^{188}Re(III)-"4+1"-MIXED-LIGAND COMPLEXES: STABILITY STUDIES AND LABELING OF BIOMOLECULES

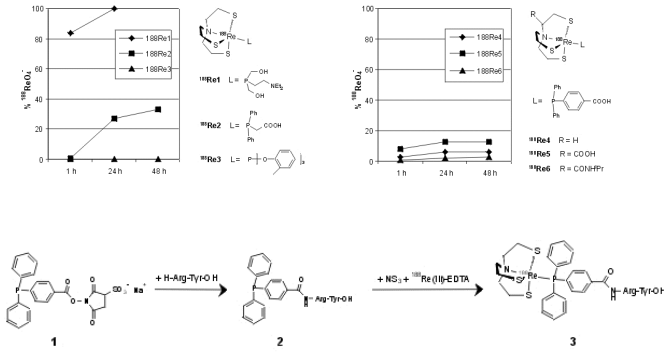
E. Schiller,[1] S. Seifert,[1] F. Tisato,[2] F. Refosco,[2] W. Kraus,[3] H. Spies,[1] T.K. Heinrich,[1]
H.-J. Pietzsch.[1]

[1]Institut fur Bioanorganische und Radiopharmazeutische Chemie, Forschungszentrum Rossendorf, Dresden, Germany; [2]Istituto di Chimica Inorganica e delle Superfici, Consiglio Nazionale delle Ricerche, Padova, Italy; [3]Bundesanstalt fur Materialforschung und - Prufung, Berlin, Germany.

In order to understand relationships between the structure of rhenium-188 '4+1' complexes and their in vitro stability we synthesized a series of rhenium model complexes and determined their stability in human plasma. Rhenium-188 '4+1' complexes were synthesized using different monodentate phosphorus(III) ligands, including both lipophilic and hydrophilic tertiary phosphines and phosphites (**^{188}Re1**-**3**) and unsubstituted tetradentate ligands and substituted NS_3 ligands bearing a carboxyl group or an isopropyl amide were used as chelators (**^{188}Re4**-**6**). As instability in aqueous solution leads always to perrhenate, the amount of $^{188}ReO_4^-$ formed after 1h, 24 h and 48 h was determined by TLC.

According to our findings **^{188}Re4** was of sufficient stability and therefore used for further investigations. We tried to find out which physico-chemical parameters of the corresponding non-radioactive rhenium complexes **Re1-6** may govern the formation of complexes of high in vitro stability.

The water-soluble N-hydroxysulfosuccinimidyl activated ester **1** is a useful compound for the conjugation of exclusively water-soluble biomolecules. Rhenium-188 labeling of the phosphine-arginine-tyrosine conjugate **2** as model compound was carried out using a labile ^{188}Re(III)-EDTA intermediate. Based on this procedure peptides and proteins shall by labeled and tested in vivo.



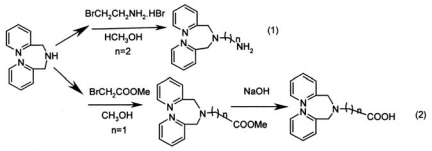
Keywords: Rhenium-188, 4+1-Complexes, Targeted Radiotherapy

SYNTHESIS AND CHARACTERIZATION OF RHENIUM TRICARBONYL COMPLEXES WITH DIFFERENT BIFUNCTIONAL TRIDENTATE NNN, NNO LIGANDS

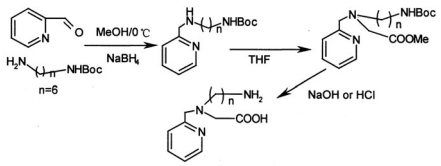
J.Y. Xia, Y. Wang, J.F. Yu, L. Tang, D.Zh. Yin.
Radiopharmaceutical Centre, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Jiading, Shanghai, China.

Discovery of new chelating ligands that bind technetium/rhenium strongly is a priority in the quest toward the design and development of site specific radiopharmaceuticals. The current study on the application of Tc/Re carbonyls in radiopharmaceuticals has been focused on the utility of specific tridentate ligand systems for achieving kinetic inertness and in vivo stability. In this paper, (Bis(2-pyridylmethyl)-amino)-ethylamine(1) (scheme 1), (Bis(2-pyridylmethyl)-amino)-acetate(2) (scheme 1) and [(6-amino-hexyl)-pyridyl-2-methyl-amino]-acetate(3) (scheme 2) that possessed an NNN, NNO donor atom sets were synthesized, which can be a good selection for these requirements.

Scheme 1



Scheme 2



The Re(I)-complexes of three ligands were prepared in good yield with $[NEt_4]_2[ReBr_3(CO)_3]$ in water at 75 °C for 3 h. The complexes were characterized by elemental analysis and spectroscopic methods.

The ^{188}Re(I)-complexes of three ligands were carried out by incubating the fac-$[^{188}Re(CO)_3(H_2O)_3]^+$ fragment for 30 min at 75 °C in PBS buffer (0.1 M NaCl/0.05 M sodium phosphate buffered, pH7.4) under nitrogen. After cooling in ice bath, complexes formations were monitored by HPLC. The HPLC analysis demonstrated that the reaction produces in the complex 1 with yield greater than 95% and its retention times was 7.5 min, which is stable for more than 12 h, and the complex 2 with yield 93% is stable for more than 8 h, its retention times was 15.5 min, while the complex 3 with yield more than 90% can be stable 10 h and its retention time was 15.4 min respectively. The identity of the ^{188}Re-complexes were proved by comparative HPLC studies using samples of the well characterized rhenium(I) complexes as reference.

In conclusion, three ligands react with fac-$[M(CO)_3(H_2O)_3]^+$ (M=^{188}Re, Re) fragment resulting in three single stable complexes, $M(CO)_3$(1), $M(CO)_3$(2) and $M(CO)_3$(3). The high affinity of the ligand systems for the mental core implies that the application of this ligand systems for the purpose of ^{188}Re radiopharmaceuticals development is promising.

Keywords: Rhenium(I), Radiopharmaceuticals, Tricarbonyl Complexes

DEVELOPMENT OF RHENIUM-188 COMPLEXES BASED ON NOVEL CHELATORS DERIVED FROM DIMERCAPTOSUCCINIC ACID (DMSA) SUITABLE FOR EASY LINKING OF BIOMOLECULES

T.K. Heinrich,[1] W. Kraus,[2] H.-J. Pietzsch,[1] C. Smuda,[1] S. Seifert,[1] H. Spies.[1]
[1]Institut fur Bioanorganische und Radiopharmazeutische Chemie, Forschungszentrum Rossendorf, Dresden, Germany; [2]Bundesanstalt fur Materialforschung und – Prufung, Berlin, Germany.

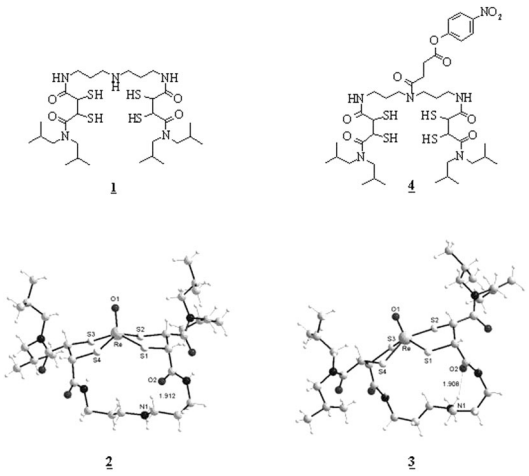
This work is part of our efforts to develop chelating agents for stable binding and easy conjugation of rhenium-188 to biologically interesting structures. Keeping in mind the high in vivo stability of $[^{188}ReO(DMSA)_2]^-$ (1) we want to exploit this coordination system for the design of $^{188}ReO(V)$ chelates which are stable towards re-oxidation to perrhenate and towards ligand exchange under all conditions of radiopharmaceutical procedures and applications.

The new type of tetradentate ligand has been synthesized by bridging two molecules of dimercaptosuccinic acid (DMSA) with an alkylene triamine chain. The resulting stereo-isomeric tetrathiolato S_4 ligand **1** forms five-coordinate oxorhenium(V) complexes **2** (*exo-cis*) and **3** (*exo-trans)* by ligand exchange reaction of $NBu_4[ReOCl_4]$ in methanol. Without addition of base the compounds will be isolated as "betain", $[ReO(S_4)]$, with the protonated nitrogen of the bridge as internal "counter ion". X-ray crystal structure determination of both stereoisomeric forms reveals the square-pyramidal coordination geometry of the $ReOS_4$ core. The orientation of the metal-oxo core is *exo* in relation to the carbamido groups in both isomers.

The activated BFCA **4** enables easy linking of biomolecules con-taining a terminal amino group. Prototypic model conjugates with tripeptides have been identified in non-radioactive form by electrospray mass spectrometry.

The Re-188 labelling procedure runs fast, in good yields and under mild conditions, making the new complexes interesting as a further access to stable rhenium-188 radiotherapeutics.

1. Blower P. J. et al., Eur. J. Nucl. Med. 1998, 25, 613-621



Keywords: Rhenium-188, Dimercaptosuccinic Acid, Targeted Radiotherapy

DIRECT METAL-CYCLIZED SOMATOSTATIN ANALOGUES: SYNTHESIS, CHARACTERIZATION AND RECEPTOR BINDING STUDIES

H.M. Bigott,[1] S. Junnotula,[2] F. Gallazzi,[2] M.R. Lewis,[3] S.S. Jurisson.[2]
[1]Life Sciences Fellowships Program, University of Missouri-Columbia, Columbia, MO, United States; [2]Department of Chemistry; [3]Department of Veterinary Medicine and Surgery.

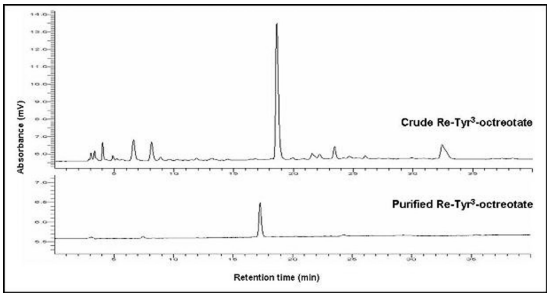
Radiometal-labeled somatostatin analogues are being developed as cancer imaging and/or radiotherapy agents through their ability to selectively target somatostatin receptor positive tumors. The vast majority of these studies have involved the bifunctional chelate approach, in which a chelating moiety is appended to the peptide sequence targeting the somatostatin receptor. While the lead compounds arising from these studies demonstrate excellent imaging characteristics, their clinical utility for therapy has been limited by relatively poor retention of radioactivity in tumors as well as nephrotoxicity due to prolonged retention in the kidneys.

We are currently exploring the direct metal cyclization of somatostatin analogues, wherein the radiometal (e.g., ^{99m}Tc, ^{188}Re) is coordinated directly into the disulfide bond of the somatostatin derivative, using a Cys-S-Tc/Re-S-Cys structure to form the cyclic peptide. Previous work with alpha-melanotropin stimulating hormone (α-MSH) receptor targeting involved both the bifunctional chelate approach (1) and direct incorporation of ^{99m}Tc or ^{188}Re into the disulfide bond of α-MSH peptides (1,2). The direct metal cyclization approach resulted in the highest tumor uptake and retention observed for any α-MSH radiometalated complex (3). This suggests the possibility that analogous structural modifications of somatostatin derivatives will result in their improved tumor retention of radioactivity and tumor imaging contrast, and will allow more effective therapy at lower doses that would spare the kidneys.

The syntheses of Re(V) complexes of several somatostatin analogues (e.g., Tyr^3-octreotate) have been achieved via transchelation reactions utilizing modified literature procedures (1,2). In short, the Re coordination products were prepared by reacting the non-cyclized peptides with the Re(V) starting material [$ReOCl_3(OPPh_3)(SMe_2)$] in aqueous methanol solution (pH ~8.5) at 65°C for 60 min. Analysis by LCMS confirmed that the main product of each reaction had the expected mass of the Re peptide complex (e.g., *m/z* = 1251 $(M+H)^+$ for Re-Tyr^3-octreotate). Reversed-phase HPLC was employed to isolate the Re-cyclized peptides, and the masses of the peak-purified products were again verified by MS. The figure shown depicts typical analytical HPLC chromatograms of Re-Tyr^3-octreotate in the crude reaction mixture (top, 18.6 min retention time) and the purified Re-Tyr^3-octreotate product (bottom). Synthesis and characterization of Re- and ^{99m}Tc-labeled somatostatin analogues will be detailed. The IC_{50} values of the purified Re-labeled derivatives, determined from competitive binding assays with ^{125}I-Tyr^3-octreotide in AR42J rat pancreatic tumor cells, will also be presented.

Acknowledgments

This research was supported through a University of Missouri-Columbia Life Sciences Postdoctoral Fellowship and by US DOE grant number DE-FG02-01ER63192.



References

1. Giblin MF, Jurisson SS and Quinn TP. *Bioconjugate Chem.* 1997; 8: 347-353.
2. Giblin MF, Wang N, Hoffman TJ, Jurisson SS and Quinn TP. *Proc. Natl. Acad. Sci. USA* 1998; 95: 12814-12818.
3. Cheng Z, Chen J, Quinn TP and Jurisson SS. *Cancer Res.* 2004; 64: 1411-1418 and references therein.

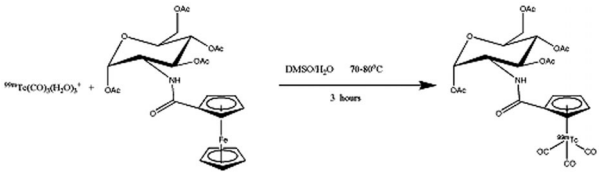
Keywords: Somatostatin Analogues, Metal Cyclization, Rhenium-188 / Technetium-99m

CYCLOPENTADIENYLTRICARBONYL RHENIUM(I) AND TECHNETIUM(I) COMPLEXES CONTAINING PENDANT GLUCOSE DERIVATIVES

M.J. Adam,[1] C.L. Fisher,[1,2] C. Orvig,[2] C. Ewart,[1,2] B.O. Patrick.[2]
[1]Chemistry, TRIUMF, Vancouver, BC, Canada; [2]Chemistry, University of British Columbia, Vancouver, BC, Canada.

The development of metal complexes for medicinal applications is a rapidly expanding area of chemistry. However, organometallic species for use in medicine have remained relatively unexplored until recently. One particular organometallic fragment that has aroused interest in nuclear medicine is the neutral $CpM(CO)_3$ core (Cp = cyclopentadienyl, M = Tc(I) or Re(I). ^{99m}Tc, a gamma emitter, is the most widely used radioisotope in nuclear imaging, while Re has two beta particle emitting radioisotopes of interest for therapy. The $CpM(CO)_3$ core is ideal for *in vivo* use by virtue of its stability, small size, and lipophilicity. Biological selectivity with these complexes can be achieved by attaching biomolecules to the Cp ring via a linker. Complexes of this nature, containing peptides, proteins and fatty acids, have been noted in the literature. [1]

Our research has focused on the labelling of carbohydrates with Re and Tc as potential radiopharmaceuticals. One approach we have taken is to investigate the conversion of ferrocenyl-carbohydrate derivatives to the corresponding carbohydrate-$CpM(CO)_3$ compounds. We initially attempted to apply the Double Ligand Transfer reaction (DLT)[2] to this conversion without sucesss, since the conditions were too harsh for the carbohydrate groups. More recently a single ligand transfer procedure has been reported[3] for the direct synthesis of $CpM(CO)_3$ compounds from ferrocenyl derivatives. This convenient method uses the direct reaction of the aqueous *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ reagent with ferrocenyl derivatives. We now report the application of this method to the synthesis of carbohydrate-$CpM(CO)_3$ compounds from the corresponding ferrocenyl compounds. Initially we have synthesized the Tc-99m glucosamine derivative in 54% radiochemical yield as shown in figure 1.



Briefly, the acetylated glucosamine ferrocenyl derivative was heated in DMSO/water for 3 hours at 70-80°C with the *fac*-Tc reagent. Product analysis was performed on a gradient HPLC system and compared to the non radioactive Re compound. Conversion of a variety of other sugar-ferrocenyl compounds, including unprotected sugar derivatives, is currently underway. Optimization of reaction conditions is also underway and will be reported.

Support for this project has been provided by the National Sciences and Engineering Research Council of Canada and MDS Nordion. We would also like to thank Mallinckrodt Inc. for generously providing the Isolink boranocarbonate kits.

1. T. Spradau and J. Katzenellenbogen. *Bioconjugate Chem.* 1998, 9, 765-772.
2. T. Spradau and J. Katzenellenbogen. *Organometallics* 1998, 17, 2009-2017.
3. S. Masi, S. Top, L. Boubekeur, G. Jaouen, S. Mundwiler, B. Spingler, and R. Alberto. *Eur. J. Inorg. Chem.* 2004, 2013-2017.

Keywords: Carbohydrates, Technetium and Rhenium, Cp-M-Tricarbonyl

RADIOLABELLING OF GLYCOSYLATED SOMATOSTATIN WITH TECHNETIUM-99m

C. He,[1] S. Zhai,[2] H. Cui,[1] J. Du.[1,2]
[1]Department of Isotopes, China Institute of Atomic Energy, Beijing, China; [2]Medical Isotopes Research Center, Peking University, Beijing, China.

Somatostatin influences tumour growth, tumour progression and metastasis through direct and indirect mechanisms by its interaction with somatostatin receptors. Radiolabeled somatostatin analogues have been used for systemic radiotherapy and for diagnostic investigations. Since natural somatostatin has too short biological half-life to be clinically useful, a number of somatostatin derivatives with extended half-life have been developed.

Glycosylated somatostatin that natural somatostatin-14 (SMS) conjugated to dextran (Dx) of strictly defined molecular weight has shown an enhanced in vivo stability and retained high affinity to all five somatostatin receptor subtypes[1,2,3]. The somatostatin-dextran (SMS-Dx) has been successfully radiolabeled with ^{99m}Tc by using direct dextran labeling[4] and ^{99m}Tc(I)-carbonyl approach[5], but the serum stability of radiolabeled conjugate was not satisfied. Herein, we report synthesis of new chelator MAG2Lys, the radiosyntheses of ^{99m}Tc-MAG2Lys-Dx-SMS 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 MAG2Lys at 5°C for 10 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 MAG2Lys in final conjugate was determined by spectrophotometric method. The molar ratio of MAG2Lys/dextran and SMS/dextran in final MAG2Lys-Dx-SMS conjugate was 2 and 4 respectively.

The MAG2Lys-Dx-SMS conjugate was then radiolabeled with ^{99m}Tc in the presence of $SnCl_2$ in 0.2 M acetate buffer, pH 5.0 for 10 min at room temperature. The labeling yield was determined by ITLC-SG with 85% methnol 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 ^{99m}Tc-MAG2Lys-Dx-SMS was prepared in high yield (>80%). The radiochemical purity determination demonstrated the stability of the conjugate over a time course of 24 h in saline and serum.

In summary, the glycosylated somatostatin conjugate MAG2Lys-Dx-SMS was successfully labeled with ^{99m}Tc 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.

This work was supported by the National Natural Science Foundation of China (No. 30370421).

References:
1. Holmberg A. et al. Antibody Immunoconj Radiopharm 1994; 7:253-259.
2. Behe M. et al. Medical Oncology 2001; 18:59-64.
3. Wulbrand U. et al. Cancer 2002; 94(S4):1293-1297.
4. Holmberg et al. Cancer Res 1995; 55(S): 5710-5713.
5. Du J et al. Appl Radia Isot 2001; 55:181-187.

Keywords: Somatostatin, Dextran, Technetium-99m

SYNTHESIS OF DICARBA ANALOGUES OF OCTREOTIDE USING TANDEM HOMOGENEOUS CATALYSIS

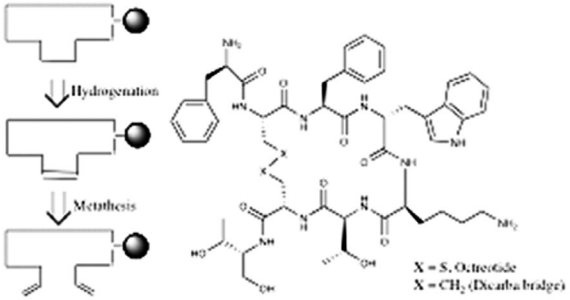
A.N. Whelan,[1] J. Elaridi,[1] S.V. Smith,[2] R.J. Mulder,[3] A.J. Robinson,[1] J. Sosabowski.[4]
[1]Chemistry, Monash University, Clayton, Victoria, Australia; [2]Materials and Engineering Science, Australian Nuclear Science and Technology Organisation (ANSTO), Menai, New South Wales, Australia; [3]Molecular Sciences, CSIRO, Clayton, Victoria, Australia; [4]Department of Nuclear Medicine, St Bartholomew's Hospital, West Smithfield, London, United Kingdom.

Cystine bridges are common structural motifs in naturally occurring cyclic peptides. In some cases, the cystine bridge constitutes part of a peptide binding domain or active site where the disulfide bridge may act as a reactive functional group and undergo reduction within the cell to release metal-chelating thiol groups. In other cases, however, the cystine bridge serves only a skeletal, structural role to maintain secondary and tertiary structure and may be replaced with non-reducible structural mimics without significantly affecting biological activity.

Octreotide is a synthetic cyclic octapeptide with one cystine bridge.[1] When labelled with ^{111}In it is marketed as OctreoScan®, an imaging agent for neuroendocrine tumours. Particular attention is now being focussed on ^{99m}Tc and ^{188}Re radiolabelled analogues. The incorporation of these isotopes into cystine-containing peptides is often complicated by the need for a reduction step for the generation of Tc and Re in the (III) - (V) oxidation states required for complexation to the ligand. This reduction step can lead to concomitant reduction of the disulphide bridge resulting in loss of receptor binding affinity.

This paper will discuss the development of a highly generic, efficient and regioselective method for the preparation of dicarba analogues of cystine containing peptides.[2] Six dicarba analogues of somatostatin, including octreotide, vapreotide and lanreotide, have been synthesised using tandem sequences of homogeneous metal catalysed reactions (Figure 1). In each case, the cysteine pair was replaced with non-proteinaceous allylglycine and subjected to Ru-catalysed metathesis.[3] The resultant unsaturated cyclic peptides were then reduced with a homogeneous Rh-phosphine catalyst. All reactions were quantitative and each of the peptides were obtained in 20-46% yield in >98% purity. Competition experiments using ^{111}In-DOTA-Octreotate as radioligand with increasing concentrations of dicarba octreotide analogues have been conducted with sst2-expressing cells and will be reported. Significant findings include higher binding affinity for *unsaturated* (C=C) versus *saturated* (C-C) dicarba analogues. We believe that our results will find widespread interest amongst groups involved in peptidomimetic and radiopharmaceutical research.

1. Bauer, W., Briner, U., Doepfner, W., Haller, R., Huguenin, R., Marbach, P., Petcher, T.J., Pless, J. Life Sci. 1982, 31, 1133-1140.
2. Whelan, A.N., Elaridi, J., Harte, M., Smith, S.V., Jackson, W.R., Robinson, A.J. Tetrahedron Letters, 2004, 45, 9545-9547.
3. Grubbs, R.H. Tetrahedron, 2004, 60, 7117-7140.



Keywords: Cystine Replacement, Dicarba Analogues, Octreotide Analogues

REFORMULATION OF ^{99m}Tc-RP527

N. Wang, A. Cagnolini, J. Chen, K.E. Linder, L.E. Lantry, P. Wedeking, A. Nunn, H. Fan, R. Swenson.
Discovery Research, Bracco Research USA, Princeton, NJ, United States.

^{99m}Tc-RP527(^{99m}TcOMe$_2$N-Gly-Ser-Cys-Gly-Ava5-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH$_2$) is a novel ^{99m}Tc labeled peptide for the in vivo detection of tumors that express the Gastrin-Releasing Peptide Receptor (GRP-R). This receptor is over expressed in several types of cancer, including prostate, breast and small cell lung cancer. A clinical trial was performed by Resolution Pharmaceuticals with ^{99m}Tc-RP527(ACM protected) prepared using a 4-vial frozen kit containing 200 µg of ligand and purified using HPLC to remove radioactive impurities and unchelated ligand prior to injection. We developed a two vial formulation containing only 4 µg of unprotected ligand that does not require HPLC purification and yields an RCP > 90% at six hours post-reconstitution. The components of the formulation are as follows: Vial 1: 4 µg of unprotected RP527, 40 µg of $SnCl_2{\cdot}2H_2O$, 1.3 mg of Gluconic acid, sodium salt, 20 mg of gentisic acid, sodium salt, 2.5 mg of hydroxypropyl-γ-cyclodextrin, pH adjusted to 3 - 4; Vial 2: 300 µL of EtOH. Frozen kits prepared with this formulation gave RCP values of 93% at 18 minutes and 91% at 6 hours post reconstitution. Biodistribution studies were conducted in nude PC-3 tumor bearing mice administered 5 µCi of ^{99m}Tc-RP527 via i.v. tail vein.

Biodistribution of ^{99m}Tc-RP527

Organ	60 min	24 hours
Blood (% ID/g)	0.55 ± 0.10	0.18 ± 0.23
Liver (% ID/g)	9.5 ± 3.2	2.0 ± 0.3
Kidneys (% ID/g)	4.8 ± 2.4	0.82 ± 0.14
Pancreas (% ID/g)	21 ± 7	1.8 ± 0.2
PC-3 tumor (% ID/g)	2.3± 0.4	1.2 ± 0.2
Skin (% ID/g)	0.42 ± 0.07	0.06 ± 0.008
Muscle (% ID/g)	0.21 ± 0.06	0.01 ± 0.01
GI tract (% ID/g)	32.3 ± 5	0.87 ± 0.46
Bladder/urine (% ID)	39.66 ± 9.8	0.005 ± 0.007

This formulation comprises 30% (300 µl) of EtOH, which is utilized to improve recovery of the ^{99m}Tc complex from the vial. Two non-ionic detergents, Cyclohexyl-n-methyl-β-D-maltoside and n-Hexyl-β-D-Glucopyranoside were tested to substitute for EtOH, since EtOH can not be freeze-dried with the other formulation components. Moreover the use of a solid compound instead of EtOH would reduce the number of vials from two to one, reducing production costs considerably. These detergents were chosen because they do not dissolve biological membranes, are water-soluble and contain a sugar moiety that has potential ligand exchange properties. Recovery and RCP obtained substituting the EtOH with 50-100 mg of these glucosides was equivalent to that obtained using EtOH.

The fact that ^{99m}Tc-RP527 has a mixed hepatobiliary-renal clearance pathway, while ^{177}Lu-AMBA, Bracco's radiotherapeutic agent currently in development has a renal one precludes its use to directly determine dosimetry data for radiotherapy.

However, due to good tumor targeting this compound could be used for patient screening and to monitor patient response to therapy.

This formulation method appears to be general and could be applied to other N_3S Tc chelated peptides, especially where low ligand amounts are required.

Keywords: Reformulation, GRP, Technetium-99m

STABILIZATION OF TECHNETIUM-99m RADIOPHARMACEUTICALS USING HYDROPHILIC THIOETHERS

J.E. Cyr, D.A. Pearson, J. Lister-James, L. Dinkelborg.
Radiopharmaceuticals Research, Schering AG, Berlin, Germany.

Autoradiolysis is a common problem in Tc-99m radiopharmaceuticals leading to shortened shelf life and poorer radiochemical purity. There are some well-recognized excipients that are useful against radiolysis, for example, gentisic acid[1] and ascorbic acid.[2] However, it is noteworthy that these anti-radiolytics often cannot be used directly in a Tc-99m kit formulation, possibly because they also compete for the Tc-99m in the radiolabeling reaction. Thiols such as cysteine are also well-known antioxidants[3] which likely function as free radical scavengers,[4] and therefore could be useful as anti-radiolytics in radiopharmaceutical formulations. However, thiols are also often especially good competing ligands for the Tc(V) core. In formulation studies of the lung cancer imaging agent Tc-99m depreotide (NeoTect/NeoSpect) gentisic acid, ascorbic acid, and cysteine added into the kit prior to labeling produced substantially lower radaiochemical yields compared to no-additive control preparations. Some other well-known phenolic antioxidant excipients such as BHT are effective free radical scavengers, but are not suited for radiopharmaceutical formulations due to limited water solubility.

We have observed that hydrophilic thioethers can be used directly in Tc-99m kit formulations to stabilize the radiolabeled product. For example, the Tc-99m depreotide kit formulation containing 4 mg of L-methionine had radiochemical purity (RCP) ³ 92% out to 6.5 hours, whereas the same formulation without methionine had RCP = 80% at 6.5 hours. Other types of Tc-99m compounds were shown to be stabilized by L-methionine, including the GP IIb/IIIa receptor-binding peptide Tc-99m P748, the GP IIb/IIIa receptor-binding benzodiazepine derivative Tc-99m P424, and the model chelator peptide Tc-99m P1300 (P1300 = Bz-bDpr-Lys-Cys-Lys.amide) possessing the C-terminal chelator amino acid sequence from depreotide. In addition to peptide-based N_3S chelates, simple N_2S_2 BAT chelates were also found to benefit from addition of L-methionine.

Other hydrophilic thioethers including for example 2-(ethylthio)ethylamine, methioninol, and 3-methylthio-1,2-propanediol were also found to stabilize Tc-99m preparations, indicating that the active structural feature of these compounds is the thioether moiety.

The mechanism of stabilization of the thioethers is likely scavenging of oxidizing free radicals produced during water radiolysis, generating a sulfur-centered radical cationic methionine species.[5]

Conclusion: Hydrophilic thioethers are effective anti-radiolytic stabilizers for Tc-99m formulations.

1. U.S. patent 5,384,113; Jan. 24, 1995
2. Liu, S, Ellers, CE, and Edwards, DS, *Bioconj. Chem.,* 2003, **14**, 1052.
3. a) Yu-Chang, Jw, and Musetta, AH, *J. Parenteral Sci. Tech.*, 1988, **42 (Supplement)**, S4; b) Gulizar, A., *Yonsei Med. Journal*, 2004, **45**, 776.
4. Russell, J, et al. J. Pharm. Biomed. Anal., 1994, **12**, 863.
5. Lam, XM, Yang, JY, and Cleland, JL, *J. Pharm Sci*., 1997, **86**, 1250.

Keywords: Technetium-99m, Formulation, Thioether

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

J. Zhang,[1,2] X. Wang,[1,2] Z. Song,[2] J. Chu.[2]
[1]Key Laboratory of Radiopharmaceuticals, Ministry of Education, Beijing, China; [2]Department of Chemistry, Beijing Normal University, Beijing, China.

Introduction

In the last two decades, great advances in myocardial perfusion imaging have been achieved, such as ^{99m}Tc-MIBI[(1)], ^{99m}Tc-tetrofosmin[(1)] and $^{99m}TcN(NOET)_2$[(2)]. However, the persistent liver uptake observed for these heart imaging agents are their common defects. Recent years, a class of asymmetrical nitrido ^{99m}Tc heterocomplex as heart imaging agents have been reported[(3)]. These complexes are monocationic and are represented by the general formula $[^{99m}TcN(PNP)(L)]^+$, where L is the monoanionic form of a dithiocarbamate ligand of the type $[R^1(R^2)NC(=S)S]^-$, PNP is a diphosphine ligand of the type $[R^3{}_2(P)(CH_2)_2]_2NR^4$. In order to extend the investigation of the biological properties of the class of asymmetrical nitrido ^{99m}Tc heterocomplexes, we report here the synthesis and biodistribution of the $[^{99m}TcN(PNP5)(DMCHDTC)]^+$(DMCHDTC: 2,3-dimethyl cyclohexyl dithiocarbamate) complex. This complex exhibited significant heart localization and good heart/liver, heart/lung and heart/blood ratios, suggesting its potentiality as a new myocardial perfusion imaging agent.

Experimental

The complex was prepared as follows: 1ml of saline containing $[^{99m}TcO_4]^-$ was added to a kit containing 0.05mg of stannous chloride dihydrate, 5.0mg of succinic dihydrazide(SDH), 5.0mg of propylenediamine tetraacetic acid(PDTA). The mixture was kept at room temperature for 15min. To the resulting solution, 1.0mg of PNP5(dissolved in 0.3ml of ethanol) and 1.0mg of DMCHDTC ligand(dissolved in 0.3ml saline) were added simultaneously. The vial was heated at 100ºC for 15min.

The RCP of the product was evaluated by TLC and the TLC was performed on a polyamide strip and eluted with saline:acetone=1:1(V/V). The Rf values for some selected complexes are follows: $^{99m}TcO_4^-$ 0.1, $^{99m}TcO_2.nH_2O$ 0.1, $[^{99m}TcN]_{int}^{2+}$ 0.9-1.0, ^{99m}TcN-DMCHDTC 0.1, ^{99m}TcN-PNP5 0.9-1.0, $[^{99m}TcN(PNP5)(DMCHDTC)]^+$ 0.5-0.7.

Biodistribution studies were carried out in mice in compliance with national laws related to the conduct of animal experimentation.

Results and discussion

From Table 1, the complex was seen to have a significant heart uptake and good retention. The liver, lung and blood clearances were fast so that the heart/liver, heart/lung and heart/blood ratios were increasing. The heart/liver, heart/lung and heart/blood ratios of the complex were 1.24, 3.62 and 23.05 at 60min post-injection, suggesting it will be a potential myocardial imaging agent.

Conclusion

The novel complex has been successfully prepared through an efficient method, which can be easily utilized for the preparation of a radiopharmaceutical through a freeze-dried kit formulation. The significant heart localization, good retention and high target/non-target ratio in mice of the complex exhibited favorable properties, justifying further investigation.

Acknowledgements:

The work was financially supported by National Natural Science Foundation of China(20201004) and by Beijing Committee of Science and Technology(2004A20).

References:

1 Schwaiger M, Melin J. Lancet. 1999; 354: 661

2 Pasqualini R, Duatti A, Bellande E, et al. J. Nucl. Med. 1994; 35: 334

3. Boschi A, Uccelli L, Bolzati C, et al. Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine. 2002; 6:431

Table 1 Biodistribution in mice of [99mTcN(PNP5)(DMCHDTC)]+ (x±s, n=3)(ID%/g)

	5min	30min	60min
heart	14.47±0.39	12.23±3.43	8.76±0.63
liver	57.29±1.12	11.99±1.73	7.07±1.09
lung	11.73±0.40	3.73±0.91	2.42±0.73
kidney	77.64±3.86	51.94±3.86	38.07±2.01
brain	0.23±0.05	0.10±0.05	0.06±0.03
blood	4.16±0.53	0.67±0.09	0.38±0.03

Keywords: Technetium-99m, [99mTcN]2+core, Myocardial Imaging

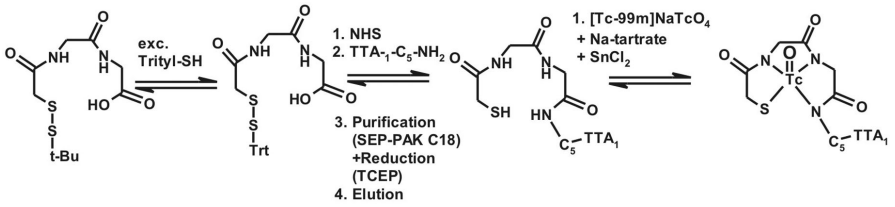
S-TRITYL PROTECTED MERCAPTOACETYL-GLYCYL-GLYCINE – A USEFUL PURIFICATION HANDLE FOR OLIGONUCLEOTIDE-CHELATOR CONJUGATES AS PRECURSORS FOR RADIOLABELING OF APTAMERS WITH Tc(V) AND Re(V)

M. Friebe,[1] M. Hecht,[2] K. Graham,[1] A.W. Stephens,[1] C.S. Hilger,[1] B. Johannsen,[2] L.M. Dinkelborg.[1]

[1]Research Laboratories of, Schering AG, Berlin, Germany; [2]Institute of Bioinorganic and Radiopharmaceutical Chemistry, Forschungszentrum Rossendorf, Dresden, Saxony, Germany.

Introduction: Radioactively labeled aptamers have the potential to be useful tools for diagnostic imaging of disease specific targets in nuclear medicine. Aptamers are characterized by its oligonucleotide nature, a molecular weight between peptides and antibodies and high affinity binding to a wide variety of extracellular protein targets. TTA-1 is a fully synthetic modified RNA with a low nM affinity and high selectivity for the extracellular matrix protein tenascin-C.[1] TTA-1 bears a C_5-alkyl linked amino group at 5′ position for conjugation of metal chelators and a 3′-3′ cap for protection from biodegradation. The mercaptoacetyl-glycyl-glycine (MAG-2) chelating unit has been widely used as a chelator for Tc-99m and Re-188 radiolabeling.[2,3] An easy method to separate the TTA-1-MAG-2 conjugate from unreacted TTA-1-C_5-NH_2 would be of interest in order to achieve Tc-99m radiolabeling with high specific radioactivity.

Materials & Methods: ^{t}Bu-MAG-2 was synthesized according to the literature.[4] Radiolabeling was performed at pH 8.3 and 95°C. Results: The trityl-S-S-CH_2-CO-NH-glycine-glycine group attached



to the TTA-1-C_5-NH2 allowed for an easy separation of this conjugate from the unreacted starting material using a C-18-SEP-PAK-cartridge, the unreacted TTA-1-C_5-NH_2 eluted from the cartridge, whereas the TTA-1-MAG-2-S-trityl was retained. An *in-situ* deprotection was carried out with (Tris[2-carboxyethyl]phosphine (TCEP) and the pure unprotected TTA-1-MAG-2 (-SH) was eluted from the cartridge. The Tc-99m radiolabeling was quantitative with a specific activity of 10 mCi/nmol.

Discussion/Conclusion: The bulky and lipophilic trityl-S-protecting group provides an excellent purification handle for oligonucleotide-chelator conjugates. The *in-situ* deprotection on the purification cartridge is time-effective and preserves the sulfhydryl group unoxydized for radiolabeling with Tc-99m at a high specific radioactivity. A retained binding affinity of K_D 2 x 10^{-9} for the resulting Tc complex indicates an excellent compatibility of this method with oligonucleotides.

1.Hicke B.J. et al [2001] J. Biol. Chem. 276: 48644
2.Johannsen B. et al [1993] Inorg. Chim. Acta 210:209
3.Guhlke S. et al [1998] J. Nucl. Med. 39:105
4.Hilger C.S et al [1999] Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine p. 557, SGE-Editoriali Padova

Keywords: Oligonucleotides, Tc(V)/Re(V), Aptamers

CYCLOPENTADIENYL RHENIUM (TECHNETIUM) TRICARBONYL COMPLEXES INTEGRATED IN ESTROGEN RECEPTOR (ER) LIGANDS FOR ER+ TUMOR IMAGING

N.C. Ackroyd, J.A. Katzenellenbogen.
Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, United States.

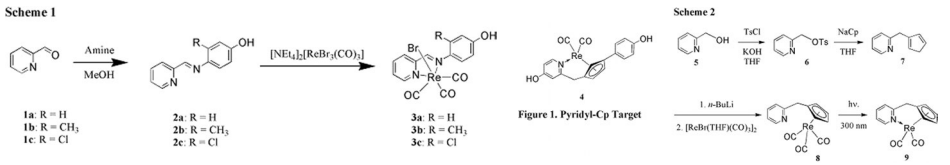
The estrogen receptor (ER), which is over-expressed in many breast cancers, is a medically relevant target for imaging and has been imaged with the fluorine-18 labeled ER ligand, 16a-[^{18}F]fluoroestradiol (FES).[1] The low cost and widespread availibility of the gamma-emitting isotope ^{99m}Tc makes it attractive for use in SPEC imaging; however, the high molecular weight and polarity of tethered metal complexes has thus far made it difficult to image of nuclear receptors such as ER using this isotope. Previous work on ^{99m}Tc-labeled ER ligands suggests that an integrated design, in which the technetium radio–metal label forms a part of the core structure of the receptor ligand, will display the requisite in vivo stability, as well as the potential for high binding affinity to ER; an example is shown below.[2] It is with the goal in mind of making the imaging of ER in breast tumors more widely available and less expensive that we are studying the design and synthesis of novel ER ligands labeled with ^{99m}Tc.

The approach taken has been the chemical synthesis of multi-dentate ligands capable of semi-encapsulating Re(I) carbonyl complexes in a manner that buries the metal center within the structure of the receptor ligand. Our current ligand designs include a substituted pyridine bearing a second coordinating site, either an imine[3,4] or cyclopentadiene[2], which causes the ligand to bend around the metal center. A synthetic sequence leading to these novel organometallic species is shown below. The binding affinity to ER, relative to estradiol (E2) is measured using a competitive radiometric binding assay.

An initial series of pyridyl imines has been prepared; though only complexed in a bidentate fashion, the metal center appears to be stable. The best of these compounds (the chloro-substituted **3c**) exhibited significant ER binding affinty. Incorporation of a second hydroxyl on the pyridine ring is expected to result in higher affinities. The pyridyl cyclopentadienyl system was prepared by first forming a yellow cyclopentadienyl-rhenium-tricarbonyl complex, followed by the photolysis of a carbon monoxide with the concomitant formation of a pyridyl-nitrogen to rhenium bond to form a red solid. The cyclized complex was characterized using HRMS, as well as a downfield shift of the carbonyl ^{13}C-NMR signal.

Acknowledgements. This work was supported by a grant from the Department of Energy (DE FG02 86ER60401) and the Department of Defense Breast Cancer Research Program (BC021658)

(1) Mintun, M. A.; Welch, M. J.; Siegel, B. A.; Mathias, C. J.; Brodack, J. W.; McGuire, A. H.; Katzenellenbogen, J. A. *Radiology* **1988**, *169*, 45-8.
(2) Mull, E. S.; Sattigeri, V. J.; Rodriguez, A. L.; Katzenellenbogen, J. A. *Bioorganic & Medicinal Chemistry* **2002**, *10*, 1381-1398.
(3) Wang, W.; Spingler, B.; Alberto, R. *Inorganica Chimica Acta* **2003**, *355*, 386-393.
(4) Shavaleev, N. M.; Bell, Z. R.; Accorsi, G.; Ward, M. D. *Inorganica Chimica Acta* **2003**, *351*, 159-166.



Keywords: Estrogen Receptor, ER+ Tumors, Technetium Complexes

η^5-TYPE ORGANOMETALLIC COMPOUNDS AND THE PREPARATION OF Tc RADIOTRACERS

A. Green,[1] O.O. Sogbein,[1] R. Chankalal,[1] A.S. Louie,[1] J.F. Valliant.[2]
[1]Chemistry, McMaster University, Hamilton, ON, Canada; [2]Chemistry and Medical Physics, McMaster University, Hamilton, ON, Canada.

Since the discovery of a means to prepare $[^{99m}Tc(CO)_3(OH_2)_3]^+$,[1] a vast amount of research has gone into developing methods to synthesize organometallic compounds as synthons for preparing new radiopharmaceuticals. We have recently discovered a new and mild labeling strategy that enables η^5-sandwich type complexes of $[M(CO)_3]^+$ (M = Re, ^{99m}Tc) to be prepared in water in very high yields.[2]

The labeling strategy, which is a vast improvement over existing methodologies, involves the use of aqueous fluoride ion in combination with the $[M(CO)_3]^+$ core.[2] Using carboranes, the new methodology facilitated the synthesis of η^5-Tc and Re complexes of a series of uniquely functionalized ligands in high yield. These include organometallic derivatives of carbohydrates, peptides, and serotonin antagonists.

Factors which influence the yield of the reaction at the macroscopic scale and the tracer level were investigated revealing interesting aspects of Tc(I) chemistry that have yet to be reported. Details of the synthesis of the organometallic-carborane derivatives and selected *in vivo* imaging experiments will be presented.

References:

(1) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Abram, U.; Kaden, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987-7988.

(2) Sogbein, O. O.; Merdy, P.; Morel, P.; Valliant, J. F. *Inorg. Chem.* **2004**, *43*, 3032-3034.

Keywords: Technetium, Organometallic, Carboranes

SYNTHESIS OF NOVEL *FAC*-[^{99M}Tc(CO)$_3^+$]-LABELED (±)-NICOTINE AND A-84543 ANALOGS AS POTENTIAL $\alpha_4\beta_2$ NEURONAL NICOTINIC RECEPTOR IMAGING AGENTS

D. Papagiannopoulou,[1] D.J. Kramer,[2] A. Mamhood,[1] A. Davison,[2] A.G. Jones.[1]
[1]Radiology, Harvard Medical School, Boston, MA, United States; [2]Chemistry, Massachussetts Institute of Technology, Cambridge, MA, United States.

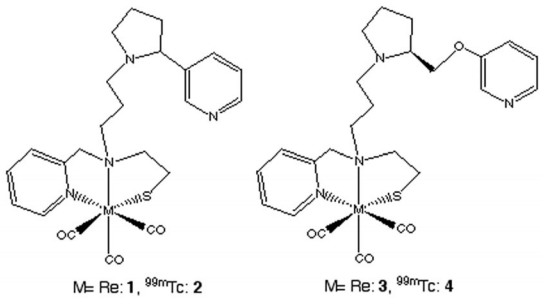
^{99m}Tc is the most widely available γ-emitting radionuclide used for in-vivo imaging in nuclear medicine. In this study, we present the initial results of our efforts to develop ^{99m}Tc-based complexes targeted to the $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptors (nAChR) as diagnostic agents for neurodegenerative diseases[1]. Research efforts in the past decade have led to the development of a number of potent $\alpha_4\beta_2$ specific nAChR ligands, such as 3-(2-pyrrolidinyl)-pyridine (nicotine, K_i=1nM) and 3-(2-(S)- pyrrolidinyl-methoxy)-pyridine (A-84543, K_i=0.15nM)[2].

We have designed and synthesized novel neutral rhenium(I) and technetium(I) tricarbonyl complexes of the type *fac*-M[NN(R)S](CO)$_3$, with the (2-mercaptoethyl)picolylamine (MEPA) tridentate ligand[3] of the general formula 2-C_5H_5N-CH_2-N((CH_2)$_3$R)(CH_2)$_2$SH, where R is an $\alpha_4\beta_2$ nAChR binding moiety. In complexes **1** (M=Re) and **2** (M=^{99m}Tc), R is (±)-nornicotine and in complexes **3** (M=Re) and **4** (M=^{99m}Tc), R is an A-84543 analog. In both cases, an N-propyl linker was used to tether the pharmacophore moiety to the chelate via the pyrrolidinyl nitrogen (Figure 1).

The rhenium complexes (**1, 3**) were synthesized by ligand exchange reaction from the (NEt_4)$_2$[$ReBr_3$(CO)$_3$] precursor with a 50% isolated yield and were characterized spectroscopically via FTIR, ^{1}HNMR and HRMS. The ^{99m}Tc complexes (**2, 4**) were synthesized from the precursor ^{99m}Tc(H_2O)$_3$(CO)$_3^+$ in high radiochemical yield (>92%). The identity of the ^{99m}Tc complexes (**2, 4**) was established by HPLC analyses via co-injection with their rhenium analogs (**1, 3**). The purified technetium complexes exhibit high *in vitro* stability (>95%) at pH 7.4 at 40 °C during a 4-hr incubation period. The partition coefficients (logP_{oct}) of the ^{99m}Tc-complexes in 1-octanol/PBS pH=7.4 were also determined to be 0.418 and 0.664 for **2 and 4** respectively. Preliminary tissue distribution data in healthy mice revealed that both complexes display a whole brain uptake of 0.15% ID/g at an early time point of 15 min post injection. Although the values are relatively low, additional in-vivo studies need to be conducted to determine if the radioactivity accumulation observed in the brain is attributable to receptor-specific binding in regions of high receptor density.

REFERENCES:

1. Sihver W., Nordberg A., Langstrom B., Mukhin A.G., Koren A.O., Kimes A.S., London E.D. Behav. Brain Res. 113: 143 (2000)
2. Abreo M.A., Lin N-H., Garvey D.S., Gunn D.E., Hettinger A-M., Wasicak J.T., Pavlik P.A., Martin Y.C., Donnelly-Roberts D.L., Anderson D.J., Sullivan J.P., Williams M., Arneric S.P., Holladay M.W. J. Med. Chem. 39: 817 (1996)
3. Kramer D.J., Davison A., Davis W.M., Jones A.G. Inorg. Chem. 41: 6181 (2002)



ACKNOWLEDGEMENTS:

This work was funded by USPHS 5RO1 EB002104 (formerly 5 RO1 CA34970).

Keywords: Technetium, Nicotinic Acetylcholine Receptors, Tc(CO)^3 Complexes

ETHER LIGAND COMPLEXES OF $Tc(CO)_3^+$ FOR CARDIAC IMAGING

K.P. Maresca,[1] D. Keith,[1] F.J. Femia,[1] J.F. Kronauge,[1] N. Lazarova,[2] J.A. Zubieta,[2] J.W. Babich.[1]
[1]R & D, Molecular Insight Pharmaceuticals, Cambridge, MA, United States; [2]Chemistry, Syracuse University, Syracuse, NY, United States.

Clinically approved ^{99m}Tc-cardiac blood flow tracers are lipophilic cations, which appears to be a required characteristic for uptake and retention in the myocardium.[1] Ether substitutents are the structural component commonly employed to modulate lipophilicty in these agents (e.g., Cardiolite, BMS Imaging and Myoview, Amersham Health). We have previously described a novel series of cationic ^{99m}Tc-tricarbonyl complexes of ether derivatized di-(pyridylmethyl)amine (DPA) and di-(imidazolemethyl)amine (DIA) that display accumulation in the rat heart.[2] Here we expand this series in an attempt to understand the structural requirements for improved cardiac uptake and retention. Eight Tc-99m complexes, plus Cardiolite™ were evaluated.

Di-(pyridylmethyl)amine (DPA) and di-(imidazolemethyl)amine (DIA) ligands were functionalized using ether substituents. Complexes of the $^{99m}Tc(CO)_3L$, were readily formed by reaction of $^{99m}[Tc(CO)_3(H_2O)_3]^+$[3] with the ether containing tridentate ligands. The ^{99m}Tc-tricarbonyl complexes were stable in vitro against molar excesses of cysteine and histidine. Log P values were measured for the complexes and calculated for the free ligands. HPLC retention was also measured as surrogate for Log P.

RESULTS AND DISCUSSION

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared using $Na^{99m}TcO_4$ and commercially available Isolink™ kits (Mallinckrodt). The HPLC column employed was a Vydac C18 (25 cm x 4.6 mm x 5 um) column.

Derivatization of the DPA and DIA ligands was affected using ether-containing alkyl bromides. The DIA was prepared by reductive alkylation. The reaction pathways were straightforward with fair yields. The resulting derivatized tridentate chelates, depicted in **Figure 1**, were characterized using both ^{1}H NMR and GC/MS.

As shown in **Table 1**, the smallest complex, the ethyl ethoxy derivative TEC-1, demonstrated the most favorable ratios of HT/BL and HT/LIV at 120 minutes. The replacement of the ethyl-ethoxy on TEC-1 with the dimethoxy (TEC-2), while doubling the total number of ethers actually decreased %ID/g in the heart (from 0.89% to 0.56% at 5′) and all the associated ratios HT/BL, HT/LIV, and HT/LU. In contrast, substituting with the ethyl-diethoxy moiety in TEC-3 (increasing log P from -0.07 to 0.27) increases the %ID/g in the heart, with a maximum of 1.56% at120 minutes compared to TEC-1's 0.84%. Despite this increase in heart uptake, the related ratios of HT/BL, HT/LIV, and HT/LU do not substantially increase due to an increase in overall retention for TEC-3. TEC-3 does demonstrate the highest HT/LIV at 5 minutes, 2.1. The same pattern of SAR, where diethoxy increased %ID/g in the heart verses methoxy, continued with the DIA complexes (TEC-4 vs TEC-5 and -6) as well as the aromatic ethers TEC-7 and TEC-8.

In summary, a convenient method for the preparation of ether-containing Tc-99m complexes as agents has been developed. The series, while small, suggests this new class of compound may have potential as ^{99m}Technetium (I) heart imaging agents. A broader series of compounds will be prepared to further characterize the structural requirements needed for an improved cardiac tracer.

1. Marshall, R.C., Barnett, C.A. *et al*., Circulation (1990) 82: 998-1007.
2. Maresca, K.P., Babich, JW *et al*. [abs] J. Soc. Nucl. Med. (2004) P703.
3. Alberto, R., Schubiger, A. P. *et al*. J. Am. Chem. Soc. (2001) 123: 3135.

Keywords: Technetium-Tricarbonyl, Ethers, Heart Agents

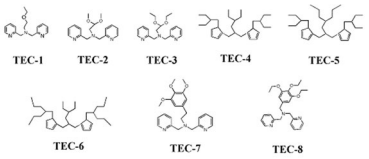


Table 1. Tc-99m-ether biodistribution summary.

TEC#	1	2	3	4	5	6	7	8	Cardiolite™‡
%ID/G HT 5'	0.89	0.56	0.96	0.70	1.13	0.87	0.61	1.10	2.65
%ID/G HT 120'	0.84	0.78	1.56	0.90	1.04	0.99	0.46	1.08	
%ID/G BL 5'	**.051**	**0.16**	**0.11**	**0.13**	**0.24**	**0.16**	**0.2**	**0.10**	**0.06**
%ID/G BL 120'	**0.013**	**0.06**	**0.033**	**0.016**	**0.022**	**0.015**	**0.013**	**0.02**	
HT/BL 5'	17.5	3.45	8.2	5.26	4.82	5.37	3.5	11.1	44.2
HT/BL 120'	66.8	13.9	43.9	54.4	48.6	66.1	36.6	50.9	
HT/LIV 5'	**1.75**	**0.78**	**2.1**	**0.65**	**0.58**	**0.38**	**0.18**	**0.27**	**4.2**
HT/LIV 120'	**15.1**	**4.1**	**12.3**	**0.83**	**3.21**	**4.33**	**4.9**	**4.8**	
HT/LU 5'	2.71	1.6	2.8	2.64	3.06	3.13	1.43	2.7	3.5
HT/LU 120'	3.47	3.6	5.0	4.37	5.23	3.93	3.1	3.0	
HPLC Rt*	**8.8**	**7.4**	**16.3**	**25.3**	**77.4**	**>110**	-	-	**32.5**
Log P'†	**-0.07**	**0.0**	**0.27**	**0.37**	**1.05**	**1.73**	-	-	**0.85**
Log P''	**2.06**	**2.01**	**2.69**	**0.48**	**1.16**	**1.83**	**3.51**	**4.25**	-
M.W.	**271**	**287**	**315**	**442**	**470**	**498**	**393**	**422**	**777**
# Ethers	**1**	**2**	**2**	**6**	**6**	**6**	**3**	**3**	**6**

HT = Heart, BL = Blood, LIV = Liver, LU = Lung, Rt = Retention time.
* HPLCs were performed on Vydac C18 columns (25 cm x 4.6 cm x 5 µm) using an isocratic method 45%B, 1 ml/min. for 110'. The solvents employed were (A) = triethylammonium phosphate buffer (pH 2.5) and (B) = methanol.
† Log P was calculated for the Tc-99m complexes using Octanol / PBS (pH7.2) n =5.
** Log P was calculated for ligands using ChemDraw Ultra 7.0.
‡ Cardiolite was performed at 15 minutes.

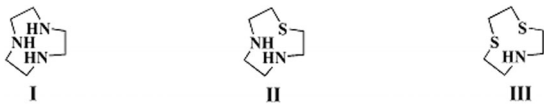
NOVEL TACN COMPLEXES AS ESTROGEN RECEPTOR LIGANDS FOR TUMOR IMAGING

M.L. Nickels, J.A. Katzenellenbogen.
Department of Chemistry, University of Illinois Urbana-Champaign, Urbana, IL, United States.

Breast cancer is the most prevalent form of cancer in U.S. women and the second leading cause of cancer-related deaths.[1] Early detection of breast cancer is vital to the survival of the patient. Studies have shown that of the 64% of women diagnosed at a localized stage of breast cancer, 97% have a likelihood of survival over a five-year period. This is not the case if the cancer has spread regionally or distantly; the numbers now become 75% and 20%, respectively, over a five-year period.[2] Just as early detection of tumors is essential for survival, so determination of estrogen receptor levels is vital for making the appropriate treatment choices of endocrine therapy vs. radiation and/or cytotoxic chemotherapy. While imaging estrogen receptor levels in tumors can be done using F-18 labeled estrogens, it is important to develop receptor imaging agents labeled with the widely available and less expensive technetium-99m radionuclide. We are exploring the design and evaluation of metal carbonyl complexes of cyclic tridentate ligands as potential ligands for the estrogen receptor.

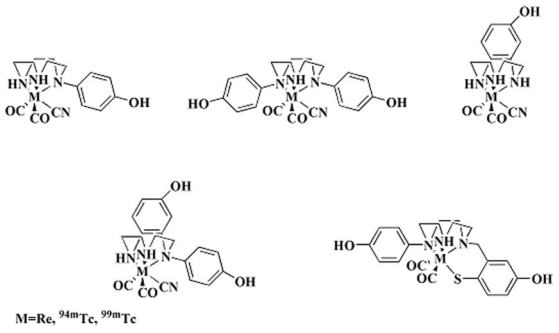
The cyclic chelates we have explored are based on 1,4,7-triazacyclononane (tacn) (**I**), 1,4-diaza-7-thiacyclononane (**II**) and 7-aza-1,4-dithiacyclononane (**III**) (Figure 1).

Figure 1.



Ligands **I, II** and **III** have been extensively studied for their strong chelating ability to a variety of metals, making them promising candidates for radiometal labeling.[3] The tridentate character of these ligands provides a unique way of incorporating radioactive metals, such as ^{94m}Tc or ^{99m}Tc, into the estrogen receptor by developing analogs in which the metal complex forms are an integral part of the receptor ligand (integrated design).[4] To adapt this metal chelate system for receptor binding, we are preparing derivatives in which the donor nitrogen atoms or bridging dimethylene units are substituted with groups that are typically found in non-steroidal estrogens. Several of them are illustrated in Figure 2. Substitution of the available nitrogens is generally accomplished by reaction with a suitable electrophile under basic conditions. More elaborate approaches have also been employed to obtain analogs similar to the compounds shown in Figure 2.

Figure 2.



(1) Skaddan, M. B.; Wust, F. R.; Jonson, S.; Syhre, R.; Welch, M. J.; Spies, H.; Katzenellenbogen, J. A. *Nuclear Medicine and Biology* **2000**, *27*, 269-278.
(2) Anonymous; American Chemical Society: 2004; Vol. 2004.
(3) Briellmann, M.; Kaderli, S.; Meyer, C. J.; Zuberbuhler, A. D. *Helvetica Chimica Acta* **1987**, *70*, 680-9.
(4) Minutolo, F.; Katzenellenbogen, J. A. *Journal of the American Chemical Society* **1998**, *120*, 13264-13265.

Keywords: Metal Chelate, Estrogen Receptor, PET Imaging

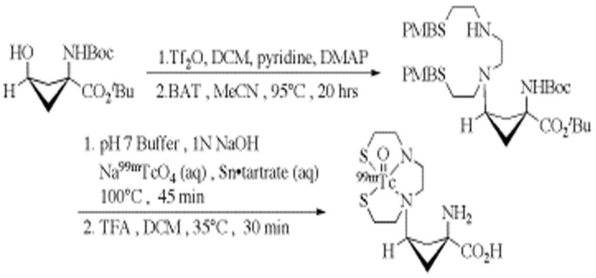
SYNTHESIS, RADIOLABELING, AND *IN VITRO* CHARACTERIZATION OF *SYN* AND *ANTI* ^{99m}Tc ACBC•BAT AS POSSIBLE TUMOR IMAGING AGENTS

A.M. Giamis,[1] V.M. Camp,[1] Z. Zhang,[2] A.O. DePompei,[1] W. Yu,[1] R.J. Voll,[1] J.E. McConathy,[1] M.M. Goodman.[1]
[1]Division of Radiological Sciences, Emory University Hospital, Atlanta, GA, United States; [2]Department of Neurosurgery, Emory University Hospital, Atlanta, GA, United States.

The non-natural non-metabolized amino acids *syn* and *anti* 1-[N-[(1,1-dimethylethoxy)carbonyl]amino]-3-[N,N′-bis[2-[[(4-methoxyphenyl)methyl]thio]ethyl]-1,2-ethanediamine]-1-cyclobutanecarboxylic acid 1,1-dimethylethyl ester (**ACBC•BAT**) were synthesized, radiolabeled with ^{99m}Tc, deprotected and evaluated *in vitro* as potential SPECT tumor imaging agents against human tumor cell lines (A549 - lung, MDA MB468 - breast, DU145 - prostate, SKOV3 - ovarian, U87 - glial blastoma).

Starting from *syn* and *anti* 1-[N-[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxyoxy-1-cyclobutanecarboxylic acid methyl esters, prepared and reported earlier by this group (Shoup, *et al*, J. Labelled Cpd. Radiopharm **1999**, *42*, 215-25). The alcohol at C3 was converted to the corresponding trifluoromethanesulfonate which was subsequently displaced by N,N′-bis[2-[[(4-methoxyphenyl)methyl]thio]ethyl]-1,2-ethanediamine (**BAT**) to afford *syn* and *anti* 1-[N-[(1,1-dimethylethoxy)carbonyl]amino]-3-[N,N′-bis[2-[[(4-methoxyphenyl)methyl]thio]ethyl]-1,2-ethanediamine]-1-cyclobutanecarboxylic acid methyl esters (**ACBC•BAT**) as precursors to the radiolabeling.

Each of the protected amino acid diastereomers were dissolved in pH 7 phosphate buffer. A solution of sodium pertechnatate (^{99m}Tc) was added followed by saturated stannous tartrate solution. The pH of the resulting solution was adjusted to 12 by the addition of sodium hydroxide. After heating for as long as an hour, the pH was neutralized and the complex was extracted into dichloromethane. Removal of the BOC and methyl ester protecting groups was accomplished by reaction with trifluoroacetic acid. Chromatographic purification provided *syn*- and *anti*-^{99m}Tc **ACBC•BAT** complexes in high yield with radiochemical purity over 99% measured by radiometric TLC.



The results of cell assays were reported in the table below, with cell uptake from 1.3 to 20.9 % CPM / 1e6 cells (Table 1). These findings suggested that these *syn*- and *anti*- ^{99m}Tc **ACBC•BAT** amino acids enter these tumor cells *in vitro* primarily *via* ACS amino acid transport. These results support the candidacy of *syn*- and *anti*-^{99m}Tc **ACBC•BAT** as SPECT imaging agents. Research supported by Nihon Medi-Physics Co., Ltd.

Table 1		**A549**	**DU145**	**SKOV3**	**MDA MB468**	**U87**
Syn- 99mTc ACBC•BAT	**Control**	16.09	17.54	11.73	16.09	20.88
	BCH	15.00	16.01	11.81	15.00	19.99
	MeAIB	12.54	15.46	10.92	12.54	16.76
	ACS	9.72	9.94	6.90	9.72	12.61
Anti- 99mTc ACBC•BAT	**Control**	6.04	4.84	2.73	3.51	3.65
	BCH	6.41	4.67	2.03	3.13	2.84
	MeAIB	6.31	4.35	2.34	3.27	4.06
	ACS	4.83	3.01	1.30	2.21	1.83

Acknowledgement: Nihon Medi-Physics Co., Ltd. has license. Emory University may receive royalties.

Keywords: ACBC•BAT, Technetium-99m, Tumor Imaging

A ^{99m}Tc-TRICARBONYL COMPLEX WITH A CONJUGATE OF 2-PICOLYLAMINE-N-ACETIC ACID AND 2-(4-CHLOROPHENYL)TROPANE AS A POTENTIAL DOPAMINE TRANSPORTER TRACER AGENT

D. Kieffer, T. de Groot, H. Vanbilloen, B. Cleynhens, D. Rattat, G. Bormans, A. Verbruggen.
Laboratory of Radiopharmaceutical Chemistry, University Hospital Gasthuisberg, Leuven, Belgium.

Several ligands have been proposed for exploration of the dopaminergic transport system by PET and SPECT. ^{123}I-labelled ioflupane, also called ^{123}I-FP-CIT and commercially available under the name DatSCAN™, is an example of such a dopamine transporter tracer agent that now is being clinically used for SPECT in mainly the European countries. However, the suboptimal availability of the iodine-123 radioisotope is a limitation to the worldwide application of this tracer agent.

Also many ^{99m}Tc-complexes have already been proposed for imaging of DAT sites such as [^{99m}Tc]-TRODAT-1, [^{99m}Tc]-Technepine and a so-called [^{99m}Tc]-integrated-tropane-BAT, but the in vivo brain uptake and localization of the iodine-123 radiotracers is clearly superior. In recent years, also a few ^{99m}Tc-tricarbonyl tropanes have been described, in which a diligand is linked with a phenyltropane moiety. As far as information is available, their brain uptake was limited.

In this study, we developed a ^{99m}Tc-tricarbonyl labelled tropane in which a 4-chlorophenyltropane is linked to a tridendate ligand system, namely N-(2-picolylamine-N-acetic acid) which is known to form a stable and neutral complex with the $^{99m}Tc(CO)_3^+$ core. The new tracer agent was characterized using LC-MS and its biological characteristics were evaluated.

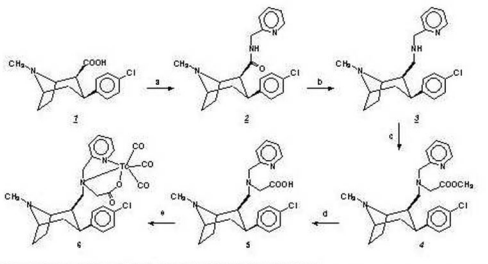
The aryltropane ligand was synthesized as outlined in Scheme 1 starting from 3b-(4-chlorophenyl)-N-methyl tropane-2b-carboxylic acid (**1**). The structure of the ligand was confirmed by NMR and mass spectrometry.

For radiolabelling, the precursor *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared using an Isolink kit and then reacted with the ligand (**5**) or its ester precursor (**4**) at 70 °C in phosphate buffer 0.5 M (pH 4, 7, 9 or 11) for 20 min. HPLC analysis of the labeling mixtures showed in each of the tested reaction conditions the formation of two main radiochemical species with a retention time of 15.3 min and 16.0 min, respectively. Optimal labeling yields (>85 %) were obtained at pH 7, even when the ester intermediate (**4**) was used as starting material.

Radio-LC-MS analysis of the reaction mixture showed that these two compounds were most probably two isomers, as they had the same mass (theoretical mass 596.0768 Da, found 596.0801 Da) which corresponds to the calculated mass of compound (**6**). Most probably, coordination of the tricarbonyl core of **5** is exclusively tridentate (via the acid function, the ternary amine and the pyridine nitrogen).

Biodistribution of the two HPLC isolated isomers was studied in normal mice at 2 and 60 min post injection. Most importantly, none of these radiolabelled compounds showed brain uptake. In addition, after incubation of the new ^{99m}Tc complexes with brain slices from healthy mice followed by autoradiographic examination, no significant binding to the striatum could be detected.

This indicates that the newly developed ^{99m}Tc-tricarbonyl tropane-PAA derivatives do not bind to the dopamine transporter and are not suitable as potential DAT tracer agents.



Scheme 1. Preparation of the conjugate and its labeling with ^{99m}Tc.
(a) oxalyl chloride, 2-aminopicoline, Et_3N, RT (93%); (b) BH_3.THF 1 M, reflux (90%); c) $BrCH_2COOMe$, NEt_3, MeOH RT (54%); (d) 1 M NaOH, RT 60 min; (e) phosphate buffer, $[^{99m}Tc(CO)_3(OH_2)_3]^+$, 70 °C 20 min

Keywords: Technetium-99m, Tricarbonyl, Dopamine

PREPARATION AND EVALUATION OF INDIRECTLY ^{99m}Tc-LABELED ANTI-HUMAN GASTRIC CANCER MONOCLONAL ANTIBODY 3H11

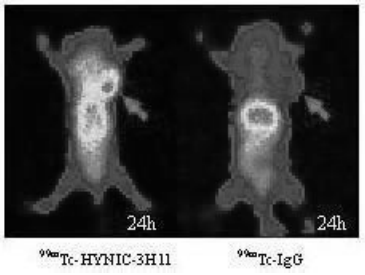
B. Jia,[1] Z. Liu,[1] H. Zhao,[1] Z. Yang,[2] F. Wang.[1]
[1]Medical Isotopes Research Center, Peking University, Beijing, China; [2]Nuclear Medicine, School of Clinical Oncology, Peking University, Beijing, China.

Gastric cancer is one of the most devastating diseases with the higher mortality in China. Most of patients are in processive stage when they have the first physical examination, and not sensitive to radiotherapy and chemotherapy. The radical operation for the patients is not satisfactory either. So it is necessary to seek new means for the early diagnosis and therapy of patients. The radioimmunoimaging of directly labeled anti-human gastric cancer monoclonal antibody (mAb) 3H11 with ^{131}I and ^{99m}Tc was reported. Indirect labeling of ^{99m}Tc with S-hydrazinonicotinamide (HYNIC) as a bifunctional chelating agent is of excellent *in vitro* stability and tumor targeting than direct labeling of ^{99m}Tc.

A HYNIC solution was added to a mAb 3H11 solution, leading to a final molar ratio of 8:1 (HYNIC/mAb 3H11). The reaction mixture was stirred at 4 centigrade for 5 h. The HYNIC-3H11 conjugate was dialyzed in 20 mM Citrate Buffer (containing 100 mM NaCl), pH 7.4 and pH 5.2 respectively, twice for 8 h. ELISA result revealed that no significant change of immunoreactivity was observed after conjugating. 0.2 mg of HYNIC-3H11 conjugate was labeled with 1110MBq ^{99m}Tc using tricine as a coligand at room temperature for 30 min. The labeling yield was more than 90%, and the radiochemistry purity was more than 99% after purification by Sephadex G-25 PD-10 column. The specific activity of final product was 3700MBq/mg mAb 3H11.

The stability of ^{99m}Tc-HYNIC-3H11 was monitored in saline and serum at 37 centigrade during 48 h period. In addition, diethylenetriamine pentaacetic acid (DTPA) and cysteine challenge assays were performed. The preparation showed a good stability.

The biodistribution in BALB/c nude mice with human gastric cancer M85 cells and the eliminated parameters from blood pool in BALB/c mice were determined. Pattern of blood clearance of ^{99m}Tc-HYNIC-3H11 was defined as two-compartment model, with $T_{1/2a}$ and $T_{1/2b}$ calculated to be 1.5 h and 38.2 h, respectively. The biodistribution results showed that ^{99m}Tc-HYNIC-3H11 was well accumulated in tumor, and the radioactive ratios of tumor to blood, tumor to muscle and tumor to stomach were 1.9, 21.4 and 15.1 at 24 h postinjection. Comparing to control ^{99m}Tc-IgG, The clear images of xenografted tumors were obtained at 24 h postinjection. ^{99m}Tc-HYNIC-3H11 is promising for the early diagnosis of patients with gastric cancer.



Keywords: Technetium-99m Labeling, Monoclonal Antibody, Imaging

^{99m}Tc-LABELED STILBENES AS POTENTIAL IMAGING AGENTS FOR β-AMYLOID PLAQUES IN THE BRAIN

H.F. Kung, M.-P. Kung, C. Hou, Z.-P. Zhuang.
Radiology, University of Pennsylvania, Philadelphia, PA, United States.

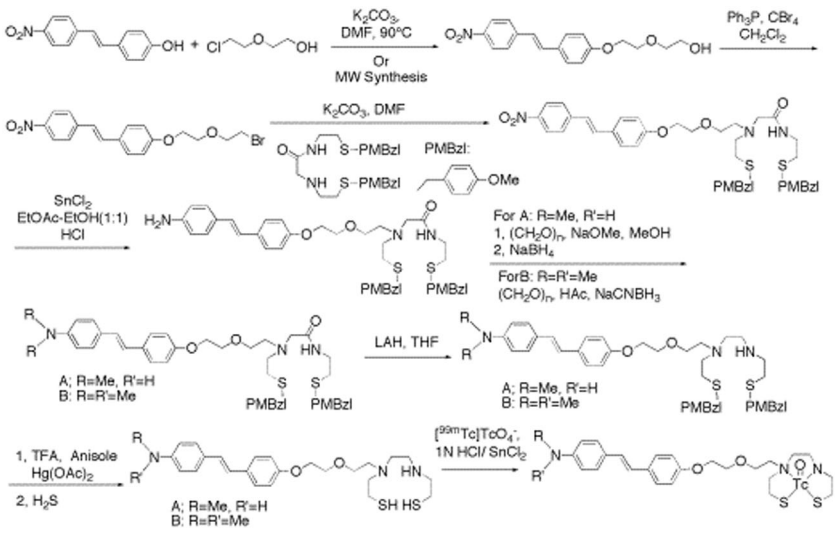
Development of SPECT imaging agents based on Tc-99m targeting Abeta plaques is useful for diagnosis of Alzheimers disease (AD). A stilbene derivative, [^{11}C]SB-13, showing promise in detecting senile plaques present in AD patients has been reported previously1,2. Based on the 4-amino-stilbene core structure we have added substituted groups through which a chelating group, N2S2, was conjugated. We report herein a series of Tc-99m labeled stilbene derivative conjugated with a TcO[N2S2] core.

The syntheses of stilbenes containing a N2S2 chelating ligand are achieved by the scheme shown above. Lipophilic 99mTc stilbene complexes were successfully prepared and purified through HPLC. Preliminary results of in vitro labeling of brain sections from transgenic mice showed very promising plaque labeling. These 99mTc stilbene derivatives are warranted for further evaluations as potential imaging agents targeting amyloid plaques.

References:

(1) Verhoeff, N. P.; Wilson, A. A.; Takeshita, S.; Trop, L.; Hussey, D.; Singh, K.; Kung, H. F.; Kung, M.-P.; Houle, S. Am. J. Geriatr. Psychiatry 2004, 12, 584-595.

(2) Ono, M.; Wilson, A.; Nobrega, J.; Westaway, D.; Verhoeff, P.; Zhuang, Z. P.; Kung, M. P.; Kung, H. F. Nucl. Med. Biol. 2003, 30, 565-571.



Acknowledgement: supported by a grant from department of energy

Keywords: SPECT Imaging, Alzheimer's Disease, Brain Section Labeling

MULTIMERIC FORMS OF P-160, A PEPTIDE IDENTIFIED BY PHAGE DISPLAY

S. Kraemer,[1] V. Askoxylakis,[1] J. Zhang,[2] S. Zitzmann,[3] M. Eisenhut,[4] U. Haberkorn,[1] W. Mier.[1]
[1]Nuklearmedizin, Universitatsklinikum Heidelberg, Heidelberg, Germany; [2]Institute for Biological Sciences, National Research Council, Ottawa, Canada; [3]Klinische Kooperationseinheit Nuklearmedizin, Deutsches Krebsforschungszentrum, Heidelberg, Germany; [4]Radiopharmazeutische Chemie, Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Novel tumor specific peptides can be identified by the screening of phage display libraries. However, when applying the peptide identified problems arise because of the low enzymatic stability of small peptides. In contrast larger peptide conjugates are known to demonstrate a higher stability against peptidases. In analogy to the constructs used for immunization multivalent constructs are available by coupling of the monomers to a multivalent core such as keyhole limpet hemocyanin or serum albumin. As an alternative to peptide-protein conjugates multiple antigen peptides can be used. These consist of two or four copies of the peptide attached to a branched lysine core. These molecules ought to be large enough to lower the rate of degradation and might allow multiple interactions with several binding sites on one target cell.

A solid phase peptide synthesis (SPPS) method utilizing Fmoc-Lys(Fmoc)-OH building blocks was performed to synthesize the cores in a stepwise, head-to-tail approach. The linear peptide sequences VPWMEPAYQRFL (1) were assembled stepwise on these templates by standard Fmoc/t-buty/trityl protection chemistry to build up the full-length peptide chains. Finally the peptides were linked to 6-BOC-hydrazino-pyridine-3-carboxylic acid (BOC-HYNIC) using HATU 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate as the coupling reagent. Final side-chain removal and cleavage from the resin yielded the peptides that were finally isolated by reverse-phase HPLC.

Using the strategy described, the peptides were readily obtained. Problems were encountered for the modification with HYNIC, the deprotection step led to the formation of TFA-adducts which were difficult to be separated because of the large size of the conjugates. However, an improved deprotection protocol allowed the synthesis of HYNIC derivatives in good yields. A high labeling efficiency can be obtained in the labeling of the with 99m-Tc using Tricine as a coligand.

The stepwise synthesis approach gives access to defined and pure oligomeric neuroblastoma-binding peptides. However, extensive purification is required to obtain the purified constructs. It has to be considered that the constructs are 60-mers (tetramer) which is the limit of peptide size accessible by standard peptide synthesis. The determination of the pharmacokinetic properties of the oligomerized derivatives are currently on the way.

(1) Zhang J, Spring H, Schwab M.: Neuroblastoma tumor cell-binding peptides identified through random peptide phage display. Cancer Lett 2001 171 153-64.

Keywords: Tumor Targeting, Peptide, Phage Display

PRELIMINARY STUDY ON THE $^{99m}Tc(CO)_3$-PNP5 COMPLEX

J. Chu,[1] D. Kong,[2] B. Li,[2] X. Wang.[2]
[1]College of Science, Beijing University of Chemical Technology, Beijing, China; [2]Department of Chemistry, Beijing Normal University, Beijing, China.

Introduction

In recent years, many investigators focused on various ^{99m}Tc-carboxyl complexes[1]. Especially, the discovery of $^{99m}Tc(CO)_3$-MIBI accelerated the development of compounds of this type. The purpose of this study is to synthesize the $^{99m}Tc(CO)_3$-PNP5 complex and to study its biological characters and improved properties by adding Tween-80[2].

Methods

The ligand bis(dimethoxypropylphosphinoethyl)-ethoxyethylamine (PNP5) was synthesized by procedures reported elsewhere[3, 4]. The $^{99m}Tc(CO)_3$-PNP5 complex was prepared according to the following procedure: $Na^{99m}TcO_4$ (2.0mL, 37-370MBq) was added to a sealed vial full of carbon monoxide and containing 5.0mg of $NaBH_4$, 4.0mg of Na_2CO_3 and 15.0mg of potassium sodium tartrate. The mixture was heated at 100° for 15 min and then cooled to room temperature. To the resulting solution, 1.0mL phosphate buffer (0.050M, pH7.4) was added, and the pH was adjusted to 7.4 with dilute hydrochloric acid. And then, 0.50mg of PNP5 (dissolved in 0.10mL of ethanol) was added and the vial was heated at 100° for 15 min. The final labelling yield was analyzed by thin-layer chromatography (TLC) of polyamide film with acetonitrile as mobile phase. The stability of $^{99m}Tc(CO)_3$-PNP5 was studied at room temperature.

According to the above-mentioned method, 4.0mL solution of $^{99m}Tc(CO)_3$-PNP5 (7.4MBq/mL) was prepared. 2.0mL of it was added to a vial containing 50mg Tween-80 and the mixture was labelled as $^{99m}Tc(CO)_3$-PNP5(T), and the residual solution was labelled as $^{99m}Tc(CO)_3$-PNP5 all the same. In vivo distribution studies of them were carried out in ICR mice contrastively. 0.1mL of the tracer solution was injected via a tail vein. The mice were sacrificed at 5, 30 and 60 min post-injection. The organs of interest were collected, weighed and measured for radioactivity.

Results and discussion

The structure of PNP5 was determined by IR, NMR and MS. $^{99m}Tc(CO)_3$-PNP5 was prepared through ligand exchange reaction with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ intermediate. Its labelling yield was more than 90%, which stayed over 90% after 6h. It indicated that $^{99m}Tc(CO)_3$-PNP5 was stable.

Results of biodistribution in mice showed that $^{99m}Tc(CO)_3$-PNP5 possess high initial liver uptake and low uptake in blood and lungs. The activity in liver washed out rapidly and the heart-to-liver ratio (0.43, 1.40 and 3.76 at 5, 30 and 60 min post-injection respectively) rose obviously. However, its myocardial uptake (8.63, 6.42 and 7.07 %ID/g at 5, 30 and 60 min post-injection respectively) was disappointing. Compared with $^{99m}Tc(CO)_3$-PNP5, $^{99m}Tc(CO)_3$-PNP5(T) had higher myocardial uptake (16.04, 13.72 and 9.39 %ID/g at 5, 30 and 60 min post-injection respectively), lower liver uptake, and better heart-to-liver ratio (1.29, 3.80 and 4.00 at 5, 30 and 60 min post-injection respectively). It indicated that the biological properties of $^{99m}Tc(CO)_3$-PNP5 were improved obviously by adding Tween-80 in favor of myocardial imaging.

Conclusions

As a new compound, $^{99m}Tc(CO)_3$-PNP5 has been prepared successfully. The biodistribution in mice showed that its properties are unideal and could be improved obviously by adding Tween-80. $^{99m}Tc(CO)_3$-PNP5(T) indicated its potential as a myocardial perfusion imaging agent and further study is needed.

References

1. Waibel R, et al. Nature Biotechnology, 17:897(1999)
2. Zhang Xianzhong. Doctoral Dissertation, Chem. Dept. Beijing Norm. Univ., (2002)
3. Boschi A, et al. Nucl. Med. Commun., 23:689(2002)
4. Bianchini C, et al. Organometallics, 14:1489(1995)

Keywords: Technetium-99m tricarbonyl-PNP5, Biodistribution, Tween-80

PREPARATION, CHARACTERIZATION AND BIODISTRIBUTION OF A NEW TECHNETIUM-99m COMPLEX WITH HYNIC(TRICINE)-AMDP

L. Liu,[1] H. Guo,[1] Z. Tang,[1,2] J. Lu,[1,2] X. Wang.[1,2]
[1]Department of Chemistry, Beijing Normal University, Beijing, China; [2]Key Laboratory of Radiopharmaceuticals, Ministry of Education, Beijing, China.

Objective: ^{99m}Tc-MDP is the most widely used bone imaging agent today but it is still not perfect. Its soft tissue uptake is too high and the time between injection and imaging is a little longer. In order to solve this problem, many efforts have been done recently[1]. We have designed and synthesized a new Technetium-99m complex by using HYNIC (hydrazinopyridine-3-carboxylic acid) as a bifunctional chelating agent, so the bisphosphonate part of the complex can be freed and its affinity to the bone can been improved.

Methods: AMDPE (aminobisphosphonate tetraethyl ester) and BocHYNIC were synthesized respectively according to Darko Kantoci[2] and Abrams[3]. After another two steps we got the target product HYNIC-AMDP. The preparation of ^{99m}Tc (tricine)-HYNIC-AMDP complex was as follow: In a 10ml vial containing 1mg of HYNIC-AMDP, 2mg of Sodium citrate, 50mg of tricine and 0.1mg of $SnCl_2 \cdot 2H_2O$, the pH value was 7-8. Then $^{99m}TcO_4^-$(1-2mL, 1.85~185MBq) was added to that vial and it was kept at R.T. for 0.5h.The radiochemical purity (RCP) was evaluated by TLC on polyamide film with saline as mobile phase. The lipophilicity was also determined.

Biodistribution studies were carried out in mice. ^{99m}Tc complex (~725kBq,0.1mL) was injected through the tail vein. The mice were sacrificed at different time and the organs of interested were weighted and counted in a NaI well-type gcounter.

Results: The RCP of ^{99m}Tc-complexes was ranged between 90%~98%. The logP (partition coefficient)values for the ^{99m}Tc (tricine)-HYNIC-AMDP and ^{99m}Tc-AMDP were –2.77 and –1.53, respectively.The biodistribution of these two complexes in mice are shown in Table 1.Compared with ^{99m}Tc-AMDP, the target complex shows high bone uptake, good retension and far more blood clearance speed. And it also exhibited much higher T/NT ratios,especially the bone/muscle and bone/blood ratios.

Conclusion: The novel ^{99m}Tc(tricine)-HYNIC-AMDP complex has been successfully synthesized and characterized. The complex showed favorable biological properties, suggesting that it could be potentially useful as a bone imaging agent.

Acknowledgements: The work was financially supported by Ministry of Science and Technology of the People's Republic of China. The authors thank Dr.Yang Jianquan for his help with the animal experiments.

Reference

1.Kristin Verbeke. et al. Bioconjugate Chem. 13: 16-22 (2002)
2.Darko kantoci. et al. Synth Comm. 26: 2037-2043 (1996)
3.Michael J. Abrams et al. J Nucl Med. 31: 2022-2028 (1990)

Biodistribution of 99mTc-complexes in mice (%ID/g, n=3)

Tissues	COmplex	Post-injection time/hour			
		0.5h	1.0h	2.0h	3.0h
Bone	A*	44.18±8.58	35.08±4.92	36.71±4.99	32.41±4.28
	B*	9.05±1.65	7.59±1.60	6.87±0.19	5.54±1.23
Blood	A	0.70±0.01	0.38±0.02	0.21±0.03	0.18±0.01
	B	1.59±0.79	0.35±0.08	0.18±0.02	0.53±0.17
Muscle	A	0.55±0.06	0.68±0.05	0.26±0.02	0.16±0.01
	B	0.28±0.10	0.18±0.09	0.33±0.15	0.08±0.01
Liver	A	2.19±0.39	2.28±0.44	1.36±0.39	1.30±0.12
	B	26.93±2.81	31.89±2.82	29.06±1.81	28.53±1.97
Lungs	A	0.56±0.19	0.41±0.09	0.29±0.04	0.21±0.10
	B	10.55±2.90	6.08±1.27	1.53±0.33	1.40±0.38
Kideys	A	3.24±0.56	1.76±0.30	1.12±0.20	0.59±0.15
	B	8.45±2.48	7.33±2.30	9.13±1.87	8.22±1.99

*A: 99mTc (tricine)-HYNIC-AMDP, *B: 99mTc-AMDP

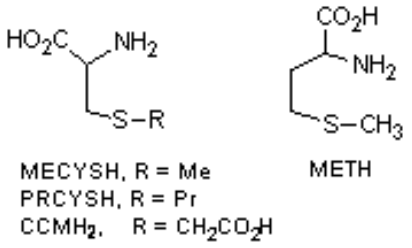
Keywords: Technetium-99m Complex, HYNIC-AMDP, Bone Imaging Agent

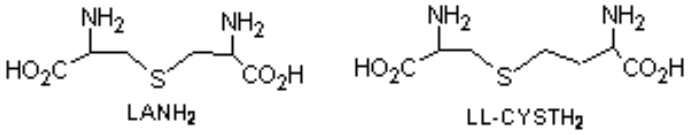
DEVELOPMENT OF RENAL RADIOPHARMAEUTICLS OF DIPEPTIDES CONTAINING THIOETHER FUNCTION WITH THE $\{^{99m}Tc(CO)_3\}^+$ CORE

H. He,[1] M. Lipowska,[1] X. Xu,[1] L.G. Marzilli,[2] A. Taylor.[1]
[1]Radiology, Emory University, Atlanta, GA, United States; [2]Chemistry, Louisiana State University, Baton Rouge, LA, United States.

Parallel studies of the preparation of Re and ^{99m}Tc agents are useful in interpreting the nature of ^{99m}Tc radiopharmaceuticals. We have been interested in developing radiopharmaceuticals possessing high renal clearance. We have explored $^{99m}Tc(CO)_3$ complexes of amino acids with a thioether function as renal function imaging agents. We first examined the coordination of $Re(CO)_3$ with MECYSH, PRCYSH and METH (see Chart). These ligands were shown to coordinate tridentately to the $\{Re(CO)_3\}^+$ core via the thioether, the primary amine and the carboxylate. We also synthesized and characterized $[Re(CO)_3(CCMH)]$ ($CCMH_2$ = S-carboxymethyl-L-cysteine, see Chart), which has a dangling α-carboxylic instead of SCH_2CO_2H. The biodistribution of $[^{99m}Tc(CO)_3(CCM)]^-$ was evaluated and the agent showed high renal excretion.[1]

One of the best renal agents, $^{99m}Tc(V)$-EC, has a $HO_2C\text{-}CH_2\text{-}NH\text{-}Tc\text{-}NH\text{-}CH_2\text{-}CO_2H$ sequence with two dangling carboxylate groups. Here we introduce two dipeptide ligands, lanthionine (3,3′-thiodialanine, $LANH_2$) and L-cystathionine (S-(2-amino-2-carboxyethyl)-L-homocysteine, LL-$CYSTH_2$) (see Chart), also with two carboxylate functions. We will present the synthesis and structural characterization of $[Re(CO)_3(LANH_2)]$ and $[Re(CO)_3(LL\text{-}CYSTH_2)]$. Both $LANH_2$ and LL-$CYSTH_2$ coordinate via two amino and thioether groups, leaving two carboxylate groups dangling. Meso-$LANH_2$ was found to form two isomers due to the presence of the prochiral sulfur. One major isomer of $[Re(CO)_3(LL\text{-}CYSTH_2)]$ was isolated in the reaction of LL-$CYSTH_2$ with the $[Re(CO)_3(H_2O)_3]^+$precursor. Biodistribution studies of $[^{99m}Tc(CO)_3$ (LL-CYST)$]^-$ will be presented and compare with those of $[^{99m}Tc(CO)_3(CCM)]^-$ and $[^{99m}Tc(CO)_3$ (LAN)$]^-$.





Reference:
1. Lipowska M, He H, Malveaux E, Xu X, Marzilli L G, Taylor A, *J Nucl Med* 2004; **45:** 461P.

Keywords: Technetium and Rhenium Tricarbonyl, Renal Function, Dipeptide

THE STUDY OF ^{99m}Tc MOEDP AS A NEW BONE IMAGING AGENT

L. Liu,[1] X. Wang.[1,2]
[1]Department of Chemistry, Beijing Normal University, Beijing, China; [2]Key Laboratory of Radiopharmaceuticals, Ministry of Education, Beijing, China.

Objective: After intravenous administration, ^{99m}Tc-diphosphonates show a fairly rapid blood clearance, the uptake in bone is almost 1h p.i., but clearance from soft tissue occurs more slowly[1]. A considerable time interval is required after injection of the radiopharmaceutical before bone scanning can be started. This necessary delay between injection and imaging is a serious inconvenience for the nuclear medicine personnel and for the patients, especially for children. In the present study, we synthesized the new diphosphonates ligand 2-methoxylethylenebisphosphonate (MOEDP) which was used for the ^{99m}Tc labeling for development of a new bone imaging agent.

Methods: The initial material methylenebis(phosphonate)ester was synthesized according to Osmo [2]. According to the base-catalyzed reaction of a methylenebis(phosphonate)ester with paraformaldehyde and the deprotection of the diphosphonate ester by bromotrimethylsilicane[3], the new ligand MOEDP was obtained. Direct labeling of MOEDP with ^{99m}Tc was performed by addition of 1mL of eluate containing 1.85-185MBq ^{99m}Tc to a labeling vial which containing 5mg of MOEDP, 2mg of Sodium citrate, and 0.1mg of $SnCl_2 \cdot 2H_2O$, pH6~7, and it was kept at room temperature for 15 min. The final radiochemical purity (RCP) was analyzed by thin-layer chromatography (TLC) of polyamide film with saline as mobile phase. The stability of ^{99m}Tc-MOEDP was studied at room temperature.

In vivo distribution studies of ^{99m}Tc-MOEDP were carried out in ICR mice. The 0.1mL of the diluted tracer solution (7.4MBq/mL) was injected via a tail vein. The mice were sacrificed by decapitation at fixed time intervals and the organs of interested were weighted and counted in a NaI well-type γcounter.

Results: The structure of MOEDP was determined by ^{1}HNMR. The RCP of ^{99m}Tc–MOEDP was more than 95%. The stability study of ^{99m}Tc–MOEDP indicated its radiochemistry purity (89.1%) was close to 90% after 6h.

The biodistribution of ^{99m}Tc–MOEDP were shown in Table 1. It demonstrated that ^{99m}Tc–MOEDP had the high bone uptake, and improving with the time going, as well as the ratios between of bone and other organ.

Conclusion: The novel ^{99m}Tc-MOEDP complex has been successfully prepared and characterized. The biodistribution showed that ^{99m}Tc–MOEDP was a very promising bone imaging agent. Further studies were in progressing.

Acknowledgements: The work was financially supported by Ministry of Science and Technology of the People's Republic of China. The authors thank Dr. Yang Jianquan for his help with the animal experiments.

Reference

1.Fogelman I. Eur. J. Nucl. Med., 7: 506 (1982)
2.Osmo E.O., Hormi, et al. Synth Comm., 20: 1865-1867(1990)
3.Charles R., Degenhardt, Don C. Burdsall. J. Org. Chem., 51: 3488-3490(1986)

Biodistribution of 99mTc–MOEDP in ICR mice(%ID/g, n=3)

Tissues	Post-injection time/hour				
	0.5	1.0	1.5	2.0	3.0
Bone	17.52±1.62	16.48±1.19	15.29±3.94	26.55±4.40	18.26±4.32
Blood	0.80±0.05	0.31±0.04	0.26±0.07	0.18±0.01	0.14±0.02
Muscle	0.38±0.13	0.43±0.05	0.14±0.04	0.09±0.01	0.07±0.01
Liver	1.42±0.11	0.71±0.13	0.53±0.06	0.31±0.01	0.11±0.02
Lung	0.85±0.02	0.45±0.04	0.28±0.08	0.24±0.03	0.20±0.01
Kidney	3.85±0.70	2.39±0.21	1.94±0.19	1.87±0.21	0.50±0.08

Keywords: Bone Imaging Agent, Technetium-99m-MOEDP, 2-Methoxylethylenebisphosphonate

INFLUENCE OF ARYL SUBSTITUENTS ON THE REACTIVITY OF ^{99m}Tc COMPLEXES OF TETRAFLUORPHENOL-3,5-DIAMINOBENZOATE DERIVATIVES

O. Calderon Sanchez,[1] C. Bauer,[2] A. Mohammed,[1] U. Haberkorn,[1] W. Mier,[1] M. Eisenhut.[2]
[1]Department of Nuclear Medicine, University of Heidelberg, Heidelberg, Germany; [2]Department of Radiopharmaceutical Chemistry, German Cancer Research Center, Heidelberg, Germany.

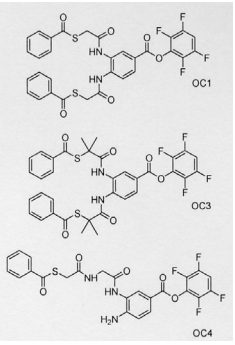
Objectives: The labeling of proteins with ^{99m}Tc provides a technology often applied for tumor imaging. Several methods have been developed to improve the binding of complexed ^{99m}Tc to antibodies, among which postconjugation labeling plays a preferential role. Compared with this route, the complexation of ligands before being attached to e.g. lysine residues of antibodies is not as attractive because of the time involved. The advantage of precomplexation, however, resides in the minimal ligand load. High ligand/antibody ratios necessary for good labeling yields may lead to reduction of the antibody's binding affinity. This work aims at the comparison of ^{99m}Tc-complexation and complex-conjugation characteristics of 2,3,5,6-tetrafluorphenyl esters (TFP) of *N-(S*-benzoylthioacetyl)glycylglycyl-*p*-aminobenzoic acid (OC2) with ligands deriving from TFP-3,5-diaminobenzoates (OC1, OC3 and OC4).

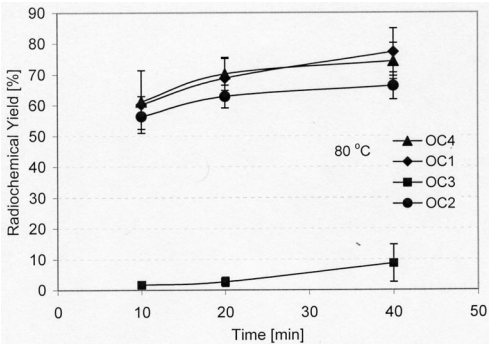
Methods: The ligands summarized in the formula scheme (Fig. 1) were synthesized, characterized for their identity and complexed with ^{99m}Tc using the Sn(II)gluconate method. The radiochemical yields and proof of TFP-ester stability were analyzed with HPLC under time and temperature control. Time course performance and pH-dependencies of the conjugation reactions were subsequently carried out with the anti EGF-receptor antibody (EMD72000, Merck KGaA). The complexation and conjugation results were compared with ^{99m}Tc-OC2 (1).

Results: The complexation yields depended strongly on temperature and duration of reaction: heating OC1 at 60, 80 und 100°C for 10 min yielded 33, 60, 67% RCY and 51, 61, 65% RCY for OC4. Figure 2 summarizes the complexation yields obtained with OC1-4 at 80°C. OC1 and OC4 showed little better complexations yields than OC2. However, ^{99m}Tc complexation yields of OC3, the tetramethyl derivative of OC1, was found far below the the other ligands. The HPLC purified, ligand free ^{99m}Tc complexes of OC1 and OC4 were conjugated at pH 10 with the anti EGF-receptor antibody. After 20 min reaction time at 30°C they showed labeling yields of 14 (OC1) and 7% (OC4); OC2 for comparison gave 50%. Raise of temperature to 40°C increased the yield to 28 und 10%, respectively.

Conclusion: Compared with OC2 the ^{99m}Tc complexation of OC1 and OC4 was improved. The low complexation yield plexes ^{99m}Tc-OC1 and -OC4 were rather inactive in respect to their conjugation efficiency. The influence of the meta positioned R-CO-NH substituent which is participating in ^{99m}Tc-complex formation may explain the reduction of reactivity at the electrophilic center of the activated ester function.

1. O. Calderon Sanchez et al. Bioconjugate Chemistry 2003;14:1209-1213.





Keywords: Technetium-99m Complex Formation, Activated Ester Reactivity, Influence of aryl Substituents

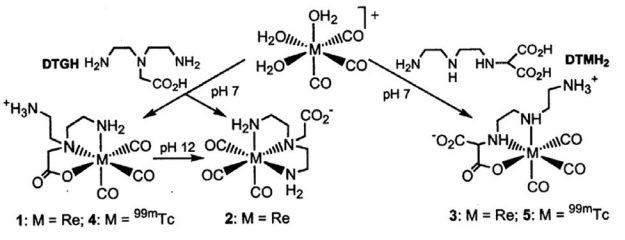
DEVELOPMENT OF BIFUNCTIONAL CHELATES WITH ALIPHATIC AMINE DONORS FOR THE COMPLEXATION WITH THE $\{M(CO)_3\}^+$ CORE (M = Re, ^{99m}Tc)

M. Lipowska,[1] H. He,[1] X. Xu,[1] L.G. Marzilli,[2] A. Taylor.[1]
[1]Radiology, Emory University, Atlanta, GA, United States; [2]Chemistry, Louisiana State University, Baton Rouge, LA, United States.

The labeling of small bioactive peptides with ^{99m}Tc has been a major goal in nuclear medicine.[13] Our goal is to develop a ^{99m}Tc tracer for renal imaging. There are two strategies for ^{99m}Tc radiopharmaceutical design: integrated or bifunctional approaches. The integrated approach involves direct labeling with incorporation of ^{99m}Tc into the structure of peptide. In contrast, the bifunctional approach allows retention of the receptor binding affinity by connecting a radionuclide to the targeting molecule through bifunctional chelate (BFC). Careful selection of BFC, in which one functionality is covalently attached to the targeting molecules while the other stabilizes the nuclide, is required to assure stability and sufficient labeling with high yield. We studied two polyamines, *N,N*-bis(2-aminoethyl)glycine (DTGH) and diethylenetriamine-*N*-malonic acid ($DTMH_2$), that coordinate tridentately to the $\{M(CO)_3\}^+$ core (M = Re, ^{99m}Tc). These ligands have the diethylenetriamine backbone and one or two auxiliary acetate donors. The combination of the terminal amine group and an acetate group allows insertion of the chelate at any position along the peptide sequence, or alternatively allows facile conjugation of biomolecules through formation of amide links.

All products of the reaction of the $[Re(CO)_3(H_2O)_3]^+$ [/sup]precursor were isolated and characterized by elemental analysis and NMR spectroscopic and X-ray methods. The symmetrical DTGH ligand gave two products: one with a $Re(CO)_3(N_2O)$ (**1**) coordination sphere and the other with a $Re(CO)_3(N_3)$ (**2**) coordination sphere, in which all three amine groups are donors and the $CH_2CO_2^-$ moiety is the pendant arm. The $Re(CO)_3(N_2O)$ product undergoes complete conversion to $Re(CO)_3(N_3)$ at high pH. The isomers reach equilibrium (**1:2**, 30:70) after 5 days at pH 7. $DTMH_2$ gave only one major product, $Re(CO)_3$(DTMH) (**3**). X-ray diffraction methods revealed that, in the distorted octahedral, DTMH coordinates with an N_2O donor set (the two neutral secondary amines and one terminal carboxylate group). The second carboxylate group and the $-(CH_2)_2NH_2$ group are dangling. The pendant free primary amino group can be regarded as mimicking the amino group of lysine. At physiological pH this amino group is protonated and thus positively charged.

DTGH was labeled with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, but because of the HPLC separation conditions (pH 2.5) only one product, $^{99m}Tc(CO)_3$(DTG) (**4**), the analog of **1**, was isolated. $^{99m}Tc(CO)_3$(DTMH) (**5**) was also synthesized and both **4 and 5** were evaluated in rats. Biodistribution studies showed that **5** had higher renal excretion (activity in the urine as a percent of ^{131}I-orthoiodohippurate (OIH is an internal control) was 83 ± 4% at 60 min) than for **4** (73% ± 5%), similar kidney retention at 60 min (**4**: 3.0%, **5**: 3.4% (of injected dose)), and reduced liver uptake (**5**: 4.3%, **4**: 13%). The higher renal excretion and reduced hepatic uptake of **5** can be attributed to an additional free carboxylic acid. This features merits further testing of $^{99m}Tc(CO)_3$(DTMH) in humans.



This work was supported by NIH Grant DK38842

1. Liu, S.; Edwards, D. S. *Chem. Rev.* **1999**, *99*, 2235-2268.
2. Fichna, J.; Janecka, A. *Biocojugate Chem.* **2003**, *14*, 3-17.
3. Stephenson, K.A.; Zubieta, J.; Banerjee, S.R. *Bioconjugate Chem.* **2004**, *15*, 128-136.

Keywords: Technetium(I) and Rhenium(I), Tricarbonyl Complexes, Amino Acids

PREPARATION, CHARACTERIZATION AND BIODISTRIBUTION OF ^{99m}Tc EDP AS A NEW BONE IMAGING AGENT

L. Liu,[1] X. Wang.[1,2]

[1]Department of Chemistry, Beijing Normal University, Beijing, China; [2]Key Laboratory of Radiopharmaceuticals, Ministry of Education, Beijing, China.

Objective:Radionuclide bone imaging is the most common clinical investigation in nuclear medicine. At present the complexes of ^{99m}Tc-MDP[1] and ^{99m}Tc-HMDP are the most widely used tracer agents for clinical radioisotopic bone scanning, whereas they have a number of suboptimal properties. In this study, we synthesized the new ligand Ethenylidenebisphosphonate(EDP)and made the ^{99m}Tc labeling and biodistribution study.

Methods: The initial material methylenebis(phosphonate)ester was synthesized according to published procedures[2]. The new ligand EDP was synthesized successfully by the following procedures. The procedures involved the base-catalyzed reaction of a methylenebis(phosphonate) ester with paraformaldehyde, acid-catalyzed elimination of methanol and the deprotection of the diphosphonate ester by bromotrimethylsilicane[3].

Labeling of EDP with ^{99m}Tc was performed as followed: To a labeling vial containing 5mg of EDP,2mg of sodium citrate and o.1mg of $SnCl_2 \cdot 2H_2O$ 1mL of eluate containing 1.85-185 MBq ^{99m}Tc was added and it was kept at room temperature for 15 min.The radiochemical purity (RCP) of the final labeling product was determined by thin-layer chromatography (TLC) on polyamide film with saline as mobile phase. The stability of ^{99m}Tc-MOEDP was studied at room temperature.

In vivo distribution studies of ^{99m}Tc-EDP were carried out in ICR mice. The ^{99m}Tc-EDP was injected through the tail vein in a volume of 0.1 mL (0.74 MBq). The mice were killed by decapitation at fixed time intervals and the tissues harvested were weighted and counted in a NaI well-type γcounter.

Results: The structure of EDP was determined by ^{1}HNMR. The RCP of ^{99m}Tc–EDP was more than 95%. The stability study of ^{99m}Tc–EDP indicated its radiochemistry purity was more than 90% after 6h.

The biodistribution of ^{99m}Tc–EDP in ICR mice were shown in Table 1. In mice, ^{99m}Tc–EDP showed very promising characteristics with the high femur uptake and the high ratios of femur to other organs which improving with the time going, and rapid blood clearance.

Conclusion: The novel ^{99m}Tc-EDP complex has been successfully prepared and characterized. On the basis of ^{99m}Tc–EDP provided these excellent biological properties, we thought it was worth to further studied to develop it as a new bone imaging agents. Further studies on ^{99m}Tc–EDP were being conducted and would be reported in subsequent course.

Acknowledgements: The work was financially supported by Ministry of Science and Technology of the People's Republic of China. The authors thank Dr. Yang Jianquan for his help with the animal experiments.

Reference

1. Suubrahamian, G., McAfee, et al. J. Nucl. Med., 16: 744-755(1975)

2.Osmo E.O., Hormi, et al. Synth Comm., 20: 1865-1867(1990)

3.Charles R., Degenhardt, Don C. Burdsall. J. Org. Chem., 51: 3488-3490(1986)

Table 1. Biodistribution of 99mTc–EDP in ICR mice(%ID/g, n=3)

Tissues	Post-injection time/hour				
	0.5	1.0	1.5	2.0	3.0
Bone	13.30±1.22	14.82±1.23	16.28±1.22	22.20±3.80	19.64±3.78
Blood	0.61±0.08	0.28±0.06	0.21±0.01	0.19±0.03	0.16±0.01
Muscle	0.42±0.02	0.11±0.03	0.06±0.01	0.04±0.01	0.03±0.01
Liver	1.07±0.21	0.61±0.12	0.27±0.01	0.14±0.04	0.13±0.02
Lungs	0.54±0.06	0.36±0.08	0.22±0.05	0.18±0.08	0.16±0.02
Kidney	2.39±0.39	1.75±0.44	1.55±0.31	1.36±0.24	1.09±0.12

Keywords: Bone Imaging Agent, Technetium-99m EDP, Ethenylidenebisphsphonate

^{99m}Tc-LABELLED BIGUANIDE DERIVATIVES: CHEMICAL SPECIATION MODELLING OF AND EVALUATION IN VERVETS

J.M. Wagener,[1] J.R. Zeevaart,[1] M. Neves,[2] M.C. Costa,[3] C. Rodrigues,[3] M.K. Dithebe,[1] O. Knoesen,[1] W.K. Louw.[1]

[1]Radiochemistry, Necsa, Pretoria, South Africa; [2]de Quimica, ITN, Sacavem, Portugal; [3]Tecnologia de Industrias Quimicas, INETI, Lisboa, Portugal.

^{99m}Tc-DMSA is known to be a safe and effective agent for static renal imaging. It however has a long uptake time, which is a limiting factor in diagnostic procedures and also leads to a relatively high radiation dose to patients. There is a constant search for possible new renal imaging agents with good resolution, kidney/liver contrast and low radiation dose to all organs. Biguanides are used for therapy of non-insulin-dependent diabetes mellitus with the liver as target organ. They inhibit gluconeogenesis in the liver and kidney. Biguanide derivatives such as, ^{99m}Tc-Dimethyl Biguanide (DMBG), -Biuret (BIU), -2-Imino-4-Thiobiuret (ITB) and -Carboxy-Biguanide (CBIG), show on theoretical grounds, potential as alternative kidney-imaging agents instead of ^{99m}Tc-DMSA. ^{99m}Tc-DMBG and ^{99m}Tc-DMSA were reported to have distinct renal and urinary excretion profiles in rabbits. ^{99m}Tc-DMBG was cleared faster and showed better contrast in the whole-body images for the same acquisition times [1], therefore ^{99m}Tc-DMBG has promising practical and dosimetric features as an alternative renal imaging agent to ^{99m}Tc-DMSA.

The formation constants of the ligands and important blood plasma metal ions complexes have been determined potentiometrically using ESTA (Equilibrium Simulation by Titration Analysis). These results were added to the ECCLES (Evaluation of Constituent Concentration in Large Equilibrium Systems) database, a thermodynamic model of blood plasma, to calculate the *in vivo* speciation of the complexes and predict the possible biodistribution of the metal ions [2, 3,]. ^{99m}Tc systems cannot be modelled due to its large variety of oxidation states. The ligands, DMBG, BIU, ITB and CBIG, have nitrogen donor atoms, which are expected to complex ^{99m}Tc while the blood plasma model shows no complexation of the ligand by the metal ions in blood plasma. It is thus deducted that ^{99m}Tc will remain complexed to the ligands in blood plasma. [2,3,4]. Therefore no or little side effects relating to mobilisation of blood plasma metal ions once the radiopharmaceutical has been administered are expected. It is also expected that these ligands will clear rapidly from the blood plasma because of the increase in lipophilicity of the biguanide derivatives. Pre-clinical tests were done to conclude the study and confirm the theoretical results comparing the performance of the new ligands to the gold standard, DMSA. The vervets showed kidney and gallbladder uptake for the labeled biguanide derivates.

References:

1 Neves M, *et al*, Nucl.Med.Biol., **26**, 79-83 (1999)

2 Dithebe M, "Metal ion speciation in blood plasma incorporating 2-imino-4-thiobiuret as a potential kidney imaging agent in nuclear medicine," MSc thesis University of the North West, 2000.

3 Edwin M, "Biuret and biguanide as potential kidney imaging agents," MSc thesis, University of the North West, 2000.

4 Neves M *et al*, Nucl.Med.Biol., **26**, 85-89 (1999)

Keywords: Kidney Agents, Imaging, Biguanide

A NEW RADIO-TLC METHOD FOR THE QUANTIFICATION OF [^{18}F]ALTANSERIN IN HUMAN PLASMA

D. Bier, M.H. Holschbach, M. Lang, H.H. Coenen.
Forschungszentrum Juelich GmbH, Institut fuer Nuklearchemie, Juelich, Germany.

The 5-HT$_{2A}$ antagonist [^{18}F]altanserin (**1**), is routinely used in human PET studies. One problem associated with its quantification in plasma is the fast blood clearance of the tracer resulting in low radioactivity plasma levels. Classical HPLC analyses as described in the literature [1, 2] are lengthy and not suitable for routine use, standard radio-TLC procedures result in poor counting statistics.

The aim of the study was to develop a fast, robust and reliable radio-TLC method allowing precise quantification of unchanged **1** in human plasma during PET examinations. The recovery of **1** from plasma was examined using a set of 10 different deproteinizing extraction solvents. High recoveries were found for acetonitrile (107 ± 10%) and dichloromethane (DCM, 96 ± 5%). Various chromatographic phases (Si60, RP18, Cellulose, ALOX-N, ALOX-60) and eluents were examined. ALOX-N in combination with an eluent consisting of chloroform (95) / 2-propanol (5) / trifluoroacetic acid (0.2) (v / v / v) and chamber saturation gave a good separation of **1** (R_f 0.77) from the putative metabolite [^{18}F]altanserinol (**2**) (R_f 0.5). Since n.c.a. **1** is a labile compound that rapidly decomposes on most polar solid phases, carrier of altanserin (2.5 µg / 100 µL sample) was added. The instability of n.c.a. **1** could also be shown in incubation experiments with chromatographic phases; this has potential impact on solid phase purification steps during the radiosynthesis of n.c.a. **1**. Furthermore decomposition of n.c.a. **1** was observed in inorganic buffers (phosphate-, borate buffer pH 7.4). In contrast n.c.a. **1** proved to be stable for at least three hours in pooled human serum (n = 10).

Finally a routine procedure using DCM as an extraction solvent was adopted. For each time point human plasma (300 µL) was spiked with carrier altanserin, extracted with DCM (150 µL), and an aliquot of the DCM layer (100 µL) was spotted onto a ALOX-N TLC plate with a Hamilton syringe which was slowly emptied under a gentle flow of warm air, giving a spot with a diameter of max. 5 mm containing the radioactivity of 200 µL of the parent plasma sample. For each time point plasma aliquots (20 µL) of unextracted plasma were applied to the upper part of the TLC plate as references. After development of the plate, the radioactive distribution was read out using an InstantImager. Calculation of % unchanged **1** (%A) was performed according to the formula:

% A = (100 x AC) / (10 x PC)

AC = counts corresponding to **1**; PC = counts corresponding to the 20 µL plasma sample

Besides its simplicity, speed and reliability this procedure has the following two advantages: first, the obtained data need not be corrected for decay and second, a plasma activity / time curve is obtained without additional measurements.

[1] Lemaire C. et al., J. Nucl. Med. 32, 2266-2272 [1991]

[2] Tan P.Z. et al., Nuclear Med. Biol. 26, 601-608 [1999].

Keywords: Fluorine-18 Altanserin, Plasma Analysis, Radio-TLC

STUDY ON TWO ^{99m}Tc-NITRIDE HETEROCOMPLEXES AS NOVEL POTENTIAL MYOCARDIAL PERFUSION IMAGING AGENTS

J. Chu,[1] B. Li,[2] D. Kong,[2] J. Zhang,[2] X. Wang.[2]
[1]College of Science, Beijing University of Chemical Technology, Beijing, China; [2]Department of Chemistry, Beijing Normal University, Beijing, China.

Introduction

Recent studies showed that ^{99m}Tc-nitride heterocomplexes, [^{99m}TcN(PNP)(DTC)]$^+$, containing a terminal technetium-nitrogen multiple bond coordinated to one diphosphine ligand (PNP) and one dithiocarbamate ligand (DTC) have potential to be used as new myocardial perfusion imaging agents[1,2]. This article reported the preparation and biodistribution of two new ^{99m}Tc-nitride heterocomplexes towards the goal of developing an ideal myocardial perfusion imaging agent.

Methods

The ligand bis(dimethoxypropylphosphinoethyl)-ethoxyethylamine (PNP5) was synthesized by procedures reported elsewhere[2,3]. The DTC ligands, N-ethoxy-N-ethyl dithiocarbamate (NOEt) and N-diethyl dithiocarbamate (DEDC), were obtained by published procedures[2]. [^{99m}TcN(PNP5)(DTC)]$^+$ complexes were prepared according to the procedure reported in [1,2]. The radiochemical purity (RCP) was analyzed by thin-layer chromatography (TLC) of polyamide film with mixture of acetone and saline (V/V=1:6) as mobile phase.

In vivo distribution studies of [^{99m}TcN(PNP5)(NOEt)]$^+$ and [^{99m}TcN(PNP5)(DEDC)]$^+$ were carried out in ICR mice. 0.1mL of the diluted tracer solution (7.4MBq/mL) was injected via a tail vein. The mice were sacrificed at 5, 30 and 60 min post-injection. The organs of interest were collected, weighed and measured for radioactivity.

Based on the results in mice, characters of [^{99m}TcN(PNP5)(NOEt)]$^+$ were further studied in dog. After 1.0mL solution of it (370MBq) was injected via a tongue vein, time-activity curves (0-40 min) for tissue uptake and myocardial imaging at 40, 60, 90 and 120 min were investigated in dog with SPECT apparatus.

Results and discussion

The structure of ligands was determined by IR, NMR and MS. [^{99m}TcN(PNP5)(NOEt)]$^+$ and [^{99m}TcN(PNP5)(DEDC)]$^+$ were prepared through ligand exchange reaction and their RCPs were more than 90%. Results of biodistribution in mice indicated that [^{99m}TcN(PNP5)(NOEt)]$^+$ and [^{99m}TcN(PNP5)(DEDC)]$^+$ possessed similar properties. They had high myocardial uptake (23.94±1.21 and 24.47±2.70 %ID/g at 5 min post-injection respectively), good heart retention and low uptake in liver, lungs and blood. They were cleared from non-target tissues rapidly and the ratios of target to non-target were excellent. For example, the ratios of heart to liver were 1.61, 3.06 and 5.89 for [^{99m}TcN(PNP5)(NOEt)]$^+$ and 2.64, 2.89 and 4.35 for [^{99m}TcN(PNP5)(DEDC)]$^+$ at 5, 30 and 60 min post-injection respectively. Studies in dog showed that [^{99m}TcN(PNP5)(NOEt)]$^+$ possessed high heart uptake and low uptake in lungs. The activity in liver washed out rapidly and the ratio of heart to liver can exceed 1.0 at 22min post-injection. Moreover, clear SPECT images obtained at 40, 60, 90 and 120 min indicated [^{99m}TcN(PNP5)(NOEt)]$^+$ may be used for myocardial imaging.

Conclusions

New heterocomplexes [^{99m}TcN(PNP5)(NOEt)]$^+$ and [^{99m}TcN(PNP5)(DEDC)]$^+$ have been prepared successfully. The biodistribution showed that they possess favorable biologic properties and could be promising heart perfusion tracers.

Acknowledgements

The authors thank Prof. Wang Jincheng and his colleagues of Beijing Anzhen Hospital for their help with the SPECT imaging.

References

1. Boschi A, et al. Nucl. Med. Commun., 23:689(2002)
2. Boschi A, et al. J. Nucl. Med., 44:806(2003)
3. Bianchini C, et al. Organometallics, 14:1489(1995)

Keywords: Technetium-99m-Nitride Heterocomplex, Myocardial Perfusion Imaging Agent, Biodistribution