has only recently been determined. Two types of models are of interest - structural and functional models.

1.4.1 Structural models

The challenge for a structural model is to arrive at a complex with the correct relative orientation of all the different ligands as observed in the active site of the metalloenzyme. A number of molecular models to probe the nature of the active site of [Fe]-hydrogenase have been synthesised over the years (Figure 1.5). The nuclear resonance vibrational spectroscopy (NRVS) data for the dicarbonyl diphosphine dithiolate Fe(II) complex **1** was found to be in good agreement with the IR and EXAFS results for [Fe]-hydrogenase.³⁷ The CO stretching frequencies of compound **3** was compared with those for the isolated cofactor and enzyme.³⁸ From the latter it was concluded that the oxidation state of the iron centre in both the isolated cofactor and the enzyme was Fe(II). Compound **5** served as an IR and Mossbauer spectroscopic model for the iron centre in [Fe]-hydrogenase.³⁹ Compound **8** was prepared to correlate its CO exchange and isomerization reactivity with that for the natural [Fe]-hydrogenase.⁴⁰ It was found that extrinsic CO did not exchange with the CO ligands, consistent with observations for the [Fe]-hydrogenase. The compounds **12a** and **12b** were used to reconstitute [Fe]-hydrogenase from the apoenzyme.⁴¹ It was found that while the reconstituted [Fe]-hydrogenase using **12a** could activate molecular hydrogen, that using **12b** could not. Both **12a** and **12b** were inactive for the activation of molecular hydrogen in the absence of the apoenzyme, demonstrating that the protein has an essential role for the activation of molecular hydrogen. The difference in activity between **12a** and **12b** also suggests that the 2-hydroxy group on the pyridine ligand has a crucial role. The [Fe]-hydrogenase reconstituted from **12a** also shows the highest turnover frequency among all known hydrogen activation catalysts.

Figure 1.5. Structural models of the active site of the [Fe]-hydrogenase.

1.4.2 Functional models

Although all the complexes in Figure 1.9 have similar geometric and electronic structures to the active site of the [Fe]-hydrogenase, they all failed to activate molecular hydrogen. Two iron-based complexes, which are structural mimics, have been reported for the hydrogenation of commercially available organic compounds instead of the biological substrate (Figure 1.6).^{42,43} The first example, described by Hu and co-workers in 2016, is compound **13** which activates molecular hydrogen (50 bars) for the hydrogenation of an aldehyde.⁴² The efficiency of the complex was low due to decomposition. Even more recently, compound **14** which features an anthracene scaffold, was shown to abstract a C-H hydride from imidazolidine and mediates hydrogen evolution (Scheme 1.6).⁴³

Figure 1.6. Functional models of the active site of the [Fe]-hydrogenase.

Scheme 1.6. Mechanism of hydrogen evolution by model complex **14**.

1.5 Aim and objectives of this project

Hydrogen, the most abundant element in the universe, has many industrial uses,⁴⁴ and is an important alternative energy source to replace fossil fuels.⁴⁵ It is thus important to be able to produce hydrogen gas efficiently. Besides steam reforming of natural gas,⁴⁶ water electrolysis, photo-electrolysis, photo-biological production and high-temperature water decomposition, hydrogenases are also a potential candidate for the production of hydrogen intended for a hydrogen economy.¹¹ For example, type-II hydrogenase can produce hydrogen from water with a turnover frequency (TOF) in the order of $10,000\text{ s}^{-1}$ and hence are of interests as a promising approach to renewable hydrogen production.¹⁰

From the previous section, it is clear that while a number of Fe-based structural models of the active site of [Fe]-hydrogenase have been reported, none containing the homologues ruthenium and osmium are known. Ruthenium and osmium metal complexes are generally more stable than their iron counterparts, and many are known to activate molecular hydrogen and catalyze hydrogen transfer reactions.^{47,48,49,50,51} The construction of structural mimics containing ruthenium and osmium metals should therefore contribute towards a better understanding of the nature of the active site in [Fe]-hydrogenase and its catalytic action.

In this thesis, the aim is to synthesise, and investigate the chemistry of, ruthenium and osmium metal complexes which are structural mimics of the [Fe]-hydrogenase. Specifically, the following are to be carried out:

1. Construct and characterize ruthenium and osmium structural mimics of the active site of the [Fe]-hydrogenase,
2. Study their structural, physical and chemical properties, and
3. Examine their catalytic potential for hydrogen generation and related hydrogenation reactions.

In addition, the idea of encapsulation of a metal centre with a protein as is found in metalloenzymes, is also extended to encapsulation with silica via a single source precursor.

1.6 References

1. Thauer, R. K. *Microbiology+* **1998**, *144* (9), 2377-2406.
2. Lubitz, W.; Ogata, H.; Rüdiger, O.; Reijerse, E. *Chem. Rev.* **2014**, *114* (8), 4081-4148.
3. Stephenson, M.; Stickland, L. H. *Biochem. J.* **1931**, *25* (1), 205.
4. Elsdon, S. *Trends Biochem. Sci.* **1981**, *6*, 251-253.
5. Frey, M. *Chembiochem* **2002**, *3* (2-3), 153-160.
6. Vignais, P. M.; Billoud, B.; Meyer, J. *FEMS Microbiol. Rev.* **2001**, *25* (4), 455-501.
7. Drennan, C. L.; Peters, J. W. *Curr. Opin. Struc. Biol.* **2003**, *13* (2), 220-226.
8. Jugder, B.-E.; Chen, Z.; Ping, D. T. T.; Lebhar, H.; Welch, J.; Marquis, C. P. *Microb. Cell Fact.* **2015**, *14* (1), 42.
9. Greening, C.; Berney, M.; Hards, K.; Cook, G. M.; Conrad, R. *P. Natl. Acad. Sci.* **2014**, *111* (11), 4257-4261.
10. Madden, C.; Vaughn, M. D.; Díez-Pérez, I.; Brown, K. A.; King, P. W.; Gust, D.; Moore, A. L.; Moore, T. A. *J. Am. Chem. Soc.* **2011**, *134* (3), 1577-1582.
11. Kim, D.-H.; Kim, M.-S. *Bioresour. Technol.* **2011**, *102*, 8423-8431.
12. Albracht, S.; Graf, E.-G.; Thauer, R. *FEBS Lett.* **1982**, *140* (2), 311-313.
13. Cao, Z.; Hall, M. B. *J. Am. Chem. Soc.* **2001**, *123* (16), 3734-3742.
14. Zirngibl, C.; Hedderich, R.; Thauer, R. *FEBS Lett.* **1990**, *261* (1), 112-116.
15. Zirngibl, C.; Dongen, W.; Schworer, B.; Bunau, R.; Richter, M.; Klein, A.; Thauer, R. K. *FEBS J.* **1992**, *208* (2), 511-520.
16. Hartmann, G. C.; Santamaria, E.; Fernández, V. M.; Thauer, R. K. *J. Biol. Inorg. Chem.* **1996**, *1* (5), 446-450.
17. Shima, S.; Thauer, R. K. *Chem. Rec.* **2007**, *7* (1), 37-46.
18. Pilak, O.; Mamat, B.; Vogt, S.; Hagemeyer, C. H.; Thauer, R. K.; Shima, S.; Vonnrhein, C.; Warkentin, E.; Ermler, U. *J. Mol. Biol.* **2006**, *358* (3), 798-809.
19. Lyon, E. J.; Shima, S.; Boecher, R.; Thauer, R. K.; Grevels, F.-W.; Bill, E.; Roseboom, W.; Albracht, S. P. *J. Am. Chem. Soc.* **2004**, *126* (43), 14239-14248.
20. Lyon, E. J.; Shima, S.; Buurman, G.; Chowdhuri, S.; Batschauer, A.; Steinbach, K.; Thauer, R. K. *FEBS J.* **2004**, *271* (1), 195-204.
21. Huhmann-Vincent, J.; Scott, B. L.; Kubas, G. J. *Inorg. Chim. Acta* **1999**, *294* (2), 240-254.

22. Thauer, R. K.; Klein, A. R.; Hartmann, G. C. *Chem. Rev.* **1996**, 96 (7), 3031-3042.
23. Shima, S.; Pilak, O.; Vogt, S.; Schick, M.; Stagni, M. S.; Meyer-Klaucke, W.; Warkentin, E.; Thauer, R. K.; Ermler, U. *Science* **2008**, 321 (5888), 572-575.
24. Shima, S.; Lyon, E. J.; Thauer, R. K.; Mienert, B.; Bill, E. *J. Am. Chem. Soc.* **2005**, 127 (29), 10430-10435.
25. Hiromoto, T.; Ataka, K.; Pilak, O.; Vogt, S.; Stagni, M. S.; Meyer-Klaucke, W.; Warkentin, E.; Thauer, R. K.; Shima, S.; Ermler, U. *FEBS Lett.* **2009**, 583 (3), 585-590.
26. Tard, C.; Pickett, C. J. *Chem. Rev.* **2009**, 109 (6), 2245-2274.
27. Snyder, B. S.; Rao, C. P.; Holm, R. H. *Aust. J. Chem.* **1986**, 39 (7), 963-974.
28. Fox, S.; Wang, Y.; Silver, A.; Millar, M. *J. Am. Chem. Soc.* **1990**, 112 (8), 3218-3220.
29. Shima, S.; Vogt, S.; Göbels, A.; Bill, E. *Angew. Chem.* **2010**, 122 (51), 10113-10117.
30. Shima, S.; Lyon, E. J.; Sordel-Klippert, M.; Kauß, M.; Kahnt, J.; Thauer, R. K.; Steinbach, K.; Xie, X.; Verdier, L.; Griesinger, C. *Angew. Chem.* **2004**, 116 (19), 2601-2605.
31. Kubas, G. J. *Chem. Rev.* **2007**, 107 (10), 4152-4205.
32. Fairhurst, S.; Henderson, R.; Hughes, D.; Ibrahim, S.; Pickett, C. *J. Chem. Soc. Chem. Comm.* **1995**, (15), 1569-1570.
33. Matsumoto, T.; Kabe, R.; Nonaka, K.; Ando, T.; Yoon, K.-S.; Nakai, H.; Ogo, S. *Inorg. Chem.* **2011**, 50 (18), 8902-8906.
34. Summers, P. A.; Calladine, J. A.; Ghiotto, F.; Dawson, J.; Sun, X.-Z.; Hamilton, M. L.; Towrie, M.; Davies, E. S.; McMaster, J.; George, M. W. *Inorg. Chem.* **2015**, 55 (2), 527-536.
35. Ott, S.; Kritikos, M.; Åkermark, B.; Sun, L. *Angew. Chem.* **2003**, 115 (28), 3407-3410.
36. Charreteur, K.; Capon, J. F.; Gloaguen, F.; Pétilion, F. Y.; Schollhammer, P.; Talarmin, J. *Eur. J. Inorg. Chem.* **2011**, 2011 (7), 1038-1042.
37. Guo, Y.; Wang, H.; Xiao, Y.; Vogt, S.; Thauer, R. K.; Shima, S.; Volkers, P. I.; Rauchfuss, T. B.; Pelmeshnikov, V.; Case, D. A. *Inorg. Chem.* **2008**, 47 (10), 3969-3977.
38. Zhong, W.; Zampella, G.; Li, Z.; De Gioia, L.; Liu, Y.; Zeng, X.; Luo, Q.; Liu, X. *J. Organomet. Chem.* **2008**, 693 (25), 3751-3759.
39. Chen, D.; Ahrens-Botzong, A.; Schünemann, V.; Scopelliti, R.; Hu, X. *Inorg. Chem.* **2011**, 50 (11), 5249-5257.
40. Chen, D.; Scopelliti, R.; Hu, X. *Angew. Chem. Int. Ed.* **2010**, 49 (41), 7512-7515.
41. Shima, S.; Chen, D.; Xu, T.; Wodrich, M. D.; Fujishiro, T.; Schultz, K. M.; Kahnt, J.;

- Ataka, K.; Hu, X. *Nat. Chem.* **2015**, 7 (12), 995.
42. Xu, T.; Yin, C.-J. M.; Wodrich, M. D.; Mazza, S.; Schultz, K. M.; Scopelliti, R.; Hu, X. *J. Am. Chem. Soc.* **2016**, 138 (10), 3270-3273.
 43. Seo, J.; Manes, T. A.; Rose, M. J. *Nat. Chem.* **2017**, 9 (6), 552-557.
 44. Corneil, H. G.; Heinzelmann, F. J. *Acs. Sym. Ser.* **1980**, 5, 67-94.
 45. Dutta, S. *J. Ind. Eng. Chem.* **2014**, 20 (4), 1148-1156.
 46. Soltani, R.; Rosen, M.; Dincer, I. *Int. J. Hydrogen Energ.* **2014**, 39 (35), 20266-20275.
 47. Rohmann, K.; Kothe, J.; Haenel, M. W.; Englert, U.; Hölscher, M.; Leitner, W., *Angew. Chem. Int. Ed.* **2016**, 55 (31), 8966-8969.
 48. Chen, M.-H.; Lin, J.-H.; Li, C.-R.; Gliniak, J.; Chang, C.-H.; Peng, C.-S.; Chen, Y.-T.; Jen-Shiang, K. Y.; Wu, T.-K. *Int. J. Hydrogen Energ.* **2017**, 42 (15), 9784-9794.
 49. Naziruddin, A. R.; Huang, Z.-J.; Lai, W.-C.; Lin, W.-J.; Hwang, W.-S. *Dalton Trans.* **2013**, 42 (36), 13161-13171.
 50. Bolano, T.; Esteruelas, M. A.; Fernandez, I.; Onate, E.; Palacios, A.; Tsai, J.-Y.; Xia, C. *Organometallics* **2015**, 34 (4), 778-789.
 51. Sullivan, B. P.; Caspar, J. V.; Meyer, T. J.; Johnson, S. *Organometallics* **1984**, 3 (8), 1241-1251.

Chapter 2: Ruthenium Structural Mimics of the [Fe]-Hydrogenase

As described in chapter 1, the construction of structural and functional models of the active site of [Fe]-hydrogenase has gained much interest over the last few years.^{1,2} The currently accepted model for the active site of [Fe]-hydrogenase consists of an iron centre coordinated by a thiolate ligand, two *cis*-carbonyl ligands and a chelating five-membered metallocyclic acyl pyridinyl ligand with an as-yet unidentified ligand which is most probably a solvent molecule (Figure 2.1).³

Figure 2.1. Current model of the active site of [Fe]-hydrogenase.

Mimics comprising iron complexes containing CO, thiolate, and pyridine ligands, have been found to display similar hydrogen activation property as the [Fe]-hydrogenase (Figure 2.2).^{4,5,6,7} Since ruthenium complexes are also known to catalyse hydrogen activation and hydrogen transfer reactions,^{8,9,10} it would therefore be worth developing ruthenium structural analogues of [Fe]-hydrogenase and study their reactivity as a hydrogenase mimic.

Figure 2.2. Functional models for the activation of hydrogen.

The synthetic challenge is to arrive at a complex with the correct relative orientation of all the different ligands. A number of studies on the synthesis of molecular models which mimic natural [Fe]-hydrogenase have already been published.^{11,12,13,14} A recent study showed that the

reaction of $\text{Fe}(\text{CO})_4\text{Br}_2$ with 2-aminopyridine gave $[\text{FeBr}(\text{2-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(\text{MeCN})]$, **5a'**, and its subsequent reaction with 2,6-dimethylbenzenethiol afforded $[\text{Fe}(\text{2-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{SC}_6\text{H}_3\text{Me}_2)(\text{CO})_2(\text{MeCN})]$, **7a'** (Scheme 2.1).¹⁵

Scheme 2.1. Synthetic route for the preparation of structural model of the active site of [Fe]-hydrogenase.

It can be expected that the ruthenium analogue should be accessible from $\text{Ru}(\text{CO})_4\text{Br}_2$. An attempt was therefore made at the synthesis of the ruthenium structural analogue $[\text{Ru}(\text{2-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{SC}_6\text{H}_3\text{Me}_2)(\text{CO})_2(\text{MeCN})]$, **7a** of the [Fe]-hydrogenase in three steps starting from $\text{Ru}(\text{CO})_4\text{Br}_2$ (**1a**).

2.1 Ruthenium structural mimics

2.1.1 Reaction of **1a** with 2-aminopyridine

The starting compound **1a** is available *via* a literature method from $\text{Ru}_3(\text{CO})_{12}$.¹⁶ The reaction of **1a** with an aminopyridine, **2n** in dichloromethane at 0 °C led to noticeable evolution of carbon monoxide and afforded $[\text{RuBr}_3(\text{CO})_3][\text{2-NH}_2\text{C}_5\text{H}_2\text{NHR}_1\text{R}_2]$, **3n** which was isolated from the yellow supernatant, and $[\text{RuBr}_2(\text{2-NHC}(\text{O})\text{C}_5\text{H}_2\text{NR}_1\text{R}_2)(\text{CO})_2][\text{2-NH}_2\text{C}_5\text{H}_2\text{NHR}_1\text{R}_2]$, **4n** which was obtained as a white precipitate (Scheme 2.2). This procedure followed that for the corresponding iron analogue $[\text{FeBr}_2(\text{2-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2][\text{2-NH}_2\text{C}_5\text{H}_4\text{NH}]$, **4a'** although the iron analogue for **3a**, *viz.*, $[\text{FeBr}_3(\text{CO})_3][\text{2-NH}_2\text{C}_5\text{H}_4\text{NH}]$, **3a'** was not reported.¹⁵

Installation of the 2-aminopyridine on **1a** involves direct coordination of pyridine to the ruthenium centre followed by intramolecular nucleophilic attack of the pendant amine group on a metal-bound carbonyl ligand to afford the carbamoyl complex **4n** (Scheme 2.3).¹³ The infrared spectra of the complexes **3n** showed three bands in the carbonyl region, consistent with a facial arrangement of three terminal carbonyl ligands.¹⁶ Their mass spectra also showed presence of the cation and anion in their corresponding detection mode. Thus the [*fac*-

$\text{RuBr}_3(\text{CO})_3^-$ molecular ion was observed at m/z 424.63 in the negative ion mode. The structure of this has been confirmed by a single crystal X-ray crystallographic study on complex **3a**. The formation pathway for **3n** is unclear, although it must involve some disproportionation reaction.

Scheme 2.2. Synthesis of **3n** and **4n**.

Scheme 2.3. Mechanism for the formation of carbamoyl complex.

The solid-state IR spectra of **4n** showed two stretches in the carbonyl region for the two *cis*-terminal carbonyl ligands, and a strong carbamoyl (C=O) peak at 1600-1650 cm^{-1} .^{10,16,17} All four complexes are very soluble in acetonitrile, less soluble in acetone, methanol and tetrahydrofuran. Their mass spectra also showed the cation and anion in their corresponding detection modes. The structure of **4a** was confirmed by a single crystal X-ray diffraction study; an ORTEP plot showing the molecular structure of the complex anion is given in Figure 2.3. It is isostructural with the known Fe analogue **4a'**; the carbamoyl pyridinyl forms a planar five-membered (RuNCNC) metallacycle with the ruthenium center.¹⁵ The Ru-C (carbamoyl) bond length is comparable to those found in other ruthenium(II) carbamoyl compounds.^{18,19} The two bromide ligands are in a mutually *cis* geometry, as are the two carbonyl ligands, and the carbamoyl pyridinyl unit has the pyridine *trans* to a CO and the carbamoyl *trans* to a Br. The Ru1-Br2 bond is significantly longer than the Ru1-Br1 bond ((2.6376(6) Å) and 2.5828(6) Å, respectively) and may be attributed to a stronger trans influence of the carbamoyl compared to the carbonyl ligand.¹³

Figure 2.3. ORTEP plot of the anion of **4a**. The counter cation and all hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru1-N1 = 2.130(4), Ru1-Br1 = 2.5828(6), Ru1-Br2 = 2.6376(6), Ru1-C1 = 1.874(5), Ru1-C2 = 1.887(5), Ru1-C8 = 2.010(5); C8-Ru1-N1 = 79.5(2), C8-Ru1-C2 = 90.6(2).

2.1.2 Reaction of **4n** with acetonitrile

Dissolution of the white precipitates **4n** in acetonitrile yielded the neutral compounds $[\text{RuBr}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_2\text{NR}_1\text{R}_2)(\text{CO})_2(\text{MeCN})]$, **5n** as white crystalline solids upon concentration (Scheme 2.4). This is also in accordance with the literature method for the synthesis of the corresponding iron analogue $[\text{FeBr}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(\text{MeCN})]$, **5a'**; ¹⁵ the more labile bromide *trans* to the carbamoyl ligand is replaced by acetonitrile.

Scheme 2.4. Synthesis of **5n**.

These complexes have all been completely characterized spectroscopically and in the case of **5a**, **5b** and **5d**, by single crystal x-ray diffraction studies as well. These complexes are all chiral but, as may be expected, are obtained as racemates (both the Δ and Λ forms); they crystallized on centrosymmetric space groups. An ORTEP plot showing the molecular structure of **5a** is given in Figure 2.4. A common atom labelling Scheme together with a table of selected bond parameters of **5a**, **5b** and **5d** are given in Table 2.1. All the ligands which were observed in **4n** retained their relative position in **5n** except that one of the bromide ligands has been substituted by an acetonitrile. The steric effect of the methyl substitution in the 6-position of the pyridine ring in **5b** is apparent from the larger angle between the pyridine ring and the acetonitrile ligand (N1-Ru-N2 of 101.6(1) $^\circ$ compared to 92.73(8) $^\circ$ and 93.9(3) $^\circ$ in **5a** and **5d**, respectively).

The effect of substitution at the 6-position of the aminopyridyl ligand (substituent R₂) also manifested itself in the spectroscopic characteristics of the complexes. Thus, while the IR spectrum of **5b** showed only two bands for the CO stretches, that for **5a**, **5c** and **5d** showed four, suggesting the presence of isomers in the latter complexes. This was also corroborated by

the ^1H NMR spectra, which showed two sets of peaks for **5a**, **5c** and **5d** but only one for **5b**. Pickett, *et. al.* have described the presence of three isomers for the iron analogue of **5a**, viz., **5a'**, on the basis of the IR spectral characteristics and a computational study.¹⁵

Figure 2.4. ORTEP plot of the complex **5a**.

Table 2.1. Common atom labelling Scheme and selected bond parameters for **5a**, **5b** and **5d**.

Parameters	Complexes		
	5a	5b	5d
	$R_1 = R_2 = \text{H}$	$R_1 = \text{H}, R_2 = \text{Me}$	$R_1 = \text{OH}, R_2 = \text{H}$
Ru-N1	2.110(2)	2.163(3)	2.118(7)
Ru-N2	2.182(2)	2.185(3)	2.189(8)
Ru-Br	2.560(3)	2.537(5)	2.535(1)
Ru-C1	1.893(3)	1.872(5)	1.863(9)
Ru-C2	1.886(2)	1.898(3)	1.899(9)
Ru-C3	2.015(2)	2.004(5)	2.034(9)
N1-Ru-N2	92.73(8)	101.6(1)	93.9(3)
N2-Ru-C2	95.52(8)	89.2(1)	95.5(3)
N1-Ru-C3	80.07(8)	79.8(1)	80.8(3)

difference agrees with the experimental ratio of 100:0 obtained from the NMR spectrum. The solid-state structure obtained for **5a** corresponds to the major isomer (isomer 1) while this ambiguity does not exist for **5b**.

A V-T ^1H NMR study conducted on **5a** shows broadening and coalescence of the resonances at higher temperatures (Figure 2.6). This is indicative of an isomerization process that is slow on the NMR timescale. The exchange rate and activation energy barrier estimated from the coalescence of the ~ 8.35 ppm doublet at 70°C are 12 s^{-1} and 75 kJ mol^{-1} , respectively. In contrast, the V-T ^1H NMR spectra of **5b** showed that the resonances did not broaden significantly, with the exception of the NH resonance (Figure A2.2); this is consistent with the observation that one isomer predominates.

Figure 2.6. The V-T ^1H NMR spectra of **5a**.

2.1.3 Reactions of **5a** and **5b** with thiophenols

In order to complete the synthesis of the structural mimic **7n**, attempts at the reaction of **5a** with thiophenols **6** were made but this failed to give any product even after stirring for 2 d at ambient temperature. In the presence of one equivalent of base (trimethylamine), dimeric $[\{\text{Ru}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(\mu^2\text{-SAr})\}_2]$ (**8a** or **8b**) was obtained instead (Scheme 2.5). Presumably, the reactions proceeded via the corresponding monomeric species **7n**, which were identified in solution by high resolution mass spectrometry but were too unstable to be isolated. Similar results were obtained from the reaction of **5b** with *p*-tolyl-SH but with 2,6-xylyl-SH, the reaction stopped at the monomer **7d**, which could be isolated. The stability of **7d** may be ascribed to steric protection afforded by the methyl groups at the 6- and 2,6-positions of the pyridine and phenyl rings, respectively. Attempts to cleave the Ru-S bridges in **8a-c** by reaction with phosphines (PMe_3 and PPh_3) failed, this is similar to that reported for the dimeric iron compound $[\{\text{Fe}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(\mu^2\text{-SC}_2\text{H}_4\text{OH})\}_2]$.¹⁵

Scheme 2.5. Synthesis of structural mimic **7d**.

Complex **7d** is the first known ruthenium-based structural mimic of the [Fe]-hydrogenase active site. It has been completely characterized, including by single crystal X-ray crystallography, and the ORTEP plot showing its molecular structure is given in Figure 2.7.

Figure 2.7. ORTEP plot of the structural mimic **7d**. All the hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru-N1 = 2.194(2), Ru-N2 = 2.171(2), Ru-S = 2.4564(7), Ru-C1 = 1.886(3), Ru-C2 = 1.876(3), Ru-C3 = 2.022(2), C3-Ru-N1 = 79.80(9), N1-Ru-N2 = 99.00(8), N2-Ru-C2 = 91.9(1).

The infrared spectrum of **7d** shows two intense absorption bands at 2041 and 1965 cm^{-1} for the *cis*-dicarbonyl ligands, which are shifted to lower wavenumbers with respect to the parent complex **5b** [2062 and 1989 cm^{-1}] but are still within the range for ruthenium(II) dicarbonyl complexes.^{10,17} This suggests that the ruthenium center in **7d** is more electron-rich than in **5b**.¹¹ The ruthenium center is six-coordinate, with the solvent site remaining occupied by a bound acetonitrile ligand. The Ru-S bond length of 2.4564(7) Å is close to literature values typical for such bonds [2.30–2.50 Å].^{20,21,22}

The dimeric complexes **8a–c** have also been characterized spectroscopically and structurally by single crystal X-ray crystallography. While all three are symmetrical $\text{Ru}^{\text{II}}\text{--Ru}^{\text{II}}$ homodimers bridged by two thiolate ligands, **8a** and **8b** are centrosymmetric but **8c** has approximate C_2 symmetry; the ORTEP plots of **8b** and **8c** are given in Figure 2.8. The dimers

8a and **8b** are achiral (they are centrosymmetric) and, although **8c** is chiral, the crystal is a racemic twin. The difference in the structure adopted by **8c** may be ascribed to steric interaction between the 6-methyl group on the pyridine ligand and the thiolate group. Their ^1H NMR spectra showed only one set of peaks; this is similar to that reported for the dimeric iron compound $[\{\text{Fe}(\text{COMe})(\text{CO})_2(\mu^2\text{-SPyMe})\}_2]$ for the [Fe]-hydrogenase model complexes, although they are supposed to show mixture of sets due to different diastereomers.¹⁴

Figure 2.8. ORTEP plots of **8b** (top) and **8c** (bottom).

A common atom labelling Scheme, together with a table of selected bond parameters, for **8a-c** are given in Table 2.2. The range of Ru-S bond lengths observed is similar to that for Ru-S bridged complexes reported in the literature (2.30-2.50 Å),^{20,21,22} but that *trans* to the carbamoyl ligand is significantly lengthened, consistent with the stronger *trans* influence of this ligand compared to the carbonyl ligand.^{14,15}

Table 2.2. Common atom labelling Scheme and selected bond parameters for **8a-c**.

Complex	8a	8b	8c*
Ar	<i>p</i> -tolyl	2,6-xylyl	<i>p</i> -tolyl
Ru-N	2.146(4)	2.148(2)	2.198(5); 2.175(5)
Ru1-S1	2.471(1)	2.4596(6)	2.446(2); 2.447(2)
Ru1-S2	2.517(1)	2.5240(6)	2.504(2); 2.550(2)
Ru-CO	1.888(6); 1.878(5)	1.884(3); 1.878(2)	1.866(8) - 1.891(7)
Ru-C(O)NH	2.044(5)	2.029(2)	2.017(8); 2.006(6)
N-Ru-C(O)NH	79.5(2)	79.2(1)	78.9(2); 79.5(2)
S-Ru-S	80.71(4)	77.12(2)	80.56(6); 79.66(6)

*exhibits racemic twinning.

2.1.4 Reaction of **7d** with phosphines

Compound **7d** contains a labile acetonitrile ligand which may be replaced by stronger ligands such as organophosphines. Indeed, the dropwise addition of PMe_3 to an acetonitrile solution of **7d** at ambient temperature afforded **9a** within 5 min (Scheme 2.6). Diffraction-quality yellow crystals of **9a** were obtained by slow diffusion of the above solution at room temperature; the ORTEP plot showing its molecular structure, together with selected bond parameters, are given in Figure 2.9.

Scheme 2.6. Synthesis of **9a**.

Complex **9a** is also a close structural mimic for the active site of the [Fe]-hydrogenase. The absorption bands for the *cis*-dicarbonyl ligands are shifted to even lower wavenumbers [2039 and 1972 cm^{-1}] compared to those for **5b** and **7d**, consistent with the stronger electron-donating ability of PMe_3 . On the other hand, the Ru-P bond [$2.4638(8)\text{ \AA}$] is on the longer end of typical Ru-P bond lengths [$2.306(1)$ - $2.410(1)\text{ \AA}$],^{21,23} and may be attributed to a stronger *trans* influence of the carbamoyl compared to the carbonyl ligand.¹³

Figure 2.9. ORTEP plot of the complex **9a**. All the hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru-N = 2.200(2), Ru-P = 2.4638(8), Ru-S = 2.4686(8), Ru-C1 = 1.882(4), Ru-C2 = 1.857(4), Ru-C3 = 2.057(3), C3-Ru-N = 77.96(9), N-Ru-P = 104.52(6), P-Ru-C2 = 87.6(1).

2.2 Other attempts at the synthesis of structural mimics

2.2.1 From the reaction of phosphine derivatives with thiols

Attempts to synthesize stable structural mimics from **5a** and **5b** were also made through the reaction of their phosphine-substituted derivatives. The monosubstituted derivatives **10a**, **10b** and **10c** were readily obtained through their reactions with one equivalent of phosphine (PMe_3 or PPh_3) at ambient temperature (Scheme 2.7).

Scheme 2.7. Synthesis of **10a-c**.

All three complexes have been completely characterized spectroscopically and structurally by single crystal X-ray crystallography. An ORTEP plot showing the molecular structure of **10a** is given in Figure 2.10; selected bond parameters, together with a common atom labelling Scheme, for them are collected in Table 2.3. Similar to **9a**, the Ru-P bond length in these complexes are significantly longer than those the range (2.306(1)-2.410(1) Å) reported for the analogues $[\text{RuCl}(\text{CO})(\text{PCy}_3)[\eta^2-(\text{CH}_2-\text{C}_6\text{H}_2\text{Me}_2)-(\text{N}_2\text{C}_3\text{H}_4)(\text{C}_6\text{H}_2\text{Me}_3)]]$ and $[\text{RuCl}_2(\text{CO})_2(\text{PPh}_3)_2]$.^{21,23} This can again be attributed to the stronger *trans* influence of the carbamoyl group.¹³

The reaction of **10c** with **6b** indeed afforded **9a** (Scheme 2.8) except that unlike the reactions above, it proceeded slowly, taking about 7 h at ambient temperature (Figure 2.11).

Scheme 2.8. Reaction of **10c** with **6b**.

Figure 2.10. ORTEP plot of the complex **10a**.

Table 2.3. Common atom labelling Scheme and selected bond parameters for **10a**, **10b** and **10c**.

Parameters	Complexes		
	10a*	10b	10c
	R ₂ = H, L = PPh ₃	R ₂ = H, L = PMe ₃	R ₂ = Me, L = PMe ₃
Ru-N	2.144(5)	2.121(2)	2.181(2)
Ru-P	2.476(1)	2.4252(9)	2.4844(6)
Ru-Br	2.5423(8)	2.5889(5)	2.5720(4)
Ru-C1	1.932(7)	1.880(3)	1.865(3)
Ru-C2	1.893(7)	1.886(3)	1.879(2)
Ru-C3	2.067(6)	2.077(3)	2.061(2)
C3-Ru-N	79.0(2)	78.9(1)	78.23(8)
N-Ru-P	99.3(1)	96.45(7)	106.23(5)
P-Ru-C2	88.9(2)	89.23(9)	86.93(8)

*exhibits twinning.

Figure 2.11. IR spectral monitoring of the reaction of **10c** with **6b**.

The analogous reaction with **10a**, on the other hand, afforded a new compound **11a** together with **8b** instead (Scheme 2.9). Compound **11a** has been completely characterized spectroscopically and structurally as well; the ORTEP plot showing its molecular structure is given in Figure 2.12. As may be expected the trans influence of the carbamoyl ligand led to a lengthening of the Ru-Br bond length (2.6631(6) Å *cf* 2.5423(8) Å in **10a**).

Scheme 2.9. Reaction of **10a** with **6b**.

Figure 2.12. ORTEP plot of the complex **11a**. All the hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru–N = 2.151(4), Ru–P1 = 2.3790(13), Ru–P2 = 2.3814(13), Ru–Br = 2.6631(6), Ru–C1 = 1.852(5), Ru–C2 = 2.005(5), C2–Ru–N = 79.20(18), C2–Ru–C1 = 91.6(2), N–Ru–Br = 89.05(11).

Complex **11a** most likely resulted from the disproportionation of **10a**, and this was investigated through monitoring a solution of the latter at ambient temperature by $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy (Figure 2.13). The initial spectrum showed a resonance at 10.4 ppm ascribable to **10a**, as well as a very weak resonance at -5.00 ppm corresponding to free PPh_3 .²⁴ Both resonances slowly decreased and disappeared, and a new peak at 32.4 ppm, ascribable to **11a**, emerged. An IR spectrum taken of the mixture after 12 h showed the presence of carbonyl peaks for **5a**. Similarly, monitoring the reaction of **5a** with one equivalent of PPh_3 by $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy showed the formation of **10a** within 15 min, but this slowly disproportionated into a mixture of **5a** and **11a** over 12 h. This disproportionation is similar to that reported for the corresponding iron analogue $[\text{FeBr}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(\text{MeCN})]$, **5a'**, which gave a mixture of the mono- and di-substituted products $[\text{FeBr}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(\text{PPh}_3)]$, **10a'** and $[\text{FeBr}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})(\text{PPh}_3)_2]$, **11a'**, respectively.¹³

2.2.2 Attempt at installing the thiolate ligand first

An alternative synthetic pathway to the [Fe]-hydrogenase structural mimic through installation of the thiolate ligand before subsequent reaction with 2-aminopyridine was also made. The reaction of **1a** with **6b** resulted in the formation of the dimeric complex **12** together with disulphide (Scheme 2.10). An attempt to cleave the Ru-S bridges in **12** by reaction with PMe₃ was unsuccessful.

Scheme 2.10. Reaction of **1a** with thiol.

Compound **12** has been completely characterized spectroscopically and by a single crystal x-ray structural study; the ORTEP plot showing its molecular structure is given in Figure 2.14. It consists two crystallographically independent asymmetric molecules. Selected bond parameters of each independent molecule are collected in Table 2.4 together with a common atom labelling scheme. Each molecule contains an Ru^I-Ru^I homodimer bridged by two thiolate ligands in a butterfly structure.

Figure 2.14. ORTEP plot of the complex **12**.

Table 2.4. Common atom labelling scheme and selected bond parameters for two independent molecules of **12**.

Parameters	12	
	A	B
Ru1-S1	2.447(1)	2.450(2)
Ru1-S2	2.441(2)	2.442(2)
Ru1-Ru2	2.6211(6)	2.6214(6)
Ru2-S1	2.435(1)	2.441(1)
Ru2-S2	2.457(2)	2.452(2)
Ru1-C1	1.909(7)	1.894(5)
Ru1-C2	1.905(5)	1.907(7)
Ru1-C3	1.945(5)	1.959(6)
Ru2-C4	1.882(6)	1.900(7)
Ru2-C5	1.888(5)	1.900(5)
Ru2-C6	1.972(7)	1.972(7)

2.2.3 An attempt with a chloro derivative

An attempt at the synthesis of a chloro analogue of **5a** was made via $\text{Ru}(\text{CO})_4\text{Cl}_2$, **1b** (Scheme 2.11). As in the case of **5a**, the complex **5e** exists as a mixture of two isomers. Its reaction with one equivalent of PPh_3 gave the di-substituted derivative **11b**, which has been completely characterized, including x-ray crystallographically; a common atom labelling scheme together with selected bond parameters of **11b** and **11a** are collected in Table 2.5. Its subsequent reaction with **6b** failed to give any product except the disulphide of **6b**.

Scheme 2.11. Synthesis of chloro derivative.

Table 2.5. Common atom labelling scheme and selected bond parameters for **11a** and **11b**.

Parameters	11a	11b
Ru-N	2.151(4)	2.146(3)
Ru-P1	2.379(1)	2.377(1)
Ru-P2	2.381(1)	2.371(1)
Ru-X	2.6632(6)	2.508(1)
Ru-C1	1.853(6)	1.844(5)
Ru-C2	2.004(5)	2.008(4)
C2-Ru-N	79.2(2)	79.5(1)
N-Ru-X	89.0(1)	87.48(8)
X-Ru-C1	100.1(2)	103.1(1)

2.3 Reaction of **5n** with papain

The successful reaction of **5n** with thiols suggested that they may also be incorporated into an enzyme via reaction with a cysteine residue. Hence, an attempt was made at the conjugation of **5n** onto papain as the protein host (Scheme 2.12).²⁵

Scheme 2.12. Synthesis of mimic of [Fe]-hydrogenase.

Papain, from *Carica papaya*, possesses a free sulfhydryl (Cys25) and an imidazole (His159) moiety in its active site (Figure 2.15).²⁵ The Cys25 and His159 residues are in close proximity through H-bonding between their heteroatoms, with a separation of ~ 3.65 Å, and they are mainly responsible for the peptidase activity of papain.²⁶ The peptidase activity, i.e., the cleavage of peptide bonds, involves deprotonation of Cys25 by His159 acting as an internal base, followed by nucleophilic attack of Cys25 on the carbonyl carbon of the peptide backbone (Scheme 2.13).

Figure 2.15. (a) X-ray structure of papain. (b) A close-up structure on the active site of papain.

Scheme 2.13. Mechanism of peptidase activity of papain.

As preliminary studies, the reactions of N-acetyl-L-cysteine-methyl ester and imidazole with **5b** in acetonitrile were first carried out giving **8e** and **10d**, respectively (Scheme 2.14). Both products were characterized by IR and ^1H NMR spectroscopy, and mass spectrometry, and the data were in accord with their formulations. In analogy to **8a-c**, the formation of **8e** presumably proceeded via an intermediate **7e**.

Scheme 2.14. Reaction of **5b** with cysteine and imidazole.

A reaction of **5b** with imidazole, followed by the addition of N-acetyl-L-cysteine-methyl ester, also gave **8e** as the major product (Scheme 2.15). This suggests that coordination of the imidazole to ruthenium, like acetonitrile, is labile. It also suggests that other functional groups containing an NH moiety are not likely to be reactive to the ruthenium complex. Furthermore, since the reaction proceeded without the need of an external base, it suggests that imidazole can act as an internal base for the reaction.

Scheme 2.15. Reaction of **5b** with cysteine and imidazole in presence of water.

A similar reaction of **5b** in acetonitrile with an aqueous solution mixture of N-acetyl-L-cysteine-methyl ester and imidazole also afforded **8e**, suggesting that complex **5b** is stable in aqueous medium. It can therefore be expected that the reaction of **5b** with papain should afford the monomeric bioconjugated product **13b**; since Cys25 resides in the pocket of papain, formation of the monomer instead of the dimer may be favored by steric crowding around the ruthenium center.

2.3.1 Kinetic studies

The conjugation of papain with **5n** may be assessed through measurement of the hydrolysis rate of N- α -carbobenzoxy-glycine p-nitrophenyl ester (ZGlyONP).²⁷ Papain catalyses the hydrolysis of ZGlyONP to afford p-nitrophenol and N-[(benzyloxy)carbonyl]glycine in a pH 7.8 buffer medium (Scheme 2.16). Conjugation of **5n** to the active site of papain will lead to a decrease in its hydrolytic activity and hence of p-nitrophenol; the latter can be monitored spectrophotometrically ($\lambda_{\text{max}} = 415 \text{ nm}$).

Scheme 2.16. Hydrolysis of ZGlyONP.

For the measurements, ZGlyONP (100 μM) was used with a mixture of papain (0.59 μM) and **5n** (19 μM) which has been incubated for various lengths of time; the absorbance at 415 nm was measured over a period of 2 min. The rate of hydrolysis decreased with the incubation time of papain with **5n**. A plot of the logarithm of the hydrolysis rate (V_0) as a function of incubation time was linear, indicating first-order kinetics for the reaction between papain and **5n** (Figure 2.16); the slope defined the pseudo-first order rate constant k_{obs} of the reaction. The rate was almost zero with 3 days of incubation, indicating complete inhibition. The rate constants for all the ruthenium complexes are very close to each other, suggesting similar behavior towards papain and also successful conjugation.

Figure 2.16. Graph of ZGlyONp hydrolysis rate (V_0) vs incubation time for **5a**, **5b**, **5c** and **5d** (top), and the pseudo-first order rate constants (k_{obs}) obtained.

As commercial samples of papain contain impurities that may affect conjugation, measurements were also made with affinity-purified papain; the rate of hydrolysis for affinity-purified papain (0.06 μM) conjugated with **5b** (19 μM) decreased much faster than that without purification, with activity dropping to almost zero with 2 h incubation (Figure 2.17). Similar

inhibitions were observed for **5b-d**. An attempt at conjugation with the iron analogue **5a'** was unsuccessful due to decomposition in aqueous medium.

Figure 2.17. Graph of % activity of papain vs incubation time.

The bioconjugate **13b** was further characterized by FT-IR and UV spectroscopy, and mass spectrometry. The FT-IR measurements of the ruthenium complex **5b** and its papain bioconjugate **13b** were carried out via ATR on samples deposited onto nitrocellulose membranes. These membranes are transparent only between 1800 and 2200 cm^{-1} . The ATR spectrum of bioconjugate **13b** shows two characteristic carbonyl bands (2064 and 2000 cm^{-1}) which are shifted to lower wavenumbers than in **5b** (Figure 2.18). The C $\equiv$ N stretch of metal-bound acetonitrile cannot be assigned as the acetonitrile ligand is very labile; in the solid state and in the absence of acetonitrile as solvent, it is easily lost. The mass spectrum shows molecular ion peaks assignable to **13b**, as well as [**13b**+MeOH] and [**13b-5b**] fragments (Figure 2.19). The UV-visible spectrum of bioconjugate **13b** shows two peaks, at 279 and ~318 nm (Figure 2.20). The former may be ascribed to papain,²⁶ and the latter to the ruthenium centre.

Figure 2.18. Infrared spectra of **5b** (gray dotted line) and **13b** (black full line). Solutions were deposited on nitrocellulose membrane and dried in air.

Figure 2.19. Mass spectrum of **13b**.

Figure 2.20. An overlay of the UV-visible spectra of **5b** (blue), papain (black) and **13b** (red).

2.3.2 Computational studies

As an aid to verification of the formation of a bioconjugate, a computational study on a series of complexes which are very close in structure to the expected bioconjugate **13b** was carried out (Figure 2.21). The synthesis of **15b** will be described in the next chapter; **10d** was obtained from the reaction of **5b** with imidazole, and its subsequent reaction with 2,6-dimethylbenzenethiol afforded **9b**. The IR and ^1H NMR spectra of **9b** showed that it existed as two isomers, and a computational study showed that isomer 2 was the more stable, by 1.2 kJ/mol; the isomeric ratio (40:60) calculated from the free energy difference was consistent with the experimental ratio of 45:55 obtained from the NMR spectrum.

Figure 2.21. Structural model for the active site of the [Fe]-hydrogenase.

The computed and experimental (ATR) IR spectra for all the complexes are shown in Table 2.6. Using the difference in frequency between the two CO stretches for comparison, it is observed that the experimental and computed values for the bioconjugate are close to those for **7d**, isomer 2 of **9b**, and **15b**, suggesting that the structure of **13b** is similar to these, i.e., it is coordinated by a thiolate (S-donor, cysteine) as well as an imidazole (N-donor, histidine) ligand.

Table 2.6. ν_{CO} (cm^{-1}) for all the complexes.

Compounds	Experimental (ATR)		Theoretical (Gas phase)		
	ν_{CO}	$\Delta\nu$	ν_{CO}	$\Delta\nu$	ν_{CO} (scaled)*
5b	1977, 2060	83	1981, 2057	76	1982, 2058
10d	1975, 2049	74	1962, 2051	89	1963, 2052
7d	1960, 2038	78	1975, 2043	68	1976, 2044
9b (Isomer 1)	1959, 2039	80	1956, 2036	80	1957, 2037
9b (Isomer 2)	1984, 2056	72	1976, 2038	62	1977, 2039
15b	1983, 2043	60	1979, 2052	73	1980, 2053
Bioconjugate 13b	2000, 2064	64	-	-	-

*Scale factor: $\nu_{\text{CO}}(\text{obs})/\nu_{\text{CO}}(\text{calc}) = 1.0004$ (obtained by averaging over all ir values of all compounds).

2.4 Conclusion

Ruthenium-based structural mimics of the active site of [Fe]-hydrogenase, $[\text{Ru}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(o,o\text{-Me}_2\text{-C}_6\text{H}_3\text{S})(\text{L})]$ (where $\text{L} = \text{MeCN}$ or PMe_3), have been synthesized; bulky ligands around the ruthenium center, in particular substitution at the 6-position of the pyridine ligand, are required to prevent dimerisation (Scheme 2.17). This could be relevant to the chemistry of the natural [Fe]-hydrogenase as it also possesses a substituent at the same position.

Scheme 2.17. Synthesis of structural mimic.

Although awaiting confirmation, embedding of a close mimic into papain has also been achieved, in which the ruthenium center appeared to have been anchored via the cysteine and an imidazole residue of the active site responsible for its peptidase activity.

2.5 Experimental section

2.5.1 General procedures: All experiments were performed under an argon atmosphere using standard Schlenk techniques except bio-conjugation experiment. All chemicals were obtained from Sigma Aldrich and were used as received whereas papain was obtained from Calbiochem and purified it before use for the bio-conjugation. It was purified by gel filtration on a gel desalting column (Hiprep desalt 26/10, GE Healthcare) using 0.15 M NaCl solution as an eluent. Absorbance spectroscopy for the bio-conjugation was recorded using Varian Cary 50 spectrometer. ESI-MS analyses for the bio-conjugation were carried on LTQ-Orbitrap. Solvents that were used for reactions were distilled over the appropriate drying agents under argon before use except bio-conjugation experiment. Purification of compounds was carried out by column chromatography on silica gel. Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer. Solution spectra were recorded in a solution IR cell with NaCl windows and a path length of 0.1 mm at a resolution of 2 cm⁻¹, except for the bioconjugation experiments in which the solution spectra were recorded using an ATR attachment. Solid-state spectra were recorded as KBr pellets. HRMS were recorded in ESI mode on a Waters UPLC-Q-ToF MS mass spectrometer. Nominal masses were recorded in ESI mode on a Thermo Deca Max mass spectrometer with an ion-trap mass detector. ¹H, ¹³C{¹H} and ³¹P{¹H} NMR spectra were obtained on a JEOL 400 MHz spectrometer at room temperature unless otherwise stated. Chemical shifts for ¹H and ¹³C{¹H} were referenced with respect to the residual resonances of the respective deuterated solvents (¹H NMR: δ_H = 7.26 for CDCl₃, δ_H = 2.05 for (CD₃)₃CO, δ_H = 1.94 for CD₃CN, ¹³C NMR: δ_C = 77.0 for CDCl₃, δ_C = 29.8 for (CD₃)₃CO, δ_C = 1.32 for CD₃CN). Chemical shifts for ³¹P{¹H} were referenced with respect to 85% aqueous H₃PO₄.

2.5.2 Synthesis of 3-5

A solution of Ru(CO)₄Br₂ (0.53 mmol) and **2n** (0.8 mmol) in dichloromethane (10 mL) were stirred for 1h at 0 °C. The mixture was subsequently left to stir for another 30 min at room temperature to give a white precipitate which was collected via filtration and washed with hexane (20 mL) followed by dichloromethane (40 mL) to afford analytical pure **4n**. The yellow supernatant was rotary evaporated to dryness and the residue re-crystallized from dichloromethane/hexane to afford yellow crystals of **3n**. Dissolution of **4n** in acetonitrile (30 mL) followed by slow evaporation of the solvent at room temperature afforded **5n** as white crystals. Experimental and characterization data are given in Tables 2.7-2.10.

Table 2.7. Yield, HRMS and IR data for **3n** and **4n**.

Compound	Yield (mg, mmol, %)	*HRMS (m/z) ([M+H] ⁺ for 3n and [M] ⁻ for 4n) Found (calculated)	ν_{CO} (cm ⁻¹) **
3a	188, 0.36, 32	95.4419 (95.4462)	2125 (s), 2059 (s), 2009 (w)
3b	146, 0.28, 30	109.0766 (109.0769)	2125 (s), 2061 (s), 1996 (s)
3c	197, 0.33, 34	153.0664 (153.0668)	2123 (s), 2052 (s), 2002 (s)
3d	125, 0.23, 44	112.3858 (112.3822)	2120 (s), 2050 (s), 2006 (s)
4a	237, 0.45, 48	Found: 438.7878 Calculated for C ₈ H ₇ N ₂ O ₃ Br ₂ Ru: 438.7867	2061 (s), 1999 (s)
4b	159, 0.29, 54	Found: 452.7867 Calculated for C ₉ H ₇ N ₂ O ₃ Br ₂ Ru ¹⁰⁴ : 452.7878	2046 (s), 1973 (s)
4c	240, 0.38, 60	Found: 496.7929 Calculated for C ₁₀ H ₉ N ₂ O ₅ Br ₂ Ru: 496.7922	2056 (s), 1984 (s)
4d	132, 0.32, 55	Found: 454.3363 Calculated for C ₈ H ₅ N ₂ O ₄ Br ₂ Ru: 454.3381	2056 (s), 1988 (s)

*Calculated values are for ¹⁰²Ru unless otherwise indicated. For all **3n**, [M]⁻ observed at 422.6471; calculated for C₃O₃Br₃Ru: 422.6436.

** All **3** measured in DCM and all **4**-measured using KBr.

Elemental analyses:

4a: Found C 27.4, H 1.8, N 8.4; Calculated for C₁₃H₁₂N₄O₃Br₂Ru.CH₂Cl₂: C 27.2, H 2.3, N 9.1.

4b: Found C 32.56, H 2.50, N 10.11; Calculated for C₁₅H₁₆N₄O₃Br₂Ru: C 32.10, H 2.87, N 9.98.

Table 2.8. Yield, MS and IR data for **5n**.

Compound	Yield (mg, mmol, %)	MS (m/z)		ν_{CO} (ACN, cm^{-1})
		*HRMS ⁺ ([M+H] ⁺)	MS ⁺	
5a	29, 0.07, 72	Found: 399.8876 Calculated for C ₁₀ H ₉ N ₃ O ₃ BrRu: 399.8871	361 [M-MeCN+H], 278 [M-MeCN-Br]	2061 (s), 1999 (s), 1989 (s)
5b	24, 0.06, 58	Found: 413.9021 Calculated for C ₁₁ H ₁₁ N ₃ O ₃ BrRu: 413.9027	372 [M-MeCN+H] 293 [M-MeCN-Br]	2061 (s), 1988 (s)
5c	22, 0.05, 46	Found: 459.8926 Calculated for C ₁₂ H ₁₁ N ₃ O ₅ BrRu: 459.8936	417 [M-MeCN+H] 337 [M-MeCN-Br]	2064 (s), 2003 (s), 1994 (s)
5d	14, 0.03, 34	Found: 417.8826 Calculated for C ₁₀ H ₉ N ₃ O ₄ BrRu ¹⁰⁴ : 417.8831	375 [M-MeCN+H] 295 [M-MeCN-Br]	2060 (s), 1996 (s), 1986 (s)

* Calculated values are for ¹⁰²Ru unless otherwise indicated.

Elemental analyses:

5a: Found C 26.90, H 1.86, N 7.21; Calculated for [C₁₀H₈N₃O₃BrRu-CH₃CN] = C₈H₅N₂O₃BrRu: C 26.83, H 1.41, N 7.82.

5b: Found C 31.70, H 2.40, N 10.48; Calculated for C₁₁H₁₀N₃O₃BrRu: C 31.98, H 2.44, N 10.17.

Table 2.9. ¹H NMR data for **4n**.

Compound	¹ H NMR (<i>d</i> ₆ -acetone)
4a	9.42 (br, 1H, NH), 9.26-9.25 (br, 2H, NH), 8.14-8.07 (m, 2H, PyH), 7.82-7.78 (m, 2H, PyH), 7.27-7.25 (d, 1H, <i>J</i> = 9.2 Hz, PyH), 7.10-7.04 (m, 2H, PyH), 6.95-6.93 (m, 1H, PyH)
4b	9.51-9.52 (br, 1H, NH), 7.93-7.89 (t, 1H, <i>J</i> = 9.2 Hz, PyH), 7.69-7.65 (t, 1H, <i>J</i> = 8.2 Hz, PyH), 7.60-7.62 (br, 2H, NH), 7.00-6.98 (d, 1H, <i>J</i> = 8.7 Hz, PyH), 6.93-6.91 (d, 1H, <i>J</i> = 8.2 Hz, PyH), 6.86-6.80 (m, 2H, PyH), 2.94 (s, 3H, Me), 2.58 (s, 3H, Me)
4c	9.48-9.47 (br, 1H, NH), 9.33-9.32 (br, 2H, NH), 8.20 (s, 1H, PyH), 7.92 (s, 1H, PyH), 7.37-7.35 (d, 1H, PyH), 6.99-6.97 (d, 1H, PyH), 3.42 (s, 3H, Me), 3.95 (s, 3H, Me)
4d	9.14-9.13 (d, 1H, <i>J</i> = 5.5 Hz, PyOH), 8.23-8.22 (br, 1H, NH), 7.95-7.91 (t, 1H, <i>J</i> = 6.9 Hz, PyH), 7.86-7.84 (m, 1H, PyH), 7.11-7.06 (m, 3H, PyH)

Table 2.10. ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR data for **5n**.

Compound	^1H NMR (d_3 -acetonitrile)	$^{13}\text{C}\{^1\text{H}\}$ NMR (d_3 -acetonitrile)
5a	<i>Isomer 1</i> : 8.54-8.552 (br, 1H, NH), 8.37-8.36 (d, 1H, $J = 5.5$ Hz, PyH), 7.84-7.80 (m, 1H, PyH), 7.03-7.00 (m, 2H, PyH). <i>Isomer 2</i> : 9.10-9.09 (d, 1H, $J = 5.5$ Hz, PyH), 8.71-8.69 (br, 1H, NH), 7.89-7.85 (m, 1H, PyH), 7.06-7.03 (m, 2H, PyH).	<i>Isomer 1</i> : 195.8 (CO), 195.4 (CO), 191.2 (NHCO), 157.5, 147.5, 140.6, 116.5, 109.3. <i>Isomer 2</i> : 198.8 (CO), 198.2 (CO), 195.6 (NHCO), 158.0, 148.6, 141.2, 116.8, 109.5
5b	8.52-8.50 (br, 1H, NH), 7.67-7.63 (t, 1H, $J = 7.8$ Hz, PyH), 6.88-6.86 (t, 1H, $J = 7.8$ Hz, PyH), 6.82-6.80 (d, 1H, $J = 8.2$ Hz, PyH), 2.65 (s, 3H, Me), 1.97 (s, 3H, Me).	(d_6 -acetone): 195.1 (CO), 191.8 (CO), 159.5 (NHCO), 158.4, 140.2, 117.2, 116.6, 106.9, 25.5.
5c	9.24-9.23 (d, 1H, $J = 6.0$ Hz, PyH), 8.87-8.85 (br, 1H, NH), 8.71-8.69 (br, 1H, NH), 8.51-8.50 (d, 1H, $J = 6.0$ Hz, PyH), 7.51-7.49 (m, 2H, PyH), 7.48-7.46 (m, 2H, PyH), 3.94 (s, 3H, CH_3), 3.92 (s, 3H, CH_3).	199.1 (CO), 192.8 (CO), 162.5 (NHCO), 159.4, 149.7, 149.2, 114.6, 114.1, 109.3, 51.5
5d	9.17 (br, 1H, NH), 8.98-8.96 (m, 1H, PyH), 7.96-7.94 (m, 1H, PyH), 7.31 (br, 1H, OH), 6.58-6.56 (br, 1H, OH), 6.50-6.44 (m, 4H, PyH)	Not measured

2.5.3 Synthesis of **7** and **8**

In a typical reaction, triethyl amine (7 μ L, 0.05 mmol) and **6a** (0.05 mmol) were added into a solution of **5a** (0.05 mmol) in acetonitrile (5 mL). The resulting solution was then stirred for 2 h at room temperature, which gave a white precipitate over a few minutes. The resulting solution was then analyzed by mass spectrometry to identify the formation of **7a**. The white precipitate was separated via filtration and washed with acetonitrile (20 mL) to afford analytical pure **8a**.

An analogous procedure was followed for the syntheses of **7b-c** and **8b-c**.

Triethyl amine (7 μ L, 0.05 mmol) and **6b** (0.05 mmol) were added into a solution of **5b** (0.05 mmol) in acetonitrile (5 mL). The resulting solution was then stirred for 5 min at room temperature. Slow evaporation of the solvent at room temperature afforded analytical pure **7d** as yellow crystals.

Characterisation data are given in Tables 2.11 and 2.12.

2.5.4 Synthesis of **9a**

A sample of PMe_3 (5 μ L, 0.05 mmol) was added into a solution of **7d** (20 mg, 0.05 mmol) in acetonitrile (5 mL). The resulting mixture was then stirred for 30 min at ambient temperature and slow evaporation of the solvent at room temperature afforded **9a** as yellow crystals. **9a** (29 mg, 0.07 mmol, 72 %). ν_{CO} (ACN, cm^{-1}): 2039 (s), 1972 (s). ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$): δ 8.63 (br, 1H, NH), 7.58-7.54 (m, 1H, $J = 7.8$ Hz, PyH), 7.07-7.05 (d, 2H, $J = 7.8$ Hz, ArH), 6.99-6.97 (m, 1H, ArH), 6.89-6.87 (d, 1H, $J = 7.8$ Hz, PyH), 6.60-6.58 (d, 1H, $J = 7.8$ Hz, PyH), 2.23 (s, 6H, Me), 1.96 (s, 3H, Me), 1.70 (s, 9H, Me) ppm. ^{31}P NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$): δ -32.5 (s, PMe_3). HRMS $^+$ (m/z): found 507.0436 $[\text{M}+\text{H}]^+$, calculated for $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_3\text{SPRu}^{102}$: 507.0445 $[\text{M}+\text{H}]^+$.

2.5.5 Synthesis of **10n**

In a typical reaction, PPh_3 (0.05 mmol) was added into a solution of **5a** (0.05 mmol) in acetonitrile (5 mL). The resulting mixture was then stirred for 30 min at ambient temperature. Slow evaporation of the solvent at room temperature afforded **10a** as white crystals after 2 d.

An analogous procedure was followed for the syntheses of **10b** and **10c** using PMe_3 (0.05 mmol) instead of PPh_3 .

Characterisation data for **10n** are given in Tables 2.13 and 2.14.

Table 2.11. Yield, MS and IR data for **7n** and **8n**.

Compound	Yield (mg, mmol, %)	MS (m/z)		ν_{CO} (ACN, cm^{-1})
		HRMS ⁺ ([M+H] ⁺)	MS ⁺	
8a	25, 0.03, 60	Found 805.9443 Calculated for $\text{C}_{30}\text{H}_{27}\text{N}_4\text{O}_6\text{S}_2\text{Ru}^{101}\text{Ru}^{102}$: 805.9471	806 [M+H] 775 [M-CO]	2052 (m), 2036 (s), 1976 (m), 1948 (s)
8b	27, 0.03, 68	Found 833.9730 Calculated for $\text{C}_{32}\text{H}_{26}\text{N}_5\text{O}_8\text{S}_2\text{Ru}^{98}$: 833.9717	834 [M+H] 805 [M-CO]	2044 (s), 2015 (w), 1977 (s), 1945 (w)
8c	30, 0.04, 74	Found 832.9595 Calculated for $\text{C}_{31}\text{H}_{28}\text{N}_5\text{O}_6\text{S}_2\text{Ru}^{99}\text{Ru}^{104}$: 832.9626	832 [M+H] 804 [M-CO]	2044 (s), 2016 (s), 1970 (s), 1941 (m)
7d*	13, 0.03, 55	Found 472.0539 Calculated for $\text{C}_{19}\text{H}_{22}\text{N}_3\text{O}_3\text{SRu}^{102}$: 472.0520	471 [M+H] 443 [M-CO]	2046 (s), 1978 (s)

*Compounds **7a-c** were characterized by nominal mass MS as follows: **7a**: 465 [M+Na]⁺; **7b**: 457 [M+H]⁺; **7c**: 480 [M+Na]⁺.

Elemental analyses:

8b: Found C 46.33, H 3.33, N 6.98, S 7.72; Calculated for $\text{C}_{32}\text{H}_{28}\text{N}_4\text{O}_6\text{S}_2\text{Ru}_2$: C 46.26, H 3.40, N 6.74, S 7.72.

7d: Found C 48.47, H 4.46, N 9.21, S 6.73; Calculated for $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_3\text{SRu}$: C 48.50, H 4.07, N 8.93, S 6.81.

Table 2.12. ¹H NMR data for **7n** and **8n**.

Compound	¹ H NMR (<i>d</i> ₆ -dmsO)
8a	10.70 (br, 1H, NH), 8.35-8.34 (d, 1H, <i>J</i> = 5.04 Hz, PyH), 8.00-7.95 (m, 1H, PyH), 7.47-7.45 (d, 2H, <i>J</i> = 8.72 Hz, PhH), 7.16-7.14 (d, 1H, <i>J</i> = 8.24 Hz, PyH), 7.05-7.03 (d, 2H, <i>J</i> = 5.04 Hz, PhH), 6.93-6.89 (m, 1H, <i>J</i> = 5.04 Hz, PyH), 2.25 (s, 3H, Me)
8b	9.36-9.35 (d, 1H, NH), 7.77-7.74 (m, 1H, PyH), 7.67-7.62 (m, 2H, ArH), 7.48-7.43 (m, 2H, PyH), 7.27-7.24 (m, 1H, PyH), 6.96-6.88 (m, 3H, ArH), 2.20 (s, 6H, Me)
8c	10.50 (br, 1H, NH), 7.80-7.77 (t, 1H, <i>J</i> = 8.2 Hz, PyH), 7.52-7.51 (d, 2H, <i>J</i> = 8.2 Hz, PhH), 7.03-7.01 (d, 2H, <i>J</i> = 7.8 Hz, PhH), 6.90-6.91 (d, 1H, <i>J</i> = 4.9 Hz, PyH), 6.82-6.80 (d, 1H, <i>J</i> = 8.2 Hz, PyH), 2.49 (s, 3H, Me), 2.24 (s, 3H, Me)
7d	(<i>d</i> ₃ -acetonitrile): 8.56 (br, 1H, NH), 7.75-7.71 (t, 1H, <i>J</i> = 7.4 Hz, PyH), 7.03-7.01 (d, 2H, <i>J</i> = 7.8 Hz, PyH), 6.94-6.87 (m, 3H, PyH), 2.48 (s, 3H, Me), 2.28 (s, 6H, Me)

Table 2.13. Yield, MS and IR data for **10n**.

Compound	Yield (mg, mmol, %)	MS (m/z)		ν_{CO} (ACN, cm^{-1})
		HRMS ⁺ ([M+H] ⁺)	MS ⁺ (m/z)	
10a	21, 0.03, 68	Found 622.9905 Calculated for $\text{C}_{26}\text{H}_{20}\text{N}_2\text{O}_3\text{BrPRu}^{104}$: 622.9930	622 [M+H] 544 [M-Br]	2058 (s), 1988 (s)
10b	29, 0.07, 72	Found 436.9074 Calculated for $\text{C}_{11}\text{H}_{15}\text{N}_2\text{O}_3\text{BrPRu}^{104}$: 436.9058	436 [M+H] 357 [M-Br]	2052 (s), 1982 (s)
10c	34, 0.09, 82	Found 450.9232 Calculated for $\text{C}_{12}\text{H}_{17}\text{N}_2\text{O}_3\text{BrPRu}^{104}$: 450.9214	450 [M+H] 370 [M-Br]	2052 (s), 1983 (s)

Elemental analyses:

10a: Found C 51.03, H 2.82, N 6.26; Calculated for $\text{C}_{26}\text{H}_{20}\text{N}_2\text{O}_3\text{PBrRu} \cdot \text{CH}_3\text{CN}$: C 50.84, H 3.50, N 6.35.

10b: Found C 30.96, H 3.21, N 6.87; Calculated for $\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_3\text{BrPRu}$: C 30.43, H 3.25, N 6.45.

10c: Found C 32.45, H 2.99, N 5.98; Calculated for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_3\text{PBrRu}$: C 32.16, H 3.60, N 6.25.

Table 2.14. ^1H and $^{31}\text{P}\{^1\text{H}\}$ NMR data for **10n**.

Compound	^1H NMR (CDCl_3)	$^{31}\text{P}\{^1\text{H}\}$ (CDCl_3)
10a	8.91-8.90 (d, 1H, $J = 6.8$ Hz, PyH), 7.85-7.84 (d, 1H, $J = 6.0$ Hz, PyH), 7.72-7.63 (m, 6H, PhH), 7.46-7.40 (m, 9H, PhH), 7.06-7.03 (d, 1H, $J = 8.2$ Hz, PyH), 6.54-6.50 (m, 1H, PyH)	10.39 (PPh ₃)
10b	9.63 (br, 1H, NH), 8.29-8.28 (d, 1H, $J = 6.0$ Hz, PyH), 7.89-7.87 (t, 1H, $J = 1.4$ Hz, PyH), 7.23-7.21 (d, 1H, $J = 8.2$ Hz, PyH), 7.00-6.98 (t, 1H, $J = 1.4$ Hz, PyH), 1.77-1.74 (d, 9H, Me)	-28.90 (PMe ₃)
10c	9.58 (br, 1H, NH), 7.70-7.66 (t, 1H, $J = 7.3$ Hz, PyH), 6.98-6.95 (d, 2H, $J = 8.7$ Hz, PyH), 6.92-6.90 (d, 1H, $J = 7.3$ Hz, PyH), 2.67 (s, 3H, Me), 1.68-1.66 (s, 9H, Me)	-34.32 (PMe ₃)

2.5.6 Synthesis of 11a

Triethyl amine (7 μ L, 0.05 mmol) and **6b** (0.05 mmol) were added into a solution of **10a** (20 mg, 0.05 mmol) in acetonitrile (5 mL). The resulting mixture was then stirred for 12 h at ambient temperature which afforded **8b** and **11a** (based on IR spectrum). The crude mixture was then evaporated in vacuum. The residue was separated by column chromatography on silica gel with dichloromethane/ethyl acetate (v/v = 8/2) as eluent to afford **11a** (35 mg, 0.06 mmol, 51 %). ν_{CO} (ACN, cm^{-1}): 1947 (s). ^1H NMR (400 MHz, CDCl_3): δ 8.91-8.90 (m, 1H, PyH), 7.85-7.84 (m, 1H, PyH), 7.72-7.63 (m, 6H, PhH), 7.46-7.40 (m, 9H, PhH), 7.06-7.03 (d, 1H, PyH), 6.54-6.50 (m, 1H, PyH) ppm. $^{31}\text{P}\{^1\text{H}\}$ NMR (400 MHz, CDCl_3): 33.08 ppm. HRMS⁺ (m/z): found 856.0802 $[\text{M}+\text{H}]^+$, calculated for $\text{C}_{45}\text{H}_{36}\text{N}_3\text{O}_2\text{PBrRu}^{96}$: 856.0804 $[\text{M}+\text{H}]^+$.

Characterisation data for **8b** are given in Tables 2.11 and 2.12.

The analogous reaction with **10c** (0.05 mmol) afforded **9a** whose characterisation data are given in subsection 2.5.3.

2.5.7 Synthesis of 12

A sample of **6a** (4 μ L, 0.025 mmol) was added into a solution of **1a** (10 mg, 0.025 mmol) in acetonitrile (20 mL). The resulting mixture was stirred for 2 h at ambient temperature. The solvent was then evaporated in vacuum. The residue was purified by column chromatography on silica gel with dichloromethane/hexane (v/v = 8/2) as an eluent, which afforded **12** (35 mg, 0.06 mmol, 51 %) as yellow crystals suitable for x-ray diffraction study. ν_{CO} (DCM, cm^{-1}): 2085 (w), 2057 (s), 2009 (w). ^1H NMR (400MHz, CDCl_3): 7.19-7.15 (t, 2H, J = 6.9 Hz, ArH), 7.09-7.07 (d, 1H, J = 8.2 Hz, ArH), 2.62 (s, 6H, Me) ppm. HRMS⁺ (m/z): found 646.8690, calculated for $\text{C}_{22}\text{H}_{18}\text{O}_6\text{S}_2\text{Ru}_2^{104}$: 646.8659 $[\text{M}+\text{H}]^+$. Found C 41.81, H 2.09, S 9.66; Calculated for $\text{C}_{22}\text{H}_{18}\text{O}_6\text{S}_2\text{Ru}_2$: C 40.99, H 2.81, S 9.95.

2.5.8 Synthesis of 5e

PhICl_2 (24 mg, 0.02 mmol) was added to a solution of $\text{Ru}_3(\text{CO})_{12}$ (11 mg, 0.02 mmol) in dichloromethane (5 mL) at 0 $^\circ\text{C}$. The mixture was stirred for 1 h at 0 $^\circ\text{C}$ then 2-amino pyridine (9 mg, 0.1 mmol) was added and the mixture left to stir for another 30 min at 0 $^\circ\text{C}$. The mixture was subsequently left to stir for another 30 min at room temperature. The white precipitate was separated via filtration and washed with dichloromethane followed by acetonitrile. Evaporation of solvent yielded the white solid **5e**. ν_{CO} (ACN, cm^{-1}): 2061 (s), 2041 (m), 1987 (s) 1965 (m). HRMS⁺ (m/z): found 354.4631, calculated for $\text{C}_{10}\text{H}_8\text{N}_3\text{O}_3\text{ClRu}^{102}$: 354.4672 $[\text{M}+\text{H}]^+$.

2.5.9 Reaction of **5e** with **PPh₃**

A sample of **PPh₃** (13 mg, 0.05 mmol) was added into a solution of **5e** (18 mg, 0.05 mmol) in acetonitrile (5 mL). The resulting mixture was then stirred for 30 min at ambient temperature and slow evaporation of the solvent at room temperature afforded **11b** as white crystals. **11b** (18 mg, 0.02 mmol, 44 %). ν_{CO} (ACN, cm^{-1}): 1951 (s). ^1H NMR (400 MHz, CDCl_3): δ 7.55-7.50 (m, 9H, PhH), 7.35-7.33 (m, 1H, PyH), 7.28-7.26 (m, 1H, PyH), 7.22-7.18 (m, 6H, PhH), 6.31-6.29 (d, 1H, $J = 8.2$ Hz, PyH), 5.91-5.87 (m, 1H, PyH) ppm. ^{31}P NMR (400 MHz, CDCl_3): 34.8 ppm. MS^+ (m/z): 811 $[\text{M}+\text{H}]^+$. HRMS^+ (m/z): found 810.2826, calculated for $\text{C}_{43}\text{H}_{35}\text{N}_2\text{O}_2\text{ClP}_2\text{Ru}^{102}$: 810.2852 $[\text{M}+\text{H}]^+$

2.5.10 Reaction of **5b** with **6c**

A sample of **6c** (4.3 mg, 0.02 mmol) and triethyl amine (2.8 μl , 0.02 mmol) were added into a solution of **5b** (10 mg, 0.02 mmol) in acetonitrile (5 ml). The resulting mixture was then stirred for 30 min at ambient temperature. The solution volume was then reduced in vacuum to approximately 1 ml. After standing for 3d, complex **8e** (9.0 mg, 0.01 mmol, 48 %) was obtained as a yellow crystalline solid. ν_{CO} (ACN, cm^{-1}): 2048 (m), 2034 (s), 1983 (m), 1971 (s), 1747 (m), 1681 (m), 1616 (m). ^1H NMR (300 MHz, CD_3CN): δ 9.02-8.99 (br, 1H, NH), 8.89-8.87 (br, 1H, NH), 7.71-7.66 (m, 1H, PyH), 6.90-6.86 (m, 1H, PyH), 6.73-6.68 (m, 1H, PyH), 4.68-4.66 (m, 1H, CH), 4.44-4.39 (m, 2H, CH_2), 3.59 (s, 3H, Me), 2.21 (s, 3H, Me), 1.88 (s, 3H, Me) ppm. HRMS^+ (m/z): found 960.9688, calculated for $\text{C}_{30}\text{H}_{34}\text{N}_6\text{O}_{12}\text{S}_2\text{Ru}_2^{104}\text{Na}$: 960.9667 $[\text{M}+\text{Na}]^+$.

2.5.11 Synthesis of **10d**

A sample of imidazole (1.6 mg, 0.02 mmol) was added into a solution of **5b** (10 mg, 0.02 mmol) in methanol (5 ml). The resulting mixture was then stirred for 30 min at ambient temperature. The solution volume was then reduced in vacuum to approximately 1 ml. After standing for 3d, complex **10d** (3.0 mg, 0.01 mmol, 34 %) was obtained as a yellow crystalline solid. ν_{CO} (MeOH , cm^{-1}): 2062 (s), 1998 (s). ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$): δ 11.95 (br, 1H, NH), 9.66 (br, 1H, NH), 8.24 (s, 1H, CH), 7.71-7.67 (t, 1H, $J = 7.8$ Hz, PyH), 7.25-7.20 (d, 2H, $J = 10.5$ Hz, CH), 6.93-6.87 (m, 2H, PyH), 3.06 (s, 3H, Me) ppm. HRMS^+ (m/z): found 440.0812, calculated for $\text{C}_{12}\text{H}_{11}\text{N}_4\text{O}_3\text{BrRu}^{104}$: 440.0828 $[\text{M}+\text{H}]^+$. Found C 34.37, H 2.80, N 13.59; Calculated for $\text{C}_{12}\text{H}_{11}\text{N}_4\text{O}_3\text{BrRu} \cdot \text{C}_2\text{H}_3\text{N}$: C 34.94, H 2.93, N 14.55.

2.5.12 Reaction of **5b** with **6c** and imidazole in aqueous medium

A mixture of **6c** (4.3 mg, 0.02 mmol) and imidazole (1.6 mg, 0.02 mmol) in water (5 ml) was added into a solution of **5b** (10 mg, 0.02 mmol) in acetonitrile (5 ml). The resulting mixture was then stirred for 2 h at ambient temperature. The solution volume was then concentrated and analyzed by ATR-IR spectroscopy.

2.5.13 Synthesis of **9b**

A sample of imidazole (1.6 mg, 0.02 mmol) was added into a solution of **5b** (10 mg, 0.02 mmol) in methanol (5 ml). The resulting mixture was then stirred for 30 min at ambient temperature. 2,6-dimethylbenzenethiol (2.4 μ L, 0.02 mmol) and triethylamine (2.7 μ L, 0.02 mmol) were added into the above solution and stirred the resulting mixture for 15 min at ambient temperature. The solvent was then evaporated in vacuum which afforded yellow crystalline solid of **9b** (8.0 mg, 0.02 mmol, 84 %). ν_{CO} (MeOH, cm^{-1}): 2050 (s), 1988 (s). ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$): δ 9.72 (br, 1H), 9.54 (br, 1H), 8.88 (br, 1H), 8.35 (br, 1H), 8.22 (s, 1H), 8.19 (s, 1H), 7.69-7.65 (m, 1H), 7.55-7.51 (m, 1H), 7.38-7.36 (d, 2H, $J = 11$ Hz), 7.23-7.20 (d, 2H, $J = 7.8$ Hz), 6.95-6.85 (m, 4H), 6.73-6.64 (m, 6H), 3.07 (s, 3H), 3.04 (s, 3H), 2.27 (s, 6H), 2.21 (s, 6H) ppm. HRMS $^+$ (m/z): found 498.5256, calculated for $\text{C}_{20}\text{H}_{21}\text{N}_4\text{O}_3\text{SRu104}$: 498.5233 $[\text{M}+\text{H}]^+$.

2.5.14 Preparation of papain samples

To get rid of the low molecular weight contaminants, papain is submitted to gel filtration chromatography according to the following procedure.

2.5.14.1 Purification by gel filtration (GF)^{27,28}

Papain (55 mg solid) was dissolved in water (5.5 ml). In some cases, 30 mM mercaptoethanol (11 μ l) was added to activate the enzyme. Gel filtration was carried out by injecting the solution (4 to 5 ml) onto a gel filtration column (Hiprep desalt 26/10, GE Healthcare). Species were eluted with 0.15 M NaCl at 5 ml/min and 4-ml fractions were collected. The fractions containing the proteins were pooled and assayed for their protein content at 280 nm ($\epsilon = 46,000 \text{ M}^{-1}.\text{cm}^{-1}$). The concentration of thiol was measured by the 4,4'-dithiodipyridine (DTDP) assay. The hydrolytic activity of the protein sample was assayed on the chromogenic substrate ZGlyONp.

2.5.14.2 Purification by affinity chromatography²⁹

The peptide GGYR (Bachem) was attached to CNBr-activated Sepharose (Sigma) via its terminal NH₂ group. The resin (20 ml) was packed into a column (XK 16, GE Healthcare) and conditioned with 20 mM EDTA, 10 mM mercaptoethanol pH 4.3 (buffer 3) at 1 ml/min. In the meantime, papain (200 mg solid, Calbiochem) was dissolved in 25 mM EDTA, 30 mM mercaptoethanol pH 4.3 (20 ml) and the solution was incubated for 30 min. The solution (18 ml) was injected into the column. The resin was successively washed with 2 volumes of buffer 3, then 2 volumes of 5 mM EDTA pH 6.0. Papain was finally eluted with 2 volumes of water.

2.5.15 Reaction between papain sample resulting from GF and 5n at the preparative scale

Papain (0.5 ml of a 31.4 μ M solution in water; final concentration = 15.7 μ M) was mixed with 50 mM phosphate buffer pH 7.8 (0.5 ml) and complex **5n** (50 μ l of a 10 mM solution in MeCN; final concentration = 500 μ M). 20 μ L of the mixture was used to measure the hydrolytic activity of ZGlyONP (50 μ L) in 430 μ L of phosphate buffer pH 6.5 at various times and the remaining mixture was incubated at RT for 50 h. The remaining hydrolytic activity of the mixture on ZGlyONP was 20% of the starting activity. N-ethylmaleimide (50 μ l of a 20 mM solution in water; final concentration = 1 mM) was added to block the remaining activity. After 30 min, the remaining hydrolytic activity of the mixture on ZGlyONP was 13% of the starting activity. The mixture was submitted to gel filtration on a dextran desalting column (10 ml, Thermo scientific). Species were eluted with water. 1-ml fractions were collected and their absorbance at 280 and 313 nm measured with a spectrophotometer. The fractions containing the protein were pooled and the solution was submitted to diafiltration on Amicon Ultra 4 (10 kDa cutoff, Millipore) to remove remaining unbound complex (4 cycles of concentration / dilution). The concentrate (0.22 ml, [papain] = 23 μ M) was further concentrated by ultrafiltration on Vivaspinn 500 (10 kDa cutoff, Sartorius) to a final volume of 20 μ l.

2.5.16 Reaction between affinity-purified papain and **5n** at the preparative scale

Papain (1.6 ml of a 3.2 μ M solution in water; final concentration = 2.56 μ M) was mixed with 50 mM phosphate buffer pH 7.8 and **5n** (20 μ l of a 10 mM solution; final concentration = 100 μ M). 20 μ L of the mixture was used to measure the hydrolytic activity of ZGlyONP (50 μ L) in 430 μ L of phosphate buffer pH 6.5 at various times. The remaining mixture was incubated at RT for 18 h and submitted to gel filtration on a dextran desalting column (10 ml, Thermo scientific). Species were eluted with water. 1-ml fractions were collected and their absorbance at 280 nm measured with a spectrophotometer. The fractions containing the protein were pooled and the solution (3 ml; 1.25 μ M) was concentrated by ultrafiltration on Amicon Ultra 4 (10 kDa cutoff, Millipore) to a final volume of 0.22 ml. A control experiment using affinity-purified papain was also carried out to measure the hydrolytic activity of ZGlyONP.

2.5.17 Computational studies

The computational studies were carried out using density function theory (DFT), utilizing the hybrid functional of Truhlar and Zhao (M06).^{27,28,29,30,32} The basis set used was def2TZVP,³¹ for the transition metals, and 6-311+G(2d,p) for the light atoms. For calculations involving solvated species, the polarized continuum model (PCM) was employed. Spin-restricted calculations were used for structural optimization, harmonic frequency calculations and to evaluate zero-point energy (ZPE) corrections. Optimized geometries were characterized as equilibrium structures with all real frequencies. All calculations were performed using the Gaussian 09 suite of programs.³² The coordinates for parent complexes were taken from the crystal structure and each of the other isomers was modified from this.

2.5.18 X-Ray crystallographic studies

Diffraction-quality crystals were grown from DCM (**3a** and **4a**), MeCN (**5a-b**, **5d**, **8a-c**, **9a**, **10a-c**), or DCM/hexane (**11a-b** and **12**). The selected crystals were mounted on quartz fibers. X-ray diffraction data were collected at 103(2) K on a Bruker X8 APEX system, using either Cu (**5d** and **10a**) or Mo (the rest) K α radiation, with the SMART suite of programs.³³ Data processing and correction were made for Lorentz and polarization effects with SAINT,³⁴ and for adsorption effects with SADABS.³⁵ Twinning was observed for the crystals of **8c** and **10a**; the twin laws and cell parameters were determined using the program CELL_NOW,³⁶ and the reflection data processed and corrections applied using the program TWINABS.³⁷ All the structural solutions and refinements were carried out with the SHELXTL suite of programs.³⁸ The structures were solved by direct methods to locate the heavy atoms, followed by successive difference maps for the light, non-hydrogen atoms.

Solvates were found in **10a** (MeCN), **11a** (CHCl₃ and 0.25 hexane), **11b** (2 x MeCN) and **11c** (0.1 DCM). Disorder of a carbonyl and the bromide ligand was found for **5b** which were modelled with two sites each, with occupancies summed to unity. Two of the phenyl rings in **11a** were also disordered; each was modelled with two sites, one of equal occupancies and the other with occupancies summed to unity. Appropriate restraints were placed on all disordered parts. All non-hydrogen atoms were refined with anisotropic displacement parameters in the final model. All the organic hydrogen atoms were placed in calculated positions and refined with a riding model. The crystallographic data are summarized in Tables 2.15-18.

Table 2.15. Crystal and refinement data for **3a**, **4a**, **5a** and **5b**.

Complex	3a	4a	5a	5b
Empirical formula	C ₈ H ₇ Br ₃ N ₂ O ₃ Ru	C ₁₃ H ₁₂ Br ₂ N ₄ O ₃ Ru	C ₁₂ H ₁₁ BrN ₄ O ₃ Ru	C ₁₁ H ₁₀ BrN ₃ O ₃ Ru
Formula weight	519.96	533.16	440.23	413.20
Crystal system	Monoclinic	Monoclinic	Triclinic	Monoclinic
Space group	C 2/c	P 2 ₁ /n	P $\bar{1}$	P 2 ₁ /n
a, Å	22.190(4)	7.3536(5)	7.0469(2)	11.3743(7)
b, Å	9.2188(14)	13.6633(9)	9.9374(3)	9.1237(5)
c, Å	13.717(2)	16.5687(10)	12.2509(4)	14.6542(9)
α , deg	90	90	108.6197(9)	90
β , deg	94.252(4)	93.385(2)	100.6851(9)	110.109(4)
γ , deg	90	90	101.0785(10)	90
Volume, Å ³	2798.3(8)	1661.83(19)	769.25(4)	1428.05(15)
Z	8	4	2	4
ρ_c , Mg/m ³	2.468	2.131	1.901	1.922
μ , mm ⁻¹	9.684	5.770	3.630	3.902
F(000)	1936	1024	428	800
Crystal size, mm ³	0.340 x 0.340 x 0.360	0.16 x 0.12 x 0.06	0.36 x 0.36 x 0.30	0.200 x 0.220 x 0.240
Reflections collected	42435	20692	32811	29734
Independent reflections	10362 [R(int) = 0.0361]	4296 [R(int) = 0.0476]	4928 [R(int) = 0.0352]	6340 [R(int) = 0.1123]
Max. and min. transmission	0.1370 and 0.1280	0.7234 and 0.4587	0.4089 and 0.3549	0.5090 and 0.4540
Data / restraints / parameters	4310 / 0 / 158	4296 / 0 / 214	4928 / 0 / 215	6340 / 83 / 205
Goodness-of-fit on F ²	1.100	1.093	1.276	1.079
Final R indices [I>2sigma(I)]	R1 = 0.0557, wR2 = 0.1095	R1 = 0.0352, wR2 = 0.0859	R1 = 0.0201, wR2 = 0.0585	R1 = 0.0447, wR2 = 0.0662
R indices (all data)	R1 = 0.0934, wR2 = 0.1243	R1 = 0.0713, wR2 = 0.0983	R1 = 0.0255, wR2 = 0.0754	R1 = 0.0764, wR2 = 0.0769
Largest diff. peak and hole, e.Å ⁻³	2.212 and -1.820	0.909 and -0.912	0.927 and -0.685	0.875 and -0.800

Table 2.16. Crystal and refinement data for **5d**, **7d**, **8a** and **8b**.

Complex	5d	7d	8a	8b
Empirical formula	C ₁₀ H ₈ BrN ₃ O ₄ Ru	C ₁₉ H ₁₉ N ₃ O ₃ RuS	C ₃₀ H ₂₄ N ₄ O ₆ Ru ₂ S ₂	C ₃₂ H ₂₈ N ₄ O ₆ Ru ₂ S ₂
Formula weight	415.17	470.50	802.79	830.84
Crystal system	Monoclinic	Monoclinic	Monoclinic	Triclinic
Space group	P 2 ₁ /n	P 2 ₁ /n	P 2 ₁ /c	P $\bar{1}$
a, Å	7.31150(10)	7.4279(2)	10.7820(9)	8.1126(3)
b, Å	11.5978(2)	16.2154(4)	11.0144(12)	9.0321(3)
c, Å	15.2727(2)	15.9289(4)	13.0500(11)	10.9347(3)
α , deg	90	90	90	80.1023(12)
β , deg	93.4830(8)	90.8020(9)	101.800(5)	85.2609(8)
γ , deg	90	90	90	81.0634(12)
Volume, Å ³	1292.69(3)	1918.39(9)	1517.0(2)	778.43(5)
Z	4	4	2	1
ρ_c , Mg/m ³	2.133	1.629	1.757	1.772
μ , mm ⁻¹	13.596	0.951	1.184	1.157
F(000)	800	952	800	416
Crystal size, mm ³	0.040 x 0.060 x 0.120	0.310 x 0.100 x 0.100	0.040 x 0.040 x 0.060	0.126 x 0.131 x 0.238
Reflections collected	11336	58011	25244	35954
Independent reflections	2287 [R(int) = 0.0526]	4752 [R(int) = 0.0619]	3115 [R(int) = 0.1019]	7522 [R(int) = 0.0452]
Max. and min. transmission	0.6120 and 0.2920	0.91 and 0.78	0.9540 and 0.9320	0.8680 and 0.7700
Data / restraints / parameters	2287 / 0 / 174	4752 / 0 / 248	3115 / 0 / 204	7522 / 0 / 210
Goodness-of-fit on F ²	1.112	1.172	1.088	1.111
Final R indices [I>2sigma(I)]	R1 = 0.0572, wR2 = 0.1915	R1 = 0.0303, wR2 = 0.0633	R1 = 0.0440, wR2 = 0.1115	R1 = 0.0427, wR2 = 0.0806
R indices (all data)	R1 = 0.0602, wR2 = 0.1956	R1 = 0.0361, wR2 = 0.0652	R1 = 0.0712, wR2 = 0.1360	R1 = 0.0605, wR2 = 0.0889
Largest diff. peak and hole, e.Å ⁻³	2.200 and -2.401	0.616 and -0.817	1.210 and -1.489	1.814 and -1.206

Table 2.17. Crystal and refinement data for **8c**, **9a**, **10a** and **10b**.

Complex	8c	9a	10a	10b
Empirical formula	C ₃₂ H ₂₈ N ₄ O ₆ Ru ₂ S ₂	C ₂₀ H ₂₅ N ₂ O ₃ PRuS	C ₂₈ H ₂₃ BrN ₃ O ₃ PRu	C ₂₂ H ₂₈ Br ₂ N ₄ O ₆ P ₂ Ru ₂
Formula weight	830.84	505.52	661.44	868.38
Crystal system	orthorhombic	Monoclinic	Monoclinic	Monoclinic
Space group	P 2 ₁ 2 ₁ 2 ₁	P 2 ₁ /n	P 2 ₁ /c	P 2 ₁ /c
a, Å	10.6574(3)	8.3913(2)	8.5355(2)	15.5360(7)
b, Å	16.0828(4)	19.8911(4)	24.5578(6)	7.5748(4)
c, Å	19.4466(4)	13.6636(3)	12.5616(3)	13.2957(6)
α, deg	90	90	90	90
β, deg	90	103.5335(7)	91.7402(12)	109.3279(17)
γ, deg	90	90	90	90
Volume, Å ³	3333.16(14)	2217.30(8)	2631.86(11)	1476.48(12)
Z	4	4	4	2
ρ _c , Mg/m ³	1.656	1.514	1.669	1.953
μ, mm ⁻¹	1.080	0.896	7.469	3.880
F(000)	1664	1032	1320	848
Crystal size, mm ³	0.080 x 0.120 x 0.320	0.060 x 0.070 x 0.200	0.120 x 0.140 x 0.420	0.060 x 0.240 x 0.320
Reflections collected	36227	53395	30257	30220
Independent reflections	10049 [R(int) = 0.1003]	7044 [R(int) = 0.0786]	4662 [R(int) = 0.1979]	5380 [R(int) = 0.0895]
Max. and min. transmission	0.9190 and 0.7240	0.9480 and 0.8410	0.4680 and 0.1450	0.8010 and 0.3700
Data / restraints / parameters	10049 / 0 / 420	7044 / 0 / 259	4662 / 0 / 335	5380 / 0 / 175
Goodness-of-fit on F ²	1.127	1.079	1.053	1.019
Final R indices [I>2σ(I)]	R1 = 0.0636, wR2 = 0.0802	R1 = 0.0447, wR2 = 0.0662	R1 = 0.0847, wR2 = 0.2252	R1 = 0.0440, wR2 = 0.1072
R indices (all data)	R1 = 0.0954, wR2 = 0.0896	R1 = 0.0764, wR2 = 0.0769	R1 = 0.0861, wR2 = 0.2278	R1 = 0.0578, wR2 = 0.1169
Largest diff. peak and hole, e.Å ⁻³	1.381 and -1.282	0.875 and -0.800	1.617 and -1.539	2.030 and -2.344

Table 2.18. Crystal and refinement data for **10c**, **11a**, **11b** and **12**.

Complex	10c	11a	11b	12
Empirical formula	C ₁₂ H ₁₆ BrN ₂ O ₃ PRu	C _{45.75} H _{38.75} BrCl _{3.75} N ₂ O ₂ P ₂ Ru	C ₄₇ H ₄₁ ClN ₄ O ₂ P ₂ Ru	C ₂₂ H ₁₈ O ₆ Ru ₂ S ₂
Formula weight	448.22	1024.39	892.30	644.62
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	P 2 ₁ /c	P 2 ₁ /n	P 2 ₁ /n	P 2 ₁ /n
a, Å	15.3586(4)	13.6855(5)	14.4524(16)	20.7246(15)
b, Å	8.0246(2)	18.5067(6)	19.558(3)	10.8643(6)
c, Å	13.6194(3)	18.9718(6)	15.295(2)	22.3813(14)
α, deg	90	90	90	90
β, deg	108.4861(8)	101.0290(15)	105.836(4)	110.249(2)
γ, deg	90	90	90	90
Volume, Å ³	1591.93(7)	4716.3(3)	4159.2(9)	4727.9(5)
Z	4	4	4	8
ρ _c , Mg/m ³	1.870	1.443	1.425	1.811
μ, mm ⁻¹	3.602	1.498	0.562	1.490
F(000)	880	2064	1832	2544
Crystal size, mm ³	0.050 x 0.249 x 0.258	0.080 x 0.140 x 0.300	0.080 x 0.160 x 0.180	0.040 x 0.120 x 0.140
Reflections collected	34156	88531	54349	48674
Independent reflections	5075 [R(int) = 0.0558]	12109 [R(int) = 0.1060]	9578 [R(int) = 0.1534]	12360 [R(int) = 0.0762]
Max. and min. transmission	0.8400 and 0.4570	0.8900 and 0.6620	0.9560 and 0.9060	0.9430 and 0.8190
Data / restraints / parameters	5075 / 0 / 185	11740 / 719 / 697	9578 / 0 / 516	9673 / 0 / 585
Goodness-of-fit on F ²	1.010	1.117	1.019	1.068
Final R indices [I>2σ(I)]	R1 = 0.0269, wR2 = 0.0627	R1 = 0.0579, wR2 = 0.1546	R1 = 0.0511, wR2 = 0.0933	R1 = 0.0439, wR2 = 0.1014
R indices (all data)	R1 = 0.0394, wR2 = 0.0708	R1 = 0.0949, wR2 = 0.1905	R1 = 0.1152, wR2 = 0.1173	R1 = 0.0711, wR2 = 0.1312
Largest diff. peak and hole, e.Å ⁻³	1.051 and -1.246	2.320 and -2.108	0.772 and -1.013	0.714 and -1.005

2.6 References

1. Li, B.; Liu, T.; Popescu, C. V.; Bilko, A.; Darensbourg, M. Y. *Inorg. Chem.* **2009**, *48* (23), 11283-11289.
2. Schilter, D.; Camara, J. M.; Huynh, M. T.; Hammes-Schiffer, S.; Rauchfuss, T. B. *Chem. Rev.* **2016**, *116* (15), 8693-8749.
3. Hiromoto, T.; Ataka, K.; Pilak, O.; Vogt, S.; Stagni, M. S.; Meyer-Klaucke, W.; Warkentin, E.; Thauer, R. K.; Shima, S.; Ermler, U. *FEBS Lett.* **2009**, *583* (3), 585-590.
4. Seo, J.; Manes, T. A.; Rose, M. J. *Nat. Chem.* **2017**, *9* (6), 552-557.
5. Xu, T.; Yin, C.-J. M.; Wodrich, M. D.; Mazza, S.; Schultz, K. M.; Scopelliti, R.; Hu, X. *J. Am. Chem. Soc.* **2016**, *138* (10), 3270-3273.
6. Kalz, K. F.; Brinkmeier, A.; Dechert, S.; Mata, R. A.; Meyer, F. *J. Am. Chem. Soc.* **2014**, *136* (47), 16626-16634.
7. Song, L.-C.; Hu, F.-Q.; Wang, M.-M.; Xie, Z.-J.; Xu, K.-K.; Song, H.-B. *Dalton Trans.* **2014**, *43* (21), 8062-8071.
8. Rohmann, K.; Kothe, J.; Haenel, M. W.; Englert, U.; Hölscher, M.; Leitner, W. *Angew. Chem. Int. Ed.* **2016**, *55* (31), 8966-8969.
9. Chen, M.-H.; Lin, J.-H.; Li, C.-R.; Gliniak, J.; Chang, C.-H.; Peng, C.-S.; Chen, Y.-T.; Jen-Shiang, K. Y.; Wu, T.-K. *Int. J. Hydrogen Energ.* **2017**, *42* (15), 9784-9794.
10. Naziruddin, A. R.; Huang, Z.-J.; Lai, W.-C.; Lin, W.-J.; Hwang, W.-S. *Dalton Trans.* **2013**, *42* (36), 13161-13171.
11. Chen, D.; Scopelliti, R.; Hu, X. *Angew. Chem. Int. Ed.* **2010**, *49* (41), 7512-7515.
12. Wright, J. A.; Turrell, P. J.; Pickett, C. J. *Organometallics* **2010**, *29* (23), 6146-6156.
13. Turrell, P. J.; Hill, A. D.; Ibrahim, S. K.; Wright, J. A.; Pickett, C. J. *Dalton Trans.* **2013**, *42* (22), 8140-8146.
14. Chen, D.; Scopelliti, R.; Hu, X. *J. Am. Chem. Soc.* **2009**, *132* (3), 928-929.
15. Turrell, P. J.; Wright, J. A.; Peck, J. N.; Oganessian, V. S.; Pickett, C. J. *Angew. Chem. Int. Ed.* **2010**, *49* (41), 7508-7511.
16. Johnson, B.; Johnston, R.; Lewis, J. *J. Chem. Soc. A* **1969**, 792-797.
17. Messerle, B. A.; Tolhurst, V. A.; Turner, P. *Eur. J. Inorg. Chem.* **2003**, *2003* (2), 293-300.
18. Tomon, T.; Koizumti, T. A.; Tanaka, K. *Angew. Chem. Int. Ed.* **2005**, *44* (15), 2229-2232.
19. Teo, A. K. L.; Fan, W. Y. *Chem. Comm.* **2014**, *50* (54), 7191-7194.

20. Nagarajaprakash, R.; Ramakrishna, B.; Mahesh, K.; Mobin, S. M.; Manimaran, B. *Organometallics* **2013**, 32 (24), 7292-7296.
21. Hirano, M.; Togashi, S.; Ito, M.; Sakaguchi, Y.; Komine, N.; Komiya, S. *Organometallics* **2010**, 29 (14), 3146-3159.
22. Zhang, Q.-F.; Lai, C.-Y.; Wong, W.-Y.; Leung, W.-H. *Organometallics* **2002**, 21 (19), 4017-4020.
23. De Araujo, M. P.; Porcu, O. M.; Batista, A. A.; Oliva, G.; Souza, D.-H. F.; Bonfadini, M.; Nascimento, O. R. *J. Coord. Chem.* **2001**, 54 (1), 81-94.
24. Schraml, J.; Čapka, M.; Blechta, V. *Magn. Reson. Chem.* **1992**, 30 (6), 544-547.
25. Chevalley, A.; Cherrier, M. V.; Fontecilla-Camps, J. C.; Ghasemi, M.; Salmann, M. *Dalton Trans.* **2014**, 43 (14), 5482-5489.
26. Haquette, P.; Salmann, M.; Svedlung, K.; Martel, A.; Rudolf, B.; Zakrzewski, J.; Cordier, S.; Roisnel, T.; Fosse, C.; Jaouen, G. *ChemBiochem* **2007**, 8 (2), 224-231.
27. Kirsch, J. F.; Igelström, M. *Biochemistry-us* **1966**, 5 (2), 783-791.
28. Riener, C. K.; Kada, G.; Gruber, H. J. *Anal. Bioanal. Chem.* **2002**, 373 (4), 266-276.
29. Funk, M.; Nakagawa, Y.; Skochdopole, J.; Kaiser, E. *Chem. Biol. Drug Des.* **1979**, 13 (3), 296-303.
30. Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.: Theory, Computation, and Modeling (Theor. Chim. Acta)* **2008**, 120 (1), 215-241.
31. Ehlers, A.; Böhme, M.; Dapprich, S.; Gobbi, A.; Höllwarth, A.; Jonas, V.; Köhler, K.; Stegmann, R.; Veldkamp, A.; Frenking, G. *Chem. Phys. Lett.* **1993**, 208 (1-2), 111-114.
32. Gaussian09, R., 02, MJ Frisch et al., Gaussian. Inc., Wallingford CT, **2009**.
33. SMART version 5.628; Bruker AXS Inc., Madison, Wisconsin, USA, **2001**.
34. SAINT+ version 6.22a, Bruker AXS Inc., Madison, Wisconsin, USA, **2001**.
35. Sheldrick, G. M. SADABS; University of Göttingen, Göttingen, Germany, **1996**.
36. Sheldrick, G. M. CELL_NOW; University of Göttingen, Göttingen, Germany, **2008**.
37. Sheldrick, G. M. TWINABS; University of Göttingen, Göttingen, Germany, **2008**.
38. SHELXTL version 5.1; Bruker AXS Inc., Madison, WI, USA, **1997**.

Chapter 3: A Series of Group-VIII Structural Mimics Based on 2-Mercaptopyridine

As described in chapter 2, formation of dimer or monomer from the reaction of **5b** with thiol **6n** depended on steric crowding around the ruthenium center; the dimeric species **8c** was obtained with the less sterically encumbered *p*-thiocresol whereas monomeric species **7d** was obtained with the sterically more demanding 2,6-dimethylbenzenethiol (Scheme 3.1). Presumably, dimerisation in the second reaction was prevented by the steric hindrance around the ruthenium center.

Scheme 3.1. Synthesis of structural mimic using thiol.

Another approach to prevent dimerisation is to block the 6th coordination site of the ruthenium atom with an inert ligand. One possibility is with the bidentate chelating thiol (2-mercaptopyridine) instead of the monodentate thiol **6n**. Structural mimics containing this ligand, *viz.*, [Fe(2-CH₂C(O)C₅H₃NR₂)(CO)₂(2-SC₅H₃NR₃)], R₂ = R₃ = Me (**15c'**) and R₂ = OMe, R₃ = Me (**15d'**) have recently been described (Figure 3.1).^{1,2} It was thus envisaged that use of the bidentate 2-mercaptopyridine ligand may also afford stable ruthenium structural mimics.

$R_2 = R_3 = \text{Me}$ (**15c'**)

$R_2 = \text{OMe}, R_3 = \text{Me}$ (**15d'**)

Figure 3.1. Reported structural models containing a bidentate 2-mercaptopyridine ligand.¹

3.1 Synthesis of structural mimics based on 2-mercaptopyridine

The ruthenium structural mimics $[\text{Ru}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_3\text{NR}_2)(\text{CO})_2(2\text{-SC}_5\text{H}_4\text{N})]$, $R_2 = \text{H}$ (**15a**) and $R_2 = \text{Me}$ (**15b**) were synthesized from **5a** and **5b**, respectively, by treatment with 2-mercaptopyridine in acetonitrile at room temperature (Scheme 3.2). Presumably, the reaction proceeded through nucleophilic substitution of bromide ligand (Br^-) by the sulphur atom, followed by substitution of the acetonitrile ligand by chelating attack of the nitrogen atom, of 2-mercaptopyridine ($\text{C}_5\text{H}_4\text{NS}^-$) anion. Both complexes have been isolated and characterized spectroscopically and crystallographically, and they are structurally similar to the reported iron analogues **15c'**, **15d'**.

Scheme 3.2. Synthesis of **15a/b**.

Their infrared spectra showed two intense absorption bands [(2041(s), 1965(s) for **15a** and 2042(s), 1972(s) cm^{-1} for **15b**)] for the *cis* dicarbonyl ligands, which is within the range for ruthenium(II) dicarbonyl complexes,^{3,4} and for the amide group [1607(m) for **15a** and 1608(m) for **15b** cm^{-1}]. Their ^1H NMR spectra showed broad singlets for the NH protons between 8 to 10 ppm. However, while the spectrum for a solution of **15b** showed two sets of peaks in a 38:62 ratio, that for **15a** showed only one set initially but a second set slowly emerged on standing at

ambient conditions, with the final ratio at 20:80 after 4 d (Figure 3.2). Crystallisation of **15a** from tetrahydrofuran gave diffraction-quality yellow crystals with ^1H NMR spectra corresponding to the second product, and the crystallographic analyses showed that it was the isomeric form **16a** (Figure 3.2). The ^1H NMR spectrum of **15a** showed a signal at 8.25 ppm which is downfield shifted to 9.0 ppm in **16a** during isomerization. This signal is assignable to the 6-H (H_d) of the amino-pyridine ligand, which is close to the mercaptopyridine ligand. In the case of **15/16b**, the crystallographic analyses showed the structure of both the isomeric forms **15b** and **16b**. The ^1H NMR spectrum of **15/16b** showed signals for **16b** appear at more downfield region than that of **15b**, which has been confirmed by comparing its values with that of the reported iron analogues **15/16d'**;¹⁶ as well as with that of computed chemical shift values.

Figure 3.2. ^1H NMR spectra [in $(\text{CD}_3)_2\text{CO}$] of **15a** showing its isomerisation at room temperature to **16a**, and of crystals of **16a**.

An ORTEP plot showing the molecular structure of **16b** is given in Figure 3.4a. It is an isomeric form of **15b** in which the relative positions of the nitrogen and sulfur atoms of the 2-mercaptopyridine ligand differ. The crystallographic studies also show that **15b**, **16a** and **16b** all exist as dimers through two intermolecular hydrogen bonds between the amide groups of two monomeric units. The presence of dimers through H-bonding was corroborated by the mass spectrometry. Fragments corresponding to dimers (m/z 779.45 and 827.45) were observed in the mass spectra together with those for the monomeric units (m/z 389.83 and 403.93, respectively). The former peaks disappeared when tetrafluoroboric acid was added to the sample for mass spectrometry, suggesting that the dimers were the result of H-bonding, presumably via the amide NH group, which were cleaved upon protonation.

Osmium complexes are known to catalyse hydrogen activation and hydrogen transfer reactions.^{7,8} In general, osmium complexes tend to exhibit slower kinetics compared to the ruthenium analogues and are therefore useful for such studies. An attempt at the preparation of the osmium mimic $[\text{Os}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(2\text{-SC}_5\text{H}_4\text{N})]$, **15a''** using a similar route to that for **15a** proved not viable. An alternative route via the trifluoroacetate complex **17''**, which is available *via* a literature method from $\text{Os}_3(\text{CO})_{12}$,^{9,10} produced a yellow solid **15a''**, the osmium analogue of **15a** (Scheme 3.3).

Scheme 3.3. Synthesis of osmium structural mimic.

Presumably, substitution of the trifluoroacetate ligand by pyridine at the osmium center was followed by intramolecular attack of the primary amine group at an osmium-bound carbonyl ligand (Scheme 3.4).¹¹ This is the first known example of the formation of a five-membered osmocyclic carbamoyl pyridinyl ring $[\text{Os}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})]$.

Scheme 3.4. Proposed mechanism for the formation of osmium structural mimic.

Complex **15a''** has been completely characterized spectroscopically and crystallographically. As for the ruthenium analogue **15a**, it undergoes isomerisation in solution; the ^1H NMR spectrum showed only one set of resonances initially, but a second set slowly emerged on standing at ambient conditions, with the final ratio at 15:85 after 14 d (Figure 3.3).

Figure 3.3. ^1H NMR spectra [in $(\text{CD}_3)_2\text{CO}$] of **15a''** showing its isomerisation at room temperature to **16a''** and ^1H NMR spectrum of crystals of **16a''**.

Crystallisation of **15a''** from tetrahydrofuran gave diffraction-quality yellow crystals with ^1H NMR spectra corresponding to the second product, and the crystallographic analysis showed that it was the isomeric form **16a''**; the ORTEP plot showing its molecular structure is given in Figure 3.4b. Similar to the ruthenium analogues **16a** and **16b**, it exists as dimers through intermolecular hydrogen bonding between the amide groups of two monomers.

The above results show that for the ruthenium and osmium mimics, the isomer obtained in the solid state (**16a** and **16a''**) has the sulphur atom of the 2-mercaptopyridine ligand trans to the acetyl ligand. This is in contrast to the reported iron mimics **15c'** and **15d'**, in which the nitrogen atom of the 2-mercaptopyridine ligand is trans to the acetyl ligand. In order to make a better comparison, exact iron analogue, i.e., $[\text{Fe}(2\text{-NHC}(\text{O})\text{C}_5\text{H}_4\text{N})(\text{CO})_2(2\text{-SC}_5\text{H}_4\text{N})]$, was prepared via **4a'** which was in turn available from $\text{Fe}(\text{CO})_5$ (Scheme 3.5).¹² A single crystal X-ray diffraction study carried out showed that it was **15a'**, i.e., the analogue of **15c'** and **15d'**; the ORTEP plot showing its molecular structure is given in Figure 3.4c. It clearly shows that the nitrogen atom of the 2-mercaptopyridine ligand is trans to the acetyl ligand. Like the ruthenium and osmium analogues, it exists as dimers through two intermolecular hydrogen bonding between the amide groups of two molecules, with a $\text{C}=\text{O}\cdots\text{H}$ distance of 1.93 Å. It is thus clear that the favored isomer for ruthenium and osmium is different from that for iron.

Scheme 3.5. Synthesis of **15a'**.

3.2 Solid state structures of **15n** and **16n**

Selected bond parameters and a common atom labeling scheme for all the newly-synthesized complexes, viz., **16a**, **15b**, **16b**, **16a''** and **15a'**, as well as the reported close analogue **15c'**, are collated in Table 3.1. As already mentioned above, the synthesized complexes exist as dimers through two intermolecular hydrogen bonding between the amide groups of two monomers. Although each monomeric unit is chiral (Δ or Λ form), the dimers either have crystallographically imposed centrosymmetry (**16a**, **15b**, **16a''** and **15a'**) or pseudo-centrosymmetry (**16b**). The C=O...H distances of 1.88 to 2.20 Å, the longest being for **16a''**, are within the range (1.5-2.2 Å) observed for medium H-bonding interactions.^{5,6} The steric effect of a methyl group at the 6-position of the pyridine ring in **16b** can be seen in the increase in the Ru-N1 and N1-Ru-N2 bond parameters [Ru-N1 = 2.18(1) Å and N1-Ru-N2 = 100.5(2)°, respectively] compared to those in **16a** [Ru-N1 = 2.115(3) Å and N1-Ru-N2 = 66.4(1)°, respectively]. This steric interaction of alkyl groups has also been observed in the reported iron complex **15c'**; the Fe-N bond parameters in **15c'** [Fe-N1 = 2.083 Å, Fe-N2 = 2.110 Å and N1-Fe-N2 = 98.48°, respectively] are greater than the corresponding ones in **15a'** [Fe-N1 = 1.998(3) Å, Fe-N2 = 2.036(3) Å and N1-Fe-N2 = 94.0(1)°, respectively]. Hence, steric effects play a big role on the structural parameters and properties of the compounds and this must have implications in the design of a structural mimic for [Fe]-hydrogenase. The stronger trans influence of the carbamoyl ligand compared to the carbonyl ligand leads to significant elongation of the M-N2 bonds over the M-N1 bonds (2.036(3) vs 1.998(2) and 2.200(2) vs 2.152(2), for **15a'** and **15b**, respectively), and for the Ru-S bond in **16a** and **16b** compared to that in **15b**.

Figure 3.4. ORTEP plots of (a) **16b**, (b) **16a''** and (c) **15a'** showing hydrogen bonding between two monomeric units. All hydrogen atoms except those involved in the hydrogen bonding have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level.

Table 3.1. Common atom labelling scheme and selected bond parameters for **16a**, **15b**, **16b**, **16a''**, **15a'** and **15c'**.

Bond length (Å) and angle (°)	15a'	16a	15b	16b		16a''	15c'*
				Molecule 1	Molecule 2		
M	Fe	Ru	Ru	Ru		Os	Fe
R ₂ , R ₃	H, H	H, H	Me, H	Me, H		H, H	Me, Me
C(C3)=O...H	1.93	1.88	1.98	1.939	1.960	2.20	-**
M-N1	1.998(3)	2.115(3)	2.152(2)	2.15(1)	2.18(1)	2.133(9)	2.083(5)
M-N2	2.036(3)	2.116(4)	2.200(2)	2.113(7)	2.112(9)	2.13(1)	2.110(5)
M-S	2.3797(9)	2.520(1)	2.4754(6)	2.574(3)	2.569(3)	2.503(6)	2.376(2)
M-C1	1.774(3)	1.884(5)	1.875(2)	1.88(1)	1.89(1)	1.89(2)	1.776(6)
M-C2	1.785(4)	1.878(4)	1.873(3)	1.89(1)	1.88(1)	1.88(1)	1.778(7)
M-C3	1.935(3)	2.021(4)	2.020(2)	2.021(9)	2.027(9)	2.06(1)	1.946(6)
N1-M-C3	82.7(1)	79.5(2)	79.28(9)	79.7(3)	79.4(3)	78.7(4)	83.3(2)
C3-M-C2	87.5(1)	92.9(2)	90.40(10)	90.4(4)	91.3(4)	93.4(5)	87.8(3)
S-M-N1	88.84(8)	94.7(1)	88.36(5)	98.0(2)	100.5(2)	93.4(3)	88.1(1)
S-M-N2	70.13(8)	66.4(1)	66.63(6)	65.2(2)	65.4(3)	66.8(3)	69.6(1)
N2-M-N1	94.0(1)	86.2(1)	101.42(8)	87.5(3)	88.4(3)	85.0(4)	98.5(2)

*NH is replaced by a CH₂ group in **15c'**.

****15c'** did not exist as dimer through intermolecular hydrogen bonding.

3.3 Solution structures of **15n** and **16n**

As indicated above, the solid-state structures obtained for the Fe, Ru and Os analogues corresponded to different isomers; structure **15n** for Fe and Ru, and, structure **16n** for Ru and Os. A comparison of the ^1H NMR spectra of freshly prepared samples with those left standing for a period of time at room temperature suggests that the two isomers exhibited different patterns for their resonances which can be used to differentiate between them. In all cases, there is evidence of isomerization; in the case of **16a'** the resonances broadened after standing for 2 d (even under an inert atmosphere), indicating decomposition (Figure 3.5). Although these isomers could be separated on a TLC plate as close-moving bands, their IR and NMR spectra showed the presence of a mixture in a constant ratio, suggesting that the isomerisation was slow but dynamic. In the case of **15b/16b**, for which the ^1H NMR spectrum showed them to be present in $\sim 3:2$ ratio, a variable temperature experiment did not show any significant signs of broadening even at elevated temperatures, pointing to a slow isomerization.

Figure 3.5. ^1H NMR spectra [in $(\text{CD}_3)_2\text{CO}$] of **15a'** showing its isomerization at room temperature to **16a'**.

These observations are reminiscent of that for the known iron analogue **15d'** which has been reported to isomerise via an intermediate **I**, but only upon UV irradiation (Scheme 3.6).¹

Scheme 3.6. Isomerization under presence of uv light.

A computational study was carried out to obtain the free energies for the two isomers. The isomeric ratios based on the differences in their free energies were in excellent agreement with the experimentally observed ratios (Table 3.2).

Table 3.2. Computed free energy and isomeric ratios of the complexes **15n** and **16n**.

Complex	Calculated $\Delta G^\circ(\mathbf{16n}) - \Delta G^\circ(\mathbf{15n})$ (kJ/mol)	Ratio of 15n:16n	
		Calculated	Experimental
15a'/16a'	-3.7	20:80	25:75
15a/16a	-4.5	15:85	20:80
15a''/16a''	-5.8	10:90	15:85
15b/16b	-0.8	45:55	40:60
15c'/16c'	+15.0	100:0	-
15d'/16d'	+27.0	100:0	-

The computational studies suggest that **16n** are more stable than **15n** for all the three metals, with a clear trend of an increasing energy gap from iron (**15/16a'**) through ruthenium (**15/16a**) to osmium (**15/16a''**). The greater stability of **16n** compared to **15n** may be attributed to placement of the weaker σ -donating thiolate trans to the strongly σ -donating carbamoyl ligand. Interestingly, this energy gap is much smaller (-0.8 kJ/mol) for **15/16b**. In comparison, **15d'** is more stable by +27.0 kJ/mol than **16d'**, and the large energy gap is consistent with the report that this isomerisation required uv light. This complex differs significantly from the other complexes but, together with **15/16b**, they demonstrate that introducing a methyl substituent at the 6-position of the pyridine ligand can significantly affect the relative stability as well as the structural parameters of the complexes. The latter effect is reflected in the M-N1, M-N2, M-S, S-M-N1 and N1-M-N2 bond parameters which are increased significantly from R = H to R = alkyl (Table 3.3), and are also consistent with the experimentally observed trend in the solid-state structures.

Table 3.3. Common atom labeling ORTEP plots showing optimized structures of the two isomers for all the complexes.

Parameters	15a'	15a	15b	15a''	15d'
M	Fe	Ru	Ru	Os	Fe
R ₂ ,R ₃	H,H	H,H	Me,H	H,H	OMe, Me
M-N1	2.021	2.149	2.207	2.169	2.075
M-N2	2.028	2.207	2.233	2.202	2.078
M-S	2.390	2.506	2.504	2.529	2.399
S-M-N1	88.15	86.73	87.17	85.71	87.99
N2-M-N1	90.72	89.94	98.11	89.46	94.33

	16a'	16a	16b	16a''	16d'
	Fe	Ru	Ru	Os	Fe
	H,H	H,H	Me,H	H,H	OMe, Me
	2.028	2.155	2.203	2.178	2.109
	2.000	2.136	2.143	2.157	2.030
	2.428	2.578	2.619	2.578	2.482
	94.17	92.12	99.39	91.23	105.94
	86.77	84.75	86.89	83.92	85.97

The isomerization in **15d'** has been proposed to involve migratory de-insertion of the acyl to a carbonyl and alkyl following CO loss. Although the mechanism for the isomerisation here has not been studied, it is unlikely that an analogous migratory de-insertion may occur involving high energy bond breaking. Instead, a Ray-Dutt twist pathway is more likely (Scheme 3.7).

Scheme 3.7. Proposed mechanism for the isomerization.

3.4 Reactivity of the complex **15a** with phosphines

Due to ring strain, 4-membered rings are generally unstable. In contrast, a number of studies revealed that there are a number of different ruthenium complexes containing 4-membered metalla-heterocyclic ring which are unexpectedly stable.^{13,14} In order to verify the statement for our synthesized ruthenium complexes, attempts at the reaction of **15a** with PMe_3 were made which instantly gave **18a** via substitution of the nitrogen atom of 2-mercaptopyridine ligand at ambient temperature (Scheme 3.8). The compound **18a** has been characterized spectroscopically and by single crystal x-ray structural studies as well. ORTEP plots showing the molecular structure of **18a** together with selected bond parameters are given in Figures 3.7. As may be expected the trans influence of the carbamoyl ligand led to a lengthening of the Ru- PMe_3 bond length (2.423(3) Å *cf* 2.3790(13) Å in **11a**).

Scheme 3.8. Reaction of **15a** with PMe_3 .

Figure 3.6. ORTEP plot of complex **18a**. All the hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru–N = 2.149(7), Ru–P = 2.423(3), Ru–S = 2.435(3), Ru–C1 = 1.878(11), Ru–C2 = 1.879(13), Ru–C3 = 2.050(10), C3–Ru–N = 79.6(3), C3–Ru–C2 = 91.0(4), N–Ru–P = 97.0(2).

Similar to the disproportionation behavior of **10a** described in chapter 2 (Section 2.21), compound **18a** also underwent disproportionation into a mixture of **11c** and **15a** over 17 h (Scheme 3.9) under ambient condition. The di-substituted compound **11c** and **15a** were then further converted to mono-substituted **19a** over 4 d at ambient temperature. The reactions were investigated through monitoring a solution of **18a** by $^{31}\text{P}\{^1\text{H}\}$ NMR spectroscopy (Figure 3.7). The initial spectrum showed a resonance at -24.2 ppm ascribable to **18a**. The resonance slowly decreased and disappeared, and a new peak at -5.6 ppm, ascribable to **11c**, emerged over 2 h. The peak at -5.6 ppm also decreased and disappeared, and another new peak at 14.7 ppm, ascribable to **19a**, emerged over 4 d. Analogous products were also obtained by reacting **15a** with PBU_3 but only at higher temperature (75 °C), suggesting moderate stability of the 4-membered metalla-heterocyclic ring in **15a** compared to the previously reported ruthenium complexes.¹³ All four compounds have been characterized spectroscopically and in the case of **19a**, **11c** and **11d**, by single crystal x-ray structural studies as well. ORTEP plots showing the molecular structure of **19a** and **11d**, together with selected bond parameters, are given in Figures 3.8 and 3.9, respectively.

Scheme 3.9. Disproportionation of **18**.

Figure 3.7. $^{31}\text{P}\{^1\text{H}\}$ NMR spectra [in CD_3CN] of **18a** showing its disproportionation at room temperature into **11c** and **15a**.

Figure 3.8. ORTEP plot of complex **19a**. All the hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru–N1 = 2.132(4), Ru–N2 = 2.127(4), Ru–P = 2.296(1), Ru–S = 2.551(2), Ru–C2 = 1.832(5), Ru–C3 = 2.011(5), C3–Ru–N1 = 79.3(2), C3–Ru–C2 = 93.6(2), S–Ru–N1 = 91.9(1), N1–Ru–N2 = 87.2 (1), S–Ru–N2 = 66.1(1).

Figure 3.9. ORTEP plot of complex **11d**. All the hydrogen atoms have been omitted for clarity. Thermal ellipsoids are drawn at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru–N1 = 2.144(2), Ru–P1 = 2.3714(9), Ru–P2 = 2.3645(9), Ru–S = 2.4984(8), Ru–C2 = 1.857(3), Ru–C3 = 2.033(3), C3–Ru–N1 = 78.5(1), C3–Ru–C2 = 91.7(1), S–Ru–N1 = 89.36(7).

3.5 Conclusion

Structural mimics for the active site of [Fe]-hydrogenase, of the type $[M(2\text{-NHC(O)C}_5\text{H}_3\text{NR})(\text{CO})_2(2\text{-SC}_5\text{H}_4\text{N})]$ [$M = \text{Fe, Ru and Os}$] have, for the first time, been prepared for all three group VIII metals. They were found to exist as isomers; in all cases, the initial (kinetic) product was **15n**, which isomerized slowly in solution to **16n**. The thermodynamically most stable isomer depended on the nature of the metal and the substituent on the N,S ligand. Thus the relative stability of **16n** over **15n** increased from Fe through Ru to Os, but this was decreased upon substitution at the 6-position of the pyridine ligand and could be ascribed to increased steric repulsion from the S atom (Figure 3.10).

Figure 3.10. Computed relative thermodynamic stability of the isomers **15n** and **16n**.

In the solid state, they were found to exist as dimers through two intermolecular hydrogen bonds between the amide ($\text{O}=\text{C}-\text{NH}$) groups of the monomers. It could be envisaged that this H-bonding interaction would be of some consequence, for instance, in folding if these complexes were incorporated into a protein. This would also be in contrast to the active site of natural [Fe]-hydrogenase; the acyl ($\text{O}=\text{C}-\text{CH}_2$) in place of the amide group could not possess such H-bonding interactions.

3.6 Experimental section

3.6.1 General procedures: All experiments were performed under an argon atmosphere using standard Schlenk techniques. Chemicals were obtained from Sigma Aldrich and were used as received. Solvents that were used for reactions were distilled over the appropriate drying agents under argon before use. Purification of compounds was carried out by column chromatography on silica gel. Infrared spectra were recorded on a Bruker Alpha FTIR spectrometer. Solution spectra were recorded in a solution IR cell with NaCl windows and a path length of 0.1 mm, at a resolution of 2 cm^{-1} . HRMS were recorded in ESI mode on a Waters UPLC-Q-ToF MS mass spectrometer. ^1H , $^{13}\text{C}\{^1\text{H}\}$ and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra were obtained on a JEOL 400 MHz spectrometer at room temperature unless otherwise stated. Chemical shifts for ^1H and $^{13}\text{C}\{^1\text{H}\}$ were referenced with respect to the residual resonances of the respective deuterated solvents (^1H NMR: $\delta_{\text{H}} = 7.26$ for CDCl_3 , $\delta_{\text{H}} = 2.05$ for $(\text{CD}_3)_3\text{CO}$, $\delta_{\text{H}} = 1.94$ for CD_3CN , ^{13}C NMR: $\delta_{\text{C}} = 77.0$ for CDCl_3 , $\delta_{\text{C}} = 29.8$ for $(\text{CD}_3)_3\text{CO}$, $\delta_{\text{C}} = 1.32$ for CD_3CN). Chemical shifts for $^{31}\text{P}\{^1\text{H}\}$ were referenced with respect to 85% aqueous H_3PO_4 .

3.6.2 Synthesis of **15a/b** and **16a/b**

Sodium hydride (5 mg, 0.22 mmol) and 2-mercaptopyridine (24 mg, 0.22 mmol) were added into a solution of **5a** (0.25 mmol) in acetonitrile (5 mL). The resulting mixture was then stirred for 2h at ambient temperature. The solvent was then evaporated in vacuum. The residue was purified by column chromatography on silicagel with dichloromethane/ethyl acetate (v/v = 8/2) as an eluent, which afforded **15a** as yellow solid. A sample of **15a** (20 mg) was dissolved in tetrahydrofuran (2 mL) and kept the resultant solution at room temperature for 2 d which afforded yellow crystals of **16a**.

An analogous procedure was followed for the synthesis of **15b** and **16b**.

Characterization data are given in Tables 3.4-3.5.

Table 3.4. Yield, HRMS and IR data for **15a/b** and **16a/b**.

Compounds	Yield (mg, mmol, %)	*HRMS ⁺ (m/z) [(M+H) ⁺] found (calculated)	FT-IR (ACN, cm ⁻¹)
15a	18, 0.02, 88	389.9443, calculated for C ₁₃ H ₁₀ N ₃ O ₃ SRu : 389.9489	2050 (s), 1985(s), 1621 (s)
15b	32, 0.08, 36	403.9625, calculated for C ₁₄ H ₁₂ N ₃ O ₃ SRu : 403.9643	2051 (s), 1983 (s), 1634 (m)
16a	70, 0.18, 72	389.9486, calculated for C ₁₃ H ₁₀ N ₃ O ₃ SRu : 389.9489)	2049 (s), 1985 (s), 1621 (m)
16b	41, 0.10, 46		2051 (s), 1983 (s), 1634 (m)

* Calculated values are for ¹⁰²Ru unless otherwise indicated

Elemental analyses:

15a: Found C 43.16, H 3.54, N 8.73, S 6.07; Calculated for C₁₃H₉N₃O₃SRu.C₄H₈O₂: C 42.85, H 3.60, N 8.82, S 6.73.

15b: Found C 41.83, H 2.44, N 10.55, S 8.63; Calculated for C₁₄H₁₁N₃O₃SRu: C 41.79, H 2.76, N 10.44, S 7.97.

Table 3.5. ¹H and ¹³C{¹H} NMR data for **15a/b** and **16a/b**.

Compounds	¹ H NMR (CDCl ₃)	¹³ C NMR ((CD ₃) ₂ CO)
15a	8.57 (br, 1H, NH), 8.16-8.14 (m, 1H, PyH), 7.79-7.74 (m, 1H, PyH), 7.53-7.48 (m, 1H, PyH), 7.27-7.26 (m, 1H, PyH), 7.04-7.01 (m, 2H, PyH), 6.87-6.80 (m, 2H, PyH)	202.2 (NHCO), 197.5 (terminal CO), 193.1 (terminal CO), 180.7, 158.3, 150.1, 145.6, 140.2, 136.1, 126.9, 118.1, 116.4, 109.3.
15b	8.38 (br, 1H, NH), 8.02-8.01 (m, 1H, PyH), 7.56-7.51 (m, 1H, PyH), 7.36-7.34 (m, 1H, PyH), 7.27-7.23 (m, 1H, PyH), 6.80-6.75 (m, 2H, PyH), 6.67-6.62 (m, 1H, PyH), 2.08 (s, 3H, Me).	
16a	8.94-8.92 (m, 1H, PyH), 8.76 (br, 1H, PyH), 7.78-7.74 (m, 1H, PyH), 7.53-7.50 (m, 1H, PyH), 7.37-7.34 (m, 1H, PyH), 6.96-6.92 (m, 1H, PyH), 6.89-6.86 (m, 1H, PyH), 6.84-6.83 (m, 1H, PyH), 6.74-6.71 (m, 1H, PyH)	201.6 (NHCO), 198.4 (terminal CO), 196.7 (terminal CO), 182.0, 158.4, 148.1, 148.0, 141.0, 136.5, 128.1, 117.4, 116.5, 109.2
16b	8.64 (br, 1H, NH), 7.70-7.69 (m, 1H, PyH), 7.56-7.51 (m, 1H, PyH), 7.28-7.24 (m, 1H, PyH), 6.89-6.82 (m, 2H, PyH), 6.76-6.75 (m, 1H, PyH), 6.69-6.64 (m, 1H, PyH), 3.06 (s, 3H, Me)	

3.6.3 Synthesis of **15a''** and **16a''**

A sample of **17''** (50 mg, 0.10 mmol), 2-aminopyridine (10 mg, 0.10 mmol) and toluene (10 ml) were placed into a 30 mL microwave vial. The vial was sealed and then subjected to microwave radiation at a holding temperature of 170 °C for 4 h, to afford a pale-yellow solution. The solvent was then evaporated in vacuum. The residue was purified by column chromatography on silica gel with DCM/ EA (v/v = 6:4) as an eluent, which afforded **15a''** as yellow solid (23 mg, 0.05 mmol, 48%). FT-IR (KBr, cm⁻¹): 2028 (s), 1951 (s), 1620 (m). ¹H NMR (400 MHz, CD₃CN): δ 9.62 (br, 1H, NH), 8.38-8.36 (m, 1H, PyH), 7.88-7.83 (m, 1H, PyH), 7.64-7.58 (m, 1H, PyH), 7.33-7.29 (m, 1H, PyH), 7.25-7.21 (m, 1H, PyH), 7.16-7.19 (m, 1H, PyH), 6.88-6.83 (m, 1H, PyH), 6.78-6.75 (m, 1H, PyH). HRMS⁺ (m/z): found 480.0012 [M+H]⁺, calculated for C₁₃H₁₀N₃O₃SOs¹⁹⁰ : 480.0058.

A sample of **15a''** (10 mg) was dissolved in dichloromethane and kept the resultant solution at 0°C 2 d, which afforded crystals of **16a''** suitable for x-ray diffraction study. FT-IR (KBr, cm⁻¹): 2027 (s), 1950 (s), 1622 (m). ¹H NMR (400 MHz, CD₃CN): δ 9.00-8.97 (m, 1H, PyH), 8.90 (br, 1H, NH), 7.79-7.75 (m, 1H, PyH), 7.61-7.59 (m, 1H, PyH), 7.41-7.37 (m, 1H, PyH), 7.06-7.04 (m, 1H, PyH), 6.94-6.91 (m, 1H, PyH), 6.83-6.76 (m, 2H, PyH). ¹³C NMR (400 MHz, CDCl₃): 193.4 (NHCO), 182.5 (terminal CO), 178.8 (terminal CO), 177.6, 168.9, 148.4, 147.5, 140.8, 136.0, 129.7, 117.8, 116.9, 108.6 ppm. HRMS⁺ (m/z): found 480.0056 [M+H]⁺, calculated for C₁₃H₁₀N₃O₃SOs¹⁹⁰ : 480.0058.

3.6.4 Synthesis of **15a'** and **16a'**

Triethylamine (0.03 mL, 0.21 mmol) and 2-mercaptopyridine (22 mg, 0.21 mmol) were added into a solution of **4a'** (100 mg, 0.21 mmol) in acetonitrile (10 mL). The resulting mixture was then stirred for 2h at ambient temperature to give a brown solution. The solvent was then evaporated in vacuum. The residue was dissolved in dichloromethane (5 mL) and placed onto a silica column. Elution with dichloromethane/acetone (v/v = 20/1) developed a yellow band, which afforded **15a'** as orange crystals (27 mg, 0.08 mmol, 38%). Found C 45.53, H 2.30, N 12.33, S 9.93; Calculated for C₁₃H₉N₃O₃SFe: C 45.50, H 2.64, N 12.25, S 9.34. FT-IR (ACN, cm⁻¹): 2036 (s), 1971 (s), 1601 (m). ¹H NMR (400 MHz, CD₃CN): δ 8.66 (br, 1H, NH), 8.20-8.18 (m, 1H, PyH), 7.77-7.72 (m, 1H, PyH), 7.55-7.71 (m, 1H, PyH), 7.16-7.14 (d, 1H, PyH), 7.07-7.01 (m, 2H, PyH), 6.83-6.78 (m, 2H, PyH). ¹³C NMR (400 MHz, CDCl₃): 183.5 (NHCO), 180.2 (terminal CO), 179.2 (terminal CO), 158.1, 150.5, 149.0, 145.9, 139.1, 135.8,

126.6, 118.3, 117.0, 109.5 ppm. HRMS⁺ (m/z): found 343.9796, calculated for C₁₃H₁₀N₃O₃SFe: 343.9792.

A sample of **15a'** (10 mg) was dissolved in acetonitrile and kept the resultant solution at room temperature 1 d, which afforded **16a'**. ¹H NMR (400 MHz, CD₃CN): δ 9.80 (br, 1H, NH), 9.02-9.01 (m, 1H, PyH), 7.74-7.68 (m, 1H, PyH), 7.64-7.63 (m, 1H, PyH), 7.52-7.49 (d, 1H, PyH), 7.44-7.34 (m, 1H, PyH), 7.04-6.98 (m, 1H, PyH), 6.79-6.74 (m, 1H, PyH), 6.64-6.00 (m, 1H, PyH) ppm.

3.6.5 General procedure for the reaction of **15a** with PMe₃

A sample of PMe₃ (30 μl, 0.2 mmol) was added to a solution of **15a** (78 mg, 0.2 mmol) in acetonitrile (10 ml). The resulting mixture was then stirred for 1 h at ambient temperature. Slow evaporation of the solvent at room temperature afforded **18a** as yellow crystals after 2 d.

Experimental and characterization data are given in Tables 3.6-3.7.

A sample of PMe₃ (30 μl, 0.2 mmol) was added to a solution of **15a** (78 mg, 0.2 mmol) in acetonitrile (10 ml). The resulting mixture was then stirred for 12 h at ambient temperature. The solvent was then evaporated in vacuum. The residue was dissolved in dichloromethane (5 mL) and placed onto a silica column. Elution with dichloromethane/methanol (9.5:0.5, v/v) developed two yellow bands, top one afforded **19a** and bottom one afforded **11c** as yellow powder.

Experimental and characterization data are given in Tables 3.6-3.7.

3.6.6 General procedure for the reaction of **15a** with PBu₃

A sample of Pbu₃ (20 μl, 0.2 mmol) was added to a solution of **15a** (78 mg, 0.2 mmol) in acetonitrile (10 ml). The resulting mixture was then stirred for 20 h at 75 °C. The solvent was then evaporated in vacuum. The residue was dissolved in dichloromethane (5 mL) and placed onto a silica column. Elution with ethylacetate developed two yellow bands, top one afforded **19b** and bottom one afforded **11d** as yellow powder. **19b** (80 mg, 0.14 mmol, 70%). ν_{CO} (THF, cm⁻¹): 1957 (s). ³¹P NMR (400 MHz, CDCl₃): δ 8.16 (s, PMe₃) ppm. HRMS⁺ (m/z): calculated for 562.2142 [M+H]⁺, found 562.2122 [M+H]⁺. **11d** (30 mg, 0.04 mmol, 20%). ν_{CO} (THF, cm⁻¹): 1947 (s). ³¹P NMR (400 MHz, CDCl₃): δ 3.48 (s, PMe₃) ppm. HRMS⁺ (m/z): found 765.7743 [M+H]⁺, calculated for 765.7723.

Table 3.6. Yield, HRMS and IR data for **18a**, **19a** and **11c**.

Compounds	Yield (mg, mmol, %)	HRMS ⁺ (m/z) ([M+H] ⁺) found (calculated)	FT-IR (THF cm ⁻¹)
18a	70, 0.15, 74	465.9929, calculated for C ₁₆ H ₁₉ N ₃ O ₃ PSRu: 465.9928	2048 (s), 1982 (s)
19a	37, 0.08, 42	437.9984, calculated for C ₁₅ H ₁₉ N ₃ O ₂ PSRu: 437.9979	1955 (s)
11c	27, 0.05, 26	513.7718, calculated for C ₁₈ H ₂₈ N ₃ O ₂ P ₂ SRu: 513.7713	1945 (s)

* Calculated values are for ¹⁰²Ru unless otherwise indicated

Elemental analyses:

19a: Found C 41.31, H 3.07, N 9.36, S 5.54; Calculated for C₁₆H₁₈N₃O₃PSRu: C 41.38, H 3.91, N 9.05, S 6.90.

Table 3.7. ¹H and ³¹P NMR data for **18a**, **19a** and **11c**.

Compounds	¹ H NMR (CDCl ₃)	³¹ P NMR (CDCl ₃)
18a	9.39-9.37 (br, 1H, NH), 8.17-8.16 (d, 1H, <i>J</i> = 6.0 Hz, PyH), 8.13-8.11 (d, 1H, <i>J</i> = 0.92 Hz, PyH), 7.84-7.80 (m, 1H, PyH), 7.24-7.22 (m, 1H, PyH), 7.17-7.13 (m, 2H, PyH), 6.97-6.93 (m, 1H, PyH), 6.72-6.69 (m, 1H, PyH), 1.67-1.64 (d, 9H, <i>J</i> = 8.2 Hz, Me)	-25.1
19a	9.04-9.02 (d, 1H, <i>J</i> = 5.5 Hz, PyH), 8.93-8.90 (br, 1H, NH), 7.61-7.57 (m, 1H, PyH), 7.57-7.52 (m, 1H, PyH), 7.16-7.12 (m, 1H, PyH), 6.93-6.91 (d, 1H, <i>J</i> = 8.2 Hz, PyH), 6.87-6.85 (d, 1H, <i>J</i> = 8.2 Hz, PyH), 6.80-6.77 (m, 1H, PyH), 6.50-6.47 (m, 1H, PyH), 1.23 (s, 9H, Me)	14.7
11c	8.94-8.92 (br, 1H, NH), 8.72-8.71 (d, 1H, <i>J</i> = 5.5 Hz, PyH), 8.18-8.17 (d, 1H, <i>J</i> = 3.7 Hz, PyH), 7.61-7.59 (t, 1H, <i>J</i> = 7.4 Hz, PyH), 7.49-7.47 (d, 1H, <i>J</i> = 8.2 Hz, PyH), 7.20-7.19 (m, 1H, <i>J</i> = 6.7 Hz, PyH), 6.91-6.89 (d, 1H, <i>J</i> = 8.2 Hz, PyH), 6.83-6.79 (t, 1H, <i>J</i> = 6.4 Hz, PyH), 6.70-6.67 (t, 1H, <i>J</i> = 5.5 Hz, PyH), 1.19 (s, 18H, Me)	-5.6

3.6.7 Computational studies

The computational studies were carried out using density function theory (DFT), utilizing the hybrid functional of Truhlar and Zhao (M06).¹⁵ The basis set used was def2TZVP,¹⁶ for the transition metals, and 6-311+G(2d,p) for the light atoms. For calculations involving solvated species, the polarized continuum model (PCM) was employed. Spin-restricted calculations were used for structural optimization, harmonic frequency calculations and to evaluate zero-point energy (ZPE) corrections. Optimized geometries were characterized as equilibrium structures with all real frequencies. All calculations were performed using the Gaussian 09 suite of programs.¹⁷ The coordinates for parent complexes were taken from the crystal structure and each of the other isomers was modified from this.

3.6.8 Crystallographic studies

Diffraction-quality crystals were grown from THF (**16a** and **16a''**), an acetone/DCM (**15a'**) or a DCM/EA (**16b**) mixture, and mounted on quartz fibers. X-ray diffraction data were collected at 103(2) K on a Bruker X8 APEX system, using Mo K α radiation, with the SMART suite of programs.¹⁸ Data processing and correction were made for Lorentz and polarization effects with SAINT,¹⁹ and for adsorption effects with SADABS.²⁰ Structural solution and refinement were carried out with the SHELXTL suite of programs.²¹ The structures were solved by direct methods to locate the heavy atoms, followed by successive difference maps for the light, non-hydrogen atoms. THF solvates were found for **16a** and **16a''**, with that in the latter exhibiting disorder which was modeled with two sites, with occupancies summed to unity. The molecule of **16a''** also exhibited disorder of both the organic ligands, which were modelled with two sites each with their occupancies summed to unity. A disordered DCM solvate was found for **18a**; the disorder was modelled over three locations. Appropriate restraints were placed on all disordered parts. All non-hydrogen atoms were refined with anisotropic displacement parameters in the final model. All the organic hydrogen atoms were placed in calculated positions and refined with a riding model. The crystallographic data for **15a'**, **16a**, **16b** and **16a''**, and for **11c**, **11d** and **18a**, are summarized in Table 3.8 and 3.9, respectively.

Table 3.8. Crystal and refinement data for **15a'**, **16a** and **15b**.

Complex	15a'	16a	15b
Empirical formula	C ₁₃ H ₉ N ₃ O ₃ SFe	C ₁₃ H ₉ N ₃ O ₃ SRu.C ₄ H ₈ O	C ₁₄ H ₁₁ N ₃ O ₃ SRu
Formula weight	343.14	460.46	402.39
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	P2 ₁ /c	P $\bar{1}$	P 1 21/c 1
a, Å	14.4937(16)	8.9181(18)	15.2122(4)
b, Å	7.0729(8)	9.4837(19)	7.7502(2)
c, Å	13.5049(15)	11.951(2)	12.8631(3)
α , deg	90	89.310(11)	90
β , deg	93.177(4)	70.975(10)	94.2485(11)
γ , deg	90	76.551(11)	90
Volume, Å ³	1382.3(3)	927.1(3)	1512.36(7)
Z	4	2	4
ρ_c , Mg/m ³	1.649	1.649	1.767
μ , mm ⁻¹	1.254	0.985	1.189
F(000)	696	464	800
Crystal size, mm ³	0.160 x 0.120 x 0.040	0.420 x 0.420 x 0.400	0.060 x 0.080 x 0.220
Reflections collected	17842	30503	23388
Independent reflections	4431 [R(int) = 0.0779]	4158 [R(int) = 0.0898]	7299 [R(int) = 0.0566]
Max. and min. transmission	0.95 and 0.78	0.6940 and 0.6820	0.9320 and 0.7800
Data / restraints / parameters	4431 / 0 / 190	4154 / 0 / 238	7299 / 0 / 203
Goodness-of-fit on F ²	1.036	1.106	1.026
Final R indices [I>2sigma(I)]	R1 = 0.0553, wR2 = 0.1151	R1 = 0.0371, wR2 = 0.0769	R1 = 0.0451, wR2 = 0.0774
R indices (all data)	R1 = 0.0989, wR2 = 0.1349	R1 = 0.0578, wR2 = 0.0897	R1 = 0.0795, wR2 = 0.0912
Largest diff. peak and hole, e.Å ⁻³	1.147 and -0.536	1.345 and -0.829	1.326 and -1.272

Table 3.9. Crystal and refinement data for **16b**, **16a''** and **18a**.

Complex	16b	16a''	18a
Empirical formula	C ₂₈ H ₂₂ N ₆ O ₆ Ru ₂ S ₂	C ₁₇ H ₁₇ N ₃ O ₄ SOs	C ₁₇ H ₂₂ N ₃ O ₄ PSRu
Formula weight	804.77	549.59	496.47
Crystal system	Orthorhombic	Triclinic	Triclinic
Space group	P c a 2 ₁	P $\bar{1}$	P $\bar{1}$
a, Å	14.5359(5)	8.7626(9)	8.2139(7)
b, Å	12.0357(5)	9.0312(9)	12.5608(14)
c, Å	17.3015(7)	12.0826(11)	19.8742(19)
α , deg	90	88.739(3)	90.776(3)
β , deg	90	73.350(3)	97.718(2)
γ , deg	90	86.469(3)	93.283(3)
Volume, Å ³	3026.9(2)	914.34(16)	2028.1(3)
Z	4	2	4
ρ_c , Mg/m ³	1.766	1.996	1.626
μ , mm ⁻¹	1.188	7.115	0.982
F(000)	1600	528	1008
Crystal size, mm ³	0.140 x 0.220 x 0.320	0.100 x 0.210 x 0.320	0.020 x 0.040 x 0.120
Reflections collected	25072	27656	7612
Independent reflections	10008 [R(int) = 0.1161]	5808 [R(int) = 0.1152]	96.9%
Max. and min. transmission	0.8510 and 0.7020	0.5360 and 0.2090	0.9810 and 0.8910
Data / restraints / parameters	10008 / 1 / 399	5808 / 1328 / 471	7612 / 0 / 498
Goodness-of-fit on F ²	0.982	1.025	1.073
Final R indices [I>2sigma(I)]	R1 = 0.0642, wR2 = 0.1338	R1 = 0.0488, wR2 = 0.1024	R1 = 0.0716, wR2 = 0.1047
R indices (all data)	R1 = 0.1048, wR2 = 0.1596	R1 = 0.0859, wR2 = 0.1212	R1 = 0.1230, wR2 = 0.1243
Largest diff. peak and hole, e.Å ⁻³	3.235 and -1.128	2.145 and -2.112	1.129 and -0.985

Table 3.10. Crystal and refinement data for **11c**, **11d** and **19a**.

Complex	11c	11d	19a
Empirical formula	C ₁₈ H ₂₇ N ₃ O ₂ P ₂ RuS. 0.1CH ₂ Cl ₂	C ₃₆ H ₆₃ N ₃ O ₂ P ₂ RuS	C ₃₀ H ₃₆ N ₆ O ₄ P ₂ Ru ₂ S ₂ .CH ₂ Cl ₂
Formula weight	1041.97	764.96	957.77
Crystal system	Trigonal	Monoclinic	Monoclinic
Space group	P $\bar{3}$	P 2 ₁ /c	C2/c
a, Å	17.7042(7)	15.5007(7)	27.593(3)
b, Å	17.7042(7)	10.4578(3)	8.2794(8)
c, Å	12.8694(6)	24.7720(10)	17.4248(18)
α , deg	90	90	90
β , deg	90	92.923(2)	90.5233(19)
γ , deg	120	90	90
Volume, Å ³	3493.3(3)	4010.4(3)	3980.5(7)
Z	6	4	4
ρ_c , Mg/m ³	1.486	1.267	1.598
μ , mm ⁻¹	0.941	0.555	1.120
F(000)	1597	1624	1928
Crystal size, mm ³	0.380 x 0.300 x 0.240	0.060 x 0.100 x 0.120	0.200 x 0.140 x 0.120
Reflections collected	63984	11328	20159
Independent reflections	4793 [R(int) = 0.0847]	99.4%	3779 [R(int) = 0.0422]
Max. and min. transmission	0.81 and 0.69	0.9670 and 0.9360	0.88 and 0.76
Data / restraints / parameters	4793 / 2 / 262	11328 / 0 / 415	3779 / 4 / 232
Goodness-of-fit on F ²	1.172	1.003	1.051
Final R indices [I > 2 σ (I)]	R1 = 0.0378, wR2 = 0.0820	R1 = 0.0486, wR2 = 0.0848	R1 = 0.0385, wR2 = 0.0948
R indices (all data)	R1 = 0.0637, wR2 = 0.0986	R1 = 0.1018, wR2 = 0.1051	R1 = 0.0438, wR2 = 0.0987
Largest diff. peak and hole, e.Å ⁻³	1.086 and -0.519	0.693 and -0.698	2.098 and -1.232

3.7 References

1. Chen, D.; Scopelliti, R.; Hu, X. *Angew. Chem. Int. Ed.* **2010**, *49*, 7512.
2. Song, L.-C.; Hu, F.-Q.; Wang, M.-M.; Xie, Z.-J.; Xu, K.-K.; Song, H.-B. *Dalton Trans.* **2014**, *43*, 8062.
3. Naziruddin, A. R.; Huang, Z.-J.; Lai, W.-C.; Lin, W.-J.; Hwang, W.-S. *Dalton Trans.* **2013**, *42*, 13161.
4. Messerle, B. A.; Tolhurst, V. A.; Turner, P. *Eur. J. Inorg. Chem.* **2003**, *2003*, 293.
5. Gilli, P.; Pretto, L.; Bertolasi, V.; Gilli, G. *Account Chem. Res.* **2008**, *42*, 33.
6. Jeffrey, G. A.; Takagi, S. *Accounts Chem. Res.* **1978**, *11*, 264.
7. Bolano, T.; Esteruelas, M. A.; Fernandez, I.; Onate, E.; Palacios, A.; Tsai, J.-Y.; Xia, C. *Organometallics* **2015**, *34*, 778.
8. Sullivan, B. P.; Caspar, J. V.; Meyer, T. J.; Johnson, S. *Organometallics* **1984**, *3*, 1241.
9. Deeming, A. J.; Randle, N. P.; Bates, P. A.; Hursthouse, M. B. *J. Chem. Soc. Dalt.* **1988**, 2753.
10. Deeming, A. J.; Meah, M. N.; Randle, N. P.; Hardcastle, K. I. *J. Chem. Soc. Dalt.* **1989**, 2211.
11. Turrell, P. J.; Hill, A. D.; Ibrahim, S. K.; Wright, J. A.; Pickett, C. J. *Dalton Trans.* **2013**, *42*, 8140.
12. Turrell, P. J.; Wright, J. A.; Peck, J. N.; Oganessian, V. S.; Pickett, C. J. *Angew. Chem. Int. Ed.* **2010**, *49*, 7508.
13. Andreu, P. L.; Cabeza, J. A.; Fernandez-Colinas, J. M.; Riera, V. *J. Chem. Soc. Dalton. Trans.* **1990**, 2927.
14. Dwivedi, S. D.; Dubey, S. K.; Singh, A. K.; Pandey, K. K.; Pandey, D. S. *Inorg. Chim. Acta* **2010**, *363*, 2095.
15. Zhao, Y.; Truhlar, D. G. *Theor. Chem. Acc.: Theory, Computation, and Modeling (Theor. Chim. Acta)* **2008**, *120*, 215.
16. Ehlers, A.; Böhme, M.; Dapprich, S.; Gobbi, A.; Höllwarth, A.; Jonas, V.; Köhler, K.; Stegmann, R.; Veldkamp, A.; Frenking, G. *Chem. Phys. Lett.* **1993**, *208*, 111.
17. Gaussian09, R., 02, MJ Frisch et al., Gaussian. Inc., Wallingford CT, **2009**.
18. SMART version 5.628; Bruker AXS Inc., Madison, Wisconsin, USA, **2001**.
19. SAINT+ version 6.22a, Bruker AXS Inc., Madison, Wisconsin, USA, **2001**.
20. Sheldrick, G. M. SADABS; University of Göttingen, Göttingen, Germany, **1996**.
21. SHELXTL version 5.1; Bruker AXS Inc., Madison, WI, USA, **1997**.

Chapter 4: Catalytic Studies

Group VIII metal complexes are well-known to catalyze a wide range of organic transformation reactions.^{1,2,3} It is thus reasonable to expect that the compounds which have been synthesized would exhibit catalytic activity. In this chapter, the catalytic activity of the complexes **5a**, **5b** and **15n** are examined (Figure 4.1).

Figure 4.1. Synthesized structural mimics and precursors.

As structural mimics of [Fe]-hydrogenase, the complexes may act similarly thus the reactions which are related to the activity of [Fe]-hydrogenase are described first (section 4.1). This is followed by two other reactions which are more remotely related – the hydrolysis of organosilanes for the production of hydrogen (section 4.2), and the hydrocarboxylation of terminal alkynes (section 4.3).

4.1 Hydrogenases activity

Biological [Fe]-hydrogenase (Hmd) catalyzes the reduction of the carbocationic substrate, *N*⁵,*N*¹⁰-methenyltetrahydromethanopterin (methenyl-H₄MPT⁺) through heterolytic cleavage of dihydrogen into H⁺ and H⁻ (Scheme 4.1).⁴ As the natural substrate is hard to come by, it is useful to examine a close analogue instead. The essential structural feature of the substrate is the imidazolinium ring. Progressive simplification of the substrate leads to either a symmetrically or unsymmetrically N-disubstituted imidazolinium (Scheme 4.2). Hydrogenation of such a simplified substrate would be an interesting approach to assess functional models of the [Fe]-hydrogenase. Unfortunately, **15a** (9 mol%) failed to hydrogenate both (1-ethyl-3-methyl)imidazolium chloride and 1,3-bis(dimethyl)imidazolinium tetrafluoroborate under a dihydrogen atmosphere (2 bars) at room temperature even after 2 d.

Scheme 4.1. Reversible reduction of methenyl-H₄MPT⁺.

Scheme 4.2. Simplified model of biological substrate and its reduction.

A second approach taken to assess the catalytic activity of the complexes **15n** was that of electrocatalytic hydrogen production. Unlike for the two other types of hydrogenases, electrocatalytic hydrogen production with [Fe]-hydrogenase model complexes have not been explored much, beyond a recent report on iron model complexes (Figure 4.2).^{5,6,7}

Figure 4.2. Reported functional mimics.

4.1.1 Electrochemical properties of **15n**

The cyclic voltammograms of **15a'**, **15a** and **15a''** in acetonitrile shows multiple irreversible oxidation and reduction waves, with the first oxidation potential increasing in the order **15a'** < **15a** < **15a''**, and the first reduction potential in the reverse trend (Figure 4.3).

Figure 4.3. Cyclic voltammograms of 1.0 mM **15a'** (blue trace), **15a** (red trace) and **15a''** (black trace) with 0.1 M *n*-Bu₄NPF₆ in CH₃CN recorded at a scan rate of 0.1 V s⁻¹, at 25±2 °C, with a 1 mm diameter planar glassy carbon working electrode (left). Electrochemical data of all compounds (right).

The very similar oxidation potentials for the three complexes suggest that oxidation probably occurred at the thiolate S atom, while the similar peak heights for the first oxidation and reduction waves indicate that the same number of electrons are transferred. The oxidized and reduced species, however, are electrochemically active and undergo further oxidation/reduction to as-yet unidentified species. A comparison with the previously reported [Fe]-hydrogenase model complexes [Fe(2-CH₂C(O)C₅H₃NR₂)(CO)₂(2-SC₅H₄N)] (R₂ = CH₂OCOMe (**15e'**) and R₂ = CH₂OCOPh (**15f'**)) suggests that their electrochemical behavior are very similar (Figure 4.3); all the complexes display irreversible first oxidation and reduction waves.⁵

A controlled potential electrolysis (CPE) experiment for both oxidation and reduction processes were carried out on **15a** to measure the number of electrons exchanged during both the first reduction and oxidation processes. The cyclic voltammograms and the current versus time plot obtained before and after exhaustive first oxidation of **15a** are shown in Figure 4.4. The current versus time plot shows that one electron per molecule was transferred from **15a**.

Figure 4.4. (a): Cyclic voltammograms of 1.0 mM **15a**/0.5 M $n\text{-Bu}_4\text{NPF}_6$ in CH_3CN prior to bulk oxidation (red) and after exhaustive oxidation (black). Scans recorded at a scan rate of 0.1 V s^{-1} at $25 \pm 2^\circ \text{C}$ with a 1 mm diameter planar glassy carbon working electrode. (b): Current vs time data obtained during the exhaustive oxidation of **15a** at +1.55 V vs. reference electrode.

Similar to the oxidation process above, cyclic voltammograms and the current versus time plots obtained before and after exhaustive first reduction of **15a** are shown in Figure 4.5. The current versus time plot indicates that one electron per molecule was transferred to **15a**.

Figure 4.5. (a): Cyclic voltammograms of 1.0 mM **15a**/0.5 M $n\text{-Bu}_4\text{NPF}_6$ in CH_3CN prior to the bulk reduction (red) and after exhaustive reduction (black). Scans recorded at a scan rate of 0.1 V s^{-1} at $25 \pm 2^\circ \text{C}$ with a 1 mm diameter planar glassy carbon working electrode. (b): Current vs. time data obtained during the exhaustive reduction of **15a** at -1.75 V vs. reference electrode.

4.1.2 Electrocatalytic hydrogen production

The electrocatalytic efficiencies of **15a'**, **15a** and **15a''** for the production of hydrogen with trifluoroacetic acid (TFA) as the proton source were also examined. The current at the reduction potentials increased, and continued to increase considerably, with the addition of more TFA (Figure 4.6). Such a striking change in the acid-induced current is indicative of an electrocatalytic proton reduction process.^{5,8} A TOF of 2.4 h^{-1} at an overpotential of -0.21 V was measured for **15a'**, which is lower than that of reported complexes **15e'** (10.3 h^{-1} at -0.14 V) and **15f'** (14.5 h^{-1} at -0.15 V).⁵ The overpotential for **15a'** was taken as the difference between the first reduction potential (-1.90 V) and the applied potential (-2.11 V), and the estimated catalytic current at this same overpotential were -72 , -36 and $-43 \mu\text{A}$ for **15a'**, **15a** and **15a''**, respectively (grey dotted lines, Figure 4.6). The efficiency for hydrogen production based on the catalytic current produced thus show that **15a'** was much more efficient than the other two complexes. The evolved hydrogen gas was confirmed from a combination of CPE and GC experiments. Although the mechanism for the electrocatalytic proton reduction has not been studied in detail, it is probably similar to that reported for the other hydrogenases model complexes (Scheme 4.3).^{9,10,11}

Scheme 4.3. Proposed mechanism for the electrocatalytic proton reduction.

Figure 4.6. Cyclic voltammogram of (a) **15a'** (b) **15a** and (c) **15a''** (1.0 mM)/ $n\text{-Bu}_4\text{NPF}_6$ (0.1 M) in CH_3CN recorded at a scan rate of 0.1 V s^{-1} at $25 \pm 2^\circ \text{C}$ with a 1 mm diameter planar glassy carbon working electrode at different concentrations of trifluoroacetic acid (red: 0 mM, blue: 2 mM, green: 5 mM, pink: 10 mM and black: 20 mM).

4.2 Hydrolysis of organosilanes

For the hydrogen economy, besides production is the problem of storage.^{12,13} One potential solution is to store the hydrogen in hydride form. One such are organosilanes which are able to release hydrogen through catalytic hydrolysis.¹⁴ These produce organosilanols as the only by-product, which are valuable building blocks for the polymer and materials industry, and in various organic transformations including olefin-hydroformylation and Heck-type coupling reactions.^{15,16,17} A recent study reported that the iron complex **4a'** was an efficient catalyst for hydrogen generation from the hydrolysis of organosilanes (Scheme 4.4).¹⁸ It can therefore be expected that the ruthenium analogues **5a** and **5b** may also perform the same catalytic reaction.

Scheme 4.4. Hydrolysis of tertiary silane by reported complex **4a'**.

A strong effervescence was immediately observed when phenylsilane was added into a wet acetonitrile solution of **5a** at room temperature (Scheme 4.5). It produced significantly more gas in the presence of excess water. No gas evolution was observed with anhydrous acetonitrile or when either phenylsilane or **5a** was removed.

Scheme 4.5. Hydrolysis of primary silane by **5a**.

The identity of the evolved gas was not confirmed by spectroscopically but assumed to be dihydrogen in parallel with the literature.^{19,20} The organosilane and water supplied one H atom each,¹⁸ and the by-product was organosilanol. In the case of phenylsilane, the phenylsilanetriol by-product has been characterized by ¹H NMR spectroscopy and mass

spectrometry. It was obtained quantitatively as a white powder after removal of the solvent. The reaction also proceeded in other solvents such as tetrahydrofuran.

Complex **5a** was effective for the hydrolysis of a wide range of organosilanes, and exhibited much faster rates for primary silanes (RSiH_3) than for tertiary silanes (R_3SiH); the yield was 99% in 20 s for phenylsilane (Table 4.1). Similar results were observed using **5b** as the catalyst. Optimization of the catalyst loading for the hydrolysis of phenylsilane showed that 3 mol-% was optimal (Table 4.2).

Table 4.1. Hydrolysis of various organosilane catalyzed by **5a** and **5b**.

Entry	Silane	5a		5b	
		Time (min)	H ₂ yield (%)	Time (min)	H ₂ yield (%)
1	PhSiH₃	20 sec	99	20 sec	99
2	Ph(CH ₂) ₃ SiH ₃	10	96	10	95
3	CH ₃ (CH ₂) ₅ SiH ₃	22	96	15	85
4	Ph ₂ SiH ₂	30	91	20	99
5	(o-ClC ₆ H ₄ CH ₂) ₂ SiH ₂	40	93	35	88
7	(ⁱ Pr) ₃ SiH	20	96	20	85
8	Et ₃ SiH	15	99	20	97
9	*PMHS	120	80	120	86

*PMHS = Poly(methylhydrosiloxane)

Although several transition metal-based catalysts, such as, rhodium,²¹ iridium,²² rhenium,²³ and ruthenium,^{16,19} have been reported for silane hydrolysis, none of them show such a fast rate; this is the first example in which hydrolysis is completed within a few seconds.

It is also the first example in which the catalyst is more selective towards primary over tertiary silanes. A comparison of the turnover frequency, against that of several heterogeneous catalysts reported recently for the hydrolysis of phenylsilane,^{24,25} shows that **5a** and **5b** perform better (Table 4.3). The recyclability of **5a** and **5b** was also verified; the yield showed a decrease of ~30% after the 5th cycle (Figure 4.7).

Table 4.2. Optimisation of catalyst loading of **5a** and **5b** in the hydrolysis of phenylsilane.

Entry	Loading	Yield (%) - 5a	Yield (%) - 5b
1	1 mol-%	76	80
2	2 mol-%	85	88
3	3 mol-%	99	99

Table 4.3. Catalytic performance of various catalysts in the hydrolysis of phenylsilane.

Catalyst	Time (min)	H ₂ (%)	TOF (hr ⁻¹)
5a	20 sec	99	5940
5b	20 sec	99	5940
Carbon Nanotube–Gold Nanohybrids ²⁵	90	97	647
Nanoporous Gold ²⁰	360	80	2.67
Nanoporous Silver ²⁴	120	50	2.50

Figure 4.7. Recyclability of **5a** (left) and **5b** (right) for the hydrolysis of phenylsilane.

4.3 Hydrocarboxylation of terminal alkynes

The catalytic hydrocarboxylation of terminal alkynes is an organic transformation of great interest as the intra- and intermolecular versions give rise to enol lactones and β -oxo-esters, respectively (Scheme 4.6).

Scheme 4.6. Hydrocarboxylation reaction of terminal alkynes.

Many natural products containing enol lactones show biological activity.^{26,27,28} While some contain an exocyclic substructure,²⁹ others contain an endocyclic ring.³⁰ Exocyclic enol lactones are useful intermediates in the synthesis of more complex natural products such as parthenolide, helenalin and arglabin (Figure 4.8). Similarly, β -oxo-esters are also important substrates in pharmaceutical chemistry because the acetonide ester motif can be found in several natural compounds and biologically active steroids.^{31,32,33}

Figure 4.8. Structures of parthenolide, helenalin and arglabin.

The cyclization of α,ω -acetylenic acid represents an effective synthetic approach to the enol lactone system.^{34,28} Only two ruthenium complexes, viz., $[\text{TpRu}\{\text{PhCNC}(\text{Ph})\text{CCPh}\}(\text{PMe}^i\text{Pr}_2)]$ and $[\text{TpRuH}(\text{PPh}_3)_2]$, have been reported for this reaction. The former gives the endocyclic enol lactone (anti-Markovnikov) and the latter a mixture of the exocyclic (Markovnikov) and endocyclic enol lactones,³⁵ but neither gives the

exocyclic enol lactone regioselectively. It was found that complex **15a** catalyzes the cyclization of 5-hexynoic acid to the corresponding exocyclic enol lactone **23b**, regioselectively and exclusively (Scheme 4.7). The cyclization takes place smoothly in refluxing toluene within 12 h and with 1.4% catalyst loading. The product **23b** was characterized by ^1H NMR and mass spectrometry and matched the reported values.³⁵

Scheme 4.7. Cyclization of α,ω -acetylenic acid.

With alkynoic acids [$\text{HOOCCH}_2(\text{CH}_2)_n\text{CCH}$ ($n = 1, 2, 3, 7$)] of different chain lengths, it was observed that the exocyclic enol lactones **23n** were obtained for $n = 1$ -3 whereas the endocyclic enol lactone **24n** resulted for $n = 7$ under the same reaction conditions; a control reaction without **15a** afforded no products (Table 4.4).³⁵ The cyclization was not stereoselective for the larger cyclic enol lactone, giving a mixture of the Z- and E-enol lactones. The yield also decreased with the increase in ring size; high yields were obtained for the five and six-membered ring systems. This may be ascribed to the higher thermodynamic stability of these rings compare to the other ring sizes. An attempt at the cyclization of 5-hexynoic acid with **15a'** was unsuccessful due to decomposition at higher temperature, whereas **15a''** showed similar catalytic efficiency to **15a** (Table 4.4). Optimization of the catalyst loading for **15a** shows that 1.4 mol-% was optimal (Table 4.5).

Several ruthenium complexes have been reported to catalyze the intermolecular hydrocarboxylation of terminal alkynes to β -oxo-esters,^{37,38,39} and **15a** was also found to catalyze the reaction of propargyl alcohol with benzoic acid, with Na_2CO_3 as a cocatalyst,³⁷ to afford 2-oxopropyl benzoate regioselectivity in 54% yield (Scheme 4.8).

Scheme 4.8. Intermolecular hydrocarboxylation of terminal alkynes.

Table 4.4. Catalyzed cyclization of alkynoic acids to enol lactones.

Entry	Catalyst	n	Product ratio 23n:24n	Total yield (%) ^a
1	15a	0	0	0
2	15a	1	100:0	93
3	15a	2	100:0	98
4	15a	3	100:0	57
5	15a	7	0:100 ^b	80
6	15a''	1	100:0	62
7	15a''	2	100:0	77

^aAll yields are based on NMR measurements with anisole as internal standard. ^bAs a mixture of Z/E isomers in the ratio 55:45.

Table 4.5. Optimization of catalyst loading for **15a** using 5-hexynoic acid.

Entry	Catalyst	Yield (%) ^a
1	0.3 mol-%	62
2	0.8 mol-%	80
3	1.4 mol-%	98

^aAll yields are based on NMR measurements with anisole as internal standard.

The catalytic cycles for the intramolecular reactions presumably follow that given in the literature (Scheme 4.9).^{36,37} Initial partial decoordination of the 2-mercaptopyridine ligand in **15a** allows for coordination of the C≡C triple bond of the alkynoic acid. An intramolecular nucleophilic attack by the carboxylate group at the α- or β-position of the metal-bound alkyne moiety is followed by reductive elimination to afford either the exocyclic or endocyclic product, respectively; while an intermolecular nucleophilic attack by a carboxylate group at the α-position of the metal-bound alkyne leads to a β-oxo-esters. The change in selectivity with the chain length may have to do with the accessibility to the carboxylate, of the α- or β-position of the metal-bound alkyne; with a shorter chain, the α-position is more accessible and formation of a terminal alkenyl complex is also favoured, while this is decreased with a longer chain length.

Scheme 4.9. Proposed catalytic cycle for the intramolecular hydrocarboxylation of terminal alkyne.

4.4 Conclusion

The ruthenium complex **15a** was found not to catalyse the H₂-reduction of imidazolium but this may be because the latter is not a good model for the substrate (methenyl-H₄MPT) of natural [Fe]-hydrogenase. Nevertheless, these group VIII metal complexes **15** were found to be efficient catalysts for electrocatalytic hydrogen generation, with the iron complex **15a'** being more efficient than either the ruthenium complex **15a** or osmium complex **15a''**.

On the other hand, **15a** and **15a''** were found to be efficient and regioselective catalysts for the cyclisation of α,ω -acetylenic acids to exocyclic enol lactones. The complexes **5a** and **5b** were also found to catalyse the very rapid hydrolysis of silanes to produce hydrogen gas (TOF $\sim 6000 \text{ h}^{-1}$ over 20 s), and showed selectivity for primary over tertiary silanes.

4.5 Experimental section

4.5.1 General procedures: All experiments were performed under an argon atmosphere using standard Schlenk techniques. Chemicals were obtained from Sigma Aldrich and were used as received. Solvents that were used for reactions were distilled over the appropriate drying agents under argon before use. Purification of compounds was carried out by column chromatography on silica gel. Infrared spectra were recorded on a Bruker Alpha FTIR spectrometer. Solution spectra were recorded in a solution IR cell with NaCl windows and a path length of 0.1 mm, at a resolution of 2 cm^{-1} . HRMS were recorded in ESI mode on a Waters UPLC-Q-ToF MS mass spectrometer. ^1H , $^{13}\text{C}\{^1\text{H}\}$ and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra were obtained on a JEOL 400 MHz spectrometer at room temperature unless otherwise stated. Chemical shifts for ^1H and $^{13}\text{C}\{^1\text{H}\}$ were referenced with respect to the residual resonances of the respective deuterated solvents (^1H NMR: $\delta_{\text{H}} = 7.26$ for CDCl_3 , $\delta_{\text{H}} = 2.05$ for $(\text{CD}_3)_3\text{CO}$, $\delta_{\text{H}} = 1.94$ for CD_3CN , ^{13}C NMR: $\delta_{\text{C}} = 77.0$ for CDCl_3 , $\delta_{\text{C}} = 29.8$ for $(\text{CD}_3)_3\text{CO}$, $\delta_{\text{C}} = 1.32$ for CD_3CN). Chemical shifts for $^{31}\text{P}\{^1\text{H}\}$ were referenced with respect to 85% aqueous H_3PO_4 . Electrochemical measurements were made using a computer-controlled Eco ChemieAutolab PGSTAT302N potentiostat in a three-electrode cell with a 1 mm diameter planar glassy carbon disk (Cypress Systems) working electrode, a platinum wire (Metrohm) auxiliary electrode, and a silver wire (Cypress Systems) miniature reference electrode connected to the main solution via a salt bridge containing 0.5 M $n\text{-Bu}_4\text{NPF}_6$ in CH_3CN . Gas chromatography measurements were made using Agilent 7890A gas-chromatograph with a thermal conductivity detector (TCD), a 5 Å molecular sieve column (2 mm $\times$ 5 m), and argon as the carrier gas.

4.5.2 Electrochemical and electro catalytic experiments

A solution of 0.1 M $n\text{-Bu}_4\text{NPF}_6$ in MeCN was used as an electrolyte in each of electrochemical and electro catalytic experiments. Prior to each scan, the electrolyte solutions used for voltammetric analysis were de-oxygenated by purging with high purity argon gas, and the working electrodes were cleaned by polishing with alumina oxide (grain size 0.3 μm) slurry on a Bueller Ultra-pad polishing cloth rinsing with ultrapure water, acetone and then dried. Accurate potentials were obtained by using ferrocene as an internal reference, which was added to the solutions at the end of the measurements. All voltammetric experiments were performed at room temperature ($25 \pm 2\text{ }^\circ\text{C}$) inside a Faraday cage.

4.5.3 Controlled Potential Electrolysis

Bulk controlled potential electrolysis (CPE) experiments were performed in a two-compartment CPE cell divided by a sintered glass frit with porosity no. 5 (1.0-1.7 μm). Identically sized glassy carbon cylindrical rods (length 6 cm, outer diameter 1.8 cm and internal diameter 1.2 cm) were employed as the working and auxiliary electrodes and symmetrically arranged with respect to each other. A silver wire (Cypress Systems) miniature reference electrode connected to the main solution via a salt bridge containing 0.5 M *n*-Bu₄NPF₆ in CH₃CN was positioned close to within 2 mm of the surface of the working electrode. Approximately 25 mL volumes of solutions were used in each electrode compartment; they were simultaneously deoxygenated and stirred using a stream of argon gas bubbles. All CPE experiments were performed at room temperature (25 $\pm$ 2 $^{\circ}\text{C}$). The number of electrons transferred during the bulk oxidation process may be calculated from $Q = nNF$ equation, where N is the number of moles of starting compound, n is the number of electrons transferred, Q is the charge (coulombs) and F is the Faraday constant (96485 C mol⁻¹).

4.5.4 CPE-GC analysis for measurement of hydrogen

The CPE was performed with a solution of complex **15a'** (1mM) in anhydrous ACN, tetrabutyl ammonium hexafluoro phosphate (0.1 M), trifluoro acetic acid (20 mM). The solution was bubbled with nitrogen gas prior to the experiment. A constant potential of -1.71 V w.r.t SCE was applied for a period of 30 min. The gas was collected from the cell using a syringe fitted with a lock and analyzed by gas chromatography. The amount of hydrogen produced was measured to be 5.9 μmol after deducting the background reaction which corresponds to 2.4 h⁻¹ TOF.

4.5.5 General procedure for the hydrolysis of organosilane

A sample of organosilane (1 mmol) was added into an acetonitrile (2 mL) solution containing the catalyst (3 mol-%) and excess H₂O (10 mmol, 0.07 mL) at 25 $^{\circ}\text{C}$. The reaction mixture was stirred, and immediately evolution of gas was observed. Hydrogen gas was collected and measured using an upward delivery tube set up with an inverted measuring cylinder filled with water. After removal of solvent afforded the desired product.

Phenylsilanetriol, **21a** (154 mg, 1 mmol, 99 %). ¹H NMR (400 MHz, (CD₃)₂CO): 7.72-7.70 (m, 2H, ArH), 7.36-7.30 (m, 3H, ArH), 5.52 (s, 3H, OH).

4.5.6 General procedure for the lactonization of α,ω -acetylenic acid

A sample of α,ω -acetylenic acid (0.9 mmol, 0.1 ml), Na_2CO_3 (0.04 mmol, 4.1 mg) and the catalyst **15a** (0.01 mmol, 5 mg) were dissolved in toluene (5 ml). The resulting mixture was then stirred for 12 h at 100 °C. After cooling to room temperature, removal of solvent afforded the desired product.

23a

An analytically pure product **23a** was isolated by column chromatography using hexane/dichloromethane (9:1, v/v) as the eluent. (0.85 mmol, 93%). ^1H NMR (400 MHz, CDCl_3): δ 4.74-4.72 (m, 1H, J = 2.3 Hz, CH), 4.30-4.29 (m, 1H, J = 1.8 Hz, CH), 2.87-2.82 (m, 2H, CH_2), 2.66-2.62 (m, 2H, CH_2).

Literature values: ^1H NMR (400 MHz, CDCl_3): δ 4.77-4.72 (br, 1H, CH), 4.34-4.27 (br, 1H, CH), 2.92-2.83 (m, 2H, CH_2), 2.72-2.62 (m, 2H, CH_2).³³

23b

An analytically pure product **23b** was isolated by column chromatography using hexane/dichloromethane (9.5:0.5, v/v) as the eluent. (0.89 mmol, 98%). ^1H NMR (400 MHz, CDCl_3): δ 4.58-4.59 (d, 1H, J = 1.8 Hz, CH), 4.26-4.25 (d, 1H, J = 1.4 Hz, CH), 2.61-2.57 (m, 2H, CH_2), 2.46-2.43 (m, 2H, CH_2), 1.88-1.80 (m, 2H, CH_2).

Literature values: ^1H NMR: δ 4.62 (br, 1H, CH), 4.28 (br, 1H, CH), 2.61 (m, 2H, CH_2), 2.47 (m, 2H, CH_2), 1.86 (m, 2H, CH_2).³⁵

23c

An analytically pure product **23c** was isolated by column chromatography using hexane/diethyl ether (8:2, v/v) as the eluent. (0.52 mmol, 57%). ^1H NMR (400 MHz, CDCl_3): δ 4.84-4.82 (br, 1H, CH), 4.68-4.66 (br, 1H, CH), 2.29-2.24 (m, 2H, CH_2), 2.01-2.05 (m, 2H, CH_2), 1.78-1.72 (m, 2H, CH_2), 1.44-1.39 (m, 2H, CH_2).

Literature values: ^1H NMR: δ 4.82 (br, 1H, CH), 4.68 (br, 1H, CH), 2.40-2.00 (m, 4H, CH_2), 1.8-1.5 (m, 4H, CH_2).³⁵

24d-Z-isomer ^1H NMR (400 MHz, CDCl_3): δ 6.99-6.97 (d, 1H, CH), 4.85-4.80 (m, 1H, CH), 2.17-2.06 (m, 4H, CH_2), 1.64-1.42 (m, 12H, CH_2).

Literature values: ^1H NMR: δ 7.00 (br, 1H, CH), 4.78 (br, 1H, CH), 2.58-2.00 (m, 4H, CH_2), 1.70-1.25 (m, 12H, CH_2).³⁵

24d-E-isomer ^1H NMR (400 MHz, CDCl_3): δ 7.06-7.02 (d, 1H, CH), 5.38-5.35 (d, 1H, CH), 2.40-2.27 (m, 4H, CH_2), 1.35-1.22 (m, 12H, CH_2).

Literature values: ^1H NMR: δ 7.06 (br, 1H, CH), 5.40 (br, 1H, CH), 2.58-2.00 (m, 4H, CH_2), 1.70-1.25 (m, 12H, CH_2).³⁵

4.5.7 Preparation of 2-oxopropyl benzoate

A sample of propargyl alcohol (1.3 mmol, 0.08 ml), benzoic acid (0.9 mmol, 111 mg), Na_2CO_3 (0.04 mmol, 4.1 mg) and the catalyst **15a** (0.01 mmol, 5 mg) were dissolved in toluene (5 ml). The resulting mixture was then stirred for 12 h at 100 °C. After cooling to room temperature, removal of solvent afforded a crude oil. The crude was purified by column chromatography, with hexane/diethyl ether (1:1, v/v) as eluent, to afford the desired product as white crystalline powder (90 mg, 0.5 mmol, 54 %). ^1H NMR (400 MHz, CDCl_3) δ : 8.09–8.07 (m, 2H, ArH), 7.59–7.55 (m, 1H, ArH), 7.46–7.42 (m, 2H, ArH), 4.86 (s, 2H, CH_2), 2.21 (s, 3H, Me).

4.6 References

1. Bauer, I.; Knölker, H.-J. *Chem. Rev.* **2015**, *115*(9), 3170-3387.
2. Naota, T.; Takaya, H.; Murahashi, S.-I. *Chem. Rev.* **1998**, *98* (7), 2599-2660.
3. Chelucci, G.; Baldino, S.; Baratta, W. *Accounts Chem. Res.* **2015**, *48* (2), 363-379.
4. Lyon, E. J.; Shima, S.; Buurman, G.; Chowdhuri, S.; Batschauer, A.; Steinbach, K.; Thauer, R. K. *FEBS J.* **2004**, *271* (1), 195-204.
5. Song, L.-C.; Hu, F.-Q.; Wang, M.-M.; Xie, Z.-J.; Xu, K.-K.; Song, H.-B. *Dalton Trans.* **2014**, *43* (21), 8062-8071.
6. Crouthers, D. J.; Denny, J. A.; Bethel, R. D.; Munoz, D. G.; Darensbourg, M. Y. *Organometallics* **2014**, *33* (18), 4747-4755.
7. Ekström, J.; Abrahamsson, M.; Olson, C.; Bergquist, J.; Kaynak, F. B.; Eriksson, L.; Sun, L.; Becker, H.-C.; Åkermark, B.; Hammarström, L. *Dalton Trans.* **2006**, (38), 4599-4606.
8. Borg, S. J.; Behrsing, T.; Best, S. P.; Razavet, M.; Liu, X.; Pickett, C. J. *J. Am. Chem. Soc.* **2004**, *126* (51), 16988-16999.
9. Canaguier, S.; Fourmond, V.; Perotto, C. U.; Fize, J.; Pécaut, J.; Fontecave, M.; Field, M. J.; Artero, V. *Chem. Comm.* **2013**, *49* (44), 5004-5006.
10. Tran, P. D.; Barber, J. *Phys. Chem. Chem. Phys.* **2012**, *14* (40), 13772-13784.
11. Lansing, J. C.; Camara, J. M.; Gray, D. E.; Rauchfuss, T. B. *Organometallics* **2014**, *33* (20), 5897-5906.
12. Han, W.-S.; Kim, T.-J.; Kim, S.-K.; Kim, Y.; Kim, Y.; Nam, S.-W.; Kang, S. O. *Int. J. Hydrogen Energ.* **2011**, *36* (19), 12305-12312.
13. Becker, L. Cambridge Scientific Abstracts, 2001.
14. Tour, J. M.; Cooper, J. P.; Pandalwar, S. L. *J. Org. Chem.* **1990**, *55* (11), 3452-3453.
15. Chandrasekhar, V.; Boomishankar, R.; Nagendran, S. *Chem. Rev.* **2004**, *104* (12), 5847-5910.
16. Lee, M.; Ko, S.; Chang, S. *J. Am. Chem. Soc.* **2000**, *122* (48), 12011-12012.
17. Murugavel, R.; Voigt, A.; Walawalkar, M. G.; Roesky, H. W. *Chem. Rev.* **1996**, *96* (6), 2205-2236.
18. Teo, A. K. L.; Fan, W. Y. *Chem. Comm.* **2014**, *50* (54), 7191-7194.
19. Tan, S. T.; Kee, J. W.; Fan, W. Y. *Organometallics* **2011**, *30* (15), 4008-4013.
20. Asao, N.; Ishikawa, Y.; Hatakeyama, N.; Yamamoto, Y.; Chen, M.; Zhang, W.; Inoue, A. *Angew. Chem. Int. Ed.* **2010**, *49* (52), 10093-10095.

21. Yu, M.; Jing, H.; Fu, X. *Inorg. Chem.* **2013**, 52 (19), 10741-10743.
22. Lee, Y.; Dong, S.; Kim, S.; Han, H.; Chang, S.; Lee, P. H. *J. Org. Chem.* **2004**, 69 (5), 1741-1743.
23. Ison, E. A.; Corbin, R. A.; Abu-Omar, M. M. *J. Am. Chem. Soc.* **2005**, 127 (34), 11938-11939.
24. Li, Z.; Zhang, C.; Tian, J.; Zhang, Z.; Zhang, X.; Ding, Y. *Catal. Commun.* **2014**, 53, 53-56.
25. John, J.; Gravel, E.; Hagège, A.; Li, H.; Gacoin, T.; Doris, E. *Angew. Chem.* **2011**, 123 (33), 7675-7678.
26. Amos, R. A.; Katzenellenbogen, J. A. *J. Org. Chem.* **1978**, 43 (4), 560-564.
27. Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Gilmore, C. J.; Restivo, R. J.; Bryan, R. F. *J. Am. Chem. Soc.* **1973**, 95 (4), 1335-1336.
28. Cavicchioli, M.; Bouyssi, D.; Goré, J.; Balme, G. *Tetrahedron lett.* **1996**, 37 (9), 1429-1432.
29. Mason, C.; Edwards, K.; Carlson, R.; Pignatello, J.; Gleason, F.; Wood, J. *Science* **1982**, 215 (4531), 400-402.
30. Tori, M.; Shiotani, Y.; Tanaka, M.; Nakashima, K.; Sono, M. *Tetrahedron Lett.* **2000**, 41 (11), 1797-1799.
31. Blithe, D. L.; Nieman, L. K.; Blye, R. P.; Stratton, P.; Passaro, M. *Steroids* **2003**, 68 (10), 1013-1017.
32. Scheid, G.; Kuit, W.; Ruijter, E.; Orru, R. V.; Henke, E.; Bornscheuer, U.; Wessjohann, L. A. *Eur. J. Org. Chem.* **2004**, 2004 (5), 1063-1074.
33. Lu, H.-L.; Wu, Z.-W.; Song, S.-Y.; Liao, X.-D.; Zhu, Y.; Huang, Y.-S. *Org. Process Res. Dev.* **2014**, 18 (3), 431-436.
34. Krafft, G. A.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1981**, 103 (18), 5459-5466.
35. Jiménez-Tenorio, M.; Puerta, M. C.; Valerga, P.; Moreno-Dorado, F. J.; Guerra, F. M.; Massanet, G. M. *Chem. Comm.* **2001**, (22), 2324-2325.
36. Chan, D. M.; Marder, T. B.; Milstein, D.; Taylor, N. J. *J. Am. Chem. Soc.* **1987**, 109 (21), 6385-6388.
37. Jeschke, J.; Gäbler, C.; Korb, M.; Rüffer, T.; Lang, H. *Eur. J. Inorg. Chem.* **2015**, 2015 (18), 2939-2947.
38. Mitsudo, T.; Hori, Y.; Yamakawa, Y.; Watanabe, Y. *J. Org. Chem.* **1987**, 52 (11), 2230-2239.
39. Devanne, D.; Ruppin, C.; Dixneuf, P. H. *J. Org. Chem.* **1988**, 53 (4), 925-926.

Chapter 5: Single Source Precursor for Silica Encapsulation

As discussed in the previous chapters, hydrogenases are a class of metalloenzymes which are characterized by protein encapsulated metal centres in their active sites,^{1,2,3,4,5} and the extension of this to artificial metalloenzymes has grown over the last few decades.^{6,7,8} This idea of encapsulation of an active metal centre may also be extended to a silica cage, which would allow stabilization of the embedded metal centre (Figure 5.1). Silica derivatives have found use in nanoscience and nanotechnology, as well as in natural system.^{9,10} They can be produced with an ordered and controllable porous structure, with high surface area and thermal stability, making them suitable as catalyst supports.^{11,12,13} Embedding metal nanoparticles in them provides good dispersion of the metal and increases access of the substrate to the active site.^{14,15}

Figure 5.1. Metal encapsulated in a protein (left),¹⁶ and in silica (right).

Heterogeneous or heterogenised catalysts are generally preferred because of the ease of separation from the reaction mixture. They typically comprise a metal-based catalyst anchored onto a supporting material.¹⁷ The metals are deposited via reduction of one of its salts, decomposition, or grafting of an organometallic precursor, onto silica or zeolites.^{16,17} Organometallic ruthenium compounds are useful as precursors for the deposition of metal complexes and of metallic nanoparticles with a variety of size distribution; they are used in a wide variety of applications including catalysis, biomedicine, sensing, and adsorption.^{17,18,19} In this chapter, the idea is to develop a single source precursor that can lead to a metallic catalyst on a support. This idea will be tested through the hydrolysis of a ruthenium complex with a ligand that carries siloxyl (SiOR) groups followed by calcination (Scheme 5.1).

Scheme 5.1. Schematic representation of the synthesis of ruthenium on silica support.

5.1 Synthesis and characterization of a single source precursor

The first synthetic challenge is to prepare a ruthenium complex containing a siloxyl moiety as a stable single source precursor. One possibility is through the reaction of the OH-functionalized bi-metallic ruthenium complex $[\text{Ru}_2(\text{CO})_4(\text{PPh}_3)_2(\mu\text{-OOCCH}_2\text{OH})_2]$, **30** with triethoxychlorosilane $[\text{ClSi}(\text{OEt})_3]$ to afford $[\text{Ru}_2(\text{CO})_4(\text{PPh}_3)_2(\mu\text{-OOCCH}_2\text{OSi}(\text{OEt})_3)_2]$, **31**. Compound **30** is accessible from $\text{Ru}_3(\text{CO})_{12}$ via a similar route to the known analogues $[\text{Ru}_2(\text{CO})_4(\text{PPh}_3)_2(\mu\text{-OOCR})_2]$ ($\text{R} = \text{Ph}$, ^tBu);²⁰ treatment of $\text{Ru}_3(\text{CO})_{12}$ with three equivalents of glycolic acid followed by PPh_3 in refluxing acetonitrile afforded **30**. Subsequent reaction with triethoxychlorosilane in the presence of triethylamine afforded the ruthenium siloxyl complex **31**, together with $[\text{HNEt}_3\text{Cl}]$; a similar reaction with trimethylchlorosilane afforded the TMS analogue **32** (Scheme 5.2). All three complexes **30-32** have been characterized spectroscopically and crystallographically; an ORTEP plot showing the molecular structure of **31** is given in Figure 5.2, and selected bond parameters and a common atom labeling Scheme for all three are collated in Table 5.1.

The IR spectra of all three compounds show two stretches for the COO group at $\sim 1605(\text{s})$ and $\sim 1435(\text{m}) \text{ cm}^{-1}$, with $\Delta\nu \sim 170 \text{ cm}^{-1}$, which is indicative of a chelating carboxylate group.²¹ The stretches for their carbonyl ligands [for example, $2027(\text{s})$, $1983(\text{m})$ and $1955(\text{s}) \text{ cm}^{-1}$ for **31**] are also similar to each other and with that for the known compound $\text{Ru}_2(\text{CO})_4(\mu\text{-OOC}^t\text{Bu})_2(\text{PPh}_3)_2$ [$2026(\text{s})$, $1985(\text{m})$, $1953(\text{s}) \text{ cm}^{-1}$].²⁰

Scheme 5.2. Synthesis of single source precursor.

Figure 5.2. ORTEP plots of **31** with thermal ellipsoids drawn at the 50% probability level.

All three complexes have a sawhorse geometry, with the PPh₃ ligands occupying the axial positions, and isostructural with the known analogue Ru₂(CO)₄(μ-OOC^tBu)₂(PPh₃)₂.²⁰ The bond parameters are comparable to those found in reported ruthenium analogues.^{20,22}

Table 5.1. Common atom labelling Scheme and selected bond parameters for **30**, **31** and **32**.

Bond length (Å) and angle (°)	30	31	32
R	H	Si(OEt) ₃	SiMe ₃
Ru1-Ru2	2.7423(5)	2.7290(6)	2.7236(6)
Ru1-P1	2.411(1)	2.4318(8)	2.4401(8)
Ru1-C1	1.831(6)	1.843(2)	1.848(2)
Ru1-C2	1.841(5)	1.837(4)	1.843(3)
Ru1-O1	2.116(3)	2.133(1)	2.130(2)
Ru1-O2	2.148(3)	2.118(3)	2.121(2)
Ru2-P2	2.430(1)	2.4411(8)	2.4340(8)
Ru2-C3	1.828(5)	1.843(3)	1.847(3)
Ru2-C4	1.849(4)	1.844(3)	1.841(3)
Ru2-O3	2.141(3)	2.129(2)	2.127(2)
Ru2-O4	2.125(3)	2.145(2)	2.128(2)
Ru1-Ru2-O3	83.12(8)	82.14(6)	84.05(5)
Ru1-Ru2-O4	84.28(9)	83.56(6)	81.62(5)
Ru2-Ru1-O1	82.47(9)	82.20(6)	81.52(5)
Ru2-Ru1-O2	83.22(9)	83.49(6)	83.94(5)

5.2 Formation of ruthenium supported on silica

In order to obtain ruthenium supported on silica, an attempt at the base catalyzed hydrolysis of **31** followed by calcination was made; the calcination of organometallic precursors have been shown to give well-dispersed metallic nanoparticles with a narrow size distribution.^{23,24,25,26} Treatment of a solution of **31** in a water-ethanol mixture at 60 °C with aqueous ammonia (pH = 12) afforded a brown gelatinous precipitate (**A**) suspended in a yellow supernatant (Scheme 5.3). Presumably, hydrolysis and co-condensation of the siloxyl moieties occurred.¹⁸ A thermo-gravimetric analysis (TGA) on **A** shows that weight loss reached a plateau at ~ 400 °C (Figure 5.3). Thus, **A** was calcined under vacuum at 450 °C to afford a black crystalline powder **B**.

The TEM image of **B** reveals the presence of well-dispersed dark spots of ruthenium nanosized particles on the silica substrate (Figure 5.4 top), and an SEM elemental mapping (Figure 5.4 bottom) as well as an energy dispersive X-ray (EDX) analysis (Figure A5.5 (b)), confirmed the presence of O, Si and Ru atoms; the carbon coated copper grid interfered with the analysis for carbon.

Scheme 5.3. Synthetic route for the preparation of ruthenium on silica support.

Figure 5.3. TGA analysis of **A** using 12.5570 mg of sample heated at 20 °C per minute under N₂ atmosphere.

Figure 5.4. Electron micrographs of **B**. Top: TEM image. Bottom: (a) SEM image and (b-e) X-ray elemental mapping for oxygen (O), ruthenium (Ru), silicon (Si) and carbon (C).

The X-ray photoelectron spectra (XPS) of **B** for oxygen (O), ruthenium (Ru), silicon (Si), carbon (C) and phosphorous (P) are shown in Figure 5.5, together with those for the material (**C**) obtained from the calcination of **31** under the same conditions. It is clear that,

unlike **C**, the spectrum for **B** does not show any phosphorous present. The Ru3d_{3/2} peak is difficult to resolve as it overlaps with the C1s peak.^{18,27} The Ru3d_{5/2} peak for **B** is noisy and broad, at ~281.5 eV. This can be assigned to an Ru^{II} species; the Ru3d_{5/2} peak is more clearly seen at 280.6 eV in **C**, which can be assigned to an Ru^I species.^{27,28} Interestingly, an IR spectrum of **B** showed peaks in the carbonyl region indicating residual CO ligands (Figure 5.6), while that for **C** did not show any.

Figure 5.5. XPS spectra of **B** (top row) and **C** (bottom row) for oxygen (O), ruthenium (Ru), silicon (Si) carbon (C) and phosphorous (P).

Figure 5.6. IR spectrum of **B**.

5.3 Catalytic studies

The catalytic activity of **B** for the cyclopropanation of alkenes with diazoacetate was assessed; this reaction has been reported to be catalyzed by an analogue of **31**, viz., $[\text{Ru}_2(\text{CO})_4(\mu\text{-OCOMe})_2(\text{NCMe})_2]$.²² The cyclopropanation of styrene with ethyl diazoacetate using **B** as catalyst afforded a mixture of the cyclopropanes **34** and **35** in a ratio of 68:32 (Scheme 5.4).

Scheme 5.4. Cyclopropanation of styrene with ethyl diazoacetate.

The reaction can also proceed with **31**, **C**, or **31** anchored onto MCM41 (**D**) as catalysts, under the same conditions; the catalytic efficiencies are summarized in Table 5.2. The catalyst **D** is a heterogenised version of **31** on MCM41 following the literature method.^{29,30} The data suggests that the efficiency is significantly lower with the calcined catalysts; presumably because the ruthenium is in a more highly oxidized form in these.

Table 5.2. Catalytic efficiencies of all the catalysts for the cycloaddition reaction between styrene and ethyl diazoacetate.

Catalyst	B	31	C	D
Yield	28%	86%	32%	72%
<i>E</i> : <i>Z</i> ratio	68:32	75:25	60:40	65:35

In the course of the catalytic studies, it was found that the hydrolysed material **A** obtained from **31** did not give rise to the same cyclopropanation reaction but directed the reaction to the formation of the pyrazoline **36a** instead. This is the first observation of metal-catalyzed pyrazoline formation via what is formally a [2+3] cycloaddition reaction. Optimization of the solvent and reaction temperature showed that 1,2-dichloroethane and 50 °C were optimal (Table 5.3).

Table 5.3. Optimization for the catalyzed formation of pyrazoline **36a**.

Entry	Solvent	Yield (%)*
1	DCE	57
2	THF	30
3	DMF	20
4	Dioxane	15
5	EtOH	0

Entry	Temperature (°C)	Yield (%)*
1	25	5
2	40	30
3	50	57
4	60	55
5	70	30
6	80	23

*All yields are based on NMR measurements with anisole as internal standard. Reaction conditions: styrene (17 mmol), ethyl diazoacetate (1 mmol), **A** (55 mg) in 5 mL of solvent.

A limited set of substrate scope reactions was also performed with different derivatives of styrene (Table 5.4). It showed that the best conversion was obtained using styrene as the substrate. The recyclability of **A** was tested under the same conditions over five successive catalytic cycles, and the yield was maintained over all five cycles (Figure 5.7). This suggests that there was no leaching of the ruthenium nanoparticles during the catalytic reactions. It also indicates higher stability of the nanoparticles.

Table 5.4. Substrate scope for the catalyzed cycloaddition reaction.

Entry	Aryl alkene	Product	Yield (%)
1		(36a)	57%
2		(36b)	40%
3		(36c)	38%

*All yields are based on NMR measurements with anisole as internal standard. Reaction conditions: Alkene (17 mmol), ethyl diazoacetate (1 mmol), **A** (55 mg) in DCE (5 mL).

Figure 5.7. Recycling of **A** for the cycloaddition reaction.

5.4 Characterization of A

The unique catalytic activity of **A** is surprising and so attempts were made to characterize it spectroscopically. The TEM image shows spherical particles in the sub-micrometer size range (Figure 5.8 top), and the presence of C, O, Si and Ru were confirmed by both EDX analysis (Figure A5.5 (a)) as well as an SEM elemental mapping (Figure 5.8 bottom).

Figure 5.8. Electron micrographs of **A**. Top: TEM image. Bottom: (a) SEM image and (b-e) X-ray elemental mapping for oxygen (O), ruthenium (Ru), silicon (Si) and carbon (C).

Presence of these elements was also supported by the XPS spectra (Figure 5.9), although there were no clear Ru peaks. Unlike the case for **C**, there was no phosphorus detected, which suggests that the phosphine ligands were lost during hydrolysis. An IR spectrum also showed the presence of carbonyl ligands (Figure 5.10). It is therefore proposed that **A** contains an Ru^{II} species.^{28,31}

Figure 5.9. XPS spectra of **A** of all constituting elements oxygen (O) (a), ruthenium (Ru)-carbon (C) (b), silicon (Si) (c) and phosphorous (P) (d).

Figure 5.10. IR spectrum of **A**.

5.5 Conclusion

Encapsulation of ruthenium onto silica surface was attempted via hydrolysis and calcination from a single-source precursor $[\text{Ru}_2(\text{CO})_4(\mu\text{-O}_2\text{CCH}_2\text{OSi}(\text{OEt})_3)_2(\text{PPh}_3)_2]$ (**31**) (Scheme 5.5). The resulting material was an active catalyst for the cyclopropanation of styrene with ethyl diazoacetate, in the same way as the precursor **31**. Surprisingly, the intermediate material obtained from the hydrolytic first step catalyzed the reaction towards the formation of pyrazoline via a [2+3] cycloaddition.

Scheme 5.5. Synthetic route for the preparation of ruthenium on silica support.

5.6 Experimental section

5.6.1 General procedure: All experiments except hydrolysis reaction were performed under an argon atmosphere using standard Schlenk techniques. All chemicals were obtained from Sigma Aldrich and were used as received whereas $\text{Ru}_3(\text{CO})_{12}$ was purchased from Oxkem and used as supplied. Solvents that were used for reactions were distilled over the appropriate drying agents under argon before use. Purification of compounds was carried out by column chromatography on silica gel. Infrared spectra were recorded on Bruker Alpha FT-IR spectrometer. Solution spectra were recorded in a solution IR cell with NaCl windows and a path length of 0.1 mm, at a resolution of 2 cm^{-1} . Solid-state spectra were recorded as KBr pellets. HRMS were recorded in ESI mode on a Waters UPLC-Q-ToF MS mass spectrometer. ^1H , $^{13}\text{C}\{^1\text{H}\}$ and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra were obtained on a JEOL 400 MHz spectrometer at room temperature unless otherwise stated. Chemical shifts for ^1H and $^{13}\text{C}\{^1\text{H}\}$ were referenced with respect to the residual resonances of the respective deuterated solvents (^1H NMR: $\delta_{\text{H}} = 7.26$ for CDCl_3 , $\delta_{\text{H}} = 2.05$ for $(\text{CD}_3)_3\text{CO}$, $\delta_{\text{H}} = 1.94$ for CD_3CN , ^{13}C NMR: $\delta_{\text{C}} = 77.0$ for CDCl_3 , $\delta_{\text{C}} = 29.8$ for $(\text{CD}_3)_3\text{CO}$, $\delta_{\text{C}} = 1.32$ for CD_3CN). Chemical shifts for $^{31}\text{P}\{^1\text{H}\}$ were referenced with respect to 85% aqueous H_3PO_4 . XPS was performed with a monochromatic Mg X-ray radiation source and a Phoibos 100 spectrometer (SPECS Germany). All XPS spectra were reference to adventitious carbon at 284.5 eV. XPS spectra were resolved and binding energies calculated using XPS peak 4.1 software with respect to the adventitious carbon C1s peak set at 284.5 eV. TEM analysis were carried using JEOL JEM-1400 operated at 100 kV. Samples were dispersed on carbon grids after sonicating the samples in ethanol for 0.5 h. SEM coupled with EDX studies was carried out using JEOL JSM-7600F microscope with an accelerating voltage of 5kV. Before performing the SEM measurements, the sample were prepared by depositing dilute solution of materials on carbon coated copper grids, followed by drying and coating them with a thin layer of Pt to increase the contrast. TGA was carried out on a TGA Q500 instrument. Calcination was carried out on a CARBOLITE tube furnace instrument.

5.6.2 Synthesis of 30

A sample of glycolic acid (9 mg, 0.11 mmol) was added into a solution of $\text{Ru}_3(\text{CO})_{12}$ (20 mg, 0.03mmol) in acetonitrile (20 ml). The resulting mixture was refluxed for 12 h to give a yellow solution. A sample of PPh_3 (25 mg, 0.09mmol) was added to the above solution and the solution was refluxed for another 4 h to give an intense yellow solution. Evaporation under vacuum afforded a yellow residue, and the residue was purified by column chromatography with

dichloromethane as eluent afforded **30** as a yellow powder (196 mg, 57%). ν_{CO} (DCM, cm^{-1}): 2028 (s), 1984 (m), 1956 (s). ^1H NMR (400 MHz, CDCl_3): δ 7.51-7.46 (m, 6H, ArH), 7.44-7.39 (m, 9H, ArH), 3.68-3.67 (d, 2H, $J = 5.0$ Hz, CH_2), 1.50-1.47 (t, 1H, $J = 5.0$ Hz, OH) ppm. ^{31}P NMR (400 MHz, CDCl_3): δ 19.0 ppm. HRMS⁺ (m/z): calcd for $\text{C}_{47}\text{H}_{38}\text{O}_{10}\text{PRu}^{96}\text{Ru}^{102}$, 991.0322 $[\text{M}+\text{H}]^+$, found 991.0339 $[\text{M}+\text{H}]^+$. Found C 53.46, H 2.71; Calculated for $\text{C}_{44}\text{H}_{36}\text{O}_{10}\text{P}_2\text{Ru}_2$: C 53.44, H 3.67.

5.6.3 Synthesis of **31**

Triethyl amine (6 μl , 0.03 mmol) was added to a solution of **30** (15 mg, 0.015 mmol) in tetrahydrofuran (5 ml). The resulting mixture was stirred for 15 min at 25 °C. Chlorotriethoxysilane (6 μl , 0.03 mmol) was added and the mixture stirred for 30 min, filtered and evaporated under vacuum to afford **31** as a yellow powder (10 mg, 0.007 mmol, 47 %). ν_{CO} (DCM, cm^{-1}): 2027 (s), 1983 (m), 1955 (s). ^1H NMR: δ 7.58-7.54 (m, 6H, ArH), 7.39-7.37 (m, 9H, ArH), 3.93 (s, 4H, CH_2), 3.65-3.59 (q, 6H, $J = 6.9$ Hz, CH_2), 1.06-1.03 (t, 9H, $J = 5.0$ Hz, CH_3). ^{31}P NMR (400 MHz, CDCl_3): δ 12.3 ppm. HRMS⁺ (m/z): calcd for $\text{C}_{52}\text{H}_{57}\text{O}_{11}\text{P}_2\text{Ru}_2^{100}\text{Si}_2$, 1316.9567 $[\text{M}+\text{H}]^+$, found 1316.9563 $[\text{M}+\text{H}]^+$. Found C 48.36, H 3.43; Calculated for $\text{C}_{56}\text{H}_{64}\text{O}_{16}\text{Si}_2\text{P}_2\text{Ru}_2$. CH_2Cl_2 : C 48.96, H 4.76.

5.6.4 Synthesis of **32**

Compound **32** was prepared using an analogous procedure except that the crude product was purified by column chromatography with DCM as eluent. This afforded **32** as a yellow powder (29 mg, 0.02 mmol, 84 %). ν_{CO} (DCM, cm^{-1}): 2026 (s), 1985 (m), 1953 (s). ^1H NMR (400 MHz, CDCl_3): δ 7.58-7.54 (m, 6H, ArH), 7.39-7.35 (m, 9H, ArH), 3.73 (s, 2H, CH_2), -0.17 (s, 9H, Me). ^{31}P NMR (400 MHz, CDCl_3): δ 12.8 ppm. HRMS⁺ (m/z): calcd for $\text{C}_{51}\text{H}_{55}\text{O}_{11}\text{P}_2\text{Ru}^{98}\text{Ru}^{100}\text{Si}_2$, 1137.0915 $[\text{M}+\text{H}]^+$, found 1137.0923 $[\text{M}+\text{H}]^+$.

5.6.5 Hydrolysis of **31**

Complex **31** (200 mg, 0.15 mmol) was dissolved in EtOH (20 mL) and then H_2O (15 mL) was added to the above solution. The mixture was stirred at room temperature for 30 min under vigorous stirring. The pH of the reaction mixture was around 7.5 at this point, which was adjusted to *ca.* 12 by the addition of NH_3 (2 mL) solution, stirred for another 12 h at room temperature, and then heated at 60 °C for 72 h. The brown colored gel (55 mg, 16 % obtained from ICPMS) was recovered by centrifugation at 4000 rpm, washed several times with water

followed by ethanol, dichloromethane and dried under vacuum for overnight. ν_{CO} (KBr, cm^{-1}): 2057 (w), 2067 (w), 1987 (m), 1930 (s), 1920 (s).

5.6.6 Calcination

A sample of **A** (170 mg) was calcined at 450 °C under vacuum for 30 min to afford a black crystalline powder of **B** (128 mg).

Similar calcination of **31** (200 mg) afforded a black crystalline powder of **C** (112 mg).

5.6.7 Synthesis of D

MCM41 (120 mg) was added to a solution of **31** (30 mg) in toluene (5 ml). The resulting mixture was refluxed for 12 h, which afforded gel type yellow solid. This was recovered by centrifugation at 4000 rpm, washed several times with dichloromethane and dried overnight under vacuum. ν_{CO} (KBr, cm^{-1}): 2020 (s), 1975 (m), 1951 (s).

5.6.8 General procedure for catalytic reactions

Styrene (0.2 ml, 10 mmol), ethyl diazoacetic acid (0.2 ml, 2 mmol) and **31** (10 mol %)/**A-D** (55 mg) were added in dichloroethane (10 ml). The resulting mixture was then stirred for 2 d at 50 °C. After cooling to room temperature, the catalyst and solution were separated by high speed centrifugation. Then, the removal of solvent afforded the desired product.

The identities of the products **34** and **35** were confirmed through matching their ^1H NMR spectra with the reported values.^{32,33}

Yellow oil. ^1H NMR (400 MHz, CDCl_3): δ 7.29-7.24 (m, 3H, PhH), 7.11-7.10 (m, 2H, PhH), 4.20-4.14 (q, 2H, CH_2), 2.53-2.49 (m, 1H, CH), 1.92-1.88 (m, 1H, CH), 1.62-1.57 (m, 1H, CH), 1.30-1.27 (m, 1H, CH), 1.29-1.26 (t, 3H, Me). MS^+ (m/z): 190 $[\text{M}]^+$.

Literature values: ^1H NMR (200 MHz, CDCl_3): δ 7.33-7.08 (m, 5H, PhH), 4.18 (q, 2H, CH_2), 2.52 (m, 1H, CH), 1.90 (m, 1H, CH), 1.60 (m, 1H, CH), 1.31 (m, 1H, CH), 1.28 (t, 3H, CH_3).³²

Yellow oil. ^1H NMR (400 MHz, CDCl_3): δ 7.22-7.17 (m, 3H, PhH), 7.09-7.08 (m, 2H, PhH), 3.90-3.85 (q, 2H, CH_2), 2.58-2.54 (m, 1H, CH), 2.11-2.05 (m, 1H, CH), 1.74-1.69 (m, 1H, CH), 1.35-1.31 (m, 1H, CH), 0.99-0.95 (t, 3H, Me). MS^+ (m/z): 190 $[\text{M}]^+$.

Literature values: ^1H NMR (300 MHz, CDCl_3): δ 7.3 (m, 4H, PhH), 7.18 (m, 1H, PhH), 3.87 (q, 2H, CH_2), 2.67-2.52 (m, 1H, CH), 2.06 (m, 1H, CH), 1.70 (m, 1H, CH), 1.40-1.23 (m, 1H, CH), 0.95 (t, 3H, CH_3).³³

Colorless oil (57 %). ^1H NMR (400 MHz, CDCl_3): δ 7.33-7.29 (m, 3H, ArH), 7.27-7.21 (m, 2H, ArH), 6.40 (s, 1H, NH), 4.99-4.93 (t, 1H, CH), 4.29-4.24 (q, 2H, CH_2), 3.37-3.30 (m, 1H, CH_2), 2.93-2.86 (m, 1H, CH_2), 1.33-1.29 (t, 3H, J = 6.9 Hz, Me). MS^+ (m/z): 218 $[\text{M}+\text{H}]^+$.

Yellow oil (35 %). ^1H NMR (400 MHz, CDCl_3): δ 7.33-7.22 (m, 2H, ArH), 6.96-6.89 (m, 2H, ArH), 6.41 (s, 1H, NH), 4.99-4.93 (t, 1H, CH), 4.33-4.29 (q, 2H, CH_2), 3.38-3.31 (m, 1H, CH_2), 2.93-2.86 (m, 1H, CH_2), 1.38-1.33 (t, 3H, J = 7.3 Hz, Me). MS^+ (m/z): 252 $[\text{M}+\text{H}]^+$.

Yellow oil (38 %). ^1H NMR (400 MHz, CDCl_3): δ 6.33 (s, 1H, NH), 4.95-4.90 (q, 1H, CH), 4.70-4.66 (q, 1H, CH_2), 4.35-4.31 (q, 1H, CH_2), 4.22-4.17 (q, 2H, CH_2), 1.65-1.59 (t, 3H, Me). MS^+ (m/z): 309 $[\text{M}+\text{H}]^+$.

5.6.9 Crystallographic studies

Diffraction-quality crystals of **30** and **31** were grown from dichloromethane, and **32** was grown from acetonitrile at 0 °C and mounted on quartz fibers. X-ray diffraction data were collected at 103(2) K on a Bruker X8 APEX system, using Mo K α radiation, with the SMART suite of programs.³⁴ Data processing and correction were made for Lorentz and polarization effects with SAINT,³⁵ and for adsorption effects with SADABS.³⁶ Structural solution and refinement were carried out with the SHELXTL suite of programs.³⁷ The structures were solved by direct methods to locate the heavy atoms, followed by successive difference maps for the light, non-hydrogen atoms.

Solvates were found for the crystals of **31** (DCM) and **32** (ACN). The ACN solvate in the latter exhibited disorder which was modelled with four alternative sites; the occupancies refined to a total of ~0.66. There was disorder (~5%) of the ruthenium atom positions in **30**, which were modelled with two alternative positions each. Appropriate restraints were placed on all disordered parts.

Table 5.5. Crystal and refinement data for **30-32**.

Complex	30	31	32
Empirical formula	C ₄₄ H ₃₆ O ₁₀ P ₂ Ru ₂	C ₅₆ H ₆₄ O ₁₆ P ₂ Ru ₂ Si ₂ .0.66CH ₂ Cl ₂	C ₅₀ H ₅₂ O ₁₀ P ₂ Ru ₂ Si ₂ .CH ₃ CN
Formula weight	988.81	1369.38	1174.23
Crystal system	Monoclinic	Triclinic	Triclinic
Space group	P2 ₁ /c	P 1	P 1
a, Å	10.4234(9)	12.6618(4)	10.5028(3)
b, Å	19.0090(13)	13.7592(4)	13.6946(4)
c, Å	20.6686(15)	20.8909(6)	20.5244(6)
α, deg	90	99.0370(16)	72.1988(16)
β, deg	99.086(3)	98.5968(17)	85.7229(17)
γ, deg	90	117.3761(14)	71.1619(14)
Volume, Å ³	4043.9(5)	3088.13(16)	2659.21(14)
Z	4	2	2
ρ _c , Mg/m ³	1.624	1.473	1.466
μ, mm ⁻¹	0.886	0.701	0.729
F(000)	1992	1403	1200
Crystal size, mm ³	0.30 x 0.26 x 0.24	0.200 x 0.120 x 0.080	0.280 x 0.300 x 0.360
Reflections Collected	16524	109581	49736
Independent reflections	7147 [R(int) = 0.0456]	19922 [R(int) = 0.0584]	17090 [R(int) = 0.0484]
Max. and min. transmission	0.82 and 0.73	0.94 and 0.87	0.8220 and 0.7790
Data / restraints / parameters	7147 / 0 / 532	19922 / 34 / 751	17090 / 0 / 629
Goodness-of-fit on F ²	1.192	1.060	1.164
Final R indices [I>2σ(I)]	R1 = 0.0365, wR2 = 0.1046	R1 = 0.0412, wR2 = 0.0937	R1 = 0.0340, wR2 = 0.0924
R indices (all data)	R1 = 0.0509, wR2 = 0.1320	R1 = 0.0688, wR2 = 0.1091	R1 = 0.0495, wR2 = 0.1117
Largest diff. peak and hole, e.Å ⁻³	1.338 and -0.860	1.347 and -1.208	0.860 and -1.244

5.7 References

1. Vallee, B. L.; Williams, R. P. *Natl. Acad. Sci. Usa.* **1968**, *59* (2), 498-505.
2. Ulmer, D.; Vallee, B. *Adv. Chem.* **1971**, *10*, 187-218.
3. Zhang, S.; Zhao, Y. *Chem. Comm.* **2012**, *48* (80), 9998-10000.
4. Riordan, J.; Vallee, B. *Adv. Exp. Med. Biol.* **1974**, 33-57.
5. Bos, J.; Roelfes, G. *Curr. Opin. Chem. Biol.* **2014**, *19*, 135-143.
6. Lewis, J. C. *Acs Catal.* **2013**, *3* (12), 2954-2975.
7. Dydio, P.; Key, H.; Nazarenko, A.; Rha, J.-E.; Seyedkazemi, V.; Clark, D.; Hartwig, J. *Science* **2016**, *354* (6308), 102-106.
8. Ueno, T.; Tabe, H.; Tanaka, Y. *Chem.–Asian J.* **2013**, *8* (8), 1646-1660.
9. Athinarayanan, J.; Periasamy, V. S.; Alshatwi, A. A. *J. Mater. Sci-Mater. M.* **2014**, *25* (7), 1637-1644.
10. Dolatabadi, J. E. N.; de la Guardia, M. *Trac-Trend Anal. Chem.* **2011**, *30* (9), 1538-1548.
11. Hu, S. H.; Gao, X. *Adv. Funct. Mater.* **2010**, *20* (21), 3721-3726.
12. Tang, F.; Li, L.; Chen, D. *Adv. Mater.* **2012**, *24* (12), 1504-1534.
13. Jia-Hu, G.; Yu-Cun, L.; Tao, C.; Su-Ming, J.; Hui, M.; Ning, Q.; Hua, Z.; Tao, Y.; Wei-Ming, H. *RSC Adv.* **2015**, *5* (56), 44990-44997.
14. Castillo, R.; Koch, B.; Ruiz, P.; Delmon, B. *Stud. Surf. Sci. Catal.* **1995**, *91*, 291-298.
15. Pope, D.; Walker, D.; Whalley, L.; Moss, R. *J. Catal.* **1973**, *31* (3), 335-345.
16. Xuereb, D. J.; Raja, R. *Catal. Sci. Technol.* **2011**, *1* (4), 517-534.
17. Davies, G.-L.; O'Brien, J.; Gun'ko, Y. K. *Sci. Rep.* **2017**, *7*, 45862.
18. Jana, A.; Mondal, J.; Borah, P.; Mondal, S.; Bhaumik, A.; Zhao, Y. *Chem. Comm.* **2015**, *51* (53), 10746-10749.
19. Rosso-Vasic, M.; De Cola, L.; Zuilhof, H. *J. Phys. Chem. C* **2009**, *113* (6), 2235-2240.
20. Chor, B. Y.; Ganguly, R.; Leong, W. K. *J. Organomet. Chem.* **2016**, *802*, 15-20.
21. Deacon, G.; Phillips, R. *Coordin. Chem. Rev.* **1980**, *33* (3), 227-250.
22. Maas, G.; Werle, T.; Alt, M.; Mayer, D. *Tetrahedron* **1993**, *49* (4), 881-888.
23. Shimizu, T.; Ota, M.; Sato, Y.; Inomata, H. *Chem. Eng. Res. Des.* **2015**, *104*, 174-179.
24. Cao, A.; Lu, R.; Veser, G. *Phys. Chem. Chem. Phys.* **2010**, *12* (41), 13499-3510.
25. Ballinger, B.; Motuzas, J.; Miller, C. R.; Smart, S.; Da Costa, J. C. D. *Sci. Rep.* **2015**, *5*, 8210.
26. Zheng, S.; Zhu, K.; Li, W.; Ji, Y. *New J. Chem.* **2017**, *41*, 5752-5763.

27. Bianchi, C.; Ragaini, V.; Cattania, M. *Mater. Chem. Phys.* **1991**, 29 (1-4), 297-306.
28. Morgan, D. J. *Surf. Interface Anal.* **2015**, 47 (11), 1072-1079.
29. Dragutan, I.; Dragutan, V. *Platin. Met. Rev.* **2008**, 52 (3), 157-162.
30. Ying-Min, Y.; Jin-Hua, F.; Yi-ping, Z.; Xiao-ming, Z. *J. Fuel Chem. Technol.* **2006**, 34 (6), 700-705.
31. Folkesson, B. *Acta Chem. Scand* **1973**, 27 (1), 19.
32. Galardon, E.; Maux, P. L.; Toupet, L.; Simonneaux, G. *Organometallics* **1998**, 17 (4), 565-569.
33. Rosenberg, M. L.; Langseth, E.; Krivokapic, A.; Gupta, N. S.; Tilset, M. *New J. Chem.* **2011**, 35 (10), 2306-2313.
34. SMART version 5.628; Bruker AXS Inc., Madison, Wisconsin, USA, **2001**.
35. SAINT+ version 6.22a, Bruker AXS Inc., Madison, Wisconsin, USA, **2001**.
36. Sheldrick, G. M. SADABS; University of Göttingen, Göttingen, Germany, **1996**.
37. SHELXTL version 5.1; Bruker AXS Inc., Madison, WI, USA, **1997**.

Chapter 6: Conclusion

In this work, two classes of organometallic compounds of group VIII metal complexes were examined as structural mimics for the active site of the [Fe]-hydrogenase. The first contained a thiolato ligand and was of the general formula $[\text{Ru}(2\text{-NHC(O)C}_5\text{H}_3\text{NMe})(\text{CO})_2(o,o\text{-Me}_2\text{-C}_6\text{H}_3\text{S})(\text{L})]$ and the second contained a bidentate mercaptopyridine ligand and was of the general formula $[\text{M}(2\text{-NHC(O)C}_5\text{H}_4\text{N})(\text{CO})(2\text{-SC}_5\text{H}_4\text{N})(\text{L})]$.

There were a number of synthetic challenges to overcome in order that the different ligands could be incorporated at the correct relative orientation. One of these was that appropriate substitutions at the 6 and 2,6-positions of the pyridine and thiol ligands, respectively, were critical. In the absence of these substitutions, dimerization occurred (Scheme 6.1). This suggested that steric crowding around the metal centre played a crucial role, and paralleled that of the chemistry of the [Fe]-hydrogenase; the accepted structure of the [Fe]-hydrogenase possessed a substituent at the 6-position of the pyridine ligand.

Scheme 6.1. Synthesis of structural mimic **7d**.

The mercaptopyridine complexes did not suffer from this propensity towards dimerization. They were found, however, to be able to exist in isomeric forms in solution. A computational study suggested that the relative thermodynamic stability of the isomers depended on the nature of the metal but, more importantly, that substitution at the 6-position of the pyridine ligand had a profound on the relative stability of the isomers (Figure 6.1). This must have implications in the design of structural mimics of [Fe]-hydrogenase.

Figure 6.1. Computed relative thermodynamic stability of the isomers **15n** and **16n**.

These structural mimics **15/16** were also found to be good electrocatalysts for proton reduction, with the iron complex being much more efficient than the ruthenium or osmium analogues. The precursors to the ruthenium mimics, *viz.*, [RuBr(2-NHC(O)C₅H₃NR)(CO)₂(MeCN)] (**5a**, R = H; **5b**, R = Me) also catalysed the hydrolysis of silanes to produce hydrogen gas. These behaviour were more closely related to the function of the [NiFe] and [FeFe]-hydrogenases rather than the [Fe]-hydrogenase.

Preliminary investigations on the synthesis of a mimic of the entire [Fe]-hydrogenase enzyme were also carried out via bioconjugation onto papain. Suitable precursors were the complexes **5n**, and the evidence pointed to a mononuclear ruthenium being anchored via the cysteine and imidazole residues of the active site responsible for peptidase activity (Figure 6.2). This lack of dimerization and isomerisation in **13n** pointed to the importance of the protein cage and in turn, a species such as **7d** could be viewed to have achieved this through steric hindrance via substitutions at the pyridine and thiolate ligands.

Figure 6.2. Papain bioconjugates **13n**.

The idea of encapsulation of ruthenium within a cage was also extended to that of a silica cage through the hydrolysis (to **A**) and calcination (to **B**) of a single source precursor $[Ru_2(CO)_4(\mu-O_2CCH_2OSi(OEt)_3)_2(PPh_3)_2]$, **31**. Surprisingly, while **B** and the precursor **31** were active catalysts for the cyclopropanation of styrene with ethyl diazoacetate, the hydrolysis product **A** directed the reaction towards a [2+3] cycloaddition instead (Scheme 6.2).

Scheme 6.2. Catalytic reaction by ruthenium on silica support.