P178 ZOLEDRONIC ACID LABELED WITH SOFT BETA EMITTERS ^{47}Sc AND ^{177}Lu FOR BONE PAIN THERAPY

M. NEVES [1], I. ANTUNES [1], A. MAJKOWSKA [2] and A. BILEWICZ [2]

[1]Nuclear and Technological Institute, Sacavem, Portugal; [2]Radiochemistry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland

Introduction: Bisphosphonates (BP's) have a strong affinity for calcium phosphates and for hydroxyapatite. Because of their chemical structure and the characteristic P-C-P- bond, which is non-biodegradable and non-hydrolysed in vivo, BP's have been the chosen molecules for bone scanning imaging. BP's are also able to coordinate β-emitter radionuclides as ^{153}Sm and ^{186}Re and found application in clinical nuclear medicine for bone metastasis therapy (Re-186-HEDP-Metastron and Sm-153-EDTMP-Quadramet). Until now BP's were labeled with β emitters with emission of relatively high energy β particles like ^{153}Sm, ^{188}Re and ^{166}Ho. Due to long distance interaction of hard β particles the degradation of bone marrow is observed. Therefore, instead of hard b emitters we plan to apply BP's labeled with soft β emitters e.g. ^{47}Sc and ^{177}Lu. The distance of particles emitted by such emitters in bone is much shorter, less than 1 mm, therefore bone marrow will be not affected.

Experimental: The sodium salt of zoledronic acid (1-Hydroxy-2-imidazol-1-yl-phosphonoethyl) was purchased as a gift from Novartis Pharma AG. Due to relatively short half live of ^{47}Sc the labeling of BP's was studied using γ-emitted ^{46}Sc ($T_{1/2}$=83.8 d) radiotracer. ^{46}Sc and ^{177}Lu were obtained by neutron irradiation of Sc_2O_3 and Lu_2O_3 targets at a neutron flux $3x10^{14}$ n/cm^2/s for 6 h. The specific activities of the radionuclides were 100 MBq/mg for ^{46}Sc and 700 MBq/mg for ^{177}Lu. The radiolabelling efficiency and stability evaluation of the radiocomplexes were accomplished by ascending instant thin layer chromatography using ITLC-SG (Polygram,Macherey-Nagel) strips developed with the mobile phase: H_2O/NH_3 (25:1). Adsorption of the ^{46}Sc and ^{177}Lu-BP's complexes onto hydroxyapatite (HA) was accomplished following an adaptation of previously described methods [1].

Results and Discussion: The ^{46}Sc and ^{177}Lu zoledronate complexes migrate (Rf 0.8–1.0), while ionic ^{46}Sc and ^{177}Lu and colloidal radioactive forms remains at the origin. The labeling efficiency of zoledronate with ^{177}Lu and ^{46}Sc calculated from ITLC studies indicates that ^{46}Sc forms much stronger complexes with zoledronate than ^{177}Lu. This is result of much smaller ionic radius of Sc^{3+} than Lu^{3+}.

The adsorption of labeled zoledronate on HA was studied. The maximum percentage adsorption values, higher than 98%, were achieved by ^{46}Sc and ^{177}Lu-zoledronate using amounts of hydroxyapatite higher then 10 mg.

Reference: [1] Neves M., Gano L., Pereira N., Costa M.C., Costa M.R., Chandia M., Rosado M., Fausto R. Nucl.Med.Biol. 29, 329 (2002).

Acknowledgement: The work was supported by EC program Transfer of Knowledge (POL-RAD-PHARM) and Portuguese FCT (FEDER and POCI/QUI/55508/2004).

Keywords: Bisphosphonates, Sc-47, Lu-177

P179 TRI AND TETRAAZA DERIVATIVE COMPLEXES OF ^{47}Sc AS PRECURSORS FOR THERAPEUTIC RADIOPHARMACEUTICALS

A. MAJKOWSKA and A. BILEWICZ

Radiochemistry, Institute of Nuclear Chemistry and Technology, Warsaw, Poland

Introduction: The medium energy beta emitter ^{47}Sc is a promising therapeutic radioisotope for the curative treatment of cancer using labeled biomolecules. It has a half-life of $T_{1/2}$ =3.35 days and a maximum beta energy of 600 keV, resulting in a short range of radiation in tissue. The decay is accompanied by the emission of low energy gamma radiation with Eg =159 keV suitable for simultaneous imaging. ^{47}Sc is could be produced by irradiation of ^{47}Ti target with high energy neutrons (En$>$1 MeV). Sc^{3+} has ionic radius 74.5 pm (CN=6) and is chemically similar to Ga^{3+}, In^{3+}, Y^{3+} and heaviest lanthanides therefore ligands developed for these cations should be suitable for chelating ^{47}Sc. In order to find the best chelators for attaching ^{47}Sc to biomolecules the formation of Sc^{3+} complexes with 1,4,7,10-tetraazacyclododecane-1,7-bis acetic acid (DO2A), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-1,4,7 triaacetic acid (NOTA), were studied by capillary and paper electrophoresis and thin layer chromatography.

Experimental: The DOTA and DO2A ligands were purchased from Macrocyclics. The NOTA ligand was synthesized by a reaction of 1,4,7-triazacyclononane trihydrochloride with bromoacetic acid. The purity of the product was checked by MS and NMR methods. The Sc^{3+} complexes with macrocyclic ligands were synthesized by mixing 1.5 mM aqueous solution of the ligand with 1.5 mM aqueous solution of Sc^{3+}. The stability constants of scandium macrocyclic complexes were determined using capillary electrophoresis. When this reaction reached equilibrium (8 days), the small sample of solution was injected into the capillary to detect the concentrations of complex and ligands, and the stability constants were calculated from the free ligand concentration. The complexes were crystallized and structures were characterized by X-ray diffraction and NMR. Formation kinetics and stability of Sc^{3+} complexes were studied using a gamma-emitted ^{46}Sc ($T_{1/2}$=83.8 d) radiotracer.

Results and Discussion: Taking into account stepwise protonation constants the stability constants of Sc(DOTA), $\log K_{ScDOTA}$=24.2, Sc(NOTA) $\log K_{ScNOTA}$=15.6 and Sc(NOTA), $\log K_{ScNOTA}$=11.8 were determined. The results indicate that Sc^{3+}, similarly to lanthanides and Y^{3+}, forms most stable complexes with DOTA ligand.

The electrophoresis and ion exchange adsorption indicate that Sc^{3+} forms with DOTA anionic complexes, with NOTA neutral complexes and with DO2A cationic complexes. In DOTA complexes, Sc^{3+} exhibits CN=8, while in NOTA and DO2A exhibit CN=6.

Conclusion: The high thermodynamic stability and inertness of the Sc^{3+} DOTA complexes indicate that ^{47}Sc labeled biomolecules could be alternative to ^{177}Lu radiopharmaceuticals.

Acknowledgement: The research was supported by a EC grant Transfer of Knowledge Pol-Rad-Pharm.

Keywords: Sc-47, Sc-44, Therapeutic Radiopharmaceuticals

P180 PREPARATION AND QUALITY CONTROL OF ^{123}I-ANGIOTENSIN II

A. SATTARI

Cyclotron and Nuclear Medicine Department, Nuclear Medicine Research Center, Karaj, Tehran, Islamic Republic of Iran

Introduction: Angiotensin is an oligopeptide in the blood that causes vasoconstriction, increased blood pressure, and release ofaldestrone from the adrenal cortex. Angiotensin I is converted to angiotensin II through removal of two terminal residues by the Angiotensin converting enzyme (ACE, or kininase), which is found predominantly in the capillaries of the lung. ACE is a target for inactivation by ACE inhibitore drugs, which decrease the rate of angiotensin II production.

Experimental: Angiotension II is a soluble powder in water. A concentration of 2 mMol/ml of that solution was prepared and stored at a temperature of -8°C. 200μl of Iodogen solution added to 20 ml chloroform. The prepared solution was evaporated in a reaction vial. 200 μl buffer (0.5M KH_2PO_4), 20 μl angiotension II and 20 μl ^{123}I (home made Iodine-123) were added to the reaction vial. After10 minutes solution removed from reaction vial and passed through a Hypersil BDC C18 column lot no. 5/120/5646.

Results and Discussion: A sample of extracted solution from the column was selected for determination of radiochemical purity by HPLC. An eluent solution containing KH_2PO_4 0.0I M, pH 6.5 and 25% EtOH, was used. Rotation times for free Iodine5 minutes and for ^{123}I-Angiotension 10 minutes were obtained. In several preparations, radiochemical purity more than 95%±2 was obtained. For bio-distribution study the ^{123}I-Angiotension solution was injected to mouse and in different interval times 1, 5, 10, 20, 60 and 180 minutes organ counts was performed. The organ count carried out at 1 and 5 minutes after injection were showed a high amount activity in stomach and small intestinal. Bio-distribution studies were resulted that ^{123}I-Angiotension is broken after injection with in few minutes by the enzymes in blood. There was no significant difference between blank groups (^{123}I and buffer) and study group (^{123}I-Angiotension) was observed 1 and 3 hours after injection.

Keywords: 123I-Angiotensin II Preparation, 123I-Angiotensin II Quality Control, 123I-Angiotensin II Biodistribution, 123I-Angiotensin II Iodogen, 123I-Angiotensin II Labelling

P181 A NOVEL TRIAZOLE DERIVATIVE: ^{90}Y-LABELING, RADIOCHEMICAL AND BIOLOGICAL EVALUATION

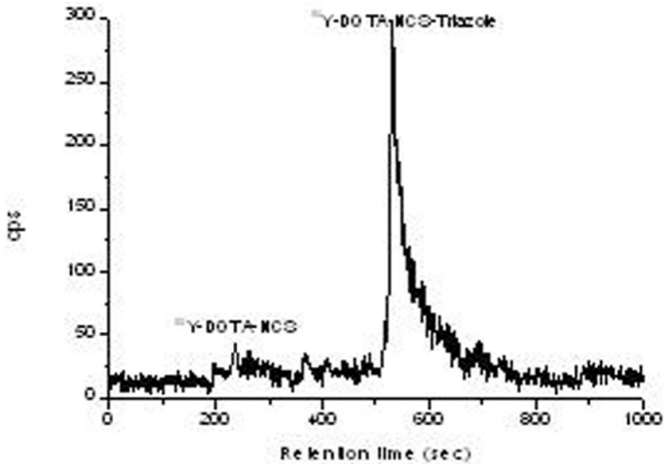
A. KORDE [1], U. PANDEY [1], D. SATPATI [1], Y. MODHWE [2], V. VELINGKAR [2] and M. VENKATESH [1]

[1]Radiopharmaceuticals, Bhabha Atomic Research Centre, Mumbai, Maharashtra, India; [2]Pharmaceutical Chemistry, K.M.K. College of Pharmacy, Mumbai, Maharashtra, India

Introduction: ^{90}Y is widely used for therapy due to its high energy β^- rays and availability from ^{90}Sr/^{90}Y generator. 1,2,4-Triazole derivatives possess a variety of pharmacological activities, including cytotoxicity. A triazole derivative, was synthesized and radiolabeled with ^{90}Y with an aim to achieve tumor therapy by synergistic effect of the β^- radiation and cytotoxicity of the triazole.

Experimental: 4-p-amino phenyl-1(5,5-diphenyl imidazolidone-2'-yl)-5-thio-1,2,4 triazolidine-3-one, the target molecule was synthesized in 5 steps. The intermediates and the final derivative were characterized by ^{1}H NMR spectra. The triazole derivative was then conjugated with DOTA-NCS. DOTA-NCS-triazole was labeled with ^{90}Y, by incubation with ^{90}Y acetate at pH 5.0, at 80°C for 30 min. ^{90}Y-DOTA-NCS and ^{90}Y-DOTA-NCS-triazole were characterized by C18 reverse phase HPLC, using H_2O and acetonitrile with 0.1% TFA for elution in the gradient mode at 1 mL/min. Cytotoxicity studies of the ^{90}Y labeled product in human colon cancer cell line (HT29) was carried out by MTT assay while *in vitro* cell uptake was determined in AR42J cells. Pharmacokinetics and tumor uptake were studied *in vivo* in murine fibrosarcoma model.

Results and Discussion: The triazole and DOTA-NCS-triazole could be synthesized in high purity and yields. ^{90}Y-triazole derivative could be prepared in >95% yield, using 100 μg of DOTA-NCS-triazole and 37 MBq of ^{90}Y acetate. In the HPLC, ^{90}Y-DOTA-NCS-triazole eluted with R_t of 9.1 min. and ^{90}Y-DOTA-NCS at 4 min. (fig.). ^{90}Y-DOTA-NCS-triazole was stable in PBS at 25°C for 48 h. ^{90}Y-DOTA-NCS-triazole was toxic to HT 29 cells even at 10^{-7} M while cold triazole exhibited the same level of cytotoxicity at 10^{-3} M. In AR42J cells, ^{90}Y-DOTA-NCS-Triazole conjugate exhibited uptake of 34.8±2.2% corresponding to 1 μg of triazole which reduced to 28.8±0.6% in presence of 100 μg of cold triazole. Biodistribution studies showed ~ 80% renal clearance within 3 h p.i with very low activity in major organs. Tumor uptake was ~0.9%/g at 3 h p.i. and ~0.4%/g at 24 h p.i.



Conclusion: A novel ^{90}Y-triazole derivative was prepared in good yield and stability. Although the product exhibited good cytotoxicity in cancer cells, higher tumor accumulation *in vivo* is warranted for further evaluation as a cancer therapeutic agent.

Acknowledgement: Authors are greatful to Dr. H.D. Sarma for help in animal experiments.

Keywords: Yttrium-90, Triazole

P182 UTILIZATION OF AN IODOVINYL UNIT IN THE RADIOIODINATION OF A SMALL PEPTIDE

D.D. ROSSOUW

Radionuclide Production, iThemba Labs, Faure, South Africa

Introduction: The utilization of prosthetic groups in the radioiodination of proteins has been prompted, amongst others, by the *in vivo* deiodination of proteins labelled in the tyrosine unit [1]. The conjugating agent *N*-succinimidyl iodobenzoate has been used mostly in the radioiodination of monoclonal antibodies[2,] but more recently also in the radioiodination of a small peptide [1,3]. The iodovinyl unit has been used to prepare iodovinyl antibody conjugates[4], but has, to author's knowledge, never been applied to peptide labelling. This study describes the methodology that was used to prepare an ^{123}I-labelled vinyl conjugate of a small chemotactic peptide.

Experimental: The 2,3,5,6-tetrafluorophenyl-5-(tributylstannyl)-4-pentenoate (TFP-TBS-PEA) precursor was synthesized and subsequently labelled, with few modifications, according to a literature procedure [4]. Labelling mixtures were purified on a 100 mg C18 mini-column and the labelled product eluted with dimethylformamide (250 μl). The DMF eluate was mixed with 5 μl of a 10 mM solution of the peptide in DMF and 2 μl *N,N*-diisopropylethylamine, the mixture stirred for 30 minutes at room temperature, diluted with water and subsequently purified on a 500 mg C18 mini-column. The column was eluted with mixtures of 0.1% trifluoroacetic acid/acetonitrile, water and subsequently water/ethanol to elute the labelled conjugate.

Results and Discussion: Recovery yields of the ^{123}I-TFP-PEA synthon ranged between 60 and 75%, depending on the amount of precursor used. Due to the volatile nature of the labelled synthon, it was eluted from the C18 mini-column with the subsequent conjugation solvent (DMF) in order to avoid an evaporation step. Minimal amounts of peptide and base were needed to ensure a complete consumption of the labelled synthon after 30 minutes. Two relatively polar radiochemical impurities appeared in the radio-HPLC chromatogram. One of them was directly proportional to the amount of activity used. The formation of this impurity could also be partially suppressed by minimizing the volume of the reaction mixture and the amount of base. Both impurities were quantitatively and selectively removed during the purification step. Recovery yield of the labelled conjugate in the water/ethanol mixture (best scenario at high activity labelling) was 65–70% based on the labeled synthon input activity, resulting in an overall conjugate yield of 40–50%.

Conclusion: A labelled small peptide-iodovinyl conjugate was successfully prepared. Reaction conditions and purification procedures are being optimised in order to optimize yield and chemical purity.

References: [1] Al-Jammaz I. et al. *Appl. Radiat. Isot.*, **57**, 743, (2002). [2] Wilbur D.S. et al. *J. Nucl. Med.* **30**, 216, (1989). [3] Pozzi O.R. et al. *Appl. Radiat. Isot.*, **64**, 668, (2006). [4] Hadley S.W. and Wilbur D.S. *Bioconjugate Chem.*, **1**, 154, (1990).

Keywords: 123I, Peptide, Iodovinyl, Conjugate, In Vivo Deiodination

P183 ADDITION OF ORGANIC SOLVENTS MIGHT INHIBIT RADIOIODINATIONS IN Cu(I)-ASSISTED NUCLEOPHILIC EXCHANGE

J.L.H. EERSELS and J.D.M. HERSCHEID

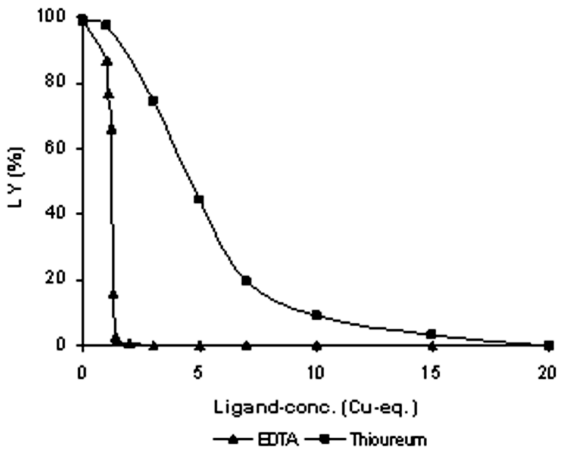
Nuclear Medicine & PET Research, VU University Medical Centre, Amsterdam, Netherlands

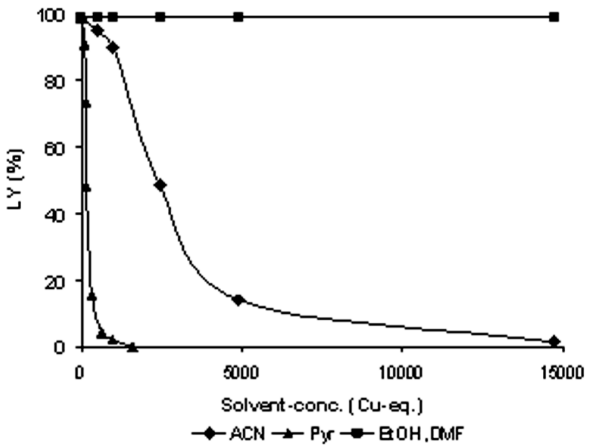
Introduction: In view of a comparative, mechanistic study of radioiodination in aqueous and non-aqueous reaction conditions with the Cu(I)-assisted nucleophilic method, we have evaluated the presence of several commonly-used organic solvents (ACN, EtOH, DMF and pyridine) on the labelling yield in aqueous medium. In addition, these effects were compared with the 'expected outcomes' when typical ligands (EDTA, thiourea) were added in the reaction mixture.

Experimental: To a 2 mL vial were added 2 mg of MIBG. $\frac{1}{2}H_2SO_4$, 5 mg of gentisic acid, 0.1 mg of $CuSO_4.5H_2O$, a certain amount of competitor and 40-60 MBq of nca $Na^{123}I$; all from in advance prepared stock-solutions. The mixture was brought to a constant end-volume of 1 mL with water. After flushing for 15 minutes with N_2, the vial was heated at 100°C for 40 min. The radiochemical yield and purity were then analyzed by RP-HPLC.

Results and Discussion: The effects of addition of competitors on the labelling yield are shown in graphs 1 and 2 for ligands and organic solvents, respectively. Additions are expressed in amounts of Cu-equivalents.

In all experiments no labelled side-products were observed. While addition of EtOH or DMF (even up to 70 vol% - data not shown) has no lowering effect on the labelling yield, an amount of only 50 μL of the organic solvent ACN decreases the labelling yield already to 50%.





Conclusion: ACN and pyridine form a complex with Cu^+ and thus inhibit to a certain extent the nucleophilic, Cu^+-catalyzed radioiodination reactions. EtOH and DMF have no binding affinities for the cuprous ion, and do not interfere the labelling reaction. The selective complexation of the cuprous ion by some organic solvents is well-documented in literature. As such EtOH and DMF can be used in mixed-solvent conditions for the labelling of water-insoluble substrates. The nitrogen donor atom is a 'softer' atom than oxygen with better complexing properties and often present in coordinating groups or ligands.

Keywords: Radioiodination, Copper(I)-Catalyzed

P184 RADIOIODINATED OF TETRHYDROPALMATINE AND ITS BIODISTRIBUTION IN MICE

H. YU [1,2], C. TAN [1], X. LIN [1], L. ZHANG [1], B.O. CHENG [1], G. CAO [1] and Z. WANG [2]

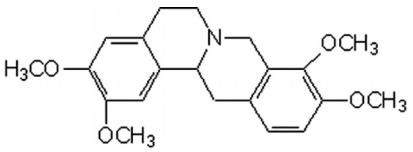
[1]Department of Research, Jiangsu Institute of Nuclear Medicine, Wuxi, Jangsu, China; [2]School of Chemical and Material Engineering, Southern Yangtze University, Wuxi, Jiangsu, China

Introduction: Tetrahydropalmatine (THP,See Fig), an active principle isolated from the natural product Corydalis yanhusuo, possesses pharmacological activities, such as analgesic, sedative, tranquilizing, hypnotic, antihypertensive and hypo-locomotion actions. A range of analytical methods are required for the measurement of the drug and in biological fluids and organs. However, TLC lacks quantitative precision, while HPLC presents lower efficient and time-consuming. Therefore, it is necessary to establish a rapid and effective method for quantitative analysis of THP.

Radioiodination is one of the extensively used methods for labeling compounds containing nucleophilic group. The principle of labeling is based on the "in situ" oxidation of iodide to atomic iodine and its substitution into phenol rings located in the ortho position of nucleophilic group, such as the hydroxyl group. This paper describes the preparation and pharmacokinetics in mice of radioiodinated THP.

Experimental: The preparation of radioiodinated THP was carried out by Chloramine-T method using iodine-131. The radiolabeled compound was characterized by polyamide TLC with Hexane: chloroform:methanol=2:3:0.5(V/V)and isolated using extraction with chloroform. Biodistribution studies were carried out in KM mice. At designated time after i.v. administration(5μci/200μl of 131I-THP), the animals(n=6 for each time point) were sacrificed. Blood samples and tissues of interested were removed, weighted and radioactivity was counted. The percent injected dose per gram (%ID/g) was calculated for each sample.

Results and Discussion: The radiochemical yield of ^{131}I-THP was about 75% and its radiochemistry purity exceeded 97% after extraction. Three weeks later, the radiochemistry purity maintained 95%. Its biodistribution displayed high uptakes and slow clearance in fat and brain with 3.2 and 1.7%ID/g at 5 min, 2.7 and 1.3%ID/g at 30 min post injection, respectively. Among the brain, ^{131}I-THP revealed a proceeding higher uptake in the parietal lobe and cerebellum throughout the time course (2.1 and 2.0%ID/g at 5 min, 2.6 and 2.3%ID/g at 10 min, respectively).



Conclusion: These preliminary results suggest that THP, is hydrophobic and can penetrated the intact blood–brain barrier, labeled with radiotracers has great promise to assess quantitative trace information on physiological effects in vivo.

Keywords: Tetrahydropalmatine, Radioiodinated, Biodistribution

P185 RADIOIODINATION AND ASTATINATION OF BIOMOLECULES VIA TRIAZENE

J. KADERAVEK [1], L. LESETICKY [1], J. KOZEMPEL [1] and O. LEBEDA [2]

[1]Organic and Nuclear Chemistry, Charles University in Prague, Prague, Czech Republic; [2]Nuclear Physics Institute, Academy of Sciences of the Czech Republic, Rez near Prague, Czech Republic

Introduction: The triazene group decomposition - the Wallach's reaction - offers a mild method for radiohalogene atom (^{18}F, ^{123}I, ^{211}At...) incorporation in modified aromatic ring. This reaction requires an acid catalyst, but for complicated bioactive molecules (monoclonal and/or polyclonal antibodies, larger peptides...) the presence of an acid could be fatal, and then can be an cation exchange resin, which is more fine, as an acid catalyst used. The presence of an aromatic ring bearing the triazene group in a bioactive molecule can be accomplished by simple active ester conjugation with an amino- group in the bioactive molecule.

Experimental: Radioiodination and astatination reactions were carried out. As the model substrate were 2-piperidino-1-diazenyl benzoate glycine ethyl ester, 2-piperidino-1-diazenyl benzoate phenylalanine methyl ester and 2-piperidino-1-diazenyl benzoate phenylalanyl-leucyl-glycine ethyl ester used, and the astatination reaction was accomplished with modified IgM.

Typical astatination procedure: To the solution of ^{211}At (2 μl, 2 MBq) in 100 μl of dry acetonitrile was added 0,5 mg of sodium sulphite. After 5 minutes apropriate substrate (0,03 mmol) and cation exchange resin (20 mg) or TFA (5 μl, 0,05 mmol) were added too. TLC analysis was accomplished after 30 minutes of sonication with autoradiographic scanner.

The complete synthesis sequence is shown in Figure 1.

Results and Discussion: The radiohalogenation and astatination of modified bioactive molecules were carried out with satisfactory radiochemical yields. Both types of acid catalysis can be used, the cation exchange resin in H^+ form offers very mild condition for sensitive molecules.

Conclusion: Wallach's acid catalyzed triazene group decomposition with halogen species X^- incorporation into an aromatic ring was accomplished with relatively high (radio)chemical yields. The application of sodium sulphite as reducing agent in astatination reaction increased radiochemical yield remarkably (85–88%). This fact speaks for astatine speciation changes – other astatine forms (At^+, At^{3+}, At^{5+} ...) were reduced to their nucleophilic form At^-, needed for Wallach's reaction.

Keywords: Labelling, Radioiodination, Astatination, Triazene, Wallach's Reaction

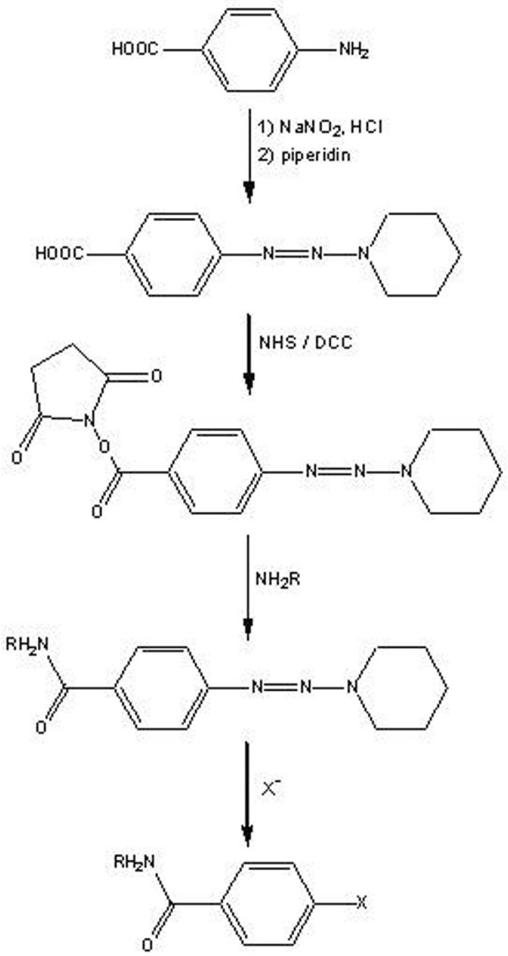


Fig. 1

P186 CHEMICAL AND BIOLOGICAL EVALUATION OF ^{153}Sm AND ^{166}Ho COMPLEXES WITH TETRAETHYLESTER OF DOTP (DOTP^{OEt})

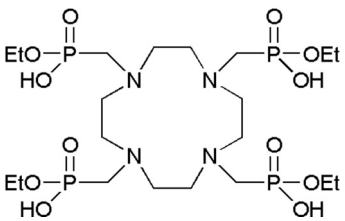
M. FÖRSTERVÁ [1,2], F. MARQUES [3], Z. JANDUROVÁ [2], L. GANO [3], P. HERMANN [2], F. MELICHAR [1] and I. SANTOS [3]

[1]Radiopharmaceutical Department, Nuclear Physics Institute, AS CR, Rez, Czech Republic; [2]Department of Inorganic Chemistry, Univerzita Karlova, Prague, Czech Republic; [3]Química Inorgânica e Radiofarmaceutica, ITN, Sacavém, Portugal

Introduction: Bifunctional ligands such as DTPA or DOTA have been used in radiotherapy and radiodiagnosis for a long time. A key parameter for these applications is a fast and efficient complexation of a suitable radioisotope. Acyclic DTPA forms complexes immediately; complexation with macrocyclic DOTA is much slower. However, complexes with macrocyclic ligands present better kinetic and thermodynamic stability. In general, the most suitable central ions for DOTA-like ligands are trivalent lanthanides and indium. The ^{153}Sm and ^{166}Ho isotopes were selected for this study due to their decay properties which are convenient for radiotherapy. In our DOTA derivative, acetates were replaced by four methylphosphonic acid monoethyl ester pendant arms (DOTP^{OEt}).

Experimental: DOTP^{OEt} was synthesized by Mannich reaction and alkaline hydrolysis. ^{153}Sm and ^{166}Ho were produced by thermal neutron bombardment of isotopically enriched $^{152}Sm(NO_3)_3$ or natural $Ho(NO_3)_3$, respectively. The properties of the radioactive complexes were evaluated through the study of: complexation conditions, *in vitro* stability, charge, lipo/hydrophilicity, human serum protein binding, hydroxyapatite binding and biodistribution in CD-1 Charles River mice. The radiolabelling efficiency and stability evaluation of the radiocomplexes were accomplished by ascending ITLC using silica gel strips. Radioactive distribution on the ITLC strips was detected using a γ detector coupled to a radiochromatogram scanner. The radioactivity in other samples was measured by a γ counter.

Results and Discussion: The ligand under study exhibits a fast complex formation at optimized conditions (pH 6, 40°C, 10 min). Resulting complexes are stable both *in vitro* and *in vivo*. Both complexes are highly hydrophilic with an overall negative charge and no significant human serum protein or hydroxyapatite binding. ^{153}Sm-DOTP^{OEt} complex shows a rapid clearance from most organs including blood and muscle and rapid total excretion.



Conclusion: The fast complexation rate, stability and the biological behaviour of ^{153}Sm/^{166}Ho –DOTP^{OEt} make these complexes promising as potential therapeutic agents when linked to a carrier biomolecule to target selectively a diseased site.

Acknowledgement: We thank AS CR, GRICES and COST D38 Action for support.

Keywords: Phosphonate Complexes, Macrocyclic Complexes, 166Ho and 153Sm, Biodistribution, Dota Analogues

P187 SYNTHESIS AND EVALUATION WITH MICROPET OF L-6-(^{124}I)IODODOPA AS A POTENTIAL PET IMAGING AGENT

P. PANICHELLI [1], D. MARTINI [1], M. COLONNA [1], L. CASTIGNANI [1], C. MALIZIA [1], G. VALENTINI [1] and C. NASUTI [2]

[1]ACOM, Montecosaro, Macerata, Italy; [2]Biology, University of Camerino, Camerino, Macerata, Italy

Introduction: 6-[^{18}F]Fluoro-3,4dihydroxy-L-phenylalanine(6-[^{18}F]F-L-DOPA)is normally used as useful radiopharmaceutical for evaluating cerebral dopamine metabolism in human and neuroendocrine tumors with positron emission tomography. At the same,6 [^{124}I]I-L-DOPA appears to be a suitable PET tracer for the same selective measurement of cerebral DOPA transport, having a low affinity for dopamine metabolism.

Experimental: L-6[^{124}I]IODODOPA was synthesized using three different precursor:

- Regioselective radioiodination of N-trifluoroacetyl 3,4-dimethoxy-6-trifluoroacetoxymercurio-L-Phenylalanine-ethyl ester under no carrier added condition gave L-6[^{124}I]IODODOPA
- By the exchange of 124I for bromine on L-6-bromodopa
- Regioselective radioiododestannylation of N-Formyl-3,4-di-tert-butoxycarbonyloxy-6-(trimethylstannyl)-L-phenylalanine ethyl ester under no carrier added condition gave L-6[^{124}I]IODODOPA

The in vivo evaluation is performed using these three different precursor in three groups of nude mice (5 mice each group) with subcutaneous tumor-tissue injection. We executed biodistribution study using YAPPET micropet system and autoradiography.

Results and Discussion: The labelling efficiency was in the three different labelling, respectively:

- More than 85%with radiochemical purity of over 95%.(for the iododermercuration).
- About 50% with radiochemical purity of over 95% (in the exchange of bromodopa).
- About 40% with radiochemical purity of 99% (for the iododestannylatyon).

L-6[^{124}I]IODODOPA showed high brain accumulation and rapid blood clearance. The mice slices studies indicates high affinity of L-6[^{124}I]IODODOPA for carrier-mediated and stereoselective active transport system. The tissue homogenate analysis revealed that most of the accumulated radioactivity was intact L-6[^{124}I]IODODOPA.

Conclusion: The best labelling efficiency is by iododemercuration. L-6[^{124}I]IODODOPA appears to be a suitable PET tracer for the selective measurements of cerebral L-amino acid transport and for the studies of neuroendocrines tumors. In principle, the application of an easier labelling, a shorter time of synthesis, and a major radiochemical yield, could suggest to produce radio-halogenated radiopharmaceuticals with more long lifetime and with the same ability for tools of diagnosis with PET.

Keywords: 124I, Iododopa, Radioiodination, MicroPET, Fluorodopa

P188 MULTIMERIC DOTA SCAFFOLDS FOR THE CONJUGATION TO PROTEINS

S. KRÄMER [1], C. WAENGLER [1], M. EISENHUT [2], U. HABERKORN [1] and W. MIER [1]

[1] Abteilung für Nuklearmedizin, University of Heidelberg, Heidelberg, Germany; [2] Radiopharmaceutical Cemistry, German Cancer Research Centre (DKFZ), Heidelberg, Germany

Introduction: In addition to the tissue specific uptake, the therapeutic success of a tumor specific agent is determined by the cytotoxic effect that can be attained per carrier molecule. The number of receptors expressed on the tumor tissue is limiting the amount of conjugate, that can be administered. If the protein causes pharmacological effects, only very small amounts of protein may be tolerated. Hence the efficacy of the conjugate is determined by the number of the β-emitters per carrier molecule. In addition to the efforts being made to ameliorate the targeting of the protein, the transport capacity of the carrier should thus be increased. We have recently developed a strategy for the conjugation of several DOTA chelates per peptide carrier (Mier W, Graham KAN, Wang Q et al. Synthesis of peptide conjugated chelator oligomers for endoradiotherapy and MRT imaging. Tetrahedron Lett 2004; 45: 5453-5).

Experimental: The syntheses of four different p-nitrobenzoyl-[Lys(Mtt)]n-resins (n = 1,3,6,9) was performed by Fmoc solid-phase peptide synthesis on a Rink Amide AM Resin using HBTU/DIPEA activation. After selectively removing the Mtt-protecting groups with 2% TFA in DCM, tris-tBu-DOTA was attached to the lysine side chains. The products were detached from the resins and deprotected by TFA treatment. After HPLC purification, the aromatic nitro groups were converted into amino groups by catalytic hydrogenation using a 20% Pd/C catalyst. The reaction of the amines with thiophosgene yielded the corresponding isothiocyanates.

Results and Discussion: We were able to synthesize p-isothiocyanatobenzoyl-[Lys(DOTA)]n-amides with one, three, six or nine lysine spacers in acceptable yields (6-31% overall) at high purity. As the best results for the hydrogenation step can be obtained with a heterogene catalyst the reductions had to be performed in solution. Preparative HPLC was required to obtain the pure intended compounds. The purity of the desired products was determined by HPLC and ESI mass spectrometry.

Conclusion: The synthesis of multimeric chelators is challenging. It could be shown that DOTA oligomers can be obtained by solid phase synthesis protocols. The activation with thiophosgene can be performed in solution to yield the activated isothiocyanates. These products are stable when stored at -18°C. Conjugation and labelling reactions with tumour targeting proteins are currently in progress.

Keywords: Targeting, Chelator Oligomers, DOTA

P189 ^{177}LuDOTMP – A NEW ALTERNATIVE TO METASTATIC BONE PAIN PALLIATION? FORMULATION STUDIES

M. CHANDIA[1], A. KETRING[2] and C. CUTLER[2]

[1]Comision Chilena de Energia Nuclear, Santiago, Chile; [2]Missouri University Research Reactor, MO, USA

Introduction: The bisphosphonates (BPh) remain the most widely used and effective antiresorptive agents. Moreover, in recent years numerous derivatives of tetraphosphonates or cyclic compounds with incorporated phosphonate functionalities in their structures have been prepared because of their widespread interest in biomedicine.The therapeutic properties of β emitters such as ^{153}Sm, ^{166}Ho and ^{177}Lu have been reported in the literature. The radioisotope ^{177}Lu has nuclear properties, short beta penetration range, long half-life which accommodates transportation to remote areas and a huge cross section that facilitates production in most research reactors, that make it suitable for use in the palliative therapy of pain due to skeletal metastasis.

Aim: To compare the chemical and stability of DOTMP (1,4,7,10-cyclododecyltetraaminetetramethylenephosphonic acid) and EDTMP (ethylenediamine tetramethylenephosphonic acid) radiolabeled with ^{177}Lu.

Experimental: ^{177}Lu in 0.05M of HCl was produced at the Research Reactor Center at the University of Missouri-Columbia. Radiolabeling of DOTMP and EDTMP with ^{177}Lu with varying ligand concentrations at different pHs (5.0-11.0) and temperatures (25°C-100°C) was evaluated to determine which would result in the highest yield and stability. Quality Control methods were investigated comparing ITLC-SG and small columns containing sephadex that separates the radiolabeled complex from free metal and radiocolloid. Stability of the complexes was determined by a hydroxyapatite challenge and in rat serum. Biological distribution at 15 min, 30 min, 120min, 240 min and 1440 min was obtained.

Results and Discussion: High radiochemical purity was achieved as a function of pH and varying the ratio of ligand to metal, range of 98.75% and 99.94%. The compound DOTMP was stable out to 6 days. The comparative results showed the labeling and stability of DOTMP to be better than those for EDTMP when labeled with ^{177}Lu. Quality control was most accurately and easily determined by utilizing the sephadex column. TLC's analysis resulted in streaking and complex decomposition and thus were not reproducible or reliable in our hands.

Conclusion: It is possible to produce tetraazacyclododecanes labeled with ^{177}Lu with a chemical purity over 99.5%. The method is easy, fast and the best results were obtained for pH between 7.0-9.0; the product ^{177}Lu-DOTMP has excellent stability in vitro, can be made in high yields with lower ligand:metal ratios and exhibits higher bone uptake in comparison with ^{177}Lu-EDTMP. Quality control is best determined utilizing a sephadex column.

Acknowledgement: At International Atomic Energy Agency Fellowship Code No: CHI05001.

Keywords: ^{177}Lu, Radiopharmaceuticals, Therapeutic, DOTMP, EDTMP

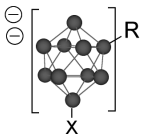
P190 PREPARATION, RADIOIODINATION AND EVALUATION OF AMINE- AND CARBOXYLATE-CONTAINING DECAHYDRODECABORATE(2-) DERIVATIVES TO MINIMIZE RESIDUALIZATION IN TISSUES

D.S. WILBUR, M.K. CHYAN, D.K. HAMLIN and M.A. PERRY

Radiation Oncology, University of Washington, Seattle, WA, USA

Introduction: Studies have shown that the decahydrodecaborate(2-) moiety, **1**, can be used as a pendant group for rapid and efficient radiohalogenation of biomolecules. Importantly, astatination of biomolecules using conjugates containing **1** provides radiolabeled molecules that are stable to in vivo deastatination. Studies have also shown that the decaborate(2-) cage molecules can be retained (residualized) in tissues. This is problematic with regards to radiation doses to tissues such as kidney and liver. In this investigation several derivatives of decaborate(2-) were synthesized, radioiodinated, and evaluated in biodistribution studies to determine if amine or carboxylate functionalities affect retention in tissues.

Experimental: **1** was carbonylated by reaction with oxalyl chloride to give **2**. **3** was prepared by reaction with CH_3CN and acid, followed by hydrolysis of the resultant acetamide. **2** was modified by reaction with amine-containing compounds to provide **4**, **5**, **7–10**. **3** was modified by reaction with succinic anhydride to form **6**. Decaborate derivatives **4–10** were radioiodinated using chloramine-T in 5% HOAc/H_2O or N-chlorosuccinimide in 5% HOAc/MeOH. Biodistributions of radioiodinated derivatives were conducted in athymic mice at 1 & 4 h pi.



● = B or BH

1. R = H; X = H
2. R = CO^+; X = H
3. R = NH_3^+; X = H

X = H or ^{125}I

4. R = $CONHCH_2CO_2H$
5. R = $CONHCH_2CO_2Me$
6. R = $NHCOCH_2CH_2CO_2H$

7. R = $CONHNH_2$
8. R = $CONHCH_2CH_2NMe\text{-}CH_2CH_2NH_2$
9. R = $CONHCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$
10. R = $CONH(CH_2)_3(OCH_2CH_2)_3CH_2NH_2$

Results and Discussion: Syntheses of **2**-**10** were readily conducted based on literature procedures. Radioiodinations gave isolated yields of 10–80%. Blood concentrations of the radioiodinated compounds ranged from 1%ID/g (**4**) to 6.4%ID/g (**6**) @ 1h pi and 0.06%ID/g (**6**) to 0.28%ID/g (**8**) @ 4 h pi. None of the compounds were retained in lung, kidney or spleen. Most of the compounds had relatively rapid clearance from liver. However, the ester **5** had significant retention in liver (10.7%ID/g @ 1h vs. 7.75%ID/g @ 4h). The neutral compound, **8**, also had slow release from liver (7.86%ID/g @ 1h vs. 3.75%ID/g @ 4h). All radiolabeled compounds were very stable to in vivo dehalogenation.

Conclusion: Most of the amine and carboxylate-containing derivatives cleared rapidly through the renal system, with no apparent residualization in tissues at times studied. Of the compounds studied, the glycine adduct **4**, tetramine adduct **9** and trioxadiamine adduct **10** have the most favorable pharmacokinetic behavior. Continuing studies will be conducted to derivatize the decaborate(2-) compounds for biomolecule conjugation.

Acknowledgement: We thank NIH (1R01 CA113431) for funding the studies.

Keywords: Radiohalogenation, Decaborate Derivatives, At-211, In Vivo Stability, Biomolecule Pendant Groups

P191 A SINGLE PROSTHETIC GROUP FOR LABELING BIOMOLECULES WITH TECHNETIUM AND IODINE

T. GULLON [1], A.E. GREEN [1], A.F. ARMSRTONG [1], P.W. CAUSEY [2], A.S. LOUIE [1] and J.F. VALLIANT [1]

[1]Chemistry, McMaster University, Hamilton, ON, Canada; [2]McMaster Nuclear Reactor, Hamilton, ON, Canada

Introduction: Designing targeted radiopharmaceuticals often entails the use of a bifunctional prosthetic group or chelate to covalently link radiohalogens and radiometals respectively to the targeting vector of interest. If a situation arises where it is desirable to change the nature of the isotope between the two classes (halogen and metal), then it is often necessary to switch the nature of the bifunctional ligand. It would be advantageous to have access to bifunctional synthons that can be labeled with both radiometals and radiohalogens.

Results and Discussion: Our group has developed a strategy for preparing Tc and Re organometallic complexes of carboranes in water at the macroscopic and tracer levels.[1] Carboranes are clusters consisting of carbon, boron and hydrogen that bind Tc and Re in a manner similar to that of cyclopentadiene. Using microwave heating, Tc and Re complexes of a range of carborane derivatives can be synthesized directly from $[M(CO)_3(H_2O)_3]^+$ (M= Re, Tc).[2] This includes mono- and di-substituted compounds containing aryl, pyridyl, amino and carbohydrate substituents. Following a procedure recently reported by Wilbur et al.[3], these carborane ligands can also be labeled with radioiodine to form stable species containing robust B-I bonds.

Carboranes are one of the very few synthons that can be labeled with both radiometals and radiohalogens without significantly modifying the core ligand system. The synthesis of a novel series of Tc labeled and iodinated carborane derivatives was achieved in good to excellent yield. Of particular focus was the synthesis and radiolabeling of carbohydrate and aryl carborane derivatives that can be used as synthons for the preparation of targeted radiopharmaceuticals.

Conclusion: Carboranes, including bi- and tri-functional derivatives, can be labeled with both technetium and iodine in high yield making the clusters highly versatile synthons for the preparation of targeted radiopharmaceuticals.

Acknowledgement: NSERC of Canada for funding.

References: [1] Sogbein O.O., Merdy P., Morel P. and Valliant J.F. *Inorg. Chem.* **2004**; *43*:3032-4. [2] Green A.E.C., Causey P.W., Louie A.S., Armstrong A.F., Harrington L.E. and Valliant J.F. *Inorg Chem* **2006**; *45*:5727-9. [3] Wilbur D.S., Hamlin D.K., Chyan M., Kegley B.B., Quinn, J. and Vessella R.L. *Bioconjugate Chem.* **2004**; *15*:601-16.

Keywords: Technetium, Iodine, Carboranes, Bioconjugation

P192 PREPARATION AND ANALYSIS OF 2-,3-,4-^{211}At-BENZENE DERIVATIVES BY Cu^{+}-ASSISTED HALOGEN EXCHANGE

S.S. SRIYAPUREDDY, G.J. MEYER, D. KRULL, K.H. MATZKE and W.H. KNAPP

Klinik f. Nuklearmedizin, Medizinische Hochschule Hannover, Hannover, Germany

Introduction: Astatine forms relatively weak aliphatic carbon bonds. Therefore astatinated benzene derivatives have usually been used as linkers in biologic applications to enhance the stability of astatinated peptides. The most prominent linker used so far is 3-211At-succinimidyl-benzoate (SAB), which is usually prepared by electrophilic substitution from the corresponding trimethyl-tin precursor. In order to evaluate different linker systems we aimed for the production and identification of 2-,3-, and 4- 211At-benzoic acid, 2-,3-, and 4- 211At-benzaldehyde, 4-211At-nitrobenzene and 2-, and 4- 211At-phenylalanine.

Experimental: 211At was produced at the cyclotron of the MHH and isolated by means of a dry-distillation method from the target. The production of all astatinated benzene derivatives was achieved by Cu+ assisted nucleophilic halogen exchange reaction from the corresponding iodine compounds at 120°C for 60 min under strong reducing conditions. All iodinated precursors, except o-I-phenylalanine, were purchased commercially. o-I-phenylalanine was prepared from o-Br-phenylalanine using the same reaction procedure and was purified by means of semi preparative HPLC. Identification of all astatinated compounds was carried out by analysis of the elution sequence of the corresponding F-, Cl-, Br-, and I-benzene derivatives. For comparison 3-211At-benzoic acid and 4-211At-nitrobenzene were prepared by destannylation of the corresponding trimethyl-tin derivatives also, using 30 min reaction time with no addition of either oxidizing agents or acid.

Results and Discussion: The Cu+ assisted nucleophilic halogen exchange resulted in radiochemical yields of 80%±10% for all 211At-benzene derivatives. Destannylation reactions gave yields of 70±15%. After HPLC-purification the final products were obtained with > 99% purity. Because of some losses during purification the final yield was approximately 60MBq from 100MBq 211At. HPLC-analysis showed that the elution of o-211At-benzaldehyde and o-211At-phenylalanine followed the elution sequence of ortho-halogenated benzaldehydes and ortho-halogenated phenylalanines (F<Cl<Br<I<At), however the elution of all other 211At-benzene derivatives did not follow the elution sequence of their homologous derivatives (F<Cl<Br<I) but eluted before the homologous iodine compound.

Conclusion: The nucleophilic Cu+ assisted halogen exchange reaction is very effective for the astatination of deactivated benzene derivatives. The method provides sufficient yields for therapeutic labelling applications. 2-, and 4-211At-phenylalanines are substrates for the amino acid transporter at Glioma cells and therefore should be further evaluated for therapeutic applications.

Keywords: 211 Astatine, Labelling of Benzene Derivatives, Cu Assisted Halogen Exchange, Nucleophilic Substitution