

Inorganic and Radiopharmaceutical Chemistry

Isabel Rego Santos

Apart from the continuing improvement in equipment and technology, the success of **Nuclear Medicine** is strongly dependent on the availability of powerful probes for molecular imaging and/or targeted therapy. The Radiopharmaceutical Chemistry group does **basic/applied-oriented research and technology transfer** to find radioactive probes for **molecular imaging and/or targeted radiotherapy**. This is a multidisciplinary task based on innovative organic and coordination chemistry, bioconjugation, radiochemistry, radiopharmacy and cellular biology knowledge afforded by modern genomics/proteomic research. We have implemented and developed expertise on these fields, as well as facilities to carry on such activities. This year, our facilities have been expanded with a new laboratory and with the implementation of the **Western Blot** technique.

Our main achievements on **Radiopharmaceutical Sciences** are described in this report. However, we would like to emphasize the following points:

Research

1- The complex **ISATechII** has been **selected by a Pharmaceutical Company** to enter into **clinical evaluation, as a myocardial imaging probe**. We consider this a tremendous achievement for **ITN and for the Portuguese Science and Technology**.

2- Radioactive **metal complexes**, bearing fluorescent DNA intercalators, **target the nucleus** of tumour cells, confirming the utility of **multimodal probes** for nuclear/optical imaging and radionuclide therapy.

3- The **first organometallic complexes** inhibiting nitric oxide synthase (*i*NOS) were isolated, characterized and evaluated.

4- A favourable biological profile (**bone uptake and pharmacokinetics**) has been found for a ^{99m}Tc-**bisphosphonate**, making this complex very promising as a vector to deliver cytotoxic agents to the bone, while minimizing the toxic effects on normal tissues.

Education and Training

1- Graduation: teaching of Radiopharmacy at ESTSeL and Faculty of Pharmacy/University of Lisbon. Under a protocol, students of the Nuclear Medicine Course/ESTeSL are trained in our group (two weeks/year).

2- Post-graduation: organization, teaching and coordination of the **Master Course Biomedical Inorganic Chemistry: Diagnostic and Therapeutical Applications** (3th Edition) (DR n° 123, 26/05/04, II série). This Master course has been adjusted and approved to run under the **Bologna Agreement**. During 2007, we have also **participated** in PhD Teaching Programs organized by other Universities and Associated Laboratories.

3- International level: teaching in the European Radiopharmacy Course, INSTN.

4- Young scientists: Twenty two are trained in the group, playing a major role in our projects.

Expertise Provided: Nuclear Medicine Centers, Portuguese Medicines Evaluation Agency, IAEA and Foreign Science Foundations.

Financial support: Mallinckrodt Medical B.V. (a Covidien Company), FCT, CIMAGO/FLAD and EC/COST RTD ACTIONS.

Publications: Peer-review International Journals - 16; Patents - 2; Chapters in Books - 4; Proceedings/Reports- 7; Thesis: PhD - 1, MSc - 5.

Research Team

Researchers

I. SANTOS, Princ., Agregação, Group Leader
A. PAULO, Princ.
J.D.G. CORREIA, Princ.
M.P.C. CAMPELLO, Aux.
M.C. OLIVEIRA, Aux.
L.GANO, Aux.
F. MARQUES, Aux.
P. RAPOSINHO, Aux.
C.FERNANDES, Assistant

Technical Personnel

A. RODRIGUES, technical assistant
E. CORREIA, technical assistant

Students

L.MARIA, Pos-Doc., FCT grant
R. GARCIA, Pos-Doc., FCT grant
A. DATTA, Pos-Doc., FCT grant
R. VITOR, PhD student, FCT grant
S. LACERDA, PhD student, FCT grant
C. XAVIER, PhD student, FCT grant
E. PALMA, PhD student, FCT grant
T. ESTEVES, PhD student, FCT grant
C. NETO, PhD student, FCT grant
B. OLIVEIRA, MSc student
M. BALBINA, MSc student QIR/RPI
Z. BARBOSA, MSc student, QIR/IBILI
S. CUNHA, MSc student
F. LUCENA, MSc student
F. SILVA, MSc student
F. FIGUEIRA, MSc student
M. MORAIS, MSc student
G.CLEMENTE, MSc student
C. MOURA, MSc student
M. VIDEIRA, BIC grantee, POCl
B. MARTINS, undergraduate student
A. SANTOS, undergraduate student

Myocardial Imaging Agent: From Basic/Applied–Oriented Research to Technology Transfer

L. Maria, C. Fernandes, R. Garcia, L. Gano, A. Paulo, I. Santos

Objectives

Nuclear Cardiology is an important noninvasive tool for the clinical evaluation of patients with known or suspected Coronary Artery Disease (CAD), one of the leading causes of death in western countries. The main goal of this project is to find good performing myocardial imaging probes for Nuclear Cardiology.

Results

Among heart diseases, CAD is a leading cause of premature and permanent disability. Detection of perfusion abnormalities when the CAD patients are still asymptomatic would be of considerable benefit to avoid myocardial infarction and to apply therapeutic regimes, before irreversible myocardial damage occurs. This early diagnosis can be achieved with Nuclear Cardiology if a good performing nuclear myocardial probe is available, *i.e.* a probe with a high first-pass extraction, a high heart uptake, a stable retention, and high heart/liver and heart/lung ratios. ^{99m}Tc -MIBI, despite its widespread clinical application, does not meet the requirements of an ideal myocardial probe due to its relatively low first-pass excretion and high liver uptake. Thus, there is a significant interest on finding myocardial probes to overcome such disadvantages.

Our group has introduced novel cationic ^{99m}Tc complexes for myocardial imaging (Figure 1).

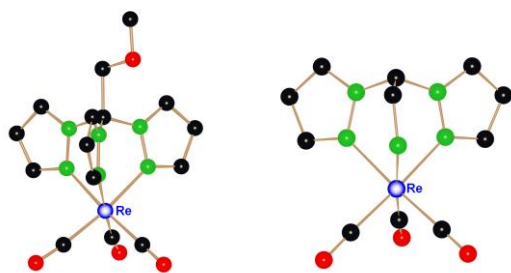


Fig. 1. Lead structures of myocardial imaging agents

Animal studies showed that the pharmacokinetics of the complexes can be modulated by introducing different functional groups in different positions of the tridentate chelators. Among several complexes, **ISATechI** and **ISATechII** were selected as the most promising for further studies (Figure 2). Results from Single Photon Emission Computed Tomography (SPECT) imaging studies in Sprague-Dawley rats have definitively shown that **ISATechII** was an excellent complex, presenting a significant and fast heart uptake, a stable retention and an extremely fast liver clearance. Clear images of the heart could be obtained with **ISATechII**, within a short time post-injection (Figure 3).

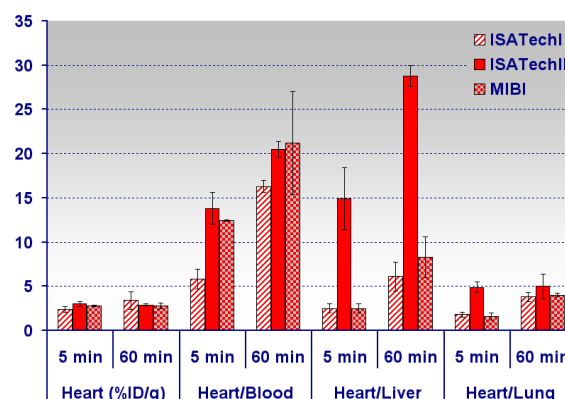


Fig. 2. Relevant target/non target ratios

Due to the excellent pre-clinical results, the complex **ISATechII** has been selected for clinical trials. GMP preparation of **ISATechII** and toxicity studies are underway.

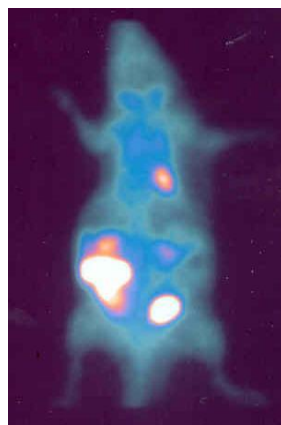


Fig. 3. Rat image 5 minutes post-injection

Published work:

I. Santos, A. Paulo, Tricarbonyl Complexes with Tridentate Chelators for Myocardium Imaging, *PCT/EP2007/010216*.

L. Maria, S. Cunha, M. Videira, L. Gano, A. Paulo, I. C. Santos, I. Santos, Rhenium and Technetium Tricarbonyl Complexes Anchored by Pyrazole-based Tripods: Novel Lead Structures for the Design of Myocardial Imaging Agents, *Dalton Trans.* (2007) 3010-3019. Selected as a **HOT ARTICLE**,

www.rsc.org/Publishing/Journals/dt/HotArticles.asp

Top Ten most popular *Dalton Transactions* article (July)

May Copsey, A clearer view of heart disease: Versatile pyrazole ligands offer a promising future for heart imaging radiotracers, *Chem. Science* (2007) 4.

www.rsc.org/Publishing/ChemScience/Volume/2007/08/Index.asp

Mixed-Ligand Re/^{99m}Tc Complexes for the Labelling of Biomolecules with Clinical Relevance

M. Videira, A. Paulo, I. Santos

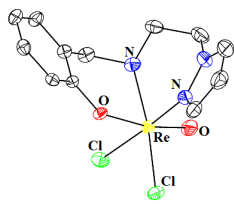


Fig. 1

The finding of novel metal fragments suitable for the radiolabelling of biologically relevant molecules remains a topic of utmost importance in modern radiopharmaceutical chemistry. To achieve such goal we have focused on mixed-ligand oxorhenium (V) complexes containing the NNO/OO donor atom set (Figure 2) which were prepared by reacting the corresponding oxorhenium dichloride (Figure 1) with adequate bidentate co-ligands. All the novel Re(V) oxocomplexes were fully characterized by the common analytical techniques, namely by multinuclear NMR and X-ray crystallography. The possibility of preparing other mixed-ligand complexes anchored by related ligands of different denticity is under evaluation, aiming to select the most promising compounds to pursue with the studies at the no-carrier added level (^{99m}Tc).

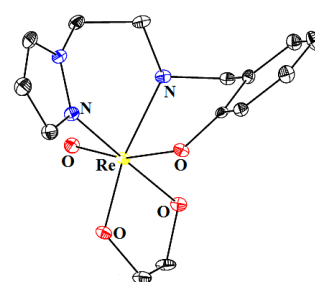


Fig.2

Gallium (III) Metallopharmaceuticals for Chemotherapy and/or Molecular Imaging

F. Silva, R. Garcia, F. Marques, A. Paulo, I. Santos

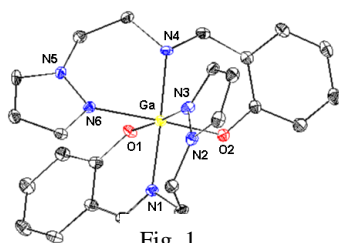


Fig. 1

A DOTA-like macrocyclic ligand was coupled to quinazoline derivatives (Figure 2) and used to prepare ⁶⁷Ga complexes which are being evaluated as biomarkers for *in vivo* molecular imaging of epidermal growth factor receptors (EGFR) overexpressed in cancer cells.

Based on tridentate pyrazolyl-containing chelators and on a DOTA-like macrocyclic ligand we have synthesized novel Ga(III) complexes having in mind their use as cytotoxic metallopharmaceuticals and/or as radioactive probes (⁶⁷Ga/⁶⁸Ga) for *in vivo* nuclear imaging. The pyrazolyl-containing chelators allowed the stabilization of non-radioactive homoleptic complexes (Figure 1) which in some cases displayed cytotoxic activity against murine or human tumour cells, appearing therefore as useful tools for the design of antitumor drugs.

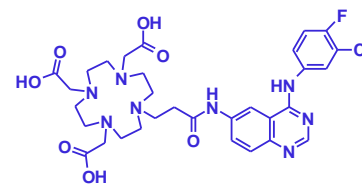
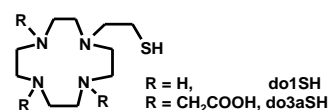


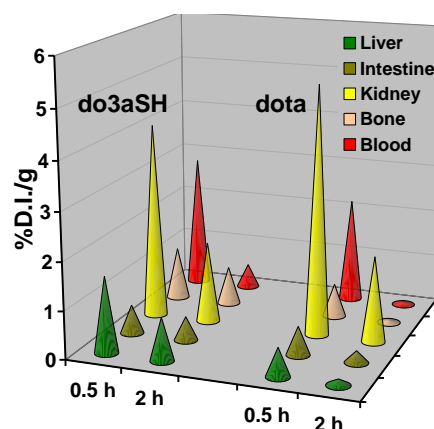
Fig.2

Macrocyclic Ligands with Sulphur Donor Atoms for Labelling Biomolecules with Radiometals

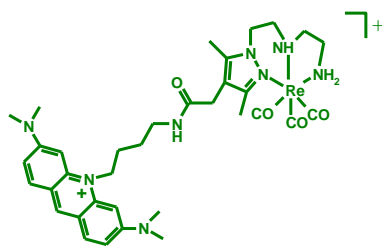
 S. Lacerda, M.P.C. Campello, F. Marques, L. Gano, I. Santos, S. Juran,¹M. Walther,¹H.-J. Pietzsch¹


Macrocyclic bifunctional chelators are widely used for labelling biomolecules with radiometals aiming their use for *in vivo* diagnosis and antitumor therapy. As part of our ongoing efforts on this field, we have explored dota-like macrocyclic ligands having pendant thiol arms (do1SH/do3aSH) for metal coordination and coupling to biomolecules. The novel ligand do3aSH was successfully used to synthesize complexes with radiolanthanides (¹⁵³Sm and ¹⁶⁶Ho) and copper (^{64/67}Cu). All of these radiocomplexes have been obtained in high radiochemical yield and present good *in vitro* stability. Biodistribution studies in mice have shown that ¹⁵³Sm-do3aSH displays a favourable biological profile, following a similar trend to the well-established ¹⁵³Sm-dota complex. These findings prompted us to explore the coupling of biomolecules through the thiol group, which is currently underway.

Macrocyclic bifunctional chelators are widely used for labelling biomolecules with radiometals aiming their use for *in vivo* diagnosis and antitumor therapy.

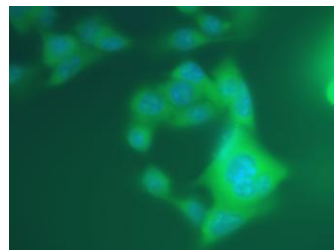

¹ Institut für Radiopharmazie, Forschungszentrum Dresden-Rossendorf, Germany

Multifunctional Organometallic Complexes for Nuclear Targeting

 R. F. Vitor, T. Esteves, C. Xavier, F. Marques, A. Paulo, I. Santos, G. G. Martins¹


Recently, we have shown that Re(I)^{99m}Tc(I) tricarbonyl complexes anchored by pyrazolyl-diamine ligands containing an anthracen-9-yl group at the 4- position of the azole ring are able to target the nucleus of murine B16F1 melanoma cells. These promising results prompted us to evaluate related complexes bearing acridine moieties. By focusing on acridine derivatives, we have taken into account their characteristic fluorescence which provides a sensitive spectroscopic handle to study its interaction with DNA and to

follow *in vitro* the cellular localization of their compounds. Currently, we are evaluating the influence of the different chromophores (anthracenyl vs acridinyl moiety) in the interaction of the compounds with DNA and on their ability to target the nucleus and to induce cell death.


¹ Instituto Gulbenkian da Ciência, Oeiras, Portugal

Peptide Nucleic Acids Labeled with the *fac*-[^{99m}Tc(CO)₃]⁺ Moiety for Monitoring the Endogenous Gene Expression

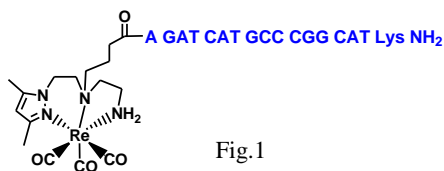
 C. Xavier, C. Giannini¹, S. Maiorana¹, R. Alberto², I. Santos


Fig. 1

The main goal of this research is to find novel ^{99m}Tc probes for non-invasive imaging of endogenous gene expression, using the antisense approach. Taking into account the advantages of PNAs (Peptide Nucleic Acids) compared to normal oligonucleotides, we have synthesized a 16-mer PNA antisense sequence (*N*- A GAT CAT GCC CGG CAT-C), complementary to a region of the *N-myc* mRNA

which is amplified in human neuroblastoma. This 16-mer sequence was coupled to a bifunctional pyrazolyl-diamine chelator and the resulting conjugate was used to synthesize a Re(I) tricarbonyl complex (Figure 1). UV melting temperature experiments confirmed that the functionalized Re(I) complex recognizes the complementary DNA sequence (Figure 2). These results encourage further studies at the no-carried added level in order to evaluate the ability of the ^{99m}Tc congener to recognize *in vitro* and *in vivo* *N-myc* mRNA.

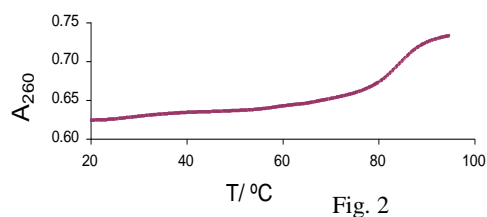


Fig. 2

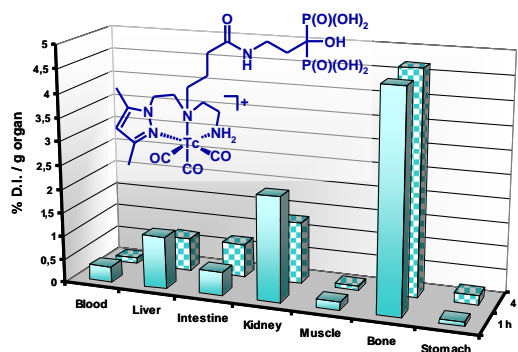
¹ Department of Organic and Industrial Chemistry, University of Milan, Italy;

² Institute of Inorganic Chemistry, University of Zurich, Switzerland

^{99m}Tc(CO)₃-labeled bisphosphonates for bone targeting: radiosynthesis and biological assessment

E. Palma, J. D. G. Correia, B. L. Oliveira, L. Gano, I. Santos

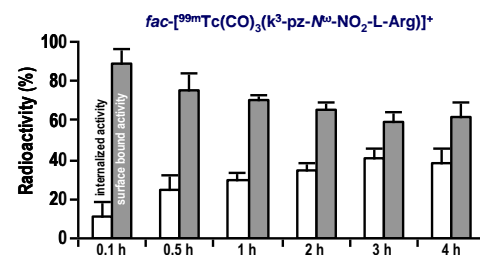
Aiming at the development of new ^{99m}Tc(CO)₃-based agents with higher affinity for bone, we prepared a new bioconjugate (**pz-pamidronate**) which comprises a pyrazolyl-diamine backbone (**pz**, for metal stabilization) and a pamidronate unit for bone targeting. This bioconjugate allowed the synthesis of *fac*-[^{99m}Tc(CO)₃(k³-**pz-pamidronate**)]⁺ which has been identified by HPLC comparison with the fully characterized Re(I) surrogate. Biodistribution studies in mice showed that this radiocomplex presented a fast rate of blood clearance and a high rate of total radioactivity excretion, occurring primarily through the renal-urinary pathway. The relevant bone uptake (4.7±0.9 % ID/g organ, 4 h p.i.) and the high stability observed encourage further studies in order to assess the usefulness of [^{99m}Tc(CO)₃(k³-**pz-pamidronate**)]⁺ as bone-imaging agent and for application in dual therapy.



$^{99m}\text{Tc}(\text{I})$ -complexes containing L-Arg analogues for probing inducible nitric oxide synthase (iNOS) *in vivo*

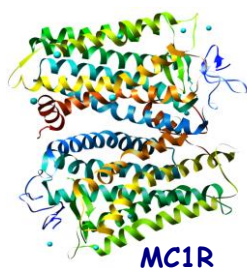
 B. L. Oliveira, J. D. G. Correia, P. D. Raposinho, I. Santos, C. Cordeiro,¹ A. P. Freire¹

Following our studies on the design of radioactive compounds for probing inducible nitric oxide synthase (iNOS) levels *in vivo*, we introduced bioorganometallic complexes of the type *fac*-[M(CO)₃(k³-pzL)] (M = ^{99m}Tc , Re; **pzL** = bifunctional pyrazolyl-containing chelator with a pendant substrate/inhibitor of NOS). Testing of the catalytic activity of iNOS *in vitro* revealed that the Re(I) complexes bearing the *N*⁰-Me-L-Arg (*K*_i = 36 μM) and *N*⁰-NO₂-L-Arg (*K*_i = 84 μM) units presented considerable inhibitory action, being unique examples of organometallic compounds able to inhibit that enzyme. Internalization studies in B16F1 murine melanoma cells showed that the highest internalization level was observed for the $^{99m}\text{Tc}(\text{I})$ complex with the unit *N*⁰-NO₂-L-Arg. In conclusion, $^{99m}\text{Tc}(\text{CO})_3$ -labeled L-Arg analogues may hold potential for monitoring increased levels of iNOS *in vivo*.

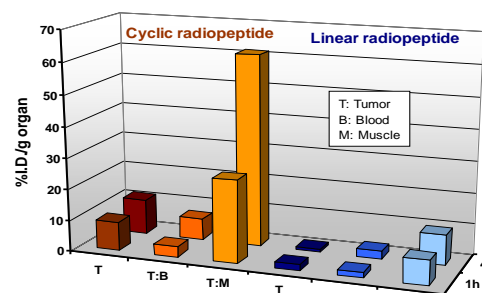


¹ Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Portugal.

Melanoma targeting with $^{99m}\text{Tc}(\text{CO})_3$ -labeled α -MSH analogues

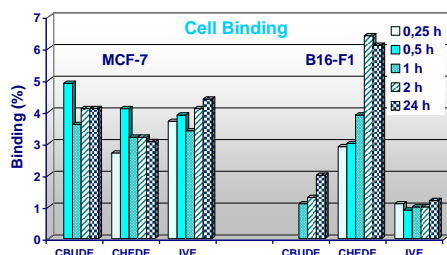
 P. Raposinho, C. Xavier, J. D. G. Correia, I. Santos, S. Falcão¹, P. Gomes¹


Linear and cyclic radiolabeled α -melanocyte-stimulating hormone (α -MSH) analogs were evaluated as radioactive probes to target the melanocortin-1 receptor (MC1R) overexpressed in melanoma. The effect of lactam-based cyclization on the tumor-seeking properties of α -MSH analogues was assessed by comparing the biological properties of the $^{99m}\text{Tc}(\text{CO})_3$ -labeled cyclic peptide conjugate *pz*- β Ala-Nle-*cyclo*[Asp-His-*D*Phe-Arg-Trp-Lys]-NH₂ (*pz* = pyrazolyl-containing chelator) with those of the corresponding linear analogue. The cyclic radioconjugate presented a remarkable internalization and cellular retention in murine melanoma B16F1 cells. Accordingly, a higher MC1R-mediated tumor uptake and retention was obtained in melanoma-bearing mice for the cyclic radioconjugate (11.31 ± 1.83% ID/g, 4 h) as compared to the linear radiopeptide.



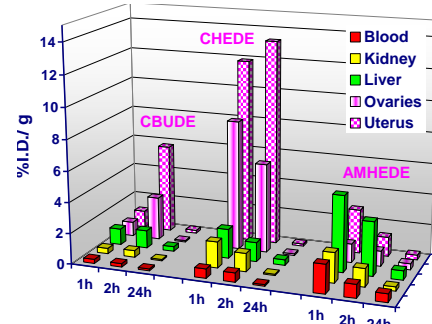
¹ CIQUP, Faculdade de Ciências, Universidade do Porto, Portugal.

Novel Estrogen Receptor Ligands as Potential Probes for Targeted Tumour Imaging and Therapy

 C. Neto, M. C. Oliveira, L. Gano, F. M. Marques, I. Santos, T. Yasuda¹, T. Thiemann¹, F. Botelho², C. Oliveira²


Unlike ^{125}I -CHEDE, cellular accumulation of ^{125}I -CBUDE was higher in ER (+) MCF-7 cells than in ER (-) B16F1 cells as observed for 17 α -iodovinyloestradiol (IVE). Consistently, ^{125}I -CBUDE demonstrated a high, selective and receptor-mediated target tissue uptake in immature female rats. These results encourage us to pursue studies with related compounds bearing different functionalized 7-alkyl chains.

As part of our ongoing work on specific ligands for targeted therapy and/or imaging of estrogen receptor positive (ER+) breast tumours a series of radioiodinated 7-alkyl-17 α -iodovinyloestradiols with either a cyano (^{125}I -CHEDE, ^{125}I -CBUDE) or an amide (^{125}I -AMHEDE) terminal group were prepared and their potential as SPECT biomarkers was evaluated.



¹ Interdisciplinary Graduate School of Engineering Science, Kyushu University, Japan.

² CIMAGO, FMUC, Coimbra