Technetium Complex of Tricine: Useful Precursor for the ^{99m}Tc Labelling of Hydrazino Nicotinamide Modified Proteins. LARSEN, S.K†, CALDWELL, G.‡, HIGGINS III, J.D.†, ABRAMS, M.J.†, and SOLOMON, H.F.‡ †Johnson Matthey, Inc., West Chester, PA 19380; ‡R.W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477.

The high biological specificity of certain macromolecules, such as an antibody, has been used to target specific in vivo sites for diagnostic imaging or therapy with radioisotopes. The labelling of macromolecules with ^{99m}Tc can be classified into three principal methods: direct labelling, bifunctional chelates, and preformed chelates. The method currently preferred in our research is to reduce pertechnetate ion in the presence of a chelating precursor to form a labile ^{99m}Tc-precursor complex which in turn reacts with the metal binding group of a bifunctionally modified protein to form a ^{99m}Tc-protein conjugate. Previously, we have described the use of ^{99m}Tc-glucoheptonate to radiolabel polyclonal IgG which has been modified with hydrazinonicotinamide (SHNH) groups[1]. However, this requires a 60 minute incubation period and specific activities <25 mCi/mg to achieve radiolabelling yields >90%. We now present the use and characterization of the polyhydroxyamino acid tricine complex of technetium as a more efficient and facile labelling precursor for ^{99m}Tc labelling of SHNH modified proteins.

Tricine, tris(hydroxymethyl)methylglycine, and analogues thereof, can be formulated in aqueous solutions, pH 6-8, with stannous chloride reducing agent for the spontaneous formation of ^{99m}Tc-precursors. Analysis for the formation of "Tc-tricine" is performed on ITLC-SG strips similarly to ^{99m}Tc-glucoheptonate analysis, using saline for the quantitation of TcSncolloid at the origin and methylethylketone for the quantitation of pertechnetate at the solvent front. Solutions of "Tc-tricine" (36 mg/ml precursor, 50 μg/ml stannous chloride, pH 6.0), under similar conditions to ^{99m}Tc-glucoheptonate, radiolabelled SHNH modified IgG >90% in minutes at room temperature. Fig. 1. Additionally, solutions of "Tc-tricine" can achieve >90% radiolabelling of IgG-SHNH at specific activities >150 mCi/mg protein as compared to Tc-glucoheptonate. Fig. 2. The utility of quantitative radiolabelling at high specific activities is more important in the labelling of SHNH modified small molecules. Lastly, "Tc-tricine" radiolabelled IgG-SHNH shows the same *in vitro* stability as Tc-glucoheptonate radiolabelled IgG-SHNH.

The chemical nature of the "Tc-tricine" complex formed and the resulting hydrazine moieties have been partially characterized by traditional spectroscopies. We have formulated the "Tc-tricine" as the monoanionic species $TcO(tricine)_2^-$. At the carrier added level "Tc-tricine" can be synthesized by the addition of excess TBA·tricine to $^{99}TcOCl_4$·TBA in MeOH or by the addition of a stannous/tricine solution to $^{99}TcO4^-$ in water. The resulting golden brown solutions are chromatographically and electrophoretically equivalent to carrier free ^{99m}Tc-tricine. Solutions of "Tc-tricine" are unstable to oxidation in the absence of excess tricine or reducing agent to stabilize them. This complicates their analysis which must be done in situ. The carrier added species ^{99}Tctricine·TBA analyzed by negative mode FAB-MS shows a molecular ion peak at m/z 469 which has been assigned the formula $TcO(tricine)_2^-$. Additionally, ^{13}C NMR of ^{99}Tctricine·TBA in D_2O shows clearly a downfield shift for all ligand resonances except the carboxylate carbon, indicative of ligand coordination through the amine and one hydroxymethyl group but suggesting the carboxylate oxygens are uncoordinated.

Reaction of ^{99}Tctricine-TBA with two equivalents of SHNH-phenylalanine-methyl ester results in a deep burgundy solution which can be cleaned up on a C18 solid phase extraction column. The resulting solid analyzed by negative mode FAB-MS produced molecular ion peaks at m/z 587 assigned as Tctricine(SHNHPheMe)$^-$ and at m/z 901 assigned as Tctricine(SHNHPheMe)$_2^-$.

The nature of the Tc-tricine interaction as well as the Tc-hydrazine interaction in these conjugates will be discussed. Attempts to crystallize these compounds have not been succesful to date.

1) D.A.Schwartz, M.J. Abrams, M.M. Hauser, F.E. Gaul, S.K. Larsen, D. Rauh and J.A. Zubieta: Bioconjugate Chem. 2:333-336 (1991).

Fig. 1 Technetium Labelling of IgG-SHNH

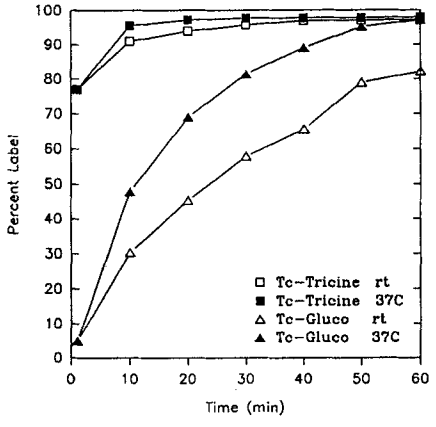
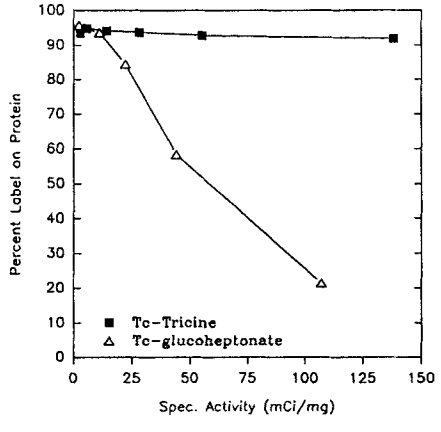


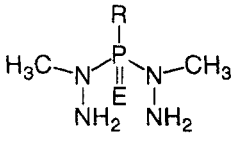
Fig. 2 Protein Radiolabelling of Two Tc-Precursors vs. Specific Activity.

37C; 60 min.; IgG-SHNH

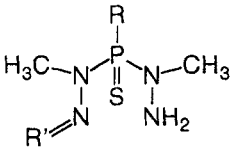


Unsymetrical Phosphorus Hydrazide Chelating Systems as Potential BFCA's for Proteins and Peptides, VOLKERT, W.A. HOFFMAN, T.J., CORLIJA, M., SINGH, P.R., JIMENEZ, H., KATTI, K.K., KATTI, K.V., LUSIAK, P., KETRING, A.R., HOLMES, R.A. Research Service, H.S. Truman Memorial Veterans Hospital and the Departments of Radiology, Chemistry and Research Reactor, University of Missouri, Columbia, MO 65211

Development of BFCAs that are capable of selectively chelating ^{99m}Tc under mild conditions after conjugation is an attractive approach for ^{99m}Tc labeling of large or small biomolecules (e.g., MAbs or small peptide receptor agents, respectively). Schwartz, et al [1] have demonstrated the advantages of using this strategy using aryl-hydrazido-based BFCAs. Recent studies in our laboratory demonstrate the potential of bis-hydrazido-phosphorus (BHP) ligands to form unique and chemically flexible BFCAs that complex ^{99m}Tc and other metals in high yields [2-3]. One type of BHP derivative (based on the monohydrazidophosphinesulfide [MHPS] backbone) was prepared as a potential BFCA and shown to complex ^{99m}Tc.



BHP (E=O,S)



MHPS

The following two MHPS ligands were synthesized, characterized and used in this study: **MHPS I** (R = Ph and R' = -CHPhCOOH) and **MHPS II** (R = Ph and R' = -CHPhCH = CHCOOH). These two model MHPS ligands were synthesized by Schiff base coupling of the corresponding p-carboxybenzaldehyde. Following the reaction of phenyl-BHP-sulfide (PBHPS) with the corresponding aldehyde (in 1:1 molar reactant mixtures) in abs. EtOH, the MHPS ligands were separated from the two other BHPS impurities (i.e., unreacted PBHPS and the di-Schiff base PBHPS) by column chromatographic procedures (e.g., **MHPS 1** was separated by flash chromatography (Silica gel) using a solvent system of 6:4:1 hexane:ethyl acetate:methanol). The purity of the MHPS ligands were ascertained by ^{1}H, ^{31}P, ^{13}C NMR, IR spectroscopy and C, H and N analysis (e.g, for **MHPS 1**; Anal. Calcd for $C_{16}H_{19}N_4O_2PS$: C, 53.03; H, 5.28; N, 15.46. Found: C, 53.44; H, 5.45; N, 15.31).

^{99m}Tc-MHPS complexation was accomplished by two approaches: 1) by moderate HCl acidification of solutions containing **MHPS I** or **MHPS II** and $^{99m}TcO_4^-$ or 2) by transchelation. ^{99m}Tc complexation in HCl in the absence of any other reducing agents (e.g., Sn II) was normally accomplished by mixing 0.2 ml 0.1 mg/ml **MHPS I** or **MHPS II** in EtOH with 0.2 ml of 0.1M HCl in 0.9% aqueous saline containing ≥ 10 mCi (370 MBq) $^{99m}TcO_4^-$ at 90-95°C for 15 min. The reversed phase HPLC chromatogram shown in Figure 1 shows that only one major hydrophobic ^{99m}Tc product is formed. Paper electrophoresis demonstrates that this complex is also neutral. No significant decomposition of either ^{99m}Tc-**MHPS I** or -**MHPS II** is observed for ≥24 hr in 0.1M bicarbonate buffer at pH 7-7.5 at room temperature. Complexation yields of ≥ 90% and specific activities ≥ 100 Ci/mmole were obtained. The structure of Tc-MHPS chelates are not known, however, they can be assumed to be similar to the neutral-lipophilic Re-BHPS and -MHPS chelates formed by the identical HCl acidification procedure. The structure of these Re complex involves coordination of the Re(V) mono-oxo

core via both hydrazido-N atoms and the S-atom to generate a complex with a 1:1 ligand:metal ratio [4].

Formation of ^{99m}Tc-**MHPS I** or **MHPS II** chelates is also accomplished by transchelation, similar to the approaches used for labeling DADT [5] or SHNH [1] BFCAs. Exchange labeling in non-aqueous solutions can be effected by using ^{99m}Tc-HMPAO. The HPLC chromatograms in Figure 2 demonstrate transmetallation of ^{99m}Tc from ^{99m}Tc-HMPAO to ^{99m}Tc-**MHPS II** (retention times; 8.5 min and 7.1 min respectively). The HPLC conditions used for these analyses were the same as described in Figure 1. In this study, 1 mg/ml of **MHPS II** was incubated with ^{99m}Tc-d,l-HMPAO dissolved in acetonitrile for 1 hr at room temperature producing >85% yields of ^{99m}Tc-**MHPS II**. Similar results were obtained using ^{99m}Tc-glucoheptonate (^{99m}Tc-GH) in aqueous solutions at pH 5.5-7 at an exchange rate that was approximately 4 fold slower than observed with ^{99m}Tc-HMPAO. However, recent studies with other water soluble weak ^{99m}Tc chelates showed that exchange rates in aqueous solutions could be accelerated markedly.

The -COOH group on the MHPS ligands facilitate conjugation of this BFCA or the "preformed" radionuclide chelates to biomolecules (e.g., peptides, antibodies, etc.). To demonstrate the feasibility of conjugating the uncomplexed ligand, the -COOH group on **MHPS II** was activated using isobutylchloroformate (IBCF). The IBCF-activated **MHPS II**, reacted with n-butylamine in acetonitrile, produced the n-butylamine conjugate in >80% yield. The C,H,N analytical data are consistent with the chemical constitution of this conjugate. In addition, ^{1}H NMR spectrum showed that the free hydrazido -NH_2 group (i.e., 4.2 ppm, w) in the MHPS ligand is not altered upon IBCF activation or following coupling to the primary amine. The ^{13}C-NMR the conjugation reaction caused resonance of the carboxyl group carbon (i.e., 171.2 ppm) to change to 164.5 ppm confirming formation of the amido group. Conjugation of "preformed" ^{99m}Tc-**MHPS I** to polyclonal IgG was also accomplished by IBCF activation. The activated ^{99m}Tc complex was added to 1.0 mg/ml of IgG at pH 9.2 and incubated for 30 min at room temperature. The conjugation yield was approximately 70%. Following separation of unconjugated ^{99m}Tc via size exclusion chromatography, the ^{99m}Tc-labeled IgG was shown to be stable in saline for ≥ 24 hr at pH 7.0.

The results of these studies demonstrate the potential of MHPS ligands for use as BFCAs for attaching ^{99m}Tc to biomolecules. The ability of using MHPS conjugates for labeling small receptor avid peptides at mildly acidic HCl solutions without the presence of any other reducing agent is particularly attractive. In addition, MHPS BFCAs provides opportunities for labeling small peptides or other molecules in non-aqueous environment. The ability to form ^{99m}Tc-MHPS chelates via transchelation also permits ^{99m}Tc labeling of MHPS conjugated biomolecules in either aqueous or non-aqueous systems, using appropriate donor chelates, under mild conditions.

References:

1. Schwartz DA, Abrams MJ. *Bioconj Chem* 2: 333, (1991).
2. Volkert WA, Singh PR, Katti KK, Ketring AR, Katti KV. *J Labelled Comp* XXXII, 15, (1993).
3. Katti KV, Singh PR, Barnes CL. *Inorg. Chem.* 31: 4588, (1992).
4. Katti KV, Singh PR, Volkert WA, Ketring AR, Katti KK. *Appl Radiat Isot* 43:1151 (1992).
5. Baidoo KE, Scheffel U, Lever SZ. *Cancer Res* 50:799s (1990).

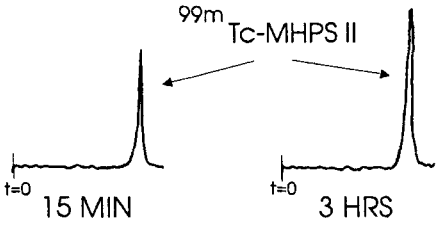


Figure 1. HPLC of ^{99m}Tc-**MPHS II** performed using a Hamilton PRP-1 column and a gradient elution system where mobile phase A = 100% 0.2M NaH_2PO_4 and B = 100% acetonitrile. The gradient was initiated after 2 min of 100% A (FR = 1 ml/min) and changed linearly to 100% B over a 5 min interval.

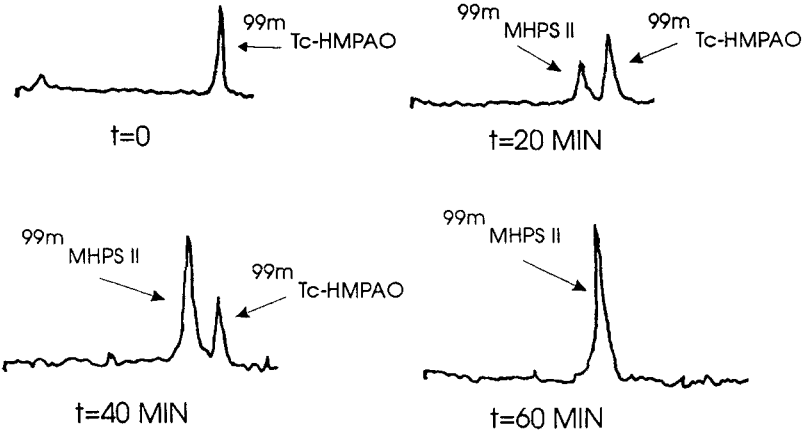


Figure 2. Sequence of reversed phase of HPLC chromatograms (same column and conditions for HPLC operations as described in Figure 1) demonstrating exchange of ^{99m}Tc from ^{99m}Tc-d,l-HMPAO to ^{99m}Tc-**MHPS II**.

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Incorporation of a Glucose Moiety in a Technetium-99m Nitrido Compound. UCCELLI, L; DUATTI, A*; PASQUALINI, R; MARCHI, A; GIGANTI, M; PIFFANELLI, A. Laboratorio di Medicina Nucleare, Università di Ferrara, 44100 Ferrara, Italy; Dipartimento di Chimica Fisica ed Inorganica, Università di Bologna, 40136 Bologna, Italy; CIS bio international, Gif-sur-Yvette, France.

Glucose is a fundamental substrate for brain and heart metabolism. It is reasonable to speculate that the incorporation of a sugar moiety into the chemical structure of a radiopharmaceutical, without affecting its biological activity, would allow the preparation of a tracer for monitoring metabolic pathways. This goal is particularly difficult to obtain with technetium-99m as a result of large perturbations exerted by this metal on the molecular structure of the labelled substrate, which generally undergoes a change in its biological properties. However, the preparation of a metabolic tracer labelled with Tc-99m is always desirable due to the ideal properties of this radionuclide.

We have previously reported the synthesis of a ^{99}Tc complex containing a Schiff-base derivative of glucose (1). The preparation at tracer level of the same compound gave poor results because of the decomposition of the glucose-derived ligand during the reaction with pertechnetate and stannous ions. In order to make as simple as possible the coordination of the sugar moiety to the technetium center, we investigated the possibility to incorporate glucose derivatives in Tc-99m radiopharmaceuticals by simple substitution reactions onto a preformed intermediate. The recent availability of an efficient synthesis of the stable $[Tc{\equiv}N]^{2+}$ core at tracer level (2), forced us to use this group as starting substrate for the synthesis of the final Tc-99m compound. The first attempt was carried out with the sodium salt of the derivative 1-thio-β-D-glucose having a deprotonated thiol group in place of a hydroxylic group in the sugar ring. This choice of the ligand was suggested by the high affinity of thiol groups towards the $[Tc{\equiv}N]^{2+}$ core. The overall reaction scheme was as follows:

a) $^{99m}TcO_4^- + H_2NN(CH_3)C(=S)SCH_3 + HCl + [P(C_6H_4SO_3)_3]Na_3 \rightarrow [^{99m}TcN]_{int}$

b) $[^{99m}TcN]_{int} + LHNa \rightarrow [^{99m}TcN(L)_2]^{2-}$
where LHNa = 1-thio-β-D-glucose sodium salt.

Step (a) was performed at 100 °C for 15 min, and led to the formation of the technetium nitrido intermediate $[^{99m}TcN]_{int}$. Step (b) was carried out at neutral pH and at room temperature. The formation of the substituted complex took place in 10 min and the final yield was 96 %. The product was characterized by ion exchange chromatography, electophoresis and by comparison with the Tc-99 analog prepared trhough the reaction:

$$^{99}TcNCl_2(PPh_3)_2 + LHNa \rightarrow [^{99}TcN(L)_2]^{2-}.$$

The ^{99}Tc complex possesses a square pyramidal geometry with an apical Tc≡N group and two bidentate ligand molecules coordinated in the basal plane through the negative thiol sulfur atom and the adjacent deprotonated hydroxylic oxygen atom of the ring. The resulting complex is dianionic.

Injection in rats of the ^{99m}Tc complex showed that it was eliminated predominantly through the kidneys, a behavior which should be expected for anionic species. However, although the clinical utility of this compound appears to be limited, these results indicate that the incorporation of a sugar derivative in a ^{99m}Tc radiopharmaceutical is feasible through a simple and efficient procedure.

References

1. Duatti A., Marchi A., Magon L., Deutsch E., Bertolasi V., Gilli G. - Inorg. Chem. 26: 2182(1987).
2. Pasqualini R., Bellande E., Comazzi V., Duatti A., Marchi A. - Appl. Radiat. Isot. 43: 1329(1992).

Novel Homo- and Heterodimeric Bis-bidentate $(NS)_2$ Technetium(V) Complexes. An Approach to Metal Complexes that Mimic Steroid Hormones.

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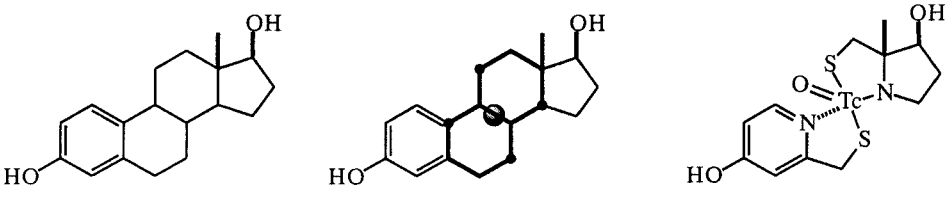
In our effort to develop ligands for steroid receptors labeled with ^{99m}Tc that can be used to image receptor-positive breast tumors, we have recently described the preparation of two tetradentate rhenium and technetium complexes conjugated to a synthetic progestin.[1,2] Despite having an overall size nearly twice that of a normal steroid, these complexes preserve nanomolar binding affinity for the progesterone receptor. However, the size of these conjugates appears to be a factor that impairs their receptor-selective distribution in vivo.

In conceiving of an approach to reduce the size of such metal labeled steroids, we have changed the design from a "conjugated" structure, in which the metal binding chelate is attached to a steroid molecule, to a more "integrated" arrangement, in which the metal binding unit is an integral core of a skeleton that mimics the molecular structure of the steroid in both size and shape.[3] An example of such a metal complex **2**, related to the steroid hormone estradiol **1**, is shown in Figure 1; the template overlay of the bisamine bisthiol metal complex and the decalin system of the B and C rings of the steroid, is shown in the middle structure (heteroatoms are shown as dark dots and the metal as a larger circles).

Figure 1. Design of Bis-bidentate Tc-Complexes Mimicking Estrogens.

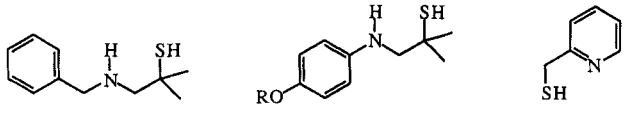


1 **2**

In contrast to most complexes labeled with ^{99m}Tc which are tetradentate, the bis-bidentate complex **2** has two separate bis-bidentate amino thiol components. In order to mimic natural compounds such as hormones, bis-bidentate Tc-99m ligands may be more useful than tetradentate ligands, and as they can be "fine tuned" to design desired complexes.[3] Key issues in the preparation of such bis-bidentate complexes would be: (1) Can a stable complex be formed from two bis-bidentate ligands? (2) Can a mixed or "hetero" complex be formed in preference to a "homo" complex? (3) Will the complex adopt a geometry with the matched heteroatoms in a *trans* configuration (vs *cis*)?

We have prepared a number of new complexes of oxotechnetium and oxorhenium from a variety of bis-bidentate ligands (Figure 2) and studied their structure and stability as models for hormone mimics such as **2**.

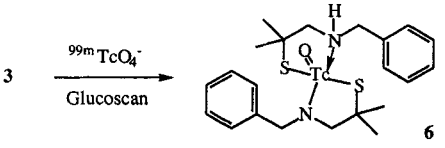
Figure 2. Bidentates Ligands.



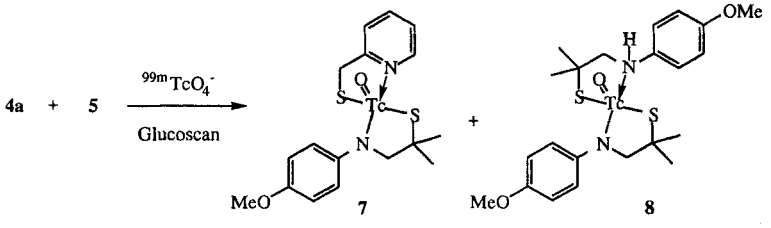
3 **4a**, R = CH_3 **4b**, R = H **5**

While several homodimeric bis-bidentate complexes have been reported in the literature, there has not been a report of the synthesis of a heterodimeric complex. We have prepared several homo- and heterodimeric complexes (Scheme 1 and 2). Most reactions provided good radiochemical yields of ca. 40-50% (isolated by HPLC, decay uncorrected), with high radiochemical purity.

Scheme 1. Typical Homodimeric Complexation Reaction.



Scheme 2. Typical Heterodimeric Complexation Reaction.



[^{99m}Tc]**7** : [^{99m}Tc]**8** = 50:1

In vivo Stability Study of ^{99m}Tc Complex:

Compound [^{99m}Tc]**7** was reasonably stable in vivo, while homodimeric compounds [^{99m}Tc]**6** and [^{99m}Tc]**8** were very stable in organic solvent, but quite unstable in 20% EtOH/saline solution. Compound [^{99m}Tc]**7** as a 20% EtOH/saline solution was used to carry out in vivo stability studies in rats (Figure 3), with 20% of [^{99m}Tc]**7** remaining intact in the blood at 2 h post injection.

Figure 3. Percent of Heterodimeric [99mTc]**7** Remaining Unmetabolized in vivo in Blood.

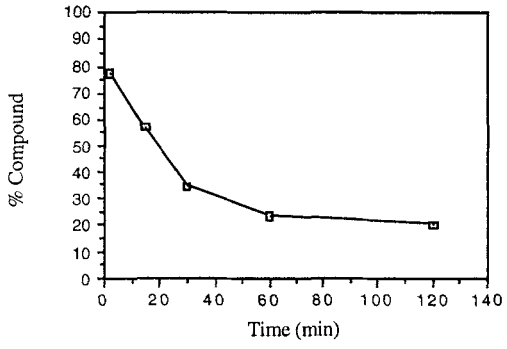


Table 1. Biodistribution of Heterodimeric Bis-bidentate in Sprague Dawley Rats (150 g, n = 5) Following Intervenous Injection.

	% Injected Dose Per Gram of Tissue				
	2 min	15 min	30 min	60 min	120 min
Blood	0.68 ± 0.09	0.32 ± 0.03	0.21 ± 0.04	0.20 ± 0.02	0.19 ± 0.00
Lung	4.23 ± 2.53	0.83 ± 0.15	0.43 ± 0.05	0.34 ± 0.06	0.22 ± 0.03
Liver	4.12 ± 0.61	3.69 ± 0.52	2.99 ± 0.79	3.31 ± 0.43	2.89 ± 0.32
Kidney	3.12 ± 0.51	1.29 ± 0.18	1.07 ± 0.22	1.59 ± 0.09	1.78 ± 0.22
Muscle	0.36 ± 0.10	0.38 ± 0.08	0.26 ± 0.03	0.17 ± 0.04	0.11 ± 0.04
Fat	0.24 ± 0.15	0.51 ± 0.20	0.60 ± 0.13	1.07 ± 0.37	1.13 ± 0.21
Heart	3.28 ± 0.82	0.72 ± 0.07	0.39 ± 0.07	0.31 ± 0.02	0.15 ± 0.01
Brain	1.35 ± 0.19	0.62 ± 0.06	0.28 ± 0.06	0.18 ± 0.02	0.13 ± 0.01

Conclusion.

This work indicates that some bis-bidentate ^{99m}Tc complexes can be prepared in high yield and have the potential to be radiopharmaceuticals. It is noteworthy that the heterodimeric bis-bidentate [^{99m}Tc]**7** shows high initial uptake in rat heart and brain (Table I).

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References

1. a) DiZio, J. P.; Fiaschi, R.; Davison, A.; Jones, A. G.; Katzenellenbogen, J. A. *Bioconjugate Chem.* **1991**, *2*, 353. b) DiZio, J. P.; Anderson, C. J.; Davison, A.; Ehrhardt, G. J.; Carlson, K. E.; Welch, M. J.; Katzenellenbogen, J. A. *J. Nucl. Med.* **1992**, *33*, 558.
2. O'Neil, J. O.; Anderson, C. J.; Carlson, K. E.; Welch, M. J.; Katzenellenbogen, J. A. *J. Nucl. Med.* **1993**, *34*, 18p.
3. Chi, D. Y.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1993**, submitted.

Chemistry of the First Class of Brain Imaging Agents Containing the Tc≡N Multiple Bond.

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Technetium complexes containing the Tc≡N triple bond have been recently introduced into the field of diagnostic nuclear medicine after the advent of a new, improved synthesis of the $[Tc(V)\equiv N]^{2+}$ group at tracer level, and in sterile and apyrogen conditions (1, 2). The application of this synthetic procedure to the preparation of Tc-99m nitrido radiopharmaceuticals with dithiocarbamate ligands, $[R(R')NCS_2]^-$, has led further to discover that the resulting neutral, disubstituted complexes, $^{99m}TcN[R(R')NCS_2]_2$, localize selectively in the myocardium of several animal species and of humans (3, 4). It was found that the heart uptake and clearance of these radiopharmaceuticals were strongly affected by the nature of the lateral groups R and R' bound to the $>NCS_2$ moiety. In particular, derivatives having lateral acyclic alkyl groups were found to exhibit the highest myocardial uptakes, while the introduction of lateral alkoxy groups gave rise to faster washouts from the heart.

We investigated the effect on the biodistribution properties of these complexes occurring after the introduction of ester groups on the ligand side chain. The synthesis of the final complexes was carried out as outlined in the following scheme:

a) $^{99m}TcO_4^- + H_2NN(CH_3)C(=S)SCH_3 + HCl + [P(C_6H_4SO_3)_3]Na_3 \rightarrow [^{99m}TcN]_{int}$;

b) $[^{99m}TcN]_{int} + R(R')NCS_2Na \rightarrow {}^{99m}TcN[R(R')NCS_2]_2$
where R = Me, Et, and R' = $CH_2C(=O)OH$;

c) $^{99m}TcN[R(R')NCS_2]_2 + CH_3I \rightarrow {}^{99m}TcN[R(R'')NCS_2]_2$
where R'' = $-CH_2C(=O)OCH_3$.

The overall reaction proceeded through the first formation of a prereduced technetium nitrido intermediate (a), which was successively used to afford the disubstituted complex $^{99m}TcN[R(R')NCS_2]_2$ having two lateral carboxylate groups (b); the diester derivative was finally obtained by *in situ* esterification of the two carboxylate groups with methyl iodide. The resulting diester product was purified by ion exchange chromatography in order to separate it from the unreacted dicarboxylate complex and the monoester derivative. The radiochemical purity of the diester complexes, checked by TLC analysis, was always over 95 %, and their *in vitro* stability greater than 4 hours.

The molecular structure of the complexes $^{99m}TcN[R(R'')NCS_2]_2$ [R'' = $-CH_2C(=O)OCH_3$] was established by comparison with the ^{99}Tc analogs characterized by IR, ^1H NMR and mass spectra. The Tc atom lies in a square pyramidal arrangement of ligands, with an apical Tc≡N bond and two dithiocarbamate ligands coordinated in the basal plane through the four sulfur atoms of the two $-C(=S)S^-$ groups. The complexes possess a vanishing net charge.

The biological distribution properties of the diester complexes were surprising when compared with the distributions of the analogous bis(dithiocarbamato) nitrido Tc(V) derivatives having lateral alkyl or alkoxy groups. Injection in Cynomologus monkeys showed that the diester compounds are retained into the cerebral region yielding very contrasted images similar to those obtained with ^{99m}Tc-HMPAO. ROI analysis gave high values of brain uptake, retention time and blood/brain ratio. No residual heart uptake was observed with these derivatives. In contrast, distribution studies in rats showed that the diester complexes did not localize in the brain of these animals, but remained associated with the blood pool probably through the linkage to plasma proteins. This fact could be attributed to the hydrolysis of the two ester groups occurring in rats, which causes their rapid conversion to the corresponding carboxylic groups as reported for the complex ^{99m}Tc-ECD.

The results presented here suggest that the basic molecular building block $TcNS_4$, composed by the $[Tc{\equiv}N]^{2+}$ core bound to four sulfur atoms in a square pyramidal geometry, plays a role in the chemistry of technetium nitrido complexes similar to that held by the $TcON_2S_2$ unit in the chemistry of technetium oxo complexes (5). In fact, the great stability of the $TcNS_4$ arrangement towards oxidation-reduction and substitution reactions allows the facile modification of the side-chain functional groups by both ligand variation and *in situ* reactions onto preformed complexes. This fact would prove of great importance for the development of technetium radiopharmaceuticals capable to interact with specific biological substrates.

References

1. Duatti A., Marchi A. and Pasqualini R. - J. Chem. Soc., Dalton Trans. 3729(1990).
2. Pasqualini R., Bellande E., Comazzi V., Duatti A. and Marchi A. - Appl. Radiat. Isot. 43: 1329(1992).
3. Pasqualini R. and Duatti A. - J. Chem. Soc., Chem. Commun. 1354(1992).
4. Duatti A., Marchi A., Pasqualini R., Comazzi V. and Bellande E. J. Nucl. Med. 32: 925(1991).
5. Dewanjee M.K. - Semin. Nucl. Med. 20: 5(1990).

Preparation and Evaluation of Tc-99m complexes of Aromatic Bridged Amine-amide-thiol-thioether N_2S_2 Ligands

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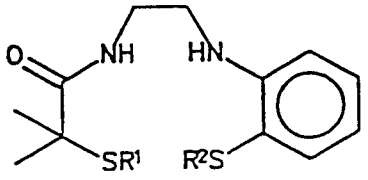
Tc-99m Complexes of several bis(amine)-bis(thiol) (N_2S_2) ligands have been prepared and evaluated as brain imaging agent (1,2,3,4). In our search for a Tc-99m labeled brain perfusion imaging agent, we investigated a new class of chelating ligands based on an amine-amide-thiol-thioether core (I). These compounds are similar to amide-thiol-thioether ligands reported previously (5) but differ in that they possess an aromatic ring bridging a nitrogen and sulfur atom, and contain an amine and an amide functionality. We reported here the synthesis and biodistribution of the Tc-99m complexes of a series of S-substituted derivatives.

The ligands were synthesized using 2-S-(4-methoxybenzylthio)-2-methylpropanoic acid as the starting material. It was converted to the acyl chloride and condensed with glycine to give 2-N-(2-S-(4-methoxybenzylthio)2-methyl-1-oxopropyl)aminoacetic acid (A). Removal of the methoxybenzyl protecting group gave 2-N-(2-mercapto-2-methyl-1-oxopropyl)aminoacetic acid. This intermediate was then reacted with a series of alkyl halides to give the desired thioethers. Condensation with 2-S-(triphenylmethylthio)aniline gave the diamides. Treatment with borane-THF complex successfully cleaved the triphenylmethyl from sulfur and the amide on the aromatic side of the molecule resulting in the desired final products (Ia-d). Ligands with the general structure (II) were synthesized by converting compound (A) to the acyl chloride and subsequent condensation with amines to give diamides. The aromatic amides were then selectively reduced with borane-THF complex.

The Tc-99m complexes were prepared by incubating the ligands with $Na^{99m}TcO_4$ in ethanol in the presence of $SnCl_2$ at room tempeture for 15 min. The product was purified through C-18 Sep-pak with 50% EtOH/saline solution. The radiochemical purity and yields of the complexes were determined by TLC and HPLC.

Biodistribution of these complexes was studied in ether anesthetized rats. Each rat was injected in the tail vein with 0.1 to 0.5 mCi of the Tc-99m labeled complex in 0.2 ml 50% EtOH/saline solution. Rats were sacrificed by exsanguination at predetermined time points, organs were removed and the radioactivity was counted. High brain uptake and rapid washout was observed for the aliphatic S-alkylated derivatives. On the other hand, the aromatic S-alkylated derivatives showed high brain uptake with good retention. The brain uptake of the S-methyl derivative (IIa) was 1.69% dose at 5 min and 1.74% at 30 min. Thus this class of ligand may be promising as a functional brain imaging agents.

References
1. Kung H.F. et al - J. Nucl Med. 25:326 (1984).
2. Lever S.Z., Burns, H.D., et al - J. Nucl. Med. 26:1287 (1985).
3. Vallabhajosula S., Zimmerman R.E., - J. Nucl. Med. 30:599 (1989).
4. McBride B.J., Baldwin R.M., et al - J. Med. Chem. 36:81 (1993).
5. Bryson N., Dewan J.C., - Inorg. Chem. 27:2154 (1988).



Ia: $R^1 = CH_3$, $R^2 = H$
Ib: $R^1 = CH_2CH_3$, $R^2 = H$
Ic: $R^1 = CH_2CH_2CH_3$, $R^2 = H$
Id: $R^1 = CH_2CH_2CH_2CH_3$, $R^2 = H$
IIa: $R^1 = H$, $R^2 = CH_3$
IIb: $R^1 = H$, $R^2 = CH_2CH_2CH_3$

Table I: Brain Uptake of Tc-complexes in Rats

Tc-99m Complexes	Time (min)	Brain uptake %dose	Brain/ Blood
Ia	5	2.33	4.23
	30	0.47	1.54
Ib	5	2.55	5.19
	30	0.51	1.36
Ic	5	1.55	4.34
	30	0.46	1.83
Id	5	1.43	3.11
	30	0.50	1.66
IIa	5	1.69	1.04
	30	1.73	1.17
IIb	5	1.27	1.21
	30	1.24	1.23

A New Approach to Cell Labeling with Technetium-99m: Synthesis, Cell Labeling and *in-vitro* Evaluation of Cellular Function.

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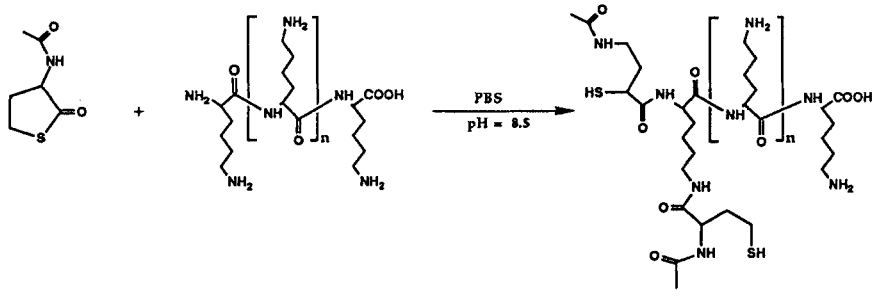
Current methods of human blood cell labeling in nuclear medicine rely on two radiopharmaceuticals, ^{111}In-oxine and ^{99m}Tc-HMPAO. The proposed mechanism for labeling cells with these neutral lipophilic agents is passive diffusion of the agent across the cell membrane and intracellular decomposition resulting in entrapment of the radiolabel within the cell.

The new approach described here is based on the mechanism of nonspecific adsorptive endocytosis.[1] Most mammalian cells have negatively charged cell surfaces, a property that may be exploited to radiolabel cells via an electrostatic attraction using soluble, radiolabeled polycationic species. If the cells possess endocytotic mechanisms, the adsorbed agent may be internalized and trapped within the cell.

In order to use polycationic poly-amino acids such as poly-Lysine for cell labeling with ^{99m}Tc, it is necessary to introduce strong ^{99m}Tc-chelating groups onto the poly-Lysine backbone. We have developed a mild and convenient method for the synthesis of such modified poly-Lysine (MPL) by conjugating thiol groups onto the poly-Lysine backbone utilizing N-acetyl homocysteine thiolactone. ^{99m}Tc labeling of MPL is conveniently achieved by transchelation with ^{99m}Tc-glucoheptonate resulting in an efficiency of >95%.

Evaluation of cell uptake of ^{99m}Tc-MPL by various human blood cell populations indicates maximal uptake of 60-80% by the various white cell subpopulations, and only 35% uptake with red cells (Table 1). Stability of the ^{99m}Tc-MPL radiolabel on cells incubated in serum at 37°C is good, with only 5% elution observed in 2 h. Since this mechanism for cell labeling is based on the initial electrostatic adsorption of the polycationic ^{99m}Tc-MPL on the negatively charged cell membrane, inhibition of the uptake is observed in the presence of polyanionic species such as heparin and serum albumin. Assessment of various functions of cells radiolabeled with ^{99m}Tc-MPL indicates that proliferative capacity in response to IL-2, cytolytic capacity, and adhesion functions are not altered by the radiolabeling procedure.

The method described should prove useful in the development of ^{99m}Tc-labeled blood cells not only for infection imaging but also for following immune cell traffic *in vivo*.



TL PL MPL

Table 1

Cells	Cell conc. (million/mL)	[99mTc-MPL] (μM)	% Uptake
Mixed Leukocytes	30	0.4	67.4 ± 3.5
PBMC	30	0.4	75±5
Granulocytes		0.5	74±2.8
RBCs	50	0.4	35±5

(1) Ryser H.J.P. and Shen W.C. (1986) Drug-polylysine conjugates: their potential for chemotherapy and for the study of endocytosis. In: Targeting Drugs and Synthetic Systems (Edited by Gregoriadis G., Senior J., and Poste G.), pp. 103-121. Plenum Press, New York.

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UPTAKE OF Tc-99m-SESTAMIBI BY STEREOTACTICALLY IMPLANTED 9L GLIOSARCOMA TUMOR IN THE RAT.

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There is considerable interest in the development of new radiopharmaceuticals for the differentiation of recurrent tumor from the effects of therapy (e.g., radiation necrosis). If these new radiopharmaceuticals are metabolic rather than simply perfusion agents, they also open up the possibility of using SPECT for tumor staging. Two agents that appear promising for this application are ^{99m}Tc-MIBI and ^{201}Tl. There are several reports of the uptake of ^{99m}Tc-MIBI by tumors (1), and the accumulation of ^{201}Tl in tumors was first described shortly after its introduction for the evaluation of myocardial perfusion (2). Both ^{99m}Tc-MIBI and ^{201}Tl are of particular interest for the evaluation of brain tumors because of their very low uptake by normal brain, and several clinical studies have suggested that ^{201}Tl might be of value in the staging of brain tumors (3-5). More recently, we have investigated the use of ^{99m}Tc-MIBI for the evaluation of pediatric brain tumors (6, 7). Despite this, there are remarkably few reports in which the biodistribution of these tracers has been measured in animal tumor models (8,9). In the only report of the biodistribution of ^{99m}Tc-MIBI in an animal tumor model, the tumor was implanted in the thigh (9). A model in which the tumor is stereotactically implanted in the brain should, however, be more clinically relevant. The present study was, therefore, undertaken to measure any differences in uptake of ^{99m}Tc-MIBI and ^{201}Tl by the 9L gliosarcoma tumor when the tumor was implanted in the rat's brain rather than the thigh.

9L gliosarcoma cells (40,000) were stereotactically implanted in the right caudate nucleus of CD Fisher rats (male, 100-125 g) (10). The tumors were allowed to grow until symptoms of neurological impairment appeared (approx. 2 weeks). At this time the tumors were 1 - 4 mm in diameter. The animals were injected (iv) with a mixture of ^{99m}Tc-MIBI and ^{201}Tl, sacrificed 15 min post-injection, and the tumor and selected organs excised. None of the excised tumors were found to be necrotic. The tissue samples were weighed, assayed for ^{99m}Tc and ^{201}Tl, and the percent injected dose of each tracer in each tissue sample calculated by comparison of the counts in the tissue samples to standards. The ^{201}Tl assays were performed after decay of ^{99m}Tc and the ^{99m}Tc assays were corrected for crossover of ^{201}Tl. Data analysis was conducted using the program "InStat" (GraphPad, San Diego, CA). Student's t-test was used to test the significance of differences when the variances were equal and the Mann-Whitney test was used when the variances of the two samples were not equal.

The biodistribution at 15 min. post-injection are summarized in Table 1 and are expressed as the mean and standard error ($n=5$). The percent injected dose per gram ^{99m}Tc-MIBI in the tumor and normal (contralateral) brain is 0.23 ± 0.08 and 0.017 ± 0.010. The corresponding values for ^{201}Tl are $0.984\pm0.306\%$ and $0.112\pm0.036\%$, respectively. For both ^{99m}Tc-MIBI ($p=0.016$) and ^{201}Tl ($p=0.0079$), the uptake of the tracer by the tumor is significantly greater than the uptake by the contralateral normal brain. The tumor-to-normal brain ratios for ^{99m}Tc-MIBI and ^{201}Tl in the stereotactic model are 13 and 8.8, respectively, compared to 6.4 and 6.5 for ^{99m}Tc-MIBI and ^{201}Tl when the tumor was implanted in the thigh (9).

Comparison of the biodistribution of ^{99m}Tc-MIBI and ^{201}Tl in the subcutaneously (thigh) and stereotactically implanted 9L gliosarcoma models reveals differences only in the results for ^{99m}Tc-MIBI uptake in the brain ($p = 0.014$) and ^{201}Tl uptake in the heart ($p = 0.0066$). For all other tissues, including the tumor, the p values were greater than 0.05, suggesting that the location of the tumor did not affect the biodistribution of either radiopharmaceutical. In particular, the differences in uptake of the two radiopharmaceuticals by the two tumor models were very small: $p = 0.54$ and $p = 0.66$ for ^{99m}Tc-MIBI and ^{201}Tl, respectively. This result suggests that the "thigh" model can be used to screen new agents for tumor avidity with minimal concern about the possible effect of site differences.

These results confirm our earlier report (9) that, under identical perfusion conditions, ^{99m}Tc-MIBI should be an effective radiopharmaceutical for evaluation of brain tumor with tumor-to-normal ratios being as least as high as that of ^{201}Tl.

Table 1. Biodistribution of ^{99m}Tc-MIBI and ^{201}Tl.

Tissue	^{99m}Tc-MIBI		^{201}Tl	
	% i.d./g	S.E.	% i.d./g	S.E.
Tumor	.23	.08	.98	.31
Brain	.018	.010	.11	.04
Blood	.12	.03	.12	.03
Muscle	.28	.05	.44	.24
Heart	5.0	.9	5.4	.7

REFERENCES:

1. Piwnica-Worms D. and Holman B.L. - J. Nucl. Med. 31:1166(1990)
2. Cox P., Belfer A., and von der Pompe W. - Br. J. Radiol. 49:767(1976)
3. Black K.L., *et al.* - J. Neurosurg. 71:342(1989)
4. Kaplan W.D., *et al.* - J. Nucl. Med. 28:47(1987)
5. Kim K.T., *et al.* - J. Nucl. Med. 31:965(1990)
6. O'Tuama L.A., Packard A.B., and Treves, S.T. - J. Nucl. Med. 31:2040(1990)
7. O'Tuama L., *et al.* - J. Nucl. Med. (in press)
8. Ando A., *et al.* - Eur. J. Nucl. Med. 12:567(1987)
9. Packard A.B., *et al.* - Nucl Med. Biol. (in press)
10. Wen P., Lampson M.A., and Lampson, L.A. - J. Neuroimmun. 36:57(1992)

Comparison of labelling characteristics of Tc99m-ethylene dicysteine and its monoacid derivative Tc99m-ethylene cysteamine cysteine

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L,L-ethylene dicysteine (L,L-EC), a diamino dithiol tetraligand substituted with two carboxylic acid functions, forms a very stable complex with ^{99m}Tc [1,2,3]. In order to study the importance of the 2 COOH-groups for the Tc-chelating properties and for the biological properties of the ^{99m}Tc-complexes, we have synthesized the monoacid derivative ethylene cysteamine cysteine (ECC, **2**) of **1** and compared the technetium chelating strength of the diacid **1** and the monoacid **2**.

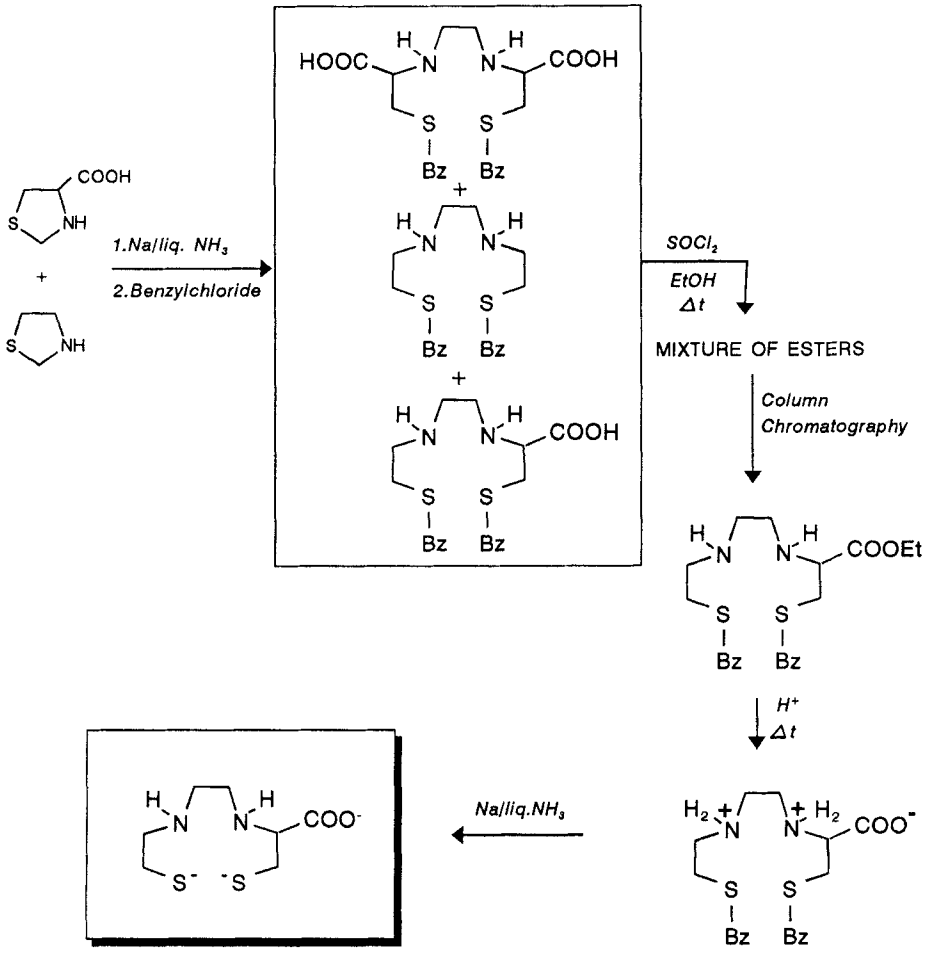
2 was obtained besides **1** and ethylene dicysteamine (**3**) by reductive dimerization of equimolecular amounts of thiazolidine and L-thiazolidine-4-carboxylic acid using sodium in liquid ammonia in analogy with the synthesis of EC[4] (Scheme 1). After the dimerization an excess of benzylchloride was added and the precipitate obtained after acidification was filtered off. The crude mixture was suspended in a cold solution of thionylchloride in ethanol and refluxed overnight. The monoester was purified and isolated by column chromatography on silicagel. Hydrolysis of the pure monoester in concentrated hydrochloric acid yielded pure S-benzyl protected **2**. The S-benzyl protective groups were removed by the action of sodium in liquid ammonia to yield pure **2**.

Labelling of **2** with technetium-99m was studied by a direct labelling method at room temperature at pH 6 to 12 (ligand + $SnCl_2$ + TcO_4^-) and by exchange labelling (100°C-10') in the presence of 100 µg $SnCl_2$ + 15 mg tartaric acid at pH 6 to 10. Whatever labelling method was used, two diastereomeric ^{99m}Tc-complexes of **2** (A and B, in the order of elution on HPLC) were formed and isolated by reverse phase HPLC separation of the reaction mixtures after labelling. The relative amount of A and B was highly dependent on the pH during the direct or exchange labelling reaction (figure 1). Electrophoresis of ^{99m}Tc-**1**, ^{99m}Tc-**2** (A+B) and ^{99m}Tc-MAG3 at pH 6 and 12 revealed a charge of -1 for ^{99m}Tc-**2** and -2 for the other agents. HPLC chromatograms of the reaction mixture after direct labelling of 1 mg **2** at different pH values (pH 6-10) are compared in figure 2. The radiochemical purity exceeds 97% when labelling is performed at pH≥10, but is clearly lower after labelling at lower pH as it has also been observed for EC[1]. To compare the Tc-chelating strength of EC and ECC, mixtures of equimolecular amounts of **1** and **2** were labelled with Tc99m using the exchange or direct labelling method at pH 6-12. After exchange labelling the ratio ^{99m}Tc-EC/^{99m}Tc-ECC was each time about 1.5 whereas after direct labelling the ratio varied from 3 to 1.7 (table 1).

On the basis of the results we can conclude that the monoacid derivative of EC exhibits weaker chelating properties than EC. Compared to MAG3 however, both diamino dithiol tetraligands exhibit a stronger chelating strenght (results not shown). It thus appears that the presence of one carboxyl group in **1** is sufficient to obtain a stable complex with technetium, but the Tc-binding properties are highly dependent on the pH. On the other hand, it is clear that the presence of two negatively charged carboxylate groups enhances the Tc-binding properties, probably by promoting the deprotonation of the amines. Some of the isomers of ^{99m}Tc-ECC were found to have excellent renal excretion characteristics in mice and a baboon. ECC has also been studied as a potential Tc-chelating moiety after coupling to peptides. The results of these studies will be reported in separate papers.

References

1. Verbruggen A.M., Nosco D.L., Van Nerom C.G., Bormans G.M., Adriaens P.J., De Roo M.J. - J Nucl Med 1992; 33: 551-557
2. Crombez D., Cleynhens B., Bormans G., De Roo M., Verbruggen A. - Eur J Nucl Med 1991; 8:605 (abstract)
3. Qu T., Wu Y., Wang X., Liu Y. and Ye Y. - Proceedings of the Topical Symposium on the Behaviour and Utilisation of Technetium '93, Sendai, Japan, p. 71
4. Blondeau P., Berse C., Gracel D. - Can J Chem 1967; 45: 49-52



Scheme 1: Synthesis of ethylene cysteamine cysteine (**2**)

Table 1. Exchange and direct labelling at different pH of equimolecular amounts of 1 and 2. Results are given as percentage of total activity eluted from the HPLC-column

	Exchange labelling			Direct labelling		
	^{99m}Tc-EC	^{99m}Tc-ECC	Rest	^{99m}Tc-EC	^{99m}Tc-ECC	Rest
pH 12	-	-	-	73	24	3
pH 10	54	35	11	47	25	27
pH 8	57	39	4	28	16	58
pH 6	56	35	19	-	-	-

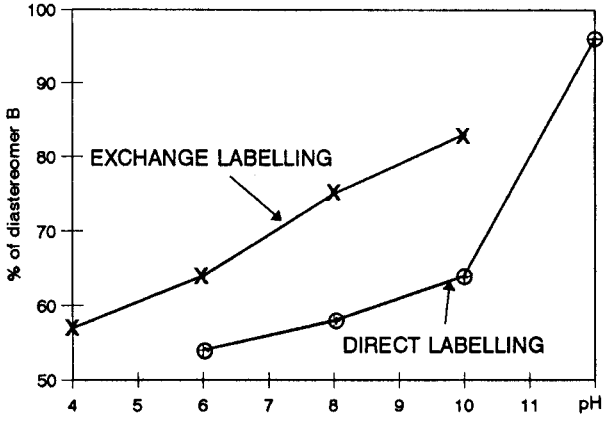


Figure 1. Percentage of diastereomer B of ^{99m}Tc-2 formed after labelling at different pH values

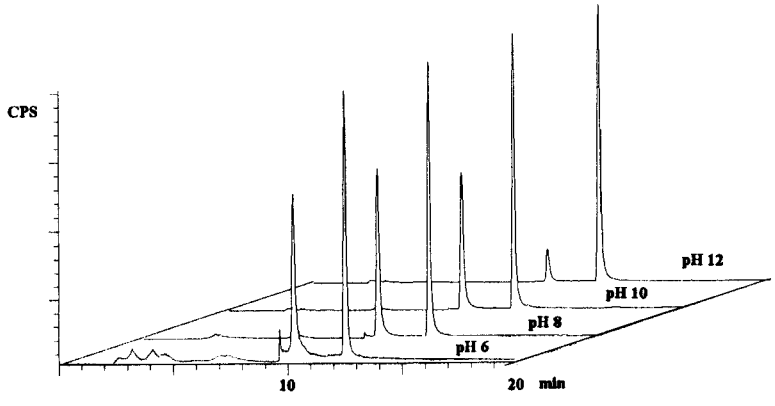


Figure 3. Radio-HPLC-chromatograms of ^{99m}Tc-2 after direct labelling at different pH

QUANTITATING TECHNETIUM PHARMACOKINETICS WITH PET, SPECT AND BETA SPECTROSCOPY

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Introduction.

This work addresses three separate technical developments, each extending the concept of quantitative pharmacokinetics to the study of new Tc-labeled imaging agents:

- non-invasive PET with ^{94m}Tc, now made from enriched $^{94}MoO_3$,
- single photon imaging with a CAMAC / Mac gamma camera interface, currently tracking the kinetics of new ^{99m}Tc agents co-injected with 2-^{18}FDG for metabolic co-registration in open-chested swine preparations,
- fast clarance measurements of $\beta^{\pm}$ emitting test agents in open chested canine myocardia with a solid state detector.

Each of these techniques acts to buttress the basic pharmacology needed to bring a new ^{99m}Tc-imaging agent to the clinical stage.

$^{94m}TcO_4^-$ from enriched $^{94}MoO_3$: Getting it right this time.

On a milliCurie-administered basis, ^{99m}Tc dominates the field of diagnostic radiopharmaceuticals by almost two orders of magnitude. In spite of this, the advent of a new ^{99m}Tc agent is deprived of the advantages enjoyed by any PET compound, whose *in vivo* concentrations can be directly followed quantitatively in a non-invasive imaging study. Technetium-94m ($t_{1/2}$ = 53 min; β^+) would extend this advantage to the Tc-chemist, giving him the same opportunity to measure tissue concentrations in $\mu Ci/cm^3$, kinetic data that is indispensible for compartmental modeling to understand the mechanism of drug action. Tc-94m is produced by the $^{94}Mo(p,n)^{94m}Tc$ reaction on natural molybdenum at 11 MeV, albeit accompanied by a half dozen radionuclidic contaminants as shown in Table I. The ^{99m}Tc observed is the result (1) of the $^{100}Mo(p,2n)\ ^{99m}Tc$ reaction.

In the case of a slowly clearing agent, a threefold reduction in absorbed radiation dose results from elimination of the A ≠ 94 technetium contaminants, possible with enriched ^{94}Mo targetry. Early attempts at ^{94m}Tc / ^{94}Mo separation by sublimation from MoO_3 proved unstable. The ^{94m}Tc recovery / ^{94}Mo loss ratio is sharply peaked between 780 and 800°C, and difficult to monitor in ≈mm^2 beam strikes or post-bombardment in a muffle furnace. Now, the ^{94}Mo inventory (≈50 mg, $6/mg) is recrystallized from ammoniacal solution in the bottom of a 50 mm deep, 14 mm diameter glassy carbon crucible shown in Figure 1. This thermal contact suffices to remove the beam power (8 μA at 11 MeV) in vacuo, with thin target (ΔE_p = 2 MeV) yields of ≈ 4 mCi/μA at the end of a 53-minute irradiation. The MoO_3 target material is dissolved in 30% H_2O_2, made alkaline with conc NH_4OH, and the $^{94m}TcO_4^-$ extracted into MEK. The aqueous ^{94}Mo - ammonium molybdate is later recrystallyzed in the target vessel used for the next irradiation with > 90% mass recovery. The $^{94m}TcO_4^-$ / MEK is washed 3 times with 1N NaOH, blown dry and reconstituted as $^{94m}TcO_4^-$ in physiological saline, ready for introduction to the ligand under study. The chemical separation is reliable, and takes ≈ 30 minutes from EOBwith ≈80% yield, decay corrected. Myocardial imaging agents currently under study include Teboroxime, SestaMIBI and the investigational hypoxia indicator BMS 181321.

A gamma camera / computer interface for the acute animal preparation.

The open-chested animal prep offers the basic researcher total control over the myocardial circulation, and acts as a near-perfect test-bench for planar, single photon imaging of new tracers of myocardial function under controlled ischemic conditions. The absence of collateral circulation in the swine heart makes it a suitable model to study the sequelae of myocardial ischemia (2).Test agents labeled with Tc-99m (eg. 50 mCi of BMS 181321) are introduced just upstream of the coronary arteries, co-injected with several mCi of F-18 2FDG to insure complete "physiological co-registration".

A GE Maxi 400 gamma camera head, equipped with a modified pinhole collimator, sends the analog (X^+,X^-,Y^+,Y^-) signals off to a CAMAC interface for quad conversion, buffering and block transfer to a Mac Quadra 950. The three addresses: E = (X^++X^-+Y^++Y^-), X=(X^+-X^-)/E, and Y =(Y^+-Y^-)/E, are formed in real time for histogramming into the three-space arrays N(X,Y,E) with (256 x 256 x 64) dispersion. All hardware is commercially available NIM and CAMAC standard. Software makes use of the high level K-Max (Sparrow) and DIPs (Hayden) languages. Dynamic image acquistion covers the 75 minutes post-injection period, after which the pig is euthanized, the lesioned vascular bed is delineated with an injection of india ink and the excised myocardium is sectioned. The dozen heart

slices are arranged on the collimator face for static imaging of the ^{99m}Tc and ^{18}F distributions. Finally, a Canon video still camera captures a visual record, with the black ischemic tissue borders clearly delineated in each slice. This visual record, imported into the DIPs data analysis package, guides the exact circumscription of the ROI's in the two radionuclide images, co-registered by $^{99m}Tc/^{18}F$/ink fiducial point sources in the image. Imaging actual, excised 2-D tissue slices provides the gold standard of transverse section isolation that SPECT can only aspire to. The *ex vivo* , multi-isotope imaging procedure is closer to slicing the heart into a thousand cubes, and analyzing them in a thousand well counter / MCA's in parallel.

Beta spectroscopy to follow fast tissue clearance kinetics of technetium agents.
The short range (≈ few mm) of beta particles in tissue, and ease of collimation, can be put to advantage in tracking the passage of a tracer, introduced as a δ- function into the arterial input of the tissue bed. The desired gamma insensitivity is assured by a thin detector of low Z, such as the ruggedized silicon surface barrier (Survivor, Ortec EG&G) detector with an area of 100 mm^2 and depletion depth of ≈300 μm, thick for electrons below 200 keV. The attenuation of betas fortuitously (3) follows an exponential relation $I / I_o = \exp[-(\mu/\rho)(\rho t)]$ with a mass attenuation coefficient (cm^2/g) empirically related to the beta end point energy E_{max}(MeV) as $\mu/\rho = 17/E^{1.14}$.

The open chested canine prep is fully instrumented and flow controlled, with an injection port directly upstream of the left anterior descending coronary artery, permitting the injection of a 200 μl bolus of test tracer in less than a second. The time dependence of the beta activity passing through the myocardial tissue bed downstream ranges from a slightly broadened δ-function for non-extracted tracers, to an admixture of a δ-and-step functions for partially extracted tracers (imperfect μ-spheres), to the monoexponential clearance of freely diffusable indicators. Table II lists the tracers being investigated in this acute canine prep, arranged in order of increasing extraction from zero (red blood cells) to near-unity (O-14 water and dissolved fluoromethane). The cardiac flow agent Teboroxime was labeled sequentially with ^{99m}Tc and ^{94m}Tc, with identical time courses observed. The 140 keV conversion electron (10%) from ^{99m}Tc decay affords surprisingly good β/γ discrimination, and is then easily thresholded out for the subsequent ^{94m}Tc-BATO study. The advantages of beta detection in isolated organ preps (4) has largely gone unappreciated. The unexpected result that these advantages can be extended to ^{99m}Tc opens up new opportunities for probing tissue response with exquisite temporal and spatial resolution.

Conclusions.
The three technical developments discussed here can provide the technetium pharmacologist with a bridge between "cut-and-count" with laboratory animals and qualitative SPECT imaging in man. The quantitation of ^{94m}Tc / PET, the physiological co-registration of multi-isotope (^{99m}Tc / ^{18}F) studies, and the detail achievable with beta detection all promote the rational drug design of tomorrow's ^{99m}Tc imaging agents.

Acknowledgements.
The authors gratefully acknowledge the financial support of Bristol -Myers Squibb and the National Institutes of Health (R29 HL 47003 and CA 09206). Special thanks are due to the Cardiovascular Research Team for their competence and good spirits.

References.

1. Almeida GL, Helus F. On the production of ^{99}Mo and ^{99m}Tc by cyclotron. Radiochem. Radioanal.Letters. **28**(3), 205-214 (1977)
2. Liedtke AJ, DeMaison L, Eggleston AM, Cohen LM, Nellis SH, Changes in substrate metabolism and effects of excess fatty acids in reperfused myocardium. Circ Res **62**, 535-542 (1988).
3. Evans RD, The Atomic Nucleus, McGraw Hill Book Co, New York, 628 (1955).
4. Sasaki Y, Pitt B. Measurement of regional myocardial blood flow in dogs using a catheter semiconductor radiation detector. Cardiovascular Research. **10**, 499-502 (1976)

Table I.
Technetium radionuclidic contaminants from the ^{nat}Mo(p,n) reaction.

Reaction	Abundance*	$t_{1/2}$	Yield#	Absorbed radiation dose‡
^{92}Mo(p,n)^{92}Tc	15%	4 m	1 mCi/μA	liver, < 1 mrad
^{94}Mo(p,n)^{94m}Tc	9%	53 m	3.4	intestinal tract, ≈800 mrad
^{94}Mo(p,n)^{94}Tc	9%	4 hr	0.7	upper large intestine, 200 mrad
^{95}Mo(p,n)^{95}Tc	16%	20 hr	4.3	lower large intestine, 170 mrad
^{95}Mo(p,n)^{95m}Tc	16%	61 d	1.5	lower large intestine, 2 mrad
^{96}Mo(p,n)^{96}Tc	17%	4 d	10	lower large intestine, 400 mrad
^{100}Mo(p,2n)^{99m}Tc	10%	6 hr	0.08 (ref 1.)	upper large intestine, < 1 mrad.

* Abundance in % occuring in natural molybdenum.
\# At end of saturated bombardment on natural molybdenum, $11 > E_p > 8$ MeV.
‡ to stated target organ, from Tc contaminants accompanying 1 mCi of ^{94m}Tc-BATO, injected 1 hour post EOB

Table II

Radiotracers used to probe extraction in the canine myocardial

Agent	$t_{1/2}$	Extraction	E_β(max)
^{11}C-RBC's	20 m	0	962 keV
$^{82}Rb^+$	75 s	0.4	3200
^{62}Cu-PTSM	10 m	0.5	2934
$^{13}NH_4^+$	10 m	0.6	1190
^{99m}Tc-BATO	6 hr	0.8	120 (discrete line)
^{94m}Tc-BATO	53 m	0.8	2474
$H_2{}^{14}O$	71 s	0.9	1810
$CH_3{}^{18}F$	110 m	1.0	635

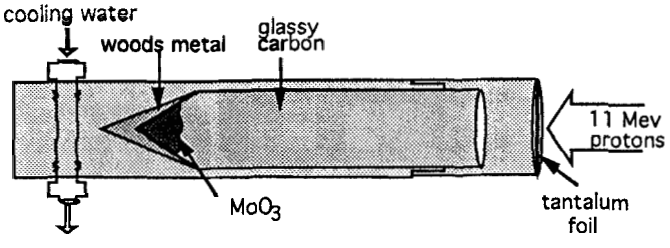

Fig. 1. Glassy carbon lined target chamber for the irradiation of enriched $^{94}MoO_3$.

^{99}TcΞN/^{99m}TcΞN-COMPLEXES WITH AMINOACIDS. SYNTHESIS, AND CRYSTAL STRUCTURE OF [^{99}TcNCl(CYS-OEt)(PPh_3)]

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Transition metal complexes with aminoacids and peptides have been synthesized and structurally characterized for studing their interactions with proteins and antibodies. In particular, neutral, stable and lipophilic technetium complexes have been widely studied as potential brain perfusion agents and the complex [^{99}TcO(L,L-ECD)] (L,L-ECD = N,N'-1,2-ethylenediyl-bis-L-cysteinediethylester) has been characterized by X-Ray analysis and the corresponding ^{99m}Tc-complex proposed as a marker of regional cerebral blood flow[1]. More recently, a ^{99m}Tc-cysteine complex has been evaluated in animal distribution studies for tumor diagnosis but its chemical structure has not been determined[2].

We have synthesized and characterized nitrido-technetium complexes with aminoacids: [TcNCl(CYS-OEt)(PPh_3)] (CYS-OEt= L-cysteine-ethyl ester), [TcNCl(CYS)(PPh_3)] (CYS= L-cysteine) and [TcN$(CSA)_2$] (CSA= cysteamine) and the X-ray crystal structure of [TcNCl(CYS-OEt)(PPh_3)] determined. The complex presents a distorted square pyramidal geometry where the Tc atom is displaced from the basal plane by 0.594(1) Å; the TcΞN distance of 1.605(3) Å is typical of all nitrido compound of Technetium(V)[3]. The [^{99}TcN-(aminoacid)] complexes were prepared by substitution reactions on the complex [$TcNCl_2(PPh_3)_2$] (yield ≥ 90%). These compounds were also obtained starting from [$AsPh_4$][$TcNCl_4$] but the presence of PPh_3 was required to reduce the core $[TcΞN]^{3+}$ to $[TcΞN]^{2+}$.

The ^{99m}TcΞN-complexes were prepared following the procedure for the preparation of [^{99m}TcΞN]-intermediate species[4]. The pH was adjusted to 7.4 (HCO_3^-/CO_3^{2-} buffer), an aqueous solution of the ligand (10 mg mL^{-1}) was added to the vial and the temperature raised to 70°C for 15 min. The chromatographic analysis revealed the formation of products which were different if compared to those obtained at ^{99}Tc-level and in addition, electrophoresis showed their anionic character.

References

(1) (a) Edwards, D.S.; Cheesman, E.H.; Watson, M.W.; Maheu, L.J.; Nguyen, S.A.; Dimitre, L.; Nason,T.; Watson, A.D.; Walovitch, R.; **Technetium and Rhenium in Chemistry and Nuclear Medicine, 3**, Nicolini, M.; Bandoli, G.; Mazzi, U.; Cortina International, Verona, Raven Press, 1990, pag. 433; (b) Verbruggen, A.; Bormans, G.; Van Nerom, C.; Cleynhens, B.; Crombez, D.; De Roo,M.; **ibid.** pag. 445.

(2) Ozker, K.; Urgancioglu, I.; Sungur, A.; Amac, A.; Hoskan, N.; Ardogil, N. **Appl. Radiat. Isot. 1992, 43**, 1517.

(3) (a) Marchi, A.; Duatti, A.; Rossi, R.; Magon, L.; Pasqualini, R.; Bertolasi, V.; Ferretti, V.; Gilli, G. **J. Chem. Soc. Dalton Trans.** 1988, 1743; (b) Marchi, A.; Garuti, P.; Duatti, A.; Magon, L.; Rossi, R.; Ferretti, V.; Bertolasi, V. **Inorg. Chem. 1990, 29**, 2091; (c) Marchi, A.; Rossi, R.; Magon, L.; Duatti, A.; Casellato, U.; Graziani, R; Vidal, M.; Riche, F. **J. Chem. Soc. Dalton Trans. 1990**, 1935.

(4) Pasqualini, R.; Comazzi, V.; Bellande, E.; Duatti, A.; Marchi, A. **Appl. Radiat. Isot. 1992, 43**, 1329.

Diaminodisulfide (DADS) Ligands. Useful Alternatives to Diaminodithiol (DADT) Ligands for Technetium and Rhenium.

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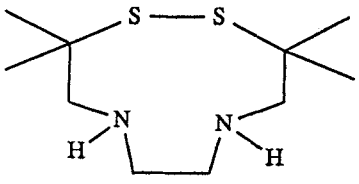
Mono-N-substituted DADS ligands presented the advantages of stability and easy purification compared to the DADT ligands. The commonly used $NaBH_4$ reduction of the di-imine precursor (1) to the DADS instead results predominantly in a bicyclic product (2). This useful intermediate is easily substituted on the 2° amine (3) followed by reduction to give mono-N-substituted DADT ligands (4). These are used to form functionalised, neutral, lipophilic Technetium and Rhenium complexes (5). We used this method to prepare a range of N-substituted complexes[2]. However the N substituted DADT ligands (4) possessed several poor characteristics. The free bases were unstable and the salts were hygroscopic, some very highly. Consequently, the DADT ligands were difficult to purify and characterise and had limited shelf life. Bodor et al obtained[3] obtained the DADS ligands via an intermediate amino borane internal complex (6 and 7) from the bicyclic precursor using borane generated $NaBH_4$ and BF_3/Et_2O complex. Using commercially available BH_3/THF we also obtained the monocyclic DADS. In the BH_3/THF reduction of (2) the intermediate borane complex was also isolated but was different from that reported by Bodor - with a higher melting point 149 -152°C to 52 - 54°C. The structure of the complex was determined to be (8) from I-R data and X-ray crystallography.

Labelling of the DADS ligand with ^{99m}Tc was carried out using $NaBH_4$ as reducing agent. The DADS ligand was also used to label with ^{188}Re in a one pot reaction using dithiothreitol to cleave the disulfide bond[4].

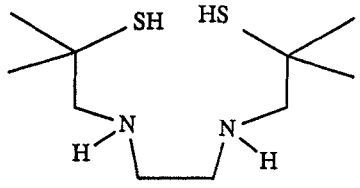
This method provides advantages in the preparation of ^{99m}Tc, ^{188}Re and ^{186}Re labelled compounds over the present DADT ligands.

References

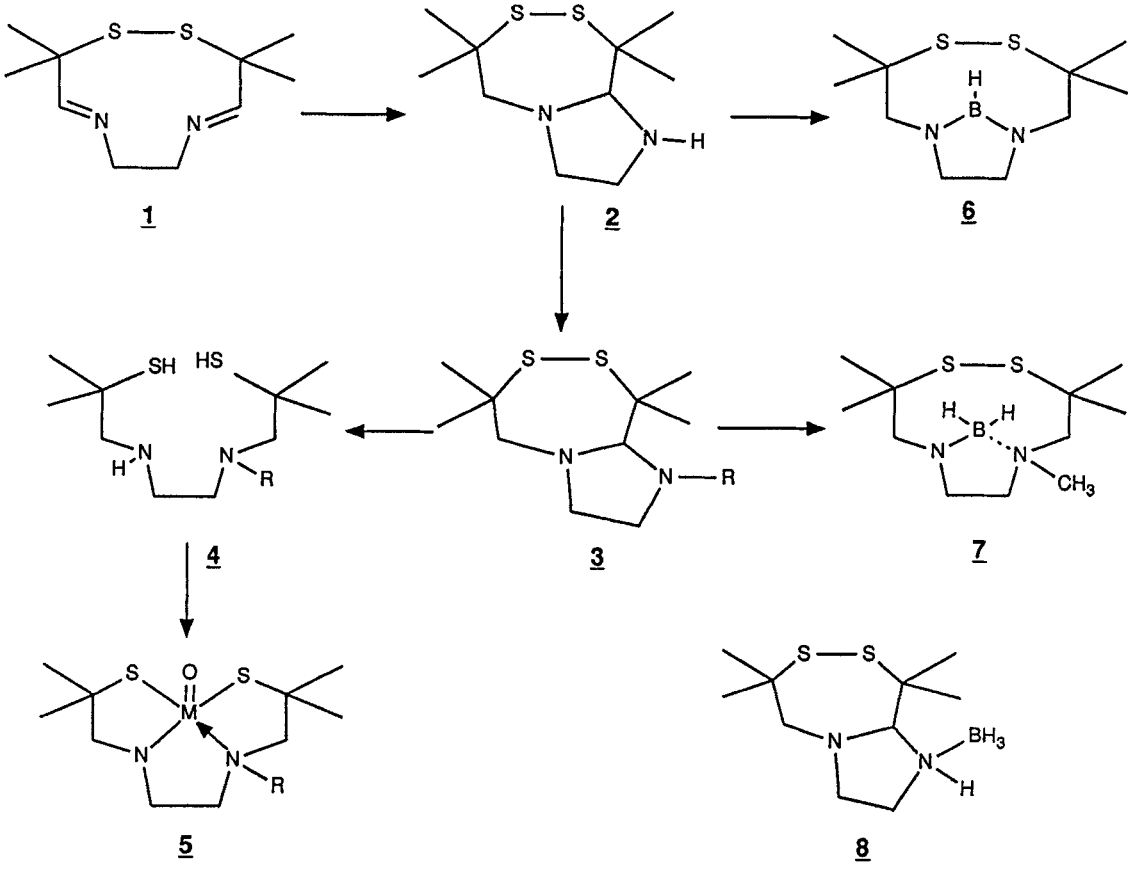
1. Joshua, A.V., Scott, J.R., Sondhi, S.M., Ball, R.G., and Lown, J.W., *J. Org. Chem.* 52: 2447 (1987).
2. Jackson, T.W., Kojima, M., and Lambrecht, R.M., *Radiochim. Acta.* In press.
3. Bodor, N., Koltai, E., and Pro'kai, L., *Tetrahedron* 48 (23): 4767(1992).
4. Eisenhut, M., Brandau, W., and Missfeldt, M., *Nucl. Med. Biol.* 16(8): 805(1989).



DADS



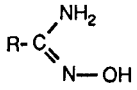
DADT

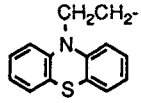
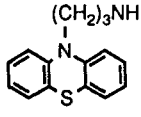
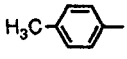


Preparation and HPLC Analysis of ^{99m}Tc Complexes with Hydroxamamides.
Ozeki, K.; Kimura, Y. ; Nakajima, K. ; Morita, J. ; Harada, K. ; Nakayama, M. ; Sugii, A.; Okabayashi, I. Faculty of Pharmaceutical Sciences,Kumamoto University,Kumamoto and Niigata University of Pharmaceutical Sciences,Niigata,Japan.

The study on a coordination site capable of binding ^{99m}Tc is essential for the development of novel ^{99m}Tc labeled functional imaging agents. We reported that a hydroxamamide group is a promising chelating moiety to form a stable complex with ^{99m}Tc.(1) In this study, sereval hydroxamamide (Ham) derivatives were synthesized to prepare different ^{99m}Tc complexes in the charge, molecular weight, and lipophilicity.(Table1) These ligands formed rapidly the complexes with ^{99m}Tc at greater than 95% yield at room temperature. The results of cellulose acetate electrophoresis indicated that the ^{99m}Tc complexes were uncharged or positively charged.

Table.1 Hydroxamamide derivatives in this study



R	Abbreviation	R	Abbreviation
CH_3CH_2—	$HamC_3$		PTZHam
$(CH_3)_2NCH_2CH_2$—	DAHam		
	BHam		PTZNHam
	pTHam		

All ^{99m}Tc complexes were resolved into two components(A and B) in a reversed phase HPLC analysis by isocratic elution method using a mixture of methanol and pH 7.0 phosphate buffer. (Fig.1) In each ^{99m}Tc complex, the component A decreased with an increase of reaction time, and the other component B increased. After each component was fractionated by using the HPLC system, each fraction was left for 3 h and re-analyzed. In the radiochromatogram of each fraction, no peak was obserbed except for the peaks based on both components. These information obtained from the HPLC analysis demonstrates the high stablitity of the ^{99m}Tc-hydroxamamides. When two Ham derivatives having different lipophilicity were simultaneously used as ligands, the formation of a mixed ligand ^{99m}Tc complex was ascertained by HPLC analysis with gradient elution method. As show in Fig.2, This mixed ligand complex was also resolved into two components. This fact indicates that the ^{99m}Tc complex is composed of Ham and ^{99m}Tc in a 2:1 molar ratio. These complexes indicated each ^{99m}Tc complex presented independent biodistribution pattern in mice. The variations of *in vivo* characteristics reflects on the difference in properties (lipophilicity, charge, and molecular weight) and shows the stability of ^{99m}Tc-hydroxamamides *in vivo*.

1)Nakayama M. Saigo H. Kai E. Koda A. Ozeki H. Harada K. Tomiguchi S. O. Kojima A. Hara M. Nakajima R and Takahasi M. Nucl. Med. Commun.13: 445(1992)

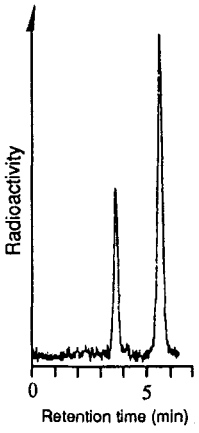


Fig.1 Typical chromatogram of ^{99m}Tc-BHam
Column : Lichrospher RP-18 (125 x 4 mm I.D.)
Eluent : MeOH : 0.01 M phosphate buffer = 40 : 60
Flow rate :1 ml/min

Table.2 Complex yield of component A and B

	Complex yield of component A and B (%)							
	^{99m}Tc-HamC$_3$[1]		^{99m}Tc-BHam[2]		^{99m}Tc-pTHam[3]		^{99m}Tc-PTZHam[4]	
Incubation time	A	B	A	B	A	B	A	B
15min	50.1	49.6	57.9	38.1	47.1	63.5	49.2	45.9
30min	33.7	65.2	50.3	45.0	36.5	63.5	56.6	43.3
60min	22.5	76.5	40.3	55.4	29.2	70.8	47.1	52.9
120min	18.0	82.0	31.2	64.9	22.5	77.4	35.1	64.9
Separation factor	1.56		1.69		1.41		1.92	

Eluent : MeOH : 0.01 M phosphate buffer(pH7)=10 : 90[1],40 : 60[2],60 : 40[3],80 : 20[4]

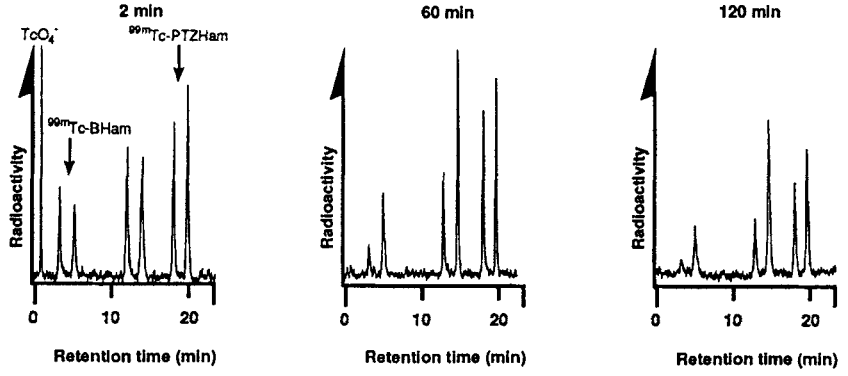


Fig.2 Chrmatograms of ^{99m}Tc mixed ligand complex
Ligand concn. : BHam 1.25 X10^{-4} M, PTZHam 3.75 X10^{-4} M

Chemical Characteristics of Tc-99m Labeled Ethylene Imine Phenols

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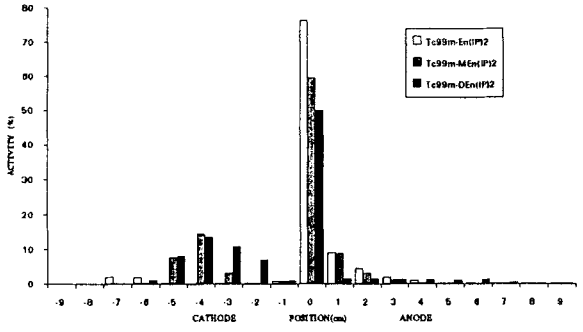
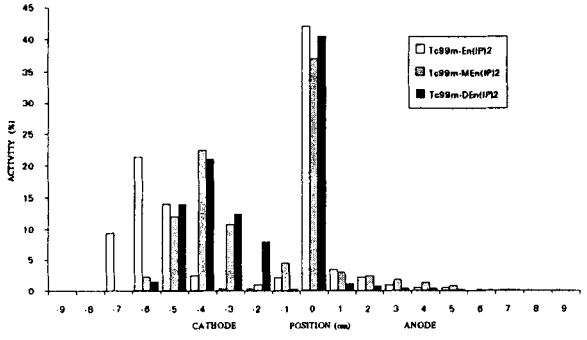
Ethyleneimine phenol [$En(IP)_2$][IUPAC named 1,2-bis(2-hydroxybenzylimino) ethane] and its derivatives, dimethyl ethyleneimine phenol [$MEn(IP)_2$] and 1,2-dimethyl ethyleneimine phenol [DEn(IP)2] were synthesized from condensation of one mole of 1,2-diamino ethane, methyl-1,2-diamino ethane and 1,1-methyl-1,2-diamino ethane, correspondingly, with two moles of salicylaldehyde. These chelates were complexed to Tc-99m using Sn^{2+}($1x10^{-4}$M) reduction of $^{99m}TcO_4^-$ in the presence of the ligands($1x10^{-3}$M) under the varying buffer solutions. The Tc-99m complexes, ^{99m}Tc-$En(IP)_2$, ^{99m}Tc-$MEn(IP)_2$, or ^{99m}Tc-$DEn(IP)_2$ formed were characterized simultaneously by electrophoresis, high performance liquid chromatography (HPLC) and solvent extraction. The electrophoresis results show that particularly in the medium of 0.5M borate emerge all of the Tc-99m complexes with cationic character. Whereas, all of the other media of buffers including 0.5M acetate, 0.5M phosphate, 0.5M bicarbonate, etc., investigated make the Tc-complexs to be exclusive of cationic species.

The Tc-99m labeled ethyleneimine phenols in the borate medium were designed for a more detailed study owing to its potential as a useful radiopharmaceutical in nuclear medicine. Complexation of Tc-99m with $En(IP)_2$, $MEn(IP)_2$, and $DEn(IP)_2$ were conducted in different pH solutions, including pH6, pH7 and pH8, in the same borate medium. The ionic character of the Tc-99m complexes was found to be very much pH dependent; the fraction of cationic species increases with increasing the pHs and on the contrary, the fraction beyond the cationic species, i.e., of neutral species, increases with decreasing the pHs, for each Tc-99m complex (see Fig. 1). In comparing the cases among the three Tc-99m complexes, ^{99m}Tc-$DEn(IP)_2$ exists with more cationic character than ^{99m}Tc-$MEn(IP)_2$ which again appears with more cationic character than ^{99m}Tc-$En(IP)_2$ (see Fig. 1). The characters of the Tc-99m complexes formulated in the different pH solutions were also confirmed by an ion-pair HPLC analysis (E.Merck Lichropher 100 RP C-18 column $CH_3COONa/CH_3CN/H_2O$ as gradient eluant) (see Fig. 2, the last peak being the cationic species). The cationic Tc99m-complex species can be separated and purified by chloroform/buffer extraction. The pure cationic Tc-99m complexes in the aqueous phases have been taken for biodistribution study by means of rats and indicated the potentiality as a renal static imaging agent. In the animal study, it is interesting to note that the trend of the renal uptake and retention for the three ^{99m}Tc complexes is the same as their performance of cationic character, i.e. ^{99m}Tc-$DEn(IP)_2$ > ^{99m}Tc-$MEn(IP)_2$ > ^{99m}Tc-$En(IP)_2$(see Fig. 3).



(a)



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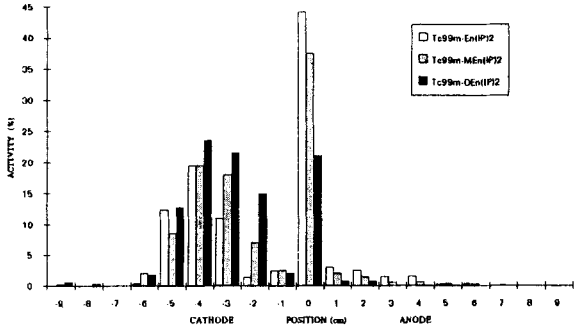
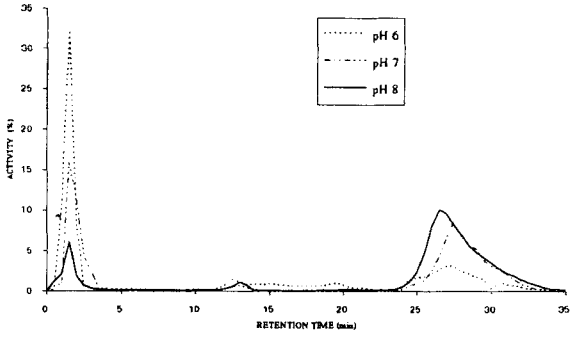
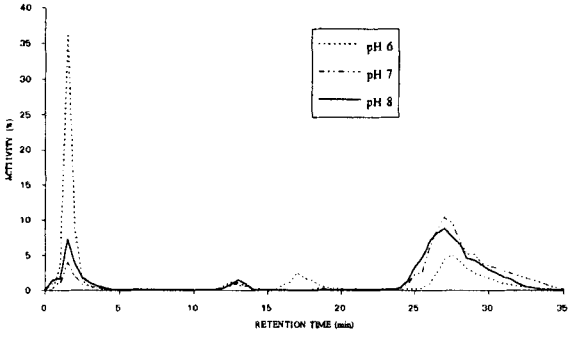


(c)

Fig. 1 Electrophoregrams for ^{99m}Tc-En(IP)$_2$, ^{99m}Tc-MEn(IP)$_2$ and ^{99m}Tc-DEn(IP)$_2$ in (a) pH6, (b) pH7 and (c) pH8 solution under the 0.5M borate medium.



(a)



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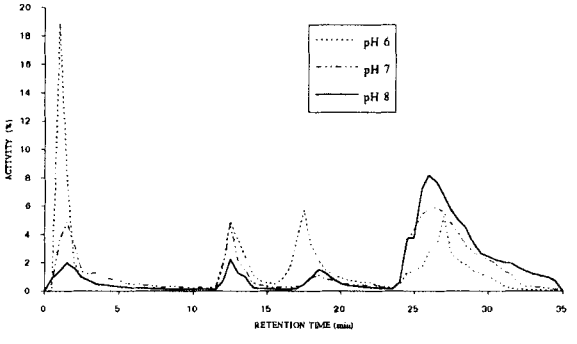
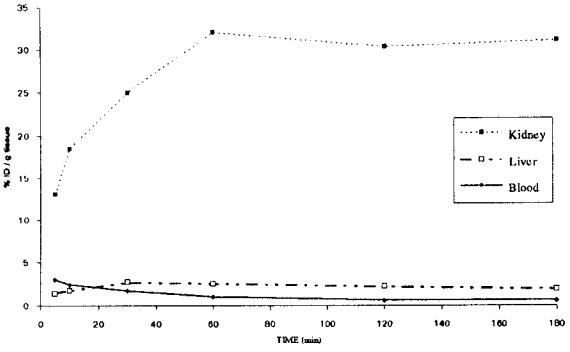
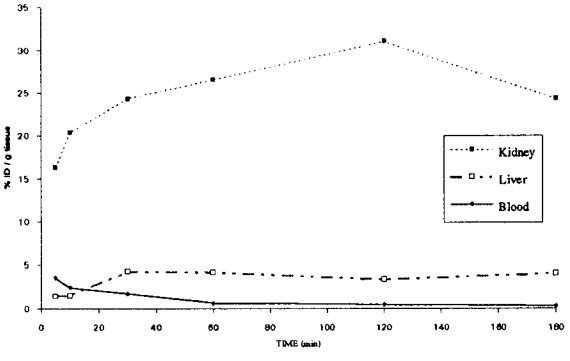


(c)

Fig. 2 HPLC Chromatograms for (a) ^{99m}Tc-En(IP)$_2$, (b) ^{99m}Tc-MEn(IP)$_2$ and (c) ^{99m}Tc-DEn(IP)$_2$ in pH6, pH7 and pH8 solution under the 0.5M borate medium.



(a)



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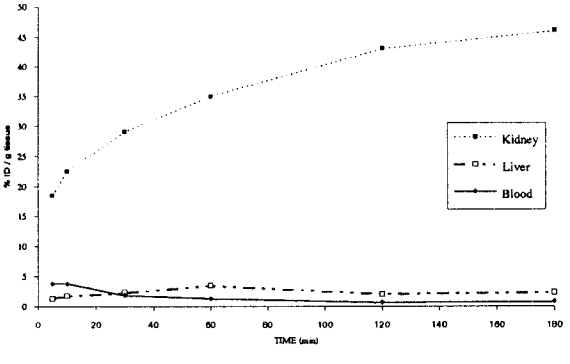

(c)

Fig. 3 Biodistribution test by rats for (a) ^{99m}Tc-En(IP)$_2$, (b) ^{99m}Tc-MEn(IP)$_2$ and (c) ^{99m}Tc-DEn(IP)$_2$ in pH8 solution under the 0.5M borate medium.

FORMATION OF LIPOPHILIC Tc-99m SPECIES DURING STANNOUS TIN REDUCTION OF Tc-99m PERTECHNETATE: Preliminary Results

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During the investigation of labeling lipophilic compounds with Technetium 99m using stannous chloride as the reductant, it was observed that Tc-99m pertechnetate is reduced to lipophilic form by stannous tin without any lipophilic complexing agent present. The following work was carried out to determine the conditions required for the formation of lipophilic Tc-99m species.

One ml of Tc-99m pertechnetate (2-5 mCi) in saline was mixed with 1 ml each of (1) 100% ethanol, (2) 50% ethanol and 50% saline (0.9%), (3) 0.9% saline, (4) 50% ethanol 50% 0.1M sodium acetate pH 5.5 or (5) 0.1M sodium acetate pH 5.5. To each of the mixtures, 5 ug-20 ug of $SnCl_2$ $2H_2O$ dissolved in 10 ul of 0.3M HCl was added. The mixture was heated in a boiling water bath for 15 minutes and cooled for 15 min at room temperature.

To quantitatively determine the Tc-99m lipophilic fraction in the reaction mixture, solvent extractions were performed. Aliquots of the above reaction mixtures were mixed with saline and extracted with (1) octanol, (2) chloroform, (3) ethyl acetate and (4) dichloromethane. Extractions were performed at 15', 1 hr and 3 hrs after the reaction to determine the stability of lipophilic Tc-99m components in the reaction mixture. The results are shown in Tables 1 and 2.

It is obvious that there is formation of lipophilic Tc-99m component(s) even when only 5 ug of stannous chloride were used for reduction. With 10 ug of stannous chloride, quantitative conversion (95-97%) of Tc-99m pertechnetate to lipophilic form occurred as determined by extraction into chloroform or methylene chloride. The formed lipophilic Tc-99m is stable in aqueous solution at least 3 hours after preparation. Lipophilic Tc-99m species prepared as above using stannous tin and heating may be useful to prepare new Tc-99m labeled radiopharmaceuticals especially lipid soluble compounds. Also bioactive peptides, receptor imaging agents may be labeled with the reduced Tc-99m lipophilic species in very high specific activities.

At present we do not know what these Tc-99m species are and whether stannous tin is a part of the Tc-99m lipophilic species. Further experiments to characterize the Tc-99m lipophilic species are in progress.

Table 1: Solvent Extraction Data for Tc-99m Lipophilic Species Produced by Stannous Tin Reduction of Pertechnetate in Various Reaction Media. To 1 ml of Tc-99m Pertechnetate 5 ug - 20 ug $SnCl_2H_20$ in HCl was added with ethanol, saline or 0.1M pH 5.5 NaAC in water. Heated for 15 min in boiling water.

	15 min post reaction					1 hr post reaction				
Quantity of $SnCl_2$ $2H_20$ ug	Percent Activity Extracted from Saline into				Quantity of $SnCl_2$ $2H_20$ ug	Percent Activity Extracted from Saline into			
	Octanol	Chloroform	Ethyl Acetate	Methylene Chloride		Octanol	Chloroform	Ethyl Acetate	Methylene Chloride
	Reaction Mixture: 50% ethanol in saline					Reaction Mixture: 50% ethanol in saline			
5	66.3	94.4	56.6	95.0	5	59.9	97.3	55.3	95.7
10	77.0	95.0	80.8	93.9	10	81.5	95.5	83.2	93.0
20	49.1	97.5	52.6	97.4	20	47.3	97.3	35.1	97.4
	Reaction Mixture: 25% ethanol in saline					Reaction Mixture: 25% ethanol in saline			
5	69.3	88.5	66.5	82.9	5	70.8	93.3	67.5	89.7
10	66.9	92.4	62.2	92.1	10	75.0	91.3	64.2	86.8
20	41.2	89.7	18.7	87.6	20	41.8	93.5	20.9	90.9
	Reaction Mixture: 25% ethanol, 25% 0.1M NaAC in saline					Reaction Mixture: 25% ethanol, 25% 0.1M NaAC in saline			
5	52.3	89.3	31.0	81.1	5	54.4	85.0	31.0	80.0
10	47.9	96.4	27.4	90.7	10	47.4	96.6	27.6	89.2
20	32.0	96.8	1.9	90.9	20	24.6	91.6	2.1	90.7
	Reaction Mixture: 100% saline					Reaction Mixture: 100% saline			
5	43.5	89.9	39.5	75.6	5	42.2	86.1	36.6	78.5
10	30.0	92.3	21.8	79.4	10	29.9	86.1	22.3	83.7
20	25.1	90.0	17.4	84.2	20	19.2	99.0	16.3	82.7
	Reaction Mixture: 50% 0.1M NaAC in saline					Reaction Mixture: 50% 0.1M NaAC in saline			
5	30.9	89.6	18.7	76.9	5	32.5	86.7	23.1	77.9
10	20.8	95.6	9.2	85.0	10	19.1	96.3	10.0	84.4
20	17.9	98.3	1.6	80.3	20	19.2	98.5	1.6	82.7

Table 2: Solvent Extraction Data for Tc-99m Lipophilic Species Produced by Stannous Tin Reduction of Pertechnetate in Various Reaction Media. To 1 ml of Tc-99m Pertechnetate 5 ug - 20 ug $SnCl_2H_20$ in HCl was added with ethanol, saline or 0.1M pH 5.5 NaAC in water. Heated for 15 min in boiling water.

3 hrs post reaction

Quantity of $SnCl_2$ $2H_20$ ug	Percent Activity Extracted from Saline into			
	Octanol	Chloroform	Ethyl Acetate	Methylene Chloride
	Reaction Mixture: 50% ethanol in saline			
5	59.5	97.6	53.2	97.8
10	83.5	93.9	83.8	88.2
20	30.1	97.7	31.0	96.3
	Reaction Mixture: 25% ethanol in saline			
5	71.2	93.9	70.4	90.1
10	70.7	93.4	63.1	88.1
20	38.5	93.9	21.1	90.4
	Reaction Mixture: 25% ethanol, 25% 0.1M NaAC in saline			
5	49.8	84.0	34.2	82.7
10	47.7	95.8	30.2	94.0
20	19.5	93.8	1.7	86.5
	Reaction Mixture: 100% saline			
5	43.6	87.2	40.9	81.3
10	32.7	81.8	24.6	76.7
20	28.0	95.6	16.9	81.0
	Reaction Mixture: 50% 0.1M NaAC in saline			
5	34.3	86.2	24.3	85.3
10	20.2	96.5	12.7	87.1
20	17.1	84.2	1.8	80.1

Diaminedithiol Conjugates of Benzovesamicol: Synthesis and *In Vivo* Evaluation. DEL ROSARIO[†*], R.B.; JUNG[†], Y-W; BAIDOO[††], K.E.; LEVER[††], S.Z.; and WIELAND[†], D.M. Division of Nuclear Medicine[†], University of Michigan Medical Center, Ann Arbor, MI 48109-0552 and and Division of Radiation Health Sciences[††], The Johns Hopkins University, Baltimore, MD 21205-2179

(-)-2R,3R-5-[^{123}I]-Iodobenzovesamicol (Scheme **1**, X = I) is a stereospecific radiotracer for mapping cholinergic neurons in the brain undergoing clinical trials as a SPECT clinical agent for the study of Alzheimer's disease (1,2,3). Additional *in vivo* studies of the neuronal mapping potential of a series of iodobenzovesamicols have revealed that considerable bulk tolerance exists in positions 5, 6 and 7 of BVM (1,2,3).

These observations led to the hypothesis that a technetium-99m chelate group might be introduced in the 5 position of BVM without adversely affecting its *in vivo* neuronal mapping characteristics ([^{99m}Tc]-**1**, Scheme **1**). To this end, we have synthesized two diaminedithiol (DADT) conjugates of BVM via attachment of the chelate at the 5 position. DADT-BVMs **1** and **2** were both synthesized starting from 5-aminobenzovesamicol (**4**). DADT-BVM **1** was prepared by coupling of 5-aminomethylbenzovesamicol with the previously reported BCA thiolactone reagent (5) (Scheme **1A**). The synthesis of DADT-BVM **2** involved condensation of BVM with 3,3,10,10-tetramethyl-1,2,-dithia-5,8,-diazacyclodecane (6) as a key step (Scheme **1B**).

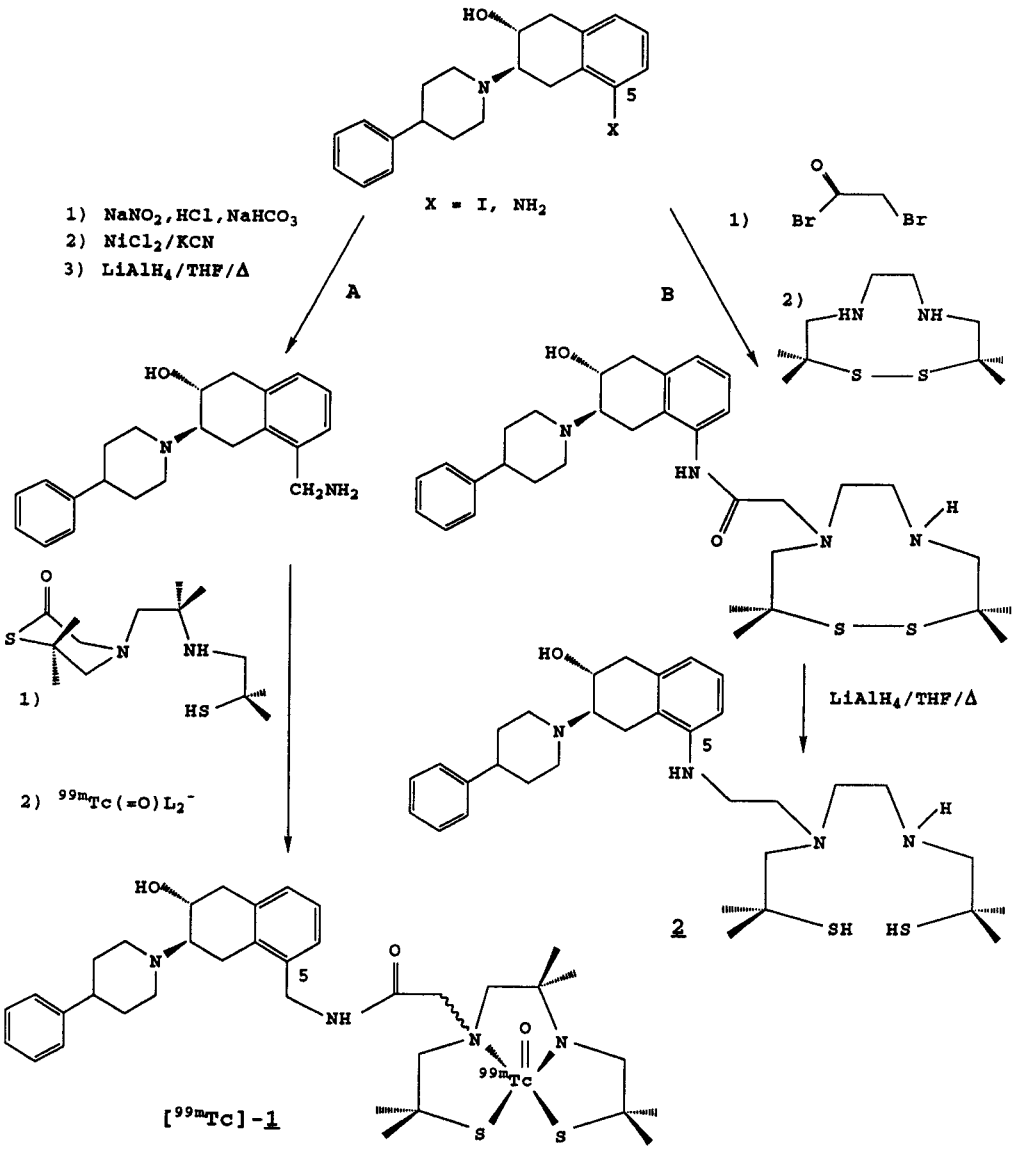
^{99m}Tc radiolabeling of DADT BVMs **1** and **2** with ^{99m}Tc glucoheptonate gave a 4.7:1 and 12: 1 mixture of two ^{99m}Tc complexes respectively as determined by HPLC in 20-70% radiochemical yield. Biodistribution data of the major [^{99m}Tc]-**1** and [^{99m}Tc]-**2** products in CD-1 mice (n=4-5) showed very little uptake and no regional selectivity in the mouse brain. For whole brain, the % injected dose values were 0.12% and 0.04% for [^{99m}Tc]-**1**, and 0.09% and 0.06% for [^{99m}Tc]-**2** at t = 5 and 30 minutes post injection. For *in vitro* studies, DADT BVM **1** was also radiolabeled with ^{99}Tc glucoheptonate. The major [$^{99m/99}$Tc]-**1** product exhibited a log P = 3.13 ± 0.06 (S.D.) with an IC_{50} = 140-280 nM vs. (-)-N-[^{3}H]methyl-5-aminobenzovesamicol.

In vitro and *in vivo* data for ^{99m}Tc-**1** and **2** suggest that its low uptake and retention in brain are probably due to a combination of both increased size and reduced binding affinity. These results help define the limitations imposed on the design of a ^{99m}Tc labeled vesamicol analog for *in vivo* cholinergic nerve mapping. Future efforts will be guided by these constraints. This work was funded by N. I. H. Grants Nos. NS 25656 and CA32845 and the University of Michigan Memorial-Phoenix Project 790-364417.

References

1. Jung, Y. W., Van Dort, M. E., Gildersleeve, D. L. and Wieland, D. M. (1990) *J. Med. Chem.* **33**, 2065-2068.
2. Jung, Y-W., Van Dort, M.E., Gildersleeve, D.L., Kuhl, D.E. and Wieland, D.M. (1993) *J. Label. Cmpds. Radiopharm.* **32**, 307-310.
3. Kuhl, D.E., Koeppe, R.A., Fessler, J.A., Minoshima, S., Ackermann, R.J., Carey, J.E., Frey, K.A. and Wieland, D.M. (1993) *J. Nucl. Med.* **34**, 25.
4. Rogers, G.A., Parsons, S.M., Anderson, D.C. et al. (1989) *J. Med. Chem.* **32**, 1217-1230.
5. Baidoo, K. E. and Lever, S.Z. (1990) *Tet. Letters*, 5701-5704.
6. Shiba, K., Mori, H., Matsuda, H., Tsuji, S., Kinuya, K. and Hisada, K. (1991) *Nucl. Med. Biol.* **42**, 1159-1164.

Scheme 1


X = I, NH2
1) NaNO2, HCl, NaHCO3
2) NiCl2/KCN
3) LiAlH4/THF/Δ
A
B
1)
2)
LiAlH4/THF/Δ
2) 99mTc(=O)L2-
[99mTc]-1
2

Labeling Antisense Deoxyoligonucleotide with Tc-99m and Hybridization with c-myc Oncogene MRNA in P388 Leukemic Cells. M.K. DEWANJEE, A.K. GHAFOURIPOUR, M. SUBRAMANIAN, M. HANNA, M. KAPADVANJWALA, A.N. SERAFINI, S. EZUDDIN, D. LOPEZ, G.N. SFAKIANAKIS. University of Miami, Miami FL & BRI, Rockville MD.

The c-myc oncogene is amplified in leukemia, lymphoma, breast and small cell lung cancer, making the c-myc mRNA a suitable target-receptor for following the progression of malignancy by noninvasive imaging (1-4). The radiolabeling of antisense deoxyoligonucleotide derivatives with I-125 and In-111 were described previously (1-2). The antisense probe works as "molecular velcro", sticking to the specific complementary sequence of oncogene or other sequence of interest. The deoxyoligonucleotides specific for binding mRNAs of histone2/4, proliferating cell nuclear antigen (PCNA), actin, cyclin2 and c-myc oncogene were modified at the 5'terminus for conjugation with paramethoxyphenylisothiocyanate for radioiodination and polyaminopolycarboxylate for chelation with In-111 radionuclide. In this study, we extended radiolabeling techniques further by chelating the most useful and versatile radionuclide of Tc-99m. Both the phosphodiester and phosphorothioate derivatives [diethylenetriaminepentaacetate (DTPA)- and tetraethylene-hexaamineoctaacetate-(LiLo)benzylisothio-cyanates] were labeled by transchelation with oxo-technetium cation produced by the stannous glucoheptonate (Ca-glucoheptonate: 50 mg and stannous chloride dihydrate 0.7-1.1 mg, Merck Frosst Canada Inc.) reconstituted with 1-2 ml of sterile saline. The 15-mer oligonucleotide (ON) sequence was synthesized, aminolinked [sense (SN) and antisense (AS) phosphodiester (O) and monothioester (S)] and coupled to DTPA-isothiocyanate and aliquots (10μg) were lyophilized. Fifty-hundred mCi of Tc-99m pertechnetate (Mo-99-->Tc-99m generator, Medi-Physics, Inc.) were incubated with 50-100 mCi of pertechnetate in saline at room temp. Unbound Tc-99m was removed by gel-filtration (Sephadex G-25) with 0.1 M metal-free phosphate buffered saline (pH=7.05). The labeling efficiency (>95%) was checked with paper and high pressure liquid chromatography (Waters, Model 600E, MA). The retention times of the Tc-99m oligonucleotide and pertechnetate were 16.1 and 18.6 min. respectively.

The kinetics of hybridization was studied by incubation of cytoplasmic extracts of c-myc mRNA (supernatant of homogenate of P388 leukemic cells), with Tc-99m labeled sense and antisense probes. The mRNA-probe complex, unbound radiolabeled oligonucleotide and degradation products, were analyzed by the HPLC analysis (size exclusion, TSK-300 column) technique; the retention time of the probe-mRNA complex, probe and probe-fragments was 8.0, 16.1 and 17.0 minutes respectively. The integrated area under the curve permitted the study of the kinetics of hybridization of antisense probe with c-myc mRNA. The uptake (%) of antisense probe by P388 cells, reaches a saturation level at 45 min of incubation. Like the cellular uptake, the binding of antisense Tc-99m probe with mRNA reaches a saturation value at 45 minutes of incubation. On the other hand, there is very little hybridization of the Tc-99m labeled sense probe with the c-myc mRNA. The low rate constant

values for the sense probe also indicated lower level of binding. This new HPLC technique for hybridization study, may permit evaluation of other amplified oncogenes (ras, erbB2, myc) with radiolabeled antisense probes in cancer cells and tumor tissues in live tumor-grafted animals and cancer patients.

References

1. Dewanjee M.K. Ghafouripour A.K. Werner R.K. Serafini A.N. Sfakianakis G.N. - Development of sensitive radioiodinated antisense oligonucleotide probes by conjugation technique. Bioconj Chem 2: 195-200(1992)
2. M.K. Dewanjee, G.K. Panoutsopoulos, A.K. Ghafouripour, M. Kapadvanjwala, T. Ionescu, A. Ganju-Krishan, S. Glenn, T. Boothe, R. Werner, R. Vargas-Cuba, A.N. Serafini, G.N. Sfakianakis. - Metabolic fate of indium-111 labeled anti-sense deoxyoligonucleotide (ASDON) in a pig model. Proc. Miami Bio/Technology Winter Symposium, January 17-22, 1993, p 112.
3. Dewanjee M.K. - The chemistry of ^{99m}Tc-labeled radiopharmaceuticals. Sem Nucl Med XX, 5-27(1990)
4. Dewanjee M.K. - Radiololabeled antisense probes: diagnosis and therapy. Diagnostic Oncology (supplement) (1993)
Supported by NHLBI (HL47201, Shannon Award), NIH-NS22603-08, Baxter Healthcare Corporation and DOE grant DE-FG05-88ER60728.

Figure 1. General scheme of radiolabeling of antisense deoxyoligonucleotide probes with DTPA-derivatives for labeling with Tc-99m radionuclide.

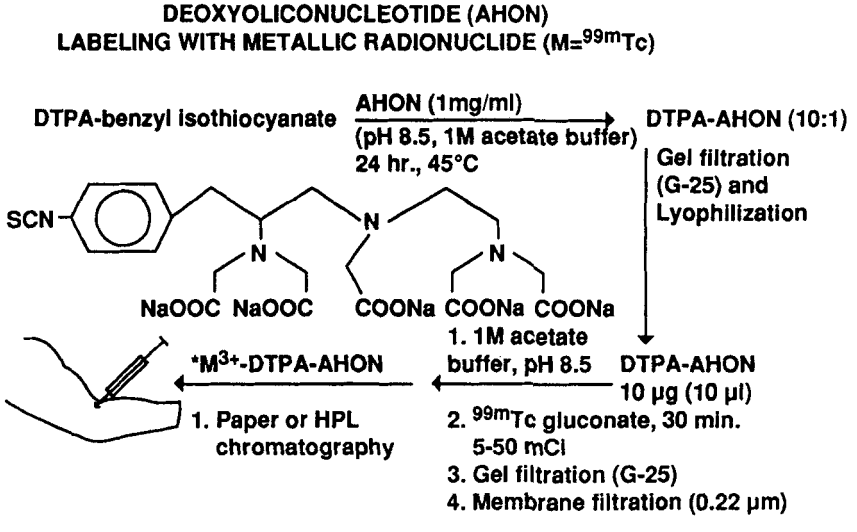
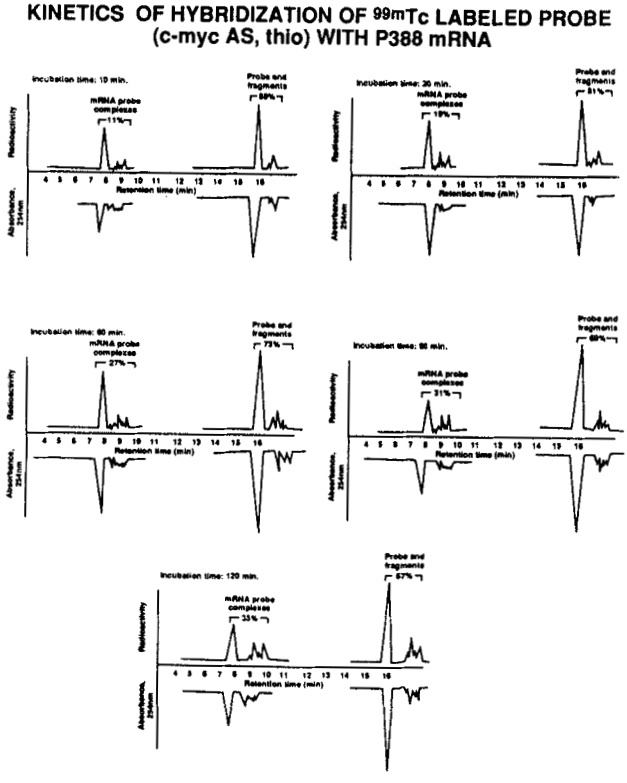


Figure 2. Kinetics of intracellular hybridization of c-myc oncogene mRNA with Tc-99m labeled antisense deoxyoligonucleotide (TCASDON) probes in leukemic cells (P388).



Tc-99m Labeled Peptides: Site and Nature of Radiolabeling and Its Effect on Pharmacokinetics and Biodistribution.

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In the last 2 decades, a number of radiolabeled antibodies and their fragments were evaluated as diagnostic tracers in nuclear medicine. A major limitation of these agents is the fact that they are high molecular weight substances and clearance characteristics were not greatly altered by chemical modification of the carrier or changing the radiolabel. It has been shown recently that small peptides with specific binding to target tissue offer many advantages of radiolabeling as well as alteration of clearance characteristics by incorporation of appropriate chelating agents in the structure. It has been shown that radiolabeled peptides show great potential as diagnostic tracers for imaging atherosclerosis, infection and tumor in vivo. (1-4).

We have developed a number of small synthetic peptides for different imaging studies and radiolabeled these molecules with Tc-99m using various types of chelators which vary the charge and lipophilicity of the Tc-99m peptide complex. The nature and location of chelator group within a peptide molecule greatly influences the pharmacokinetics and biodistribution of the tracer. The labeling conditions may also affect the integrity of the peptide. We have chosen to use bisamide bisthiol and bisamine bisthiol types of N_2S_2 and triamide thiol type N_3S chelators which can be labeled with Tc-99m in near quantitative yield within 15 minutes at either room temperature or at 100°C by ligand exchange with a labile intermediate such as Tc-99m glucoheptonate. Several examples are discussed here.

Peptides containing chelators for Tc-99m were prepared by solid phase peptide synthesis on an ABI 431A synthesizer using Fmoc chemistry and were purified by reverse-phase HPLC using a Waters Delta Pak C18 column and a gradient of acetonitrile in water containing 0.1% TFA. Peptide structures were confirmed by FABMS. The radiochemical yield/purity of the Tc-99m labeled peptides was determined by reverse-phase HPLC and TLC.

SP-4 is a synthetic oligopeptide (17 amino acids) and was shown to localize in atherosclerotic lesions (1,4). The C-terminal of SP-4 was coupled to a chelating agent based on an N_2S_2 ligand (P215) or an N_3S ligand (P199). The peptides were then labeled with Tc-99m by exchange labeling using Tc-99m glucoheptonate. ^{99m}Tc-P199 contains an anionic complex while ^{99m}Tc-P215 contains a neutral and lipophilic complex. In rabbits, plasma clearance of these two tracers (Figure 1) was quite similar but much faster compared to radioiodinated SP-4 (which was not coupled to any chelator group). The biodistribution and tissue uptake and clearance of these two tracers were significantly different (Table 1).

To develop Tc-99m labeled peptides for imaging infection, analogs of peptides known to be chemotactic for neutrophils were modified to contain chelating moieties for

Tc-99m. P84 and P322 are chemotactic peptides derived from platelet factor 4, each containing a bisamide bisthiol chelator (known to form anionic technetium complexes). P322 is an extended form of P84 containing additional charged amino acids (23 versus 18 aminoacids) designed to enhance renal clearance. P304 is an formyl-Met-Leu-Phe type tetrapeptide containing a lipophilic bisamine bisthiol chelator (known to form neutral technetium complexes). The three Tc-99m labeled peptides showed marked differences in tissue distribution, clearance, (Table 2) as well as cell binding and infection localization.

1. Shih.-L, Lees R.S., Chang M., Lees A.M.- Proc.Natl.Acad.Sci. USA. 87: 1436(1990)
2. Fischman A.J., et al- J.Nucl.Med. 32: 483(1991)
3. Bakker W.H., et al- Life Sciences 49: 1593(1991)
4. Vallabhajosula S., et al- J Nucl Med. 32: 1842(1991)

TABLE 1: Biodistribution of Tc-99m Labeled Peptides For Imaging Atherosclerosis

Organ	% I.D. per organ at 4 hour post injection	
	Tc-99m-P199*	Tc-99m-P215*
Blood	0.26 ± 0.07	0.09 ± 0.06
Lung	0.28 ± 0.02	0.08 ± 0.01
Liver	2.14 ± 0.19	27.6 ± 4.70
Kidney	14.1 ± 2.60	1.34 ± 0.14
Spleen	0.19 ± 0.02	0.06 ± 0.03
Gut	21.5 ± 2.72	43.2 ± 3.10
Bladder and Urine	43.8 ± 2.64	9.32 ± 4.40

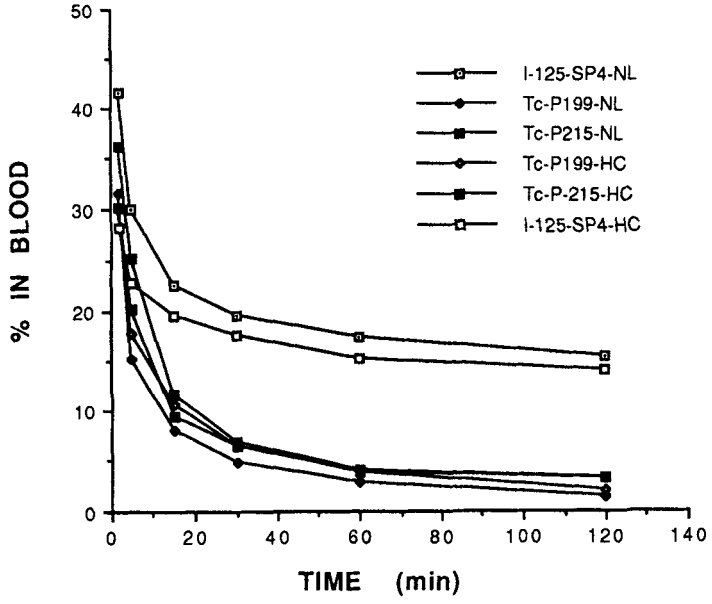
* Mean ± S.D. (n = 5)

TABLE 2: Biodistribution of Tc-99m Labeled Peptides For Imaging Infection

Organ	% I.D. per organ at 4 hour post injection		
	Tc-99m-P84*	Tc-99m-P322*	Tc-99m-P304*
Blood	0.78 ± 0.11	3.10 ± 0.49	0.44 ± 0.05
Lung	1.33 ± 0.15	0.35 ± 0.03	0.43 ± 0.06
Liver	2.97 ± 0.32	7.04 ± 0.73	5.25 ± 0.35
Kidney	5.18 ± 0.76	34.4 ± 1.80	0.88 ± 0.39
Spleen	0.24 ± 0.05	0.16 ± 0.02	0.28 ± 0.06
Gut	46.9 ± 3.24	8.40 ± 2.56	80.4 ± 5.10

* Mean ± S.D. (n = 5)

Figure 1: BLOOD CLEARANCE OF RADIOLABELED PEPTIDES IN RABBITS



NL: Normal rabbits

HC: Hypercholesterolemic rabbits

Evaluation of ^{99m}Tc-mercaptoacetyl-albumin and ^{99m}Tc-dimercaptopropionyl-albumin in a volunteer as blood pool agents for ventriculography.

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From a practical point of view, technetium-99m labelled human serum albumin (^{99m}Tc-HSA) is a much safer and easier-to-prepare radiopharmaceutical for radionuclide ventriculography as compared to ^{99m}Tc-labelled autologous red blood cells (^{99m}Tc-RBCs). However, the relatively rapid diffusion of the activity out of the vascular compartment and the significant renal elimination[1,2] make ^{99m}Tc-HSA less attractive as a blood pool agent.
To improve the labelling strength of HSA, and in this way enhance the blood retention, we have derivatized HSA by introduction of some mercaptoacetyl (MA) or 2,3-dimercaptopropionyl (DMP) side chains on the lysine residues of albumin according to an earlier described procedure[3].

After purification, the resulting MA-HSA and DMP-HSA can easily be labelled at room temperature by simple addition of Sn^{2+} ions and pertechnetate.
After a thorough evaluation in mice and rabbits[3], the FPLC-purified ^{99m}Tc-MA-HSA and ^{99m}Tc-DMP-HSA preparations were tested in a healthy volunteer and compared to the commonly used radiopharmaceuticals for ventriculography: ^{99m}Tc-RBCs and ^{99m}Tc-HSA (reconstituted from a Technescan-HSA kit, Mallinckrodt Medical, The Netherlands).
The radiolabelled preparations were diluted with saline to a concentration of 3.7 MBq/ml and 0.5 ml was injected via an arm vein. At fixed time intervals, 2-ml whole blood samples were obtained to construct the time-activity curves for the four preparations (fig.1). The activity in the blood at 5 min p.i., i.e. after sufficient mixing of the injected substances with the blood, was equalled to 100% and the activities at the other time intervals were related to this value. After injection of ^{99m}Tc-HSA, the activity in the blood decreases continuously during the two hours of investigation. Introduction of mercaptoacetyl groups results in a ^{99m}Tc-preparation with a clearly higher retention in the blood. As could be expected, introduction of a diligand ^{99m}Tc-binding system (DMP-HSA) yields an even more stable ^{99m}Tc-chelate than when a monoligand was introduced (MA-HSA). Indeed, the blood retention of ^{99m}Tc-DMP-HSA is almost identical to that of ^{99m}Tc-RBCs.
The four preparations exhibit a bi-exponential clearance from the intravascular compartment with a minor fast component and a major slow component. The T1/2 values and proportions of both components are presented in table 1. Introduction of side chains on albumin results in an obvious increase in T1/2 of the slow component: from 4.3 h for conventional ^{99m}Tc-HSA to 10.1 h for ^{99m}Tc-MA-HSA and 75.3 h for ^{99m}Tc-DMP-HSA which is even longer than the T1/2 of ^{99m}Tc-RBCs.
The volunteer was asked to void at 30 min, 60 min and 120 min after injection and the percentage of injected dose excreted at these time intervals was determined without correction for the postvoid residual urine. The cumulative urinary excretion of the ^{99m}Tc-activity after administration of these preparations is shown in figure 2. Two hours after injection, the percentage of injected dose excreted in the urine was less than 3% for ^{99m}Tc-MA-HSA, ^{99m}Tc-DMP-HSA and ^{99m}Tc-RBCs. However, when ^{99m}Tc-HSA was used, 4.5% of the injected dose was already excreted during the first 30 min and 17% after 2 h.

These results clearly show that a real improvement in vascular retention has been achieved by the introduction of a number of mercaptoacetyl and, especially, dimercaptopropionyl side chains on albumin. It can be reasonably expected that derivatization with a tri- or tetraligand system will result in an even more stable ^{99m}Tc-labelled derivatized albumin preparation.

Table 1. T1/2 and proportion of the fast and slow component of the different preparations.

	fast component		slow component	
	T1/2 (min)	proportion (%)	T1/2 (h)	proportion (%)
^{99m}Tc-HSA	9.86	25.31	4.31	74.69
^{99m}Tc-MA-HSA	9.47	17.94	10.09	82.86
^{99m}Tc-DMP-HSA	5.96	16.75	75.35	83.25
^{99m}Tc-RBCs	4.76	13.00	31.00	87.00

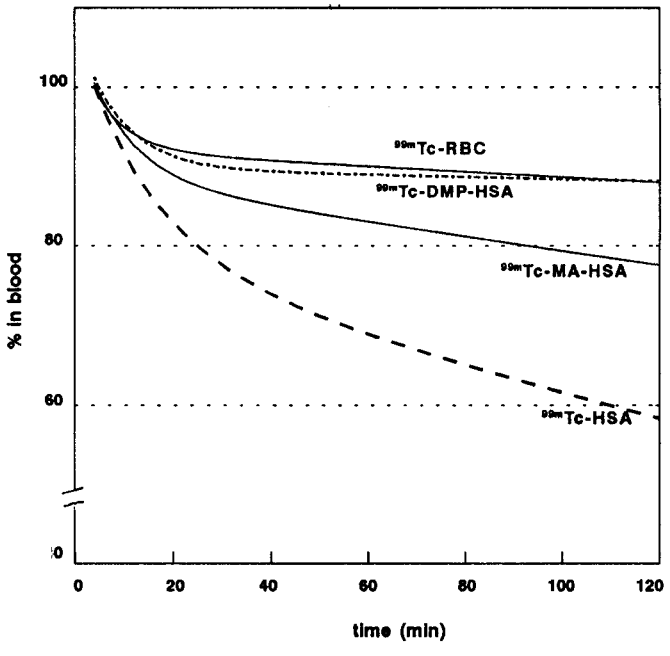

Fig.1. Blood time-activity curves of the tested agents in the same volunteer

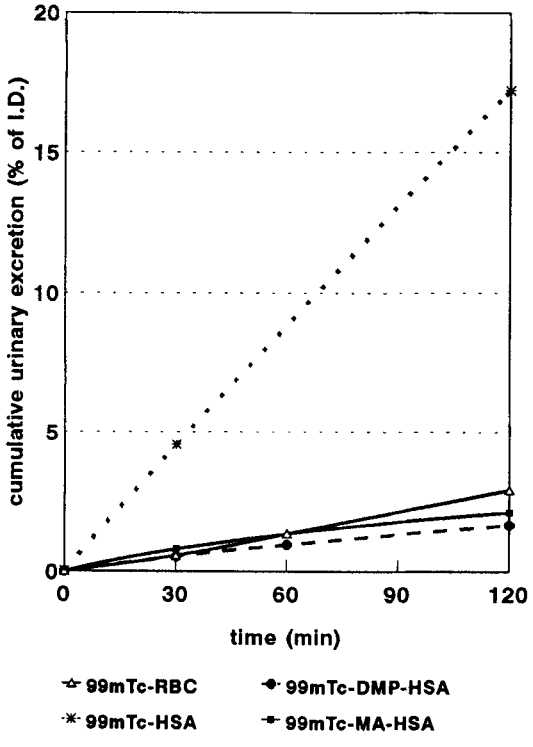

Fig.2. Cumulative urinary excretion of the tested agents in a volunteer

References

1. Thrall J.H., Freitas J.E., Swanson D., Rofers W.L., Clare J.M., Brown M.L., Pitt B. - J Nucl Med 19:796 (1978)

2. Atkins H.L., Klopper J.F., Ansari A.N. - Clin Nucl Med 5:166 (1980)

3. Verbeke K, Vanbilloen H, De Roo M, Verbruggen A. - Eur J Nucl Med 19:1993 (in press)

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Comparison of different methods to improve the ^{99m}Tc-chelating strength of human serum albumin

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^{99m}Tc-labelled autologous red blood cells (^{99m}Tc-RBCs) are considered as the most appropriate radiopharmaceutical for radionuclide ventriculography, although they suffer from some practical disadvantages such as occasional low labelling efficiency (in vivo labelling), risk of viral contamination due to handling of blood samples and the time consuming labelling procedure (in vitro labelling). Human serum albumin (HSA) is easy to label with ^{99m}Tc but does not form a stable complex. This results in a rapid loss of the label and diffusion of the activity out of the vascular compartment.

To improve the labelling strength of HSA, we have derivatized albumin in different ways, namely by coupling of albumin with DTPA and by the introduction of a number of mercaptoacetyl or 2,3-dimercaptopropionyl side chains .

DTPA-albumin was prepared by reaction of albumin with DTPA-cyclic anhydride at pH 7.0 as described by Hnatowich et al[1]. Mercaptoacetyl and dimercaptopropionyl groups were introduced using respectively N-succinimidyl S-acetylthioacetate (SATA) and N-succinimidyl 2,3-di(S-acetylthio)propionate (SATP) (fig.1) according to an earlier described procedure[2] (fig.2). After deprotection with hydroxylamine and purification, the resulting mercaptoacetyl-HSA (MA-HSA) and dimercaptopropionyl-HSA (DMP-HSA) can easily be labelled at pH 7.0 by simple addition of Sn^{2+} and pertechnetate (≥90% labelling yield). Labelling of DTPA-HSA with ^{99m}Tc under the same conditions resulted in an 80% labelling yield. However, FPLC-analysis on a Superdex 200 HR column (Pharmacia, Sweden) revealed a high amount of polymeric albumin (up to 30%). Literature data and our own results indicate a faster blood clearance of polymeric forms of albumin. Therefore, DTPA-albumin was considered as a less suitable HSA-derivative for stable labelling with ^{99m}Tc and was not tested furthermore.

Earlier results have shown the importance of an optimal number of introduced side chains for both stable labelling and minor alteration of the biological properties of albumin preparations. The best results with respect to blood retention were obtained after derivatization of albumin with SATA in a 1:25 molar ratio or reaction with SATP in a 1:8 molar ratio. This corresponds with the introduction of 6 mercaptoacetyl groups or 2 dimercaptopropionyl groups (determined using Ellman's reagent[3,4]).

The stability of the optimal ^{99m}Tc-MA-HSA and ^{99m}Tc-DMP-HSA preparations was tested by cysteine challenge experiments. Monomeric ^{99m}Tc-MA-HSA and ^{99m}Tc-DMP-HSA were isolated by FPLC and incubated with cysteine in two molar ratios (1:300 and 1:30,000). After 3 h, the various preparations were tested in mice using ^{125}I-HSA as an internal biological standard. The results of the biodistribution studies are shown in table 1 and 2. In the case of ^{99m}Tc-MA-HSA, the urinary excretion increases and the blood retention decreases significantly after incubation with cysteine, which indicates a rather high exchange with cysteine and, consequently, a relatively weak bond between ^{99m}Tc and MA-HSA. On the other hand, the results obtained with ^{99m}Tc-DMP-HSA are indicative of a much stronger bond between ^{99m}Tc and the albumin derivative. After incubation with cysteine in a 1:300 molar ratio, only a slight decrease in blood retention was observed. This could have been expected as the introduced dimercaptopropionyl groups create a diligand chelating system whereas the mercaptoacetyl groups only form a monoligand system.

According to these results, derivatization with the dimercaptopropionyl side chain seems to be an attractive and efficient method to modify albumin and possibly other proteins for stable labelling with ^{99m}Tc.

References

1. Hnatowich D.J., Layne W.N., Childs R.L., Lanteigne D., Davis M.A., Griffin T.W., Doherty P.W. - Science 220:613-615 (1983)
2. Verbeke K., Vanbilloen H., De Roo M., Verbruggen A. - Eur J Nucl Med 19 (1993) (in press)
3. Ellman G.L. - Arch. Biochem. Biophys. 74:443-450 (1958)
4. Ellman G.L. - Arch. Biochem. Biophys. 82:70-77 (1959)

[a] Research assistant of the Belgian National Fund for scientific research

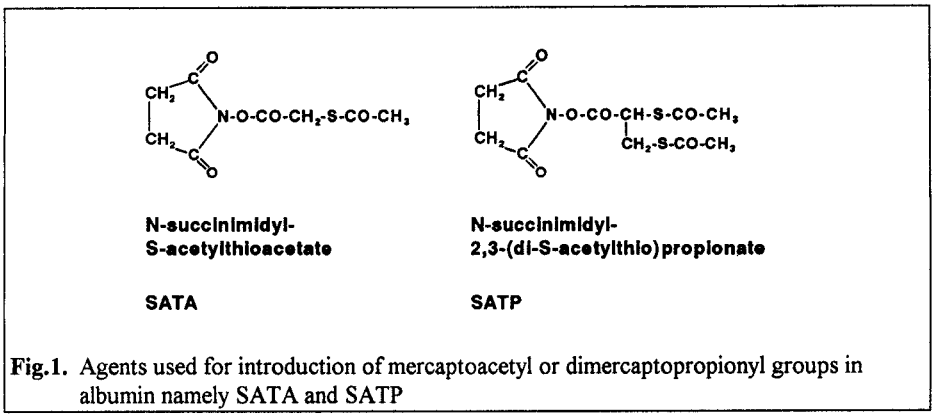

Fig.1. Agents used for introduction of mercaptoacetyl or dimercaptopropionyl groups in albumin namely SATA and SATP

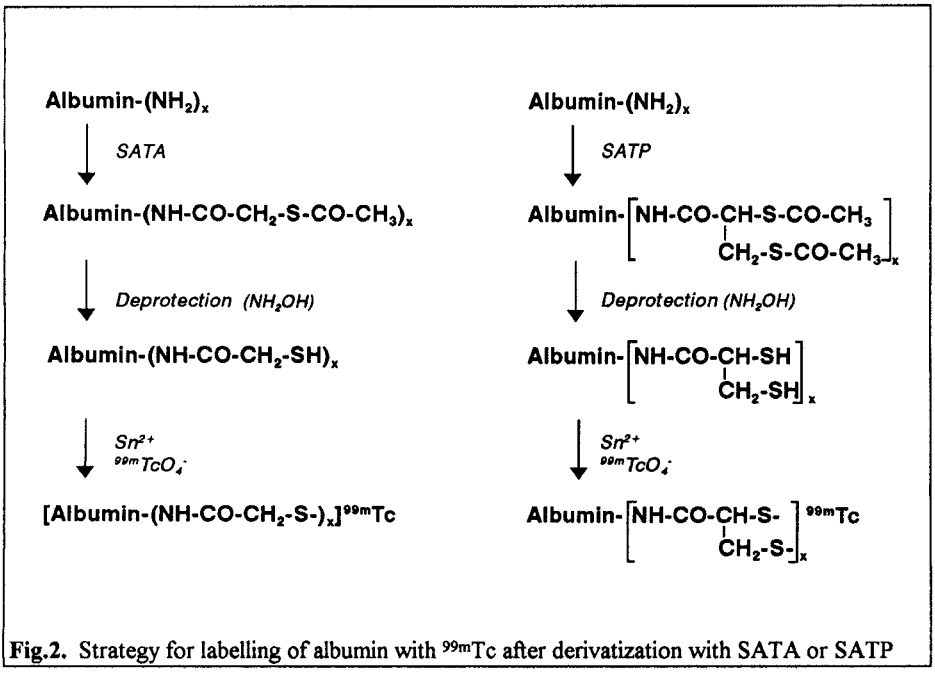

Fig.2. Strategy for labelling of albumin with ^{99m}Tc after derivatization with SATA or SATP

Table 1. Influence of the presence of cysteine on the biodistribution of ^{99m}Tc-MA-HSA and ^{99m}Tc-DMP-HSA in mice (n=2) at 10 min and 60 min p.i.

	% of injected dose in organs					
	blood		liver		urine + kidneys	
	10'	60'	10'	60'	10'	60'
^{99m}Tc-MA-HSA	75.1	64.6	13.5	14.6	4.5	4.8
^{99m}Tc-MA-HSA: cysteine (1:300)*	61.7	46.1	9.9	10.1	18.3	31.8
^{99m}Tc-MA-HSA: cysteine (1:30,000)*	31.5	23.2	19.5	22.8	24.3	55.7
^{99m}Tc-DMP-HSA	78.3	71.5	10.9	10.3	5.0	6.6
^{99m}Tc-DMP-HSA: cysteine (1:300)*	77.3	69.2	10.3	9.6	8.1	12.5
^{99m}Tc-DMP-HSA: cysteine (1:30,000)*	64.8	55.2	12.5	12.9	13.9	16.8
^{125}I-HSA	84.2	77.7	11.0	9.5	4.3	4.0

* after an incubation of 3 h

Kinetic Behavior of Dilute Aqueous Solution of ^{99}Tc-HMPAO Diastereoisomers

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The Ceretec (Kit product of Amersham International plc Buckinghamshire, U.K.) has been widely employed for the diagnosis of regional cerebral blood flow in the patient with neurological disorders and for labelling of leukocyte (in vitro)[1,2]. However, it should be pointed out that this agent also showed some drawbacks, i.e. the reconstituted solution of the Ceretec for intraveneous injection or in vitro leukocyte labelling has to be used up within a maximum of 30 minutes after reconstitution, and any unused material must be discarded, because it was found that the concentration fo ^{99m}TcO-d,l-HMPAO in the reconstituted solution is decreasing considerably with time[3,4].

A number of workers have devoted their efforts to exploer the causes of the instability inherent to the reconstituted solution. However, so far not much convincing finding has been obtained. It must be pointed out that in most of investigation, only the Cereter kit was used to prepare the ^{99m}TcO-d,l-HMPAO with ca 20mCi of pertechnetate solution. Thus only a very trace amount of the ^{99m}TcO-d,l-HMPAO in a very small volume of solution (ca 0.5ml) was used during their course of study. It was noted that the starting concentration of ^{99m}TcO-d,l-HMPAO used for decomposition-rate study was estimated to be ca 10^{-8} M.

In an attempt to obtain much better understanding on the aspect of stability of ^{99m}Tc-HMPAO a large amount of d,l-HMPAO and

meso-HMPAO were obtained in our labortory by improved method, their characteristic properties such as I R, N M R m.p., UV-visible spectra were measured. A large quautity of ^{99}TcO-HMPAO diasteroisomer were preared[6], their physical and chemical properties were charaterized. Stock solutions of ^{99}TcO-HMPAO diastereoisomers were perpared in chloroform, acetonitrile and mixture of acetonitrile and water (1:1). The stability of ^{99}TcO-HMPAO diastereoisomers were studied by measuring the decomposition rate of the complexes. Changes in the concentration of complexes were followed by UV-visible spectrophotometry and the temperature were controled at 25.0±0.1℃ in air.

In this study the following kinetic runs were performed:

1. Heterogeneous system:
   (a) ^{99}TcO-HMPAO in $CHCl_3$was extraced with concentrated base solution (1.0M)
   (b) ^{99}TcO-HMPAO in $CHCl_3$ was extracted with weak acid solution (pH=6 to pH=1)
   (c) ^{99}TcO-HMPAO in $CHCl_3$ was extracted with stronger acid solution (0.05M $HClO_4$ to 0.5M $HClO_4$).
2. Homogeneous system
   ^{99}TcO-HMPAO in the mixture of acetonitril and water (1:1) was reacted with different acidic solution (0.01 to 0.5 M $HClO_4$)
3. Temperature effect on reaction rates (25℃,36℃,35℃,40℃)
   With all kinetic data collected a plausible reaction mechanism will be suggested and all of the rate constants will be presented to support the proposed mechanism.

Reference:

1. R. D. NerincKx, R. C. Harrison, A. M. Foster, et.al. J. Nucl, Med. 28 559(1987).
2. P.F. sharp, F.N. Smith, H.G. Gemmell, el.al., J. Nucl, Med, 27 171(1986).
3. J. C. Hung, M. Corlija, W. A. volkert, R. A. Holmes, J. Nucl. Med. 29 1968(1968).
4. H. J. Ding and S.J. Yeh, Intl. J. Appl. Radiat, Isot. 43 1013 (1992)
5. S. Jurisso, E.O. Schlemper, D. E. Toutner et. al. Inong, Chem. 25 543(1986).

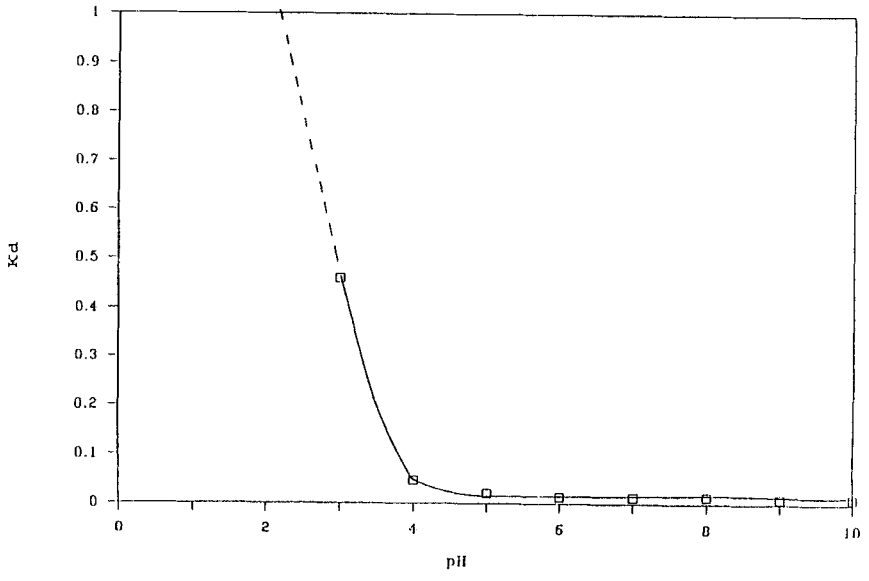

Fig1. Rate of Concentration Decrease in ^{99}TcO-d,l-HMPAO Solution

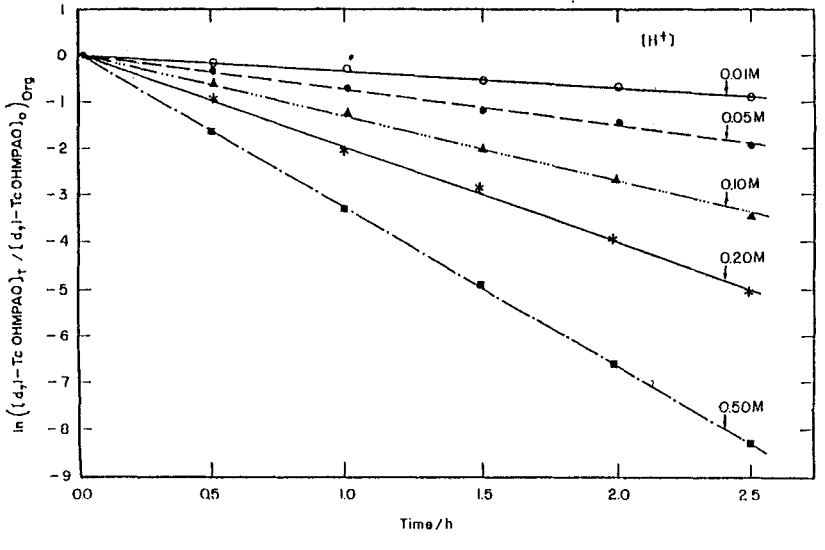

Fig2. ^{99}TcO-d,l-HMPAO in Chloroform Various Perchloric acid.

EXAMINATION OF ^{99}Tc-HMPAO COMPLEX FORMATION IN AQUEOUS MEDIA

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^{99m}Tc-hexamethyl propyleneamine oxime (HMPAO) is a well-known cerebral radiopharmaceutical, introduced in 1987 (1). Its only disadvantage is a limited shelf-life after reconstitution, so many researchers tried to solve this problem (2-9). Nevertheless, the chemistry of ^{99m}Tc-HMPAO in aqueous solution is not still understood. While ^{99}Tc-HMPAO complexes were synthesized, isolated and their structure in solid state established (10), the reaction of ^{99m}Tc complex formation and interconversion in aqueous solution, as well as interaction with tin is not still elucidated.

The aim of this work was to study complex formation of HMPAO ligand and the long-living isotope Tc-99 in aqueous solution as a model system for ^{99m}Tc-radiopharmaceutical.

Materials and Methods

4,8-diaza-3,6,6,9-tetramethyl-undecane-2,10-dione bisoxime (HMPAO) was synthesized in our Laboratory in gram quantities (yield 45-50%) according to Neirinckx et al. (1,11). The starting substances, purchased from Aldrich Cnem. Co., Milwaukee, WI, USA were previously purified: 2,3-butanedione monoxime by recrystallzation from aqueous solution, while 2,2-dimethyl -1,3-propanediamine by distillation (purity of both was checked by gas chromatography). Diastereoisomers dl- and meso- were separated by successive recrystallizations from acetonitrile and ethylacetate. Their chemical purtiy was checked by melting point determination, by normal phase HPLC (column Supelco LC-Si, Gland, Switzerland) with UV-detector set at 210 nm and with ammonia-methanol eluant, as well as by spectroscopy (IR, ^{1}H- and ^{13}C-NMR). For our spectrophotometric study, meso-HMPAO with melting point 145^{o}C was used.

Interaction of Tc-99 with meso-HMPAO was examined in neutral, alkaline (pH 9-9.5) and acidic (pH 2) media with constant concentrations of the ligand ($1x10^{-3}$M) and Tc-99 ($1x10^{-4}$M), while Sn/II/ concentrations were varied so that molar ratio of Sn:Tc was 0.5:1; 1:1; 1.5:1; 2:1; 2.5:1, and 3:1.

Technetium-99 was used in the form of NH_4TcO_4 purchased from the Radiochemical Centre Amersham, England. A stock solution of NH_4TcO_4 (concentration reported by the producer) was made by dilution and standardized by measuring ultraviolet absorbance at 248 nm using a molar extinction coefficient of 6220 $M^{-1}cm^{-1}$ (13) or 5690 at 244 nm and 2170 at 287 nm, respectively (12,13).

Stannous chloride dihydrate (Merck, Darmstadt, Germany) was used as a reductant; also all other reagents were of analytical grade of purity.

Ultraviolet and visible spectra were recorded from 190 to 500 nm using spectrophotometer UVIKON 810/820 (Kontron, Austria) in a 10 mm quartz cell.

The complex formation was the most clearly expressed in alkaline solutions. Due to this fact and especially to their importance for radiopharmaceutical application, complex formation in alkaline media was investigated by continual variation method (14). Solution mixtures were prepared so that reactant concentrations varied in the following ranges: HMPAO $1x10^{-4}$M to $9x10^{-4}$M; Sn/II/ $1.35x10^{-3}$M to $1.5x10^{-4}$M, and $^{99}TcO_4$ $9x10^{-4}$M to $1x10^{-4}$M. This was achieved by mixing 10-x mL of $1x10^{-3}$M HMPAO and x mL of equi-volume Sn-Tc mixtures (molar ratio Sn:Tc=1.5:1; concentrations $SnCl_2$ $7.5x10^{-3}$M and Tc $5x10^{-3}$M), then pH adjusted to 9.5.

Results and discussion

On the basis of the spectrophotometic study, the complex formation between HMPAO ligand and technetium (previously reduced by stanous ions) was found out and confirmed. The complexation in alkaline and neutral media takes place, whereas for acidic medium it is not certain.

^{99}Tc-HMPAO complex is yellow coloured, characterized by absorption maximum at 265-275 nm and by an inflection at 325-340 nm.

In order to avoid ambiguous results, we checked absorption sectra of the

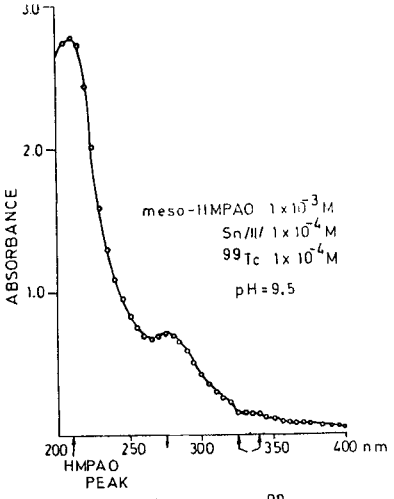

Fig. 1. UV-SPECTRUM OF ^{99}Tc-HMPAO

following solutions (in the concentration range and wavelength range of interest): HMPAO , $SnCl_2$-HMPAO, TcO_4^-, $SnCl_2$-TcO_4^-, and TcO_4^--HMPAO, so possible interference of the mentioned components was excluded.

An example of the recorded spectra is shown in Fig. 1.

By plotting a relationship between absorbance values at 335 nm and molar ratio, it was found out that for all spectra the molar radio Sn:Tc=1.5:1 is a characteristic one, so it can be supposed that Tc/IV/ is in the complex in neutral and alkaline medium. This conclusion should be approved by another method, e.g. redox potentiometric titration.

^{99}Tc-HMPAO complex composition was investigated using the Job's method and the plotted curve is shown in Fig. 2.

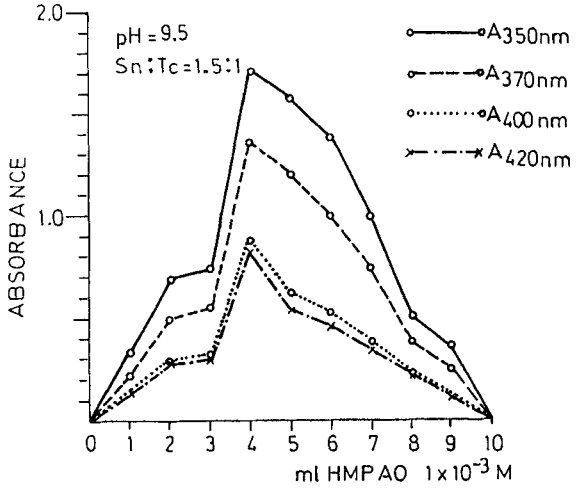

Fig. 2. JOB'S CURVE

From Job's curve it can be concluded: a) in relationship with all chosen absorbances, it is clear that a complex is formed with molar ratio HMPAO:Tc=2:3;

b) in relationship to A_{420} and A_{400}, it can be seen an inflection at 5 mL, corresponding to HMPAO:Tc=1:1.

It can be considered as a logic possibility because the ligand has two OH (oxime) groups, and also the coordination is possible at imine nitrogen atoms.

REFFERENCES

1. Neirinckx R., Canning L., Piper I., Nowotnik D., Pickett R., Holmes R., Volkert W., Forster A., Weisner P., Marriott J., and Chaplin S. - J.Nucl.Med. 28:191(1987)
2. Hung J., Corlija M., Volkert W., Holmes R.-J.Nucl.Med. 29:1568 (1988)
3. Karesh S.-J.Nucl.Med.Technol. 17:215(1989)

4. Ballinger J.-J.Nucl.Med. 31:1892(1990)
5. Hung J., Volkert W., Holmes R.-Nucl.Med.Biol. 16:675(1989)
6. Ponto J.-Am.J.Hosp.Pharm. 47:2511(1990)
7. Ballinger J., Gulenchyn K., Reid R.-J.Nucl.Med. 31:118(1990)
8. Ballinger J. and Gulenchyn K.-Appl.Radiat.Isot. 42:315(1991)
9. Tubergen K., Corlija M., Volkert W., Holmes R.-J.Nucl.Med. 32:111(1991)
10. Jurisson S., Schlemper E., Troutner D., Canning L., Nowotnik D., Neirinckx R.-Inorg.Chem. 25:543(1986)
11. Canning L., Nowotnik D., Neirinckx R., Piper I. - Europen Patent 0 194 843, March 1986, Amersham
12. Rulfs C.-CRC Critical Reviews inAnalytical Chemistry, p.335 CRC Press, Cleveland, OH, 1970
13. Mullen P., Schwochau K., Jorgensen C. - Chem.Phys.Letters 3:49 (1969)
14. Job P. - Ann.Chim. 9:113(1928)

Synthesis of BAT-Ligands with Quaternary Amino Groups and Myocardial Uptake Studies.

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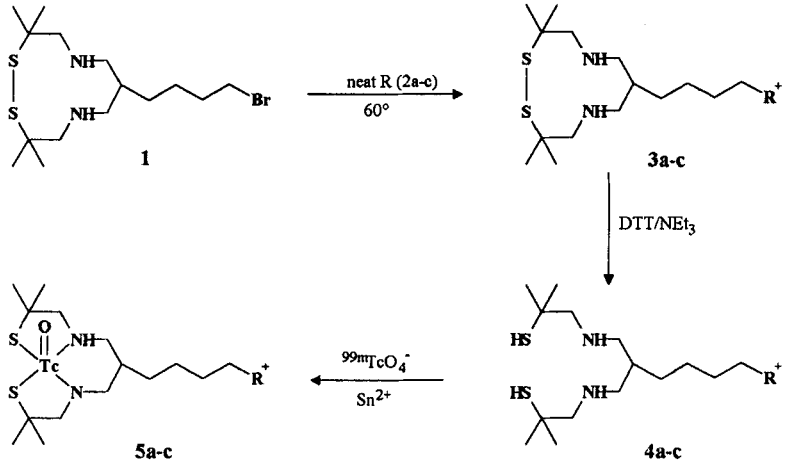
The synthesis of cationic ^{99m}Tc complexes for myocardial-perfusion studies in man is currently an active area of radiopharmaceutical research. Until now ^{99m}Tc complexes with various isonitrile and phosphine ligands have shown the greatest potential as a substitute for $^{201}Tl^{+}$ [1,2]. The positive charge of these complexes is due to the oxidation state at which the respective ligands stabilize the central technetium atom. For comparative reasons we chose a ligand which forms neutral technetium complexes and provides a positive charge by a quaternary ammonium function located on a side chain. Although this concept was already mentioned in the literature[3] we introduce three examples of new ^{99m}Tc bis-(aminoethanethiol) complexes (^{99m}Tc BAT complexes) exhibiting a positively charged nitrogen on a side chain. The biodistribution studies, we performed with mice, showed positive myocardial uptake.

As outlined in the reaction scheme the synthesis started with the previously introduced BAT-ligand synthon 7-(4'-bromobutyl)-3,3,11,11-tetramethyl-1,2-dithia-5,9-diazacycloundecane (**1**)[4]. Excess amounts of the respective nucleophiles **2a-c** (R=pyridine (**2a**), triethyl amine (**2b**), dimethylglycine ethylester (**2c**)) were reacted at 60°C under nitrogen for 2h with the neat synthon **1**.



1 **3a-c** **4a-c** **5a-c**

R = **2a** **2b** **2c**

Evaporation of the volatile reactants and recrystallisation from EtOH/Et_2O afforded the corresponding quaternary ammonium salts **3a-c**. In order to receive the corresponding bifunctional BAT ligands, the quaternary ammonium salts **3a-c** were treated under nitrogen over night at ambient temperature with excess dithiothreitol (DTT) in acetonitrile in the presence of TEA. The ligands **4a-c** were precipitated as the respective hydrochloride salts from Et_2O. Their identity were proved by elemental and spectroscopical analyses.

^{99m}Tc complexation was performed using the standard tin-reduction method: 10 µl 50 mM solution of the respective ligands were mixed with 0.5 µl 5 mM Sn(II)-tartrate as a reducing agent. After addition of 200 µl [^{99m}Tc]pertechnetate (200-500 MBq) and 5′ reaction time the ^{99m}Tc-BAT complexes **5a-c** were ready for analysis and organ distribution studies. The HPLC analysis revealed >95% complexation yield and the electrophoresis proved the positive charge of the complex. The 24 h stability of all complexes were >95%.

The organ distribution studies in NMRI mice are summarized in the table below. Among the three compounds tested, the pyridyl substituted ^{99m}Tc BAT complex **5a** showed the highest heart uptake. By contrast with the other two complexes **5b-c** the myocardial activity of ^{99m}Tc complex **5a** persisted almost constant over one hour. Within that time span the blood values decreased to a heart/blood ratio of 22. After 60′ the heart/lung and heart/liver ratio amounted to 2 and 0.2, respectively.

ORGAN UPTAKE (% INJ. DOSE/G) OF ^{99m}Tc-BAT COMPLEXES WITH QUATERNARY AMMONIUM GROUPS 5a-c (MEDIANS OF 3 MICE)

Subst.	Compl.	TIME	Blood	Heart	Lung	Spleen	Liver	Kidney	Muscle	Brain
PYR	**5a**	5′	1.492	3.604	4.177	1.082	21.706	34.680	0.484	0.157
		60′	0.155	3.353	1.890	0.724	19.943	5.602	0.168	0.031
TEA	**5b**	5′	3.638	2.992	7.195	1.792	21.045	29.512	0.540	0.208
		60′	1.396	1.559	3.256	2.358	15.702	5.431	0.208	0.084
DMGEE	**5c**	5′	1.511	1.493	2.305	0.726	20.660	36.659	0.368	0.102
		60′	0.151	0.894	0.770	0.348	5.613	5.017	0.048	0.020

References

1. Jones A.G., Abrams M.J., Davison A. et al. - Int.J.Nucl.Med.Biol. 11: 225 (1984)
2. Kelly J.D., Foster A.M., Higley B. et al. - J.Nucl.Med. 34: 222 (1983)
3. Griffith D.V., Tonkinson D.J., Burke J.F. et al. - Technetium and Rhenium in Chemistry and Nuclear Medicine 3, Cortina Int., Verona, 1990, pp 353
4. Eisenhut M., Brandau W., Mißfeldt M. - Int.J.Nucl.Med.Biol. 16: 805 (1989)

Tc-99m Labeled Dimethyl Ethyleneimine Phenol as a Potential Renal Static Imaging Agent

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1,1-Dimethyl ethyleneimine phenol [$DEn(IP)_2$][IUPAC named 1,1-dimethyl-1,2-bis-(2-hydroxybenzylimino) ethane] was complexed to Tc-99m using Sn^{2+}($1x10^{-4}$M) reduction of $^{99m}TcO_4^-$ in the presence of the ligand ($1x10^{-3}$M) under the buffer solution of 0.5M borate adjusted at pH 8. The Tc-99m labeled complex, ^{99m}Tc-$DEn(IP)_2$ formed was found to contain a large fraction (>70%) of a cationic species with a small fraction (<10%) of a neutral species. The cationic ^{99m}Tc-$DEn(IP)_2$ species could be separated and purified by chloroform/buffer extraction. The aqueous solution was confirmed to represent the pure cationic ^{99m}Tc-$DEn(IP)_2$ by electrophoresis (0.01N $NaHCO_3$,500V)(see Fig.1) and by ion-pair HPLC (E. Merck Lichropher 100 RP C-18 column; CH_3COONa /CH_3CN /H_2O as gradient eluant)(see Fig.2, the last peak being the cationic species)

It is of our interest to further investigate the cationic ^{99m}Tc-$DEn(IP)_2$ if it is a useful radiopharmaceutial in nuclear medicine. From a preliminary biodistribution test by rats using the cationic ^{99m}Tc-$DEn(IP)_2$, a rapid uptake and high retention (>70% of the total activity injected at 24h) in the kidneys but with a high blood clearance was noticed. This biological characteristics of the cationic ^{99m}Tc-$DEn(IP)_2$ seems to be similar to that of ^{99m}Tc-DMSA(dimercaptosuccinic acid) which is currently used as a renal static imaging agent. A detailed biological study for the cationic ^{99m}Tc-$DEn(IP)_2$ was conducted in parallel with ^{99m}Tc-DMSA for comparison. Table 1 is the result of biodistribution tests by rats both for ^{99m}Tc-$DEn(IP)_2$ and for ^{99m}Tc-DMSA, indicating that both ^{99m}Tc-labeled complexes have a similar high and delayed uptake by the kidneys and high kidney/blood and kidney/liver ratios beneficial to γ imaging. Tables 2 and 3 are the results of blood distribution for ^{99m}Tc-$DEn(IP)_2$ and ^{99m}Tc-DMSA, respectively. Aliquot s of plasma separated from the blood of rats were centrifuged by 5k cutoff filter to divide the protein-bound and free species in the plasma. ^{99m}Tc-$DEn(IP)_2$ was observed to exhibit as ^{99m}Tc-DMSA a high-percentage protein-bound species , which may be the substrate for renal tubular fixation leading to the high renal retention of the new ^{99m}Tc labeled complex. However, it is interesting to note that ^{99m}Tc-$DEn(IP)_2$ may be the first cationic ^{99m}Tc labeled compound discovered as the possible renal static imaging agent, which is absolutely different in charge from the anionic ^{99m}Tc-DMSA.

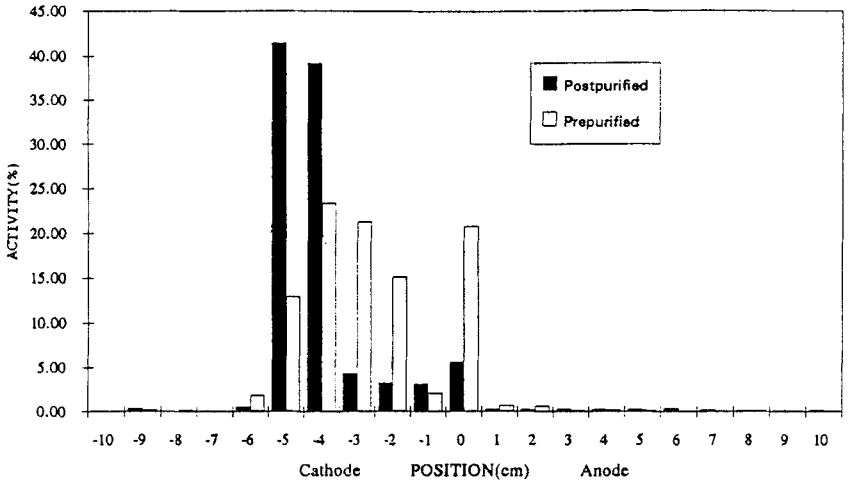

Figure 1. Electrophoregrams of ^{99m}Tc-DEn(IP)$_2$ prepurified and postpurified.

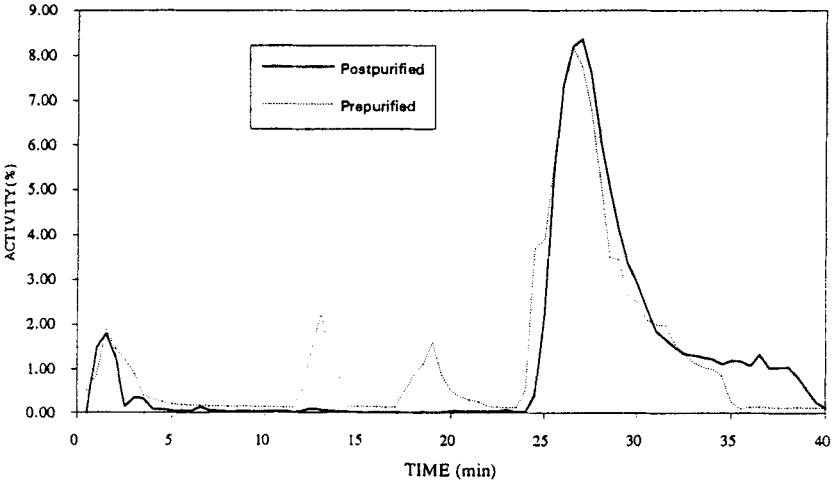

Figure 2. Chromatograms of ^{99m}Tc-DEn(IP)$_2$ prepurified and postpurified.

Table 1. Biodistribution test by rats for ^{99m}Tc-DEn(IP)$_2$ and ^{99m}Tc-DMSA.

	^{99m}Tc-DEn(IP)$_2$			^{99m}Tc-DMSA		
	0.5h	2.0h	24h	0.5h	2.0h	24h
Kidney*	14.8 (0.97)	31.7 (1.16)	53.0 (1.37)	29.5 (0.92)	41.5 (0.09)	41.5 (0.14)
Blood*	2.0 (0.02)	1.2 (0.35)	0.3 (0.06)	4.5 (0.92)	1.8 (0.09)	0.3 (0.14)
Liver*	1.9 (0.26)	3.2 (0.51)	1.4 (0.09)	2.0 (0.49)	1.0 (0.11)	1.5 (0.03)
Kidney/Blood	9.17	22.00	194.20	8.75	21.40	137.62
Kidney/Liver	8.03	10.11	40.12	11.92	38.67	18.56

*The values are indicated as %ID/g tissue with mean (S.D.) from 3 rats.

Table 2. Blood distribution for ^{99m}Tc-DEn(IP)$_2$

Time	Blood cell(%)	Plasma(%)	
		Protein-bound	Free
0.5h	21.2	49.5	28.9
	(1.7)	(5.1)	(4.9)
2.0h	31.2	57.1	2.0
	(4.1)	(6.9)	(4.5)
24.0h	44.5	52.7	6.74
	(2.0)	(7.5)	(0.54)

*Each value is indicated with mean(S.D.) from 3 rats.

Table 3. Blood distribution for ^{99m}Tc-DMSA

Time	Blood cell(%)	Plasma(%)	
		Protein-bound	Free
0.5h	15.5	85.3	2.5
	(3.1)	(3.5)	(0.7)
2.0h	21.2	59.5	19.3
	(1.6)	(8.1)	(6.9)
24.0h	21.1	66.1	17.7
	(2.1)	(3.6)	(4.1)

*Each value is indicated with mean(S.D.) from 3 rats.

Modified Synthesis, Characterization and Evaluation of Thiolactones.

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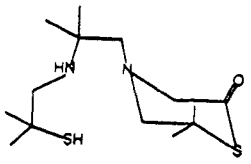
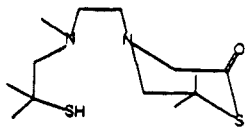
Recently Lever and coworkers have developed (1, 2) the use of thiolactones as a new class of bifunctional chelating agents for incorporating Tc-99m in the bioactive molecules. Their synthesis of the thiolactone-HM (hexamethyl), A, required the condensation of a diamine with a dithiodialdehyde to give a bisimine. The imine was reduced with lithium aluminum hydride and thioalkylated with p-methoxybenzyl chloride. N-alkylations were conducted with ethyl bromoacetate in the presence of potassium carbonate. The cleaving of p-methoxybenzyl group was achieved using HF. Thiolactone-PM (pentamethyl), B, was synthesized by methylation of a bicyclic imidazolidino[1,2-d]dithiazepine, C.



A



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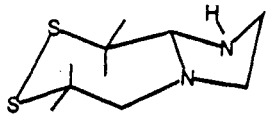


C

We recently reported another method of synthesis of bisaminothiol ligands (3) using the condensation of acid chloride with the diamines. Using the same synthetic strategy we have synthesized both A and B starting from 2-methyl-[2-(p-methoxymercaptobenzyl)]propionyl chloride. The condensation of two equivalents of acid chloride with the appropriate diamine resulted in bisdiamides. The diamides were reduced using borane-tetrahydro- furan to give the corresponding methylene derivatives. N-alkylation of the reduced bisamide was carried out using ethyl bromoacetate in DMF. The deblocking of p-methoxybenzyl protecting groups was achieved using trifluoroacetic acid, methane sulfonic acid in the presence of anisole as a radical scavenger to give thiolactone-HM and thiolactone-PM in high yields. The synthesis is depicted in scheme 1. All products were characterized using routine spectroscopic methods. This newer approach provides a single method for synthesizing both thiolactones HM and PM, and avoids the use of the detonating gas, HF for the deprotection step.

One hundred ul of alcoholic solution (1 mg/ml) of thiolactone -HM, a required quantity of Tc-99m and 50 ul saturated aqueous solution of stannous tartarate were incubated at 22 °C for 10 minutes. Greater than 90% of Tc-99m was extractable in $CHCl_3$. The chloroform layer was evaporated to dryness, the complex was dissolved in 100 microliter of 50% EtOH and incubated with $3X10^7$ freshly harvested human leukocytes suspended in 0.5 ml of 0.9 % NaCl (4).

Approximately 50% of radioactivity was associated with cells out of which less than 20% was bound to contaminating RBC. After a 3 hour incubation of labeled cells in plasma at 37 °C, greater than 50% radioactivity was still associated with WBC.

Thiolactone-HM was also used to label with Tc-99m, human polyclonal immunoglobulin and anti-nuclear antibody, TNT-1, obtained from Dr. Epstein at USC. The procedure of Baidoo et al. was employed (5). Briefly, the antibody (MAb, 100 ug -1 mg) in 0.25 mM borate buffer pH-9 was incubated at 22 °C for 2 hours with 100 M excess of thiolactone-HM in DMF. Excess reagents were removed by molecular filtration (Centricon-30, Amicon, MA). The conjugated MAb was washed with 0.02 M phosphate buffer at pH-7.0, aliquoted, and stored for the future use. Tc-99m labeling was accomplished by exchange with freshly prepared Tc-99m glucoheptonate at 37 °C for 1 hr. Approximately 50% of Tc-99m was bound to MAb.

The in vitro stability and tissue distribution of Tc-99m-TNT-1 in mice bearing experimental embryonal carcinoma were studied and compared to those with Tc-99m-TNT-1 prepared by our ascorbic acid reduction mediated direct method (6). Results in Table 1 and 2 with the conjugate are closely similar to those obtained by the direct method.

In summary, 1) we have developed a simple and reliable method for synthesis of thiolactones, 2) thiolactone-HM can be labeled with Tc-99m, 3) Tc-99m-thiolactone-HM can be used to label WBC and monoclonal antibodies and 4) the results obtained with thiolactone labeled MAb are similar to Tc-99m MAb labeled by the direct method. Work in part was supported by NIH CA-RO1-51960 (MLT) and DOE-FG02-92ER 61485 (MLT).

References:

1) K.E. Baidoo, S.Z. Lever. Bioconjugate Chemistry, 1990: 2; 132-137.

2) K.E. Baidoo, S.Z. Lever. Tetrahedron Letter, 1990: 31; 5701-5704.

3) C.S. John, Y. Ohmomo, H.F. Kung, C.H. Paik, R.C. Reba. J. Labelled Compds. Radiopharms. 1991: 30; 26-28.

4) M.L. Thakur, J.P. Lavender, R.N. Arnot et al. J. Nuc. Med. 1977: 18; 1014-1021.

5) K.E. Baidoo, U. Scheffel, S.Z. Lever. Cancer Research, 1990: 50; 7995-8003.

6) M.L. Thakur, J.D. DeFulvio. J. Immun. Methods, 1991: 137; 217-224.

Table 1. Stability Studies

(% Tc-99m associated with proteins at 37°C)

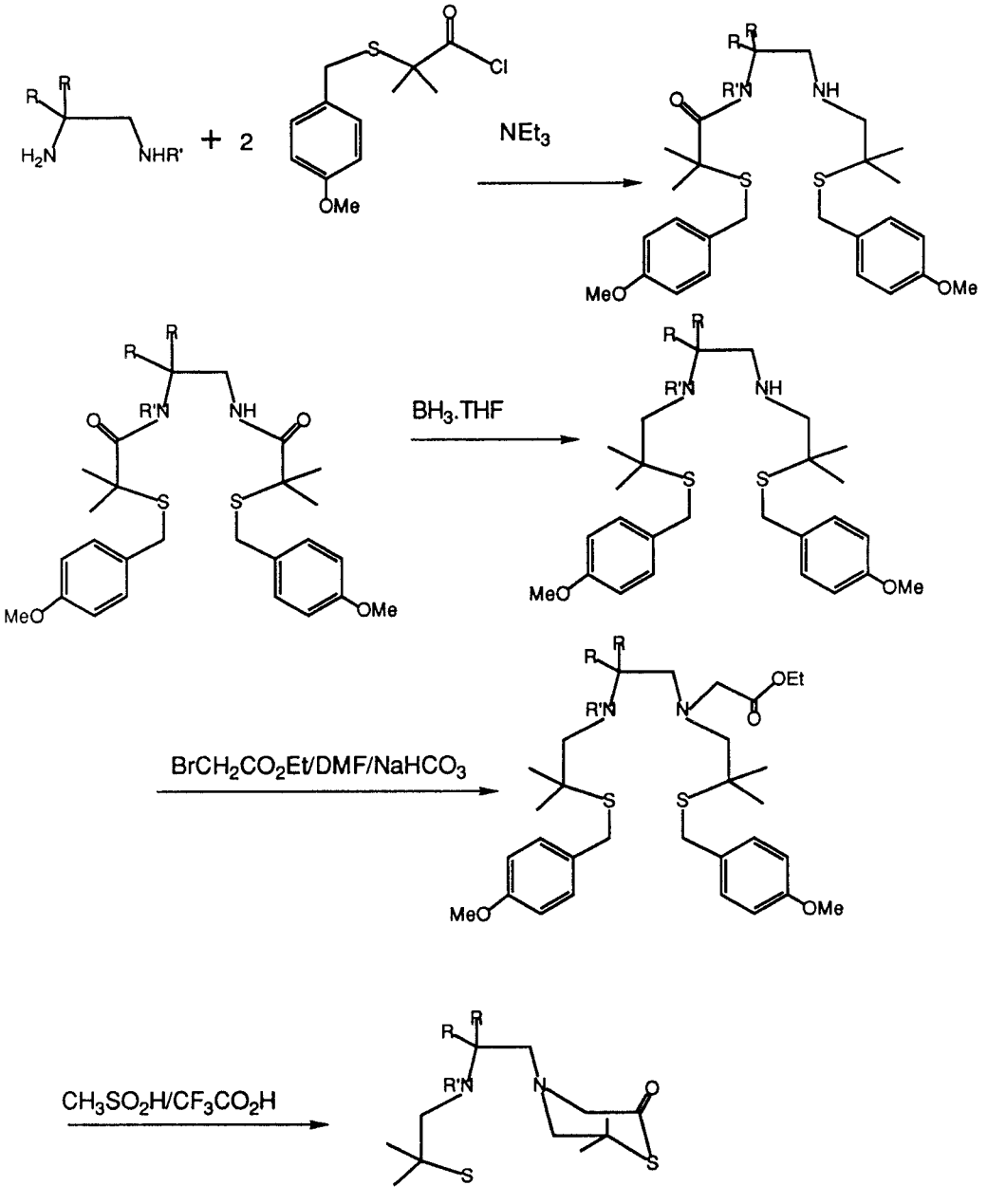
	^{99m}Tc-HIgG (AA Method)*				^{99m}Tc-HIgG-N_2S_2#			
	0.5 hr	2 hr.	3 hr.	4 hr.	1 hr.	2 hr.	3 hr.	4 hr.
1:100 Cysteine	70	48	43	38	68	55	49	48
1:250 Cysteine	65	41	40	34	56	48	44	41
1:100 DTPA	97	96	95	95	85	84	85	82
1:250 DTPA	97	96	96	94	80	78	79	80
1:100 HSA	98	94	91	88	90	87	90	85
1:250 HSA	88	78	76	74	90	86	80	82

*AA: Ascorbic Acid; #N_2S_2: Thiolactone-HM

Table 2. Four Hr. Biodistribution of ^{99m}Tc-Labeled MAb in mice (%ID/organ; N=5)

	^{99m}Tc-TNT-1-AA*	^{99m}Tc-TNT-1-N_2S_2#
Muscle	0.33 (+/-) 0.07	0.54 (+/-) 0.1
Intestine	1.4 (+/-) 0.4	2.6 (+/-) 0.7
Heart	2.07 (+/-) 1.4	1.1 (+/-) 0.1
Lung	2.9 (+/-) 0.7	5.4 (+/-) 1.1
Blood	4.8 (+/-) 1.5	4.4 (+/-) 0.4
Spleen	32.8 (+/-) 3.7	34.8 (+/-) 5.0
Kidney	14.2 (+/-) 1.3	17.9 (+/-) 2.2
Liver	23.1 (+/-) 5.0	32.9 (+/-) 5.8
Tumor	1.7 (+/-) 0.77	1.80 (+/-) 0.2

AA*: Ascorbic Acid; #N_2S_2: Thiolactone-HM



Scheme 1. Modified Synthesis of Thiolactones.

THE INFLUENCE OF COPPER (II) ION ON THE COMPLEX OF ^{99m}Tc-MAG_3

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It has already been known that glycine and it's derivative could form complex with copper ion which is one of the essential element in human body. The ^{99m}Tc-MAG_3 should be, therefore, affected by the ion in human body as well.

The objective of this study is to determine the formation constant of copper ion with MAG_3 and observed the possibility of the reaction between this ion and ^{99m}Tc-MAG_3.

Synthesis of ^{99m}Tc-MAG_3 has been carried out in the same way as Brandua's method(1988).The labelling MAG_3 kit shows higher RCP than 95% of ^{99m}Tc-MAG_3.

To determine the formation constant and species distribution of MAG_3 and Cu-MAG_3 has been performed by using potentiometric data and then calculated by SUPERQUAD program.The results are shown in figure 1.1, 1.2 and table 1.

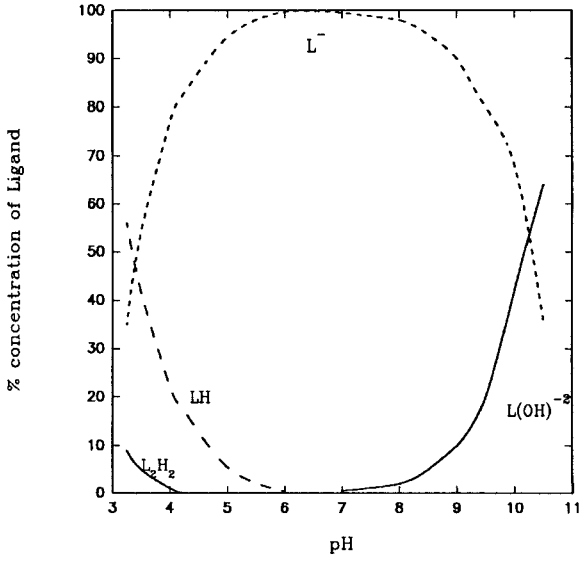

FIGURE 1.1 THE SPECIES DISTRIBUTION OF MAG_3

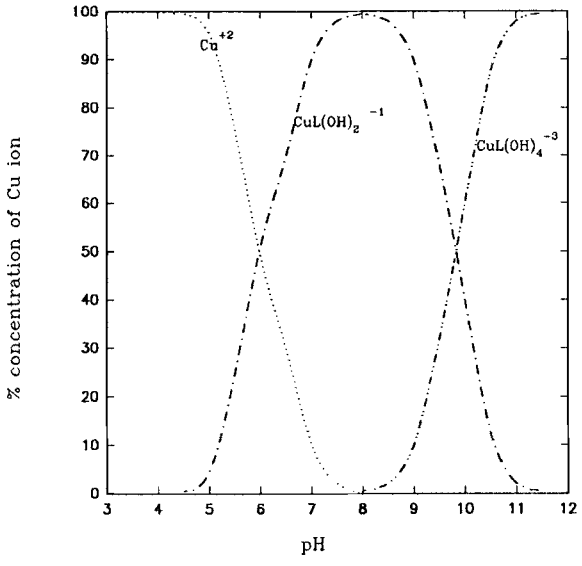

FIGURE 1.2 THE SPECIES DISTRIBUTION OF Cu-MAG_3

TABLE 1 THE EQUILIBRIUM CONSTANT OF MAG_3 AND Cu-MAG_3

FORMATION EQUATION	Log K
$2LH \text{ ------> } L_2H_2$	2.21 ± 0.05
$LH \text{ ------> } L^- + H$	-3.47 ± 0.01
$L^- + H_2O \text{ ------> } L(OH)^{2-} + H$	3.34 ± 0.02
$Cu^{2+} + L^- + 2HO^- \text{ ------> } [CuL(HO)_2]^-$	18.71 ± 0.11
$Cu^{2+} + L^- + 4HO^- \text{ ------> } [CuL(HO)_4]^{-3}$	26.68 ± 0.16

The radiochemical purity of ^{99m}Tc-MAG_3 during 30 minutes after adding 90 - 900 μg of copper ion at pH 7.0 - 7.5, decreased with slope as shown in table 2 and it's also shown that, copper ion can reduce RCP of ^{99m}Tc - MAG_3 (see figure 2). Therefore, it is possible that the same radiopharmaceutical phenomena can happen in human body as well.

TABLE 2 THE DECREASING SLOPE OF RCP.OF ^{99m}Tc-MAG$_3$ BY COPPER ION

Cu (μg.)	Slope
0.00	-0.055
90.00	-0.055
225.00	-0.061
445.00	-0.082
675.00	-0.096
900.00	-0.110

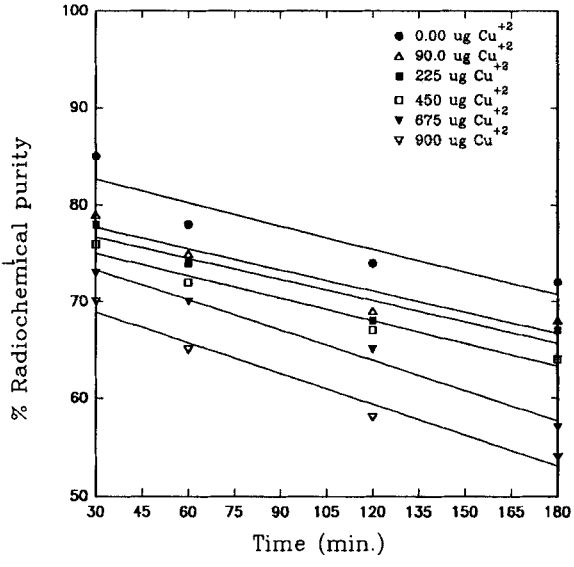

FIGURE 2 INFLUENCE OF COPPER ION ON RADIOCHEMICAL PURITY OF ^{99m}Tc-MAG$_3$

Plasma proteins and red blood cells labeled with technetium-99m: role of precipitating agents.

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The nature, the oxidation state and the binding type of the technetium-99m (^{99m}Tc) in several ^{99m}Tc-complexes are not known with certainty. This is also true for ^{99m}Tc-labeled plasma proteins (PP) and red blood cells (RBC). In this communication we present the results obtained with ^{99m}Tc-labeled PP or RBC that were treated with various precipitating agents: hydrochloric acid-HCl, trichloroacetic acid-TCA, mercuric chloride-$HgCl_2$, acetone and ethanol.

Table I shows that the precipitating action in the ^{99m}Tc-labeled RBC in ethanol and acetone are similar as well as in TCA and HCl. However, in $HgCl_2$ this action seems to be different.

Table I - % of radioactivity in insoluble fractions (IF) obtained from ^{99m}Tc-labeled RBC precipitated with different agents

$SnCl_2$ (uM)	Precipitating agents				
	Ethanol	Acetone	TCA	HCl	$HgCl_2$
0 (control)	10.9	10.5	11.9	11.5	48.8
0.065	14.4	12.1	14.3	11.9	50.9
0.650	36.1	37.2	23.6	24.4	-
6.500	98.6	99.1	72.2	76.4	86.4
65.000	98.9	99.2	77.7	74.9	87.5
650.000	97.8	98.5	76.5	75.9	87.9

Related to PP the results showed (results not shown) that the precipitating action for ^{99m}Tc-labeled PP in ethanol and HCl are the same as well as in acetone and TCA. The precipitating action of $HgCl_2$ in PP seems to be different from RBC. The radioactivity is mainly in SF in ^{99m}Tc-labeled PP and in ^{99m}Tc-labeled RBC the radioactivity is in the IF.

The results showed that ^{99m}Tc-binding RBC is more resistant to acetone and to ethanol treatment than to TCA and to HCl ones. This suggests a probable hidrophobic character for this RBC-binding. ^{99m}Tc-binding PP is more resistant to trichloroacetic acid and to acetone treatment than to ethanol and to HCl ones.

The fixation mechanism of ^{99m}Tc to PP and/or the binding characteristics may be different from RBC, once the results with precipitating agents are not the same. The sensibility to ethanol and HCl treatments shows that ^{99m}Tc-binding to RBC is stronger than to PP. For ^{99m}Tc-labeled PP the highest resistance is observed in acetone and TCA. The values in HCl and ethanol are similar, but minor than in acetone and TCA and greater than in $HgCl_2$.

Preparation of ^{99m}Tc-sucralfate for ulcer detection.
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Sucralfate is a basic aluminun salt of sucrose octasulfate which has been used to treat peptic ulcer disease. The drug binds to the exposed proteins of the defective mucosa, inhibits the formation of the pepsin-protein-complex, forms a viscous protective barrier at the ulcer site, counteracts the protease and lipase enzymes of *Helicobacter pylori* and has been labeled with technetium for the imaging of gastroduodenal ulcers and active inflammatory bowel disease (1-6).

The complicated radioactive labeling process *in vitro* implies first the labeling of human serum albumin and the *in vivo* method is time consumming (4,7). We considered the possibility that the labeling could be easily done in one step using $SnCl_2 \cdot 2H_2O$ as the reducing agent.

Therefore, the object of this paper was to find the best labeling method to obtain a ^{99m}Tc-sucralfate complex for clinical use.

For the one step kit preparation the ratio of reducing agent to technetium and of sucralfate was assessed and the amount of free $^{99m}TcO_4^-$ was determined by ascending Whatman N° 1 paper chromatography in acetone. The stability of the complex at different pH, water dilution, mixing time, and its competence with proteins and pepsin as well as the shelf life of the kit were evaluated.

To determine the best reagent proportion the $SnCl_2 \cdot 2H_2O$ was diluted in 0.5 N HCl as follows: 0.01, 0.02, 0.1, 0.5, 1.0, 2.0, and 5 mg/mL. The activity of technetium used was 18.5 and 37 MBq for all the *in vitro* tests and 555 MBq for the maximum amount that could be used for a scan. The sucralfate varied from 5 to 100 mg. Each preparation was repeated 5-15 times. The results are sumarized in TABLES 1-3.

TABLE 1.
LABELING EFFICIENCY (%)
WITH DIFFERENT CONCENTRATIONS OF $SnCl_2 \cdot 2H_2O$

mg/mL 0.5 N HCl	%
0.01	50.0
0.02	49.6
0.1	61.7
0.5	98.0
1.0	98.5
2.0	99.7
3.0	68.3

sucralfate 10 mg, pH 4

TABLE 2
LABELING EFFICIENCY (%)
WITH DIFFERENT ACTIVITY OF $^{99m}TcO_4^-$

MBq	%
18.5	97.7
37.0	98.2
111.0	98.2
185.0	98.6
555.0	95.5

$SnCl_2 \cdot 2H_2O$ 1 mg, sucralfate 20 mg, pH 4

TABLE 3
LABELING EFFICIENCY (%)
WITH DIFFERENT AMOUNTS OF SUCRALFATE

mg	%
5.0	98.4
10.0	97.5
20.0	98.8
50.0	95.2
100.0	79.3

$SnCl_2 \cdot 2H_2O$ 1mg, pH 4

The kit prepared with 1 mg $SnCl_2$ $2H_2O$ and 20 mg of sucralfate was stable >95 % at 18-22°C in both the presence and absence of light for 12 days.

The mixing period for the radiopharmaceutical was varied from 5 to 60 minutes. The labeling after 5 minutes was 98.8 % and after 60 minutes it was 97.6%. With a 5 minute mixing time it was stable for 6 hours. It was also important to know if this preparation would be stable in the stomach at an acid pH and also in the intestine at a basic pH. The effect of pH is shown in TABLE 4.

TABLE 4
LABELING EFFICIENCY (%), EFFECT OF pH

pH	%
1	75.7
2	97.2
4	98.8
6	97.3
8	97.1
10	88.4

$SnCl_2 \cdot 2H_2O$ 1mg, sucralfate 20 mg

The label of the radiopharmaceutical is not lost *in vitro* to 35-200 mg of proteins for up to 30 minutes at pH 4 nor to one gram of pepsin during 15 minutes at the same pH, as shown in TABLE 5.

TABLE 5
EFFECT OF PROTEIN AND PEPSIN
ON THE LABELING EFFICIENCY (%)

pH	TIME minutes	PROTEIN mg	%	1 g PEPSIN %
4	5	35	97.7	99.0
4	15	35	97.8	97.9
4	5	100	97.0	
4	30	100	98.1	92.5
4	5	200	97.8	
4	30	200	97.2	
4	120			91.4
6	5	35	96.8	
6	5	35	96.0	
8	5	35	93.7	
8	15	35	94.6	

$SnCl_2 \cdot 2H_2O$ 1 mg, sucralfate 20 mg

Sucralfate $C_{12}H_{14}O_{11}[SO_3 \cdot AL_2(OH)_5 \cdot H_2O]_8$ molecular weight 2086.74, a white amorphous powder is soluble in dilute solutions of HCl and NaOH. The S and O atoms bind with the reduced technetium to form the stable complex. A one step kit was labeled under various circunstances proving that it is stable, easy to prepare with a labeling efficiency >97 % and diluted in 20 mL of tap water is well tolerated by the patient.

The radiopharmaceutical thus prepared is now on a trial nuclear medicine study to determine the scan's sensibility and specificity in comparison with barium X rays and endoscopy, in the diagnosis of peptic ulcer and for inflammatory bowel disease.

References:

1. Morikawa H., Miyake M. and Iwai S.- *J Chem Soc Faraday Trans* 77:629-39(1980)
2. Nagashima R.- *J Clin Gastroenterol* 3:103-10(1981)
3. Slomiany B., Piotrowski J. and Slomiany A.- *Am J Gastroenterol* 87:595-9(1992)
4. Vasquez T.E., Bridges R.L., Braunstein P., Jansholt A.L. and Meshkinpour H.- *Radiology* 148:227-31(1983)
5. Puttemans N., Lambert M. *et al.*- *J Nucl Med* 28:521(1987)
6. Vasquez T.E., Pretorius H.T. and Greenspan G.- *Nucl Med Comm* 8:327-34(1987)
7. Pera A., Seevers R.H. *et al.*- *Radiology* 156:783-6(1985)

A new radiopharmaceutical for bone tumor therapy——the experimental study of 186 Re—HEDP

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Several radiotracers or their labelled compounds have been used to palliate extreme skeletal pain caused by disseminated bone metastases for many years, but none has achieved widespread clinical application. Rhenium is a beta emitters with excellent physical properties that may be useful for the formulation of radiotherapeutic agents. It has a short half life (90. 6. h.), with moderate—energy beta particles that penetrate over a short range of tissue and a gamma ray which is well suited to image.

Preparation of $NH_4^{186}ReO_4^-$

$NH_4^{186}ReO_4^-$ was prepared by neutron irradiation of ^{185}Re, enriched to 99%, in the Southwest Institute of Nuclear Physics and Chemistry of Engineering Physics Academic of China, using a thermal flux of 5×10^{13} n $cm^{-2}\cdot S$. After 120 hours in a titanium can, it can be dissolved in 3Mol/L HNO_3 and taken to dryness, added to 25% $NH_3\cdot H_2O$, taken to dryness again, and then dissolved by DDH_2O.

Preparation of ^{186}Re—HEDP Complex

The Preparation was performed by the reduction method. 1 ml HEDP solution were added to $SnCl_2\cdot H_2O$ and $NH_4^{186}ReO_4^-$, respectively. After dissolution and formation of the ^{186}Re—HEDP complex, the solution was filtered throuqh 0. 22um membrance and sterilised by autoclaving.

Analysis of ^{186}Re—HEDP Complex

Injection of ^{186}Re—HEDP is a colourless, tasteless, no bacteria and clear solution. The pH of solution was 5. Labelling yield was greater than 95%.

^{186}Re—HEDP complex can be stored in the formulation vial at room temperature and retains its stability for 24 hours. If it can be isolated from air or added to Vitc, the labelling efficiency was still greater than 95% for 120 hours.

The Pyrogen experiments of ^{186}Re—HEDP

Before injection ^{186}Re—HEDP, 3 rabbits (weiqht2. 2kg)were fed singly for 1 meek, and then taken the basic temperature. After injection ^{186}Re—HEDP 30 min, 60min. and 180min, we

took the temperature respectively. The Pyrogen experiments conformed to the pharmacopoeia of people's republic of China (1985 edition).

The Toxic test of HEDP(table 1)

Table 1: the toxic test in Kuming mice[a] of ^{186}Re—HEDP

	n	medicine(dose)	times[b]	(histology observation all brain, heart, liver, Kindney)
control	10	G.N.S(0.9%)		normal
experinents	10	HEDP(1mg)	800	nomal

a after fed 10 days

b be equal to human dose

The effects of high dose ^{186}Re—HEDP on animals (Table 2)

Table 2: The effects of high dose* ^{186}Re—HEDP on animals (n=5)

	RBC ($\times10^{12}$/L)	Hb (g/L)	WBC ($\times10^{9}$/L)	platelet ($\times10^{9}$/L)
control	5.4	137.0	7.6	172.2
experiments				
7d	5.0	134.0	6.8	164.9
15d	5.1	135.0	6.4	168.4
30d	5.5	136.0	7.7	171.4
120d	5.4	138.0	8.1	175.0

* be equal to 40 timer human dose

The biodistribution of ^{186}Re—HEDP in rats (Table 3)

Table3: Biodirtribution of ^{186}Re—HEDP in rats (n=5)

Time (h)	blood	heart	liver	kidney	muscle	bone
1	13.3±0.1*	1.1±0.2	7.8±2.4	15.2±4.40	5.2±1.5	108.9±18.3
3	1.7±0.5	0.8±0.3	2.5±1.1	81.2±20.3	2.0±0.3	288.8±35.1
24	1.5±0.3	0.5±0.2	1.2±0.4	35.1±11.3	1.8±0.2	249.0±27.2
48	0.8±0.3	0.2±0.1	0.7±0.2	28.2±13.5	1.2±0.2	213.5±21.3
96	0.4±0.2	0.2±0.1	0.2±0.1	9.2±1.3	0.4±0.1	135.7±24.2
120	0.1±0.01	0.1±0.01	0.1±0.01	0.5±0.2	0.1±0.01	105.1±18.4

* % dose/g tissue ×100

The whole bone imaging of ^{186}Re—HEDP in animal

After injection 186Re—HEDP (500μCi, 18. 5MBq) 0. 5h, 1h, 3h and 96h, the whole bone imaging of the same New Zealand white rabbit was taken by SPECT. The picture of bone imaging was wonderfal, The skull, extremities, articulatis and vertebra were clear (Fig 1).

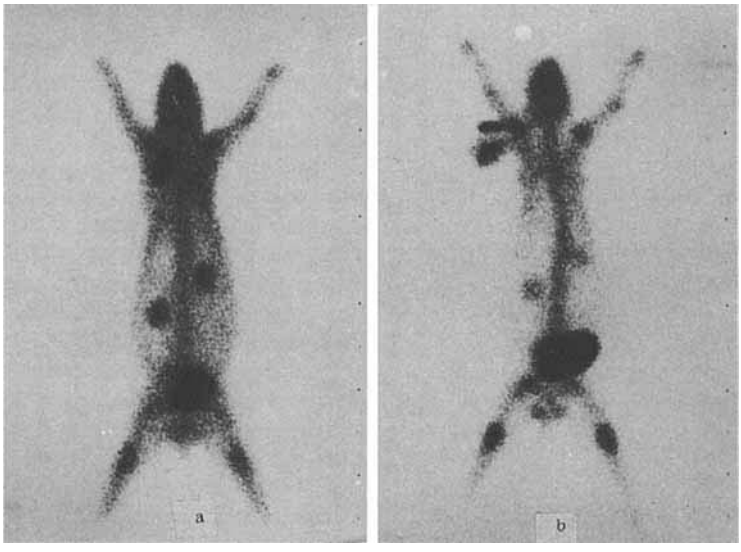

Fig1; bone imaging of ^{186}Re—HEDP in rabbit

a affer injection ^{186}Re—HEDP 30 min.

b affer injection ^{186}Re—HEDP 60 min.

Experimental Studies of Pantavalent Technnetium-99m-Dimercaptosuccinic Acid As the Hepatic Tumor Imaging Agent

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ABSTRACT Previous literature have negated the application of 99mTc(V)-DMS to liver and other internal organs cancer imaging[1、2].

To test better tumor/liver ratio in tunor-bearing mice, the present study was carried out in various conditions to clarify the applicability of 99mTc(V)-DMS in imaging of hepatic tumor . Various experimental conditions were tested by varying kinds of experimental tumor, concentration of the ligand, time post-injection, and pH(pH 8-9). In the present study, we modified the original labelling methods[3] described by yokoyama et al and observed the biodistribution of the labelling compounds obtained under different experimental conditions in tumor-bearing mice . And then we applied the 99mTc(V)-DMS to the SPECT imaging of 35 patients with hepatic carcinoma , and of these, there were 88.5% true positives. The results showed that the modification can apparently elevate the tumor/liver ratio to 2.5-3.0. Consequently the labelling compounds can be used for both primary and metastatic cancer in liver. The data of pH test revealed that the labelling rate is highest at pH 8.25-8.45, the labelling compound obtained under pH8.45 has the maximum dissociation. For the positive correlation reported by Horiuch et al[4] between ability to dissociate and tumor affinity of the 99 mTc(V)-complex, in this study the dissociable ability of Tc(V)-DMS is must obvious at pH8.45. Data of the experiment suggested that pH 8.45 should be optimal to ensure intense tumor affinity.

The results demonstrated that 99mTc(V)-DMS can be used as an effective imaging agent for hepatic cancer.

Acknowledgments The part work of this study was finished in Department of Radiopharmaceutical chemistry , Faculty of pharmaceutical Sciences of Kyoto University. The authors are grateful to Professor Yokoyama and Ms. Horiuchi for their important direction.

REFERENCES

1. Ohta-H; Endo-K, et al: Nucl-med-Commun. 1988 Fed;9(2): 105—116
2. Ohta-H; Endo-K, et al: J-Nucl_Med. 1988 Jum;29(6):1130-5
3. Yokoyama A, hata N, Horiuchi K, et al: Int Nucl Med Biol, 12:273.1985
4. Horiuchi K, Yomoda A, Endo k, Torizuka K: Tc(V)-DMS tumour imaging agent:Tc-complex dissociation eguilibria, a relevant factor in tumor localisation. In: Nicolini M, Bandol G, Mazzi U, eds. Technetium in chemistry and nuclear medicine 2. New York: Raven Press; 1986:155-159

Table 1. Radiobiodistribution in Hepatoma H 22-Bearing mice and Ehrlich carcinoma-bearing mice

	Blood	Tumor	Liver	Kidney	tumor/blood	tumor/liver
Hepatoma H22	0.059±0.019	0.18±0.08	0.06±0.01	0.6±0.02	3.05	2.9
Ehrlich tumor	0.07 ±0.02	0.18±0.01	0.05±0.01	0.6±0.1	2.40	3.4

%dose/g tissue

Mean± 1SD for 5 animals per point

Table 2. Optima imaging time for excellent tumor/liver ratio

time after injection	blood	tumor	liver	kidney	Tu/Bl	Tu/li
1 hours	0.10±0.01	0.15±0.06	0.06±0.01	0.45±0.18	1.50	2.38
2 hours	0.11±0.04	0.21±0.04	0.06±0.01	0.69±0.11	1.90	3.13
3 hours	0.04±0.02	0.09±0.02	0.03±0.01	0.26±0.06	2.50	3.16
4 hours	0.03±0.01	0.06±0.03	0.02±0.01	4.40±0.05	2.61	3.00

% dose/g tissue

Mean ± 1SD for 5 animals per point

^{99m}Tc(v)-DMSA Hepatic Cabcincma Image And Observation Of Prelimnary Clinic Application. CAL ZONGPING; **LI SHAOLIN ET. AL. 181.HanYu Road, Chongqing. Depart of Nuclear Medicine of Chongqing Canccer Institute, Chongqing, P.R. China; **Department of Basic Nuclear Medicine of Chongqing Uuiversity of Medical Science, Chongqing, P.R. China.

The tumor affinity of ^{99m}Tc(c)-DMSA has been reported at home and abroad. It is charactorized With fast blood clearance and short imaging time.[1] But Ohta. et al[2] trported that there is no result or bad effect in the tumor imaging of lung and liver and other internal organs besides it can be used in the tumor of heads and necks. In our cuntry professor Tan Tianzhi et al[3] reported that the tumor/liver ratio of ^{99m}Tc(v)-DMSA is 0.29 in the distribution ratio of tumor-carrying rats. Our experimental research proves that the tumor/liver ratio can be iucreased through some methods, which makes it possible that ^{99m}Tc(v)-DMSA can be used in the imaging of liver cancer.
Condition of marks: PH ranges are controlled strictly to make the PH value of reactive liquid between 8.2 to 8.28. The reagents are freshly made. A special drug ahould be put into the reactive liquid at 20mg/ml. The concentration of DMAS is 6.0×10^{-3}M, $SnCl_2 2H_2O$ is 1.0×10^{-4}M. Tumor/Liver ratio is 2.36 ± 0.48, which has been proved by the experiments for the distribution of liver tumor which is insides rats.
55 cases of patientes with hepatic diseases receive 99mTc(v)-DMSA images. There are 35 cases of liver malignant tumor in this group. Among them there are 29 cases of primary hepatic cell carcinoma, 4 cases of secondary liver cancer and 2 cases of post-operative relapse with primary hepatic cell carcimoma. In 35 cases of liver malignant tumor, 31 cases (88.57%) are positive in the imaging of ^{99m}Tc(v)-DMSA SPECT. There are 20 cases of benign hepatic diseases. Among them, one false positive cases is spongy heamengioma of left liver.

In the ^{99m}Tc(v)-DMSA liver cancer imaging in the group, the sensitivity is 88.57%, the specificty is 95%, the accurate rata is 90.90%, the positive calculating value is 96.86% and the negative calculating value is 82.61%. However in the same group the sensitves of Ultrasonic, TCT, AFP are 57.89%, 69.23% and 71.43% respectively. Referances:

(1). Yokoyama A, Hata N, Horiuchi K, et al. The design of a pentravalent 99mTc-dimercaptosuccinate complex as a tumor imaging agent. Int J Nucl Med Biol 1985; 12: 273-279.

(2). Ohta H, Endo K, et al. Clinical evaluation of tumor imaging using 99mTc-dimercaptosuccinic acid a new tumer-seeking agent. Nucl Med Commun 1988 Feb; 9(2): 105-116.

(3). Tan Tianzhi, et al. Prepation of tumor imaging agent kit 99mTc-DMSA. Chese Journal of Nuclear Medicine 1990; 10(1): 43.

(4). John C, Watakinson, etal. Technetium-99mTc(v)-dimercaptosuccinate acid uptake in patients with head and neck squamous carcinoma: experience in imaging. J Nucl Med 1989; 30: 170-180.

(5). Ohta H, Yamamoto K, et al. A new imaging agent for medull carcinoma of the thyroid. J Nucl Med 1984; 25: 323-325.

(6). AW SE, Sumdram FX, Goh AS. 99mTechnetium(v) dimercaptosuccinic acid (99mTc(v)-DMSA) as a tumir seeking agent in nasopharyngeal carcinoma. Ann Acad Med 1986; 15: 499-501.

(7). Teates CD, Eray ST Williamson BR. Tumor detection with 67Ga-citrate: a literature survey (1970-1978). Clin Nucl Med 1978; 3: 456-460.

Approaches to the Synthesis of [^{11}C]Olefins and Derivatives as Novel Labelling Agents

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Studies with positron emission tomography (PET) require a wide range of molecules to be labelled with carbon-11 ($t_{1/2}$ = 20.4 min; β^+ = 99.8%), which is mainly produced in high radioactivities from the $^{14}N(p,\alpha)^{11}C$ reaction, as either no-carrier-added [^{11}C]methane or [^{11}C]carbon dioxide. ^{11}C-Carboxylations of organometallic reagents, especially organolithiums and Grignard reagents, are generally very efficient reactions and by choice of conditions may be controlled to give one of a variety of labelled products, including ^{11}C-labelled carboxylic acids, alcohols and ketones (for a recent review see reference 1). Treatment of the initially formed ^{11}C-carboxylated adducts with lithium aluminium hydride or phthaloyl chloride are also efficient routes to ^{11}C-labelled alcohols[2] or acid chlorides[3], respectively. The synthesis of ^{11}C-labelled olefins and derivatives, which are potentially useful labelling agents (*e.g.* in ring closure reactions) has hardly been addressed. Here we report on preliminary studies of the feasibility of preparing ^{11}C-labelled olefins via the ^{11}C-carboxylation of methylmagnesium bromide, reduction to ^{11}C-labelled alcohols and dehydration over heated alumina.

The carbonation of methylmagnesium bromide with cyclotron-produced [^{11}C]carbon dioxide under mild conditions gives a simple adduct which may be converted into [1-^{11}C]ethanol by lithium aluminium hydride and hydrolysis (Figure 1).[2] Under more severe conditions the adduct may react sequentially with methylmagnesium bromide, giving [2-^{11}C]acetone and [2-^{11}C]*t*-butanol after simple hydrolysis (Figure 1).[2,4,5] Initially, we explored the preparation of [^{11}C]ethylene from [1-^{11}C]ethanol by dehydration over heated alumina, as follows.

[^{11}C]Carbon dioxide, produced by the proton irradiation of nitrogen, was concentrated cryogenically by passage into a stainless steel loop immersed in liquid argon. Typically, the [^{11}C]carbon dioxide was transferred by a slow stream of nitrogen into methylmagnesium bromide (0.3 mmol) in diethyl ether (300 μL) under nitrogen for 2 min at room temperature. Lithium aluminium hydride (1.2 mmol) in THF (600 μL) was then added under nitrogen and the reaction mixture heated to 75 °C for 6 min. The reaction mixture was then cooled and water (400 μL) added. A slow stream of nitrogen was the passed through the reaction mixture while it was heated to 100 °C to carry volatile products into a trap of methanol. GC with TCD and radioactivity detection established that [1-^{11}C]ethanol was the main volatile radioactive product from these reaction conditions. The main impurity was [2-^{11}C]*iso*-propanol, presumably formed by reduction of [2-^{11}C]acetone (Figure 1). Under more severe carboxylation conditions [2-^{11}C]*iso*-propanol was found to be the main radioactive product and some [2-^{11}C]*t*-butanol was also observed.

In attempts to prepare [^{11}C]ethylene, the [1-^{11}C]ethanol was carried in a slow stream of nitrogen from the heated (100 °C) reaction mixture through a heated (600 °C external temperature) column of alumina (100 mesh, 99.99%) and into a trap containing bromine (1M) in carbon tetrachloride (400 μL). Some of the key experimental parameters (*e.g.* furnace temperature) were varied from run to run. Each run was carried out in a remotely-controlled experimental apparatus as depicted in Figure 2 and required approximately 20 min from the end of radionuclide production. Trapped products were analyzed by GC on a 60/80 Carbopack B column with 1% SP 1000 (10' x 1/8") using a temperature gradient (150 °C for 10 min, rising by 20 °C/min to 190 °C and held for 20 min) with TCD and radioactivity detection.

In these analyses [^{11}C]1,2-dibromoethane (retention time, 13 min) has been observed as the major radioactive product (> 70% of trapped radioactivity), so providing direct evidence for the production of [^{11}C]ethylene from [1-^{11}C]ethanol (retention time, 1 min) (Figure 3). The major byproduct is identified as [2-^{11}C]1,2-dibromopropane (retention time, 17 min) so providing evidence for the generation of [2-^{11}C]propylene by the dehydration of [2-^{11}C]*iso*-propanol, the already noted

byproduct of [1-^{11}C]ethanol production. Unreacted [1-^{11}C]ethanol and [2-^{11}C]*iso*-propanol were not found to any significant extent in these analyses, suggesting efficient conversion of ^{11}C-labelled alcohols to ^{11}C-labelled alkenes. However, it is found that at least 40% of the labelled alcohols do not pass through the alumina column. In some experiments as much as 70% of the radioactivity was retained by the alumina catalyst. This may be due to polymerisation of the generated ^{11}C-labelled alkenes at the high temperatures examined so far. Further optimisation is required to obtain efficient transfer of radioactivity through the heated alumina. At present we are using commercial alumina; we are especially exploring the use of the less readily available highly pure γ-form which should have less tendency to promote undesirable polymerisation reactions.[6]

[1-^{11}C]Ethanol is expected to be prepared free of other labelled alcohols by further optimisation so increasing the potential radiochemical yield of [^{11}C]ethylene. Also by appropriate choice of reaction conditions and by varying the organo and halo groups in the organomagnesium halide,[2] or by using an organolithium,[7] it should prove possible to prepare a wide range of ^{11}C-labelled alkenes and their derivatives, essentially in the same apparatus. The easily derived ^{11}C-labelled 1,2-dibromoalkane are expected to have direct utility for labelling tracers and radioligands for positron emission tomography. Further work is in progress to explore these possibilities.

References

1. R.E. Ehrenkaufer and J.G. Scripko, in *New Trends in Radiopharmaceutical Synthesis, Quality Assurance and Regulatory Control*, Plenum Press, New York, 1991, pp 247–257.
2. M.E. Raichle, J.O. Eichling, M.J. Straatmann, M.J. Welch, K.B.Larson and M.M. Ter-Pogossian, *Am J. Physiol.*, 1976, 230, 543.
3. Luthra S.K., Pike V.W. and Brady F., *Appl. Radiat. Isot.*, 1990, 41, 471.
4. Pike V.W., Eakins M.N., Allan R.M., and Selwyn A.P., 1982, *Int. J. Appl. Radiat. Isot.*, 35, 505.
5. Någren K and Långström B., *J. Labelled Compd. Radiopharm.*, 1988, 26, 83
6. A. Shintaro, T. Katsuo and I. Toshiyaki, *European Pat. Appl. 1992, No 0498543A1.*
7. Berger G. Mazière M., Prenant C. and Comar D., *Int. J. Appl. Radiat. Isot.*, 1980, 31, 577.

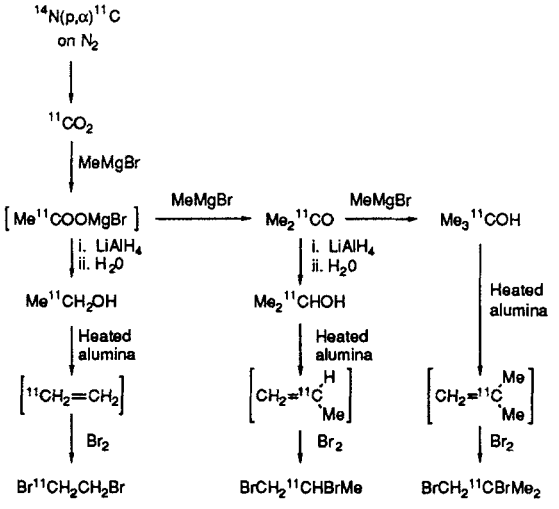

Figure 1. Routes to ^{11}C-labelled alkenes and derivatives via the [^{11}C]carboxylation of methyl magnesium bromide

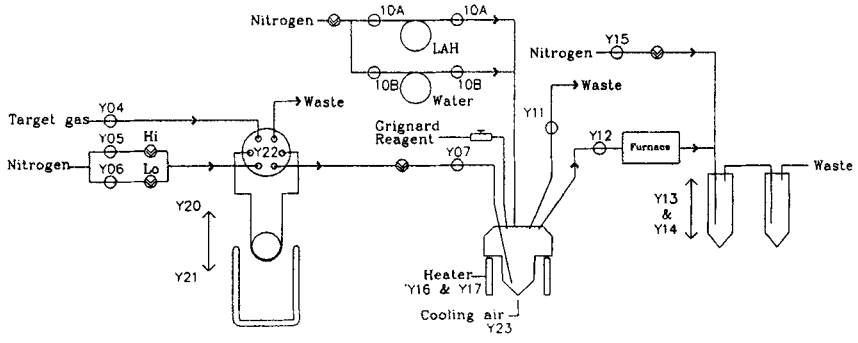

Figure 2. Remotely controlled experimental apparatus for the investigation of the preparation of [^{11}C]olefins and their dibromo adducts. (Y and A numbers refer to remotely controlled valves)

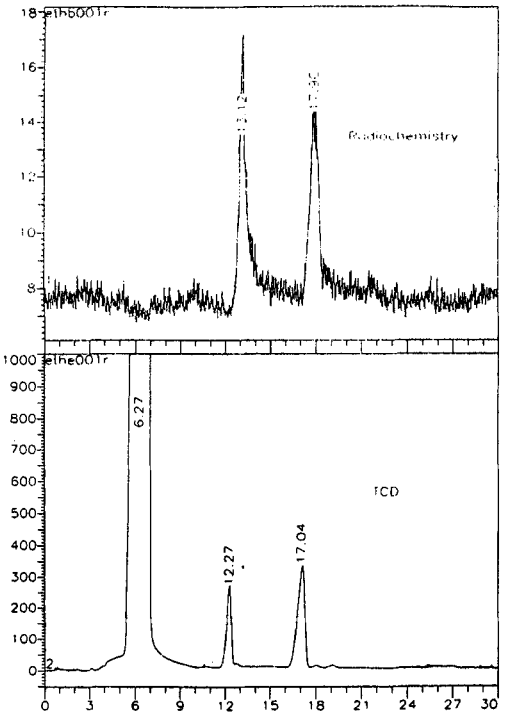

Figure 3. GC analysis (see text for conditions) of the products formed by passing [1-^{11}C]ethanol through heated alumina and into a 1M-bromine solution in carbon tetrachloride — top panel, [^{11}C]1,2-dibromoethane (13.12 min) and [2-^{11}C]1,2,-dibomopropane (17.90 min) — bottom panel 1,2-dibromoethane (12.27 min) and 1,2-dibromopropane (17.04 min).

^{11}C-labelled Bifunctional Precursors for use in Wittig Reactions

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Many drugs and other biologically interesting compounds contain carbon-carbon double bonds. A versatile method of forming carbon-carbon double bonds is to use the Wittig reaction. Earlier the only available ^{11}C-labelled Wittig reagent was [^{11}C]methyltriphenylphosphorane (1), which makes it possible to prepare molecules containing a labelled methylene group.

In this report the synthesis of [^{11}C]cyanoalkyltriphenylphosphoranes (figure 1) via two different synthetic routes are presented. These multifunctional ^{11}C-labelled precursors (1, 2), can be used in Wittig reactions and makes it possible to prepare molecules with a labelled nitrile group three or four methylene groups away from the carbon-carbon double bond.

$Ph_3\overset{+}{P}-\overset{-}{C}H(CH_2)_{n-1}{}^{11}CN$ n = 3 or 4

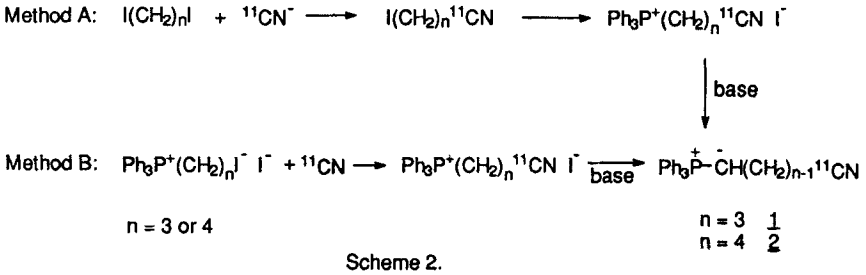
[^{11}C] cyanoalkyltriphenylphosphoranes

figure 1.

[^{11}C]Cyanoalkyltriphenylphosphoranes (1 and 2), scheme 2.

Method A: The ^{11}C-labelled iodoalkylnitriles where prepared by a substitution reaction with [^{11}C]cyanide and the corresponding diiodoalkane (2). Triphenylphosphine was added in 1,2-dichlorobenzene and the mixture was heated. To generate the phosphorane the epoxide epichlorohydrin was added as a precursor of the base. The epoxide opens in the reaction with the free iodide ions, produced in the substitution reaction, giving a basic alkoxide ion. The amount of base versus the amount of phosphorus ylid is therefor controlled *in situ* (3).

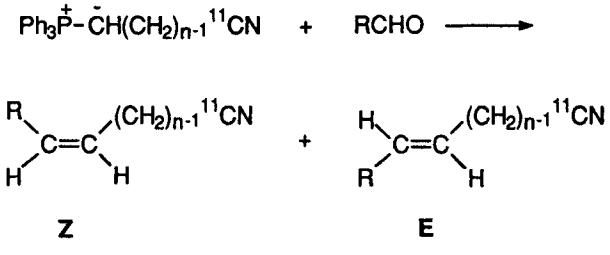
Method B: In this method the [^{11}C]nitrile group was introduced by a substitution reaction with [^{11}C]cyanide directly on the corresponding iodoalkyltriphenylphosphonium salts. The products 1 and 2 were obtined after heating the reaction mixture in the presence of epichlorohydrin as a precursor of the base, see "Method A".



Scheme 2.

Synthesis of alkenes from the [^{11}C]cyanoalkyltriphenylphosphoranes (1 and 2), scheme 3.

For a model study we chose some alifatic and aromatic aldehydes to study the reactivity and utility of the Wittig reagents, scheme 3. The reactions were carried out in one-pot, adding the aldehyde and epichlorohydrin to the solution with the labelled phosphonium salt. The aldehydes used were: bensaldehyde, 3-nitrobensaldehyde, cinnamaldehyde, valeraldehyde and nonyl aldehyde.



Scheme 3.

The ^{11}C-labelled Wittig reagents 1 and 2 synthesized by Method A, scheme 2, were produced in 80-98 % radiochemical yield in 15 minutes. By Method B the yields were 75-98 % in 10 minutes, counted from the end of [^{11}C]cyanide production. Reaction with the aldehydes gave the corresponding alkene in high yields, 92-98 % radiochemical yield after ca. 10 minutes. The E:Z ratios were determined. When the Wittig reaction is carried out under salt-free reaction conditions (no Lithium-salts present), the Z-alkene is the major product.

The high yields in the synthesis of the reagents 1 and 2 and their reaction with aldehydes, makes it interesting to further investigate the utility of these multifunctional precursors in the preparation of ^{11}C-labelled alkenes. We are also developping methods for producing other precursors for Wittig reactions, for example (4-carboxybutyl) triphenylphosphorane.

References

1. Kihlberg T., Gullberg P. and Långström B., J. Labell. Radiopharm. 10 (1990) 1115-1120.
2. Hörnfeldt K., Antoni G. and Långström B., Acta Chem. Scand. 46 (1992) 87-91.
3. Buddrus J. and Kimpenhaus W., Chem. Ber. 106 (1973) 1648-1660.

[^{11}C]-Cyano-biphenyl from [^{11}C]-copper(I)cyanide. Model for the construction of the tetrazole moiety of labelled Angiotensin II antagonists for PET.

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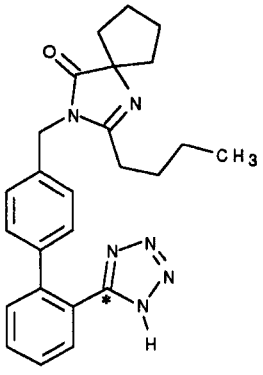
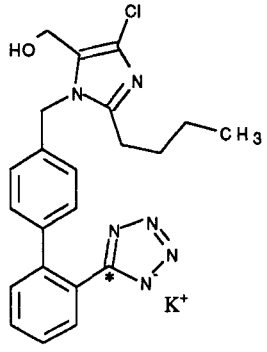
Over the past decade, inhibition of the renin-angiotensin system has emerged as a promising therapeutic approach. The peptide-hormone angiotensin II (AgII) exerts potent inotropic and chronotropic effects on mammalian cardiac tissue. AgII plays a role in preserving the function of the failing heart. In addition to its direct effects on myocardial contractility (as proven in denervated heart), AgII has a substential effect on cardiac function through the sympathetic cardiac nerves. AgII also stimulates protein synthesis and cell growth. Therefore, the study of AgII receptors during heart failure, heart growth, hypertrophy and ventricular remodelling after myocardial infarction is of interest. High-affinity low-capacity membrane AgII-receptor-binding sites have been identified. Most of the effects are mediated through the AT1 receptor while the role of the AT2 receptor remains unclear.

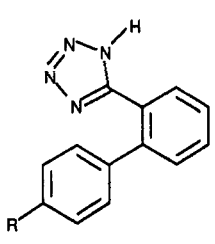


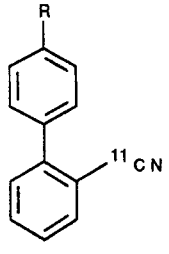
SR-47436



DUP-753

Recently, many nonpeptide Ag II antagonists have been developed by different companies. They all have the biphenyl-tetrazole entity in common as for example in SR-47436 and DUP-753.





As a first step towards their [^{11}C]-labelling, the synthesis of [^{11}C]-cyano-biphenyl is presented. The radiosynthesis follows scheme **I**. [^{11}C]-HCN was obtained by a rapid on-line synthesis from [^{11}C]-CH_4 and ammonia passed through a quartz tube containing platinum at 990°C (**1**). The [^{11}C]-CH_4 was produced by the (p,α) reaction with 20 MeV protons on nitrogen containing 5 % of hydrogen.

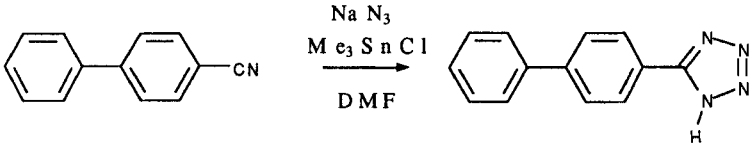
Scheme I:

$$N_2+H_2 \longrightarrow {}^{11}CH_4 \longrightarrow H^{11}CN \longrightarrow Cu^{11}CN \longrightarrow Ar\text{-}^{11}CN$$

For the radiosynthesis, [^{11}C]-HCN was trapped in a vial at -15°C to eliminate the excess of ammonia. After warming to room temperature, an aqueous solution of $CuSO_4$ and $Na_2S_2O_5$ (100µl) was added into the vial containing [^{11}C]-HCN and the mixture was heated at 60°C for 5 min. After this, 200µl of biphenyl halide in DMF was added into the vial and the reaction mixture was heated at 180°C for 5 min. For reversed phase HPLC analysis, solvents were evaporated, the vial was cooled to room temperature and 500µl of methanol was added. About 30% of [^{11}C]-cyano-biphenyl was obtained after 15min (decay corrected). Classical conditions (**2**) for the synthesis of copper(I) cyanide were adapted for [^{11}C]-HCN and experimental conditions are shown in table **1**.

This substitution of aromatic halide via the copper [^{11}C]-cyanide pathway was found to be useful in synthetizing easily aromatic-[^{11}C]-cyanide as precursor for aromatic-[^{11}C]-tetrazoles.The (1H-tetrazol-5-yl)biphenyl was obtained via the classical method : scheme **II**, (**3**).

Scheme II:



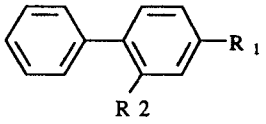
Synthesis conditions :
In a vial, at 1µmole of cyano-biphenyl in solution in DMF (100µl) were added 20µl of trimethyltin chloride in DMF (10µmole) and 20µl of sodium azide in water(10µmole). The reaction mixture was heated 5min at 140°C. The (1H-tetrazol-5-yl)biphenyl was obtained with 30% yield(calculated by reversed phase HPLC).

Table 1 :

Table of parameter studies for radiolabelling of halogeno-biphenyl

[^{11}C]	$CuSO_4$ µmole	$Na_2S_2O_5$ µmole	precursor µmole	solvent µl DMF	*yield %
			(I)		
$K^{11}CN$	1	0.9	19	200	---
$H^{11}CN$	1.08	0.9	19.5	200	22
			(II)		
$K^{11}CN$	0.8	1	20	200	---
$H^{11}CN$	1.05	0.9	23	200	34

* decay corrected



(I) $R_1 = Br$, $R_2 = H$

(II) $R_1 = H$, $R_2 = Br$

(1) Mestelan,G.; Crouzel,C.; and Comar,D.. Synthesis and distribution Kinetic in animals of [α-^{11}C]-3,4-dimethoxyphenethylamine. Int. J. Nucl. Med. Biol., 4, 185-193, 1977.

(2) Friedman,L.; and Shechter,H.. Dimethylformamide as a useful solvent in preparing nitriles from aryl halides and cuprous cyanide; improved isolation techniques. J. Org.Chem., 26, 2522-2524, 1960.

(3) Carini,D.; Duncia,J.; Aldrich,P.; Chiu, A.; Johnson,A.; Pierce,M.; Price,W.; Santella III,J.; Wells,G.; Wexler,R.; Wong,P.; Yoo,S.-E.; and Timmermans,P..Nonpeptide angiotensin II receptor antagonists: the discovery of a series of N-(biphenylylmethyl)imidazoles as potent, orally active antihypertensives. J. Med. Chem., 34, 2525-2547, 1991.

Synthesis of Carbon-11 labelled lower chloromethanes ; Application in a methylenation reaction. CROUZEL C. and HINNEN F. - Service Hospitalier Frédéric Joliot - DRIPP, CEA, 91406 ORSAY-France.

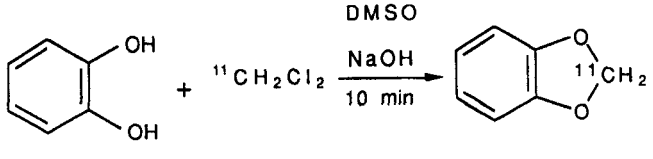
We have shown that the catalyst $CuCl_2$ on pumice stone allows, depending on the temperature, for either [^{11}C] carbon tetrachloride (380° C) or [^{11}C] chloroform (330° C) in the reaction of Cl_2 with $^{11}CH_4$ (1,2).

At temperatures below 300° C, this catalyst gives labelled monochloromethane or dichloromethane but with modest yields (20 to 25 % with respect to $^{11}CH_4$). In our search for catalyst more selectively in favour of the lower chloromethanes we studied the chlorination of [^{11}C] methane with 0.5 % Pt on Al_2O_3 as well as with supported Lewis acid halides-oxyhalides such as $FeOCl/Al_2O_3$ and $ZrOF_2/Al_2O_3$ as catalysts (3). These catalysts were prepared from γ - Al_2O_3 with either $FeCl_3$ or ZrF_4 and were studied in term of amount of catalyst, temperature, chlorine concentration and carrier gas flowrate.

In the majority of experiments only monochloromethane and dichloromethane were formed with on some occasions some traces of chloroform.

The best yield of $^{11}CH_2Cl_2$ is obtained with 700 mg of 10 % ZrF_4 on Al_2O_3 at 310° C with carrier gas flowrate of 30 ml/min. (He).Under these conditions 30-35 % of the total radioactivity is in the $^{11}CH_2Cl_2$ form with 20 % $^{11}CH_3Cl$. A decrease of temperature and an increase of flowrate reverse the proportions, very little $^{11}CO_2$ is formed (<5 %). The various chlorinated derivatives of $^{11}CH_4$ were analysed by gas chromatography (Delsi Nermag Apparatus, Columm Hayasep Q, 80-100 mesh, 2m x 1/8", stainless steel, 180° C, with helium as carrier gas, 50ml/min). tr-CH_4,CO_2 = 0.6 min, tr-CH_3Cl = 1.3 min, tr-CH_2Cl_2 = 3.2 min, tr-$CHCl_3$ = 6.2 min, tr-CCl_4 = 9.5 min.

The separation $^{11}CO_2/^{11}CH_4$ can be carried out on the same column at 30° C.
As an example of a methylenation reaction, $^{11}CH_2Cl_2$ was used on catechol (4).



Catechol 1,3-benzodioxole

t°	Yield %
100°	17
120°	15
140°	48
160°	56

20 minutes after EOB, 150-200 mCi (5,55-7.4 GBq) [^{11}C] Benzo-1,3 - dioxole are obtained with a specific activity of 0.6-1 Ci/μmole (22,2-37 GBq/μmole).

The identification of the [^{11}C] product was carried out by HPLC, Si 60 column, 7 μm, (25 x 0.9cm), solvent: cyclohexane -AcOEt flowrate 4ml/min, U.V. 254 nm Tr = 5.5 mn.

REFERENCES :

1 - Crouzel C., Amano R. and Fournier D. Appl. Radiat. Isot. 38, 669-670, (1987).
2 - Landais P. and Crouzel C. Appl. Radiat. Isot. 38, 297-300, (1987).
3 - Olah G.A., Gupta B. , Farina M. and al. J. Am. Chem. Soc. 107, 7097-7105, 1985.
4 - Bonthrone W. and Cornforth J. W. J. Chem. Soc. (C) 1202-1204, (1969).

^{11}C-Alkylation on Al_2O_3/MF : a Useful Method for Rapid Labelling

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^{11}C-methylation is one of the most widely used method for radiopharmaceutical labelling with ^{11}C. It consists usually of adding [^{11}C]methyl iodide prepared separately to a solution of the NOR derivative, the chemical reaction taking place at given time and temperature. The HPLC system is necessary to separate the unreacted precursor and the radiochemical and chemical impurities from the desired compounds. These steps are generally time consuming, difficult and expensive to automate.
Alkylation on solid support (1, 2) is an approach which allows labelling at high yield and high regioselectivity in a short reaction time (3, 4). Alcalino fluoride supported on alumina (5-7) is a well know reagent in non radioactive chemistry used for alkylation. The system presents the following characteristics :

- a large surface area leading to high reaction rates and high yields
- the presence of pores and of the alcalino metal constrains the substrate and the reactant resulting in high regioselectivity
- important adsorption of the NOR derivatives ensuring easy purification like a simple filtration.

From a mecanistic point of view, the interfacial reaction involved in the alkylation on solid support is illustrated in Scheme 1. The fluorides solubilized in water establish strong hydrogen bonds with the hydroxyl groups at the surface of the alumina. The alcalino metal stays near the fluoride as well as a part of the water in the coordination sphere of the ion. The fluoride anions can also establish an hydrogen bond with the organic NOR derivatives. The result of this is a transfer of the electron density from the fluoride anion to the nucleophilic site of the NOR derivative allowing the reaction with [^{11}C]methyl iodide. The methylated product leaves the support because no interaction can be realised between the fluoride and the methyl group (5, 6).
The preliminary results of the use of alcalino fluoride on alumina for alkylation with [^{11}C]methyl iodide are reported here. Important parameters governing the reaction were tested on [^{11}C]flumazenil (8) a routinely used molecule in the Cyclotron Research Center of Liège. Two different techniques were used to study the ^{11}C-methylation of NOR RO 15.1788 on Al_2O_3/MF: the "on column" reaction and the "in suspension" method.

"On column" method

The "on column" reaction was realised by passing [^{11}C]methyl iodide in acetonitrile (500 µl) at room temperature through a pre-HPLC column filled with the support Al_2O_3/MF-NOR RO 15.1788 prepared according to J.Yamawaki *et al.* (7). A simple elution with acetonitrile led to a solution of [^{11}C]flumazenil and more than 93 % of the NOR derivative remained on the support. Different parameters were shown of prime importance: the amount of water on the support, the alcalino metal associated to fluoride and the pore size.

Amount of water

This parameter was studied by using a support dried in an oven at different temperatures. The results are summarized in Table 1. The best yields were obtained with a support (CsF/Al_2O_3) dried in an oven during 24 hours at 150 °C.

Alcalino metal
The key role of the alcalino metal associated to fluoride was shown by the results presented in Table 2. No alkylation was detected with Li and Na. On the other hand a large variation of the results was obtained with Rb and Cs.

Pore size
Different types of alumina were used to show the effect of the pore size. It appeared that the reaction with the alumina which had the smallest pores had the highest regioselectivity and the highest yield (a large surface reaction area increases the yield).

"In suspension" method
To increase the yield of the reaction by rising the diffusion of the [^{11}C]methyl iodide, a second technique was investigated: the "in suspension" reaction. This procedure was realised by trapping the [^{11}C]methyl iodide in a suspension of Al_2O_3/KF-NOR RO 15.1788 in acetonitrile. The mixture was heated at 110 °C for about 20 seconds. The [^{11}C]RO 15.1788 was separated by a simple filtration. The radiochemical yield obtained was about 94 % (EOB) and more than 99 % of the NOR RO 15.1788 involved in the reaction were retained on the support (Table 3) resulting in a solution of acetonitrile containing [^{11}C]flumazenil and the unreacted [^{11}C]methyl iodide. After classical Sep-Pak purification and sterilisation, an injectable solution of [^{11}C]flumazenil presenting radiochemical and chemical purities > 99 % and a specific activity > 1 Ci/μmol EOS (average on twenty runs) was obtained without HPLC purification.

A small amount of NOR RO 15.1788 remains in the injectable solution (Table 3). This quantity is still superior to what can be obtained after HPLC but the system is not completely optimized. With a specific activity of 1Ci/μmol at the end of synthesis, the amount of NOR RO 15.1788 is much smaller than the RO 15.1788 and it becomes possible to use this process for routine productions.

The interfacial reaction using MF-alumina for the methylation of NOR RO 15.1788 is characterized by high yield, high rate of reaction and high regioselectivity. More than 99 % of the NOR derivative are easily removed by a simple filtration. The total preparation time from [^{11}C]methyl iodide can be reduced to 8 minutes including purifications and formulation. A study of the different parameters of the reaction has been done and is being pursued. The interfacial reaction is currently tested on other molecules, the goal being to generalize the method.

1 McKillop A. and Young W.D. - Synthesis 401 (1979)
2 McKillop A. and Young W.D. - Synthesis 481 (1979)
3 Jewett D.M. - J. Label. Compds. Radiopharm. 30: 171 (1991)
4 Iwata R., Pascali C., Yuasa M., Yanai K., Takahashi T., Ido T. - Appl. Radiat. Isot. 43: 1083 (1992)
5 Posner G.H. - Angew. Chem. Int. Ed. Eng. 1: 487 (1978)
6 Clark J.H. Chem.Rev. 80: 429 (1980)
7 Yamawaki J. and Takahashi A. - Chem. Lett. 755 (1979)
8 Mazière M., Hantraye Ph., Prenant C., Sastre J., Comar D. - Appl. Radiat. Isot. 35: 973 (1984)

Scheme 1

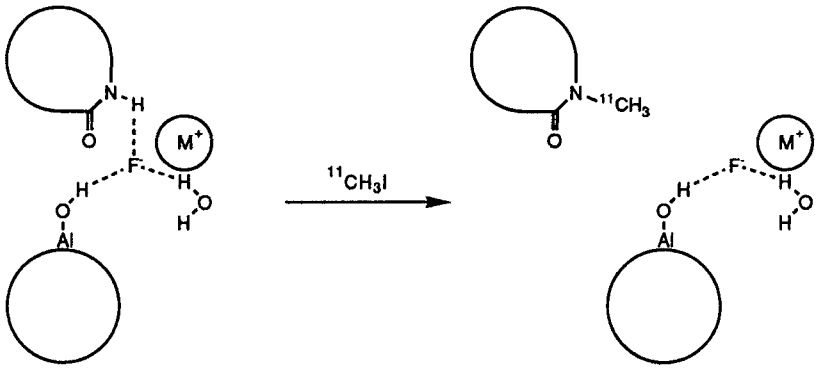

Table 1

Temperature (°C)	100	150	200	250	300
Yield % (EOB)	17	62	53	43	8

Table 2

Cation	Li	Na	K	Rb	Cs
Yield % (EOB)	0	0	44 (± 8)	6 (± 4)	62 (± 20)

Table 3

Quantity of NOR RO 15.1788 at the beginning of the reaction (mg)	Preparation time from [^{11}C]methyl iodide (min)	Radiochemical yield EOS (%)	NORRO 15.1788 in the injectable solution (µg)	Percentage of NORRO 15.1788 adsorbed after filtration
0,2	8	55	1,60	99,19
0,3	8	57	0,84	99,72
0,4	8	28	1,12	99,28
0,5	8	57	1,40	99,72
0,6	8	54	0,84	99,86
0,7	8	42	0,70	99,90
1,0	8	50	0,89	99,99
	8	49	1,05	99,67

Preparation, Handling and Reactivity of [^{11}C]Methyl Triflate for Fast Carbon-11 Radiomethylations: Some New Results.

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[^{11}C]Methyl triflate (1) is more reactive than [^{11}C]methyl iodide yet sufficiently stable to be handled without special precautions. C-, O- and S- methylations were accomplished under conditions applicable to PET. Reactions were usually instantaneous at or below room temperature, thus no heating or confinement of the reaction mixture was required. Desmethyl raclopride was O-methylated to [^{11}C]raclopride at -20° in dimethylacetamide. Norepinephrine was N-methylated to [^{11}C]epinephrine in DMF. Homocysteine was S-methylated to [^{11}C]methionine in water at 0°. Dimethylacetamide and propylene carbonate are preferred solvents since they are relatively unreactive toward methyl triflate. Diisopropyl ethylamine is preferred as a base since it reacts relatively slowly with methyl triflate. Triisopropylamine (2) is even less reactive but not commercially available. Amines in the form of halide salts may be unsatisfactory as substrates, since halide anions may react with methyl triflate to yield less reactive methyl halides.

[^{11}C]Methyl triflate can be concentrated from a stream of N_2 by trapping it on carbon yarn at -60°. It is sufficiently unreactive toward dry glass, Teflon, polyolefins, stainless steel, etc. that no special apparatus is necessary. Methyl iodide was converted to methyl triflate by supported silver triflate at 180° even in the presence of small amounts of water. Methyl bromide was just as reactive as methyl iodide for the formation of methyl triflate over supported silver triflate at 180°. Methyl chloride was less reactive. Microscopic observation revealed that the silver triflate was coated with a molten phase at 180°. Thus the conversion is probably a gas-liquid phase reaction. Silver triflate dissolved in a eutectic melt composed of equimolar amounts of Na, K and Li triflates was an effective reagent for the conversion of methyl iodide. In addition to graphitized carbon Teflon powder and carbon fiber were found to be adequate as inert supports for silver triflate. The apparent specific activity of [^{11}C]raclopride was lower when the conversion of [^{11}C]methyl iodide to [^{11}C]methyl triflate was done at 220° instead of 180°.

1. Jewett, D.M. - Appl. Radiat. Isot. 43:1383 (1992).

2. Bock, H., Goebel, I., Zdenek, H., Liedle, S. and Oberhammer, H. - Angew. Chem. Int. Ed. Engl. 30:187 (1991).

Palladium Promoted One-pot Synthesis of ^{11}C-labelled Benzamide Compounds.

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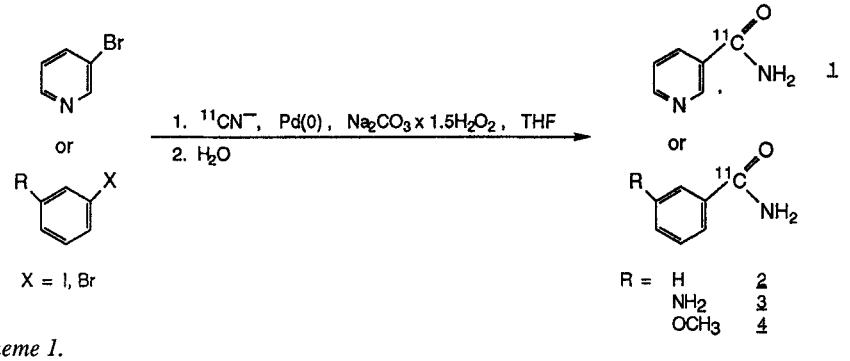
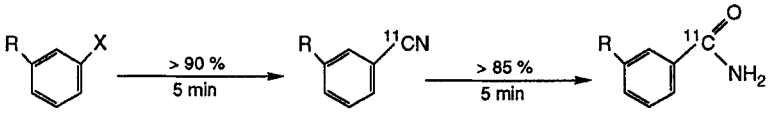
Poly (ADP-ribose) polymerase catalyses the synthesis of Poly (ADP-ribose) from NAD^+, thereby releasing the DNA from histones to a transcriptionally and reparation active form (1). In order to establish if *in vivo* and *in vitro* studies of this enzyme is feasible with positron emission tomography (PET), the poly (ADP-ribose) synthetase inhibitors benzamide, 3-aminobenzamide, 3-methoxybenzamide and nicotinamide were labelled with carbon-11 in the amide group. In previous work we have been studying the cyanation of aromatic compounds, employing the transition metals chromium or palladium, to obtain [cyano- ^{11}C]benzonitriles (2). We here present the application of the Pd(0) catalysed reaction of hydrogen [^{11}C]cyanide with aromatic substrates in the rational and simple one-pot procedure for synthesis of ^{11}C-labelled amides (Scheme 1).



Scheme 1.

The first reaction step in the preparation of the ^{11}C-labelled amides 1-4, was the metal catalysed carbon-carbon bond forming reaction of the hydrogen[^{11}C]cyanide with an aromatic halide. The products formed were [cyano-^{11}C]benzonitriles (Scheme 2) or 3-[^{11}C]cyanopyridine. High radiochemical yields, usually >90 % as determined by analytical HPLC, were obtained in a few minutes reaction time. In the second reaction step conversion of the nitriles to [carboxy-^{11}C]benzamides was achieved by reaction with hydrogen peroxide which was liberated *in situ*, by water, from sodium percarbonate. The radiochemical yields for the conversion varied between 86-99 % .



Scheme 2.

The total synthesis time including reversed phase HPLC purification was 25-30 minutes. The radiochemical yield, counted from when hydrogen [^{11}C]cyanide was trapped, was 45-70 %. The specific radioactivity was in the order of 2-3 Ci/μmol, and radiochemical purity higher than 99% . The position of label was confirmed, and the product identity verified, by

conducting a combined $^{13}C/^{11}C$-synthesis of 3-methoxy benzamide 4 , and analysing the product by GC-MS, ^{1}H- and ^{13}C NMR. The identity of compounds 1-3 was assessed by analytical HPLC before and after addition of reference substance.

As the aim of this work was to employ the ^{11}C-labelled aromatic amides in PET investigations, the palladium content in the final product was an important aspect. To ensure that the residue of the metal catalyst had been separated from the product, fractions collected after different purification procedures were analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES). The best result was achieved when the reaction mixture was subjected to solid phase extraction prior to semi preparative HPLC. The palladium concentration in the final formulated product solution (5 ml) was approximately 50 ng/ml, which is close to detection limit. Following intravenous administration the LD_{50} for $PdCl_2$ in rat has been determined to 3 mg/kg (3).

PET studies on the distibution of these ^{11}C-labelled inhibitors of poly (ADP-ribose) synthetase were performed in rhesus monkey . Different kinetic patterns were observed, giving important pharmacokinetic information. Further work is, however , necessary for the assessment if these compounds are also able to indicate the enzyme activity of poly (ADP-ribose) synthtase. The ability to employ the palladium promoted ^{11}C-labelling reaction in synthesis of more potent poly (ADP-ribose) synthetase inhibitors is therefore now in progress.

1. Boulikas T. - Anticancer Research 11; 489 (1991)
2. Andersson Y., Malmborg P., and Långström B. - J. Lab. Comp. and Radiopharmaceuticals 30; 144 (1991)
3. Moore W., Hysell D., Hall L., Campbell K., and Stara J. - Environmental Health Perspectives 10; 63 (1975)