P128 PREPARATION AND EVALUATION OF (^{55}Co)-2-ACETYLPYRIDINE THIOSEMICARBAZONE COMPLEX FOR PET TUMOR IMAGING

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Introduction: Due to the interesting anti-proliferative properties of cobalt-thiosemicarbazone complexes, the production of a ^{55}Co-labeled thiosemicarbazone, *i.e.* 2-acetylpyridine thiosemicarbazone (APTS) was investigated (Fig. 1).

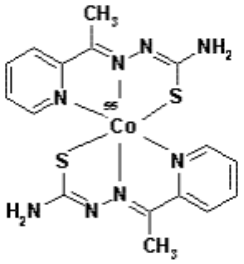

Fig. 1

Experimental: Co-55 ($T_{1/2}$=17.53 h) was produced by 150 μA irradiation of a natural nickel target by 15 MeV protons. The ^{55}Co was separated from the irradiated target material using a two step method with a radiochemical yield of >95%. For the labeling optimization, the ^{55}Co-chloride was mixed with 2-acetylpyridine thiosemicarbazone for 30 min at room temperature to yield [^{55}Co]-APTS with a radiochemical yield of higher than 99%.

Results and Discussion: RTLC and HPLC showed a radiochemical purity of more than 99% after C_{18} column chromatography. A specific activity of about 10-20 Ci/mmol was obtained. The final solution was diluted in normal saline to 5% ethanolic solution ready for biological evaluation. The stability of the final product was checked in the absence and presence of human serum at 37°C for up to 24 h. The partition co-efficient of the final complex was also determined. The sterile solution was finally administered to fibrosarcoma-bearing mice through their tail vein followed by co-incidence imaging (Fig. 3).

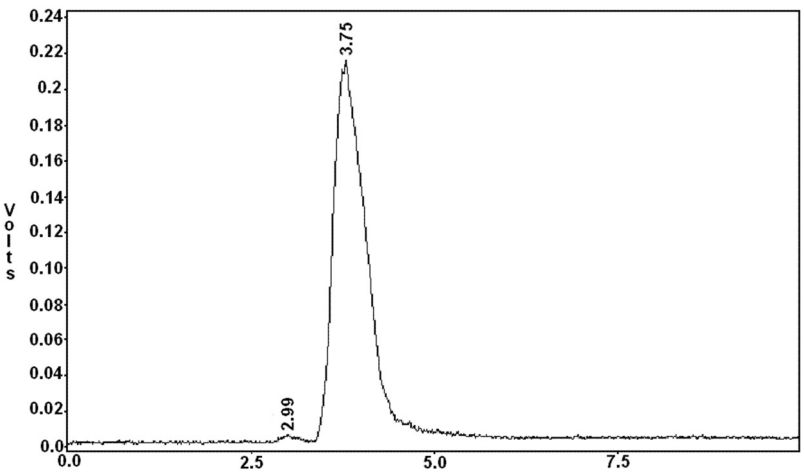

Fig. 2

Fig. 3

Conclusion: The final radiotracer showed a significant tumor uptake in the neck region, as well as liver and GI system. the initial data has encouraged us to investigate the imaging properties of this new tracer.

Keywords: Cobalt-55, Cobalt Thiosemicarbazonato Complex, Anti-Tumor Activity, Tumor Imaging, PET

P129 PREPARATION AND EVALUATION OF (^{201}Tl)(III)-DTPA COMPLEX FOR CELL LABELING

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Introduction: Due to interesting physical properties and wide availability of thallium-201 as a SPECT radionuclide, the incorporation of this nuclide into DTPA for cell labeling was targeted (Fig 1.).

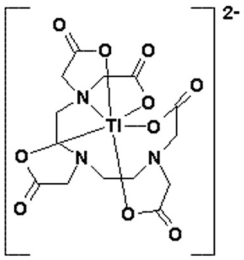

Fig. 1

Experimental: Thallium-201 ($T_{1/2}$=3.04 d) in Tl^{+} form was converted to Tl^{3+} cation in presence of O_3/6M HCl and di-isopropyl ether, controlled by RTLC/gel electrophoresis methods. The final evaporated activity reacted with cDTPA in normal saline to yield [^{201}Tl](III)DTPA at room temperature after 0.5 h followed by solid phase extraction purification using C_{18} Sep-Pak column.

Results and Discussion: A radiochemical yield>95% was gained. Radiochemical purity of more than 99% was obtained using RTLC (Fig. 2) with specific activity of about 260 GBq/mmol. The stability of the tracer was checked in the final product in presence of human serum at 37°C up to 3 days as well as partition coefficient measurements. The labeled compound was used in red blood cell (RBC) labeling. The cell uptake ratio was determined at 4, 25 and 37°C up to 3 hours (Fig. 3).

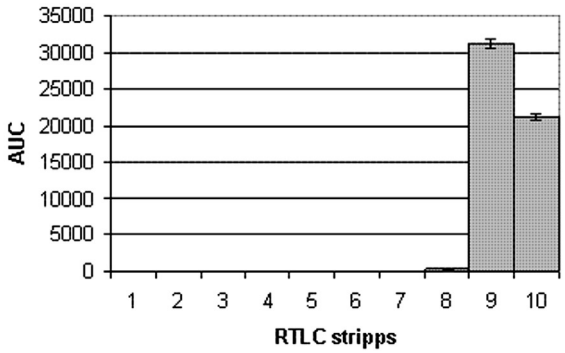

Fig. 2

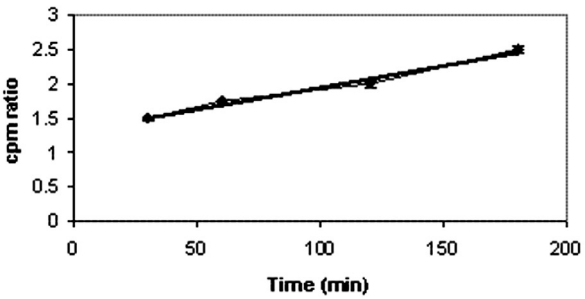

Fig. 3

Conclusion: High stability of Tl(III)DTPA complex and availability of Tl-201 in many countries, makes it a facile, non-expensive and potent cell labeling agent for use in the detection of inflammation (using WBC), acute thrombosis (using platelet) and GI bleeding (using RBC).

Acknowledgement: The authors wish to thank Dr A. Sattari and other colleagues in the radioisotope production team at NRCAM.

Keywords: Tl(III)-201, Radiolabeling, Cell Labeling, Radiopharmacy, Satbility

P130 PREPARATION, QUALITY CONTROL AND BIODISTRIBUTION STUDIES OF (^{67}Ga)-DOTA-ANTI-CD20

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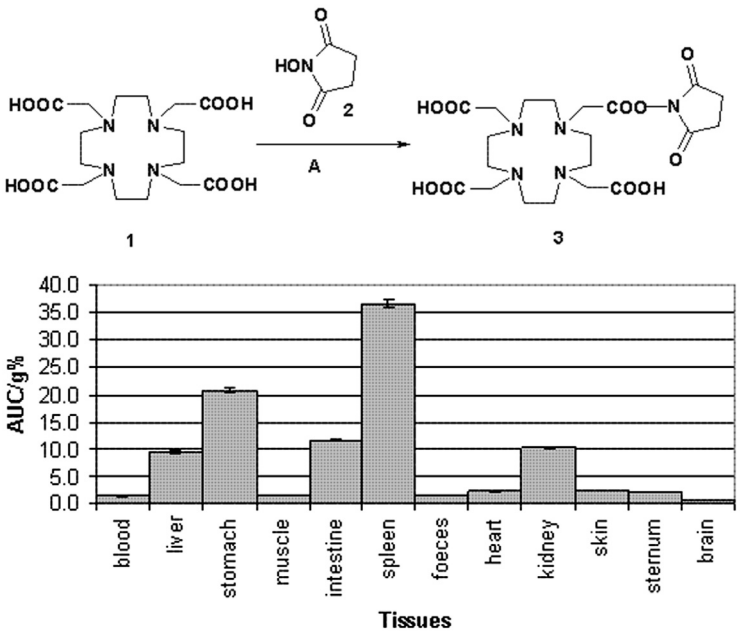
Introduction: Rituximab was successively labeled with [^{67}Ga]-gallium chloride. The macrocyclic bifunctional chelating agent, N-succinimidyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA-NHS) was prepared at 25°C using DOTA, N-hydroxy succinimide (NHS) in CH_2Cl_2.

Experimental: DOTA-Rituximab was obtained by the addition of 1 ml of a Rituximab pharmaceutical solution (5 mg/ml, in phosphate buffer, pH=7.8) to a glass tube pre-coated with DOTA-NHS (0.01-0.1 mg) at 25°C with continuous mild stirring for 15 h. Radiolabeling was performed at 37°C in 3h.

Results and Discussion: Radio-thin layer chromatography showed an overall radiochemical purity of 90-95% at optimized conditions (specific activity =30 GBq/mg, labeling efficacy; 82%). The final isotonic ^{67}Ga-DOTA-rituximab complex was checked by gel electrophoresis for radiolysis/chemolysis control. Radio-TLC was performed to ensure the formation of only one species followed by filtration through a 0.22 μ filter. Preliminary in vivo studies in a normal rat model were performed in order to determine complex distribution of the radioimmunoconjugate up to 28h.



Conclusion: [^{67}Ga]-DOTA-rituximab is a good probe for bio-dosimtery of therapeutic rituximab conjugates.

Keywords: Rituximab, CD-20, DOTA-NHS, Radiolabeling, Quality Control

P131 RADIOACTIVE SYNTHESIS OF $^{188}Re(CO)_3$-β-ELEMENE DERIVATIVES

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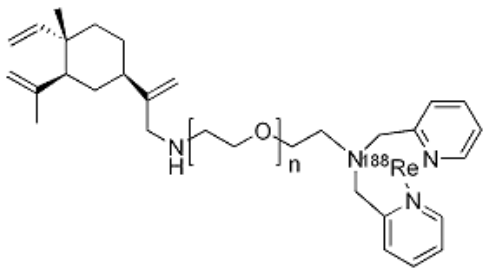
Introduction: In the past years, the bioinorganic chemistry of rhenium has been developed toward novel applications in therapeutic nuclear medicine. Interests in this field originate from the β particle emitting radioisotopes for therapeutic applications. Furthermore, due to the generator technique, ^{188}Re is nowadays readily available carrier-free at any time.

β-elemene, a Traditional Chinese Medicine, exhibits anticancer effects in human and murine tumor cells in vitro and in vivo and has substantial clinical activity against various tumors without severe side effects. No bone marrow suppression and drug resistance have been observed in the clinical studies; on the contrary, patient immunity was improved during the therapy with β-elemene. In this paper $^{188}Re(CO)_3$ β-elemene derivative was prepared.

Experimental: The first step we synthesizd the β-elemene derivatives, prepared the nonradioactive complex containing β-elemene and $Re(CO)_3$ moiety, followed by radioactive synthesis of β-elemene-$^{188}Re(CO)_3$ conjugate under the same conditions compared with that of nonradioactive synthesis. From HPLC analysis the two complexes have the same retention time.

Results and Discussion: The n = 0, 2, 8, when their water solubility increases, some better property is expected. The overall yield of radioactive synthesis is up to 80% and the purity of final conjugates is up to 95% after isolated by HPLC. The evaluation of their in vitro and in vivo biological behavior is in progress. Some leading research reported β-elemene derivatives with better water solubility will increase their antitumor activity both in vitro and in vivo.



Conclusion: For the first time we prepared a series of ^{188}Re β-elemene derivatives, they are expected to have selective uptake by tumor cells and no specific tissue accumulation in healthy animals; may be it will be developed to be therapeutic radiopharmaceutical in the future.

Acknowledgement: The project was supported by Hundred Talent Program of Chinese Academy of Sciences and Shanghai Institute of Applied Physics, Chinese Academy of Sciences.

Keywords: β-Elemene, 188Re, Radioactive Synthesis

P132 PREPARATION OF ^{68}Ga-LABELLED RITUXIMAB

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Introduction: Rituximab is a monoclonal Ab directed against the CD20 surface antigen which is overexpressed by certain B-cell lymphomas like NHL. Therefore Rituximab, labelled with the positron emitter ^{68}Ga, could be potentially used both for diagnostic and dosimetric purposes in the mentioned malignancies.

Experimental: First into the commercially available MoAb Rituximab (Mabthera®) was introduced the linker molecule DTPA or DOTA, respectively. The DTPA-Ab was prepared by twice addition of 300μg DTPA-dianhydride to 10mg Ab and incubation for 1h with subsequent gel filtration using 0.5M acetate buffer, pH 5.4. The DOTA-Ab was prepared by addition of 14.2mg DOTA, 6.5mg Sulfo-NHS and 0.6mg EDC, dissolved in water, to 4.5mg Ab with subsequent incubation for 20h (4°C, pH 8.5, stirring) and afterwards gel filtration using 0.25M ammonium acetate. Then the DTPA-Ab (250μg) and the DOTA-Ab (280μg) were labelled with 0.1ml ^{68}Ga-$GaCl_3$ (up to 100MBq) in acetate buffer, pH 5.4, for 30min at 37°C. Quality control was performed first by TLC using 0.1M sodium citrate and 1M ammonium acetate/methanol (1+1), respectively, and second by SEC-HPLC using 0.1M phosphate buffer, pH 7.4.

Results and Discussion: The DTPA-labelled Ab could be labelled with up to 80% labelling yield, whereas the DOTA-labelled Ab could be labelled with up to 90% yield. Due to time consuming gel filtration overall yields were 5-11MBq since convenient radiochemical purities could only be obtained at small starting activities (50-70MBq resp. 0.1ml). Higher activities resulted in significant amounts of free ^{68}Ga-$GaCl_3$ which could not be removed quantitatively from the preparations. A critical parameter was the volume of ^{68}Ga-actvitity so that this problem could be eventually overcome by concentration of ^{68}Ga-$GaCl_3$.

Conclusion: Due to better ^{68}Ga-incorporation the DOTA-Ab has greater potential then the DTPA-Ab. Beause of restricted facilities (no eluate concentraton) the preparations can only be conducted in small scale at the moment which would be sufficient for animal experiments but not for humans.

Keywords: ^{68}Ga, Rituximab, Anti-CD20, NHL, Diagnosis

P133 CHARACTERIZATION OF (^{64}Cu)Cu-ATSM FOR HYPOXIC TISSUE IMAGING

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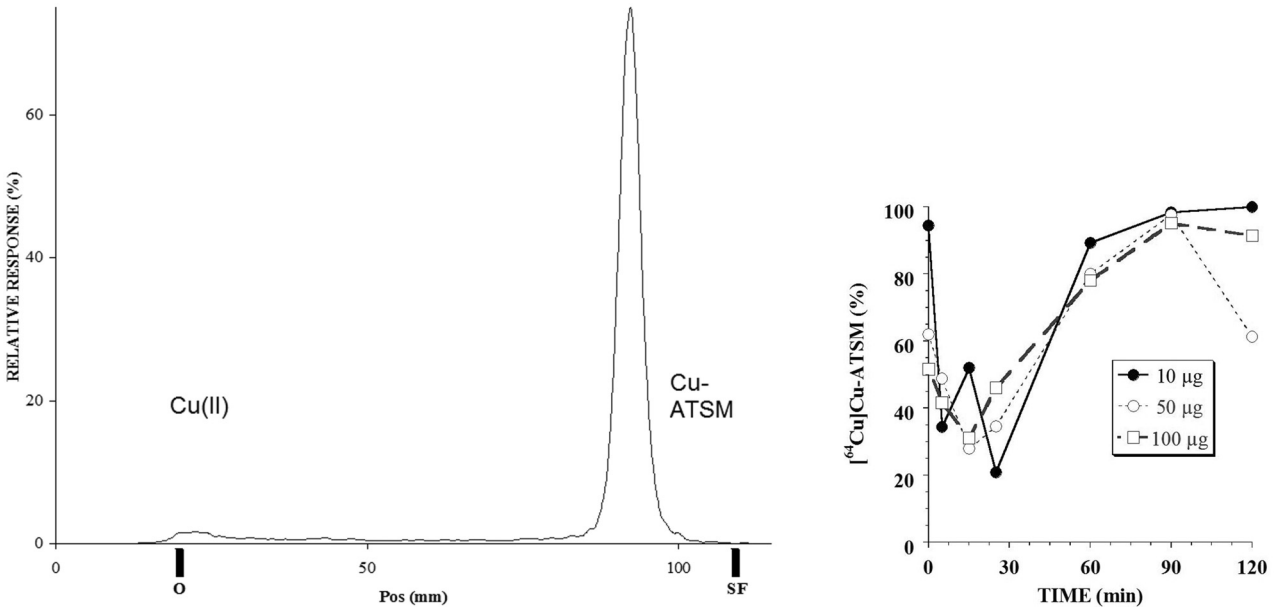
Introduction: [^{64}Cu]Cu-ATSM has been utilized as a possible hypoxic tissue imaging agent and is made generally following a procedure recommended by Washington University. We investigated the conditions for labeling the tracer for our microPET imaging studies, looking at reaction rate at different precursor concentrations by chromatographic analysis.

Experimental: The base conditions were approximately 74 MBq (2 mCi) [^{64}Cu]$CuCl_2$ reacted with 10 mg ATSM in 10 mL DMSO in 150 mL 1 M NaOAc buffer, incubated at room temperature, and analyzing the reaction by thin layer chromatography on silica gel with 100% ethyl acetate. We found that, in our hands, the reaction proceeded slowly at room temperature, and required over 60 min reaction time to achieve greater than 90% incorporation. Labeling yields were similar at ATSM concentrations from 10 to 100 μg (Figure 2).



Figures: Reaction of [^{64}Cu]$Cu(OAc)_2$ with ATSM. Fig. 1 (left). TLC of reaction mixture after 90 min at room temperature. Fig. 2 (right). Course of reaction at three different ATSM concentrations.

Results and Discussion: The kinetic data suggest that there exist dynamic interactions between [^{64}Cu]Cu(II) ion and coordinating ligands available in the environment such as Cl^-, OAc^-, H_2O, DMSO, and ATSM during the first hour of mixing. In some experiments, an unidentified second species was observed from the early incubation mixtures during the TLC analysis.

Conclusion: When not disturbed, [^{64}Cu]Cu-ATSM complex became stable after 60 minute incubation in the 1M NaOAc buffer.

Acknowledgement: National Institute of Health (NCI R25T-CA092043) and Vanderbilt University, Department of Radiology & Radiological Sciences.

Keywords: Copper-64, ATSM, Radiolabeling, Positron Emission Tomography, Hypoxia

P135 PREPARATION OF THE ^{177}Lu-DOTA-TATE, ^{188}Re-DOTA-TATE AND ^{188}Re-TATE. IN VITRO COMPARATIVE EVALUATION

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Introduction: This study describes the radiolabelling methods of DOTA-Tyr3-Octreotate (DOTA-TATE) and Tyr3-Octreotate (TATE) derivative octreotides with the β-particle emitting radionuclides, ^{177}Lu (670 mm tissue mean range) and ^{188}Re (3500mm tissue mean range) and the bioaffinity to the somatostatin receptors of ^{188}Re-DOTA-TATE and ^{188}Re-TATE in comparative evaluation with ^{177}Lu-DOTA-TATE as radiobiopharmaceutic for targeting of somatostatin receptors overexpressed in micromethastasis.

For radiolabelling procedures the optimal values of β-emitters to peptide molar ratios, pH, temperature and incubation time were established taking into account the chemical structures of biovector and the chemical properties of radiometals.

Experimental: The DOTA-TATE in acetate buffer 0.4M pH 4.5 was labelled with $^{177}LuCl_3$ in 0.05N HCl (DOTA-TATE to ^{177}Lu molar ratio of 3.7). The reaction mixture was incubated 30 minutes at 80°C. After incubation and cooling was added HABA (3-hydroxy-4 aminobenzoic acid) as radiolitic stabilizer.

The ^{188}Re radiolabelling procedure of TATE, required the reducing of –S-S– bonds with 2-ME to $–S^-$ tiol groups as coordinating sites to bind the rhenium atoms in a reduced oxidation state. The reduced peptide was labelled with prereduced 188Re in presence of HEDP/Gentisic acid/$SnCl_2$ (25/10/2.5 massic ratios) and 3pH

The ^{188}Re-DOTA-TATE was obtained using the Ca Gluconate (CaG) as coligand in ^{188}Re transchelation to the DOTA cycle. The mixture of ^{188}Re and formulated DOTA-TATE in solution of CaG/Gentisic acid/$SnCl_2$ (10/10/2.5massic ratios) 5pH, incubated for 90 minutes at 90°C lead to ^{188}Re-DOTA-TATE as final product.

In vitro receptor binding assay of ^{177}Lu-DOTA-TATE, ^{188}Re-DOTA-TATE and ^{188}Re-TATE was performed using rat brain cortex membrane. The results of experimental works regarding the competitive and saturation binding assays were mathematical processed for obtaining of the IC_{50} and K_D values for each radioproduct.

Results and Discussion: The obtained results show that radiochemical purity of ^{177}Lu-DOTA-TATE, ^{188}Re-DOTA-TATE and ^{188}Re-TATE is higher then 95%. The specific IC_{50} and K_D values for each radiobiomolecule are comparable and show high binding affinity of them for the somatostatin receptors.

Conclusion: These results are promising for to start the in vivo investigations regarding the radiopharmacological aspects and therapeutically dose effects of ^{188}Re-DOTA-TATE and ^{188}Re-TATE.

Acknowledgement: The authors thanks for Financial Support regarding the realisation of this study in the frame work "VIASAN" National Researche Programm for Health.

Keywords: Octreotate, Radiolabelling, ^{177}Lu, ^{188}Re, Bioaffinity

P136 A NEW FATTY ACID CYSTEINE CONJUGATE FOR ASYMMETRIC (^{99m}TcN(PNP))$^{2+}$SYNTHON LABELING FOR POSSIBLE USE IN METABOLIC CARDIAC IMAGING

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Introduction: ^{123}I labeled fatty acids and ^{18}F-FDG are the two radiopharmaceuticals currently used for myocardial metabolic imaging. Limited availability and short half-life of these cyclotron-produced radionuclides drives the quest for cost effective and characteristically ideal ^{99m}Tc-based agents for this purpose.

^{99m}Tc-nitrido ([^{99m}Tc≡N]$^{+2}$) core, isoelectronic to the conventional [^{99m}Tc=O]$^{+3}$ core, has affinity for soft donor atoms such as S and P, and forms complexes with square pyramidal geometry. In recent times, ^{99m}Tc-nitrido core has been studied extensively. Using this core, a large number of asymmetric [2+2] complexes with a long chain bidentate phosphorous (PNP) lipophilic backbone occupying two basal (cis) positions leaving the other two for a suitable ligand with N, O and S donors have been prepared and have shown excellent *in-vivo* biological characteristics. Cysteine group involving either NH_2 and S^- or COO^- and S^- have been found to form stable asymmetric [2+2] ^{99m}Tc-nitrido complexes with the new [^{99m}TcN(PNP)]$^{2+}$ core. In the present work, a fatty acid-cysteine conjugate has been prepared and labeled with [^{99m}TcN(PNP)]$^{2+}$ core, with an aim to prepare a fatty acid analog labeled with ^{99m}Tc.

Experimental: The amino group of cysteine moiety was linked to one of the carboxylic acids of hexadecanedioic acid. S-trityl cysteine was esterified as reported using ethyl p-toluene sulphonate in dry ethanol. The amine group of protected cysteine derivative was then conjugated with a hexadecanedioic acid in 1:1 ratio in presence of dicyclohexylcarbodiimide in dry dichloromethane. This fatty acid trityl cysteine ester derivative was then deprotected to generate free acid and thiol group suitable for complexing with [^{99m}TcN(PNP)]$^{2+}$ core. The intermediates and final ligand were characterized by ^{1}H-NMR spectroscopy.

[^{99m}TcN]$^{2+}$ core was prepared using succinic dihydrazide, stannous chloride and sodium pertechnetate, to which the PNP (PNP6) in ethanol was added and the reaction was carried out at 25°C for 30 min to give new [^{99m}TcN(PNP)]$^{2+}$ core. The fatty acid cysteine derivative was then added to the reaction mixture and heated at 100° C for 30 min to obtain the required complex.

Results and Discussion: The synthetic modifications resulted in high yields of pure derivatives. The radiolabelling yield was found to be more than 70%, as estimated by HPLC. The radiolabeled molecule could be prepared in >95% purity by using Sep-pak.

Conclusion: A novel ^{99m}Tc-fatty acid derivative is prepared in good yields and may have potential for metabolic imaging of heart.

Keywords: 99mTc-Nitrido Core, Fatty Acid, Meatbolic Cardiac Imaging

P137 ^{86}Y-LABELLED HUMAN SERUM ALBUMIN MICROSPHERES (DOTA-HSAM): *IN VIVO* STABILITY DEPENDS ON SURFACE STRUCTURE OF THE SPHERES

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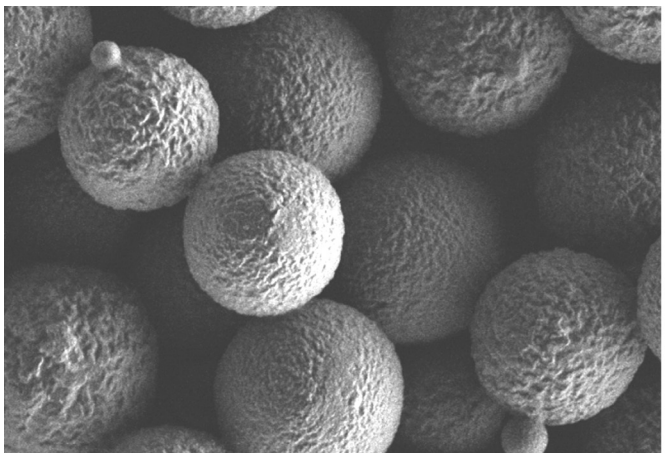
Introduction: Radiolabelled particles are an attractive tool in the therapy of malignancies of the liver. We consider particles manufactured from denatured human serum albumin (HSAM) as useful carriers of therapeutic radionuclides, e.g. Y-90.

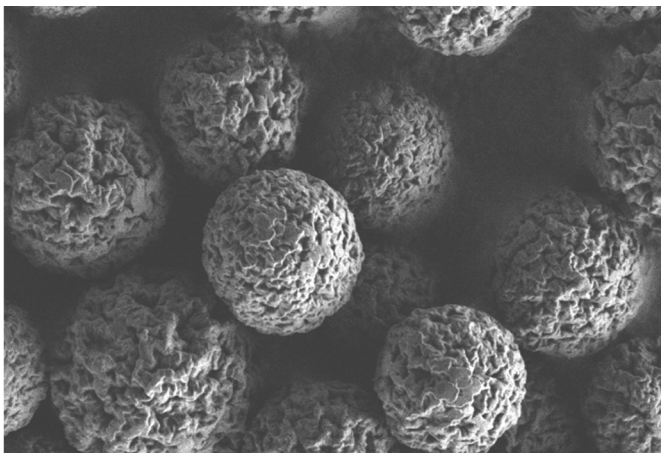
Experimental: Two batches of HSAM (ROTOP Pharmaka GmbH; diameter: 20–30 μm) with smooth or rough surface, as determined by REM, were used for our studies. HSAM were functionalized by coupling DOTA via its isothiocyanate derivative in aqueous solution at pH 8.5 to lysine ε-amino groups on the surface of the spheres. Stability of Y-86 labelled DOTA-HSAM was investigated both *in vitro* (DTPA challenge) and *in vivo* (i. v. injection into the tail vein of healthy Wistar rats).

Results and Discussion: Approximately 25 DOTA molecules per molecule HSA could be attached as estimated from elemental analysis of DOTA-HSAM. Y-86 labelling was performed under optimized conditions in 96 ± 1% yield. No significant differences between smooth- and rough-surfaced HSAM were found for both, DOTA coupling and Y-86-labelling. In DTPA challenge experiments 98 ± 0% of the radioactivity were still particle-associated after 24 hours incubation at 37°C. In the *in vivo* experiments radiolabelled smooth and rough microspheres were completely trapped in the lungs. After 48 h the two batches differed significantly in their biodistribution pattern. For the clearance of radioactivity from the lungs decay-corrected half-lives of 85 h (rough microspheres) and 187 h (smooth microspheres) were calculated.

Biodistribution of Y-86-DOTA-HSA microspheres 48 h p. i.

	Kidney (% ID)	Lung (% ID)	Urine (% ID)
Smooth Microspheres	5.69 ± 1.99	85.51 ± 4.49	6.06 ± 4.52
Rough Microspheres	9.45 ± 3.38	69.33 ± 13.74	8.74 ± 6.66





Conclusion: For the preparation of HSA-derived microspheres for radiotherapeutic application smooth-surfaced spheres are superior to rough spheres due to their higher *in vivo* stability.

Acknowledgement: QSA Global for providing $^{86}YCl_3$.

Keywords: Microspheres, Yttrium-86, In Vivo Stability

P138 SYNTHESIS, CHARACTERIZATION AND PRELIMINARY BIOLOGICAL EVALUATION OF (^{64}Cu)THHYLENEDICYSTEINE-DEOXYGLUCOSE

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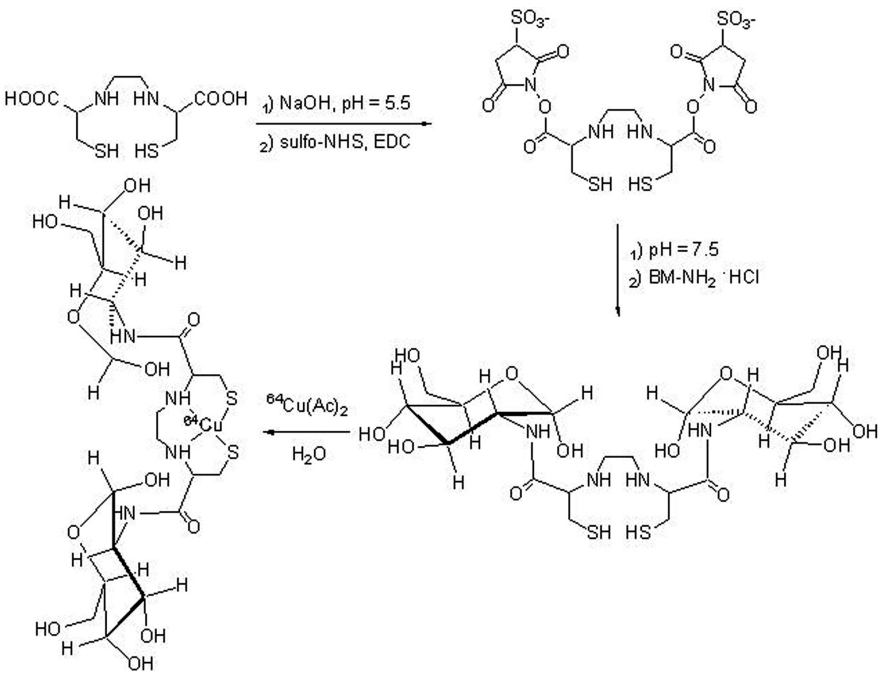
Introduction: To evaluate PET imaging of [^{64}Cu]ethylenedicysteine-deoxyglucose [^{64}Cu]ECDG, an interesting copper compound. This copper complex has much interest as delivery agent for radioactive copper in new copper compound and for functional metabolic imaging potentialities.

Experimental: The ligand ethylenedicysteine-deoxyglucose (ECDG) was synthesized by means of reacting ethylenedicysteine, with glucosamine, with carbodiimide as the coupling agent. Radiosynthesis of [^{64}Cu]ECDG was achieved by means of adding the required amount of ECDG and $^{64}CuCl_2$.

Results and Discussion: We report the synthesis and applications of [^{64}Cu]ECDG complex. The [Cu] ECDG complex was characterised by melting point, elemental analysis, ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectroscopy, FT-IR spectroscopy and ESI-MS. [^{64}Cu]ECDG was determined via Radio-Thin-Layer Chromatography to have a radiochemical purity of 95%. The specific radioactivity was calculated to be 0.5Ci/mmol. To determinate biodistribution [^{64}Cu]ECDG was injected through the tail vein in three groups of five swiss Albino mice each (male) (30-35 g). The animal were sacrificed by cardiectomy under slight ether anaesthesia at predetermined time intervals (1, 15 and 60 min.). The organs of interest were excised, wighed and the radioactivity counted in gamma counter. Several tumoral cell lines were injected into 18 nude mice, the animals were then injected with [^{64}Cu]ECDG or [^{18}F]FDG (0.074MBq per mice). The Micropet studies were executed using a Yappet System (ISE) after 5 minutes, 6 hours and 24 hours after injection. Through micropet analysis we have compared Micropet Images of the animal group injected [^{64}Cu]ECDG, indicated as group 1, and the animal group injected [^{18}F]FDG, indicated as group 2.



Conclusion: The [^{64}Cu]ECDG can be synthesized at room temperature starting from ethylenedicysteine-deoxyglucose and $^{64}CuCl_2$. Decay-corrected radiochemical yields of [^{64}Cu]ECDG based on ^{64}[Cu] were 80% ± 5% (n=10). MicroPET imaging studies in nude mice have evidenced similarities between [^{64}Cu]ECDG and [^{18}F]FDG uptake in tumors, and study findings supported the potential use of [^{64}Cu]ECDG as a functional imaging agent.

Acknowledgement: The authors thank S. Pezzuto and A. Nasini for excellent technical assistance; S. Moscatelli for helpful discussions.

Keywords: Copper 64, MicroPET, Deoxyglucose, Ethhylenedicysteine, Biodistribution

P139 ORGANOMETALLIC TECHNETIUM-99m RGD-PEPTIDE CONJUGATES WITH TUNABLE LIPOPHILICITY

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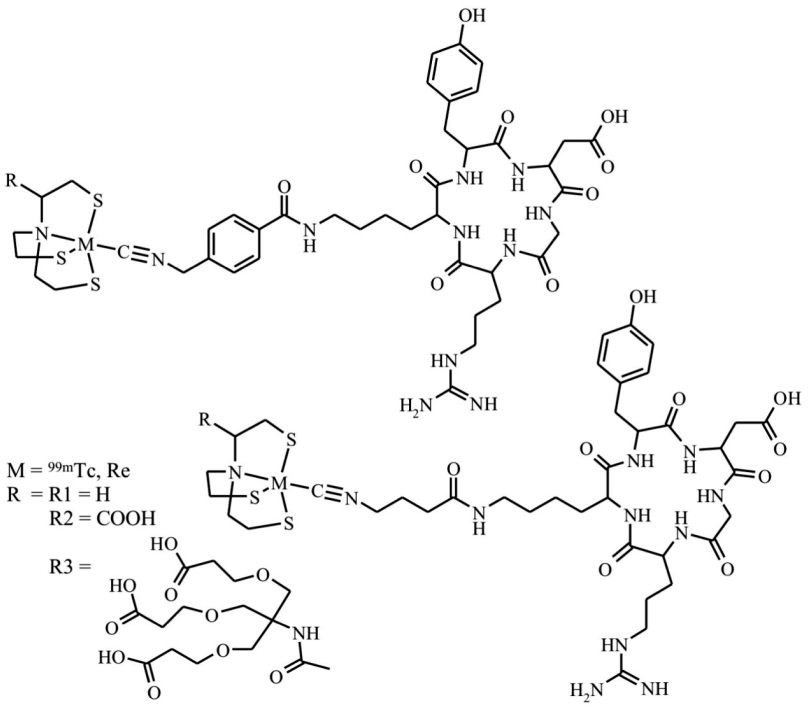
Introduction: Radiolabeled RGD-peptides have been studied for in vivo targeting of integrin receptors which are over-expressed in carcinomas [1]. The high pharmacological influence of the radiometal complex and limitations of various labelling concepts are reasons for exploring new approaches. The so-called "4+1" mixed-ligand chelate system, [Tc(NS_3)(CN-R)], is an interesting alternative due to high labelling efficiency and in vivo stability [2]. Here we present the application of this concept to the labelling of the peptide c(RGDyK).

Experimental: The figure shows the labelled peptides, corroborated upon coinjection with the corresponding rhenium compounds. The RGD-peptide, serving as monodentate ligand, was functionalized with 4-(isocyanomethyl)benzoic acid (L1) and 4-isocyanobutanoic acid (L2). ^{99m}Tc labelling was performed starting from ^{99m}Tc-EDTA followed by ligand exchange reaction with a mixture of the appropriate isocyanide-modified peptide and a tetradentate ligand (NS_3R). The resulting series of ^{99m}Tc peptide conjugates [^{99m}Tc(NS_3R)(L-c(RGDyK))] with R = R1, R2, R3 and L = L1, L2 was evaluated in biodistribution studies using tumour-bearing mice.

Results and Discussion: The radiochemical yield was about 60% after labelling 0.05-0.1 mg of isocyanide-modified peptide. Purification by HPLC resulted in radiochemical purity > 95%. The stability of the ^{99m}Tc conjugates in PBS was higher than 90% after 24 h. Distribution ratios (logD, octanol/PBS, pH 7.4) covered the range from -0.59 ± 0.14 for [^{99m}Tc(NS_3R1)(L1-c(RGDyK))] to -3.3 ± 0.2 for [^{99m}Tc(NS_3R3)(L2-c(RGDyK))]. The compounds exhibited only low tumour uptake and a fast hepatobiliary elimination. Substitution of the tripodal chelator bearing R1 or R2 by the carboxyl group-bearing ligand NS_3R3 resulted in a predominant urinary excretion.



Conclusion: The "4+1" mixed-ligand approach enables the ^{99m}Tc-labelling of c(RGDyK). The chelate unit dominates the biodistribution profile which could be influenced by varying the tripodal chelator.

References: [1] Haubner, R., Wester, H.-J. Curr. Pharm. Design 10 (2004) 1439-1455. [2] Seifert, S. et al., Bioconjugate Chem. 15 (2004) 856-863.

Acknowledgement: IAEA, CSIC, FMP-CHLCC, Lab. de vacunas recombinantes, Fac. de Medicina, Uruguay for financial support.

Keywords: Tc '4+1' Complexes, Tc-Labeled Peptide, RGD Peptide, Isocyano-Modified Peptide

P140 A SYNTHETIC PROTOCOL TO DAPS, CHELATORS FOR POTENTIAL RADIOPHARMACEUTICAL APPLICATION

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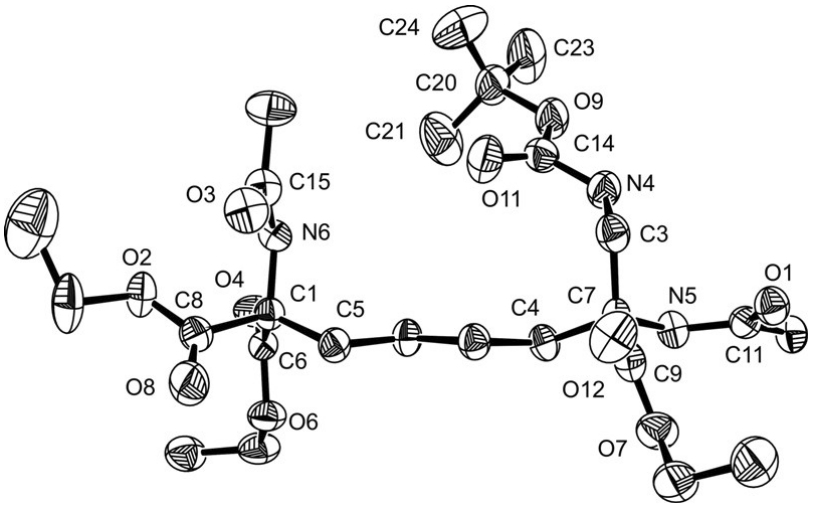
Introduction: Dap (α, β-diamino propionic acid) and its derivatives have stimulated the research interest from both chemist and biochemist because of their unique structural role played in molecules of biological significance. In the radiopharmaceutical context, it has been demonstrated by our group that it could be employed as tripodal ligand and efficiently labeled with ^{99m}Tc(I)/Re(I) tri-carbonyl in aqueous phase to form the corresponding complexes withstanding the competition of Histidine or Cysteine. However, the cost and difficulty of its derivatization prohibit the further exploration. In this report, a new synthetic strategy was employed to overcome the above mentioned barrier towards **Dap**.

Experimental: Ethyl acetamidocyanoacetate (**I**) was reduced to Ac-Dap(Boc)-OEt (**II**) with $NaBH_4/NiCl_2$ at 0°C in one step with the yield 80%. The α-derivatized **Dap** was exemplified by first coupling **I** to $Br(CH_2)_4C(NHAc)(COOEt)_2$ (**III**) with NaOEt, and then the resulting product was reduced with the same method to afford the corresponding **Dap** derivative (**IV**) (ORTEP drawing was shown below with ellipsoid of 50% probability and H atoms were omitted for clarity) with 57% overall yield.

Results and Discussion: During the reduction from **I** to **II**, all function groups are kept intact except the targeted Cyano group, and the transformation is much cheaper and efficient by all means. Compound **I** was very conveniently derivatized at α-carbon. **III** was a good starting material for both racemic and enantiopure α-amino acid (**AA**). The α-substituent had no interference upon the selective reduction of cyano group. This method could be easily applied to the preparation of other α-subsitituted **Dap** derivatives from different demands.

In this case, **IV** can be further deprotected by usual methods to obtain the free **Dap**-**AA** chelator, the $Re(I)(CO)_3$ complex of which was found to exhibit the first example of recognition and transportation of small chelator-metal conjugate by LAT1.



Conclusion: In summary, we have found a straightforward and efficient method to produce **Dap** its α-derivatives in a way of low cost. And hence this strategy is laying out a wider road towards the exploration of **Dap** chemistry.

Acknowledgement: We thank Mallinckrodt Medical (Petten) for financial support and Dr. H. Knight and Dr. B. Spinger for their helpful discussion.

Keywords: Diamino Propionic Acid, 99mTc(I)/Re(I) Labeling, Crystal Structure, Synthesis

P141 LABELING DOTATOC USING A DILUTE SOLUTION OF ^{90}Y CHLORIDE

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Introduction: Therapy with Y-90 labeled peptides is being actively investigated in certain neuroendocrine tumors. Labeling of the peptide is usually performed in house starting from Y-90 Chloride. There are a small number of manufacturers who provide this radioisotope. For practical reasons, our group is currently utilizing a formulation of Y-90 utilized for the labeling of the approved monoclonal antibody Zevalin(TM). This formulation is more dilute (approximately 50-80 mCi/mL) compared to other commercially available and more widely used forms (approximately 10 times more concentrated). The aim of this work is to optimize labeling conditions for this which for us is currently the only available source of Y-90.

Experimental: DOTATOC was dissolved at a concentration of 2mg/mL (approximately 1 mM) in ultrapure water. Immediately prior to use the Y-90 chloride solution was buffered with varying amounts of gentisic acid (0.35 M) sodium acetate (0.4 M) buffer system. Radiometal and peptide were mixed at a ratio ranging from 0.5 to 1 Ci/mg. Reactions were performed in a heating block with temperatures ranging from 95 to 125°C or in a water bath at 95°C for different times in conical 1 or 2 mL glass vials or in the Y-90 source vial itself. The labeled peptide was then purified on a C18 cartridge (Sep-pak light). Reversed phase HPLC and Silica Gel TLC were utilized to assess radiochemical purity.

Results and Discussion: The overall yield of radiolabeled peptide varied between 50 and 99%. Incubation in a water bath for 30 min at 95° appeared to be sufficient for labeling. No improvement in yield was observed using higher temperatures or longer times of incubation. Incubation in the water bath was also more efficient than the heating block perhaps for the achievement of more homogeneous temperatures inside the vials. Radiometal to peptide ratios of 0.5 or 1 Ci/mg gave similar yields in parallel experiments. Conditions that appear to favor yield are reduction of the incubation volume by dividing the reaction into several vials although this implies increased handling times and radiation exposure to personnel. Performing the reaction in the source vial also favored higher yields however this involves removing approximately half the volume of the Y-90 solution prior to performing the reaction and in one instance this was not effective giving a 50% yield.

Conclusion: DOTATOC labeling with low concentration Y-90 chloride is feasible however it appears less effective and not as reproducible as with higher concentration formulations. The use of higher concentration formulations of Y-90 is more desirable.

Keywords: Yttrium Labeling, DOTATOC, Radiolabeled Peptide Therapy, Quality Control, Labelling Efficiency

P142 SYNTHESIS AND BIODISTRIBUTION OF NOVEL (^{99m}TcN(PNP))$^{2+}$ METAL FRAGMENT LABELED GLUCOSE-DITHIOCARBAMATE DERIVATIVES

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Introduction: Carbohydrates are of primary importance as energy sources for living organisms and thus highly evolved transport and metabolic pathways have been developed for utilization. The use of radiolabelled carbohydrate derivatives takes advantage of these pathways to track energy metabolism as well as imaging regions of interest *in vivo*. A new class of Glucose-Dithiocarbamate ligands and their technetium complexes have been studied.

Experimental: Fivel Glucose-Dithiocarbamate ligands L_n (L_1~L_5) have been synthesized and the [^{99m}TcN(L_6) (L_n)]$^+$ (L_n= Glucose derivatives containing dithiocarbamates; L_6=PNP bisphosphine) complexes (Fig.1) were prepared in two steps according to literature procedure[1,2,3]. The radiochemical purity was assessed by ITLC and Radio-HPLC with a C-8 reverse phase column and a gradient elution (0–5min: 70% solvent A(0.1% TFA buffer) and 30% solvent B (acetonitrile); 5–25min: solvent B from 30% to 90% and solvent A from 70% to 10%. the flow rate: 1.0ml/min). Biodistribution studies were performed in normal mice.

Results and Discussion: The radiochemical purity (RCP) was >90% for all the complexes. The retention time were 17.1min, 17.6min, 18.3min, 18.7min and 19.1min for [^{99m}TcN (L_1)(L_6)]$^+$, [^{99m}TcN (L_2)(L_6)]$^+$, [^{99m}TcN (L_3)(L_6)]$^+$, [^{99m}TcN (L_4)(L_6)]$^+$ and [^{99m}TcN (L_5)(L_6)]$^+$, respectively. The results from biodistribution studies demonstrated all these cationic complexes showing similar biodistribution characteristics with a lower initial heart uptake and a rapid clearance from blood, muscle, liver and other tissues in normal mice.

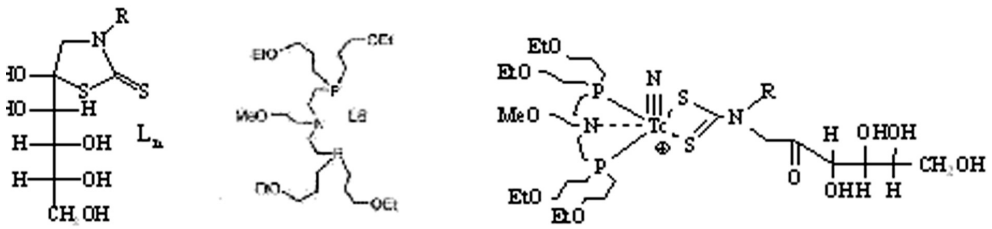

Fig.1. L_n, L_6 and the complex [^{99m}TcN(L_6) (L_n)]$^+$
(L_n=L_1~L_5; L_1: R= -Me; L_2: R= -Et; L_3: R= -i-Pr; L_4: R= -Pr; L_5: R= -i-Bu)

Conclusion: Five new Glucose-Dithiocarbamate ligands L_n (L_1~L_5) and [^{99m}TcN(L_6) (L_n)]$^+$ have been accomplished. The distribution of these ^{99m}Tc complexes was evaluated in mice.

References: [1] Hatada K, Riou LM, Ruiz M, et al. J Nucl Med, 45:2095–101, 2004. [2] Zhang JB, Wang XB, Li CY. Nucl Med Biol, 29:665–9, 2002. [3] Shuang Liu, Zhengjie He, Wen-Yuan Hsieh, et al. Nucl Med Biol, 33: 419–432, 2006.

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Keywords: Glucose, Dithiocarbamate, 99mTcN(PNP) Complexes, Radiopharmaceuticals

P143 A BIS(2-PYRIDYLMETHYL) DERIVATIVE OF 1,4,7-TRIAZACYCLONONANE AS HIGHLY HYDROPHILIC CHELATING AGENT FOR COPPER

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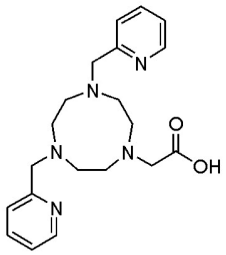
Introduction: Highly thermodynamically stable Cu(II) complexes are required for the development of new copper(II) target-specific radiopharmaceuticals. In this perspective, azamacrocycle derivatives [1], and in particular a 1,4,7-triazacyclononane derivative containing two pyridine pendant arms [2] is a potentially promising ligand candidate. The further addition of a carboxylic group to this chelating agent could provide an attractive lead for the labelling of biomolecules with copper radionuclides.

Experimental: A new ligand, 2-[4,7-bis(2-pyridylmethyl)-1,4,7-triazacyclononan-1-yl]acetic acid (**1**), has been synthesised, and the X-ray structure of a corresponding copper(II)-complex determined. A bioconjugate (**2**) was obtained by coupling a modified bombesin derivative [3] to **1** (HBTU, DIPEA, DMF, r.t.). **2** was purified by semi-preparative HPLC. The free ligand **1** and the bioconjugate **2** were labelled with ^{64}Cu. To 100 μl of ligand solution (0.0001 M ligand dissolved in 0.05 M NH_4OAc), 200 kBq of $^{64}CuCl_2$ was added. Labelling yields were studied by TLC.

Results and Discussion: The structure of [**1** [suB] Cu]$^{2+}$ was solved by X-ray single-crystal diffraction and indicates that the ligand is able to bind Cu^{2+} in a hexacoordinate manner. A high complex stability ($logK_1 > 20$) was determined. The bioconjugation of **1** to a bombesin derivative was successfully achieved via amide coupling to give **2**. The free ligand **1** and the bioconjugate **2** were labelled with ^{64}Cu and the resulting complexes were found to be stable in the presence of a large excess of competing ligands such as cyclam and glutathione as well as in rat plasma. These ^{64}Cu complexes are highly hydrophilic ($logD_{o/w} < -2.3$).



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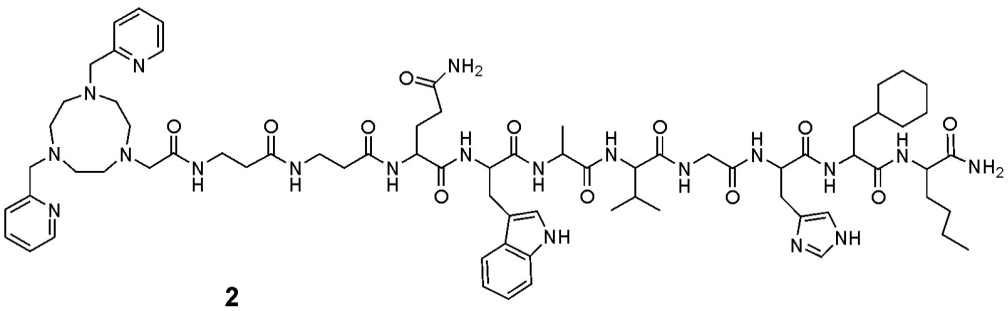

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Conclusion: A bis(2-pyridylmethyl) derivative of 1,4,7-triazacyclononane **1** and its bombesin bioconjugate **2** can readily form ^{64}Cu complexes with high stability. The bifunctional chelating agent **1** may be an attractive candidate for developing new copper radiopharmaceuticals.

References: [1] K.P. Guerra *et al.*, *Dalton Trans.*, **2006**, 4124-4133. [2] G.A. McLachlan *et al.*, *Inorg. Chem.* **1994**, *33*, 4663-4668. [3] E. Garcia Garayoa *et al.*, *Quart. J. Nucl. Med. Molec. Imaging*, **2006**, *50*, 15-16.

Keywords: Azamacrocycle, BFA, CU-64, Bombesin, Bioconjugation

P144 PHARMACOLOGICAL EFFECTS OF BACKBONE MODIFICATION ON ^{99m}Tc-LABELED BOMBESIN-LIKE PEPTIDES

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Introduction: Overexpression of gastrin-releasing peptide receptors (GRPrs) in several types of human cancer cells including prostate, breast, small cell lung and pancreatic cancers render them interesting targets for tumor imaging and therapy. Bombesin, a tetradecapeptide, is an analogue of human gastrin releasing peptide binding to GRPrs with high affinity and specificity. Goal of the present study was to introduce modifications at the spacer's site and evaluate the resulting new 99mTc-labeled bombesin analogues in comparison to GGC-Aca-BN[2-14]-NH2. This last analogue in which the spacer Aca represents 6-amino-hexanoic acid has been already studied at our laboratories.

Experimental: Solid phase peptide synthesis (SPPS) was applied to produce GGC-X-BN[2-14]-NH2 conjugates, where the spacer group X either does not exist (X=0) or is X=Ornithine-Ornithine-Ornithine. These conjugates were purified (>95%), by semi-preparative RP-HPLC and characterized by LC-MS-ESI. According to the labeling protocol, sodium gluconate was used as an intermediate exchange ligand for 99mTc pertechnetate, which was reduced by stannous ions.

Results and Discussion: The labeling yield, identified by RP-HPLC was high, even at very low peptide' concentrations, for both studied derivatives. Biodistribution differences between these two analogues, also in relation to GGC-Aca-BN[2-14]-NH2 were observed in normal female Swiss mice. Thus, the radiolabeled bombesin analogue without the spacer presented similar uptake in pancreas compared to GGC-Aca-BN[2-14]-NH2, and its main excretion was through the urinary system, unlike the GGC-Aca-BN[2-14]-NH2 which is eliminated to some extent also via the hepatobiliary system. Concerning the new derivative GGC-Ornithine-Ornithine-Ornithine-BN[2-14]-NH2 its uptake in pancreas was significantly greater than the other two analogues and was mainly excreted via the renal system. The elimination of the lipophilic spacer (Aca) seems to affect biokinetics; moreover, its replacement by a hydrophilic spacer (Ornithine-Ornithine-Ornithine) seems to enhance the in vivo uptake and the specificity for GRPrs.

Conclusion: Stabilized bombesin analogues including the above spacer (Ornithine-Ornithine-Ornithine) in their molecule look promising as radiopharmaceuticals for tumor imaging and/or therapy of GRP receptor-positive tumors. Extensive chemical and radiochemical investigation, detailed in vitro investigation as well as further in vivo evaluation in normal rodents and in experimental pathological models are in progress.

P145 DOES THE CYCLIC NITROGEN ATOM CONTRIBUTE TO COORDINATION OF TECHNETIUM-HYNIC COMPLEXES?

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Introduction: Hydrazinonicotinamide (HYNIC) has been established as a useful bifunctional complexing agent for technetium-99m (^{99m}Tc) but the exact chemical nature of the complexes formed remains uncertain. In order to explore the role of the cyclic nitrogen atom we have prepared conjugates of HYNIC and hydrazinobenzoic acid (HYBA) with a model peptide and radiolabelled these with ^{99m}Tc using three well-established co-ligand systems, EDDA, tricine and tricine/nicotinic acid (NA).

Experimental: We compared the physicochemical properties of the complexes produced using a variety of techniques.

Results and Discussion: For each co-ligand system, HPLC analysis of the radiolabelled HYBA conjugates showed the presence of a greater number of species than the HYNIC conjugate. When labelled with Tc-99m**,** HYNIC conjugates form fewer and more stable species than the corresponding HYBA conjugates. LC-MS analysis showed that all complexes contained one hydrazine moiety bound to Tc. LC-MS also suggests that complexes formed with the HYNIC conjugate contain a smaller number of coordinating co-ligand molecules than the HYBA conjugate indicating that HYNIC is able to more effectively satisfy the coordination requirement of technetium perhaps by binding in chelating mode. In studies designed to test the stability of the complexes formed, all of the HYNIC derivatives showed higher stability than the corresponding HYBA derivatives, and protein binding of the HYBA complexes in serum was greater than that of HYNIC (See Table 1).

Table 1. Stability of radioligand in human serum

	Conjugate/Co-ligand System					
	HYNIC Tricine	HYBA Tricine	HYNIC Tricine/NA	HYBA Tricine/NA	HYNIC EDDA	HYBA EDDA
% Peptide Bound						
0 hr	98.4	94.0	99.1	93.8	100.0	100.0
1 hr	92.5	63.2	67.8	71.6	86.8	76.9
3 hr	83.4	53.4	61.6	60.5	76.2	60.8
5 hr	90.2	63.6	55.7	63.1	72.2	53.1
% Protein Binding						
1 hr	17.9	17.5	4.8	22.9	6.5	7.0
3 hr	17.3	30.5	9.0	25.0	13.7	7.1
5 hr	18.5	36.8	7.4	23.3	11.5	12.7

Conclusion: These results indicate that the nitrogen atom of the cyclic ring of HYNIC plays an important role in the complexation of the technetium-99m atom, either by direct co-ordination or by an indirect influence on the hydrazine moiety.

Acknowledgement: This study was financially supported by EPSRC and Cancer Research UK.

Keywords: Technetium, HYNIC

P146 PREPARATION OF ^{64}Cu BIACETYL BIS-(N^4-ALKYLTHIOSEMICARBAZONE) LIGANDS FROM ISOTHIOCYANATES

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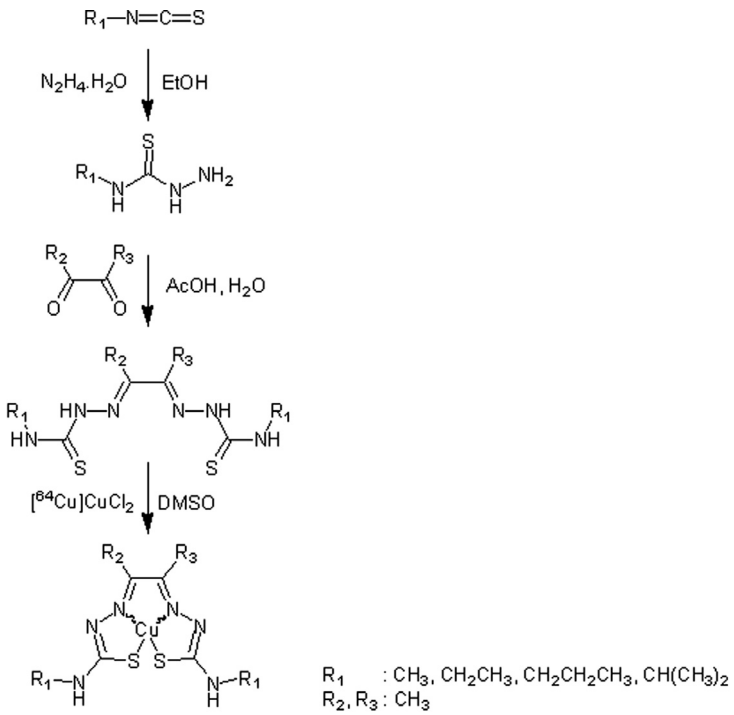
Introduction: Bis-thiosemicarbazone complexes of copper radionuclides (^{61}*Cu*, ^{62}*Cu*, ^{64}*Cu*) derived from vicinal diones are known as hypoxia selective or blood perfusion agents for PET (eg. *Cu*-ATSM, *Cu*-PTSM). We have modified the method of Gingras et al. [1] for the preparation of variously N^4-alkylated ligands, changing the starting material to isothiocyanates.

Experimental: Synthetic steps are shown in scheme 1. Reagents were used as shipped, without further purification (Sigma-Aldrich and Lachema, Czech Republic). All ligands were characterised by ^{1}H, ^{13}C-NMR, MS (APCI$^+$) and IR spectra and by elemental analyses. Ligands were labelled by ^{64}*Cu* afterwards. ^{64}*Cu* have been produced both by the activation of $^{nat.}$*Cu* in LVR-15 reactor (ÚJV Ređ, Czech rep.) and by the $^{nat.}$*Zn(d,x)*^{64}*Cu* reaction on U120M cyclotron (NPI ASCR). [^{64}Cu]$CuCl_2$ was isolated as described previously [2]. Radiochemical yields were determined by thin layer chromatography. TLC plates were measured on Instan-Imager scanner (Packard, USA).

Results and Discussion: The synthesis of biacetyl bis-(N^4-monoalkylated thiosemicarbazone) ligands was performed in two steps. This allows faster preparation of thiosemicarbazone ligands from corresponding isothiocyanates in good overall yield (about 70% isolated yield). Labelling yields were high (>95%) in less than 5 min. for both n.c.a. and reactor made ^{64}*Cu*.



Conclusion: We have developed simplified method for the preparation of bis-thiosemicarbazone ligands for labelling with copper isotopes. Reported method allows the preparation of ligands with wide structural modifications on N^4-nitrogen, starting from commercially available isothiocyanates.

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Acknowledgement: Authors gratefully acknowledge the financial support of the Academy of Sciences of the Czech republic (grant No. IAA400480616).

Keywords: Thiosemicarbazones, 64Cu, Copper Complexes, Isothiocyanates

P147 BISPIDINES AS A NEW CLASS OF CHELATING AGENTS FOR COPPER RADIONUCLIDES

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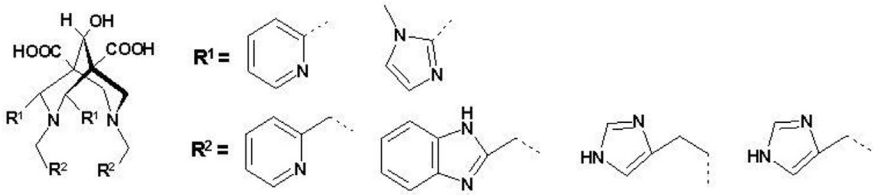
Introduction: The synthesis, characterization and evaluation of novel hexadentate bispidine derivatives containing pyridine and/or imidazole units as donor groups are presented. Bispidine (= 3,7-diazabicyclo[3.3.1]-nonane) ligands show unique complexation behaviour towards transition metals [1,2]. The high thermodynamic stability of the complexes with Cu(II) offers the possibility to apply such complexes for diagnostic (64Cu) and therapeutic (67Cu) purposes [3]. Moreover the bispidine structure opens suitable chemical approaches to connect bio-molecules onto the skeleton, an important feature in view of the targeting of such complexes.

Experimental: The ligands were prepared by two consecutive Mannich condensations according to the known procedure [1]. Cyclic voltammetry (CV) measurements were recorded on a BAS 100B instrument with a standard three-electrode cell at 25°C in degassed water in an Ar atmosphere. Bispidines were labelled with 67Cu using 67CuCl2. To 200 μl of the ligand solution (10-4 M ligand in 0.05 M MES/NaOH buffer, pH = 5.4) 250 kBq of 67CuCl2 were added. 67Cu-labelling yields were studied by TLC using RP18 TLC plates being developed in acetonitrile/water (0.1%TFA) = 4/1.

Results and Discussion: CV measurements were performed in order to estimate the stability of the copper(II) bispidine complexes. Strongly negative redox potentials were found for all compounds investigated indicating the high stability of the Cu(II) complexes [2]. Labelling experiments of the new bispidines with 67Cu and 64Cu indicate a rapid formation of radiocopper complexes under mild conditions in almost quantitatively yields.



Conclusion: The radiocopper complexes were found to be stable in the presence of a high excess of competing ligands, and showed a high in vitro stability in rat plasma up to 24 h. Studies on the bioconjugation of the bispidine 64Cu complexes are now in progress.

Acknowledgement: Roger Schibli (Paul Scherrer Institute, Villigen, Switzerland) is gratefully acknowledged for providing copper-67.

References: [1] P. Comba, W. Schiek, Coord. Chem. Rev. 2003, 238-239, 21-29. [2] C. Bleiholder et al. Inorg. Chem. 2005, 44, 8145-8155. [3] M.J. Welch, C.S. Redvanly, Eds., Handbook of Radiopharmaceuticals: Radiochemistry and Applications, J. Wiley & Sons, Chichester, 2003.

Keywords: Bispidine Ligands, Copper, In Vitro Stability, Cyclic Voltammetry

P148 THE STABILITY *IN VITRO* AND RADIOLABELING OF ANTISENSE OLIGODEXYNUCLEOTIDE WITH ^{188}Re

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Introduction: Antisense oligodeoxynucleotides(ASONs) are potential clinical therapeutic agents for malignant tumors because they may regulate the expression of activated oncogenes in tumor cells via specific mRNA base pairing and degradation. ASONs conjugated to a suitable bifunctional chelating agent and radiolabelled with a β-emitting radionuclide could become useful in the therapy of cancers. ^{188}Re (β; Emax, 2.12Mev; γ Photon, 155keV; 15%) is a very attractive radioisotope for radioimmunotherapy (RIT). The high-energy b-particals emitted by ^{188}Re have a relatively longer average path length (about 2.2 mm in tissues), with 17h half-life.

A 18-mer phosphorothioate ASON, sequence complementary PDGFG-βmRNA in rats, (5' TAT CAC TCC TGG AAG CCC 3', MW:7399), with a primary amine on the 5' end, was purchased from Shanghai Biological Engineering Limited Company.

Experimental: S-Bz-MAG_2-TFP ester was obtained by using S-benzoyl mercaptoacetyldiglycine (S-Bz-MAG_2) and 2,3,5,6-Tetrafluorophenol (TFP), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) at pH 6, isolated by Sep-Pak C18 column. Then the S-Bz-MAG_2-TFP ester was conjugated with ASON at pH 9.

After purification by Sep-Pak C18 column, the conjugate was dissolved in 0.5mL 0.1mol·L-1 tartrate buffer, containing 4 mg $SnCl_2 \cdot 2H_2O$, 4 mg ascorbic acid, and 0.5mL 37–370 MBq/mL ^{188}Re solution. The mixture was incubated in a boiling water bath, for 20 min. The labeling yield was determined using ITLC.

Determination: The radiolabelling yield and radiochemical purity of labeled ASONs were determinated by two-strip silica gel paper chromatography (PC), The PC strip was developed with methylethylketone for the detection of ^{188}Re-MAG_2-ASONs (Rf=0) and hydrolyzed rhenium ($^{188}ReO_2$) (Rf=0), while PC developed with normal saline was used for detection of hydrolyzed rhenium ($^{188}ReO_2$) (Rf=0). Stability of ^{188}Re-MAG_2-ASONs: 0.3 mL saline or 0.3mL fetal calf serum was added to the dried ^{188}Re-MAG_2-ASONs at room temperature, respectively. The radiochemical purity was determined 4h after purification.

Results and Discussion: The radiolabelling yield of S-Bz-MAG_2-TFP was more than 83%. The radiochemical purity of ^{188}Re-MAG_2-ASONs was 92% in saline, 83% in fetal calf serum respectively.

Conclusion: Optimum labeling conditions for ^{188}Re-MAG_2-ASONs were obtained, resulting in high radiolabelling yield and good in vitro stability.

Keywords: Antisense Oligonucleotide (ASON), 188Re, ASON Radiolabelling, 188Re ASON Stability

P149 SYNTHESIS OF TECHNETIUM(I)-99m PENTACARBONYL COMPLEX

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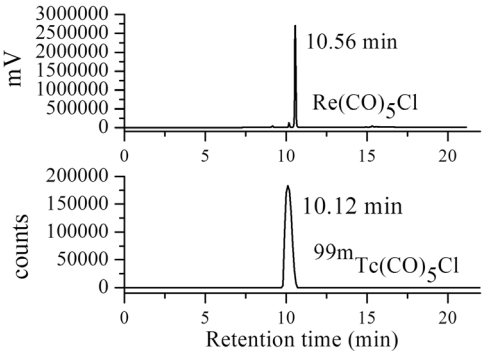
Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, China

Introduction: Pentacarbonyl halides of technetium, $[^{99}Tc(CO)_5]X$ (X= Cl, Br, I) have been synthesized by the reaction of the halogens with ditechnetium decacarbonyl [1]. Unfortunately, the synthesis of starting material $Tc_2(CO)_{10}$ required high temperatures, long reaction times and high pressures. Rhenium (I) pentacarbonyl halides have been prepared directly from $[ReO_4]^-$ also at elevated temperature and pressure[2]. Safety reasons are often a hindrance in the preparation of Pentacarbonyl halides. We present herein the preparation and characterization of $[^{99m}Tc(CO)_5]^+$ directly from $[^{99m}TcO_4]^-$ under mild conditions.

Experimental: Sodium [^{99m}Tc]-pertechnetate was dried in an oil bath to remove water. After cooling down to room temperature, formic acid and conc. H_2SO_4 were added. The mixture was heated at 150~160°C in a sealed vial for 30 min. The vial was opened carefully and the mixture was extracted with CH_2Cl_2.

$$HCOOH \xrightarrow[\Delta]{\text{conc. } H_2SO_4} CO\uparrow + H_2O\,, \quad [^{99m}TcO_4]^- + CO \xrightarrow{150\sim160^\circ C} [^{99m}Tc(CO)_5]^+$$

Characterization of pentacarbonyl complexes was performed on reverse phase HPLC. Mobile phase consisted of H_2O (solvent A) and acetonitrile (solvent B). The HPLC gradient system: 30% B with a linear gradient to 100% B from 0 to 10 min with no change in the composition from 10 to 20 min. The flow rate was 1 mL/min. The sample (5 μL) was injected into the column and the elution was monitored by observing the UV profile at 254 nm for rhenium compound and radio-trace for technetium-99m compound.



Results and Discussion: The retention times of technetium-99m and rhenium compounds were 10.12 and 10.56 min, respectively. Based on HPLC analysis, we presumed the technetium-99m complex should be pentacarbonyl chloride by comparison of the retention time of corresponding rhenium complex. Furthermore, $[Tc(CO)_5]^+$ could be converted to $[^{99m}Tc(CO)_3(OH_2)_3]^+$ in refluxing water. The mechanism of carbonylation reaction and its future application as a precursor will be studied.

Acknowledgement: This work was supported by the National Natural Science Foundation of China (NSFC 20471011).

References: [1] Hileman JC, et al. *Inorg. Chem.* 1, 25 (1962). [2] Shtemenko AV, et al. *Ukrainskii Khimicheskii Zhurnal.* 61, 11 (1995).

Keywords: Technetium-99m, Rhenium, Pentacarbonyl

P150 NOVEL ^{99m}Tc-AGENTS FOR CELL LABELLING: BIODISTRIBUTION IN NORMAL MICE AND PRELIMINARY *IN VITRO* CELL LABELLING

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Introduction: A simple and efficient method for stem cell labeling was described using a lipophilic long chain ester, hexadecyl-4-[^{18}F]fluorobenzoate (Ma et al., Nucl Med Biol, 2005;32:701-705). Labelling was hypothesized to result from absorption into the cell membrane by virtue of the lipophilic tail. However, because of the short half-life of fluorine-18 these cells can not be tracked for a long period and labeling with ^{99m}Tc would be an advantage for longer cell tracking.

Experimental: Three novel ^{99m}Tc(CO)$_3$ compounds (anionic (A), neutral (B) and cationic (C)) also containing a lipophilic hexadecyl tail were synthesized. We studied their biodistribution in normal mice and performed a preliminary evaluation of their usefulness for in vitro labelling of mixed blood cells. ^{99m}Tc-d,l-HMPAO (D) and [^{18}F]FDG (E) were used as a reference. Labelling efficiency was measured after incubation of mixed cells for 15 min with the respective tracers in saline and retention of the radionuclide after incubation of labelled cells for 1 h in plasma was measured.

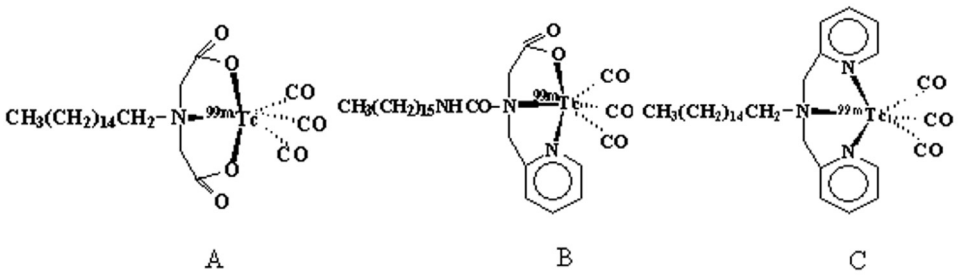

Fig. 1. Proposed structures of the three ^{99m}Tc-(CO)$_3$ complexes.

Results and Discussion: Radiolabelling yields of complexes A, B and C were 99%, 89% and 99%, respectively. After IV injection in mice, the neutral B showed predominant liver clearance while anionic A and cationic C showed in addition some renal clearance. Cell labelling efficiency and retention of radiolabel in cells are shown in the table.

Table 1. Labelling efficiency and retention of tracers

	A	B	C	D	E
Cell labelling efficiency (after 15 min in saline)	57.5	70.5	65.0	69.4	40.8
Retention of radiolabel in cells after 1 h in plasma	40.8	39.7	78.7	88.8	76.9

Conclusion: Cell labelling efficiency ranged from 40 to 70%. Of the three new ^{99m}Tc-complexes, the positive complex C (log P 1.25) showed the most efficient retention (80%) in blood cells whereas cells labelled via B (log P 2.57) or A (log P 2.15) lost about 60% of the radionuclide to the plasma in 1 h. Further evaluation is ongoing.

Acknowledgement: This study was funded in part by the EC - FP6-project DiMI, LSHB-CT-2005-512146.

Keywords: 99mTc-Tricarbonyl, Cell Labelling, SPECT

P151 AN INTEGRATED DESIGN FOR TECHNETIUM-99m RADIOPHARMACEUTICALS USING A PYRIDYL {NNS} CHELATION CORE

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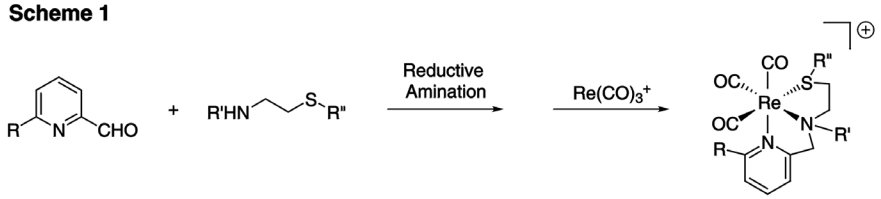
Introduction: Conventionally, the development of receptor binding Tc(I) radiopharmaceuticals involves a pendant motif. This design utilizes a bifunctional chelator that both coordinates to the metal centre and covalently binds to a biomolecule of interest, placing the metal complex at a location removed from the bioactive site. In contrast to this traditional approach we have developed an integrated peptide-based radiopharmaceutical design, which incorporates the $Tc(CO)_3$ complex directly into the peptide backbone making the labeling element a critical component for receptor binding.

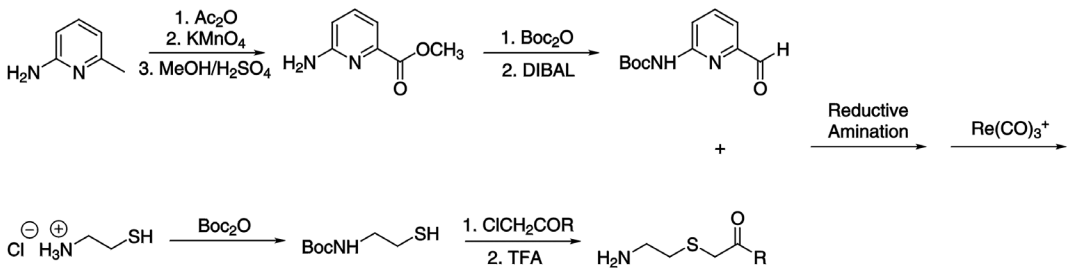
Experimental: The synthesis of a model {NNS} chelator began with commercially available 2-pyridinecarboxaldehyde and cysteamine, giving the desired Re complex in 5 steps. A functionalized chelation system was then synthesized starting from commercially available 2-amino-6-methylpyridine and cysteamine via a convergent route giving the desired {NNS} chelate in 12 steps. All products were fully characterized by NMR spectroscopy and mass spectrometry.

Results and Discussion: A model system was constructed to confirm the suitability of a pyridyl {NNS} donor set as a chelation core for Re/Tc tricarbonyl complexes (Scheme 1). 2-Pyridinecarboxaldehyde and a substituted cysteamine underwent reductive amination followed by reaction with $[NEt_4]_2[Re(Br)_3(CO)_3]$ to produce the model Re complex. A second synthetic route utilized a disubstituted pyridine to enable further functionalization and peptide incorporation (Scheme 2). For the N-terminus component, 2-amino-6-methylpyridine was acetylated followed by oxidation to the carboxylic acid. Esterification and amide cleavage under acidic conditions produced a methyl ester, which was Boc-protected and then reduced to the aldehyde. For the C-terminus, cysteamine was Boc-protected followed by a nucleophilic substitution reaction with 2-chloroacetamide ($R=NH_2$) and subsequent Boc-deprotection. A reductive amination of the resultant substituted cysteamine and Boc-protected aldehyde followed by metal coordination generated the desired Re metal complex.

Scheme 1



Scheme 2



Conclusion: An {NNS} chelation system was designed and synthesized enabling the Re/Tc tricarbonyl core to be incorporated into the backbone of peptide structures.

Acknowledgement: Lawson Health Research Institute; CIHR – Strategic Training Program.

Keywords: Tc(I)/Re(I), Radiopharmaceuticals, Peptides, Tricarbonyl Complexes, Integrated Design

P152 PENDANT ^{99m}Tc(I) TRICARBONYL TRIDENTATE COMPLEXES OF CARBOHYDRATES AS POTENTIAL SPECT IMAGING AGENTS

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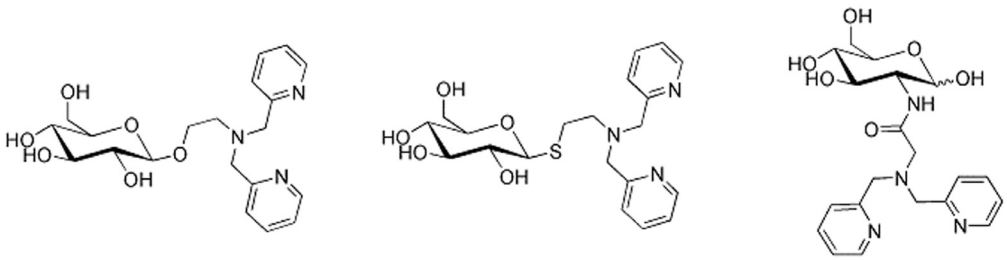
Introduction: In our program to prepare radiometal carbohydrate conjugates for imaging and therapy we report here the synthesis and evaluation in tumour bearing mice of three Tc(I) 2,2'-Dipicolylamine (DPA) complexes with ^{99m}Tc. The DPA derivatives are linked to the glucose either through the glucosamine nitrogen or through a C1 glycosidic O-ether or S-ether linkage as shown in the structures.

Experimental: ^{99m}Tc was carried out with Isolink kits giving radiochemical yields in excess of 90%. Briefly, a 1 mL solution of Na[$^{99m}TcO_4$] was added to an IsoLink kit and the vial heated to reflux for 20 min. Upon cooling, 1 mL of a 0.1 M HCl solution was added to adjust the pH to 9-10. Labelling was achieved by mixing an aliquot (200 mL) of the $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor with 1 mL of a 0.1 mM solution of one of the ligands and incubating at 75°C for 30 min.

Results and Discussion: Tumour:blood and tumour:muscle ratios were determined for each mouse, the results averaged and the standard deviations calculated. Over time, the activity in both the blood and tumour decreases. For the thio-glucosed complex and glucose complex the activity clears faster from the blood than the tumour tissue, thus the blood:tumour ratio increases, peaking at the last time point measured (2 h) with values of 1.2 and 2.6, respectively. The higher activity in the tumour compared to in the blood at the 2 h time point suggests that the tumour will be observable. For the glucosamine derivative the tumour:blood ratio remains between 0.5 and 0.7 throughout, indicating that the blood and tumour activity are clearing at the same rate. Planar SPECT imaging also confirms these results and the tumor is clearly visible with all three compounds.



Conclusion: Three tridentate glucose-metal conjugates were readily prepared in high chemical and radiochemical yields with ^{99m}Tc. Increasing tumour to blood ratios with time for the glucose and thio-glucose derivavtives suggest that these may be useful in tumour imaging.

Acknowledgement: The authors would like to thank the Natural Sciences and Engineering Council of Canada (NSERC) and TRIUMF for financial support.

Keywords: Technetium, Sugar Metal Complexes, Tc-Tricarbonyl, Tridentate Ligands

P153 MICROPET EVALUETION OF ^{64}Cu-ASPARAGINE IN GLIOBLASTOMA

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Introduction: Our **aim** is to evaluate the metabolic substrate activation of asparagine in glioblastoma. This could open new strategy in diagnosis and therapy of this illness. We didn't find literature data that showed asparagine accumulation in glioblastoma. Otherwise there are some papers that describe Cu64-peptide accumulation in brain tumors.

Experimental: We report the synthesis and applications of copper-64 compound with asparagina$_3$ which can be synthesized at room temperature starting from asparagine in buffer acetate pH 5.5 and $^{64}CuCl_2$.

Decay corrected radiochemical yields of compound based on ^{64}[Cu]Copper were 80% ± 5% (n=12) by using a subsequent purification on Sep-Pak Plus C18.

MicroPET imaging studies in nude mice have evidenced the potentialities of these complexes for tumor imaging and therapy.

We injected cultured cells U-87 MG of glioblastoma in subcutaneous of two groups of 20 mice: first group of nude mice and second group of normal rats. We isolated after two weeks 10 animals for group where the tumors were well visible and in these we executed a micropet analysis after injection of Cu64-asparagine (100-200 microCi).

We used for Copper production a cyclotron of 18 Mev (IBA) with a Nickel solid target. The micropets studies were executed using a Yappet system (ISE) after 5 min, 6 h, and 24 h after injection in tumors regions. After 30 minutes were executed a whole body scan to study the biodistribution of tracer.

Results and Discussion: All tumors showed a rapid uptake and a slow washout of tracer. This is very important to show that glioblastoma needs asparagines for metabolic cells activities. For this reason a therapeutic approach could be possible with asparaginase in those tumors that consume asparagines.

The first thing that we must verify is the crossing of BB barrier of Cu64-asparagina and our first results, in two mice where the U-87 MG cells were injected inside the brain, seems to show a crossing of this tracer inside the brain tumor.

Conclusion: Further study is needed to verify the BB cross barrier and the possibility of new therapeutic development in glioblastoma, however the system asparagines/asparaginase seems to be very promising in glioblastoma and could be studied also in other forms of tumors.

Acknowledgement: The authors thank G. Paparelli for excellent technical assistance; S. Moscatelli for helpful discussions.

Keywords: Copper-64, MicroPET, Asparagina, Glioblastoma, PET

P154 LABELING OF BOMBESIN ANALOGS WITH β-EMITERS FOR TARGETED THERAPY

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Introduction: Bombesin, a 14-amino acid peptide, is the amphibian analog to mammalian Gastrin Releasing Peptide. Since GRP receptors are over expressed on a variety of human tumors like prostate, radiolabeled bombesin analogs have also demonstrated high affinity to a number of these receptors. Aim of the present study was to develop new Bombesin (BN)-like peptides labeled with the radioisotopes ^{90}Y, ^{177}Lu, ^{186}Re and ^{188}Re, with high specific activity, for possible therapeutic application. These radioisotopes have attractive nuclear characteristics such as the β-particle emission useful for targeted therapy and favorable half life. ^{177}Lu and ^{188}Re also emit γ-radiation, suitable for simultaneous external imaging. The bombesin derivatives under study are: GGC-*Aca*-QRLGNQWAVGHLM-$CONH_2$ (BN1.1) and DOTA-*Aca*-QRLGNQWAVGHLM-$CONH_2$ (DOTA-BN) where *Aca* is 6-amino-hexanoic acid. BN1.1 labeled with Tc-99m has been already extensively investigated, was also labeled with ^{186}Re and ^{188}Re, through a Re-gluconate complex; DOTA-BN was labeled with ^{90}Y and ^{177}Lu, the chelating agent DOTA has been proven suitable for labeling with M^{+3} radiometals.

Experimental: Carrier-free ^{188}Re and ^{90}Y were used, the first was produced from a ^{188}W/^{188}Re generator, and the second was obtained by solid phase extraction from ^{90}Sr. Carrier-added ^{177}Lu and ^{186}Re were obtained by neutron irradiation of enriched targets. We have studied the labeling conditions in relation to a number of parameters which influence the labeling yield such as pH, molar ratio, reducing agents concentration and incubation conditions. The stability of the labeled species was also studied. Radiolabeling yield was checked by HPLC, by ITLC-SG and by Sep Pak separation.

Results and Discussion: The labeling yield obtained for all the labeled derivatives was higher than 95%, resulting to a single product with high radiochemical purity. The radiolabeled species are stable for at least 24 hours post labeling.

Conclusion: Previous studies of the bombesin analog BN1.1 demonstrated a selective targeting behavior in biological models when labeled with ^{99m}Tc. We extended this study using a similar bombesin analog and the radioisotopes ^{186}Re, ^{188}Re, ^{90}Y and ^{177}Lu, in order that the labeled products can be applied in therapy. The radiolabeling process for all derivatives has yielded stable radiolabeled biomolecules of high radiochemical purity. *In vitro* studies and further radiobiological evaluation in normal and pathological models are under way.

Acknowledgement: This work has been partly supported by the research grant No. 2 P05A 02428 of the Polish Ministry of Science and Higher Education.

Keywords: Gastrin Realising Peptide, Bombesin, Rhenium 186/188, Yttrium-90, Lutetium-177

P155 AUTOMATED MICROWAVE SUPPORTED SYNTHESIS OF ^{68}Ga-LABELLED PEPTIDES FOR TUMOR IMAGING

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Introduction: At the German Cancer Research Center (DKFZ), the synthesis of Ga-68 labelled radiotracers such as DOTA-TOC and the bombesin analogue BZH3 (DOTA-Bom) is based on the use of a Ge-68/Ga-68 generator system already developed in 1981. Commercially available is a generator type for elution with 0.1M HCl. The aim of this work was the construction of a fully automated synthesis module for the rapid and convenient preparation of the radiotracers useable with both generator types. The main focus herein was to reduce the radiation load of the operator and to obtain reproducible yields. The implementation of a laboratory microwave for the labelling step - as recommended by I. Velikyan et al. - should shorten the synthesis time and enhance the chemical and radiochemical purity.

Experimental: The synthesis module was assembled from parts of a kit developed by Scintomics (Fürstenfeldbruck, Germany) and a laboratory microwave (CEM, Kamp-Lintfort, Germany). The Ge-68/Ga-68 generator for elution with 0.1M HCl was purchased from Cyclotron Co., Ltd (Obninsk, Russia).

The Obninsk generator is eluted with 0.1M HCl and by means of a gradient mixer the eluate was concentrated to 5-6M HCl using 30% HCl as the second solvent. The Heidelberg generator is directly eluted with 5.5M HCl. The [Ga-68]$GaCl_4^-$ is in both cases trapped on an anion exchange resin (AG-1X8, BioRad, München, Germany). 95±3% of the Ga-68 activity could be trapped on the anion exchange resin. After drying the resin in a continuous helium flow, 84±3% of the activity was eluted with 200±14μL water into the reaction vessel containing the precursor and buffer. The optimal pH range of 4.1-4.8 was maintained using Hepes buffer and small amounts of NaOH. Possible ionic impurities such as [Ga-68]Ga^{3+} were removed with a SepPak C-18 or C-8 cartridge (Waters, Eschborn, Germany). The radiolabelled peptide was eluted from the cartridge with 1.0 mL ethanol. After ethanol evaporation the product was transferred into isotonic saline and sterile filtered.

Results and Discussion: This module setup allows the labelling of several DOTA peptide derivatives in high yields and purity. In the case of Ga-68-DOTA-TOC, for example, radiochemical yields of 98.1±0.7% were obtained after 2 minutes at 95°C. The radiotracers were produced in high specific activities and good yields with or without a further purification step after synthesis times of 8 or 19 min, respectively.

Conclusion: The module setup allows the convenient and fast automated labelling of DOTA-derivatized peptides in high specific activities with Ga-68 for PET.

Keywords: Gallium-68, Microwave, Automatization, PET

P156 A NEW CONVENIENT STRATEGY FOR THE SYNTHESIS OF HIGH AFFINITY RECEPTOR MULTIMERS FOR NON INVASIVE DIAGNOSTICS AND ENDORADIOTHERAPY

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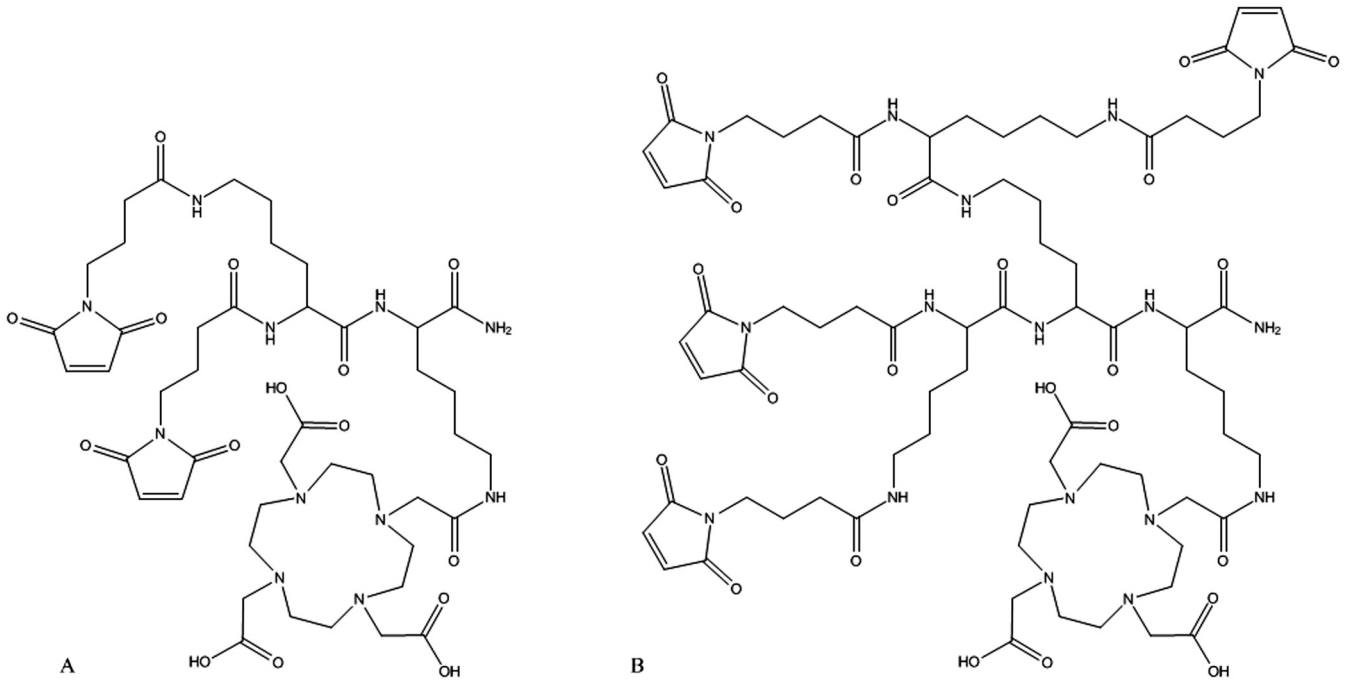
Introduction: Receptor ligand multimers derivatized with radionuclide chealting macrocycles are of increasing interest for high affinity receptor ligands applicable in oncologic diagnostics and endoradiotherapy. We report the synthesis of a dendritic polylysine crosslinker containing 2 and 4 maleimido functions respectively which was derivatized with DOTA for the complexation of various radiometals. The crosslinkers were coupled to different peptides such as a cyclic RGD-containing peptide, Tyr^3-Octreotate and Bombesin derivatized N-terminal with SATA.

Experimental: The peptides and the crosslinkers (Fig. 1) were synthesized following a standard Fmoc peptide synthesis protocol using 4eq. of the (amino)acids and HBTU (3.9eq.) for activation. The synthesis of the peptides was followed by their di- and tetramerization. These multimerizations were carried out by dissolving 2 equivalents per maleiimido function of the respective peptide in phosphate buffer (0.1M, pH 7.2, 30mg/mL) (in some cases DMSO had to be added to achieve a solution). This solution was then added to the crosslinker dissolved in phosphate buffer (0.1M, pH 7.2, 20mg/mL). To this mixture 100 μL of a 2% hydroxylamine solution in water were added to remove the acetyl protecting group and to allow the reaction of the free thiols with the maleiimido functions of the dendritic structure. The reaction is finished instantaneously and the purification was carried out by HPLC. The yields of the coupling reactions were between 73 and 96%.

The dimers and tetramers were dissolved in water (1nmol/μL). The labelling with gallium-68 was carried out using an automated synthesis module. Therefore, 10nmol of the multimer, 210μL Gallium-68 in 5.5M HCl, 10μL sodium hydroxide solution (30%) and 65mg HEPES were added to adjust the pH to 4.2. The mixture was then reacted in a microwave oven for 3 minutes and purified via a C-18 cartridge (Waters). After evaporation of the ethanol, dilution and sterile filtration the radiochemical yields were 62-75% after 20 minutes.



Conclusion: The new strategy allows the rapid and convenient synthesis of multimers suitable for labelling with radiometals for both PET-diagnosis and endoradiotherapy.

Keywords: Multimer, Endoradiotherapy, PET, Peptide

P157 GALLIUM-68 COMPLEXES WITH TRIPODAL NS_3 LIGANDS: IN VITRO AND IN VIVO STABILITY STUDIES

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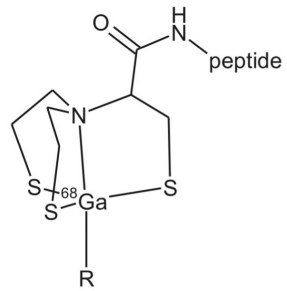
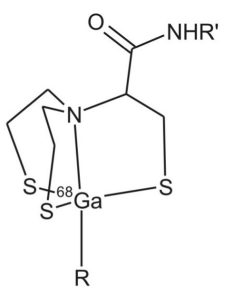
Introduction: We report on formation and structures of ^{68}Ga "4+1" complexes, [Ga(NS_3)R], with the tripodal ligand tris(2-mercaptoethyl) amine (NS_3) and water or secondary amines as co-ligands (R). To improve the bio-behavior of such compounds new hydrophilic NS_3 chelators have been introduced. Challenge experiments with transferrin have been performed to study the influence of the co-ligand on the in vitro stability of the gallium complexes. Their in vivo stability was evaluated in the rat using HSA microspheres.

Experimental: ^{68}Ga was eluted from a $^{68}Ge/^{68}Ga$ generator (Obninsk, Russia) as $GaCl_3$ in hydrochloric solution (0.1 M) and added to the aqueous solution of the oxalate salt of the NS_3 ligand in presence of the corresponding amine. Ga complexes were formed in aqueous solution at ambient temperature. Challenge experiments were carried out at 37°C with the plasma protein transferrin (5 mg/ml), the exchange was controlled by thin layer chromatography on silicagel (Merck) and methanol as eluent.

Results and Discussion: The labelling procedure with ^{68}Ga generator eluate runs in high yield under weakly alkaline conditions within some minutes. It is not necessary to heat the reaction mixture so that sensitive molecules can also be labelled. Challenge experiments with apotransferrin were carried out for several hours. Amine-coordinated complexes as well as non-amine compounds showed high in vitro stability within 240 minutes. The in vivo stability of the complexes was evaluated in the rat using HSA microspheres. ^{68}Ga-labelled HSA microspheres were completely accumulated in the lung. In small animal PET studies no significant loss of ^{68}Ga activity was observed within three hours.



R = H_2O, imidazole-bearing modifier (e.g. PEG)



R = imidazole-bearing peptide
R' = modifier (e.g. PEG)

Conclusion: The '4+1' mixed-ligand approach enables the ^{68}Ga-labelling of biomolecules under physiological conditions. The lipophilicity of the complexes can be controlled by introducing pharmacological modifiers (e.g. PEG, glucosamine, crown ether) into the tetradentate ligand and/or derivatization of the monodentate ligand. Moreover, the described approach allows the functionalization of a biomolecule with the tripodal as well as with the monodentate ligand.

Keywords: Gallium Complexes, Tripodal Ligand, In Vitro Stability, In Vivo Stability, Tris(2-Mercaptoethyl) Amine

P158 PREPARATION OF A NOVEL ($^{99m}Tc(CO)_3$)-LABELED 2-NITROIMIDAZOLE DERIVATIVE AS A POTENTIAL MARKER OF TUMOR HYPOXIA

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Introduction: Hypoxia plays a major contributing role toward the resistance of tumors to both radiation and chemotherapy. Hence detection and quantification of hypoxia is an important issue of considerable interest and is being actively pursued. The inherent drawbacks in the available invasive techniques are major impediments which can be circumvented by using radiopharmaceuticals in addressing the problem in a non invasive way. The bioreductive breakdown of 2-nitroimidazoles provides a way for the in vivo labeling of hypoxic cells. In the present work, 2-nitroimidazole chemically conjugated with a bifunctional chelator viz, a diethylenetriamino derivative, has been chosen as the vector to target tumour hypoxia. The resultant nitroimidazole-diethylenetriamine derivative has been labeled with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core.

Experimental: Diethylenetriamine was converted into the corresponding diphthalimido derivative on reaction with phthalic anhydride in acetonitrile. Subsequent N-alkylation of diphthalimido protected diethylenetriamine derivative with 1,3-dibromopropane in presence of Hugin's Base resulted in compound **1** (Fig. 1). The second step involved the N-alkylation of the NH- of 2-nitroimidazole with **1** and deprotection of the two primary amine groups in the final step to yield the target ligand (**2**) (Fig. 2).

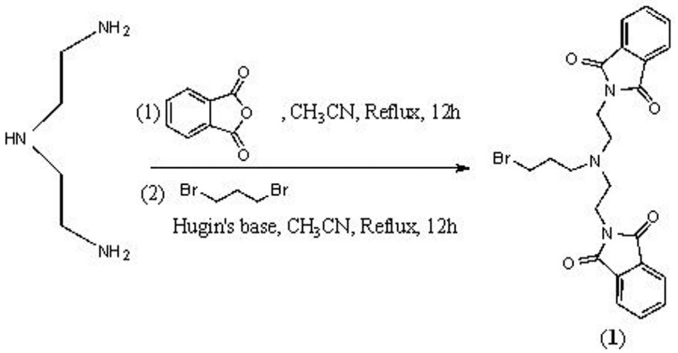

Fig. 1

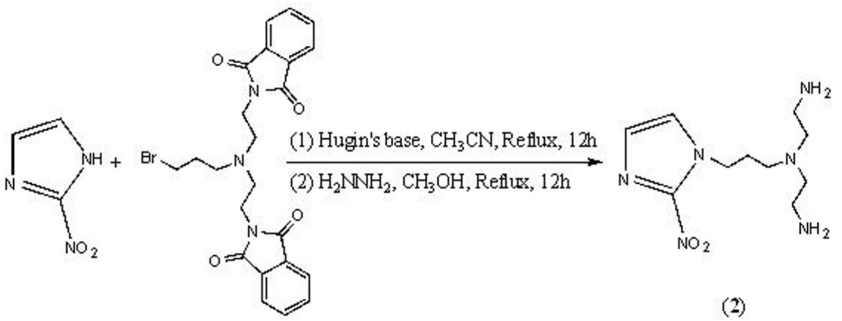

Fig. 2

Results and Discussion: The ligand could be synthesized in an overall yield of 70% and was characterized by ^{1}H-NMR, IR and mass spectral analysis. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core was prepared in situ following a reported procedure. The 2-nitroimidazole complex was prepared by adding 0.1 mg of the ligand in 0.5 mL of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core and heating the mixture at 80° C for 30 min. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core and the prepared complex was characterized by HPLC.

Conclusion: $^{99m}Tc(CO)_3$-labeled -2-nitroimidazole complex was synthesized in over 95% complexation yield. Bioevaluation of the 2-nitroimidazole complex will be carried out in suitable animal model.

Acknowledgement: The authors gratefully acknowledge the encouragement and support provided by Dr. V. Venugopal, Director, Radiochemistry and Isotope Group, BARC.

Keywords: 2-Nitroimidazole, 99mTc-Tricarbonyl, Hypoxia, Tumor

P159 SYNTHESIS AND EVALUATION OF $^{99m}Tc(CO)_3$-(*N,N*-DIACETYLOXYAMINO)PENTADECANOIC ACID

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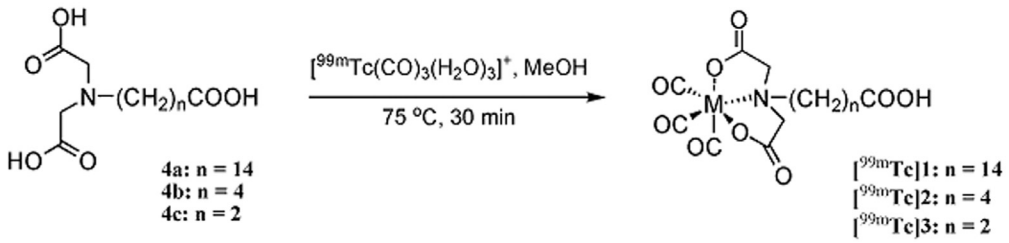
Introduction: Long chain fatty acid is known to be the main energy source for the myocardium. ^{99m}Tc-labeled fatty acid analogs are attractive, due to ready availability and ideal physical properties of ^{99m}Tc (141 keV, $t_{1/2}$=6 h). Although many efforts were made to develop ^{99m}Tc-labeld long chain fatty acid analogs, these radiotracers showed poor myocardial uptake, which may have been due in part to the relatively large size of the ^{99m}Tc cores. With a strategy for development of $^{99m}Tc(CO)_3$-labeled long chain fatty acid analogs, we chose an amino-*N,N*-diacetic acid system that can be successfully coordinated to ω-position of fatty acid. In this study, we synthesized a tridentate $^{99m}Tc(CO)_3$-(*N,N*-diacetyloxyamino)pentadecanoic acid ([^{99m}Tc]**1**) as a novel radiotracer for evaluation of fatty acid metabolism.

Experimental: The precursor **4a**, ω-(*bis*-carboxymethyl-amino)pentadecanoic acid, was prepared in high yield in 5 steps from ω-pentadecalactone. $Re(CO)_3$-(*N,N*-diacetyloxyamino)pentadecanoic acid ([Re]**1**), the cold standard, was synthesized by reacting the precursor **4a** with $(NEt_4)_2[Re(CO)_3Br_3]$ in methanol at 75°C for 2 h. [^{99m}Tc]**1** was prepared as the synthesis of [Re]**1** using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ at 75°C for 30 min, and purified using a C18 Sep-Pak cartridge.



Results and Discussion: Radiochemical yield of [^{99m}Tc]**1** was 80-82%, and its radiochemical purity measured by HPLC was higher than 98%. In vitro stability of [^{99m}Tc]**1** was measured in human serum at 37°C, and the TLC results showed that [^{99m}Tc]**1** was stable over 3 h (>90%). Tissue distribution studies were carried out in mice, and the maximum heart to blood uptake ratio was 1.3 at 5 min after a tail-vein injection. Radioactive metabolites in urine samples of mice at 30 min postinjection were analyzed by HPLC and corresponded to a 1:3 ratio of $^{99m}Tc(CO)_3$-5-(*N,N*-diacetyloxyamino) pentanoic acid ([^{99m}Tc]**2**) to $^{99m}Tc(CO)_3$-3-(*N,N*-diacetyloxyamino))propionic acid ([^{99m}Tc]**3**), indicating that [^{99m}Tc]**1** was mainly metabolized to [^{99m}Tc]**3** via β-oxidation.

Conclusion: These results suggest that [^{99m}Tc]**1** is a promising radiotracer for evaluation of fatty acid metabolism.

Keywords: 99mTc(CO)3-(N,N-Diacetyloxyamino)Pentadecanoic Acid, Fatty Acid, β-Oxidation

P160 16-CYCLOPENTADIENYL TRICARBONYL ^{99m}Tc-16-OXO-HEXADECANOIC ACID IS METABOLIZED BY β-OXIDATION IN THE MYOCARDIUM

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Introduction: Fatty acids are important substrates for energy metabolism in the myocardium under aerobic conditions. In the myocardium, long chain fatty acids are metabolized by β-oxidation, and disturbances in this metabolism often reflect myocardial damage. In the present study, we prepared 16-cyclopentadienyl tricarbonyl ^{99m}Tc 16-oxo-hexadecanoic acid ([^{99m}Tc]CpTT-16-oxo-HDA, **1**), evaluated its property as a substrate for β-oxidation in the myocardium and compared its metabolite with the proposed radioactive metabolite.

Experimental: Incorporation of ^{99m}Tc into the precursor by a double ligand transfer reaction was carried out in the presence of $Cr(CO)_6$ and $CrCl_3$ at 180°C for 1 h. The resulting methyl ester of **1** was hydrolyzed in 0.3 N NaOH-methanol (1:3) at 70°C for 5 min, which was then purified by HPLC. Re complex was also synthesized for identification of **1**. The proposed metabolite, [^{99m}Tc]CpTT-4-oxo-butyric acid, was synthesized via the same route as the synthesis of **1**. In vitro stability of **1** was determined in human serum at 37°C. In tissue distribution studies, mice were injected with **1** via a tail vein. At 1, 5, 10, and 30 min postinjection, samples of blood, heart, lung, liver, kidney, and stomach were removed, weighed, and counted. For analysis of metabolites, mice were injected with **1** and samples of the heart were collected at 5 and 30 min postinjection, homogenized, and centrifuged. The supernatants were analyzed by HPLC.

Results and Discussion: Overall radiochemical yield of **1** was 25-30% (decay-corrected) and radiochemical purity was higher than 98% as determined by HPLC and TLC. Complex **1** was stable when it was incubated in human serum (>93% for 3 h). In tissue distribution studies, heart uptake of **1** (9.03%ID/g at 1 min and 5.41%ID/g at 5 min post-injection) was higher than other ^{99m}Tc-labeled fatty acid analogs, and its heart to blood uptake ratios were 3.27 at 10 min and 3.76 at 30 min post-injection. When the samples of the heart were analyzed, a major radioactive metabolite was detected on HPLC, which was identified as ^{99m}Tc-CpTT-4-oxo-butyric acid. This result demonstrated that **1** was metabolized to [^{99m}Tc]CpTT-4-oxobutyric acid after six cycles of β-oxidation.

Conclusion: Although direct comparisons in %ID/g of radiotracers cannot be made, due to different experiment conditions and animal species used for the studies, **1** appears superior to other ^{99m}Tc-labeled fatty acid analogs, in terms of high heart uptake, high heart to blood and heart to liver uptake ratios.

Keywords: Cyclopentadienyl Tricarbonyl Tc-99m, Fatty Acid, β-Oxidation, Myocardium

P161 USE OF COORDINATING AMINO ACID SEQUENCES TO DESIGN AN OPTIMAL TECHNETIUM BINDING SITE IN HYNIC-CONJUGATED PEPTIDES

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Introduction: Electrospray mass spectrometry of salmon calcitonin labelled with Tc-99 via HYNIC with tricine as co-ligand showed only one tricine bound to technetium, whereas typically two are observed.[1] We speculated that this was due to participation of a neighbouring histidine in the coordination sphere of technetium. We therefore investigated the role of neighbouring amino acid side chains in coordinating with HYNIC-bound technetium, both to assess the risk that Tc-labelling using HYNIC might alter peptide conformation, and to assess the potential for the design of enhanced Tc-binding sequences by judicious placing of coordinating amino acids in the sequence.

Experimental: A series of tripeptides and tetrapeptides incorporating HYNIC-lysine both with and without a histidine at different positions in the sequence were prepared. The peptides were radiolabelled with Tc-99m and Tc-99 with tricine, tricine/nicotinic acid or ethylenediamine diacetic acid as co-ligand, and the labelled species examined by HPLC, cysteine challenge, bovine serum albumin challenge, and electrospray mass spectrometry.

Results and Discussion: With the exception of histidine located at the N-terminus, histidine located immediately on either side HYNIC-lysine markedly enhanced stability to cysteine challenge and BSA binding and contained only one tricine, or one tricine and one nicotinic acid, or one EDDA. Peptides without histidine or with neighbouring N-terminal histidine were less stable and contained two tricine, or one tricine and two nicotinic acid, or two EDDA ligands. The mass spectra also suggested that glutamate participated in Tc-binding but there was no corresponding enhancement of stability.

Conclusion: Coordination of neighbouring histidine side chains to Tc occurs and could therefore alter peptide or protein conformation on labelling. This should be considered when designing Tc-labelled peptides. Including histidine in a neighbouring position to HYNIC-lysine enhances stability of the labelled peptide and reduces the demand of the metal centre for binding to additional co-ligands. This approach holds promise for the optimal design of HYNIC-containing binding sites for peptide labelling by use of suitable amino acid sequences.

References: [1] Greenland WEP, Howland K, Hardy J, Fogelman I, Blower PJ, *J Med Chem.*, **2003**, 46:1751-1757.

Acknowledgement: This research was funded by the UK EPSRC.

Keywords: Technetium, Hynic, Peptide, Histidine

P162 LABELLING OF $(DOTA)_n$-OCTREOTATE DERIVATIVES WITH ^{68}Ga

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Introduction: Radiometal(III)-labelled peptidic derivatives of somatostatin provide substantial impact in nuclear medicine diagnosis and therapy. Recently, ^{68}Ga has contributed to the molecular imaging of endocrine tumors mainly using DOTA-octreotide derivatives. An important characteristic of positron emitting ^{68}Ga is its availability via the $^{68}Ge/^{68}Ga$ generator system. However, the specific activities achieved both by conventional or microwave-supported heating are limited. Further increase in specific activities might be achieved by introducing $(DOTA)_n$-octreotide derivatives with n=2 or 4. In this study, the labelling yields, kinetics and specific activities of DOTA-octreotide, $(DOTA)_2$-Lys-Sar_5-octreotate, $(DOTA)_4$-apg_3-octreotate and $(DOTA)_4$-apg_3-sar_5-octreotate with ^{68}Ga have been investigated.

Experimental: Peptides were synthetised on solid phase using the Fmoc strategy. Pentasarcosine was used as primary spacer and aminopropylglycine (apg) to introduce di- and tetravalency. DOTA was coupled as the tris (tBu) ester. After full assembly the peptide was cyclised on resin, cleaved and deprotected followed by HPLC-purification.

Pre-concentration and purification of initial $^{68}Ge/^{68}Ge$ generator eluate was performed utilizing a miniaturized column with cation exchanger and HCl/acetone media. Purified fraction was used for labelling of DOTA-octreotate derivatives.

First, various amounts of 3.5, 7.0, 10.5, 14.0 nmol of DOTA-Tyr^3-octreotide (DOTATOC) were used. Next, 3.5 nmol of $(DOTA)_n$ (n=2, 4) peptides, 3.5 nmol each were compared. Labelling was performed at 100°C for up to 15 min using pure water/HCl solution and pH 2.3. Reaction yields were determined using TLC.

Results and Discussion: SPPS gave <10% overall yield of n=2, 4 DOTA derivatives. Reproducible and high yields for ^{68}Ga-labelling of DOTATOC are obtained using 15 or 20 μg after 3-10 min reaction time, for example. At amounts of 5 μg (3.5 nmol), yields are significantly lower even at 15 min reaction time. For $(DOTA)_n$ derivatives at 3.5 nmol, both yields and kinetics of ^{68}Ga labelling improve according to the number of DOTA chelators. Specific activities increase by factors of about 2 (n=2) and 3 (n=4) at 3, 10 and 15 min reaction time, and of about 2-3 (n=2) and 5-6 (n=4) at 1 min, respectively.

Conclusion: Labelling of DOTATOC is defined by its amount and kinetics. At low peptide amounts, ^{68}Ga labelling yields are low due to the amounts of metallic impurities present. This is compensated by increasing number of DOTA chelators per peptide. Labelling yields, kinetics and specific activities are higher if compared to n=1 analogs. Provided that binding affinities and pharmacokinetics of the $(DOTA)_n$-octreotide derivatives (n=2 or 4) are adequate, these derivatives are superior.

Keywords: Ge/Ga-Generator, DOTA-TOC

P163 TRIFLUOROACETYL-HYNIC PROTECTING GROUP FOR HYNIC: APPLICATION IN ^{99m}Tc PEPTIDE RADIOLABELLING

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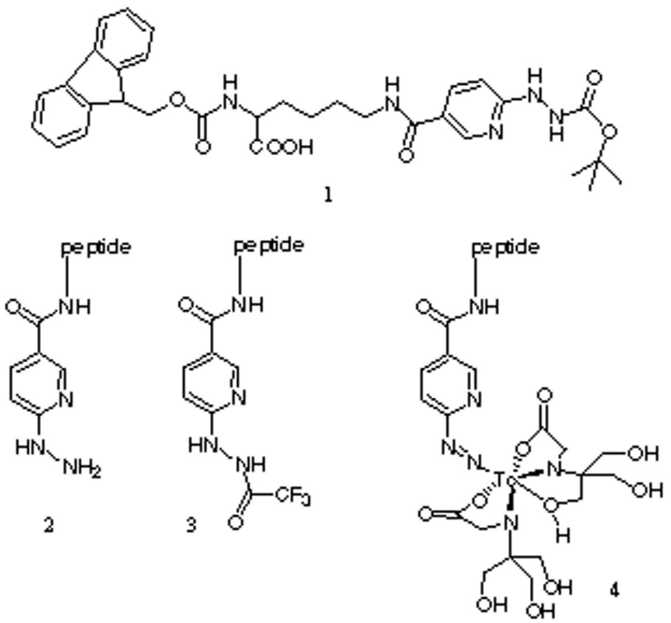
Results and Discussion: Fmoc-lysine-HYNIC-Boc **1** is an amino acid analogue for solid phase synthesis of peptide radiopharmaceuticals for ^{99m}Tc labelling.[1] Using HPLC-ESMS we have shown that during solid phase peptide synthesis, deprotection of nanogastrinHYNIC-peptide and decoupling from the resin with trifluoroacetic acid gives, initially, unprotected HYNIC-peptide **2**, which is converted to trifluoroacetylHYNIC-peptide **3** upon prolonged incubation (>50 h) under decoupling conditions.[2] The trifluoroacetylHYNIC group is hydrolysed under typical ^{99m}Tc labelling conditions to yield peptide **4**, rendering an additional deprotection step prior to peptide radiolabelling unnecessary. Furthermore, the trifluoroacetyl group offers significant protection to the HYNIC functionality in presence of acetaldehyde[3] leading to high efficiency ^{99m}Tc peptide radiolabelling. These findings will enhance the versatility and utility of the HYNIC group in the trifluoroacetylated form in the synthesis of peptide radiopharmaceuticals for ^{99m}Tc labelling.



Keywords: 99mTc, Site-Specific Peptide Radiolabelling, Trifluoroacetyl-HYNIC, Protecting Group

P164 A COMPARISON OF $^{64}Cu^{2+}$ COMPLEXATION BY POLYAZA AND POLYAZACARBOXYLATE MACROCYCLIC AND BISCYCLIC LIGANDS

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Introduction: The use of bi-functional ligands for ^{64}Cu radiolabelling of targeting agents is generally based on its parent ligand forming stable complexes with the Cu^{2+} ion. The thermodynamic stability of these complexes is generally determined at millimolar concentrations of ligand under standard state conditions (i.e. constant ionic strength of I=0.1M at 25.0°C). Labelling with $^{64}Cu^{2+}$ for imaging is conducted at lower concentrations and often in the presence of competing metal ions. This study investigates the formation and dissociation kinetics of $^{64}Cu^{2+}$ complexes of a series of polyaza and polyazacarboxylate macrocyclic and biscyclic ligands (see Figure 1) at ≤ micromolar concentrations over a range of pH and in the presence and absence of competing metal ions.

Experimental: The ligands were purchased from Macrocyclic Inc or synthesized by methods described previously.[1] Equimolar concentration of Cu^{2+} ions doped with ^{64}Cu (10^{-6}-10^{-8}M) and each ligand were reacted in buffer (pH 3 to 9) at ~25°C.[1] Complexation of Cu^{2+} was monitored by instant thin layer chromatography (ITLC) by sampling at various time intervals. Mobile phases were developed and validated for each ligand system.

Results and Discussion: Figure 2 shows the effect of pH on the complexation of $^{64}Cu^{2+}$ with each ligand at 30 mins. The data demonstrates the optimum pH for Cu^{2+} complexation for each ligand is different. Pre-organisation of nitrogen donor groups in the biscyclic systems provided for comparatively faster complexation of Cu^{2+} over a wider range of pH. $^{64}Cu^{2+}$ complexes formed and exposed to excess (10-1000 fold) of Zn^{2+} and Cu^{2+} ions. No loss of $^{64}Cu^{2+}$ ion from the complexes was evident in presence of Zn^{2+}. In contrast in the presence of excess Cu^{2+}, there was evidence of $^{64}Cu^{2+}$ exchange after 5 hours at room temperature for many of the systems.

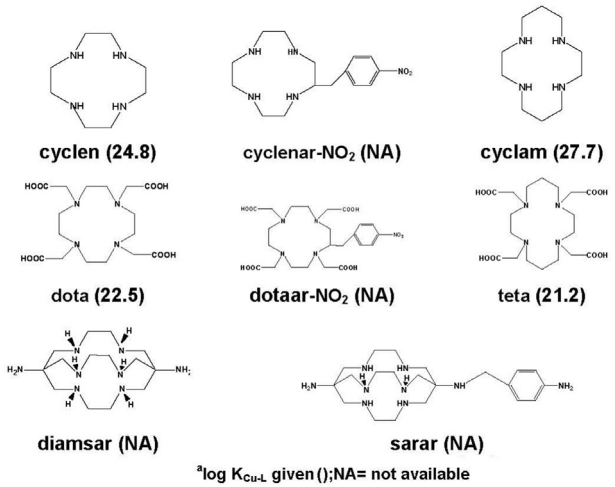

Fig. 1. Polyaza and polyazacarboxylate macrocyclic and biscyclic ligands.

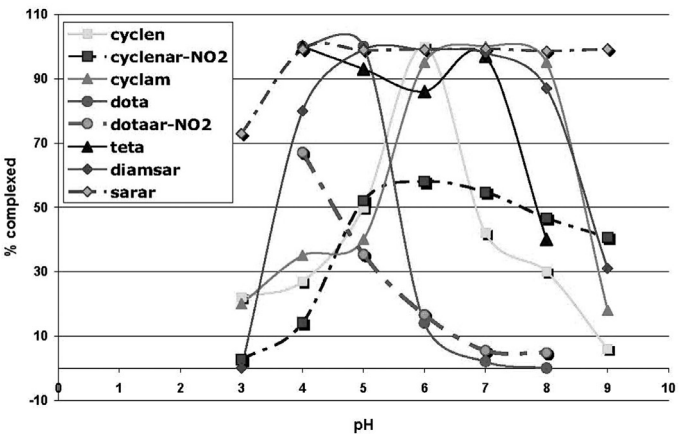

Fig. 2. $^{64}Cu^{2+}$ complexation by polyaza and carboxylate ligands at varying pH at 30 min.

Conclusion: This data provides some explanation for the varied performance of ligands used for $^{64}Cu^{2+}$ labelling reported in the literature. It provides additional knowledge required for ligand design and their application in the development of novel ^{64}Cu imaging agents for the future.

Reference: [1] Di Bartolo, Sargeson, Donlevy and Smith, *J. Chem. Soc.*, *Dalton Trans.*, 2001, 2303–2309.

Keywords: Cu-64, Radiolabelling, Complexation, Polyazaligands, PET

P165 *IN VIVO* EVALUATION OF COPPER-64-LABELED CROSS-BRIDGED TETRAAZAMACROCYCLIC COMPLEXES WITH AMIDE AND/OR CARBOXYLATE FUNCTIONAL GROUPS

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Introduction: The use of ^{64}Cu-labeled small molecule complexes conjugated to proteins and peptides has become increasingly common over the past two decades. The "cross-bridged" cyclam complex ^{64}Cu(II)-4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (^{64}Cu-CB-TE2A) has demonstrated improved *in vivo* stability compared to ^{64}Cu-TETA, even when CB-TE2A is conjugated to a peptide. This enhanced stability has led to the synthesis and *in vivo* evaluation of several new CB-TE2A analogues with monoamide, diamide, and/or monocarboxylate coordinating pendant arms.

Experimental: All ^{64}Cu complexes were prepared according to literature procedures, and biodistribution studies were conducted using normal rats. Animals were sacrificed at selected time points post-injection (p.i.), organs of interest were removed, weighed, and counted. The %ID/g and %ID/organ were determined.

Results and Discussion: This study was conducted to assess the *in vivo* stability of the ^{64}Cu-complexes of a series of CB-TE2A analogues with varied coordinating pendant arms: CB-TEAMA (one carboxylate and one amide), CB-TE1A (one carboxylate), CB-TE1AM (one amide) and CB-TE2AM (two amides). Table 1 describes the clearance properties of each ^{64}Cu complex from the blood, liver and kidney at 24 hours p.i. All ^{64}Cu complexes are effectively cleared from the blood, but activity remains highest in the liver and the kidney suggesting hepatobiliary and renal excretion are the primary routes of removal. Complexes having only one amide pendant group clear the blood well, but the most optimal clearance properties are in complexs with at least one carboxylate arm.

Table 1. Clearance properties (%ID/g) of cross-bridged ligands at 24 h post-injection

^{64}Cu-Ligand	Blood	Liver	Kidney
CB-TE2A	0.0032±0.0006	0.024±0.0021	0.094±0.011
CB-TEAMA	0.0032±0.0004	0.2226±0.03	0.1633±0.0232
CB-TE1A	0.0018±0.0003	0.537±0.0073	0.0491±0.0027
CB-TE1AM	0.0265±0.0032	1.765±0.1806	1.8142±0.1638
CB-TE2AM	0.0028±0.0005	2.1235±0.1953	8.5943±1.4579

Conclusion: The presence of at least one carboxylate arm in cross-bridged cyclam chelators appears vital for the stability of these ^{64}Cu-complexes.

Acknowledgement: This research is supported by NCI grants R01 CA93375 (GRW, EHW, CJA), F32 CA115148 (TJW), R24 CA86307 (^{64}Cu production) and DOD grant W18XWH-04-1-0396 (ALF).

Keywords: Copper-64, Bifunctional Chelator, Cross-Bridged Macrocycles, In Vivo Stability

P166 NEW MACROCYCLIC DIAMIDE LIGAND SYSTEMS: POTENTIAL BIFUNCTIONAL LIGANDS FOR COPPER- AND GALLIUM-BASED PET IMAGING AGENTS

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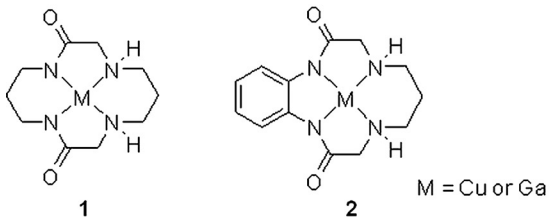
Introduction: A series of macrocyclic tetradentate diamide ligands have been synthesised as potential bifunctional chelators for various metals of radiopharmaceutical interest including Cu(II) and Ga(III). The ligands were synthesised using peptide coupling methodologies and naturally occurring amino acids including lysine and glutamic acid. These amino acid side chains provide amine and carboxylate functional groups for subsequent linkage to biologically active molecules. The Cu(II) and Ga(III) complexes have been synthesised where the amide nitrogen atoms of the ligand are deprotonated and coordinated.

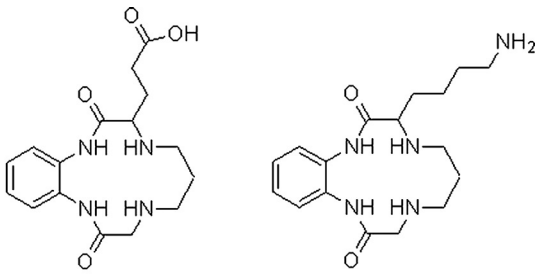
Experimental: We are interested in the development of a range of polydentate ligand systems for metals such as Cu(II) and Ga(III) which, when bound to biologically active molecules (BAM's), may be used as targeted PET imaging agents. One of our major research focuses has been Cu(II) complexes of *bis*(thiosemicarbazone) ligands for imaging hypoxic tissues and via appropriate conjugation as targeted imaging agents.[1-3] We are also interested in the development of macrocyclic bifunctional ligands for radiopharmaceutical applications. Macrocycles often form complexes with high kinetic stability, making them ideal candidates for use as imaging agents targeted to extra-cellular sites.

Results and Discussion: The symmetric diamide ligands **1** and **2** have been synthesised by treatment of the appropriate diamine with two equivalents of 2-chloroacetyl chloride followed by a ring closing reaction with 1,3-diaminopropane. The Cu(II) and Ga(III) complexes of these ligands were synthesised by treatment of a mixture of the ligand and metal chloride with sodium methoxide allowing for deprotonation and binding of the amide nitrogen atoms (Fig. 1).]Further functionalisation of these systems, providing sites for conjugation to BAM's, and allowing modulation of lipophilicity may be readily achieved by the incorporation of appropriate amino acids into the above structural cores using peptide coupling techniques e.g. **3** and **4**. Compounds **3** and **4** were synthesised by the asymmetric coupling of the Cbz protected amino acids glutamic acid (**3**) or lysine (**4**) and glycine to the diamine: *o*-phenylenediamine. The Cbz protecting groups were then cleaved followed by a ring closing reaction using 1,3-dibromopropane (Fig. 2). The incorporation of glutamic acid and lysine provide romote carboxylate and amine functional groups for later conjugation to BAM's.



1 **2**

Fig. 1



3 **4**

Fig. 2

References: [1] Christlieb, M.; Dilworth, J.R., *Chem.-A Eur. J.* **2006**, 12, (24), 6194-6206. [2] Cowley, A.R.; Dilworth, J.R.; Donnelly, P.S.; White, J.M., *Inorg. Chem.* **2006**, 45, (2), 496-498. [3] Cowley, A.R.; Davis, J.; Dilworth, J.R.; Donnelly, P.S.; Dobson, R.; Nightingale, A.; Peach, J.M.; Shore, B.; Kerr, D.; Seymour, L., *Chem. Commun.* **2005**, (7), 845-847.

Keywords: PET, Macrocycle, Copper, Gallium

P167 TRIAZOLE BASED CHELATING SYSTEMS FOR THE Tc/Re-TRICARBONYL CORE

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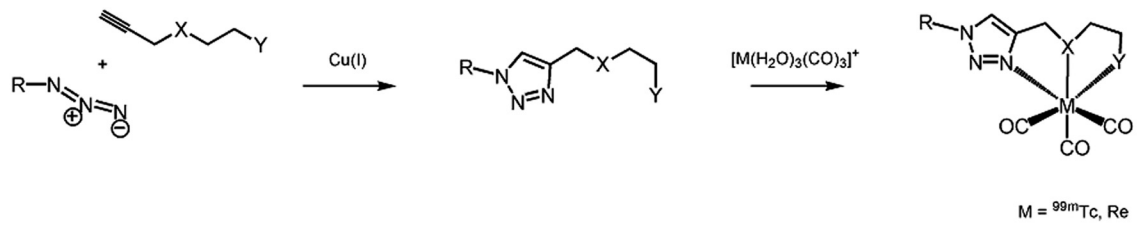
Introduction: A significant synthetic effort is usually necessary to install chelating systems into molecules of radiopharmaceutical interest. We have recently demonstrated that click chemistry (the Cu(I) catalyzed cycloadditon of alkynes and azides) offers an easy way to synthesize histidine-like chelating systems suitable for the $M(CO)_3$ core (M = ^{99m}Tc, Re) [Mindt et al. JACS 2006]. The aim of the current investigation was to expand this approach and synthesize a series of alkynes which when reacted with an azide derivative form novel triazole containing, tridentate chelating systems suitable for labelling with $[M(CO)_3(H_2O)_3]^+$.

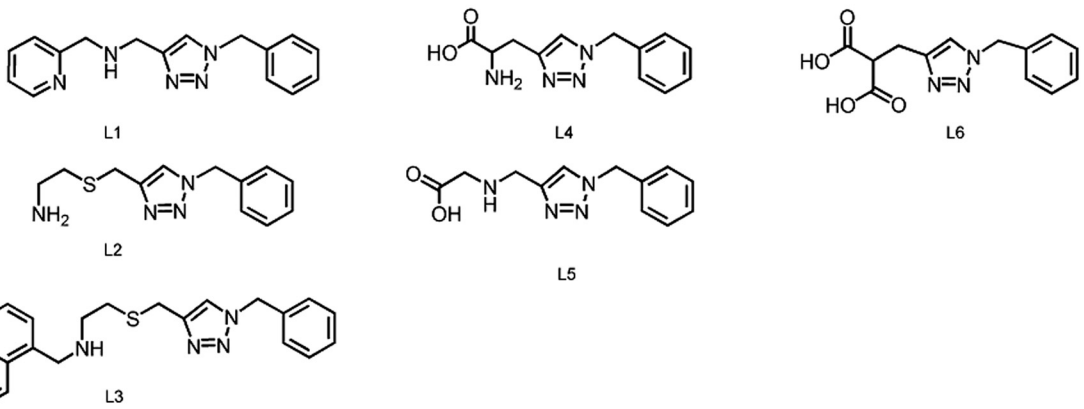
Experimental: The model ligands L1-L6 were synthesized by reaction of the corresponding alkynes with benzyl azide and a catalytic amount of Cu(I) in water/tBuOH (yields > 80%). $Re(CO)_3$ complexes were prepared by heating the ligands with $[Re(CO)_3Br_3][NEt_4]_2$ in MeOH/water mixtures for 1-4 hours. The complexes were characterized by NMR, mass spectrometry, IR spectroscopy and elemental analysis. ^{99m}Tc complexes were characterized by comparison of their gamma HPLC traces with the UV HPLC traces of the analogous Re complexes.

Results and Discussion: The model ligands L1-L6 were synthesized efficiently in a single step. Triazole formation was achieved without the protection of other functional groups, which is likely to simplify the installation of such chelates into future radiotracers. Ligands L1, L2 and L3 gave rise to cationic complexes when reacted with $[M(CO)_3(H_2O)_3]^+$, whereas L4 and L5 formed neutral complexes and L6 a negatively charged complex. L3 incorporates a pendant aromatic ring system with the potential to intercalate into DNA. Spectroscopic analyses of the Re complexes of L1-L6 revealed in all cases a tridentate coordination to the metal centre. The concentration dependence of $^{99m}Tc(CO)_3$ complex formation showed that with ligands L1, L4 and L5 > 90% labelling could be achieved at a ligand concentration of $\sim 10^{-6}$ M, whereas L6 was less efficient (90% yield at $\sim 10^{-2}$ M).

General scheme for ligand and complex formation



Triazole based ligands



Conclusion: Using click chemistry we have developed a straightforward method to incorporate a series of different chelating systems into azide containing (bio)molecules. Chelating systems can be easily compared and their properties tailored to the (bio)molecule of interest. We are currently installing these ligand systems into tumour affine peptides to investigate the effect of the chelating system on their biodistribution.

Keywords: Technetium, Rhenium, Tricarbonyl, Click Chemistry, Bifunctional Chelators

P168 ^{99m}Tc-PN$_2$S-PEG$_{5000}$-UBI$_{29-41}$ AS INFECTION TRACER: COMPARISON WITH DIRECT ^{99m}Tc-LABELLING APPROACH

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Introduction: Ubiquicidin (UBI) 29-41 fragment binds preferentially with the anionic microbial cell membrane at the site of infection. The peptide, even when directly labeled with ^{99m}Tc, targeted bacterial cells in experimental animals. Biodistribution studies showed rapid clearance of this agent mainly through the kidneys. Here ^{99m}Tc-PN$_2$S-PEG$_{5000}$-UBI$_{28-41}$ production and biostribution profile in mice are reported and compared with previous results.

Experimental: N-(N-(3-diphenylphosphino propionyl) glycyl)-S-tritylcysteine ligand (PN$_2$S) was conjugated to the heterobifunctional methoxy-poly(ethylene glycol)-amine 5 kDa (PEG$_{5000}$) and then UBI$_{28-41}$ peptide was joined to obtain PN$_2$S-PEG$_{5000}$-UBI$_{28-41}$. PN$_2$S-PEG-UBI was added to ^{99m}TcO$_4^-$ saline (50MBq) + 15ml of SnCl$_2$ (1mg/ml in 0.1M HCl) adjusted at pH 2. The solution, left for 45 min at 45°C, was filtered (Sep-PakC18). Radiochemical purity and stability studies were performed by using RP-HPLC on ^{99m}Tc-PN$_2$S-PEG-UBI diluted 1:50 with both 0.9% saline and 0.1 M phosphate buffer (pH= 7) and incubated for 6 h at 20°C. Biodistribution studies of ^{99m}Tc-PN$_2$S-PEG-UBI were performed in healthy BALC/c mice (20.5 g. b.w.) and in infected S.Aureus Swiss mice (20.5 g. b.w.).

Results and Discussion: The radiochemical purity of ^{99m}Tc-PN$_2$S-PEG-UBI resulted to be 98.1% (a single pick at r.t. = 17min with RP-HPLC). The product was stable for up to 6 hr-incubation in saline or in phosphate buffer and showed a radiochemical purity higher than 95%. Biodistribution studies in healthy mice showed ^{99m}Tc-PN$_2$S-PEG high activity in urine to suggest that is cleared through kidneys. However the presence of UBI induced a reduced plasma half-life and an higher liver uptake. Biodistribution studies of ^{99m}Tc-PN$_2$S-PEG-UBI in infected animals showed some activity at the infection sites, no significantly different from using ^{99m}Tc-UBI$_{28-41}$.

Conclusion: A high radiochemically pure and stable ^{99m}Tc-PN$_2$S-PEG-UBI has been obtained. The biodistribution studies of the PEGylated tracer in healthy mice revealed a high liver and bladder uptake that limits its application for the abdominal area. Studies in infected animals showed similar epatobiliary and renal clearance as well to be accumulated in infection sites. Furthermore no statistical difference was found on the accumulation of ^{99m}Tc-PN$_2$S-PEG-UBI and ^{99m}Tc-UBI$_{28-41}$ at the infection sites. The introduction of the spacer PEG$_{5000}$ does not improve the detection of infection sites but does not influence the UBI biospecificity.

Keywords: Technetium-99m, UBI, Infection, PEG, PN2S

P169 ^{99m}Tc-LABELLING OF RECOMBINANT COAGULATION FACTOR VIIa WITH FAC-^{99m}Tc(CO)$_3$-CORE: A RADIOPHARMACEUTICAL POTENTIAL FOR IMAGING IN ACUTE BLEEDING LESIONS

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Introduction: Tissue factor (TF) is an integral membrane glycoprotein. It acts as the cellular receptor for coagulation factor VII (FVII) as well as for the activated form, FVIIa. Vessel wall injury exposes TF to coagulation factors in the blood, and coagulation is initiated when FVII binds to the newly exposed TF and is activated to FVIIa. The TF/FVIIa complex may, after ^{99m}Tc labelling, provide an ideal marker of vascular injury potentially applicable in diagnostic imaging of acute gastrointestinal (GI) bleeding.

Experimental: *Fac*-^{99m}Tc(CO)$_3$(H$_2$O)$_3^+$ (0.5 mL, 166 MBq) was added to a solution of rFVIIa (0.6 mL, 1.39 mg/mL) and stirred at 45°C for 30 minutes. Purification was performed on a PD-10 column. In vitro test of the biological activity of ^{99m}Tc(CO)$_3$-rFVIIa was obtained in pull down experiments with the Sepharose 4B coupled to the extracellular domain of TF (residues 1-209) and mouse monoclonal anti-human FVIIa antibody (F1A2). Experiments to validate unspecific binding were performed with Sepharose 4B coupled with an unrelated antibody or with glycine-blocked Sepharose 4B.

Results and Discussion: Recombinant FVIIa (rFVIIa) was radiolabelled with technetium-99m in a direct labelling reaction using fac-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$. Incorporation of radioactivity depended strongly on rFVIIa concentration and temperature. After purification ^{99m}Tc(CO)$_3$-rFVIIa was obtained in 46% radiochemical yield and in >95% radiochemical purity. ^{99m}Tc(CO)$_3$-rFVIIa was analyzed by HPLC and tested for TF and FVII antibody binding in pull down experiments. The pull down experiments showed that the biological activity of the radiolabelled product remained intact - also after storage for 24 hours in the formulation mixture as well as in human serum samples. Computer modelling analysis of the FVIIa structure was applied for probing for energetically favourable binding sites for the ^{99m}Tc(CO)$_3^+$-core. Two candidate sites for ^{99m}Tc(CO)$_3^+$-stabilizing ligand structures in FVIIa were identified by probing of a FVIIa model structure with the ^{99m}Tc(CO)$_3^+$-moiety.

Conclusion: ^{99m}Tc(CO)$_3$-rFVIIa represents a novel promising tool for the diagnosis of acute GI bleeding lesions.

Acknowledgement: The authors thank Mallinckrodt Medical, Netherlands for providing Isolink® kits.

Keywords: Factor VIIa, Technetium, Tricarbonyl, GI Bleeds

P170 ^{105}Rh COMPLEXES WITH P/S/N CONTAINING CHELATES AND THEIR POTENTIAL APPLICATION TO RADIOTHERAPY

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Introduction: Rh-105 is a reactor-produced beta-emitting radionuclide with very appealing nuclear properties ($t_{1/2}$= 35.5 h, β_{max}= 0.57 MeV, γ=319 keV (19%)) for radiotherapy. Rh-105 is readily produced in high specific activity and "no-carrier added" levels by indirect (n,γ) activation of an isotopically enriched ^{104}Ru target. The low spin d^6 Rh(III) complexes exhibit high kinetic inertness, insuring the *in vivo* stability of the complexes in the biological milieu, a determining factor that enhances the suitability of the radiotherapeutic agent. Various ligands (S/N/P donors) were evaluated as bifunctional chelating agents for Rh(III).

Experimental: Tetrathioether (S_4), dithiaphosphine (S_2P), diaminediphosphine (N_2P_2) and dithiadiphosphine (S_2P_2) ligands have been synthesized and characterized (NMR, MS). The Rh(III) complexes have been synthesized and fully characterized (NMR, MS, elem. anal. and/or X-ray diffraction). The radiotracer Rh-105 analogues of each complex were synthesized and characterized by TLC, HPLC and for stability in serum. Some were evaluated in vivo in normal mice.

Results and Discussion: The tetradentate (S_4, N_2P_2, S_2P_2) ligands formed either *cis*- or *trans*-$[RhCl_2L]^+$ complexes, depending on their chain length, while the tridentate (S_2P) ligands formed *fac*-$[RhCl_3L]$ complexes. The primary differences were in the optimal conditions found for the syntheses of the radiotracer complexes (pH, temperature, time, ligand concentration). Radiochemical yields (50-95%) depended on the particular complex. All complexes were stable in serum at 37°C over several days. The S_4, S_2P and N_2P_2 complexes of Rh-105 were evaluated in normal mice. Clearance was through the renal and/or hepatobiliary system, dependent on the complex lipophilicity.

Conclusion: Thioether (S_4) ligands resulted in high yields (>95%) but reaction temperatures of 90°C are required. Phosphine-amine (N_2P_2) ligands allowed lower reaction temperatures (65°C), but the amines required higher reaction pH resulting in lower yields (50-70%). The phosphine-thioether (S_2P and S_2P_2) ligands, for which the radiochemistry is currently underway, may result in the best possible combination of high yields and lower reaction temperatures.

Acknowledgement: We thank the DOE (DE-FG02-01ER63192) for financial support.

Keywords: Rhodium-105, Bifunctional Chelates, Radiotherapy, Rhodium(III)

P171 NEW BIFUNCTIONAL CHELATORS FOR TUMOUR TARGETED DELIVERY OF COPPER-64 FOR POSITRON EMISSION TOMOGRAPHY

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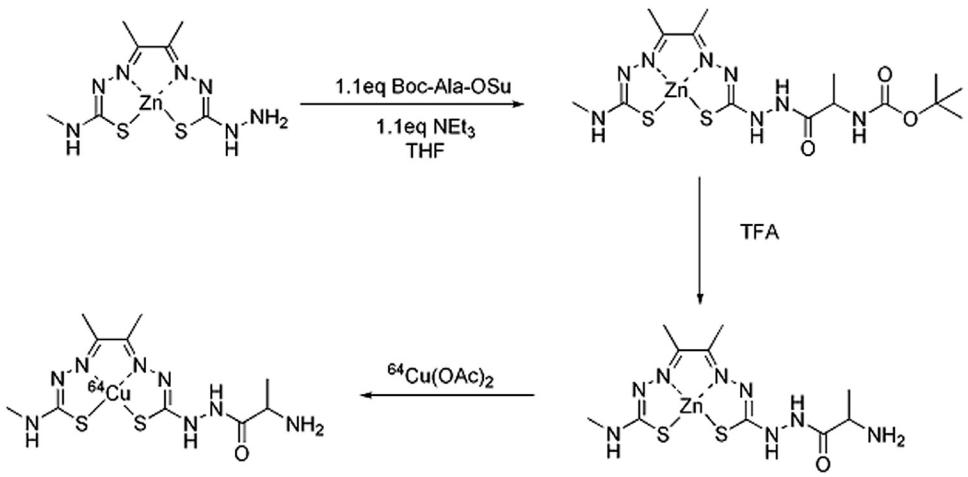
Introduction: Positron emission tomography provides information on the nature of a tumour, and is routinely used for planning the treatment of cancer. For example, tracers have been developed for assessment of hypoxia and rate of glucose metabolism [1]. Bifunctional chelators (BFCs) consist of a ligand for a radionuclide, a linker group and a targeting group which is designed to accumulate the BFC at the cancerous site and clear rapidly from other tissues. Copper-64 has a half-life of 12.7 hours and is suited for radiopharmaceuticals with relatively slow target accumulation such as labelled proteins, as well as smaller tracer compounds. The half-life allows time for preparation of the copper-64 at an offsite cyclotron, synthesis and purification of the radiopharmaceutical, transportation to a patient, and accumulation at the target, with sufficient activity remaining to produce good images.

Experimental: Amino acid derivatised *bis*(thiosemicarbazone) ligands based on hypoxia selective CuATSM [2] have been synthesised via peptide coupling reactions.

Results and Discussion: Functionalisation with amino acids alters the physical properties of zinc and copper *bis*(thiosemicarbazonato) complexes; in particular their solubility in water, which is much greater than the parent compound, CuATSM. Radiolabelling experiments show that the copper-64 complexes can be prepared either via transmetallation from zinc(II) species or by direct complexation with the pro-ligand. The cellular uptake and localisation of the fluorescent zinc(II) complexes can be observed by confocal microscopy [3]. The amino acid functionality can be used as a solubilising linker to conjugate more amino acids and other biological molecules for receptor targeting.The syntheses of these complexes and full characterisation using a range of spectroscopic and electrochemical techniques are presented.



Conclusion: The amino acid functionalised complexes broaden the scope of use of copper *bis*(thiosemicarbazonato) complexes, introducing the possibility of receptor targeting via conjugation of peptides.

Acknowledgement: HMB would like to thank GSK for providing a studentship.

References: [1] O. Couturier et al, Eur. J. Nuc. Med. Mol. Imaging 2004, 31(8), 1182. [2] Y. Fujibayashi et al, J. Nuc. Med. 1997, 38(7), 1155. [3] A. Cowley at al, Chem. Comm. 2005, 7, 845.

Keywords: Copper-64, Bis(Thiosemicarbazones), Positron Emisson Tomography, Bioconjugation, Hypoxia

P172 LOOKING TO THE NEXT GENERATION OF TECHNETIUM INSTANT KITS. ADVANCES IN RAPID AND MULTI-STEP SYNTHESIS Tc AND Re RADIOPHARMACEUTICALS

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Introduction: Instant kits helped make ^{99m}Tc the most widely used radionuclide in diagnostic medicine as it provided a way for hospital-based radiopharmacies to produce agents reproducibly in high yield and purity. Recently, there has been a shift away from local production towards contracting large central radiopharmacies to supply unit doses. This change creates an opportunity to revisit the paradigm by which Tc-radiopharmaceuticals are produced as a way to expand the realm and mitigate some of the existing limitations of Tc radiopharmaceutical chemistry.

Unlike ^{18}F-based tracers which usually require a purification step, Tc-based agents are expected to be accessible in a single synthetic step at high yield. The need to use single step labeling procedures with yields >95% limits the range of compounds that can be tagged by Tc and the maximal achievable effective specific activity. What is needed is a move away from instant kits towards "Tc-synthesis boxes" which can facilitate automated multi-step synthesis of Tc radiopharmaceuticals.

To bring about this change requires further exploration of Tc chemistry to develop easily automated multi-step synthetic protocols that are rapid, high yielding and adaptable to classes of targeting vectors that are difficult to prepare using traditional instant kits. Tc(I) and Re(I) complexes are ideally suited in this regard as they are generally inert making them amenable to a wide range of reaction conditions, bioconjugation protocols and purification methods.

Results and Discussion: Beginning with MO_4^- (M = ^{99m}Tc, $^{186/188}$Re) we are able to perform multi-step labeling and conjugation procedures using the $[M(CO)_3]^+$ core and a bis-pyridyl- chelate in less than 90 minutes (purified) in extremely high yields. The procedure includes preparation of $[M(CO)_3(H_2O)_3]^+$, formation of a bifunctional chelate complex, conversion to an active ester and finally coupling to a targeting vector.

Conclusion: Microwave heating, inline purification protocols and the $[M(CO)_3]^+$ core can be used to prepare targeted radiopharmaceuticals that are beyond the current scope of traditional instant kits. Tc and Re labeling experiments including conjugation to a range of substrates and the nature of the purification protocols support the hypothesis that this approach can be adapted to central radiopharmacy production. The proposed paradigm shift is important given the paucity of new Tc-based radiopharmaceuticals that have received approval for clinical use.

Keywords: Technetium, Kits, Microwave, Rhenium, Bioconjugation

P173 PREPARATION AND COMPARISON OF THREE DIFFERENT KIT FORMULATION IN ^{99m}Tc LABELING OF ANTIMICROBIAL PEPTIDE UBI 29-41 FOR INFECTION IMAGING

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Introduction: UBI 29-41 (a derivative of antimicrobial peptide ubiquicidin) labelled with ^{99m}Tc is reported to discriminate between bacterial infections and sterile inflammatory processes. In this study we prepared and compared UBI labelled with ^{99m}Tc by different kit formulations. We tested three lyophilized kits, one based on the direct labelling method with only $SnCl_2$ as reducing agent and two based on 6-hydrazinopyridine-3-carboxylic acid (HYNIC) via tert-butoxy carbonyl (BOC) and tricine as a coligand with or without ethylenediaminodiaceticacid (EDDA).

Experimental: Linear fragment of UBI 29-41 was synthesised by solid phase peptide synthesizing method. After cleavage from the resin, peptide was conjugated with BOC-HYNIC in solution. With deportation and purification of peptide conjugate, three lyophilized kits were prepared as following: kit 1: 20 μg UBI 29-41, 5 μg $SnCl_2$, pH = 9; kit 2: 20 μg UBI 29-41, 20 μg $SnCl_2$, 15 mg tricine, pH = 5.2; kit 3: 20 μg UBI 29-41, 20 μg $SnCl_2$, 15 mg tricine, 5 mg EDDA, pH = 7. After addition of $^{99m}TcO_4^-$ solution the kits were labeled under specific conditions and ITLC, HPLC analysis was performed. The binding of labeled UBI to bacteria, S. aureus, was estimated and stability experiments in human serum were performed. The accumulation in infected tissues was studied using ex vivo counting and scintigraphy.

Results and Discussion: Peptides UBI 29-41 and conjugate of UBI 29-41 with HYNIC were prepared with high purity (>97%). Radiochemical analysis indicated rapid and high labeling yield (90-95%) for all three kits, the labeled compounds being stable for 24 h in vitro in human serum (RCP for kit 1 >80%, kit 2 >70%, kit 3 >90%). The kit 2 showed the highest binding of 80% of the added activity to bacteria, that of which were 70% for kit 1 and 60% for kit 3 and were reduced to 25%, 20%, 10% in present of 50 fold cold peptide UBI 29-41. After injection into infected mice, all tracers were rapidly removed from the circulation by renal excretion (35-40%ID/g after 1 h p.i), but blood activity of kit 2 (2.5%ID/g) remained higher than kit 1(1.5%ID/g) and kit 3 (1%ID/g). Specific accumulation in infected thigh muscles as indicated by a T/NT ratio was 4.06% (kit 2), 3.34% (kit 1), 3.07% (kit 3). Data show that although kit 3 is more stable, using EDDA in formulation binding to bacteria and T/NT in mice is the lowest. Also in comparison with kit 2 and probably due to taking place of ^{99m}Tc at or nearby a biologically active site of peptide, kit 1 have a lower binding to bacteria and T/NT in mice.

Conclusion: The HYNIC-UBI 29-41 labeled in presence of tricine as a coligand (kit 2) showed the most promising results for further in vivo evaluation.

Keywords: UBI 29-41, HYNIC, KIT, 99mTc, Infection

P174 ENZYMATIC FUNCTIONALIZATION AND RADIOLABELING OF A TUMOR AFFINE MONOCLONAL ANTIBODY USING TRANSGLUTAMINASE

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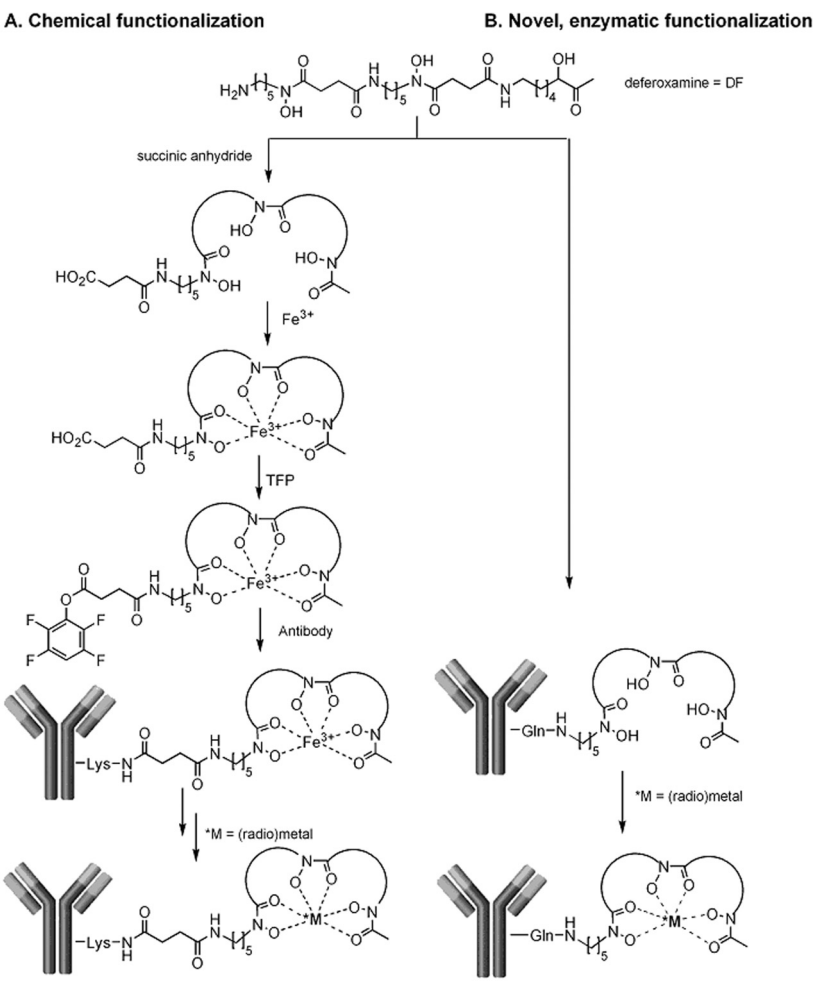
Introduction: Antibodies (mAb) functionalized with the chelator deferoxamine (DF) and radiolabeled with ^{89}Zr showed promising clinical results (Börjesson et al. Clin Cancer Res, 2006). However, functionalization of mAbs with DF via the lysine (Lys) side-chains by chemical methods is rather cumbersome (five-step synthesis, Fig. 1A).

Transglutaminase (TGase) form stable, isopeptidic bonds between the side chain of glutamine (Gln) and Lys. Deferoxamine has a 5-amion pentyl residue, which mimics the side chain of Lys. The aim of the current study was to demonstrate, that functionalization of mAbs with DF can be achieved in a single step using the enzymatic activity of TGases.

Experimental: For this studies the tumor affine mAb chCE7agl (MW ~150 kDa) has been used. DF and bacterial TGase (MW ~35 kDa) were obtained from commercial sources. 0.2 mM DF has been reacted with 1 mg/mL chCE7agl using 1 U/mL BTGase for 1 h under physiological conditions. The functionalized protein was purified by size exclusion column chromatography (Sephadex PD10). The DF/protein ratio was determined spectrophotometrically at 430 nm in presence of Fe(III). The DF-chCE7agl immunoconjugate was radiolabeled with ^{67}Ga (DF is also a good chelator for e.g. Fe and Ga). The radiolabeled product was purified by Sephadex PD10 and characterized by means of TLC.

Results and Discussion: The chemical methodology requires the inversion of the coupling functionality of deferoxamine for coupling toLys. The enzymatic approach allows the use of unmodified DF when coupled to Gln. The chCE7agl-DF conjugate was formed in a one-step procedure using BTGase under physiological conditions (Fig. 1B). The DF/protein ratio was calculated to be 2-3 on average (for the chemical method the ratio was found to be 1 only). The chCE7agl-DF was radiolabeled with ^{67}Ga. The yields were moderate. However, addition of excess DF to ^{67}Ga-DF-chCE7agl revealed only slight demetallation (10% of radioactivity), proving the stability and specificity of the radiolabeling.



Conclusion: TGases offer a most elegant approach for the functionalization of mAbs with DF. The method is devoid of the use of protection/deprotection strategies or reactive groups. With same methodology it should be possible to introduce other bifunctional chelating agents. Corresponding experiments as well as further in vitro and in vivo experiments are currently in progress.

Keywords: Radioimmunoconjugate, Transglutaminase, Deferoxamine, Ga-67

P175 DEVELOPMENT OF RENAL $^{99m}Tc(CO)_3$ AGENT WITH $CMMSH_3$

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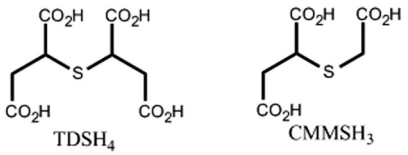
Introduction: In a search for superior ^{99m}Tc renal imaging agents with high specificity for renal excretion and rapid clearance from the bloodstream, we have been exploring possible radiopharmaceuticals containing a $\{^{99m}Tc(CO)_3\}$ core. Toward this end, highly hydrophilic small ligands are ideal to produce promising ^{99m}Tc agents. We are exploring ligands $CMMSH_3$ and $TDSH_4$ (see chart) in our studies. These ligands feature the binding sites of two carboxyl groups and a thioether function available to the coordination of the $\{^{99m}Tc(CO)_3\}$ core.

Experimental: A mixture of $CMMSH_3$ or $TDSH_4$ and $[^{99m}Tc(CO)_3(CO)_3]^+$ was heated at 70°C (pH 7) for 1h. The products were purified by HPLC. Bidistribution studies were performed on Sprague-Dawley rats, using ^{131}I-orthoiodohippurate as an internal control. The animals were sacrifices at 10 and 60 min (n =5). Parallel studies for the preparation of Re analogues were also carried out to understand the chemistry of the radioactive ^{99m}Tc species.



Results and Discussion: The labeling of $CMMSH_3$ and $TDSH_4$ with the $[^{99m}TcCO)_3(CO)_3]^+$ precursor gives the products in high yield (almost quantitatively). NMR showed the products of Re analogues were composed of diasteroisomers. Because of the limitation of HPLC separation condition, the diasteroisomers formed in both reactions cannot be separated by preparative HPLC. These ^{99m}Tc products were tested in rats. Biodistribution studies showed that the $[^{99m}Tc(CO)_3(CMMS)]^{2-}$ agents had excellent renal excretion [activity in the urine as a percent of ^{131}I-orthoiodohippurate (OIH is an internal control) was 98±1% at 60 min], while $[^{99m}Tc(CO)_3(TDS)]^{3-}$ agents had lower renal excretion (68±8% at 60 min).

Conclusion: The $[^{99m}Tc(CO)_3(CMMS)]^{2-}$ agents showing high renal excretion, are warranted for further evaluations as potential renal imaging agent.

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Keywords: Technetium-99m, Rhenium, Carbonyl, Imaging, Renal

P176 ^{111}In- AND ^{64}Cu-DOTA-LABELED E. COLI HEAT STABLE ENTEROTOXIN ANALOG POSSESSING A POLYETHYLENE GLYCOL SPACER GROUP

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Introduction: A peptide comprising residues 5-19 (Y^5CCELCCNPACTGCF^{19}) of the E. coli heat-stable enterotoxin (ST_h) and an N-terminal polyethylene glycol spacer moiety was synthesized and N-terminally labeled with the DOTA chelating group.

Experimental: The resulting molecule, DOTA-$(AEEA)_3$-F^{19}-ST_h(5-19), was radiolabeled using both $^{111}InCl_3$ and $^{64}CuCl_2$, and the radioconjugates were characterized by RP-HPLC, in vitro receptor binding assay, and in vivo biodistribution assays.

Results and Discussion: The DOTA-PEG-ST_h conjugate was efficiently labeled with both ^{111}In and ^{64}Cu, with higher radiochemical yields obtained in ^{64}Cu labeling reactions. Competitive binding analyses of $(AEEA)_3$-F^{19}-ST_h(5-19), DOTA-$(AEEA)_3$-F^{19}-ST_h(5-19), and ^{nat}In-DOTA-$(AEEA)_3$-F^{19}-ST_h(5-19) demonstrated IC_{50} values ranging between 3.3 ± 0.3–9.1 ± 3.5 nM. Direct binding studies of ^{111}In- and ^{64}Cu-labeled compounds to T84 cells in vitro demonstrated receptor-specific internalization of each compound into cells over the course of 100 minutes, although both internalization and total binding were reduced for the ^{64}Cu-labeled peptide. The ^{111}In-labeled peptide demonstrated 3 to 6-fold lower tumor uptake at 4 hr pi in SCID mice bearing T84 human colorectal cancer tumor xenografts than has been observed previously with similar ST_h constructs (here, 0.27 ± 0.03%ID/g), suggesting that replacement of the 4 N-terminal amino acid residues of wild-type ST_h with the PEG spacer described here negatively impacts tumor localization in vivo. Uptake in nontarget tissues was minimal for the ^{111}In-labeled peptide (0.01 ± 0.002, 0.04 ± 0.004, and 0.01 ± 0.001%ID/g at 4 hr pi for blood, liver, and muscle, respectively, n=3), and >97% ID was excreted into urine at this timepoint. We had hypothesized that the rapid excretion into urine typical of radiolabeled ST_h analogs would serve to minimize in vivo dissociation of ^{64}Cu from the DOTA chelator seen in other contexts. In fact, in vivo biodistribution studies of the ^{64}Cu-labeled peptide in CF-1 normal mice demonstrated that dissociation of ^{64}Cu from the DOTA chelator remains a significant problem, with uptake in blood, liver, and muscle of 0.34 ± 0.22, 2.76 ± 0.26, and 0.20 ± 0.04 respectively at 4 hr pi, and 84.7 ± 4.6% ID in urine (n=5).

Conclusion: Further research into the development of a ^{64}Cu-ST_h PET radiopharmaceutical is currently underway.

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Keywords: E. coli Heat-Stable Enterotoxin, Copper-64, Indium-111, Colorectal Cancer

P177 PHOSPHONATE-COMPLEXES OF GALLIUM-68 FOR BONE TUMOR IMAGING

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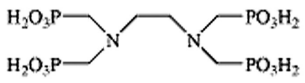
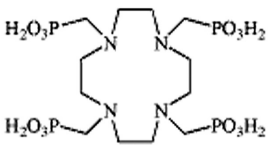
Introduction: As Tc-99m-phosphonates are well established tracers for the diagnoses of bone metastases using SPECT, analogue attempts for PET using the $^{68}Ge/^{68}Ga$ generator would be potentially useful. Therefore molecules containing phosphonates with binding affinities to apatite and being adequate complexing agents for Ga^{3+} could be interesting targeting vectors for the synthesis of generator-based PET-tracers for skeletal imaging. The aim of the study was to synthesis complexes with different types of phosphonate ligands in order to understand the rational of ^{68}Ga-phosphonates related to apatite binding.

Experimental: ^{68}Ge (T1/2 = 270 d) provides the positron emitter ^{68}Ga (T1/2 = 68 min) as an easily available and relatively inexpensive source of a PET nuclide. ^{68}Ge is fixed on a solid phase of TiO_2. Through HCl ^{68}Ga is eluted from the generator and immobilized on an acidic cation exchanger. Impurities such as Zn, Fe and Ti as well as ^{68}Ge generator breakthrough are removed by a mixture of acetone/HCl (N1). Subsequently, ^{68}Ga is eluted in 400 μL of a second mixture of acetone/HCl (N2) from the cation exchanger.

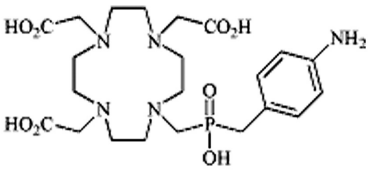
As proof-of-concept, the ligands EDTMP, DOTP and DO3AP-ABn have been selected. ^{68}Ga labeling is performed in 400 μL Na-HEPES buffer by adding the ^{68}Ga fraction of N2. Through variation of reaction time, temperature, pH and amount of the ligands, optimum reaction parameters for complex formation were tested. Analyses of radiochemical yield are carried out by TLC. Binding studies on synthetic apatite were applied to simulate the binding of the ^{68}Ga-phosphonates to bone structures.



EDTMP



DOTP



DO3AP-ABn

Results and Discussion: The elution of ^{68}Ga and the on line-processing of the eluate are performed within 5 min only. Labeling proceeds at temperatures between 25 and 60°C within 2 to 10 min. Ligands are used in nanomole amounts only and the radiochemical yields are 50 to 95%. The most promising complex concerning synthesis is EDTMP with a radiochemical yield of 95% in 5 min. First apatite binding assays show strong and fast binding of ^{68}Ga-EDTMP and ^{68}Ga-DOTP, while ^{68}Ga-DO3AP-ABn is not binding.

Conclusion: Syntheses of ^{68}Ga complexes are performed within 20 min after elution of the generator. First evaluations on apatite show high binding in a short time for both ^{68}Ga-EDTMP and the macrocyclic ^{68}Ga-DOTP. Preliminary μ-PET imaging on rats demonstrated bone uptake in vivo for ^{68}Ga-EDTMP and ^{68}Ga-DOTP. Interestingly, ligands containing phosphonates are not a guarantee for fast and high binding to bone.

Keywords: Germanium-68/Gallium-68 Generator, Skeletal Imaging, Phosphonate Ligands