New bifunctional chelators for copper and rhenium for radiopharmaceutical applications

Jon Dilworth

University of Oxford

Kings College September 2007
Contents

Technetium and rhenium chemistry
- Metal-nitrogen multiple bonds?
- New structural motifs, fluorescence?
- Redox active Re complexes?

Copper chemistry
- Bis(thiosemicarbazone) complexes
- Redox resistant macrocyles
'HYNIC' vs 'SHYNIC'

'HYNIC'
- Uncertain structures
- Suitable co-ligands?
- Suitable for Re?

'SHYNIC'
- Very stable, high yields
- Effective for Re
- Use with metals such as Cu
Structure of \([\text{ReO(SHYNIC)}_2]\)

Cowley, Dilworth and Donnelly, Dalton Trans 2004
Radiolabelling of $[\text{ReO(SHYNIC)}_2]$ 

- $[^{188}\text{ReO}_4]^-$ + SnCl$_2$/tartaric acid + S-HYNIC-$H_3$.
- After 1 hour essentially 100 % yield. No perrhenate remains.
A new bifunctional chelator for Re and Tc

X. Sun and J.R. Dilworth 2007

Unpublished results
Some structural motifs in Re chemistry

Re(V)

Re(V)

Re(I)

‘3 + 1’ approach – Spies et al
Pyrazolylacetate Re Complexes Directly in "One-Pot" Reactions From Perrhenate

L : R = H, Me, Ph, PhOMe

Also for diazenide cores with Re(III)
Fluorescent with appropriate pyrazole substituents
Also for 99m-Tc

Towards targeting of pyrazolylacetate complexes in 'one-pot' synthesis route

Redox Reactive complexes with thiosemicarbazones

Tridentate thiosemicarbazones are extremely potent inhibitors of ribonuclease reductase – used therapeutically

First reduction relatively close to Cu(ATSM) – redox activation?

Complexes very stable in serum over many hrs

Cowley, Dilworth, Donnelly, Dalton Trans 2003
Copper coordination chemistry

Thiosemicarbazone complexes

New reduction resistant macrocycles
What does the chemistry of these complexes tell us about possible mechanisms for hypoxic selectivity?

How far can these complexes be modified without compromising their hypoxic selectivity? Can they be optimised for different degrees of hypoxia?
DFT calculations on Cu(II)bis(thosemicarbazones)

Strong distortion on reduction

pKₐ substituent dependent

Complete assignment of UV/vis using TD-DFT

Calculation of solution redox potentials

Substitution at exocyclic N causes minimum perturbation

HOMO

LUMO

LUMO antibonding for Cu-N and Cu-S

HOMO-LUMO gap correlates with redox potential

*Dalton Transactions 2006, 496*
Spectroelectrochemical studies on [Cu(ATSM)]

Successive in situ UV/vis spectra for reduction of Cu(ATSM) showing isobestic points. Much more complex in presence of water/acid

Time resolved spectrum showing rapid reformation of Cu(II) via diffusion of O₂ into cell
Proposed mechanism for hypoxic selectivity

Consistent with spectroelectrochemistry, CV and DFT calculations
Criteria for modification of ATSM

• Retain redox characteristics - no changes to backbone - use exocyclic nitrogens
• Retain as much of ATSM structure as possible - single site modification
• Simple, rapid, reproducible and flexible chemistry
• Stability at least equivalent to that of Cu(ATSM)
• Facility to attach functional targeting or probe molecules - sugars, proteins, 2-nitroimidazoles, fluorophores, 18-F radiolabel, cytotoxins etc
New bifunctional thiosemicarbazones

MeHN

+ 

NH₂₃

MeHN

- NH₂

ATSMa

- NH₂

ATSMen

- NH₂

ATSMac
More extreme modifications: a new hybrid thiosemicarbazone ligand

Cu(II) complex strongly fluorescent!

Cowley, Dilworth and Donnelly, Inorg Chem 2006
Synthesis of Zn(ATSMA) and glucose conjugates

Route now available to attach F-sugars direct to MeNH group

UV/vis of transmetallation

Cu analogue

Inorg Chem 2007, 465
Solid phase purification of derivatised [Cu(ATSM)]

Also effective for transmetallation of macrocycles with Cu and for Tc and Re based radiolabelling

P. Barnard, S. Bayly, H. Betts, J. Holland, unpublished 2007
Uptake of a glucose conjugate in IGROV cells

\[
\text{OH} \quad \text{OH}
\]

\[
\text{H} \quad \text{H}
\]

\[\text{ex} = 560 \text{ nm, } \text{em} = 605 \text{ nm}\]

\[\text{ex} = 360 \text{ nm, } \text{em} = 530 \text{ nm}\]

Coronal slice through rat which received 11.52 MBq $^{64}$CuATSE-G showing distribution of activity (same slice for both time points):

A: over first 60 min after injection  
B: 60 - 120 min after injection
Synthesis of acetophenone-imine conjugate

CV indicates reversible couple with little shift from Cu(ATSM)
Stable in serum for several hours
Hypoxic selectivity of imine conjugate

Biodistribution data for imine conjugate vs Cu(ATSM)

Versus Cu(ATSM) at 60 min
Aminoacid and peptide conjugates

ZnATSM/A

\[ \text{ZnATSM/A} \rightarrow \text{NEt}_3 \rightarrow \text{THF} \rightarrow \text{ZnATSM/A-Ala-Boc} \]

Helen Betts, unpublished 2007
PET images of ATSM conjugates in tumour bearing rats

Conjugate of ATSMA with alanine

Conjugate of ATSMA with glucose

Images by M. Christlieb and D. Honess, GCI, 2007
Fluorescence studies of anthracene imine

Fluorescence spectrum of anthracenealdehyde

Fluorescence of anthracene imine adduct many times more intense than for ATSM - FRET occurring from anthracene to zinc. Copper analogue also fluorescent

M. Theobald, unpublished, 2007
**CV and structure of Cu diamide macrocycle**

High yield radiolabelling with 64-Cu

Very stable in serum and with glutathione and reductive challenges

Forms stable Ga complex

*P. Barnard, unpublished 2007*
Bifunctional diamide macrocycles

\[
\text{Bifunctional diamide macrocycles}
\]

![Chemical reaction diagram showing the synthesis of bifunctional diamide macrocycles.

1. Reaction of an aromatic diamine with an amine acid (Z-NH₂CO₂H) using DCC.
2. Transformation of the resulting diamide into a macrocycle using BOP.
3. Reduction of the macrocycle with H₂/Pd/C.
4. Substitution with 1,2-dibromoethane to form the final product.

R = CH₂CH₂COO'Bu
R = CH₂CH₂CH₂CH₂NHBoc]
Summary

- Synthesis and characterisation of new bifunctional chelators for Tc and Re
- Studies of fundamental chemistry of Cubis(thiosemicarbazones) – insights into mechanism of action in vivo
- Conjugation via exocyclic nitrogen to modify biological behaviour
- Use of fluorescence to study cellular uptake and distribution
- Synthesis and characterisation of new redox resistant Cu diamide macrocycles
Dr Andrew Cowley, Prof D. Kerr, Dr G Churchill, Dr F Aigbirhio, Dr M. Christlieb, Dr D Honess,

Funding: EPSRC, GE Healthcare, GSK, CRUK, DTI, Siemens Molecular Imaging