Chemistry of Metal Radionuclides (Rb, Ga, In, Y, Cu and Tc)

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The doubter is a true man of science; he doubts only himself and his interpretations, but he believes in science.

Claude Bernard

12.1 Introduction

Clearly, and extensive knowledge, experience, and understanding of the metal chemistry at the tracer level, would enable us to develop a number of new molecular imaging radiotracers based on β+ emitting radiometals. In nuclear medicine, ^{99m}Tc continues to be the most widely used diagnostic radionuclide because of its ideal nuclear properties ($T_{½}$ = 6 h and 140 keV γ photon) and its ready availability as a generator produced radionuclide. In the last three decades, a number of ^{67}Ga and ^{111}In labeled radiopharmaceuticals (based on chelates, peptides, antibodies) have been developed for both planar and SPECT imaging studies. In addition, a number of therapeutic radiopharmaceuticals have been developed based on beta emitting radiometals (^{90}Y, ^{177}Lu and ^{67}Cu), however, many of these agents are still under active clinical evaluation. The extensive knowledge, experience, and understanding of the metal chemistry at the tracer level, would enable us to develop a number of new molecular imaging radiotracers based on β^+ emitting radiometals. The advantages of metal labeled molecular imaging radiotracers can be summarized as follows:

- Easy availability: ^{66}Cu and ^{68}Ga generators are available for easy in house preparation based on kit production. Cyclotron production of metallic nuclides has been optimized using medical cyclotrons using primarily (*p*,*n*) nuclear reactions
- 40 year experience with metal-labeled SPECT tracers
- Ability to label target specific biomolecules (peptides and proteins)
- Availability of radionuclide pairs for imaging and therapy (^{68}Ga/^{67}Ga ^{62}Cu/^{64}Cu/^{67}Cu, ^{110}In/^{111}In, ^{86}Y/^{90}Y)
- High SA of radiometal
- High SA of metal-labeled peptide or protein
- High in vivo stability of metal-labeled tracers
- Favorable radiation dosimetry

12.1.1 Physical and Chemical Characteristics of Metals

Some of the important physical properties and the electron configuration of various metals useful in developing molecular imaging probes are summarized in Table 12.1. Among these metals, gallium, and indium belong to group IIIB, while yttrium belongs to group IIIA of the periodic table (Fig. 3.3). All other metals, useful for developing radiopharmaceuticals, are transition metals with complex coordination chemistries.

Radioisotopes of various metals useful for PET and SPECT imaging studies are listed in Tables 12.2 and 12.3. The selection of a radiometal for labeling a specific peptide or protein is dependent on several factors, such as physical half-life, SA, type(s) of decay and emission(s), energy of the emission(s), and cost and availability. In addition, pharmacokinetics, drug delivery of the radiometal-complex to the target site, and clearance of the radiometal complex from both the target and nontarget tissues, are all important factors that determine the selection of an appropriate radiometal in tracer development.

Among the β^+ emitting metallic nuclides, ^{64}Cu ($T_{½}$ = 12.6 h), ^{66}Ga ($T_{½}$ = 9.45 h), ^{86}Y ($T_{½}$ = 14.74 h), and

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Table 12.1 Physical properties and electron configuration of metals

	Element					
Physical property	Cu	Ga	Y	Zr	In	Tc
Atomic number	29	31	39	40	49	43
Atomic radius (pm)	128	122	181	160	163	136
Ionic radius (pm)	2^+, 72	3^+, 62	3^+, 106	2^+, 109	3^+, 92	7^+, 56
Electron structure	[Ar] $3d^{10}$ $4s^1$	[Ar] $3d^{10}$ $4s^2$ $4p^1$	[Kr] $4d^1$ $5s^2$	[Kr] $4d^1$ $5s^2$	[Kr] $4d^{10}$ $5s^2$ $5p^1$	[Kr] $4d^5$ $5s^2$
Electronegativity	1.90	1.81	1.22	1.65	1.78	1.9
Oxidation state	+1, +2	+3	+3	+4, +2	+3	−1 to +7

Table 12.2 Important radioisotopes of metals useful for PET and SPECT

	Stable isotopes		Radioactive isotopes				
Metal	Nuclide	%	Nuclide	$T_{½}$ (h)	Decay	β (% emission)	SA (Ci μmole^{-1})
Copper	^{63}Cu	69.17	^{60}Cu	0.39	EC, β^+	β^+ (93)	
	^{65}Cu	30.83	^{61}Cu	3.32	EC, β^+	β^+ (62)	
			^{62}Cu	0.163	EC β^+	β^+ (98)	19,310
			^{64}Cu	12.80	EC, β^+, β^-	β^+ (19), β^- (40)	245
			^{67}Cu	61.92	β^-, γ	β^- (100)	
Gallium	^{69}Ga	60.10	^{66}Ga	9.45	EC, β^+	β^+ (62)	331
	^{71}Ga	30.90	^{67}Ga	78.24	EC, γ		40
			^{68}Ga	1.14	EC, β^+	β^+ (90)	2,766
Rubidium	^{85}Rb	72.16	^{82}Rb	75 s	EC, β^+	β^+ (96)	
	^{87}Rb	27.84					
Yttrium	^{89}Y	100	^{86}Y	14.74	EC, β^+	β^+ (34)	213
			^{90}Y	64.08	β^-	β^- (100)	
Zirconium	^{90}Zr	51.45	^{89}Zr	78.48	EC, β^+	β^+ (23)	39.9
	^{91}Zr	11.22	^{97}Zr	16.80	β^-, γ	β^- (100)	
	^{92}Zr	17.15					
	^{94}Zr	17.38					
	^{96}Zr	2.80					
Indium	^{113}In	4.3	^{110}In	1.1	EC, β^+, γ	β^+ (71)	
	^{115}In	95.7	^{111}In	67.2	EC, γ		47
Technetium			^{94m}Tc	0.88	EC, β^+	β^+ (72)	
			^{99m}Tc	6.01	IT		522

Table 12.3 Positron emitting radiometals with potential clinical utility

	Stable isotopes		Radioactive isotopes			
Metal	Nuclide	%	Nuclide	$T_{½}$ (h)	β^+ Decay (%)	β^+ E_{max} (MeV)
Scandium	^{45}Sc	100	^{44}Sc	3.92	β^+ (95)	1.47
Titanium	^{48}T1	73.8	^{45}Ti	3.09	β^+ (86)	1.04
Cobalt	^{59}Co	100	^{55}Co	17.5	β^+ (77)	1.50
Strontium	^{88}Sr	87.9	^{83}Sr	32.4	β^+ (24)	1.15
Iron	^{56}Fe	91.72	^{52}Fe	8.275	β^+ (55.5)	0.80

^{89}Zr ($T_{½}$ = 3.27 days) are more appropriate for development of commercial PET radiopharmaceuticals that can be transported across the country. For most of these metallic radionuclides, cyclotron production methods have been optimized, using medical cyclotrons using primarily (*p*,*n*) nuclear reactions (Table 12.4). Also, the ^{82}Sr($T_{½}$ = 25 days)→^{82}Rb ($T_{½}$ = 1.25 months) generator (cardioGen-82®) has been FDA

Table 12.4 The most common nuclear reactions for the production of positron emitting radiometals

Radiometal	Nuclear reaction	Target abundance (%)	Useful energy range (MeV)
^{44}Sc	^{44}Ca (*p*,*n*) ^{44}Sc	2.086	≈ 11
^{60}Cu	^{60}Ni (*p*,*n*) ^{60}Cu	26.16	
^{61}Cu	^{61}Ni (*p*,*n*) ^{61}Cu	1.25	9–12
^{64}Cu	^{64}Ni (*p*,*n*) ^{64}Cu	0.91	8–15
^{66}Ga	^{66}Zn (*p*,*n*) ^{66}Ga	27.8	8–15
^{67}Ga	^{68}Zn (*p*,2*n*) ^{67}Ga	19.0	12–22
^{86}Y	^{86}Sr (*p*,*n*) ^{86}Y	9.86	10–15
^{89}Zr	^{89}Y (*p*,*n*) ^{89}Zr	100	≈ 14
^{94m}Tc	^{94}Mo (*p*,*n*) ^{94m}Tc	9.12	10–15
^{110}In	^{110}Cd (*p*,*n*) ^{110}In	12.5	10–20
^{111}In	^{112}Cd (*p*,2*n*)^{111}In	24.0	12–22

approved for myocardial perfusion studies. The two nuclides with short half-lives, ^{68}Ga ($T_{½}$ = 68.3 min) and ^{62}Cu ($T_{½}$ = 9.76 min), can be produced on demand from generator systems without the need for an on-site cyclotron. Interestingly, the short half-life positron emitting nuclides ^{110}In and ^{94m}Tc may also have potential utility in developing specific targeted molecular imaging probes with relatively faster blood clearance, similar to ^{68}Ga labeled agents.

12.1.1.1 Specific Activity of Radiometals

The SA of the radiometal is an indicator of potency; the higher the SA of the radiometal, the higher is the SA of the radiometal-labeled biomolcule. The theoretical SA of carrier-free radiometals, useful for developing PET and SPECT radiotracers is shown in Table 12.2. The practical SA that can be achieved by cyclotron production or by generator, however, depends on many other factors. In general, SA of all β⁺ emitting radiometals is much higher than the corresponding SPECT nuclides, except for ^{89}Zr. Also, the SA of ^{68}Ga (2.766 Ci nmol^{-1}) is even much higher than that of ^{18}F (1.71 Ci nmol^{-1}).

The maximum theoretical SA of ^{64}Cu is ~4,000 mCi μg^{-1}, but the cyclotron production of ^{64}Cu achieves a maximum SA of ~200 mCi μg^{-1} at EOB (McCarthy et al. 1997). In practice, purity control difficulties in solid target production often cause much lower SA to be delivered due to cold Cu contamination. In comparison, the SA of generator-produced ^{62}Cu is >70,000 mCi μg^{-1}, and levels approaching this maximum can be routinely achieved.

12.1.1.2 Decay Characterisitics

The intensity of β⁺ emission from a radionuclide, or branching ratio directly affects the rate of true coincidences, because lower β⁺ decay fraction results in fewer annihilation events per MBq (Williams et al. 2005). Also, the β⁺ must slow down and rest before it can annihilate with an electron. Thus, annihilation takes place in a spherical volume whose radius depends on the energy. Consequently with PET, positrons with lower energy will have shorter range in tissue and higher expected spatial resolution.

Another important consideration is the emission of γ photons associated with certain positron emitters. The number of photons, amount of energy, and the abundance (%) for several radiometals useful for PET are shown in Table 12.5. Except for ^{62}Cu, ^{64}Cu and ^{68}Ga, all other radiometals have significant gamma emissions. Radionuclides, such as ^{66}Ga, ^{86}Y, ^{89}Zr and ^{124}I, have a very high proportion of γ emission compared to the intensity of β⁺ emission. Detection of gamma photons or scattered photons, along with annihilation photons, may reduce the coincidence count rate performance (true counts) in several different ways (Robinson et al. 2004; Haddad et al. 2008). Also, the associated gamma emission will have a significant impact on the radiation dose to the patient, radiation exposure, and burden to the technical staff (Williams et al. 2005).

Table 12.5 β⁺ Emitting radionuclides and associated γ emissions

	β⁺ decay		Major γ energy (KeV) and abundance (%)		
Nuclide	(%)	E_{mean} (MeV)	1	2	3
^{18}F	96.73	0.2498			
^{62}Cu	97.2	1.316			
^{64}Cu	17.4 and 39 (β⁻)	0.2782 0.1902	1346 (0.47)		
^{66}Ga	50.0 3.8	1.9 0.397	1039 (37)	2751 (23)	4.295 (4)
^{68}Ga	88.0	0.836	1077 (3)		
^{86}Y	11.9 5.6 3.6	0.535 0.681 0.883	443 (17)	627 (33)	1.076 (83)
^{89}Zr	22.74	0.3955	908 (100)		
^{94m}Tc	67.0	1.0942	871 (94.2)	1522 (4.5)	1869 (5.7)
^{82}Rb	83.3 11.7	1.535 1.638	777 (13.4%)		

On the basis of a model incorporating radionuclide decay properties of copper radioisotopes and scanner parameters for GE Advance scanner, it has been shown that spatial resolution, sensitivity, scatter fraction, and noise-equivalent count rate (NEC) depend very much on the branching ratio and β^+ range (or spatial resolution) of the radionuclide (Table 12.6). Compared to ^{18}F, the sensitivity of the PET scanner (cpm Bq^{-1} mL^{-1}) for ^{64}Cu is significantly decreased (5.44 vs 0.98). In addition, predicted variation of NEC depends on the activity concentration (KBq mL^{-1}) (Williams et al. 2005). As a result, it is essential to administer approximately 5 times more ^{64}Cu activity in order to achieve similar NEC to that of ^{18}F. Since β^+ energy is similar for both these radionuclides, spatial resolution with ^{64}Cu is close to that of ^{18}F. On the basis of the data in Table 12.7, one can expect that for ^{68}Ga, sensitivity would be similar to that of ^{18}F.

Table 12.6 PET scanner sensitivity as a function of β^+ decay fraction and spatial resolution

Nuclide	β^+ Decay fraction	β^+ Energy E_{max} (KeV)	Spatial resolution (mm)		Sensitivity
			Tangential	Radial	Cps Bq^{-1} mL^{-1}
^{15}O	1.0	1,723	5.8	6.0	5.62
^{18}F	0.97	635	4.7	5.0	5.44
^{62}Cu	0.97	2,925	7.2	7.4	5.5
^{60}Cu	0.92	2,194	6.3	6.6	5.23
^{61}Cu	0.62	1,159	5.1	5.4	3.43
^{64}Cu	0.17	657	4.7	5.0	0.98
^{68}Ga	0.90	1,900	–	–	≈ 5.0[a]

The table modified from Williams et al. 2005
[a]Sensitivity for ^{68}Ga is approximated based on the data for other radionuclides

Table 12.7 Bifunctional chelating agents

	Diethylenetriamene-pentaacetic acid	DTPA
Polyaminocaboxylic acids	Ethylenediaminetetraacetic acid	EDTA
Macrocyclics	1,4,7,10-tetraazacyclodo-decane-N,N‴,N‴,N⁗-tetraacetic acid	DOTA
	1,4,8,11-tetraazacyclotetrado-decane N,N″,N‴,N⁗-tetraacetic acid	TETA
	1,4,7,-triazacyclododecane-N,N″,N‴,N⁗-tetraacetic acid	NOTA
	SAR ????	SAR
Others	Bis(thiosemicarbazone)	BTS
	Propyleneamine oxime	PnAO
	Diaminedthiol	DADT
	Mercaptoacetylglycyl-glycylglycine	MAG_3

12.2 Chelation Chemistry of Radiometals

12.2.1 Chelating Agents

In 1970s, ligands known as *bifunctional chelating agents* (BFC) were introduced to complex radiometals such as ^{111}In and ^{67}Ga. Various BFCs (Table 12.7) have been designed and synthesized over the years to develop a wide variety of radiopharmaceuticals based on radiometals.

A *ligand* is a neutral molecule or an ion having a lone pair of electrons that can be donated to form a bond with a metal ion. A chelating agent (or a chelate) is a molecule containing more than one ligand or an atom (such as N, O, and S) that can donate a lone pair of electrons. The chelating agents listed in Table 12.7 all contain N and O atoms that can form coordinate covalent bonds with central metal ions.

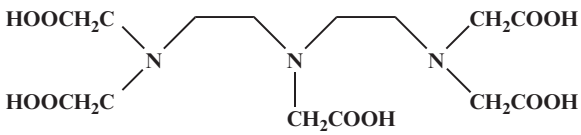
Chelates, such as EDTA and DTPA, are open chain polyaminopolycarboxylic acids, while NOTA, DOTA and TETA are cyclic polyaminoploycarboxylates (or macrocyclics) consisting of triaza or tetraaza macrocycle ranging from a 9 to 14 membered ring size. BFCs based on bis(thiosemicarbozone) or BTS containing N and S atoms have been developed specifically to bind Cu metal. Similarly, BFCs such as PnAOs, DADTs and MAG3 containing N and S atoms were designed to develop ^{99m}Tc radiopharmaceuticals. A wide variety of derivatives of the BFCs have been designed to improve the in vivo stability and optimize the kinetics of a specific metal–chelate complex. The structures of various BFCs are shown in Figs. 12.1 and 12.2

In coordination compounds, the metal ions have two types of valence; *primary valence* (also known as oxidation state) refers to the ability of metal ion to form ionic bonds with oppositely charged ions, while *secondary valence* (also known as coordination number) refers to the ability of a metal ion to bind to Lewis bases (ligands) to form complex ions. Therefore, the

coordination number is the number of bonds formed by the metal ion with the atoms (that can donate a pair of electrons) in a chelating agent. This number varies from 2 to 8, depending on the size, charge and electron configuration of the metal ion. The ligand geometric arrangements of coordination compounds can be linear, square planar, tetrahedral or octahedral depending on the coordination number. Transition metals (Cu, Y, Tc) usually form coordination compounds.

The BFCs contain a side chain for conjugation to a peptide or protein. The side chain can be attached to the carbon backbone of the chelate (C-functionalized chelate), or by substitution to one of the nitrogen atoms in the molecule. C-functionalized chelating agents are preferable and provide greater stability to the metal–chelate complex, since all the nitrogen and oxygen atoms will be available for coordination with the metal ion. It is also preferable to conjugate the chelating agent to the peptide or protein of interest first, before complexation with the radiometal.

Diethylenetriamenepentaacetic acid (DTPA)



Ethylenediaminetetraacetic acid (EDTA)

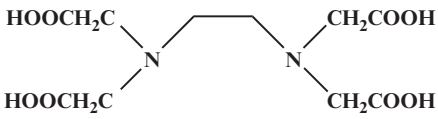


Fig. 12.1 Bifunctional open chelating agents (DTPA and EDTA)

12.2.2 Stability of Metal–Ligand Complex

As shown in Table 12.1, except for Ga and In, all other metals are transition metals. The elctronegativity and oxidation state play a major role in the formation of metal–ligand complexes. Since metal ions form insoluble hydroxides in water at physiological pH, direct labeling of peptides and proteins with metallic radionuclides is relatively very difficult. Chelating agents can complex and stabilize the metal. Therefore, it is necessary to first attach a chelating agent, by covalent bonds, to a peptide or protein in order to develop radiometal labeled peptides and proteins as imaging agents.

The metal ions dissolved in water are complexed to form aqua ions. However, in the presence of a chelating agent or the ligand (L) with greater affinity for the metal

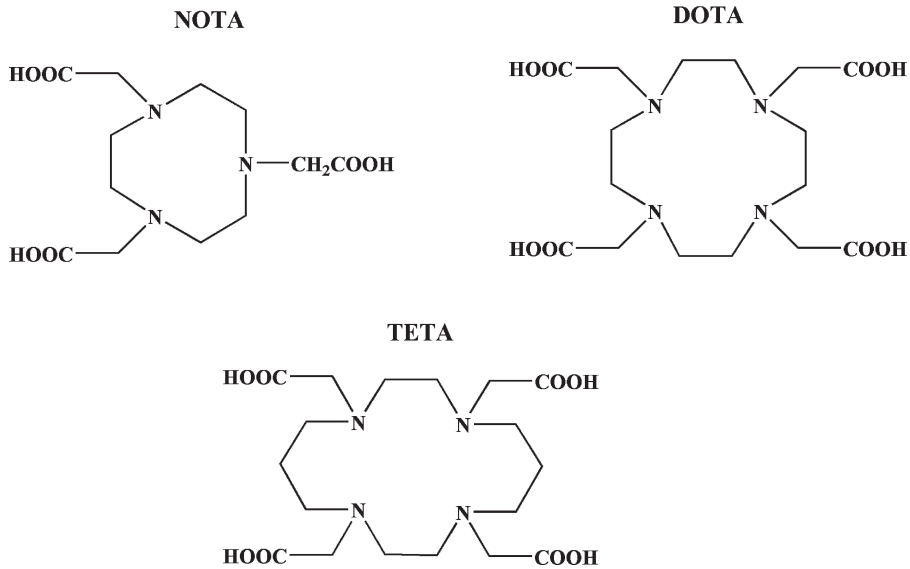


Fig. 12.2 Bifunctional macrocyclics (NOTA, DOTA and TETA)

than the affinity of OH^- ion for the metal, the formation of metal–chelate complex is preferred, as shown below.

$$M+L \leftrightarrow ML \quad (12.1)$$

$$K_S = \frac{[ML]}{[M][L]} \quad (12.2)$$

In the above equation, [ML] represents the concentration of the metal–ligand complex, while [M] and [L] represent the concentrations of the free metal and the free ligand. The stability of the metal–ligand complex is defined by the stability constant (K_s) when the system reaches an equilibrium between interacting chemical species (Brunner et al. 1995). The higher the value of K_s, the greater the thermodynamic stability of the metal–ligand complex (Table 12.8). The values of K_s (such as 10^4 or 10^{30}) are normally represented as log K_s values (such as 4 and 30).

It is important to appreciate that the stability constant can only reveal the direction of the reaction (formation or dissociation), but not the rate of the reaction. For example, when a purified metal–ligand complex is injected into the circulation, the rate of dissociation of the complex may be significantly increased due to extreme dilution of the complex. Therefore, the *kinetic stability* of the metal–ligand complex is very important under in vivo conditions where competing ions and ligands may augment the transchelation of the radiometal (Brunner et al. 1995).

The K_s values are usually determined for reaction in ideal conditions of buffer, pH and temperature, and do not necessarily reflect the stability of metal–ligand complex in vivo. A quantity known as *conditional stability constant* can be measured or estimated as a function of pH and in the presence of different amounts of other competing ligands.

Table 12.8 Stability constants of metal–ligand complex

	Ga	In	Y	Cu	Fe
DTPA	25.5	29.0	22.1	21.4	
EDTA	21.7	21.7		18.8	
DOTA	21.3	23.9	24.9	22.2	
NOTA	30.98				
TETA				21.6	
Citrate	10.02	6.18			
OH–	39.4	36.9			
HSA				16.2	
Transferrin	19.75	18.3			21.44

as $Ga(OH)_4^-$ or $In(OH)_4^-$

12.3 Chemistry of Gallium, Indium and Yttrium

Both gallium and indium have a filled *d* shell and three electrons in the outermost shell (Table 12.1). In contrast, yttrium has an incomplete *d* shell with one electron and two electrons in the outermost shell. However, for these three metals (M), the most important oxidation state is M^{III}. Their coordination chemistry is somewhat similar however, due to small differences in their ionic radii and electronegativities, minor, but significant differences do exist in their chemistries. These three metals also share chemical characteristics with ferric ion (Fe^{3+}). This similarity with ferric ion is important in the development of radiopharmaceuticals with Ga, In, and Y since iron is an essential element in the human body and a number of iron binding proteins, such as transferrin (in blood), exist to transport and store iron in vivo. As a result, the atoms of iron always compete with these radiometals for specific binding with proteins, such as transferrin, lactoferrin and ferritin, in vivo (Weiner and Thakur 2003).

The aqueous chemistry of Ga, In and Y is dominated by their ability to form strong complexes (both soluble and insoluble) with the hydroxyl ion. The fully hydrated (hexaaquo) M^{3+} ions are only stable under acidic conditions. As the pH is raised above 3, these three metals form insoluble hydroxides ($M(OH)_3$). A variety of OH intermediates are formed as a function of pH and the mass of the metal. Among these three metals, gallium is more amphoteric than indium and yttrium. As a result, at physiological pH, gallium exists predominantly as a soluble species, $[Ga(OH)_4]^-$ (gallate) (Green and Welch 1989). With indium, soluble $[In(OH)_4]^-$ starts forming only at pH values higher than 7.0. Similar to Ga and In, with Y only the trivalent ion is stable in aqueous solution at acidic pH; the ionic radius of Y is larger than Ga and In, Y binds larger number of water molecules.

The total solubility of these three metals at the physiological pH is very limited; very high SAs of radiometals are needed to keep them soluble in water. However, it is a common practice to add weak chelating agents (such as citrate, acetate or tartrate ion) to complex the metal and prevent precipitation at the neutral pH. For example, ^{67}Ga is used in the clinic as ^{67}Ga-citrate. Following intravenous administration, ^{67}Ga binds to transferrin in plasma and transported to

tumors and infectious foci as "Ga-transferrin complex" (Vallabhajosula et al. 1980).

The coordination chemistry of the metallic radionuclide will determine the geometry and stability of the "metal–chelate complex." Different metallic radionuclides have different coordination chemistries and require BFC with different donor atoms, and chelator frameworks. Both, Ga and In are classified as hard acids and prefer hard bases (Weiner and Thakur 2003). It has been shown that in +3 oxidation state, both Ga and In form thermodynamically stable complexes with either 4, 5, or 6 coordinate ligands, with 6-coordiante being the most stable, while Y prefers octadentate coordinating ligands. The advantage of using the acyclic chelators (DTPA and EDTA) is their extremely fast and high radiolabeling efficiency under mild conditions and greater thermodynamic stability; however, their kinetic lability often results in the dissociation of the radiometal. The macrocyclic chelates (NOTA, DOTA, and TETA), however, provide greater thermodynamic stability as well as kinetic stability. While Ga and In form greater thermodynamically stable complexes with NOTA, DTPA and EDTA, Y prefers DOTA. The labeling kinetics of DOTA-based BFCs is usually slow, and much more dependent on the radiolabeling conditions, including the DOTA-conjugate concentration, pH, reaction temperature, heating time, buffer agent and concentration, and presence of other metallic impurities such as Fe^{3+} and Zn^{2+} (Kukis et al. 1998).

12.3.1 ^{68}Ga-Labeled Radiopharmaceuticals

The recent development of ^{68}Ga-PET is a true landmark in molecular imaging that will allow for the use of diverse molecules and receptor analogues in clinical practice. The inherent superiority of PET imaging is a clear advantage compared to SPECT. Also the feasibility of using the ^{68}Ge/^{68}Ga generator, a round the clock for more than a year, is extremely cost-effective negating the need for on-site cyclotron (AL-Nahhas et al. 2007; Dijkgraaf et al. 2007; Maecke and Andre 2007)

12.3.1.1 ^{68}Ga Generator

The ^{68}Ga generator was first developed in the 1960s for brain imaging studies (Yano and Anger 1964). Subsequent generators (Fig. 12.3) utilized ^{68}Ge germanate adsorbed on tin dioxide and ^{68}Ga was eluted with HCl (Loc'h et al. 1980; Schuhmacher and Maier-Borst 1981). The use of a relatively high concentrations

Fig. 12.3 Gallium-68 generators: Obninsk (**a**) generator (10–100 mCi ^{68}Ge) and AGG100 (**b**) generator (10–50 mCi) supplied by The Eckert and Ziegler Isotope Products (EZIP)

of HCl (1.0 N) presents a problem due to the volatility of $GeCl_4$ and the subsequent spread of airborne, long-lived ^{68}Ge contamination. In addition, ^{68}Ga is eluted in a large volume of acid (>5 mL), containing metal impurities that are known to bind with high affinity to DOTA. A commercial generator (Fig. 12.3) is available based on the use of TiO_2 as an inorganic matrix to immobilize ^{68}Ge in the oxidation state IV+. Consequently, ^{68}Ga (III) can be easily separated by eluting it with dilute HCl. It has also been reported that the SA of the generator eluted ^{68}Ga can be as high as 27 Ci μmol^{-1} (Breeman and Verbruggen 2007). These generators, however, are not necessarily optimized for the synthesis of ^{68}Ga-labeled radiopharmaceuticals. The eluates have rather large volumes with a pH of 1, a breakthrough of ^{68}Ge, increasing with time or frequency of use, and impurities such as stable Zn(II), Ti(IV), Fe(III). In order to avoid these impurities, additional concentration and purification can be performed using a miniaturized column with organic cation-exchanger resin and hydrochloric acid/acetone eluent (Zhernosekov et al. 2007). The processed ^{68}Ga fraction can be directly transferred to solutions containing labeling precursors such as DOTATOC. Labeling yields of >95% and specific activities of (50–500 MBq nmol^{-1}) can be obtained under optimized conditions. Further, fully automated synthesis modules have been developed to prepare ^{68}Ga radiopharmaceuticals for clinical use (Azhdarinia et al. 2007; Decristoforo et al. 2007; Velikyan et al. 2008).

^{68}Ga Labeled Biomolecules

Somatostatin receptor binding peptide octreotide and its analogs (Chap. 15), DOTATOC (Fig. 12.4), DOTATATE and DOTANOC have all been labeled with ^{68}Ga and evaluated to determine the diagnostic potential in patients with neuroendocrine tumors (Antunes et al. 2007; Lucignani 2008). Preclinical studies have demonstrated that $^{67/68}$Ga-DOTA-octapeptides show distinctly better preclinical pharmacological performance than the ^{111}In-labeled peptides, especially on SST2-expressing cells and the corresponding animal models (Antunes et al. 2007). ^{68}Ga-octreotide analogs may be excellent candidates for further development for use in clinical studies.

Bombesin (BBN), an amphibian analog of the mammalian gastrin-releasing peptide, is a tetradecapeptide neurohormone that binds to gastrin-releasing peptide receptors (GRPr) that are expressed in a variety of

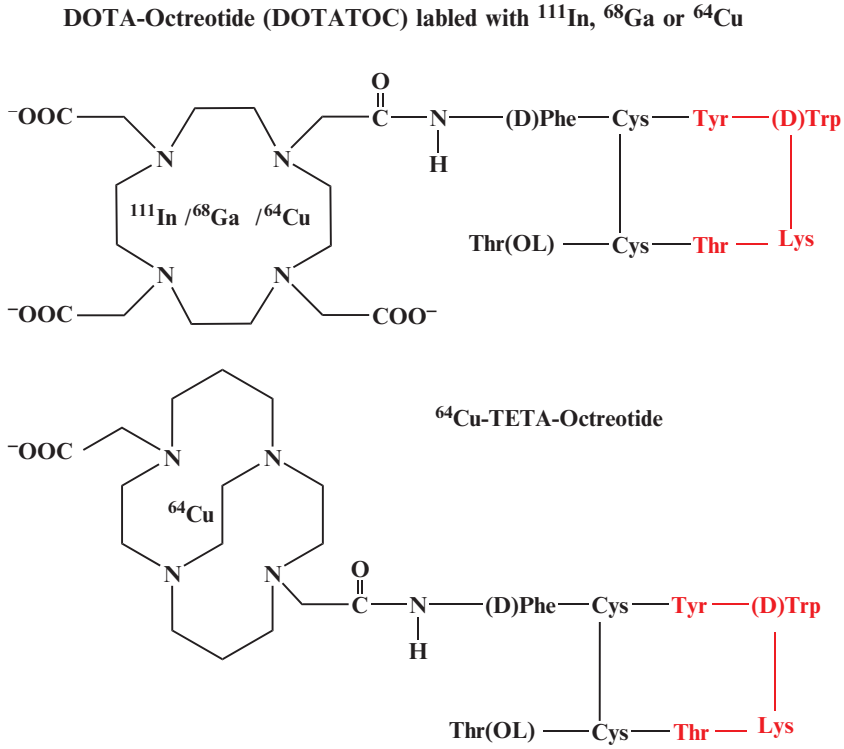


Fig. 12.4 Radiolabeled octreotide analogs for imaging neuroendocrine tumors expressing somatostatin receptors

cancers, including breast, lung, pancreatic, and prostate cancer. Recently, a novel bombesin analog, DOTA-PEG(4)-BN(7–14) (DOTAPESIN), has been developed to synthesize radiolabeled analogs for diagnosis and radionuclide therapy of prostate and other human cancers, which overexpress bombesin receptors (Zhang et al. 2007). In preclinical studies, ^{68}Ga-DOTAPEPSIN has demonstrated significant tumor targeting and potential for noninvasive imaging studies of prostate cancer.

12.4 Transition Metals

In the last few decades, several radioisotopes of transition metals such as ^{99}Mo, ^{99m}Tc, ^{59}Fe, and ^{90}Y have been used extensively in nuclear medicine. A number of positron emitting radiometals such ^{44}Sc, ^{45}Ti, ^{52}Fe, ^{55}Co, ^{64}Cu, ^{86}Y, ^{89}Zr, and ^{94m}Tc have also been found to have suitable radioactive decay and emission characteristics for PET imaging studies.

Since the tracer chemistry of ^{86}Y is similar to that of ^{67}Ga and ^{111}In, ^{86}Y labeled peptides and antibodies have also been evaluated as potential diagnostic PET imaging agents. While the potential utility of ^{86}Y-labeled peptides for PET imaging studies in patients has been documented (Lövqvist et al. 2001), the physical half-life of ^{86}Y is suboptimal (not long enough) to follow the in vivo kinetics of labeled mAbs. It is more appropriate to use long-lived positron emitters (half-lives of days) for developing immuno-PET to allow optimal target/background ratios with radiolabeled mAbs. ^{89}Zr may be more appropriate since it has the ideal physical half-life ($T_{½}$ = 3.27 days), which is compatible with the time needed for intact mAbs to achieve optimal tumor/background ratios (Verel et al. 2003, 2005). The use of shorter-lived positron emitters, such as ^{86}Y and ^{64}Cu, however, does provide an attractive opportunity for the development of radiolabeled mAb fragments for use with immuno-PET.

The radioisotopes of copper, such as ^{62}Cu, ^{64}Cu and ^{67}Cu, have attracted considerable attention because the emission properties of these radionuclides offer the potential to develop both diagnostic and therapeutic radiopharmaceuticals (Blower et al. 1996). ^{64}Cu labeled porphyrin and ^{67}Cu-citrate were introduced almost 40 years ago as tumor imaging agents (Bases et al. 1963; Raynaud et al. 1973). Also, ^{67}Cu labeled antibodies have been extensively evaluated for radioimmunotherapy (DeNardo et al. 1991). Following the development of the high SA (>10 Ci μmol^{-1}) production of ^{64}Cu, based on enriched ^{64}Ni target and proton bombardment, using a biomedical cyclotron, there has been renewed interest in the development of molecular imaging probes based on ^{64}Cu (McCarthy et al. 1997). In addition, the production of large quantities of ^{60}Cu and ^{61}Cu, have also been optimized, based on enriched Ni targets (McCarthy et al. 1999).

12.4.1 Chemistry of Copper

The chemistry of copper is dominated by two oxidation states, I and II (Anderson et al. 2003). Copper salts form the aqua ion $[Cu(OH)_6]^{2+}$. The compounds of Cu (I) oxidation state are unstable in aqueous solution and readily oxidize to Cu(II), which can form 4, 5, or 6 coordination bonds with ligands. In the Cu (II) oxidation state, the metal binds strongly with N- and S-containing molecules forming coordination complexes. Complex formation with chelating agents occurs at pH < 7 because formation of insoluble $Cu(OH)_2$ is not a major concern.

The ability to fully exploit Cu radionuclides for PET tracer development is limited, at least in part, by the high lability of the Cu(II) complexes (i.e., high k_d). As a result, the stability of the Cu complexes is not very high and there is a significant loss of Cu from the BFC, especially in vivo (Anderson et al. 2003). There are several mechanisms by which Cu can be removed from a ligand, in vivo. These include reduction to Cu(I) with subsequent demetallation and metabolic degradation of the ligand itself. In circulation, Cu binds to the human serum albumin (HSA), which typically exists at a concentration of 5×10^{-4}M. Since the concentration of HSA is relatively high compared to the mass of Cu, the amount of Cu that is transferred from the chelator to the endogenous proteins is of major concern. Therefore, the choice of the BFC used for labeling copper radionuclides to peptides and proteins is very important. The choice of BFC really depends on the blood clearance kinetics of the target specific molecule (peptide, protein or nanoparticle) to be labeled with the copper radionuclide.

In order to bind radionuclides of copper to peptides and antibody molecules, macrocyclic chelators, such as TETA have been developed (Blower et al. 1996; Anderson et al. 2001). However, Cu (II)-TETA complexes are not optimal as imaging agents since they are not stable in vivo (Bass et al. 2000). Recently, a new class of bicyclic tetraazamacrocycles (Fig. 12.5), the

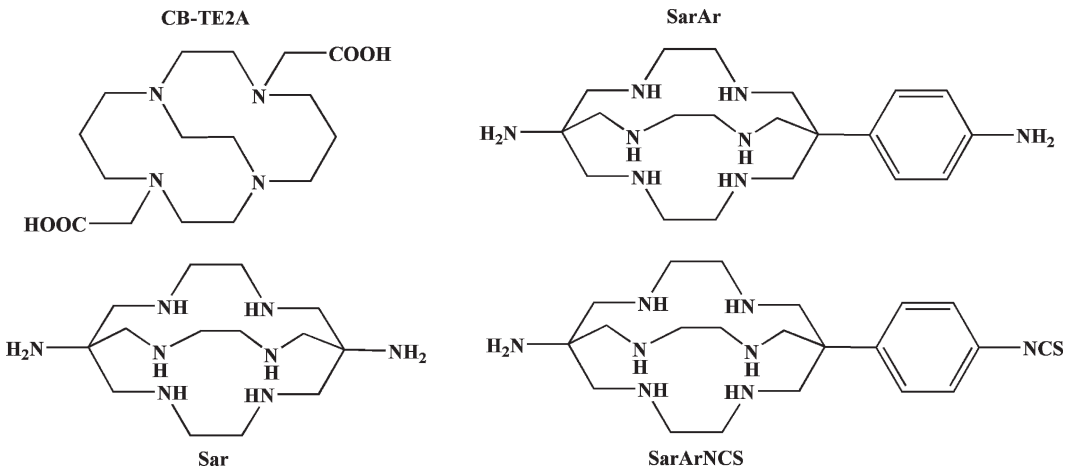


Fig. 12.5 Bifunctional aacrocyclics for complexing copper radionuclides. The ethylene "crossbridged" cyclam (CB-cyclam) derivative (CB-TE2A) and hexa-aza cages (Sar, SarAr, and SarArNCS)

ethylene "crossbridged" cyclam derivatives (CB-2ETA), were developed which form highly kinetically stable complexes with Cu(II) and are less susceptible to transchelation in vivo (Boswell et al. 2004; Wadas et al. 2007). Similarly, another series of TETA analogs known as hexa-aza-cryptand ligands SarAr and SarArNCS were also reported to form strong and stable Cu (II) complexes by wrapping the Cu atom more tightly (Di Bartolo et al. 2001; Smith 2007). This approach does not, however, take into account other factors that may affect complex stability in vivo, such as the chelate ring size, chelate flexibility, and ring substitution.

12.4.1.1 ^{62}Cu-BTS Complexes

^{62}Cu is a decay product of the parent ^{62}Zn ($T_{½}$ = 9.13 h). In a ^{62}Cu generator, an acidic solution of ^{62}Zn is loaded onto a Dowex anion-exchange column and the daughter ^{62}Cu ($T_{½}$ = 9.76 min) can be eluted from the generator with 0.1 N HCl containing NaCl (100 mg mL^{-1}) and with or without carrier $CuCl_2$ (1 µg mL^{-1}) (Robinson et al. 1980). A commercial generator has been used to develop radiopharmaceuticals for clinical evaluation (Haynes et al. 2000). An improved and fully remote-controlled ^{62}Zn/^{62}Cu generator based on a cation exchanger for clinical use was recently discussed (Fukumura et al. 2006).

On the basis of the Cu (II)-bis(thiosemicarbazone) or Cu-BTS complex, several PET radiopharmaceuticals (Fig. 12.6) have been developed (Anderson et al.

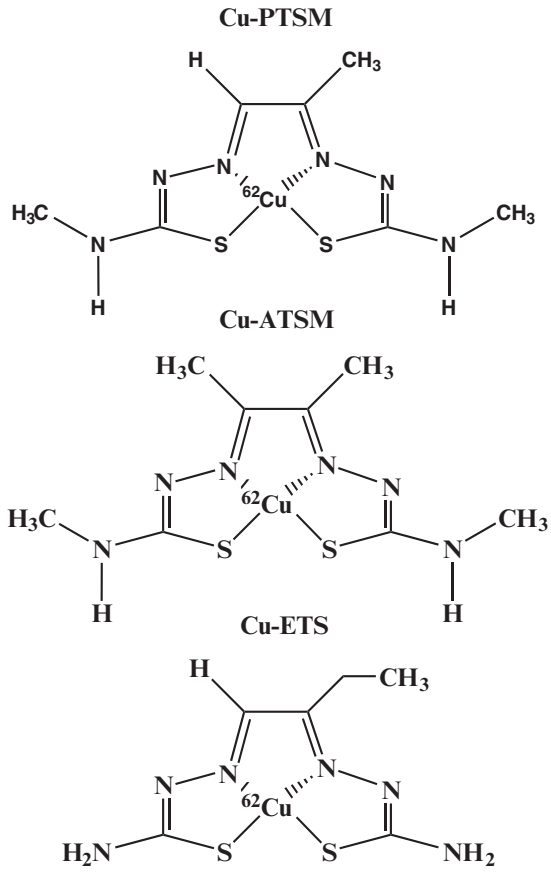


Fig. 12.6 Cu (II)-bis(thiosemicarbazone) or Cu-BTS complexes: ^{62}Cu-PTSM and ^{62}Cu-ETS for designed to measure myocardial blood flow while ^{62}Cu-ATSM has preferential uptake in hypoxic tissue

2003). Among these tracers, ^{62}Cu-pyruvaldehyde-bis(N^4-methylthiosemicarbazone (Cu-PTSM) and ^{62}Cu-diacetyl-bis(N^4-methylthiosemicarbazone) (Cu-ATSM) (Mathias et al. 1990; Lewis and Welch 2001) have been extensively evaluated in patients. Cu-PTSM was designed as a tracer to measure blood flow, while Cu-ATSM has preferential uptake in hypoxic tissue. These agents are neutral, lipophilic, and rapidly diffuse into the cells. Subsequently, the intracellular enzymes, which will bind to macromolecules within the cell reduce the complex to the Cu(I) oxidation state. While Cu-PTSM has demonstrated significant potential for imaging myocardial blood flow, its very high binding to albumin in the circulation impairs its ability for quantitative determination of myocardial perfusion (Basken et al. 2008). A second generation of Cu-BTS complex, known as ^{62}Cu-ETS, with minimal plasma protein binding is currently under clinical evaluation.

12.4.1.2 ^{64}Cu Labeled Peptides and Proteins

A second class of radiopharmaceuticals, based on biomolecules such as peptides and proteins, are being developed using ^{64}Cu and ^{67}Cu. Attachment of Cu radionuclides to molecular probes requires the use of a BFC, which is used to connect a radionuclide and a biological molecule. The two most common chelators studied thus far have been the macrocyclic ligands TETA and DOTA. Since the 1980s, mAbs labeled with ^{67}Cu have been evaluated extensively as therapeutic agents, however, ^{64}Cu labeled peptides have attracted much attention recently as radiopharmaceuticals due to their relatively low immunogenicity, good pharmacokinetic properties, and binding affinities (Fichna and Janecka 2003).

^{64}Cu Labeled Peptides and Proteins

The somatostatin receptor binding peptide, octreotide, was, recently, conjugated with TETA and labeled with ^{64}Cu (Anderson et al. 2001). Preliminary preclinical studies have demonstrated that the ^{64}Cu-TETA-octreotide detects more tumor lesions than the clinically approved ^{111}In-DTPA-octreotide. In addition, ^{64}Cu-TETA-octreotide significantly inhibits the growth of somatostatin receptor positive tumors in animal models. Similar results have been documented with a number of other ^{64}Cu labeled octreotide analogs.

The cyclic peptide Arg–Gly–Asp (RGD) binds to cancer cells and/or neoplastic vascular endothelial cells via the $\alpha_v\beta_3$ integrin receptor. RGD has been labeled with ^{64}Cu as ^{64}Cu-DOTA-RGD and ^{64}Cu-DOTA-pegylated RGD peptide for PET imaging studies (Li et al. 2008). The introduction of a bifunctional polyethylene glycol moiety between DOTA and RGD led to some improved in vivo kinetics of the resulting radiotracer, compared to that of ^{64}Cu-DOTA-RGD, but the insertion of a long PEG also reduced the receptor binding affinity to some extent.

Bombesin analogs radiolabeled with ^{64}Cu, which contain various aliphatic linkers placed between the BBN peptide and the DOTA-chelator, have previously been evaluated for tumor imaging studies. Since new chelation systems, such as CB-TE2A (Fig. 12.5) have been reported to significantly stabilize the ^{64}Cu labeled complexes in vivo, BBN analogs of ^{64}Cu-CB-TE2A chelate complex, such as ^{64}Cu-CB-TE2A-8-AOC-BBN(714)NH_2 has been recently prepared and evaluated in preclinical studies (Garrison et al. 2007; Prasanphanich et al. 2007). These studies have demonstrated very high selectivity and affinity for the GRPr.

The EphA2 receptor tyrosine kinase is significantly overexpressed in a wide variety of cancer types. High EphA2 expression has been correlated with increased metastatic potential and poor patient survival. Further, a humanized mAb (IC1) specific for both human and murine EphA2, has been labeled with ^{64}Cu using DOTA and evaluated for tumor imaging studies in animal models with different EphA2 expression levels. Quantitative radioimmuno-microPET imaging studies have demonstrated excellent correlation between tumor uptake and receptor expression (Cai et al. 2007a). ^{64}Cu-DOTA-cetuximab, a chimeric mAb specific for epidermal growth factor receptors (EGFR) has also been prepared and evaluated in preclinical studies (Cai et al. 2007b). The success of EGFR-positive tumor imaging using ^{64}Cu-DOTA-cetuximab can be used in the clinic to characterize the pharmacokinetics, select the right population of patients for EGFR-targeted therapy, monitor the therapeutic efficacy of antiEGFR treatment, and to optimize the dosage of either cetuximab alone or cetuximab in combination with other therapeutic agents (Lucignani 2008).

12.4.2 ^{89}Zr-Labeled mAbs

Preclinical and clinical studies have also previously demonstrated the diagnostic potential of immuno-PET

based on ^{86}Y- or ^{124}I-labeled mAbs. While ^{86}Y is an ideal radiometal for developing antibody-based radiopharmaceuticals, the physical characteristics (short half-life, positron energy, and gamma emission) are not ideal for PET radioimmuno imaging studies. Because iodine-labeled proteins are dehalogenated in vivo, ^{124}I is, therefore, not ideal for preparing radioiodinated mAbs.

Further, since the intact antibodies need 2–3 days to penetrate a solid tumor, the selection of the radionuclide with suitable half-life is essential. ^{89}Zr, a transition metal has an ideal physical half-life (78.4 h) and appropriate β^+ energy (E_{mean} = 0.39 MeV) for PET imaging studies (DeJesus et al. 1990). The most preferred method of production is based on the reaction ^{89}Y(p,n)^{89}Zr using ^{89}Y (natural abundance 100%) foil as the target material. Subsequently, ^{89}Zr is purified by anion-exchange chromatography. Zr complexes with hydroxamates in acidic solutions (>1N HCl), compare to other metallic impurities (Fe, Al, Y), which do not interact. The most common and convenient chemical form is ^{89}Zr in oxalic acid (0.5 M) with a purity >99.99% (Verel et al. 2003).

It has been shown that the methanesulfonate salt of desferrioxamine or desferal (DF) is an ideal BFC to complex ^{89}Zr since it forms stable bonds with the three hydroxamate groups (Meijs et al. 1992). For the coupling of DF to mAbs, a modified form of DF, *N*-(*S*-acetyl)mercaptoacetyldesferrioxamine B (SATA-Df) can be used. Conjugation to mAb can be performed following modification of lysine groups of mAb into maleimide groups (Meijs et al. 1992). Also, a conjugation method based on the reaction of an active 2,3,5,6-tetrafluorophenol-chelate ester (TFP-chelate ester) with the lysine moieties of mAb, results in a stable amide bond as the linker unit. This method provides optimal control over the number of groups conjugated to the mAb and has been used for the production of radioimmuno-PET (Verel et al. 2003). Since direct conjugation of mAbs with TFP-chelate ester is not possible, first blocking it with iron is an essential step for developing a postconjugation labeling method. Following removal of the iron with EDTA, the radiolabeling of mAb involves the transchelation of ^{89}Zr from oxalate to DF coupled mAb at a physiological pH (Fig. 12.7). Further, because the ^{89}Zr labeling procedure uses lysine residues of the mAb for the stable coupling of the chelate moiety, the method is applicable to any mAb, as well as to mAb fragments or peptides that contain a lysine group.

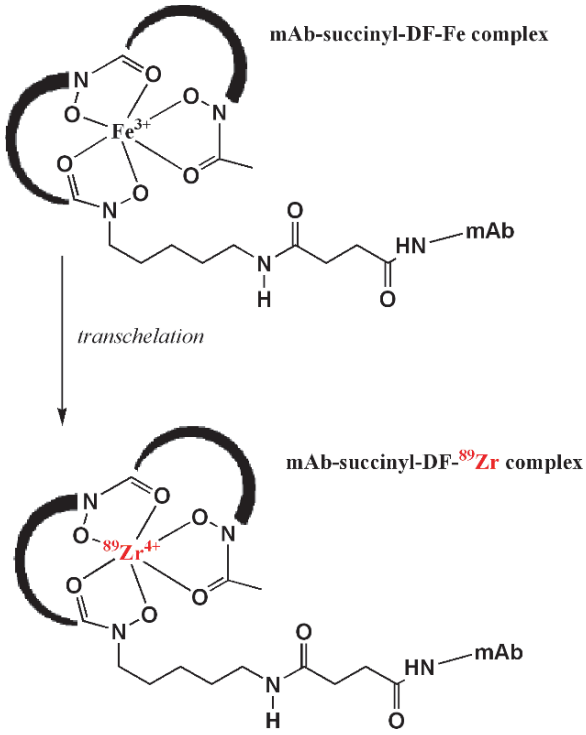


Fig. 12.7 Labeling monoclonal antibodies (mAb) with ^{89}Zr using desferal (DF) with its three hydroxamate groups. First step involves conjugation of mAb to DF and subsequent labeling with Fe to prepare mAb-succinyl-DF-Fe complex. Final step involves transchelation and labeling to obtain mAb-succinyl-DF-^{89}Zr complex

12.4.3 Technetium Chemistry

Technetium (Tc) was first discovered in 1937 by Emilio Segre and Carlo Perrier. Because it was artificially produced by bombarding molybdenum with deuterons, the name technetium for this new element was derived from the Greek word *technetos*, meaning artificial. Trace amounts of ^{99}Tc, however, were isolated from a uranium-rich ore in 1961 and totay more than 20 isotopes of technetium are known, all of which are radioactive. The most useful isotope, the metastable ^{99m}Tc ($T_{½}$ = 6.01 h), decays by isomeric transition to the relatively long-lived ^{99}Tc ($T_{½}$ = 2.1 × 10^5 year) following emission of a 140 KeV gamma photon. As previously noted, in 1958 scientists at the Brookhaven National Laboratory (BNL) reported the development of the first ^{99m}Tc generator based on the parent radioisotope ^{99}Mo. Since the 1970s, ^{99m}Tc radiopharmaceuticals have played a major role in the advancement of nuclear

medicine as a diagnostic specialty. Also, since the SA ^{99m}Tc can be very high (599 Ci μmol^{-1}), it is an excellent nuclide for developing molecular imaging radiopharmaceuticals for SPECT. ^{94m}Tc, a positron emitting radionuclide ($T_{½}$ = 53 min) with even higher theoretical SA, may also have significant potential for developing radiopharmaceuticals for use with PET.

Tc is a second row group VII transition metal that is capable of multiple oxidation sates (−1 to +7). In aqueous solution, the pertechnetate anion, $^{99m}TcO_4^-$, is the most stable chemical species with a +7 oxidation state. Because of the similar size and charge as that of iodide (I^-), the in vivo distribution of pertechnetate is similar to that of an iodide ion (Deutsch et al. 1983). However, because pertechnetate is chemically stable and inert, it can not bind directly to any organic molecule or chelate. Following reduction by appropriate reducing agents, pertechnetate can be transformed into lower oxidation states that are chemically more reactive. Several reducing agents have been investigated with stannous chloride ($SnCl_2$) being the most widely used agent for preparing complexes of Tc(V) and Tc(I), while boronhydrides are used to prepare organometallic Tc(I) complexes. During reduction by the stannous ion (Sn^{2+}), in an appropriate buffer and pH, the presence of a ligand stabilizes Tc in its lower oxidation state. In a specific Tc-complex, the oxidation state of Tc, however, depends on the chelate and pH (Deutsch et al. 1983). As a transition metal, Tc can adopt a large number of coordination geometries, depending on the donor atoms and the type of the chelating agent. Several donor atoms, such as N, S, O and P, geometrically arranged in a chelating molecule, can form coordination complexes with technetium. A number of ligands, such as DTPA, Dimercaptosuccinic acid (DMSA), iminodiacetic acid (IDA) derivatives (such as HIDA, DISIDA, BrIDA), phosphates, and phophonates (such as PYP, MDP, EHDP) have been labeled with ^{99m}Tc, and routinely used for diagnostic imaging studies in nuclear medicine.

12.4.3.1 Tc(V) Complexes

The radiopharmaceutical chemistry of Tc(V) is dominated by the $\{TcO\}^{3+}$ core, which is stabilized by a wide range of donor atoms (N, S, O), but has a preference for thiolate, amido, and alkoxide ligands. Several tetraligand chelates designed to bind to Tc(V) are typically form complexes (such as N_2S_2, N_3S, N_3O, and N_4) having square pyramidal geometries (Fig. 12.8). In Ceretec™, the hexamethylpropyleneamineoxime (HMPAO) ligand forms a neutral square pyramidal complex with the $\{^{99m}TcO\}^{3+}$core, while Neurolite™ consists of a chelate (ECD) which is made up of two cysteine ethyl ester units that form a neutral complex with the same core. Following diffusion into the brain

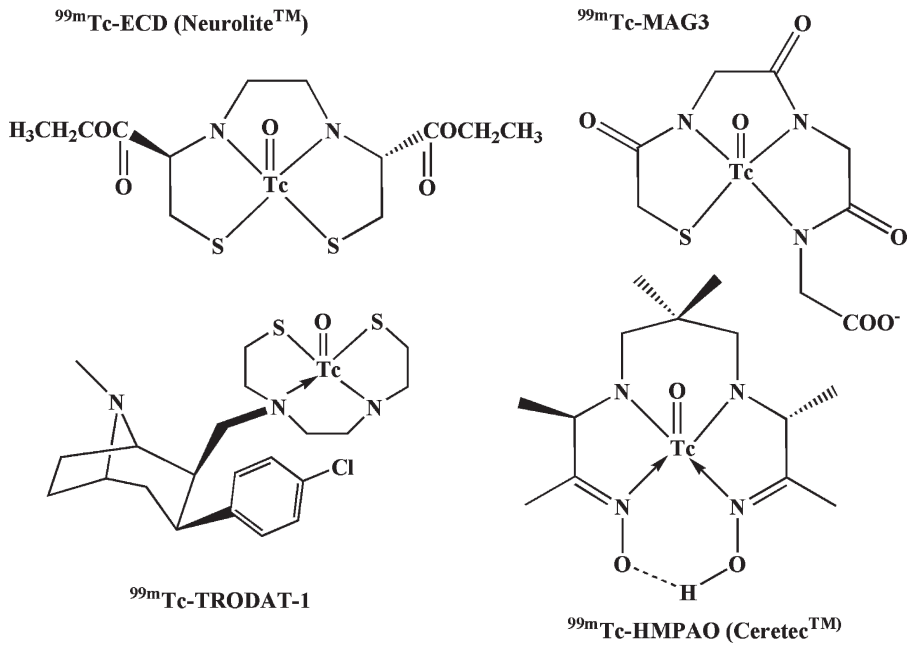


Fig. 12.8 Tc(V) complexes (such as N_2S_2, N_3S, N_3O, and N_4) having square pyramidal geometries that are routinely used for clinical imaging studies based on SPECT

cells, the ester group is hydrolyzed by an esterase, and the ^{99m}Tc-complex is trapped within the cell. The ^{99m}Tc complex of mercaptoacetyltriglycine (MAG3) also forms a square pyramidal complex with Tc(V) with the basal plane consisting of three nitrogen atoms and one sulfur donor atom. The carboxylic acid group does not coordinate to the metal center and is believed to help facilitate excretion via the kidneys.

^{99m}Tc-TRODAT-1 is a conjugate of a cocaine derivative with a ^{99m}Tc(V)oxo-diaminodithiol (N_2S_2) complex developed by Kung and coworkers for the diagnosis of Parkinson's disease based on SPECT (Meegalla et al. 1996; Kung et al. 1996). This is one of the most successful ^{99m}Tc labeled receptor imaging agents in clinical use, to date.

An important consideration when preparing complexes of Tc(V) is the formation of stereoisomers due to the fact that the substituents of the stereogenic center can be located *syn* (same side) or *anti* (opposite side) to the Tc-oxo bond. These compounds are also called diastereomers.

12.4.3.2 Tc(I) Organometallic Complexes

Complexes containing direct metal–carbon bonds are generally classified as organometallic compounds, which traditionally are prepared under strictly anhydrous conditions. Tc isonitrile complexes of the type $[Tc(CNR)_6]^+$ are widely used to assess cardiac function and, unlike most organometallic compounds, can be prepared in high yield in aqueous solutions.

The hexakis-((2-methoxy-2-methyl-1-propyl) isonitrile) complex of ^{99m}Tc(I) (Tc-Sestamibi, or Cardiolyte®) is the most prominent example of monoligand complexes, in which six methoxy isobutyl isonitrile (MIBI) groups bind to a single Tc (I) atom. A copper adduct is used as the precursor for ^{99m}Tc labeling and to prevent premature degradation of the reactive isonitrile ligands.

Tc-Tricarbonyl Core, $[Tc(CO)_3]^+$

A major advancement in Tc chemistry has been the discovery that a highly adaptable tricabonyl Tc core makes it possible to prepare organometallic complexes in aqueous solution (Alberto et al. 1999). In an effort to develop new organometallic precursors, for the preparation of ^{99m}Tc-complexes, investigators shown

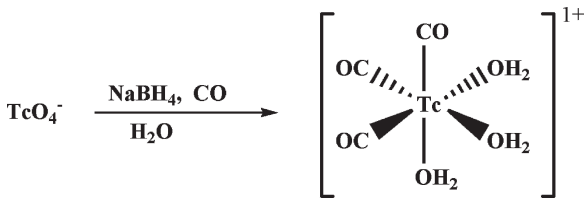


Fig. 12.9 The preparation of Tc-tricarbonyl core by treating ^{99m}Tc peertechnetate with sodium borohydride in the presence of carbon monoxide (CO) gas or potassium boranocarbonate ($K_2H_3BCO_2$)

that, by treating pertechnetate (TcO_4^-) with sodium borohydride ($NaBH_4$) in the presence of carbon monoxide (CO) gas, one can produce the reactive Tc(I) species, $Tc(CO)_3(OH_2)_3]^+$ (Fig. 12.9) (Waibei et al. 1999; Alberto et al. 1999). In this complex, the three facially oriented water molecules are sufficiently labile so that they can be readily displaced by a variety of mono-, bi- and tridentate ligands. Since it is difficult to work with CO gas, the technology is based on the use of a solid reagent, potassium boranocarbonate ($K_2H_3BCO_2$), which acts as both, a reducing agent and a source of CO gas (Alberto et al. 2001). The kit is available from Mallinckrodt (Tyco) Medical under the trade name Isolink. Further, it has been shown that both, bidentate and tridentate chelates bind rapidly to the $[Tc(CO)_3]^+$ core on a macroscopic scale and at the tracer level.

12.4.3.3 ^{99m}Tc-Labeled Biomolecules

The ability to incorporate ^{99m}Tc or the cyclotron produced ^{94m}Tc into tracer molecules, such as peptides and proteins, is of significant importance for the development of radiolabeled molecular imaging probes. In the 1990s several approaches have been developed to label peptides and proteins with ^{99m}Tc (Liu and Edwards 1999). New directions in developing chelators for developing ^{99m}Tc-chelate-biomolecule complex has been reviewed recently (Banergee et al. 2005). Three important labeling methods which have been developed are:

- The MAG_3-based bifunctional chelates
- The *N*-oxysuccinimidylhydrazino-nicotinamide system and
- The recently described single amino acid chelates for the $[Tc(CO)_3]^{1+}$core

The ^{99m}Tc(V)O complex of mercaptoacetyltriglycine (MAG_3H_5), was developed by Fritzberg et al., in 1986

as an anionic kidney-imaging agent. The parent ligand is readily derivatized as the *S*-acetyl MAG_3-ethyl ester, containing a *p*-isothiocyanatobenzyl substituent, or as the *S*-acetyl MAG3-hydroxysuccinimidyl ester for conjugation to biomolecules (Ram and Buchsbaum 1994). An alternative pendant approach to radiolabeling is provided by the *N*-oxysuccinimidylhydrazinonicotinamide (HYNIC) as a bifunctional chelator (Babich and Fischman 1995).

The $[Tc(CO)_3]^{1+}$ carbonyl core offers the possibility of radiolabeling by appending a labeling group to the biomolecule (peptide or protein) by means of tridentate chelators or a combination of bidentatate and monodentate ligands. The carbonyl chelators, diaminoproprionic acid (DAP), retroNα-carboxymethyl histidine, and L-propargyl glycine are water-soluble, and the aqua groups readily undergo ligand exchange. These chelators are readily attached to the N-terminus of a peptide or to an orthogonally deprotected amino group side chain during chemical synthesis and bind ^{99m}Tc(I) carbonyl aquaions. Further improvements in the radiolabeling of proteins with the ^{99m}Tc using the tricarbonyl core can be accomplished by introducing thiol groups to protein structure by preparing derivatives with mercaptobutyrimidyl groups (MBG), which can be generated following reaction with 2-iminothiolane. It has been shown that the addition of three MBG groups could double the radiolabeling yields to more than 90% in a short time, at room temperature (Biechlin et al. 2005).

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