SYNTHESIS OF RHENIUM AND TECHNETIUM COMPLEXES OF GLUCOSE DERIVATIVES

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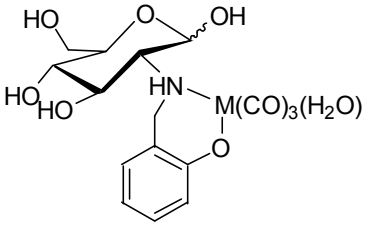
Keywords: rhenium, technetium, glucose, radiopharmaceutical synthesis

Synthesis and characterization of novel rhenium(I) and technetium(I) compounds containing glucose derivatives will be presented. The labelling of carbohydrates is of great interest in the development of new drugs as possible diagnostic and therapeutic agents. With this objective, two separate routes to several different neutral complexes containing the (M = Re^{I}, Tc^{I}) core have been investigated.

In one route, glucose derivatives containing potentially monoanionic chelating ligands with either N,O (amine phenol) or O,O (pyridinone) donor sets were synthesized. These ligands were allowed to react with *fac*-$[M(OH_2)_3(CO)_3]^+$ precursors to form the target molecule.[1] Rhenium complexes have been prepared (see example below) and radiolabelling studies with ^{99,99m}Tc are currently underway.



In the second approach, the target glucose derivatives contain the {*fac*-$M(CO)_3$} core chelated to an acylcylclopentadienyl unit. Their synthesis was attempted using the double ligand transfer reaction (DLT).[2] To accomplish this several ferrocenoyl-sugar derivatives were prepared. Also, as an indirect approach to the DLT reaction we prepared tricarbonyl(methoxycarbonylcyclopenta-dienyl)rhenium(I) as a starting material.[3] Subsequent reactions with this material were carried out to attach a glucose derivative to the cyclopentadienyl ring.

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SYNTHESIS, ^{99m}TC COMPLEXATION AND PROTEIN CONJUGATION OF 2,3,5,6-TETRAFLUOROPHENYL *N*-(*S*-BENZOYLTHIOACETYL)GLYCYLGLYCYL-p-AMINO-BENZOATE

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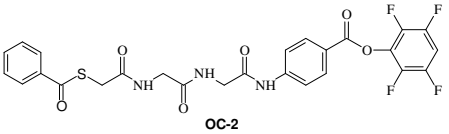
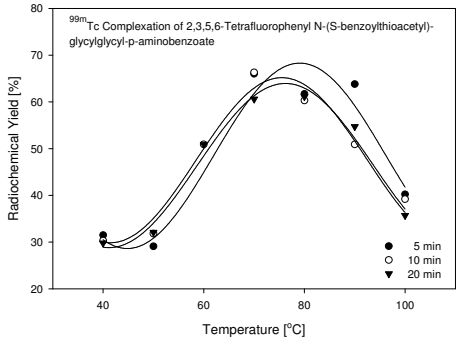
Keywords: ^{99m}Tc complex, activated ester, precomplexation route, protein labeling

The labelling of proteins with ^{99m}Tc represents a technology often applied for tumor imaging. Among those ligands which are used for the preconjugation route BAT-type N_2S_2 ligands, HYNIC and His_6 tags are the most prominent examples which provide stable complexation sites in proteins. There are various arguments supporting this approach saying that preformed chelator-tagged protein can be hold on stock and that ^{99m}Tc complexation is fast and efficient. Complexation of ligands preceding conjugation on the other hand using e.g. 4,5-bis(ethylcarbonylmercaptoaceta-mide)pentanoic acid[1] is associated with laborious manipulations which, however, is compensated by the very small complex/chelator load. This feature may be termed as a non-chelator(carrier) added preparation. High chelators/protein ratio may have negative influence on the binding characteristics. Here we describe a novel, activated heterobifunctional complex ligand which proved stable against hydrolysis during ^{99m}Tc complexation.



OC-2

Synthesis of OC-2: 5 mmol *S*-benzoyl mercaptoactetyldiglycine-*N*-hydroxysuccinimide ester[2] and 5 mmol *p*-aminobenzoic acid were dissolved in 35 mL CH_3CN and the solution was refluxed 5 hr forming a white precipitate. 100 mL CH_3CN was added and refluxed for additional 30 min.The reaction mixture was filtered warm, and the product was washed with CH_3CN to obtain *N*-(*S*-benzoylthioacetyl)glycylglycyl-*p*-aminobenzoic acid as a white solid (73%); m.p.: 266-268°C (dec). 2.5 mmol of this compound and 5 mmol 2,3,5,6-tetrafluorophenol were dissolved in 20 mL DMF and the solution was cooled in an ice-water bath. 5 mmol *N*,*N'*-dicyclohexylcarbodiimide dissolved in 5 mL DMF was added dropwise over 30 min. The reaction mixture was stirred at 0°C for 2 hr and then at room temperature overnight. After removal of insoluble *N*,*N'*-dicyclohexylurea by filtration the solvent was evaporated *in vacuo*. The residue was recrystallized twice from iPrOH yielding OC-2 (34%); m.p.:243-245°C (dec). **Complexation:** 100 µl 2 mM OC-2 in DMF was mixed with 100 µl $^{99m}TcO_4^-$ eluate (300 MBq) and 2 µl 50 mM $SnCl_2$ in 0.2 M K-gluconate solution. This solution was heated for 20 min at 80°C and separated by RP18 HPLC. As shown above the RCY of complexation is temperature dependent showing a maximum between 70-80°C almost independent of reaction time. **Conjugation:** The HPLC eluate was evaporated to dryness and reacted at pH 9-9.5 with 1 mg of an anti-EGF receptor antibody (60µM). 46% RCY was obtained at 30°C after 15 min. **Conclusion:** OC-2 is a novel activated heterobifunctional chelator which is stable against hydrolysis during ^{99m}Tc complexation. Conjugation of ^{99m}Tc-OC-2 with a monoclonal antibody yielded a no carrier added labelling product.



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THE STUDY OF A NEW ^{99m}Tc NITRIDO COMPLEX FOR BRAIN IMAGING

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Keywords: Technetium-99m, $[^{99m}TcN]^{2+}$core, brain imaging, biodistribution

In the development of novel ^{99m}Tc nitrido complexes, we have synthesized several ^{99m}Tc nitrido complexes. The purpose of this report was to introduce one of them the bis(N-cyclopentyl dithiocarbamato) nitrido technetium complex ^{99m}TcN(CPEDTC)$_2$ (CPEDTC: N-cyclopentyl dithiocarbamato). The bis(N-cyclopentyl dithiocarbamato) nitrido technetium complex ^{99m}TcN(CPEDTC)$_2$ was synthesized by the reduction of $^{99m}TcO_4^-$ into $[^{99m}Tc\ N]^{2+}$ with stannous chloride in the presence of succinic dihydrazide and propylenediamine tetraacetic acid, followed by the addition of sodium N-cyclopentyl dithiocarbamate monohydrate. The radiochemical purity (RCP) of the product was over 90% as measured by thin layer chromatography(TLC). It was stable over 6h at room temperature. Its partition coefficient indicated it was a good lipophilic complex. The electrophoresis results showed the complex was neutral. The biodistribution results in mice indicated that ^{99m}TcN(CPEDTC)$_2$ was significantly retained into the brain. The brain uptake(ID%/g) is 3.58, 5.26 and 3.73 and the brain/blood ratio is 0.79, 1.69 and 1.59 at 5, 30 and 60min post-injection respectively. These results suggested potential usefulness of the complex ^{99m}TcN(CPEDTC)$_2$ as a new brain perfusion imaging agent.

RADIOLABELING OF GLYCOSYLATED SOMATOSTATIN WITH INDIUM-111

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Keywords: Somatostatin, Dextran, Indium-111, Radiolabeling

Somatostatin influences tumor growth, tumor progression and metastasis through direct and indirect mechanisms by its interaction with somatostatin receptors (SMSR) [1]. This forms the basis for its use in the therapy of somatostatin receptor positive tumors. Radiolabeled somatostatin analogues can additionally be used for systemic radiotherapy and for diagnostic investigations. Since natural somatostatin has too short biological half-life ($t_{1/2}$ <3 min) to be clinically useful, a number of somatostatin derivatives with extended half-life have been developed [2].

Recently, we have designed, synthesized and characterized a glycosylated somatostatin that natural somatostatin-14 (SMS) conjugated to dextran (Dx) of strictly de?ned molecular weight, which shown a long *in vivo* half-life and high affinity to all five somatostatin receptor subtypes [3, 4, 5]. Due to the unique and promising properties of somatostatin-dextran (SMS-Dx), we are developing the radiolabeled SMS-Dx conjugate for the diagnosis and targeted radiotherapy of somatostatin receptor positive tumors. Herein, we report radiosyntheses of ^{111}In-DTPA-SMS-Dx and *in vitro* investigation of this conjugate.

Dextran-10 was oxidized with sodium periodate at room temperature to yield reactive aldehyde groups. The activated dextran was subsequently reacted with somatostatin and 2-(p-NH_2-Bz)-6-methyl-DTPA (1B4M-DTPA) at 5 C for 4 h. The conjugate was then stabilized by reducing Schiff bases with sodium cyanoborohydirde for another 2.0 h and purified with a Sephadex G25 column. The concentration of somatostatin and DTPA in final conjugate was determined by spectrophotometric method [6]. The molar ratio of DTPA/dextran and SMS/dextran in final DTPA-SMS-Dx conjugate was 2.5 and 4 respectively.

The DTPA-SMS-Dx conjugate was then radiolabeled with ^{111}In in 0.4 M acetate buffer, pH 5.5 for 20 min at room temperature. The labeling yield was determined by ITLC-SG with EDTA 4 mM, pH 4.5 as solvent. The radiolabeled conjugate was purified on a Sephadex G25 column. The radiolabeled and purified conjugate was analyzed by HPLC with a Superdex 75 column with both on-line radioactivity and UV detection.

The conjugate ^{111}In-DTPA-SMS-Dx was prepared in high yield (>95%). The radiochemical purity determination demonstrated the stability of the conjugate over a wide range of pH values over a time course of 24 h in saline and serum.

In summary, the glycosylated somatostatin conjugate was successfully labeled with ^{111}In in high yield and stability. The studies to evaluate the receptor binding affinity of radiolabeled conjugates and their biodistribution in animal tumor xenograft models are in progress.

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SYNTHESIS OF MODIFIED BISPYRAZOLYLACETIC ACIDS AND THEIR COMPLEXES WITH $Tc(CO)_3^+$ FRAGMENT

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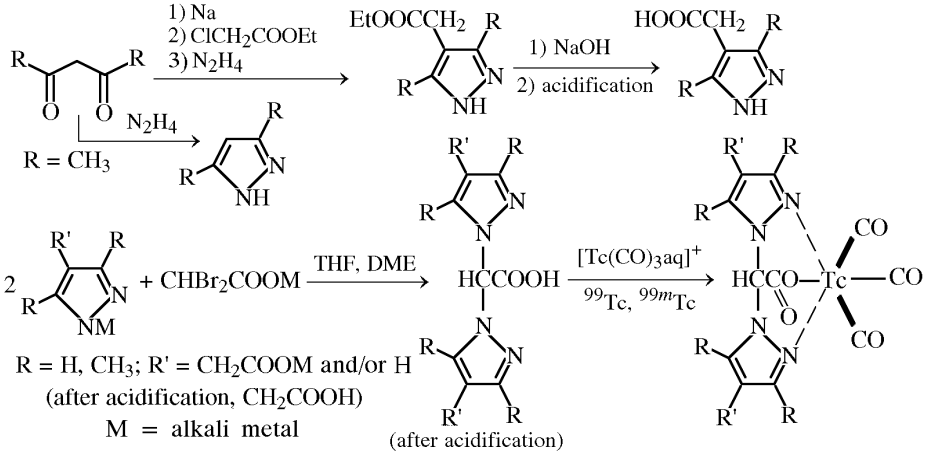
Keywords: Technetium; Carbonyl complexes; Bispyrazolylacetates

Development of new heart imaging agents by labeling of fatty acids with ^{99m}Tc is an urgent problem. A suitable precursor for this purpose is $Tc(CO)_3(H_2O)_3^+$. One of promising tripodal chelators for linking this species to fatty acids is bispyrazolylacetic acid (HL). Since we failed to prepare this acid in a good yield by the known heterogeneous procedure [1], we developed a new homogeneous procedure for preparing this acid in which the initial pyrazole was preliminarily converted to its sodium salt, soluble in the nonaqueous reaction medium, by treatment with NaH. In this stage of our study, instead of fatty acids we used acetic acid as a model. The carboxymethyl group was introduced into the ligand as follows: acetylacetone was converted to the 3-(ethoxycarbonylmethyl) derivative and then to the corresponding substituted pyrazole, which, after saponification, was brought into the reaction with lithium dibromoacetate. The acetic acid derivative containing two modified pyrazole substituents was thus prepared. However, the compounds containing simultaneously the modified and nonmodified pyrazole groups are of more interest. A procedure was developed for preparing such compounds by gradual substitution of bromine atoms in dibromoacetic acid.



$Tc(CO)_3L$ complexes were isolated and characterized by NMR and IR spectroscopy. Complexation of $Tc(CO)_3$ with bispyrazolylacetic acid in aqueous solutions was studied by ^{99}Tc NMR spectroscopy. This chelator completely binds $Tc(CO)_3^+$ in aqueous solution (1:1 Tc:ligand ratio, concentration ca. 10^{-3} M) and is not displaced by 10-fold histidine excess. $Tc(CO)_3Pz_2CHCOO$ is not bound by serum proteins. In the case of ^{99m}Tc, complexation was observed at the ligand concentration of about 10^{-5} M.

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SYNTHESIS AND EVALUATION OF $^{99m}Tc(CO)_3(N_2O)$-PENTADECANOIC ACID

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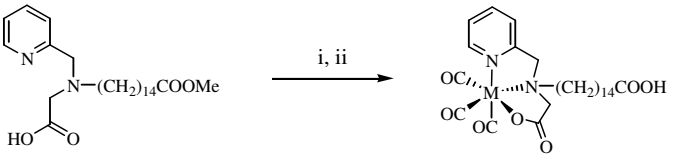
Keywords: $^{99m}Tc(CO)_3(N_2O)$-pentadecanoic acid, Fatty acid, Myocardial imaging

Long chain fatty acids serve as the main energy source for the myocardium. Although many efforts were made to develop ^{99m}Tc-labeled long chain fatty acids due to ideal physical properties and ready availability of ^{99m}Tc, conventional synthetic methods did not provide suitable radioligands for myocardial imaging. In this study, we synthesized a N_2O tridentate $^{99m}Tc(CO)_3(N_2O)$-pentadecanoic acid ([^{99m}Tc]**1**) as a novel radioligand for myocardial imaging.

The pyridinyl precursor of methyl pentadecanoate was prepared in high yield in 8 steps from ω-pentadecalactone. $Re(CO)_3(N_2O)$-pentadecanoic acid ([Re]**1**), the cold standard, was synthesized by reacting $(NEt_4)_2[Re(CO)_3Cl_3]$ with the pyridinyl precursor in methanol at rt for 2 h, followed by hydrolysis in 0.4 N NaOH-MeOH under reflux for 1 h. [^{99m}Tc]**1** was prepared as in the synthesis of [Re]**1** using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ at 75 °C for 30 min followed by hydrolysis at 75 °C for 15 min, and then purified by HPLC at a flow rate of 4 mL/min using a 70:24:6:0.06 mixture of CH_2Cl_2:hexane:2-propanol:HOAc. The desired fraction eluted at 24-26 min was collected, concentrated and redissolved in 5% ethanol-saline. Bovine serum albumin (5%) was added to the final solution at 50 °C to facilitate the myocardial uptake of the fatty acid. In vitro stability of [^{99m}Tc]**1** was measured in human serum at 37 °C for 12 h using radio-TLC. Dynamic images were obtained in SD rats using a gamma camera.



Reagents: i) M = Re ([Re]**1**): $(NEt_4)_2[Re(CO)_3Cl_3]$, MeOH, rt, 120 min; M = ^{99m}Tc ([^{99m}Tc]**1**): $[^{99m}Tc(CO)_3(H_2O)_3]^+$, saline, 75 °C, 30 min. ii) 0.4 N NaOH-MeOH

[^{99m}Tc]**1** was synthesized in 89-92% radiochemical yield and with radiochemical purity higher than 99%. [Re]**1** was used for the identification of [^{99m}Tc]**1** based on the similar chemical properties between ^{99m}Tc and Re complexes, although their HPLC retention times were not identical. [^{99m}Tc]**1** was shown to be stable (>98%) over 12 h when incubated in human serum. Dynamic images of the rats showed rapid accumulation of the radioactivity in the liver and kidneys with low uptake in the myocardium. ROIs were drawn on the liver and the heart, and their count/pixel ratios were obtained as 2.2:1, 10.7:1, and 27.5:1 at 1, 2 and 5 min postinjection, respectively. There was no significant uptake in the thyroid or salivary gland, indicating that ^{99m}Tc pertechnetate was not regenerated during the time of the study.

In conclusion, the results show that the [^{99m}Tc]**1** may not be a suitable candidate for myocardial imaging. This study describes the first example of the preparation and biological evaluation of $^{99m}Tc(CO)_3$-fatty acid. Further studies are warranted to lower the lipophilicity and to minimize the size of the ^{99m}Tc core.

"2+1" DITHIOCARBAMATE-BASED $M(CO)_3$ COMPLEXES (M = Tc, Re) FOR LABELING BIOMOLECULES

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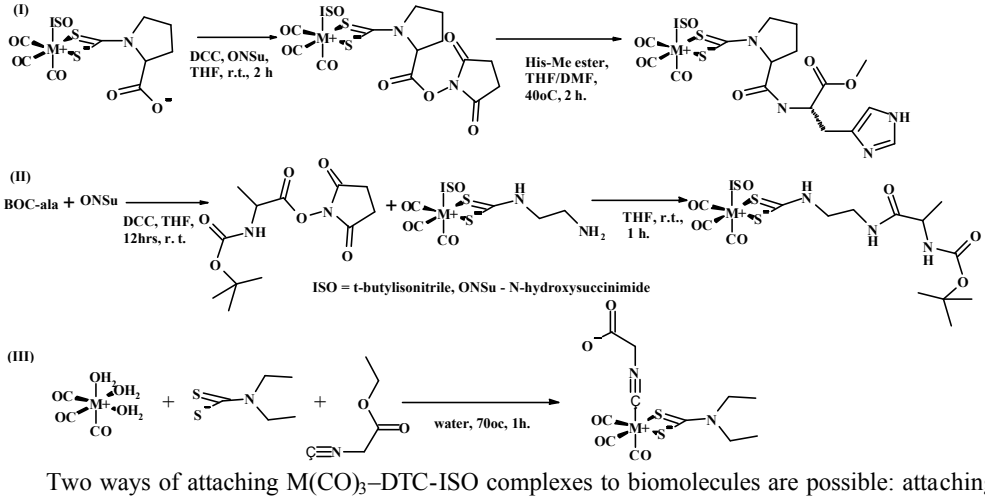
Keywords: technetium, rhenium, carbonyl, dithiocarbamates, amino acids

Over the last few years, the chemistry of $M(CO)_3^+$ (M = Tc, Re) species has been intensively developed and at present a premium has been placed on the identification of suitable chelation systems forming strong complexes with $M(CO)_3^+$ species and on the procedures for attaching of these chelation units to biomolecules. Previously [1] we reported that dithiocarbamate (DTC) chelation unit forms strong complexes with $M(CO)_3^+$ species. The common disadvantage of bidentate ligands is the presence of free coordination site, which can be substituted with a stronger donor ligand. Our goal was to develop methods for introduction of "2+1" dithiocarbamate-based $M(CO)_3$ fragment into a peptide chain. Firstly, we tested a series of monodentate ligands (imidazole, phosphines, isonitriles (ISO)) to find an optimal "2+1" DTC based chelation system. Experiments were carried on n.c.a. level of $^{99m}Tc(CO)_3$ and it was found that isonitrile-dtithiocarbamate system is most preferable. The resulting complex was formed at concentration of DTC and ISO ligands at10^{-5}M and is stable *in vitro* at least for 24 hrs.

We developed a pre-labeling procedure for attaching DTC chelation unit to biomolecules (amino acids was chosen as model compounds) using the activated esters method.



Two ways of attaching $M(CO)_3$–DTC-ISO complexes to biomolecules are possible: attaching via free carboxylic group (reaction I) and via free amino group (reaction II). The $M(CO)_3$ fragment can be also attached through monodentate coordinated ISO ligand (reaction III). Ethyl isocyanoacetate could be readily hydrolysed and the free carboxylic group could be coupled to amino groups. Thus, the proposed strategy of attaching $M(CO)_3$ –DTC-ISO complexes is promising for labeling of peptides. In addition, it should be noted, that the direct reaction of CS_2 with various amino acids is possible, but in the case of real pepides the side reactions can occur. Further work is in progress to optimize this methodology.

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(2-BENZIMIDAZOLYLMETHYLTHIO)ACETIC ACID: A NOVEL NSO LIGAND FOR THE *fac*-$[M(CO)_3]^+$ CORE (M = Re, ^{99m}Tc)

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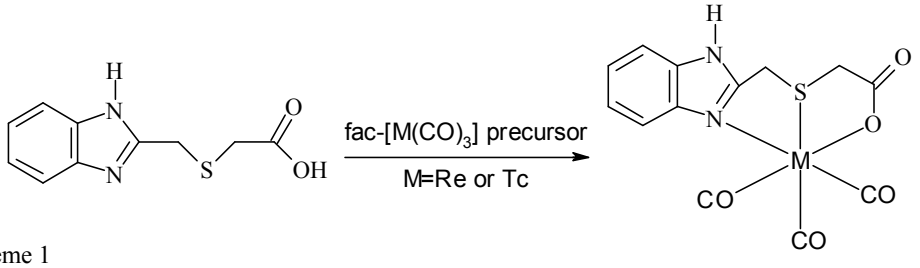
Keywords: Rhenium(I), Technetium(I), tridentate ligand, imidazole derivatives

Discovery of new chelating agents that bind technetium/rhenium strongly is a priority in the quest toward the design and development of site specific radiopharmaceuticals. The current emphasis on the application of Tc/Re carbonyls in nuclear medicine has demanded the utility of specific tridentate ligand systems for achieving kinetic inertness and *in vivo* stability. (2-Benzimidazolylmethylthio)acetic acid (Scheme 1) which possessed an NSO donor atom set can be a good fit for these requirements. Furthermore, the t nitrogen on the imidazole ring and the phenyl ring are sites for derivatization. In this preliminary investigation into the chemistry of the ligand with respect to the *fac*-$[M(CO)_3]^+$ core (M = ^{99m}Tc, Re), the Re and the ^{99m}Tc were synthesized.



Scheme 1

The Re(I)-complex was prepared in good yield by refluxing the ligand with $[NEt_4]_2[ReBr_3(CO)_3]$ in acetonitrile and equimolar amounts of NaOH for 2 hours. The complex was characterized by elemental analysis, spectroscopic methods and crystallographic analysis. The X-ray analysis of the complex showed that the ligand acts as monoanionic tridentate ligand (NSO) and occupies the three remaining sites of the *fac*-$[Re(CO)_3]^+$ core.

The ^{99m}Tc complex was carried out by incubating the *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor for 20 min at 70^o C with 0.4 mg of the ligand in a saturated solution of sodium chloride at pH 1. Then, the pH of the solution was adjusted to 8 and after standing for 30 min at room temperature the reaction mixture was analyzed by HPLC. The HPLC analysis demonstrated that the reaction produces in a single complex with yields greater than 90 %, which is stable for more than 6 h. The identity of the ^{99m}Tc complex was established by comparative HPLC studies using sample of the well characterized rhenium(I) complex as reference.

In conclusion, (2-benzimidazolylmethylthio)acetic acid reacts with *fac*-$[M(CO)_3]^+$ core resulting in a single stable complex, $M(CO)_3$(NSO). The high affinity of the ligand for the metal core coupled with the capability in derivatization imply that exploitation of this ligand system for the purpose of ^{99m}Tc-radiopharmaceuticals development is promising.

SYNTHESIS, RADIOLABELING AND EVALUATION OF ^{99m}Tc-COMPLEXES CONTAINING ß-METHYL PHENYL PENTADECANOIC ACID

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Keywords: Fatty acid, ^{99m}Tc, synthesis, biodistribution

Objective To study the correlation between structures and myocardial uptake.

Methods Four new ligands of the derivatives of ß-methyl-15-phenylpentadecanoic acid (BMPPA-NS?BMPPA-NTA?CACPPA and BMPPA-DTPA) were synthesized. ^{99m}Tc labeling of each ligand was prepared by adding freshly eluted $^{99m}TcO_4^-$ into the aqueous solution containing stannous chloride and a little alcohol. The resulting solution was heated in boiling water for about 15min. The partition coefficients(PC) of these four ligands were performed by adding each ^{99m}Tc labeled ligand in the mixture of phosphate buffer(3.0g, 0.1mol/L, pH7.00 and pH7.40) and n-octanol(3g). PC was expressed as logPC=counts(n-octanol)/counts(buffer). The biodistribution of these four ligands were tested in SD rats. Each ^{99m}Tc labeled ligand was injected through tail veins into fasted rats (180~200g, divided into 8 groups, 3 for each). The uptake of each organ was expressed as a fraction of injected dose per gram.

Results The chemical structures of four ligands and all intermediates were characterized by IR, elemental analysis, ^{1}HNMR and MS. The labeling yields were higher than 80% confirmed by TLC and HPLC, and radiochemical purity was higher than 95% after extraction. PC were 1.18, 1.12, 1.04 and 1.00 at pH 7.00, and 1.16, 1.14, 1.06 and 1.02 at pH 7.40, respectively. Biodistribution of these four ligands showed that the maximal myocardial uptakes were 0.91ID%/g, 0.78ID%/g, 0.68ID%/g and 0.38ID%/g, respectively.

Conclusions The value of PC may have positive correlation to the myocardial uptake of the ligand based on the similar structure. In order to explore ^{99m}Tc labeled derivative of ß-methyl-15-phenylpentadecanoic acid, research on reducing the hydrophilic group and adding an hydrophobic group should be emphasized.

DOUBLE – LABELLING OF PENTETREOTIDE USING 99mTc AND 111InCl

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The detection of Neuro-endocrine tumour by radiolabelled Pentetriotide – 111InCl is receiving a great deal of interest. Our Aim is to investigate a method of labelling of Pentreteotide using regularly available radionuclide pertechnetate. TcO4- is a transition metal that emits Gamma energy of ideal Characteristic for the detection using conventional gamma camera.

Methods: Commercially prepared Pentetreotide 111InCl was labelled with pertechnetate using SnCl2 as a reducing agent. The conjugates were separated through Sephadex G-50 and purified by G-25 and HPLC at flow rate 1 ml / min. UV absorbance at 280 nm confirmed the molecular weight of the pentreteotide.

The labelling efficiencies were determined by using instant thin - layer chromatography (ITLC) LE > 90 % and 97% respectively using 99mTc and 111InCl.

Normal rabbit images showed similar destribution with Pentetreotide using 99mTc and 111InCl though renal uptake excretion was high with Pentreteotide –99mTc.

Conclusion: Our in vitro experiments using different methods of separation is in favour of a strong and stable radiolabelled pentreteotide with 99mTc which offers cheap and potentially sensitive and selective means whereby diagnostic doses of radionuclides can be specifically delivered to the tumour site.

TRICARBONYL TECHNETIUM (I) COMPLEXES WITH DIFFERENT DIPHOSPHATE LIGANDS

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Keywords: Tc-carbonil, labelling, diphosphate ligands

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ has proved to be an excellent agent for labelling different kind of ligands. It can be formed in high yield directly from generator eluted pertechnetate in aqueous solution. As three coordinated waters are labile, they could be exchanged readily with a variety of mono-, bi- and tridentate ligands forming complexes.

A number of ^{99m}Tc-phosphate compounds, made by adding $^{99m}TcO_4^-$ to a kit, have been applied for bone imaging. There are a lot of literature data about forming mixed-metal complexes containing Tc (III), Tc (IV), Tc (V) and Sn(II) in undetermined proportions. The subject of this paper was the labelling of some diphosphate ligands with $[^{99m}Tc(CO)_3(H_2O)_3]^+$: pyrophosphate (PyP), imidodiphosphat (IDP), methane-diphosphonic acid (MDP), 3,3-diphosphono-1, 2-propanedicarboxylic acid (DPD) and 1-hydroxyethane-1, 1-diphosphonic acid (HEDP). All experiments were carried out with carbonyl labelling agent Isolink ^{TM} (Mallinckrodt Medical B.V.) and carbonyl precursor prepared in NCRS "Democritos".

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared from carbonyl labelling agent according a direction for use. Before use the basic solution was neutralised. Reaction products, prepared from different ligand solutions and Tc-carbonyl, were analysed by HPLC equipped with UV and -detector, on Alltech C-18 column, 250 mm x 10mm, 0.7 ml/min with TEAP 0.05 M, methanol and water. Runs were isocratic.

These diphosphate ligands showed different bonding for tricarbonyl technetium (I) precursor, so different phosphate ligands coplexed to Tc-carbonyl have shown different retention times. For example, retention time for HEDP was 12 min and retention time for DPD was 15 min. Obtained results have confirmed that in investigation conditions (pH 5.5 and with heating) the best labelling with HEDP and DPD, until the labelling efficiency of other ligands is negligible. There was no labelling in acid medium (pH 2.5) and without heating.

INITIAL STUDIES OF A SINGLE AMINO ACID CHELATE (SAAC): ^{99m}Tc(I)-DpK

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Key Words: Tc-99m, Technetium Tricarbonyl, Amino Acids

Technetium tricarbonyl chemistry has recently been the subject of much interest in radiopharmaceutical development. Previous studies on the coordination chemistry of the $\{M(CO)_3\}^{1+}$ core has established amine, aromatic heterocyclic amine, and carboxylate donors as very effective chelating ligands. These observations led to the design of tridentate chelators constructed as extensions of the side chain of amino acids. Such amino acid analogs provide a tridentate donor set for chelation and an amino acid functionality for attachment to biomolecules. We recently developed a family of such single amino acid chelates (SAAC) that serve this function and can be readily incorporated into peptides via solid phase synthesis techniques. As part of these continuing studies, we report here on the initial synthesis and Tc-99m labeling of [Tc-99m$(CO)_3${-*N,N*-di(pyridine-2-methyl)-lysine}] (**Tc-(I)-dpK**), a model SAAC, and its evaluation in rats.

[e-{*N,N*-di(pyridyl-2-methyl)}a-(fmoc)lysine]: The Fmoc-lysine, 2-pyridinecarboxaldehyde and sodium triacetoxyborohydride were mixed in 1,2-dichloroethane. The suspension was stirred at ambient temperature under an argon atmosphere for 1 hr. The reaction mixture was partitioned between chloroform and water. The residue was purified through a pad of silica gel using methanol-chloroform to provide the product in 85 % yield. Fmoc-deprotection employed stirring 4-dimethylaminopyridine in DMF/methanol at 25 C for 12 hrs. Structural confirmation was performed by ^{1}H and ^{13}C NMR.

[Re(CO)$_3${h^3-e-[(*N,N*-di(pyridyl-2-methyl)]a (fmoc) lysine}][Br]: The Re(I) complex was synthesized by refluxing $[NEt_4]_2[ReBr_3(CO)_3]$ with the fmoc-DpK in methanol for 4 hours and concentrated. The residue was dissolved in chloroform, washed with water, dried ($NaSO_4$) and evaporated to dryness to give a colorless product. ESMS m/z = 900.

Tc-99m labeling: $[^{99m}Tc(CO)_3(H_2O)_3]^+$was heated with DpK in 0.5 ml (1mg / ml) of methanol at 100 C for 30 minutes. Purity, analyzed via C18 HPLC, showed >99% RCY. In challenge experiments the HPLC purified product demonstrated no degradation in either 100mM Cysteine or Histidine in PBS pH 7.2 at 37 C for 18 hrs. Labeling yields of > 50% RCY, were achievable at levels as low as 2 g / ml.

Animal Studies: The biodistribution of Tc-99m-DpK was investigated in male rats (Sprague Dawley, n = 5 / timepoint, ~180 gms). The compound was injected via the tail vein in saline (10 Ci / 100 l). Animals were sacrificed at 5, 30, 60 and 120 minutes p. i..

Table 1. *Selected Biodistribution results of Tc99m-DpK Complex, expressed as Average %ID/g ± (SEM).*

Organ	5 Min ± (SEM)	30 Min. ± (SEM)	60 Min. ± (SEM)	120 Min. ± (SEM)
Blood	0.579 ± 0.051	0.069 ± 0.009	0.025 ± 0.005	0.013 ± 0.001
Liver	3.359 ± 0.442	2.748 ± 0.113	2.590 ± 0.077	2.119 ± 0.062
Kidney	6.053 ± 1.027	4.948 ± 0.106	4.931 ± 0.430	3.888 ± 0.419
GI	0.491 ± 0.081	0.886 ± 0.065	1.462 ± 0.085	2.725 ± 0.565

Summary: The SAAC labeling proceeded in high yield and was stable to excess histidine and cysteine challenges for more than 18 hours. Biodistribution studies showed major accumulation in kidney and liver only, at early timepoints. Activity decreased in all tissues as a function of time, except in the GI tract, which increased with time. These experiments suggest SAAC, particularly dpK, is a potential enabling technology for combinatorial peptide library synthesis, as well as the labeling of other important biomolecules.

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SYNTHESIS OF MODIFIED BISPYRAZOLYLACETIC ACIDS AND THEIR COMPLEXES WITH $Tc(CO)_3^+$ FRAGMENT

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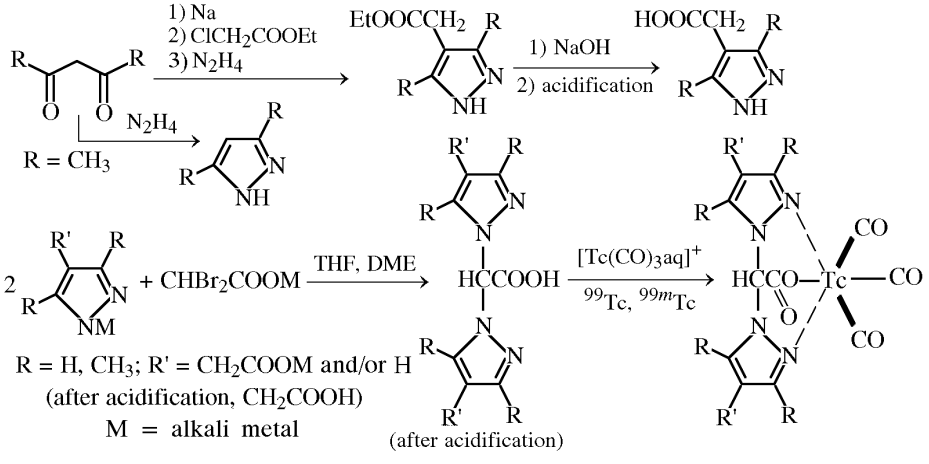
Keywords: Technetium; Carbonyl complexes; Bispyrazolylacetates

Development of new heart imaging agents by labeling of fatty acids with ^{99m}Tc is an urgent problem. A suitable precursor for this purpose is $Tc(CO)_3(H_2O)_3^+$. One of promising tripodal chelators for linking this species to fatty acids is bispyrazolylacetic acid (HL). Since we failed to prepare this acid in a good yield by the known heterogeneous procedure [1], we developed a new homogeneous procedure for preparing this acid in which the initial pyrazole was preliminarily converted to its sodium salt, soluble in the nonaqueous reaction medium, by treatment with NaH. In this stage of our study, instead of fatty acids we used acetic acid as a model. The carboxymethyl group was introduced into the ligand as follows: acetylacetone was converted to the 3-(ethoxycarbonylmethyl) derivative and then to the corresponding substituted pyrazole, which, after saponification, was brought into the reaction with lithium dibromoacetate. The acetic acid derivative containing two modified pyrazole substituents was thus prepared. However, the compounds containing simultaneously the modified and nonmodified pyrazole groups are of more interest. A procedure was developed for preparing such compounds by gradual substitution of bromine atoms in dibromoacetic acid.



$Tc(CO)_3L$ complexes were isolated and characterized by NMR and IR spectroscopy. Complexation of $Tc(CO)_3$ with bispyrazolylacetic acid in aqueous solutions was studied by ^{99}Tc NMR spectroscopy. This chelator completely binds $Tc(CO)_3^+$ in aqueous solution (1:1 Tc:ligand ratio, concentration ca. 10^{-3} M) and is not displaced by 10-fold histidine excess. $Tc(CO)_3Pz_2CHCOO$ is not bound by serum proteins. In the case of ^{99m}Tc, complexation was observed at the ligand concentration of about 10^{-5} M.

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SYNTHESIS OF DOTATATE DERIVATIVES WITH AN INTERCALATING MOIETY

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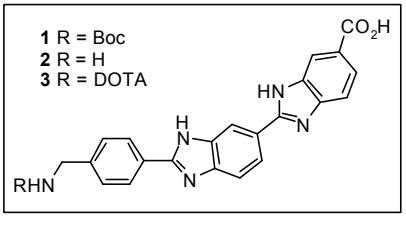
Keywords: Peptides, nuclear localisation, radiotherapy, intercalators, somatostatin

When conjugated to 'therapeutic' nuclides such as ^{90}Y, somatostatin receptor binding peptides have been shown to be successful for the therapy of receptor expressing tumours. The most successful radiotherapeutic is ^{90}Y-DOTATOC. However, the therapeutic value of this treatment is limited by the side effects in non-target tissues such as the kidneys. The effectiveness treatment modality could be further increased with conjugates which enable the nuclear targeting of the low energy emitting radioactive isotopes.

Intercalators, planar aromatic compounds, are able to interact with DNA by sandwiching themselves between the stacked bases at right angles to the long axis of the helix. The bis-benzimidazole dyes, Hoechst 33258 and 33342, have been shown to bind to the minor groove of DNA in A-T rich regions. Under certain circumstances, Auger-electron-emitting radionuclides can be extremely radiotoxic and produce extensive DNA damage. The degree of damage appears to depend upon the location of the decaying atom. Consequently, Auger electron-emitting radioisotopes, such as ^{195m}Pt, ^{114m}In, ^{111}In, ^{99m}Tc, ^{67}Ga and ^{51}Cr are known to be highly cytotoxic when localised in cell nuclei due to highly localised energy deposition by low energy Auger electrons. In addition binding to the DNA might increase the retention in the receptor expressing tissues.

The bis-benzimidazole intercalating moiety **1** was prepared using variations on the literature methods[1,2]. Compound **1** was coupled under normal SPPS conditions to the carrier peptide, Tyr^3-octreotate. Next the chelating agent (DOTA) needed to be attached to the intercalating moiety. First, the free amine derivative **2** was prepared from by its treatment with trifluoroacetic acid. This was coupled in solution to DOTA tris-*t*-butyl ester to give **3**. This was then coupled to the Tyr^3-octreotate using SPPS. The conjugates were purified by RP-HPLC and characterised by mass spectrometric and chromatographic methods. These conjugates can be efficiently labelled with radionucildes as exemplified with ^{111}In. Using fluorescence microscopy, the cellular uptake of these conjugates was investigated.



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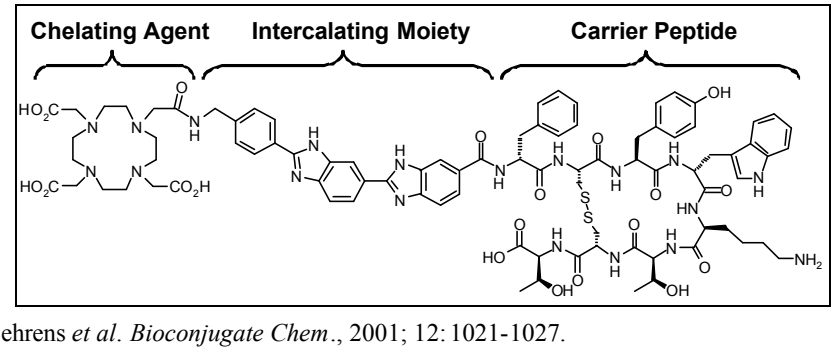

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PREPARATION OF INDIUM PENTETATE COMPLEX (^{111}IN-DTPA)

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Key words: ^{111}In, production, separation of ^{111}In, ^{111}In-DTPA

Indium is produced through irradiation of natural or enriched Cadmium-112 by proton and or deuteron beams on a Cyclotron. A number of compound prepare with Indium have been evaluated for tumor localization studies. One of them is ^{111}In-DTPA complex that is used for cerebral spinal fluid studies.

Indium-111 was produced on the, C30-Cyclotron by the *^{nat}Cd(p, xn) reaction.* For the In-111 production experiments at 20 MeV, natural cadmium targets were bombarded with 150-μA current. After bombardment, the In-111 was separated from the cadmium target and other contamination in one step procedure using a cationic-resin column. Indium 114 as a main radionuclide impurity was less than 2.6±0.7%, and the radionuclide purity was more than 97.3±0.2%.

In the present study, DTPA has been chelated with ^{111}In by employing various methods and then tested for its stability in-vitro during storage and in human plasma. Three models for the preparation of ^{111}In -DTPA were used. At the first method anhydride DTPA Anhydride was hydrolyzed in acidic medium and indium chloride was added in the solution and pH adjusted to 7. In the second method DTPA anhydride was hydrolyzed in basic medium and indium acetate was added to the solution and in the last method calcium three sodium DTPA was dissolved in the water and indium acetate added to the solution. In each method, labeling efficiency and radiochemical purity were determined by chromatography system one, two and three days after preparation. After three days the stability of the complex in the room temperature was confirmed.

PRODUCTION OF CU-64 FROM NATURAL NICKEL

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Keywords: Cu-64, Cyclotron byproduct, Natural Nickel

Copper-64 ($T_{1/2}$ = 12.7h) is an intermediate half-lived positron emitting radionuclide that is a useful radiotracer for positron emission tomography (PET) as well as a promising radiotherapy agent for biomedical studies and treatment of cancer.

It has been suggested that it may be possible to produce Cu-64 from natural Nickel on a cyclotron (Cyclone-30). We have irradiated natural Nickel coated on gold foil with a copper substrate. Nickel has been electroplated successfully at thickness of 80μm, the shuttle geometry designed such that the targets saw the beam at 6°, it means the surface increased to 800μm. An initial production run at 23 MeV proton energy with 50 μAh for 1hour was performed. The irradiated target was dissolved off the gold foil in 25 ml of 9.0 N warm nitric acid. The solution was then evaporated to dryness. The residual was dissolved in 20ml of 6N hydrochloric acid. The Cu-64 was separated from the Nickel target using anionic exchange resin as a byproduct of Co-57. HPGe detector obtained the activity of Cu-64 15 mCi with the yield of 300 μCi/μAh. This method allows the production of Co-57 and Cu-64 using only one target.