Recent advances in \(^{99m}\text{Tc}\) radiopharmaceuticals

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\(^{99m}\text{Tc}\) radiopharmaceuticals play an important role in widespread applications of nuclear medicine. When \(^{99m}\text{Tc}\) radiopharmaceuticals first came into use, major efforts were directed toward the development of \(^{99m}\text{Tc}\) 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 \(^{99m}\text{Tc}\) radiopharmaceuticals for assessment of regional cerebral and myocardial blood flow. Recent efforts have been directed toward the design of \(^{99m}\text{Tc}\)-labeled compounds for estimating receptor or transporter functions. A number of bifunctional chelating agents that provide \(^{99m}\text{Tc}\) labeled proteins and peptides of high \textit{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 \(^{99m}\text{Tc}\) radiopharmaceutical design. In this manuscript, recent progress in \(^{99m}\text{Tc}\) radiopharmaceuticals is reviewed with the major emphasis laid on key innovations in this field to provide the \(^{99m}\text{Tc}\) radiopharmaceuticals available today.

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

1. INTRODUCTION

Technetium-99m (\(^{99m}\text{Tc}\)) 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 \(^{99}\text{Mo}\)-\(^{99m}\text{Tc}\) generator systems. In addition, development of \(^{99m}\text{Tc}\) radiopharmaceuticals for tumor imaging paves the way for therapeutic radiopharmaceuticals with high energy beta emitters \(^{186}\text{Re}\) and \(^{188}\text{Re}\) 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 \(^{99m}\text{Tc}\) 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 \(^{99m}\text{Tc}\)-dithiosemicarbazone complex (a \(^{99m}\text{Tc}\)-N\(_2\)S\(_2\) type complex) with a glucose backbone (Fig. 1A) could penetrate the intact blood-brain barrier of laboratory animals.\(^{1,2}\) They also reported that \(^{99m}\text{Tc}\)-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 \(^{99m}\text{Tc}\) 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\(_2\)S\(_2\) 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, \(^{99m}\text{Tc}\)-hexakis(2-methoxy-isobutylisonitrile).\(^5\) These chemical studies stimulated the development of another class of mononuclear
In this paper, progress in developing 99mTc radiopharmaceuticals for perfusion imaging is first mentioned, then, 99mTc radiopharmaceuticals for receptor and transporter functions follow, since the chemistry developed for 99mTc perfusion agents provided the basic structures for designing the latter. Progress in protein- and peptide-based 99mTc radiopharmaceuticals is then briefly reviewed. In the last part of this review, new approaches for chemical design of new radiopharmaceuticals are described.

2. 99mTc RADIOPHARMACEUTICALS FOR PERFUSION IMAGING

2-1. Regional Cerebral Blood Flow

To determine regional cerebral blood flow, coordination molecules (ligands) were required to provide 99mTc 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 99mTc complexes. These include diaminodithiolate (DADT or BAT), bis(thiosemicarbazone) derivatives (DTS), propylene amine oxime (PnAO), and bis(thiosemicarbazone) complexes of dithiocarbamate and trans-dioxo Tc(V) complex of cyclam, which provided a good basis for the development of currently available 99mTc radiopharmaceuticals.

Fig. 1 99mTc complexes that paved the way for currently available 99mTc radiopharmaceuticals. (A) 99mTc-dithiosemicarbazone (N₂S₂) derivative of glucose, (B) 99mTc-dithiosemicarbazone (N₂S₂) derivatives of tertiary amine, (C) oxo99Tc(V) complex of containing a 99TcON₃S₂ core, and (D) hexakis(isocyanide) complex of 99Tc(I).

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

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

etrate 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 99mTc complexes in the brain. The success in measuring cerebral blood flow by radioiodinated compounds with amine derivatives such as [123I]N-isopropyl-iodoamphetamine ([123I]IMP) and [123I]N,N,N’-trimethyl-N’-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propane diamine ([123I]HIPDM) stimulated an incorporation of an amine functional group to the neutral and lipophilic mononuclear 99mTc complexes. This chemical modification provided 99mTc complexes with longer cerebral residence times (e.g., an N-piperidinylethyl derivative of 99mTc-DADT). However, such chemical modification resulted in the formation of two 99mTc complexes with different biological characteristics. Chemical studies with 99Tc revealed that the two 99mTc 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 99mTcO³⁺ core constituted a major disadvantage in this chemical design of 99mTc radiopharmaceuticals for cerebral perfusion agents. In 1989, Walovitch et al. found that N,N’-1,2-ethanediylbis-L-cysteine diethylster (ECD) (Fig. 4A) overcame the problem associated with the isomer formation and provided excellent uptake and retention characteristics for SPECT imaging of the brain.

Meanwhile, based on previous findings that 3,3’-(1,3-
propanediylidimino)bis(3-methyl-2-butano) dioxime (PnAO) (Fig. 2B) forms macrocyclic complexes with Ni^{2+} and Co^{3+}.\textsuperscript{16} Fair et al. synthesized and characterized a technetium complex of PnAO as a neutral complex with a square pyramidal structure.\textsuperscript{17} Volkert et al. found that \textsuperscript{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.\textsuperscript{11} In a search for a ligand which not only transports \textsuperscript{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).\textsuperscript{18} \textsuperscript{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 \textsuperscript{99m}Tc-HM-PAO to more hydrophilic secondary complexes. Further studies showed that intracerebral glutathione (GSH) may play an important role in the \textit{in vivo} conversion of \textsuperscript{99m}Tc-HM-PAO to hydrophilic forms and may be the mechanism for trapping the \textsuperscript{99m}Tc complex in brain and other cells.\textsuperscript{19}

Morgan et al. developed another type of neutral, lipophilic \textsuperscript{99m}Tc complex with \(N\)-(2-(1H pyrolylmethyl) \(N\)- (4-pentene-3-one-2))ethane-1,2-diamine (MRP-20) as the ligand.\textsuperscript{20,21} The chemical structure of this complex is shown in Figure 4C. \textsuperscript{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 \textsuperscript{99m}Tc complexes for brain perfusion were recently developed by Mastrostamatis et al., who prepared mixed ligand \textsuperscript{99m}Tc complexes of the general formula \textsuperscript{99m}TcOL\(_1\)L\(_2\)\(_H\), where L\(_H\) is an \(N\)-substituted bis(2-mercaptoethylamine) and L\(_H\) is a monodentate thiol.\textsuperscript{22} The tridentate ligands react with the \textsuperscript{99m}TcO\(_{3+}\) core, leaving open one \(cys\) site to the oxo group in the coordination sphere of the metal. The neutral mixed ligand \textsuperscript{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 \textsuperscript{99m}Tc-glucoheptonate as precursor and equimolar quantities of the two ligands. Most of the \textsuperscript{99m}Tc complexes in this series showed high initial brain uptake, and this uptake remained nearly constant for more than 30 min.\textsuperscript{23} The retention mechanism of \textsuperscript{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\textsuperscript{−}, and formation of the hydrophilic [\textsuperscript{99m}Tc(SNS/SG) metabolite.\textsuperscript{24,25} Since the GSH concentration in the brain decreases in the diseased state, \textsuperscript{99m}Tc complexes of this type may also be useful to assess intracerebral GSH levels by selecting appropriate tridentate and monodentate ligands.

2-2. Myocardial Perfusion Agents

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

The first cationic \textsuperscript{99m}Tc chelate that exhibited significant myocardial uptake in animals was Tc complexes of \(tr\)-[\textsuperscript{99m}Tc(DIARS)\(_2\)X\(_2\)]\(^+\), where DIARS represents the \(o\)-phenylenebis(dimethylarsine) ligand and X represents chloride or bromide,\textsuperscript{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)ethylene (DMPE) provided cationic \textsuperscript{99m}Tc complexes of \(tr\)-[\textsuperscript{99m}Tc(DMPE)\(_2\)]\(^+\) (Fig. 6B).\textsuperscript{27} Although this \textsuperscript{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 \textit{in vivo} reduction of the cationic \textsuperscript{99m}Tc complexes to their neutral \textsuperscript{99m}Tc(II) form, [\textsuperscript{99m}Tc(II)(DMPE)\(_2\)]\(^0\).\textsuperscript{28} In order to avoid the deleterious effects of \textit{in vivo} reduction, a new class of non-reducible Tc cation was developed (Fig. 6C),\textsuperscript{29} which provided diagnostic accuracy comparable to that of \textsuperscript{201}Tl.

\begin{center}
\textbf{Fig. 5} The “3 + 1” mixed ligand concept for \textsuperscript{99m}Tc brain perfusion agent.
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\textbf{Fig. 6} \textsuperscript{99m}Tc cationic complexes with diarsine or diphosphine ligand for measuring myocardial perfusion. (A) [\textsuperscript{99m}Tc(III)(DIARS)\(_2\)Cl\(_2\)]\(^+\), (B) [\textsuperscript{99m}Tc(III)(DIMPE)\(_2\)Cl\(_2\)]\(^+\), (C) [\textsuperscript{99m}Tc(III)(N,N’-ethylenebis(acetylacetone-iminato))bis(triphenylphosphine)]\(^+\), (D) [\textsuperscript{99m}Tc(I)(DMPE)]\(^+\), (E) [\textsuperscript{99m}Tc(V)O\(_2\)(DMPE)\(_2\)]\(^+\), and (F) [\textsuperscript{99m}TcO\(_2\)(tetrofosmin)]\(^+\).
\end{center}
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(diethylidithiocarbamato)nitrido technetium(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 redistibution 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

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.
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 \(\textit{in vivo}\) 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 to minimize 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 \(\text{N}_2\text{S}_2\) 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 presynaptic 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 alkylation of 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 \(\omega\)-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-quinuclidinyl-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 \(\text{N}_2\text{S}_2\) 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
are required for optimizing 99mTc chelate structures, this approach may pave another way to designing 99mTc radiopharmaceuticals for targeted imaging.

The “3 + 1” mixed ligand complex was also applied to 99mTc 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 IC50 values between 10 and 50 nM. Meeglla et al. applied this approach to prepare a 99mTc-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. 99mTc POLYNUCLEAR COMPLEXES

99mTc polynuclear complexes constitute another approach for targeted imaging. 99mTc 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 Ca2+ in the basic bone structure hydroxyapatite, which render the radiopharmaceuticals useful for visualizing various skeletal cancers.59,60 The first 99mTc-bisphosphonate to be developed was the chelate with tripolyphosphate.61 Soon it was demonstrated that the functional species in the 99mTc complexes would be a 99mTc-pyrophosphate. Although 99mTc-pyrophosphate is still used, most 99mTc-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 99mTc bone imaging agents are shown in Figure 12A–12D.

It is known that bisphosphonates have strong binding affinity for Ca2+. Bisphosphonates also act as bidentate chelating agents to form 99mTc polynuclear complexes.62 In addition, the reaction of Co(ethylene diamine)2 (bisphosphonate) complexes with Ca2+ indicated that a coordinated bisphosphonate can bind Ca2+ very strongly in an aqueous solution.63 These studies suggested that 99mTc-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 99mTc center and calcium on bone matrix. High accumulation of 99mTc bisphosphonate to cancerous bone promotes palliative treatment of bone metastases with 186Re complexes of bisphosphonate.64–67

In 99mTc bisphosphonate complexes, bisphosphonate ligands play an important role in bone localization. On the other hand, a 99mTc-labeled tumor seeking agent was developed using dissociation characteristic of 99mTc polynuclear complex per se. Based on the findings of hydrolytic polynucleation of gallium citrate as relevant to tumor cell accumulation,68 99mTc 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 99mTc-DMS indicated that the increase in the tumor uptake of 99mTc 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 99mTc-DMS when the animals were loaded with a large amount of glucose to facilitate glycoctylation in the tumor cells.72,73 These findings suggested the following mechanisms of tumor localization of 99mTc-DMS: upon administration of 99mTc-DMS into the blood stream, 99mTc complex equilibria are altered by dilution with plasma, and depolymerization or dissociation occurs, inducing the formation of active 99mTc species or dissociated 99mTc 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 medullary thyroid carcinomas.\textsuperscript{74–79} These findings stimulated the synthesis of the $^{186/188}$Re analog of DMS for targeted radiotherapy.\textsuperscript{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).\textsuperscript{81–84} Antibody fragments showed much faster pharmacokinetics and even distribution in the tumor mass in a size-related manner.\textsuperscript{85,86} The successful visualization of somatostatin-positive tumor by $^{111}$In-labeled synthetic somatostatin analog $^{111}$In-DTPA-o-Phe\textsubscript{1}-octreotide\textsuperscript{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 species to amino acid residues of the proteins constitutes a convenient way to prepare $^{99m}$Tc-labeled proteins.\textsuperscript{89} This procedure, however, is not applicable to small molecular weight peptides without appropriate chelating sites. In addition, \textit{in vivo} dissociation of initially bound $^{99m}$Tc species from the protein molecules was demonstrated\textsuperscript{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,\textsuperscript{91} since the chelator had been applied as the BCA for $^{111}$In labeling of proteins.\textsuperscript{92} However, this procedure induced intramolecular cross-linking, which reduces the stability of the radiolabel, as demonstrated in $^{111}$In-DTPA-conjugated proteins.\textsuperscript{93} In order to prepare $^{99m}$Tc labeled proteins with high \textit{in vivo} stability without reducing the bioactivity of the original proteins, a large number of BCAs have been developed.  

\textit{p}-Carboxyethylphenylglyoxal di(N-methylthiosemicarbazone) (Fig. 13B) was the first BCA that satisfied the two criteria of $^{99m}$Tc labeled antibodies.\textsuperscript{94} A variety of BCAs followed that possess $\text{N}_2\text{S}_2$, $\text{N}_3\text{S}$ or $\text{N}_4$ coordination molecules (Fig. 13C–13E) to provide stable $^{99m}$Tc-labeled proteins without inducing their biological activities.\textsuperscript{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\textsubscript{3}S\textsubscript{2} chelating molecule to an antibody fragment, which necessitated a deprotection step before $^{99m}$Tc complexation reaction.\textsuperscript{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-hydradzinonicotinamide (HYNIC; Fig. 14A)\textsuperscript{101} and a bis(hydroxyamamide) derivative (Fig. 14B).\textsuperscript{102,103}
ies, 101, 104, 105 chemotactic peptide, 106–108 somatostatin analogs 109–113 and oligonucleotides. 114 Previous studies show that HYNIC acts as a monodentate 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-containing 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 radiocchemical 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 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-$^{[125]}$Ibenzoic acid to release meta-$^{[125]}$Iiodohippuric 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-$^{[125]}$Iiodohippuric 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. 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.
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 co-workers recently published a new atomospheric synthesis for complexes [MX₃(CO)₃]^+(M = Tc or Re; X = Cl or Br).¹³² These complexes were water-soluble and remained stable in aqueous solution as [M(OH₂)₃(CO)₃]^+. They further reported one-step synthesis of the complexes [M(OH₂)₃(CO)₃]^+(M = ⁹⁹mTc and ¹⁸⁶Re) by direct reduction from [⁹⁹mTc(pertechnetate or perrhenate with sodium borohydride in the presence of carbon monoxide in aqueous solution with over 95% yields.¹³³ ⁹⁹mTc(OH₂)₃(CO)₃ formed stable complexes with histidine and a histidine-conjugated peptide (neurotensin) with high yields and specific activity. The resulting ⁹⁹mTc(CO)₃-conjugated histidine and peptide remained stable in vivo.¹³⁴ ⁹⁹mTc(OH₂)₃(CO)₃ also formed a neutral complex with picolinamidine-NN-diaceotic 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 ⁹⁹mTc complex applicable to the design of a variety of new ⁹⁹mTc radiopharmaceuticals.¹³⁵ More recently, Dyszlewski et al. developed a freeze-dry formulation for the preparation of the ⁹⁹mTc(CO)₃(OH)₃ core in aqueous solution.¹³⁶ Cyclopentadienyl (Cp) ⁹⁹mTc(I) and ¹⁸⁶Re(I) tricarbonyl complexes [CpM(CO)₃] (M = Tc or Re) constitute another interesting precursors for ⁹⁹mTc 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 ⁹⁹mTc(I).¹³⁷ The CpTc(CO)₃ was applied to labeling proteins, peptides¹³⁸,¹³⁹ and an estradiol derivative.¹⁴⁰ Although further studies are required to prepare the ⁹⁹mTc(I) complex with high radiochemical yields under simple procedures, this class of organometallic ⁹⁹mTc(I) complexes may also provide a variety of molecules that are thought “impossible” to radiolabel with ⁹⁹mTc without impairing their bioactivity. The organometallic ⁹⁹mTc complexes also exhibit chemical and biological characteristics similar to those of ¹⁸⁶/¹⁸⁸Re counterparts, which renders the organometallic approach attractive to the design and development of therapeutic agents with ¹⁸⁶/¹⁸⁸Re as the radionuclides.

7. CONCLUSION

In this manuscript, recent progress in developing ⁹⁹mTc 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 ⁹⁹mTc radiopharmaceuticals that are now used in clinical studies. Future advances in technetium chemistry will also provide ⁹⁹mTc 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-


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