PRODUCTION OF ^{96}Tc, A LONG LIVED SUBSTITUTE FOR ^{99m}Tc.

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Technetium-96 decays by electron capture with a 4.35 d half life and primary gamma rays which are easily detectable: 778.5 keV (100%), 812.8 (82%), and 850.3 (99%). These properties make it valuable as a stand-in for ^{99m}Tc in in-vitro or animal studies beyond the useful life of ^{99m}Tc. We have known for some time (1,2) that a small quantity of ^{96}Tc is coproduced with ^{97}Ru by the irradiation of Rh foil with 200 MeV protons at the Brookhaven Linac Isotope Producer (BLIP). It was also noticed as an impurity by Lagunas Solar et al. (3) in the 67 MeV proton irradiation of Rh. Technetium-96 prepared with the BLIP was used for the first time in a recent collaborative study with the University of Cincinnati to compare the long term behavior of bone agents Tc-HEDP and ^{186}Re-HEDP in rats (4). We report here the method of production and isolation of ^{96}Tc from a Rh target.

Technetium-96 has been produced by the ^{93}Nb(α,n), ^{96}Mo(p,n), and ^{96}Mo(d,2n) reactions (5,6,7). Nevertheless, the irradiation of Rh is an attractive route because of the coproduction of ^{97}Ru, a useful radionuclide for various imaging applications. In these experiments we bombarded high purity Rh foil with ~90 MeV protons rather than 200 MeV typically used to prepare ^{97}Ru. Early unpublished data from several irradiations at the Indiana University Cyclotron (8) showed that the yield of ^{96}Tc was better at approximately 80 MeV with acceptable ^{97}Ru production. The chosen energy was the closest to this value that was compatible with the standard target array at BLIP. The target foil, 0.025 cm thick and measuring 2.5 x 2.5 cm in area, is clipped onto a stainless steel backing plate for insertion into a BLIP target holder, placed in a water gap between larger disk targets. Production irradiations generally occur over a weekend. After bombardment, the target is transferred to a processing hot cell and dissolved by a.c. electrolysis in a small cell made of glass. The Rh foil is used as one of the electrodes, a graphite rod the other electrode, and the electrolyte is 6N HCl. In early versions of this cell the square Rh foil was clipped to a Rh wire at a corner and the liquid level kept below the point of contact. Unfortunately, this always left the corner undissolved and therefore was a major source of lost yield (up to 20%). An improved design was recently developed in which the Rh foil is held by a clamp consisting of two graphite rods with a small graphite disk (acting as a fulcrum) between them. A rubber "O" ring placed below the disk supplies tension to hold the Rh between the ends of the graphite rods. In this manner the entire Rh foil can be immersed in the electrolyte. Also, to obtain high current density and even surface electrical fields, the other graphite rod electrode is encased to within ~1cm of the bottom with shrink tubing. Current densities of 0.3A/cm^2 dissolve the target in 12-15h. Only negligible amounts of undissolved target fragments still remain. This solution is sucked by vacuum into a flask where it is evaporated to near dryness to remove the HCl and 3 ml of water are added. The solution is transferred by pressure to a distillation flask to which is added an oxidizing mixture of 3 ml of 12 N H_2SO_4 and 3 ml of $KMnO_4$. Ruthenium-97 is distilled as RuO_4 which is collected in a vessel containing 1:1 HCl: EtOH to give a solution containing predominantly ruthenium (III) chloride. The overall ^{97}Ru recovery is about 90%. To separate the Tc, the rhodium sulfate residue is transferred to a separatory funnel and 11 N NaOH added to make the solution strongly basic. Technetium (present as pertechnetate) is separated nearly quantitatively by repeated extractions with methyl ethyl ketone, using bubbled

N_2 to mix the phases. The organic phase is evaporated and the ^{96}Tc recovered in 0.05 N NH_4OH. The apparatus to perform these separations remotely in a hot cell is shown schematically in Figure 1.

The radionuclides detected in the target after a brief 15 minute irradiation are listed in Table 1. The final product contains only $^{95,95m,96}Tc$. The preliminary yield of ^{96}Tc from a 0.025cm thick target was 1.8mCi/μA at end of bombardment (EOB). This is not the true nuclear yield achievable because the beam spot area is considerably larger than the target area. A large beam spot is necessary at BLIP to reduce the power density deposited in our thick salt targets and thus prevent their melting. After correcting for the fraction of the beam actually hitting the Rh target, a production rate of 12.0 mCi/μA is obtained even in this relatively thin target. Although the small area target has given adequate quantity of ^{97}Ru, a larger, thicker target could be accommodated to produce more ^{96}Tc if necessary. After a 4 day irradiation at approximately 90 MeV the ratios $^{95}Tc/^{96}Tc$ and $^{95m}Tc/^{96}Tc$ were 3.9 and 2.3×10^{-2} respectively at EOB. After processing and shipment the $^{95}Tc/^{96}Tc$ ratio declines to 0.5. This ratio can be controlled by adjusting the proton energy, the length of irradiation and the decay time after EOB, even though ^{95}Tc does not interfere in tracer applications of ^{96}Tc. Studies to further speed the processing and optimize yield are underway. Technetium-96 as ammonium pertechnetate is now available to the nuclear medicine community during the BLIP operating cycle.

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Table 1. Radionuclides identified in solution of Rh target which was irradiated with ~90 MeV protons for 15 minutes.

Radionuclide	$t_{1/2}$	Radionuclide	$t_{1/2}$
^{100}Pd	3.7 d	^{97}Ru	2.88 d
^{101}Pd	8.5 d	^{93}Tc	2.75 h
^{99}Rh	16.1 d	^{94}Tc	4.88 h
^{99m}Rh	4.7 h	^{95}Tc	20 h
^{100}Rh	20.8 h	^{95m}Tc	61 d
^{101m}Rh	4.34 d	^{96}Tc	4.35 d
^{94}Ru	51.8 m	^{90}Mo	5.67 h
^{95}Ru	1.65 h	^{93m}Mo	6.95 h

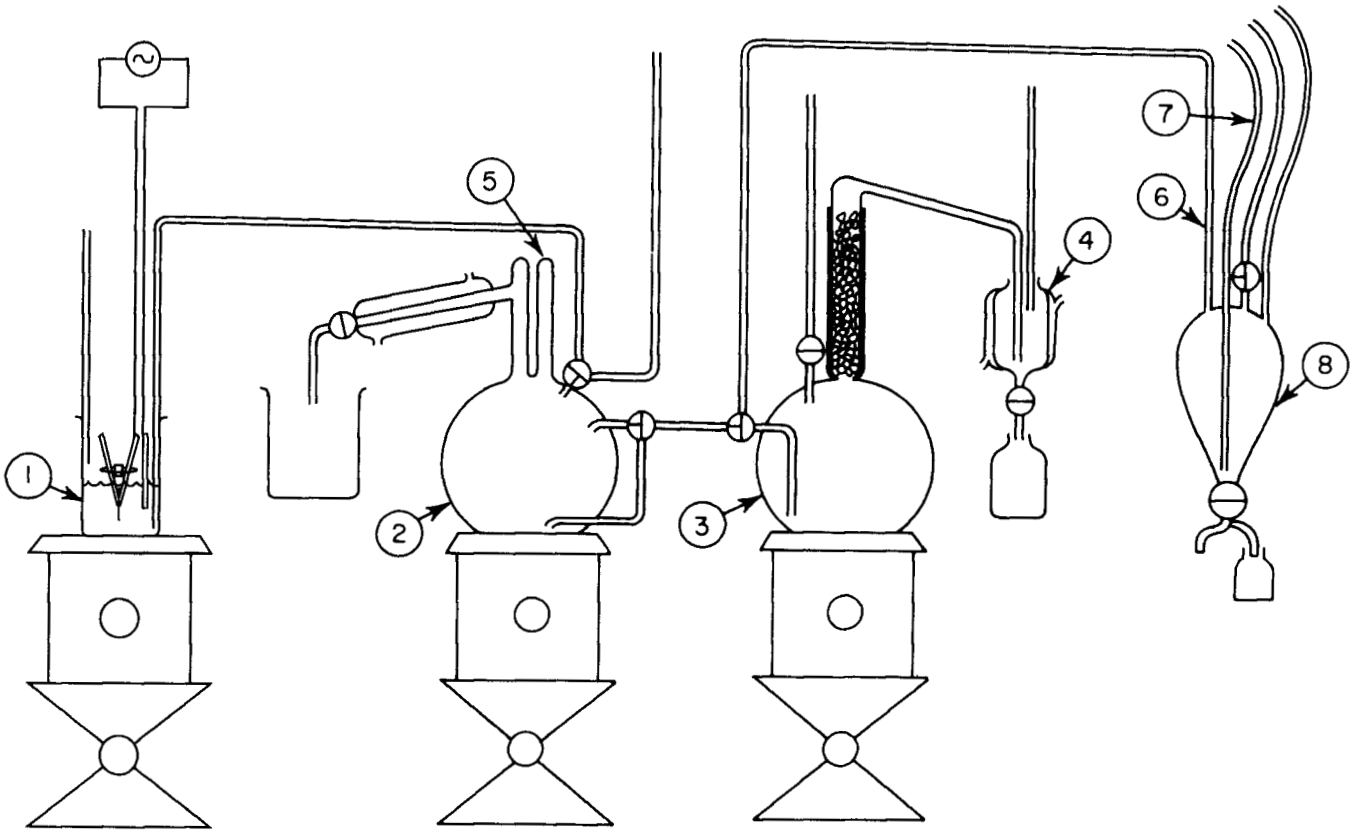

Fig. 1 Apparatus for remote hot cell separation of ^{97}Ru and ^{96}Tc from a Rh target. (1) a.c. electrolysis cell with graphite clamp; (2) HCl distillation flask; (3) Ru distillation flask; (4) ^{97}Ru collection vessel cooled by ice water; (5) thermocouple well; (6) ^{96}Tc transfer line; (7) air bubbler; (8) ^{96}Tc extraction vessel. (adapted from ref. 2.)

Structural Analysis of $Tc^{V}O-N_2S_2$ Complexes.

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In developing new ^{99m}Tc brain perfusion agents based on complexes containing a $Tc^{V}O$-bisaminothiol (BAT) core, formation of syn and anti isomers is common when pendant side chains are incorporated into the BAT backbone (1-4). It is important to identify structural features of these isomers contributing to disparity in biodistribution. Three molecules, [TcO(BAT-TE)], and the syn and anti isomers of [TcO(PIP-PH)], were prepared and characterized by elemental analysis, mass spectroscopy, nmr, ir, uv-visible spectroscopy and X-ray crystallography.

Structural features of these complexes can be summarized as the following:

1. The X-ray crystallography studies show that all of these neutral BAT complexes have highly distorted square pyramidal geometry about the Tc with an oxygen in the apical position.

2. The piperidinyl ring of the pendant side chain of both isomers is in the chair conformation.

3. The distance between the apical oxygen and the heterocyclic nitrogen of the piperidinyl ring is 3.597Å for the syn isomer and 5.461Å for the anti isomer. This indicates that the 3 dimensional structure of the syn isomer is more compact than the anti isomer.

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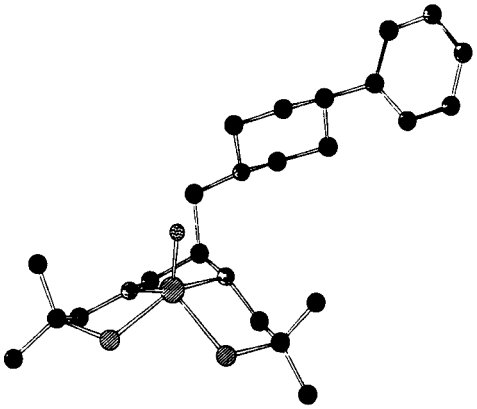
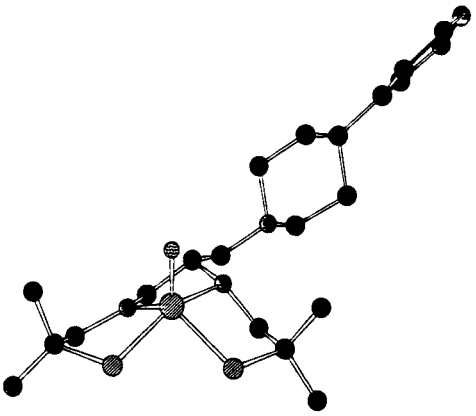
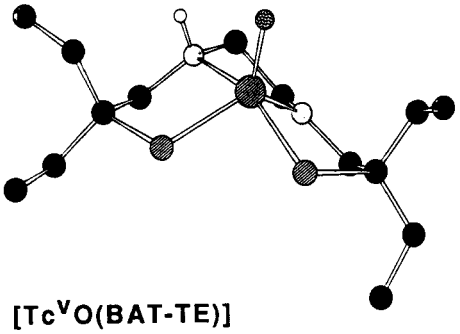


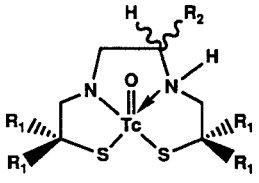
[$Tc^{V}O$(PIP-PH)] Syn-



[$Tc^{V}O$(PIP-PH)] Anti-



[$Tc^{V}O$(BAT-TE)]



COMPOUNDS	R_1	R_2
[$Tc^{V}O$(BAT-TE)]	Et	H
[$Tc^{V}O$(PIP-PH)]	Me	CH_2–N⟨piperidine⟩–Ph

RADIOPHARMACEUTICAL FOR RENAL FUNCTION STUDIES.

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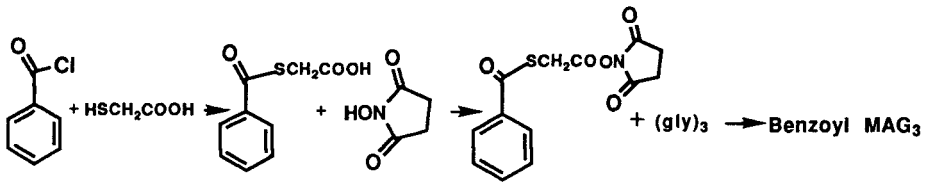
$^{99m}TcMAG_3$, a potential replacement for ^{123}I and ^{131}I o-iodiohippuran in renal function studies (1), is now available via a cold kit formulation that provides a product of excellent radiochemical purity and stability (2). The chemistry that underlies this kit formulation includes (i) characterization of the structure and properties both of $TcMAG_3$ and of Tc-containing impurities present in early kit formulations, (ii) a new approach to the synthesis of the MAG_3 ligand to eliminate the source of potential radiolabeled impurites (3), and (iii) detailed modifications of earlier generation kit formulations.

$TcMAG_3$ has been characterized by structural and chemical investigations. This anionic complex contains a mono-oxo Tc(V) core and is remarkably robust. It is stable even when heated in solutions of strong acid, base or DTPA. The Tc(V) core is reduced only by very strong reductants in the presence of excess halide ion, and undergoes only slow aerobic oxidation in aqueous solution.

Certain of the radiolabeled impurities found in early kit formulations of $^{99m}TcMAG_3$ were shown to exhibit biodistributions that interfered with renal function studies. Structural and chemical characterization of these radiolabled impurities demonstrated that they arose from "cold" impurities present in the MAG_3 ligand itself. A particularly troublesome impurity is the methyl ester of MAG_3 which is generated as a by-product during the original MAG_3 ligand synthesis. To avoid the genration of radiolabeled impurities resulting form the "cold" impurites in the kit, a new synthetic route to MAG_3 was developed. This new synthesis avoids the tedious Schotten-Bauman reaction used in earlier work (1b), and is based upon reaction of an activated ester of S-benzoylmercaptoacetic acid with triglycine. The basic reaction is the same as one used by Brandau, et al in a synthesis of benzoyl MAG_3 published earlier (1a).



Other radiolabelled impurities were shown to arise from the chemistry of the $^{99m}TcMAG_3$ complex itself. Specifically, the reduction of the $^{99m}Tc^{V}$=O core of the $^{99m}TcMAG_3$ gives rise to $^{99m}Tc^{IV}MAG_3$, which can then decompose to other radiolabelled impurities including "reduced, hydrolyzed 99m-technetium". Management of this chemistry required careful choice and control of reductant (stannous ion); in the final kit formulation, air is added to the kit after reconstitution in order to remove excess stannous ion.

Additional improvements over early kit formulation for $^{99m}TcMAG_3$ were largely based on (i) studies delineating the rates and mechanism of the reaction of the benzoyl-protected MAG_3 ligand with various ^{99m}Tc-TL complexes (where TL represents a transfer ligand), (ii) studies evaluating conditions which affect the formation of ^{99m}Tc-TL complexes from the $^{99m}TcO_4^-$ and (iii) studies evaluating conditions which affect the stability of the final $^{99m}TcMAG_3$ product. The final formulation is based on a judicious balance of MAG_3, tartrate (the TL), Sn(II) and H^+ concentrations, as well as of the time and temperature of heating.

Development of the cold kit formulation for $^{99m}TcMAG_3$ provides a notable example of the effective application of the principles of inorganic chemistry to radiopharmaceutical development.

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ELECTROCHEMISTRY OF BORON-CAPPED ^{99}Tc-DIOXIME COMPLEXES.

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Boronic Acid Adducts of Technetium Dioxime (BATO) complexes have recently been introduced as perfusion imaging agents (1,2). These complexes contain three bidentate dioxime ligands capped by a boronic acid moiety, and an anionic "axial" ligand (X), which occupies a seventh coordination site (3). We report here the cyclic voltammetric behavior of several BATO complexes, $TcX(dioxime)_3BR$ and the uncapped *tris*-dioxime species, $TcCl(dioxime)_3$ (4), (dioxime = dimethylglyoxime [DMG], cyclohexanedionedioxime [CDO]).

Results for a typical chloro-BATO compound in DMF (N,N-dimethylformamide) are shown in Figure 1. The first reduction (at E_{pc}~-1.3V in Figure 1) has been determined to be a two-electron process (5) for the bromo and chloro species. On the return anodic sweep, the associated re-oxidation peak at E_{pa}~-0.7V is too far removed to correspond to the oxidation half of a reversible couple. These results are characteristic of a chemically irreversible two-electron reduction in which a chemical change follows (or accompanies) the initial electron-transfer, producing a species which is easier to reduce ($E_1^o \leq E_2^o$) (6). A further irreversible reduction process is observed near the cathodic limit (E_{pc}~-2.65V). These three redox processes (irreversible initial reduction, re-oxidation, and further reduction) were found in all BATO compounds studied (7), and peak potential values for each are given in Table 1.

It is immediately evident that whereas peaks 2 (re-oxidation) and 3 (further reduction) remain relatively fixed, there is considerable variation in the position of peak 1 (the major two-electron reduction). Peak 1 E_{pc} data for the chloro BATOs are tabulated below. The following trends were noted: CDO complexes in general reduce at a potential that is 60-90mV more positive than the analogous DMG species. Changing substituents on the boron cap has little effect on peak 1; chloro-DMG and chloro-CDO BATOs reduce at approximately E_{pc}= -1.34 and -1.29V respectively. The exceptions are the aryl-substituted complexes, exhibiting slightly more positive peak potentials. The effect of changing the axial ligand is shown in the tabulated results for phenyl-capped compounds. Bromo-substituted BATOs are easier to reduce (ΔE_{pc}~0.5V) than chloro, while hydroxy-substitution yields a species more difficult to reduce (7).

Chloro BATOs	Epc (V)	
Boronic Acid Cap	DMG	CDO
BOH	-1.34	-1.28
BMe	-1.37	-1.28
BEt	-1.35	
B2MP*	-1.36	
BBu	-1.35	-1.30
BPh	-1.31	-1.25
uncapped (tris)	-1.38	-1.34

*2MP = 2-methylpropyl

Phenyl-capped BATOS			
	Epc (V)		
Dioxime	Cl	Br	OH
DMG	-1.31	-0.83	
CDO	-1.25	-0.76	-1.77

The *tris*-dioxime complexes exhibit the same features as the BATO compounds (an additional small further reduction is also seen) indicating that none of the BATO redox processes are centered on the boronic acid cap.

It has been noted that the failure of cationic metal complexes as myocardial imaging agents may be due to their reduction in vivo (8), and therefore reduction potential is an important factor in predicting activity. Since the reduction behavior of the chloro BATO complexes does not vary significantly, it appears any observed differences in BATO biodistribution are not similarly attributable to a reduction process.

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5. This was determined by comparison of polarographic diffusion limited currents of the BATO reduction to that of the one-electron Ru(III)/Ru(II) couple of $Ru(acac)_3$.

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7. It should be noted that the initial reduction of the hydroxy species studied is slightly different: it involves two separate irreversible one-electron processes ($E_1° > E_2°$) rather than a single two-electron process.

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Figure 1. Cyclic Voltammogram of $TcCl(DMG)_3BPh$ (0.27mM) in DMF. Supporting electrolyte: 0.1M tetrabutylammonium tetrafluoroborate. Hanging mercury drop working electrode vs. $Ag/AgNO_3$ reference. Scan rate = 100 mV/sec.

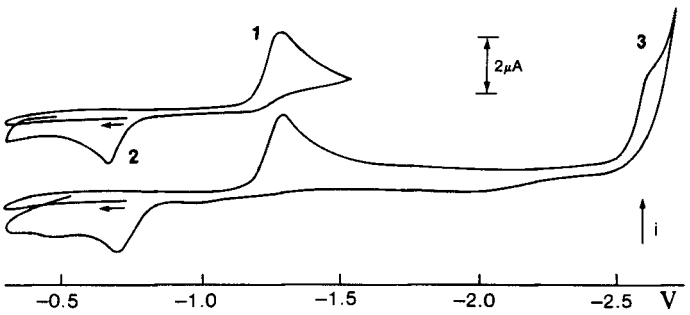

Table 1 Cyclic Voltammetry Data for BATO compounds in DMF. Scan rate = 100 mV/sec. All solutions are 0.2-0.7mM in sample and 0.1M in tetrabutylammonium tetrafluoroborate. Hanging mercury drop working electrode vs. $Ag/AgNO_3$ reference. Peaks 1 and 3; $E_p = E_{pc}$ = peak potential, cathodic; Peak 2; $E_p = E_{pa}$ = peak potential, anodic.

$TcX(dioxime)_3B\text{-}R$

		Peak Potentials (Ep) in Volts					
		DMG			CDO		
X	R	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
Cl	OH	-1.33	-0.7	-2.66	-1.28	-0.71	-2.66
Cl	Me	-1.35	-0.7	-2.69	-1.29	-0.72	-2.68
Cl	Et	-1.34	-0.71	-2.67			
Cl	2MP*	-1.35	-0.7	-2.67			
Cl	Bu	-1.35	-0.7	-2.69	-1.30	-0.71	-2.68
OH	Bu	-1.84, -2.56**	-0.79	-2.68			
Cl	Ph	-1.30	-0.69	-2.64	-1.24	-0.69	-2.65
Br	Ph	-0.82	-0.71	-2.64	-0.76	-0.7	-2.69
OH	Ph				-1.76, -2.46**	-0.79	-2.64
Cl	uncapped (tris)	-1.38	-0.7	-2.22, 2.70	-1.34	-0.7	-2.18,-2.68

*2MP = 2-methylpropyl

**See reference 7

MIXED COMPLEXES OF TECHNETIUM AND TIN

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The majority of Tc-99m radiopharmaceuticals are synthesized by Sn(II) reduction of $[^{99m}TcO_4]^-$ in the presence of excess ligand. There is a high probability that in these preparations the excess ligand can also complex the Sn(II) reactant and/or the Sn(IV) product. In fact, the incorporation of tin during the formation of Tc-99m radiopharmaceuticals has been postulated for the ^{99m}Tc-BATO class of complexes based on isolation of a $[^{99}Tc(oxime)_3(\mu\text{-}OH)SnCl_3]$ intermediate in the synthesis of the ^{99}Tc-BATO analogs from $[^{99}TcO_4]^-$(1). Tin incorporation has also been suggested for ^{99m}Tc-diphosphonate agents, where it is hypothesized that these agents may contain Sn-diphosphonate polymers that have been tagged with Tc-99m(2). In this paper, we report the crystal structures of two members of a new class of mixed Tc/Sn complexes wherein Tc(IV) and Sn(IV) are randomly substituted for one another in the crystal lattice.

Both these mixed Tc/Sn complexes result from the preparation of Tc(IV) coordination complexes using stannous ion to reduce $[^{99}TcO_4]^-$. The first is $[M(IV)Br_6]^{2-}$ which can consistently be crystallized with a Sn/Tc ratio of 2/1 ratio when Sn(II) is employed as a reductant in the presence of excess HBr. Similarly, the synthesis of $[^{99}Tc(IV)(4\text{-}OMeSAL)_3TAME]^+$ yields a homogeneous crystalline mixture of $[Sn(IV)(4\text{-}OMeSAL)_3TAME]^+$ and $[^{99}Tc(IV)(4\text{-}OMeSAL)_3TAME]^+$ in a 4/1 ratio. Notably, Tc(IV) and Sn(IV) have effectively the same atomic radius (0.65Å and 0.69Å respectively), and thus are effectively identical with respect to size and charge. For this reason the two metal centers are structurally indistinguishable in the two cases described above. For example, the presence of tin is not readily apparent in either of the mixed metal Sn(IV)/Tc(IV) complexes using standard characterization techniques such as single crystal X-ray structural analysis, uv/vis/ir spectroscopy, or reverse phase HPLC analysis. The presence of tin was confirmed, and the Sn/Tc ratios were determined, by electron microprobe techniques. It is important to note that since scanning electron microscopy is not a standard mode of analysis used for Tc-99 complexes, researchers should be aware of the liklihood of isostructural Sn(IV) and/or Sn(II) complexes being present in Tc preparations when stannous ion is used as a reductant. In conclusion, the chemistry of tin must be carefully considered in the syntheses and characterization of Tc-99 complexes, as well as in the design and development of Tc-99m radiopharmaceuticals.

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The Synthesis and Characterization of $[M(C_8H_5N_2N{=}NH)_3](BPh_4)$, {where M = Tc or Re} tris-Diazene Chelate Complexes of Technetium(I) and Rhenium(I).

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The reaction of ammonium pertechnetate or tetrabutylammonium perrhenate with the chelating organohydrazine hydralazine hydrochloride gives the Tc(I) {or Re(I)} cationic *tris*-chelate complex $[M(C_8H_5N_2N{=}NH)_3]^+$, which is isolated as the tetraphenylborate salt. (See figure 1.) The reaction thus involves a six electron reduction of the pertechnetate (or perrhenate) to the cationic Tc(I) complex by three organohydrazine molecules which are each concomitantly oxidized by two electrons to coordinate as diazene chelates, with nitrogen-nitrogen double bonds. (See figure 2.) These dark green complexes are freely soluble in ethanol but decompose in solution in the absence of excess ligand. The green color is gradually lost when column chromatography is attempted on the complex, as the excess hydralazine is removed. The tetraphenylborate salt is only marginally soluable in aqueous media, which inhibits their migration in electrophoresis experiments. They can be isolated as the PF_6^- or BF_4^- salts, but the hydrolytic stability in these forms is greatly reduced, as evident from the loss of the characteristic green color in solution. The infrared spectra of these complexes show no peak associated with $\nu(M{=}O)$, nor associated with $\nu(C{=}N)$ or $\nu(N{=}N)$, indicative of exclusive chelated organodiazene ligation. A weak absorption near 3250 cm^{-1} is associated with the $\nu(N\text{-}H)$ from the organodiazene ligands. The FAB(+) mass spectra of the complexes show the free parent ions for both rhenium and technetium complexes. For the technetium complex M^+ = 573 m/z and for the rhenium complex M^+ = 661 m/z. The technetium complex's mass spectrum also shows a peak of 417 m/z associated with a fragment from the loss of one of the three chelated diazene ligands. The ^{99}Tc NMR spectroscopy shows the technetium complexes resonance occurring in the established Tc(V) region, at ~1620 ppm. This downfield shift is attributed to a para-magnetic shielding effect. These complexes are electrochemically active. Each complex exhibits a pair of reversible oxidations. One of the two observed processes is likely ligand based, since it occurs at very similar $E_{1/2}$ values for the rhenium and technetium complexes.

The title complex can also be prepared from Tc99m-pertechnetate, by the analogous synthetic route. HPLC traces monitoring for activity establish that only the *tris*-diazene complex is present during the course of the preparation. The lipophilic cation can be easily separated from unreacted pertechnetate on reverse-phase C_{18} chromatagraphy cartridges (Seppak). Electronic spectroscopy establishes the high wavelength absorbance maxima for the technetium species at 540 nm and 370 nm. Preliminary biodistribution data are presented in Tables I and II below.

Biodistributions were done on female Wistar rats, with three rats sacrificed at each time point. Injection was intravenous into the leg vein, under ether anesthetic. They were sacrificed by cardiac puncture. Normal organs were immediately excised, and the carcass was not counted. Organs were weighed and counted whole in an automatic γ-counter. Standards were prepared from the original injection. Each animal was injected with 15 mC_i of activity in a volume of 0.3 ml containing ethanol. Table I lists the biodistribution results at four time points, with average counts listed for the major organs of interest. Heart to blood ratios are listed in Table II.

Table I: Biodistribution Data for $[Tc(C_8H_5N_2N{=}NH)_3]^+$.

time(min)	kidneys	liver	stomach	intestine	spleen	lungs	blood	heart
2	1.79	3.72	0.15	0.17	2.24	2.81	1.87	1.65
5	2.30	3.96	0.18	0.33	2.86	2.73	1.44	1.29
30	1.32	2.70	0.39	1.34	2.41	1.18	0.38	0.97
240	1.11	1.58	0.24	3.61	1.08	0.48	0.17	0.44

Table II: Heart to Blood Ratios and other data of interest.

time(min)	heart/blood	heart/lungs	heart/liver
2	0.882	0.587	0.444
5	0.896	0.473	0.326
30	2.553	0.882	0.359
240	2.588	0.917	0.278

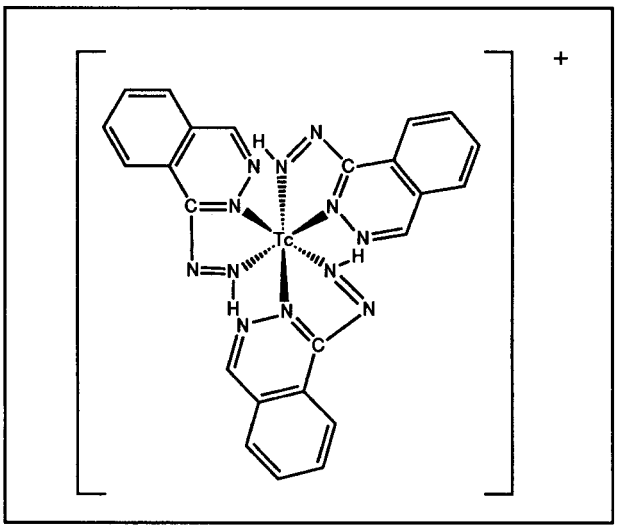

Figure 1.

Proposed structure of the *tris*-diazene complex $[Tc(C_8H_5N_2N{=}NH)_3]^+$ showing a facial arrangement of N-H moieties. Actual coordination geometry is probably a distorted trigonal prism rather than octahedral as depicted here.)

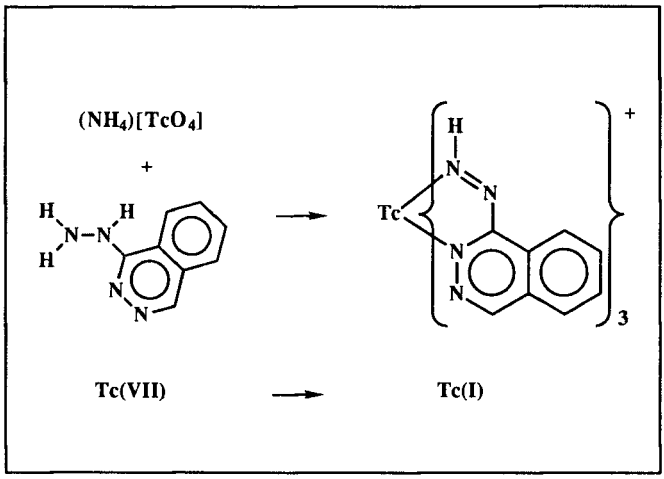

Figure 2.

Reaction scheme for the reduction of pertechnetate to the Tc(I) complex $[Tc(C_8H_5N_2N{=}NH)_3]^+$.

A NEW METHOD FOR THE PREPARATION OF Tc-99m RADIOPHARMACEUTICALS CONTAINING THE Tc≡N MULTIPLE BOND.

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A method for the preparation of ^{99m}Tc-radiopharmaceuticals containing the Tc≡N group was first proposed by Baldas (1), and is based on the following reaction scheme:

$$[^{99m}TcO_4]^- + HCl + NaN_3 \xrightarrow{\text{evaporation}} [^{99m}TcNCl_4]^- \xrightarrow{\text{ligand}} [^{99m}TcN\text{-complexes}] \quad (1).$$

This method presents two serious drawbacks, which severely limits its application to diagnostic nuclear medicine: (i) it requires to evaporate the reaction solution to dryness before the addition of the ligand, a procedure which does not permit a careful control of the sterility and apyrogenicity of the radiopharmaceutical preparartion, and (ii) the stability of the complex $[^{99m}TcNCl_4]^-$ is limited to values of pH<4, considering that in neutral and basic solutions this species was observed to transform rapidly in $[^{99m}TcO_4]^-$ (2).

We report here a new method for the preparation of the Tc≡N group at the non carrier added (NCA) level, which avoids all problems associated with Baldas'method, and is, thus, perfectly applicable to the nuclear medicine practice. The basic reaction is:

$$[^{99m}TcO_4]^- + HCl + NH_2N(R)\text{-}C(=S)SCH_3 + PPh_3 \xrightarrow{r.t.} [^{99m}TcN]_{int} \quad (2).$$

After adjustment of the pH to the required value, the addition of the ligand to the same reaction solution containing the intermediate technetium-nitrido species leads to the final ^{99m}TcN-radiopharmaceutical.

The S-methyl ester of dithiocarbazic acid, $NH_2N(R)\text{-}C(=S)SCH_3$, plays the role, in the reaction, of N^3-donor, while the presence of PPh_3 appears as fundamental for obtaining the reduced Tc≡N group. It was found that all the derivatives containing the functional arrangement $\text{-}(R)N\text{-}N(R')\text{-}C(=S)SCH_3$ behave as good sources of nitrido nitrogen atoms in reaction (2), when PPh_3 is present.

The chromatographic characterization of the species $[^{99m}TcN]_{int}$ demonstrated that it is not a single compound, but a mixture of complexes all containing the Tc≡N bond: addition of a suitable ligand to reaction (2), indeed, gave rise, invariably, to one single product containing the Tc≡N moiety. These results have been obtained using a number of ligands such as chelating amines, cyclam, dithiocarbamates, 8-quinolinethiolato, Schiff bases. The nature of the resulting complexes have been established by comparison of the chromatographic behavior of the products prepared through reaction (2) at the NCA level, with that of the products prepared at the carrier added level through the same reaction (3).

1. Baldas J. and Bonnyman J. Int. J. Appl. Radiat. Isot. 36, 133, 919 (1985).
2. Alagui A., Apparu M., D'Hardemare A. D. M., Riche F. and Vidal M. Int. J. Appl. Radiat. Isot. 40, 813 (1989).
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Synthesis and Characterization of a ^{99}Tc-Porphyrin Complex.

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Porphyrins are perhaps the most important family of naturally occurring chelating agents yet, despite the tremendous biological importance of these ligands, there are few examples of Tc-porphyrin complexes. There are, to date, only two reports of ^{99}Tc-porphyrins (1,2) and no reports of well-characterized ^{99m}Tc-porphyrins. Of the two ^{99}Tc-porphyrins that have been prepared, the first, $Tc(CO)_3TPP$ (H_2TPP = tetraphenylporphyrin) was prepared from $Tc_2(CO)_{10}$ and H_2TPP in refluxing decalin (1). In the second case, Lawrence, et al. prepared $TcO(OAc)OEP$ (H_2OEP = octaethylporphyrin) in low yield by refluxing NH_4TcO_4 and H_2OEP in glacial acetic acid (2).

In the course of our investigations of Tc-ß-diketonato complexes, we have investigated the use of $Tc(acac)_3$ (Hacac = 2,4-pentanedione) as a starting material for the synthesis of Tc-porphyrins. Although ß-diketonato complexes are among the more convenient starting materials for the synthesis metalloporphyrins (3) because of their ready solubility in the same organic solvents that are required to dissolve the porphyrin ligands, studies of this reaction with technetium have not been previously reported.

Heating a mixture of $^{99}Tc(acac)_3$ and H_2TPP in phenol at 180-190 °C for several days caused the purple solution of the porphyrin to become deep brown. Removal of the phenol (by extraction with 1 M NaOH or sublimation) left a very dark residue that was purified by column chromatography on silica gel using toluene and methylene chloride as eluents. Three major bands were eluted from the column corresponding to unreacted $^{99}Tc(acac)_3$ and H_2TPP and a Tc-TPP complex. The Tc-TPP product was further purified by reprecipitation from methylene chloride/hexane. The uv-visible spectrum of this material contained two bands in the 450-700 nm region and a Soret band at 390 nm that was shifted from the 405 nm band in H_2TPP starting material. Changes of this type are typical of those that occur on metallation of porphyrins. The infrared spectrum included bands at 940 cm^{-1} (Tc=O) as well as others in regions typical of coordinated OPh^- and the TPP ligand. The largest peak in the mass spectrum (FAB^+) occurred at m/z=727 ($[TcOTPP]^+$) and no peak was observed that corresponded to TcO(OPh)TPP. This is not, however, sufficient evidence to prove the absence of phenoxide as an axial ligand because these ligands frequently do not appear in the mass spectrum even if present in the complex (3). We have, therefore, tentatively assigned the structure of this complex as [TcO(OPh)(TPP)] pending continuing efforts to obtain a single crystal suitable for x-ray structure determination.

This work was supported by DOE grant DE-FG02-86ER-60460.

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Synthesis and Characterization of a ^{99m}Tc-Porphyrin Complex.

A.B. Packard

SUPPORTING DATA

The uv-visible spectrum of the reaction mixture began as a typical four band spectrum in the 450-700 nm region plus the Soret band at 405 nm. The spectrum of the product contain only 2 bands in the 400-700 nm region (500 and 600 nm) and the Soret is of decreased intensity and is shifted to 390 nm. These changes are typical of those that occur when the ligand is deprotonated and a metal inserted (3).

The infrared spectrum of the reprecipitated product is similar to that of the porphyrin starting material (H_2TPP). Important differences include the absence of a peak at 3310 cm^{-1} (N-H in H_2TPP) and a new peak at 940 cm^{-1} which has been assigned as Tc=O. This second peak corresponds to a value of 962 cm^{-1} reported by Lawrence, et al for the complex TcO(OAc)OEP (H_2OEP = octaethylporphyrin) (2). Other peaks of interest in the spectrum of the Tc-TPP product include those at 1560, 1470, 1210, and 752 cm^{-1}. These peaks are typical of those observed for coordinated phenoxide (-OPh) and may be tentatively assigned as due to C=C (1560, 1470 cm^{-1}), C-O (1210 cm^{-1}), and C-H (753 cm^{-1}) (4)

The mass spectrum was recorded in the FAB^+ mode using NBA as the solvent. The most prominent feature of the spectrum occurs at m/z=727 and corresponds to $Tc(O)TPP^+$. The next most abundant peak occurs at m/z=711 which is equivalent to the loss of the oxo group from the technetium. A peak of equal intensity occurs at m/z=651 and corresponds to the loss of a phenyl ring from the periphery of the porphyrin ligand. There was no peak at m/z=821 which would arise from $TcO(OPh)TPP\text{-}H^+$. This pattern is similar to that observed for Re(O)(OPh)(OEP) (4).

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SYNTHESIS OF Tc(III) COMPLEXES WITH A P,N BIDENTATE PHOSPHINO AMINE LIGAND.

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Despite many Tc complexes with mono- and di-phosphines are known, only little attention has been payed to complexes with chelating phosphines containing, together with the phosphorus atom, other different coordinating groups.

In a first survey of the reactivity of this type of ligands, we have reported(1) on the preparation of stable Tc(III)99/99m complexes containing three phosphino carboxylate ligands such as Ph_2P-R-COOH, shortly PCOOH, where R=*o*-C_6H_4, C_2H_4 and CH_2. The peculiarity of these $Tc(PCOO)_3$ complexes is the simplicity of the synthesis on the "no carrier added" Tc-99m level and the reductant of pertechnetate is the ligand itself. Hence, the interest in studying this series of ligands for the synthesis of lipidic complexes usable as radiopharmaceuticals. Neutral Tc(III) complexes were obtained, acting each ligand as mono negative.

In this comunication we report the preliminary results on the reactivity of the bidentate (*o*-aminophenyl)diphenylphosphine, (PNH_2). The higher basicity of the amino group, with respect to the carboxylic one, adds the possibility for the ligand to function as neutral. As consequence, we should expect positive charged Tc(III) complexes.

On reacting, at reflux in ethanol, a mixture of solid NH_4TcO_4 and the ligand PNH_2 for 30 min., a dark powder (A) was recovered from a purple solution.

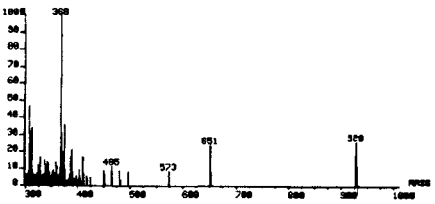

Figure 1

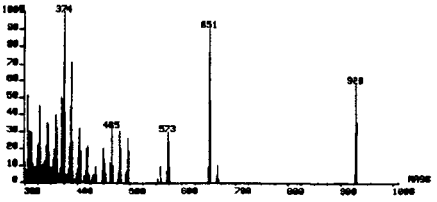

Figure 2

Figure 1 shows the FAB+ mass spectrum of this crude reaction product. The peak at m/z=928 is attributable to the species $[Tc(PNH)_3+1]^+$, in which three ligands have been coordinated. The peak at m/z=651 is due to the loss of one ligand. When the reaction

was carried out at room temperature, in acid medium (by CF_3COOH), blue crystals (B) separated from the blue solution. The i.r. spectrum shows a ν(C=O) at 1665 cm^{-1}, attributable to $[CF_3COO]^-$, which is not present in solid A spectrum. The mother liquors, upon addition of a methanolic solution of $NaBPh_4$, yield the blue $[BPh_4]^-$ complex salt.

Figure 2 reports the FAB+ spectrum of B; it shows a peak at m/z=928 and a fragmentation pattern similar to which of compound A. Both the compounds contain three ligands bonded to the Tc atom. However, condutivity measurements reveal that B is conducting in acetonitrile and the value of 128 Ω^{-1} cm^2 $mole^{-1}$ is in the expected range of mono electrolites. The positive nature of the charge was established, since the complex is retained in a Accel Cm Sep-Pak cartridge (Waters). The compound A is, instead, non conducting and, also not retained in the cationic exchange cartridge.

Solution magnetic susceptibility measurements using the Evans method show for both the complexes A and B values of 2.46 and 2.49 B.M. for the magnetic moment, which is in agreement with 2 unpaired electrons corresponding to a Tc(III) d_4 (t_{2g}^4) configuration in an octahedral environment.

On the basis of these findings we can assume the following formulation for the complexes:

$[Tc^{III}(PNH)_3]$ A

$[Tc^{III}(PNH)_2(PNH_2)]^+$ B

The mono positive charge of complex B can be explained by admitting that two ligands are bonded to the Tc atom through their mono-deprotonated amino group, and the third ligand through a neutral one. Complex A is formed only in basic conditions, i.e. those created by the ligand itself, or by adding a base. Furthermore, the complex B can be converted in A just by addition of a base [NaOH or $N(C_2H_5)$]. In the same manner complex A can be converted in B by addition of a non-coordinating acid.

In conclusion, we have synthetized two pH dependent Tc(III) complexes. It has to be studied whether, by varying the basicity of the amino group a lipidic complex, able to cross the BBB, with pKa around 7, may be prepared and, this property used for brain retention.

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PREPARATION AND CHARACTERIZATION OF MULTIDENTATE AMINETHIOL LIGANDS LABELED WITH TECHNETIUM-99m.

C.S. John, [1]E.O. Schlemper, P. Hosain, L. Cioloca, C.H. Paik, and R.C. Reba. Division of Nuclear Medicine, George Washington University, Washington, D.C., [1]Department of Chemistry, University of Missouri-Columbia, Columbia, MO.

In order to develop new technetium labeled lipophilic brain and heart blood flow imaging agents we have started to study multidentate aminethiol ligands. A triaminetrithiol (TATT) derived from 1,4,7-triazacyclononane and a tetraaminetrithiol (TEATT) derived from tris(2-aminoethyl)amine have been synthesized and characterized. Tc-99m labeling is achieved in high yields using tin(II)chloride or tin(II)tartarate as a reducing agent. The resulting complexes are highly lipophilic and have good in-vitro stability. In order to study the chemistry at macromolecular level the reaction of TATT with ammonium pertechnetate in the presence of tin(II)tartarate has been studied. The complex formed is extracted in chloroform and purified by passing through a silica gel column and eluting with 90/10 : chloroform/methanol. A dark brown band is collected which yields brown crystals upon removal of the solvents. This complex is characterized by single crystal X-ray diffraction method, IR and FAB mass spectroscopy. An ORTEP diagram of tin(IV) complex is shown in figure 1.

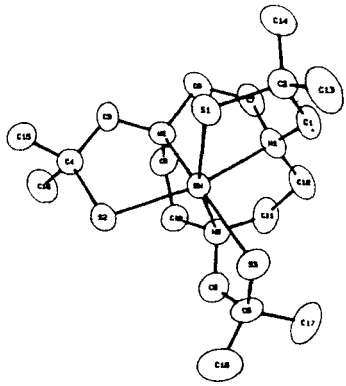

Figure 1. ORTEP diagram of Sn-N_3S_3

It is clear that the oxidation of tin(II) to tin(IV) has occured during the complexation process. Tin is coordinated to three nitrogen and three sulfur in a distorted octahedral environment. The three thiols are deprotonated and pertechnetate ion is found as the counteranion. IR spectrum shows a strong absorption at 900 cm^{-1} which is consistent with the Tc=O stretching frequency. The FAB mass spectrum shows the parent ion at 510 (M^+) that corresponds to $C_{18}H_{36}N_3S_3{}^{120}Sn$ and the appropriate isotopic distribution for the incorporation of one tin. There is no peak observed for the analogus technetium-N_3S_3 complex.

Supporting data for: Preparation and characterization of multidentate aminethiol ligands labeled with technetium-99m.

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Table 1. Bond distances in angstroms.

Sn-S1	2.411
Sn-S2	2.415
Sn-S3	2.435
Sn-N1	2.301
Sn-N2	2.292
Sn-N3	2.328

Table 2. Bond Angles in Degrees

S1-Sn-S2	103
S1-Sn-S3	101
S1-Sn-N1	83
S1-Sn-N3	159
S1-Sn-N2	96
S3-Sn-N1	160

DCM Data Spectrum Data File: csj1 22-DEC-89 11:23
Sample: ST. JOHN, N3 S3 TC, MEOH/GLY
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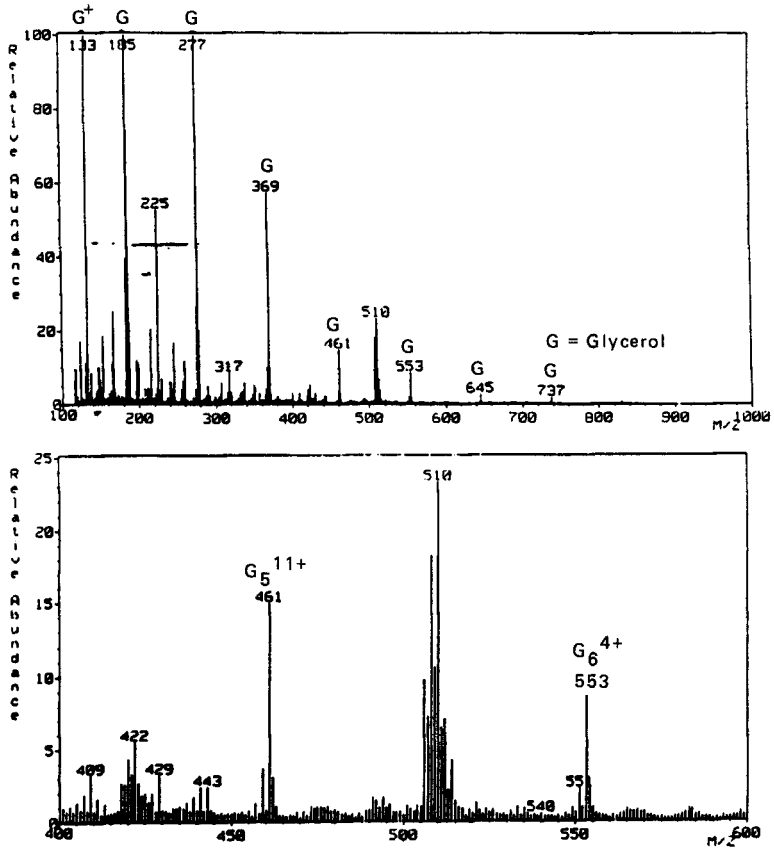
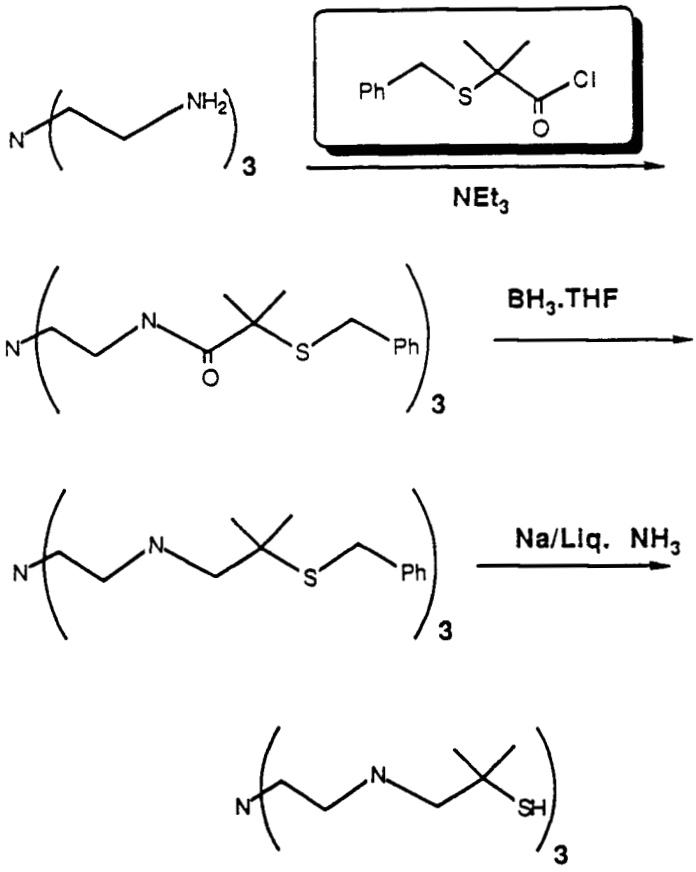
Total Peaks: 763 Norm.: Max. Scan Mode: MF (Positive) Centroid



Synthesis of Tetraaminetrithiol:



Using the similar approach triaminetrithiol was prepared.

DIRECT LABELLING OF MERCAPTOTRIAMIDES AND DIMERCAPTODIAMIDES WITH TECHNETIUM-99m AT ROOM TEMPERATURE.

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Tc-99m labelled dimercaptodiamides (e.g. Tc-99m DADS (I), Tc-99m CO_2DADS (II)) and mercaptotriamides (e.g. Tc-99m MAG3 (III), Tc-99m MAGAG (IV)) have been developed in an attempt to find a Tc-99m labelled substitute for I-131 hippuran. III has become now a practical and efficient renal function agent with widespread use. II and III are also being explored as Tc-99m complexing moiety in conjugates with proteins for immunoscintigraphy (1,2). The preparation of the Tc-99m complexes of these types of ligands requires a boiling step in order to remove the thiol-protecting group and to promote the rate of the exchange reaction. This can be considered as a practical inconvenience in routine clinical practice.

We have now developed a labelling method that allows direct labelling of CO_2DADS, MAG3 and MAGAG at room temperature (RT) using the deprotected ligands. Their S-benzyl- and S-benzoyl protected precursors were synthesized by published procedures. S-benzoyl group(s) were removed by alkaline hydrolysis of the thio-ester function in 0.1N NaOH for 10 min at room temperature. Free ligands obtained in this way were used as such without isolation or purification. Cleavage of the S-benzyl group(s) was performed by treatment of the precursor with Na in ammonia followed by isolation of the ligand. Deprotected CO_2DADS obtained by this procedure was stored for 1 year under N_2 atmosphere and normal labelling results were observed despite the presumed instability of free thiol groups.

Labelling with Tc-99m at RT was accomplished by the addition of 1 ml phosphate buffer, 50-200 µg $SnCl_2$ in 25 µl EtOH and 2 ml generator eluate to 0.5 mg-2 mg of the deprotected ligand and incubation for 1 min-60 min. RP-HPLC analysis on Hypersil ODS with gradient elution revealed that different Tc-99m compounds are formed in function of pH. Only labelling at pH 11 or higher afforded radiochemical species with identical retention times on RP-HPLC as the corresponding Tc-complexes (II, III and IV), prepared by exchange labelling of the S-benzoyl or S-benzyl protected precursors (fig. 1,2,3).

In most labelling conditions varying amounts of side-products were initially formed besides the desired Tc-99m complex, but the radiochemical purity increased in function of time. This indicates the initial formation of rather weak Tc-99m complexes which are gradually converted to the final more stable oxotechnetium (V) chelates. The initial percentage of impurities and the rate of their disappearance was found to be dependent on incubation temperature and pH (fig. 4,5). The amount of ligand or $SnCl_2$ did not affect labelling kinetics in the tested conditions. Up to now radiochemical purity was always highest using deprotected ligands obtained from S-benzyl precursors.

It can be concluded that mercaptoamide containing ligands can be labelled successfully with technetium-99m at room temperature at pH>10. However, more developmental work is still required to improve radiochemical purity and to confirm the identy of the Tc-99m complexes obtained by direct labelling. Investigation of the toxicological aspects is also necessary if studies in humans would be considered.

1. Lister-James J., Weber R., Boutin R. et al. J. Nucl. Med., 30, 793, (1989).
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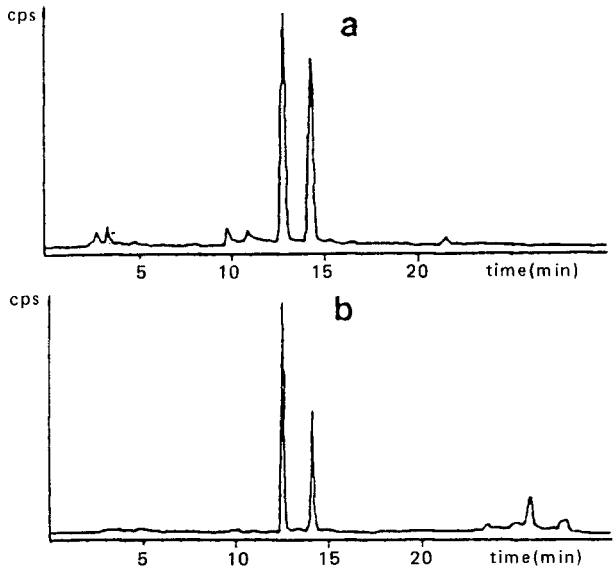

Figure 1. HLPC chromatograms of ^{99m}Tc-CO_2-DADS obtained by direct labelling at room temperature (a) and by exchange labelling of the S-benzyl-protected precursor (b) (HPLC conditions see below)

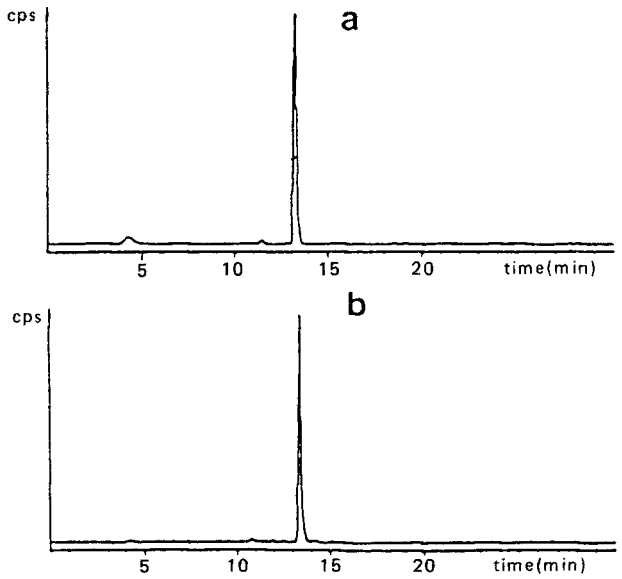

Figure 2. HPLC chromatogram of ^{99m}Tc-MAG_3 obtained by direct labelling at room temperature (a) and by exchange labelling of the S-benzyl-protected precursor (b) (HPLC conditions see below)

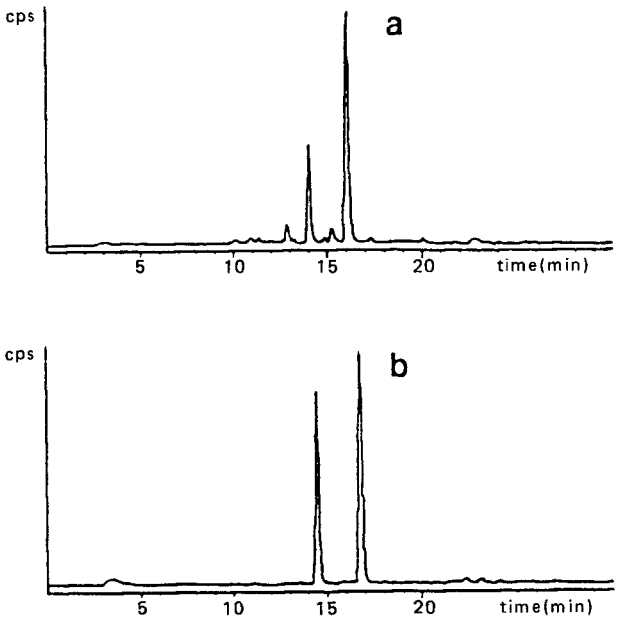

Figure 3. HPLC chromatograms of ^{99m}Tc-MAGAG obtained by direct labelling at room temperature (a) and by exchange labelling of the S-benzyl-protected precursor (b)

HPLC CONDITIONS: Hypersil C18, flow 1ml/min with phosphate buffer/EtOH gradient

TIME	0'	15'	20'	25'
Phosphate buffer pH 5.85,0.025M	100	80	50	50
EtOH	0	20	50	50

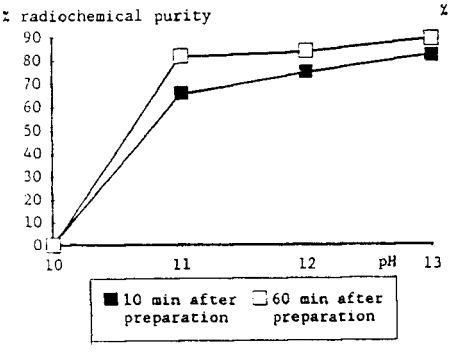

Figure 5. Effect of incubation pH on the radiochemical purity of ^{99m}Tc-MAG_3 obtained by direct labelling

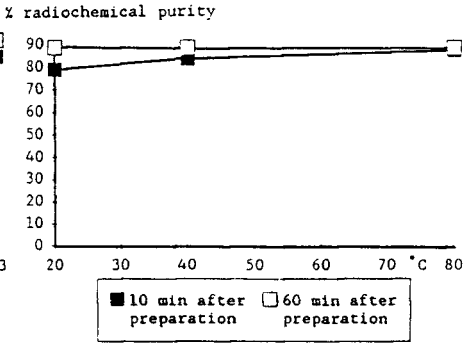

Figure 4. Effect of incubation temperature on the radiochemical purity of ^{99m}Tc-MAG_3 obtained by direct labelling

FLUID SHEAR ON THE LOSS OF Tc-99m FROM Tc-LABELED RBCs IN A DOG MODEL.

Sumit Dewanjee, Mrinal K. Dewanjee, Mansoor Kapadvanjwala, George. N. Sfakianakis
Department of Radiology and Biomedical Engineering. University of Miami School of Medicine, Miami, FL

Red cells deform under high fluid shear -stress in all vessels; in addition, during traversal through microvascular beds of capillaries, bifurcations, confluxes and sinusoids, they almost fold to pass the narrow channels of sinusoids. At low flow, they form aggregates (rouleaux) via the adhesive macromolecules forming the cell-cell bridge. The shear rate, representing laminar velocity change/radial distnace is directly proportional to flow and inversely proportional to the radius. The shear rate is responsible for the tumbling as the blood cells undergo streamline flow. The viscosity (shear stress/shear rate) of blood (non-Newtonian fluid) decreases with increase of shear rate. At high shear stress, the lipid membrane stretches and leaks resulting in the loss of small molecules. In Tc-99m labeled red cells, about 20-30% of Tc-99m binds to small molecules; only 4-6% of these labeled small molecules are lost resting red cells within a period of 24 hours. However, a major part is released in vivo. Our studies indicate that due to the random nature of labeling, this type of loss will be true for all oxo-metallic cations (Tc=O, Cr and V=O).

The loss of Tc-99m from Tc-RBC due to glomerular filtration is under-estimated in patients; this loss of Tc-99m labeled small molecules (TCLSM) results from continuous elution from the labeled RBCs (LRBC). We studied the effect of fluid shear on the loss of Tc-99m from LRBC using a Grabowsky flow chamber.

Sn-pyrophosphate (5 ug/kg) was injected in the dog and allowed to circulate for 30 min., and then 40 ml of blood was withdrawn to run the flow chamber. Tc-99m pertechnetate was added to the blood and incubated for 10 min and washed. Tc-RBCs were allowed to flow at a rate of 2, 4 and 5 ml/min (shear of 740, 1550 and 1937 sec-1) at 37° C. The diagram for the flow-loop containing the peristaltic pump and the flow chamber is shown in Figure 1. The results (mean $\pm$SD) of Tc retained in LRBCs at different flow (shear) rates are as follows:

ml/min	**10'**	**15'**	**20'**	**30'**
0	98.2$\pm$3.7	97.7$\pm$1.4	98.6$\pm$0.3	95.8$\pm$3.3
2	94.1$\pm$4.8	96.0$\pm$2.8	95.8$\pm$2.8	95.3$\pm$3.3
5	92.8$\pm$1.9	95.4$\pm$2.6	93.0$\pm$5.4	91.2$\pm$4.1

Simultaneously Tc-99m loss for Tc-RBC labeled in vivo was measured at 1, 3 and 24 hours. 6% and 1% of Tc-99m were eluted from labeled RBC at 1 and 24 hours, on the other hand (1-4)% was eluted from the resting LRBCs. Gel filtration of hemolysate before and after shear-stress indicates that this loss is mainly due to TCLSM, suggesting that physiological shear and effusion of TCLSM result in vivo loss of Tc-99m from Tc-RBC.

Due to the randomness of chelation of oxo-technetium cation with the hemoglobin and other small molecules, labeling of pretinned cells always results in binding of Tc-99m with 20-30%

of Tc to small molecules. Due to charge on these molecules they do not diffuse out of the membrane under resting condition. The discrepancy was finally resolved from the shear-stressed labeled RBCs. Similar results were also found for the Tc-99m labeled platelets.

References
1. Dewanjee M.K. J. Nucl. Med. 15, 703-706 (1974).
2. Dewanjee M.K. Wahner H. Radiology 132, 711-716 (1979).
3. Dewanjee M.K. Rao S.A. Penniston J.T. J. Labelled Compd. Radiopharm. 19, 193-195 (1983) .
4. Srivastava S.C, Babich D., Richards P. J. Nucl. Med. 24, 128 (1983).
5. Mackey S.T., Dewanjee M.K., Chowdhury S. J. Labelled Compd. Radiopharm. 1985; 21, 1058-1060.(1985).
6. Joist J.H., Baker R.K. Blood 58, 350-363 (1981).

Acknowledgements
The supports of Department of Energy grant DE-FG05-88ER60728, Florida High Technology and Industry Council, Baxter-Bentley Corporation are gratefully acknowledged.

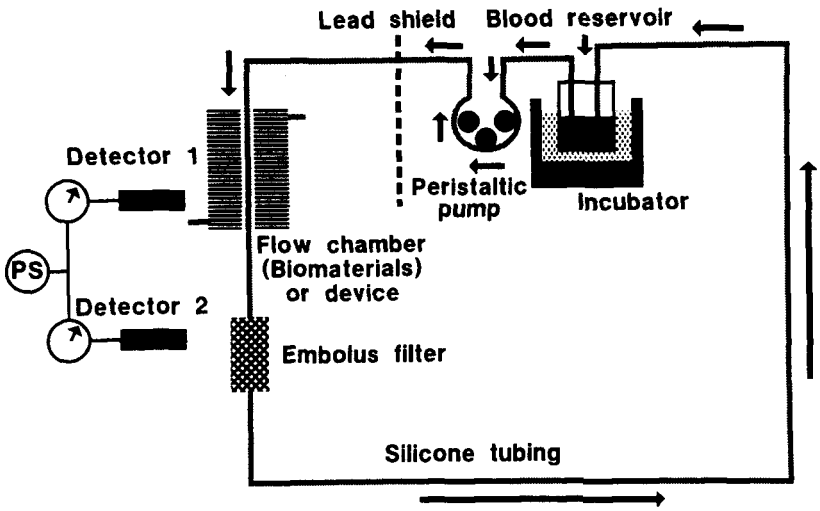

Figure 1. The flow-loop for quantitation of Tc-99m loss from labeled red cells.

A NEW VERSATILE METHOD FOR THE SYNTHESIS OF AMINETHIOL LIGANDS.

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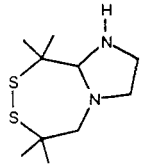
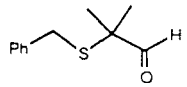
[1]University of Pennsylvania, Philadelphia, PA 19014.

There is a current interest in the design of new aminethiol ligands labeled with technetium-99m for use as regional cerebral blood flow, myocardial blood flow agents and for antibody labeling (1-4).

The classical method of synthesis of the diaminedithiol (DADT) ligands involves the condensation of a primary amine, for example ethylenediamine, with dithiadialdehyde to give 1,2-dithia-5,8-diazacyclodeca- 4,8-dienes (bis(imine)). The bis(imine) is reduced with a mild reducing agent such as sodium borohydride to yield 1,2-dithia-5,8-diazacyclodecane (1). It has been shown by Joshua (5) that sodium borohydride reduction of bis(imine) results in intramolecular reductive cyclization to provide bicyclic imidazolidines **1**. Lever (4) and coworker took advantage of the naked proton on the nitrogen of bicyclic compound and alkylated the secondary nitrogen for further synthetic elaboration. Recently Mach (6) reported the preparation of 2-methyl -2-benzylthiopropionaldehyde **2** for the preparation of diaminethiol ligands.



1



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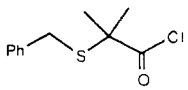


3

We report here a versatile method for the preparation of a variety of aminethiol ligands. The key intermediate for the synthesis is the acid chloride **3**. The precursor for the synthesis of **3** is 2-methyl-2-mercaptobenzylpropionic acid. Benzylmercaptan is allowed to react with ethyl-2-bromoisobutyrate to yield ethyl-(2-methyl-2-mercaptobenzyl)propionate which is hydrolysed to give acid. The acid is readily converted to acid chloride in almost quantitative yields with thionyl chloride. After removal of the volatiles from reaction mixture, the acid chloride is added to amines in the presence of triethylamine as acid scavenger. The resulting amide is reduced with borane and the debenzylation with sodium/liquid ammonia gives the free mercaptan compound.

This method is a general method for preparation of aminethiol ligands. All the steps involved in the synthesis give excellent yields. We have synthesized mono and disubstituted diaminedithiol ligands and multidentate aminethiol ligands as well.

1. Kung H.F., et al. J. Nuc. Med., 25, 326-332 (1984).
2. Lever S.Z., et al. J. Nuc. Med., 26, 1287-1294 (1985).
3. John C.S., et al. J. Nuc. Med., 30, 773 (1989) (abstract).
4. Lever S.Z., et al. Tet. Lett., 29, 3219-3222 (1988).
5. Joshua A.V., et al. J. Org. Chem., 52, 2447-2451 (1987).
6. Mach R.H., et al. Tet. Lett., 30, 4069-4071 (1989).

Supporting data for:

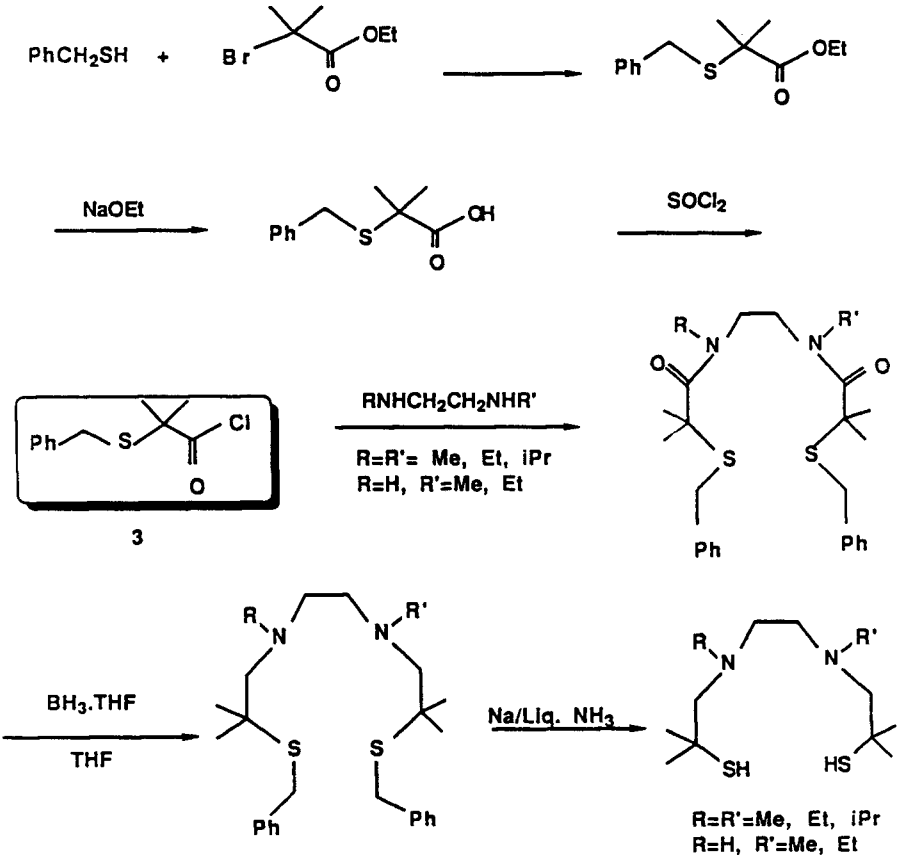
A NEW VERSATILE METHOD FOR THE SYNTHESIS OF AMINETHIOL LIGANDS.

C.S.John, [1]Y. Ohmomo, [1]H.F. Kung, C.H. Paik and R.C. Reba

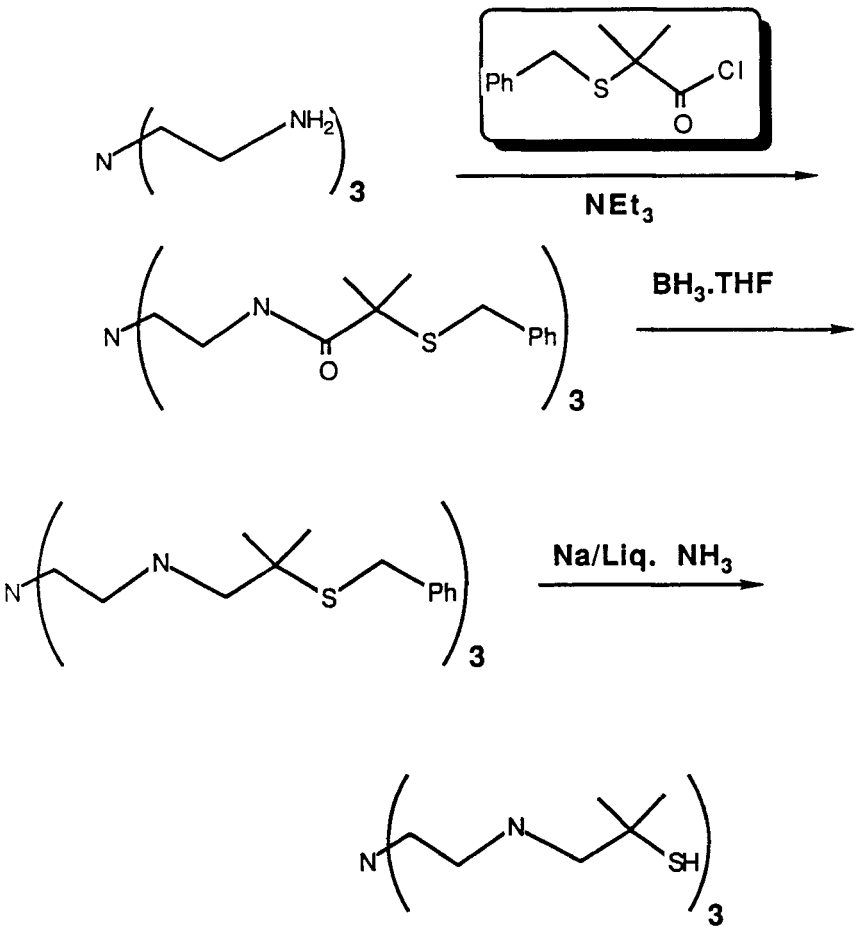
The George Washington Univ. Medical Center, Washington, DC 20037.

[1]University of Pennsylvania, Philadelphia, PA 19014.

The alkylated and dialkylated diaminedithiols are readily synthesized in high yields using the following reaction sequences:



Supporting data for:
A NEW VERSATILE METHOD FOR THE SYNTHESIS OF AMINETHIOL LIGANDS.
C.S.John, Y. Ohmomo, H.F. Kung, C.H. Paik and R.C. Reba.
The George Washington University Medical Center, Washington, DC. 20037
University of Pennsylvania, Philadelphia, PA. 19014



Using the similar approach triaminetrithiol was prepared starting from 1,4,7,-triazacyclononane.

Metal-N_2S_2 Complexes in Radiopharmaceutical Development

H. F. Kung, B.-L. Liu, L.C. Francesconi, Y. Ohmomo, J. Billings, M.P. Kung. Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104

Bis-aminoethanethiol (N_2S_2, BAT) ligands are very robust in their coordination chemistry with metals. The BAT ligands not only form neutral complexes with $Tc^{V}O^{+3}$ (1-4) and In^{+3} (5), but also complex with Ga^{+3} and Cu^{+2}, leading to plus one charged compounds in aqueous solutions. The overall net charge of the complexes appears to be determined by the charge of metal in the center core and also by the ionization of the hydrogens at four possible sites, two S-H and two N-H groups (Figure 1). The reason that Tc-99m BAT complexes are neutral is that three hydrogens (two SH groups and one NH group) are ionized. The other metals (Cu, In and Ga) appear to form similar complexes; however, only two S-H groups in these complexes are ionized. It is important to recognize that the formation of the complex and the ionizability of the S-H and N-H groups will determine the final net charge of the complex. Using BAT-TECH ligand as an example, complexes of $Tc^{V}O^{+3}$, In^{+3}, Ga^{+3}, and Cu^{+2} with zero and plus one net charges were formed. Biodistribution studies of these complexes in animals (Table 1) demonstrated the expected biodistribution based on the net charge and lipid-solubility. With different substitution groups on the ligand system the lipid-solubility and stability of the final complex can also be adjusted. The complexes demonstrate the versatility of this ligand system and the potential of using this ligand system for fine tuning the physico-chemical properties of the final complex. It may be feasible to use this ligand system to develop new radiopharmaceuticals.

Table 1
Biodistribution of M-BAT-TECH in rats after an iv injection
(% dose/organ)

	2 min			30 min		
Organ	$Tc^{V}O^{+3}$	Ga^{+3}	Cu^{+2}	$Tc^{V}O^{+3}$	Ga^{+3}	Cu^{+2}
Blood	10.18	10.2	18.4	1.97	3.58	10.4
Heart	1.68	1.68	1.67	0.20	0.52	1.81
Liver	21.52	21.5	25.3	18.1	33.5	27.8
Brain	2.23	0.02	0.08	0.16	0.01	0.07

References:

1. Walovitch R.C., Hill T.C., Garrity S.T. et al. J. Nucl. Med. 30:1892 (1989)
2. Scheffel U., Goldfarb H.W., Lever S.Z. et al. J. Nucl. Med. 29:73 (1988)
3. Kung H..F, Guo Y.-Z., Yu C.-C. et al. J. Med. Chem. 32:433 (1989)
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5. Liu B.-L., Kung H.F., Jin Y.T. et al. J. Nucl. Med. 30:367 (1989)

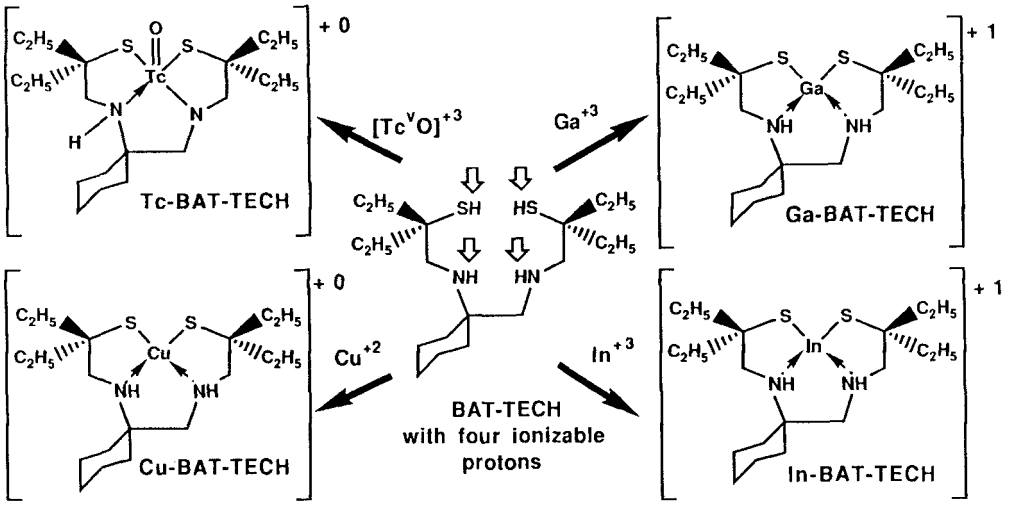


Figure 1. Formation of M-N2S2 complexes

Mass Spectral Analysis of N2S2 Ligands Used For Complexation of Tc-99m.

R.H. Mach and H.F. Kung+
Cerebrovascular Research Center and +Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104.

Bifunctional chelates such as bis(aminoethanethiol) (BAT) and propylenediaminodithiol (PAT) are gaining an increasing importance in diagnostic nuclear medicine since both ligands form a neutral and lipid soluble complex with ^{99m}Tc (1-3). As part of our recent studies in developing ^{99m}Tc-based agents for myocardial imaging, we observed an anomaly with respect to the mass spectral (MS) data of a series of BAT-fatty acid analogs: fast atom bombardment MS produced a molecular ion peak consistent with the structure $[M-2H]H^+$. To determine if this was a general trend with N2S2 ligands, we conducted a series MS studies under a variety of experimental conditions. The results of this study can be summarized as follows:

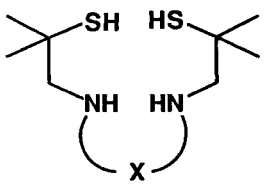
1) N2S2 ligands present as the hydrochloride salt tend to give a true molecular ion peak with chemical ionization (CI) MS; in each case the protonated form of the molecular ion peak was observed (MH^+).

2) N2S2 ligands present as the free base have a tendency to form an $[M-2H]H^+$ molecular ion peak with CI-MS, which is consistent with the oxidation of the dithiol to the corresponding disulfide; electron impact MS gave the unprotonated $[M-2H]^+$ molecular ion peak. An exception to this was ethyl cysteinate dimer (L,L-ECD) (4), which gave an MH^+ peak as both the salt and free base.

3) CI-MS of N2S2 ligands (salt form) containing 3° thiols (TM, PAT-HM) gave primary fragmentation patterns of $[M-34]H^+$ (loss of H2S) and $[M-76]H^+$ (loss of 2-mercaptopropane).

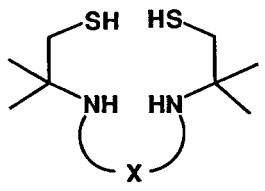
4) CI-MS of N2S2 ligands (salt form) containing 1° thiols (TMR, reverse PAT-HM, and reverse PAT-TM) gave two primary fragmentary patterns: $[M-2]H^+$ (disulfide formation) and $[M-48]H^+$ (loss of CH3SH). However, ECD displayed the following fragmentation pattern: $[M-34]H^+$ (loss of H2S) and $[M-74]H^+$ (loss of COOEt).

The above data demonstrates the observed molecular ion peak and fragmentation pattern of N2S2 chelates can be a function of both the structure and chemical form of the ligand. The formation of an $[M-2H]H^+$ "molecular ion" peak is a feature of some (but not all) N2S2 ligands in the free base form, and may lead to an erroneous structure assignment if proper care is not taken when analyzing the mass spectral data.



TM: X = CH_2CH_2

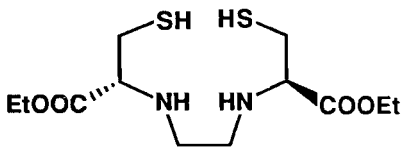
PAT-HM: X = $CH_2C(CH_3)_2CH_2$



Reverse TM: X = CH_2CH_2

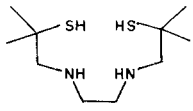
Reverse PAT-HM: X = $CH_2C(CH_3)_2CH_2$

Reverse PAT-TM: X = $CH_2CH_2CH_2$



L,L,-ECD

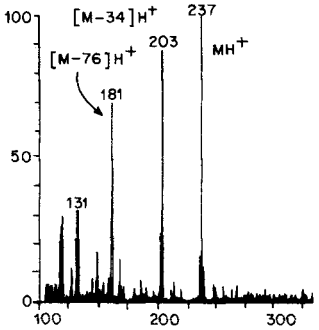
1) Kung, H.F., Molnar, M., Billings, J.B., Wicks, R., and Blau, M. J. Nucl. Med. 25, 326-332 (1984).
2) Lever, S.Z., Burns, H.D., Kervitsky, T.M., et. al. J. Nucl. Med. 26, 1287-1294 (1985).
3) Mach, R.H., Kung, H.F., Guo, Y.-Z., et. al. Nucl. Med. Biol., in press.
4) Walovitch, R.C., Hill, T.C., Garrity, S.T., et. al. J. Nucl. Med. 30, 1892-1901 (1989).



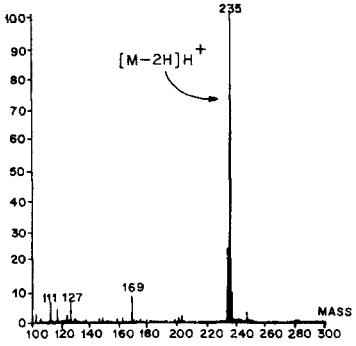
$C_{10}H_{24}N_2S_2$

TM

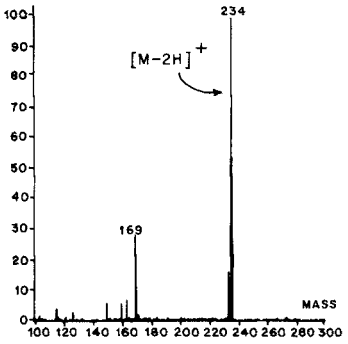
MW = 236



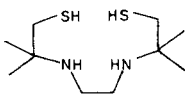
a) Conditions: Chemical ionization MS, HCl salt



b) Conditions: Chemical ionization MS, free base



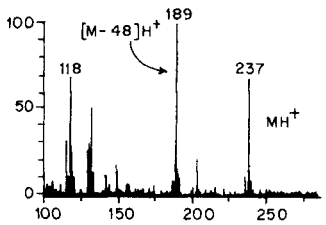
c) Conditions: Electron impact MS, free base



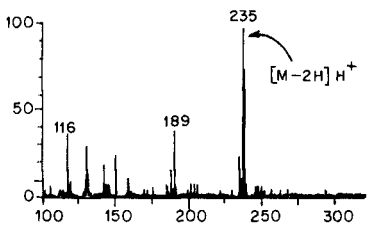
$C_{10}H_{24}N_2S_2$

Reverse-TM

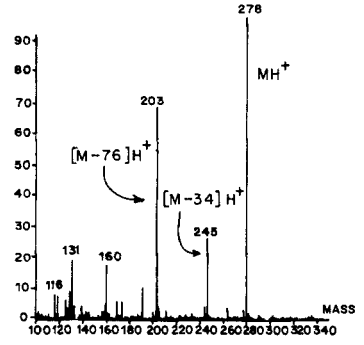
MW = 236



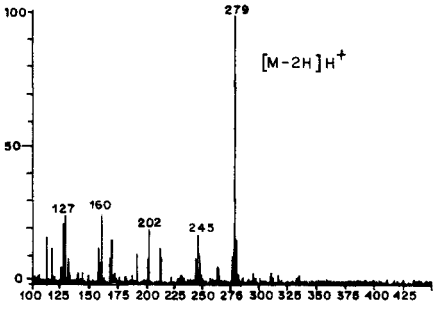
a) Conditions: Chemical ionization MS, HCl salt



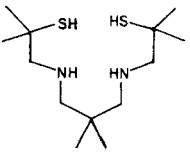
b) Conditions: Chemical ionization MS, free base



a) Chemical ionization
MS, Hcl salt



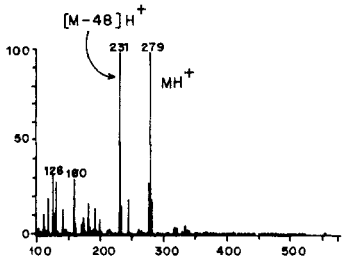
b) Chemical ionization
MS, free base



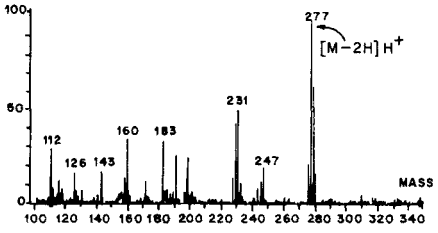
PAT-HM

MW = 278

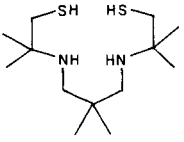
$C_{13}H_{30}N_2S_2$



a) Chemical ionization
MS, HCl salt



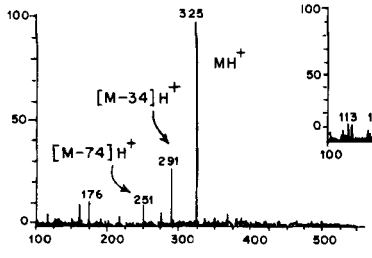
b) Chemical ionization
MS, free base



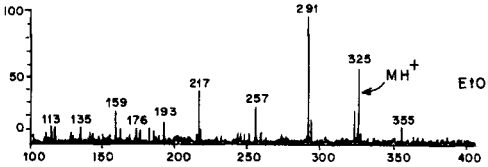
reverse PAT-HM

MW = 278

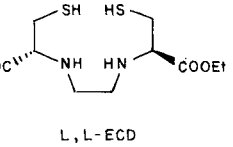
$C_{13}H_{30}N_2S_2$



a) Chemical ionization
MS, HCl salt



b) Chemical ionization
MS, free base



L, L-ECD

MW = 324

$C_{12}H_{24}N_2S_2O_4$

SYNTHESIS AND ^{99m}Tc LABELING OF NEW TRIPEPTIDES AS POSSIBLE HIPPURAN REPLACEMENTS

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The 99mTc complex of N-(S-benzoylmercaptoacetyl)glycylglycylglycine (MAG_3) has shown promise as a substitute for o-Iodohippuran in renal imaging studies (1-5). However in clinical comparisons with ^{131}I-Hippuran, the renal tubular extraction of ^{99m}Tc-MAG_3 was only 66% of that of Hippuran. Verbruggen, et al. [6, 7] prepared analogs of MAG_3 substituting glycine with alanine and with serine. These compounds, however, resulted in ^{99m}Tc-labeled isomers with differing renal extraction rates [7]. Both amino acids have an asymmetric center which explains some of the isomers. We used the non-asymmetric β-alanine to obviate this problem. We have synthesized a series of eight tripeptides based on the combinations of β-alanine (BA) and glycine (G). In addition to MAG_3, which we used as the model compound, the following combinations were possible and all were synthesized and labeled with Tc99m: MAG_2BA, MAGBAG, $MAGBA_2$, $MABAG_2$, MABAGBA, $MABA_2G$ and $MABA_3$. The synthesis of all compounds was carried out using a succinimidyl active ester method (Figure 1). The mercapto groups were protected by benzoylation. The synthesis were designed to avoid the use of alcohol and thus prevent the formation of possible ester contaminants. Initial 99mTc labeling was carried out by first removing the S-benzoyl protective group via hydrolysis followed by stannous reduction in the presence of TcO_4^-. All of the compounds formed anionic complexes. All the Tc-99m labeled compounds were analyzed by HPLC (C18 colum, 5% Ethanol in 0.05 m phosphate buffer pH 6.5) to identify useful compounds and to determine labeling efficiency.

Of this group, MAG_2BA, $MABAG_2$, MABAGBA and MAG_3 were made into freeze dried kits containing 200 g of the benzoylated derivative, 10 ug of $SnCl_2 2H_2O$ and 20-200 mg

of sodium tartarate or sodium gluconate at pH 5.5. To label the kit, $^{99m}TcO_4^-$ was added in small volume and the vial was heated in a boiling water bath for 10 minutes. This method gave

Supporting Data

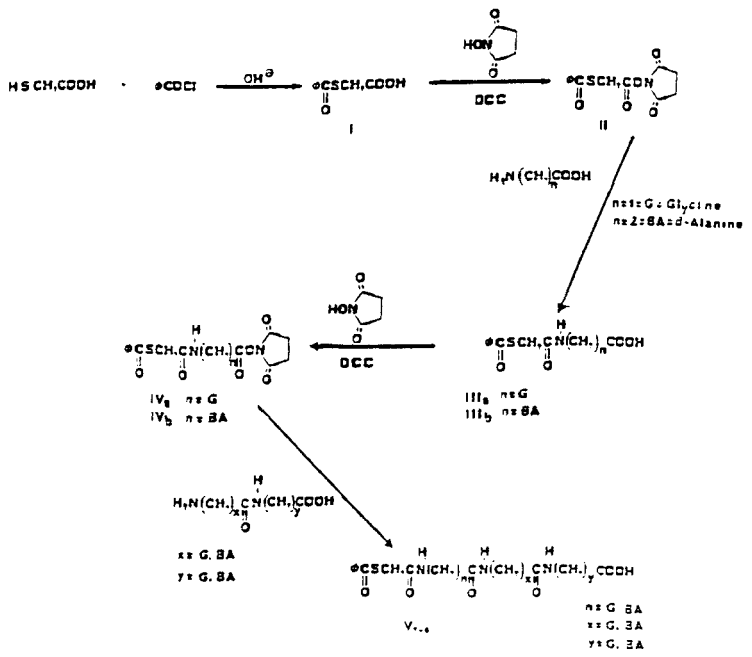

FIGURE 1. Synthesis of MAG_3 Analogs.

Analytical Data For Compounds Prepared

CPD.	M.P.	I.R.	C	H	N	S
(V_1) MAG_3	197.0-199.0	3320, 3260, 1750 2520-2720 (Quartet) 1750, 1670, 1630, 1565	49.34 48.95	4.67 4.72	11.44 11.51	9.73 9.61
(V_2) MAG_2BA	185.0-189.0	3300, 3180, 1705 1650, 1560	50.38 50.11	5.02 5.05	11.02 11.07	8.40 8.25
(V_3) MAGBAG	176.5-177.5	3320, 3260, 1730 1700, 1670, 1640, 1500	50.38 50.58	5.02 5.03	11.02 10.97	8.40 8.37
(V_4) $MAGBA_2$	176.5-180.0	3320, 3250, 1705 1665, 1640, 1550	51.63 51.73	5.25 5.56	10.63 10.68	8.11 8.35
(V_5) $MABAG_2$	203.0-205.0	3300, 3080 2540-2730 (Quartet) 1750, 1660, 1640, 1570	50.38 50.62	5.02 5.03	11.02 10.92	8.41 8.22
(V_6) MABAGBA	185.0-186.0	3290, 3080, 1700 1660, 1640, 1500	51.63 51.37	5.35 5.35	10.63 10.50	8.11 7.98
(V_7) $MABA_2G$	192.0-193.5	3290, 3080, 1710 1660, 1630, 1550	51.63 51.63	5.25 5.58	10.63 10.34	8.11 8.33
(V_8) $MABA_3$	214.0-216.0	3290, 3060, 1710, 1670, 1640, 1545	52.80 53.05	5.56 5.88	10.26 10.30	7.83 8.04

quantitative labeling >95% as determined by RPTLC (Analtech C18 PLATE, 0.01M phosphate buffer pH 6.0). In this system pertechnetate, Tc–tartarate and Tc–gluconate had an Rf of 0.9 to 1.0 whereas the labeled compounds had Rf values of 0.35 to 0.6.

Careful evaluation in experimental animals are necessary to determine if any of these compounds are better than Tc–99m MAG_3.

This work was supported in part by USPHS Grant AM–33357 awarded by the National Institutes of Diabetes, Digestive and Kidney Diseases.

References:

1. AR Frizberg, S Kasina, D Eshima, et al. Synthesis and biological evaluation of Tc–99m MAG_3 as a hippuran replacment. **J Nucl Med** 27(1);111–116;1986.

2. W Brandau, B Bubeck, M Eisenhut, et al. Technetium 99m labeled renal function and imaging agents III: Synthesis of 99mTc–MAG_3 and biodistribution of by–products. **Appl Radiat Isotopes** (Int. J Radiation Appl Inst Part A) 39:121–129;1988.

3. A Taylor, JA Zifer, A Steves, et al. Clinical comparision of I–131 orthoiodohippurate and the kit formulation of Tc–99m mercaptoacetyl triglycine. **Radiology** 170:721–725;1989.

4. HM Abdel–Dayem, S Sadek, R Al–Bahar, et al. Comparison of Tc–99m mercaptoacetyl triglycine and 131 I–orthoiodo hippurate in determination of effective renal plasma flow (ERPF). **Nucl Med Comm** 10:99–107;1989.

5. CL Maini, P Antonacci, A Sargiotto, et al. Dynamic renal scanning using 99mTc–MAG_3 in man. **Euro Jl Nucl Med** 15:635–640;1989.

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7. A Verbruggen, G Bormans, B Cleynhens, et al. Relation between stereochemical configuration and biodistribution of the isomers of ^{99m}Tc–mercaptoacetyl glycyl alanyl glycine (99mTc–MAGAG) in a baboon. **J of Labelled Compounds and Radiopharmaceuticals** 25:436–439;1989.

OPTIMALIZATION OF THE LABELLING OF ETHYLENEDICYSTEINE (EC) WITH TECHNETIUM-99m

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In a recent study we have found that the complex of technetium-99m with L,L- or D,D- ethylenedicysteine (Tc-99m EC, fig. 1) is excreted rapidly and efficiently in the urine after I.V. injection in mice, baboons and human volunteers. Uptake or retention in other organs is minimal. Therefore Tc-99m EC can be considered as a potential alternative for Tc-99m MAG_3 for renal function studies.

In order to develop a reliable labelling kit for the rapid reconstitution of Tc-99m EC at room temperature we have studied the influence of labelling conditions on the radiochemical purity and labelling efficiency.

Ethylenedicysteine was synthesized by a dimerization reaction on thiazolidine-4-carboxylic acid following a published procedure (1). Labelling with technetium-99m was performed by mixing 1 mg ligand, dissolved in 1 ml 0.05M phosphate buffer (pH 3 to pH 13), 100 µg $SnCl_2.2H_2O$ in 25 µl 0.05 N HCl and 1 ml generator eluate (10 mCi Tc-99m) followed by incubation for 10 min at room temperature.
At pH < 6 the preparation became turbid, probably due to the reduced solubility of EC at low pH. However, after filtration through a 0.22 µm membrane filter the filtrate contained over 90% of the activity.

Simple and rapid TLC or PC methods were developped (fig. 2) to determine the presence of pertechnetate or colloidal TcO_2 in the reaction mixtures.
However, these common radiochemical impurities were not found in noticeable amounts in any of the reaction mixtures. Analysis for other radiochemical impurities was performed by RP-HPLC on a Hypersil ODS column (250 mm X 4.6 mm) eluted with gradient mixtures of 0.0125 M phosphate buffer pH 2.5 (A) and 30% ethanol in this phosphate buffer (B) (0-10 min : 0 to 30% B, 10 min-20 min : 30% B). Some HPLC chromatograms are shown in fig. 3.

Labelling at pH 11 or 12 results in the formation of a single radiochemical species (yield 98-99%) which shows the mentioned favourable renal excretion characteristics. This Tc-99m complex has the same retention time on HPLC as the product formed by alkaline or enzymatic hydrolysis of the two ester functions in the brain perfusion agent Tc-99m ECD. During labelling at lower pH a second Tc-99m complex is formed in relative amounts which increase with decreasing pH. This complex is cleared more slowly from the plasma and its urinary excretion is clearly lower as compared to the complex formed at high pH.

Table 1 shows the yield of the desired Tc-99m EC complex in function of the pH during labelling. It appears that Tc-99m EC for renal function studies can be prepared with high radiochemical purity by addition of Tc-99m generator eluate to a labelling kit containing 1 mg of EC and 100 µg $SnCl_2$ at pH 11-12. Such labelling kits can be stored in lyophilized form for several months without clear reduction of the labelling yield. To obtain a preparation suitable for intravenous injection, the reaction mixture can be neutralized by the addition of a small volume of 0.5 M H_3PO_4. Radiochemical purity is not affected by this neutralization step and the preparation remains stable for at least 15 hours (fig. 3).

1. Blondeau P., Berse C. and Gravel D., Can. J. Chem., 45, 49-52 (1967).

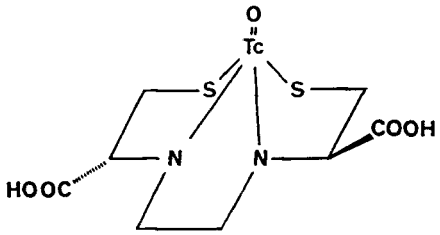

Fig. 1 : Structure of Tc-ethylenedicysteine (Tc-EC).

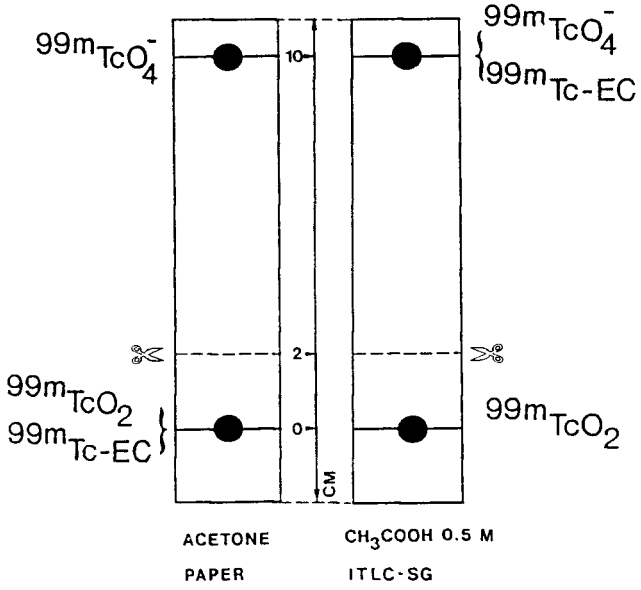

Fig. 2 : TLC and PC methods for rapid quality control of ^{99m}Tc-EC.

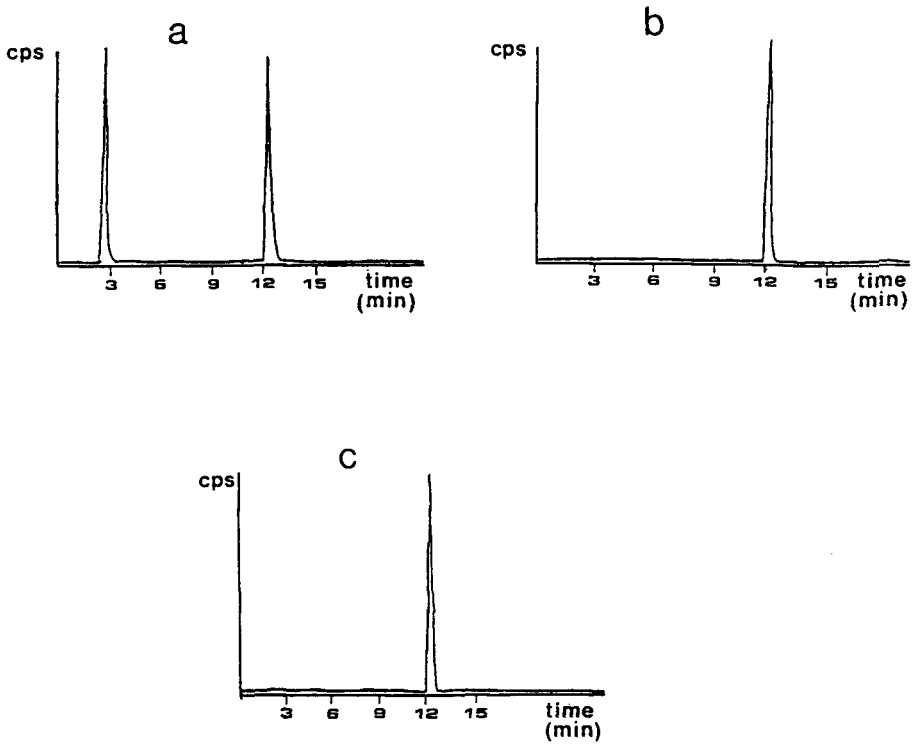

Fig. 3 : HPLC-chromatogram of ^{99m}Tc-EC
a. labelled at pH 8
b. labelled at pH 12 and a adjusted to pH 6.5 with 0.5 M H_3PO_4
c. preparation b after 15 hours.

Table 1 : Influence of pH in labelling mixture on the yield of the ^{99m}Tc-EC complex with favourable renal excretion characteristics.

pH	% ^{99m}Tc-EC	pH	% ^{99m}Tc-EC
6	42.7	10	97.1
7	47.6	11	98.3
8	61.9	12	99.6
9	69.1	13	98.8

MONOAMIDE MONOAMINE DITHIOLATE LIGANDS (MAMA) AS CHELATING AGENTS FOR TECHNETIUM: KINETIC AND MECHANISTIC STUDIES OF COMPLEX FORMATION

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Bifunctional chelating agents containing diamide dithiolate (DADS) donor atoms have been shown to be useful for labeling of monoclonal antibodies (1). The development involved the synthesis of a number of derivatives of 4,5-bis(mercaptoacetamido)pentanoate that were shown to form Tc-N_2S_2-antibody conjugates (2). These conjugates are very stable in vivo and in vitro. Despite the high stability of these complexes, the slow kinetics of chelation of DADS (when certain sulfur protecting groups are used) ligands required the preparation of Tc-N_2S_2-antibody conjugates in two steps. In the first step, Tc-N_2S_2 chelate is prepared at high temperature (75°-100°C) at acidic pH. In the second step the chelate is conjugated to the antibody at basic pH. An N_2S_2 chelating agent that retained stable binding of Tc and had faster chelation kinetics would enable specific chelation of ligand attached to the protein and thus a simpler labeling process.

As an approach to N_2S_2 ligands with rapid chelation kinetics, we have developed a new class of N_2S_2 ligands containing one amide, one amine and two thiolate donor atoms (MAMA-S_2). The ligands were synthesized with a variety of sulfur protecting groups which include acid and metal labile groups. The ligands were labeled at pH range 3-7 at temperatures ranging from 37° to 75° by exchange reaction with Tc-99m gluconate. The results are summarized in Table I.

Replacement of the amide nitrogen in the ligand I by amine nitrogen (as in IIa) increases the labeling yield at 37°C by three fold. The ligand IIb labeled faster than the ligand IIa indicating a more facile metal assisted cleavage of the S-benzyl group. These results clearly show that MAMA ligands label technetium under milder conditions compared to DADS ligands. The difference in the yield of the complex **4** with IIb and IIc indicate the position of the S-benzyl group with respect to location of the amine is important. Though the same chelate (Figure 1, complex **4**) was formed in both cases at high temperature, the kinetics and mechanism of the reaction appears to be different.

To investigate the reaction mechanism, we have prepared and isolated the kinetically and thermodynamically stable complexes of ligands IIb and IIc with Tc-99. The reaction of ligand IIb with Tc-99 gluconate at pH=2-3 at room temperature produced a yellow color, whereas the reaction of IIc gave the yellow color only on heating. The intermediate and end products were isolated and purified. The HPLC and FAB mass spectral data indicate that in the case of ligand IIb, an intermediate complex **2** (Figure 1) was formed at room temperature, which is converted to the more stable complex **4** upon heating. No such intermediate (complex **3**) was observed in the reaction of IIc with Tc-99 gluconate at room temperature, but heating the reaction mixture to higher temperature lead directly to the formation of the complex **4**.

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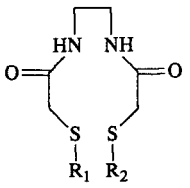
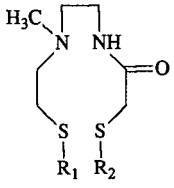
1. A. R. Fritzberg, P. G. Abrams, P.L. Beaumier, S. Kasina, A. C. Morgan, T. N. Rao, J. A. Sanderson, A. Srinivasan, D. S. Wilbur and J.-L. Vanderheyden. Proc. Natl. Acad. Sci. USA, 85, (1988) 4025.
2. S. Kasina, J.-L. Vanderheyden and A. R. Fritzberg. Sixth International Symposium on Radiopharmaceutical Chemistry. 1986. p 269 (abstract).

TABLE I

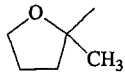


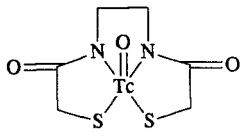
Ligand I
(DADS)



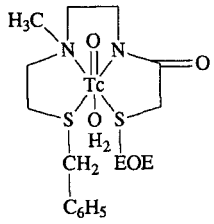
Ligand II
(MAMA)

Ligand	R_1	R_2	% complex 4 formed at pH	75°	37°
I	EOE	EOE	3	80[a]	16[a]
IIa	EOE	EOE	3	83	57
IIb	$CH_2C_6H_5$	EOE	3	98	50
IIc	EOE	$CH_2C_6H_5$	3	60	22
IId	$CH_2C_6H_5$	Me-THF	7	88	57

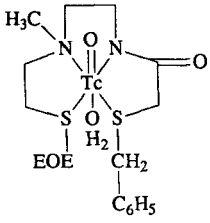
[a] Complex 1 (Figure 1) ; EOE =  ; MeTHF = 



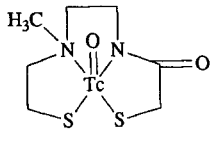
Complex 1



Complex 2
(Kinetic
Intermediate)



Complex 3



Complex 4
(Thermodynamically
stable product)

Figure 1

COMPLEXES OF TECHNETIUM-99m WITH TETRAGLYCINE AND TETRA-L-ALANINE AND THEIR BIODISTRIBUTION IN MICE.

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Tc-99m MAG3 (I) has been proven to be a practical and useful tracer agent for the evaluation of renal function. To investigate whether the thiol group is essential in I for efficient renal excretion by tubular secretion, we have studied the labelling characteristics and biological behaviour of derivatives of I in which the thiol is replaced by an amino function : Tc-99m tetraglycine (II) and its tetramethyl substituted derivative Tc-99m tetra-L-alanine (III) (fig. 1).

Tetraglycine (G4) and tetra-alanine (A4) can be labelled with technetium-99m in high yield by either direct labelling or exchange labelling. Direct labelling at room temperature is achieved by the addition of 1 ml NaOH 0.1N, 100 µg $SnCl_2$ in 25 µl EtOH and 2 ml generator eluate (20-100 mCi Tc-99m) to a vial containing 2 mg of the ligand. For exchange labelling a mixture containing 2 mg of the ligand, 5 mg sodium tartrate, 100 µg $SnCl_2$ in 25 µl EtOH and 2 ml generator eluate is heated during 10 min.

Analysis of the Tc-99m G4 labelling mixtures by gradient RP-HPLC showed the presence of a single peak which elutes somewhat earlier than I in the same HPLC conditions (fig. 2). Biodistribution of HPLC-purified Tc-99m G4 in mice at 10 min and 30 min p.i. showed that its urinary excretion is substantially lower and its hepatic uptake clearly higher as compared to Tc-99m MAG3 (table 1). At 30 min p.i. stomach uptake was about 5% of I.D., probably due to the presence of Tc-99m TcO_4^-, which possibly is formed by in vivo degradation of the Tc-99m G4 complex. No pertechnetate was however observed after prolonged storage of the isolated peak at pH 5.8. Labelling of L-A4 with Tc-99m yielded 2 peaks on RP-HPLC (IIIa and IIIb), probably corresponding with syn and anti diastereomers of the Tc-99m A4 complex. Because of the presence of 4 additional methyl groups, lipophilicity and retention time in HPLC is higher for IIIa and IIIb as compared to Tc-99m G4.

Exchange labelling at pH 6 afforded IIIa and IIIb in relative amounts of respectively 65% and 35% while direct labelling at room temperature in alkaline medium yielded mainly (>95%) IIIa. After isolation and storage at pH 5.8 IIIa converted slowly to IIIb (figure 3), whereas IIIb remained stable in function of time. It appears that IIIb has a higher chemical stability at this pH whereas IIIa is formed preferentially in the alkaline conditions of direct labelling. Biodistribution studies in mice showed that IIIa is rapidly cleared by the hepatobiliary system, whereas activity in kidneys and bladder is minimal (table 2). On the other hand IIIb is cleared by both the hepatobiliary and the renal system in almost equal proportions. Activity is slowly transferred from the liver to the G.I.T. for both products. In contrast to our findings with Tc-99m G4, no stomach activity was observed which suggests that IIIa and IIIb are more stable in vivo than II.

It will be interesting to determine the exact configuration of these Tc-99m G4 and Tc-99m A4 complexes and to find out why and in which way such complexes can convert to the other isomer.

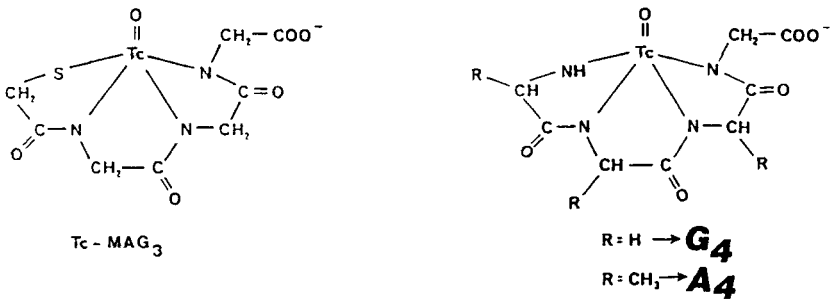

Figure 1. Proposed structure of Tc-G_4 and Tc-A_4 compared to the structure of Tc-MAG_3

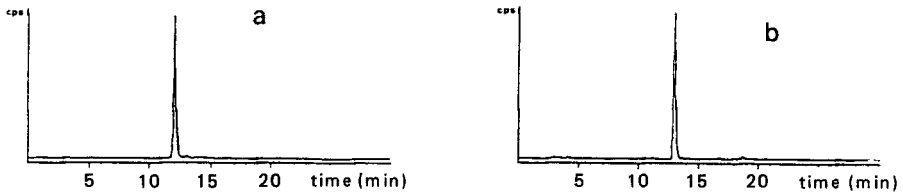

Figure 2. HPLC chromatograms of ^{99m}Tc-G_4 (a)(obtained by direct labelling) and ^{99m}Tc-MAG_3 (b)
HPLC conditions are listed below

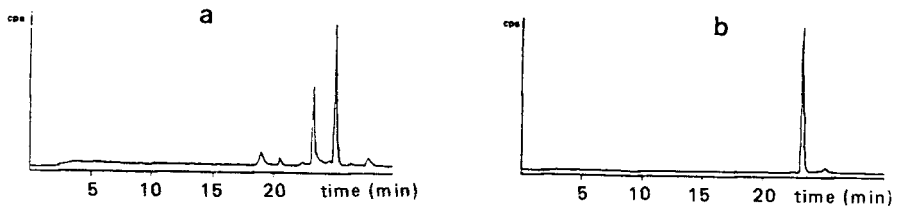

Figure 3. HPLC chromatograms of ^{99m}Tc-A_4 obtained by exchange labelling (a) and by direct labelling. (b)

HPLC CONDITIONS: Hypersil ODS C18, flow 1ml/min with phosphate buffer/EtOH gradient.

TIME	0'	15'	20'	25'
Phosphate buffer pH 5.85, 0.025M	100	80	50	50
EtOH	0	20	50	50

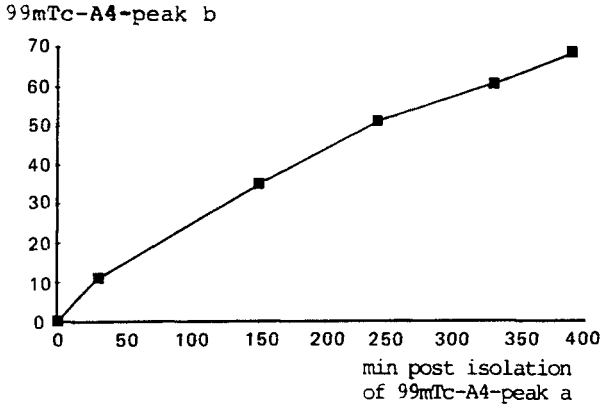

figure 4. % of 99mTc-A4-peak b formed by the conversion of HPLC isolated 99mTc-A4-peak a in function of time

Table 1. Biodistribution in mice at 10 min and 30 min p.i. of 99mTc-G4 compared to the biodistribution of 99mTc-MAG3 (values listed are % of i.d.)

	^{99m}Tc-G_4		^{99m}Tc-MAG_3	
	10'	30'	10'	30'
Bladder	69.8	73.7	74.4	89.0
Kidneys	6.0	2.6	9.0	1.8
Liver	7.2	5.0	3.9	1.3
G.I.T.	2.1	2.7	2.1	4.5
Stomach	0.7	4.9	0.2	0.1
Blood	2.6	2.2	1.8	0.5

Table 2. Biodistribution in mice at 10 min and 30 min p.i. of 99mTc-A4-peak a and 99mTc-A4-peak b. (values listed are % of i.d.)

	^{99m}Tc-A4 peak a		^{99m}Tc-A4 peak b	
	10'	30'	10'	30'
Bladder	13.4	49.6	0.8	1.0
Kidneys	23.6	2.8	0.6	0.1
Liver	47.2	28.4	79.7	73.2
G.I.T.	6.4	15.5	13.0	24.0
Stomach	0.3	0.1	0.3	0.3
Blood	1.3	0.5	0.1	0.1

CRYPTATE COMPLEXES FOR GENERATOR-BASED IMAGING OF BLOOD FLOW

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Generator-based radiopharmaceuticals have been the mainstay of clinical nuclear medicine, but have played only a minor role in the development of PET. There are, however, a number of practical generator systems for bringing short-lived positron emitters into the PET suite. Generators for Rb-82 and Ga-68 have been developed commercially but are used only as ions or simple chelates. Our laboratory has been evaluating cryptands as ligands for use with positron emitters. Cryptands (*e.g.* [2.2.2]) are macrobicyclic molecules with polyether linkages between nitrogen bridgeheads (3 linkages with 2 ethers each in [2.2.2]). Our hypothesis was that a metal ion would be held in this small lipophilic cage and be electronically shielded and thus freely diffusible. We have studied a series of metal complexes with cryptand [2.2.2] and two benzyl (B) substituted forms, [2.2.2]B and [2.2.2]BB.

Proton NMR was used to demonstrate the formation of cryptates of 15 various cations with [2.2.2]. Complex formation withstood competition from ions common in physiological solutions. However, the potential for cryptates to dissociate in vivo, and the absence of chromophores or sensitive methods for detection, necessitated labeling cryptand [2.2.2] with tritium so that we could follow the radioactive metal ion as well as the tritiated ligand in vitro and in vivo. As a simple model of the anticipated freely-diffusible character of cryptates, we have measured their partition coefficients (PC) in chloroform/water and in red cells/saline. This has required the use of tritium and radioactive metal tracers and uv, nmr, and ICP spectroscopy as analytical tools to follow both the ligand and the included metal ion.

Cryptands in metal-free solutions had partition coefficients of 4, 9 and 16 for [2.2.2] and its B and BB forms, respectively. This relative scaling between the substituted cryptates was maintained nearly quantitatively for the series of metal complexes ranging from Li^+ to Pr^{+++}; however, the cryptates were consistently less lipophilic than their cryptands. The PCs in red cells were 2 and 6 for the B and BB forms, respectively, but ranged around unity for several divalent and trivalent cations. Silver

complexes were the only ones which showed a substantial amount of metal cryptate in the chloroform phase, suggesting that Ag^+ represents a special case.

We conclude from these results that cryptates do not effectively shield the charge of included metal ions and that larger complexes able to shield charge will be required to make freely-diffusible flow tracers from generator-based metal ions. The earlier report (1) of blood flow measured with Ag^+[2.2.2] was probably because of the uniquely soft character of silver complexes with N-containing ligands, and is not representative of the general potential of this class of radiopharmaceuticals.

This work was supported by NIH grant HL35343.

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^{99m}Tc-MRP20: The development of a neutral lipophilic complex as a brain perfusion agent.

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MRP20 is a ligand designed to complex technetium with the formation of a neutral Tc(V) complex, TcOL. It is one of a series of ligands (the general structure is given in figure 1) which react under similar conditions with pertechnetate to give a neutral, lipophilic product and several of these new complexes have been assessed for their potential as rCBF tracers. ^{99m}Tc-MRP20 is currently under investigation in Phase II clinical trials and has been fully characterised by ^{99}Tc as [TcO(MRP20)][1].
Some of the derivatives of the MRP series which have been prepared are detailed in table 1.

These were systematically studied for radiopharmaceutical and biological behaviour, the latter as a full biodistribution study in rats followed by a SPECT dog study on the more interesting complexes. The lipophilicity of all ligands and complexes was determined by shake-flask and reverse phase HPLC methods[2]. Values ranged from Log P = 0.93 (MRP23) to 2.1 (MRP22), with the Log p of MRP20 being 1.84. The % injected dose retained in the brain at 30 minutes p.i. varied from 0.12% (MRP22) to 2.24% (MRP20). All the complexes are unstable and decomposed by one of two routes, either by re-oxidation to pertechnetate (MRP30 and MRP31) or with the formation of a secondary, more hydrophilic complex. This latter is probably formed by

the hydrolysis of the azomethine moiety as the same decomposition species has been identified in several of the derivatives.

^{99m}Tc-MRP20 has been evaluated in eight adult male volunteers[3]. Four of the eight subjects underwent whole body studies at 15min, 1, 2, 4, 7 and 24h post injection, the other four underwent SPECT imaging at similar time points. The distribution in the brain was not seen to change over the 24h and uptake into the brain reached a maximum within one minute of injection. The % injected dose in the brain was calculated to be 5.2% (s.d. 1.6) at 15 min p.i. and 4.9% (s.d. 1.5) at 7h p.i. The whole body studies showed considerable retention of the tracer in the skeletal muscles (20% i.d.) and > 2% was retained in the myocardium.

In conclusion therefore, we believe ^{99m}Tc-MRP20 shows promise as a cerebral blood flow tracer and that the ligand structure is sufficiently flexible that other interesting, potentially useful complexes may be designed. We are currently investigating a second generation molecule that shows improved biological handling in animals.

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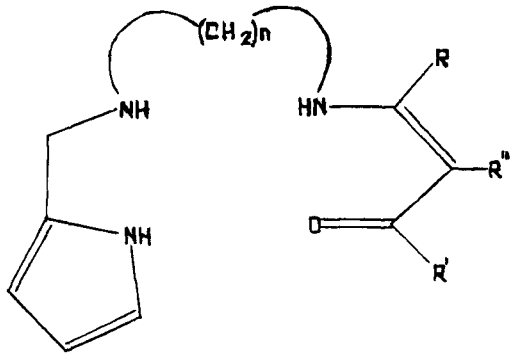

Figure 1: General structure of the MRP series of ligands.

MRP20 n = 2 R = R' = Me

MRP21 n = 2 R' = Me, R = CF_3

MRP22 n = 2 R = R' = ^{t}Bu

MRP23 n = 2 R = R' = Me

MRP27 n = 2 R = R' = Et

MRP28 n = 2 R = R' = nPr

MRP30 n = 3 R = R' = Me

MRP31 n = 3 R' = Me, R = CF_3

R'' = H except MRP23, where R'' = Me.

Table 1: Some derivatives that have been prepared and studied.

STUDIES ON THE STABILITY OF Tc-d,l-HMPAO IN AQUEOUS SOLUTIONS.

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^{99m}Tc-d,l-HMPAO (^{99m}Tc-d,l-hexamethylpropyleneamine oxime), a cerebral perfusion imaging agent, must be administered within 30 min of reconstitution of the freeze-dried Ceretec (Amersham, Int'l) kit because of its limited stability in aqueous solutions [1]. The mechanism(s) responsible for the observed rate of *in vitro* conversion of the lipophilic chelate (LC) to hydrophilic species are unknown, however, the presence of 1 mM gentisic acid at physiological pH substantially reduces the rate of the LC conversion reaction(s) [2]. This suggest that limitations on stability of ^{99m}Tc-d,l-HMPAO are related more to the reaction(s) of the LC with small quantities of reactants in solution rather than the inherent instability of the chelate.

Since the rate of LC dissociation is related to the radioactivity concentration, radiolytically induced decomposition could play an important role in the conversion reactions. This possibility was investigated by irradiating aqueous solutions at pH 6.5-7.8 containing ether extracted ^{99m}Tc-d,l-HMPAO with ^{60}Co γ-rays. The solutions were analyzed using HPLC, TLC and paper chromatography. The results of these studies demonstrate that ^{99m}Tc-d,l-HMPAO [Tc-HMPAO $\sim 10^{-8}$M] is very sensitive to radiolysis (Figure 1) with only approximately 2500 cGy needed to produce 50% dissociation of the LC [i.e., $k_d = (2.8 \pm 0.8) \times 10^{-4}$ cGy^{-1}]. In contrast, ^{99m}Tc-meso-HMPAO irradiated under the same conditions with ^{60}Co γ-rays is 15% as sensitive as the d,l-isomer [Figure 1]. A comparison of the rate of LC conversion with internal doses delivered by self-irradiation to solutions containing 6 mCi (222 MBq) per ml indicates that $\geq$ 65% of ^{99m}Tc-d,l-HMPAO is radiolytically induced. The

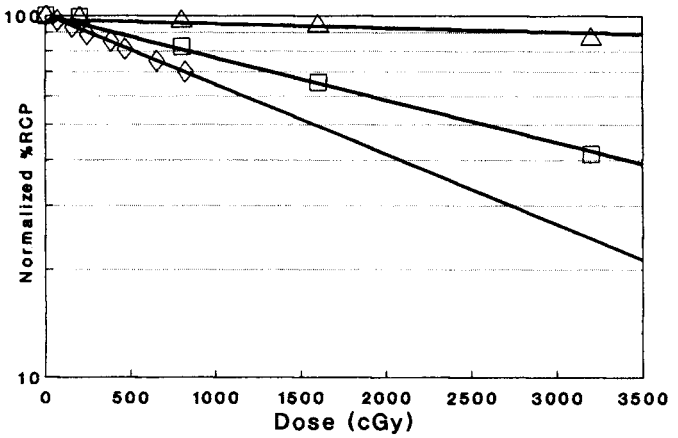

FIGURE 1. Plot of ln (Normalized Radiochemical Purity) of ^{99m}Tc-HMPAO vs. radiation dose to solutions irradiated with ^{60}Co γ-rays. The decomposition rate constant (k_d calculated from these plots are: 0.000043 cGy for ^{99m}Tc-meso-HMPAO ($\triangle$) and 0.00028 cGy^{-1} for ^{99m}Tc-d,l-HMPAO ($\square$). For comparison, the k_d estimated for solutions containing 6 mCi ^{99m}Tc-d,l-HMPAO (not irradiated with ^{60}Co γ-rays) from self-irradiation doses (calculated by MIRD approach) is approximately 0.0004 cGy^{-1} ($\langle\rangle$).

primary reactions producing LC dissociation are most likely oxidative in nature since $^{99m}TcO_4^-$ was found to be the major hydrophilic product.

In a second series of experiments, Tc-d,l-HMPAO was prepared by adding only the long-lived ^{99}Tc (as pertechnetate) to freeze-dried Ceretec vials. In the absence of ^{99m}Tc, the internally delivered radiation dose (cGy) was minimal. The radiochemical purity (RCP) of the LC in the solutions as a function of time was measured as % extraction of ^{99}Tc into diethyl ether. Samples containing 1 x 10^{-7}M and 5 x 10^{-7}M ^{99}Tc-d,l-HMPAO dissociated at a much slower rate [Table 1] than ^{99m}Tc-d,l-HMPAO solutions [3]. Specifically, the RCP was 84-85% after 4 hrs incubation at 22°C (averaging approximately 2% per h) and dropped to only 73-75% after 24 h (Table 1).

TABLE 1. Effect of Incubation Time on Radiochemical Purity (RCP) of ^{99}Tc-d,l-HMPAO[a] in 0.9% Aqueous NaCl

Conditions	Radiochemical Purity(%)[b] Time (h)				
	0h	1h	2h	4h	24h
1x10^{-7}M Tc-HMPAO					
22°C	93	90	88	84	75
4°C		93	92	90	81
5 x 10^{-7}M Tc-HMPAO					
22°C	93	89	88	85	73
4°C		90	90	93	93

a. ^{99}Tc-d,l-HMPAO was made by reconstituting Ceretec with 5 ml of 0.9% aqueous NaCl containing $^{99}TcO_4^-$.

b. RCP was measured by determining the % ^{99}Tc extracted from 1 ml of the aqueous phase from the vial into 1 ml of diethylether.

The rate of LC dissociation was reduced even further when solutions were stored for 24h at 4°C (Table 1). These results demonstrate that some conversion of ^{99}Tc-d,l-HMPAO to non-lipophilic products does occur over 24 hr, however, the rate of this conversion is far lower than with ^{99m}Tc-d,l-HMPAO stored under the same conditions. These observations are consistent with the radiolysis experiments providing further support to the hypothesis that radiolytically induced reactions play an important role in promoting ^{99m}Tc-d,l-HMPAO dissociation. The specific reactive radiolytically produced intermediates (such as peroxides, OH• or $O_2^{\bullet-}$ free radicals) responsible for producing ^{99m}Tc-d,l-HMPAO dissociation are not known, however, it is likely that gentisic acid provides stabilization by intercepting these intermediates before they can interact with the LC.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF THREE ACETAL ISONITRILES LABELLED WITH TECHNETIUM-99m.

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[^{99m}Tc(I)]-hexakis-2-methoxy-isobutylisonitrile has proven to be a clinically useful tracer agent in nuclear cardiology. In order to study the influence of different functional groups in such isonitrile ligands on the heart uptake and excretion rate of their Tc-99m complexes we have synthesized three acetal isonitriles for labelling with technetium-99m : isocyanoacetaldehyde dimethyl acetal (I), isocyanoacetaldehyde diethyl acetal (II) and 4-isocyanobutyraldehyde diethyl acetal (III, table 1). The ligands were synthesized by conversion of the appropriate aminoaldehyde dialkyl acetal to its N-formamide derivative followed by dehydratation of the reaction product to the isonitrile (scheme 1). Their chemical identity was confirmed by ^{13}C and ^{2}H-NMR.

Labelling with technetium-99m was performed by heating the ligand (1mg/1ml EtOH) with 2 ml generator eluate (370-740 MBq Tc-99m) in the presence of dithionite (5mg/0.25 ml water) as reducing agent. The Tc-99m complexes were analyzed for radiochemical purity on ITLC-SG, eluted with 20% NaCl and on paper eluted with acetone. Paper electrophoresis (300V, 30 min) in a methanol-0.025 M phosphate buffer pH 7.4 mixture (70:30 V:V) was used to elucidate the cationic charge. $CHCl_3$/water partition coefficients were determined to estimate the lipophilicity (table 2).

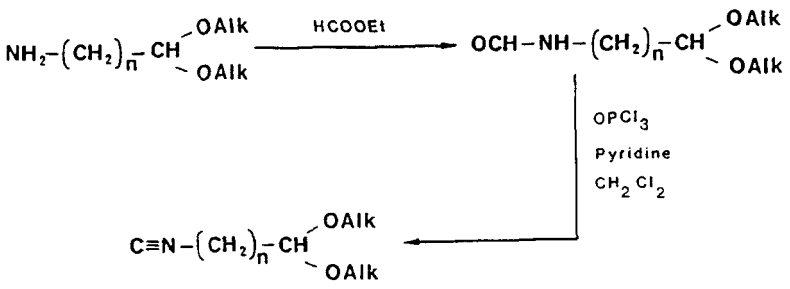
HPLC-peak isolation for biodistribution studies in mice was carried out on a Hypersil ODS (7 µm) column (250 mm x 4.6 mm) eluted with gradient mixtures of 0.05M NH_4OAc pH4 and acetone (0'-10' : 0-90% acetone; 10'-20' : 90% acetone).

At 2 min p.i. all complexes show a reasonable heart uptake in mice, from 42% to 64% of the Tc-99m MIBI value. Activity washes out gradually from the heart although over 50% of the initial heart uptake remains after 30 min. Tc-99m-I, which is slightly more polar than Tc-99m MIBI, approaches the parent compound to the highest degree, but extraction from the blood is slower. On the other hand, it is cleared from the liver to the intestines and urine more efficiently than Tc-99m MIBI from 10-30 min p.i. (table 3). This indicates that the presence of a dimethyl acetal as compared to a methyl ether in MIBI promotes the excretion of the Tc-99m complex, possibly by the faster in vivo conversion to more polar metabolite(s).

After I.V. injection of Tc-99m-I in a baboon, the heart is clearly visualized. Activity in the liver clears to the bile and intestines. At 30 min p.i. activity is most pronounced in the gallbladder and bladder.

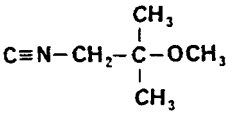
As can be derived from the partition coefficients and the HPLC retention times (table 3), complex II and III are more lipophilic and this results in a high and persistent uptake in the liver, a slow clearance from the blood and a lower uptake in the myocardium.

It is concluded that further investigation of Tc-99m acetal isonitriles may be useful to develop a heart perfusion agent that is cleared more efficiently and rapidly from the liver than it is the case with Tc-99m MIBI.

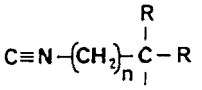


Scheme 1. Synthetic route for the preparation of acetal isonitriles

TABLE 1. Structure of MIBI and the three acetal isonitriles



2-METHOXY-ISOBUTYLISONITRILE (MIBI)



Ligand	n	R
I	1	OCH_3
II	1	OC_2H_5
III	3	OC_2H_5

TABLE 2. Migration (in cm) towards the cathode during paper electrophoresis of Tc-99m MIBI and the Tc-99m acetal isonitriles (conditions see text)

Tc-99m-MIBI	3.9
Tc-99m-I	3.5
Tc-99m-II	3
Tc-99m-III	2.5

TABLE 3. Comparison of chemical and biological properties of the Tc-99m complexes with MIBI and the acetal isonitriles

	MIBI	I	II	III
HPLC retention time (min)	13.5	12.7	15.9	16.1
Partition Coefficient Chloroform/water	87.9	84.4	91.6	96.8
% of I.D at 10 min p.i. in				
Heart	1.0	0.6	0.5	0.2
Liver	22.6	24.6	46.4	63.4
Intestines	17.9	18.7	9.3	04.8
Blood	1.6	10.1	27.8	11.7
Lungs	0.8	0.4	2.3	4.8
Kidneys+Urine	21.8	21.5	10.0	6.6

PREPARATION AND BIODISTRIBUTION OF COPPER-67 COMPLEXES WITH TETRADENTATE SCHIFF-BASE LIGANDS

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The zinc-62/copper-62 radionuclide generator is a potential source of a positron-emitting radiolabel for facilities that lack a cyclotron but would like to employ positron emission tomography (PET) for diagnostic imaging studies. We have previously reported (1-3) studies of copper-labeled copper(II) *bis*(thiosemicarbazone) complexes aimed at identification of possible copper-62 tracers for cerebral and myocardial blood flow. In these studies we have also tried to define structure-activity relationships for tracer uptake and retention in tissues of interest. The most promising tracer identified to date, Cu(PTSM), remains under investigation as a multi-organ PET perfusion tracer. Our investigations are now extending to other classes of tetradentate ligands that can provide uncharged copper complexes, in order to refine our understanding of the physicochemical criteria for blood-brain barrier (BBB) passage and tracer trapping (and to potentially identify superior tracers). We report here the synthesis and biodistribution of the copper-67 complexes of a series of tetradentate Schiff-base ligands.

The ligands were synthesized by condensation of ethylenediamine (en), 1,2-propanediamine (pn), 1,3-propanediamine (tn), or 2,2-dimethyl-1,3-propanediamine (2,2-Me_2tn) with either salicylaldehyde (sal), 4-methoxysalicylaldehyde (4-MeOsal), 2-hydroxyacetophenone (2-HO-aceptoph), or acetylacetone (acac). The fourteen [Cu-67]-Schiff-base complexes studied (Figures 1 and 2) were readily obtained by reaction of the acetate-buffered divalent Cu-67 ion with an excess of the tetradentate ligand dissolved in ethanol. In all cases the radiochemical purity of the product exceeded 95% as assessed by thin layer chromatography on silica gel plates eluted with ethyl acetate. Octanol/water partition coefficients (*log P*) were measured by repeated partitioning of each tracer between 1-octanol and isotonic TRIS buffer (pH 7.0). The measured *log P* values increase as expected with increasing alkyl substitution of the basic ligand frameworks (Figures 1-2) and lie in the $0.5 < log\ P < 3.0$ range where high cerebral uptake might be expected following intravenous injection (2-4).

The biodistribution of each of these [Cu-67]-Schiff-base complexes was determined in rats following injection (0.1-0.2 mL, 1-3 μCi) into the femoral vein. Tables 1 and 2 show the brain and blood levels of these copper tracers found at 1 minute, 5 minutes, and 2 hours post-injection. All the tracers studied penetrate the blood-brain barrier to some extent, but some far better than others. For closely related compounds, observed brain uptake tends to increase with increasing lipophilicity; however, *log P* is clearly not the sole determinant of high brain uptake. Substantial variations are also observed in the tendency of these compounds to be retained or cleared from brain tissue. Work remains in progress to elucidate the underlying chemical processes involved.

Support for this work was provided by the National Cancer Institute (RO1-CA46909), the National Heart, Lung, and Blood Institute (KO4-HL01801), and the U.S. Department of Energy (DE-FG02-89ER60868).

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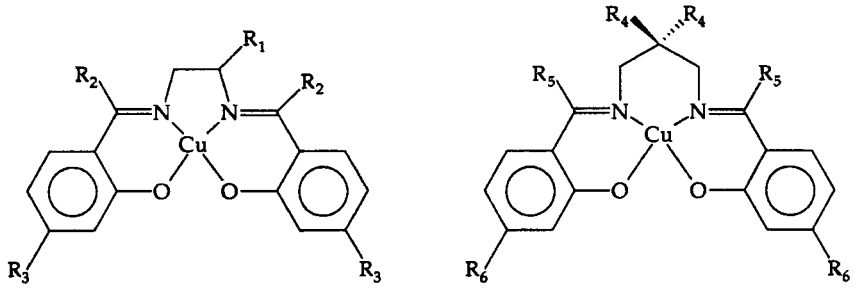
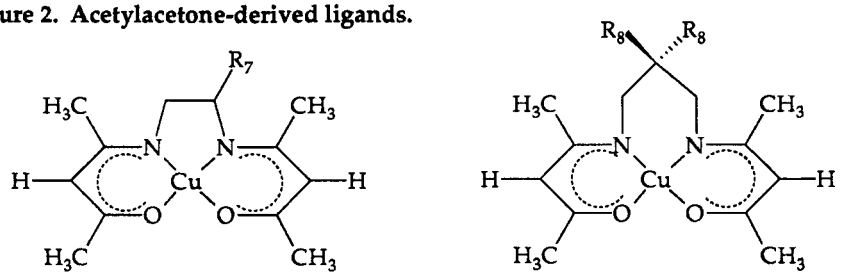
Figure 1. Salicylaldehyde and 2-hydroxyacetophenone derived ligands.



Ligand	R_1	R_2	R_3	R_4	R_5	R_6	*log P*
$(sal)_2$ en	H	H	H	-	-	-	1.7
$(sal)_2$ pn	CH_3	H	H	-	-	-	2.0
$(sal)_2$ tn	-	-	-	H	H	H	1.8
$(sal)_2$(2,2-Me_2 tn)	-	-	-	CH_3	H	H	2.6
$(4\text{-MeOsal})_2$ en	H	H	OCH_3	-	-	-	2.4
$(4\text{-MeOsal})_2$ tn	-	-	-	H	H	OCH_3	2.6
$(4\text{-MeOsal})_2$(2,2-Me_2 tn)	-	-	-	CH_3	H	OCH_3	3.2
$(2\text{-HO acetoph})_2$ en	H	CH_3	H	-	-	-	2.0
$(2\text{-HO acetoph})_2$ pn	CH_3	CH_3	H	-	-	-	2.2
$(2\text{-HO acetoph})_2$ tn	-	-	-	H	CH_3	H	2.15
$(2\text{-HO acetoph})_2$(2,2-Me_2 tn)	-	-	-	CH_3	CH_3	H	3.0

Figure 2. Acetylacetone-derived ligands.



Ligand	R_7	R_8	*log P*
$(acac)_2$ en	H	-	1.7
$(acac)_2$ pn	CH_3	-	2.1
$(acac)_2$(2,2-Me_2 tn)	-	CH_3	2.8

Table 1. Brain Levels of Radiocopper Afforded by ^{67}Cu-Schiff-Base Complexes in Rats*

	% Injected Dose per Gram (n = 3 or 4)		
Ligand	1 minute	5 minutes	2 hours
$(acac)_2$ en	0.68 ± 0.08	0.52 ± 0.06	0.41 ± 0.02
$(acac)_2$ pn	0.43 ± 0.04	0.29 ± 0.02	0.14 ± 0.01
$(acac)_2$(2,2-Me_2 tn)	1.2 ± 0.3	0.95 ± 0.07	0.93 ± 0.26
$(sal)_2$ en	0.35 ± 0.07	0.14 ± 0.04	0.07 ± 0.02**
$(sal)_2$ pn	0.63 ± 0.24	0.10 ± 0.01	0.012 ± 0.001
$(sal)_2$ tn	1.02 ± 0.47	1.11 ± 0.22	1.20 ± 0.34
$(sal)_2$ (2,2-Me_2 tn)	1.44 ± 0.39	0.50 ± 0.06	0.43 ± 0.05
$(4\text{-MeOsal})_2$ en	0.44 ± 0.13	0.31 ± 0.03	0.044 ± 0.005
$(4\text{-MeOsal})_2$ tn	0.34 ± 0.11	0.37 ± 0.08	0.52 ± 0.07
$(4\text{-MeOsal})_2$ (2,2-Me_2 tn)	1.13 ± 0.14	0.45 ± 0.07	0.21 ± 0.03
$(2\text{-HO acetoph})_2$ en	0.30 ± 0.02	0.29 ± 0.08	0.22 ± 0.04
$(2\text{-HO acetoph})_2$ pn	0.38 ± 0.11	0.17 ± 0.02	0.10 ± 0.01
$(2\text{-HO acetoph})_2$ tn	0.07 ± 0.02	0.06 ± 0.01	0.05 ± 0.01
$(2\text{-HO acetoph})_2$ (2,2-Me_2 tn)	0.11 ± 0.02	0.07 ± 0.01	0.07 ± 0.01

Table 2. Blood Levels of Radiocopper Afforded by ^{67}Cu-Schiff-Base Complexes in Rats*

	% Injected Dose per Gram (n = 3 or 4)		
Ligand	1 minute	5 minutes	2 hours
$(acac)_2$ en	1.28 ± 0.07	0.74 ± 0.03	0.51 ± 0.03
$(acac)_2$ pn	1.48 ± 0.02	0.96 ± 0.13	0.43 ± 0.04
$(acac)_2$(2,2-Me_2 tn)	2.04 ± 0.74	0.70 ± 0.01	0.43 ± 0.05
$(sal)_2$ en	0.58 ± 0.19	0.36 ± 0.05	0.26 ± 0.05**
$(sal)_2$ pn	0.52 ± 0.13	0.20 ± 0.02	0.10 ± 0.01
$(sal)_2$ tn	1.05 ± 0.20	0.61 ± 0.08	0.51 ± 0.08
$(sal)_2$ (2,2-Me_2 tn)	0.48 ± 0.12	0.22 ± 0.02	0.35 ± 0.04
$(4\text{-MeOsal})_2$ en	1.32 ± 0.11	1.08 ± 0.21	0.26 ± 0.01
$(4\text{-MeOsal})_2$ tn	1.29 ± 0.13	0.79 ± 0.17	0.44 ± 0.05
$(4\text{-MeOsal})_2$ (2,2-Me_2 tn)	0.53 ± 0.14	0.23 ± 0.03	0.32 ± 0.03
$(2\text{-HO acetoph})_2$ en	1.16 ± 0.05	0.67 ± 0.05	0.38 ± 0.03
$(2\text{-HO acetoph})_2$ pn	1.47 ± 0.14	0.77 ± 0.09	0.48 ± 0.10
$(2\text{-HO acetoph})_2$ tn	1.60 ± 0.43	0.93 ± 0.13	0.63 ± 0.04
$(2\text{-HO acetoph})_2$ (2,2-Me_2 tn)	1.74 ± 0.22	0.84 ± 0.05	0.52 ± 0.04

**ca* 200g
***15 minutes

PROPOSED CARBANION FORMATION IN THE EXCHANGE REACTION OF THE F-18 LABELED INHALATION ANESTHETICS. M.R SATTER and R.J. NICKLES. University of Wisconsin, Madison WI.

The molecular mechanism of action of the inhalation anesthetics remains a mystery despite their use in surgical procedures for well over a century. The theories of anesthesia quoted most often have a common foundation in the correlation discovered between anesthetic potency and lipid solubility whereby the product of MAC * and the corresponding gas/olive oil partition coefficient varies less than twofold over a 70,000-fold range of anesthetising partial pressures. This data suggests that anesthetic action is inextricably tied to lipid solubility and that these agents operate primarily on the lipid fraction of brain tissue. However, recent evidence reveals that inhalation anesthetics can act on the nonpolar fractions of membrane bound proteins. Firestone et al [1] found that Halothane at physiologically relevant concentrations reduced by ~ 50% the specific binding of a labeled activating ligand (tritiated-phorbol dibutyrate) to Protein Kinase-C. Protein Kinase-C is an enzyme found in abundance in the synapses of the mammalian brain which upon activation phosphorlyate proteins that modulate key steps in synaptic transmission [2].

We have labeled four inhalation anesthetics via a net [F-18] F^- for [F-19] F^- exchange reaction in order to monitor the biodistribution, uptake and clearance kinetics of these agents in the human brain at subanesthetic concentrations with PET scanning. Aqueous [F-18] F^- produced by 11 MeV proton irradiation of a thick 400 μL [O-18] H_2O target, is azeotropically dehydrated in CH_3CN with the bicyclic aminopolyether Kryptofix 222. The $[K/2.2.2]^+$ F^- phase transfer catalyst is dissolved in 1 ml of CH_3CN + 100 μL of anesthetic and refluxed (95 °C/1.5 atm) in an acylation vial for 10 minutes. The separation of the anesthetics from the reaction mixture is accomplished via solvent extraction with water. All reaction products were identified with capillary radio-GC, proton and fluorine NMR spectroscopy, and radiometric partition coefficients measured in olive oil (37°C). We postulate the reaction mechanism involves the formation of a carbanion resulting from the loss of an α-proton. The carbanion stability is attributed to the adjoining trifluoromethyl moiety, a halogen (X) and a functional group (Y). The subsequent loss of a fluoride ion from the CF_3 group leads to alkene formation, enabling fluoride ions to exchange in the solvent:

$$CF_3CHXY \text{ <-------> } CF_3CXY + H^+ \text{ <-------> } CF_2{=}CXY + F^-$$

Substituting CD_3CN for CH_3CN as solvent and with [F-19] F^- from KF (0.25 mM), proton NMR spectra show that a deuterium for α-proton exchange occurs with the anesthetics Isoflurane and Halothane, precluding an E2 elimination pathway. Spectroscopy is being done to discover if a correlation exists between the hydrogen bond shift of the α-proton and the radiochemical yield. The hydrogen bond shift, a measure of acidity, is defined as the difference in the proton chemical shift of the infinitely diluted unassociated anesthetic in cyclohexane and that of the infinitely diluted hydrogen bonded anesthetic in methanol. Anesthetics labeled to date include Isoflurane -$CF_3CHClOCF_2H$ [99% radiochemical purity, 50% radiochemical yield], Halothane-$CF_3CHBrCl$ [85%, 40%], CF_3CH_2I-[75%, 70%] and CF_3CH_2Br-[85%, 60%]. The anesthetic Desflurane-$CF_3CHFOCF_2H$, identical to Isoflurane except for fluorine occupancy at the halogen X position, does *not* exchange label. Apparently, carbanion stability requires that the halogen X accomodate a suitable fraction of the electron density. The difference in reactivities of these similar compounds can be explained by the fact that fluorine has no empty d-orbitals to accomodate the carbanion electron.

Dynamic PET studies in normal subjects with labeled Halothane and Isoflurane, modelled as inert, diffusible tracers, show a strikingly similar cerebral biodistribution *in vivo*. Presently, an effort is underway to acquire anatomical MRI fat images to overlay with the anesthetic PET brain:blood partition coefficient images. This work may establish whether lipid solubility is the sole driving force behind the kinetics of these agents in the brain.

* MAC is the mimimun alveolar concentration at 1 atmosphere that prevents a response to a noxious stimulus (skin incision) in 50% of subjects.

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Partition Coefficients in Olive Oil

	N	oil/gas	SD
Isoflurane			
stable	30	196	±4.7
radioactive	8	202	±6.8
Halothane			
stable	21	91.6	±2.1
radioactive	9	94.7	±4.8

Fig. 1 Partition coefficient measurements of stable and labeled anesthetics in olive oil at 37°C.

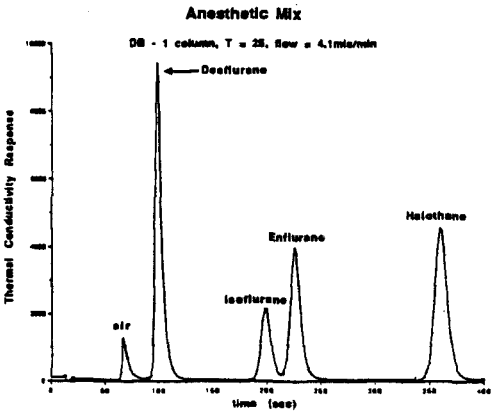
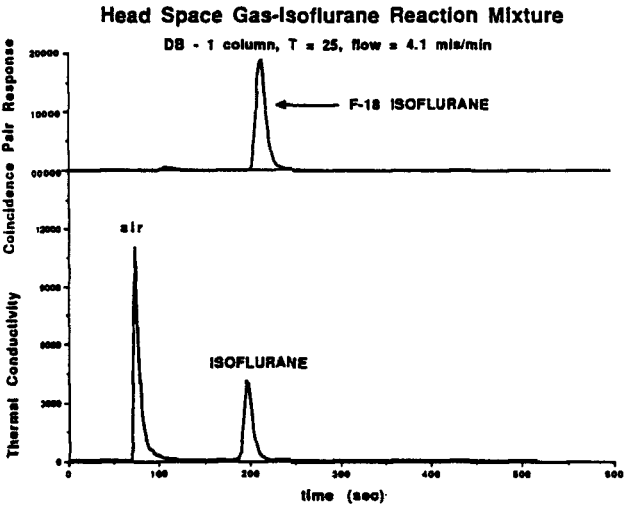


Fig. 2 Capillary gas chromatograph of a mixture of anesthetics. Note: the column [DB-1, 5 μM, 30M] completely resolves the anesthetic isomers Isoflurane ($CF_3CHClOCF_2H$) and Enflurane ($CHClFCF_2OCF_2H$).

Fig. 3 Chromatograph of the head space gas following the F-18 for F-19 exchange reaction with Isoflurane. A delay time of 14 seconds exists between the thermal conductivity detector and the 2 NaI(Tl) 5X2 detectors in coincidence. Post reaction, the mixture is passed through a C-18 Sepak cartridge. Following a 10 milliliter water rinse to remove acetonitrile and Kryptofix 222, Isoflurane is eluted with 2 mls of tetrahydrofuran (THF).



SYNTHESIS AND EVALUATION OF LOW MOLECULAR WEIGHT COMPLEXES OF GALLIUM AND INDIUM WITH DIFFERENT OVERALL CHARGES.

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We and others have synthesized and evaluated several series of ligands with potential as radiopharmaceuticals with gallium and indium.(1-4) These ligands (L) have different types of structures, but, in general, the overall charge of the metal(M)-L complex is negative or zero. We prepared a series of substituted **PLED** (N,N'-bis(pyridoxyl)- ethylenediamine-N,N'-diacetic acid) and **HBED** (N,N'-bis[2-hydroxybenzyl]ethylene- diamine-N,N'-diacetic acid) radiometal complexes where lipophilic groups were attached to the aromatic rings in an attempt to alter the overall charge of the molecule, and found that the addition of lipophilic groups significantly altered the biodistribution of these complexes.(2) In the present study we have prepared three ligands, N-(2-hydroxybenzyl)-N'-pyridoxyl-ethylenediamine-N,N'-diacetic acid (**HBPLED**); N-(2-hydroxy-3,5-dimethylbenzyl)-N'-(3-hydroxy-1,2,5-trimethyl-4-pyridylmethyl)ethylenediamine-N,N'-diacetic acid (**Me_4HBPLED**); and N,N'-bis(3-hydroxy-1,2,5-trimethyl-4-pyridylmethyl) ethylenediamine-N,N'-diacetic acid (**DMPLED**) shown in Scheme 1. Their formal charges are -1 for HBPLED, 0 for Me_4HBPLED, and +1 for DMPLED. The purpose of the study was to evaluate similar overall structures with different formal charges and the effect on biodistribution.

HBPLED was synthesized by condensing salicylaldehyde with N-acetylethylenediamine to give the Schiff base which is then hydrogenated to N-acetyl-N'-(o-hydroxybenzyl) ethylenediamine. After deacetylation with HCl the resulting amine was condensed with pyridoxal to produce a second Schiff base, which was then hydrogenated to give N-(o-hydroxybenzyl)-N'-pyridoxylethylenediamine, which was then alkylated with sodium bromoacetate to yield HBPLED.

Me_4HBPLED The double Schiff base and hydrogenation procedure described above is employed with first, 2,4-dimethylphenol-6-carboraldehyle, and second, deoxypyridoxal, followed by alkylation with sodium bromoacetate to give N-2-hydroxy-3,5-dimethylbenzyl-N'-deoxypyridoxyl-ethylenediamine-N,N'-diacetic acid. This product was is then methylated with methyl iodide to give Me_4HBPLED.

DMPLED Ethylenediamine was converted to the bis Schiff base with deoxypyridoxal, which was hydrogenated; that product was alkylated with bromoacetate to give the diacid. The latter is methylated with methyl iodide to yield DMPLED.

The M-L complexes were prepared with In-111, Ga-67,68, and/or Fe-59. In all cases, the L was dissolved in water (1mg/ml). An aliquot of L solution was mixed with the radiometal diluted in 0.4M sodium acetate (pH 6.8). The final M-L complex solution was adjusted to pH 6-7, if necessary. Electrophoresis of the radioactive complexes on cellulose acetate strips after 45 min at 4mA constant current was performed.

Biodistribution studies of In-111 and Ga-68 complexes with HBPLED, Me_4HBPLED, and DMPLED were carried out in adult female Sprague Dawley rats (~200g). The radioactive complexes were injected in the femoral vein of the anesthetized (ether inhalation) rat. The rats were allowed to recover, and 1,5,15, or 60 min later reanesthetized and sacrificed by

decapitation. Blood, lung, heart, liver, spleen, kidney, fat, muscle, bone, bladder, and brain were removed, weighed, and counted.

Although these molecules have different formal overall charges, the biological behavior of all three complexes with indium are very similar, where the radioactivity cleared through the kidney. Even though the formal charges on these M-L complexes are different, various specific areas of the molecule will have positive charges and negative charges. This appears to be the more important in determing the biological distribution and clearance route rather than the overall charge. This work suggests that different approaches will be necessary in order to significantly alter the biodistribution of gallium and indium complexes.

This work was supported in part by NIH grant CA42925.

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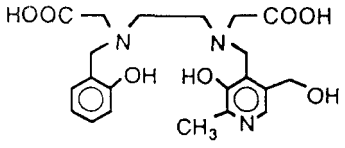
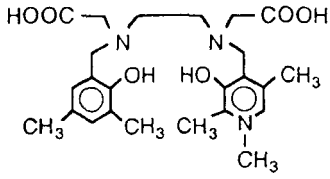
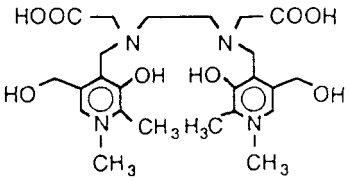


HBPLED



Me_4HBPLED



DMPLED

CHARACTERIZATION AND SUBCELLULAR LOCALIZATION OF Tc-MIBI IN HEART TISSUE USING NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND ENERGY DISPERSIVE ELECTRON MICROSCOPY.

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The interspecies variation in biodistribution and mixed reports on the "trapping" mechanism for different lipophilic cationic technetium myocardial imaging agents have complicated the development of these compounds. Understanding the biodistribution of these radiopharmaceuticals or the rational design of an improved agent requires a knowledge its chemical identity in vivo and the mechanism of localization. We have studied the chemical integrity in vitro and subcellular distribution for some technetium cations using the techniques of ^{99}Tc NMR spectroscopy and electron probe X-ray microanalysis (EPXMA).

^{99}Tc NMR spectra were obtained on a Varian VXR-500 spectrometer using $^{99}TcO_4^-$ as the external reference which resonates at 112.508 MHz.[1] Measurements were obtained on minced samples of a Langendorf perfused rat heart after injection of $Tc(MIBI)_6^+$. Spherical clusters of 11 day old embryonic chick heart cells (size 50-100 um diameter)[2] were incubated for 60 min @ 37 °C in 4 mL of modified Earle's solution containing 9.3 uM $^{99}Tc(MIBI)_6^+$ and $^{99m}Tc(MIBI)_6^+$ to quantitate cellular uptake.[3] Individual aggregrates were rapidly frozen in liquid propane, cryosectioned at -140°C to a thickness of 1200-1500 angstroms and freeze dried on a liquid nitrogen cooled copper block for 48 h @ 10^{-3} Torr.[4] EPXMA was performed on the sections in a transmission electron microscope equipped with a scanning device and a collimated 30 mm^2 Si(Li) energy dispersive X-ray detector and multichannel analyser. X-ray spectra were obtained with a small square raster (0.1-0.06 um^2) at 100,000 to 150,000x magnification with an accelerating voltage of 80 kV and beam current of 1.0 nA. Quantitation of the characteristic X-ray energy peak at K_α^1 = 18.41 KeV for technetium was performed using the Hall continuum normalization technique.[4]

^{99}Tc-NMR analysis of perfused heart tissue after loading with $Tc(MIBI)_6^+$ confirmed a single Tc(I) species present with a resonance at -1950.3 ppm vs TcO_4^-. This signal was identical to the injected compound and did not exhibit the significant line broadening predicted for binding to a macromolecule. Microanalysis for technetium from regional X-ray spectra showed mitochondrial Tc to be between 10-30 mmoles/Kg dry wt and cytoplasmic Tc to be less than 1 mmole/Kg dry wt. By combining the results of these two techniques we conclude that under equilibrium conditions, $Tc(MIBI)_6^+$ is concentrated in mitochondria probably as the free lipophilic cation.

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3-HYDROXY-4-PYRONES: EVALUATION OF A NEW CLASS OF BIDENTATE LIGANDS FOR THE MEMBRANE TRANSPORT OF GALLIUM AND INDIUM.

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Institute of Cancer Research and Royal Marsden Hospital, Sutton and Kings College*, Chelsea, U.K.

A class of bidentate ligands has been described based on 3-hydroxy-2-methyl-4-pyrone(HP) which possess the ability to bind iron(III) and the Group III metals Al(III), Ga(III), and In(III) forming neutral 3:1(ligand-metal) complexes(1). The lipophilicity of this class of chelate may be controlled by alkyl substitution at the 2 position so as to control transmembrane movement of the metal-ligand complex. In respect to radiopharmaceutical design the alkyl substituted series allows for a novel investigation of the structure-activity relationship involved in cell labelling and brain uptake index studies.

The aims of this work were to investigate the feasibility of using HP for cell labelling with Ga and In and to establish the relationship between cell labelling efficiency and the lipophilic character of the alkyl series described in figure 1.

The HPs used in this study were prepared according to a previously published method (1). Stock solutions of the HP derivatives were prepared at a concentration of 0.01 Molar in 20mM HEPES/0.8% saline, pH 7.4-7.5. The Ga-67-GaCl3 and In-111-InCl3 were obtained commercially and adjusted to a final HCl concentration of 0.04N. The Ga-67-(HP)3 and In-111-(HP)3 were prepared by the dropwise addition of the more concentrated HP solution to an acidic solution of the radiometal. The final pH was adjusted to 7.0.

All cell labelling experiments were carried out using blood from normal human volunteers. For these initial studies erythrocytes and leukocytes were labelled with Ga-67-(HP)3 and In(HP)3, respectively. The following parameters were studied in relation to labelling efficiency; the presence of plasma, ligand concentration, incubation time, and cell volume. Partition coefficients(n-octanol/HEPES-saline,pH7.4) were determined the Ga-HP complexes using the shake-flask method.

The partition coefficients for the Ga-67-tris(HP) complexes are given in table 1. The effect of ligand concentration on uptake of Ga-67 into erythrocytes at 15 minutes is shown in figure 2. Figure 3. shows the effect of ligand concentration on uptake of In-111 into leukocytes at 15 minutes. In both cases the uptake of radiometal is rapid, reaching a maximum within 5 minutes for the butyl and isopropyl derivatives while the ethyl and methyl derivatives reach a maximum only after 15 minutes. In the presence of plasma minimal labelling yields were obtained indicating that the HPs do not bind Ga or In strongly enough to resist loss of metal to transferrin. However, in the absence of plasma, high labelling efficiency can be accomplished with both the isopropyl- and butyl-HP derivatives with cell volume having little or no effect on labelling yield. Using this small alkyl series it was possible to demonstrate a relationship between lipophilicity and labelling efficiency (figure 4). The trend indictaes a minimal lipophilic nature is required for uptake to occur while labelling efficiency probably reaches a maximum at

log P of 2. Future work will focus on a larger alkyl series in order to more clearly define the relationship between log P and cell labelling yield as well as the clinical utility of these novel ligands for imaging studies.

REFERENCES

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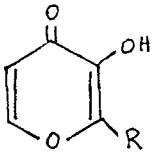
Figure 1. Structure of 3-hydroxy-2-alkyl-4-pyrones used in this study.



R= CH_3, methyl-HP
R= C_2H_5, ethyl-HP
R= $CH(CH_3)_2$, isopropyl-HP
R= $(CH_2)CH_3$, butyl-HP

Table 1. Partition coefficients of Ga-67-(HP)3 derivatives.

methyl-HP	0.17
ethyl-HP	4.22
isopropyl-HP	20.9
butyl-HP	72.5

Figure 2. The effect of ligand concentration on the uptake of Ga-67 into human erythrocytes.

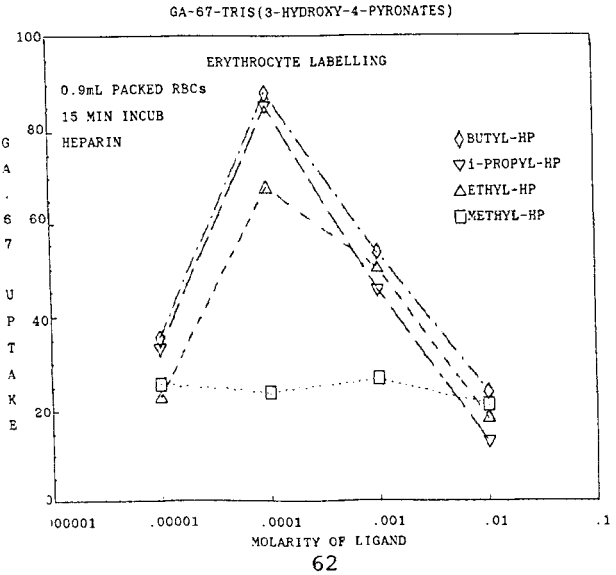


Figure 3. The effect of ligand concentration on the uptake of In-111 into human leukocytes.

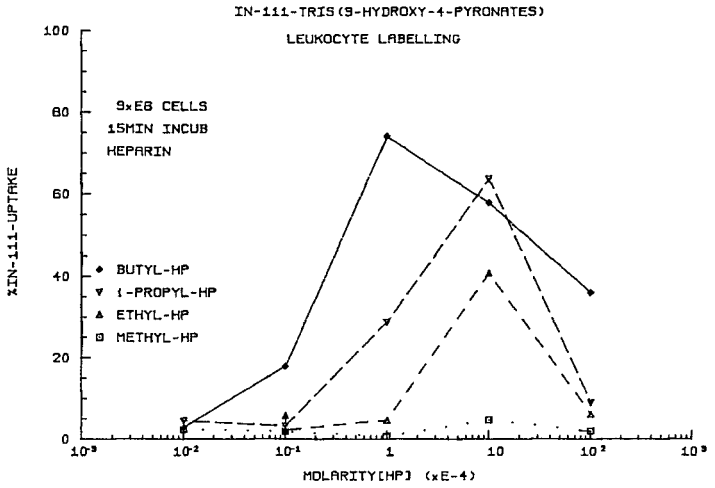
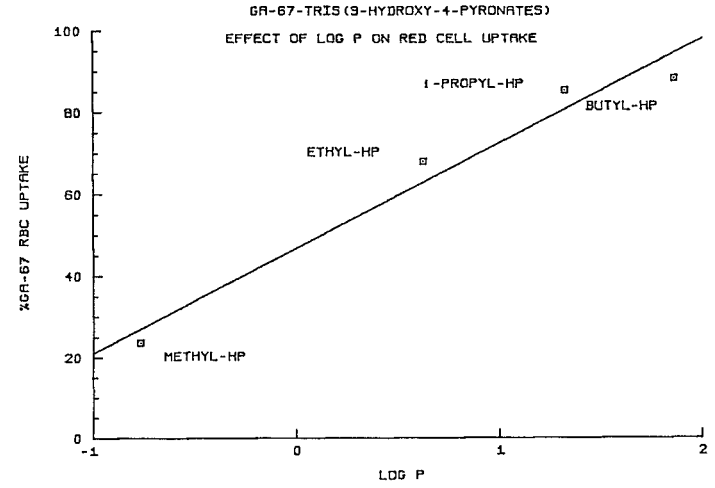


Figure 4. Lipophilicity of Ga-67-tris(3-hydroxy-4-pyronates) and the maximal labelling efficiency of erythrocytes.



PREPARATION AND EVALUATION OF COPPER-67 LABELED COPPER(II) *BIS*(THIOSEMICARBAZONE) DERIVATIVES AS POTENTIAL BLOOD FLOW TRACERS

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The clinical use of positron emission tomography (PET) for diagnostic imaging would be facilitated by the development of radiopharmaceuticals labeled with generator-produced positron-emitting radionuclides by eliminating the need for an in-house cyclotron. Copper-62, derived from the zinc-62/copper-62 generator and having a ten minute half-life, is a radiolabel suitable for PET perfusion studies. We have previously reported on a series of lipophilic copper-labeled Cu(II) *bis*(thiosemicarbazone) ketoaldehyde derivatives as potential cerebral and myocardial perfusion tracers. Of the compounds studied, Cu(II) pyruvaldehyde *bis*(N^4-methylthiosemicarbazone), Cu(PTSM), remains the most promising, exhibiting "microsphere-like" retention in tissues (1). In order to define further the structural and physicochemical properties required of a metal-containing perfusion tracer, a series of *bis*(thiosemicarbazone) derivatives of diketones and dialdehydes was synthesized, their [^{67}Cu]-labeled Cu(II) complexes formed, and their biodistributions studied in rats.

The dicarbonyl compounds and their *bis*(thiosemicarbazone) derivatives were synthesized by literature methods (2,3). The copper-67 labeled complexes of these ligands were formed by a procedure involving the addition of a saturated DMSO solution of ligand to *ca* 30 μCi [^{67}Cu]$CuCl_2$ followed by ethanol and propylene glycol to solubilize the complexes. The solutions were then diluted to 5-10% ethanol concentration with normal saline and filtered through a 0.22 μm filter. The radiochemical purity of the products was determined by thin layer chromatography on silica gel plates eluted with ethyl acetate. All the copper-67 labeled complexes had a radiochemical purity of $\geq$ 93%. Octanol/water partition coefficients were measured. The compounds were lipophilic having log P values of 0.9 to 2.6.

The biodistributions of the copper-67 labeled Cu(II) *bis*(thiosemicarbazone) complexes were determined in rats following femoral vein injection of 1 - 2 μCi (0.1 - 0.2 mL) of tracer. The percent injected dose per organ was calculated for each tissue (Tables 1 and 2). These complexes cleared from the blood and showed varying patterns of tissue uptake and retention in the brain and heart. The N^4-methyl substituted *bis*(thiosemicarbazone) complexes, Cu(1,2-CyTSM), Cu(1,3-CyTSM), Cu(1,4-CyTSM), Cu(2,5-HxTSM), Cu(1,4-BuTSM), all crossed the blood-brain barrier at one minute post-injection, but only Cu(1,4-CyTSM) and Cu(1,4-BuTSM) were retained. The myocardial uptake and retention of these compounds followed a similar trend. The N^4-unsubstituted *bis*(thiosemicarbazone) derivatives, Cu(1,2-CyTS), Cu(1,3-CyTS), Cu(1,4-CyTS), and Cu(2,5-HxTS) penetrate the blood-brain barrier less efficiently. The *in vivo* behavior of these complexes remains under investigation.

Support for this work was provided by the National Cancer Institute (Grant #RO1-CA46909), the National Heart, Lung, and Blood Institute (Grant #KO4-HL01801) and a Purdue Research Foundation David Ross Fellowship.

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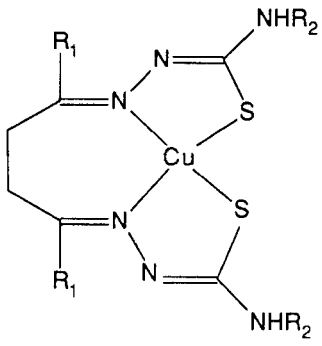
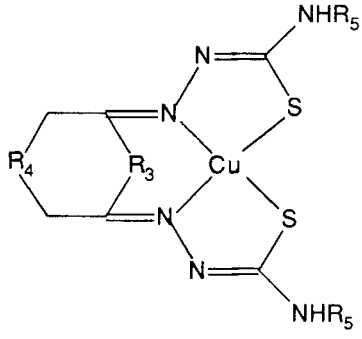
Figure 1. Structures of Copper(II) *Bis*(thiosemicarbazone) Complexes and Measured Octanol/Water-Partition Coefficients (*P*)





Complex	R_1	R_2	R_3	R_4	R_5	*log P*
Cu(1,4-BuTSM)	H	CH_3	-	-	-	1.62
Cu(2,5-HxTS)	CH_3	H	-	-	-	1.40
Cu((12,5-HxTSM)	CH_3	CH_3	-	-	-	2.10
Cu(1,2-CyTS)	-	-	-	C_2H_4	H	1.61
Cu(1,2-CyTSM)	-	-	-	C_2H_4	CH_3	2.62
Cu(1,3-CyTS)	-	-	CH_2	CH_2	H	1.37
Cu(1,3-CyTSM)	-	-	CH_2	CH_2	CH_3	2.04
Cu(1,4-CyTS)	-	-	C_2H_4	-	H	1.40
Cu(1,4-CyTSM)	-	-	C_2H_4	-	CH_3	2.03

Table 1. Brain Levels of Copper-67 Afforded by Copper(II) *Bis*(thiosemicarbazone) Complexes in the Rat (167-233g)

Complex	% Injected Dose per Organ (n = 3 or 4) 1 minute	5 minutes	2 hours
Cu(1,2-CyTS)	0.50 ± 0.22	0.54 ± 0.16	0.47 ± 0.05
Cu(1,3-CyTS)	0.16 ± 0.05	0.07 ± 0.01	0.08 ± 0.01
Cu(1,4-CyTS)	0.30 ± 0.14	0.15 ± 0.04	0.13 ± 0.05
Cu(2,5-HxTS)	0.31 ± 0.02	0.26 ± 0.02	0.18 ± 0.04
Cu(1,2-CyTSM)	3.32 ± 0.39	1.04 ± 0.26	0.45 ± 0.05
Cu(1,3-CyTSM)	1.13 ± 0.10	0.85 ± 0.19	0.69 ± 0.20
Cu(1,4-CyTSM)	3.87 ± 0.63	3.08 ± 0.88	2.84 ± 0.89
Cu(2,5-HxTSM)	1.84 ± 0.26	1.79 ± 0.03	1.36 ± 0.65
Cu(1,4-BuTSM)	2.22 ± 0.45	2.16 ± 0.61	2.65 ± 0.76

Table 2. Heart Levels of Copper-67 Afforded by Copper(II) *Bis*(thiosemicarbazone) Complexes in the Rat (167-233g)

Complex	% Injected Dose per Organ (n = 3 or 4) 1 minute	5 minutes	2 hours
Cu(1,2-CyTS)	0.6 ± 0.1	0.5 ± 0.1	0.36 ± 0.01
Cu(1,3-CyTS)	1.0 ± 0.2	0.5 ± 0.1	0.4 ± 0.02
Cu(1,4-CyTS)	1.2 ± 0.5	0.8 ± 0.2	0.6 ± 0.3
Cu(2,5-HxTS)	1.4 ± 0.3	1.4 ± 0.1	0.9 ± 0.2
Cu(1,2-CyTSM)	1.6 ± 0.5	0.4 ± 0.1	0.26 ± 0.02
Cu(1,3-CyTSM)	1.7 ± 0.2	0.8 ± 0.2	0.6 ± 0.1
Cu(1,4-CyTSM)	3.6 ± 0.9	3.1 ± 0.7	2.7 ± 1.1
Cu(2,5-HxTSM)	2.3 ± 0.3	2.2 ± 0.1	1.9 ± 0.5
Cu(1,4-BuTSM)	2.6 ± 0.5	2.3 ± 0.3	2.4 ± 1.1

Improved Synthesis of (^{15}O)Butanol for Clinical Use

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Since KABALKA et al. first reported the preparation of (^{15}O)butanol in 1984 (1) several modifications of the original method (reaction of molecular oxygen with tributylborane) have been published (2-5). Following the example of BERRIDGE et al. (5) we used a SEP-PAK alumina cartridge as support for 0.1 ml of tributylborane. However, with a procedure similar to the one of BERRIDGE (scheme 1) we could not avoid contamination of our sample by boric acid derivatives despite the use of cartridges containing up to 1.3 g of reverse-phase material. Therefore we added a column (25 x 3 mm) containing anion exchange resin to the outlet of the C_{18} cartridge (SEP-PAK, contents 300 - 360 mg of material). The use of a column loaded with AG 1 resin (OH^--type) proved successful as reported by TAKAHASHI et al. (3). In our experiments a resin with high exchange capacity and small particle size (in order to reduce dead volume), such as Serdolit AS-6, 0.1 - 0.2 mm (OH^--type), gave better results. Ion retardation resins (tested with AG-11 A8) were not effective.

Description of the synthetic procedure (scheme 1). Target gas (0.2% O_2 in N_2), initially with a flow of 3000 ml/min, is passed through the alumina cartridge. The trapping product is hydrolyzed by 6.5 ml of sterile water and simultaneously the reaction products are washed onto the C_{18} cartridge. The resulting 4.3 - 4.5 ml of eluate contain the (^{15}O)H_2O almost completely. The C_{18} cartridge and the resin column are eluted with 6.2 ml of 5% ethanol in water into a vessel containing 0.3 ml of sterile concentrated saline to give 6.5 ml of an isotonic solution. Sterile filtration yields 6 ml of a pyrogen free injectable solution.

Yields. 106 ± 11 (n=19) mCi are produced from a 10 min, 25 µA, 8.5 MeV deuteron bombardment. The entire procedure requires 2.75 min from EOB. About 1700 ml of target gas are passed through the alumina cartridge within 1 minute to finally result in 9.5 - 14 mg butanol in the injectable solution. Specific activity at time of injection (40 mCi): 0.2 - 0.3 Ci/mmol.

Radiochemical yields range from 57 - 67% (corrected to EOB). From these values combined with the ratio of input volume of $(^{15}O)O_2$ and isolated quantity of butanol a $(^{15}O)O_2$-trapping yield of 79 - 92% can be calculated.

Purity. Radioactive by-products are $(^{15}O)H_2O$ with a negligible amount of $\leq$ 0.5% and 4 - 5 % of sec-butanol formed from sec-butyl groups in the reagent. Boric acid derivatives, which represent the only non-radioactive impurities, could be reduced to the low amount of typically 40 µg of boron per sample (ICP analysis). HPLC analyses were performed with a Merck Lichrosorb RP-18 column (250 x 4 mm), eluent CH_3CN/H_2O = 15:85 and a flow rate of 2.0 ml/min. Retention times were: $(^{15}O)H_2O$ 1.1 min, sec-butanol 2.7 min, butanol 3.3 min, boron compounds 4.4 - 4.6 min. Refractive index and radioactivity detectors were used. A typical chromatogram is depicted in Fig. 1.

Analysis before injection. In order to check the purity of the produced solution before injection we used a short HPLC column (Merck Lichrospher RP-18, 50 x 4 mm), eluent CH_3CN/H_2O = 10:90 and a flow rate of 2.5 ml/min. Retention times were: $(^{15}O)H_2O$ 0.25 min, sec-butanol 0.8 min, butanol 1.0 min, boron compounds 1.4 - 1.5 min (Fig. 2).
If the analysis is performed immediately after butanol synthesis, the result can be given within 2 min or even faster, when the flow rate is increased further.

Sequential PET studies. The method described allows sequential production of injectable solutions using the same apparatus. Provided that the removable cartridges and the resin columns are prepared in advance and can easily be exchanged before each production interval, time intervals of about 17 min can be achieved (a waiting period of 10 min for reduction of radiation included).

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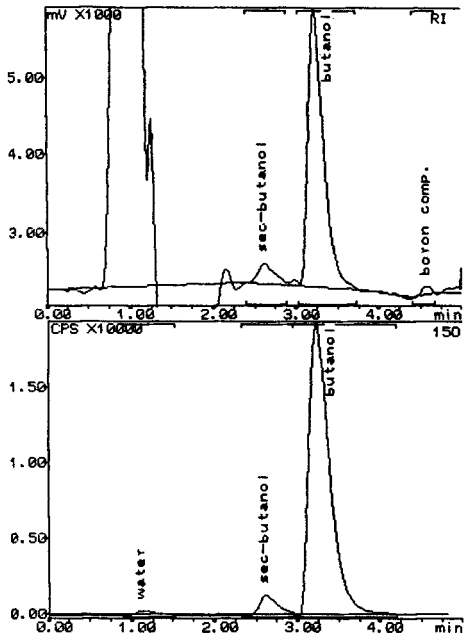


Fig. 1: HPLC analysis of an injectable (^{15}O)butanol solution performed with Lichrosorb RP-18 (250 x 4 mm) column. Above: Refractive index detector. Below: Radioactivity detector

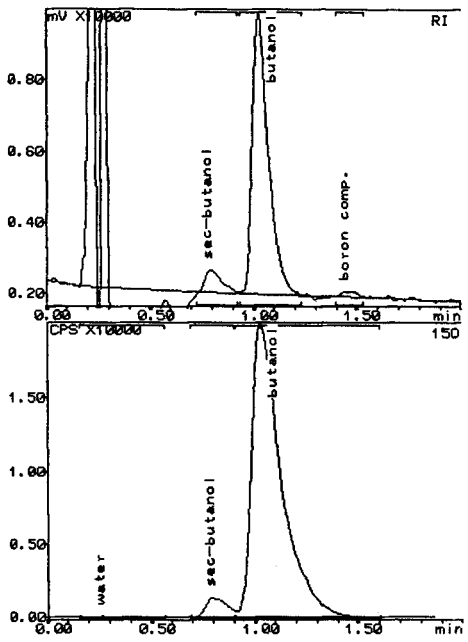
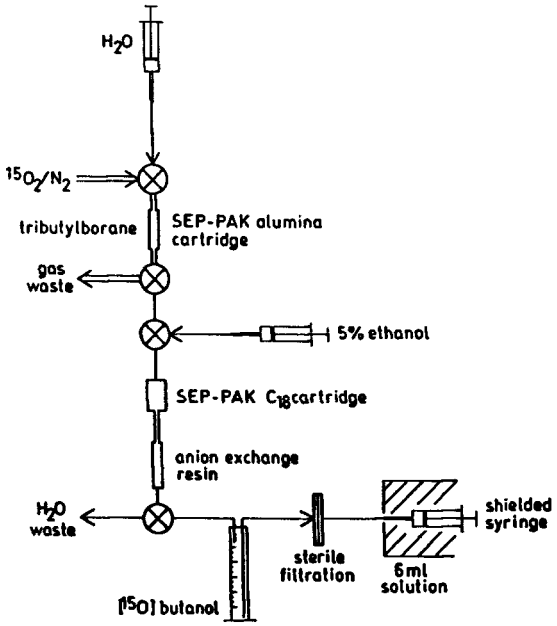


Fig. 2: Similar sample as in Fig. 1, analyzed with Lichrospher RP-18 (50 x 4 mm) column. Above: Refractive index detector. Below: Radioactivity detector.



Scheme 1. Synthesis of [^{15}O] butanol ready for injection

Synthesis and Characterization of a New Gallium N_2S_2 Complex for Myocardial Imaging.

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Gallium complexes, labeled with Ga-68, are potential radiopharmaceuticals for imaging by positron emission tomgraphy (PET). The $^{68}Ge/^{68}Ga$ generator with the 275 day parent and 68 min daughter half-life is convenient for preparation of useful radiopharmaceuticals for routine clinical use(1). One of the major challenges of developing new myocardial perfusion tracers based on this radionuclide is the formation of suitable complexes with appropriate lipid-solubility. We describe the synthesis and chemistry of a new gallium N_2S_2 complex, which displays the desired physico-chemical characteristics (Figure 1).

Gallium (Ga^{3+}) reacts with bisaminoethanethiol (BAT) ligands in millimolar quantities under aqueous acidic conditions (pH3) to form a neutral, lipophilic complex (Figure 1). The complex is crystallized from acetone and aqueous acid. Clear, colorless crystals can be obtained by slow evaporation of an acetone/ HCl solution. Conductivity data, elemental analysis data, infrared, proton NMR spectroscopy and mass spectral measurements are consistent with the formulation [Ga(BAT-TECH)Cl]. Examination of the IR and NMR data suggest that, as with the Tc=O analogs of the BAT ligands, complexation occurs by gallium binding to the two sulfur atoms and the two nitrogen atoms. The IR data of the complex show no band attributable to the SH stretching frequency, which appears as a strong band at 2420 cm^{-1} in the spectrum of the ligand. The proton NMR data (27°C, $CDCl_3$) show resonances for all of the diasterotopic methylene protons adjacent to the nitrogen atoms of the ligand backbone. The observation of well resolved resonances of the individual methylene protons, similar to that observed for proton NMR of the Tc=O complexes of N_2S_2 ligands, suggests that the gallium atom binds to the nitrogen atoms and may impose a rigid structure on an otherwise flexible ligand. Conductivity measurements (0.42 $ohm^{-1}\,cm^2\,mol^{-1}$;10^{-3} M, acetonitrile) indicate that the molecule is neutral in this solvent. The mass spectral data

(chemical ionization) show a molecular ion cluster at m/z= 465 with an isotope distribution pattern in agreement with $[M+H]^+$ (where M= [Ga(BAT-TECH)Cl]) and fragmentation from this molecular ion to give $[M-Cl]^+$, M/Z=430, indicating the cleavage of the chloride ion.

At tracer concentrations, the aqueous chemistry has been studied using [^{67}Ga] gallium citrate as the starting material. The net charge in an aqueous solution was found to be +1(Fig. 2). The ratio of ligand to ^{67}Ga was determined to be 1:1(Fig. 3). Presumably, under the conditions required for the preparation of the ^{67}Ga complex, the cationic $[^{67}Ga(BAT\text{-}TECH)]^+$ species is formed. Using reverse phase HPLC (PRP-1 column, ; mobile phase=90/10 CH_3CN/ 5mM DMGA, pH=7 ; flow rate 1 mL/min), $[^{67}Ga(BAT\text{-}TECH)]^+$ elutes at 6.5 min, suggesting that the complex is very lipid-soluble. Preliminary animal biodistribution studies in rats with $[^{67}Ga(BAT\text{-}TECH)]^+$ (Table 1) and imaging study in a monkey with $[^{68}Ga(BAT\text{-}TECH)]^+$ demonstrate that the agent is localized in myocardium. The data suggest that this series of GaN_2S_2 complexes may be potentially useful for developing new radiopharmaceuticals for PET imaging.

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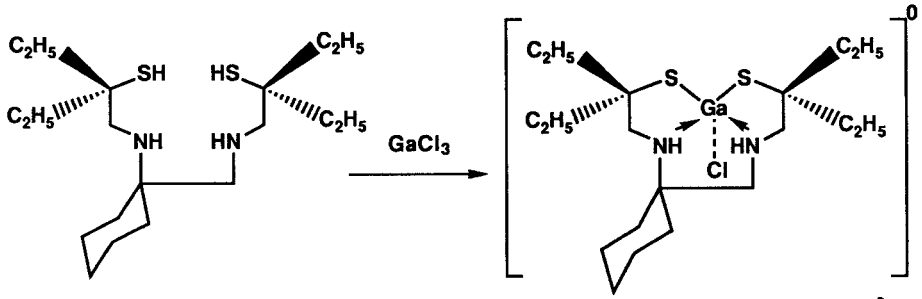
Table 1

Biodistribution of [^{67}Ga]BAT-TECH in rats after an iv injection (% dose/organ)

Organ	2 min	30 min	60 min
Blood	10.18 ± 0.30	3.58 ± 0.08	4.54 ± 1.10
Heart	1.68 ± 0.12	0.52 ± 0.08	0.26 ± 0.02
Muscle	13.89 ± 3.21	21.14 ± 2.18	10.79 ± 1.85
Lung	2.07 ± 0.07	0.46 ± 0.09	0.37 ± 0.009
Kidney	6.94 ± 0.31	2.00 ± 0.10	1.06 ± 0.14
Spleen	0.50 ± 0.06	0.15 ± 0.009	0.11± 0.001
Liver	21.52 ± 1.11	33.54 ± 4.42	46.41 ± 2.39
Skin	5.44 ± 1.65	7.56 ± 1.60	5.78 ± 0.92
Brain	0.02 ± 0.004	0.01 ± 0.001	0.01 ± 0.002

Figure 1.



BAT-TECH $[Ga(BAT\text{-}TECH)Cl]^0$

Determination of net charge of Ga-(BAT-TECH):

$$\left[[^{67}Ga]\,BAT\text{-}TECH\right]^{+X} + RH_X \rightleftharpoons R\text{-}\left[[^{67}Ga]\,BAT\text{-}TECH\right]^{+} + xH^+$$

RH: cation exchange resin

The equilibrium constant = K

$$K = \frac{R\text{-}\left[[^{67}Ga]\,BAT\text{-}TECH\right]^{+}\left[H^+\right]^X}{\left[[^{67}Ga]\,BAT\text{-}TECH\right]^{+X}_{aq}\left[RH_X\right]}$$

Distribution Coefficient ▫ D

$$D = \frac{R\text{-}\left[[^{67}Ga]\,BAT\text{-}TECH\right]^{+}}{\left[[^{67}Ga]\,BAT\text{-}TECH\right]^{+X}_{aq}}$$

$$\log D = \log K + \log\left[RH_X\right] + xpH \qquad \log D \text{ ▫ } xpH + C$$

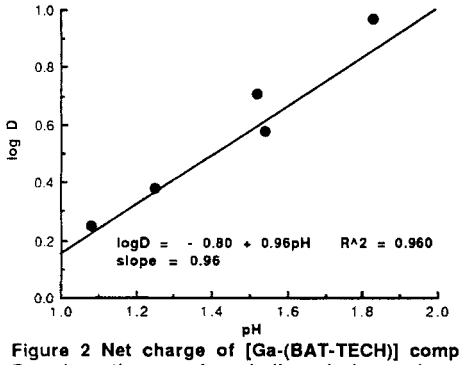


Figure 2 Net charge of [Ga-(BAT-TECH)] complex Based on the equations indicated above, the net charge is plus one

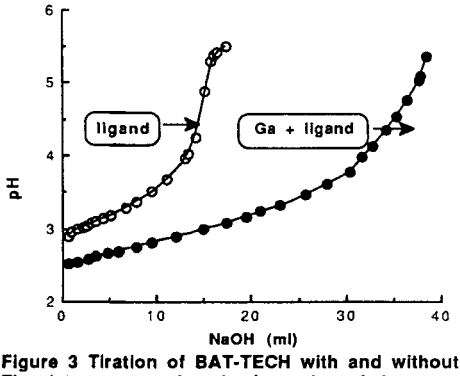


Figure 3 Tiration of BAT-TECH with and without Ga The data suggest that the formation of the complex is Ga/ligand=1:1

SYNTHESIS, CHARACTERIZATION AND BIODISTRIBUTION OF SOME NEW [^{99}Tc] CARBONYL COMPLEXES.

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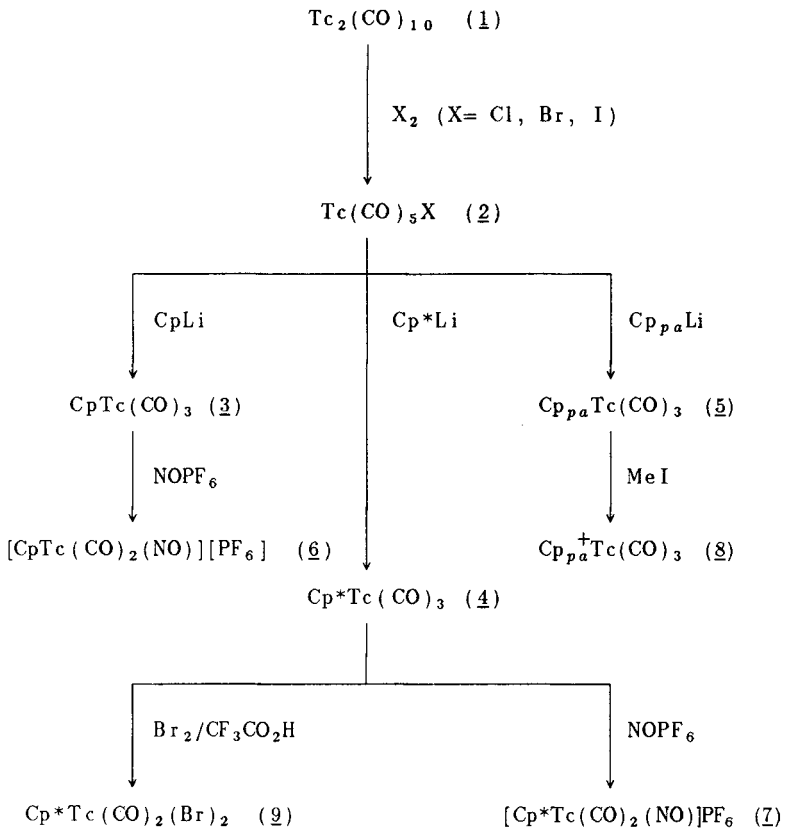
As a part of a program aimed at the synthesis and evaluation as possible radiopharmaceuticals of technetium complexes in which the metal atom is in a low oxidation state, we have prepared and characterized a number of technetium carbonyl complexes starting from $Tc_2(CO)_{10}$ ([^{99}Tc]-1).

Using the long-lived isotope ^{99}Tc, a novel procedure for the preparation of the starting material [^{99}Tc]-1 has been elaborated. It is based on reduction of ammonium pertechnetate with sodium amalgam in THF and it allows us to work under much less drastic conditions of temperature and CO pressure than those previously reported (1). The carbonyl halides of technetium, $Tc(CO)_5X$ (X= Cl, Br, I) ([^{99}Tc]-2) have been synthesized by the action of the halogens on [^{99}Tc]-1 according to known procedures (2).

A series of cyclopentadienyl complexes of general formula $Cp'Tc(CO)_3$, where Cp' stands for C_5H_5, $C_5(CH_3)_5$ and $C_5(CH_3)_4[(CH_2)_3N(CH_3)_2]$ ([^{99}Tc]-3,4,5) has been prepared as well as their nitrosyl derivatives ([^{99}Tc]-6,7), the quaternary salt ([^{99}Tc]-8) of the aminocyclopentadienyl ligand, and the dicarbonyl complex [^{99}Tc]-9 (Scheme 1).

Scheme 1



Cp= C_5H_5

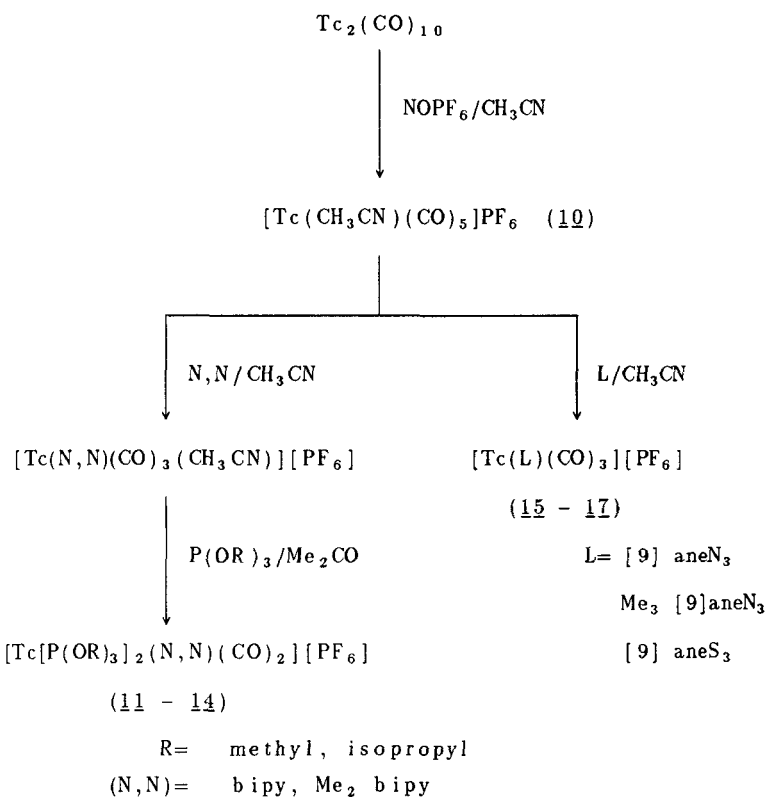
Cp*= $C_5(CH_3)_5$

Cp_{pa}= $C_5(CH_3)_4[(CH_2)_3N(CH_3)_2]$

Cp_{pa}^{+}= $C_5(CH_3)_4[(CH_2)_4N^{+}(CH_3)_3]\ I^{-}$

Ditechnetium decacarbonyl, ([^{99}Tc]-1), reacts instantaneously with $NOPF_6$ in acetonitrile to give $[Tc(CH_3CN)(CO)_5][PF_6]$ ([^{99}Tc]-10) quantitatively. The latter constitutes an excellent starting material for the preparation of unipositive lipophilic compounds, ([^{99}Tc]-11-17), since it reacts with a variety of bi- and tridentate ligands containing N, P, or S atoms (Scheme 2). The resulting cationic complexes are promising candidates for heart delineation (see an example in Table I).

Scheme 2



A selection of these compounds has been tested in guinea pigs to evaluate their potential as radiopharmaceuticals. Results of these experiments will be presented.

Table II shows the main carbonyl stretching frequencies of some of the complexes prepared.

Table I Biodistribution stuy of $[Tc[P(O-^{i}Pr)_3]_2(Me_2bipy)(CO)_2]PF_6$ in male guinea pigs. Uptake of ^{99}Tc/g of organ.

	5 min	30 min
Liver	10.14 ± 0.17	9.70 ± 2.34
Kidney	11.31 ± 0.52	12.47 ± 0.77
Lung	1.53 ± 0.23	3.14 ± 0.50
Muscle	0.54 ± 0.29	0.39 ± 0.05
Heart	1.12 ± 0.20	1.78 ± 0.40
Brain	0.22 ± 0.11	0.12 ± 0.02
Plasma	1.17 ± 0.25	1.23 ± 0.22

Table II Infrared data for carbonyltechnetium complexes in the range 4000 200 cm^{-1}

Compound	$\nu(CO)$ (cm^{-1})	
$Cp^*Tc(CO)_3$	2020 m	1925 s
$Cp^*Tc(CO)_2(NO)^+$	2085 m	2035 m
$Cp_{pa}Tc(CO)_3$	2010 m	1910 s
$Cp^*Tc(CO)_2(Br)_2$[a]	2030 s	1970 m
$Tc(CH_3CN)(CO)_5^+$	2065 s	2040 m
$([9]ane\ N_3)Tc(CO)_3$	2025 m	1920 s
$Tc[P(O-^{i}Pr)_3]_2(Me_2bipy)(CO)_2^+$	1950 m	1880 m

Measured for solutions in acetonitrile. [a] Measured in CH_2Cl_2.

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A Simple Colorimetric Method For Determination of the Specific Activity of Spallation-Produced Copper-67 (Cu-67) Using the *Bis*-(N^4-methyl)Thiosemicarbazone (TSC) Derivative of Phenylglyoxal.

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Copper-67 (Cu-67) is a useful radioisotope for therapeutic applications, and multi-millicurie levels are available in HCl solution from high-energy spallation of zinc targets from Brookhaven National Laboratory (BNL) and Los Alamos National Laboratory (LANL) on a seasonal basis. Carrier-free Cu-67 is available from reactor production by the Zn-67(n,p)Cu-67 route (1,2), with subsequent separation from the target material by solvent extraction and/or cation exchange chromatography. Although the reactor route is an attractive method for production of carrier-free Cu-67, the production yields are much lower than from spallation, and Cu-67 from this method is considerably more expensive. Spallation-produced Cu-67 can have low specific activity because the target contains undesirable levels of Cu-63 and Cu-65. Low specific activity may interfere with antibody radiolabeling, where high specific activity is often desired. The availability of a simple, inexpensive method to determine the specific activity of Cu-67 solutions would thus be useful.

Bis-TSC derivatives of 1,2-ketoaldehydes and 1,2-diketones form complexes (Fig. 1) with Cu(II) which have a strong absorbance in the visible region (3). The bifunctional *bis*-TSC ligands are also being developed for radiolabeling antibodies with Cu-64 and Cu-67 (4,5). We have developed a simple colorimetric method to determine the specific activity of Cu-67 solutions using the *bis*-TSC derivative of phenylglyoxal (PG-TSC) which has a maximal absorbance (Fig. 2) at 480 nm ($\epsilon = 3.5 \times 10^3$). The analysis is perfomed by the direct addition of a sample of the Cu-67 HCl solution to an excess of the PG-TSC ligand in ethanol-sodium acetate buffer. The absorbance is determined at 480 nm after 15 min at room temperature. The standard curve (Fig. 3) is linear (r=1.00) up to 40 ppm (40 μg/mL) with a lower limit of detection of 0.4 ppm using a Carey 219 spectrophotometer. Analysis of samples from BNL and LANL gives good agreement with values determined by Isotope Coupling Plasma (ICP) analysis (Table I). The PG-TSC derivative of zinc(II) chloride was also evaluated (100-fold excess) and was shown to have no interference on the 480 nm absorbance of the Cu(II) complex (2.5 μg/mL). These results demonstrate that this simple technique can be used with an ultraviolet spectrophotometer for the rapid, routine analysis of Cu-67 solutions from spallation production with no special sample preparation.

Table I. Analysis of Spallation-Produced Copper-67 Solutions

Copper-67 Solution			Specific Activity (mCi/μg)*	
Source	Date Processed	Date Analyzed at ORNL	PG-TSC Analysis	ICP Analysis
BNL, in 0.1 N HCl	5-30-89	7-26-89	7.7	6.0
LANL, in 2 N HCl				
Sample I	6-13-89	6-20-89	4.8	4.5
Sample II	7-13-89	8-18-89	8.8	4.4
Sample III	8-3-89	8-31-89	6.1	6.9
Sample IV	8-29-89	9-25-89	11.7	9.3

*The specific activity values are decay corrected to the end of bombardment dates.

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Acknowledgements

Research at ORNL supported by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. Research by T. W. Lee was supported by the INER of Taiwan and was an activity between ORNL and INER under the American Institute in Taiwan/Coordination Council for North American Affairs Agreement for Civil Nuclear Cooperation.

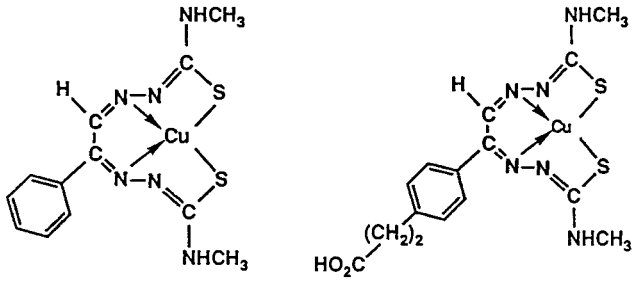

Figure 1. Chemical structures of Cu(II) complexes of the *bis*-(N^4-methyl)thiosemicarbazone derivatives of (A) phenylglyoxal (PG-TSC) and (B) p-carboxyethylphenylglyoxal.

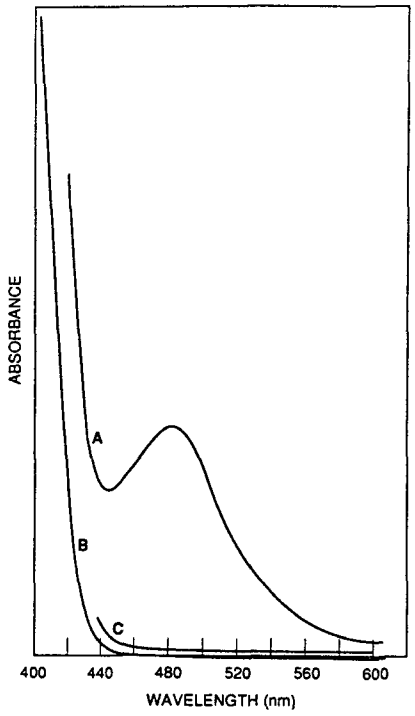

Figure 2. Ultraviolet spectra of (A) the Cu(II) PG-TSC complex, (B) the Zn(II) PG-TSC complex and (C) the PG-TSC ligand in the ethanol-HCl-acetate buffer assay mixture.

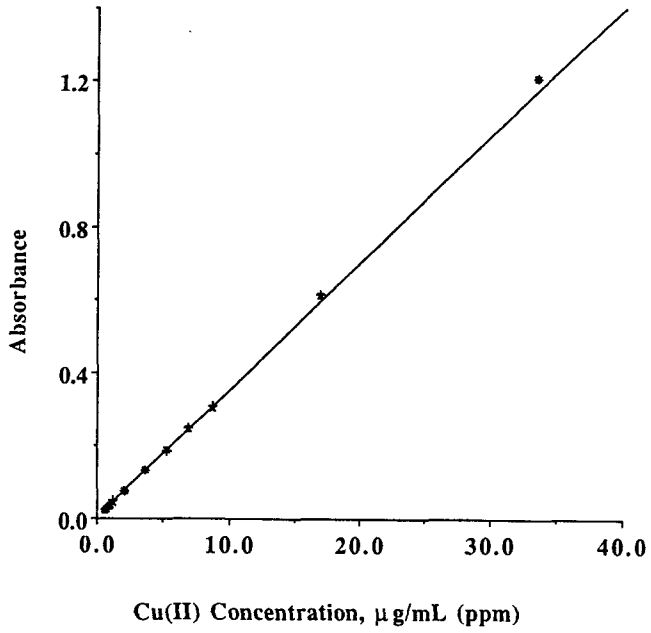

Figure 3. Calibration curve of the 480 nm absorbance of the Cu(II) PG-TSC complex in ethanol-HCl-acetate buffer solution.

MYOCARDIAL RETENTION AND LIGAND STRUCTURE OF CATIONIC TECHNETIUM DIOXIME COMPLEXES

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We have synthesized and evaluated new cationic $^{99}Tc/^{99m}Tc$ dioxime complexes with various vicinal dioxime ligands for assessing systematically the effects of carbon chain length, position isomerism of the dioxime groups, and the introduction of a terminal methoxy group on the myocardial uptake. The complexes were obtained by the reduction of pertechnetate with borohydride in presence of the ligands followed by HPLC separation. Anion chromatography, paper electrophoresis and FAB mass spectrometry revealed the cationic character. The mass peaks and the elemental analyses are consistent with the structure of six coordinate complexes of Tc(V).

The organ distribution of the dioxime complexes in mice as a function of the ligand carbon chain length, using 2,3-pentane-, 2,3-hexane, and 2,3-octane dione dioxime for complex formation, showed increasing uptake in the myocardium from 3.8 to 5.5 % dose/g at 1 min p.i. with increasing lipophilicity. The myocardial washout appeared to be less pronounced for the octane dioxime complex and the blood clearance was relatively fast resulting in a heart/blood ratio of 1.8 at 100 min p.i.

Evaluating the biodistribution of isomeric complexes formed with asymmetric or symmetric dioxime ligands, as in the case of 2,3-hexane- and 3,4-hexane dione dioxime, revealed with 5.0 vs. 2.8 % dose/g at 1 min. p.i. a remarkably higher myocardial uptake of the complex containing asymmetric ligands.

To improve the myocardial retention of the dioxime complexes we introduced a terminal methoxy group into 2,3-pentane- and 2,3-hexane dione dioxime. The typical change observed was a considerable increase of the activity in the liver and simultaneously in the kidneys, whereas the uptake in the myocardium remained almost unaffected.

Taking into account the heart/blood, heart/liver and heart/lung distribution ratios the dioxime complexes of 2,3-hexane- and 6-methoxy-2,3-hexane dione yielded the best results in this study. However, carbon chain length, position isomerism, and a terminal methoxy group cause only a limited effect on the myocardial retention of the cationic Tc dioxime complexes.

PREPARATION AND BIOLOGICAL EVALUATION OF COMPLEXES OF TECHNETIUM-99m WITH COMPOUNDS RELATED TO BOTH MAG3 AND HIPPURIC ACID.

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Radioiodinated o-iodohippuric acid (OIH) is the golden standard for radioisotopic renal function studies. During the past ten years a large number of Tc-99m labelled compounds have been evaluated as a potential substitute for OIH. One of them, Tc-99m MAG3, has become at this moment the renal function agent of choice for daily practice in many Nuclear Medicine departments.

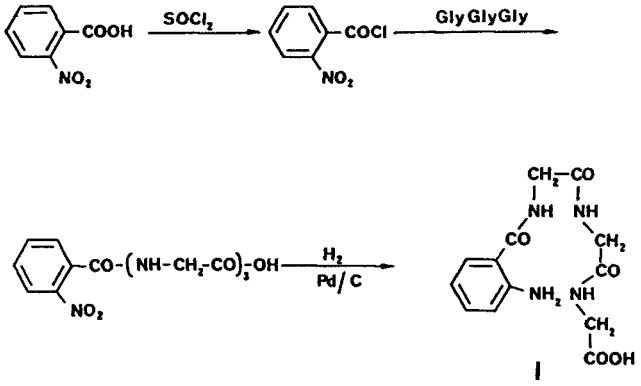
We have now synthesized three ligands for complexation with technetium, which can be considered as derivatives of both mercaptoacetyltriglycine and hippuric acid : 2-aminobenzoyltriglycine (I), 2-mercaptobenzoyltriglycine (II) and mercaptoacetylglycylglycyl-p-aminohippuric acid (III). The synthetic pathway for the preparation of the 3 ligands is presented in scheme 1 to scheme 3.

2-Aminobenzoyltriglycine was obtained by catalytic reduction of the 2-nitro precursor which was prepared by reaction of 2-nitrobenzoylchloride with triglycine.
The derivative of thiosalicylic acid (II) was synthesized with a S-benzyl protective group. Thiosalicylic acid ethyl ester was reacted with benzylchloride, hydrolyzed to the free acid, converted to the acid chloride and finally coupled to triglycine.
The derivative of MAG3 in which the terminal glycine moiety is replaced by 4-aminohippuric acid (PAHA) was also synthesized with a S-benzyl protective group. S-benzylmercaptoacetyldiglycine was converted to the reactive N-hydroxysuccinimide ester and then coupled to PAHA.

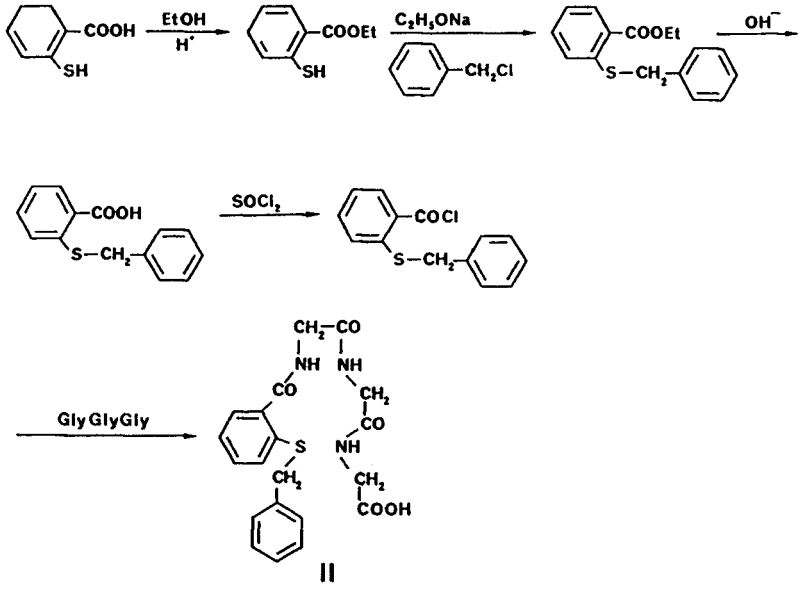
The new ligands were labelled with technetium-99m by exchange labelling at neutral pH in the presence of sodium tartrate. The reaction mixtures were analyzed by RP-HPLC on Hypersil ODS eluted with gradient mixtures of a 0.0125 M phosphate buffer pH 5.85 and ethanol. For each product one main radiochemical species was observed with a clearly longer retention time than that of Tc-99m MAG3 in the same HPLC-system. 2-Aminobenzoyltriglycine was labelled also directly by mixing 1 mg ligand, 1 ml NaOH 0.1N, 100 µg $SnCl_2.2H_2O$ and 2 ml generator eluate at room temperature. Radiochemical purity of the reaction product of this direct labelling method was clearly superior to that of the same product obtained by exchange labelling.

Biodistribution studies in mice (table 1) showed that Tc-99m I and Tc-99m II are extracted rapidly and efficiently from the blood by both the kidneys and the liver. They are finally transported to the intestines and urine in roughly equal amounts. Compared to Tc-99m MAG3 they accumulate to a substantially higher degree in the hepatobiliary system, which can be attributed to their higher lipophilicity, probably due to the presence of an aromatic nucleus.
Tc-99m III is extracted very slowly from the blood by the liver and to a lower degree also by the kidneys. This Tc-99m complex contains a carbonylglycine moiety, which is the side-chain of OIH, but not the oxotechnetium-glycine sequence (TcO-G) that is present in Tc-99m I, Tc-99m II and also in Tc-99m MAG3 and Tc-99m CO_2DADS. The biological behaviour of Tc-99m III can be an indication that the TcO-G moiety is required for efficient interaction with the receptor proteins in the kidneys and/or liver, that are responsible for the extraction from the plasma.

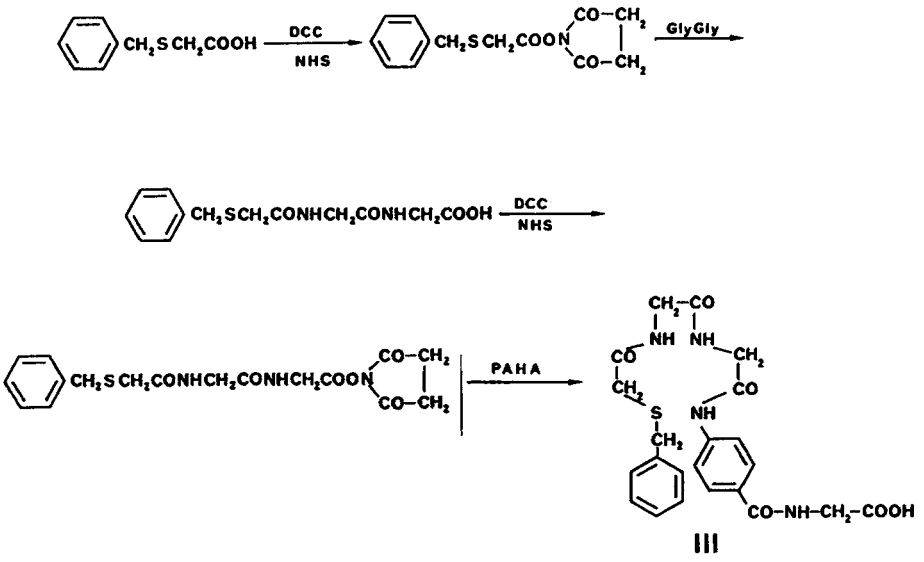
It is concluded that the incorporation of an aromatic nucleus in Tc-99m MAG3 enhances too much lipophilicity and therefore such Tc-99m complexes are not suitable as renal function agents.



Scheme 1. Synthesis of 2-aminobenzoyltriglycine (I)



Scheme 2. Synthesis of S-benzyl-2-mercaptobenzoyltriglycine (II)



Scheme 3. Synthesis of S-benzylmercaptoacetylglycylglycyl-p-aminohippuric acid. (III)

Table 1. Biodistribution in mice (n=3) of the new Tc-99m complexes

Organ uptake as % of injected dose

	Tc-99m I	Tc-99m II		Tc-99m III
	10'	10'	30'	10'
KIDNEYS	1.7	3.7	0.4	7.3
URINE	38.9	24.2	39.4	10.3
LIVER	24.8	31.5	9.0	10.2
INTESTINES	14.7	23.6	42.5	15.9
BLOOD	0.9	0.9	0.3	38.3

IS SYN OR ANTI ORIENTATION OF THE OXOTECHNETIUM AND CARBOXYL GROUP IN Tc-99m RENAL FUNCTION AGENTS AFFECTING THE RENAL EXCRETION RATE?

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Tc-99m L,L-ethylenedicysteine (Tc-99m L,L-EC,I) and Tc-99m D,D-EC (II) are cleared efficiently by active renal tubular transport in mice, baboons and humans. It can be assumed that I and II possess the structure proposed in fig. 1, as I is also formed by enzymatic or alkaline hydrolysis of the two ester functions of the brain perfusion agent Tc-99m ECD, the structure of which is well characterized (1). Therefore, I and II share with other actively secreted renal function agents such as Tc-99m MAG3 and Tc-99m CO_2DADS the oxotechnetium-glycine (TcO-G) moiety in their structure (fig. 1). This TcO-G sequence resembles structurally the carbonylglycine side-chain of hippuran and could be the site of interaction with the tubular transport receptor according to Despopoulos' theory (2).

The isomer of Tc-99m CO_2DADS with a syn orientation of the TcO and carboxyl group is cleared more efficiently than the anti isomer by active renal tubular transport (3). This agrees well with the assumption that the TcO and COO^- groups are both involved in the interaction with the tubular receptor protein. In Tc-99m MAG3 the terminal carboxylate can freely rotate and thus it can be oriented in the same direction as the TcO-group. In I and II one of the carboxyl groups is oriented syn with respect to the TcO group. On basis of these data one can suppose that a relation exists between the syn or anti orientation of the TcO and carboxylate group on the one side and the efficiency of the tubular excretion rate of these Tc-99m complexes on the other side.

To further elucidate the value of this hypothesis we have studied the isomers of Tc-99m meso-EC, in which both carboxyl groups are oriented either syn or anti with respect to the TcO-core. Theoretically the meso-isomers are formed together with I and II upon labelling of racemic EC with Tc-99m (fig. 2).

Labelling of racemic EC with Tc-99m was performed by mixing 1 mg ligand, 1 ml phosphate buffer, 100 µg $SnCl_2.2H_2O$ and 2 ml generator eluate. Reaction mixtures were analyzed and purified by RP-HPLC. The chromatograms show the presence of 3 peaks (A,B,C - fig. 3) in relative amounts which vary in function of the pH during labelling. Retention time (Rt) of peak B is identical as Rt of I and II. This lets suppose that peak A and C are the syn and anti isomers of meso Tc-99m EC, but the exact configuration has not yet been determined.

Biodistribution of HPLC-isolated peaks A, B and C was studied in mice at 10 min and 30 min p.i. with OIH as internal biological standard. No difference could be observed between A, B or C with respect to renal excretion, uptake in liver and intestines or retention in blood. A, B and C are excreted rapidly in the urine with only low retention in kidneys, liver or intestines (table 1).

The biological characteristics of the respective isomers of Tc-99m EC in mice do not support the hypothesis that a syn orientation of the TcO and COOH group is a general requirement for all discussed Tc-99m complexes for efficient renal excretion by active tubular transport.

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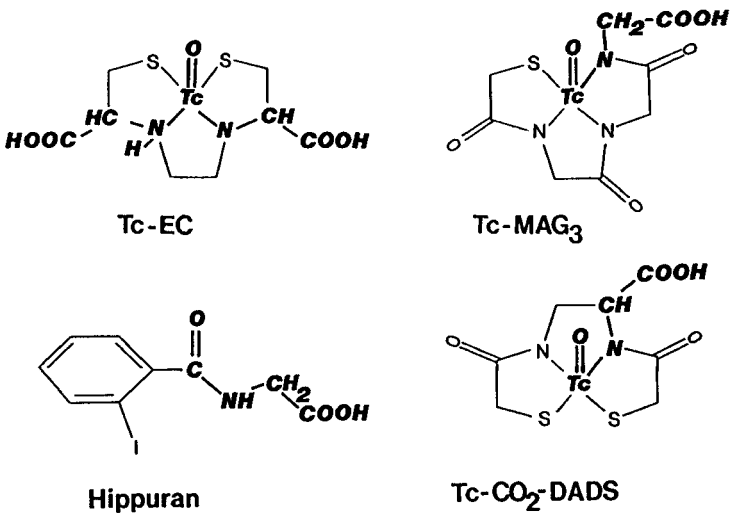

Figure 1. Similarities in the structure of Tc-ECD, Tc-MAG3, Tc-CO2DADS and hippuran.

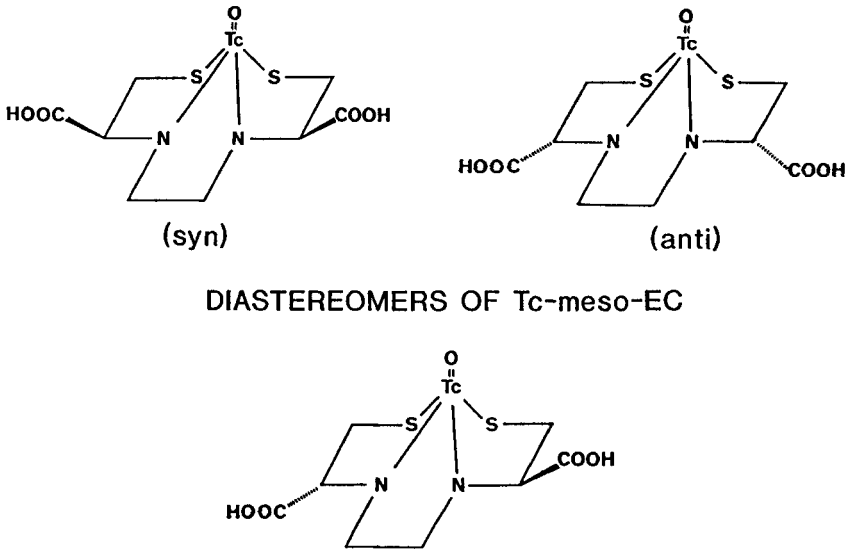

Figure 2. Isomers of Tc-99m ethylenedicysteine formed during labelling of racemic EC.

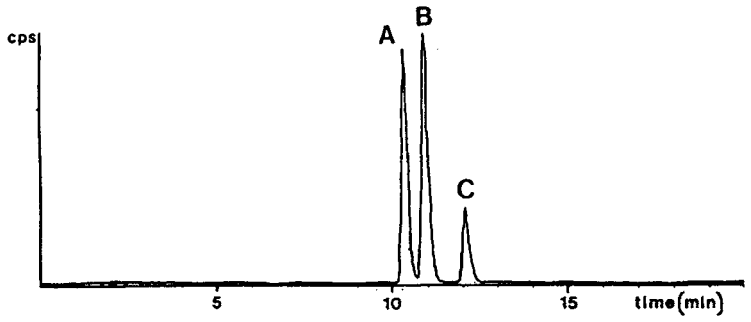

Figure 3. HPLC-chromatogram after labelling racemic EC with Tc-99m at pH 12. Stationary phase: Hypersil ODS (7µm), 250 mm x 4.4 mm. Mobile phase: gradient-mixtures of 0.0125M phosphate buffer pH 2.5 (A) and 30% ethanol in A (B). 0'-10' = 0-30%B
10'-20'= 30%B

Table 1. Biodistribution in mice (n=3) of peak A,B and C obtained upon labelling of racemic ethylenedicysteine with Tc-99m

Organ uptake as % of injected dose at 10 min p.i.

	A	B	C	OIH
Kidneys	4.7	4.3	4.4	3.9
Urine	70.2	67.8	66.3	67.5
Liver	3.5	4.1	2.0	2.6
Intestines	1.4	1.8	1.7	2.4
Blood	3.0	4.4	4.4	5.1

DIRECT SIMULTANEOUS PRODUCTION OF [^{15}O]WATER AND [^{13}N]AMMONIA OR [^{18}F]FLUORIDE ION BY 26 MeV PROTON BOMBARDMENT OF A DOUBLE CHAMBER WATER TARGET

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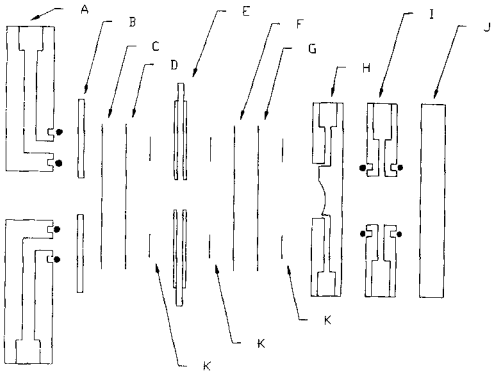
A simple pressurized double liquid chamber target was developed to provide the option for simultaneous production of [^{15}O]H_2O and either ^{13}N or ^{18}F using a single proton beam. Irradiation of natural water in the front chamber produced [^{15}O]H_2O by the ^{16}O(p,pn)^{15}O reaction directly. Large doses of sterile [^{15}O]H_2O (0.5-1.0 Ci, >99.95% radionuclidic purity, free of activated metal species) were routinely prepared in <1 min time from end of 20 μA bombardments using a mixed bed of 3:1:1 AG1: AG50: Chelex 100 ion exchange resins (reuseable) for in-line purification of the water as it left the target. Proton energies were ~17-20 MeV on exit from the front chamber.

The rear silver liquid target was identical to one previously reported for production of [^{18}F]fluoride ion (1). It was threefold thick to 17 MeV protons in water and it efficiently produced either ^{13}N by the ^{16}O(p,α)^{13}N reaction or [^{18}F]fluoride ion by the ^{18}O(p,n)^{18}F reaction (1, 2). Both front and rear chambers were pressurized with ≥ 6 atm of gas to suppress boiling at high beam currents. The rear chamber pressure was > the front chamber during irradiation to insure that the septal foils bowed outward to maintain proper rear target thickness. Using hydrogen as the overpressure gas on the rear chamber and an in-line anion exchange (AG1) column radiochemical cleanup (2), high yields of sterile, aqueous [^{13}N]NH_3 (40-200 mCi; 20μA) were produced directly from the rear chamber at the same time as [^{15}O]H_2O was produced from the front chamber.

The combination of this target system and a cyclotron capable of generating 26-30 MeV protons provides great flexibility, simplicity and economy for rapid, high volume production of [^{15}O]H_2O, [^{13}N]NH_3, and [^{18}F]FDG, the three best validated and most widely used radiopharmaceuticals in clinical PET, without the need for particle changes or dedicated oxygen-15 producing machines.



Exploded side view of double chamber target. **A**. beamline mounting flange w/ water channels, O-rings and air jets (not shown) for foil cooling. **B**. Front target foil sealing plate. **C**. Havar foil 0.025 mm. **D**. Al foil 0.01 mm. **E**. front target body w/ fill and vent tubes shown. **F**. Havar 0.025 mm. **G**. Ag 0.025 mm. **H**. Silver rear target body w/fill and vent ports. **I**. disk for circulating cooled water w/ inlet and outlets. **J**. back plate. **K**. Ag crush seals (0.25 mm).

This work was supported by DOE DE FG02-87ER60561 and NIH NS15655

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Development of Solid Targetry for the Production of Positron-Emitting Radionuclides with a Tandem Cascade Accelerator.

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The development of a low energy particle accelerator, such as the Tandem Cascade Accelerator (TCA),[1] for the production of positron-emitting radionuclides for PET studies imposes several constraints on the target used to produce the isotopes. At low energies, commonly used target foils (aluminum, silver, Havar™) would severely reduce the particle energy impinging on the target material, resulting in a significant reduction in the radioisotope yield. This problem can be circumvented by the use of foils made from elements of low atomic number (e.g. diamond, beryllium) and/or the use of solid targets which would eliminate the need for target windows. It has been shown[2] that clinically significant yields of the commonly used PET isotopes (^{11}C, ^{13}N, ^{15}O, ^{18}F) can be produced from solid targets: ^{11}C from a boron-containing target, ^{13}N from a graphite or carbide target, ^{18}F from an enriched oxide target, and ^{15}O from an inorganic nitride. At low bombarding energies (3.7 MeV) and high currents (0.2-1.0 mA), 1-2 Curies of these radioisotopes can be produced.

Our investigations into the use of solid targets to produce PET isotopes has begun with the study of the potential of various inorganic nitrides as suitable solid targets for the production of oxygen-15-labeled radiopharmaceuticals. Using the Allis-Chalmers cyclotron at Washington University, the following nitrides were bombarded with 6.2 MeV deuterons at currents ranging from 1 to 30 microamps and irradiation times from a few seconds to 20 minutes: aluminum, boron, lithium, calcium, and magnesium. In all of the nitrides studied, the half-life of the radionuclide produced in the solid was found to agree with that of oxygen-15 (122 sec). Furthermore, comparative low current (1-2 μA) irradiations on these materials have shown that the decay-corrected yield of oxygen-15 agrees with the relative nitrogen content in each of the nitrides. Higher current irradiations, however, show a deviation from this observation in that lithium and calcium nitride produce significantly less oxygen-15 than expected, a result most likely due to insufficient heat transfer, resulting in a release of radioactivity from the solid. An interesting observation was found with aluminum nitride: similar yields of oxygen-15 were produced from either a commercial hot-sintered pellet or a pellet pressed from powder. Comparative bombardments on aluminum nitride in air and *in vacuo* indicate that the oxygen-15 species produced is bound to the solid and not released during bombardment (Figure 1).

The nitrides studied are known to react with aqueous media to produce gaseous ammonia and the metal hydroxide, however, only the nitrides of lithium and calcium demonstrated the ability to completely dissolve in strong acid or base, a necessary criteria in order to extract the oxygen-15 species from the solid target. Although it has been reported that aluminum nitride will dissolve in hot concentrated potassium hydroxide or phosphoric acid,[3] this material, either as a powder or hot sintered pellet, did not dissolve adequately in these media to release the oxygen-15 species from the solid matrix. The release of carbon-14 activity from neutron-irradiated aluminum nitride following rapid heating in a

stream of oxygen has been reported[4], and this approach is being investigated. It has been determined via radio-gas chromatography that the radioactive species formed upon the dissolution of deuteron-irradiated lithium and calcium nitride is oxygen-15-labeled water.

The use of solid targets for the production of PET radioisotopes with low energy particles appears promising in view of the above results. Furthermore, the facile release of the radionuclide from some of these solid targets into a useful radiopharmaceutical demonstrates the potential utility that these targets can have in the routine production of large quantities of PET radiopharmaceuticals from a low energy particle accelerator.

This work was supported by NIH grants 2R44CA43672 and HL 13851.

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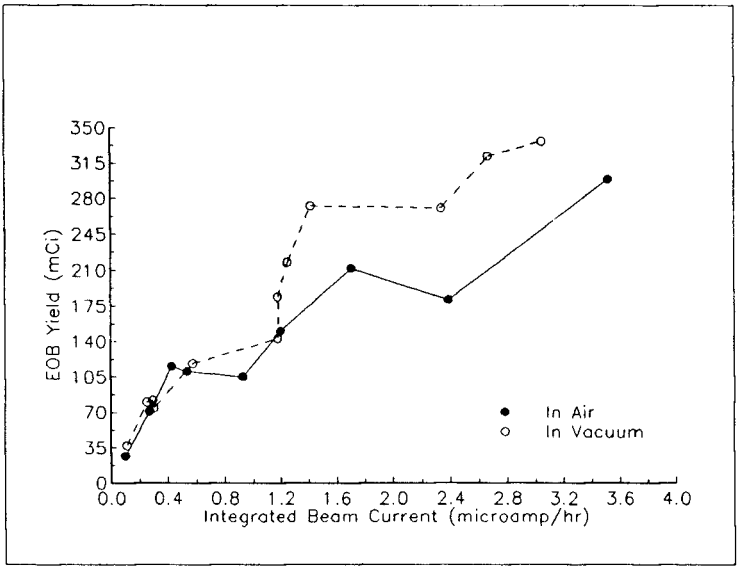

Figure 1. Decay-corrected yield of oxygen-15 from an AlN pellet bombarded with 6.2 MeV deuterons in air and *in vacuo*.

ELECTROPHILIC F-18 FROM AN 11 MeV PROTON CYCLOTRON FOR THE RADIOLABELING OF PRESYNAPTIC DOPAMINERGIC TRACERS.

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The increasing study of presynaptic dopaminergic physiology by positron emission tomography (PET) concomitant with the proliferation of small proton cyclotrons has emphasized a void in accelerator targetry for the reliable production of electrophilic ^{18}F produced from O-18. As the most common syntheses of the presynaptic dopaminergic tracers require $[^{18}F]F_2$, those institutions with proton cyclotrons have been, to date, incapable of producing 6-$[^{18}F]$Fluoro-L-DOPA (6-FD) or fluorinated DOPA analogs for PET studies. While targetry developments for the production of $[^{18}F]F_2$ from cyclotrons with deuteron capabilities are well advanced (1, 2, 3, 4), targetry technique for the production of $[^{18}F]F_2$ from protons is still in its infancy (5, 6).

At the University of Wisconsin, two targets with conically shaped target chambers (one nickel-volume=22 ml, the other gold-plated aluminum volume=14 ml) designed for use on a CTI 114 RDS 11 MeV proton cyclotron have been constructed and studied for their ability to produce $[^{18}F]F_2$. Target performance was tested while operating under two different irradiation protocols: a one-step approach bombarding a gaseous mixture of $^{18}O_2+F_2$, and a two-step approach consisting of an ^{18}F production irradiation of $^{18}O_2$, its quantitative cryorecovery for subsequent reuse, followed by an $[^{18}F]F_2$ recovery irradiation of $Kr+F_2$. From these targets, activities of 200 mCi of usable $[^{18}F]F_2$ were recovered at specific activities of 3 Ci/mmole with 75 minute, 10μA proton irradiations.

The economic two-step protocol as performed on the nickel target proved the most reliable source of $[^{18}F]F_2$. The target chamber was passivated with pure F_2 at 2 atmospheres and 200°C for 24 hours to create a 0.1 μM nickel fluoride passivation layer which proved critical for efficient trapping of ^{18}F on the target walls prior to the recovery irradiation. With 5 minute, 5 μA $^{18}O(p,n)^{18}F$ production irradiations, the nickel target yielded 65% of its activity, virtually all in the form of $[^{18}F]F_2$, when followed by a 5 minute-5 μA recovery bombardment of $Kr+F_2$. More rigorous irradiation conditions (>300 μA-min) resulted in lower fractional yields (30-50%). Repeated recovery irradiations of $Kr+F_2$ removed a constant fraction of available ^{18}F activity from the walls in the form of $[^{18}F]F_2$ until the walls were depleted, demonstrating that virtually no ^{18}F is irreversibly bound to the nickel fluoride walls and that simple exchange kinetics are involved. Varying the F_2 carrier concentration from 0 - 80 μmoles resulted in a gradual increase in $[^{18}F]F_2$ yield. Yields of 15% with carrier F_2 levels of 10 μmoles achieved maximal specific activity levels. Beam alignment proved critical, as even minimal grazing of the target chamber walls drastically reduced yields. Theoretical considerations implicated light production in the target for catalyzing the critical $^{18}F/^{19}F$ exchange reactions. The one-step method for $[^{18}F]F_2$ production with both the nickel and gold-plated targets achieved ≈90% yields for 5 minute-5 μA irradiations. These outstanding yields, however, decreased to levels corresponding to the two-step results at integrated currents over 300μA-min. Proton beam grazing the walls of the small 14 ml gold-plated target chamber may have been responsible for the poor yields of this target as compared to results of a similar gold-plated target (6).

Synthesis of 6-$[^{18}F]$fluoro-L-DOPA (6-FD) by the fluoro-demercuration method of Luxen (7) challenged the labeling properties of electrophilic $[^{18}F]F_2$ gas mixtures eluted from the targets under the two irradiation protocols. The $Kr+[^{18}F]F_2$ eluted from the two-step method achieved the expected 12% radiochemical yield from $[^{18}F]F_2$ while experience with ^{18}F activity eluted with oxygen from the single step protocol suffered from lower yields, particularly during the solvent evaporation step. Several novel fluorinated presynaptic dopaminergic tracers, $[^{18}F]$fluoro-m-tyrosine and $[^{18}F]$fluoromethylene-m-tyrosine have been routinely synthesized through direct fluorination reactions using electrophilic fluorine from both targets.

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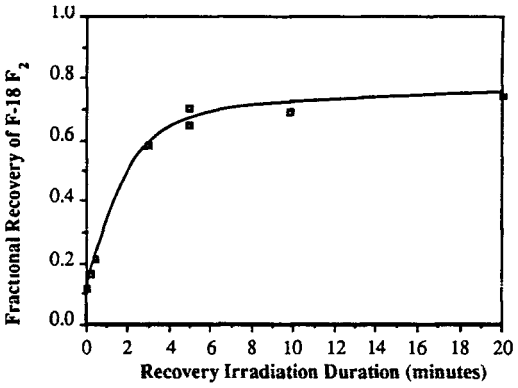

Figure 1--Figure 5.6--Fractional recovery of $^{18}F_2$ as a function of irradiation duration for a constant 5 μA recovery irradiation of Kr+F_2. The ^{18}F production conditions were a constant 5 μA for 5 minutes for all data points.

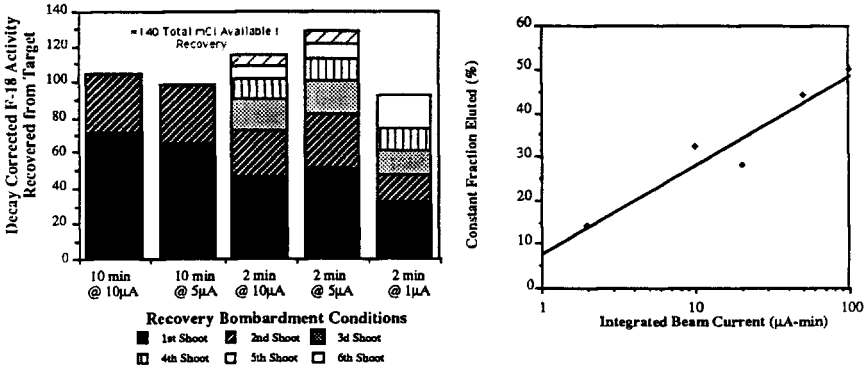

Figure 2--Activity eluted from the nickel target for successive recovery irradiations using the two-step $^{18}F_2$ production scheme. This data strongly suggests that nearly all the ^{18}F produced in the target is available for exchange-induced recovery in the form of $^{18}F_2$.

Beam Current	Duration	Soda Lime	Head Gas	Acetate Column	Acetyl Hypofluorite
5 μA 5 min	18.8 mCi	350 μCi	---	---	
5 μA	5 min	13.1 mCi	80 μCi	---	---
10 μA	75 min	17.4 mCi	14 mCi	79 mCi	89 mCi
10 μA	75 min	2 mCi	1.8 mCi	87 mCi	82 mCi
5 μA 10 min	18.5 mCi	1.5 mCi	---	---	
10 μA	5 min	20.3 mCi	160 μCi	---	---
10 μA	42 min	10 mCi	4.7 mCi	34 mCi	34 mCi
10 μA	45 min	1 mCi	5 mCi	36 mCi	28 mCi

Figure 3--Single irradiation protocol results with the nickel target (**Bold Face = Double irradiation protocol** provided for comparison sake) under similar irradiation conditions.

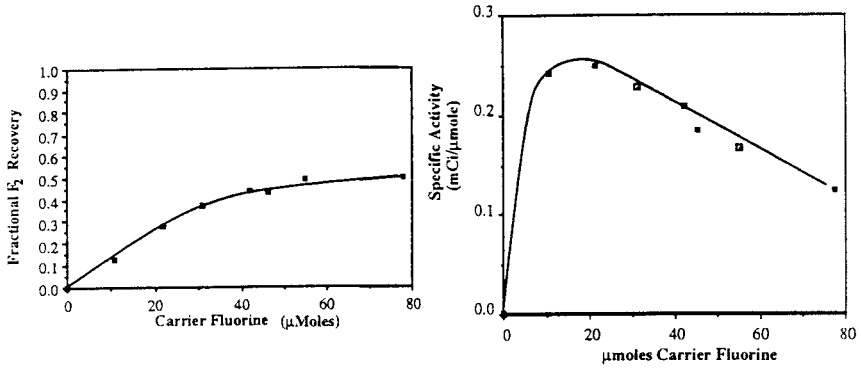

Figure 4--(Left) Fractional $^{18}F_2$ recovery as a function of carrier fluorine for 5 minute, 5 μA recovery bombardments. Target performance was sub-optimal during these experiments. (Right) Specific activity of the recovered $^{18}F_2$ product as a function of carrier fluorine during the recovery irradiation. The gain in yield achieved by increasing carrier concentration is only achieved through the sacrifice of specific activity. These data were measured for 5 minute, 5 μA production and recovery irradiations. Specific activity, of course, rises significantly with the elution of higher activities of $^{18}F_2$ which results from longer and higher current production irradiations. Specific activities of $^{18}F_2$ near 3 Ci/mmole have been achieved using the two-step method for $^{18}F_2$ production.Figure 4-The 6-FD synthesis using $[^{18}F]F_2$ from the nickel and gold plated targets.

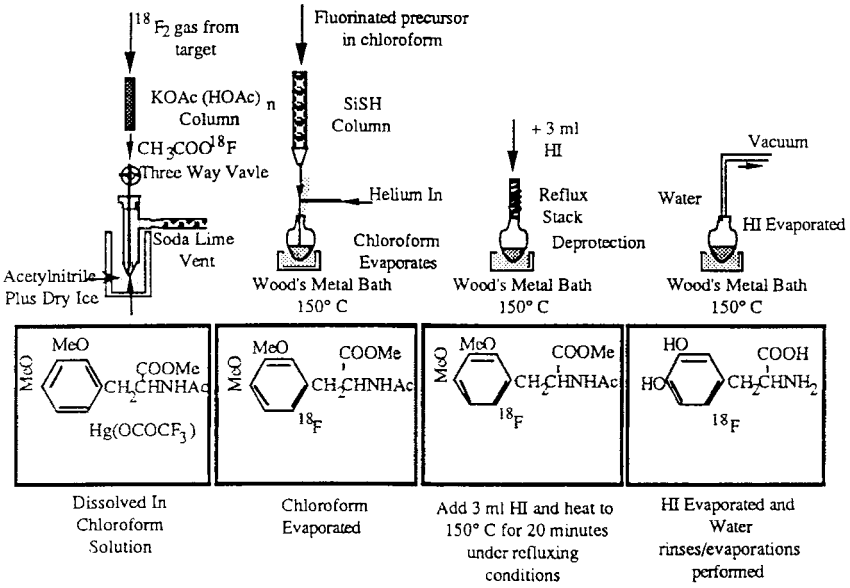

Figure 5--Schematic of the fluoro-demercuration chemistry used to synthesize 6-FD

PERFORMANCE OF A HIGH-LEVEL COPPER-62 GENERATOR AND REMOTE SYSTEM FOR [Cu-62]-Cu(PTSM) SYNTHESIS

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The development of radiopharmaceuticals labeled with generator-produced positron-emitting copper-62 (9.7 minute half-life) could facilitate more widespread use of positron emission tomography (PET) by reducing the need for nuclides that require in-house cyclotron production. We have previously reported (1-6) the use of longer-lived copper isotopes (Cu-67 and Cu-64) to screen copper compounds as potential tracers for cerebral and myocardial blood flow and have identified the copper(II) complex of pyruvaldehyde *bis*(N^4-methylthiosemicarbazone), Cu(PTSM), as a promising agent for these applications (Figure 1). We have now constructed Zn-62/Cu-62 generators at high activity levels (>300 mCi) and report here a remote control system for rapidly synthesizing [Cu-62]-Cu(PTSM) from the acidic generator eluent.

Zinc-62 (9.26 hour half-life) can be readily obtained by the Cu-63 (p, 2n) Zn-62 nuclear reaction using a medium energy cyclotron. We have employed a generator system based on that described by Robinson (7). The Zn-62 was loaded onto a prepurified Dowex 1 X 8 (200-400 mesh) anion exchange column (4 cm X 0.7 cm diameter or 2 cm X 1.0 cm diameter) from which the Cu-62 daughter can be selectively and efficiently eluted with 2N HCl. Over an 18-20 hour time frame after loading, generator performance was evaluated concurrently with its use to provide Cu-62 for Cu(PTSM) PET studies. Following elution of the system void volume, > 80% of available Cu-62 was obtained in 2-3 mL of eluate. Zinc-62 breakthrough was typically < 0.001% of eluted copper-62 activity.

The lead-shielded remote system for [Cu-62]-Cu(PTSM) synthesis consists of a set of five pneumatic 3-way valves that can be controlled remotely to effect transfer of reagents to and from the synthesis vessel and purification apparatus (Figure 2). The generator is eluted with positive pressure using a syringe charged with 2N HCl and attached to the column inlet. The column void volume is directed to a waste container and the eluent stream then switched to the reaction vessel for collection of the Cu-62 activity (2 or 3 mL). The eluate is then buffered to pH 5 by addition of two equivalents of 3N NaOAc solution. Next 1.5 μg of the H_2(PTSM) ligand in *ca* 0.1 mL ethanol is added and the solution mixed for 2 minutes by remote activation of the vortex mixer. The reaction mixture is then passed through a C_{18}-SepPak cartridge by pressurizing the reaction vessel with air and opening the valve connecting this cartridge to the reaction vessel dip-tube. The lipophilic Cu(PTSM) complex is retained on the C_{18}-SepPak and, following a water wash, can be recovered in a volume of 0.2-0.3 mL by elution with ethanol. After dilution with saline to a 5% alcohol concentration the product is filtered through a sterile 0.2 μm fluoropolymer membrane filter prior to use in PET imaging studies. The product was obtained with > 98% radiochemical purity, as demonstrated

by thin layer chromatography on silica gel plates eluted with ethyl acetate (Figure 3). The remote system allows [Cu-62]-Cu(PTSM) synthesis in under 8 minutes with *ca* 40% radiochemical yield (based on Cu-62 activity available at end of elution, without decay correction).

Support for this work was provided by the National Cancer Institute (R01-CA46909), the National Heart, Lung, and Blood Institute (K04-HL01801), and the U.S. Department of Energy (DE-FG02-87ER60512).

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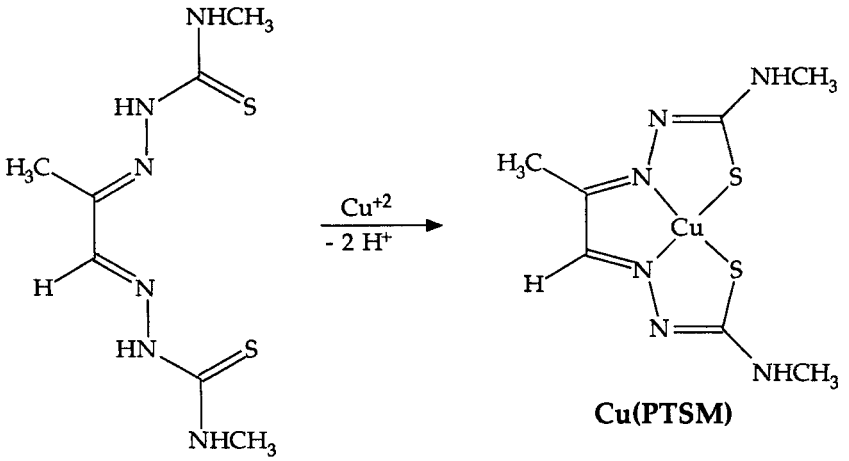
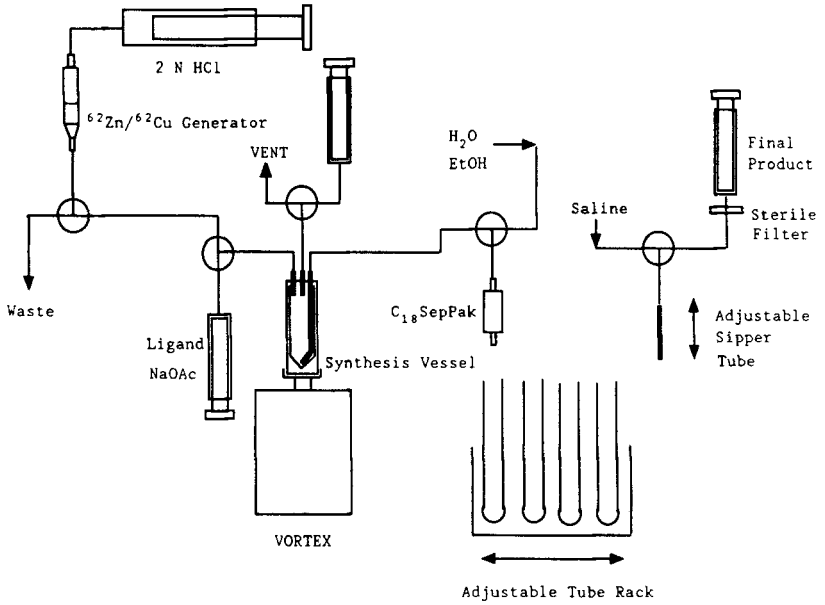
Figure 1. Cu(PTSM) synthesis and structure.



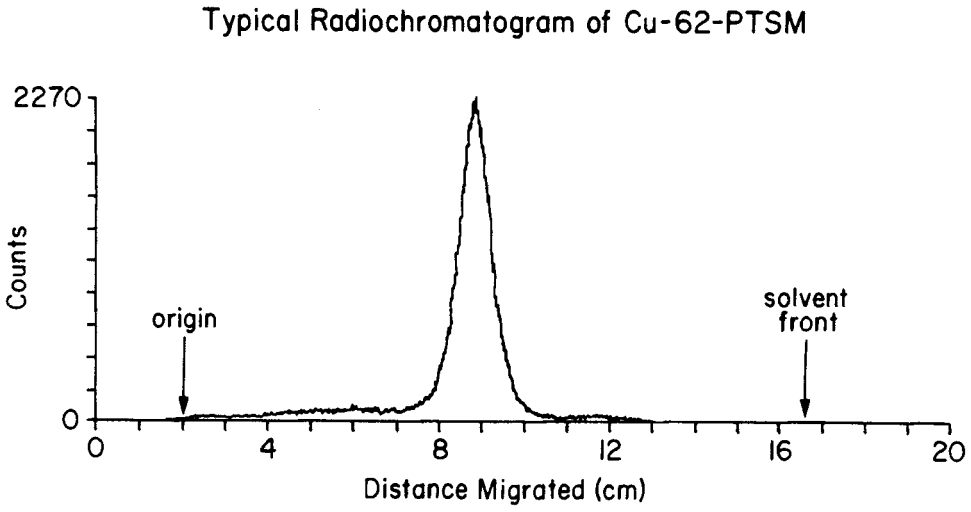
H_2(PTSM) Cu(PTSM)

Figure 2.



Remote System for the Production of ^{62}Cu-PTSM

Figure 3.



PRODUCTION OF ^{110}In FOR PET INVESTIGATION VIA Cd(^{3}He,xn)^{110}Sn-^{110}In REACTION WITH LOW ENERGY CYCLOTRON.

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The ^{110}In is a positron emitting (62%) radionuclide with a half-life of 69 m. With the increasing use of positron emission tomography in nuclear medicine, compounds labelled with ^{110}In could be potentially useful for imaging with PET scanners, for example for diagnosing the rejection of transplanted organs, when repeated investigation with short interval is necessary to see the effect of the used therapy.

The only practical way to produce ^{110}In without producing its 4.9 h half-life, non-positron emitter isomeric state leads through its parent nuclei, the ^{110}Sn ($T_{1/2}$ = 4.11 h) which decays only to the ground state of ^{110}In. Until now the ^{110}Sn has been produced by proton particle irradiation of natural In target (1). However, this method of production demands a high energy cyclotron (E_p= 80 MeV). With low and middle energy cyclotrons for practical production two other possibilities exist: the (p,xn), (p,pxn) reactions on Sn above 20 MeV and the (^{3}He,xn), (^{4}He,xn) reactions on Cd for lower energy accelerators. In this work we investigated the Cd(^{3}He,xn)^{110}Sn process which can be performed using our low energy cyclotron.

Excitation function was measured by stacked-foil technique for the reaction of Cd(^{3}He,xn)^{110}Sn on natural Cd in the energy range of 16.3 to 27 MeV using the MGC-20 cyclotron of ATOMKI, Debrecen. Commercially available high purity cadmium foils (Goodfellow, Cambridge, England) and samples, prepared via electrolytic deposition of Cd on Ni foils, were used as target materials. The excitation function measurement was carried out by activation method using gamma-ray spectroscopy. The experimental technique and the data evaluation were similar to (2). The cross section obtained are shown in Fig.1. The abundance of the target material and the Q-values of the contributing nuclear reactions are summarized in Table 1. In the investigated energy range the ^{110}Cd(^{3}He,3n)^{110}Sn reaction appears to be dominant. The calculated thick target yield of ^{110}Sn amounts to 1860 MBq/C (~180 μCi/μAh in the energy range of 16.3 to 27 MeV. The levels of the radioactive impurities of ^{108}Sn, ^{109}Sn, ^{111}Sn, ^{113}Sn and ^{113m}Sn at EOB depend on the irradiation time and the isotopic abundance of the target.

For production the irradiated Cd of 0.1 g/cm target was dissolved in 9 M HBr, evaporated to dryness and picked up in 5 ml 9 M HBr. After an optimal cooling period of ~3 h, the main part of the contaminating Sn isotopes decayed out expect ^{113}Sn ($T_{1/2}$ = 115 d). For separation of the directly produced and decay product In isotopes from Cd matrix and Cd and Sn radioisotopes cation exchange method was chosen (3). DOWEX 50 WX-2 (100-200 mesh) resin was used to select the In isotopes from Cd and from the main parts of the Sn isotopes. The solution was led through the column of 4 mm i.d. and 4 cm lenght with 1 ml/min volume speed. The column was washed with 10 ml of 9 M HBr to remove the Sn remained on the resin as much as

possible and without removing In isotopes. Because of the not too low distribution coefficient of Sn(IV) at this molarity we reduced it to Sn(II) where this coefficient is smaller, therefore no significant part of Sn remained on the column. 174 min later when the ^{110}In activity reached the maximal value in the filtrate, the solution was led again through another column. To remove the ^{110}In activity the resin was eluted with 3 M HBr and the filtrate was evaporated to dryness. The ^{110}In activity (A) at the end of the radiochemical process can be given by

$$A = bC$$

where C is the EOB activity of ^{110}Sn and b= 0.35 which includes the cooling times and the efficiency of the radiochemical separation. ^{110}In-oxine was prepared using the method of Thakur (4) for labelling cellular blood components.

This method for producing ^{110}In with low energy cyclotron seems to be simple and quick. The shape of the excitation function for ^{110}Sn and the isotopic abundance of the target used suggest that yield could be significantly increased using higher entrance energies and enriched target.

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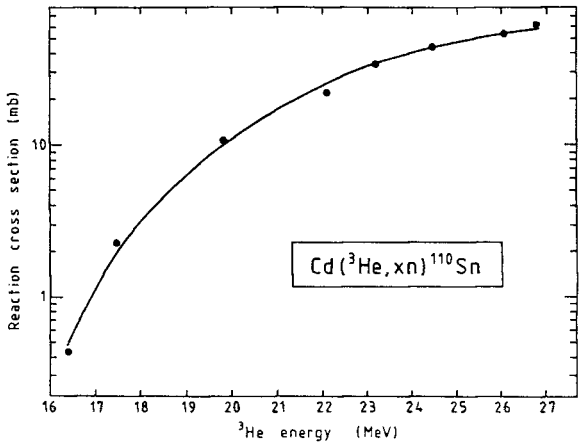

Fig.1. Excitation function of Cd(^{3}He,xn)^{110}Sn reaction measured on natural Cadmium.

Table 1. Nuclear processes contributing to the formation of ^{110}Sn.

Reaction	Q (MeV)	Isotopic Abundance of the Target (%)
^{108}Cd(^{3}He,n)^{110}Sn	+ 3.4	0.89
^{110}Cd(^{3}He,3n)^{110}Sn	-13.8	12.5
^{111}Cd(^{3}He,4n)^{110}Sn	-20.8	12.8

CHEMICAL PROCESSING FOR THE PRODUCTION OF CARRIER FREE SELENIUM-73 FROM GERMANIUM AND ARSENIC TARGETS AND SYNTHESIS OF L-[^{73}Se]SELENOMETHIONINE.

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The positron emitter ^{73}Se ($t_{1/2}$: 7.2 h, β^+ 65%) remains a potentially useful tracer for positron emission tomography (PET) studies. The limited use of ^{73}Se for PET applications results from various factors and especially from the poor availability of ^{73}Se in sufficient amounts in the no-carrier-added (nca) state at the end of the radiochemical processing. Intensive work has been reported by various authors on the production of ^{73}Se but the major part of their investigations was limited to cross section measurements (1-3) and to a smaller extent, to medium scale purification (1-4).

Establishing the radiochemical conditions for nca ^{73}Se production and its separation from both arsenic and germanium targets were the goals of the present work. A simple wet chemical procedure was developed, optimized and used for the preparation of nca ^{73}Se in high chemical and radiochemical purities. A recovery procedure for the target material was developed in order to allow the use of ^{70}Ge enriched material. The entire process was used on more than 20 production runs in order to prepare L-[^{73}Se]selenomethionine via a fast chemical synthesis (5) for human studies.

The ^{75}As(p,3n)^{73}Se reaction was investigated with the external proton beam from the CGR MeV 930 S (IRE Fleurus Belgium). Irradiations were performed at four different energies : 65, 60, 55 and 50 MeV. The arsenic target was prepared by compacting metallic arsenic powder (2150 mg) in a gold plated stainless steel backing resulting in an adhering solid pellet of 685 mg/cm^2. This target appeared to be suitable for irradiation with beam intensities greater than 10 µA for more than 1 h and also for subsequent chemical separation.

The ^{nat}Ge(^{4}He,xn)^{73}Se reaction was used with the external ^{4}He beam at the energy of 26 MeV available from the CGR MeV 520 machine at the Cyclotron Center of Liege. The germanium target consisted in a 105 mg/cm^2 thick layer of 400 mg powdered sodium metagermanate (Na_2GeO_3) placed in the recess of a gold plated copper backing and fritted under pressure. Sodium metagermanate was evaluated because of its rapid solubilisation in water after a long period of bombardement (up to 100 µA.h). Although sodium metagermanate presented a lower thermal conductivity than GeO_2, beam intensities of 10 µA have been used for several hours without problems.

The production rates of ^{73}Se and radionuclidic impurities obtained for proton and alpha bombardments are shown in Table 1. As previously reported (3). ^{75}As(p,3n) is the method of choice if a suitable proton energy is available; for a compact cyclotron, the ^{70}Ge(^{4}He,n) reaction can be used with the target recovery procedure described below.

The wet chemical processing consisted of dissolution of the target (water for Na_2GeO_3, KOH-H_2O_2 for arsenic), addition of conc. HCl (up to a final concentration of 20% HCl), extraction of $GeCl_4$ with CCl_4 (recovery of the target material in the case of the germanium target), reduction to elemental selenium by SO_2 at 85°C and extraction of elemental selenium with benzene. No-carrier-added elemental selenium was obtained after washing the organic phase with water, drying over $MgSO_4$ and evaporation of the solvent. Radiochemical yields were 75±5% (EOB corrected) in less than 90 min and the radiochemical and chemical purities were higher than 99.8 and 99.9% respectively.

Typical conditions for the production of 30 mCi (EOS) of L-[^{73}Se]selenomethionine with a specific activity of 5 Ci/mmol (EOS) were 55 MeV protons (10 µA) for 1 h leading to 180-200 mCi (EOB) of ^{73}Se, after chemical purification 150-160 mCi (EOB) of nca elemental ^{73}Se

were obtained. The chemical synthesis was realized as described elsewhere (5). The overall yield including separation of the isotope, synthesis and purification of the final compound was 40% (EOS) after a total processing time of 7 h (bombardment included).

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TABLE : 1

Production yields (EOB) of ^{72}Se, ^{73}Se and ^{75}Se for the $^{75}As(p, xn)$, $^{nat}Ge(^{4}He,xn)$ and $^{70}Ge(^{4}He,n)$ (1) nuclear reactions. The percentage of each radionuclidic impurity with respect to the ^{73}Se production rate is given in parenthesis.

		mCi/μAh		
Ep (MeV)	ΔE (MeV) in target	^{72}Se	^{73}Se	^{75}Se
65	58-50	0.15 (1.5%)	10	0.004 (0.04%)
60	52-43	0.10 (0.65%)	15.3	0.005 (0.03%)
55	45-35	0.02 (0.10%)	20	0.006 (0.03%)
50	39-28	0.001 (0.005%)	17.4	0.04 (0.23%)
Eα (MeV) (^{nat}Ge)				
26	25-10	0.0011 (0.55%)	0.2	0.0064 (3.2%)
(^{70}Ge)				
26	26-10		1	

PRODUCTION OF ^{73}Se VIA $^{70}Ge(\alpha,n)$-PROCESS AT A COMPACT CYCLOTRON.

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The radioisotope ^{73}Se ($T_{\frac{1}{2}}$ = 7.1 h; EC = 35%; β^+ = 65%; E_{β^+} = 1.32 MeV) is a potentially interesting sulphur analogue for application in positron emission tomography (PET). It can be produced via (p,3n) and (d,4n) reactions on ^{75}As [cf. 1,2] or ^3He- and α-particle induced reactions on Ge isotopes [cf. 3,4]. The method of choice, however, is the $^{75}As(p,3n)$-reaction [cf. 2,4] provided a cyclotron with high energies is available. At a medium-sized compact cyclotron the $^{70}Ge(\alpha,n)$-process might be worth considering. Due to the expected low yield the development of a high current target was considered mandatory.

An alloy of Cu and Ge was prepared with Ge content ranging between 28 and 70%. In high current irradiation tests using extracted beams the alloy with Ge content between 28 and 40% was found to be most suitable since it could easily withstand power densities of up to 1.5 kW cm^{-2}. For irradiations with internal beams, however, a Cu_3Ge intermetallic compound obtained via simultaneous electro-deposition of Ge and Cu (from an alkaline bath) on a Cu-backing proved to be ideal. The thickness of the layer, which is a critical factor, could be easily controlled. A target thickness of 20 mg Cu_3Ge/cm^2 is needed to cover the energy range E_α = 28 → 10 MeV in internal irradiations at an angle of 6.2° [cf. 5]. The compound contains 28% Ge and was found to withstand internal beam currents of about 80 μA.

A thermochromatographic technique was developed to separate radio-selenium from the irradiated internal target. The distillation yield at 900 °C (at a constant He flow rate of 4 l/h) was > 90% and radioselenium could be taken up from the quartz tube by H_2O_2. Using 97% enriched ^{70}Ge the experimental thick target yield of ^{73}Se in high current irradiations was found to be about 400 μCi/μAh, amounting to about 40% of the theoretical value. A 2 h irradiation at 60 μA could lead to 50 mCi ^{73}Se (at EOB). The radionuclidic impurities detected were ^{72}Se (0.5%), ^{75}Se (0.1%), ^{66}Ga (0.3%) and ^{65}Zn (0.5%).

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EXCITATION FUNCTIONS OF (p,2n) AND (p,pn) REACTIONS ON HIGHLY ENRICHED ^{124}Xe WITH SPECIAL REFERENCE TO THE PRODUCTION OF ^{123}I.

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For the production of medically important SPECT radioisotope ^{123}I ($T_{½}$ = 13.2 h) the $^{123}Xe \rightarrow {}^{123}I$ generator method is commonly used. Two reactions, viz. $^{127}I(p,5n)^{123}Xe$ and $^{127}I(d,6n)^{123}Xe$, have been applied for a long time. In recent years the $^{124}Xe(p,x)^{123}I$-process involving highly enriched ^{124}Xe has been gaining considerable significance since it can be used at a medium-sized cyclotron and leads to the highest purity of ^{123}I. The nuclear data information available for this process, however, was scarce. Differential yield data were reported [1-3] but are somewhat discrepant. We report here on the excitation functions of the important reaction channels $^{124}Xe(p,2n)^{123}Cs$ and $^{124}Xe(p,pn)^{123}Xe$.

Stainless steel cells (vol. 8 ml) having thin Al windows were filled with enriched ^{124}Xe to a pressure of 0.5 bar and Cu monitor foils placed in front and at the back of each cell. Due to the short half-life of ^{123}Cs ($T_{½}$ = 5.8 min) only one cell was irradiated at a time. Irradiations were done at the Jülich Isochronous Cyclotron (JULIC) and the Compact Cyclotron (CV 28). The primary incident proton energies were 45, 30, 24, 22 and 20 MeV. Measurements were performed using 20 and 99.8% enriched ^{124}Xe. In the case of 20% enrichment, the irradiated cell was evacuated and its inner wall rinsed with water containing $CsNO_3$. The solution was subjected to γ-ray spectroscopy. With 99.8% enriched gas, however, no chemical separation was prformed and counting of the cell was done directly.

The measured excitation functions covering an energy range of 17.5 to 44 MeV are shown in Fig. 1. Evidently the $^{124}Xe(p,2n)$-process is much stronger than the $^{124}Xe(p,pn)$-channel; beyond 36 MeV, however, the two processes have almost equal cross sections. The excitation function of the (p,2n) process is rather flat between 23 and 29 MeV and amounts to about 600 mb. The optimum energy range for the production of ^{123}I is E_p = 29 → 23 MeV and the calculated thick target yield of ^{123}I at 6.6 h after EOB amounts to about 11.5 mCi/μAh.

The differential yields of ^{123}I were determined experimentally earlier using ^{nat}Xe as target material [2]. We now measured the yields using highly enriched ^{124}Xe. Furthermore, the yields were calculated from the excitation functions determined in this work. All the three sets of yield data agree within an error of 15%. We therefore believe our yield values to be more accurate than the literature data.

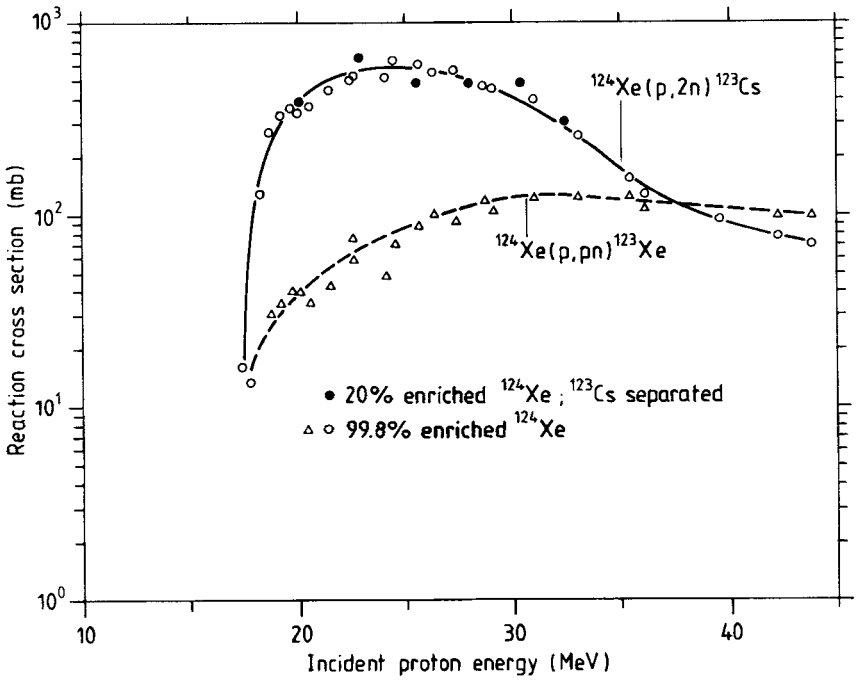

Fig. 1: Excitation functions of (p,2n) and (p,pn) reactions on ^{124}Xe

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Production of Iodine-123 from Xenon-124: Comparison of results from different laboratories.

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Iodine-123 is an important isotope in clinical nuclear medicine. The production of very pure I-123 has been achieved with the use of highly isotopically enriched Xe-124. This target material has a high initial investment, but can be reused with little or no denegration of the product isotopic purity. Three different laboratories have carried out cross-section measurements for the reaction and have obtained somewhat different results (1,2,3). In order to investigate the apparent discrepencies, we have carried out a set of measurements for the reaction using different target holders and isotopic purities of the Xenon-124. We have found that there is an effect in the recovery of the I-123 depending on the type of target holder used. A cell of quartz had the lowest retention of the iodine, with a target holder of aluminum only slightly less efficient. The material which retained the most I-123 was Havar. The exact chemical form of the iodine in the target is unclear.

The yield of this reaction at 6.6 hrs after end of irradiation for 99.9% enriched and for 40% enriched xenon-124, corrected to 100% enrichment for comparison, is given in Figure 1. The yields obtained in the other studies compared to our results obtained in two different types of targets are presented in Figure 2. This production method produces iodine of very high isotopic purity as is shown in Figure 3 which is the gamma spectrum of the iodine produced with 99+% istopically enriched xenon-124. This work supported by DOE.

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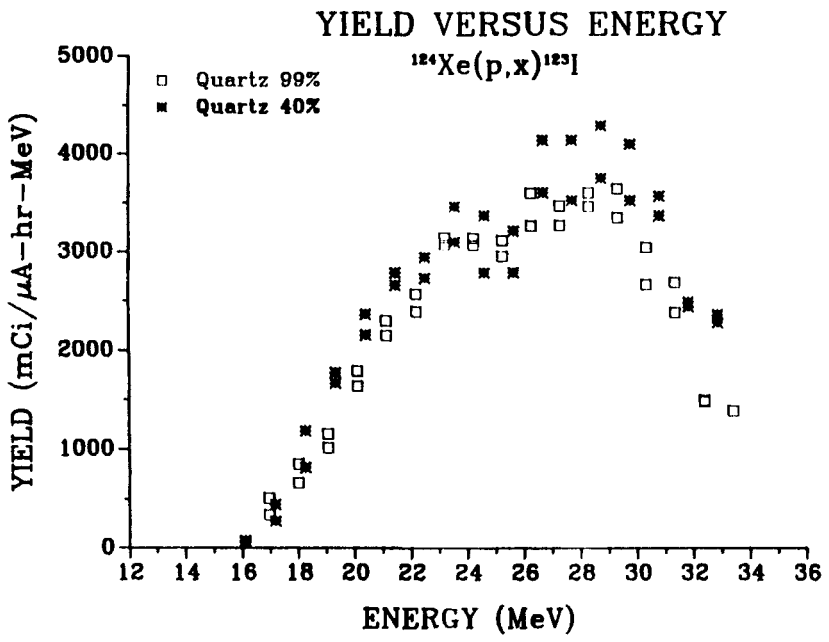

Figure 1

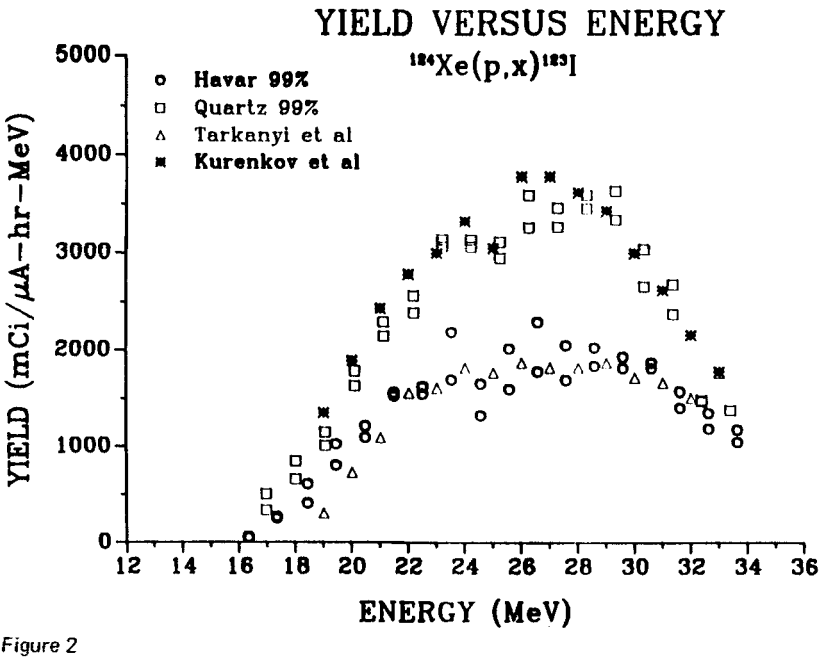

Figure 2

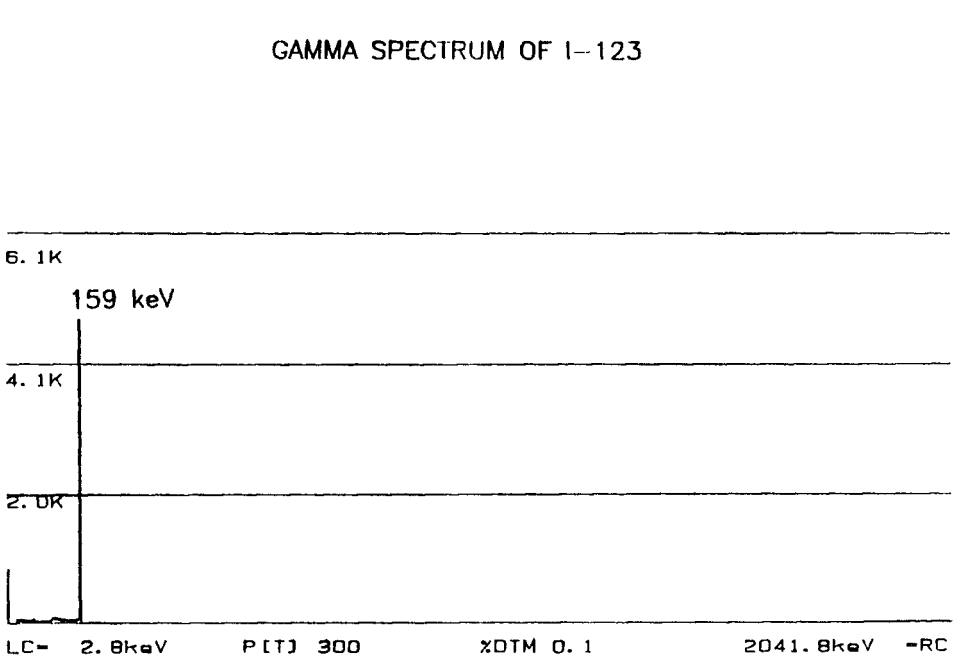
GAMMA SPECTRUM OF I-123
6. 1K
159 keV
4. 1K
2. 0K
LC- 2. 8keV
P[T] 300
%DTM 0. 1
2041. 8keV
-RC

Figure 3

COPRODUCTION OF COPPER-64 AND COPPER-67 WITH GALLIUM-67 USING PROTONS ON ZINC-68.

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There is a growing interest in the use of radiopharmaceuticals labelled with copper, as illustrated by the development of Cu(PTSM) labelled with copper-62, a positron-emitter for use with PET(1). Due to the short half life of copper-62($t_{\frac{1}{2}}$=9.7 min), developmental chemistry is best accomplished with radioisotopes of longer half-lives. Several which are available are Cu-67 ($t_{\frac{1}{2}}$=61.9 hr), Cu-64($t_{\frac{1}{2}}$=12.7 hr), Cu-61($t_{\frac{1}{2}}$=3.4 hr), and Cu-60(23.2 min).

In order to have a copper radioisotope available in our facility with a half-life longer than Cu-62 and without having to perform additional cyclotron irradiations, we have investigated the simultaneous production of Cu-64 and Cu-67 with Ga-67 produced during the irradiation of isotopically enriched Zn-68 with protons. Previously, it has been shown that Cu-64 and Cu-67 are produced during the irradiation of natural zinc with 16 MeV deuterons(2). The production rates for Cu-67 and Cu-64 were 0.123±0.003 and 143±12 uCi/uA•hr, respectively.

Our facility currently produces between 40 and 50 curies of Ga-67 per week. Normally, Zn-68 as metal is plated on a copper surface and irradiated with 26.5 MeV protons. After irradiation the zinc is removed from the target with 7N HCl. The volume is measured and the Ga-67 is extracted with isopropyl ether. The 7N HCl solution containing the Zn-68 is saved for recovery. For this investigation, initially, several samples of the recovery solutions were examined by HPLC to confirm the presence of gallium(as Ga-67), copper and zinc. A Dionex IonPac-CS5 column eluted with 0.006M pyridine dicarboxylic acid at pH=4.0(LiOH) at 1.5 ml/min was used. The method of detection was UV-visible at 520 nm using post column derivitization with 0.2 mM 4-(2-pyridylazo)resorsinol (PAR), 2M NH_4OH and 1M NH_4OAc at a flow rate of 0.7 ml/min. This method provided a separation and confirmed the presence of Ga-67 and the copper radioisotopes. Samples from the HPLC containing the copper radioisotopes were assayed using a Ge(Li) detector and indicated that both Cu-64 and Cu-67 were being formed. Once the presence of Cu-64 and Cu-67 were confirmed, recovery solutions were analyzed as follows: A sample was assayed with a Ge(Li) detector to determine the amount of Cu-64 using the 1346 keV peak(0.48% abundant). Counting errors were usually in the range of ±3-5%. Traces of Ga-67 remained, thus at this point an accurate determination of the Cu-67 could not be made. To get an accurate analysis of the Cu-67, the Cu-64 and Cu-67 were completely separated from the Ga-67 using a AG1-X8 column eluted with 3.5N HCl. Selected elution samples were checked for Ga-67 by looking for the 393 keV and the 887 keV peaks. Those which showed complete separation from Ga-67 were pooled, concentrated, and then assayed. The amount of Cu-67 was determined and by comparison to the Cu-64, the amount of Cu-67 in the original sample was calculated. The production rate of Cu-67 and Cu-64 were 1.57±0.55 and 329±68 uCi/uA • hr, respectively. Therefore, in a typical run, the total amounts produced are 7.8 mCi of Cu-67 and 1650 mCi of Cu-64. Because the Zn-68 is plated onto copper, the amount of carrier copper in the recovery solutions were on the order of 5-10 mg. The Cu-67 is produced by the Zn-68(p,2p)Cu-67 reaction. A potential reaction for the production of Cu-64 is Zn-68(p, n)Cu-64. At this point the Cu-65(p,pn)Cu-64 reaction cannot be ruled out, but does not appear to explain the total amount of Cu-64 being produced. This method appears to be suitable for the production of copper radioisotopes for in-house use and for preliminary investigations.

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A Radioisotope Production Facility using 70-120 MeV Protons

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The TRIUMF Radioisotopes for Medicine (TRIM) facility consists of a dedicated beamline operating between 70-120 MeV, a switching magnet with 5 target stations and a hot cell for solid target transfers. The proton beam is delivered to this facility by extracting part of the beam in the 500 MeV cyclotron. Both the energy and the intensity of the beam are adjustable independently of two other simultaneous beams used for physics research.

Four radioisotope production target systems can accept beam intensities from 10 to 100 uA. The fifth branch of the switchyard is under development for proton therapy (see figure 1).

The first radioisotope production target is molten LiBr for the production of radiokryptons and their daughter radiobromines. Incident protons are degraded to 50 MeV to enhance production of ^{77}Kr/Br(1).

The second production target is molten NaI (2) for the production of radioxenons and iodines at energies between 70 and 86 MeV. The principle products are ^{123}Xe for ^{123}I and ^{122}Xe for the ^{122}Xe/^{122}I generator(3).

The third system is designed for irradiating encapsulated solid target materials. Proton energies between 40 and 110 MeV are available by degradation in water. Targets can be inserted from a remote hot cell and extracted at the end of bombardment without interrupting normal operating of the cyclotron. The capsules are attached to a float and moved from the hot cell to the bombardment position on a column of water which can be varied in height. The capsule is immersed in water during irradiation. Water behind the capsule serves as a beam stop for transmitted beam. An adjustable sheet of flowing water in front of the capsule degrades the beam energy to the desired energy. This facility has been used to produce a number of positron generators such as ^{82}Sr/^{82}Rb,^{128}Ba/^{128}Cs and ^{52}Fe/^{52m}Mn(4-6).

The fourth target is molten cesium for the production of ^{127}Xe with proton energies between 85 and 105 MeV (7). Noble gas species are continuously flushed from targets 1,2, and 4 to remote processing facilities.

The administration at TRIUMF is seeking to attract external research groups to evaluate these and other radioisotopes for new applications in the biomedical sciences. Collaborative work is already underway using the positron emitter generators produced on this facility. Suggested guidelines for the organization of a users groups will be discussed.

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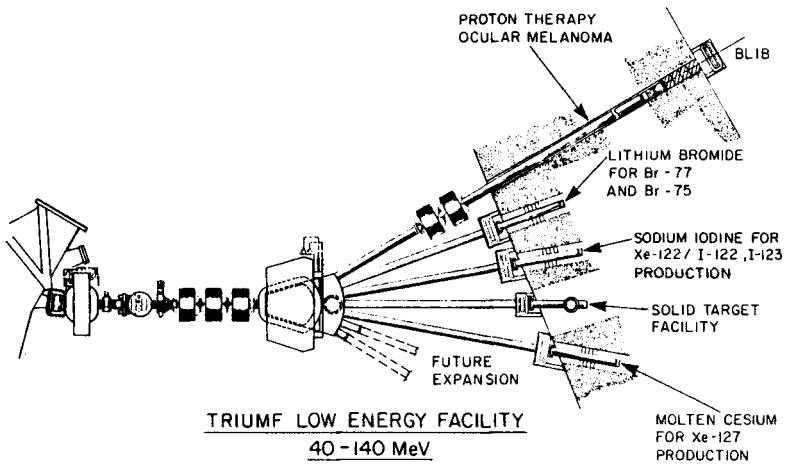

Figure 1 The TRIUMF low energy beam line configuration.

TRIM Products

Generators		Isotopes	
Species Parent / Daughter	Half-Life Parent / Daughter	Species	Half-Life
^{82}Sr / Rb	25 d / 76 s	^{52}Fe	8.3 h
^{128}Ba / Cs	2.4 d / 3.6 m	^{67}Cu	62 h
^{52m}Fe / Mn	5.6 d / 21 m	^{127}Xe	36 d
^{122}Xe / I	20 h / 3.6 m		
^{118}Te / Sb	6 d / 3.5 m		

Table 1 Typical radionuclides produced by the TRIUMF low energy facility.

Cross-section measurements for the $^{13}C(p,n)^{13}N$ and $^{12}C(d,n)^{13}N$ nuclear reactions.

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Nitrogen-13 is one of the positron emitting isotopes which has been used in several chemical forms for Positron Emission Tomography. As particle accelerators of lower energy come into use, it is important to be able to accurately predict the production capability of the accelerator for the major positron emitting isotopes. The data for the $^{13}C(p,n)^{13}N$ and the $^{12}C(d,n)^{13}N$ nuclear reaction cross-sections were not complete. We have therefore measured the cross-sections for these reactions over the energy range between threshold and 20 MeV utilizing a gaseous carbon dioxide target and a Tandem Van de Graaff accelerator.

The threshold for the $^{13}C(p,n)^{13}N$ nuclear reaction is at 2.0 MeV with a maximum of the cross-section at 6.5 MeV. Figure 1 shows the cross-section as a function of energy. The threshold for the $^{12}C(d,n)^{13}N$ reaction was at 0.2 MeV with a maximum at 1.4 MeV. Figure 2 is the cross-section for this reaction as a function of energy. These data are in general agreement with the previously published data for these reactions over smaller energy intervals.

This work supported by DOE.

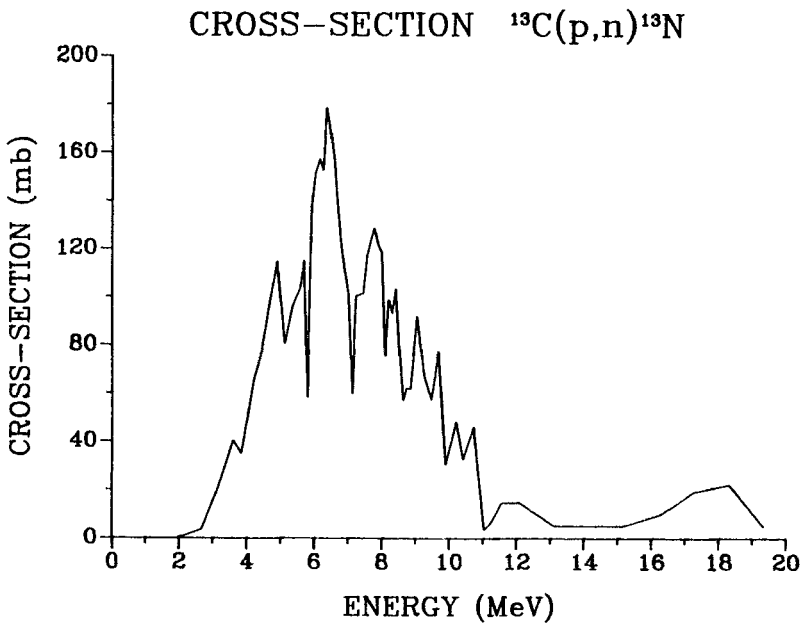

Figure 1

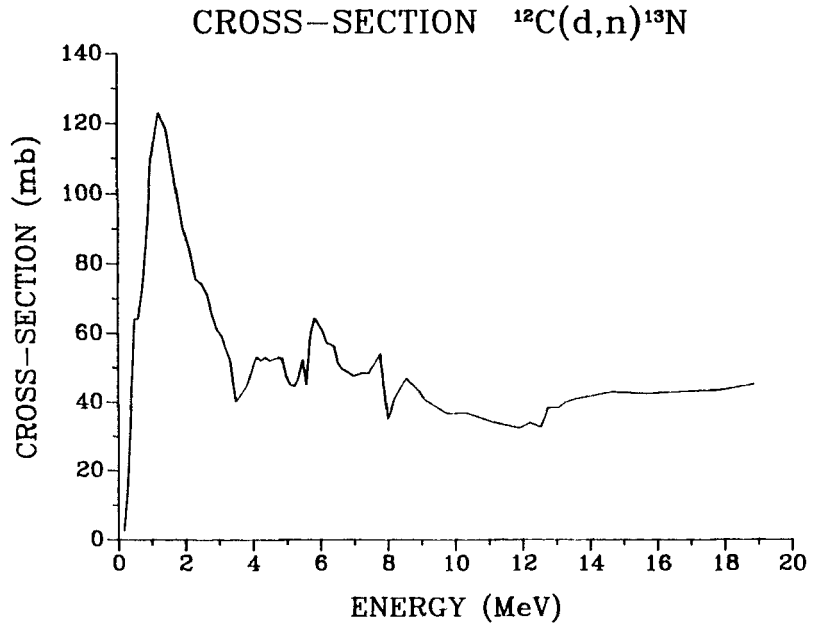

Figure 2

DEVELOPMENT OF AN INEXPENSIVE PROGRAMABLE LOGIC CONTROLLER FOR CLINICAL GASES REGULATION

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To meet the requirements of the PET clinicians at our new facility, we had to develop a regulation system for the production of radioactive clinical gases such as $^{15}O_2$, $C^{15}O$, $C^{15}O_2$, ^{11}CO. The aim was to obtain a constant radioactive output at a determined flow rate, in the PET camera room located 30 m from the cyclotron.

Although the target output for a given beam intensity is stable, a need for a dilution arises; moreover, a regulation system gives a better security.

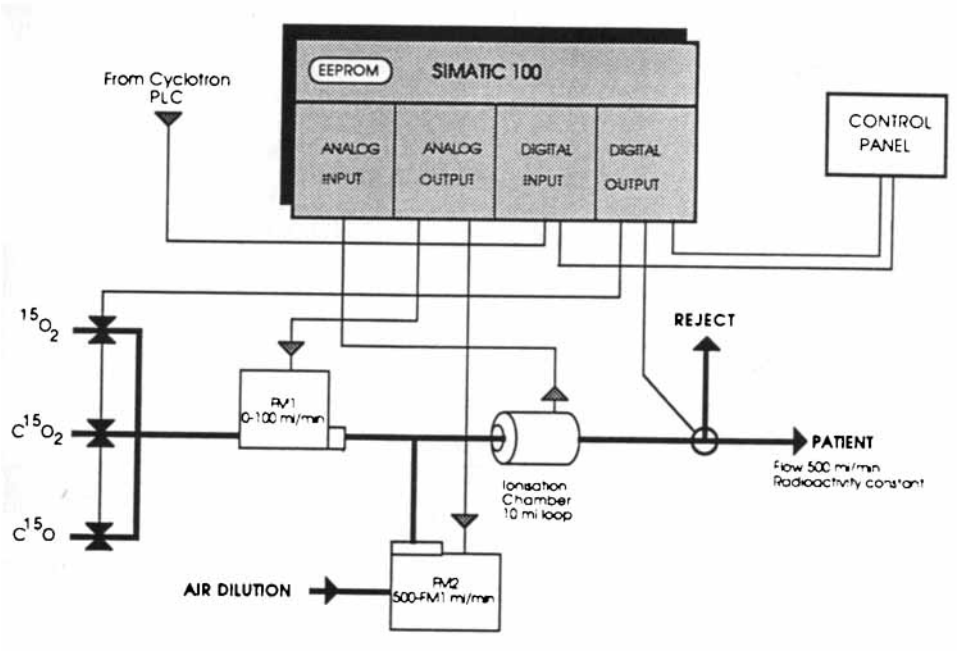
The small PLC we choose for this job is a Siemens SIMATIC 100, providing interesting possibilities of adding different interfaces: it is equipped with one module x 2 analog inputs, one module x 4 analog outputs, one module x 8 digital inputs, one module x 8 digital outputs.

The input radioactivity signal is provided by a small finger ionisation chamber (40 x 10 mm) located inside a 10 ml loop of the final diluted gas; two analog outputs are controlling two regulating flowmeter Brooks TR 5850 (0-100 ml/min for activity, 0-500 ml/min for air dilution). The digital inputs are connected to the cyclotron, accessing to informations such as nature of gas, beam on, position of electrovalves, and to the control panel in the PET camera room. Digital outputs control the switching valves (gases, patient/reject) and the lamps in the control panel. (see scheme 1). The total cost of this equipment is below 1500 US $.

Programing is done easily by simple instructions such as loading, transfering registers; instructions are grouped in functionnal blocks and the resulting program (<100 instructions) is stored in the system EEPROM. The regulation algorithm we use for the moment is the simplest one, i.e. direct control of the activity input flowmeter by comparison of the radioactivity after dilution to the required flow rate to the preset radioactivity level. The system regulates $^{15}O_2$ to 22 µCi/ml and $C^{15}O_2$ to 20 µCi/ml, at a constant flow rate of 500 ml/min; the stabilities at the patient output (PET Camera) are better than 3 %. These levels are easily modified by a new programation. The system authorizes commutation of the radioactive flow to the patient only when regulation is achieved, approximately 3 to 4 minutes after switching the active flow to the camera room.

Radioactive clinical gases are controlled prior to administration by GC/Radiochromatography using a dual channel integrator; a trace of the QC, the activity output and the proportional regulating electrovalve position is recorded in the patient file.

Over eight months of extensive use, this system has proven very satisfactory; a wide variation of the beam current on target is allowed and a very short interruption of bombardment can even pass unnoticed for the terminal user, as the regulation system will counteract to correct for the diminution of incoming activity.


From Cyclotron PLC
EEPROM
SIMATIC 100
ANALOG INPUT
ANALOG OUTPUT
DIGITAL INPUT
DIGITAL OUTPUT
CONTROL PANEL
$^{15}O_2$
$C^{15}O_2$
$C^{15}O$
PV1 0-100 ml/min
REJECT
PATIENT
Flow 500 ml/min
Radioactivity constant
Ionisation Chamber 10 ml loop
AIR DILUTION
PV2 500-FM1 ml/min

IRRADIATION OF WATER TO IMPROVE ^{15}O SPECIFIC ACTIVITY

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We have investigated a flow-through water target for production of high specific activity $O^{15}O$ and $C^{15}O$ by the $^{16}O(p,pn)$ reaction. Because we share a cyclotron with neutron therapy, we have only 50 MeV H^+ available during the day. We currently use a 0.36L O_2 target which yields 0.5 Ci of $O^{15}O$ with a specific activity of 9.5 Ci/mole (EOB) in a thin target for 3 min/45 MeV/30μA. The disadvantage of the O_2 target for making ^{15}O is that the product has low specific activity and cannot be used for CO. Both Welch (1) and Piltingsrud (2) described higher specific activity $[^{15}O]O_2$ by the (γ,n) reaction on $[^{16}O]$water using high current electron accelerators to produce photons. Welch found up to half of the ^{15}O in O_2 which could be released by boiling the target water. Our hypothesis is that irradiation of water with a high H^+ current will leave the nucleogenic molecules in the gas phase and conveniently separable from the water used for the target.

Our target system included a high speed pump to recirculate water between a 2L reservoir with gas sparging, and the Al target body. This pump kept the pressure above 1 atm. The target front piece had a 2 cm dia. X 0.4 cm thick window for degrading protons to 35 MeV, and a back chamber 6 cm deep by 5 cm dia. Water entered and exited at the back of the target and cooled the window. The window thickness was chosen to produce ^{15}O, ^{11}C and ^{13}N in a single target; although, we could select for specific production of ^{15}O or ^{11}C by varying this thickness. The target has been tested with currents up to 62 μA. Water was sampled from the target outflow and was counted before and after boiling, to assay for activity in dissolved gases. Gases produced in the target were carried away with the sparging gas and were either trapped and analyzed by gas chromatography or trapped on ascarite for measurement of $[^{11}C]CO_2$. All samples were assayed for radioactivity over time and analyzed by nonlinear regression to determine the mCi of each isotope.

The amount of ^{15}O produced was consistent with a thick target. The fraction of ^{15}O recovered as O_2

gas was a linear function of current, with 0.38 mCi (EOB) recovered at 9.8 μA and 98 mCi at 62 μA. Oxygen gas production also depended upon the presence of some dissolved O_2 in the target water. Helium sparging decreased the recovery of O_2 to <0.2%, regardless of current. Recovery of $[^{15}O]O_2$ was the same, whether the source of O_2 was air (1 atm, 35°C) or sparging with O_2 under increased pressure. Some ^{11}C and ^{13}N was also produced. The ^{11}C was recovered as CO_2, with essentially no ^{11}C left in the water, regardless of current. All of the ^{13}N remained in the water.

We conclude that production of $[^{15}O]O_2$ requires more carrier O_2 than radiolysis can supply. The addition of a small amount of carrier O_2 allows for recovery of 50 mCi as oxygen gas with 40 μA/ 3 min and 100 mCi with 62 μA/ 3 min. The specific activity is ~1.5 Ci/mmol. We are encouraged by these results to measure production at higher flows and with increased current.

This work was supported by NIH grant CA42045.

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