Paper B1

TECHNETIUM-99m COMPLEXES OF 3-HYDROXY-4-PYRIDINONE LIGANDS

D.S. Edwards*, M.J. Poirier* and C. Orvig#
* DuPont-Merck Pharmaceutical Co., N. Billerica, MA
# The Department of Chemistry, University of British Columbia, Vancouver, Canada.

Kanvinde et. al. (1) have recently reported the synthesis of a series of technetium-99m-3-hydroxy-4-pyrone complexes. The rabbit biodistribution of one of these cationic technetium complexes shows remarkably high heart uptake (4.3% i.d./g for the Kojic Acid complex). We have been investigating the chemistry and biodistribution of a series of N-substituted-3-hydroxy-2-methyl-4-pyridinone complexes of aluminum, gallium and indium.(2) The formation constants of the pyridinone complexes significantly exceed that of the analogous pyrone complexes; and the incorporation of a substituent on the ring nitrogen permits the systematic variation of their lipophilicity. Therefore, we have investigated the chemical and biological properties of technetium-99m-3-hydroxy-2-methyl-4-pyridinone complexes.

The N-alkyl-3-hydroxy-4-pyridinone ligands (alkyl = ethyl, isopropyl, isobutyl, benzyl and cyclohexyl) were synthesized by the reaction of the respective primary amine with maltol, following the procedure of Kontoghiorges et. al. (3) The ligands were characterized by IR, ^{1}H and ^{13}C NMR and elemental analysis.

The ^{99m}Tc-pyridinone complexes were synthesized by heating $^{99m}TcO_4^-$ in the presence of the ligand and sodium bisulfite. The complexes were characterized by TLC, HPLC and electrophoresis. Radiochemical purity of > 90% was obtained and was maintained for at least 4 hours. Relevant analytical data are shown in Table 1. The analogous reaction using $[NH_4][^{99}TcO_4]$ yields an orange crystalline solid that has been characterized by IR and FABMS. The structure of $^{99}TcL_3^+$ is shown in Figure 1.

The biodistribution of the ^{99m}Tc-pyridinone complexes were determined in guinea pigs (n= 3) and the results are presented in Table 2. The heart uptake of the complexes ranges from 0.04% i.d./g for the cyclohexyl complex to 0.7% for the benzyl complex. (For comparison, Cardiolite® has heart uptake of 1.2% i.d./g. in guinea pigs.) Heart uptake rises with increasing lipophilicity in the series ethyl, isobutyl, benzyl, but falls dramatically at the cyclohexyl complex. Liver uptake is generally high for all the complexes and only the cyclohexyl complex exhibits rapid blood clearance. Next, we plan to synthesize and test the para-methoxyphenyl (pap) complex (the $Ga(pap)_3$ complex (4) shows rapid heart uptake and blood clearance in rabbits and dogs) as well as investigate the effect of adding functionality to the alkyl chain.

(1) Kanvinde, M.H., Basmadjian, G.P., Mills, S.L., Kale, N.J., J. Nucl. Med., **1990**, *31*, 908(Abstract). Kanvinde, MM.H., Kale, N.J., Basmadjian, G.P., ACS National Meeting, 1991.

(2) Matsuba, C.A., Nelson, W.O., Rettig, S.J., Orvig, C., Inorg. Chem., **1988**, *27*, 3935. Clevette, D.J., Lyster, D.M., Nelson, W.O., Rihela, T., Webb, G.A., Orvig, C., Inorg. Chem., **1990**, *29*, 667. Zhang, Z., Rettig, S.J., Orvig, C., Inorg. Chem., **1991**, *30*, 509.

(3) Kontoghiorghes, G.J., Sheppard, L., Inorg. Chim. Acta, **1987**, *136*, L11.

(4) Zhang, Z., Lyster, D.M., Webb, G.A., Orvig, C., Nuc. Med. Biol., **1992**, in press.

FIGURE 1. Structure of ^{99m}Tc-pyridinone Complexes

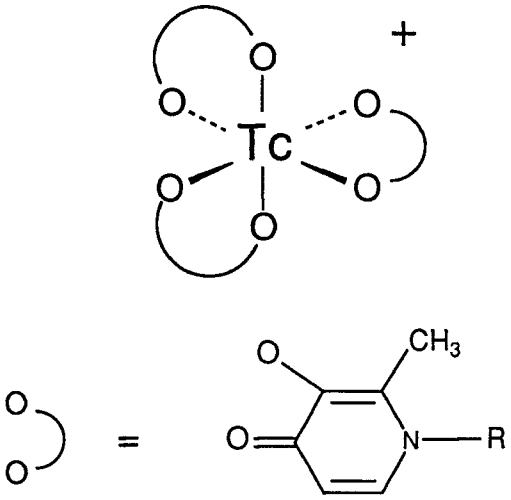


TABLE 2. Guinea Pig Biodistribution Data on ^{99m}Tc-pyridinone Complexes

	R = C_2H_5	*i*-C_4H_9	$CH_2C_6H_5$	C_6H_{11}
Heart: t = 5 min	0.21±0.02	0.47±0.04	0.54±0.04	0.04±0.001
t = 15 min	0.15±0.03	0.46±0.04	0.70±0.13	0.03±0.005
t = 60 min	0.06±0.01	0.40±0.05	0.66±0.08	0.04±0.002
Lung: t = 5 min	0.35±0.03	0.45±0.05	0.27±0.02	1.10±0.33
t = 15 min	0.24±0.08	0.33±0.07	0.32±0.06	0.50±0.09
t = 60 min	0.10±0.02	0.19±0.02	0.26±0.02	0.36±0.04
Liver: t = 5 min	0.79±0.12	1.85±0.35	3.08±0.49	1.08±0.16
t = 15 min	0.69±0.06	2.19±0.26	2.72±0.56	0.47±0.05
t = 60 min	0.87±0.11	1.49±0.29	3.08±0.59	0.23±0.01
Blood: t = 5 min	0.50±0.03	0.27±0.31	0.17±0.03	0.005±0.001
t = 15 min	0.31±0.10	0.07±0.01	0.14±0.02	0.003±0.001
t = 60 min	0.11±0.02	0.13±0.01	0.08±0.01	0.001

(A biodistribution study was not performed on the isopropylpyridinone complex.)

TABLE 1. Analytical Data on ^{99m}Tc-pyridinone Complexes

R =	TLC Rf	HPLC Ret. Time	Charge
C_2H_5	0.86	3.30	+
i-C_3H_7	0.78	7.28	+
i-C_4H_9	0.67	10.59	+
$CH_2C_6H_5$	0.61	16.27	+
C_6H_{11}	0.53	25.57	+

TLC: Whatman C_{18}plates, 4:3:2:1 solvent mixture (CH_3CN: MeOH: 0.5 M NH_4OAc: THF)

HPLC: Hamilton PRP-1 column, gradient method 40/60 to 80/20 CH_3CN(0.1% TFA)/H_2O (0.1% TFA) over 20 min.

Electrophoresis: Whatman 31 Et CHR paper, 0.70 MeOH/0.025M phosphate buffer (pH 7), 250 V, 30 min.

Paper B2

PREPARATION AND BIODISTRIBUTION IN RATS OF BIS(DITHIOCARBAMATO) NITRIDO TECHNETIUM (V)RADIOPHARMACEUTICALS: INFLUENCE OF LIGAND SIDE CHAINS ON HEART UPTAKE.

Bellande[a],E.,Brucato[a],V.,Comazzi[a],V.,Lainé[a],J.,Lecayon[a],M.,Duatti[b],A.,Pasqualini[a],R.

a)Cis bio international,B.P. 32,91192 Gif sur Yvette,France.
b)Dipartimento di Chimica Fisica ed Inorganica,Università di Bologna,40136 Bologna,Italy.

Technetium radiopharmaceuticals containing the $[Tc{\equiv}N]^{2+}$ core are now easily prepared after the advent of a new improved procedure for obtaining the $Tc{\equiv}N$ multiple bond at no-carrier-added level,in sterile and apyrogenic conditions (1).This method makes use of tertiary phosphines as reducing agents,and of S-methyl dithiocarbazate $[H_2N\text{-}N(CH_3)\text{-}C(=S)SCH_3]$ as N^{3-} donating ligand.

The first class of technetium nitrido radiopharmaceuticals showing myocardial uptake has been recently reported (2).This class includes neutral complexes containing two monoanionic dithiocarbamate ligands, $[R(R')NCS_2]^-$,coordinated to the Tc^{+5} ion of the $[Tc{\equiv}N]^{2+}$ core in the basal plane of a square pyramidal arrangement.Two terms of this class,namely the compounds $[^{99m}TcN(Et_2NCS_2)_2]$ and $\{^{99m}TcN[Et(EtO)NCS_2]_2\}$,showed good quality images in human volunteers.We report here the preparation and biodistribution in rats of ^{99m}TcN-dithiocarbamate complexes bearing symetrical side chain modifications on the ligand $[R_2N\text{-}CS_2]^-$.

Synthesis of the ligands. The ligands were prepared according to the general scheme:

$$R_2NH + CS_2 \longrightarrow R_2NCS_2Na$$

The reactions were carried out in EtOH or Et_2O,in presence of NaOH,and the final products were characterised by the usual techniques (NMR,IR,MS,elemental analysis).

Labeling procedures. The reaction includes two steps .The first step carried out at 100° C leads to the formation of intermediate nitrido species through the reaction:

$$[^{99m}TcO_4]^- + HCl + [P(m\text{-}C_6H_4SO_3)_3]Na_3 + H_2N\text{-}N(CH_3)\text{-}C(=S)SCH_3 \longrightarrow [^{99m}TcN]_{int}$$

After cooling,the pH was raised to 8.0, and 10 mg of the appropriate dithiocarbamate ligand added to the reaction solution,which was further left to stand for 10 min at room temperature.The radiochemical purity was checked by TLC on silica gel using CH_2Cl_2 as eluant.The yields were always greater than 93%.

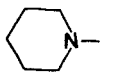
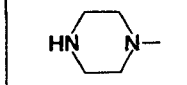
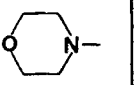
Animal studies. Adult male rats were injected with ~50 KBq of activity and,after sacrifice,the principal organs and the blood were collected 5 and 60 min p.i..

Table I reports the values of the activity,expressed as % of ID in the whole organ or fluid samples,at 5 and 60 minutes p.i. for the various ^{99m}TcN-dithiocarbamate compounds.

1) Duatti,A.,Marchi,A.and Pasqualini,R.*J.Chem.Soc.,Dalton Trans.*,3729-3733 (1990)
2)Duatti,A.,Marchi,A.,Pasqualini,R.;Comazzi,V.,Bellande,E.*J.Nucl Med.*.**32**;(5) 925 (1991)

Table I % of I.D. in the whole organ or fluid 5 and 60 minutes p.i.

R / ORGANS	CH_3		C_2H_5		nC_3H_7		nC_4H_9		$OH(CH_2)_2$	
	5 mn.	60 mn.	5 mn.	60 mn.	5 mn.	60 mn.	5 mn.	60 mn.	5 mn.	60 mn.
Liver	17,3	28,1	16,9	26,5	27,2	27,8	47,8	52,8	25,1	6,7
Kidneys	5,2	2,0	4,7	3,0	2,9	2,1	1,4	1,2	22,8	4,3
Lungs	4,4	8,0	9,5	1,2	2,7	0,7	2,3	2,0	0,5	0,2
Heart	2,3	0,6	2,4	1,3	1,57	1,0	0,8	0,6	0,27	0,1
Blood	3,18	2,16	7,92	2,22	3,6	1,5	11,4	4,08	6,6	2,82

R / ORGANS										
	5 mn.	60 mn.	5 mn.	60 mn.	5 mn.	60 mn.	5 mn.	60 mn.	5 mn.	60 mn.
Liver	45,4	28,1	31,2	39,0	57,5	50,3	30,3	26,7	66,4	49,6
Kidneys	0,4	5,6	1,5	0,8	1,4	2,3	7,2	4,7	1,6	2,7
Lungs	2,1	0,5	1,9	2,6	4,6	2,7	4,3	2,8	1,4	0,8
Heart	0,1	0,05	0,8	0,27	0,4	0,2	1,1	0,5	0,16	0,15
Blood	0,96	2,34	1,38	0,36	1,26	1,32	10,2	6,06	3,9	4,5

Paper B3

SYNTHESIS OF TECHNETIUM NITRIDO RADIOPHARMACEUTICALS WITH HMPAO

Licia Uccelli,[a] Adriano Duatti,[b] Roberto Pasqualini,[c] Melchiorre Giganti,[a] Adriano Piffanelli,[a] Luciano Magon[d]

[a)] Laboratorio di Medicina Nucleare, Università d Ferrara, 44100 Ferrara, Italy. [b)] Dipartimento di Chimica Fisica ed Inorganica, Università di Bologna, 40136 Bologna, Italy. [c)] CIS Bio international, 91192 Gif-sur-Yvette, France. [d)] Dipartimento di Chimica, Università di Ferrara, 44100 Ferrara, Italy.

The $[Tc{\equiv}N]^{2+}$ core is isoelectronic with the $[Tc{=}O]^{3+}$ core which is present in the molecular structure of a number of ^{99m}Tc-radiopharmaceuticals currently in use in the clinical practice. It has been observed that the stability of the Tc≡N bond in various complexes is greater than that of the oxo analogue (1). This suggest that the stable $[Tc{\equiv}N]^{2+}$ core may be used to obtain new classes of ^{99m}Tc-radiopharmaceuticals, and part of the current effort in exploring technetium nitrido chemistry is devoted toward the preparation of nitrido analogues of oxo compounds which have proven useful in diagnostic nuclear medicine.

The complex ^{99m}TcO(HMPAO) (HMPAO = hexamethylpropyleneamine oxime) is an efficacious cerebral perfusion imaging agent (2) whose structure comprises the trianionic tetradentate HMPAO ligand bonded to the $[Tc{=}O]^{3+}$ core in the basal plane of a square pyramidal arrangement. However, this compound does not possess a high stability *in vitro*, and undergoes a relatively rapid decomposition which in pratical terms means that ^{99m}TcO(HMPAO) must be administered within 30 min of its formulation (3). The inherent *in vitro* instability of ^{99m}TcO(HMPAO) is a major limitation to the potential clinical utility of this compound.

We report here the synthesis of the technetium nitrido analogue of ^{99m}TcO(HMPAO), where the oxo group has been replaced by a nitride nitrogen atom (N^{3-}). The aim of this study was to obtain, through this replacement, a compound having the same square pyramidal structure of ^{99m}TcO(HMPAO), but showing a higher *in vitro* stability.

Synthesis. The nitrido analogue of ^{99m}TcO(HMPAO) was prepared using two different routes. The first procedure utilized a new efficient method for preparing the Tc≡N group at no-carrier-added level that was recently introduced into the field of nuclear medicine (4). The whole synthesis is illustrated in following reaction scheme:

$$[^{99m}TcO_4]^- + HCl + [P(m\text{-}C_6H_4SO_3)_3]Na_3 + H_2N\text{-}N(CH_3)\text{-}C(=S)SCH_3 \rightarrow [^{99m}TcN]_{int} + HMPAO \rightarrow [^{99m}TcN(HMPAO)]^{+,0,-}$$

This preparation was carried out in two steps. The first step was accomplished at 100 °C and gave rise to the formation of a technetium nitrido intermediate $[^{99m}TcN]_{int}$. After cooling, the pH was raised to the neutral value and HMPAO was added to the same reaction solution. The final product was obtained, at room temperature, in 15 min through a simple exchange reaction onto the prereduced intermediate $[^{99m}TcN]_{int}$. The second procedure is showed in the following reaction scheme:

$$^{99m}TcO(HMPAO) + H_2N\text{-}N(CH_3)\text{-}C(=S)SCH_3 \rightarrow [^{99m}TcN(HMPAO)]^{+,0,-}.$$

^{99m}TcO(HMPAO) was obtained from a commercial kit formulation and, after addition of S-methyl dithiocarbazate, the whole reaction was carried out in a single step, at 100 °C, within 15 min.

The identity of the resulting compounds was established by HPLC and ion exchange chromatography. These analyses revealed that three main products were obtained using the two procedures illustrated above, corresponding to the formation of neutral, monoanionic and monocationic complexes. The overall net charge of the complexes appears to be determined by the charge of the $[Tc{\equiv}N]^{2+}$ core and also by the ionization of the hydrogens at three possible sites, two NH and one OH groups. It is likely that an equilibrium exists in solution beteween the three forms, but this remains to be elucidated. Preliminary biodistributions in rats showed that only a small part of the total injected activity was localized in the cerebral region, indicating that the replacement of the oxo group by the nitride group affects also the biological properties of the radiopharmaceutical. This fact is probably related to the non-vanishing net charge of the two complexes $[^{99m}TcN(HMPAO)]^{+,-}$.

1) Baldas, J., Bonnyman, J. *Int. J. Appl. Radiat. Isot.* **36**, 133-139.

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

3) Hung, J.C., Corlija, M., Volkert, W.A., Holmes, R.A. *J. Nucl. Med.* **29**, 1568-1576 (1988).

4) Duatti, A., Marchi, A., Pasqualini, R. *J. Chem. Soc., Dalton Trans.* 3729-3733 (1990).

Paper B4

A NEW TC-99M LABELED BRAIN SCANNING AGENT WITH ELIMINATION OF CHIRAL CENTERS IN THE COORDINATION SPHERE WITH A COMPACT NINE-MEMBER CORE.

Mrinal K. Dewanjee, Abdol K. Ghafouripour, William. I. Ganz, Aldo N. Serafini, George N. Sfakianakis. Departments of Radiology, University of Miami School of Medicine, Miami, Florida, U.S.A.

INTRODUCTION: A new Tc-99m labeled brain scanning agent was developed by the elimination of chiral centers in the coordination sphere with a compact nine-member core (1-5).

EXPERIMENTAL PROCEDURES: The DPEDO core was synthesized by the 3 step procedure starting from benzaldehyde. The benzaldoxime was synthesized by the addition of benzaldehyde to hydroxylamine hydrochloride in acid media at room temperature and extracted with methylene chloride and dried. Benzohydroxyiminoyl chloride was synthesized from N-chlorosuccinimide and benzaldoxime in dimethyl formamide. After extraction and drying, benzohydroximoyl chloride was conjugated with ethylenediamine (5) and the core of 3,6-diaza-2,7-diphenyl-octane 2,7-dione bisoxime (diphenyl ethylenediamine dioxime: DPEDO) was synthesized. The Sn(II)-DPEDO kit was prepared by mixing aliquots of solutions of DPEDO(100 μg) and $SnCl_2,2H_2O$ (10 μg) and freeze-dried. After reconstituting the kit with 10-50 mCi of Tc-99m pertechnetate, HPLC chromatography was carried out. A labeling efficiency of >95% was found.

RESULTS AND DISCUSSION: Intravenous administration in mice, rats and Yorkshire pigs demonstrated high extraction in cerebral cortex like Tc-99m HMPAO. This new Tc-99m complex was also found to label platelets and white cells with a higher labeling efficiency than Tc-99m HMPAO. We have thus synthesized a new lipid-soluble complex that permits both the studies of brain imaging and cell-labeling with Tc-99m radionuclide. The compact DPEDO core also permits higher transchelation at a faster rate than HMPAO, as observed by Tc-99m bound cytoplasmic proteins in platelets and red cells.

REFERENCES
1. Dewanjee MK, Rao SA, Penniston JT: Mechanism of red blood cell labeling with Tc-99m pertechnetate and the role of cation pumps at the RBC membrane on distribution and binding of Sn^{2+} and Tc-99m with membrane proteins and hemoglobin. J. Labelled Compd. Radiopharm. 19: 193-195(1983).
2. Dewanjee MK, Rao SA, Didisheim P: Indium-111 tropolone, a new high-affinity platelet label: Preparation and evaluation of labeling parameters, J. Nucl. Med. 22: 981-987(1981).
3. Becker W, Borner W, Kromer EP, et al: Tc-99m-HMPAO: A new platelet labeling compound? Eur. J. Nucl. Med. 13: 267-268(1987).
4. Neirinckx RD, Canning LR, Piper IM, Nowotnik DP, Pickett RD, Holmes RA, Volkert WA, Forster AM, Weismer PS, Marriott JA, Chaplin SB: Technetium-99m, d,l-HMPAO: A new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. J. Nucl. Med. 28:191-202(1987).
5. Johnson JE, Ghafouripour AK, Arfan M, Todd SL, Sitz DA. Mechanism of amine and amide ion substitution reaction at the carbon-nitrogen double bond. J. Org. Chem. 50: 3348-3355(1985).

ACKNOWLEDGEMENT.
Supported by grants from Department of Energy (DOE FG-05-88ER60728), Florida High Technology and Industry Council and Baxter Healthcare Corporation.

Paper B5

Synthesis and Biological Evaluation of Dicarboxylate Technetium-99m-Diamide-Dimercaptide Complexes as Potential Renal Imaging Agents.

Daniel J. Canney, Jeffrey J. Billings, Yu-Zhi Guo, Lynn C. Francesconi and Hank F. Kung.
Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104 USA.

Ortho-iodohippurate (OIH) has been viewed as the gold standard for radioisotopic renal function studies. However, Tc-99m-mercaptoacetylglycylglycylglycine (MAG_3) is a promising new renal radiopharmaceutical that has found widespread clinical utility.[1] Recently, a potential addition to the renal radiopharmaceutical armamentarium has been reported, namely, Tc-99m-ethylenedicysteine (Tc-99m-EC; Figure 1).[2] This *diamine* dimercaptide (N_2S_2) Tc-99m complex contains 2 carboxylate moieties and is rapidly and efficiently excreted in the urine of mice, baboons, and human volunteers. The goal of the present study was to prepare *diamide* dimercaptide analogs of Tc-99m-EC with carboxylate groups at different positions on the chelate ring and to evaluate them as potential renal imaging agents.

Schemes I and II illustrate the synthetic routes utilized in the preparation of ligands **4**, **5**, and **7**. Both routes afforded the desired ligands in modest overall yields (22% and 20%, respectively). Radiolabeling with Tc-99m, thiol deprotection and ester hydrolysis of test compounds were performed *in situ* (1N NaOH) in the presence of sodium dithionite. Purification was accomplished by reverse-phase HPLC (PRP-1, Hamilton Inc.; 85% K_2PO_4 buffer (20 mM; pH = 7) /15 % ethanol) and resulted in only a single component. The Tc-99-**7** complex was also shown to coelute with Tc-99m-**7** using RP-HPLC (retention time = 5.2 min). Compounds **5**, and **7** possess carboxylate moieties on the carbon atom α to the sulfur, while **4** contains an acetate at that position. Compound **5** also possesses an extended chelate ring as compared to **4**, and **7**. Biodistribution studies revealed slow renal excretion rate for the test complexes as compared to OIH (Tables I and II). After 1 h, the urinary excretion of **4**, **5** and **7**, was approximately 22%, 22%, and 32%, respectively, compared to 85 to 86% for OIH. Slow blood disappearance, high muscle uptake, and modest hepatobiliary excretion of Tc-99m **4**, **5** and **7** were also observed. Because distinct species variability regarding the renal excretion rates of similar complexes have been reported, planar imaging studies of Tc-99m complexes of **4**, **5**, and **7** were performed with monkeys. While differences in biodistribution between rats and monkeys for individual agents were apparent, poor renal excretion rates were observed for each of these agents, accompanied by high activity in the liver, heart, and muscle tissue (data not shown). Additionally, blood disappearance appeared to be slow as evidenced by high background activity. Lastly, protein binding of these complexes and that of MAG_3 in both rat and monkey plasma was determined (Table III). The plasma protein binding values obtained for Tc-99m complexes of **4**, **5**, and **7** were similar to that determined for Tc-99m-MAG_3.

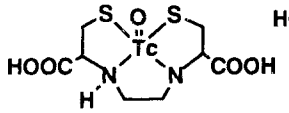
This work further demonstrates that efficient renal handling of Tc-99m N_2S_2 complexes is extremely sensitive to small structural modifications. The dicarboxylate N_2S_2 complexes evaluated here are poor candidates as renal radiopharmaceuticals.

[1] Bubeck, B., et al. *J. Nucl. Med.* 31: 1285(1990).

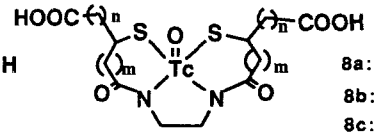
[2] Van Nerom, C. et al. *J. Labelled Compd. Radiopharm.* 30: 37(1991), abstr.

Acknowledgements. This work was supported by a grant from Nihon Medi-Physics.

Figure 1.



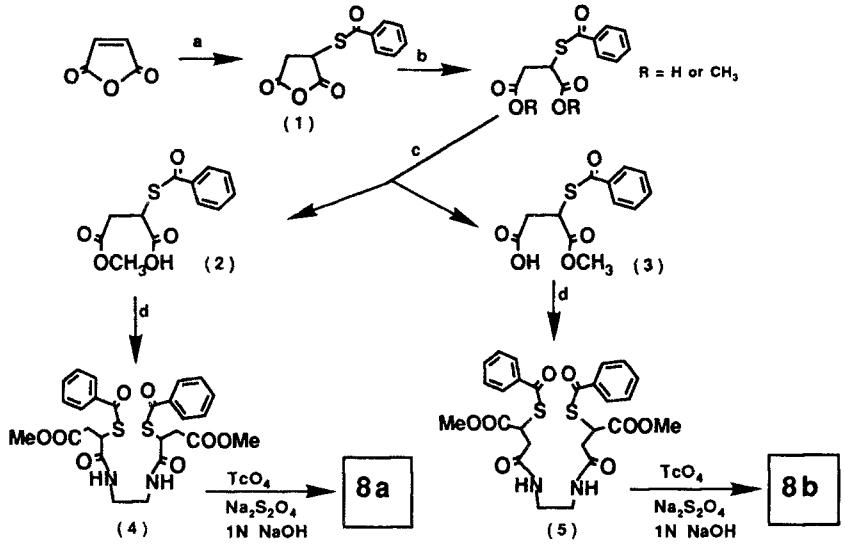
Tc-99m EC



8a: n=1, m=0
8b: n=0, m=1
8c: n=0, m=0

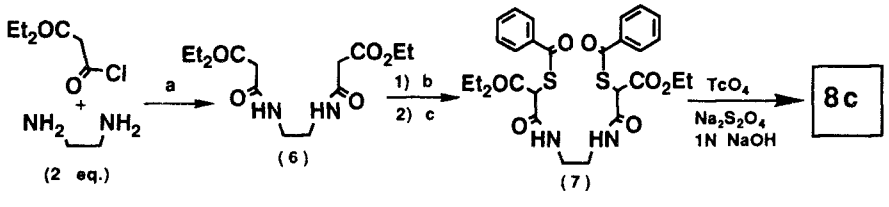
Tc-99m 8a, b, and c.

Scheme I. Synthesis of dimethyl 3,10-bis(benzoylthio)-4,9-dioxo-5,8-diazododecanedioate (4) and dimethyl 2,11bis(benzoylthio)-4,9-dioxo-5,8-diazododecanedioate (5).



a) Thiobenzoic acid, dibenzoylperoxide, Et_2O, reflux; b) $NaOCH_3$, CH_3OH, -78 °C;
c) Column chromatography (20% hexanes/ET_2O & 1% AcOH) d) NHS, DCC, THF, 0 °C.

Scheme II. Synthesis of Diethyl 2,9-Bis(benzoylthio)-3,8-dioxo-4,7-diazadecanedioate (7).



a) CH_2CH_2, 0 °C; b) NBS, HBr, CH_2CH_2, r.t.; c) Sodium thiobenzoate, EtOH, r.t.

Table I. Comparison of Rat Biodistribution Data for Tc-99m N_2S_2 Dicarboxylate Complexes with I-131-OIH.[a]

Tc-99m Complex	min	kidney	blood	liver	muscle	intestines	heart
4	10	4.3 ± 0.7	26.7 ± 1.5	5.0 ± 0.4	26.9 ± 1.4	1.7 ± 0.1	0.4 ± 0.03
	60	2.1 ± 0.1	16.4 ± 0.5	3.1 ± 0.3	21.1 ± 2.1	1.3 ± 0.5	0.2 ± 0.02
OIH	10	6.9 ± 3.0	5.3 ± 0.7	2.5 ± 0.9	9.9 ± 0.8	1.4 ± 0.9	0.09 ± 0.01
	60	0.6 ± 0.2	0.6 ± 0.03	0.3 ± 0.1	1.6 ± 0.3	0.8 ± 0.4	< 0.01 %
5	10	5.3 ± 1.8	26.2 ± 1.2	5.0 ± 0.1	22.8 ± 2.3	1.4 ± 0.1	0.4 ± 0.01
	60	1.9 ± 0.1	15.2 ± 0.8	3.0 ± 0.3	20.4 ± 1.8	1.7 ± 0.2	0.3 ± 0.05
OIH	10	9.9 ± 5.2	4.4 ± 0.2	3.3 ± 1.1	7.9 ± 1.1	1.6 ± 1.3	0.1 ± 0.01
	60	0.5 ± 0.1	0.5 ± 0.04	0.5 ± 0.3	1.6 ± 0.4	1.6 ± 1.2	0.01 ± 0.002
7	10	6.0 ± 0.8	24.0 ± 1.3	4.9 ± 0.9	23.6 ± 3.6	1.6 ± 0.3	0.4 ± 0.02
	60	4.0 ± 0.7	13.1 ± 1.2	5.0 ± 0.3	21.5 ± 5.4	1.3 ± 0.1	0.2 ± 0.01
OIH	10	11.4 ± 2.3	4.6 ± 0.5	2.0 ± 1.0	7.6 ± 0.4	1.7 ± 0.3	0.08 ± 0.01
	60	0.9 ± 0.3	0.2 ± 0.09	0.4 ± 0.2	2.0 ± 0.1	1.6 ± 1.1	< 0.01%

[a] Values are mean ± S.D. % injected dose for 3-4 rats. Corrections were made for I-131 spillover into Tc-99m channel.

Table II. Comparison of Urinary Excretion of Tc-99m Complexes of Compounds 4, 5, and 7 with I-131-OIH in Rats.

Tc-99m Complex	Time (min., post injection)					
	10	20	30	40	50	60
4	5.8 ± 0.7	5.0 ± 0.4	4.0 ± 0.5	2.9 ± 0.3	2.4 ± 0.7	1.9 ± 0.4
OIH	50.3 ± 5.3	18.4 ± 3.7	8.21 ± 0.5	4.1 ± 0.5	2.5 ± 0.2	1.5 ± 0.3
5	6.5 ± 0.5	4.6 ± 0.5	3.3 ± 0.2	2.9 ± 0.2	2.3 ± 0.1	1.9 ± 0.2
OIH	54.8 ± 3.3	14.7 ± 3.0	7.4 ± 0.7	4.6 ± 0.2	2.6 ± 0.3	1.8 ± 0.3
7	7.4 ± 0.9	7.9 ± 2.6	5.5 ± 0.8	4.9 ± 0.8	3.7 ± 0.3	2.7 ± 0.7
OIH	46.6 ± 4.1	20.5 ± 6.9	8.7 ± 0.1	6.2 ± 1.9	3.1 ± 0.3	1.8 ± 0.5

[a] Values are mean ± S.D. % injected dose for 3-4 rats. Corrections were made for I-131 spillover into Tc-99m channel.

Table III. Comparison of plasma protein binding in rats and monkey of N_2S_2 dicarboxylate Tc-99m complexes and Tc-99m-MAG3.[a]

Tc-99m Complex	Rat Plasma	Monkey Plasma
MAG3	30.3 ± 1.7	35.2 ± 9.0
5	22.0 ± 1.0	26.5 ± 0.5
4	31.1 ± 1.2	26.2 ± 1.3
7	21.4 ± 0.4	21.9 ± 1.2

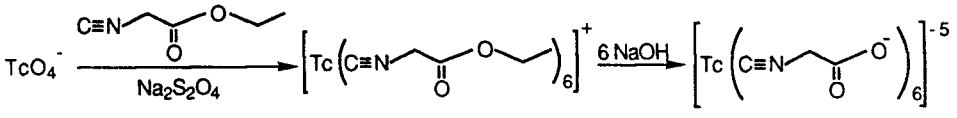
[a] Standard trichloroacetic acid precipitation method. Values are mean ± S.D. for 3 determinations.

SYNTHESIS AND BIOLOGICAL STUDIES OF A NEW RENAL FUNCTION AGENT: [HEXAKIS(CARBOXYMETHYLISOCYANIDE)TECHNETIUM]$^{-5}$

James F. Kronauge, Eva Barbarics, Alan Davison* and Alun G Jones
The Joint Program in Nuclear Medicine, Harvard Medical School and The Brigham and Women's Hospital, Department of Radiology, Boston MA 02115. *The Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

The technetium(I) hexakis isonitrile complexes possess a readily attainable and thermodynamically stable central technetium core. The large variation in alkyl groups available as substituents on the isonitrile ligand affords the ability to custom design the biological behavior of the molecule. Incorporation of an ester group on the isonitrile maintains the neutral change of the ligand and yields a hydrophobic cationic complex when coordinated to technetium(I). Hydrolysis of the ester group to the free acid produces a symmetrical hydrophilic molecule with a large negative charge.

The most hydrophilic derivative of this class of penta-anionic complexes [Technetium (Carboxymethylisocyanide)$_6$]$^{-5}$, [Tc(CNCH$_2$COO$^-$)$_6$]$^{-5}$ (Tc-CAMI) was synthesized from both $^{99}TcO_4^-$ and $^{99m}TcO_4^-$ with an excess of $Na_2S_2O_4$ and carboethoxy methylisocyanide in water/ethanol (1:1) followed by hydrolysis with sodium hydroxide.



Polyanions are typically difficult to characterize however the technique of ^{99}Tc-NMR demonstrated the unique resonance frequency of -1937 ppm (relative to $^{99}TcO_4^-$) for the cationic technetium(I) ester species with the six subsequent small incremental changes (+4 ppm) associated with hydrolysis of each of the coordinated ligands and the concurrently increasing negative charge. Also decreasing the pH, to induce protonation of the free acid groups, shifted the resonance frequency back to that of the original cationic compound.

Simultaneous biodistributions of Tc-CAMI and ^{131}I-Hippuran following coinjection into mice were performed at increasing times post injection with results expressed as percent injected dose and dose per gram in tables 1 and 2. For both compounds more than 80 percent of the dose was in the urine by one hour. Dynamic gamma camera imaging over one hour was used to compare the pharmacokinetics of Tc-CAMI with ^{131}I-Hippuran, Tc-DTPA and Tc-MAG$_3$ with the curves shown in Fig 1. For the simultaneous imaging of ^{131}I-Hippuran and Tc-CAMI, T_{MAX} was very similar 135 s versus 155 s respectively. However renal clearance was significantly longer for Tc-CAMI at 855 s vs 375 s for Hippuran. A summary of the uptake and clearance values for the four agents is presented in table 3. A large intraspecies variation in clearance rates was observed for Tc-CAMI which was probably due to different responses to anesthesia. The highly symmetrical structure of this penta-anionic complex may make it uniquely suitable as a renal function imaging agent.

Table 1. Biodistribution of [^{99m}Tc(Carboxymethylisocyanide)$_6$]$^{+5}$ and (^{131}I) o-Iodohippuran in Mice as % of Injected Dose (*Mean + S.D, n=6)

		5 min*	20 min*	60 min*	120 min*
Blood	Tc-CAMI	9.71 ± 0.90	4.21 ± 1.24	0.38 ± 0.07	0.13 ± 0.02
	I-Hippuran	4.97 ± 0.50	1.04 ± 0.36	0.23 ± 0.04	0.23 ± 0.08
Urine	Tc-CAMI	36.48 ± 0.90	67.10 ± 3.59	81.97 ± 2.51	83.57 ± 3.37
	I-Hippuran	38.91 ± 1.65	71.01 ± 3.24	83.66 ± 2.31	84.73 ± 2.45
Liver	Tc-CAMI	3.40 ± 0.36	1.95 ± 0.24	0.62 ± 0.08	0.50 ± 0.05
	I-Hippuran	3.48 ± 0.72	1.04 ± 0.23	0.19 ± 0.04	0.20 ± 0.04
Kidneys	Tc-CAMI	5.26 ± 0.31	2.77 ± 0.48	0.71 ± 0.09	0.48 ± 0.10
	I-Hippuran	5.08 ± 0.71	1.15 ± 0.50	0.12 ± 0.03	0.13 ± 0.08
Stomach	Tc-CAMI	0.77 ± 0.23	0.51 ± 0.18	0.79 ± 1.31	1.21 ± 1.17
	I-Hippuran	0.66 ± 0.14	0.94 ± 0.17	1.37 ± 1.37	1.83 ± 1.45
Intestine	Tc-CAMI	5.30 ± 0.42	2.94 ± 0.54	1.67 ± 0.89	5.37 ± 2.69
	I-Hippuran	3.29 ± 0.34	1.33 ± 0.28	0.78 ± 0.44	2.29 ± 1.47

Table 2. Biodistribution of [^{99m}Tc(Carboxymethylisocyanide)$_6$]$^{+5}$ and (^{131}I) o-Iodohippuran in Mice as % of Injected Dose per Gram (*Mean ± S.D, n=6)

		5 min*	20 min*	60 min*	120 min*
Blood	Tc-CAMI	7.11 ± 0.66	3.07 ± 1.02	0.28 ± 0.04	0.09 ± 0.02
	I-Hippuran	3.65 ± 0.45	0.76 ± 0.29	0.16 ± 0.02	0.16 ± 0.06
Liver	Tc-CAMI	2.75 ± 0.44	1.57 ± 0.20	0.53 ± 0.09	0.40 ± 0.06
	I-Hippuran	2.79 ± 0.68	0.84 ± 0.18	0.16 ± 0.03	0.16 ± 0.03
Kidneys	Tc-CAMI	14.94 ± 1.66	7.70 ± 1.20	2.16 ± 0.26	1.50 ± 0.31
	I-Hippuran	14.43 ± 2.53	3.12 ± 0.98	0.38 ± 0.09	0.37 ± 0.22

Table 3. Dynamic Gamma-Camera Pharmacokinetic Data in Rabbits for Various Renal Function Agents

	Heart		Right Kidney		Left Kidney		Liver	
	T_{Max} [sec]	$t_{1/2}$ [sec]	T_{Max} [sec]	$t_{1/2}$ [sec]	T_{Max} [sec]	$t_{1/2}$ [sec]	T_{Max} [sec]	$t_{1/2}$ [sec]
Tc-CAMI*	17	88	218	588	213	530	37	563
I-Hippuran	35	200	135	375	135	385	40	240
Tc-DTPA	15	210	150	630	150	780	30	480
Tc-MAG_3	20	60	240	258	240	258	60	260

* Mean value of 3 animals

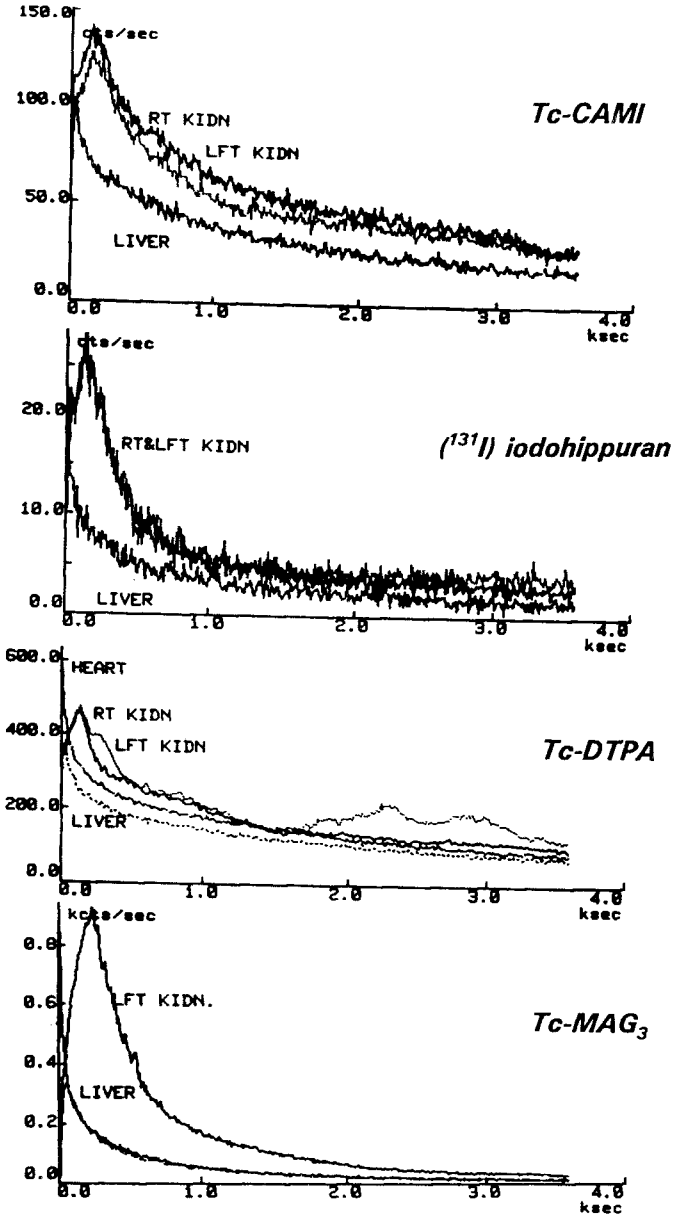


Figure 1. Time activity curves for the kidneys and liver after intravenous injection of Tc-CAMI, (^{131}I) Iodohippuran, Tc-DTPA and Tc-MAG_3 in rabbits

Paper B7

A Reduction of pI Prevents Kidney Uptake of Avidine.
J. M. Jeong, S. Kinuya, C. H. Paik, T. Saga, V. K. Sood, R. D. Neumann, J. C. Reynolds and J.A. Carrasquillo. Nuclear Medicine, NIH, Bethesda, MD 20892 and Radiopharmaceutical Chemistry, George Washington Univ Med Ctr, Washington,DC 20037.

One approach to use a Biotin-Avidine binding system in vivo tumor detection is to inject a radiolabeled avidine after biotin-antitumor antibody conjugates were preinjected and bound to the tumor 1(1). We have attempted to optimize a Tc-99m labeling method of avidine to use for tumor detection. A problem we have encountered was that the labeled avidine produced a very high liver and kidney uptake. It was suspected that the pI 10 of avidne was responsible for the high organ uptakes because this high pI makes avidine highly positively charged at physiological pH. To investigate the above hypothesis, we have reduced the pI of avidine to 4.0-4.8 by acylation reaction with succinic anhydride. The conjugation of succinic acid did not affect the biotin-binding activity as determined by a competitive binding assay using I-125 labeled avidine as a radio-ligand and Agarose-biotin beads as a binding system. Avidine, streptavidine and the modified avidine were labeled with Tc-99m as follows: Biocytin was first conjugated to Bz-MAG3 by acylation reaction with N-hydroxysuccinimide ester of Bz-MAG3 in DMF (2). Bz-MAG3-biocytin was labeled with Tc-99m with a >95 % yield using a method similar to Fritzberg (3). Tc-99m MAG3-biocytin was then incubated with avidine, streptavidine and avidine-succininc acid with Tc-99m-MAG3-biocytin at a molar ratio of 1 for 1 hr at room temperature. The labeling yields were quantitative.

Tc-99m-MAG3-biocytin bound avidine, streptavidine and avidine-succinic acid were purified by size exclusion HPLC and injected into a tail vein of mice for in-vivo comparison. The Tc-99m labeled avidine was eliminated from blood very rapidly and taken up primarily by liver and kidneys: at 15 min 2 % ID remained in the total blood, 57 % in liver,and 29 % in kidneys. The liver and kidney radioactivities decreased to 13 and 2 %, respectively at 21 hr. The intestinal activity was less than 9 % ID throughout the time period. The Tc-99m labeled Avidine-succinic acid produced a much higher blood activity (30 % ID) at 15 min whereas the kidney uptake was negligible (2 % ID). The initial liver activity was similar to the unmodified avidine activity but removed from liver faster so that the activity remaining at 21 hr was 4 % ID. The intestinal activity was similar to that of avidin. The whole body clearance of the modified avidine (T1/2=14 hr) was slightly faster than that of the native avidine (T1/2=17 hr). This result indicates that the kidney uptake occurs primarily through a charge interaction and the binding of labeled avidine to the kidneys can be prevented by a reduction of pI to 5. The liver uptake, however, was not decreased substantially by the pI reduction although the chemical modification increased the clearance rate of the activity from the liver. Comparison with the biodistribution of the labeled streptavidine (pI 6.5), which does not have a carbohydrate moiety, indicates that the major cause of the liver uptake of avidine is the binding of the carbohydrate moieties of avidine to hepatocytes.

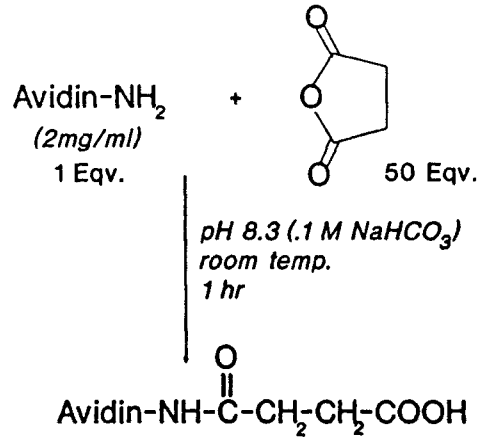
References

1. Hnatowich D.J., Virzi F., Rusckowski M., J. Nucl. Med., 28, 1294-1302 (1987).

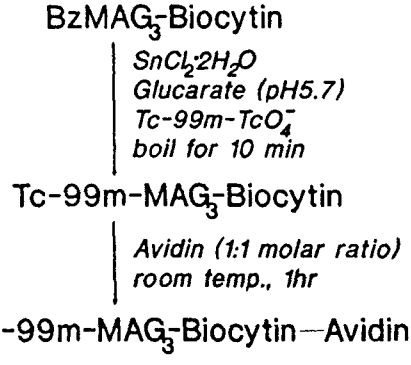
2. Jeong J.M., Kinuya S., Paik C.H., et al. Submitted to 9th International Symposium on Radiopharmaceutical Chemistry to be held in Paris France, April 6-10, 1992.

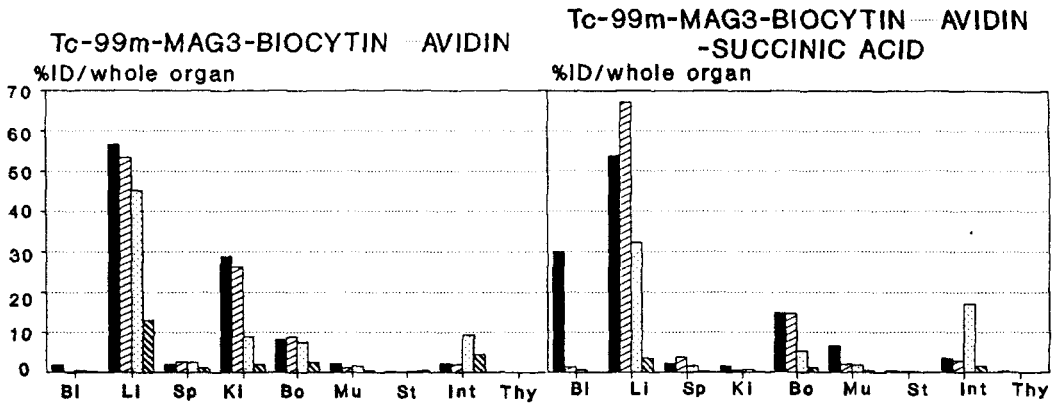
3. Fritzberg A.R., Kasina S., Eshima D., et al. J. Nucl. Med., 27, 111-116 (1986).

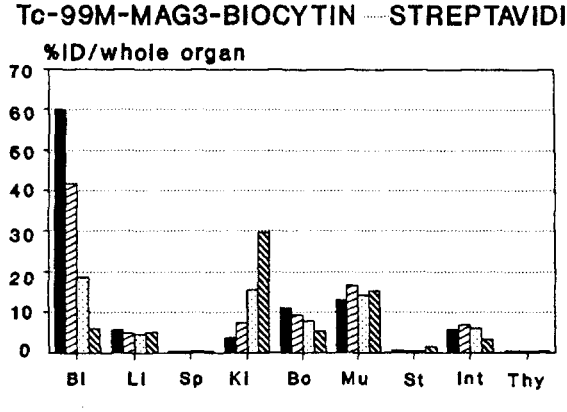
Conjugation of Succinic Acid to Avidin

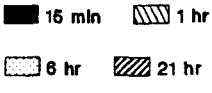


Labeling of Avidin with Tc-99m









Bl, Blood; Li, Liver; Sp, Spleen; Ki, Kidney; Bo, Bone; Mu, Muscle; St, Stomach; Int, Intestine; Thy, Thyroid.

TECHNETIUM-99M-LABELED CHEMOTACTIC PEPTIDES VIA THE HYDRAZINO NICOTINAMIDE DERIVATIVE FOR IMAGING FOCAL SITES OF INFECTION.

JW Babich, MJ Abrams*, D Schwartz*, D Kroon#, M Pike, HW Strauss, RH Rubin, AJ Fischman.
Division of Nuclear Medicine, Dept. of Radiology, Massachusetts General Hospital, Boston, MA, *Johnson Matthey, West Chester, PA, #RW Johnson Pharmaceutical Research Institute, Raritan, NJ.

We have previously shown that In-111-labeled chemotactic peptides (CTPs) were effective agents for the external imaging of focal sites of infection in rats(1). However, the short biological half-time of the peptides makes In-111 a poor choice for imaging. Tc-99m is ideal for labeling CTPs due to its short physical $T_{1/2}$, high specific activity, excellent imaging properties, low cost and widespread availability. The active ester of hydrazino-nicotinic acid (HyNic) has been successfully used as a direct protein conjugate capable of facilitating quantitative Tc-99m labeling from simple complexes possessing the Tc(v) oxo core (2). In this study we have applied HyNic derivatization to CTPs and investigated the biological activity, labeling chemistry and infection localizing ability of these novel CTPs.

Methods: The following HyNic containing CTPs were synthesised using the Merrifield solid phase technique and purified by HPLC: (A); f-Met-Leu-Phe-(N -HyNic)Lys, (B); f-Met-Leu-Phe-$NH(CH_2)_6NH$(HyNic), (C); f-Met-Leu-Phe-(N -HyNic)-D-Lys-NH_2, and (D); f-Nle-Leu-Phe-(N -HyNic)Lys-NH_2. Biological activity was evaluated by assaying superoxide production and determining the binding affinity to human PMNs via competitive binding of ForML[^{3}H]F as previously described(1). Tc-99m labelling experiments were performed with the intention of maximizing the specific activity. Imaging experiments were performed in rats with focal E. Coli infections in the thigh. HyNic-CTPs were labeled with Tc-99m by incubating equal volumes of Tc-99m-glucoheptonate (DuPont) (>150 mCi/mL) and HyNic-CTPs (>40 ug/mL in pH 5.2 acetate)) together at room temp. for 1 hour. Radiochemical purity (RCP) of the Tc-GH was determined by ITLC-SG using both acetone and saline. The Tc-99m-GH RCP was always >95%. Peptide labeling was determined ITLC-SG and acetone:water (9:1). Tc-99m incorporation into the peptide was ca. 95% at 1 hour.

Results: The HyNic-CTPs presented here all demonstrated binding affinities for the chemoattractant receptor below 20 nM. All HyNic-CTPs produced superoxide with potencies similar to ForMLF. Tc-99m-labeling of HyNic-CTPs was near quantitative when final CTP conc. was >20 ug/mL. These initial experiments indicate it is possible to label chemotactic peptide to specific activities above 2000 mCi/uMole. Tc-99m-CTPs were shown to localise as well as In-111-labeled CTP in focal E. Coli infections.

REFS.1) Fischman AJ, et al, J Nucl Med 1991;32:483-491.
2) Abrams MJ, et al, J Nucl Med 1990; 31:2022-2028.

SYNTHESES OF QNB BORONIC ACID STEREOISOMERS AND THEIR BATO COMPLEXES: POTENTIAL ^{99m}Tc mACh RECEPTOR BINDING TRACERS

P. Nanjappan, K. Ramalingam, S.Jurisson, J. Pirro, R. Di Rocco, R.K. Narra, D.P. Nowotnik, A.D. Nunn. Bristol-Myers Squibb Pharmaceutical Reseach Institute, New Brunswick, NJ 08903, USA.

Changes in muscarinic acetylcholinergic receptor (mAChR) density in the brain have been associated with several neurodegenerative disorders, including Alzheimer's disease (1). Therefore, a radiotracer which is labeled with ^{99m}Tc and binds to the mAChR might be useful for the routine evaluation of receptor density. To achieve this goal, we have investigated new technetium BATO (BATO=Boronic Acid adduct of Technetium diOxime) complexes which are covalently linked to the mAChR antagonist, QNB (3-quinuclidinyl benzilate). This was achieved by the addition of a *p*-boronic acid group to one of the phenyl rings of QNB (QNB boronic acid; a compound described as its racemate by Kabalka et al (2)).

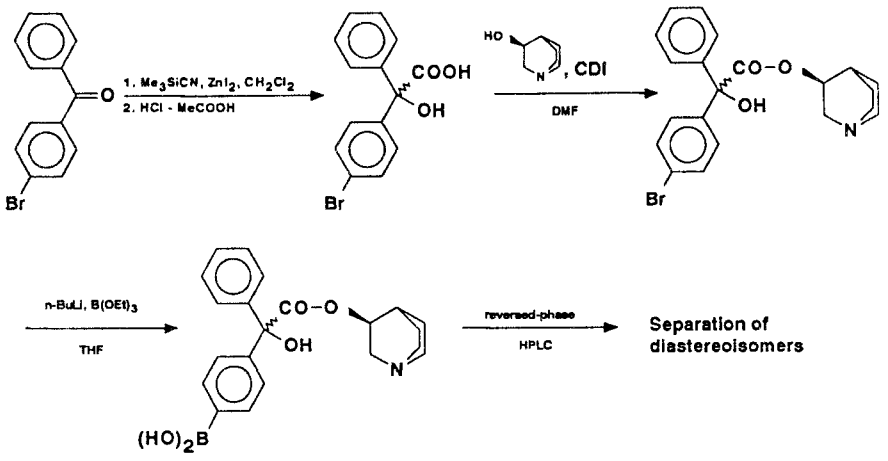
It is known that receptor binding can be influenced by the stereochemical configuration. QNB boronic acid contains 2 chiral centers, so it was necessary to prepare all four stereoisomers of QNB boronic acid. We report here on the synthesis of the four QNB boronic acid stereoisomers. The initial route was based on that described for iodo-QNB (3). However, we modified the procedure to reduce the number of steps, and, by use of preparative HPLC to separate diastereoisomers, only two syntheses were needed to produce all four isomers.

The affinity binding constants (K_a) of the four stereoisomers of QNB boronic acid were determined for both rat caudate putamen and rat heart receptor preparations. The results show that R-quinuclidinyl isomer has about a 55 fold higher affinity than does the S isomer. The stereochemistry at the benzilate center does not appear to be as important; the R-benzilate has about a 5 fold higher affinity than does the S isomer.

The QNB BATO complexes were prepared by 'capping' the $^{99/99m}$TcCl(DMG)$_3$ complex (4,5) with the QNB boronic acid. Initial in vitro receptor binding studies with these complexes suggest high non-specific binding, probably resulting from the high lipophilicity of these complexes. These results are similar to those reported for syn-Tc-DADT-QNB (6), which also displayed predominantly non specific binding. However, the in vivo heart time-activity profile (in rats) for the (S)QN(R)B-BATO indicated possible receptor binding (the % id in heart increases with time, 0-240 min). By comparison, the other stereoisomeric complexes displayed more conventional wash-in/wash-out profiles, typical for non-retained complexes. Other differences in the biodistribution of the stereoisomeric complexes were also observed, e.g. the blood and liver time/activity profiles.

These data indicate that these complexes show some initial promise for imaging the mAChR, but improvements are required; in particular, derivatives with lower non-specific binding are required.

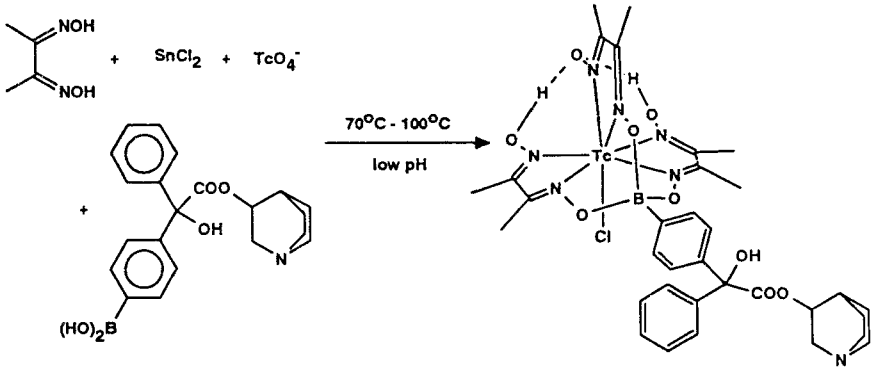
Syntheses of the QNB boronic acid stereoisomers



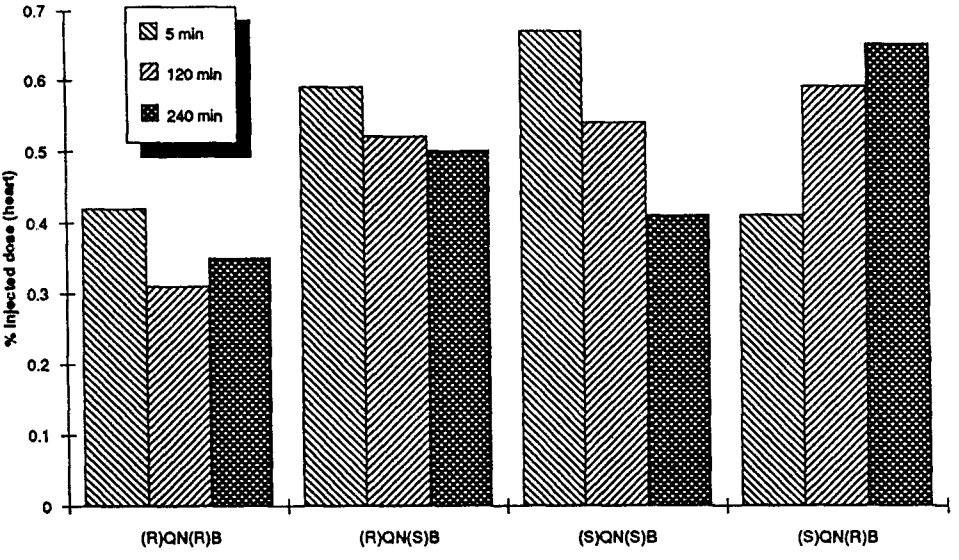
In vitro receptor binding of the QNB boronic acid stereoisomers:

Compound	K_a (caudate putamen)	K_a (heart)
R,S-QNB	3.24 (±1.21) x 10^9	1.55 (±0.42) x 10^9
(R)QN(R)B-B(OH)2	1.44 (±0.40) x 10^8	5.10 (±1.63) x 10^7
(R)QN(S)B-B(OH)2	2.42 (±0.34) x 10^7	9.78 (±2.49) x 10^6
(S)QN(R)B-B(OH)2	3.36 (±1.11) x 10^6	6.14 (±0.64) x 10^5
(S)QN(S)B-B(OH)2	5.51 (±0.28) x 10^5	1.90 (±0.25) x 10^5

Syntheses of the QNB-BATOs:



Heart time-activity profiles of the QNB-BATO stereoisomers (rats)



References:

1. Holman BL, Gibson RE, Hill TC, et al. *JAMA* 1985; 254: 3063-3066.

2. Kabalka GW, Gai Y-Z, Mathur S. *Nucl. Med. Biol.* 1989; 16: 359-360.

3. Cohen VI, Rzeszotarski WJ, Gibson RE, Fan LH, Reba RC. *J. Pharm. Sci.* 1989; 78: 833-836.

4. Treher EN, Francesconi LC, Gougoutas JZ, Malley MF, Nunn AD. *Inorg. Chem.* 1989; 28: 3411-3416.

5. Linder KE, Malley MF, Gougoutas JZ, Unger SE, Nunn AD. *Inorg. Chem.* 1990; 29: 2428-2434.

6. Lever SZ, Wagner HN. In: Nicolini M, Bandoli G, Mazzi U, Nicolini M, Bandoli G, Mazzi U,eds. *Technetium and rhenium in chemistry and nuclear medicine 3*. Verona, Italy: Cortina International; 1990:649-659.

Paper B10

SYNTHESIS AND TECHNETIUM-99m LABELLING OF 2-ALKOXYISOBUTYLISONITRILE

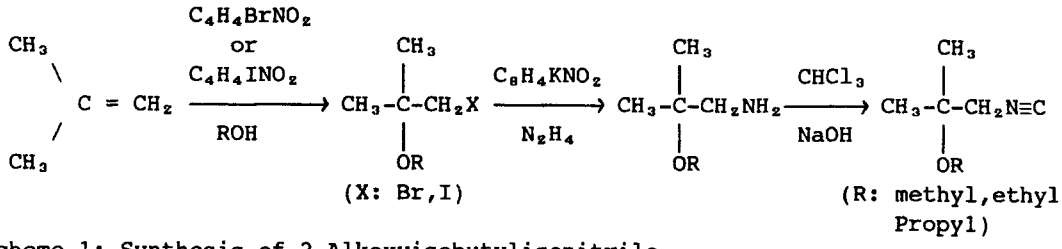
Te-Wei Lee, Chang-Shinn Su, Lie-Hang Shen, and Gann Ting

INSTITUTE OF NUCLEAR ENERGY RESEARCH
P.O.BOX 3-27 LUNGTAN TAIWAN, R.O.C.

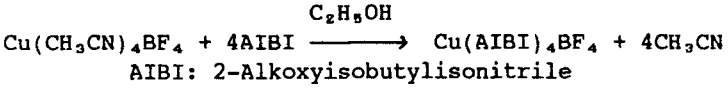
^{99m}Tc labelled isonitrile compounds have been proven to be a myocardial perfusion agent[1-3]. The synthesis of isonitrile ligand has been published literature[4-5]. We developed a novel process for synthesizing of 2-alkoxyisobutylisonitrile. Isobutylene was used as the starting material for synthesis of 2-alkoxyisobutylisonitrile. The haloalkoxylation of isobutylene in alcohol medium gives 2-alkoxyisobutylhalide which is then converted to 2-alkoxyisobutylamine. In the basic condition, the reaction of 2-alkoxyisobutylamine with chloroform produces 2-alkoxyisobutylisonitrile (scheme 1). Their chemical identity was confirmed by IR, ^{13}CNMR and ^{1}HNMR methods.

The ligands were labelled with technetium-99m by exchange labelling of stable tetrakis(2-alkoxyisobutylisonitrile) copper (I) complex. Tetrakis(2-alkoxyisobutylisonitrile) copper(I) complex can be prepared by the exchange of acetonitrile molecules in tetrakis(acetonitrile) copper(I) complex with isonitrile ligands(scheme 2). These products were also analyzed by elemental analysis, IR, ^{13}CNMR and ^{1}HNMR. The radiochemical purity of ^{99m}Tc-2-alkoxyisobutylisonitrile was determined by usine ITLC-SG strips method[6].

Reference:
1. Holman B.L., Jones A.G., Lister-James J., Davison A., Abrams M.J., Kirshenbaum J.M., Tumeh S.S, and English R.J., J. Nucl. Med. 25: 1350(1984).
2. Holman B.L., Sporn V., Jones A.G., Sia B., Perez-Balino N., Devison A., Lister-James J., Kronauge J. F., Mitta A.E.A., Camin L. L., Campbell S., Williams S.J., and Carpenter A.T., J. Nucl. Med. 28:13 (1987)
3. Okada R.D., Glover D., Gaffney T., and Williams S., Circulation V. 77:491(1988).
4. Bergstein P.L., Subramanyam V. European Pat. 233368.
5. van Wyk A.J., van Aswegen A., Knoesen O., Fourie P.J., Koekemoer J., Herbst C.P., Otto A.C., and Lotter M.G. Appl. Radiat. Isot. 42:687(1991).
6. Proulx A., Ballinger J.R., Otto A.C. and Lotter M.G., Appl. Radiat. Isot. 40:95(1989).



Scheme 1: Synthesis of 2-Alkoxyisobutylisonitrile



Scheme 2: Preparation of Tetrakis(2-Alkoxyisobutylisonitrile)copper(I) Complex

Paper B11

Convenient Synthesis of Analogs of N-(S-Benzoylmercaptoacetyl)glycylglycylglycine

C. H. Yang, C. C. Chen and M. K. Chen, Department of Chemistry
Chung-Yuan Christian University, Chung-Li, Taiwan, R.O.C.

G. Ting, and T. W. Lee, Institue of Nuclear Energy Research,
Lung-Tan, Taiwan, R.O.C.

The Tc-99m Complex of N-(S-Benzoylmercaptoacetyl)glycylglycylglycine (S-Bz-MAG_3) is used as a renal imaging agent. However, the renal tubular extraction of Tc-99m-MAG_3 was only 66% of that of I-131-OIH[1]. Therefore, It is desirable to prepare analogs of S-Bz-MAG_3 in the hope of finding complex with even better properties for renal imaging. Fritzberg et al.[2] had prepared a series of S-Bz-MA-Gly-Gly-XXX using tripeptides, Gly-Gly-XXX, as the starting materials.Similarly, Verbruggen et al.[3] also prepared S-Bz-MA-XXX-YYY-ZZZ using tripeptides, XXX-YYY-ZZZ, as the starting materials. In our approach, succinimidyl active ester of S-Benzoylmercatoacetylglycine and S-Benzoyl-mercaptoacetyl-β-alanine were first prepared and coupled to the more easily available dipeptides, Gly-XXX, to pepare a series of analogs, S-Bz-MA-Gly-Gly-XXX and S-Bz-MA-β-Ala-Gly-XXX. In total, ten analogous compounds were synthesized by this scheme, a reflection of the versatility of this method. Although in preparation of S-Bz-MA-Gly and S-Bz-MA-β-Ala, methanol was used, no ester contaminant[4] from this step was detected in the final product as observed by HPLC.
The evaluation of Tc-99m complexes of these compounds for renal imaging is currently undergoing.

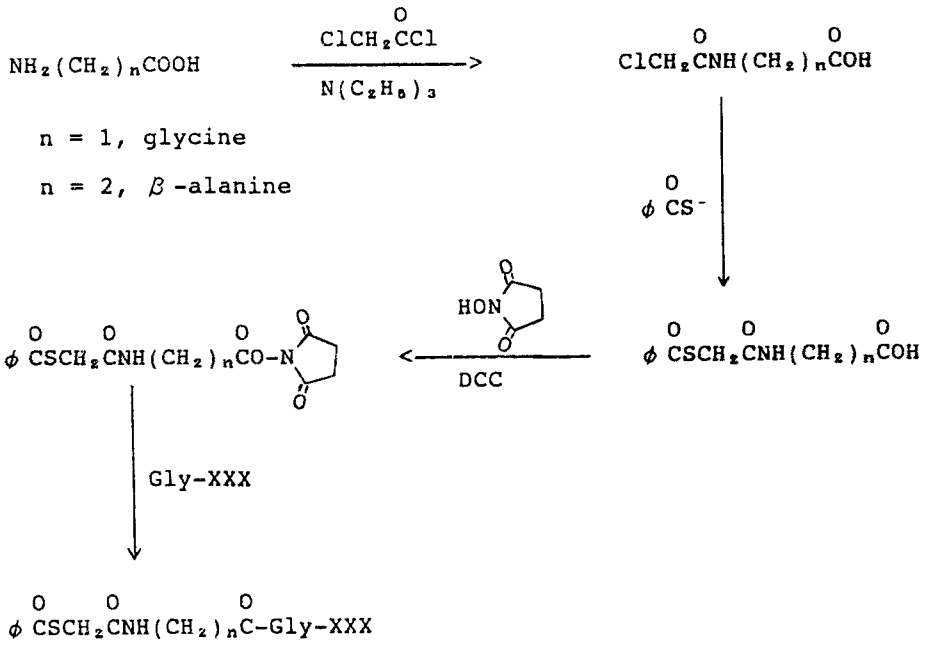
References:

1. Taylor, A. et al., Radiology, 170, 721 (1989).

2. Fritzberg, A. R., Eur. Pat. Appl. 0173424 (1986)

3. Verbruggen, A. M., Eur. Pat. appl. 0250013 (1987)

4. Nosco, D.L., et al., J. Labelled compounds and Radiopharmaceuticals 30, 6 (1991).



Synthesized Analogs:

	M.P. (° C)
1. S-Bz-MA-Gly-Gly-Gly	197 - 198
2. S-Bz-MA-Gly-Gly-Leu	205 - 208
3. S-Bz-MA-Gly-Gly-Phe	198 - 200
4. S-Bz-MA-Gly-Gly-β-Ala	199 - 202
5. S-Bz-MA-Gly-Gly-Gly-Gly	246 - 248
6. S-Bz-MA-β-Ala-Gly-Gly	215 - 216
7. S-Bz-MA-β-Ala-Gly-Leu	220 - 224
8. S-Bz-MA-β-Ala-Gly-β-Ala	192 - 193
9. S-Bz-MA-β-Ala-Gly-Gly-Gly	236 - 238
10. S-Bz-MA-β-Ala-Gly-Phe	210 - 214

Paper B12

中国原子能科学研究院

INSTITUTE OF ATOMIC ENERGY
P.O.BOX275,BEIJING
PEOPLE'S REPUBLIC OF CHINA

PREPARATION OF ^{99m}Tc-LABELLED d,l-DIASTEREOISOMER OF HM-PAO FOR CEREBRAL BLOOD FLOW IMAGING(1)

Lanqin Bai, Jinjie Huang, Souzhen Bai, and Lun Xiao (2)
China Institute of Atomic Energy. P.O.Box 275,Beijing 102413,China

The d,l-disatereoisomer of hexamethyl propyleneamine oxime (HM-PAO) was labelled with Tc-99m for cerebral perfusion imaging. Improvements of the synthesis and isolation of HM-PAO resulted in pure d,l-HM-PAO.

^{1}H NMR spectroscopy was found to be able to differentiate effectively between d,l- and meso- HM-PAO isomers in the diastereoisomeric mixture, and provide a rapid as well as simple analysis of the purity of the fractions containing one or both of these diastereoisomers. The purity of d,l-HM-PAO analysed by ^{1}H NMR was consistently in excess of 99%.

The Tc-99m complex of d,l-HM-PAO was formed by injecting five milliliters of sodium pertechnetate (Tc-99m, 74 - 740 MBq/ml) into a sealed 10 ml glass vial containing a freeze dried formulation of 1.0 mg of d,l-HM-PAO, 8 μg of stannous chloride dihydrate, and 4.5 mg of sodium chloride under an atomsphere of nitrogen. The vial was shaken to dissolve the solid contents. The radiochemical purity (RCP) of the complex was assayed by thin layer chromatography using three systems of developing reagents. The initial RCP was consistently in excess of 90%.

The biodistribution of the primary complexes in mouse showed 2.24%I.D. brain uptake 2 mins after injection. The retention of activity in the brain was high,up to 97% at 1 hr postinjection, and 72% at 24 hrs postinjection. The biodistribution of the primary of the complex of meso-HM-PAO showed mouse brain uptaking of 1.93% I.D. at 2 mins postinjection. The retention was lower, 44% of the brain uptake at 1 hr postinjection, and 25% at 24 hrs postinjection.

---

(1) Partially supported by IAEA.

(2) To whom all correspondence should be addressed.

中国原子能科学研究院

INSTITUTE OF ATOMIC ENERGY
P.O.BOX275,BEIJING
PEOPLE'S REPUBLIC OF CHINA

The analytical results of the diastereoisomers are shown below:

M.P.: d,l- 133.1 - 133.4°C meso- 147.0 - 148.0°C

IR: 3310 cm^{-1}(OH), 3200-3100 cm^{-1}(OH,NH),
1380-1370 cm^{-1}(CH_3-C-CH_3)

Elemental analysis of $C_{13}H_{28}N_4O_2$(Mol. wt. 272):
Calculated C 57.3, H 10.4, N 20.6
Found C 57.4, H 10.5, N 20.6

MS: m/e = 272(M^+)

^{1}H NMR assignments, shown in Table 1.

Table 1. Assignments, chemical shifts(δ,ppm) and coupling constants(J,Hz) of HM-PAO diastereoisomers

Isomer	Me^1	Me^2	Me^3	CH_2N	CHMe
d,l	0.7880	1.0802, 1.0668	1.6469	2.1779, 2.0739	3.1294
	s	d,J=6.71	s	ABq,J=11.56	q,J=6.77
meso	0.7804, 0.7775	1.0774, 1.0640	1.6470	2.1766, 2.0756	3.1423
	s	d,J=6.71	s	ABq,J=10.90	q,J=6.71

Note: Measured in DMSO at 25°C, 500 MHz. s -- singlet, d -- doublet, ABq -- AB quartet.

Paper B13

Optimum Sythesis Condition for Technetium-99m Labelled DTPA-HSA and Its Behaviour

Tang H., Ding J. H., Lin Q.F., Fan G.Y., Bai L.Q.
Isotope Department of China Institute of Atomic Energy
P.O. Box 275 (58). Beijing 102413, P.R. China

It is worthy to be considered to improve the in vivo stability of radionuclides labelled protein by bifunctional chelators conjugation [1,2]. The conjugation of DTPA to HSA using cyclic DTPA dianhydride (CDTPAA) and the labeling of DTPA-HSA with Technetium-99m have been investigated in our lab. . The effects of pH, stannous concentration and DTPA:HSA (reaction molar ratio) is represented here, and blood clearance of labelled complexes in mice was compared.

The sythesis of CDTPAA was carried out according to Eckelman et al [3]. We found the existence of unexpected impurity in the product with 500 MHz NMR., that is undetectable with 60 MHz NMR [1], and the impurity can be minimized in drier cythesis condition such as in winter in our normal chem. lab. . (See Fig. 1.2)

The relation between DTPA/HSA moler ratio and Tc-99m-complexes yield was research by labeling the mixture solution of HSA and free -DTPA with Technetium-99m in Clark-Lubs buffer (CLB) at pH 5. HSA (purified previously by sephadex G 50 column) concentration is 10 mg/ml, $SnCl_2 \cdot 2H_2O$ 0.15 umol/ml . (See Fig. 3.) Effect of pH on the formation of Tc-99m-complexs was determined by labeling the mixture of HSA and free -DTPA with Technetium-99m in CLB ,pH 3-7, HSA 10 mg/ml , DTPA/HSA =5 molar ratio,$SnCl_2$ 0.15 umolar/ml. It shows that Tc-99m-DTPA yield > 80% at pH 5-7. (Fig. 4.). The dependence of Tc-99m-DTPA yield on Sn/DTPA was researched by labeling the mixture of HSA and free-DTPA in CLB at pH 5 , HSA 10mg/ml, DTPA/HSA=2.5. It shows that Sn/DTPA < 1 is the suitable labeling condition.

The preparation of DTPA-HSA is carried out by addtion of CDTPAA, DTPA/HSA 1,3,5,10, to HSA 10mg/ml at pH 5 in CLB., then purified by sephadex G50 column (1×30). The purified DTPA-HSA is labelled by $Na^{99m}TcO$ elution, $SnCl_2 \cdot 2H_2O$ 0.3 umol (Fig. 6) and $SnCl_2 \cdot 2H_2O$ 0.1,0.5, 0.7 umol when DTPA/HSA=5. (Fig.7). The blood clearance in mice of there various labelled compounds have been researched. It seems that the DTPA/HSA reaction molor ratio (from 1-10) and Tin concentration (0.1-0.7 umol when DTPA/HSA=5) does not effect much on the behaviour of these labelled compounds in mice blood.

Reference:

1. D.J.Hnatowich, W.W.Layne and R.L.Childs Appl.Radiat. Isot 33.327 (1982).
2. Mrinal K.Dewanjee Seminars in Nuclear Medicine 20.5-27 (1990).
3. Eckelman W.C., Karesh S.M and Reba R.C. J.Pharm. Sci. 54.704 (1975).

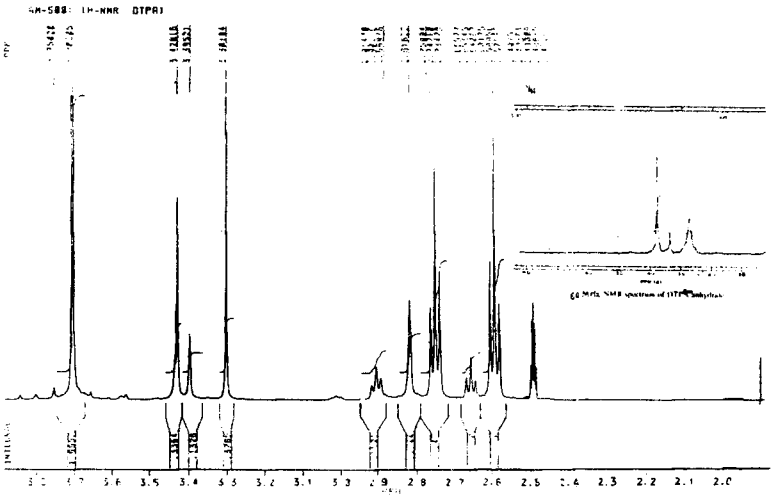


Fig.1. 500 & 60 MHz NMR Spectrum of Product of CDTPAA containing inpurity.

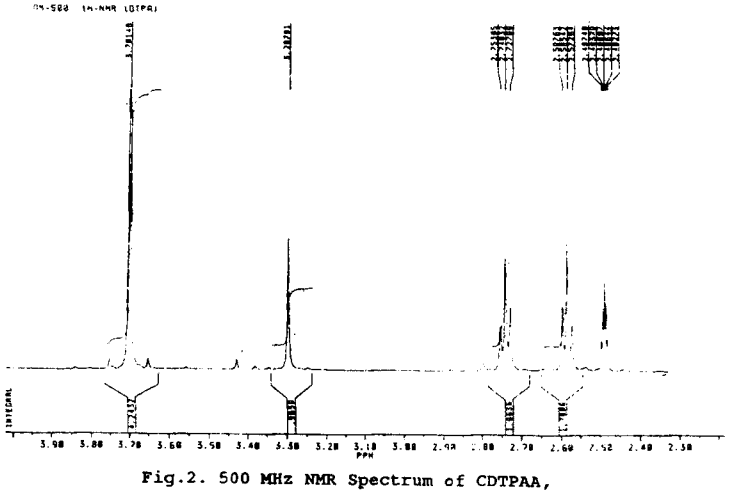


Fig.2. 500 MHz NMR Spectrum of CDTPAA, impurity minimized.

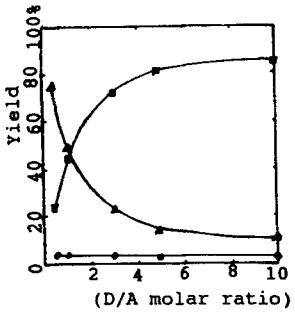


Fig.3

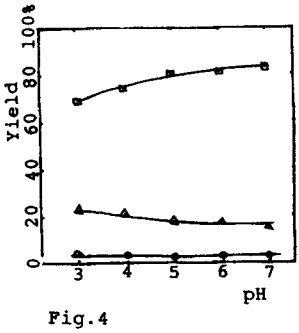


Fig.4

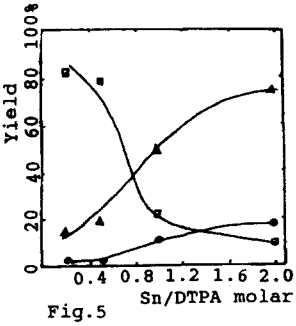


Fig.5

Fig.3-5.
Effect of DTPA/HSA molor ratio pH and Sn/DTPA molar ratio on Tc-99m-complex yield, respectively.

Tc-99m-DTPA
Tc-99m-HSA
colloid

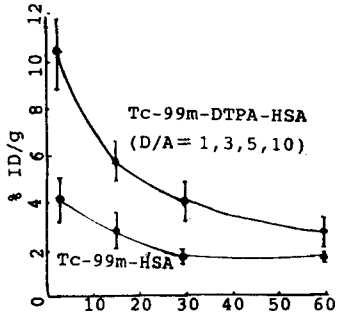


Fig.6.
Blood clearance in mice for Tc-99m-DTPA-HSA, formed at different D/A reaction molar ratio.

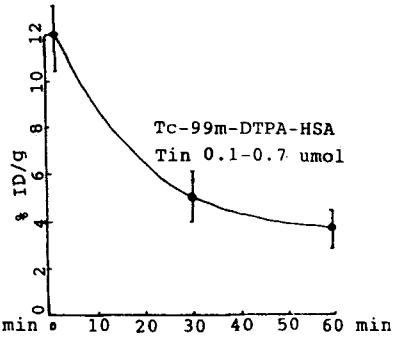


Fig.7.
Blood clearance in mice for Tc-99m-DTPA-HSA,different Sn/DTPA.

Paper B14

SYNTHESIS OF Tc-99m LABELLED RANITIDINE

Du Jin, Jin Xiao-Hai, Yaun Song-Ying
Isotope Department of China Institute of Atomic Energy,
P.O. Box 275 (58), Beijing 102413, P.R. China

Ranitidine hydrochloride is a highly potent H_2 receptor antagonist of value in treatment of peptic ulceration. Tc-99m-ranitidine may be used for the dynamic study of ranitidine drug in vivo, specific organ localization based on its special drug-receptor interaction. But the ranitidine(R) can not be labelled with Tc-99m directly. In order to label ranitidine with Tc-99m, we developed a method of Tc-99m indirect labelled ranitidine and studied labelling conditions of this method and factors of influedcing labelling efficiency. First of all, functional groups (NO_2) of ranitidine was reduced with $SnCl_2$ under special conditions and then DTPA cyclic anhydride was conjugated with it. The reaction mixture was purified to separate free DTPA from R-CDTPA by dialysis method, and then R-CDTPA was labelled with Tc-99m. The labelling yield was determined by paper chromatography method, radiochemical purity of Tc-99m-R-CDTPA is more than 99%.

Labelling procedure: 1g ranitidine was reduced with $SnCl_2$ under special conditions, the DTPA cyclic anhydride was synthesized according to the method of C.H. Paik et al. Preparaing ranitidine-DTPA conjugates in 0.05N PBS (pH 7.4) at molar ratio 1:2, the conjugates was purified by Sephadex G-20 to remove free DTPA. The pure conjugates was labelled with Tc-99m solution. The radiolabelled ranitidine was analyzed by paper chromatography and devoloped system of acetate buffer solution (pH 6) was used. Rf of the labelling ranitidine is 0.9.

The effect of pH values of reaction medium, various quantities of R-CDTPA, reaction time and temperature on labelling efficiency was studied in detail and optimum values were obtained. The variation of the labelling efficiency with pH, mass of R-CDTPA, reaction time and temperature are showen in Fig 1-4. The effect of pH values was very important, as Fig 1. shows, the best pH values of labelling medium is 5-6. The stability of Tc-99m-R-DTPA is given in Table 1.

The final results indicated that radiochemical purity of Tc-99m-R-DTPA is more than 99%. The initial results of clinical experiments have shown that the dynamic studies of ranitidine drug in vivo are very satisfied.

Supporting data for: Synthesis of Tc-99m Labelled Ranitidine

Du Jin, Jin Xiao-Hai, Yaun Song-Ying
Isotope Department of China Institute of Atomic Energy,
P.O. BOx 275 (58), Beijing 102418, P.O. China

Table. 1 The stability of Tc-99m-R-DTPA

Time (hr)	radiochemical purity
6	98%
12	97%
18	96%
24	96%

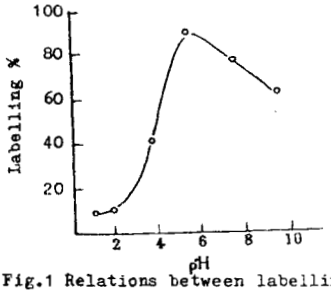


Fig.1 Relations between labelling % and the pH values

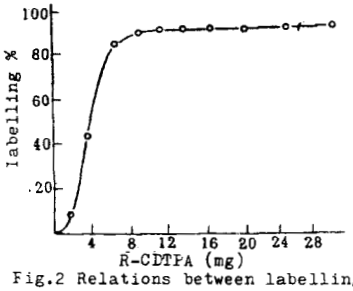


Fig.2 Relations between labelling % & mass of ranitidine-CDTPA

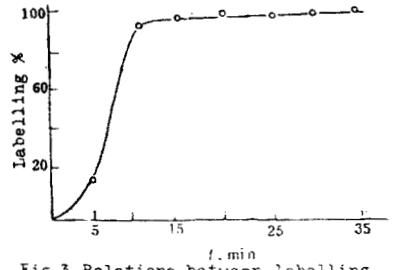


Fig.3 Relations between labelling & reaction time

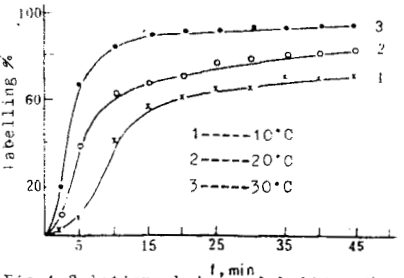


Fig.4 Relations between labelling % and temperature

Paper B15

EFFECT OF STEREOISOMERS (D,L AND MESO) OF TC-99M HMPAO ON PLATELET LABELING AND KINETICS IN BEAGLE DOGS: COMPARISON WITH IN-111 LABELED PLATELETS.

Mrinal K. Dewanjee, Abdol K. Ghafouripour, Mansoor Kapadvanjwala, William. I. Ganz, Aldo N. Serafini, George N. Sfakianakis. Departments of Radiology and Biomedical Engineering, University of Miami School of Medicine, Miami, Florida, U.S.A.

INTRODUCTION. The clearance of stereoisomer of (d,l) Tc-99m HMPAO from the brain was found slower than that of meso isomer (1-3). We wanted to test the hypothesis, whether this difference of cerebral-clearance also holds true for Tc-99m HMPAO labeled autologous platelets. We carried out the platelet-kinetics with both stereoisomers.

EXPERIMENTAL PROCEDURES: HMPAO stereoisomers were synthesized and separated by fractional crystallization (8X). Sn(II)-HMPAO kits (50 μg of HMPAO and 8 μg of $SnCl_2$, $2H_2O$) were prepared and lyophilized. Forty three milliliters of blood were collected in 7 ml of ACD anticoagulant and platelet-labeling was carried out in ACD-saline media with 40-50 mCi of freshly eluted Tc-99m pertechnetate from Mo-99-->Tc-99m generator (Medi-Physics Inc.). Time-dependence of platelet-uptake was studied by incubation of aliquots ($1.2X10^9$, triplicate) of platelets with stereoisomers of Tc-99m HMPAO (100 μCi). Platelet-kinetics was repeated five times in two conditioned Beagle dogs after injection of 30-35 mCi of Tc-99m labeled autologous platelets. Serial blood samples (7 ml) were obtained by veni-puncture at 10, 30, 120, 240, 320 minutes, 24, 48 and 72 hours. The blood samples were centrifuged and radioactivity in plasma and cell pellet was determined with a gamma-counter. After lysing the labeled platelets the Tc-99m radioactivity in the cytoplasmic proteins and organelles was determined with a gamma-counter; 50-60% of the radioactivity in the cytoplasm was protein-bound.

RESULTS AND DISCUSSION: The values of mean and standard deviation of labeling efficiency, platelet-survival time (exponential) and recovery were calculated and shown in Table 1. The kinetics of uptake and platelet-retention (d,l: 70%; meso: 64%) was slightly higher for d,l stereoisomer; although the results are not statistically significant. The in vivo results indicate that the survival time is almost similar for meso and d,l stereoisomer, suggesting that for cell-labeling with HMPAO the separation of stereosiomer before kit preparation may not be necessary. The platelet survival times with both stereoisomers were significantly lower than In-111 labeled platelets, suggesting continuous loss of Tc-99m labeled small molecules from Tc-99m labeled platelets.

Table 1. Comparative evaluation of platelet survival studies in Beagle dogs with stereoisomers of Tc-99m HMPAO and In-tropolone

	^{99m}Tc-HMPAO (meso)	^{99m}Tc-HMPAO (d,l)	^{111}In-Tropolone	
Labeling eff(%)	54.8 ± 7.6	59.6 ± 6.5	75.6 ± 11.4	
Recovery(%)	46.7 ± 5.0	43.3 ± 12.0	56.4 ± 10.6	
Survival time (hr)	31.6 ± 3.1	29.5 ± 3.3	46.3 ± 6.7	

REFERENCES

1. Dewanjee MK, Rao SA, Didisheim P: Indium-111 tropolone, a new high-affinity platelet label: Preparation and evaluation of labeling parameters, J. Nucl. Med. 22: 981-987(1981).
2. Becker W, Borner W, Kromer EP, et al: Tc-99m-HMPAO: A new platelet labeling compound? Eur. J. Nucl. Med. 13: 267-268(1987).
3. Neirinckx RD, Canning LR, Piper IM, Nowotnik DP, Pickett RD, Holmes RA, Volkert WA, Forster AM, Weismer PS, Marriott JA, Chaplin SB: Technetium-99m, d,l-HMPAO: A new radiopharmaceutical for SPECT imaging of regional cerebral blood perfusion. J. Nucl. Med. 28:191-202 (1987).

ACKNOWLEDGEMENT.
Supported by grants from Department of Energy (DOE FG-05-88ER60728), Florida High Technology and Industry Council and Baxter Healthcare Corporation.

Paper B16

LIVER CANCER IMAGING AGENT —— ^{99m}Tc-N-PYRIDOXYL-5-METHYLTRYPTOPHAN (^{99m}Tc-5-PMT) DERIVATIVES ^{99m}Tc-6-PMT AND ^{99m}Tc-7-PMT SYNTHESES AND BIODISTRIBUTIONS

Y.G. Zhou, Z.L. Xiao and J.Z. Chen
Dept. of Radiopharmacy, School of Pharmacy,
Shanghai Medical University, Shanghai 200032, P.R.China

^{99m}Tc labelled N-pyridoxyl-5-methyltryptophan(^{99m}Tc-5-PMT) is a hepatobiliary imaging agent with a very rapid blood clearance and hepatobiliary transit and has a very low rate of urinary secretion(1). Sn-5-PMT has been prepared as a kit and applied clinically as an imaging agent for the diagnosis of liver cancer and metastatic hepatic tumour localization. The 61% hepatocellular carcinoma showed positive images. The smallest tumour which could be identified was 2cm in diameter with a tumour/liver ratio of 3.33(2). In our continuing study of the relation between pharmaceutical structure and biodistribution, we have prepared two ^{99m}Tc-5-PMT derivatives: Tc-99m-N-pyridoxyl-6-methyl-tryptophan(^{99m}Tc-6-PMT) and Tc-99m-N-pyridoxyl-7-methyl-tryptophan(^{99m}Tc-7-PMT), and compared them on the basis of pharmacokinetics and biodistributions in rats.

N-pyridoxyl-6-methyl-tryptophan(6-PMT) and N-pyridoxyl-7-methyl-tryptophan(7-PMT) have been synthesized using the sodium borohydride reduction of a Schiff base condensed from pyridoxyl-hydrochloride and 6-methyl-DL-tryptophan(7-methyl-DL-tryptophan), as described in our previous synthesis of 5-PMT(2). The 6-PMT and 7-PMT were identified by their elemental analyses and NMR spectra. The yield of these compounds was 90% in both cases. 6-PMT m.p.=234-236(decomp), 7-PMT m.p.=186-188(decomp).

Preparation of Sn-6-PMT and Sn-7-PMT kit: A mixture of 100mg of 6-PMT(or 7-PMT), 6mg of $SnCl_2 \cdot 2H_2O$ and 2.5mg of L-(+)-ascorbic acid were added to 20ml of nitrogen-purged 0.05N HCl solution. The solution was then adjusted to pH 8.3-8.5 with 0.45N sodium hydroxide solution before passing it through a 0.22μm Millipore filter and collecting it in sterile vials. Each kit contained 5mg of 6-PMT(or 7-PMT), 0.3mg of $SnCl_2 \cdot 2H_2O$ and 0.12mg of L-(+)-ascorbic acid in a total volume of 2ml. These solutions were lyophilized, then stored at -25^{0}C.

Preparation of ^{99m}Tc-6-PMT and ^{99m}Tc-7-PMT: The ^{99m}Tc-6-PMT(or ^{99m}Tc-7-PMT) was prepared by adding 1mCi of Na $^{99m}TcO_4$ solution to the kit with subsequent heating at 100^{0}C in a water bath for 10 min.. The label was analyzed by ITLC on silica-gel plates, developed in the following solvent system: Ethyl acetate:2-Butanone:DMSO:H_2O (20:10:4:0.7, v/v). The ^{99m}Tc-6-PMT(or ^{99m}Tc-7-PMT) labelling efficiency was found to be greater than 96%.

Biodistributions of ^{99m}Tc-6-PMT and ^{99m}Tc-7-PMT were carried out in rats. The results of the biodistribution are presented in Table 1 and Table 2 respectively. The fastest hepatobiliary transit, lower blood concentration, the weak excretion from the kidneys and more concentrated in intestine have been observed. ^{99m}Tc-6-PMT and ^{99m}Tc-7-PMT have been studied and compared with ^{99m}Tc-5-PMT on the basis of pharmacokinetics and biodistribution in rats. They are potentially useful for the imaging of hepatobiliary and liver cancer.

1. Kato-Azuma M.J. Nucl, Med. 23, 517 (1982)
2. ZHOU Y.G., ZHU T., ZHAO H.Y., WU Z.M., SHI Q.X. and LIANG F.H. Nucl. Med. Biol. Int. J. Radit. Appl. Instrum. Part B 14, 467 (1987)

TABLE 1. Organ Distribution of ^{99m}Tc-6-PMT in Rats Various Time After i.v. Injection

Organ	Liver	Muscle	Kidneys	Stomach	Intestine	Blood
Time						
5'	49.6±3.7	0.1±0.1	4.2±0.5	0.9±0.1	21.8±6.6	2.2±0.9
	7.1±0.5	0.3±0	3.3±0.4	0.3±0.1	2.5±1.0	2.3±0.5
15'	19.7±1.6	0.2±0.1	3.5±0.8	6.8±6.4	39.5±2.5	1.0±0.4
	3.5±0.2	0.4±0.1	2.8±0.8	4.9±4.3	4.7±0.5	1.1±0.1
30'	9.9±0.4	0.3±0	5.2±0.1	6.2±5.2	86.4±4.1	0.4±0.1
	1.6±0.2	0.4±0.1	3.8±0.1	2.2±1.6	10.2±0.2	0.6±0
60'	5.2±0.5	0.1±0.1	4.6±0.1	1.1±0.3	84.5±10.7	0.6±0.7
	0.6±0.4	0.2±0.1	3.1±0.3	0.6±0.2	10.5±2.4	0.8±0.8
120'	4.5±1.1	0.3±0.3	5.3±1.1	0.3±0.1	90.6±20.0	0.1±0.1
	0.8±0.2	0.8±0.7	4.1±0.9	0.2±0	17.0±4.2	0.3±0.1

Above line: %dose/organ ± SD.
Below line: %dose/organ/gram ± SD
Three rats in each group

TABLE 2. Organ Distribution of ^{99m}Tc-7-PMT in Rats Various Time After i.v. Injection

Organ	Liver	Muscle	Kidneys	Stomach	Intestine	Blood
Time						
5'	16.4±4.4	0.5±0.3	2.1±0.3	1.1±0.9	23.8±4.6	0.7±0.1
	2.2±0.8	0.4±0.2	1.5±0.4	0.5±0.6	3.0±0.9	0.6±0.3
15'	7.0±1.5	0.5±0.5	1.4±0.4	1.3±1.0	49.3±6.6	0.3±0.1
	0.9±0.2	0.3±0.3	1.1±0.3	0.5±0.5	6.8±1.2	0.5±0.2
30'	4.7±0.5	0.2±0.3	0.8±0.2	0.2±0.1	42.5±11.9	0.2±0.1
	0.7±0.1	0.3±0.3	0.6±0.1	0.1±0	4.9±1.3	0.2±0
60'	3.8±0.7	0.9±0.8	0.8±0.1	0.3±0.1	53.8±3.7	0.2±0.1
	0.6±0.2	0.8±0.7	0.6±0.1	0.1±0	6.2±0.9	0.2±0.1
120'	3.5±0.2	0.1±0	0.8±0.1	0.1±0	54.5±4.3	0.1±0
	0.6±0.1	0.1±0	0.6±0.3	0.03±0	7.2±1.0	0.1±0

Paper B17

[1] Coulais Y., [1]Guiraud R., Cros G., Darbieu M.H. and[3] Pasqualini R.

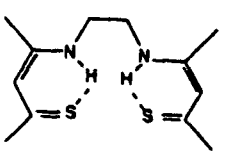
[1]Laboratoire de Biophysique et de Médecine Nucléaire Toulouse -Purpan ,133 Route Narbonne, 31062 Toulouse Cedex.

[2]Laboratoire de Chimie de Coordination du CNRS, UP 8241 lié par convention à l'Université Paul Sabathier et à l'Institut National Polytechnique, 205 Route de Narbonne ,31077 Toulouse Cedex.

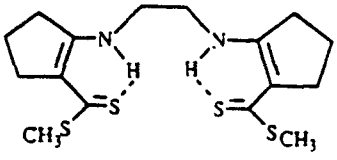
[3]CIS bio international, B.P. 32 - 91192 Gif-sur-Yvette Cedex

SYNTHESIS, CHARACTERIZATION AND BIODISTRIBUTION OF NEW 99m Tc NITRIDO COMPLEXES WITH UNSATURATED N_2S_2 SCHIFF BASE

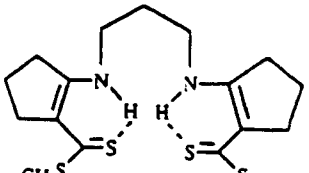
In a recent work we have shown that tetradendate N_2S_2 Schiff base ligands (H2L) give neutral nitrido complexes [NTc(V)L] with ^{99} Tc and ^{99m}Tc isotopes(1),(2). The biodistribution in mice and rats show the ability of such lipophilic complexes to cross the blood brain barrier.



H_2L



H_2L^1



H_2L^2

In the aim to improve both uptake and / or the retention in brain tissue we have studied the complexation of ^{99}Tc and ^{99m}Tc with a new class of unsaturated Schiff bases (H_2L^1) and (H_2L^2) bearing two dithio carboxilic methylester groups. A stable nitrido complexe $N^{99}Tc(V)L^1$ has been isolated and ^{1}H N.M.R. analysis confirms the double deprotonation of ligands leading to the same N_2S_2 donor set as H_2L. Stables lipophilic nitrido ^{99m}Tc complexes were prepared in a good yield using the labelling procedure previously described(3) .For H_2L^1 complexes biodistribution in rats show an important heart uptake with a good retention .SPECT studies on a dog reveal also a good heart uptake but with a faster rate of wash out. The significantly enhanced heart uptake for H_2L^1 complexes may be du to thiomethyl groups.

organs*	H2L	H2L1	H2L2	H2L	H2L1	H2L2
	5 minutes			30 minutes		
blood	0.82	0.41	0.57	0.44	0.27	0.37
liver	2.27	3.90	2.33	1;05	1.78	1.88
intestin	1.13	1.13	0.28	1;02	1.12	0.68
heart	0.80	2.25	1.09	0.34	2.26	0.73
lungs	0.76	3.73	4.44	0.37	5.64	4.78
kidneys	2.01	2.00	1.14	1.78	1.74	0.91
brain	0.19	0.26	0.12	0.05	0.28	0.15

*Biodistribution of nitrido 99mTc complexes: dose %/gram. (average of three rats)

1) Tisato F., Mazzi U.,Bandoli G.,Cros G., Darbieu M.H., Coulais Y. and Guiraud.R J.Chem. Soc. Dalton Trans. - 130, (1991)

2)Coulais Y., Guiraud R., Cros G. and Darbieu M.H. - unpublished work

3) Duatti A ., Marchi A. and Pasqualini R. -J.Chem. Soc. Dalton Trans.3729,(1990)

Synthesis of a Tc-99m MAG3-Biotin Conjugate as a Biliary Agent. J. M. Jeong, S. Kinuya, C. H. Paik, T. Saga, V. K. Sood, R. C. Neumann, J. C. Reynolds and J. A. Carrasquillo. Nucl Med, NIH, Bethesda, MD 20892 and Radiopharmaceutical Chem, George Washington Univ Med Ctr, Washington, DC 20037.

Biliary excretion is known to be the main route of elimination for anions, cations, and nonionized molecules containing both polar and lipophilic groups and molecular weights larger than 300 (1,2,3). In the present abstract, we report the synthesis and biodistribution of a Tc-99m biliary agent which is a simple 1:1 Tc-99m:chelator complex with the net charge of -2 and a lipophiilc moiety. This biliary agent was synthesized by acylation of biocytin with N-hydroxy succinimide ester of benzoylmercaptoacetyl glycylglycylglycine (Bz-MAG3) in dimethylformamide. This reaction resulted in the conjugation of Bz-MAG3 to biotin through a lycine spacer. The addition of tetrahydrofuran to the reaction mixture precipitated out the crude products. They were then washed with water to remove cyclohexylurea and unreacted reagents. The purified product was identified by nmr using biocytin and Bz-MAG3 as reference samples. The melting point was 171-173 °C. Bz-MAG3-Biocytin was labeled with Tc-99m by a method similar to Fritzberg's (4). The labeling yield was >95 % determined by reverse phase TLC (Analtech, Newark, DE) developed with 0.01 M sodium phosphate buffer at pH 6.7. In this system, Rf values of Tc-99m Biocytin-MAG3, Tc-99m MAG3 and Tc-99m pertechnetate were 0, 0.6 and 0.9, respectively. The labeled product was futher purified with a C_{18} SEP-PAK cartridge (Waters, Milford, MA). The cartridge was first eluted with a solvent mixture containing 5% ethanoland 95 % 0.01 M sodium phosphate at pH 6.7 to eliminate polar Tc-99m impurities from the cartridge. The desired product was then eluted out with ethanol and diluted 500 times with saline for the biodistribution studies. As a control, Tc-99m-N(2,6-diisopropylphenyl-carbamoylmethyl)-iminodiacetic acid (Tc-99m DISIDA) was prepared from a HEPATOLITE kit (Du Pont,MA). The labeling yield was quantitative and this control biliary agent was used without futher purification.

The Tc-99m radiopharmaceuticals (2 uCi, 200 ul) were injected into a tail vein of mice. Mice were sacrificed at 2, 5, 15, 30 and 60 min. The biodistributions of Tc-99m MAG3-Biocytin were very similar to those of Tc-99m DISIDA at the time intervals. The new biliary agent cleared very rapidly from blood: at 5 min, less than 3 % of the injected dose remained in the total blood. It was mainly taken up by liver and excreted rapidly through biliary system. More than 80 % of the injected activity was accumulated in intestine within 30 min. The activities accumulated in kidneys, stomach and spleen were negligible, and less than 10 % of the injected activity was excreted in urine during a 60 min period.

It is interesting to note that Tc-99m MAG3 and C-14 -biotin are both eliminated from body mainly through renal excreton but the increase of the molecular weight of Tc-99m MAG3 by the conjugation of biocytin causes it to be eliminated through biliary system even though the net charge of the complex at physiological pH is -2 which is the same as Tc-99m MAG3.

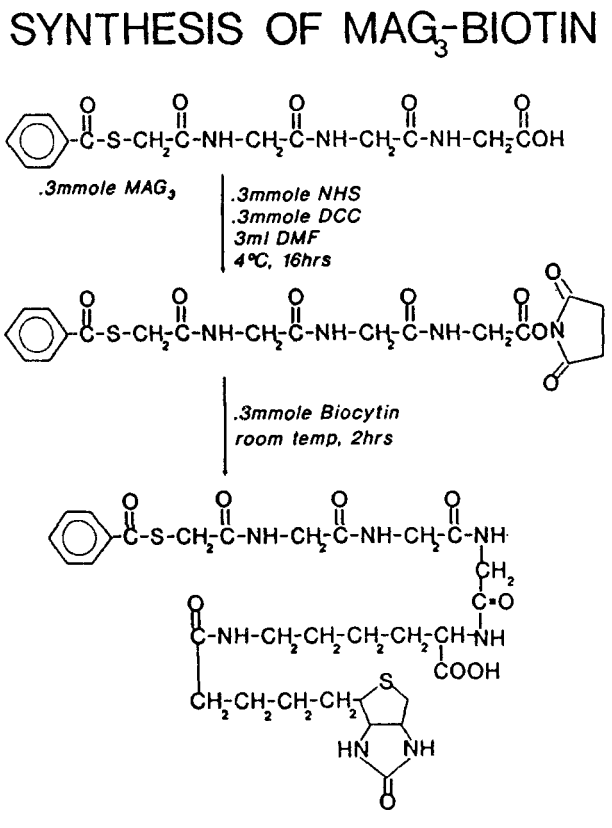
References

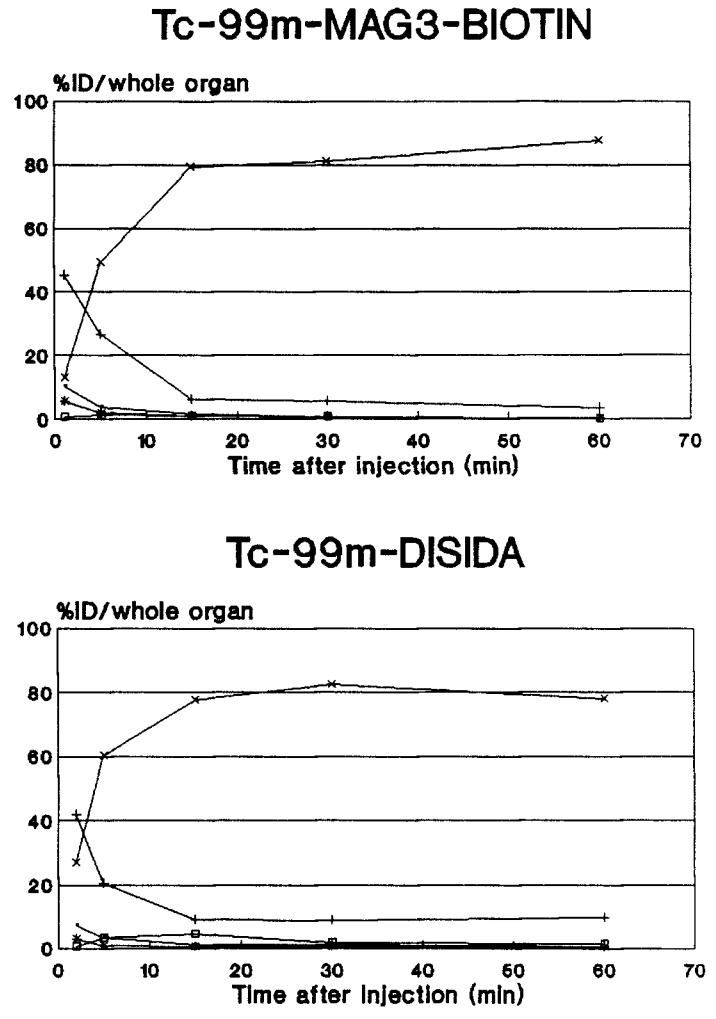
1. Loberg M.D., Cooper M., Harvey E., et al. J. Nucl. Med., 17, 633-638 (1976).

2. Wistow B.W., Subramanian G., Van Heertum R.L., et al. J. Nucl. Med., 18, 455-461 (1977).

3. Chervu L.R., Nunn A.D., Loberg M.D. Seminars Nucl. Med., 12, 5-17 (1982).

4. Fritzberg A.R., Kasina S., Eshima D., et al. J. Nucl. Med., 27, 111-116 (1986).




Tc-99m-MAG3-BIOTIN
%ID/whole organ
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Time after injection (min)
Tc-99m-DISIDA
%ID/whole organ
100
80
60
40
20
0
0
10
20
30
40
50
60
70
Time after injection (min)
Blood
Liver
Kidney
Stomach
Intestine

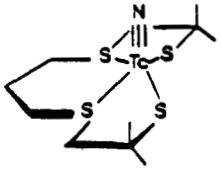
Paper B19

TcO^{3+} AND TcN^{2+} CORES COMPLEXATION BY DITHIOETHERDITHIOLS BIODISTRIBUTION IN MICE

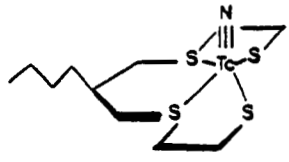
S. DROUILLARD[1], A; ALAGUI[2], M. APPARU[1], M. COMET[1], R. PASQUALINI[3], M. VIDAL[1]

1. GERMAB - LEDSS IV : UJF. Chimie Recherche, BP 53X, 38041 Grenoble Cedex - FRANCE.
2. Université CADI AYYAD, Faculté des Sciences, Chimie, BP S15, Marrakech - MAROC.
3. CIS bio international : BP 6, 91192 Gif-sur-Yvette - FRANCE.

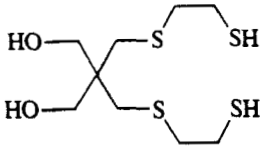
Complexation of the ^{99m}TcNcore by tetradentate ligands possessing two labile hydrogen atoms must lead to stable neutral complexes. These species may constitute useful tracers in the case of a myocardial uptake study. With this objective in mind we initiated a general study of the complexation of the $^{99m}TcN^{2+}$ core by dithioetherdithiols. Here we present our initial results.



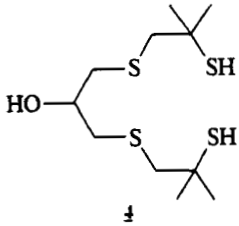
1-TcN



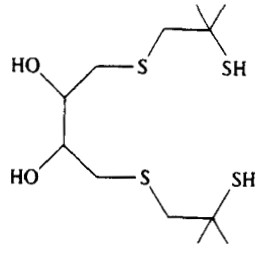
2-TcN



3

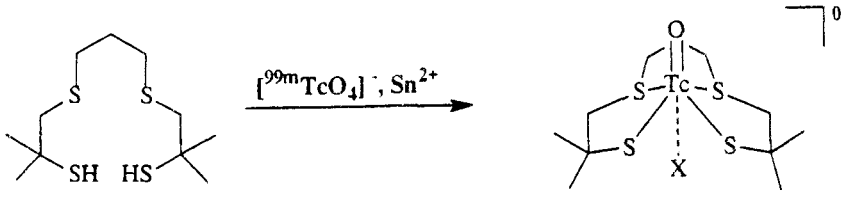


4



Complexes of ^{99m}Tc with 1 and 2 were prepared with $[^{99m}TcNCl_4]^-$ or $^{99m}TcNCl_2[P(CH_2CH_2CN)_3]_2$ as an intermediate. The only observed species results from a double deprotonation. These complexes being too lipophilic, we prepared hydroxylated 4S ligands 3, 4, 5. We report the synthesis of these compounds and the results of complexation of the TcN core by these tetradentates.

Surprisingly, the complexation of ^{99m}TcO by 1 led to the formation of an uncharged complexe. One may suppose the following structure due to the fixation of a negative ligand *trans* to the Tc=O bond.



X = Cl^-, OH^-

The biodistribution of the different complexes was studied in Swiss mice. The 4S-^{99m}TcO complexe prepared from 1 showed a myocardial uptake higher than that observed with RP30-a product presently used in scintigraphy. Moreover, one must notice that, as a consequence of their lipophilic character, the complexes of the dithioetherdithiols 1 and 2 show a high hepatic uptake unlike the chelates of the hydroxylated 4S ligands which show a high renal clearance.

Paper B20

Tc-99m AND In-113m COMPLEXES WITH DTPA DIAMIDES; BIODISTRIBUTION OF Tc-99m-DTPA(NH-HIPP)$_2$

V. A. Brattsev, I. N. Milovskaya, E. A. Terentyeva, Yu. P. Arkhapchev*

Institute of Atomic Energy, Moscow, 123182, USSR;
*** Cardiology Center, Moscow, 121552, USSR**

It is well known that Tc-99m and In-113m form stable anionic complexes with DTPA, which are broadly used in the diagnostic nuclear medicine. High stability and presence of "spare" carboxylic groups on the outer sphere of the complexes suggest a tempting opportunity to design new radiopharmaceuticals by linking those groups to some biologically active species proved useful in diagnoses.

For such approach we have studied the synthesis of diamides of DTPA (1) by interaction of DTPA dianhydride (2) in anhydrous pyridine with some primary and secondary amines, such as para-nitroaniline, diethylamine and para-aminohippuric acid. The prepared diamides: DTPA(NHC$_6$H$_4$NO$_2$)$_2$, DTPA(NEt$_2$)$_2$, and DTPA(NH-Hipp)$_2$ have been isolated in a crystalline form and characterized by melting points, elemental analysis data, PMR- and IR-spectra.

In-113m complexes have been prepared by mixing aqueous solutions of the DTPA diamides neutralized with NaOH to pH 7.5-8.5 with In-113m solution in 0.02 M HCl. Tc-99m complexes have been prepared from the same solutions of DTPA diamides, to which small amount of $SnCl_2 \cdot 2H_2O$ have been added, by mixing with pertechnetate solution. The composition of the thus prepared radioactive solutions have been studied by electrophoresis on plastic plates with agarose gel in tris-barbiturate buffer with pH 8.6, 400-500 V, 8-15 min, with subsequent autoradiography on X-ray films and count rate measuring of the radioactive spots.

All Tc-99m and In-113m complexes with amido derivatives of DTPA are stable till at least pH 8.6. Complexes with N-aryl amides are all anionic (transfer to anode, no radioactivity on the start), probably due to participation of the amide nitrogen in complexation after ionization of NH bond. In these cases several isomeric complexes are likely formed. Only with DTPA(NEt$_2$)$_2$ both Tc-99m and In-113m form each single complex which stays on the start during electrophoresis. In this case there is only one possibility to form an octahedral complex, and such Tc complex should contain technetium in the valence state Tc(3+).

Biodistribution studies of Tc-99m-DTPA(NH-Hipp)$_2$, designed as a substitute for I-131-OIHA, unfortunately, demonstrated its inadequate behavior in animals.

Table 1. Biodistribution of Tc-99m-DTPA(NH-Hipp)$_2$ in comparison with I-131-OIHA, and Tc-99m-MAG$_3$ (3) (rats, % dose/organ).

	Min	Blood	Liver	Kidneys	Intestine	Urine
Tc-99m-DTPA(NH-Hipp)$_2$	2	2.5	3.2	7.3	-	-
	60	4.1	1.3	3.1	3.5	64.5
I-131-OIHA	10	4.1	2.1	2.6	1.1	74.1
	120	0.2	0.1	0.1	0.3	94.7
Tc-99m-MAG$_3$	10	2.6	2.9	3.5	1.1	79.9
	120	0.1	0.1	0.1	1.2	98.5

In conclusion: DTPA diamides are easily formed from various amines and DTPA dianhydride. They can form stable complexes with Tc-99m and In-113m and thus can be a basis for design and development of new radiopharmaceuticals.

1. Bodanszky M. et al. - J. Amer. Chem. Soc. 99:235 (1977).
2. Hnatowich D. et al. - J. Nucl. Med. 22:810 (1981).
3. Fritzberg A. et al. - J. Nucl. Med. 27:111 (1986).

Paper B21

SYNTHESIS, LABELING WITH Tc-99m AND BIOLOGICAL EVALUATION OF NEW LIGANDS RELATED TO BOTH MAG_3 AND HIPPURIC ACID.

D. Stepniac-Biniakiewicz[1], H. Vavouraki[2], S. Mastrostamatis[2], E. Chiotellis[2], E. Deutsch[1].
[1]Biomedical Chemistry Research Center, Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221, USA.
[2]Radiopharmaceuticals Lab., NCSR "DEMOKRITOS", 15310 Ag. Paraskevi, Attiki, GREECE.

^{99m}Tc-labeled dimercaptoamides (e.g. ^{99m}Tc-CO_2DADS) and dimercaptotriamides (e.g. ^{99m}Tc-MAG_3) have been developed in an attempt to find a ^{99m}Tc-labeled replacement for IOH. ^{99m}Tc-MAG_3 has become the renal agent of choice and it is in widespread clinical use. However, clinical comparisons show that the tubular extraction of ^{99m}Tc-MAG_3 is only 66% of that of Hippuran (1-3), and thus there is still a need for improved ^{99m}Tc-agents.Substituted analogs of MAG_3 as well as few other structure-related compounds, differing in renal extraction rates are currently under evaluation (4,5). In order to come to a better understanding of the interaction of hippurate-like substances with the renal tubular transport mechanism we designed two new compounds related to both MAG_3 and Hippuric Acid: N-(S-benzoylmercaptoacetyl)glycyl-p-aminohippuric acid (MAGHA) and N-(S-benzoylmercaptoacetyl)glycylglycylglycyl-p-aminohippuric acid (MAG_3HA). In this paper we report their synthesis, labeling with technetium-99m and preliminary results of biodistribution studies in rats.

Different pathways for the preparation of MAGHA are presented in scheme 1.The amino group of p-aminohippuric acid (PAHA) is less nucleophilic than the amino group of common amino acids, and this influences the course of the coupling reaction. Both the yield and the purity of MAGHA obtained according to route 1, which utilizes the highly reactive Z-Glycine Anhydride, are higher than for route 2, in which PAHA is reacted with N-hydroxysuccinimide ester. The MAG_3HA was synthesized in a similar manner from glycyl-p-aminohippuric acid (GHA).

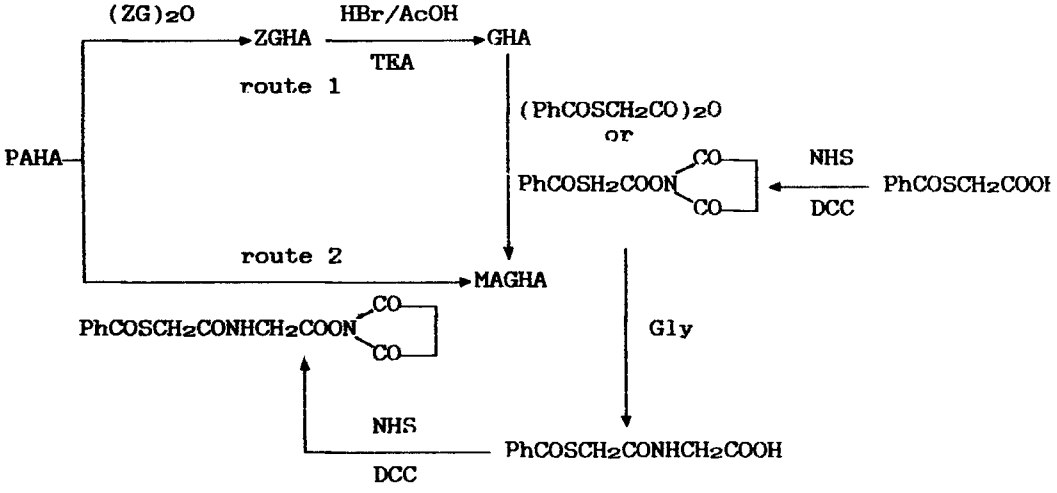
Both new ligands were labeled directly with Tc-99m by mixing 1-2 mg of ligand dissolved in 50 µl of 1N NaOH with 20 µl of sodium dithionite solution (100 mg/ml H_2O) and then adding generator-derivated $^{99m}TcO_4^-$. After heating at 100°C for 15 min the pH of the final solution was adjusted to 7.0-7.5. The reaction mixtures were analyzed by reversed phase HPLC on a C18 column using a gradient of 0.01M phosphate buffer and methanol. ^{99m}Tc-MAGHA elutes at 17.9 min while ^{99m}Tc-MAG_3HA elutes at 17.5 min; both elute in radiochemical purities and yields >90%.

The results of biodistribution studies in rats are presented in Table 1. While both complexes are taken up by the kidney, the ^{99m}Tc-MAG_3HA clears from the kidney more rapidly and more extensively than does the simpler MAGHA complex. Concomitantly,^{99m}Tc-MAG_3HA clears more rapidly from the blood and the liver and thus it is clearly the superior agent. Comparison with the biodistributions of related ^{99m}Tc-agents (6) indicate that ^{99m}Tc-MAG_3HA is a promising agent, and that this class of compounds warrants further investigation. We are thus

D. Stepniac-Biniakiewicz et al.

initiating a study to firmly establish the structures of ^{99m}Tc-MAG$_3$HA and ^{99m}Tc-MAGHA; these data will provide the basis for the development of structure-activity relationships which should lead to improved ^{99m}Tc-agents containing both MAG$_n$ and HA moieties.

REFERENCES
1. A. Fritzberg, S.Kasina, D.Eshima et al., J.Nucl.Med.,27:111-16,1986
2. A. Taylor, J.A.Zifer, A.Stevens et al., Radiology, 170:721-25,1989.
3. HM. Abdel-Dayem, S.Sadek, R.Al-Bahar et al., Nucl.Med.Comm., 10:99-107, 1989.
4. A. Verbruggen, G. Bormans, B.Cleynhes et al., J.Label.Comp.Radioph. 25:436-39,1989.
5. M. Subhami et al., Technetium and Rhenium in Chemistry and Nuclear Medicine. Nicolini,Bandoli,Mazzi (eds) p. 453,1990.
6. M. Subhani, H.VanBilloen, B. Cleynhes et al., J.Label.Comp.Radioph. 30:83-5,1991.



Scheme 1.: Synthetic pathways to N-(S-mercaptoacetyl)glycyl-p-amino-hippuric acid (MAGHA).

Table 1. Biodistribution in rats (n=3 or 4) of the new 99m-Tc-complexes. Organ uptake as % of injected dose/g tissue.

	99m-Tc-MAGHA		99m-Tc-MAG$_3$HA	
Time	15'	60'	15'	60'
Blood	0.554	0.224	0.447	0.084
Kindey	1.36	1.06	0.641	0.156
Liver	2.53	0.59	1.77	0.163

Paper B22

PAPER WITHDRAWN

W.T. HUANG, K.S. LIN, M.K. HSIEH, J.M. LO.

PAPER WITHDRAWN

PAPER WITHDRAWN