Copper Chelation Chemistry and its Role in Copper Radiopharmaceuticals

T.J. Wadas[1], E.H. Wong[2], G.R. Weisman[2] and C.J. Anderson[1,*]

[1]*Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110, USA and* [2]*Department of Chemistry, University of New Hampshire, Durham, NH 03824, USA*

Abstract: Molecular imaging is an important scientific discipline that plays a major role in clinical medicine and pharmaceutical development. While several imaging modalities including X-ray computed tomography (CT) and magnetic resonance imaging (MRI) generate high-resolution anatomical images, positron emission tomography (PET) and single photon emission computed tomography (SPECT) offer insight into the physiological processes that occur within a living organism. Of these two nuclear medicine imaging techniques, PET has advantages with respect to sensitivity and resolution, and this has led to the production and development of many positron emitting radionuclides that include non-traditional radionuclides of the transition metals. Copper-64 ($t_{1/2}$ = 12.7 h, β^+: 17.4%, $E_{\beta+max}$ = 656 keV; β^-: 39%, $E_{\beta-max}$ = 573 keV) has emerged as an important positron emitting radionuclide that has the potential for use in diagnostic imaging and radiotherapy. However, ^{64}Cu must be delivered to the living system as a stable complex that is attached to a biological targeting molecule for effective imaging and therapy. Therefore, significant research has been devoted to the development of ligands that can stably chelate ^{64}Cu. This review discusses the necessary characteristics of an effective ^{64}Cu chelator, while highlighting the development and evaluation of ^{64}Cu-complexes attached to biologically-targeted ligands.

Key Words: Copper-64, bifunctional chelator, positron emission tomography, radiopharmaceutical, macrocycle.

INTRODUCTION

Molecular imaging has emerged as an important scientific discipline, which has had a tremendous impact on clinical medicine and pharmaceutical development over the last decade. It allows physicians to generate high-resolution images of the human body non-invasively, diagnose illness and prescribe treatment regimens based upon them. In addition, scientists involved with drug discovery are able to accelerate the screening processes in pre-clinical drug development. Molecular imaging can aid in facilitating determinations of desirable or undesirable pharmacological side effects, analyze interactions between a drug or drug candidate with its desired target, and evaluate delivery, absorption, distribution, metabolism and elimination in a living system [1-3].

Several imaging modalities that are currently used in diagnostic medicine and pharmaceutical development are computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT) and positron emission tomography (PET). CT and MRI are primarily anatomical imaging techniques that provide superior resolution, while PET and SPECT are nuclear medicine imaging modalities that image physiological processes that are occurring within the living system. Of the latter two, PET has advantages with respect to sensitivity and resolution. While each modality provides important information to the investigator, dual modality imaging like PET/CT or SPECT/CT integrates the high-resolution anatomical images with physiological information and enables the investigator to identify the physiological basis of disease and correlate it with the anatomical image.

*Address correspondence to this author at the Mallinckrodt Institute of Radiology, Washington University School of Medicine, 510 S. Kingshighway Blvd., Campus Box 8225, St. Louis, MO 63110, USA; Tel: 314-362-8427; Fax: 314-362-9940; E-mail: andersoncj@wustl.edu

PET has become a widely used diagnostic imaging tool by clinicians in the United States with over 1 million PET imaging procedures performed in 2001 [4]. The pharmaceutical industry has also begun applying PET imaging to drug discovery using rodent and other non-human models of human disease. In pharmaceutical research and development, however, human PET scanners are inadequate in terms of spatial resolution for imaging rodents and smaller animals. To address this issue, small animal PET systems have been developed with the ability to image rodents and generate reconstructed images with a resolution as low as 1mm. However, generating images of superior quality is not a trivial task and the continual development of systems with increased sensitivity and spatial resolution is a serious research question that is beyond the scope of this review. A complete discussion regarding the development and implementation of small animal PET systems can be found in the literature [4, 5].

PET imaging requires the delivery of a pharmacologically significant molecule containing a positron-emitting radionuclide to a tissue or organ of interest. As the radionuclide decays it ejects a positron from its nucleus which travels a short distance before being annihilated with an electron to release two 511 keV gamma rays 180° apart (Fig. (**1**)). The emitted gamma rays of these coincident annihilation events are captured by the detectors of the PET scanner, and after sufficient acquisition time the data are reconstructed to yield images of the radiotracer's location within the organism.

Selection of the proper radionuclide in radiopharmaceutical design is critical and depends upon several factors. The half-life of the radionuclide should allow for sufficient uptake and decay to yield considerable contrast and quality images [6]. The energies of the radionuclide emission should

1381-6128/07 $50.00+.00

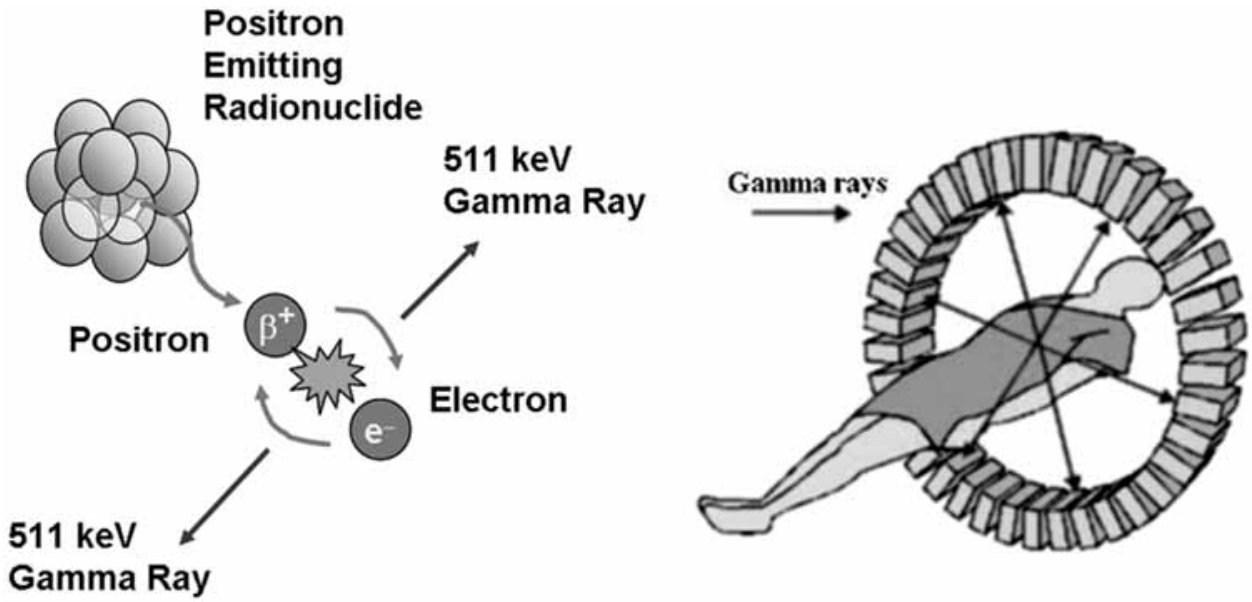


Fig. (1). A positron emitting radionuclide emits a positively charged electron (β^+) and combines with a negatively charged electron in tissue. The collision between the positron and the electron results in the emission of two 511 keV photons approximately 180° apart. A PET scanner detects the coincident 511 keV photons.

be appropriate for proper detection by the equipment while cost and availability are also important considerations. The radionuclides currently used, including ^{15}N, ^{11}C, ^{15}O, ^{18}F, ^{76}Br and ^{124}I are incorporated into radiotracers using traditional organic synthetic techniques and a comprehensive review of their production, isolation and chemistry is available [7]. Recently, non-traditional PET radionuclides, particularly those of the transition metals, have gained considerable interest because of increased production and availability. While radionuclides like ^{94m}Tc, ^{66}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{45}Ti and $^{60/61/62/64}$Cu have been prepared and used as imaging or radiotherapeutic agents, significant research effort has been devoted to the copper radionuclides because they offer a varying range of half-lives and positron energies as depicted in Table **1** [8]. Finally, the well-established coordination chemistry of copper allows for its reaction with a wide variety of chelator systems that can potentially be linked to antibodies, proteins, peptides and other biologically relevant small molecules.

COORDINATION CHEMISTRY OF COPPER

The aqueous solution coordination chemistry of the transition metal copper is limited to its three accessible oxidation states (I-III) [9-12]. The lowest oxidation state, Cu(I) has a diamagnetic d^{10} configuration and forms complexes without any crystal-field stabilization energy. Complexes of this type

Table 1. Decay Characteristics of Copper Radionuclides

Isotope	$t_{1/2}$	β^- MeV (%)	β^+ MeV (%)	EC (%)	γ MeV (%)
^{60}Cu	23.4 min	---	2.00 (69%) 3.00 (18%) 3.92 (6%)	7.4%	0.511 (186%) 0.85 (15%) 1.33 (80%) 1.76 (52%) 2.13 (6%)
^{61}Cu	3.32 h	---	1.22 (60%)	40%	0.284 (12%) 0.38 (3%) 0.511 (120%)
^{62}Cu	9.76 min	---	2.91 (97%)	2%	0.511 (194%)
^{64}Cu	12.7 h	0.573 (39.6%)	0.655 (17.4%)	41%	0.511 (34.8%) 1.35 (0.6%)
^{67}Cu	62.0 h	0.395 (45%) 0.484 (35%) 0.577 (50%)	---	---	0.184 (40%)

are readily prepared using relatively soft polarizable ligands like thioethers, phosphines, nitriles, isonitriles, iodide, cyanide and thiolates. A broad range of coordination geometries is observed. Cu(I) complexes are biologically relevant because they are able to reductively activate molecular oxygen (O_2). However, due to the lability of most Cu(I) complexes, they typically lack sufficient kinetic stability for radiopharmaceutical applications.

Copper (II) exists as a d^9 metal center of borderline softness which favors amines, imines, and bidentate ligands like bipyridine to form square planar, distorted square planar, trigonal pyramidal, square pyramidal, as well as distorted octahedral geometries. Jahn-Teller distortions in six-coordinate Cu(II) complexes are often observed as an axial elongation or a tetragonal compression. Due to the presence of some crystal-field stabilization energy, Cu(II) is generally less labile toward ligand exchange and is the best candidate for incorporation into radiopharmaceuticals.

A third oxidation state Cu(III) is relatively rare and difficult to attain without the use of strong π-donating ligands. These complexes usually adopt a square planar geometry due to the d^8 Cu(III) electron configuration. A review has been published describing the chemistry of this oxidation state [13].

PRODUCTION OF COPPER RADIONUCLIDES

One of the major challenges in the production of radionuclides for use as biological tracers or diagnostic imaging and targeted radiotherapy applications is the production of radionuclides with high specific activity, i.e. a high amount of radioactivity with the lowest possible amount of non-radioactive isotopes. Preparing high specific activity copper radionuclides is an even bigger challenge, since copper is ubiquitous in the environment. For all of the copper isotopes described below, a non-copper target is used to produce no-carrier-added copper radionuclides (i.e. no unlabeled copper used in the production process). In using a target having a different atomic number, a chemical separation of the copper radionuclide from the target material is possible. In addition to using non-copper target material, the experimental conditions for preparing the target and separating the copper radionuclides from the target must be as metal-free as possible. Since the production of copper radionuclides (^{60}Cu, ^{61}Cu, ^{62}Cu and ^{67}Cu) has been covered extensively in the literature [14-18], this section of the article will briefly describe the most current production methods of ^{64}Cu.

PRODUCTION OF ^{64}Cu

Copper-64 can be effectively produced by both reactor-based and accelerator-based methods. One method of ^{64}Cu production is the ^{64}Zn(n,p)^{64}Cu reaction in a nuclear reactor [19]. Most reactor-produced radionuclides are produced using thermal neutron reactions, or (n,γ) reactions, where the thermal neutron is of relatively low energy, and the target material is of the same element as the product radionuclide. For producing high-specific activity ^{64}Cu, fast neutrons are used to bombard the target in a (n,p) reaction. This method enabled the production of high specific activity ^{64}Cu at the Missouri University Research Reactor (MURR) in amounts averaging 9.25 GBq (250 mCi) [19].

Smith and coworkers separated large amounts of ^{64}Cu by-product from cyclotron production of ^{67}Ga *via* the ^{68}Zn (p,2n)^{67}Ga reaction at the National Medical Cyclotron, Sydney, Australia [20]. This mode of production has the advantage of being very economical and allows for production of very large amounts (> 111 GBq (> 3 Ci)) of reasonably high specific activity material (~ 31.8 TBq/mmol (~ 860 Ci/mmol)). The disadvantage is that on-demand production would be problematic, since the major radionuclide produced is longer-lived ^{67}Ga ($T_{1/2}$ = 72 h).

The production of no-carrier-added ^{64}Cu *via* the ^{64}Ni(p,n) ^{64}Cu reaction on a biomedical cyclotron was proposed by Szelecsenyi *et al.* In this study small irradiations were performed demonstrating the feasibility of ^{64}Cu production by this method [21]. Subsequent studies by McCarthy *et al.* were performed, and this method is now used to provide ^{64}Cu to researchers throughout the United States [22]. The following paragraphs will outline the methods for production of large amounts (up to 37 GBq (1 Ci)) of ^{64}Cu for diagnostic imaging and cancer therapy applications.

The target for producing ^{64}Cu is enriched ^{64}Ni (99.6%) (ISOFLEX, San Francisco, CA; ~$18/mg). The ^{64}Ni (typically 10-50 mg) is prepared as previously described [22] and electroplated onto a gold disk (ESPI Metals, Ashland, OR) using a procedure modified from Piel *et al.* [23]. At Washington University School of Medicine, ^{64}Cu is produced on a Cyclotron Corporation CS-15 cyclotron using 15.5 MeV protons (15-45 μA beam current) by the ^{64}Ni(p,n)^{64}Cu reaction. After bombardment, the ^{64}Cu is separated from the target nickel in a one-step procedure using an ion-exchange column. Typically, 18.5 GBq (500 mCi) ^{64}Cu are produced with a 40 mg ^{64}Ni target and a bombardment time of 4 h. The specific activity of the ^{64}Cu ranges from 47.4 to 474 GBq/μmol (1280 to 12,800 mCi/μmol). The typical yields for ^{64}Cu productions are 0.2 mCi/μA·h per mg ^{64}Ni. The enriched ^{64}Ni can be 85-95% recovered as previously described and re-used for future bombardments, which contributes to the cost-efficiency of this method of ^{64}Cu production [22].

Recently, Obata *et al.* reported the production of ^{64}Cu on a 12 MeV cyclotron, which is more representative of the modern cyclotrons currently in operation [24]. They utilized very similar methods to those previously published [22]. A remote system was described for separation of the ^{64}Cu from the ^{64}Ni target.

CHELATING LIGANDS FOR ^{64}Cu

Utilizing ^{64}Cu of high specific activity with an efficient chelator is critical in achieving high uptake of the copper radionuclide in the tissue or organ of interest while minimizing the non-selective binding or incorporation into non-target organs or tissues. The stability of the radio-copper complex *in vivo* is a critical factor for optimal ligand design. Ligands that can form radiocopper complexes with superior kinetic inertness to Cu(II) decomplexation (proton-assisted as well as transchelation or transmetallation) are ideal since this is more significant than thermodynamic stability after the radiocopper complex is injected into a living organism [25, 26]. As reduction of Cu(II) to Cu(I) and subsequent Cu(I) loss may also be a pathway for loss of radio-copper, resistance of the radio-copper complex to Cu(II)/Cu(I) reduction

as well as reversibility can also be important [27]. In addition, rapid complexation kinetics are also essential to allow for the facile formation of the radio-copper complex. Finally, chelators also must be designed with available functional groups that allow them to be covalently linked to targeting peptides, proteins, and antibodies. Such a ligand acts as a bifunctional chelator (BFC) consisting of the metal complexing ligand and a functional group for attachment to the targeting molecule as depicted in Fig. (**2**) [8, 26].

Some of the earliest BFC ligand systems used were acyclic polyamine carboxylate ligands which include EDTA (**1**), DTPA (**2**) and their derivatives (Fig. (**3**)) [28-35]. Despite high thermodynamic stabilities (log $K_{Cu\text{-}EDTA}$ = 18.7; log $K_{Cu\text{-}DTPA}$ = 21.4), serum stability measurements using the ^{67}Cu labeled ligands revealed that the ^{67}Cu complexes were not stable in human serum for long periods [34]. By day three of the study, ^{67}Cu-(*p*-NO_2-(benzyl)-EDTA and ^{67}Cu-DTPA-N-butylamide had retained only 18% and 35% respectively of the ^{67}Cu originally incorporated. Attempts to increase complex stability by the incorporation of rigidifying groups into the polyamine carboxylate backbone or by conjugating it to antibodies were made but neither approach met with success [36]. Other acyclic ligands that have demonstrated moderate success as ^{64}Cu chelators include N_2O_2 donors, Schiff-base ligands, dithiocarbamates, diphosphine ligands and *bis*-thiosemicarbazones, though their potential has only been partially realized [37-40]. For example, the diphosphine ligands shown in Fig. (**3**) (structures **4**-**7**) form lipophilic cationic complexes with Cu(I). These complexes were determined to be substrates for p-glycoprotein, thereby having potential as PET agents for imaging multi-drug resistance [41]. These low molecular weight complexes showed sufficient *in vivo* stability and rapid blood clearance that resulted in significant tumor to blood ratios.

Bis-thiosemicarbazone ligands like H_2ATSM (**8**) and H_2PTSM (**9**) also chelate ^{64}Cu and the resulting complexes are utilized as blood flow and hypoxia imaging agents [37-40, 42-53]. However, their usefulness results from ^{64}Cu-complex instability once inside the heart or hypoxic tissue, and the chemistry that makes this possible has been well documented. Lewis *et al.* have shown that ^{64}Cu-ATSM is taken up into hypoxic tumor cells preferentially over normoxic tumor cells and has the potential to be an effective radiotherapeutic treatment in cancer therapy [46]. Multiple and single dose regimens of ^{64}Cu-ATSM were effective at significantly increasing the survival rates of GW39 tumor-bearing hamsters over their control counterparts. In addition, no overt toxicity was observed and significant survival benefit was seen at less than the maximum tolerated dose (MTD). These encouraging results have stimulated the synthesis and evaluation of other *bis*-thiosemicarbazone and *bis*-salicylaldimine ligands for the complexation of ^{64}Cu for the selective targeting of hypoxic tissues [37].

CHELATORS BASED ON CYCLAM AND CYCLEN BACKBONES

To improve the *in vivo* stability of ^{64}Cu complexes researchers have turned to tetraazamacrocyclic ligands with pendant arms that utilize both the macrocyclic and chelate effects to enhance stability. By far the most extensively used class of chelators for ^{64}Cu has been the macrocyclic polyaminocarboxylates shown in Fig. (**4**). These systems have been thoroughly investigated, and *in vitro* and *in vivo* testing have shown them to be superior to acyclic chelating agents for ^{64}Cu [31]. This enhanced stability is most likely due to the greater geometrical constraint incorporated into the macrocyclic ligand that enhances the kinetic inertness and thermodynamic stability of their ^{64}Cu complexes [54-56]. Two of the most important chelators studied were DOTA (**21**) and TETA (**22**). While DOTA has been used as a BFC for ^{64}Cu, its promiscuity as a chelating agent, and its relative instability when compared to TETA have made it only a marginal ^{64}Cu chelator [57-62]. The tetraazamacrocyclic ligand TETA therefore, has been extensively used as a radioligand for ^{64}Cu, and successful derivatization of this ligand has allowed researchers to conjugate it to antibodies, proteins, and peptides [34, 63-75].

Although ^{64}Cu-TETA complexes are more stable than ^{64}Cu-DOTA and ^{64}Cu-labeled complexes of acyclic ligands, their instability *in vivo* has been well documented. Using metabolism studies, Bass *et al.* demonstrated that when ^{64}Cu-TETA-OC was injected into normal Sprague-Dawley rats, nearly 70% of the ^{64}Cu from ^{64}Cu-TETA-OC was transchelated to a 35 kDa species believed to be superoxide dismutase (SOD) in the liver 20 h post-injection [76]. These results are supported by the observations of Boswell *et al.*

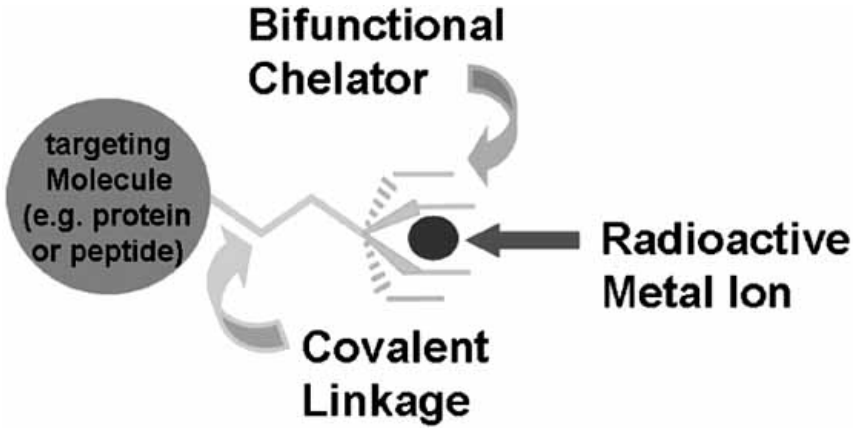


Fig. (2). A schematic of a radiopharmaceutical showing a bifunctional chelator to complex the radioactive metal ion that is attached to a molecule of pharmacological importance (small molecule, peptide or protein).

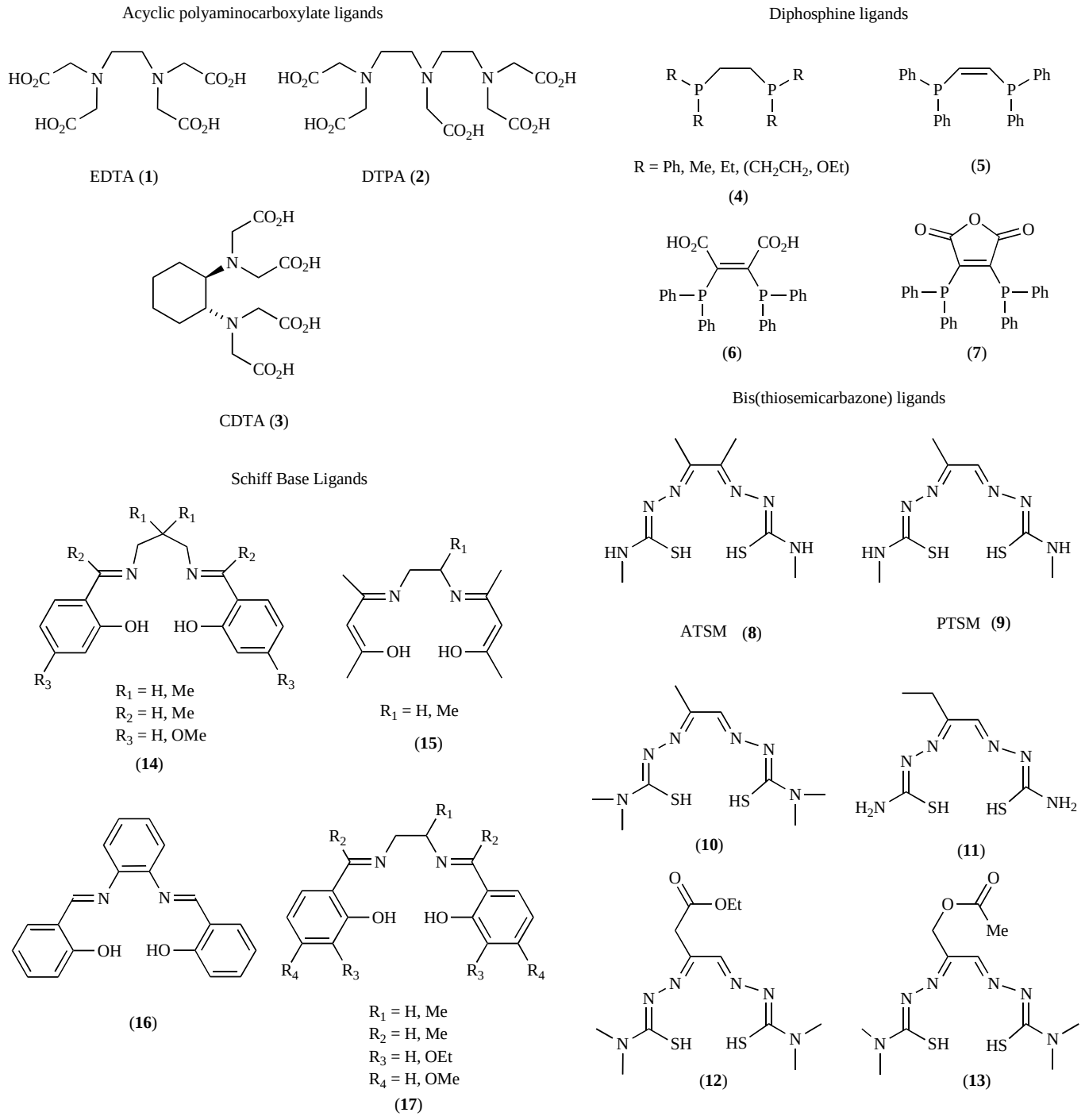
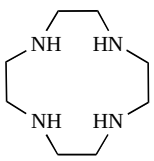


Fig. (3). Representative acyclic chelators for ^{64}Cu complexation.

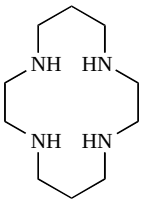
[77]. Additionally, Mirick *et al.* reported the dissociation of ^{67}Cu from the TETA derivative BAT (**24**) and its transchelation to ceruloplasmin during radioimmunotherapy trials in lymphoma patients [78]. Clinical PET studies using ^{64}Cu-TETA-OC also resulted in slow blood clearance and accumulation of ^{64}Cu in the liver over time [79].

One class of cyclic polyamine ligands showing relatively high stability *in vitro* and *in vivo* are the methylenephosphonate pendant armed tetraazamacrocylic ligands. Kotek and coworkers have synthesized and structurally characterized a series of these ligands, which when complexed to Cu(II) display a high degree of kinetic inertness [80]. Stability studies demonstrated that several of these Cu(II) complexes are stable between 30 and 210 days in 1.0 M acid. In addition, a ligand with a single methylenephosphonate pendant arm has been designed which selectively complexes Cu(II) over other metal ions making it attractive for ^{64}Cu radiopharmaceutical applications [81]. Several related phosphonate pendant-armed ligands (**20**) shown in Fig. (**4**) demonstrated high stability in rat serum and were also observed to have high uptake in bone suggesting their usefulness as bone imaging or

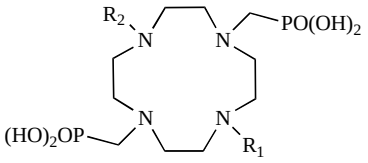
Cyclic Polyamines and their derivatives



Cyclen (**18**)



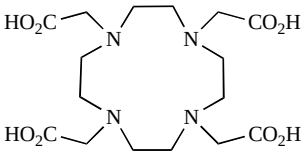
Cyclam (**19**)



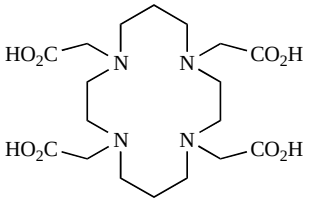
DOXP (X = 2: $R_1 = R_2 = H$;
X = 3: $R_1 = H$, $R_2 = CH_2PO(OH)_2$;
X = 4: $R_1 = R_2 = CH_2PO(OH)_2$)

(**20**)

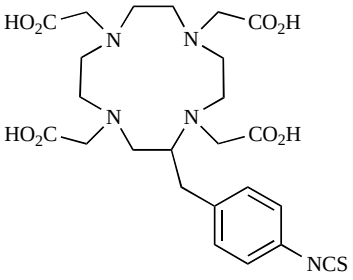
Cyclic Polyaminocarboxylates and their derivatives



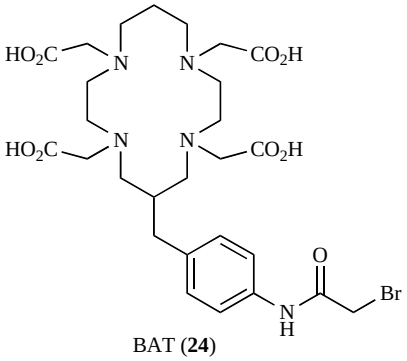
DOTA (**21**)



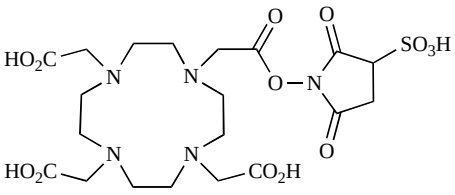
TETA (**22**)



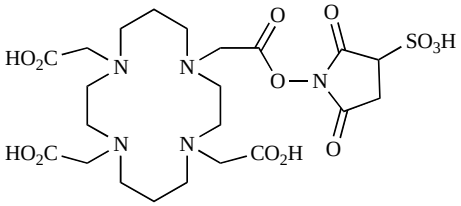
DOTA-NCS (**23**)



BAT (**24**)



DOTA-NHS (**25**)



TETA-NHS (**26**)

Fig. (4). Representative tetraazamacrocyclic chelators and their derivatives for ^{64}Cu complexation.

bone palliation agents, but as of this writing, there are no reports in the literature on the use of this class of chelators attached to targeting molecules [82].

Despite the considerable efforts made by researchers to use tetraaza-tetracarboxylate macrocyclic ligands as effective BFCs for ^{64}Cu, it is evident that the *in vivo* instability of these ^{64}Cu complexes emphasizes the need for more stable ^{64}Cu chelators. With this in mind, several new ligand systems, including those based upon the *cis, cis*-1,3,5-triamino-cyclohexane scaffold, the sarcophagine ligands and the cross-bridged (CB) tetraazamacrocycles have been developed to complex ^{64}Cu more stably.

THE TRIAMINOCYCLOHEXANE LIGANDS

Until recently, ligands based upon the *cis, cis*-1,3,5-triamino-cyclohexane scaffold have been difficult to prepare

in purities and yields high enough for physical characterization and biological evaluation. Bowen *et al*. first published an improved synthesis of the *cis, cis*-1,3,5-triamino-cyclohexane from the commercially available tricarboxylic acid [83]. Reaction with the appropriate carboxaldehyde followed by reduction leads to the formation of the chelators shown in Fig. (**5**). Brechbiel and coworkers have exploited this chemistry to generate a series of chelators that contain thiophenes and substituted pyridyl and imidazole rings [84]. While these systems have been synthesized and explored as chelation chemotherapeutics or anti-angiogenic therapies, a few reports have evaluated their potential as ^{64}Cu chelators [84-91]. Park *et al*. studied the natural Cu(II) complexes of N,N', N"-tris-(2-pyridylmethyl)-1,3,5-*cis,cis*-triaminocyclohexane (tachpyr) (**29**) and N, N', N"-tris-(6-methyl-2-pyridylmethyl-1,3,5-*cis,cis*-triaminocyclohexane (tachpyr-(6-Me)) (**31**) in solution, in the solid state and examined their serum stability as copper chelators using ^{64}Cu [91]. Synthesis of the $^{64/67}$Cu-tachpyr and $^{64/67}$Cu-(tachpyr-(6-Me)) complexes were achieved in 100% yields after 60 minutes at 37°C. Even though both ligands demonstrated facile complexation to $^{64/67}$Cu, they exhibited differing stability in human serum. The ^{67}Cu-tachpyr ion was stable over a period of 7 days, while significant dissociation of ^{67}Cu from the ^{67}Cu-(tachpyr-(6-Me)) complex occurred after only an hour at physiological temperatures.

In a second report, Ma *et al*. further investigated the utility of *cis,cis*-1,3,5-triaminocyclohexane derivatives by creating several new derivatives of the original tachpyr ligand and a new set of tachpyr analogs containing imidazole rings yielding the ligand *cis,cis*-1,3,5-triaminocyclohexane-N,N', N"-*tris*(2-methyl-N- methyl imidazole) (IM) (**30**) and its

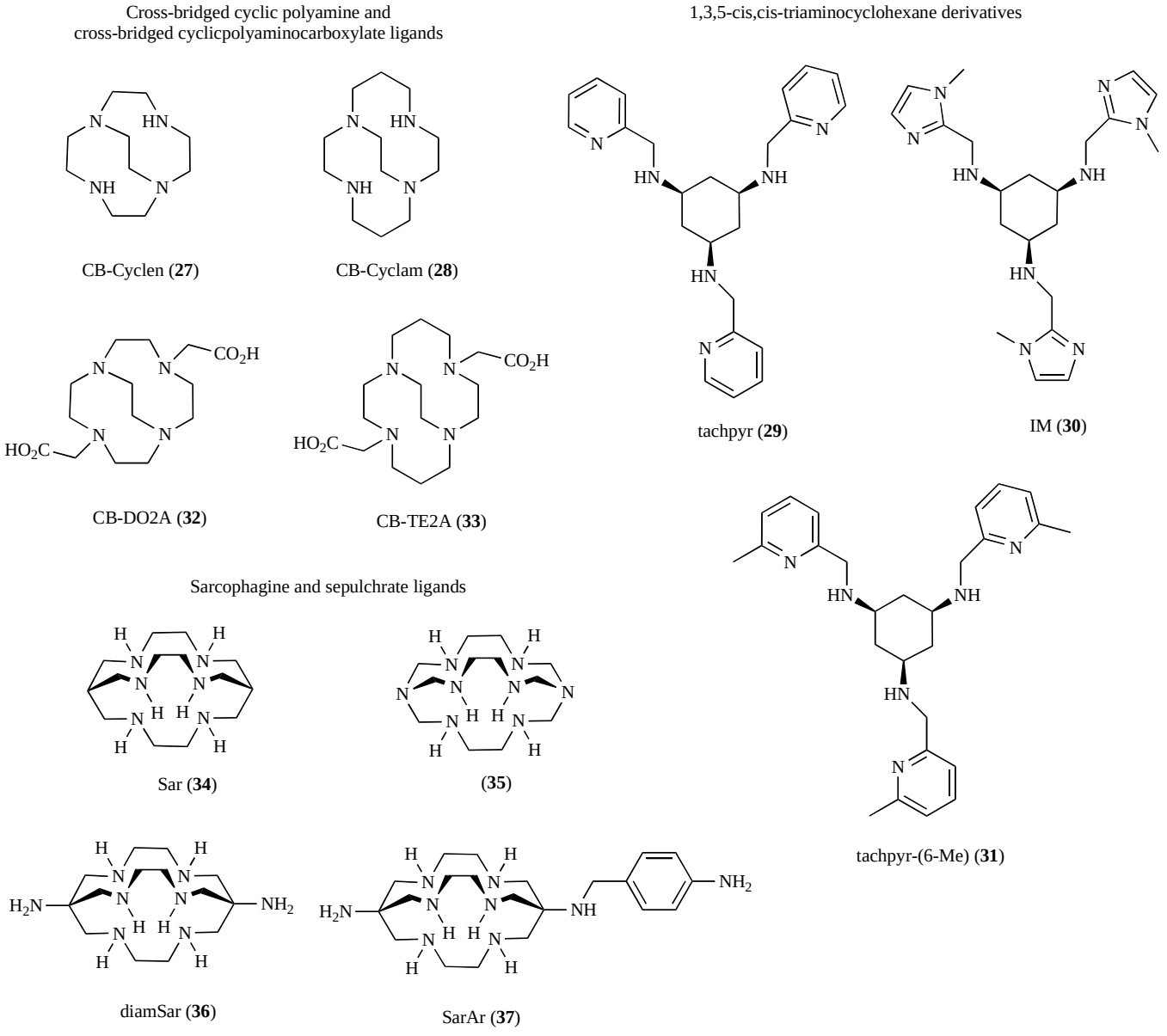


Fig. (5). Chelators currently being evaluated for ^{64}Cu complexation

derivatives [84]. All ligands were evaluated as radiocopper chelators and interestingly, the authors described variable formation kinetics based upon these ligand systems. For example, if the aliphatic secondary amines remain unsubstituted in either ligand system, then copper complexation is instantaneous with yields and purity of the radiocopper complexes approaching 100%. However, if these secondary aliphatic amines are substituted to form tertiary amines, then radiocopper complexation is slow and incomplete even after 2 h at 37°C. When all of the complexes were screened against an excess of EDTA, the $^{64/67}$Cu-tachpyr and the $^{64/67}$Cu-IM complexes were determined to be the most stable. In addition, only those complexes synthesized with secondary aliphatic amines in the ligand structure or those synthesized without methyl groups in the 6-position of the pyridyl rings were stable to transchelation by EDTA. In cross-ligand exchange experiments using ^{67}Cu-TETA, tachpyr was able to transchelate 43% and 92% of ^{67}Cu originally coordinated to TETA after 24 h and 96 h, respectively while IM was able to transchelate 63% and 95% of ^{67}Cu associated with TETA after the same periods. Conversely, TETA was able to transchelate only 7% and 30% of the ^{67}Cu in ^{67}Cu-tachpyr and only 3% of the ^{67}Cu in ^{67}Cu-IM. In addition, human serum stability experiments demonstrated that no activity was transferred to serum over a period of 14 days confirming the kinetic inertness of the radiocopper complexes.

Significant effort and resources have been utilized to synthesize and characterize these ligands, but before they can be safely used as ^{64}Cu radioligands their *in vivo* stability and biodistribution remain to be determined. Further, only one attempt to date has generated a system that can be covalently linked to monoclonal antibodies, and significant effort to modify these ligand systems has been needed in order to generate useful BFC's for conjugation to proteins or peptides or other small molecules [90, 92]. Loss of molecular symmetry, complications due to cross-linking and introduction of useful functional groups at specific locations on the ligand system remain as challenges that impede their use as ^{64}Cu radioligands for imaging and radiotherapy [84].

THE HEXAAMINE SARCOPHAGINE CHELATORS

Another class of ligands that has gained attention as potential ^{64}Cu chelators are the hexaazamacrobicyclic cage type ligands, which are based upon the sepulchrate (**35**) or sarcophagine cage motifs shown in Fig. (**5**) and whose syntheses were first described by Sargeson and co-workers [93, 94]. Both cage systems are synthesized by reaction of the inert tris-ethylenediamine cobalt (III) complex with formaldehyde followed by reaction with ammonia/formaldehyde or nitromethane/formaldehyde under basic conditions to generate the sepulchrate or sarcophagine (Sar) (**34**) ligands, respectively. When care is taken to use the chiral Co(III) *tris*-ethylenediamine complex, only one of the possible 16 isomers forms in the reaction, but the cobalt (III) metal center must be removed using cyanide ion or concentrated HBr to make the free ligand available as a chelator for other metal ions. These ligands can be prepared in high yields and high purity at low cost. Sar analogs substituted at the apical bridgehead carbons with a variety of functional groups including NH_2, OH, Cl, and NO_2 or other alkyl or aryl organic groups have also been synthesized.

Thermodynamic stability data of these cage complexes have been difficult to obtain, but it was shown that the Hg(II)-Sar complex is much more stable than Hg(II)-DOTA, Hg(II)-TETA or Hg(II)-cyclam [95]. This suggests that the hexaazamacrobicyclic complex resists metal dissociation from the cage ligand as a function of the ligand structure. It was hypothesized that when one of the chelating nitrogen atoms of the cage dissociates from the metal center, the topological constraint induced by the ligand does not allow it to move very far away from the metal center, effectively ensuring its facile re-coordination [94].

To date only one report that describes the complexation, stability, and biodistributions of the ^{64}Cu sarcophagine complexes has been published. DiBartolo *et al.* investigated a family of Sar derivatives with various functional groups at the apical sites, while the SarAr ligand (**37**) was used to determine the ^{64}Cu complexation rates from pH 4-9 [96]. From the data presented, complexation was 100% complete within several minutes at 25 °C over the entire pH range. Serum stability experiments conducted using human plasma demonstrated that the ^{67}Cu-Sar complex had exceptional stability with 98% of the associated activity remaining complexed after 7 days. In addition, biodistribution data was collected using ^{64}Cu-Sar, ^{64}Cu-diamSar (**36**) and ^{64}Cu-SarAr in Balb/c mice. All three complexes cleared from the blood rapidly and uptake was low in bone, heart, stomach, spleen, muscle, lungs, and the gastrointestinal tract. Liver clearance was observed to be good over the 30-minute time course of this study demonstrating that the ^{64}Cu complexes are initially stable *in vivo*, but clearance of all three ^{64}Cu complexes is much slower through the kidney. Activity levels increased in the case of the ^{64}Cu-Sar (**34**) complex, though this type of accumulation is not uncommon for positively charged complexes. Finally, the SarAr chelator has been covalently attached to whole and fragmented B72.3 murine antibodies and labeled with ^{64}Cu for imaging of mice bearing LS-174T colon carcinoma tumors [3]. High tumor-to-blood ratios were achieved with effective localization at the tumor was suggestive of *in vivo* stability of this radioimmunoconjugate.

While these preliminary investigations have demonstrated the potential of the sarcophagine ligand as ^{64}Cu chelators, more *in vivo* experimentation is warranted. Although the initial clearance rates at 30 minute are promising, a more rigorous examination of the biodistribution of these complexes out to 24 h would help to better determine the stability of these complexes. This is especially important since clearance of the complexes occurs through the kidney, which is often a dose-limiting organ.

THE CROSS-BRIDGED TETRAAMINE LIGANDS

Recently, the class of ethylene cross-bridged cyclam (**28**) and cyclen (**27**) ligands and their pendant armed derivatives have attracted attention as chelators for radiocopper. These ligands were first conceived of and synthesized by Weisman and coworkers in the 1990's [97, 98]. The highly-basic tetraamine macrobicyclic ligands were designed to complex selected metal cations like Li^+, Cu^{2+}, and Zn^{2+} within their clamshell-like clefts. Numerous copper complexes of these and related ligands have since been prepared and studied by Wong, Weisman and coworkers as well as other research groups [99-107]. With available structural data, the expected

cis-folded binding conformation of these chelators has been confirmed in all cases. Attachment of two carboxymethyl pendant arms to CB-cyclam (**28**) to give CB-TE2A (**33**) further ensures complete envelopment of a six-coordinate Cu(II) as shown in Fig. (**6**). While the measurement of stability constants of Cu-CB complexes have been limited by the proton-sponge nature of these chelators, available data for Cu-CB-cyclam (log K_f = 27.1) revealed very similar values to unbridged Cu-cyclam (log K_f = 27.2) and related complexes [108]. On the other hand, their kinetic inertness, especially in aqueous solution, has been shown to be truly exceptional [27]. Proton-assisted decomplexation is a convenient indicator of solution inertness. Under *pseudo*-first order conditions of high acid concentration (e.g. 5 M HCl), decomplexation half-lives can provide a comparative gauge. Remarkable resistance of Cu-CB complexes toward such processes has recently been demonstrated by Woodin *et al.* [27]. As shown in Table **2**, Cu-CB-cyclam is almost an order of magnitude more inert than Cu-cyclam in 5 M HCl at 90°C, while Cu-CB-TE2A is 4 orders of magnitude more inert. Impressively, the latter complex resists acid decomplexation even better than the fully-encapsulated sarcophagine complex Cu(II)-diamSar . It was confirmed that both the cross-bridged cyclam backbone as well as presence of two enveloping carboxymethyl arms are required for this unusual kinetic inertness.

With respect to ease of Cu(II)/Cu(I) reduction, cyclic voltammetric studies of Cu(II) complexes of a variety of tetraazamacrocyclic complexes revealed that Cu-CB-TE2A (**38**) is not reduced in 0.1 N sodium acetate until a relatively negative potential of -1.07 V (*vs.* Ag/AgCl) [27]. Further, unlike the Cu-DOTA (**41**), Cu-TETA (**40**) and Cu-diamSar cyclic voltammograms, this reduction is *quasi*-reversible, suggesting the innate ability of the cross-bridged cyclam ligand to adapt to a geometry suitable for Cu(I) coordination [27] (Gustafson L, Wong Edward H. Unpublished results. 2006). Both Cu-CB-TE2A's resistance to proton-assisted decomplexation and Cu(II)/Cu(I) reduction suggest that the ligand may be an especially promising chelator candidate for radiocopper(II).

Sun *et al.* evaluated the biological stability of a series of ligands that were derivatives of the CB-macrocycles shown in Fig. (**5**). The ^{64}Cu complexes were formed under carrier-added and no-carrier-added conditions in less than 2 h at 55°C [108]. Serum stability experiments indicated that these complexes are stable in rat serum out to 24 h. Results of biodistribution studies of these ^{64}Cu complexes in female Sprague Dawley rats were highly dependant upon the chelator. The complex ^{64}Cu-CB-TE2A was determined to be the most stable and was cleared most rapidly from the blood, liver, and kidney.

Boswell *et al.* directly compared the *in vivo* stability of CB-TE2A and CB-DO2A (**32**) which are analogues of TETA and DOTA respectively, and have developed the analytical conditions to purify and isolate the kinetic ^{64}Cu-CB-complexes and their metabolic analytes [109, 110]. Both CB-ligands were labeled in high radiochemical purity at 95°C using ethanol and cesium carbonate, followed by the addition of $^{64}CuCl_2$. These relatively harsh conditions were needed to ensure that the competing reactions between ^{64}Cu and any trace impurity ligands were suppressed. The biodistribution of the ^{64}Cu-CB-DO2A (**41**) complex was also completed and compared to that of ^{64}Cu-DOTA. At 4 h p.i., ^{64}Cu-CB-DO2A demonstrated significantly better clearance properties than the ^{64}Cu-DOTA analogue. Metabolism studies in normal rats were also conducted and demonstrated that the CB-ligands are less susceptible to ^{64}Cu transchelation than their non-cross bridged analogues. By 4 h ^{64}Cu-CB-TE2A underwent significantly less transchelation in the liver than ^{64}Cu-TETA (13% *vs.* 75%), while ^{64}Cu-CB-DO2A underwent less transchelation than ^{64}Cu-DOTA (61% *vs.* 90%).

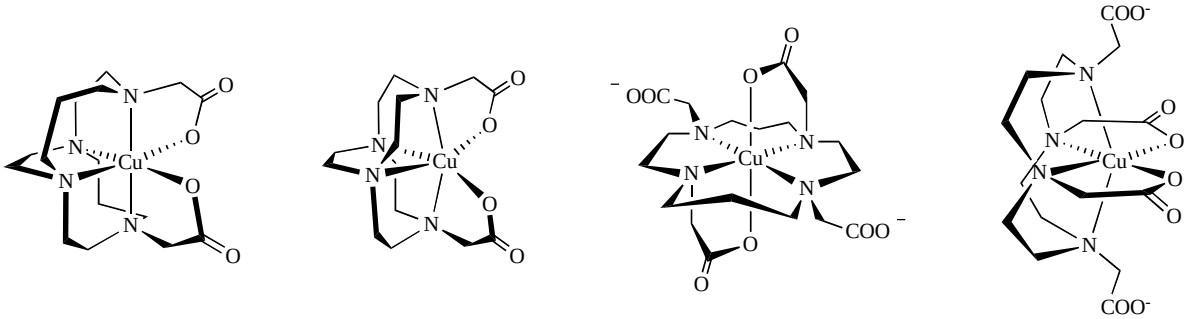


Fig. (6). A comparison of the structures of Cu(II) complexes of the CB ligands CB-TE2A and CB-DO2A with those of TETA and DOTA based on published crystallographic data from references [55-56, 107-108].

Table 2. Pseudo-First Order Half-Lives for Acid-Decomplexation of Cu(II) Complexes

Ligand	CB-TE2A	diamSar	CB-cyclam	TETA	DOTA	cyclam
5M HCl 90 °C	154(6) h	40(1) h	11.7(1) min	4.5(5) min	<3 min	<3 min
12M HCl 90 °C	1.6(2) h	<3 min	<3 min	<3 min	<3 min	<3 min

^{64}Cu-CB-TE2A was clearly the most stable of all of the ^{64}Cu complexes tested and this was most evident at 20 h, where only 24% of the injected ^{64}Cu was transchelated to proteins; in contrast, 92% of the ^{64}Cu associated with TETA was transchelated. In addition, a survey of biodistribution data of several ^{64}Cu-tetraazamacrocycles in normal rats reveals that ^{64}Cu-CB-TE2A has superior clearance properties as shown in Fig. (**7**) [77, 108, 111]. These data correspond with the *in vitro* data, and demonstrate the enhanced *in vivo* stability that the ethylene cross-bridge and carboxylate pendant arms provide to the tetraazamacrocycles.

Studies also focused upon modifying these CB chelators for conjugation to small molecules and peptides [112, 113]. Sprague *et al.* confirmed that CB-TE2A will be a valuable BFC for ^{64}Cu. In this study, CB-TE2A was conjugated to the somatostatin analogue Y3-TATE and directly compared to the ^{64}Cu-TETA-Y3-TATE conjugate [113]. ^{64}Cu-CB-TE2A-Y3-TATE was radiolabeled in high radiochemical purity with specific activities of 48.1-188.7 MBq/μg (1.3-5.1 mCi/μg) of peptide without the need for harsh labeling conditions. Biodistribution studies using AR42J tumors implanted in male Lewis rats revealed that this complex had greater affinity for somatostatin-positive tissues compared to the TETA conjugate. Accumulation of ^{64}Cu-TE2A-Y3-TATE was lower at all time points in blood and liver, and less accumulation was observed in the kidney at earlier time points when compared to ^{64}Cu-TETA-Y3-TATE. These data suggest that the ^{64}Cu-CB-TE2A-Y3-TATE is more resistant to transchelation than the TETA analogue. Further, the biodistribution results are corroborated by microPET imaging as depicted in Fig. (**8**).

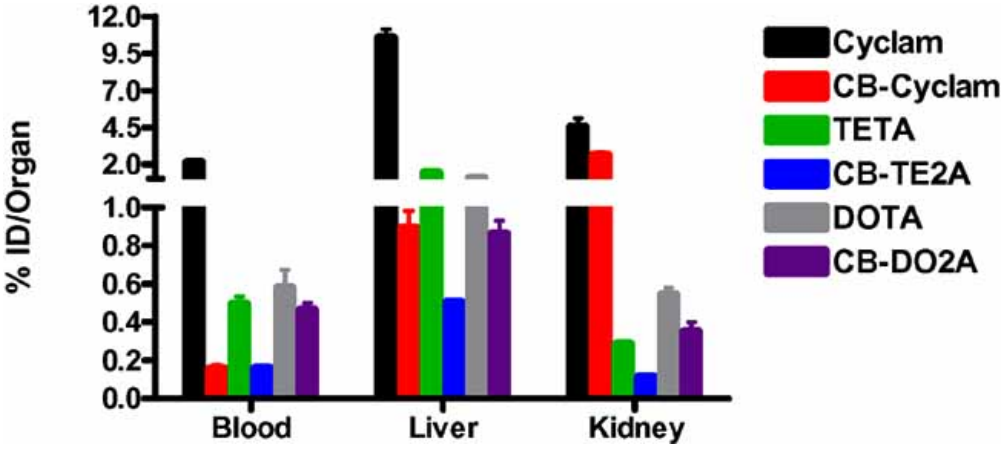


Fig. (7). Biodistribution data of selected ^{64}Cu-tetraazamacrocycles at 24 h p.i. in normal rats. Adapted from references [108, 111-112].

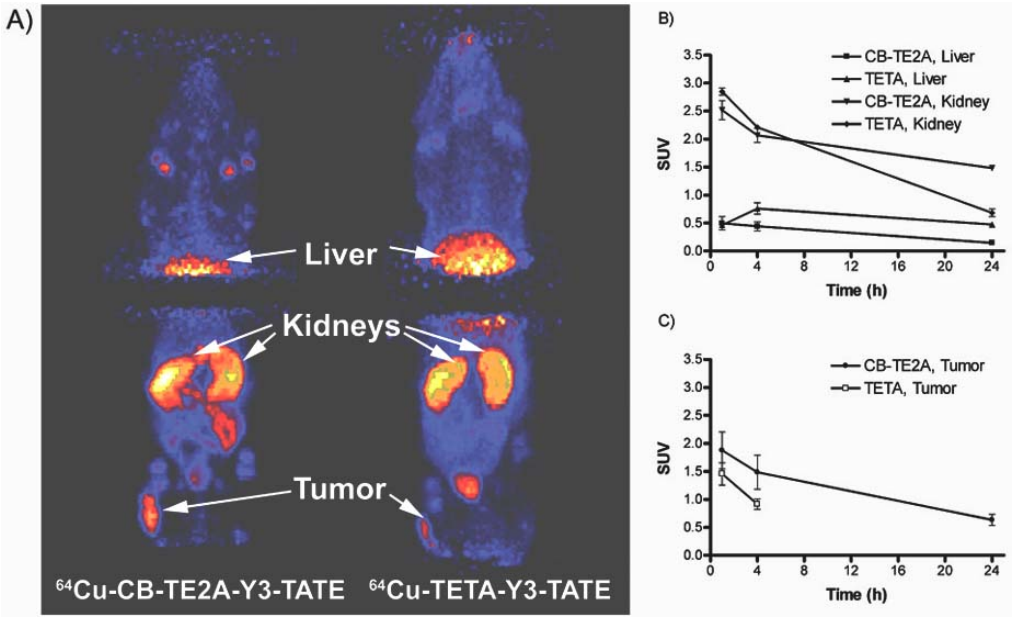


Fig. (8). (**A**) MicroPET image of male Lewis rats bearing a somatostatin positive AR42J tumor in the right hind flank. The rat on the left side of the figure was imaged with ^{64}Cu-CB-TE2A-Y3-TATE while the rat on the right was imaged with ^{64}Cu-CB-TETA-Y3-TATE. The tumor on the rat imaged with ^{64}Cu-CB-TE2A-Y3-TATE is much more visible than in the rat imaged with ^{64}Cu-TETA-Y3-TATE. (**B**) Comparison of liver and kidney uptake over time between the two radiotracers based on SUV analysis. (**C**) A comparison of the accumulation of both radiotracers in AR42J tumors over time based on SUV analysis. Reprinted from reference [113].

Additionally, Archibald and coworkers have developed a synthetic strategy to create a CB-TE2A-biotin conjugate [112]. However, the separation of diastereomers, which are a result of this strategy, may limit its potential application as a BFC for ^{64}Cu.

Since the use of CB-TE2A as a BFC necessitated the conversion of one of its carboxymethyl arms into a peptide linkage to the targeting moiety, there was initial concern about detrimental effects on the resulting ^{64}Cu-complex's resistance to radiocopper loss through solution decomplexation and/or Cu(II)/Cu(I) reduction. The biological data above allayed this concern by confirming the satisfactory behavior of its bioconjugate. Parallel acid-decomplexation and electrochemical studies of model copper complexes of CB-cyclam with only a single carboxymethyl or mixed carboxylmethyl/amide arms supported these results (Southwick E, Peng Y, Widger P, Weisman Gary R, Wong Edward H. Unpublished results. 2006). While shorter acid-decomplexation half-lives and less negative Cu(II) reductions were indeed observed, these complexes remained significantly more inert than their unbridged analogs such as Cu-TETA. This argues well for the development of a second-generation of CB-chelators with even more improved radiocopper binding properties.

CONCLUSION

The development, production, and use of ^{64}Cu as a radionuclide for diagnostic imaging and therapy have greatly increased over the last decades. Because of the choice of copper isotopes with variable emission types and energies, it has become essential to develop ligand systems that can stably complex ^{64}Cu to form kinetically inert radiometal complexes. To accomplish this goal, the importance of improved ligand design and synthesis as well as the employment of rigorous physical and biological screening processes to evaluate their *in vivo* effectiveness cannot be overstated. Indeed, these will continue to be essential for the future development of ^{64}Cu radiopharmaceuticals to become practical diagnostic imaging and radiotherapeutic agents.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge NIH grants R01 CA093375 (to EHW, CJA and GRW) and F32 CA115148 (to TJW).

ABBREVIATIONS

ATSM = Diacetyl *bis*(N^4-methylthiosemicarbazone)

β^- = Negatron (beta minus)

β^+ = Positron (beta plus)

BAT = Bromoacetamidobenzyl

BFC = Bifunctional chelator

CB = Cross-bridged

CB-DO2A = 4,10-*bis*(carboxymethyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane

CB-TE2A = 4,11-*bis*(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane

Ci = Curie

CT = Computerized tomography

diamSar = 3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine

DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

DTPA = Diethylenetriaminepentaacetic acid

EDTA = Ethylenediaminetetraacetic acid

GBq = Gigabecquerel

h = Hour(s)

IM = *cis*,*cis*-1,3,5-triamino-cyclohexane-N,N',N"-*tris*(2-methyl-N- methyl imidazole)

MTD = Maximum tolerated dose

mCi = Millicurie

mmole = Millimole

OC = Octreotide

PET = Positron emission tomography

p.i. = Post injection

PTSM = Pyruvaldehyde *bis*(N^4-methylthiosemicarbazone)

RIT = Radioimmunotherapy

Sar = 3,6,10,13,16,19-hexaazabicyclo[6.6.6] eicosane

SarAr = 1-N-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine

SOD = Superoxide dismutase

SPECT = Single photon emission computed tomography

SUV = Standard uptake value

tachpyr-(6-Me) = N, N', N"-tris-(6-methyl-2-pyridylmethyl-1,3,5-*cis*,*cis*-triamino-cyclohexane

tachpyr = N,N',N"-tris-(2-pyridylmethyl)-1,3,5-*cis*,*cis*-triamino-cyclohexane

TE2A = 1,4,8,11-tetraazacyclotetradecane-1,8-diacetic acid

TETA = 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid

μA = Microamp

V = Volts

Y3-TATE = Tyrosine-3-octreotate

REFERENCES

References 114-116 are related articles recently published in Current Pharmaceutical Design.

[1] Finklstein SN, Sinskey AJ, Cooper SM. Medical imaging in drug discovery part II. PharmaGenomics 2004; 4: 20-4.

[2] Mountz JD, Hsu H-C, Wu Q, Liu H-G, Zhang H-G, Mountz JM. Molecular imaging: new applications for biochemistry. J Cell Biochem Suppl 2002; 39: 162-71.

[3] Smith SV. Molecular imaging with copper-64. J Inorg Biochem 2004; 98: 1874-901.

[4] Rowland DJ, Lewis JS, Welch MJ. Molecular imaging: The application of small animal positron emission tomography. J Cell Biochem Suppl 2002; 39: 110-5.

[5] Chatziioannou AF. Molecular imaging of small animals with dedicated PET tomographs. Eur J Nucl Med 2002; 29: 98-114.

[6] McQuade P, Rowland DJ, Lewis JS, Welch MJ. Positron-emitting isotopes produced on biomedical cyclotrons. Curr Med Chem 2005; 12: 807-18.

[7] Welch MJ, Redvanly CS. Ed, Handbook of radiopharmaceuticals: radiochemistry and applications. Weinheim, John Wiley & Sons. 2003.

[8] Anderson CJ, Welch MJ. Radiometal-labeled agents (non-Technetium) for diagnostic imaging. Chem Rev 1999; 99: 2219-34.

[9] Linder MC. Biochemistry of copper, New York, Plenum Press. 1991.

[10] Linder MC, Hazegh-Azam M. Copper biochemistry and molecular biology. Am J Clin Nutri Suppl 1996; 63: 797s-811s.

[11] Peisach J, Aisen P, Blumberg WE. Ed. Biochemistry of copper. New York, Academic Press. 1966.

[12] Frieden E. Perspectives on copper biochemistry. Clin Physiol Biochem 1986; 4: 11-9.

[13] Kaim W, Rall, J. Copper-a "modern" bioelement. Angew Chem Int Ed Engl 1996; 35: 43-60.

[14] Barnes J, Thomas K, Bentley G, Steinkruger F, O'Brien HJ. Copper-67 production and recovery from Zno targets. J Nucl Med 1982; 23: 102.

[15] Bormans G, Janssen A, Adriaens P, Crombez D, Witsenboer A, De Goeij J, *et al.* A ^{62}Zn/^{62}Cu generator for the routine production of ^{62}Cu-PTSM. Appl Radiat Isot 1992; 43: 1437-41.

[16] Kastleiner S, Coenen HH, Qaim SM. Possibility of production of ^{67}Cu at a small-sized cyclotron *via* the (p,α)-reaction on enriched ^{70}Zn. Radiochimica Acta 1999; 84: 107-10.

[17] McCarthy DW, Bass LA, Cutler PD, Shefer RE, Klinkowstein RE, Herrero P, *et al.* High purity production and potential applications of copper-60 and copper-61. Nucl Med Biol 1999; 26: 351-8.

[18] Mirzadeh S, Mausner LF, Srivastave SC. Production of no-carrier added Cu-67. Appl Radiat Isot 1986; 37: 29-36.

[19] Zinn KR, Chaudhuri TR, Cheng TP, Morris JS, Meyer WA. Production of no-carrier-added Cu-64 from zinc metal irradiated under boron shielding. Cancer 1994; 73: 774-8.

[20] Smith SV, Waters DJ, Di Bartolo N. Separation of ^{64}Cu from ^{67}Ga waste products using anion exchange and low acid aqueous/organic mixtures. Radiochimica Acta 1996; 75: 65-8.

[21] Szelecsenyi F, Blessing G, Qaim SM. Excitation function of proton induced nuclear reactions on enriched ^{61}Ni and ^{64}Ni: possibility of production of no-carrier-added ^{61}Cu and ^{64}Cu at a small cyclotron. Appl Radiat Isot 1993; 44: 575-80.

[22] McCarthy DW, Shefer RE, Klinkowstein RE, Bass LA, Margenau WH, Cutler CS, *et al.* The efficient production of high specific activity Cu-64 using a biomedical cyclotron. Nucl Med Biol 1997; 24: 35-43.

[23] Piel H, Qaim SM, Stocklin G. Excitation functions of (p,xn)-reactions on ^{nat}Ni and highly enriched ^{62}Ni: possibility of production of medically important radioisotope ^{62}Cu on a small cyclotron. Radiochimica Acta 1992; 57: 1-5.

[24] Obata A, Kasamatsu S, McCarthy DW, Welch MJ, Saji H, Yonekura Y, *et al.* Production of therapeutic quantities of ^{64}Cu using a 12 MeV cyclotron. Nucl Med Biol 2003; 30: 535-9.

[25] Meares CF. Chelating agents for the binding of metal ions to antibodies. Nucl Med Biol 1986; 13: 311-18.

[26] Sun X, Anderson CJ. Production and applications of copper-64 radiopharmaceuticals. Methods Enzymol 2004; 386: 237-61.

[27] Woodin KS, Heroux KJ, Boswell CA, Wong EH, Weisman GR, Niu W, *et al.* Kinetic inertness and electrochemical behavior of copper(II) tetraazamacrocyclic complexes: possible implications for *in vivo* stability. Eur J Inorg Chem 2005; 4829-33.

[28] Hnatowich DJ, Layne WW, Childs RL, Lanteigne D, Davis MA. Radioactive labeling of antibody: a simple and efficient method. Science 1983; 220: 613-5.

[29] Parker D. Tumor targeting with radiolabelled macrocycle-antibody conjugates. Chem Soc Rev 1990; 19: 271-91.

[30] Kukis DL, Li M, Meares CF. Selectivity of antibody-chelate conjugates for binding copper in the presence of competing metals. Inorg Chem 1993; 32: 3981-2.

[31] Cole WC, DeNardo SJ, Meares CF, McCall MJ, DeNardo GL, Epstein AL, *et al.* Serum stability of ^{67}Cu chelates: comparison with ^{111}In and ^{57}Co. Nucl Med Biol 1986; 13: 363-8.

[32] Meares CF, Wensel TG. Metal chelates as probes of biological systems. Acc Chem Res 1984; 17: 202-9.

[33] Yeh SM, Sherman DG, Meares CF. A new route to "bifunctional" chelating agents: conversion of amino acids to analogs of ethylenedinitrilotetraacetic acid. Anal Biochem 1979; 100: 152-9.

[34] Moi MK, Meares CF, McCall MJ, Cole WC, DeNardo SJ. Copper chelates as probes of biological systems: stable copper complexes with a macrocyclic bifunctional chelating agent. Anal Biochem 1985; 148: 249-53.

[35] Meares CF, McCall MJ, Reardan DT, Goodwin DA, Diamanti CI, McTigue M. Conjugation of antibodies with bifunctional chelating agents: isothicyanate and bromoacetamide reagents, methods of analysis, and subsequent addition of metal ions. Anal Chem 1984; 142: 68-78.

[36] Cole WC, DeNardo SJ, Meares CF, McCall MJ, DeNardo GL, Epstein AL, *et al.* Comparative serum stability of radiochelates for antibody radiopharmaceuticals. J Nucl Med 1987; 28: 83-90.

[37] Blower PJ, Lewis JS, Zweit J. Copper radionuclides and radiopharmaceuticals in nuclear medicine. Nucl Med Biol 1996; 23: 957-80.

[38] Lewis JS, McCarthy DW, McCarthy TJ, Fujibayashi Y, Welch MJ. The evaluation of ^{64}Cu-diacetyl-*bis*(N^4-methylthiosemicarbazone) (^{64}Cu-ATSM) *in vivo* and *in vitro* in a hypoxic tumor model. J Nucl Med 1999; 40: 177-83.

[39] Fujibayashi Y, Cutler CS, Anderson CJ, McCarthy DW, Jones LA, Sharp T, *et al.* Comparative imaging studies of ^{64}Cu-ATSM a hypoxia imaging agent and C-11-acetate in an acute myocardial infarction model: *ex vivo* imaging in rats. Nucl Med Biol 1999; 26: 117-21.

[40] Fujibayashi Y, Taniuchi H, Yonekura Y, Ohtani H, Konishi J, Yokoyama A. Copper-62-ATSM: A new hypoxia imaging agent with high membrane permeability and low redox potential. J Nucl Med 1997; 38: 1155-60.

[41] Lewis JS, Dearling JLJ, Sosabowski JK, Zweit J, Carnochan P, Kelland LR, *et al.* Copper *bis*(diphosphine) complexes: radiopharmaceuticals for the detection of multi-drug resistance in tumors by PET. Eur J Nucl Med 2000; 27: 638-46.

[42] Dearling JLD, Lewis JS, Mullen GED, Rae MT, Zweit J, Blower PJ. Design of hypoxia-targeting radiopharmaceuticals: selective uptake of copper-64 complexes in hypoxic cells *in vitro*. Eur J Nucl Med 1998; 25: 788-92.

[43] Dearling JLJ, Lewis JS, Welch MJ, McCarthy DW, Blower PJ. Redox-active complexes for imaging hypoxic tissues: structure-activity relationships in copper(II)*bis*(thiosemicarbazone) complexes. Chem Commun 1998; 22: 2531-3.

[44] Dearling JLJ, Lewis JS, Mullen GED, Welch MJ, Blower PJ. Copper *bis*(thiosemicarbazone) complexes as hypoxia imaging agents: structure-activity relationships. J Biol Inorg Chem 2002; 7: 249-59.

[45] Lewis JS, Connett JM, Garbow JR, Buettner TL, Fujibayashi Y, Fleshman JW, *et al.* Copper-64-pyruvaldehyde-*bis*(N4-methylthiosemicarbazone) for the prevention of tumor growth at wound sites following laparoscopic surgery: monitoring therapy response with microPET and magnetic resonance imaging. Cancer Res 2002; 62: 445-9.

[46] Lewis JS, Laforest R, Buettner TL, Song S-K, Fujibayashi Y, Connett JM, *et al.* Copper-64-diacetyl-*bis*(N^4-methylthiosemicarbazone): an agent for radiotherapy. Proc Natl Acad Sci USA 2001; 98: 1206-11.

[47] Laforest R, Dehdashti F, Lewis JS, Schwarz SW. Dosimetry of $^{60/61/62/64}$Cu-ATSM: a hypoxia imaging agent for PET. Eur J Nucl Med Molec Imag 2005; 32: 764-70.

[48] McQuade P, Martin KE, Castle TC, Went MJ, Blower PJ, Welch MJ, *et al.* Investigation into ^{64}Cu-labeled *bis*(selenosemicarbazone) and *bis*(thiosemicarbazone) complexes as hypoxia imaging agents. Nucl Med Biol 2005; 32: 147-56.

[49] Obata A, Kasamatsu S, Lewis JS, Furukawa T, Takamatsu S, Toyohara J, *et al.* Basic characterization of ^{64}Cu-ATSM as a radiotherapy agent. Nucl Med Biol 2005; 32: 21-8.

[50] Obata A, Yoshimoto M, Kasamatsu S, Naiki H, Takamatsu S, Kashikura K, *et al.* Intra-tumoral distribution of ^{64}Cu-ATSM: a comparison study with FDG. Nucl Med Biol 2003; 30: 529-34.

[51] Aft RL, Lewis JS, Zhang F, Kim J, Welch MJ. Enhancing targeted radiotherapy by Copper(II)diacetyl- *bis*(N4-methylthiosemicarbazone) using 2-deoxy-d-glucose. Cancer Res 2003; 63: 5496-504.

[52] Lewis JS, Sharp TL, Laforest R, Fujibayashi Y, Welch MJ. Tumor uptake of copper-diacetyl-*bis*(n4-methylthiosemicarbazone): effect of changes in tissue oxygenation. J Nucl Med 2001;42: 655-61.

[53] Lewis JS, McCarthy DW, McCarthy TJ, Fujibayashi Y, Welch MJ. Evaluation of ^{64}Cu-ATSM *in vitro* and *in vivo* in a hypoxic tumor model. J Nucl Med 1999; 40: 177-83.

[54] Busch DH. The compleat coordination chemistry: one practitioner's perspective. Chem Rev 1993; 93: 847-60.

[55] Riesen A, Zehnder M, Kaden TA. Cu-TETA crystal structure. Acta Crystallogr 1988; C44: 1740-2.

[56] Riesen A, Zehnder M, Kaden TA. Cu-DOTA crystal structure. Helv Chim Acta 1986; 69: 2067-73.

[57] Wu Y, Zhang X, Xiong Z, Cheng Z, Fisher Darrell R, Liu S, *et al*. microPET imaging of glioma integrin $\alpha_v\beta_3$ expression using $^{(64)}$Cu-labeled tetrameric RGD peptide. J Nucl Med 2005; 46: 1707-18.

[58] McQuade P, Miao Y, Yoo J, Quinn TP, Welch MJ, Lewis JS. Imaging of melanoma using ^{64}Cu- and ^{86}Y-DOTA-ReCCMSH(Arg11), a cyclized peptide analogue of α-MSH. J Med Chem 2005; 48: 2985-92.

[59] Chen X, Hou Y, Tohme M, Park R, Khankaldyyan V, Gonzales-Gomez I, *et al.* Pegylated Arg-Gly-Asp peptide: ^{64}Cu labeling and PET imaging of brain tumor $\alpha_v\beta_3$-integrin expression. J Nucl Med 2004; 45: 1776-83.

[60] Chen X, Park R, Tohme M, Shahinian AH, Bading JR, Conti PS. MicroPET and autoradiographic imaging of breast cancer α_v-integrin expression using ^{18}F- and ^{64}Cu-labeled RGD peptide. Bioconjugate Chem 2004; 15: 41-9.

[61] Chen X, Sievers E, Hou Y, Park R, Tohme M, Bart R, *et al.* Integrin $\alpha_v\beta_3$-targeted imaging of lung cancer. Neoplasia 2005; 7: 271-9.

[62] Li WP, Lewis JS, Srinivasan A, Schmidt MA, Anderson CJ. Copper-64/61 and iodine-125-labeled DOTA-D-TYR1-octreotate: a new somatostatin analaog for labeling with metals and halogens. J Labelled Compd Radiopharm 2001; 44(suppl. 1): S948-S50.

[63] Anderson CJ, Schwarz SW, Connett JM, Cutler PD, Guo LW, Germain CJ, *et al.* Preparation, biodistribution and dosimetry of copper-64-labeled anti-colorectal carcinoma monoclonal antibody fragments 1A3-F(ab')$_2$. J Nucl Med 1995; 36: 850-8.

[64] Anderson CJ, Sherman ELC, McCarthy DW, Bass LA, Jones LW, Cristel ME, *et al.* Radiotherapy studies of Cu-64-labeled TETA-octreotide in tumor-bearing rats. J Nucl Med 1996; 37: 128P.

[65] Anderson CJ, Jones LA, Bass LA, Sherman ELC, McCarthy DW, Cutler PD, *et al.* Radiotherapy, toxicity and dosimetry of copper-64-TETA-octreotide in tumor-bearing rats. J Nucl Med 1998; 39: 1944-51.

[66] Anderson CJ, Dehdashti F, Cutler PD, Schwarz SW, Laforest R, Bass LA, *et al.* Copper-64-TETA-octreotide as a PET imaging agent for patients with neuroendocrine tumors. J Nucl Med 2001; 42: 213-21.

[67] Cutler PD, Schwarz SW, Anderson CJ, Connett JM, Welch MJ, Philpott GW, *et al.* Dosimetry of copper-64-labeled monoclonal antibody 1A3 as determined by PET imaging of the torso. J Nucl Med 1995; 36: 2363-71.

[68] Dehdashti F, Anderson CJ, Trask DD, Bass LA, Schwarz SW, Cutler PD, *et al.* Initial results with PET imaging using Cu-64-labeled TETA-octreotide in patients with carcinoid tumor. J Nucl Med 1997; 38: 103P.

[69] DeNardo S, DeNardo G, Kukis D, Mausner L, Moody D, Meares C. Pharmacology of Cu-67-TETA-Lym-1 antibody in patients with B cell lymphoma. Anti Immunocon Radiopharm 1991; 4: 36.

[70] Lewis JS, Srinivasan A, Schmidt MA, Anderson CJ. *In vitro* and *in vivo* evaluation of ^{64}Cu-TETA-Tyr^3-octreotate: a new somatostatin analog with improved target tissue uptake. Nucl Med Biol 1998; 26: 267-73.

[71] Lewis JS, Srinivasan A, Schmidt MA, Schwarz SW, Jones LA, Anderson CJ. Radiotherapy and dosimetry of Copper-64-TETA-Tyr3-octreotate in a somatostatin receptor positive tumor bearing animal model. J Nucl Med 1998; 39: 104P.

[72] Lewis JS, Lewis MR, Srinivasan A, Schmidt MA, Wang J, Anderson CJ. Comparison of four ^{64}Cu-labeled somatostatin analogs *in vitro* and in a tumor-bearing rat model: evaluation of new derivatives for positron emission tomography imaging and targeted radiotherapy. J Med Chem 1999; 42: 1341-7.

[73] Lewis MR, Boswell CA, Laforest R, Buettner TL, Ye D, Connett JM, *et al.* Conjugation of monoclonal antibodies with TETA using activated esters: biological comparison of ^{64}Cu-TETA-1A3 with ^{64}Cu-BAT-2IT-1A3. Cancer Biother Radiopharm 2001; 16: 483-94.

[74] Philpott GW, Schwarz SW, Anderson CJ, Dehdashti F, Connett JM, Zinn KR, *et al.* RadioimmunoPET: detection of colorectal carcinoma with positron-emitting copper-64-labeled monoclonal antibody. J Nucl Med 1995; 36: 1818-24.

[75] Wang M, Caruano AL, Lewis MR, Ballard L, Kelly K, Vanderwaal RP, *et al*. Subcellular fractionation of copper-64-TETA-octreotide in AR42J rat pancreatic tumor cells. Quart J Nucl Med 2001; 45: S12.

[76] Bass LA, Wang M, Welch MJ, Anderson CJ. *In vivo* transchelation of Copper-64 from TETA-octreotide to superoxide dismutase in rat liver. Bioconjugate Chem 2000; 11: 527-32.

[77] Boswell CA, Sun X, Niu W, Weisman GR, Wong EH, Rheingold AL, *et al*. Comparative *in vivo* stability of copper-64-labeled cross-bridged and conventional tetraazamacrocyclic complexes. J Med Chem 2004; 47: 1465-74.

[78] Mirick GR, O'Donnell RT, DeNardo SJ, Shen S, Meares CF, DeNardo GL. Transfer of copper from a chelated ^{67}Cu-antibody conjugate to ceruloplasmin in lymphoma patients. Nucl Med Biol 1999; 26: 841-5.

[79] Anderson CJ, Dehdashti F, Cutler PD, Schwarz SW, Laforest R, Bass LA, *et al.* ^{64}Cu-TETA-octreotide as a PET imaging agent for patients with neuroendocrine tumors. J Nucl Med 2001; 42: 213-21.

[80] Kotek J, Lubal P, Hermann P, Cisarova I, Lukes I, Godula T, *et al.* High thermodynamic stability and extraordinary kinetic inertness of copper(II) complexes with 1,4,8,11-tetraazacyclotetradecane-1,8-*bis*(methylphosphonic acid): example of a rare isomerism between kinetically inert penta- and hexacoordinated copper(II) complexes. Chem--Eur J 2003; 9: 233-48.

[81] Fuezerova S, Kotek J, Cisarova I, Hermann P, Binnemans K, Lukes I. Cyclam (1,4,8,11-tetraazacyclotetradecane) with one methylphosphonate pendant arm: a new ligand for selective copper(II) binding. Dalton Trans 2005; 2908-15.

[82] Sun X, Wuest M, Kovacs Z, Sherry AD, Motekaitis R, Wang Z, *et al. In vivo* behavior of copper-64-labeled methanephosphonate tetraazamacrocyclic ligands. J Biol Inorg Chem 2003; 8: 217-25.

[83] Bowen T, Planalp RP, Brechbiel MW. An improved synthesis of cis,cis-1,3,5-triaminocyclohexane: synthesis of novel hexadentate ligand derivatives for the preparation of gallium radiopharmaceuticals. Bioorg Med Chem Lett 1996; 6: 807-10.

[84] Ma D, Lu F, Overstreet T, Milenic DE, Brechbiel MW. Novel chelating agents for potential clinical applications of copper. Nucl Med biol 2002; 29: 91-105.

[85] Turner J, Koumenis C, Kute TE, Planalp RP, Brechbiel MW, Beardsley D, *et al.* Tachpyridine, a metal chelator, induces G2 cell-cycle arrest, activates checkpoint kinases, and sensitizes cells to ionizing radiation. Blood 2005; 106: 3191-9.

[86] Torti SV, Ma R, Venditto VJ, Torti FM, Planalp RP, Brechbiel MW. Preliminary evaluation of the cytotoxicity of a series of tris-2-aminoethylamine (Tren) based hexadentate heterocyclic donor agents. Bioorg Med Chem 2005; 13: 5961-7.

[87] Chong H-S, Torti SV, Ma R, Torti FM, Brechbiel MW. Synthesis and potent antitumor activities of novel 1,3,5-cis,cis-triaminocyclohexane n-pyridyl derivatives. J Med Chem 2004; 47: 5230-4.

[88] Ye N, Park G, Przyborowska AM, Sloan PE, Clifford T, Bauer CB, *et al.* Nickel(II), copper(II) and zinc(II) binding properties and cytotoxicity of tripodal, hexadentate tris(ethylenediamine)-analogue chelators. Dalton Trans 2004; 1304-11.

[89] Zhao R, Planalp RP, Ma R, Greene BT, Jones BT, Brechbiel MW, *et al.* Role of zinc and iron chelation in apoptosis mediated by tachpyridine, an anti-cancer iron chelator. Biochem Pharmacol 2004; 67: 1677-88.

[90] Chong H-S, Torti FM, Torti SV, Brechbiel MW. Synthesis of 1,3,5-cis,cis-triaminocyclohexane n-pyridyl derivatives as potential antitumor agents. J Org Chem 2002; 67: 8072-8.

[91] Park G, Dadachova E, Przyborowska A, Lai S-j, Ma D, Broker G, *et al.* Synthesis of novel 1,3,5-cis,cis-triaminocyclohexane ligand based Cu(II) complexes as potential radiopharmaceuticals and correlation of structure and serum stability. Polyhedron 2001; 20: 3155-63.

[92] Brady ED, Chong H-S, Milenic DE, Brechbiel MW. Development of a spectroscopic assay for bifunctional ligand-protein conjugates based on copper. Nucl Med Biol 2004; 31: 795-802.

[93] Geue RJ, Hambley TW, Harrowfield JM, Sargeson AM, Snow MR. Metal ion encapsulation: cobalt cages derived from polyamines, formaldehyde, and nitromethane. J Am Chem Soc 1984; 106: 5478-88.

[94] Sargeson AM. The potential for the cage complexes in biology. Coord Chem Rev 1996; 151: 89-114.

[95] Grondahl L, Hammershoi A, Sargeson AM, Thoem VJ. Stability and kinetics of acid- and anion-assisted dissociation reactions of hexaamine macrobicyclic mercury(II) complexes. Inorg Chem 1997; 36: 5396-403.

[96] Di Bartolo NM, Sargeson AM, Donlevy TM, Smith SV. Synthesis of a new cage ligand, SarAr, and its complexation with selected transition metal ions for potential use in radioimaging. J Chem Soc Dalton Trans 2001; 2303-9.

[97] Weisman GR, Rogers ME, Wong EH, Jasinski JP, Paight ES. Cross-bridged cyclam. protonation and Li+ complexation in a diamond-lattice cleft. J Am Chem Soc 1990; 112: 8604-5.

[98] Weisman GR, Wong EH, Hill DC, Rogers ME, Reed DP, Calabrese JC. Synthesis and transition-metal complexes of new cross-bridged tetraamine ligands. J Chem Soc Chem Commun 1996; 947-8.

[99] Hubin TJ, McCormick JM, Collinson SR, Busch DH, Alcock NW. Ultra rigid cross-bridged tetraazamacrocycles as ligands-the challenge and the solution. Chem Commun 1998; 1675-6.

[100] Springborg J. Adamanzanes-bi-and tricyclic tetraamines and their coordination compounds. Dalton Trans 2003; 1653-65.

[101] Hubin TJ, Tyryshkin N, Alcock NW, Busch DH. An o-xylyl cross-bridged 5,12-dioxocyclam. Acta Crystallogr Sect C 1999; C55: 1888-9.

[102] Hubin TJ, Alcock NW, Busch DH. A cyclam-like macrocycle side-bridged by a propyl chain. Acta Crystallogr Sect C 1999; C55: 2188-90.

[103] Hubin TJ, Alcock NW, Morton MD, Busch DH. Synthesis, structure, and stability in acid of copper(II) and zinc(II) complexes of cross-bridged tetraazamacrocycles. Inorg Chim Acta 2003; 348: 33-40.

[104] Hubin TJ. Synthesis and coordination chemistry of topologically constrained azamacrocycles. Coord Chem Rev 2003; 241: 27-46.

[105] Niu W, Wong EH, Weisman GR, Zakharov LN, Incarvito CD, Rheingold AL. Copper(II) and zinc(II) complexes of amide pendant-armed cross-bridged tetraamine ligands. Polyhedron 2004; 23: 1019-25.

[106] Hubin TJ, Alcock NW, Busch DH. Copper(I) and copper(II) complexes of an ethylene cross-bridged cyclam. Acta Crystallogr 2000; C56: 37-9.

[107] Wong EH, Weisman GR, Hill DC, Reed DP, Rogers ME, Condon JS, *et al.* Synthesis and characterization of cross-bridged cyclams and pendant-armed derivatives and structural studies of their copper(II) complexes. J Am Chem Soc 2000; 122: 10561-72.

[108] Sun X, Wuest M, Weisman GR, Wong EH, Reed DP, Boswell CA, *et al.* Radiolabeling and *in vivo* behavior of copper-64-labeled cross-bridged cyclam ligands. J Med Chem 2002; 45: 469-77.

[109] Boswell CA, McQuade P, Weisman GR, Wong EH, Anderson CJ. Optimization of labeling and metabolite analysis of copper-64-labeled azamacrocyclic chelators by radio-LC-MS. Nucl Med Biol 2005; 32: 29-38.

[110] Boswell CA, Sun X, Niu W, Wong EH, Weisman GR, Rheingold AL, *et al*. Comparative *in vivo* stability of copper-64-labeled cross-bridged and conventional tetraazamacrocyclic complexes. J Med Chem 2004; 47: 1465-74.

[111] Jones-Wilson TM, Deal KA, Anderson CJ, McCarthy DW, Kovacs Z, Motekaitis RJ, *et al.* The *in vivo* behavior of copper-64-labeled azamacrocyclic compounds. Nucl Med Biol 1998; 25: 523-30.

[112] Lewis EA, Boyle RW, Archibald SJ. Ultrastable complexes for *in vivo* use: a bifunctional chelator incorporating a cross-bridged macrocycle. Chemical Commun 2004; 2212-3.

[113] Sprague JE, Peng Y, Sun X, Weisman GR, Wong EH, Achilefu S, *et al.* Preparation and biological evaluation of copper-64-labeled Tyr3-octreotate using a cross-bridged macrocyclic chelator. Clin Cancer Res 2004;10: 8674-82.

[114] Dolle F. Fluorine-18-labelled fluoropyridines: advances in radiopharmaceutical design. Curr Pharm Des 2005; 11(25): 3221-35.

[115] Landini L, Santarelli MF, Positano V, Leone A. Molecular imaging: its application in cardiovascular diagnosis. Curr Pharm Des 2005; 11(17): 2225-34.

[116] Yoshinaga K, Chow BJ, dekemp RA, Thorn S, Ruddy TD, Davies RA, *et al.* Application of cardiac molecular imaging using positron emission tomography in evaluation of drug and therapeutics for cardiovascular disorders. Curr Pharm Des 2005; 11(7): 903-32.