Published in final edited form as:
Q J Nucl Med Mol Imaging. 2008 June ; 52(2): 185–192.

Cross-bridged Macrocyclic Chelators for Stable Complexation of Copper Radionuclides for PET Imaging

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Abstract

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 non-standard positron-emitting radionuclide for PET imaging of diseased tissues. A significant challenge of working with copper radionuclides is that they must be delivered to the living system as a stable complex that is attached to a biological targeting molecule for effective imaging and therapy. Significant research has been devoted to the development of ligands that can stably chelate ^{64}Cu, in particular, the cross-bridged macrocyclic chelators. This review describes the coordination chemistry and biological behavior of ^{64}Cu-labeled cross-bridged complexes.

Introduction

Non-traditional positron-emitting radionuclides, particularly those of the transition metals, have gained considerable interest for imaging with positron emission tomography (PET) because of increased production and availability. 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**[1]. In addition, 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 copper is limited to its three accessible oxidation states (I-III) [2-4]. 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 are readily prepared using relatively soft polarizable ligands like thioethers, phosphines, nitriles, isonitriles, iodide, cyanide and thiolates; however, Cu(I) complexes are typically not used for *in vivo* imaging due to their lability. Copper (II) is a d^9 metal of borderline softness which favors amines, imines, and bidentate ligands like bipyridine to form square planar, distorted square planar, trigonal pyramidal, square

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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.

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 [5]. 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 250 mCi [5].

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 [6]. Subsequent studies by McCarthy *et al*. were performed, and this method is now used to provide ^{64}Cu to researchers throughout the United States [7]. 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 [8]. They utilized very similar methods to those previously published [7]. 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 a chelator that forms a stable complex *in vivo* 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. Ligands that can form radio-copper complexes with high 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 radio-copper complex is injected into a living organism [9, 10]. Reduction of Cu(II) to Cu(I) and subsequent Cu(I) loss may also be a pathway for loss of radio-copper, and resistance of the radio-copper complex to Cu(II)/Cu(I) reduction as well as reversibility can also be important [11]. 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.

Chelators Based on Cyclam and Cyclen Backbones

The most widely used chelators for attaching ^{64}Cu to biological molecules are 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. (**1**). 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 [12]. 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 [13-15]. Two of the most important chelators studied were DOTA (**1**) and TETA (**2**). While DOTA has been used as a BFC for ^{64}Cu, its ability to bind many different metal ions, and its decreased stability compared to TETA make it less than ideal [16-21]. The tetraazamacrocyclic ligand TETA therefore, has been extensively used as a chelator for ^{64}Cu, and successful derivatization of this ligand has allowed researchers to conjugate it to antibodies, proteins, and peptides [22-32].

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. Bass *et al*. demonstrated that when ^{64}Cu-TETA-octreotide was injected into normal Sprague-Dawley rats, nearly 70% of the ^{64}Cu from ^{64}Cu-TETA-octreotide was transchelated to a 35 kDa species believed to be superoxide dismutase (SOD) in the liver 20 h post-injection [33]. These results are supported by the observations of Boswell *et al*. [34].

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 inert ^{64}Cu chelators. With this in mind, new ligand systems, including those based upon the cross-bridged (CB) tetraazamacrocycles have been developed to complex ^{64}Cu more stably.

The Cross-bridged Tetraamine Ligands (Figure 2)

This class of chelators was first conceived of and synthesized by Weisman and Wong and coworkers in the 1990's [35, 36], and they were originally designed to complex 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 [37-42]. With available structural data, the expected *cis*-folded coordination geometry of these chelators has been confirmed in all cases. Attachment of two carboxymethyl pendant arms to CB-cyclam (**8**) to give CB-TE2A (**10**) further ensures complete envelopment of a six-coordinate Cu(II) as shown in Fig. (**3**).

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 non-bridged Cu-cyclam (log K_f = 27.2) and related complexes [43]. On the other hand, their kinetic inertness, especially in aqueous solution, has been shown to be truly exceptional [11, 44]. 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 [11, 44]. 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.

The effect of pendant arm length on acid stability was also investigated. Heroux et al. compared the properties of Cu(II) complexes of cross-bridged cyclam and cyclen having *N*-carboxyethyl pendant arms (CB-DO2LA (**14**) and CB-TE2LA (**15**)) to the corresponding CBTE2A and CB-DO2A complexes [44]. The inertness of Cu-CB-TE2LA in 5 N HCl at 90°C of 100 h was very high compared to other chelators, though not quite as good as 154 h for Cu-CB-TE2A (Table 2).

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 (**16**) is not reduced in 0.1 N sodium acetate until a relatively negative potential of −1.07 V (vs. Ag/AgCl) [11]. Further, unlike the Cu-DOTA (**19**), Cu-TETA (**18**) 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 [11] (Gustafson L, Wong Edward H. Unpublished results. 2006). The reduction of the Cu-CB-TE2LA was also quasi-reversable, but was 400 mV more easily reduced than Cu-CB-TE2A [44]. Both resistance to proton-assisted decomplexation and the Cu(II)/Cu(I) reduction of Cu-CB-TE2A suggest that the ligand may be an especially promising chelator candidate for $^{64/67}$Cu(II).

Biological stability of several of the ^{64}Cu complexes of the CB-macrocycle ligands shown in Fig. (**2**) has been investigated [34, 43, 48]. The ^{64}Cu complex of CB-TE2A was formed under carrier-added and no-carrier-added conditions in less than 2 h at 55°C [43]. 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 dependent 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 the ^{64}Cu complexes of CB-TE2A and CB-DO2A (**9**), which are analogues of TETA and DOTA respectively, and developed the analytical conditions to purify and isolate the kinetic ^{64}Cu-CB-complexes and their metabolic analytes [34, 45]. 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 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%). ^{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. (**4**) [34, 43, 46]. 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 [47]. 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 [47]. ^{64}Cu-CB-TE2A-Y3-TATE was radiolabeled in high radiochemical purity with specific activities of 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.

To further examine the stability of cross-bridged macrocycles with one carboxylate and one amide group, a series of Cu(II) complexes of cross-bridged macrocyclic chelators (CBTEAMA (**11**), CB-MeTEAMA (**12**), and PhTEAMA (**13**)) were synthesized and evaluated as model compounds for ^{64}Cu-CB-TE2A-peptide conjugates. The biological behavior of the ^{64}Cu complexes was evaluated in normal rats [48]. These agents showed rapid blood clearance and relatively low liver uptake at 24 h post-injection, demonstrating that one carboxylate is likely to be sufficient for *in vivo* stability. In addition, Cu-TEAMA showed a highly negative, quasi-reversible reduction potential (−0.96 V) (Wong et al., unpublished results), which is consistent with the *in vivo* results.

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 is highly essential for the future development of ^{64}Cu radiopharmaceuticals as practical diagnostic imaging and/or radiotherapeutic agents.

Acknowledgements

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

Abbreviations

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
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
h	hour(s)
mCi	millicurie
mmole	millimole
OC	octreotide
PET	positron emission tomography
p.i.	post injection
SOD	superoxide di smutase
SUV	Standard uptake value
TETA	1,4,8,11-tetraazacyclotetradecane-1,4,8, 11-tetraacetic acid
V	volts
Y3-TATE	tyrosine-3-octreotate

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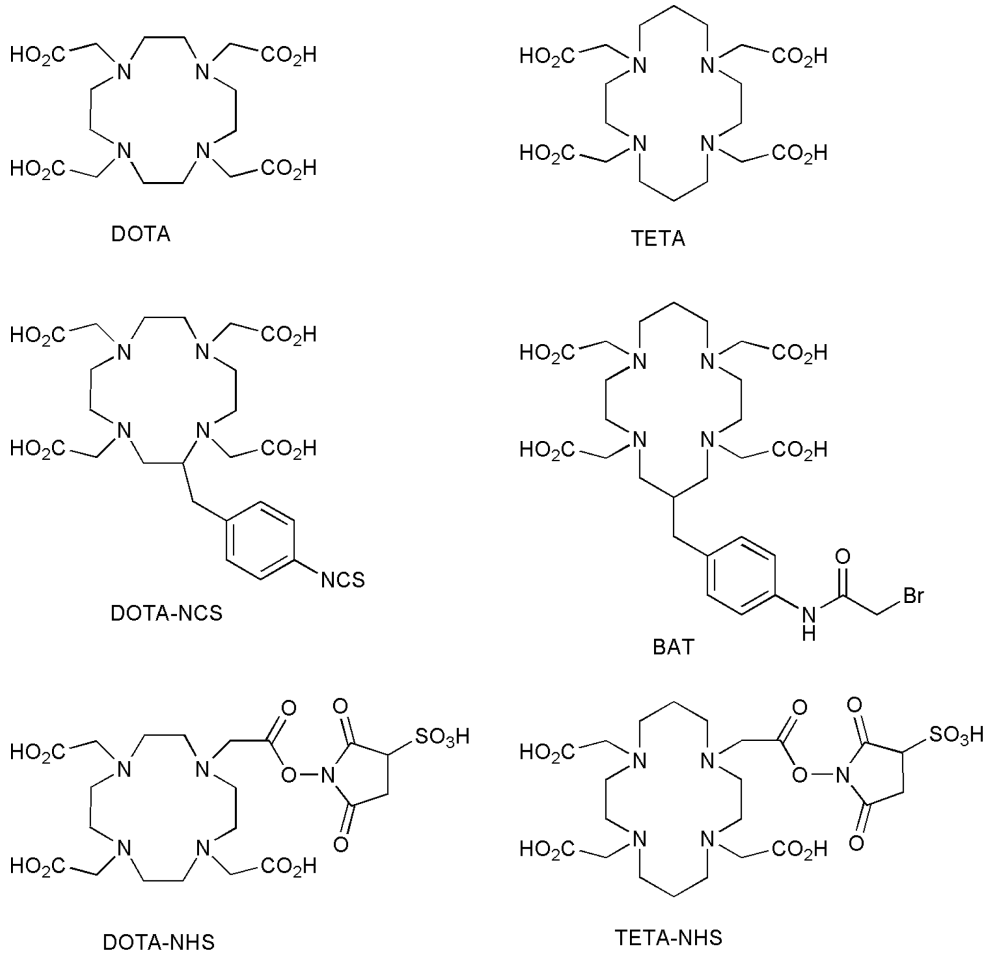

Figure 1.
Cyclic polyaminocarboxylates and their derivatives.

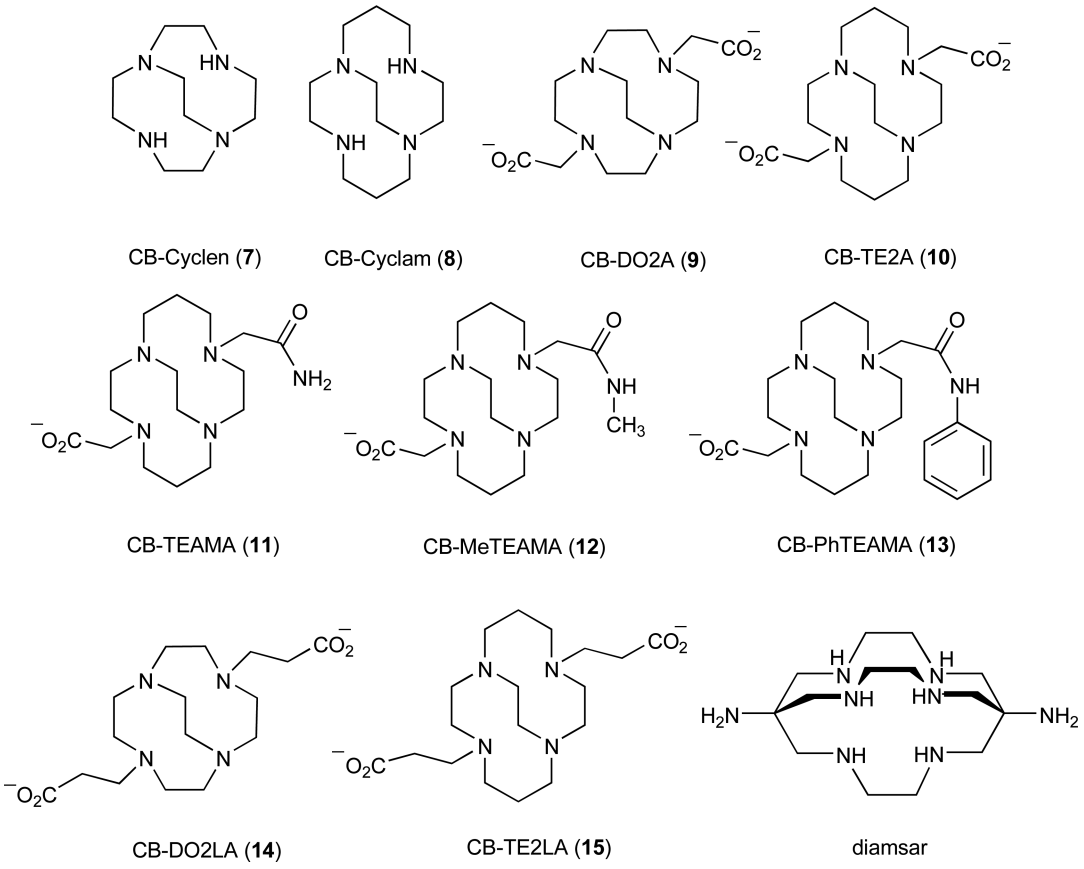

Figure 2.

Cross-bridged macrocyclic complexes and analogs compared to the sarcophogene chelator, diamsar.

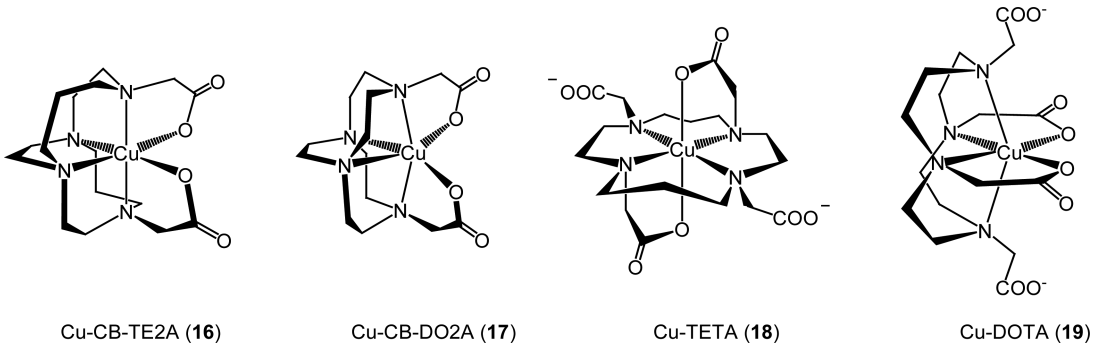

Figure 3.

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 [14, 15, 34, 42].

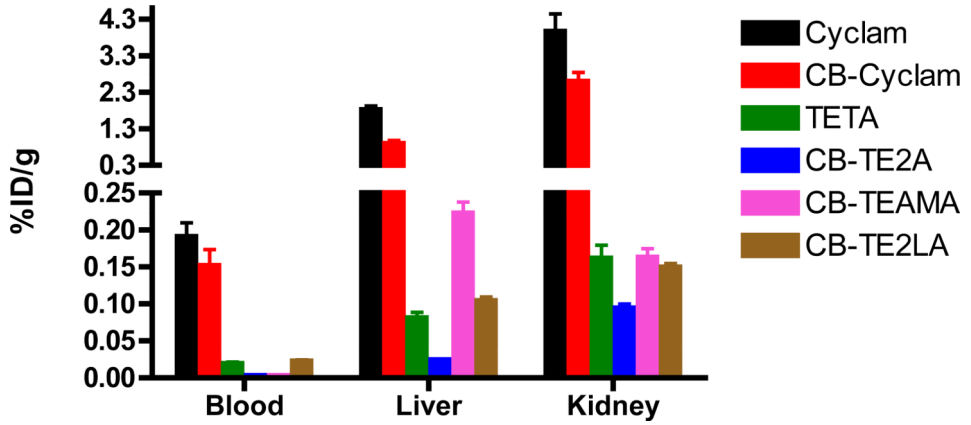

Figure 4.

Biodistribution data of selected ^{64}Cu-labeled cyclam and bridged cyclam analogs at 24 h p.i. in normal rats. Adapted from references [43, 44, 46, 48].

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%)	7.0%	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 (38.4%)	0.655 (17.8%)	43.8%	0.511 (35.6%) 1.35 (0.6%)
^{67}Cu	62.0 h	0.395 (45%) 0.484 (35%) 0.577 (20%)	---	---	0.184 (40%)

Table 2

Pseudo-first order half-lives for acid-decomplexation and reduction potentials of Cu(II) complexes (all values are from Heroux et al. [44] unless otherwise noted).

Chelator	5 M HCl 90°C	12 M HCl 90°C	E_{red} (V) vs Ag/AgCl
DOTA	<3 min	<3 min	−0.94 (irrev.)[a]
cyclam	<3 min	<3 min	−0.68 (quasi-rev)
TETA	4.5(5) min	<3 min	−1.18 (irrev.)
CB-cyclam	11.7(1) min	<3 min	−0.52 (quasi-rev)
CB-TE2A	154(6) h	1.6(2) h	−1.08 (quasi-rev.)
CB-DO2A	< 3 min	n.d.	−0.92 (irrev.)
CB-TE2LA	100 h	39(5) min	−0.68 (quasi-rev.)
CB-DO2LA	< 3 min	n.d.	−0.78 (irrev.)
CB-TEAMA	n.d.	n.d.	−0.96 (quasi-rev.)
diamsar	40(1) h	< 3 min	−1.1 (irrev)

[a] [34]