Summer Internship Report – Medicinal and Bioinorganic Group (TUM)

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Internship Dates: 3rd June 2025 – 30th July 2025

Supervisor: Professor Angela Casini

Abstract

During my summer internship in the Medicinal and Bioinorganic Chemistry group led by Professor Angela Casini at the Technical University of Munich (TUM), I gained exposure to synthetic bioinorganic chemistry and medicinal chemistry strategies. My work focused on working with PhD students across different projects in three key areas: (i) the design and characterization of ruthenium (Ru) complexes as potential bioactive catalysts, (ii) the use of solid-phase peptide synthesis (SPPS) to construct peptide sequences and cyclic structures relevant for drug development, and (iii) the development of metal-based mass tags for mass spectrometry imaging (MSI) of proteins.

In the ruthenium complex project, I worked with Riccardo Scotti to synthesize and analyze picolinamide and picolinic acid Ru complexes, and their hydride derivatives, to explore how subtle ligand variations can tune reactivity and biological activity. In the SPPS project with Felix Böhm, I contributed to the stepwise assembly of peptide sequences, on-resin cyclization, and test cleavages. Finally, in the mass tag project with Melina Rumpf, I synthesized indole–pyridine derivatives, carried out nitro-to-amine reductions, and prepared biotin-based linkers to develop novel Ru-containing tags with unique isotopic signatures for enhanced protein imaging.

Through these experiences, I gained a deeper understanding of how metal complexes, peptides, and analytical tagging strategies intersect in modern anticancer research. The projects provided me with hands-on training in synthesis, purification, and characterization, and offered insight into how chemical design decisions translate into biological function. This report provides an overview of the scientific background, experimental contributions, and reflections on skills acquired, concluding with perspectives on how these research directions may evolve.

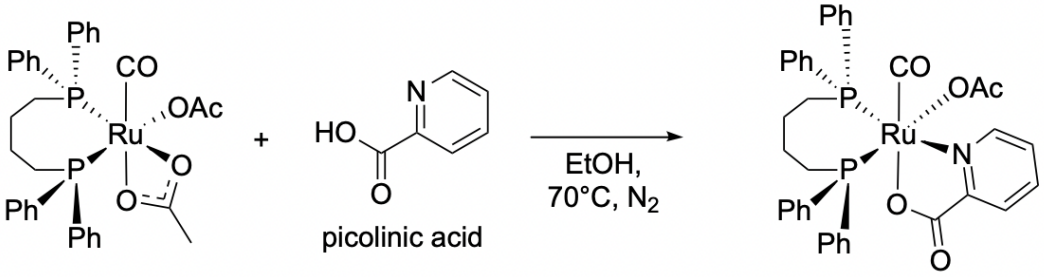
Ruthenium Complexes (Riccardo Scotti)

Ruthenium complexes are attractive candidates in anticancer research due to their versatile coordination chemistry and ability to undergo redox reactions.[1] They can interact with biomolecules such as DNA, enzymes, and proteins, making them promising alternatives to platinum-based drugs.[1]

I worked with PhD student Riccardo Scotti on his research into organometallic ruthenium complexes as bioactive catalysts. Specifically, we synthesized picolinamide and picolinic acid Ru-complexes, which have shown potential to induce reductive stress in cancer cells, specifically, acting as a reducing agent to NAD+. These complexes are designed to act as catalytic therapeutics, leveraging the redox properties of Ru. I assisted with their preparation, hydride formation studies, and subsequent NMR analyses. This experience deepened my understanding of how subtle ligand modifications influence not only the stability and redox chemistry of the complexes but also their potential biological activity.

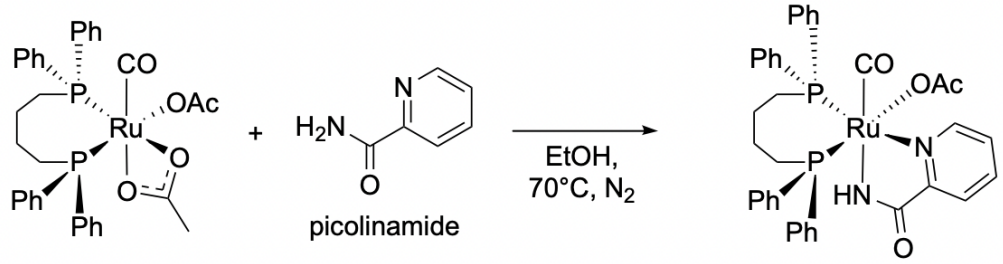
A reaction of a Ru–phosphine precursor with picolinic acid in a 1:1 ratio in dry ethanol at 70°C under nitrogen atmosphere for 24 hours. The 1:1 ratio is critical as to only have one acetate ligand be substituted, not both. The product was precipitated with dichloromethane (DCM) and diethyl ether (Figure 1).

Figure 1: Synthesis of Ru–picolinamide complex (Ru-pico)



The same procedure was carried out with a Ru-phosphine precursor except using picolinamide instead of picolinic acid under the same reaction conditions (Figure 2).

Figure 2: Synthesis of Ru–picolinic acid complex (Ru-pica)



After the Ru-pica and pico complexes were purified, their respective hydride Ru-complexes were then synthesized. The reaction carried out in an NMR tube since the complex was synthesized in a small amount. The Ru-pico/pica complex was added to its respective NMR tube along with dimethylformamide (DMF) and deuterium oxide. 10 equivalents of sodium formate were then added. To purify, the product was filtered with hexane to get rid of potential oxides (Figure 3 and 4).

Figure 3: Hydride formation of Ru–picolinamide complex

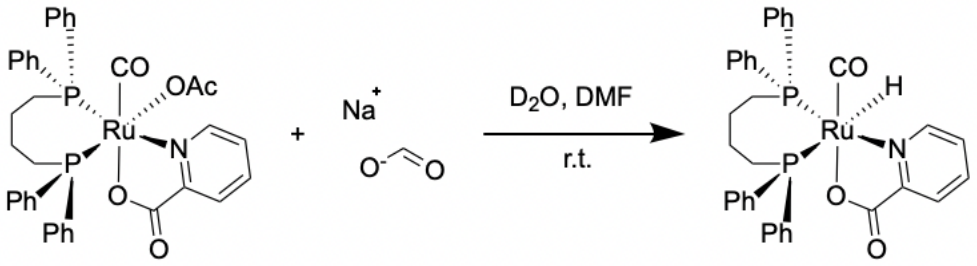
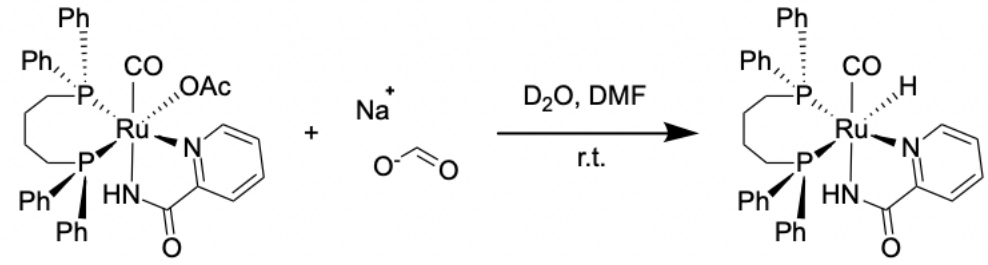


Figure 4: Hydride formation of Ru– picolinic complex



The investigations undertaken confirmed that the pico and pica-hydride Ru complexes could be successfully synthesized. NMR analyses confirmed the hydride pico and pica products were formed as a doublet of doublet could be seen around -5ppm in the 1H-NMRs. Furthermore, it was concluded that the pica Ru-complex was found to be yellow in color before the hydride formation then turned colorless. Additionally, the pica complex is more reactive because of the amide bond hence the subsequent hydride complex was formed faster than the pico complex. The pico complex, on the other hand, was found to form the hydride complex slower and was colorless for the whole synthesis. It was also discovered that both final hydride complexes are soluble in ether and hence cannot be used for precipitation as a purification method.

The work demonstrated that ligand environment strongly governs the reactivity and stability of Ru complexes. Going forward, the design of ligand frameworks could be expanded to tune catalytic properties, improve biocompatibility, and explore applications in redox-based cancer therapeutics.

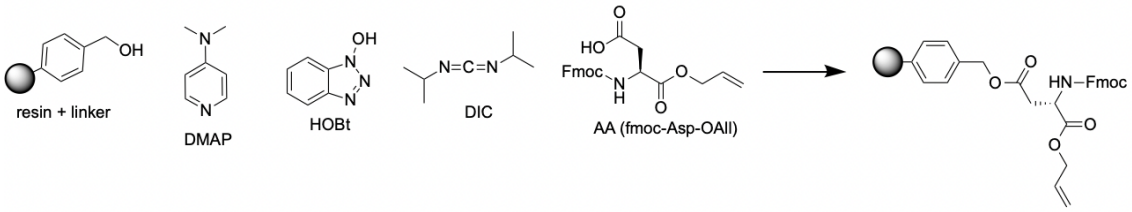
Solid-Phase Peptide Synthesis (Felix Böhm)

SPPS is a robust method for assembling peptides on a resin support, enabling rapid synthesis and purification. In the Casini group, SPPS is used to generate bioactive peptides, linkers, and hybrid molecules relevant for drug development and mechanistic studies.

In the work done with PhD student Felix Böhm, the side chain of the amino acid aspartic acid was directly bound to the linker of the resin. A series of deprotecting the base-labile Fluorenylmethyloxycarbonyl (Fmoc) protecting group on the N-terminus and coupling a new amino acid was repeated until the desired peptide sequence was achieved. An on-resin cyclization was attempted as well.

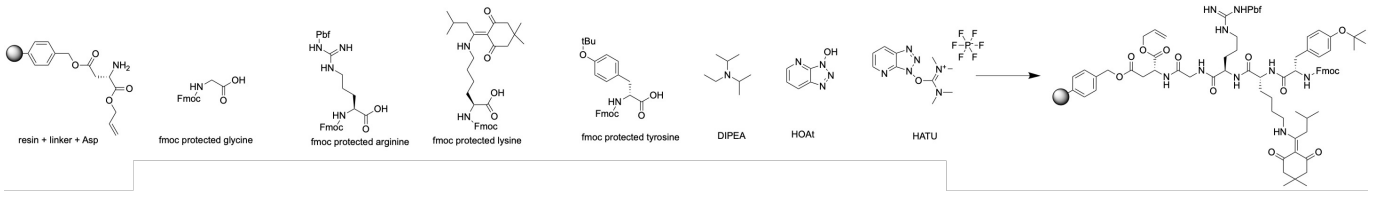
To start the peptide synthesis, 50mg of resin was added to a filter syringe which was then swelled with washes of DCM and DMF. 4-Dimethylaminopyridine (DMAP), hydroxybenzotriazole (HOBt), N,N'-Diisopropylcarbodiimide (DIC), as well as the Fmoc-protected aspartic acid were added to the syringe which was then shaken for 3 hours. Washes of DCM and DMF were repeated (Figure 5). The Fmoc-group was deprotected with a 50% solution of piperidine.[2]

Figure 5: Steglich esterification to couple aspartic acid



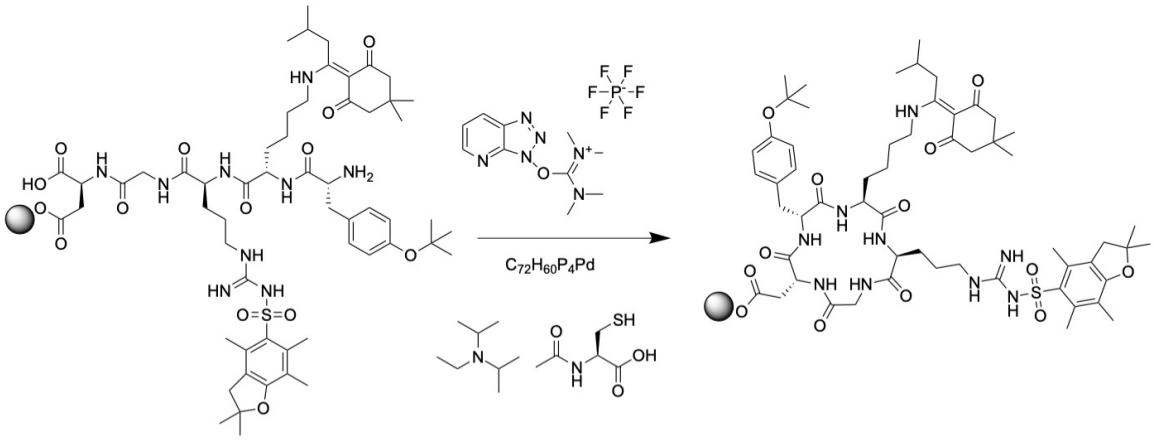
Next, two equivalents of glycine, two equivalents of HOAt, and 1.98 equivalents of HATU were dissolved in DMF and five equivalents of DIPEA. This mixture was then added to the filter syringe and left to shake for 1 hour. The syringe was washed with DCM and DMF and glycine's Fmoc protection group was deprotected with 50% piperidine. This process was repeated with amino acids arginine, lysine, and tyrosine until the desired amino acid sequence was achieved[2] (Figure 6).

Figure 6: Coupling of GRKy amino acid sequence



Finally, an on-resin cyclization was performed with a palladium complex and n-acetylcysteine (NAC). The mixture was left to shake for 2 hours then washed with DMF and methanol. A deprotection with 50% piperidine was then performed, along with DCM and DMF wash steps. Finally, HATU and DIPEA were added to the syringe and shaken for 1 hour (Figure 7). Cleavage from the resin was performed using Trifluoroacetic acid (TFA), triisopropylsilane, and water.[2] The peptide was filtered from the cleavage cocktail and subsequent mass spectrometry was performed.

Figure 7: On-resin cyclization



Mass spectrometry data proved the on-resin cleavage was relatively successful, with main peaks in the correct region of around 1118 amu. Two couplings of gold complexes were also attempted but were deemed unsuccessful according to mass spectrometry data from the test cleavage. Another test cleavage was done, and more mass spectrometry data was gathered using different solvent mixtures, however, the results were the same. It is unclear what product was formed after the attempted coupling of the gold complexes.

SPPS provided valuable training in peptide chemistry and highlighted challenges in yield and selectivity. Future improvements could involve optimizing protecting group strategies, testing alternative coupling reagents, and applying SPPS-derived peptides in bioinorganic conjugation strategies.

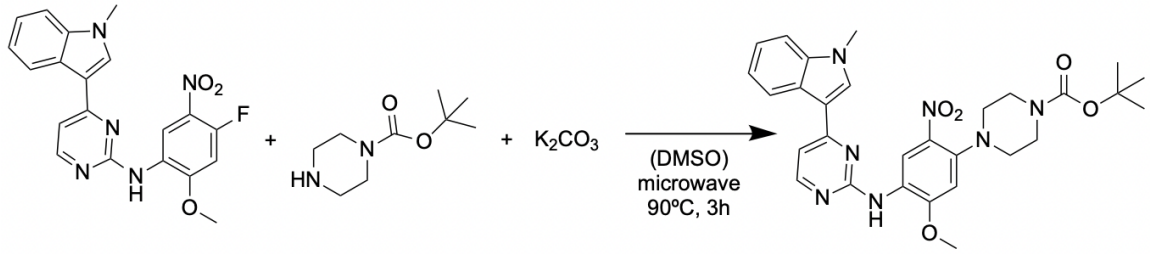
Mass Tags (Melina Rumpf)

Mass tags are isotopically distinct or chemically unique moieties incorporated into molecules to facilitate detection by MSI.[3] MSI enables the spatial mapping of biomolecules in tissues, making it a powerful tool for investigating disease mechanisms and drug localization.[4] However, not all molecules are easily detectable due to poor ionization or low abundance, hence why mass tag labelling strategies have been developed.[5]

During my internship, I contributed to work on metal-based mass tags with PhD student Melina Rumpf. This project aimed to develop and test novel ruthenium-based tags, which take advantage of their unique isotopic signatures to improve imaging sensitivity and multiplexing capabilities. I learned how metal-based tags are synthesized and conjugated to biomolecular scaffolds.[6]

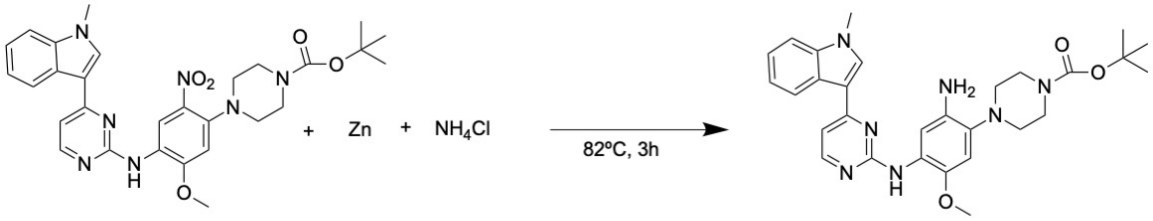
The first reaction performed involved the nucleophilic aromatic substitution of a para-fluoro, para-nitro substituted indole–pyridine scaffold with Boc-protected piperazine under microwave heating conditions (Figure 8). The resulting derivative represents a mass taggable handle. The product was purified using flash chromatography.

Figure 8: Indole–Pyridine Derivative



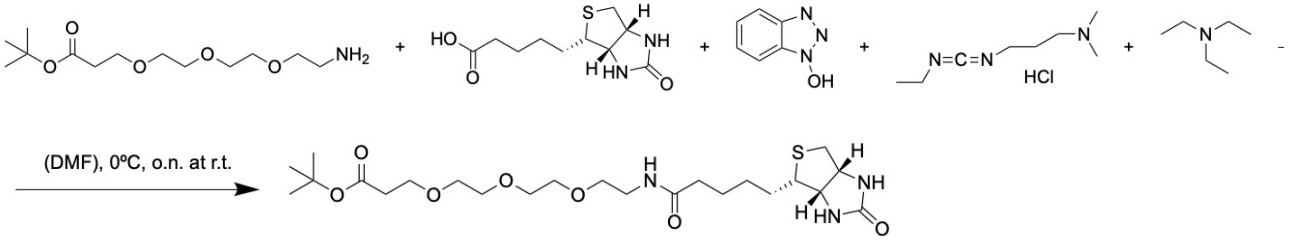
The nitro group of the product in Figure 8 was then reduced to an amine group using zinc and ammonium chloride. This was done so that the amine group is more available to attack new groups on other compounds. The reaction was carried out in a mixture of ethanol and water and was later purified using flash chromatography (Figure 9). A color change could be seen with the starting reactant being dark orange and the product being a light yellow color.

Figure 9: Reduction of Nitro Group to Amine



A linker for mass tag usage was synthesized using biotin. The reaction was carried out at 0ºC overnight in DMF. A deprotection of the Boc protection group was then performed using TFA in a solution of DCM (Figure 10).

Figure 10: Linker Coupling Reaction



The linker coupling reaction (Figure 10) had to be performed again because the wanted product could not be distinguished in 1H-NMR analyses. After performing NMRs of the starting reagents, it was discovered that the biotin had been degraded after prolonged opening. A new reagent was purchased, and the reaction was performed again, leading to the desired linker product.

The work highlighted how synthetic chemistry can enhance analytical imaging by improving detection sensitivity and multiplexing. A promising avenue for further development is the integration of click chemistry into mass tag design.[7] Click reactions, such as azide–alkyne cycloadditions, offer highly selective and bioorthogonal ways to attach tags to biomolecules. This could enable faster, more efficient, and site-specific conjugation of mass tags to proteins or peptides directly in biological samples.[7] Future directions may therefore include developing new cleavable linkers, incorporating click chemistry handles into mass tag scaffolds, and expanding metal-based tags to multiplexed proteomic applications.

Techniques and Skills Learned

- Synthetic organic and organometallic chemistry workflows
- Solid-phase peptide synthesis (SPPS)
- Purification methods (column chromatography, HPLC, flash chromatography)
- Analytical characterization (NMR, MS)
- Laboratory safety and solvent handling
- Scientific communication and electronic notebook use
- Microwave-assisted reactions

Conclusion and Future Work

This internship provided valuable exposure to interdisciplinary research at the interface of bioinorganic and medicinal chemistry. I gained both practical laboratory skills and conceptual insights into drug design, peptide synthesis, and the role of metal complexes in cancer therapy. Future work could involve tailoring Ru complexes for catalytic therapeutic applications and evaluating them in cellular assays, optimizing SPPS strategies for the efficient synthesis of cyclic and metal-conjugated peptides, and developing next-generation mass tags for high-resolution, multiplexed MSI. Collectively, these efforts highlight the potential of combining synthetic chemistry with biological insight to advance anticancer research.

References

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