Bioconjugation of triosmium carbonyl clusters

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2017

Tan, Y. Q. (2017). Bioconjugation of triosmium carbonyl clusters. Doctoral thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/69452>

<https://doi.org/10.32657/10356/69452>

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2016.

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A thesis submitted in fulfilment of the
requirements for the degree of Doctor of
Philosophy

Acknowledgements

There are many people who have contributed in some way or other to the work written in this dissertation. I would first like to thank those of you who I have not mentioned in name, but have supported me through my graduate studies – you know who you are! Your constant moral support is appreciated and has kept me going through the tough times.

First and foremost, I would like to express my extreme gratitude towards my supervisor, A/P Leong Weng Kee for his constant guidance and advice. It has been a long and sometimes, winding road for me to get here, and I really could not have made it without his support and guidance. Thank you for being patient with me and really instilling in me an appreciation for true scientific research.

Secondly, I would like to thank my co-supervisor, A/P Ang Wee Han at the National University of Singapore, who started me off on this research path during my FYP days. I am grateful for all the timely help, advice and support for my project. Thank you for always welcoming me back to the group even when I have been away for several years.

I would also like to extend my appreciation to my collaborators in the University of Ulm – Prof. Ute Kaiser and Anita for their assistance and expertise in carrying out the TEM imaging of DNA. A special thank you to Dr. Kuan Seah Ling who helped me settle down during my stay in Ulm.

To all my fellow graduate students – Shirley, Joey, Jing Wei, Yingzhou, Yongjia, Bo Yang, Zhiyong, Chandan, Sock Li, Liu Yu, Garvin, Kumaran and Marcus– thank you for all the assistance and invaluable discussions we have had. To the undergraduates and exchange students – Shi Ning, Shin Yiing, Poh, Eric, Carmen, Yu Han, Edward, Holly, Chloe, Aaron and Wei Xiang – you inject a liveliness into this rather serious environment. Also, a shoutout to my NUS labmates – MJ, Daniel and Siew Qi – who have helped me through my work in NUS.

I would also like to thank the technical staff of NTU – Ee Ling, Derek, Wen Wei, Joan, Dr Rakesh and Dr Li – for all the help in completing the analysis of my compounds. The financial support from the NTU research scholarship over the four years is also gratefully acknowledged.

I thank my close friends in Singapore: Teddi, Shwu Yan, my roommate Pey Hua – for all the encouragement, and for putting up with me through all my crazy antics. Last but not least to my family – Mum, Dad and my brother – my deepest gratitude for all the support, encouragement and for always providing me a home to seek comfort and solace from the very stressful times here. I would not be here without you all. Thank you. ♥

Table of Contents

Title	Page
Acknowledgements	i
Table of Contents	ii
Abstract	vii
List of Figures	viii
List of Schemes	xii
List of Tables	xiv
Abbreviations	xv
Molecular numbering scheme	xvii
 Chapter 1. Organometallic Bioconjugates	 1
1.1 Bioconjugation of Organometallic Species	2
1.1.1 Conjugation to Nucleic Acids	2
1.1.2 Conjugation to Proteins	6
1.1.3 Conjugation to Sugars and Carbohydrates	10
1.1.4 Conjugation to Lipids and Steroids	13
1.1.5 Conjugation to vitamins	14
1.2 Bioconjugation of Osmium Carbonyl Clusters	18
1.3 Conjugation Methods	20
1.4 Aim and Objectives	22
1.5 References.	23
 Chapter 2. Conjugation of Osmium Carbonyl Clusters to Nucleic Acids	 28
2.1 Conjugation of Nucleosides	28
2.2 Synthetic Strategy	29
2.3 Functionalization of Thymidine	31
2.3.1 Modification of Thymidine at the N ³ Position	31
2.3.2 Modification of Thymidine at the C ⁵ Position	32
2.3.3 Modification of the Ribose Sugar	33
2.4 Triosmium Carbonyl Cluster – Nucleoside Conjugates	34
2.4.1 Direct ligand displacement reaction	34
2.4.2 Copper-catalyzed azide-alkyne cycloaddition reaction	35
2.5 Conclusion	40

2.6 Experimental	41
2.6.1 Experimental procedure	41
2.6.1.1 N-alkylation of Thymidine (a-b, g)	41
2.6.1.2 Synthesis of Azide Derivatives (c-d)	42
2.6.1.3 Synthesis of Thiol Derivatives (e-f)	42
2.6.1.4 Sonogashira Coupling (h-k)	43
2.6.1.5 Synthesis of 5-ethynyl-2'-deoxyuridine (l)	43
2.6.1.6 Substitution of 5'-O-DMT-5-butynyl tosylate -2'-deoxyuridine (m-n)	43
2.6.1.7 Synthesis of 5-methylanhydrouridine (p)	43
2.6.1.8 Synthesis of $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-S}(\text{CH}_2)_3\text{-thymidine})$ (6e)	44
2.6.1.9 Synthesis of $\text{Os}_3(\text{CO})_{11}(5\text{-CH}_2\text{CN-5'-O-DMT-2'-deoxyuridine})$ (10g)	44
2.6.1.10 Synthesis of $\text{Os}_3(\mu\text{-H})(\text{CO})_{10}(\mu\text{-N}_3\text{H-(CH}_2)_3\text{-Thymidine-5'-O-DMT})$ (11d)	44
2.6.1.11 Synthesis of $\text{Os}_3(\mu\text{-H})_2(\text{CO})_9(\mu\text{-N(CH}_2)_3\text{-Thymidine-5'-O-DMT})$ (13d)	44
2.6.1.12 Synthesis of Carboxylate Bridged Clusters (7L1 – L2)	45
2.6.1.13 Synthesis of 3-(diphenylphosphino)-(CH ₂) ₂ COO(CH ₂) ₃ Br (P1)	45
2.6.1.14 Synthesis of $\text{Os}_3(\text{CO})_{12-n}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{Br})_n$ (8 – 9P1)	46
2.6.1.15 Synthesis of $\text{Os}_3(\text{CO})_{12-n}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)_n$ (8 – 9P2)	46
2.6.1.16 Click Chemistry Reaction	46
2.6.1.17 Synthesis of $\text{Os}_6(\text{CO})_{20}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{X})$ (15P3-4)	47
2.6.1.18 Synthesis of $\text{Os}_6(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{OOC}(\text{CH}_2)_5\text{N}_3]$ (15P5)	47
2.6.2 Experimental Data	48
2.6.2.1 Nucleoside Derivatives	48
2.6.2.2 Phosphine Ligands and Triosmium Carbonyl Clusters	54
2.6.2.3 Hexaosmium Carbonyl Clusters	57
2.6.2.4 Nucleoside-Cluster Conjugates	58
2.7 References	65

Chapter 3. Application of Osmium Carbonyl Cluster – Nucleoside Conjugates	66
3.1 As Therapeutic Agents	66
3.2 As SERS Imaging Agents	68
3.3 As TEM Imaging Agents	71
3.3.1 Conjugation of Osmium Carbonyl Clusters to DNA	72
3.3.2 TEM Imaging of Osmium Carbonyl – DNA Conjugates	75
3.4 Conclusion	76
3.5 Experimental	77
3.5.1 Cell Based Assays And Uptake Studies	78
3.5.1.1 Cell Culture	78
3.5.1.2 MTS Assay of Conjugates	78
3.5.1.3 Cell Uptake of Cluster 9I-2	79
3.5.1.4 SERS Imaging of Cancer Cells	79
3.5.2 DNA conjugation	79
3.5.2.1 PCR Optimization using Taq DNA Polymerase	79
3.5.2.2 PCR using Modified Nucleotide EdUTP and Taq DNA Polymerase	80
3.5.2.3 PCR Optimization using Pwo DNA Polymerase.	80
3.5.2.4 PCR using Modified Nucleotide EdUTP and Pwo DNA Polymerase	81
3.5.2.5 Gel Electrophoresis	81
3.5.2.6 Conjugation of Osmium Carbonyl Clusters to DNA	81
3.5.2.7 Preparation of DNA Samples for MALDI-TOF Analysis	81
3.5.2.8 Preparation of TEM Samples	82
3.6 References	83
 Chapter 4. Protonation of Disubstituted Triosmium Carbonyl Clusters	 84
4.1 Hydrogen Bonding	87
4.2 Conformational Change	93
4.2.1 Orthogonal Functionalization	96
4.2.2 Conjugation for Conformational Studies	100
4.3 Conclusion	103
4.4 Experimental	104
4.4.1 Experimental Procedure	104

4.4.1.1 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOC}(\text{CH}_3)_3$ (P7)	104
4.4.1.2 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NHCOCH}_2\text{CH}_2\text{PPh}_2$ (P8)	104
4.4.1.3 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NHBoc}$ (P9)	104
4.4.1.4 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NH}_2$ (P10)	105
4.4.1.5 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}$ (N-hydroxysuccinimide) (P11)	105
4.4.1.6 Preparation of $\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_3\text{Br}]$ (8P3)	105
4.4.1.7 Preparation of Symmetric Disubstituted Triosmium Carbonyl Clusters $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{R})_2$	105
4.4.1.8 Preparation of $\text{Os}_3(\text{CO})_{10}(\mu\text{-}$ $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NHCOCH}_2\text{CH}_2\text{PPh}_2)$ (9P8)	106
4.4.1.9 Preparation of Asymmetric Disubstituted Triosmium Carbonyl Clusters $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{R}^1)(\text{PPh}_2\text{R}^2)$	106
4.4.1.10 Preparation of azide-functionalized clusters $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{R}^1)(\text{PPh}_2\text{R}^2)$	107
4.4.1.11 Preparation of cis-cis $[\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOH})][\text{PF}_6]$ $([\mathbf{9P6}+\text{H}]^+)$	107
4.4.1.12 Preparation of $[\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-}$ $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NHCOCH}_2\text{CH}_2\text{PPh}_2)][\text{PF}_6]$ ($[\mathbf{9P8}+\text{H}]^+$)	107
4.4.1.13 CuAAC Reaction With Propargylamine (9L3)	108
4.4.1.14 Preparation of Cis-Cis Chelated Cluster ($[\mathbf{9L3}+\text{H}]^+$)	108
4.4.1.15 CuAAC Reaction With DL-Propargylglycine (9A1)	108
4.4.1.16 Conjugation of Pentapeptide Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (9A2)	108
4.4.1.17 Conjugation of Heptapeptide Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pra- NHMe (9A3)	109
4.4.2 Computational studies	109
4.4.3 Crystallographic Analysis	109
4.4.4 Experimental data	111
4.5 References	120

Chapter 5. Disubstituted Phosphine Clusters as pH Sensors	122
5.1 Screening Triosmium Carbonyl Clusters for pH Sensing	124
5.2 Anchoring Disubstituted Phosphine Clusters on Gold Surfaces	127
5.3 Phosphine-Substituted $\mu\text{-S}$ Clusters for pH Sensing	131

5.4 Conclusion	137
5.5 Experimental	138
5.5.1 Experimental Procedure	139
5.5.1.1 Screening the Protonation of Triosmium Carbonyl Clusters by Infrared Spectroscopy	139
5.5.1.2 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$ (P14)	139
5.5.1.3 Reaction of Cluster 2 with Phosphine P14	139
5.5.1.4 Preparation of $\text{Os}_3(\text{CO})_{11}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH})$ (8P14)	139
5.5.1.5 Preparation of $\text{Os}_3(\text{CO})_{10}[\text{PPh}_2\text{CH}_2\text{CH}_2\text{COO}(\text{N-}$ hydroxysuccinimide)] ₂ (9P11)	140
5.5.1.6 Reaction of NHS Ester Derivatives with $\text{H}_2\text{N-R-SH}$	140
5.5.1.7 Preparation of $\text{Os}_3(\text{CO})_{10}[\text{PPh}_3][\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}(\text{NHS})]$ (9P11.13)	140
5.5.1.8 Preparation of $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-S}(\text{CH}_2)_2\text{SH}](\text{PR}_3)$ (17P17-18)	140
5.5.1.9 Immobilization of 17P17 on Solid Support	141
5.5.1.10 Monitoring The Response Of Clusters 17P17 and 17P18 toward Acid	141
5.5.1.11 Incubation of Clusters with Gold Nanoparticles	142
5.5.2 Experimental Data	143
5.6 References	148
Chapter 6. Conclusion	149
6.1 References	155

Abstract

This thesis describes the conjugation of triosmium carbonyl clusters to biomolecules, in particular, nucleosides and oligopeptides. Several different approaches were employed for this conjugation, including the copper-catalyzed 1,3-dipolar azide-alkyne cycloaddition (CuAAC) reaction. Various triosmium carbonyl clusters were successfully conjugated to nucleosides, and the conjugation was also extended to hexaosmium carbonyl clusters. The effect of bioconjugation on the biological activity of the cluster was examined, with several clusters showing promising anti-proliferative activity. The CuAAC reaction was also utilized for the labelling of oligopeptides with osmium carbonyl clusters for TEM imaging.

Oligopeptides were successfully conjugated to functionalized disubstituted phosphine clusters via simultaneous biorthogonal reactions. These oligopeptide-cluster conjugates underwent stereochemical changes upon protonation, and the effect of these changes on the structure and conformation of the oligopeptide were investigated. Cluster protonation was also investigated for development into SERS-based intracellular pH sensors.

List of Figures

Figure	Title	Page
1.1	Organometallic complexes in clinical trials and on the market.	1
1.2	Nucleotides and their structure.	2
1.3	The conjugation of fluorescent rhenium complexes to thymidine.	3
1.4	Conjugation of organometallic species at the nucleobase.	4
1.5	Conjugation of technetium and rhenium carbonyl complexes at the ribophosphate backbone.	4
1.6	Conjugation of PNA to nickel and chromium complexes	5
1.7	Conjugation of ferrocene to peptide nucleic acids.	6
1.8	Conjugation of ferrocene to amino acids through various coupling reactions.	7
1.9	Conjugation of cobaltocenium and molybdenum complexes to targeting peptides.	8
1.10	Conjugation of peptides via the single amino acid chelate method (SAAC).	9
1.11	Ring slip reaction between the sandwich ruthenium complex and the aromatic peptide residues.	10
1.12	Metal – carbohydrate coordination complexes.	10
1.13	Technetium-99m conjugated to glucose and their derivatives.	11
1.14	Rhenium and iridium – glucose conjugates.	12
1.15	Functionalization of glucose for reaction with dicobalt hexacarbonyl.	12
1.16	Fatty acid and phospholipid conjugates.	13
1.17	Organometallic conjugates of steroids.	14
1.18	Conjugation of estradiol to chromium carbonyl via a η^6 interaction.	14
1.19	Displacement of labile ligands by vitamin B12.	15
1.20	Conjugation of rhenium (bis-quinoline) carbonyl moieties to vitamin B12.	16
1.21	Derivatization of the glutamic acid terminal of folic acid for conjugation.	17
1.22	Iridium and rhodium pyridylbenzaldehyde complexes for conjugation with biotin.	18
1.23	False color infrared image (left) and 3-d display (right) of oral mucosa cells treated with the triosmium carbonyl cluster.	19
1.24	Functionalized triosmium carbonyl clusters and their applications in bioassays.	19

1.25	Triosmium and triruthenium carbonyl clusters conjugated to maltol and sugar derivatives.	20
1.26	Bioorthogonal reactions for the conjugation of metal fragments to biomolecules.	21
1.27	Direct ligand displacements for the conjugation of organometallic fragments to biomolecules.	22
2.1	Organometallic – thymidine conjugates.	28
2.2	Potential modifications of thymidine.	29
2.3	Direct ligand replacements of triosmium carbonyl derivatives with different ligands.	30
2.4	Bioorthogonal click chemistry reaction.	30
3.1	Triosmium carbonyl – nucleoside conjugates used for cell viability assays.	66
3.2	Cell viability assay of the conjugates carried out on breast cell lines at 20 mM concentrations.	67
3.3	Cell uptake studies using cluster 9I-2 .	68
3.4	Infrared spectrum of cell pellets after incubation with cluster 9P2 and 9I-2 .	69
3.5	ICP-MS concentrations of cell pellets after incubation with clusters 9P2 and 9I-2 .	69
3.6	Non-functionalized molecules which show SERS enhancement.	70
3.7	Bright field image of MDA-MB-231 cells incubated with cluster 9I-2 (left) and their SERS measurement (right).	71
3.8	Conjugation of DNA to osmium carbonyl clusters.	72
3.9	Gel electrophoresis of PCR using <i>Taq</i> polymerase with the modified nucleotide EdUTP.	73
3.10	Gel electrophoresis of PCR carried out using the <i>Taq</i> polymerase (left) and <i>Pwo</i> polymerase (right) on the 5kb template using a 1.2% agarose gel.	73
3.11	TEM imaging of osmium carbonyl conjugated DNA strands.	74
3.12	DNA sequences and modifications made for conjugation to osmium carbonyl clusters.	74
3.13	MALDI-TOF analysis of the 19-mer DNA strand conjugated with cluster 15P5 .	75
3.14	TEM images of DNA conjugated with cluster 15P5 , deposited on graphene coated TEM grids.	76

4.1	Isomers of the disubstituted triosmium carbonyl cluster.	84
4.2	Computational studies on cluster $[9P+H]^+$.	86
4.3	Bond angles of the protonated <i>cis</i> – <i>cis</i> isomer and $[Os_3(\mu-H)(CO)_{10}(NCCH_3)_2]^+$.	86
4.4	<i>Cis-trans</i> isomerization of $RuCl_2(dppm)_2$.	87
4.5	Isomerization of $[4Fe-4C]$ clusters.	87
4.6	Hydrogen bonding in the protonated <i>cis-cis</i> isomer	88
4.7	Changes in the hydride resonance of the 1H NMR spectrum upon conversion of cluster $[9P7+H]^+$ to cluster $[9P6+H]^+$.	89
4.8	1H NMR spectra of cluster 9P6 (top) and cluster $[9P6+H]^+$ in $CDCl_3$ (bottom).	90
4.9	1H NMR spectrum of cluster 9P8 (top) and $[9P8+H]^+$ (bottom) in $CDCl_3$.	91
4.10	ORTEP representation of cluster $[9P8+H]^+$.	92
4.11	Typical hydrogen bond geometry for amide groups.	92
4.12	Top view and side-on view of the computationally optimized structure of the simplified <i>cis-trans</i> isomer of 9P8 .	93
4.13	Expected inter- and intrastrand interactions in a cluster-oligopeptide conjugate.	94
4.14	Expected inter- and intrastrand interactions in the chelated cluster-oligopeptide conjugate.	95
4.15	Targeted conjugation of oligopeptides to disubstituted triosmium carbonyl clusters.	96
4.16	1H NMR spectra of clusters 9L3 (top) and $[9L3+H]^+$ (bottom) in $CDCl_3$.	99
4.17	1H NOESY spectra of the neutral cluster 9L3 .	100
4.18	1H and $^{31}P\{^1H\}$ NMR spectra of cluster $[9A3+H]^+$.	103
5.1	Infrared spectrum of neutral disubstituted triosmium carbonyl phosphine cluster 9P13 and its protonated form.	122
5.2	Color change upon conversion of cluster 9P13 to cluster $[9P13+H]^+$.	122
5.3	Small molecule intracellular pH probes.	123
5.4	Triosmium carbonyl clusters as intracellular pH sensors	124
5.5	Infrared spectra of clusters 8P13 upon protonation.	125
5.6	Infrared spectra of clusters 9P13 upon protonation.	125
5.7	Infrared spectra of cluster 16P13 upon protonation.	126
5.8	NMR spectra of cluster $[9P13+H]^+$ upon standing.	126

5.9	Anchoring the substituted phosphine clusters onto the BMFON substrate.	129
5.10	Raman spectrum of clusters 9P15 , 9P16 and 9P13.15 anchored onto the BMFON chip.	129
5.11	Preparation of cluster nanoparticle conjugates for SERS measurements.	130
5.12	Uv-vis absorption spectra of non-conjugated gold nanoparticles, and gold nanoparticles incubated with cluster 6L6 .	130
5.13	Uv-vis absorption of gold nanoparticles incubated with cluster 8P14 and 9P15 at 50 μ M.	131
5.14	Phosphine-substituted μ -S clusters.	131
5.15	^1H NMR spectra of cluster 17P17 in CDCl_3 .	132
5.16	Hydride region of the ^1H NMR spectra and $^{31}\text{P}\{^1\text{H}\}$ NMR spectra of cluster 17P17 upon protonation.	134
5.18	Infrared spectrum of cluster 17P17 upon addition and removal of trifluoroacetic acid.	135
5.19	Uv-vis absorption spectra of gold nanoparticles incubated with cluster 17P17 .	135
5.20	Raman spectrum of cluster 17P17 anchored on the BMFON substrate and its infrared spectrum recorded in cyclohexane.	136
5.21	Infrared spectrum in absorbance mode of cluster 17P17 at different pH.	137
5.22	Effective range of clusters 17P17 and 17P18 , monitored by IR spectroscopy.	137
6.1	Applications of triosmium carbonyl – nucleoside conjugates	150
6.2	Applications of the protonation of the disubstituted phosphine clusters.	152
6.3	Strategies to improve the solubility of triosmium carbonyl clusters.	153
6.4	Maleimide-thiol reaction for conjugation of oligopeptides to triosmium carbonyl clusters.	154

List of Schemes

Scheme	Title	Page
2.1	Synthesis of N ³ -alkylated thymidine derivatives.	31
2.2	Synthesis of 5-alkyl-2'-deoxyuridine derivatives by Sonogashira coupling.	32
2.3	Functionalization of the 5-alkyl-2'-deoxyuridine derivatives.	33
2.4	Functionalization of thymidine at the 2'-position.	34
2.5	Synthesis of triosmium carbonyl-nucleoside conjugates by direct ligand displacement.	35
2.6	Synthesis of azide and alkyne-functionalized clusters.	36
2.7	Functionalization of phosphines with azide groups	36
2.8	Synthesis of azide functionalized triosmium carbonyl bis phosphine clusters.	37
2.9	Click chemistry reactions of osmium carbonyl clusters.	38
2.10	Functionalization of hexaosmium raft clusters.	40
2.11	Conjugation of hexaosmium carbonyl cluster 15P6 by the click chemistry reaction.	40
4.1	Preparation and protonation of disubstituted phosphine clusters.	84
4.2	Protonation of the disubstituted triosmium carbonyl cluster.	85
4.3	Preparation of carboxylic acid functionalized cluster 9P6 .	88
4.4	Preparation of chelated cluster 9P8 and its protonation.	90
4.5	Conjugation of cluster 9P2 to DL-propargylglycine.	95
4.6	Preparation of amine-functionalized phosphine.	96
4.7	Reaction of cluster 2 with a 1:1 ratio of phosphines P1 and P10 .	97
4.8	Synthesis of orthogonally functionalized cluster 9P2.10 .	97
4.9	Preparation of the orthogonally functionalized cluster 9P2.6 and 9P11.12 .	98
4.10	Reaction of cluster 9P2.6 with propargylamine.	98
4.11	Conjugation of cluster 9P11.12 to the pentapeptide Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (A2).	101
4.12	Conjugation of cluster 9P11.12 to heptapeptide Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pra-NHMe and its protonation to cluster [9A3 +H] ⁺ .	102
5.1	Synthesis of substituted triosmium carbonyl clusters for preliminary studies.	124

5.2	Reaction of triosmium carbonyl clusters with phosphine P14 .	127
5.3	Synthesis of symmetric thiol-functionalized disubstituted triosmium carbonyl phosphine clusters.	128
5.4	Synthesis of asymmetric thiol-functionalized disubstituted triosmium carbonyl phosphine cluster.	128
5.5	Reaction of clusters 8P with 1,2-ethanedithiol.	132
5.6	Reaction of clusters 9P with 1,2-ethanedithiol.	133
6.1	Conjugation of triosmium carbonyl clusters to nucleotides.	149
6.2	Conjugation of oligopeptides to orthogonal disubstituted phosphine cluster.	151
6.3	Isomeric distribution upon protonation of disubstituted phosphine derivatives.	151

List of Tables

Table	Title	Page
2.1	Yields of conjugate 7I using different solvents for the click chemistry reaction.	39
2.2	N-alkylation of thymidine.	41
2.3	Substitution with sodium azide.	42
2.4	Preparation of thiol-functionalized thymidine derivatives.	42
2.5	Sonogashira coupling with 5-iodo-2'-deoxyuridine.	43
2.6	Substitution of 5'-O-DMT-5-butynyl tosylate -2'-deoxyuridine.	43
2.7	Synthesis of carboxylate-bridged triosmium carbonyl clusters at room temperature.	45
2.8	Synthesis of carboxylate-bridged triosmium carbonyl clusters using reflux.	45
2.9	Synthesis of substituted phosphine clusters.	46
2.10	Synthesis of azide-functionalized substituted phosphine clusters.	46
2.11	Synthesis of cluster-nucleoside conjugates by the click chemistry reaction.	47
2.12	Synthesis of phosphine-substituted hexaosmium carbonyl clusters	47
3.1	Cycling parameters for PCR using <i>Taq</i> DNA polymerase.	80
3.2	Cycling parameters for PCR using <i>Pwo</i> DNA polymerase.	80
4.1	Synthesis of symmetric disubstituted triosmium carbonyl clusters.	106
4.2	Synthesis of asymmetric disubstituted triosmium carbonyl clusters.	106
4.3	Synthesis of azide-functionalized clusters.	107
4.4	Crystallographic data for cluster [9P8 +H] ⁺ .	110
5.1	Functionalization of disubstituted phosphine clusters with thiol groups.	140
5.2	Preparation of clusters 17P .	141
5.3	Monitoring the response of the cluster at different pH.	141

List of Abbreviations

ATR	Attenuated Total Reflectance	TEM	Transmission Electron Microscopy
Boc	tert-butoxycarbonyl	THF	Tetrahydrofuran
BMFON	Bimetallic Film over Nanoparticle	TLC	Thin Layer Chromatography
COE	Cyclooctene	TMNO	Trimethylamine-N-oxide
Cp	Cyclopentadienyl	TOF	Time of Flight
CuAAC	Copper-catalyzed azide-alkyne cycloaddition	Biological Terms	
DCC	Dicyclohexylcarbodiimide	Ala	Alanine
DCM	Dichloromethane	Cbz	Carboxybenzyl
DMSO	Dimethylsulfoxide	CD	Circular Dichroism
DMF	Dimethylformamide	dATP	Deoxyadenosine triphosphate
DMT	Dimethoxytrityl	dCTP	Deoxycytosine triphosphate
dppm	Diphenylphosphino methane	dGTP	Deoxyguanosine triphosphate
ESI	Electrospray Ionization	dTTP	Deoxythymidine triphosphate
HBTU	(2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)	DMEM	Dulbecco's Modified Eagle's Medium
Hex	Hexane	EdUTP	5-Ethynyl-deoxyuridine triphosphate
MALDI	Matrix-assisted Laser Desorption Ionization	Gly	Glycine
MeOH	Methanol	Ile	Isoleucine
MS	Mass Spectrometry	Lys	Lysine
NHS	N-hydroxysuccinimide	MTS	(3-(4,5-dimethylthiazol-2-yl)-5-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
NMR	Nuclear Magnetic Resonance	PBS	Phosphate Buffer Saline
SERS	Surface-Enhanced Raman Spectroscopy	PCR	Polymerase Chain Reaction
		Phe	Phenylalanine
		PNA	Peptide Nucleic Acid
		Pra	Propargylglycine

Infrared Spectroscopy

ν_{CO}	Carbonyl stretching frequency
vs	Very strong
s	Strong
m	Medium
w	Weak
sh	Shoulder

NMR Spectroscopy

s	Single
δ	Chemical shift
d	Doublet
J	Coupling constant
t	Triplet
m	Multiplet
quin	Quintet
br	Broad

Molecular Numbering Scheme

i) Nucleosides

- a**, $R_1 = \text{H}$, $R_2 = (\text{CH}_2)_3\text{Br}$
b, $R_1 = \text{DMT}$, $R_2 = (\text{CH}_2)_3\text{Br}$
c, $R_1 = \text{H}$, $R_2 = (\text{CH}_2)_3\text{N}_3$
d, $R_1 = \text{DMT}$, $R_2 = (\text{CH}_2)_3\text{N}_3$
e, $R_1 = \text{H}$, $R_2 = (\text{CH}_2)_3\text{SH}$
f, $R_1 = \text{DMT}$, $R_2 = (\text{CH}_2)_3\text{SH}$
g, $R_1 = \text{DMT}$, $R_2 = \text{CH}_2\text{CN}$

- h**, $R_1 = \text{H}$, $R_2 = \text{SiMe}_3$
i, $R_1 = \text{DMT}$, $R_2 = \text{SiMe}_3$
j, $R_1 = \text{DMT}$, $R_2 = (\text{CH}_2)_2\text{OTs}$
k, $R_1 = \text{DMT}$, $R_2 = (\text{CH}_2)_3\text{COOH}$
l, $R_1 = \text{H}$, $R_2 = \text{H}$
m, $R_1 = \text{DMT}$, $R_2 = (\text{CH}_2)_2\text{N}_3$
n, $R_1 = \text{DMT}$, $R_2 = (\text{CH}_2)_2\text{Br}$

p

ii) Phosphine ligands

- P1**, R = (CH₂)₂COO(CH₂)₃Br
P3, R = (CH₂)₃Br
P4, R = (CH₂)₃OH
P6, R = (CH₂)₂COOH
P7, R = (CH₂)₂COOC(CH₃)₃
P9, R = (CH₂)₂CONH(CH₂)₂NHBoc
P10, R = (CH₂)₂CONH(CH₂)₂NH₂
P11, R = (CH₂)₂CO(NHS)
P14, R = (CH₂)₃SH

iii) Clusters

- 1**, L₁ = CO, L₂ = CH₃CN
2, L₁ = L₂ = CH₃CN
3, L₁ = CO, L₂ = C₈H₁₄
4, L₁ = L₂ = C₈H₁₄

Every — bond to osmium represents a CO ligand

5

6L4, R = C₆H₅
6L5, R = (CH₂)₃P(C₆H₅)₂
6L6, R = (CH₂)₂SH

7L1, R = -C≡CH
7L2, R = -(CH₂)₅N₃

8P1, R¹ = R² = Ph, R³ = (CH₂)₂COO(CH₂)₃Br
8P2, R¹ = R² = Ph, R³ = (CH₂)₂COO(CH₂)₃N₃
8P3, R¹ = R² = Ph, R³ = (CH₂)₃Br
8P13, R¹ = R² = R³ = Ph
8P14, R¹ = R² = Ph, R³ = (CH₂)₃SH
8P17, R¹ = R² = R³ = CH₃
8P18, R¹ = R² = R³ = O(CH₃)

9P1, R¹ = R² = Ph, R³ = (CH₂)₂COO(CH₂)₃Br
9P2, R¹ = R² = Ph, R³ = (CH₂)₂COO(CH₂)₃N₃
9P6, R¹ = R² = Ph, R³ = (CH₂)₂COOH
9P7, R¹ = R² = Ph, R³ = (CH₂)₂COOC(CH₃)₃
9P11, R¹ = R² = Ph, R³ = (CH₂)₂CO(NHS)
9P13, R¹ = R² = R³ = Ph
9P15, R¹ = R² = Ph, R³ = (CH₂)₂CONH(CH₂)₂SH
9P16, R¹ = R² = Ph, R³ = (CH₂)₂CONH(C₆H₅)SH
9P17, R¹ = R² = R³ = CH₃
9P18, R¹ = R² = R³ = O(CH₃)

- 9P1.6,** $R^1 = (CH_2)_2COO(CH_2)_3Br$, $R^2 = (CH_2)_2COOH$
9P1.10, $R^1 = (CH_2)_2COO(CH_2)_3Br$, $R^2 = (CH_2)_2CONH(CH_2)_2NH_2$
9P2.6, $R^1 = (CH_2)_2COO(CH_2)_3N_3$, $R^2 = (CH_2)_2COOH$
9P2.10, $R^1 = (CH_2)_2COO(CH_2)_3N_3$, $R^2 = (CH_2)_2CONH(CH_2)_2NH_2$
9P11.12, $R^1 = (CH_2)_2CO(NHS)$, $R^2 = (CH_2)_3N_3$,
9P11.13, $R^1 = (CH_2)_2CO(NHS)$, $R^2 = Ph$
9P13.15, $R^1 = Ph$, $R^2 = (CH_2)_2CONH(CH_2)_2SH$

15P3, X = Br
15P4, X = OH

15P5

16P13

[16P13+H]⁺

17P17, R = CH₃
17P18, R = O(CH₃)

[17P17+H]⁺

iv) Conjugates

6e

7c

7l

8l

9I-1

9I-2

10g

11d

13d

15I

9A1-1

9A1-2

9A2

9A3

Chapter 1. Organometallic bioconjugates

For a long time, it was thought that organometallic complexes were incompatible with biological systems due to their sensitivity to moisture and air.¹ This was despite the existence of metal-centered complexes such as the cobalt-centered vitamin B12 in nature. This perspective changed drastically, however, and has led to the development of a field now known as bioorganometallics. The term bioorganometallics refers to the biological study of complexes containing a direct metal-carbon bond.² It not only refers to the study of naturally-occurring organometallic complexes but also encompasses synthetic complexes which may be applied in biological systems. Current applications of these complexes include their use as therapeutic and diagnostic agents, some of which have entered clinical trials and some successfully marketed (Figure 1.1).

Figure 1.1 Organometallic complexes in clinical trials and on the market.

Many of the organometallic complexes which have been used for biological applications involve conjugation to molecules with known biological activity. For example, the family of ferrocifens used in the treatment of breast cancer, is essentially a conjugate of ferrocene and the known anti-cancer drug, tamoxifen. Currently in clinical trials, ferrocifens show improved activity as compared to tamoxifen.³ Such conjugates allow the properties of both the organometallic fragment and biological agent to be combined, leading to improvement in the distribution and compatibility of the organometallic species with biological systems. Besides being used as therapeutic and diagnostic agents, organometallic bioconjugates have also found applications in bioinspired asymmetric catalysis and in the sensing of biological analytes.^{2,4-7}

1.1 Bioconjugation of Organometallic Species

Bioconjugation refers to the formation of a covalent bond between two fragments, one of which is usually a biomolecule. The synthetic methodologies which have been adopted for bioconjugation depends on the nature of the biomolecule and the fragment which is to be combined. In the case of organometallic bioconjugates, the synthetic route is often determined by the reactivity and stability of the organometallic fragment. Organometallic fragments such as ferrocene, technetium radionuclides and rhenium luminescent agents have been conjugated to different classes of biomolecules.

1.1.1 Conjugation to Nucleic Acids

Nucleic acids are essential to living organisms as they are the building blocks which make up our genetic code. The modification and labelling of nucleic acids have been widely explored to gain a better understanding into the cellular machinery and for the development of new therapeutic agents. For example, the conjugation of nucleic acids to organic-based fluorophores and labels are tools which are commonly utilized to locate a specific gene or sequence in a long strand of DNA.⁸⁻¹⁰ Conjugation of these nucleic acids have been carried out at various sites of the nucleic acid structure, such as at the 5' and 3' position of the ribose backbone (Figure 1.2).

Figure 1.2 Nucleotides and their structure.

With organometallic fragments, conjugation and modification of oligonucleotides have most commonly been carried out at the thymine nucleobase. This is most probably due to the greater synthetic accessibility of thymine derivatives. Alkylation of thymine derivatives at the N³ position of the nucleobase can be achieved by deprotonation of the amide, followed by reaction with alkyl halides.¹¹ This route has been adopted for conjugation of the rhenium and

technetium tricarbonyl cores to thymidine.^{12,13} The alkylation at the N³ position of thymidine allows functionalization of the nucleoside with a primary amine group. Subsequent reaction of the amine-functionalized nucleoside with chelators allows for the conjugation of thymidine with rhenium and technetium tricarbonyl (Figure 1.3). These conjugates are recognized by the human thymidine kinase type 1 (hTK1) enzyme which is responsible for the phosphorylation of thymidine at the 5'-OH position.¹⁴ Overexpression of the hTK1 enzyme in cancer cells causes the accumulation of the conjugates and hence the application of these conjugates as fluorescent and radioimaging agents.

Figure 1.3 The conjugation of fluorescent rhenium complexes to thymidine.

Nucleosides have also been conjugated to cobalt carbonyl and ferrocene through modification of the nucleobase, and these have been shown to inhibit cancer cell growth.¹⁵ The bis-conjugation of adenine, thymine and uracil with ferrocene derivatives has also been utilized for self-assembly of materials directed by base pairing.¹⁶⁻¹⁸ The direct bonding of deoxyadenosine to the $[(Cp)^*Rh]$ half-sandwich complex has also been extensively studied by Fish and co-workers as supramolecular hosts of aromatic organic substrates (Figure 1.4).¹⁹⁻²¹

Figure 1.4 Conjugation of organometallic species at the nucleobase.

Besides conjugation through the nucleobase, modifications of the nucleoside on the ribose sugar have also been carried out (Figure 1.5).^{22,23} For example, the conjugation of technetium carbonyl to the nucleoside thymidine has been carried out by amide coupling at the 5' position. These conjugates have been used for applications in radioimaging.

Figure 1.5 Conjugation of technetium and rhenium carbonyl complexes at the ribophosphate backbone.

The replacement of the deoxyribosephosphate backbone with a N-[(2-aminoethyl)glycine] chain has yielded a non-natural DNA polymer now known as peptide nucleic acid (PNA) which shows strong binding with DNA and RNA.²⁴ Modification of PNAs with

ferrocene, nickel and chromium carbonyl half sandwich complexes has been carried out via different synthetic routes (Figure 1.6).^{25,26}

Figure 1.6 Conjugation of PNA to nickel and chromium complexes

Ferrocenyl derivatives in particular have been used for conjugation to PNAs due to their potential in being used as DNA biosensors.¹⁸ Insertion of the ferrocenyl moiety into the PNA backbone has been demonstrated using solid phase peptide synthesis. This involves the formation of a peptide bond utilizing the ferrocenyl carboxylic acid derivative.²⁷ Insertion of the ferrocenyl derivative was also achieved utilizing the ferrocene-azide derivative in a copper-catalyzed azide alkyne click chemistry (CuAAC) reaction with the PNA backbone (Figure 1.7).²⁸

Figure 1.7 Conjugation of ferrocene to peptide nucleic acids.

1.1.2 Conjugation to Proteins

Structurally, proteins are built from amino acids joined covalently by a peptide linkage. They are the working engines of the body, carrying out a vast array of functions. Nature further diversifies the function of proteins by conjugating them to other molecules to form lipoproteins, phosphoproteins, cytochromes and glycoproteins. Synthetically, proteins and their building blocks have been conjugated to fluorophores, DNA molecules and drug molecules as cellular probes, therapeutic agents and material building blocks.²⁹⁻³¹

The conjugation of organometallic fragments to amino acids, short peptides and proteins has been carried out with several different metals, namely, iron, rhenium, manganese and platinum. In principle, conjugation of the organometallic fragment can be carried out on several different parts of the peptide - the N- and C-termini, as well as the α -side chain.²⁷ The conjugation of ferrocene, in particular, has been used widely due to its stability and electrochemical properties. Ferrocene-amino acid conjugates have been synthesized using the formation of a peptide bond with ferrocene-carboxylic acid or ferrocene-aldehyde derivatives (Figure 1.8).³²⁻³⁶ The reaction proceeds under relatively mild conditions in the presence of standard coupling reagents. Such conjugates have been studied for their potential as biosensors.

Co - SVT-40T conjugate

Mo - enkephalin conjugate

Figure 1.9 Conjugation of cobaltocenium and molybdenum complexes to targeting peptides

A majority of the reported organometallic-peptide conjugates utilize derivatization of a lysine residue for conjugation. This approach is known as the single amino acid chelate (SAAC) strategy. Using this method, chelating ligands may be attached on natural or unnatural amino acids which are inserted away from the targeting sequence of the peptide. The reductive alkylation of the lysine residues, in particular, has been found to be particularly useful and high yielding for the incorporation of chelating ligands.⁴² For example, the chemotactic peptide N-formyl-Met-Leu-Phe (fMLF) has been conjugated to the rhenium and radioactive technetium $M(CO)_3$ core by incorporation of a lysine residue at a region away from the targeting sequence.^{43,44} The conjugate possesses better affinity for granulocytes as compared to the parent peptide. The SAAC strategy has generally been shown to be useful for the preparation of a library of peptides for conjugation to the metal core (Figure 1.10).

Figure 1.10 Conjugation of peptides via the single amino acid chelate method (SAAC).

While the methods described above have been effective for the conjugation of short oligopeptides, the labelling of larger protein structures with an organometallic fragment provides an even bigger challenge as conjugation may affect the bioactivity and structure of the protein. A potential method is the direct coordination of the metal center via a η^6 -binding with aromatic residues on the protein. For example, UV irradiation of sandwich complexes with labile aromatic rings such as $[(\eta^5\text{-Cp})\text{Ru}(\eta^6\text{-naphthalene})]^+$ leads to displacement of the naphthalene ring by aromatic residues on the protein.⁴⁵ This method has been utilized for the labelling of the protein mellitin with a ruthenium sandwich compound at a tryptophan residue (Figure 1.11). Although the presence of the metal did not affect the tertiary structure of the protein, the coordination causes a weakening of the hydrophobic interactions with the tryptophan residue, leading to a collapse of the quaternary structure.⁴⁶

Figure 1.11 Ring slip reaction between the sandwich ruthenium complex and the aromatic peptide residues.

1.1.3 Conjugation to Sugars and Carbohydrates

The coordination of carbohydrates to metal ions via the alcohol and ketone groups was investigated in the early years of bioinorganic chemistry. Despite the large number of functional groups in these molecules, their weak donor ability renders the complexes rather unstable in neutral aqueous solutions.⁴⁷ Deprotonation of the alcohol groups and the addition of anchoring groups improved the coordinating ability of carbohydrates and more stable complexes were formed with harder metal ions (Figure 1.12).⁴⁸⁻⁵⁰ Most of these bioconjugates are Werner-type coordination complexes.

Figure 1.12 Metal – carbohydrate coordination complexes.

Conjugation to organometallic species is commonly achieved via alkylation of a hexose precursor with diols such as ethylene glycol, followed by subsequent functionalization with different groups for coordination to the organometallic precursor (Figure 1.13).^{51,52}

Figure 1.13 Technetium-99m conjugated to glucose and their derivatives.

In an attempt to emulate the success of radioactive ¹⁹F-labelled glucose in PET imaging, the conjugation of radioactive technetium and rhenium carbonyl cores to glucose derivatives have also been examined. These have been carried out, mostly through derivatisation with chelating ligands.^{52,53} Unfortunately, most of the prepared conjugates have shown rather limited uptake by the highly specific glucose transporters (GLUT) in the cells.⁵⁴ Conjugation of glucose and other sugars such as galactose to other organometallic imaging agents such as fluorescent iridium complexes has also been carried out using linkers carrying chelating ligands.^{55,56} The iridium polypyridyl – glucose conjugate, in particular, has shown good uptake by the GLUT and these have been used for the preferential imaging of cancer cells (Figure 1.14).

Figure 1.14 Rhenium and iridium – glucose conjugates.

Besides conjugation with imaging agents, sugar molecules have also been used to improve the cytotoxicity and uptake of therapeutic agents. It has been established that at high concentrations, certain sugar molecules can induce apoptosis. For example, sugar conjugates of platinum, palladium and cobalt have shown promising *in vitro* activity.⁵⁷ The well-known affinity of the dicobalt hexacarbonyl fragment for the alkyne moiety was exploited in its conjugation to fructose. This was carried out via the alkylation of a hydroxyl group on an acetal-protected precursor with propargyl bromide (Figure 1.15). Selective deprotection of the acetal groups prior to coordination of the dicobalt carbonyl complex yielded three fructose-conjugated products which showed moderate anti-proliferative activity toward cancer cells.⁵⁸

Figure 1.15 Functionalization of glucose for reaction with dicobalt hexacarbonyl.

1.1.4 Conjugation to Lipids and Steroids

Lipids, which include fats, steroids, glycerides, fat-storing vitamins, phospholipids and waxes, play a vital role in energy storage and cell signalling, and are also a vital component of the cellular membrane. Fatty acids, in particular, have often been conjugated to organometallic species. Structurally, a fatty acid consists of long saturated or unsaturated aliphatic chains containing a carboxylic acid group. Conjugation to fatty acids is usually carried out by derivatization of the long aliphatic chains with other functional groups for reaction with an organometallic species.

For example, saturated undecanoic acid was functionalized with a terminal thiol group for reaction with a triosmium dodecacarbonyl derivative. Although the fatty acid derivative carries two reactive groups (-SH and -COOH), there is clear affinity of the thiol group for the soft metal centre since the reaction yields the μ -S conjugate preferentially.⁵⁹ This method was also extended to the synthesis of an organometallic conjugate of a phospholipid (Figure 1.16). The derivatization of fatty acids with other groups, such as, isocyanide and cyclopentadiene, have also been carried out for conjugation to the radioactive technetium-99m core as a myocardial imaging agents.⁶⁰⁻⁶²

Figure 1.16 Fatty acid and phospholipid conjugates.

Steroids are the cell signalling molecules in the body and are involved in the activation of steroid hormone receptors. The core structure of steroids consists of four fused rings arranged in a specific configuration. Conjugation of these steroids to organometallic species mostly involves derivatization of the side chains and functional groups of the fused ring structure. Functionalization of the side chains of steroids such as cholesterol, mestranol and estradiol with various groups allows for conjugation to organometallic species (Figure 1.17).⁶³⁻

Figure 1.17 Organometallic conjugates of steroids.

Coordination of organometallic fragments to steroids may also be carried out via one of the aromatic rings on the fused structure. For example, heating a mixture of an estradiol derivative with Cr(CO)₆ yielded two diastereomers which could be separated by chromatographic methods.⁶⁶ A series of these chromium carbonyl–estradiol conjugates, with different functional groups and spacers, were prepared via this method (Figure 1.18). Some of these derivatives have shown specific and reversible binding to estrogen receptors detected through infrared spectroscopy, even at low concentrations.⁶⁷

Figure 1.18 Conjugation of estradiol to chromium carbonyl via a η^6 interaction.

1.1.5 Conjugation to Vitamins

Unlike the above classes, vitamins are not classified based on their structure but on their chemical and biological functions, which are very diverse. For example, vitamins C and E function as antioxidants while the vitamin B complexes are enzyme cofactors. Historically, it was the discovery of the cobalt-centered vitamin B12 which fuelled the development of bioorganometallics.²

Vitamin B12 consists of a cobalt center coordinated to a planar tetra-pyrrole corrin ring and is found in several physiological forms, such as, methylcobalamin and adenosylcobalamin. Modification of the functional groups around the corrin ring has been shown to affect the affinity and uptake of vitamin B12 derivatives into the cells, while conjugation at the axial sites affects the affinity to a lesser degree. Technetium and rhenium carbonyls have therefore been conjugated to vitamin B12 via the axial cyano ligand.^{68,69} The aqua ligand in organometallic precursors such as $M(L)(H_2O)(CO)_3$ [L = tightly bound bidentate ligand] can be replaced by a cyano ligand upon reaction with vitamin B12 to yield conjugates of the type Co-CN-M (Figure 1.19). Conjugation of a fluorescent rhenium (bis-quinoline) derivative has also been carried out at the 5'-hydroxyl group of the ribose moiety, and these conjugates have been used effectively for the fluorescent imaging of cancer cells (Figure 1.20).^{70,71}

Figure 1.19 Displacement of labile ligands by vitamin B12.

Figure 1.20 Conjugation of rhenium (bis-quinoline) carbonyl moieties to vitamin B12.

Folic acid has been conjugated to known therapeutic and imaging agents. The overexpression of folate receptors in many types of cancers increases the need and demand of folates in rapidly dividing cells, making it a good targeting strategy for therapeutic and imaging agents, a considerable number of which have made it to the market. These include conjugates of cytotoxic platinum complexes, as well as fluorescent and radioactive rhenium and technetium complexes, to target cancer cells.⁷²⁻⁷⁴ Conjugation of these organometallic species has been carried out via functionalization of the C-terminus of the glutamic acid with chelating ligands, much like that described in Section 1.1.2 (Figure 1.21).^{73,75}

Figure 1.21 Derivatization of the glutamic acid terminal of folic acid for conjugation.

Although biotin is one of the essential vitamins in the body, it is perhaps more well-known in biochemistry for its strong affinity for the protein, streptavidin. The rapid and strong interaction of biotin and streptavidin is commonly used for the purification of biomolecules as well as in biological assays.⁷⁶ The development of fluorescent biotinylating agents is of interest as it allows for the spectrofluorometric determination and measurement of the biotin-streptavidin interaction. The conjugation of fluorescent organometallic complexes such as the iridium and rhodium polypyridyl complexes to biotin has been demonstrated and the avidin binding of these conjugates have been measured as well.^{77,78} Lo and co-workers have made use of the pyridylbenzaldehyde (pba) ligand and its facile reaction with amine-functionalized ligands for the conjugation of biotin to organometallic species.⁷⁹ A series of conjugates with different linkers and spacers were prepared by reacting the $[M_2(pba)_4Cl_2]$ precursors, with the amine-functionalized biotin derivatives using this method (Figure 1.22).

Figure 1.22 Iridium and rhodium pyridylbenzaldehyde complexes for conjugation with biotin.

1.2 Bioconjugation of Osmium Carbonyl Clusters

Although most of the organometallic conjugation methods have been developed for mononuclear organometallic complexes, the conjugation of metal carbonyl clusters to biomolecules has become of interest in recent years since the application of these clusters in biological systems have become more apparent. One of the earliest conjugates reported is the fatty acid conjugated triosmium carbonyl cluster described in section 1.1.4, used in mid-infrared cellular imaging (Figure 1.23).⁵⁹ These infrared imaging agents have advantages over their organic counterparts since the carbonyl stretches of the metal carbonyl cluster are found in a region relatively free from interference. Recent work has also demonstrated the utility of such metal carbonyl clusters as photoacoustic imaging agents.⁸⁰

Figure 1.23 False color infrared image (left) and 3-d display (right) of oral mucosa cells treated with the triosmium carbonyl cluster.

Although triosmium carbonyl clusters have been used as infrared imaging agents, the resolution of this technique limits its application for cellular imaging.^{81,82} This may be countered by utilizing its complement, Raman spectroscopy. Enhancement of the cluster's Raman stretches may be achieved by immobilization of metal carbonyl clusters on surface-enhanced Raman spectroscopy (SERS) – active surfaces such as gold nanoparticles.⁸² Clusters functionalized with boronic acid and carboxylic acid moieties have been utilized effectively for the detection of glucose and cancer biomarkers as well (Figure 1.24).^{83,84} Functionalization of the cluster was carried out by preparation of thiolato-bridged clusters, which have demonstrated good stability in biological systems.

Figure 1.24 Functionalized triosmium carbonyl clusters and their applications in bioassays.

Metal carbonyl clusters such as the $\text{Os}_3(\text{CO})_{10}(\text{NCCH}_3)_2$ have also shown potential as therapeutic agents.^{85,86} The conjugation of triosmium and ruthenium carbonyl clusters to naturally occurring organic compounds have yielded complexes which show good cytotoxicity against cancer cell lines. Example of such clusters are the triosmium and triruthenium carbonyl clusters which contain at least one bridging maltolato ligands (Figure 1.25).^{87,88} Conjugation with sugars such as glucofuranoside may also be achieved by substitution with sugar-functionalized phosphines (Figure 1.24); this yields a series of complexes which exhibit anti-angiogenic properties.⁸⁹

Figure 1.25 Triosmium and triruthenium carbonyl clusters conjugated to maltol and sugar derivatives.

1.3 Conjugation Methods

From the examples presented above, it is apparent that the bioconjugation of organometallic complexes often requires the functionalization of one, or both the metal fragment, and the biomolecule of interest. This functionalization allows orthogonal reactions to be utilized to conjugate the two fragments. One such reaction which has been utilized rather frequently is the amide coupling reaction. This has been especially useful for the conjugation of peptides and vitamins such as folic acid, since these molecules have free amine and carboxylic acid groups (Figure 1.26).

Another useful, but less utilized orthogonal reaction which has been employed is the copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC). Since the alkyne and azide functional group is less common in biological systems, the reaction is especially useful to achieve specific conjugation of a biomolecule with the metal fragment (Figure 1.26).

Amide coupling reaction

Folic acid - Tc conjugate

Rhenium - Vitamin B12 conjugate

Mo - enkephalin conjugate

Copper-catalyzed azide-alkyne cycloaddition reaction (CuAAC)

Figure 1.26 Bioorthogonal reactions for the conjugation of metal fragments to biomolecules.

Besides the bioorthogonal reactions discussed above, it is also apparent that suitably functionalized biomolecules can be utilized for direct ligand displacement with metal precursors. However, this is largely dependent on the reactivity of the metal precursor. For example, the functionalization of various biomolecules with chelating ligands has been rather successful for conjugation to technetium and rhenium carbonyl complexes. Triosmium carbonyl precursors may also react with suitably functionalized biomolecules to afford stable bridged products (Figure 1.27).

Figure 1.27 Direct ligand displacements for the conjugation of organometallic fragments to biomolecules.

1.4 Aim and Objectives

From the discussion above, the bioconjugation of organometallic species is an important strategy to improve the activity and accumulation of the organometallic species in the biological system. Our group has been working on the application of osmium carbonyl clusters as bioimaging and therapeutic agents. A necessary prerequisite for further development of these lies in the development of bioconjugation methods for the metal carbonyl clusters. To date, osmium clusters have only been conjugated to biomolecules such as fatty acids, cholesterol and amino acids.^{59,90,91} The aim of the project described in this thesis is to examine the bioconjugations of triosmium carbonyl clusters to other classes of biomolecules, in particular:

- i) Nucleoside and nucleotides, and
- ii) Oligopeptides

The potential applications of these conjugates, as well as the effect of the clusters on the structure of the biomolecule, are also to be examined.

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Chapter 2. Conjugation of Osmium Carbonyl Clusters to Nucleic Acids

As discussed in chapter 1, nucleosides have been conjugated to organometallic species via various synthetic routes for both therapeutic and imaging applications (Figure 2.1). Thymidine, in particular, is often used for conjugation because of its synthetic accessibility and potential for biological applications. In most of these conjugates, thymidine acts as a targeting agent, since rapidly dividing cells have an increased demand for nucleic acids. Enzymes such as thymidine kinase are overexpressed in diseased cells, causing an increased demand and uptake of thymidine by these cells.¹

Figure 2.1 Organometallic – thymidine conjugates.

2.1 Conjugation of Nucleosides

In principle, it is possible for thymidine to react directly with an activated organometallic species. However, this is often not the case. For example, the reaction of thymidine with the triosmium carbonyl cluster $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-OH})$ does not yield any product.² Functionalization or modification of the nucleoside is therefore necessary to improve its reactivity with the organometallic fragment.

Conjugations and modifications on the nucleobase, thymine, can be carried out at the N³, C⁵ or C⁶ position (Figure 2.2).³ Alkylation of the N³ position is one of the more commonly used conjugation methods since this can occur readily under basic conditions.⁴ However, conjugation at this position interferes with hydrogen bonding of the nucleobase.¹ This may be bypassed by modification at the C⁵ position through C-C coupling reactions. Oxidation of the alkene bond may also be carried out for functionalization at the C⁵ and C⁶ carbons, much like in the synthesis of thymidine glycol.⁵ However, this would result in the loss of aromaticity and would yield epimers (Figure 2.2).

Figure 2.2 Potential modifications of thymidine.

Modifications to the ribose ring can also be carried out at multiple sites. Deprotonation of the hydroxyl group activates the 3' and 5' positions toward modifications (Figure 2.2).^{3,6} However, this may not be very desirable since both these groups are involved in forming phosphodiester bonds in oligonucleotides. Derivatization at these positions may thus affect recognition of the nucleoside derivatives by enzymes such as thymidine kinase in the cell. Functionalisation at the 2' position may be carried out as an alternative, although this may affect the conformation of the sugar ring.⁷

2.2 Synthetic Strategy

The conjugation of biomolecules to organometallic species also depends on the reactivity of the organometallic species with various types of ligands. It is therefore essential to consider the reactions that trisium carbonyl cluster derivatives may participate in.

The conjugation of trisium carbonyl clusters to thymidine may be carried out via two methods as discussed in Section 1.3. The first involves a direct ligand displacement reaction between a functionalized nucleoside and an activated trisium carbonyl cluster. For example, the bis(acetonitrile) derivative **2** is known to react with thiols and carboxylic acids to form stable bridged clusters.^{8,9} Other activated clusters such as the cyclooctene cluster **3** and unsaturated cluster **5** have also been known to react with azides, alkynes and nitriles (Figure 2.3).¹⁰⁻¹²

Every — bond to osmium denotes a CO ligand

Figure 2.3 Direct ligand replacements of triosmium carbonyl derivatives with different ligands.

A second conjugation method involves the use of biorthogonal reactions such as the peptide coupling reaction and the copper-catalyzed Huisgen 1,3-dipolar azide-alkyne cycloaddition – the CuAAC “click” reaction. The click chemistry reaction proceeds between azides and terminal alkynes to yield the regiospecific 1,4 – disubstituted tetrazole product under relatively mild conditions (Figure 2.4). Azide and alkyne groups are not naturally found in biological systems, making this reaction especially useful for the labelling of biomolecules.¹³ Earlier work has shown that the click chemistry reaction is also effective for the conjugation of triosmium carbonyl clusters with nucleobases, thus it may be adopted for the conjugation of triosmium carbonyl clusters with thymidine.²

Figure 2.4 Bioorthogonal click chemistry reaction.

2.3 Functionalization of Thymidine

In both synthetic approaches discussed above, the functionalization and modification of thymidine is required. Since it is known that trisium carbonyl cluster derivatives can react with azides, alkynes, thiols and phosphines, the modification of thymidine with these functional groups at different positions of the nucleoside was examined.

2.3.1 Modification of Thymidine at the N³ Position

Alkylation of thymidine at the N³ position can be carried out by reaction with a bromoalkane under basic conditions.¹ Derivatives, **a**, **b** and **g** were obtained in moderate yields using similar reaction conditions (Scheme 2.1). However, attempts to synthesize the carboxylic-acid functionalized derivative were rather unsuccessful as the basic conditions led to the deprotonation of 4-bromobutyric acid. Switching to the sodium 4-bromobutyrate salt also failed to yield the desired product.

Scheme 2.1 Synthesis of N³-alkylated thymidine derivatives.

Subsequent reaction of the bromo-functionalized thymidine derivative **a-b** with nucleophiles such as sodium azide yield the azide-functionalized derivative **c-d** in good yields.

However, the thiolation reaction of this derivative using hexamethyldisilathiane gives derivatives **e-f** in moderate yields only (Scheme 2.1).

2.3.2 Modification of Thymidine at the C⁵ Position

Modifications on the nucleobase at the C⁵ position were carried out using C-C coupling reactions on the commercially available 5-iodo-2'-deoxyuridine derivative. The Sonogashira coupling reaction yielded the alkyne functionalized derivatives **h-k** in moderate yields. (Scheme 2.2)

Scheme 2.2 Synthesis of 5-alkyl-2'-deoxyuridine derivatives by Sonogashira coupling.

Cleavage of the trimethylsilyl group from the coupling product **h** under basic conditions yields the 5'-ethynyl-2'-deoxyuridine derivative **i** which has been reported to show anti-proliferative activity against the MCF-7 cell line.¹⁴ Derivative **j** was further reacted to obtain the azide functionalized analogue (Scheme 2.3). However, attempts to obtain the thiol derivative **o** were largely unsuccessful. Prior conversion of derivative **j** to the bromide-functionalized derivative **n** also did not yield the desired product **o**. This may be due to the radical catalysed thiol-yne reaction which potentially yields a dimeric species.¹⁵ Attempts to avoid the thiol-yne reaction by shortening the reaction time and excluding light from the reaction were not successful as well (Scheme 2.3).

Scheme 2.3 Functionalization of the 5-alkyl-2'-deoxyuridine derivatives.

2.3.3 Modification of the Ribose Sugar

Modifications on the 2' site of the ribose ring may be introduced via the ring opening reaction of the 2,2'-anhydro-5-methyluridine derivative **p** in the presence of a borane complex.¹⁶ Such reactions have been reported for the modification at the 2' position with ethers and tertiary amines.¹⁷⁻¹⁹ However, attempted reactions of **p** with functionalized alcohols in the presence of borane were largely unsuccessful, and the majority of the starting material was recovered. The use of high pressure vessels as described in the literature was also not effective (Scheme 2.4).

Scheme 2.4 Functionalization of thymidine at the 2'-position.

2.4 Triosmium Carbonyl Cluster – Nucleoside Conjugates

Reaction of the functionalized nucleosides with triosmium carbonyl clusters were next examined.

2.4.1 Direct Ligand Displacement Reaction

Weakly coordinated ligands in suitable triosmium carbonyl derivatives such as clusters **2**, **3** and **5**, can be displaced by stronger ligands. The nucleoside derivative **g** reacted with cluster **3** but not with **1** or **2**, to afford the conjugate **10g**. This was only characterised via IR spectroscopy as it could not be isolated due to the weakly coordinating nature of the nitrile group. Nucleoside **d**, on the other hand, reacted readily with the unsaturated cluster **5** to yield the μ -azenido cluster **11d**. Thermolysis of this cluster yielded the μ_3 -NR cluster **13d**, driven by the evolution of nitrogen (Scheme 2.5).¹⁰

Scheme 2.5 Synthesis of triosmium carbonyl-nucleoside conjugates by direct ligand displacement.

2.4.2 Copper-Catalyzed Azide-Alkyne Cycloaddition Reaction

Triosmium carbonyl clusters for the click chemistry reaction need to be stable for use with biological molecules. The carboxylate bridged clusters **7L1** and **7L2** were prepared in moderate yields from the reaction of cluster **2** with propionic acid and 6-azidohexanoic acid, respectively. A higher-yielding alternative route was via the trimethylamine-N-oxide (TMNO) activation of $\text{Os}_3(\text{CO})_{12}$ (Scheme 2.6).

Scheme 2.6 Synthesis of azide and alkyne-functionalized clusters.

The synthesis of clusters with an azide-functionalized phosphine was not as straight forward. Functionalization of the free phosphine with an azide group was hindered by interference from the spontaneous Staudinger ligation reaction (Scheme 2.7). Thus installation of the azide group was carried out on a suitably functionalized phosphine derivative of the cluster.

Scheme 2.7 Functionalization of phosphines with azide groups.

Reaction between the phosphine **P1** and the “lightly-stabilised” clusters, **1** and **2**, readily afforded the derivatives **8P1** and **9P1** in good yields. The reaction of clusters **8P1** and **9P1** in a slight excess of sodium azide yielded clusters **8P2** and **9P2** in moderate yields (Scheme 2.8). Stoichiometric control of this reaction is important as **8P1** and **9P1** are susceptible to attack by strong nucleophiles such as the azide anion.

Scheme 2.8 Synthesis of azide functionalized triosmium carbonyl bis phosphine clusters.

As mentioned above, the conjugation of thymine derivatives to triosmium carbonyl clusters via the CuAAC reaction has previously been carried out successfully using copper iodide and 2,6-lutidine.² A similar attempt here yielded the 1,4-substituted tetrazole conjugates **7c**, **7l** and **8l** in moderate to low yields (Scheme 2.9). Attempts to increase the yield by increasing the amount of catalyst used led to decomposition of the triosmium carbonyl cluster. The conjugation reaction was also carried out on the disubstituted cluster **9P2**; since this cluster has two sites for the click reaction to occur, two conjugated products, **9l-1** and **9l-2**, were obtained.

Scheme 2.9 Click chemistry reactions of osmium carbonyl clusters.

Since the click chemistry reaction is commonly used in biological systems, the conjugation of triosmium carbonyl clusters to nucleosides was also tested under aqueous conditions. However, due to their limited solubility in aqueous systems, the clusters were first dissolved in water-miscible solvents before being added into the reaction mixture. Copper(II) sulfate was used in conjunction with sodium ascorbate to generate the catalytic copper(I) species in situ. The reaction between cluster **7L2** and nucleoside **I** was studied as the model reaction.

Table 2.1 Yields of conjugate **7I** using different solvents for the click chemistry reaction.

Solvent	Catalyst	Additive	Yield
DMSO	CuSO ₄ ·5H ₂ O	Sodium ascorbate	-
THF	CuSO ₄ ·5H ₂ O	Sodium ascorbate	4.23 %
ACN	CuSO ₄ ·5H ₂ O	Sodium ascorbate	-
DMF	CuSO ₄ ·5H ₂ O	Sodium ascorbate	12.3%
DMF	CuSO ₄ ·5H ₂ O, NBu ₄ I (phase transfer)	Sodium ascorbate	2.44%

Cluster **7L2** shows limited solubility in aqueous systems, precipitating from solution upon addition into the reaction mixture. This leads to rather low yields of the conjugate **7I**, the highest being obtained with DMF as the solvent for cluster **7L2**. An attempt with a phase transfer reagent led to cluster decomposition.

The click chemistry reaction was also extended to hexaosmium carbonyl raft clusters. These were first derivatised with a phosphine ligand carrying a bromide functionality (Scheme 2.10). Unlike the triosmium derivatives, however, nucleophilic substitution using stoichiometric amounts of sodium azide was not successful. As such, the azide functional group

was introduced using an ester coupling reaction instead, this yielded the azide-functionalized cluster **15P5**.

Scheme 2.10 Functionalization of hexaosmium raft clusters.

The reaction of the functionalized cluster **15P5** with nucleoside **I** using click chemistry conditions yielded the hexaosmium conjugate **15I** in moderate yields, thus demonstrating the utility of the click chemistry reaction for the conjugation of osmium carbonyl clusters to nucleosides (Scheme 2.11).

Scheme 2.11 Conjugation of hexaosmium carbonyl cluster **15P6** by the click chemistry reaction

2.5 Conclusion

In this chapter, the conjugation of triosmium carbonyl clusters to nucleosides was explored. Functionalization of the nucleosides was necessary for conjugation and this was

carried out mainly at the N³ and C⁵ position of thymidine. A number of triosmium carbonyl – thymidine conjugates were prepared via the direct ligand displacement and click chemistry reaction. The click chemistry reaction proved to be a particularly useful conjugation method, although yields were moderate at best. The potential applications of these conjugates are studied in the next chapter.

2.6 Experimental

Syntheses were carried out using analytical grade, anhydrous solvents and standard Schlenk techniques under an inert argon atmosphere, unless otherwise stated. DCM was dried over calcium hydride while THF was dried using sodium with benzophenone as an indicator. All TLC separations were carried out using Merck 60 F₂₅₄ 20 x 20 cm silica gel TLC plates. Compounds **1**,²⁰ **2**,²⁰ **3**,²¹ **5**,²² **14**,²³ **P3**,²⁴ 5'-O-(p,p-dimethoxytrityl)-2'-deoxythymidine (DMT-thymidine)²⁵ were synthesized according to literature methods.

¹H NMR spectra were recorded on a Bruker AV 300 or BBFO 400 NMR spectrometer. Chemical shifts were referenced against the residual ¹H resonance of the deuterated solvent. Solution IR spectra were obtained using a Bruker Alpha FTIR spectrometer at a resolution of 2 cm⁻¹ using a solution IR cell with NaCl windows and a pathlength of 0.1 mm unless stated. Mass spectrometric data was obtained using methanol as solvent, on a Waters Q-ToF Premier Mass spectrometer or a Thermo Finnigan LCQ Fleet Mass Spectrometer.

2.6.1 Experimental Procedure

2.6.1.1 N-alkylation of Thymidine (a-b, g)

The bromoalkane was added to a solution of the thymidine derivative and NaH (3 eq.) in DMF (5 mL). The reaction mixture was left to stir at room temperature for 16 h. The solvent was removed *in vacuo* and the residue resuspended in water (20 mL). The aqueous layer was extracted with ethyl acetate (3 x 15 mL). The organic extracts were combined, dried over MgSO₄, and the solvent removed *in vacuo*. The residue was purified by column chromatography using DCM : Methanol as an eluant.

Table 2.2 N-alkylation of thymidine.

	Thymidine (mmol)	R-Br (mmol)	Yield (%)
a , R = (CH ₂) ₃ Br	0.826	1.656	51.3%
b , R = (CH ₂) ₃ Br	0.110	0.275	61.6 %
g , R = CH ₂ CN	0.147	0.370	99.3%

2.6.1.2 Synthesis of Azide Derivatives (c-d)

Sodium azide and the thymidine-N³-bromopropene (**a** or **b**) derivative were dissolved in anhydrous DMF (5 mL) and degassed via three cycles of freeze-pump-thaw. The mixture was heated at 80°C for 16 hours. The solvent was removed in vacuo, resuspended in water (20 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were washed with saturated sodium chloride (20 mL) and dried over MgSO₄. The residue was purified by column chromatography using DCM : Methanol as an eluant.

Table 2.3 Substitution with sodium azide.

	N ³ -bromopropene derivative (mmol)	NaN ₃ (mmol)	Yield (%)
c	0.172	0.461	64.5
d	0.077	0.153	95.3

2.6.1.3 Synthesis of Thiol Derivatives (e-f)

Hexamethyldithiasilane and tert-butyl ammonium fluoride (2 eq.) were added to a solution of thymidine-N³-bromopropene (**a** or **b**) in THF (2 mL) in a Wheaton vial at 0°C. The reaction mixture was degassed with argon and warmed to room temperature. The reaction was stirred at room temperature for 2 hours. The solution was removed in vacuo and the residue dissolved in ethyl acetate (20 mL) and washed with saturated ammonium chloride (2 x 15 mL). The organic extracts were dried over MgSO₄ and the solvent removed in vacuo to yield a pungent pale yellow oil. The crude product was purified by column chromatography using DCM : MeOH as an eluant.

Table 2.4 Preparation of thiol-functionalized thymidine derivatives.

	N ³ -bromopropene derivative (mmol)	Hexamethyldithiasilane (mmol)	Yield (%)
e	0.318	0.382	79.6
f	0.089	0.135	86.5

2.6.1.4 Sonogashira Coupling (h-k)

To a solution of the 5'-iodo-2'-deoxyuridine derivative, Pd(PPh₃)₄ (0.05 eq.) and CuI (0.1 eq.) in DMF (8 mL) in a Carius tube was added the alkyne and triethylamine (2.5 eq.). The solution was degassed by 3 cycles of freeze-pump-thaw and heated at 60°C for 16 h. The solvent was removed in vacuo and the residue purified via column chromatography using a gradient elution of DCM : MeOH.

Table 2.5 Sonogashira coupling with 5-iodo-2'-deoxyuridine.

	5-iodo-2'- deoxyuridine (mmol)	R $\equiv$ (mmol)	Yield (%)
h , R = SiMe ₃	1.41	2.12	86.0
i , R = SiMe ₃	0.23	0.46	89.1
j , R = (CH ₂) ₂ OTs	0.31	0.61	93.1
k , R = (CH ₂) ₅ COOH	0.15	0.31	40.8

2.6.1.5 Synthesis of 5-ethynyl-2'-deoxyuridine (l)

Compound **h** (40 mg, 0.123 mmol) was taken up in a suspension of K₂CO₃ (25.6 mg, 0.185 mmol) in methanol (5 mL) and stirred at room temperature for 2 h. The solvent was removed in vacuo and the residue purified by column chromatography using DCM : MeOH (9:1, v/v). Yield : 38.7 % (12.1 mg).

2.6.1.6 Substitution of 5'-O-DMT-5-butynyl tosylate -2'-deoxyuridine (m-n)

The sodium salt was added to a solution of derivative **j** in DMF (7 mL) in a Carius tube. The solution was degassed by 3 cycles of freeze-pump-thaw and heated at 60 °C for 16 h. The solvent was removed in vacuo and the residue partitioned between water (20 mL) and DCM (3 x 15 mL). The organic extracts were combined, dried over MgSO₄ and the solvent removed in vacuo. The residue was purified by column chromatography using a gradient eluant of DCM : MeOH.

Table 2.6 Substitution of 5'-O-DMT-5-butynyl tosylate -2'-deoxyuridine.

	Derivative j (mmol)	NaX (mmol)	Yield (%)
m , X = N ₃	0.13	0.37	75.5
n , X = Br	0.27	0.67	81.0

2.6.1.7 Synthesis of 5-methylanhydrouridine (**p**)

Diphenyl carbonate (124 mg, 0.58 mmol) was added to a solution of 5-methyluridine (100 mg, 0.387 mmol) and NaHCO_3 (325 mg, 3.87 mmol) in DMF (8 mL) and the mixture was left to reflux under an inert atmosphere for 5 h. The solution was cooled to room temperature, poured into cold water (20 mL) and extracted with ethyl acetate (3 x 15 mL). The organic extracts were combined, dried over MgSO_4 , filtered and the solvent removed in vacuo. The residue was purified by column chromatography using a gradient elution of DCM : MeOH (100 % to 92 % DCM, v/v). Yield : 16.5% (51.3 mg).

2.6.1.8 Synthesis of $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-S}(\text{CH}_2)_3\text{-thymidine})$ (**6e**)

Cluster **2** (150 mg, 0.161 mmol) and nucleoside **e** (60 mg, 0.193 mmol) were dissolved in dry THF (7 mL) and stirred at room temperature for 16 hours. The solvent was removed in vacuo and the crude reaction mixture was purified by thin layer chromatography using an eluant of DCM : ethyl acetate (9:1, v/v). Two yellow bands were obtained, of which the second band was identified as the product. Yield : 46.4% (87.0 mg).

2.6.1.9 Synthesis of $\text{Os}_3(\text{CO})_{11}(5\text{-CH}_2\text{CN-5'-O-DMT-2'-deoxyuridine})$ (**10g**)

A solution of compound **g** (8.31 mg, 0.014 mmol) in DCM (1 mL) was added to a solution of $\text{Os}_3(\text{CO})_{11}(\text{COE})$ (**3**) in DCM (3 mL). The solution was stirred at room temperature for 20 minutes and monitored by IR spectroscopy. The solvent was removed in vacuo. No further purification was carried out. Yield : Not calculated (Product not purified).

2.6.1.10 Synthesis of $\text{Os}_3(\mu\text{-H})(\text{CO})_{10}(\mu\text{-N}_3\text{H}(\text{CH}_2)_3\text{-Thymidine-5'-O-DMT})$ (**11d**)

Compound **d** (18 mg, 0.029 mmol) was added to a solution of cluster **5** (20 mg, 0.025 mmol) in toluene (5 mL) and left to stir under an inert atmosphere for 17 hours. The solvent was removed in vacuo and the product was used as is without purification. Crude yield : 141.0% (50.5 mg).

2.6.1.11 Synthesis of $\text{Os}_3(\mu\text{-H})_2(\text{CO})_9(\mu\text{-N}(\text{CH}_2)_3\text{-Thymidine-5'-O-DMT})$ (**13d**)

Compound **11d** (50.5 mg, 0.034 mmol) was dissolved in toluene (5 mL) in a Carius tube and degassed via three cycles of freeze-pump-thaw. The mixture was heated at 100°C for 24 hours. The solvent was removed in vacuo and the residue taken up in DCM. The crude product was purified by thin layer chromatography using DCM : ethyl acetate (85: 15, v/v) as an eluent. Two yellow bands were obtained, of which the first was identified as the product. Yield : 8.97 % (1.7 mg).

2.6.1.12 Synthesis of Carboxylate Bridged Clusters (**7L1** – **7L2**)

Route A

The carboxylic acid was added to a solution of cluster **2** in THF (5 mL). The mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue was purified by TLC using DCM : Hexane as the eluant. Three yellow bands were obtained, of which the second was recovered and identified as the product.

Table 2.7 Synthesis of carboxylate-bridged triosmium carbonyl clusters at room temperature.

	Cluster 2 (mmol)	R-COOH (mmol)	Yield (%)
7L1 , R = -C≡CH	0.032	0.162	25.0
7L2 , R = -(CH ₂) ₅ N ₃	0.128	0.384	27.3

Route B

A solution of TMNO (2.2 eq.) in ACN (10 mL) was added to a solution of Os₃(CO)₁₂ in ACN (40 mL) and the mixture was refluxed for 4 hours with monitoring by IR spectroscopy. To this mixture was then added the carboxylic acid and the mixture was refluxed for an additional 2 hours. The solvent was removed in vacuo and the residue redissolved in minimal amounts of DCM for purification by TLC using DCM : Hexane as an eluant. The second yellow band was recovered and identified as the product.

Table 2.8 Synthesis of carboxylate-bridged triosmium carbonyl clusters using reflux.

	Os ₃ (CO) ₁₂ (mmol)	R-COOH (mmol)	Yield (%)
7L1 , R = -C≡CH	0.066	0.13	26.3
7L2 , R = -(CH ₂) ₅ N ₃	0.13	0.95	34.0

2.6.1.13 Synthesis of 3-(diphenylphosphino)-(CH₂)₂COO(CH₂)₃Br (**P1**)

To a solution of 3-(diphenylphosphino)-propionic acid (**P6**) (100 mg, 0.387 mmol) and 4-(dimethylamino)pyridine (2.5 mg, 0.02 mmol) in DCM (5 mL) was added 3-bromopropanol (107.6 mg, 0.774 mmol) and 1,3-dicyclohexylcarbodiimide (87.9 mg, 0.426 mmol). The mixture was stirred at room temperature for 16 h. The crude mixture was passed through a short column of silica using an eluant of DCM. The solvent was removed in vacuo and the collected fraction was used as is. Yield : 97.4 % (143 mg).

2.6.1.14 Synthesis of $Os_3(CO)_{12-n}(PPh_2CH_2CH_2COOCH_2CH_2CH_2Br)_n$ (**8** – **9P1**)

To a solution of cluster $Os_3(CO)_{12-n}(NCCH_3)_n$ in THF (5 mL) was added phosphine **P1** and the reaction was stirred at room temperature for 2 hours. The solution was concentrated and the residue purified by column chromatography using an eluant of DCM : Hex. The major yellow band (**8P1**) or orange band (**9P1**) was recovered using DCM and identified as the product.

Table 2.9 Synthesis of substituted phosphine clusters.

	$Os_3(CO)_{12-n}(ACN)_n$ (mmol)	Phosphine P1 (mmol)	Yield (%)
8P1 , n = 1	0.054	0.065	57.8
9P1 , n = 2	0.072	0.16	67.3

2.6.1.15 Synthesis of $Os_3(CO)_{12-n}(PPh_2CH_2CH_2COOCH_2CH_2CH_2N_3)_n$ (**8** – **9P2**)

Sodium azide was added to a solution of **8P1** or **9P1** in DMF (7 mL) and the reaction was stirred at room temperature for 4 hours with monitoring by TLC. The solvent was removed in vacuo and the residue dissolved in minimal amounts of DCM. Purification was carried out using TLC using DCM : Hex as an eluant. The second yellow band was recovered using DCM and identified as the product.

Table 2.10 Synthesis of azide-functionalized substituted phosphine clusters.

	8P1 or 9P1 (mmol)	NaN_3 (mmol)	Yield (%)
8P2 , n = 1	0.04	0.038	20.4
9P2 , n = 2	0.032	0.059	10.8

2.6.1.16 Click Chemistry Reaction

To a solution of the functionalized cluster and nucleoside in DMF (5 mL) was added CuI (0.02 eq.) and 2,6-lutidine (0.05 eq.) and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue purified by TLC using an eluant of DCM : Methanol (95 : 5, v/v).

Table 2.11 Synthesis of cluster-nucleoside conjugates by the click chemistry reaction.

	Cluster (mmol)	Nucleoside (mmol)	Yield (%)
7c	0.037	0.046	39.0
7l	0.025	0.029	11.1
8l	0.020	0.021	8.5
9l – 1			13.0
9l – 2	0.009	0.02	31.1
15l	0.012	0.017	9.6

2.6.1.17 Synthesis of $Os_6(CO)_{20}(PPh_2CH_2CH_2CH_2X)$ (**15P3-4**)

To a solution of cluster **14** in THF (5 mL) was added the phosphine (**P3** or **P4**) and the mixture was stirred at room temperature for 2 hours. The solvent was concentrated and the residue purified by column chromatography using an eluant of DCM : Hexane (1:4, v/v). The major dark blue band was recovered and identified as the product.

Table 2.12 Synthesis of phosphine-substituted hexaosmium carbonyl clusters.

	Cluster 14 (mmol)	Phosphine (mmol)	Yield (%)
15P3 , X = Br	0.057	0.125	29.7
15P4 , X = OH	0.046	0.125	22.2

2.6.1.18 Synthesis of $Os_6(CO)_{20}[PPh_2(CH_2)_3OOC(CH_2)_5N_3]$ (**15P5**)

To a solution of cluster **15P4** (20 mg, 0.0103 mmol) in DCM (5 mL) was added 6-azidohexanoic acid (2.4 mg, 0.0154 mmol), 1,3-dicyclohexylcarbodiimide (2.5 mg, 0.0124 mmol) and 4-dimethylaminopyridine (1 mg, 0.0793 mmol) and the mixture was stirred at room temperature for 2 hours. The solvent was removed in vacuo and the residue purified by column chromatography using and eluant of DCM : Hexane (2:3, v/v). The first blue band was collected and identified as the product. Yield = 51.3% (11.0 mg).

2.6.2 Experimental Data

2.6.2.1 Nucleoside Derivatives

3-(3-bromopropyl)-2'-deoxythymidine
X = Br, R = H (**a**)

^1H NMR (CDCl_3) δ / ppm:
1.91 (s, 3H, H7), 2.13 – 2.22 (m, 2H, H9), 2.30 – 2.33 (m, 2H, H2') 3.40 (t, 2H, H8, J = 6.8 Hz), 3.79 – 3.94 (m, 2H, H5'), 3.98 – 3.98 (m, 1H, H3'), 4.04 (t, 2H, H10, J = 6.8 Hz), 4.52(br s, 1H, H4'), 6.20 (t, 1H, H1', J_H = 6.8 Hz), 7.52 (s, 1H, H6).

^{13}C NMR (CDCl_3) δ / ppm :
13.1 (C7), 30.4, 30.7, 40.1 (C9, C10, C8) 61.9 (C2'), 70.9 (C4'), 86.9, 87.1 (C3', C5'), 109.9, 134.9 (C5, C6), 150.8, 164.1 (C4, C2).

3-(3-bromopropyl)-5'-(4,4'-dimethoxytrityl)-2'-deoxythymidine
X = Br, R = DMT (**b**)

^1H NMR (CD_3OD) δ / ppm :
1.44(s, 3H, H7), 2.10 – 2.19 (quin, 2H, H9, J = 6.7 Hz), 2.34 – 2.37 (m, 2H, H2'), 3.35 – 3.40 (m, 2H, H5'), 3.42 – 3.46 (t, 2H, C8, J = 6.6 Hz), 3.76 (s, 6H, DMT) 4.02 – 4.06 (m, 3H, H10, H4'), 4.52 – 4.55 (m, 1H, H3'), 6.31 – 6.35 (t, 1H, H1', J = 6.45 Hz), 6.84 – 6.87 (m, 4H, DMT), 7.20 – 7.32 (m, 7H, DMT), 7.41 – 7.44 (m, 2H, DMT), 7.72 (s, 1H, C6).

3-(3-azidopropyl)-2'- deoxythymidine X = N ₃ , R = H (c)	¹ H NMR (CD ₃ OD) δ/ ppm: 1.83 – 1.91 (m, 5H, H7, H9), 2.16 – 2.33 (m, 2H, H2'), 3.37 (t, 2H, H8, J = 6.8 Hz), 3.72 – 3.83 (m, 2H, H5'), 3.90 – 3.93 (m, 1H, H3'), 4.02 (t, 2H, H10, J = 6.9 Hz), 4.38 – 4.42 (m, 1H, H4'), 6.30 (t, 1H, H1, J = 6.6 Hz), 7.84 (d, 1H, H6, J = 1.2 Hz). ¹³ C NMR (CD ₃ OD) δ/ ppm : 11.8 (C7), 26.7, 38.7, 39.9 (C8, C9, C10), 49.1 (C1'), 61.3 (C2'), 70.7 (C4'), 85.8, 87.5 (C3', C5'), 109.3, 135.2 (C5, C6), 150.9 (C4'), 164.0 (C2').
3-(3-azidopropyl)-5'-(4,4'- dimethoxytrityl) - 2' – deoxythymidine X = N ₃ , R = DMT (d)	¹ H NMR (CD ₃ OD) δ/ ppm : 1.44 (s, 3H, H7), 1.80 – 1.89 (m, 2H, H9), 2.33 – 2.37 (m, 2H, H2'), 3.32 – 3.44 (m, 4H, H5', H8), 3.75 [s, 6H, -OCH ₃ (DMT)] 3.97 – 4.02 (m, 3H, H10, H4'), 4.50 – 4.57 (m, 1H, H3'), 6.34 (t, 1H, H1', J = 6.6 Hz), 6.82 – 6.85 (m, 4H, DMT), 7.20 – 7.32 (m, 7H, DMT), 7.41 – 7.44 (m, 2H, DMT), 7.72 (s, 1H, H6). IR (CH ₂ Cl ₂) ν/cm ⁻¹ : 2100 (-N ₃ stretch). ESI-MS <i>m/z</i> : 606.09 [M+Na] ⁺ .

3-(3-propylthiol)-2' – deoxythymidine X = SH, R = H (e)	¹ H NMR (CD ₃ OD) δ/ ppm: 1.91 (s, 3H, H7), 2.22 – 2.29 (m, 4H, H2', H9), 3.75 – 3.83 (m, 2H, H8), 3.91 – 3.92 (m, 1H, H3'), 4.02 – 4.05 (m, 2H, H10), 4.41- 4.38 (m, 1H, H4'), 6.30 (t, 1H, H1', J = 6.6 Hz), 7.89 (s, 1H, H6). ¹³ C NMR (CD ₃ OD) δ/ ppm: 13.3 (C7), 30.9, 37.3, 41.4 (C8, C9, C10), 62.7 (C2'), 72.0 (C4'), 87.1, 88.8 (C3', C5'), 110.7, 136.5 (C5, C6), 152.3, 165.4 (C=O).
3-(3-propylthiol)-5'-(4,4'- dimethoxytrityl) - 2' – deoxythymidine X = SH, R = DMT (f)	¹ H NMR (C ₆ D ₆) δ/ ppm : 1.43 (t, 1H, -SH, J = 10.8 Hz), 1.64 (s, 3H, H7), 1.83 (quin, 2H, H9, J = 5.3 Hz), 2.03 – 2.07 (m, 2H, H2'), 2.18 (q, 2H, H10, J = 10 Hz), 3.29 – 3.30 [m, 7H, H5', -OCH ₃ (DMT)], 3.44 – 3.49 (m, 1H, H5'), 3.86 – 3.87 (m, 1H, H3'), 4.01 (t, 2H, H8, J = 5 Hz) 4.15 – 4.16 (m, 1H, H3'), 6.60 (t, 1H, H1', J = 4.8 Hz), 6.75 – 6.78 (m, 4H, DMT), 7.03 – 7.08 (m, 2H, DMT), 7.36-7.55 (m, 8H, DMT, H6).
3-acetonitrile-5'-O-DMT-2'- deoxythymidine R = DMT (g)	¹ H NMR (CDCl ₃) δ/ppm : 1.44 (s, 3H, H7), 2.33 – 2.39 (m, 2H, H2'), 2.45 – 2.50 (m, 1H, H2'), 3.38 – 3.51 (m, 2H, H5'), 3.79 [s, 6H, -OCH ₃ (DMT)], 4.11 – 4.12 (m, 1H, H3'), 4.62 – 4.64 (m, 1H, H4'), 4.77 – 4.86 (m, 2H, H8), 6.43 – 6.46 (m, 1H, H1'), 6.84 – 6.86 (m, 4H, DMT), 7.22 – 7.42 (m, 7H, DMT), 7.42 – 7.74 (m, 2H, DMT), 7.74 (s, 1H, H6). ESI-MS <i>m/z</i> : 606.09 [M+Na] ⁺ .

5-trimethylsilylacetylene – 2'- deoxyuridine X = SiMe ₃ , R = H (h)	¹ H NMR (CDCl ₃) δ/ppm : 0.02 (s, 9H, SiMe ₃), 2.32 – 2.43 (m, 1H, H2'), 2.45 – 2.50 (m, 1H, H2'), 3.85 – 3.97 (m, 2H, H5'), 4.03 – 4.06 (s, 1H, H3'), 4.57 – 4.60 (m, 1H, H4'), 6.17 (t, 1H, H1', J = 6.4 Hz), 7.94 (s, 1H, H6). ESI-HRMS m/z : 275.06 [M+Na] ⁺
5-trimethylsilylacetylene – 5'- O - (4,4 – dimethoxytrityl) 2'- deoxyuridine X = SiMe ₃ , R = DMT (i)	¹ H NMR (C ₆ D ₆) δ/ppm : 0.00 (s, 9H, SiMe ₃), 2.16 – 2.23 (m, 1H, H2'), 2.50 – 2.55 (m, 1H, H2'), 3.27 – 3.41 (m, 2H, H5'), 3.76 [s, 6H, -OCH ₃ (DMT)] 4.12 – 4.13 (m, 1H, H3'), 4.45 – 4.46 (m, 1H, H4'), 6.28 – 6.31 (m, 1H, H1'), 6.83 – 6.85 (m, 4H, DMT), 7.17 – 7.47 (m, 7H, DMT), 7.60 – 7.61 (m, 2H, DMT), 8.04 (s, 1H, H6).
5-ethynyl-2'- deoxyuridine X = H, R = H (l)	¹ H NMR (D ₂ O) δ/ppm : 2.36 – 2.53 (m, 2H, H2'), 3.69, (s, 1H, C≡CH), 3.81 – 3.93 (m, 2H, H5'), 4.09 – 4.12 (s, 1H, H3'), 4.50 – 4.54 (m, 1H, H4'), 6.29 (t, 1H, H1', J = 6.8 Hz), 8.34 (s, 1H, H6). ¹³ C NMR [(CD ₃) ₂ C=O] δ/ ppm: 39.8 (C2'), 61.4 (C5'), 75.0, 83.5 (C3', C4'), 83.5, 86.3, 87.4 (C1', C10, C11), 98.8 (C7), 145.8 (C6), 151.0 (C4, C5).

5-hexynoic acid -5'-O-DMT-2'-
deoxyuridine (k)

^1H NMR (C_6D_6) δ/ppm :

1.62 – 1.69 (m, 2H, H₉), 2.14 – 2.27 (m, 6H, H₂', H₁₀, H₁₁), 3.74 [s, 6H, -OCH₃ (DMT)], 4.07 – 4.08 (m, 1H, H₃'), 4.47 (br s, 1H, H₄'), 6.26 (t, 1H, H₁', J = 6.6 Hz), 6.79 – 6.82 (m, 4H, DMT), 7.14 – 7.19 (m, 1H, DMT), 7.23 – 7.33 (m, 6H, H₁₀, H₁₁), 7.40 – 7.43 (m, 2H, H₁₂), 7.88 (s, 1H, H₆).

ESI-MS m/z : 663.05 [$\text{M}+\text{Na}$]⁺, 1302 [$2\text{M}+\text{Na}$]⁺, 639 [$\text{M}-\text{H}$]⁻, 1279 [$2\text{M}-\text{H}$]⁻.

5-butynyl tosylate-5'-O-DMT-2'-
deoxyuridine

^1H NMR (CDCl_3) δ/ppm :

2.27 – 2.32 (m, 2H, H₂'), 2.35 – 2.41 (m, 2H, H₉), 2.42 (s, 3H, -OTs), 3.31 – 3.71 (m, 2H, H₅'), 3.69 – 3.75 (m, 2H, H₁₀), 3.77 [s, 6H, -OCH₃ (DMT)], 4.09 – 4.10 (m, 1H, H₃'), 4.56 – 4.57 (m, 1H, H₄'), 6.30 – 6.33 (m, 1H, H₁'), 6.80 – 6.83 (m, 4H, DMT), 7.16 – 7.19 (m, 1H, DMT), 7.23 – 7.30 (m, 8H, DMT), 7.31 – 7.40 (m, 2H, -OTs), 7.69 – 7.71 (m, 2H, -OTs), 8.09 (s, 1H, H₆).

ESI-MS m/z : 775.03 [$\text{M}+\text{Na}$]⁺.

5-azidobutynyl -5'-O-DMT-2'-
deoxyuridine

X = N₃ (**m**)

¹H NMR (C₆D₆) δ/ppm :

2.08 – 2.17 (m, 4H, H2', H9), 2.49 – 2.51 (m, 1H, H2), 2.65 (t, 2H, H10, J = 7.4 Hz), 3.29 – 3.33 (m, 1H, H5'), 3.39 [s, 6H, -OCH₃, (DMT)], 3.47 – 3.52 (m, 1H, H5'), 4.20 – 4.21 (m, 1H, H3'), 4.42 (br s, 1H, H4'), 6.45 (t, 1H, H1'), 6.84 – 6.86 (m, 4H, DMT), 7.08 – 7.12 (m, 1H, DMT), 7.23 – 7.27 (m, 2H, DMT), 7.49 – 7.60 (m, 4H, DMT), 7.60 – 7.62 (m, 2H, DMT), 8.17 (s, 1H, H6).

5-bromobutynyl -5'-O-DMT-2'-
deoxyuridine

X = Br (**n**)

¹H NMR (C₆D₆) δ/ppm :

2.19 – 2.21 (m, 1H, H2'), 2.42 – 2.46 (m, 2H, H9), 2.56 – 2.58 (m, 1H, H2'), 2.83 – 2.87 (m, 2H, H10), 3.39 [s, 6H, -OCH₃ (DMT)], 3.42 – 3.47 (m, 2H, H5'), 4.29 (br s, 1H, H3'), 4.57 (br s, 1H, H4'), 6.47 – 6.50 (m, 1H, H1'), 6.84 – 6.86 (m, 4H, DMT), 7.08 – 7.10 (m, 1H, DMT), 7.33 – 7.35 (m, 2H, DMT), 7.48 – 7.50 (m, 4H, DMT), 7.61 – 7.64 (m, 2H, DMT), 8.20 (s, 1H, H6), 10.59 (br s, -NH).

5-Methyl-1,2-anhydrouridine (**p**)

¹H NMR (CD₃OD) δ/ppm :

1.86 (s, 3H, H7), 3.78 – 3.95 (m, 2H, H5'), 4.11 – 4.12 (m, 1H, H4'), 4.48 – 4.52 (m, 1H, H2'), 6.15 (d, 1H, H1', J = 4.8 Hz), 7.89 (s, 1H, H6).

2.6.2.2 Phosphine Ligands and Triosmium Carbonyl Clusters

$\text{PPh}_2[(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$ (**P1**)

^1H NMR (CDCl_3) δ/ppm :

2.15 (quin, 2H, H4, $J = 6.3$ Hz), 2.34 – 2.46 (m, 4H, H1, H2), 3.44 (t, 2H, H5, $J = 6.4$ Hz), 4.20 (t, 2H, H3, $J = 6$ Hz), 7.32 – 7.52 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ/ppm : -15.1.

$\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-COOC}\equiv\text{CH})$ (**7L1**)

^1H NMR (CDCl_3) δ/ppm :

-10.44 (s, 1H, $\mu\text{-H}$), 2.81 (s, 1H, $\text{C}\equiv\text{CH}$).

IR (CH_2Cl_2) ν/cm^{-1} : 2115 (w), 2078 (s), 2066 (s), 2028 (s), 2017 (sh), 1985 (w).

$\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-COO}(\text{CH}_2)_5\text{N}_3]$ (**7L2**)

^1H NMR (CDCl_3) δ/ppm :

-10.5 (s, 1H, $\mu\text{-H}$), 1.24 – 1.27 (m, 2H, H4), 1.41 (quin, 2H, H5, $J = 7.6$ Hz), 1.50 – 1.57 (m, 2H, H3), 2.22 (t, 2H, H6, $J = 7.4$ Hz), 3.24 (t, 2H, H1, $J = 6.8$ Hz).

^{13}C NMR (CDCl_3) δ/ppm :

24.8 (C3), 26.2 (C4), 28.4 (C2), 36.9 (C5), 51.2 (C6), 174.1, 174.2, 175.9, 181.6, 182.9 ($-\text{CO}$), 191.5 (C1).

$\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$

X = Br (**8P1**)

^1H NMR (CDCl_3) δ/ppm :

2.12 (quin, 2H, H4, $J = 6.3$ Hz), 2.20 – 2.26 (m, 2H, H1), 2.95 – 3.0 (m, 2H, H2), 3.39 (t, 2H, H5, $J = 6.6$ Hz), 4.15 (t, 2H, H3, $J = 6.0$ Hz), 7.43 – 7.50 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ/ppm : -9.8.

IR (CH_2Cl_2) ν/cm^{-1} : 2107 (m), 2054 (s), 2032 (sh), 2018 (vs), 1987 (m), 1977 (sh).

$\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{N}_3]$

X = N_3 (**8P2**)

^1H NMR (CDCl_3) δ/ppm :

1.85 (quin, 2H, H4, $J = 8.5$ Hz), 2.17 – 2.27 (m, 2H, H1), 2.93 – 3.01 (m, 2H, H2), 3.33 (t, 2H, H5, $J = 6.6$ Hz), 4.10 (t, 2H, H3, $J = 6.0$ Hz), 7.47 – 7.61 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ/ppm : -9.2.

IR (CH_2Cl_2) ν/cm^{-1} : 2107 (m), 2054 (s), 2031 (sh), 2018 (vs), 1981 (m), 1977 (sh).

$\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{Br})_2$ ^1H NMR (CDCl_3) δ/ppm :

X = Br (**9P1**)

2.11 (quin, 2H, H4, J = 6.2 Hz), 2.21 (br s, 2H, H1), 2.95 (br s, 2H, H2), 3.38 (t, 2H, H5, J = 6.6 Hz), 4.13 (t, 2H, H3, J = 6 Hz), 7.44 – 7.46 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ/ppm : -14.9, -11.5, -10.2.

$\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)_2$ ^1H NMR (CDCl_3) δ/ppm :

X = N_3 (**9P2**)

1.84 (quin, 2H, H4, J = 6.4 Hz), 2.22 (br s, 2H, H1), 2.96 (br s, 2H, H2), 3.32 (t, 2H, H5, J = 6.6 Hz), 4.08 (t, 2H, H3, J = 6.2 Hz), 7.44 – 7.46 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR δ/ppm : -15.0, -11.5, -10.3.

ESI-MS m/z : 1533.81 $[\text{M}+\text{H}]^+$.

2.6.2.3 Hexaosmium Carbonyl Clusters

X = Br (**15P3**)

^1H NMR (CDCl_3) δ /ppm :

1.72 – 1.77 (m, 2H, H2), 2.75 – 2.81 (m, 2H, H1), 3.35 (t, 2H, H3), 7.43 – 7.55 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -9.6.

X = OH (**15P4**)

^1H NMR (CDCl_3) δ /ppm :

1.45 – 1.49 (m, 2H, H2), 2.68 – 2.72 (m, 2H, H1), 3.60 (br s, 2H, H3), 7.46 – 7.50 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -8.4.

$\text{Os}_6(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{OOC}(\text{CH}_2)_5\text{N}_3]$ (**15P5**)

^1H NMR (CDCl_3) δ /ppm :

1.33 – 1.56 (m, 4H, H2, H7), 1.60 – 1.67 (m, 6H, H1, H6, H8), 2.26 – 2.33 (m, 2H, H9), 2.62 – 2.68 (m, 2H, H9), 3.26 (t, 2H, H5, $J = 6.6$ Hz), 4.01 (t, 2H, H3, $J = 5.6$ Hz), 7.42 – 7.82 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -8.7.

2.6.2.4 Nucleoside-Cluster Conjugates

$\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-S}(\text{CH}_2)_3\text{thymidine}]$ (**6e**)

^1H NMR (CDCl_3) δ /ppm :

-17.46 (s, 1H, $\mu\text{-H}$), 1.91 – 2.02 (m, 5H, H7, H9), 2.28 – 2.49 (m, 4H, H2', H10), 3.82 – 3.95 (m, 2H, H5'), 4.00 – 4.05 (m, 3H, H3', H8), 4.61 (br s, 1H, H4'), 6.12 (t, 1H, H1', $J = 6.8$ Hz), 7.29 (s, 1H, H6).

IR (DCM) ν/cm^{-1} : 2108 (w), 2068 (s), 2058 (m), 2020 (s), 1999 (m), 1979 (w).

ESI-HRMS (MeOH) m/z : 1168.76 $[\text{M}+\text{H}]^+$.

$\text{Os}_3(\text{CO})_{11}[\text{NC}(\text{CH}_2)_3\text{thymidine}]$ (**10g**)

IR (DCM) ν/cm^{-1} : 2053 (s), 2041 (s), 2016 (sh), 2009 (s), 1984 (m).

$\text{Os}_3(\mu\text{-H})(\text{CO})_{10}(\mu\text{-N}_3\text{H}-(\text{CH}_2)_3\text{-Thymidine-5'-O-DMT})$
(**13d**)

^1H NMR (CDCl_3) δ/ppm :

-12.7 (d, 1H, $\mu\text{-H}$, $J = 0.8$ Hz), 1.59 (s, 3H, H7), 1.98 – 2.04 (m, 2H, H9), 2.28 – 2.41 (m, 2H, H2'), 3.35 – 3.49 (m, 2H, H5'), 3.783 [s, 6H, (DMT, $-\text{OCH}_3$)], 3.88 – 4.04 (m, 5H, H4', H8, H10), 4.55 (br s, 1H, H3'), 6.39 – 6.41 (m, 1H, H1', $J = 6.75$ Hz), 6.85 – 6.88 (m, 4H, DMT), 7.26 – 7.30 (m, 7H, DMT), 7.39–7.41 (m, 2H, DMT), 7.92 – 7.93 (m, 1H, H6).

^{13}C NMR (CDCl_3) δ/ppm :

13.2(C7), 30.9 (C4), 28.4 (C2), 36.9 (C5), 51.2 (C1), 174.1, 174.2, 175.9, 181.6, 182.9 ($-\text{CO}$), 191.5 (C1).

IR (toluene) ν/cm^{-1} : 2107 (w), 2065 (s), 2029 (s), 1996 (w), 1983 (w), 1971 (w).

$\text{Os}_3(\mu\text{-H})_2(\text{CO})_9(\mu\text{-N}(\text{CH}_2)_3\text{-Thymidine-5'-O-DMT})$
(13d)

^1H NMR (CD_3OD) δ /ppm :

-18.8 (s, 2H, $\mu\text{-H}$), 1.41 (s, 3H, H7), 2.07 – 2.12 (m, 2H, H9), 2.32 – 2.37 (m, 2H, H2'), 3.37 – 3.42 (m, 2H, H5'), 3.78 [s, 6H, $-\text{OCH}_3$ (DMT)], 4.01 – 4.13 (m, 4H, H4', H10), 4.51 – 4.60 (m, 1H, H3), 6.33 – 6.38 (t, 1H, H1', $J = 6.75$ Hz), 6.85 – 6.88 (m, 4H, DMT), 7.23 – 7.32 (m, 7H, DMT), 7.41 – 7.44 (m, 2H, DMT), 7.78 (s, 1H, H6).

IR (CH_2Cl_2) ν/cm^{-1} : 2113 (w), 2076(m), 2049 (s), 2031 (w), 1997(m), 1981 (w).

Conjugate of $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-COOC}\equiv\text{CH})$ and 3-(3-azidopropyl)-2'-deoxythymidine **(7c)**

^1H NMR [$(\text{CD}_3)_2\text{C=O}$] δ /ppm :

-10.0 (s, 1H, $\mu\text{-H}$), 2.22 – 2.25 (m, 4H, H2', H9), 2.80 (s, 3H, H7), 3.77 – 3.82 (m, 2H, H5'), 3.93 – 3.97 (m, 3H, H3', H10), 4.44 – 4.49 (m, 3H, H4', H8), 6.31 (t, 1H, H1', $J = 6.6$ Hz), 7.83 (d, 1H, H6, $J = 0.8$ Hz), 8.41 (s, 1H, H11).

^{13}C NMR [$(\text{CD}_3)_2\text{C=O}$] δ /ppm :

12.4 (C9), 38.1 (C7), 40.4 (C2'), 61.8 (C3'), 71.0 (C8), 85.7 (C4'), 87.8 (C5'), 108.9 (C1'), 128.4 (C6), 134.9 (C5), 140.9 (C12), 150.9 (C11), 163.1 (C4), 174.6, 174.7, 175.0, 176.6, 178.2 ($-\text{C}\equiv\text{O}$), 182.1 (C13), 182.7 (C2).

IR (MeOH) ν/cm^{-1} : 2114 (w), 2077(s), 2064 (s), 2026 (vs), 198 (w).

ESI-HRMS (MeOH) m/z : 1247.97 $[\text{M}+\text{H}]^+$.

Conjugate of $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-COO}(\text{CH}_2)_5\text{N}_3]$ and 5-ethynyl-2'-deoxyuridine (**7l**)

^1H NMR $[(\text{CD}_3)_2\text{C}=\text{O}]$ δ/ppm :

1.26 – 1.29 (m, 2H, H11), 1.49 (quin, 2H, H10, $J = 7.5$ Hz), 1.84 – 1.93 (m, 2H, H12), 2.28 – 2.37 (m, 4H, H2', H9), 3.75 – 3.86 (m, 2H, H5'), 4.01 – 4.03 (m, 1H, H3'), 4.32 (t, 2H, H13, $J = 7.2$ Hz), 6.39 (t, 1H, H1', $J = 6.8$ Hz), 8.23 (s, 1H, H6), 8.74 (s, 1H, H8).

IR (MeOH) ν (cm^{-1}) : 2113 (w), 2076 (vs), 2063 (s), 2025 (vs), 2017 (sh), 1981 (m).

ESI-HRMS (MeOH) m/z : 1261.98 $[\text{M}+\text{H}]^+$.

Conjugate of $\text{Os}_3(\text{CO})_{11}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)$ and 5-ethynyl-2'-deoxyuridine (**8l**)

^1H NMR (CDCl_3) δ/ppm :

2.17 – 2.28 (m, 4H H9, H12), 2.41 – 2.42 (m, 2H, H2'), 2.94 – 3.01 (m, 2H, H11), 3.84 – 4.07 (m, 5H, H2', H5', H10), 4.38 (t, 2H, H8, $J = 6.8$ Hz), 4.65 (br s, 1H, 4'), 6.33 (t, 1H, H1', $J = 6.6$ Hz), 7.45 – 7.50 (m, 10H, $\text{H}_{\text{aromatic}}$), 8.17 (s, 1H, H6), 8.72 (s, 2H, H8, -NH).

^{31}P NMR (CDCl_3) δ/ppm : -9.8.

IR (CH_2Cl_2) ν/cm^{-1} : 2107 (m), 2054 (s), 2032 (sh), 2018 (vs), 1987 (w), 1976 (w).

ESI-HRMS m/z : 1474.07 $[\text{M}+\text{H}]^+$.

Conjugate of

$\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)_2$ and 5-ethynyl-2'-deoxyuridine (**9I-1**)

^1H NMR (CDCl_3) δ /ppm :

1.83 (quin, 2H, H17, $J = 6$ Hz), 2.17 – 2.22 (m, 6H, H2', H13, H14), 2.41 – 2.43 (m, 2H, H18), 2.96 (br s, 4H, H12, H15), 3.32 (t, 2H, H16, $J = 6.6$ Hz), 3.84 – 4.09 (m, 5H, H3', H5', H16), 4.38 (t, 2H, H9, $J = 6.6$ Hz), 4.66 – 4.67 (m, 1H, H4'), 6.34 (t, 1H, H1', $J = 6.6$ Hz), 7.45 (br s, 20H, $\text{H}_{\text{aromatic}}$), 8.18 (s, 1H, H6), 8.63 (br s, 1H, -NH), 8.74 (s, 1H, H8).

^{31}P NMR (CDCl_3) δ /ppm : -14.8, -11.5, -10.1.

IR (MeOH) ν/cm^{-1} : 2083 (w), 2026 (m), 1999 (s), 1967 (m), 1954 (sh).

ESI-HRMS (MeOH) m/z : 1787.19 $[\text{M}+\text{H}]^+$.

Conjugate of

$\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)]$ and 5-ethynyl-2'-deoxyuridine (**9I-2**)

^1H NMR $[(\text{CD}_3)_2\text{C}=\text{O}]$ δ/ppm :

2.17 – 2.37 (m, 6H, H2', H10, H14), 3.10 – 3.12 (m, 2H, H13), 3.79 – 3.87 (m, 2H, H5'), 4.01 – 4.05 (m, 3H, H3', H11), 4.48 (t, 2H, H9, $J = 6.8$ Hz), 4.53 – 4.57 (m, 1H, H4'), 6.40 (t, 1H, H1', $J = 6.8$ Hz), 7.51 – 7.58 (m, 10H, H_{aromatic}), 8.24 (s, 1H, H6), 8.74 (s, 1H, H7).

^{31}P NMR (CDCl_3) δ/ppm : -14.1, -11.3, -9.7.

IR (MeOH) ν/cm^{-1} : 2083 (w), 2026 (m), 1999 (s), 1967 (m), 1954 (sh).

ESI-HRMS (MeOH) m/z : 2039.34 $[\text{M}+\text{H}]^+$.

Conjugate of $\text{Os}_6(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{OOC}(\text{CH}_2)_5\text{N}_3]$ and
5-ethynyl-deoxyuridine (**15l**)

^1H NMR (CDCl_3) δ /ppm :

1.37 – 1.44 (m, 4H, H11, H16), 1.61 – 1.66 (m, 6H, H17, H10, H12), 2.27 (t, 2H, H9, $J = 7.0$ Hz), 2.30 – 2.36 (m, 2H, H2'), 2.93 – 2.98 (m, 2H, H15), 3.83 (br s, 2H, H5'), 4.02 (br s, 1H, H3'), 4.07 (t, 2H, H13, $J = 5.8$ Hz), 4.42 – 4.45 (m, 2H, H15), 4.55 (br s, 1H, H4'), 6.40 (t, 1H, H1', $J = 7.0$ Hz) 7.60 (br s, 10H, $\text{H}_{\text{aromatic}}$), 7.99 (s, 1H, -NH), 8.25 (s, 1H, H6), 8.75 (s, 1H, H8).

^{31}P NMR (CDCl_3) δ /ppm : -8.2.

ESI-HRMS m/z : 2337.16 $[\text{M}+\text{H}]^+$.

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Chapter 3. Application of Osmium Carbonyl Cluster – Nucleoside Conjugates

Triosmium carbonyl clusters have been used effectively as both therapeutic agents and SERS imaging agents.¹⁻⁴ A strategy to improve on these applications is through conjugation to biomolecules such as nucleosides and peptides, which may lead to improved cellular uptake and targeting. Conjugates of triosmium carbonyl clusters to nucleosides have been prepared successfully from the ligand displacement and CuAAC reactions in Chapter 2. The potential of these conjugates in improving the applications of the triosmium carbonyl clusters was therefore evaluated.

3.1 As Therapeutic Agents

Nucleosides and their analogues have long been used as therapeutic agents since they have shown good anti-viral and anti-proliferative activity.⁵⁻⁷ For example, nucleoside analogues such 5-ethynyl-2'-deoxyuridine **1**, have exhibited anti-proliferative activity against certain cancer cells.⁸ Triosmium carbonyl clusters such as **2** have also been shown to exhibit anti-cancer activity, particularly against the breast cancer cell lines.^{1,4} The anti-proliferative activity of several triosmium carbonyl nucleoside conjugates (Figure 3.1) against the MDA-MB-231 cell line was therefore investigated and the results are shown in Figure 3.2.

Figure 3.1 Triosmium carbonyl – nucleoside conjugates used for cell viability assays.

In general, the conjugates showed improved activity compared to the parent nucleosides. Of these, however, only **6e** and **7l** show comparable activity and selectivity to **2**. The anti-proliferative activity of **2** has been attributed to the presence of labile ligands which enable it to bind to cellular targets.⁹ However, the cytotoxicity of **6e** and **7l** point to a rather different mechanism since the thiol and carboxylate ligands are strongly bound to the metal centres.

Figure 3.2 Cell viability assay of the conjugates carried out on breast cell lines at 20 mM concentrations.

3.2 As SERS Imaging Agents

In contrast to therapeutic agents, molecules used for cell imaging should show low cytotoxicity but should have good solubility for uptake into cells. The doubly conjugated cluster **9I-2**, in particular, fulfils these criteria and shows good potential as an imaging agent. The uptake of cluster **9I-2** into cancer cells was first evaluated by infrared spectroscopy and ICP-OES of the obtained cell pellet after incubation (Figure 3.3).

Figure 3.3 Cell uptake studies using cluster **9I-2**.

From the measurements of the obtained cell pellet, there is a higher uptake of the nucleoside conjugated cluster **9I-2** compared to its precursor **9P2** (Figure 3.4). In addition, the cluster **9I-2** also shows preferential uptake into the MDA-MB-231 breast cancer cells as compared to the normal MCF-10A breast epithelial cells (Figure 3.5).

Figure 3.4 Infrared spectrum of cell pellets after incubation with cluster **9P2** and **9I-2**.

Figure 3.5 ICP-MS concentrations of cell pellets after incubation with clusters **9P2** and **9I-2**.

The immobilization of SERS reporters onto gold solid supports is often carried out by appending thiol- or amine-functionalized molecules onto the gold surface.¹⁰ While its mechanism has not been fully understood, it is likely that chemical bonds to the solid support are not necessary for SERS enhancement to occur. For example, SERS enhancement has been observed for non-functionalized molecules such as carbon dioxide, phenol and crystal violet, which may interact with the SERS active surfaces through π -interactions and physisorption (Figure 3.6).¹¹⁻¹⁴

Figure 3.6 Non-functionalized molecules which show SERS enhancement.

The triosmium carbonyl cluster **5** has also been used for live cell SERS imaging upon immobilization to gold nanoparticles despite being a non-functionalized molecule, suggesting that the interaction of the triosmium carbonyl framework with a gold support is sufficient for SERS enhancement.² However, as cluster **5** is coordinatively unsaturated, it could have reacted with the gold surface, thereby giving rise to its SERS enhancement. Nevertheless, the potential of cluster **9I-2** as a SERS imaging agent was evaluated, despite its lack of thiol or amine functional groups.

Bright field images show the accumulation of gold nanoparticles in the cells. Although the cells were incubated with cluster **9I-2** for 24 hours, no SERS enhancement was observed and only the carbon dioxide signal was detected in the 1800 – 2200 cm⁻¹ window (Figure 3.7). The lack of SERS enhancement for cluster **9I-2** suggests that chemical bonds between the cluster with the gold surface may be needed to produce a SERS signal. This is corroborated by the observation that incubation of cluster **9I-2** with gold nanoparticles did not result in any SERS enhancement. It is thus likely that the lack of SERS enhancement for cluster **9I-2** is due to its failure to be chemically appended to the solid surface. This also suggests that cluster **5** is indeed chemically bonded to the gold nanoparticle surface to produce the SERS enhancement necessary for the live-cell imaging.

Figure 3.7 Bright field image of MDA-MB-231 cells incubated with cluster **9I-2** (left) and their SERS measurement (right).

3.3 As TEM Imaging Agents

The imaging of DNA using transmission electron microscopy (TEM) is of great interest as it provides an opportunity to observe the macromolecule in its native state. Unfortunately, since DNA consists of lighter atoms such as carbon, nitrogen and oxygen, they are virtually invisible under the electron microscope and cannot be imaged directly. To aid in the visualization of these DNA strands, heavy metals such as osmium, platinum and uranium are typically used to stain the macromolecule prior to imaging. However, such staining techniques may affect the structure of the DNA strands and introduce artefactual damage to the sample.¹⁵ Covalent attachment via conjugation methods are preferable as such methods are milder and can take place under biological conditions.

3.3.1 Conjugation of Osmium Carbonyl Clusters to DNA

The CuAAC reaction between the 5-ethynyl-2'-deoxyuridine derivative **1** and the azide-functionalized osmium clusters presents an opportunity to covalently attach heavy metal clusters onto an oligonucleotide under relatively mild conditions. This requires the introduction of an alkyne functional group into an oligonucleotide by solid phase oligonucleotide synthesis or the polymerase chain reaction (PCR).

Introduction of the alkyne group into a DNA strand by PCR was carried out by replacement of 2'-deoxythymidine triphosphate (dTTP) with modified 5-ethynyl-2'-deoxyuridine triphosphate (EdUTP). This allows for the incorporation of the alkyne-functionalized nucleotide into the DNA strand (Figure 3.8). The PCR reaction was first optimized using the *Taq* polymerase enzyme on a 200 base pair (bp) template.

Figure 3.8 Conjugation of DNA to osmium carbonyl clusters.

Gel electrophoresis of the PCR-amplified DNA suggest that a complete replacement of the parent dTTP with EdUTP was not tolerated by the enzyme. Amplification of the DNA strand was achieved only when a mixture of dTTP : EdUTP in a 4 : 1 ratio was employed (Figure 3.9). Conjugation of the purified DNA was initially carried out using cluster **7L2**. However, TEM imaging of the conjugated DNA was rather unsuccessful. Utilizing a traditional TEM microscope, images of 0.5 μm resolution were obtained and this was insufficient for the observation of the

280 base pair DNA strands. The amplification and conjugation was then carried out on a longer 5 kilobase DNA template.

Figure 3.9 Gel electrophoresis of PCR using *Taq* polymerase with the modified nucleotide EdUTP. PCR was carried out on the 200 base pair DNA template using 2.5% agarose gel.

Optimization of the PCR on the long 5kb DNA template using *Taq* polymerase was unsuccessful, indicating that the enzyme is not tolerant towards the modified EdUTP nucleotide, even when dTTP : EdUTP was used in a 2 : 1 ratio. Replacement of *Taq* polymerase with a more tolerant *Pwo* polymerase improves the amplification of the DNA template when the modified nucleotide is used (Figure 3.10). The amplified DNA product can be observed when 80% of EdUTP is used to replace the natural nucleotide. Conjugation of the 5kb DNA to the more polar cluster **8P2** did not result in any change in electrophoretic mobility.

Figure 3.10 Gel electrophoresis of PCR carried out using the *Taq* polymerase (left) and *Pwo* polymerase (right) on the 5kb template using a 1.2% agarose gel.

Cluster **8P2** and the hexaosmium carbonyl cluster **15P5** were also conjugated to shorter DNA strands with the interest of developing sequence-specific probes. The clusters were conjugated to two separate but complementary DNA strands of different lengths – both of which contain a single alkyne modification (Figure 3.11). In principal, imaging of the annealed strands should show two clusters of different density at a fixed distance (Figure 3.12).

Figure 3.11 TEM imaging of osmium carbonyl conjugated DNA strands.

Figure 3.12 DNA sequences and modifications made for conjugation to osmium carbonyl clusters.

The conjugated product of cluster **15P5** with the 20-mer DNA strand was analysed by MALDI-TOF. A peak at $m/z = 4095$ was observed and this may be attributed to the $[(M+15P5)/2]^-$ product, indicating that the conjugation of cluster **15P5** with the single stranded DNA was successful (Figure 3.13). The low intensity of the signal may be attributed to possible fragmentation and poor incorporation of the conjugated product during sample preparation, since conjugation to the cluster may affect the polarity of the DNA molecule.

Figure 3.13 MALDI-TOF analysis of the 19-mer DNA strand conjugated with cluster **15P5**.

3.3.2 TEM Imaging of Osmium Carbonyl – DNA Conjugates

Although TEM imaging of stained DNA has been carried out using traditional TEM microscopy, these methods involve relatively high energy electron beams in order to achieve atomic resolution. However, these high energy electron beams cause damage to biological structures, leading to a loss of information on the native structure of DNA.

One method to reduce the amount of radiation damage to the specimen is to embed the DNA specimen between layers of a solid support. Graphene has emerged as the ultimate solid support for TEM imaging in recent years as graphene-sandwiched specimen have shown greater resistance to ion radiation damage, without any great effect on the resolution of the images.¹⁶ Similar methods may be applied to the imaging of DNA by TEM to reduce the amount of radiolysis of the specimen. The conjugated DNA was deposited between two TEM grids coated with graphene to create a “sandwiched” sample.

From the general overview and layout of the sample, no distinctive DNA structures were detected (Figures 3.14a and 3.14b). Magnification of the area reveals several dark spots, which may indicate that heavier atoms are present in the sample. Unfortunately, stacked

carbon atoms from organic molecules may also appear as dark spots in the micrograph. Heavy atoms may be distinguished from stacked light atoms by changing the focus of the microscope. Figures 3.14c and 3.14d, taken 150 s apart with a change of focus, show that there are significant beam-induced changes in the sample. Thus it was not possible to determine if the spots which have been observed are due to the heavy atoms or due to the presence of carbonaceous material.

Figure 3.14 TEM images of DNA conjugated with cluster **15P5**, deposited on graphene coated TEM grids.

3.4 Conclusion

Triosmium carbonyl–nucleoside clusters have been evaluated as therapeutic and imaging agents. In general, the conjugates demonstrate improved solubility and cell uptake in vitro. Two of the conjugates exhibit promising anti-proliferative activity, although their mode of action requires further investigation. Imaging of cancer cells using SERS was also attempted but met with difficulties in achieving significant SERS enhancement.

The CuAAC conjugation was also utilized for the labelling of DNA strands with triosmium and hexaosmium carbonyl clusters. Following unsuccessful attempts at DNA

imaging using conventional TEM methods, the conjugates were imaged by sandwiching between graphene layers but this proved inconclusive due to the presence of carbonaceous material resulting from the sample preparation. Improvements on the sample preparation methods are therefore required. The use of low-voltage scanning transmission electron microscopy (STEM) may also be useful since heavy atoms are more easily discriminated with that technique.

3.5 Experimental

All DNA amplification reactions were carried out on a MJ Research Peltier thermal cycler system. PCR reagents were obtained from New England Biolabs unless stated. DNA primers were obtained by custom synthesis by Integrated DNA Technologies (IDT DNA). The modified nucleotide, 5-ethynyl-2'-deoxyuridine triphosphate (EdUTP) was purchased from Jena Bioscience. Gel electrophoresis reagents were obtained from 1st Base unless stated. SYBR Safe DNA gel stain was obtained from Invitrogen. GeneRuler ready-to-use 100 bp DNA ladder and 6x loading dye were purchased from ThermoScientific. All DNA purification reagents were purchased from Qiagen.

Cell culture media Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Biowest while the DMEM-F12 mixture was obtained from Lonza. Phosphate buffer saline (PBS), foetal bovine serum (FBS) and trypsin-EDTA solution (0.25%) were obtained from Gibco Life Technologies. MTS Cell Proliferation reagent was obtained from BioVision Incorporated.

DNA quantification was carried out on a Shimadzu BioSpec Nano UV-vis spectrometer at an absorbance of 260 nm. All gels were imaged using the G:Box Chemi HR16 gel documentation system. MALDI-Tof sample preparation were carried out as described using the ammonium form of the Dowex 50WX8 cation exchange resin.¹⁷ The analyses were carried out with a Bruker Autoflex TOF instrument equipped with a smartbeam laser, using a linear negative method.

TEM investigations were performed with a FEI Titan 80-300 transmission electron microscope fitted with a CEOS CETCOR third order spherical aberration corrector and a Gatan UltraScan 1000XP CCD camera. The microscope was operated at an electron acceleration voltage of 80 kV and the electron source extraction voltage was set to 2 kV reducing the primary electron energy distribution to 0.5 eV.

Cell uptake measurements using infrared spectroscopy were carried out in the solid state using the attenuated total reflection (ATR) mode. ICP-OES measurements of the cell pellet were carried out using a Thermo Scientific iCAP 6500 system using hydrochloric acid digestion. The Raman spectral measurements were carried out using a Renishaw InVia Raman (UK) microscope with a Peltier cooled CCD detector and an excitation wavelength at 633 nm, where the laser beam is directed to the sample through a 50× objective lens, which was used to excite the sample and also to collect the return Raman signal. All Raman spectra were processed with WiRE3.0 software. The maximum laser power at the sample was measured to be 6.2mW and the exposure time was set at 10s throughout the measurements. Prior to each measurement, the instrument was calibrated with a silicon standard whose Raman peak is centered at 520 cm⁻¹.

3.5.1 Cell Based Assays and Uptake Studies

3.5.1.1 Cell Culture

For routine cell culture, MDA-MB-231 breast cancer cells were maintained in cell culture media consisting of high glucose DMEM (90%), fetal bovine serum (FBS) (9%) and penicillin-streptavidine (1%) whereas the MCF-10A epithelial cell line was grown in cell culture media of DMEM-F12 (92%), fetal bovine serum (7.65%), Gentamicin (0.4%) with additives of insulin (0.05%), EGF (0.01%) and hydrocortisone (0.002%). Cells were grown in T25 or T75 cell culture flasks kept at 37°C with 5% CO₂ unless stated. Upon reaching confluence, cell detachment was carried out by first washing the cells with PBS, followed by incubation of the cells with trypsin for 2-3 minutes. The cell pellet was obtained by centrifugation at 150 x g for 5 min and resuspended in media for sub-culturing.

3.5.1.2 MTS Assay of Conjugates

Cells were cultured at 5⁴ cells per well and maintained in serum free media overnight before treatment with the conjugates. The osmium carbonyl clusters were first dissolved in sterile dimethylsulfoxide (DMSO) to make 20 mM stock solutions. They were then diluted with serum-free media, with final concentrations of 20 µM used for cytotoxicity tests. The cells were then treated with the indicated concentrations of osmium carbonyl clusters in triplicates and incubated for 24 h, after which the MTS reagent (20 µL) was added to each well. This was left to incubate at 37°C in 5% CO₂ for 2 h. The absorbance at 490 nm was then measured and the cell proliferation relative to the control sample was calculated.

3.5.1.3 Cell Uptake of Cluster **9I-2**

Cells were cultured in 100 mm petri dishes and grown to confluence before incubation in serum free media for 24 h. Cells were treated with the triosmium carbonyl cluster **9I-2** or **9P2** (40 mM) dissolved in DMSO diluted to a final concentration of 40 μ M for 12 h. The cells were washed thoroughly with PBS (3x) and H₂O (3x) before trypsin detachment from the dishes. The cell pellet was obtained by centrifugation at 150 x g for 5 min before resuspension in serum-free media for cell counting. From the suspension, 20³ cells were removed, pelleted by centrifuging at 150 x g for 5 min and washed with PBS containing 10% DMSO (3x). The cell pellet was directly used for IR measurements using ATR.

For ICP measurements, the cell pellet was first digested in a 10% HCl solution and centrifuged to remove the remaining cell residue. The samples were diluted to a final concentration of 2% HCl for the ICP-OES measurements.

3.5.1.4 SERS Imaging of Cancer Cells

The MDA-MB-231 cells were cultured at 10⁶ cells per well in chambered glass slides and maintained in FBS-free media overnight at 37 °C in 5% CO₂. Cells were treated with the triosmium carbonyl cluster **9I-2** dissolved in DMSO diluted to a final concentration of 20 μ M for 12 h. The cells were washed thoroughly using PBS (3x) and H₂O (3x) before incubation with gold nanoparticles (500 μ L/well) for 4h. The cells were washed thoroughly with H₂O (3x) and fixated with methanol at 0°C.

3.5.2 DNA Conjugation

3.5.2.1 PCR Optimization using *Taq* DNA Polymerase

Routine PCR optimization was carried out by preparing a master mix solution containing 1x reaction buffer, 200 μ M deoxynucleotide triphosphates (dATP, dCTP, dTTP and dGTP), 0.2 μ M forward primer, 0.2 μ M reverse primer, 1.25 units *Taq* DNA polymerase and this was topped up with DNA-se free water to a final volume of 50 μ L per reaction. PCR was carried out in triplicates by addition of the master mix solution (50 μ L) into the PCR tubes, followed by the template DNA (787 ng). A negative control was also prepared using the master mix solution (50 μ L), without the template DNA. Cycling parameters were as follows :

Table 3.1 Cycling parameters for PCR using *Taq* DNA polymerase.

1) Initial denaturation	95°C for 30s
2) Denaturation	95°C for 30 s
3) Annealing	58°C for 1 min
4) Extension	68°C for 30s
5) Amplification	Repeat steps 2-4 for 40 cycles
6) Final extension	68°C for 5 min

3.5.2.2 PCR using Modified Nucleotide EdUTP and *Taq* DNA Polymerase

A mastermix solution containing 1x reaction buffer, 200 μ M deoxynucleotide triphosphates (dATP, dCTP, and dGTP), 0.2 μ M forward primer, 0.2 μ M reverse primer, 1.25 units *Taq* DNA polymerase was prepared and this was topped up with DNA-se free water to a final volume of 49 μ L per reaction. Solutions of dTTP : EdUTP (1 μ L) at the indicated ratios were added to each PCR tube. Template DNA (787 ng) was added to each tube except one which would be used as a control experiment. Cycling parameters were as described in 3.5.2.1 (Table 3.1).

3.5.2.3 PCR Optimization using *Pwo* DNA Polymerase.

Routine PCR optimization was carried out by preparing a master mix solution containing 1x reaction buffer, 200 μ M deoxynucleotide triphosphates (dATP, dCTP, dTTP and dGTP), 0.3 μ M forward primer, 0.3 μ M reverse primer, 0.5 units *Pwo* DNA polymerase and this was topped up with DNA-se free water to a final volume of 25 μ L per reaction. PCR was carried out in triplicates by addition of the master mix solution (25 μ L) into the PCR tubes, followed by the template DNA (100 ng). A negative control was also prepared using the master mix solution (50 μ L), without the template DNA. Cycling parameters were as follows:

Table 3.2 Cycling parameters for PCR using *Pwo* DNA polymerase.

1) Initial denaturation	92°C for 30 s
2) Denaturation	92°C for 30 s
3) Annealing	51°C for 30 s
4) Extension	72°C for 5 min 40 s
5) Amplification	Repeat steps 2-4 for 30 cycles
6) Final extension	68°C for 5 min

3.5.2.4 PCR using Modified Nucleotide EdUTP and Pwo DNA Polymerase

A mastermix solution containing 1x reaction buffer, 200 μ M deoxynucleotide triphosphates (dATP, dCTP, and dGTP), 0.3 μ M forward primer, 0.3 μ M reverse primer, 1.25 units DNA polymerase was prepared and this was topped up with DNA-se free water to a final volume of 24.5 μ l per reaction. Solutions of dTTP : EdUTP (0.5 μ L) at the indicated ratios were added to each PCR tube. Template DNA (100 ng) was added to each tube except one which would be used as a control experiment. Cycling parameters were as described in 3.5.2.3 (Table 3.2).

3.5.2.5 Gel Electrophoresis

Agarose gel was made by dissolving agarose powder in 1x Tris Acetate EDTA (150 ml) by microwaving for 2 mins, with swirling every 30 s, after which the SYBR Safe DNA stain (20 μ l) was added. The solution was cooled to approximately 80°C, poured into a gel caster and left to solidify for 30 mins at room temperature. After loading the wells with a mixture of the DNA sample (10 μ l) and 6x loading dye (2 μ l), the gel was run at a constant voltage of 120 V for 2 hours in 1x TAE buffer.

3.5.2.6 Conjugation of Osmium Carbonyl Clusters to DNA

A solution of the osmium carbonyl cluster in DMSO (1 mM) (2 eq.) was added to a solution of DNA in Tris-EDTA (TE) buffer in a 1.5 mL microtube. Subsequently, a solution of CuSO₄ (0.02 mg/mL) (0.02 eq) and sodium ascorbate (0.02 mg/mL) (0.05 eq) in DNase-free water was added to the microtube and each tube was topped up to a final volume of 20 μ L. The reaction was left on an orbital shaker overnight at room temperature for 16 hrs. The solution was then dialyzed against TE buffer (3 x 500mL) to remove excess reactants. Concentration of DNA was measured using UV-Vis absorption.

3.5.2.7 Preparation of DNA Samples for MALDI-TOF Analysis

A matrix solution was prepared by mixing 3-hydroxypicolinic acid [50 mg/mL in H₂O:ACN (1:1, v/v)] and diammonium hydrogen citrate (100 mg/mL in H₂O) in a 10:1 (v/v) ratio. To this solution (5 μ L) was added the conjugated single stranded DNA (2 μ L) and several grains of the ammonium form of the ion exchange resin. This mixture was mixed thoroughly and incubated at room temperature for 15 mins, after which the mixture (1 μ L) was applied to the target and left to dry at room temperature. The MALDI-TOF analysis was carried out using a linear method in the negative ion mode.

3.5.2.8 Preparation of TEM Samples

Prior to sample preparation, two graphene grids were cleaned by activated carbon at 270°C for 30 min. Residual activated carbon was blown off the grids before sample addition. To the first graphene-coated gold grid was dropped 3-5 μL of the DNA solution (1 $\mu\text{L}/\text{mL}$) and the second grid was placed on top of the first. The sample was left to dry at room temperature for 20 min before TEM analysis.

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Chapter 4. Protonation of Disubstituted Triosmium Carbonyl Clusters

Phosphine ligands in substituted triosmium carbonyl clusters almost invariably occupy equatorial positions.¹⁻⁴ In disubstituted clusters, these phosphine ligands can be arranged in a *cis*- or *trans*- position with respect to the Os-Os bond between the phosphine-substituted osmium atoms. This gives rise to three possible isomers (Figure 4.1).

Figure 4.1 Isomers of the disubstituted triosmium carbonyl cluster.

The preparation of such disubstituted triosmium carbonyl phosphine clusters is carried out by the reaction of “lightly-stabilized” triosmium carbonyl clusters with an excess of the tertiary phosphine. The ligand displacement proceeds rapidly and the reaction reaches completion within two hours (Scheme 4.1).

Scheme 4.1 Preparation and protonation of disubstituted phosphine clusters.

In general, only two isomers (the *cis-trans* and the *trans-trans* isomers) have been observed to be in dynamic equilibrium in solution.⁵ If the two phosphine ligands are the same, this gives rise to three resonances in the ³¹P NMR spectrum. Computational studies were carried out on simplified models of these two isomers to examine the energetics of this equilibrium. The computed Gibbs’ free energies show that in the neutral form, the *trans-trans* isomer is the most thermodynamically stable isomer. However, the *cis-trans* isomer is only 1.8 kJ/mol higher in energy. Although this energy gap is dependent on the R substituent, it is small enough to allow the fast equilibrium between the two isomers to be experimentally observed. The mechanism of this isomerization is thought to involve a turnstile rotation about the Os atom carrying the phosphine ligand, giving rise to the fast equilibrium between the two isomers.⁶

Protonation of the neutral cluster **9P** can be carried out by the addition of trifluoroacetic acid or tetrafluoroboric acid to a chloroform solution of the neutral cluster. Isolation of this protonated species is carried out upon anion exchange with NH_4PF_6 (Scheme 4.2).

Scheme 4.2 Protonation of the disubstituted triosmium carbonyl cluster.

Like the neutral disubstituted cluster, the phosphines can adopt a *cis*- or *trans*- position with respect to the protonated Os-Os bond between the phosphines. However, the distribution of isomers changes significantly upon protonation. The major isomer adopts the *cis-cis* configuration and is observed as a single, high intensity resonance in the ^{31}P NMR spectrum. Two other peaks of low and equal intensity are also observed and these are assigned to the *cis-trans* isomer, generally present as 20% of the major isomer.⁷

These observations are supported by computational studies. The computed energies show that the *cis-cis* isomer is now the most thermodynamically stable, while the *trans-trans* isomer is the highest in energy (Figure 4.2). In addition to this, the isomerisation energy between the two most stable isomers is increased upon protonation. Equilibrium therefore favours the *cis-cis* isomer, while the *cis-trans* isomer is observed only at very low concentrations.

Figure 4.2 Computational studies on cluster $[\mathbf{9P}+\text{H}]^+$, when R = H.

The exact reason for the stability of the *cis-cis* isomer is not fully understood. However, a comparison between the solid state structures of *cis-cis* $[\mathbf{9P(iii)}+\text{H}]^+$ and the geometrically similar $[\text{Os}_3(\mu\text{-H})(\text{CO})_{10}(\text{NCCH}_3)_2]^+$ show that they have similar Os($\mu\text{-H}$)Os-L (where L = CO or PR_3) angles (Figure 4.3).^{7,8} These bond angles are indicative of the steric crowding at the equatorial sites – a larger bond angle would indicate repulsion of the ligands at the *cis* position with respect to the protonated Os-Os bond edge. The equatorial carbonyl ligands in $[\text{Os}_3(\mu\text{-H})(\text{CO})_{10}(\text{NCCH}_3)_2]^+$ are relatively unhindered and are not sterically bulky. The similar bond angles it thus suggests that the *cis* sites in the protonated phosphine disubstituted clusters are also not crowded and may therefore be preferred.⁷

Figure 4.3 Bond angles of the protonated *cis-cis* isomer (left) and $[\text{Os}_3(\mu\text{-H})(\text{CO})_{10}(\text{NCCH}_3)_2]^+$ (right).

The isomerisation upon protonation of the triosmium carbonyl cluster effectively brings the two phosphine ligands closer together; the P...P distance for this geometry has been found to vary from ~ 3.2 to ~ 5.7 Å.^{7,9-13} This implies that the distance between the substituents on the phosphines is reduced, allowing for intramolecular interactions between them to be possible. It is therefore interesting to examine if this stereochemical change upon protonation may be used to effect conformational changes in a biomolecule such as an oligopeptide.

Such “triggered” isomerization has also been observed, for example, in the $\text{RuCl}_2(\text{dppm})_2$ complex which undergoes *cis-trans* isomerization upon photochemical, redox or thermal activation (Figure 4.4).^{14,15}

Figure 4.4 *Cis-trans* isomerization of $\text{RuCl}_2(\text{dppm})_2$.

Another example is a set of tetrairon clusters which shows isomerisation about the metal core (Figure 4.5).^{16,17} Isomerization of these [4Fe-4C] clusters occurs spontaneously upon standing in acetonitrile, via cleavage of the carbon-carbon bond. Further chemical oxidation leads to reversible scission of the metal-metal bond.¹⁸

Figure 4.5 Isomerization of [4Fe-4C] clusters.

4.1 Hydrogen Bonding

Intramolecular interactions in the protonated *cis-cis* isomer $[\mathbf{9P(iii)}+\text{H}]^+$ may be investigated through the presence of distance-dependent interactions such as hydrogen bonding. Hydrogen bond distances are typically less than 2.5 Å. Thus in the neutral *cis-cis* and *cis-trans* isomers of cluster **9P**, hydrogen bonding is not expected between the phosphine

substituents. Upon protonation, this distance is reduced and the substituents could be close enough for hydrogen bonding (Figure 4.6).

Figure 4.6 Hydrogen bonding in the protonated *cis-cis* isomer

Disubstituted phosphine clusters functionalized with hydrogen bonding moieties such as carboxylic acids and amides were prepared for the study (Scheme 4.3). Although cluster **9P6** was observed as the major product in the crude NMR spectrum, it was only isolated in low yields. In an attempt to improve the isolated yield, a synthesis via deprotection of the tert-butyl ester protected cluster **9P7** was also attempted. However, the ^1H NMR spectrum taken shortly after the addition of TFA to cluster **9P7** showed the presence of the distinct triplet hydride signal at -19 ppm, indicating protonation at the metal-metal bond to form $[\mathbf{9P7}+\text{H}]^+$. This resonance shifted upfield upon standing for 24 hours, indicating subsequent deprotection of the tert-butyl ester (Figure 4.7). Cluster $[\mathbf{9P6}+\text{H}]^+$ was isolated from the reaction mixture after anion exchange.

Scheme 4.3 Preparation of carboxylic acid functionalized cluster **9P6**.

Figure 4.7 Changes in the hydride resonance of the ¹H NMR spectrum upon conversion of cluster **[9P7+H]⁺** (●) to cluster **[9P6+H]⁺** (▲).

Hydrogen bonding can be observed by a shift in the ¹H resonances, depending on the strength of the interaction.¹⁹⁻²¹ A comparison between the ¹H NMR spectra of the neutral cluster **9P6** with its protonated form **[9P6+H]⁺** show a slight downfield shift in the resonances (Figure 4.8). The NMR spectra of both clusters, however, show a broadened signal at ~4.6 ppm. This, together with the absence of a residual water resonance, is indicative of proton exchange or hydrogen bonding between the cluster and water. Thus, while intramolecular hydrogen bonding between the two chains is not detected, intermolecular hydrogen bonding may be present.

Figure 4.8 ^1H NMR spectra of cluster **9P6** (top) and cluster $[\mathbf{9P6+H}]^+$ in CDCl_3 (bottom).

From the above, it is apparent that it will be difficult to distinguish between intra- and intermolecular hydrogen bonding; the flexibility and free rotation of the phosphine ligands in **9P6** allows for intermolecular hydrogen bonding. In an attempt to reduce this, the chelated cluster **9P8** and its protonated form $[\mathbf{9P8+H}]^+$ were prepared (Scheme 4.4).

Scheme 4.4 Preparation of chelated cluster **9P8** and its protonation.

A comparison between the ^1H NMR spectra of **9P8** and $[\mathbf{9P8+H}]^+$ show a sharpening and a general downfield shift of the resonances upon protonation (Figure 4.9). The chemical

shifts of these resonances are independent of the concentration of the solution, indicating the absence of intermolecular bonding.

Figure 4.9 ^1H NMR spectrum of cluster **9P8** (top) and $[\mathbf{9P8+H}]^+$ (bottom) in CDCl_3 .

A diffraction quality single crystal of cluster $[\mathbf{9P8+H}]^+$ was obtained and an x-ray crystallographic analysis shows that it has the expected *cis-cis* orientation of the phosphorus atoms with respect to the Os-Os bond [$\angle\text{P1-Os1-Os2} = 115.51(3)^\circ$ and $\angle\text{P2-Os2-Os1} = 125.51(3)^\circ$]. As has been observed previously, the Os-Os bond chelated by the hydride is significantly longer than the other two Os-Os bonds [$\text{Os1-Os2} = 3.0575(3) \text{ \AA}$, cf. $\text{Os1-Os3} = 2.8847(3) \text{ \AA}$ and $\text{Os2-Os3} = 2.8985(3) \text{ \AA}$].⁷ Perhaps more interesting is the orientation of the N2 amide proton to the carboxyl group O1 in the solid state (Figure 4.10).

Figure 4.10 ORTEP representation of cluster $[9P8+H]^+$. Hydrogen atoms (except hydride and amide hydrogens) have been omitted for clarity. Thermal ellipsoids are drawn at 50% probability level.

Crystallographic studies on small molecule amides have shown that the typical bond distance and angle between the H-acceptor and donor are 1.5 – 2.5 Å and 130 – 170°, respectively, although the ranges may be wider for proteins (Figure 4.11).²²⁻²⁴ In cluster $[9P8+H]^+$, the N2-H--O interatomic distance is 2.13 Å and the angle is 148.8°, which fall within the range for a hydrogen bond involving an amide group. It is thus possible that there is an intramolecular hydrogen bond between the amide group and the carboxyl oxygen O1 in cluster $[9P8+H]^+$.

Figure 4.11 Typical hydrogen bond geometry for amide groups.

Computational studies were carried out on a simplified model of cluster **9P8**, in which the phenyl groups on the phosphines have been replaced with hydrogen atoms. In the *trans-trans* isomer, the phosphine substituents are too far apart and therefore, hydrogen bonding interactions may be less significant. Calculations were therefore carried out for the *cis-trans* isomer. The optimized structure of the *cis-trans* isomer shows that the carboxyl groups and amide protons are facing away from each other (Figure 4.12). In addition, the interatomic distance (5.3 Å) between the amide proton and the carboxyl group exceeds the typical length of a hydrogen bond. This suggests that hydrogen bonding is not present in the neutral *cis-trans* isomer and that the intramolecular hydrogen bond is only present upon protonation of the cluster.

Figure 4.12 Top view (left) and side-on view (right) of the computationally optimized structure of the simplified *cis-trans* isomer of **9P8**. Hydrogen atoms, except the amide and phosphine hydrogens, have been omitted for clarity. Groups which are expected to show hydrogen bonding, but do not, have been highlighted.

4.2 Conformational Change

The possibility of using the stereochemical change in clusters **9P** to effect a conformational change in a peptide was next examined through a cluster-oligopeptide conjugate. Such conformational changes are typically examined via ^1H NOESY NMR spectroscopy and circular dichroism (CD), supported by computational studies.^{25,26} In the neutral form, the cluster-oligopeptide conjugate should exhibit only intrastrand NOE interactions. Protonation of the cluster should bring the substituents close enough, such that

both intra- and interstrand NOE interactions are present (Figure 4.13). In order to differentiate between these NOE interactions, the triosmium carbonyl cluster can be conjugated to two different oligopeptides.

Figure 4.13 Expected inter- and intrastrand interactions in a cluster-oligopeptide conjugate.

As has been observed above, the flexibility of the phosphine substituents, as well as the dynamic equilibrium between isomers of the disubstituted phosphine cluster, may make it difficult to study these interactions because of the additional possibility of intermolecular interactions. This can be restricted with the use of chelated cluster-oligopeptide conjugates (Figure 4.14).

Figure 4.14 Expected inter- and intrastrand interactions in the chelated cluster-oligopeptide conjugate.

The CuAAC reaction has been successfully used for the conjugation of triosmium carbonyl clusters to nucleosides. It was employed again for the conjugation of oligopeptides, although this required the use of alkyne- or azide-modified amino acids. The CuAAC conjugation was first tested using cluster **9P2** and DL-propargylglycine. The conjugation proceeded at both azide groups and yielded clusters **9A1-1** and **9A1-2**, which were observed spectroscopically (Scheme 4.5).

Scheme 4.5 Conjugation of cluster **9P2** to DL-propargylglycine.

It is apparent that the presence of two similar functional groups in the cluster may yield the doubly conjugated product, which is undesirable for the preparation of the chelated oligopeptide conjugate. Orthogonal functional groups were thus introduced on the

disubstituted triosmium carbonyl cluster. Since peptides have free amine and carboxylic acid groups, a useful orthogonal reaction to the CuAAC reaction was the amide coupling reaction (Figure 4.15).

Figure 4.15 Targeted conjugation of oligopeptides to disubstituted triosmium carbonyl clusters.

4.2.1 Orthogonal Functionalization

Phosphines functionalized with amines were prepared via amide coupling with the Boc-protected ethylene diamine. The phosphine **P10** was isolated in moderate yields using silica passivated with triethylamine (Scheme 4.6).

Scheme 4.6 Preparation of amine-functionalized phosphine.

Introduction of orthogonal functional groups on the disubstituted phosphine cluster calls for the synthesis of asymmetric disubstituted phosphine clusters. This could be achieved by the reaction of cluster **2** with a 1:1 mixture of the two desired phosphines (Scheme 4.7). In principle, the reaction should yield products **9P1**, **9P1.10** and **9P10** in equal amounts. However, it was found that the reaction yielded cluster **9P1** as the major product while the desired mixed phosphine cluster **9P1.10** was only obtained in relatively low yields (4.7%).

Scheme 4.7 Reaction of cluster **2** with a 1:1 ratio of phosphines **P1** and **P10**.

A more successful route to **9P2.10** was via TMNO-activated substitution of the mono-substituted cluster **8P1** in the presence of phosphine **P10**. Subsequent reaction with sodium azide yielded the orthogonal cluster **9P2.10**, although it was not isolated successfully from its precursor **9P1.10** (Scheme 4.8). Considering that cluster **9P1.10** cannot undergo the CuAAC reaction, no further purification was attempted.

Scheme 4.8 Synthesis of orthogonally functionalized cluster **9P2.10**.

A similar reaction sequence was used for the orthogonal functionalization of the disubstituted phosphine cluster with a carboxylic acid group (**9P2.6**) and its activated form, the N-hydroxysuccinimide ester (NHS) (**9P11.12**); both clusters were obtained in moderate yields (Scheme 4.9).

Scheme 4.9 Preparation of the orthogonally functionalized cluster **9P2.6** and **9P11.12**.

The ability of cluster **9P2.6** to undergo the orthogonal conjugation reactions was investigated by its reaction with propargylamine. This was carried out via a stepwise amide coupling reaction followed by the CuAAC reaction to yield the chelated cluster **9L3**. Cluster **9L3** was then protonated to the *cis-cis* isomer **[9L3+H]⁺** (Scheme 4.10). Similar results were obtained using cluster **9P11.12** via a one-step reaction under CuAAC conditions. Clusters **9L3** and **[9L3+H]⁺** are considered as asymmetric chelated clusters and can be used for preliminary conformational studies by ¹H NOESY NMR.

Scheme 4.10 Reaction of cluster **9P2.6** with propargylamine.

In general, the ¹H resonances shift downfield upon protonation to **[9L3+H]⁺**. Upon close inspection, the resonance assigned to the amide proton shows a significant downfield shift upon protonation (Figure 4.16). This may be attributed to the protonation of the amide group or the presence of hydrogen bonding with the carboxyl group due to conformational change. Considering that no changes in the integration ratios and mass spectrum were observed for cluster **[9L3+H]⁺**, it is likely that hydrogen bonding causes the downfield shift of the amide

proton. This, along with the upfield shift of proton H7, suggests a possible change in the conformation of the phosphine substituents.²⁷⁻³¹

Figure 4.16 ¹H NMR spectra of clusters **9L3** (top) and **[9L3+H]⁺** (bottom) in CDCl₃.

Analysis of clusters **9L3** and **[9L3+H]⁺** were also carried out using a ¹H NOESY experiment. A majority of the NOE cross peaks in cluster **9L3** remain unchanged upon protonation. In the neutral cluster, NOE cross peaks of the amide proton with its neighbouring H2 and H3 protons are observed (Appendix 4.3.7). Upon protonation, an additional interaction is observed, between the amide proton and the tetrazole proton H4 (Figure 4.17). This indicates a possible conformation change, which affects the amide proton upon protonation, which enables it to interact with its neighbouring protons.

Figure 4.17 ^1H NOESY spectra of the protonated cluster $[\mathbf{9L3+H}]^+$. Crosspeaks highlighted in green correspond to the NOE interactions of the amide proton which are not observed in the neutral species.

4.2.2 Conjugation for Conformational Studies

The above protocol was next applied on the orthogonal disubstituted cluster **9P11.12** for conjugation to an oligopeptide. The study on cluster **9P8** has shown that the phosphine centres can be chelated by aliphatic chains consisting of 10 atoms. Thus, **9P11.12** was conjugated to a pentapeptide with a lysine and a propargylglycine (Pra) residue as conjugation sites. Under CuAAC conditions, the conjugate **9A2** was obtained and isolated successfully; mass spectrometric analysis of the product shows the presence of a single pentapeptide in the molecule, along with the expected loss of the NHS ester (Scheme 4.11).

Scheme 4.11 Conjugation of cluster **9P11.12** to the pentapeptide Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (**A2**).

However, upon addition of trifluoroacetic acid to the reaction mixture, no change in the ^1H and ^{31}P NMR spectra was observed, indicating that protonation of the cluster did not occur. This could be due to the steric properties of the pentapeptide **A2**. Firstly, the double bond character of the amide bond may render the peptide backbone more rigid, making it unable to bend to the *cis-cis* conformation.^{26,32} Secondly, the steric bulk of the peptide may also have hindered the conversion to the more congested *cis-cis* conformation. To verify this hypothesis, two less bulky residues were added to the peptide sequence; the heptapeptide **A3** was conjugated to cluster **9P11.12** under similar reaction conditions to yield the chelated cluster **9A3** (Scheme 4.12).

Scheme 4.12 Conjugation of cluster **9P11.12** to heptapeptide Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pr-NHMe and its protonation to cluster **[9A3+H]⁺**.

The ¹H and ³¹P NMR spectra of the crude mixture upon the addition of trifluoroacetic acid show the presence of a triplet hydride resonance, along with the phosphorus resonances corresponding to the *cis-cis* isomer (Figure 4.18). This protonated species could not be isolated, however, and the effect of this isomerisation on the peptide conformation could not be determined.

Figure 4.18 ^1H (top) and $^{31}\text{P}\{^1\text{H}\}$ (bottom) NMR spectra of cluster $[\mathbf{9A3+H}]^+$.

4.3 Conclusion

In this chapter, the effects of protonation of the disubstituted phosphine clusters and the resulting stereochemical change have been investigated. It was found that the protonation of the cluster could induce distance-dependent interactions such as intramolecular hydrogen bonding. Although solid state structures indicate the presence of an intramolecular hydrogen bond upon protonation, NMR spectroscopic studies were not as conclusive. The presence of hydrogen bonding could be investigated further by conjugation of the disubstituted phosphine cluster to more extensive hydrogen bonded systems such as DNA and polyamides.

The ability of the disubstituted phosphine cluster to change the conformation of its substituents was also investigated. Conformational studies carried out on a smaller system indicate that the conformation of the substituents does change upon protonation. Attempts to extend these conformational studies to larger oligopeptides were hampered largely by difficulties with purification and hence characterisation.

4.4 Experimental

Syntheses were carried out using analytical grade, anhydrous solvents and standard Schlenk techniques under an inert argon atmosphere, unless otherwise stated. DCM was dried over calcium hydride while THF was dried using sodium with benzophenone as an indicator. All TLC separations were carried out using Merck 60 F₂₅₄ 20 x 20 cm silica gel TLC plates. Boc-ethylenediamine was prepared according to literature procedures.³³ Oligopeptides were obtained by solid phase peptide synthesis from GL Biochem.

¹H NMR spectra were recorded on a Bruker AV 300 or BBFO 400 NMR spectrometer. Chemical shifts were referenced against the residual ¹H resonance of the deuterated solvent. Solution IR spectra were obtained using DCM on a Bruker Alpha FTIR spectrometer at a resolution of 2 cm⁻¹ using a solution IR cell with NaCl windows and a pathlength of 0.1 mm unless stated. Mass spectrometric data was obtained using methanol as solvent, on a Waters Q-ToF Premier Mass spectrometer or a Thermo Finnigan LCQ Fleet Mass Spectrometer.

4.4.1 Experimental Procedure

4.4.1.1 Preparation of *PPh₂CH₂CH₂COOC(CH₃)₃* (**P7**)

To a solution of 3-(diphenylphosphino)-propionic acid (**P6**) (200 mg, 0.774 mmol) and 4-(dimethyl)aminopyridine (20 mg, 0.155 mmol) in dichloromethane (5 mL) was added *t*-butanol (150 μ L, 1.55 mmol) and 1,3-dicyclohexylcarbodiimide (239 mg, 1.16 mmol). The solution was left to stir at room temperature for 16 h. The crude solution was filtered through silica using DCM as a solvent and the product was used as is without further purification. Yield: 191.9 mg (78.9%)

4.4.1.2 Preparation of *PPh₂CH₂CH₂CONHCH₂CH₂NHCOCH₂CH₂PPh₂* (**P8**)

Ethylenediamine (35 mg, 0.583 mmol) was added to a solution of 3-(diphenylphosphino)-propionic acid (**P6**) (300 mg, 1.162 mmol), HBTU (485 mg, 1.278 mmol) and diisopropylethylamine (164 mg, 1.278 mmol) in DMF (6 mL). The mixture was stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the residue purified by column chromatography using an eluant of DCM:MeOH (95:5, v/v). Yield: 58.3% (366 mg).

4.4.1.3 Preparation of *PPh₂CH₂CH₂CONHCH₂CH₂NHBoc* (**P9**)

Diisopropylamine (0.12 mL, 0.708 mmol) was added to a solution of boc-ethylene diamine (68 mg, 0.424 mmol), 3-(diphenylphosphino)-propionic acid (**P6**) (91 mg, 0.354 mmol)

and HBTU (200 mg, 0.531 mmol) in DMF (5 mL) and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue partitioned between DCM (3 x 20 mL) and water (20 mL). The organic extracts were combined, dried over MgSO_4 , filtered and the solvent removed in vacuo. The crude product was purified by column chromatography using a gradient eluant of DCM:MeOH (98% to 95% DCM, v/v). Yield: Not calculated.

4.4.1.4 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{NH}_2$ (**P10**)

Phosphine **P9** (126 mg, 0.315 mmol) was added to a solution of trifluoroacetic acid (2 mL) in DCM (2 mL) and stirred at room temperature for 1 h. The solvent was removed in vacuo and the residue redissolved in DCM (25 mL) and extracted with saturated NaHCO_3 (2 x 15 mL). The organic layer was dried over MgSO_4 , filtered and the solvent removed in vacuo. The crude product was passed through a short column of silica using a gradient eluant of DCM : MeOH (100 % to 96% DCM, v/v) with 1% triethylamine as an eluant. Yield: 55.4% (52.4 mg)

4.4.1.5 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}$ (*N*-hydroxysuccinimide) (**P11**)

To a solution of 3-(diphenylphosphino)-propionic acid (**P6**) (200 mg, 0.968 mmol) and *N*-hydroxysuccinimide (133.7 mg, 1.162 mmol) in DCM (5 mL) was added 1,3-dicyclohexylcarbodiimide (227 μL , 1.45 mmol). The mixture was stirred at room temperature for 2 h, after which the crude mixture was filtered through silica and the filtrate was dried in vacuo. The crude product was used without further purification. Yield: 97.9% (337 mg).

4.4.1.6 Preparation of $\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_3\text{Br}]$ (**8P3**)

To a solution of cluster **1** (120 mg, 0.130 mmol) in THF (5 mL) was added the phosphine **P3** (48 mg, 0.157 mmol) in THF (2 mL). The mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo and the residue purified by column chromatography using an eluant of DCM : Hexane (1:4, v/v). Yield: 55.2% (85 mg)

4.4.1.7 Preparation of Symmetric Disubstituted Triosmium Carbonyl Clusters $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{R})_2$

To a solution of cluster **2** in THF (5 mL) was added the phosphine in THF (2 mL). The mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo and the residue purified by chromatographic methods using an eluant of DCM : Hexane.

Table 4.1 Synthesis of symmetric disubstituted triosmium carbonyl clusters.

	Cluster 2 (mmol)	Phosphine (mmol)	Yield (%)
9P6 , R = (CH ₂) ₂ COOH	0.053	0.143	17.3
9P7 , R ⁻ = (CH ₂) ₂ COOC(CH ₃) ₃	0.110	0.253	71.9

4.4.1.8 Preparation of Os₃(CO)₁₀(μ-PPh₂CH₂CH₂CONHCH₂CH₂NHCOCH₂CH₂PPh₂) (**9P8**)

To a solution of Os₃(CO)₁₀(ACN)₂ (100 mg, 0.107 mmol) in THF (5 mL) was added phosphine **P8** (75 mg, 0.139 mmol). The mixture was left to stir at room temperature for 2 h. The crude solution was concentrated and purified by TLC using an eluant of DCM : ethyl acetate (1:1, v/v). The major orange band was recovered and identified as the product. Yield: 29.5% (41 mg)

4.4.1.9 Preparation of Asymmetric Disubstituted Triosmium Carbonyl Clusters Os₃(CO)₁₀(PPh₂R¹)(PPh₂R²)

To a solution of Os₃(CO)₁₁(PPh₂R¹) and the phosphine PPh₂R² in ACN at 0°C was added dropwise TMNO (0.95 eq.) in ACN. The mixture was stirred with warming to room temperature for 4 h. The mixture was concentrated and the residue purified by column chromatography using an eluant of DCM.

Table 4.2 Synthesis of asymmetric disubstituted triosmium carbonyl clusters.

	Os ₃ (CO) ₁₁ (PPh ₂ R ¹) (mmol)	PPh ₂ R ² (mmol)	Yield (%)
9P1.6 , R ¹ = (CH ₂) ₂ COO(CH ₂) ₃ Br, R ² = (CH ₂) ₂ COOH	0.095	0.145	41.2
9P1.10 , R ¹ = (CH ₂) ₂ COO(CH ₂) ₃ Br, R ² = (CH ₂) ₂ CONH(CH ₂) ₂ NH ₂	0.063	0.095	38.1

4.4.1.10 Preparation of azide-functionalized clusters $Os_3(CO)_{10}(PPh_2R^1)(PPh_2R^2)$

Sodium azide was added to a solution of the bromide derivative in DMF and the reaction was stirred for 4 h at room temperature. The solvent was removed in vacuo and the residue purified by column chromatography using an eluant of DCM : Hex (95:5, v/v).

Table 4.3 Synthesis of azide-functionalized clusters.

	Bromide derivative (mmol)	NaN ₃ (mmol)	Yield (%)
9P2.6 , R ¹ = (CH ₂) ₂ COO(CH ₂) ₃ N ₃ , R ² = (CH ₂) ₂ COOH	0.039	0.037	65.2
9P2.10 , R ¹ = (CH ₂) ₂ COO(CH ₂) ₃ N ₃ , R ² = (CH ₂) ₂ CONH(CH ₂) ₂ NH ₂	0.011	0.012	Not isolated
9P11.12 R ¹ = (CH ₂) ₂ CO(NHS) R ² = (CH ₂) ₃ N ₃	0.026	0.024	57.3

4.4.1.11 Preparation of *cis-cis* $[Os_3(CO)_{10}(PPh_2CH_2CH_2COOH)][PF_6]$ (**[9P6+H]⁺**)

To a solution of the neutral cluster **9P7** (64 mg, 0.043 mmol) in CDCl₃ (0.4 mL) in an NMR tube was added CF₃COOH (0.013 mL, 0.17 mmol) in CDCl₃ (0.3 mL). The mixture was left to stand for 24 h at room temperature and monitored at 3 h intervals by ¹H NMR. The solvent was then removed and the residue redissolved in MeOH (3 mL). To this solution, was added NH₄PF₆ (15 mg, 0.092 mmol) and the mixture was stirred at room temperature for 16 h. The crude mixture was concentrated and adsorbed onto silica for separation using column chromatography using an eluant of DCM : MeOH (9:1, v/v). Yield: 43.2% (24.9 mg)

4.4.1.12 Preparation of $[Os_3(CO)_{10}(\mu-H)(\mu-$

To a solution of cluster **9P8** in CDCl₃ (11 mg, 0.008 mmol) was added TFA (10 μL, 0.08 mmol). The mixture was left to stand at room temperature for 15 min. After monitoring by ¹H and ³¹P NMR, the reaction was dried in vacuo and the residue redissolved in MeOH (5 mL), to which NH₄PF₆ (6.5 mg, 0.04 mmol) was added. The reaction was stirred at room temperature for 16 h. The solvent was removed and the product extracted using DCM. The residue was purified using TLC using an eluant of DCM : MeOH (92:8, v/v). The major yellow band was recovered and identified as the product. Yield: Not calculated.

4.4.1.13 CuAAC Reaction With Propargylamine (**9L3**)

To a solution of cluster **9P2.6** (35 mg, 0.024 mmol) in DMF was added HBTU (14 mg, 0.036 mmol), DIPEA (8.2 μ L, 0.048 mmol) and propargylamine (3 μ L, 0.036 mmol). The mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue purified by column chromatography using an eluant of DCM : ethyl acetate (95 : 5, v/v). The collected fraction was then reacted with CuI (1 mg, 0.005 mmol) and 2,6-lutidine (2 μ L, 0.013 mmol) in THF (5 mL) for 16 h. The mixture was concentrated and purified by TLC using an eluant of DCM : ethyl acetate (92 : 8, v/v) The band at R_f = 0.3 was collected and identified as the product. Yield: 27.7% (10.2 mg)

4.4.1.14 Preparation of Cis-Cis Chelated Cluster ([**9L3**+H]⁺)

To a solution of the neutral cluster **9L3** (37 mg, 0.025 mmol) in CDCl₃ (0.4 mL) in an NMR tube was added CF₃COOH until a color change was observed. The mixture was left to stand for 15 min at room temperature and the solvent removed in vacuo. The residue was redissolved in MeOH (7 mL) and NH₄PF₆ (10 mg, 0.063 mmol) was added to the solution. The mixture was left to stir at room temperature for 16 h. The crude mixture was concentrated and separated by column chromatography using an eluant Of DCM : MeOH (9:1, v/v). Yield: 9.3% (3.5 mg)

4.4.1.15 CuAAC Reaction With DL-Propargylglycine (**9A1**)

To a solution of cluster **9P2** (30 mg, 0.020 mmol) in THF was added DL-propargylglycine (3.5 mg, 0.029 mmol), CuI (1 mg, 0.004 mmol) and 2,6-lutidine (2 μ L, 0.01 mmol). The mixture was left to stir at room temperature for 16 h. The solvent was removed in vacuo and the crude mixture was analyzed by ¹H NMR. Yield: Not calculated.

4.4.1.16 Conjugation of Pentapeptide Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (**9A2**)

To a solution of cluster **9P11.12** (57 mg, 0.039 mmol) in DMF (5 mL) was added the pentapeptide **A2** (20 mg, 0.027 mmol), CuI (1.5 mg, 0.008 mmol) and 2,6-lutidine (2.3 μ L, 0.02 mmol) the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue washed with methanol (3 x 5 mL). The washings were combined and concentrated for purification by column chromatography using DCM : MeOH (95:5, v/v) as an eluant. Yield: 5% (3.1 mg)

4.4.1.17 Conjugation of Heptapeptide Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pra-NHMe (**9A3**)

To a solution of cluster **9P11.12** (42 mg, 0.028 mmol) in DMF (5 mL) was added the heptapeptide **A3** (15 mg, 0.017 mmol), CuI (1.1 mg, 0.006 mmol) and 2,6-lutidine (1.6 μ L, 0.014 mmol). The mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue washed with methanol (3 x 5 mL). The washings were combined and concentrated for purification by column chromatography using DCM : MeOH (95:5, v/v) as an eluant. Yield: 4% (2.5 mg)

4.4.2 Computational studies

DFT calculations were performed with the Gaussian 09W suite of programs,³⁴ utilizing the hybrid functional of Truhlar and Zhao (M06).³⁵ The LanL2DZ (Los Alamos effective core potential double- ζ) basis set, along with f- type polarization functions were employed for the Os atoms, while the 6-311+G(2d, p) basis set was used for the lighter atoms.^{36,37} Spin-restricted calculations were used for geometry optimization. Harmonic frequencies were then calculated to characterize the stationary points as equilibrium structures with all real frequencies, and to evaluate zero-point energy (ZPE).

4.4.3 Crystallographic Analysis

Crystals of [**9P8**+H]⁺ were grown by slow evaporation of a DCM/ Hexane solution. The crystals chosen were mounted on a quartz fiber. X-ray diffraction data were collected on a Bruker X8 APEX Single Crystal X-ray Diffractometer at 223(2) K, using Mo K α radiation, with the SMART suite of programs.³⁸ Data was processed and corrected for Lorentz and polarization effects with SAINT and for absorption effects with SADABS.³⁹ Hydrogen atoms were placed in calculated positions and refined with a riding model. Appropriate restraints were placed on all disordered parts. Structural solution and refinement were performed with SHELXTL suite of programs.⁴⁰ The structures were solved by direct methods or by Paterson methods to locate the heavy atoms, followed by successive difference maps for the light, non-hydrogen atoms. The metal hydrides were placed by potential energy calculations using the XHYDEX program,⁴¹ with fixed isotropic thermal parameters, while organic hydrogen atoms were placed in calculated positions, assigned isotropic thermal parameters and refined with a riding model. Crystallographic data is as below:

Table 4.4 Crystallographic data for cluster [9P8+H]⁺.

Empirical formula	C43 H37 Cl2 F6 N2 O12 Os3 P3	
Formula weight	1622.16	
Temperature	223(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P $\bar{1}$	
Unit cell dimensions	a = 11.9917(7) Å	$\alpha = 70.0772(17)^\circ$
	b = 15.2972(8) Å	$\beta = 80.9048(19)^\circ$
	c = 16.0614(9) Å	$\gamma = 68.4613(18)^\circ$
Volume	2574.7(2) Å ³	
Z	2	
Density (calculated)	2.092 Mg/m ³	
Absorption coefficient	7.662 mm ⁻¹	
F(000)	1532	
Crystal size	0.36 x 0.36 x 0.32 mm ³	
Theta range for data collection	2.03 to 31.23°.	
Reflections collected	70458	
Independent reflections	16595 [R(int) = 0.0742]	
Max. and min. <i>transmission</i>	0.1930 and 0.1690	
Data / restraints / parameters	16595 / 0 / 652	
Goodness-of-fit on F ²	1.072	
Final R indices [I > 2sigma(I)]	R1 = 0.0391, wR2 = 0.0891	
R indices (all data)	R1 = 0.0625, wR2 = 0.1001	
Largest diff. peak and hole	1.776 and -3.607 e.Å ⁻³	

4.4.4 Experimental data

$\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOC}(\text{CH}_3)_3$ (**P7**).

^1H NMR (CDCl_3) δ /ppm :

1.43 (s, 9H, H3), 2.30 – 2.32 (m, 4H, H1, H2), 7.32 – 7.46 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -14.9.

$\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NHCO}(\text{CH}_2)_2\text{PPh}_2$
(**P8**)

^1H NMR (CDCl_3) δ /ppm :

2.22 – 2.36 (m, 8H, H1, H2, H7, H8), 3.31 – 3.32 (m, 4H, H4, H5), 6.24 (br s, 2H, H3, H6), 7.29 – 7.41 (m, 20H, $\text{H}_{\text{aromatic}}$)

^{31}P NMR (CDCl_3) δ /ppm : -15.9.

$\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NHBoc}$ (**P9**)

^1H NMR (CDCl_3) δ /ppm :

1.26 (s, 9H, H7), 2.21 – 2.29 (m, 2H, H1), 2.35 – 2.39 (m, 2H, H2), 3.25 (br s, 2H, H5), 3.29 – 3.33 (m, 2H, H4), 4.90 (br s, 1H, H6), 6.13 (br s, 1H, H3), 7.33 – 7.46 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -15.0.

$\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2$ (**P10**)

^1H NMR (CDCl_3) δ /ppm :

2.21 – 2.29 (m, 2H, H1), 2.32 – 2.38 (m, 2H, H2), 2.75 (t, 2H, H5, $J = 6$ Hz), 3.22 (q, 2H, H4, $J = 6$ Hz), 6.29 (br s, 1H, H3), 7.30 – 7.42 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -15.6.

$\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}(\text{N-hydroxysuccinimide})$

(**P11**)

^1H NMR (CDCl_3) δ /ppm :

2.41 – 2.46 (m, 2H, H2), 2.63 – 2.71 (m, 2H, H1), 2.82 (s, 4H, H3, H4), 7.34 – 7.46 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -15.0.

$\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_3\text{Br}]$ (**8P3**)

^1H NMR (CDCl_3) δ /ppm :

1.71 – 1.80 (m, 2H, H2), 2.75 – 2.81 (m, 2H, H1), 3.36 (t, 2H, H3, $J = 6.4$ Hz), 7.47 – 7.50 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -10.5.

$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COOH}]_2$ (**9P6**)

$\text{P}^1 = \text{P}^2 =$

^1H NMR (CDCl_3) δ /ppm :

2.26 – 2.28 (m, 2H, H1), 2.97 – 2.98 (m, 2H, H2), 7.45 – 7.50 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -15.5, -11.9, -10.2.

$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COOC}(\text{CH}_3)_3]_2$

(**9P7**)

$\text{P}^1 = \text{P}^2 =$

^1H NMR (CDCl_3) δ /ppm :

1.43 (s, 9H, H3), 2.10 (br s, 2H, H1), 2.91 (br s, 2H, H2), 7.39 – 7.45 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -14.8, -11.6, -10.1.

$\text{Os}_3(\text{CO})_{10}[\mu^-$

$\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NHCO}(\text{CH}_2)_2\text{PPh}_2]$

(9P8)

$\text{P}^1 = \text{P}^2 =$

^1H NMR (CDCl_3) δ /ppm :

2.12 – 2.14 (m, 4H, H2, H7), 3.12 – 3.15 (m, 4H, H1, H8), 3.36 (br s, 4H, H4, H5), 6.50 (br s, 2H, H3, H6) 7.42 – 7.43 (m, 20H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -11.3, -9.8, -8.3

$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$

$[\text{PPh}_2(\text{CH}_2)_2\text{COOH}]$ **(9P1.6)**

$\text{P}^1 =$

$\text{P}^2 =$

^1H NMR (CDCl_3) δ /ppm :

2.10 (quin, 2H, H4, $J = 6.2$ Hz), 2.21 – 2.23 (m, 4H, H1, H6), 2.95 (br s, 4H, H2, H7), 3.38 (t, 2H, H3, $J = 6.4$ Hz), 4.12 – 4.15 (m, 2H, H5), 7.44 – 7.46 (m, 20H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -15.5, -14.8, -11.9, -11.5, -10.7, -10.3.

$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$
 $[\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2]$ (**9P1.10**)

$\text{p}^1 =$

^1H NMR (CDCl_3) δ/ppm :

2.07 – 2.21 (m, 4H, H1, H4, H6), 2.74 (t, 2H, H10, $J = 6\text{Hz}$), 3.17 – 3.22 (m, 2H, H9), 3.36 – 3.39 (m, 2H, H5), 4.12 (t, 2H, H3, $J = 6.2\text{ Hz}$), 5.82 (br s, 1H, H8), 7.41 – 7.44 (m, 20H, $\text{H}_{\text{aromatic}}$)

^{31}P NMR (CDCl_3) δ/ppm : -14.5, -11.5, -11.2, -10.1

$\text{p}^2 =$

$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{N}_3]$

$[\text{PPh}_2(\text{CH}_2)_2\text{COOH}]$ (**9P2.6**)

$\text{P}^1 =$

$\text{P}^2 =$

^1H NMR (CDCl_3) δ /ppm :

1.86 (quin, 2H, H4, $J = 6.4$ Hz), 2.25 (br s, 4H, H1, H6), 2.97 (br s, 4H, H2, H7), 3.34 (t, 2H, H5, $J = 6.6$ Hz), 4.10 (t, 2H, H3, $J = 6.2$ Hz), 7.48 (br s, 20H, $\text{H}_{\text{aromatic}}$)

^{31}P NMR (CDCl_3) δ /ppm : -15.5, -14.8, -12.0, -11.5, -10.7, -10.3.

$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_3\text{N}_3]$

$[\text{PPh}_2(\text{CH}_2)_2\text{CO}(\text{NHS})]$ (**9P11.12**)

$\text{P}^1 =$

$\text{P}^2 =$

^1H NMR (CDCl_3) δ /ppm :

1.48 (br s, 2H, H2), 2.46 (br s, 2H, H1), 2.68 (br s, 2H, H4), 2.81 (br s, 4H, H6, H7), 3.00 (br s, 2H, H5), 3.27 (t, 2H, H3, $J = 6.0$ Hz), 7.46 – 7.47 (br s, 20H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -15.6, -14.6, -12.2, -11.4, -10.4.

ESI-MS : 1478.1 $[\text{M}+\text{H}]^+$.

Peptide conjugate **9A2**

^{31}P NMR (DMSO) δ/ppm : -9.3, -11.1, -13.1.

ESI-HRMS (MeOH) m/z : 2119.5 $[M+Na]^+$

Peptide conjugate **9A3**

³¹P NMR (DMSO) δ/ppm : -16.1, -15.9, -13.7, -11.0, -9.4.

ESI-MS (MeOH) m/z : 2225.6 $[M+H]^+$.

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Chapter 5. Disubstituted Phosphine Clusters as pH Sensors

The stereochemical change in the disubstituted triosmium carbonyl phosphine clusters upon protonation leads to not only changes in the NMR spectra,¹ but also changes in their infrared (IR) and UV-vis spectra (Figures 5.1 and 5.2). These spectrochemical changes present an opportunity to utilize the cluster as a pH-responsive agent. Other triosmium carbonyl clusters, such as $\text{Os}_3(\text{CO})_{12}$, can also be protonated but require highly acidic conditions.¹⁻³

Figure 5.1 Infrared spectrum of neutral disubstituted triosmium carbonyl phosphine cluster **9P13** and its protonated form (right).

Figure 5.2 Color change upon conversion of cluster **9P13** (orange) to cluster **[9P13+H]⁺** (yellow).

The protonation of the cluster is dependent on the electron density of the metal-metal bond – more electron rich clusters would be protonated more easily. The electron density of

the metal cluster can be tuned by altering its ligand sphere. It is expected that the substitution of carbonyl groups on the cluster with more electron-donating ligands will increase the electron density on the metal cluster.² It is therefore possible to have triosmium carbonyl clusters which can be protonated at different pH.

Substituted triosmium carbonyl clusters such as cluster **9P** show good stability and low cytotoxicity in biological systems (Chapter 3). Therefore, they may be developed as potential intracellular pH sensors. Although numerous pH-responsive agents have been developed to date, only a few have been successfully used as intracellular pH sensors.⁴⁻⁷ Intracellular pH sensing is of great interest since many cell functions and processes are regulated by the acidity of the intracellular environment. Several inorganic complexes such as the iridium polypyridyl, rhenium and ruthenium bipyridine systems have been developed as intracellular pH sensors; these utilize fluorescence as the detection method (Figure 5.3).^{8,9}

Figure 5.3 Small molecule intracellular pH probes.

Although protonation can, and has previously been, detected by IR, its use in biological systems is not feasible due to interference from water. A good alternative is its complement, Raman spectroscopy. In recent years, triosmium carbonyl clusters immobilized on SERS-active surfaces have been utilized effectively in bioassays.^{10,11} A similar concept may be utilized for the development of pH sensors using SERS, although this would require prior functionalization of the clusters with thiol groups for its immobilization onto gold solid supports (Figure 5.4).

Figure 5.4 Triosmium carbonyl clusters as intracellular pH sensors.

5.1 Screening Triosmium Carbonyl Clusters for pH Sensing

In principle, pH responsive probes should demonstrate a fast conversion to a stable protonated species. Although the protonation of substituted phosphine clusters has been established, detailed studies on its reaction time and stability of the protonated product have not been carried out. Protonation of mono-, di- and tri-substituted phosphine clusters of triosmium carbonyl were thus compared to the μ -S clusters, which are also known to be protonated.² Clusters **8P13**, **9P13** and **6L4** were prepared for the preliminary investigations by reaction with the “lightly-stabilized” clusters **1** and **2**. The tri-substituted phosphine cluster **16P13** was prepared following literature methods by reflux of $\text{Os}_3(\text{CO})_{12}$ with triphenylphosphine (Scheme 5.1).²

Scheme 5.1 Synthesis of substituted triosmium carbonyl clusters for preliminary studies.

The response time of these clusters was examined by monitoring the protonation over 15 mins by infrared spectroscopy. Due to the limited solubility of the clusters in aqueous systems, the protonation was carried out in organic solvents, using trifluoroacetic acid. Although changes are observed for all the substituted phosphine clusters, only the di- and tri-

substituted phosphine clusters **9P13** and **16P13** show significant changes after 5 mins (Figures 5.5 – 5.7).

Figure 5.5 Infrared spectra of clusters **8P13** upon protonation.

Figure 5.6 Infrared spectra of clusters **9P13** upon protonation.

5.2 Anchoring Disubstituted Phosphine Clusters on Gold Surfaces

Since the disubstituted phosphine cluster has shown good potential to be developed as pH-responsive agents, they can be further developed for pH sensing by SERS. This would require immobilization onto gold surfaces for Raman signal enhancement. It has been shown previously that physical adsorption of the disubstituted phosphine cluster is insufficient for signal enhancement to occur (Chapter 3). Thus the clusters would have to be chemically bonded to the gold surface; functionalization of the disubstituted phosphine clusters with thiol groups are required.

The functionalization of disubstituted phosphine clusters with thiol groups was first attempted by preparation of the thiol-functionalized phosphine **P14**. However, the reaction of phosphine **P14** with the lightly stabilized triosmium cluster **2** yielded the μ -S bridged cluster **6L5** instead. An attempt to prevent the formation of **6L5** through reaction with cluster **1** still afforded **6L5** as the major product, with the desired mono-substituted cluster **8P14** as a minor product (Scheme 5.2).

Scheme 5.2 Reaction of triosmium carbonyl clusters with phosphine **P14**.

The alternative strategy of functionalizing the phosphine ligands in a disubstituted phosphine cluster with a thiol group was explored. This was achieved by reaction of the activated cluster **9P11** with 4-aminothiophenol and cysteamine. Symmetric, as well as asymmetric disubstituted phosphine clusters were prepared via similar methods in moderate yields (Schemes 5.3 and 5.4).

Scheme 5.3 Synthesis of symmetric thiol-functionalized disubstituted triosmium carbonyl phosphine clusters.

Scheme 5.4 Synthesis of asymmetric thiol-functionalized disubstituted triosmium carbonyl phosphine cluster.

Initial attempts to chemically append clusters **9P15**, **9P16** and **9P13.15** to gold nanoparticles were unsuccessful, as no SERS enhancement was observed. This could be due to the limited solubility of the clusters in aqueous media, causing it to precipitate before attachment to the gold surface. In order to overcome this, attempts were made to immobilize the clusters onto a bimetallic film over nanoparticle chip (BMFON).

The BMFON substrate comprises a gold and silver bimetallic coat over polystyrene nanostructures deposited on a glass slide.¹² Such substrates have demonstrated good stability and strong SERS enhancement of reporters anchored onto its surface.¹³ For example,

triosmium carbonyl clusters bonded to BMFON substrates have been utilized for the detection of biomarkers.¹¹ In addition to its stability, the BMFON chip is more tolerant toward organic solvents as compared to colloidal gold. Attempts to anchor clusters **9P15**, **9P16** and **9P13.15** onto the BMFON substrate was carried out in dichloromethane (Figure 5.9).

Figure 5.9 Anchoring the substituted phosphine clusters onto the BMFON substrate.

However, the SERS spectra show a lack of significant Raman signals in the 1800 – 2000 cm^{-1} region (Figure 5.10). The lack of signals is surprising considering that clusters functionalized with thiol groups should exhibit a significant enhancement of its carbonyl stretches. A survey of literature showed that aggregation of nanoparticles to an optimal particle size is important in order to achieve good enhancement.¹⁴⁻¹⁷ This aggregation is influenced by SERS reporters bonded to the surface of the nanoparticles. It is therefore likely that the clusters interfered with the aggregation of the gold nanoparticles. Since aggregation of the gold nanoparticles should exhibit a red shift in the absorption maximum of the colloidal solution, it is possible to monitor the aggregation by UV-Vis spectroscopy. The influence of the phosphine substituted clusters on the nanoparticle aggregation was thus investigated.

Figure 5.10 Raman spectrum of clusters **9P15**, **9P16** and **9P13.15** anchored onto the BMFON chip.

In a typical preparation, a solution of the cluster in DMSO was added to the colloidal gold solution and left to incubate for an hour. Excess cluster was removed by multiple cycles of washing and centrifugation (Figure 5.11). This procedure was carried out with **8P14** and **9P15**, as well as with **6L6** which has previously shown good SERS enhancement.¹⁸

Figure 5.11 Preparation of cluster nanoparticle conjugates for SERS measurements.

The non-conjugated colloidal gold nanoparticles show an absorption maximum at ~535 nm. After three cycles of centrifugation, the absorption intensity of the suspension decreases due to precipitation of the nanoparticles, although no absorption at 700 nm is observed. The nanoparticle conjugates of cluster **6L6** exhibit similar absorption spectra, although a slight red-shift in its absorption maximum is observed, suggesting some aggregation (Figure 5.12)

Figure 5.12 Uv-vis absorption spectra of non-conjugated gold nanoparticles (left), and gold nanoparticles incubated with cluster **6L6** (right).

Surprisingly, gold nanoparticle-conjugates of the phosphine substituted clusters **8P14** and **9P15** show a decrease in the absorption at 535 nm, and no absorption observed at 700

nm. In the case of the disubstituted phosphine cluster **9P15**, the absorption drops drastically and the colloidal solution becomes almost colorless after three cycles of centrifugation (Figure 5.13). The loss of the distinct colloidal gold absorption is an indication that cluster **9P15** probably reacts with the gold nanoparticles, leading to their breakdown. This may account for the lack of Raman signal enhancement.

Figure 5.13 Uv-vis absorption of gold nanoparticles incubated with cluster **8P14** and **9P15** at 50 μ M.

5.3 Phosphine-Substituted μ -S Clusters for pH Sensing

Bridged μ -S clusters such as **6L6** show good SERS enhancement making them attractive as an alternative platform for pH sensing. However, they are not protonated easily and hence their basicity has to be increased, for example, by substitution with phosphines. The protonation of phosphine-substituted μ -S clusters, such as, **17P** and **18P** has been reported previously (Figure 5.14).^{19,20} These clusters can thus serve as pH-responsive agents, if appropriately functionalized for attachment to the gold surface.

Figure 5.14 Phosphine-substituted μ -S clusters.

8P17, R = CH₃
8P18, R = O(CH₃)

17P17, R = CH₃
17P18, R = O(CH₃)

In principle, the thiolato bridge may be formed between the unsubstituted osmium centers or at the phosphine-substituted osmium atom, giving rise to two possible regioisomers, **i** and **ii**. However, a singlet is observed in the hydride region of the ^1H NMR spectrum of **17P17**, which suggests that the thiol ligand bridges the unsubstituted osmium atoms. The pattern of the CO stretches in the infrared spectrum is also similar to that of other $[\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SR})(\text{PR}_3)]$ clusters which are known to adopt structure **ii**.²⁰

Similar reaction conditions were also adopted for the synthesis of the disubstituted clusters **18P**. Once again, the thiolato bridge can be formed between the phosphine-substituted osmium centres or across the unique osmium centre, giving rise to two possible regioisomers (and their stereoisomers).²⁰ Several hydride peaks were observed in the ¹H NMR

spectrum of the crude solution, corresponding to these isomers. However, they could not be isolated by chromatographic methods (Scheme 5.6).

Scheme 5.6 Reaction of clusters **9P** with 1,2-ethanedithiol.

The protonation of cluster **17P17** was next examined. Upon addition of trifluoroacetic acid, an additional doublet is observed in the hydride region of the ¹H NMR spectrum, along with a shift in the ³¹P resonance (Figures 5.16). These indicate a complete conversion to the protonated species, with the protonation across one of the unbridged Os-Os bond edges. Perhaps more interestingly, the protonation appears to be reversible, since the doublet hydride signal is lost upon removal of the acid from the solution. Although a slight shift in the hydride resonance is observed, the ³¹P{¹H} NMR spectrum indicates a conversion back to the neutral species.

Figure 5.16 Hydride region of the 1H NMR spectra (top) and $^{31}P\{^1H\}$ NMR spectra (bottom) of cluster **17P17** upon protonation. Resonances marked (*) are likely due to the decomposition of the cluster.

Further investigations on the reversibility of this protonation were also carried out by addition of a base to a solution of cluster $[17P17+H]^+$. The infrared spectra indicate that the proton can be removed to form the neutral species **17P17** once more (Figure 5.18).

Figure 5.18 Infrared spectrum of cluster **17P17** upon addition (blue) and removal of trifluoroacetic acid (red). All spectra were recorded in dichloroethane.

In contrast to the di-substituted phosphine cluster **9P15**, when **17P17** is incubated with gold nanoparticles, a broad absorption at 700 nm appears almost immediately upon addition of the cluster. Incubation for a longer period results in a significant decrease in the absorption at 535 nm, and the broad absorption at 700 nm shifts to longer wavelengths (Figure 5.19). These observations suggest that cluster **17P17** induces the aggregation of nanoparticles when in excess. On the other hand, the Raman spectrum of cluster **17P17** anchored onto BMFON shows significant enhancement of the carbonyl stretch at 2077 cm^{-1} (Figures 5.20). This shows that it can make a suitable SERS-based pH-responsive agent.

Figure 5.19 Uv-vis absorption spectra of gold nanoparticles incubated with cluster **17P17** ($50\mu\text{M}$).

Figure 5.20 Raman spectrum of cluster **17P17** anchored on the BMFON substrate (top) and its infrared spectrum recorded in cyclohexane (bottom).

The pH-response range of clusters **17P17** and **17P18** were measured as solutions in 1,2-dichloroethane due to their poor solubility in aqueous medium. Their protonation with trifluoroacetic acid could be followed by changes in both their infrared and Uv-vis spectra. The IR spectrum of **17P17** in the 2150 – 2100 cm^{-1} region (Figure 5.21) can be integrated to provide a measure of the response of the cluster to the acid. The plots of the response which shows that the effective range of cluster **17P18** (pH 0.6 – 1.6) is slightly lower than that of cluster **17P17** (pH 1 – 2) (Figure 5.22). Similar results were obtained from the UV-vis spectra. This is as expected for the less electron-donating $\text{P}(\text{OMe})_3$ in **17P18** compared to the more electron-donating PMe_3 in **17P17**. Nevertheless, both clusters are only effective as pH sensors in the pH 1 – 2 range. As intracellular pH sensors, this is rather undesirable since the pH of cells typically range from pH 5– 7.

Figure 5.21 Infrared spectrum in absorbance mode of cluster **17P17** at different pH.

Figure 5.22 Effective range of clusters **17P17** (red) and **17P18** (blue), monitored by IR spectroscopy. Integration was carried out from 2101 – 2156 cm^{-1} for both clusters.

5.4 Conclusion

In this chapter, the spectral changes accompanying the protonation of phosphine-substituted triosmium carbonyl clusters was explored for development into a SERS-based pH sensing platform. Disubstituted phosphine clusters were found to exhibit interaction with the

gold surface, which limited their use, although the exact nature of this interaction needs further investigation. On the other hand, phosphine-substituted thiolato-bridged triosmium carbonyl clusters were found to be reversibly protonated and could be successfully anchored onto the BMFON surface. Unfortunately, the effective pH range was very low, making them unsuitable for intracellular pH sensing. Nevertheless, it was also shown that the electron density of the cluster, and hence the pH range, could be tuned through the nature of the phosphine ligand.

5.5 Experimental

Syntheses were carried out using analytical grade, anhydrous solvents and standard Schlenk techniques under an inert argon atmosphere, unless otherwise stated. DCM was dried over calcium hydride while THF was dried using sodium with benzophenone as an indicator. All TLC separations were carried out using Merck 60 F₂₅₄ 20 x 20 cm silica gel TLC plates. Compounds **6L4**,²¹ **8P13**, **8P17**, **8P18**,² **9P13**,²² **16P13**,¹⁹ were synthesized according to literature methods. Gold nanoparticles (60 nm) were purchased from Synergy Scientific.

¹H NMR spectra were recorded on a Bruker AV 300 or BBFO 400 NMR spectrometer. Chemical shifts were referenced against the residual ¹H resonance of the deuterated solvent. Solution IR spectra were obtained using DCM on a Bruker Alpha FTIR spectrometer at a resolution of 2 cm⁻¹ using a solution IR cell with NaCl or CaF₂ windows and a pathlength of 0.1 mm unless stated. Mass spectrometric data was obtained using methanol as solvent, on a Waters Q-ToF Premier Mass spectrometer or a Thermo Finnigan LCQ Fleet Mass Spectrometer. All UV-Vis absorption spectra were measured on a Shimadzu UV 3600 UV-Vis-NIR Spectrometer in dichloroethane for the clusters or water for the nanoparticle-cluster conjugates, unless stated.

Raman spectral measurements were carried out using a Renishaw InVia Raman (UK) microscope with a Peltier cooled CCD detector and an excitation wavelength at 785 nm, where the laser beam is directed to the sample through a 50× objective lens, which was used to excite the sample and also to collect the return Raman signal. All Raman spectra were processed with WiRE3.0 software. The maximum laser power at the sample was measured to be 6.2 mW and the exposure time was set at 10s throughout the measurements. Prior to each measurement, the instrument was calibrated with a silicon standard whose Raman peak is centered at 520 cm⁻¹.

5.5.1 Experimental Procedure

5.5.1.1 Screening the Protonation of Triosmium Carbonyl Clusters by Infrared Spectroscopy

Trifluoroacetic acid (20 μ L, 0.261 mmol) was added to a solution of clusters (0.005 mmol) in DCM (5 mL). The mixture was stirred at room temperature with regular monitoring by infrared spectroscopy every 5 min for 15 min using the CaF_2 cell.

5.5.1.2 Preparation of $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$ (**P14**)

Thiourea (37.3 mg, 0.490 mmol) was added to a solution of phosphine **P3** (100 mg, 0.327 mmol) in DMF (5 mL) and the solution was degassed by 3 cycles of freeze-pump-thaw. The mixture was heated at 100°C for 16 h. A 2M NaOH solution (2 mL) was added to the mixture and degassed again by 3 cycles of freeze-pump-thaw. The mixture was heated once more for 2 hours at 100°C. The solvent was removed in vacuo and the residue partitioned between sat NH_4Cl (20 mL) and DCM (2 x 15 mL). The organic extracts were combined and washed once more with water (20 mL), dried over MgSO_4 , filtered and dried in vacuo. The residue was purified by column chromatography using an eluant of DCM : Hexane (2 : 3, v/v). Yield : 35 mg (41.3%)

5.5.1.3 Reaction of Cluster **2** with Phosphine **P14**

A solution of phosphine **P14** (32.1 mg, 0.123 mmol) was added to a solution of cluster **2** (50 mg, 0.054 mmol) in THF (5 mL) at 0°C and the mixture was stirred for 2 h with warming to room temperature. The solvent was removed in vacuo and the residue purified by TLC using an eluant of DCM : Hex (2 : 3, v/v). The major yellow band was recovered and identified as cluster **6L5** instead.

5.5.1.4 Preparation of $\text{Os}_3(\text{CO})_{11}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH})$ (**8P14**)

A solution of phosphine **P14** was added to a solution of cluster $\text{Os}_3(\text{CO})_{11}(\text{ACN})$ (**1**) in THF at 0°C and the mixture was stirred for 2 h with warming to room temperature. The solvent was removed in vacuo and the residue purified by TLC using an eluant of DCM : Hex (2 : 3, v/v). The major yellow band was recovered and identified as cluster **6L5** while the minor yellow band was identified as cluster **8P14**. Yield : Not calculated

5.5.1.5 Preparation of $Os_3(CO)_{10}[PPh_2CH_2CH_2COO(N\text{-hydroxysuccinimide})]_2$ (**9P11**)

Phosphine **P11** (90.3 mg, 0.254 mmol) was added to a solution of $Os_3(CO)_{10}(ACN)_2$ (100 mg, 0.107 mmol) in THF. The mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo and the crude mixture was purified by column chromatography using DCM : ethyl acetate as an eluant (95 : 5, v/v). Yield : 135 mg (80.8%)

5.5.1.6 Reaction of NHS Ester Derivatives with $H_2N\text{-R-SH}$

To a solution of the cluster precursor in THF in a Carius tube was added $H_2N\text{-R-SH}$. The mixture was degassed by 3 cycles of freeze-pump-thaw and was heated at 60°C for 2h. The solvent was removed in vacuo and the residue purified by column chromatography using an eluant of DCM : ethyl acetate.

Table 5.1 Functionalization of disubstituted phosphine clusters with thiol groups.

	Cluster precursor (mmol)	$H_2N\text{-R-SH}$ (mmol)	Yield (%)
9P15 , R = $(CH_2)_2$	0.029	0.061	18.8
9P16 , R = C_6H_4	0.045	0.063	39.7
9P11.15 , R = $(CH_2)_2$	0.025	0.032	38.0

5.5.1.7 Preparation of $Os_3(CO)_{10}(PPh_3)[PPh_2CH_2CH_2CO(NHS)]$ (**9P11.13**)

To a solution of cluster **8P13** (50 mg, 0.044 mmol) and phosphine **P11** (20.3 mg, 0.057 mmol) in DCM (10 mL) was added a solution of TMNO (3.2 mg, 0.042 mmol) in ACN (20 mL). The mixture was stirred at room temperature for 16 h and the solvent was removed in vacuo. The residue was purified by column chromatography using DCM as an eluant. Yield : 56.8% (36.6 mg)

5.5.1.8 Preparation of $Os_3(CO)_{10}(\mu\text{-H})[\mu\text{-S}(CH_2)_2SH](PR_3)$ (**17P17-18**)

A solution of TMNO (1.2 eq) in ACN (5 mL) was added dropwise to a solution of $Os_3(CO)_{11}(PR_3)$ and 1,2-ethanedithiol (1.5 eq.) in DCM (5mL) and the mixture was stirred at room temperature for 3 hours with bubbling of argon. The solvent was removed in vacuo and the residue redissolved in a minimal amount of DCM. The crude solution was purified by TLC using an eluant of DCM : Hexane (2 : 3, v/v). The major yellow band ($R_f = 0.46$) was recovered using dichloromethane.

Table 5.2 Preparation of clusters **17P**.

	Os ₃ (CO) ₁₁ (PR ₃) (mmol)	Yield (%)
17P17 , R = CH ₃	0.026	35.6
17P18 , R = O(CH ₃)	0.032	30.6

5.5.1.9 Immobilization of **17P17** on Solid Support

Two BMFON chips were immersed in a 5 mM solution (500 μ L) of cluster **17P17** in dichloromethane (DCM) for 2 hours at room temperature. The chips were then washed with DCM (2x) and left to dry at ambient temperature, after which, the SERS measurement was taken.

5.5.1.10 Monitoring The Response Of Clusters **17P17** and **17P18** toward Acid

Stock solutions of trifluoroacetic acid (10 μ L/mL), and of clusters **17P17** and **17P18** (0.5 mM), in dichloroethane (DCE) were prepared. The TFA stock solution was added to the cluster in the following amounts and the solution was topped up to a final volume of 500 μ L. The solution was left to stir at room temperature for 5 mins after which, the IR or UV-Vis spectra of the crude solution was measured.

Table 5.3 Monitoring the response of the cluster at different pH.

Cluster (0.5 mM) (μ L)	CF ₃ COOH (10 μ L/mL) (μ L)	DCE (mL)	Final Volume (mL)	[H ⁺] (mmol/L)	pH
100.0	1.6	898.4	1.0	0.2	3.68
100.0	4.0	896.0	1.0	0.5	3.28
100.0	10.0	890.0	1.0	1.3	2.88
100.0	48.0	852.0	1.0	6.3	2.20
100.0	96.0	804.0	1.0	12.5	1.90
100.0	190.0	710.0	1.0	24.8	1.60
100.0	286.0	614.0	1.0	37.4	1.43
100.0	384.0	516.0	1.0	67.4	1.17
100.0	584.0	326.0	1.0	76.3	1.12
CF ₃ COOH (μ L)					
100.0	10.0	980.0	1.0	130.7	0.88
100.0	20.0	960.0	1.0	261.4	0.58
100.0	30.0	940.0	1.0	392.1	0.41

* Concentration of H⁺ calculated assuming complete dissociation of CF₃COOH

5.5.1.11 Incubation of Clusters with Gold Nanoparticles

Stock solutions (50 mM) of the clusters in DMSO were prepared. To a colloidal suspension of gold nanoparticles (1 mL) was added the stock solution of the cluster to a final concentration of 50 μ M. The mixture was incubated on an orbital shaker for 1 hour, after which the UV-vis spectrum was measured. The suspension was pelleted by centrifugation at 4000 rpm for 5 mins. The pellet was re-suspended in H₂O (1 mL) and the Uv-vis spectrum was measured. Two more cycles of centrifugation and resuspension were repeated.

5.5.2 Experimental Data

$\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$ (**P14**)

^1H NMR (CDCl_3) δ /ppm :

1.35 (t, 1H, H4, $3J = 8\text{Hz}$), 1.73 – 1.79 (m, 2H, H2), 2.15 – 2.19 (m, 2H, H1), 2.61 – 2.66 (m, 2H, H3), 7.34 – 7.46 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -17.0

$\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{CH}_2\text{PPh}_2)$ (**6L5**)

^1H NMR (CDCl_3) δ /ppm :

-17.4 (s, 1H, $\mu\text{-H}$), 1.73 – 1.75 (m, 2H, H2), 2.13 – 2.17 (m, 2H, H1), 2.42 – 2.45 (m, 2H, H3), 7.33 – 7.41 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -16.9.

$\text{Os}_3(\text{CO})_{11}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH})$ (**8P14**).

^1H NMR (CDCl_3) δ /ppm :

1.49 – 1.53 (m, 2H, H2), 2.50 – 2.56 (br s, 2H, H1), 2.72 – 2.78 (m, 2H, H3), 7.46 – 7.49 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -9.9.

$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2\text{CH}_2\text{CH}_2\text{COO}(\text{N-hydroxysuccinimide})]_2$ (**9P11**)

^1H NMR (CDCl_3) δ /ppm :

2.46 (br s, 2H, H1), 2.82 (s, 4H, H3, H4), 3.00 – 3.02 (m, 2H, H2), 7.47 – 7.49 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -10.7, -12.1, -15.4.

$\text{Os}_3(\text{CO})_{10}(\text{PPh}_3)(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}(\text{NHS}))$ (**9P11.13**)

^1H NMR (CDCl_3) δ /ppm :

2.46 (br s, 2H, H1), 2.80 (br s, 4H, H3, H4), 3.02 (br s, 2H, H2), 7.37 – 7.51 (m, 25 H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -14.7, -12.5, -11.2, -10.4, -9.4

$\text{Os}_3(\text{CO})_{10}(\text{PPh}_3)$

$(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{SH})$ (**9P13.15**)

^1H NMR (CDCl_3) δ/ppm :

1.24 – 1.29 (m, 1H, -SH), 2.08 – 2.09 (m, 2H, H1), 2.55 – 2.60 (m, 2H, H4), 3.01 (br s, 2H, H2), 3.30 – 3.35 (m, 2H, H3), 6.23 (m, 1H, -NH), 7.40 – 7.45 (m, 25 H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ/ppm : -12.9, -9.9, -5.3, -1.7

ESI-HRMS (MeOH) m/z : 1432.04 $[\text{M}+\text{H}]^+$

$\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{SH})_2$

(**9P15**)

^1H NMR (CDCl_3) δ/ppm :

1.28 – 1.30 (m, 1H, H6), 2.08 – 2.12 (m, 2H, H2), 2.56 – 2.61 (m, 2H, H5), 3.03 (br s, 2H, H1), 3.31 – 3.36 (m, 2H, H4), 5.76 (br s, 1H, H3), 7.43 – 7.45 (m, 10H, $\text{H}_{\text{aromatic}}$)

^{31}P NMR (CDCl_3) δ/ppm : -9.4, -11.1, -13.9

ESI-HRMS (MeOH) m/z : 1487.06 $[\text{M}+\text{H}]^+$

$\text{Os}_3(\text{CO})_{10} [\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONH}(\text{C}_6\text{H}_4)\text{SH}]_2$

(9P16)

^1H NMR (CDCl_3) δ /ppm :

2.26 – 2.27 (m, 2H, H1), 3.11 (br s, 2H, H2), 3.78 (br s, 1H, -SH), 6.54 – 6.56 (m, 2H, H4), 7.22 – 7.25 (m, 3H, H3, -NH), 7.31 – 7.49 (m, 10H, $\text{H}_{\text{aromatic}}$).

^{31}P NMR (CDCl_3) δ /ppm : -9.3, -11.2, -13.9.

ESI-HRMS (MeOH) m/z : 1583.08 $[\text{M}+\text{H}]^+$

$\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{CH}_3)_3]$

(17P17)

^1H NMR (CDCl_3) δ /ppm :

-17.07 (s, 1H, $\mu\text{-H}$), 1.66 (t, 1H, -SH, $^3J = 8.4$ Hz), 1.86 (d, 9H, -CH_3 , $J = 10$ Hz), 2.51 – 2.55 (m, 2H, H1) 2.23 – 2.73 (m, 2H, H2).

^{31}P NMR (CDCl_3) δ /ppm : - 53.5.

IR (cyclohexane) ν/cm^{-1} : 2082 (w), 2050 (s), 2024 (m), 2000 (s), 1985 (m), 1963 (w), 1957 (w).

ESI-HRMS (MeOH) m/z : 994.86 $[\text{M}+\text{H}]^+$

$\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{OCH}_3)_3]$

(17P18)

^1H NMR (CDCl_3) δ /ppm :

-17.25 (s, 1H, $\mu\text{-H}$), 1.68 (t, 1H, -SH , $^3J = 8.4$ Hz), 2.50 – 2.56 (m, 2H, H1) 2.23 – 2.73 (m, 2H, H2), 3.59 (d, 9H, -OCH_3 , $J = 12$ Hz).

^{31}P NMR (CDCl_3) δ /ppm : 101.6.

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Chapter 6. Conclusion

In this work, the conjugation of triosmium carbonyl clusters to two classes of biomolecules was explored.

The conjugation of triosmium carbonyl clusters to nucleosides was achieved via two routes –direct ligand displacement and the CuAAC click chemistry reaction (Scheme 6.1). Both methods required prior functionalization of the nucleoside, at the C⁵ and N³ position of the thymidine. The CuAAC reaction, in particular, proved to be rather versatile and was also extended for the conjugation of a hexaosmium carbonyl raft cluster.

Direct ligand displacement

Copper-catalyzed azide-alkyne cycloaddition (CuAAC)

Scheme 6.1 Conjugation of triosmium carbonyl clusters to nucleotides.

The potential of these conjugates as therapeutic and imaging agents were examined. In general, the conjugation was found to improve the solubility and uptake of the clusters into cancer cells, and several of the conjugates showed improved anti-proliferative activity (Figure 6.1a). Imaging of cancer cells by SERS was also attempted although this was met with some difficulty in the immobilization of the cluster onto gold nanoparticles (Figure 6.1b).

The success of the CuAAC reaction for the conjugation of triosmium and hexaosmium carbonyl clusters to nucleosides was also utilised in the labelling of oligonucleotides with these clusters (Figure 6.1c). TEM imaging of the labelled DNA strands on graphene was attempted, although this was hindered by the presence of carbonaceous material on the graphene surface.

a) Therapeutic agents

b) SERS imaging agents

c) TEM imaging

Figure 6.1 Applications of triosmium carbonyl – nucleoside conjugates.

Triosmium carbonyl clusters were also conjugated to oligopeptides via the CuAAC reaction. The reaction was found to be rather tolerant to the amide coupling reaction and these two reactions simultaneously for the conjugation of a single oligopeptide to a disubstituted phosphine triosmium carbonyl cluster (Scheme 6.2).

Scheme 6.2 Conjugation of oligopeptides to orthogonal disubstituted phosphine cluster.

Disubstituted phosphine derivatives of triosmium carbonyl clusters can exist in different isomeric forms which differ in the relative stereochemistry of the two phosphine ligands. This isomer distribution can be changed upon protonation (Scheme 6.3)

Scheme 6.3 Isomeric distribution upon protonation of disubstituted phosphine derivatives.

The protonation of these clusters was used to effect conformational changes and intramolecular interactions in the cluster-oligopeptide conjugate (Figure 6.2). Protonation was found to induce intramolecular interactions between the substituents on the two phosphines, as well as conformational changes on the substituents.

The protonation of the cluster was also explored for the development of SERS-based pH sensors. Phosphine substituted derivatives of a thiolato-bridged cluster allowed for anchoring onto a SERS-active solid support (BMFON) via the thiolato ligand. Although the protonation currently occurs at pH 1 – 2, this may be tuned via the electronic properties of the donor ligands. More electron-donating ligands such as N-heterocyclic carbenes may thus be explored in the future.

Figure 6.2 Applications of the protonation of the disubstituted phosphine clusters.

From this work, it is apparent that there are several aspects which are important for successful and effective bioconjugation to be carried out. Firstly, the reactivity and stability of the organometallic species need to be taken into consideration; many of these complexes decompose in aqueous or polar solvent systems which are favourable for the biomolecule. This was especially pronounced in the direct ligand displacement reactions, which employ reactive clusters which are susceptible to nucleophilic attack by polar organic solvents.

Secondly, most of the triosmium carbonyl clusters have poor solubility in aqueous and polar solvent systems. This has led to poor yields, even with excess amounts of the cluster. The solubility of the cluster in aqueous systems therefore, needs to be improved for in situ conjugation

to biomolecules. This may be carried out via two methods. The first approach involves functionalization of the cluster with water soluble ligands such as the TPPTS ligand (Figure 6.3). The second approach involves the preparation of cationic clusters, such as the protonated substituted phosphine clusters, which are more polar and therefore, more soluble in polar solvent systems.

i) Water soluble ligands

ii) Preparation of cationic clusters for conjugation

Figure 6.3 Strategies to improve the solubility of triosmium carbonyl clusters.

The CuAAC reaction has been employed rather successfully for the conjugation of triosmium carbonyl clusters to both oligopeptides and nucleotides. It is also orthogonal to other reactions such as the amide coupling reaction. However, this reaction requires the biomolecule to be functionalized with the appropriate azide or alkyne group for in situ conjugation, although advances have been made in the introduction of unnatural amino acids and nucleotides into biological systems in recent years which.¹⁻³ Nevertheless, other conjugation reactions such as the maleimide-thiol reaction should also be explored for the in situ conjugation of triosmium carbonyl clusters to oligopeptides (Figure 6.4).

Figure 6.4 Maleimide-thiol reaction for conjugation of oligopeptides to triosmium carbonyl clusters.

We have also shown in this work that the conversion between two isomeric forms of phosphine disubstituted triosmium carbonyl clusters upon protonation can be used in a number of applications. One such application which may be pursued further is that of SERS-based pH sensors, although the clusters in this work had pH response in the low pH range. Another application was in the protonation of these clusters to effect intramolecular interactions and conformational changes in oligopeptides. However, the clusters used in this study were protonated at rather low pH and tuning the pH response to a biologically relevant range would be desirable.

Fortunately, we have shown that it is indeed possible to tune the pH response through the electronic properties of the ligands. For example, ligands which are strong electron donors such as N-heterocyclic carbenes, may lower the pH response. In addition, the number of substitutions on the cluster can also be increased to shift the pH response of the cluster. However, the chemistry of such compounds are relatively new and needs to be established.^{4,5} Alternative triggers to protonation to effect these structural changes may also be worth exploring. Indeed, the idea of a stereochemical change in a metal cluster which can be triggered by a simple chemical reaction to effect structural changes in a larger molecule should have a wider potential.

6.1 References

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Bioconjugation of Triosmium Carbonyl Clusters

- Appendices and Supporting Information -

Table of Contents

Title	Page
Table of Contents	ii
Chapter 2. Conjugation of Osmium Carbonyl Clusters to Nucleic Acids - Appendix	1
2.1 Nucleosides	1
2.1.1 3-bromopropyl-2'-deoxythymidine (a)	1
2.1.2 3-bromopropyl-5'-O-DMT-2'-deoxythymidine (b)	2
2.1.3 3-azidopropyl-2'-deoxythymidine (c)	2
2.1.4 3-azidopropyl-5'-O-DMT-2'-deoxythymidine (d)	3
2.1.5 3-(3-propylthiol)-2'-deoxythymidine (e)	5
2.1.6 3-(3-propylthiol) – 5'-O-DMT – 2' –deoxythymidine (f)	6
2.1.7 3-(acetonitrile) – 5'-O-DMT – 2' –deoxythymidine (g)	6
2.1.8 5-trimethylsilylacetylene – 2'- deoxyuridine (h)	7
2.1.9 5-trimethylsilylacetylene – 5'-O-DMT-2'- deoxyuridine (i)	8
2.1.10 5-butynyl tosylate-5'-O-DMT-2'-deoxyuridine (j)	8
2.1.11 5-hexynoic acid -5'-O-DMT-2'-deoxyuridine (k)	9
2.1.12 5-ethynyl-2'- deoxyuridine (l)	10
2.1.13 5-azidobutynyl -5'-O-DMT-2'-deoxyuridine (m)	11
2.1.14 5-bromobutynyl -5'-O-DMT-2'-deoxyuridine (n)	11
2.1.15 5-Methyl-1,2-anhydrouridine (o)	12
2.2 Phosphine ligands	12
2.2.1 $\text{PPh}_2[(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$ (P1)	12
2.2.2 $\text{PPh}_2(\text{CH}_2)_3\text{Br}$ (P3)	13
2.2.3 $\text{PPh}_2[(\text{CH}_2)_3\text{OH}]$ (P4)	15
2.3 Triosmium carbonyl clusters	16
2.3.1 $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-COOC}\equiv\text{CH})$ (7L1)	16
2.3.2 $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-COO}(\text{CH}_2)_5\text{N}_3]$ (7L2)	17
2.3.3 $\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$ (8P1)	19
2.3.4 $\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{N}_3]$ (8P2)	20
2.3.5 $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{Br})_2$ (9P1)	21
2.3.6 $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)_2$ (9P2)	22
2.4 Hexaosmium carbonyl clusters	24

2.4.1	$\text{Os}_6(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{Br}]$ (15P3)	24
2.4.2	$\text{Os}_6(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{OH}]$ (15P4)	25
2.4.3	$\text{Os}_6(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{OOC}(\text{CH}_2)_5\text{N}_3]$ (15P5)	26
2.5	Nucleoside Conjugates	28
2.5.1	$\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-S}(\text{CH}_2)_3\text{thymidine}]$ (6e)	28
2.5.2	$\text{Os}_3(\text{CO})_{11}[\text{NC}(\text{CH}_2)_3\text{thymidine}]$ (10g)	31
2.5.3	$\text{Os}_3(\mu\text{-H})(\text{CO})_{10}(\mu\text{-N}_3\text{H}-(\text{CH}_2)_3\text{-Thymidine-5'-O-DMT})$ (11d)	31
2.5.4	$\text{Os}_3(\mu\text{-H})_2(\text{CO})_9(\mu\text{-N}(\text{CH}_2)_3\text{-Thymidine-5'-O-DMT})$ (13d)	33
2.5.5	Conjugate of $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-COOC}\equiv\text{CH})$ and 3-(3-azidopropyl)-2'-deoxythymidine (7c)	34
2.5.6	Conjugate of $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-COO}(\text{CH}_2)_5\text{N}_3]$ and 5-ethynyl-2'-deoxyuridine (7l)	37
2.5.7	Conjugate of $\text{Os}_3(\text{CO})_{11}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)$ and 5-ethynyl-2'-deoxyuridine (8l)	40
2.5.8	Conjugate of $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)_2$ and 5-ethynyl-2'-deoxyuridine (9l-1)	42
2.5.9	Conjugate of $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)]$ and 5-ethynyl-2'-deoxyuridine (9l-2)	45
2.5.10	Conjugate of $\text{Os}_6(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{OOC}(\text{CH}_2)_5\text{N}_3]$ and 5-ethynyl-2'-deoxyuridine (15l)	48
Chapter 3.	Application of Osmium Carbonyl Cluster – Nucleoside Conjugates - Appendix	51
3.1	MTS assay	51
3.2	ICP-MS of cell pellets	51
Chapter 4.	Protonation of Disubstituted Triosmium Carbonyl Cluster - Appendix	52
4.1	Phosphine ligands	52
4.1.1	$\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOC}(\text{CH}_3)_3$ (P7).	52
4.1.2	$\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NHCO}(\text{CH}_2)_2\text{PPh}_2$ (P8)	53
4.1.3	$\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NHBoc}$ (P9)	54
4.1.4	$\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2$ (P10)	55
4.1.5	$\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}(\text{N-hydroxysuccinimide})$ (P11)	56
4.2	Symmetric substituted triosmium carbonyl clusters	58

4.2.1	$\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_3\text{Br}]$ (8P3)	58
4.2.2	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COOH}]_2$ (9P6)	59
4.2.3	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COOC}(\text{CH}_3)_3]_2$ (9P7)	60
4.2.4	$\text{Os}_3(\text{CO})_{10}[\mu\text{-PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NHCO}(\text{CH}_2)_2\text{PPh}_2]$ (9P8)	61
4.3	Assymmetric substituted triosmium carbonyl clusters	62
4.3.1	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}] [\text{PPh}_2(\text{CH}_2)_2\text{COOH}]$ (9P1.6)	62
4.3.2	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}] [\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2]$ (9P1.9)	63
4.3.3	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{N}_3] [\text{PPh}_2(\text{CH}_2)_2\text{COOH}]$ (9P2.6)	64
4.3.4	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}] [\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2]$ (9P2.10)	65
4.3.5	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_3\text{Br}] [\text{PPh}_2(\text{CH}_2)_2\text{CO}(\text{NHS})]$ (9P3.11)	66
4.3.6	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_3\text{N}_3] [\text{PPh}_2(\text{CH}_2)_2\text{CO}(\text{NHS})]$ (9P11.12)	69
4.3.7	Conjugate of 9P11.12 with propargylamine (9L3)	71
4.4	Protonation of disubstituted phosphine triosmium carbonyl clusters	73
4.4.1	$\{\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COOH}]+\text{H}\}^+$ [9P6+H] ⁺	73
4.4.2	$\{\text{Os}_3(\text{CO})_{10}[\mu\text{-PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NHCO}(\text{CH}_2)_2\text{PPh}_2]+\text{H}\}^+$ [9P8+H] ⁺	75
4.4.3	Conjugate of 9P11.12 and propargylamine [9L3+H] ⁺	76
4.5	Oligopeptides and their conjugates	79
4.5.1	Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (A2)	79
4.5.2	Conjugate of 9P11.12 and Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (9A2)	80
4.5.3	Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pra-NHMe (A3)	85
4.5.4	Conjugate of 9P11.12 and Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pra-NHMe (9A3)	86
4.5.5	Protonation of cluster 9A3	92
Chapter 5. Disubstituted Phosphine Clusters as pH Sensors - Appendix		94
5.1	Monitoring response time and stability of substituted clusters by NMR and IR spectroscopy	94
5.1.1	$\text{Os}_3(\text{CO})_{11}(\text{PPh}_3)$ (8P13)	94
5.1.2	$\text{Os}_3(\text{CO})_{10}(\text{PPh}_3)_2$ (9P13)	96
5.1.3	$\text{Os}_3(\text{CO})_9(\text{PPh}_3)_3$ (16P13)	99
5.1.4	$\text{Os}_3(\text{CO})_{10}(\mu\text{-SPh})$ (6L4)	101
5.2	Phosphine ligands and substituted phosphine clusters	103
5.2.1	$\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$ (P14).	103
5.2.2	$\text{Os}_3(\text{CO})_{11}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH})$ (8P14)	104
5.2.3	$\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{CH}_2\text{PPh}_2)$ (6L5)	105
5.2.4	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2\text{CH}_2\text{CH}_2\text{COO}(\text{N-hydroxysuccinimide})]_2$ (9P11)	107

5.2.5	$\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{SH})_2$ (9P15)	108
5.2.6	$\text{Os}_3(\text{CO})_{10}[\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONH}(\text{C}_6\text{H}_4)\text{SH}]_2$ (9P16)	110
5.2.7	$\text{Os}_3(\text{CO})_{10}(\text{PPh}_3)[\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}(\text{NHS})]$ (9P11.13)	112
5.2.8	$\text{Os}_3(\text{CO})_{10}(\text{PPh}_3)(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{SH})$ (9P13.15)	113
5.2.9	$\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{CH}_3)_3]$ (17P17)	115
5.2.10	$\text{Os}_3(\text{CO})_{11}[\text{P}(\text{OCH}_3)_3]$ (8P18)	118
5.2.11	$\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{OCH}_3)_3]$ (17P18)	119
5.3	Protonation of cluster 17P17	121
5.4	Monitoring the response of the cluster at different pH	125
5.4.1	$\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{CH}_3)_3]$ (17P17)	125
5.4.2	$\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{OCH}_3)_3]$ (17P18)	127

Chapter 2. Conjugation of Osmium Carbonyl Clusters to Nucleic Acids - Appendix

2.1 Nucleosides

2.1.1 3-bromopropyl-2'-deoxythymidine (a)

^1H NMR (CDCl_3)

^{13}C NMR (CDCl_3)

2.1.2 3-bromopropyl-5'-O-DMT-2'-deoxythymidine (b)

^1H NMR (CDCl_3)

2.1.3 3-azidopropyl-2'-deoxythymidine (c)

^1H NMR [$(\text{CD}_3)_2\text{C}=\text{O}$]

^{13}C NMR $[(\text{CD}_3)_2\text{C}=\text{O}]$

2.1.4 3-azidopropyl-5'-O-DMT-2'-deoxythymidine (d)

^1H NMR (CD_3OD)

^{13}C NMR (CD_3OD)

Mass Spectrum

2.1.5 3-(3-propylthiol)-2'-deoxythymidine (e)

^1H NMR (CDCl_3)

^{13}C NMR (CDCl_3)

2.1.6 3-(3-propylthiol) – 5'-O-DMT – 2'-deoxythymidine (f)

^1H NMR (C_6D_6)

2.1.7 3-(acetonitrile) – 5'-O-DMT – 2'-deoxythymidine (g)

^1H NMR (CDCl_3)

Mass Spectrum

2.1.8 5-trimethylsilylacetylene – 2'- deoxyuridine (h)

¹H NMR (CDCl₃)

2.1.9 5-trimethylsilylacetylene – 5'-O-DMT-2'- deoxyuridine (i)

^1H NMR (CDCl_3)

2.1.10 5-butynyl tosylate-5'-O-DMT-2'-deoxyuridine (j)

^1H NMR (CDCl_3)

2.1.11 5-hexynoic acid -5'-O-DMT-2'-deoxyuridine (k)

^1H NMR (CDCl_3)

^{13}C NMR (CDCl_3)

2.1.12 5-ethynyl-2'- deoxyuridine (I)

^1H NMR (D_2O)

^{13}C NMR [$(\text{CD}_3)_2\text{C}=\text{O}$]

^1H NMR (CDCl_3) ¹H NMR (C₆D₆)

2.1.15 5-Methyl-1,2-anhydrouridine (o)

^1H NMR (CD_3OD)

2.2 Phosphine ligands

2.2.1 $\text{PPh}_2[(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$ (P1)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

2.2.2 $\text{PPh}_2(\text{CH}_2)_3\text{Br}$ (P3)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

^{13}C NMR (CDCl_3)

2.2.3 $PPh_2[(CH_2)_3OH]$ (**P4**)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

Mass spectrum

2.3 Triosmium carbonyl clusters

2.3.1 $Os_3(CO)_{10}(\mu-H)(\mu-COOC\equiv CH)$ (**7L1**)

1H NMR ($CDCl_3$)

FTIR (CH₂Cl₂)

2.3.2 Os₃(CO)₁₀(μ-H)[μ-COO(CH₂)₅N₃] (7L2)

¹H NMR (CDCl₃)

^{13}C NMR (CDCl_3)

FTIR (CH_2Cl_2)

2.3.3 $\text{Os}_3(\text{CO})_{11}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}]$ (**8P1**)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

FTIR (CH₂Cl₂)

2.3.4 Os₃(CO)₁₁[PPh₂(CH₂)₂COO(CH₂)₃N₃] (**8P2**)

¹H NMR (CDCl₃)

^{31}P NMR (CDCl_3)

2.3.5 $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{Br})_2$ (9P1)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

2.3.6 $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)_2$ (9P2)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

FTIR (CH_2Cl_2)

2.4.1 $Os_6(CO)_{20}[PPh_2(CH_2)_3Br]$ (15P3)

2.4.2 $\text{Os}_5(\text{CO})_{20}[\text{PPh}_2(\text{CH}_2)_3\text{OH}]$ (**15P4**)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

¹H NMR (CDCl₃)

^{13}C NMR (CDCl_3)

Mass spectrum

2.5 Nucleoside Conjugates

2.5.1 $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})[\mu\text{-S}(\text{CH}_2)_3\text{thymidine}]$ (**6e**)

^1H NMR (CDCl_3)

^{13}C NMR (CDCl_3)

Mass Spectrum

C₂₃H₂₀N₂O₁₅Os₃S
YQ-N2 3 (0.083) Cm (1:7)

Page 1

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

9887 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

Elements Used:
C: 20-23 H: 18-20 N: 0-2 O: 12-15 S: 0-1 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3
190Os: 0-3 192Os: 0-3

C23H20N2O15Os3S
YQ-N2 9 (0.222)

1: TOF MS ES+
5.06e+000

Minimum:		10.0	10.0	-1.5																
Maximum:				50.0																
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula													
1168.9420	1168.9320	10.0	8.6	18.5	14.4	0.0	C23	H19	N2	O15	S	190os	1920s2							

FTIR (CH₂Cl₂)

2.5.2 $Os_3(CO)_{11}[NC(CH_2)_3\text{thymidine}]$ (**10g**)

2.5.3 $Os_3(\mu-H)(CO)_{10}(\mu-N_3H-(CH_2)_3\text{-Thymidine-5'-O-DMT})$ (**11d**)

1H NMR ($CDCl_3$)

FTIR (CH₂Cl₂)

2.5.4 $Os_3(\mu-H)_2(CO)_9(\mu-N(CH_2)_3\text{-Thymidine-5'-O-DMT})$ (**13d**)

1H NMR ($CDCl_3$)

FTIR (CH₃CN)

2.5.5 Conjugate of Os₃(CO)₁₀(μ-H)(μ-COOC≡CH) and 3-(3-azidopropyl)-2'-deoxythymidine (7c)

¹H NMR [(CD₃)₂C=O]

FTIR (MeOH)

Mass Spectrum

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

242 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 26-26 H: 21-22 N: 5-5 O: 17-17 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3 190Os: 0-3 192Os: 0-3

C26H21N5O7Os3

Thy-N3 10 (0.240)

1: TOF MS ES+
1.51e+001

Minimum:								
Maximum:	5.0	5.0	-1.5	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
1247.9796	1247.9795	0.1	0.1	21.5	13.5	0.7	C26 H22 N5 O17 190Os2 192Os	
	1247.9799	-0.3	-0.2	21.5	13.5	0.7	C26 H22 N5 O17 188Os 192Os2	

2.5.6 Conjugate of $Os_3(CO)_{10}(\mu-H)[\mu-COO(CH_2)_5N_3]$ and 5-ethynyl-2'-deoxyuridine (71)

¹³C NMR [(CD₃)₂O]

Mass Spectrum

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
 242 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
 Elements Used:
 C: 27-27 H: 23-24 N: 5-5 O: 17-17 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3 190Os: 0-3
 192Os: 0-3

C₂₇H₂₃N₅O₁₇S₃
Edu-click-hexa 7 (0.157)

1: TOF MS ES+
6.73e+001

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

Mass spectrum

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
 Element prediction: Off
 Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

7499 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 38-40 H: 32-33 N: 3-5 O: 16-18 P: 1-1 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3
 190Os: 0-3 192Os: 0-3

C40H32N5O18Os3P
 TYQ-4 6 (0.138)

1: TOF MS ES+
 5.00e+000

Minimum:				-1.5					
Maximum:		100.0	50.0	50.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula		
1473.0330	1473.0339	-0.9	-0.6	30.5	12.2	0.7	C40	H33	N5 O18 P 189Os 190Os 192Os
	1473.0346	-1.6	-1.1	30.5	12.2	0.7	C40	H33	N5 O18 P 187Os 192Os2

2.5.8 Conjugate of $Os_3(CO)_{10}(PPh_2CH_2CH_2COOCH_2CH_2CH_2N_3)_2$ and 5-ethynyl-2'-deoxyuridine (9I-1)

1H NMR ($CDCl_3$)

^{31}P NMR (CDCl_3)

^{13}C NMR (CDCl_3)

FTIR (MeOH)

2.5.9 Conjugate of $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}_3)$ and 5-ethynyl-2'-deoxyuridine (9I-2)

^1H NMR $[(\text{CD}_3)_2\text{C}=\text{O}]$

^{31}P NMR $[(\text{CD}_3)_2\text{C}=\text{O}]$

^{13}C NMR $[(\text{CD}_3)_2\text{C}=\text{O}]$

Mass spectrum

C68564N10O24Os3P2
TYQ-N1 3 (0.082) Cm (1:11)

1: TOF MS ES+
273

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

126230 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 68-68 H: 64-65 N: 8-10 O: 22-24 P: 1-2 127I: 0-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3 190Os: 0-3 192Os: 0-3

C68564N10O24Os3P2

TYQ-N1 3 (0.082) Cm (1:7) [click](#)

1: TOF MS ES+
2.42e+001

Minimum:					-1.5					
Maximum:		5.0	10.0		50.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
2034.2434	2034.2393	4.1	2.0	45.5	46.9	1.6	C68 H65 N10 O24 P2 189Os3			
	2034.2383	5.1	2.5	45.5	46.9	1.6	C68 H65 N10 O24 P2 186Os 189Os 192Os			
	2034.2379	5.5	2.7	45.5	46.9	1.6	C68 H65 N10 O24 P2 187Os 188Os 192Os			
	2034.2375	5.9	2.9	45.5	46.9	1.6	C68 H65 N10 O24 P2 187Os 190Os2			
	2034.2373	6.1	3.0	45.5	46.9	1.6	C68 H65 N10 O24 P2 186Os 189Os 190Os			

FTIR (MeOH)

2.5.10 Conjugate of $Os_6(CO)_{20}[PPh_2(CH_2)_3OOC(CH_2)_5N_3]$ and 5-ethynyl-deoxyuridine (**15I**)

¹H NMR [(CD₃)₂S=O]

^{31}P NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^{13}C NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

TYQ os6 raft e-dU click 211015 ^{13}C NMR BBFO2 DMSO 211015

C52H38N5O27Os6P
TYQ-OS6-3-2 3 (0.082) Cm (1:14)

1: TOF MS ES+
25.2

100
0.16 raft conjugate

2312.0940

2311.0435
2309.1311
2308.1309
2336.1104
2307.1462
2337.1689
2340.1294
2306.1306
2391.2292
2393.1445
2305.0564
2277.0479
2274.0637
2077.0325
2098.8362
2074.4080
2099.0503
2215.6702
2404.0344
2404.0815
2504.4211
2515.1038
2639.2378
2675.3181
2702.8220
2765.1772

%

m/z

m/z	Relative Intensity (%)
2074.4080	~5
2077.0325	~10
2098.8362	~10
2099.0503	~5
2215.6702	~5
2274.0637	~10
2277.0479	~15
2305.0564	~10
2306.1306	~10
2307.1462	~10
2308.1309	~15
2309.1311	~20
2311.0435	~30
2312.0940	100
2336.1104	~25
2337.1689	~10
2340.1294	~10
2391.2292	~10
2393.1445	~10
2404.0344	~10
2404.0815	~5
2504.4211	~5
2515.1038	~5
2639.2378	~5
2675.3181	~5
2702.8220	~5
2765.1772	~5

Page 1

Tolerance = 10.0 PPM / DBE: min = -10.0, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
30827 formula(e) evaluated with 15 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 52-52 H: 38-39 O: 27-27 31P: 0-2 184Os: 0-6 186Os: 0-6 187Os: 0-6 188Os: 0-6 189Os: 0-6
190Os: 0-6 192Os: 0-6

C52H38N5O27Os6P
TYQ-OS6-3-2 17 (0.395)

1: TOF MS ES+
1.01e+000

[illegible]

Chapter 3. Application of Osmium Carbonyl Cluster – Nucleoside Conjugates - Appendix

3.1 MTS assay

Compound	MDA-MB-231			MCF-10A		
Blank	1.446695	1.358323	1.574970	1.363089	1.464393	1.327984
2	0.224506	0.222491	0.234180	0.688064	0.711133	0.678536
e	1.077989	1.044581	1.145289	N/A		
6e	0.216352	0.216892	0.210765	0.771314	0.777834	0.768305
g	0.715047	0.700469	0.780143	N/A		
10g	0.281645	0.272433	0.290149	0.796208	0.831776	0.723825
c	1.076985	0.840488	1.093914	N/A		
7c	0.597441	0.592805	0.622330	0.689569	0.692578	0.668004
l	1.458988	1.457880	1.537082	N/A		
7l	0.217433	0.279158	0.210765	0.740221	0.740722	0.717151
8l	1.113563	1.051391	1.241536	1.115346	1.144935	1.022066
9l-1	1.126633	1.446520	1.110954	1.241224	1.286359	1.150953
9l-2	1.094915	1.277746	1.002733	1.174524	1.178536	1.036610

3.2 ICP-MS of cell pellets

	MDA-MB-231			MCF-10A		
Cluster 9P2	9.99	13.46	6.28	N/A		
Cluster 9l-2	625.94	638.92	481.91	121.64	121.96	97.63

Chapter 4. Protonation of Disubstituted Triosmium Carbonyl Cluster - Appendix

4.1 Phosphine ligands

4.1.1 $PPh_2CH_2CH_2COOC(CH_3)_3$ (P7).

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

4.1.2 $PPh_2(CH_2)_2CONH(CH_2)_2NHCO(CH_2)_2PPh_2$ (**P8**)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

4.1.3 $PPh_2(CH_2)_2CONH(CH_2)_2NHBoc$ (P9)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

4.1.4 $PPh_2(CH_2)_2CONH(CH_2)_2NH_2$ (P10)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

^{13}C NMR (CDCl_3)

4.1.5 $\text{PPh}_2\text{CH}_2\text{CH}_2\text{CO}(\text{N-hydroxysuccinimide})$ (P11)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

^{13}C NMR (CDCl_3)

4.2 Symmetric substituted triosmium carbonyl clusters

4.2.1 $Os_3(CO)_{11}[PPh_2(CH_2)_3Br]$ (**8P3**)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

4.2.2 $\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COOH}]_2$ (**9P6**)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

4.2.3 $Os_3(CO)_{10}[PPh_2(CH_2)_2COOC(CH_3)_3]_2$ (**9P7**)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

4.2.4 $Os_3(CO)_{10}[\mu\text{-}PPh_2(CH_2)_2CONH(CH_2)_2NHCO(CH_2)_2PPh_2]$ (**9P8**)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

4.3 Asymmetric substituted triosmium carbonyl clusters

4.3.1 $Os_3(CO)_{10}[PPh_2(CH_2)_2COO(CH_2)_3Br][PPh_2(CH_2)_2COOH]$ (**9P1.6**)

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

^{13}C NMR (CDCl_3)

4.3.2 $\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}] [\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2]$ (9P1.9)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

4.3.3 $\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{N}_3][\text{PPh}_2(\text{CH}_2)_2\text{COOH}]$ (9P2.6)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

4.3.4 $\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{Br}] [\text{PPh}_2(\text{CH}_2)_2\text{CONH}(\text{CH}_2)_2\text{NH}_2]$ (9P2.10)

^1H NMR (CDCl_3)

³¹P NMR (CDCl₃)

4.3.5 $Os_3(CO)_{10}[PPh_2(CH_2)_3Br][PPh_2(CH_2)_2CO(NHS)]$ (**9P3.11**)

¹H NMR (CDCl₃)

^{31}P NMR (CDCl_3)

^{13}C NMR (CDCl_3)

Minimum:					-1.5																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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4.3.6 $\text{Os}_3(\text{CO})_{10}[\text{PPh}_2(\text{CH}_2)_3\text{N}_3][\text{PPh}_2(\text{CH}_2)_2\text{CO}(\text{NHS})]$ (**9P11.12**)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

Mass spectrum

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

41838 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 43-44 H: 34-36 N: 0-4 O: 12-14 P: 1-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3

190Os: 0-3 192Os: 0-3

C₄₄H₃₄N₄O₁₄S₃P₂

TYQ-3 6 (0.139)

1: TOF MS ES+
3.04e+000

Minimum: -1.5
Maximum: 100.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1479.0419	1479.0439	-2.0	-1.4	33.5	13.4	0.0	C ₄₄ H ₃₅ N ₄ O ₁₄ P ₂ 190Os 192Os ₂

4.3.7 Conjugate of **9P11.12** with propargylamine (**9L3**)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

^1H NOESY NMR (CDCl_3)

Mass spectrum

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
 2769 formula(e) evaluated with 6 results within limits (up to 50 best isotopic matches for each mass)
 Elements Used:
 C: 46-46 H: 38-39 N: 4-4 O: 13-13 P: 1-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3
 190Os: 0-3 192Os: 0-3

C43H38N4O13Os3P2
YQ-C5 3 (0.082) Cm (1:10)

1: TOF MS ES+
4.04e+002

Minimum:																		
Maximum:		10.0	10.0		-1.5													
					50.0													
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula											
1485.0739	1485.0743	-0.4	-0.3	33.5	28.9	1.7	C46	H39	N4	O13	F2	1870s	1890s	1920s				
	1485.0744	-0.5	-0.3	33.5	28.9	1.7	C46	H39	N4	O13	F2	1860s	1920e2					
	1485.0737	0.2	0.1	33.5	29.0	1.8	C46	H39	N4	O13	F2	1890e2	1900s					
	1485.0727	1.2	0.8	33.5	29.0	1.8	C46	H39	N4	O13	F2	1860s	1900s	1920s				
	1485.0721	1.8	1.2	33.5	29.0	1.8	C46	H39	N4	O13	F2	1880e2	1920s					
	1485.0716	2.3	1.5	33.5	29.1	1.9	C46	H39	N4	O13	F2	1880s	1900e2					

4.4.1 $\{Os_3(CO)_{10}[PPh_2(CH_2)_2COOH]+H\}^+$ (**9P6+H**)⁺

Chemical structure of the Os_3 complex is shown above the spectrum. The structure features three osmium (Os) atoms in a triangular core, bridged by three hydride (H) atoms. Each osmium atom is also bonded to a phenylphosphine (Ph-P) group and a carboxylic acid (HO-C(=O)-CH₂-) group. The counterion is $[\text{PF}_6]^-$.

³¹P NMR (CDCl₃)

4.4.2 $\{Os_3(CO)_{10}[\mu-PPh_2(CH_2)_2CONH(CH_2)_2NHCO(CH_2)_2PPh_2]+H\}^+ [9P8+H]^+$

1H NMR ($CDCl_3$)

^{31}P NMR (CDCl_3)

4.4.3 Conjugate of **9P11.12** and propargylamine [**9L3**+H]⁺

^1H NMR (CDCl_3)

³¹P NMR (CDCl₃)

¹H NOESY NMR

Mass Spectrum

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
 2773 formula(e) evaluated with 6 results within limits (up to 50 best isotopic matches for each mass)
 Elements Used:
 C: 46-47 H: 38-39 N: 4-4 O: 13-13 P: 1-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3
 190Os: 0-3 192Os: 0-3

C₄₆H₃₉N₄O₁₃S₃P₂
YQ-C4 12 (0.277)

1: TOF MS ES+
4.05e+000

Minimum:										-1.5
Maximum:		10.0	10.0							50.0
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
1487.0767	1487.0767	0.0	0.0	33.5	13.4	1.7	C46	H39	N4	O13 P2 1890s2 1920s
1487.0757	1.0	0.7	33.5	13.4	1.7	C46	H39	N4	O13 P2 1860s 1920s2	1920s
1487.0747	2.0	1.3	33.5	13.4	1.7	C46	H39	N4	O13 P2 1880s 1900s	
1487.0743	2.4	3.5	33.4	13.4	1.7	C46	H39	N4	O13 P2 1900s3	
1487.0629	13.8	9.3	34.5	13.6	1.9	C47	H39	N4	O13 P2 1840s 1870s2	
1487.0624	14.3	9.6	34.5	13.6	1.9	C47	H39	N4	O13 P2 1840s2 1900s	

4.5.1 Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (**A2**)

$^1\text{H NMR } [(\text{CD}_3)_2\text{S=O}]$

^{13}C NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

4.5.2 Conjugate of 9P11.12 and Cbz-Lys-Phe-Ile-Ser-Pra-NHMe (9A2)

^1H NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^{13}C NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^1H NOESY NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^1H COSY NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

Mass Spectrum

4.5.3 Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pra-NHMe (A3)

^1H NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^{13}C NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

4.5.4 Conjugate of 9P11.12 and Cbz-Lys-Phe-Ile-Gly-Ala-Ser-Pra-NHMe (**9A3**)

^1H NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^{31}P NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^{13}C NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

^1H NOESY $[(\text{CD}_3)_2\text{S}=\text{O}]$

^1H COSY $[(\text{CD}_3)_2\text{S}=\text{O}]$

Mass Spectrum

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 20.0 PPM / DBE: min = -10.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
63641 formula(s) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 82-83 H: 90-92 N: 10-12 O: 20-21 P: 1-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3
190Os: 0-3 192Os: 0-3

C87H95N13O24Os3P2
TYQ-8-P 70 (1.542)

1: TOF MS ES+
1.01e+000

4.5.5 Protonation of cluster **9A3**

¹H NMR [(CD₃)₂S=O]

TYQ peptide 7 mer conjugate_TFA 280915 1H NMR BBF01 400 DMSO 280915

^{31}P NMR $[(\text{CD}_3)_2\text{S}=\text{O}]$

Chapter 5. Disubstituted Phosphine Clusters as pH Sensors - Appendix

5.1 Monitoring response time and stability of substituted clusters by NMR and IR spectroscopy

5.1.1 $\text{Os}_3(\text{CO})_{11}(\text{PPh}_3)$ (**8P13**)

IR spectra

^1H NMR (CDCl_3) (5 mins)

^{31}P NMR (CDCl_3) (5 min)

^1H NMR (CDCl_3) (24 h)

^{31}P NMR (CDCl_3) (24 h)

5.1.2 $\text{Os}_3(\text{CO})_{10}(\text{PPh}_3)_2$ (**9P13**)

IR monitoring

^1H NMR (CDCl_3)(5 min)

^{31}P NMR (CDCl_3) (5 min)

^1H NMR (CDCl_3)(24 h)

^{31}P NMR (CDCl_3) (24 h)

5.1.3 $\text{Os}_3(\text{CO})_9(\text{PPh}_3)_3$ (**16P13**)

IR monitoring

^1H NMR (CDCl_3) (5 min)

^{31}P NMR (CDCl_3) (5 min)

^1H NMR (CDCl_3) (24 h)

^{31}P NMR (CDCl_3) (24 h)

5.1.4 $\text{Os}_3(\text{CO})_{10}(\mu\text{-SPh})$ (**6L4**)

IR monitoring

^1H NMR (CDCl_3) (5 min)

^1H NMR (CDCl_3) (24 h)

5.2 Phosphine ligands and substituted phosphine clusters

5.2.1 $PPh_2CH_2CH_2CH_2SH$ (P14).

1H NMR ($CDCl_3$)

^{31}P NMR ($CDCl_3$)

^{13}C NMR (CDCl_3)

5.2.2 $\text{Os}_3(\text{CO})_{11}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SH})$ (8P14)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

5.2.3 $\text{Os}_3(\text{CO})_{10}(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{CH}_2\text{PPh}_2)$ (6L5)

^1H NMR (CDCl_3)

³¹P NMR (CDCl₃)

5.2.4 $\text{Os}_3(\text{CO})_{10}[\text{PPh}_2\text{CH}_2\text{CH}_2\text{COO}(\text{N-hydroxysuccinimide})]_2$ (**9P11**)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

^{13}C NMR (CDCl_3)

5.2.5 $\text{Os}_3(\text{CO})_{10}(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{SH})_2$ (**9P15**)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

Mass spectrum

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
25125 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 44-44 H: 140-141 N: 2-2 O: 11-12 P: 0-2 S: 0-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3
189Os: 0-3 190Os: 0-3 192Os: 0-3

C₄₄H₄₀N₂O₁₂S₃P₂S₂
TYQ-P2 3 (0.083) Cm (1:8)

1: TOF MS ES+
1.11e+001

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1488.0339	1488.0388	-4.9	-3.3	29.5	23.6	0.0	C44 H41 N2 O12 P2 S2 189Cs 192Os2

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

Mass Spectrum

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

70309 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 52-52 H: 41-41 N: 2-2 O: 11-12 P: 0-2 S: 0-2 127I: 0-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3 190Os: 0-3 192Os: 0-3

C52H40N2O12Os3P2S2

YQ-P1 10 (0.239)

1: TOF MS ES+
3.04e+000

Minimum:				-1.5					
Maximum:				50.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula		
1581.0251	1581.0330	-7.9	-5.0	37.5	22.0	1.4	C52 H41 N2 O12 P2 S2 190Os3		
	1581.0334	-8.3	-5.2	37.5	22.0	1.4	C52 H41 N2 O12 P2 S2 189Os 190Os 192Os		
	1581.0344	-9.3	-5.9	37.5	22.0	1.4	C52 H41 N2 O12 P2 S2 186Os 192Os2		
	1581.0354	-10.3	-6.5	37.5	22.0	1.4	C52 H41 N2 O12 P2 S2 189Os2 192Os		

5.2.7 $Os_3(CO)_{10}(PPh_3)[PPh_2CH_2CH_2CO(NHS)]$ (9P11.13)

^{31}P NMR (CDCl_3)

5.2.8 $\text{Os}_3(\text{CO})_{10}(\text{PPh}_3)(\text{PPh}_2\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2\text{SH})$ (9P13.15)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

Mass spectrum

Elemental Composition Report

PPh₃-SH C2

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

22154 formula(e) evaluated with 4 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 44-45 H: 32-35 N: 0-1 O: 10-11 S: 0-1 P: 1-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3

189Os: 0-3 190Os: 0-3 192Os: 0-3

C45H35NO11Os3P2S

YQ-C2 3 (0.082) Cm (1:8)

1: TOF MS ES+
1.56e+002

Minimum:					-1.5			
Maximum:	10.0	10.0			50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
1428.0132	1428.0106	2.6	1.8	33.5	44.3	1.3	C45 H34 N 011 S P2 189Os2 192Os	
	1428.0096	3.6	2.5	33.5	44.4	1.3	C45 H34 N 011 S P2 186Os 192Os2	
	1428.0086	4.6	3.2	33.5	44.5	1.4	C45 H34 N 011 S P2 188Os 190Os 192Os	
	1428.0082	5.0	3.5	33.5	44.5	1.5	C45 H34 N 011 S P2 190Os3	

5.2.9 Os₃(CO)₉[μ-H](μ-SCH₂CH₂SH)[P(CH₃)₃] (17P17)

¹H NMR (CDCl₃)

³¹P NMR (CDCl₃)

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Tolerance = 10.0 PPM
Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

2311 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)

Elements Used:

Elements Used: C: 13-14 H: 15-16 O: 8-9 P: 0-1 S: 1-2 184Os: 0-3 186Os: 0-3 187Os: 0-3 188Os: 0-3 189Os: 0-3

190Os: 0-3 192Os: 0-3

C14H15O9O53PS2

C14H15O9Os3PS2
TYQ-6 3 (0.084) Cm (1:7)

1: TOF MS ES+
2.48e+001

Minimum:						-1.5							
Maximum:		100.0		10.0		50.0							
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula						
992.8774	992.8751	2.3	2.3	10.5	15.7	1.3	C14	H16	O9	P	S2	1890s2	1920s
	992.8742	3.2	3.2	10.5	15.7	1.4	C14	H16	O9	P	S2	1860s	1920s2
	992.8731	4.3	4.3	10.5	15.8	1.4	C14	H16	O9	P	S2	1880s	1900s 1920s
	992.8727	4.7	4.7	10.5	15.8	1.5	C14	H16	O9	P	S2	1900s3	

Infrared spectrum

5.2.10 $\text{Os}_3(\text{CO})_{11}[\text{P}(\text{OCH}_3)_3]$ (**8P18**)

¹H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

5.2.11 $\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{OCH}_3)_3]_3$ (17P18)

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

5.3 Protonation of cluster 17P17

Protonation

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

Infrared spectrum

Removal of acid

^1H NMR (CDCl_3)

^{31}P NMR (CDCl_3)

Infrared spectrum

Overall IR spectrum

5.4 Monitoring the response of the cluster at different pH

5.4.1 $Os_3(CO)_9(\mu-H)(\mu-SCH_2CH_2SH)[P(CH_3)_3]$ (**17P17**)

Infrared spectrum

UV-vis spectrum

Effective range

Infrared spectrum

pH	Percentage Area Integrated
3.7	1.69367
2.9	1.87212
2.2	3.1526
1.9	3.70633
1.6	6.00886
1.4	7.37127
1.3	11.23458
1.1	12.78234
0.6	16.16433

UV-vis spectrum

pH	Absorbance
3.7	0.55200
3.3	0.52500
2.9	0.53800
2.2	0.51800
1.9	0.43650
1.6	0.25450
1.4	0.19300
1.3	0.12600
1.1	0.10600
0.9	0.05500
0.6	0.04550

5.4.2 $\text{Os}_3(\text{CO})_9(\mu\text{-H})(\mu\text{-SCH}_2\text{CH}_2\text{SH})[\text{P}(\text{OCH}_3)_3]$ (**17P18**)

Infrared spectrum

UV-vis spectrum

Effective range

Infrared spectrum

pH	Percentage Area Integrated
2.9	1.59027
2.2	2.23256
1.9	2.60937
1.6	3.57747
1.4	4.79763
1.3	5.49620
1.1	7.67664
0.9	11.48773
0.6	15.41801
0.4	15.38276

UV-vis spectrum

pH	Absorbance
2.9	0.495
2.2	0.4875
1.9	0.443
1.6	0.417
1.4	0.3465
1.3	0.337
1.1	0.237
0.9	0.203
0.6	0.1065
0.4	0.0975