Crosslinking Allosteric Sites on the Nucleosome Core Particle

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par

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Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

Marie Curie
Acknowledgements

First and foremost I would like to thank my scientific advisor, Professor Paul Dyson, for giving me the opportunity to work as a Ph.D. student in his group. I am grateful for the challenging project he assigned me as well as the freedom he gave me to explore and develop different approaches to the various experimental endeavours. Thank you for your support and guidance, especially in circumnavigating synthetic roadblocks.

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Last but definitely not least, a massive thank you to my family. Thank you for your never ending support and humour over the past four years and indeed my life! I wouldn’t have made it without you all!
Abstract

Designing compounds that bind to the acidic patch of the nucleosome core particle (NCP) is an emerging therapeutic approach that can inhibit the binding of key chromatin factors. Pretreatment of human ovarian carcinoma, A2780, cells with RAPTA-T, an antimetastatic agent which also binds to the acidic patch, sensitises the cells to auranofin, an antiarthritic agent, resulting in a three-fold increase in gold(I)-chromatin adducts. This synergism is accompanied by an allosteric relationship between RAPTA-T and auranofin. The binding of RAPTA-T to the carboxylate E61/E64 residues on the H2A protein (RU1) and imidazole H106 and carboxylate E102 residues of the H2B dimer (RU2) triggers a series of allosteric modifications that opens the previously inaccessible symmetry-related imidazole sites, H3 H113 (AU1) and H113' (AU1'), on the H3-H4 tetramer. This series of systematic alterations in the inter-helical orientations of the H2A and H3/H3' α-helices connecting the RU1/RU2 and AU1/AU1' binding sites result in more compact AU1/AU1' sites that favours auranofin binding. Based on this allosteric effect, multi-nuclear homo- and heterometallic drugs were designed and synthesised with the aim of targeting the RAPTA-T and auranofin sites on the NCP.

Chapter 1 is an introduction into crosslinking anticancer agents inspired by cisplatin, RAPTA-T and auranofin. Both homo- and heterometallic complexes possessing the ability to form DNA-DNA, protein-protein and DNA-protein crosslinks are discussed.

Chapter 2 follows an investigation into the impact of linker length on the cytotoxicity of homobimetallic ruthenium(II) and gold(I) complexes inspired by RAPTA-C and auranofin. As the lipophilicity of polyethylene glycol linkers plateaus between PEG₆ and PEG₁₀, the impact of linker length on the cytotoxicity of the resulting dinuclear complexes, independent of increasing lipophilicity, can be observed.

Chapter 3 discusses the design, synthesis and in vitro cytotoxicity of a series of heterobimetallic ruthenium(II)-gold(I) complexes linked via a bis-phosphine ligand bearing different lengths of polyethylene glycol chains. The affinity of the complexes for histidine residues was assessed on t-histidine and the 1-16 sequence of the amyloid β protein using mass
spectrometry, demonstrating that the complexes have the potential bind to the RU2, AU1 and AU1’ sites on the NCP.

Chapter 4 describes the synthesis of a heterobimetallic ruthenium(II)-gold(I) complex linked via the arene of the RAPTA-moiety with a long polyethylene glycol linker. The interactions of the complex on the NCP were studied using X-ray crystallography and molecular dynamic simulations revealing that the complex can crosslink the RU1 and AU1 binding sites. A trinuclear diruthenium(II)-gold(I) complex, that has the potential to crosslink the RU1, RU2 and AU1 binding sites, is also described.

Chapter 5 explores the possibility of igniting a similar allosteric effect as RAPTA-T using alternative molecules. Residues 1-23 Kaposi’s sarcoma-associated herpesvirus (KSHV) latency-associated nuclear antigen (LANA) and residues 535-551 prototype foamy virus (PFV) structural protein (GAG) bind to the acidic patch of the NCP. Gold(I)-peptide conjugates containing these peptide sequences were prepared to assess if the binding of the peptide can cause a structural alteration in the NCP that allows the gold(I) centre to bind to the AU1 or AU1’ binding site.

Keywords

RAPTA-T; Auranofin; Chromatin; Nucleosome Core Particle; Allosteric Effects; Synergism; Crosslinking.
Zusammenfassung


Kapitel 1 ist eine Einführung in vernetzende Antikrebsmittel, die von Cisplatin, RAPTA-T und auranofin inspiriert sind. Sowohl homo- als auch heterometallische Komplexe, die die Fähigkeit besitzen, DNA-DNA, Protein-Protein und DNA-Protein Vernetzungen zu bilden, werden diskutiert.

Kapitel 3 diskutiert das Design, die Synthese und In-vitro-Zytotoxizität einer Reihe von hetero-bimetallischen Ruthenium(II)-Gold(I)-Komplexen, die über einen Bisphosphinliganden mit unterschiedlichen Längen von Polyethylenlycolketten verknüpft sind. Die Affinität von Komplexen für Histidinreste wurde mit t-Histidin und der 1-16-Sequenz des Amyloid-β-Proteins unter Verwendung von Massenspektrometrie untersucht, was zeigt, dass die Komplexe das Potenzial haben, an die RU2, AU1 und AU1' Stellen am NCP zu binden.

Kapitel 4 beschreibt die Synthese eines Ruthenium(II)-Gold(I)-Komplexes, der über den Arylliganden der RAPTA-Einheit mit einem langen Polyethylenlykol-Linker verknüpft ist. Die Wechselwirkungen des Komplexes am NCP wurden mittels Röntgenkristallographie und molekulardynamischer Simulationen untersucht, die zeigen, dass der Komplex die Bindungsstellen RU1 und AU1 vernetzen kann. Ein dreikerniger Diruthenium(II)-Gold(I)-Komplex, der die Bindungsstellen RU1, RU2 und AU1 vernetzen kann, wird ebenfalls beschrieben.

Kapitel 5 untersucht die Möglichkeit, einen ähnlichen allosterischen Effekt wie RAPTA-T mit alternativen Molekülen aus zu lösen. Die Reste 1-23 des Kaposi-Sarkom-assoziierten Herpesvirus (KSHV), Latenz-assoziiertes Kernantigen (LANA) und die Reste 535-551 des Prototyp-Foamy-Virus (PFV) Strukturprotein (GAG) binden an die sauren Stellen des NCPs. Gold(I)-Peptidkonjugate, die diese Peptidsequenzen enthalten, wurden hergestellt, um zu bewerten, ob die Bindung des Peptids eine strukturelle Veränderung im NCP verursachen kann, die es dem Gold(I)-Zentrum ermöglicht, an die AU1 oder AU1' Bindungsstelle zu binden.

Schlüsselwörter

RAPTA-T; Auranofin; Chromatin; Nucleosome Core Partikel; Allosterische Effekte; Synergismus; Vernetzung.
**Resumé**

La conception de composés qui se lient au patch acide de la particule nucléosomique (PN) est une nouvelle approche thérapeutique permettant l’inhibition de la liaison de facteurs cruciaux de la chromatine. Le prétraitement des cellules carcinome ovarien humain, A2780, avec RAPTA-T, un agent antimétastatique qui se lie également au patch acide, sensibilise les cellules à l’auranofin, un agent antiarthritique, multipliant par trois la formation d’adduits or (I)-chromatine. Cette synergie s’accompagne d’une relation allosterique entre RAPTA-T et auranofin. La liaison de RAPTA-T aux résidus carboxylates E61/E64 sur la protéine H2A (RU1) et les résidus imidazole H106 et carboxylate E102 du dimère H2B (RU2) déclenche une série de modifications allosteriques qui ouvre les sites imidazole précédemment inaccessibles pour cause de symétrie, H3 H113 (AU1) et H113’ (AU1’), sur le tétramère H3-
H4. Cette série de modifications systématiques dans les orientations inter-hélicoïdales des hélices a H2A et H3/H3’ reliant les sites de liaison RU1/RU2 et AU1/AU1’ aboutit à des sites AU1/AU1’ plus compacts qui favorisent la liaison à l’auranofin. Sur la base de cet effet allosterique, des médicaments homo et hétérométalliques multinucléaires ont été conçus et synthétisés dans le but de cibler de manière conjointe les sites RAPTA-T et auranofin sur la PN.


Le chapitre 2 relate une étude sur l’impact de la longueur du linker sur la cytotoxicité des complexes homobimétalliques d’ruthénium(II) et d’or(I) inspirés de RAPTA-C et d’auranofin. Puisque la lipophilie des composés atteint un plateau entre les linkers PEG6 et PEG10, on peut observer l’impact de la longueur de la liaison sur la cytotoxicité des complexes dinucléaires résultants, indépendamment de ce facteur.

Le chapitre 3 examine la conception, la synthèse et la cytotoxicité in vitro d’une série de complexes hétéro-bimétalliques ruthénium(II)-or(I) liés par un ligand biphasphine et
différentes longueurs de chaînes de polyéthylèneglycol. L’affinité des complexes pour les résidus d’histidine est évaluée sur la ε-histidine et sur la séquence 1-16 de la protéine β-amyloïde par spectrométrie de masse, démontrant que les complexes peuvent se lier aux sites RU2, AU1 et AU1’ de la PN.

Le chapitre 4 décrit la synthèse d’un complexe hétérobimétallique de ruthénium(II)-or(I) lié via le fragment arène du composé RAPTA, avec un long linker polyéthylène glycol. Les interactions du complexe sur la PN sont étudiées par cristallographie aux rayons-X et simulations de dynamique moléculaire, révélant que le complexe peut réticuler les sites de liaison RU1 et AU1. Un complexe trinucléaire diruthénium(II)-or(I), qui a le potentiel de réticuler les sites de liaison RU1, RU2 et AU1, est également décrit.

Le chapitre 5 explore la possibilité d’utiliser un effet allostérique similaire à celui de RAPTA-T, en utilisant des molécules alternatives. Les résidus 1-23 de l’antigène nucléaire associé à la latence (LANA) de l’herpès-virus associé au sarcome de Kaposi (KSHV) et les résidus 535-551 de la protéine structurale (GAG) prototype du virus foamy (PFV) prototype se lient au patch acide du PN. Des conjugués peptid-or(I) contenant ces séquences peptidiques sont préparés pour évaluer si la liaison du peptide pourrait provoquer une altération structurelle du PN permettant à la partie or(I) de se lier au site de liaison AU1 ou AU1’.

**Mots clés**

RAPTA-T; Auranofin; chromatine; particule nucléosomique; relation allostérique; synergie; réticulation.
Abbreviations

° degrees
°C degrees Celcius
Å Ångström
A2780 human ovarian carcinoma cell line
A2780cisR cisplatin resistant human ovarian carcinoma cell line
A549 human lung carcinoma cell line
518A2 human melanoma cell line
Ala or A alanine
5'-AMP adenosine 5'-monophosphate
AMP antimicrobial peptide
Arg or R arginine
Asn or N asparagine
Asp or D aspartic acid
AU1 auranofin binding site containing H113 on the NCP
AU1' auranofin binding site containing H113' on the NCP
AUF auranofin
auranofin (1-thio-β-D-glucopyranose-2,3,4,6-tetraacetato-S)(triethylphosphine)gold(I)
BBR3005 \[
[\{trans-PtCl(NH_3)_2\cdot H_2N(CH_2)_6NH_2\}]^{2+}
\]
BBR3171 \[
[\{cis-PtCl(NH_3)_2\cdot H_2N(CH_2)_6NH_2\}]^{2+}
\]
BBR3464 \[
\{trans-PtCl(NH_3)_2\cdot H_2N(CH_2)_6NH_2\}\cdot \mu \cdot \{trans-Pt(NH_3)_2\cdot H_2N(CH_2)_6NH_2\}\]
CBS chromatin binding sequence
Caki-1 human renal cancer cell line
cisplatin cis-diaaminedichloroplatinum(II)
CLC-3 chloride channel protein
5'-CMP cytidine 5'-monophosphate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNE-2</td>
<td>human nasopharyngeal carcinoma cell line</td>
</tr>
<tr>
<td>CYP-3</td>
<td>cyclophilin 3</td>
</tr>
<tr>
<td>Cys or C</td>
<td>cysteine</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>dd</td>
<td>doublet of doublets</td>
</tr>
<tr>
<td>d(ApG)</td>
<td>adenine-guanine, DNA sequence</td>
</tr>
<tr>
<td>d(GpG)</td>
<td>guanine-guanine, DNA sequence</td>
</tr>
<tr>
<td>d(GpTpG)</td>
<td>guanine-thymine-guanine, DNA sequence</td>
</tr>
<tr>
<td>d(GpXpG)</td>
<td>guanine-unidentified nucleobase-guanine, DNA sequence</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DDB2</td>
<td>damaged DNA-binding protein 2</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-diisopropylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dppm</td>
<td>bis(diphenylphosphino)ethane</td>
</tr>
<tr>
<td>EDCI</td>
<td>N-ethyl-N'-(3-dimethaminopropyl)carbodiimide hydrochloride</td>
</tr>
<tr>
<td>EDT</td>
<td>ethanedithiol</td>
</tr>
<tr>
<td>en</td>
<td>ethylene diamine</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>electron spray ionisation mass spectrometry</td>
</tr>
<tr>
<td>ESI(+)</td>
<td>electron spray ionisation operated in the positive mode</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>EtO</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>Equiv</td>
<td>equivalent(s)</td>
</tr>
<tr>
<td>FANCD2</td>
<td>Fanconi anemia gene</td>
</tr>
<tr>
<td>FMOC</td>
<td>9-fluorenylmethoxycarbonyl chloride</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>GAG</td>
<td>prototype foamy virus structural protein</td>
</tr>
<tr>
<td>Gln or Q</td>
<td>glutamine</td>
</tr>
<tr>
<td>Glu or E</td>
<td>glutamic acid</td>
</tr>
<tr>
<td>Gly or G</td>
<td>glycine</td>
</tr>
<tr>
<td>5'-GMP</td>
<td>guanosine 5'-monophosphate</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptors</td>
</tr>
<tr>
<td>GRPR</td>
<td>gastrin releasing peptide receptor</td>
</tr>
<tr>
<td>H2A, H2B, H3, H4</td>
<td>histone proteins</td>
</tr>
<tr>
<td>HATU</td>
<td>1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxide hexafluorophosphate</td>
</tr>
<tr>
<td>HBL-100</td>
<td>human breast cancer cell line</td>
</tr>
<tr>
<td>HCT-116</td>
<td>human colon cell line</td>
</tr>
<tr>
<td>HeLa</td>
<td>human epitheloid cervix carcinoma cell line</td>
</tr>
<tr>
<td>HEK-293</td>
<td>human embryonic kidney cell line</td>
</tr>
<tr>
<td>HepG2</td>
<td>human liver hepatocellular carcinoma cell line</td>
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<tr>
<td>HEWL</td>
<td>hen egg white lysozyme</td>
</tr>
<tr>
<td>His or H</td>
<td>histidine</td>
</tr>
<tr>
<td>H2O</td>
<td>water</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>HT-29</td>
<td>human colorectal adenocarcinoma cell line</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz (s-1)</td>
</tr>
<tr>
<td>IC50</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IHF</td>
<td>integration host factor</td>
</tr>
<tr>
<td>K</td>
<td>kelvin</td>
</tr>
<tr>
<td>Lys or L</td>
<td>lysine</td>
</tr>
<tr>
<td>IMes</td>
<td>1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene)</td>
</tr>
<tr>
<td>IM-MS</td>
<td>ion mobility mass spectrometry</td>
</tr>
<tr>
<td>m</td>
<td>milli/multiplet</td>
</tr>
</tbody>
</table>
M  molar/ molecule
Met or M  methionine
mg  milligram
μg  microgram
mL  millilitre
μL  microlitre
mM  millimolar
mmol  millimole
m/z  mass-to-charge ratio
Jurkat  T-cell leukaemia
K2CO3  potassium carbonate
Kd  dissociation constant
KP1019  hexacoordinate indazolium trans-[tetrachloridobis(1H-indazole)ruthenate(III)]
KSHV  Kaposi’s Sarcoma-associated herpesvirus
L1210  mouse lymphocytic leukaemia cells
LANA  latency-associated nuclear antigen
Log P  octanol-water partition coefficient
NaCl  sodium chloride
NCP  nucleosome core particle
NAMI-A  imidazolium trans-[tetrachloride(dimethylsulfoxide- S)(1H-imidazole) ruthenate(III)]
NF-κB  key transcriptional activator of the Fanconi anemia pathway
NHC  N-heterocyclic carbene
NKP1019  hexacoordinate sodium trans-[tetrachloridobis(1H-indazole)ruthenate(III)]
NMR  nuclear magnetic resonance
NSHC  N, S-heterocyclic carbene
NTR1-3  neurotensin receptors 1-3
MBHA  4-methylbenzhydrylamine
<table>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>MD</td>
<td>molecular dynamics</td>
</tr>
<tr>
<td>MIA PaCa-2</td>
<td>human pancreas carcinoma cell line</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide</td>
</tr>
<tr>
<td>Orn</td>
<td>ornithine</td>
</tr>
<tr>
<td>OM</td>
<td>organometallic moiety</td>
</tr>
<tr>
<td>p</td>
<td>para</td>
</tr>
<tr>
<td>Pan-MPP</td>
<td>pan-matrix metalloproteinases</td>
</tr>
<tr>
<td>pBR322</td>
<td>supercoiled plasmid DNA</td>
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<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PET3</td>
<td>triethylphosphine</td>
</tr>
<tr>
<td>PFV</td>
<td>prototype foamy virus</td>
</tr>
<tr>
<td>Phe or F</td>
<td>phenylalanine</td>
</tr>
<tr>
<td>PPh3</td>
<td>triphenylphosphine</td>
</tr>
<tr>
<td>Ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>Pro or P</td>
<td>proline</td>
</tr>
<tr>
<td>PTA</td>
<td>1,3,5-triaza-7-phosphaadamantane</td>
</tr>
<tr>
<td>q</td>
<td>quintet</td>
</tr>
<tr>
<td>RCC1</td>
<td>regulator of chromatin condensation 1</td>
</tr>
<tr>
<td>RAED</td>
<td>([((\eta^6\text{-arene})\text{RuCl}_2(\text{ethylene diamine})]^+))</td>
</tr>
<tr>
<td>RAPTA</td>
<td>([((\eta^6\text{-arene})\text{RuCl}_2(1,3,5\text{-triaza-7-phosphaadamantane})])</td>
</tr>
<tr>
<td>Rf</td>
<td>retardation factor</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>reverse phase high performance liquid chromatography</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
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<tr>
<td>RU1</td>
<td>RAPTA-T binding site containing E61 and E64 on the NCP</td>
</tr>
<tr>
<td>RU2</td>
<td>RAPTA-T binding site containing E102 and H106 on the NCP</td>
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<tr>
<td>sept</td>
<td>septet</td>
</tr>
<tr>
<td>Ser or S</td>
<td>serine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>SGC-7901</td>
<td>human gastric carcinoma cell line</td>
</tr>
<tr>
<td>SRL</td>
<td>sarcin ricin loop</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>t1/2</td>
<td>half life</td>
</tr>
<tr>
<td>T47D</td>
<td>human breast cancer cell line</td>
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<tr>
<td>TBTU</td>
<td>O-(benzotiazol-1-yl)-N,N,N',N'-tetramethyl uronium tetrafluoroborate</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
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<td>TFA</td>
<td>trifluoroacetic acid</td>
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<td>THA</td>
<td>5,8,9,10-tetrahydroanthracene</td>
</tr>
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<td>Tht</td>
<td>tetrahydrothiophene</td>
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<tr>
<td>Thr or T</td>
<td>threonine</td>
</tr>
<tr>
<td>TIS</td>
<td>triisopropylsilane</td>
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<td>TLC</td>
<td>thin layer chromatography</td>
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<td>5'-TMP</td>
<td>thymidine 5'-monophosphate</td>
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<td>Trp or W</td>
<td>tryptophan</td>
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<tr>
<td>TrX</td>
<td>thioredoxin reductase</td>
</tr>
<tr>
<td>TSC</td>
<td>thiosemicarbazone</td>
</tr>
<tr>
<td>Tyr or Y</td>
<td>tyrosine</td>
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<tr>
<td>5'-UMP</td>
<td>uridine 5'-monophosphate</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>ultraviolet visible</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
</tr>
<tr>
<td>XPC</td>
<td>xeroderma pigmentosum complementation group C</td>
</tr>
</tbody>
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Chapter 1
Metal-Based Crosslinking Anticancer Agents
1.1 Introduction

Non-covalent DNA-protein crosslinks are vital for cell survival. Fundamental control of DNA replication, transcription and cellular responses to DNA damage is mediated by the binding of regulatory proteins to chromosomal DNA.\(^1\) Despite their fundamental importance, disruptive DNA lesions, caused by the covalent entrapment of proteins on DNA, present major physical challenges to the DNA repair mechanisms of the cell.\(^2\) These covalent DNA-protein crosslinks, involving DNA lesions, can be induced by both endogenous and exogenous factors including formaldehyde, acetaldehyde, ionising radiation,\(^3\) the abortive action of some enzymes and chemotherapeutic drugs.\(^4\)

Chemotherapeutic drugs targeting DNA must compete with DNA lesion repair mechanisms to achieve cell death. Human cells are remarkably adept at identifying and repairing DNA damage using an arsenal of enzymatic tools and repair mechanisms.\(^5\) Considered the most toxic of DNA lesions, replication associated DNA double strand breaks occur when DNA lesions obstruct replication fork progression in the S-phase of the cell cycle.\(^6\)\(^7\) Studies have shown that DNA crosslinks are most critical in the S-phase of proliferating cells.\(^8\) The repair mechanism used by the cell is dependent on the nature of the double strand break. Non-homologous end joining is the most common repair mechanism for most direct double strand breaks, whereas replication associated breaks are typically repaired by homologous recombination and other related replication repair pathways.\(^9\)\(^10\) More commonly, chemotherapeutic drugs covalently bind to DNA, chemically modifying the nucleobases and interfering with replication fork progression. Monofunctional alkylating agents possess one active site and therefore can modify a single nucleobase. In contrast, bifunctional alkylating agents pose a more severe challenge to replication repair mechanisms via the formation of DNA-protein crosslinks or alternatively intra- or inter-strand DNA crosslinks which can severely block replication forks.\(^11\)

Attacking this pathway comes with repercussions. Enhanced repair of DNA-chemotherapeutic agent crosslinks can result in clinical acquired resistance (Figure 1.1).\(^12\)\(^13\) The source of such resistance has been linked to the overexpression of P-glycoprotein, a transmembrane drug efflux pump, as well as p53-triggered upregulation of nucleotide excision repair genes, xeroderma pigmentosum complementation group C (XPC) and damaged DNA-
binding protein 2 (DDB2).[14][15] Interestingly, DNA repair pathways can also be manipulated in order to sensitise cells to DNA crosslinking therapeutic agents.[16] The Fanconi anemia pathway coordinates the DNA repair pathways, including homologous recombination and nucleotide excision repair, responsible for resolving DNA crosslinks.[17] Hyperactivation of the Fanconi anemia genes have been linked to melphalan (an alkylating agent) resistance in multiple myeloma.[18] However, a study found that the resistance can be circumnavigated by inhibiting key transcriptional activator, NF-κB, of the Fanconi anemia pathway.[19] Manipulating the monoubiquitination of Fanconi anemia gene, FANCD2, through the inhibition of the Nedd8-conjugation system can also sensitise the cell to crosslinking agents.[20]

![Diagram](image.png)

Figure 1.1. The formation of DNA crosslinks and the development of resistance.

DNA crosslinks can be categorised into two groups; (i) short range crosslinks that can link a maximum of three nucleobases on a single DNA strand or crosslink the two strands of double stranded DNA or (ii) long range crosslinks that can link more distant nucleobases or even two lengths of double stranded DNA (Figure 1.2). Each category offers different challenges to the cell repair pathway.
1.2 Platinum-Based Crosslinking Anticancer Agents

Cis-diamminedichloroplatinum(II), known as cisplatin (Figure 1.3), is a bifunctional alkylating agent with short range crosslinking capabilities. Approved for clinical use in 1978, cisplatin is used to treat a variety of cancers including lung, ovarian, brain, breast cancers and carcinoma.\[21\] Activated by the cell cytoplasm, the labile chloride ligands dissociate and water molecules associate forming a strong electrophile with the capacity to react with the nucleophilic groups of proteins and DNA. Binding to the purine residues of DNA, cisplatin forms predominantly 1,2-intrastrand crosslinks (d(GpG) and d(ApG)) between purine residues that ultimately block cell division and result in apoptosis. The formation of 1,3-intrastrand d(GpXpG) adducts and short-range interstrand crosslinks can also occur.\[21\] The shorter 1,2-d(GpG) intrastrand crosslinks induce a more severe bend in DNA, with values ranging from ca. 60° to 80°,\[22,23\] compared to 1,3-d(GpTpG) crosslinks where the bend observed was ca. 30°.\[24\] 1,3-d(GpTpG)-cisplatin DNA adducts containing the cis-[Pt(NH$_3$)$_2$]$^{2+}$ fragment could influence the rotational positioning of nucleosomal DNA forcing it to adopt an asymmetric arrangement with respect to the histone core. The position of the 1,3-d(GpTpG) adduct within
the nucleosome may offer protection from repair mechanisms due to the proximity of the histone core. However, crosslinked cisplatin-DNA adducts are susceptible to DNA repair mechanisms which results in clinical acquired resistance in tumors. The emergence of resistance alongside the severe side effects of cisplatin treatment prompted the development of novel organometallic drugs with differing modes of action.

Figure 1.3. Cisplatin and its derivatives, carboplatin and oxaliplatin, alongside polynuclear platinum(II) complexes inspired by cisplatin.

Cisplatin and its derivatives, oxaliplatin and carboplatin (Figure 1.3), share similar mechanisms of action and consequently similar resistance issues, whereas multinuclear platinum complexes, with their scope for long-range crosslinks, offer an alternative approach to platinum cancer therapy. The dinuclear platinum complexes \( \{\text{trans-PtCl(NH}_3)\}_2\text{H}_2\text{N(CH}_2)\text{6NH}_2\}^{2+} \) and \( \{\text{cis-PtCl(NH}_3)\}_2\text{H}_2\text{N(CH}_2)\text{6NH}_2\}^{2+} \) (BBR3005 and BBR3171, respectively, Figure 1.3) and the trinuclear platinum complex \( \{\text{trans-PtCl(NH}_3)\}_2\mu-\{\text{trans-Pt(NH}_3)\}_2\text{H}_2\text{N(CH}_2)\text{6NH}_2\}_4^{4+} \) (BBR3464, Figure 1.3) were developed with the expectation of possessing novel modes of DNA binding. Although the DNA interstrand crosslinks formed by trans-platinum complexes BBR3005 and BBR3464 possess similar
structural profiles, they differ in interstrand crosslinking efficiency. BBR3464 crosslinked 20% of plasmid pSP73 DNA (linearised by EcoRI) compared to 70-90% by BBR3005. These values are significantly higher than the ca. 6% reported for cisplatin and, in the case of BBR3464, comparable to the 10-20% of trans-diamminedichloroplatin (transplatin).

Despite the structural similarities, the localisation of a +2 charge on the central \([\text{trans-Pt(NH}_3)_2\{\text{H}_2\text{N(}CH_2)_6\text{NH}_2\}_2]\) unit of BBR3464 drastically increases the cytotoxicity of the compound compared to BBR3005. The high charge of BBR3464 is also linked to its rapid binding to DNA (t_{1/2} = ca. 40 minutes) due to charge-mediated pre-association. Binding in a bifunctional fashion, BBR3464 forms long-range inter- and intrastrand DNA crosslinks with the platinated guanine bases being one or more base pairs apart. Interestingly, delocalised BBR3464 long-range intrastrand crosslinks are equally, if not more, probable than intrastrand crosslinks. Further studies revealed that BBR3464 preferentially forms 1,4-interstrand crosslinks, consequently binding to guanine residues separated by two base pairs, in both the 3'-3' and 5'-5' directions. The 1,4-interstrand crosslinks are more resistant to DNA repair mechanisms, including nucleotide excision repair, than BBR3464 intrastand adducts. The 1,4-interstrand BBR3464 adducts distort DNA resulting in a directional bend of helical axis and local unwinding of the duplex. In addition to the 1,4-interstrand crosslinks, BBR3464 is capable of long range 1,6-interstrand crosslinks where the charged central \([\text{PtN}_4]\) unit rests in the minor groove.

Following structure-activity studies, BBR3464 was selected for clinical trials, predominantly due to possessing \textit{in vitro} activity that is 2-3 fold magnitude higher than cisplatin against cisplatin resistant cell lines. Pre-clinical trials revealed activity in p53 mutant tumours and minimal induction of p53 following BBR3464 treatment. However, despite passing Phase I trials with promising activity against pancreatic, non-small cell lung, ovarian cancer and melanoma, BBR3464 failed the subsequent Phase II studies. Minimal response was observed in patients with small cell lung cancer or gastric and gastro-esophageal adenocarcinoma.
1.3 Ruthenium-Based Crosslinking Anticancer Agents

As in the case of polynuclear cisplatin derivatives, ruthenium anticancer agents followed a similar route in the search for novel binding modes. Leading ruthenium(III) compounds, imidazolium \textit{trans}-[tetrachloride(dimethylsulfoxide-\text{S})(1\textit{H}-imidazole)ruthanate(III)] (NAMI-A, Figure 1.4), \cite{41,42} hexacoordinate indazolium \textit{trans}-[tetrachloridobis(1\textit{H}-indazole)ruthanate(III)] (KP1019, Figure 1.4) \cite{43-45} and its sodium salt analogue (NKP1339, Figure 1.4) \cite{46} have completed phase I and phase I/II clinical trials. Preclinical studies revealed the anti-metastatic effect of NAMI-A, \cite{47,48} and proteins are considered the primary biomolecular targets. \cite{49,50}

Figure 1.4. Leading ruthenium(III) anticancer agents, NAMI-A, KP1019 and NKP1019, and dinuclear ruthenium(III) complexes, 1-3, inspired by NAMI-A.
Despite DNA not being considered the primary target of the ruthenium(III) complexes,\textsuperscript{[51]} complexes such as NAMI-A are capable of binding to DNA following the hydrolysis of at least one labile chloride ligand.\textsuperscript{[52]} NAMI-A was even shown to induce DNA-protein crosslinks in isolated nuclei.\textsuperscript{[53]} Dinuclear derivatives of NAMI-A have been developed, i.e. (NH₄)\textsuperscript{[RuCl\{dimethylsulfoxide-S\}\{µ-pyrazine\}\{RuCl\{dimethylsulfoxide-S\}(dimethylsulfoxide-O)\}]} (1, Figure 1.4), Na\textsubscript{2}[{RuCl\{dimethylsulfoxide-S\}\{µ-(4,4'-bipyridine)\}]} (2, Figure 1.4) and Na\textsubscript{2}[{trans-RuCl\{dimethylsulfoxide-S\}\{µ-[4,4'-(1,2-ethanediyl)bispyridine]\}]} (3, Figure 1.4), possessing different spacers. The dinuclear complexes, 1-3, appear to have similar impact on the cell cycle as NAMI-A. The complexes were found to be ineffective in the treatment of a primary tumour when tested \textit{in vivo} using the murine MCA carcinoma model that spontaneously metastasises in the lungs. However, 3 showed strong anti-metastatic properties comparable to NAMI-A at a 3.5 fold lower dosage (measured in moles of ruthenium). The total number of lung metastases was reduced by 85 % with shrinking of the remaining metastases by an average of 95 % in weight compared to controls. Compound 3 is also an effective inter-strand DNA crosslinking complex in linearised plasmidic DNA. Compared to NAMI-A, 3 can form inter-strand DNA crosslinks at a faster rate (\(t_{1/2} = 2.5\) h for 3 versus \(t_{1/2} = 4.5\) h for NAMI-A) and to a higher degree (after 48 h, ca. 10.5 % ruthenium bound for 3 versus ca. 4.7 % for NAMI-A).\textsuperscript{[54][55]}

The ability to control the target of ruthenium(II)-arene complexes was discovered through the comparison of the RAPTA and RAED families of complexes. The RAPTA complexes, possessing a general structure of \([\eta^6\text{-arene}]\text{RuCl}(1,3,5\text{-triaza-7-phosphaadanantane})\] are renowned for their general low toxicity and range of attractive properties including antimetastasis and antiangiogenesis.\textsuperscript{[56]} RAED complexes, with the general structure \([\eta^7\text{-arene}]\text{RuCl}(\text{ethylenediamine})]^+\), are markedly more toxic with a cytotoxicity against primary tumours that is analogous to cisplatin.\textsuperscript{[57][58]} Despite the structural similarities, the RAPTA and RAED families possess different targets. The positively charged RAED-C, \([\eta^8\text{-p-cymene}]\text{RuCl}(\text{ethylenediamine})]\text{PF}_6\) (Figure 1.5), possesses an affinity for DNA, binding preferentially to N7 on the guanine bases.\textsuperscript{[57–59]} RAPTA complexes, such as \([\eta^7\text{-p-cymene}]\text{RuCl}(1,3,5\text{-triaza-7-phosphaadanantane})\] (RAPTA-C, Figure 1.5) preferentially to bind to proteins.\textsuperscript{[60]}
The nucleosome core particle (NCP) is the repeating unit of chromatin comprised of an octamer of pairs of H2A, H2B, H3 and H4 histone proteins encircled by 145-147 base pairs of DNA.\textsuperscript{[61]} The proximity of DNA and protein makes it a good model for a competition assay between DNA and protein. X-ray crystallographic studies performed on the NCP revealed that RAPTA-C forms three specific histone protein adducts sites on the exposed face of the H2A-H2B dimer where a high abundance of glutamate and aspartic residues are located.\textsuperscript{[62]} In contrast, although it also shares a histone binding site with RAPTA-C, RAED-C forms DNA adducts at two pseudo-symmetry related adduct sites (SHL ±1.5) via monofunctional coordination to the 5′-guanine N7 atom of an AGG unit. This preferential binding was further observed \textit{in vitro} where 77\% of RAED-C chromatin adducts are formed on DNA whilst 61\% of RAPTA-C chromatin adducts are formed on the histone proteins.\textsuperscript{[63]} Thus the structure and properties of the leg ligand in ruthenium(II)-arene complexes has the ability to direct the primary target of the complex.
The nature of the arene moiety can influence the interactions of a ruthenium(II)-arene complex with DNA. Replacing the \( \eta^6 \)-cymene moiety of RAED-C with bulky, hydrophobic biphenyl, dihydroanthracene or tetrahydroanthracene (THA) results in non-covalent interactions between the arene and DNA in addition to the typical Ru-N7 guanine interaction.\(^{[59]}\) This additional interaction has a strong impact on the cytotoxicity, \([\eta^6\text{-THA}]\text{RuCl}({\text{ethylenediamine}})\text{PF}_6\) (RAED-THA, Figure 1.5) is 20 times more cytotoxic than RAED-C.\(^{[57][58][64]}\) X-ray crystallographic studies on the NCP revealed RAED-THA binds at the same SHL \( \pm 1.5 \) sites as RAED-C on the DNA. However, the [RAED-THA]-DNA adducts also include a one-stranded intercalation and increased DNA distortion.\(^{[65]}\) In fact, this bimodal DNA binder has been shown to withstand DNA repair mechanisms \textit{in vitro} more efficiently than either RAED-C or cisplatin.\(^{[64]}\)

Inspired by the positive attributes of RAED-THA, a dinuclear RAED complex of the structure \([((\eta^6\text{-biphenyl})\text{RuCl}({\text{en}}))\text{-}(\text{CH}_2)_6]^2+\) (4, Figure 1.5) was developed linking two RAED-biphenyl units by a long alkyl chain which allows the metal centres to act as independent units. Containing four stereogenic centres, 4 can adopt ten different conformations with the \( R^\alpha, R^\delta \) configuration found to be thermodynamically favoured. 4 forms DNA crosslinks with efficiency comparable to cisplatin. 1,3-d(GpXpG) interstrand crosslinks alongside 1,2-d(GpG) and 1,3-d(GpTpG) intrastrand crosslinks were observed on site-specifically ruthanated 20-mer oligonucleotides. The binding of 4 induces significant unwinding of plasmid DNA with an angle of 31°, over twice that of its monomer (14°) and inhibits DNA-directed RNA synthesis \textit{in vitro}.\(^{[66]}\)

A series of dinuclear ruthenium(II)-arene complexes (5\textit{a-f}, Figure 1.5), inspired by the RAPTA family and linked via the arene, was synthesised exploring the influence of conformation on the cytotoxicity and binding. The conformation of the ruthenium centres is controlled by the stereochemical configuration of the 1,2-diphenylethylenediamine linker. Complexes 5\textit{a} and 5\textit{c}, with the 1,2-diphenylethylenediamine linker in the RR and SS configurations, possess a closed structure whilst 5\textit{b}, with the 1,2-diphenylethylenediamine linker in the RS configuration, and 5\textit{d-f}, with the more flexible alkyl and polyethylene glycol chains, have open conformations. The closed conformations, 5\textit{a} and 5\textit{c}, were found to be the most cytotoxic against the tested cell lines, A2780, A2780cisR and HEK-293. As previously stated, the RAPTA complexes, bearing their 1,3,5-triaza-7-phophaaadamantane ligand, bind
preferentially to proteins.\textsuperscript{(63)} Despite this preference, interactions with nucleobase guanosine 5’-monophosphate (5’-GMP) were observed in both 1:1 and 2:1 (5’-GMP:complex) ratios. 5a, 5c and 5d formed 1:1 (5’-GMP:complex) adducts where all four labile chloride ligands have been lost, suggesting that both ruthenium centres are bound to the same 5’-GMP. The incubation of 5a-d with 13-mer 5’-ATACATCGTACAT-3’ lead to 1:1 adducts in which the metal complex had lost all chloride ligands. However, higher order adducts were not observed.\textsuperscript{(67)}

Mass spectrometry analysis revealed interactions between 5a-d and \(\epsilon\)-histidine where 1:1 and 2:1 (\(\epsilon\)-histidine:complex) adducts were observed. Analysis of 5a-d complexes binding to the amyloid \(\beta\)-protein (residues 1–16, H-Asp\(^1\)-Ala\(^2\)-Glu\(^3\)-Phe\(^4\)-Arg\(^5\)-His\(^6\)-Asp\(^7\)-Ser\(^8\)-Gly\(^9\)-Tyr\(^10\)-Glu\(^11\)-Val\(^12\)-His\(^13\)-His\(^14\)-Gln\(^15\)-Lys\(^16\)-OH), performed using electron transfer dissociation fragmentation, indicated that the dinuclear complexes were able to cross link histidine residues His\(^6\), His\(^13\) and His\(^14\). Ion mobility mass spectrometry (IM-MS) revealed split distributions in the arrival time distributions of the (5a-5d)-peptide adducts suggesting that the complexes crosslink the peptide at His\(^6\)-His\(^13\) and His\(^6\)-His\(^14\), which leads to the formation of adducts with two different sizes (Figure 1.6).\textsuperscript{(67)}

![Figure 1.6. Dinuclear ruthenium(II) complexes, 5a, 5b and 5c, crosslinking the amyloid \(\beta\)-protein (residues 1–16, H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-OH).](image-url)
X-ray crystallographic and molecular dynamic studies performed on the nucleosome core particle showed that ruthenium(II) complexes possessing both the closed and open conformations (5a-5f, Figure 1.5) crosslink the RU1 and RU2 binding sites on the highly electronegative acidic patch of the NCP. Initially, substantial adduct formation is observed at the RU1 site where the ruthenium centre, which has lost the labile chloride ligands, coordinates to the E61 and E64 carboxylate side chains of the H2A protein. Once a high occupancy of RU1 is achieved, it is followed by considerable adduct formation in the RU2, where the ruthenium centre coordinates to carboxylate E102 and histidine H106 residues on the H2B protein. Therefore, the coordination of the dinuclear complexes mirrors those of mononuclear complexes, RAPTA-T and RAPTA-C, wherein the RU1 site is initially favoured and its occupation stabilises the formation of adducts at the RU2 sites by providing addition hydrophobic interactions.\[63][64]

The flexibility of the linker and the subsequent conformation of the dinuclear complexes dictate which, if any, sites are crosslinked on the NCP. Although the linker could not be resolved for 5d-f, bearing the more flexible linkers C2, C10 and PEG, the RU1 and RU2 adducts are well defined. The linkers of the more rigid dinuclear complexes, 5a-b, were resolved and the linking of the RU1 and RU2 site is clearly visible in the crystal structure. 5a (RR) and 5c (SS) possess the more closed conformation, where the ruthenium centres remain on the same face of the linker, allowing the complexes to crosslink the RU1 and RU2 sites. In contrast, the ruthenium centres reside on opposing faces of the linker in 5b (RS). Therefore, although 5b initially binds to the RU1 site, similar to the rest of the series, the second ruthenium centre is unable to orientate itself in a way that binding to the RU2 site is feasible. Instead, the second ruthenium centre of 5b binds to the more distant E91 histone side chain on the H2A protein.\[69]

The biological consequences of these dinuclear ruthenium(II) crosslinking agents contrast to those of cisplatin and RAPTA-C as the dinuclear complexes, 5a-f, neither arrest specific cell cycle phases nor cause DNA damage response. However, they do induce a misfolding of chromatin fibre and block the binding of the acidic patch binding protein, the regulator of chromatin condensation 1 (RCC1) resulting in an irreversible, anomalous state of condensed chromatin that eventually results in apoptosis.\[69]
A series of dinuclear ruthenium(II)-p-cymene complexes, where two \{(\eta^6-p-isopropyltoluene)RuCl[3-oxo-O]-2-methyl-4-pyridinonato-O,\} units are linked via flexible alkyl chains of varying lengths, (CH$_2$)$_n$ (where n = 4, 6, 8, 12), were developed (6, Figure 1.7). The monomeric example of this complex was shown to coordinate to 9-ethylguanine suggesting that the target of the dinuclear complex could be DNA.$^{[6]}$ A direct correlation was observed between the linker length, lipophilicity and cytotoxicity of the complexes. The monomer was inactive against the A2780 cell line up to 50 µM$^{[6]}$ whereas the dinuclear complex bearing the longest chain (n = 12) possesses an IC$_{50}$ of 1.5 ± 0.3 µM.$^{[7]}$ The dinuclear complexes also show good activity against oxaliplatin-resistant cells lines, in some cases with a 10-fold increase in cytotoxicity.$^{[8]}$ Complexes, 6, were shown, via ESI-MS, to interact with transferrin when incubated in a 1:1 (complex:protein) ratio. Interestingly, 1:2 (complex:protein) adducts were not observed suggesting that the complexes, 6, do not crosslink two transferrin molecules. The complexes, 6, were shown to rapidly coordinate to nucleotides guanosine 5'-monophosphate (5'-GMP) and adenosine 5'-monophosphate (5'-AMP), but no interaction was observed with cytidine 5'-monophosphate (5'-CMP), uridine 5'-monophosphate (5'-UMP) or thymidine 5'-monophosphate (5'-TMP). The complex, 6 (n = 6), was incubated with a 13-mer oligonucleotide and 1:1 adducts were observed.$^{[9]}$ Interestingly, during a more detailed binding study, the 6 bearing the longest spacer ( n = 12), has a different mode of binding and can form a high degree of DNA-protein and DNA duplex crosslinking in a model involving Klenow fragment from DNA polymerase I (KF) and linker histone H1. The extent of DNA-protein crosslinks is dependent on spacer length, and the formation of these so-called “suicide lesions” could inhibit the binding of vital repair proteins and transcription factors.$^{[10]}$

Figure 1.7. Structures of dinuclear ruthenium complexes, 6 and 7, and mononuclear derivative, 8.
Dinuclear ruthenium(II) complexes, of the structure \([\eta^6-p\text{-cymene})\text{Ru(N,S-TSC)}]_2(\text{PF}_6)_6\) (where TSC = thiosemicarbazone) (7, Figure 1.7), exhibit cytotoxicities superior to those of cisplatin and oxaliplatin against nasopharyngeal carcinoma (CNE-2) and gastric carcinoma (SGC-7901) cell lines. The mono and dinuclear complexes show differing modes of DNA binding. Whilst the mononuclear complex (8, Figure 1.7) induces significant unwinding of supercoiled DNA, the dinuclear complex, 7, lengthens the supercoiled form of pBR322 plasmid DNA and significantly reduces DNA mobility without causing obvious unwinding. This suggests that binding mode of 7 is DNA base pair intercalation.\(^{[74]}\)

Based on dinuclear iron analogues,\(^{[75]}\) dinuclear double stranded metallosupramolecular ruthenium complexes with the general cyclindrical structure \([\text{Ru}_2\text{Cl}_4\text{L}_2]\) (where L = bisazopyridine bearing a di(4-phenyl)methane spacer) were also designed to target DNA.\(^{[76]}\) A dinuclear ruthenium(II) triple-stranded helicate \([\text{Ru}_2\text{L}_3]^{4+}\), where L = bis(pyridylimine) ligand, with luminescent properties was also prepared. Aided by its strongly positive charge, the \([\text{Ru}_2\text{L}_3]^{4+}\) compound was shown to bind non-covalently to the major groove of the negatively charged DNA. Despite the non-covalent nature of the interaction of \([\text{Ru}_2\text{L}_3]^{4+}\) with DNA, its cytotoxicity against human breast cancer cell lines, HBL-100 and T47D, were just 2-5 fold lower than cisplatin which covalently binds to DNA.\(^{[77]}\) Further studies revealed that \([\text{Ru}_2\text{L}_3]^{4+}\) unwinds negatively supercoiled plasmid DNA and possesses an identical binding mode to the \([\text{Fe}_2\text{L}_3]^{4+}\) analogue suggesting the cage itself controls the binding. However, upon irradiation with visible and UVA light, \([\text{Ru}_2\text{L}_3]^{4+}\) induces single-strand DNA breaks preferentially at guanine residues.\(^{[78]}\)

1.4 Gold-Based Crosslinking Anticancer Agents

Gold(I) and gold(III) complexes have a high affinity for thiol-containing residues such as cysteines and selenocysteines. Thioredoxin reductase (TrX) and glutathione reductase, two key enzymes in the regulation of cellular redox balance-associated cell growth, contain cysteine residues in their active sites and consequently can be inhibited by gold anticancer agents.\(^{[79-81]}\) At the forefront of gold-based anticancer medicines is the gold(I) complex,
auranofin $\left[\text{1-thio-}\beta\text{-d-glucopyranose-2,3,4,6-tetraacetato-S}(\text{triethylphosphine} \text{gold(I)})\right]$ (Figure 1.5), a gold(I) compound being repositioned since it was originally developed and approved for the treatment of rheumatoid arthritis.\textsuperscript{[82-84]} Auranofin has been shown to selectively inhibit the mitochondrial form of thioredoxin reductase consequently inducing apoptosis.\textsuperscript{[85]} Additionally, its ability to inhibit pro-inflammatory pathways makes it an interesting candidate to tackle a myriad of ailments.\textsuperscript{[86]}

Figure 1.8. Structure of auranofin and its binuclear derivative bis(diphenylphosphino)ethanegold(I) chloride, 9.

Gold(I) complexes, such as auranofin, possess one labile or “sacrificial” ligand (i.e. can be described as monofunctional whereas compound with two labile ligands are referred to as bifunctional), are unable to crosslink biomolecules but instead form a single covalent bond. In addition to interactions with thiol-containing enzymes, gold(I) complexes have been shown to bind to histidine residues. A crystal structure has been solved showing the gold(I) complex, Au(P\text{Et}_3)\text{Cl} where the chloride has dissociated, binding to the histidine residue present in cyclophilin 3 (CYP-3) despite the presence and availability of two thiol-containing cysteine residues, Cys163 and Cys168.\textsuperscript{[87]} This is not a stand alone finding, gold(I) complexes binding with histidines have also been observed with ribonuclease A and hen egg white lysozyme.\textsuperscript{[88][89]}

In fact, x-ray crystallographic studies of the nucleosome core particle unearthed an allosteric relationship between RAPTA-T, $\left[\eta_6^\prime\text{-p-toluene}\text{RuCl}_2(1,3,5\text{-triaza-7-phosphaadamantane})\right]$, and auranofin. Interestingly, no evidence of binding was observed after of 4 days of incubation with the nucleosome core particle with auranofin alone. However, when used in combination with RAPTA-T, ruthenium adducts are observed occupying the RU1 and RU2 binding site on the H2A histone protein and auranofin binding is observed at two symmetry-related locations, AU1 and AU1'. Within the AU1 and AU1' binding sites, the sugar-thiolate of auranofin has dissociated and the gold centre coordinates to the imidazole delta nitrogen.
atoms of the histidine residues, H113 and H113’, located on the H3/H3’ histone proteins. The binding is stabilised by the extensive hydrophobic interactions between surrounding H3/H3’ residues and the triethylphosphine group of auranofin. Molecular dynamic simulations revealed that binding of RAPTA-T causes a series of small structural changes resulting in a kink in the long α-helix of the H2A protein which opens up the AU1 and AU1’ sites sufficiently to favour auranofin binding.\(^{[68]}\)

Although dinuclear gold(I) complexes have been extensively studied, their ability to crosslink either proteins or DNA has been scarcely explored.\(^{[90]}\) The dinuclear complex, bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride (9, Figure 1.8), was found to produce a concentration dependent increase in DNA single-stranded breaks in mouse lymphocytic leukaemia (L1210) cells. The cells were unable to repair the breaks and the quantity of DNA single-stranded breaks increased when the cells were further incubated in complex-free medium. Significant numbers of DNA-protein crosslinks were observed in addition to the DNA single-stranded breaks at low concentrations of 9. As the concentration of 9 was increased, the quantity of DNA-protein crosslinks decreased until only DNA single-stranded breaks were evident. 9 was shown to inhibit the uptake of \(^{[3}H\) thymidine, \(^{[3}H\) uridine and \(^{[3}H\) leucine into DNA, RNA and protein in B16 melanoma cells. The inhibition was both concentration and time-dependent but was seen to be of greater impact to protein biosynthesis where the complex rapidly shut down leucine incorporation.\(^{[91]}\) However, in contrast, no interaction was observed between dinuclear gold(I) alkynyl complexes bearing PTA or DAPTA ligands and plasmid pBR322 DNA.\(^{[92]}\) Other dinuclear alkynyl gold(I) complexes were found to inhibit thioredoxin reductase to a comparable extent as auranofin. However, unlike auranofin, no complex-thioredoxin reductase adducts were observed.\(^{[93]}\)

1.5  Heterobimetallic Crosslinking Anticancer Agents

Due to the myriad of targets of gold(I) complexes, they are frequently used in heterobimetallic combinations.\(^{[94]}\) Combining gold(I) with ruthenium(II) or platinum(II) in one complex could lead to heterobimetallic complexes with the ability to form protein-protein,
DNA-DNA and DNA-protein crosslinks. However, although there are examples of heterobimetallic complexes in literature, their crosslinking capabilities have been scarcely explored.

Ruthenium-platinum complexes could potentially crosslink DNA and proteins. A platinum(II)-ruthenium(III) heterobimetallic complex (10, Figure 1.9), inspired by transplatin and NAMIA-A, has been synthesised. 10 was shown to significantly reduce the electrophoretic mobility of sarcin-ricin loop (SRL) RNA compared to cisplatin and the monomeric ruthenium(III) control (11, Figure 1.9). The impact on RNA mobility could be a consequence of two metal centres interacting with the RNA in close proximity. The significant increase in inhibition compared to 11 implies that the introduction of the platinum(II) moiety implicates a chelating effect where the binding of the platinum centre to RNA favourably positions the ruthenium for additional interactions. Similar to cisplatin, 10 shows a preference for purine rich residues, however, it requires a higher degree of three-dimensional accessibility due to its bulkier size. In vitro, 10 has been shown to interfere significantly with ribosomal activity and the synthesis of the dihydrofolate reductase (DHFR) enzyme, essential for de novo synthesis of purines, consequently disrupting the translation initiation process. [96]

A series of heterobimetallic ruthenium(II)-gold(I) complexes have undergone extensive structure-activity experiments. \([\eta^6-p\text{-cymene}]\text{RuCl}_2(\mu\text{-dppm})\text{AuCl}]\) (12, Figure 1.10) and \([\eta^6-p\text{-cymene}]\text{RuCl}_2(\mu\text{-dppm})\text{Au(S-thiazoline)}]\) (13, Figure 1.10) (where dppm = 1,1-bis(diphenylphosphino)methane) were synthesised and no interaction was observed between
the complexes and supercoiled plasmid pBR322 DNA. RuAu-1 and RuAu-2 exhibit a comparable inhibition of cathepsin B, ca. 4.6 times more effective than the mononuclear [([η⁶-p-cymene]RuCl₂(τ¹-dppm))] complex. Protein binding studies, analysed by ESI-MS, found that 12 and 13 possess a high affinity for cytochrome c and RNase via the ruthenium moiety. Fewer adducts were observed between the complexes and hen egg white lysozyme (HEWL). It appears that the gold(I) moiety is not involved in protein binding and, consequently, crosslinks were not detected.\textsuperscript{[96]}

Figure 1.10. Heterobimetallic ruthenium(II)-gold(I) complexes, 12, 13 and 14, inspired by RAPTA-C and auranofin.

With the indication that the labile ligand of the gold(I) complex could be influential to the activity, a series of complexes of general formula [([η⁶-p-cymene]RuCl₂(μ-dppm)Au(NHC)]ClO₄ (where NHC = a N-heterocyclic carbene ligand) bearing side groups of differing bulkiness (IPr = 1,3-diisopropylbenzene, IMes = mesitylene, Icy = cyclohexane and goofy = isopropyl groups) was prepared. The complexes display selectivity towards renal (Caki-1) and colon (HCT-116) cancer cell lines compared to non-tumoral human embryonic kidney (HEK-293) cells, and are more cytotoxic than cisplatin and the mononuclear derivatives against the cancerous cell lines. These heterobimetallic complexes were not found to interact with plasmid pBR322 DNA. However inhibition of thioredoxin reductase activity was observed in Caki-1 cells treated with [([η⁶-p-cymene]RuCl₂(μ-dppm)Au(NHC-iPr)]ClO₄.\textsuperscript{[97]}

From this series of ruthenium(II)-gold(I) complexes bearing N-heterocyclic carbene ligands, [([η⁶-p-cymene]RuCl₂(μ-dppm)Au(IMes)]ClO₄ (where IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene) (14, Figure 1.10) was selected for further evaluation. Consistent with the parent drugs, RAPTA/RAED and auranofin,\textsuperscript{[98]} RANCE-1 induced apoptosis in Caki-1 cells. RANCE-1 has the ability to significantly inhibit migration and
invasion in 2D scratch and 3D Transwells assays as well as showing impressive antiangiogenic properties. The vascular endothelial growth factor (VEGF), a key mediator of angiogenesis, is strongly inhibited by RANCE-1 alongside pan-matrix metalloproteinases (pan-MPP) and pan-cathepsin, but it is a less efficient inhibitor of thioredoxin reductase in Caki-1 cells than auranofin.\textsuperscript{[99]} Despite the extensive study of the biological properties of RANCE-1, there has been no investigation into its crosslinking capabilities.

![Chemical structures](image)

**Figure 1.11.** Heterobimetallic platinum(II)-gold(I) complexes, 15 and 16, inspired by auranofin and cisplatin.

Platinum-gold heterobimetallic complexes have been developed but little in depth analysis on biological targets has been performed.\textsuperscript{[100]} Platinum(II)-gold(I) complexes, combining the structures of cisplatin and auranofin, linked via a dipyridylamine-phosphine ligand (15, Figure 1.11), possess the ability to overcome cisplatin resistance in human ovarian carcinoma (A2780cisR) cells. However, although binding between the platinum moiety and model nucleobase 9-ethylguanine was observed, no interaction was observed between the complex and plasmid BR322 DNA.\textsuperscript{[101]} The encapsulation of related complex, \([\text{PPh}_3\text{Au}(\mu-2-(2'-pyridyl)-benzimidazole)Pt((\text{Me})(\text{DMSO}))][\text{PF}_6]\) (16, Figure 1.11) in a ferritin cage led to the decomposition of the complex, but did indicate the preferred binding of the two metals. The platinum centres remain trapped within the protein but to not coordinate to any side groups or residues, whereas the gold coordinates to the cysteine residues, Cys126.\textsuperscript{[102]}
1.6 Concluding Remarks

The lack of investigation into the crosslinking capabilities of platinum(II), ruthenium(II) and gold(I) heterobimetallic complexes provides much scope for expansion. The well-defined binding preferences of the parent drugs, cisplatin, RAPTA and auranofin enable the design of complexes that possess the capability to form DNA-DNA, protein-protein and DNA-protein crosslinks. Focusing on the combination of RAPTA-T and auranofin, the combination of these parent drugs has pronounced advantageous properties. Pretreatment of the ovarian carcinoma (A2780) cells sensitises the cells to auranofin with a decrease in cell viability observed alongside a three-fold increase in auranofin chromatin uptake observed compared to treatment with auranofin alone. This synergism is accompanied by an allosteric effect observed on the nucleosome core particle. The binding of RAPTA-T at the E61/E64 carboxylate residues on the H2A histone protein (RU1) and H106 imidazole and E102 carboxylate groups on the H2B protein (RU2) enables AUF to bind to two previously inaccessible symmetry-related histidine residues H113 (AU1) and H113’ (AU1’) on the H3 histone proteins. As previously described, the binding of RAPTA-T to the RU1 or RU2 site ignites a series of small structural alterations resulting in a kink within the long α-helix of the H2A protein that opens the AU1/AU1’ binding sites to favour auranofin binding.\[68\]

The primary aim of this thesis is to design and synthesise a heterometallic ruthenium(II)-gold(I) complexes capable of crosslinking these allosteric sites. The ruthenium moiety, closely resembling RAPTA-T, must ignite the allosteric effect by binding to the RAPTA-T sites, RU1 or RU2, and simultaneously bind to the newly available auranofin site, AU1 or AU1’, using a gold(I) moiety attached to the terminus of a long, flexible linker.
1.7 References


Chapter 2
Influence of Linker Length on the Cytotoxicity of Homo-Dinuclear Ruthenium(II) and Gold(I) Complexes.

Reprinted with permission from “Batchelor L.K., Paunescu, E., Soudani M., Scopelliti R., Dyson, P. J. (2017), Influence of Linker Length on the Cytotoxicity of Homobinuclear Ruthenium(II) and Gold(I) Complexes, Inorganic Chemistry. DOI: 10.1021/acs.inorgchem.7b01082”. Copyright enquiries should be directed to the ACS.

The work described was completed in collaboration with Emilia Paunescu who synthesised the gold complexes, Mylène Soudani who performed the cytotoxicity evaluation on the gold complexes and Rosario Scopelliti who resolved the crystal structures.
2.1 Introduction

The clinical success of cisplatin resulted in considerable efforts being directed towards the development of other platinum-based therapeutics.\[^1\] However, the need to overcome the adverse side effects and intrinsic or acquired resistance to these compounds led to the investigation of alternative metals for their therapeutic potential.\[^2\] Ruthenium(III) complexes, imidazolium \([\text{trans-tetrachloro}(1H\text{-imidazole})(\text{S-dimethylsulfoxide})\text{ruthenate(III)}]\),\[^3\] and indazolium \([\text{trans-tetrachloro}(1H\text{-indazole})\text{ruthanate(III)}]\) (KP1019)\[^4–6\] and its sodium analogue (NKP1339)\[^7\], have completed phase I and phase I/II clinical trials. Ruthenium(II) organometallic compounds have also attracted attention as they exhibit a number of promising pharmacological properties.\[^8–10\] For example, the so-called RAPTA complexes (Figure 2.1),\[^11\] of general formula \([\eta^6\text{-arene}]\text{RuX}_2(\text{PTA})\] (PTA = 1,3,5-triaza-7-phosphaadamantane), and the RAED complexes, \([\eta^6\text{-arene}]\text{RuCl(en)}\] (en = ethylene diamine),\[^12\] have been particularly well studied for their anticancer properties. RAPTA-C\[^13\] and RAED-C,\[^14\] (where \(\text{C} = p\text{-cymene}\)) along with their derivatives exhibit an array of promising \textit{in vitro} and \textit{in vivo} properties.\[^9,12,13,15–19\] Interestingly, crystallographic studies on the nucleosome core particle have shown that the choice of ligand strongly influences the biomolecular target of ruthenium(II)-arene complexes with RAED-C preferentially binding to DNA and RAPTA-C binding to the histone proteins.\[^20,21\] Mononuclear gold(I)-phosphine complexes have been evaluated for anticancer properties and exhibit promising activity.\[^22–25\] Auranofin (1-thio-\(\beta-D\)-glucopyranose-2,3,4,6-tetraacetato-S)(triethylphosphine)gold(I), which is used clinically for the treatment of rheumatoid arthritis,\[^25,26\] is currently being repositioned as an anticancer drug.\[^27–32\] Similar to RAPTA complexes,\[^33,34\] auranofin preferentially binds to cysteine-rich proteins such as thioredoxin reductase (TrX).\[^35–37\]

Multinuclearity, i.e. covalently connecting two or more metal centres via an appropriate linker, emerged as an approach to introduce new modes of action to overcome resistance in chemotherapy. The trinuclear platinum compound, \([\{\text{trans-PtCl(NH}_3\}_2-\mu-(\text{trans-Pt(NH}_3\}_2\{\text{H,N(CH}_2\}_6\text{NH}_2\}_2)]^{4+}\) (BBR3464, Figure 2.1), can overcome cisplatin-resistance and it exhibits a profile of antitumor efficacy distinct from that of cisplatin in a number of preclinical models.\[^39\] However, despite successfully passing Phase I clinical trials, BBR3464 failed a Phase II evaluation with only a minor response observed in small lung cancer and gastric/gastro-esophageal adenocarcinoma.\[^40,41\]
A growing number of multinuclear ruthenium(II) and gold(I) complexes have also been reported. Interests in homo-bimetallic ruthenium(II) complexes have focused on structure-reactivity investigations and the use of bioactive bridging ligands such as thiosemicarbazones. The influence of spacer length on in vitro anticancer activity has previously been explored using bis(pyridinone)alkane linkers \((\eta^6-\text{p-cymene})\text{Ru}(O,O-\text{C}_6\text{H}_3\text{N}(\text{CH}_2)_n\text{N}O_2\text{C}_6\text{H}_3O_2\text{Ru}(\eta^6-\text{p-cymene}) (n = 3, 6, 12, \text{RU1, Figure 2.1})

Both proteins and DNA were identified as possible targets for the dinuclear Ru(II) complexes, which hydrolyse rapidly to form the active diaqua species. Importantly, the cytotoxicity of the complex with the longest bridging ligand was attributed to the ability of the complex to form both DNA-DNA and DNA-protein crosslinks. A RAED-type binuclear complex, \([(\text{Ru}(\eta^6-\text{biphenyl})\text{Cl}(\text{en}))_2\text{CH}_2]^{2+}\), similarly bearing an alkyl linker, has been shown to form interstrand DNA crosslinks. Flexible alkyl spacers used in an acylpyrazolonato-bridged ruthenium(II) complexes led to complexes with a higher cytotoxicity than related compounds with rigid phenyl spacers. Attempts were made to investigate polyethylene glycol linkers, however, the bis(nicotinate)/(isonicotinate) ligands were unstable in solution.

Homo-binuclear ruthenium(II) complexes linked by different stereochemically configured 1,2-diphenylethylenediamine spacers exhibit open and closed conformations. The dinuclear complexes are considerably more cytotoxic than the monomers, but did not display cancer cell selectivity. Consequently, a strategy was subsequently developed to generate dinuclear ruthenium(II) complexes directly in cancer cells.

Significant efforts have also been devoted to the development of bis-phosphine gold(I) complexes. However, little attention has been paid to the nature of the linker in homo-binuclear gold(I) complexes. A series of \([(\text{AuCl})_2(\text{Ph}_2\text{P}(\text{CH}_2)_n\text{PPh}_2))] (where n = 1-6) was prepared and evaluated in vitro against murine B16 melanoma cells (AU1, Figure 2.1). The cytotoxicity initially decreases with linker length, n = 1 (6 \(\mu\)M) and n = 2 (8 \(\mu\)M), up to n = 3 where a plateau is reached (n = 3-6, IC_{50} = 2-3 \(\mu\)M). Alkyl linkers were also assessed in phosphine-bridged dinuclear Au(I) alkynyl complexes linked via \((\text{CH}_2)_n\) (where n = 1 and 4) groups. More recently, investigations into lipophilicity have included the exchange of hydrophobic PPh\textsubscript{3} ligands with more lipophilic PEt\textsubscript{3} ligands in dinuclear phosphine-gold(I) sulfanylcarboxylate complexes resulting in lower IC_{50} values against selected cell lines.
Other investigations include optimisation of TrX inhibition bridging bis(N-heterocyclic carbene) ligands, alkynyl ligands and thiocarbonates.

As the use of alkyl chains produce a general correlation between linker length and cytotoxicity correlating with increasing lipophilicity, we decided to evaluate the effect of polyethylene glycol chains. Herein, we report the synthesis, structural characterisation and antiproliferative activity of two series of dinuclear complexes based on ruthenium(II) and gold(I) systems. The bis-ruthenium(II) and bis-gold(I) centres are linked via polyethylene glycol chains of varying length while maintaining the basic structure of the parent drugs, RAPTA-C and auranofin. This strategy allows the influence of linker length on cytotoxicity to be studied and compared to their monometallic precursors.

![Figure 2.1. Selected examples of dinuclear complexes inspired by well-known monometallic drugs.](image)

### 2.2 Results and Discussion

Two series of homo-binuclear ruthenium(II)-p-cymene, 1b-7b, and gold(I), 1d-7d, derivatives were prepared using the routes shown in Scheme 2.1. Universal bis-phosphine ligands, 1a-7a, were synthesised via an esterification reaction between 4-(diphenylphosphino)benzoic acid and the appropriate ethylene glycol in the presence of N-
ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), a coupling agent, and 4-dimethylaminopyridine (DMAP), a basic catalyst. Ligands 1a-7a were isolated by chromatographic purification in moderate yields (36-64%). The bis-ruthenium(II)-p-cymene complexes 1b-7b were obtained in high yields (96-98%) in a single step from the reaction of the appropriate ligands 1a-7a with the \([\eta^6\text{-p-cymene}]\text{RuCl}_2\)_2 dimer, under inert conditions in dry CH$_2$Cl$_2$ (Scheme 2.1). The binuclear Au(I)-\(\beta\)-\(D\)-thioglucose-tetraacetate complexes 1d-7d were prepared following a two-step strategy (Scheme 2.1). The intermediate binuclear Au(I)-chloride complexes, 1c-7c, were obtained in good yields (95-98%) from the direct reaction of the appropriate bis-phosphine ligands 1a-7a with Au(I)Cl(tht) (tht = tetrahydrothiophene), freshly prepared following an adapted literature procedure.[70],[71] Subsequent reaction of 1c-7c with the \(\beta\)-\(D\)-thioglucose-tetraacetate ligand under basic conditions (K$_2$CO$_3$) in acetone or EtOH/H$_2$O affords the desired Au(I) complexes 1d-7d in good yields (84-96%).[71],[72]

![Scheme 2.1: Synthesis of the bis-phosphine ligands (1a-7a), bis-Ru(II)-p-cymene (1b-7b) complexes, bis-Au(I)-Cl intermediates (1c-7c) and the bis-Au(I)-\(\beta\)-\(D\)-thioglucose-tetraacetate complexes (1d-7d), n = 1-6 and 8.](image_url)
Mononuclear complexes were also prepared to provide a structure-activity comparison between the binuclear complexes and the parent drugs, RAPTA-C and auranofin. The mononuclear Ru(II)-p-cymene (8b and 9b) and Au(I) (8d and 9d) complexes, containing 4-(diphenylphosphino)benzoic acid and triphenylphosphine ligands, were prepared from the direct reaction of the phosphines with the \([([\eta^2-\text{p-cymene}]\text{RuCl}_2)]_2\) dimer and Au(I)Cl(tht) precursors, respectively (Scheme 2.1).

All compounds were characterised by \(^1\text{H}\), \(^{31}\text{P}\) and \(^{13}\text{C}\) NMR spectroscopy, mass spectrometry and elemental analysis. In the \(^{31}\text{P}\) NMR spectra the phosphine ligands 1a-7a produce a singlet between -4.99 and -5.08 ppm. In the binuclear complexes, the peaks shift to higher frequencies with singlet resonances observed between 24.94-25.00 ppm for 1b-7b, 33.01-33.14 ppm for 1c-7c and 38.71-38.79 ppm for 1d-7d, confirming coordination of phosphorus centres to the metal ions. The mononuclear complexes also give rise to singlets observed in the same range as the binuclear species, i.e. at 24.18 ppm for 8b, at 25.28 ppm for 9b, at 33.19 ppm for 8c, 33.21 ppm for 9c, 38.83 ppm for 8d and 38.82 ppm for 9d.

The \(^1\text{H}\) NMR spectra of the ligands 1a-7a and the complexes also show distinct differences. The multiplet corresponding to eight protons ortho to the C-P bond on the phenyl rings shifts from 7.29-7.39 ppm to 7.76-7.83 ppm \((\Delta\delta \approx 0.4 \text{ ppm})\), with a larger shift to 7.85-7.98 ppm \((\Delta\delta \approx 0.6 \text{ ppm})\) observed for the four ortho protons on the functionalised ring. Similarly, a shift of \(\Delta\delta \approx 0.2 \text{ ppm}\) to higher frequencies is observed, from 7.29-7.39 ppm \((1a-7a)\) to 7.45-7.60 ppm \((1c-7c\text{ and }1d-7d)\), corresponding to the 12 protons ortho to the C-P bond in the
gold(I) complexes. Complex 8b possesses two multiplets in the aromatic region; 7.77-7.85 ppm, corresponding to the six phenyl protons \textit{ortho} to the C-P bond, and 7.46-7.32 ppm corresponding to the nine \textit{meta-} and \textit{para-}protons. In contrast, the $^1$H NMR spectra of complexes 8c and 8d contain only one aromatic multiplet that corresponds to all fifteen protons of the triphenylphosphine ligand (8c 7.42-7.58 ppm and 8d 7.42-7.59 ppm). Complex 9b possesses a multiplet at 7.97-8.00 ppm corresponding to the 4 protons of the functionalised ring whereas in 9c and 9d the multiplet corresponding to the two protons \textit{ortho} to the C-P bond are observed at 8.05-8.19 ppm in 9c and 7.81-7.90 ppm in 9d.

Coordination of the ligands to the metal ions is denoted by the peaks corresponding to the carbon atoms directly connected to the phosphorous centre shifting to lower frequencies (with increased $^1J_{C,P}$ coupling constants) in the $^{13}$C NMR spectra. In the bis-Ru(II)-$\rho$-cymene complexes 1b-7b a shift of $\Delta\delta_C \approx 4.9$ ppm and coupling of $\Delta^1J_{C,P} \approx 28$ Hz was observed for the two C-P carbons on the functionalised ring and $\Delta\delta_C \approx 3$ ppm and $\Delta^1J_{C,P} \approx 34$ Hz for the peaks corresponding to the four C-P carbons on the phenyl rings. An analogous effect was observed for the bis-gold(I) complexes, for 1c-7c there is a shift of $\Delta\delta_C \approx 9.6$ ppm and coupling of $\Delta^1J_{C,P} \approx 45$ Hz, corresponding to the two C-P carbons on the functionalised ring and a shift of $\Delta\delta_C \approx 8.5$ ppm and $\Delta^1J_{C,P} \approx 52$ Hz representing the four C-P carbons on the phenyl rings. A
similar, but slightly less pronounced effect, is observed in the $^{13}$C NMR spectra of 1d-7d and in the mononuclear complexes similar changes are also observed. The impact of increasing PEG chain length on the electronic environment of the metal centers is negligible with all coupling constants and $^{31}$P peaks remaining consistently similar throughout the series. The most abundant peaks observed in the ESI mass spectra may be assigned to [M+H]$^+$ and [M+Na]$^+$ ions for ligands 1a-9a, as [M-Cl]$^+$ ions for 1b-9b, as [M+Na]$^+$ ions for 1c-8c, for 9c as [M-H]$^-$. ions, for 1d-7d as [M-Cl]$^+$ ions and for 8d-9d as [M+H]$^+$ ions.

Table 2.1. Comparison of selected bond lengths (Å) and angles (°) in RAPTA-C$^{[a]}$ and 9b.

<table>
<thead>
<tr>
<th></th>
<th>RAPTA-C$^{[a]}$</th>
<th>9b$^{[b]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru-η$^6$</td>
<td>1.692, 1.701</td>
<td>1.709, 1.723, 1.706, 1.714</td>
</tr>
<tr>
<td>Ru-P</td>
<td>2.296(2), 2.298(3)</td>
<td>2.363(2), 2.3691(19), 2.364(2), 2.3651(19)</td>
</tr>
<tr>
<td>Ru-Cl$^{[a]}$</td>
<td>2.421, 2.426</td>
<td>2.420, 2.425, 2.420</td>
</tr>
<tr>
<td>Cl-Ru-Cl</td>
<td>87.25(8), 88.97(9)</td>
<td>89.85(7), 88.52(7), 89.50(7), 87.51(6)</td>
</tr>
<tr>
<td>P-Ru-Cl$^{[a]}$</td>
<td>85.26, 84.04</td>
<td>89.16, 88.78, 89.91, 89.35</td>
</tr>
</tbody>
</table>

$^{[a]}$ In the crystal of RAPTA-C there are two independent molecules in the asymmetric unit.

$^{[b]}$ In 9b there are four independent complexes in the asymmetric unit.

The solid-state structures of 9b and 9c were established by single crystal X-ray diffraction analyses confirming the expected molecular structures. Single crystals of 9b were grown via the slow evaporation of chloroform from a concentrated solution of 9b (Figure 2.2). 9b contains four independent molecules in each asymmetric unit compared to the two found for RAPTA-C.$^{[73]}$ Key bond parameters are compared with those of RAPTA-C (Table 2.1) and, overall, the arrangement around the Ru(II) centre is remarkably similar with 9b adopting the familiar half-sandwich three-legged piano-stool geometry. The mean Ru-η$^6$ distance is longer in 9b (1.706-1.723 Å) compared to RAPTA-C (1.692-1.701 Å) and the same trend is observed for the Ru-P bond lengths (9b 2.363(2)-2.3691(19) Å and RAPTA-C: 2.296(2)-2.298(3) Å). The average Ru-Cl bond lengths and Cl-Ru-Cl angles are similar for both complexes, however a difference is observed in the average P-Ru-Cl angles with those in 9b (88.78-89.91°) being consistently larger than in RAPTA-C (84.04-87.25°). In the crystal of 9b intermolecular H-bonds between the carboxylic acid groups are observed leading to the formation of homodimeric assemblies.
The slow diffusion of THF into a saturated solution of 9c in CDCl₃ afforded crystals suitable for X-ray diffraction analysis (Figure 2.3). The bond parameters around the Au(I)-centre in 9c are presented in Table 2.2 and compared with those of auranofin. The Au-P bond distance in 9c (2.233(9) Å) is comparable to the value observed in auranofin (2.259 Å) (Table 2.2). Despite the different labile ligands, i.e. thiol vs. chloride, the Au-Cl bond in 9c (2.286(10) Å) is a similar length to the Au-S bond in auranofin (2.293 Å). However, the nature of the labile ligand influences the angle around the gold(I) centre, the P-Au-Cl (178.07(3)°) angle in 9c is larger than the auranofin P-Au-S angle (173.6°). The crystal network in 9c reveals dimeric arrangements due to intermolecular H-bonding interactions of the carboxylic acid group.

![Figure 2.3. Solid-state structure of 9c; thermal ellipsoids are 50% equiprobability envelopes. H-atoms and solvent molecules (THF) are omitted for clarity.](image)

Table 2.2. Comparison of selected bond lengths (Å) and angles (°) in auranofin and 9c.

<table>
<thead>
<tr>
<th></th>
<th>Auranofin</th>
<th>9c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au-P</td>
<td>2.259</td>
<td>2.233(9)</td>
</tr>
<tr>
<td>Au-S</td>
<td>2.293</td>
<td>2.286(10)</td>
</tr>
<tr>
<td>P-Au-S</td>
<td>173.6</td>
<td>178.07(3)</td>
</tr>
</tbody>
</table>

Lipophilicity has previously been correlated to increasing cytotoxicity in dinuclear ruthenium(II) complexes (RU1, Figure 2.1). Consequently, the partition coefficients (log P) of polyethylene glycol chains and alkyl chains were calculated. As expected, the lipophilicity of the alkanes increases with increasing chain length (Figure 2.4), a trend that is
transferred to bis-ruthenium complexes bearing alkyl linkers of the structure \((\eta^6-p\text{-cymene})\text{Ru(O}_2\text{C(CH}_3\text{)NC}_6\text{H}_4\text{O}_2\text{N(CH}_2\text{n})NC}_6\text{H}_4\text{O}_2\text{Ru(\eta^6-p\text{-cymene}) (n = 3, 6 and 12).}^{[45]}\) In contrast, PEG chains have limited lipophilicity with the hydrophobicity increasing with chain length up to hexaethylene glycol where a plateau is reached. According to calculations, octaethylene glycol is the most lipophilic with a log \(P\) value of -0.63, and longer PEG chains become increasingly hydrophilic. The plateau, consisting of PEG chains 6-9, have similar log \(P\) values in the range -0.62 to -0.67. Log \(P_{\text{octanol/water}}\) values were determined experimentally for 1b-7b and 1d-7d using the shake-flask method (Table 2.3).\(^7\) The log \(P\) values reside in the lipophilic range for both series. Bis-Au(I)-\(\beta\text{-D-thioglucose-tetraacetate complexes 1}_1\text{d-7d are more hydrophilic than their ruthenium counterparts 1}_1\text{b-7b due to the presence of two }\beta\text{-D-thioglucose-tetraacetate ligands. For the shorter chain lengths, 1}_1\text{b-4b and 1}_1\text{d-3d}, lipophilicity was shown to increase with increasing chain length for both series. However for complexes bearing longer chain lengths, 5}_5\text{b-7b and 5}_5\text{d-7b, the log }P\text{ values remain essentially constant despite increasing chain length which values of 1.4-1.5 and 0.3-0.4 respectively.}

![Figure 2.4](image)

**Figure 2.4.** Calculated partition coefficients for alkyl chains and polyethylene glycol chains as a function of length. The calculated Log \(P_{\text{alkyl}}\) value of methane \((n = 1)\) was omitted for clarity.

The cytotoxicity of the ligands 1a-7a, and Ru(II)-\(p\text{-cymene 1}_1\text{b-9b and Au(I)-}\beta\text{-D-thioglucose-tetraacetate 1}_1\text{d-9d complexes was assessed against human ovarian carcinoma cell lines, A2780 and A2780cisR, the latter having acquired resistance to cisplatin, and non-tumoral human embryonic kidney (HEK-293) cells using the MTT assay (Table 2.3).}
Table 2.3. Experimental Log $P_{oct/wat}$ and \textit{in vitro} antiproliferative activity of compounds 1b-9b and 1d-9d against human ovarian carcinoma (A2780), human ovarian carcinoma cisplatin resistant (A2780cisR) and human embryonic kidney 293 (HEK-293) cell lines after 72 h exposure. Values are given as the mean ± SD (μM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log $P_{oct/wat}$</th>
<th>A2780</th>
<th>A2780cisR</th>
<th>HEK-293</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>1.3 ± 0.1</td>
<td>60 ± 2</td>
<td>110 ± 3</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>2b</td>
<td>1.3 ± 0.1</td>
<td>10 ± 0.1</td>
<td>11.0 ± 0.7</td>
<td>12.2 ± 0.5</td>
</tr>
<tr>
<td>3b</td>
<td>1.6 ± 0.1</td>
<td>19.4 ± 0.3</td>
<td>37.6 ± 1.6</td>
<td>19.6 ± 1.1</td>
</tr>
<tr>
<td>4b</td>
<td>1.8 ± 0.3</td>
<td>11.3 ± 0.1</td>
<td>14.6 ± 0.3</td>
<td>14.7 ± 0.9</td>
</tr>
<tr>
<td>5b</td>
<td>1.4 ± 0.05</td>
<td>6.4 ± 0.7</td>
<td>12.9 ± 3.7</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>6b</td>
<td>1.4 ± 0.02</td>
<td>7.3 ± 0.3</td>
<td>10.5 ± 0.1</td>
<td>9.1 ± 0.1</td>
</tr>
<tr>
<td>7b</td>
<td>1.5 ± 0.02</td>
<td>11.6 ± 0.9</td>
<td>14.1 ± 7.7</td>
<td>14.2 ± 0.3</td>
</tr>
<tr>
<td>8b</td>
<td>-</td>
<td>42 ± 1</td>
<td>35 ± 7</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>9b</td>
<td>-</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>1d</td>
<td>0.4 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>2d</td>
<td>0.9 ± 0.1</td>
<td>0.22 ± 0.03</td>
<td>0.67 ± 0.02</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>3d</td>
<td>0.7 ± 0.2</td>
<td>0.19 ± 0.02</td>
<td>0.91 ± 0.01</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>4d</td>
<td>0.6 ± 0.1</td>
<td>0.19 ± 0.02</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>5d</td>
<td>0.3 ± 0.2</td>
<td>0.22 ± 0.02</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>6d</td>
<td>0.4 ± 0.3</td>
<td>0.17 ± 0.01</td>
<td>1.4 ± 0.04</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>7d</td>
<td>0.3 ± 0.2</td>
<td>0.25 ± 0.02</td>
<td>1.4 ± 0.4</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>8d</td>
<td>-</td>
<td>0.54 ± 0.07</td>
<td>1 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>9d</td>
<td>-</td>
<td>6.9 ± 0.8</td>
<td>12.0 ± 2</td>
<td>11.7 ± 0.4</td>
</tr>
<tr>
<td>(C₆H₅)₂P-C₆H₄-CO₂H</td>
<td>-</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>P(C₆H₅)₃</td>
<td>-</td>
<td>85 ± 7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cisplatin</td>
<td>-</td>
<td>1.3 ± 0.2</td>
<td>11 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>RAPTA-C</td>
<td>-</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>auranofin</td>
<td>-</td>
<td>1.3 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.9 ± 0.6</td>
</tr>
</tbody>
</table>
The cytotoxicity of 4-(diphenylphosphino)benzoic acid, triphenylphosphine, auranofin, cisplatin and RAPTA-C was evaluated for comparison purposes. Elemental analysis indicates that CH₂Cl₂ and CDCl₃ are present in some compounds, so their cytotoxicity was evaluated at IC₅₀ concentration and they were found to be inactive. All compounds were pre-dissolved in DMSO, their stability in this solvent was confirmed via ¹H and ³¹P NMR spectroscopy, before being immediately diluted into the appropriate cell culture medium. Further stability studies under pseudo-cell culture conditions of 100mM NaCl in water and 5% DMSO were conducted on 2d, 4d and 6d complexes over 72 h. The stability was monitored via ESI(+) mass spectrometry and all complexes showed good stability under these conditions.

The original RAPTA series possess low cytotoxicities with IC₅₀ values of >200 μM against a range of cell lines.¹¹ As discussed above, the structures of 9b and RAPTA-C are similar, and the similarities are reflected in their cytotoxicities with IC₅₀ values >200 μM determined against all tested cell lines. However, the mononuclear ruthenium(II) complex 8b, bearing a hydrophobic triphenylphosphine ligand, is considerably more cytotoxic than 9b with an IC₅₀ of 42 ± 1 μM. A similar trend is present with 8d and 9d, with 9d being ca. 14 fold more cytotoxic than 8d. With the free ligands, triphenylphosphine and 4-(diphenylphosphanyl)benzoic acid, also presenting respective IC₅₀ values of 85± 7 μM and >200 μM against the A2780 cell line, the difference may be attributed to differences in lipophilicity. However, the impact of global charge may be of influence.

The bis-phosphine ligands 1a-7a are inactive at concentrations of up to 500 μM on the three cell lines. The dinuclear complexes 1b-7b and 1d-7d are considerably more cytotoxic than the mononuclear complexes 9b and 9d against all cell lines. The complexes containing the monoethylene glycol linker (1b and 1d) are considerably less cytotoxic than the complexes with longer linkers (2b-7b and 2d-7d). In contrast, in a series of bis-Ru(II)-p-cymene complexes with bridging bis(nicotinate)/(isonicotinate) ligands, only the complex with the shortest monoethylene glycol linker possessed moderate activity against a range of cell lines including human melanoma (518A2) cell line (IC₅₀ = 53 ± 1 μM).⁴⁹

The bis-Ru(II)-p-cymene complexes 2b-7b exhibit IC₅₀ values in the low micromolar range against the A2780 cell line. The cytotoxicity towards the cisplatin resistant cell line (A2780cisR) remains in the low micromolar range, with up to 2-fold loss in cytotoxicity
compared to the A2780 cisplatin sensitive cell line for 3b and 5b. No selectivity was observed towards the cancer cell lines with the values obtained for the non-tumorigenic HEK-293 cell line being similar in magnitude. The cytotoxicity of these dinuclear complexes is comparable to previously investigated series including the series of rigid RAPTA-type dinuclear complexes, linked via the functionalization of the $\eta^6$-arene, where the most active complex has an IC$_{50}$ of 3.7 ± 0.6 μM against the A2780 cell line.[50]

The relationship between linker length and cytotoxicity on the A2780 cell line shows an increasing cytotoxicity with increasing linker length between 3b and 5b, which correlates with the increasing lipophilicity of the linkers (Figure 2.4). However, 2b with the diethylene glycol linker is ca. 2 fold more cytotoxic than 3b. The lipophilicity of the linker is essentially constant for compounds 6b and 7b, with the IC$_{50}$ values being 7.3 ± 0.3 and 11.6 ± 0.9 μM, respectively.

The bis-Au(I)-β-D-thioglucose-tetraacetate complexes 1d-7d are highly cytotoxic on all three tested cell lines with IC$_{50}$ values in the low micromolar range. Compounds 2d-7d are highly cytotoxic against A2780 cells (with IC$_{50}$ values between 0.17 and 0.245 μM), while being significantly less active, up to 8-fold, on the cisplatin resistant A2780cisR cell line. The IC$_{50}$ values of 1d-7d on non-tumourigenic HEK-293 cells are very similar to those on A2780cisR cells. Interestingly, no major differences are observed between the activities of the bis-Au(I) complexes 2d-7d and the mononuclear complex, 8d, containing the hydrophobic PPh$_3$ ligand, whereas 9d, with the more hydrophilic phosphine ligand i.e. (C$_6$H$_5$)$_2$P-C$_6$H$_4$-CO$_2$H, is ca. 35-fold less cytotoxic in A2780 cells and ca. 10-fold less cytotoxic in A2780cisR and HEK-293 cells. With the exception of complexes, 1d and 9d the activities are in the same order to those of auranofin.$^4$

Complexes 2d-7d display a narrow range of IC$_{50}$ values between 0.17 ± 0.01 μM and 0.25 ± 0.02 μM and, thus, there is no discernible correlation with the linker length and associated lipophilicity. However, the series is significantly more cytotoxic than [(AuCl)$_2$(Ph$_2$P-(CH$_3$)$_n$PPh$_3$)] (n = 1-6) in which the most active complexes (n = 3, 5, 6) have IC$_{50}$ values of ca. 2 μM again murine B16 melanoma cells.$^{[59]}$ A three- (n = 1) and four-fold (n = 2) increase in cytotoxicity was observed for the more lipophilic complexes.$^{[59]}$ The series is also significantly
more cytotoxic than the phosphine-bridged dinuclear Au(I) alkynyl complexes bearing alkyl linkers, where no correlation was observed between the cytotoxicity and linker length.\textsuperscript{[60]}

2.3 Concluding remarks

The synthesis of two series of homo-binuclear RAPTA-like Ru(II)-\textit{p}-cymene complexes 1\textsubscript{b}-7\textsubscript{b} and auranofin-like Au(I) complexes 1\textsubscript{d}-7\textsubscript{d}, linked \textit{via} bis-phosphine modified polyethylene-glycol chains of varying length, was successfully achieved. The antiproliferative activity of these compounds was determined against tumourigenic and non-tumourigenic cell lines and a distinct increase cytotoxicity was observed for both series compared to the mononuclear precursors 9\textsubscript{b} and 9\textsubscript{d}. There is a correlation between the lipophilicity and cytotoxicity of the bis-ruthenium(II) complexes, which reaches a plateau where the lipophilicity no longer increases with the length of the polyethylene-glycol chain, i.e. when \( n = 6 \). In contrast, the cytotoxicity of all the bis-gold(I) complexes lies within a narrow range and is not readily correlated to the linker length and associated lipophilicity.
2.4 Experimental Section

2.4.1 Materials

RuCl$_3$.3H$_2$O was purchased from Precious Metals Online. All other chemical reagents were purchased from Aldrich, AlfaAesar, Acros and TCI Chemicals and used without further purification. [Ru($\eta^6$-p-cymene)Cl$_2$]$^{78}$ and AuCl(tht)$^{70,71}$ were prepared following literature procedures. Dichloromethane was dried and degassed using a PureSolv solvent purification system (Innovative Technology INC). Thin Layer Chromatography was conducted on Merck TLC Silicagel coated aluminium sheets 60 F254 and verified by UV lamp at 254nm and KMnO$_4$ stain. Purification of ligands was achieved via manual chromatography using Silicagel (Silicycle R12030B) or a Varian 971-FP flash chromatography system using prepackaged silica gel columns (Luknova).

2.4.2 Instrumentation and Methods

$^1$H (400 MHz), $^{13}$C (101 MHz) and $^{31}$P (162 MHz) were recorded on a Bruker Avance II 400 spectrometer at 298 K. Chemical shifts are reported in parts per million (ppm) and referenced to deuterated solvent residual peaks (CDCl$_3$: $^1$H δ 7.26, $^{13}$C{${^1}$H} δ 77.16 ppm)$^{2}$ Coupling constants (J) are reported in Hertz (Hz). High-resolution electrospray ionization mass spectra (HR ESI-MS) were obtained on a Xevo G2-S QTOF mass spectrometer coupled to the Acquity UPLC Class Binary Solvent manager and BTN sample manager (Waters, Corporation, Milford, MA). Elemental analyses were carried out by the microanalytical laboratory at the the Institute of Chemical Sciences and Engineering (EPFL) using a Thermo Scientific Flash 2000 Organic Elemental Analyzer. UV-vis spectra were recorded using SpectroMax M5e multi-mode microplate reader (using SoftMax Pro software, version 6.2.2). The diffraction data of compounds 9b and 9c were measured at low temperature [100(2) K]
using Mo $K_{\alpha}$ radiation on a Bruker APEX II CCD diffractometer equipped with a kappa geometry goniometer. The datasets were reduced by EvalCCD and then corrected for absorption. The solutions and refinements were performed by SHELX. The crystal structures were refined using full-matrix least-squares based on $F^2$ with all non hydrogen atoms anisotropically defined. Hydrogen atoms were placed in calculated positions by means of the “riding” model. The log $P$ values of polyethylene glycol and alkyl linker compounds were predicted using the Virtual Computational Chemistry Lab (VCCLAB). The experimental log $P_{\text{octanol}/\text{water}}$ values were determined using the shake-flask method and the absorbance of each fraction was recorded using SpectroMax M5e multi-mode microplate reader (using SoftMax Pro software, version 6.2.2).

2.4.3 Synthesis

2.4.3.1 Synthesis of the bis-phosphine ligands 1a-7a

**General Procedure**

4-(diphenylphosphino)benzoic acid (2.2 equiv.) and EDCI (2.4 equiv.) were dissolved in CH$_2$Cl$_2$ (50 mL) and stirred under N$_2$ at r.t. for 15 min. The appropriate ethylene glycol (1.0 equiv.) and DMAP (0.4 equiv.) were added and the mixture was further stirred for 24 h at r.t. The reaction mixture was washed with H$_2$O (150 mL) and the aqueous phase was re-extracted with CH$_2$Cl$_2$ (2x100 mL). The organic phase was washed with brine (150 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The treated product was purified by flash column chromatography using adapted elution system of Hex/EtOAc or CH$_2$Cl$_2$/MeOH. The product was recovered as a colourless viscous solid.
**Compound 1a**

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.900 g, 2.938 mmol, 2.2 equiv.), monoethyleneglycol (0.075 mL, 1.345 mmol, 1 equiv.), EDCI (0.614 g, 3.203 mmol, 2.4 equiv.) and DMAP (0.065 g, 0.532 mmol, 0.4 equiv.) were stirred for 24h in CH₂Cl₂ (50 mL). The product was isolated as a colourless oil (0.216 g, 0.338 mmol, 25 %).

**Elemental Analysis (%):** calcd. for C₄₀H₃₂O₄P₂: C 75.23, H 5.05, found C 75.55, H 5.23.

**¹H NMR (CDCl₃, 400 MHz):** 7.97 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_H,P = 8.6 Hz, ¹J_H,H = 1.4 Hz), 7.29-7.40 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 4.64 (4H, s, Ar-(C=O)-O-(CH₂)₂-O).

**³¹P {¹H} NMR (CDCl₃, 162 MHz):** -4.99 (2P).

**¹³C {¹H} NMR (CDCl₃, 101 MHz):** 166.3 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_C,P = 15 Hz), 136.3 (4C, d, 4xP-(Ar)C-CH-CH-CH, ¹J_C,P = 11 Hz), 134.2 (8C, d, 8xP-(Ar)C-CH-CH-CH, ¹J_C,P = 20 Hz), 133.3 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_C,P = 19 Hz), 129.8 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_C,P = 6 Hz), 129.3 (4C, 4xP-(Ar)C-CH-CH-CH), 128.8 (8C, d, 8xP-(Ar)C-CH-CH-CH, ¹J_C,P = 7 Hz), 63.0 (2C, 2x(Ar)-(C=O)-O-(CH₂)₂-O).

**ESI-MS(+):** m/z found 639.1853 [M+H]⁺ C₄₀H₃₃O₄P₂ requires 639.1854.

**Compound 2a**

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.900 g, 2.938 mmol, 2.2 equiv.), diethyleneglycol (0.128 mL, 1.349 mmol, 1 equiv.), EDCI (0.614 g, 3.203 mmol, 2.4 equiv.) and DMAP (0.065 g, 0.532 mmol, 0.4 equiv.) were stirred for 24h in CH₂Cl₂ (50 mL). The product was isolated as a colourless oil (0.328 g, 0.480 mmol, 36 %).
Elemental Analysis (%): calcd. for C_{42}H_{36}O_{5}P_{2} C 73.89, H 5.32; found C 73.56, H 5.51.

\[^1\]H NMR (CDCl\textsubscript{3}, 400 MHz): 7.94-7.97 (4H, m, 4xO-(C=O)-(Ar)C-CH=CH=C-P, \textit{J}_{\text{HH}} = 8.5 Hz, \textit{J}_{\text{HP}} = 1.5 Hz), 7.29-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH=CH=C-P, 8xP-(Ar)C-CH=CH-CH, 8xP-(Ar)C-CH=CH-CH, 4xP-(Ar)C-CH=CH=C-H\textsubscript{2}), 4.46-4.49 (4H, m, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O), 3.82-3.87 (4H, m, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O).

\[^{31}\]P \{\[^1\]H\} NMR (CDCl\textsubscript{3}, 162 MHz): \textit{δ} = -5.08 (2P).

\[^{13}\]C \{\[^1\]H\} NMR (CDCl\textsubscript{3}, 101 MHz): 166.4 (2C, 2xO-(C=O)-(Ar)C-CH=CH=C-P, \textit{J}_{\text{CH}} = 14 Hz), 136.3 (4C, d, 4xP-(Ar)C-CH=CH-CH, \textit{J}_{\text{CP}} = 11 Hz), 134.1 (8C, d, 8xP-(Ar)C-CH=CH-CH, \textit{J}_{\text{CP}} = 20 Hz, 133.2 (4C, d, 4xO-(C=O)-(Ar)C-CH=CH=C-P, \textit{J}_{\text{CH}} = 19 Hz), 130.0 (2C, 2xO-(C=O)-(Ar)C-CH=CH=C-P, \textit{J}_{\text{CP}} = 6 Hz), 129.3 (4C, 4xP-(Ar)C-CH=CH=C-H\textsubscript{2}), 128.8 (8C, d, 8xP-(Ar)C-CH=CH=CH, \textit{J}_{\text{CH}} = 7 Hz), 69.3 (2C, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O), 64.2 (2C, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O).

ESI-MS(+): \textit{m/z} found 683.2112 [M+H]\textsuperscript{+}, 705.1931 [M+Na]\textsuperscript{+}, C_{42}H_{37}O_{5}P_{2} requires 682.6925, C_{42}H_{36}NaO_{5}P_{2} requires 705.1936.

Compound 3a

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.900 g, 2.938 mmol, 2.2 equiv.), triethyleneglycol (0.179 mL, 1.340 mmol, 1 equiv.), EDCI (0.614 g, 3.203 mmol, 2.4 equiv.) and DMAP (0.065 g, 0.532 mmol, 0.4 equiv.) were stirred for 24h in CH\textsubscript{2}Cl\textsubscript{2} (50 mL). The product was isolated as a colourless oil (0.446 g, 0.614 mmol, 46 %).

Elemental Analysis (%): calcd. for C_{44}H_{40}O_{6}P_{2} C 72.72, H 5.55; found C 72.54, H 5.58.

\[^1\]H NMR (CDCl\textsubscript{3}, 400 MHz): 7.95-7.98 (4H, m, 4xO-(C=O)-(Ar)C=CH=CH=C-P, \textit{J}_{\text{HH}} = 8.4 Hz, \textit{J}_{\text{HP}} = 1.4 Hz), 7.29-7.38 (24H, m, 4xO-(C=O)-(Ar)C-CH=CH=C-P, 8xP-(Ar)C-CH=CH-CH, 8xP-(Ar)C-CH=CH-CH, 4xP-(Ar)C-CH=CH=C-H\textsubscript{2}), 4.43-4.45 (4H, m, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O), 3.79-3.82 (4H, m, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O).

\[^{31}\]P \{\[^1\]H\} NMR (CDCl\textsubscript{3}, 162 MHz): \textit{δ} = -5.07 (2P).

\[^{13}\]C \{\[^1\]H\} NMR (CDCl\textsubscript{3}, 101 MHz): 166.4 (2C, 2xO-(C=O)-(Ar)C-CH=CH=C-P, \textit{J}_{\text{CH}} = 14 Hz), 161.3 (4C, d, 4xP-(Ar)C-CH=CH-CH, \textit{J}_{\text{CP}} = 11 Hz), 134.1 (8C, d, 8xP-(Ar)C-CH=CH-CH, \textit{J}_{\text{CP}} = 20 Hz, 133.3 (4C, d, 4xO-(C=O)-(Ar)C-CH=CH=C-P, \textit{J}_{\text{CH}} = 19 Hz), 130.1 (2C, 2xO-(C=O)-(Ar)C-CH=CH=C-P, 129.5 (4C, d, 4xO-(C=O)-(Ar)C-CH=CH=C-P, \textit{J}_{\text{CP}} = 6 Hz), 129.3 (4C, 4xP-(Ar)C-CH=CH=C-H\textsubscript{2}), 128.8 (8C, d, 8xP-(Ar)C-CH=CH=CH, \textit{J}_{\text{CH}} = 7 Hz, 69.3 (2C, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O), 64.2 (2C, 2xAr-(C=O)-O-CH\textsubscript{2}-CH=CH=O).
8xP-(Ar)C-CH-CH-CH, $\textit{J}_{P.H} = 7$ Hz), 70.9 (2C, 2x(Ar)-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$), 69.4 (2C, 2x(Ar)-(C=O)-O-CH$_2$-CH$_2$-O), 64.3 (2C, 2x(Ar)-(C=O)-O-CH$_2$-CH$_2$-O).

ESI-MS(+): $m/z$ found 727.2383 [M+H]$^+$, 749.2202 [M+Na]$^+$, C$_{44}$H$_{41}$O$_6$P$_2$ $^+$ requires 727.2378, C$_{44}$H$_{40}$NaO$_5$P$_2$ $^+$ requires 749.2198.

**Compound 4a**

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.900 g, 2.938 mmol, 2.2 equiv.), tetraethyleneglycol (0.231 mL, 1.338 mmol, 1 equiv.), EDCI (0.615 g, 3.203, 2.4 equiv.) and DMAP (0.065 g, 0.532 mmol, 0.4 equiv.) were stirred for 24h in CH$_2$Cl$_2$ (50 mL). The product was isolated as a colourless oil (0.487 g, 0.632 mmol, 47 %).

**Elemental Analysis (%):** calcd. for C$_{46}$H$_{44}$O$_7$P$_2$: C 71.68, H 5.75; found C 71.59, H 5.83.

$^1$H NMR (CDCl$_3$, 400 MHz): 7.95-8.00 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^1J_{H,H} = 8.2$ Hz, $^1J_{H,P} = 1.6$ Hz), 7.29-7.38 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 4.43-4.46 (4H, m, 2xAr-(C=O)-O-CH$_2$-CH$_2$-O), 3.77-3.80 (4H, m, 2xAr-(C=O)-O-CH$_2$-CH$_2$-O), 3.61-3.70 (8H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$).

$^{31}$P {$^1$H} NMR (CDCl$_3$, 162 MHz): -5.06 (2P).

$^{13}$C {$^1$H} NMR (CDCl$_3$, 101 MHz): 166.4 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P, $^1J_{C-H} = 14$ Hz), 136.3 (4C, d, 4xP-(Ar)C-CH-CH-CH, $^1J_{C-P} = 11$ Hz), 134.1 (8C, d, 8xP-(Ar)C-CH-CH-CH, $^1J_{C-P} = 20$ Hz), 133.3 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^1J_{C-H} = 19$ Hz), 130.2 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 129.5 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^1J_{C-P} = 7$ Hz), 129.3 (4C, 4xP-(Ar)C-CH-CH-CH), 128.8 (8C, d, 8xP-(Ar)C-CH-CH-CH, $^1J_{C-P} = 7$ Hz), 70.77, 70.79, 70.83 (4C, 2x(Ar)-(C=O)-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$), 69.3 (2C, 2x(Ar)-(C=O)-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$), 64.3 (2C, 2x(Ar)-(C=O)-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$).

ESI-MS(+): $m/z$ found 771.2630 [M+H]$^+$, 793.2451 [M+Na]$^+$, C$_{46}$H$_{44}$O$_7$P$_2$ $^+$ requires 771.2641, C$_{46}$H$_{44}$NaO$_7$P$_2$ $^+$ requires 793.2460.
Compound 5a

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.800 g, 2.612 mmol, 2.2 equiv.), pentaethyleneglycol (0.251 mL, 1.186 mmol, 1 equiv.), EDCI (0.546 g, 2.848 mmol, 2.4 equiv.) and DMAP (0.058 g, 0.475 mmol, 0.4 equiv.) were stirred for 24h in CH₂Cl₂ (50 mL). The product was isolated as a colourless oil (0.621 g, 0.762 mmol, 64%).

Elemental Analysis (%): calcd. for C₄₈H₄₈O₈P₂ C 70.75, H 5.94; found C 70.85, H 6.04.

¹H NMR (CDCl₃, 400 MHz): 7.96-7.99 (4H, m, 4xO-(C=O)-(Ar)C=CH=CH=O, ⁸Jₗ,H = 8.4 Hz, ⁸Jₗ,P = 1.6 Hz), 7.28-7.37 (24H, m, 4xO-(C=O)-(Ar)C=CH=CH=O, 8xP-(Ar)C=CH=CH-O), 3.79-3.81 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.65-3.68 (4H, m, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂), 3.61-3.64 (4H, m, 2xAr-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-CH₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): -5.08 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 166.4 (2C, 2xO-(C=O)-(Ar)C=CH=CH=O), 144.3 (2C, d, 2xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 14 Hz), 136.3 (4C, d, 4xP-(Ar)C=CH=CH-O, ¹Jₗ,P = 11 Hz), 134.1 (8C, d, 8xP-(Ar)C=CH=CH=O, ¹Jₗ,P = 20 Hz), 133.2 (4C, d, 4xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 19 Hz), 130.1 (2C, 2xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 6 Hz), 129.1 (4C, 4xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 7 Hz), 70.80, 70.76, 70.73 (6C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂).

ESI-MS(+) m/z found 815.2905 [M+H⁺], 837.2723 [M+Na⁺], C₄₈H₄₉O₈P₂⁺ requires 815.2897, C₄₈H₄₉NaO₈P₂⁺ requires 837.2722.

Compound 6a

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.800 g, 2.612 mmol, 2.2 equiv.), pentaethyleneglycol (0.251 mL, 1.186 mmol, 1 equiv.), EDCI (0.546 g, 2.848 mmol, 2.4 equiv.) and DMAP (0.058 g, 0.475 mmol, 0.4 equiv.) were stirred for 24h in CH₂Cl₂ (50 mL). The product was isolated as a colourless oil (0.621 g, 0.762 mmol, 64%).

Elemental Analysis (%): calcd. for C₄₈H₄₉O₈P₂ C 70.75, H 5.94; found C 70.85, H 6.04.

¹H NMR (CDCl₃, 400 MHz): 7.96-7.99 (4H, m, 4xO-(C=O)-(Ar)C=CH=CH=O, ⁸Jₗ,H = 8.4 Hz, ⁸Jₗ,P = 1.6 Hz), 7.28-7.37 (24H, m, 4xO-(C=O)-(Ar)C=CH=CH=O, 8xP-(Ar)C=CH=CH-O), 3.79-3.81 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.65-3.68 (4H, m, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂), 3.61-3.64 (4H, m, 2xAr-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-CH₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): -5.08 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 166.4 (2C, 2xO-(C=O)-(Ar)C=CH=CH=O), 144.3 (2C, d, 2xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 14 Hz), 136.3 (4C, d, 4xP-(Ar)C=CH=CH-O, ¹Jₗ,P = 11 Hz), 134.1 (8C, d, 8xP-(Ar)C=CH=CH=O, ¹Jₗ,P = 20 Hz), 133.2 (4C, d, 4xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 19 Hz), 130.1 (2C, 2xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 6 Hz), 129.1 (4C, 4xO-(C=O)-(Ar)C=CH=CH=O, ¹Jₗ,P = 7 Hz), 70.80, 70.76, 70.73 (6C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂).

ESI-MS(+) m/z found 815.2905 [M+H⁺], 837.2723 [M+Na⁺], C₄₈H₄₉O₈P₂⁺ requires 815.2897, C₄₈H₄₉NaO₈P₂⁺ requires 837.2722.
According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.800 g, 2.612 mmol, 2.2 equiv.), hexaethyleneglycol (0.298 mL, 1.190 mmol, 1 equiv.), EDCI (0.546 g, 2.848 mmol, 2.4 equiv.) and DMAP (0.058 g, 0.475 mmol, 0.4 equiv.) were stirred for 24h in CH₂Cl₂ (50 mL). The product was isolated as a colourless oil (0.341 g, 0.397 mmol, 33%).

Elemental Analysis (%): calcd. for C₅₀H₅₂O₉P₂ C 69.92, H 6.10; found C 70.04, H 6.13.

¹H NMR (CDCl₃, 400 MHz): 7.96-7.99 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz), 7.28-7.39 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.2 Hz).

³¹P-NMR (CDCl₃, 162 MHz): -5.06 (2P).

¹³C NMR (CDCl₃, 101 MHz): 166.3 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 14 Hz), 136.1 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 20 Hz), 133.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 19 Hz), 129.4 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 6 Hz), 129.1 (4C, 4xP-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 11 Hz), 128.7 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 7 Hz, 70.70, 70.65, 70.63, 70.59 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 7 Hz, 70.70, 70.65, 70.63, 70.59 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 7 Hz, 70.70, 70.65, 70.63, 70.59), 69.2 (2C, 2x(Ar)-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂, 2x(Ar)-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂, 2x(Ar)-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂, 2x(Ar)-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂, 2x(Ar)-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂, 2x(Ar)-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂). ESI-MS(+) m/z found 859.3168 [M+H]⁺, 881.2988 [M+Na]⁺, C₅₀H₅₃O₉P₂ requires 859.3165, C₅₀H₅₂NaO₉P₂ requires 881.2984.

Compound 7a

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.800 g, 2.612 mmol, 2.2 equiv.), octaethyleneglycol (0.440g, 1.188 mmol, 1 equiv.), EDCI (0.546 g, 2.848 mmol, 2.4 equiv.) and DMAP (0.058 g, 0.475 mmol, 0.4 equiv.) were stirred for 24h in CH₂Cl₂ (50 mL). The product was isolated as a colourless oil (0.499 g, 0.527 mmol, 45%).

Elemental Analysis (%): calcd. for C₅₄H₆₀O₁₁P₂ C 68.49, H 6.39; found C 68.16, H 6.35.

¹H NMR (CDCl₃, 400 MHz): 7.96-8.00 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.6 Hz), 7.29-7.37 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³Jₓₓ= = 8.4 Hz, ³JₓP= = 1.6 Hz).
(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 4.45-4.47 (4H, m, 2xAr-(C=O)-O-(CH2)-O-(CH2)-CH2), 3.80-3.82 (4H, m, 2xAr-(C=O)-O-(CH2)-O-(CH2)-H), 3.63-3.66 (4H, m, 2xAr-(C=O)-O-(CH2)-O-(CH2)-H), 3.60-3.62 (8H, m, 2xAr-(C=O)-O-(CH2)-O-(CH2)-H), 3.61 (8H, s, 2xAr-(C=O)-O-(CH2)-O-(CH2)-H).

31P 1H NMR (CDCl3, 162 MHz): -5.06 (2P).

13C 1H NMR (CDCl3, 101 MHz): 166.4 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 144.4 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 15 Hz), 136.5 (4C, d, 4xP-(Ar)C-CH-CH-CH, J_C,P = 11 Hz), 134.1 (8C, d, 8xP-(Ar)C-CH-CH-CH), J_C,P = 20 Hz), 133.3 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 19 Hz), 130.1 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 129.5 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 6 Hz), 129.3 (4C, 4xP-(Ar)C-CH-CH-CH), 128.8 (8C, d, 8xP-(Ar)C-CH-CH-CH, J_C,P = 7 Hz), 70.92, 70.88, 70.85, 70.81 (12C, 2x(Ar)-(C=O)-O-(CH2)-O-(CH2)-CH2), 2x(Ar)-(C=O)-O-(CH2)-O-(CH2)-CH2), 2x(Ar)-(C=O)-O-(CH2)-O-(CH2)-CH2), 2x(Ar)-(C=O)-O-(CH2)-O-(CH2)-CH2), 2x(Ar)-(C=O)-O-(CH2)-O-(CH2)-CH2), 69.4 (2C, 2x(Ar)-(C=O)-O-(CH2)-O-(CH2)-CH2).

ESI-MS(+): m/z found 947.3671 [M+H]+, 969.3488 [M+Na]+, C54H61O11P2+ requires 947.3684, C54H60NaO11P2+ requires 969.3509.

2.4.3.2 Synthesis of the bis-ruthenium complexes 1b-7b

General Procedure

[(η6-p-cymene)RuCl2]2 (1 equiv.) and the appropriate ligand 1a-7a (1 equiv.) in CH2Cl2 (12 mL) were stirred for 42 h at r.t. under N2 in the dark. The reaction evolution was monitored by 1H and 31P NMR (CDCl3). The reaction mixture was concentrated under reduced pressure and then further dried under high vacuum to yield the product as a red solid.

Compound 1b
According to the general procedure, \(((\eta^5-p\text{-cymene})\text{RuCl}_2)_2\) (0.19 g, 0.307 mmol, 1 equiv.), 1a (0.20 g, 0.307 mmol, 1 equiv.), CH\(_2\text{Cl}_2\) (12 mL), 42 h at r.t. The product was isolated as a dark red solid (0.376 g, 0.301 mmol, 98\%).

**Elemental Analysis (%)**: calcd. for C\(_{60}\)H\(_{60}\)Cl\(_4\)O\(_4\)P\(_2\)Ru\(_2\)·CH\(_2\)Cl\(_2\) C 54.84 H 4.68; found C 54.77, H 4.93, CH\(_2\)Cl\(_2\) originates from the reaction solvent.

**\(^1\)H NMR (CD\(_2\)Cl\(_2\), 400 MHz)**: 7.88-7.97 (8H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4xO-(C=O)-(Ar)C-CH-C-CH-CH-P), 7.77-7.82 (8H, m, 8xP-(Ar)C-CH-CH-CH), 5.20 (4H, d, 4xCH\(_3\)-(Ar)C-CH-CH-C, \(\nu\)J\(_{\text{HH}}\) = 6.1 Hz), 4.98 (4H, d, 4xCH\(_3\)-(Ar)C-CH-CH-C, \(\nu\)J\(_{\text{HH}}\) = 6.1 Hz), 4.58 (4H, s, 2xAr-(C=O)-O-CH\(_2\)-CH\(_2\)-O), 2.83 (2H, sept, 2x(Ar)C-CH-CH-C(CH\(_3\))\(_2\), \(\nu\)J\(_{\text{HH}}\) = 7.2 Hz), 1.84 (6H, s, 2xCH\(_2\)-(Ar)C-CH-CH-C), 1.09 (12H, d, 2x(Ar)C-CH-CH-C-CH(CH\(_3\))\(_2\), \(\nu\)J\(_{\text{HH}}\) = 7.2 Hz).

**\(^{31}\)P \{\(^1\)H\} NMR (CD\(_2\)Cl\(_2\), 162 MHz)**: 25.00 (2P).

**\(^{13}\)C \{\(^1\)H\} NMR (CD\(_2\)Cl\(_2\), 101 MHz)**: 165.8 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 139.7 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(\nu\)J\(_{\text{CP}}\) = 9 Hz), 134.3 (8C, d, 8xP-(Ar)C-CH-CH-CH, \(\nu\)J\(_{\text{CP}}\) = 9 Hz), 133.3 (4C, d, 4xP-(Ar)C-CH-CH-CH, \(\nu\)J\(_{\text{CP}}\) = 3 Hz), 130.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(\nu\)J\(_{\text{CP}}\) = 45 Hz), 128.8 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, \(\nu\)J\(_{\text{CP}}\) = 10 Hz), 120.3 (8C, d, 8xP-(Ar)C-CH-CH-C-P, \(\nu\)J\(_{\text{CP}}\) = 10 Hz), 111.46, 111.43 (2C, 2xCH\(_3\)-(Ar)C-CH-CH-C), 87.39, 87.34 (4C, 4x(CH\(_3\))\(_2\)-(Ar)C-CH-CH-C), 63.0 (2C, 2xO-(Ar)C-CH-CH-C-CH\(_2\)-O), 30.4 (2C, 2x(Ar)C-CH-CH-C-CH(CH\(_3\))\(_2\)), 19.9 (4C, 2xAr-(C=O)-O-CH\(_2\)-CH\(_2\)-O), 17.9 (2C, 2xCH\(_3\)-(Ar)C-CH-CH-C).

**ESI-MS(+)**: \(m/z\) found 1215.1104 [M-Cl]+, 1273.0695 [M+Na]+, calcd. for C\(_{60}\)H\(_{60}\)Cl\(_4\)O\(_4\)P\(_2\)Ru\(_2\)+ 1215.1119, calcd. for C\(_{60}\)H\(_{60}\)Cl\(_4\)NaO\(_4\)P\(_2\)Ru\(_2\)+ 1273.0706.

**UV-Vis**: \(\lambda_{\text{max}}\) = 250 nm, 370 nm.

\textbf{Compound 2b}

According to the general procedure, \(((\eta^5-p\text{-cymene})\text{RuCl}_2)_2\) (0.49 g, 0.808 mmol, 1 equiv.), 2a (0.55 g, 0.808 mmol, 1 equiv.), CH\(_2\text{Cl}_2\) (12 mL), 42 h at r.t. The product was isolated as a dark red solid (1.03 g, 0.791 mmol, 98\%).
Elemental Analysis (%): calcd. for C₆₂H₆₄Cl₄O₅P₂Ru₂ C 57.50, H 4.98; found C 57.76, H 5.12.

¹H NMR (CDCl₃, 400 MHz): 7.86-7.96 (8H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 7.78-7.83 (8H, m, 8xP-(Ar)C-CH-CH-CH), 7.36-7.44 (12H, m, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH₂), 5.21 (4H, d, 4xCH₃-(Ar)C-CH-CH-C, ³J_H,H = 6.2 Hz), 4.98 (4H, d, 4xCH₃-(Ar)C-CH-CH-C, ³J_H,H = 6.2 Hz), 4.45-4.42 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.83-3.81 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 2.85 (2H, sept, 2x(Ar)C-CH-CH-C-CH(CH₃)₂, ³J_H,H = 6.9 Hz), 1.85 (6H, s, 2xC H₃-(Ar)C-CH-CH-C), 1.09 (12H, d, 2x( Ar)C-CH-CH-C-CH(CH₃)₂, ³J_H,H = 6.9 Hz).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 24.95 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 166.1 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 139.5 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 43 Hz), 134.4 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ³J_C,P = 9 Hz), 133.4 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³J_C,P = 9 Hz), 131.3 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 2 Hz), 130.7 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³J_C,P = 2 Hz), 128.8 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 10 Hz), 128.3 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ³J_C,P = 10 Hz), 111.59, 111.56 (2C, 2xCH₃-(Ar)C-CH-CH-C, 96.4 (2C, 2xCH₃-(Ar)C-CH-CH-C), 89.15, 89.12 (4C, 4xCH₃-(Ar)C-CH-CH-C), 87.49, 87.43 (4C, 4xCH₃-(Ar)C-CH-CH-C), 64.5 (2C, 2xCH₃-(Ar)C-CH-CH-C), 30.4 (2C, 2x(CH₃)-(Ar)C-CH-CH-C), 17.9 (2C, 2xCH₃-(Ar)C-CH-CH-C).


UV-Vis: λ_max = 250 nm, 370 nm.

**Compound 3b**

According to the general procedure, [(η⁵-p-cymene)RuCl₂]₂ (0.15 g, 0.251 mmol, 1 equiv.), \(3a\) (0.18 g, 0.251 mmol, 1 equiv.), CH₂Cl₂ (12 mL), 42 h at r.t. The product was isolated as a dark red solid (0.33 g, 0.246 mmol, 98 %).

Elemental Analysis (%): calcd. for C₆₀H₆₂Cl₃O₇P₂Ru₂·CH₂Cl₂ C 54.82, H 4.95; found C 54.75, H 4.95, CH₂Cl₂ originates from the reaction solvent.

¹H NMR (CDCl₃, 400 MHz): 7.66-7.79 (8H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 7.37-7.49 (12H, m, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH₂), 5.19 (4H, d, 4xCH₃-(Ar)C-CH-CH-C, ³J_H,H = 6.1 Hz), 4.96 (4H, d, 4xCH₃-(Ar)C-CH-CH-C, ³J_H,H = 6.1 Hz), 4.40-4.38 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.77-3.74 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.65 (4H, s, 2xAr-(C=O)-O-CH₂-CH₂-O).
O-CH2), 2.83 (2H, sept, 2x(Ar)C-CH-CH-C-CH(CH3)2, \(J_{HH} = 6.9\) Hz), 1.83 (6H, s, 2xCH3- (Ar)C-CH-CH-C), 1.08 (12H, d, 2x(Ar)C-CH-CH-C-CH(CH3)2, \(J_{HH} = 6.9\) Hz).

\(^{31}\)P \(^1\)H NMR (CDCl3, 162 MHz): 24.94 (2P).

\(^{13}\)C \(^1\)H NMR (CDCl3, 101 MHz): 166.0 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 139.4 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{CP} = 8\) Hz), 134.3 (8C, d, 8xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{CP} = 9\) Hz), 133.4 (4C, d, 4xP-(Ar)C-CH-CH-C-P, \(J_{CP} = 10\) Hz), 128.3 (8C, d, 8xP-(Ar)C-CH-CH-C-P, \(J_{CP} = 10\) Hz), 111.50, 111.47 (2C, 2xCH3-(Ar)C-CH-CH-C), 96.4 (2C, 2xCH3-(Ar)C-CH-CH-C), 89.09, 89.07 (4C, 4xCH3-(Ar)C-CH-CH-C), 87.43, 87.38 (4C, 4xCH3-(Ar)C-CH-CH-C), 70.8 (2C, 2xCH3-(Ar)C-CH-CH-C), 69.3 (2C, 2xCH3-(Ar)C-CH-CH-C), 64.4 (2C, 2xCH3-(Ar)C-CH-CH-C), 30.4 (2C, 2xCH3-(Ar)C-CH-CH-C).

ESI-MS (+): \(m/z\) found 1303.1660 [M-Cl]+, 1361.1219 [M+Na]+, \(C_{66}H_{72}Cl_{4}O_{7}P_{2}Ru_{2}\) requires 1303.1644, \(C_{66}H_{72}Cl_{4}NaO_{7}P_{2}Ru_{2}\) requires 1361.1224.

UV-Vis: \(\lambda_{max} = 250\) nm, 370 nm.

**Compound 4b**

According to the general procedure, \([(\eta^8-p\text{-cymene})RuCl_2]_2\) (0.13 g, 0.205 mmol, 1 equiv.), 4a (0.16 g, 0.205 mmol, 1 equiv.), CH2Cl2 (12 mL), 42 h at r.t. The product was isolated as a red solid (0.275 g, 0.199 mmol, 97 %).

**Elemental Analysis (%)**: calcd. for \(C_{56}H_{52}O_{7}P_{2}Ru\cdot1/2\text{CH}_{2}\text{Cl}_{2}\): C 56.03, H 5.06; found C 55.67, H 5.16, CH2Cl2 originates from the reaction solvent.

\(^1\)H NMR (CDCl3, 400 MHz): 7.85-7.95 (8H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 7.76-7.81 (8H, m, 8xP-(Ar)C-CH-CH-C-P, 7.34-7.42 (12H, m, 8xP-(Ar)C-CH-CH-C-P), 5.19 (4H, d, 4xCH3-(Ar)C-CH-CH-C-P, \(J_{HH} = 6.2\) Hz), 4.96 (4H, d, 4xCH3-(Ar)C-CH-CH-C-P, \(J_{HH} = 6.2\) Hz), 4.41-4.38 (4H, m, 2xAr-(C=O)-O-CH2-CH2-O), 3.75-3.73 (4H, m, 4xAr-(C=O)-O-CH2-CH2-O), 3.64-3.60 (8H, m, 2xAr-(C=O)-O-CH2-CH2-O, 2xAr-(C=O)-O-CH2-CH2-O), 2.83 (2H, sept, 2xAr-(C=O)-O-CH2-CH2-O, \(J_{HH} = 6.9\) Hz), 1.83 (6H, s, 2xCH3-(Ar)C-CH-CH-C-P, 1.08 (12H, d, 2x(Ar)C-CH-CH-C-CH(CH3)2, \(J_{HH} = 6.9\) Hz).

\(^{31}\)P \(^1\)H NMR (CDCl3, 162 MHz): 24.96 (2P).

\(^{13}\)C \(^1\)H NMR (CDCl3, 101 MHz): 166.1 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 139.4 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{CP} = 8\) Hz), 134.5 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{CP} = 9\) Hz), 133.4 (4C, d, 4xP-(Ar)C-CH-CH-
According to the general procedure, \([\eta^*-p\text{-cymene}]\text{RuCl}_2\) \(0.16 \text{ g, 0.269 mmol, 1 equiv.}\), 5a \((0.22 \text{ g, 0.269 mmol, 1 equiv.})\), CHCl\(_2\) \((12 \text{ mL})\), 42 h at r.t. The product was isolated as a red solid \((0.38 \text{ g, 0.263 mmol, 98 \%})\).

**Elemental Analysis (%)**: calcd. for C\(_{68}\)H\(_{76}\)Cl\(_4\)O\(_8\)P\(_2\)Ru\(_2\)·\(\frac{1}{2}\)CH\(_2\)Cl\(_2\) C 55.98, H 5.28; found C 55.86, H 5.34; CH\(_2\)Cl\(_2\) originates from the reaction solvent.

\(^1\text{H NMR (CDCl}_3, 400 \text{ MHz)}\): 7.88-7.97 \((8\text{H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4xO-(C=O)-(Ar)C-CH-CH-C-P})\), 7.79-7.81 \((8\text{H, m, 8xP-(Ar)C-CH-CH-CH})\), 7.35-7.42 \((12\text{H, m, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH})\), 5.20 \((4\text{H, d, 4xCH}_3-(Ar)C-CH-CH-C, J_{H,H} = 6.1 \text{ Hz})\), 4.96 \((4\text{H, d, 4xCH}-(Ar)C-CH-CH-C, J_{H,H} = 6.1 \text{ Hz})\), 4.42-4.39 \((4\text{H, m, 2xAr(O=C-O)-O-CH}_2-\text{H})\), 3.77-3.74 \((4\text{H, m, 2xAr(O=C-O)-O-CH}_2-\text{H})\), 3.65-3.56 \((12\text{H, m, 2xAr(O=C-O)-O-(CH}_2)\_2-\text{H)}\), 2.83 \((2\text{H, sept, 2xAr(O=CH-CH-CH}(CH)_2))\), J\(_{H,H} = 6.9 \text{ Hz})\), 1.84 \((6\text{H, s, 2xCH}^\text{H}_-\text{(Ar)C-CH-CH-CCH(CH)}_2))\), 1.09 \((12\text{H, d, 2xAr(O=CH-CH-CCH(CH)}_2))\), J\(_{H,H} = 6.9 \text{ Hz})\).

\(^{31}\text{P \{^1\text{H}\} NMR (CDCl}_3, 162 \text{ MHz)}\): 24.96 \((2\text{P})\).

\(^{13}\text{C \{^1\text{H}\} NMR (CDCl}_3, 101 \text{ MHz)}\): 166.1 \((2\text{C, 2xO-(C=O)-(Ar)C-CH-CH-C-P})\), 139.4 \((2\text{C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, J}_{C,P} = 44 \text{ Hz})\), 134.5 \((4\text{C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P})\), 134.4 \((8\text{C, d, 8xP-(Ar)C-CH-CH-CH})\), 133.4 \((4\text{C, d, 4xP-(Ar)C-CH-CH-CH})\), J\(_{C,P} = 45 \text{ Hz})\), 131.4 \((2\text{C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, J}_{C,P} = 2 \text{ Hz})\), 130.7 \((4\text{C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, J}_{C,P} = 10 \text{ Hz})\), 128.3 \((8\text{C, d, 8xP-(Ar)C-CH-CH-CH})\), J\(_{C,P} = 10 \text{ Hz})\), 111.56, 111.53 \((2\text{C, 2xCH}_2-(Ar)C-CH-CH-C)}\), 94.6 \((2\text{C, 2xCH}-(Ar)C-CH-CH-C))\), 93.09, 89.11 \((4\text{C, 4xCH}-(Ar)C-CH-CH-C)}\).
According to the general procedure, [(η⁶-p-cymene)RuCl₂]₂ (0.16 g, 0.261 mmol, 1 equiv.), 6a (0.22 g, 0.261 mmol, 1 equiv.), CH₂Cl₂ (12 mL), 42 h at r.t. The product was isolated as a red solid (0.38 g, 0.256 mmol, 98%).

**Elemental Analysis (%)**: calcld. for C₇₀H₈₀Cl₄O₉P₂Ru₂ C 57.14, H 5.48; found 57.05, H 5.64.

**1H NMR (CDCl₃, 400 MHz)**: 7.88-7.97 (8H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 7.78-7.83 (8H, m, 8xP-( Ar)C-CH-CH-CH), 7.36-7.45 (12H, m, 8xP-( Ar)C-CH-CH-CH, 4xP-( Ar)C-CH-CH-C-P), 5.21 (4H, d, 4xCH₃-(Ar)C-CH-CH-C, 3J_H,H = 6.2 Hz), 4.97 (4H, d, 4xCH₃-(Ar)C-CH-CH-C, 3J_H,H = 6.2 Hz), 4.44-4.41 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.79-3.77 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.67-3.61 (16H, m, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2x Ar-(C=O)-O-(CH₂)₂-O-CH₂, 2x Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-CH₂, 2x Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-CH₂), 2.85 (2H, sept, 2x( Ar)C-CH-CH-C-CH(CH₃)₂, 3J_H,H = 6.9 Hz), 1.86 (6H, s, 2xC-H-(Ar)C-CH-CH-C), 1.10 (12H, d, 2x( Ar)C-CH-CH-C-CH(CH₃)₂, 3J_H,H = 6.9 Hz).

**31P {1H} NMR (CDCl₃, 162 MHz)**: 24.96 (2P).

**13C {1H} NMR (CDCl₃, 101 MHz)**: 166.1 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-CH-C-P, 3J_C,P = 44 Hz), 134.5 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 3J_C,P = 7 Hz), 134.4 (8C, d, 8xP-( Ar)C-CH-CH-C-P, 3J,C,P = 9 Hz), 133.4 (4C, d, 4xP-( Ar)C-CH-CH-C-P, 3J,C,P = 45 Hz), 131.4 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 3J,C,P = 9 Hz), 130.7 (4C, d, 4xP-( Ar)C-CH-CH-C-P, 3J,C,P = 2 Hz), 128.4 (8C, d, 8xO-(C=O)-(Ar)C-CH-CH-C-P, 3J,C,P = 10 Hz), 128.3 (8C, d, 8xP-( Ar)C-CH-CH-C-P, 3J,C,P = 10 Hz), 111.6, 111.58 (2C, 2xCH₂-(Ar)C-CH-CH-C), 96.4 (2C, 2xCH₂-(Ar)C-CH-CH-C), 89.13, 89.10 (4C, 4xCH₂-(Ar)C-CH-CH-C), 87.49, 87.44 (4C, 4xCH₂-(Ar)C-CH-CH-C), 70.82, 70.73, 70.67 (8C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂, 69.2 (2C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 64.5 (2C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 30.4 (2C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 22.0 (4C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 17.9 (2C, 2xCH₂-( Ar)C-CH-CH-C).
According to the general procedure, \([\eta^6-\tau\text{-cymene}]\text{RuCl}_2\): (0.18 g, 0.299 mmol, 1 equiv.), \(7\alpha\) (0.28 g, 0.299 mmol 1 equiv.), CH\(_2\)Cl\(_2\) (12 mL), 42 h at r.t. The product was isolated as a red solid (0.45 g, 0.287 mmol, 96%).

**Elemental Analysis (%):** calcld. for C\(_{74}\)H\(_{88}\)Cl\(_4\)O\(_{11}\)P\(_2\)Ru\(_2\):

- C 57.00, H 5.69; found C 56.95, H 5.90.

**\(^1\)H NMR (CDCl\(_3\), 400 MHz):** 7.88-7.98 (8H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 7.77-7.83 (8H, m, 8xP-(Ar)C-CH-CH), 7.36-7.45 (12H, m, 8xP-(Ar)C-CH-CH-CH), 5.21 (4H, d, 4xCH\(_3\)-(Ar)C-CH-CH-C, \(J_{H,H} = 6.2\) Hz), 4.98 (4H, d, 4xCH\(_3\)-(Ar)C-CH-CH-C, \(J_{H,H} = 6.2\) Hz), 4.44-4.42 (4H, m, 2xAr-(C=O)-O-(CH\(_2\))\(_2\)-O-CH\(_2\)-CH\(_2\)-O), 3.79-3.77 (4H, m, 2xAr-(C=O)-O-(CH\(_2\))\(_2\)-O-CH\(_2\)-CH\(_2\)-O), 3.70-3.61 (16H, m, 2xAr-(C=O)-O-(CH\(_2\))\(_2\)-O-(CH\(_2\))\(_2\)-O-CH\(_2\)-CH\(_2\)-O), 2.85 (2H, sept, 2xAr-(C=O)-O-(CH\(_2\))\(_2\)-O-(CH\(_2\))\(_2\)-O-CH\(_2\)-CH\(_2\), \(J_{H,H} = 6.9\) Hz), 1.85 (6H, s, 2xCH\(_3\)-(Ar)C-CH-CH-CH), 1.10 (12H, d, 2xAr-(C=O)-O-(Ar)C-CH-CH-C-CH(CH\(_3\))\(_2\), \(J_{H,H} = 6.9\) Hz).

**\(^{31}\)P \(\{^1\)H\} NMR (CDCl\(_3\), 162 MHz):** 24.94 (2P).

**\(^{13}\)C \(\{^1\)H\} NMR (CDCl\(_3\), 101 MHz):** 166.1 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 139.4 (2C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{C,P} = 44\) Hz), 134.5 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{C,P} = 9\) Hz), 111.59, 111.56 (2C, 2xAr-(C=O)-O-(Ar)C-CH-CH-C-P, \(J_{C,P} = 9\) Hz), 89.12, 89.09 (4C, 4xCH\(_3\)-(Ar)C-CH-CH-C-P), 87.48, 87.42 (4C, 4xCH\(_3\)-(Ar)C-CH-CH-C-P), 87.48, 87.42 (4C, 4xCH\(_3\)-(Ar)C-CH-CH-C-P), 87.48, 87.42 (4C, 4xCH\(_3\)-(Ar)C-CH-CH-C-P), 70.81, 70.73, 70.67 (8C, 2xAr-(C=O)-O-(CH\(_2\))\(_2\)-O-CH\(_2\)-CH\(_2\)-CH\(_2\)-O), 64.5 (2C, 2xAr-(C=O)-O-(CH\(_2\))\(_2\)-O-CH\(_2\)-CH\(_2\)-CH\(_2\)), 30.4 (2C, 2xAr-(C=O)-O-(Ar)C-CH-CH-CH-(CH\(_3\))), 27.9 (2C, 2xCH\(_3\)-(Ar)C-CH-CH-C).
**Compound 8b**

According to the general procedure, \[{(\tau^5-p\text{-cymene})\text{RuCl}_2}\] (0.30 g, 0.490 mmol, 1 equiv.), PPh₃ (0.26 g, 0.979 mmol, 2 equiv.), CH₂Cl₂ (12 mL), 42 h at r.t. The product was isolated as a dark red solid (0.54 g, 0.950 mmol, 97%).

**Elemental Analysis (%)**: calcd. for C₂₉H₂₉Cl₂PRu C 59.16, H 5.14, found C 59.26 H 4.90.  

**¹H NMR (CDCl₃, 400 MHz)**: 6.77-7.85 (6H, m, 6xP-(Ar)C-CH-CH-CH), 7.32-7.46 (9H, m, 6xP-(Ar)C-CH-CH-CH, 3xP-(Ar)C-CH-CH-CH), 5.19 (2H, d, 2xCH₃-(Ar)C-CH-CH-C, \(^3J_{H,H} = 6.2\) Hz), 4.98 (2H, d, 2xCH₃-(Ar)C-CH-CH-C, \(^3J_{H,H} = 6.2\) Hz), 2.84 (1H, sept, (Ar)C-CH-CH-C-CH(CH₃)₂, \(^3J_{H,H} = 7.1\) Hz), 1.86 (3H, s, C-CH₃-(Ar)C-CH-CH-C), 1.09 (6H, d, (Ar)C-CH-CH-C-CH(CH₃)₂, \(^3J_{H,H} = 7.1\) Hz).

**³¹P {¹H} NMR (CDCl₃, 162 MHz)**: 24.18 (1P).

**¹³C {¹H} NMR (CDCl₃, 101 MHz)**: 134.5 (6C, d, 6xP-(Ar)C-CH-CH-CH, \(^2J_{C,P} = 9\) Hz), 133.9 (3C, d, 3xP-(Ar)C-CH-CH-CH, \(^2J_{C,P} = 2\) Hz), 128.1 (8C, d, 8xP-(Ar)C-CH-CH-CH, \(^2J_{C,P} = 10\) Hz), 111.20, 111.17 (1C, CH-(Ar)C-CH-CH-C), 89.18, 89.15 (2C, 2CH₃-(Ar)C-CH-CH-C), 87.28, 87.23 (2C, 2xCH₃-(Ar)C-CH-CH-C), 30.3 (1C, (Ar)CH₂-(Ar)C-CH-CH-C), 22.0 (2C, (Ar)CH₂-(Ar)C-CH-CH-C), 17.8 (1C, CH₂-(Ar)C-CH-CH-C).

**ESI-MS(+)**: m/z found 533.0772 [M-Cl], \(\text{C}_{29}\text{H}_{30}\text{ClPRu}^-\) requires 533.0739.

**Compound 9b**

According to the general procedure, \[{(\tau^5-p\text{-cymene})\text{RuCl}_2}\] (0.30 g, 0.490 mmol, 1 equiv.), 4-(diphenylphosphino)benzoic acid (0.30 g, 0.980 mmol, 2 equiv.), CH₂Cl₂ (12 mL), 42 h at r.t. The product was isolated as a dark red solid (0.59 g, 0.961 mmol, 98%).

**Elemental Analysis (%)**: calcd. for C₆₃H₅₃O₂PRu·1/2CHCl₃ C 52.71, H 4.42; found C 52.73, H 4.67, CHCl₃ originates from the recrystallization process.
Chapter 2

H NMR (CDCl₃, 400 MHz): 7.97-8.00 (4H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.78-7.84 (4H, m, 4xP-(Ar)C-CH-CH-CH), 7.36-7.45 (6H, m, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH), 5.22 (2H, d, 2xCH₃-(Ar)C-CH-CH-C-P, \( \Delta_H,J_{H,H} = 6.1 \) Hz), 4.99 (2H, d, 2xCH₃-(Ar)C-CH-CH-C-P, \( \Delta_H,J_{H,H} = 6.1 \) Hz), 2.84 (1H, sept, (Ar)C-CH-CH-C(CH₃)₂, \( \Delta_H,J_{H,H} = 6.9 \) Hz), 1.85 (3H, s, C-H-(Ar)C-CH-CH-C), 1.10 (6H, d, (Ar)C-CH-CH-C-CH(CH₃)₂, \( \Delta_H,J_{H,H} = 6.9 \) Hz).

\( \text{^31P} \{\text{^1H}\} \) NMR (CDCl₃, 162 MHz): 25.27 (1P).

\( \text{^13C} \{\text{^1H}\} \) NMR (CDCl₃, 101 MHz): 171.1 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 140.4 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, \( \Delta_J,J_{C,P} = 43 \) Hz), 134.5 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \( \Delta_J,J_{C,P} = 11 \) Hz), 134.4 (4C, d, 4xP-(Ar)C-CH-CH-CH, \( \Delta_J,J_{C,P} = 10 \) Hz), 133.3 (2C, d, 2xP-(Ar)C-CH-CH-CH, \( \Delta_J,J_{C,P} = 45 \) Hz), 130.7 (2C, d, 2xP-(Ar)C-CH-CH-CH, \( \Delta_J,J_{C,P} = 1 \) Hz), 130.5 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, \( \Delta_J,J_{C,P} = 10 \) Hz), 128.3 (4C, d, 4xP-(Ar)C-CH-CH-CH, \( \Delta_J,J_{C,P} = 2 \) Hz), 129.3 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \( \Delta_J,J_{C,P} = 10 \) Hz), 111.57, 111.61 (1C, CH-/(Ar)C-CH-CH-C), 89.06, 89.03 (2C, 2xCH₃-(Ar)C-CH-CH-C), 87.51, 87.46 (2C, 2xCH₃-(Ar)C-CH-CH-C), 30.4 (1C, (Ar)C-CH-CH-C(CH₃)), 22.0 (2C, (Ar)C-CH-CH-C(CH₃)), 17.9 (1C, CH-/(Ar)C-CH-CH-C).

ESI-MS(+): m/z found 577.0648 [M-Cl]⁺, 635.0228 [M+Na]⁺, C₂₉H₂₉ClO₂PRu⁺ requires 577.0637, C₂₉H₂₉Cl₂NaO₂PRu⁺ requires 635.0223.

2.4.3.3 Synthesis of the bis-gold intermediates 1c-7c

General Procedure

The appropriate ligand 1a-7a (1 equiv.), dissolved in dry CH₂Cl₂ (15 mL), was added a solution of AuCl(tht) (2 equiv.) in dry CH₂Cl₂ (10 mL) at 0°C under N₂. The reaction mixture was stirred at r.t. for 6 h and the reaction evolution was monitored by \( ^1\text{H} \) and \( ^3\text{P} \) NMR (CDCl₃). The reaction mixture was concentrated under reduced pressure and the product was washed with hexane (5x25 mL), re-solubilised in CH₂Cl₂ (50 mL) before being concentrated and further dried under high vacuum. The product was isolated as a white solid and stored at -20°C under dark.
**Compound 1c**

According to the general procedure, AuCl(tht) (0.188 g, 0.588 mmol, 2 equiv.), 1a (0.188 g, 0.294 mmol, 1 equiv.), CH₂Cl₂ (35 mL), 6 h at r.t. The product was isolated as a white solid (0.319 g, 0.289 mmol, 98%).

**Elemental Analysis (%)**: calcd. for C₄₀H₃₂Au₂Cl₂O₄P₂: C 43.54, H 2.92; found: C 43.55, H 3.02.

**¹H NMR (CDCl₃, 400 MHz)**: 8.07-8.12 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 4Jₕ,ₜ = 8.4 Hz, 4Jₜ,ₚ = 2.1 Hz), 7.45-7.60 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 4.68 (4H, s, Ar-(C=O)-O-(CH₂)₂-O).

**³¹P {¹H} NMR (CDCl₃, 162 MHz)**: 33.14 (2P).

**¹³C {¹H} NMR (CDCl₃, 101 MHz)**: 165.3 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 134.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 2Jₕ,ₚ = 59 Hz), 134.3 (8C, d, 8xP-(Ar)C-CH-CH-CH, 2Jₜ,ₚ = 14 Hz), 134.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 2Jₜ,ₚ = 14 Hz), 132.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 2Jₜ,ₚ = 3 Hz), 132.5 (4C, d, 4xP-(Ar)C-CH-CH-CH, 2Jₜ,ₚ = 3 Hz), 130.2 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 2Jₜ,ₚ = 12 Hz), 129.6 (8C, d, 8xP-(Ar)C-CH-CH-CH, 2Jₜ,ₚ = 12 Hz), 127.9 (4C, d, 4xP-(Ar)C-CH-CH-CH, 2Jₜ,ₚ = 63 Hz), 63.3 (2C, 2x(Ar)-(C=O)-O-(CH₂)₂-O).

**ESI-MS (+)**: m/z found 1067.0825 [M-Cl]⁺, 1125.0388 [M+Na]⁺, C₄₀H₃₂Au₂Cl₂O₄P₂⁺ requires 1067.0795, C₄₀H₃₂Au₂Cl₂NaO₄P₂⁺ requires 1125.0382⁺.

**Compound 2c**

According to the general procedure, AuCl(tht) (0.235 g, 0.735 mmol, 2 equiv.), 2a (0.250 g, 0.366 mmol, 1 equiv.), CH₂Cl₂ (35 mL), 6 h at r.t. The product was isolated as a white solid (0.403 g, 0.351 mmol, 96%).
Elemental Analysis (%): calcd. for C_{42}H_{36}Au_{2}Cl_{2}O_{5}P_{2}: C 43.96, H 3.16; found C 43.83, H 3.23.

{\textsuperscript{1}}H NMR (CDCl_{3}, 400 MHz): 8.06-8.10 (4H, m, 4x O-(C=O)-(Ar)C-CH-CH-C-P, \textsuperscript{3}J_{HH} = 8.2 Hz, \textsuperscript{4}J_{HP} = 2 Hz), 7.44-7.60 (24H, m, 4x O-(C=O)-(Ar)C-CH-CH-C-P, 8x P-(Ar)C-CH-H-CH, 8x P-(Ar)C-CH-H-CH, 4x P-(Ar)C-CH-C-\textit{H}) 4.48-4.50 (4H, m, 2x Ar-(C=O)-O-CH_2-CH_2-O), 3.85-3.87 (4H, m, 2x Ar-(C=O)-O-CH_2-CH_2-O).

{\textsuperscript{31}}P \{\textsuperscript{1}H\} NMR (CDCl_{3}, 162 MHz): 33.02 (2P).

{\textsuperscript{13}}C \{\textsuperscript{1}H\} NMR (CDCl_{3}, 101 MHz): 165.4 (2C, d, 2x O-(C=O)-(Ar)C-CH-CH-C-P, \textsuperscript{3}J_{CP} = 62 Hz), 134.5 (8C, d, 8x P-(Ar)C-CH-H-CH, \textsuperscript{2}J_{HP} = 14 Hz), 134.0 (4C, d, 4x O-(C=O)-(Ar)C-CH-H-CH-P, \textsuperscript{3}J_{CP} = 62 Hz), 133.2 (2C, d, 2x O-(C=O)-(Ar)C-CH-H-CH-P, \textsuperscript{3}J_{CP} = 12 Hz), 128.0 (4C, d, 4x P-(Ar)C-CH-H-CH, \textsuperscript{4}J_{CP} = 63 Hz), 129.6 (8C, d, 8x P-(Ar)C-CH-CH-H, \textsuperscript{3}J_{CP} = 12 Hz), 128.0 (4C, d, 4x P-(Ar)C-CH-H-CH, \textsuperscript{4}J_{CP} = 63 Hz), 132.5 (4C, d, 4x P-(Ar)C-CH-H-CH, \textsuperscript{4}J_{CP} = 63 Hz), 130.1 (4C, d, 4x O-(C=O)-(Ar)C-CH-C-CH-P, \textsuperscript{3}J_{CP} = 12 Hz), 69.2 (2C, 2x Ar-(C=O)-O-CH_2-CH_2-O).

ESI-MS(+): m/z found 1111.1104 [M-Cl]+, 1169.0690 [M+Na]+, C_{42}H_{36}Au_{2}Cl_{2}O_{5}P_{2}+ requires 1111.1058, C_{42}H_{36}Au_{2}Cl_{2}NaO_{5}P_{2}+ requires 1169.0644.

Compound 3c

According to the general procedure, AuCl(tht) (0.221 g, 0.692 mmol, 2 equiv.), 3a (0.250 g, 0.344 mmol, 1 equiv.), CH_{2}Cl_{2} (35 mL), 6 h at r.t. The product was isolated as a white solid (0.406 g, 0.341 mmol, 98%).

Elemental Analysis (%): calcd. for C_{44}H_{40}Au_{2}Cl_{2}O_{6}P_{2}: C 44.35, H 3.38; found C 44.30, H 3.51.

{\textsuperscript{1}}H NMR (CDCl_{3}, 400 MHz): 8.07-8.11 (4H, m, 4x O-(C=O)-(Ar)C-CH-CH-C-P, \textsuperscript{3}J_{HH} = 8.4 Hz, \textsuperscript{4}J_{HP} = 2.2 Hz), 7.45-7.60 (24H, m, 4x O-(C=O)-(Ar)C-CH-CH-C-P, 8x P-(Ar)C-CH-H-CH, 8x P-(Ar)C-CH-H-CH, 4x P-(Ar)C-CH-H-CH), 4.44-4.48 (4H, m, 2x Ar-(C=O)-O-CH_2-CH_2-O), 3.79-3.83 (4H, m, 2x Ar-(C=O)-O-CH_2-CH_2-O).

{\textsuperscript{31}}P \{\textsuperscript{1}H\} NMR (CDCl_{3}, 162 MHz): 33.07 (2P).

{\textsuperscript{13}}C \{\textsuperscript{1}H\} NMR (CDCl_{3}, 101 MHz): 165.4 (2C, d, 2x O-(C=O)-(Ar)C-CH-CH-C-P, \textsuperscript{3}J_{CP} = 62 Hz), 134.3 (8C, d, 8x P-(Ar)C-CH-H-CH, \textsuperscript{2}J_{HP} = 14 Hz), 134.2 (4C, d, 4x O-(C=O)-(Ar)C-CH-H-CH-P, \textsuperscript{3}J_{CP} = 62 Hz), 133.3 (2C, d, 2x O-(C=O)-(Ar)C-CH-H-CH-P, \textsuperscript{3}J_{CP} = 12 Hz), 128.5 (4C, d, 4x P-(Ar)C-CH-H-CH, \textsuperscript{4}J_{CP} = 63 Hz), 130.2 (4C, d, 4x P-(Ar)C-CH-H-CH, \textsuperscript{4}J_{CP} = 63 Hz), 129.5 (8C, d, 8x P-(Ar)C-CH-CH-H, \textsuperscript{3}J_{CP} = 12 Hz), 128.0 (4C, d, 4x P-(Ar)C-CH-H-CH, \textsuperscript{4}J_{CP} = 63 Hz).
O-(CH$_2$)$_2$-O-CH$_2$), 69.3 (2C, 2x(Ar)-(C=O)-O-CH$_2$-CH$_2$-O), 64.7 (2C, 2x(Ar)-(C=O)-O-CH$_2$-CH$_2$-O).

ESI-MS(+): m/z found 1155.1376 [M-Cl]$^+$, 1213.0951 [M+Na]$^+$, C$_{46}$H$_{44}$Au$_2$ClO$_7$P$_2$$^+$ requires 1155.1320, C$_{46}$H$_{44}$Au$_2$Cl$_2$NaO$_7$P$_2$$^+$ requires 1213.0906.

**Compound 4c**

According to the general procedure, AuCl(tht) (0.208 g, 0.651 mmol, 2 equiv.), 4a (0.250 g, 0.324 mmol, 1 equiv.), CH$_2$Cl$_2$ (35 mL), 6 h at r.t. The product was isolated as a white solid (0.389 g, 0.315 mmol, 97%).

**Elemental Analysis (%):** calcld. for C$_{46}$H$_{44}$Au$_2$ClO$_7$P$_2$: C 44.71, H 3.59; found C 44.78, H 3.57.

$^1$H NMR (CDCl$_3$, 400 MHz): 8.08-8.12 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^3$J$_{H,H}$ = 8.4 Hz, $^4$J$_{H,P}$ = 2 Hz), 7.46-7.60 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 4.46-4.49 (4H, m, 2xAr=C=O-O-CH$_2$-CH$_2$-O), 3.79-3.82 (4H, m, 2xAr=C=O-O-CH$_2$-CH$_2$-O), 3.61-3.72 (8H, m, 2xAr=C=O-O-CH$_2$-CH$_2$-O).

$^{31}$P $^1$H NMR (CDCl$_3$, 162 MHz): 33.01 (2P).

$^{13}$C $^1$H NMR (CDCl$_3$, 101 MHz): 165.4 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P, $^1$J$_{C,H}$ = 58 Hz), 134.3 (8C, d, 8xP-(Ar)C-CH-CH-CH, $^1$J$_{C,P}$ = 14 Hz), 134.0 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^1$J$_{C,P}$ = 14 Hz), 133.3 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, $^1$J$_{C,P}$ = 2 Hz), 132.4 (4C, d, 4xP-(Ar)C-CH-CH-CH, $^1$J$_{C,P}$ = 2 Hz), 130.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^1$J$_{C,P}$ = 12 Hz), 129.5 (8C, d, 8xP-(Ar)C-CH-CH-CH, $^1$J$_{C,P}$ = 12 Hz), 127.9 (4C, d, 4xP-(Ar)C-CH-CH-CH, $^1$J$_{C,P}$ = 63 Hz), 70.7 (4C, 2xAr=C=O-O-CH$_2$-CH$_2$-O, 2xAr=C=O-O-CH$_2$-CH$_2$-CH$_2$-O, 69.1 (2C, 2xAr=C=O-O-CH$_2$-CH$_2$-O), 64.7 (2C, 2xAr=C=O-O-CH$_2$-CH$_2$-O).

ESI-MS(+): m/z found 1199.1633 [M-Cl]$^+$, 1257.1218 [M+Na]$^+$, C$_{46}$H$_{44}$Au$_2$ClO$_7$P$_2$$^+$ requires 1199.1582, C$_{46}$H$_{44}$Au$_2$Cl$_2$NaO$_7$P$_2$$^+$ requires 1257.1168.

**Compound 5c**
According to the general procedure, AuCl(tht) (0.162 g, 0.507 mmol, 2 equiv.), 5a (0.206 g, 0.253 mmol, 1 equiv.), CH₂Cl₂ (35 mL), 6 h at r.t. The product was isolated as a white solid (0.308 g, 0.241 mmol, 95%).

Elemental Analysis (%): calcd. for C₄₈H₄₈Au₂Cl₂O₈P₂: C 45.05, H 3.78; found C 45.05, H 3.79.

¹H NMR (CDCl₃, 400 MHz): 8.09-8.12 (4H, m, 4×O-(C=O)-(Ar)C-CH-CH-C-P, J_H,H = 8.6 Hz, J_H,P = 2.4 Hz), 7.46-7.60 (24H, m, 4×O-(C=O)-(Ar)C-CH-CH-C-P, 8×P-(Ar)C-CH-CH-CH, 8×P-(Ar)C-CH-CH-CH, 4×P-(Ar)C-CH-CH-C-P), 4.47-4.49 (4H, m, 2×Ar-(C=O)-O-CH₂-CH₂-O), 3.80-3.82 (4H, m, 2×Ar-(C=O)-O-CH₂-CH₂-O, 3.66-3.68 (4H, m, 2×Ar-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 3.62-3.64 (4H, m, 2×Ar-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 3.63 (4H, s, 2×Ar-(C=O)-O-CH₂-CH₂-O-(CH₂)₂-O-CH₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 32.99 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 165.4 (2C, d, 2×O-(C=O)-(Ar)C-CH-CH-C-P), 134.3 (2C, d, 2×O-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 57 Hz), 134.3 (8C, d, 8×P-(Ar)C-CH-CH-CH, 2J_C,P = 14 Hz), 134.0 (4C, d, 4×O-(C=O)-(Ar)C-CH-CH-CH, 2J_C,P = 14 Hz), 133.3 (2C, d, 2×O-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 2 Hz), 132.4 (4C, d, 4×P-(Ar)C-CH-CH-CH, 3J_C,P = 3 Hz), 130.1 (4C, d, 4×O-(C=O)-(Ar)C-CH-CH-C-P, 1J_C,P = 12 Hz), 129.5 (8C, d, 8×P-(Ar)C-CH-CH-CH, 3J_C,P = 12 Hz), 127.9 (4C, d, 4×P-(Ar)C-CH-CH-CH, 3J_C,P = 12 Hz), 127.9 (4C, d, 4×P-(Ar)C-CH-CH-CH, 3J_C,P = 12 Hz), 70.71, 70.66 (6C, 2×(Ar)-(C=O)-O-(CH₂)₂-O-CH₂, 2×(Ar)-(C=O)-O-(CH₂)₂-O-CH₂-CH₂, 2×(Ar)-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-CH₂), 69.1 (2C, 2×(Ar)-(C=O)-O-CH₂-CH₂-O-(CH₂)₂-O-CH₂), 67.4 (2C, 2×(Ar)-(C=O)-O-CH₂-CH₂-O-(CH₂)₂-O-CH₂).

ESI-MS(+): m/z found 1243.1903 [M-Cl]⁺, 1301.1469 [M+Na]⁺ Cu₆H₈Au₂Cl₂O₄P₂⁺ requires 1243.1844, Cu₆H₈Au₂Cl₂NaO₄P₂⁺ requires 1301.1430.

Compound 6c

According to the general procedure, AuCl(tht) (0.192 g, 0.601 mmol, 2 equiv.), 6a (0.257 g, 0.299 mmol, 1 equiv.), CH₂Cl₂ (35 mL), 6 h at r.t. The product was isolated as a white solid (0.376 g, 0.284 mmol, 95%).
**Elemental Analysis (%):** calcd. for C$_{50}$H$_{52}$Au$_2$Cl$_2$O$_9$P$_2$: C 45.37, H 3.96; found C 45.44, H 3.84.

$^1$H NMR (CDCl$_3$, 400 MHz): 8.08-8.12 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^3$J$_{H,H}$ = 8.4 Hz, $^4$J$_{H,P}$ = 2 Hz), 7.45-7.59 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 4.46-4.49 (4H, m, 2xAr-(C=O)-O-CH$_2$-CH=O), 3.79-3.82 (4H, m, 2xAr-(C=O)-O-CH$_2$-CH=O), 3.65-3.68 (4H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$), 3.60-3.64 (4H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$), 3.59-3.62 (8H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$); $^{31}$P $^1$H NMR (CDCl$_3$, 162 MHz): 33.03 (2P).

$^{13}$C $^1$H NMR (CDCl$_3$, 101 MHz): 165.4 (2C, d, $^1$JC,C = 17 Hz), 133.2 (2C, d, $^1$JC,C = 17 Hz), 134.0 (4C, d, $^1$JC,C = 17 Hz), 133.4 (2C, d, $^1$JC,C = 17 Hz), 80.4 (8C, d, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH), 70.78, 70.74, 70.68 (8C, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$. 3.60-3.64 (4H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$), 3.59-3.62 (8H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$), 3.61 (8H, s, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$. 3.61 (8H, s, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$. 3.61 (8H, s, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$).

ESI-MS: m/z found 1287.2147 [M-Cl]$^+$, 1345.1719 [M+Na]$^+$, C$_{50}$H$_{52}$Au$_2$Cl$_2$O$_9$P$_2$ requires 1287.2106, C$_{50}$H$_{52}$Au$_2$Cl$_2$NaO$_9$P$_2$ requires 1345.1692.

**Compound 7c**

According to the general procedure, AuCl(tht) (0.230 g, 0.720 mmol, 2 equiv.), 7a (0.340 g, 0.359 mmol, 1 equiv.), CH$_2$Cl$_2$ (35 mL), 6 h at r.t. The product was isolated as a white solid (0.485 g, 0.344 mmol, 96%).

**Elemental Analysis (%):** calcd. for C$_{54}$H$_{60}$Au$_2$Cl$_2$O$_{11}$P$_2$: C 45.94, H 4.28; found C 45.82, H 4.04.

$^1$H NMR (CDCl$_3$, 400 MHz): 8.08-8.12 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, $^3$J$_{H,H}$ = 8.2 Hz, $^4$J$_{H,P}$ = 2 Hz), 7.45-7.59 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 4.46-4.49 (4H, m, 2xAr-(C=O)-O-CH$_2$-CH=O), 3.79-3.82 (4H, m, 2xAr-(C=O)-O-CH$_2$-CH=O), 3.65-3.68 (4H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$), 3.60-3.64 (4H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$), 3.59-3.62 (8H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$).

$^{31}$P $^1$H NMR (CDCl$_3$, 162 MHz): 33.01 (2P).

$^{13}$C $^1$H NMR (CDCl$_3$, 101 MHz): 165.4 (2C, d, $^1$JC,C = 17 Hz), 133.2 (2C, d, $^1$JC,C = 17 Hz), 134.0 (4C, d, $^1$JC,C = 17 Hz), 133.4 (2C, d, $^1$JC,C = 17 Hz), 80.4 (8C, d, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH), 70.78, 70.74, 70.68 (8C, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$. 3.60-3.64 (4H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$), 3.59-3.62 (8H, m, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$), 3.61 (8H, s, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$. 3.61 (8H, s, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$. 3.61 (8H, s, 2xAr-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$).

ESI-MS: m/z found 1287.2147 [M-Cl]$^+$, 1345.1719 [M+Na]$^+$, C$_{50}$H$_{52}$Au$_2$Cl$_2$O$_9$P$_2$ requires 1287.2106, C$_{50}$H$_{52}$Au$_2$Cl$_2$NaO$_9$P$_2$ requires 1345.1692.


CH-CH-C-P, \( J_{C,P} = 2 \text{ Hz} \), 132.4 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH, \( J_{C,P} = 12 \text{ Hz} \), 129.5 (8C, d, 8xP-(Ar)C-CH-CH, \( J_{C,P} = 2 \text{ Hz} \), 130.2 (4C, d, 4xP-(Ar)C-CH-CH, \( J_{C,P} = 12 \text{ Hz} \), 128.0 (4C, d, 4xP-(Ar)C-CH-CH, \( J_{C,P} = 63 \text{ Hz} \), 70.77, 70.72, 70.66 (12C, 2x(Ar)-(C=O)-O-(CH2)-O-CH, 2x(Ar)-(C=O)-O-(CH2)-O-CH, 2x(Ar)-(C=O)-O-(CH2)-O-CH, 2x(Ar)-(C=O)-O-(CH2)-O-CH, 69.2 (2C, 2x(Ar)-(C=O)-O-CH-CH-CH), 64.8 (2C, 2x(Ar)-(C=O)-O-CH-CH-CH).

ESI-MS(+): \( m/z \) found 1375.2667 [M-Cl]+, 1433.2239 [M+Na]+, \( \text{C}_{54}\text{H}_{60}\text{Au}_{2}\text{ClO}_{11}\text{P}_{2} \) requires 1375.2630, \( \text{C}_{54}\text{H}_{60}\text{Au}_{2}\text{ClO}_{11}\text{P}_{2} \) requires 1433.2217.

**Compound 8c**

![Compound 8c](image)

According to the general procedure, \( \text{AuCl(tht)} \) (0.318 g, 0.995 mmol, 1 equiv.), \( \text{PPh}_3 \) (0.260 g, 0.991 mmol, 1 equiv.), CHCl\(_2\) (25 mL), 4 h at r.t. The product was isolated as a white solid (0.471 g, 0.952 mmol, 96%).

**Elemental Analysis (%)**: calcd. for \( \text{C}_{18}\text{H}_{15}\text{AuClP} \) C 43.70, H 3.06, found C 44.02, H 2.74.

\(^1\text{H} \text{NMR (CDCl}_3\text{, 400 MHz)}\): 7.42-7.58 (15H, m, 6xP-(Ar)C-CH-CH-CH, 6xP-(Ar)C-CH-CH-CH, 3xP-(Ar)C-CH-CH-CH).

\(^{31}\text{P \{'H\} NMR (CDCl}_3\text{, 162 MHz)}\): 33.19 (1P).

\(^{13}\text{C \{'H\} NMR (CDCl}_3\text{, 101 MHz)}\): 134.2 (6C, d, 6xP-(Ar)C-CH-CH-CH, 6xP-(Ar)C-CH-CH-CH, 3xP-(Ar)C-CH-CH-CH, \( J_{C,P} = 14 \text{ Hz} \), 132.1 (3C, d, 3xP-(Ar)C-CH-CH-CH, \( J_{C,P} = 3 \text{ Hz} \), 129.3 (6C, d, 6xP-(Ar)C-CH-CH-CH, \( J_{C,P} = 12 \text{ Hz} \), 128.8 (3C, d, 3xP-(Ar)C-CH-CH-CH, \( J_{C,P} = 62 \text{ Hz} \).

ESI-MS(+): \( m/z \) found 517.0163 [M+Na]+, \( \text{C}_{18}\text{H}_{15}\text{AuClNaP} \) requires 517.0163.

**Compound 9c**

![Compound 9c](image)
According to the general procedure, AuCl(tht) (0.590 g, 1.846 mmol, 1 equiv.), 4-(diphenylphosphino)benzoic acid (0.564 g, 1.841 mmol, 1 equiv.), CH₂Cl₂ (25 mL), 6 h at r.t. The product was isolated as a white solid (0.962 g, 1.786 mmol, 97%).

Elemental Analysis (%): calcd. for C₁₉H₁₅AuClO₂P: C 42.36, H 2.81, found C 42.60 H 2.59.

¹H NMR (CDCl₃, 400 MHz): 8.05-8.19 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, J_H,H = 8.4 Hz, J_H,P = 2.1 Hz), 7.47-7.64 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 33.21 (1P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 170.5 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 135.8 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 58 Hz), 134.4 (4C, d, 2xP-(Ar)C-CH-CH-C-P, J_C,P = 14 Hz), 134.1 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 14 Hz), 132.4 (2C, m, O-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 12 Hz), 129.6 (4C, d, 4xP-(Ar)C-CH-CH-C-P, J_C,P = 12 Hz), 128.1 (2C, d, 2xP-(Ar)C-CH-CH-C-P, J_C,P = 61 Hz).


2.4.3.4 Synthesis of the bis-gold complexes 1d-7d

General Procedure

The appropriate bis-gold intermediate 1c-7c (1 equiv.) was added to a suspension of β-D-thioglucose tetraacetate (2 equiv.) and K₂CO₃ (4 equiv.) in degassed acetone under N₂. The reaction mixture was stirred under N₂ at r.t for 48 h in the dark and the reaction evolution was verified by ¹H and ³¹P NMR (CDCl₃). The reaction mixture was concentrated under reduced pressure, the crude was re-suspended in CH₂Cl₂ and filtered under gravity. The filtrate was concentrated under reduced pressure and further dried under high vacuum. The product was isolated as a white solid and stored at -20°C under dark.
Compound 1d

According to the general procedure, β-D-thioglucose tetraacetate (0.026 g, 0.071 mmol, 2 equiv.), K₂CO₃ (0.050 g, 0.362 mmol, 4 equiv.), 1₁c (0.100 g, 0.091 mmol, 1 equiv.) in acetone (25 mL), 48 h at r.t. dark. The product was isolated as a white solid (0.145 g, 0.0824 mmol, 90%).

Elemental Analysis (%): calcd. for C₆₈H₇₀Au₂O₂₂P₂S₂ C 46.42, H 4.01; found C 46.37, H 3.92.

¹H NMR (CDCl₃, 400 MHz): 8.09-8.12 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_HH = 8.6 Hz, ¹J_HP = 2.0 Hz), 7.46-7.63 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH, 8xP-(Ar)C-CH-CH, 4xP-(Ar)C-CH-CH-C_H), 5.12-5.17 (4H, m, S-CH-CH-O-Ac, S-CH-CH-O-Ac), 5.05 (2H, t, CH-CH-O-Ac, ³J_HH = 9.7 Hz), 5.03 (2H, t, S-CH-CH-O-Ac, ³J_HH = 9.2 Hz), 5.01 (2H, t, P-(Ar)C-CH-CH, ³J_HH = 12.2 Hz), 4.70 (2H, d, CH-O-Ac, ³J_HH = 4.7 Hz), 4.08 (2H, d, CH-O-Ac, ³J_HH = 2.3 Hz), 2.04 (6H, s, 2xCH-O-(C=O)-(CH2)2-O-Ac), 1.96 (6H, s, 2x(C=O)-CH3), 1.89 (6H, s, 2x(C=O)-CH3), 1.87 (6H, s, 2x(C=O)-CH3).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 38.71 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 170.8 (2C, 2x(C=O)-(CH2)-), 170.3 (2C, 2x(C=O)-(CH2)-), 169.7 (2C, 2x(C=O)-(CH2)-), 169.6 (2C, 2x(C=O)-(CH2)-), 165.4 (2C, 2x(C=O)-(Ar)C-CH-CH-C-P), 135.8 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J cp = 53 Hz), 134.5 (4C, d, 4xP-(Ar)C-CH-CH, ³J cp = 14 Hz), 134.2 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³J cp = 12 Hz), 132.5 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 132.1 (4C, m, 4xP-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 129.5 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ³J cp = 12 Hz), 128.8 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 123.2 (4C, m, 4xP-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 123.2 (4C, m, 4xP-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 129.5 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ³J cp = 12 Hz), 128.8 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 123.2 (4C, m, 4xP-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 123.2 (4C, m, 4xP-(Ar)C-CH-CH-C-P, ³J cp = 14 Hz), 75.9 (2C, S-CH-CH-O-Ac), 74.2 (2C, S-CH-CH-O-Ac), 69.0 (2C, 2xCH-CH-O-Ac), 63.2 (2C, 2xO-(C=O)-O-(CH2)-O-Ac), 62.9 (2C, 2xCH-CH-(C=O)-(CH2)-), 21.2 (2C, 2xCH-O-(C=O)-(CH2)-), 20.82 (2H, 2x(C=O)-(CH2)-), 20.77 (2C, 2x(C=O)-(CH2)-), 20.72 (2C, 2x(C=O)-(CH2)-).


UV-Vis: λ_max = 250 nm.
According to the general procedure, β-D-thioglucose-tetraacetate (0.107 g, 0.294 mmol, 2 equiv.), K₂CO₃ (0.082 g, 0.593 mmol, 4 equiv.), 2c (0.170 g, 0.148 mmol, 1 equiv.) in acetone (25 mL), 48 h at r.t. dark. The product was isolated as a white solid (0.223 g, 0.123 mmol, 84%).

**Elemental Analysis (%)**: calcd. for C₇₀H₇₄Au₂O₂₃P₂S₂ C 46.62, H 4.14; found C 46.74, H 4.10.

**H NMR (CDCl₃, 400 MHz)**: 8.08-8.12 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 3 JₜH,H = 8.5 Hz, 4 JₜH,P = 2 Hz), 7.47-7.65 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-C-H), 5.13–5.17 (4H, m, S-CH-CH-O-Ac, S-CH-CH-C-H-O-Ac), 5.08 (2H, dd, CH₂-O-Ac, 3 JₜH,H = 9.2 Hz), 4.48-4.50 (4H, m, 2x Ar-(C=O)-O-CH₂-CH₂-O), 4.21 (2H, dd, CH₂-O-Ac, 3 JₜH,H = 12.2 Hz, 3 JₜH,H = 4.8 Hz, 3 JₜH,H = 2.4 Hz), 4.09 (2H, dd, CH₂-O-Ac, 3 JₜH,H = 9.2 Hz), 3.75 (2H, ddd, CH-CH₂-O-Ac, 3 JₜH,H = 9.7 Hz, 3 JₜH,H = 4.8 Hz, 3 JₜH,H = 2.4 Hz), 2.04 (6H, s, 2xCH₂-O-(C=O)-(CH₃)), 1.96 (6H, s, 2x(C=O)-(CH₂)), 1.89 (6H, s, 2x(C=O)-(CH₂)).

**P {H} NMR (CDCl₃, 162 MHz)**: 38.77 (2P).

**C {H} NMR (CDCl₃, 101 MHz)**: 170.9 (2C, 2x(C=O)-CH₃), 170.4 (2C, 2x(C=O)-CH₃), 169.8 (2C, 2x(C=O)-CH₃), 169.7 (2C, 2x(C=O)-CH₃), 165.6 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 135.6 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 2 JₜC,P = 54 Hz), 134.6 (4C, d, 4xP-(Ar)C-CH-CH-C-P, 2 JₜC,P = 14 Hz), 134.2 (4C, d, 4xP-(Ar)C-CH-CH-C-P, 2 JₜC,P = 14 Hz), 128.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 2 JₜC,P = 2 Hz), 132.1 (4C, d, 4xP-(Ar)C-CH-CH-C-P, 2 JₜC,P = 2 Hz), 130.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 2 JₜC,P = 12 Hz), 129.5 (8C, d, 8xP-(Ar)C-CH-CH-C-P, 2 JₜC,P = 12 Hz), 129.0 (4C, d, 4xP-(Ar)C-CH-CH-C-P, 2 JₜC,P = 57 Hz), 83.3 (2C, 2xCH₂-O-Ac), 77.8 (2C, S-CH-CH-O-Ac), 75.9 (2C, S-CH-CH-O-Ac), 74.3 (2C, S-CH-CH-O-Ac), 69.2 (2C, 2xCH₂-O-Ac), 69.1 (2C, 2x(CH₂)-(C=O)-O-CH₂-CH₂-O), 64.6 (2C, 2x(CH₂)-(C=O)-O-CH₂-CH₂-O), 63.0 (2C, 2xCH₂-O-(C=O)-(CH₂)), 21.3 (2C, 2xCH₂-O-(C=O)-(CH₂)), 20.86 (2C, 2x(CH₂)-(CH₂)), 20.77 (2C, 2x(C=O)-(CH₂)).

**ESI-MS (+)**: m/z found 1825.2839 [M+Na⁺], 1439.2242 [M-RS⁺]. C₇₀H₇₄Au₂NaO₂₃P₂S₂⁺ requires 1825.2761, C₅₆H₅₅Au₂O₁₄P₂S⁺ requires 1439.2113.

**UV-Vis**: λₘₚₓ = 250 nm.
According to the general procedure, β-D-thioglucose-tetraacetate (0.107 g, 0.294 mmol, 2 equiv.), K₂CO₃ (0.081 g, 0.586 mmol, 4 equiv.), 3c (0.175 g, 0.147 mmol, 1 equiv.) in acetone (25 mL), 48 h at r.t. dark. The product was isolated as a white solid (0.258 g, 0.140 mmol, 95%).

Elemental Analysis (%): calcd. for C₇₂H₇₈Au₂O₂₄P₂S₂ C 46.81, H 4.26; found C 47.02, H 3.98.

¹H NMR (CDCl₃, 400 MHz): 8.09-8.12 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_H,H = 8.5 Hz, ¹J_H,P = 2.0 Hz), 7.47-7.67 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH, 8xP-(Ar)C-C-CH-CH, 4xP-(Ar)C-CH-CH-H), 5.11–5.18 (4H, m, S-CH-CH-O-Ac, S-CH-CH-CH-O-Ac), 5.07 (2H, t, C H-CH-CH₂-O-Ac ²J_H,H = 9.1 Hz), 5.04 (2H, t, S-CH-C H-O-Ac, ³J_H,H = 9.2 Hz), 4.46-4.48 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 4.22 (2H, dd, C H₂-O-Ac, ²J_H,H = 12.2 Hz, ³J_H,H = 4.8 Hz), 4.10 (2H, dd, S-CH-C H-O-Ac, ²J_H,H = 12.2 Hz, ³J_H,H = 2.4 Hz), 4.00-4.02 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.80-3.83 (4H, m, 2xAr-(C=O)-O-CH₂-CH₂-O), 3.76 (2H, ddd, CH-CH₂-O-Ac, ²J_H,H = 9.8 Hz, ³J_H,H = 4.8 Hz), 3.69 (4H, s, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂), 2.05 (6H, s, 2xCH₂-O-(C=O)-CH₃), 1.97 (6H, s, 2x(C=O)-CH₃), 1.90 (6H, s, 2x(C=O)-CH₃).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 38.79 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 170.9 (2C, 2x(C=O)-CH₃), 170.4 (2C, 2x(C=O)-CH₃), 169.8 (2C, 2x(C=O)-CH₃), 169.7 (2C, 2x(C=O)-CH₃), 169.6 (2C, 2x(C=O)-CH₃), 135.6 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_C,P = 14 Hz), 134.6 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ¹J_C,P = 14 Hz), 134.5 (2C, d, 4xP-(Ar)C-CH-CH-C-P, ¹J_C,P = 14 Hz), 133.0 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ¹J_C,P = 2 Hz), 130.2 (4C, m, 4xP-(Ar)C-CH-CH-C-P, ¹J_C,P = 12 Hz), 129.5 (8C, d, 8xP-(Ar)C-CH-CH-C-P, ¹J_C,P = 12 Hz), 129.0 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ¹J_C,P = 7 Hz), 83.3 (2C, 2xCH₂-O-Ac), 77.8 (2C, 2xCH₂-O-Ac), 75.9 (2C, 2xCH₂-O-Ac), 74.3 (2C, 2xCH₂-O-Ac), 70.8 (2C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂), 69.3 (2C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂), 69.1 (2C, 2xCH₂-O-Ac), 64.6 (2C, 2xAr-(C=O)-O-CH₂-CH₂-O), 63.0 (2C, 2xAr-(C=O)-O-CH₂-CH₂-O), 20.87 (2C, 2x(C=O)-CH₃), 19.0 (2C, 2x(C=O)-CH₃), 19.0 (2C, 2x(C=O)-CH₃).

ESI-MS(+) : m/z found 1869.3127 [M+Na]⁺, 1483.2581 [M-RS]⁺, C₇₂H₇₈Au₂O₂₄P₂S₂⁺ requires 1869.3023, C₅₈H₅₉Au₂O₁₅P₂S⁺ requires 1483.2375.

UV-Vis: λmax = 250 nm.
Compound 4d

According to the general procedure, β-D-thioglucose-tetraacetate (0.103 g, 0.283 mmol, 2 equiv.), K₂CO₃ (0.079 g, 0.572 mmol, 4 equiv.), 4c (0.175 g, 1 equiv.) in acetone (25 mL), 48 h at r.t. dark. The product was isolated as a white solid (0.233 g, 0.123 mmol, 87%).

Elemental Analysis (%): calcd. for C₇₄H₈₂Au₂O₂₅P₂S₂ C 46.99, H 4.37; found C 47.02, H 4.29.

¹H NMR (CDCl₃, 400 MHz): 8.09-8.13 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ²JH,H = 8.5 Hz, ³JH,P = 2.0 Hz), 7.46-7.64 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH, 8xP-(Ar)C-CH-CH-CH, 5.13–5.18 (4H, m, S-CH-CH-O-Ac, S-CH-CH-O-Ac), 5.09 (2H, t, CH-CH-CH₂-O-Ac, ²JH,H = 9.6 Hz), 5.04 (2H, t, S-CH-CH-O-Ac, ³JH,H = 2.0 Hz), 4.46-4.48 (4H, m, 2x Ar-(C=O)-O-(CH₂)₂-O), 4.21 (2H, dd, CH₂-O-Ac, ²JH,H = 12.3 Hz, ³JH,H = 4.8 Hz), 4.10 (2H, dd, S-CH-CH-O-Ac, ²JH,H = 12.3 Hz, ³JH,H = 2.3 Hz), 3.79-3.81 (4H, m, 2x Ar-(C=O)-O-CH₂-O-Ac), 3.75 (2H, ddd, CH-CH₂-O-Ac, ²JH,H = 9.8 Hz, ³JH,H = 4.8 Hz, ³JH,H = 2.3 Hz), 3.63-3.70 (8H, m, 2x Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂), 2.04 (6H, s, 2xCH₂-O-(C=O)-CH₃), 2.01 (6H, s, 2x(C=O)-CH₃), 1.97 (6H, s, 2x(C=O)-CH₃), 1.90 (6H, s, 2x(C=O)-CH₃).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 83.71 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 170.9 (2C, 2x(C=O)-CH₃), 170.4 (2C, 2x(C=O)-CH₃), 169.8 (2C, 2x(C=O)-CH₃), 169.7 (2C, 2x(C=O)-CH₃), 165.6 (2C, 2x(C=O)-CH₃), 155.5 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ²JCP = 54 Hz), 134.5 (4C, d, 4xP-(Ar)C-CH-CH-CH, ³JCP = 14 Hz), 134.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ²JCP = 2 Hz), 132.1 (4C, m, 4xP-(Ar)C-CH-CH-CH), 130.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ²JCP = 12 Hz), 129.5 (8C, d, 8xP-(Ar)C-CH-CH-CH, ³JCP = 12 Hz), 129.0 (4C, d, 4xP-(Ar)C-CH-CH-CH, ³JCP = 57 Hz), 83.3 (2C, 2xCH₂-O-Ac), 77.8 (2C, S-CH-CH-O-Ac), 75.9 (2C, S-CH-CH-O-Ac), 74.3 (2C, S-CH-CH-O-Ac), 70.80, 70.78 (4C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂, 69.2 (2C, 2x(Ar)-(C=O)-O-(CH₂)₂-O-CH₂, 69.1 (2C, 2xCH₂-O-(C=O)-O-(CH₂)₂-O-CH₂), 64.7 (2C, 2x(Ar)-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 63.0 (2C, 2xCH₂-CH₂-(C=O)-CH₂), 21.3 (2C, 2xCH₂-CH₂-(C=O)-CH₂), 20.86 (2C, 2x(C=O)-CH₃), 20.82 (2C, 2x(C=O)-CH₃), 20.77 (2C, 2x(C=O)-CH₃).

ESI-MS (+): m/z found 1913.3390 [M+Na⁺], 1527.2816 [M-RS⁺], C₇₄H₈₂Au₂Na₂O₂₅P₂S₂⁻ requires 1913.3285, C₆₀H₆₃Au₂O₁₆P₂S⁻ requires 1527.2638.

UV-Vis: λmax = 250 nm.
According to the general procedure, β-D-thioglucose-tetraacetate (0.100 g, 0.274 mmol, 2 equiv.), K₂CO₃ (0.076 g, 0.550 mmol, 4 equiv.), 5c (0.175 g, 0.137 mmol, 1 equiv.) in acetone (25 mL), 48 h at r.t. dark. The product was isolated as a white solid (0.253 g, 0.131 mmol, 96%).

**Elemental Analysis (%)**: calc'd. for C₇₆H₈₆Au₂O₂₆P₂S₂ C 47.16, H 4.48; found C 47.30, H 4.20.

**1H NMR (CDCl₃, 400 MHz)**: 8.09-8.13 (4H, m, 4xO-(C=O)-(Ar)-CH-CH-C-P, 3J_H,H = 8.5 Hz, 4J_H,P = 2.0 Hz), 7.46-7.64 (24H, m, 4xO-(C=O)-(Ar)-CH-CH-C-P, 8xP-(Ar)-CH-CH-CH, 8xP-(Ar)-CH-CH-CH, 4xP-(Ar)-CH-CH-CH), 5.11–5.17 (4H, m, S-CH-CH-O-Ac, S-CH-CH-CH-O-Ac), 5.08 (2H, t, CH-CH-CH₂-O-Ac, 3J_H,H = 9.6 Hz), 5.04 (2H, t, CH₂-O-Ac, 3J_H,H = 12.2 Hz, 4J_H,P = 2.0 Hz), 4.46-4.48 (4H, m, 2x Ar-(C=O)-O-CH₂-CH₂-O), 4.21 (2H, dd, CH₂-O-Ac, 2J_H,H = 12.2 Hz, 3J_H,H = 4.7 Hz), 4.10 (2H, dd, CH₂-O-Ac, 2J_H,H = 12.2 Hz, 3J_H,H = 2.3 Hz), 3.79-3.82 (4H, m, 2x Ar-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 3.66-3.68 (4H, m, 2x Ar-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 3.62 (4H, s, 2x Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-CH₂), 2.04 (6H, s, 2xCH₂-O-(C=O)-CH₃), 2.00 (6H, s, 2x(C=O)-CH₃), 1.96 (6H, s, 2x(C=O)-CH₃).

**31P {1H} NMR (CDCl₃, 162 MHz)**: 38.73 (2P).

**13C {1H} NMR (CDCl₃, 101 MHz)**: 170.8 (2C, 2x(C=O)-O-CH₃), 170.4 (2C, 2x(C=O)-O-CH₃), 169.74 (2C, 2x(C=O)-O-CH₃), 169.67 (2C, 2x(C=O)-O-CH₃), 165.6 (2C, 2xO-(C=O)-(Ar)-CH-CH-C-P), 135.4 (2C, d, 2xO-(C=O)-(Ar)-CH-CH-C-P, 2J_C,P = 54 Hz), 134.5 (4C, d, 4xP-(Ar)-CH-CH-C-P, 2J_C,P = 14 Hz), 134.1 (4C, d, 4xO-(C=O)-(Ar)-CH-CH-C-P, 2J_C,P = 14 Hz), 133.0 (2C, d, 2xO-(C=O)-(Ar)-CH-CH-C-P, 2J_C,P = 14 Hz), 132.1 (4C, d, 4xP-(Ar)-CH-CH-C-P, 2J_C,P = 14 Hz), 129.4 (8C, d, 8xP-(Ar)-CH-CH-C-P, 2J_C,P = 14 Hz), 129.0 (4C, d, 4xP-(Ar)-CH-CH-C-P, 2J_C,P = 14 Hz), 128.6 (4C, d, 4xP-(Ar)-CH-CH-C-P, 2J_C,P = 14 Hz), 128.3 (2C, 2xCH₂-O-Ac), 75.9 (2C, S-CH₂-O-Ac), 74.3 (2C, S-CH₂-O-Ac), 70.72, 70.74 (6C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 64.7 (2C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 63.0 (2C, 2xCH₂-O-Ac), 62.1 (2C, 2xAr-(C=O)-O-(CH₂)₂-O-CH₂-CH₂), 20.84 (2C, 2x(C=O)-CH₃), 20.80 (2C, 2x(C=O)-CH₃), 20.75 (2C, 2x(C=O)-CH₃).


**UV-Vis:** λₘₐₓ = 250 nm.
β-D-Thioglucose-tetraacetate (0.128 g, 0.351 mmol, 2 equiv.), K₂CO₃ (0.053 g, 0.383 mmol, 2.2 equiv.) and 6c (0.232 g, 0.175 mmol, 1 equiv.) in a mixture of H₂O/EtOH/CH₂Cl₂ (30 mL, 1:1:1 (v/v/v)) were stirred 72 h in the dark at r.t. The reaction mixture was concentrated to dryness and the crude was suspended in mixture acetone/CH₂Cl₂ (30 mL, 1:1 (v/v)), the inorganic salts were removed by filtration and the filtrate was concentrated under reduced pressure and further dried under vacuum to afford the product as a white solid (0.323 g, 0.163 mmol, 94 %).

Elemental Analysis (%): calcd. for C₇₈H₉₀Au₂O₂₇P₂S₂ C 47.33, H 4.58; found C 47.43, H 4.55.

¹H NMR (CDCl₃, 400 MHz): 8.10-8.13 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_H,H = 8.4 Hz, ⁴J_H,P = 2.0 Hz), 7.45-7.63 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ⁸J_H,P = 51.1–51.17 (4H, m, S-CH-CH-O-Ac, S-CH-CH-O-Ac), 5.08 (2H, t, C H-CH-CH₂-O-Ac, ³J_H,H = 9.1 Hz), 4.46-4.49 (4H, m, 2x Ar(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂), 3.76 (2H, ddd, C H₂-O-Ac, ³J_H,H = 9.8 Hz, ³J_H,H = 4.8 Hz), 3.66-3.68 (4H, m, 2x Ar(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂), 3.60-3.64 (4H, m, 2x Ar(C=O)-O-(CH₂)₂-O-(CH₂)₂-O-(CH₂)₂), 2.04 (6H, s, 2xCH₂-O-(C=O)-CH₂), 2.00 (6H, s, 2x(C=O)-CH₂), 1.96 (6H, s, 2x(C=O)-CH₂), 1.89 (6H, s, 2x(C=O)-CH₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 38.78 (2P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 170.8 (2C, 2x(C=O)-CH₂), 170.4 (2C, 2x(C=O)-CH₂), 169.7 (2C, 2x(C=O)-CH₂), 163.6 (2C, 2x(C=O)-CH₂), 162.6 (2C, 2x(C=O)-CH₂), 135.5 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 53 Hz), 134.5 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 133.0 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 132.1 (4C, m, 4xP-(Ar)C-CH-CH-C-P), 130.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 129.0 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 129.0 (4C, d, 4xP-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 128.4 (3C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 127.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 126.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 127.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 127.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 127.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 127.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 127.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz), 127.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, ³J_C,P = 14 Hz).

**UV-Vis**: \( \lambda_{\text{max}} = 250 \text{ nm} \).

**Compound 7d**

\( \beta \)-D-Thioglucose-tetraacetate (0.130 g, 0.357 mmol, 2 equiv.), K2CO3 (0.054 g, 0.391 mmol, 2.2 equiv.), 7c (0.252 g, 0.178 mmol, 1 equiv.) in a mixture H2O/EtOH/CH2Cl2 (30 mL, 1:1:1 (v/v/v)) were stirred 72 h in the dark at r.t.. the reaction mixture was concentrated to dryness and the crude was suspended in mixture acetone/CH2Cl2 (30 mL, 1:1 (v/v)), the inorganic salts were removed by filtration and the filtrate was concentrated under reduced pressure and further dried under vacuum to afford the product as a white solid (0.317 g, 0.153 mmol, 86 %).

**Elemental Analysis (%):** calcd. for C82H98Au2O29P2S2 C 47.63, H 4.78; found C 47.85, H 4.60.

**1H NMR (CDCl3, 400 MHz):** 8.10-8.13 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, \( \text{J}_{HH} = 8.4 \text{ Hz}, \text{J}_{HP} = 2.0 \text{ Hz} \)), 7.47-7.64 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 5.11–5.18 (4H, m, S-CH-CH-O-Ac, S-CH-CH-CH-O-Ac), 5.09 (2H, t, CH2-O-Ac, \( \text{J}_{HH} = 9.6 \text{ Hz} \)), 5.04 (2H, t, CH2-O-Ac, \( \text{J}_{HH} = 9.1 \text{ Hz} \)), 12.2 Hz, \( \text{J}_{HH} = 8.4 \text{ Hz} \)), 4.10 (2H, dd, CH2-O-Ac, \( \text{J}_{HH} = 9.1 \text{ Hz} \)), 4.10 (2H, dd, CH2-O-Ac, \( \text{J}_{HH} = 9.6 \text{ Hz} \)), 3.80-3.83 (4H, m, 2x Ar-(C=O)-O-CH2-CH2-O), 3.76 (2H, ddd, CH2-O-Ac, \( \text{J}_{HH} = 12.2 \text{ Hz} \), \( \text{J}_{HH} = 2.4 \text{ Hz} \)), 3.66-3.69 (4H, m, 2x Ar-(C=O)-O-CH2-CH2-O), 3.62-3.65 (8H, m, 2x Ar-(C=O)-O-CH2-CH2-O), 3.59-3.62 (8H, m, 2x Ar-(C=O)-O-CH2-CH2-O), 2.04 (6H, s, 2x CH2-O-(C=O)-CH2), 2.00 (6H, s, 2x CH2-O-(C=O)-CH2), 1.97 (6H, s, 2x CH2-O-(C=O)-CH2).

**31P {1H} NMR (CDCl3, 162 MHz):** 38.71 (2P).

**13C {1H} NMR (CDCl3, 101 MHz):** 170.8 (2C, 2x(C=O)-CH3), 170.4 (2C, 2x(C=O)-CH3), 165.6 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 135.4 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \( \text{J}_{CP} = 53 \text{ Hz} \)), 134.5 (4C, d, 4xP-(Ar)C-CH-CH-CH, \( \text{J}_{CP} = 14 \text{ Hz} \)), 134.4 (4C, d, 4xP-(Ar)C-CH-CH-CH, \( \text{J}_{CP} = 14 \text{ Hz} \)), 134.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, \( \text{J}_{CP} = 14 \text{ Hz} \)), 133.0 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \( \text{J}_{CP} = 2 \text{ Hz} \)), 132.1 (4C, m, 4xP-(Ar)C-CH-CH-CH), 130.1 (4C, d, 4xO-(C=O)-(Ar)C-CH-CH-C-P, \( \text{J}_{CP} = 12 \text{ Hz} \)), 129.4 (8C, d, 8xP-(Ar)C-CH-CH-CH, \( \text{J}_{CP} = 57 \text{ Hz} \)), 83.2 (2C, 2x CH2-O-Ac), 77.9 (2C, S-CH-CH-CH-O-Ac), 75.8 (2C, S-CH-CH-O-Ac), 74.2 (2C, S-CH-CH-O-Ac), 70.70, 70.68, 70.61 (8C,
2x(Ar)-(C=O)-O-(CH2)-O-CH2, 2x(Ar)-(C=O)-O-(CH2)-O-CH2-OCH3, 2x((Ar)-(C=O)-O-(CH2)-O-CH2-OCH3, 69.2 (2C, 2x(Ar)-(C=O)-O-(CH2)-O-CH2-OCH3), 69.0 (2C, 2xCH-CH-CH-O-Ac), 64.6 (2C, 2x(Ar)-(C=O)-O-CH2-CH2), 62.9 (2C, 2xCH-CH2-(C=O)-CH3), 21.2 (2C, 2xCH2-O-(C=O)-CH3), 20.82 (2C, 2x(C=O)-CH3), 20.78 (2C, 2x(C=O)-CH3), 20.73 (2C, 2x(C=O)-CH3).


UV-Vis: λmax = 250 nm.

Compound 8d

According to the general procedure, β-D-thioglucose tetraacetate (0.258 g, 0.708 mmol, 1 equiv.), K2CO3 (0.196 g, 1.418 mmol, 2 equiv.), 8c (0.350 g, 0.707 mmol, 1 equiv.) in acetone (25 mL), 24 h at r.t. dark. The product was isolated as a white solid (0.548 g, 0.666 mmol, 89%).

Elemental Analysis (%): calcd. for C32H34AuO9PS C 46.72, H 4.17, found C 46.97, H 3.85.

1H NMR (CDCl3, 400 MHz): 7.42-7.59 (15H, m, 6xP-(Ar)C-CH-CH-CH, 6xP-(Ar)C-CH-CH-CH, 3xP-(Ar)C-CH-CH-CH), 5.12–5.17 (2H, m, S-CH-CH-O-Ac, S-CH-CH-O-Ac), 5.08 (1H, t, CH-CH-CH2-O-Ac, JHH = 9.5 Hz), 5.03 (1H, t, S-CH-CH-O-Ac, JHH = 9.3 Hz), 4.19 (1H, dd, CH2-O-Ac, JHH = 12.2 Hz, JHH = 4.8 Hz), 4.09 (1H, dd, CH2-O-Ac, JHH = 12.2 Hz, JHH = 2.4 Hz), 3.74 (1H, ddd, CH-CH2-O-Ac, JHH = 9.7 Hz, JHH = 4.8 Hz, JHH = 2.4 Hz), 2.02 (3H, s, CH2-O-(C=O)-CH3), 1.99 (3H, s, (C=O)-CH3), 1.95 (3H, s, (C=O)-CH3), 1.87 (3H, s, (C=O)-CH3).

31P {1H} NMR (CDCl3, 162 MHz): 38.83 (1P).

13C {1H} NMR (CDCl3, 101 MHz): 170.9 (1C, (C=O)-CH3), 170.4 (1C, (C=O)-CH3), 169.7 (1C, (C=O)-CH3), 134.4 (6C, d, 6xP-(Ar)C-CH-CH-CH, JCP = 14 Hz), 131.7 (3C, d, 3xP-(Ar)C-CH-CH-CH, JCP = 2 Hz), 129.8 (3C, d, 3xP-(Ar)C-CH-CH-CH, JCP = 57 Hz), 129.3 (6C, d, 6xP-(Ar)C-CH-CH-CH, JCP = 11 Hz), 83.2 (1C, CH-CH3-O-Ac), 77.8 (1C, CH2-CH3-O-Ac), 75.8 (1C, CH-CH3-O-Ac), 74.3 (1C, CH-CH3-O-Ac), 69.1 (1C, CH2-CH3-(C=O)-CH3), 63.0 (1C, CH-CH3-(C=O)-CH3), 21.2 (1C, CH2-CH3-(C=O)-CH3), 20.80 (2C, 2x(C=O)-CH3), 20.75 (1C, (C=O)-CH3).

According to the general procedure, β-D-thioglucose tetraacetate (0.304 g, 0.834 mmol, 1 equiv.), K₂CO₃ (0.231 g, 1.671 mmol, 2 equiv.), 9c (0.450 g, 0.835 mmol, 1 equiv.) in acetone (35 mL), 48 h at r.t. dark. The product was isolated as a white solid (0.687 g, 0.793 mmol, 95%).

**Elemental Analysis (%)**: calcd. for C₃₃H₃₄AuO₁₁PS·CDCl₃ C 40.56 H, 3.70, found C 39.96, H 3.57, CDCl₃ originates from NMR solvent.

**¹H NMR (CDCl₃, 400 MHz)**: 7.81-7.90 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.30-7.48 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH-H), 5.06-5.13 (2H, m, S-CH-CH-O-Ac, S-CH-CH-O-Ac), 4.99 (1H, t, CH-CH-CH₂-O-Ac, J₃H,H = 9.2 Hz), 4.97 (1H, t, S-CH-CH-O-Ac, J₃H,H = 9.3 Hz), 4.07 (1H, dd, CH₂-O-Ac, J₃H,H = 12.1 Hz, J₂H,H = 5.1 Hz), 4.02 (1H, d, CH₂-O-Ac, J₃H,H = 12.1 Hz), 3.68 (1H, m, CH₂-O-Ac), 2.16 (6H, s, 2xCH₂-O-(C=O)-CH₃), 2.00 (3H, s, (C=O)-CH₃), 1.91 (3H, s, (C=O)-CH₃), 1.90 (3H, s, (C=O)-CH₃).

**³¹P {¹H} NMR (CDCl₃, 162 MHz)**: 38.82 (1P).

**¹³C {¹H} NMR (CDCl₃, 101 MHz)**: 171.8 (1C, (C=O)-CH₃), 171.4 (1C, (C=O)-CH₃), 170.2 (1C, (C=O)-CH₃), 170.1 (1C, (C=O)-CH₃), 169.8 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 141.7 (1C, m, O-(C=O)-(Ar)C-CH-CH-C-P), 134.3 (2C, d, 2xP-(Ar)C-CH-CH-CH-CH, J₃C,P = 15 Hz), 134.2 (2C, d, 2xP-(Ar)C-CH-CH-CH-CH, J₃C,P = 14 Hz), 133.7 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, J₃C,P = 15 Hz), 131.9 (3C, m, O-(C=O)-(Ar)C-CH-CH-CH-C-P, J₃C,P = 15 Hz), 129.9 (2C, m, 2xP-(Ar)C-CH-CH-CH), 129.7 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-CH, J₃C,P = 11 Hz), 129.4 (2C, d, 2xP-(Ar)C-CH-CH-CH, J₃C,P = 11 Hz), 129.3 (2C, d, 2xP-(Ar)C-CH-CH-CH, J₃C,P = 11 Hz), 83.2 (1C, CH₂-O-Ac), 77.5 (1C, S-CH-CH-O-Ac), 75.6 (1C, S-CH-CH-O-Ac), 74.1 (1C, S-CH-CH-O-Ac), 69.3 (1C, S-CH-CH-O-Ac), 63.3 (1C, S-CH-CH-O-Ac), 21.2 (1C, CH₂-O-(C=O)-CH₃), 20.8 (2C, 2x(C=O)-CH₃), 20.7 (1C, (C=O)-CH₃).

2.4.4 Stability Studies.

The stability of complexes 1b-9b and 1d-9d in DMSO-$\delta$ was assessed via $^1$H (400 MHz) and $^{31}$P (162 MHz) NMR at 298K for 20 min. The stability of complexes 2d, 4d and 6d in pseudo-cell culture conditions was assessed in aqueous 100 mM NaCl and 5% DMSO for 72 h at 298 K and monitored via ESI(+) mass spectrometry.

2.4.5 Cell culture and Cytotoxicity Studies.

The human ovarian carcinoma (A2780 and A2780cisR) cell lines were obtained from the European Collection of Cell Cultures (ECACC). The human embryonic kidney (HEK-293) cell line was obtained from ATCC (Sigma, Switzerland). Penicillin streptomycin, RPMI-1640 GlutaMAX and DMEM GlutaMAX media were obtained from Life Technologies and fetal bovine serum (FBS) was obtained from Sigma. The cells were cultured in RPMI 1640 GlutaMAX (A2780 and A2780cisR) and DMEM GlutaMAX (HEK-293) medium containing 10% heat-inactivated fetal bovine serum and 1 % penicillin-streptomycin at 37 °C and CO$_2$ (5%). The A2780cisR cell line were routinely treated with cisplatin (2 μM) in the media. Cytotoxicity was determined using the MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). Cells were seeded in flat bottomed 96-well plates as a suspension in prepared medium (100 μL aliquots and approximately 4300 cells per well) and pre-incubated for 24 h. Stock solutions of compounds were prepared in DMSO and were rapidly diluted in medium. The solutions were sequentially diluted to give a final DMSO concentration of 0.5% and final compound concentration range (0 μM to 500 μM). Cisplatin was tested as a positive control (0 μM to 100 μM). The compounds were added to the pre-incubated 96-well plates in 100 μL aliquots and the plates were incubated for 72 h. MTT (20μL, 5 mg/mL in Dulbecco’s Phosphate Buffered Saline, DPBS) was added to the cells and the plates were incubated for a further 4 h. The culture medium was aspirated, and the purple formazan crystals, formed by the mitochondrial dehydrogenase activity of vital cells, were dissolved in DMSO (100 μL per well). The absorbance of the resulting solutions, directly proportional to the number of surviving cells, was quantified at 590 nm using a SpectroMax.
M5e multimode microplate reader (using SoftMax Pro software, version 6.2.2). The percentage of surviving cells was calculated from the absorbance of wells corresponding to the untreated control cells. The reported IC_{50} values are based on means from three independent experiments, each comprising four tests per concentration level.
2.5 References


Chapter 2


Chapter 2


The work described was completed in collaboration with Daniel Ortiz who performed the mass spectrometry binding studies.
3.1 Introduction

Understanding and controlling the targets of metal-based drugs remains of great importance in the development of selective drugs. Ruthenium-based drugs possess a plethora of targets ranging from proteins to DNA, and yet directing these complexes towards a desirable target remains challenging. A degree of control can be established in ruthenium(II)-arene complexes by exchanging the bidentate ethylenediamine ligand of [(η⁶-p-cymene)Ru(en)]PF₆ (where en = ethylene diamine) (RAED-C) for the compact, water soluble PTA ligand of [(η⁶-p-cymene)RuCl₂(PTA)] (where PTA = 1,3,5-triaza-7-phosphaadamantane) (RAPTA-C, Figure 3.1), which directs the complex preferentially towards protein binding sites over those of DNA. Furthermore, DNA binding of RAED-C can be enhanced by substituting the p-cymene arene with the more hydrophobic 5,8,9,10-tetrahydroanthracene (THA) resulting in the intercalation of DNA and bimodal binding on naked DNA. On the other hand, RAPTA-C and [Ru(η⁶-toluene)(PTA)Cl₂] (RAPTA-T), which differ only by an iso-propyl group, form specific and identical adducts on histone H2A and H2B histone dimers of the nucleosome core. Notably, a series of binuclear ruthenium(II)-arene complexes are able to crosslink these binding sites inducing a state of irreversible condensed chromatin, resulting in apoptosis. The binding of RAPTA-T at these sites, consisting of two glutamic acid residues (RU1) and a glutamic acid and histidine residue (RU2), causes a series of structural changes in the nucleosome core that induce a kink in the long α-helix of the H2A histone protein. This structural alteration opens up a binding site for auranofin, a gold(I) drug of the structure (1-thio-β-D-glucopyranose-2,3,4,6-tetraacetato-S)(triethylphosphine)gold(I) (Figure 3.1), approved for the treatment of rheumatoid arthritis, that is inaccessible prior to the binding of the RAPTA-T. A synergy between the two drugs was discovered where RAPTA-T appears to sensitise the cells to auranofin resulting in a beneficial increase in tumour cell cytotoxicity and a 3 fold increase in auranofin chromatin adducts.

Heterobimetallic complexes have emerged as a promising family of complexes that can combine the attributes and targets of two metals within one structure. As well as the capacity to possess markedly higher activities than the parent drugs alone, heterometallic drugs have a myriad of potential applications. Numerous heterometallic complexes possessing photophysical properties have been considered for cellular imaging, as trackable probes and drug carriers for cytotoxic complexes. Ferrocenyl and titanocene
complexes are particularly versatile building blocks for heterometallic complexes due to their facile functionalization and favourable redox properties.\textsuperscript{[27–34]}

However, heterometallic complexes combining cisplatin-, RAPTA- and auranofin-type drugs have been scarcely explored. Ruthenium-gold and ruthenium-platinum species have been shown to possess cytotoxicities comparable to cisplatin against HeLa cells.\textsuperscript{[35]} A platinum(II)-gold(I) complex was encapsulated within a ferritin cage with the aim of enhancing its selectivity, however, the complex was unstable with the gold(I) complex binding to the protein and platinum remaining in the bulk, decreasing the efficacy of the agent.\textsuperscript{[36]} A ruthenium(II)-platinum(IV) prodrug possessing high cytotoxicity against cisplatin resistant cells was also able to inhibit cell migration.\textsuperscript{[37]}

The combination of RAPTA complexes, which possess a general low toxicity and antimetastatic properties,\textsuperscript{[38]} and auranofin, which is highly cytotoxic with anti-inflammatory properties,\textsuperscript{[39]} offers great potential. Few heterometallic complexes based on ruthenium(II)-arene compounds and auranofin have been reported. $[(\eta^6-p$-cymene)$_2$RuCl$_2$($\mu$-dppm)]Au(IMes)ClO$_4$ (RANCE-1, Figure 3.1, where dppm = diphenylphosphanylmethyl(diphenyl) phosphane and IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene), is a promising heterobimetallic complex exhibiting efficient inhibition of thioredoxin reductase (TrX), vascular endothelial growth factor (VEGF), pan-matrix metalloproteinases (pan-MPP) and pan-cathepsin. When compared to

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.1}
\caption{Parent drugs, RAPTA-C and auranofin, and selected heterobimetallic complexes.}
\end{figure}
auranofin, RANCE-1 presents similar antiproliferative activity against renal cancer cell line (Caki-1) and improved inhibition of VEGF, pan-MMP and pan-cathepsin.\textsuperscript{[40]} Replacing the auranofin-like thiolato-β-D-glucose tetraacetate ligand with a chloride ligand results in a three-fold decrease in cytotoxicity against human ovarian carcinoma (A2780) cells.\textsuperscript{[41]} Other examples include \([[(\eta^5-p\text{-cymene})\text{RuCl}_2(\mu\text{-dppm})\text{AuCl}]] (1, \text{Figure 3.1})\) and \([[(\eta^5-p\text{-cymene})\text{RuCl}_2(\mu\text{-dppm})\text{Au(S-thiazoline)}]] (2, \text{Figure 3.1}),\) which shows that differing the sacrificial ligand coordinated to the gold centre does not impact the activity in this case.\textsuperscript{[42]} However, introducing N-heterocyclic carbene ligands to the gold centre in cationic ruthenium(II)-gold(I) complexes of the same structure can enhance tumour cell selectivity.\textsuperscript{[43]}

Herein, we describe the synthesis, cytotoxicity and target binding studies of a series of heterobimetallic complexes containing RAPTA-C- and auranofin-like fragments. The design of the complexes aims to preserve the structure of the parent drugs, RAPTA-C and auranofin, as closely as possible whilst allowing flexibility to enable binding at two different and distal sites. A series of linker lengths was explored to determine the impact of the linker length on the cytotoxicity of the complexes. The ability of the complexes to bind to histidine residues was explored via mass spectrometry using both single amino acids and a fragment of the amyloid β protein.

3.2 Results and Discussion

3.2.1 Synthesis and characterisation

With the aim of targeting the binding sites of RAPTA-C and auranofin, maintaining the key structural features of the parent drugs is important. However, alterations are required in order to tether the complexes via a flexible linker with the PTA and triethylphosphine ligands, belonging to RAPTA-C and auranofin, respectively, being replaced by 4-
(diphenylphosph phosphino)benzoic acid ligands, which provide a functionalizable carboxylic acid moiety and air stability. The labile thio-β-D-glucose-2,3,4,6-tetraacetate ligand of auranofin is replaced with a labile chloride ligand. The ρ-cymene arene and the two labile chloride ligands present in RAPTA-C were maintained due to the hydrophobic interactions provided by the arene during binding and the vital role of the chlorides in the activation of the complex via aquation. Polyethylene glycol was selected as a suitable linker due to its flexibility and its higher water solubility compared to alkyl chains.

As a 4-(diphenylphosph phosphino)benzoic acid ligand is coordinated to both the ruthenium(II) and the gold(I) centres, care must be taken to achieve high selectivity in the coupling step. Manipulation of reaction stoichiometry was insufficient to control the monocoordination of either ruthenium or gold to a bis-phosphine ligand. Therefore, monophosphine ligands (1a-1d) were prepared via the esterification reaction between 1 equivalent of 4-(diphenylphosph phosphino)benzoic acid and 1.5 equivalents of the appropriate polyethylene glycol chain using N-ethyl-N’-(3-dimethylan ropyl)carbodiimide hydrochloride (EDCI) as coupling reagent and 4-(dimethylamino)pyridine (DMAP) as base catalyst (Scheme 3.1). Ligands 1a-1d (Scheme 3.1) were coordinated to the gold via a freshly prepared gold(I)-tetrahydrothiophene intermediate to yield gold(I) complexes 2a-2d in near-quantitative yields. The stability of the gold(I)-phosphine complexes allows further reactions
to take place without effecting the integrity of the complex. The second 4-(diphenylphosphosphino)benzoic acid was introduced to the gold complex using identical coupling conditions to those employed in the first synthetic step, resulting in cationic cyclic gold complexes 3a-3d. The final step introduces the ruthenium(II) centre via the coordination of one of the phosphine ligands previously interacting with the gold(I) centre.

All compounds were characterised by $^1$H, $^{31}$P and $^{13}$C NMR spectroscopy, high-resolution mass spectrometry and elemental analysis. The coordination of the ligands to the metal centres was monitored by $^{31}$P NMR spectroscopy. The phosphine ligand coordinated to the ruthenium centre produces a characteristic singlet at ca. 25 ppm, whereas the gold-phosphine peak is observed at ca. 33 ppm (cf. ca. -5 ppm for the free ligand), allowing the reactions to be easily monitored. The introduction of a second 4-(diphenylphosphosphino)benzoic acid to 2a-2d results in changes of differing magnitudes in the $^{31}$P NMR spectra, depending on the number of PEG units the complex possesses. The resulting cyclic gold(I) complexes 3a-3d present a single broad peak at 29.53 (3a), 31.78 (3b), 28.20 (3c) and 31.09 (3d) ppm. Upon the introduction of the ruthenium(II) centre, 3a-3d de-cyclise and two peaks are observed in the $^{31}$P NMR spectra, as mentioned above at ca. 25 and 33 ppm for the ruthenium(II) and gold(II) coordinated phosphine ligands, respectively (Figure 3.2).

![Figure 3.2. $^{31}$P NMR spectra (162 MHz, CDCl₃) of the cyclic gold complex 3b (top) and heterobimetallic complex 4b (bottom).]
The $^1$H NMR spectra of the target complexes 4a-4b confirmed their formation with the appearance of the distinctive $\pi$-cymene peaks including doublets at 5.20-5.22 ppm and 4.97-4.99 ppm, septet at 2.80-2.88 ppm, singlet at 1.85-1.86 ppm and a doublet at 1.09-1.12 ppm. The coordination of a phosphine ligand to the ruthenium was observed via a downfield shift of the $(Ar)$C-CH-C=CH-C-P-Ru phenyl protons from 7.28-7.46 ppm (3a-3d) to 7.77-7.83 ppm (4a-4d) as well as the O-(C=O)-(Ar)C-CH-C=P-Ru protons from 7.28-7.46 ppm (3a-3d) to 7.89-7.95 ppm (4a-4d) (Figure 3.3).

![Figure 3.3. $^1$H NMR (400 MHz, CDCl$_3$) spectra of cyclic gold complex 3b (top) and heterobimetallic complex 4b (bottom). Notable resonances are identified with coloured circles: phenyl protons (red), $\eta^1$-arene protons (blue), PEG protons (green).](image)

3.2.2 Evaluation of in vitro antiproliferative activity

The antiproliferative activity of 4a-4d was assessed using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay against cisplatin sensitive and resistant human ovarian carcinoma (A2780 and A2780cisR) and non-tumoural human embryonic kidney (HEK-293) cell lines (Table 3.1).

Cisplatin, auranofin and RAPTA-C were tested as controls.
Table 3.1. *In vitro* antiproliferative activity of 4a-4d, cisplatin, auranofin and RAPTA-C against human ovarian carcinoma (A2780), human ovarian carcinoma cisplatin resistant (A2780cisR) and human embryonic kidney 293 (HEK-293) cell lines after 72 h exposure. Values are given as the mean ± SD (μM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>PEG Units</th>
<th>A2780</th>
<th>A2780CisR</th>
<th>HEK293</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>4</td>
<td>2.5 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>4b</td>
<td>5</td>
<td>2.4 ± 0.5</td>
<td>3.1 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>4c</td>
<td>6</td>
<td>2.4 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>4d</td>
<td>8</td>
<td>1.8 ± 0.6</td>
<td>3.9 ± 0.5</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>cisplatin</td>
<td>-</td>
<td>1.9 ± 0.4</td>
<td>13.3 ± 1.2</td>
<td>9 ± 0.8</td>
</tr>
<tr>
<td>auranofin</td>
<td>-</td>
<td>1.3 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>RAPTA-C</td>
<td>-</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

The cytotoxicity of the parent complex, RAPTA-C, is low against a range of cell lines, with an IC₅₀ > 200 μM against the tested cell lines. Compounds 4a-4d possess IC₅₀ values in the low micromolar range against all tested cell lines, with values comparable to cisplatin and auranofin against the A2780 cell line and to auranofin against the A2780CisR cell line. Although the compounds overcome cisplatin resistance, they do not show selectivity towards the tumoural cell lines compared to the non-tumoural cell lines. Moreover, the length of the PEG linker has negligible impact on the cytotoxicity of the complexes, showing that the cytotoxicity of these complexes is independent of linker length, as observed with homobimetallic ruthenium(II) and gold(I) complexes.⁴⁵

Compounds 4a-4d are considerably more cytotoxic towards the A2780 cell line compared to [(1-methyl-3-(4-((4'-methyl-2,2'-bipyridin-4-yl)methylcarbamoyl)benzyl)imidazole-2-ylidine gold(I) chloride][(n⁶-p-cymene)ruthenium(II) chloride] hexafluorophosphate and [(1-methyl-3-(4-((4'-methyl-2,2'-bipyridin-4-yl)methylcarbamoyl)benzyl)imidazole-2-ylidine gold(I) (thiolato-β-d-glucose tetraacetate)][(n⁶-p-cymene)ruthenium(II) chloride] hexafluorophosphate, which possess IC₅₀ values of 63.4 ± 2.4 μM and 16.8 ± 3.1 μM respectively.⁴¹ [(n⁶-p-cymene)RuCl(μ-dppm)Au(NHC)]ClO₄ (where NHC = N-heterocyclic carbene) complexes possess cytotoxicity greater than cisplatin against human renal (Caki-1) cells with IC₅₀ values in the low micromolar range. They show good selectivity towards non-tumoural HEK-293.
cells compared to 4a-4d with IC₅₀ values of ≥73 μM. The bimetallic compounds [(η⁶-ρ-cymene)RuCl₂(μ-dppm)AuCl] (1, Figure 3.1) and [(η⁶-ρ-cymene)RuCl₂(μ-dppm)Au(S-thiazoline)] (2, Figure 3.1) show comparable cytotoxicity towards human colon (HCT-116) cells to the [Ru(ρ-cymene)Cl₂(μ-dppm)Au(NHC)]ClO₄ complexes with IC₅₀ values of 4.6 ± 0.1 μM (1) and 6.5 ± 0.1 μM (2) versus 8-10 μM for the cationic NHC complexes.[42] RANCE-1 (Figure 3.1) also presents comparable cytotoxicity to the cationic NHC complexes with an IC₅₀ of 8.7 ± 0.9 μM against Caki-1 cells which is around three-fold more cytotoxic than cisplatin but 3 fold less cytotoxic than auranofin against the same cell line.[46]

3.2.3 Amino acid and peptide binding studies

The ability of the heterobimetallic complexes to bind to the amino acid residues present in the RU1, RU2, AU1 and AU1' binding sites in the nucleosome core particle was assessed using amino acids and a model peptide. In crystallographic studies on the nucleosome core particle, auranofin binds to histidine residues whilst RAPTA-T binds to both histidine and glutamine residues.[11] Complex 4b was incubated with L-Histidine for 2 h in a 1:1 complex-amino acid ratio in unbuffered solution (98 % milliQ water, 2 % DMSO) at 310 K and the adducts analysed by mass spectrometry.[46–48] ESI-MS revealed a peak at m/z 894.6949 corresponding to the adduct [4b -3Cl + 3His +2K]⁺, in which dissociation of the three labile chloride ligands and subsequent binding of three histidine residues indicates that both the ruthenium and gold centres bind to histidines.

Peptide binding studies were performed on a fragment of the amyloid β protein (residues 1-16, H-Asp¹-Ala²-Glu³-Phe⁴-Arg⁵-His⁶-Asp⁷-Ser⁸-Gly⁹-Tyr¹⁰-Glu¹¹-Val¹²-His¹³-His¹⁴-Gln¹⁵-Lys¹⁶-OH). Complex 4a was incubated with the 16-mer for 2 h in a 1:3 complex-peptide ratio in unbuffered solution (98% milliQ, 2% DMSO) at 310 K. A 1:3 complex-peptide ratio was required to suppress the facile ionisation of the gold centre that suppresses the signal of peptide complex adducts. ESI-MS revealed 1:1 adducts of 4a and the 1-16 amyloid β peptide; 2:1 complex-peptides adducts were not observed (Figure 3.4). The loss of the three labile chloride ligands in the complex indicates that both the ruthenium and gold centres are
coordinated to at least one amino acid residue crosslinking the peptide. In order to obtain further information on the mode of binding in the 1:1 adduct observed, the \([\text{peptide} + 4a + 5H – 3Cl]\)^{5+} ion (Theoretical m/z 632.0238; Observed m/z 632.0258; -3.21 ppm) was chosen for fragmentation due to its high charge state and intact 4a adduct. Collision induced dissociation (CID), producing predominantly b- and y-type fragments, and electron-transfer dissociation (ETD) fragmentation, which breaks N-C\(\alpha\) bonds along the peptide backbone producing c- and z-type fragments, were performed. ETD fragmentation has recently been used to evaluate the binding of dinuclear ruthenium(II)-arene complexes on the amyloid \(\beta\) peptide where the metal centres were found to bind to histidine residues. The analysis of the fragments produced was performed using an online Apm2s application (available on ms.cheminfo.org), which enabled the identification both terminal and internal fragments that are otherwise difficult to identify manually.

![Figure 3.4. ESI-MS spectrum of 4a incubated with 1-16 amyloid \(\beta\)-peptide in a 1:3 complex-peptide ratio at 310 K for 2 h (Peaks of interest are labelled).](image)

As both the ruthenium and gold centres can bind to histidine, it is likely that the \([\text{peptide} + 4a + 5H – 3Cl]\)^{5+} ion represents a mixture of adducts in which the metals interact with His\(\text{6}\), His\(\text{13}\) and His\(\text{14}\) in different combinations. The unmetallated peptide fragments produced by the CID and ETD fragmentation processes reveal an interesting pattern (Figure 3.5). The unmetallated CID fragments b\(_s\)-b\(_{15}\) and y\(_{1}\)-y\(_{15}\) were observed, whereas the smaller fragments, y\(_{1}\)-y\(_{3}\) and b\(_{1}\)-b\(_{5}\), were not present. The smallest fragments, b\(_s\) and y\(_s\), consist of residues H-Asp\(^1\)-Ala\(^2\)-Glu\(^3\)-Phe\(^4\)-Arg\(^5\)-His\(^6\) and His\(^13\)-His\(^{14}\)-Gln\(^{15}\)-Lys\(^{16}\)-OH, respectively. In both directions,
the fragmentation process is interrupted at a histidine residue, His\textsuperscript{6} for b fragments and His\textsuperscript{13} for y fragments, suggesting that there is obstruction, most likely a bound metal centre, which interrupts fragmentation. The ETD fragmentation reveals an identical pattern where the unmetallated peptide fragments, c\textsubscript{6}, c\textsubscript{7}, c\textsubscript{9-13}, c\textsubscript{15} and z\textsubscript{4-z14} fragments, were observed (Figure 3.5). The smallest fragments observed, c\textsubscript{6} and z\textsubscript{4}, also consist of H-Asp\textsuperscript{1}-Ala\textsuperscript{2}-Glu\textsuperscript{3}-Phe\textsuperscript{4}-Arg\textsuperscript{5}-His\textsuperscript{6} and His\textsuperscript{13}-His\textsuperscript{14}-Gln\textsuperscript{15}-Lys\textsuperscript{16}-OH, respectively. The similarity between the CID and ETD fragmentation patterns suggest that the same obstruction at His\textsuperscript{6} and His\textsuperscript{13} impedes both fragmentation processes.

![Figure 3.5. Fragmentation of the [peptide + 4a + 5H -3Cl]\textsuperscript{5+} ion (m/z 632.0238): unmetallated CID, b (blue) and y (purple), and ETD, c (red) and z (green), fragments.](image)

Interestingly, the series of ETD peptide fragments, containing bound 4a (Figure 3.6), mirror the unmetallated ETD fragments (Figure 3.5). The fragments c\textsubscript{6-13} and z\textsubscript{5-15}, containing the complete [4a – 3Cl] adduct, are observed. The smallest fragments, c\textsubscript{6} and z\textsubscript{5},
consisting of H-Asp\(^1\)-Ala\(^2\)-Glu\(^3\)-Phe\(^4\)-Arg\(^5\)-His\(^6\) and His\(^{13}\)-His\(^{14}\)-Gln\(^{15}\)-Lys\(^{16}\)-OH residues, respectively, are identical to the non-metallated fragments. As it is plausible that both the gold and the ruthenium centres could bind to any of the His\(^6\), His\(^{13}\) and His\(^{14}\) sites, a ruthenium or gold centre bound to the His\(^6\) and His\(^{13}\) residues could be impeding further fragmentation of the peptide-4\(_a\) adduct. Metallated b- and y- type fragments containing [4\(_a\) – 3Cl] are also observed in the CID spectrum (Figure 3.6), however, the smallest fragments found are b\(_{11}\)\(^*\) and y\(_{12}\)\(^*\) and do not yield much information.

However, the observed unmetallated internal fragments only include one, if any, histidine residues. Fragments c\(_{13}\)Z\(_8\), c\(_{13}\)Z\(_7\) and c\(_{13}\)Z\(_6\) include the His\(^{13}\) residue and the c\(_7\)Z\(_{13}\), c\(_7\)Z\(_{14}\), c\(_7\)Z\(_{15}\), c\(_9\)Z\(_{12}\), c\(_9\)Z\(_{14}\), c\(_{10}\)Z\(_{15}\) and fragments include the His\(^{6}\) residue. The other unmetallated fragments observed internal fragments, c\(_{1}\)Z\(_{1}\) and c\(_{1}\)Z\(_{6}\), do not contain any histidines residues. This suggests that the histidine residues that are not included in the fragments could be bound to 4\(_a\).
In contrast, all the observed metallated CID and ETD internal fragments containing the $[4\text{a} - 3\text{Cl}]$ adducts contain at least one histidine residue (Figure 3.7). The CID internal fragments $b_y15^*$ and $b_y12^*$, contain Ala$^1$-Glu$^2$-Phe$^4$-Arg$^5$-His$^6$ and Arg$^5$-His$^6$-Asp$^7$ residues, indicating that either the gold or ruthenium centres are bound to the His$^6$ residue. On the other hand, the metallated internal ETD fragments include all three histidine residues in different combinations. Fragments $c_{11}Z_{15}^*$, $c_{11}Z_{13}^*$, $c_{10}Z_{14}^*$ and $c_{12}Z_{15}^*$ contain the His$^6$ residue, $c_{11}Z_{15}^*$ contains the His$^{13}$ and $c_{12}Z_{15}^*$ contains both His$^{13}$ and His$^{14}$. Fragments $c_{13}Z_{14}^*$, $c_{13}Z_{11}^*$, and $c_{14}Z_{15}^*$ contain all three histidine residues, His$^6$, His$^{13}$ and His$^{14}$, suggesting that the gold and ruthenium centres are both bound to the fragment via at least one histidine.

Figure 3.7. Fragmentation of the $[\text{peptide} + 4\text{a} + 5\text{H} - 3\text{Cl}]^{5+}$ ion (m/z 632.0238): metallated CID, $b_y$, (blue), and ETD, $c_z$, (red), internal fragments containing the $[4\text{a} - 3\text{Cl}]$ adduct.
3.3 Concluding Remarks

A series of heterometallic ruthenium(II)-gold(I) complexes inspired by the preferential binding of RAPTA-T and auranofin in the nucleosome core particle was synthesised with different lengths of linkers ranging from 4 to 8 PEG units. They possess cytotoxicities in the low micromolar range against A2780, A2780cisR and HEK293 cell lines. Although they do not show selectivity towards tumour cells, they do have the ability to overcome cisplatin resistance in the A2780cisR cell line. Binding studies performed on L-Histidine and the 1-16 mer amyloid β protein show that the both the ruthenium and gold centres can bind to histidine residues, suggesting that these complexes have the capability to bind to the RU2, AU1 and AU1' binding sites on the nucleosome core particle.
3.4 Experimental Section

3.4.1 Materials

All commercially available starting materials were purchased from Sigma Aldrich, TCI, ABCR and used without further purification. Ruthenium trichloride hydrate was purchased from precious metals online and used in the synthesis of the [Ru(p-cymene)Cl2]2 dimer. L-cysteine was purchased from ABCR and the 1-16 β-amyloid peptide (H-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-Gln-Lys-OH) was purchased as a trifluoroacetate salt from Bachem. Dichloromethane was purified and degassed using a PureSolv solvent purification system (Innovative Technology INC) prior to use. Reactions were monitored via thin-layer chromatography carried out on silica plates (Merck 5554) and visualised under UV radiation (254 nm). Flash column chromatography was conducted in the normal phase on a CombiFlash-EZ prep machine installed with prepacked Luknova columns and the stated eluent system.

3.4.2 Instrumentation and Methods

1H (400 MHz), 31P (101 MHz) and 13C (162 MHz) NMR characterisation was conducted on a Bruker Advance II 400 and the spectra were referenced internally to the residual solvent peak of CDCl3 (1H: 7.26 ppm, 13C: 77.16 ppm). Coupling constants (J) are reported in hertz. High-resolution ESI-MS characterisation was performed on a Xevo G2-S QTOF mass spectrometer coupled to the Acquity UPLC Class Binary Solvent manager and BTN sample manager (Waters, Corporation, Milford, MA). Elemental Analysis was conducted by the microanalytical laboratory at EPFL using a Thermo Scientific Flash 2000 organic elemental analyzer.
3.4.3 Synthesis

3.4.3.1 Synthesis of the mono-phosphine ligands 1a-1d

**General Procedure**

4-(Diphenylphosphino)benzoic acid (1 equiv.) and EDCI (1.3 equiv.) were dissolved in dry CH₂Cl₂ (3 mL) and stirred under N₂ at room temperature for 1 h. The solution was added dropwise to a solution of the appropriate ethylene glycol (1.5 equiv.) and DMAP (0.5 equiv.) in dry CH₂Cl₂ (2 mL) and the reaction stirred under N₂ at room temperature for 21 h. The reaction mixture was washed with brine (100 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved via flash column chromatography using an eluent system of C₆H₆/EtOAc and the product was isolated as a colourless oil.

**Compound 1a**

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.300 g, 0.979 mmol, 1 equiv.), EDCI (0.244 g, 1.273 mmol, 1.3 equiv.), tetraethylene glycol (0.285 g, 1.273 mmol, 0.25 mL, 1.5 equiv.) and DMAP (0.060 g, 0.490 mmol, 0.5 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as a colourless oil (0.291 g, 0.603 mmol, 62 %).

**Elemental Analysis (%)**: calcd. for C₂₇H₃₁O₆P C 67.21 H 6.48; found C 67.20 H 6.42.

**¹H NMR (CDCl₃, 400 MHz)**: 7.97-8.00 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.26-7.38 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-C-CH-CH, 2xP-(Ar)C-CH-CH-C-H), 4.46-4.49 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.80-3.83 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.60-3.71 (10H, m, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂).
((CH₂)-O)-(CH₂), Ar-(C=O)-O-((CH₂)-O)-CH₂-C₂H₅-OH), 3.57-3.60 (2H, m, Ar-(C=O)-O-((CH₂)-O)-CH₂-C₂H₅-OH).

$^{31}$P \{^1H\} NMR (CDCl₃, 162 MHz): -4.95 (1P).

$^{13}$C \{^1H\} NMR (CDCl₃, 101 MHz): 166.4 (1C, O-(C=O)-(Ar)-CH-CH-C-P), 144.3 (1C, d, O-(C=O)-(Ar)-CH-CH-C-P, \( \delta_C = 14 \) Hz), 136.3 (2C, d, 2xP-(Ar)-CH-CH-C-P, \( \delta_C = 11 \) Hz), 134.1 (4C, d, 4xP-(Ar)-CH-CH-C-P, \( \delta_C = 20 \) Hz), 133.3 (2C, d, 2xO-(C=O)-(Ar)-CH-CH-C-P, \( \delta_C = 19 \) Hz), 130.1 (1C, O-(C=O)-(Ar)-CH-CH-C-P), 129.5 (2C, d, 2xO-(C=O)-(Ar)-CH-CH-C-P, \( \delta_C = 6 \) Hz), 129.2 (2C, 2xP-(Ar)-CH-CH-C-P), 128.8 (4C, d, 4xP-(Ar)-CH-CH-C-P, \( \delta_C = 7 \) Hz), 72.6 (1C, O-CH₂-CH₂-OH), 70.8 (2C, Ar-(C=O)-O-((CH₂)₂-O)-CH₂, 70.7 (1C, Ar-(C=O)-O-((CH₂)₂-O)-CH₂), 70.5 (1C, Ar-(C=O)-O-((CH₂)₂-O)-CH₂), 69.3 (1C, Ar-(C=O)-O-((CH₂)₂-O)-CH₂), 64.2 (1C, Ar-(C=O)-O-CH₂-CH₂-OH), 2.19 (1H, bs, -OH).


**Compound 1b**

![Compound 1b](image)

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.300 g, 0.979 mmol, 1 equiv.), EDCI (0.244 g, 1.273 mmol, 1.3 equiv.), pentaethylene glycol (0.350 g, 1.469 mmol, 1.5 equiv.) and DMAP (0.060 g, 0.490 mmol, 0.5 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as a colourless oil (0.340 g, 0.646 mmol, 66%).

**Elemental Analysis (%):** calcd. For C₉₇H₁₀₃O₉P C 66.15 H 6.70; found C 66.02 H 6.66.

$^1$H NMR (CDCl₃, 400 MHz): 7.97-8.00 (2H, m, 2xO-(C=O)-(Ar)-CH-CH-C-P), 7.26-7.38 (12H, m, 2xO-(C=O)-(Ar)-CH-CH-C-P, 4xP-(Ar)-CH-CH-C-P, 4xP-(Ar)-CH-CH-C-P, 2xP-(Ar)-CH-CH-C-P), 4.45-4.48 (2H, m, Ar-(C=O)-O-CH₂-CH₂-OH), 3.80-3.83 (2H, m, Ar-(C=O)-O-CH₂-CH₂-OH), 3.60-3.71 (16H, m, Ar-(C=O)-O-CH₂-CH₂-OH, Ar-(C=O)-O-CH₂-CH₂-OH, Ar-(C=O)-O-CH₂-CH₂-OH, 2.19 (1H, bs, -OH).

$^{31}$P \{^1H\} NMR (CDCl₃, 162 MHz): -5.05 (1P).

$^{13}$C \{^1H\} NMR (CDCl₃, 101 MHz): 166.5 (1C, O-(C=O)-(Ar)-CH-CH-C-P), 144.3 (1C, d, O-(C=O)-(Ar)-CH-CH-C-P, \( \delta_C = 14 \) Hz), 136.3 (2C, d, 2xP-(Ar)-CH-CH-C-P, \( \delta_C = 11 \) Hz), 134.1 (4C, d, 4xP-(Ar)-CH-CH-C-P, \( \delta_C = 20 \) Hz), 133.3 (2C, d, 2xO-(C=O)-(Ar)-CH-CH-C-P, \( \delta_C = 19 \) Hz), 130.2 (1C, O-(C=O)-(Ar)-CH-CH-C-P), 129.5 (2C, d, 2xO-(C=O)-(Ar)-CH-CH-C-P, \( \delta_C = 6 \) Hz), 129.2 (2C, 2xP-(Ar)-CH-CH-C-P), 128.8 (4C, d, 4xP-(Ar)-CH-CH-C-P, \( \delta_C = 7 \) Hz), 72.6 (1C, O-CH₂-CH₂-OH), 70.7-7.08 (5C, Ar-(C=O)-O-((CH₂)₂-O)-CH₂, 70.5 (1C, Ar-(C=O)-O-((CH₂)₂-O)-CH₂), 69.3 (1C, Ar-(C=O)-O-((CH₂)₂-O)-CH₂), 64.2 (1C, Ar-(C=O)-O-CH₂-CH₂-OH), 2.19 (1H, bs, -OH).
According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.300 g, 0.979 mmol, 1 equiv.), EDCI (0.244 g, 1.273 mmol, 1.3 equiv.), hexaethylene glycol (0.414 g, 1.469 mmol, 0.37 mL, 1.5 equiv.) and DMAP (0.060 g, 0.490 mmol, 0.5 equiv.) in CH$_2$Cl$_2$ (5 mL). The product was isolated as a colourless oil (0.372 g, 0.652 mmol, 67%).

**Elemental Analysis (%)**: calcd. for C$_{31}$H$_{39}$O$_8$P C 65.25 H 6.89; found C 65.16 H 6.93.

**$^1$H NMR (CDCl$_3$, 400 MHz)**: 7.97-7.99 (2H, m, 2xO-(C=O)-(Ar)C-CH=CH-CH=CH), 7.29-7.38 (12H, m, 2xO-(C=O)-(Ar)C-CH=CH-CH=CH, 4xP-(Ar)C-CH=CH-CH=CH, 4xP-(Ar)C-CH=CH-CH=CH, 2xP-(Ar)C-CH=CH-CH=CH, 3.45-3.47 (2H, m, Ar-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$-CH$_2$), 3.76-3.78 (7C, Ar-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$-CH$_2$, 3.58-3.60 (20H, m, Ar-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$-CH$_2$, 2.65 (1H, bs, -OH).

**$^{31}$P {$^1$H} NMR (CDCl$_3$, 162 MHz)**: -4.84 (1P).

**$^{13}$C {$^1$H} NMR (CDCl$_3$, 101 MHz)**: 166.4 (1C, O-(C=O)-(Ar)C-CH=CH-CH=CH), 144.3 (1C, d, O-(C=O)-(Ar)C-CH=CH-CH=CH, $^{1}$J$_{CP}$ = 14 Hz), 136.3 (2C, d, 2xP-(Ar)C-CH=CH-CH=CH, $^{1}$J$_{CP}$ = 11 Hz), 134.1 (4C, d, 4xP-(Ar)C-CH=CH-CH=CH, $^{1}$J$_{CP}$ = 19 Hz), 130.2 (1C, O-(C=O)-(Ar)C-CH=CH-CH=CH), 129.5 (2C, d, 2xO-(C=O)-(Ar)C-CH=CH-CH=CH, $^{1}$J$_{CP}$ = 6 Hz), 129.3 (2C, 2xP-(Ar)C-CH=CH-CH=CH), 128.8 (4C, d, 4xP-(Ar)C-CH=CH-CH=CH, $^{1}$J$_{CP}$ = 17 Hz), 127.2 (1C, O-(C=O)-(Ar)C-CH=CH-CH=CH, $^{1}$J$_{CP}$ = 7 Hz), 70.7-70.8 (7C, Ar-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$-CH$_2$), 70.5 (1C, Ar-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$-CH$_2$), 69.3 (1C, Ar-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$-CH$_2$), 64.3 (1C, Ar-(C=O)-O-(CH$_2$)$_2$-O-CH$_2$-CH$_2$).

**HRMS (ESI(+)-QTOF)**: m/z found 571.2467 [M+H]$^+$ requires 571.2461 (ppm = 1.05).
According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.300 g, 0.979 mmol, 1 equiv.), EDCI (0.244 g, 1.273 mmol, 1.3 equiv.), octaethylene glycol (0.544 g, 1.469 mmol, 1.5 equiv.) and DMAP (0.060 g, 0.490 mmol, 0.5 equiv.) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL). The product was isolated as a colourless oil (0.309 g, 0.469 mmol, 48%).

**Elemental Analysis (\%)**: calcd for C\textsubscript{35}H\textsubscript{47}O\textsubscript{10}P.C\textsubscript{6}H\textsubscript{14} C 66.11 H 8.25, found C 66.31 H 8.02.

\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz): 7.96-7.98 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.30-7.36 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH, 4xP-(Ar)C-CH-CH, 2xP-(Ar)C-CH-CH-H), 4.44-4.46 (2H, m, Ar-(C=O)-O-CH\textsubscript{2}-CH\textsubscript{2}-O), 3.79-3.82 (2H, m, Ar-(C=O)-O-((CH\textsubscript{2})\textsubscript{2}-O)\textsubscript{3}-O-CH\textsubscript{2}-CH\textsubscript{2}-O).

\textsuperscript{31}P \{\textsuperscript{1}H\} NMR (CDCl\textsubscript{3}, 162 MHz): -5.08 (1P).

\textsuperscript{13}C \{\textsuperscript{1}H\} NMR (CDCl\textsubscript{3}, 101 MHz): 166.4 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 143.2 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, 1\textsubscript{JC,P} = 14 Hz), 135.2 (2C, d, 2xP-(Ar)C-CH-CH-C-P, 1\textsubscript{JC,P} = 11 Hz), 133.0 (4C, d, 4xP-(Ar)C-CH-CH-CH, 1\textsubscript{JC,P} = 20 Hz), 132.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 1\textsubscript{JC,P} = 19 Hz), 129.1 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 128.5 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 1\textsubscript{JC,P} = 6 Hz), 128.2 (2C, 2xP-(Ar)C-CH-CH-CH), 127.4 (4C, d, 4xP-(Ar)C-CH-CH-CH, 1\textsubscript{JC,P} = 7 Hz), 71.6 (1C, O-CH\textsubscript{2}-CH\textsubscript{2}-OH), 69.41-69.77 (12C, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}. 68.3 (1C, Ar-(C=O)-O-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2}-OH).

HRMS (ESI(+)-QTOF): m/z found 681.2808 [M+Na]\textsuperscript{+} C\textsubscript{35}H\textsubscript{47}O\textsubscript{10}PNa\textsuperscript{+} requires 681.2805 (ppm=0.44).
3.4.3.2 Synthesis of the mono-phosphine gold(I) complexes 2a-2d

General Procedure

The appropriate mono-phosphine ligand (1 equiv.) and freshly prepared AuCl(tht)\[^{[54]}\] (1 equiv.) were dissolved in CH₂Cl₂ (10 mL) and stirred under N₂ and r.t for 18 hours. The reaction mixture was concentrated to 1 mL under reduced pressure and it was purified via flash column chromatography using CH₂Cl₂/CH₃OH as eluent. The product was isolated as a colourless oil.

**Compound 2a**

According to the general procedure, 1a (0.291 g, 0.603 mmol, 1 equiv.) and AuCl(tht) (0.193 g, 0.603 mmol, 1 equiv.) in CH₂Cl₂ (10 mL). The product was isolated as a colourless oil (0.419 g, 0.587 mmol, 97 %).

**Elemental Analysis (%)**: calcd. for C₂₇H₃₁AuClO₉P C 45.36 H 4.37; found C 45.71 H 4.57.

\[^{1}\]H NMR (CDCl₃, 400 MHz): 8.02-8.04 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.38-7.51 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH, 4.38-4.40 (2H m, Ar-(C=O)-O-CH₂-CH₂-O), 3.72-3.74 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.54-3.59 (10H, m, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)-(CH₂)₂, Ar-(C=O)-O-(CH₂)-O-(CH₂)-O), 3.45-3.47 (2H, m, O-CH₂-CH₂-OH).

\[^{31}\]P \(^{1}\)H NMR (CDCl₃, 162 MHz): 32.89 (1P).

\[^{13}\]C \(^{1}\)H NMR (CDCl₃, 101 MHz): 165.1 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 134.0 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, \(^{3}\)Jₚ,C = 60 Hz), 133.9 (4C, d, 4xP-(Ar)C-CH-CH-CH, \(^{3}\)Jₚ,C = 14 Hz), 133.7 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(^{3}\)Jₚ,C = 14 Hz), 133.0 (1C, O-(C=O)-(Ar)C-CH-CH-C-P, \(^{3}\)Jₚ,C = 3 Hz), 132.2 (2C, 2xP-(Ar)C-CH-CH-CH, \(^{3}\)Jₚ,C = 3 Hz), 129.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(^{3}\)Jₚ,C = 12 Hz), 129.3 (4C, 4xP-(Ar)C-CH-CH-CH, \(^{3}\)Jₚ,C = 12 Hz), 127.5 (2C, d, 2xP-(Ar)C-CH-CH-CH, \(^{3}\)Jₚ,C = 63 Hz), 72.3 (1C, Ar-(O)-CH₂-CH₂-CH₂), 70.2-70.4 (3C, Ar-(C=O)-O-(CH₂)-O-(CH₂)-CH₂, Ar-(C=O)-O-(CH₂)-O-(CH₂)-CH₂), 70.0 (1C, Ar-(C=O)-O-(CH₂)-O-(CH₂)-CH₂), 68.8 (1C, Ar-(C=O)-O-(CH₂)-O-(CH₂)-CH₂), 64.4 (1C, Ar-(C=O)-O-(CH₂)-O-(CH₂)-CH₂), 61.3 (1C, O-CH₂-CH₂-OH).
HRMS (ESI(+)-QTOF): \( m/z \) found 737.1116 \([M+Na]^+\) \( \text{C}_{27}\text{H}_{31}\text{AuClO}_6\text{PNa}^+ \) requires 737.1110 (ppm = 0.81).

**Compound 2b**

According to the general procedure, 1b (0.340 g, 0.646 mmol, 1 equiv.) and \( \text{AuCl(tht)} \) (0.207 g, 0.646 mmol, 1 equiv.) in \( \text{CH}_2\text{Cl}_2 \) (10 mL). The product was isolated as a colourless oil (0.482 g, 0.634 mmol, 98%).

Elemental Analysis (%): calcd. for \( \text{C}_{29}\text{H}_{35}\text{AuClO}_7\text{P} \) C 45.89 H 4.65; found C 45.49 H 4.63.

\(^1\text{H} \) NMR (\( \text{CDCl}_3, 400 \text{ MHz} \)): 8.08-8.11 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.46-7.58 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH), 4.45-4.48 (2H m, Ar-(C=O)-O-CH2-CH2-O), 3.79-3.81 (2H, m, Ar-(C=O)-O-CH2-CH2-O), 3.58-3.68 (14H, m, Ar-(C=O)-O-CH2-CH2-O-CH2-CH2, Ar-(C=O)-O-CH2-CH2-O-CH2-CH2, Ar-(C=O)-O-CH2-CH2-O-CH2-CH2, Ar-(C=O)-O-CH2-CH2-O-CH2-CH2, Ar-(C=O)-O-CH2-CH2-O-CH2-CH2, Ar-(C=O)-O-CH2-CH2-O-CH2-CH2).

\(^{31}\text{P} \) \(^{1}\text{H} \) NMR (\( \text{CDCl}_3, 162 \text{ MHz} \)): 33.00 (1P).

\(^{13}\text{C} \) \(^{1}\text{H} \) NMR (\( \text{CDCl}_3, 101 \text{ MHz} \)): 165.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 134.3 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, \( \kappa_{A}=60 \text{ Hz} \)), 134.2 (4C, d, 4xP-(Ar)C-CH-CH-CH, \( \kappa_{A}=14 \text{ Hz} \)), 133.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \( \kappa_{A}=14 \text{ Hz} \)), 133.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P, \( \kappa_{A}=3 \text{ Hz} \)), 132.4 (2C, 2xP-(Ar)C-CH-CH-C-P, \( \kappa_{A}=3 \text{ Hz} \)), 130.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \( \kappa_{A}=12 \text{ Hz} \)), 129.3 (4C, 4xP-(Ar)C-CH-CH-CH, \( \kappa_{A}=12 \text{ Hz} \)), 127.9 (2C, d, 2xP-(Ar)C-CH-CH-CH, \( \kappa_{A}=12 \text{ Hz} \)), 72.5 (1C, O-CH2-CH2-CH2), 70.5-70.7 (5C, Ar-(C=O)-O-(CH3)-O-CH2-CH2, \( \kappa_{A}=63 \text{ Hz} \)), 70.3 (1C, Ar-(C=O)-O-(CH3)-O-CH2-CH2, \( \kappa_{A}=63 \text{ Hz} \)), 69.1 (1C, Ar-(C=O)-O-CH2-CH2-CH2), 64.7 (1C, Ar-(C=O)-O-CH2-CH2-CH2), 61.7 (1C, O-CH2-CH2-CH2).

HRMS (ESI(+)-QTOF): \( m/z \) found 781.1393 \([M+Na]^+\) \( \text{C}_{29}\text{H}_{35}\text{AuClO}_7\text{PNa}^+ \) requires 781.1372 (ppm = 2.69).

**Compound 2c**

HRMS (ESI(+)-QTOF): \( m/z \) found 781.1393 \([M+Na]^+\) \( \text{C}_{29}\text{H}_{35}\text{AuClO}_7\text{PNa}^+ \) requires 781.1372 (ppm = 2.69).
According to the general procedure, 1c (0.370 g, 0.652 mmol, 1 equiv.) and AuCl(tht) (0.209 g, 0.652 mmol, 1 equiv.) in CH₂Cl₂ (10 mL). The product was isolated as a colourless (0.503 g, 0.503 mmol, 96%).

**Elemental Analysis (%):**
- calcd. for C₃₁H₃₉AuClO₈P: C 46.37; H 4.90; found C 46.25; H 4.69.
- H NMR (CDCl₃, 400 MHz): 8.03-8.11 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.45-7.57 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH, 4.45-4.47 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.53-3.67 (20H, m, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₂-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₃-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₄-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₅-(CH₂)₂-OH).
- P {¹H} NMR (CDCl₃, 162 MHz): 32.98 (1P).
- C {¹H} NMR (CDCl₃, 101 MHz):
  - 13C (C=O)+(Ar)C-CH-CH-C-P, 134.2 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 14 Hz), 133.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 14 Hz), 133.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P, J_C,P = 3 Hz), 132.4 (2C, d, 2xO-(Ar)C-CH-CH-C-P, J_C,P = 14 Hz), 129.5 (4C, 4xP-(Ar)C-CH-CH-C-P, J_C,P = 14 Hz), 127.9 (2C, d, 2xP-(Ar)C-CH-CH-C-P, J_C,P = 14 Hz), 70.4-70.6 (7C, Ar-(C=O)-O-(CH₂)₂-O-CH₂-CH₂, Ar-(C=O)-O-((CH₂)₂-O)₂-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₃-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₄-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₅-(CH₂)₂-OH).
- HRMS (ESI(+)-QTOF): m/z found 825.1644 [M+Na]⁺, requires 825.1635 (ppm = 1.09).

**Compound 2d**

According to the general procedure, 1d (0.309 g, 0.469 mmol, 1 equiv.) and AuCl(tht) (0.150 g, 0.469 mmol, 1 equiv.) in CH₂Cl₂ (10 mL). The product was isolated as a colourless (0.411 g, 0.461 mmol, 98%).

**Elemental Analysis (%):**
- calcd. for C₃₅H₄₇AuClO₁₀P: C 47.17; H 5.32; found C 47.06; H 5.39.
- H NMR (CDCl₃, 400 MHz): 8.05-8.07 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.44-7.54 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH, 4.42-4.44 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.52-3.66 (20H, m, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₂-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₃-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₄-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₅-(CH₂)₂-OH).
(C=O)-O-((CH2)2-O)-((CH2)2), Ar-(C=O)-O-((CH2)2-O)-((CH2)2), Ar-(C=O)-O-((CH2)2-O)-((CH2)2).

\(^{31}\)P \(\{^1\}^H\) NMR (CDCl\(_3\), 162 MHz): 32.95 (1P).

\(^{13}\)C \(\{^1\}^H\) NMR (CDCl\(_3\), 101 MHz): 165.2 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 134.1 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, \(J_{C,P} = 60\) Hz), 134.1 (4C, d, 4xP-(Ar)C-CH-CH-C-P, \(J_{C,P} = 14\) Hz), 133.8 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{C,P} = 14\) Hz), 133.1 (1C, O-(C=O)-(Ar)C-CH-CH-C-P, \(J_{C,P} = 3\) Hz), 132.3 (2C, 2xP-(Ar)C-CH-CH-C-P, \(J_{C,P} = 3\) Hz), 130.0 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \(J_{C,P} = 12\) Hz), 129.3 (2C, 2xP-(Ar)C-CH-CH-C-P, \(J_{C,P} = 12\) Hz), 127.7 (2C, d, 2xP-(Ar)C-CH-CH-C-P, \(J_{C,P} = 63\) Hz), 74.4 (1C, O(CH2-CH2-OH), 70.4-70.5 (11C, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, 70.1 (1C, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, 68.9 (1C, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, 64.6 (1C, Ar(C=O)-O-(CH2)-O-(CH2)-CH3, 61.5 (1C, O(CH2-CH2-OH).

HRMS (ESI(+)-QTOF): \(m/z\) found 913.2172 [M+Na]\(^+\) \(C_{35}H_{47}AuClO_{10}PNa\) requires 913.2159 (ppm = 1.42).

3.4.3.3 Synthesis of the bis-phosphine gold(I) complexes 3a-3d

**General Procedure**

4-(Diphenylphosphino)benzoic acid (1.2 equiv.) and EDCI (1.5 equiv.) were dissolved in dry CH\(_2\)Cl\(_2\) (2 mL) and stirred under N\(_2\) at room temperature for 1 h. The solution was added to a solution of the appropriate mono-phosphine gold(I) complex (1 equiv.) and DMAP (0.5 equiv.) in dry CH\(_2\)Cl\(_2\) (3 mL) and the reaction stirred under N\(_2\) at room temperature for 20 h. The reaction mixture was washed with brine (40 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved via flash column chromatography using an eluent system of CH\(_2\)Cl\(_2\)/CH\(_3\)OH. The product was washed with pentane (3 x 25 mL) and isolated as a cream oil.

**Compound 3a**

![Chemical Structure](image)
According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.097 g, 0.317 mmol, 1.2 equiv.), EDCI (0.097 g, 0.397 mmol, 1.5 equiv.), 2a (0.189 g, 0.264 mmol, 1 equiv.) and DMAP (0.016 g, 0.132 mmol, 0.5 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as a cream oil (0.220 g, 0.219 mmol, 83 %).

Elemental Analysis (%): calcd. for C₄₆H₄₄AuClO₇P₂·½CH₂Cl₂ C 53.41 H 4.34; found C 53.07 H 4.46.

¹H NMR (CDCl₃, 400 MHz): 7.87-7.89 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 7.28-7.43 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 5.29 (s, residual CH₂Cl₂), 4.43-4.46 (4H m, Ar-(C=O)-O-CH₂-CH₂-O), 3.79-3.82 (4H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.66-3.68 (8H, m, 2xAr-(C=O)-O-(CH₂)₂-(CH₂)₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 29.53 (1P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 165.8 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 138.6 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 133.3 (4C, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 131.8 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 131.5 (4C, d, 4xP-(Ar)C-CH-CH-CH, 129.7 (4C, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 129.1 (8C, 8xP-(Ar)C-CH-CH-CH), 128.8 (2C, d, 2xP-(Ar)C-CH-CH-CH, 127.0-70.8 (4C, 2xAr-(C=O)-O-(CH₂)₂-(CH₂)₂), 69.2 (2C, Ar-(C=O)-O-CH₂-CH₂), 64.7 (2C, Ar-(C=O)-O-CH₂-CH₂), 53.5 (residual CH₂Cl₂).

HRMS (ESI(+)-QTOF): m/z found 967.2210 [M-Cl]+ C₄₆H₄₄AuO₇P₂ requires 967.2228 (ppm = -1.86).

Compound 3b

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.073 g, 0.237 mmol, 1.2 equiv.), EDCI (0.057 g, 0.296 mmol, 1.5 equiv.), 2b (0.150 g, 0.198 mmol, 1 equiv.) and DMAP (0.012 g, 0.099 mmol, 0.5 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as a cream oil (0.185 g, 0.177 mmol, 89 %).

Elemental Analysis (%): calcd. for C₄₈H₄₈AuClO₈P₂·½CH₂Cl₂ C 53.46 H 4.53; found C 53.27 H 4.76.

¹H NMR (CDCl₃, 400 MHz): 7.90-7.92 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 7.29-7.43 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 4.44-4.46 (4H m, Ar-(C=O)-O-CH₂-CH₂-O), 3.79-3.81 (4H, m, Ar-(C=O)-O-CH₂-CH₂-O).

Compound 3b

![Compound 3b](image-url)

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.073 g, 0.237 mmol, 1.2 equiv.), EDCI (0.057 g, 0.296 mmol, 1.5 equiv.), 2b (0.150 g, 0.198 mmol, 1 equiv.) and DMAP (0.012 g, 0.099 mmol, 0.5 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as a cream oil (0.185 g, 0.177 mmol, 89 %).
(C=O)-O-CH₂-CH₂-O), 3.54-3.59 (12H, m, 2xAr-(C=O)-O-(CH₂)₂-O-(CH₂)₂), Ar-(C=O)-O-((CH₂)₂-O)-(CH₂)₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 31.78 (1P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 165.7 (2C, 2xO-(C=O)-(Ar(C=O)-(Ar)-(CH₂)₂-(CH₂)₂-P), 137.9 (2C, d, 2xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 133.5 (4C, 4xP-(Ar)-(CH₂)₂-(CH₂)₂-P), 132.1 (2C, 2xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 131.4 (4C, 4xP-(Ar)-(CH₂)₂-(CH₂)₂-P), 130.9 (4C, d, 4xP-(Ar)-(CH₂)₂-(CH₂)₂-P, J_C,P = 40 Hz), 129.9 (4C, 4xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 129.3 (8C, 8xP-(Ar)-(CH₂)₂-(CH₂)₂-P), 127.0-70.8 (6C, 2xAr-(C=O)-O-(CH₂)₂-(CH₂)₂-P), 69.2 (2C, Ar-(C=O)-O-(CH₂)₂-(CH₂)₂-P), 64.7 (2C, Ar-(C=O)-O-(CH₂)₂-(CH₂)₂-P).

HRMS (ESI(+)-QTOF): m/z found 1011.2484 [M-Cl]⁺, requires 1011.2490 (ppm = -0.59).

Compound 3c

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.082 g, 0.269 mmol, 1.2 equiv.), EDCI (0.064 g, 0.336 mmol, 1.5 equiv.), 2c (0.180 g, 0.224 mmol, 1 equiv.) and DMAP (0.014 g, 0.112 mmol, 0.5 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as a cream oil (0.174 g, 0.150 mmol, 62%).

Elemental Analysis (%): calcd. for C₅₀H₅₂AuClO₉P₂·½C₅H₁₂: C 55.93 H 5.19; found C 56.14 H 5.04.

¹H NMR (CDCl₃, 400 MHz): 7.89-7.91 (4H, m, 4xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 7.28-7.44 (24H, m, 4xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 8xP-(Ar)-(CH₂)₂-(CH₂)₂-P, 8xP-(Ar)-(CH₂)₂-(CH₂)₂-P, 4xP-(Ar)-(CH₂)₂-(CH₂)₂-P, 5.29 (s, residual CH₂Cl₂), 4.44-4.47 (4H, m, Ar-(C=O)-O-(CH₂)₂-(CH₂)₂-O), 3.80-3.82 (4H, m, Ar-(C=O)-O-(CH₂)₂-(CH₂)₂-O), 3.59-3.68 (16H, m, 2xAr-(C=O)-O-(CH₂)₂-O-(CH₂)₂-P). 53.5 (residual CH₂Cl₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 28.20 (1P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 165.9 (2C, 2xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 138.6 (2C, 2xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), J_C,P = 32 Hz), 134.1 (8C, 8xP-(Ar)-(CH₂)₂-(CH₂)₂-P), 133.5 (4C, 4xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 131.8 (2C, 2xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 131.5 (4C, d, 4xP-(Ar)-(CH₂)₂-(CH₂)₂-P), J_C,P = 36 Hz), 131.1 (4C, 4xP-(Ar)-(CH₂)₂-(CH₂)₂-P), 129.8 (4C, 4xO-(C=O)-(Ar(C=O)-(CH₂)₂-(CH₂)₂-P), 129.2 (8C, 8xP-(Ar)-(CH₂)₂-(CH₂)₂-P), 128.8 (2C, d, 2xP-(Ar)-(CH₂)₂-(CH₂)₂-P), 127.0-70.8 (8C, 2xAr-(C=O)-O-(CH₂)₂-(CH₂)₂-P), 69.2 (2C, Ar-(C=O)-O-(CH₂)₂-(CH₂)₂-P), 64.6 (2C, Ar-(C=O)-O-(CH₂)₂-(CH₂)₂-P), 53.5 (residual CH₂Cl₂).
Compound 3d

According to the general procedure, 4-(diphenylphosphino)benzoic acid (0.066 g, 0.215 mmol, 1.2 equiv.), EDCI (0.052 g, 0.269 mmol, 1.5 equiv.), 2d (0.160 g, 0.180 mmol, 1 equiv.) and DMAP (0.011 g, 0.090 mmol, 0.5 equiv.) in CH2Cl2 (5 mL). The product was isolated as a cream oil (0.203 g, 0.172 mmol, 96 %).

Elemental Analysis (%): calcd. for C54H60AuClO11P2 C 54.99 H 5.13; found C54.64 H 5.37.

1H NMR (CDCl3, 400 MHz): 7.90-7.92 (4H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 7.30-7.46 (24H, m, 4xO-(C=O)-(Ar)C-CH-CH-C-P, 8xP-(Ar)C-CH-CH-CH, 8xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH), 5.29 (s, residual CH2Cl2), 4.44-4.47 (4H m, Ar-(C=O)-O-CH2-CH2-O), 3.80-3.82 (4H, m, Ar-(C=O)-O-CH2-CH2-O), 3.59-3.70 (24H, m, 2x Ar-(C=O)-O-(CH2)2-O-(CH2)2, 2xAr-(C=O)-O-((CH2)2-O)2-(CH2)2, 2xAr-(C=O)-O-((CH2)2-O)3-(CH2)2).

31P {1H} NMR (CDCl3, 162 MHz): 31.09 (1P).

13C {1H} NMR (CDCl3, 101 MHz): 165.7 (2C, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 137.8 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 3.6 Hz), 134.1 (8C, 8xP-(Ar)C-CH-CH-CH), 133.5 (4C, 4xP-(Ar)C-CH-CH-CH), 130.8 (4C, d, 4xP-(Ar)C-CH-CH-CH, 3.9 Hz), 129.9 (4C, 4xO-(C=O)-(Ar)C-CH-CH-C-P), 129.3 (8C, 8xP-(Ar)C-CH-CH-CH), 128.8 (2C, d, 2xP-(Ar)C-CH-CH-CH, 12 Hz), 70.6-70.8 (12C, 2xAr-(C=O)-O-(CH2)3-O-(CH2)3), 69.2 (2C, Ar-(C=O)-O-(CH2)3-O-(CH2)3), 64.7 (2C, Ar-(C=O)-O-(CH2)3-O-(CH2)3), 53.6 (s, residual CH2Cl2).

HRMS (ESI(+)-QTOF): m/z found 1143.3398 [M-Cl] C54H60AuClO11P2 requires 1143.3276 (ppm = 10.67), m/z found 1201.2952 [M+Na] C54H60AuClO11P2Na requires 1201.2863 (ppm = 7.41).
3.4.3.4 Synthesis of the heterobimetallic ruthenium(II)-gold(I) complexes 4a-4d

**General Procedure**

The appropriate bis-phosphine gold(I) complex (2 equiv.) and [Ru(η⁶-p-cymene)Cl₂]₂ (1 equiv.) were dissolved in CH₂Cl₂ (5 mL) and stirred at r.t. under N₂ for 42 h. The solvent was removed via rotary evaporation and purification was achieved via flash column chromatography using CH₂Cl₂/CH₃OH as the eluent system. The product was washed with pentane (3 x 25 mL) and it was isolated as an oily, red solid.

**Compound 4a**

According to the general procedure, 3a (0.23 g, 0.22 mmol, 2 equiv.) and [Ru(η⁶-p-cymene)Cl₂]₂ (0.69 g, 0.11 mmol, 1 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as an oily, red solid (0.064 g, 0.049 mmol, 29%).

**Elemental Analysis (%)**: calcd. for C₃₆H₃₈AuCl₂O₅P₂Ru.CH₂Cl₂: C 49.10 H 4.34; found C 49.25 H 4.37.

**¹H NMR (CDCl₃, 400 MHz)**: 8.09-8.11 (2H, m, 2x(O-(C=O)-(Ar)C-CH-CH-C-P-Au), 7.89-7.93 (4H, m, 2x(O-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2x(O-(C=O)-(Ar)C-CH-C-P-Ru), 7.77-7.82 (4H, m, 4x(Ar)C-CH-C-P-Ru), 7.47-7.56 (12H, m, 2x(O-(C=O)-(Ar)C-CH-C-P-Au, 4x(Ar)CH-CH-C-P-Au, 4x(Ar)CH-C-CH-C-P-Au, 2x(Ar)CH-C-CH-C-P-Au, 2x(Ar)CH-C-CH-C-P-Au, 3.73-7.41 (4H, m, 4x(Ar)CH-CH-C-CH-C-P-Ru, 2x(Ar)CH-C-CH-C-P-Ru), 5.20-5.21 (2H, d, 2xCH₁-(Ar)C-CH-CH-C-CH(CH₃)₂, 3JH₂H = 6.9 Hz), 4.98-4.98 (2H, d, 2xCH₁-(Ar)C-CH-CH-C-CH(CH₃)₂, 3JH₂H = 5.9 Hz), 4.45-4.48 (2H, m, Au-P-(C=O)-O(CH₂)₃-O-H₂, 4.39-4.39 (2H, m, Ru-P-Ar-(C=O)-O(CH₂)₃-O-H₂, 4.39-4.39 (2H, m, Au-P-Ar-(C=O)-O(CH₂)₃-O-H₂, Ru-P-Ar-(C=O)-O(CH₂)₃-O-H₂, Ru-P-Ar-(C=O)-O(CH₂)₃-O-H₂, 3.63-3.67 (8H, m, 2xAr-(C=O)-O(CH₂)₃-O-H₂, 2.80-2.86 (1H, sept, (Ar)C-CH-C-CH-C-CH(CH₃)), 3JH₂H = 6.9 Hz), 1.85 (3H, s, (CH₃)-(Ar)C-CH-C-CH-C), 1.09-1.10 (6H, d, (Ar)C-CH-C-CH-C-CH(CH₃)), 3JH₂H = 6.9 Hz).

**³¹P (¹H) NMR (CDCl₃, 162 MHz)**: 33.02 (Au-P, 1P), 25.03 (Ru-P, 1P).
**C \{^1\}H NMR (CDCl\textsubscript{3}, 101 MHz):** 166.1 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru), 165.4 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Au), 139.05-139.58 (2C, m, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au), 133.15-133.60 (3C, m, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au), 132.4 (2C, d, 2xRu-P-(Ar)C-CH-CH-CH, \textit{\textit{J}}_{C,P} = 3 \text{ Hz}), 131.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au), 130.7 (2C, 2xRu-P-(Ar)C-CH-CH-CH), 129.5 (4C, d, 2xAu-P-(Ar)C-CH-CH-CH, \textit{\textit{J}}_{P,C} = 12 \text{ Hz}), 128.7 (2C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru), 128.2 (4C 2xRu-P-(Ar)C-CH-CH-CH), 127.8 (2C, 2xAu-P-(Ar)C-CH-CH-CH), 111.4 (1C, CH\textsubscript{3}-(Ar)C-CH-CH-C), 96.4 (1C, CH\textsubscript{3}-(Ar)C-CH-CH-C), 89.1 (2C, CH\textsubscript{2}-(Ar)C-CH-CH-C), 87.4 (2C, CH\textsubscript{2}-(Ar)C-CH-CH-C), 70.71-70.79 (4C, Ru-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 69.2 (1C, Ru-P-Ar-(C=O)-O-CH\textsubscript{2}CH\textsubscript{2}), 69.1 (1C, Au-P-Ar-(C=O)-O-CH\textsubscript{2}CH\textsubscript{2}), 70.71-70.79 (4C, Ru-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 64.7 (1C, Au-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 64.4 (1C, Ru-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 30.4 (1C, Au-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 22.0 (2C, (Ar)C-CH-CH-C-P-Ru), 17.9 (1C, (Ar)C-CH-CH-C-P-Au).

**HRMS (ESI(+)-Orbitrap):** \textit{m/z} found 1331.1259 [M+Na]\textsuperscript{+} \textit{C}_{56}\textit{H}_{58}\textit{AuCl}_{3}\textit{NaO}_{7}\textit{P}_{2}\textit{Ru} \textit{requires} 1331.1323 (ppm = -4.78).

**Compound 4b**

According to the general procedure, 3b (0.14 g, 0.14 mmol, 2 equiv.) and [Ru\textsuperscript{η\textsuperscript{5}-p-cymene]Cl\textsubscript{2}} (0.043 g, 0.069 mmol, 1 equiv.) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL). The product was isolated as an oily, red solid (0.078 g, 0.058 mmol, 41 %).

**Elemental Analysis (%):** calcd. for \textit{C}_{58}\textit{H}_{62}\textit{AuCl}_{3}\textit{O}_{8}\textit{P}_{2}\textit{Ru}.CH\textsubscript{2}Cl\textsubscript{2} C 49.27 H 4.49; found C 49.19 H 4.51.

**\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz):** 8.10-8.12 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au), 7.90-7.95 (4H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Ru), 7.78-7.83 (2xO-(C=O)-(Ar)C-CH-CH-C-P-Au, 4x(Ar)C-CH-CH-C-P-Au), 7.53-7.57 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Ru, 4x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Ru), 7.38-7.50 (6H, m, 4x(Ar)C-CH-CH-C-P-Ru, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au), 7.38-7.50 (6H, m, 4x(Ar)C-CH-CH-C-P-Ru, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au), 7.38-7.50 (6H, m, 4x(Ar)C-CH-CH-C-P-Ru, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au), 5.21-5.22 (2H, d, 2xCH\textsubscript{2}-(Ar)C-CH-CH-C, \textit{\textit{J}}_{CH_2} = 6.2 \text{ Hz}), 4.97-4.99 (2H, d, 2xCH\textsubscript{2}-(Ar)C-CH-CH-C, \textit{\textit{J}}_{CH_2} = 5.9 \text{ Hz}), 4.46-4.49 (2H, m, Au-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 4.14-4.17 (2H, m, Ru-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 3.76-3.82 (4H, m, Au-P-Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 1.86 (3H, s, CH\textsubscript{3}-(Ar)C-CH-CH-C), 1.10-1.12 (6H, d, (Ar)C-CH-CH-C-CH(CH\textsubscript{3})\textsubscript{2}, \textit{\textit{J}}_{CH_2} = 6.9 \text{ Hz}).

**\textsuperscript{31}P \{^1\}H NMR (CDCl\textsubscript{3}, 162 MHz):** 33.02 (Au-P, 1P), 25.00 (Ru-P, 1P).
**Compound 4c**

According to the general procedure, 3c (0.20 g, 0.18 mmol, 2 equiv.) and [Ru(η⁶-p-cymene)Cl₂]₂ (0.056 g, 0.091 mmol, 1 equiv.) in CH₂Cl₂ (5 mL). The product was isolated as an oily, red solid (0.036 g, 0.025 mmol, 14 %).

**Elemental Analysis (%):** calcd. for C₆₀H₆₆AuCl₃O₉P₂Ru.C₅H₁₂ C 53.12 H 5.35; found C 52.96 H 5.35.

**¹H NMR (CDCl₃, 400 MHz):** 8.09-8.12 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au), 7.89-7.94 (4H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Ru), 7.78-7.82 (4H, m, 4x( Ar)C-CH-CH-C-P-Ru), 7.52-7.59 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au, 4x( Ar)CH-CH-C-P-Au, 4x( Ar)CH-CH-C-P-Au, 2x( Ar)CH-CH-C-P-Au), 7.37-7.49 (6H, m, 4x( Ar)CH-CH-C-P-Au, 2x( Ar)CH-CH-P-Au), 7.37-7.49 (6H, m, 4x( Ar)CH-CH-C-P-Au, 2x( Ar)CH-CH-C-P-Au), 5.20-5.22 (2H, d, 2xCH₃-(Ar)C-CH-CH-C, J>H,H = 5.9 Hz), 4.97-4.98 (2H, d, 2xCH₃-(Ar)C-CH-CH-C, J>H,H = 5.6 Hz), 4.46-4.49 (2H, m, Au-P-Ar(C=O)-O-CH₂-CH₂-O), 4.40-4.43 (2H, m, Ru-P-Ar(C=O)-O-CH₂-CH₂-O, Ru-P-Ar(C=O)-O-CH₂-CH₂-O), 3.76-3.82 (4H, m, Au-P-Ar(C=O)-O-CH₂-CH₂-O, 2xAr(C=O)-O-((CH₂)₂-O)₂-CH₂), 3.58-3.68 (16H, m, 2x Ar-(C=O)-O-(CH₂)₂, 2x Ar-(C=O)-O-(CH₂)₂), 2.83-2.86 (1H, sept, ( Ar)C-CH-CH-C-CH(CH₃)₂, J>H,H = 6.9 Hz), 1.85 (3H, s, ( CH₃-(Ar)C-CH-CH-C), 1.09-1.11 (6H, d, ( Ar)C-CH-CH-C-(CH₂)₃), J>H = 6.9 Hz).

**³¹P {¹H} NMR (CDCl₃, 162 MHz):** 33.02 (Au-P, 1P), 24.99 (Ru-P, 1P).
\[^{13}\text{C\ H}\] NMR (CDCl\textsubscript{3} 101 MHz): 166.1 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru), 165.4 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Au), 139.15-139.58 (2C, m, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, O-(C=O)-(Ar)C-CH-CH-C-P-Au), 133.15-133.63 (3C, m, O-(C=O)-(Ar)C-CH-CH-C-P-Au, 2xRu-P-(Ar)-C-CH-CH(CH)-), 132.4 (2C, d, 2xAu-P-(Ar)C-CH-CH, \[^{3}\text{J}_{\text{C,P}} = 3 \text{ Hz}\}), 131.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, O-(C=O)-(Ar)C-CH-CH-C-P-Au), 130.7 (2C, 2xRu-P-(Ar)C-CH-CH(CH)-), 130.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au, 2xRu-P-(Ar)-C-CH-CH(CH)-, \[^{3}\text{J}_{\text{C,P}} = 12 \text{ Hz}\}), 129.5 (4C, d, 4xAu-P-(Ar)C-CH-CH, \[^{3}\text{J}_{\text{C,P}} = 12 \text{ Hz}\}), 128.8 (2C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2xRu-P-(Ar)C-CH-CH(CH)-, \[^{3}\text{J}_{\text{C,P}} = 10 \text{ Hz}\}), 127.8 (2C, d, 2xAu-P-(Ar)C-CH-CH, \[^{3}\text{J}_{\text{C,P}} = 63 \text{ Hz}\}), 111.6 (1C, CH-(Ar)C-CH-CH-O), 96.4 (1C, CH-(Ar)C-CH-CH-C), 89.1 (2C, CH-(Ar)C-CH-CH-C), 87.4 (2C, CH-(Ar)C-CH-CH-C), 70.68-70.77 (8C, 4xAu-P-(Ar)-C-CH-CH(CH)-, 2xRu-P-(Ar)-C-CH-CH(CH)-, \[^{3}\text{J}_{\text{C,P}} = 63 \text{ Hz}\}), 69.2 (1C, Au-P-Ar(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 69.1 (1C, Au-P-Ar(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 64.8 (1C, Au-P-Ar(C=O)-O-(CH\textsubscript{2})\textsubscript{2}), 64.5 (1C, Au-P-Ar(C=O)-O-(CH\textsubscript{2})\textsubscript{2}), 30.4 (1C, CH-(Ar)C-CH-CH(CH\textsubscript{3})\textsubscript{2}), 22.0 (2C, (Ar)C-CH-CH-C-(CH\textsubscript{3})\textsubscript{2}), 17.9 (1C, CH-(Ar)C-CH-CH-C).

HRMS (ESI(+)-Orbitrap): \textit{m/z} found 1419.1779 [M+Na\textsuperscript{+}] \text{requires 1419.1849 (ppm = -4.93)}.

**Compound 4d**

According to the general procedure, 3\textsubscript{d} (0.21 g, 0.18 mmol, 2 equiv.) and [Ru(\eta\textsuperscript{5}-p-cymene)Cl\textsubscript{2}]\textsubscript{2} (0.056 g, 0.091 mmol, 1 equiv.) in CH\textsubscript{2}Cl\textsubscript{2} (5 mL). The product was isolated as an oily, red solid (0.031 g, 0.021 mmol, 11%).

Elemental Analysis (%): calc\textsubscript{d} for C\textsubscript{60}H\textsubscript{66}AuCl\textsubscript{3}O\textsubscript{9}P\textsubscript{2}Ru.CDCl\textsubscript{3} C 48.61 H 4.77; found C 48.95 H 4.44.

\[^{1}\text{H}\] NMR (CDCl\textsubscript{3} 400 MHz): 8.10-8.12 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au), 7.90-7.94 (4H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Ru, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Ru), 7.78-7.83 (4H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au, 4x(Ar)C-CH-CH-C-P-Au, 4x(Ar)C-CH-CH-C-P-Ru, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Ru), 7.53-7.58 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au, 4x(Ar)C-CH-CH-C-P-Au, 4x(Ar)C-CH-CH-C-P-Ru), 7.38-7.50 (6H, m, 4xAu-P-(Ar)-C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Au, 2x(Ar)C-CH-CH-C-P-Ru), 5.20-5.22 (2H, d, 2xCH\textsubscript{3}-(Ar)C-CH-CH-C, \[^{3}\text{J}_{\text{H,H}} = 6.0 \text{ Hz}\}), 4.97-4.99 (2H, d, 2xCH\textsubscript{3}-(Ar)C-CH-CH-C, \[^{3}\text{J}_{\text{H,H}} = 6.3 \text{ Hz}\}), 4.47-4.50 (2H, m, Au-P-Ar(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 4.41-4.44 (2H, m, Ru-P-Ar(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 3.77-3.83 (4H, m, Au-P-Ar(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 3.61-3.65 (24H, m, 2xAr-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, \[^{3}\text{J}_{\text{H,H}} = 6.9 \text{ Hz}\}), 1.85 (3H, s, CH-(Ar)C-CH-CH-C), 1.10-1.11 (6H, d, (Ar)C-CH-CH-C-CH(CH\textsubscript{3})\textsubscript{2}, \[^{3}\text{J}_{\text{H,H}} = 6.9 \text{ Hz}\]).
**13C {1H} NMR (CDCl3, 101 MHz):** 166.1 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru), 139.13-139.60 (2C, m, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, O-(C=O)-(Ar)C-CH-CH-C-P-Au), 133.98-134.68 (12C, m, 4xRu-P-(Ar)C-CH-CH-C-P-Ru, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au), 133.18-133.63 (3C, m, O-(C=O)-(Ar)C-CH-CH-C-P-Au, 2xRu-P-(Ar)C-CH-CH-C-P-Ru), 132.4 (2C, d, 2xRu-P-(Ar)C-CH-CH-C-P-Ru, \( ^3J_{C,P} = 3 \) Hz), 131.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P-Ru), 130.7 (2C, d, 2xRu-P-(Ar)C-CH-CH-C-P-Ru, \( ^3J_{C,P} = 3 \) Hz), 130.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P-Au, \( ^3J_{C,P} = 12 \) Hz), 129.5 (4C, d, 2xAu-P-(Ar)C-CH-CH-C-P-Au, \( ^3J_{C,P} = 12 \) Hz), 128.8 (2C, d, O-(C=O)-(Ar)C-CH-CH-C-P-Ru, \( ^3J_{C,P} = 10 \) Hz), 128.3 (4C, d, 2xRu-P-(Ar)C-CH-CH-C-P-Ru, \( ^3J_{C,P} = 10 \) Hz), 127.8 (2C, d, 2xAu-P-(Ar)C-CH-CH-C-P-Au, \( ^3J_{C,P} = 63 \) Hz), 111.6 (1C, CH3-(Ar)C-CH-CH-C-), 96.4 (1C, CH3-(Ar)C-CH-CH-C), 89.1 (2C, CH3-(Ar)C-CH-CH-C), 87.4 (2C, CH3-(Ar)C-CH-CH-C), 70.65-70.77 (12C, Ru-P-Ar-(C=O)-O-(CH2)-O-(CH2)), Au-P-Ar-(C=O)-O-(CH2)-O-(CH2), Ru-P-Ar-(C=O)-O-(CH2)-O-(CH2), Au-P-Ar-(C=O)-O-(CH2)-O-(CH2), Ru-P-Ar-(C=O)-O-(CH2)-O-(CH2), Au-P-Ar-(C=O)-O-(CH2)-O-(CH2), 69.2 (1C, Ru-P-Ar-(C=O)-O-(CH2)-C), 69.1 (1C, Au-P-Ar-(C=O)-O-(CH2)-C), 64.8 (1C, Au-P-Ar-(C=O)-O-(CH2)-C), 64.5 (1C, Ru-P-Ar-(C=O)-O-(CH2)-C), 30.4 (1C, (Ar)C-CH-CH-C-CH(3)), 22.0 (2C, (Ar)C-CH-CH-C-CH(3)), 17.9 (1C, CH3-(Ar)C-CH-CH-C-CH(3)).

HRMS (ESI(+)-Orbitrap): m/z found 1507.2302 [M+Na]+ requires 1507.2375 (ppm = 4.80).

### 3.4.4 Cell Culture and Cytotoxicity Studies

Human ovarian carcinoma (A2780 and A2780cisR) cell lines were obtained from the European Collection of Cell Cultures. The human embryonic kidney (HEK-293) cell line was obtained from ATCC (Sigma, Buchs, Switzerland). Penicillin streptomycin, RPMI 1640 GlutaMAX (where RPMI = Roswell Park Memorial Institute), and DMEM GlutaMAX media (where DMEM = Dulbecco’s modified Eagle medium) were obtained from Life Technologies, and fetal bovine serum (FBS) was obtained from Sigma. The cells were cultured in RPMI 1640 GlutaMAX (A2780 and A2780cisR) and DMEM GlutaMAX (HEK-293) media containing 10% heat-inactivated FBS and 1% penicillin streptomycin at 37 °C and CO2 (5%). The A2780cisR cell line was routinely treated with cisplatin (2 μM) in the media to maintain cisplatin resistance. The cytotoxicity was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cells were seeded in flat-bottomed 96-well plates as a suspension in a prepared medium (100 μL aliquots and approximately 4300 cells/ well) and preincubated for 24 h. Stock solutions of compounds were prepared in DMSO and were rapidly diluted in a medium. The solutions were
sequentially diluted to give a final DMSO concentration of 0.5% and a final compound concentration range (0–200 μM). Cisplatin and RAPTA-c were tested as a positive (0–100 μM) and negative (200 μM) controls respectively. The compounds were added to the preincubated 96-well plates in 100 μL aliquots, and the plates were incubated for a further 72 h. MTT (20 μL, 5 mg/mL in Dulbecco’s phosphate buffered saline) was added to the cells, and the plates were incubated for a further 4 h. The culture medium was aspirated, and the purple formazan crystals, formed by the mitochondrial dehydrogenase activity of vital cells, were dissolved in DMSO (100 μL/well). The absorbance of the resulting solutions, directly proportional to the number of surviving cells, was quantified at 590 nm using a SpectroMax M5e multimode microplate reader (using SoftMax Pro software, version 6.2.2). The percentage of surviving cells was calculated from the absorbance of wells corresponding to the untreated control cells. The reported IC50 values are based on the means from two independent experiments, each comprising four tests per concentration level.

3.4.5 Mass Spectrometry Binding Studies

3.4.5.1 Binding studies of 4b with L-Histidine

Complex 4b was incubated under agitation with L-Histidine for 2 h in a 1:1 complex-amino acid ratio in unbuffered solution (98 % milliQ water, 2 % DMSO) at 310 K. The samples were diluted first in milliQ water (factor 100) and then in CH₃OH/HCOOH (0.1 % HCOOH in CH₃OH) by a factor of 10.

3.4.5.2 Binding studies of 4a with 1-16-mer β-amylloid peptide

Complex 4a was incubated under agitation with the 16-mer β-amylloid protein for 2 h in a 1:3 complex-peptide ratio in unbuffered solution (98% milliQ, 2% DMSO) at 310 K. The
samples were diluted first in millQ water (factor 100) and then in CH₃OH/HCOOH (0.1 % HCOOH in CH₃OH) by a factor of 10.

3.4.5.3 TOF

Routine analyses were conducted on a Xevo G2-S QTOF mass spectrometer coupled to the Acquity UPLC Class Binary Solvent manager and BTN sample manager (Waters, Corporation, Milford, MA). The sample manager system temperature was maintained at 10 °C and the injection volume was 2 μL. Mass spectrometer detection was operated in positive ionization using the ZSpray™ dual-orthogonal multimode ESI/APCI/ESCi® source. The TOF mass spectra were acquired in the resolution mode over the range of m/z 50-1200 at an acquisition rate of 0.036 sec/spectra. The instrument was calibrated using a solution of sodium formate (0.01 mg/L in isopropanol/H₂O 90:10). A mass accuracy better than 5 ppm was achieved using a Leucine Enkephalin solution as lock-mass (200 pg/mL in ACN/H₂O (50:50)) infused continuously using the LockSpray source. Source settings were as follows: cone, 25V; capillary, 3 kV, source temperature, 150°C; desolvation temperature, 500°C, cone gas, 10 L/h, desolvation gas, 500 L/h. Data were processed using MassLynx™ 4.1 software and QuanLynx application for quantification.

3.4.5.4 Orbitrap

Mass spectrometry analyses were performed on a LTQ Orbitrap FTMS instrument (LTQ Orbitrap Elite FTMS, Thermo Scientific, Bremen, Germany) operated in the positive mode coupled with a robotic chip-based nano-ESI source (TriVersa Nanomate, Advion Biosciences, Ithaca, NY, U.S.A.). A standard data acquisition and instrument control system was utilised (Thermo Scientific) whereas the ion source was controlled by Chipsoft 8.3.1 software (Advion BioScience). Samples were loaded onto a 96-well plate (Eppendorf, Hamburg, Germany) within an injection volume of 5μL. The experimental conditions for the ionization voltage was +1.4kV and the gas pressure was set at 0.30 psi. The temperature of ion transfer capillary was 275 °C, tube voltages. FTMS spectra were obtained in the 200-2000 m/z range in the reduce profile mode with a resolution set to 120,000. In all spectra one microscan was
acquired with a maximum injection time value of 1000ms. For CID, ETD and HCD analysis, each precursor ion was isolated with a width window of 8. Normalised collisions energies for CID and HCD fragmentation were 30% and 18%, respectively. A total of 100 scans each consisting in 10 μscans were acquired in reduced profile mode and averaged. ETD reaction time was set at 180 ms.

3.4.5.5 General input Apm\(^2\)s parameters:

Experimental MS were exported as .txt files before drop into the Apm\(^2\)s tool.\(^{[52],[47]}\) Protons (From +1 to +5), modifiable charge (From +1 to +5) and metal adduct (C56H58AuO7P2Ru) in the different boxes of “List of groups”. Zone widths were selected based of the Ru expected isotopic pattern (-6.5 to 8.5) and the common zone parameter was fixed “as second”. Minimal similarity was set at 70%, max results at 500 and best result range at 0.

b, y and b/y (internal fragments) fragment ions were selected for the CID experiments whereas c, z and c/z were chosen for ETD.
3.5 References


The work described was completed in collaboration with Louis De Falco and Zenita Adhireksan who performed the X-ray crystallographic studies and Thibaud Von Erlach who performed the molecular dynamic simulations.
4.1 Introduction

As the repeating units of chromatin, nucleosomes represent key epigenetic targets. The nucleosome core consists of an octamer of pairs of H2A, H2B, H3 and H4 histone proteins encircled by 145-147 base pairs of DNA.\(^1\) A key protein binding motif for chromatin regulation was identified on the face of the H2A-H2B dimer where a high abundance of glutamate and aspartic residues are located.\(^{2-4}\) This area, known as the “acidic patch”, is vital for the folding of nucleosome into chromatin fibre and for the repression of transcription.\(^{5,6}\) As regulatory proteins such as the regulator of chromatin condensation 1 (RCC1) dock on this highly electronegative area,\(^4,7\) the selective binding of competing molecules could have an inhibitory effect. With the acidic patch playing host to at least two specific metal binding sites,\(^{8-13}\) binuclear ruthenium compounds (Figure 4.1) inhibit the binding of RCC1 and subsequently disrupt the folding of chromatin fibres inducing aberrant chromatin condensation.\(^{14}\)

![Figure 4.1. Dinuclear ruthenium(II) complexes which bind to the NCP.](image)

The ruthenium compound, \([\eta^2-p\text{-toluene}]{\text{Ru}}(1,3,5\text{-triaza-7-phosphaadamantane})\text{Cl}_2\)], termed RAPTA-T (Figure 4.2), is an anti-metastatic drug with inhibitory properties towards both primary and metastatic tumours.\(^{15,16}\) RAPTA-T displays strong site selectivity towards the acidic patch at binding sites consisting of carboxylate groups E61 and E64 on the H2A
protein (RU1) and imidazole H106 and carboxylate groups E102 of the H2B dimer (RU2).[13]

The RU1 and RU2 sites have been crosslinked by dinuclear RAPTA complexes linked via the arene with a thirteen-unit polyethylene glycol linker (PEG), a two-unit methylene linker (C2), a ten-unit methylene linker (C10) and a 1,2-diphenylethlenediamide linker in the RR, RS and SS configuration (Figure 4.1). The linker strongly influences the nature of the crosslinks formed. Whilst the more flexible linkers, PEG, C2 and C10, and the linkers promoting a closed conformation, RR and SS, can crosslink the RU1 and RU2 sites, the RS complex cannot. Due to the conformation of RS, the ruthenium centres reside on opposing faces relative to the linker. Therefore, once the RU1 site is occupied, the second ruthenium is in the wrong orientation to bind to the RU2 site. Instead, the second ruthenium centre binds to the distant carboxylate residue E91 on the H2A protein.[14]

Allosteric effects, discovered and established in the 1960s by Monrod, Wyman, Changeux et al,[17–19] are key mechanisms by which metabolic and cellular pathways are regulated.[20] Targeting allosteric sites has emerged as a promising therapeutic target.[21][22] A myriad of receptors and ion channels, including G protein-coupled receptors (GPCR),[23][22] are vulnerable to inhibition and regulation via allosteric effects.[24–27] Recent advances in the structural biology of GPCRs have enabled the resolution of crystal allowing the structures of bound allosteric modulators to the GPCR to be observed.[28][29] Recently, cryo-electron microscopy has been used to observe the allosteric effect of the peptide toxin, mambalgin-1, binding to chicken acid sensing ion channel.[30]

Synergism between RAPTA-T and auranofin, [(3,4,5-triacetyloxy-6-acetylxyoxymethyl,oxane-2-thio-late)Au(triethylphosphanium)] (AUF, Figure 4.2), a gold(I) anti-arthritic drug that is being repurposed as an anticancer drug,[31] was found when human ovarian carcinoma (A2780) cells pretreated with RAPTA-T are then treated with AUF, significantly impacting cell viability. Co-treatment also results in a significant increase in AUF accumulation on cellular chromatin compared to treatment with AUF alone, suggesting that the action of RAPTA-T modulates the mechanism of action of AUF.

Nucleosome core particle (NCP) crystallographic studies revealed a strong allosteric relationship between RAPTA-T and AUF where the AUF coordinates to the two symmetry-related imidazole sites, H3 H113 (AU1) and H113' (AU1'), on the H3-H4 tetramer, but only
after RAPTA-T has bound RU1 or both the RU1 and RU2 sites. The RAPTA and AUF bind sites are located at ca. 27 Å from each other. Molecular dynamics (MD) simulations showed that the binding of RAPTA-T causes a series of systematic changes in the inter-helical orientations of the H2A and H3/H3' α-helices connecting the RU1/RU2 and AU1/AU1' binding sites. These conformational changes result in a more compact AUF binding site and an increase in favorable hydrophobic interactions with the triethyl groups.\cite{13}

Based on these intriguing observations, we designed, synthesised and evaluated a hetero-bimetallic ruthenium(II)-gold(I) compound (1, see Scheme 4.1) capable of spanning the 27 Å distance between the RU1 and AU1 binding sites and inciting the allosteric effect. Herein we report crystallographic/MD studies on the NCP, confirming that 1 crosslinks the allosteric sites on the nucleosome core particle.

![Figure 4.2. Structures of RAPTA-T and AUF.](image)

### 4.2 Results and Discussion

#### 4.2.1 Dinuclear Ruthenium(II)-Gold(I) Complex

The hetero-bimetallic complex, 1, was designed to embody the structure of the parent drugs, RAPTA-T and AUF, as closely as possible to maintain their specific nucleosome binding modes. With this in mind, the ideal design involves the parent drugs linked via a
flexible linker with the ability to span $> 27 \text{ Å}$. The linker length should exceed the 27 Å necessary to allow the linker to circumnavigate the forest of amino acid side chains that separate the RAPTA-T and AUF binding sites. Polyethylene glycol (PEG) was selected as an appropriately flexible linker with the advantage of a higher hydrophilicity than comparable alkyl linkers. The RAPTA moiety was attached to the linker via the functionalization of the arene leaving the vital labile chloride and 1,3,5-triaza-7-phosphaadamantane (PTA) ligands of RAPTA-T unperturbed. AUF required further modification with its sacrificial ligand, 3,4,5-triacetoxy-6-acetylomethylxoxane-2-thiolate, replaced with a sufficiently labile chloride. 4-(diphenylphosphino)benzoic acid was selected as a functionalizable substitute to triethylphosphine due to its good stability and hydrophobic nature capable of replicating the important hydrophobic interactions observed at the AU1/AU1' binding sites.

Scheme 4.1. Synthetic route to the heterobimetallic ruthenium(II)-gold(I) compound, 1.
The heterobimetallic compound, 1, was prepared in a four-step synthesis (Scheme 4.1). The mono-substituted phosphine ligand, \( a \), was obtained via an esterification reaction between 4-diphenylphosphinobenzoic acid and hexaethylene glycol in the presence of N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 4-(dimethylamino)pyridine (DMAP). The gold(I) chloride fragment was coordinated to the phosphine ligand via a freshly prepared gold(I) intermediate, AuCl(tht) (tht = tetrahydrothiophene)\(^{[32]}\) to yield \( b \). The chain length was further extended and a primary amine, initially protected by 9-fluorenylmethoxycarbonyl chloride, introduced via an esterification reaction with 15-(9-fluorenyloxycarbonyl)amino-4,7,10,13-tetraoxa-pentadecanoic acid (with EDCI and DMAP acting as coupling reagent and catalyst). The isolation of the desired product from the gold(I) starting material was found to be problematic due to similar \( R_f \) values and, therefore, the primary amine was deprotected with 20% pipiridine in DMF to increase the polarity of the product, \( c \), allowing complete purification. The target compound, 1, was obtained via an amide coupling reaction between the primary amine and [Ru(3-(4-methylcyclohexa-1,4-dien-1-yl)propanoic acid)(PTA)(\( \mu \)-oxalato)], prepared using a literature method,\(^{[33]}\) in the presence of \( O \)-(benzotnazol-1-yl)-\( \mathcal{N},\mathcal{N},\mathcal{N}',\mathcal{N}' \)-tetramethyl-uronium tetrafluoroborate (TBTU) and \( \mathcal{N},\mathcal{N} \)-diisopropylamine (DIPEA). The oxalate dissociates during the purification and is replaced by chloride ligands sourced from concentrated NaCl\(_\text{aq} \) solution resulting in the desired homobimetallic complex, 1.

Compound 1 and all the intermediates on route to 1 were characterised by \( ^1\text{H} \), \( ^{31}\text{P} \) and \( ^{13}\text{C} \) NMR spectroscopy, mass spectrometry and elemental analysis. Characteristic changes in the \( ^{31}\text{P} \) NMR spectra confirmed the coordination of the gold centre to the diphenyl(phosphine)benzoic acid ligand with singlet resonances observed at -5.05 ppm for the free ligand and at 32.98 ppm when the phosphorus is coordinated to the gold(I) centre. The peak corresponding to the phosphorus centre (coordinated to the gold(I) ion) in the \( ^{31}\text{P} \) NMR spectra of the subsequent compounds including 1 remains in the range 32.98-33.07 ppm. The introduction of the ruthenium centre with the formation of 1 resulted in the appearance of a second singlet at -33.68 ppm in the \( ^{31}\text{P} \) NMR indicative of the phosphorus centre in the ruthenium-PTA unit. The structure of 1 is further confirmed from mass spectrometry in which a peak envelope corresponding to the compound with the loss of a chloride ligand is observed as [M-Cl\(^+\)] at m/z 1489.3344. Fragmentation was observed in the MS with the most abundant being the [M-RuCl\(_2\)PTA+Na\(^+\)] ion.
To assess the nature of the adducts formed on chromatin by 1, trials were conducted by incubating 1 with nucleosome core particle crystals for different durations and analyzed by X-ray crystallography. At the earliest time point ($t = 28$ h), occupancy of the RU1 site by the RAPTA moiety of 1 is apparent (Figure 4.3 b). The two labile chloride ligands have
dissociated enabling the ruthenium(II) centre to coordinate to the carboxylate groups E61 and E64 of the RU1 binding site. The PTA ligand and the arene, coordinated to the ruthenium, are well resolved. The first two carbons of the linker attached to the arene are also well resolved, however the ester group and adjacent PEG chain is not visible. Gold adducts, corresponding to the gold complex of 1, are also observed at AU1 and AU1' (Figure 4.3 b) indicating that the allosteric effect has been activated by the binding of the RAPTA moiety of 1 at RU1. The chloride ligand has dissociated allowing the gold(I) centre to bind to the H3 H113 histidine residue. The diphenyl(phosphine)benzoic acid ligand is clearly resolved, however, the PEG chain beyond the ester group is not visible. Additional gold adduct formation was observed at the H3 H39 histidine site situated near the entry/exit points of the nucleosome core. Incubations of longer duration (t = 120 h) lead to increased occupancy of the RU1, AU1, AU1' and H3 H39 sites as well as appearance of additional gold adducts at the H2B H46 and several guanine N7 sites on the DNA. Interestingly, in contrast to mono- and homo-dinuclear RAPTA complexes,\textsuperscript{[5,7,10,16]} no occupancy of the RU2 site is observed by 1 which can be attributed to both steric and entropic factors.

Despite the longest incubation data set containing residual electron density linking the occupied RU1 and AU1 site consistent with the presence of the PEG linker, the linker itself could not be clearly resolved (Figure 4.3 a). The ester groups, that link the resolved RAPTA and gold complexes to the PEG linker, are orientated towards each other, which is consistent with the metal centres being linked (Figure 4.3 b). Molecular Dynamic (MD) simulations were conducted to assess the binding of 1 to the NCP in an attempt to locate the linker. The longest incubation data set (t = 120 h) where the RU1 and AU1 occupancy is highest, was selected as the basis for the atomic model. Notably, the AU1 site, which resides on the same face of the nucleosome as the RU1 site, has a significantly higher apparent occupancy than the AU1' site on the opposing face. Within the AU1/AU1' sites, the phenyl substituents of the gold complex are nestled within a substantially hydrophobic recess surrounding the coordinating imidazole ND1 atom (Figure 4.3 b). According to the MD simulations, the PEG linker can easily span the ~36 Å distance between the RU1 and AU1 sites. The simulations show that the linker is highly flexible and average fluctuations of the central PEG units deviate from the average position in the order of 4-5 Å. The linker does not occupy a defined binding site but samples many different configurations avoiding the hydrophilic protein surface by forming few and transient contacts.
4.2.2 Trinuclear Diruthenium(II)-Gold(I) Complex

A trinuclear diruthenium(II)-gold(I) complex, 2, was devised in order to synthetically stabilise the fluctuating linker of 1 in the aim of resolving its structure when the trinuclear complex is bound to the NCP crystal. By binding simultaneously to both the RU1 and RU2 binding site, igniting the allosteric effect and crosslinking with the newly accessible AU1 site, the polyethylene glycol linkers could be stabilised by having three points of attachment encircling a central connection point. The metal centres of 2 are identical to those of 1 as it was shown in the previous section that they bind to the RU1 and AU1 binding sites on the NCP. A 2-hydroxymethyl-1,3-propanediol group is introduced to the linker to form a three-way central junction. The linker must be modified to introduce a three-way central junction.
The synthetic route deviates from 1 following the coordination of mono-phosphine ligand to the gold(I) centre (b, Scheme 4.2). To synthesise 2, a carboxylic acid moiety is introduced to the free hydroxyl group of the hexaethylene glycol chain via the addition of succinic anhydride under basic, triethylamine conditions. The resulting gold(I) complex, d, was coupled to 2-hydroxymethyl-1,3-propanediol group under standard ester coupling conditions of EDCI and DMAP, as coupling reagent and base, in equivalence that promote the mono-coupled product, e. Due to the low solubility of 2-hydroxymethyl-1,3-propanediol group in CH₂Cl₂, a mixture of CH₂Cl₂ and DMF was used. The synthetic route of 2 now mimics the conditions used by 1 to simultaneously lengthen the linker and introduce primary amines.
Two 15-(9-fluorenyloxycarbonyl)amino-4,7,10,13-tetraoxa-pentadecanoic acids are coupled to the free hydroxyl groups of 2-hydroxymethyl-1,3-propanediol moiety under the EDCI and DMAP coupling conditions. As with the synthesis of 1, complete purification of the resulting complex so the deprotection of the primary amines is performed immediately using 20 % pyridine in DMF. The considerable increase in polarity afforded by the deprotection of the amines allows the product, f, to be purified by flash column chromatography. In the final step, the RAPTA moieties are introduced via the coupling of the [Ru(3-(4-methylcyclohexa-1,4-dien-1-yl)propanoic acid)(PTA)(μ-oxalato)] to the gold(I) complex, f, using TBTU and DIPEA, as coupling reagent and base. The oxalate was removed via washing with concentrated brine solution and the final complex, 2, was titurated with pentane before being dried under vacuum.

![Figure 4.5. Structure of heterobimetallic trinuclear diruthenium(II)-gold(I) complex, 2.](image)

All compounds were fully characterised via $^1$H, $^{31}$P and $^{13}$C NMR spectroscopy, high-resolution mass spectrometry and elemental analysis. The coordination of the diphenyl(phosphino)benzoic acid ligand to the gold centre was tracked via $^{31}$P NMR spectroscopy and the peak remained at 32.98-33.03 ppm throughout the synthesis. The construction of the linker was followed via $^1$H and $^{13}$C NMR spectra where the appearance of the relevant proton and carbon peaks was observed at each step. Synthesis was further confirmed via ESI-MS at each step. The final complex, 2, proved challenging to analyse due to its mycellic nature caused by the addition of the two water-soluble RAPTA complexes to the water-insoluble gold-complex and linker. The characteristic peaks for RAPTA moieties are observed but at a lower integration than expected. However, a successful coupling is
indicated in the $^1$H NMR spectrum by a shift to a lower ppm for the linker protons adjacent to
the amide bond, O-CH$_2$-CH$_2$-NH-(C=O), from 3.90 ppm for O-CH$_2$-CH$_2$-NH$_2$ to 3.35-3.43
ppm for CH$_2$-CH$_2$-NH-(C=O) and from 3.18 ppm for O-CH$_2$-CH$_2$-NH$_2$ to 3.04-3.11 ppm for
O-CH$_2$-CH$_2$-NH-(C=O). The presence of the ruthenium centres in the final complex was also
confirmed in the $^{31}$P NMR spectrum where a peak at 33.02 ppm corresponds to the gold centre
and a peak at -30.73 ppm confirms the presence of the Ru-PTA bond.

4.2.3 *In Vitro* Evaluation of Di- and Trinuclear
Ruthenium(II)-Gold(I) complexes

The cytotoxicity of 1 and 2 was assessed against cisplatin sensitive and cisplatin resistant
human ovarian carcinoma (A2780 and A2780cisR) and non-tumoral human embryonic
kidney (HEK-293) cell lines using the MTT assay (Table 4.1). Auranofin was tested as a
reference with cisplatin and RAPTA-C tested as positive and negative controls.

<table>
<thead>
<tr>
<th>Compound</th>
<th>A2780</th>
<th>A2780cisR</th>
<th>HEK293</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinucler Ru(II)Au(I)</td>
<td>6.9 ± 1.3</td>
<td>8.5 ± 0.8</td>
<td>13.6 ± 1.5</td>
</tr>
<tr>
<td>Trinuclear Ru(II)$_2$Au(I)</td>
<td>7.0 ± 0.5</td>
<td>8.7 ± 0.4</td>
<td>15.6 ± 1.5</td>
</tr>
<tr>
<td>auranofin</td>
<td>1.1 ± 0.1</td>
<td>2.6 ± 0.3</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>cisplatin</td>
<td>1.6 ± 0.9</td>
<td>17 ± 1.8</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>RAPTA-C</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

1 and 2 possess comparable cytotoxicity in the low micromolar range against the selected
cell lines. With similar IC$_{50}$ values against A2780 cells, 6.9 ± 1.3 μM and 7.0 ± 0.5 μM for the
di- and trinuclear respectively, they are less cytotoxic than auranofin and cisplatin. However,
1 and 2 overcome cisplatin resistance with values of 8.5 ± 0.8 μM and 8.7 ± 0.4 μM compared

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to $17 \pm 1.8 \ \mu M$ recorded for cisplatin. Both complexes show a two-fold selectivity towards the tumoural cell line, A2780, over the non-tumoural cell line, HEK293.

The uptake of 1 and 2 onto the chromatin in A2780 cells was assessed in vitro and compared to RAPTA-T and auranofin. The A2780 cells, four flasks per compound prepared to ~80 % confluency (ca. $43 \times 10^6$ cells per flask), were incubated for 2 hours with the compounds at concentrations 500 μM RAPTA, 20 μM auranofin, 20 μM 1 and 20 μM 2. The concentrations were selected based on the IC$_{50}$ values of compounds against the A2780 with the aim of achieving an observable uptake but not to kill the cells. After 2 hours, the cells were washed with ice-cold phosphate-buffered saline solution (PBS) to remove the compounds and the chromatin was extracted using a Pierce Chromatin Prep Module (Thermo Fisher Scientific, Switzerland) according to the manufacturer’s protocol. The chromatin-content of each sample was quantified as μg of DNA content using the Quant-iT PicoGreen dsDNA assay (Invitrogen). The metal content of the samples was quantified using inductively coupled plasma mass spectrometry (ICP-MS).

The values obtained, presented in pmol of metal per μg of DNA, are a quantification of metal accumulated on chromatin but it does differentiate between adducts formed on the histone protein or those on the DNA. The values obtained for control compounds, RAPTA-T and auranofin, 130 ± 13 pmol of ruthenium per μg of DNA and 594 ± 96 pmol of gold per μg of DNA respectively, are comparable to those previously reported.$^{[13]}$ The chromatin uptake of 1 was quantified as 8.9 ± 0.9 pmol of ruthenium and 78 ± 6 pmol of gold per μg of DNA. The discrepancy in the quantity of ruthenium- and gold-chromatin adducts mirrors the observations on the NCP crystal where additional gold adducts are observed on the nucleosomal DNA.

In contrast, 2 forms over two-fold more ruthenium adducts than 1 with a value of 18.1 ± 3.3 pmol per μg of DNA recorded. This correlates to the ratio of ruthenium in the complexes themselves where one ruthenium centre resides in each molecule of 1 complex whilst two ruthenium centres are present in each molecule of 2. A significantly greater quantity of chromatin-bound gold was observed for the trinuclear complex, 8584 ± 1036 pmol per μg of DNA, which exceeds 8-fold that of the gold adducts observed after the combination of A2780 cells with RAPTA-T (500 μM) and auranofin (20 μM), 1006 ± pmol per μg of DNA.$^{[13]}$
4.3 Concluding Remarks

Herein, we have shown that it is possible to crosslink allosteric binding sites on the acidic patch of the nucleosome core particle. The hetero-bimetallic complex, 1, was inspired by the preferential and interrelated binding sites of RAPTA-T and AUF, i.e. with the ruthenium moiety bound to the RU1 site, igniting the allosteric effect and allowing the gold moiety of the same complex to bind in AU1. Although the linker could not be fully resolved in the x-ray crystallographic study, a strip of electron density was observed linking the RU1 and AU1 binding sites. Molecular dynamic simulations showed that the high flexibility of the linker meant that the position of the central PEG units could vary by up to 4-5 Å. Both gold and ruthenium adducts are observed on the chromatin of A2780 cells \textit{in vitro}. In accordance to the x-ray crystallographic study, where additional gold adducts were observed on the DNA after 120 h of incubation, the gold accumulation on chromatin was considerably higher than that of ruthenium.

A related trinuclear diruthenium(II)-gold(I) complex, 2, was synthesised in an attempt to synthetically solve the fluctuating linker issue. 1 and 2 possess comparable cytotoxicity against all tested cell lines. A 2-fold selectivity was observed towards the tumoural A2780 cell line versus the non-tumoural HEK-293 cell line. 2 formed over 2-fold more ruthenium adducts than 1 on chromatin \textit{in vitro} and 8-fold more gold adducts than RAPTA-T and auranofin used in combination.

To the best of our knowledge, crosslinking such distal allosteric sites has not been reported before. The allosteric relationship between RAPTA-T and auranofin is particularly distinctive as related ruthenium(II)-arene complexes such as RAED-C do not elicit an allosteric response. Consequently, the concept of using metal complexes that covalently bind to specific sites in proteins represents a broadly applicable approach to crosslink allosteric sites that could be extended to many other systems.
4.4 Experimental Section

4.4.1 Materials

Chemical reagents were purchased from commercial sources (Sigma Aldrich, Acros and TCI Chemicals) and used without further purification. RuCl$_3$.3H$_2$O was purchased from Precious Metals Online. [Ru(3-(4-methylcyclohexa-1,4-dien-1-yl)propanoic acid)(PTA)(μ-oxalato)] and AuCl(tht) were prepared following a literature procedures.[33] The reactions were performed under an inert atmosphere ($N_2$) using Schlenk techniques. Dry solvents, dried using a PureSolv solvent purification system (Innovative Technology Inc.), were collected and used under an inert atmosphere ($N_2$). Thin Layer Chromatography was conducted on Merck TLC silica gel coated aluminium sheets 60 F254 and verified by UV lamb at 254 nm. Purifications were achieved by column flash chromatography using a CombiFlash Rf$^+$ automated column machine operated with prepacked Luknova columns.

4.4.2 Instrumentation and Methods

$^1$H (400 MHz), $^{31}$P (162 MHz) and $^{13}$C (101 MHz) NMR spectra were recorded on a Bruker Avance II 400 spectrometer at 298 K. Chemical shifts are reported in parts per million (ppm) and referenced to deuterated solvent residual peaks (CDCl$_3$: $^1$H $\delta$ 7.26, $^{13}$C($^1$H) $\delta$ 77.16 ppm) and coupling constants ($J$) are reported in Hertz (Hz). High resolution electrospray ionization mass spectra (HR ESI-MS) were obtained on a Xevo G2-S QTOF mass spectrometer coupled to the Acquity UPLC Class Binary Solvent manager and BTN sample manager (Waters, Corporation, Milford, MA). Elemental analyses were carried out by the microanalytical laboratory at the the Institute of Chemical Sciences and Engineering (EPFL) using a Thermo Scientific Flash 2000 Organic Elemental Analyzer.
4.4.3 Synthesis

4.4.3.1 Synthesis of Dinuclear Ruthenium(II)-Gold(I) complex, 1

**Compound a**

![Chemical Structure](image)

4-(Diphenylphosphino)benzoic acid (3.00 g, 9.794 mmol, 1 equiv.) and N-(N’-3-dimethylaminopropyl)carbodiimide hydrochloride (2.44 g, 12.733 mmol, 1.3 equiv.) were dissolved in CH$_2$Cl$_2$ (30 mL) and stirred under N$_2$ and r.t for 1 hour. The reaction mixture was added dropwise to a solution of hexaethylene glycol (3.10 mL, 12.733 mmol, 1.3 equiv.) and 4-dimethylaminopyridine (0.60 g, 4.897 mmol, 0.5 equiv.), dissolved in CH$_2$Cl$_2$ (5 mL), and the reaction stirred for 18h at r.t. and under N$_2$. An aqueous work up was performed, the reaction mixture was washed with saturated NaCl solution (40 mL) and the combined organic fractions were dried over anhydrous Na$_2$SO$_3$, filtered and dried under reduced pressure. Purification was achieved via flash column chromatography with eluent 100% EtOAc and washing with pentane (3 x 30 mL). The product was isolated as a colourless oil (3.82 g, 6.697 mmol, 68%)

**Elemental Analysis (%):** calcld for C$_{31}$H$_{49}$O$_8$P.\textsuperscript{11} C: 65.98; H: 7.29; found C: 66.20 H: 7.00.

$^1$H NMR (CDCl$_3$, 400 MHz): 7.96-7.99 (2H, m, 2xO-(C=O)-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O), 7.26-7.35 (12H, m, 2xO-(C=O)-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, 4xP-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, 4xP-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, 2xP-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, 4.43-4.43 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 3.78-3.82 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O, 3.56-3.71 (20H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O, Ar-(C=O)-O-CH$_2$-CH$_2$-O, Ar-(C=O)-O-CH$_2$-CH$_2$-O, Ar-(C=O)-O-CH$_2$-CH$_2$-O, Ar-(C=O)-O-CH$_2$-CH$_2$-O).

$^{31}$P \{\textsuperscript{1}H} NMR (CDCl$_3$, 162 MHz): -5.05 (1P).

$^{13}$C \{\textsuperscript{1}H} NMR (CDCl$_3$, 101 MHz): 166.3 (1C, O-(C=O)-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, $^1J_{CP}$ = 14 Hz), 136.2 (2C, d, 2xP-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, $^1J_{CP}$ = 11 Hz), 134.0 (4C, d, 4xP-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, $^1J_{CP}$ = 20 Hz), 133.2 (2C, d, 2xO-(C=O)-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, $^1J_{CP}$ = 11 Hz), 133.0 (2C, d, 2xO-(C=O)-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, $^1J_{CP}$ = 20 Hz), 132.8 (2C, d, 2xO-(C=O)-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, $^1J_{CP}$ = 11 Hz), 132.5 (2C, d, 2xO-(C=O)-(Ar)-C(CH$_2$)$_2$O-CH$_2$-CH$_2$-O, $^1J_{CP}$ = 20 Hz).
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CH-C-P, 2JC,P = 19 Hz), 130.0 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 129.4 (2C, d, 2xO-(C=O)(Ar)C-CH-CH-C-P, 3JC,P = 6 Hz), 129.2 (2C, 2xP-(Ar)C-CH-CH-CH), 128.7 (4C, 4xP-(Ar)CCH-CH-CH, 3JC,P = 7 Hz), 72.6 (1C, Ar-(C=O)-O-((CH2)2-O)5-CH2-CH2-OH), 70.5-70.7 (7C,
Ar-(C=O)-O-(CH2)2-O-CH2-CH2-O-((CH2)2-O)4-H),
Ar-(C=O)-O-((CH2)2-O)2-CH2-CH2-O((CH2)2-O)3-H, Ar-(C=O)-O-((CH2)2-O)2-CH2-CH2-O-((CH2)2-O)3-H, Ar-(C=O)-O-((CH2)2O)3-CH2-CH2-O-((CH2)2-O)2-H, Ar-(C=O)-O-((CH2)2-O)3-CH2-CH2-O-((CH2)2-O)2-H, Ar(C=O)-O-((CH2)2-O)4-CH2-CH2-O-(CH2)2-OH, Ar-(C=O)-O-((CH2)2-O)4-CH2-CH2-O-(CH2)2OH), 70.3 (1C, Ar-(C=O)-O-(CH2)2-O-CH2-CH2-O-((CH2)2-O)4-H), 69.2 (1C, Ar-(C=O)-OCH2-CH2-O-((CH2)2-O)5-H), 64.2 (1C, Ar-(C=O)-O-CH2-CH2-O-((CH2)2-O)5-H), 61.70 (1C,
Ar-(C=O)-O-((CH2)2-O)5-CH2-CH2-OH).
HRMS (ESI-(+)): m/z found 571.2467 [M+H]+ C31H40O8P+ requires 571.2461.

Compound b

Compound a (1.30 g, 2.278 mmol, 1 equiv.) and freshly prepared AuCl(tht) (0.73 g, 2.278
mmol, 1 equiv.) were dissolved in CH2Cl2 (30 mL) and stirred under N2 and r.t for 18 hours.
The reaction mixture was concentrated in vacuo and the product was purified via flash
colomn chromatography with eluent 100% EtOAc. The product was isolated as a colourless
oil (1.46 g, 1.818 mmol, 80 %).

Elemental Analysis (%): calcd for C31H49AuClO8P C 46.37 H 4.90; found C 46.25 H 4.69.
1

3

4

H NMR (CDCl3, 400 MHz): 8.03-8.11 (2H, dd, 2xO-(C=O)-(Ar)C-CH-CH-C-P, JH,H = 8.5 Hz, JH,H =
2.0 Hz), 7.45-7.57 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH), 4.45-4.47 (2H m, Ar-(C=O)-O-CH2-CH2-O),
3.78-3.81 (2H, m, Ar-(C=O)-O-CH2-CH2-O), 3.53-3.67 (20H, m, Ar-(C=O)-O-(CH2)2-O(CH2)2, Ar-(C=O)-O-(CH2)2-O-(CH2)2-O-(CH2)2, Ar-(C=O)-O-(CH2)2-O-(CH2)2-O-(CH2)2-O(CH2)2, Ar-(C=O)-O-(CH2)2-O-(CH2)2-O-(CH2)2-O-(CH2)2-O-(CH2)2, Ar-(C=O)-O-(CH2)2-O(CH2)2-O-(CH2)2-O-(CH2)2-O-(CH2)2-O-(CH2)2.

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P {1H} NMR (CDCl3, 162 MHz): 32.98 (1P).

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C {1H} NMR (CDCl3, 101 MHz): 165.4 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 134.2 (1C, d, O(C=O)-(Ar)C-CH-CH-C-P, 1JC,P = 60 Hz), 134.2 (4C, d, 4xP-(Ar)C-CH-CH-CH, 2JC,P = 14 Hz),
133.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 2JC,P = 14 Hz), 133.3 (1C, O-(C=O)-(Ar)C-CHCH-C-P, 4JC,P = 3 Hz), 132.4 (2C, 2xP-(Ar)C-CH-CH-CH, 4JC,P = 3 Hz), 130.2 (2C, d, 2xO(C=O)-(Ar)C-CH-CH-C-P, 3JC,P = 12 Hz), 129.5 (4C, 4xP-(Ar)C-CH-CH-CH, 3JC,P = 12 Hz),
127.9 (2C, d, 2xP-(Ar)C-CH-CH-CH, 1JC,P = 63 Hz), 72.7 (1C, Ar-(C=O)-O-((CH2)2-O)5-CH2CH2-OH), 70.4-70.6 (7C, Ar-(C=O)-O-(CH2)2-O-CH2-CH2-O-((CH2)2-O)4-H), Ar-(C=O)-O((CH2)2-O)2-CH2-CH2-O-((CH2)2-O)3-H, Ar-(C=O)-O-((CH2)2-O)2-CH2-CH2-O-((CH2)2-O)3-H,
Ar-(C=O)-O-((CH2)2-O)3-CH2-CH2-O-((CH2)2-O)2-H, Ar-(C=O)-O-((CH2)2-O)3-CH2-CH2-O-

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((CH$_2$)$_2$-O)$_2$-H, Ar-(C=O)-O-((CH$_2$)$_2$-O)-CH$_2$-CH$_2$-O-(CH$_2$)$_2$-OH, Ar-(C=O)-O-((CH$_2$)$_2$-O)-CH$_2$-CH$_2$-O-(CH$_2$)$_2$-OH), 70.1 (1C, Ar-(C=O)-O-((CH$_2$)$_2$-O)-CH$_2$-CH$_2$-O-(CH$_2$)$_2$-OH), 69.1 (1C, Ar-(C=O)-O-CH$_2$-CH$_2$-O-(CH$_2$)$_5$-H), 64.7 (1C, Ar-(C=O)-O-CH$_2$-CH$_2$-O-(CH$_2$)$_5$-H), 61.6 (1C, Ar-(C=O)-O-((CH$_2$)$_5$-CH$_2$-CH$_2$-OH).

HRMS (ESI(+)); $m/z$ found 825.1644 [M+Na]$^+$ C$_{31}$H$_{39}$AuClO$_8$PNa$^+$ requires 825.1635.

**Compound c**

![Compound c structure](image)

1-(9H-Fluoren-9-yl)-3-oxo-2, 7, 10, 13, 16-pentaoxa-4-azononadecan-19-oic acid (0.50 g, 1.021 mmol, 1 equiv.) and N-(N'-3-dimethylaminopropyl)carbodiimide hydrochloride (0.25 g, 1.327 mmol, 1.3 equiv.) were dissolved in CH$_2$Cl$_2$ (20 mL) and stirred under N$_2$ and r.t for 1 hour. **Compound b** (0.82 g, 1.021 mmol, 1 equiv.) and 4-dimethylaminopyridine (0.06 g, 0.510 mmol, 0.5 equiv.) were added and the reaction stirred under N$_2$ and r.t for 18 hours. An aqueous work up was performed, the reaction mixture was washed with saturated NaCl solution (40mL) and the combined organic fractions were dried over anhydrous Na$_2$SO$_4$, filtered and dried under reduced pressure. Flash column chromatography was performed with eluent system CH$_2$Cl$_2$/CH$_3$OH and the Fmoc-protected product was obtained in a mixture with unreacted Compound b. The primary amine was deprotected with 20 % piperidine in dimethylformamide for 4 hours under N$_2$ and r.t. The dimethylformamide was removed under vacuum and flash column chromatography was performed with eluent system CH$_2$Cl$_2$/CH$_3$OH.

**Compound c** was isolated as a yellow oil (0.31 g, 0.292 mmol, 29 %).

**Elemental Analysis (%):** calc'd for C$_{42}$H$_{60}$AuClNO$_{13}$P.$\text{C}^\text{1/3}$CH$_2$Cl.$\text{C}^\text{1/3}$OH C 46.86 H 5.87 N 1.26; found C 46.73 H 6.27 N 1.29.

$^1$H NMR (CDCl$_3$, 400 MHz): 8.04-8.07 (2H, dd, 2xO-(C=O)-(Ar)c-C-H-C-c-P, $^3$J$_{HH}$ = 8.3 Hz, $^3$J$_{HH}$ = 2.3 Hz), 7.42-7.52 (12H, m, 2xO-(C=O)-(Ar)c-C-H-C-c-P, 4xP-(Ar)c-C-H-C-c-Ch, 4xP-(Ar)c-C-H-c-C-H, 4P-(Ar)c-C-c-H-c-H, 4P-(Ar)c-C-c-H-c-Ch), 4.41-4.44 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 4.17-4.20 (2H, m, Ar-(C=O)-O-((CH$_2$)$_5$-CH$_2$-O), 3.80-3.82 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 3.75-3.78 (2H, m, H$_2$N-((CH$_2$)$_4$-CH$_2$-O)-C-(C=O)), $^3$J$_{HH}$ = 6.1 Hz), 3.69-3.72 (2H, m, H$_2$N-C-H$_2$), 3.56-3.65 (30H, m, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$), $^3$J$_{HH}$ = 6.1 Hz, 5.14-5.17 (2H, m, H$_2$N-C-H$_2$), 2.57-2.60 (2H, t, H$_2$N-((CH$_2$)$_4$-CH$_2$-O)-C-(C=O)), $^3$J$_{HH}$ = 6.1 Hz).
\[^{31}\text{P}\] {\text{H}}\text{NMR (CDCl}_3\text{, 162 MHz): 33.02 (1P).}\n
\[^{13}\text{C}\] {\text{H}}\text{NMR (CDCl}_3\text{, 101 MHz): 172.3 (1C, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 60 Hz), 134.3 (4C, d, 4xP-(Ar)-O-(C}_\text{H}_2-\text{O})-O-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 14 Hz), 134.0 (2C, d, 2xO-(C}=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 14 Hz), 133.3 (1C, O-(C}=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 14 Hz), 132.4 (2C, 2xP-(Ar)-O-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 12 Hz), 129.5 (4C, 4xP-(Ar)-O-(C}=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 12 Hz), 127.9 (2C, d, 2xP-(Ar)-O-(C}=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 12 Hz), 70.6-70.8 (10C, m, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 12 Hz), 70.1-70.2 (4C, m, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, 'J_{ CP} = 12 Hz), 69.2 (1C, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, 69.1 (1C, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P, 67.0 (1C, H-N-(C}_\text{H}_2-\text{O})-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P), 66.7 (1C, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P), 64.8 (1C, Ar-(C}=\text{O})-O-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P), 64.1 (1C, H-N-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P), 40.4 (1C, H-N-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P), 35.0 (1C, H-N-(C}_\text{H}_2-\text{O}-(C)=\text{O})-(\text{Ar})C-CH-CH-C-P).

**HRMS (ESI-(+)):** m/z found 1050.3253 [M+H]\textsuperscript{+} \text{C}_{42}\text{H}_{61}\text{AuClNO}_{13}\text{P} requires 1050.3235, 1014.3508 [M-Cl]\textsuperscript{+} requires 1014.3468.

**Complex 1**

![Complex 1](image)

[Ru(3-(4-methylcyclohexa-1,4-dien-1-yl)propanoic acid)(PTA)(\text{μ-oxalato})] (0.066 g, 0.129 mmol, 1.5 equiv.) was stirred with O-(Benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate (0.050 g, 0.154 mmol, 1.8 equiv.) and N, N-diisopropylethylamine (0.055 g, 0.428 mmol, 5 equiv.) in dimethylformamide for 20 minutes under N\textsubscript{2} at r.t.. **Compound c** (0.09 g, 0.085 mmol, 1 equiv.) was added and the reaction stirred for 1 hour and reaction evolution was followed via mass spectrometry. The dimethylformamide was removed and the crude was dried under vacuum for 30 minutes. The crude was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (30mL) and washed with saturated NaCl solution (4 x 30 mL). The organic layer was stirred with saturated NaCl solution (50 mL) for 2 hours, the organic layer was then collected, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, filtered and concentrated under reduced pressure. The product was precipitated using CH\textsubscript{2}Cl\textsubscript{2}/pentane and was further washed with pentane (3 x 20 mL). The product was isolated as a red, oily solid (0.088 g, 0.057 mmol, 68%).
**Elemental Analysis (%):** calcd for C₅₈H₈₂AuCl₃N₄O₁₄P₂Ru.C₅H₁₂ C 47.36 H 5.93 N 3.45; found C 47.13 H 6.06 N 3.51.

\(^1\)H NMR (CDCl₃, 400 MHz): 9.30 (1H, bs, HN-(C=O)-CH₂-CH₂-Ar), 8.10-8.13 (2H, dd, 2xO-(C=O)-(Ar)C-CH₂-CH₂-P, J_H,H = 8.4 Hz, J_C,P = 2.2 Hz), 7.47-7.60 (12H, m, 2xO-(C=O)-(Ar)C-CH₂-CH₂-P, J_C,P = 14 Hz), 5.59-5.61 (1H (2H), d, N-(C=O)-CH₂-CH₂-(Ar)C-CH₂-CH₂-P, J_H,H = 5.6 Hz), 5.50-5.51 (1H (2H), d, N-(C=O)-CH₂-CH₂-(Ar)C-CH₂-CH₂-P, J_H,H = 5.7 Hz), 4.52-4.54 (2H (6H), m, PTA), 4.47-4.49 (2H (6H), m, PTA), 4.31-4.31 (2H (6H), m, PTA), 4.21-4.23 (2H, m, Ar-(C=O)-O-((CH₂)₂-O)₅-(C=O)), 3.81-3.83 (2H, m, Ar-(C=O)-O-((CH₂)₂-O)₅-(C=O)), 3.39-3.41 (2H, m, (C=O)-NH-(CH₂)₂-O), 2.58-2.64 (6H, m, (C=O)-N-((CH₂)₂-O)₄-CH₂-CH₂), 2.05 (1H (3H), s, (Ar)C-CH₂-CH₂-P)

\(^{31}\)P {\(^1\)H} NMR (CDCl₃, 162 MHz): 33.07 (1P, Au-P), -33.68 (1P, Ru-P).

\(^{13}\)C {\(^1\)H} NMR (CDCl₃, 101 MHz): 172.3 (1C, N-(C=O)-CH₂-CH₂-Ar), 171.6 (1C, Ar-(C=O)-O-CH₂-CH₂-O-((CH₂)₂-O)₅-(C=O)), 165.4 (1C, O-(C=O)-(Ar)C-CH₂-CH₂-P, J_C,P = 14 Hz), 134.3 (1C, d, O-(C=O)-(Ar)C-CH₂-CH₂-P, J_C,P = 58 Hz), 134.3 (4C, d, 4xP-(Ar)C-CH₂-CH₂-P, J_C,P = 14 Hz), 134.0 (2C, d, 2xO-(C=O)-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 132.4 (4C, d, 4xP-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 131.4 (4C, d, 4xP-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 129.5 (4C, d, 4xP-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 128.0 (2C, d, 2xP-(Ar)C-CH₂-CH₂-P, J_C,P = 58 Hz), 127.8 (2C, d, 2xP-(Ar)C-CH₂-CH₂-P, J_C,P = 58 Hz), 98.8 (1C, N-(C=O)-CH₂-CH₂-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 97.8 (1C, N-(C=O)-CH₂-CH₂-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 88.7 (2C, N-(C=O)-CH₂-CH₂-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 79.8-80.7 (2C, N-(C=O)-CH₂-CH₂-(Ar)C-CH₂-CH₂-P, J_C,P = 12 Hz), 66.6 (1C, (C=O)-NH-(CH₂)₂-O-((CH₂)₂-O)₅-(C=O), 64.8 (1C, Ar-(C=O)-O-CH₂-CH₂-P, J_C,P = 12 Hz), 63.8 (1C, Ar-(C=O)-O-((CH₂)₂-O)₅-(C=O)), 51.7 (3C, PTA), 39.4 (1C, (C=O)-NH-(CH₂)₂-O-((CH₂)₂-O)₅-(C=O)), 31.4 (1C, residual pentane, 29.8 (1C, C=O)-NH-(CH₂)₂-O-((CH₂)₂-O)₅-(C=O)), 22.7 (2C, residual pentane), 21.1 (1C, N-(C=O)-CH₂-CH₂-P, J_C,P = 12 Hz), 14.2 (1C, residual pentane).

HRMS (ESI-(+)): m/z found 1489.3344 [M-Cl]⁺ C₅₈H₈₂AuCl₃N₄O₁₄P₂Ru⁺ requires 1489.3389.
4.4.3.2 Synthesis of Trinuclear Diruthenium(II)-Gold(I) Complex, 2

**Compound d**

![Chemical Structure Image]

**Compound b** (0.58 g, 0.72 mmol, 1 equiv.) and succinic anhydride (0.09 g, 0.90 mmol, 1.2 equiv.) were dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (5 mL). Triethylamine (0.22 g, 2.17 mmol, 0.3 mL, 3 equiv.) was added and the reaction stirred at RT under N\textsubscript{2} for 1 h. The solvent was removed via rotary evaporation and the product purified via flash column chromatography using CH\textsubscript{2}Cl\textsubscript{2}/CH\textsubscript{3}OH as eluent. The product was washed with pentane (3 x 15 mL) and isolated as a colourless oil (0.45 g, 0.498 mmol, 69%).

**Elemental Analysis (%)**: calcld for C\textsubscript{35}H\textsubscript{43}AuClO\textsubscript{11}P C 46.55 H 4.80 found C 46.90 H 4.52.

\textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz): 8.08-8.10 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.45-7.57 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH-H), 4.45-4.47 (2H, s, Ar-(C=O)-O-CH\textsubscript{2}-CH\textsubscript{2}-O), 4.20-4.22 (2H, m, O-CH=CH-CH-O), 3.79-3.81 (2H, m, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), 3.58-3.67 (18H, m, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, 2.60 (4H, s, O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}.

\textsuperscript{31}P \{\textsuperscript{1}H\} NMR (CDCl\textsubscript{3}, 162 MHz): 32.99 (1P).

\textsuperscript{13}C \{\textsuperscript{1}H\} NMR (CDCl\textsubscript{3}, 101 MHz): 175.8 (1C, O-(CH\textsubscript{2})\textsubscript{2}-O-(C=O)-(CH\textsubscript{2})\textsubscript{2}-(C=O)-OH), 172.1 (1C, O-(CH\textsubscript{2})\textsubscript{2}-O-(C=O)-(CH\textsubscript{2})\textsubscript{2}-(C=O)-OH), 165.3 (1C, O-(C=O)-(Ar)C-CH-CH-CH-P), 134.3 (1C, d, O-(C=O)-(Ar)C-CH-CH-CH-P, \textsuperscript{1}J_{C,P} = 60 Hz), 134.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-CH-P, \textsuperscript{1}J_{C,P} = 14 Hz), 133.9 (4C, d, 4xP-(Ar)C-CH-CH-CH, \textsuperscript{2}J_{P,C} = 14 Hz), 133.2 (1C, O-(C=O)-(Ar)C-CH-CH-CH-P, \textsuperscript{1}J_{C,P} = 14 Hz), 132.4 (2C, d, 2xP-(Ar)C-CH-CH-CH-H, \textsuperscript{1}J_{P,C} = 3 Hz), 130.1 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-CH-P, \textsuperscript{2}J_{P,C} = 12 Hz), 129.5 (4C, d, 4xP-(Ar)C-CH-CH-CH, \textsuperscript{1}J_{P,C} = 12 Hz), 127.8 (2C, d, 2xP-(Ar)C-CH-CH-CH, \textsuperscript{1}J_{P,C} = 63 Hz), 70.4-70.6 (8C, Ar-(C=O)-O-(CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, Ar-(C=O)-((CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}), Ar-(C=O)-((CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}, Ar-(C=O)-((CH\textsubscript{2})\textsubscript{2}-O-(CH\textsubscript{2})\textsubscript{2}).

**HRMS (ESI(+)-QTOF)**: m/z found 901.1823 [M\textsuperscript{+}] C\textsubscript{35}H\textsubscript{43}AuClO\textsubscript{11}P\textsuperscript{+} requires 901.1819.

**Compound e**
**Compound d** (1.30 g, 1.44 mmol, 1 equiv.) and *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.36 g, 1.87 mmol, 1.3 equiv.) were dissolved in dry HCON(CH$_3$)$_2$ (6 mL) and stirred for 1 h. The solution was added dropwise to a solution of 2-hydroxymethyl-1,3-propanediol (0.23 g, 2.16 mmol, 1.5 equiv.) and 4-(dimethylamino)pyridine (0.09 g, 0.72 mmol, 0.5 equiv.) in HCON(CH$_3$)$_2$ (3 mL) and the reaction stirred for 48 h. The solvent was removed with N$_2$ and the crude was redissolved in CH$_2$Cl$_2$ (20 mL) and washed with brine (30 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered under gravity and concentrated via rotary evaporator. Flash column chromatography was performed using CH$_2$Cl$_2$/CH$_3$OH as eluent. The product was isolated as a colourless oil (0.71 g, 0.72 mmol, 50 %).

**Elemental Analysis (%)**: calc'd for C$_{39}$H$_{51}$AuClO$_{13}$P C 47.26 H 5.19; found C 47.27 H 5.36.

$^1$H NMR (CDCl$_3$, 400 MHz): 8.09-8.12 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.47-7.59 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH, 2xP-(Ar)C-CH-CH-CH), 4.46-4.49 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 4.21-4.23 (4H, m, O-CH$_2$-CH$_2$-O-(C=O)-CH$_2$, CH-CH$_2$-O-(C=O)), 3.80-3.82 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 3.61-3.71 (22H, m, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_2$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_3$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_4$-(CH$_2$)$_2$, 2xCH-CH$_2$-O), 2.63 (4H, s, O-(CH$_2$)$_2$-O-(C=O)-(CH$_2$)$_2$), 2.01 (1H, s, CH-(CH$_2$-O)$_2$);

$^{31}$P {${^1}$H} NMR (CDCl$_3$, 162 MHz): 33.01 (1P).

$^{13}$C {${^1}$H} NMR (CDCl$_3$, 101 MHz): 175.8 (1C, O-(CH$_2$)$_2$-O-(C=O)-(CH$_2$)$_2$-(C=O)-OH), 172.1 (1C, O-(CH$_2$)$_2$-O-(C=O)-CH$_2$), 165.4 (1C, O-(C=O)-ArC-CH-CH-C-P), 134.3 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 60 Hz), 134.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 14 Hz), 134.0 (4C, d, 4xP-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 14 Hz), 133.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 3 Hz), 132.4 (2C, d, 2xP-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 3 Hz), 130.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 12 Hz), 129.5 (4C, d, 4xP-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 12 Hz), 127.9 (2C, d, 2xP-(Ar)C-CH-CH-C-P, $^3$J$_{C,P}$ = 63 Hz), 70.4-70.6 (8C, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-Ar, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_2$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_3$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_4$-(CH$_2$)$_2$, 69.11, 69.13 (2C, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 64.8 (1C, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 64.0 (1C, O-CH$_2$-CH$_2$-O-(C=O)-CH$_2$), 62.8 (2C, CH$_2$-CH$_2$-CH$_2$-OH), 62.2 (2C, CH-(CH$_2$-OH)), 42.4 (1C, CH$_2$-CH$_2$-OH), 29.20, 29.26 (2C, O-(CH$_2$)$_2$-O-(C=O)-(CH$_2$)$_2$).

**HRMS (ESI(+)-QTOF)**: m/z found 1013.2310 [M+Na]$^+$ C$_{39}$H$_{51}$AuClO$_{13}$PNa$^+$ requires 1013.2319.
Compound f

1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nonaaza-4-azahentriacontan-31-oic acid (0.185 g, 0.278 mmol, 2.3 equiv.) and N-ethyl-N′(3-dimethylanilino)carbodiimide hydrochloride (0.058 g, 0.303 mmol, 2.5 equiv.) were dissolved in dry CH₂Cl₂ (5 mL) and stirred for 1 h. The solution was added to Compound e (0.120 g, 0.121 mmol, 1 equiv.) and 4-(dimethylamino)pyridine (0.007 g, 0.061 mmol, 0.5 equiv.) in CH₂Cl₂ (3 mL) and the reaction stirred for 18 h under N₂ at r.t. The reaction mixture was washed with brine (30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered under gravity and concentrated via rotary evaporation. Flash column chromatography was performed using CH₂Cl₂/CH₃OH as eluent and the Fmoc-protected product was obtained in a mixture with unreacted Compound e. The Fmoc-protected amine was deprotected with 20 % piperidine in dimethylformamide for 1 hours under N₂ and r.t. The dimethylformamide was removed under vacuum and flash column chromatography was performed with eluent system CH₂Cl₂/CH₃OH. Compound f was isolated as a yellow oil (0.071 g, 0.060 mmol, 50 %).

Elemental Analysis (%): calcd for C₇₇H₁₂₅AuClN₂O₃₁P.2CH₂Cl₂ C 47.25 H 6.48 N 1.40; found C 47.08 H 6.77 N1.46.

¹H NMR (CDCl₃, 400 MHz): 8.09-8.12 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.93 (4H, bs, 2xN(H)), 7.48-7.59 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH₂-CH₂, 4xP-(Ar)C-CH₂-CH₂, 4xP-(Ar)C-CH₂-CH₂, 4xP-(Ar)C-CH₂-CH₂), 4.47-4.49 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 4.20-4.23 (2H, m, O-CH₂-CH₂-O-(C=O)-(CH₂)₂-(C=O)), 4.12-4.14 (6H, m, CH-(CH₂-O-(C=O)), 3.90 (4H, bs, 2xO-CH₂-CH₂-NH₂), 3.80-3.82 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.55-3.74 (78H, m, Ar-(C=O)-O-(CH₂)₂-(C=O), Ar-(C=O)-O-(CH₂)₂-(C=O), Ar-(C=O)-O-(CH₂)₂-(C=O), Ar-(C=O)-O-(CH₂)₂-(C=O)), 3.18 (4H, bs, 2xO-CH₂-CH₂-NH₂), 2.57-2.64 (8H, m, CH-CH₂-O-(C=O)-(CH₂)₂-(C=O), 2xH₂N-(CH₂)₂-O-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂, 2xH₂N-((CH₂)₂-O)₂-(CH₂)₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 33.03 (1P).
$^{13}$C ($^1$H) NMR (CDCl$_3$, 101 MHz): 172.3 (1C, (C=O)-(CH$_3$)-(C=O)-CH=CH), 171.4 (2C, 2xCH-CH$_2$O-(C=O)-(CH$_3$)$_2$-O), 165.4 (1C, O-(C=O)-(Ar)C=CH-CH-C-P), 134.4 (1C, d, O-(C=O)-(Ar)C=CH-CH-C-P, $^{1}J_C,P = 60$ Hz), 134.3 (2C, d, 2xO-(C=O)-(Ar)C=CH-CH-C-P, $^{1}J_C,P = 14$ Hz), 133.3 (1C, O-(C=O)-(Ar)C=CH-CH-C-P, $^{1}J_C,P = 3$ Hz), 132.4 (2C, d, 2xP-(Ar)C=CH-CH, $^{3}J_C,P = 12$ Hz), 128.0 (2C, d, 2xP-(Ar)C=CH-CH, $^{3}J_C,P = 62$ Hz), 69.9-70.8 (36C, (Ar)-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-(C=O)), 66.9 (2C, CH-CH$_2$O-(C=O)-CH=CH), 66.5 (2C, 2xH$_2$N-CH$_2$O), 64.8 (1C, (Ar)-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$-(C=O)), 62.1 (1C, CH=CH-O-(C=O)-(CH$_2$)$_2$-(C=O)), 60.8 (2C, 2xCH-CH$_2$O-(C=O)-(CH$_2$)$_2$), 40.6 (2C, 2xH$_2$N-CH$_2$O), 37.5 (1C, C=CH-CH$_2$O-(C=O)), 35.0 (2C, 2xCH-CH=CH-(C=O)-CH=CH, 29.8 (residual H grease), 29.0 (2C, O-(CH$_2$)$_2$-O-(C=O)-(CH$_2$)$_2$-(C=O).

HRMS (ESI(+)-QTOF): m/z found 901.3904 [M-Cl+H]$^{2+}$ C$_{77}$H$_{126}$AuN$_2$O$_{31}$P$_2$ requires 901.3874.

**Complex 2**

[Ru(3-(4-methylcyclohexa-1,4-dien-1-yl)propanoic acid)(PTA)(μ-oxalato)] (0.017 g, 0.0325 mmol, 2.3 equiv.) was stirred with O-(Benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate (0.011 g, 0.0354 mmol, 2.5 equiv.) and N, N-diisopropylethylamine (0.009 g, 0.0707 mmol, 5 equiv.) in dimethylformamide (2 mL) for 45 minutes under N$_2$ at r.t. **Compound f** (0.026 g, 0.00141 mmol, 1 equiv.) was added and the reaction stirred for 1 hour. The dimethylformamide was removed and the crude was dissolved in CH$_2$Cl$_2$ (10mL) and washed with saturated NaCl solution (20 mL) under agitation for 45 minutes. The organic layer was then collected, dried over anhydrous Na$_2$SO$_4$, filtered and...
concentrated to 3 mL and filtered through a fine filter. The product washed with pentane (3 x 25 mL) and isolated as a red, oily solid (0.034 g, 0.0123 mmol, 86 %).

**Elemental Analysis (%):** calculated for C109H169AuCl5N8O33P3Ru2.CDCl3: C 44.01, H 5.76, N 3.70; found C 44.29, H 5.83, N 3.68.

**1H NMR (CDCl3, 400 MHz):**

- 10.83 (1H (2H), bs, N-H-(C=O), 8.09-8.11 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.45-7.57 12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4.83 (6H (12H), bs, PTA), 4.34-4.46 (5H (8H), m, N-(C=O)-CH2-CH2-(Ar)C-CH-CH-C-CH3, N-(C=O)-CH2-CH2-(Ar)C-CH-CH-C-CH3, 3.79-3.81 (2H, m, Ar-(C=O)-O-CH2-CH2-O, 3.59-3.73 (78H, m, Ar-(C=O)-O-CH2-CH2-O), 3.35-3.43 (4H, m, O-C H2-CH2-NH-(C=O)), 3.04-3.11 (4H, m, O-CH2-CH2-NH-(C=O)), 2.87-2.93 (4H (8H), HN-(C=O)-(CH 2)2-(Ar)), 2.56-2.65 (8H, m, CH-CH 2-O-(C=O)-(CH2)2-(C=O), 2xCH-CH 2-O-(C=O)-CH2-CH2-O), 2.37-2.42 (1H, m, (Ar)C-CH-CH-C-CH3).

**31P {1H} NMR (CDCl3, 162 MHz):**

- 33.02 (2P, Au-P), -29.01 (21P, Ru-P).

**13C {1H} NMR (CDCl3, 101 MHz):**

- 172.4 (2C, HN-(C=O)-(CH2)2-(Ar)), 172.3 (1C, (C=O)-(CH2)2-(C=O)-(CH2)2-(Ar)), 172.1 (1C, O-(CH2)2-(C=O)-(CH2)2-(Ar)), 171.3 (2C, 2xCH-CH2-O-(C=O)-(CH2)2-(Ar)), 165.4 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 134.0 (2C, d, O-(C=O)-(Ar)C-CH-CH-C-P, 1JCP = 60 Hz), 134.3 (4C, d, 4xP-(Ar)C-CH-CH-C-P, 1JCP = 14 Hz), 133.3 (1C, O-(C=O)-(Ar)C-CH-CH-C-P, 1JCP = 14 Hz), 132.4 (2C, d, 2xP-(Ar)C-CH-CH-C-P, 1JCP = 3 Hz), 132.0 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 1JCP = 12 Hz), 129.5 (4C, d, 4xP-(Ar)C-CH-CH-C-P, 1JCP = 6 Hz), 128.0 (2C, d, 2xP-(Ar)C-CH-CH-C-P, 1JCP = 6 Hz), 98.2-98.7 (4C, HN-(C=O)-(CH2)2-(C=O)-(Ar)C-CH-CH-C-P), 88.2-89.2 (4C, HN-(C=O)-(CH2)2-(C=O)-(Ar)C-CH-CH-C-P), 72.6 (6C, PTA), 70.4-70.7 (38C, HN-(C=O)-(CH2)2-(C=O)-(Ar)C-CH-CH-C-P), 69.11, 69.13 (2C, (Ar)-(C=O)-O-CH2-CH2-O, O-CH2-CH2-O-(C=O)-(CH2)2-(C=O)), 66.5 (2C, CH-CH2-O-(C=O)-(CH2)2-(C=O)), 64.8 (1C, (Ar)-(C=O)-O-CH2-CH2-O, 64.0 (1C, O-CH2-CH2-O-(C=O)-(CH2)2-(C=O)), 62.0 (1C, CH-CH2-O-(C=O)-(CH2)2-(C=O)), 61.9 (2C, 2xCH2-CH2-O-(C=O)-(CH2)2-(C=O)), 53.9 (residual CHCl3), 50.2-50.6 (6C, PTA), 42.2 (2C, O-CH2-CH2-NH-(C=O), 38.5 (2C, HN-(C=O)-CH2-CH2-(Ar)C-CH-CH-C-P), 37.4 (1C, CH-(C=O)-(C=O)), 35.0 (2C, 2xCH2-CH2-O-(C=O)-(CH2)2-(C=O)), 31.9 (residual DMF), 30.4 (2C, HN-(C=O)-CH2-CH2-(Ar)C-CH-CH-C-P), 29.6 (residual H grease), 29.0 (2C, O-(C=O)-(C=O)-(CH2)2-(C=O)), 22.8 (residual CH2Cl2), 21.1 (2C, HN-(C=O)-(CH2)2-(Ar)C-CH-CH-C-P), 14.2 (residual C5H12).

**HRMS (MALDI(+)-QTOF):**

- m/z found 1394.3649 \([M]^+\) requires 1394.3602.
4.4.4 Crystallographic Analysis of Treated Nucleosome Core Particle

X-ray crystallographic analysis was conducted using NCP assembled with recombinant Homo sapiens histones and a 145 bp DNA fragment. The hanging droplet method was used to grow NCP crystals from buffers containing MnCl₂, KCl and K-cacodylate [pH 6.0]. Crystals were harvested and transferred into a stabilization buffer (37 mM MnCl₂, 40 mM KCl, 20 mM K-cacodylate [pH 6.0], 24% 2-methyl-2,4-pentanediol and 2% trehalose). MgSO₄ was substituted in place of MnCl₂ by thorough rinsing of crystals with a magnesium buffer (10 mM MgSO₄, 20 mM K-cacodylate [pH 6.0], 24% 2-methyl-2,4-pentanediol and 2% trehalose).

To obtain structural data on 1 adduct formation in the nucleosome core, native NCP crystals were subjected to 28-, 54-, 97- and 120-hour incubation with magnesium buffer containing 1 mM 1. Treated crystals were mounted directly into a cryocooling N₂ gas stream set at −175º C. X-ray diffraction data were recorded at beam line X06DA of the Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland) with a Pilatus 2M-F detector at a wavelength corresponding to the X-ray absorption edge of gold (1.04 Å). Data were processed using the in-house processing pipeline, go.com, developed by the Swiss Light Source macromolecular crystallography beamlines. X-ray data were additionally edited with Phenix.

Initial solution of the 1-NCP adduct structure was achieved by first solving the 1.99 Å resolution structure of native NCP, assembled with recombinant Homo sapiens histones and a 145 bp DNA fragment, by molecular replacement with the 2.5 Å resolution structure of native NCP, assembled with recombinant Xenopus laevis histones and the same 145 bp DNA fragment, as the reference model. This native human-histone NCP model and the crystal structure of (Xenopus-histone) NCP containing RAPTA-C adducts (pdb code 3MNN) were subsequently used as reference models to solve the 1-NCP structure. Coot and routines from the CCP4 suite were used to conduct model building and structural refinement. Small molecule crystal structures of RAPTA compounds, [Au(2-
isopropylimidazole)(Pcyclohexyl3)\textsuperscript{[44]} and [Au(4-(diphenylphosphanyl)-benzoic acid)Cl]\textsuperscript{[45]} were used to compose stereochemical restraint parameters for the 1 adduct.

\section{4.4.5 Classical Molecular Dynamic Simulations}

Classical MD has been used to equilibrate the aforementioned systems and to perform the production runs. The AMBER force field ff14SB with the ff99bsc0\textsuperscript{[46][47]} modifications for DNA was adopted for the system. The TIP3P\textsuperscript{[48]} model has been employed for the description of explicit water molecules. Non-standard parameters for the polyethylene glycol linker have been taken from previous studies.\textsuperscript{[10][49][50]} The atomic partial charges were based on QM calculations of the investigated drug using the RESP method.\textsuperscript{[51]} The AMBER 16 package\textsuperscript{[52-55]} was used as MD engine. The system was simulated with a time step of 2 fs. Hydrogen atoms were added assuming standard bond lengths and were constrained to their equilibrium bond lengths with the SHAKE algorithm implemented in AMBER. The system was studied in the isothermal-isobaric (NPT) ensemble coupled to a Langevin thermostat at 300 K and a collision frequency of 1.0 ps\textsuperscript{-1} and a barostat at 1 atm. The Particle Mesh Ewald (PME) method was used to evaluate long-range electrostatic interactions and a cutoff of 10 Å was used to account for the van der Waals interactions, a 10 Å was also used for the short range for the PME. All the simulations were carried out with the following protocol: 1) the system was subjected to energy minimization by using the Steepest Descent algorithm; 2) the system was heated up gradually and finally thermalized at physiological temperature (310K) in the canonical ensemble (NVT) using a Langevin bath; 3) switch to NPT ensemble performing ~1.8 ns of MD at 310 K. After this initial phase, production runs were carried out in the NVT ensemble at 310 K. For the nucleosomal adduct, we collected a total of ~ 500 ns of MD simulations for the system. Coordinates of the system were collected every 20 ps for a total of ~25’000 frames. The statistical analysis was performed on the fully equilibrated second half of the trajectory of the simulation.
4.4.6 Cell Culture and Cytotoxicity Studies

Human ovarian carcinoma (A2780 and A2780cisR) cell lines were obtained from the European Collection of Cell Cultures. The human embryonic kidney (HEK-293) cell line was obtained from ATCC (Sigma, Buchs, Switzerland). Penicillin streptomycin, RPMI 1640 GlutaMAX (where RPMI = Roswell Park Memorial Institute), and DMEM GlutaMAX media (where DMEM = Dulbecco’s modified Eagle medium) were obtained from Life Technologies, and fetal bovine serum (FBS) was obtained from Sigma. The cells were cultured in RPMI 1640 GlutaMAX (A2780 and A2780cisR) and DMEM GlutaMAX (HEK-293) media containing 10% heat-inactivated FBS and 1% penicillin streptomycin at 37 °C and CO2 (5%). The A2780cisR cell line was routinely treated with cisplatin (2 μM) in the media to maintain cisplatin resistance. The cytotoxicity was determined using the 3-(4,5-dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.\[46\] Cells were seeded in flat-bottomed 96-well plates as a suspension in a prepared medium (100 μL aliquots and approximately 4300 cells/ well) and preincubated for 24 h. Stock solutions of compounds were prepared in DMSO and were rapidly diluted in a medium. The solutions were sequentially diluted to give a final DMSO concentration of 0.5% and a final compound concentration range (0–200 μM). Cisplatin and RAPTA-c were tested as a positive (0–100 μM) and negative (200 μM) controls respectively. The compounds were added to the preincubated 96-well plates in 100 μL aliquots, and the plates were incubated for a further 72 h. MTT (20 μL, 5 mg/mL in Dulbecco’s phosphate buffered saline) was added to the cells, and the plates were incubated for a further 4 h. The culture medium was aspirated, and the purple formazan crystals, formed by the mitochondrial dehydrogenase activity of vital cells, were dissolved in DMSO (100 μL/well). The absorbance of the resulting solutions, directly proportional to the number of surviving cells, was quantified at 590 nm using a SpectroMax M5e multimode microplate reader (using SoftMax Pro software, version 6.2.2). The percentage of surviving cells was calculated from the absorbance of wells corresponding to the untreated control cells. The reported IC50 values are based on the means from two independent experiments, each comprising four tests per concentration level.
4.4.7 Chromatin Uptake Study

4.4.7.1 Isolation of Cellular Chromatin

Human ovarian carcinoma (A2780) cells were grown in RPMI 1640 GlutaMAX medium, containing 10 % FBS, and 1 % penicillin/streptomycin at 37 °C and CO2 (5%). Four flasks (150 cm$^3$) per compound, containing A2780 cells grown to ~80 % confluency, were incubated with 500 μM RAPTA-T, 20 μM auranofin, 20 μM complex 1 and 20 μM complex 2 for 2 h at 37 °C and CO2 (5%). Subsequently, the cells were washed with ice cold phosphate-buffered saline (PBS) to remove excess, unbound complex. The cellular chromatin was extracted using a Pierce Chromatin Prep Module (Thermo Fisher Scientific, Switzerland) according to the manufacturer’s protocol.

4.4.7.2 Quantification of Extracted Chromosomal DNA

The chromosomal DNA content of each sample was analysed before the ICP-MS analysis. The chromosomal DNA was quantified using a PicoGreen dsDNA quantification assay (Invitrogen). PicoGreen (50 μL per well, 200 x diluted in 10 mM Tris + 1 mM EDTA buffer) was added to 50 μL of chromosomal DNA sample and the fluorescence signal was determined by spectrofluorometric analysis (484 nm excitation/520 nm emission) using a M5e multimode microplate reader (using SoftMax Pro software, version 6.2.2).

4.4.7.3 Inductively Coupled Plasma Mass Spectrometry Analysis

The samples were digested using microwave oven synthWave from Mikrowellen-systeme MWS. 80μL of each sample and 1 mL of aqua regia were placed in a quartz digestion tube and digested at 195 °C and 60 bar for 20 min. Following digestion, samples were diluted 100 times with an aqueous solution containing 1% HCl and 2 % HNO3.
ICP-MS analysis was performed using NexION 350 D instrument from Perkin Elmer. It was operated in standard mode and Y @ 2ppb was used as internal standard. The linear calibration curve was done using standard solutions of Au and Ru with concentrations ranging from 0.01 to 10 ppb, all in a 1% HCl/2 % HNO3 solution.
4.5 References


Neimanis, C. Schmithals, V. Köberle, E. Süß, S. Zeuzem, H. Stark, A. Piiper, D.


(In print).


Chapter 5
Gold(I)-Peptide Conjugates targeting the Nucleosome Core Particle

The work described was completed in collaboration with Patrick Gonschorek who synthesised the peptides.
5.1 Introduction

The attachment of organometallic moieties to bioactive peptides can result in a myriad of applications and advantageous properties.\(^1\) Antimicrobial peptides (AMPs) are cationic polypeptides that are essential for the defence of multicellular organisms against microbes, such as bacteria and fungi, by targeting their negatively charged cellular membrane.\(^2\textsuperscript{–}^4\) Introducing a toxic organometallic moiety (OM), such as ferrocene, cobaltocenium, ruthenocene or osmocene, to simple AMPs can result in organometallic antimicrobial peptides (OM-AMPs) with enhanced antimicrobial properties.\(^5\textsuperscript{–}^{11}\) In some cases, cellular uptake can be increased by introducing positively charged OMs, such as cobaltocenium or neutral ferrocenyl moieties to free simian virus nuclear localisation peptide sequence, PKKKRKV.\(^12\) Metal-peptide conjugates are also prime candidates for positron emission tomography (PET) imaging probes where labelling gastrin releasing peptide receptor (GRPR) targeting peptides with \(^{68}\text{Ga}\) or \(^{153}\text{Ga}\) are promising for the imaging of prostate cancer.\(^13\textsuperscript{,}14\)

The introduction of peptides to known metallodrug infrastructures has the potential to improve cell permeability, accumulation and selectivity.\(^13\textsuperscript{,}16\) Tumour-uptake can be increased by taking the alkyne neurotensin(8-13) fragment, a peptide that can be internalised via three neurotensin receptors (NTR 1-3) found in breast, prostate and gastrointestinal cancers, and functionalising it with an antitumoural dicolbalt hexacarbonyl complex (Co\(_2\)(CO)\(_6\)) and a ruthenocene moiety (1A, Figure 5.1).\(^17\) Platinum(IV) complexes conjugated to peptides via click chemistry have been developed with the aim of improving the selectivity of the complexes.\(^18\) The introduction of chlorotoxin, a 32 amino acid derivative of giant yellow Israeli scorpion venom, to the Pt(IV) scaffold induces a selectivity for gliomas which express the chloride channel protein (CLC-3) to which the toxin specifically binds (1B, Figure 5.1).\(^19\) Another example includes the combination of a platinum(II) complex and synthetic peptide based on the integration host factor (IHF) with the aim of increasing the specificity of DNA targeting.\(^20\)
Gold(I)-peptide conjugates have been synthesised using standard ester coupling conditions of DCC and DMAP,[21] as well as via direct [3+2] cycloaddition reactions between gold(I) azides and terminal alkynes.[22] The site of peptide attachment in gold(I) conjugates, either by the stable or sacrificial ligand, can be influential. The cytotoxicity of a gold(I)-dipeptide conjugate, in which the dipeptide (GF) is attached via a stable NHC ligand (2A, Figure 5.2), is three fold lower than that of a NHC-gold(I) conjugate, whose dipeptide (CL) is coordinated via the sacrificial cysteine thiol (2B, Figure 5.2), against epitheloid cervix carcinoma (HeLa), liver hepatocellular carcinoma (HepG2) and human colorectal adenocarcinoma (HT-29) cell lines.[23] A gold(I)-N,S-heterocyclic carbene (NSHC) conjugate, containing the peptide Boc-Gly-(Thz-Ala)-OMe (where Thz-Ala = L-Thiazolylalanine)(2C, Figure 5.2) was found to be less cytotoxic than more auranofin-like conjugates (2D, Figure 5.2) against human lung carcinoma (A549), human pancreas carcinoma (MIA PaCa-2) and T-cell leukaemia (Jurkat) cell lines.[24][25] Mitochondria targeting (phosphine)gold(I) dipeptide and tetrapeptide conjugates (2E and 2F, Figure 5.2), containing YL and YRFK derived sacrificial ligands respectively, can overcome cisplatin resistance in p53-mutant MDA-MB231 breast cancer.
cells and strongly inhibit thioredoxin reductase (TrX) which results in cell death via apoptopic pathways.\cite{26}

Figure 5.2. Selected examples of Au(I)-peptide conjugates.

As the binding of the ruthenium(II) drug, RAPTA-T,\cite{27} ignites a series of conformational changes in the long α-helix of the H2A histone protein resulting in the opening of corresponding auranofin binding sites,\cite{28} it is possible that the binding of alternative structures to the same region of the acidic patch could have a similar outcome. Kaposi's sarcoma-associated herpesvirus (KSHV) latency-associated nuclear antigen (LANA), consisting of 1162 amino acid residues, is essential for episome persistence and thus tethers the KSHV genome to mitotic chromosomes consequently secreting viral DNA into the daughter nuclei after mitosis.\cite{29} Residues 1-23 of LANA, 1-MAPPGMLRSGRSTGAPLTRGSC-23, bind to the nucleosome in a hairpin conformation to the acidic patch of the H2A-H2B histone proteins.\cite{30} A $k_d$ of $184 \pm 28.5$ nM has been reported for the binding of the 1-23 LANA peptide to the nucleosome.\cite{31} The prototype foamy virus (PFV) structural protein GAG, 535-GGYNLRPRTYQPQRYGG-551, can also bind to chromatin.\cite{32,33} PFV GAG contains a chromatin binding sequence (CBS) in the C-terminal region that interacts with the acidic patch of the H2A-H2B histone dimers in a similar mode to LANA.\cite{34} Both peptides contain
the characteristic arginine anchors required to make the necessary contacts with the carboxylate residues on the H2A-H2B acidic patch.\textsuperscript{[35]}

Herein we describe the design, synthesis and biological evaluation of four gold(I)-peptide conjugates inspired by auranofin, a gold(I) anti-arthritic drug,\textsuperscript{[36][37]} and two viral peptides, LANA and GAG, with an affinity for the acidic patch on the NCP is described. The peptides were linked to the gold(I) moiety via the C- or N-terminus to a gold(I) centre with a long, flexible polyethylene glycol linker. If the binding of the peptides to the NCP ignites a series of conformational change that opens up the AU1 and AU1' binding site, in a similar fashion to the binding of RAPTA-T, the gold(I) moiety will be able to bind consequently, crosslinking two sites on the NCP.

5.2 Results and Discussion

In order to create a compound that can simultaneously bind to the acidic patch of the NCP, in the mode of unmodified LANA and GAG, whilst possessing the ability to access the auranofin binding sites, AU1 and AU1', a long linker is required. Polyethylene glycol was selected as a suitable linker due to its flexibility and higher water solubility compared to alkyl chains.\textsuperscript{[38]} As in Chapter 4, the auranofin-like gold(I) centre was modified to contain a functionalisable, air stable 4-(diphenylphosphino)benzoic acid ligand and a labile chloride ligand. Despite the bulky nature of the 4-(diphenylphosphino)benzoic acid ligand, this complex has been shown to bind to the AU1 binding site (see Chapter 4). To ensure that the peptides bind to the NCP unhindered, the GAG peptide was not modified. However, as the LANA peptide contains a methionine residue whose thiol side group could interact with the gold complex, it was replaced with an ornithine residue.

Two synthetic routes were considered for the preparation of the gold(I)-peptide conjugates. As the peptide synthesis was performed using standard Fmoc solid state chemistry,\textsuperscript{[39]} the gold(I) complex could be considered as another amino acid and be introduced using typical amide coupling conditions employing 1-[bis(dimethylamino)methylene]-1H-1,2,3-
triazolo[4,5-b] pyridinium-3-oxide hexafluorophosphate (HATU) and N,N-diisopropylethylamine (DIPEA) (Scheme 5.1). However, as the peptide side groups are still present and the N-terminus of the peptide remains attached to the resin, a deprotection step is required. This process is problematic due to the harsh conditions required to detach the peptide from the resin. Typically, 90 % trifluoroacetic acid (TFA), 2.5 % water, 2.5 % phenol, 2.5 % ethanedithiol (EDT) and 2.5 % thioanisol is used. However, the use of thiol-containing EDT and thioanisol was not advisable as they can react with the gold centre, therefore alternative conditions were used, 95% TFA, 2.5 % triisopropylsilane (TIS) and 2.5 % water. The resulting gold(I)-peptide conjugates were purified using RP-HPLC and the relevant fractions were lyophilised. However, although the desired products were observed via high resolution mass spectrometry, the peaks were at a very low intensity and multiple unidentifiable peaks of greater intensity indicated degradation of the products.

Scheme 5.1. Attempted synthesis of the gold(I)-peptide conjugates.

In order to circumvent the problematic deprotection step involving the gold(I) centre, a synthetic route was devised in which the complex and the deprotected peptide were
conjugated using copper click chemistry. This route involves the introduction of a propargylglycine residue to the peptide and a terminal azide to the complex. The gold(I) complex, containing a long polyethylene glycol linker and a terminal azide, was prepared in five steps (Scheme 5.2). The phosphorus ligand, 4-(diphenylphosphino)benzoic acid was coupled to octaethylene glycol via an esterification reaction with EDCI and DMAP acting as coupling reagent and base. The equivalents of reagents were adapted, 1 equiv. 4-(diphenylphosphino)benzoic acid versus 1.5 equiv. octaethylene glycol, to promote the formation of the mono-phosphine product, 1. A carboxylic acid, necessary for the addition of a second octaethylene glycol to the linker, was introduced to the ligand using succinic anhydride under basic, triethylamine conditions to afford 2. The second octaethylene glycol unit was introduced under the same EDCI/DMAP coupling conditions to yield 3 substantially increasing the length of the linker. The phosphorus ligand was coordinated to the gold centre via a freshly prepared gold(I) intermediate, AuCl(tht) (where tht = tetrahydrothiophene). Finally, the terminal alkyne was introduced via an esterification reaction between 4 and 2-azidoacetic acid under EDCI/DMAP conditions in DMF to yield 5.

Scheme 5.2. Synthesis of gold(I) complex containing a terminal azide, 5.

Compounds 1-5 were fully characterised using $^1$H, $^{31}$P and $^{13}$C NMR spectroscopy, mass spectrometry and elemental analysis. Each product was purified via flash column
chromatography conducted in the normal phase using CH$_2$Cl$_2$/CH$_3$OH as eluent and isolated as viscous oils. The complexation of the phosphorus ligand to the gold(I) centre was confirmed via a downfield shift in the $^{31}$P NMR from -5.07 ppm for 3 to 32.97 ppm for 4. The successful synthesis of 5 was confirmed by NMR spectroscopy where a characteristic multiplet representing the CH$_2$-O-(C=O)-CH$_2$-N$_3$ protons appears at 4.31-4.33 ppm in the $^1$H NMR spectrum. The phosphorus peak remained at 33.00 ppm in the $^{31}$P NMR spectrum and the [M+Na]$^+$ ion was observed at m/z 1448.4567 in the ESI(+) - MS spectrum.

The LANA (L1 and L2) and GAG (L3 and L4) peptides, modified at either the N- or C-termini with a propargylglycine (Figure 5.3), were synthesised using an automatic peptide synthesiser using standard Fmoc solid phase chemistry on Rink Amide MBHA resin (25 μmol scale, 0.3 mmol/g loading). The amide coupling reactions were performed twice for each amino acid using 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium-3-oxide hexafluorophosphate (HATU) and N,N-diisopropylethylamine (DIPEA). The Fmoc protecting groups were removed using pipiridine (20 % (v/v)) in DMF and the peptides were capped using acetic anhydride (5 % (v/v)) and lutidine (6 % (v/v)) in DMF.
The peptides were deprotected and cleaved from the resin via incubation 90 % TFA, 2.5 % H<sub>2</sub>O, 2.5 % thioanisole, 2.5 % phenol and 2.5 % EDT and the peptides were purified via RP-HPLC. The successful synthesis of the peptides, L<sub>1</sub>-L<sub>4</sub>, was confirmed via high-resolution ESI(+) spectrometry with the [M + 2H]<sup>2+</sup> ion observe at m/z 1101.6240 (L<sub>1</sub>), 1101.6228 (L<sub>2</sub>), 1039.0308 (L<sub>3</sub>) and 1039.0299 (L<sub>4</sub>). The purity was assessed via analytical HPLC with purities of 63.8 (L<sub>1</sub>), 66.1 (L<sub>2</sub>), 93.7 (L<sub>3</sub>) and 81.4 % (L<sub>4</sub>).

![Scheme 5.3. Copper click reaction forming Au(I)-peptide conjugates, AuL<sub>1</sub>-AuL<sub>4</sub>.](image)

The click reactions between 5 and the appropriate peptide, L<sub>1</sub>-L<sub>4</sub>, were conducted in tert-butyl alcohol and milliQ water in the presence of 1 mol % copper(II) sulphate and 10 mol % sodium ascorbate in milliQ water (Scheme 5.3). The resulting conjugates, AuL<sub>1</sub>-AuL<sub>4</sub>, were purified via RP-HPLC and the appropriate fractions were lyophilised. The successful synthesis of the conjugates was confirmed via high resolution ESI(+) mass spectrometry where the [M-Cl+3H]<sup>4+</sup> ion was observed for each conjugate at m/z 898.9393 (AuL<sub>1</sub>), 898.9382 (AuL<sub>2</sub>), 867.6437 (AuL<sub>3</sub>) and 867.6442 (AuL<sub>4</sub>). A shift in the <sup>31</sup>P NMR spectroscopic signals was observed from 32.97 ppm for the free complex to 42.31-42.67 ppm for the conjugates indicating that the environment of the phosphorus is influenced by the introduction of the peptide. The purity of the conjugates was analysed by analytical HPLC with 89.2, 91.0, 90.1 and 85.6 % recorded for AuL<sub>1</sub>, AuL<sub>2</sub>, AuL<sub>3</sub> and AuL<sub>4</sub> respectively (Figure 5.4).
The stability of the conjugates was assessed in physiological conditions (100 mM NaCl in D$_2$O) for 72 h monitored via $^{31}$P NMR spectroscopy and ESI-(+) mass spectrometry. The gold(I) peptides showed good stability with no degradation observed. The phosphorus peak in the $^{31}$P NMR remained at 42.34-42.36 ppm, 42.64-42.71 ppm, 42.51 ppm and 42.61-42.65 ppm for $\text{AuL1}$, $\text{AuL2}$, $\text{AuL3}$ and $\text{AuL4}$, respectively. The $[\text{M-Cl+3H}]^{4+}$ peak was observed with no discernable degradation throughout the 72 h incubation.
The cytotoxicity of the gold(I)-peptide conjugates, \textit{AuL1-AuL4} (Figure 5.5), and free peptides, \textit{L1-L4} (Figure 5.3), was assessed against cisplatin sensitive and cisplatin resistant human ovarian carcinoma (A2780 and A2780cisR) and non-tumoural human embryonic kidney (HEK-293) cell lines using the MTT assay (Table 5.1). Auranofin was evaluated as a reference along side cisplatin and RAPTA-C as positive and negative controls.

The free ligands \textit{L1-L4} were inactive against all cell lines with IC\textsubscript{50} values >200 μM. Although less cytotoxic than auranofin, introducing the gold(I) complex to the peptides induces cytotoxicity of <50 μM against the tumoural cell lines, A2780 and A2780cisR, for all the conjugates. \textit{AuL1} and \textit{AuL2}, containing the LANA peptide, possess comparable cytotoxicity against all cell lines indicating that their activity is not influenced if the attachment of the linker to the C- or N-termini. In contrast, for \textit{AuL3} and \textit{AuL4}, containing the GAG peptide, the attachment of the linker does influence the cytotoxicity with \textit{AuL3}, N-terminal attachment, possessing an IC\textsubscript{50} of 40 ± 4 μM compared to 53 ± 6 μM for \textit{AuL4}, C-terminal attachment, against the A2780 cell line. Both the gold(I)-GAG conjugates, \textit{AuL3} and \textit{AuL4}, are more cytotoxic against the cisplatin resistant cell line, A2780cisR, than cisplatin sensitive A2780. \textit{AuL3} is the most cytotoxic of the gold(I) conjugates against the cisplatin resistant A2780cisR with an IC\textsubscript{50} of 36 ± 2 μM. All gold(I)-peptide conjugates show selectivity towards the tumoural cell lines with IC\textsubscript{50} values >100 μM HEK-293 cell line compared to values <50 μM against the tumoural cell lines.

Table 5.1. \textit{In vitro} antiproliferative activity of \textit{AuL1-AuL4, L1-L4}, cisplatin, auranofin and RAPTA-C against human ovarian carcinoma (A2780), human ovarian carcinoma cisplatin resistant (A2780cisR) and human embryonic kidney 293 (HEK-293) cell lines after 72 h exposure. Values are given as the mean ± SD (μM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>A2780</th>
<th>A2780cisR</th>
<th>HEK293</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{AuL1}</td>
<td>35 ± 3</td>
<td>42 ± 3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>\textit{AuL2}</td>
<td>36 ± 3</td>
<td>46 ± 3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>\textit{AuL3}</td>
<td>40 ± 4</td>
<td>36 ± 2</td>
<td>&gt;100</td>
</tr>
<tr>
<td>\textit{AuL4}</td>
<td>53 ± 6</td>
<td>50 ± 5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>\textit{L1}</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>\textit{L2}</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>
When compared to a NHC-gold(I) dipeptide conjugate with a dipeptide (GF) attached via the stable, non-sacrificial NHC ligand, the cytotoxicity, determined by a resazurin assay, was in the mid micromolar range with 52.7 ± 5.0 μM recorded against HeLa cells, 71.3 ± 5.9 μM against HepG2 and 58.0 ± 4.3 μM against HT-29 cells. Moreover, (phosphine)gold(I) complexes with di- and tetrapeptides attached via the sacrificial ligand were considerably more active against HeLa cells with IC₅₀ values ranging between 4-50 μM. The conjugates, containing YL and YRFK derived sacrificial ligands, also possess greater cytotoxicity than the free peptides with IC₅₀ values ranging between 4-50 μM against HT-29 and 2-13 μM against MCF-7 cells for the conjugates compared to 82.7 ± 11.6 μM and 62.9 ± 0.8 μM for the YL derived peptide and >400 μM for the YRFK peptide. In some cases the (phosphine)gold(I) dipeptide conjugates presented comparable cytotoxicity to the free gold complex against the selected cell lines. A NSHC-gold(I) conjugate containing the Boc-Cys-Gly-OMe dipeptide possesses a cytotoxicity of ca. 25 μM against the selected cell lines. A related cysteine-containing gold(I) dipeptide conjugates also exhibit cytotoxicity in the low micromolar range with IC₅₀ values of 1.5-15.6 μM against A549 cells, 0.4-2.2 μM against Jurkat cells and 0.1-5.4 μM against MIA PaCa-2 cells.

### 5.3 Concluding Remarks

A series of gold(I)-peptide conjugates, containing LANA and GAG peptides, were successfully synthesised using copper click chemistry. The conjugates showed good stability in pseudo-physiological conditions of 100 mM in D₂O. Clicking the gold complex 5 to the free ligands L₁-L₄ introduced cytotoxicity in the mid-low micromolar range to the inactive
peptides. For the gold(I)-LANA conjugates \textit{AuL1} and \textit{AuL2}, the attachment of the complex to the N- or C-termini of the peptide does not influence the cytotoxicity of the conjugates. Whereas the gold(I)-GAG conjugate \textit{AuL3} attached via the N-terminus showed greater cytotoxicity than \textit{AuL4} attached via the C-terminus. All conjugates showed selectivity toward the tumoural cell lines, A2780 and A2780cisR, versus the non-tumoural cell line, HEK-293. The adduct formation of the conjugates is being assessed on NCP crystals using X-ray crystallography. Preliminary results show that the peptide bind to the acidic patch of the NCP after the shortest time point (t = 24 h), however gold adduct formation at the AU1 and AU1’ sites was only evident after the longest time point (t = 72 h). Although only moderate adduct formation is observed, it appears that \textit{AUL4} (containing GAG attached via the C-terminus) produced the strongest signal in the AU1 site followed whilst the gold(I)-LANA conjugate, \textit{AUL2} (attached via the C-terminus) having the weakest signal.

5.4 Experimental Section

5.4.1 Materials

Chemical reagents were purchased from commercial sources (Sigma Aldrich, ABCR, Acros and TCI Chemicals) and used without further purification. The reactions were performed under an inert atmosphere (N\textsubscript{2}) using Schlenk techniques. Dry solvents, dried using a PureSolv solvent purification system (Innovative Technology Inc.), were collected and used under an inert atmosphere (N\textsubscript{2}). Thin Layer Chromatography was conducted on Merck TLC silica gel coated aluminium sheets 60 F254 and verified by UV lamb at 254 nm. Purifications were achieved by column flash chromatography using a Combi\textit{Flash RF}+ automated columnn machine operated with prepacked Luhnova columnns.
5.4.2 Instrumentation and Methods

$^1$H (400 MHz), $^{31}$P (162 MHz) and $^{13}$C (101 MHz) NMR spectra were recorded on a Bruker Avance II 400 spectrometer at 298 K. Chemical shifts are reported in parts per million (ppm) and referenced to deuterated solvent residual peaks (CDCl$_3$: $^1$H $\delta$ 7.26, $^{13}$C{$^1$H} $\delta$ 77.16 ppm) and coupling constants ($J$) are reported in Hertz (Hz). High resolution electrospray ionization mass spectra (HR ESI-MS) were obtained on a Xevo G2-S QTOF mass spectrometer coupled to the Acquity UPLC Class Binary Solvent manager and BTN sample manager (Waters, Corporation, Milford, MA). Elemental analyses were carried out by the microanalytical laboratory at the the Institute of Chemical Sciences and Engineering (EPFL) using a Thermo Scientific Flash 2000 Organic Elemental Analyzer.

5.4.3 Synthesis

5.4.3.1 Synthesis of Gold Complex

**Compound 1**

![Chemical Structure](image)

4-(Diphenylphosphino)benzoic acid (1.50 g, 4.90 mmol, 1 equiv.) and N-ethyl-$N'$-(3-dimethylaninopropyl)carbodiimide hydrochloride (1.22 g, 6.37 mmol, 1.3 equiv.) were dissolved in dry CH$_2$Cl$_2$ (10 mL) and stirred under N$_2$ at room temperature for 2 h. The solution was added dropwise to a solution of octaethylene glycol (2.72 g, 7.35 mmol, 1.5 equiv.) and 4-(dimethylamino)pyridine (0.30 g, 2.44 mmol, 0.5 equiv.) in dry CH$_2$Cl$_2$ (5 mL) and the reaction stirred under N$_2$ at room temperature for 20 h. The reaction mixture was
washed with brine (100 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved via flash column chromatography using an eluent system of CH$_2$Cl$_2$/CH$_3$OH. The product was washed with hexane (3 x 15 mL) and isolated as a colourless oil (1.55 g, 2.35 mmol, 48%).

**Elemental Analysis (%):** calcd for C$_{35}$H$_{47}$O$_{10}$P. C 66.11 H 8.25, found C 66.31 H 8.02.

$^1$H NMR (CDCl$_3$, 400 MHz): 7.96-7.98 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.30-7.36 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-CH-C-P, 4xP-(Ar)C-CH-CH-CH, 4xP-(Ar)C-CH-CH-CH-C-P, 2xP-(Ar)C-CH-CH-CH), 4.44-4.46 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 3.79-3.82 (2H, m, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_2$-(CH$_2$)$_2$-O, Ar-(C=O)-O-((CH$_2$)$_2$-O)$(CH_2$)$_2$-O, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_3$-(CH$_2$)$_2$-O, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_4$-(CH$_2$)$_2$-O, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_5$-(CH$_2$)$_2$-O, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_6$-(CH$_2$)$_2$-O, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_7$-(CH$_2$)$_2$-O).

$^{31}$P $^1$H NMR (CDCl$_3$, 162 MHz): -5.08 (1P).

$^{13}$C $^1$H NMR (CDCl$_3$, 101 MHz): 165.4 (1C, O-(C=O)-(Ar)C-CH-CH-C-P), 143.2 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, $^{1}J_{C,P} = 14$ Hz), 135.2 (2C, d, 2xP-(Ar)C-CH-CH-CH-C-P, $^{2}J_{C,P} = 11$ Hz, 133.0 (4C, d, 4xP-(Ar)C-CH-CH-CH, $^{3}J_{C,P} = 20$ Hz), 132.2 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, $^{4}J_{C,P} = 19$ Hz), 129.1 (1C, O-(C=O)-(Ar)C-CH-CH-CH-C-P), 128.5 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-CH-C-P, $^{5}J_{C,P} = 6$ Hz), 128.2 (2C, d, 2xP-(Ar)C-CH-CH-CH, 127.4 (4C, d, 4xP-(Ar)C-CH-CH-CH, $^{6}J_{C,P} = 7$ Hz), 71.6 (1C, O-(C=O)-(Ar)C-CH-CH-CH, 69.41-69.77 (12C, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$).


**Compound 2**

![Compound 2](image)

**Compound 1** (1.200 g, 1.823 mmol, 1 equiv.) and succinic anhydride (0.274 g, 2.735 mmol, 1.5 equiv.) were dissolved in CH$_2$Cl$_2$ (5 mL). Triethylamine (0.553 g, 5.46 mmol, 0.76 mL, 3 equiv.) was added and the reaction stirred for 1 hour at r.t. under N$_2$. Purification was achieved via flash column chromatography using an eluent system of CH$_2$Cl$_2$/CH$_3$OH and the product was washed with pentane (3 x 15 mL) and isolated as a colourless oil (0.661 g, 0.871 mmol, 48%).
Elemental Analysis (%): calcd for C_{39}H_{51}O_{13}P.  
\[ \text{C} \] 63.16 \ H 7.44 found C 62.90 H 7.73.

$^1$H NMR (CDCl$_3$, 400 MHz): 7.94-7.96 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.27-7.40 (12H, m, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_3$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_4$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_5$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$CH_2$), 4.42-4.44 (2H, m, Ar-(C=O)-O-H$_2$-CH-O), 4.18-4.44 (2H, m, CH$_2$-O-(C=O)-(CH$_2$)$_2$-(C=O)-OH), 3.79-3.81 (2H, m, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 3.59-3.67 (26H, m, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_3$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_4$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_5$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$CH_2$), 2.60 (4H, s, O-(C=O)-(CH$_2$)$_2$-(C=O)-O);

$^{31}$P \{^1$H\} NMR (CDCl$_3$, 162 MHz): -5.08 (1P);

$^{13}$C \{^1$H\} NMR (CDCl$_3$, 101 MHz): 173.9 (1C, O-(C=O)-(CH$_2$)$_2$-(C=O)-OH), 172.1 (1C, O-(C=O)-(CH$_2$)$_2$-(C=O)-OH), 143.9 (1C, d, O-(C=O)-(CH$_2$)$_2$-(C=O)-OH), 135.8 (2C, d, O-(C=O)-(CH$_2$)$_2$-(C=O)-OH), 133.6 (4C, d, 4xP-(Ar)C-CH-CH-CH, \$\delta_{JC,P} = 20$ Hz), 128.9 (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, \$\delta_{JC,P} = 20 $ Hz), 128.4 (4C, d, 4xP-(Ar)C-CH-CH-CH, \$\delta_{JC,P} = 19$ Hz), 129.7 (1C, d, O-(C=O)-(Ar)C-CH-CH-CH, \$\delta_{JC,P} = 17$ Hz), 70.15-70.36 (12C, Ar-(C=O)-O-(CH$_2$)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_2$-O-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_3$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_4$-(CH$_2$)$_2$, Ar-(C=O)-O-((CH$_2$)$_2$-O)$_5$-(CH$_2$)$_2$, 68.9 (1C, Ar-(C=O)-O-CH$_2$-CH$_2$-O), 68.7 (1C, CH$_2$-CH$_2$-O-(C=O)-(CH$_2$)$_2$-(C=O)-OH), 64.0 (1C, Ar-(C=O)-O-(CH$_2$-CH-O), 63.8 (1C, O-(C=O)-(CH$_2$)-O-(CH$_2$)-O), 29.1 (2C, O-(C=O)-(CH$_2$)-O-(CH$_2$)-O);

HRMS (ESI(+)QTOF): m/z found 781.2966 [M+Na$^+$] C$_{39}$H$_{51}$O$_{13}$PNa$^+$ requires 781.2965.

**Compound 3**

![Structure of Compound 3](image_url)  

**Compound 2** (0.660 g, 0.870 mmol, 1 equiv.) and N-ethyl-N’-(3-dimethlamino)propyl)carbodiimide hydrochloride (0.217 g, 1.131 mmol, 1.3 equiv.) were dissolved in dry CH$_2$Cl$_2$ (10 mL) and stirred under N$_2$ at room temperature for 2 h. The solution was added dropwise to a solution of octaethylene glycol (0.450 g, 1.218 mmol, 1.4 equiv.) and 4-(dimethylamino)pyridine (0.053 g, 0.435 mmol, 0.5 equiv.) in dry CH$_2$Cl$_2$ (5 mL) and the reaction stirred under N$_2$ at r.t. for 17 h. The reaction mixture was washed with brine (50 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved by flash column chromatography using an eluent system of CH$_2$Cl$_2$/CH$_3$OH and the product was isolated as a colourless oil (0.695 g, 0.625 mmol, 72 %).

Elemental Analysis (%): calcd for C$_{55}$H$_{83}$O$_{21}$P C 59.45 H 7.53; found C 59.71 H 7.71.
\[^1H\] NMR (CDCl\(_3\), 400 MHz): 7.95-7.97 (2H, m, 2\(\times\)O-(C=O)-(Ar)C-CH-CH-C-P), 7.29-7.36 (12H, m, 2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 2\(\times\)O-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 2\(\times\)P-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 2\(\times\)O-(Ar)C-CH-CH-CH), 4.43-4.46 (2H, m, Ar-(C=O)-O-CH2-CH2-O), 4.21-4.24 (4H, m, CH2-O-(C=O)-(CH2)2-(C=O)-O-CH2), 3.79-3.81 (2H, m, Ar-(C=O)-O-CH2-CH2-O), 3.59-3.69 (5\(\times\)O-(C=O)-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 2\(\times\)P-(Ar)C-CH-CH-CH), 6.01 (4H, s, O-(C=O)-(CH2)2-(C=O)-O).

\[^31P\] {\(^1H\)} NMR (CDCl\(_3\), 162 MHz): -5.07 (1P).

\[^{13}C\] {\(^1H\)} NMR (CDCl\(_3\), 101 MHz): 172.3 (2C, O-(C=O)-(CH2)2-(C=O)-O), 166.4 (1C, Ar-(C=O)-O), 144.2 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, \(\nu_C,P = 14\) Hz), 136.2 (2\(\times\)O-(C=O)-(Ar)C-CH-CH-C-P, \(\nu_C,P = 6\) Hz), 129.5 (2\(\times\)O-(C=O)-(Ar)C-CH-CH-C-P, \(\nu_C,P = 3\) Hz), 128.8 (4\(\times\)C, d, 4\(\times\)P-(Ar)C-CH-CH-CH, \(\nu_C,P = 7\) Hz), 72.7 (1C, O-(C=O)-(Ar)C-CH-CH-CH, \(\nu_C,P = 1\) Hz), 69.3 (1C, Ar-(C=O)-O-CH2-CH2-O), 69.1 (2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH, 64.3 (1C, Ar-(C=O)-O-CH2-CH2-O), 64.0 (2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH, 61.8 (1C, -O-CH2-CH2-OH), 29.1 (2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH).

HRMS (ESI(+)-QTOF): \(m/z\) found 1133.5081 [M+Na]\(^+\) \(C_{55}H_{83}O_{21}PNa\) requires 1133.5062.

**Compound 4**

**Compound 3** (0.700 g, 0.630 mmol, 1 equiv.) and freshly prepared Au(I)Cl(tht) (0.202 g, 0.630 mmol, 1 equiv.) were stirred in dry CH\(_2\)Cl\(_2\) (10 mL) for 20 h under N\(_2\), darkness and at r.t. Purification was achieved via flash column chromatography using an eluent system of CH\(_2\)Cl\(_2\)/CH\(_3\)OH, and the product was isolated as a clear oil (0.831 g, 0.619 mmol, 98%).

**Elemental Analysis (%):** calc for \(C_{55}H_{83}AuClO_{21}P\): C 49.17 H 6.23; found C 49.17 H 6.14.

\[^1H\] NMR (CDCl\(_3\), 400 MHz): 8.06-8.08 (2H, m, 2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH-C-P), 7.44-7.56 (12H, m, 2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 2\(\times\)P-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 2\(\times\)O-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH), 4.17-4.20 (4H, m, CH2-O-(C=O)-(CH2)2-(C=O)-O-CH2), 3.77-3.79 (2H, m, Ar-(C=O)-O-CH2-CH2-O), 3.54-3.67 (5\(\times\)O-(C=O)-(Ar)C-CH-CH-CH, 4\(\times\)P-(Ar)C-CH-CH-CH, 2\(\times\)P-(Ar)C-CH-CH-CH), 69.1 (2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH, 64.0 (1C, O-(C=O)-(Ar)C-CH-CH-CH, 61.8 (1C, -O-CH2-CH2-OH), 29.1 (2\(\times\)O-(C=O)-(Ar)C-CH-CH-CH).
\[ ((\text{CH}_2)\text{O})_2\text{AuP}((\text{CH}_2)\text{O})_2, \text{HO-((CH}_2\text{O})_3\text{AuP(}((\text{CH}_2\text{O})_2\text{O}))_2, \text{HO-((CH}_2\text{O})_4\text{AuP(}((\text{CH}_2\text{O})_2\text{O}))_2, \text{HO-((CH}_2\text{O})_5\text{AuP(}((\text{CH}_2\text{O})_2\text{O}))_2, \text{HO-((CH}_2\text{O})_6\text{AuP(}((\text{CH}_2\text{O})_2\text{O}))_2, \text{HO-((CH}_2\text{O})_7\text{AuP(}((\text{CH}_2\text{O})_2\text{O}))_2, \text{2.60 (4H, s, O-(C=O)-(CH}_2\text{O})_2-(C=O)-O).}

\[ ^{31}P \text{ NMR (CDCl}_3\text{, 162 MHz): } 32.97 \text{ (Au-P)}.

\[ ^{13}C \text{ NMR (CDCl}_3\text{, 101 MHz): } 172.2 \text{ (2C, O-(C=O)-(CH}_2\text{O})_2-(C=O)-O), 165.3 \text{ (1C, Ar-(C=O)-O), 134.2 \text{ (1C, d, O-(C=O)-(Ar)C-CH-CH-P, } } {\chi}_{P} = 60 \text{ Hz), 134.1 \text{ (4C, d, 4xArC-CH-CH-CH, }{\chi}_{P} = 14 \text{ Hz), 133.2 \text{ (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P, }{\chi}_{P} = 2 \text{ Hz), 132.3 \text{ (2C, d, 2xO-(C=O)-(Ar)C-CH-CH-C-P, }{\chi}_{P} = 12 \text{ Hz), 129.4 \text{ (4C, d, 4xArC-CH-CH-CH, }{\chi}_{P} = 12 \text{ Hz), 127.8 (2C, d, 2xArC-CH-CH-CH, }{\chi}_{P} = 63 \text{ Hz), 72.5 \text{ (1C, O-CH}_2\text{CH}_2\text{-OH), 70.28-70.62 (24H, Ar-(C=O)-O-(CH}_2\text{O})_2-(CH}_2\text{-O-(C=O)-(CH}_2\text{O})_2-(C=O)-O-CH}_2\text{-CH}_2\text{-O), 69.0 \text{ (3C, Ar-(C=O)-O-CH}_2\text{-CH}_2\text{-O-(C=O)-(CH}_2\text{O})_2-(CH}_2\text{-O-(C=O)-(CH}_2\text{O})_2-(C=O)-O, 64.7(1C, Ar-(C=O)-O-CH}_2\text{-CH}_2\text{-O), 63.8 (2C, CH}_2\text{-O-(C=O)-(CH}_2\text{-O-(C=O)-O-CH}_2\text{-CH}_2\text{, 61.7 (1C, -O-CH}_2\text{-CH}_2\text{-OH), 29.0 (2C, O-(C=O)-(CH}_2\text{-O-(C=O)-O).}}

HRMS (ESI(+)-QTOF): m/z found 694.2166 [M+2Na]^{+} \text{ C}_{55}\text{H}_{81}\text{AuClO}_{21}\text{PNa}_{2}^{+} \text{ requires 694.2157.}
2-azidoacetic acid (0.051 g, 0.521 mmol, 1.2 equiv.) and N-ethyl-N’-(3-dimethlaminopropyl)carbodiimide hydrochloride (0.122 g, 0.639 mmol, 1.5 equiv.) were dissolved in dry (CH₃)₂NCH (5 mL) and stirred under N₂ at room temperature for 1 h. The solution was to a solution of Compound 4 (0.572 g, 0.426 mmol, 1 equiv.) and 4-(dimethylamino)pyridine (0.026 g, 0.213 mmol, 0.5 equiv.) in dry (CH₃)₂NCH (5 mL) and the reaction stirred under N₂ at room temperature for 48 h. The (CH₃)₂NCH was removed with N₂ and the crude was dissolved in CH₂Cl₂ (30 mL), washed with brine (100 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. Purification was achieved via flash column chromatography using an eluent system of CH₂Cl₂/CH₃OH. The product was washed with pentane (3 x 50 mL) and isolated as a yellow-brown oil (0.326 g, 0.229 mmol, 54%).

(%) : calc for C₁₉₇H₂₄AuClN₃O₂₂P₂. C 50.63 H 6.78 N 2.73; found C 50.59 H 6.80 N 2.49.

¹H NMR (CDCl₃, 400 MHz): 8.08-8.11 (2H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P), 7.46-7.58 (12H, m, 2xO-(C=O)-(Ar)C-CH-CH-C-P, 4xP-(Ar)C-CH-CH-C-P), 6.19-6.22 (4H, m, CH₂-O-(C=O)-(Ar)C-CH-CH-C-P), 3.89 (2H, s, O-(C=O)-(Ar)C-CH-CH-C-P), 3.78-3.81 (2H, m, Ar-(C=O)-O-CH₂-CH₂-O), 3.59-3.74 (54H, m, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₂-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₃-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₄-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₅-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₆-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₇-CH₂, N₃-CH₂-(C=O)-O-CH₂-CH₂, N₃-CH₂-(C=O)-O-(CH₂)₂-O-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₂-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₃-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₄-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₅-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₆-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₇-CH₂).

³¹P {¹H} NMR (CDCl₃, 162 MHz): 33.00 (Au-P).

¹³C {¹H} NMR (CDCl₃, 101 MHz): 172.3 (2C, O-(C=O)-(CH₂)-(C=O)-O), 168.4 (1C, O-(C=O)-(CH₂)-(Ar)), 156.4 (1C, Ar-(C=O)-O), 153.4 (1C, d, O-(C=O)-(Ar)C-CH-CH-C-P), 134.2 (4C, d, 4P-(Ar)C-CH-CH-C-P), 129.5 (4C, d, 4P-(Ar)C-CH-CH-C-P), 127.9 (2C, d, 2P-(Ar)C-CH-CH-C-P), 121.2 (2C, d, 2P-(Ar)C-CH-CH-C-P), 70.58-70.69 (25C, Ar-(C=O)-O-(CH₂)₂-O-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₂-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₃-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₄-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₅-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₆-(CH₂)₂, Ar-(C=O)-O-((CH₂)₂-O)₇-CH₂, N₃-CH₂-(C=O)-O-CH₂-CH₂, N₃-CH₂-(C=O)-O-(CH₂)₂-O-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₂-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₃-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₄-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₅-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₆-(CH₂)₂, N₃-CH₂-(C=O)-O-((CH₂)₂-O)₇-CH₂).
HRMS (ESI(+)QTOF): m/z found 1448.4567 [M+Na]+ C_{57}H_{84}AuClN_{3}O_{22}PNa requires 1448.4536.

5.4.3.2 Synthesis of Peptides

The peptides were synthesised on an automated peptide synthesiser (Advanced ChemTech 348\textsuperscript{a} parallel peptide synthesiser (AAPPTec)) using standard Fmoc solid phase chemistry on Rink Amide MBHA resin (25 \textmu mol scale, 0.3 mmol/g loading). The amide coupling was performed twice for each for each amino acid (10 equiv.) using base NMM (10 equiv.), HATU (4 equiv.) and HOBt (4 equiv.) with an incubation of 30 minutes. The Fmoc groups were removed by incubating the resin twice with piperidine (6 equiv., 20 % (v/v)) in DMF. The resin was washed 4 times before and 5 times after Fmoc removal with DMF (3 mL). The peptides were capped using a capping mixture of acetic anhydride (5 % (v/v)) and lutidine (6 % (v/v)) in DMF.

The final peptides were deprotected and cleaved from the resin via incubation 90 % TFA, 2.5 % H_{2}O, 2.5 % thioanisole, 2.5 % phenol and 2.5 % EDT under agitation for 4 hours at room temperature. The resin was removed by vacuum filtration and the peptides were precipitated with cold diethyl ether (50 mL) at -20 °C. The precipitated peptides were collected via centrifugation, washed with cold diethyl ether (2 x 30 ml) and lyophilised.

Peptides were purified with a HPLC system (Prep LC 2535 HPLC, Waters) using a preparative C18 reverse-phase column (SunfireTM prep C18 OBD 10 \mu m, 100 Å, 19x250 mm, Waters) with a flow rate of 20 ml min\textsuperscript{-1} and a linear gradient of 10-50% v/v solvent B in 40 min (A: 99.9% v/v H2O and 0.1% v/v TFA; B: 99.9% v/v ACN and 0.1% v/v TFA). Fractions containing the desired peptide were lyophilised.
**Peptide L1**

Analytical RP-HPLC: 63.8%.

HRMS (ESI(+)-QTOF): $m/z$ found 1101.6240 [M+2H]$^2^+$ C$_{92}$H$_{162}$N$_{36}$O$_{27}$ requires 1101.6205.

**Peptide L2**

Analytical RP-HPLC: 66.1%.

HRMS (ESI(+)-QTOF): $m/z$ found 1101.6228 [M+2H]$^2^+$ C$_{92}$H$_{162}$N$_{36}$O$_{27}$ requires 1101.6205.

**Peptide L3**

Analytical RP-HPLC: 93.7%.

HRMS (ESI(+)-QTOF): $m/z$ found 1039.0308 [M+2H]$^2^+$ C$_{92}$H$_{139}$N$_{31}$O$_{25}$ requires 1039.0280.
Section 5.4.3.3 Synthesis of Gold(I)-Peptide Conjugates

**General Procedure**

Compound 5 (1.1 equiv.) and the appropriate peptide (1 equiv.) were dissolved in tert-butyl alcohol (2 mL) and milliQ water (2 mL) in the presence of 1 mol % of copper (II) sulphate and 10 mol % of sodium ascorbate. The reaction stirred vigourously at r.t. and under N₂ for 24 h. The solvent was removed with N₂. Purification was achieved via preparative HPLC using acetonitrile/water (10 %-45 %) as eluent over 30 minutes. The product was lyophilised and isolated as a white solid.

**Gold(I)-LANA conjugate: AuL₁**
According to the general procedure, **Compound 5** (0.039 g, 0.0275, 1.1 equiv.), **Peptide L1** (0.050 g, 0.0227 mmol, 1 equiv.) in tert-butyl alcohol (2 mL) and milliQ water (2 mL) in the presence of 1 mol % of copper (II) sulphate pentahydrate and 10 mol % of sodium ascorbate. The product was lyophilised and isolated as a white solid (16.06 mg, 0.00464 mmol, 20 %);

Analytical RP-HPLC: 89.2 %.

$^3$P \{^1^H\} NMR (100mM NaCl in D$_2$O 162 MHz): 42.34 (1P).

HRMS (ESI(+)-QTOF): m/z found 898.9393 [M-Cl+3H]$^+$ requires 898.9367.

**Gold(I)-LANA conjugate: AuL2**

According to the general procedure, **Compound 5** (0.039 g, 0.0275, 1.1 equiv.), **Peptide L2** (0.050 g, 0.0227 mmol, 1 equiv.) in tert-butyl alcohol (2 mL) and milliQ water (2 mL) in the presence of 1 mol % of copper (II) sulphate pentahydrate and 10 mol % of sodium ascorbate. The product was lyophilised and isolated as a white solid (21.88 mg, 0.00632 mmol, 28 %).

Analytical RP-HPLC: 91.0%.

$^3$P \{^1^H\} NMR (100mM NaCl in D$_2$O 162 MHz): 42.67 (1P).

HRMS (ESI(+)-QTOF): m/z found 898.9382 [M-Cl+3H]$^+$ requires 898.9367.

**Gold(I)-GAG conjugate: AuL3**

According to the general procedure, **Compound 5** (0.039 g, 0.0275, 1.1 equiv.), **Peptide L3** (0.050 g, 0.0227 mmol, 1 equiv.) in tert-butyl alcohol (2 mL) and milliQ water (2 mL) in the presence of 1 mol % of copper (II) sulphate pentahydrate and 10 mol % of sodium ascorbate. The product was lyophilised and isolated as a white solid (21.88 mg, 0.00632 mmol, 28 %).
According to the general procedure, Compound 5 (0.038 g, 0.0265, 1.1 equiv.), Peptide L3 (0.050 g, 0.0241 mmol, 1 equiv.) in tert-butyl alcohol (2 mL) and milliQ water (2 mL) in the presence of 1 mol % of copper (II) sulphate pentahydrate and 10 mol % of sodium ascorbate. The product was lyophilised and isolated as a white solid (25.03 mg, 0.00783 mmol, 33 %).

**Analytical RP-HPLC:** 90.1 %.

$^{31}$P $^{1}H$ NMR (100 mM NaCl in D$_2$O, 162 MHz): 42.51 (1P).

HRMS (ESI(+)-QTOF): $m/z$ found 867.6437 $[M-Cl+3H]^+$ requires 867.6404.

**Gold(I)-GAG conjugate: AuL4**

According to the general procedure, Compound 5 (0.019 g, 0.0132, 1.1 equiv.), Peptide L4 (0.025 g, 0.0120 mmol, 1 equiv.) in tert-butyl alcohol (2 mL) and milliQ water (2 mL) in the presence of 1 mol % of copper (II) sulphate pentahydrate and 10 mol % of sodium ascorbate. The product was lyophilised and isolated as a white solid (24.46 mg, 0.00765 mmol, 64 %).

**Analytical RP-HPLC:** 85.6 %.

$^{31}$P $^{1}H$ NMR (100 mM NaCl in D$_2$O, 162 MHz): 42.61 (1P).

HRMS (ESI(+)-QTOF): $m/z$ found 867.6442 $[M-Cl+3H]^+$ requires 867.6404.

### 5.4.4 Cell Culture and Cytotoxicity Studies

Human ovarian carcinoma (A2780 and A2780cisR) cell lines were obtained from the European Collection of Cell Cultures. The human embryonic kidney (HEK-293) cell line was obtained from ATCC (Sigma, Buchs, Switzerland). Penicillin streptomycin, RPMI 1640 GlutaMAX (where RPMI = Roswell Park Memorial Institute), and DMEM GlutaMAX media
(where DMEM = Dulbecco’s modified Eagle medium) were obtained from Life Technologies, and fetal bovine serum (FBS) was obtained from Sigma. The cells were cultured in RPMI 1640 GlutaMAX (A2780 and A2780cisR) and DMEM GlutaMAX (HEK-293) media containing 10% heat-inactivated FBS and 1% penicillin streptomycin at 37 °C and CO2 (5%). The A2780cisR cell line was routinely treated with cisplatin (2 μM) in the media to maintain cisplatin resistance. The cytotoxicity was determined using the 3-(4,5-dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cells were seeded in flat-bottomed 96-well plates as a suspension in a prepared medium (100 μL aliquots and approximately 4300 cells/well) and preincubated for 24 h. Stock solutions of compounds were prepared in MilliQ water and sequentially diluted in the appropriate medium to give a final compound concentration range (0−200 μM). Cisplatin and RAPTA-C were tested as a positive (0−100 μM) and negative (200 μM) controls respectively. The compounds were added to the preincubated 96-well plates in 100 μL aliquots, and the plates were incubated for a further 72 h. MTT (20 μL, 5 mg/mL in Dulbecco’s phosphate buffered saline) was added to the cells, and the plates were incubated for a further 4 h. The culture medium was aspirated, and the purple formazan crystals, formed by the mitochondrial dehydrogenase activity of vital cells, were dissolved in DMSO (100 μL/well). The absorbance of the resulting solutions, directly proportional to the number of surviving cells, was quantified at 590 nm using a SpectroMax M5e multimode microplate reader (using SoftMax Pro software, version 6.2.2). The percentage of surviving cells was calculated from the absorbance of wells corresponding to the untreated control cells. The reported IC50 values are based on the means from two independent experiments, each comprising four tests per concentration level.
5.5 References


Conclusions and Perspectives
Combining the attributes of known anticancer agents in a multinuclear scaffold can lead to compounds with crosslinking capabilities. Known anticancer agents, whose targets have been extensively explored, can inspire the design of crosslinking agents. Such combinations can provide scope for heterometallic complexes capable of forming DNA-DNA, DNA-protein and protein-protein crosslinks. RAPTA-T and auranofin provided the inspiration for the complexes described in this thesis, with the aim of crosslinking known RAPTA-T and auranofin sites on the histone proteins of the NCP.

The nature of the linker that attaches one metal centre to another can impact the properties of the resulting dinuclear complex. A plateau in the lipophilicity of polyethylene glycol chains between PEG₆ and PEG₁₀ enables the impact of increasing linker length on the cytotoxicity of bis-ruthenium(II) and bis-gold(I) complexes to be evaluated independent of increasing lipophilicity. Whilst the narrow nanomolar range cytotoxicity observed for the bis-gold(I) complexes meant that correlations were not readily assessable, a correlation exists between the lipophilicity and cytotoxicity of the bis-ruthenium(II) complexes. Therefore, if an increase in the cytotoxicity of a dinuclear ruthenium complex is desired, increasing the lipophilicity of the linker is viable method. However, increasing linker length alone, which could enable the crosslinking of more distant sites, has no discernable impact on the anti-proliferative properties of the ruthenium complex.

Heterobimetallic ruthenium(II)-gold(I) complexes bearing bis-phosphine ligands and PEG linkers of 4-8 units also possess no clear correlation between linker length and cytotoxicity. As histidine residues are present in the RU2, AU1 and AU1' binding sites on the NCP, a heterobimetallic ruthenium(II)-gold(I) complex designed to crosslink the sites should bind to histidine residues. The ruthenium(II)-gold(I) complex bearing a PEG₅ linker can bind to three histidine molecules showing that both the ruthenium and gold centres are bound. When the ruthenium(II)-gold(I) complex bearing a PEG₄ linker is incubated with the 1-16 mer amyloid β-protein, the chloride ligands dissociate and the complex binds to the peptide in a 1:1 ratio. Fragmentation of the resulting adduct in a +5 charge state using CID and ETD techniques revealed that the complex is most likely bound to the three histidine residues present in various combinations.
Conclusions and Perspectives

In order to crosslink the RU1 and AU1 sites on the NCP, the water solubility and linker length had to be increased. Linking the metal centres via the arene of the RAPTA-moiety enables the inclusion of the water-soluble PTA ligand consequently increasing the water solubility of the complex and further maintaining the RAPTA-T structure. The subsequent X-ray crystallographic studies of the dinuclear ruthenium(II)-gold(I) complex on the NCP revealed that the ruthenium moiety bound to the RU1 site, ignited the allosteric effect and enabled the gold moiety of the same complex to bind in AU1. Although the linker could not be fully resolved in the X-ray crystallographic study, a strip of electron density was observed linking the RU1 and AU1 binding sites. Molecular dynamic simulations showed that the high flexibility of the linker meant that the position of the central PEG units could vary by up to 4-5 Å which accounts for the difficulty in the resolution of the linker. The successful crosslinking of RU1 and AU1 sites is, to the best of our knowledge, the first report of one complex crosslinking distal allosteric sites.

As the binding of RAPTA-T and dinuclear ruthenium(II)-gold(I) complex to the RU1 binding site can cause a series structural alterations within the histone proteins that opens the previously inaccessible AU1 and AU1' sites, it is possible that the binding of other compounds to the acidic patch of the NCP could have a similar effect. A series of gold(I)-peptide conjugates containing the 1-23 residues of the LANA peptide and the 535-551 residues of the GAG peptide were tested on the NCP crystal. Preliminary experiments showed that the peptide bound to the NCP after 1 day of incubation and, after the longest incubation time over 3 days, low to moderate occupation of the AU1/AU1' sites were observed. This suggests that although a structural alteration occurs, subsequently opening up the AU1 and AU1' binding sites, this allosteric effect is not as favourable for the gold(I) binding as that of RAPTA-T.

Whilst, the dinuclear ruthenium(II) gold(I) complex is capable of crosslinking the RU1 and AU1 sites on the NCP, the biological implications of should be further investigated in vitro and in vivo. The crosslinking of the RU1 and AU1 sites could lead to the inhibition of key chromatin factors, such as the regulator of chromatin condensation 1 (RCC1), and should be evaluated. Furthermore, the origin of additional gold adducts observed on the DNA of the NCP after 120 h of incubation should be accounted for through further molecular dynamic simulations.
The x-ray crystallographic studies of the triruthenium(II)-gold(I) complex and the gold(I)-peptide conjugates are ongoing with the preliminary results looking promising. As LANA and GAG peptides possess an affinity for the NCP, the chromatin uptake of the gold(I)-peptide conjugates should be quantified using ICP-MS to observe the impact on gold-adduct formation on the NCP. Due to the relatively large size of the peptides, it would also be interesting to evaluate the inhibition potential of the gold(I)-peptide conjugates on the acidic patch of the NCP.

In conclusion, the use of known metal complexes, such as cisplatin, RAPTA-T and auranofin, which covalently bind to specific sites represents a broadly applicable approach to crosslink binding sites in multiple systems. Whilst heterometallic crosslinking agents remain a relatively unexplored family of anticancer agents, their novel modes of action offer a greater challenge to cell repair systems compared to mononuclear complexes and therefore they could offer a means with which to overcome the current drug resistance. Although the binding of other compounds, such as peptides, can cause structural alterations that partially open the AU1 and AU1' binding sites, it is the allosteric effect caused by the binding of RAPTA-T that is particularly distinguished. The binding of RAPTA-T to the NCP incites a very specific structural change that distinctly favours the binding of gold(I) complexes in the AU1 and AU1'. Therefore, the synergistic and allosteric properties of RAPTA-T and auranofin could find applications in drug combination therapy.
Curriculum Vitae
Curriculum Vitae

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Research Experience

- The design, synthesis of characterisation of homo- and heterobimetallic complexes containing platinum, ruthenium and gold.
- In vitro cytotoxic evaluation and uptake studies.

Education

01.2015 – present PhD - Ecole Polytechnique Fédéral de Lausanne (EPFL)
Mentor: Prof. Paul J. Dyson
Crosslinking Allosteric Sites on the Nucleosome Core Particle

10.2010 – 06.2014 MSc - Imperial College London
Degree: MSc in Chemistry with Research Abroad
Degree Class: 2:1 (Hons)

10.2005 – 06.2009 Sherborne School for Girls, Dorset UK
A-levels: Chemistry (A); Mathematics (A); Geography (A)
AS-levels: Biology (A)

Extra-Curricular Experience and Semesters Abroad

09.2015 – 09.2016 Swiss Young Chemist Association (SYCA); Administrative Assistant
Key player in organising the Swiss Snow Symposium 2016, Saas Fee

09.2013 – 09.2014 Research Project Abroad; Ecole Polytechnique Fédéral de Lausanne
Mentor: Prof. Paul J. Dyson
Synthesis and biological evaluation of ruthenium(II)-arene complexes in intra-molecular drug combinations with anti-angiogenic agents.
Grade: 1st /A; oral French language proficiency

07.2009 – 09.2010 WASHCost, Andhra Pradesh, India, Intern
Conducted water quality case studies in rural Anantapur and Kurnol.
Comparison of national, regional and private laboratories to unearth the source of the discrepancy in the analysis of identical water samples.

**Core Skills**

- Proficient in organic and inorganic synthesis in milligram and multi-gram scale
- Proficient in the synthesis under inert atmosphere (Schlenk and glove box techniques)
- Proficient in analytics (LC, HPLC, NMR, MS, IR, UV-Vis)
- Proficient in cell culture and in vitro cytotoxicity and uptake studies.

**Teaching Experience**

- Mentor for two master students during their master thesis and semester project.
- Teacher and mentor to numerous students, interns and apprentices in *in vitro* cell studies
- 710 hours of teaching experience as teaching assistant in biochemistry, organic- and inorganic synthesis.

**Languages**

- **English** (mother tongue)
- **French** (proficient in writing and speaking)
- **German** (basic knowledge)

**Publications:**

- L. K. Batchelor, P. J. Dyson, Metal-Based Crosslinking Agents, Review, *in preparation*


**Conference Contributions**

– 9th Asian Bioinorganic Chemistry, Singapore, 12.2018 (Poster Presentation)

– Swiss Snow Symposium, Saas Fee, 2018 (Oral Presentation)

– SCS fall meeting, annually occurring between 2016-2018 (Poster Presentation)

– 8th Asian Bioinorganic Chemistry, Auckland, New Zealand, 12.2016 (Oral Presentation)

**Awards and Grants**

– SNSF Travel Award 2018
**Annex: Chapter 2**

Table A2.1. Partition Coefficients (log $P$) of polyethylene glycol and alkyl chains.

<table>
<thead>
<tr>
<th>Compound</th>
<th>log $P$</th>
<th>Compound</th>
<th>log $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoethylene glycol</td>
<td>-1.53</td>
<td>Methane</td>
<td>-1.31</td>
</tr>
<tr>
<td>Diethylene glycol</td>
<td>-1.24</td>
<td>Ethane</td>
<td>1.44</td>
</tr>
<tr>
<td>Triethylene glycol</td>
<td>-1.12</td>
<td>Propane</td>
<td>2.19</td>
</tr>
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Figure A2.2. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2\textit{a} in CDCl$_3$ at RT.
Figure A2.3. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 3a in CDCl$_3$ at RT.
Figure A2.4. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 44a in CDCl$_3$ at RT.
Figure A2.5. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 5a in CDCl$_3$ at RT.
Figure A2.6. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 6a in CDCl$_3$ at RT.
Figure A2.7. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 7a in CDCl₃ at RT.
Figure A2.8. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1b in CDCl$_3$ at RT.
Figure A2.9. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2b in CDCl$_3$ at RT.
Figure A2.10. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of $3b$ in CDCl$_3$ at RT.
Figure A2.11. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 4b in CDCl$_3$ at RT.
Figure A2.12. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 5b in CDCl$_3$ at RT.
Figure A2.13. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 6b in CDCl$_3$ at RT.
Figure A2.14. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 7b in CDCl$_3$ at RT.
Figure A2.15. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 8b in CDCl$_3$ at RT.
Figure A2.16. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 99b in CDCl$_3$ at RT.
Figure A2.17. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1c in CDCl$_3$ at RT.
Figure A2.18. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2c in CDCl$_3$ at RT.
Figure A2.19. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 3c in CDCl$_3$ at RT.
Figure A2.20. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 4c in CDCl$_3$ at RT.
Figure A2.21. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 5c in CDCl$_3$ at RT.
Figure A2.22. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 6c in CDCl$_3$ at RT.
Figure A2.23. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 7c in CDCl$_3$ at RT.
Figure A2.24. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 8c in CDCl$_3$ at RT.
Figure A2.25. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 9c in CDCl$_3$ at RT.
Figure A2.26. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1d in CDCl$_3$ at RT.
Figure A2.27. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2\textit{d} in CDCl$_3$ at RT.
Figure A2.28. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 3d in CDCl$_3$ at RT.
Figure A2.29. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 4d in CDCl$_3$ at RT.
Figure A2.30. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 5d in CDCl$_3$ at RT.
Figure A2.31. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 6d in CDCl$_3$ at RT.
Figure A2.32. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 7d in CDCl$_3$ at RT.
Figure A2.33. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 8\textit{d} in CDCl$_3$ at RT.
Figure A2.33. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 9d in CDCl$_3$ at RT.
Figure A3.1. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1a in CDCl$_3$ at RT.
Figure A3.2. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1b in CDCl$_3$ at RT.
Figure A3.3. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1c in CDCl₃ at RT.
Figure A3.4. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1d in CDCl$_3$ at RT.
Figure A3.5. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2a in CDCl$_3$ at RT.
Figure A3.6. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2b in CDCl$_3$ at RT.
Figure A3.7. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of $3b$ in CDCl$_3$ at RT.
Figure A3.8. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 44b in CDCl$_3$ at RT.
Figure A3.9. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 3a in CDCl$_3$ at RT.
Figure A3.10. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 3b in CDCl$_3$ at RT.
Figure A3.11. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 3c in CDCl$_3$ at RT.
Figure A3.12. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 33d in CDCl₃ at RT.
Figure A3.13. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 4a in CDCl$_3$ at RT.
Figure A3.14. $^1\text{H}$ (top), $^{31}\text{P}$ (middle), $^{13}\text{C}$ (bottom) NMR spectra of 4b in CDCl$_3$ at RT.
Figure A3.15. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 4c in CDCl$_3$ at RT.
Figure A3.16. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 44d in CDCl₃ at RT.
Figure A3.17. Mass Spectrum of 4b and l-histadine (1:1) after 2 h incubation at 310 K.

Figure A3.18. Mass Spectrum of 4a and 1-16 amyloid β protein (1:3) after 2 h incubation at 310 K.
Figure A3.19. Mass Spectrum of the CID fragmentation of the [peptfir + 4a -3Cl +5H]^+ ion.

Figure A3.20. Mass Spectrum of the ETD fragmentation of the [peptfir + 4a -3Cl +5H]^+ ion.
Table A3.1. Mass Spectrum of the ETD fragmentation of the [peptfir + 4a -3Cl +5H]⁺ ion.

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Figure A3.21. Comparison of calculated and experimental CID peaks of the unmetallated b15, b10 and b7 fragments.

Figure A3.22. Comparison of calculated and experimental CID peaks of the unmetallated y15, y9 and y4 fragments.
Figure A3.23. Comparison of calculated and experimental CID peaks of the unmetallated b7y15, b8y15 and b15y15.

Figure A3.24. Comparison of calculated and experimental CID peaks of the metallated b15* and b11* fragments.
Figure A3.25. Comparison of calculated and experimental CID peaks of the metallated $y_{15}^*$ and $b_{13}^*$ fragments.

Figure A3.26. Comparison of calculated and experimental CID peaks of the metallated $b_{6}y_{15}^*$ and $b_{7}y_{12}^*$ internal fragments.
Figure A3.27. Comparison of calculated and experimental ETD peaks of the unmetallated c15 and c6 fragments.

Figure A3.28. Comparison of calculated and experimental ETD peaks of the unmetallated z13 and z4 fragments.
Figure A3.29. Comparison of calculated and experimental ETD peaks of the unmetallated c10z15 and c7z15 internal fragments.

Figure A3.30. Comparison of calculated and experimental ETD peaks of the metallated c15* and c8* fragments.
Figure A3.31. Comparison of calculated and experimental ETD peaks of the metallated z15* and z5* fragments.

Figure A3.32. Comparison of calculated and experimental ETD peaks of the metallated c15z11* and c10z15* fragments.
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Figure A4.1. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of a in CDCl$_3$ at RT.
Figure A4.2. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of $b$ in CDCl$_3$ at RT.
Figure A4.3. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of cc in CDCl$_3$ at RT.
Figure A4.4. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of d in CDCl$_3$ at RT.
Figure A4.5. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of ee in CDCl$_3$ at RT.
Figure A4.6. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of $f$ in CDCl$_3$ at RT.
Figure A4.7. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1 in CDCl$_3$ at RT.
Figure A4.7. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2 in CDCl₃ at RT.
Annex: Chapter 5

Figure A5.1. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 1 in CDCl$_3$ at RT.
Figure A5.2. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 2 in CDCl$_3$ at RT.
Figure A5.3. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 3 in CDCl$_3$ at RT.
Figure A5.4. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 4 in CDCl$_3$ at RT.
Figure A5.5. $^1$H (top), $^{31}$P (middle), $^{13}$C (bottom) NMR spectra of 5 in CDCl$_3$ at RT.
Figure A5.6. $^{31}$P NMR spectrum of $\text{AuL}_1$ in $\text{D}_2\text{O}$ at RT.
Figure A5.7. $^{31}$P NMR spectrum of AuL$_2$ in D$_2$O at RT.

Figure A5.8. $^{31}$P NMR spectrum of AuL$_3$ in D$_2$O at RT.
Figure A5.9. $^{31}$P NMR spectrum of AuL4 in D$_2$O at RT.
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