Diamide Macrocycles: Potential Bifunctional Ligands for Cu-64, Ga-68 and Tc-99m

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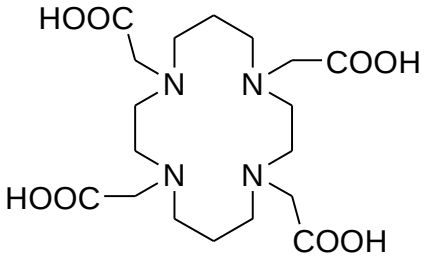
Targeted Radionuclide Therapy

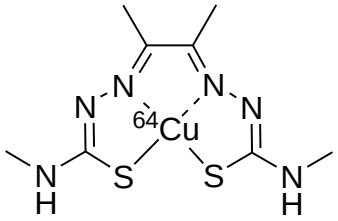
Krakow, June 24-25 2008

Radiopharmaceutical ligands

•New bifunctional ligands for Cu(II) for labeling peptides and proteins

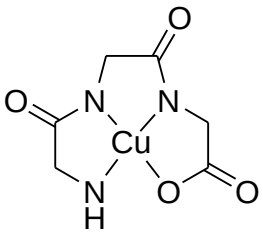


TETA

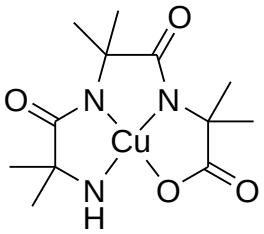


- TETA form thermodynamically stable complexes with Cu(II).
- [Cu(II)TETA] show relatively poor stability *in vivo* particularly for conjugates for peptide conjugates – ^{64}Cu(TETA-octreotide).
- The *in vivo* instability of [Cu(II)TETA] is likely to result from reduction to the Cu(I) species, which is unstable with respect to ligand dissociation.
- Reduction and complex destabilization prior to Cu(I) loss has been implicated in the selective trapping of Cu-64 from its bis(thiosemicarbazone) complex in hypoxic cells.

Radiopharmaceutical ligands

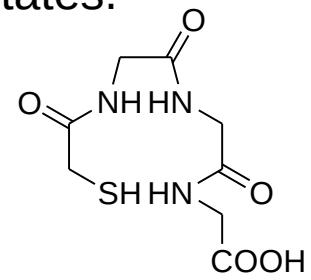


[Cu(III)Gly_3]

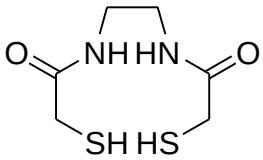


[Cu(III)Aib_3]

- Amidato-donors (deprotonated amide groups) are well known to stabilize metal ions in high oxidation states.



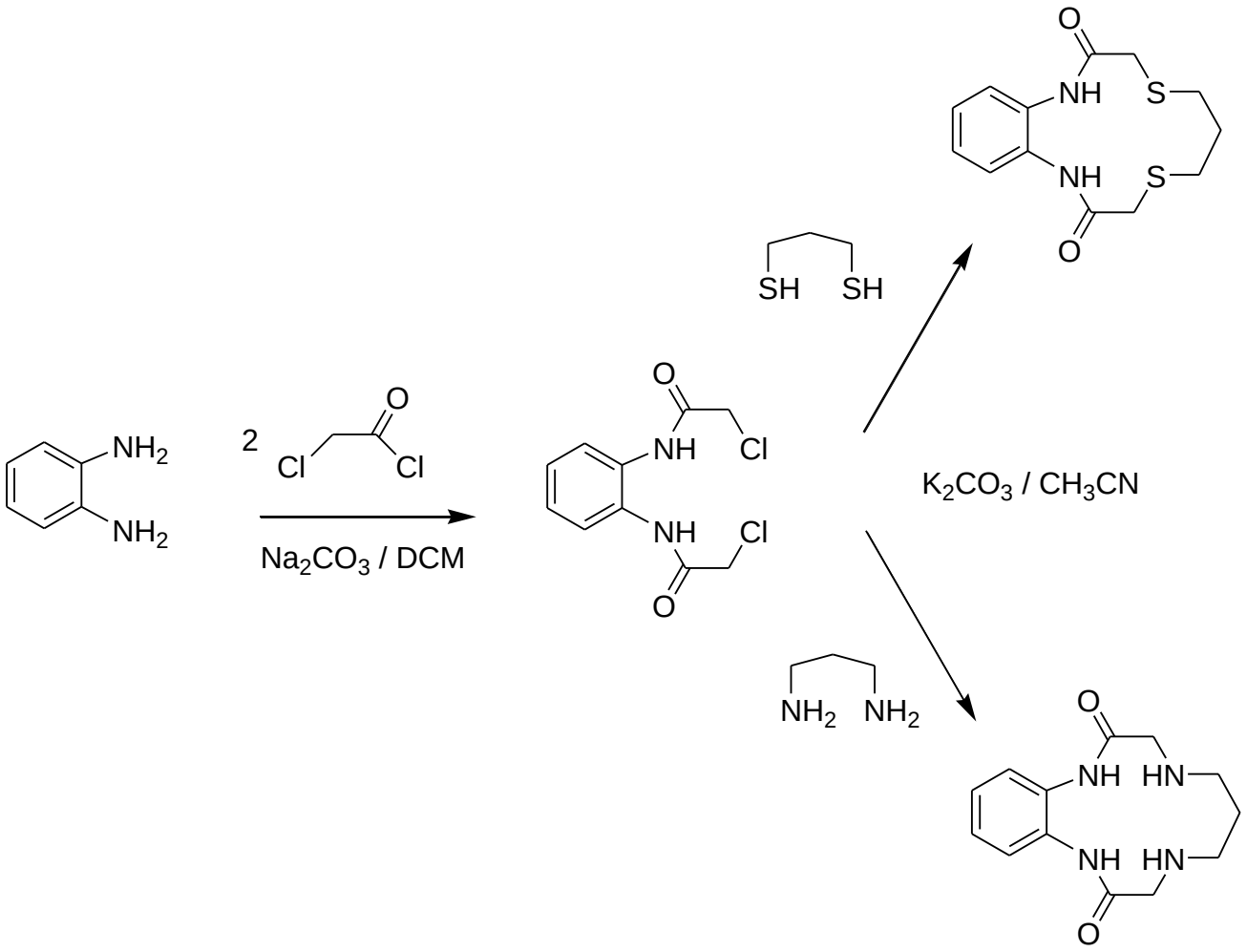
N_3S triamidethiol



N_2S_2 diamidedithiol

- N_3S and N_2S_2 ligands with amidato-donors have been successfully used with Tc-99m and Re-186.
- Macrocycles – good thermodynamic and kinetic stability and amides strong anionic donors.

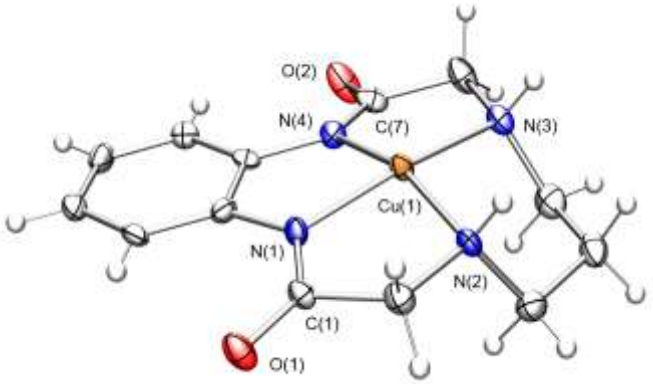
Diamide macrocycles from phenylene diamine

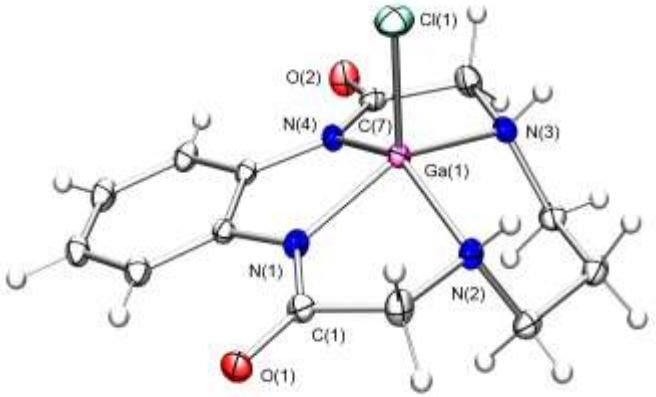


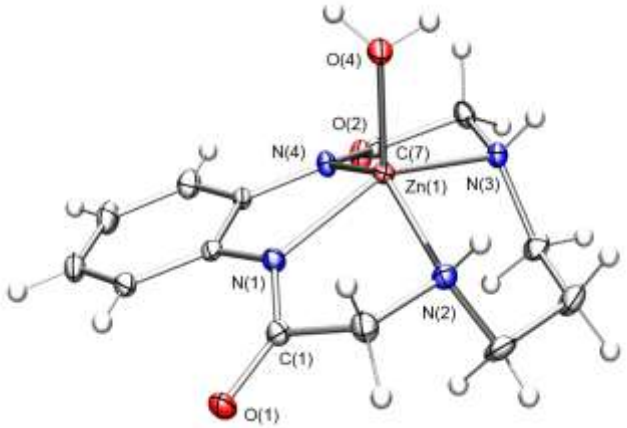
Metal complexes

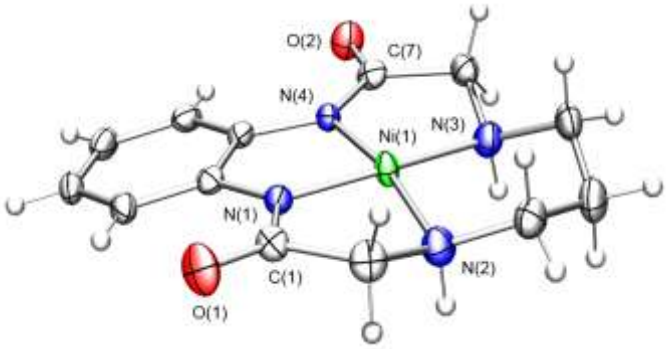
- Copper complex forms rapidly under mildly basic conditions (acetate in water).
- Zinc and gallium complexes synthesised in methanol with the strong base sodium methoxide.
- Gallium complex may be synthesised without base in methanol with microwave heating.

Structural Studies

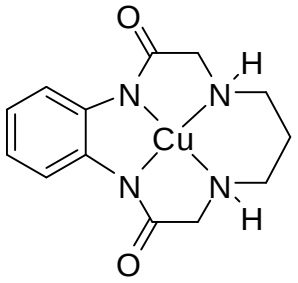


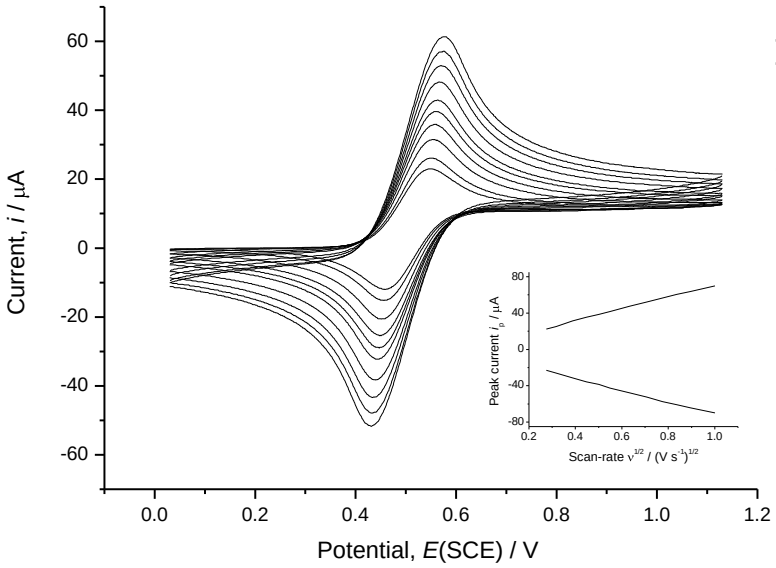


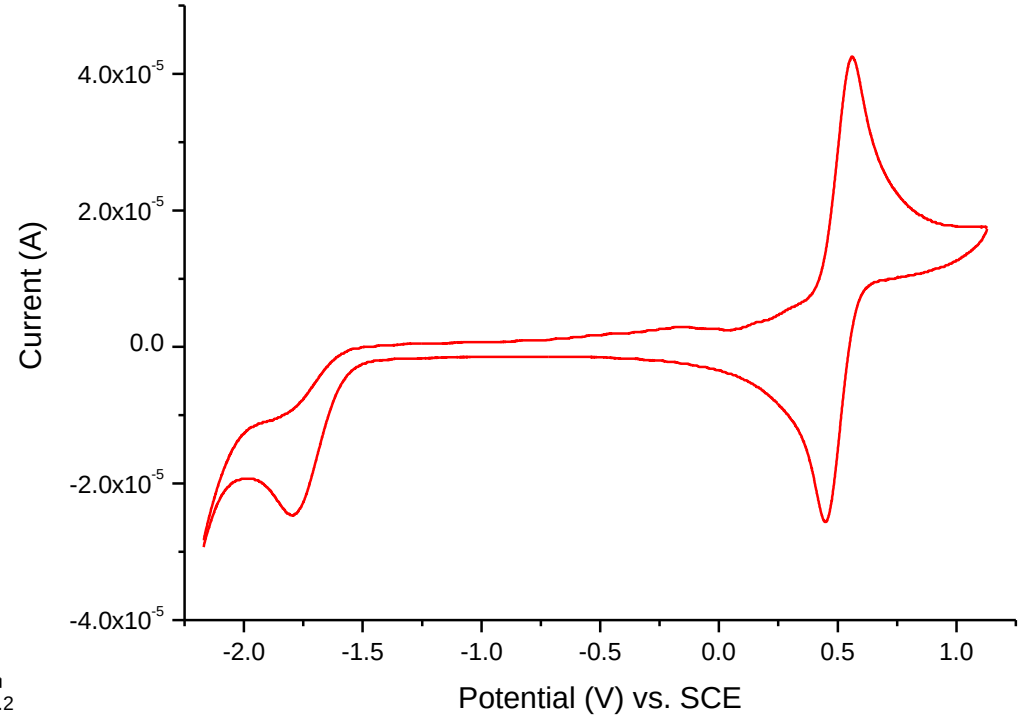




Electrochemistry

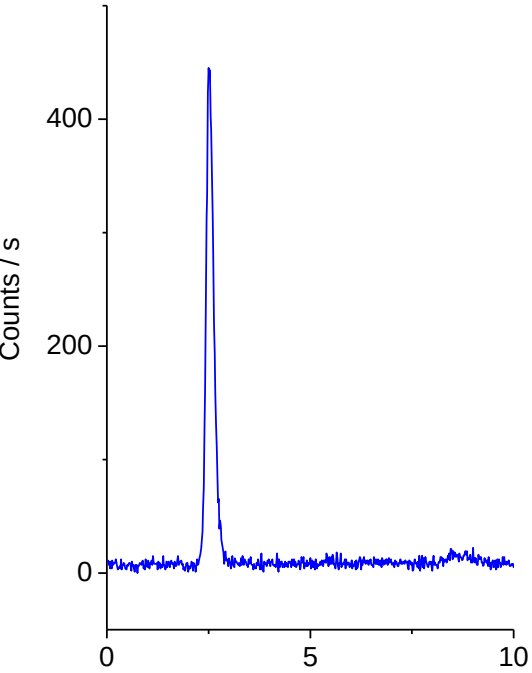


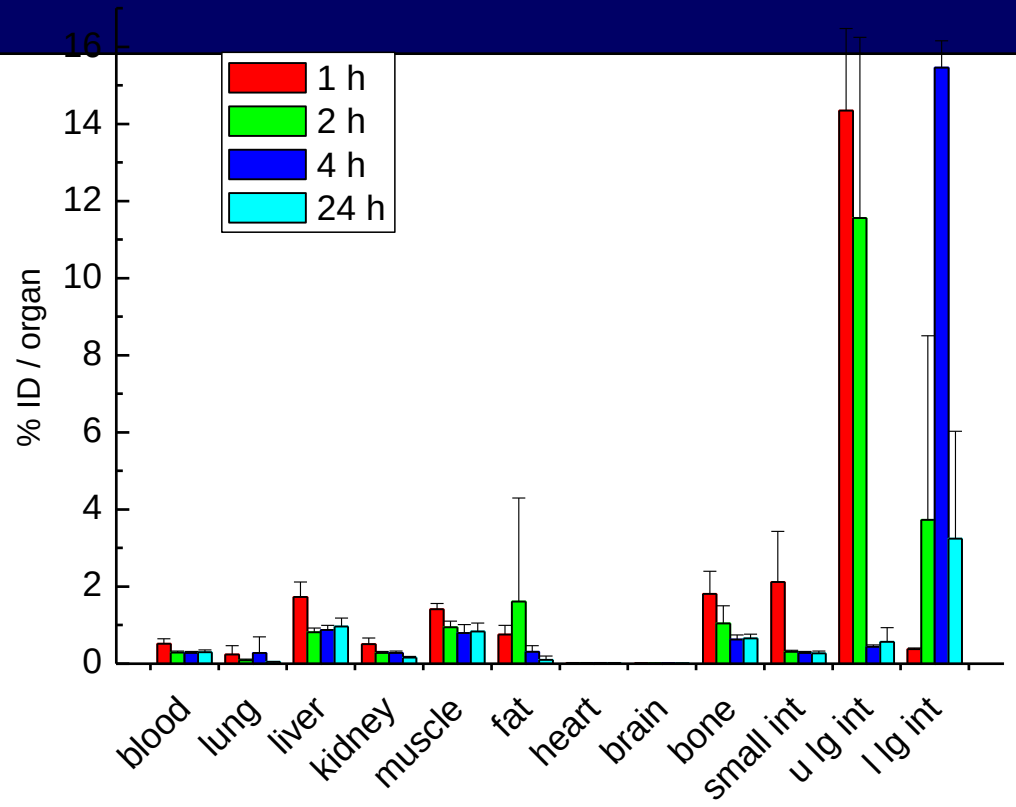




- Very negative reduction potential, inaccessible under biologically relevant conditions.

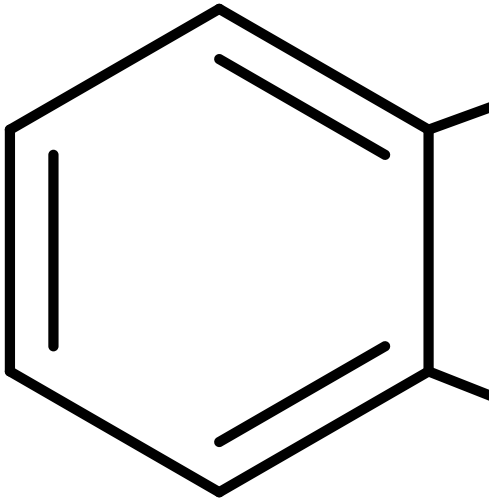
Cu-64 Biodistribution

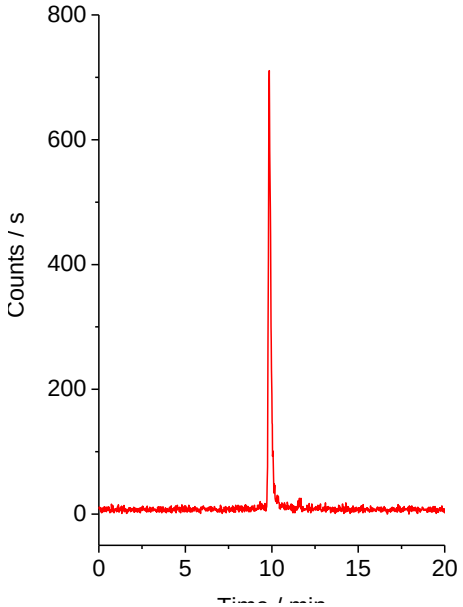




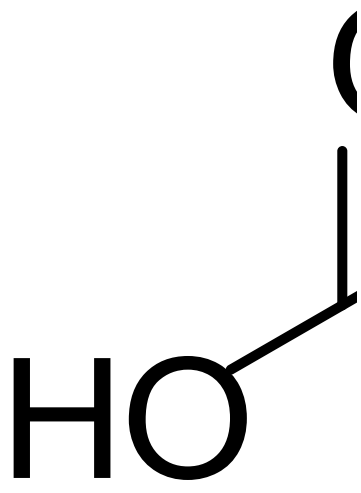
- RP-HPLC shows short retention time for Cu-64 labelled macrocycle (hydrophilic).
- Biodistribution studies in rats (Carolyn Anderson, MIR, Washington University, St. Louis), complex shows rapid excretion via liver-gastrointestinal tract.
- Complex does not dissociate in blood and is likely to be stable *in vivo*.

Amino acid based bifunctional ligands



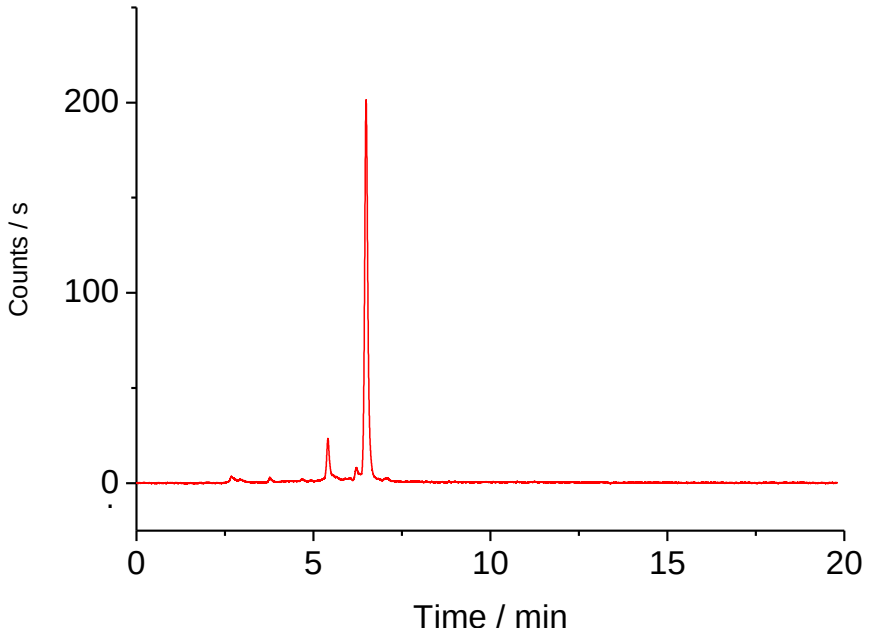


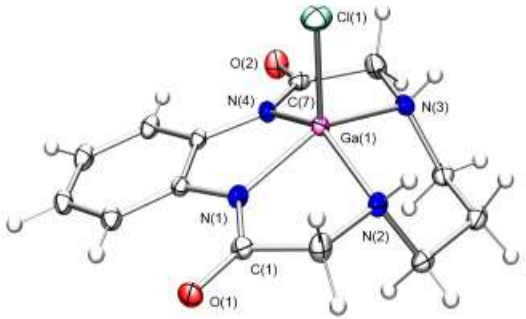
Bifunctional Ligands



- Macrocycle with free carboxylate group obtained in four steps.
- With Professor Helmut Maecke. Conjugation to Somatostatin peptides (Octreotide).
- Biodistribution studies in rats – comparison with TETA-octreotide.

Other radio-isotopes





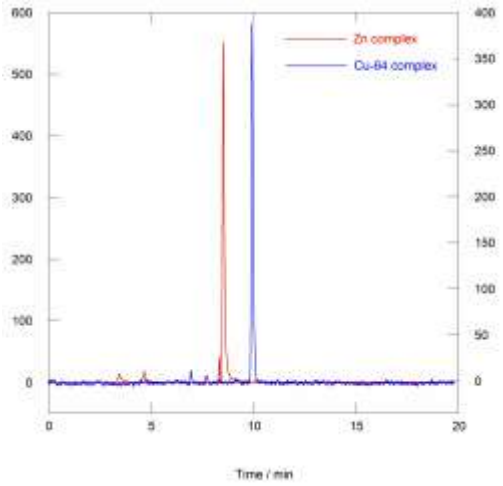
Purification of Radiopharmaceuticals

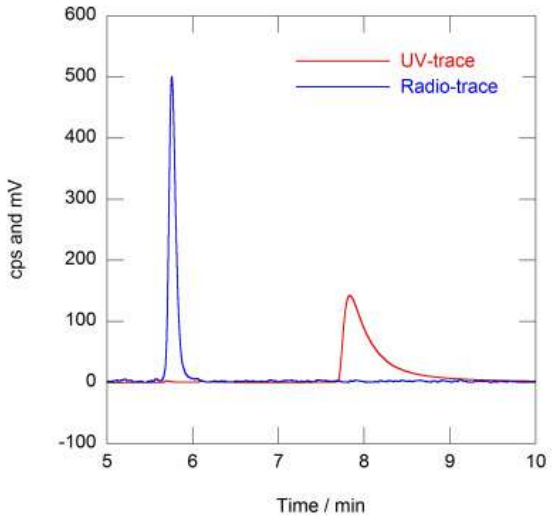
Importance of high specific activity with receptor targeting

Without separation of unlabelled ligand –poor image contrast

Radiolabelled complex separated from unlabelled ligand – improved image contrast

Purification of radio-labelled compounds on solid support

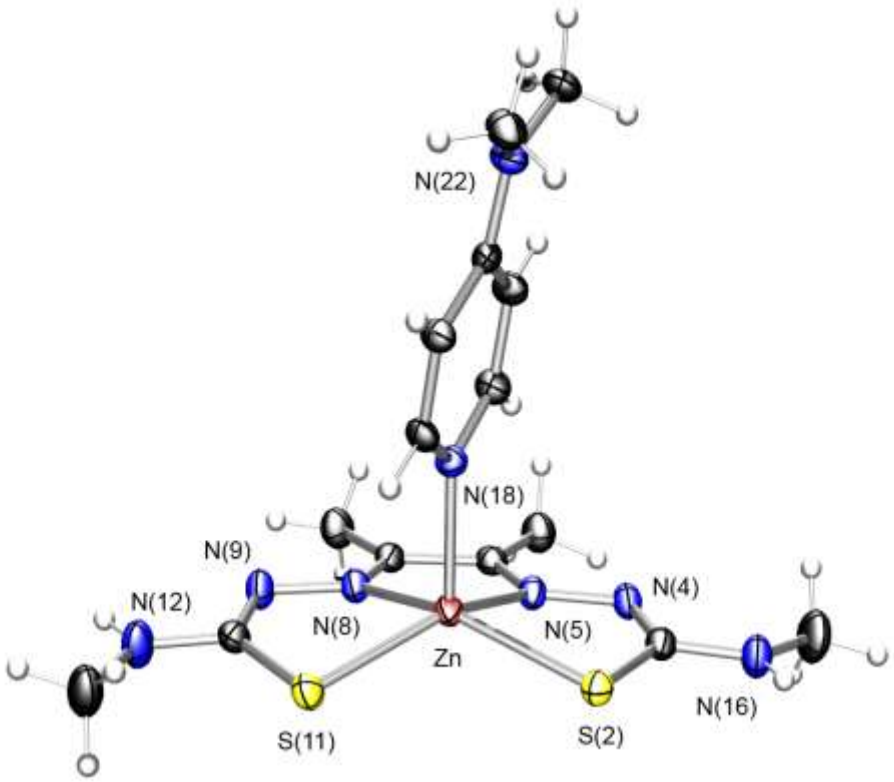




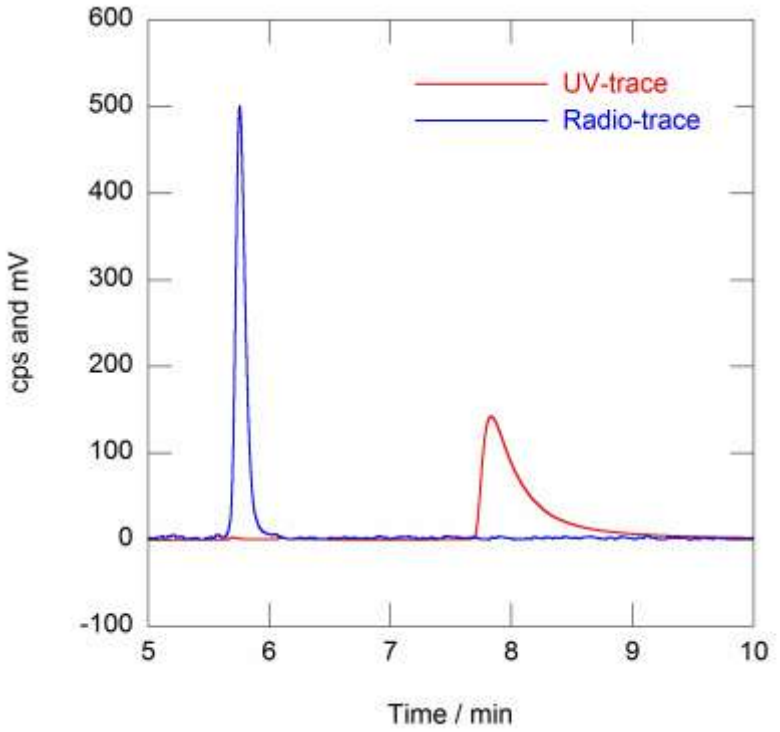
Purification of radio-labelled compounds on solid support

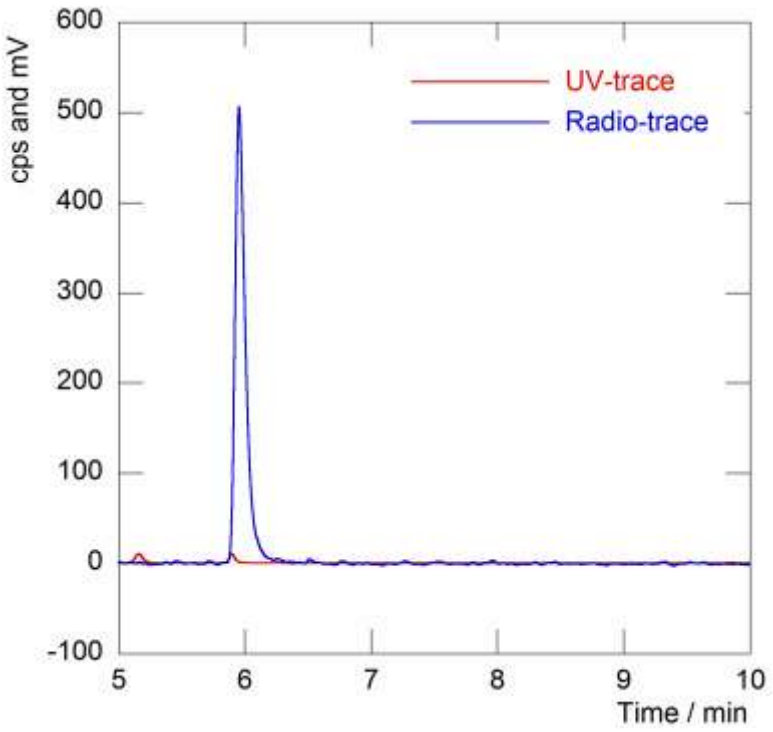
- Jahn-Teller distortion for Cu(II) (d9) results in 4-coordinate square-planar complex, which is released from beads.
- Strong trans effect of Tc=O bond weakens Tc-N DMAP bond

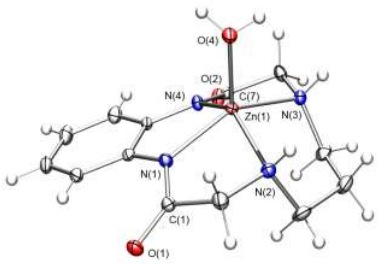
[Zn(ATSM)(DMAP)] Structure



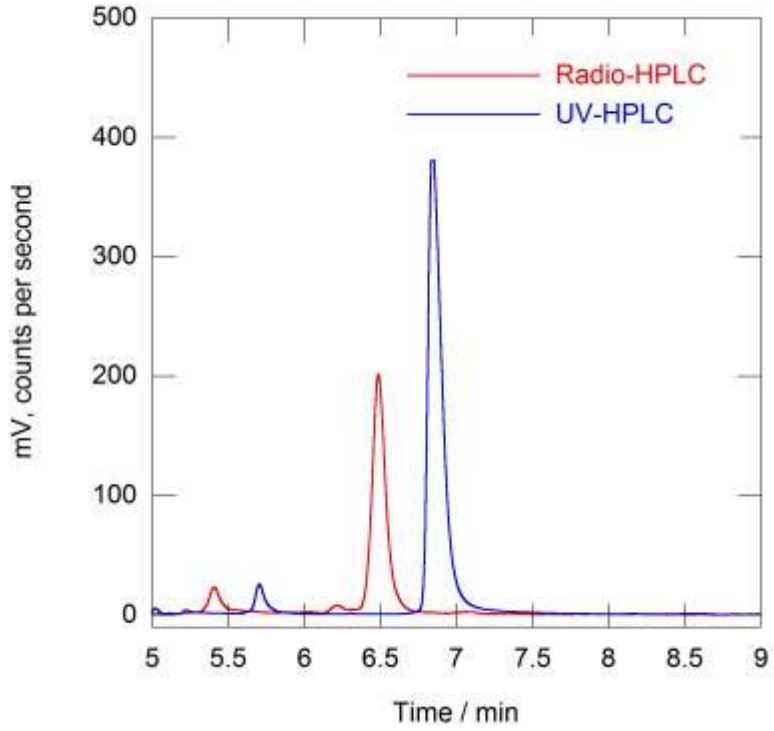
Solid Phase purification, ^{64}Cu

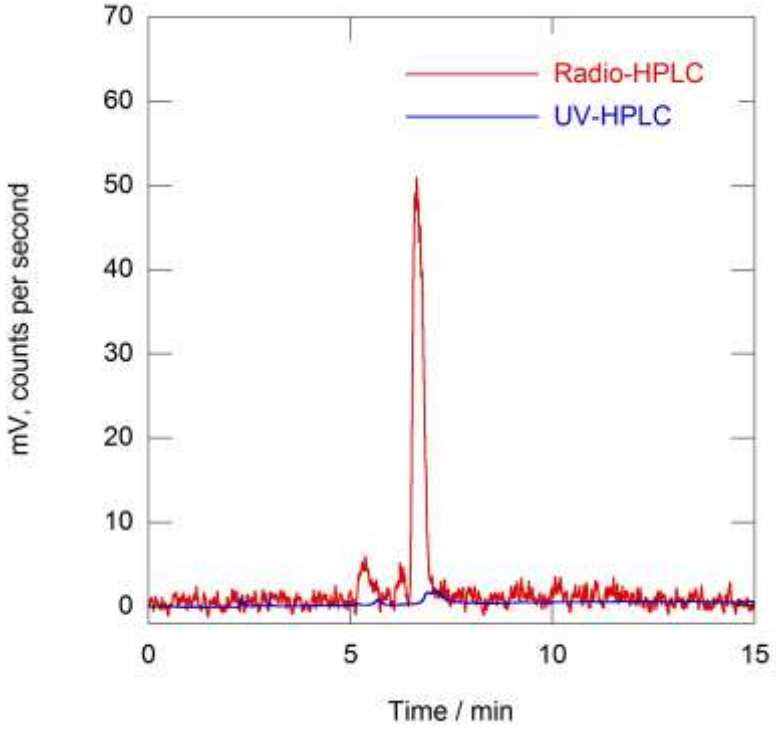






Solid Phase purification, ^{99m}Tc





Acknowledgements

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Dilworth group

Washington University in St.Louis

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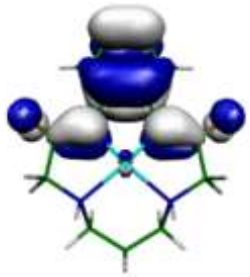
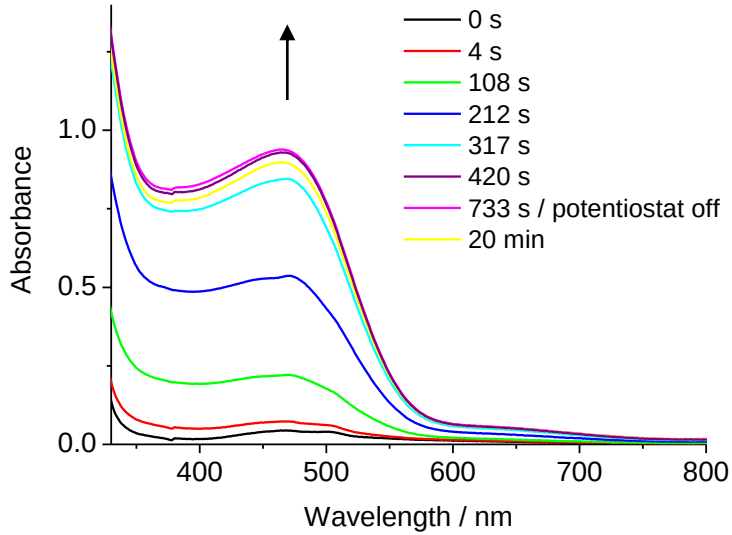
Dr Thaddeus Wadas

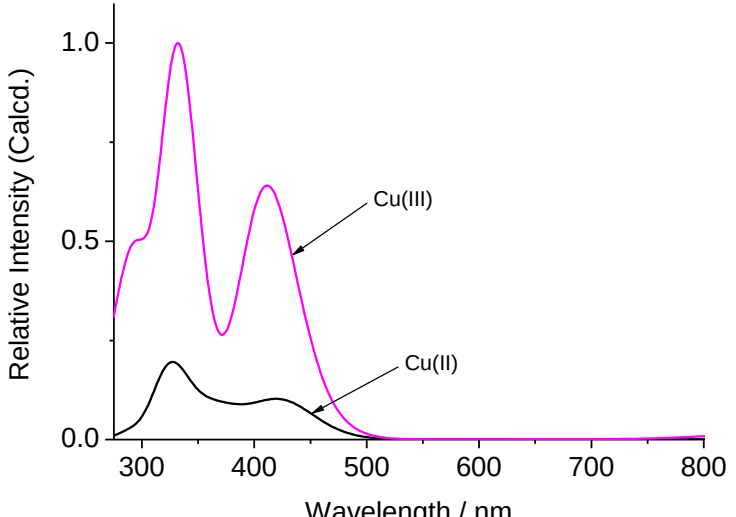
Electrochemistry

- UV-vis spectroelectrochemistry (DMF) in optically transparent thin-layer electrode quartz cell (Applied potential = 0.6 V).
- DFT calculations used to optimise structure of $[Cu(III)(mac)]^+$ in singlet and triplet states.
- Triplet state is predicted to be more stable by 40.5 $kjmol^{-1}$.
- Calculations predict an increase in absorption intensity for Cu(III) complex.



Cu(II)-macrocycle HOMO with high π-character





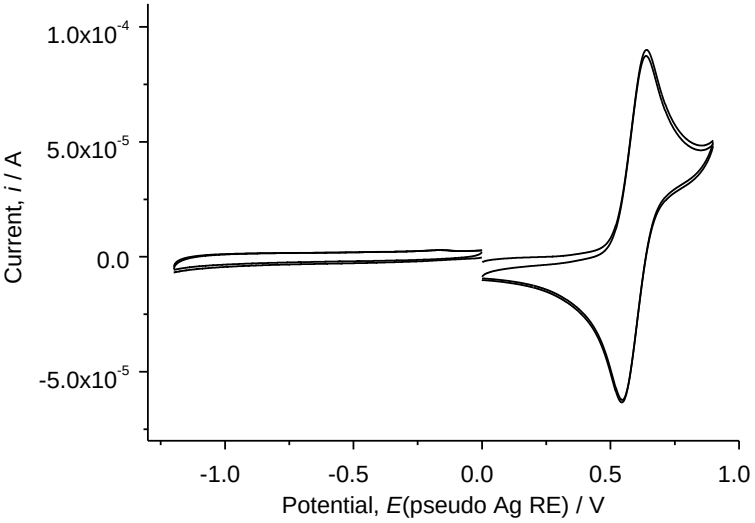
Diamide Macrocycles

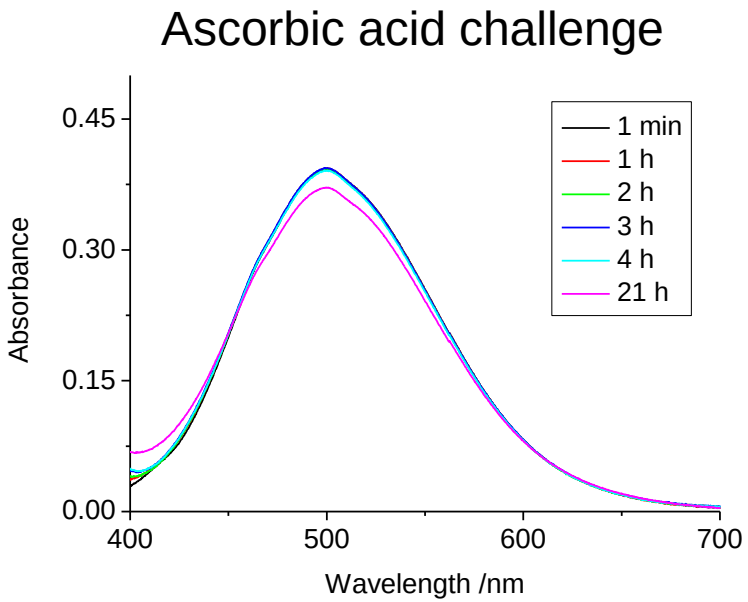
- The biodistribution of the ^{64}Cu-dioxotetraazamacrocycle complex has been evaluated using microPET in rats.
- The complex showed rapid clearance from major organs (e.g. blood, liver and kidney) indicating that the complex is stable *in vivo*.
- Cu labelling sensitive to structure, other diamide macrocycles label with Cu-64 less efficiently.

- ^{64}Cu-monooxotetraazamacrocycle showed high uptake and retention in clearance organs.

Cutler, C. S.; Wuest, M.; Anderson, C. J.; Reichert, D. E.; Sun, Y.; Martell, A. E.; Welch, M. *J. Nucl. Med. Biol.*, **2000**, 27, 375-380.

Cu(II)-dioxocyclam



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