P339 IMPROVED GAS PHASE PRODUCTION OF [11C]CH3I BY I2-CONCENTRATION-CONTROL

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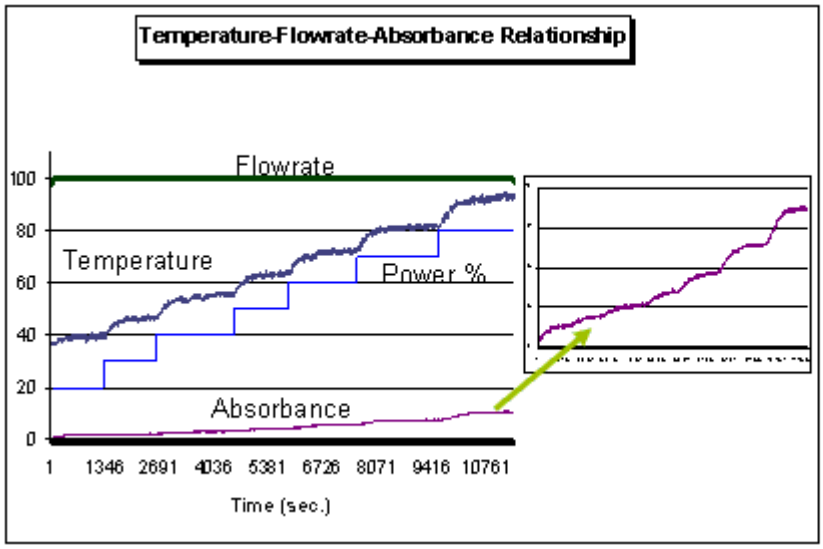
Objectives: ^{11}C-methyl iodide has been so far the most important starting material in the production of ^{11}C-labelled tracers used in investigations with Positron Emission Tomography (PET). The most commonly used method for its production is the high temperature iodination of ^{11}C-methane in a circulating gas phase, in which the ^{11}C-methyl iodide is continuously trapped on a solid phase to prevent further iodination (Larsen et al., Appl. Radiat. Isot. Vol.48, No. 2. pp. 153-157. 1997). Since it is evident that yield is dependent upon the I_2 concentration in the vapour phase in the quartz tube (Link et. al., Nuclear Medicine & Biology, Vol. 24, pp. 93-97, 1997), we report here on the development of a spectroscopic method to determine this optimal iodine vapour concentration. The heating temperature of the oven, which sublimates the iodine, is automatically adjusted to achieve and keep this concentration during the reaction period.

Methods: The concentration of I_2 in the vapour phase was measured immediately behind the iodine oven using a UV/VIS Microspectometer Module. This spectrometer is equipped with a high-grade, low-noise silicon detector and is suitable for carrying out measurements in the spectral range of 350 nm - 850 nm. Blank measurements were carried out prior to the experiments and the background was subtracted from the spectra obtained. The absorbance of I_2 is measured in the range of 510-540 nm. The I_2 absorption measurement is continuously corrected for the absorption at 770-800 nm, to compensate for non iodine vapour contaminations of the glass tube.

Results: The relationship between the heating temperature of the furnace and the I_2 absorbance was measured (Figure 1).



The I_2 Absorbance was correlated to the I_2 concentration in the vapour phase by determining the weight loss of I_2 from the oven for a given time and flow rate together with the absorption. Thus at a flow rate of 50 mL/min during 1 h, an absorption of 0.2 corresponded with the sublimation of 30mg (0.24 mMol) of iodine. In a preliminary experiment at this concentration / absorption an ^{11}C-MeI yield of 30% (not corrected for halflife) was obtained 5 min. after EOB. The optimal concentration of iodine vapour will be determined.

Conclusions: Thus the amount of iodine used can be optimized and the quarzglass tube with iodine can be used as long as the optimal vapour phase iodine concentration is reached.

P340 NEW KIT-LIKE 68GA-LABELING STRATEGY FOR PROTEINS

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Objectives: Although ^{68}Ga-labeled compounds have gained broad interest in diagnostic imaging, only few examples of ^{68}Ga-labeled proteins and other macromolecules can be found. A reason therefor is the high temperature needed for the complexation reaction of radiometal ions using the commonly applied chelator DOTA. As these high temperatures are detrimental to protein molecules, labeling using DOTA precursors needs long complexation times not compatible with the short half-life of ^{68}Ga. However, prelabelling approaches are also quite intricate and unfavorable regarding the short half-life of ^{68}Ga. Other alternatives using NOTA- and HBED-CC-derivatives for the introduction of ^{67}Ga and ^{68}Ga into proteins showed either moderate radiochemical yields complicating the radiosynthesis and requiring a final purification step or require a complicated multistep synthesis for the chelator. Another important point is the determination of the number of the introduced derivatization sites per carrier molecule as a strong structural alteration generally leads to a dramatic loss of biological activity. However, in the case of the above mentioned chelators, the number of derivatization sites is difficult to determine which further limits their application in the ^{68}Ga-labeling of proteins.

Methods: Thus, a new labeling method based on the macrocyclic chelator NOTA was developed which allows the complexation of ^{68}Ga within very short time spans at room temperature. The newly developed, unprotected NOTA-derivative contains a thiol moiety for efficient and simple introduction into arbitrary proteins and could easily be introduced in high yields and under very mild conditions (pH 7.2, room temperature, 30 minutes) into several maleimide-modified proteins (hMAb 425, human, rat and bovine serum albumin, CRM mutant of diphtheria toxin and annexin V).

Results: As these proteins strongly differ in molecular size and structure, thiol-NOTA showed to be applicable for the derivatization of a high variety of carrier proteins to be labeled with ^{68}Ga. Using this NOTA-derivative, a well-defined number of derivatization sites could be introduced into the carrier molecule resulting in only minor structural modification and a highly preserved biological activity. The NOTA-derivatized proteins could be labeled with ^{68}Ga in a one-step reaction within very short time spans of 5 to 10 minutes at room temperature in high specific activities of 20 to 45 GBq/μmol. The radiochemical purities achieved were $\geq$95% which makes further purification steps dispensable.

Conclusions: As the labeling reaction of the derivatized protein is completed within maximal 10 minutes resulting in highly pure products without purification and as the NOTA-derivatized proteins can be stored in solution at 4°C for several weeks without changes of the radiolabeled products, they could serve as labeling kits for ^{68}Ga-labeled proteins.

P341 EVALUATING BIFUNCTIONAL CHELATES FOR THE DEVELOPMENT OF GA-68 BASED RADIOPHARMACEUTICALS

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Objectives: The generator produced isotope Ga-68 ($t_{1/2}$ = 68 min) is of increasing interest for the development of new PET radiopharmaceuticals. Bifunctional chelates (BFCs) that facilitate efficient, high specific activity radiolabelling with Ga-68 and yield complexes with superior in vivo stability are needed. To this end, we undertook a systematic comparison of 4 BFCs containing different chelating moieties; Oxo (1-oxa-4,7,10-triazacyclododecane-4,7,10-triacetic acid), PCTA (3,6,9,15-tetraazabicyclo [9.3.1] pentadeca-1(15),11,13-triene-3,5,9-triacetic acid), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and NOTA (1,4,7-triazacyclononane-1,4,6-triacetic acid).

Methods: Radiolabelling under mild conditions (aqueous buffer, room temperature) was optimized for both Ga-68 and the longer lived isotope Ga-67 ($t_{1/2}$ = 3.26 d). Reactions were analyzed by HPLC. Each BFC was compared with respect to reaction time, radiochemical yield and specific activity achievable. The stability of the radiolabelled BFCs was evaluated by incubating at low pH or in the presence of transferrin, an iron transport protein known to compete for Ga in vivo.

Results: High radiochemical yields (>95%) were achieved for Ga-67 and Ga-68 radiolabelling of each of the BFCs, but the reaction times with DOTA were longer. Increasing the specific activity by lowering the amount of BFC used in the reaction, lengthened the reaction times and decreased the radiochemical yield. This was most pronounced for Ga-68 radiolabelling of DOTA where radiochemical yields fell to <30% in 20 minutes, while the 3 other BFCs gave >75% radiochemical yield in less than 5 minutes under the same conditions. Stability studies showed > 50% loss of Ga to transferrin in less than one hour from both DOTA and Oxo, while NOTA and PCTA were relatively inert. Similar stability differences were seen when incubating at pH 2.

Conclusions: PCTA, Oxo and NOTA were all more efficiently radiolabelled with Ga compared to DOTA. Significantly improved stability was observed for NOTA and PCTA, especially with respect to transmetalation to transferrin. In vivo PET imaging and biodistribution studies are planned.

P342 FUNCTIONALIZED CYSTEINE DERIVATIVES FOR LABELING TC(CO)3

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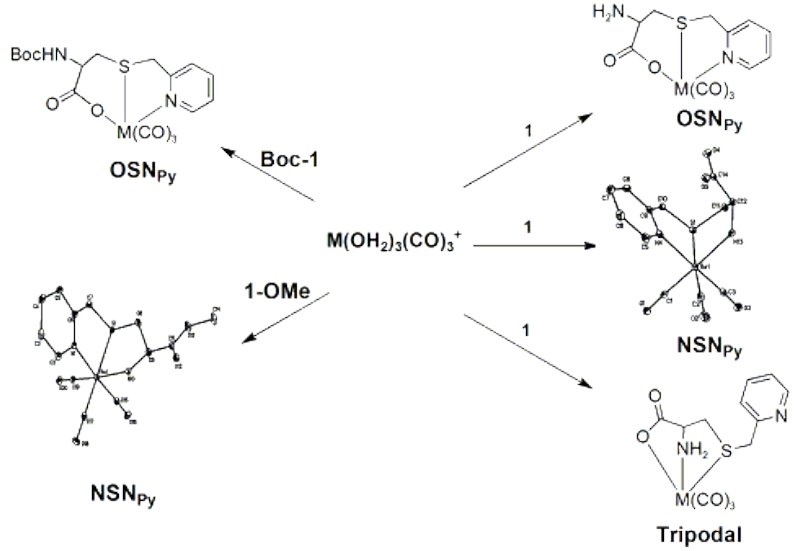
Objectives: Interest in developing new ligands capable of complexing fac-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) and coupling to biomolecules in a facile manor led the investigation of a new ligand system S-(pyridin-2-ylmethyl)-L-cysteine, 1. Ligand 1 has the unique potential of forming multiple coordination species with Re and ^{99m}Tc. Three different tridentate coordination modes were anticipated from the reaction mixture: one tripodal through the cysteine (ONS), and two linear including the S-pyridyl and cysteine (OSN_{Py}, NSN_{Py}).

Methods: Variations of S-(pyridin-2-ylmethyl)-L-cysteine ligands and Re complexes were prepared by standard synthetic routes and characterized by normal methods (NMR, elemental analysis, X-ray crystallography). ^{99m}Tc complexes were prepared by routine methods and compared to the Re complexes with UV/Vis and radiometric HPLC.

Results: From the examination of 1 with fac-$M(H_2O)_3(CO)_3^+$ (M = Re, ^{99m}Tc), only the NSN_{Py} complex was observed in the reaction mixture from the three possibilities. HPLC analysis of the reaction mixture showed a single peak, however, ^{1}H NMR evaluation yielded two linear isomers. Separation of the two species and X-ray characterization clearly indicated the formation of two species of fac-Re(NSN_{Py}-1)$(CO)_3^+$ due to the prochiral thioether and L-cysteine. These unexpected results with Re were further confirmed with protected versions at C and N terminus of 1 that specifically direct the coordination mode to either linear OSN_{Py} or NSN_{Py} complexes. Reaction of the protected OSN_{Py} ligand with Re did not yield the anticipated complex, however, the protected NSN_{Py} ligand yielded two isomeric complexes of fac-Re(NSN_{Py}-1-OMe)$(CO)_3^+$ as was observed with 1. These isomeric complexes were separated by HPLC and structurally confirmed as both complexes had intact methyl ester groups.



The S-(pyridin-2-ylmethyl)-L-cysteine ligands at 10^{-5}-10^{-6} M were also examined with $^{99m}Tc(CO)_3$. 1 and the protected NSN_{Py}-1 had similar labeling yields and chromatograms to the NSN_{Py}-Re complexes. Interestingly, the protected OSN_{Py} ligand was also labeled with $^{99m}Tc(CO)_3$, albeit with lower yields than the NSN_{Py} analogs.

Conclusions: The interactions of S-(pyridin-2-ylmethyl)-L-cysteine ligands with $Re(CO)_3$ showed a clear preference for NSN_{Py} coordination amongst the other possible modes (OSN_{Py} or tripodal). The ^{99m}Tc studies confirm the effectiveness of the ligands (>99%) at $<10^{-5}$ M of 1 and both of the protected versions of 1. Clearly, the differences in ligand interactions between Re and ^{99m}Tc are not as straightforward as anticipated.

P343 MICROWAVE ASSISTED CHELATION OF SN-117M WITH A SULFHYDRYL-REACTIVE DOTA DERIVATIVE FOR LABELING OF ANNEXIN V

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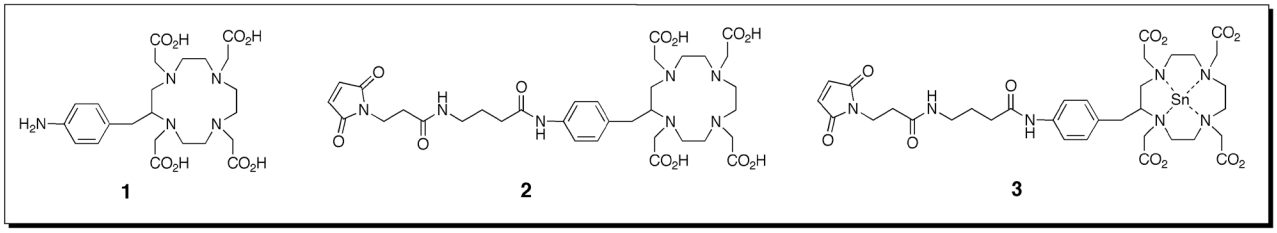
Objectives: The overall objective of the research effort was to obtain a method of labeling a protein-reactive DOTA derivative with Sn-117m for conjugation with annexin V. To accomplish that objective, it was necessary to: (1) determine conditions for labeling DOTA with non-radioactive Sn and with Sn-117m; (2) synthesize a protein-reactive DOTA derivative that was stable to the chelation conditions; (3) optimize the Sn-117m labeling conditions; and (4) conjugate the Sn-117m-DOTA derivative with annexin V.

Methods: Sn(IV) and Sn(II) reactions were conducted with aminobenzyl-DOTA, 1, under standard conditions and in a microwave reactor. Non-radioactive products were purified and characterized (NMR, MS) so they could be used as HPLC standards for Sn-117m labeled compounds. Labeling of DOTA derivatives with Sn-117m was evaluated using conditions that provided the non-radioactive products. A maleimido-DOTA derivative, 2, was synthesized and the Sn-117m labeling was evaluated using standard and microwave reactor conditions. The radiolabeling reaction mixtures were purified by HPLC. The isolated Sn-117m labeled maleimido-DOTA, 3, was reacted in PBS, pH 6.5, with a (TCEP pretreated) mutant of annexin V (#128,containing a single N-terminal cysteine) for 15 min, then purified over a Econo-Pac 10DG desalting column.



Results: Initial experiments with Sn(IV) chelation in 1 were conducted at 90°C under different buffer and pH conditions for 16 h. Unfortunately, these conditions gave no complex formation. However, use of Sn(II) in 0.05 N HCl at 90°C for 16 h provided an unstable complex, that when oxidized by H_2O_2, gave a new (stable) complex that had the correct mass for the desired compound. Optimal labeling of 2 with Sn-117m was obtained in 0.05 N HCl at 150°C for 2 min in the microwave. In one example, a 2.5 mCi reaction yielded 1.56 mCi (62%) of 3 after isolation from the HPLC effluent. Reaction of [Sn-117m]3 with annexin V and purification yielded 0.56 mCi (37%; 22% overall) of Sn-117m-labeled annexin V. This labeling procedure did not decrease the activity of annexin V in the RBC bioactivity assay.

Conclusions: A rapid microwave assisted method for labeling a maleimido-DOTA derivative with Sn-117m has been developed. Unlike chelation of non-radioactive Sn, H_2O_2 was not required to yield the stable complex. Reasonable yields were obtained for preparation of [Sn-117m]3, and modest yields were obtained for conjugation with annexin V. Additional optimization steps may provide higher overall yields.

Research Support: Research funding was provided by a gift from Clear Vascular, Inc., NY, NY. The Sn-117m was provided by Trace Life Sciences, Denton, TX.

P344 SYNTHESIS AND EVALUATION OF 99mTc(CO)3-GALALCTO-RGD

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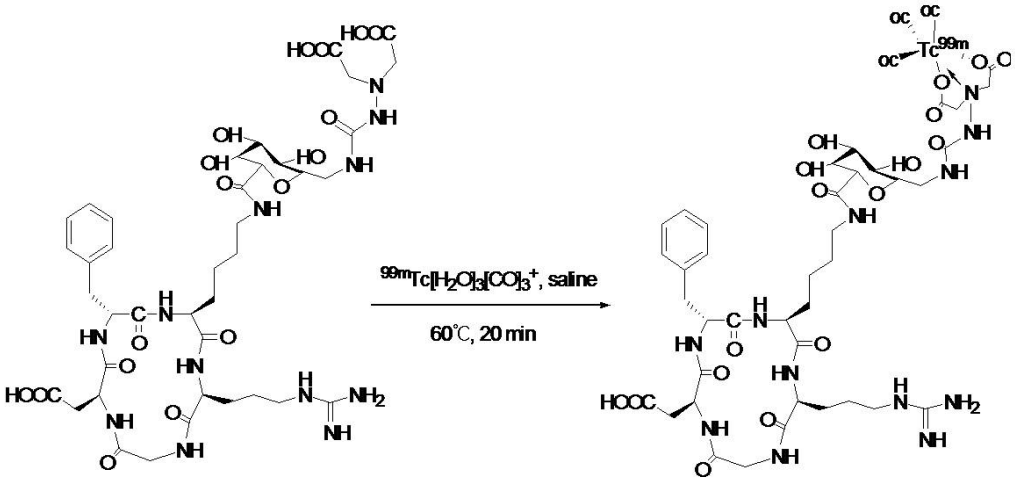
Objectives: Angiogenesis is a requirement for tumor growth and metastasis and integrin $\alpha_v\beta_3$ is highly expressed on the neovasculature of tumors. Radiolabeled arginine-glycine-aspartic (RGD) tripeptides have high affinity to integrin $\alpha_v\beta_3$ and they can be very useful radiotracer for noninvasive imaging of growing and metastatic tumors over expressed integrin $\alpha_v\beta_3$. In this study, we synthesized $^{99m}Tc(CO)_3$-galalcto-RGD as a derivatives of [^{18}F]galalcto-RGD and evaluated biological properties by in vitro tests.

Methods: The precursor was prepared in 43.8% yield in 4 steps from protected c(RGDfk) and $^{99m}Tc(CO)_3$-galacto-RGD was prepared with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and precursor(0.05~0.5 mg) at 60℃ for 20 min. After labeling, the reaction mixture was purified by HPLC. Labeling efficiency and radiochemical purity were determined by HPLC. For in vitro stability test, $^{99m}Tc(CO)_3$-galacto-RGD was added to 0.5 mL of saline and 0.5 mL of human serum and incubated at 37℃ to 6 hours. In vitro cell uptake test of $^{99m}Tc(CO)_3$-galacto-RGD was performed with A431(human epithelial carcinoma), LLC (melanomalewis lung carcinoma) and SCC7(squamous cell carcinoma) cell lines. We also prepared [^{18}F]galacto-RGD to compare in vitro tumor cell uptakes.

Results: The radiolabeling efficiency was 98.9±1.4% using 0.5 mg of precursor and radiochemical purity was higher than 98.0% and we have same radiochemical purity up to 6 hours in saline and plasma. $^{99m}Tc(CO)_3$-galacto-RGD showed 2.21, 2.16, 2.10% uptake for A431, LLC, SCC7 in the in vitro cell uptake tests and [^{18}F]galalcto-RGD also showed similar cell uptake results such as 2.08, 1.98, 2.10 for each cell line. [IMG1] [IMG2]

Conclusions: These results supported that $^{99m}Tc(CO)_3$-galacto-RGD is promising radiotracer for imaging of angiogenesis.



Scheme 1. Preparation of $^{99m}Tc(CO)_3$-galacto-RGD

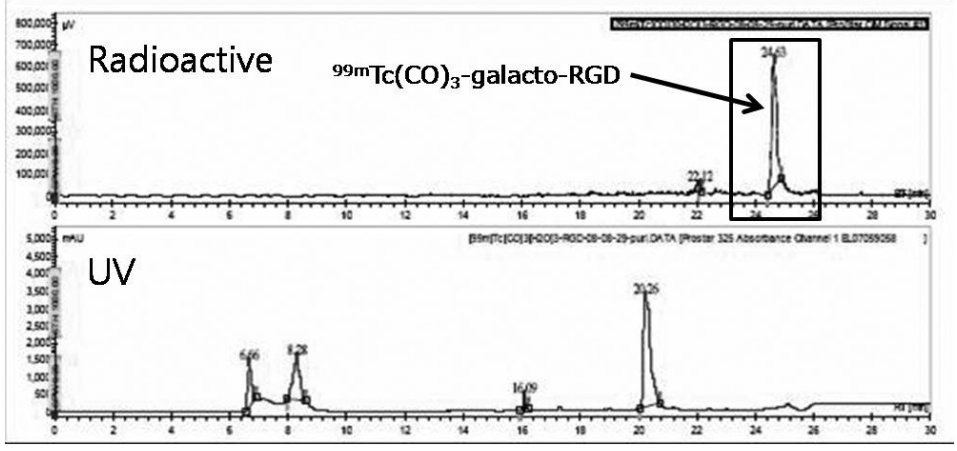


Fig 1. Chromatogram of purification

P345 MACROCYCLIC COMPLEXES OF 44/47Sc AS PRECURORS FOR RADIOPHARMACY

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Objectives: Two isotopes of scandium, ^{47}Sc and ^{44}Sc, are perspective radinuclides for radiotherapy and diagnostic imaging. ^{47}Sc decays with the half life of 3.35 days and maximum β^- energy of 600 keV. It also emits low-energy γ radiation (E_γ =159 keV) suitable for simultaneous imaging. The other scandium radionuclide - ^{44}Sc ($t_{1/2}$=3.92h) is an ideal β^+ emitter for PET diagnosis. It can be produced by ^{44}Ca(p,n)^{44}Sc nuclear reaction in small cyclotrons or as a daughter of long lived ^{44}Ti ($t_{1/2}$=60,4y) from ^{44}Ti/^{44}Sc generator. The goal of our work was to find the best ligands for attaching scandium radionuclides with biomolecules. Due to formation of thermodynamically stable and kinetically inert complexes the macrocyclic ligands were choosen: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-1,4,7 triacetic acid (NOTA), 1,4,7-triazacyclodecane-1,4,7 triacetic acid, (10 ane) 1,4,8-triazacycloundecane triacetic 1,4,8 acid (11 ane) and 1,5,9-triazacyclododecane 1,5,9 triacetic acid (11 ane).

Methods: For reasons of availability we used in our experiments the ^{46}Sc ($T_{1/2}$=83.8 d) - carrier added nuclide instead of ^{47}Sc and ^{44}Sc. Stability constants of scandium complexes were determined using HPLC method. Sc complexes were prepared by reacting macrocyclic ligands of 0.01 M concentration, respectively, with $^{46}ScCl_3$ in ammonium acetate buffer at pH=6.0. The stability constants were calculated from the ratio of Sc-L complex to free Sc peaks measured with γ detector. For comparison, in the same way stability constants of ^{177}Lu complexes were calculated. The kinetics of Sc-DOTA and Sc-NOTA complexes were measured at pH=6.0. Complex formation was determined by instant thin layer chromatography method using ITLC-SG strips developed with the mobile phase: H_2O/NH_3 (25/1).

Results: Sc^{3+} forms more stable complexes with DOTA ligand than Lu^{3+}. Also complexes of Sc with DOTA ($\log K_{Sc\text{-}DOTA}$= 27.5) are stronger by a few orders of magnitude than complexes with NOTA ($\log K_{Sc\text{-}NOTA}$=17.6) and 10 ane ($\log K_{Sc\text{-}10ane}$=14.8) ligands. The radiochemical yield of labeling (5.5 nmol of Sc^{3+} and 55 nmol of ligands) for Sc-DOTA is about 99% and it is much higher than for the Sc-NOTA complex (80%). The formation of the Sc-NOTA complex is faster than for Sc-DOTA complex. After 10 minutes the equilibrium for Sc-NOTA was reached, while for Sc-DOTA 30 minutes is needed for attaining equilibrium. Sc-DOTA and Sc-NOTA complexes exhibit high stability in human serum at 37^{0}C. After 120 hours of incubation in the serum more than 97% of Sc-DOTA remains in solution. In 0,01M PBS buffer Sc-DOTA is stable but in the case of Sc-NOTA, Sc-10 ane, Sc-11ane and Sc-12 ane complexes phosphates exchange ligands in first coordination sphere. It was found by HPLC method that Sc(DOTA) complex is more hydrophilic than Lu(DOTA) and Sc(NOTA), suggesting different coordination spheres in this complexes. The cation of Sc is smaller than Lu, so the peak of more hydrated Sc-DOTA complex appears before the peak of Lu-DOTA complex.

Conclusions: The presented results show that macrocyclic complexes of ^{44}Sc and ^{47}Sc radionuclides are attractive precursors for diagnostic and therapeutic radiopharmaceuticals.

Research Support: This part of work was carried out in the frame of grants from Ministry of Science and Higher Education in Poland no. DWM/N166/COST/2007 and N204 143 32/3547.

P346 HEPATIC ASIALOGLYCOPROTEIN RECEPTOR IMAGING USING 99mTc-DMP-NGA

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Objectives: Quantitative imaging of asialoglycoprotein receptors could estimate the function of the liver [1,2]. ^{99m}Tc labeled galactosyl-neoglycoalbumin(NGA) and diethylenetriaminepentaacetic acid galactosyl human serum albumin(GSA) have been developed for SPECT imaging and clinical used in Japan [3,4]. We have tried to improve the in vivo stability by derivatization of the neogalactosylalbumin with a limited number of dimercaptopropionyl (DMP) groups.

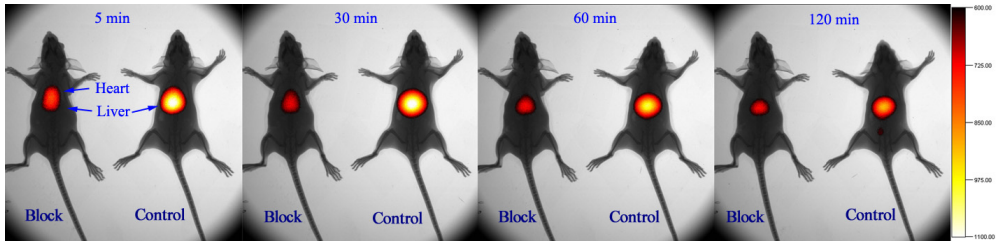
Methods: The NGA was treated with N-succinimidyl 2,3-di(S-acetylthio) propionate(SATP), and removal of the S-acetyl protective groups obtained the DMP-NGA as a novel neogalactosylalbumin derivative. DMP-NGA was directly radiolabeled with technetium-99m. Ex vivo biodistribution of ^{99m}Tc-DMP-NGA and blocking experiment (preinjecting with 10 mg/kg body weight free GSA as blocking agent) was investigated on normal mice. Images were acquired withKodak In-Vivo Imaging System FX Pro. Each mouse was injected 3.7MBq ^{99m}Tc-DMP-NGA. For comparison, the mouse with or without free GSA (10 mg/kg body weight) blocking were imaged at same conditions. For each radioautography image, regions of interest (ROIs) were drawn over each liver and the net intensity of each ROIs was calculated.

Results: The radio-labeling yield of ^{99m}Tc-DMP-NGA was about 70% (determined by ITLC) under optimized labeling conditions. After HPLC purification, the radiochemical purity of ^{99m}Tc-DMP-NGA was more than 98%. The in vitro stability study showed that it is stable over 6 h at room temperature. Ex vivo biodistribution showed that the liver accumulated (99.35±9.77)%, (74.25±3.03)% and (52.47±7.58)% of the injected dose per gram at 5, 30 and 120 min after injection, respectively.

The biodistribution of the ^{99m}Tc-DMP-NGA in normal mice. Expressed as % injected dose per gram (%ID/g). Each value represents the mean ± SD of five animals.

	5 min	5 min blocking	30 min	120 min
Heart	1.61±0.17	4.65±0.64	2.11±0.71	1.97±0.23
Liver	99.35±9.77	36.08±4.31	74.25±3.03	52.47±7.58
Lung	1.77±0.22	12.59±1.96	2.85±2.33	1.28±0.19
Kidney	3.01±0.19	9.83±1.00	5.14±0.64	6.31±1.21
Spleen	3.10±0.58	6.18±0.78	4.02±1.19	4.07±0.93
Stomach	1.58±2.00	0.84±0.16	5.53±7.40	4.00±3.16
Blood	1.04±0.05	31.72±1.24	0.89±0.19	0.61±0.01
Bone	4.28±0.94	7.91±1.92	5.39±1.03	5.54±1.08
Muscle	1.62±0.49	1.99±0.26	1.81±0.30	1.82±0.37
Intestine	2.21±0.88	3.58±1.48	7.41±5.73	3.83±1.82

The ratio of liver/kidney and liver/blood were higher than that of ^{99m}Tc-GSA. The liver uptake of ^{99m}Tc-DMP-NGA decreased obviously after block (36.08±4.31%ID/g at 5 min p.i. $P<0.0001$), indicating the specific binding to ASGP receptor. In vivo imaging studies also showed significant different liver uptake before and after inhibition with free GSA. The results of in vivo imaging studies showed that there have significant different with and without free GSA inhibition at 5, 30, 60, 120 min p.i., respectively. The net intensity ratios of normal and inhibited mice were 1.58, 2.14, 1.40 and 1.23 at 5, 30, 60 and 120 min p.i. respectively. This agreed with the result of blocking bioditribution study.



Conclusions: The bifunctional coupling agent DMP was introduced into NGA as a potential ASGP receptor agent. DMP-NGA was directly labeled with technetium-99m with moderate labeling yield. After purified with HPLC, ^{99m}Tc-DMP-NGA was get with high radiochemical purity and good in vitro stability. The promising biological properties of ^{99m}Tc-DMP-NGA indicated it could be used as a novel hepatocyte-targeting agent to evaluate hepatic function and clinical use in the future.

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P347 DESIGN, CHEMICAL SYNTHESIS AND BIOLOGICAL EVALUATION OF THREE SMALL AMPHIPHILIC RHEMIUM AND 99mTECHNETIUM TRICARBONYL COMPLEXES AS POTENTIAL IMAGING AGENTS

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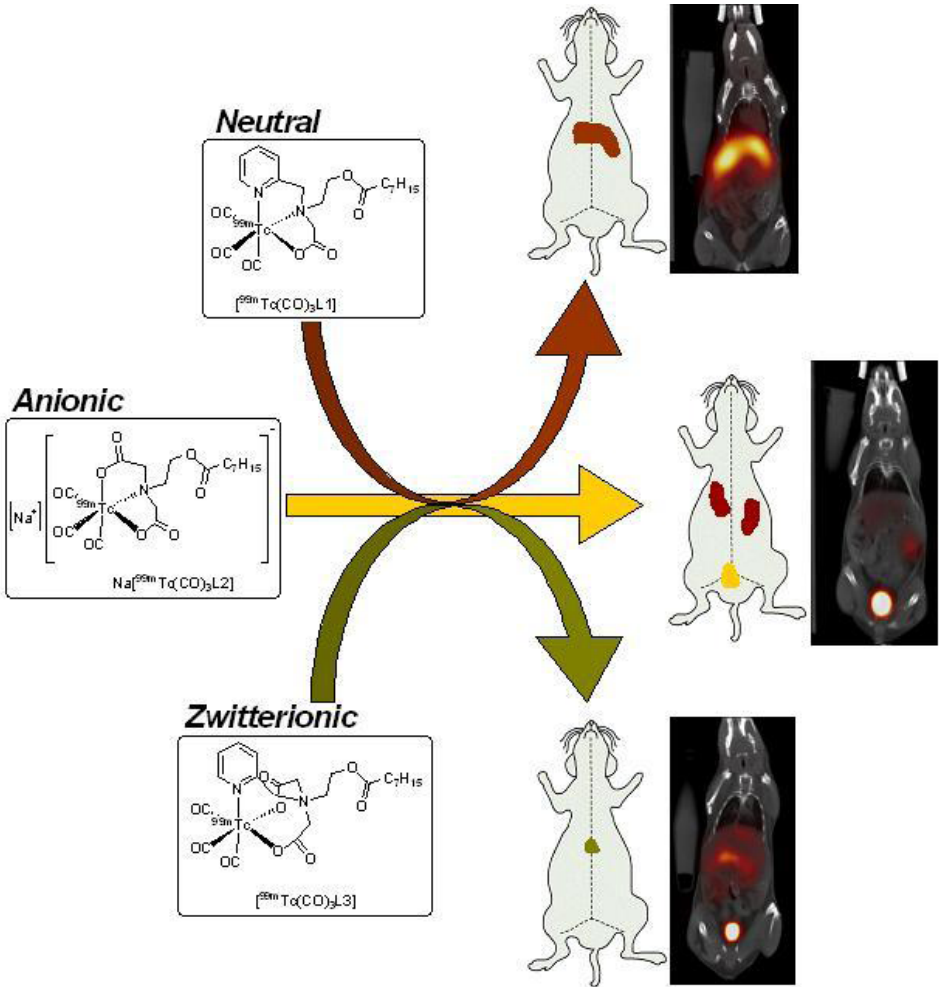
Objectives: Most recently, synthesis of novel imaging agents on the basis of ^{99m}Tc has shifted towards target-specific derivatives with one type of chelating entity for the $[^{99m}Tc(CO)_3]^+$ core, following the "Technetium-tagged" approach. In contrast to this, it was our aim to obtain and explore the in vivo properties of simple, small and non-specific "Technetium-based" amphiphilic metal complexes of the $[Tc(CO)_3]^+$ core. In an attempt to achieve this, the synthesis incorporated a mimic of the charge distribution of amphiphilic molecules essential to cell membranes. Exploring the in vivo behaviour of these compounds following a sparsely investigated approach of non-target-specific uptake of small amphiphilic molecules was of major interest.

Methods: All 3 ligand systems were synthesized via the free, C_8-chain-derivatized amine, subsequently converted by reductive amination and/or alkylation into the corresponding protected ligand, and in a final step deprotected and purified by prep. HPLC. The identity of all ligands was confirmed by standard characterization techniques. $Re(CO)_3$ complexes were synthesized and characterized as model compounds for the corresponding ^{99m}Tc complexes. The complexes $[^{99m}Tc(CO)_3L1]$ and $[^{99m}Tc(CO)_3L2]^-$ were prepared in high radiochemical yield through reaction of fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ with the ligands while $^{99m}Tc(CO)_3L3$ was produced in only 30% yield. Complex stability towards the naturally occurring amino acids cysteine and histidine was investigated. The subsequent HPLC analysis revealed no noticeable decomposition for any of the three complexes. The lipophilicity of the complexes was determined by evaluation of water/octanol partition coefficients.

Results: A tumor-bearing model was used to evaluate the imaging potential of $[^{99m}Tc(CO)_3L1]$, $[^{99m}Tc(CO)_3L2]^-$ and $[^{99m}Tc(CO)_3L3]$. Imaging was performed at four time points, biodistribution studies were performed after the final imaging time point. The in vivo distribution of $[^{99m}Tc(CO)_3L1]$ showed very fast localization of the complex in the liver, with clearance through the intestines, which is confirmed through the biodistribution data. For $[^{99m}Tc(CO)_3L2]^-$ fast processing of the compound through the kidneys, within the first 5-10 minutes after injection, and consequent localization in the bladder, followed by excretion was observable. Finally, for $[^{99m}Tc(CO)_3L3]$ 1 h post injection, high localization in the gall bladder was observed, paired with slow clearance through the intestines.

Conclusions: In conclusion, no significant tumour, brain or heart uptake was observed for any of the investigated compounds. Zwitterionic complexes not do show the same potential for myocardial uptake as do cationic complexes for this very purpose. Also, for a more efficient mimicking of phospholipid-type molecules, a technetium-tagged approach might be more suitable. Changing the charge of a small amphiphilic molecule results in highly contrasting, but still defined biodistributional behavior in an in vivo system is somewhat surprising and of potential importance in future applications.



P348 SYNTHESIS OF NOVEL 15 AND 16 C- FATTY ACID DERIVATIVES LABELED WITH [99MTcN(PNP)]2+ FOR MYOCARDIAL IMAGING

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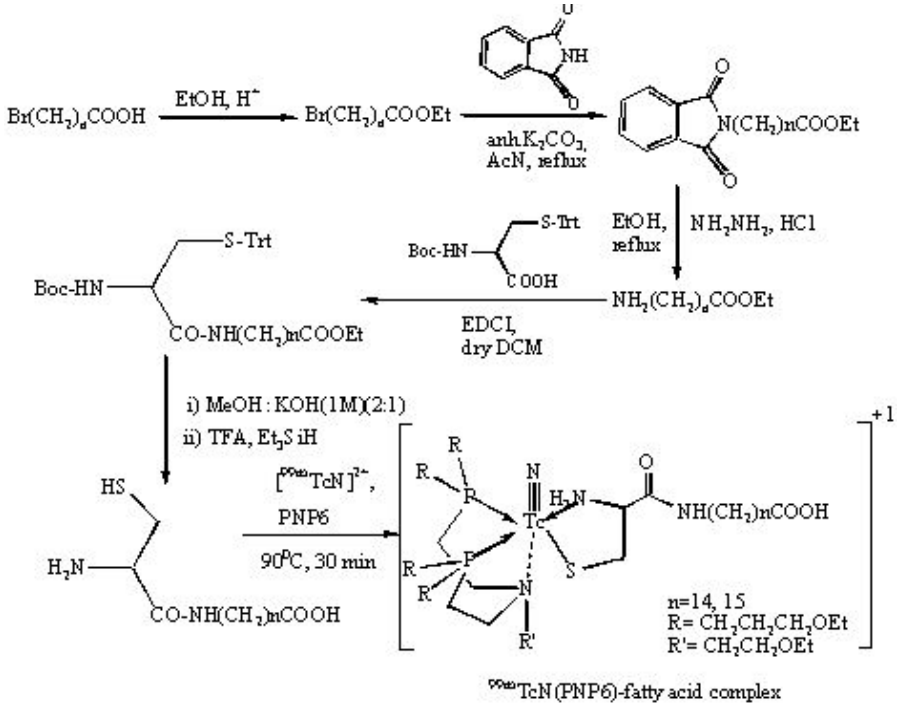
Objectives: ^{123}I-labeled fatty acids and ^{18}F-FDG are used for early detection of myocardial abnormalities in high risk patients. The inherent problems associated with the use of cyclotron produced radionuclides such as ^{123}I and ^{18}F necessitates the need for ^{99m}Tc based derivatives for the aforementioned purpose. In the present work, two novel fatty acid derivatives have been synthesized and radiolabeled with ^{99m}Tc via $[^{99m}TcN(PNP)]^{2+}$ core. Since $[^{99m}TcN(PNP)]^{2+}$ core prefers complexation with bidentate ligands having donor atoms SS/OS/NS, the envisaged strategy in the present work, is derivatization of C -15 and 16 fatty acids with cysteine as a chelating agent with NH_2 and SH as the donor groups.

Methods: The synthetic scheme followed for derivatization of the two fatty acids as well as the radiolabeling procedure with $[^{99m}TcN(PNP)]^{2+}$ core were identical. The bromo fatty acids have been derivatized at the terminal bromo residue to amino groups thereby rendering it suitable for conjugation with the carboxylic substituent of cysteine. Towards this, the first step involved ethyl ester protection of bromo fatty acid followed by conversion to the 15- and 16- phthalimido esters. The phthalimide group was then cleaved to yield the 15-and 16-amino esters. The free amino groups of amino ester derivatives were then conjugated with COOH residue of N-Boc, S-Trt cysteine in presence of N-(3-dimethylaminopropyl)-N'-ethyl carbodimide hydrochloride as a coupling agent. Hydrolysis of the ester groups resulted in formation of the free acids. The characterization of all intermediates and the final conjugates were carried using ^{1}H-NMR. In the final step, the two ligands were obtained after simultaneous Boc and trityl deprotection using trifluoroacetic acid (TFA) and triethyl silane. The target ligands, 15-cysteinyl fatty acid and 16-cysteinyl fatty acid derivatives, were used as such for complexation with $[^{99m}TcN(PNP)]^{2+}$ core independently without further characterization. The radiolabeling involves preparation of $[^{99m}TcN]^{2+}$ core using succinic dihydrazide, stannous chloride and sodium pertechnetate. To the prepared $[^{99m}TcN]^{2+}$ core, PNP6 ligand and fatty acid cysteine conjugate were added simultaneously and the reaction mixture was heated at 90°C for 30 min. The formation of the final complex was characterized by HPLC.

Results: The desired conjugates of C-15 and 16 fatty acids were obtained after purification using silica gel column chromatography in ~70% and 60% yield respectively. The final $[^{99m}TcN(PNP)]$ fatty acid complexes were positively charged and obtained as a mixture of two isomers (syn and anti) in more than 80% yields as characterized by HPLC. The bioevaluation of the radiolabeled complexes after HPLC purification will be carried out in suitable animal model.

Conclusions: Two novel ^{99m}Tc-labeled fatty acid derivatives were prepared in good radiolabeling yields and stability. The evaluation of their potential applicability in myocardial imaging is underway.

Research Support: The PNP6 ligand for these studies was kindly donated by Prof. Adriano Duatti, Univ. of Ferrara, Italy.



^{99m}TcN(PNP6)-fatty acid complex

P349 SYNTHESIS AND EVALUATION OF RHENIUM-CYCLIZED TYR-3-OCTREOTATE DERIVATIVES

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Objectives: Structure-activity studies on a series of Re-cyclized octreotide peptide derivatives for targeting somatostatin receptors led to the identification of a lead compound, Re-Tyr^3-octreotate (Re-dPhe-Cys-Tyr-dTrp-Lys-Thr-Cys-ThrOH), with good in vitro receptor affinity (29 nM IC_{50}; Bigott-Hennkens, et al. J. Med. Chem. 51:1223-1230;2008). The ^{99m}Tc-Tyr^3-octreotate analogue, however, was unstable at the radiotracer level in vivo, likely due to its 8-membered metal chelate ring (Bigott, et al. Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine, Ed., U. Mazzi, S.G.E.: Padova, 7:295-300;2006). A new series of sequence-modified peptides was developed, based on Tyr^3-octreotate, in attempt to distance the metal coordination from the key receptor-binding residues (dTrp^4-Lys^5) for improved receptor affinity and to generate more desirable 5- or 6-membered metal chelate rings for improved stability.

Methods: Linear peptide sequences, with or without synthetically modified cysteine residues, were prepared by standard solid-phase 9-fluorenylmethoxycarbonyl (Fmoc) peptide synthesis. Peptide cyclization with nonradioactive Re was carried out in transchelation reactions between the reduced peptides (i.e., containing SH groups) and either [$ReOCl_3(OPPh_3)(SMe_2)$] or [TBA][$ReOCl_4$]. Crude peptides obtained were characterized by HPLC and LC-MS and were purified by semi-preparative reversed-phase HPLC, using an in-house optimized multistep gradient. Two-dimensional NMR experiments determined the sites of Re coordination within the peptides. In vitro competitive binding assays with ^{111}In-DOTA-Tyr^3-octreotide in AR42J rat pancreatic tumor cells yielded IC_{50} values as a measure of somatostatin receptor affinity.

Results: Based on the octapeptide Re-Tyr^3-octreotate, numerous Re(V)-cyclized peptides were synthesized and evaluated for their in vitro somatostatin receptor affinities. The modifications included 1) insertion of a third Cys residue into the peptide sequence, replacement of dPhe^1 with Cys/dCys, or replacement of Cys^2 with a synthetic bidentate cysteine derivative, in order to stabilize metal binding against exchange and/or oxidation under physiological conditions; 2) acetylation of the peptide N-terminus, in order to disfavor metal coordination to the N-terminal amine; and 3) combinations thereof. In general, these modifications improved the stability of the radiometallated peptide complexes (radiotracer studies submitted as separate abstract), however, at the expense of receptor affinity (IC_{50} values determined to date increased to >100 nM).

Conclusions: Modifications made to date to the Tyr^3-octreotate sequence to distance the site of metal coordination from the receptor binding amino acids have not improved somatostatin receptor affinity. Three-dimensional molecular structure determinations of the Re-peptides, which are currently underway, will aid our quest for combining receptor affinity with stability in future peptide generations.

P350 PREPARATION, QUALITY CONTROL AND STABILITY OF 99mTc-TEPA

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Objectives: Polyamines are essential for the growth and survival of all cells with biosynthesis and transportation of polyamines being very active in tumors.^{99m}Tc-polyamines are a novel type of potent imaging agent of tumor. In this paper , we describe the labeling of triethylenetetramine(TEPA) with the most widely used imaging radionuclide, ^{99m}Tc. Effects of pH and stannous chloride amount on the radiolabeling yield were investigated. The stability of ^{99m}Tc-TEPA was also evaluated.

Methods: In the labeling of ^{99m}Tc-TEPA, TEPA (5mg in 1mL saline) was added to a vial. To determine the optimal amount of reducing agent, 0.05-50μg of $SnCl_2 \cdot 2H_2O$ was used; pH was adjusted by using HCl. After addition of all reagents, 37MBq $Na^{99m}TcO_4$ was added into the vial. The vial was incubated at room temperature for 20min. The radiolabeling yield (RLY) and the radiochemical purity (RCP) were determined by thin layer chromatography. The stability of ^{99m}Tc- TEPA was tested at 0.5,1,3 and 6h after storage at room temperature.

Results: The effect of pH on the radiolabeling yield was examined for pH 2-9. When the pH values was between 5 and 8, the RLY of ^{99m}Tc- TEPA were both more than 90%. The highest radiolabeling yield is obtained at pH 7 with 95.2%. The effect of stannous chloride on radiolabeling was studied between 0.05μg and 50μg stannous chloride. Result showed that the RLY was dependent on the amount of $SnCl_2 \cdot 2H_2O$ present in the reaction mixture. The highest labeling efficiency was obtained by using 2μg of stannous chlorides. The overall maximum efficiency is achieved at the following reaction mixture: TEPA concentration: 5mg/mL, pH 7, $SnCl_2$ concentration: 2μg/mL, reaction time: 20minutes. As a result, the RLY and RCPwere 96.1% and 97.3% receptively. The RCP of the freshly prepared ^{99m}Tc- TEPA was still greater than 95% at 6h after storage at room temperature.

Conclusions: The radiolabeling of ^{99m}Tc-TEPA was optimized in this paper. The resulting complex of ^{99m}Tc- TEPA is quite stable within 6h. In conclusion, ^{99m}Tc- TEPA may be applied for tumor imaging.

P351 A NOVEL KIT OF NMB-ABP WITH HIGH AFFINITY FOR BONE

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Objectives: The purpose of this study is to develop a new kit for ^{99m}Tc-NMB-ABP preparation and to evaluate the biodistribution in mice and SPECT imaging in rabbit of the new ^{99m}Tc-NMB-ABP complex prepared by labeling the kit for bone scanning.

Methods: A sterile kit contained NMB-ABP, reducing agent $SnCl_2$ and stabilizer was prepared. ^{99m}Tc-NMB-ABP was preparedconveniently by injection $^{99m}TcO_4^-$ into NMB-ABP kit. The toxicity of NMB-ABP kit was assessed according to the Pharmacopoeia of PR China (2000 Edition). Biodistribution experiments were performed on normal Kunming mice (20± 2g). ^{99m}Tc-NMB-ABP or ^{99m}Tc-MDP (740 kBq) was injected intravenously through the tail vein. SPECT imaging of Japanese white rabbit was done in SKYLIGHT SPECT（Philips） after administering about 74 MBq of the ^{99m}Tc-NMB-ABP or ^{99m}Tc-MDP intravenously in male white rabbit (2kg).

Results: The long-term stability of kits, stored for periods of 1 to 6 months at 2-8°C in freezer, was assessed using TCL and RP-HPLC. The labeling yields were still above 95% after 6 months storage at 2-8°C in freezer. There has no obviously difference of the six hours stability study of ^{99m}Tc-NMB-ABP, it was prepared either by labeling the kitafter 3 months storage or by labeling the new manufactured one. No drug-related signs of toxicity were noted for mice in the 3083 MBq/kg dose in the 4-day observation period drug administration and no deaths were observed after 4 days. The bone uptake of ^{99m}Tc-NMB-ABP in normal Kunming micewas as high as 34.08±11.76%ID/g (n=5) at 5min (28.73±5.54%ID/g for ^{99m}Tc-MDP), increasing to 41.71± 5.79 %ID/g at 30min(40.14±4.21%ID/g for ^{99m}Tc-MDP) and 43.93±5.44 %ID/g at 1h (43.23±2.17%ID/g for ^{99m}Tc-MDP) after injection, . The liver, blood, muscle, heart, spleen, brain and lung uptakes of ^{99m}Tc-NMB-ABP were all lower than 0.5 %ID/g at 30min after injection. The bone/blood and bone/muscle ratios were increased from 70.14 and 40.43 at 15min to 116.04 and 104.26 at 30min,respectively.Both of the ratios of bone/muscle and bone/liver of ^{99m}Tc-NMB-ABP were better than that of ^{99m}Tc-MDP. Compared to ^{99m}Tc-MDP, ^{99m}Tc-NMB-ABP had faster bone accumulation, lower liver and spleen uptake and faster elimination from liver and spleen. SPECT imaging of ^{99m}Tc-NMB-ABP in Japan white rabbit showed high bone to soft tissue ratio at 30min after injection with highly selective skeletal uptake and lower liver uptake. The images scanned at 60min showed clearly the whole skeleton, bladder and kidney and no concentration in any other organ. Most of the radiotracer was excreted by the urinary system.(Fig.1)

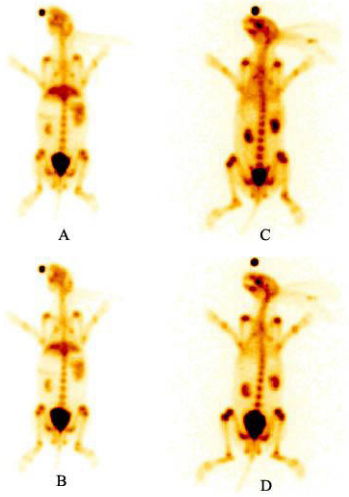

Fig 1 SPECT images of ^{99m}Tc-MDP in 30min(A), 60min(B) and ^{99m}Tc-NMB-ABP in 30min(C), 60min(D) after injection

Conclusions: In summary, we developed a novel kit for ^{99m}Tc-NMB-ABP preparation. ^{99m}Tc-NMB-ABP showed rapider and higher bone uptake, lower uptakes and rapider declines in liver and spleen when compared with ^{99m}Tc-MDP.

Research Support: The authors wish to acknowledge the support of Beijing Shihong Pharmaceutical Center for the help in preparing the NMB-ABP kits and donation of MDP kits.

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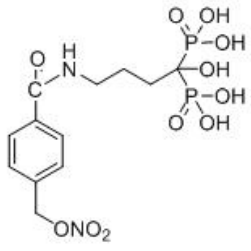

Fig 2 Chemistry structure of NMB-ABP

P352 NEW COORDINATION CHEMISTRY FOR THE DEVELOPMENT OF NEW TECHNETIUM-99M IMAGING AGENTS AND THEIR RHENIUM ANALOGUES

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Objectives: Of interest to this project is the exploitation of the radio-imaging properties of technetium-99m to trace hypoxic regions within the brain both specifically and non-invasively. As there are no non-radioactive isotopes of technetium, its congener, rhenium, is used as a surrogate for developmental synthesis and characterisation of compounds. Both the $[M(CO)_3]^+$ and $[M^V{=}O]$ (M = Re, Tc-99m) cores are stable platforms ideally suited for radiopharmaceutical application. Consequently, the objective is to synthesise rhenium complexes with systems that incorporate various stable aromatic-amine based ligands. Ultimately, a range of reducible nitro containing imidazoles, or indeed other pharmacophores, can be attached to the ligand framework to facilitate bio-reduction within cells with low oxygen concentrations.

Methods: Schiff base condensation reactions were used to prepare potential tridentate ligands for complexation to $[Re(H_2O)_3(CO)_3]Br$. For example synthesis of L^1: 2-Hydrazinopyridine (1.0 g, 9.2 mmol) was dissolved in anhydrous tetrahydrofuran (15 mL). 2-Pyridinecarboxaldehyde (0.9 mL, 9.2 mmol) and glacial acetic acid (0.5 mL, 9.2 mmol) were added and the reaction stirred at reflux for 6 h after which a yellow precipitate formed and was filtered and washed with THF. All tetradentate ligands for complexation to $[ReOCl_3(PPh_3)_2]$ were prepared in the following manner. For example synthesis of L^5: To a solution of 2-hydrazinopyridine (0.6 g, 5.0 mmol) in ethanol (5 mL) was added 2-[2-[(methylamino)thioxomethyl]hydrazinylidene] methyl ester (0.5 g, 2.5 mmol). The solution was stirred at reflux overnight then concentrated in vacuo to approximately 75% then left to solidify in the freezer. The solid was stirred in H_2O for an hour then filtered and washed with H_2O to afford a tan solid.

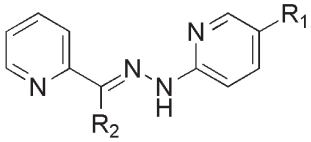

Figure 1. Proposed tridentate ligands
L^1 :R_1 = H, R_2 = H
L^2 :R_1 = CH_3, R_2 = H
L^3 :R_1 = H, R_2 = CO_2H
L^4 :R_1 = CH_3, R_2 = CO_2H

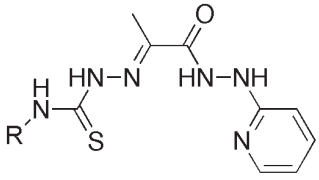

Figure 2. Proposed tetradentate ligands
L^5: R = CH_3
L^6: R = CH_2CH_3
L^7: R = C_6H_5

All ligands were characterised by ^1H and ^{13}C NMR spectroscopy, RP-HPLC, ESI-HRMS and IR. Where possible a crystal structure was obtained.

Results: It was found that upon complexation of L^1 onto rheniumtriaquatricarbonyl bromide, a possible metal mediated cyclisation was induced, forming the fused heterocycle-metal complex, bromo tricarbonyl 3-(2-pyridinyl)-1,2,4-triazolo[4,3-a] pyridine rhenium (I) (Fig. 3). Without heating, the crystal structure indicated that L^1 had coordinated as the bidentate ligand with the octahedral coordination sphere completed with bromide. The characterisation of a range of potential tetradentate ligands ligands is progressing effectively.

Conclusions: The synthesis of the tridentate ligands was successful. Interestingly, upon complexation onto the metal, the ligand either cyclised or bound in a bidentate fashion. Nevertheless, the cyclised products may be of use to further couple with a nitro-imidazole or a similarly redox active substituent. It is envisioned that upon completion of the series of tetradentate ligands they can subsequently be co-ordinated to the square planar pyramidal $[Re^V{=}O]$ core. After successful complexation, condensation with a bioreductant will be attempted, followed by the substitution of rhenium with radioactive technetium.

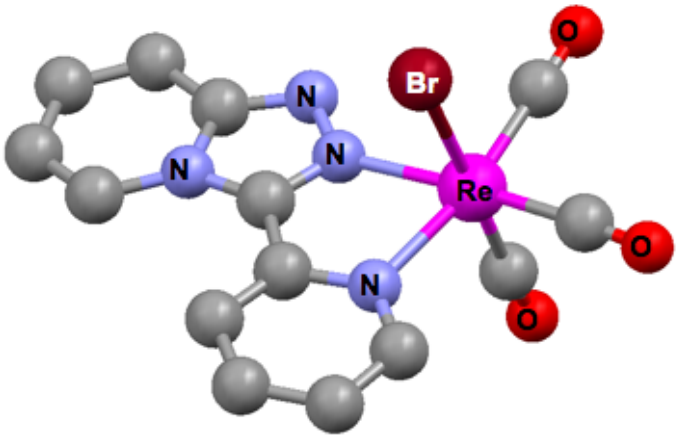

Figure 3. Crystal structure of cyclised ligand system L^1 onto $Re[(CO)_3]^+$ core

P353 SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 99mTc-LABELED NO-DONOR BISPHOSPHONATE FOR BONE SCINTIGRAPHY

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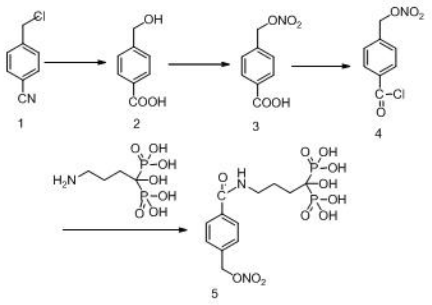
Objectives: The preparation, characterization and in vivo biodistribution of a new radiopharmaceutical ^{99m}Tc- NMB-ABP are reported for bone scintigraphy.

Methods: The chelating NMB-ABP（5） was prepared by BPs, conjugating 4-amino-1-hydroxy-butylidene-1, 1-bisphosphonate (ABP) with NO-donor moiety, 4-nitrooxymethyl benzoic acid (NMB) according to the procedure outlined in Scheme 1.



Scheme 1. Synthesis of NMB-ABP

^{99m}Tc-NMB-ABP was prepared by reduction of $^{99m}TcO_4^-$ in the presence of $SnCl_2$ and NMB-ABP. The RCP of ^{99m}Tc-NMB-ABP complex was evaluated by a combination of TLC and RP-HPLC. Paper electrophoresis were carried out using phosphate buffer (0.025M, pH=7.4) at 150v for 120min. The octanol/water partition coefficient was determined in the mixture of 1-octanol and phosphate buffer (0.025 M, pH 7.4). Plasma stability assay was performed in freshly prepared mice plasma at 37°C. At 1, 2,3,4,5 and 6 h, 10μl aliquots were drawn for TLC and RP-HPLC analyses. Plasma protein binding assay was evaluated by ultrafiltration. The hydroxyapatite-binding assay was performed according to procedures described previously with a slight modification. Hydroxyapatite powder was suspended in Tris/HCl-buffered saline. ^{99m}Tc-NMB-ABP was added to the hydroxyapatite suspension, and samples were gently shaken for 2 h at room temperature. After centrifugation at 5000 rpm for 20 min, the radioactivity of the supernatant was measured. Biodistribution experiments were performed on normal Kunming mice (20± 2g).

Results: ^{99m}Tc-NMB-ABP is a hydrophilic complex with high RCP (>95% by TLC and HPLC). ^{99m}Tc-NMB-ABP is stable in mice plasma for at least 6 h. The result of electrophoresis showed that it is a negative complex. The proportions of ^{99m}Tc-NMB-ABP bound to protein plasma was 84.37± 9.32 %. The rate of hydroxyapatite binding was increased with the amount of hydroxyapatite. The percentage of ^{99m}Tc-NMB-ABP bound to hydroxyapatite increase to 91.5% from 66.5% as the amount hydroxyapatite increase to 25mg/mL from 10mg/mL, respectively. Bone uptake of ^{99m}Tc-NMB-ABP was as high as 37.63± 1.17 %ID/g (n=3) at 30 min after injection, increasing to 40.04± 5.07 %ID/g at 60min. The radioactivities in liver, blood, muscle, heart, spleen, brain and lung were all lower than 0.5 %ID/g at 60 min after injection. The bone-to-blood ratio ,bone-to-muscle ratio and bone-to-liver ratio arrived at 100.5 , 221.7 and 116.1 at 60 min, respectively.

Conclusions: A novel NO-donor bisphosphonates, NMB-ABP, was synthesized and labeled with technetium-99m in high RCP (>95%). ^{99m}Tc-NMB-ABP is a hydrophilic and negative complex. Animal studies showed this complex had fast and high accumulation in bone and rapid clearance from blood, liver. The present findings indicate that ^{99m}Tc-NMB-ABP has great potential for bone scintigraphy.

Research Support: The authors wish to acknowledge the support of Beijing Shihong Pharmaceutical Center for the help in preparing the NMB-ABP kits and donation of MDP kits.

References: 1. Ogawa K, Mukai T, Kawai K, Takamura N, Hanaoka H, Hashimoto K, Shiba K, Mori H, Saji H. Usefulness of competitive inhibitors of protein binding for improving the pharmacokinetics of 186Re-MAG3-conjugated bisphosphonate (186Re-MAG3-HBP), an agent for treatment of painful bone metastases. Eur J Med Mol Imaging 36:151-121(2009) 2. Ogawa K, Mukai T, Inoue Y, Ono M), Saji H. Development of a novel Tc-99m-chelate-conjugated bisphosphonate with high affinity for bone as a bone scintigraphic agent. J Nucl Med 47:2042-2047(2006)

$$\text{Hydroxyapatite binding ratio (\%)} = \left(1 - \frac{\text{radioactivity of supernatant of sample}}{\text{radioactivity of supernatant of the control}}\right) \times 100$$

P354 COMPARATIVE IN VITRO EVALUATION OF DOTA-BOMBESIN ANALOG LABELLED WITH YTTRIUM-90, LUTETIUM-177, GALLIUM-68 and SCANDIUM-44

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Objectives: The specific aim of this study was to compare the labelled compounds of a DOTA-BN[2-14]NH_2, a DOTA-chelated Bombesin analog, with ^{90}Y, ^{177}Lu, ^{68}Ga and ^{44}Sc for their labelling yields, specific activity and affinity to GRP receptors. Initial in vitro and in vivo evaluation of the ^{90}Y and ^{177}Lu labelled DOTA-BN[2-14]NH_2 indicated that their binding affinities to PC-3 cells in vitro were metal-mediated. Thus it was decided to extend the study to other M^{+3} type radiometals such as ^{68}Ga and ^{44}Sc to evaluate their potential for PET imaging.

Methods: The radioisotopes ^{90}Y (N.C.A) and ^{177}Lu (C.A) were produced at IAE Radioisotope Centre Polatom, ^{68}Ga and ^{44}Sc were eluted from ^{68}Ge/^{68}Ga and ^{44}Ti/^{44}Sc semi-automated generators, respectively, provided at the Institute of Nuclear Chemistry, University of Mainz. Labelling of DOTA-BN[2-14]NH_2 with ^{90}Y and ^{177}Lu was carried out in ammonium acetate buffer, pH=5.0 with 25 min incubation at 85^{o}C. The cold complexes of ^{nat}Ga/^{nat}Sc-DOTA-BN[2-14]NH_2 were synthesized and characterized by HPLC and MS analysis. The labelling with ^{68}Ga and ^{44}Sc was studied in relation to the incubation time and to the specific activity of the final sample. The radiochemical evaluation involved ITLC-SG analysis and solid phase extraction (C18 mini columns) with concern to final specific activity of the radiolabel. The internalization and externalization studies as well as GRP receptors binding affinity assays were performed in PC-3 cells (human androgen independent prostate carcinoma ATCC CRL-1435).

Results: The specific activities achieved for ^{90}Y- and ^{177}Lu-DOTA-BN[2-14]NH_2 were 67.3 and 33.6 GBq/mmol, respectively. In case of ^{68}Ga-DOTA-BN[2-14]NH_2 it was 7.5 GBq/μmol and 10-15 min incubation was sufficient to complete radiolabelling. The obtained labeling yield was >95%, the sample was purified before its further use. The internalization rate of the ^{68}Ga-DOTA-BN[2-14]NH_2 was slower as compared with that obtained for the ^{90}Y and ^{177}Lu respective analogues, however the percentage of internalization at the end of incubation was comparable. The efflux study revealed a faster externalization rate of ^{68}Ga-DOTA-BN[2-14]NH_2 as compared with ^{90}Y- and ^{177}Lu-DOTA-BN[2-14]NH_2.

Conclusions: The observed differences in receptor affinities between DOTA-BN[2-14]NH_2 complexes with variousmetals indicate that BN[2-14]NH_2 can be a good model molecule for evaluating the biological utility of various complexing ligands, when attached to this biomolecule. Further in vitro evaluation involving cold and radiolabelled Ga and Sc complexes of DOTA-BN[2-14]NH_2 are in progress.

Research Support: COST Actions BM0607 and D38 and the research grant No 126/N-COST/1008/0 from Polish Ministry of Science and Higher Education

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P355 IMPROVED LABELING OF DTPA-CONJUGATED PEPTIDES WITH INDIUM-111 IN MES BUFFER

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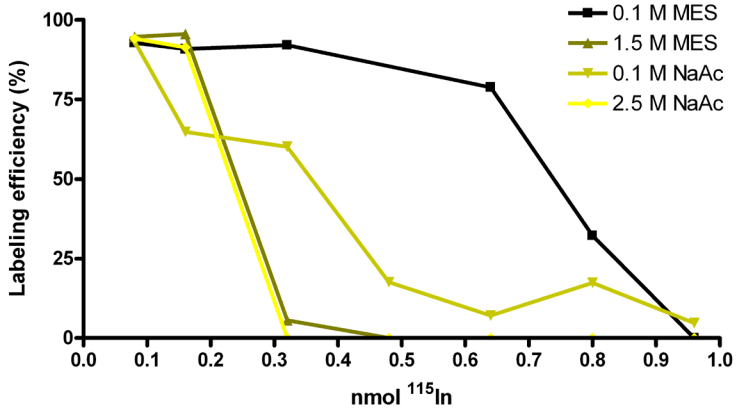
Objectives: DTPA and DOTA-conjugated peptides and antibodies labeled with ^{111}In can be used for the diagnosis of tumors. High specific activities (SA) are required to administer low amounts of tracer to patients or in animal models to prevent receptor saturation. Moreover, administration of low amounts of tracer prevent side-effects and saturation of the receptor which may lead to reduced accumulation in the target tissue. Acetate buffers are routinely used for radiolabeling with ^{111}In. In order to increase the specific activity we examined the effect of the buffer used during the labeling procedure (NaAc vs. MES buffer (2-(N-morpholino) ethanesulfonic acid), pH 5.5) as well as the volume of the labeling mixture on the maximal achievable specific activity. Cadmium, the decay product of ^{111}In, can also be incorporated in the chelator which may lead to a reduced labeling efficiency. The effect of cadmium contamination was examined for MES buffer and NaAc buffer.

Methods: Various concentrations of ^{115}In and a trace of ^{111}In-activity were added to 1 μg of DTPA-Octreotide or DTPA-Exendin-3. MES buffer or NaAc buffer (0.1 M, pH 5.5) were added to a final volume of 1200 μl. In a second experiment concentrated MES buffer (1.5 M) or NaAc buffer (2.5 M) were added to a final volume of 100 μl. After 20 min incubation at RT the radiochemical purity was determined by ITLC. Similar labeling reactions were carried out with DOTA-Octreotate, while in those experiments incubation was carried out at 95 ^{o}C for 20 min. To investigate the effect of cadmium on the labeling efficiency, various concentrations of $CdCl_2$ were added to the labeling reaction of DTPA-Octreotide with MES buffer or NaAc buffer (0.1 M).

Results: Radiolabeling of DTPA-Exendin in 0.1 M MES buffer resulted in a maximum SA of 0.72 nmol ^{115}In/nmol DTPA-Exendin-3. Radiolabeling in 1.5 M MES, 0.1 M NaAc and 2.5 M NaAc resulted in lower SA (0.21, 0.14 and 0.19 nmol ^{115}In/nmol DTPA-Exendin-3 respectively). A SA of 0.52 nmol ^{115}In/nmol DTPA-Octreotide was obtained in 0.1 M MES buffer, whereas labeling in 1.5 M MES, 0.1 M NaAc or 2.5 M NaAc resulted in a SA of 0.17 nmol^{115}In/nmol DTPA-Octreotide. The highest SA for DOTA-Octreotate was obtained in 2.5 M NaAc: 0.43 nmol ^{115}In/nmol DOTA-Octreotate. Radiolabeling in 0.1M MES, 1.5 M MES and 0.1 M NaAc resulted in lower SA (0.29, 0.32 and 0.15 nmol ^{115}In/nmol DOTA-Octreotate). The calculated specific activities in GBq/nmol are summarized in the table shown below. The presence of 0.1 nmol Cd^{2+} in the labeling mixture of DTPA-Octreotide in 0.1 M NaAc resulted in a decrease of labeling efficiency, whereas Cd^{2+} concentrations up to 100 nmol did not affect the labeling efficiency of the peptide in 0.1 M MES buffer up to 100 nmol Cd^{2+}.

Conclusions: In 0.1 M MES buffer DTPA-conjugated peptides could be labeled with ^{111}In to a specific activity that was at least 3-fold higher than that in more common buffer systems. The enhanced labeling efficiency could be due to the reduced competitive chelation of the decay product of ^{111}In, cadmium. The highest specific activity for DOTA-conjugated Octreotate was obtained in 2.5 M NaAc.

	DTPA-Exendin-3 (GBq/nmol)	DTPA-Octreotide (GBq/nmol)	DOTA-Octreotate (GBq/nmol)
0.1 M MES	1.23	0.89	0.50
1.5 M MES	0.36	0.29	0.55
0.1 M NaAc	0.24	0.29	0.26
2.5 M NaAc	0.32	0.29	0.73



P356 NEW AND INNOVATIVE BIFUNCTIONAL CHELATES FOR IMAGING AND THERAPY

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Objectives: Finding new bifunctional chelates (BFC) that can be used for both therapy and imaging is desirable in order to verify the efficient and specific targeting of a therapeutic agent. Currently, BFCs based on DOTA are considered the gold standard for such applications. However, when chelating DOTA to some isotopes the reaction kinetics are slow and often require the application of heat that may damage the function of a biological vector. We describe two new BFCs with superior properties compared to DOTA.

Methods: OXO and PCTA were labeled with Y-90, In-111 and Lu-177 and compared to DOTA. The labeling yield was assessed by ITLC and HPLC. The pH range for labeling was studied, as well as the kinetics of the reaction. The molar ratio of chelate per isotopes was optimized to yield greater than 95% chelation. The stability of each chelate in serum and under varying pH conditions was studied for 7 days. The biodistribution of the each chelate in rats (n=3/chelate) was studied using a microSPECT camera.

Results: The new BFC OXO and PCTA have faster labeling kinetics than DOTA. Molar ratios of chelate:isotope of 5:1 give labeling yields higher than 95% using In-111 and Y-90. Labelling yields above 95% can be achieved with OXO and PCTA when labeled with Lu-177 at a molar ratios 10:1 and 5:1 respectively, while DOTA requires a ratio 100:1. Y-90 labelled OXO, PCTA and DOTA showed good stability (>95% complexation) in glycine solutions at pH 4 and 6 and in serum for 10 days. In-111 labeled OXO, PCTA and DOTA showed good stability in glycine solutions at pH 2, 4 and 5 and in serum for 10 days. Lu-177 labeled OXO and DOTA showed good stability at pH 4 and 6 and in serum for 10 days; however, PCTA did not perform as well in all conditions. The new BFCs were also tested in vivo. In-111 labeled OXO had faster clearance from the liver (10 +/- 2min) compared to DOTA and PCTA (50, 135 +/- 2min).

Conclusions: The new BFCs OXO and PCTA can be labeled with SPECT and therapeutic isotopes. OXO demonstrated faster clearance in vitro. Future studies will evaluate the performance of these new BFCs once attached to biological vectors.

P357 IN VITRO AND IN VIVO EVALUATION OF [64Cu-NO2A-(X)-BBN(7-14)NH2] RADIOPHARMACEUTICALS FOR POTENTIAL PET IMAGING OF HUMAN PROSTATE CANCER

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Objectives: Gastrin-releasing peptide receptors (GRPr) are expressed in high numbers on human prostate cancer. The bombesin peptide derivative, BBN(7-14)NH_2, has high affinity and selectivity to GRPr. Therefore, Cu-64 radiolabeled BBN(7-14)NH_2 conjugates could have potential in positron-emission tomography (PET) of human prostate cancer. Our aim was to produce ^{64}Cu-NO2A-(X)-BBN(7-14)NH_2 conjugates via the bifunctional chelate approach, where X = pharmacokinetic modifier (Beta-alanine, 5-aminovaleric acid, 6-aminohexanoic acid, 8-aminooactanoic acid, 9-aminonanoic acid, or para-aminobenzoic acid), and NO2A = 1,4,7-triazacyclononane-1,4-diacetic acid.

Methods: Solid-phase peptide synthesis was used to produce (X)-BBN(7-14)NH_2 conjugates, after which the bifunctional chelating ligand, NO2A, was added via manual conjugation. Radiolabeling was performed by incubating $^{64}CuCl_2$ with NO2A-(X)-BBN(7-14)NH_2 in buffered, aqueous solution (pH ~ 7). In vitro assays of the conjugates and non-radioactive ^{63}Cu conjugates were performed in human PC-3 cells. In vivo pharmacokinetic studies of the radiolabeled conjugates were performed in normal CF-1 and PC-3 tumor-bearing SCID mice. In vivo, multimodal, molecular images were obtained of the radiolabeled conjugates in PC-3 tumor-bearing SCID mice via microPET/CT and microMRI.

Results: In vitro studies indicated idea uptake of the NO2A conjugates (1.99-6.24 nM), and ^{63}Cu-NO2A conjugates (3.16-51.81 nM) in PC-3 cells. In vivo results of the ^{64}Cu-NO2A-(X)-BBN(7-14)NH_2 conjugates at 1, 4, and 24 h p.i. showed affinity towards GRPr-positive tissue and effective clearance properties. Due to the favorable in vivo pharmacokinetic properties of ^{64}Cu-NO2A-(X)-BBN(7-14)NH_2, high-resolution multimodal, molecular imaging was performed via microPET/CT and microMRI in a PC-3 tumor-bearing SCID mouse model. High-quality target to non-target images were obtained, with the tumors clearly visible.

Conclusions: The NO2A chelator effectively stabilized Cu(II) under in vivo conditions. ^{64}Cu-NO2A-(X)-BBN(7-14)NH_2 conjugates showed affinity and specificity towards GRPr-positive tissues. High quality microPET images of PC-3 xenografted tumors in SCID mouse model were obtained, demonstrating the potential for PET imaging of GRPr-positive human prostate cancer tumors.

P358 A STRUCTURAL AND KINETIC INVESTIGATION OF SELECTED RHENIUM(I) TRICARBONYL COMPLEXES

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Objectives: Tricarbonyl triaqua complexes of Re(I) and Tc(I) have been investigated intensively[1] because of their potential use in diagnostic and therapeutic medicine. To develop a method to incorporate ^{99m}Tc and ^{188}Re into biomolecules, the reactivity of these complexes must be fully studied and understood and some predictions regarding complex formation reactions should then be possible. Of special interest is the thermodynamic and kinetic properties of H_2O substitution in $[Re(CO)_3(H_2O)_3]^+$. Bidentate ligand systems are often used to link a biological substrate to $[Re(CO)_3]^+$ leaving the third vacant position on the Re(I) core to add electron rich monodentate ligands[2]. In vivo, the third coordination site in these compounds are also exposed to possible nucleophilic attack by ligands in the blood stream such as NCS^- and halides; thus it is imperative to investigate the reactivity and kinetics of these systems. The main objectives of this study was therefore focused to specifically study the kinetic behaviour of a range of model $[Re(CO)_3]^+$ complexes, and are summarized as follows: Synthesize and characterize complexes of the type $[Re(L\text{-}L')(CO)_3X]^{n+}$ (n = 0, 1) with different N,N', N,O and O,O' donor ligand systems, ranging from 1,10 phenanthroline(phen, N, N') to picolinic acid (pico, N, O) and hydroxyflavone (Flav, O,O'). Determine the mechanism of the aqueous substitutions of $[Re(L\text{-}L')(CO)_3(H_2O)]^{n+}$ with different monodentate ligands. Study the effect of the different coordinated ligands on the rate of substitution.

Methods: Characterization of reactants and products included X-ray crystallogaphy[3-5]. The kinetic studies were carried out in methanol due to solubility problems in water.

Results: All the reactions showed simple pseudo first-order kinetics. In general, the rates of the reactions increased in the order N,N' < N,O < O,O' for neutral incoming ligands, with the highest value for k_1 being 5.1 x 10^{-3} $M^{-1}s^{-1}$ for the reaction of [Re(Flav)] with py. A general trend of an increase in rate of substitution with an increase in Re-OH_2 bond distance is observed. Also, the rates of the substitutions were dependent on the nature of the incoming monodentate ligand. Negative values for $\Delta S^{\neq}$ were observed.

Conclusions: It seems that the reactions proceeds via an I_a type mechanism. The O,O' donor ligands activate the metal centre substantially more than the N,O and N,N' ligands. The fact that the $[Re(Flav)(CO)_3(OH_2)]$ complex undergoes much faster aqua substitutions could be important. Having a potentially biologically active ligand so close to the metal centre might have some advantages in terms of radiopharmacy and needs to be investigated in future. .

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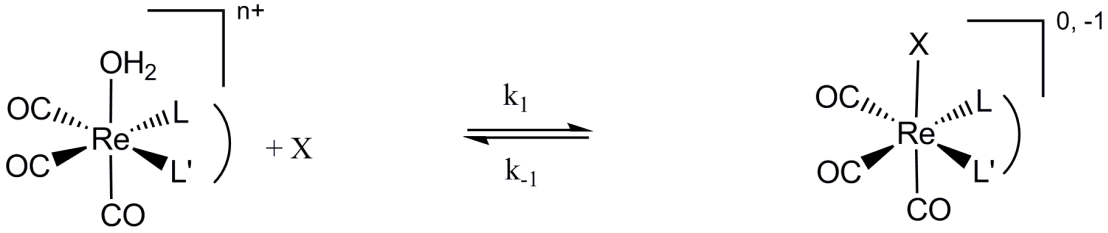

P359 A NEW STRATEGY FOR THE PREPARATION AND PURIFICATION OF TC-BASED RADIOPHARMACEUTICALS

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Objectives: In the era of molecular imaging it is often beneficial for probes to be produced in high effective specific activity. When considering metal-based agents, removal of excess ligand following radiolabeling is therefore an important issue. This is often done by HPLC for research applications, which is not suitable for routine kit-based productions. Solid-phase ligand capture methods have been developed to address this issue wherein a Cu chelate bound to an insoluble polymer is used to selectively concentrate reaction products. A complementary solution-phase strategy that reduces non-specific binding has been developed employing fluorous (perfluorinated) chemistry as a means to remove unlabeled chelate-derivatives. The "fluorous effect" allows for selective filtration of a fluorous ligand capture agent using a perfluorinated solid-phase extraction cartridge leaving the labeled product in solution.

Methods: A fluorous diacetic acid Cu (FDAC) complex was prepared and used to selectively remove excess ligand following radiolabeling of a tridentate ^{99m}Tc chelate. UV and γ HPLC are used to determine purity and concentration of unlabeled ligand.

Results: By taking advantage of the fluorous effect, a new chemoselective filtration platform has been developed. The FDAC complex coordinates to the free donor sites of the tridentate ligand. Washing with a fluorophobic solution of 20% water/methanol elutes only the ^{99m}Tc complex while the unlabeled ligand now coordinated to FDAC is retained. Greater than 99% purity with small lose to non-specific binding.

Conclusions: New fluorous technologies are convenient platforms for the preparation and purification of metal-based molecular imaging agents in high effective specific activity.

P360 NODAPA-OH AND NODAPA-NCS: MONO- AND MULTIMERIC SIX-COORDINATE Ga-CHELATORS

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Objectives: The commercially available $^{68}Ge/^{68}Ga$ radionuclide generator systems and its recent improvement concerning on-line processing and labelling, [1] may provide a beneficial complement to nuclear imaging with established, cyclotron produced PET nuclides like ^{11}C and ^{18}F. Adequate radioligand precursors meeting the coordination chemistry of gallium(III) with versatile conjugation possibilities are of high interest. The macrocyclic chelators DOTA and NOTA (Fig. 1) are established as frequently considered routes for the introduction of a ^{68}Ga-tag. Compared to open chain acyclic analogues, both provide complexes of superior kinetic and thermodynamic stability since gallium is irreversibly complexed at room temperature. DOTA remains the most frequently used chelator because of its better commercial availability and less challenging synthesis, although its six-coordinate nine-ring analogue NOTA displays higher stabilities and faster incorporation of Ga(III) at lower temperatures.Thus, we were interested in a time-saving and cost-effective access to a NOTA based versatile gallium chelator allowing convenient conjugation to various targeting molecules.

Methods: Chelators 1-3 were synthesised from 4-substituted phenylacetic acids and triazacyclononanes (TACN). To analyse whether the chain branch in one pendant arm affects the kinetic and themodynamic characteristics of [^{68}Ga]NOTA-complex formation, labelling of NODAPA-OH, NODAPA-NCS, NODAPA-NCS_2 and NODAPA-NO_2 with generator produced and purified Gallium-68 was carried out in aqueuous solution at pH = 2.8. Quality control was performed using an Agilent Zorbax C 8 column using 50 mM phosphate buffer and MeOH as eluent at 0.5 ml/min. The stability of the novel ^{68}Ga chelates was determined in a DTPA-challenge study at 25 °C and 37 °C employing 1 mM, 10 mM and 100 mM solutions of DTPA in water.Plasma protein binding and transchelation to serum proteins in vitro was examined under physiological conditions in rat plasma. 4 MBq of [^{68}Ga]NODAPA-OH were incubated in 300mL of rat plasma from male adult Wistar rats, obtained via centrifugation of full blood. Samples of 50 mL were withdrawn after 1, 30, 60, 90 and 180 min and analysed by radio-TLC.

Results: Chelators 1-3 were obtained in 22 ± 6 % overall yield. Yields for ^{68}Ga(III) complex formation were very high (85±5 % already at 1 min) and comparable to those achieved for NOTA. The DTPA challenge indicated >94% complex stability, in a similar range as the congener NOTA. In correlation to the DTPA-challenge, less than 2 % of non-[^{68}Ga]NODAPA-OH radioactivity was observed in rat plasma after 3 h.

Conclusions: Novel NOTA-based bifunctional chelators have been obtained via a simple and efficient synthesis route. Compounds 1-3 provide excellent ^{68}Ga labeling and stability parameters. While offering –NCS and –OH functionalities, covalent coupling to various potential targeting vectors is possible.

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P361 BIO-ORTHOGONAL FUNCTIONALITIES FOR TUMOR PRE-TARGETING WITH STAUDINGER LIGATION: SYNTHESIS, RADIOLABELING AND BIOLOGICAL EVALUATION

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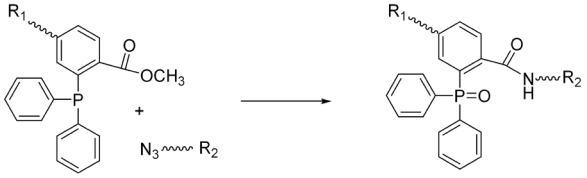
Objectives: Pre-targeting combines the excellent tumor-seeking properties of MAbs with the fast clearance of low MW molecules and is a highly sought-after approach for RIT. In recent years, the Staudinger Ligation (SL, Figure 1) between functionalized azides and phosphines was introduced as an effective strategy to perform bio-conjugations in living systems[1]. These functionalities are small, abiotic, and bio-orthogonal. Therefore, we envision the use of azide-conjugated MAbs and radiolabeled phosphines in a new concept of pre-targeted RIT. Here we present the synthesis and biological evaluation of ^{177}Lu-labeled DOTA-phosphine effector probe 1. Next, we describe the development and evaluation of a range of mono- and multivalent azide constructs designed to maximize the SL yield on a tumor-bound MAb.



R_1 = ^{177}Lu-DOTA
R_2 = MAb

Methods: Probe 1 was synthesized and labeled with ^{177}Lu. Its stability was evaluated in physiological media at different time points. The biodistribution was evaluated in normal mice (n=5) up to 2 hours p.i. A series of NHS-activated mono- and multivalent azide constructs was synthesized from linear and branched hexaethyleneglycol (HEG)-based structures and conjugated to a MAb (Rituximab). The SL reactivity of the azides alone and their corresponding MAb conjugates with ^{177}Lu-1 was studied in PBS. The most reactive MAb-azide construct was further evaluated in reactions with ^{177}Lu-1 in human serum.

Results: Probe 1 was synthesized in 6 steps in 42% overall yield. The azides were synthesized in 4-8 steps in 10-36% overall yield. The labeling of 1 with c.a. ^{177}Lu was complete after 10 min at 45 °C in acetate buffer (pH 8.5). In PBS, human serum and blood, ^{177}Lu-1 was stable for several hours, and showed only very limited oxidation of the phosphine to the inactive oxide. In normal mice, ^{177}Lu-1 cleared rapidly from the blood (3.5 min half-life) and did not accumulate in non-target organs, including liver and kidney. In model and antibody reactions in PBS the SL yield was influenced by the chemical structure and multivalency of the azide construct. Mono- and multivalent structures containing 2-azidoethyl groups and a monovalent structure terminated with a 2-azidoacetamido moiety gave modest SL yields, while a trimer functionalized with three 2-azidoacetamido moieties afforded a high reactivity. Three-to-four ^{177}Lu-1 probes reacted with one MAb-azidoacetamide trimer conjugate in PBS and fresh human serum (2 h incubation at 37 °C).

Conclusions: A novel approach to tumor pre-targeting is presented. We demonstrate that antibody-bound azides are reactive towards radiolabeled phosphines in biological media and that this reactivity can be tuned by modifying the azide structure. The validation of tumor pre-targeting with SL in mice is underway. References 1) C. S. Bertozzi, PNAS, 2006, 103, 4819

P362 REGIOSELECTIVE SYNTHESIS OF TRANS-DISUBSTITUTED CYCLAM: A VERSATILE ROUTE TO CYCLAM DERIVATIVES

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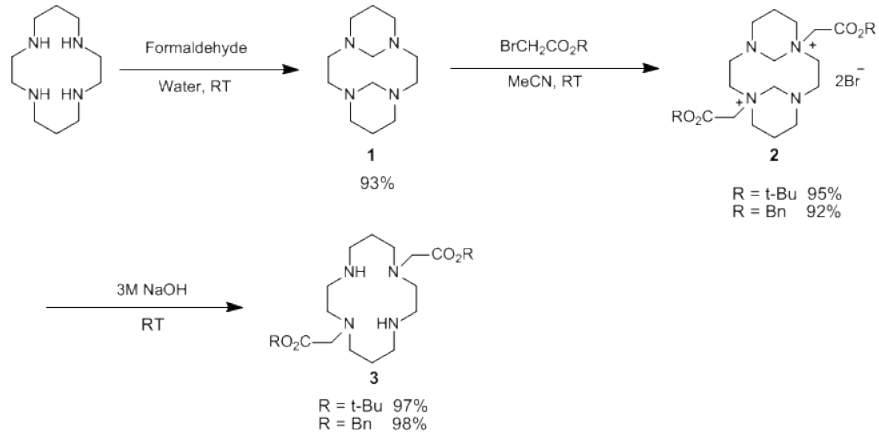
Objectives: Cyclam and its various derivatives were known to be very effective chelates for the use in diagnostic and therapeutic radiopharmaceuticals. Various synthetic methods for mono-, tri- and tetra-substituted cyclam have been reported in the literature. But very few methods have been reported towards the synthesis of N,N-disubstituted cyclam, especially trans-disubstituted cyclam, because of difficulties of regioselctive N,N-diprotection. Herein we report a simple regioselctive synthetic procedure for the trans-N,N-disubstituted cyclam, which can be converted easily to very effective bifunctional chelate.

Methods: Initially cyclam was treated with formaldehyde to give a tricyclic cyclam. Trans-dialkyaltion of this macrotricyclic compound was carried out with alkylating agents e.g. t-butylbromoacetate, benzyl bromoacetate in acetonitrile at room temperature followed by the cleavage of the bisaminal bond by treating with 3M NaOH aqueous solution at room temperature for 5h.



Results: During the alkylation of tricyclic cyclam, the disubstituted product was separated out from the reaction mixture as a solid. The pure solid was collected by simple filtration from the reaction mixture. Cleavage of the bisaminal group were carried out by using basic hydrolysis which afforded the 1,8-substituted product in excellent yields. The overall yields of the two trans-disubstituted products, t-Bu and Bn- are 86% and 84% respectively starting from cyclam.

Conclusions: We have successfully synthesized the trans-1,8-disubstituted cyclam derivatives in excellent yields. These di-substituted cyclam derivatives could be converted to other important derivatives such as tri- and tetra-substituted cyclam of interest. The final product also opens easy access toward the synthesis of TE2A just on acid hydrolysis or H_2-Pd/C reduction of the disubstituted products. Substitution of TE2A by some other linking group, which might allow conjugation with biomolecules, may lead to a very interesting bifunctional chelating agent.

P363 SYNTHESIS OF Ga-68 LABELED AMINO ACID DERIVATIVES AND TEST FOR TUMOR IMAGING

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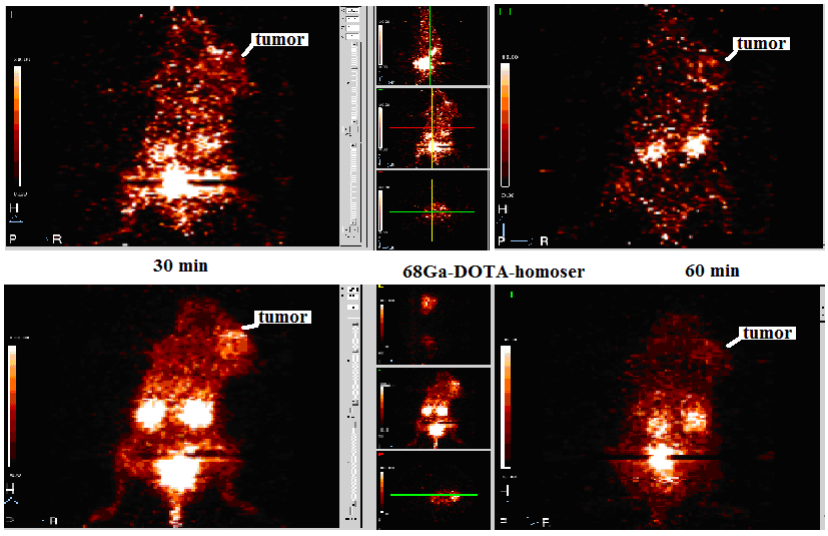
Objectives: Various amino acids labeled with 18F and 11C for positron emission tomography (PET) have been developed. However, they require expensive cyclotron and complicate synthesis procedure. We developed L-aminohomoalanine and L-lysine derivatives of NOTA and DOTA for labeling with 68Ga.



Methods: Protected γ-amino-L-homoserine was purchased, where as protected ε-amino-L-Lysine was synthesizedstarting from N-boc-Lys(Bz)-OH by esterification and cleavage of protected bezyl group via catalytic reduction . Both compounds were conjugated to NOTA and DOTA in water/acetonitrile (1:1) system using DCC as a coupling agent. Protecting groups were removed by 4M hydrochloric acid in dioxane. The final products were obtained as hydrochloric acid salts by RP-HPLC purification. NOTA-homoser, NOTA-lys, DOTA-homoser, and DOTA-lys (each 50 μg) were labeled with ^{68}Ga in pH 3.1~5.5. All reactions were performed for 10 min in boiling water bath in one step. Labeling efficiencies were checked by ITLC. Animal-PET imaging studies were performed using balb/c mice xenografted with CT-26 (mouse colon cancer).

Results: All the compounds were labeled with high yields (>98%). All labeled compounds showed high stability (>95%) at room temperature and protein binding in human serum found very low (<3% for NOTA derivatives and <15% for DOTA derivatives). A small-animal PET images for labeled compounds showed a high bladder activity at 1 h. High uptakes were found in tumors along with kidney on 2 h images comparing to other organs. NOTA derivatives showed better image than DOTA derivatives.

Conclusions: We successfully synthesized ^{68}Ga labeled amino acid derivatives for tumor PET which can be labeled by one step reaction within 10 min.

P364 NEW APPROACHES FOR REDUCTION OF PERTECHNETATE AND PERRHENATE

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Objectives: ^{188}Re is an attractive radionuclide for radiotherapy and its complexes are generally prepared using stannous ion to reduce ^{188}Re(VII) to lower valent states, similarly to ^{99m}Tc complexes. Due to the lower reduction potential of Re compared to Tc, excesses of Sn(II), ligand, heat and low pH are employed for reduction of $^{188}ReO_4^-$. Excess Sn can also bind to the ligands, effectively reducing the amount of ligand available for ^{188}Re complexation, thus negatively impacting the specific activity of the ^{188}Re conjugate. The objective of this project is to examine an alternative approach that utilizes the photocatalytic reduction of ^{99m}Tc and ^{188}Re by nanoparticulate metal oxides called polyoxometalates (POMs).

Methods: POMs undergo stepwise, multielectron redox reactions while maintaining their structural integrity. POMs can be reduced photochemically in the presence of a sacrificial organic electron donor. The reduced POMs in turn reduce high valent TcO_4^- and ReO_4^- to lower valent Tc or Re. We report on the reduction of pertechnetate and perrhenate using non-complexing POMs, Keggin ions, ($XW_{12}O_{40}^{n-}$, X=P, n=3; Si, n=4; Al, n=5), and complexing POMs, the Wells-Dawson lacunary isomer (α_2-$P_2W_{17}O_{61}$)$^{10-}$, and a "wheel" POM, $P_8W_{48}O_{184}^{40-}$. The resulting complexes have been characterized by electrochemistry, UV- Visible Spectroscopy and multinuclear NMR spectroscopy.

Results: The POM, α_2-$P_2W_{17}O_{61}^{10-}$, was reduced by light in the presence of a sacrificial electron donor to form the 2-electron reduced α_2-$P_2W_{17}O_{61}^{12-}$ (blue color). Upon addition of a solution of $^{99}TcO_4^-$ (10^{-4} M), the blue color disappeared and a red-brown color was generated. UV-Visible spectroscopy, electrochemistry and ^{31}P NMR studies show clearly that Tc(V) is generated and is complexed into the POM. The same procedure resulted in the reduction of ReO_4^-. The non-complexing Keggin POMs reduce $^{99}TcO_4^-$ to TcO_2 because there is no complexing agent in the solution. A non-complexing POM that can be reduced specifically by 2 electrons by excitation at 366 nm (O-W charge transfer band) was supported on silica. Addition of ReO_4^- and a complexing (α_2-$P_2W_{17}O_{61}^{10-}$) POM results in a reduction to Re(V) and complexation of the Re(V) into the defect of the complexing POM framework as verified by UV-Visible Spectroscopy and ^{31}P NMR Spectroscopy. Large wheel POMs are also photocatalytically reduced and in turn, reduce and complex $^{99}TcO_4^-$ and ReO_4^- into their frameworks.

Conclusions: Non-complexing and complexing Polyoxometalates (POMs), non-toxic nanoparticulate metal oxides, are photocatalysts for the clean reduction of Tc(VII) pertechnetate and Re(VII) perrhenate. The reduced metal can bind into a complexing POM or into another ligand. Supporting POMs on solid matrices will allow for clean separation of the reduced product. This strategy circumvents reduction using stannous ion.

Research Support: NIH-SCORE

References: References on influence of Sn(II) in formulations of Re-188 radiopharmaceuticals: Dadachova E, Chapman J. Tin-free ^{188}Re(V) DMSA- a potential antitumor radiopharmaceutical: Synthesis and biodistribution. ACS National Meeting. Dallas: American Chemical Society; 1998. Dadachova E, Mirzadeh S. The role of tin in the direct labeling of proteins with rhenium-188. Nucl. Med. Biol. 1997;24(6):605-608. Dadachova E, Chapman J. 188-Re(V)DMSA revisited-preparation and biodistribution of a potential radiotherapeutic agent with low kidney uptake. Nucl Med Commun. 1998;19(2):173-181. References on reduction potentials of metal ions Bratsch, S. G. J. Phys. Chem. Ref. Data 1989, 18, 1-21. Bard, A. J.; Parsons, R.; Jordan, J. Standard Potentials in Aqueous Solution; Marcel Dekker, Inc.: New York, 1985. References on use of polyoxometalates for reduction of metal ions: Finke, R. G. Transition Metal Nanoclusters: solution-phase synthesis, then characterization and mechanism of formation, of polyoxoanion-and tetrabutylammonium-stabilized nanoclusters; Marcel Dekker, Inc: New York, 2001. Lin, Y.; Finke, R. G. J. Amer. Chem. Soc. 1994, 116, 8335-8353. Mandal, S.; Selvakannan, P. R.; Pasricha, R.; Sastry, M. J. Amer. Chem. Soc. 2003, 125, 8440-8441. Mandal, S.; Rautaray, D.; Sastry, M. J. Mater. Chem. 2003, 13, 3002-3005. Mandal, S.; Mandale, A. B.; Sastry, M. J. Mater. Chem. 2004, 14, 2868-2871.

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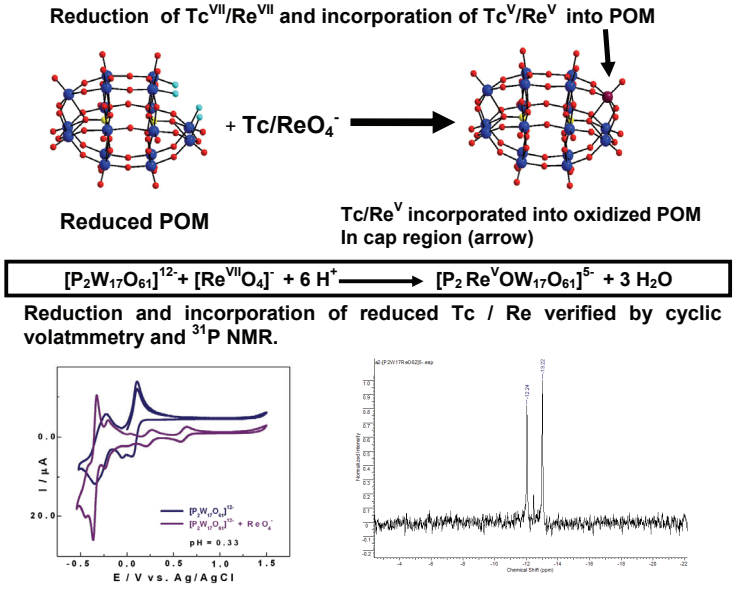

P365 SYNTHESIS OF NOTA- AND DOTA-NITROIMIDAZOLE DERIVATIVES FOR Ga-68 LABELING AND TEST AS A NEW MARKER FOR IMAGING TUMOR HYPOXIA

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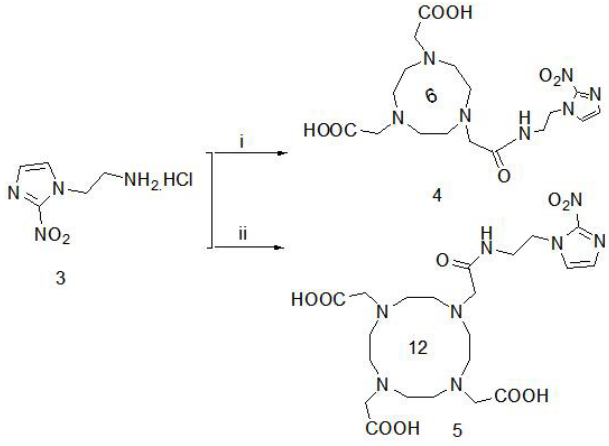
Objectives: Development of radiolabeled nitroimidazole derivatives for hypoxia imaging remains an active field of research. In earlier studies ^{67}Ga-labeled nitroimidazole has been reported as a NOTA derivative with functional group at ring methylene group, which involves difficult synthesis. To simplify this, we designed novel mono carboxylic acid modified neutral ^{68}Ga labeled NOTA-nitroimidazole derivative along with DOTA derivative which can be used as a hypoxia imaging agent for positron emission tomography (PET).

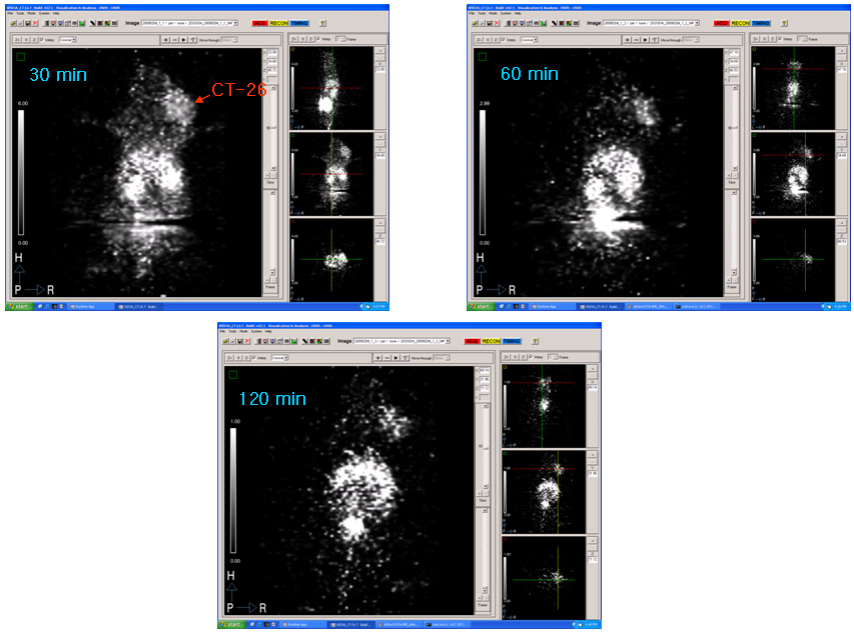


Methods: Our approach to the synthesis of ^{68}Ga labeled nitroimidazole derivatives of NOTA and DOTA involved the preparation of 2-nitroimidazole-N-ethylamine as HCl salt. 2-Nitroimidazole-N-ethyl-N-Boc-amine was prepared by conjugation of 2-(Boc-amino)ethyl bromide with 2-nitroimidazole. The subsequent deprotection of amine with 4M HCl in dioxane gave 2-nitroimidazole-N-ethylamine, conjugation with NOTA and DOTA done in water/DMF (1:1) system using DCC as a coupling agent. The final products obtained were purified by RP-HPLC and labeled with ^{68}Ga in pH 3 in boiling water bath for 10min Labling efficiencies were checked by ITLC. PET imaging studies were performed using balb/c mice xenografted with CT-26 (mouse colon cancer) under hypoxia condition.

Results: ^{68}Ga-NOTA and DOTA nitroimidazole derivatives were labeled with labeling efficiency of higher than 96%. PET images of ^{68}Ga-NOTA-imidazole showed a high tumor uptake at 30 min compared to ^{68}Ga-DOTA-imidazole.

Conclusions: We successfully synthesized ^{68}Ga labeled NOTA and DOTA nitroimidazoles for imaging tumor hypoxia, which can be labeled within 10 min. with high labeling efficiency. ^{68}Ga-NOTA-nitroimidazole was proved to be a potential hypoxic tumor PET agent.

^{68}Ga-NOTA-nitroimidazole



P366 A NOVEL APPROACH FOR THE SYNTHESIS OF RHENIUM AND TECHNETIUM-99m TRICARBONYL COMPLEXES WITH A (SS)(P) COORDINATIVE SET

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1. University of Padova, Department of Pharmacology and Anaestesiology, Padova, Italy; 2. Universitat Autonoma de Barcelona, Department of Chemistry, Bellaterra, Spain; 3. University of Padova, Department of Pharmaceutical Sciences, Padova, Italy; 4. Universitat Autonoma de Barcelona, X-ray Diffraction Service, Bellaterra, Spain

Objectives: The research aim was to develop an innovative method to synthesize tricarbonyl complexes of technetium and rhenium with the (SS)(P) coordinative set by using a transmetallation reaction with a stable Zn(II) dithiocarbamate complex. The work shows the viability of the transmetallation approach for labelling a biomolecule bioconjugated to a functionalized dithiocarbamate moiety.

Methods: The new Zn(II) dithiocarbamate complex [$Zn(SSC_7H_9NNaO_2)_2$] was synthesized starting from isonipecotic acid and fully characterized (NMR, IR, elem. anal, ESI-MS, X-ray diffraction). $^{185/187}$Re complex: [$Zn(SSC_7H_9NNaO_2)_2$] (16 μmol) solved in MeOH (6 mL) was added to a solution of[NEt_4]$_2$[fac-$Re(CO)_3Br_3$] (32 μmol) in the same solvent (1.5 mL) and the reaction was heated to reflux. After 90 min a solution of PPh_3 (32 μmol) in 6 mL of MeOH was added and the reaction was refluxed for 2 h. The precipitate was filtrated and the solvent was removed in vacuo. The obtained product was characterized by IR, NMR, ESI-MS and RP-HPLC. ^{99m}Tc complex: ^{99m}Tc-tricarbonyl complex was prepared by performing one- or two-step reaction: in the one-step reaction, a [$^{99m}Tc(CO)_3(H_2O)_3$]$^+$ solution prepared from Isolink® (20 μL, ~20 MBq, pH 7) was diluted 1:4 with MeOH, then a PPh_3 solution (10 μL, 0.2 mM) and a [$Zn(SSC_7H_9NNaO_2)_2$] solution (10 μL, 0.1 mM) in MeOH were added. The reaction was incubated at 65°C for 2 h. The two-step reaction was performed in the same conditions but firstly the solution of [$Zn(SSC_7H_9NNaO_2)_2$] was added and the reaction mixture was incubated at 65°C for 30 min; subsequently PPh_3 solution was added and the reaction was stirred at 65°C for 3 h.

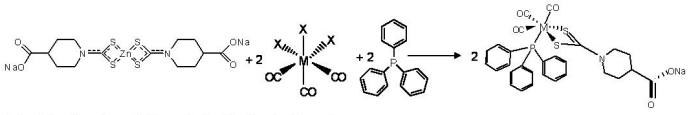

Fig. 1. *Reaction scheme for the synthesis of the tricarbonyl complexes.*

Comparative [$^{99m}Tc(CO)_3(H_2O)_3$]$^+$ labelling reactions with the sole presence of [$Zn(SSC_7H_9NNaO_2)_2$] or PPh_3 were carried out in the same conditions. All reactions were monitored by RP-HPLC.

Results: Rhenium complex [$^{185/187}Re(SSC_7H_9NNaO_2)(CO)_3(PPh_3)$] was synthesized and characterized by NMR, IR, ESI-MS and HPLC methods. All spectroscopic data support the proposed structure and are compatible with reported data for similar [2+1] complexes$_{(1)}$. The identity of the homologous ^{99m}Tc complex was established by comparative RP-HPLC studies using the characterized rhenium complex as sample reference (Table 1):

Product	M = $^{185/187}$Re	M = ^{99m}Tc
[$M(CO)_3(PPh_3)$]$^+$	15.4	15.9
[$M(CO)_3(PPh_3)_2$]$^+$	17.0	17.5
[$M(SSC_7H_9NNaO_2)(CO)_3$]	14.0	14.4
[$M(SSC_7H_9NNaO_2)(CO)_3(PPh_3)$]	15.8	16.3

Table 1.Comparison between the RP-HPLC retention times (min) of tricarbonyl complexes

Conclusions: A new approach for the synthesis of Re(I) and Tc(I) complexes has been developed. The Zn(II) dithiocarbamate complex reacts cleanly via transmetallation with the precursors to provide [$M(SSC_7H_9NNaO_2)(CO)_3(PPh_3)$] in high yield. The Zn(II) dithiocarbamate complex owns a carboxylic moiety suitable for conjugating biomolecules underlining the feasibility of this novel strategy for the production of target-specific radiopharmaceuticals. Keywords: Technetium-99m, Dithiocarbamate complexes, Tricarbonyl complexes.

References: (1) Riondato M., Camporese D., Martn D., et al., Eur. J. Inorg. Chem., 2005, 4048-4055.

P367 68GA-LABELLING OF DOTA COUPLED BIOTIN ANALOGUES AND THEIR CHARACTERIZATION

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Objectives: The aim in this study was to develop a tracer that could be used to monitor islets of Langerhans during transplantation. The biotin based tracer would suggest advantages over the currently used [^{18}F]FDG in terms of binding specificity, higher accumulation and longer residence. Applying avidin-coated islets, ^{68}Ga-labelled analogues of biotin were chosen due to the well-known strong binding between biotin and avidin ($K_d \approx 10^{-15}$ M).

Methods: Biotin analogues were first conjugated with DOTA chelator, purified by HPLC and analyzed by Nuclear magnetic resonance and Electrospray ionization mass spectrometry. Then they were used for the ^{68}Ga-labelling and complexation with gallium stable isotopes (^{69,71}Ga) for the further identification and confirmation of the final product.

Results: ^{68}Ga-labeling kinetics of three biotin analogues, with different linkers between the biotin and DOTA chelator was studied as a function of the conjugate concentration at room temperature, conventional and microwave heating. The elevated temperature (90 °C) provided near quantitative radioactivity incorporation within less than 10 min. The influence on avidine binding properties when varying the linker was investigated and compared with the native biotin. The extent of binding of the labeled compounds to avidin was 54–91% after 5 min. Blocking experiments were performed confirming the specificity of the binding of biotin analogues to avidin.

Conclusions: Three ^{68}Ga-labeled biotin-DOTA conjugates with different PEG linkers were prepared. All analogues demonstrated binding to avidin in solution. The tracers will be further evaluated in in vitro experiments of avidin-coated islets of Langerhans and in transplantation models in vivo.

P368 FIRST APPLICATION OF THE IART APPROACH WITH A NEW RE-188 LABELED BIOTIN DERIVATIVE

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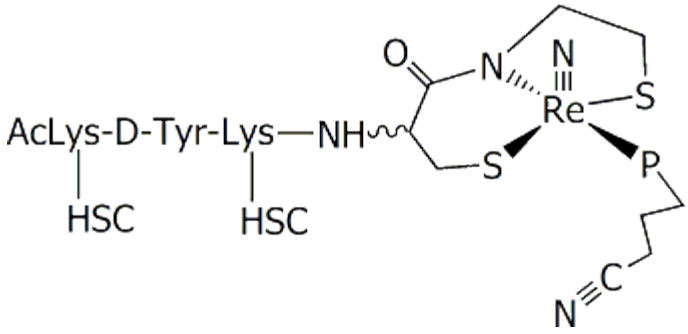
Objectives: Intraoperative Avidination for Radionuclide Therapy (IART) is a new approach for the treatment of breast cancer that relies on the avidin-biotin binding system. In this approach, the anatomic area of the tumor is filled with native avidin injected in situ by the surgeon into and around the tumor bed. Selective accumulation of avidin in residual cancerous tissue provides a target for the radiolabeled biotin injected intravenously 1 day later.

Methods: A matched pair of Tc-99m and Re-188 conjugated complexes with biotin have been prepared using the so-called '3+1 method'. Similarly, another ^{99m}Tc/^{188}Re matched pair of complexes incorporating two copies of the bivalent hapten 3-[[[2-(4-imidazolyl)ethyl]amino]carbonyl]propionylglycine haptens (histamine-succinyl-glycyl = HSG) having selective affinity for 679.1MC7 monoclonal antibody ($IgG_{1,k}$), have been prepared using the same method. In vitro and in vivo experiments in mouse models were conducted to assess affinity for avidin of the ^{99m}Tc/^{188}Re biotin conjugated complexes. Comparison with in vivo results obtained through the application of the pretargeting approach using bivalent antibodies has been also undertaken.

Results: High labeling yields were obtained in the preparation of both biotin and bivalent hapten derivatives using the 3+1 approach with Tc-99m and Re-188. A representative example of the resulting complexes is reported in the Figure. The complexes showed excellent stability toward enzyme catalyzed removal of biotin. In vitro affinity of the ^{99m}Tc/^{188}Re biotin conjugated complexes was approximately 98% of that observed for the native vitamin H. In a mouse model of localized intramuscular administration of avidin microspheres, highly selective in vivo accumulation at the avidin target site was observed after intravenous injection of radiolabeled biotin. In a mouse tumor model, efficient tumor targeting was observed with both the avidin/biotin and bivalent hapten pretargeting approaches.

Conclusions: The synthesis and first biological evaluation of new ^{99m}Tc and ^{188}Re conjugate complexes with biotin and a bivalent histamine-hemisuccinate hapten have been successfully carried out. These new matched-pair agents are of potential interest for application in the IART approach and pretargeted radioimmunotherapy.



P369 A CONVENIENT SYNTHETIC STRATEGY FOR PRODUCTION OF CLICKABLE DOTA AND TETA-DERIVED BIFUNCTIONAL CHELATES

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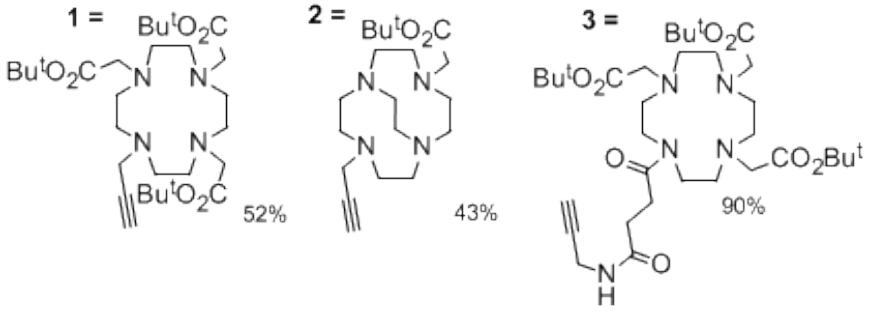
Objectives: There are a myriad of metallic radionuclides, with a diverse range of radioactive properties, suitable for the labeling of multiple peptides and antibodies. Our goal is to develop "clickable" biomolecules that can be stored, and, when required, a metal-specific bifunctional chelate can be selectively, readily and simply "clicked" onto the biomolecule prior to metal radiolabeling and application. Conventional bioconjugation methods are based on nucleophilic reactions which are intrinsically non-selective. The approach we have taken is based on the Huisgen reaction ("click" chemistry) [Kolb, H.C., Finn, M.G., Sharpless, K.B. Angew. Chem. Int. Ed. 2001, 40, 2004-2021]; this reaction allows for the selective conjugation of the peptide (pre-synthesized with a functional azide group), with a bifunctional chelate containing a C-C triple bond. While methods for attachment of the azide group to synthetic peptides have been developed [Knor, S., Modlinger, A., Poethko, T., Schottelius, M., Wester, H.-J., Kessler, H. Chem. Eur. J. 2007, 13, 6082 6090], there is lack of convenient synthetic procedures for production of bifunctional chelates containing triple bonds. In this work we aimed to develop a practical and robust synthesis of the bifunctional chelates amenable for Huisgen-based "click" chemistry.

Methods: Propargyl bromide and propargyl amine were selected as they are commercially available reactive precursors for the introduction of triple bonds into the macrocyclic structure. This allowed us to incorporate a terminal alkyne functionality, vital for the click-reaction, without lengthy synthetic procedures. The DOTA with three protected carboxylic groups and an unprotected nitrogen was available commercially. The bridged DOTA analog, with only one nitrogen atom available for substitution, was synthesized by analogy with CBTE2A [Wong, E.H., Weisman,G.R., Hill, D.C., Reed, D.P., Rogers, M.E., Condon, J.S., Fagan, M.A., Calabrese, J.C., Lam, K.-C., Guzei, I.A., Rheingold, A.L.J. Am. Chem. Soc. 2000, 122, 10561-10572].

Results: Three varieties of "DOTA" containing triple bonds have been synthesized (Figure 1). Compound 1 is the simplest DOTA derivative amenable to click-chemistry. It was synthesized in one-step from commercially available materials with 52% yield. Compound 2 represents a class of cross-bridged chelates. Synthesis of this chelate proved to be more challenging (7 steps, yield 20%), but the fact that metal complexes of cross-bridged chelates exhibit higher in vivo stability justify these efforts [3]. The synthesis of compound 3 proved to be the simplest of the three examples (90% yield, no chromatographic purification).

Conclusions: Three bifunctional chelates amenable for Huisgen based "click"-reactions were synthesized. The synthetic strategies for production of these chelates is readily transferrable to TETA-, DFO- and DTPA-based chelates, providing a versatile and robust system selective radiolabeling of molecule with stable metal-chelate systems.



P370 PRINCIPLES GOVERNING THE STABILITY OF TECHNETIUM AND RHENIUM TRIPEPTIDES

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1. Hunter College of the City University of New York, Chemistry Department, New York, NY; 2. Laboratoire de Chimie Quantique-UMR 7177, CNRS and Universit Louis Pasteur, Strasbourg, France

Objectives: Tripeptide ligands can be easily appended onto targeting vectors for complexation of ^{188}Re and ^{99m}Tc. The amino acid residues of the tripeptide impact the 1) stability of the resulting complex and the 2) diastereomer distribution. The objective of this study is to understand the molecular features of tripeptides that impact the stability of Tc/ReO species including both L- and D- amino acids.

Methods: Syntheses of macroscopic ^{99}Tc and Re and tracer ^{99m}Tc and ^{188}Re complexes. Characterization was performed using standard techniques, including X-ray crystallography, NMR and Circular Dichroism Spectroscopy. Kinetic and thermodynamic studies tracking the γ ray of ^{99m}Tc were monitored via HPLC. DFT calculations and geometry optimizations.

Results: Kinetic and thermodynamic studies show that the anti diastereomer for Tc/ReOphe-gly-cys (TcO FGC) is favored (K_{eq} = 3.6). This is modeled by Density Functional Theory (DFT) calculations and geometry optimizations on both the syn- and anti- ReO FGC diastereomers. Hydrogen bonding interactions between the amide and –yl oxygen atom, that can be observed from X-ray crystallography, contribute to the stability of the anti diastereomer. In contrast, the syn diastereomer of Re/TcO phe lys cys (MO FKC) is the more stable, also modeled by DFT. The anti-syn conversion is triggered by deprotonation of the N_{1amine} to form a strong M-N_1 bond that appears to lock in the syn conformation as determined from NMR experiments and X-ray structures. The strong M-N_1 bond appears to influence the stability of the molecule. Substitution of glutamic acid appears to stabilize a second set of diastereomers over time; mass spectrometry suggests that these diastereomers possess a N_{1amide}-Tc/Re species. Charged residues appear to impact positively the stability of the complexes according to our work and that of others.. Substitution of D- amino acids into the FKC framework reverses the HPLC profile of the ^{99m}Tc complexes and stabilizes one diastereomeric species. Synthesis, isolation of the ReOFKc (c= D-cysteine) results in two diastereomers wherein the more stable early eluting diastereomer is assigned to the structure where the ReO group is syn to the lys and phe group based on NMR, CD, among other techniques. Synthesis of the ^{99m}Tc tracer species (90^{o}C , 30 min, pH 7.4) results in one radiochemical species.

Conclusions: Tripeptide ligands containing both L- and D- amino acids form diastereomers when complexed to Tc/Re=O groups. The stability of the diastereomers is a function of the amino acid residues. In the tripeptide where there is gly in the second position, the anti diastereomer is stabilized by H-bonding of the M=O –yl oxygen with the amide functionality of the cysteine. When lys comprises the second position, the pK_a of the amine terminus is lowered and the proton is easily released, triggering a syn conformation and a stable, strong M-N_1amide bond. This chemistry follows for both L- and D- amino acids incorporated in the FKC construct.

Research Support: NIH-SCORE

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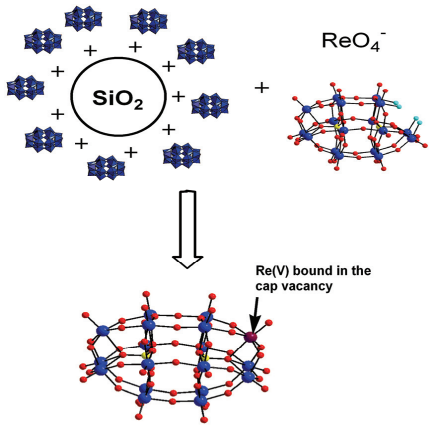

Reduction of ReO_4^- using a supported non-complexing POM.

P371 EFFECT OF DOTA POSITION ON MELANOMA TARGETING AND PHARMACOKINETIC PROPERTIES OF LACTAM BRIDGE-CYCLIZED ALPHA-MELANOCYTE STIMULATING HORMONE PEPTIDE

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Objectives: The purpose of this study was to examine the effect of DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) position on melanoma targeting and pharmacokinetic properties of lactam bridge-cyclized alpha-melanocyte stimulating hormone (α-MSH) peptide.

Methods: A novel DOTA-conjugated lactam bridge-cyclized α-MSH peptide, namely Ac-GluGlu-CycMSH[DOTA] (Ac-Glu-Glu-c[Lys(Lys-DOTA)-Nle-Glu-His-DPhe-Arg-Trp-Gly-Arg-Pro-Val]), was synthesized using standard 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry. DOTA was directly attached to the cyclic ring while the N-terminus of the peptide was acetylated to generate Ac-GluGlu-CycMSH[DOTA]. The MC1 receptor binding affinity of Ac-GluGlu-CycMSH[DOTA] was determined in B16/F1 melanoma cells. Melanoma targeting and pharmacokinetic properties of Ac-GluGlu-CycMSH[DOTA]-^{111}In were determined in B16/F1 melanoma-bearing C57 mice and compared to that of ^{111}In-DOTA-GlyGlu-CycMSH (DOTA was coupled to the N-terminus of the peptide).

Results: Ac-GluGlu-CycMSH[DOTA] displayed 0.6 nM MC1 receptor binding affinity in B16/F1 cells. Ac-GluGlu-CycMSH[DOTA]-^{111}In was readily prepared with greater than 95% radiolabeling yield. Ac-GluGlu-CycMSH[DOTA]-^{111}In exhibited higher tumor uptake (11.39±1.74 %ID/g 0.5 h post-injection) and more prolonged tumor retention (9.42±2.41 %ID/g 4 h post-injection) than that of ^{111}In-DOTA-GlyGlu-CycMSH (10.40±1.40 and 7.40±0.43 %ID/g at 2 and 4 h post-injection, respectively) in B16/F1 melanoma-bearing C57 mice. The uptake values for non-target organs were generally low except for the kidneys.

Conclusions: DOTA position exhibited profound effect on melanoma targeting and pharmacokinetic properties of Ac-GluGlu-CycMSH[DOTA]-^{111}In, providing a new insight into the design of lactam bridge-cyclized peptide for melanoma imaging and therapy.

P372 EVALUATION OF 67GA-LABELED LACTAM BRIDGE-CYCLIZED ALPHA-MELANOCYTE STIMULATING HORMONE PEPTIDES FOR MELANOMA IMAGING

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Objectives: The purpose of this study was to examine melanoma targeting and pharmacokinetic properties of ^{67}Ga-labeled lactam bridge-cyclized alpha-melanocyte stimulating hormone (α-MSH) peptides.

Methods: Two 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-conjugated lactam bridge-cyclized α-MSH peptides, namely DOTA-GluGlu-CycMSH (DOTA-Glu-Glu-c[Lys-Nle-Glu-His-DPhe-Arg-Trp-Gly-Arg-Pro-Val-Asp]) and DOTA-GlyGlu-CycMSH, were synthesized using standard 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry. The MC1 receptor binding affinities of DOTA-GluGlu-CycMSH and DOTA-GlyGlu-CycMSH were determined in B16/F1 melanoma cells. Melanoma targeting and pharmacokinetic properties of ^{67}Ga-DOTA-GluGlu-CycMSH and ^{67}Ga-DOTA-GlyGlu-CycMSH were determined and compared in B16/F1 melanoma-bearing C57 mice.

Results: DOTA-GluGlu-CycMSH and DOTA-GlyGlu-CycMSH displayed 6.7 and 0.9 nM MC1 receptor binding affinities in B16/F1 cells. Both ^{67}Ga-labeled peptides were readily prepared with greater than 95% radiolabeling yield. ^{67}Ga-DOTA-GlyGlu-CycMSH exhibited high tumor uptake (8.59±1.37 %ID/g 0.5 h post-injection) and prolonged tumor retention (5.02±1.35 %ID/g 24 h post-injection) in B16/F1 melanoma-bearing C57 mice. The uptake values for non-target organs were generally low (<0.3 %ID/g) except for the kidneys 2, 4 and 24 h post-injection. Although ^{67}Ga-DOTA-GluGlu-CycMSH displayed reduced renal uptake value (18.33±1.29 %ID/g) than that of ^{67}Ga-DOTA-GlyGlu-CycMSH (22.60±4.03 %ID/g) 4 h post-injection, the tumor uptake value of ^{67}Ga-DOTA-GluGlu-CycMSH was only 69.5% of that of ^{67}Ga-DOTA-GlyGlu-CycMSH 4 h post-injection.

Conclusions: High melanoma uptake and prolonged retention highlighted the potential of ^{67}Ga-DOTA-GlyGlu-CycMSH as an effective imaging probe for melanoma detection.

P373 IMPACT OF CHARGE AND PENDANT CARBOXYL GROUPS OF $^{99m}Tc(CO)_3$ THIOETHER-CARBOXYLATE COMPLEXES ON RENAL TUBULAR TRANSPORT

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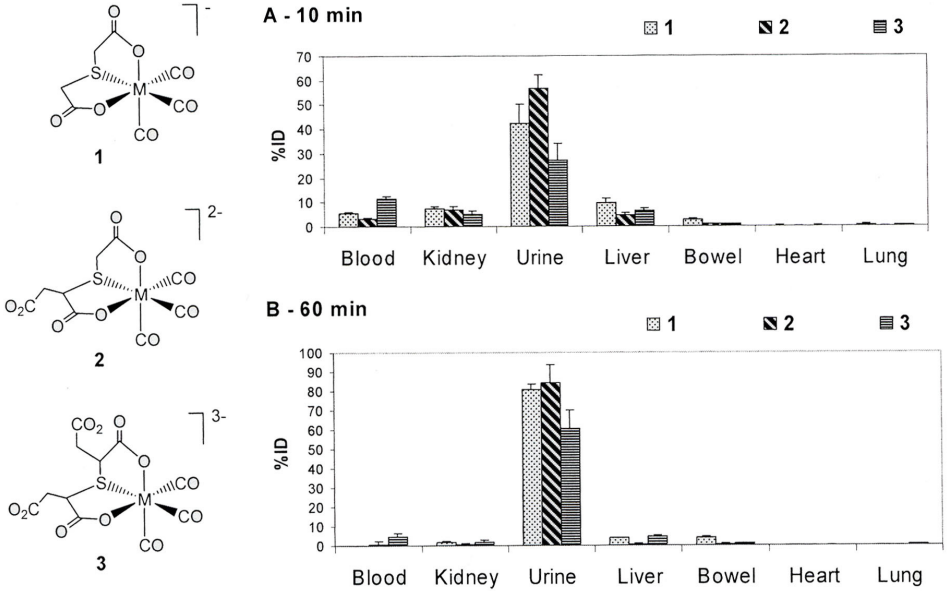
Objectives: $^{99m}Tc(CO)_3$-nitrilotriacetic acid ($^{99m}Tc(CO)_3$(NTA)) is a new, dianionic renal tubular agent with a clearance equal to that of ^{131}I-OIH in rats [1]; one charge is associated with the metal coordination sphere and the second with terminal carboxyl group. To determine the effect of the total charge and charge distribution on the pharmacokinetics of the $^{99m}Tc(CO)_3$ radiopharmaceuticals, we evaluated three $^{99m}Tc(CO)_3$-thioether-carboxylate complexes with one negative charge on the metal coordination sphere and with 0-2 pendant carboxyl groups.

Methods: Monoanionic (1), dianionic (2), and trianionic (3) $^{99m}Tc(CO)_3$-thioether-carboxylate complexes were synthesized from commercially available ligands, labeled with ^{99m}Tc using an IsoLink kit (Covidien), and evaluated in Sprague-Dawley rats (n = 5 for each time point).

Results: All three agents were efficiently prepared with radiochemical purities > 99% and showed no decomposition for 6 h at pH 7.4. All were excreted unchanged in the urine. The dianion 2 had the highest urine excretion at 10 and 60 min coupled with relatively low uptake in the liver and bowel, whereas the monanion 1 had similar activity in the urine at 60 min but higher uptake in the liver and bowel. The trianion 3 was not extracted efficiently by the kidney (%ID in the urine = 27% at 10 min and 61% at 60 min) and had relatively high retention in the blood and liver (Figs. A and B) [2].



Conclusions: While $^{99m}Tc(CO)_3$ agents 1-3 have one negative charge associated with the coordination sphere, the overall dianionic charge and one pendant carboxyl group in 2, as in $^{99m}Tc(CO)_3$(NTA) and ^{99m}TcO-MAG3, may account for its more rapid renal excretion. On the other hand, the total -2 charge of the periphery from two carboxyl groups in close proximity and the -3 net charge may have decreased the blood clearance and the rate of excretion in 3. The lack of the dangling carboxyl group in 1, which has been always considered to be essential for renal tubular receptor recognition, may slow the rate of elimination in the urine at 10 min when compared to 2, although there was no significant difference at 60 min (P = 0.43). The small size of 1 could contribute to its good renal excretion. However, the low net charge of 1 (lowest of all three agents) can increase hepatobiliary excretion or intestinal secretion. In summary, an analysis of the charge distribution suggests that a mononegative inner coordination sphere and a dianionic overall charge at physiological pH favor rapid renal tubular secretion.

Research Support: This work was supported by the NIH (R01 DK38842).

References: [1] M. Lipowska et al., J. Nucl. Med., in press. [2] H. He et al., Nucl. Med. Biol. 2007, 34: 709-716.

P374 A RADIOGALLIUM-LABELED BIFUNCTIONAL CHELATE FOR HYPOXIC TUMOR IMAGING

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Objectives: DOTA is well used as a chelator to form stable complexes with radiometals. This ligand has eight coordinating donor atoms (four nitrogens and four oxygens) and forms octa-coordinated complexes with metals in +3 oxidation state. However, recent crystallographic studies demonstrated a hepta-coordinated structure of Ga-DOTA complexes; the four nitrogens of the cyclen ring and two oxygens of the opposite carboxylate arms are coordinated to the metal. From these observations, we were able to deduce that two free carboxylate groups of the radiogallium-DOTA complex may be utilized for coupling to functional moieties that recognize molecular targets for in vivo imaging without reducing the radiogallium-complex stability. Thus, we designed 2,2'-[4,10-bis(2-{[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]amino}-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl]diacetic acid (DOTA-MN2, Figure 1), employing a metronidazole moiety as the recognition site of hypoxic lesions, based on the drug design concept of bifunctional chelates.

Methods: Coupling of DOTA-bis(tert-butyl)ester with 1-(2-aminoethyl)-2-methyl-5-nitroimidazole dihydrochloride, followed by deprotection, afforded DOTA-MN2. ^{67}Ga-labeling was carried out by reaction of DOTA-MN2 with ^{67}Ga-citrate at 95 °C. The stability of ^{67}Ga-DOTA-MN2 was investigated by incubation with mouse plasma at 37°C. To evaluate the pharmacokinetics, ^{67}Ga-DOTA-MN2 was injected intravenously into normal and NFSa tumor-bearing mice.

Results: After purification by HPLC, ^{67}Ga-DOTA-MN2 was obtained with high radiochemical purity (>96%). Radiochemical yields of the final formulated product were 35-59%. When ^{67}Ga-DOTA-MN2 was incubated with mouse plasma, the radiochemical purity was unchanged for 24 h. In normal mice, ^{67}Ga-DOTA-MN2 showed rapid blood clearance and low tissue accumulations of the radioactivity. At 24 h, 79% and 4% of the injected radioactivity of ^{67}Ga-DOTA-MN2 were recovered in the urine and feces, respectively. In cellulose acetate electrophoresis, 93% of the radioactivity excreted in the urine exhibited a similar migration distance to that of ^{67}Ga-DOTA-MN2. When injected to NFSa tumor-bearing mice, significant accumulation of the radioactivity in the tumor was observed, and the tumor-to-blood ratio increased up to 6 h. These results indicate that the drug design of ^{67}Ga-DOTA-MN2 based on bifunctional chelates makes both high in vivo stability of Ga-DOTA chelate and significant recognition of the hypoxic lesions by metronidazole molecules possible.

Conclusions: We successfully designed and synthesized a metronidazole-derivatized bifunctional chelate, ^{67}Ga-DOTA-MN2 as a hypoxic imaging agent. The present findings suggest that DOTA can be used for designing new radiogallium-labeled SPECT and PET radiopharmaceuticals with two identical or distinct recognition moieties to produce efficient interaction with target biomolecules.

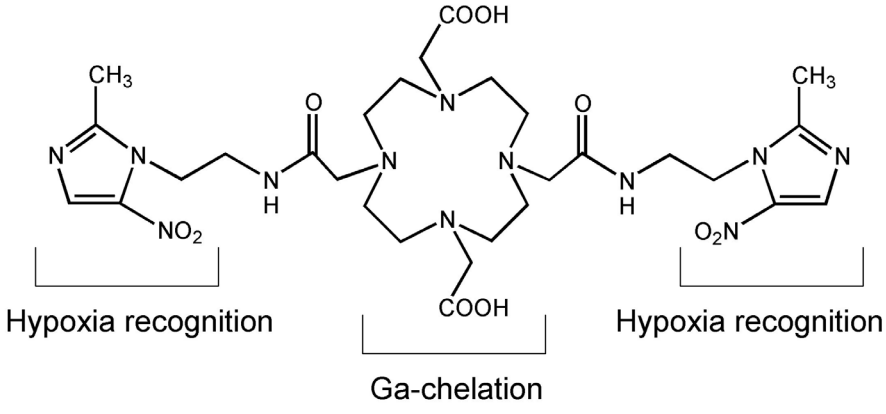

Figure 1 Chemical structure of DOTA-MN2

P375 SUGAR SUBSTITUTED BIPYRIDINE COMPLEXES OF 99M-TECHNETIUM

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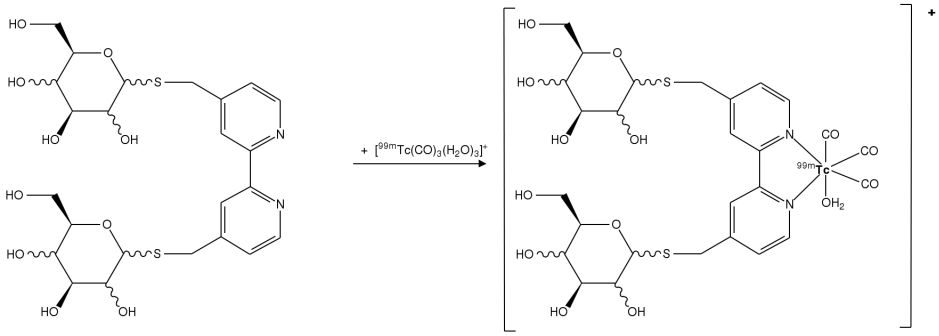
Objectives: Bipyridines functionalized with carbohydrates combine the useful properties of both components. They show versatile and stable complexation of metal ions via the bipyridyl nitrogens. Appending a carbohydrate to a metal complex may add the advantages of reducing toxicity, improving solubility and opens up the possibility of molecular targeting of carbohydrate binding domains and transport systems in cells and tissues. Therefore in this study we connected different monosaccharides S-glycosidically to 2,2'-bipyridine in order to obtain biologically stable ligands for ^{99m}Tc(I) ions. We investigated the usage of 4,4'-bis(β-D-glycopyranosyl-thiomethyl)-2,2'-bipyridine (1a) and the corresponding galactose (1b) respectively mannose (1c) derivatives as novel ligands for the complexation of ^{99m}Tc. Exemplary the ^{99m}Tc complex (2a) was used for a histidine stability test.

Methods: For the labeling reaction the Isolink®- kit was used to form the ^{99m}Tc(I)-carbonyl starting from ^{99m} TcO_4^-. To 1 mg of 1a-c 0.5 μL of the prepared ^{99m}Tc(I)-carbonyl solution were added and the mixture was heated in a glass vial at 100°C for 30 min. After cooling to room temperature the products were analyzed for their radiochemical purity (> 95%) by HPLC using the following conditions: column: RP-18e (5 μm); solvent A: triethylamine phosphate (0.05 M) pH=2.25; solvent B: methanol; flow rate: 1.2 mL/min; gradient: from 0-3 min 100 % A, 3-17 min to 100 % B, from 17-22 min 100 % B. Histidine stability test of Tc complex 2a: A solution of histidine (0.1 mM, 500 μL PBS, pH 7.4) was added to a solution of the ^{99m}Tc complex 2a (500 μL, final ligand concentration 0.05 mM). The mixture was incubated at 37°C and samples were taken at 1, 2.5, 4.5 and 24 h for HPLC analysis.

Results: Complexation of the ligands 1a-c was carried out by addition of the neutralized $[^{99m}Tc(CO)_3(H_2O)]^+$ solution to the appropriate ligand. Increased formation of by-products while heating the reaction mixtures at 100°C could not be detected and the formed complexes 2a-c are stable for several hours in this aqueous solution. The HPLC analysis shows no retransformation to $^{99m}TcO_4^-$. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (Rf = 3.2 min) reacted completely and the radiochemical impurities were lower than 5 % without an additional cleaning step. Retention times of the formed compounds 2a-c are between 11.9 and 12.4 min using the HPLC method mentioned. The in vitro stability of the formed ^{99m}Tc complexes was exemplary assessed for 2a using standard procedures by incubation with solutions of histidine in PBS buffer at 37°C. The susceptibility of the complex towards ligand exchange reactions with this amino acid was studied over a 24 h period.

Conclusions: The results clearly show that the complex possesses a good stability over a 4.5 h period. Slowly two additional signals for more lipophilic complexes appear in the HPLC. A complete ligand exchange resulting in the formation of the 99m-Tc histidine carbonyl complex $^{99m}Tc(His)(CO)_3$ (Rf = 9.9 min), which has been synthesized under the same conditions for comparison, could not be detected.



1a=β-D-gluco-
1b=β-D-galacto-
1c=β-D-manno-

2a-c

P376 99M-TECHNETIUM COMPLEXES OF SUGAR CONTAINING TRIPODAL TRIAMINES AS POTENTIAL RADIO IMAGING AGENTS

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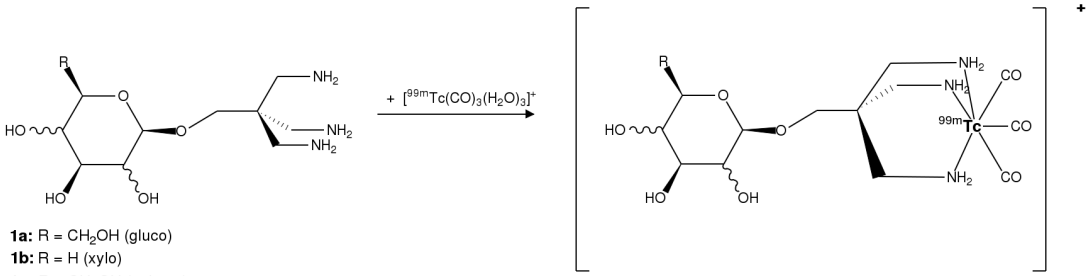
Objectives: Carbohydrates are involved in various biological processes such as polyvalent interactions; they are substrates for selective transport systems and part of antibiotic agents. Bound to a metal center they are able to improve solubility, decrease toxicity and open the possibility to target saccharide specific binding domains or metabolic pathways. Tripodal triamine ligands derived from 1,1,1-tris(aminomethyl)ethane (TAME) are known to complex a variety of metal ions. In order to take advantage of the stability of the complexes of the TAME type and to develop useful tracers for the mentioned biological phenomena, we synthesized new sugar containing tripodal triamines of the TAME type, derived from glucose 1a, xylose 1b and galactose 1c precursors and their corresponding ^{99m}Tc(I) carbonyl complexes 2a-c. The formation of the galactose substituted ^{99m}Tc(I) carbonyl complex 2c was examined and the stability against histidine over a period of 24 h could be shown by HPLC analysis.

Methods: Starting form $^{99m}TcO_4^-$ the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was generated by using the Isolink®kit and phosphate buffer was use to adjust the pH to 7. To 1 mg (3.7 μmol) of compound 1a-c 1.35 mL of the solution of ^{99m}Tc- carbonyl added and the mixture was heated at 100°C for 30 min. After cooling to room temperature the products were analyzed for their radiochemical purity (> 95%) by HPLC using the following conditions: HPLC pump: Jasco PU-1580; quaternary gradient unit: Jasco LG-1580-04; radio detector: biostep IsoScan LC; RI detector: Jasco RI 1530; column: RP-18e (5μm); solvent A: triethylamine phosphate (0.05 M) solution in water, adjusted with H_3PO_4 to pH = 2.25; solvent B: methanol; flow rate: 1.2 mL/min; gradient: from 0-3 min 100 % A, 3-17 min to 100 % B, from 17-22 min 100 % B. Histidine stability test of ^{99m}Tc complex 2c A solution of histidine (4.8 mg, 31 μmol, in 1 mL water) was added to a solution of the ^{99m}Tc complex 2c (1.35 mL, final ligand concentration 1.57 mM). The mixture was incubated at 37°C and samples were taken at 1, 2.5, 3.5, 19 and 24 h for HPLC analysis, respectively.

Results: Increased formation of by-products while heating the reaction mixtures at 100°C could not be detected and the formed complexes 2a-c were stable for several hours. The retention time of 1c was found to be 1.3 min. The HPLC analysis shows no retransformation to $^{99m}TcO_4^-$ (Rf = 1.9 min). The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ ions (Rf = 3.9 min) reacted completely and the radiochemical impurities were lower than 5% without an additional cleaning step. The preceding gradient was also used for analysis of the stability of complex 2c against ligand exchange with histidine, applying standard procedures (incubation of 2c with a 10-fold excess of histidine).

Conclusions: Even after 24 h no decomposition of the complex could be observed and the chromatogram shows the sole appearance of 2c. No formation of the ^{99m}Tc-carbonyl histidine complex (Rf = 9.9 min) or other ^{99m}Tc containing byproducts could be observed. It shows the advantage of using a tripodal triamine to complex the ^{99m}Tc-carbonyl core.



1a: R = CH_2OH (gluco)
1b: R = H (xylo)
1c: R = CH_2OH (galacto)

2a-c

P377 RADIOLABELING OF FRAGILE MACROLIGANDS WITH GA-68

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Objectives: ^{68}Ga- labeled receptor specific ligands like DOTA-conjugated peptides are commonly used for diagnostics in PET. In order to obtain a high level of specific activity of different labeled macroligands, $^{68}GaCl_3$ possessing a high purity is necessary. Routinely, we favor the cationic cleaning of the ^{68}Ge/^{68}Ga generator eluate. The ^{68}Ga eluate is cleaned and concentrated by using of a cationic ion exchanger for concentration and subsequent elution with a mixture of acetone and HCl. With the obtained $^{68}GaCl_3$ eluate it is possible to label DOTA- peptides with a relatively low molecular weight (e.g. DOTA-TATE) . Contrariwise labeling of conjugates of peptides or proteins possessing a higher molecular weight is problematic. Some of those fragile macromolecules denature in the presence of acetone. Basically, the labeling efficiency decreases dramatically because of the stronger influence of foreign ions. In this regard the most considerable contamination are iron ions because of their high impact on the radiochemical yield. In this case the known cationic cleaning is not sufficient. Already a concentration of 0.1 mg/L of iron leads to a loss of radiochemical yield of more than 60 %. On the other hand for established anionic cleaning procedures, using e.g. AG1X8, a high volume of concentrated HCl is required. Here we present a cleaning procedure along with practical results of radio labeling using a TiO_2 based commercially available generator.

Methods: The ^{68}Ga eluates and the described products were analyzed by HPLC (column: RP-18; solvent A: acetonitrile solution in water (5 %), 0.1 % TFA; solvent B: 95 % acetonitrile solution in water, 0.1 % TFA; flow rate: 1.2 mL/min; gradient: from 100% A to 100 % B). The quoted ion exchangers are commercially available (LiChrolut SCX (40-63 μm), Merck; AG1X8 Biorad).

Results: We found that some plastic adapters and the rubber inside of some magnetic valves can emit iron ions under acid conditions by simultaneous use of organic solvents. But also leakages inside the generator can be responsible for iron contaminations. By the combination of both known methods the following cleaning procedure provides a $^{68}GaCl_3$ with the required high purity. The pH value of the reaction mixture was between 3 and 4, which is optimal for the complexation. It is free of organic solvents. The generator was eluted with 5 mL 0.1 M HCl and the ^{68}Ga was collected on an SCX cartridge (ca. 50 mg SCX; reclaimable with 1 mL 4.0 M HCl / 2 mL water). Afterwards the ^{68}Ga was eluted with 0.4 mL 4.0 M HCl into a glass vial containing 0.4 mL 7.0 M HCl. Under these conditions (5.5 M HCl) ^{68}Ga-chlorid was transformed into the anionic $[GaCl_4]^-$ ion and could be collected on an AG1X8 column (ca. 30 mg, conditioned with 2 mL 4.0 M HCl). The column was dried by an inert gas steam (1-2 min) and the cartridge was eluted with 0.2 - 0.4 mL of water into a solution of the corresponding DOTA-peptide in 0.4 mL 1.5 M HEPES buffer with subsequent heating to 80 °C (5min).

Conclusions: For DOTA-TATE, -NOC, -TOC or DOTA-Affibody the procedure leads to the final products with a radiochemical purity greater than 95 % without further cleaning steps.

P378 HBED-CC-TFP2: A VERSATILE 68GA-LABELING AGENT FOR DIMERIZATION OF PEPTIDES

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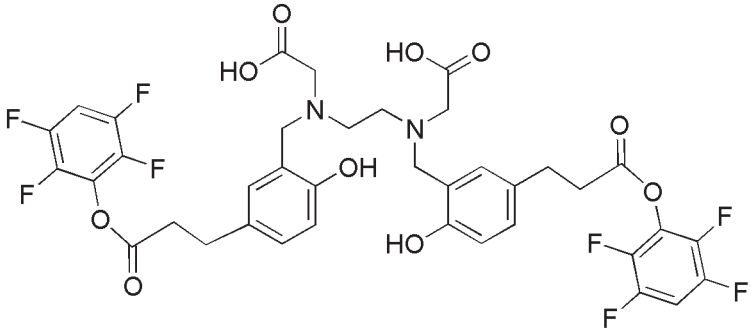
Objectives: N,N'-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-N,N'-diacetic acid (HBED-CC) have been reported as a highly efficient and gentle labeling agent for biomolecules with ^{68}Ga [1]. The acyclic structure of HBED-CC allows rapid $[^{68}Ga]Ga^{3+}$ incorporation at room temperature, and its potential for dimerization of peptides is given through the activation of carboxyethyl groups not participating in complexation.

Methods: In order to selectively protect the complexing moiety HBED-CC was complexed using $FeCl_3$. The two remaining propionic acid functions were activated by reacting $[Fe(HBED\text{-}CC)]^-$ with 100 times molar excess of TFP (2,3,5,6-tetrafluorophenol) supplemented with DIC (N,N'-diisopropylcarbodiimide). $[Fe(HBED\text{-}CC)TFP_2]^-$ was purified by preparative HPLC. The identification of the various products was performed with HPLC and MALDI-MS. Deprotection was carried out by trapping the purified product on a preconditioned RP cartridge (Waters SepPak-Classic C18) followed by flushing with 1 M HCl.

Results: Besides the two phenolic and carboxylate groups, essential for complex formation, HBED-CC has two propionic acid functions which were both selectively activated with TFP. According to HPLC $[Fe(HBED\text{-}CC)TFP_2]^-$ was formed with 27 % yield after 20 minutes at room temperature. The desired product was chemically identified by MALDI-MS. Based on HPLC the isolated yield amounted to 15 - 18 % related to initially added $[Fe(HBED\text{-}CC)]^-$. The purified $[Fe(HBED\text{-}CC)TFP_2]^-$ was either used directly for conjugation of peptides or after an HCl assisted deprotection step for conjugation of molecules not allowing further HCl treatments.

Conclusions: Taken together HBED-CC-TFP_2 is a promising agent for the dimerization of peptides or other biomolecules provding simultaneously a highly efficient complexation moiety for ^{68}Ga. HBED-CC offers excellent kinetics allowing fast and easy-to-perform labeling of even labile biomolecules at room temperature.

References: 1. Eder M, Wngler B, Knackmuss S, Legall F, Little M, Haberkorn U, et al. Tetrafluorophenolate of HBED-CC: a versatile conjugation agent for (68)Ga-labeled small recombinant antibodies. Eur J Nucl Med Mol Imaging. 2008;35:1878-86.



HBED-CC-TFP_2

P379 NOVEL TECHNETIUM-99m COMPLEXES WITH BISPHOSPHONATES AS POTENTIAL BONE SCINTIGRAPHY AGENTS

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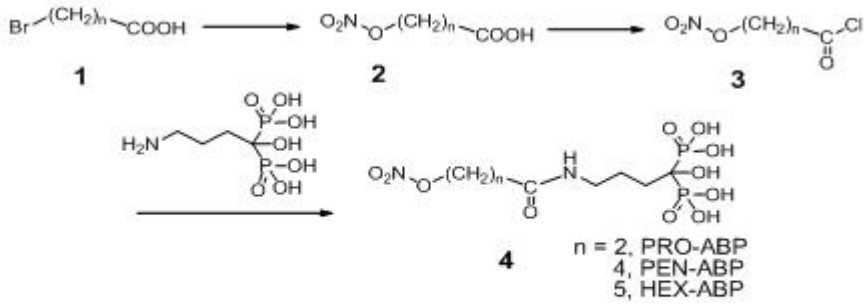
Objectives: The aim of our study is to develop ^{99m}Tc-labeled bisphosphonates for bone scintigraphy. The synthesis, radiolabeling and biodistribution of those technetium-99m complexes are reported.



Scheme1. Synthesis of some NO-BPs

Methods: Three NO-BPs (4) were synthesized by conjugating BPs with NO-donor moiety according to the procedure outlined in Scheme 1. The synthesis of nitrate (3) and its immediate precursor (2) was based on literature methods with a slight modification. ^{99m}Tc-NO-BPs was prepared by reduction of $^{99m}TcO_4^-$ in the presence of $SnCl_2$ and NO-BPs. The RCP of ^{99m}Tc-NO-ABP complex was evaluated by TLC and RP-HPLC. Paper electrophoresis were carried out using phosphate buffer (0.025M, pH=7.4) at 150v for 120min. The octanol/water partition coefficient was determined in the mixture of 1-octanol and phosphate buffer (0.025 M, pH 7.4). Biodistribution experiments were performed on normal Kunming mice (20± 2g). After administering 74 MBq of the ^{99m}Tc-NO-BPs intravenously in male Japanese white rabbit (2kg), SPECT imaging were done by using SKYLIGHT SPECT（Philips）.

Results: Three technetium-99m complexes were prepared with more than 95% of radiochemical purities. In electrophoresis, the fact that ^{99m}Tc-PRO-ABP, ^{99m}Tc-PEN-ABP and ^{99m}Tc-HEX-ABP migrated towards the anode indicated those complexes are negative. The partition coefficient of three complexes showed they are hydrophilic complexes. The bone uptakes of ^{99m}Tc-PRO-ABP, ^{99m}Tc-PEN-ABP and ^{99m}Tc-HEX-ABP were as high as 25.38± 5.09, 17.71± 1.87, 22.94± 4.13 %ID/g (n=3) at 15min after injection, increasing to 26.76±4.24, 25.49±3.86,33.06±5.63%ID/g at 1h, respectively. The radioactivities in liver, blood, muscle, heart, spleen, brain and lung were all lower than 0.5 %ID/g at 30min after injection. The bone-to-blood ratios of ^{99m}Tc-PRO-ABP increased greatly to 218.39 at 60min from 9.65 at 15min indicating the fast blood clearance. SPECT images in rabbit revealed that high bone to soft tissue ratio with ^{99m}Tc-NO-BPs occurs at 30min with highly selective skeletal uptake and lower liver uptake. The scan at 60min showed clearly the whole skeleton, bladder and kidney image and no concentration in any other organ. Most of the radiotracer was excreted by the urinary system.

Conclusions: In summary, three NO-donor bisphosphonatesare capable of forming stable ^{99m}Tc-chelates that exhibit high bone uptake and low non-target uptake. They showed great potential for bone scintigraphy since their highly selective bone uptake and rapid clearance in blood.

Research Support: The authors wish to acknowledge the support of Beijing Shihong Pharmaceutical Center for the help in preparing the PRO-ABP, PEN-ABP and HEX-ABP kits and donation of MDP kits.

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P380 STEREOSPECIFIC ONE POT DEUTERATION OF BETA-FURANOSYL AZOMYCIN NUCLEOSIDES: A MODEL REACTION FOR SYNTHESIS OF 3H-BETA-5-FAZA AND OTHER AZOMYCIN NUCLEOSIDE ANALOGUES

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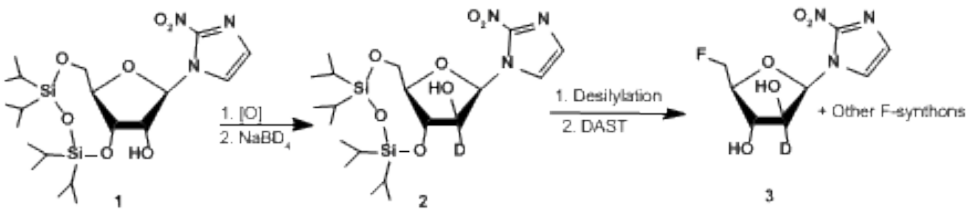
Objectives: To develop a regioselective 'one pot isotopic labeling' procedure for deuteration or tritiation of β-5-FAZA. This approach serves as a model for developing tritium (^3H) labeled products of azomycin nucleoside based radiosensitizers and hypoxia imaging agents. (1-3).

Methods: 1-β-D-(3',5'-O,O-tetraisopropyldisyloxyribofuranosyl)-2-nitroimidazole (TIPDS-β-AZR) was added to a suspension of CrO_3/Ac_2O in anhydrous pyridine at 22 °C (30 min) that yielded a C-2' keto intermediate which, on reaction with $NaBD_4$, underwent stereospecific deuteration at C-2' carbonyl to afford 1-β-D-(3',5'-O,O-tetraisopropyldisyloxy-2'-[^2H]-ribofuranosyl)-2-nitroimidazole ([^2H]-TIPDS-β-AZA). Desilylation of this product, using KF/benzoic acid, gave 1-β-D-(2'-[^2H]-arabinofuranosyl)-2-nitroimidazole[^2H]-β-AZA which, on DAST-assisted fluorination, afforded 1-β-D-(5'-deoxy-5'-fluoro-2'-[^2H]-arabinofuranosyl)-2-nitroimidazole ([^2H]-β-5-FAZA).



Results: Current work describes the site-specific stereoselective synthesis of isotopically labeled azomycin β-nucleoside, (2'-[^2H]-β-AZA). The deuterated, silylated intermediate was purified on a silica gel column using hexanes/ethyl acetate (8:2; v/v), and [^2H]-TIPDS-β-AZA was recovered in 70% yield. This intermediate was desilylated using KF/benzoic acid to afford [^2H]-β-AZA (78% yield) and elaborated to the corresponding fluoro analogue (2'-[^2H]-β-5-FAZA) by DAST-assisted fluorination (20% yield). The overall isolated yield of fluorinated products was ~55%.

Conclusions: A 'site-specific (at C-2')' stereoselective one pot synthesis' of deuterium labeled [^2H]-β-5-FAZA has been developed. This procedure can be adapted to synthesize ^3H-labeled (fluorinated) β-azomycin arabinofuranosyl nucleosides in satisfactory yield, for long-duration biological evaluations are not possible with ^{18}F-labeled analogues.

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P381 SYNTHESIS AND BIOLOGICAL EVALUATION OF TECHNETIUM-99M LABELLED DDNP DERIVATIVES AS POTENTIAL BETA-AMYLOID IMAGING AGENTS

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Objectives: Up to now, a great deal of moleculars were reported to target beta-amyloid. But most of them were labelled by ^{18}F or ^{11}C for positron emission tomography (PET), however ^{99m}Tc labelled radioactive probes for beta-amyloid were rarely reported. FDDNP namely 2-(1-(6-((2-fluoroethyl)(methyl)amino)naphthalen-2-yl)ethylidene)malononitrile and FENE namely 1-(6-((2-fluoroethyl)(methyl)amino)naphthalen-2-yl)ethanone are two probes with high affinity for both Aβ plaques and neurofibrillary tangles. In this study two neutral ^{99m}Tc labelled DDNP and ENE conjugated to a monoamine-monoamide (MAMA) ligand were synthesised and their biological characteristics were evaluated.

Methods: The final ^{99m}Tc labelled DDNP and ENE were prepared by ligand exchange reaction employing the precursor ^{99m}Tc-glucoheptonate (GH). To evaluate the affinity to Aβ plaques. Fluorescent staining assay were performed on the brain slices (5 μm) of a Tg C57 (APP, PSP 12 month old) mouse. The biodistribution were carried out in ICR normal mice (18–22 g), which were sacrificed at the specific time after injection of the purified(^{99m}Tc-DDNP 99.18%, ^{99m}Tc-ENE 93.24%) complex (260KBq).

Results: The corresponding rhenium complex of DDNP and ENE selectively stained the amyloid plaques in the brain slices of the transgenic mouse at the concentration of 1μM (Fig 1).

It was found that ^{99m}Tc-ENE displayed a fairly good initial brain uptake in normal mice (0.65% ID/g at 2min postinjection) with a reasonable washout of the radioactivity from the brain (0.19 %ID/g at 2h postinjection) while ^{99m}Tc-DDNP showed a low brain uptake (0.28 %ID/g at 2min) (Tab 1).

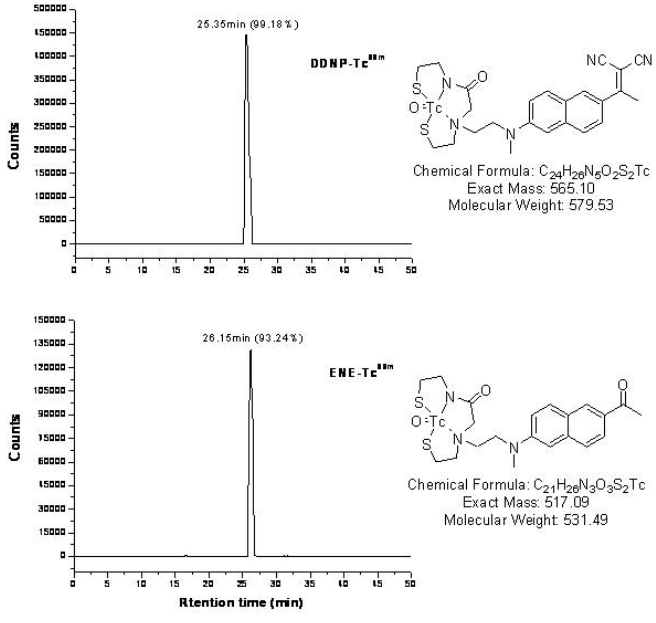
Table 1. Biodistribution of ^{99m}Tc-DDNP and ^{99m}Tc-ENE in normal mice (n = 4)

Tissue	Time after injection (min) 2	10	30	60	120
^{99m}Tc-DDNP					
Blood	6.35±0.59	2.43±0.39	1.71±0.31	1.14±0.13	0.73±0.08
Heart	10.77±1.1	5.47±0.43	2.94±0.46	1.86±0.29	1.25±0.3
Liver	18.31±3.23	21.12±3.83	16.95±1.89	15.48±3.63	13.9±3.61
Spleen	3.57±0.44	3.21±0.35	2.34±0.29	1.5±0.23	1.08±0.23
Lung	10.59±1.74	4.67±0.85	3.24±0.43	2.29±0.31	1.47±0.21
Kidney	11.01±1.88	8.56±0.84	5.93±0.87	4.18±0.47	3.14±0.74
Brain	0.28±0.03	0.21±0.02	0.17±0.03	0.12±0.02	0.1±0.04
^{99m}Tc-ENE					
Blood	5.81±0.95	2.55±0.81	2.07±0.19	1.64±0.27	1.22±0.38
Heart	6.88±1.03	3.84±0.56	2.85±0.25	2.26±0.52	1.35±0.4
Liver	9.31±0.96	12.49±1.85	13.85±1.25	12.79±2.97	8.63±2.09
Spleen	2.78±0.41	2.98±0.36	2.51±0.3	2.13±0.21	1.21±0.23
Lung	8.27±2.03	4.31±0.48	3.17±0.13	2.66±0.4	1.5±0.34
Kidney	6.56±0.63	4.67±0.71	4.12±0.62	3.66±0.52	2.24±0.53
Brain	0.65±0.09	0.6±0.04	0.44±0.02	0.36±0.06	0.19±0.03

logP values for ^{99m}Tc-DDNP and ^{99m}Tc-ENE were 1.70 and 1.89 respectively.

Conclusions: Both ^{99m}Tc and Re complexes of DDNP and ENE were successfully synthesized and their preliminary evaluation results demonstrated that they can bind to Aβ plaques on the brain slices of transgenic mouse. In addition, ^{99m}Tc-ENE showed fairly high initial brain uptake and medium washout. These results encourage us to further study of their derivatives as imaging agents for Aβ plaques in the brain.

Research Support: This work was financially supported by NSFC (20871021).



P382 IN VITRO STABILITY STUDIES OF TYR-3-OCTREOTATE ANALOGUES CYCLIZED VIA TC-99m COORDINATION

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Objectives: Development of radiolabeled Tyr^3-octreotate analogues is of interest for targeting receptor-positive somatostatin tumors for diagnostic and radiotherapeutic purposes. We are investigating a direct radiolabeling approach for incorporation of the radiometal into the peptide sequences, where Tyr^3-octreotate analogues are cyclized via coordination of ^{99m}Tc instead of the usual disulfide bridge. The main goal of using this approach is to obtain stable radiometal coordination with higher tumor uptake and retention compared to bifunctional radiometal chelation approaches.

Methods: Various Tyr^3-octreotate analogues were synthesized via standard solid-phase 9-fluorenylmethoxycarbonyl (Fmoc) peptide synthesis and purified via semi-preparative RP-HPLC. Seven of the purified analogues were radiolabeled with ^{99m}Tc by first reducing $^{99m}TcO_4^-$ with $SnCl_2$ and temporarily chelating the resulting Tc(V) monooxo metal center with glucoheptonate followed by transchelation to the peptide. Confirmation of ^{99m}Tc cyclization of each analogue was verified via co-injection of the nonradioactive Re-cyclized analogue on RP-HPLC (synthesis and in vitro receptor binding studies are reported in a separate abstract). In vitro stability of the ^{99m}Tc-cyclized Tyr^3-octreotate analogues was determined by incubating the radiolabeled analogues in PBS, mouse serum, and cysteine solutions (10 mM cysteine concentration, followed by 1 mM concentration if instability was observed at the 10 mM level) at physiological conditions (pH 7.4 and 37 °C). The percent of intact peptide was obtained by testing aliquots of the incubated solutions at various time points (0, 1, 2, 4 and 24 h) with radio-RP-HPLC and radio-TLC.

Results: Coordination of ^{99m}Tc in an N_2S_2 system is stable in mouse serum yet unstable in PBS at physiological conditions, where the +5 metal center (^{99m}Tc) is oxidized to $^{99m}TcO_4^-$, as indicated by the elution time on the RP-HPLC. Further, the Tc N_2S_2 system is stable against cysteine challenge at 1 mM, but shows oxidation when incubated in 10 mM cysteine solution. However, coordination of ^{99m}Tc in an NS_3 system proved to be more stable under all conditions tested to date. Complete comparison of all seven ^{99m}Tc-cyclized Tyr^3-octreotate analogues is currently underway.

Conclusions: Tyr^3-octreotate analogues with differences in their sequences, and consequently their metal coordination sites, were radiolabeled with ^{99m}Tc and evaluated for their in vitro stability. Coordination of ^{99m}Tc via an NS_3 system appears to more stable than N_2S_2 systems. Further studies are underway to confirm these findings.

P383 SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL CATIONIC Tc(I)TRICARBONYL COMPLEXES AS POTENTIAL RADIOTRACERS

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Objectives: A novel amide ligand with conjugate phenol-based and imidazole was synthesized from salicylic aldehyde. Here, we report the work carried out on complexation studies of the amide ligand via ^{99m}Tc(I)–tricarbonyl synthon and bioevaluation to study its biological activity.

Methods: The synthesis pathways are represented in figure 1. The intermediate products are I: 4-hydroxy-3-nitrobenzaldehyde; II: 4-(hydroxymethyl)-2-nitrophenol; III: 4-(bromomethyl)-2-nitrophenol; IV: 4-((1H-imidazol-1-yl)methyl)-2-nitrophenol; V: 4-((1H-imidazol-1-yl)methyl)-2-aminophenol; VI: pyridine-2,6-dicarbonyl dichloride; VII: N^2,N^6-bis(5-((1H-imidazol-1-yl)methyl)-2-hydroxyphenyl)pyridine-2,6-dicarboxamide. Synthesis of cationic complexe $[^{99m}Tc(CO)_3(L)]^+$(L=VII) was through direct reduction of $^{99m}TcO_4^-$ with sodium borohydride in aqueous solution in the presence of carbon monoxide. And the cationic ^{99m}Tc(I)–tricarbonyl complexe was purified by HPLC. Biodistribution studies were performed using mice.

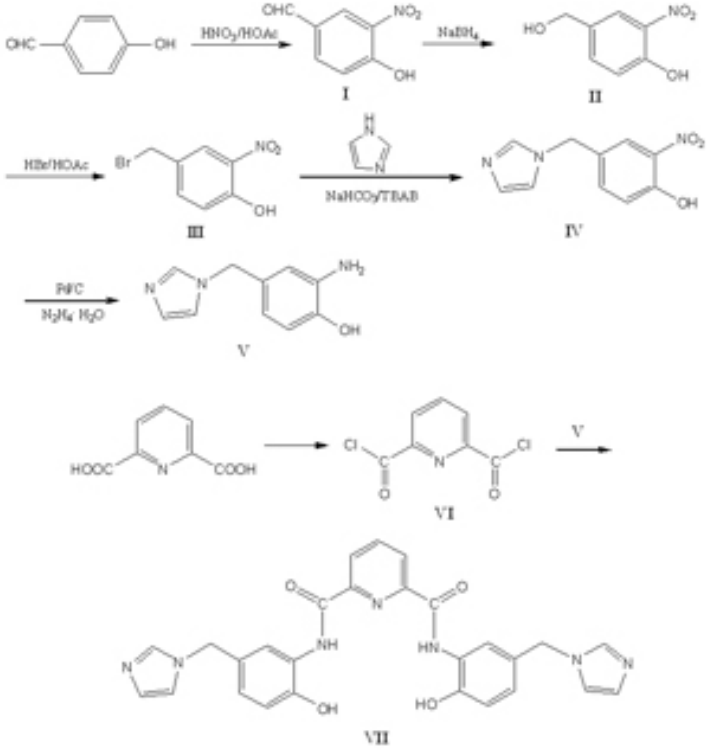
Results: The radiochemical purity of $[^{99m}Tc(CO)_3(L)]^+$ (L=VII) was 98%. Table 1 summarize its organ uptake (expressed as % ID/g). Biodistribution studies in animal model showed high blood uptake, the amide ligand has little potential as radiotracer. Based on the results, further studies on modification of the amide ligand for preparation of ^{99m}Tc tricarbonyl complexes is needed. We will use amino acid instead of 4-((1H-imidazol-1-yl)methyl)-2-aminophenol to obtain rapid clearance from the blood, muscle, liver and lungs. The experiments are in progress.

Conclusions: The amide ligand could be labeled successfully with ^{99m}Tc via tricarbonyl synthon. Further studies on modification of the amide ligand for preparation of ^{99m}Tc tricarbonyl complexes is under research.

Research Support: National Natural Science foundation of China (No 20371009 and 20671014) and Beijing Key Subject Program.

Table 1 Selected biodistribution data for $[^{99m}Tc(CO)_3(L)]^+$ (L=VII) in mice

Organ	5min	30min	60min	120min
Heart	4.20±0.86	3.88±0.40	3.39±0.90	2.15±0.13
Liver	7.12±0.43	7.74±0.52	7.67±1.08	7.27±0.45
Lungs	8.06±3.09	7.56±2.50	6.09±1.69	5.52±0.60
Spleen	2.42±1.05	1.15±0.50	1.22±0.43	1.14±0.20
Kidneys	10.05±1.65	9.97±1.03	8.42±0.86	6.39±0.97
Brain	0.41±0.10	0.25±0.05	0.27±0.06	0.19±0.02
Muscle	1.23±0.24	1.04±0.06	0.88±0.14	0.44±0.08
Intestine	1.95±0.59	1.79±0.54	1.78±0.14	1.38±0.03
Blood	12.85±1.15	10.01±1.08	8.79±0.39	6.77±0.25



P384 RADIOLABELLING OF NEUROTENSIN AGONIST AND ANTAGONIST WITH 177Lu

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Objectives: Neurotensin (NT) is a tri-decapeptide specific to human body, with a very short biologic half live that causes lack of balance at specific receptors saturation level which can generate tumor diseases (cancer).The aim of study was to develop and optimize the radiolabelling methods of neurotensin agonist (NT) and antagonist (SR48692) with 177Lu.

Methods: Taking into account the multitude of variables that must be considered, some relating to the radioisotope, and others to the biological carrier, was selected the indirect radiolabeling method using the DOTA-Neurotensin (DOTA-NT) purchased from specialized company piChem, DOTA-SR48692 and TRITA-SR48692 bioconjugates which were synthesized in our laboratory using the following method: the mixture of SR48692/DMSO and DOTA or TRITA/0.1M NaHCO3 pH=8.8, in 1:1.5 molar ratio, was taked at 25^0C for 6h. The DOTA-SR48692 and TRITA-SR48692 were purified by Sephadex-25 gel chromatography and lyophilized. In this study, we have developed a simple and efficient procedure for labeling with 177Lu of mentioned bioconjucates. The samples consisting of 10 -100μg bioconjugates in 50-500μL of 0.4M acetate buffer and 4.5 pH were labeled with 10-100 mCi of $177LuCl_3$ in 0.05N HCl. The optimal values for obtaining the maximum complexation yield are: 3.7 DOTA-NT/177Lu, 5.8 DOTA-SR48692/Lu and 5.2 TRITA-SR48692/Lu molar ratios, 90 min. incubation time at 90 ^{0}C in 0.4M acetate buffer 4.5 pH. After incubation of the samples in the specific conditions of temperature and cooling, were added the different concentrations of HABA (3-hydroxy-4 aminobenzoic acid) or GA (gentisic acid) as radiolitic stabilizer. The identification and characterization of DOTA-NT, DOTA-SR48692 and TRITA-SR48692 bioconjugates were effected by IR spectroscopy. The TLC and HPLC methods were used for evaluation of the radiochemical purity of 177Lu-DOTA-NT and 177Lu-DOTA-SR48692 selected radiobioconjugates.

Results: The IR spectroscopic study indicates the well-defined band at 2140 $cm^{-1}$and 1438 cm^{-1} in the DOTA-SR48692 bioconjugate spectra The obtained results regarding quality control of radiobioconjugates show radiochemical purity higher than 97% for 177Lu-DOTA-NT and higher than 94.2% for 177Lu-DOTA-SR48692 and good stability in the 0.9%NaCl up to 24hours at room temperature.

Conclusions: In this work we report an efficient procedure for the preparation of the radiobioconjugates, 177Lu-DOTA-NT and 177Lu-DOTA-SR48692 in weakly acidic solutions suitable for in vivo injection.. With all the results described in this study we can start the in vitro and in vivo researches for to define a new radiopharmaceutical product for targeted therapy of neuroendocrine tumors.

P385 NEW COPPER-64 COMPLEXES FOR IMAGING HYPOXIA

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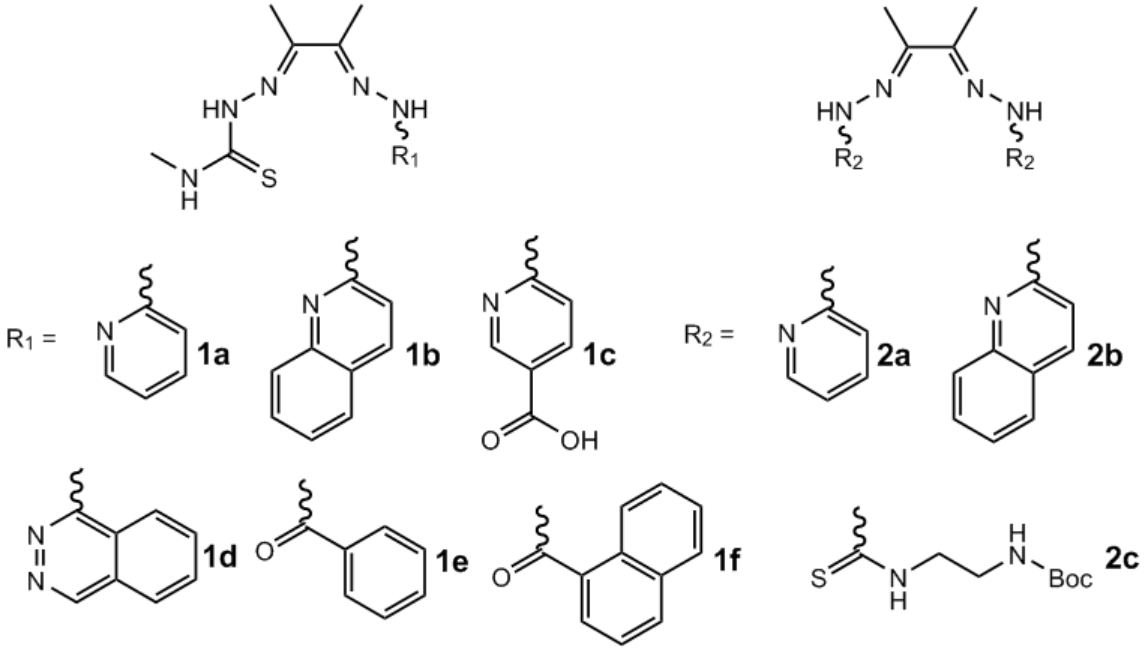
Objectives: Diacetyl bis(N^4-methylthiosemicarbazonato) copper(II) (CuATSM) is a known hypoxia selective complex, and the effects of changing the substituents on the backbone and pendant arms have been extensively studied. However there has been little work in exploring the chemical and biological effects of changing one or both of the donor groups to the copper. A recent study has shown that two new copper(II) thiosemicarbazone-pyridylhydrazine hybrid complexes undergo quasi-reversible reductions at biologically accessible potentials. These complexes, along with a series of other hybrid copper complexes based on the CuATSM system, have been synthesised to explore the effects of changing one or both of the chelating groups. Here we report initial chemical and biological experiments to ascertain their suitability as hypoxia selective agents, alongside work on developing new 'bifunctional' bis(thiosemicabazone) ligands.



Methods: Cyclic voltammetry measurements in dimethylformamide (DMF) were run to study the Cu(II)/Cu(I) redox potential along with stability studies in water, PBS and human serum. The ligands were radiolabelled with ^{64}Cu and logP and serum stability studies were undertaken. In order to ascertain their potential as hypoxia markers cellular uptake was measured in vitro at various oxygen concentrations.

Results: The copper complexes of 1a-1f and 2c undergo quasi-reversible reductions at biologically accessible potentials (-0.68 V to -0.54 V) with 2c having the same reduction potential as CuATSM, whilst 2a undergoes a quasi-reversible reductions at -0.82 V and 2b undergoes an irreversible reduction at -0.92 V. Stability studies show the complexes are more stable than CuATSM after incubation in human serum at 37 ℃ for 6 hrs, and all ligands except 2b were successfully radiolabelled with ^{64}Cu with high radiochemical yields (>95%). 1c showed higher uptake in anoxic vs normoxic HeLa cells whilst 2c showed no oxygen dependence.

Conclusions: The chemical data suggests all compounds except 2a and 2b should be hypoxia selective because of their biologically accessible Cu(II)/Cu(I) redox potentials. Biological data, however, shows that hypoxia selectivity is persevered if only one 'half' of the Cu-ATSM system is modified, whilst changing both halves removes it. This suggests at least one methylthiosemicarbazone moiety needs to be present to provide hypoxia selectivity. We theorize that the moiety is required to allow enzymatic reduction of the complex to take place within biological systems, and work is underway to explore this process.

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P386 STUDIES ON 99mTc-LABELED BISPHOSPHONATES AS POTENTIAL BONE IMAGING AGENTS

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Objectives: The purpose of this study was to synthesize and evaluate novel ^{99m}Tc- bisphosphonates labeled with 1-hydroxy-2-(1-methylimidazol-2-yl)ethylidene-1,1-bisphosphonic acid (HMIBP), 2-(2-ethylicimidazol-1-yl)-1-hydroxyethane-1,1-bisphosonic acid (EIDP), and 2-(1H-1,2,4-triazol-1-yl)-1-hydroxyethane-1,1-bisphosphonic acid (TADP) as potential bone imaging agents in comparison with the currently used agent ^{99m}Tc-MDP (methylenediphosphonic acid).

Methods: HMIBP, EIDP, and TADP were synthesized and radiolabeled with ^{99m}Tc, respectively. The radiolabeling yield (RLY) and radiochemical purity (RCP) were determined by thin-layer chromatography (TLC) with developing systems of (1) acetone: physiological saline = 2:1 and (2) purified water. The in vitro stabilities of the freshly prepared ^{99m}Tc-HMIBP, ^{99m}Tc-EIDP, and ^{99m}Tc-TADP were determined from evaluating their RCP every 1 h at room temperature (25±2 °C). Imaging and biodistribution studies in mice were completed with the ^{99m}Tc-labeled complexes. The samples of different organs were countered by a well-type γ-counter to determine residual activity in different organs. Tissue concentrations were calculated and expressed as percent uptake of injected dose per gram (%ID/g). Bone-to-organ uptake ratios were determined from the %ID/g values.

Results: Both labeling yields and radiochemical purities of ^{99m}Tc-HMIBP, ^{99m}Tc-EIDP, and ^{99m}Tc-TADP were more 95%, and they were stable enough at room temperature. Bone scanning images in the first 60 min showed that bone uptakes of ^{99m}Tc-HMIBP, ^{99m}Tc-EIDP, and ^{99m}Tc-TADP were 7.45±1.14 %ID/g, 23.76±2.36 %ID/g, and 12.58±3.06 %ID/g, respectively, while that of ^{99m}Tc-MDP was 4.79 %ID/g. The corresponding bone-to-muscle uptake ratios were 28.65, 95.04, 96.77, and 39.95 for ^{99m}Tc-HMIBP, EIDP, TADP, and MDP; and the bone-to-blood uptake ratios were 62.08, 33.94, 20.62, and 17.31. The 2 h post injection bone uptakes of three new complexes were 5.87±2.43 %ID/g, 11.17±3.71 %ID/g, and 8.53±0.01 %ID/g, respectively, while that of ^{99m}Tc-MDP was 3.87 %ID/g. The bone-to-muscle uptake ratios were 28.65, 65.71, 106.63, 44.63, and bone-to-blood uptake ratios were 62.08, 39.89, 26.66, 26.22.

Conclusions: In comparison with ^{99m}Tc-MDP, the significantly higher bone uptake of ^{99m}Tc-HMIBP, ^{99m}Tc-EIDP, and ^{99m}Tc-TADP, combined with the quicker clearance from soft tissues and better bone imaging make them desirable agents for further studies.

Research Support: This work was supported by the National Natural Science Foundation of China (No. 20801024) and WU JIEPING Medical Foundation (No. 320.6750.08056).

P387 SUBSTITUENT EFFECT ON THE STRUCTURES AND PROPERTIES OF 99mTc-BISPHOSPHONATE COMPLEXES

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Objectives: The purpose of this work was tolook for novel potential bone imaging agents in ^{99m}Tc-bisphosphonate complexes and investigate the effect of different substitutions at the bridge carbon atom of methylenediphosphonic acid (MDP) on the structures and biological properties. So, a series of ^{99m}Tc-complexes with different bisphosphonate ligands were designed, synthesized, and studied.

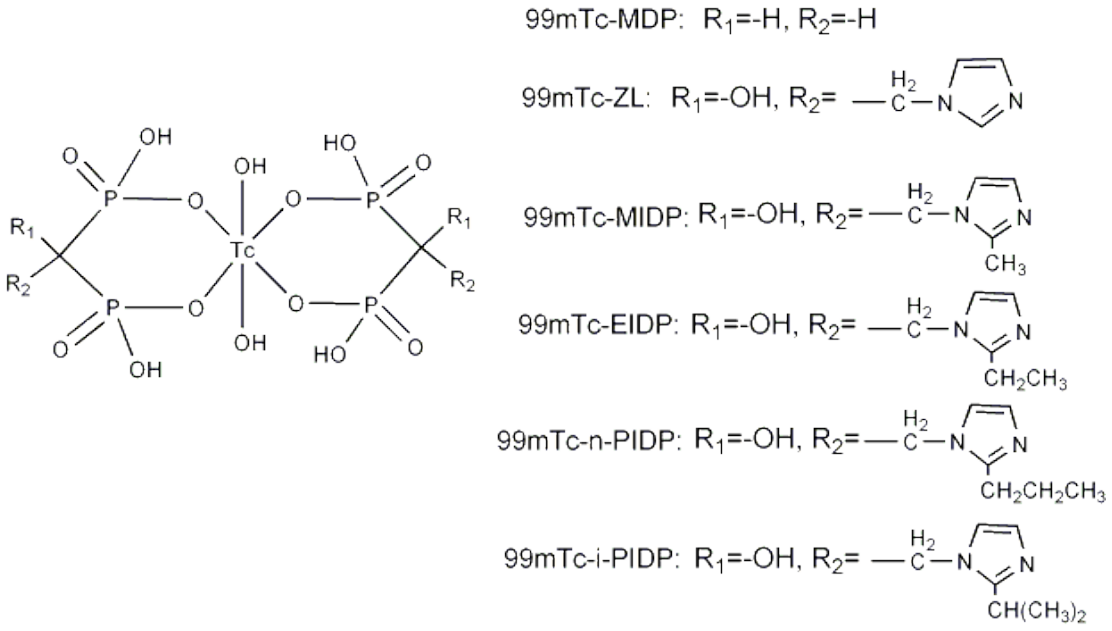

FIGURE 1 99mTc complexes labeled with bisphosphonic acid

Methods: Hyperchem7.5 and Gaussian03 programs were employed to design and model ^{99m}Tc-complexes labeled with ZL (2-(imidazol-1-yl)-1-hydroxyethane-1,1-bisphosphonic acid), MIDP (2-(2-methylicimidazol-1-yl)-1-hydroxyethane-1,1-bisphosonic acid), EIDP (2-(2-ethylicimidazol-1-yl)-1-hydroxyethane-1,1-bisphosonic acid), n-PIDP (2-(2-propylicimidazol-1-yl)-1-hydroxyethane-1,1-bisphosonic acid) and i-PIDP (2-(2-isopropylicimidazol-1-yl)-1-hydroxyethane-1,1-bisphosonic acid) using molecular mechanics (MM) and density functional theory (DFT) methods, respectively. Different conformational and geometric isomers within a single simulation were searched. Geometric and electronic properties were studied to assess the biological behavior. The ligands were further synthesized and labeled with ^{99m}Tc. Radiolabeling yield and radiochemical purity were measured by thin-layer chromatography. Biodistribution and bone imaging in mice were carried out with the prepared complexes. Tissue concentrations were calculated and expressed as percent uptake of injected dose per gram (%ID/g). Bone-to-organ uptake ratios were determined from the %ID/g values.

Results: Reasonable geometries of target materials were determined from theoretical computations and the obtained parameters (E_T, E_{HOMO}, E_{LUMO}, ΔE_{L-H}, Θ_{Tc}, Θ_{R1}, Θ_{R2} and μ) were correlated with the biological behavior. ^{99m}Tc-ZL, MIDP and EIDP were prepared successfully and other two complexes were in experiment. On the whole, biological properties of ^{99m}Tc-EIDP were larger than those of ^{99m}Tc-MIDP, ^{99m}Tc-ZL and ^{99m}Tc-MDP.

TABLE 1 Biodistribution in rats of ^{99m}Tc-MDP, ^{99m}Tc-ZL, ^{99m}Tc-MIDP and ^{99m}Tc-EIDP (x±•, n=5)

Uptake	^{99m}Tc-MDP		^{99m}Tc-ZL		^{99m}Tc-MIDP		^{99m}Tc-EIDP	
	60min	120min	60min	120min	60min	120min	60min	120min
$a_{m,bone}$/%ID·g^{-1}	4.79	3.87	9.78±0.93	10.05±0.49	11.68±1.22	14.22±0.45	23.76±2.36	11.17±3.71
$a_{m,□}/a_{m,□}$	17.31	26.22	23.29	28.71	18.54	47.4	33.94	39.89
$a_{m,□}/a_{m,□}$	39.95	44.63	31.55	47.84	41.71	61.83	95.04	65.71

Conclusions: The combination of experimental studies with theoretical calculations or simulations helps to select a subgroup of target structures of potential bone imaging agents for synthesis. ^{99m}Tc-EIDP may be a superior agent for bone imaging worthy of further studies, in comparison with ^{99m}Tc-MDP, ^{99m}Tc-MIDP and ^{99m}Tc-ZL.

Research Support: This work was supported by the National Natural Science Foundation of China (No. 20801024) and WU JIEPING Medical Foundation (No. 320.6750.08056).

P388 PREPARATION AND BIOLOGICAL EVALUATION OF 99mTc-ZL AND 99mTc-TADP AS BONE IMAGING AGENTS

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Objectives: Two complexes 99mTc-ZL [2-(imidazol-1-yl)-1-hydroxyethane-1,1-bisphosphonic acid] and 99mTc-TADP [2-(1H-1,2,4-triazol-1-yl)-1-hydroxyethane-1,1-bisphosphonic acid] were prepared and studied. The SPECT imaging was carried out to evaluate their feasibilities as novel bone imaging agents and explore the difference in their biological properties.

Methods: ZL and TADP were synthesized and radiolabeled with 99mTc, respectively. The radiochemical purity (RCP) and radiolabeling yield (RLY) of 99mTc-ZL and 99mTc-TADP were determined by TLC (thin-layer chromatography) method with systems of (1) acetone: physiological saline = 2:1 and (2) purified water. The in vitro stability was assessed by measuring the RCP every 1h at room temperature. Biodistribution studies of 99mTc-ZL and 99mTc-TADP in mice were investigated and compared with that of 99mTc-MDP. The SPECT imaging of rabbit was carried out with 99mTc-ZL and 99mTc-TADP using Philips SKYLight ECT. The whole-body image was observed for 3 h. The uptakes of femur, muscle beside femur, and other soft tissues were obtained, and the bone-to-soft-tissues uptake ratios were calculated from the same ROI. The bone scans were collected at 15, 30, 60, 90, 120, 140, 160, and 180 min, respectively.

Results:ZL and TADP were synthesized and radiolabeled with 99mTc, respectively. The radiochemical purity (RCP) and radiolabeling yield (RLY) of 99mTc-ZL and 99mTc-TADP were determined by TLC (thin-layer chromatography) method with systems of (1) acetone: physiological saline = 2:1 and (2) purified water. The in vitro stability was assessed by measuring the RCP every 1h at room temperature. Biodistribution studies of 99mTc-ZL and 99mTc-TADP in mice were investigated and compared with that of 99mTc-MDP. The SPECT imaging of rabbit was carried out with 99mTc-ZL and 99mTc-TADP using Philips SKYLight ECT. The whole-body image was observed for 3 h. The uptakes of femur, muscle beside femur, and other soft tissues were obtained, and the bone-to-soft-tissues uptake ratios were calculated from the same ROI. The bone scans were collected at 15, 30, 60, 90, 120, 140, 160, and 180 min, respectively.

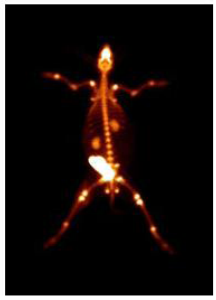

Figure 1 Rabbit Bone Imaging of ^{99m}Tc-TADP at 1h

Conclusions: When a carbon atom of imidazole at the bridge carbon atom of methylenediphosphonic acid (MDP) was substituted by nitrogen atom, i.e. triazole substituted imidazole (from 99mTc-ZL to 99mTc-TADP), higher bone uptake, quicker clearance in soft tissues, and better bone imaging were obtained. So, 99mTc-TADP was worthy of further investigation and it would be used as a novel excellent bone imaging agent for skeletal imaging in future.

Research Support: This work was supported by the National Natural Science Foundation of China (No. 20801024) and WU JIEPING Medical Foundation (No. 320.6750.08056).

P389 THE PREPARATION AND BIOEVALUATION OF 99mTc LABELED HYNIC-NGA AS POTENTIAL HEPATIC ASGP RECEPTOR IMAGING AGENTS

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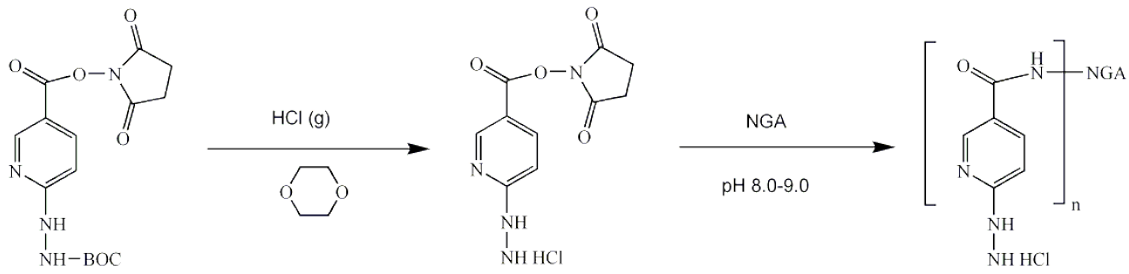
Objectives: Quantitative imaging of asialoglycoprotein (ASGP) receptors could estimate the function of the liver. Several years ago, Ono et al. [1] reported the preparation of ^{99m}Tc-HYNIC-NGA (NGA=galactosyl-neoglycoalbumin) by using tricine as co-ligand. Here we tried to label HYNIC-NGA with ^{99m}Tc by using bicine or HEDTA as co-ligand. The ^{99m}Tc complexes were prepared in high yield and their biological properties were evaluated in mice.

Methods: HYNIC-NGA was obtained by treating NGA with succinimidyl 6-hydrazinopyndine-3-carboxylate hydrochlorid (SHYNIC) (scheme 1) and radiolabeled with technetium-99m directly by using bicine or HEDTA as co-ligand to get ^{99m}Tc-bicine/HYNIC-NGA or ^{99m}Tc-HEDTA/HYNIC-NGA. The labeling conditions were optimized and the labeling yield was determined by ITLC. Ex vivo biodistribution of ^{99m}Tc-bicine/HYNIC-NGA and ^{99m}Tc-HEDTA/HYNIC-NGA was investigated in normal mice.



Scheme 1. The synthetic route of HYNIC-NGA

Results: The labeling yields of ^{99m}Tc-bicine/HYNIC-NGA and ^{99m}Tc-HEDTA/HYNIC-NGA were above 80% under the optimized conditions. After purified with HiTrap Desalting column, the final radiochemical purities of these ^{99m}Tc complexes were in excess of 95%. Ex vivo biodistribution showed that the liver uptake of ^{99m}Tc-HEDTA/HYNIC-NGA was 86.57±5.68%ID/g, 74.63±8.74%ID/g and 45.11±4.21 %ID/g at 5, 30 and 120 min after injection, respectively. The liver uptake of ^{99m}Tc-bicine/HYNIC-NGA was 56.58±6.57%ID/g, 38.06±3.35%ID/g and 23.17±4.96%ID/g, respectively. In the blocking experiment (by preinjecting 10 mg/kg body weight free GSA as blocking agent), the liver uptake of ^{99m}Tc-HEDTA/HYNIC-NGA was decreased obviously to 43.34±6.41%ID/g at 5 min p.i. which indicated that the complex of ^{99m}Tc-HEDTA/HYNIC-NGA has specific binding to ASGP receptor.

The biodistribution of ^{99m}Tc-bicine/HYNIC-NGA and ^{99m}Tc-HEDTA/HYNIC-NGA in normal mice. Expressed as % injected dose per gram (%ID/g). Each value represents the mean ± SD

	^{99m}Tc-bicine/ HYNIC-NGA	^{99m}Tc-bicine/ HYNIC-NGA	^{99m}Tc-bicine/ HYNIC-NGA	^{99m}Tc-HEDTA/ HYNIC-NGA	^{99m}Tc-HEDTA/ HYNIC-NGA	^{99m}Tc-HEDTA/ HYNIC-NGA	^{99m}Tc-HEDTA/ HYNIC-NGA
Tissue	5 min	30 min	120 min	5 min	5 min blocking	30 min	120 min
Heart	1.33±0.09	0.56±0.17	0.33±0.06	0.59±0.11	5.27±1.57	0.55±0.14	0.77±0.18
Liver	56.58±6.57	38.06±3.35	23.17±4.96	86.57±5.68	43.34±6.41	74.63±8.74	45.11±4.21
Lung	2.72±0.39	1.06±0.08	0.56±0.18	1.02±0.44	14.87±5.79	0.69±0.19	0.74±0.15
Kidney	9.13±1.27	10.13±1.01	7.54±1.04	1.86±0.14	9.62±1.63	2.19±0.24	4.32±0.23
Spleen	1.47±0.23	0.80±0.07	0.59±0.09	1.21±0.26	5.91±1.20	0.99±0.15	1.52±0.23
Stomach	0.69±0.07	1.17±0.32	1.12±0.18	0.37±0.23	0.54±0.12	0.67±0.21	0.69±0.18
Blood	3.14±0.35	1.13±0.11	0.48±0.06	0.54±0.08	30.08±8.21	0.32±0.03	0.17±0.0 2
Bone	1.35±0.29	0.92±0.17	0.79±0.08	1.07±0.12	5.86±2.42	0.86±0.24	1.47±0.46
Musle	0.69±0.11	0.24±0.09	0.25±0.08	0.51±0.19	1.12±0.32	0.91±0.19	0.68±0.33
Intestine	1.74±1.93	7.01±6.67	1.14±0.28	0.66±0.57	1.02±0.28	5.36±5.43	1.60±1.02

Conclusions: The two novel complexes ^{99m}Tc-bicine/HYNIC-NGA and ^{99m}Tc-HEDTA/HYNIC-NGA were prepared with high radiochemical purity. ^{99m}Tc-HEDTA/HYNIC-NGA showed much higher liver uptake than that of ^{99m}Tc-bicine/HYNIC-NGA. The furthermore blocking study of ^{99m}Tc-HEDTA/HYNIC-NGA showed that it has specific binding to ASGP receptor. The promising biological properties of ^{99m}Tc-HEDTA/HYNIC-NGA indicated it could be developed as novel hepatocyte-targeting agent to evaluate hepatic function in the future.

Research Support: This work was supported in part by grants from the National Natural Science Foundation of China (20401004).

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P390 LABELING AND STABILITY STUDIES OF 44SC-DOTATOC

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Objectives: The radioisotope ^{44}Sc is a positron emitter, which is of interest for medical imaging using PET radiopharmaceuticals because of its 3.93 h half-life and 94% b^+ branching. The aim of this work was to find the optimum conditions for labeling a DOTA-conjugated octreotide (DOTATOC) with ^{44}Sc and to check the stability of obtained conjugate.

Methods: The ^{44}Sc was eluted from the recently developed ^{44}Ti/^{44}Sc generator and on-line post-processed on the AG 50W-X8 resin . Finally around 160 MBq ^{44}Sc was obtained in 3 mL of 0.25 M ammonium acetate buffer, pH = 4.0. This solution was used for labeling of DOTATOC directly. Optimisation of the reaction conditions was performed varying period and temperature of heating, addition of various amounts of DOTATOC and pH of the reaction mixture. The influence of microwave heating on the time and radiolabeling of DOTATOC with ^{44}Sc was determined under the best conditions found during the optimization experiments. Radiochemical analysis of ^{44}Sc-DOTATOC was accomplished using silica-coated TLC plates (Silica-gel 60) and 4 different developing solutions: a) 0.1 M sodium citrate pH = 4.0; b) 0.1 M sodium acetate pH = 4.0 c) 5% NaCl / MeOH (3:1); d) 5% NaCl / MeOH / 25% NH_3 (3:1:1). ^{44}Sc-DOTATOC was purified on a C-18 cartridge and eluted with 400 mL of pure ethanol. Stability of ^{44}Sc-DOTATOC was checked at room temperature and at 37ºC in EtOH, 0.9% NaCl, PBS (pH=7.4) and in the presence of the metal cations Fe^{3+}, Ca^{2+}, Mg^{2+} and Cu^{2+} at 10^{-2} M concentration. The purified ^{44}Sc-peptide was diluted at least 20 times by the solution in which stability was checked.

Results: The overall radiolabeling yield was >96% when 35 mL (24.5 nmol) of DOTATOC was added to 3 mL of the ^{44}Sc eluate (pH = 4.0) and heated in the oil-bath for 25 minutes at 95ºC. Changing the pH below 3 or increasing to > 5 resulted in a drop of labeling yield. Microwave-assisted labeling speeded up ^{44}Sc complexation with DOTATOC. After 1 minute of microwave heating reaction, the yield was >95% and increased up to 98% during next 2 min. Purification on the small C-18 cartridge recovered the ^{44}Sc-conjugate in 400 mL of pure EtOH with the radiochemical purity higher than 99%. Stability studies of around 160 MBq ^{44}Sc-DOTATOC in 400 mL of EtOH showed high stability of the labeled conjugate during at least 7 h. ^{44}Sc-DOTATOC was also stable during 8 h studies in 0.9% NaCl and PBS at 37ºC. The obtained ^{44}Sc-DOTATOC seems to be kinetically very inert. It was stable even after 25 h incubation at 37ºC in the presence of metal cations at concentration 10^{-2} M.

Conclusions: Optimum labeling of DOTATOC with ^{44}Sc in >96% yield were obtained for 24.5 nmol of DOTATOC and 3 mL of ^{44}Sc eluate at pH = 4.0. The studies show high stability of the obtained ^{44}Sc-DOTATOC conjugate in different media. The potential diagnostic radiopharmaceutical was obtained after purification with more than 99% radiochemical purity. After membrane sterilization and dilution in saline it is ready for further cell and animal studies.

P391 SYNTHESIS OF NEW [Y-90]-DOTA BASED MALEIMIDES FOR THE PRELABELING OF THIOL-BEARING L-OLIGONUCLEOTIDES AND PEPTIDES

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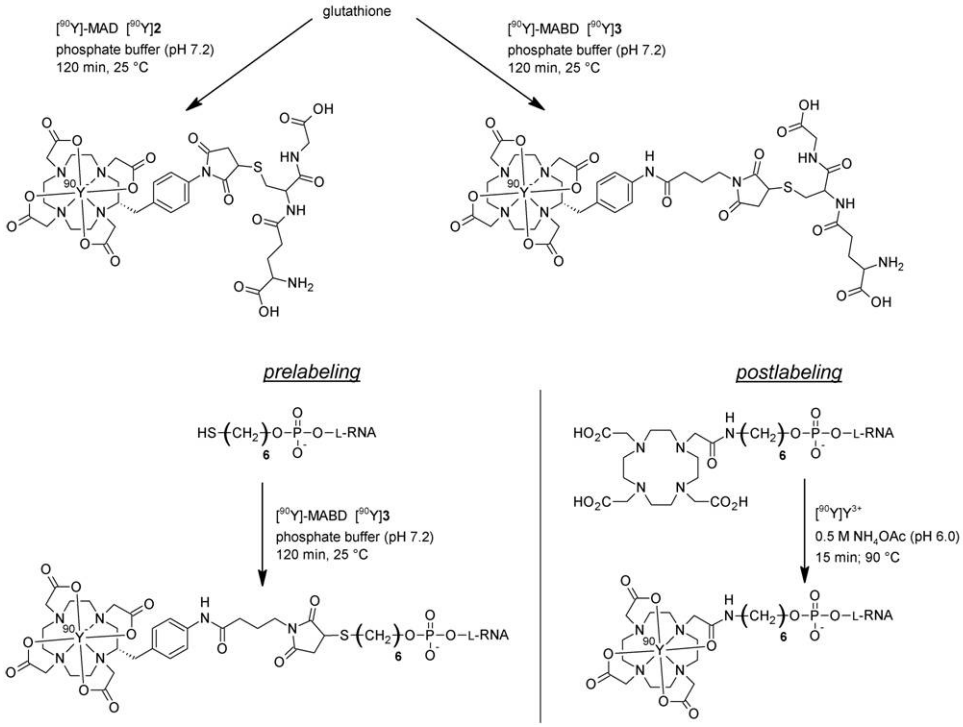
Objectives: A common chelator for radioisotopes of Y and the lanthanides is DOTA. However, the elevated temperatures necessary to achieve sufficient radiochemical yields may be a drawback, especially for the radiolabeling of thermally sensitive molecules such as DOTA-modified antibodies. A promising alternative to the "direct" radiolabeling of DOTA conjugates ("postlabeling") is the use of so-called "prelabeling" agents. Here, we present the synthesis of two novel [^{90}Y]-DOTA-based maleimide reagents, [^{90}Y]2 and [^{90}Y]3, suitable for the mild radiolabeling of thiol-bearing peptides or thiol-modified L-RNAs. Application and reactivity of both maleimide reagents were evaluated by the labeling of glutathione (GSH) and a thiol-modified 12mer L-RNA as model substances.

Methods: L-RNA [sequence: 5'-(1-hydroxy-7,8-dithia-tetradecyl) UGACUGACUGAC-3', MW 4124] was synthesized at NOXXON Pharma AG (Germany). (S)-p-NH_2-Bn-DOTA was purchased from Macrocyclics (USA). ^{90}Y was purchased as [^{90}Y]YCl_3 from QSA Global GmbH (Germany). Measurements of ^{90}Y were done in the ^{90}Y channel of a dose calibrator ISOMED 2000 (Nuklear-Medizintechnik Dresden, Germany) by measuring the bremsstrahlung. The compounds were characterized by HPLC, gel electrophoresis and mass spectrometry.

Results: A straightforward method to synthesize [^{90}Y]-MAD [^{90}Y]2 and [^{90}Y]-MABD [^{90}Y]3 is to initially complex [^{90}Y]Y^{3+} with (S)-p-NH_2-Bn-DOTA and to subsequently transform the purified complex [^{90}Y((S)-p-NH_2-Bn-DOTA)]$^-$ into the corresponding maleimides by using activating agents. The scheme illustrates the subsequent preparation of [^{90}Y]-MAD-GSH and [^{90}Y]-MABD-GSH and the ^{90}Y-labeling of an L-RNA via the pre- and postlabeling approach. In comparison to the N-aryl maleimide [^{90}Y]-MAD, N-alkyl maleimide [^{90}Y]-MABD showed an increased hydrolytic stability at pH $\geq$ 7. A slightly higher reactivity was found for [^{90}Y]-MAD by prelabeling of 0.1 and 1 μg glutathione, respectively in phosphate buffer (pH 7.2) at room temperature. In terms of high radiochemical yields, the direct radiolabeling of DOTA-L-RNA with [^{90}Y]YCl_3 proved to be more suitable than the prelabeling of the thiol-modified 12mer L-RNA derivative with [^{90}Y]-MABD.



Conclusions: We could demonstrate the applicability of maleimide reagents [^{90}Y]-MAD and [^{90}Y]-MABD for the prelabeling approach. Both reagents showed a high potential for that purpose. Concerning high radiochemical yields, the direct labeling of DOTA-l-RNA with [^{90}Y]YCl_3 proved to be more efficient than the prelabeling of the thiol-modified 12mer l-RNA with [^{90}Y]-MABD at low activity levels. With regard to ^{90}Y-labeling of thermally sensitive molecules prelabeling could have more advantages than the direct radiolabeling, due to the milder labeling conditions.

P392 SYSTEMATIC GALLIUM-68 LABELING STUDIES EXEMPLIFIED FOR A NOVEL DO2A-TYROSINE DERIVATIVE

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Objectives: The synthesis of radio-pharmaceuticals with high specific activities requires a time-efficient and high yield-labeling procedure [1], ideally avoiding final chromatographic separation. A lot of different parameters affect the ^{68}Ga-labeling yields of macrocyclic labeling precursors. On the example of a novel DO2A-amino acid derivative (DO2A-(butyl-L-tyrosine)$_2$) we performed systematical labeling studies in order to quantify the influence of reaction time, temperature of the reaction mixture and amount of precursor. Different pH-values were examined in presence and absence of buffer. Labeling yields of the pure labeling precursor were compared with those of its TFA-salt. Furthermore, a well working, time-effective solid phase extraction method was investigated to avoid a time-wasting HPLC-run to remove possible impurities. With all these optimization-steps the labeling yields and the specific activities of the products should be significantly increased.

Methods: The established ^{68}Ge/^{68}Ga-generatorpost-processing utilizing a cation-exchange resin was carried out prior to every labeling process [2]. The labeling experiments were carried out with the resulting ^{68}Ga fraction N2 in 5 ml labeling solution, like water or buffer, using different amounts of generator-eluate and labeling precursor at various temperatures. The mixture of labeling solution and precursor was preheated, then the ^{68}Ga was added. Different pH-values were reached, depending on the volume of generator eluate and the labeling solution. A reaction under high pressure conditions was performed, too. The TFA-salts, as received from the HPLC, were removed from the labeling precursors using and ion-exchange resin. The labeling yields of the desalted compounds were compared to the TFA-salts. Different solid-phase-extraction cartridges were tested for the purification of the product.

Results: Under the optimized conditions we achieved labeling yields of more than 99 %. Furthermore we established a solid-phase extraction method, to obtain, absolutely independent of the labeling yield, a radiochemical purity of more than 97 % ready to inject. The time for the total labeling and purification process including the ^{68}Ga-post-processing was reduced to only 13 minutes. The method showed a very high reproducibility and provides a low radiation dose.

Conclusions: With all the optimizations we established a method to receive very high labeling yields in very short times. We achieved high specific activities up to 100 GBq/μmol ready to inject in a multi injection vial after just 13 minutes. This method is well suited for the production of radiopharmaceuticals to be used in further evaluations, e.g. small animal PET-studies.

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P393 NEW OCTREOTIDE DERIVATIVES FOR 99m-Tc-TRICARBONYL LABELING TO TARGET SOMATOSTATIN SST2 RECEPTOR

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Objectives: Octreotide, a cyclic somatostatin analogue, exhibits high affinity for somatostatin receptors and its indium-111 labeled variety (Octreoscan®) has been widely applied in diagnosis and staging of somatostain-receptor positive tumors. As an alternative for octreotide conjugation, we have designed new $^{99m}Tc(CO)_3$ analogs via histidine coordination. The histidine conjugated octreotides offer potential advantages-convenient preparation and minimal interruption of peptide's biologic activity. In this study, two new octreotitde derivatives, His-octreotide and His_3-octreotide, were synthesized and tested for their binding affinity. Their ability for coupling with $^{99m}Tc(CO)_3$ was also examined.

Methods: The two octreotide derivatives, His-octreotide and His_3-octreotide, were synthesized by a solid phase peptide synthetic method employing with Fmoc (9-fluorenylmethoxy-carbonyl) strategy. The peptides were purified by HPLC and their molecular weights were identified by MALDI-TOF/TOF. The receptor binding affinity of the two new peptides were tested against human somatostatin sst_{2a} receptor using [^{125}I]Tyr^{11}-Somatostatin 14 as the radioligand and octreotide as the control. $[^{99m}Tc(CO)_3(OH_2)_3]^+$, prepared with IsoLink Kit (Mallinckrodt), was used as a precursor for ^{99m}Tc(I)-His-octreotide and ^{99m}Tc(I)-His_3-octreotide. A minimal amount of ~5μg His-octreotide or His_3-octreotide was incubated with $[^{99m}Tc(CO)_3(OH_2)_3]^+$ at 75 ℃ to give products without further purification. The radiochemical purity and stability in normal saline and rat plasma were both determined by ITLC.

Results: The molecular weights of His-octreotide and His_3-octreotide were determined to be 1,156 and 1,430 $[M+H]^+$, respectively. In in vitro receptor binding assay, both His-octreotide and His_3-octreotide were found to exhibit similarly high affinities for human somatostatin sst_{2a} receptor with IC_{50} at 1.83 nM and 2.11 nM, respectively (Fig. 1). The radiochemical purities of both ^{99m}Tc(I)-His-octreotide and ^{99m}Tc(I)-His_3-octreotide were ≧90% and they remained stable in normal saline for at least 8 hour at room temperature. However, ^{99m}Tc(I)-His-octreotide somewhat more degraded rapidly in rat plasma at 37 ℃ (83.6% and 65.2% at 1 min and 2 h, respectively). In the same condition, ^{99m}Tc(I)-His_3-octreotide showed slightly better stability (76.6% and 65.5% at 1 min and 4 h, respectively).

Conclusions: We demonstrated the feasibility and advantages of utilizing $^{99m}Tc(CO)_3$ technology to label octreotide derivatives, His-octreotide and His_3-octreotide. The result has illustrated that His-octreotide and His_3-octreotide maintained high binding affinities for human somatostatin sst_{2a} receptor. Both ^{99m}Tc(I)-His-octreotide and ^{99m}Tc(I)-His_3-octreotide were easily prepared and were stable in normal saline for a long period. Their stability in rat plasma was less than optimal but sufficient for further in vivo testing and imaging studies.

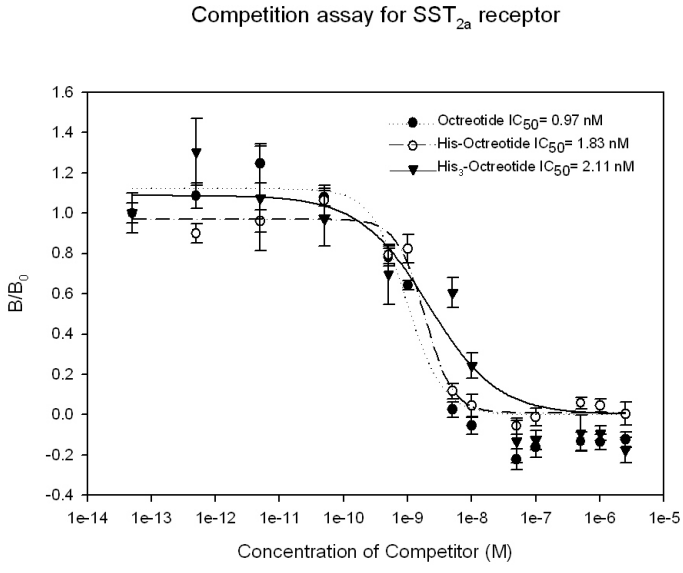

Fig. 1 Comparative displacement curves of [^{125}I]Tyr^{11}-Somatostatin 14 from octreotide (●), His-octreotide (○), and His_3-octreotide (▼) in human somatostatin sst_{2a} receptor. IC_{50} values were: octreotide, 0.97 nM, His-octreotide, 1.83 nM and His-octreotide, 2.11 nM.

P394 SYNTHESIS AND 68Ga-RADIOLABELLING OF N3S3, N3O3 AND NO3-TYPE BIFUNCTIONAL CHELATORS

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Objectives: In the present years, the positron-emitter ^{68}Ga undergoes a renaissance as generator-derived PET-nuclide for clinical routine. This is due to the recent improvements in generator performance of commercially available ^{68}Ge/^{68}Ga-generator systems, and post-processing of generator eluents. The latter includes purification of the ^{68}Ga by separation of metal contaminants as well as eluate concentration for labelling purpose. Thereby, a chemical generator system is transformed into a medical one. However, the application of ^{68}Ga(III) for radiolabelling is somewhat limited to aqueous media and most reports on Ga-radiochemistry are concerned with polyamino-polycarboxylate chelators. Consequently, several groups have reported on lipophilic Ga-chelates, dedicated for molecular imaging. However, most of the earlier approaches did not specifically target bifunctional chelators, but elucidated the synthesis of rather lipophilic complex-precursors. This report is concerned with ^{68}Ga-labelling and lipophilicity of mono- and bifunctional chelator-derivatives.

Methods: The synthesis of a novel bifunctional N_3S_3-type chelator, derived from 1,4,7-triazacyclononane, initial ^{68}Ga-radiolabelling and the determination of stability and lipophilicity of the compound are described. For comparison, the Ga-complex of tris-mercaptoethyl-1,4,7-triazacyclononane was also studied. Furthermore tetra- (NO_3) and hexadentate (N_3O_3) bifunctional chelators bearing phenol-donors were synthesised, labelled and their octanol/water partition coefficient was assessed experimentally.Ga was eluted with different acetone–based, non-aqueous solvent systems providing n.c.a. ^{68}Ga(acac)$_3$ as labelling synthon. ^{68}Ga-labelling was performed in chloroform in a focused microwave synthesis system.

Results: The ^{68}Ga complexes of various mono and bifunctional chelators with lipophilic properties have been screened for their log P values. The results indicate, that these precursors indeed form stable lipophilic radiochelates. The bifunctional analogues of these complexes enable conjugation of targeting vectors for molecular imaging.

Conclusions: The ^{68}Ga complexes of various mono and bifunctional chelators with lipophilic properties have been screened for their log P values. The results indicate, that these precursors indeed form stable lipophilic radiochelates. The bifunctional analogues of these complexes enable conjugation of targeting vectors for molecular imaging.

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