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DEVELOPMENT OF NEW RADIOPHARMACEUTICALS

FINAL REPORT OF A RESEARCH CO-ORDINATION MEETING
ORGANIZED BY THE
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FOREWORD

The possibilities to design and prepare better and more organ-specific radiopharmaceuticals for diagnostic nuclear medicine has increased dramatically in the recent past with a deeper understanding of the relationships between chemical structure and biological activity. Whereas most of the research is performed in well-funded laboratories of industrialized countries, there are several developing countries with adequate resources and experise as to undertake fruitful research in the field of radiopharmacy.

With the aim of promoting advanced research in radiopharmacy by developing new radiodiagnostics agents, in particular, hepatobiliary imaging agents labelled with ^{99m}Tc, and to facilitate exchange of information, the IAEA has established in 1983 the present Research Co-ordination Programme (CRP) with a duration of five years. The report includes detailed results obtained by all participants as well as novel preparation procedures for some of the newest and more promising radiopharmaceuticals developed under the auspices of the CRP. The extensive bibliographic reference listing is considered another important information of particular value for scientists in developing countries who do not always have access to updated scientific information sources.

The Agency wishes to thank all the scientists who contributed to the success of this CRP.

EDITORIAL NOTE

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CONTENTS

1. INTRODUCTION

The general aim of this Coordinated Research Programme (CRP) was to contribute to the efforts toward the development of new radiopharmaceuticals with improved characteristics for in vivo studies in nuclear medicine. To this effect, it was decided that during the first phase of the Programme, the research should focus primarily on the development of new hepatobiliary imaging agents, or to further test existing ones labelled with ^{99m}Tc and other radionuclides.

The scientists participating in the CRP were asked to concentrate their research efforts on the chemical and biological aspects of the laboratory preparation of radiopharmaceuticals including synthesis and physicochemical characterization of the individual compounds, formulation and testing of kits suitable for labelling with ^{99m}Tc, development or improvement of analytical techniques and protocols for testing the labelled products as well as biodistribution and in vivo kinetics studies in laboratory animals.

Whenever possible, the research was oriented towards the development of simple, reliable and economical procedures and techniques suitable for adoption to local conditions prevailing in developing countries. While recognizing the fact that new radiopharmaceuticals will not be fully developed until extensive studies in humans are successfully concluded; activities such as the determination of toxicity, physiological behavior, dosage levels and extensive clinical trials in humans were not considered essential to the scope and immediate objectives of the CRP.

Research Contracts and Research Agreements were concluded with scientists from Argentina, Australia, the People's Republic of China, Greece, India, Japan, Yugoslavia, the United States of America and Uruguay. Three Research Coordination Meetings were held: 17-19 October 1984, Kyoto, Japan; 10-13 November 1986, Buenos Aires, Argentina; and 12-15 September 1988, Athens, Greece.

Under the auspices of the CRP, new potential hepatobiliary imaging agents based on ^{99m}Tc complexes derivatives of salicylaldehyde, O-aminophenol, phthalein or fluorescein, including various iminodiacetic acid

derivatives were prepared and evaluated further. It was found that several of the ^{99m}Tc-complexes of salicylaldehyde and O-aminophenol are specifically excreted via the hepatobiliary system while their high lipophilicity may lead to radiopharmaceuticals for positive brain imaging. Bifunctional chelating agents containing on one side the structure of phthalein and on the other side the structure of IDA have generated a new hepatobiliary family of agents. Several halogenated IDA derivatives have been synthesized and characterized and their biological behavior was compared to the non-halogenated compounds. The bromo and iodo derivatives, namely, ^{99m}Tc-mebrofenin and ^{99m}Tc-iodofenin showed excellent properties for hepatobiliary imaging.

Phenolic aminocarboxylate chelates of ^{67}Ga were developed as hepatobiliary radiopharmaceuticals with longer half lives, useful in clinical situations where delayed excretion of tracer makes the short half-life of ^{99m}Tc-agents disadvantageous.

Taking advantage of the great deal of experience gained with the work on hepatobiliary agents and their lipophilic properties, some participants had also carried out research toward the development of brain and heart imaging agents. A new class of isonitriles were synthesized using a substituted aromatic ring attached to the isonitrile functional group. Animal biodistribution studies show localization in the myocardium. Cationic complexes using dialkyl and pyrrolidinyl bis-tertiary amino alkyl amides were also synthesized. These compounds failed to localize in the myocardium of experimental animals confirming the observation that cationic character alone is insufficient for myocardial deposition.

Several thiosemicarbazone complexes have been synthesized and studied as potential brain imaging agents as well as aminothiol derivatives.

One research contract was concerned with the use of Mössbauer Spectroscopy techniques to study the valence state and relative ratios of Sn(II) and Sn(IV) in lyophilized kits of ^{99m}Tc for hepatobiliary imaging. Another research contract dealt with the improvement of labelling techniques of bioactive protein molecules such as human fibrinogen.

The Final Report includes details of the research work carried out by all the participants. It provides an account of the techniques and methodology used as well as an assessment of the experimental results. The names of the scientists who participated in the CRP are also included.

2. RESULTS OF THE RESEARCH

This section of the report is divided into five subject groups. Results generated from the agreement holders and contractors were combined in each section. Important new developments are well documented in these sections. Relevant data on 1) chemical syntheses, 2) radiolabelling, 3) quality control and 4) biodistribution studies are also included here. Supplemental data on these subjects can be found in the Appendices in which only unpublished and difficult-to-locate data are included. Care has been taken to delete any material already published in major journals.

2.1 HEPATOBILIARY IMAGING AGENTS

The work performed on the development of new hepatobiliary agents can be summarized as follows: new ligands were synthesized and labelled with ^{99m}Tc, ^{67}Ga or ^{111}In. The new compounds were derivatives of salicylaldehyde, O-aminophenol or various iminodiacetic (IDA) derivatives containing phenylcarbamoyl-substituted phthalein and fluorescein rings.

These new complexes were characterized by various analytical methods and their biological behaviour was studied in different animal models. Comparative studies were also performed in human volunteers.

Derivatives of ethylenediamine di [o-hydroxyphenylacetic acid] EDDHA and N,N' - bis [2-hydroxybenzyl] ethylenediamine N,N' - diacetic acid (HBED) were synthesized by Hunt et al. (1)

The following ^{67}Ga-EDDHA complexes were prepared: t-butyl, octyl, chloro and bromo. The gallium-67 complexes of bromo and dibromo-HBED were also prepared.

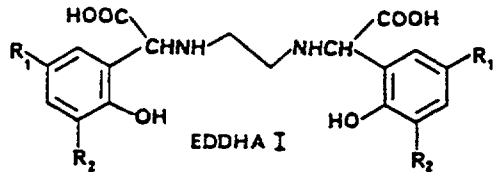
Additionally, the same ligands were labelled with ^{111}In and studied comparatively in animals. ^{67}Ga and ^{111}In-labelled EDDHA and HBED derivatives were developed to investigate chemical conditions in which delayed excretion occurs, e.g., differential diagnosis of neonatal hepatitis and biliary atresia.

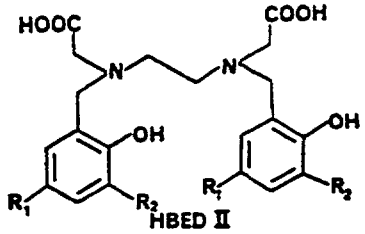
Biodistribution studies in mice showed that all compounds had a high hepatobiliary specificity, comparable to some of the ^{99m}Tc agents. Halogen substituents gave compounds with the best characteristics, with ^{67}Ga-Br-EDDHA having 79% of the injected dose in the intestines, 3.9% in the liver and 2.9% in the urine at one hour. The results for ^{67}Ga-Br-HBED were 65.8% 22.3% and 0.4% respectively, indicating a retarded rate of excretion compared with the Br-EDDHA complex.

Indium-111 EDDHA's had substantial hepatic retentions particularly with the alkyl substituted complexes and were inferior to their gallium counterparts. Indium-111 was a better label for the HBED ligands; the dichloro complex had the greatest amount in the bile of all the compounds tested (88.4%) and low urinary excretion (2.75%).

The structure of the basic ligands synthesized are shown in Figure 1, while comparative animal data of the various complexes are shown in Table 1. Advice received from paediatric nuclear medicine physicians in Australia was that indium-111 dichloro-HBED would be useful in the investigation of neonatal jaundice.

Another class of compounds ^{99m}Tc-complex derivatives of salicylaldehyde and o-aminophenol were evaluated in animals by Chiotellis et al. (2).





EDDHA = Ethylenediamine di[o-hydroxyphenylacetic acid], also as
EHPG = N,N'-ethylene bis[2-hyroxyphenylglycine]

HBED = N,N'- bis[2-hydroxybenzyl] ethylenediamine N,N'-diacetic acid

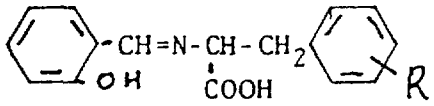
FIGURE 1.

TABLE 1

COMPARATIVE DATA IN MICE, 1-HOUR POST INJECTION
(% dose in organ)

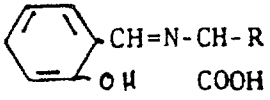
Complex	Blood	Liver	GIT+GB	Kidneys	Urine
^{111}In - EDDHA					
EDDHA	1.0	3.4	63.7	3.2	23.1
DIMETHYL	1.3	36.6	28.1	3.5	13.6
T-BUTYL	7.9	38.2	20.7	4.0	9.7
CHLORO	0.8	6.9	74.3	2.2	8.1
DICHLORO	0.4	21.3	71.7	1.8	1.1
BROMO	2.4	11.4	60.9	5.3	8.8
^{111}In - HBED					
HBED	0.3	7.5	74.2	1.0	14.8
DIMETHYL	0.4	3.6	85.6	0.6	8.8
T-BUTYL	1.8	11.8	20.3	1.2	49.3
CHLORO	0.5	2.3	61.1	1.3	28.3
DICHLORO	0.2	5.5	88.4	0.2	2.7
BROMO	0.6	9.8	76.5	4.9	7.6
^{67}Ga - EDDHA					
EDDHA	1.1	21.6	27.6	5.6	23.0
DIMETHYL	0.3	5.1	81.0	1.5	8.5
T-BUTYL	2.3	9.0	79.5	1.1	8.4
CHLORO	0.4	4.0	85.5	1.8	6.8
DICHLORO	0.3	6.3	85.4	1.4	0.8
BROMO	1.1	3.9	85.2	0.7	2.9
^{67}Ga - HBED					
HBED	0.3	1.9	67.4	0.4	18.9
DIMETHYL	0.2	5.2	74.7	0.5	9.8
T-BUTYL	2.3	4.3	75.4	1.1	6.9
CHLORO	0.5	12.4	75.6	0.9	5.8
DICHLORO	0.3	10.2	81.4	0.9	2.2
BROMO	0.4	17.6	71.2	0.4	7.3

Schiff-base compounds, derivatives of salicylaldehyde and o-amino phenol, were synthesized, labelled with ^{99m}Tc and studied in experimental animals (mice and rats). The compounds synthesized are grouped to the following general formula:



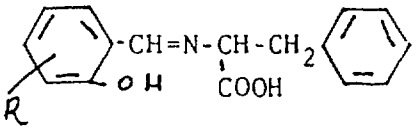
Type A

R=H, F, (o, m, p)
p-I, p-OCH_3



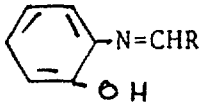
Type C

R=Tr, Nor-L, iso-L
Cyclo-L, Al, Me



Type B

R=4-OCH_3, 5-OCH_3
O-NO_2, 4-OH



Type D

R=p-methoxy-phenyl
p-dimethylaminophenyl
p-nitrophenyl
o-hydroxyphenyl
o-chloro-p-hydroxy-phenyl-
naphthyl

The Schiff-bases were characterised using elemental analysis and NMR. The labelling was carried out with ^{99m}Tc, using the stannous chloride method, for derivatives of Types A, B, and C, in water solution, while for Type D, in organic solvent. Radiochemical control of the ^{99m}Tc-labelled derivatives was performed by ITLC chromatography and electrophoresis. All the derivatives showed a high yield of labelling over 90%. The partition coefficient of the ^{99m}Tc-complexes was determined using the octanol-water phase system.

^{99m}Tc-complexes of Types A, B, and C were administered in mice at several time intervals and tested as hepatobiliary radiopharmaceuticals. Most of the complexes were specifically concentrated in the liver and excreted into the intestines. Hydrophilic complexes were also excreted via the urinary system.

The rate of the hepatic extraction varied with the substituent. Not great differences of the biodistribution were observed regarding the ^{99m}Tc-complexes of compounds A and B.

A study of the possible structure of the ^{99m}Tc-salicylidene phenylalanine was also performed using ^{113}Sn ($SnCl_2$).

Considerable work was performed on the sythesis and evaluation in animal species and human volunteers of a great variety of carbamoyl-IDA derivatives. Thus Subramanian and colleagues synthesized halogenated IDA-derivatives: meta-iodo-2,6 dimethyl HIDA, meta-iodo-2,6 diethyl HIDA and meta-iodo-2,4,6 trimethyl HIDA (Fig. 2).

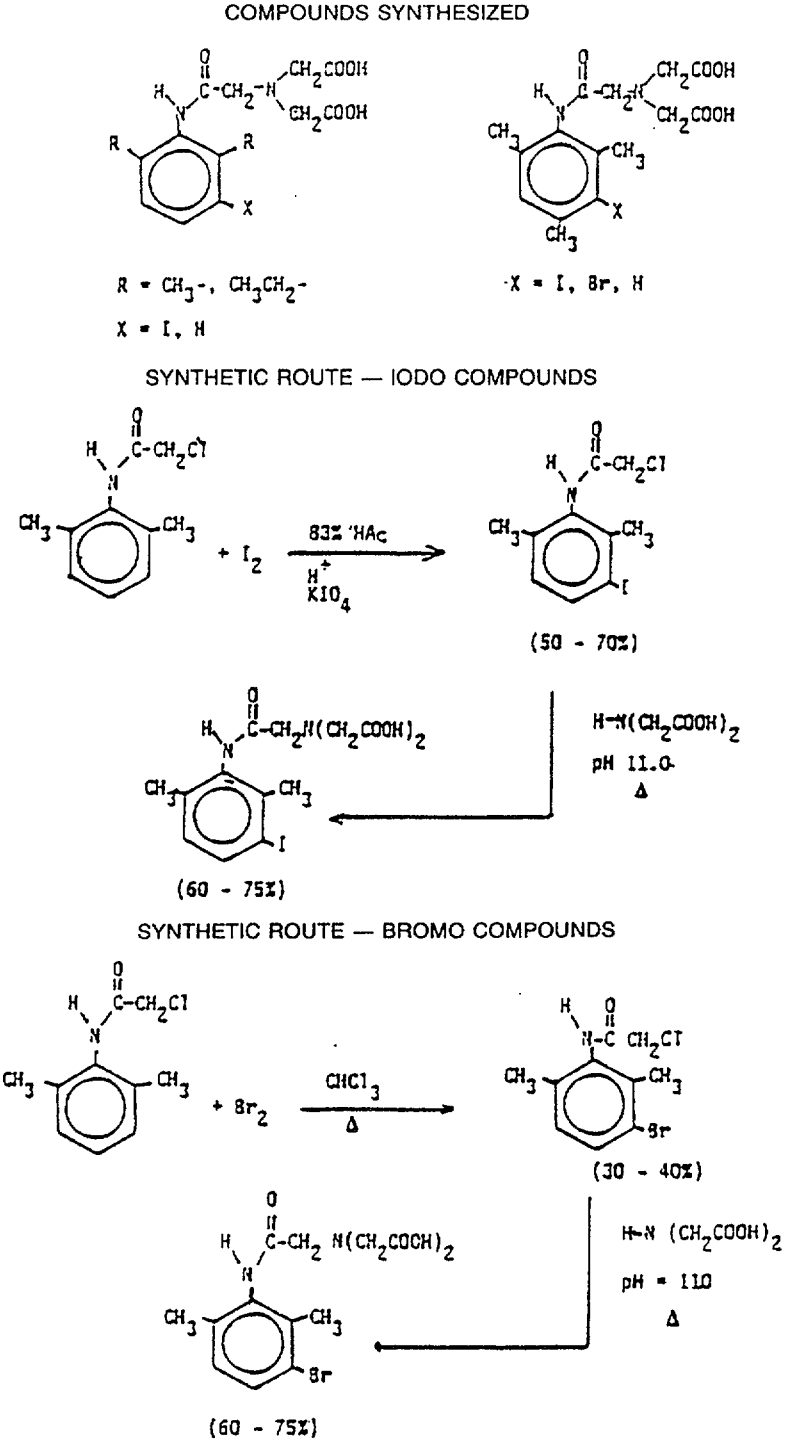


FIGURE 2.

These compounds were purified by multiple crystallizations and their chemical structures were verified by elemental analysis, IR and NMR spectra. Freeze dried kits were formulated with these new molecules using stannous chloride for subsequent labelling with ^{99m}Tc. Quality control tests were performed for determining radiopharmaceutical purity. Biodistribution studies in rabbits were conducted using these compounds both by scintigraphic imaging and organ assay after injecting the animals with the ^{99m}Tc agents. For comparison purposes, the above HIDA-derivatives without iodine on the phenyl ring and another new compound, 3 bromo, 2,4,6 trimethyl HIDA were also prepared and evaluated in a similar manner. The new halogenated HIDA's showed considerable improvement in biodistribution in rabbits as compared to the non-halogenated compounds (Table 2).

TABLE 2

Tc-99m HIDA'S IN RABBITS
PERCENT DOSE IN WHOLE ORGAN

COMPO	BLOOD			
	5'	15'	30'	60'
DIMETHYL	13.2	3.5	2.4	1.3
IODO DIMETHYL	3.6	1.7	0.4	0.9
DIMETHYL	7.4	2.6	1.3	2.2
IODO DIMETHYL	3.3	1.7	0.9	0.3
TRIMETHYL	7.9	3.4	2.7	2.5
IODO TRIMETHYL	3.1	0.7	0.4	0.4
BROMO TRIMETHYL	1.5	0.7	0.2	0.5

Tc-99m HIDA'S IN RABBITS
PERCENT DOSE IN WHOLE ORGAN

COMPO	GIT-GB			
	5'	15'	30'	60'
DIMETHYL	31.3	69.7	74.9	78.3
IODO DIMETHYL	62.7	81.6	90.1	86.0
DIMETHYL	29.5	56.9	80.3	81.1
IODO DIMETHYL	39.4	70.7	88.1	90.1
TRIMETHYL	29.5	68.0	81.3	81.2
IODO TRIMETHYL	50.9	85.2	90.3	92.7
BROMO TRIMETHYL	67.0	87.7	93.6	92.3

Tc-99m HIDA'S IN RABBITS
PERCENT DOSE IN WHOLE ORGAN

COMPO	LIVER			
	5'	15'	30'	60'
DIMETHYL	31.1	9.3	2.9	1.0
IODO DIMETHYL	21.4	6.0	1.2	0.9
DIMETHYL	37.2	21.9	3.3	1.3
IODO DIMETHYL	46.7	19.0	5.1	1.6
TRIMETHYL	32.3	10.6	1.7	1.0
IODO TRIMETHYL	35.8	6.3	2.4	0.7
BROMO TRIMETHYL	22.9	4.0	0.5	0.5

Tc-99m HIDA'S IN RABBITS
PERCENT DOSE IN WHOLE ORGAN

COMPO	URINE			
	5'	15'	30'	60'
DIMETHYL	0.5	5.2	10.0	10.3
IODO DIMETHYL	1.0	2.4	2.3	5.9
DIMETHYL	1.8	3.6	3.9	7.2
IODO DIMETHYL	0.3	1.2	0.9	1.4
TRIMETHYL	1.8	3.3	5.1	8.1
IODO TRIMETHYL	0.1	0.3	0.3	0.7
BROMO TRIMETHYL	0.3	0.6	0.4	1.3

Comparative clinical studies of the various ^{99m}Tc complexes were performed in human volunteers (Figs. 4,5,6 and 7). From this group, compounds containing halogen substituted phenyl group, Iodo diethyl, Iodo trimenthyl and Bromo trimethyl derivatives proved to be superior to others. All three agents have desirable biological properties in a hepatobiliary radiopharmaceutical. Between these three compounds, comparative clinical studies have shown that any one of the three agents could be successfully used for hepatobiliary studies in man.

Great variety of carbamoyl-IDA derivatives were also synthesized and evaluated biologically by A.E. Mitta and collaborators.

The following compounds were developed:

I 2,6 dimethylphenylcarbamoylmethylimiodiacetic.
II 2,6 diisopropylphenylcarbamoylmethylimiodiacetic.
III 2,6 diethylphenylcarbamoylmethylimiodiacetic.
IV 4n-butylphenylcarbamoylmethylimiodiacetic.
V 2,6 diisopropylphenylcarbamoylethyliminodiacetic.
VI 4n butylphenylcarbamoylethyliminodiacetic.
VII 4n butylphenylcarbamoylethylideniminodiacetic.
VIII Benzyliminodiacetic.
IX N(4 Iodine - 2,6 diethylphenylcarbamoylmethyliminodiacetic labelled with 14C).
X 4 Iodine - 2,4,6 trimethylphenylcarbamoylmethyliminodiacetic.
XI 3 Bromine 2,4,6 trimethylphenylcarbamoylmethyliminodiacetic.
XII 3 Iodine - 2,6 diethylphenylcarbamoylmethyliminodiacetic.
XIII 4 Iodine - 2,6 diethylphenylcarbamoylmethyliminodiacetic.

The synthesis of some of the above-mentioned compounds was improved by modification of reported methods (3,4). Evaluation of the ^{99m}Tc-complexes was performed in various animal and human models (5,6).

Chiotellis and collaborators prepared also ^{99m}Tc-N(3,4,5-triiodo)-acetanilido-iminodiacetic (^{99m}Tc-TIIDA). This new complex was totally excreted via the hepatocytes in healthy animals but proved less effective in animals with depressed hepatobiliary excretion when compared with other halogenated IDA-derivatives (7).

Noronha's group studied extensively the "in vitro" and "in vivo" pharmacokinetic parameters involved in normal and also in patho-physiological conditions. Several ^{99m}Tc-TIIDA derivatives were tested in animal model system with a) altered thyroid status, b) fatty liver, c) hepatitis and d) cirrhosis.

It was found that alteration in thyroid status resulting in the hypothyroid state caused a marked reduction in the rate of hepatobiliary excretion especially at early time periods. However, this physiologic condition did not affect the amount of the tracer being transported by the hepatobiliary route. In contrast, the rate of hepatobiliary excretion was unaffected in animals rendered hyperthyroid. The effect of altered thyroid status on the relative mobilities/transit times of both fast and slow hepatobiliary agents was also investigated.

A condition of fatty liver was induced in experimental rats by feeding them solely on cooked (boiled) eggs. The lipid content of the liver in these rats increased three-fold, but this did not affect the hepatobiliary clearance characteristics of ^{99m}Tc-4 Butyl-IDA - an agent of greater lipophilicity than other ^{99m}Tc-HIDA agents.

Hepatitis (similar to acute human viral hepatitis) was induced in rats by injecting them (i.p.) with D+galactosamine 25h prior to study. It was found that this condition (in which membrane lipoproteins of hepatocytes are affected) caused a considerable retention of the tracer by the liver. The more lipophilic ^{99m}Tc-4-Butyl-IDA was retarded to a greater extent than the less lipophilic ^{99m}Tc-2,6 Dimethyl-IDA (^{99m}Tc-HIDA).

Liver injury akin to cirrhosis was induced in rats by the combined action of phenobarbitone (administered orally) and inhalation of CCl_4 vapours (3-5 min twice a week for 10 weeks). It was noted that under these pathophysiological conditions the biokinetics of ^{99m}Tc-Diisopropyl-HIDA and ^{99m}Tc-4-Butyl-IDA were not significantly affected.

Another group of compounds which were studied was iminodiacetic and derivatives containing a skeleton of Phthalein or Fluorescein. The development of these compounds by Yokoyama and collaborators was based

on the concept of bifunctional radiopharmaceuticals. Among various fluorescein-IDA derivatives (Calcein) and phthalein-IDA derivatives [phthalein complexone (PC), phenolphthalein complexone (PPC) and thymolphthalein complexone (TPC)] labelled with ^{99m}Tc, following the methodology described for the low hydrolyzed state of ^{99m}Tc with preferential bile excretion (1), ^{99m}Tc-PC [3,3'-bis (N,N-di (carboximethyl) aminomethyl)-o-cresol phthalein] showed a very good labelling efficiency (over 91%) with the highest hepatobiliary excretion over 61% in 1 hr (rat bile cannulation studies). Mice biodistribution supported this result. Serial scanning obtained in rabbits injected with ^{99m}Tc-PC, showed beautiful gall bladder visualization after 20-25 min. This excellent hepatobiliary excretion behaviour is considered as reflecting the chemical structure of phthalein structure, like the well known BSP agent.

Further functionality of the new hepatobiliary with regard to 131-BSP and ^{99m}Tc-HIDA was tested by competitive biliary clearance studies carried out in the presence of various concentration of BSP. Also, an assay on the hepatic cytoplasmic protein involved in the uptake of ^{99m}Tc-PC was considered and from fractionation studies on Sephadex G-75, it became clear that the participation of the organic anion binding protein, namely, fraction Y (ligandin) in the hepatobiliary uptake of ^{99m}Tc-PC, is compatible with the BSP transport system but different from the ^{99m}Tc-HIDA. Thus, great evidence for the functionality of the conceptual approach used on designing this new ^{99m}Tc-PC hepatobiliary agent containing the BSP skeleton has been achieved. The excellent hepatobiliary performance observed in animal studies offers great potentiality as for future clinical studies.

The structure of the above-mentioned derivatives is presented in Figure 3, while the animal data are presented in Tables 3, 4.

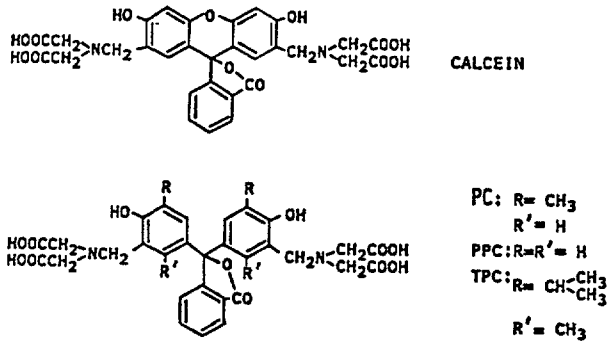


FIGURE 3.

TABLE 3

MICE BIODISTRIBUTION STUDIES OF ^{99m}Tc-PC

		10 min	30 min	60 min	180 min
Blood	a)	4.89 (0.54)	3.17 (0.41)	2.20 (0.18)	1.66 (0.20)
	b)	———	———	———	———
Kid.	a)	4.20 (0.49)	3.74 (0.21)	3.08 (0.47)	2.45 (0.20)
	b)	1.97 (0.32)	1.72 (0.34)	1.31 (0.20)	1.08 (0.02)
Liv.	a)	5.29(1.08)	3.19 (0.34)	2.41 (0.23)	2.04 (0.17)
	b)	9.20 (0.66)	4.68 (0.15)	3.94 (2.79)	2.89 (0.12)
Int.	a)	6.03(1.46)	9.71 (1.27)	10.51(0.88)	10.71 (1.94)
	b)	27.30 (5.41)	38.40 (0.85)	43.85 (2.79)	47.36 (4.71)
Stom.	a)	1.97 (2.64)	1.15 (1.12)	0.89 (0.63)	0.70 (0.44)
	b)	0.86 (0.89)	0.52 (0.45)	0.50 (0.38)	0.31 (0.15)
G.B.	a)	419.71(307.52)	161.10(96.16)	199.50(70.72)	96.72(37.07)
	b)	2.09 (0.37)	2.66 (0.75)	3.10 (0.86)	3.32 (1.86)

*Means (±s.d.) for four mice. G.B.= gall bladder.
a) % dose per gram
b) % dose per organ.

TABLE 4

LIVER HOMOGENATE FRACTION ANALYSIS

Post-Injection Time (min)	Liver cytoplasmic fraction*/Homogenate (%)**			
	2	5	8	15
^{99m}Tc-PC	76.0	79.2	84.1	74.2
^{99m}Tc-HIDA	89.9	86.6	89.3	83.7
^{131}I-BSP	58.0	66.4	74.4	66.4

(*) From 25% liver homogenate centrifuged at 105,000 g (av), 2 h.
(**) Each value is the mean for three rats.

RAT LIVER CYTOPLASMIC PROTEIN BINDING

Radiopharmaceuticals	% Bound to Liver Cytoplasmic Protein (5 min post-injection)	
	Ultrafiltration (PM 10)	Gel Filtration (G-75)
^{99m}Tc-HIDA	22.6 (4.3)	12.6 (7.6)
^{99m}Tc-PC	76.7 (1.7)	72.1 (6.0)
^{131}I-BSP	96.8 (1.1)	---------

* Each value is the mean (±s.d.) for 3-6 rats.

Clinical data are shown in Figures 4, 5, 6 and 7.

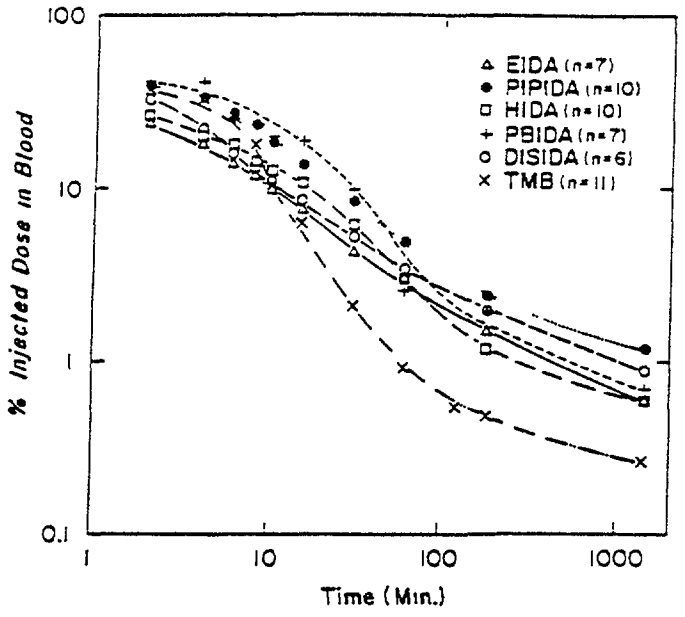


FIGURE 4. Blood clearance of ^{99m}Tc-IDA agents in normal subjects.

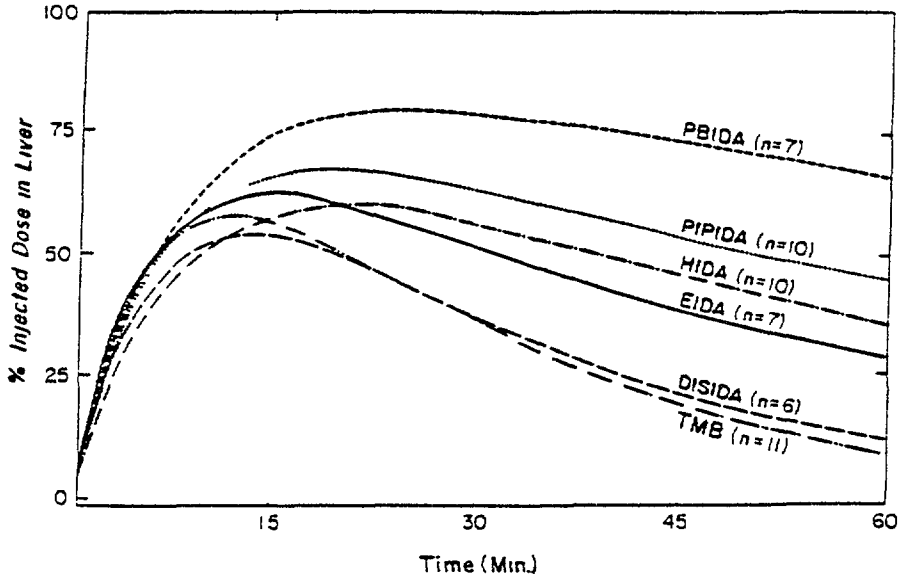


FIGURE 5. Hepatic uptake and excretion for ^{99m}Tc-IDA agents in normal subjects.

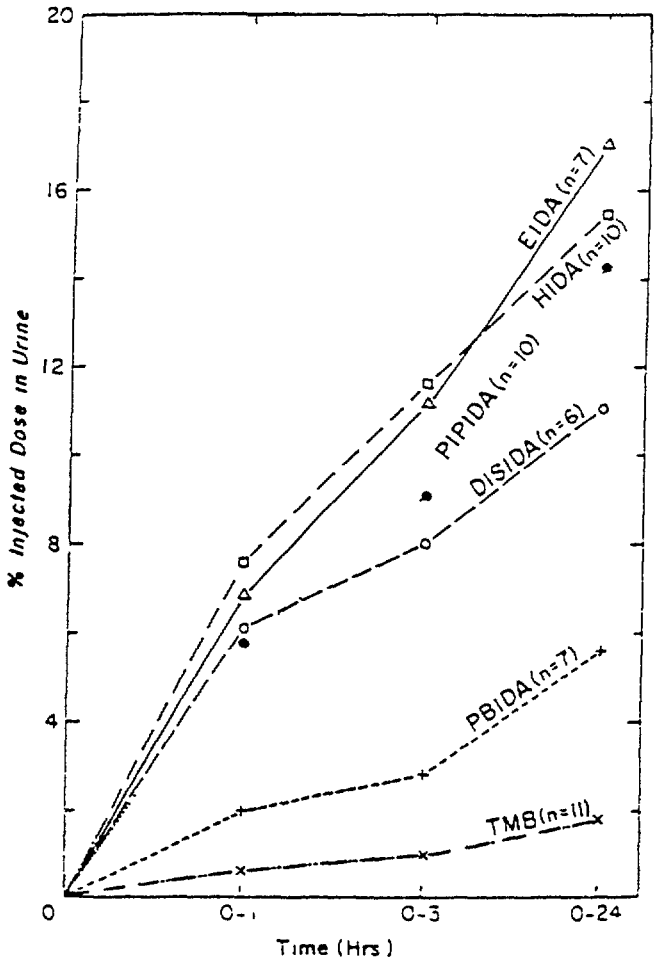


FIGURE 6. Urinary excretion of ^{99m}Tc-IDA agents in normal subjects.

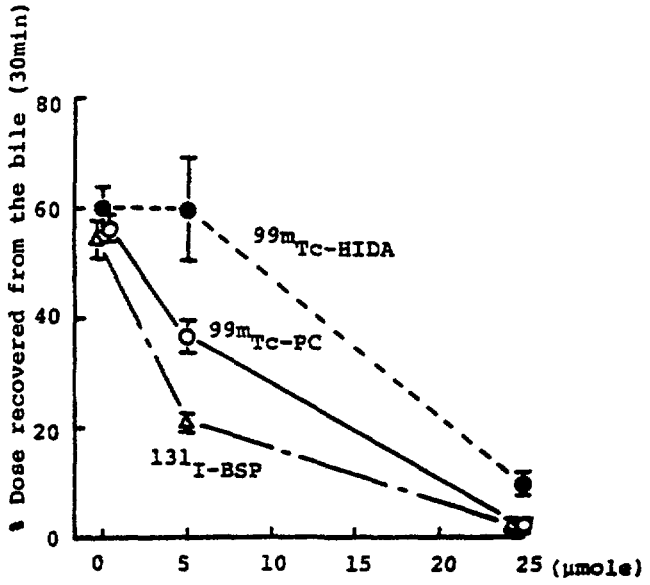


FIGURE 7.

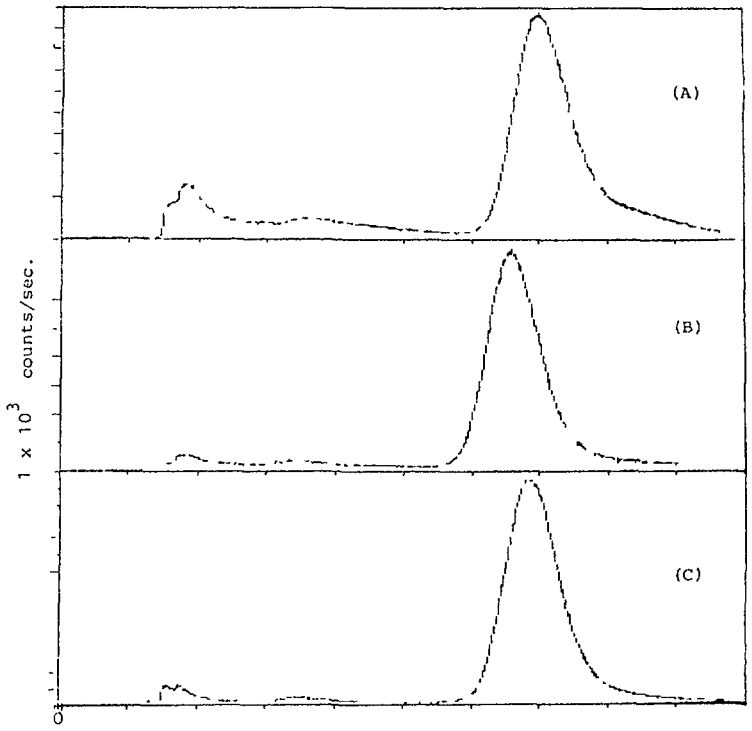
The evaluation of some new compounds synthesized by other participants of the CRP was done by A. León, S. Verdera and collaborators. The hepatobiliary agents studied were several phenylcarbamoyl IDA derivatives: (inluding Mebrofenin, Iodofenin, Cl-Bimida) and Phthalein Complexone. The investigations carried out allow to define the optimal kit formulation in a non-lyophilized form, control limits for their release, shelf-life under two different storage conditions and their "in vitro" stability. Comparative analysis of the biological behavior on experimental animals of these labelled molecules and some IDA-homologues, demonstrated that the halogenated IDA derivatives, Mebrofenin and Iodofenin, were the best compounds, with the greatest hepatobiliary excretion and the least renal elimination. That was confirmed by studies carried out in normal volunteers (6,8,9,10).

The use of non-conventional labelling methods was proved feasible: the electrolytic method for the chosen derivatives (diethyl, diisopropyl and p-n-butyl-IDA and Mebrofenin) (11) and the solid phase technique for Cl-Bimida.

The non-linear model $Ae^{kt} + B$ applied for the kinetic analysis of Iodofenin, Mebrofenin, some IDA-derivatives and phthalein complexone proved that a bicompartimental model was appropriate for the explanation of the experimental results obtained.

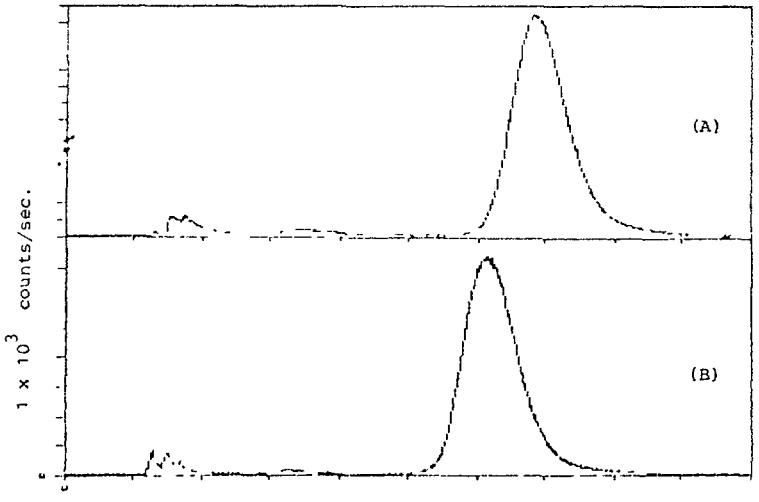
HPLC analysis of halogenated hepatobiliary agents was used to establish purity, lipophilicity, kinetics of the labelling reaction, "in vivo" stability and animal metabolites. The results obtained may indicate a bichelate structure for all the ^{99m}Tc-complexes studied as has been proposed by Loberg and Fields for ^{99m}Tc-HIDAs.

Good stability of ^{99m}Tc-Mebrofenin and ^{99m}Tc-Iodofenin was confirmed. Their kinetic study reveals that in both cases, complexation takes place via an intermediate which seems to disappear after 20 minutes. Similar behavior of these compounds to their biliary rat excretion products assumes that they are excreted essentially unchanged. Figures 8-11 show the reverse-phase HPLC analysis with 0.025M buffer phosphate pH 6.8 - methanol 50:50. Tables 5-6 show biodistribution in mice injected with the ^{99m}Tc-halogenated derivatives as well as biliary excrete of rats and mice.



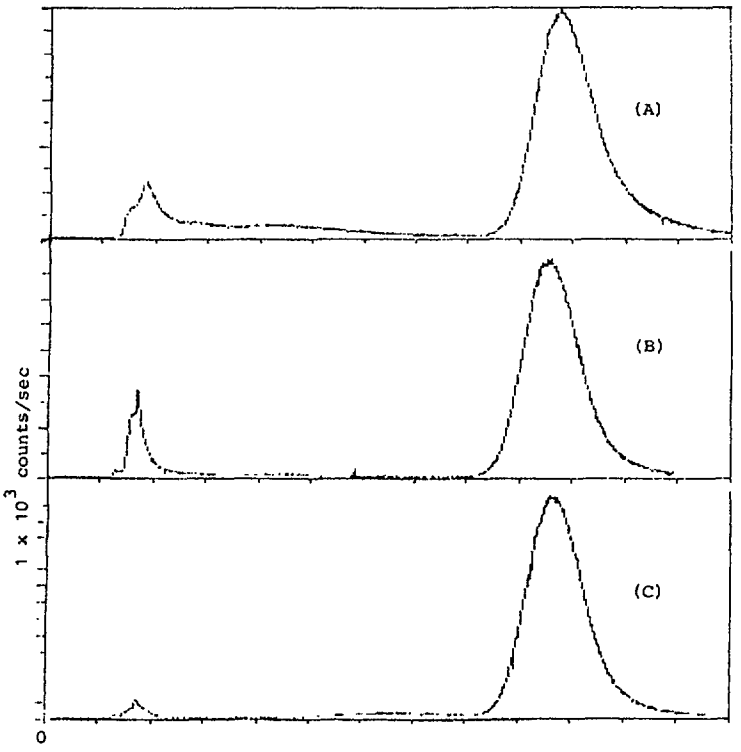
Retention time (sec. x 10^3)

FIGURE 8. HPLC (radiometric detection) of ^{99m}Tc-Mebrofenin at incubation time of (A) 0 min., (B) 15 min. and (C) 30 min.



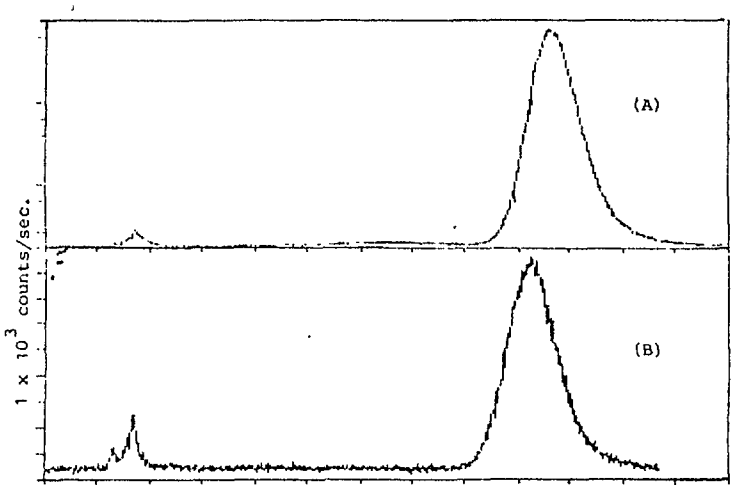
Retention time (sec. x 10^3)

FIGURE 9. HPLC (radiometric detection) of (A) ^{99m}Tc-Mebrofenin at incubation time from 30 minutes and (B) biliary rat excreta.



Retention time (sec. x 10^3)

FIGURE 10. HPLC (radiometric detection of ^{99m}Tc-Iodofenin at incubation times of (A) 0 min., (B) 15 min. and (C) 30 min.



Retention time (sec. x 10^3)

FIGURE 11. HPLC (radiometric detection) of (A) ^{99m}Tc-Iodofenin at 30 min. incubation time and (B) biliary

TABLE 5

BIODISTRIBUTION IN MICE INJECTED WITH ^{99m}Tc-MEBROFENIN AND BILIARY EXCRETA OF RATS (T = 30 min; % I.D. mean ± s.d.)

ORGAN	^{99m}Tc-MEBROFENIN (n = 10)	BILIARY EXCRETA (n = 3)
Blood	3,9 ± 3,9	4,3 ± 2,3
Liver	4,9 ± 2,3	3,3 ± 1,4
Intestines + Gallbladder	75,6 ± 6,8	72,0 ± 13,9
Urine	2,1 ± 2,1	2,1 ± 0,6

TABLE 6

BIODISTRIBUTION IN MICE INJECTED WITH ^{99m}Tc-IODOFENIN AND BILIARY EXCRETA OF RATS (T = 30 min; % I.D. mean ± s.d.)

ORGAN	^{99m}Tc-IODOFENIN (n = 10)	BILIARY EXCRETA (n = 3)
Blood	5,1 ± 4,9	0,90 ± 0,68
Liver	4,5 ± 1,4	0,89 ± 0,51
Intestines + Gallbladder	80,5 ± 2,8	96,2 ± 0,5
Urine	2,1 ± 1,9	0,44 ± 0,3

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2.2 HEART IMAGING AGENTS

At the present time, ^{99m}Tc MIBI has been developed as a heart imaging agent and is being evaluated as one of the most important radiopharmaceuticals. Because many problems remain in its practical use, the following studies on the development of ^{99m}Tc labelled heart imaging agent were carried out.

Subramanian et al. synthesized a new class of isonitriles using an aromatic ring (with substituents) attached to the isonitrile functional groups, Fig. 12. Appendix I gives more details on synthetic methods.

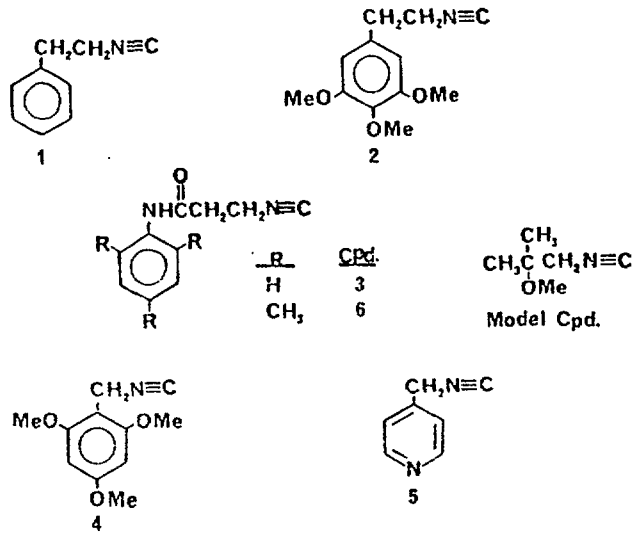


FIGURE 12.

The following labelling method was optimized for these compounds;

1) Dissolve isonitrile in ethanol.
   Take 300 ug in 60 ul of ethanol.

2) Add 300 ug formamidine sulfinic acid in 0.3 ml of NaAc pH 5.5.

3) Add 1.8 ml of ^{99m}Tc pertechnetate.

4) Heat for 10 minutes in boiling water bath.

The quality of ^{99m}Tc labelled compounds was determined by HPLC: analytical condition and Rf values of ^{99m}Tc-complex and pertechnetate are, as follows:

Reverse phase Whatman KC18-F
Solvent : Methanol : ACN : 0.5M NH_4 acetate : THF
as 3 : 3 : 2 : 2
Tc complex R_f = 0.5 - 0.60
Pertechnetate R_f = 0.9 - 0.95

Biodistribution studies performed in rabbits demonstrated that aromatic isonitriles localized in the myocardium of experimental animals similar to the best aliphatic isonitriles. Scintigraphic result using ^{99m}Tc complex of Compound 2 is shown in Fig. 1 of Appendix II.

In addition, Subramanian et al. elucidated the reproducible synthetic methods for MIBI (Appendix I).

Hunt et al. studied the development of a new series of cationic complex for heart imaging, using dialkyl and pyrrolidinyl bis-tertiary amino alkyl amides (Fig. 13).

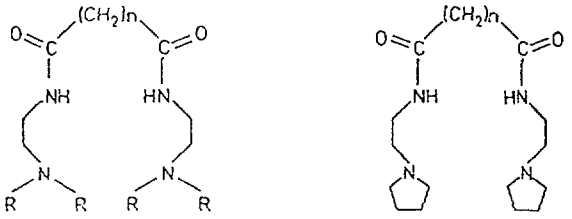


FIGURE 13.

According to the method reported by Philips et al., the synthesis of (A) and (B) was carried out by the reaction of dicarboxylic acid methyl or ethyl ester with the appropriate substituted aminoethyl amine. ^{99m}Tc labelling of these compounds was achieved using a variety of reducing agents such as sodium dithionite, stannous tartrate and others yielding ^{99m}Tc complexes which were cationic on electrophoresis.

The biodistribution results in rats of ^{99m}Tc labelled N,N'-bis (dimethylaminoethyl) succinamide (Fig. 13(A), n = 2) and N,N'-bis (pyrrolidinoethyl) oxalamide revealed that despite being cationic, the ^{99m}Tc complexes failed to localize in the myocardium confirming that cationic character alone is insufficient for myocardial deposition (APPX). Further studies on relationship between the structure of ^{99m}Tc complexes and biodistribution are now in progress.

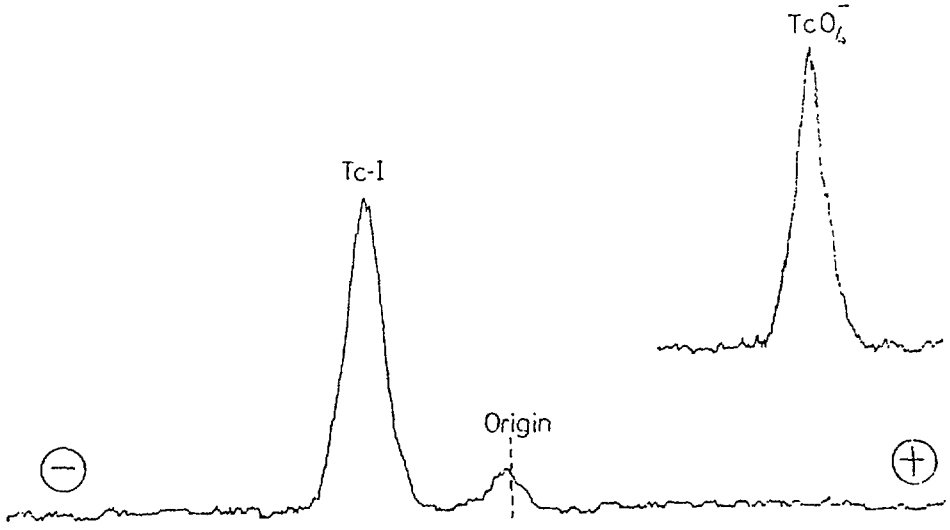


FIGURE 13 (A).

2.3 BRAIN IMAGING AGENTS

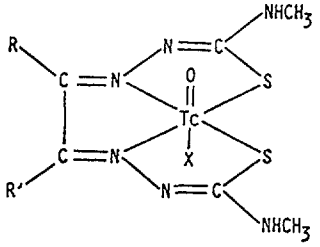
The changed emphasis of the CRP following the first and second coordination meetings to include brain imaging agents, has led to the development by several participants in the program of new structural classes of ^{99m}Tc chelates for this organ as well as new and more facile synthetic methods for brain radiopharmaceuticals already in clinical use.

In addition, quality control procedures for ^{99m}Tc-HMPAO have been examined in detail and improved.

(a) Thiosemicarbazone Complexes of ^{99m}Tc

Following the initial success in imaging the brain using ^{99m}Tc-glucosothiosemicarbazone, Yokoyama et. al. have synthesized additional dithiosemicarbazone (DTS) ligands with the objective of forming Tc complexes of low toxity with higher stability and lipophilicity. The approach was to synthesize 5-6-5 membered ring systems as shown below (II) which has enhanced metal chelate stability compared to the 5-5-5 system (I) (Fig. 14).

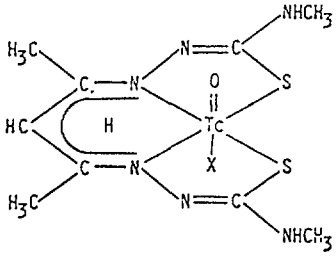
5,5,5-membered rings



D A T S I

[diacetyl-bis(N-methyl-thiosemicarbazone)]

5,6,5-membered rings



P E T S II

[pentane-2,4-dione-bis(N-methylthiosemicarbazone)]

FIGURE 14.

PETS was synthesized by the condensation of acetylacetone with N-methylsemicarbazide in the presence of ethylenediamine. Tc-99m labelling was carried out by the stannous method at pH 8 and the neutral chelates formed were extracted by n-hexane. The reverse phase HPLC analysis revealed a formation of two kinds of neutral chelates, that is, (a) fast and (b) slow-eluting chelates; both presented very high partition coefficients (octanol), 260(a) and 820(b), respectively, and great stability in mice sera. Biodistribution in mice showed the presence of very high radioactivity in brain, % injected dose per organ of 3.44 for (a) and 4.92 for (b) at 2 min postinjection, indicating a formation of neutral and compact chelate of (a) and (b). Lipophilicity and stability of these new ^{99m}Tc PETS chelates were much higher than any other 5-5-5 membered ^{99m}Tc DTS derivatives reported.

Thus, the increased ring size, 5-6-5 ring structure conferred a more stable character to DTS chelates. As for the structure of (a) and (b) chelates, preliminary studies strongly suggest the formation of Tc(V)-octahedral and Tc(V) squarepyramidal chelates, respectively. Better characterization of these chelates and development of new bifunctional radiopharmaceuticals are in progress.

(b) Improved Synthesis and Isolation of HMPAO

Because ^{99m}Tc uptake in the brain depends on the ^{99m}Tc-complex of dl-HMPAO rather than the meso complex, an improved method for the synthesis and isolation of this ligand was reported by Subramanian as developed by Schneider in his laboratories.

The details of the synthesis are reported in Appendix lII and have the advantage of improved yields over the previously reported method.

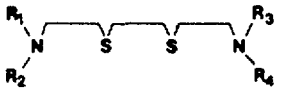
As the efficient separation of the meso from the dl forms is best performed by repeated recrystallisation, or preparative HPLC, the classical procedure of handpicking the morphologically different crystals under magnification was resorted to. Separated crystals were pooled and recrystallised to constant melting points which

was the critical parameter. This low cost separation method is advantageous for laboratories not having advanced instrumentation (prep. HPLC) or skilled staff.

(c) ^{99m}Tc Complexes with $N_X S_2$ Ligands

Following the initial work by Kung et al. (1,2) on bis aminoethane dithiol complexes of ^{99m}Tc for brain imaging, Chiotellis et al. (3) have prepared a large number of aminothiol derivatives containing different substituents in order to optimise brain uptake and retention. Reference (4) gives the most recent results.

The structure of the two classes of compounds studies are shown in Figures 15 and 16.



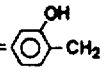
$R_1=R_2=R_3=R_4=H$		[1]	$R_1=R_2=R_3=H$	$R_4=n-C_4H_9$	[2]
$R_1=R_2=R_3=H$	$R_4=i-C_4H_9$	[3]	$R_1=R_2=R_3=H$	$R_4=$	[4]
$R_1=R_2=R_3=H$	$R_4=$ 	[5]			
$R_1=R_2=R_3=H$	$R_4=$ 	[6]			
$R_1=R_3=H$	$R_2=R_4=C_2H_5$	[7]	$R_1=R_3=H$	$R_2=R_4=$ 	[8]
$R_1, R_3=R_2, R_4=$ 		[9]			
$R_1=R_3=H$	$R_2=R_4=$ 	[10]			

FIGURE 15. Derivatives of 1,8-diamine-3,6-dithio-octane.

The synthetic methods used to prepare these compounds as well as comparative biodistribution data in mice are included in Appendix IV, as described by Chiotellis et al. The labelling with technetium was performed using sodium borohydride. The technetium complex was extracted with organic solvent and purified by HPLC. Most derivatives were analysed in more than one radioactive fraction with different retention times. The fractions were

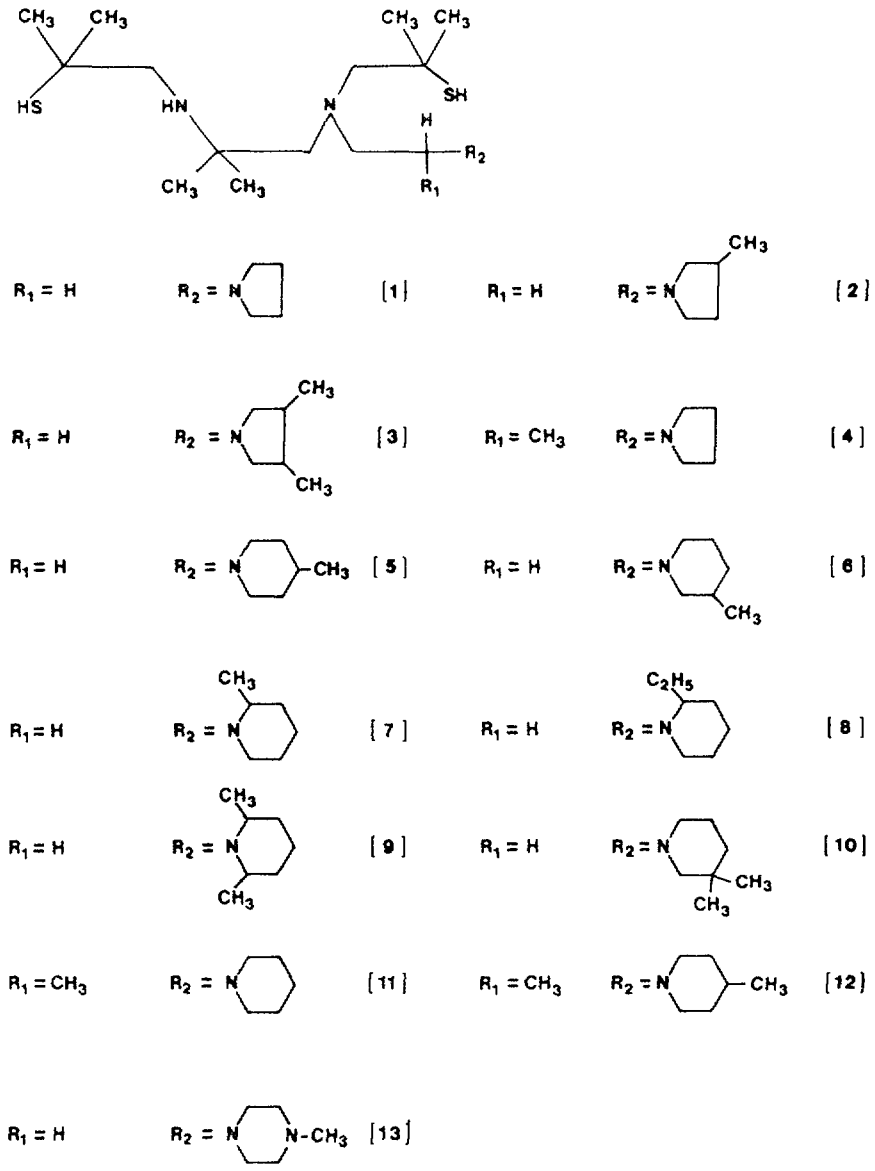


FIGURE 16. Aminothiol derivatives (Group B).

biodistributed in experimental animals. Certain technetium complexes of group A derivatives showed considerable retention in brain tissue.

Complexes of group B showed significant cerebral uptake, while retention time in brain cells varied with the substituent. Representative technetium complexes of group B were further evaluated in dogs in comparison to commercial brain agents. Chemical and biological data are presented in the figures and tables in the appendix.

The best compounds of all those evaluated were the methyl pyrrolidine-substituted and the methyl piperidine-substituted aminodithiols, however, both compounds had faster washout times from the brain compared with ^{99m}Tc-HMPAO.

(d) ^{99m}Tc-d,l-HMPAO

Because it is very expensive, a reproducible synthetic method for the parent ligand is presented. Different d,l-HMPAO/$SnCl_2.2H_2O$ ratios for freeze-dried kit formulations were studied. The composition recommended to obtain the highest radiochemical yield is the following: 250 µg d, l-HMPAO and 5 µg $SnCl_2.2H_2O$. Kit stability: 2 months stored at 20°C (Room Temperature). The optimum labelling conditions recommended are: to the freeze-dried kit, add no more than 20 mCi of ^{99m}Tc-pertechnetate, diluted to 5 ml with saline solution purged with N_2. Vortex mix it for 3 minutes.

Use the eluate from a ^{99m}Tc-generator which was previously eluted within 24 hrs and not more than 2 hrs following elution. Labelling of d,l-HMPAO produces a mixture composed of the desired lipophilic ^{99m}Tc-d,l-HmPAO complex, free $^{99m}TcO_4^-$, reduced-hydrolyzed ^{99m}Tc and a less lipophilic secondary ^{99m}Tc-complex. Physicochemical and biological methods were further evaluated by A. León, E.S. Verdera and collaborators.

The chromatographic procedure reported by Neirinckx et al. (JNM 28:191-202, 1987) allows the quantitative determination of the three potential radiochemical impurities (using 3 chromatographic systems). This procedure takes a long time to arrive to the final result, therefore, other methods for a quicker routine quality control of the labelled product were established. The following is a recommended method to obtain a direct and reproducible measurement of the lipophilic complex, in a short time:

1. Whatman 31ET - $CHCl_3$
2. Whatman 31ET - diethylether
   colloid, $^{99m}TcO_4^-$ - and ^{99m}Tc-secondary complex remain at origin and the ^{99m}Tc lipophilic complex runs with Rf 0.8-1.0 (system 1) and 0.7-1.0 (system 2).
3. Octanol and ethylacetate extractions.

A clear decomposition of the primary complex after one hour post-labelling was observed. The secondary complex increases with time, (11% at 1st hour, 14% at 3 hours). $^{99m}TcO_4^-$ at the same time showed an increase of more than 4 times. It is recommended to use the radiopharmaceutical within 30 minutes of reconstitution.

A latest report (J.N.M: 29:981, 1988) indicates that addition of microgram quantities of gentisic acid within 30 seconds of labelling prolongs the life of the lipophilic component to several hours. HPLC analysis was also developed using Varian Micro Pak MCH-5-n capp column and 0.025M phosphate buffer pM 6.8 - methanol mixture (25:75) as solvent.

HPLC analysis demonstrates the presence of at least two different radioactive impurities: free ^{99m}Tc eluted with void volume and the other with a retention time shorter than that corresponding to the main product, which is assumed to be the secondary complex. This method provides a quality control information, with a good correlation with results obtained by Neirinckx's system.

Electrophoresis analysis suggested that both ^{99m}Tc-complexes are neutral. As a result of the electric field applied, instability of ^{99m}Tc-d,l-HMPAO increases with time.

Biodistribution studies performed in mice showed brain uptake immediately after injection, (1.4 ± 0.3% at 1 min) remaining constant within the time studied (1 hour). Urinary system is the main excretion route but activity also clears through hepatabiliary pathway. From the in vitro results obtained it could be proposed that the mechanism of retention in the brain of the ^{99m}Tc-d, l-HmPAO results from the in vivo conversion of the primary complex, not only to the secondary complex but also to ^{99m}Tc-pertechnetate.

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2.4 MÖSSBAUER SPECTROSCOPY STUDIES OF COMPOUNDS IMPORTANT FOR RADIOPHARMACEUTICALS

The lyophilized complexes of different Tin(II) compounds constitute the basic part of most kits used for preparations of radiopharmaceuticals. Better knowledge of these tin compounds may be considered as indispensable for good kit formulation.

Mössbauer spectroscopy may give information about tin valence states and its distribution in lyophilized tin complexes formed under different conditions.

The main scope of this project is the application of Mössbauer spectroscopy techniques to study compounds important for radiopharmaceuticals. Lyophilized stannous chloride-ascorbic acid preparations and lyophilized N-(2,6 dimethylacetanilido) iminodiacetic acid (HIDA) and N-(2,6 disopropylacetanilido) iminodiacetic acid (DISIDA) have been examined by Mössbauer spectroscopy.

Part 1: Lyophilized Mixture of Stannous Chloride and Ascorbic Acid

Ascorbic acid is one of the basic constituents of ^{99m}Tc(Sn)-N-pyridoxylaminates kits and it may be used as antioxidant in other kits. Mössbauer spectra of lyophilized stannous chloride-ascorbic acid mixture was prepared by J. Stevovic at pH 10.0 and at pH 2.56 are presented on Fig. 17 and Fig. 18, respectively. The parameters of hyperfine interaction are given in Table 7.

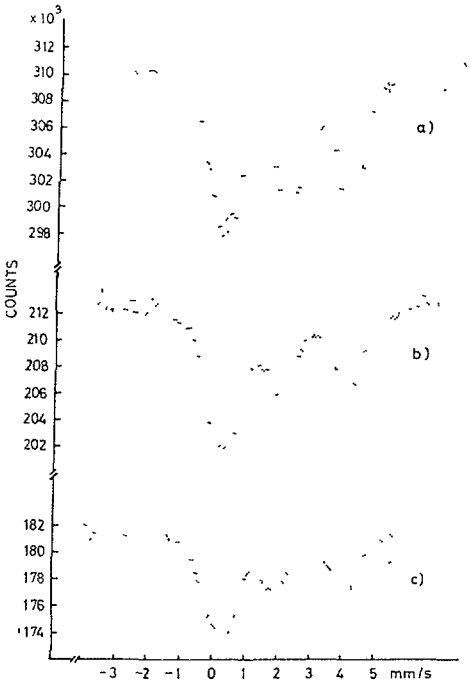

FIGURE 17.
Mössbauer spectra: $SnCl_2.2H_2O$-ascorbic acid preparation, lyophilized (pH 10.0)
Sn:ascorbic acid mole ratio: a)1.48, b)1.32, c)1:16

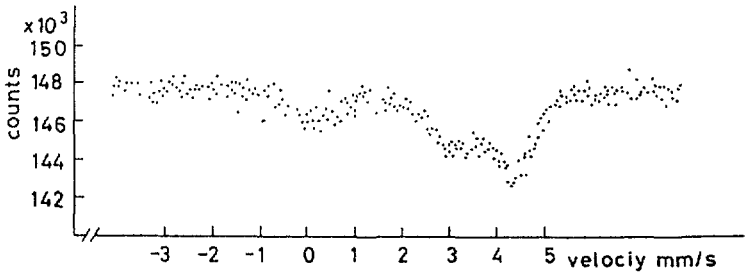

FIGURE 18.
Mössbauer spectrum: $SnCl_2.2H_2O$-ascorbic acid preparation, lyophilized (pH 2.56), Sn:ascorbic acid mole ratio 1:32

TABLE 7

SUMMARY OF MÖSSBAUER PARAMETERS FOR
LYOPHILIZED STANNOUS CHLORIDE-ASCORBIC ACID PRODUCTS

mole ratio	pH	Sn(II) IS mm/s	Sn(II) QS mm/s	Sn(IV) IS mm/s	Sn(II) %	Sn(IV) %
1:48	10.0	2.97	1.93	0.16	60	40
1:32	10.0	2.92	2.07	0.16	53	47
1:16	10.0	2.88	2.08	0.16	50	50
1:32	2.56	3.62	1.16	0.16	78	22

Mössbauer spectra of lyophilized products prepared from stannous chloride and ascorbic acid solutions at different mole ratios (1:16, 1:32, 1:48) at pH 10.0 show that tin appears in two oxidation states: as Sn(II) with the isomeric shift of 2.92 mm/s and the quadrupole splitting of 2.0 mm/s and as Sn(IV) with the IS of 0.16 mm/s. The relative distribution of tin between Sn(II) and Sn(IV) was evaluated as 60-50% and 40-50% respectively for different mole ratios of reactants. It shows that the ascorbic acid alone could not be considered as a very efficient protector for stannous tin in the initial alkaline solution (pH 10.0).

The Mössbauer spectrum of lyophilized product prepared from initially acidic solutions (pH 2,56, mole ratios of reactants 1:32) shows the Sn (II) with the IS of 3.62 mm/s and the QS of 1.16 mm/s and the Sn(IV) with the IS of 0.16 mm/s.

Part 2: Lyophilized Mixture of Stannous Chloride and IDA Derivatives

Lyophilized Sn(II) complexes of two IDA derivatives: N-(2,6 dimethyl-acetanilido) iminodiacetic acid (HIDA) and N-(2,6 diisopropylacetanilido) iminodiacetic acid (DISIDA) have been examined by Mössbauer spectroscopy by J. Stevovic and B. Zmbova.

Mössbauer spectra of lyophilized stannous chloride-HIDA mixture and lyophilized stannous chloride-DISIDA mixture are presented in Figs. 19 and 20, respectively, and the parameters of hyperfine interaction are given in Table 8.

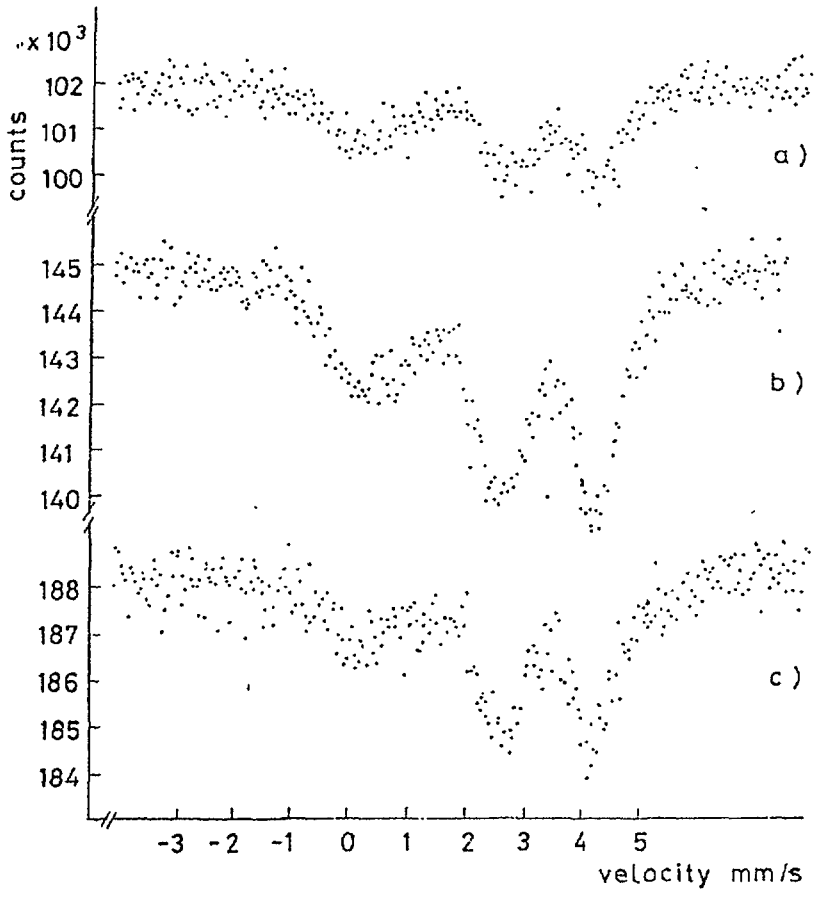

FIGURE 19. Mössbauer spectra: Sn(II)-HIDA preparations, liophilized Sn:HIDA mole ratio: a)1:8.8, b)1:4.4, c)1:2.25

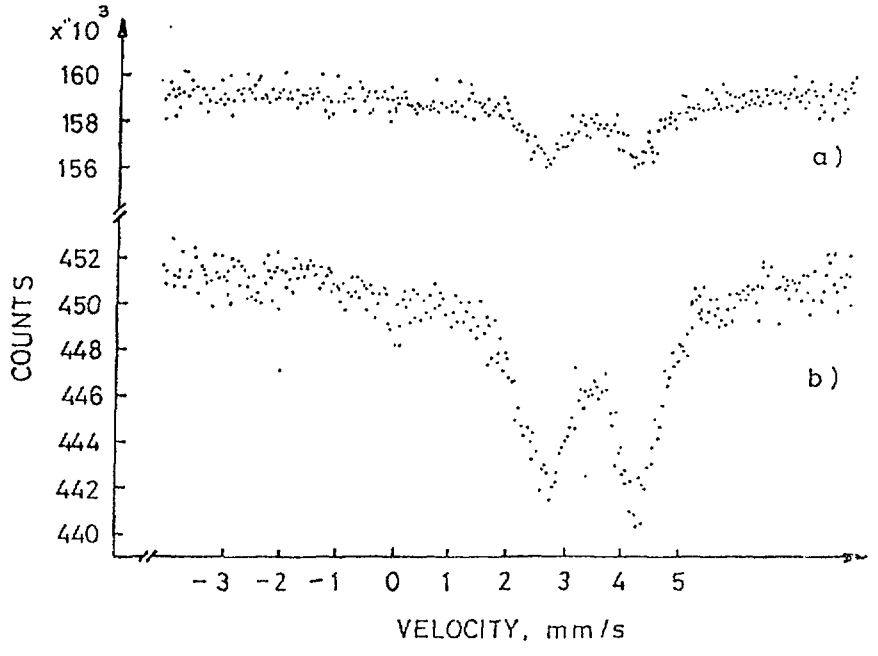

FIGURE 20. Mössbauer spectra: Sn(II)-DISIDA preparation, lyophilized Sn:DISIDA mole ratio: a) 1:4, b) 1:2.4

TABLE 8

SUMMARY OF MÖSSBAUER PARAMETERS FOR
LYOPHILIZED STANNOUS CHLORIDE-IDA DERIVATIVES

Products	mole ratio	Sn(II) IS mm/s	Sn(II) QS mm/s	Sn(IV) IS mm/s	Sn(II) %	Sn(IV) %
Sn-HIDA	1:2.25	3.32	1.53	0.18	82	18
Sn-HIDA	1:4.4	3.32	1.56	0.24	77	23
Sn-HIDA	1:8.8	3.33	1.40	0.18	72	28
Sn-DISIDA	1:2.4	3.33	1.56	-		
Sn-DISIDA	1:4	3.32	1.54	-		

Mössbauer spectra of lyophilized preparations prepared from fresh stannous chloride and HIDA solutions at different mole ratios (1:2.25, 1:4.4, 1:8.8) at pH 5.5 show that tin appears in two oxidation states: one of them was identified as Sn(II) with the IS of 3.32 mm/s and QS of 1.50 mm/s; the other was Sn(IV) with the IS of 0.20 mm/s. The relative distribution of tin between two valence states Sn(II) and Sn(IV) were evaluated as 82-75% and 18-25%, respectively, for preparation with different mole ratios of reactants.

The Mössbauer spectra of lyophilized products prepared from fresh stannous chloride and DISIDA solutions (mole ratios 1:2.4, 1:4) at pH 5.5 reveal that tin appears as Sn(II) with the IS of 3.32 mm/s and the QS of 1.54 mm/s.

High yields of stannous tin in lyophilized Sn-DISIDA and Sn-HIDA preparations indicate that they are reliable for preparations of corresponding ^{99m}Tc-radiopharmaceuticals.

2.5 CHEMICAL STUDIES ON DTPA-CONJUGATED MONOCLONAL ANTIBODY OF HUMAN FIBRINOGEN LABELLED WITH RADIOINDIUM FOR THROMBOSIS SCINTIGRAPHY

INTRODUCTION

The uses of monoclonal antibody radiopharmaceuticals in radioimmunodiagnosis and radioimmunotherapy are of major research interest (1-3). Although monoclonal antibodies labelled with radioiodine are still in

use, they have unfortunate tendency to deiodinate in-vivo and often their immunospecificity is reduced by the harsh conditions of iodination, thus limiting their utility.

Alternatively, monoclonal antibodies are being labelled with metal ion radionuclides using bifunctional chelating agents covalently coupled to the protein. Under this project, Li Yongjian and Lan Lixiang have developed a method to couple diethylenetriamine- pentaacetic acid (DTPA) to a monoclonal antibody and successfully radiolabelled it in high yield with indium-114m and indium-111.

MATERIALS AND METHODS

The radionuclide indium-114m was supplied by the Chinese Institute of Atomic Energy, and indium-111 was produced by the cyclotron of SINR (Shanghai) and iodine-125 obtained from Amersham Co. Monoclonal antibody of $IgG_{2\alpha}$ against human fibrinogen (MoAb) with specificity to bind thrombus was developed and supplied by the Shanghai Institute of Cancer Research. The antigen human fibrinogen of 90% clotting activity (hFbg) and a secondary antibody, mouse $IgG_{2\alpha}$ immuned sheep anti-serum (PoAb) with a titer of 1:32, were supplied by the Shanghai Research Institute of Biological Products. All other reagents and solvents were of reagent grade.

Preparation of bicyclic diethylenetriaminepentaacetic anhydride (BC-DTPA). DTPA was first precipitated from its saturated solution by equal volume of ethanol, washed with acetone and ether, dried at 50°C for 2 h. BC-DTPA was synthesized by a method similar to that of Eckelman (4). 39.5 g (0.1 M) DTPA in 50 ml pyridine and 40.8 g (0.4 M) acetic anhydride were mixed and stirred at 65°C for 24 h. The precipitate was removed by filtration, washed with acetic anhydride, then quickly with anhydrous ether and dried at 50-60°C. Its m.p. 179-180°C was in good agreement with that reported before (5). The IR spectra showed the carbonyl bands at 1820 cm^{-1}, 1773 cm^{-1} without the hydroxyl bands 3200-3700 cm^{-1} of DTPA, a strong band at 1639 cm^{-1} reflected its central carboxyl group was able to ionize, thus it was amphoteric. The NMR spectra also showed the expected chemical shifts at δ = 4.1 ppm, δ = 3.6 ppm. It was confirmed to be bicyclic structure with no mixed anhydrides. It was stable at room temperature in dry atmosphere and showed no detectable change for several months.

Conjugation of BC-DTPA with MoAb. Aliquots of BC-DTPA suspension in chloroform (1 mg/ml) in test tubes were dried by purified nitrogen stream. MoAb solutions (0:25-2.6 mg/ml) buffered by 0.1 M PBS (pH 6-9), were added and shaked at room temperature for different time intervals (5-60 min). Hydrolyzed BC-DTPA by boiling in PBS was prepared in the same way for use as control. The DTPA-conjugated MoAb (DTPA-MoAb) was separated by Sephadex G-50 column pre-equilibrated with 3% BSA (pH 7.4) at 4°C. DTPA-MoAb was measured spectrophotometrically at 280 nm UV.

Determination of antigen binding capacity. ^{125}I-hFbg was prepared by a modified chloramine-T method (6). The immunological specificity of MoAb and DTPA-MoAb toward PoAb was tested by double agar diffusion method using PoAb dilution from 1:1 to 1:32 and incubation at 37°C for 24 h. Both MoAb and DTPA-MoAb were immunoreactive to PoAb at dilutions 1:1-1:8.

The antigen binding capacity was tested as follows: 100 µl of MoAb, DTPA-MoAb and its hydrolysate at dilutions 0.01 µg/ml-100 µg/ml in 0.1 M PBS (pH 7.4) was added 100 µl (150,000 cpm)^{125} I-hFbg and 100 µl (2 mg/ml, 0.05 M PBS pH 7.4) human gamma-globulin incubated 2.5 h at 37°C then stood overnight at 4°C. To each sample was added 100 µl (1:2 dilution) PoAb, mixed and incubated under 37°C with frequent shaking, added 21% polyethylenglycol, counted T, centrifuged (4000 rpm, 20 min), washed once by physiological saline and counted B. By plotting B/T % vs antibody concentration the relative antigen binding capacity was found.

Determination of DTPA bound per MoAb. DTPA was determined indirectly through indium molecules it chelated. DTPA-MoAb and indium in the ratio M(MoAb):M(In)=1/10 in solution and stood at room temperature for 30 min with frequent shaking, added appropriate amount [M(EDTA):M(In)=2] of EDTA (0.1 mg/ml) stood 10 min, separated by Sephadex G-50 column (diameter 0.7x20 cm, treated as above) and assayed.

Labelling of DTPA-MoAb with ^{114m}In (^{111}In). Using the optimal conditions obtained above: DTPA/MoAb (c/p)=20, MoAb concentration= 0.5-1.0 mg/ml, pH 7.4 (PBS), reaction time 30 min at room temperature, it was dialysized at 37°C to test stability.

RESULTS AND DISCUSSION

Monoclonal antibody labelling with radioindium via DTPA-conjugation is performed quickly (in few minutes) under mild conditions (pH 7.4, room temperature). The immunological activity was noticeable affected by the number of DTPA bound to each MoAb (Fig. 5), this is equal to the ratio of indium molecules chelated per molecule of MoAb in DTPA-MoAb [M(In)/M(MoAb)]. The ratio M(In)/MMoAb was determined by the conjugation parameters: the proportion of DTPA to MoAb (c/p) used, the MoAb concentration (μg/ml), pH of the system and conjugation time (T_c). The influence of c/p on In/MoAb was effective, and noticeable at c/p $\leq$ 20 (Fig. 21); the influence of MoAb concentration was proportional but less effective (Fig. 22); the influence of pH has an optimum (around pH = 7.8) (Fig. 23); the M(In)/M(MoAb) increased quickly with T_c within 20 minutes then kept constant (Fig. 24). The hydrolysis of DTPA-MoAb did not affect its antigen binding capacity (Fig. 25).

The radioindium labelled DTPA-MoAb preserved 89% of original immunological activity with its indium bound firmly to the molecule, more than 96% of initial radioactivity was retained after 24 h stability test. The average number of DTPA per molecule of MoAb was determined to be 1.6, this is very close to that reported by Hnatowich (7).

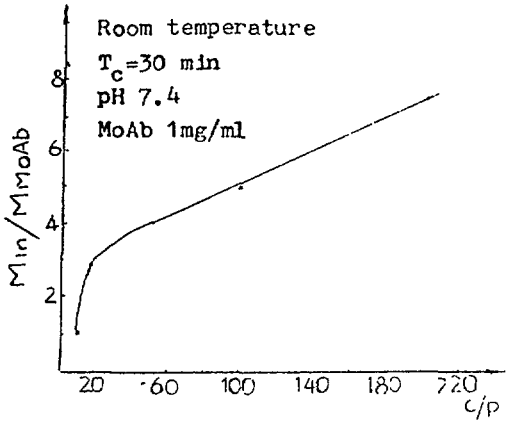

FIGURE 21. Influence of c|p.

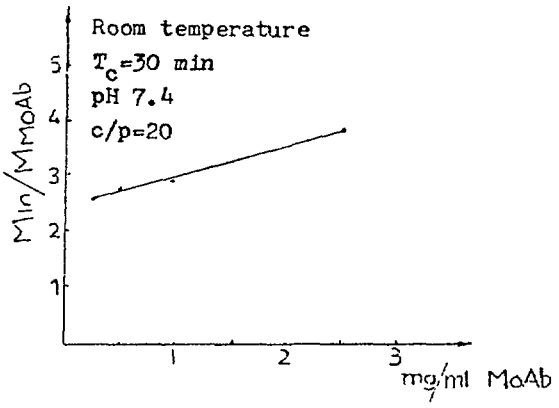

FIGURE 22. Influence of MoAb concentration.

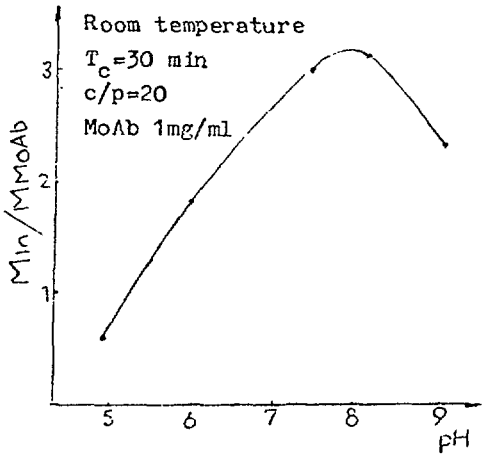

FIGURE 23. Influence of pH.

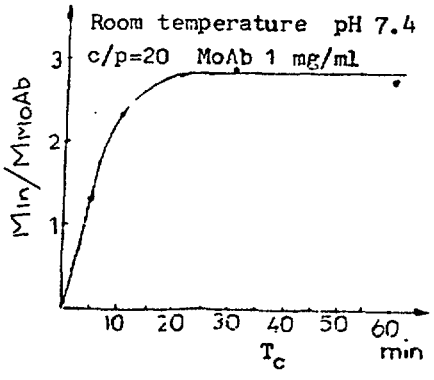

FIGURE 24. Influence of time.

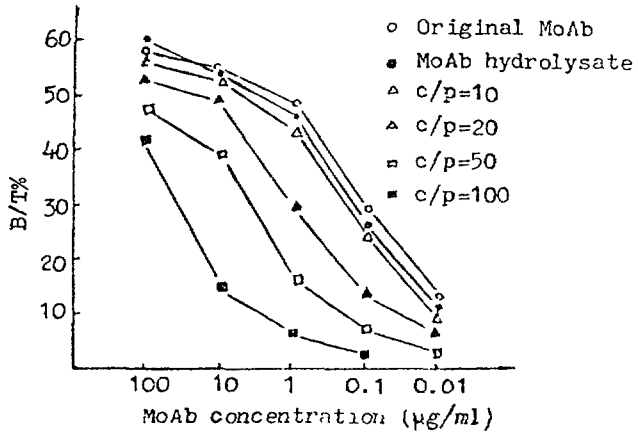

FIGURE 25.

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3. NEW COMPOUNDS STUDIED UNDER THE CRP

3.1 HEPATOBILIARY AGENTS

^{99m}Tc Agents

- N(4 Iodine - 2.6 diethylphenylcarbamoylmethyliminodiacetic labelled with 14C).
- 4 Iodine - 2,6 diethylphenylcarbamoylmethyliminodiacetic.
- N(3,4,5-triiodo)-acetanilido-iminodiacetate (^{99m}Tc-TIIDA)
- ^{99m}Tc-Diacetyl-bis (N-methylthiosemicarbazone) (^{99m}Tc-DATS)
- ^{99m}Tc-Pentanane-2,4-dione bis (N-methylthiosemicarbazone) (^{99m}Tc-PETS).
- ^{99m}Tc-3,3-Dimethyl-pentane-2,4-dione-bis (N-methylthiosemicarbazone) (^{99m}Tc-DM-PETS).
- Aromatic Isonitriles.
- N-(2,6-diisopropyl phenylcarbamoyl 1-ethyl)-IDA, (IDA-5)
- N(4-butyl " " ")-IDA, (IDA-6)
- N(4-butyl " " 2-ethyl)-IDA, (IDA-7)
- 3-bromo 2,4,6-trimethyl phenylcarbamoyl methyl) IDA (mebrofenin)
- 3-iodo " " " " ") IDA (iodofenin)
- (3-iodo-2,6-dimethyl phenylcarbamoyl methyl) IDA

^{67}Ga Agents

- ^{67}Ga-ethylenediamine di (O-hydroxyphenylacetic acid), ^{67}Ga-EDDHA
- ^{67}Ga-dimethyl EDDHA
- ^{67}Ga-t-butyl EDDHA
- ^{67}Ga-chloro EDDHA
- ^{67}Ga-dichloro EDDHA
- ^{67}Ga-bromo EDDHA
- ^{67}Ga-N,N'-bis (2-hydroxybenzyl) ethylenediamine N,N'-diacetic acid, (^{67}Ga-HBED)
- ^{67}Ga-dimethyl HBED
- ^{67}Ga-t-butyl HBED
- ^{67}Ga-chloro HBED
- ^{67}Ga-dichloro HBED
- ^{67}Ga-bromo HBED

^{111}In Agents

- ^{111}In-ethylene diamine di (o-hydroxyphenylacetic acid), ^{111}In-EDDHA
- ^{111}In-dimethyl EDDHA
- ^{111}In-t-butyl EDDHA
- ^{111}In-chloro EDDHA
- ^{111}In-dichloro EDDHA
- ^{111}In-bromo EDDHA
- ^{111}In N,N'-bis (2-hydroxybenzyl)ethylenedimine N,N'-diacetic acid,(^{111}In-HBED)
- ^{111}In-dimethyl HBED
- ^{111}In-t-butyl HBED
- ^{111}In-chloro HBED
- ^{111}In-dichloro HBED
- ^{111}In-bromo HBED

3.2 BRAIN AGENTS

^{99m}Tc-diaminotetramethyl dithiol, (DADT)

^{99m}Tc-hexylamine DADT

-aniline DADT

-dipropylamine DADT

-morpholino DADT

-iperidino DADT

-pyrrolidino DADT

-methyl pyrrolidino DADT

-amino ethyl piperidino DADT

^{99m}Tc-long chain aminodithiols (substituted)

3.3 HEART AGENTS

N,N-bis (dimethylaminoethyl)-succinamide

N,N-bis (pyrrolidinoethyl)-oxalamide

4. FUTURE DIRECTIONS AND RECOMMENDATIONS

Standardization of chemical synthesis, quality control, biological distribution of new radiopharmaceuticals can be achieved by collaboration and cooperation of inter-regional laboratories. Necessary and sufficient information on methodology, usually generated by CRPs like this one, may help very much in solving problems in the preparation of clinically useful radiopharmaceuticals in developing countries. It is found desirable to continue the organization of similar CRPs.

The participants in this program also feel that more information is needed on technetium chemistry and particularly on the chemical structure of the various complexes. The above interaction is very important for understanding biological behaviour of technetium complexes and therefore would be valuable for designing new technetium radiopharmaceuticals.

Therefore it may be very desirable to institute a new program on exchange of information on an international level perhaps through a CRP.

Further CRPs on nuclear medicine and radiopharmaceuticals on specific topics in these areas of radiopharmaceutical chemistry (Tc, In, etc.) would be of value to the IAEA Member States. Also, frequent specific purpose seminars on radiopharmaceutical conducted by the IAEA will be valuable on an international level especially in developing countries if the meetings are conducted in different regions of the world on a rotating basis. It is desirable to have these meetings in developing countries to generate enthusiasm on a regional level.

5. ACCOMPLISHMENTS OF THE CRP

(1) During the CRP, an exchange of scientific information between research agreement holders and research contract recipients was achieved. Also, this exchange of information was facilitated in the regional countries.

(2) Research projects generated under the CRP were used to train professionals (fellowship programme) and, in some cases, graduate students for doctoral dissertations in collaboration with local universities.

(3) The CRP program facilitated a significant increase in the scientific research level of some of the regional countries and also helped to transfer the technology to local countries in that region.

(4) Relevant information on clinical studies using new radiopharmaceutical was passed on to the interested countries.

(5) Radiopharmaceutical candidate chemicals developed and synthesized under the CRP were distributed to various countries.

(6) It is recognised that the research work performed under the research contract program could not have been realized without the financial support of the Agency.

Appendix I

PREPARATION OF 2-METHOXYISOBUTYLISOCYANIDE AND ITS CUPROUS CHELATE (MIBI)

N-(2-Methylallyl)formamide (1)

Ethylformate, 37.0g (0.5 moles) and 2-methylallylamine, 35.6g (0.5 moles) were refluxed gently for 17 hours. Distillation yielded 36.9g (79.7%) of (1) with a boiling point of 90-93° at 0.75 mm. Infrared: 3280 (NH), 3060 (amide overtone), 3040 (olefin) 1660 (NHC=O), 1520 (amide II) cm^{-1}.

N-(2-methoxyisobutyl) formamide (2)

Anhydrous methanol, 195 ml and mercuric acetate 61.5g (0.193 moles) were placed into a 1-liter flask and dissolved. Then over 5 minutes were added compound (1), 19.1g (0.193 moles). The reaction mixture was stirred at 25°C for 45 minutes. During this time the gummy precipitate that formed initially went into solution. The reaction mixture was placed into an ice bath and a prechilled mixture consisting of methanol, 400 ml and sodium hydroxide pellets, 46.4g (1.16 moles) was added over about 15 minutes. Finally, sodium borohydride, 3.65g (0.097 moles) was added keeping the temperature less than 20°C. The reaction mixture was then stirred 10 hours at 0-5°C. The supernatant liquid was decanted, through a celite coated filter, from the elemental mercury that had formed. Water, 400 ml, was added and the solution extracted 4 times with chloroform. Drying ($MgSO_4$) followed by distillation yielded 15.9g (62.8%) of (2) with a boiling point of 109-115°C at 1.5mm. Infrared: 3300 (NH), 3060 (amide overtone), 1670 (NHC=O), 1530 (amide II), 1160, 1180, 1070 (OCH_3) cm^{-1}.

2-methoxyisobutylisocyanide (3)

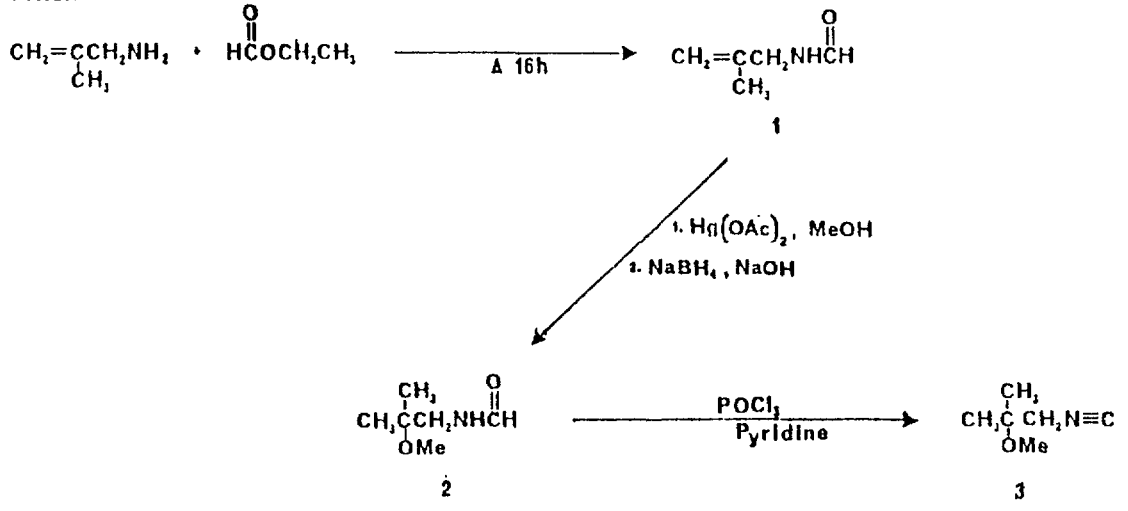
Compound (2), 15.9g (0.121 moles), pyridine, 63 ml (0.75 moles) and benzene, 37 ml were placed into a 200 ml flask in an ice bath. When the temperature reached 0-5°C there was added over 45 minutes phosphorous oxychloride, 11.1g (0.073 moles). The reaction mixture was heated at 65°C

for 45 minutes, chilled to 0-5°C and poured into 100 ml ice water. The organic layer was removed and the aqueous layer extracted 3 times with benzene. The combined benzene extracts were washed 2 times with water, dried ($MgSO_4$) and distilled to yield 6.4g (46.7%) of (3) with a boiling point of 55-57°C at 4.0 mm. Infrared: 2150 (N=C), 1180, 1160, 1070 ($-OCH_3$) cm^{-1}. Elemental analyses: C, H, N calc. 63.68, 9.80, 2.38, Found: 63.79, 9.91, 12.48.

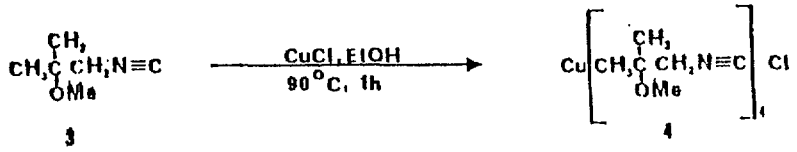
Tetra (2-methoxyisobutylisocyano)Copper (I) chloride (4)

Compound (3), 0.5g (4.42 mmole), anhydrous ethanol, 1.0 ml and anhydrous cuprous chloride 0.109g (1.10 mmoles) were placed into a screw capped vial and heated in a 90°C oil bath for 1 hour and then filtered once through paper and then through a 0.22μ filter. The solvent was evaporated to yield 0.62g (100%) of compound (4). Infrared: 3320 (OH), 2190 (chelated N≡C), 1180, 1160, 1070 ($-OCH_3$) cm^{-1}. Elemental analyses: C, H, N, Cl calc. 52.25, 8.04, 1016, 6.43, Found: 51.15, 7.91, 8.89, 5.52 TLC-Whatman KC18F plate with 3:3:2:2, methanol, acetonitrile, tetrahydrofuron, 0.5m NH_4OAc gave single spot Rf 0.83.

Scheme I

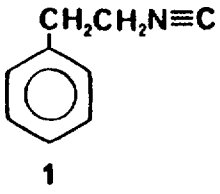


Scheme II

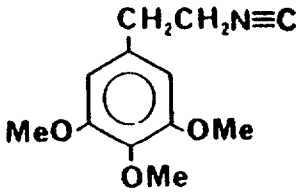


Appendix II

SYNTHESIS AND EVALUATION OF ^{99m}Tc COMPLEXES OF AROMATIC ISOCYANIDES AS POTENTIAL MYOCARDIAL IMAGING AGENTS



1



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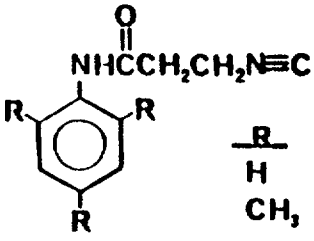
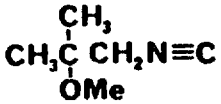
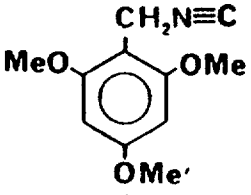


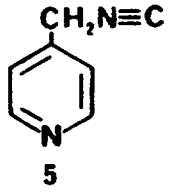
R	Cpd.
H	3
CH_3	6



Model Cpd.



4



5

LABELING METHOD

300 µg ISONITRILE IN 60 µl ETHANOL

300 µg FSA 1 mg/ml NaAC,pH 5.5

1.8 ml PERTECHNETATE

HEAT 100° 10 minutes

QUALITY CONTROL

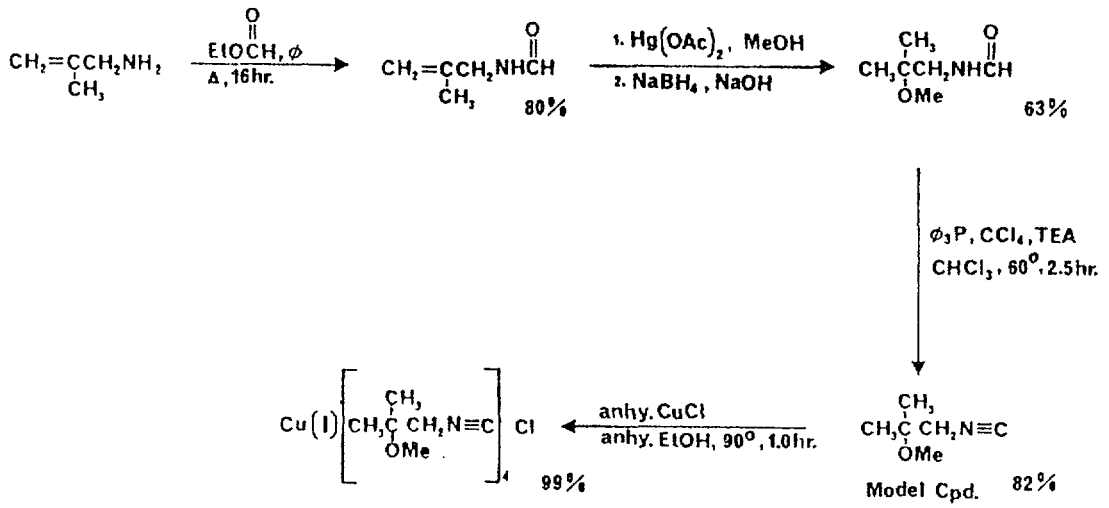
RPTLC - WHATMAN KC18-F

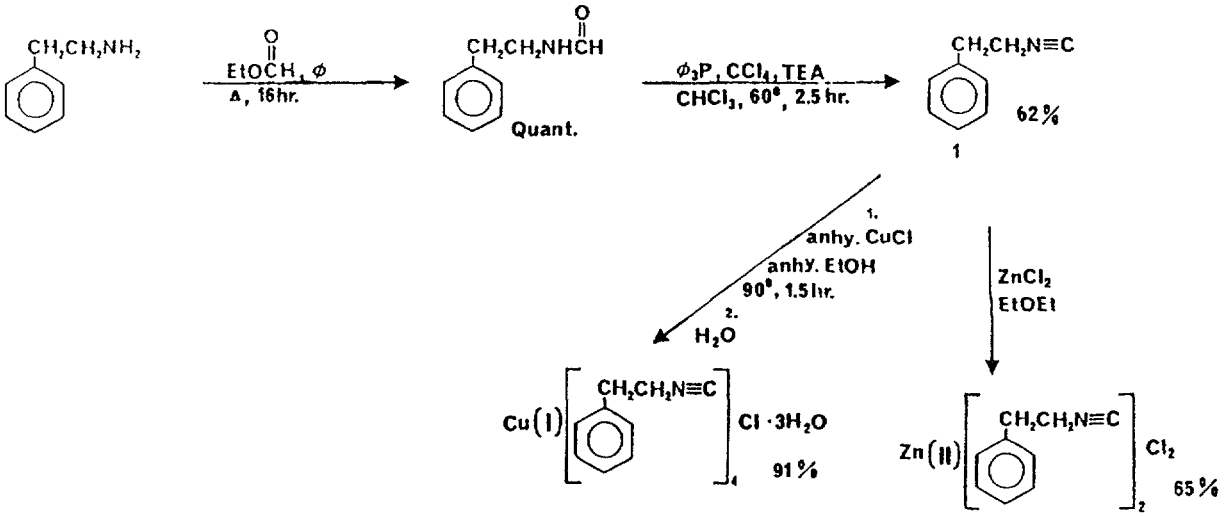
SOLVENT: MEOH: ACN: 0.5M NH_4AC, THF

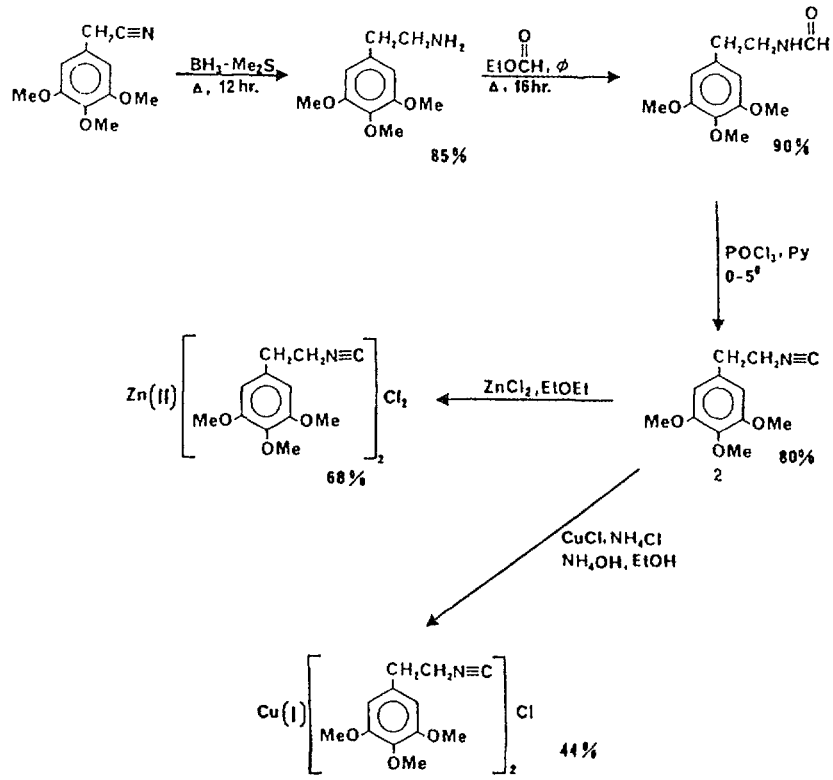
3:3:2:2

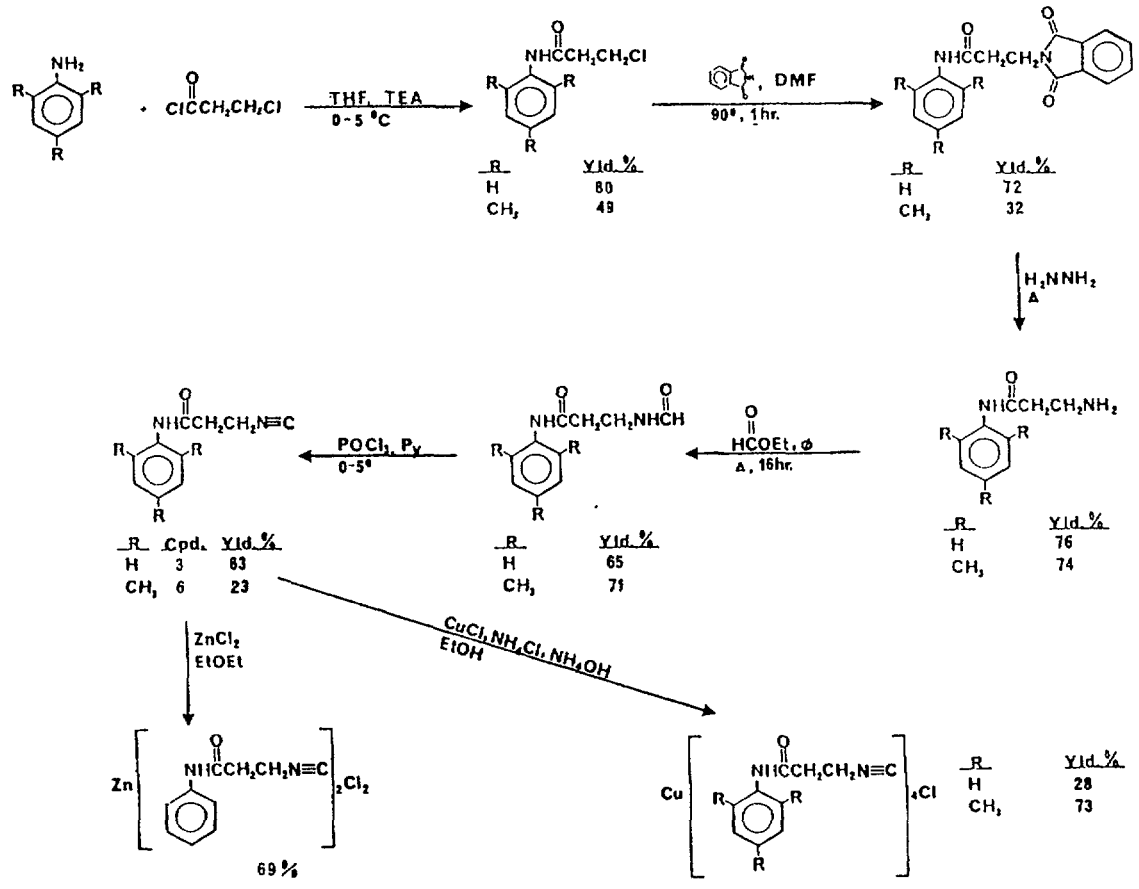
RF: Tc COMPLEX 0.54 (3,4,5, METHOXY)

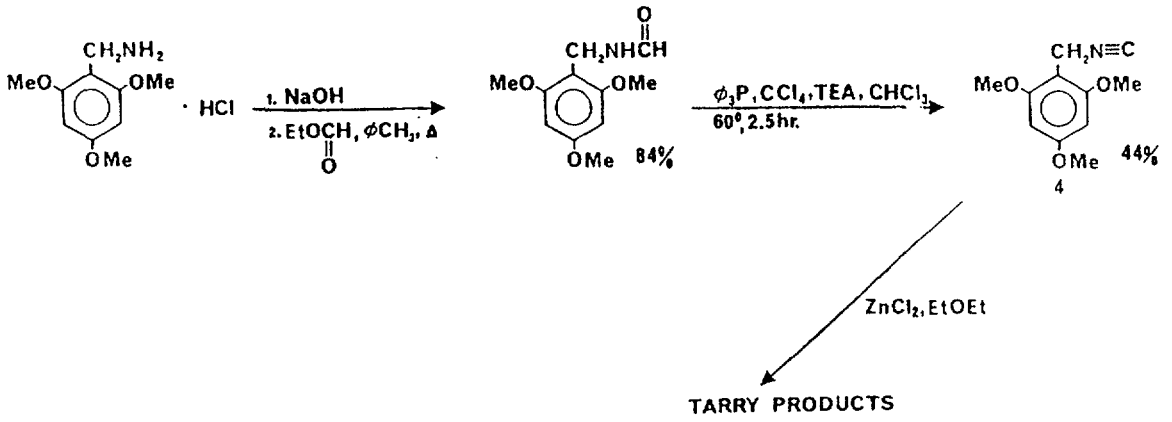
TcO_4 0.90-0.95


EtOCH, φ
Δ, 16hr.
80%
1. Hg(OAc)2, MeOH
2. NaBH4, NaOH
63%
φ3P, CCl4, TEA
CHCl3, 60°, 2.5hr.
anhy. CuCl
anhy. EtOH, 90°, 1.0hr.
99%
Model Cpd. 82%


EtOCH, φ
Δ, 16hr.
Quant.
φ3P, CCl4, TEA
CHCl3, 60°, 2.5 hr.
62%
1
1. anhy. CuCl
anhy. EtOH
90°, 1.5 hr.
2. H2O
ZnCl2
EtOEt
91%
65%


CH2C≡N
BH3-Me2S
Δ, 12 hr.
CH2CH2NH2
85%
EtOCH, φ
Δ, 16hr.
CH2CH2NHCH
90%
MeO
OMe
POCl3, Py
0-5°
CH2CH2N≡C
80%
2
ZnCl2, EtOEt
Zn(II)
Cl2
68%
CuCl, NH4Cl
NH4OH, EtOH
Cu(I)
Cl
44%


NH2
ClCCH2CH2Cl
THF, TEA
0-5 °C
NHCCH2CH2Cl
DMF
90°, 1hr.
NHCCH2CH2N
R Yld. %
H 80
CH3 49
R Yld. %
H 72
CH3 32
H2NNH2
Δ
NHCCH2CH2NH2
R Yld. %
H 76
CH3 74
HCOEt, φ
Δ, 16hr.
NHCCH2CH2NHCH
R Yld. %
H 65
CH3 71
POCl3, Py
0-5°
NHCCH2CH2N≡C
R Cpd. Yld. %
H 3 63
CH3 6 23
ZnCl2
EtOEt
Zn
Cl2
69%
CuCl, NH4Cl, NH4OH
EtOH
Cu
4Cl
R Yld. %
H 28
CH3 73


CH2NH2
MeO
OMe
OMe
· HCl
1. NaOH
2. EtOCH, φCH3, Δ
O
CH2NHCH
O
84%
φ3P, CCl4, TEA, CHCl3
60°, 2.5hr.
CH2N≡C
44%
4
ZnCl2, EtOEt
TARRY PRODUCTS


99m-Tc
RN≡C
6


CH3
CH3CN≡C
CH3
CH3
CH3C CH2N≡C
OMe

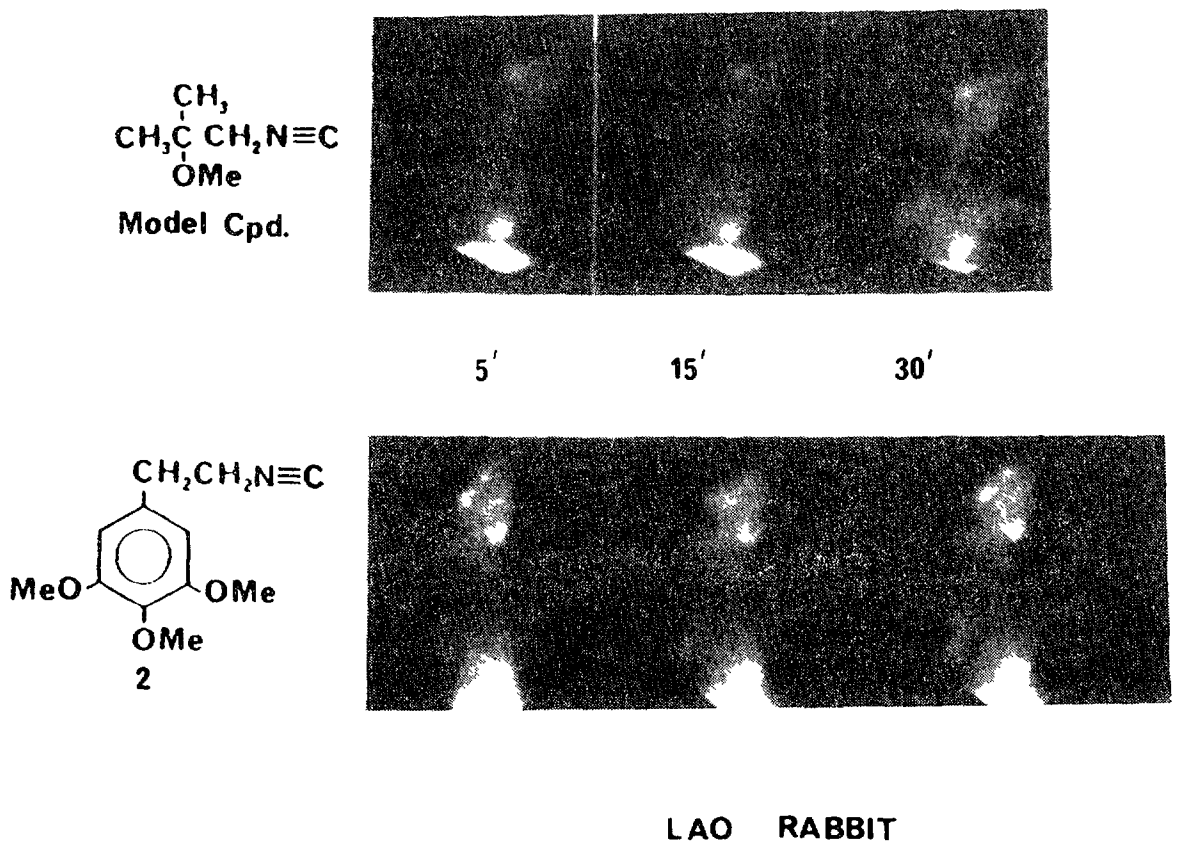

CH3
CH3C CH2N≡C
OMe
Model Cpd.
5'
15'
30'
CH2CH2N≡C
MeO
OMe
OMe
2
LAO RABBIT

FIGURE 1.

Appendix III

SYNTHESIS AND ISOLATION OF dl-HMPAO

4,8-Diaza-3,6,6,9-tetramethylundecane-3,8-diene-2,10-dione dioxime (I). 2,3-Butanedione monoxime, 72.0g (0.71 moles), p-toluenesulfonic acid monohydrate, 0.15g, and benzene, 725 ml were placed into a 2 liter three necked flask equipped with a mechanical stirrer, 250 ml dropping funnel, Dean-Stark trap, Firestone valve for nitrogen and a heating mantle.

Heating to reflux was started and over about one hour a solution consisting of 2,2-dimethyl-1,3-propanediamine, 31.7g (0.31 moles) and benzene, 75 ml were added. The reaction mix was refluxed for a total of 21 hours then stirred 12 hours at room temperature. 99.1% of the theoretical amount of water was recovered. The reaction mixture was chilled at 4°C for 16 hours. The crystals that formed were collected, washed with cold benzene and finally recrystallized from acetonitrile with a carbon treatment. The yield was 47.8g (57.5% of theory) of compound (I) with a mp of 135.5-137.5 versus the literature value of 132.0-134.0 (1). The infrared spectrum gave absorptions at 3260 (-OH) and 1640 (-N=C, C=NOH) cm^{-1}.

dl/meso - 4,8-diaza - 3,6,6,9-tetramethylundecane-2,10-dione dioxime (II). Anhydrous ethanol, 1200 ml, and compound (I), 130.0g (0.484 moles) were placed into a 2 liter round bottom flask. The resulting solution was chilled to 0-5°C and over one hour were added in small portions, sodium borohydride, 18.31g (0.484 moles). The temperature was maintained at 0-5°C throughout the addition.

The reaction mixture was stirred at 0-5°C for two hours. Then water, 400 ml, was added and the reaction mixture stirred an additional hour at 0-5°C. Stirring was continued for one more hour as the reaction mixture was allowed to warm up to room temperature.

Most of the ethanol was removed under vacuum and sufficient cold water was added to render the resulting slurry stirrable. The pH of the slurry was checked and adjusted to 11.0 if necessary. It was then chilled in an ice bath, filtered, washed with cold water and dried under vacuum. The product was crystallized one time from acetonitrile (1200 ml) to give 62.2g (47.2%) of material with a melting point of 119.5-132.0°C.

Separation of the dl diastereoisomer from the meso diastereoisomer of compound (II). The diastereoisomers were separated by multiple fractional crystallizations of compound II, 62.2g, using ethyl acetate as solvent. For the most part a concentration of 20 mg/ml gave the best results in terms of diastereoisomer separation.

A thin layer chromatographic method was developed to assess the progress of the separation. By running a series of standards, this method was judged capable of detecting as little as 1 to 2% of the meso diastereoisomer contaminating the dl diastereoisomer.

Whatman KC18F glass reverse phase plates were used as the solid phase. The corresponding plates without the fluorescent screen (KC18) will not effect separation. Also the C18 reverse phase plates from Analtech with or without the fluor do not effect diastereoisomeric separation.

The mobile phase consisted of an aqueous solution containing a 4:1 ratio of 75% methanol and 28% ammonium hydroxide.

2 ul and 4 ul portions of the sample at a concentration of 20 mg/ml in ethyl acetate were spotted. The front was allowed to travel 10-11 cm. The dried plate was visualized using minhydrin which was applied by spraying and then allowed to air dry completely before developing with a heat gun. The dl distereoisomer had the higher R_f value with the relative R_f values being dependent on the ratio of concentrations of the dl/meso diastereoisomers. A series of standard samples was prepared containing varying ratios of the

dl/meso diastereoisomers. A curve was prepared relating the relative R_f values of the dl and meso diastereoisomers to the known ratio of dl to meso. This allowed an estimation of the isomeric purity of the dl diastereoisomer. See Figure 1.

A total of 5.78g of the pure dl diastereoisomer was recovered (18.6% of theory).

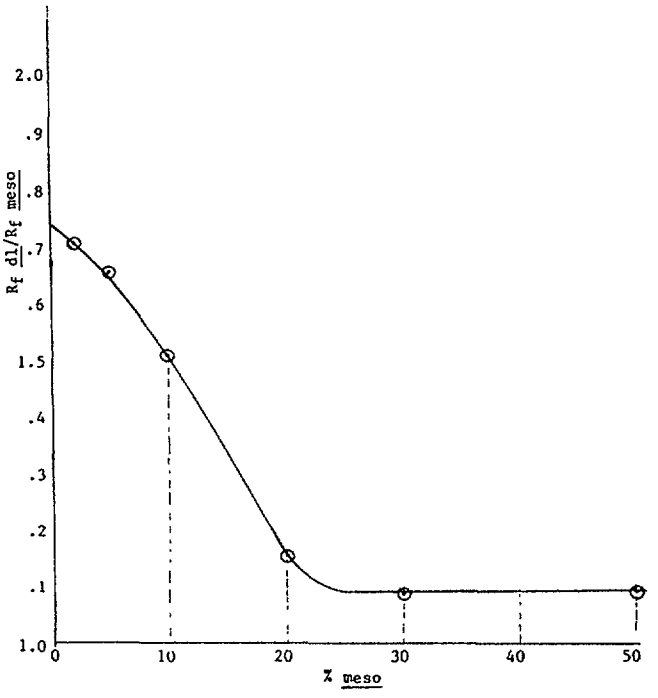


Figure 1. TLC data. Plot of R_f dl/R_f meso vs % meso.

Analytical Data

Appearance: Short hexagonal prisms as compared to the long needles of the meso form.

Melting Point: 131.0-131.5 on a single crystal versus a literature value of 128.0-130.0 (1).

Infrared Spectral Data: 3310 (-NH), 3190,3080 (-OH) cm^{-1}.

TLC: One spot R_f 0.55. Identical with Amersham material.

Elemental Analysis: For $C_{13}H_{28}N_4O_2$

	C	H	N
Calculated:	57.32	10.36	20.57
Found:	57.41	10.59	20.65

NMR: See Figures 2,3,4,5.

Isomer	ppm	multiplicity	assignment
dl	0.784	Singlet	$(CH3)_2$ - C_6
	1.070	Doublet, (5=7.0)	CH_3 - C_3, C_9
	1.643	Singlet	CH_3 - C_2, C_{10}
	2.110	AB Quartet	H - C_5, C_7
	3.122	AB Quartet	H - C_3, C_9
	3.340	Singlet	H - N_4, N_8
	10.275	Singlet	H - Oxime
meso	0.775	Singlet	$(CH_3)_2$ - C_6
	0.777	Singlet	$(CH_3)_2$ - C_6
	1.066	Doublet (5=7.0)	CH_3 - C_3, C_9
	1.642	Singlet	CH_3 - C_3, C_{10}
	2.110	AB Quartet	H - C_5, C_7
	3.122	AB Quartet	H - C_3, C_9
	3.340	Singlet	H - N_4, N_8
	10.270	Singlet	H - Oxime

Also included are the expanded spectra for the two C_6 methyl groups. The meso somer shows the expected splitting induced by asymmetric centers at C_3 and C_9.

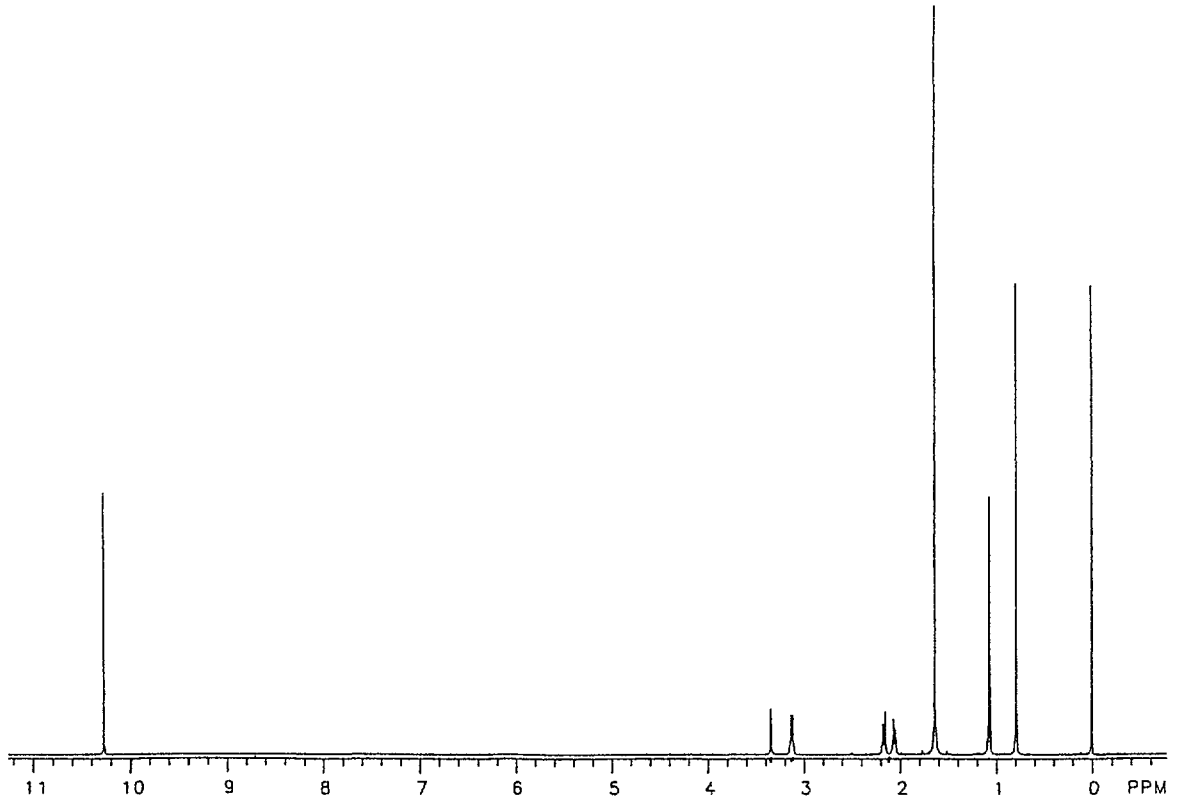


Figure 2. Proton magnetic resonance spectrum of dl-HMPAO.

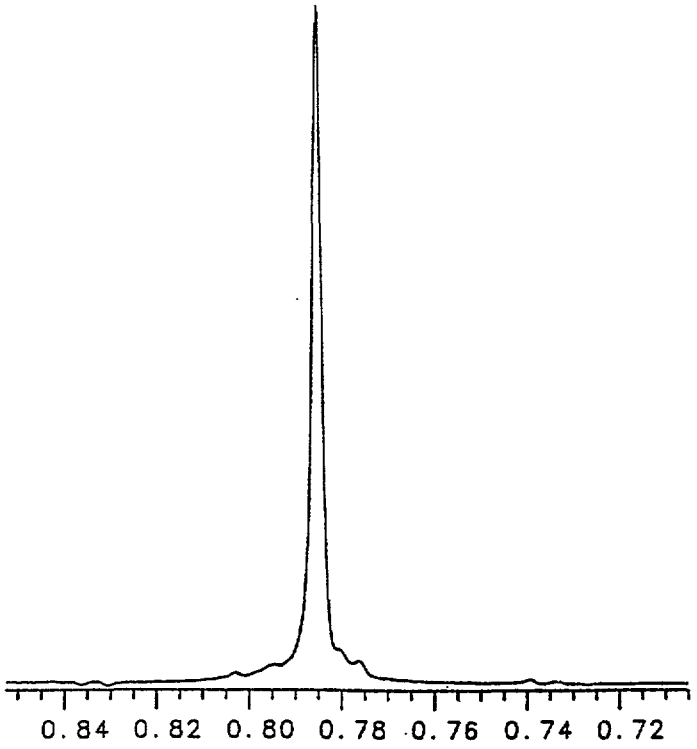


Figure 3. Proton magnetiç resonance spectrum of C_6-dimethyl protons of dl-HMPAO. Singlet demonstrates equivalence of these protons which would be expected for the dl enantiomeric pair.

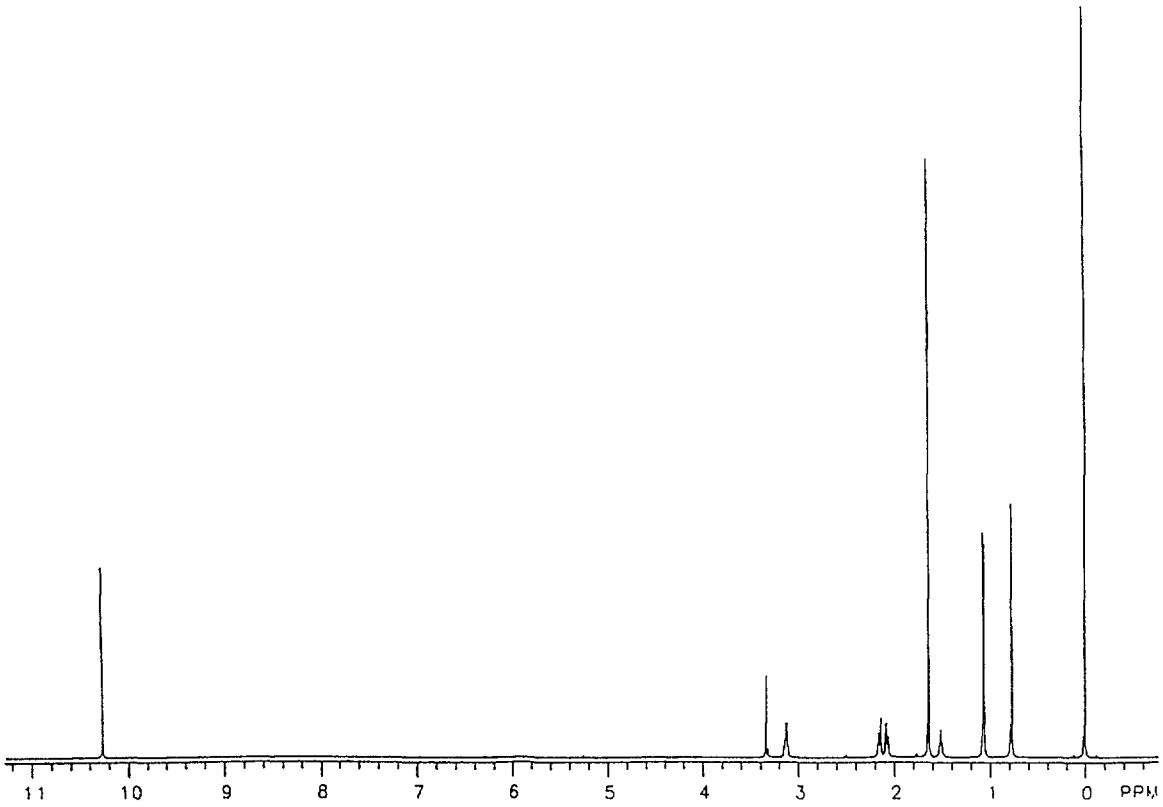


Figure 4. Proton magnetic resonance spectrum of meso-HMPAO.

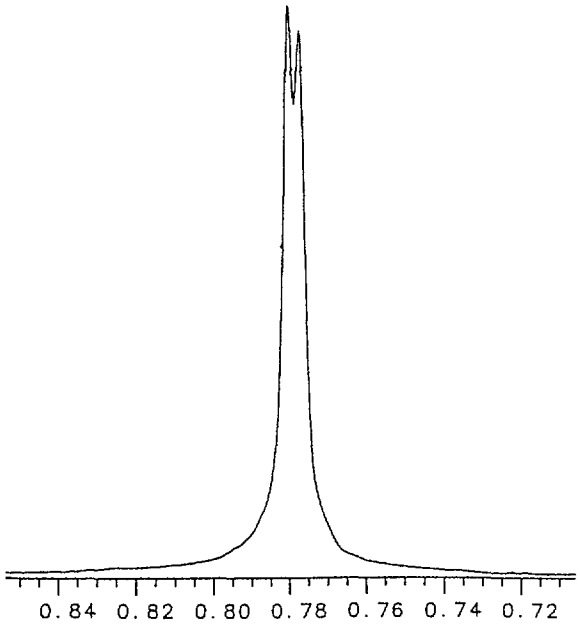


Figure 5. Proton magnetic resonance spectrum of C_6-dimethyl protons of meso-HMPAO. Doublet demonstrates the nonequivalence of these Protons which would be expected for the meso diasterioisomer.

REFERENCE

1. R.D. Neirinckx, L.R. Canning, I.M. Piper, et al., J. Nucl. Med. 28, 191-202 (1987).

Appendix IV

SYNTHESIS OF 1,8-DIAMINE-3,6-DITHIO-OCTANE AND ITS DERIVATIVES

DERIVATIVES OF 1,8-DIAMINE-3,6-DITHIO-OCTANE

SYNTHESIS

MONOSUBSTITUTTED COMPOUNDS

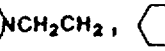
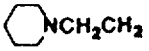
$$RNHCH_2-CH_2-S-CH_2-CH_2-S-CH_2-CH_2-NH_2$$

$$HCl + H_2NCH_2CH_2Cl + HOCH_2CH_2SH \xrightarrow{KOH/EtOH} HOCH_2CH_2SCH_2CH_2NH_2 \xrightarrow{SOCl_2/CHCl_3}$$

$$ClCH_2CH_2SCH_2CH_2NH_2 + HCl \xrightarrow{Na/\ EtOH} ClCH_2CH_2SCH_2CH_2NH_2 \quad (1)$$

$$RNH_2 + \text{S}\!\!\diagup\!\!\diagdown \xrightarrow{Ph} RNHCH_2CH_2SH \xrightarrow{Na/\ EtOH} NaSCH_2CH_2NHR \xrightarrow{(1)}$$

$$RNHCH_2CH_2SCH_2CH_2SCH_2CH_2NH_2$$

R: i-Bu, n-Bu, Ph,  , 

BISUBSTITUTTED COMPOUNDS

$$R_1R_2NCH_2CH_2SCH_2CH_2SCH_2CH_2NR_1R_2$$

$$2R_2NCH_2CH_2Cl + HCl \xrightarrow{Na/\ EtOH} 2R_2NCH_2CH_2Cl$$

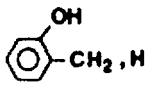
$$HSCH_2CH_2SH \xrightarrow{Na/\ EtOH} NaSCH_2CH_2SNa$$

$$R_1R_2NCH_2CH_2SCH_2CH_2SCH_2CH_2NR_1R_2$$

R_1, R_2 : H, H Et, H 

$$2RCH=O + H_2NCH_2CH_2SCH_2CH_2SCH_2CH_2NH_2 \xrightarrow{EtOH} RCH=NCH_2CH_2SCH_2CH_2SCH_2CH_2N=CHR$$

$$\xrightarrow{NaBH_4/\ EtOH} RCH_2NHCH_2CH_2SCH_2CH_2SCH_2CH_2NHCH_2R$$

R_1, R_2 : c-hexyl, H 

BIODISTRIBUTION IN MICE

%Dose per g

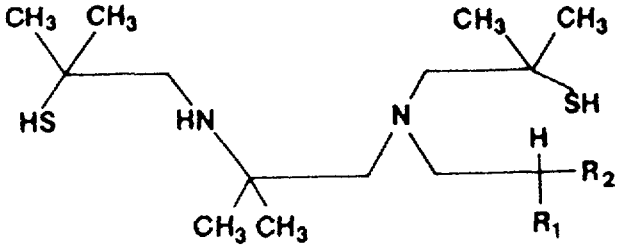
$R_1R_2NCH_2-CH_2-S-CH_2-CH_2-S-CH_2-CH_2-NR_1R_2$

R_1	R_2	FR.	PC.	BLOOD		BRAIN		LIVER		KIDNEYS		LUNGS		BRAIN/BLOOD	
				2min	15min	2min	15min	2min	15min	2min	15min	2min	15min	2min	15min
H	H	A	0.30	19.382	10.293	0.799	0.787	12.849	14.380	21.356	27.525	19.930	17.001	0.040	0.048
C_2H_5	H	A	3.15	27.092	16.490	0.646	0.406	16.490	9.249	11.044	8.403	29.509	12.535	0.024	0.036
c-hexyl	H	B	6.60	13.309	4.871	1.355	0.418	20.674	14.364	14.437	7.857	19.843	8.544	0.102	0.086
		D	49.30	9.078	3.598	5.936	3.114	13.278	14.279	12.908	11.953	O.R.	O.R.	0.655	0.865
		C	20.90	25.071	13.341	1.453	0.526	15.633	16.684	8.987	23.963	35.710	21.662	0.058	0.039
		D	19.80	15.714	10.044	8.434	4.038	13.488	21.441	13.131	4.545	O.R.	O.R.	0.537	0.402
		F	35.40	28.616	17.468	1.245	0.802	15.356	27.943	7.064	10.081	48.653	51.746	0.043	0.046
	H	C	43.44	9.109	3.803	2.303	2.022	10.976	12.349	8.616	9.357	73.588	91.019	0.253	0.532
		D	31.80	16.005	4.826	1.986	1.280	12.322	12.798	10.309	12.476	72.249	38.928	0.124	0.265

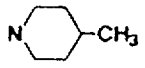
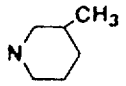
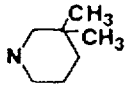
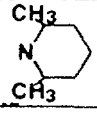
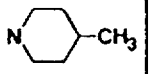
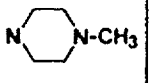
$RNHCH_2-CH_2-S-CH_2-CH_2-S-CH_2-CH_2-NH_2$

R	FR.	PC.	BLOOD		BRAIN		LIVER		KIDNEYS		LUNGS		BRAIN/BLOOD	
			2min	15min	2min	15min	2min	15min	2min	15min	2min	15min	2min	15min
i-C_4H_9	B	22.90	16.124	6.236	1.736	1.160	11.592	22.189	10.926	16.124	67.289	47.854	0.108	0.186
	C	72.90	13.473	10.852	1.031	0.718	13.995	12.137	12.518	10.060	36.949	38.409	0.076	0.066
	E	8.30	6.678	3.532	0.293	0.208	10.509	15.157	13.556	11.892	28.437	17.360	0.044	0.059
n-C_4H_9	A	3.60	20.644	12.853	0.634	0.420	11.894	9.537	7.101	6.418	15.062	11.051	0.031	0.033
	B	40.00	11.804	3.670	4.035	5.013	6.424	16.249	7.652	28.761	O.R.	87.595	0.342	1.366
	C	103.70	12.936	7.269	2.612	1.901	7.559	7.661	9.668	9.558	73.774	37.446	0.202	0.261
Ph	A	11.30	19.301	14.099	0.612	0.478	11.570	16.694	8.092	13.479	56.354	47.375	0.032	0.034
	C	19.40	12.138	7.445	1.300	0.836	17.482	25.168	9.624	10.295	57.958	41.173	0.072	0.112
	A	7.40	36.133	15.238	0.889	0.545	19.557	11.083	14.417	10.690	20.911	11.021	0.025	0.036
	D	26.50	27.586	8.184	2.429	1.705	14.382	17.936	8.266	22.638	94.562	41.571	0.088	0.208
	E	23.20	15.089	6.796	1.493	1.436	11.765	17.089	12.918	22.778	O.R.	43.933	0.098	0.210
	A	28.90	41.039	28.487	1. 3	1.003	19.200	19.502	15.717	.272	29.101	23.822	0.028	0.035

BIODISTRIBUTION IN MICE OF AMINODITHIOLS



% DOSE PER QR

R_1	R_2	P.C.	Time min	Blood	Brain	Liver	Kindneys	Lungs	Br/Bl
H		155	2	2.627	12.394	8.101	14.063	57.635	4.718
			15	1.021	5.333	19.759	14.440	15.414	5.223
H		112	2	1.745	7.507	5.699	10.460	50.090	4.521
			15	0.729	1.891	13.722	7.685	10.750	2.605
H		94	2	10.520	5.383	11.406	7.130	33.166	0.516
			15	4.111	0.894	23.166	8.193	12.580	0.216
H		210	2	2.285	5.010	8.471	12.057	60.856	1.928
			15	0.942	1.604	18.351	10.826	15.346	1.703
H		26	2	2.165	4.282	8.714	10.526	22.802	2.001
			15	0.868	1.145	13.392	3.977	3.272	1.348
H		42	2	2.173	6.811	6.556	6.163	11.473	3.216
			15	1.301	0.639	11.815	3.084	4.133	0.498
CH_3		658	2	1.354	2.811	18.188	11.607	7.678	2.077
			15	0.608	1.603	19.873	6.404	5.341	2.636
CH_3		50	2	2.149	2.070	16.569	12.563	24.820	0.96
			15	0.763	1.207	18.500	6.570	5.556	1.582
H		69	2	1.941	6.054	4.173	4.792	13.523	3.092
			15	1.934	0.790	18.020	3.491	4.286	0.421

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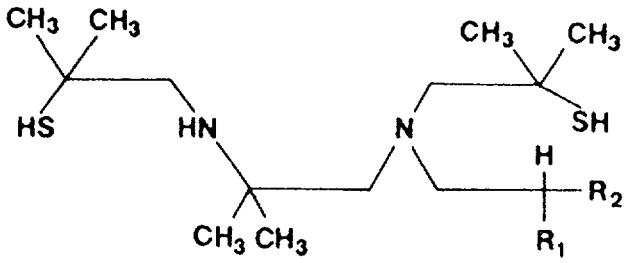
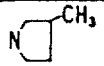
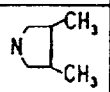


% DOSE PER QR

R_1	R_2	P.C.	Time min	Blood	Brain	Liver	Kindeys	Lungs	Br/Bl
H		29	2	1.918	4.691	3.691	8.535	77.244	2.446
			15	1.153	3.571	11.549	14.895	32.407	3.097
H		49	2	1.683	5.994	5.687	13.020	67.139	3.561
			15	0.891	2.873	14.535	8.733	15.910	3.224
H		235	2	2.106	5.903	8.484	18.218	84.705	2.803
			15	1.556	2.814	22.427	15.876	37.628	1.808
CH_3		191	2	2.916	9.048	12.249	17.397	82.499	3.100
			15	1.318	2.724	20.172	18.408	17.310	2.394

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