Comparative in Vivo Behavior Studies of Cyclen-Based Copper-64 Complexes: Regioselective Synthesis, X-ray Structure, Radiochemistry, log *P*, and Biodistribution

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The in vivo behavior of copper(II)−cyclen complexes was modified via substitution of the parent ligand with two different substituents, 4-*tert*-butylbenzyl and acetate. This was achieved by using same synthetic strategy (regioselective protection/first alkylation/deprotection/second alkylation) to give nine cyclen derivatives. The X-ray structure of [Cu(**2c**)Cl]$^+$ (**2c** = 1-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane) showed that the chlorine ion from the reaction mixture occupied the remaining apical position of a square pyramidal coordination environment of these Cu−cyclen complexes. Eight out of nine compounds were labeled with ^{64}Cu in high radiochemical purity. log *P* measurements showed that the lipophilicities of the copper complexes were increased dramatically by attaching hydrophobic substituents on the nitrogen atoms of cyclen. Conversely, as the number of acetate groups increased, the lipophilicity was decreased. The biodistribution of Cu−cyclen complexes was found to be influenced mostly by the overall charge of the complexes rather than their lipophilicity. Positively charged (+2) complexes showed high blood retention at early time points with sluggish clearance from liver by 24 h. The attachment of even one acetate group onto cyclen accelerated blood and liver clearance dramatically compared to +2 charged Cu(II) complexes. Neutral trans-substituted Cu−**4** showed the best clearance and lowest retention of doses from all organs most time, followed by −1 charged complex Cu−**2**. Trans-substituted complexes structure isomers Cu−**3** and Cu−**4** showed better clearance and lower retention from all organs than their cis-counterparts Cu−**5** and Cu−**6**.

Introduction

One of the most important ligands in medicinal inorganic chemistry is 1,4,7,10-tetraazacyclododecane (cyclen). Two derivatives of this macrocycle, DOTA and DO3A, both of which are functionalized with acetate groups, are used to form stable metal complexes with gadolinium, copper, indium, gallium, yttrium, and other metals.[1−6] These complexes are used heavily in the fields of medical imaging and radiotherapy.[7] In addition to these uses, tetraazamacrocycles have recently found applications as both antitumor drugs and anti-HIV agents.[8,9]

To utilize these compounds in medicine, derivatization of the parent macrocycle is required in order to impart specific physical properties and to meet specific biological requirements, such as localization to a specific organ or receptor. Of particular importance is the regioselective introduction of one or more acetate groups on the *N* atoms, as these will typically determine the formal charge and core structure of the final metal complex, affecting both the kinetic inertness and thermodynamic stability under physiological conditions.[10] Systematic studies of the biodistribution of radiometal complexes can aid in the design of new radiopharmaceuticals and bifunctional chelates.

Regioselective substitution of the four nitrogen atoms of cyclen is a crucial step for new cyclen-based radiometal complexes and bifunctional chelating agents. For example, alkylation of cyclen with acetate groups is an effective route to prepare complexes for attachment to biomolecules such as peptides and antibodies or to neutralize the charge of the coordinated metal. In many applications it would be advantageous to have two different types of substituents, such as charged and lipophilic groups. Mixing substituent groups with different properties has potential for fine-tuning the properties of the metal ion complex,[11] enabling us to do systematic studies of the relationship between structure and physiological properties.[11,12]

We are particularly interested in understanding the in vivo behavior of ^{64}Cu-labeled complexes. Copper-64 has favorable properties as a radionuclide for use in both positron emission tomography (PET) imaging and targeted radiotherapy due to its medium-lived half-life ($t_{1/2}$ = 12.7 h) and two different decay modes (β^+ 19%, β^- 39%).[1,7,13−17]

In a previous communication,[18] we presented a facile synthetic strategy involving various protecting groups leading to all possible cyclen substitution patterns involving two different alkylating agents, including at least one acetate group. We reported methods for the regioselective protection of cyclen leading to four different substitution patterns mono, cis-di, trans-di, and tri. The monoprotected cyclen compound, mono-*N*-Cbz-cyclen was also prepared in a very effective way using two protecting groups consecutively.

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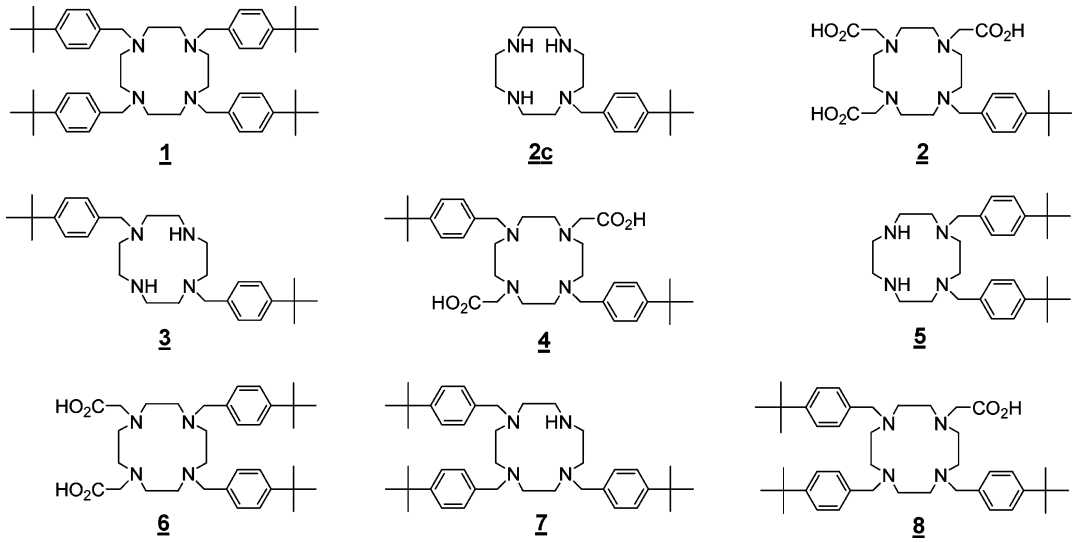


Figure 1. Nine newly synthesized cyclen-based ligands.

Jones-Wilson et al. reported the in vivo behavior of ^{64}Cu-labeled azamacrocyclic complexes.[19] Biodistribution studies of Cu(II) complexes of several tetraaza macrocycles such as cyclen, DO2A, DOTA, cyclam, etcyclam, and TETA provided a rough idea about the relationship between structure and biological behavior. It was demonstrated that the biodistribution of copper-64 complexes correlates with differences in the size of the macrocycle backbone and the formal charge of the complex. The +2 charged complexes were shown to have significantly higher uptake in liver and kidneys than neutral and −2 charged complexes.

Herein, we report in detail synthetic procedures leading to the regioselective *N*-alkylation of cyclen using different protecting groups and all possible isomers with two different substituents. We also report the X-ray crystal structure of Cu(II) complex (**2c**). Eight out of nine compounds were labeled with copper-64 in high yield and were subject to log *P* measurements in order to determine the relationship between structures of metal complexes and their lipophilicity. Biodistribution studies of the radiolabeled complexes revealed a strong relationship between in vivo distribution and the overall charge of the copper complexes.

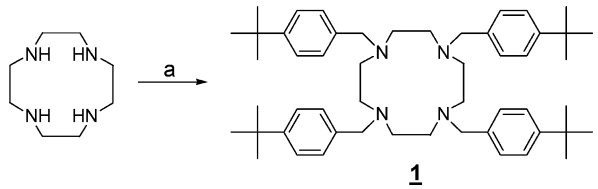
Results and Discussion

Synthesis. To compare the in vivo behavior of copper complexes with different overall charges and lipophilicities, we synthesized nine new *N*-substituted cyclen compounds with various numbers and substitution patterns of 4-*tert*-butylbenzyl, which is very hydrophobic, and acetate (CH_2CO_2H). The nine newly synthesized cyclen-based ligands are shown in Figure 1.

1,4,7,10-Tetra(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (**1**) was prepared by reacting cyclen with 4 equiv of 4-*tert*-butylbenzyl bromide in the presence of *N*,*N*-diisopropylethylamine in good yield. (Scheme 1) This general alkylation method was utilized to substitute the nitrogen atoms of cyclen to prepare all other *N*-substituted cyclen compounds.

While the synthesis of tetra-*N*-homo-substituted derivatives such as **1** is straightforward, the preparation of partially substituted or hetero-substituted cyclen compounds is synthetically challenging and more complicated.[20] To achieve selective *N*-functionalization, various approaches, such as direct functionalization, cyclization of *N*-alkylated precursors, and regioselective *N*-protection, have been devised.[20] Herein, we utilized a strategy of regioselective protection/first alkylation/deprotection/second alkylation to obtain a total of eight new regioselectively *N*-substituted cyclen derivatives.[18] In this strategy, the proper choice of protecting group for regioselective blocking of four identical nitrogen atoms of cyclen is the most critical step. The protecting groups are required to be introduced regioselectively in high yield, stable to a wide range of reaction conditions, and easily cleaved under mild reaction conditions without attacking other functional groups.[21]

Scheme 1[a]

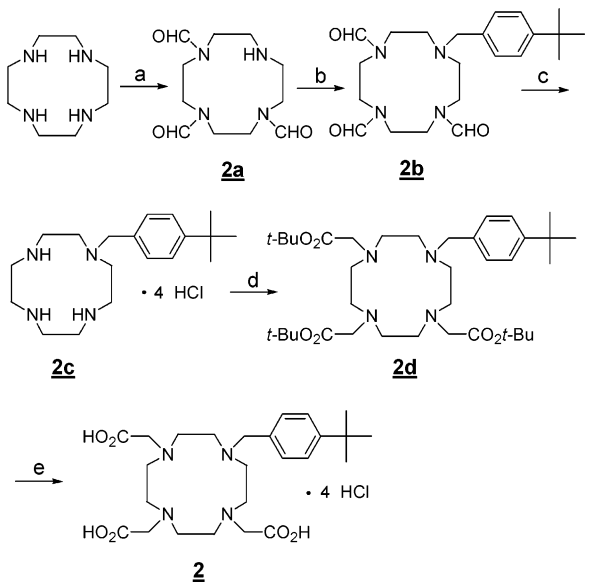


[a] Reagents and conditions: (a) 4-*tert*-butylbenzyl bromide, (*i*-Pr)$_2$NEt, MeCN, 60 °C.

In this work varying numbers of nitrogen atoms of cyclen were protected selectively and the remaining available nitrogen atoms were alkylated with hydrophobic 4-*tert*-butylbenzyl groups and then the protecting groups were cleaved to give mono-, trans-di- and cis-di-, and trialkylated cyclen compounds (**2c**, **3**, **5**, **7**). A second alkylating agent, *tert*-butyl bromoacetate, was introduced on nitrogen atoms that were previously blocked by protecting groups. Cleavage of the *tert*-butyl groups gave four hetero-substituted cyclen derivatives having at least one acetic acid group (**2**, **4**, **6**, **8**) (Schemes 2−5).

A triprotected cyclen compound, 1,4,7-triformylcyclen (**2a**), was synthesized by modification of the reported procedure.[22] A mixture of cyclen and 6 equiv of chloral hydrate in ethanol was stirred for 4 h at 60 °C. The solution was concentrated in vacuo, and the crude product was isolated via chromatography. After introduction of a 4-*tert*-butylbenzyl group using the general

Scheme 2[a]



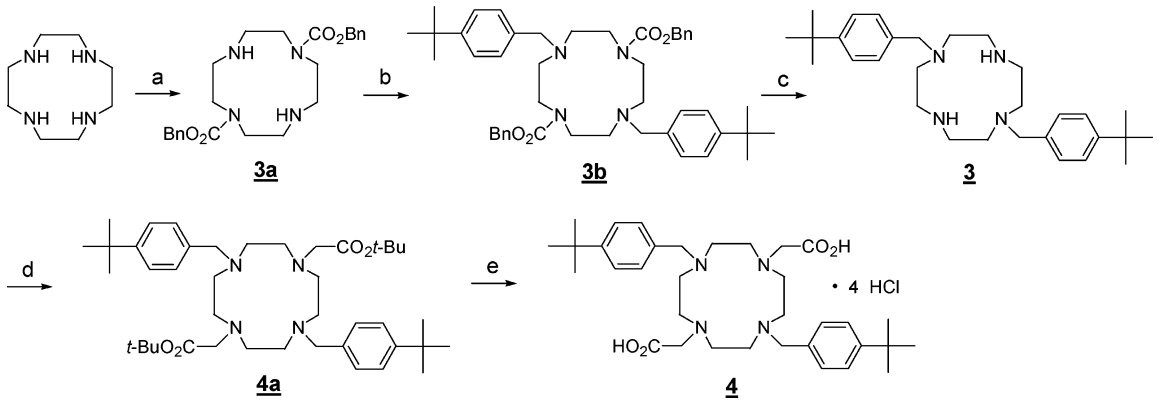
[a] Reagents and conditions: (a) $Cl_3CCH(OH)_2$, EtOH, 60 °C; (b) 4-*tert*-butylbenzyl bromide, $(i\text{-Pr})_2$NEt, MeCN, 60 °C; (c) 2 M HCl, 60 °C; (d) *tert*-butyl bromoacetate, $(i\text{-Pr})_2$NEt, MeCN, 60 °C; (e) 6 M HCl, reflux.

alkylation procedure, the formyl groups were easily removed using 2 M HCl solution to give monoalkylated cyclen compound (**2c**·4HCl). Further alkylation with *tert*-butyl bromoacetate on the deprotected amine nitrogens was carried out. A large excess of *N,N*-diisoproylethylamine (10 equiv) was used to scavenge HCl salt of reactant **2c** and byproduct HBr. After column purification of **2d**, the *tert*-butyl group was easily hydrolyzed by 6 M HCl to give the DO3A analogue **2** in hydrochloric acid form (Scheme 2). Alternatively, the *tert*-butyl groups can also be easily cleaved by treatment with TFA to give neutral product.[23]

Schemes 3 and 4 illustrate the syntheses of regioselective disubstituted cyclen derivatives. Two possible regioselective protections of two amine nitrogens in cyclen were achieved by treating cyclen with benzyl chloroformate or diethyl oxalate to give 1,7-dibenzyloxycarbonylcyclen[24] and cyclenoxamide,[25] respectively. After the initial alkylation, the Cbz and oxalate groups were deprotected by hydrogenation over Pd/C and strong base treatment (10 M NaOH) to give trans- and cis-disubstituted intermediate (with respect to the relative positions of tertiary nitrogen atoms) cyclen derivatives, respectively (**3** and **5**). The current synthesis of **3** is much higher yielding than the previously reported method,[26] which employed ethyl carbamate instead of Cbz as the protecting group. Ethyl carbamate groups were deprotected under very harsh condition (KOH, hydrazine, reflux in ethylene glycol) to give product **3** in a yield of 10%, which is much lower than the current 96% yield. Two structural isomers can be clearly assigned by ^{13}C NMR spectroscopy. D_{2h} symmetry of trans-disubstitued cyclen (**3**) gave only two signals (46.0, 51.9 ppm) for eight cyclen ring carbons in the ^{13}C NMR spectrum, while cis-isomer **5** showed four peaks (46.6, 46.9, 50.5, 50.8 ppm) for ring CH_2 atoms due to the C_{2v} symmetry of **5** in solution. The second alkylation and subsequent hydrolysis of the *tert*-butyl groups used the same methods as previously described and gave two DO2A derivatives (**4** and **6**) as the hydrochloric acid salts, which were further recrystallized from diethyl ether.

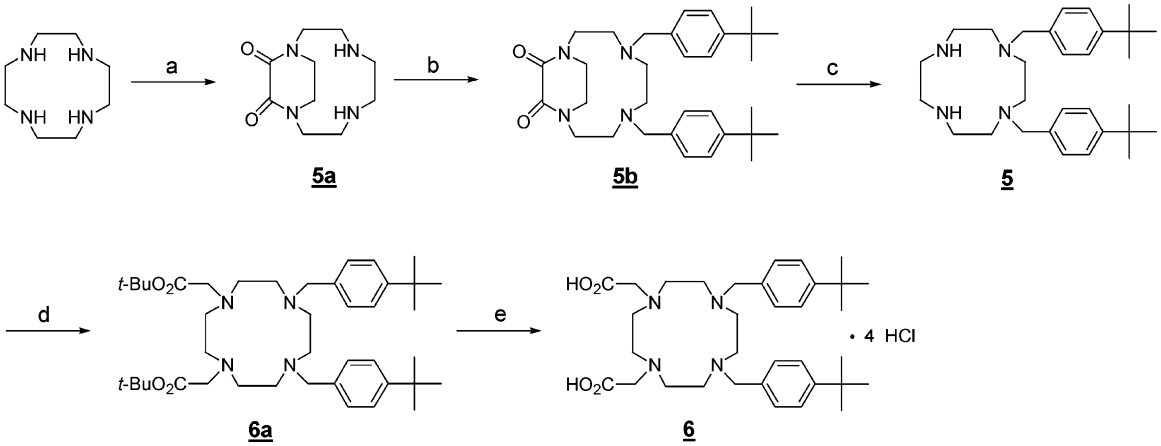
The ligands **7** and **8** were synthesized as shown in Scheme 5. A monoprotected cyclen, mono-*N*-Cbz-cyclen (**7b**) was prepared in high yield by employing two consecutive protections of all four nitrogen atoms in cyclen with formyl and Cbz groups followed by selective removal of the three formyl groups and purification by simple recrystallization.[18] In brief, it is important to keep the pH basic (up to 10) and to use excess benzyl chloroformate (4.5 equiv) during the second protection step. Selective deprotection of the formyl groups using mild acid (1 M HCl, 50 °C) afforded the monoprotected cyclen compound **7b** as the 3HCl salt. Our method for **7b** has several advantages as compared to the Kimura group's procedure.[12] In their paper, less than 3 equiv of di-*tert*-butyl dicarbonate compared to cyclen was added slowly to the reaction mixture and the product was purified from less protected and overprotected side products to give 3Boc-cyclen.[20,27] After a second protection of cyclen with Cbz, the product was purified again by column chromatography. Their overall yield from cyclen is 50%, which is much lower than the 80% overall yield achieved with our methodology.[12] Typical alkylation on the three available nitrogen atoms followed by deprotection of the Cbz group by hydrogenation over Pd/C gave trialkylated cyclen **7**. The *tert*-butyl groups of **8a** were cleaved by TFA in quantitative yield to afford **8**.

Scheme 3[a]



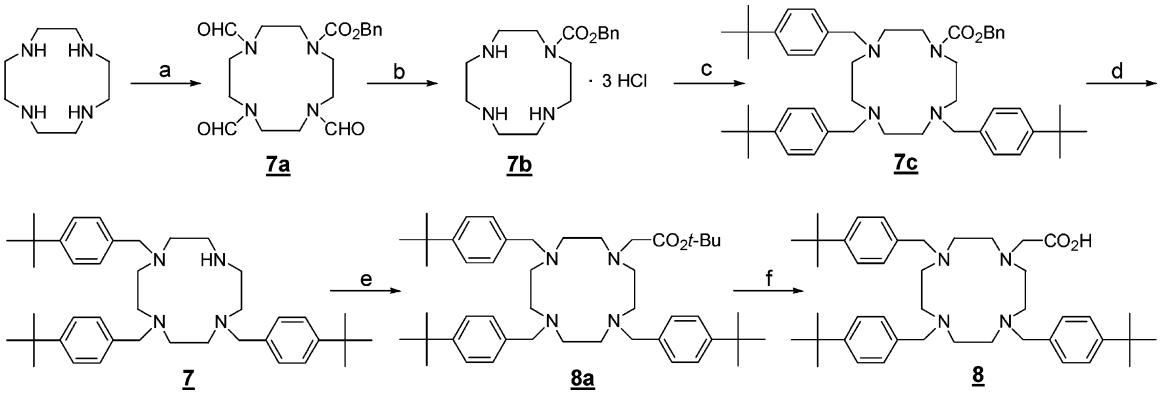
[a] Reagents and conditions: (a) benzyl chloroformate, pH 2−3, H_2O; (b) 4-*tert*-butylbenzyl bromide, $(i\text{-Pr})_2$NEt, MeCN, 60 °C; (c) H_2, 10% Pd/C, EtOH; (d) *tert*-butyl bromoacetate, $(i\text{-Pr})_2$NEt, MeCN, 60 °C; (e) 6 M HCl, reflux.

Scheme 4[a]



[a] Reagents and conditions: (a) diethyl oxalate, EtOH; (b) 4-*tert*-butylbenzyl bromide, (*i*-Pr)$_2$NEt, MeCN, 60 °C; (c) NaOH, EtOH, reflux; (d) *tert*-butyl bromoacetate, (*i*-Pr)$_2$NEt, MeCN, 60 °C; (e) 6 M HCl, reflux.

Scheme 5[a]



[a] Reagents and conditions: (a) (1) $Cl_3CCH(OH)_2$, EtOH, 60 °C, (2) benzyl chloroformate, pH 4−10, H_2O; (b) 1 M HCl, 50 °C; (c) 4-*tert*-butylbenzyl bromide, (*i*-Pr)$_2$NEt, MeCN, 60 °C; (d) H_2, 10% Pd/C, EtOH; (e) *tert*-butyl bromoacetate, (*i*-Pr)$_2$NEt, MeCN, 60 °C; (f) TFA.

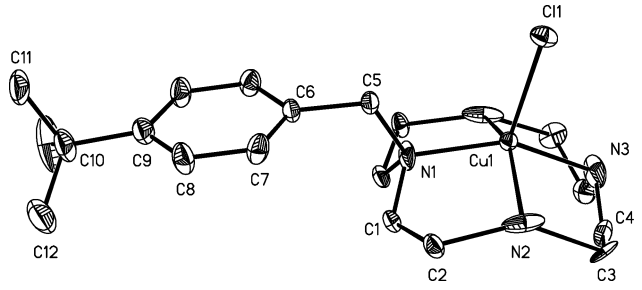


Figure 2. X-ray structure of [Cu(**2c**)Cl]$^+$(ClO$_4$)$^-$. Hydrogen atoms and perchlorate ion have been omitted for clarity. Crystallographic atom numbering is used. Displacement ellipsoids are scaled to the 30% probability level.

X-ray Structure Determination and Visible Absorption Spectra. The reaction of **2c**·4HCl with copper(II) perchlorate gave [Cu(**2c**)Cl]$^+$(ClO$_4$)$^-$, which was then subjected to X-ray diffraction analysis. The crystal structure of [Cu(**2c**)Cl]$^+$ is given in Figure 2. One chloride ion was found to coordinate to copper and one perchlorate anion counterbalanced the charge of the cationic copper complex. The Cu(II) ion is coordinated to four N atoms, and a Cl$^-$ ion occupies the apical position in a square pyramidal coordination structure. The bond distances and angles around the Cu ion indicate geometry close to an ideal square pyramid.[28−32] The preference of square pyramidal coordination geometry of cyclen-based copper complex with axial ligation[28,29,32] strongly implies that ^{64}Cu-labeled **1**, **3**, **5**, and **7** complexes also have square pyramidal coordination environment. An X-ray structure of [Cu(**3**)Cl]$^+$Cl$^-$,[33] prepared from reaction of $CuCl_2$ and ligand **3**, also supports this preference of a square pyramidal coordination geometry with Cl$^-$ ion in an apical position. The cyclen ring backbone is bent away from the plane of the nitrogen atoms in a direction opposite to that of the copper ion. The 4-*tert*-butylbenzyl group attached to the one *N* atom of cyclen ring is directed away from the cyclen ring with the benzyl group somewhat above the copper ion and the C5−C6 bond in benzyl group roughly parallel to the Cu1−N1 bond (Figure 2).

Table 1. Electronic Absorption Data of [Cu(**2c**)Cl]$^+$(ClO$_4$)$^-$

solvent	λ_{max} (nm)	ϵ_{max} (M^{-1} cm^{-1})
H_2O	594	312
$MeNO_2$	705	303
MeCN	710	356

Visible absorption spectra of [Cu(**2c**)Cl]$^+$(ClO$_4$)$^-$ were measured in three different solvents, and the result is shown in Table 1. When Cu(II) complexes of cyclen derivatives having no other possible Cu(II) coordination substituent on *N* atoms are dissolved in aqueous solution, the apical ligand is replaced with aqua ligand,[19,34,35] and this replacement can be detected by a shift of the absorption band.[36,37] The visible absorption maxima λ_{max}

Table 2. Labeling Conditions and Radiochemical Purities for Complexing ^{64}Cu to ligands **1** through **8**

ligand	solvent (μL) (ethanol:buffer[a])	temp (°C)	rxn time (min)	radiochem purity (%)	R_f
1	300:100	60	60	95	0.69[c]
2	0:300	60	60	90	0.79[c]
3	100:100[d]	40	60	97	0.15[e]
4	100:300	60	30	93	0.70[c]
5	50:80f	rt	420	98	0.35[g]
6	100:270	60	60	95	0.39[c]
7	100:160	30	30	98	0.45[g]
8	50:100	30	15	100	0.35[g]

[a] 0.1 M ammonium acetate, pH 6.4. [b] Silica gel, MeOH:ethyl acetate = 1:2. [c] Silica gel, H_2O:MeOH = 1:2. [d] 0.1 M ammonium citrate, pH 6.5. [e] Silica gel, H_2O:MeOH = 1:100. [f] H_2O. [g] RP-8, H_2O:MeCN = 1:9.

for [Cu(**2c**)Cl]$^+$(ClO_4)$^-$ in nitromethane and acetonitrile was observed at a higher wavelength, 705 and 710 nm, respectively, than that in water, indicating the presence of the axial chloride ion coordinated to copper ion. In aqueous solution, the λ_{max} was observed at 594 nm, which is comparable with the reported values of [Cu(cyclen)(H_2O)]$^+$ at 599[34] and 600 nm.[36] This confirms the displacement of the Cl^- ion by H_2O molecule in aqueous solution.[37]

Radiochemistry. All eight compounds were successfully labeled in more than 90% radiochemical purity, most in greater than 95% yield. The labeling conditions were optimized to give the best labeling yield depending on each ligand. The labeling solvents, temperatures, incubation times, radiochemical purities, R_f values, and TLC developing solvents are summarized in Table 2. Ethanol/buffer mixtures were used for radiolabeling, except for ligand **2**, due to the low solubility of cyclen derivatives and their copper complexes in pure aqueous solution. Four different TLC systems were used to check radiochemical yield. The developing solvent was chosen on the basis of the polarity of ^{64}Cu-labeled complexes and therefore their R_f values. Interestingly, ^{64}Cu complexes of ligands **5**, **7**, and **8** were found not to be stable on a normal phase silica gel TLC plate during development. Reverse phase C8 TLC plates were used instead for **5**, **7**, and **8**. Ligand **5** was mixed at room temperature with $^{64}CuCl_2$ in ethanol/water mixture solvent and allowed to stand for 7 h at room temperature to give the best labeling yield. The free authentic $^{64}CuCl_2$ and ^{64}Cu−acetate moved further, R_f = 0.66 and 0.64, respectively, under this reverse-phase C8 TLC condition. The ^{64}Cu−acetate remained on silica gel TLC at the origin, R_f = 0.0, under all other developing solvent conditions.

Ligand **2c** proved refractory to radiolabeling with ^{64}Cu. Varying the labeling conditions by changing the buffer, solvent, temperature, reaction time, and TLC conditions all failed to give a satisfactory radiochemical yield. The best radiochemical yield of 83% was achieved by adding 1 equiv of cold $CuCl_2$ relative to the ligand and shaking the mixture for 30 min at 30 °C in 0.1 M ammonium acetate solution (pH 6.4) [R_f = 0.24; RP-8, MeCN:H_2O (9:1)]. No further study was carried out with this labeled compound due to relatively low radiochemical yield.

Lipophilicity of Complexes. The log *P* (octanol/water partition coefficient) values of the eight radiolabeled compounds were determined, to investigate the effect lipophilicity has on the in vivo behavior. Average log *P* values of three measurements of three consecutive back-extractions are shown in Figure 3, along with the overall formal charges. The overall formal charge of the Cu(II) complexes were calculated on the basis of the number of acetate groups attached to the cyclen ring.[19]

Four complexes (**1**, **3**, **5**, **7**) in the first row of Figure 3 are all assigned to be positively charged (+2). As confirmed in the visible absorption study, in aqueous solution cyclen-based Cu(II) complexes without acetate groups have a square pyramidal coordination geometry with a water molecule in the apical position. Therefore, the overall charge of copper complexes is +2. Previously this overall charge assignment was also measured directly by electrophoresis in the case of [Cu(cyclen)(H_2O)]$^{2+}$.[19] In these four cases, the lipophilicity of the copper complexes are expected to be determined solely by the number of hydrophobic 4-*tert*-butylbenzyl groups rather than the charge, because all four complexes have the same positive overall charge. As the number of *tert*-butylbenzyl substituents is increased from two (trans-di) to three and to four, log *P* values decrease, which indicates that the lipophilicity of the complexes was decreased. The log *P* value of Cu−**5**, which has two substituents in cis nitrogen position of cyclen, is slightly lower than Cu−**7**, which has three substituents (2.62 vs 2.73). Complex Cu−**1**, which has four substituents, is most hydrophilic, while Cu−**3**, which has two substituents, is most hydrophobic among the four +2 compounds. This unexpected result could be explained by considering the charge distribution over the whole structure of Cu(II) complexes.[19,38] X-ray crystal structures of [Cu(**2c**)Cl]$^+$ and [Cu(**3**)Cl]$^+$ [33] and literature structures[32] showed that substituents on the ring nitrogens are directed away from the 12-membered ring and are all oriented on the same side of the plane containing the four nitrogens. The four ethylene groups of the cyclen ring are all oriented opposite the coordinated Cl ion, relative to the nitrogen plane. As the number of substituents on the cyclen ring increase, the dipole moment of this series of Cu(II) complexes seems to increase, making the compound more hydrophilic. Complexes Cu−**3** and Cu−**5** are structural isomers in which only the substituent position is different. However, the log *P* value of trans-substituted isomer Cu−**3** (3.19) is much higher than that of cis-substituted isomer Cu−**5** (2.62). Two pendant arms on one side of cyclen might make dipole moments of cis-substituted isomer bigger than that of the trans-isomer. The symmetry within complexes seems to play a great role in determining their lipophilicity.

In the case of the parent Cu−cyclen complex, octanol−water partition coefficients could not be determined because the ^{64}Cu-labled cyclen could not be back-extracted into the octanol layer.[19]

Overall charges of the other four complexes (**2**, **4**, **6**, **8**) shown in the second row of Figure 3 were assigned depending on the number of acetate groups present. Up to two acetate groups per cyclen ring can coordinate to the copper to give a five- or six-coordinated metal complex.[39,40] Charge assignment of Cu−cyclen complexes based on the number of acetate groups was experimentally confirmed using the electrophoresis

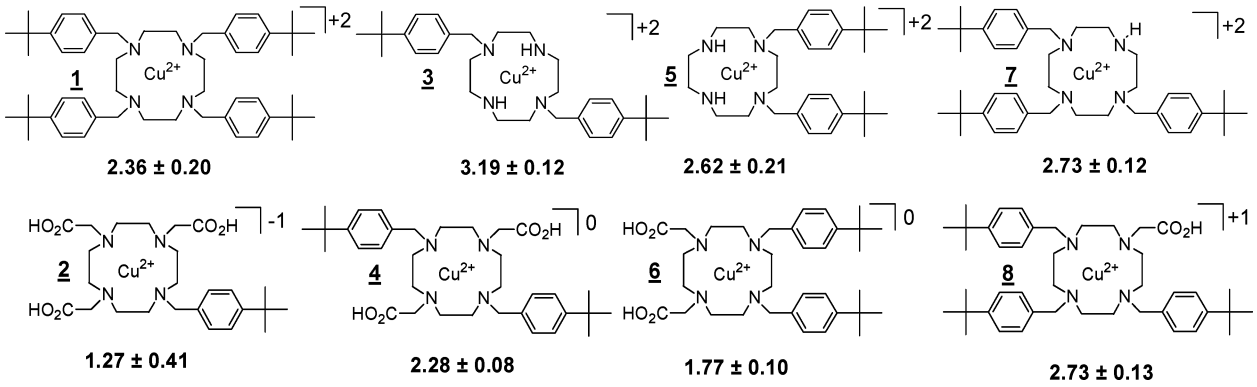


Figure 3. log *P* values and overall charges of ^{64}Cu-labeled complexes.

migration method.[19] Complex Cu−**8** has one acetate group and therefore was assigned a +1 charge. The log *P* value of Cu−**8** is exactly the same as that of its counterpart, Cu−**7**, which does not have an acetate group. However, as the overall charge of the complexes is changed from positive to neutral and negative, the lipophilicity is decreased significantly. Compounds Cu−**4** and Cu−**6**, which are assigned as neutral, have much lower log *P* values than their counterparts Cu−**3** and Cu−**5**, which do not have acetate groups. The log *P* values of Cu−**4** and Cu−**6** were decreased by 29% and 32%, respectively, from their counterparts, Cu−**3** and Cu−**5**. The same trend, in which the cis-isomer has a lower log *P* value than the trans-isomer, was observed with structural isomers Cu-**4** and Cu-**6**. Trans-substituted isomer Cu−**4** has a higher log *P* value than cis-substituted isomer Cu−**6**. Negatively charged complex Cu−**2** has the lowest log *P* value, 1.27. As the overall charge was changed from neutral to −1, the log *P* was decreased by a similar amount as from +1 to neutral. The influence of the overall charge of the copper complex on the log *P* value is clearly seen in these complexes. Hydrophilicity is increased and the log *P* value decreased as the number of acetate groups is increased up to three, changing the charge from +1 to neutral and −1. The addition of one acetate group on a cyclen nitrogen atom was found to have a marginal influence on the lipophilicity of copper(II) cyclen complexes. Symmetry within the complex also plays an important role in determining the lipophilicity of structural isomers.

Biodistribution Studies. To elucidate the relationship between structure and in vivo behavior, eight ^{64}Cu-labeled compounds were subjected to biodistribution studies. Labeled compounds were injected into female Sprague−Dawley rats, and blood, liver, kidney, muscle and heart were harvested at 15 min and 4 and 24 h postinjection. Biodistribution results are summarized in Table 3. All eight compounds show different uptake and clearance patterns; a comparison of blood, liver, and kidney clearance patterns for ^{64}Cu-labeled **1**−**8** is shown in Figure 4.

From the blood clearance comparison, a dependence on the overall charge is clearly seen. All four +2 complexes, Cu−**1**, Cu−**3**, Cu−**5**, and Cu−**7**, show much higher blood uptake than other four compounds at early time points (15 min). However, the activities cleared dramatically by 4 h to render comparable amounts of activity to those of the other four compounds. In the cases of Cu−**3**, Cu−**5**, and Cu−**7**, blood activities at 24 h are slightly higher compared to those at 4 h. This may be due to metabolic degradation of the ^{64}Cu-labeled complexes[19,41] with subsequent incorporation of the ^{64}Cu into copper-containing proteins such as superoxide dismutase or cerruloplasmin. Blood levels of the other four compounds, which have at least one acetate group, were cleared within the first 15 min, which implies that the complexes do not dissociate in the blood.[19,42,43] But blood levels did not lower much further out to 24 h. Even though the differences in blood levels of Cu−**2**, Cu−**4**, Cu−**6**, and Cu−**8** are very small, neutral complex Cu−**4**

Table 3. Biodistribution[a] of ^{64}Cu-Labeled **1**−**8** in Mature Female Sprague−Dawley Rats

organ	Cu−**1**	Cu−**2**	Cu−**3**	Cu−**4**
		15 min		
blood	3.92 ± 0.611	0.37 ± 0.043	2.75 ± 0.290	0.16 ± 0.002
liver	6.60 ± 1.061	2.28 ± 0.226	4.73 ± 0.834	1.26 ± 0.001
kidney	1.77 ± 0.120	2.64 ± 0.307	1.55 ± 0.155	3.66 ± 0.389
muscle	0.11 ± 0.035	0.12 ± 0.011	0.08 ± 0.009	0.06 ± 0.003
heart	0.64 ± 0.062	0.15 ± 0.021	0.55 ± 0.050	0.18 ± 0.008
		4 h		
blood	0.96 ± 0.089	0.21 ± 0.032	0.10 ± 0.006	0.15 ± 0.012
liver	4.93 ± 0.620	0.91 ± 0.037	6.96 ± 0.370	0.69 ± 0.007
kidney	5.31 ± 0.629	3.42 ± 0.390	2.98 ± 0.386	2.17 ± 0.644
muscle	0.14 ± 0.022	0.04 ± 0.004	0.03 ± 0.003	0.04 ± 0.009
heart	0.49 ± 0.058	0.13 ± 0.017	0.11 ± 0.026	0.15 ± 0.009
		24 h		
blood	0.55 ± 0.048	0.19 ± 0.021	0.18 ± 0.006	0.12 ± 0.008
liver	2.66 ± 0.487	0.80 ± 0.046	5.82 ± 0.425	0.43 ± 0.055
kidney	2.62 ± 0.681	1.63 ± 0.326	2.60 ± 0.142	0.79 ± 0.194
muscle	0.10 ± 0.008	0.03 ± 0.002	0.05 ± 0.008	0.03 ± 0.001
heart	0.44 ± 0.022	0.16 ± 0.005	0.16 ± 0.012	0.16 ± 0.013
organ	Cu−**5**	Cu−**6**	Cu−**7**	Cu−**8**
		15 min		
blood	2.34 ± 0.345	0.54 ± 0.028	6.56 ± 0.621	0.49 ± 0.060
liver	5.89 ± 1.246	4.43 ± 0.731	6.58 ± 0.931	5.19 ± 0.540
kidney	3.25 ± 0.382	4.55 ± 0.423	1.54 ± 0.118	1.82 ± 0.142
muscle	0.16 ± 0.025	0.15 ± 0.002	0.08 ± 0.015	0.08 ± 0.007
heart	1.70 ± 0.267	0.30 ± 0.007	1.15 ± 0.074	0.32 ± 0.035
		4 h		
blood	0.22 ± 0.040	0.51 ± 0.051	0.64 ± 0.148	0.38 ± 0.024
liver	7.16 ± 1.323	2.15 ± 0.113	5.65 ± 0.798	2.80 ± 0.483
kidney	5.92 ± 1.377	6.11 ± 1.126	9.59 ± 0.809	3.93 ± 0.449
muscle	0.09 ± 0.013	0.09 ± 0.006	0.11 ± 0.004	0.07 ± 0.003
heart	0.30 ± 0.037	0.34 ± 0.037	0.44 ± 0.054	0.27 ± 0.008
		24 h		
blood	0.31 ± 0.051	0.42 ± 0.032	0.65 ± 0.114	0.33 ± 0.063
liver	3.64 ± 0.371	1.80 ± 0.208	3.24 ± 0.538	1.83 ± 0.451
kidney	3.27 ± 1.026	2.91 ± 0.424	3.92 ± 1.339	2.09 ± 0.628
muscle	0.09 ± 0.007	0.09 ± 0.004	0.13 ± 0.006	0.07 ± 0.008
heart	0.36 ± 0.031	0.42 ± 0.039	0.57 ± 0.052	0.31 ± 0.039

[a] Data are expressed as the %ID/g ± SD with four animals per data point.

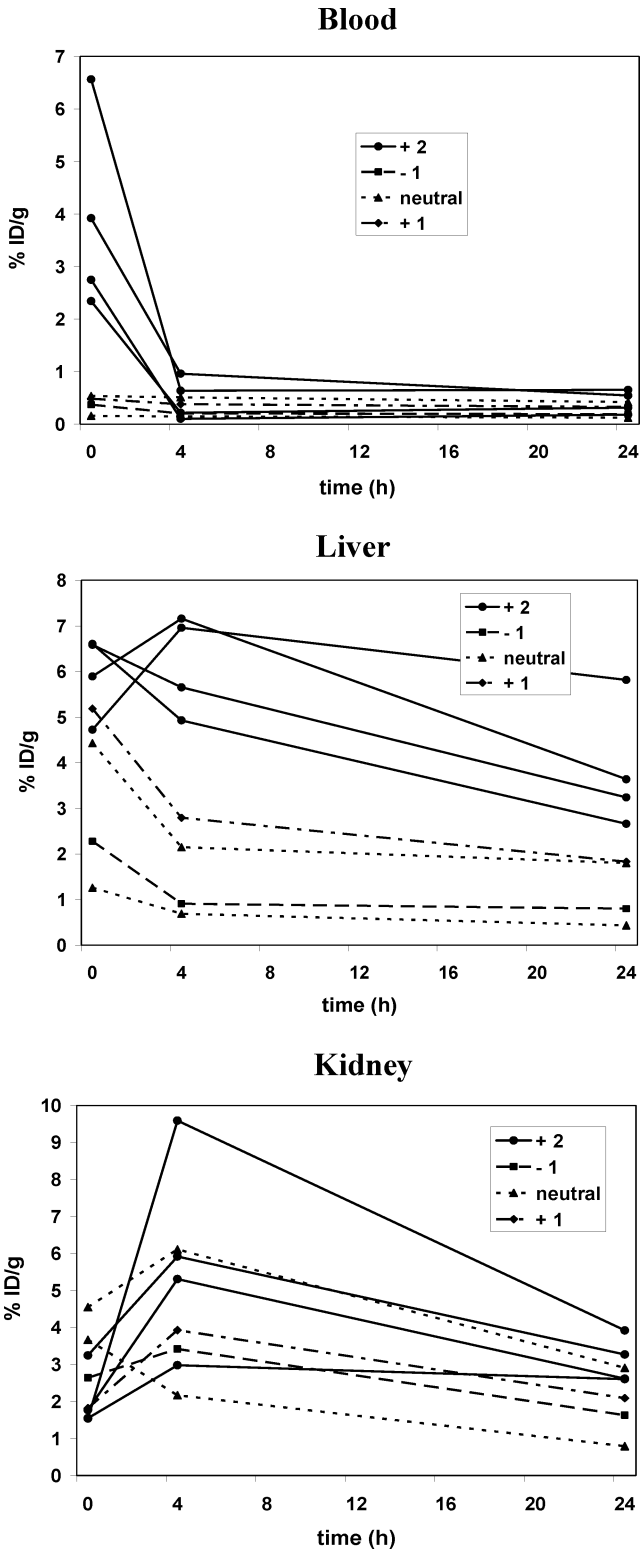


Figure 4. Comparison of blood, liver, and kidney clearance for ^{64}Cu-labeled complexes.

and −1 charged Cu−**2** showed the best blood clearance. Trans-substituted isomers Cu−**3** and Cu−**4** showed better blood clearance than their cis-substituted isomers Cu−**5** and Cu−**6**, respectively.

Comparison of liver clearance of ^{64}Cu-labeled **1**−**8** also clearly showed a charge dependence for the copper complexes. All four +2 charged compounds Cu−**1**, Cu−**3**, Cu−**5**, and Cu-**7** showed high liver uptakes at early time points and very slow clearance by 24 h. Among those four +2 charged complexes, disubstituted cyclen complexes Cu−**3** and Cu−**5** continued to accumulate in liver over time and showed the highest uptake by 4 h postinjection. However, the most lipophilic complex Cu−**3** showed severe retardation of liver clearance at 24 h. The liver activity of Cu−**3** decreased by 16% between 4 and 24 h, while Cu−**5** decreased by 49% over the same time span. In contrast, Cu−**1** and Cu−**7** cleared out from liver continuously by 24 h with similar clearance rates. Cu−**1**, which has the lowest log *P* value among these four complexes, showed the best liver clearance at 4 and 24 h time points. High liver accumulation at early time points followed by slow clearance of +2 charged azamacrocyclic complexes were consistent with other literature reports.[19,41,44]

Negatively charged complex Cu−**2** and trans-substituted neutral complex Cu−**4** showed very fast clearance through liver by 15 min. Liver uptakes of both compounds are less than 0.91% injected dose (ID)/g at 4 h. Cu−**4** had the fastest liver clearance at all time points. Cis-substituted neutral complex Cu−**6** and +1 charged Cu−**8** cleared rapidly from the blood with an initial high liver uptake followed by rapid washout. From 46 to 51% of the 15 min dose cleared from liver in 4 h, but further washout was retarded to give 1.8% ID/g for both compounds at 24 h postinjection. In comparison, trans-substituted isomer Cu−**4** showed much faster liver clearance than cis-substituted isomer Cu−**6** at the same time points, as seen with the blood clearance.

Kidney uptake and clearance of cyclen-based copper complexes showed a more complex pattern than found in the blood and liver, but they did not show a clear charge dependence. All compounds except Cu−**4** slowly accumulated in kidney over time and showed highest uptake at 4 h and then slowly cleared out by 24 h. In particular, +2 charged ^{64}Cu-labeled **1**, **3**, and **7** showed very low initial kidney uptake at 15 min (≤4% ID/g) with continuous accumulation of activity in kidney achieving a maximum at 4 h. Cu−**7** showed a 6-fold increase in the kidney level by 4 h compared to 15 min dose, consistent with the observed decrease in the blood level. Cu−**3** that has highest log *P* value showed only 13% further clearance of 4 h dose in 24 h.

Cis-substituted neutral complex Cu−**6** and +1 charged Cu−**8** complexes also showed slow initial uptake with continuous accumulation of dose up to 4 h followed by a slow clearance out to 24 h. As in the blood and liver, negatively charged Cu−**2** and especially trans-substituted neutral complex Cu−**4** showed faster kidney clearance than the other six compounds. Despite a preference for hepatic clearance, neutral compound Cu−**4** showed good kidney clearance without retardation over time to end up with 0.79% ID/g at 24 h postinjection.

Muscle and heart uptake for all complexes were minimal and no significant uptake in the myocardium was found for any of the radiometal complexes. Only Cu−**4** and Cu−**5** showed a heart/blood ratio >1 at 4 and 24 h, but their percent ID/g values were less than 0.3. Other organs, such as spleen, lung, fat, bone, and brain, were harvested in some cases to see whether any tissue specificity existed. No significant uptake was found outside of the clearance organs. The brain/blood ratios of complexes Cu−**2** and Cu−**6** were less than 0.2 at all

Table 4. Biodistribution[a] of ^{64}Cu-Labeled Cyclen, DO2A, and DOTA in Mature Female Sprague−Dawley Rats[19]

	15 min	2 h	24 h
		Cyclen	
blood	0.58 ± 0.065	0.17 ± 0.038	0.14 ± 0.012
liver	1.24 ± 0.166	0.85 ± 0.274	0.65 ± 0.020
kidney	7.69 ± 1.058	5.91 ± 1.748	1.62 ± 0.550
		DO2A	
blood	0.51 ± 0.062	0.04 ± 0.008	0.04 ± 0.008
liver	0.24 ± 0.028	0.15 ± 0.021	0.11 ± 0.017
kidney	2.23 ± 0.182	0.67 ± 0.050	0.24 ± 0.076
		DOTA	
blood	0.52 ± 0.110	0.06 ± 0.020	0.04 ± 0.015
liver	0.24 ± 0.079	0.15 ± 0.013	0.15 ± 0.025
kidney	1.77 ± 0.272	0.78 ± 0.050	0.36 ± 0.054

[a] Data are expressed as the %ID/g ± SD with four or five animals per data point.

time points, which indicates that these compounds did not cross the blood−brain barrier.

The effect on in vivo behavior by attaching hydrophobic pendants on *N* atoms of cyclen ring is clearly seen by comparison with cyclen-based copper complexes that have only acetate group as substituents. Jones-Wilson et al. reported the in vivo behavior of ^{64}Cu-labeled azamacrocyclic complexes.[19] Biodistribution data of Cu(II) complexes of cyclen, DO2A, and DOTA are re-expressed as percentage of injected dose per gram in Table 4 for direct comparison with current data. All eight compounds reported here have much higher log *P* values than those of Cu(II) complexes of cyclen, DO2A, and DOTA. The radiolabeled cyclen, DO2A, and DOTA even could not be back-extracted into the octanol layer from the aqueous layer.[19]

The copper complex of cyclen showed fast kidney uptake at early time points with rapid clearance through the kidneys. 79% of kidney dose at 15 min cleared in 24 h. The percentage of injected dose per gram in kidney is 6 times as high as that of the liver at 15 min. In contrast, Cu complexes of **1**, **3**, **5**, and **7**, which have at least two lipophilic substituents showed much higher liver uptake (%ID/g) compared to kidney at early time points. The values of 4.7−6.6% ID/g for liver were 1.8−4.3 times higher than those of kidney at 15 min. Furthermore, kidney uptake was increased over time up to 4 h, with more activity in kidney found at 24 h rather than 15 min for copper complexes of **1**, **3**, **5**, and **7**. Blood clearance is also changed dramatically by attaching lipophilic substituents on cyclen *N* atoms. Cu−cyclen complex cleared out from blood as fast as Cu−DO2A and Cu−DOTA. However, all four +2 charged complexes, which have 4-*tert*-butylbenzyl groups as substituents, showed high blood levels at 15 min (2.3−6.6% ID/g) with fast clearance by 4 h.

DO2A and DOTA complexes with Cu(II) showed faster clearance and lower retention from all organs at all time points compared to Cu−cyclen. DO2A analogues Cu−**4** and Cu−**6** cleared much faster from blood circulation and liver at early time points and showed much lower retention in liver at all times than copper complexes of **1**, **3**, **5**, and **7**. Complexes Cu−**4** and Cu−**6** showed much higher retention in liver and kidney than Cu−DO2A at all times points. Five and 18 times higher activities of Cu−**4** and Cu−**6**, respectively, were found in liver compared to that of Cu−DO2A at 15 min. Less than 25% of the dose of blood at 15 min cleared by 24 h in the cases of Cu−**4** and Cu−**6**, which is much smaller than 92% of Cu−DO2A.

Conclusion

In summary, a total of nine cyclen derivatives were synthesized successfully using the same synthetic strategy. Regioselective alkylation to afford mono, cis- and trans-di-, tri-, and tetra-substituted cyclen compounds were achieved through thoughtful choice of protecting group. A second alkylation with acetate groups gave all possible isomers with two different substituents such as DO1A, cis-, and trans-DO2A, and DO3A derivatives in reasonable yield. X-ray structure of copper(II) complex with mono-substituted cyclen **2c**, $[Cu(\mathbf{2c})Cl]^+$, showed that chloride ion from the reaction mixture occupied the remaining fifth position to give a square pyramidal coordination environment with four nitrogen atoms and chloride ion in the apical position.

Eight out of nine cyclen ligands were labeled with ^{64}Cu in high radiochemical yield. To give best labeling yield, labeling conditions were optimized. Partition coefficients (log *P*) as measured by octanol/ammonium acetate buffer were determined to range from 1.27 to 3.19. Lipophilicity of Cu(II) complexes was increased by attaching hydrophobic substituents on cyclen *N* atoms. However, as the number of substituents was increased, lipophilicity was decreased instead. Charge distribution and symmetry within the complex play important roles in determining log *P* in a series of complex with the same charge. Trans-alkylated isomers showed much higher log *P* value than their cis-alkylated counterparts. As the number of acetate groups was increased, the overall charge was changed from positive to neutral and negative, and lipophilicity was decreased. However, the change from +2 charged to +1 charged complex by adding one acetate group is insignificant.

The biodistribution of Cu−cyclen derivatives was significantly altered by the number of lipophilic substituents and overall charge of complexes. All eight hydrophobic compounds were excreted mostly through the liver rather than kidneys. Plus-two-charged complexes showed high blood retention at early time points with sluggish clearance from liver by 24 h. The attachment of even one acetate group on cyclen *N* atoms accelerated blood and liver clearance dramatically compared to +2 charged Cu(II) complexes. Kidney accumulation was continued up to 4 h for most complexes except Cu−**4** and slowly cleared by 24 h. Neutral trans-substituted DO2A derivative showed best clearance and lowest retention of doses from all organs most time, followed by negatively charged complex. Trans-substituted complexes showed better clearance and lower retention from all organs than their cis-counterparts. The attachment of a substituent except acetate group on cyclen rendered higher retention of activity in organs at 24 h compared to those of Cu−cyclen and Cu−DO2A.

Experimental Section

General Methods and Materials. 1,4,7,10-Tetraazacyclododecane (cyclen) was purchased from Strem Chemicals (Newburyport, MA), and all other solvents and reagents were obtained from Aldrich and used as received without any further purification. Water was distilled and then deionized (18 MΩ/cm^2) by passing through a Milli-Q water filtration system (Millipore Corp., Bedford, MA). ^{1}H and ^{13}C NMR

spectra were measured using a Varian Gemini 300 instrument, and chemical shifts are reported in ppm on the δ scale relative to TMS or solvent peak. Proton chemical shifts are annotated as follows: ppm (multiplicity, coupling constant (Hz), integral). Elemental microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Mass spectra were obtained from Washington University Mass Spectrometry Resource. A Perkin-Elmer Lambda 25 UV/Vis spectrometer was used to record absorption spectra.

Copper-64 was prepared on the Washington University School of Medicine Cyclotron CS-15 cyclotron by the ^{64}Ni(p,n)^{64}Cu nuclear reaction at a specific activity range of 50−200 mCi/μg as previously described.[17] EM Science silica gel 60 F_{254} and Whatman C18 silica gel TLC plates were purchased from Fisher Scientific (Pittsburgh, PA), and EM Science C8 TLC plates RP-8 were purchased from Alltech (Deerfield, IL). Radio-TLC was accomplished using a Bioscan 200 imaging scanner (Bioscan, Inc., Washington, DC). Radioactivity was counted with a Beckman Gamma 8000 counter containing a NaI crystal (Beckman Instruments, Inc., Irvine, CA)

Ligand Synthesis. 1,4,7-Triformyl-1,4,7,10-tetraazacyclododecane[22] (**2a**), 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane[24] (**3a**), and 1,4,7,10-tetraazabicyclo[8.2.2]tetradecane-11,12-dione)[25] (cyclenoxamide, **5a**) were prepared by literature methods.

1,4,7,10-Tetra(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (1). To a solution of 300 mg of cyclen (1.74 mmol) in 30 mL of acetonitrile were added 3033 μL of *N,N*-diisopropylethylamine (17.41 mmol) and 1408 μL of 4-*tert*-butylbenzyl bromide (7.66 mmol) consecutively. The reaction mixture was warmed to 60 °C and allowed to stir for 24 h. The precipitated white solid was filtered, washed with 10 mL of acetonitrile, and dried in air (1091 mg, 83%): ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 36H), 2.70 (s, 16H), 3.43 (s, 8H), 7.25 (d, J = 8.4 Hz, 8H), 7.37 (d, J = 8.4 Hz, 8H); ^{13}C NMR ($CDCl_3$) δ 31.6 (C*C*H$_3$), 34.5 (*C*CH$_3$), 53.5 (cyclen ring *C*H$_2$), 59.7 (*C*H$_2$Ph), 125.2, 128.6, 137.4, 149.4. Anal. Calcd for $C_{52}H_{76}N_4$: C, 82.48; H, 10.12; N, 7.40. Found: C, 82.51; H, 10.19; N, 7.56.

1,4,7-Triformyl-10-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (2b). To a solution of 1,4,7-triformyl-1,4,7,10-tetraazacyclododecane (2624 mg, 10.24 mmol) in 80 mL of acetonitrile were added *N,N*-diisopropylethylamine (3567 μL, 20.48 mmol) and 4-*tert*-butylbenzyl bromide (1881 μL, 10.24 mmol). The reaction was stirred at 60 °C for 2 days. After evaporation of solvent, the residue was dissolved in 30 mL of water, extracted by methylene chloride (3 × 20 mL), dried by $MgSO_4$, and concentrated to give a yellow oil. Crude product was recrystallized from diethyl ether to give off-white powder (3157 mg, 77%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (s, 9H), 2.66−2.80 (m, 4H), 3.28−3.73 (m, 12H), 3.78 (s, 2H), 7.11 (dd, J = 11.0, 11.1 Hz, 2H), 7.33 (dd, J = 11.0, 11.1 Hz, 2H), 7.88−8.18 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.4 (C*C*H$_3$), 34.6 (*C*CH$_3$), 42.2, 43.3, 43.7, 44.1, 44.6, 45.4, 46.2, 46.8, 46.9, 48.1, 49.0, 50.3, 52.1, 54.2, 54.4, 54.8, 55.3, 56.5, 56.7, 57.4, 125.4 & 125.7 (Ph ring *C*H), 130.1 & 130.3 (Ph ring *C*H), 132.0 & 132.3 (Ph ring *C*), 151.0 & 151.3 (Ph ring *C*), 163.3, 163.4, 163.5, 163.6 & 163.8 (N*C*HO); HRMS (FAB) *m/z* 409.2789 ([M + Li]$^+$, $C_{22}H_{34}N_4O_3Li$, calcd 409.2791). Anal. Calcd for $C_{22}H_{34}N_4O_3 \cdot H_2O$: C, 62.83; H, 8.63; N, 13.32. Found: C, 63.79; H, 8.92; N, 12.50.

1-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane·4HCl (2c·4HCl). 1,4,7-Triformyl-10-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (3039 mg, 7.55 mmol) was dissolved in 30 mL of 2 M HCl and the solution was heated to 60 °C and allowed to stir for 18 h. Solvent was evaporated under reduced pressure at 50 °C to give an off-white solid. Ethanol (50 mL) was added and the solution was agitated vigorously for 4 h. White power was collected, washed by ethanol and ether, and dried in air to give pure product as the HCl salt (2217 mg, 63%): ^{1}H NMR (D_2O) δ 1.27 (s, 9H), 2.90 (t, J = 5.1 Hz, 4H), 2.98 (br s, 4H), 3.11 (t, J = 5.1 Hz, 4H), 3.18 (br s, 4H), 3.83 (s, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H); ^{13}C NMR (D_2O) δ 33.5 (C*C*H$_3$), 37.1 (*C*CH$_3$), 45.2, 45.4, 47.0, 51.5, 59.9 (*C*H$_2$Ph), 129.1, 133.3, 133.5, 155.6; HRMS (FAB) *m/z* 319.2868 ([M + H]$^+$, $C_{19}H_{35}N_4$, calcd 319.2862). Anal. Calcd for $C_{19}H_{34}N_4 \cdot 4HCl$: C, 49.15; H, 8.25; N, 12.07. Found: C, 48.11; H, 8.43; N, 11.33.

1,4,7-Tris(*tert*-butoxycarbonylmethyl)-10-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (2d). To a solution of 1-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane·4HCl (499 mg, 1.07 mmol) in 30 mL of acetonitrile were added *N,N*-diisopropylethylamine (1497 μL, 8.60 mmol) and *tert*-butyl bromoacetate (634 μL, 4.27 mmol). The reaction mixture was slowly heated to 60 °C and allowed to stir for 23 h. After evaporation of solvents in vacuo, the residue was dissolved in 30 mL of ethyl acetate and washed by H_2O and brine and then dried by $MgSO_4$. Crude product was washed through an alumina column using methylene chloride and then eluted using MeOH:CH_2Cl_2 (1:20) to give a pale oil (579 mg, 74%): ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 9H), 1.23 (s, 18H), 1.25 (s, 9H), 2.63 (br s, 6H), 2.87−2.94 (br m, 8H), 3.23 (s, 2H), 3.40 (br s, 4H), 3.47 (br s, 2H), 4.47 (s, 2H), 7.23 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 27.8 (OC*C*H$_3$), 27.9 (O*C*CH$_3$), 30.9 (PhC*C*H$_3$), 34.4 (Ph*C*CH$_3$), 47.2, 50.4, 50.9, 52.1, 53.4 (N*C*H$_2$Ph), 55.3 (*C*H$_2$CO$_2$), 55.5 (*C*H$_2$CO$_2$), 55.7 (*C*H$_2$CO$_2$), 81.3 (O*C*CH$_3$), 81.5 (O*C*CH$_3$), 81.9 (O*C*CH$_3$), 125.7 (Ph ring *C*H), 127.2 ((Ph ring *C*), 131.0 (Ph ring *C*H), 152.6 (Ph ring *C*), 168.9 (*C*O$_2$), 169.4 (*C*O$_2$), 169.9 (*C*O$_2$).

1,4,7-Tris(carboxymethyl)-10-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (2·4HCl). HCl solution (6 M, 25 mL) was added to 187 mg (0.28 mmol) of 1,4,7-tri(*tert*-butoxycarbonylmethyl)-10-(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane. The flask was slowly heated to 110 °C and allowed to stir for 10 h. After evaporation of all solvents, the crude product was recrystallized from ethanol/diethyl ether to give solid **2** as the salt (4HCl) (145 mg, 80%): ^{1}H NMR (D_2O, 300 MHz) δ 1.28 (s, 9H), 3.05−3.16 (br m, 10H), 3.41 (br m, 6H), 3.49 (br s, 4H), 4.06 (br s, 2H), 4.46 (br s, 2H), 7.47 (d, J = 8.1 Hz, 2H), 7.54 (br d, J = 8.1 Hz, 2H); ^{13}C NMR (D_2O, 75 MHz) δ 33.3 (C*C*H$_3$), 37.1 (*C*CH$_3$), 51.0 (br), 51.2 (br), 52.6 (b) 54.4 (br), 56.1 (*C*H$_2$CO$_2$), 58.0 (*C*H$_2$CO$_2$), 60.4 (*C*H$_2$Ph), 129.7 (Ph ring *C*H), 133.5 (Ph ring *C*H), 133.8 & 133.9 (Ph ring *C*), 157.1 (v br, Ph ring *C*), 176.1 (v br, CO$_2$).

1,7-Bis(benzyloxycarbonyl)-4,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (3b). To a mixture of 235 mg of 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (0.53 mmol) and 929 μL of *N,N*-diisopropylethylamine (5.33 mmol) in 40 mL of acetonitrile was added 216 μL of 4-*tert*-butylbenzyl bromide (1.17 mmol). The resulting mixture was warmed slowly to 60 °C and allowed to stir overnight. The solution was concentrated in vacuo to give clear liquid. Crude product was extracted by 3 × 20 mL of methylene chloride, washed by 20 mL of brine, and dried by magnesium sulfate, and the solvent was removed under reduced pressure. The residue was purified via column chromatography on alumina, eluting with ethyl acetate/hexane (1:3) to afford a clear oil (343 mg, 88%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (s, 18H), 2.64 (br d, J = 21 Hz, 8H), 3.31−3.47 (br m, 8H), 3.55 (br s, 4H), 4.89 (br s, 4H), 7.18 (br m, 10H), 7.27 (m, 8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.3 (C*C*H$_3$), 34.3 (*C*CH$_3$), 45.0, 46.6, 47.6, 54.4 (N*C*H$_2$Ph), 59.2, 59.9, 60.2, 66.8 (O*C*H$_2$Ph), 125.1, 127.8, 128.0, 128.9, 135.8, 136.8, 149.7, 156.4 (N*C*O$_2$). Anal. Calcd for $C_{46}H_{60}N_4O_4$: C, 75.37; H, 8.25; N, 7.64. Found: C, 75.18; H, 8.53; N, 7.36.

1,7-Bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (3). To a solution of 1,7-bis(benzyloxycarbonyl)-4,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (976 mg, 1.33 mmol) in 50 mL of ethanol was added 10% Pd on activated carbon (523 mg). The reaction was stirred under hydrogen gas at room temperature for 10 h. Pd catalyst was filtered off through a Celite pad and the filtrate was concentrated under reduced pressure at 40 °C to give clear oil (529 mg, 96%): ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 18H), 2.70 (s, 16H), 3.67 (s, 4H), 7.31 (d, J = 8.3 Hz, 4H), 7.41 (d, J = 8.3 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 31.4 (C*C*H$_3$), 34.5 (*C*CH$_3$), 46.0, 51.9, 60.1 (*C*H$_2$Ph), 125.5, 128.8, 136.2, 150.3; HRMS (FAB) *m/z* 465.3955

([M + H]$^+$, $C_{30}H_{49}N_4$, calcd 465.3957). Anal. Calcd for $C_{30}H_{48}N_4 \cdot 2H_2O$: C, 71.95; H, 10.47; N, 11.19. Found: C, 70.51; H, 10.44; N, 11.56.

1,7-Bis(*tert*-butoxycarbonylmethyl)-4,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (4a). To a solution of 1,7-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (194 mg, 0.42 mmol) in 50 mL of acetonitrile were added *N,N*-diisopropylethylamine (727 μL, 4.17 mmol) and *tert*-butyl bromoacetate (154 μL, 1.04 mmol). The reaction mixture was slowly heated to 60 °C and allowed to stir for 18 h. After evaporation of solvents in vacuo, the residue was dissolved in 20 mL of Na_2CO_3 solution, extracted by methylene chloride (3 × 20 mL), washed by brine, dried over $MgSO_4$, and concentrated to give a clear oil. Crude product was filtered through an alumina column using methylene chloride and then eluted using ethyl acetate to give pure product as a clear oil (168 mg, 58%): ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 18H), 1.40 (s, 18H), 2.63 (m, 8H), 2.87 (m, 8H), 3.14 (s, 4H), 3.55 (s, 4H), 7.36 (s, 4H), 7.37 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 28.3 (OC*C*H$_3$), 31.6 (PhC*C*H$_3$), 34.6 (Ph*C*CH$_3$), 52.5, 52.8, 56.5 (*C*H$_2$Ph), 59.8 (*C*H$_2$CO$_2$), 80.7 (O*C*CH$_3$), 125.2, 128.9, 137.4, 149.8, 171.7 (CH$_2$*C*O$_2$). Anal. Calcd for $C_{42}H_{68}N_4O_4$: C, 72.79; H, 9.89; N, 8.08. Found: C, 71.08; H, 9.80; N, 7.97.

1,7-Bis(carboxymethyl)-4,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane·4HCl (4·4HCl). HCl solution (6 M, 35 mL) was added to 168 mg (0.24 mmol) of 1,7-bis(*tert*-butoxycarbonylmethyl)-4,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane. The flask was slowly heated to 110 °C and allowed to stir for 18 h. After evaporation of all solvents, the crude product was recrystallized from diethyl ether to give white crystalline solid **4** as a salt (4HCl) (125 mg, 71%):^{1}H NMR (D_2O, 300 MHz) δ 1.28 (s, 18H), 2.82 (s, 4H), 2.91 (br d, J = 15.6 Hz, 4H), 3.08 (br d, J = 15.6 Hz, 4H), 3.42 (br s, 8H), 4.45 (s, 4H), 7.51 (d, J = 8.0 Hz, 4H), 7.58 (d, J = 8.0 Hz, 4H); ^{13}C NMR (D_2O, 75 MHz) δ 30.0 (C*C*H$_3$), 33.9 (*C*CH$_3$), 47.9, 49.9, 53.2 (*C*H$_2$CO$_2$), 57.8 (*C*H$_2$Ph), 125.4, 126.6, 130.8, 154.4, 173.9 (CH$_2$*C*O$_2$); HRMS (FAB) *m/z* 581.4069 ([M + H]$^+$, $C_{34}H_{53}N_4O_4$, calcd 581.4067). Anal. Calcd for $C_{34}H_{52}N_4O_4 \cdot 4HCl$: C, 56.20; H, 7.77; N, 7.71. Found: C, 56.45; H, 8.32; N, 7.91.

4,7-Bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazabicyclo[8.2.2]-tetradecane-11,12-dione (5b). To a solution of 463 mg of 1,4,7,10-tetraazabicyclo[8.2.2]tetradecane-11,12-dione (cyclenoxamide) (2.04 mmol) in 30 mL of acetonitrile were added 3553 μL of *N,N*-diisopropylethylamine (20.4 mmol) and 825 μL of 4-*tert*-butylbenzyl bromide (4.49 mmol). The flask was stirred at 60 °C overnight. All solvent was evaporated in vacuo. The residue was dissolved again in 30 mL of water, extracted by 3 × 20 mL of methylene chloride, washed by 10 mL of brine, dried by $MgSO_4$, and concentrated to give pale yellow foam, which was recrystallized in diethyl ether to give white crystalline solid (807 mg, 76%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (s, 18H), 2.25 (m, 2H), 2.42 (br d, J = 14.4 Hz, 2H), 2.59 (m, 4H), 2.89 (m, 2H), 3.38 (m, 4H), 3.53 (m, 2H), 4.12 (br d, J = 13.8 Hz, 2H), 4.32 (br s, 2H), 7.07 (d, J = 7.4 Hz, 4H), 7.30 (d, J = 7.4 Hz, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.4 (C*C*H$_3$), 34.5 (*C*CH$_3$), 47.6, 49.7, 52.5, 55.7, 58.6 (*C*H$_2$Ph), 125.3, 129.5, 135.6, 150.3, 160.0 (N*C*O). Anal. Calcd for $C_{32}H_{46}N_4O_2$: C, 74.09; H, 8.94; N, 10.80. Found C, 74.42; H, 9.15; N, 10.79.

1,4-Bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (5). A slurry of 113 mg of 4,7-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazabicyclo[8.2.2]tetradecane-11,12-dione (0.22 mmol) in a mixed solvent of 15 mL of 10 M NaOH and 10 mL of ethanol was refluxed for 18 h. After removing the solvent, 50 mL of water was added and the mixture was extracted by 4 × 20 mL of methylene chloride. The organic layers were dried over $MgSO_4$ and concentrated over a vacuum. The crude product was purified by filtration on alumina (ethyl acetate, methanol) to give a clear oil (91 mg, 90%): ^{1}H NMR ($CDCl_3$) δ 1.28 (s, 18H), 2.66 (s, 4H), 2.81 (m, 4H), 2.88 (m, 4H), 2.99 (s, 4H), 3.62 (s, 4H), 7.09 (d, J = 8.1 Hz, 4H), 7.31 (d, J = 8.1 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 31.4 (C*C*H$_3$), 34.5 (*C*CH$_3$), 46.6, 46.9, 50.5, 50.8, 55.9 (*C*H$_2$Ph), 125.5, 129.7, 134.1, 150.7. Anal. Calcd for $C_{30}H_{48}N_4 \cdot 4H_2O$: C, 67.13; H, 10.52; N, 10.44. Found: C, 65.27; H, 9.34; N, 9.69.

1,4-Bis(*tert*-butoxycarbonylmethyl)-7,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (6a). To a solution of 287 mg of 1,4-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (0.62 mmol) in 30 mL of acetonitrile were added 645 μL of *N,N*-diisopropylethylamine (3.70 mmol) and 201 μL of *tert*-butyl bromoacetate (1.36 mmol). The reaction mixture was slowly heated to 60 °C with stirring for 24 h. After evaporation of solvent in vacuo, 30 mL of water was added. The reaction mixture was extracted with methylene chloride (4 × 20 mL). The organics were dried over $MgSO_4$, concentrated, and then purified by column chromatography on alumina using ethyl acetate/ethanol (10:1) as eluent to give pale yellow oil (207 mg, 48%): ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 18H), 1.45 (s, 18H), 2.63 (br s, 8H), 2.91 (br s, 8H), 3.26 (s, 4H), 3.46 (s, 4H), 7.28 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 28.4 (OC*C*H$_3$), 31.6 (PhC*C*H$_3$), 34.5 (Ph*C*CH$_3$), 52.3 (2C), 52.6, 52.7, 56.6 (*C*H$_2$CO$_2$), 59.8 (*C*H$_2$Ph), 80.6 (O*C*CH$_3$), 125.0, 128.8, 137.0, 149.5, 171.4 (CH$_2$*C*O$_2$).

1,4-Bis(carboxymethyl)-7,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane·4HCl (6·4HCl). HCl solution (6 M, 30 mL) was added to 1,4-bis(*tert*-butoxycarbonylmethyl)-7,10-bis(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (207 mg, 0.30 mmol). The flask was refluxed for 12 h. All solvent was evaporated under reduced pressure and crude product was recrystallized from diethyl ether to afford white crystalline solid **6** as a salt (4HCl) (179 mg, 83%): ^{1}H NMR (CD_3OD, 300 MHz) δ 1.22 (s, 18H), 3.30−3.50 (br m, 20H), 3.95 (br s, 4H), 7.39 (s, 8H); ^{13}C NMR (CD_3OD, 75 MHz) δ 31.7 (C*C*H$_3$), 35.6 (*C*CH$_3$), 50.5 (br, cyclen ring *C*H$_2$), 51.4 (br, cyclen ring *C*H$_2$), 51.7 (br, cyclen ring *C*H$_2$), 54.9 (*C*H$_2$CO$_2$), 58.5 (*C*H$_2$Ph), 127.4 (Ph ring *C*), 127.5 (Ph ring *C*H), 131.9 (Ph ring *C*H), 153.8 (Ph ring *C*); HRMS (FAB) *m/z* 581.4074 ([M + H]$^+$, $C_{34}H_{53}N_4O_4$, calcd 581.4067).

1,4,7-Triformyl-10-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (7a). A mixture of cyclen (800 mg, 4.64 mmol) and chloral hydrate (3047 mg, 18.42 mmol) was dissolved in 30 mL of ethanol. The flask was stirred at 60 °C for 4 h. The reaction mixture was concentrated under vacuum to dryness and dissolved in 30 mL of water again (pH 9, measured using pH paper). Benzyl chloroformate (1 mL) was added and the reaction was stirred for 1 h. The pH of the solution was adjusted to pH 10 from pH 4 by using saturated Na_2CO_3 solution, and then 1 mL of benzyl chloroformate was added again. After 1 h of stirring, the pH of the solution was adjusted to pH 10 again and 1 mL of benzyl chloroformate was added. The reaction mixture was allowed to stir overnight. The aqueous solution was extracted by methylene chloride (4 × 20 mL). The combined organic layer was washed by saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated under vacuum to give a clear oil. The crude product was recrystallized from diethyl ether and dried under reduced pressure to give a hygroscopic white powder (1782 mg, 98%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.02−3.65 (m, 16H), 5.07 (s, 2H), 7.27 (br s, 5H), 7.88−8.09 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 43.0, 43.7, 43.8, 44.1, 44.7, 45.0, 45.8, 46.7, 46.9, 47.6, 48.1, 48.8, 49.4, 49.8, 50.1, 50.4, 50.5, 50.8, 52.2, 53.5 (cyclen ring *C*H$_2$), 67.5, 67.6 (*C*H$_2$Ph), 127.9, 128.3, 128.6, 129.0 (Ph ring *C*H), 135.8 (Ph ring *C*), 155.9, 156.8, 157.5 (*C*O$_2$Ph), 163.0, 163.4, 163.6, 163.8, 163.9, 164.3, 164.6, 165.1, 165.7, 165.9 (N*C*HO); HRMS (ESI) *m/z* 413.1791 ([M + Na]$^+$, $C_{19}H_{26}N_4O_5Na$, calcd 413.1801). Anal. Calcd for $C_{19}H_{26}N_4O_5 \cdot H_2O$: C, 55.87; H, 6.91; N, 13.72. Found: C, 55.31; H, 6.68; N, 13.04.

1-(Benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane·3HCl (7b·3HCl). 1,4,7-Triformyl-10-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (731 mg, 1.87 mmol) was dissolved in 40 mL of 1 M HCl solution and the solution was stirred at 50 °C for 5 h. Solvent was evaporated completely under vacuum at 60 °C to give a white solid. Crude product was refluxed in 20 mL of ethanol, cooled to room temperature, filtrated, washed with 5 mL of ether, and dried in air. Excess ether was added to the ethanol filtrate to see the cloudiness of the solution. Precipitated white powder was collected,

washed by a small amount of ether, and dried in air as a second crop (633 mg, 81%): ^{1}H NMR (D_2O, 300 MHz) δ 3.19 (br s, 12H), 3.69 (br t, J = 5.1 Hz, 4H), 5.17 (s, 2H), 7.44 (br s, 5H); ^{13}C NMR (D_2O, 75 MHz) δ 45.8, 47.5, 48.2, 49.5, 71.6 (CH_2Ph), 131.4, 131.8, 131.9, 138.7, 161.4 (CO_2CH_2); HRMS (FAB) *m/z* 307.2139 ([M + H]$^+$, $C_{16}H_{27}N_4O_2$, calcd 307.2134). Anal. Calcd for $C_{16}H_{26}N_4O_2$·3HCl: C, 46.22; H, 7.03; N, 13.47; Cl, 25.58. Found: C, 45.67; H, 7.42; N, 12.94; Cl, 25.34.

1-(Benzyloxycarbonyl)-4,7,10-tris(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (7c). *N,N*-diisopropylethylamine (6995 μL, 40.16 mmol) was added to a slurry of 1-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane·3HCl (2269 μL, 5.46 mmol) in 40 mL of acetonitrile to make the mixture dissolved clearly. 4-*tert*-Butylbenzyl bromide (3229 μL, 17.57 mmol) was added to the solution dropwise at room temperature. The reaction mixture was slowly heated to 60 °C and allowed to stir for 15 h. Precipitated white powder was collected, washed by a small amount of acetonitrile and ether, and dried to give the first crop (394 mg). The yellow filtrate was concentrated to dryness, and the residue was dissolved in 5 mL of toluene and then excess hexane was added to precipitate the yellow oil, which was recrystallized in ether to give a white powder (1218 mg): yield 40%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (s, 9H), 1.31 (s, 9H), 1.32 (s, 9H), 2.57 (br s, 8H), 2.88 (br t, J = 5.7 Hz, 2H), 3.09 (br t, J = 5.7 Hz, 2H), 3.40 (s, 2H), 3.41 (s, 2H), 3.55 (s, 2H), 3.62 (br t, J = 5.7 Hz, 2H), 3.73 (br t, J = 5.7 Hz, 2H), 4.93 (s, 2H), 7.10−7.34 (m, 17H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.56 & 31.63 (CCH_3), 34.53 & 34.61 (CCH_3), 46.9, 48.0, 52.2, 52.5, 52.7, 53.4 & 53.5 (NCH_2Ph), 59.0, 59.5, 59.9, 67.0 (OCH_2Ph), 125.1 & 125.2 (NCH_2Ph ring *C*H), 127.9, 128.0, 128.5, 128.9 & 129.0 (NCH_2Ph ring *C*H), 136.6 & 136.7 (OCH_2Ph ring *C*H), 136.9 & 137.2 (NCH_2Ph ring *C*), 149.6 & 149.7 (NCH_2Ph ring *C*), 156.6 (NCO_2); HRMS (FAB) *m/z* 745.5403 ([M + H]$^+$, $C_{43}H_{65}N_4O_2$, calcd 745.5421). Anal. Calcd for $C_{49}H_{68}N_4O_2$: C, 78.99; H, 9.20; N, 7.52. Found: C, 78.47; H, 9.40; N, 7.49.

1,4,7-Tris(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (7). To a solution of 1-(benzyloxycarbonyl)-4,7,10-tris(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (265 mg, 0.35 mmol) in 30 mL of ethanol was added 10% Pd/C (250 mg). The reaction mixture was stirred under hydrogen gas at ambient pressure for 24 h. Pd catalyst was filtered off by a pad of Celite and the filtrate was concentrated under vacuum to give a clear oily solid (186 mg, 87%). A microanalysis sample was prepared by recrystallization from methylene chloride/ether: ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 9H), 1.31 (s, 18H), 2.78 (br s, 12H), 2.96 (br s, 6H), 3.47 (s, 2H), 3.74 (s, 4H), 6.64 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 4H), 7.44 (d, J = 8.1 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 31.4 & 31.5 (CCH_3), 34.5 & 34.6 (CCH_3), 49.2, 49.6, 50.3, 51.3, 62.5 (NCH_2Ph), 125.2 & 125.4 (Ph ring *C*H), 129.48 & 129.52 (Ph ring *C*H), 132.3 & 135.5 (Ph ring *C*), 150.1 & 151.0 (Ph ring *C*); HRMS (ESI) *m/z* 611.5045 ([M + H]$^+$, $C_{43}H_{65}N_4O_2$, calcd 611.5053). Anal. Calcd for $C_{41}H_{62}N_4$·H_2O: C, 78.29; H, 10.26; N, 8.91. Found: C, 75.26; H, 10.78; N, 11.90.

1-(*tert*-Butoxycarbonylmethyl)-4,7,10-tris(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (8a). To a solution of 1,4,7-tris(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (426 mg, 0.70 mmol) were added *N,N*-diisopropylethylamine (242 μL, 1.39 mmol) and *tert*-butyl bromoacetate (155 μL, 1.05 mmol). The flask was heated slowly to 60 °C and allowed to stir for 16 h. After evaporation of solvent in vacuo, 20 mL of water was added and then crude product was extracted by 3 × 20 mL of methylene chloride, dried over $MgSO_4$, and concentrated to give pale oil, which was further purified by column chromatography over alumina using ethanol:chloroform (1:20) as eluent to give clear oil (268 mg, 53%): ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H), 1.35 & 1.37 (s, 18H), 1.44 & 1.45 (s, 9H), 2.69 (br s, 8H), 2.93 (br m, 6H), 3.17 & 3.21 (s, 2H), 3.42 (s, 2H), 3.51 (s, 4H), 3.58 (s, 2H), 7.22−7.42 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 28.5 ($OCCH_3$), 31.6 & 31.7 ($PhCCH_3$), 34.56 & 34.61 ($PhCCH_3$), 52.6, 52.87, 52.93, 53.02, 53.10, 53.22, 56.56, 56.68, 59.70, 59.88, 80.62 ($OCCH_3$), 125.04 & 125.12 (Ph ring *C*H), 128.68 & 128.81 (Ph ring *C*H), 137.19 & 137.33 (Ph ring *C*), 149.38 & 149.48 & 149.66 (Ph ring *C*), 171.47 & 171. 54 (CO_2). Anal. Calcd for $C_{47}H_{72}N_4O_2$·H_2O: C, 75.96; H, 10.04; N, 7.54. Found: C, 74.57; H, 10.06; N, 7.60.

1-(Carboxymethyl)-4,7,10-tris(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (8). 1-(*tert*-Butoxycarbonylmethyl)-4,7,10-tris(4-*tert*-butylbenzyl)-1,4,7,10-tetraazacyclododecane (36 mg, 0.05 mmol) was dissolved in 25 mL of trifluoroacetic acid and the solution was stirred at room temperature for 18 h. After evaporation of solvent, the residue was dissolved in 10 mL of methylene chloride, washed by water (10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated to give clear oil (33 mg, 99%): ^{1}H NMR ($CDCl_3$, 300 MHz, no HCl) δ 1.28 (s, 27H), 2.75 (br s, 6H), 2.82 (br s, 6H), 3.12 (br s, 4H), 3.45 (s, 2H), 3.56 (s, 2H), 3.63 (br s, 4H), 6.90 (d, J = 7.8 Hz, 2H), 7.21−7.30 (m, 6H), 7.35 (d, J = 7.8 Hz, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz, no HCl) δ 31.5 (CCH_3), 34.7 (CCH_3), 50.4, 50.6, 51.8, 54.7, 57.4, 57.7, 60.2, 125.5 & 125.7 (Ph ring *C*H), 129.8 & 130.0 (Ph ring *C*H), 131.7 & 133.4 (Ph ring *C*), 151.0 & 151.2 (Ph ring *C*), 171.6 (CO_2); HRMS (FAB) *m/z* 669.5112 ([M + H]$^+$, $C_{43}H_{65}N_4O_2$, calcd 669.5108).

Copper(II) Complex of 2c, $[Cu(2c)Cl]^+(ClO_4)^-$. To a aqueous solution (10 mL) of $Cu(ClO_4)_2$·$6H_2O$ (122 mg, 0.33 mmol) was added a water solution (10 mL) of **2c**·4HCl (139 mg, 0.30 mmol). The mixture was stirred at room temperature overnight. Blue precipitates were collected by a sintered glass funnel and washed with small amount of water, ethanol, and ether, then dried in air (120 mg, 78%). A single crystal for X-ray structure determination was grown by vapor diffusion of ether into a DMF solution of **2c**: HRMS (FAB) *m/z* 416.1779 ([M − (ClO_4)]$^+$, $C_{19}H_{34}ClCuN_4$, calcd 416.1768). Anal. Calcd for $C_{19}H_{34}Cl_2CuN_4O_4$: C, 44.14; H, 6.63; N, 10.84. Found: C, 44.14; H, 6.90; N, 10.51.

CAUTION! Perchlorate salts of metal complexes with organic ligands are potentially explosive.

X-ray Crystallography. Crystals of appropriate dimension were mounted on glass fibers in random orientation. Preliminary examination and data collection was performed at −55 °C using a Bruker SMART Charge Coupled Device (CCD) Detector system single-crystal X-ray diffractometer equipped with a sealed tube X-ray source using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Preliminary unit cell constants were determined with a set of 45 narrow frames (0.3° in ϖ) scans. The data set collected consists of 4028 frames of intensity data collected with a frame width of 0.3° in ϖ and counting time of 30 s/frame at a crystal to detector distance of 4.950 cm. The double pass method of scanning was used to exclude any noise. The collected frames were integrated using an orientation matrix determined from the narrow frame scans. SMART and SAINT software packages (Bruker Analytical X-ray, Madison, WI, 1998) were used for data collection and data integration. Analysis of the integrated data did not show any decay. Final cell constants were determined by a global refinement of *xyz* centroids from all data. Collected data were corrected for systematic errors using SADABS (Blessing, R. H. *Acta Crystallogr*. **1995**, *A51*, 33−38) based upon the Laue symmetry using equivalent reflections. Structure solution and refinement were carried out using the SHELXTL-PLUS software package (Sheldrick, G. M., Bruker Analytical X-ray Division, Madison, WI, 2000). The structures were solved by direct methods and refined successfully in the space groups *Pnma*. Full matrix least-squares refinement was carried out by minimizing $\sum w(F_o^2 - F_c^2)^2$. The non-hydrogen atoms were refined anisotropically to convergence. The hydrogen atoms were treated using appropriate riding model (AFIX m3).

Radiochemistry. ^{64}Cu chloride was converted to ^{64}Cu acetate or citrate by stirring with 30−100 μL of 0.1 M ammonium acetate, pH 6.4, or 0.1 M ammonium citrate, pH 6.5, except for ligand **5**, where ^{64}Cu chloride was simply diluted by water. ^{64}Cu acetate or citrate or chloride (0.7−2.3 mCi) was added to a ligand solution containing 0.3−1.7 mg of ligand in 100−300 μL of a mixture of ethanol and buffer (see Table 1 for specific reaction conditions for each ligand). The amount of ethanol used was determined on the basis of solubilities of ligand and copper complex of ligand. For ligand **2**, only 0.1 M

ammonium acetate, pH 6.4, was used as labeling solvent. The reaction mixture was incubated at temperature in the range from room temperature to 60 °C. Reaction times ranged from 15 min to 7 h. The radiochemical purity was determined by radio-TLC using silica gel plates (for ligand **1**, **2**, **3**, **4**, **6**) or reverse phase C8 plates (for **5**, **7**, **8**). Developing solvents were chosen depending on the TLC plate and the polarity of ^{64}Cu-labeled complex.

Determination of Partition Coefficients. The partition coefficients were determined by adding 2−20 μL of ^{64}Cu-labeled complex (10−39 μCi) to a solution containing 1.5 mL of octanol and 1.5 mL of buffer solution (0.01 M ammonium acetate, pH 7.4), which are obtained from saturated octanol buffer solutions. The resulting solutions were then vortexed for 1 min and centrifuged for 5 min at 5000 rpm. A 1 mL aliquot of the octanol layer was removed, back-extracted with 1 mL of buffer, vortexed, and centrifuged for 2 min at 5000 rpm. Aliquots (150 μL) of octanol and buffer were removed, weighed, and counted (first partition coefficient). A 700 μL aliquot of the previous octanol layer was removed and back-extracted again with 700 μL of buffer, vortexed, and centrifuged as before. Aliquots (150 μL) of octanol and buffer were removed, weighed, and counted (second partition coefficient). A 400 μL aliquot of octanol layer of second back-extraction was removed and back-extracted one more time with 400 μL of buffer, vortexed, and centrifuged as before. Aliquots (150 μL) of octanol and buffer were removed, weighed, and counted (third partition coefficient). The partition coefficient was calculated as a ratio of counts in the octanol fraction of unit mass to counts in the buffer fraction of unit mass per back-extraction. The average log *P* value of the three back-extractions is reported.

Biodistribution Studies. The radioactive complexes were diluted with saline for injection, except for ^{64}Cu−**1** and ^{64}Cu−**3**, where 0.1 M ammonium acetate buffer (pH 6.4) was used instead. Mature female Sprague−Dawley rats (n = 4 per time point) weighing 170−240 g were anesthetized and injected with 17−32 μCi of activity in a volume of 100−160 μL via the tail vein. At selected time points postinjection (15 min, 4 h, and 24 h), rats were sacrificed. Organs of interest (liver, kidney, muscle, heart, and blood) were removed, weighted, and counted. The percent of injected dose per gram (%ID/g) and percent injected dose per organ (%ID/organ) were calculated by comparison to weighed, counted standard solutions. All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University's Animal Studies Committee.

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Supporting Information Available: X-ray crystallographic files for Cu−**2c**, including crystal data and structure refinement, atomic coordinates, and equivalent isotropic displacements parameters, bond lengths and angles, anisotropic displacement paramagnets, hydrogen coordinates, and projection plot with 30% probability ellipsoids. This material is available free of charge via the Internet at http://pubs.acs.org.

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