Inorganic and Radiopharmaceutical Chemistry

Isabel Santos

The *Inorganic and Radiopharmaceutical Chemistry Group* (**IRC**) was established on January 2000, after a reorganization of the scientific activities in the Chemistry Sector. This reorganization tried to reinforce the organic and inorganic expertise of the previous radiopharmacy team, as recommended by the international advisory board which has been evaluating the ITN activities in 1997. Therefore, the *Inorganic and Radiopharmaceutical Chemistry Group* is formed by researchers from the previous radiopharmacy team and by some researchers and students with expertise in inorganic chemistry, previously working in the Inorganic and Organometallic Chemistry group.

The main goal of the *Inorganic and Radiopharmaceutical Chemistry Group* is the design and synthesis of new and stable radioactive compounds which exhibit biologically useful properties for imaging and/or therapy. To relate the structure and the physico-chemical properties of these compounds to their biological behavior is also one of our goals. This multidisciplinary research area involves organic and inorganic synthesis, radiochemistry and biological studies *in vitro* and in animal models. **Our research work intends to contribute** for the development of new radiopharmaceuticals, for providing specific expertise to national organizations and for training in chemistry and radiopharmacy.

Taking into account the Nuclear Medicine needs, the background of the chemists, radiochemists and radiopharmacists involved in this team, the facilities of ITN and the interest of some National Universities and Institutes on the specificity of the IRC, we have established, during 2000**, two main research topics**:

1.- Metal-*d* and - *f* - Based Drugs
2.- Halogen-Based Drugs.

The metal-based drugs research involves mainly basic chemistry, radiochemistry and biological evaluation of Re, Tc and Ln, as these elements have radionuclides relevant for therapy and/or imaging. To design biochemically acceptable complexes is necessary to develop different building blocks for labelling biomolecules while maintaining their specificity for the receptors. This involves the evaluation of complexes with metals in different oxidation states and with distinct cores using different ligands and approaches for the targeting of biomolecules.

For **Re** and **Tc** different new building blocks have been found, with the metals in the (I) and (V) oxidation state and stabilized by boron containing ligands or by heterofunctionalized phosphines. These new building blocks present physico-chemical characteristics promissing for the labelling of biomolecules with affinity for central nervous system receptors.

For **Ln** we have been evaluating different chelators. With ^{153}Sm, prepared using the available conditions at RPI, new compounds with macrocycles containing carboxylate and phosphonate pendant arms have also been prepared.

The halogen–based drugs research involves the synthesis of new triphenylethylene derivatives to be evaluated as therapeutic or imaging agents for breast tumors.

Suitable precursors for the synthesis of halogenated TAM analogues and their α –hydroxylated derivatives have been prepared.

Close links have been established with other National and International Universities and Research Institutes through doctoral students, lecture courses and joint research projects.

In February 2000, research projects on the above mentioned scientific areas have been submitted for funding to the Foundation for Science and Technology (FCT). We acknowledge the funding of three of the six submitted projects. We also acknowledge the Portuguese Foundation for Science and Technology for several Doctoral, Post-Doctoral and Visiting-Scientist research grants, and Action Cost B12.

Adjustments and rearangements are still needed in the group**.** In fact, a better focus of our research is necessary as well as a clear definition of research strategies for the biochemists (one PhD and a ITN/R.A./PhD student at the University of Genève, since 1997). However, for some scientific decisions we need to know clearly the funding policy of ITN. The funding from FCT is desirable but we can not be based exclusively on it. The achievements attained, so far, have been possible due to the commitment of staff and students and of colleagues inside and outside ITN. All of them are acknowledged.

Research Team

Researchers

- Isabel Santos, Inorganic Chemist (Principal Researcher) (**Group Leader**)
- António Rocha Paulo, Inorganic Chemist (Aux. Researcher), (Re and Tc/Boron Containing Ligands)/Professor ESTSL
- Maria Paula Campello, Inorganic Chemist (Aux. Researcher), (Ln Complexes /Macrocyclic Ligands, ITN/ ITQB)
- Maria Cristina Melo e Silva*, Radiochemist (Aux. Researcher), (Triphenylethylene Derivatives, Halogen Labelling, ITN/IST)
- Lurdes Gano**, Pharmacist (Aux. Researcher), Biological Studies, Animal Models
- Maria Fernanda Marques, Biochemist (Aux. Researcher)
- Paula Raposinho, Biochemist (Research Assistant, since 1994), doing PhD in Switzerland since 1997
- Célia Fernandes, Radiochemist, Research Assistant, doing PhD in ITN since 1999
- João G. Correia, ITN contract (Re and Tc /Heterofunctionalized Phosphines)
- Yong-Xing, PRAXIS Post-Doctoral, Oct. 2000-Oct 2001
- Rudolf Herrmann, PRAXIS Visit. Scientist, Oct. 1999- Oct.2001 (Org. Chem., ITQB/ITN)

* PhD equivalent, February 2000.
** PhD July 2000.

Students

- Leonor Maria (PRAXIS **PhD)**, Feb. 1999 - 2003
- Raquel Garcia (PRAXIS Grad. Student), Mar. 1999- Mar. 2001
- Elisa Vaz Morgado de Palma (PRAXIS Grad. Student), Oct. 2000 – Oct. 2003
- Ana Silva, IST/(**BSc** Last year Student), Oct. 2000

Technical Personnel

- Amadeu Rodrigues, Maintenance/Ventilation/ Equipment
- Elisabete Correia, Biological Studies
- Helena Sousa, Laboratories Support

Publications

Journals:	11	and 1 in press
Proceedings:	1	
Conf. Communications:	13	
PhD Theses:	2	

Funding

	$\times 10^3$ PTE
Research Projects(a):	3879
Services:	595
TOTAL:	4474

(a)

	$\times 10^3$ PTE
- Compounds of f-elements. Synthesis, Characterization and Reactvity Studies (PRAXIS/2/2.1//QUI/454/94) – (1997 – 2000) (7500×10^3 PTE) ITN/Co-ordinator**: Isabel Santos**.....	750
- Rhenium Complexes with Nitrogen Donor Ligands (PRAXIS/P/QUI/10047/1998) – (1999 – 2001) ($18\,479 \times 10^3$ PTE $\rightarrow$ ITN/8703×10^3 PTE) ITN/Co-ordinator**: Isabel Santos**, Partner: ITQB (Carlos Romão)	2610
- 17α-[^{125}I] Iodovinil Steroids Substituted at 7α: Investigation of a New Series of Iodine [^{125}I]-Labelled Estrogens as Potential Imaging Agents for Estrogen Receptor-Positive Breast Cancers (PRAXIS/PSAU/C/SAU/89/96) – (1997 – 2000) ($18\,000 \times 10^3$ PTE $\rightarrow$ ITN/10650×10^3 PTE) ITN/Co-ordinator**: Luciana Patrício**, Partner: Univ. Coimbra, AIBILI	—
- Development of ^{99m}Tc labelled somatostatin and evaluation of their radiochemical and biological behaviour (IAEA POR/8972/R3) ITN/Co-ordinator**: Lurdes Gano**	519

Re and Tc Carbonyl Complexes Containing B-H…M Agostic Interactions as Building Blocks for the Design of Radiopharmaceuticals

Raquel Garcia, Yong Heng Xing, António Paulo, Ângela Domingos[1], Isabel Santos, Kirstin Orter[2], Roger Alberto[2]

Objectives

This project aims to provide novel chelators, based on monoanionic boron containing ligands, useful for the labelling of biomolecules with the *fac*-$[M(CO)_3]^+$ moieties.

Results

The novel complexes $[M\{\kappa^3\text{-}H(\mu\text{-}H)B(tim^{Me})_2\}(CO)_3]$ (M = Re (**1**), ^{99}Tc (**2**)), including an agostic B-H-M bond, have been prepared and fully characterized, including by X-ray diffraction analysis that confirmed the presence of the agostic interactions (Figure 1) [1]. The analogous ^{99m}Tc complex, $[^{99m}Tc\{\kappa^3\text{-}H(\mu\text{-}H)B(tim^{Me})_2\}(CO)_3]$ (**2a**), was prepared under the

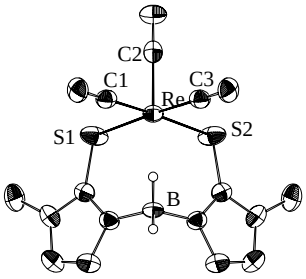


Figure 1

standard conditions of radiopharmaceutical preparation and with high specific activity. The high stability and lipophilicity of **2a** might open the way to new classes of radiopharmaceuticals as required for *i.e.* ^{99m}Tc labeled CNS-receptor ligands. A targeting molecule can be coupled to dihydrobis(methimazolyl)borate either by direct attachment to the boron or through derivatization of the thioimidazole moiety.

A thioimidazole containing an antagonist of CNS serotoninergic receptors has been prepared (Figure 2). This derivatized thioimidazole will be used in the synthesis of poly(methimazolyl)borate ligands. We are also exploring the direct attachment of the biomolecule to the boron. In order to assess the influence of the replacement of one B-H by an aliphatic or aromatic fragment, the model complexes $[Re\{\kappa^3\text{-}R(\mu\text{-}H)B(tim^{Me})_2\}(CO)_3]$ (R = Me (**3**), Ph (**4**)) have been synthesized and fully characterized [2]. These complexes also display a remarkably stable agostic B-H-Re interaction and, therefore, the attachment of bulkier groups to the boron doesn't interfere with the coordination mode of this type of ligands.

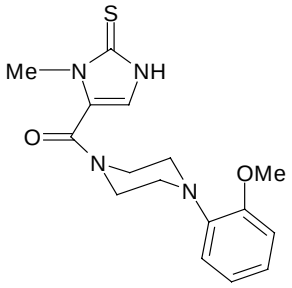


Figure 2

Published (or in press) work

[1] Garcia, R., Paulo, A., Domingos, A., Santos, I., Ortner, K., Alberto, R., Re and Tc Complexes Containing B-H…M Agostic Interactions as Building Blocks for the Design of Radiopharmaceuticals, *J. Am. Chem. Soc.* **122** (2000) 11240-11241.

[2] Santos, I., Re and Tc Complexes with Boron Containing Ligands for Specific Radiotracers, *University of Paris- Sud, Orsay, France,* February, 2000 (invited conference).

Further work

Complexes **3** and **4** will be prepared at the *n.c.a* level. Their biodistribution and *in vitro* and *in vivo* stabilities will be compared with those of complex **1**, in order to evaluate the influence of the substituents in the radiochemical and biological properties of the complexes. Hydro(methymazolyl)borates bearing CNS receptor avid molecules will be synthesized and used to prepare Re and Tc complexes. At macroscopic level, the binding affinity of the complexes to selected CNS receptors will be determined. The best performing complexes will be prepared at *n.c.a* level and their biological properties evaluated.

[1] Inorganic and Organometallic Chemistry Group, ITN.
[2] Institute of Inorganic Chemistry, University of Zürich, Switzerland.

Mixed Organometallic Re and Tc Complexes for the Labelling of Biomolecules

Raquel Garcia, Yong Heng Xing, António Paulo, Ângela Domingos[1], Isabel Santos

Objectives

This project aims to apply mixed complexes of the type $[M\{R_2B(tim^{Me})_2\}(L)(CO)_3]$ (M = Re, ^{99}Tc, ^{99m}Tc) in the labelling of biologically active molecules. This approach is expected to profit from coordinating groups already available in the target biomolecule.

Results

Complexes $[Re\{\kappa^3\text{-}R(\mu\text{-}H)B(tim^{Me})_2\}(CO)_3]$ (R = H (**1**), Me (**2**), Ph (**3**)) react with unidentate substrates leading to mixed complexes of the type $[Re\{\kappa^2\text{-}R(H)B(tim^{Me})_2(L)\}(CO)_3]$ (L = aromatic or aliphatic amines, imidazoles, isonitriles and phosphines) [1]. In the case of unidentate substrates, such as amines and phosphines, this cleavage shows a reversible character. By contrast, when complex **1** is treated with 1,2-(diphenylphosphino)ethane and 1,2-ethylenediamine the cleavage is irreversible leading to stable dimeric and monomeric complexes, respectively (Figures 1 and 2).

Communication

[1] Garcia, R., Paulo, A., Domingos, A., Santos, I., Rhenium(I) Tricarbonyl Complexes with poly(methimazolyl)borates, *XVII Encontro Nacional da SPQ*, Lisbon, March 2000.

Further work

To study by NMR the kinetics and thermodynamics of the Re-B-H agostic interaction clevage, in order to have a better insight into mechanistic aspects and to rationalize the choice of the more adequate co-ligands. New ligands of the type $[R_2B(tim^{Me})_2]^-$ (R = alkyl, aryl) will be evaluated as bidentate anchors, aiming to prevent the formation of the agostic interaction. At a later stage, the studies will be carried out at *n.c.a.* level. The most promising systems, in terms of kinetics and stability, will be evaluated in the labelling of selected biologically relevant molecules.

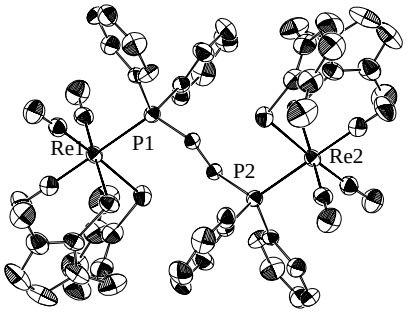


Figure 1

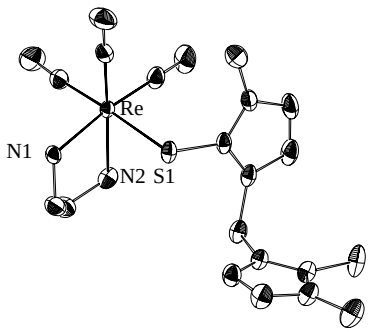


Figure 2

[1] Inorganic and Organometallic Chemistry Group, ITN.

Heterofunctionalized Phosphines with PNO and PNS Donor Atom Sets: Oxorhenium(V) Mixed-Ligand Complexes

João D. G. Correia, Ângela Domingos[1], Isabel Santos

Objectives

Evaluation of the coordination capability of heterofunctionalized phosphines for preparing Re(V) mixed ligand complexes.

Results

The heterofunctionalized phosphine ligands with PNN and PNO donor atom sets, developed in our group, revealed a great versatility in terms of charge and denticity towards the $[Re=O]^{3+}$ core [1,2]. Oxorhenium(V) mixed-ligand complexes of the ″3+2″ and ″3+1″ type have been isolated and fully characterized [2,3,4]. Having in mind the great versatility of the chelate ligands, and choosing appropriate reaction conditions, we extended the use of the PNO and PNS ligands to the preparation and characterisation of model complexes of the ″3+1″ type (**1**-**5**, Scheme 1). The liphophilicity of these compounds led us to prepare and to characterize an oxorhenium(V) mixed-ligand complex (**6**, Scheme 1) containing a o-methoxyphenylpiperazine moiety, which is the main farmacophore of several 5-HT_{1A} receptor antagonists.

Published (or in press) work

[1] Correia, J.D.G., Domingos, Â., Santos, I., Neutral Trichlorooxorhenium(V) Complexes Containing New Heterofunctionalized Phosphine Ligands of the Type PN_2 and PNO, *Eur. J. Inorg. Chem.* (2000) 1523-1529.

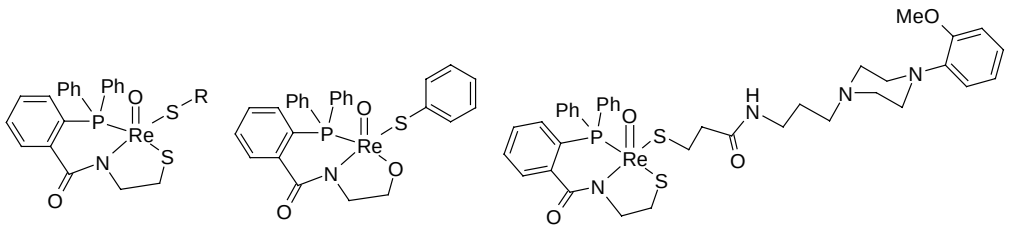
[2] Correia, J.D.G., Domingos, Â., Paulo, A., Santos, I., Novel Six-co-ordinate Oxorhenium Complexes with Ligands Containing PN_2 and PNO Donor Atom Sets: Syntheses and Structural Characterization, *J. Chem. Soc., Dalton Trans.* (2000), 2477-2482.

[3] Correia, J.D.G., Domingos, Â., Santos, I., Bolzati, C., Refosco, F., Tisato, F., Synthesis and Structural Analysis of Mono-Oxo Re(V) Complexes with Phosphino-Carboxylato Ligands, *Inorg. Chim. Acta*, in press.

[4] Correia, J.D.G., Specific Radiopharmaceuticals of Re and Tc: Synthesis of New Ligands of the PNO, PNS and PN_2 Type; Chemical and Radiochemical Studies, Conference in the Chemistry Department, ITN, July, 2000.

Further work

Evaluation of the binding affinity of complex **6**. Depending on the results, new model compounds will be synthesized and characterized in order to maintain the affinity of the biomolecule. Some basic studies on the 3+2 approach will be done. The liphophilicity of the compound will be modulated by modifying the heterofunctionalized phosphines.



1 (R = HPNS)
2 (R = C_6H_5)
3 (R = 4-NH_2-C_6H_5)
4 (R = CH_2CH_2COOH)

5

6

Scheme 1

[1] Inorganic and Organometallic Chemistry Group, ITN.

Tc(V) Oxocomplexes with the PNO/S and PNS/S Donor Atom Sets. Labelling of a $5HT_{1A}$ Receptor-Binding Ligand

Célia Fernandes, João D. G. Correia, Isabel Santos, Sepp Seifert[1], Hartmut Spies[1], R. Syhre[1]

Objectives

Preparation, characterisation and biological evaluation of Tc(V) mixed-ligand complexes using PNO and PNS type ligands as tridentate chelates and monothiol co-ligands. Labelling of a $5\text{-}HT_{1A}$ receptor ligand (derivatized aryl-piperazine) with ^{99m}Tc using the '3+1' approach.

Results

For a possible medical application, we evaluated the utility of the heterofunctionalized phosphines of the PNO and PNS type for preparing Tc complexes at the *non carrier added level (n.c.a. level)*. Within the scope of a short term scientific mission (STSM/COST Action B12) of C. Fernandes to FZR, Germany, we prepared complexes of the type ^{99m}TcO(PNS/RS) (R=thiophenol, **1**; benzylmercaptane, **2**; cyclohexanethiol, **3**; mercaptopropionic acid, **4**; mercaptoacetyldiglycine, **5**; and glutathione, **6**) with yields between 30-80%. Using the tridentate PNO ligand and thiophenol we prepared also the ^{99m}TcO(PNO/SPh) (**7**) complex (*ca*. 65% yield). These compounds were characterized by comparison of the HPLC data with the values obtained for the corresponding Re compounds, previously prepared [1-3]. **1** and **7** were chosen for stability studies, which were undertaken in aqueous solutions (saline, 0.01 M PBS pH 7.4) and in glutathione challenge experiments. Based on stability, complex **1** was chosen for biodistribution studies in rats. In spite of the low fixation in brain (0.14±0.03% injected dose ± s.d., 5 min p.i.), 120 min after injection no significant decrease of activity is observed in brain (0.11±0.02% injected dose ±s.d.). The promising results led us to label a $5HT_{1A}$ receptor-binding ligand with ^{99m}Tc (Figure 1, 80% yield). The ^{99m}Tc-complex with the biomolecule was identified by comparing its HPLC profile with that of the analogous Re complex [4].

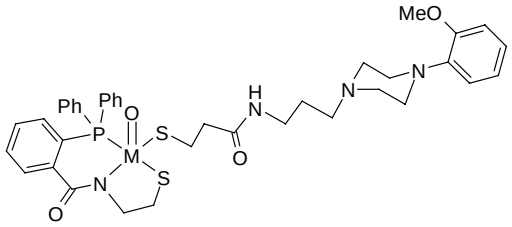


Figure 1. M = **Re** – HPLC R.T.: 8.2 min (UV/Vis, 254 nm)
M = **^{99m}Tc –** HPLC R.T.: 8.1 min (γ detection)

Published (or in press) work

[1] Correia, J.D.G., Domingos, Â., Paulo, A., Santos, I., Novel Six-co-ordinate Oxorhenium Complexes with ligands containing PN_2 and PNO Donor Atom Sets: Syntheses and Structural Characterization, *J. Chem. Soc., Dalton Trans*. (2000), 2477-2482.

[2] Correia, J.D.G., Domingos, Â., Santos, I., Novel Oxorhenium Complexes with PNO/S and PNS/S Donor Atom Sets, manuscript in prepartion.

[3] Fernandes, C., Final Report to M.C., COST Action B12, July, 2000.

[4] Santos, I., Re and Tc Complexes with Hetero-functionalized Phosphines for in vivo Assessment of Biological Functions, *Montréal University, Montréal, Canada, November*, 2000 (invited conference).

Further work

Improvement of the labelling yields of the biomolecule, stability studies, determination of the binding affinity to specific receptors and biodistribution studies. Using the same approach, other biomolecules should be labeled.

[1] Institute of Bioinorganic and Radiopharmaceutical Chemistry, Forschungszentrum Rossendorf (FZR), Dresden, Germany.

Novel Re(I) and Tc(I) Tricarbonyl Complexes With Phosphorus Containing Ligands. Labelling of a $5HT_{1A}$ Receptor-Binding Ligand

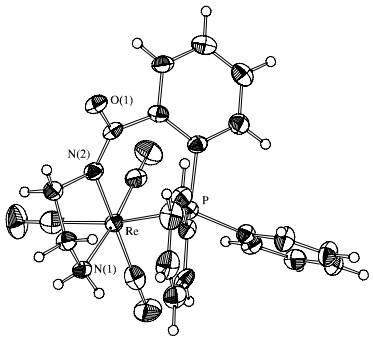
João D. G. Correia, Ângela Domingos[1], Isabel Santos, Roger Alberto[2], Kirstin Ortner[2]

Objectives

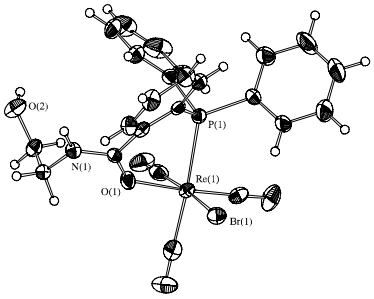
Evaluation of the coordination abilities of the H_2PNO and HPNN ligands towards the organometallic moiety *fac*-$[M(CO)_3]^+$ (M= Re, Tc) and development of efficient labelling procedures with ^{99m}Tc.

Results

Within the scope of a Short Term Scientific Mission (STSM/COST Action B12) of J. D. G. Correia to the University of Zurich, we synthesized and characterized, at a macroscopic level, the following complexes: $[M(\eta^3\text{-}PN_2)(CO)_3]$ (**1**) or $[M(\eta^2\text{-}H_2PNO)X(CO)_3]$ (**2**) (X = Br, Cl) (Figure 1).



$[Re(CO)_3(\eta^3\text{-}PNN)]$



$[Re(CO)_3Br\ (\eta^2\text{-}H_2PNO)]$

Figure 1

The corresponding ^{99m}Tc-complexes were obtained in moderate to high yields (50-98%), and in the case of $[^{99m}Tc(\eta^2\text{-}H_2PNO)X(CO)_3]$ an high stability in phosphate buffer pH 7.7 at 37º C was observed, even after 24 h [1]. Based on these results a $5HT_{1A}$ receptor-binding ligand was derivatized and the chemistry of this biomolecule was studied at the macroscopic and at *n. c. a* level. An ORTEP view of the rhenium complex is depicted in Figure 2. The ^{99m}Tc-complex with the biomolecule was identified by comparing its HPLC profile with that of the analogous Re complex (**M = Re** – HPLC R.T.: 23.0 min.; **M = ^{99m}Tc** – HPLC R.T.: 22.9 min.).

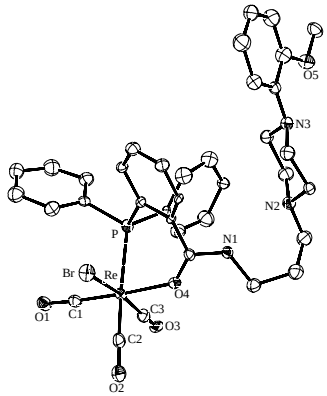


Figure 2

Internal Report

[1] Correia J.D.G., Final Report to M.C., COST Action B12, May, 2000.

Further work

Improvement of the labelling conditions, binding affinity to specific receptors and biodistribution studies will be done. New receptor–binding ligands have to be tested.

[1] Inorganic and Organometallic Chemistry Group, ITN.
[2] Institute of Inorganic Chemistry, University of Zürich, Switzerland.

Rhenium Complexes with Nitrogen Donor Ligands

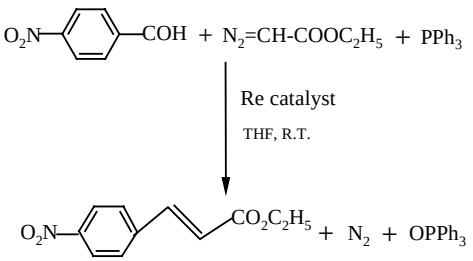
Raquel Garcia, António Paulo, Ângela Domingos[1], Isabel Santos,
Isabel Lucas[2], C.C. Romão[2]

Objectives

This project studies the chemistry of Re(V) and Re(VII) oxocomplexes stabilized with nitrogen donor ligands, aiming to control their reactivity in stoichiometric and catalytic reactions, through the use of ligands with different stereochemical and electronic properties.

Results

The coordination chemistry and reactivity of rhenium oxocomplexes with tetrakis(pyrazolyl)borate continued to be explored during this year [1]. The already available complexes $[ReO_3\{\kappa^3\text{-}B(pz)_4\}]$ (**1**), $[ReO(\mu\text{-}O)\{\kappa^3\text{-}B(pz)_4\}]_2$ (**2**), $[ReOCl_2\{\kappa^3\text{-}B(pz)_4\}]$ (**3**), $[ReO(OMe)_2\{\kappa^3\text{-}B(pz)_4\}]$(**4**) and *trans*-$[ReO_2\{\kappa^3\text{-}B(pz)_4\}(L)_2]$ (L = py (**5**), 4-NMe_2py (**6**), 1-Meimz (**7**)) were evaluated as catalysts in aldehyde olefination reactions (Scheme 1). The reactions were followed by GC chromatography, after appropriate quenching of the reaction mixtures. The tested compounds didn´t show any catalytic activity, even when reactions were run at high temperatures. These catalytic experiments were run at ITQB in collaboration with the group of Prof. Carlos Romão [2].



Scheme 1

The use of coordinative unsaturated Re(VII) oxocomplexes, such as five-coordinated $[ReO_3\{\kappa^2\text{-}R_2B(pz)_2\}]$ (R = alkyl, aryl), was expected to enhance the catalytic activity of Re complexes in aldehyde olefination. Thereby, the synthesis of this type of complexes has been attempted by reacting Re_2O_7 or $ClReO_3$ with $Na[R_2B(pz)_2]$ (R = Et, Ph). These reactions were accompanied by degradation of the ligands, and the only species identified was $(pzH_2)^+ReO_4^-$ which was characterized by X-ray diffraction analysis.

Published (or in press) work

[1] Paulo, A., Domingos, A., Garcia, R., Santos, I., Cationic Re(V) Oxo Complexes with Poly(pyrazolyl)borates: Synthesis, Characterization, and Stability, *Inorg. Chem.* **39** (2000) 5669-5674.

[2] Santos, I., Re Organocompounds: Synthesis, Characterization and Potential Applications, *5th International Symposium on Organo-Metal Complexes*, Baoding, China, August, 2000 (invited)

Further work

The funding of this project by FCT ends by April 2001. Meanwhile, it will be attempted the synthesis of other coordinatively unsaturated Re(VII) oxocomplexes, such as pz*ReO_3, and their catalytic activity will be evaluated in aldehyde olefination reactions.

[1] Inorganic and Organometallic Chemistry Group, ITN.
[2] Organometallic Chemistry and Catalysis Group, ITQB, Oeiras.

Radiopharmaceuticals Based on Lanthanide Macrocyclic Complexes

M.P.C.Campello, L. Gano, F. Marques, I. Santos, M. A. Gouveia[1], E. Martinho[2], R. Herrmann, P. Antunes[3], R. Delgado[3]

Objective

Search of bifunctional chelates for the synthesis of lanthanide macrocyclic complexes potentially useful for bone pain palliation and/or therapy.

Results

^{153}Sm, obtained using the available conditions at the RPI, have been used to prepare complexes with macrocycles containing acetate and phosphonate pendant arms. Two of these macrocycles were available at the group of R. Delgado [1, *Helv. Chim. Acta* **73 (**1990) 140-148 and *J. Chem. Soc.,Dalton Trans.* (1999) 3253-3265], and are presently under stability studies.

Other two ligands, having the same macrocyclic backbone but different number and position of the acetate and phosphonate arms are in the last steps of synthetic preparation.

With the present study the number and relative position of the phosphonate and acetate arms in the framework of one 14-membered tetraazamacrocycle will be analysed.

Another synthetic approach is under progress in order to prepare macrocycles with improving coordinating properties for lanthanides. To increase the rigidity of the chelates, aromatic amines have been used as building blocks. However, the known cyclisation methods to obtain macrocyclic compounds have failed. Some other strategies have been explored and are under evaluation.

Published (or in press) work

[1] Costa, J., Delgado, R., Drew, M. G. B., Félix, V., and André Saint-Maurice, A New Redox-Responsive 14-membered Tetraazamacrocycle with Ferrocenylmethyl Arms as Receptor for Sensing Transition-metal ions. Crystal X-ray structures of the copper(II), nickel(II) and zinc(II) complexes., *J. Chem. Soc.,Dalton Trans.*, (2000) 1907-1916.

Further Work

Continuation of the organic synthetic work for preparing macrocycles containing acetate and phosphonate pendant arms and evaluation of their thermodynamic and kinetic stabilities with lanthanides. Improvement of the labelling conditions in the case of the macrocycles with phosphonate pendant arms and stability studies. Evaluation of the utility of these compounds for biological applications.

[1] Cultural Heritage and Sciences, ITN.
[2] Research Portuguese Reactor, ITN.
[3] Institute of Chemical and Biological Technology (ITQB).

Evaluation of the genotoxic and antiestrogenic potential of triphenylethylene derivatives and their metabolites

M. M. Marques[1], F. Marques, F. A. Beland[2], M. A. Santos[1], M. C. Melo Silva, L. Gano, J. J. Pedroso Lima[3], G. G. Costa[1], A. C. Santos[3]

Objectives

Following previous work on tamoxifen and metabolites and on radiolabelling (Beland, F.A., *et al.*, *Carcinogenesis* **20** (1999) 471-477 and [1,2]) our main goal is the synthesis of halogenated triphenylethylene derivatives as alternatives to tamoxifen (TAM) for the chemoprevention of breast cancer. These compounds will be labeled and evaluated for estrogen receptor (ER) imaging of human breast cancer.

Results

The halogenated TAM analogue, toremifene, to be used as a standard compound, was prepared by way of a McMurry reaction, involving the crossed reductive coupling of an alkylated 4-hydroxybenzophenone derivative with β-chloropropiophenone. A mixture of E and Z isomers are obtained, which are not easily separated by conventional chromatographic techniques.

Alternative starting materials, more suitable to isomer separation, have been tested. Thus, β-hydroxytamoxifen, a convenient precursor for the synthesis of the halogenated TAM analogues, was also synthesised by the same methodology, starting from β-hydroxypropiophenone. The resulting mixture of isomers was easily separated by chromatography, yielding the desired Z isomer in reasonable yield.

The synthesis of α-hydroxylated derivatives, the putative metabolites that form DNA adducts, was also tested by using the corresponding conjugated dienes as precursors. These dienes were obtained by coupling bromo-olefins with vinyltributyltin, using tetrakis(triphenylphosphine)palladium (0) as the catalyst.

Published (or in press) work

[1] Costa, G.G., Hamilton, L.P., Beland, F.A., Marques, M.M., Characterization of the Major Adduct Formed by α-hydroxy-*N*-desmethyltamoxifen *in vitro* and *in vivo*, *Chem. Res. Toxicol*. **13** (2000) 200-207.

[2] Melo e Silva, M.C., Patrício, L., Gano, L., Sá e Melo, M.L., Inohae, E., Mataka, S., Thiemann, T., Synthesis and Biological Evaluation of Two New Radiolabelled Estrogens [^{125}I](*E*)-3-methoxy-17α-iodovinylestra-1,3,5(10),6-tetraen-17β-ol and [^{125}I](*Z*)-3-methoxy-17α-iodovinylestra-1,3,5(10), 6-tetraen-17β-ol, *Appl. Radiat. Isot.* **54**/**2** (2000) 227-239.

Further work

Some of the new halogenated compounds will be evaluated as alternatives to the current TAM therapy by comparison of the kinetic stability and the DNA binding capacity of their α-functionalized derivatives to those of the corresponding TAM metabolites. Their antiestrogenic and antitumor properties will be assessed by inhibition of breast tumour MCF-7 cell proliferation. The potential use of these compounds as imaging agents will also be evaluated by their *in vitro* ER binding affinity, as well as the *in vivo* tissue uptake of the corresponding radiolabelled derivatives.

[1] Structural Chemistry Center, IST, Lisboa.
[2] Division of Biochemical Toxicology, NCTR, Jefferson, AR, USA.
[3] Light and Image Biomedical Research Institute (IBILI), Coimbra.

Regulation of the Gonadotropic Axis by NPY and Melanocortin Peptides

Paula Raposinho, Michel Aubert[1]

Objectives

Evaluation of the role of neuropeptide Y (NPY) and melanocortin peptides for the regulation of the gonadotropic axis in the rat.

Results

Five receptors subtypes for NPY (Y1-Y5) have been identified. Using NPY agonists with different selectivity for the different receptors subtypes, we have shown that intracerebroventricular NPY administration inhibits LH secretion in castrated rats and produces an important hypogonadism in intact male rats by Y5-receptor subtype mediation. However, a Y1 receptor participation was not excluded. Recently, we studied the effect of a 7-day chronic central NPY infusion in wild-type and NPY-Y1 receptor knockout mice. In both, we observed an increase of food intake and an inhibition of gonadotropic axis (decreases in pituitary, seminal vesicles and testis weight, and testosterone plasma levels), suggesting that the Y1 receptor subtype is not involved in the inhibition of this axis by NPY. On the other hand central administration of NPY stimulates LH release in sex steroid-intact females rats. We have shown that the NPY Y1-antagonist/Y4-agonist 1229U91 strongly stimulates LH and FSH release in normal male rats and we suggested the Y4-receptor as the subtype implicated in the stimulatory effect of NPY in the regulation of gonadotropic axis [1]. To sustained this hypothesis we injected 1229U91 to wild-type and NPY-Y1 receptor knockout mice. In both, females and males, 1229U91 stimulates LH secretion in a dose-dependent manner which confirms that this stimulation does not results from an antagonistic action on the Y1 receptor, thus demonstrating the Y4 pathway of stimulation of gonadotropic axis [2].

As the melanocortins, by MC4-receptor, and NPY pathways probably interact in the control of feeding we compared the obesity syndrome generated by a 7-day central infusion of either NPY or the MC4-R antagonist SHU9119. Both peptides generated a similar hyperphagia and obesity. By contrast NPY produced a severe hypogonadism but no significant effects on reproduction function were seen with SHU9119, suggesting divergent hypothalamic pathways regulating food intake and neuroendocrine functions [3]. We also evaluated the effects of chronic, central MT-II (an MC4-R agonist) infusion given either alone or in association with NPY. We observed that MT-II fully suppressing the orexigenic but not the endocrine effects of NPY.

Published (or in press) work

[1] Raposinho, P.D., Broqua, P., Hayward, A., Akinsanya, K., Galyean, R., Schteingart, C., Junien, J.-L., Aubert, M.L., Stimulation of the gonadotropic axis by the Neuropeptide Y receptor-Y1 antagonist /Y4 agonist 1229U91 in the male rat, *Neuroendocrinology* **71** (2000) 2-7.

[2] Raposinho P.D., Pedrazzini, T., Tommaselli, G., Aubert, M.L., The neuropeptide Y Y1 antagonist/Y4 agonist 1229U91 stimulates LH secretion through NPY Y4 receptors: Assessment using NPY Y1-deficient mice, Poster presented in *The Endocrine Society 82nd Annual Meeting* (Juin), Toronto.

[3] Raposinho, P.D., Castillo, E., D'Alleves, V., Broqua, P., Pralong, F.P., Aubert, M.L., Chronic blockade of the melanocortin 4 receptor subtype leads to obesity independently of neuropeptide Y action, with no adverse effects on the gonadotropic and somatotropic axes, *Endocrinology* **141** (2000) 4419-27.

Further work

To confirm the role of Y5-receptor on the inhibition of gonadotropic axis by NPY studying the effects of both chronic infusion of NPY on Y5-knockout intact male mice and acute injection in castrated mice. To analyze, *in vivo*, the role of the Cocaine and Amphetamine Regulated Transcript (CART), a neuroendocrine factor that *in vitro* mediates the stimulatory action of leptin on the reproductive axis.

[1] Division d'Endocrinologie et de Diabétologie Pédiatriques (DEDP), Université de Genève, Suisse.

Compounds of f-Elements: Synthesis, Characterization and Reactivity Studies

Leonor J. Maria, Ângela Domingos[1], Adelino Galvão,[2] Isabel Santos

Objectives

To study the basic chemistry of U(III) with boron containing ligands, nitrogen or sulphur donors, with different electronic and steric properties.

Results

As shown before, $UI_3(THF)_4$ reacts with K[$H_2B(^iPr_2$-pz)2], in the molar ratio 1:3, leading to the complex [U{κ^3-H(μ-H)B($^iPr_2$-pz)2}3] (**1**), which has a limited reactivity [1, 2, 3]. In order to get more reactive species, the chemistry of this metal was explored with the more bulky nitogen donor K[HB(iPr_2-pz)$_3$] and with the sulphur donor [RHB(SImz)$_2$] (R=Ph, H), which certainly will lead to less substituted species.
Using the hydrotris(pyrazolyl)borate the compounds isolated were: [UI_2{κ^3-HB($^iPr_2$-pz)$_3$}(L)] (L=3,5$^iPr_2$-pzH, **2;** $OPPh_3$, **3**), and [UI_2{κ^3-HB($^iPr_2$-pz)$_3$}$(L)_2$] (L=THF, **4;** C_5H_5N, **5**) [4]. The characterization of some of them includes X-ray crystallographic analysis. For **2** an interesting interaction between the metal and one hydrogen atom of the 3-^{i}Pr group of the pyrazolyl ring in the axial position was observed and confirmed by EHMO calculations (Figure 1a). Using the dihydrobis(tioimidazolyl)borate ligand was possible to coordinate two ligands to the metal and the first monomeric U(III) cation with sulphur donor boron containing ligands, [U{κ^2-H_2B(Simz)$_2$}$(THF)_3]^+[BPh_4]^-$ (**6**), was fully characterized (Figure 1b).

Published (or in press) work

[1] Maria, L., Domingos, A., Santos, I, Chemistry of U(III) Dihydrobis(pyrazolyl)borates, *XVII Encontro Nacional da Sociedade Portuguesa de Química*, Lisboa, Portugal, March, 2000.

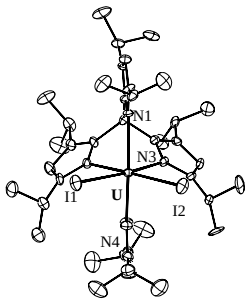
[2] Giuseppone, N., Collin, J., Santos, I, Enantioselective Diels-Alder Reactions Catalyzed by Samarium Iodo Binaphtoxides**,** *Tetrahedron Letters* **41** (2000) 639-642**.**

[3] Collin, J., Maria, L., Santos, I, Uranium Iodides as Catalysts for Diels-Alder Reactions, *Journal of Molecular Catalysis A: Chemical* **160** (2000) 263-267**.**

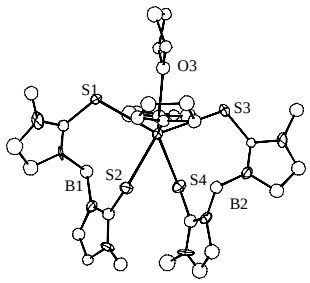
[4] Maria, L., Domingos, A., Galvão, A., Santos, I, Synthesis and Structure of U(III) Complexes with [HB(iPr_2-pz)$_3$], *4th International Conference on f-Elements,* Madrid, Spain, September, 2000.

Further work

- To study the reactivity of compound **4**, in stoichiometric reactions and to explore the chemistry of **6.** To select the best system for the synthesis of heterobimetallic complexes of U and Re.



(a)



(b)

Figure 1. ORTEP Diagrams of **2** (a) and of the cation **6** (b)

[1] Inorganic and Organometallic Chemistry Group, ITN.
[2] Structural Chemistry Center, IST, Lisboa.