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Chemistry of technetium radiopharmaceuticals 1: Chemistry behind the development of technetium-99m compounds to determine kidney function

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Abstract

The chemistry behind the development of technetium-99m (Tc-99m) complexes useful for determining renal (kidney) function is discussed. The design of these compounds so that they will be (1) stable in vivo, (2) actively taken up by the kidney and (3) excreted from the body without being metabolized is also discussed. Such issues as the purity of the product ($>90\%$ yield required), in vitro stability, and the requirement for ease of preparation are also mentioned. The development and chemistry of commercialized Tc-99m renal function radiopharmaceuticals are covered as are the interplay of the chemistry of Tc-99m and it macroscopic daughter isotope, Tc-99g. Basic technetium chemistry as well as renal physiology and function are also discussed briefly. An attempt is made to highlight areas where further work is needed.

Keywords: Technetium-99m; Renal physiology; Purity of product

1. Introduction

The major challenge for chemists designing compounds (radiopharmaceuticals) to be used in diagnostic nuclear medicine is creating agents that will (1) go to the target organ quickly, (2) go there as a high percentage of the injected radiation and (3) clear from the body quickly once the test is completed. For most applications in diagnostic nuclear medicine the use of the isotope Tc-99m is preferred. Tc-99m is a short-lived isotope, present in chemically microscopic amounts and has minimally damaging radioactive emission that is close to optimal for use with today's imaging instruments. Thus a great deal of the chemistry done in the design of diagnostic radiopharmaceuticals has been technetium chemistry.

One of the organs that can be studied effectively by nuclear medicine is the kidney, where it is important to determine kidney function as well as morphology. For this reason a great deal of time has been spent by scientists around the world in the design and synthesis of ligands and their respective Tc-99m complexes towards the goal of designing the perfect kidney function- and structure-identifying agents.

Methods for determining kidney viability in humans include determining clearance from the blood of (endogenous or exogenous) serum creatinine [1], inulin or *p*-aminohippuric acid [2]. All require the collection of urine or blood samples and the serum creatinine measurement is only accurate to within 10–15% of the actual renal clearance rate [3] and potentially less accurate in patients with significant renal impairment. A diagnostic agent that would allow collection of functional information about the kidney was needed. X-ray contrast agents (usually salts of 1,3,5-triiodobenzoic acid) have been shown to be cleared rapidly through the kidney and were possible choices when used in conjunction with computed tomography (CT) [4]. Unfortunately, X-ray contrast agents, which have to be given in large quantities to be effective, have been shown to sometimes have kidney toxicity, especially in patients with renal disease [5]. Recently, macroscopic amounts of anionic magnetic

resonsance (MR) contrast agents such as Gd-DTPA (Magnevist®) also have been shown to clear rapidly from the blood via the kidneys [6,7]. Still, a simple, rapid test of kidney function requiring only small amounts of the testing agent would be preferred. Radiopharmaceuticals (radioisotope-containing compounds) seemed to be the natural choice due to the small chemical quantity necessary to obtain information about kidney function and structure. Also, the ability of radiopharmaceuticals to easily measure function without perturbing the organ of interest has been a strength of nuclear medicine agents, setting them apart from X-ray and MR contrast agents, whose basic strength lies in giving structural information. The only drawbacks to the use of radiopharmaceuticals to measure kidney function were (1) the potentially high radiation dose to the patient if the radioisotope was not excreted quickly from the body and (2) the lack of rapid availability of most radioisotopes and, therefore, most radiopharmaceuticals except those containing Tc-99m.

A number of reviews have been written on the use of radiopharmaceuticals to measure kidney function [8–12]. The foremost of these with regard to insight into structure-activity relationships is that of Verbruggen and DeRoo [8]. Although compounds containing isotopes such as I-131 and I-123, Cr-51, and Cu-62 described in these reviews have been proposed or actually used for measuring renal function [12], most of the work has been done on designing Tc-99m-containing compounds for this purpose.

The chemistry behind the development of Tc-99m radiopharmaceuticals that were designed to measure renal function and structure is the topic of this review.

2. Technetium chemistry

2.1. Basic chemistry

Many reviews have been written on the basic chemistry of technetium [13–19] with the book by Steigman and Eckelman [13] being an excellent compilation of technetium chemistry as it applies specifically to nuclear medicine. Technetium exists commonly as two isotopes: Tc-99g and Tc-99m. The macroscopic Tc-99g, a byproduct from fusion reactors, is used to study the chemistry of technetium and verify the structures of radiopharmaceuticals derived from the other important isotope of technetium, Tc-99m. Tc-99m is produced by the decay of Mo-99 and decays to Tc-99g. It is available commercially via elution (with 0.9% saline) of alumina columns that have Mo-99 molybdate (MoO_4^{2-}) adsorbed to them. The normal concentration of total technetium (Tc-99g plus Tc-99m) in a typical elution is 10^{-7} to 10^{-9} M, making the standard methods of characterization used for macroscopic chemicals impractical for Tc-99m complexes.

The radioactive half-life of Tc-99m is 6.02 h and when it decays it releases a gamma ray of 142 KeV. This half-life is good for most nuclear medicine procedures and the gamma energy is ideal for most common nuclear medicine scanners. The radioactive half-life of Tc-99g is 212 000 years and it decays with the release of a

relatively low energy β particle. The ready availability of macroscopic amounts of Tc-99g, its low energy decay and its incredibly long half-life make this isotope a good one with which to do standard, inorganic chemistry. However, these same properties also make it difficult to work with in most academic, industrial and radiopharmacy settings. Due to its long half-life, radioactive contamination with Tc-99g in solution or in the solid form can create extremely challenging cleanup and disposal problems. This has made it very difficult to convince various radiation safety organizations at universities, companies and hospitals that the benefits of doing chemistry with Tc-99g outweigh the risks of contamination and the problems with disposing of long-lived radioactive waste. Thus, only a few investigators have ever worked with any large quantities of Tc-99g and only a small fraction of the Tc-99m complexes synthesized have ever been characterized using Tc-99g. Even fewer have been characterized by X-ray crystallography. Recently, the use of FAB mass spectrometry on HPLC-separated products containing Tc-99m spiked with micromolar Tc-99g has gained favor as a compromise to limit the potential contamination from Tc-99g and still offer molecular weight and some structural (through fragmentation patterns) information. However, in many cases stereochemistry and connectivity are not clear from these mass spectral data or even from their combination with HPLC retention times and electrophoresis data, two other common methods for characterizing Tc-99m complexes. Despite the efforts of a number of dedicated inorganic chemists with access to Tc-99g, much technetium radiopharmaceutical research is still being done as it was 30+ years ago, exclusively with Tc-99m. This severely limits the ability of chemists to modify ligand structures in any systematic way as they can only postulate the structures of the complexes they are synthesizing.

Many generalities exist in technetium chemistry [13,14] and, although many exceptions exist, this review will focus on the rules rather than those exceptions. In aqueous solution technetium can exist in any oxidation state from VII to I. The chemistry is relatively straightforward with the final oxidation state of the technetium being determined to a great extent by the ligand environment. Starting with ligands that use simple sigma donation from nitrogen, oxygen and sulfur leads almost exclusively to Tc(V) complexes. Standard chelate chemistry rules apply with respect to stability of these complexes. That is, stability goes in the order of tetradentate > tridentate > bidentate > monodentate ligands. Also, ligands containing thiol groups are preferred and are much more stable than ligands containing alcohols. Tc(V) complexes are almost exclusively square pyramidal when the overall charge on the complex is negative or neutral. These complexes contain an essentially square planar four donor atom arrangement and a Tc=O axial group. When the overall charge on the Tc(V) complex is positive, the geometry is usually octahedral with a *trans* O=Tc=O core and four equatorial, neutral ligands. Thus, neutral ligands such as amines produce cationic complexes and anionic ligands such as carboxylates, deprotonated amides or thiols produce complexes that are anionic. Inclusion of mixed ligand systems involving sigma donors and pi acceptors such as phosphines, isonitriles, carbonyls, imines, aromatics and other conjugated systems lead to Tc(I), Tc(II), Tc(III) or Tc(IV) complexes. The need for chelation decreases

with decreasing oxidation state due primarily to the incorporation of the more tightly held pi backbonding ligands and many Tc(I) complexes are made up completely or partly of monodentate ligands. Many Tc(III), (II) and (I) complexes tend to be octahedral although some reports of 7-coordinate species do exist [20]. The complexes tend, by the nature of the low oxidation state of the metal, to be inherently either neutral or cationic and are almost never anionic, unless the ligands have pendant groups that provide negative charge. Ligand systems involving exclusively pi back-bonding ligands usually lead to complexes of Tc(I). Thus, in many cases, one can almost dictate the oxidation state and coordination geometry of the Tc simply by appropriate ligand design.

The synthesis of Tc-99m complexes usually involves three components: pertechnetate (TcO_4^-), a reducing agent (R) and a ligand (L). Examples of common reducing agents include metal species such as Sn(II), Fe(II), Cu(I) as well as non-metallic reducing agents such as dithionite, borohydride, formamidine sulfinic acid and hydrazine. All of these are 1- or 2-electron reductants [13]. In some cases the ligand can also act as the reductant. This is true especially when phosphines are used either alone [21,22] or in conjunction with other ligands [23,24]. In many cases the reduction can be characterized as an oxygen abstraction reaction and this is especially true when a phosphine or Sn(II) is used as the reducing agent.

In the formation of a Tc-99m complex, pertechnetate is probably first reduced to a metastable Tc species which is then captured by the ligand (Eq. (1)). If the ligand does not form a strong enough complex with Tc or if the complexation reaction is slow compared to the rate of reduction, there is a chance that the metastable, reduced form of Tc will degrade into the thermodynamically stable and relatively unreactive TcO_2 (reduced, hydrolyzed technetium) or possibly $(Tc,R)O_2$, if the reducing agent is a metal such as Sn(II). This TcO_2 formation probably proceeds through a disproportionation reaction of the reduced intermediate to TcO_2 (Tc(IV)) and TcO_4^- (Tc(VII)).

If the ligand of interest does not react fast enough with the reduced, metastable Tc species, it is sometimes possible to form an intermediate complex with a weak ligand (L′). That ligand is then displaced in a standard ligand replacement reaction by the ligand of choice (L) (Eq. (2)). The ligand L′ is generally referred to as a transfer ligand. Examples of transfer ligands include carbohydrates such as glucoheptonate and tartrate and simple oxygen donors such as ethylene glycol [13].

In a number of cases the reduction probably proceeds in steps (Eq. (3)). Since most of the reductants can only reduce the Tc by one or two oxidation states at a time, this latter case usually applies when the Tc is being reduced to oxidation states of lower than Tc(V).

$$TcO_4^- + R \rightarrow [Tc]_{reduced} \xrightarrow{TcO_2} TcL \tag{1}$$

$$TcO_4^- + R + L' \rightarrow TcL' + L \rightarrow TcL \tag{2}$$

$$TcO_4^- + R + nL \rightarrow [TcL_n]^m + R \rightarrow [TcL_n]^{m-p} \tag{3}$$

2.2. Radiopharmaceuticals and proposed radiopharmaceuticals with Tc-99m

Examples of Tc-99m radiopharmaceuticals and their medical uses are given in Table 1 and their structures shown in Fig. 1a–g.

Aside from the products listed in Table 1 and Fig. 1, there are numerous other Tc-99m complexes that have shown promise in animal models but have not yet been developed fully into useable products in humans. A number of these are listed in Table 2 and their structures are shown in Fig. 2a–h.

3. Basic renal physiology and function

3.1. Renal physiology

Many basic reviews and textbooks exist on the anatomy and function of the kidney [8–12,36–38], especially in relation to contrast imaging agents [8–12]. The current review will briefly cover the anatomy of the kidney with emphasis on how the function of the kidney is related to its structure.

The kidney contains three major anatomical units: the cortex, the medulla and the excretory channels (Fig. 3). The medulla, located toward the center of the kidney, contains 6–10 triangular pyramidal structures. The cortex, which originates at the outside of the kidney, surrounds these pyramids and has two different zones: (a) the pyramids of Ferrein which lie between the medullar pyramids and the kidney surface and (b) a granular tissue which fills in the rest of the area around the two types of pyramidal structures. Also contained within the kidney but not usually considered part of the above tissues are the excretory channels of the kidney leading eventually to the ureter and the bladder. The main structural and functional unit of

Table 1
Tc-99m radiopharmaceuticals of various, common oxidation states

Oxidation State	Structure	Name	Use	Reference
VII	TcO_4^-	Sodium pertechnetate	Check for blood-brain barrier breach	–
VI	None	–	–	
V	Fig. 1a	Technescan-MAG3®	Check for kidney function	[25]
	Fig. 1b	Myoview®	Check for heart muscle viability	[23]
	Fig. 1c	Neurolite®	Brain perfusion imaging	[26]
	Fig. 1d	Ceretec®	Brain perfusion imaging	[27]
IV	None	–	–	
III	Fig. 1e	Cardiotec®	Check for heart muscle viability	[20]
	Fig. 1f	Technescan Q12®	Check for heart muscle viability	[24]
II	None	–	–	
I	Fig. 1g	Cardiolite®	Check for heart muscle viability	[28]

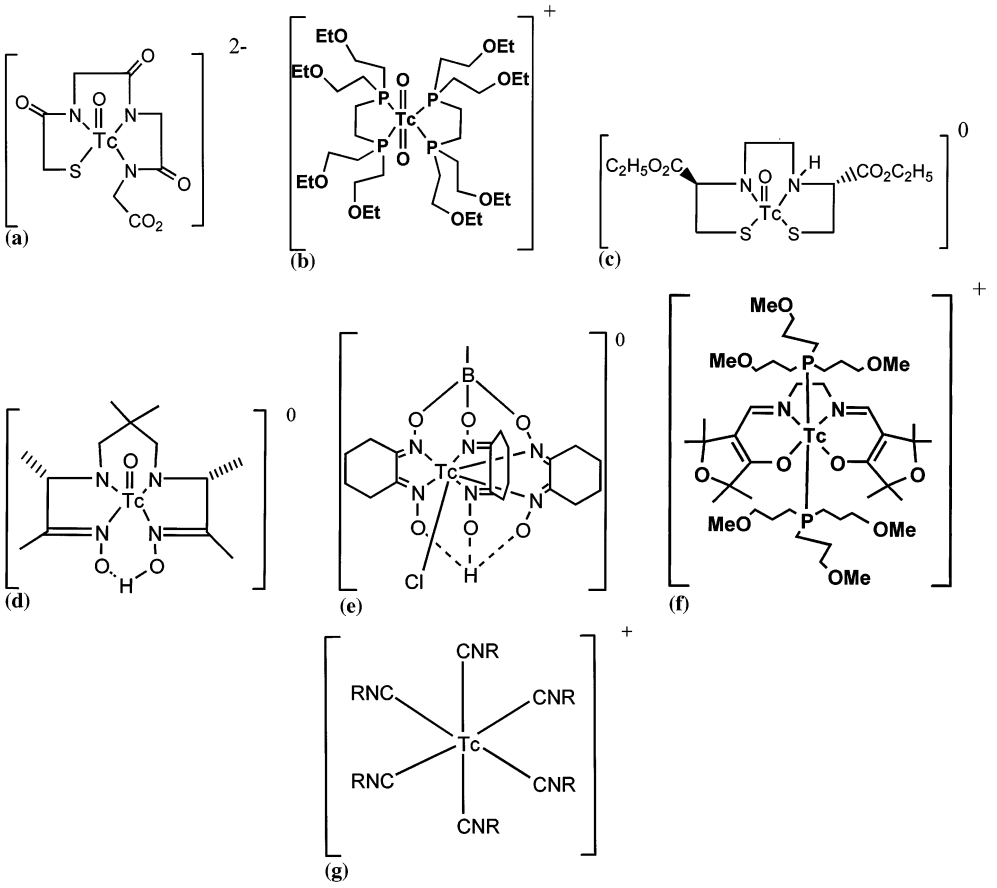

Fig. 1. Structures of some common, marketed radiopharmaceuticals.

the kidney is the nephron. There is a dispute about the number of nephrons in an average adult human kidney but a good estimate appears to be 1.3–1.5 million. The nephron consists of six major, connected segments (tubules) that originate in the cortex with the glomerulus and end with the collecting tubule near the apex of the medulla. The apex of the medulla is called the papilla and is capped by the calyces which begin the excretory channels that eventually lead to the bladder.

3.2. Renal function

The kidneys of a resting adult receive over 1 l min^{-1} of arterial blood, about 25% of the cardiac output. Blood flow goes to and from both the medulla and the cortex. About 20% of the blood is filtered by the glomeruli via a cluster of highly permeable capillaries. This filtrate from the blood is extremely low in lipids and

proteins of molecular weight (MW) greater than 70 000 and contains small (< 5000 MW), hydrophilic molecules (e.g. creatinine, amino acids) and salts (e.g. Na^+, urea, water, K^+, H^+, Mg^{2+}, Ca^{2+}, HPO_4^{2-}, Cl^-, HCO_3^-, SO_4^{2-}, uric acid). This also implies that small molecules that are relatively tightly bound (by non-covalent interactions) to high molecular weight plasma proteins will not be filtered by the glomerulus. As this filtrate passes along the tubular segments of nephron about 75% of the solutes are reabsorbed through the tubular capillaries into the plasma almost immediately. The total surface area of the tubular capillaries is about 15 times that of the glomerular capillaries, and about 80% of the renal blood flow is exposed through these capillaries, thus providing a 4-fold greater opportunity for clearance of compounds from the blood via these capillaries than via the capillaries in the glomeruli. As the filtrate moves through these tubules some substances are added to the filtrate (removed from the blood) and some substances are reabsorbed into the general circulation via the capillaries that service those tubules. These processes can occur either by active (receptor-mediated) or passive (diffusive) transport. Compounds that are highly protein bound must be actively transported from these capillaries to the filtrate. Eventually, the filtrate leaving the kidney tissues and entering the excretory channels has been reduced from about 120 to 1–2 l of urine.

The kidney's action as an excretory organ, that is, the ability to remove excess quantities of small hydrophilic molecules and ions from the blood, is the process that most contrast agents measure. Three basic measurements are done by these types of agents: glomerular filtration rate (GFR, the rate of uptake of compounds by the glomeruli), effective renal plasma flow (ERPF, a measure of active transport of compounds) and morphology (or mass). According to what was said above, radioactive compounds that measure GFR must be small and hydrophilic and must have clearance properties close to the gold standard, inulin. That is, these compounds must not have much affinity for plasma proteins and must not be reabsorbed into the blood, metabolized or be toxic to the kidney. A radioactive agent that measures ERPF should be rapidly taken up (secreted) by the tubules either by passive or active transport (preferably by active transport). Preferred compounds are ideally small, hydrophilic, non-metabolizeable and non-toxic although they can and possibly should have affinity for blood proteins. They should

Table 2
Tc-99m complexes tested as radiopharmaceuticals

Class or compound	Oxidation state	Structure	Use	Reference
Tc phosphites	I	Fig. 2a	Heart perfusion agent	[29]
Tc arenes	I	Fig. 2b	Heart perfusion agent	[30]
Tc sal_3TAMEs	IV	Fig. 2c	Heart perfusion agent	[31]
Tc phospines	I,III	Fig. 2d,e	Heart perfusion agents	[32]
Tc EC	V	Fig. 2f	Kidney function agent	[33]
Tc-tretrapeptides	V	Fig. 2g	Kidney function agents	[34]
Tc≡N compounds	III,IV,V,VI	Fig. 2h	Various	[35]

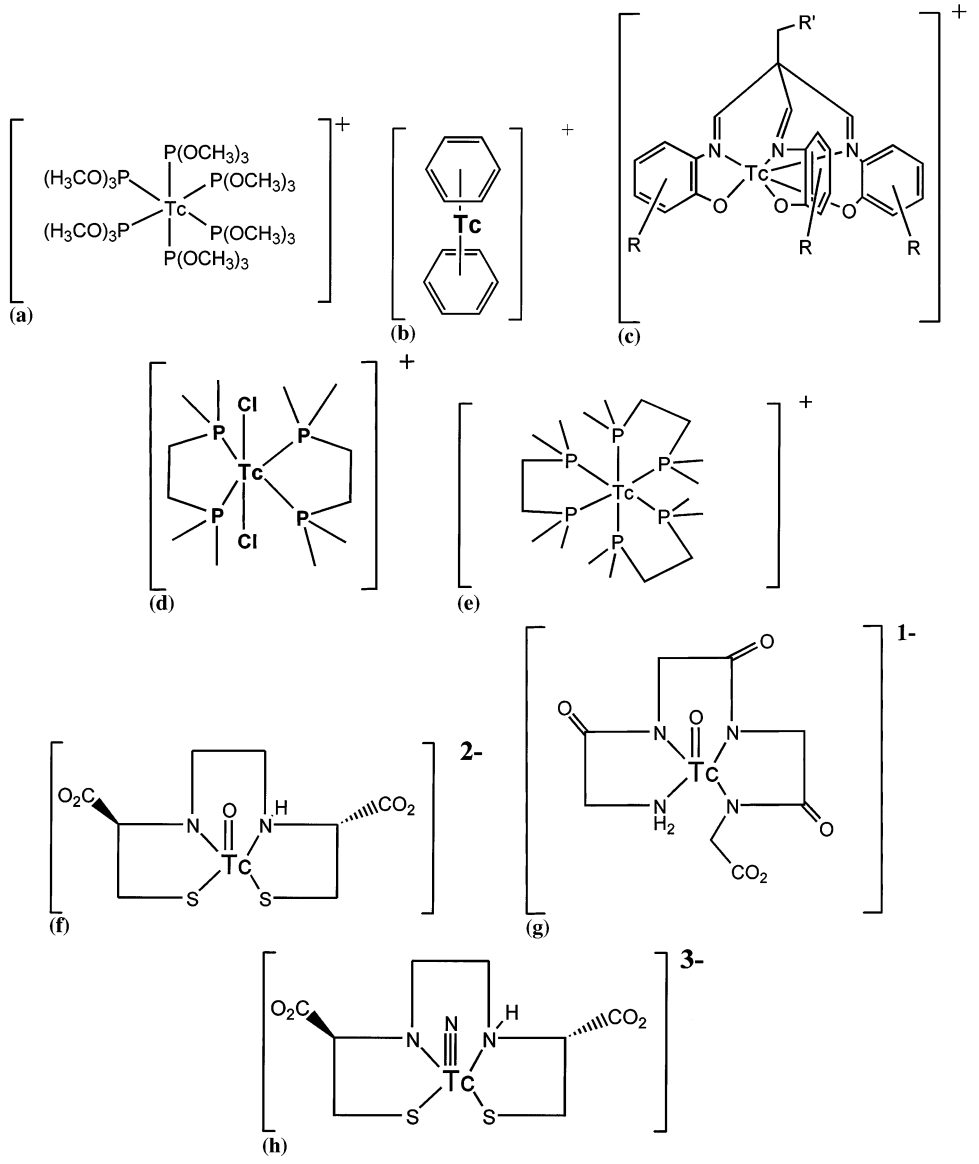

Fig. 2. Structures of Tc complexes tested as radiopharmaceuticals.

have clearance properties close to the gold standards for these measurements, *p*-aminohippuric acid (PAH) [2] or radiolabeled *o*-iodohippuran (OIH) [9]. Radioactive agents that measure renal mass must be able to become fixed to renal tubules by one of two mechanisms: (1) by having a high affinity for tubular wall proteins or (2) by being relatively weak metal complexes where the metal can be transmetallated into renal tubule proteins.

At this point it should be noted that many compounds are cleared from the blood by both glomerular filtration and tubular secretion. I-131 OIH, for example, is cleared by both mechanisms with 20% of the clearance by glomerular filtration and 80% by active tubular secretion, numbers not surprising given the ratio of blood flow described above. Some compounds like Tc-99m DTPA [39] are only glomerularly filtered and may take longer to clear, leading to high background radioactivity in the patient and in the resulting nuclear medicine images. Some compounds like Tc-99m MAG_3 [25] are only cleared by active transport through the tubules and so only have about 80% of the clearance rate of OIH.

With this foundation we can now talk about the chemistry that has led to the development of agents to meet the needs of medical doctors who want information about kidney function.

4. Chemistry in the development of Tc-99m-based kidney imaging agents

4.1. General criteria

In order for a Tc-99m compound to be effective as an agent to measure renal function it must meet certain criteria:

1. It must be hydrophilic and clear rapidly from the blood stream almost exclusively (> 98%) through the kidneys.
2. It must be stable in vivo for its entire biological lifetime.
3. It must be stable in vitro for at least 6–8 h.

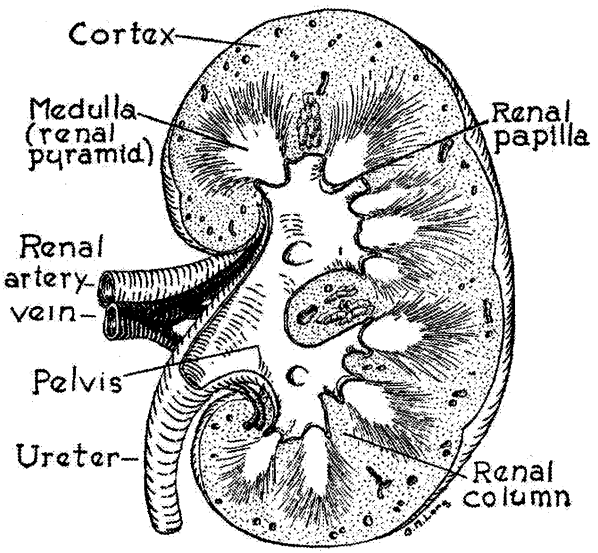

Fig. 3. Basic kidney structure from a cross sectional view (Reprinted by Permission of ArtToday®, copyright www.arttoday.com).

4. It must be able to be prepared in less than 15 min with a purity of $>90\%$.
5. If it must be prepared at the site of use, the non-radioactive components should be lyophilizable and preparation should be accomplished by simply adding the technetium 99m-containing solution to the lyophilized material with or without subsequent heating of the mixture.

All of the above criteria must be met using a solution (eluted from a Mo/Tc generator) where the concentration of technetium is as low as 10^{-9} M (1/100 the concentration of hydroxide ion in pH 7 water). Such stringent criteria have made the development of such an agent very challenging.

The properties described above can be broken into two types: those that can be controlled by the chemist (hydrophilicity, ease and cheapness of synthesis and in vitro stability of the complex) and those that cannot be controlled completely by the chemist (in vivo stability of the complex, rapid extraction from the blood by the kidneys and clearance from the body). As in the development of all radiopharmaceuticals before the advent of the more recent combinatorial chemistry methods, the chemist could only hypothesize what structures would be effective in performing the required tasks, synthesize those structures and then, using those initial results, modify the structure to optimize the desired behavior of the compound.

4.2. Renal imaging agents of Tc-99m

The history of the development of Tc-99m-based kidney imaging agents follows the pattern described above. The early non-radioactive (X-ray) contrast media and radioactive (radioiodinated X-ray contrast agents, Cr-51 EDTA, I-131 iodohippuran) contrast media used for kidney imaging were based on hydrophilic, anionic small molecules [11]. Thus, the early development of Tc-99m-based kidney imaging agents was based on generation of these types of molecules. A partial list of the Tc-99m complexes that have been proposed as renal imaging agents is shown in Table 3. Most of the ligands used in these complexes were designed by either trial and error over a number of classes of compounds or by analogy to compounds that had shown previously renal uptake and clearance. These complexes can be broken down into three classes: (a) anionic; (b) anionic with non-coordinated carboxylate(s) and (c) cationic. The remainder of this review deals with compounds that fall into these three classes.

4.2.1. Simple anionic technetium complexes

The first Tc-99m-based kidney imaging agent was Tc-99m sodium pertechnetate, Na^+ TcO_4^-. This compound, the precursor to all Tc-99m radiopharmaceuticals, was cleared to too great an extent through the liver and so was abandoned. Soon thereafter, other anionic Tc complexes using DTPA (diethylenetriaminepentaacetic acid) and DMSA (dimercaptosuccinic acid) as ligands were synthesized [2,8–12]. Products derived from these two compounds are still used in many hospitals with the former being used to determine kidney function by measuring GFR and the latter to delineate kidney structure [9]. The chemical structure of the DMSA agent has not been elucidated completely. Although some Tc-99g work has been done

Table 3
Selected ligands complexed to Tc-99m where the resulting complex has been proposed as either static (A) or dynamic (GFR (B) or active tubular secretion (C)) renal imaging agent[a]

Ligand	Type	Reference	Ligand	Type	Reference
DTPA	B*	[12,39]	CO_2–DADS and derivatives	A	[65,66]
2,3-Dimercaptosuccinic acid (DMSA)	A*	[12,40]	Cyclam derivatives	C*	[67]
Gluconic acid, sodium salt	A*	[12,41,78]	Diaminocyclohexane (DACH)	C*	[68]
Glucoheptonic acid, sodium salt	A*	[12,42,78]	2,3-Diaminopropionic acid	C	[69]
Fe+DTPA	A	[12]	Tc,Cu citrate	C	[70]
Fe+ascorbate	A	[12]	Pyrrolidino-methyl tetracycline	A	[71]
Mercaptoacetylglycylglycylglycine (MAG_3)	C*	[25]	*N*,*N*′-[2-hydroxybuyl]ethylene-diamine (ethambutol)	A	[72]
MAG_3-related compounds	C*	[8,43–50]	8-Keto-7-aza-2-amino-4,10-di-thia-decanoic acid (CO_2–DAMTE)	B,C	[73]
N,*N*′-bis(mercaptoacetyl)ethylene-diamine (DADS) and derivatives	C*	[51–53]	3-Hydroxy-2-methyl-4-pyridinones	C	[74]
Ethylenedicysteine (EC) and derivatives	C*	[33,54–56]	1,2-Dihydroxypropyl-1-phosphonate	A*	[75]
Cysteine	A*	[57]	Thiodyglycollic acid	A	[76]
o-, *m*- And *p*-aminohippuric acids	C	[58]	Gentamycin	A	[77]
Cystine	A	[59]	Various hydroxycarboxylic acids	A	[78]
Dimercaptoproprionic acid	A	[60]	Various di- and tetra amines	A*	[79]
Penicillamine	A*	[61]	Metallothioninein	A	[80]
Thiomalic acid	A	[62]	Carboxymethylisocyanide	C	[81]
Malate	A?	[63]	Carboxycysteamines	C?	[82]
Cysteine acetazolamide	?	[63]	Dimethylglyoxime	C*	[83,84]
Salicylurate	?	[63]			
N-(mercaptoacetyl)glycine	A	[64]			

[a] Note: Ligands that have also complexed to Tc-99g and characterized, are noted by an asterisk.

[40b] which seems to favor a standard square pyramidal Tc(V) structure, it is not clear that the radiopharmaceutical actually contains just this compound. Work has shown that by varying the Sn(II) content and pH of the preparation, the biodistribution (and, by inference, the product distribution) was changed [8,10]. Different products are also formed when *meso*- or D,L-DMSA are used [40a]. These findings, combined with the huge excess of ligand compared to Tc and the penchant for thiolato groups to form bridging, multimeric metal complexes might indicate that polymeric species containing Tc, DMSA and the reducing agent Sn(II) may be formed, similar to what happens when diphosphonates are used as ligands for Tc in the presence of stannous ion [14,85]. The Tc-99g versus Tc-99m results for DMSA point out that it is not just enough to see what product is formed with Tc-99g. That product must also be compared directly with the Tc-99m product to insure products formed under such different conditions of ligand/metal concentration ratio are, indeed, the same compound. This is usually done by co-injection on an HPLC system and comparison of the UV (Tc-99g) and radiometric (Tc-99m) retention times.

The chemical structure of Tc–DTPA has also not been determined. However, literature evidence from two Tc–EDTA complexes, one of which is an oxo bridged dimer of Tc(III) or Tc(IV) [86] and the other is a Tc(V) species with pair of Tc(O)EDTA ions bridging a barium ion [13,14], gives an indication that the Tc-99m DTPA may be in the III, IV or V oxidation state and that the product may, like the DMSA complexes, be reaction condition-specific. This is indeed found in Tc-99m DTPA preparations where different products seem to be produced under different sets of reaction conditions [2,8,39].

More recent work involved the synthesis of Tc-99m derivatives of hippuran [58]. The intent was to design a complex that was similar to hippuran but with a radioactive isotope (Tc-99m) attached. It was concluded that the requirements for attachment of technetium had so changed the hippuran moiety that it no longer was recognized by the active transport mechanism [8]. This effect had been seen previously in the development of Tc-99m-iminodiacetic acid hepatobiliary (liver) imaging agent (TcHIDA). This lidocaine derivative was intended, when coordinated to technetium, to be a myocardial imaging agent. Instead, coordination changed the conformation and look of the molecule so drastically that the Tc complex had no heart uptake but a rapid clearance from the liver into the gall bladder and the bile [11].

As was the case with the discovery of many of the early Tc-based radiopharmaceuticals, the methods of synthesis of the complexes listed above were developed before their utility was established. Also, as mentioned above, the structures of the compounds were never fully understood and thus the chemistry which led to the agent of interest was also a mystery. With no systematic chemical approaches employed to design the optimal agent, agents were developed only sporadically and improvements over early versions of agents were only accomplished by trial and error based on assumptions of structure that were often incorrect.

In the 1970s and early 1980s two inorganic chemists, Edward Deutsch and Alan Davison, began to apply systematic inorganic approaches to development of

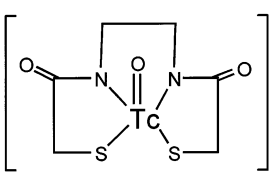

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Fig. 4. Structure of [Tc (*N*,*N*′-bis(mercaptoacetamido)ethylenediamine)], (TcDADS) complex.

Tc-based radiopharmaceuticals. The desired properties of the agent (e.g. charge, size, lipophilicity/hydrophilicity) were first determined and then, using what was known about Tc chemistry, appropriate ligands were designed to produce an agent with the given properties. The chemical structure of the Tc-99m products were then verified using Tc-99g which, in turn, allowed more efficient and meaningful structure–activity studies to be conducted. This approach was employed in the development of renal imaging agents by Davison and his group and, later, by Alan Fritzberg and co-workers.

The initial work with DTPA and DMSA showed that ligands that produced small, anionic, hydrophilic complexes also gave complexes that cleared predominantly through the kidneys. Davison's group first looked at classes of ligands that would (1) form stable, discreet complexes and (2) form small anionic complexes. They built on their previous work involving a number of different types of ligands [87–90] to discover a new class of complexes. The so-called Tc–DADS (diamidodisulfhydryl) complexes (Fig. 4) were reported by Davison and his group in 1982 [51–53]. Davison applied the principals of inorganic chemistry to generate a tetradentate, potentially tetraanionic ligand which would occupy the four coordination sites in the plane of the square pyramidal geometry that characterizes anionic, Tc(V)=O complexes of such ligands. Although no crystal structure has been reported for this compound it has been characterized by NMR, mass spectral and UV/vis data [51]. This ligand has some of the properties of the DMSA ligand including two mercapto groups to aid in forming a stable complex in good yield. As mentioned previously mercapto groups bind very tightly to Tc(V). To avoid the problems that probably plague the Tc–DMSA system (i.e. multiple and potentially polymeric products) Fritzberg chose to cap the thiols with easily removed benzoyl protecting groups [52,90]. This lessened the possibility of undesired products being formed such as $[Tc_2O_2(DADS)_4]^{2-}$, where the DADS ligands coordinate solely through their sulfur atoms [91] (Fig. 5).

In incorporating these protecting groups the rate of deprotection/chelation with the DADS ligand was now probably slower than the rate of reduction of the Tc. Thus the investigators incorporated a transfer ligand (gluconate) into the reaction. When the Tc–DADS complex was injected into animals it was found to be excreted almost exclusively by the kidneys. Fritzberg and his team then further evaluated the compound in animals and humans [52] and found that, unlike Tc–DTPA, which was cleared by glomerular filtration, the Tc–DADS compounds were cleared by active transport by renal tubular cells, similar to what is seen with radioiodinated

OIH, but without the tubular retention that Tc–DMSA undergoes [52]. Although the Tc–DADS complexes were not as effective as I-131 OIH [51,52] it was clear that these researchers were headed in the correct direction and were able to measure something not previously measurable with a Tc-99m radiopharmaceutical, active renal transport. However, when Davison [51,90] and Fritzberg [52] explored other compounds in this class they found that no compound in this class performed better than the original DADS ligand.

4.2.2. Anionic complexes with non-coordinating carboxylates

In the 1960s Despopoulos [92] proposed, based on studies of renal clearance of organic anions that for hippurate-like substances $RC(O)NX(CH_2)_nCOO^-$ ($X = H$ or CH_3; $n = 1$ to 5), that three structural factors were needed for active transport of compounds of this type. He postulated that the two oxygens of a carboxylate anion and an oxygen from an adjacent amide were necessary for this type of receptor-mediated transport. In an attempt to modify the DADS ligand, Fritzberg attached a non-coordinating carboxylate group to the carbon backbone to DADS to generate CO_2–DADS [52, 65a] generating a compound more similar to those Despopoulos had studied. These complexes, in which the Tc=O group replaces the amide carbonyl in the Despopoulos model, proved to be an improvement over the original DADS complex. The Tc-99m complex of CO_2–DADS gave the best results seen to that point for rapid renal clearance and excretion into the bladder [65a]. Unfortunately, attaching the carboxylate produces a chiral carbon which, combined with the Tc(V)=O core, results in a pair of diastereomers (referred to as A and B) (Fig. 6) the structures of which have been confirmed by X-ray crystallography [65c].

These isomers represent the *syn* and *anti* configurations, respectively, of the carboxylate with respect to the Tc=O group. The *syn* isomer showed renal clearance rates very similar to I-131 OIH. This is in line with Despopoulos' prediction if the Tc=O group replaces the amide carbonyl. Unfortunately, attempts to control the synthesis of the complex to virtually exclude isomer B were unsuccessful with the

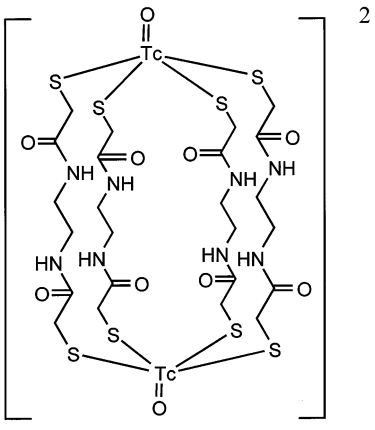
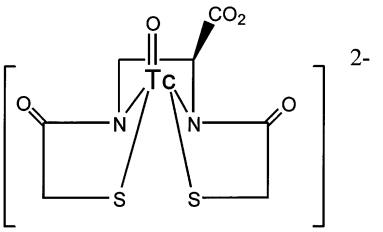


Fig. 5. Structure of $[Tc_2O_2(DADS)_4]^{2-}$ from [91].



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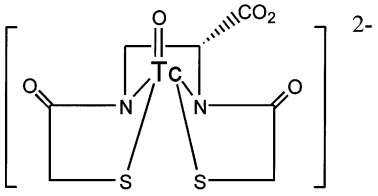


B

Fig. 6. *Syn* and *anti* configurations of Tc(CO_2DADS) complexes.

best ratio obtained being 80% A:20% B. Given the need for simplicity of radiopharmaceutical preparations, any pre-injection purification would have made the agent cumbersome and time-consuming to prepare. Also, the possibility of prep-to-prep variability in the yield of diastereomers could have called into question the results obtained when using such an agent. Thus, this class of compounds was never evaluated completely. Nevertheless, the use of an essentially -4 ligand that also contained a non-coordinating carboxylate as a chelating agent for the Tc=O core was established as a viable route for generation of a Tc-99m kidney function agent. The work that has been done with derivatives of the orginal CO_2–DADS did show that the structural requirements for effective renal clearance of CO_2–DADS-type ligands may be more stringent than that described by Despopoulos. The derivative where the carboxylate is moved to the carbon adjacent to one of the mercapto groups (proposed structure, Fig. 7) did not show the same renal clearance rate as Tc-CO_2-DADS isomer A [55a]. The Tc-99g complex was not synthesized, so the exact nature of the coordination of the ligand to technetium has not been

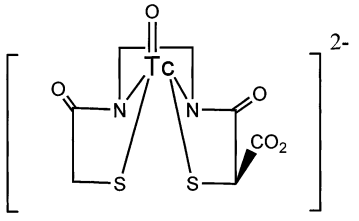


Fig. 7. Configuration of one diastereomer of a derivative of Tc–CO_2DADS.

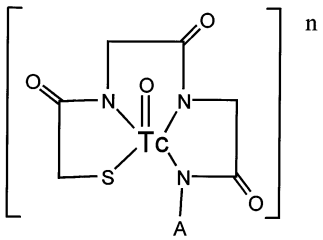


Fig. 8. Generalized structure for mercaptoacetylglycylglycylpeptide structures.

confirmed. Thus the comparison of this complex to that obtained with Tc-99m CO_2-DADS is based solely on the assumption that the structure of the complexes are the same.

Verbruggen et al. did further work to separate enantiomers of both the D- and L-isomers of Tc–CO_2–DADS [65b]. No Tc-99g work was done but biodistributions of the HPLC-purified products seemed to support the earlier work by Fritzberg's group.

Fritzberg's group built on the CO_2-DADS work by designing and synthesizing a number of mercaptoacetyl-containing tripeptides [43]. They showed that, by using the mercapto group of mercapto acetate as one of the terminal coordinating groups in their tetradentate modified tripeptide ligand and modifying the terminal peptide on the other end of the molecule (Group A, Fig. 8), they could produce stable Tc complexes all of which had a free carboxylate but did not have the chirality problems seen with CO_2–DADS. As they varied the peptide A, the rate of renal clearance was glycine > alanine > glycylglycine > phenylalanine. This indicated that the more lipophilic the terminal peptide was, the less rapidly and efficiently was the compound cleared from the kidney. They also showed that clearance went glycine > asparagine > glutamine > aspartic acid > glutamic acid indicating that incorporation of either amides or additional carboxylates did not significantly enhance renal clearance. The best agent in this class, $TcMAG_3$ (commercialized as TechnescanMAG3®), was shown to be very effective at measuring kidney function using imaging or even simple blood draws [25]. This agent is currently the gold standard for radiopharmaceutical kidney function studies.

Most of the above ligands reported by Fritzberg [47] contain benzoyl-protected thiols. The low product yields reported for these compounds (55–95%) are potentially due to the lack of inclusion of a transfer ligand. The yields may actually be increased somewhat by the use of dithionite instead of Sn(II) as the reductant as Sn(II) probably better mediates the formation of TcO_2. It has also been found that using free thiol MAG_3 produces good yields of $TcMAG_3$ [93], even with extremely small quantities of ligand [94]. However, the potential for production of impurities increases [95]. Also, if ligand synthesis or reaction conditions are not optimized, other impurities can result [96]. One of these impurities has been proposed to contain more than one MAG_3 unit per Tc with the multiple MAG_3 units being bound through their thiol atoms (Fig. 9) [102]. This compound, not exactly the

same but similar to that found in the Tc-99g synthesis of the DADS complex (Fig. 5 [91]) would be a −5 anion in vivo if the proposed structure is correct. This impurity has been shown to be more hydrophilic by reverse phase HPLC [8,50,95] than the −2-charged $TcMAG_3$ ion. There is considerable evidence that some of these impurities may revert back to the thermodynamically more stable $TcMAG_3$ complex (Fig. 1a), showing the power of the chelate effect even in the presence of potentially four strong Tc–S bonds.

The MAG_3 (mercaptoacetylglycylglycylglycine) ligand contains one terminal thiol coordinating atom and three amido coordinating atoms (Fig. 1a). The crystal structure data of Tc-99g complex of MAG_3 (crystallized with the carboxylate protonated) and other evidence indicates that all of the donor atoms (two amide nitrogens and the thiol sulfur) are deprotonated when complexed to the Tc [97]. The non-coordinating carboxylate group is provided by the terminal glycine and isomers can exist in different conformations with respect to the Tc=O group (Fig. 10), similar to what was seen with the $Tc–CO_2DADS$ complex.

Rao et al. reported the crystal structure of the Re(V) complex of MAG_3 (with the carboxylate protonated). The structure shows the anti conformation [98] (Fig. 10b) and the Tc crystal structure shows the *syn* conformation of the protonated free carboxylate (Fig. 10a). Marzilli et al. [99] proposed from molecular mechanics calculations that there was very little difference in energy in the gas phase between these two isomers for the Tc complex and that the preferred crystal structure conformation for the Tc complex was distorted by intermolecular hydrogen bonding caused by the molecule being crystallized with the carboxylate protonated. Nosco et al. were able to 'freeze out' interesting conformations by replacing the terminal carboxylate hydroxy group of MAG_3 with a bulkier methoxy group of the mercaptoacetyl-glyclglycyl methyl glycinate ligand (MAG_3OMe) [97]. The resulting polymorphs (1 and 2), which differ by whether the methoxy group is either parallel or perpendicular to the Tc=O bond, have been characterized crystallographically. In $TcMAG_3OMe$ polymorph 1, where no hydrogen bonding of the type Marzilli proposed is possible, a similar conformation to that seen in $TcMAG_3$ has been adopted as defined by the torsion angles N10–C1–C12–O13. Thus, while the calculations presented in the Marzilli paper may indeed be accurate in assuming

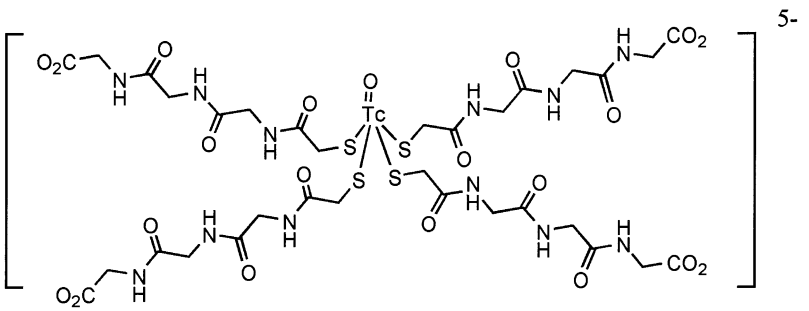
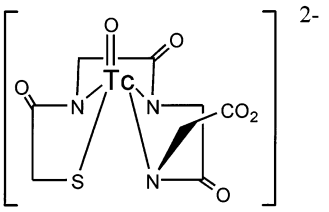
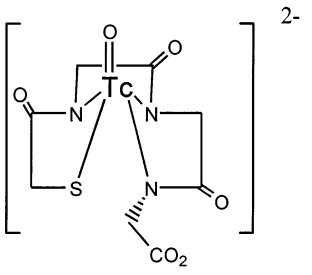


Fig. 9. Proposed structure for impurity from free thiol $TcMAG_3$ preparation.



A



B

Fig. 10. Two possible conformations of carboxyl group relative to Tc=O bond in $TcMAG_3$.

that there is very little energy difference between the $TcMAG_3$ *syn* and *anti* conformations, it is much more likely that the conformation seen in the crystal structure is due to a great extent to crystal packing forces. As a lesson that has been learned by a number of chemists, however, it is important to remember that conformations seen in the crystal phase must be used only semi-quantitatively since different conformations can be favored in solution. This is especially true in this case where the complex is crystallized in a different protonated state than what is seen in solution.

Verbruggen et al. reported separation of what were proposed to be the enantiomers of the Tc-99m MAG_3 complex [100]. However, these researchers were not able to see any difference in the biodistributions of the compounds they separated, possibly due to the ability of the pendant carboxylate to orient itself parallel to the Tc=O bond either by rearranging to the *syn* conformation or by bond rotation in the *anti* form allowing the carboxylate to point roughly parallel to the Tc=O.

As would be expected by it's lipophilicity as well as by the lack of a free carboxylate, the anionic $TcMAG_3OMe$ complex, which can be produced as a by-product of making Tc-99m MAG_3 in methanol or directly from the MAG_3OMe ligand [50,97], was not rapidly cleared from the bloodstream and showed much more liver uptake than was seen with $TcMAG_3$. In fact, studies showed that a significant portion of clearance through the kidneys was probably due to $TcMAG_3$, related to a de-esterification reaction of the methoxy group *in vivo*, probably mediated by blood esterases [50].

The synthesis of Tc-99m MAG_3 proceeds through an intermediate complex involving the transfer ligand tartrate. The mechanism of deprotection of benzoylMAG_3 is still not clear. It has been proposed by Nosco et al. [50,97] that coordination of the sulfur atom of MAG_3 to the technetium proceeds via deprotection (hydrolysis to produce benzoic acid and free thiol MAG_3) of a small amount of ligand when the reaction solution is heated. The free thiol MAG_3 then replaces the tartrate. (Note: It has been shown that trace amounts of benzoic acid are present in the reaction solution after the reaction has take place.) Other investigators have claimed that the coordination of the MAG_3 ligand can occur first followed by Tc-assisted cleavage of the C(O)–S bond [101]. Verbruggen et al. [102] have prepared MAG_3 with *S*-benzoyl, *S*-benzyl and *S*-benzamidomethyl protecting groups and found, not surprisingly, that the order of reactivity was *S*-benzoyl > *S*-benzmidomethyl > *S*-benzyl. Unfortunately this does not prove the mechanism as both normal and metal-assisted deprotection would probably follow the same pattern. The true mechanism has still not been proven for the benzoyl or for other protecting groups used or proposed for MAG_3-like ligands and would be an interesting mechanistic study.

Tc-99m MAG_3 has been reported to have anywhere between 50 and 80% of the clearance rate of I-131 OIH [25], presumably due to the fact that it is only cleared via active transport. Since the original goal was to look for a Tc-99m complex that was equal to or superior to OIH in renal clearance, a number of research groups have continued to look for compounds that have $> 100\%$ of the clearance rate of OIH. This compound would probably have to be cleared by both filtration and active transport processes.

Verbruggen's group has been the most prolific at attempting to improve upon $TcMAG_3$, synthesizing and testing in animals over 50 derivatives related to the MAG_3 structure [44–47]. This research has provided much insight into the structural factors affecting the renal clearance of Tc(V) complexes. As with most investigators, however, in most cases they synthesized just Tc-99m complexes, making absolute structure confirmation impossible.

One derivative synthesized by Verbruggen's group, mercaptoacetylglycylalanylglycine (MAGAG), (Fig. 11, as the benzoyl protected ligand) [45] can produce two pairs of diastereomers due to the optically active carbon manifested in the methyl group of the alanine and the inherent optically active Tc=O, square pyramidal center [8]. These two diastereomeric pairs, in similar fashion to the CO_2–DADS compounds discussed above, showed different rates of renal clearance. Although the actual configuration of the complexes were not verified by synthesis of the Tc-99g analogs the authors postulated structures based on whether D- or L-alanine

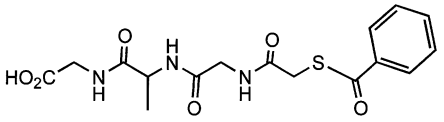


Fig. 11. Structure of benzoyl-protected MAGAG.

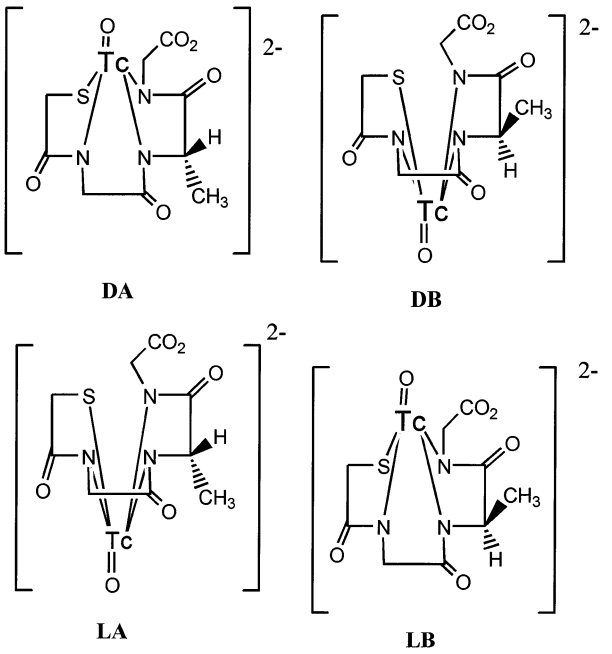


Fig. 12. Structures of diastereomers in TcMAGAG complexes proposed by Verbruggen [45].

was used and by the HPLC retention time of the peaks with isomer A eluting first and isomer B eluting next. The proposed structures, designated DA, DB, LA and LB are shown in Fig. 12. The authors suggested that the enhanced renal clearance of isomers DA and DB over LA and LB are the result of the methyl group of the alanine being *syn* to the Tc=O group in the L isomers and *anti* in the D isomers. If this is indeed true and Despopoulos' contention holds, it is powerful evidence that the renal clearance of these compounds is receptor-mediated and that it is a very stereoselective process.

Other modifications of the MAG_3 structure have also been attempted. When the methylene groups in the ligand backbone of MAG_3 are replaced by ethylene groups (Fig. 13) the ability of the ligand to form stable Tc complexes lessens [49,103].

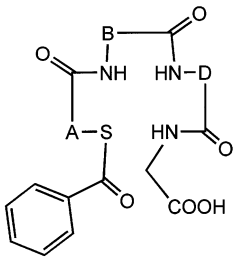


Fig. 13. Generalized structure for benzoyl MAG_3 derivatives with extra CH_2 groups inserted into the chelate ring.

Table 4
Synthesis results using MAG_3-like ligands to form technetium-99m complexes

Ligand	Yield of Tc-99m complex[X] (%)	Comments
I, $A = CH_2CH_2$, $B = C = CH_2$	93	Similar to $TcMAG_3$ formation
II, $A = C = CH_2$, $B = CH_2CH_2$	74	Similar to $TcMAG_3$ formation
III, $A = B = CH_2$, $C = CH_2CH_2$	34	Unstable product, long HPLC t_R
IV, $A = B = CH_2CH_2$, $C = CH_2$	0	No complex formation seen
V, $A = C = CH_2CH_2$, $B = CH_2$	0	No complex formation seen
VI, $B = C = CH_2CH_2$, $A = CH_2$	0	No complex formation seen
VII, $A = B = C = CH_2CH_2$	0	No complex formation seen

[X] Based on reverse phase (C18) HPLC analysis (EtOH/aqueous Na phosphate pH 7 buffer) and TLC (C-18 plates, aqueous methanol eluent) to determine % total Tc-99m in complex.

Table 4 describes the synthetic results when these ligands were used to complex Tc-99m. The ligands that formed Tc-99m complexes were also used to prepare Tc-99g complexes. The results would appear to support the necessity of having at least 2 of the 3 chelate rings in these tetradentate ligands be five-membered rings in order to form a stable complex. A peculiar result was generated with the Tc-99m complex of ligand III. This complex was not stable and disproportionated or simply oxidized back to pertechnetate. The HPLC retention time of the product formed from ligand III is much longer than the those of the complexes of ligands I and II. This supports that the complex of this ligand is an entirely different chemical entity. Complexes of similar retention time eluted under similar conditions have been seen when an excess of MAG_3 (free sulfhydryl) is present during complexation to Tc-99 (Fig. 9) [50,93,102]. However, in the case of MAG_3 these complexes eventually resolve to $TcMAG_3$, presumably because of the thermodynamic stability of the $TcMAG_3$ and its three five-membered chelated rings. In the case of ligand III, this resolution does not take place and the complex seems to oxidize back to pertechnetate. The authors were not able to isolate a stable Tc-99g product from ligand III and so have not been able to elucidate the structure of the complex produced with this ligand.

A possible explanation for the nature of the compound produced with ligand III is that this complex contains a MAG_3 ligand that retains its benzoyl group. As discussed above, it is not clear whether MAG_3 coordinates to Tc before or after it loses its benzoyl protecting group (Fig. 14) [97,101].

If benzoylMAG_3-like ligands do coordinate starting with the amido group and/or carboxylate group on the end of the molecule opposite the sulfhydryl (Fig. 14b) it is possible that formation of a six-membered chelate ring so early in the chelation

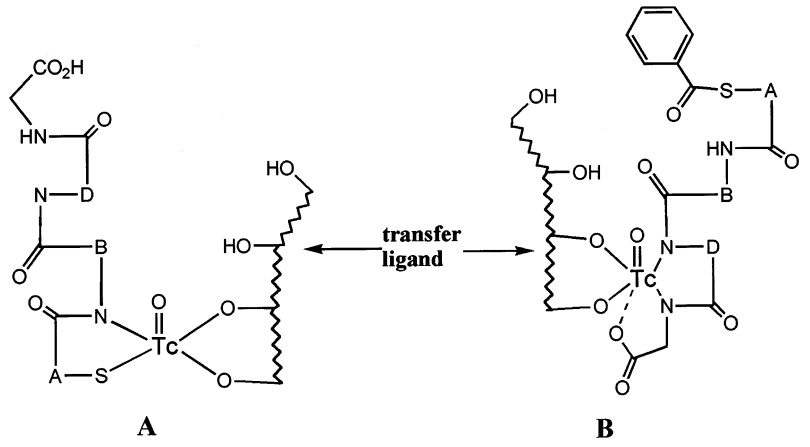


Fig. 14. Two possible initial steps in the displacement of transfer ligands in $TcMAG_3$ complex formation.

process is not favored. All but one of ligands III–IV would have a six-membered chelate ring in this position and this might explain, along with the number (2 or 3) of six-membered chelate rings, why ligands III–VII did not form stable complexes under a variety of reaction conditions.

Tc-99m complexes of I and II showed good renal clearance rates with little or no liver uptake. Compound I had a clearance rate most similar to MAG_3 (90% of $TcMAG_3$ clearance in baboons) and compound II having a slightly slower rate (80% of $TcMAG_3$). Their Tc-99g complexes gave predominant FAB mass sprectral parent peaks of the correct molecular weight [103].

Among all the derivatives synthesized by Verbruggen's group there is one that produced very interesting and somewhat unexpected in situ chemistry. When tetra-L-alanine was reacted with Tc-99m one product was produced which slowly transformed into another, more stable product [104]. The use of Tc-99g showed that this was a simple ring closure involving the non-coordinated, terminal carboxylate and the coordinated, terminal amine (Fig. 15). This type of reaction, though possible with most of the Tc-tri- and tetrapeptides discussed, was only seen in this

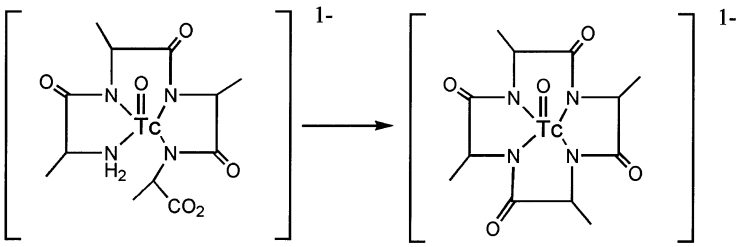


Fig. 15. Cyclization of Tc(tetra-L-alanine) complex.

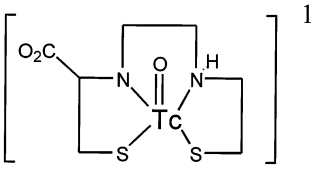


Fig. 16. Structure of TcECC (ethlene cysteamine cysteine).

complex. Neither the open chain nor the macrocyclic Tc-99m complexes had outstanding renal clearance characteristics and so were not pursued. Nevertheless, this remains as one of the few examples of technetium facilitating a template synthesis and true macrocycle formation.

Shortly after $TcMAG_3$ was shown to be effective at measuring kidney function it was proposed by Nosco and Verbruggen [33] that Tc(V) complexes that (1) contained an inherently neutral, O=TcL core and (2) had been modified by inclusion of one or more non-coordinating carboxylate groups to the ligand(s) should produce a complex very similar in charge, size, hydrophilicity and shape to $TcMAG_3$ and thus might generate alternative and potentially better kidney function agents. The first compound in this series to be tested was the technetium complex of the ethyl cysteinate dimer (Tc-EC, Fig. 2f) which contains one of its carboxylates on the same side of the molecule as the Tc=O group. The ligand is a tetradentate diamine dithiolate ligand which, when the pendant carboxylates are esterified (Fig. 1c), has been shown to produce an overall neutral, lipophilic technetium complex which effectively crosses the blood-brain barrier [26]. The de-esterified complex, Tc-EC, on the other hand, is very hydrophilic and has been shown in animal and human studies to be similar to $TcMAG_3$ in measuring renal clearance [54]. Verbruggen has, in similar compounds, shown that removal of one of the carboxylates (ECC, Fig. 16) [56], the esterification of one of the carboxylates (Fig. 17) [55a], or the addition of methyl groups to the EC backbone [55b,c] results in compounds

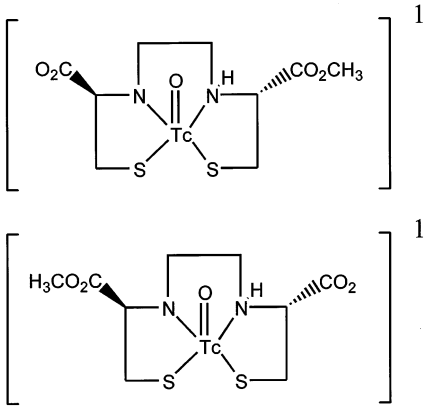


Fig. 17. Structure of possible mono de-esterified derivatives of Tc–ECD.

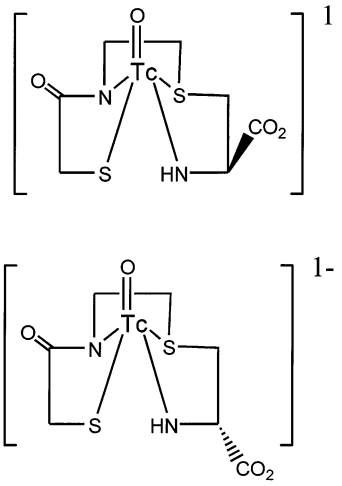


Fig. 18. Proposed possible structures of Tc–CO_2–DAMTE.

with much slower renal clearance than Tc-EC. This would seem to indicate that receptor mediated clearance may, indeed, be sensitive to the -2 charge of the Tc-EC along with the pendant *syn* carboxylate. The ultimate resolution of this work still awaits the synthesis of Tc-99g analogs of these complexes to confirm the structures proposed.

Brandau et al. have synthesized a Tc–EC-like complex (Tc–CO_2–DAMTE) which has the proposed structure shown in Fig. 18 [73]. This complex should be neutral (neutral amine) or monoanionic (amine deprotonated) and should have the same diastereomers that were present in Tc–CO_2–DADS. Experimental results did not confirm the presence of two isomers with Tc–CO_2–DAMTE and may indicate inefficient separation of those isomers by HPLC or a predominance of one isomer over the other, something not achievable with Tc–CO_2–DADS. Test results in humans showed promise as this agent was actively taken up and excreted through the kidneys. However, similar to previous findings for Tc–EC, the agent appeared to be inferior when tested in rats. Moreover, these initial results showed that this compound was inferior to $TcMAG_3$ and Tc–EC. No Tc-99g complex was reported so the exact structure (and the orientation of the pendant carboxylate) was undetermined. Without structural confirmation the results obtained with the Tc-99m complex are not absolutely interpretable. Other structures besides the one proposed by the author are possible, including coordination by the carboxylate with or without coordination (or even deprotonation) of the terminal amine. Although not directly comparable, coordination by a pendant carboxylate in these types of ligands has been documented at least three previous times. Rao et al. showed that, for the Re–DADS a reversal of the HPLC retention time was seen for isomers A and B [65e]. The authors ascribed this to coordination of the carboxylate to the Re in the *anti* isomer. In the case of Re(V)EC, one of the carboxylates coordinates to the Re, giving a six-coordinate complex (Fig. 19) [105,106]. Finally, Edwards et al.

[26] also reported evidence for this in the IR for the Tc-99g-EC complex but this was never confirmed by X-ray crystal structure. Clearly, this is a complicated process in solution with the *anti* carboxylate of these complexes showing some ability to coordinate. Evidence exists that coordination by the *anti* carboxylate in M–EC (M = Tc, Re) complexes occurs transiently in solution and that, for Re, it is a dominant form in the solid state [106]. Although there are differences in the chemistry of Tc and Re and direct analogies cannot be drawn, how much the solution geometry of technetium complexes with pendant carboxylates is affected by this type of coordination is still unknown.

The attachment of pendant carboxylates to ligands known to form stable technetium complexes has been proposed by Nosco as a means to generate novel, effective radiopharmaceuticals to measure kidney function [107]. Recently, one such study using a Tc(I) complex having the formula $[Tc(CNCH_2CO_2)_6]^{5-}$ (Fig. 1g, R=$CH_2CO_2^-$) has reported that this compound has rapid renal clearance which proceeds through an active transport mechanism [81]. No Tc-99g complex has been reported for this agent

Although it became apparent that the pendant carboxylate is essential to develop an anionic kidney function agent based on technetium, the above work [81] indicates that it has not yet been established that the Tc=O moiety was also necessary to produce such an agent. Verbruggen and co-workers [35] have recently demonstrated that replacing the Tc=O with a Tc≡N group dramatically reduces the ability of such agents to be rapidly cleared from the blood by the kidneys and thus makes them less than optimal agents to measure kidney function. The technetium in nitrido complexes which include sulfur-containing ligands has been shown to be Tc(V) [110b], giving a $[TcN]^{2+}$ core and an overall −3 charge to the complex (Fig. 2h). Further evidence for this charge comes from the fact that the Tc≡N complex (proposed −3 overall charge) appears to be more negatively charged at neutral pH than the Tc=O complex (proposed −2 overall charge) as noted by its greater anionic mobility during electrophoresis. Verbruggen's result with Tc(N)–EC has been reinforced by work done with Tc≡N complexes of DTPA, glucoheptonate and CO_2–DADS where the resulting complexes were shown to be not nearly as effective

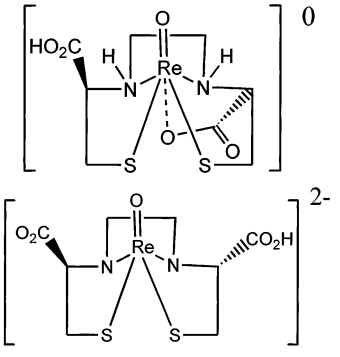


Fig. 19. Structures of crystallized Re(V)EC complexes.

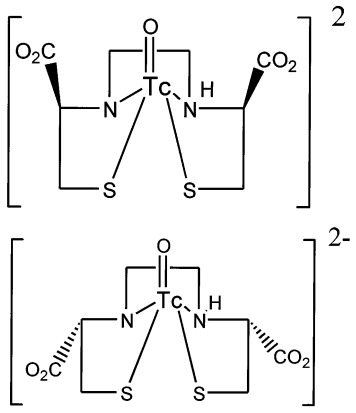


Fig. 20. Conformation of diastereomers of Tc–*meso*–EC.

renal agents as are their Tc=O analogs [108,109]. It has been impossible at this time to determine if this is solely from the receptor not recognizing the Tc≡N complexes or if it is simply from the fact that complexes with Tc≡N groups are more lipophilic than their Tc=O analogs [110]. Further studies are needed to elucidate the differences in the Tc≡N and Tc=O systems that result in different rate clearance rates and, potentially, mechanism(s) of excretion.

Finally, Verbruggen [111] has presented work that casts doubt on the necessity of a pendant carboxylate *syn* to the Tc=O group. His group synthesized Tc-99m complexes of the *meso*-EC ligand (Fig. 20) where both of the carboxylates should be pointed *syn* or *anti* to the Tc=O group and reported no difference in the biodistribution of the two isomers.

As with past experiments using Tc-99m, this work awaits the synthesis and characterization of Tc-99g analogs to help clarify the Tc-99m results. Given the findings of Marzilli's and Fritzberg's groups [65e,105,106], and the coordinating possibilities of *anti* carboxylates, the Tc-99g characterization of the Tc-*meso*-EC compounds is crucial towards the proof or disproof of Despopoulos's theory as it pertains to these types of complexes. However, even if the above result is verified by the Tc-99g, structures it may simply mean that the active transport mechanism is so sensitive that it will not respond as effectively to two carboxylates being on the same side of the molecule as the Tc=O group as it will to one. This result suggests that further work is needed to help identify the true uptake mechanism for these types of agents.

4.2.3. Cationic technetium complexes as renal imaging agents

Another approach taken recently was to use cationic complexes of Tc as renal function agents. This strategy was based on reports that hydrophilic cationic species were also cleared effectively from the blood through the kidneys [36]. Deutsch [67] has proposed that this mechanism may lead to new classes of renal function agents. These agents may be especially useful for patients with uremia [67], a condition in

which anions (salts of weak acids) are not actively transported from the blood by the kidney. A number of other groups have also published results on the use of cationic Tc-99m complexes as renal agents [79]. These results indicate that these agents may have utility to measure renal function and/or delineate kidney structure. However, a more systematic study of the factors that favor active transport and filtration of Tc-99m hydrophilic, cationic complexes will be necessary to determine if these compounds can ever approach the renal clearance properties of OIH or even $TcMAG_3$ or even address a significant clinical need that would justify their commercialization.

5. Summary

Tc-99m radiopharmaceuticals for renal imaging are being used more now than they ever have been. Although the current agents are excellent in determining how the kidney is functioning, work described in this review has shown that many groups are interested in improving on agents such as TechnescanMAG3®. For such work to be successful a more efficient marriage of Tc-99m and Tc-99g chemistry will have to take place. One without the other will probably not lead to a systematic scientific discovery because varying ligand structure without some minimal verification of complex structure is not wise. This review has also indicated that there is significant Tc-99g chemistry still to be done on already existing radiopharmaceuticals and on Tc-99m complexes of proposed radiopharmaceuticals. As that work is done there is a distinct possibility that some inconsistencies that apparently exist in the biohandling of certain of the Tc-99m complexes mentioned in this review could be better explained.

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References

[1] (a) J. Kampmann, K. Siersbaek, M. Kristensen, H. Molholm, Acta Med. Scand. 196 (1974) 517. (b) A.S. Levey, R.D. Perrone, N.E. Madias, Ann. Rev. Med. 39 (1988) 465.
[2] A. Fritzberg, J. Nucl. Med. Tech. 12 (1984) 177.
[3] B. Hood, P.-O. Attman, J. Ahlman, R. Jagenburg, Scand. J. Urol. Nephrol. 5 (1971) 154.
[4] D.P Swanson, M. Weingarden, in: D.P. Swanson, H.M. Chilton, J.H. Thrall (Eds.), Pharmaceuticals in Medical Imaging, MacMillan Publishing, NY, 1990, p. 78.
[5] M.R. Prince, C. Arnoldus, J.K. Frisoli, J. Magn. Reson. Imaging 6 (1996) 162.
[6] J.A. Frank, P.L. Choyke, H.A. Austin III, M.E. Girton, G. Weiss, Invest. Radiol. 26 (1991) S134.

[7] P.L. Choyke, J.A. Frank, M.E. Girton, Radiology 170 (1989) 713.
[8] A.M. Verbruggen, M.J.K. De Roo, in: A.D. Nunn (Ed.), Radiopharmaceuticals: Chemistry and Pharmacology, Marcel Dekker, NY, 1992, p. 365.
[9] M.D. Blaufox, J. Nucl. Med. 32 (1991) 1301.
[10] M. Villa, J.C. Saccavini, A. Bardy, Dev. Nucl. Med. 10 (1986) 351.
[11] D. Eshima, A.R. Fritzberg, A. Taylor Jr, Sem. Nucl. Med. 20 (1990) 28.
[12] J.A. Ponto, H.M. Chilton, N.A. Watson Jr, in: D.P. Swanson, H.M. Chilton, J.H. Thrall (Eds.), Pharmaceuticals in Medical Imaging, MacMillan, p, NY, 1990, p. 501.
[13] J. Steigman, W.C. Eckelman, The Chemistry of Technetium in Medicine, National Research Council, National Academy Press, Washington, DC, 1992, p. 1.
[14] E. Deutsch, K. Libson, S. Jurisson, L.F. Lindoy, Prog. Inorg. Chem. 30 (1983) 75.
[15] K. Schwochau, U. Pleger, Radiochim. Acta 63 (1993) 103.
[16] S.K. Shukla, G.B. Manni, C. Cipriani, Inorg. Chim. Acta 92 (1984) 83.
[17] M.K. Dewanjee, Sem. Nucl. Med. 20 (1990) 5.
[18] E. Deutsch, K. Libson, Comments Inorg. Chem. 3 (1984) 83.
[19] E. Deutsch, K. Libson, J.-L. Vanderheyden, A. Ketring, H.R. Maxon, Nucl. Med. Biol. 13 (1986) 465.
[20] K.E. Linder, M.F. Malley, J.Z. Gougoutas, S.E. Unger, A.D. Nunn, Inorg. Chem. 29 (1990) 2428.
[21] J.D. Kelly, A.M. Forster, B. Higley, C.M. Archer, F.S. Booker, L.R. Canning, K.W. Chiu, B. Edwards, H.K. Gill, M. McPartlin, K.R. Nagle, I.A. Latham, R.D Pickett, A.E. Storey, P.M. Webbon, J. Nucl. Med. 34 (1993) 222.
[22] J.L. Vanderheyden, A.R. Ketring, K. Libson, E. Deutsch, Inorg. Chem. 23 (1984) 3184.
[23] T. Konno, J.R. Kirchhoff, W.R. Heneman, E. Deutsch, Inorg. Chem. 28 (1989) 1174.
[24] (a) C. Rossetti, G. Paganelli, G. Vanoli, C. DiLeo, M. Kwiatkowski, F. Zito, F. Colombo, C. Bonino, A. Carpanelli, E. Deutsch, F. Fazio, J. Nucl. Med. 35 (1994) 1571. (b) M. Marmion, S.R. Woulfe, W.L. Neumann, D.L. Nosco, E.A. Deutsch, Nucl. Med. Biol., in press.
[25] A.R. Fritzberg, S. Kasina, D. Eshima, D.L. Johnson, J. Nucl. Med. 27 (1986) 111.
[26] D.S. Edwards, E.H. Cheesman, M.W. Watson, L.J. Maheu, S.A Nguyen, L. Dimitre, T. Nason, A.D. Watson, R. Walovitch, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 3, Cortina International, Verona, Raven Press, NY, 1990, p. 433.
[27] S. Jurisson, E.O Schlemper, D.E. Troutner, L.R. Canning, D.P. Nowotnik, R.D. Neirinckx, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium in Chemistry and Nuclear Medicine 2, Cortina International, Verona, Italy, 1987, p. 37.
[28] F.J.T. Wackers, D.S. Berman, J. Maddahi, D.D. Watson, G.A. Beller, H.W. Strauss, C.A. Boucher, M. Picard, B.L. Holman, R. Fridrich, E. Inglese, B. Delaloye, A. Dichof-Delaloye, L. Camin, K. McKusick, J. Nucl. Med. 30 (1989) 301.
[29] M. Jehangir, G. Subramanian, J.G. McAfee, F.D. Thomas, G. Gagne, C. Zapf-Longo, C. Ritter-Hirincirik, J. Nucl. Med. 26 (1985) 83.
[30] D.W. Wester, J.R. Coveney, D.L. Nosco, M.S. Robbins, R.T. Dean, J. Med. Chem. 34 (1991) 3284.
[31] M.E. Marmion, S.R. Woulfe, W.L. Neumann, G. Pilcher, D.L. Nosco, Nucl. Med. Biol. 23 (1996) 567.
[32] (a) J.-L. Vanderheyden, K. Libson, D.L. Nosco, A.R. Ketring, E. Deutsch, Int. J. App. Radiat. Isot. 34 (1983) 1611. (b) J.-L. Vanderheyden, M.J. Heeg, E. Deutsch, Inorg. Chem. 24 (1985) 1666. (c) J.-L. Vanderheyden, A.R. Ketring, K. Libson, M.J. Heeg, L. Roecker, P. Motz, R. Whittle, R.C. Elder, E. Deutsch, Inorg. Chem. 23 (1984) 3184.
[33] A.M. Verbruggen, D.L. Nosco, C.G. Van Nerom, G.M. Bormans, P.J. Adriaens, M.J. DeRoo, J. Nucl. Med. 33 (1992) 551.
[34] H. van Billoen, G. Bormans, J. Heylen, M. Hoogmartens, M. De Roo, A. Verbruggen, J. Label. Comp. Radiopharm. 30S (1991) 42.
[35] K.O. Mang'era, H.P. Vanbilloen, E. Ballande, R. Pasqualini, A.M. Verbruggen, Nucl. Med. Biol. 23 (1996) 987.

[36] J. Hamburger, G. Richet, J. Crosnier, J.L. Funck-Brentano, B. Antoine, H. Ducrot, J.P. Mery, H. de Montera, Nephrology, vol. 1, W.B. Saunders, Philadelphia, 1968.
[37] L.P. Sulliva, J.J. Grantham, Physiology of the Kidney, Lea and Febiger, Philadelphia, 1982.
[38] D. Jensen, The Principles of Physiology, Appleton-Century-Crofts, NY, 1982, p. 711.
[39] H.L. Atkins, K.G. Cardinale, W.C. Eckelman, Radiology 98 (1971) 674.
[40] (a) O.D. Awh, J.R. Kim, S.C. Shim, Nucl. Med. Comm. 10 (1989) 449. (b) H. Spies, D. Scheller: Inorg. Chim. Acta 116 (1986) 1.
[41] R.E. Boyd, J. Robson, F.C. Hunt, P.J. Sorby, I.P.C. Murray, W.J. McKay, Brit. J. Radiol. 46 (1973) 604.
[42] R.W. Arnold, G. Subramanian, J.G. McAfee, R.J. Blair, F.D. Thomas, J. Nucl. Med. 16 (1975) 357.
[43] D. Eshima, A. Taylor Jr, A.R. Fritzberg, S. Kasina, L. Hansen, J.F. Sorenson, J. Nucl. Med. 28 (1987) 1180.
[44] H.P. Vanbilloen, M.J. De Roo, A.M. Verbruggen, Eur. J. Nucl. Med. 23 (1996) 40.
[45] (a) A. Verbruggen, P. Dekempeneer, B. Cleynhens, M. Hoogmartens, M. De Roo, in: H.A.E. Schmidt and D. Emrich (Eds.), Nuklear Medizin-Clinical Demands on Nuclear Medicine, Schattauer Verlag, Stuttgart, New York, 1987, p. 512. (b) A. Verbruggen, G. Bormans, B. Cleynhens, A. Vancruys, M. Hoogmartens, M. De Roo, J. Label. Comp. Radiopharm. 26 (1989) 436; (c) A. Verbruggen, G. Vormans, A. Vandecruys, L. Verhaegen, P. Devos, M. De Roo, in: H.A.E. Schmidt and G.L. Buraggi (Eds.), Nuclear Medicine: Trends and Possibilities in Nuclear Medicine, Schattaer Verlag, Stuttgart, 1989, p. 440.
[46] H.H. Vanbilloen, N.A. Dezutter, B.J. Cleynhens, A.M. Verbruggen, Eur. J. Nucl. Med. 24 (1997) 1374.
[47] (a) G. Bormans, PhD Thesis, U. Leuven, 1990. (b) S. Okarvi, P. Adriens, H. Vanbilloen, S. Ons, M. De Roo, A. Verbruggen, J. Nucl. Med. 19 (1992) 741. (c) H. Van Billoen, G. Bormans, J. Heylen, M. Hoogmartens, M. De Roo, A. Verbruggen, J. Label. Comp. Radiopharm. 30S (1991) 42. (d) G. Bormans, B. Cleynhens, M. Hoogmarten, M. De Roo, A. Verbruggen, Nucl. Med. Biol. 19 (1992) 375. (e) J. Vanbilloen, M. Hoogmartens, M. De Roo, A. Verbruggen, Eur. J. Nucl. Med. 18 (1991) 686, (f) T. Vandebrouck, B. Cleynhens, C. Van Nerom, G. Bormans, M. De Roo, A. Verbruggen, Euro J. Nucl. Med. 18 (1991) 674. (g) H. Vanbilloen, G. Bormans, C. Van Nerom, M. De Roo, A. Verbruggen, Eur. J. Nucl. Med. 18 (1991) 674. (h) M.S. Okarvi, P. Adriaens, C. Van Nerom, G. Bormans, M. De Roo, A. Verbruggen, Eur. J. Nucl. Med. 18 (1991) 672. (i) B. Cleynhens, H. Vanbilloen, M. Hoogmartens, M. De Roo, A. Verbruggen, Eur. J. Nucl. Med. 18 (1991) 605. (j) A. Verbruggen, B. Cleynhens, P. Adriaens, M. Hoogmartens, M. De Roo, J. Nucl. Med. 28S (1987) 731. (k) H.P. Vanbilloen, G.M. Bormans, M.J. De Roo, A.M. Verbruggen, Nucl. Med. Biol. 22 (1995) 325. (l) M. Subhani, H. Vanbilloen, B. Cleynhens, G. Bormans, C. Van Nerom, D. Crombez, M. De Roo, A. Verbruggen, J. Label. Comp. Radiopharm. 30S (1990) 83. (m) B. Cleynhens, P. Adriaens, C. Boonen, H. Vanbilloen, C. Van Nerom, A.M. Verbruggen, J. Nucl. Biol. Med. 38S1 (1994) 69. (n) G. Bormans, B. Clleynhens, M. Hoogmartens, M. De Roo, A. Verbruggen, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 3, Cortina International, Verona, Raven Press, NY, 1990, p. 661. (o) M. Subhani, B. Cleynhens, G. Bormans, M. Hoogmartens, M. De Roo, A. Verbruggen, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 3, Cortina International, Verona, Raven Press, NY, 1990, p. 453. (p) B. Cleynhens, H. Vanbilloen, C. Boonen, M. De Roo, A.M. Verbruggen, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 4, SGE Ditoriali, Padua, Italy, 1995, p. 445.
[48] (a) K.M. Miller, J.R. Coveney, D.L. Nosco, M.S. Robbins, J. Nucl. Med. 30 (1989) 937. (b) L. Hansen, A. Taylor Jr, L.G. Marzilli: Metal Based Drugs 1 (1993) 31. (c) D. Nosco, J. Coveney, D. Helling, J. MacDonald, R. Rajagopalan, S. Wallach, J. Nucl. Med. 36 (1989) 937.
[49] D. Nosco, B. Weber, R. Rajagopalan, A. Verbruggen, G. Bormans, Eur. J. Nucl. Med. 18 (1991) 606.
[50] D.L. Nosco, J.R. Coveney, D.W. Pipes, M.S. Robbins, J. Nucl. Med. 35 (1988) 801.

[51] (a) A.G. Jones, A. Davison, M.R. LeTegola, J.W. Brodack, C. Orvig, M. Sohn, A.K. Toothaker, C.J.L. Lock, K.J. Franklin, C.E. Costello, S.A. Carr, K. Biemann, M.L. Kaplan, J. Nucl. Med. 23 (1982) 801. (b)D. Brenner, A. Davison, J. Lister-James, A.G. Jones, Inorg. Chem. 23 (1984) 3793.
[52] S. Kasina, A.R. Fritzberg, D.L. Johnson, D. Eshima, J. Med. Chem. 29 (1986) 1933.
[53] A. Davison, A.G. Jones, C. Orvig, M. Sohn, Inorg. Chem. 20 (1981) 1629.
[54] C.G. Van Nerom, G.M. Bormans, M.J. De Roo, A.M. Verbruggen, Eur. J. Nucl. Med. 20 (1993) 831.
[55] (a) A. Verbruggen, G. Bormans, C. Van Nerom, B. Cleynhens, D. Crombez, M. De Roo, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 3, Cortina International, Verona, Raven Press, NY, 1990, p. 445. (b) B. Cleynhens, H. Van Billoen, N. Dezutter, C. Boonen, M. De Roo, A. Verbruggen, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 4, SGE Ditoriali, Padua, 1995, p. 351. (c) H. Vanbilloen, B. Cleynhens, E. Seuntjens, M. De Roo, A. Verbruggen, Eur. J. Nucl. Med. 21 (1994) 807.
[56] B. Cleynhens, H. Vanbilloen, E. Seuntjens, M. De Roo, A. Verbruggen, J. Label. Comp. Radiopharm. 35 (1994) 19.
[57] (a) S. Sanyal, S. Banerjee, Nucl. Med. Biol. 17 (1990) 757. (b) A.Y. Wang, R.-S. Liu, Nucl. Med. Biol. 7 (1995) 937.
[58] (a) L.R. Chervu, B.M. Sundoro, M.D. Blaufox, J. Nucl. Med. 25 (1984) 1111. (b) B. Zmbova, D. Djokic, V. Bogdanova, I. Tadzar, B. Ajdinovic, M. Rastovac, Appl. Radiat. Isot. 40 (1989) 225. (c) K.K. Bhargava, Z. Zhang, S.B. Chun, L.R. Chervu, M.D. Blaufox, J. Label. Compounds Radiopharm. 9 (1988) 943.
[59] (a) M. Misra, H.S. Sarkar, M. Chakravarty, S. Sanyal, S. Ganguly, B.R. Sarkar, J. Chatterjee, P. Dobe, S. Ghosh, S. Banerjee, Nucl. Med. Comm. 15 (1994) 878. (b) M. Chattopadhyay, K.N. Jalan, A.K. Pal, S.N. Banerjee, Nucl. Med. Biol. 15 (1988) 535.
[60] A. Tanaka, T. Machida, M. Miki, T. Shimada, Eur. J. Nucl. Med. 8 (1983) 317.
[61] (a) A. Taylor Jr, L.B. Talner, J. Nucl. Med. 19 (1978) 178. (b) D.L. Johnson, A.R. Fritzberg, B.L. Hawkins, S. Kasina, D. Eshima, Inorg. Chem. 23 (1984) 4204.
[62] P.L. Hagan, D.M. Chauncey Jr, S.E. Halpern, P. Ayres, J. Nucl. Med. 18 (1977) 353.
[63] M. Ueda, Nippon Hinyokika Gakkai Zasshi 68 (1977) 758.
[64] L. Gianolli, F. Dosio, M. Matarrese, F. Columbo, C. Cutler, D. Stepniak-Biniakiewicz, E. Deutsch, Nucl. Med. Biol. 23 (1996) 927.
[65] (a) W.C. Klingensmith, A.R. Fritzberg, V.M. Spitzer, D.L. Johnson, C.C. Kuni, M.R. Williamson, G. Washer, R. Weil III, J. Nucl. Med. 25 (1984) 42. (b) G. Bormans, B. Cleynhens, D. Jose, M. Hoogmartens, M. De Roo, A. Verbruggen, Nucl. Med. Biol. 17 (1990) 499. (c) T.N. Rao, D. Adhikesavalu, A. Camerman, A.R. Fritzberg, J. Am. Chem. Soc. 112 (1990) 5798. (d) R.F. Schneider, G. Subramanian, T.A. Feld, J.G. McAfee, C. Zapf-Longo, E. Palladino, F.D. Thomas, J. Nucl. Med. 25 (1984) 223. (e) T.N. Rao, D.I. Brixner, A. Srinivasan, S. Kasina, J.-L. Vanderheyden, D.W. Wester, A.R. Ftitzbergh, Appl. Radiat. Inst. 42 (1991) 525.
[66] D.J. Carney, J. Billings, L.C. Francesconi, Y.Z. Guo, B.S. Haggerty, A.L. Rheingold, H.F. Kung, J. Med. Chem. 36 (1993) 1032.
[67] K.M. Herzog, E. Deutsch, K. Deutsch, E.B. Silberstein, R. Sarangarajan, W. Cacini, J. Nucl. Med. 33 (1992) 2190.
[68] A.K. Padhy, K.K. Solanki, K.E. Britton, J. Bomanji, T. Chaiwatanratan, C.C. Nimmon, Eur. J. Nucl. Med. 17 (1992) 717.
[69] M. Chattopadhyay, K.N. Jalan, A.K. Pal, S.N. Banerjee, Nucl. Med. Biol. 14 (1987) 599.
[70] A.M. Al-Hilli, N.H. Agha, S.Y. Al-Ani, J. Radioanal. Nucl. Chem. 89 (1985) 55.
[71] Y.F. Shafiq, M.H.S. Al-Hissoni, M.H. Jassim, Nucl.-Med. 27 (1988) 154.
[72] J.E. Causse, R. Pasqualini, B. Cypriani, R. Weil, R. van Der Valk, P. Bally, A. Dupuy, I. Couret, M. Benbarek, B. Descomps, Appl. Radiat. Isot. 41 (1990) 493.
[73] W. Brandau, S. Fischer, C. Puskas, A. Stenstraser, L. Kuhlmann, O. Schober, Nucl. Med. Biol. 21 (1994) 1073.
[74] (a) D.S. Edwards, S. Liu, D.M. Lyster, M.J. Poirier, C. Vo, G.A. Webb, Z. Zhang, C. Orvig, Nucl. Med. Biol. 20 (1993) 857. (b) D.S. Edwards, S. Liu, M.J. Poirier, Z. Zhang, G.A. Webb, C. Orvig, Inorg. Chem. 33 (1994) 5607.

[75] I. Castanheira, C. Sawas-Dimopoulou, A. Paulo, M. Neves, L. Patricio, Nucl. Med. Biol. 20 (1993) 279.
[76] (a) D. Nowotnik, R.D. Pickett, C.D.R. Allen, Eur. J. Nucl. Med. 11 (1985) 285. (b) N. Ramamoorthy, S.A. Balakrishnan, S.M. Gaitonde, P.M. Pandey, U.N. Nayak, M.C. Patel, R.B. Patel, P. Ramanathan, J. Radioanal. Nucl. Chem. 134 (1989) 423.
[77] S.H. Al-Kouraishi, J. Radioanal. Nucl. Chem. 125 (1988) 203.
[78] (a) I. Hoffmann, R. Muenze, I. Dreyer, R. Dreyer, J. Radioanal. Chem. 74 (1982) 53. (b) L.L.-Y. Hwang, N. Ronca, N.A. Solomon, J. Steigman, Int. J. Appl. Radiat. Isot. 36 (1985) 475.
[79] (a) C. Van Nerom, I Van den Bergh, C. Boonen, M. De Roo, A. Verbruggen, Eur. J. Nucl. Med. 19 (1992) 739. (b) W. Brandau, M. Eisenhut, D.M. Taylor, K. zum Winkel, Nuklearmedizin Suppl. (Stuttgart), 21 (1984) 827. (c) P. Blauenstein, P.A. Schubiger, J. Muller, O. Gasser, G. Pfeiffer, G. Anderegg, K.Zollinger, Z. Proso, J. Label. Comp. Radiopharm. 21 (1984) 1066. (d) K.K. Solanki, A.E. Theobald, K.E. Britton, J. Nucl. Med. 32 (1991) 1102. (e) K.K. Solanki, J. Bomanji, C.C. Nimmon, M. Carroll, A.E.Theobald, K.E. Britton, J. Nucl. Med. 32 (1991) 1105.
[80] B. Li, Y. Wu, Y. Liu, J. Li, Y. Dong, C. Wang, B. Ru, BB. Han, J. Tian, Nucl. Sci. Tech. 3 (1992) 268.
[81] S. Verdera, J.J. Lopez, R. Tagle, F. Garcia, G. Fernandez, M. Keuchkerian, C. Heuguerot, A.G. Jones, J.F. Kronauge, J. Nucl. Biol. Med. 38S1-4 (1994) 79.
[82] S. Mansur, M. Jehangir, S. Stathaki, M. Papadopoulos, S. Mastrostamatis, E. Chiotellis, Nucl. Med. Biol. 20 (1993) 819.
[83] V.N. Adonaylo, A. Stahl, C.O. Canellas, A.B. Pomilio, A.A. Vitale, J. Label. Comp. Radiopharm. 33 (1993) 443.
[84] E. Deutsch, R.C. Elder, B.A. Lange, M. Vaal, D.G. Lay, Proc. Natl. Acad. Sci. USA 73 (1976) 4287.
[85] K. Libson, E. Deutsch, B.L. Barnett, J. Am. Chem. Soc. 102 (1980) 2476.
[86] H.B. Burgi, G. Anderegg, P. Blauenstein, Inorg. Chem. 20 (1981) 3829.
[87] A. Davison, C. Orvig, H.S. Trop, M. Sohn, B.V. DePamphilis, A.G. Jones, Inorg. Chem. 19 (1980) 1988.
[88] A. Davison, B.V. DePamphilis, A.G. Jones, K.J. Franklin, C.J.L. Lock, Inorg. Chim. Acta 128 (1987) 161.
[89] A. Davison, B.V. DePamphilis, R. Faggiani, A.G. Jones, C.J.L. Lock, C. Orvig, Can. J. Chem. 63 (1985) 319.
[90] N. Bryson, J. Lister-James, A.G. Jones, W.M. Davis, A. Davison, Inorg. Chem. 29 (1990) 2948.
[91] N. Bryson, D. Brenner, J. Lister-James, A.G. Jones, J.C. Dewan, A. Davison, Inorg. Chem. 28 (1989) 3825.
[92] A.J. Despopoulos, Theor. Biol. 8 (1965) 163.
[93] B. Noll, B. Johannsen, K. May, H. Spies, Appl. J. Radiat. Isot. 43 (1992) 899.
[94] B. Johannsen, B. Noll, K.-H. Heise, K. May, R. Syhre, H. Reis, D. Strangfeld, L. Bruch, D. Modersohn, Med. Nucl. 2 (1990) 115.
[95] B. Johannsen, B. Noll, K.H. Heise, K. May, H. Spies, I. Hoffmann, S. Hoffmann, D. Klotzer, H. Reis, L. Bruch, D. Modersohn, Isoprentaxis 26 (1990) 97.
[96] W. Brandau, B. Bubeck, M. Eisenhut, D.M. Taylor, Appl. J. Radiat. Isot. 39 (1988) 121.
[97] G. Grummon, R. Rajagopalan, G. Palenik, A. Koziol, D.L. Nosco, Inorg. Chem. 34 (1995) 1764.
[98] T.N. Rao, D. Adhikesavalu, A. Camerman, A.R. Fritzberg, Inorg. Chim. Acta 180 (1991) 63.
[99] L. Hansen, R. Cini, A. Taylor Jr, L.G. Marzilli, Inorg. Chem. 31 (1992) 2801.
[100] A. Verbruggen, G. Bormans, B. Cleynhens, M. Hoogmartens, A. Vandecruys, M. De Roo, in: H.A.E. Schmidt, G.L. Buraggi (Eds.), Nuclear Medicine-Trends and Possibilities in Nuclear Medicine, Schattauer Verlag, Stuttgart, 1989, p. 436.
[101] (a) S. Kasina, J.A. Sanderson, J.N. Fitzner, A. Srinivasan, T.N. Rao, L.J. Hobson, J.M. Reno, D.B. Axworthy, P.L. Beaumier, A.R. Fritzberg, Bioconj. Chem. 9 (1998) 108. (b) A. Srinivasan, US Patent No. 5 021 556, 1991.
[102] G. Bormans, B. Cleynhens, P. Adriaens, H. Vanbilloen, M. De Roo, A. Verbruggen, Nucl. Med. Biol. 22 (1995) 339.
[103] D.L. Nosco, R. Rajagopalan, R. Weber, in preparation.

[104] G. Bormans, O.M. Peeters, H. Vanbilloen, N. Blaton, A. Verbruggen, Inorg. Chem. 35 (1996) 6240.
[105] L.G. Marzilli, M.G. Banaszczyk, L. Hansen, Z. Kuklenyik, R. Cini, A. Taylor Jr, Inorg. Chem. 34 (1995) 3579.
[106] L.G. Marzilli, L. Hansen, Z. Kuklenyik, R. Cini, M.G. Banazczyk, A. Taylor Jr, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 4, SGE Ditoriali, Padua, Italy, 1995, p. 27.
[107] (a) D.L. Nosco, US Patent 5 104 638, 1990. (b) D.L. Nosco, US Patent 5 116 598, 1992.
[108] T. Takayama, Y. Kani, T. Sekine, K. Yoshihara, H. Kudo, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 4, SGE Ditoriali, Padua, Italy, 1995, p. 33.
[109] K.O. Mang'era, E. Bellande, H. Vanbilloen, R. Pasqualini, A.M. Verbruggen, in: M. Nicolini, G. Bandoli, U. Mazzi (Eds.), Technetium and Rhenium in Chemistry and Nuclear Medicine 4, SGE Ditoriali, Padua, Italy, 1995, p. 401.
[110] (a) J. Baldas, J. Bonnyman, Int. J. Appl. Radiat. Isot. 36 (1985) 919. (b) J. Baldas, J. Bonnyman, Nucl. Med. Biol. 15 (1988) 451.
[111] A. Verbruggen, G. Bormans, C. Van Nerom, B. Cleynhens, D. Osiadacz, M. De Roo, J. Label. Comp. Radiopharm. 30 (1991) 86.