**Paper I 1**

First Examples of Heteroatomic Chelation of $ReO_4^-/^{188}ReO_4^-/^{99m}TcO_4^-$ and $PdCl_4^{2-}/^{109}PdCl_4^{2-}$ With a Phosphine-Phosphineoxide (or a Phosphinimine) Bridged Bifunctional System. [1]K.V. Katti, [2]P.R. Singh, [3]K.K. Katti, [3]K. Kopicka, C.E. Barnes, [4]W.A. Volkert and [3]A.R. Ketring [1]Department of Radiology, [2]Department of Chemistry, [3]Research Reactor, University of Missouri and [4]Research Services, H.S. Truman Memorial VA Hospital, Columbia, Missouri 65211

Small molecule $^{99m}Tc$- and $^{186}Re/^{188}Re$-radiopharmaceuticals have attracted a great deal of interest as diagnostic and therapeutic agents in recent years. The ability of small ligands to readily form well-defined chelates with these radionuclides in high yields and with good stability are important factors in developing new radiopharmaceuticals. Small chelating heterodifunctional ligands hold potential to form highly stable complexes with these metals. The strongly chelating heteroatomic ligands of the type shown in Fig. 1 are versatile because a systematic alteration of the coordinating unit E can be performed by controlled oxidation of one of the phosphorus centers in $R_2P\text{-}CH_2\text{-}PR_2$ (e.g. where R=Ph (dppm) or Me) [1].

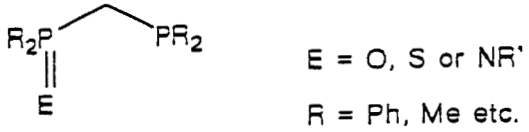



Fig. 1

In this paper we report: (a) the synthesis of a new metallacyclic compound of Re(V): $OPPh_2CH_2PPh_2ReOCl_3$ (2) from $NH_4ReO_4$ and $0PPh_2CH_2PPh_2$ (1) (b) extension of this chemistry to generate $^{99m}Tc$ and $^{188}Re$ analogues of 2. The interaction of $NH_4ReO_4$ with (1) (PCPO) in HCl resulted in the formation of 2 in quantitative yields. Compound 2 was characterized by spectroscopic ($^1H$, $^{31}P$ NMR and IR) and C,H,N,Cl analytical data and its structure was confirmed by single crystal x-ray diffraction analysis. The ORTEP plot of 2 is shown in Figure 2.

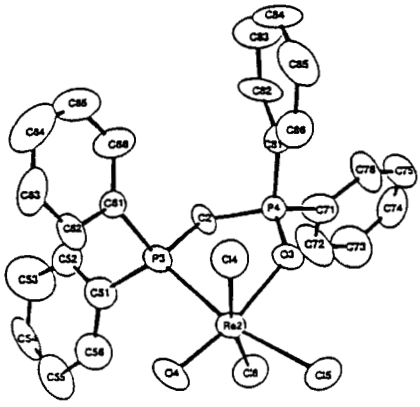
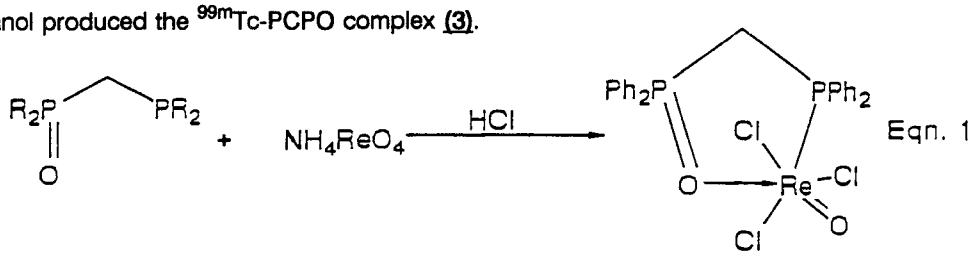


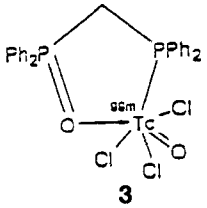

Fig. 2

The reaction shown in equation 1 can be readily extended to generate $^{99m}Tc$ and $^{188}Re$ complexes with the PCPO ligand. Typically, the $^{99m}Tc$ complex was prepared by adding 2-3 MBq of $^{99m}TcO_4^-$ in 0.2 ml of the eluant from $^{99}Mo/^{99m}Tc$ generator which was acidified by a 20 µl of conc. HCl to generate $^{99m}TcOCl_4^-$. Simple mixing of this solution with the PCPO ligand (1) dissolved in absolute ethanol produced the $^{99m}Tc$-PCPO complex (3).




1 2

The corresponding $^{188}Re$ analogue of 3 was prepared by an identical method as described above using 2-3 MBq of $^{188}ReO_4^-$ in 0.2 ml of the eluant from a $^{188}W/^{188}Re$ generator. The structures of the $^{99m}Tc$ (3) and $^{188}Re$-PCPO complexes (4) as inferred from the reaction shown in eqn (1) is given below:

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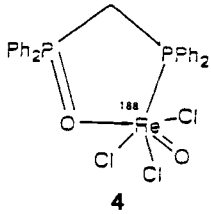


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The labelling efficiencies of $^{99m}TcO_4^-$ and $^{188}ReO_4^-$ with the PCPO ligand 1 were 97 ± 1.3% and 95 ± 1.1% to produce 3 and 4, respectively. Reversed phase HPLC analysis using a Hamilton PRP1 column and a mobile phase of 80:20, $CH_3CN:H_20$, of 3 and 4 indicated them to be of single chemical species. ITLC, paper electrophoresis (300 V for 1 hr, pH = 7-7.5) and high extraction efficiency of these chelates into chloroform (Table 1) clearly established the $^{99m}Tc$ and $^{188}Re$ complexes (3 and 4, respectively) as neutral and lipophilic in nature. The ability of a ligand that is isoelectronic with PCPO (i.e., PCPN - Figure 3) to chelate $^{99m}Tc$ and $^{188}Re$ was shown to form complexes with properties similar to $^{99m}Tc/^{188}Re$-PCPO.

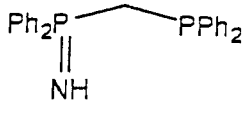
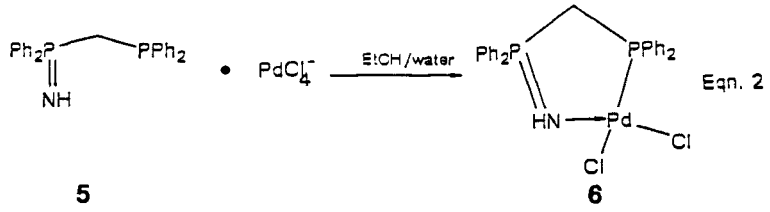



Fig. 3

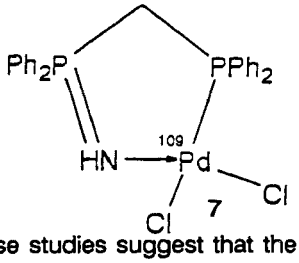
The PCPN and PCPO ligands also present the possibility of using the combination of the reactivities of the π-acid phosphine and the σ donor nitrogen to stabilize electron rich late-transition

metal radionuclides such as $^{109}Pd$ and $^{105}Rh$. The reaction of Pd(II) with PCPN (5) shown in eqn. 2 proceeds smoothly to chelate 6 in quantitative yields in aqueous media. The structure of 6 was confirmed by x-ray diffraction analysis [2]. The application of the reactivity of 5 with the corresponding $^{109}PdCl_4^{2-}$ radioisotope precursor produced 7 in 97 ± 1.2% yields at neutral pH.




5 6

Typically, complex 7 was prepared by mixing ($10^{-3}$M) ethanolic solution of 5 with an aqueous solution of $10^{-5}$M $^{109}PdCl_4^{2-}$ (obtained from the University of Missouri Research Reactor) at neutral pH and incubated at 25°C for 30-45 min. The details of the radiochemical yields are given in Table 1. Analysis by ITLC using radiographic scanning and extraction into $CHCl_3$ clearly indicated the $^{109}Pd$ complex 7 as neutral and lipophilic. Paper electrophoresis of 7 at 300 v for 1 hr at pH 7-7.5 further confirmed it to be a neutral species.

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In summary, the results of these studies suggest that the PCPO and PCPN heterdifunctional ligands are capable of forming stable chelates with early and late transition metal radionuclides. The yields of the radiochemical purity of the resultant $^{99m}Tc/^{188}Re/^{109}Pd$ chelates are high. The data indicate the potential utility of these types of chelating agents in formulating new diagnostic or therapeutic radiopharmaceuticals.

Table 1.

| Complex | Radiochemical Purity[a] 1 hr | Radiochemical Purity[a] 24 hr | Chloroform/saline Partition Coefficient Ratio |
|---|---|---|---|
| $^{99m}Tc$-PCPO (3) | 97 ± 1.3 | 94 ± 1.2 | 15.27 ± 0.61 |
| $^{188}Re$-PCPO (4) | 97 ± 1.1 | 95 ± 1.4 | 17.06 ± 2.19 |
| $^{109}Pd$-PCPN (7) | 93 ± 1.2 | 92 ± 1.2 | 47.52 ± 1.73 |

(a) RPC determined by paper and thin layer chromatography. Chelates were incubated for 1-24 hr at 25°C in 0.9% NaCl-EtOH (1:1) at pH 6.5 to 7. Values are mean ± SD.

References

1. Katti, KV, Cavell, RG. Comments Inorg Chem No. 10, 53, 1990.
2. Katti, KV, Cavell, RG. Inorg. Chem, 29, 808, 1990.

SYNTHESIS AND EVALUATION OF 4-ISOTHIOCYANATO- AND 4-BROMOACETAMIDO-CYCLOHEXYL EDTA (4-ICE AND 4-BACE) AS BIFUNCTIONAL CHELATING AGENTS

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Bifunctional chelating agents which incorporate rigidity into their structure generally produce radioimmunoconjugates with higher in-vivo stability. Rigidity can be introduced into a ligand by cyclization of a straight chained molecule to give a macrocycle or by replacing a flexible carbon chain with part of a rigid structure. Following the latter approach we have previously shown (1,2) that the monoanhydride of trans-1,2-diaminocyclohexane N,N,N',N' tetraacetic acid (CDTAMA) produced $^{111}In$, $^{203}Pb$, and $^{57}Co$ labeled immunoconjugates with better biodistribution in mice compared to the DTPA dianhydride procedure. However, a disadvantage of the CDTAMA method is that the sixth coordination site of the ligand is consumed in the conjugation step.

We now report the synthesis of 4-isothiocyanato- and 4-bromoacetamido-trans-1,2-diaminocyclohexane N,N,N',N' tetraacetic acid (4-ICE and 4-BACE), the conjugation of these ligands to anticolon ca 17-1A IgG and antiCEA $F(ab')_2$, and the biodistribution in mice of their $^{111}In$, $^{57}Co$, $^{203}Pb$, $^{47}Sc$, $^{153}Sm$, $^{67}Cu$, and $^{88}Y$ labeled immunoconjugates. The synthesis of 4-ICE and 4-BACE is outlined in Figure 1. Trans-1,2-diaminocyclohex-4-ene (1) was prepared by the procedure of Witiak et al.(3) and converted to its di-t-butoxycarbonyl derivative 2. Mercuration-amidation of the double bond of 2 gave acetamide 3 of undetermined stereochemistry at carbon 4. Hydrolysis of the t-Boc groups of 3 gave diamine 4 which was alkylated with ethyl bromoacetate to give 5. Hydrolysis of the amide and ester groups of 5 gave 6. Conversion of 6 to its tetralithium salt followed by treatment with thiophosgene in methanol gave 4-ICE. Reaction of 6 with pentamethyl piperidine (PMP) and N-succinimidyl bromoacetate gave 4-BACE. Conjugation of 4-ICE and 4-BACE to 17-1A and antiCEA at 37°C overnight using an antibody concentration of 15-20 mg/ml and a ligand to antibody molar ratio of 25:1 gave an average of two ligands per antibody.

Biodistributions in tumor xenografted nude mice with 4-ICE and 4-BACE are summarized in Figures 2 and 3. With antiCEA $F(ab')_2$, $^{111}In$ and $^{57}Co$ gave similar distributions with high tumor uptake and low non-target tissue uptake. However $^{203}Pb$ gave lower tumor uptake with high kidney and bone retention. With 17-1A IgG, $^{111}In$, $^{57}Co$, and $^{47}Sc$ gave similar distributions with high tumor uptake which is associated with prolonged blood retention, and low non-target tissue uptake. Conversely, $^{88}Y$ had lower tumor uptake with higher bone-uptake. $^{67}Cu$ and $^{153}Sm$ labeled conjugates exhibited high liver uptake in normal mice and poor stability in serum (data not shown).

In conclusion, it appears that the use of 4-ICE and 4-BACE as bifunctional chelating agents is limited to metals with small ionic radii which form 6-coordinate octahedral complexes with polyaminocarboxylates. While the 4-ICE/4-BACE biodistributions with larger metals which prefer higher coordination numbers are in general better than those of DTPA (data not shown), the rigidity of the 4-ICE/4-BACE system cannot adequately overcome the lack of coordination sites.

1. Mease, R.C. et al. J. Label. Comp. Radiopharm. 30: 319 (1991).
2. Srivastava, S.C. et al. J. Nucl. Med. 32: 1023 (1991).
3. Witiak, D.T. et al. J. Med. Chem. 30: 1327 (1987).

Work supported by the US Department of Energy under contract #DE-AC02-76CH00016 and NIH Grant # CA 51958.

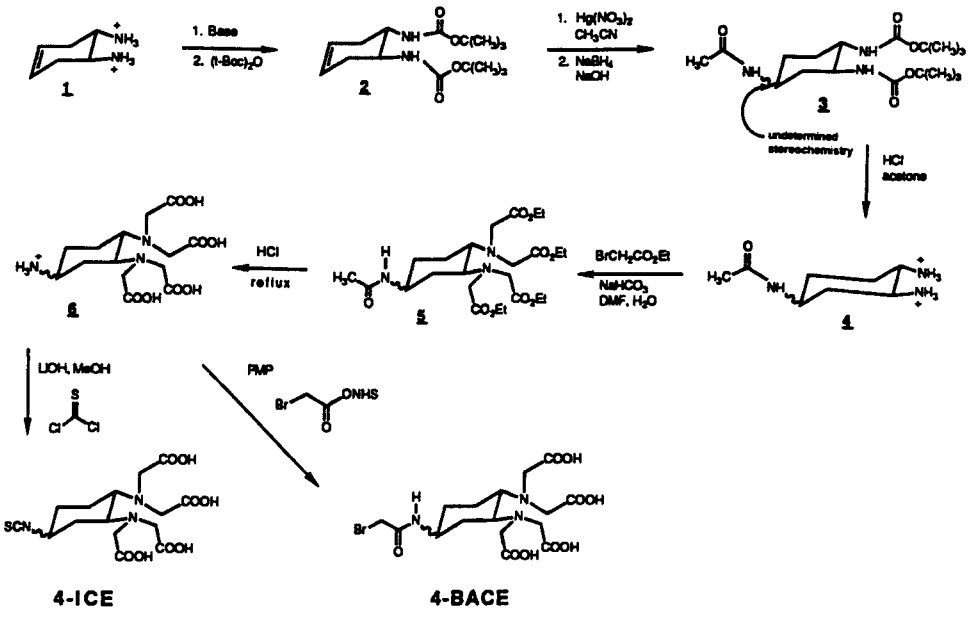


**Figure 1. Synthesis of 4-ICE and 4-BACE**

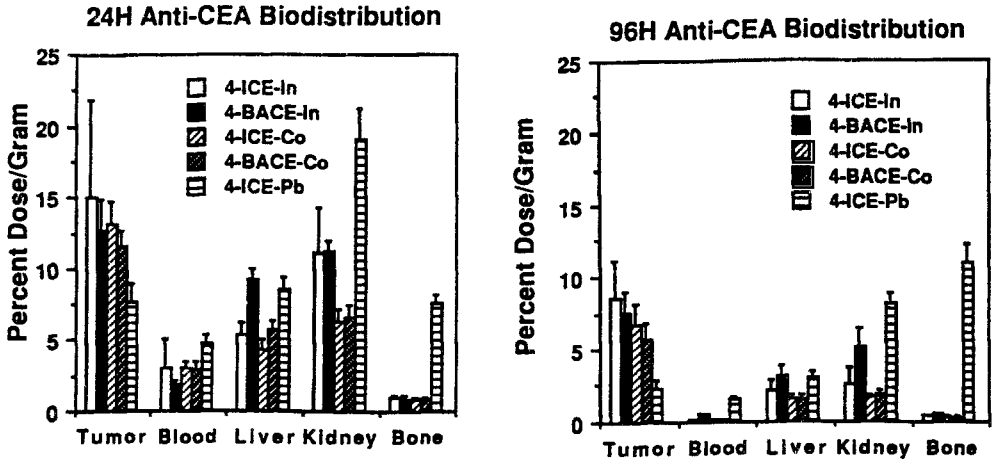


Figure 2. Biodistribution of anti-CEA F(ab')$_2$ immunoconjugates in LS 174 T xenografted nude mice

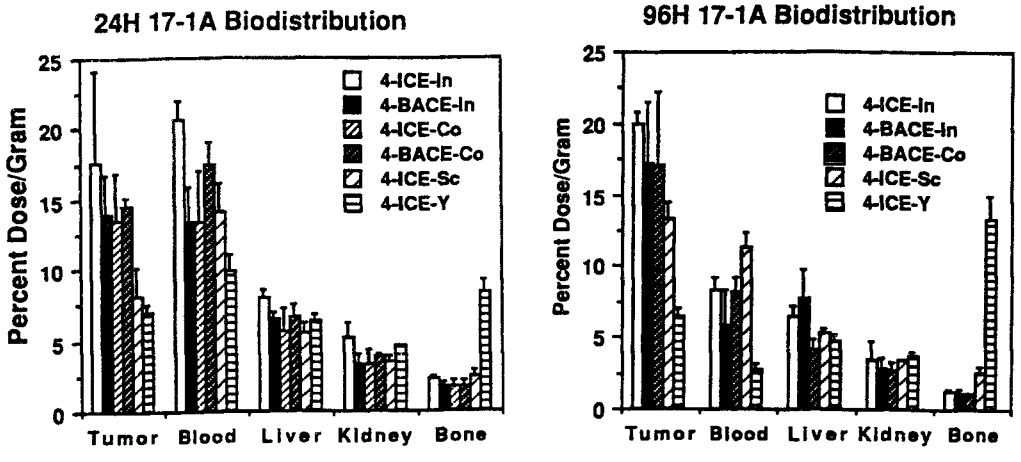


Figure 3. Biodistribution of 17-1A IgG immunoconjugates in SW 948 xenografted nude mice.

Paper I 3

Cationic Gallium(III) Schiff-Base Complexes that are Retained in Myocardium: Potential Agents for Imaging the Heart with Gallium-68 and PET.

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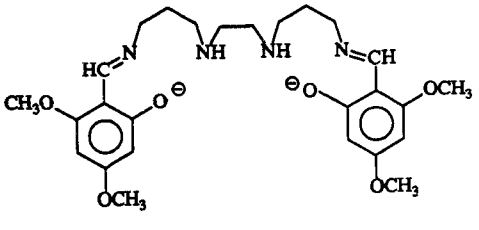
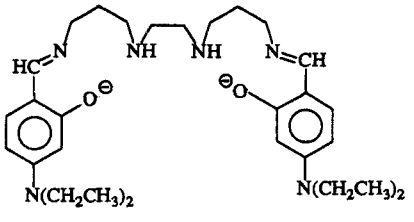
The $^{68}Ge/^{68}Ga$ parent/daughter generator is attractive as a potential radionuclide source for PET facilities operated in locations without a cyclotron. A number of $^{68}Ga$-radiopharmaceuticals have been described that can be employed for imaging the heart with PET (1-4). However, despite high heart uptake and excellent heart/blood ratios (2,3), all are cleared fairly rapidly from the myocardium. Thus, the pharmacokinetics of these tracers prevents complete exploitation of the 68 minute half-life of the gallium-68 radiolabel (e.g. by allowing long image acquisition periods and/or tracer administration at a site remote from the camera). We report here the synthesis and biodistribution of cationic $^{67}Ga$-Schiff base complexes that exhibit myocardial uptake upon intravenous injection into rats followed by prolonged retention of the gallium radiolabel in the heart.

Gallium-67 complexes of the hexadentate *N,N'-bis*(3-aminopropyl)ethylenediamine *bis*(4,6-dimethoxysalicylaldimine) and *bis*(4-diethylaminosalicylaldimine) ligands (I and II) were prepared by reaction of no-carrier-added [$^{67}Ga$]-gallium(III) acetylacetonate with the corresponding *tris*(salicylaldimines) in hot ethanol. The radiochemical purity of these $^{67}Ga$-radiotracers exceeded 99% based on thin layer chromatography using a $C_{18}$-silica gel plate eluted with methanol ($R_f$ values of 0.10 and 0.12 for the $^{67}Ga$-complexes of I and II, respectively). Cellulose acetate



$\{[(4,6\text{-MeO})_2\text{sal}]_2\text{-}3,2,3\text{-tet}\}^{2-}$

**I**



$\{(4\text{-deaSal})_2\text{-}3,2,3\text{-tet}\}^{2-}$

**II**

Table 1. Biodistribution of $^{67}Ga[(4,6\text{-}MeO_2Sal)_2 - 3,2,3\text{-}tet]^{1+}$ in Rats (178-217 g) Following Intravenous Injection.

| | % Injected Dose Per Gram of Tissue | | | |
|---|---|---|---|---|
| | 1 min | 5 min | 15 min | 120 min |
| Blood | 0.66 ± 0.14 | 0.11 ± 0.01 | 0.07 ± 0.01 | 0.03 ± 0.00 |
| Heart | 1.52 ± 0.24 | 1.45 ± 0.20 | 1.22 ± 0.12 | 1.37 ± 0.12 |
| Lungs | 0.81 ± 0.16 | 0.55 ± 0.05 | 0.42 ± 0.06 | 0.45 ± 0.11 |
| Liver | 4.72 ± 0.82 | 4.32 ± 0.53 | 2.19 ± 0.21 | 0.33 ± 0.01 |
| Spleen | 0.61 ± 0.09 | 0.52 ± 0.15 | 0.43 ± 0.06 | 0.37 ± 0.06 |
| Kidney (1) | 6.40 ± 0.92 | 6.10 ± 0.53 | 4.22 ± 0.30 | 2.77 ± 0.24 |
| Brain | 0.03 ± 0.01 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| *Heart/Blood* | *2.34 ± 0.36* | *13.28 ± 2.19* | *18.10 ± 0.91* | *45.58 ± 3.96* |
| *Heart/Lung* | *1.88 ± 0.18* | *2.64 ± 0.34* | *2.98 ± 0.53* | *3.27 ± 1.32* |
| *Heart/Liver* | *0.32 ± 0.03* | *0.34 ± 0.04* | *0.56 ± 0.10* | *4.12 ± 0.44* |
| | n = 7 | n = 7 | n = 4 | n = 4 |

Table 2. Biodistribution of $^{67}Ga[(4\text{-}deaSal)_2 - 3,2,3\text{-}tet]^{1+}$ in Rats (175-212 g) Following Intravenous Injection.

| | % Injected Dose Per Gram of Tissue | | | |
|---|---|---|---|---|
| | 1 min | 5 min | 15 min | 120 min |
| Blood | 0.80 ± 0.01 | 0.39 ± 0.05 | 0.20 ± 0.03 | 0.10 ± 0.01 |
| Heart | 0.98 ± 0.07 | 0.91 ± 0.06 | 0.90 ± 0.06 | 0.92 ± 0.10 |
| Lungs | 1.16 ± 0.06 | 0.89 ± 0.19 | 0.70 ± 0.07 | 0.53 ± 0.06 |
| Liver | 4.15 ± 0.25 | 3.96 ± 0.28 | 3.21 ± 0.25 | 1.07 ± 0.10 |
| Spleen | 1.22 ± 0.47 | 1.19 ± 0.17 | 1.27 ± 0.17 | 0.99 ± 0.18 |
| Kidney (1) | 7.22 ± 0.48 | 7.11 ± 0.89 | 6.82 ± 0.53 | 5.69 ± 0.75 |
| Brain | 0.04 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.01 |
| *Heart/Blood* | *1.22 ± 0.08* | *2.36 ± 0.25* | *4.54 ± 0.31* | *9.21 ± 1.12* |
| *Heart/Lung* | *0.85 ± 0.10* | *1.05 ± 0.19* | *1.29 ± 0.05* | *1.74 ± 0.35* |
| *Heart/Liver* | *0.24 ± 0.03* | *0.23 ± 0.02* | *0.28 ± 0.04* | *0.86 ± 0.18* |
| | n = 4 | n = 4 | n = 4 | n = 3 |

electrophoresis studies demonstrate that these $^{67}$Ga-tracers are cationic, as would be expected for $Ga^{3+}$ complexes with $N_4O_2^{2-}$ ligands. Despite their cationic nature, the $^{67}$Ga complexes of I and II are lipophilic with *log P* values of 1.7 and 2.5, respectively (where *P* is the octanol/water partition coefficient).

The biodistribution of each of these radiotracers was determined following intravenous injection into the femoral vein of ether anesthetized rats (Tables 1 and 2). Both radiopharmaceuticals afford significant myocardial uptake of activity and in both cases the $^{67}$Ga-activity is completely retained in the heart from 1 minute to 2 hours post-injection. The $^{67}$Ga complex of I exhibits the best myocardial uptake with 1% of the injected dose in the heart and excellent heart/blood ratios beyond 5 minutes post-injection.

Efforts remain underway to evaluate these and related gallium complexes as potential $^{68}$Ga-radiopharmaceuticals for imaging the heart with PET.

Acknowledgement

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References

1. Green M.A. - *J. Nucl. Med.* **31**:1641 (1990).
2. Green M.A., Mathias C.J., Neumann W.L., *et. al.* - *J. Nucl. Med.* **32**:944 (1991).
3. Kung H.F., Liu B.L., Mankoff D., *et. al.* - *J. Nucl. Med.* **31**:1635 (1990).
4. Green M.A., Welch M.J., Mathias C.J., *et. al.* - *J. Nucl. Med.* **26**:170 (1985).

**New Ligands for the Formation of Highly Stable Complexes of Trivalent Metal Ions**

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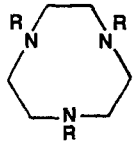
Recently it has been shown in our laboratory that highly stable Fe(III) complexes are formed by the hexadentate ligand N,N',N"-tris(3-hydroxy-6-methyl-2-pyridylmethyl)-1,4,7-triazacylcononane, **1**, TCN-HP.[1,2,3] Apparently, the o-hydroxypyridyl group is effective for hard metal ions since its hydroxy group provides a phenolate type donor but has a relatively low pK (because of the pyridine nitrogen) and does not involve the high degree of hydrogen ion competition characteristic of phenolic groups. It was therefore decided to synthesize a number of macrocyclic derivatives containing hydroxypyridyl groups in which both the size and number of donor groups in the macrocyclic ring are varied, and the number of hydroxypyridyl groups is also changed. The following six ligands have been synthesized.



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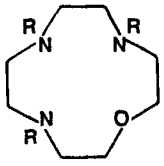

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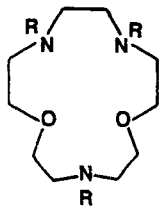

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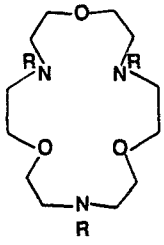

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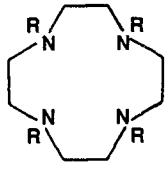

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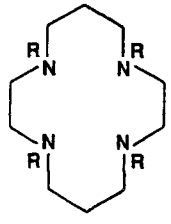
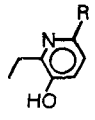


**6** 8, -4

R =



In ligands **1-6**, the second number is the total number of donor groups provided by the ligand, and the third number is the negative charge of the fully dissociated ligand. Thus ligands **1-4** would be expected to form neutral complexes with Ga(III), In(III) and Gd(III) ions while ligands **5** and **6** would form negative complexes with these ions. The Ga(III) ion which is close in ionic radius to the Fe(III) ion, would be expected to form its most stable complex with **1**. On the other hand the In(III) ion, which is larger, may be able to take advantage of all seven of the donor groups provided by **2**. The Gd(III) ion, which has a coordination number of 8 or 9, would prefer the ligands with more donor groups. On the basis of ion size, ring size, coordination number, and charge neutralization effects, one would expect the order of stability of the Gd(III) chelates to be: **4** > **5** > **6**, **3** > **2** > **1**.

The corresponding stability constants, measured potentiometrically and spectrophotometrically, will be compared with the predicted values. Plans will be described for covalent attachment of the Ga(III) and In(III) complexes to antibodies.

**Acknowledgement**

This work was supported by NIH Grants CA-42925 and HL-42780.

**References**

1. A. E. Martell, R. J. Motekaitis and M. J. Welch - J. Chem. Soc. Chem. Commun.: 1748(1990).
2. Y. Sun, A. E. Martell, J. H. Reibenspies and M. J. Welch -Tetrahedron **47**: 357(1991).
3. R. J. Motekaitis, Y. Sun and A. E. Martell - Inorg. Chim. Acta, 1991 (in press).

Paper I 5

# $^{99m}$Tc and $^{153}$Sm labeling and Biodistribution in Rats of two new Cyclic tetraphosphonates: 1,4,8,11-Tetraazacyclodecane-N,N',N",N" tetramethylene phosphonic acid (TTMP) and 1,2,4,5-Benzenetetramethylene Phosphonic acid (BTMP).

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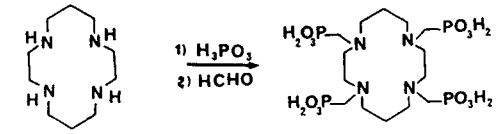
$^{99m}$Tc labeled phosphonates are widely used as skeletal imaging agents.Although the exact mechanism of the uptake is not entirely known,it appears to be dependent on the phosphonate structure.

More recently phosphonate complexes with emitting radionuclides (like $^{153}$Sm-EDTMP or $^{186}$Re-EHDP) were tested as radiopharmaceuticals showing useful properties in pain relief of bone metastasis.

We studied the reactivity towards $^{99m}$Tc and $^{153}$Sm and the biodistributions in rats of two new cyclic tetraphosphonate ligands:1,4,8,11 Tetraazacyclotetradecane-N,N'-N"-N" tetramethylene phosphonic acid (TTMP) and 1,2,4,5, Benzenetetramethylene phosphonic acid (BTMP).

**Syntesis of ligands:**

Ligands were synthesized as outlined in the following scheme:




**$^{99m}$Tc labeling:**

Labeling was carried out in the usual way by adding $^{99m}TcO_4^-$ to a solution of tetraphosphonate stannous complex at room temperature.

The relative concentration of $Sn^{2+}$ ,ligands and pH were optimised.The final radiochemical purity expressed as percent of reduced non hydrolysed Tc was greater than 93%.

**$^{153}$Sm labeling:**

$^{153}$Sm in 0.05N HCl solution was added to a buffered solution of Tetraphosphonates at room temperature.

Chromatography results showed no free Sm.

Gel permeation analysis

The molecular sizes of radiolabeled phosphonates were analysed by gel filtration using a TSK2000 column.The estimated molecular wight for all compounds were about 10 Kd.

Animal Biodistributions

Normal male rats were injected with the two tetraphosphonates labeled with $^{99m}Tc$ and $^{153}Sm$ and with $^{99m}TcHMDP$ , $^{153}SmEDTMP$ which were used as reference.
We report here the values of uptake of the most significant organs two hours after the injection.The results are expressed as % of I.D. in the whole organ.
Table I.

The $^{99m}Tc$ tetraphosphonate complexes show good uptake in bone and good bone/muscle ratios,comparable to $^{99m}TcHMDP$ biodistribution.
The $^{153}Sm$ complexes concentrate in bone,but show also high liver uptake and they do not seem to be good candidates for human trials.

| ORGANS | $^{99m}Tc$ -complexes | | | $^{153}Sm$-complexes | | |
|---|---|---|---|---|---|---|
| | TTMP | BTMP | HMDP | TTMP | BTMP | EDTMP |
| Liver | 0.3 | 0.3 | 0.6 | 13.9 | 14.1 | 0.93 |
| Kidneys | 1.7 | 2.8 | 0.9 | 1.4 | 2.9 | 0.63 |
| Tot.muscle* | 0.9 | 0.7 | 0.3 | 1.8 | 2.3 | 0.52 |
| Tot.blood * | 0.8 | 0.6 | 0.5 | 2.2 | 2.3 | 0.13 |
| Tot. bone* | 26.7 | 43.3 | 45.2 | 41.6 | 41.7 | 50.3 |
| Urines | 44 | 29 | 36 | 25 | 22 | 44 |

Table I: % of I.D. in the whole organ or fluid 2 hours p.i.
* Total organs or fluid calculated as percentage of the whole body weight.Standard deviations are omitted (n=3)

Paper I 6

## SYNTHESIS OF $^{67}$Ga-DEFEROXAMINE-DIGOXIN BIFUNCTIONAL CONJUGATE

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The cardiac glycosides exert a powerful cardiotonic action on the heart muscle and digoxin is one of the most widely used drugs. However, in clinical use, digitalis intoxication has been a prevalent adverse drug reaction. Thus, our interest has been focused on the development of digoxin-based radiopharmaceuticals for the in vitro and in vivo diagnosis of patients requiring the use of digitalis. In this study, synthesis of metal-radionuclide labeled digoxin was attempted for in-vitro radioimmunoassay of digoxin as well as in-vivo $Na^+,K^+$-ATPase imaging.

Digoxin, which contains three sugar groups, does not have metal chelating site, so that $^{67}$Ga-deferoxamine ($^{67}$Ga-DF) was selected as a metal nuclide-bifunctional chelating agent system. It is well known that steroid molecule as well as the first sugar group is important for keeping the binding ability (association and dissociation constants) of digoxin to the $Na^+,K^+$-ATPase, so that binding of DF to digoxin was performed at the terminal (third) sugar position.

The terminal digitoxose of digoxin was first cleaved by periodate oxidation, then bound with carboxymethoxylamine and the product coupled with deferoxamine. Conjugation level of the DF/DIG was approximately 2, determined by stoichiometry. The DF-DIG retained the antigenicity, although deferoxamine ,a relatively large molecule, was introduced to digoxin molecule (deferoxamine: M.W.= 560.71, digoxin: M.W.= 780.95). The $^{67}$Ga labeling of DF-DIG was easily performed by simple mixing with a purified $^{67}$Ga for one hour (1). The $^{67}$Ga-DF-DIG showed enough binding affinity to anti-digoxin antibody and the standard curve of $^{67}$Ga-RIA showed good linearity between the percent bound / total counts and the concentration of digoxin. As for $Na^+,K^+$-ATPase imaging, preliminary study was performed in guinea pigs. $^{67}$Ga-DF-DIG showed relatively high myocardial accumulation. Thus, it was concluded that $^{67}$Ga-DF-DIG as a metal-labeled bifuctional radiopharmaceutical could be successfully synthesized.

1. Furukawa T.,et al.- Chem.Pharm.Bull. 38:2285(1990)

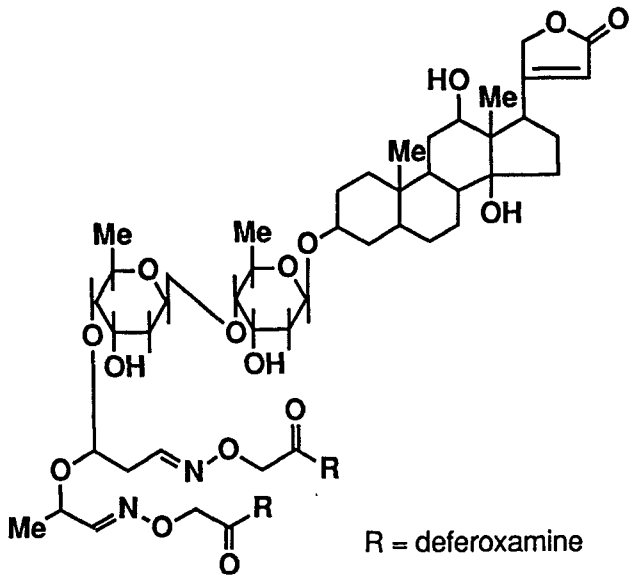


Fig. 1 Chemical structure of deferoxamine-digoxin conjugate (DF-DIG)

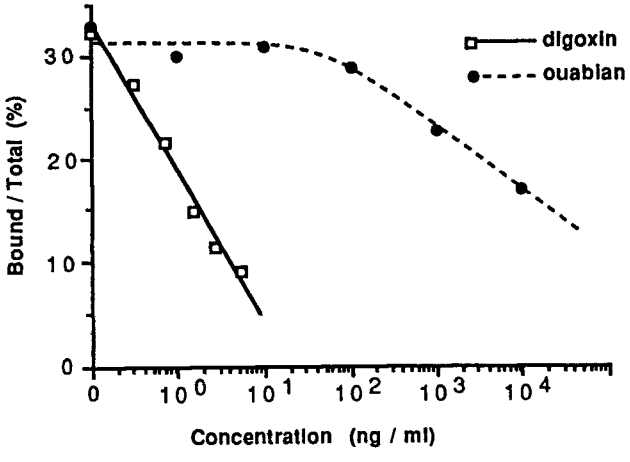


Fig. 2 Specificty of Ga-67 RIA system

## A Gallium Labelled Desferrioxamine/Somatostatin Analogue (SDZ 216-927).

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Somatostatin analogues have been radiolabelled and evaluated with both $^{123}I$(1,2) and $^{111}In$(3,4). However both these radionuclides have halflives very much greater than the known biological halflives of the respective somatostatin analogues. In this work we present a new somatostatin analogue - Desferrioxamine/SDZ 216-927 (DFOSMS) - which has been labelled and evaluated for both $^{67}Ga$ and $^{68}Ga$.

Serum stability studies with the gallium complexes of both DFO and DFOSMS demonstrated a greater stability than N,N'bis[2-hydroxybenzyl]ethylenediamine-N,N'diacetic acid (HBED) and Bis-(2-aminoethyl)-amine-N,N,N',N'',N''-pentaacetic acid (DTPA) or their bifunctional derivatives. Scatchard analysis of the binding of $^{67}$GaDFOSMS with rat cortex membranes was comparable to the original octreotide (Sandostatin®) ($K_d$ values 1.2 nM and 0.4 nM respectivly).

Radiolabelling of the DFOSMS conjugate with either $^{67}Ga$ or $^{68}Ga$ can be achieved in a one pot reaction which requires no further clean up before administration. The $^{67}$GaDFOSMS can be prepared with a greater than 99.9% incorporation of $^{67}Ga$ within 1 hour. Specific activities of 40 MBq $^{67}Ga$/µg DFOSMS can be stored at 4° C for 24 hrs with no decomposition observed.

Animal studies with $^{67}$GaDFOSMS demonstrated a fast tumour uptake in rats bearing Somatostatin receptor positive endocrine pancreatic or exocrine pancreatic tumours. One hour post injection the accumulation of activity in the tumour, blood and liver was 0.62, 0.10 and 0.146 %ID/g respectively with the

corresponding 4 hour figures 0.64, 0.029 and 0.12 %ID/g. The accumulation at the tumour could be prevented by an earlier (one hour) injection of DFOSMS to saturate the somatostatin receptors. The gallium labelled DFOSMS is almost exclusively excreted via the kidneys and metabolite analysis shows the labelled conjugate to be excreted intact.

Animal PET studies with $^{68}$GaDFOSMS in rats bearing somatostatin receptor positive endocrine pancreatic tumours showed the rapid accumulation of activity at the tumour site in under 1 minute with a maximum activity after 30 min. The $K_{off}$ value was 3.5 x $10^{-5}$ $s^{-1}$ which corresponds to a halflife of 6 hours.

References.

1 J.C.Reubi, W.H.Häcki and S.W.J.Lamberts. J.Clin Endocrinol Metab 65, 1127-1134 (1987).

2 E.P.Krenning, W.H.Bakker, W.A.P.Breeman, J.W.Koper, P.P.M.Kooij, L.Ausema, J.S.Lameris, J.C.Reubi and S.W.J.Lamberts. Lancet, 1, 242-244 (1989).

3 W.H.Bakker, R.Albert, C.Bruns, W.A.P.Breeman, L.J.Hofland, P.Marbach, J.Pless, S.W.J.Lamberts, T.J.Visser and E.P.Krenning. Life Sciences (Submitted for publication).

4 W.H.Bakker, E.P.Krenning, J.C.Reubi, W.A.P.Breeman, B.Setyono -Han, M.de Jong, P.P.M.Kooij, C.Bruns, P.M.van Hagen, P.Marbach, T.J.Visser, J.Pless and S.W.J.Lamberts. Life Sciences (Submitted for publication).

# Structure And Chemical Characteristics Of Neutral Rhenium Diamine Dithiol (DADT) Complexes

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Radioisotopes of rhenium, $^{186}$Re and $^{188}$Re, have been shown to be two of the most promising candidates for tumour targeting radiotherapy. The reason for selection of radiorhenium over other radionuclides such as $^{90}$Y is that rhenium has similar chemical properties to $^{99m}$Tc. There have also been considerable advances recently with $^{99m}$Tc-labelled monoclonal antibodies which suggest that radiorhenium should be evaluated as a label in prospective therapeutic applications.

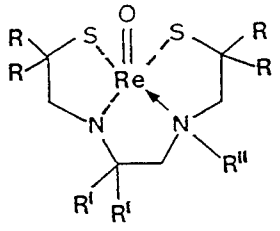
Our first approach for tumour targeting therapy was focused on preparing DADT complexes of rhenium, because many technetium compounds have been reported with ligands based on DADT chelating groups. Among the Re=O[DADT] complexes prepared by us to examine the chemical characteristics for further design of Re-radiopharmaceuticals, Re=O[TE], Re=O[TEDM] and Re=O[HM] (Fig. 1.) demonstrated less lipophilic character, although the similar $^{99m}$Tc=O[DADT] complexes showed high lipophilic character.[1] The IR spectra of these rhenium complexes showed that the Re=O stretching vibration shifted to low wave number in comparison with that of Re=O[NMTM](syn) complex[2] (Table 1). This suggested that the shift is caused by hydrogen bonding with an amine hydrogen.

The best way to verify this hypothesis, is X-ray structural determination. Suitable crystals of Re=O[TE] were grown for crystallographic analysis. The ORTEP diagram of Re=O[TE] indicates that the two Re=O[TE] formed a dimer through the hydrogen bonds between each oxygen of Re=O and hydrogen of protonated nitrogen. Both Re=O bond lengths in the dimer, 1.709Å, are greater than that previously observed[3] for Re=O[2,3-bis(mercaptoaetamido)propionic acid], 1.662Å. One of the nitrogens in Re=O[TE] is deprotonated, evident from the difference of approximately 0.23Å between the two Re-N bonds. These results show that the dimer of Re=O[TE] complexes is neutral.

Acknowledgment. We thank Mr D. Craig, University of New South Wales, for the x-ray crystal structure determination.

1. Kung H.F., Molnar M., Billings J., Wicks R. and Blau M. - J.Nucl.Med. 25: 326 (1984).
2. Mahmood A., Baidoo K.E. and Lever S.Z. - Technetium and Rhenium in Chemistry and Nuclear Medicine 3, Eds. Nicolini M., Bandoli G. and Mazzi U., 119, Raven Press, New York (1990).
3. Rao T.N., Adhikesavalu D., Camerman A. and Fritzberg A.R. - J.Am.Chem.Soc. 112: 5798 (1990).

Fig. 1. Re=O [DADT] Complexes




| DADT | R | R′ | R″ |
|---|---|---|---|
| TE | Et | H | H |
| TEDM | Et | Me | H |
| HM | Me | Me | H |
| NMTM | Me | H | Me |

Table 1. IR absorption of Re=O[DADT] complexes

| DADT | $cm^{-1}$ |
|---|---|
| TE | 920 |
| TEDM | 919 |
| HM | 922 |
| NMTM(syn) | 942* |

*lit.[3]; 942 $cm^{-1}$

**Paper I 9**

## A GALLIUM-68 LABELED CHEMOTACTIC PEPTIDE ANALOGUE FOR IMAGING FOCAL SITES OF BACTERIAL INFECTION BY PET.

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Identification of the site and extent of suspected infection via nuclear medicine imaging procedures has been hampered by the slow localization *in vivo* of currently-available radiotracers. This is especially problematic in the application of positron emission tomography, since the commonly-used PET radionuclides have physical half-lives less than two hours.

A possible solution to this problem is to radiolabel leukocyte chemoattractant peptides, which bind to circulating granulocytes as well as to leukocytes already present at the site of infection (1-3), thereby shortening the interval between radiotracer injection and lesion detection. Such a chemotactic peptide is N-formyl-methionyl-leucyl-phenylalanine, which binds with high affinity to receptors on white blood cell membranes. Recently, a series of indium-111 labeled derivatives of this peptide have been evaluated as potential radiopharmaceuticals for $\gamma$-scintigraphy (4). One of these, a derivative of formyl-norleucyl-leucyl-phenylalanyl-norleucyl-tyrosyl-lysine (For-NleLFNleYK), showed the greatest target-to-blood localization ratio *in vivo*.

With the goal of producing a generator-available positron-emitting radio-pharmaceutical that would permit the superior resolution and quantitative capability of PET to be applied to imaging sites of bacterial infection, we have investigated the utility of a gallium-68 ($\beta^+$, $t_{1/2}$ = 68 min) labeled chemotactic peptide. In particular, For-NleLFNleYK-DTPA was radiolabeled with gallium-68 and its localization *in vivo* examined using a rat model of sepsis.

Gallium-68 was eluted from $^{68}Ge$ bonded to an $SnO_2$ column (DuPont Medical Products) using 1 M HCl (5), boiled to dryness in a borosilicate glass test tube under a stream of nitrogen, and re-dissolved in 0.4 M NaOAc for transchelation with For-NleLFNleYK-DTPA for 30 minutes at ambient temperature. Purification of the labeled product was achieved using Sephadex G-50 gel permeation chromatography followed by sterile filtration through a Millex-GV 0.22 $\mu$ filter unit. For 0.5 mg of labeling substrate and an overall preparation interval of 60 min, a radiochemical yield of 38-40% of For-NleLFNleYK-[$^{68}Ga$]DTPA was achieved.

The gallium-68 labeled chemotactic peptide was injected via tail vein into the tail vein of control and septic rats. Sepsis was induced using a cecal ligation and perforation technique (6). Control rats underwent an abdominal laparotomy and cecal manipulation. The radiotracer localized *in vivo* within septic tissue; at 60-120 minutes post injection the labeled peptide localization within cecum was 2.8-3.2 times greater for animals in the septic group relative to the control rats. As has been reported for

the corresponding indium-111 labeled derivative of this chemotactic peptide (4), the greatest concentration of radiotracer was in the kidneys.

These promising results encourage the further development of gallium-68 labeled chemotactic peptides as PET radiopharmaceuticals.

This work was supported in part by DOE grant DE-FG02-87 ER60512.

1. Showell HJ, Freer JR, Zigmond SH, et al. *J Exper Med* 143: 1154 (1976).
2. Schiffmann E, Corcoran BA, Wahl SM. *Proc Natl Acad Sci USA* 72: 1059 (1975).
3. Williams LT, Snyderman R, Pike MC, Lefkowitz RJ. *Proc Natl Acad Sci USA* 74: 1204 (1977).
4. Fischman AJ, Pike MC, Kroon D, et al. *J Nucl Med* 32: 483 (1991).
5. Loc'h C, Maziere B, Comar D. *J Nucl Med* 21: 171 (1980).
6. Hotchkiss RS, Song S-K, Ling CS. *Am J Physiol* 258: R21 (1990).

## MAPPING MRNAS FOR HISTONE4 AND NMDA IN PIG AND HUMAN BRAIN WITH IN-111 LABELED ANTI-SENSE OLIGONUCLEOTIDES BY IN-SITU HYBRIDIZATION

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**INTRODUCTION.** The beta-emitting anti-sense oligo-nucleotide probes (P-32, S-35) and non-radioactive probes (biotin and digoxin) are used widely in in vitro hybridization experiments (1-3). The gamma-emitting indium-111-labeled anti-sense oligonucleotide probes were developed for imaging histone4-mRNA and NMDA transcripts as a marker for functioning and ischemic neurons (4-7).

**EXPERIMENTAL PROCEDURES.** The 25-mer nucleotide sequence for mRNAs for histone4, a nucleoprotein and NMDA receptor was selected from the GenBank. The aminohexyl-oligonucleotide (AHON) was synthesized by conjugating the aminohexyl moiety (Aminolink-2, Applied Biosystems) to the 5′ end of oligonucleotide and coupled with diethylenetriamine-pentaacetate-isothiocyanate, DTPAI (1/50) and 20 μg of DTPA-AHON were chelated with 37-370 MBq (1-10 mCi) of In-111-Chloride. The free In-111 was removed by gel-filtration (Sephadex G-25, Pharmacia Fine Chemicals). The brain of pigs (Yorkshire) was harvested and fixed with a solution of 40% formaldehyde, glacial acetic acid and methanol (1/1/8:vol/vol) for 3 days at room temperature. Thin coronal sections (1 mm) were prepared, washed free of residual fixative with citrate-buffered saline (2x SSC) and incubated for 24 hours with 50 μCi of In-111 AHON. The unbound In-111 probe was removed by three washings of citrate-buffered saline. The slices were placed one cm away from the face of the gamma camera (Pho-gamma V, Siemens Inc.) fitted with a pin-hole collimator and interfaced with the MDS computer. After imaging, the slices were sponged and placed on the saran wrap to prevent chemography and Kodak X-O-Mat film was apposed for contact autoradiography for 1 day. The ratios of radioactivity of gray and white matter were determined with the window of gamma counter (Searle 1185) set for In-111 channel.

**RESULTS AND DISCUSSION.** The scintiphotos and autoradiographs clearly demonstrated neuronal mRNAs for NMDA and histone4 in the hippocampus and cerebral cortex. The results of (mean±S.D.) relative radioactivity ratio of grey matter/white matter for histone4 mRNA was 5±1. The AHON could be labeled with In-111 at high specific activity (30-100 μCi/μg) and labeling efficiency (70-80)%; the gamma camera imaging and autoradiography with the In-111 labeled AHON tracer could be carried out in a period of 3-4 days. The gamma-emitting probes provided a simple and sensitive tool for mapping the neuronal mRNAs. This in-situ hybridization technique could be applied for mapping mRNAs, specific for neurotransmitters and membrane proteins of all tissues and of all species including tissues from human patients.

**REFERENCES**

1. Venezeky D.L., Angerer L.M. and Angerer R.C. Cell 24: 385 (1981).
2. Lum J.B. BioTechniques 4: 32-39(1986).
3. Sambrook J., Fritsch E.F. and Maniatis T. In Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York. Vol 2, pp 11.3-11.58(1989).
4. Dewanjee M.K., Ghafouripour A.K., Werner R.K., Serafini A.N., Sfakianakis G.N. Bioconj. Chem. 2: 195-200(1991).
5. Dewanjee M.K. Radioiodination: Theory, Practice, and Biomedical Applications. Kluwer Academic Publishers Inc. Boston (1991) and Dewanjee, M.K. Ind. J. Nucl. Med. 4(3): 162-174(1989).
6. Mirzadeh S, Brechbiel MW, Atcher RW, Gansow OA. Bioconjugate Chem. 1: 59-65(1990).
7. Moriyoshi K, Masu M, Ishii T, et al. Molecular cloning and characterization of the rat NMDA receptor. Nature 354: 31-37 (1991).

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

Paper I 11

# THE STABILITY OF $^{90}$Y-PROTEIN-COMPLEXES

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The chemical and biological properties of $^{90}$Y, applied as citrate complex, are very similar to those of the radioalanthanides, such as $^{167}$Tm and $^{169}$Yb, which are known to show both a bone and a tumour affinity (1).

In former experiments,we could prove a relationship between the extend of the binding of the radiolanthanides to albumine in the blood and their tumour accumulation. The stabilities of the corresponding lanthanide-albumine complexes were dependent on the ionic radii of the lanthanides (2).

Therefore, the aim of this study is to investigate by which proteins $^{90}$Y is bound and transported in blood, and to determine the stability constants of the yttrium-protein complexes formed in blood.

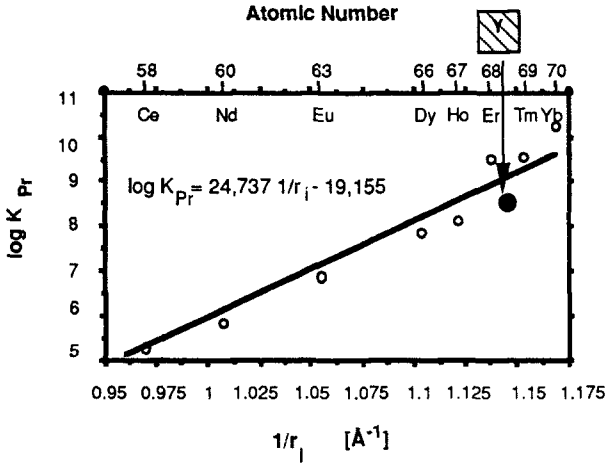


Figure 1
Dependence of log $K_{Pr}$ on the ionic radii of the central ions in lanthanide- and yttrium-citrate complexes

After incubation of both serum and HSA-solutions with $^{90}$Y-citrate the binding constant of the corresponding yttrium-protein complexes formed in the protein solutions under physiologic conditions, pH:7.4, temperature: 310 K (37 °C), isotonic ionic strength: 0.15 mole/l, was determined by means of equilibrium dialysis:The association constants of the yttrium-protein complexes formed both in serum and HSA-solutions are equal within the experimental error.

Further, a serum fractionation with alcohol proved also that the albumin fraction of serum is responsible for the yttrium binding in blood. In conclusion, serum albumine binding predominates for yttrium also.

The association constant of the yttrium albumine was determined to be $(8.5\pm1.0)\cdot10^8$ l/mole (log $K_{Pr}$ = 8.53±0.1. This association constant adapts very good to the dependence on the ionic radii as observed for the stabilities of the lanthanide-albumine complexes (Figure 1).

Considering the distinct albumine binding we assume that $^{90}$Y, applied as water soluble complex, shows also an affinity to soft-tissue-tumours, as known from the radiolanthanides.

1. Schomäcker, K. et al. - Isotopenpraxis 26:, 145(1990)
2. Schomäcker, K. et al. - Appl. Radiat. Isot. 39: 261(1988)

**Paper I 12**

# NEW TUMOUR-AFFINE RADIOACTIVE YTTRIUM COMPOUNDS

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The aim of the study was to find the optimal chemical form of yttrium compounds for tumour visualization and therapy.

We could favour among the radiopharmaceuticals tested untill now on tumour bearing mice the M-EDTMP-NTA-Ca-complexes (EDTMP-NTA: (Ethylenediaminetetramethylenephosphate/Nitrilotriacetate) (M: $^{169}$Yb, $^{87}$Y, $^{167}$Tm).

Table 1 shows the results of biokinetic investigations on melanoma bearing mice after intraperitoneal injection of both $^{87}$Y- or $^{88}$Y-EDTMP-NTA and -citrate.

Table 1

Biokinetic data after i.p. injection of $^{87,88}$Y-citrate and -EDTMP-NTA-Ca

| Time p.i. [h] | Muscle | Femur | Liver | Spleen | Intes-tine | Lung | Blood | Tumour |
|---|---|---|---|---|---|---|---|---|
| **Melanoma, Intraperitoneal application, $^{87}$Y-Citrate** | | | | | | | | |
| 0.166 | 2.21 | 4.51 | 8.56 | 3.38 | 4.83 | 8.81 | 25.32 | 6.97 |
| 0.500 | 1.16 | 10.87 | 9.32 | 2.09 | 3.40 | 5.57 | 16.26 | 6.89 |
| 2.000 | 0.45 | 24.64 | 8.90 | 0.73 | 1.91 | 3.22 | 4.48 | 3.04 |
| 4.000 | 0.45 | 23.34 | 8.21 | 0.95 | 4.47 | 1.89 | 1.27 | 6.95 |
| 24.000 | 0.10 | 19.77 | 5.69 | 0.87 | 0.73 | 1.14 | 0.14 | 1.81 |
| 48.000 | 0.21 | 18.72 | 2.70 | 0.63 | 0.35 | 0.49 | 0.03 | 0.72 |
| **Melanom, Intraperitoneal application, $^{87}$Y-NTA-EDTMP-Ca** | | | | | | | | |
| 0.166 | 0.39 | 3.10 | 2.13 | 0.45 | 1.84 | 4.23 | 13.47 | 6.69 |
| 0.500 | 0.21 | 3.02 | 2.99 | 0.77 | 1.93 | 2.61 | 8.32 | 7.16 |
| 2.000 | 0.32 | 3.85 | 3.63 | 0.89 | 2.00 | 1.91 | 3.55 | 8.68 |
| 4.000 | 0.17 | 2.86 | 3.79 | 0.82 | 2.54 | 0.98 | 0.95 | 8.31 |
| 24.000 | 0.08 | 2.32 | 3.465 | 0.76 | 2.49 | 0.68 | 0.23 | 8.11 |
| 48.000 | 0.11 | 1.98 | 3.14 | 0.71 | 2.34 | 0.91 | 0.01 | 5.74 |

The results show that liver and bone accumulate the most radioactivity after injection of the both compounds but the radioactivity level is lower after the EDTPM-NTA-administration on unchanged tumour radioactivities. To evaluate the radiation burden in these organs compared with that in the tumour for the injection of the corresponding $^{90}$Y-compounds, we used the "MIRD"-model. Table 2 shows the results:

Table 2

Radiation burden after i.p. injection of $^{90}$Y as either Citrate or EDTMP-NTA-Ca

| Radioactive compound | D [mSv/ MBq$_{appl}$] | $D_{Tumour}/D_{Skeleton}$ | $D_{Tumour}/D_{Liver}$ | $A_{appl}$ for $D_{Tumour}$ of 60 Gy MBq | mCi |
|---|---|---|---|---|---|
| Y-Citrat, Melanoma, i.p. | | 1.9 | 8.7 | 3619 | 98 |
| Tumour | 16.58 | | | | |
| Skeleton | 8.74 | | | | |
| Liver | 2.13 | | | | |
| Y-NTA-EDTMP-Ca, Melanoma, i.p. | | 98.9 | 39.0 | 831 | 23 |
| Tumour | 72.22 | | | | |
| Skeleton | 0.73 | | | | |
| Liver | 1.85 | | | | |

The dose ratios after application of Y-EDTMP-NTA-Ca are rather sufficient compared to the corresponding therapeutic methods currently used in the nuclear medical practice.

They encouraged us to carry out first therapautic investigations on melanoma bearing mice and dogs. A single dose of 37 MBq/kg body weight of our radioactive complex which contained instead of $^{87}$Y the radionuclide $^{90}$Y led to a disappearance of the tumours in the mice and to both a tumour regression and a clear change of the morphology of the tumour in the dog.

**Paper I 13**

## Preparation of Bone Tumor Treatment Agent of $^{90}$Y-EDTMP

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The radionuclide $^{90}$Y is widely used in nuclear medicine for clinical therapy since first $^{90}$Sr-$^{90}$Y generator has been developed by BNL (Brookhaven National Laboratory). The $^{90}$Y for the therapeutic nuclide has many advantages, i.e., suitable half-life(64h), intermediate β-ray energy(2.274MeV), stable daughter and suitable cheimcal properties. In addition, the radionuclide may be eluted from $^{90}$Sr -$^{90}$Y generator.

Because of $^{90}$Y-EDTMP (ethylene diamion tetramethylene phosphonate) superior biolocation, it was suggested as a potential agent to treat metastatic bone cancer, from a series of aminophosphonate complexs of $^{90}$Y. The studies on its synthesis and the behavior in animal have been carried out since 1990 in our laboratory. The main results are given in this article.

As shown in Fig.1, the labelling efficiency is more than 98% under condition of PH value from 3 to 9 and Fig.2 showed that when the concentration of EDTMP is up to 5mg/ml, the labelling efficiency is more than 98% at room temperature for 5-10min.

As shown in Fig.3 , the Rf value of $^{90}YCl_3$ and $^{90}$Y-EDTMP is 0.13 and 1.0 respectively in 10% $NH_4Ac$ solution with ascending chromatography development. From Table 1 and Fig.4, it may be found that $^{90}$Y-EDTMP has excellent biological properties including fast blood clearance, high bone uptake and lower residual activity in the liver and muscle. Meanwhile, the study of stability in vitro showed that the radiochemical purity was more than 98% after 3 days.

The toxicity trials showed that there were demonstrable changes of erythrocytes, leukocytes and thromobcytes in rabbit (3.5Kg) when the injected doses reached 100 times than that of a normal patient (3.7MBq/Kg). But they recovered from 4 weeks postinjection. An intensive investigation is under way to evaluation the use as radiotherapeutic bone tumor agent.

Table 1: %Injected Doses Still Present in Entire Organ

%I.D. in Organ ( mean±s.d. )

| Organ | 1min | 30min | 60min | 120min | 180min |
|---|---|---|---|---|---|
| Bone | 23.91±8.73 | 48.80±3.02 | 48.41±0.51 | 38.73±1.52 | 36.30±4.18 |
| Kindey | 4.54±0.93 | 0.26±0.05 | 0.20±0.00 | 0.16±0.05 | 0.13±0.06 |
| Liver | 2.23±0.71 | 0.07±0.01 | 0.02±0.01 | 0.03±0.01 | 0.04±0.02 |
| Lung | 2.30±0.43 | 0.08±0.03 | 0.01±0.01 | 0.01±0.01 | Background |
| Spleen | 0.18±0.05 | 0.02±0.01 | 0.01±0.01 | 0.01±0.01 | Background |
| Muscle | 26.33±3.72 | 2.10±1.78 | 1.05±0.52 | 0.74±0.35 | 0.31±0.18 |
| Blood | 38.89±8.74 | 0.32±0.00 | 0.43±0.09 | 0.10±0.10 | 0.04±0.02 |

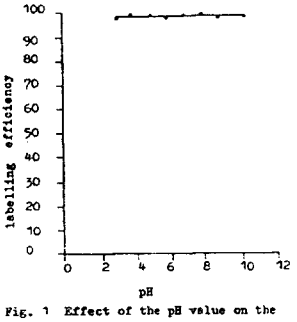



Fig. 1 Effect of the pH value on the labelling efficiency

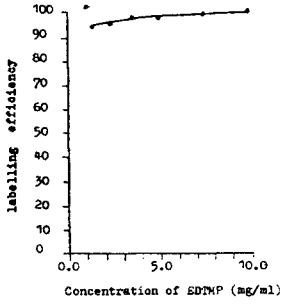
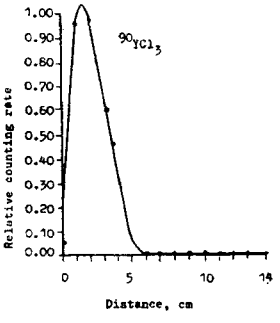



Fig. 2 Effect of the concentration of EDTMP on labelling efficiency



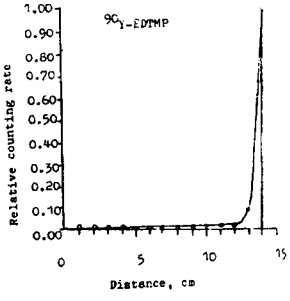
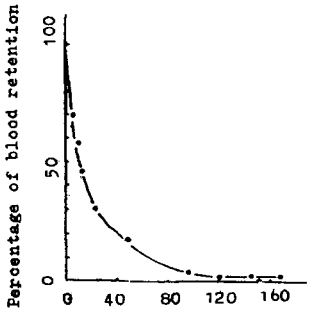




Fig. 3 Paper chromatogram of $^{90}YCl_3$ and $^{90}Y$-EDTMP




Figer 4. The blood clearance in rabbit with $^{90}Y$-EDTMP

Investigation of Dialkyl Gallium(III) Complexes as Potential Radiopharmaceuticals

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The development of radiopharmaceuticals labeled with generator-produced gallium-68 ($t_{1/2}$ = 68 minutes) could facilitate more widespread use of positron emission tomography (PET) in medical diagnosis. Efforts to develop new gallium-labeled radiopharmaceuticals have traditionally focused on the chemical similarity of the aqueous gallium(III) ion to high-spin iron(III). The present investigation has been undertaken to determine whether a fundamental difference between Ga(III) and Fe(III) chemistry, namely the aqueous stability of the covalent gallium-carbon bond, can be exploited in radiopharmaceutical design and development. We report here the synthesis of carrier-added [$^{67}$Ga]-dimethylgallium(III) acetylacetonate, $Me_2Ga(acac)$, and the determination of its biodistribution in rats following intravenous injection.

Gallium-67 labeled $Me_2Ga(acac)$ was synthesized at a specific activity of 2.8 mCi/mmol by reaction of gallium(III) chloride with excess methyllithium in diethylether (equation 1). After hydrolysis of the intermediate $Me_3Ga{\cdot}Et_2O$ to $[Me_2Ga(OH)_2]^{1-}$ the aqueous phase was adjusted to pH 7 and the $Me_2Ga(OH)(H_2O)$ (equation 2) extracted into $Et_2O$. Acetylacetone (acacH) was added to the combined ether extracts (equation 3), which were subsequently washed with water to remove any excess acacH. The ether was then evaporated and the [$^{67}$Ga]-$Me_2Ga(acac)$ purified by sublimation.

$$GaCl_3 + \text{xs } LiCH_3 \xrightarrow{Et_2O} (CH_3)_3Ga{\cdot}Et_2O \xrightarrow{H_2O} [(CH_3)_2Ga(OH)_2]^{1-} + CH_4 \quad (1)$$

$$[Me_2Ga(OH)_2]^{1-} \overset{H^+}{\rightleftharpoons} Me_2Ga(OH)(H_2O) \overset{H^+}{\rightleftharpoons} [Me_2Ga(H_2O)_2]^{1+} \quad (2)$$

$$Me_2Ga(OH)(H_2O) + acacH \rightleftharpoons Me_2Ga(acac) + 2\ H_2O \quad (3)$$

The [$^{67}$Ga]-$Me_2Ga(acac)$ was found to migrate as a single radioactive peak ($R_f$ = 0.8) on thin layer chromatography using $C_{18}$-silica gel eluted with 85 mL EtOH : 15 mL $H_2O$ : 1 mL HOAc.

The chemical purity of the carrier-added product was confirmed by 500 MHz $^{1}$H-NMR spectroscopy. The product was found to be moderately lipophilic with a measured octanol/water partition coefficient $P = 56$.

The biodistribution of the carrier-added [$^{67}$Ga]-$Me_2$Ga(acac) was determined following intravenous injection into rats as a 5% ethanol: 20% propylene glycol solution in saline, each animal receiving a dose of *ca* 2.6 μCi in 0.15 mL (Table 1). The tracer was found to clear rather slowly from the blood. At 1 minute post-injection 67 ± 4% of the injected dose remained in the blood, dropping to 31 ± 5% at 5 minutes, 10 ± 1.6% at 30 minutes, and 8.4 ± 1.5% at 2 hours post-injection. Over this time period the tracer progressively accumulated in the liver, rising from 12.3 ± 1.7% of the injected dose at 1 minute, to 24.8 ± 1.7% at 5 minutes, and to *ca* 50 ± 4% of the injected dose at 60 minutes and 2 hours post-injection. Myocardial levels of $^{67}$Ga were fairly constant over a 2 hour time period post-injection; however the high blood and liver activity levels would preclude the use of this tracer to image the heart.

Table 1. Biodistribution Data for [$^{67}$Ga]-$Me_2$Ga(acac) in Rats* Following Intravenous Injection

| | % Injected Dose Per Gram of Tissue | | | | |
|---|---|---|---|---|---|
| | 1 minute | 5 minutes | 30 minutes | 60 minutes | 120 minutes |
| Blood | 6.09 ± 0.22 | 2.85 ± 0.40 | 0.95 ± 0.19 | 0.68 ± 0.16 | 0.75 ± 0.14 |
| Heart | 1.28 ± 0.41 | 1.24 ± 0.16 | 1.15 ± 0.05 | 1.06 ± 0.08 | 1.06 ± 0.16 |
| Lungs | 3.42 ± 0.41 | 1.94 ± 0.25 | 0.95 ± 0.13 | 0.69 ± 0.10 | 0.70 ± 0.09 |
| Liver | 1.90 ± 0.45 | 3.88 ± 0.53 | 7.02 ± 0.66 | 8.12 ± 0.50 | 8.11 ± 0.46 |
| Spleen | 1.23 ± 0.33 | 1.37 ± 0.15 | 0.65 ± 0.09 | 0.50 ± 0.09 | 0.51 ± 0.06 |
| Kidney | 2.08 ± 0.14 | 2.67 ± 0.24 | 2.54 ± 0.42 | 2.35 ± 0.17 | 2.27 ± 0.12 |
| Brain | 0.14 ± 0.01 | 0.10 ± 0.02 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.10 ± 0.04 |
| | n = 4 | n = 4 | n = 4 | n = 4 | n = 4 |

*Size of rats: 150-166 g

Addition of [$^{67}$Ga]-$Me_2$Ga(acac) to rat blood (hematocrit = 45%) *in vitro* resulted in association of 80-90% of the radioactivity with red blood cells. Pre-mixing of the [$^{67}$Ga]-$Me_2$Ga(acac) with plasma did not alter the red cell binding of the $^{67}$Ga radioactivity, indicating that plasma protein adducts of the $Me_2Ga^+$ fragment ($Me_2Ga$←protein) are either not formed or are kinetically labile. When the [$^{67}$Ga]-$Me_2$Ga(acac)-labeled RBC were re-equilibrated with fresh plasma, 5-10% of the $^{67}$Ga radioactivity was extracted into the plasma phase in each of two consecutive washes, indicating that the red cell association of the $^{67}$Ga is not irreversible. This red cell binding of the tracer may explain the slow clearance of $^{67}$Ga from the blood following intravenous injection of [$^{67}$Ga]-$Me_2$Ga(acac).

Efforts remain underway to examine other diakyl gallium species and to develop a synthetic method for preparing these materials at higher specific activity.

Acknowledgement

Support for this work was provided by a grant, RO1-CA46909, awarded by the National Cancer Institute.

Paper I 15

# The Chemical Fate of $^{212}$Bi-DOTA Formed by $\beta^-$ Decay of Aqueous $^{212}$Pb(DOTA)$^{2-}$

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The increasing use of inert metal complexes in radioimmunotherapy prompted us to explore the potential use of $^{212}$Pb chelates [1,3]. Here, we report a study of the chemical fate of the $^{212}$Bi-DOTA complex formed by $\beta^-$ decay of $^{212}$Pb(DOTA)$^{2-}$ ($H_4$DOTA = 1,4,7,10,-tetraazacyclododecanetetraacetic acid) [3]. To assure that both parent and daughter complexes were thermally inert, kinetics studies were performed with $^{203}$Pb(II) and $^{206}$Bi(III) which showed that both lead and bismuth complexes with DOTA undergo chemical exchange only very slowly in aqueous solution at pH 4-10. To quantitate the amount of $^{212}$Bi released, $^{212}$Pb-DOTA was prepared and allowed to come to transient equilibrium with the bismuth daughter. The the ionic and DOTA-complexed species were then separated, and their activities determined.

The $^{212}$Pb(DOTA) complex was prepared *in situ* by mixing 1.30 μmol DOTA with sufficient radioactive metal ion solution ( ~ 100 μCi of $^{212}$Pb and Pb carrier), to have a 10% excess of metal ion. Dilute NaOH was added until pH 9 was reached, and the solution heated at 50°C for 5 min. The reaction mixtures were then neutralized to pH~6 with $HNO_3$ and purified of excess metal salts or metal hydroxides by passage through a 3 x 30 mm column of Chelex-100 with water elution (2 mL). Collected eluant was combined in a flask with 72.5 mg $NH_4I$, and the volume brought to 5 mL with water to give a 0.026 mM solution of radioactive complex in 0.1 M $NH_4I$, pH 5-6. After waiting three hours to insure that transient equilibrium was established between $^{212}$Pb and $^{212}$Bi, 250 μL aliquots of the solution were applied to the second Chelex-100 columns (3x20 mm, pre-equilibrated with 0.1 M $NH_4I$) for separation of free metal and metal complex species. The Pb(DOTA)$^{2-}$ and Bi(DOTA)$^-$ complexes were eluted with 1.75 ml of 0.1 M $NH_4I$, and free metals were stripped with 2 ml of 0.1 M $Na_2EDTA$.

As illustrated in Figure 1, the radioactive decay of both the $NH_4I$ and EDTA fractions was followed over 20 hr to allow deconvolution of the two component decay/growth curves. In the $NH_4I$ fraction, the activity of $^{212}$Bi grows to a maximum, transient equilibrium value, and thereafter decays with the half-life of $^{212}$Pb parent. Activity of $^{212}$Bi in the EDTA fraction, the uncomplexed component of the reaction solution, is seen to decay over two orders of magnitude with the half-life of $^{212}$Bi and then tails and decays with the half-life of residual $^{212}$Pb. A separate 250 μL aliquot of the reaction mixture was similarly counted to serve as a standard (*vide infra*) for determination of the $^{212}$Bi lost in the $^{212}$Bi/$^{212}$Pb-DOTA transition.

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For three experiments, the average value of breakup was 36±2% which demonstrates that substantial disruption of the metal complex occurred. When extrapolated values for activities of $^{212}$Bi in DOTA and free in solution are obtained by least square method, we observe that 37±1% of the $^{212}$Bi formed from $^{212}$Pb-DOTA is measured to be lost from the ligand, in good agreement with the earlier value.

Results of this investigation and those of an earlier study [2] clearly demonstrate that both Bi(DOTA)$^{-}$ and Pb(DOTA)$^{-2}$ are kinetically inert to exchange or hydrolysis in aqueous solutions at pH 4.5-7. Yet, our current studies show that about 35% of $^{212}$Bi-DOTA formed from $\beta^{-}$ decay of $^{212}$Pb-DOTA is dissociated to Bi(III) ions. The possible sources of $^{212}$Bi daughter atoms in the uncomplexed (or free-ion) form are outlined in Figure 2. Our kinetic data prove that all pathways involving acid/base dissociations and isotopic exchange reactions account for no more than 2% of free bismuth under solution pH conditions where $^{212}$Pb/$^{212}$Bi-DOTA transition experiments were performed [2]. Since all free $Pb^{2+}$ was removed in the preparation of Pb(DOTA)$^{2-}$ for these experiments, and because concentrations of carrier free Bi(III) are $<10^{-8}$ M, the amount of free bismuth ions formed in cross exchange reactions between Bi(III) and Pb-DOTA, and $Pb^{+2}$ and Bi-DOTA is also negligible. Therefore, we must attribute the release of $^{212}$Bi from DOTA complex almost entirely to consequences of the radiative event. By considering the various extranuclear processes responsible for kinetic and electronic excitation of the $^{212}$Bi daughter, breakup of the $^{212}$Bi-DOTA complex is ascribed to the internal conversion of $\gamma$-rays emitted by the excited $^{212}$Bi nuclide. The origin and relative importance of various extranuclear processes will be discussed.

Taken together, studies to date show that simple polydentate metal binding does not fully prevent metal complex dissociation following $\beta^{-}$ decay of the central metal ion. Little evidence is available to elucidate the chemistry which accompanies this breakup. Both simple metal-complex dissociation and reactions leading to fragmentation of the ligand seem possible. In the current study, we found that, although the $^{212}$Pb(DOTA)$^{-}$ complex might be biomedically useful in some special circumstances, such as when the complex is rapidly internalized inside a cell by antibody catabolism, inorganic chemistry has yet to provide ligands fully adequate to satisfy the stringent demands placed on a metal chelate system to be used for $^{212}$Pb radioimmunotherapy. The methods described here should be useful in evaluating new ligands designed to retain $^{212}$Bi formed by $^{212}$Pb(II) $\beta^{-}$decay.

1. Gansow, O. A., M. W. Brechbiel, S. Mirzadeh, D. Colcher and M. Roselli, "Chelates and antibodies: Current methods and new directions". In *Cancer Imaging with Radiolabeled Antibodies*, D. M. Goldenberg editor, Kluwer Academic Publishers (1990).

2. Kumar, K., Magerstadt, M. and Gansow, O. A. (1989) Lead(II) and Bismuth(III) Complexes of the Polyazacycloalkane-N-acetic Acids NOTA, DOTA and TETA. *J. Chem. Soc. Chem. Commun.* 145.

3. Mirzadeh, S., Kumar, K. and Gansow, O. A. (1992) The Chemical Fate of $^{212}$Bi-DOTA Formed by $\beta$- Decay of Aqueous $^{212}$Pb(DOTA)$^{2-}$, *Radiochimica Acta*, in press.

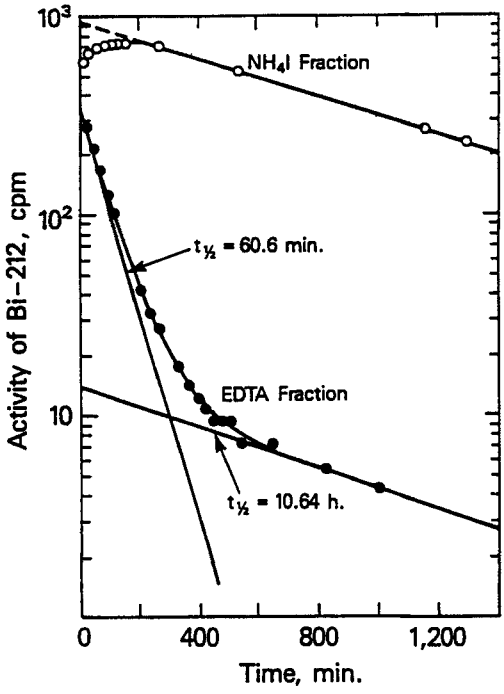


Figure 1. Post separation decay/growth of $^{212}Bi$ radioactivity in fractions obtained by Chelex-100 ion exchange chromatography of a 250 $\mu$L aliquot of a solution initially containing only $Na_2[Pb(DOTA)]$ doped with $^{212}Pb$. The $NH_4I$ fraction contains DOTA-complexed metal ions; the EDTA fraction contains metal ions not complexed to DOTA and eluted by use of 0.1 M $Na_2EDTA$.

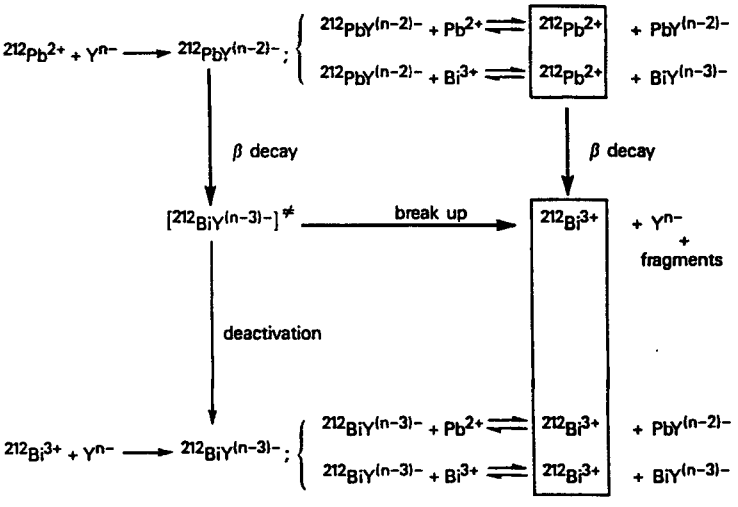


Figure 2. Possible reaction pathways leading to uncomplexed $^{212}Bi$ in an aqueous solution at pH 5-6 initially containing only $Na_2[Pb(DOTA)]$ doped with $^{212}Pb$.

**Paper I 16**

PREPARATION OF CHROMATOGRAPHICALLY AND ELECTROPHORETICALLY PURE TUMOUR-AFFINE GALLIUM-67 SPECIES IN AQUEOUS SOLUTION FOR TUMOUR DETECTION

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An ideal radiopharmaceutical for tumour detection or therapy should be able to seek out cancer sites and bind strongly to the tumour tissue, leaving healthy organs free of radioactivity. For the preparation of such radiopharmaceuticals, radionuclides in the oxidation state 3+ were found (1) to be most suitable because they easily produce radionuclide species which readily undergo biochemical reactions in vivo. Radionuclides of IIIA group elements and of lanthanides have proved useful for diagnostic and therapeutic nuclear medicine. High charge density, 4.84, Ga-67 and Ga-68 have been used in tumour detection. Due to the lack gallium radionuclide for therapy, cold gallium complexes are under study for the systemic tumour therapy(2). For past several years we have been studying the tumour affinity of Ga-67 species present in commercial Ga-67 citrate solutions and trying to find conditions under which Ga-67 becomes tumour-affine. Great difficulty was met during the comparison and interpretation of results, because all laboratories of our group do not use Ga-67 citrate from the same supplier. Unfortunately, the concentration of Ga-67 complexing citrate ion varies greatly (from 1.75 to 50 mg/ml) in the solutions used for tumour detection (3), and hence also the ionic nature of Ga-67 species administered to the patient. We analysed, therefore, each Ga-67 citrate sample received before its administration in tumour-bearing subjects. The analyses were done chromatographically and electrophoretically. In all cases total-body radionuclide distribution was studied. Ga-67 preparations,low in sodium citrate concentration, contain cationic,mainly hydrolysed, and some anionic Ga-67 species. High sodium citrate concentration solutions contain mainly citratogallate-67, and small amount of hydrolysed Ga-67 species. Tumour affinity of chromatographically and electrophoretically pure Ga-67 was studied by radionuclide distribution studies in different tumour-bearing subjects injected with pure Ga-67 preparations. These studies permited us to classify tumour into cationic Ga-67 affine and anionic Ga-67 affine groups (4), which are at present as follows:

I. Cationic Ga-67-Affine Tumours: Lung tumour, Hodgkin's disease, neuroblastoma, thyroid tumour, lymphoma, myeloma, bone metastases from soft tissue primaries, Morris hepatoma-3924A, ....

II. Anionic Ga-67-Affine Tumours: Melanoma, mammary tumours, osteosarcoma, Ewing's sarcoma, fibrosarcoma, liposarcoma, metastases in soft tissues, ....

We are treating tumours with tumour-affine cold Ga and Y-90 preparations.

1. Shukla S.K. and Cipriani C. - Metal Ions in Biology and Medicine, John Libbey Eurotext, Paris, 1990, pp. 533-537.
2. Collery Ph., Morel M., Millart H., Desoize B., Cossart C., Perdu D., Vallerand H., Bouana J.C., Pechery C., Etienne J.C., Choisy H. and Dubois de Montrenaud J.M. - Metal Ions in Biology and Medicine, John Libbey Eurotext, Paris, 1990, pp. 437-442.
3. Shukla S.K., Blotta I., Cipriani C. and Manni G.B. - Progr. Radiopharmacol. 4, 271 (1985).
4. Shukla S.K., Cipriani C., Baziotis N., Hermes R. and Argirò G. - Book of Abstracts, 20th Ann. Meeting of the Australian and New Zealand Society of Nuclear Medicine, Christchurch School of Medicine, Aug. 21-23, 1989, p. 27.

# THE FEASIBILITY OF $^{45}$Ti-LABELLED CHEMOTHERAPEUTICS. A STUDY TO PRODUCE [$^{45}$Ti]$TiCl_4$ AND $^{45}$Ti-LABELLED BUDOTITANE.

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The proper nuclear characteristics of $^{45}$Ti suggest this radioisotop for positron emission tomography. It decays by at least 84% positron emission to $^{45}$Sc with an interestingly long half-life of about 3.1h. Its $E\beta^+_{max}$ is 1.02 MeV which is between $^{11}$C and $^{13}$N. The preparation of $^{45}$Ti via the $^{45}$Sc(p,n)$^{45}$Ti nuclear reaction has been investigated in the early times of PET [1,2] demonstrating high production rates in the range of 330 MBq/uAH to 650 MBq/μAh. This information led to the objective to trace the behaviour of titanium chemotherapeutic agents in-vivo during cancer treatment, by spiking a therapeutic dose of an antineoplastic bis-β-diketonato titanium complex **1** [3] with the radioactive derivative. For that purpose a procedure was required which delivered covalent $TiCl_4$ in almost quantitative yield from irradiated $Sc^0$ or $Sc_2O_3$. We indeed obtained this valuable precursor to budotitane **1** with a 92% yield and within 35 min. Its preparation and its utilization in the synthesis of antitumour-active titanium complexes according to figure 1 will be discussed.

It is obtained in a carrier added procedure from a $Sc/TiO_2$ matrix in presence of graphite as reducing agent in a surprisingly low stream of $Cl_2$ using a quartz distillation apparatus. Coproduced $ScCl_3$ precipitates as a white solid immediately at room temperature and is therefore easily separated from the product. Trapping of the $TiCl_4$ vapour in ethanolic sodium ethanolate delivers directly the tetraethanolato derivative for further reaction with the β-dicarbonyl compound.

[1] Merrill J.C., Lambrecht R.M. and Wolf A.P. Int. Appl. Radiat. Isot. 29: 115 (1978).

[2] Ishiwata K., Ido T., Monma M. Murakami M., Kameyama M., Fukuda H. and Matsuzawa T., J. Label. Compd. Radiopharm. 19: 1539 (1982).

[3] Keppler B.K. and Heim M.E., CIPS DRUGS OF THE FUTURE 13: 637 (1988).

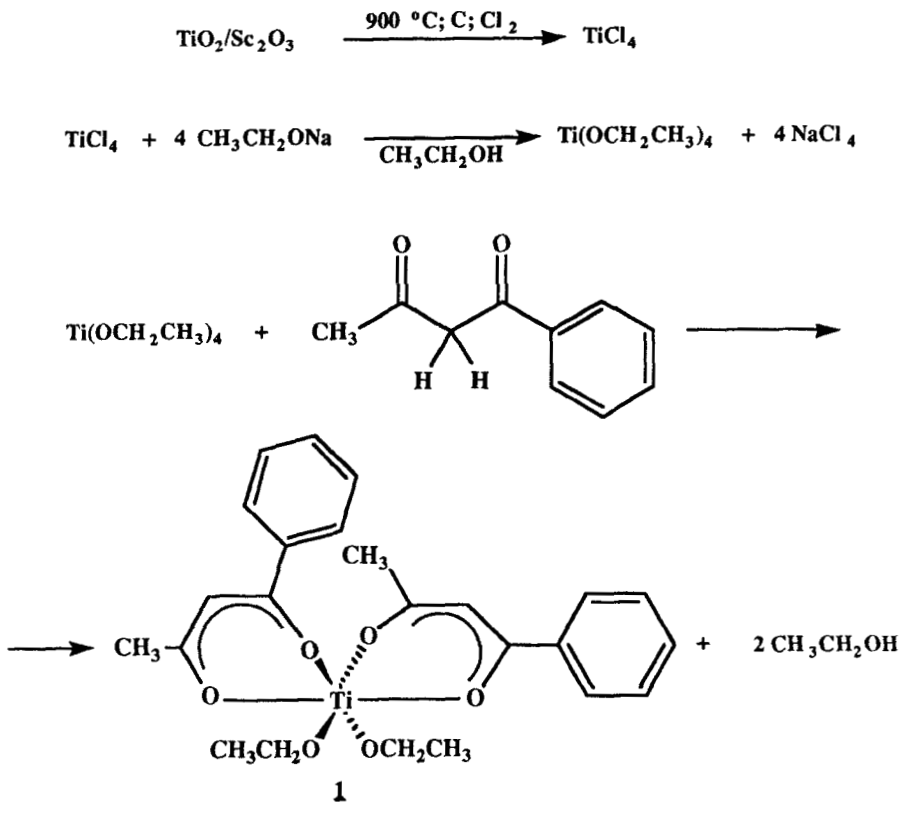


**Figure 1:** General procedure for the preparation of bis-β-diketonato titanium complexes.