Synthesis, Characterization and In Vivo Studies of Cu(II)-64-Labeled Cross-Bridged Tetraazamacrocycle-amide Complexes as Models of Peptide Conjugate Imaging Agents

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Copper-64, a positron emitter suitable for positron emission tomography (PET), demonstrates improved in vivo clearance when chelated by the cross-bridged tetraazamacrocycle CB-TE2A compared to TETA. Good in vivo clearance was also observed for ^{64}Cu-CB-TE2A conjugated to a peptide, which converts one coordinating carboxylate pendant arm to an amide. To better understand the in vivo stability of peptide-conjugated CB-TE2A, cross-bridged monoamides were synthesized. Crystal structures of ^{nat}Cu(II)-CB-TEAMA and ^{nat}Cu(II)-CB-PhTEAMA revealed hexadentate, distorted octahedral coordination geometry. In vivo biodistribution showed clearance of all ^{64}Cu-radiolabeled cross-bridged monoamides from liver and bone marrow such that uptake at 24 h was <10% of uptake at 30 min. In contrast, >60% of 30 min uptake from ^{64}Cu-TETA was retained in these tissues at 24 h. Clearance of ^{64}Cu-cross-bridged monoamides from nontarget organs suggests good in vivo stability, thus supporting the use of CB-TE2A as a bifunctional chelator without modifications to the macrocycle backbone.

Introduction

Copper-64 is a promising radionuclide for use in positron emission tomography (PET[a]) imaging as well as radiotherapy due to its half-life ($t_{1/2}$ = 12.7 h) and decay characteristics (β^+ 17.4%, $E_{\beta+\max}$ = 656 keV; β^- 39%, $E_{\beta-\max}$ = 573 keV); it can be produced in high yield and high specific activity on a biomedical cyclotron.[1−9] Increasing use of ^{64}Cu and other copper radioisotopes in nuclear medicine has generated a need for strongly complexing chelators. Kinetic stability of Cu(II) complexes has been shown to be more predictive of in vivo stability than thermodynamic stability.[10,11] Macrocyclic chelators of Cu(II) such as TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid; Figure 1) demonstrate higher kinetic and thus in vivo stability relative to acyclic chelators such as EDTA (ethylene diamine tetraacetic acid).[10−13] However, biodistribution and metabolism studies in rats using TETA have demonstrated significant transchelation of ^{64}Cu to superoxide dismutase in liver and albumin in blood resulting in high background radioactivity.[14,15]

Several "cross-bridged" tetraamine ligands, where an ethylene ($-CH_2CH_2-$) bridge connects two nonadjacent nitrogens, have been reported by Weisman and Wong.[16−18] A series of cross-bridged Cu(II) complexes have been prepared and characterized. Cross-bridged chelates were shown to have all four nitrogen lone pairs convergent on a cleft to coordinate Cu(II). Octahedral coordination geometry with the Cu(II) fully enveloped by the chelator was achieved by the addition of two carboxylate pendant arms to give Cu(II)-CB-TE2A (4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane; Figure 1).[18] Recently, we have established the exceptional kinetic inertness of this complex to aqueous acid decomplexation.[19] Busch and colleagues have reported additional cross-bridged Cu(II) complexes that also demonstrate high kinetic stability.[20−22] Studies in our laboratory have confirmed improved in vivo stability of ^{64}Cu-CB-TE2A compared to that of ^{64}Cu-TETA.[14,18,23]

Targeting radioactive metals to tumors or other tissues of interest can be accomplished by conjugation of a bifunctional chelator to a peptide or monoclonal antibody.[24−26] Conjugation of a macrocyclic chelator such as TETA converts one carboxylate arm of the chelator to an amide by reaction with either the N-terminus of a peptide or a lysine side chain. However, conjugation of CB-TE2A to a peptide results in a single free carboxylate to coordinate Cu(II); it was initially hypothesized that this would decrease its in vivo stability. To overcome this potential problem, Lewis et al. synthesized a CB-TE2A derivative in which a biotin molecule is covalently attached to the macrocycle backbone, leaving both carboxylate arms intact.[27] However, no kinetic or in vivo stability studies of Cu(II)-CB-TE2A-Bz-biotin were reported. We reported the in vivo biodistribution of ^{64}Cu-CB-TE2A conjugated to the cyclic somatostatin analogue Y3-TATE (Tyr3-octreotate, fCYwKTCT-OH).[28] Favorable clearance properties of ^{64}Cu-CB-TE2A-Y3-TATE in liver, blood, and bone marrow were all suggestive of improved in vivo stability of the ^{64}Cu-CB-TE2A-Y3-TATE relative to ^{64}Cu-TETA-Y3-TATE.[28]

Small molecule metabolites of ^{64}Cu- and ^{67}Cu-radiolabeled peptides or antibodies have not been well characterized due to the poor in vivo stability of Cu(II) chelates. As stated above,

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[a] Abbreviations: CB-TE2A, 4,11-bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane; CB-TEAMA, 4-acetamido-11-carboxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane; CB-MeTEAMA, 11-methylcarbamoylmethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadec-4-yl)-acetic acid; CB-PhTEAMA, 11-phenylcarbamoylmethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadec-4-yl)-acetic acid; CH_3CN, acetonitrile; D-Phe[1]-octreotide, fCFwK-TCT-OH; EDTA, ethylene diamine tetraacetic acid; ID, injected dose; logP, partition coefficients; PBS, phosphate buffered saline; PET, positron emission tomography; PI, post-injection; RT, retention time; TFA, trifluoroacetic acid; TETA, 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid; Y3-TATE, Tyr3-octreotate, fCYwKTCT-OH.

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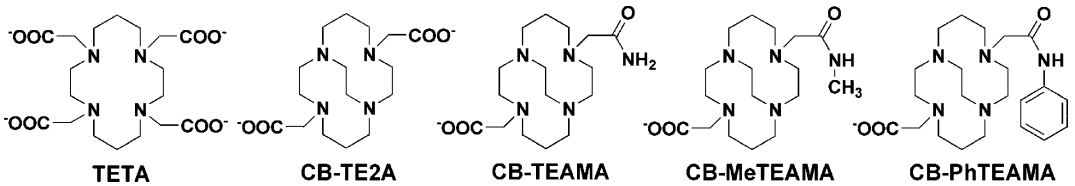
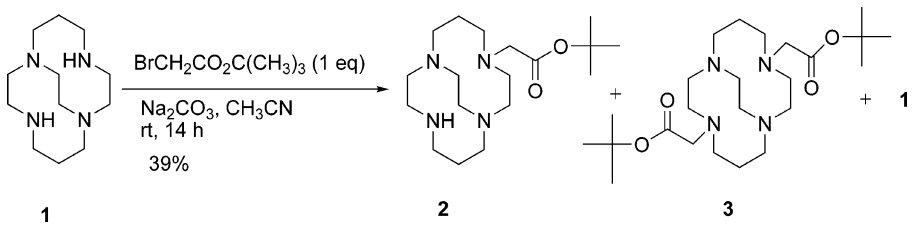


Figure 1. Structures of TETA, CB-TE2A, CB-TEAMA, CB-MeTEAMA, and CB-PhTEAMA.

Scheme 1. Synthesis of **2**



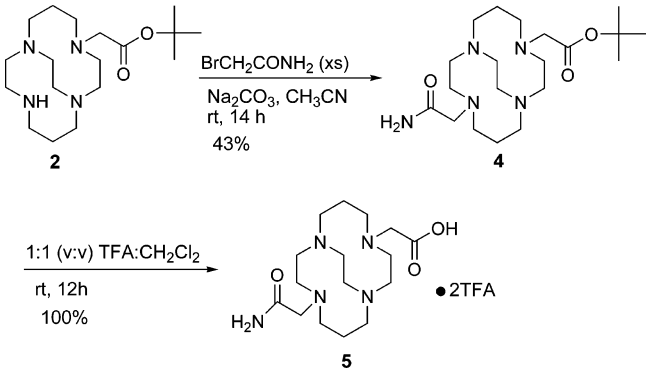
the primary radiometabolites of ^{64}Cu-TETA and ^{67}Cu-TETA-conjugated peptides and antibodies in liver and blood result from the dissociation of $^{64/67}$Cu from a peptide/antibody conjugate and subsequent binding to proteins.[15,29] This results in retention of the transchelated ^{64}Cu in nontarget tissues such as liver.[15] However, the final metabolite of ^{111}In-DTPA-D-Phe^1-octreotide (fCFwKTCT-OH), where ^{111}In-DTPA forms a relatively stable chelate, has been identified as ^{111}In-DTPA-D-Phe.[30] Similarly, the kidney metabolite of ^{64}Cu-chelate antibody conjugates was reported to be ^{64}Cu-chelate-lysine.[31] It is likely that the peptide portion of ^{64}Cu-CB-TE2A-Y3-TATE will be degraded to yield ^{64}Cu-CB-TE2A-D-Phe. To better understand the high in vivo stability of ^{64}Cu-CB-TE2A-Y3-TATE, three simplified monoamide, monocarboxylate analogues of the CB-TE2A chelator were synthesized (Figure 1). In this study, the in vivo stabilities of ^{64}Cu-CB-TEAMA, ^{64}Cu-CB-MeTEAMA, and ^{64}Cu-CB-PhTEAMA were compared to those of ^{64}Cu-TETA and ^{64}Cu-CB-TE2A.

Results

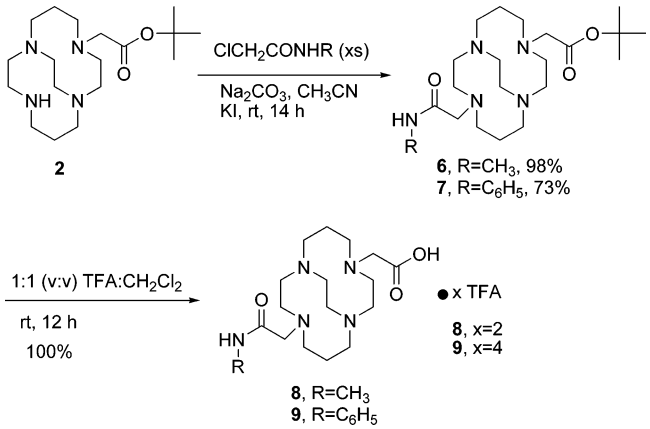
Synthesis of Ligands CB-TEAMA, CB-MeTEAMA, and CB-PhTEAMA. The title ligands were synthesized via synthetic intermediate **2**, which was prepared by statistical alkylation of cross-bridged cyclam **1**, as shown in Scheme 1. Thus, **1** was alkylated with 1 equiv of *t*-butyl bromoacetate in CH_3CN at room temperature to give a mixture consisting of **2** (45%-50% by NMR), dialkylated byproduct **3** (the precursor to **CB-TE2A**), and unreacted **1**. The desired mono-*t*-butyl ester **2** was isolated by flash chromatography in 39% yield. Treatment of **2** with an excess of 2-bromoacetamide in CH_3CN at room temperature gave cross-bridged cyclam **4** in 43% yield after workup (Scheme 2). Compound **4** was subsequently quantitatively deprotected in 1:1 (v/v) CF_3CO_2H(TFA)/CH_2Cl_2 to salt **5**·2TFA. Similarly, intermediates **6** and **7** were prepared by alkylation of **2** with 2-chloro-*N*-methylacetamide and 2-chloro-*N*-phenylacetamide in yields of 98 and 73%, respectively (Scheme 3). TFA deprotection of **6** and **7** gave **8** ([CB-MeTEAMA]H) and **9** ([CB-PhTEAMA]H) in quantitative yields as TFA salts.

Copper(II) Complexes of CB-TEAMA and CB-PhTEAMA. The copper(II) complexes of CB-TEAMA and CB-PhTEAMA were prepared and fully characterized as described in the Experimental Section. Their X-ray crystal structures are shown in Figure 2. In both complexes, Cu(II) is fully enveloped by four nitrogens from the ligand cleft and two oxygens, one from each pendant arm, resulting in a distorted octahedral geometry. Amide coordination is through the amide pendant arm's carbonyl oxygen. For Cu(II)-CB-TEAMA, the Jahn−Teller distortion is along the N(4)−Cu−O(1) axis, with elongated Cu−N and Cu−O bond lengths of 2.196(6) and 2.401(5) Å, respectively. Strong equatorial coordination is provided by N(2), N(3), O(2), and N(5). The *trans*-N(2)−Cu−N(5) and *cis*-N(3)−Cu−N(4) angles of 174.3(2) and 87.1(2)° are consistent with a reasonable ligand cleft−Cu(II) fit.

Scheme 2. Synthesis of H[CB-TEAMA]·2TFA



Scheme 3. Synthesis of H[CB-MeTEAMA]·2TFA and H[CB-PhTEAMA]·4TFA



For Cu(II)-CB-PhTEAMA, the Jahn−Teller elongation is along the N(1)−Cu−O(2) axis, with long Cu−N and Cu−O bonds of 2.205(3) and 2.367(3) Å, respectively. The equatorial coordination plane consists of N(2), O(3), N(3), and N(4). The strongly bonded carboxylate is indicated by the short Cu−O(3) distance of 1.950(3) Å. The intra-cleft *trans*-N(2)−Cu−N(3) angle of 176.5(1)° and *cis*-N(1)−Cu−N(4) angle of 88.5(1)° also confirm the good fit of Cu(II) within this cross-bridged cyclam ligand cavity.

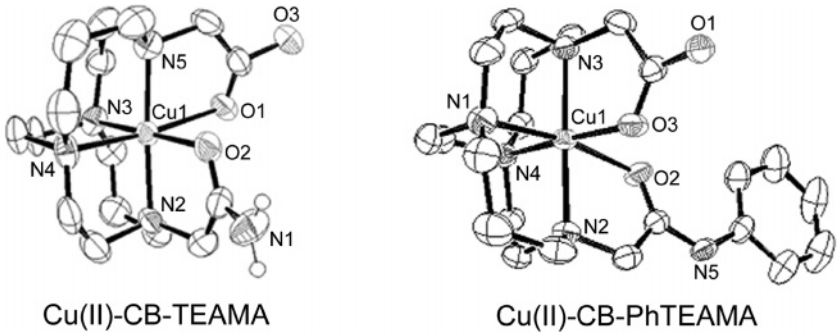


Figure 2. ORTEP drawing of the Cu-CB-TEAMA and Cu-CB-PhTEAMA complexes. Hydrogens were omitted for clarity except on the primary amide.

Table 1. Partition Coefficient for Each ^{64}Cu Complex

chelate	LogP
^{64}Cu-TETA	-3.58 ± 0.18
^{64}Cu-CB-TE2A	-2.07 ± 0.24
^{64}Cu-CB-TEAMA	-2.35 ± 0.06
^{64}Cu-CB-MeTEAMA	-2.08 ± 0.08
^{64}Cu-CB-PhTEAMA	-1.35 ± 0.05

Solid-state IR as well as solution electronic spectral data are fully consistent with these structures. The asymmetric carboxylate stretch for Cu-CB-PhTEAMA is found at 1628 cm^{-1}, while the amide carbonyl stretch appears at 1677 cm^{-1}. Corresponding bands are observed at 1627 and 1677 cm^{-1} for Cu−CB−TEAMA. Both complexes exhibit a single broad $d-d$ band at 648 nm ($\epsilon = 55$ M^{-1}cm^{-1}) and 625 nm ($\epsilon = 35$ M^{-1}cm^{-1}), respectively, in their aqueous solution electronic spectra.

Radiochemistry. Consistent with previously reported results for radiolabeling CB-TE2A with ^{64}Cu(II),[14] radiolabeling CB-TEAMA, CB-MeTEAMA, and CB-PhTEAMA in aqueous solution (0.1 M NH_4OAc, pH 8.0) resulted in the formation of several kinetically favored impurities, as evidenced by the presence of multiple peaks on radio-HPLC. However, using basic conditions in ethanol with Cs_2CO_3, as described by Boswell et al. for radiolabeling CB-TE2A,[14] single peaks were observed on radio-HPLC for ^{64}Cu-CB-TEAMA (RT = 17.1 min, 2.2−3.2 mCi/μmol), ^{64}Cu-CB-MeTEAMA (RT = 18.4 min, 53−73 mCi/μmol), and ^{64}Cu-CB-PhTEAMA (RT = 26.9 min; 51−74 mCi/μmol).

Partition Coefficients. The partition coefficients (octanol/PBS) were determined for each complex to evaluate relative lipophilicity (Table 1). The order of lipophilicity was as follows: ^{64}Cu-CB-PhTEAMA > ^{64}Cu-CB-MeTEAMA ∼ ^{64}Cu-CB-TE2A > ^{64}Cu-CB-TEAMA > ^{64}Cu-TETA.

Biodistribution Studies. To examine the in vivo properties of ^{64}Cu-CB-TEAMA, ^{64}Cu-CB-MeTEAMA, and ^{64}Cu-CB-PhTEAMA, biodistribution studies were carried out in male Lewis rats. For comparison, biodistribution was also performed for ^{64}Cu-TETA and ^{64}Cu-CB-TE2A. Figure 3 shows the radioactivity uptake to 24 h postinjection (PI) in several organs. All five complexes cleared rapidly from blood between 30 min and 2 h PI (<15% of 30 min activity remaining at 2 h). However, between 2 and 24 h, blood-associated radioactivity decreased significantly more for each monoamide cross-bridged complex than for ^{64}Cu-TETA or ^{64}Cu-CB-TE2A (% 2 h uptake remaining at 24 h = 100 × [% ID/g_{24h}/% ID/g_{2h}]; ^{64}Cu-TETA, 80.5 ± 12.5%; ^{64}Cu-CB-TE2A, 61.9 ± 7.5%; ^{64}Cu-CB-TEAMA, 23.4 ± 5.4%; ^{64}Cu-CB-MeTEAMA, 17.2 ± 6.3%; ^{64}Cu-CB-PhTEAMA, 19.3 ± 3.5%; $p < 0.01$). Kidney uptake decreased slowly between 30 min and 24 h for all five complexes such that 24 h kidney uptake was reduced to <10% of the 30 min uptake value. This resulted in an excretion of the complexes in the urine (% ID in urine, 0−24 h PI, ^{64}Cu-TETA, 71.6 ± 5.3% ID; ^{64}Cu-CB-TE2A, 74.5 ± 6.1% ID; ^{64}Cu-CB-TEAMA, 66.9 ± 8.3% ID; ^{64}Cu-CB-MeTEAMA, 63.0 ± 12.2% ID; ^{64}Cu-CB-PhTEAMA, 44.6 ± 2.1% ID).

In the liver, ^{64}Cu-CB-MeTEAMA, initially (30 min PI) had 9.3-fold higher uptake than ^{64}Cu-TETA ($p < 0.01$; Figure 3). Although not statistically significant, liver uptake was 7.4- and 5.8-fold higher for ^{64}Cu-CB-TEAMA and ^{64}Cu-CB-PhTEAMA, respectively, compared to ^{64}Cu-TETA. It was expected that liver uptake would reflect lipophilicity as determined by partition coefficients. Consistent with this hypothesis, all cross-bridged monoamides showed significantly higher 30 min liver uptake than the more hydrophilic ^{64}Cu-TETA. However, 30 min liver uptake of ^{64}Cu-CB-TE2A was even lower than for ^{64}Cu-TETA, despite a similar partition coefficient to ^{64}Cu-CB-MeTEAMA. This may be explained by the complex in vivo bioavailability, which reflects ongoing excretion as ^{64}Cu-CB-TE2A clears rapidly via the kidneys.

By 24 h, the liver uptake fell to 23.4 ± 5.0% for ^{64}Cu-CB-TE2A, 6.7 ± 0.9% for ^{64}Cu-CB-TEAMA, 7.8 ± 5.5% for ^{64}Cu-CB-MeTEAMA, and 1.3 ± 0.3% for ^{64}Cu-CB-PhTEAMA compared to 30 min uptake (Figure 3). In contrast, ^{64}Cu-TETA was retained in the liver to a much greater extent than cross-bridged complexes, resulting in a decrease in liver uptake to 66.2 ± 11.6% of 30 min uptake at 24 h ($p < 0.001$). Similarly, bone marrow uptake fell significantly between 30 min and 24 h for ^{64}Cu-CB-TE2A, ^{64}Cu-CB-TEAMA, and ^{64}Cu-CB-PhTEAMA. Although marrow uptake for ^{64}Cu-CB-MeTEAMA does appear to decrease between 30 min and 24 h, this decrease is not statistically significant ($p = 0.06$) due to high variability in 30 min uptake. In contrast, no significant change in marrow uptake was observed for ^{64}Cu-TETA over this period.

To further evaluate uptake and excretion of these ^{64}Cu complexes by the liver, radioactivity uptake in the gut was examined (Figure 4). Gastrointestinal tract biodistribution was performed without the removal of fecal contents. The GI tract was divided into four sections: stomach, proximal intestine, middle intestine, and distal intestine. The distal intestine included the cecum to the rectum; the remaining intestine (stomach to cecum) was divided in half to give the proximal and middle regions. For all cross-bridged complexes, minimal stomach uptake, high 30 min proximal intestine uptake that decreased with time, and increasing uptake in more distal parts of the intestine with time were suggestive of excretion by the liver into bile, with subsequent clearance as feces. At all time-points, intestinal uptake was highest for ^{64}Cu-CB-PhTEAMA, followed by ^{64}Cu-MeTEAMA and ^{64}Cu-CB-TEAMA. This pattern of uptake was not observed for ^{64}Cu-TETA. Radioactivity in the feces was <0.1% ID at 30 min, 1 h, and 2 h PI for all complexes.

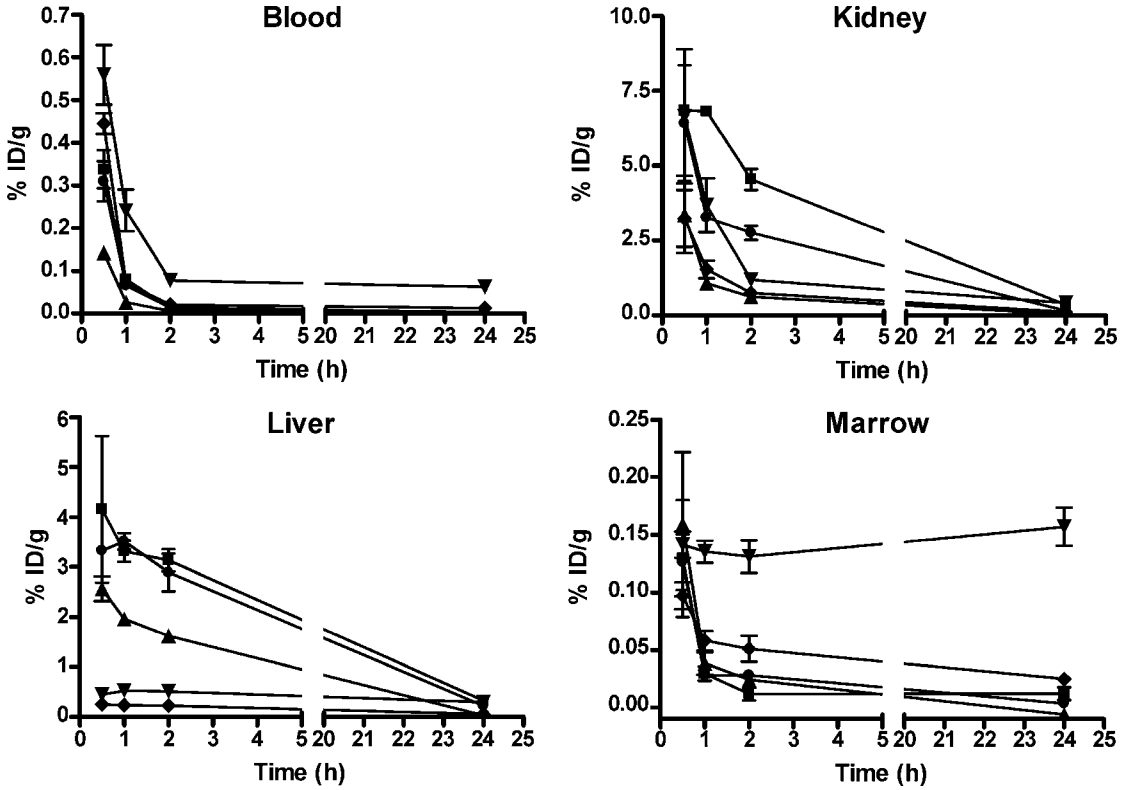


Figure 3. Selected organ biodistribution of ^{64}Cu-TETA (▼), ^{64}Cu-CB-TE2A (◆),^{64}Cu-CB-TEAMA (●), ^{64}Cu-CB-MeTEAMA (■), ^{64}Cu-CB-PhTEAMA (▲) in 33−40 day old, male Lewis rats (30 min to 24 h, $n = 4$; ^{64}Cu-TETA 1 h marrow, $n = 3$, excluded by Q-test; ^{64}Cu-CB-TE2A 2 h, $n = 3$, animal died prior to sacrifice). Note differences in scale.

By 24 h PI, amounts of ^{64}Cu excreted as feces were as follows: ^{64}Cu-TETA = 3.1 ± 0.7% ID, ^{64}Cu-CB-TE2A = 3.5 ± 0.9% ID, ^{64}Cu-CB-TEAMA = 6.8 ± 1.8% ID, ^{64}Cu-CB-MeTEAMA = 11.2 ± 1.2% ID, and ^{64}Cu-CB-PhTEAMA = 39.3 ± 3.7% ID.

Discussion

The group at Washington University has previously shown that the ^{64}Cu-labeled cross-bridged chelator CB-TE2A has favorable biodistribution properties and good in vivo stability relative to ^{64}Cu-TETA.[14,23] Subsequently, CB-TE2A was conjugated to the peptide Y3-TATE to evaluate its use as a bifunctional chelator.[28] Despite conversion of one carboxylate arm of CB-TE2A to a secondary amide upon peptide conjugation, it was found that ^{64}Cu-CB-TE2A-Y3-TATE had superior in vivo clearance properties, suggestive of improved in vivo stability relative to ^{64}Cu-TETA-Y3-TATE.[28] To investigate the in vivo biodistribution characteristics of the conjugated cross-bridged chelate independent of a peptide, three monoamide derivatives of CB-TE2A have been synthesized as models of a CB-TE2A-peptide conjugate.

High kinetic stability of a Cu(II) chelate has been shown to be predictive of in vivo stability.[10,11] It has been shown that the half-life of Cu(II)-CB-TE2A in 5 M HCl (90 °C) is approximately 2000-fold longer than for Cu(II)-TETA, indicating much higher kinetic stability.[32] Preliminary kinetic stability studies of Cu(II)-CB-TEAMA revealed that the harsh conditions caused hydrolysis of the amide bond, resulting in conversion back to Cu(II)-CB-TE2A. It was, therefore, not possible to determine directly the kinetic stability of these Cu(II) cross-bridged monoamide complexes.

Spectral data as well as X-ray crystal structures of both Cu(II)-CB-TEAMA and Cu(II)-CB-PhTEAMA confirmed their distorted octahedral coordination geometry as previously observed for Cu(II)-CB-TE2A.[18] For both monoamides, the amide pendant arm was coordinated via the carbonyl oxygen. Although coordination geometries may change in solution, this increases the likelihood that a peptide-conjugated Cu(II)-CB-TE2A could also retain this distorted octahedral coordination geometry, thus accounting for the favorable in vivo clearance properties seen with ^{64}Cu-CB-TE2A-Y3-TATE.[28]

Somewhat surprisingly, the logP values of ^{64}Cu-CB-MeTEAMA (−2.08 ± 0.08) and ^{64}Cu-CB-TE2A (−2.07 ± 0.24) were shown to be very similar. It was expected that the additional methyl group on ^{64}Cu-CB-MeTEAMA would result in a more lipophilic complex than ^{64}Cu-CB-TE2A. However, the difference in charge (^{64}Cu-CB-MeTEAMA = +1, ^{64}Cu-CB-TE2A = 0) likely balances out the additional methyl group, resulting in similar partition coefficients. This is in contrast to a previously reported logP value for ^{64}Cu-CB-TE2A (−2.42 ± 0.04).[23] However, the radiolabeling method for ^{64}Cu-CB-TE2A has since been optimized, and the ligand used in the original study may have contained trace radio-impurities such as ^{64}Cu-TE3A (predicted charge = −1),[33] which would be less lipophilic than ^{64}Cu-CB-TE2A. The order of lipophilicity for the remaining complexes was consistent with predictions.

Copper-64-CB-TEAMA, ^{64}Cu-CB-MeTEAMA, and ^{64}Cu-CB-PhTEAMA cleared over time from blood, liver, and marrow despite initial high uptake in some organs. Previous studies have associated retention of radioactivity in nontarget tissues with poor in vivo stability and transchelation of ^{64}Cu to proteins.[3,15] High liver uptake at 30 min, with subsequent excretion into feces, was not unexpected for ^{64}Cu-CB-PhTEAMA given the structure and lipophilicity. Liver uptake and fecal clearance were similar for ^{64}Cu-CB-MeTEAMA and ^{64}Cu-CB-TEAMA, with fecal clearance being significantly less for both compared to ^{64}Cu-CB-PhTEAMA ($p < 0.001$). To account for the relatively high initial liver uptake with resultant low fecal excretion, we

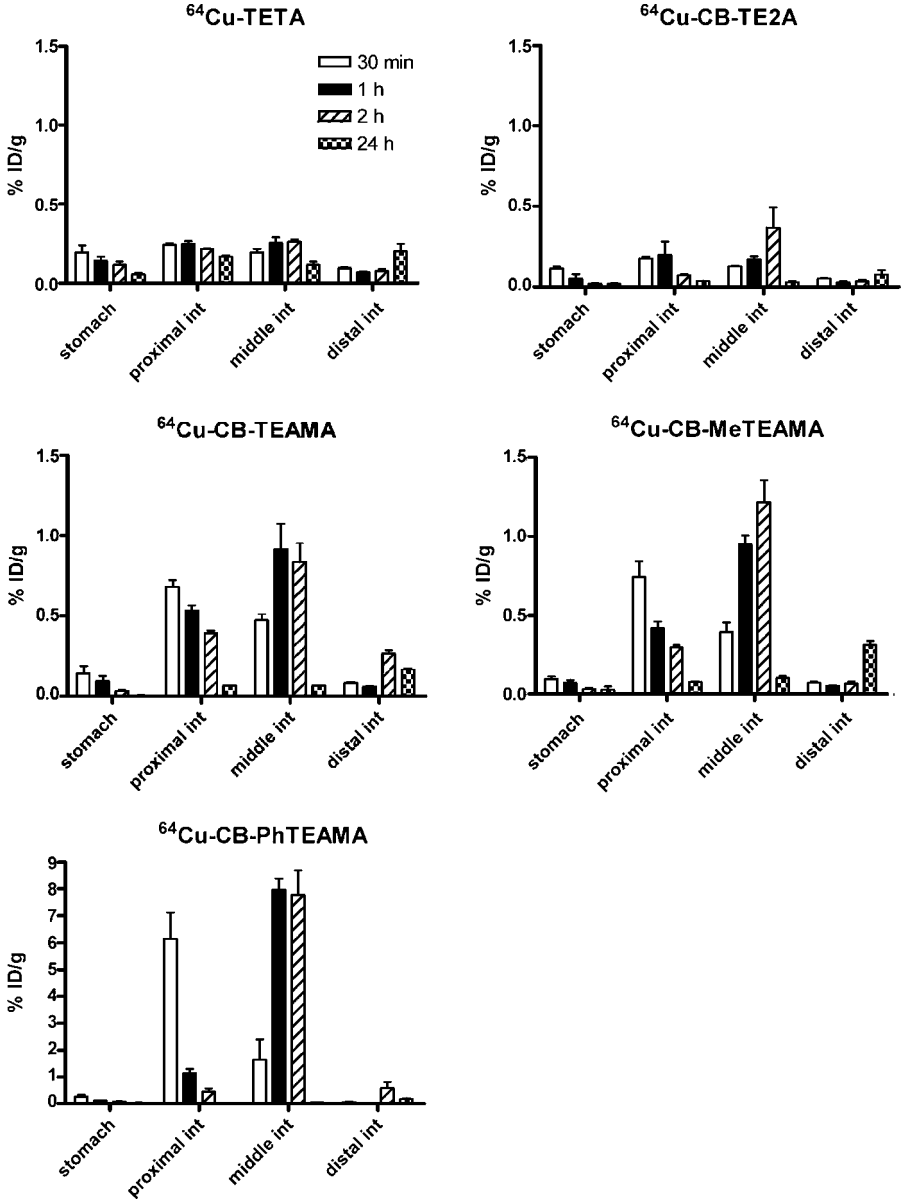


Figure 4. Gut biodistribution of ^{64}Cu-TETA, ^{64}Cu-CB-TE2A, ^{64}Cu-CB-TEAMA, ^{64}Cu-CB-MeTEAMA, and ^{64}Cu-CB-PhTEAMA in 33 to 40 day old, male Lewis rats (30 min to 24 h, $n = 4$; ^{64}Cu-CB-TE2A 2 h, $n = 3$, animal died prior to sacrifice). Intestines were removed and divided into three parts for counting without removal of fecal contents. Note differences in scale.

propose that ^{64}Cu-CB-TEAMA and ^{64}Cu-CB-MeTEAMA may have undergone enzymatic modification in the liver, such as hydrolysis of the amide to form ^{64}Cu-CB-TE2A. This might allow secretion of the radio-metabolite(s) into blood, with subsequent clearance via the kidneys.

The observation that all cross-bridged complexes demonstrate continuous clearance from blood, liver, and bone marrow is highly suggestive of improved in vivo stability compared to ^{64}Cu-TETA, as was shown previously for ^{64}Cu-CB-TE2A.[14,23] These data further support the observation that CB-TE2A can be used as a bifunctional chelator for ^{64}Cu-radiolabeled peptides without additional modifications to the macrocycle backbone. However, no studies have been conducted to examine the in vivo stability properties of ^{64}Cu-CB-TE2A conjugates to larger macromolecules, which would require imaging at later time points.

Experimental Section

Materials and Methods. Caution! Although we did not experience any difficulties, metal perchlorate salts with organic ligands and in organic solvents are potentially explosive and should be prepared and handled only in small quantities and with great care. All work involving the use of radioactive materials at Washington University is conducted under Radiation Safety Committee approved authorizations in accordance with the University's Nuclear Regulatory Commission license and Missouri State registrations.

Copper-64 was produced on a CS-15 biomedical cyclotron at Washington University School of Medicine according to published procedures.[9] Copper chloride ($CuCl_2$) was purchased from Johnson Matthey (West Deptford, NJ). All other chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All solutions were prepared using ultrapure water (18 MΩ/cm^2 resistivity). IR spectra were recorded on a Nicolet MX-1 FT spectrophotometer. UV−vis data were collected using a Cary 50 Bio UV−vis spectrophotometer. Elemental analyses were performed at Atlantic Microlab, Inc. (Norcross, GA). Analytical reversed-phase HPLC was performed on a Waters 600E (Milford, MA) chromatography system with a Waters 991 photodiode array detector and an Ortec Model 661 radioactivity detector (EG&G Instruments, Oak Ridge, TN). Radioactive samples were counted using a Beckman 8000

automated well-type gamma counter (Fullerton, CA). ES-MS was accomplished using a Waters Micromass ZQ (Milford, MA). Male Lewis rats (21 d old, 40−50 g) were purchased from Charles River Laboratories (Boston, MA).

Ligand Synthesis. Synthetic reactions were run under a nitrogen atmosphere. Solvents were removed by rotary evaporation under reduced pressure (water aspirator) and vacuum line (mechanical pump). 1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane (cross-bridged cyclam **1**) was prepared as previously reported.[18]

4-Carbo-*t*-butoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane (2). 1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane (**1**; 100 mg, 0.441 mmol) was dissolved in dry CH_3CN (4 mL), sodium carbonate (47.0 mg, 0.441 mmol) was added in one portion, and *t*-butyl bromoacetate (0.065 mL, 0.44 mmol) was added in one portion by syringe. The solution was stirred for 14 h at room temperature, followed by solvent removal. NMR indicated this crude product to be a mixture of monoester **2**, diester **3**, and residual tetraamine **1**. Purification by flash chromatography (SiO_2, MeOH/CH_2Cl_2 = 1.5:10) yielded an oil that was dissolved in water (4 mL), adjusted to pH 14 (solid KOH), and extracted with benzene (3 × 50 mL). The combined extracts were dried (Na_2SO_4) and the solvent was removed to yield **2** as a light yellow oil (60.0 mg, 0.172 mmol, 39%): ^{1}H NMR (C_6D_6, 500 MHz) δ 1.20−1.32 (m, 2H), 1.38 (s, 9H, C(C*H*$_3$)$_3$), 1.41−1.52 (m, 2H), 1.94−2.01 (dm, 1H, J = 15.6 Hz), 2.08−2.14 (dm, 1H, J = 14.7 Hz), 2.19 (ddd, 1H, J = 13.4, 10.0, 3.4 Hz), 2.24−2.29 (dm, 1H, J = 12.9 Hz), 2.30−2.40 (m, 3H), 2.45 (td, 1H, J = 13.2, 3.7 Hz), 2.53 (td, 1H, J = 11.5, 5.4 Hz), 2.58−2.65 (ddm, 1H, J = 13.4, 5.9 Hz), 2.65−2.80 (m, 5H), 2.85−2.94 (m, 1H), 2.97−3.04 (m, 1H), 3.00 and 3.11 (AB, 2H, J = 16.4 Hz), 3.15−3.26 (m, 2H), 3.41−3.48 (m, 1H), 4.69 (m, 1H, NH); ^{13}C{^{1}H} NMR (C_6D_6, 100.5 MHz) δ 25.73, 28.21 (C(*C*H$_3$)$_3$), 28.35, 48.79, 49.03, 49.07, 50.20, 53.67, 55.63, 55.71, 57.36, 57.59, 59.37, 60.75, 79.92 (O*C*(CH$_3$)$_3$), 171.24 (C═O); IR (neat) 1158, 1214, 1255, 1296, 1366, 1391, 1458, 1492, 1736(C═O), 2801, 2914, 3236 (NH) cm^{-1}; HRFABMS (M + H)$^+$ exact mass calcd for $C_{18}H_{33}N_4O_2$, 341.2917; found, 341.2915 (error −0.1 mmu/−0.3 ppm).

4-Acetamido-11-carbo-*t*-butoxymethyl-1,4,8,11-tetraazabicyclo-[6.6.2]hexadecane (4). 4-Carbo-*t*-butoxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**2**; 30 mg, 0.086 mmol) was dissolved in dry CH_3CN (4 mL). Na_2CO_3 (9.2 mg, 0.086 mmol) was then added, followed by 2-bromoacetamide (12 mg, 0.086 mmol). The mixture was stirred for 14 h at room temperature and then solvent was removed. The residue was dissolved in water (2 mL), adjusted to pH 14 (solid KOH) with cooling, and extracted with benzene (2 × 20 mL). The combined extracts were dried (Na_2SO_4) and solvent was removed to give **4** as an oil (15.0 mg, 0.0372 mmol, 43%): ^{1}H NMR (C_6D_6, 400 MHz) δ 1.01−1.40 (m, 4H), 1.39 (s, 9H, C(C*H*$_3$)$_3$), 2.00 (dt, 1H, J = 12.3, 4.1 Hz), 2.05−2.68 (m, 13H), 2.68−2.86 (m, 3H), 2.79 and 3.04 (AX, 2H, J = 16.4 Hz), 3.02 and 3.14 (AB, 2H, J = 16.4 Hz), 3.20 (td, 1H, J = 8.0, 4.1 Hz), 3.36 (td, 1H, J = 12.1, 4.5 Hz), 4.08 (ddd, 1H, J = 13.3, 8.2, 3.9 Hz), 6.37 (br s, 1H), 6.42 (br s, 1H); ^{13}C{^{1}H} NMR (C_6D_6, 100.5 MHz) δ 28.04, 28.22, 28.40. 51.67, 52.27, 53.46, 53.86, 55.89, 56.76, 57.24, 57.52, 57.55, 58.65, 59.94, 61.06, 79.96, 171.18, 174.44; IR (CCl_4) 3448, 2978, 2922, 2809, 1739, 1689, 1368, 1155, 1125 cm^{-1}; HRFABMS (M + H)$^+$ exact mass calcd for $C_{20}H_{40}O_3N_5$, 398.3131; found, 398.3106 (error −2.5 mmu/−6.3 ppm).

4-Acetamido-11-carboxymethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane·2TFA (5·2TFA). 4-Carbo-*t*-butoxymethyl-11-acetamido-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (**4**; 15 mg, 0.0372 mmol) was dissolved in a mixture of CF_3CO_2H (TFA) and CH_2Cl_2 (TFA/CH_2Cl_2 = 1:1, 6 mL), and the solution was stirred for 14 h at room temperature. Solvent was then removed to give **5** as a TFA salt (2 equiv TFA calculated on the basis of mass; 20 mg): ^{1}H NMR (D_2O, 400 MHz, *H*OD peak set to 4.80) δ 1.72−1.80 (dm, 2H, J = 17.4 Hz), 2.30−2.45 (m, 2H), 2.70−2.84 (m, 2H), 3.00−3.36(m, 16H), 3.42−3.72 (m, 4H), 3.56 and 4.08 (AX, 2H, J = 17.4 Hz), 3.63 and 4.08 (AX, 2H, J = 16.1 Hz); ^{13}C{^{1}H} NMR (D_2O, 100.5 MHz) δ 19.46, 19.47, 47.56, 47.82, 48.20, 48.30, 52.91, 53.23, 54.98, 55.11, 58.11, 58.13, 58.70, 58.93, 116.4 (q, J = 291.4 Hz, *C*F$_3$COOH), 163.1 (q, J = 35.2 Hz, CF$_3$*C*OOH), 170.05, 171.77; IR (CH_3CN) 3681, 3540, 3164, 3003, 2944, 1694, 1444, 1422, 1376, 1202, 1039, 918 cm^{-1}; HRFABMS, *m*/*z* (M + H)$^+$ exact mass for $C_{16}H_{35}N_5O_3$, 342.2505; found, 342.2500 (error −0.5 mmu/−1.4 ppm).

4-Carbo-*t*-butoxymethyl-11-(*N*-methylacetamido)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (6). Monoarmed ligand **2** (18 mg, 0.052 mmol) was dissolved in dry CH_3CN (4 mL), Na_2CO_3 (13 mg, 0.1226 mmol), KI (23 mg, 0.1385 mmol), and 2-chloro-*N*-methyl acetamide (13 mg, 0.1209 mmol) were added sequentially, and the solution was stirred for 14 h at room temperature. Solvent was removed to yield crude product as a solid, which was dissolved in water (2 mL) and adjusted to pH 3 (3 M HCl with cooling). The aqueous phase was extracted with benzene (2 × 25 mL), and the retained aqueous phase was adjusted to pH 14 with solid KOH (with cooling). The basic aqueous phase was extracted with benzene (2 × 25 mL), the combined extracts were dried (Na_2SO_4), and the benzene was removed to yield **6**, an oil (21 mg, 0.051 mmol, 98%, >94% purity by ^{13}C NMR): ^{1}H NMR (C_6D_6, 400 MHz) δ 1.05−1.40 (m, 4H), 1.40 (s, 9H, C(C*H*$_3$)$_3$), 2.02−2.16 (m, 2H), 2.17−2.36 (m, 2H), 2.36−2.66 (m, 8H), 2.64 (d, 3H, J = 5.1 Hz), 2.68−2.94 (m, 5H), 2.86 and 3.07 (AB, 2H, J = 16.2 Hz), 3.02 and 3.15 (AB, 2H, J = 16.4 Hz), 3.24 (td, 1H, J = 12.1, 4.3 Hz), 3.40 (td, 1H, J = 11.9, 4.3 Hz), 4.11 (ddd, 1H, J = 16.6, 10.0, 4.3 Hz), 6.59 (br s, 1H, NH); ^{13}C{^{1}H} NMR (C_6D_6, 100.5 MHz) δ 25.77, 28.32, 28.53, 28.58. 51.96, 52.70, 53.83, 54.02, 56.29, 57.18, 57.76, 57.95, 58.81, 60.23, 61.58, 80.34, 171.53, 171.69; IR (CCl_4) 3407, 2918, 2809, 1739, 1684, 1559, 1457, 1368, 1155, 1125 cm^{-1}; HRFABMS (M + H)$^+$ exact mass calcd for $C_{21}H_{42}N_5O_3$, 412.3288; found, 412.3274 (error −1.4 mmu/−3.3 ppm).

(11-Methylcarbamoylmethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadec-4-yl)-acetic acid·2TFA (8·2TFA). 4-(Carbo-*t*-butoxymethyl)-11-(*N*-methylacetamido)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane **6** (14 mg, 0.034 mmol) was dissolved in a mixture of TFA and CH_2Cl_2 (TFA/CH_2Cl_2 = 1:1, 8 mL), the solution was stirred for 16 h, and the solvent was removed to yield **8** as a TFA salt (20 mg): ^{1}H NMR (400 MHz, D_2O, *H*OD peak set at 4.80) δ 1.70−1.80 (dm, 2H, J = 17.0 Hz), 2.30−2.46 (m, 2H), 2.70−2.85 (m, 2H), 2.74 (s, 3H, NC*H*$_3$), 3.00−3.38 (m, 14H), 3.44−3.76 (m, 4H), 3.57 and 4.11 (AX, 2H, J = 17.4 Hz), 3.58 and 4.06 (AX, 2H, J = 16.1 Hz); ^{13}C{^{1}H} NMR (100.5 MHz, D_2O) δ 19.41 (2C), 25.95, 47.51, 47.87, 48.26, 48.32, 52.82, 53.29, 55.13 (2C), 58.07 (2C), 58.61, 58.93, 116.43 (q, J_{CF} = 292.2 Hz, *C*F$_3$COOH), 163.05 (q, J_{CF} = 35.2 Hz, CF$_3$*C*OOH), 167.65, 171.74; IR (CH_3CN) 3638−3538 (br), 3164, 3002, 2944, 1682, 1444, 1418, 1376, 1201, 1132, 1039 cm^{-1}; HRFABMS *m*/*z* (M + H)$^+$ exact mass calcd for $C_{17}H_{34}N_5O_3$, 356.2662; found, 356.2664 (error +0.2 mmu/+0.5 ppm). The product is a di-TFA salt on the basis of the mass (no evidence of impurities by NMR).

4-(Carbo-*t*-butoxymethyl)-11-(*N*-phenylacetamido)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (7). Monoarmed ligand **2** (20 mg, 0.058 mmol) was dissolved in dry CH_3CN (4 mL), and Na_2CO_3 (74 mg, 0.698 mmol), KI (13.4 mg, 0.0807 mmol), and 2-chloro-*N*-phenylacetamide (37 mg, 0.218 mmol) were added in single portions. The solution was stirred for 14 h at room temperature and solvent was removed by rotary evaporation to yield a solid, which was dissolved in water (2 mL). The pH was adjusted to 3 (3 M HCl with cooling), and the acidic solution was extracted with benzene (2 × 25 mL). The retained aqueous phase was adjusted to pH 14 (solid KOH with cooling) and extracted with benzene (2 × 25 mL), the combined benzene extracts were dried (Na_2SO_4), and the solvent was removed to yield **7** as an oil (20 mg, 0.042 mmol, 73%): ^{1}H NMR (C_6D_6, 500 MHz) δ 1.03−1.40 (m, 4H), 1.40 (s, 9H, C(C*H*$_3$)$_3$), 2.00−2.03 (m, 2H), 2.08 (dt, 1H, J = 13.0, 3.9 Hz), 2.12−2.23 (m, 2H), 2.24−2.35 (m 2H), 2.48−2.55 (m, 2H), 2.55−2.66 (m, 4H), 2.71 (td, 1H, J = 8.8, 3.6 Hz), 2.76−2.86 (m, 2H), 2.85 and 3.09 (AX, 2H, J = 16.4 Hz), 2.97 (td, 1H, J = 11.7, 4.2 Hz), 3.01 and 3.13 (AB, 2H, J = 16.4 Hz), 3.25 (td, 1H, J = 13.2, 4.6 Hz), 3.33 (td, 1H, J = 12.0, 4.4 Hz), 4.17−4.24 (m, 1H), 6.92 (tt, 1H, J = 8.6, 1.0 Hz), 7.14−7.22 (m,

Table 2. Crystal Data for [Cu-CB-TEAMA]ClO_4·$NaClO_4$ and [Cu-CB-PhTEAMA]ClO_4·MeOH

	[Cu−CB-TEAMA]ClO_4·$NaClO_4$	[Cu−CB-PhTEAMA]ClO_4·MeOH
empirical formula	$C_{16}H_{28}Cl_2CuN_5NaO_{11}$	$C_{23}H_{36}ClCuN_5O_8$
formula weight	623.86	609.56
temperature (K)	213(2)	100(2)
crystal system	orthorhombic	orthorhombic
space group	$P2_12_12_1$	*Pbca*
unit cell dimensions (Å)	$a = 10.011(5)$	$a = 21.134(5)$
	$b = 12.346(6)$	$b = 10.7258(16)$
	$c = 20.652(11)$	$c = 23.111(4)$
volume (Å^3)	2553(2)	5238.6(15)
Z	4	4
density (calcd; Mg/m^3)	1.623	1.546
absorption coefficient (mm^{-1})	1.145	0.993
crystal size (mm^3)	0.20 × 0.20 × 0.05	0.40 × 0.30 × 0.08
crystal color, habit	blue, plate	blue, plate
reflections collected	12 979	22 661
independent reflections	4363 [*R*(int) = 0.0415]	4613 [*R*(int) = 0.0432]
completeness to theta = 25.00°	99.1%	99.0%
data/restraints/parameters	4363/0/325	4613/0/343
goodness-of-fit on F^2	1.029	1.049
final *R* indices [I > 2sigma(*I*)] (%)	$R1 = 6.57$, $wR2 = 16.83$	$R1 = 5.42$, $wR2 = 13.03$
absolute structure parameter	0.51(3)	NA
largest diff. peak, hole (e.Å^{-3})	0.893, −0.476	1.038, −0.461

2H), 7.91 (dd, 2H, $J = 8.6$, 1.0 Hz), 9.12 (br s, 1H, N*H*); $^{13}C\{^1H\}$ NMR (C_6D_6, 100.5 MHz) δ 28.30, 28.57(C(CH_3)$_3$), 28.69, 51.87, 52.49, 53.84, 54.10, 56.13, 57.22, 57.46, 58.03, 58.36, 59.43, 60.40, 62.44, 80.39, 119.31, 124.24, 129.84, 139.48, 169.63, 171.49; IR (neat) 703, 739,1126, 1156, 1265, 1368, 1443, 1521, 1601, 1684, 1732, 2818, 2925, 2979, 3053, 3308 cm^{-1}; HSFABMS (M + H) exact mass calcd for $C_{26}H_{44}N_5O_3$, 474.3444; found, 474.3440 (error −0.4 mmu/−0.8 ppm).

(11-Phenylcarbamoylmethyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadec-4-yl)-acetic acid·4TFA (9·4TFA). 4-(Carbo-*t*-butoxy-methyl)-11-(*N*-phenylacetamido)-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane (**7**; 140 mg, 0.296 mmol) was dissolved in a mixture of TFA and CH_2Cl_2 (TFA/CH_2Cl_2 = 1:1, 20 mL), and the solution was stirred for 16 h. Solvent was then removed to yield **8** as a tetra-TFA adduct on the basis of mass (255 mg, quant; no impurities by NMR): ^1H NMR (CD_3CN, 500 MHz, C*H*D$_2$CN central peak set to 1.94) δ 1.63−1.72 (m, 2H), 2.20−2.34 (m, 2H), 2.68−2.76 (dm, 2H, $J = 12.5$ Hz), 2.94−3.02 (dm, 2H, $J = 14.7$ Hz), 3.03−3.22 (m, 12H), 3.27 (td, 1H, $J = 12.9$, 3.2 Hz), 3.40−3.61 (m, 4H), 3.62−3.72 (m, 2H), 4.05 (A of AX, 1H, $J = 16.6$ Hz), 4.23 (A of AX, 1H, $J = 16.6$ Hz), 7.12−7.17 (m, 1H), 7.31−7.36 (m, 2H), 7.57−7.61 (m, 2H), 10.02 (s, 1H, NH), 10.90 (br s, 2H), 11.06 (br s, 3H); $^{13}C\{^1H\}$ NMR (CD_3CN, 125.7 MHz, *C*D$_3$CN central peak set to 1.39) δ 20.73, 20.81, 48.77, 48.98, 49.39, 49.43, 53.73, 53.91, 56.66, 57.70, 59.42, 60.40, 117.25 (q, $J_{CF} = 290.5$ Hz, *C*F$_3$-CO$_2$), 121.24, 125.88, 129.94, 138.79, 160.95 (q, $J_{CF} = 36.4$ Hz, CF$_3$*C*O$_2$), 166.73, 171.91; IR (CH_3CN) 3648, 3607, 3550, 3002, 2944, 1680, 1632, 1446, 1428, 1375, 1201, 1039, 918, 749 cm^{-1}; HRFABMS $(M + H)^+$ exact mass calcd for $C_{22}H_{36}N_5O_3$, 418.2818; found, 418.2811 (error −0.7 mmu/−1.7 ppm).

Synthesis of Cu-CB-PhTEAMA. An amount of **9**·4TFA (0.082 g, 0.99 mmol) was dissolved in 3 mL methanol. Copper perchlorate hexahydrate (0.056 g, 0.15 mmol) dissolved in 2 mL of methanol was added to give a light-blue solution. NaOH (6 equiv, 0.40 mL, 1.0 M) was added and the light-blue suspension was refluxed for 5 h to give a clear solution. Cooling to room temperature gave a light-blue precipitate that was filtered off and dried under reduced pressure to give 0.052 g (59% yield) of the complex. X-ray quality crystals were grown from a methanol solution of this product by ether diffusion. IR: 1677 (s, CO), 1628 (s, COO), 1116 (vs, ClO_4) cm^{-1}. Visible electronic spectrum (H_2O): λ_{max} 648 nm ($\epsilon = 55$ M^{-1}cm^{-1}). Anal. Calcd for $CuC_{22}H_{34}N_4O_3(ClO_4)(H_2O)$: C, 44.22; H, 6.07; N, 11.72; Cl, 5.93. Found: C, 44.47; H, 5.80; N, 11.68; Cl, 5.98.

Synthesis of Cu−CB-TEAMA. An amount of **5**·2TFA (0.076 g, 0.19 mmol) was stirred in 10 mL of TFA overnight. After removal of volatiles under reduced pressure, the oily residue was taken up in 10 mL of water and its pH adjusted to approximately 8. Copper perchlorate hexahydrate (0.080 g, 0.22 mmol) was dissolved in 5 mL of water and added to give a light-blue solution. This was heated to 80 °C overnight to give a dark-blue solution. After cooling to room temperature, this was evaporated to dryness under reduced pressure. The residue was treated with absolute ethanol and centrifuged to remove a small amount of insoluble material. Ether diffusion into the supernatant yielded 32 mg (31%) of the desired complex. X-ray quality crystals were grown from an acetonitrile solution of this product by ether diffusion. IR: 3475−3380 (w, br), 1677 (s, CO), 1628 (s, COO), 1098 (vs, ClO_4) cm^{-1}. Visible electronic spectrum (H_2O): λ_{max} 648 nm ($\epsilon = 55$ M^{-1}cm^{-1}). Anal. Calcd for $CuC_{16}H_{30}N_5O_3(ClO_4)$·$0.3NaClO_4$: C, 35.58; H, 5.60; N, 12.96; Cl, 8.53%. Found: C, 35.52; H, 5.73; N, 12.89; Cl, 8.54%.

X-ray Crystallography. Crystallographic data are collected in Table 2. Both space groups were uniquely determined from systematic absences. Data collections for both structures were performed on a Bruker D8 platform diffractometer equipped with an APEX CCD detector using MoK(α) radiation. Data were corrected for absorption using empirical methods (SADABS). All nonhydrogen atoms were refined with anisotropic thermal parameters and hydrogen atoms were placed in idealized locations, except for those associated with the hydroxyl proton in the solvent MeOH for the PhTEAMA complex. Those hydrogens were ignored, but were included in computations of intensive properties. For the CB-TEAMA complex structure, refinement of the Flack parameter yielded a value of 0.51, which is interpreted as an indication that crystallization occurred as a racemic twin (further refinement with TWIN and BASF = 0.51 yielded identical results). All software was obtained from the SMART, SAINT, and SHELXTL Bruker libraries (Bruker-AXS, Madison, WI).

Radiolabeling with ^{64}Cu. Cu(II) complexes of CB-TEAMA, CB-MeTEAMA, and CB-PhTEAMA were prepared according to the method described previously for radiolabeling CB-TE2A-Y3-TATE with ^{64}Cu.[28] Briefly, $CuCl_2$ + ^{64}Cu (30 μCi) was added to each monoamide (1:1) in NH_4OAc, pH 8.0. The reaction mixture was heated for 30 min at 95 °C. Complex formation was followed by radio-TLC. After sufficient time for radio-decay, formation of the correct complex was demonstrated by ES-MS (Cu(II)-CB-PhTEAMA ($C_{22}H_{34}CuN_5O_3Cu$) calcd *m*/*z* (M^+), 479.20; found, 479.08; Cu(II)-CB-MeTEAMA ($C_{17}H_{32}CuN_5O_3Cu$) calcd *m*/*z* (M^+), 417.18; found, 417.04). HPLC analysis of the monoamide complexes was accomplished using an Agilent Technologies C8 column (3.0 × 150 mm; Palo Alto, CA)[33] with the following gradient:

solvent A, H_2O (0.1% formic acid); solvent B, CH_3CN (0.1% formic acid; solvent B); 0% B to 50% B in 40 min (0.5 mL/min flow rate).

All monoamides, as well as CB-TE2A, were radiolabeled (no carrier added) with ^{64}Cu according to the method described by Boswell et al. for formation of ^{64}Cu-CB-TE2A.[14] TETA was radiolabeled with ^{64}Cu in 0.5 M NH_4OAc, pH 6.5 (30 min at RT). Radio-HPLC for ^{64}Cu-CB-TEAMA, ^{64}Cu-CB-MeTEAMA, and ^{64}Cu-CB-PhTEAMA was performed as described above. For ^{64}Cu-CB-TE2A and ^{64}Cu-TETA, radio-HPLC was performed using an isocratic method (0.1% formic acid in H_2O).

Partition Coefficients. The partition coefficients (logP) of ^{64}Cu-TETA, ^{64}Cu-CB-TE2A, ^{64}Cu-CB-TEAMA, ^{64}Cu-CB-MeTEAMA, and ^{64}Cu-PhTEAMA were determined using previously described methods.[19] Briefly, 4 μL of ^{64}Cu-labeled complex was added to 500 μL octanol + 500 μL PBS (obtained from a saturated octanol−PBS solution, $n = 4$). Solutions were shaken for 1 h at room temperature. For each sample, 50 μL was removed from each phase and counted separately in a gamma counter. The partition coefficient was calculated as a ratio of counts in the octanol fraction to counts in the PBS fraction.

Biodistribution. All animal experiments were performed in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University's Animal Studies Committee. Male Lewis rats (33−40 d old) were injected intravenously with ^{64}Cu-radiolabeled complexes (43 μCi, 1 nmol ligand) via the tail vein. Tissue biodistribution data were obtained at 0.5, 1, 2, and 24 h PI according to previously described methods.[5] Rats in the 24 h group were maintained in metabolism cages; food and water were administered ad libitum. Urine and feces were collected.

Statistical Methods. All data are presented as mean ± standard deviation. For statistical classification, one-way ANOVA was used to determine statistically significant differences between the five complexes. Subsequently, Tukey's multiple comparison test (post-test) was used to compare individual data sets. Student's t-test (two-tailed, unpaired) was used to compare individual time points for a single complex. All statistical analysis was performed using GraphPad PRISM (San Diego, CA). All p values less than 0.05 were considered significant.

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Supporting Information Available: X-ray crystallographic information for Cu-CB-TEAMA and Cu-CB-PhTEAMA, including experimental crystal data, refinement details, final atomic position parameters, atomic thermal parameters, and complete bond distances and angles. This material is available free of charge via the Internet at http://pubs.acs.org.

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