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Recent advances in ^{99m}Tc radiopharmaceuticals

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^{99m}Tc radiopharmaceuticals play an important role in widespread applications of nuclear medicine. When ^{99m}Tc radiopharmaceuticals first came into use, major efforts were directed toward the development of ^{99m}Tc radiopharmaceuticals for bone imaging and for the excretory functions of the liver and kidneys. In the past 20 years, a significant advance has been made in technetium chemistry, which provided ^{99m}Tc radiopharmaceuticals for assessment of regional cerebral and myocardial blood flow. Recent efforts have been directed toward the design of ^{99m}Tc-labeled compounds for estimating receptor or transporter functions. A number of bifunctional chelating agents that provide ^{99m}Tc labeled proteins and peptides of high *in vivo* stability with high radiochemical yields have also been developed. More recently, organometallic technetium and rhenium compounds have been introduced as another class of ^{99m}Tc radiopharmaceutical design. In this manuscript, recent progress in ^{99m}Tc radiopharmaceuticals is reviewed with the major emphasis laid on key innovations in this field to provide the ^{99m}Tc radiopharmaceuticals available today.

Key words: technetium-99m, radiopharmaceutical, bifunctional chelating agent, conjugated design, integrated design, peptides, proteins

1. INTRODUCTION

Technetium-99m (^{99m}Tc) is one of the most desirable radionuclides for external imaging in diagnostic nuclear medicine, due to the emission of gamma ray of optimal energy (140 keV), a suitable half-life (6 h), and availability from ^{99}Mo-^{99m}Tc generator systems. In addition, development of ^{99m}Tc radiopharmaceuticals for tumor imaging paves the way for therapeutic radiopharmaceuticals with high energy beta emitters ^{186}Re and ^{188}Re because of similar chemical properties between technetium and rhenium.

Technetium (Z = 43) is situated in the middle of the second-row transition series and has no stable isotopes, which retarded the development of its chemistry. At the initial stage of ^{99m}Tc radiopharmaceutical development, major efforts were focused on imaging bone and excretory organs such as the liver and kidney, since it was thought that Tc is a foreign substance and recognized as such by the body. In 1982, Yokoyama et al. reported that a ^{99m}Tc-dithiosemicarbazone complex (a ^{99m}Tc-N_2S_2 type complex) with a glucose backbone (Fig. 1A) could penetrate the intact blood-brain barrier of laboratory animals.[1,2] They also reported that ^{99m}Tc-dithiosemicarbazone complexes appended with a tertiary (Fig. 1B) or a quarternary amine group showed myocardial uptake.[1,3] These findings encouraged further efforts to develop ^{99m}Tc radiopharmaceuticals for brain and myocardial functions. Meanwhile, the Davison and Jones group made another important finding in technetium chemistry, demonstrating that pentavalent oxotechnetium (5+) forms a five-coordinated mononuclear complex of high stability with a N_2S_2 ligand (Fig. 1C).[4] They also developed the first organometallic low-oxidation state Tc(I) hexakis(isonitrile) complex (Fig. 1D), which constituted a prototype compound for the currently used myocardial perfusion agent, ^{99m}Tc(I) hexakis(2-methoxyisobutylisonitrile).[5] These chemical studies stimulated the development of another class of mononuclear

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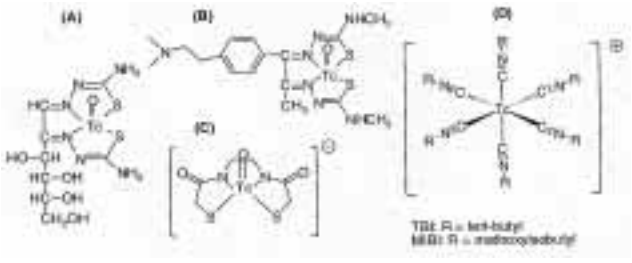

Fig. 1 ^{99m}Tc complexes that paved the way for currently available ^{99m}Tc radiopharmaceuticals. (A) ^{99m}Tc-dithiosemicarbazone (N_2S_2) derivative of glucose, (B) ^{99m}Tc-dithiosemicarbazone (N_2S_2) derivatives of tertiary amine, (C) oxo^{99}Tc(V) complex of containing a $^{99}TcON_2S_2$ core, and (D) hexakis(isocyanide) complex of ^{99}Tc(I).

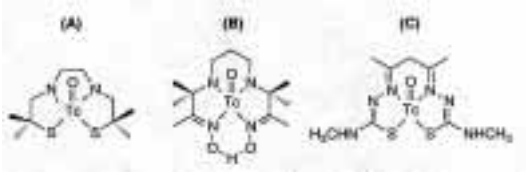

Fig. 2 Chemical structures of neutral, lipophilic and stable mononuclear ^{99m}Tc complexes that cross the intact blood-brain barrier. (A) ^{99m}Tc complexes of diaminodithiolate (DADT or BAT), (B) propylene amine oxime (PnAO), and (C) bis(thiosemicarbazone).

technetium complexes[6,7] including nitrido ^{99m}Tc complexes of dithiocarbamate and trans-dioxo Tc(V) complex of cyclam, which provided a good basis for the development of currently available ^{99m}Tc radiopharmaceuticals.

In this paper, progress in developing ^{99m}Tc radiopharmaceuticals for perfusion imaging is first mentioned, then, ^{99m}Tc radiopharmaceuticals for receptor and transporter functions follow, since the chemistry developed for ^{99m}Tc perfusion agents provided the basic structures for designing the latter. Progress in protein- and peptide-based ^{99m}Tc radiopharmaceuticals is then briefly reviewed. In the last part of this review, new approaches for chemical design of new radiopharmaceuticals are described.

2. ^{99m}Tc RADIOPHARMACEUTICALS FOR PERFUSION IMAGING

2-1. Regional Cerebral Blood Flow

To determine regional cerebral blood flow, coordination molecules (ligands) were required to provide ^{99m}Tc complexes that can penetrate the intact blood-brain barrier (BBB) in response to the blood flow. A variety of ligands were developed that form mononuclear, neutral, lipophilic and stable ^{99m}Tc complexes. These include diaminodithiolate (DADT or BAT),[8] bis(thiosemicarbazone) derivatives (DTS),[9,10] propylene amine oxime (PnAO),[11] as illustrated in Figure 2. Although all ^{99m}Tc complexes prepared from these ligands showed to penetrate the intact BBB with significant uptake in the brain, they were also marked by rapid wash out from the brain, due to a lack of an appropriate functional group that fixed the ^{99m}Tc complexes in the brain. The success in measuring cerebral blood flow by radioiodinated compounds with amine derivatives such as [^{123}I]*N*-isopropyl-iodoamphetamine ([^{123}I]IMP) and [^{123}I]*N,N,N′*-trimethyl-*N′*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propane diamine ([^{123}I]HIPDM)[12,13] stimulated an incorporation of an amine functional group to the neutral and lipophilic mononuclear ^{99m}Tc complexes. This chemical modification provided ^{99m}Tc complexes with longer cerebral residence times (e.g., an *N*-piperidinylethyl derivative of ^{99m}Tc-DADT). However, such chemical modification resulted in the formation of two ^{99m}Tc complexes with different biological characteristics.[14] Chemical studies with ^{99}Tc revealed that the two ^{99m}Tc complexes were *syn* and *anti* isomers (one isomer has the amine group located *syn* to the Tc oxo core whereas the other has the amine group located *anti* to the Tc oxo core), as shown in Figure 3, so that the formation of isomers in relation to the central $^{99m}TcO^{3+}$ core constituted a major disadvantage in this chemical design of ^{99m}Tc radiopharmaceuticals for cerebral perfusion agents. In 1989, Walovitch et al. found that *N,N′*-1,2-ethanediylbis-L-cysteine diethylester (ECD) (Fig. 4A) overcame the problem associated with the isomer formation and provided excellent uptake and retention characteristics for SPECT imaging of the brain.[15] Metabolic studies showed that the brain retention of ^{99m}Tc-ECD would be the result of the rapid *in vivo* brain metabolism to a more polar monoester-monoacid metabolite which is trapped in the brain. Although high initial brain uptake was observed with ^{99m}Tc-D,D-ECD isomer, rapid elimination from the brain was observed, suggesting enzyme-mediated hydrolysis of the ester in ^{99m}Tc-ECD. Clinical studies showed comparable results to those obtained by PET brain perfusion agent.

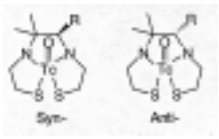

Fig. 3 Two stereoisomers of *N*-substituted ^{99m}Tc complexes of DADT (BAT).

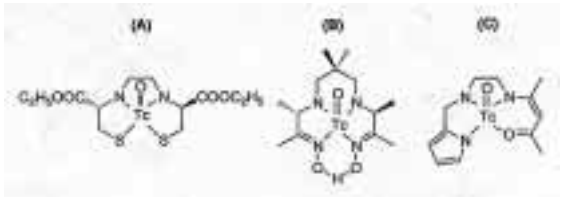

Fig. 4 Chemical structures of brain perfusion agents. (A) ^{99m}Tc-L,L-ECD, (B) ^{99m}Tc-HM-PAO, and (C) ^{99m}Tc-MRP-20.

Meanwhile, based on previous findings that 3,3′-(1,3-

propanediyldiimino)bis(3-methyl-2-butanone)dioxime (PnAO) (Fig. 2B) forms macrocyclic complexes with Ni^{2+} and Co^{3+},[16] Fair et al. synthesized and characterized a technetium complex of PnAO as a neutral complex with a square pyramidal structure.[17] Volkert et al. found that ^{99m}Tc-PnAO passively penetrated the intact blood-brain-barrier in laboratory animals with a first-pass efficiency of 80%, followed by rapid wash out from the brain.[11] In a search for a ligand which not only transports ^{99m}Tc across the BBB, but allows the radiotracer to be retained with a fixed distribution for a time sufficient to permit SPECT imaging, Neirinckx et al. selected d,l-diastereoisomer of hexamethyl propyleneamine oxime (HM-PAO) (Fig. 4B).[18] ^{99m}Tc-HM-PAO showed rapid uptake in the brain, followed by high retention in the brain up to 24 h postinjection, due to the conversion of lipophilic ^{99m}Tc-HM-PAO to more hydrophilic secondary complexes. Further studies showed that intracerebral glutathione (GSH) may play an important role in the *in vivo* conversion of ^{99m}Tc-HM-PAO to hydrophilic forms and may be the mechanism for trapping the ^{99m}Tc complex in brain and other cells.[19]

Morgan et al. developed another type of neutral, lipophilic ^{99m}Tc complex with *N*-(2-(1H pyrolylmethyl)*N′*-(4-pentene-3-one-2))ethane-1,2-diamine (MRP-20) as the ligand.[20,21] The chemical structure of this complex is shown in Figure 4C. ^{99m}Tc-MRP-20 showed good brain uptake retention after administration. It was suggested that MRP-20 would be hydrolyzed to tridentate ligand in the brain, which would account for the brain retention of the complex.

Another class of neutral, lipophilic ^{99m}Tc complexes for brain perfusion were recently developed by Mastrostamatis et al., who prepared mixed ligand ^{99m}Tc complexes of the general formula $^{99m}TcOL^1L^2$, where L^1H_2 is an *N*-substituted bis(2-mercaptoethylamine) and L^2H is a monodentate thiol.[22] The tridentate ligands react with the $^{99m}TcO^{3+}$ core, leaving open one *cys* site to the oxo group in the coordination sphere of the metal. The neutral mixed ligand ^{99m}Tc(V) complexes can be formed by occupation of the open site by a monodantate ligand, as shown in Figure 5. The complexes were formed by ligand exchange reaction with ^{99m}Tc-glucoheptonate as precursor and equimolar quantities of the two ligands. Most of the ^{99m}Tc complexes in this series showed high initial brain uptake, and this uptake remained nearly constant for more than 30 min.[23] The retention mechanism of ^{99m}Tc complexes of this type would involve rapid nucleophilic attack by intracerebral glutathione (GSH) on the complex metal center, substitution of the monodentate ligand by GS-, and formation of the hydrophilic [^{99m}Tc](SNS/SG) metabolite.[24,25] Since the GSH concentration in the brain decreases in the diseased state, ^{99m}Tc complexes of this type may also be useful to assess intracerebral GSH levels by selecting appropriate tridentate and monodentate ligands.

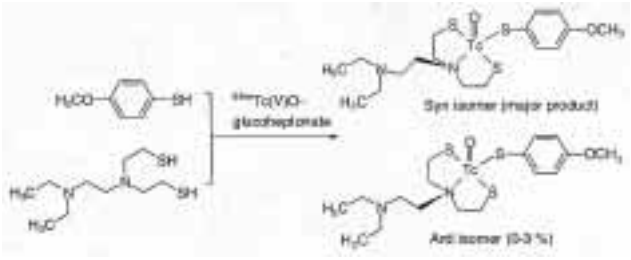

Fig. 5 The "3 + 1" mixed ligand concept for ^{99m}Tc brain perfusion agent.

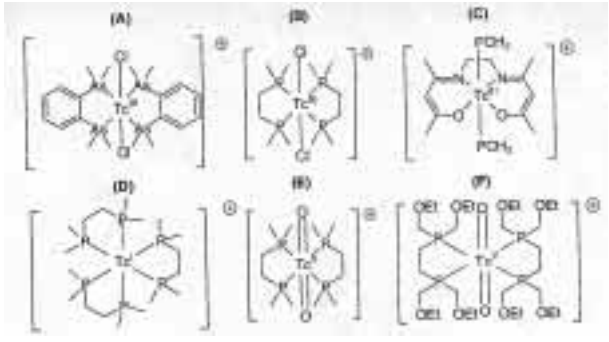

Fig. 6 ^{99m}Tc cationic complexes with diarsine or diphosphine ligand for measuring myocardial perfusion. (A) $[^{99m}Tc(III)(DIARS)_2Cl_2]^+$, (B) $[^{99m}Tc(III)(DIMPE)_2Cl_2]^+$, (C) [^{99m}Tc(III)(*N,N′*-ethylenebis(acetylacetoneiminato))bis(triphenylphosphine)]$^+$, (D) $[^{99m}Tc(I)(DMPE)_3]^+$, (E) $[^{99m}Tc(V)O_2(DMPE)_2]^+$, and (F) $[^{99m}TcO_2(tetrofosmin)]^+$.

2-2. Myocardial Perfusion Agents

In a search for ^{99m}Tc radiopharmaceuticals that provide diagnostic information compatible to those of ^{201}Tl, efforts were made to synthesize cationic ^{99m}Tc complexes with appropriate lipophilicity so that the complexes can reflect myocardial blood flow.

The first cationic ^{99m}Tc chelate that exhibited significant myocardial uptake in animals was Tc complexes of *tr*-$[^{99m}Tc(DIARS)_2X_2]^+$, where DIARS represents the *o*-phenylenebis(dimethylarsine) ligand and X represents chloride or bromide,[26] as shown in Figure 6A. Since the lipophilicity of the complexes was considered too high, emphasis shifted to less lipophilic analogs. The displacement of the arsine ligands with diphosphine ligands such as 1,2-bis(dimethylphosphino)ethane (DMPE) provided cationic ^{99m}Tc complexes of *tr*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ (Fig. 6B).[27] Although this ^{99m}Tc complex accumulated in the myocardium of laboratory animals, low myocardial uptake with intense hepatic activity levels of this complex hampered its further clinical application. This interference appeared to be caused by *in vivo* reduction of the cationic ^{99m}Tc complexes to their neutral ^{99m}Tc(II) form, $[^{99m}Tc(II)(DMPE)_2Cl_2]^0$.[28] In order to avoid the deleterious effects of *in vivo* reduction, a new class of non-reducible Tc cation was developed (Fig. 6C),[29] which provided diagnostic accuracy comparable to that of ^{201}Tl

for overall coronary disease detection and detection of individual coronary artery stenosis in patients.[30]

During the studies, it was also found that besides the ^{99m}Tc(III) complex, DMPE ligand formed $[^{99m}Tc(I)(DMPE)_3]^+$ (Fig. 6D) and $[^{99m}Tc(V)O_2(DMPE)_2]^+$ (Fig. 6E) complexes.[31] Kelly et al. explored the possibility that the limitations encountered with the ^{99m}Tc(III) DMPE complex might be overcome by the introduction of hetero-atomic functions to modify non-target uptake. They synthesized cationic and lipophilic ^{99m}Tc complex of diphosphine ligand, 1,2-bis[bis(2-ethoxyethylene) phosphino]ethane (tetrofosmin) with a $^{99m}TcO_2$ core as shown in Figure 6F.[32] Preclinical studies showed that ^{99m}Tc-tetrofosmin had good heart uptake and retention, together with rapid clearance from the liver, lung and blood. Clinical studies also showed that ^{99m}Tc-tetrofosmin produced high quality myocardial images from 5 min to several hours postinjection.[33]

Another type of cationic ^{99m}Tc complex was developed by Jones et al. who prepared organometallic ^{99m}Tc(I) hexakis(isonitrile) complexes.[34,35] The Tc(I) oxidation state is particularly advantageous because of the kinetic inertness inherent in its low-spin d_6 configuration. After extensive structure-distribution studies of the isonitrile derivatives, hexakis(2-methoxy-isobutylisonitrile; MIBI) technetium(I) shown in Figure 1D was found to improve the biodistribution properties when compared with the prototype compound, hexakis(tert-butylisonitrile) technetium(I), due to rapid clearance of radioactivity from the liver and lung. This could be attributed to the metabolism of the ether groups in MIBI ligand to hydrophilic hydroxyl groups. Further studies showed that the fundamental myocellular uptake mechanism of ^{99m}Tc-MIBI involves passive distribution across plasma and mitochondrial membranes and that at equilibrium ^{99m}Tc-MIBI is sequestered within mitochondria by the large negative transmembrane potentials.[36–38]

While most of the myocardial perfusion agents possess cationic and lipophilic characteristics, another effort was made to design myocardial perfusion agents with neutral and lipophilic ^{99m}TcN complexes. The first complex with a TcN triple bond was reported by Baldas et al. in 1981, who synthesized and characterized bis(diethyldithiocarbamato)nitridotechnetium(V) complex as shown in Figure 7A.[6] Pasqualini et al. investigated the chemical and biological properties of ^{99m}TcN complexes of dithiocarbamate, and found that the ^{99m}TcN complexes were prepared through a simple procedure involving the initial reaction of $^{99m}TcO_4^-$ with *S*-methyl *N*-methyl dithiocarbazate, in the presence of a reducing agent such as $SnCl_2$, followed by the addition of the sodium salt of the ligand. The resulting complexes remained stable over a period of 6 h and the complexes localized selectively in the myocardial of experimental animals.[39] They also found that ^{99m}TcN complex of bis(*N*-ethyl, *N*-ethoxy dithiocarbamate) (NOET) (Fig. 7B) exhibited redstribution after reflow, which provided diagnostic information similar to that of ^{201}Tl,[40,41] although, ^{99m}Tc-NOET had subcellular distribution profiles different from those of ^{201}Tl.[42]

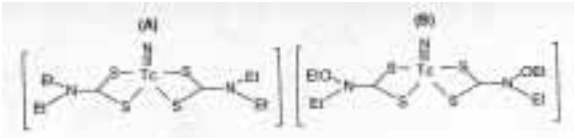

Fig. 7 Chemical structures of Tc(V)-nitrido complexes. (A) bis(diethyldithio-carbamato)nitrido technetium(V) and (B) ^{99m}Tc-NOET.

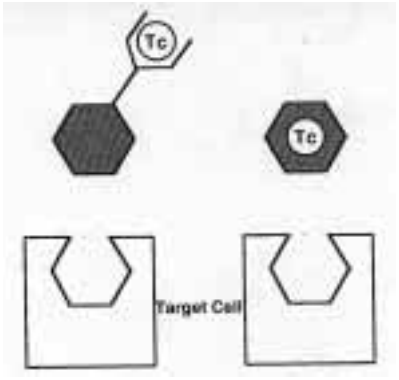

Fig. 8 Schematic drawing of two chemical approaches to design ^{99m}Tc-labeled receptor-specific radiopharmaceuticals.

3. ^{99m}Tc RADIOPHARMACEUTICALS FOR TARGETED IMAGING

A large number of organic compounds labeled with ^{11}C, ^{18}F and ^{123}I have been designed and synthesized as radiopharmaceuticals for targeted imaging of receptors, transporters and enzymes. Owing to progress in technetium chemistry, further efforts were directed toward the design and development of ^{99m}Tc radiopharmaceuticals. The chemical design of ^{99m}Tc compounds for targeted imaging is conceptually divided into two approaches.

One constitutes conjugating inert ^{99m}Tc complexes to carrier molecules that possess high and specific binding abilities to target cells. The carrier molecules vary form high molecular weight polypeptides such as monoclonal antibodies to small molecular weight compounds such as receptor antagonists. Another strategy involves ^{99m}Tc complexes that acquire biological abilities to localize the target tissues after complexation with appropriate ligands that do not localize to their targets in the absence of the Tc. The former approach is named bifunctional radiopharmaceutical or conjugated (or tethered) design, and the latter is called integrated design. The two approaches of ^{99m}Tc radiopharmaceuticals are schematically shown in Figure 8 with receptor-specific ^{99m}Tc complexes as models.

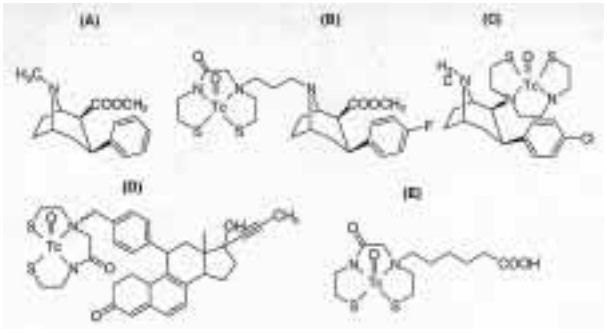

Fig. 9 Conjugated design of ^{99m}Tc-labeled compounds. Dopamine transporter imaging agents (B) Technephine and (C) TRODAT-1 were designed by replacing methyl or carboxylate group of cocaine (A). Conjugated design was also applied to prepare (D) steroid hormone analog and (E) medium chain fatty acid analog recognized as substrate for energy production.

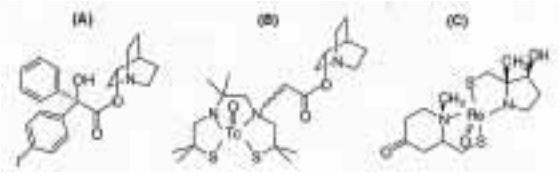

Fig. 10 Integrated design of ^{99m}Tc-labeled compounds. ^{99m}Tc labeled QNB analog (B) for muscarinic acetylcholine receptor was synthesized by replacing iodobenzen group of iodinated QNB (A) with a neutral and lipophilic ^{99m}Tc-DATD chelate. The B and C rings of steroid hormone were mimicked with Re chelate ring structure after complexation with two NS ligands (C).

3-1. Bifunctional Radiopharmaceuticals (Conjugated or Tethered Design)

This design involves the attachment of a stable and neutral ^{99m}Tc chelate to a compound that possesses a specific localization mechanism to target tissues (e.g. receptors, transporters and enzymes). This approach is analogous to the strategy employed for proteins and peptide labeling with ^{99m}Tc. The potential of this chemical design was indicated by Yokoyama et al., who succeeded in manipulating the *in vivo* behavior of ^{99m}Tc-dithiosemicarbazone (DTS) complexes by varying functional groups appended to the ligand skeleton.[43–45] When the chemical design is further applied to small molecular weight compounds, strategic replacement of ^{99m}Tc chelate moiety at a sterically tolerant site of mother molecule plays a critical role in order in minimizing the loss of the original bioactivity. Representative ^{99m}Tc compounds based on the chemical design were clearly demonstrated by dopamine transporter imaging agents simultaneously reported by two research groups.[46,47] Both tropane derivatives were designed to conjugate a ^{99m}Tc chelate of N_2S_2 ligand to a tropane molecule at different sites (Technephine: Fig. 9B and TRODAT-1; Fig. 9C). In biodistribution studies, both compounds cross the intact BBB and bind to the dopamine presynatpic transporter, indicating that both sites in tropane molecule possess steric tolerance to substitution with neutral and lipophilic ^{99m}Tc chelate. Specific uptake of TRODAT-1 in dopamine transporter located in the basal ganglia was demonstrated in human studies.[47] A recent clinical study suggested that TRODAT-1 may serve as a useful imaging agent for the early detection of Parkinson's disease.[48]

Katzenellenbogen and co-workers synthesized a series of progestins conjugated with neutral ^{99m}Tc chelates at positions known to tolerate sizeable substitution.[49,50] They first conjugated a ^{99m}Tc-DADT ligand to progestin by alkylating one of the secondary amines of the ligand. Besides the formation of isomers, this compound suffered from high non-specific binding to sites in liver and adipose tissue, presumably due to high lipophilicity of the resulting compound.[50] In a follow-up study, they reduced the lipophilicity of the whole molecule by replacing the ^{99m}Tc-DTDT complex with ^{99m}Tc-MAMA (monoamine monoamide dithiols) chelate (Fig. 9D). This compound showed higher affinity and had specific receptor-mediated uptake in rat uterus. However, high uptake of this compound was still observed in non-target tissues such as the liver.[51]

The conjugated design was also applied to ^{99m}Tc compounds for metabolic studies. Yamamura et al. conjugated the MAMA ligand to the ω-position of hexanoic acid (HA), as shown in Figure 9E. They showed that ^{99m}Tc-MAMA-HA was metabolized by beta-oxidation to ^{99m}Tc-MAMA-butyric acid in the liver, indicating that the ^{99m}Tc complex was recognized as a substrate for energy production by hepatocytes.[52]

3-2. Integrated Design

The integrated design constitutes another approach in designing and developing ^{99m}Tc radiopharmaceuticals for targeted imaging. In this design, a neutral, lipophilic and stable ^{99m}Tc chelate is mimicked as a part of the lipophilic moiety of parent compounds. By incorporating the ^{99m}Tc unit into the bioactive molecule, the steric bulk and the resulting mass of the ^{99m}Tc-based ligand are much lower than those of a comparable conjugated ^{99m}Tc compounds. Based on this concept, a ^{99m}Tc complex of *R*-3-quinuclidinl-benzilate (QNB) derivatives (Fig. 10B) was designed as a ligand for muscarinic acetylcholine receptor in the hope that ^{99m}Tc-DADT ligand could act as the diphenyl methanol portion of QNB (Fig. 10A).[53] In animal studies, this complex penetrated the BBB and showed some limited affinity for the receptor. A heterodimer of two different amino thiol derivatives was also applied to design ^{99m}Tc-labeled steroid analogs by forming a mixed ligand chelate (Fig. 10C).[54,55] It was thought that the general structure of the N_2S_2 complex of oxotechnetium(V) could replace the BC ring system of steroid so that a metal complex system of considerable size and shape resembling a steroid may be prepared. Although further studies

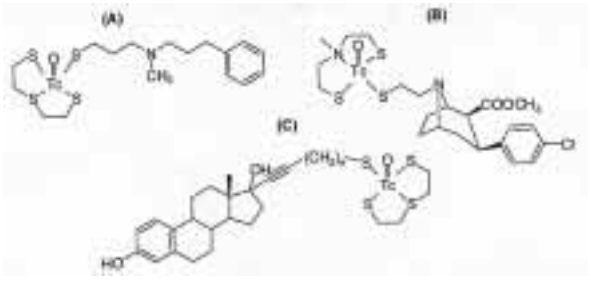

Fig. 11 Integrated design of ^{99m}Tc-labeled compounds by "3 + 1" mixed ligand approach for imaging (A) serotonin receptor, (B) dopamine transporter, and (C) estrogen receptor.

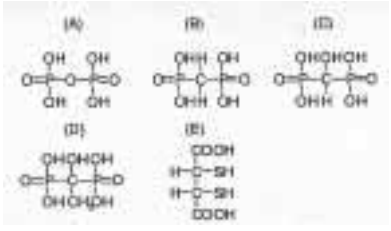

Fig. 12 Chemical structures of representative ligands for ^{99m}Tc polynuclear complexes. (A) pyrophosphate, (B) methylenediphosphonate, (C) hydroxymethylenediphosphonate, (D) 1-hydroxyethylidenediphosphonate, and (E) dimercaptosuccinic acid.

are required for optimizing ^{99m}Tc chelate structures, this approach may pave another way to designing ^{99m}Tc radiopharmaceuticals for targeted imaging.

The "3 + 1" mixed ligand complex was also applied to ^{99m}Tc radiopharmaceutical design for targeted imaging. Starting from the lead structure of ketanserin, a prototypic serotonin (5-HT) antagonist, Johannsen et al. synthesized a new oxotechnetium(V) complex.[56] A lipophilic complex consisting of a small S4 thiolate/thioether chelate unit, protonable nitrogen-containing spacer, and simple benzyl moiety (Fig. 11A) significantly inhibited the specific binding of ketanserin with IC_{50} values between 10 and 50 nM. Meeglla et al. applied this approach to prepare a ^{99m}Tc-tropane analog for dopamine transporter imaging (Fig. 11B).[57] This approach was also applied to such rhenium complexes as estrogen and mimics (Fig. 11C).[58]

4. ^{99m}Tc POLYNUCLEAR COMPLEXES

^{99m}Tc polynuclear complexes constitute another approach for targeted imaging. ^{99m}Tc complexes of bisphosphonate (diphosphonate), the most widely used radiopharmaceuticals in clinical studies, involve representative compounds of this class. They accumulate in bone, especially at sites of high calcium turnover in new actively growing or cancerous bone with easily accessible unsaturated coordination sphere of Ca^{2+} in the basic bone structure hydroxyapatite, which render the radiopharmaceuticals useful for visualizing various skeletal cancers.[59,60] The first ^{99m}Tc-bisphosphonate to be developed was the chelate with tripolyphosphate.[61] Soon it was demonstrated that the functional species in the ^{99m}Tc complexes would be a ^{99m}Tc-pyrophosphate. Although ^{99m}Tc-pyrophosphate is still used, most ^{99m}Tc-labeled bone agents utilize bisphosphonate ligands, where the metabolically unstable P-O-P backbone of pyrophosphate is replaced with a stable P-C-P backbone. Representative bisphosphonate ligands currently used for ^{99m}Tc bone imaging agents are shown in Figure 12A–12D.

It is known that bisphosphonates have strong binding affinity for Ca^{2+}. Bisphosphonates also act as bidentate chelating agents to form ^{99m}Tc polynuclear complexes.[62] In addition, the reaction of Co(ethylenediamine)$_2$ (bisphosphonate) complexes with Ca^{2+} indicated that a coordinated bisphosphonate can bind Ca^{2+} very strongly in an aqueous solution.[63] These studies suggested that ^{99m}Tc-bisphosphonate complexes accumulate on bone by binding of the coordinated bisphosphonate to calcium in the bone matrix. Thus, the ultimate chemical rationale for bone imaging would be the ability of bisphosphonates to bridge the ^{99m}Tc center and calcium on bone matrix. High accumulation of ^{99m}Tc bisphosphonate to cancerous bone promotes palliative treatment of bone metastases with ^{186}Re complexes of bisphosphonate.[64–67]

In ^{99m}Tc bisphosphonate complexes, bisphosphonate ligands play an important role in bone localization. On the other hand, a ^{99m}Tc-labeled tumor seeking agent was developed using dissociation characteristic of ^{99m}Tc polynuclear complex per se. Based on the findings of hydrolytic polynucleation of gallium citrate as relevant to tumor cell accumulation,[68] ^{99m}Tc polynuclear complexes were designed as radiopharmaceuticals for tumor imaging. After screening of various ligands, dimercaptosuccinic acid (DMS; Fig. 12D) was found to be suitable as a ligand to form a pentavalent technetium polynuclear complex.[69] Studies on tumor accumulation of ^{99m}Tc-DMS indicated that the increase in the tumor uptake of ^{99m}Tc DMS was observed by dilution-induced complex equilibrium displacement.[70,71] A good correlation was also indicated between regional pH distribution of the tumor and regional radioactivity localization; the enhanced radioactivity localization was observed in lower pH tumor tissues. This was supported by a significant increase in tumor accumulation of ^{99m}Tc-DMS when the animals were loaded with a large amount of glucose to facilitate glycocylation in the tumor cells.[72,73] These findings suggested the following mechanisms of tumor localization of ^{99m}Tc-DMS: upon administration of ^{99m}Tc-DMS into the blood stream, ^{99m}Tc complex equilibria are altered by dilution with plasma, and depolymerization or dissociation occurs, inducing the formation of active ^{99m}Tc species or dissociated ^{99m}Tc species capable of crossing the tumor cell membrane and being retained within the cells. The lower pH environment of tumor cells would act as a

trigger to promote the formation of the activated (or dissociated) ^{99m}Tc species. Numerous clinical studies have indicated the usefulness of ^{99m}Tc-DMS for imaging medulally thyroid carcinomas.[74–79] These findings stimulated the synthesis of $^{186/188}$Re analog of DMS for targeted radiotherapy.[80]

5. BIFUNCTIONAL CHELATING AGENTS FOR ^{99m}Tc-LABELED PROTEINS AND PEPTIDES

Radiolabeled proteins have been extensively investigated for imaging thrombosis, infection, inflammation and tumors. Recent genetic engineering has provided a new type of antibody fragment that consists of antibody variable heavy and light regions linked with a short peptide or a disulfide bond to form a single molecule of low molecular weight (LMW).[81–84] Antibody fragments showed much faster pharmacokinetics and even distribution in the tumor mass in a size-related manner.[85,86] The successful visualization of somatostatin-positive tumor by ^{111}In-labeled synthetic somatostatin analog ^{111}In-DTPA-D-Phe^1-octreotide[87,88] stimulated the applications of small molecular weight peptides as alternative vehicles to deliver radioactivity to target tissues. The rapid pharmacokinetics of LMW polypeptides and peptides is well matched to the physical half-life of ^{99m}Tc, and efforts were focused on developing radiolabeling procedures suitable for the biomolecules with ^{99m}Tc. In this section, the advent in chemistry for labeling LMW polypeptides and peptides with ^{99m}Tc is briefly reviewed.

Two procedures have been developed to prepare ^{99m}Tc labeled proteins. The direct coordination of reduced ^{99m}Tc specie(s) to amino acid residues of the proteins constitutes a convenient way to prepare ^{99m}Tc-labeled proteins.[89] This procedure, however, is not applicable to small molecular weight peptides without appropriate chelating sites. In addition, *in vivo* dissociation of initially bound ^{99m}Tc species from the protein molecules was demonstrated[90] so that although easy to prepare even as a kit formulation, only a limited number of studies were performed to prepare ^{99m}Tc-labeled LMW polypeptides and peptides by this procedure.

Another approach involves the attachment of strong chelating groups to protein molecules through the use of the bifunctional chelating agent (BCA), a molecule consisting of a chelating molecule (e.g., DTS, DADT, DADS) and a binding moiety (e.g., carboxylic acid) available to conjugate with polypeptides and peptides of interest. A cyclic derivative of diethylenetriaminepentaacetic acid (cDTPA; Fig. 13A) was introduced as a BCA to prepare ^{99m}Tc-labeled proteins,[91] since the chelator had been applied as the BCA for ^{111}In labeling of proteins.[92] However, this procedure induced intramolecular cross-linking, which reduces the stability of the radiolabel, as demonstrated in ^{111}In-DTPA-conjugated proteins.[93] In order to prepare ^{99m}Tc labeled proteins with high *in vivo* stability without reducing the bioactivity of the original proteins, a large number of BCAs have been developed. *p*-Carboxyethylphenylglyoxal di(*N*-methylthiosemicarbazone) (Fig. 13B) was the first BCA that satisfied the two criteria of ^{99m}Tc labeled antibodies.[94] A variety of BCAs followed that possess N_2S_2, N_3S or N_4 coordination molecules (Fig. 13C–13E) to provide stable ^{99m}Tc-labeled proteins without inducing their biological activities.[95–99] Although the thiol groups in most of the BCA facilitate the formation of mononuclear ^{99m}Tc complexes with high stability, the presence of the free thiol groups may restrict the conjugation reactions with polypeptides since the thiol groups in the chelators may undergo exchange reactions with disulfide bonds in the proteins. To avoid such undesirable side-reactions, protection of the thiol groups in the chelators was performed in conjugation of a N_3S chelating molecule to an antibody fragment, which necessitated a deprotection step before ^{99m}Tc complexation reaction.[100] Thus, thiol-free chelating agents that provide ^{99m}Tc complexes with high stability and high specific activities under mild conditions were required to prepare ^{99m}Tc-labeled LMW polypeptides and peptides of interest for diagnostic applications. Two types of thiol-free chelating agents have now become available; 2-hydrazinonicotinamide (HYNIC; Fig. 14A)[101] and a bis(hydroxyamamide) derivative (Fig. 14B).[102,103]

HYNIC has extensively been applied to ^{99m}Tc labeling of bioactive molecules such as monoclonal antibod-

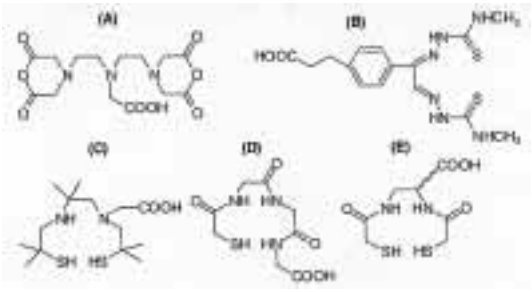

Fig. 13 Bifunctional chelating agents for ^{99m}Tc labeling of proteins. (A) cyclic DTPA dianhydride, (B) CE-DTS, (C) N_2S_2 diaminedithiol (DADT) derivative, (D) N_3S triamidethiol, derivative, and (E) N_2S_2 diamidedithiol (DADS) derivative.

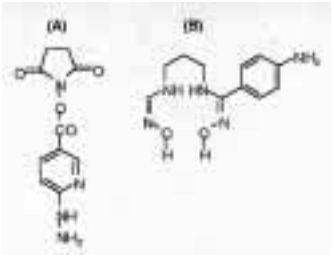

Fig. 14 Thiol-free bifunctional chelating agents for ^{99m}Tc labeling of proteins. (A) *N*-hydroxysuccinimidyl hydrazinonicotinate and (B) bis(hydroxyamamide) derivative.

ies,[101,104,105] chemotactic peptide,[106–108] somatostatin analogs[109–113] and oligonucleotides.[114] Previous studies show that HYNIC acts as a monodantate or bidentate ligand to form a mixed ligand complex with ^{99m}Tc in the presence of two tricine molecules as coligands.[115,116] However, ^{99m}Tc-labeled HYNIC-conjugated peptides included multiple radioactive species, presumably due to different bonding modalities of either the hydrazine moiety of the HYNIC or the two tricine coligands (Fig. 15).[116] In addition, ^{99m}Tc-labeled HYNIC-conjugated peptides and proteins showed slow elimination rates from the blood and persistent localization of the radioactivity in the excretory organs such as the liver and kidney, due to the progression of an exchange reaction of one tricine coligand with proteins in plasma and lysosomes.[117,118] Several coligands were developed to improve the problems associated with ^{99m}Tc-HYNIC labeled peptides when tricine was used as coligand. Recent studies demonstrated the utility and advantages of a ternary ligand system ^{99m}Tc (HYNIC-peptide) (tricine) (L) (L = water soluble phosphine or imine-*N*-consisting heterocycles) (Fig. 16).[119,120] The resulting ^{99m}Tc-HYNIC-peptides contained less radioactive species[119,120] and were eliminated more rapidly from the blood after administration.[121]

Thus, the progress in bifunctional chelating agents now allows us to prepare ^{99m}Tc-labeled proteins and peptides in high radiochemical yields without impairing the original bioactivity of parental biomolecules. These findings may also pave the way to prepare $^{186/188}$Re-labeled proteins and peptides for targeted radiotherapy. Nevertheless, ^{99m}Tc labeled LMW polypeptides and peptides suffer from high and persistent localization of the radioactivity in the kidney, as has been observed with ^{111}In-labeled proteins and peptides. Such radioactivity localization compromises diagnostic accuracy and limits therapeutic application with $^{186/188}$Re as the radionuclides. In other words, ^{99m}Tc-labeled LMW polypeptides and peptides would become much more attractive in diagnostic nuclear medicine if the undesirable radioactivity localization in the kidney could be overcome.

Recently it has become apparent that the persistent localization of the radioactivity after administration of radiolabeled LMW polypeptides and peptides was attributable to slow elimination rates of radiometabolites generated after lysosomal proteolysis of the parental peptides in renal cells.[122–126] Efforts were made to facilitate the elimination of the radiometabolites from the lysosomal compartment to urine through the use of cleavable linkages between antibody fragments and radiolabeled compounds.[100,127,128] Although the rationale behind the radiochemical design is strongly supported by the metabolic studies mentioned above, this approach may also impair target radioactivity levels when applied to LMW polypeptides and peptides that are internalized into target cells.

Meanwhile, studies of renal handling of peptides show that some glomerularly-filtered peptides are hydrolyzed

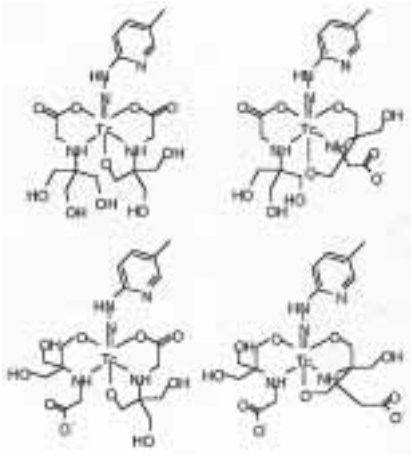

Fig. 15 Possible coordination isomers from the tricine coligand and the hydrazine moiety of HYNIC ligand.

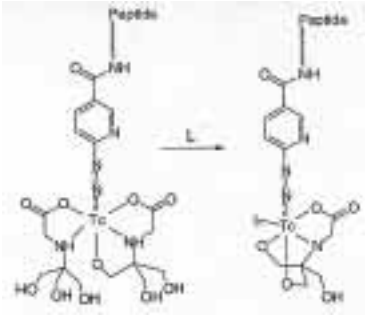

Fig. 16 Possible reaction of ^{99m}Tc(HYNIC)(tricine)$_2$ with imine-N containing heterocyclic coligand (L) to form a ternary mixed ligand of ^{99m}Tc(HYNIC)(tricine)L.

to free amino acid during a short contact time with the brush border enzymes present on the lumen of renal tubules.[129] This suggests that the renal radioactivity levels of peptides would be reduced if radiometabolites of urinary excretion are liberated from parental peptides by the action of brush border enzymes before the peptides are taken up by renal cells. Based on this hypothesis, a glycyl-lysine linker was placed between Fab fragment and *meta*-[^{125}I]benzoic acid to release *meta*-[^{125}I]iodohippuric acid at the renal brush border, since the glycyl-lysine sequence is a substrate of a brush border enzyme. The chemical design of this approach is shown in Figure 17. Animal studies demonstrated that the glycyl-lysine bond was selectively cleaved in the kidney and the resulting radiometabolite, *meta*-[^{125}I]iodohippuric acid, was rapidly excreted in the urine. This resulted in significantly lower radioactivity levels in the kidney from an early postinjection time onward when compared with directly radioiodinated Fab.[130] Metabolic studies reconfirmed that release of radioiodinated hippuric acid from the antibody fragments took place in the membrane fractions of the renal cells.[131] These findings would provide a good basis for designing ^{99m}Tc-labeled LMW polypeptides and pep-

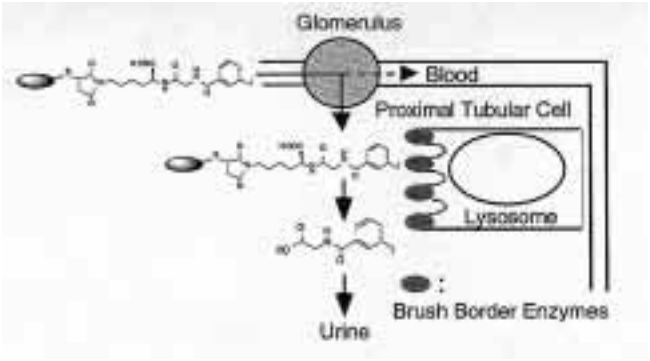

Fig. 17 Chemical design of radiolabeled low molecular weight polypeptides for low renal radioactivity. The chemical linkage (Glycyl-lysine) is cleaved by brush border enzymes present on the lumen of renal tubules before the polypeptide is internalized into renal cells. The resulting iodohippuric acid is rapidly excreted into urine.

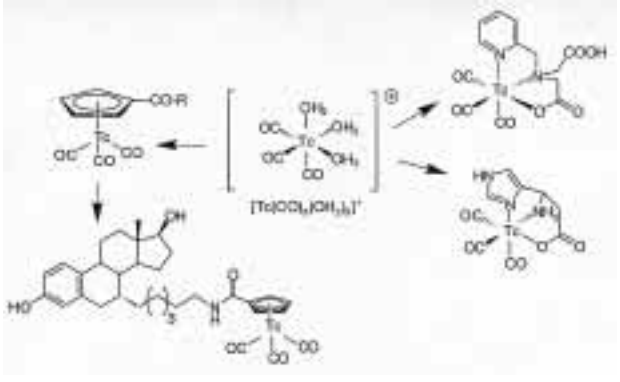

Fig. 18 Chemical reactions of an organometallic technetium(I) complex, $[^{99m}Tc(CO)_3(OH_2)_3]^+$, with a variety of ligands for radiopharmaceutical design.

tides that manifest target-selective radioactivity localization.

6. ORGANOMETALLIC $Tc(CO)_3$ COMPOUNDS

Tremendous efforts are being made to apply organometallic technetium and rhenium complexes to radiopharmaceutical design. Organometallic technetium complexes attracted little attention until the development of Tc(I) isonitrile complexes,[5] which stimulated the chemistry of low oxidation state Tc(I) complexes. Alberto and coworkers recently published a new atomospheric synthesis for complexes $[MX_3(CO)_3]^+$ (M = Tc or Re; X = Cl or Br).[132] These complexes were water-soluble and remained stable in aqueous solution as $[M(OH_2)_3(CO)_3]^+$. They further reported one-step synthesis of the complexes $[M(OH_2)_3(CO)_3]^+$ (M = ^{99m}Tc and ^{186}Re) by direct reduction from [^{99m}Tc]pertechnetate or perrhenate with sodium borohydride in the presence of carbon monoxide in aqueous solution with over 95% yields.[133] $^{99m}Tc(OH_2)_3(CO)_3$ formed stable complexes with histidine and a histidine-conjugated peptide (neurotensin) with high yields and specific activity. The resulting $^{99m}Tc(CO)_3$-conjugated histidine and peptide remained stable *in vivo*.[134] $^{99m}Tc(OH_2)_3(CO)_3$ also formed a neutral complex with picolinamine-*N*,*N*-diacetic acid (PADA) with one carboxylate remaining uncoordinated (Fig. 18). This complex also remained stable in serum or in the presence of cysteine, which rendered the ^{99m}Tc complex applicable to the design of a variety of new ^{99m}Tc radiopharmaceuticals.[135] More recently, Dyszlewski et al. developed a freeze-dry formulation for the preparation of the $^{99m}Tc(CO)_3(OH)_3$ core in aqueous solution.[136]

Cyclopentadienyl (Cp) ^{99m}Tc(I) and ^{186}Re(I) tricarbonyl complexes $[CpM(CO)_3]$ (M = Tc or Re) constitute another interesting precursors for ^{99m}Tc radiopharmaceuticals (Fig. 18). $CpTc(CO)_3$ is a kinetically inert, lipophilic core to which biomolecules can be appended through modification of the Cp ring. Spradau et al. reported a unique synthetic procedure for $CpRe(I)(CO)_3$ by means of a double ligand transfer reaction, which can be applicable to ^{99m}Tc(I).[137] The $CpTc(CO)_3$ was applied to labeling proteins, peptides[138,139] and an estradiol derivative.[140] Although further studies are required to prepare the ^{99m}Tc(I) complex with high radiochemical yields under simple procedures, this class of organometallic ^{99m}Tc(I) complexes may also provide a variety of molecules that are thought "impossible" to radiolabel with ^{99m}Tc without impairing their bioactivity. The organometallic ^{99m}Tc complexes also exhibit chemical and biological characteristics similar to those of $^{186/188}$Re counterparts, which renders the organometallic approach attractive to the design and development of therapeutic agents with $^{186/188}$Re as the radionuclides.

7. CONCLUSION

In this manuscript, recent progress in developing ^{99m}Tc radiopharmaceuticals was briefly reviewed. There is much work that was left out of this review because of space and time limitation. For the past 20 years, great progress in technetium chemistry has contributed to the development of a variety of ^{99m}Tc radiopharmaceuticals that are now used in clinical studies. Future advances in technetium chemistry will also provide ^{99m}Tc radiopharmaceuticals useful for diagnostic nuclear medicine. At the same time, significant knowledge has become available in the design of radiopharmaceuticals based on their characteristics of high sensitivity and extremely low substance amount. The metabolic trapping strategy constitutes a representative approach to the design. This approach has already been applied for quantitative assessment of enzymatic activities in the brain.[141] Chemical design of radiolabeled polypeptides that can reduce renal radioactivity levels may constitute another example of the radiopharmaceuti-

cal design. Future combination of the radiopharmaceutical design and technetium chemistry may pave the way to molecular imaging of a variety of disorders with ^{99m}Tc radiopharmaceuticals. The development of a new ^{99m}Tc radiopharmaceutical is a multidisciplinary effort and should need the collaboration of scientists in a variety of chemical and nuclear medicine fields. Without their joint efforts, nuclear medicine would not be where it is today, nor will it progress.[142]

REFERENCES

1. Yokoyama A, Arano Y, Hosotani T, Yamada A, Horiuchi K, Yamamoto K, et al. Introducing new ^{99m}Tc-bifunctional radiopharmaceutical containing dithiosemicarbazone chelate group. *The Third World Congress in Nuclear Medicine and Biology*, Paris; Pergamon Press, 1982: 1097–1100.
2. Yokoyama A, Yamada A, Arano Y, Horiuchi K, Yamamoto K, Torizuka K. Tc-99m bifunctional radiopharmaceutical from a glucose derivatives: A potential agent for brain study. In: Deutsch E, Nicolini M, Wagner Jr H, eds. *Technetium in Chemistry and Nuclear Medicine*, New York; Raven Press, 1983; 1051–1053.
3. Yokoyama A, Hosotani T, Arano Y, Horiuchi K, Yamamoto K, Torizuka K. Development of Tc-99m-phenylalkylamine bifunctional radiopharmaceuticals for myocardial imaging. In: Deutsch E, Nicolini M, Wagner Jr H, eds. *Technetium in Chemistry and Nuclear Medicine*, New York; Raven Press, 1983; 1067–1069.
4. Davison A, Jones AG, Orvig C, Sohn M. A new class of oxotechnetium (5+) chelate complexes containing a $TcON_2S_2$ core. *Inorg Chem* 1981; 20: 1629–1632.
5. Abrams MJ, Davison A, Jones AG, Costello CE, Pang H. Synthesis and Characterization of hexakis(alkylisocyanide) and hexakis(arylisocyanide) complexes of Technetium(I). *Inorg Chem* 1983; 22: 2798–2800.
6. Baldas J, Bonnyman J, Pojer PM,Williams GA. Synthesis and structure of bis(diethyldithiocarbamato)nitridotechnetium (V): A technetium-nitrogen triple bond. *J Chem Soc Dalton* 1981; 1798–1981.
7. Zuckman SA, Freeman GM, Troutner DE, Volkert WA, Holmes RA, Derveer DGV, et al. Preparation and X-ray structure of trans-dioxo(1,4,8,11-tetraazacyclotetradecane) technetium (V) perchlorate hydrate. *Inorg Chem* 1981; 20: 2286–2289.
8. Kung HF, Molnar M, Billings J, Wicks R, Blau M. Synthesis and biodistribution of neutral lipid-soluble Tc-99m complexes that cross the blood-brain barrier. *J Nucl Med* 1984; 25: 326–332.
9. Arano Y, Yabuki M, Yahata T, Horiuchi K, Yokoyama A. Stable and lipophilic technetium-99m dithiosemicarbazone complexes with 5-6-5 membered chelate ring structure. *Chem Pharm Bull Tokyo* 1990; 38: 3099–3101.
10. Arano Y, Yabuki M, Jones AG, Yokoyama A. Characterization of technetium-99m complexes of pentane-2,4-dione bis(*N*-methylthiosemicarbazone). *Chem Pharm Bull Tokyo* 1991; 39: 104–107.
11. Volkert WA, Hoffman TJ, Seger RM, Troutner DE, Holmes RA. ^{99m}Tc-propylene amine oxime (^{99m}Tc-PnAO); a potential brain radiopharmaceutical. *Eur J Nucl Med* 1984; 9: 511–516.
12. Winchell HS, Baldwin RM, Lin TH. Development of I-123 labeled amines for brain studies: Localization of I-123 Iodophenylalkylamines in rat brain. *J Nucl Med* 1980; 21: 940–946.
13. Kung HF, Tramposch KM, Blau M. A new brain perfusion imaging agent: [I-123]HIPDM: *N,N,N″*-trimethyl-*N′*-(2-hydroxy-3-amethyl-5-iodobenzyl)-1,3-propanediamine. *J Nucl Med* 1983; 24: 66–72.
14. Epps LA, Burns HD, Lever SZ, Goldfarb H, Wagner Jr H. Brain imaging agents: Synthesis and characterization of (*N*-pipeperidylethyl)hexamethyl diaminodithiolate oxo technetium (V) complexes. *Appl Radiat Isot* 1987; 38: 661–334.
15. Walovitch RC, Hill TC, Garrity ST, Cheesman EH, Burgess BA, O'Leary DH et al. Characterization of technetium-99m-L,L-ECD for brain perfusion imaging, Part 1: pharmacology of technetium-99m ECD in nonhuman primates. *J Nucl Med* 1989; 30: 1892–1901.
16. Murmann RK. The interaction of 2-methyl-2-amino-3-butanone oxime with some transition metal. *J Amer Chem Soc* 1957; 70: 521–526.
17. Fair K, Troutner DE, Schiemper EO, Muramann RK, Hoppe ML. Oxo[3,3′-(1,3-propanediyldiimino)bis(3-methyl-2-butanone oximato)(3–)-*N,N′,N″,N‴*]technetium(V), $[TcO(C_{13}H_{25}N_4O_2]$. *Acta Cryst* 1984; C40: 1544–1546.
18. Neirinckx RD, Canning LR, Piper IM, Nowotink DP, Pickett RD, Holmes RA, et al. Technetium-99m d.l-HMPAO: A new radiopharmaceutical for SPECT imaging of regional cerebral blood flow. *J Nucl Med* 1987; 28: 191–202.
19. Neirinckx RD, Burke JF, Harrison RC, Forster AM, Andersen AR, Lassen NA. The retention mechanism of technetium-99m-HM-PAO: intracellular reaction with glutathione. *J Cereb Blood Flow Metab* 1988; 8: S4–12.
20. Morgan GF, Deblaton M, Vandenbroeck P, Bastin B, Pirotte R, Michel P, et al. Synthesis and biological studies of neutral technetium-(V) complexes containing NNOS donor sets. *Nucl Med Biol* 1992; 19: 65–72.
21. Morgan GF, Deblaton M, Clemens P, Broeck PVD, Bossuyt A, Thornback JR. Technetium-99m-MRP20, a potential brain perfusion agent: *In vivo* biodistribution and SPECT studies in non-primate animals. *J Nucl Med* 1991; 32: 500–505.
22. Mastrostamatis SG, Papadopoulos MS, Pirmettis IC, Paschali E, Varvarigou AD, Stassinopoulou CI, et al. Tridentate ligands containing the SNS donor atom set as a novel backbone for the development of technetium brain-imaging agents. *J Med Chem* 1994; 37: 3212–3218.
23. Pirmettis IC, Papadopoulos MS, Chiotellis E. Novel ^{99m}Tc aminobisthiolato/monothiolato "3 + 1" mixed ligand complexes: structure-activity relationships and preliminary *in vivo* validation as brain blood flow imaging agents. *J Med Chem* 1997; 40: 2539–2546.
24. Pelecanou M, Piemettis CC, Nock BA, Papadopoulos M, Chiotellis E, Stassinopoulou CI. Interation of [ReO(SNS)(S)] and [^{99m}TcO(SNS)(S)] mixed ligand complexes with glutathione: isolation and characterization of the product. *Inorg Chim Acta* 1998; 281: 148–152.
25. Nock BA, Maina T, Yannoukakos D, Pirmettis IC,

Papadopoulos MS, Chiotellis E. Glutathione-mediated metabolism of technetium-99m SNS/S mixed ligand complexes: a proposed mechanism of brain retention. *J Med Chem* 1999; 42: 1066–1075.
26. Deutsch E, Glavan KA, Sodd VJ, Nishiyama H, Ferguson DL, Lukes SJ. Cationic Tc-99m complexes as potential myocardial imaging agents. *J Nucl Med* 1981; 22: 897–907.
27. Nishiyama H, Adolph RJ, Deutsch E, Sodd VJ, Libson K, Gerson MC, et al. Effect of coronary blood flow on uptake and washout of Tc-99m DMPE and Tl-201. *J Nucl Med* 1982; 23: 1102–1110.
28. Vanderheyden JL, Heeg MJ, Deutsch E. Comparison of the chemical and biological properties of trans-$(Tc(DMPE)_2Cl_2)^+$ and 1,2-bis(dimethylphosphino) ethane. Single-crystal structural analysis of trans-$(Re(DMPE)_2Cl_2)PF_6$. *Inorg Chem* 1985; 24: 1666–1673.
29. Jurisson S, Dancey KP, McPartlin M, Tasker PA, Deutsch E. Synthesis, characterization, and electrochemical properties of technetium complexes containing both tetradentate schiff base and monodentate tertiary phosphine ligands: Single-crystal structure of trans-(*N,N′*-ethylene-bis(acetylacetone iminato)bis(triphenyl-phosphine) technetium (III) hexafluorophosphate. *Inorg Chem* 1984; 23: 4743–4749.
30. Gerson MC, Lukes J, Deutsch E, Biniakiewicz D, Washburn LC, Elgazzar AH, et al. Comparison of technetium-99m-Q_3 and thallium-201 for detection of coronary artery disease in humans. *J Nucl Med* 1994; 35: 580–586.
31. Vanderheyden JL, Ketering AR, Libson K, Heeg ZJ, Roecker L, Motz P, et al. Synthesis and characterization of cationic technetium complexes of 1,2-bis(dimethylphosphino)ethane (DMPE). Structure determinations of trans-$[TcV(DMPE)_2(OH)(O)]$ $(F_3CSO_3)_2$, trans-$[TcIII(DMPE)_2Cl_2]F_3CSO_3$, and $[TcI(DMPE)_3]^+$ using X-ray diffration, EXAPS, and ^{99}Tc NMR. *Inorg Chem* 1984; 23: 3184–3191.
32. Kelly JD, Forster AM, Higley B, Archer CM, Booker FS, Canning LR, et al. Technetium-99m-tetrofosmin as a new radiopharmaceutical for myocardial perfusion imaging. *J Nucl Med* 1993; 34: 222–227.
33. Higley B, Smith FW, Smith T, Gemmell HG, Das Gupta P, Gvozdanovic DV, et al. Technetium-99m-1,2-bis[bis(2-ethoxyethyl) phosphino]ethane: human biodistribution, dosimetry and safety of a new myocardial perfusion imaging agent. *J Nucl Med* 1993; 34: 30–38.
34. Jones AG, Davison A, LaTegola MR, Brodack JW, Orvig C, Sohn M, et al. Chemical and *in vivo* studies of the anion oxo[*N,N′*-ethylenebis(2-mercaptoacetimido)technetate (V). *J Nucl Med* 1982; 23: 801–809.
35. Holman BL, Jones AG, Lister James J, Davison A, Abrams MJ, Kirshenbaum JM, et al. A new Tc-99m-labeled myocardial imaging agent, hexakis(t-butylisonitrile)-technetium(I) [Tc-99m TBI]: initial experience in the human. *J Nucl Med* 1984; 25: 1350–1355.
36. Piwnica-Worms D, Kronauge JF, Chiu ML. Enhancement by tetraphenylborate of technetium-99m-MIBI uptake kinetics and accumulation in cultured chick myocardial cells. *J Nucl Med* 1991; 32: 1992–1999.
37. Piwnica-Worms D, Kronauge JF, Chiu ML. Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. Mitochondrial and plasma membrane potential dependence. *Circulation* 1990; 82: 1826–1838.
38. Chiu ML, Kronauge JF, Piwnica-Worms D. Effect of mitochondrial and plasma membrane potentials on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium(I) in cultured mouse fibroblasts. *J Nucl Med* 1990; 31: 1646–1653.
39. Pasqualini R, Duatti A, Bellande E, Comazzi V, Brucato V, Hoffschir D, et al. Bis(dithiocarbamato) nitrido technetium-99m radiopharmaceuticals: a class of neutral myocardial imaging agents. *J Nucl Med* 1994; 35: 334–341.
40. Ghezzi C, Fagret D, Arvieux CC, Mathieu JP, Bontron R, Pasqualini R, et al. Myocardial kinetics of TcN-NOET: a neutral lipophilic complex tracer of regional myocardial blood flow. *J Nucl Med* 1995; 36: 1069–1077.
41. Fagret D, Marie PY, Brunotte F, Giganti M, Le Guludec D, Bertrand A, et al. Myocardial perfusion imaging with technetium-99m-Tc NOET: comparison with thallium-201 and coronary angiography. *J Nucl Med* 1995; 36: 936–943.
42. Uccelli L, Giganti M, Duatti A, Bolzati C, Pasqualini R, Cittanti C, et al. Subcellular distribution of technetium-99m-N-NOEt in rat myocardium. *J Nucl Med* 1995; 36: 2075–2079.
43. Hosotani T, Yokoyama A, Arano Y, Horiuchi K, Saji H, Torizuka K. Search for Tc-99m labeled DTS bifunctional radiopharmaceutical: role of functional groups in myocardial accumulation. *Appl Radiat Isot* 1986; 37: 505–511.
44. Hosotani T, Yokoyama A, Arano Y, Horiuchi K, Saji H, Torizuka K. Design of bifunctional radiopharmaceutical for the development of Tc-99m complex for myocardial imaging agents. *Nucl Med Biol* 1986; 13: 606–609.
45. Hosotani T, Yokoyama A, Arano Y, Horiuchi K, Wasaki H, Saji H, et al. In the procurement of a neutral and compact monomeric complex of dithiosemicarbazone (DTS) derivative: Tc-99m-KTS. *Int J Nucl Med Biol* 1985; 12: 431–437.
46. Madras BK, Jones AG, Mahmood A, Zimmerman RE, Garada B, Holman BL, et al. Technepine: a high-affinity 99m-technetium probe to label the dopamine transporter in brain by SPECT imaging. *Synapse* 1996; 22: 239–246.
47. Kung HF, Kim H-J, Kung M-P, Meegalla SK, Plösl K, Lee H-K. Imaging of dopamine transporters in humans with technetium-99m TRODAT-1. *Eur J Nucl Med* 1996; 23: 1527–1530.
48. Huang WS, Lin SZ, Lin JC, Wey SP, Ting G, Liu RS. Evaluation of early-stage Parkinson's disease with ^{99m}Tc-TRODAT-1 imaging. *J Nucl Med* 2001; 42: 1303–1308.
49. Dizio JP, Anderson CJ, Davison A, Ehrhardt GJ, Carlson KE, Welch MJ, et al. Technetium-labeled and rhenium-labeled progestins—synthesis, receptor binding and *in vivo* distribution of an 11 beta-substituted progestin labeled with technetium-99 and rhenium-186. *J Nucl Med* 1992; 33: 558–569.
50. DiZio JP, Fiaschi R, Davison A, Jones AG, Katzenellenbogen JA. Progestin-rhenium complexes: metal-labeled steroids with high receptor binding affinity, potential receptor-directed agents for diagnostic imaging or therapy. *Bioconjugate Chem* 1991; 2: 353–366.
51. O'Neil JP, Carlson KE, Anderson CJ, Welch MJ,

Katzenellenbogen JA. Progestin radiopharmaceuticals labeled with technetium and rhenium: synthesis, binding affinity, and *in vivo* distribution of a new progestin N_2S_2-metal conjugate. *Bioconjugate Chem* 1994; 5: 182–193.
52. Yamamura N, Magata Y, Arano Y, Kawaguchi T, Ogawa K, Konishi J, et al. Technetium-99m-labeled medium-chain fatty acid analogues metabolized by beta-oxidation: radiopharmaceutical for assessing liver function. *Bioconjugate Chem* 1999; 10: 489–495.
53. Lever SZ, Baidoo KE, Mahmood A, Matsumura K, Scheffel U, Wagner Jr H. Novel technetium ligands with affinity for the muscarinic cholinergic receptor. *Nucl Med Biol* 1994; 21: 157–164.
54. Chi DY, Katzenellenbogen JA. Selective formation of heterodimeric bis-bidentate aminothiol-oxometal complexes of rhenium(V). *J Am Chem Soc* 1993; 115: 7045–7046.
55. Chi DY, Oneil JP, Anderson CJ, Welch MJ, Katzenellenbogen JA. Homodimeric and heterodimeric bis(amino thiol) oxometal complexes with rhenium(V) and technetium(V)—control of heterodimeric complex formation and an approach to metal complexes that mimic steroid hormones. *J Med Chem* 1994; 37: 928–937.
56. Johannsen B, Scheunemann M, Spies H, Brust P, Wober J, Syhre R, et al. Technetium(V) and rhenium(V) complexes for 5-HT2A serotonin receptor binding: structure-affinity considerations. *Nucl Med Biol* 1996; 23: 429–438.
57. Meegalla S, Plossl K, Kung MP, Chumpradit S, Stevenson DA, Frederick D, et al. Tc-99m-labeled tropanes as dopamine transporter imaging agents. *Bioconjugate Chem* 1996; 7: 421–429.
58. Skaddan MB, Katzenellenbogen JA. Integrated "3 + 1" oxorhenium(V) complexes as estrogen mimics. *Bioconjugate Chem* 1999; 10: 119–129.
59. Francis MD, Slough CL, Tofe AJ. Factors affecting uptake and retention of technetium-99m-diphosphonate and 99m-pertechnetate in osseous, connective and soft tissues. *Calcif Tissue Res* 1976; 20: 303–311.
60. Silberstein EB, Francis MD, Tofe AJ, Slough CL. Distribution of ^{99m}Tc-Sn diphosphonate and free ^{99m}Tc-pertechnetate in selected soft and hard tissues. *J Nucl Med* 1975; 16: 58–61.
61. Subramanian G, McAfee JG. A new complex of ^{99m}Tc for skeletal imaging. *Radiology* 1971; 98: 192–196.
62. Libson K, Deutsch E, Barnett BL. Structural characterization of a ^{99}Tc-diphosphonate complex. Implications for the chemistry of ^{99m}Tc skeletal imaging agents. *J Am Chem Soc* 1980; 102: 2476–2478.
63. Jurisson SS, Benedict JJ, Elder RC, Deutsch E. Calcium affinity of coordinated diphosphonate ligands. Single-crystal structure of $[(en)_2Co(O_2P(OH)CH_2P(OH)O_2]ClO_4$ H_2O. Implication for the chemistry of technetium-99m-diphosphonate skeletal imaging agents. *Inorg Chem* 1983; 22: 1332–1338.
64. Israel O, Keidar Z, Rubinov R, Iosilevski G, Frenkel A, Kuten A, et al. Quantitative bone single-photon emission computed tomography for prediction of pain relief in metastatic bone disease treated with rhenium-186 etidronate. *J Clin Oncol* 2000; 18: 2747–2754.
65. Kucuk NO, Ibis E, Aras G, Baltaci S, Ozalp G, Beduk Y, et al. Palliative analgesic effect of Re-186 HEDP in various cancer patients with bone metastases. *Ann Nucl Med* 2000; 14: 239–245.
66. Liepe K, Franke WG, Kropp J, Koch R, Runge R, Hliscs R. Comparison of rhenium-188, rhenium-186-HEDP and strontium-89 in palliation of painful bone metastases. *Nuklearmedizin* 2000; 39: 146–151.
67. Deklerk JMH, Vanhetschip AD, Zonnenberg BA, Vandijk A, Quirijnen JMSP, Blijham GH, et al. Phase I study of rhenium-186-HEDP in patients with bone metastases originating from breast cancer. *J Nucl Med* 1996; 37: 244–249.
68. Glickson JD, Pitner TP, Webb J, Gams RA. Hydrogen-1 and gallium-71 nuclear magnetic resonance study of gallium citrate in aqueous solution. *J Am Chem Soc* 1975; 97: 1679–1683.
69. Yokoyama A, Hata N, Horiuchi K, Masuda H, Saji H, Ohta H, et al. The design of a pentavalent ^{99m}Tc-dimercaptosuccinate complex as a tumor imaging agent. Int *J Nucl Med Biol* 1985; 12: 273–279.
70. Horiuchi K, Yokoyama A, Endo K, Torizuka K. Tc(V)-DMS tumor imaging agent: Tc-complex dissociation equilibra, a relevant factor in tumor localization. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium in Chemistry and Nuclear Medicine*, Verona; Cortinal International, 1986; 155–159.
71. Horiuchi K, Yomoda I, Ohta H, Endo K, Yokoyama A. Search for polynuclear pentavalent technetium complex of dimercaptosuccinic acid [Tc(V)-DMS] tumour localization mechanism. I. medullary thyroid carcinoma animal model. *Eur J Nucl Med* 1991; 18: 796–800.
72. Horiuchi K, Saji H, Yokoyama A. pH sensitive properties of Tc(V)-DMS: analytical and *in vitro* cellular studies. *Nucl Med Biol* 1998; 25: 689–695.
73. Horiuchi K, Saji H, Yokoyama A. Tc(V)-DMS tumor localization mechanism: a pH-sensitive Tc(V)-DMS-enhanced target/nontarget ratio by glucose-mediated acidosis. *Nucl Med Biol* 1998; 25: 549–555.
74. Shinozaki T, Hirano T, Watanabe H, Aoki J, Takagishi K. Tc-99m (V) dimercaptosuccinic acid scintigraphy for bone and soft tissue lesions. *Clin Nucl Med* 2000; 25: 637–640.
75. Sahin M, Basoglu T, Bernay I, Yapici O, Canbaz F, Yalin T. Evaluation of metastatic bone disease with pentavalent ^{99}Tc^{m}-dimercaptosuccinic acid: a comparison with whole-body scanning and 4/24 hour quantitation of vertebral lesions. *Nucl Med Commun* 2000; 21: 251–258.
76. Shikare S, Bashir K, Menon PS, Bapat RD, Tilve GH. Detection of medullary carcinoma of thyroid, with liver metastasis, using ^{99m}Tc DMSA(V) scintigraphy. *J Postgrad Med* 1995; 41: 12–13.
77. Yuksel D, Ilgan S, Arslan N, Ugur O, Ozturk E, Bayhan H. The role of Tc-99m (V) DMSA scintigraphy in the evaluation of superscan on bone scintigraphy. *Clin Nucl Med* 2000; 25: 193–196.
78. Wang SJ, Lin WY, Wey SP, Shen LH, Ting G. Pentavalent Tc-99m dimercaptosuccinic acid imaging of hepatocellular carcinoma. *Neoplasma* 1999; 46: 246–248.
79. Ohta H, Endo K, Fujita T, Nakajima T, Sakahara H, Torizuka K, et al. Imaging of soft tissue tumors with Tc(V)-99m dimercaptosuccinic acid. A new tumor-seeking agent. *Clin Nucl Med* 1984; 9: 568–573.
80. Horiuchi K, Saji H, Yokoyama A. Carrier effect on radiolabeling the polynuclear pentavalent rhenium-186 com-

plex of dimercaptosuccinic acid at alkaline pH: ^{186}Re(V)-DMS. *Nucl Med Biol* 1999; 26: 771–779.
81. Pantoliano MW, Bird RE, Johnson LS. Conformational stability, folding and ligand-binding affinity of single-chain Fv immunoglobulin fragments expressed in *Escherichia coli*. *Biochemistry* 1991; 30: 10117–10125.
82. Whitlow M, Bell BA, Feng SL, Filpula D, Hardman KD, Hubert SL, et al. An improved linker for single-chain Fv with reduced aggregation and enhanced proteolytic stability. *Protein Engineering* 1993; 6: 989–995.
83. Yazaki PJ, Shively L, Clark C, Cheung CW, Le W, Szpikowska B, et al. Mammalian expression and hollow fiber bioreactor production of recombinant anti-CEA diabody and minibody for clinical applications. *J Immunol Methods* 2001; 253: 195–208.
84. Yazaki PJ, Wu AM, Tsai SW, Williams LE, Ikler DN, Wong JY, et al. Tumor targeting of radiometal labeled anti-CEA recombinant T84.66 diabody and t84.66 minibody: comparison to radioiodinated fragments. *Bioconjugate Chem* 2001; 12: 220–228.
85. Yokota T, Milenic DE, Whitlow M, Schlom J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. *Cancer Res* 1992; 52: 3402–3408.
86. Yokota T, Milenic DE, Whitlow M, Wood JF, Hubert SL, Schlom J. Microautoradiographic analysis of the normal organ distribution of radioiodinated single-chain Fv and other immunoglobulin forms. *Cancer Res* 1993; 53: 3776–3783.
87. Bakker WH, Albert R, Bruns C, Breeman WAP, Hofland LJ, Marbach P, et al. <In-111-DTPA-D-Phe^1>-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors—synthesis, radiolabeling and *in vitro* validation. *Life Sci* 1991; 49: 1583–1591.
88. Bakker WH, Krenning EP, Reubi JC, Breeman WAP, Setyonohan B, Dejong M, et al. *In vivo* application of <In-111-DTPA-D-Phe^1>-octreotide for detection of somatostatin receptor-positive tumors in rats. *Life Sci* 1991; 49: 1593–1601.
89. Mather SJ, Ellison D. Reduction-mediated technetium-99m labeling of monoclonal antibodies. *J Nucl Med* 1990; 31: 692–697.
90. Sakahara H, Saga T, Endo K, Hattori N, Hosono M, Kobayashi H, et al. *In vivo* instability of reduction-mediated ^{99m}Tc-labeled monoclonal antibody. *Nucl Med Biol* 1993; 20: 617–623.
91. Childs RL, Hnatowich DJ. Optimum conditions for labeling of DTPA-coupled antibodies with technetium-99m. *J Nucl Med* 1985; 26: 293–299.
92. Hnatowich DJ, Layne WW, Childs RL, Lanteigne D, Davis MA, Griffin TW, et al. Radioactive labeling of antibody: a simple and efficient method. *Science* 1983; 220: 613–615.
93. Arano Y, Uezono T, Akizawa H, Ono M, Wakisaka K, Nakayama M, et al. Reassessment of diethylenetriaminepentaacetic acid (DTPA) as a chelating agent for indium-111 labeling of polypeptides using a newly synthesized monoreactive DTPA derivative. *J Med Chem* 1996; 39: 3451–3460.
94. Arano Y, Yokoyama A, Furukawa T, Horiuchi K, Yahata T, Saji H, et al. Technetium-99m-labeled monoclonal antibody with preserved immunoreactivity and high *in vivo* stability. *J Nucl Med* 1987; 28: 1027–1033.
95. Baidoo KE, Scheffel U, Lever SZ. ^{99m}Tc Labeling of Proteins: Initial evaluation of a novel diaminedithiol bifunctional chelating agent. *Cancer Res* 1990; 50: 799s–703s.
96. Eisenhut M, Lehmann WD, Becker W, Behr T, Elser H, Strittmatter W, et al. Bifunctional NHS-BAT ester for antibody conjugation and stable technetium-99m labeling: conjugation chemistry, immunoreactivity and kit formulation. *J Nucl Med* 1996; 37: 362–370.
97. Fritzberg AR, Abrams PG, Beaumier PL, Kashina S, Morgan AC, Rao TN, et al. Specific and stable labeling of antibodies with technetium-99m with a diamide dithiolate chelating agent. *Proc Natl Acad Sci USA* 1988; 85: 4025–4029.
98. Liu S, Edwards DS, Looby RJ, Poirier MJ, Rajopadhye M, Bourque JP, et al. Labeling cyclic glycoprotein IIb/IIIa receptor antagonists with Tc-99m by the preformed chelate approach: effects of chelators on properties of [Tc-99m]chelator-peptide conjugates. *Bioconjugate Chemistry* 1996; 7: 196–202.
99. Maina T, Stolz B, Albert R, Bruns C, Koch P, Macke H. Synthesis, radiochemistry and biological evaluation of a new somatostatin analogue (SDZ 219-387) labelled with technetium-99m. *Eur J Nucl Med* 1994; 21: 437–444.
100. Weber RW, Boutin RH, Nedelman MA, Lister James J, Dean RT. Enhanced kidney clearance with an ester-linked ^{99m}Tc-radiolabeled antibody Fab′-chelator conjugate. *Bioconjugate Chem* 1990; 1: 431–437.
101. Abrams MJ, Juweid M, tenKate CI, Schwartz DA, Hauser MM, Gaul FE, et al. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. *J Nucl Med* 1990; 31: 2022–2028.
102. Xu LC, Nakayama M, Harada K, Nakayama H, Tomiguchi S, Kojima A, et al. Synthesis and evaluation of hydroxamamide-based tetradentate ligands as a new class of thiol-free chelating molecules for ^{99m}Tc radiopharmaceuticals. *Nucl Med Biol* 1998; 25: 295–303.
103. Xu LC, Nakayama M, Harada K, Kuniyasu A, Nakayama H, Tomiguchi S, et al. Bis(hydroxamamide)-based bifunctional chelating agent for ^{99m}Tc labeling of polypeptides. *Bioconjugate Chem* 1999; 10: 9–17.
104. Callahan RJ, Barrow SA, Abrams MJ, Rubin RH, Fischman AJ. Biodistribution and dosimetry of technetium-99m-hydrazino nicotinamide IgG: comparison with indium-111-DTPA-IgG. *J Nucl Med* 1996; 37: 843–846.
105. Ultee ME, Bridger GJ, Abrams MJ, Longley CB, Burton CA, Larsen SK, et al. Tumor imaging with technetium-99m-labeled hydrazinonicotinamide-Fab′ conjugates. *J Nucl Med* 1997; 38: 133–138.
106. van der Laken CJ, Boerman OC, Oyen WJ, van de Ven MT, Edwards DS, Barrett JA, et al. Technetium-99m-labeled chemotactic peptides in acute infection and sterile inflammation. *J Nucl Med* 1997; 38: 1310–1315.
107. Babich JW, Tompkins RG, Graham W, Barrow SA, Fischman AJ. Localization of radiolabeled chemotactic peptide at focal sites of Escherichia coli infection in rabbits: evidence for a receptor-specific mechanism. *J Nucl Med* 1997; 38: 1316–1322.

108. Babich JW, Solomon H, Pike MC, Kroon D, Graham W, Abrams MJ, et al. Technetium-99m-labeled hydrazino nicotinamide derivatized chemotactic peptide analogs for imaging focal sites of bacterial infection. *J Nucl Med* 1993; 34: 1964–1974.
109. Pearson DA, Lister James J, McBride WJ, Wilson DM, Martel LJ, Civitello ER, et al. Somatostatin receptor-binding peptides labeled with technetium-99m: chemistry and initial biological studies. *J Med Chem* 1996; 39: 1361–1371.
110. Vallabhajosula S, Moyer BR, James JL, McBride BJ, Lipszyc H, Lee H, et al. Preclinical evaluation of technetium-99m-labeled somatostatin receptor-binding peptides. *J Nucl Med* 1996; 37: 1016–1022.
111. Decristoforo C, Mather SJ. Preparation, ^{99m}Tc-labeling, and *in vitro* characterization of HYNIC and N_3S modified RC-160 and [Tyr^3]octreotide. *Bioconjugate Chem* 1999; 10: 431–438.
112. Decristoforo C, Mather SJ. 99m-Technetium-labelled peptide-HYNIC conjugates: effects of lipophilicity and stability on biodistribution. *Nucl Med Biol* 1999; 26: 389–396.
113. Decristoforo C, Mather SJ. Technetium-99m somatostatin analogues: effect of labelling methods and peptide sequence. *Eur J Nucl Med* 1999; 26: 869–876.
114. Hnatowich DJ, Winnard P Jr, Virzi F, Fogarasi M, Sano T, Smith CL, et al. Technetium-99m labeling of DNA oligonucleotides. *J Nucl Med* 1995; 36: 2306–2314.
115. Larsen SK, Solomon HF, Caldwell G, Abrams MJ. [^{99m}Tc]tricine: a useful precursor complex for the radiolabeling of hydrazinonicotinate protein conjugates. *Bioconjugate Chem* 1995; 6: 635–638.
116. Liu S, Edwards DS, Looby RJ, Harris AR, Poirier MJ, Barrett JA, et al. Labeling a hydrazino nicotinamide-modified cyclic IIb/IIIa receptor antagonist with ^{99m}Tc using aminocarboxylates as coligands. *Bioconjugate Chem* 1996; 7: 63–71.
117. Ono M, Arano Y, Uehara T, Fujioka Y, Ogawa K, Namba S, et al. Intracellular metabolic fate of radioactivity after injection of technetium-99m-labeled hydrazino nicotinamide derivatized proteins. *Bioconjugate Chem* 1999; 10: 386–394.
118. Ono M, Arano Y, Mukai T, Uehara T, Fujioka Y, Ogawa K, et al. Plasma protein binding of ^{99m}Tc-labeled hydrazino nicotinamide derivatized polypeptides and peptides. *Nucl Med Biol* 2001; 28: 155–164.
119. Liu S, Edwards DS, Barrett JA. ^{99m}Tc labeling of highly potent small peptides. *Bioconjugate Chem* 1997; 8: 621–636.
120. Liu S, Edwards DS, Harris AR. A novel ternary ligand system for ^{99m}Tc-labeling of hydrazino nicotinamide-modified biologically active molecules using imine-N-containing heterocycles as coligands. *Bioconjugate Chem* 1998; 9: 583–595.
121. Ono M, Arano Y, Mukai T, Fujioka Y, Ogawa K, Uehara T, et al. ^{99m}Tc-HYNIC-derivatized ternary ligand complexes for ^{99m}Tc-labeled polypeptides with low *in vivo* protein binding. *Nucl Med Biol* 2001; 28: 215–224.
122. Wu C, Jagoda E, Brechbiel M, Webber KO, Pastan I, Gansow O, et al. Biodistribution and catabolism of Ga-67-labeled anti-Tac dsFv fragment. *Bioconjugate Chem* 1997; 8: 365–369.
123. Rogers BE, Franano FN, Duncan JR, Edwards WB, Anderson CJ, Connett JM, et al. Identification of metabolites of ^{111}In-diethylenetriaminepentaacetic acid-monoclonal antibodies and antibody fragments *in vivo*. *Cancer Res* 1995; 55: 5714s–5720s.
124. Akizawa H, Arano Y, Uezono T, Ono M, Fujioka Y, Uehara T, et al. Renal metabolism of ^{111}In-DTPA-D-Phe^1-octreotide *in vivo*. *Bioconjugate Chem* 1998; 9: 662–670.
125. Arano Y. Strategies to reduce renal radioactivity levels of antibody fragments. *Q J Nucl Med* 1998; 42: 262–270.
126. Akizawa H, Arano Y, Mifune M, Iwado A, Saito Y, Uehara T, et al. Significance of ^{111}In-DTPA chelate in renal radioactivity levels of ^{111}In-DTPA-conjugated peptides. *Nucl Med Biol* 2001; 28: 459–468.
127. Bridger GJ, Abrams MJ, Padmanabhan S, Gaul F, Larsen S, Henson GW, et al. A comparison of cleavable and noncleavable hydrazinopyridine linkers for the Tc-99m labeling of Fab′ monoclonal antibody fragments. *Bioconjugate Chemistry* 1996; 7: 255–264.
128. Arano Y, Wakisaka K, Akizawa H, Ono M, Kawai K, Nakayama M, et al. Assessment of the radiochemical design of antibodies with a metabolizable linkage for target-selective radioactivity delivery. *Bioconjugate Chem* 1998; 9: 497–506.
129. Silvernagl S. The renal handling of amino acids and oligopeptides. *Physiol Rev* 1988; 68: 911–1007.
130. Arano Y, Fujioka Y, Akizawa H, Ono M, Uehara T, Wakisaka K, et al. Chemical design of radiolabeled antibody fragments for low renal radioactivity levels. *Cancer Res* 1999; 59: 128–134.
131. Fujioka Y, Arano Y, Ono M, Uehara T, Ogawa K, Namba S, et al. Renal metabolism of 3′-iodohippuryl N^{ε}-maleoyl-L-lysine (HML)-conjugated Fab fragments. *Bioconjugate Chem* 2001; 12: 178–185.
132. Alberto R, Schibli R, Egli A, Schubiger PA, Herrmann WA, Artus G, et al. Metal carbonyl syntheses XXII. Low pressure carbonylation of $[MOCl_4]^-$ and $[MO_4]^-$: the technetium(I) and rhenium(I) complexes $[NEt_4]_2[MCl_3(CO)_3]$. *J Organomet Chem* 1995; 493: 119–127.
133. Alberto R, Schibli R, Egli A, Schubiger AP. A novel organometallic aqua complex of technetium for the labeling of biomolecules: synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from $[^{99m}TcO_4^-]$ in aqueous solution and its reaction with a bifunctional ligand. *J Am Chem Soc* 1998; 120: 7987–7988.
134. Egli A, Alberto R, Tannahill L, Schibli R, Abram U, Schaffland A, et al. Organometallic ^{99m}Tc-aquaion labels peptide to an unprecedented high specific activity. *J Nucl Med* 1999; 40: 1913–1917.
135. Schibli R, La Bella R, Alberto R, Garcia-Garayoa E, Ortner K, Abram U, et al. Influence of the denticity of ligand systems on the *in vitro* and *in vivo* behavior of ^{99m}Tc(I)-tricarbonyl complexes: a hint for the future functionalization of biomolecules. *Bioconjugate Chem* 2000; 11: 345–351.
136. Dyszlewski ME, Bushgman MJ, Alberto R, Brodack JW, Knight H, MacDonald J, et al. Kit formulation and preliminary toxicity of $[^{99m}Tc(CO)_3]^+$ intermediate: a novel technetium radiopharmaceutical platform. *J Labelled Compds Radiopharm* 2001; 44, Suppl 1: S483–S485.

137. Spradau TW, Katzenellenbogen JA. Preparation of cyclopentadienyltricarbonylrhenium complexes using a double ligand-transfer reaction. *Organometallics* 1998; 17: 2009–2017.
138. Spradau TW, Katzenellenbogen JA. Protein and peptide labeling with (cyclopentadienyl)tricarbonyl rhenium and technetium. *Bioconjugate Chem* 1998; 9: 765–772.
139. Spradau TW, Edwards WB, Anderson CJ, Welch MJ, Katzenellenbogen JA. Synthesis and biological evaluation of Tc-99m-cyclopentadienyltricarbonyltechnetium-labeled octreotide. *Nucl Med Biol* 1999; 26: 1–7.
140. Skaddan MB, Wust FR, Jonson S, Syhre R, Welch MJ, Spies H, et al. Radiochemical synthesis and tissue distribution of Tc-99m-labeled 7alpha-substituted estradiol complexes. *Nucl Med Biol* 2000; 27: 269–278.
141. Tanaka N, Fukushi K, Shinotoh H, Nagatsuka S, Namba H, Iyo M, et al. Positron emission tomographic measurement of brain acetylcholinesterase activity using *N*-[^{11}C]methylpiperidin-4-yl acetate without arterial blood sampling: methodology of shape analysis and its diagnostic power for Alzheimer's disease. *J Cereb Blood Flow Metab* 2001; 21: 295–306.
142. Jurisson S, Berning D, Jia W, Ma D. Coordination compounds in nuclear medicine. *Chem Rev* 1993; 93: 1137–1156.