Inorganic Chemistry

Article

pubs.acs.org/IC

Evaluation of the H_2dedpa Scaffold and its cRGDyK Conjugates for Labeling with ^{64}Cu

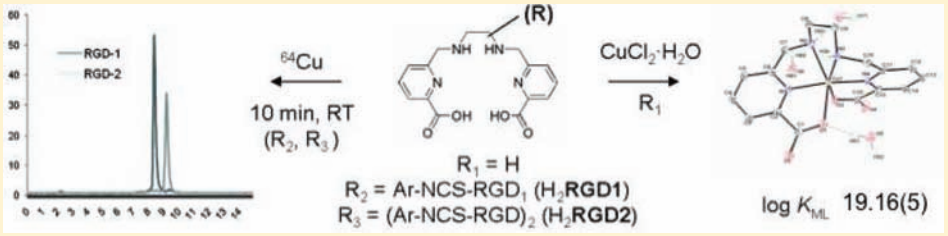
Eszter Boros,[†,‡] Jacqueline F. Cawthray,[†] Cara L. Ferreira,[§] Brian O. Patrick,[†] Michael J. Adam,*[,‡] and Chris Orvig*[,†]

[†]Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC, V6T 1Z1, Canada

[‡]TRIUMF, 4004 Wesbrook Mall, Vancouver, BC, V6T 2A3, Canada

[§]NORDION, 4004 Wesbrook Mall, Vancouver, BC, V6T 2A3, Canada

Supporting Information



ABSTRACT: Studies of the acyclic ligand scaffold H_2**dedpa** and its derivatives with the peptide cRGDyK for application in copper radiopharmaceuticals are described. Previously shown to be a superb ligand for $^{67/68}$Ga, the chelate is now shown to coordinate ^{64}Cu in its derivatized and nonderivatized forms rapidly under mild reaction conditions (10 min, RT, pH 5.5 10 mM sodium acetate buffered solution). The hexadentate, distorted octahedral coordination of H_2**dedpa** is confirmed in the corresponding solid state X-ray crystal structure of [Cu(**dedpa**)]. Cyclic voltammetry determined the reduction potential of [Cu(**dedpa**)] to be below values found for common bioreductants. Reduction and reoxidation were irreversible but reproducible, indicating a potential change of coordination mode upon reduction of Cu(II) to Cu(I). The thermodynamic stability constant log K_{CuL} was determined to be 19.16(5), comparable to other frequently used ^{64}Cu chelates. Serum stability of the ^{64}Cu labeled chelate revealed only 3% transchelation/association to serum proteins after 2 h, while the conjugates reveal 10% ([Cu(**RGD1**)]) and 6% ([Cu(**RGD2**)]) transchelation at the same time point.

A variety of Cu isotopes applicable to diagnostic imaging and radiotherapy have been the subject of extensive research. ^{64}Cu ($t_{1/2}$ = 12.7 h, β^+ 17.4%, E_{max} = 0.656 MeV, β^- 39%, E_{max} = 0.573 MeV) is of current interest for both positron emission tomography (PET) and radiotherapy.[1] Its longish half-life is applicable to developing PET agents with larger biomolecules, such as monoclonal antibodies, that may require longer circulation times before imaging to achieve optimal target uptake.[2] While not available as a generator-produced isotope, the longer half-life allows for shipment of this cyclotron-produced isotope over longer distances.[3]

As opposed to the nonredox active Ga(III), mainly two oxidation states (I, II) are relevant for copper in aqueous solution. The d^9 Cu(II) configuration is predominant in aqueous solution and prefers square planar and Jahn–Teller distorted octahedral geometries. The ligand exchange kinetics of Cu(II) are particularly rapid, therefore kinetically inert ligand systems with strong crystal field stabilization for complexation are preferred for incorporation into radiopharmaceuticals.[4] Cu(II) is a borderline soft metal center, with preferences for donor atoms similar to the preferences of Ga(III): amines, imines, pyridines, and carboxylates.[5]

Among the bifunctional ligand systems investigated and previously reported for the purpose of targeted delivery of ^{64}Cu, a strong preference for hexadentate cage-like polyaza macrocyclic ligands can be found.[1] Preferred properties for good ^{64}Cu bifunctional chelates include complex stability against blood serum challenge over the course of 24 h and in vivo inertness toward transchelation; the latter can be monitored by liver uptake, negative complex reduction potentials well below −0.4 V (NHE),[4,6] and reversibility of the reduction reaction without loss of ligand.[7] Cu(II) is preferentially reduced to give a 1+, 1−, or neutral overall charge of the metal complex in order to minimize high kidney uptake and accelerate excretion. Mild labeling conditions are preferred, despite the long half-life, because heat- and pH-sensitive biomolecules such as antibodies are often used with this isotope.[1] Thermodynamic stability is a consideration when designing Cu(II) chelates; however, it does not necessarily correlate with in vivo complex stability.[4] In Figure 1 and Table 1, a brief summary of important chelate structures (for ^{64}Cu)

Received: March 2, 2012
Published: May 14, 2012

dx.doi.org/10.1021/ic300482x | *Inorg. Chem.* 2012, 51, 6279–6284

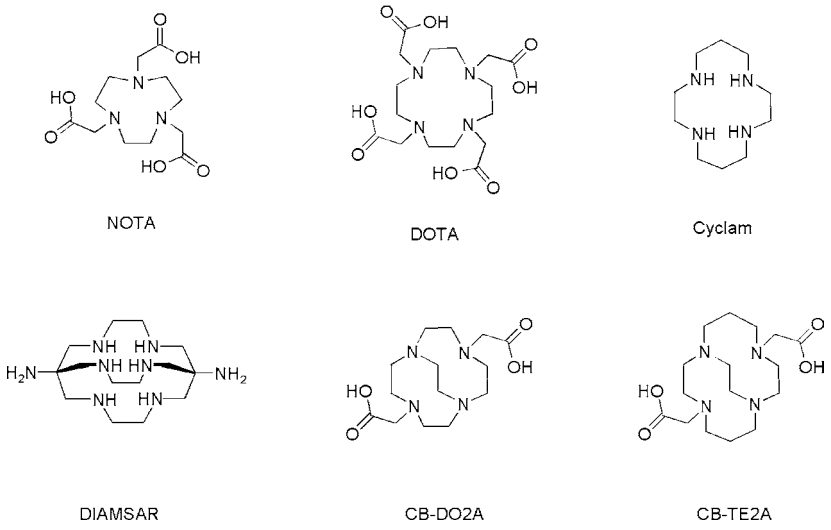


Figure 1. Structures of selected known chelate systems for labeling with ^{64}Cu.

Table 1. Summary of Relevant Properties of Previously Investigated Chelates for Labeling of ^{64}Cu

ligand system	labeling conditions	log K_{ML}[a]	E_{red} (vs NHE)
NOTA[8]	30 min, room temp	21.63	−0.7 (irrev)
DOTA[9]	5 min, room temp	22.7	−0.74 (irrev)
Cyclam[10]	10 min, room temp	27	−0.48 (irrev)
DIAMSAR[11]	5−20 min, room temp	nd	−0.90 (irrev)
CB-TE2A[12]	1 h, 95 °C	nd	−0.88 (q-rev)
CB-DO2A[12]	30 min, 80 °C	nd	−0.72 (irrev)

[a] K_{ML} = [ML]/[M][L]

and some of their relevant characteristics such as labeling conditions, reduction potential and thermodynamic stability constant (log K_{ML}) are shown. Recently published comprehensive reviews provide a more in-depth analysis of this field.[3]

In this work, we utilized the H_2**dedpa** chelate for rapid chelation of ^{64}Cu under mild labeling conditions, as well as in vitro serum stability. The [Cu(**dedpa**)] complex is further investigated for its structural properties via solid state structure determination and electrochemical properties using cyclic voltammetry. The ^{64}Cu complexes of two **dedpa**$^{2-}$ conjugates bearing cRGDyK ([Cu(**RGD1**)] and [Cu(**RGD2**)], Figure 2) are also investigated for their labeling properties and in vitro serum stability. cRGDyK is a small cyclic peptide targeting $\alpha_v\beta_3$ integrin, a receptor which is highly expressed on activated endothelial and tumor cells.[13] cRGDyK conjugates have been widely investigated for the targeting of tumors in nuclear medicine[14−16] and hence serve as a convenient tool to evaluate the tumor targeting properties of novel radiolabeled conjugates such as [Cu(**RGD1**)] and [Cu(**RGD2**)]. It was our goal to investigate the potential of the H_2dedpa chelate to serve as a multipurpose ligand system, capable of coordination of two PET nuclides: ^{64}Cu and ^{68}Ga.

EXPERIMENTAL SECTION

Materials and Methods. Electrospray ionization mass spectrometry (ESI-MS) spectra were recorded on a Micromass LCT instrument at the Department of Chemistry, University of British Columbia. IR spectra were collected neat in the solid or liquid state on a Thermo Nicolet 6700 FT-IR spectrometer and UV−vis spectra in solution with a Hewlett-Packard 8453 spectrophotometer. High-performance liquid chromatography (HPLC) analysis of nonradioactive complexes, and monitoring decomplexation in 6 M HCl at 90 °C were undertaken on a Phenomenex Synergi 4 μm Hydro-RP 80A column (250 mm × 4.6 mm) on a Waters WE 600 HPLC system equipped with a 2478 dual-wavelength absorbance UV detector run using the Empower software package. HPLC analysis or purification of nonradioactive peptides was undertaken on a Phenomenex Jupiter 5 μm C18 300 Å (100 × 4.6 mm). HPLC solvents consisted of 0.1% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B). A linear gradient method (100% solvent A to 100% solvent B over 30 min) was used.

^{64}Cu was obtained as a dilute HCl solution (Nordion); it was commercially available from Nordion at the time of the ^{64}Cu experiments. Product specification reported the specific activity of the ^{64}Cu to be >5000 Ci/g with <0.2 μg Cu per mCi. The HPLC system used for analysis of the ^{64}Cu labeled compounds consisted of a Waters Alliance HT 2795 separation module equipped with a Raytest Gabbistar NaI detector and a Waters 996 photodiode array (PDA) detector. Radiolabeling yields for the ^{64}Cu labeled compounds were determined using a Waters XBridge BEH130 4.6 mm ×150 mm column. Samples were analyzed with a linear gradient method (95% solvent A to 100% solvent B over 30 min). If not mentioned otherwise, cRGDyK was acquired from Peptides International (Louisville, KY) in

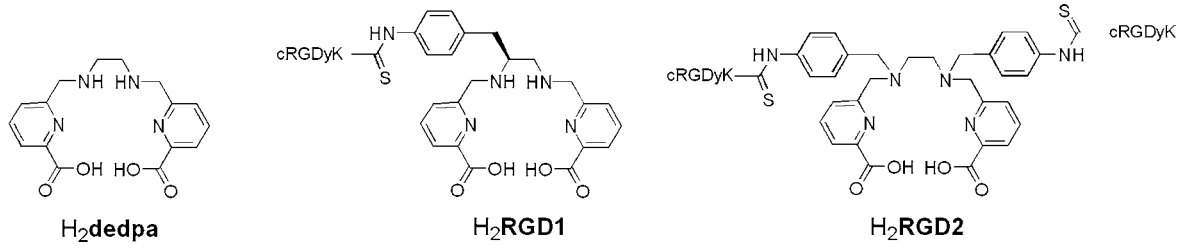


Figure 2. Ligand systems investigated in this study.

dx.doi.org/10.1021/ic300482x | Inorg. Chem. 2012, 51, 6279−6284

25 mg aliquots. Syntheses of conjugates H_2**RGD1** and H_2**RGD2** were executed as previously reported.[17]

1,2-[{6-(Carboxylato)pyridin-2-yl}methylamino]ethane Copper(II) Trihydrate, [Cu(dedpa)]·3H_2O. H_2dedpa·2 HCl[18] (5.8 mg, 0.023 mmol) was dissolved in 1 mL H_2O. $CuCl_2·H_2O$ (2.4 mg, 0.023 mmol) was dissolved in a 1:1 mixture of CH_3OH/H_2O (2 mL total volume) and added dropwise to the ligand solution. The color of the mixture was a greenish blue indicating coordination of the ligand. The pH was adjusted to 6−7 by dropwise addition of an aqueous solution of 0.1 M NaOH. Subsequently, the solvent was removed and the blue crude residue was redissolved in minimal amounts of H_2O and loaded onto a C18 Waters sep-pak column cartridge. The column was flushed with 20 mL H_2O and the complex was eluted with EtOH as a clear blue solution, which was set aside for slow evaporation in the fume hood. IR (neat, cm^{-1}): 1621 (w), 1574 (s, br), 1410 (m), 1362 (m). HR-ESI-MS calcd. for $C_{16}H_{16}CuN_4NaO_4$: 414.0365, found: 414.0374 $[M + Na]^+$. Elemental analysis: calcd. (found) for $[Cu(dedpa)]·3H_2O$: C 43.09 (42.58), H 4.97 (4.67), N 12.56 (12.04). UV−vis (sodium acetate buffer 10 mM, pH 4.4): λ = 212 (sh) ε = 23,865 M^{-1} cm^{-1}, 261 (sh) (10,400), 267 (11240), 275 (sh) (8805).

Solid State Crystal Structure Determination. A blue prism crystal of $C_{16}H_{16}Cu\ N_4O_4·3H_2O$ having approximate dimensions of 0.11 mm × 0.15 mm × 0.21 mm was mounted on a glass fiber. All measurements were made on a Bruker APEX DUO diffractometer with graphite monochromated Mo−Kα radiation. Data were collected and integrated using the Bruker SAINT[19] software package. The linear absorption coefficient, μ, for Mo−Kα radiation is 12.07 cm^{-1}. Data were corrected for absorption effects using the multiscan technique (SADABS[20]), with minimum and maximum transmission coefficients of 0.808 and 0.876, respectively. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods. The material crystallizes with three molecules of H_2O in the asymmetric unit. All non-hydrogen atoms were refined anisotropically. All hydroxyl hydrogen atoms and N−H hydrogen atoms were located in difference maps and refined isotropically. The hydrogen atoms of each water molecule were disordered in three orientations, each orientation taking advantage of a possible hydrogen bond interaction. The excellent quality of the data made it possible to refine the positions and occupancies of the hydrogen fragments on each water molecule; however, the isotropic thermal parameters were constrained to be 1.5 times the thermal parameter of its associated oxygen. All other hydrogen atoms were placed in calculated positions. Finally, the material crystallizes as a racemic twin, in an approximately 80:20 ratio between the two twin volumes.

Cyclic Voltammetry Measurements on [Cu(dedpa)]. Cyclic voltammetry was carried out using an Autolab potentiostat. A glassy carbon electrode (MF 2012, 3 mm diameter) was used as the working electrode, the reference electrode was Ag/AgCl (sat.), and the counter electrode was platinum mesh. Cyclic voltammetry was carried out in 0.1 M NaOAc, pH 7 (adjusted with glacial acetic acid), with a 0.1 M solution of [Cu(dedpa)] at scan rates of 100 and 10 mV/s. A 100 mV/s scan was applied to a solution of solvent between 2 and −2 V in order to establish solvent reduction and oxidation limits. These were found to be 0.5 to −1.25 V and should not lead to solvent redox behavior. Subsequently, the ligand or complex was dissolved in the previously measured solvent solution and the cyclic voltammogram was measured within a variety of electrochemical potential windows (1.5 to −1.25 V, as well as 0.5 to −0.5 V). It was found that the one electron reduction of the copper complex could be observed at −1.12 V (normal hydrogen electrode, NHE: 0.92 V) with reoxidation at 0.02 V (NHE: 0.22). The afforded voltammogram is reproducible upon cycling (see the Supporting Information).

Acid Stability of [Cu(dedpa)]. A solution of 6 M HCl (10 mL) was heated to 90 °C. [Cu(**dedpa**)] (20 mg) was added into the solution and aliquots of 10 μL were removed from the solution at time points 0, 3, 5, 10, 20, and 30 min and injected onto the HPLC. Disappearance of the initial peak indicative of [Cu(**dedpa**)] was monitored ($t_{1/2} \leq 5$ min).

Thermodynamic Stability. The experimental procedures and details of the apparatus closely followed those previously reported for H_2**dedpa** with Ga(III).[21] As a result of the strength of the binding of **dedpa**$^{2-}$ to Cu(II), the complex formation constants could not be determined directly; the ligand−ligand competition method using the known competitor Na_2H_2EDTA was used. All experiments were performed at 25 °C in 0.15 M NaCl as supporting electrolyte. Cu(II) ion solutions were prepared by dilution of the appropriate atomic absorption standard (AAS) solution. The exact amount of acid present in the copper standard was determined by titration of an equimolar solution of Cu(II) and Na_2H_2EDTA. The amount of acid present was determined by Gran's method.[22] Calibration of the electrode was performed prior to each measurement by titrating a known amount of HCl with 0.1 M NaOH. Calibration data were analyzed by standard computer treatment provided within the program MacCalib[23] to obtain the calibration parameters *E*0 and p*K*w. Equilibration times for titrations were 10 min for metal complexation titrations. Ligand and metal concentrations were in the range of 0.8 mM to 1.0 mM for potentiometric titrations. The data were treated by the program Hyperquad2008.[24] The four successive proton dissociation constants, corresponding to hydrolysis of $Cu(II)_{aq}$ ion, included in the calculations were taken from the work of Baes and Mesmer.[25] All values and errors represent the average of at least three independent experiments.

Molecular Modeling. Molecular modeling was performed using the Gaussian 09[26] and GaussView packages. Density functional theory, with the B3LYP functional, employing the 6-31+G(d,p) basis set, was used to obtain the optimized geometry and the electron density. Solvent (water) effects were described through a continuum approach by means of the IEF PCM as implemented in G09. The electrostatic potential was mapped onto the calculated electron density surface.

Radiolabeling Procedure. $^{64}Cu^{2+}$ in dilute HCl (100 μL, 0.7 mCi) was added to 900 μL of a 10^{-5} M solution of H_2**dedpa** in 10 mM sodium acetate solution (pH 5.5) and left to react for 10 min at RT. The reaction progress was monitored by analytical HPLC to evaluate yield of the coordination reaction: [^{64}Cu(**dedpa**)] t_R on HPLC: 5.2 min (gradient: A: 0.1% TFA in H_2O, B: CH_3CN 5−100% B linear gradient 30 min), yield: 99%. Ligand concentrations as low as 10^{-6} M were capable of coordinating the radionuclide with a 63% yield (under these labeling conditions). To evaluate complex stability, an aliquot (500 μL) of the reaction mixture was added to a solution of mouse serum (500 μL). The mixture was incubated at 37 °C and analyzed by PD-10 size exclusion columns after 2 and 24 h (a 15 min time point was included for the conjugates). Aliquots of the reaction mixtures were loaded onto the prerinsed PD-10 columns (total loading volume: 2.5 mL). Subsequently, the column was rinsed with 3.5 mL phosphate buffered saline (PBS). The resulting eluent was collected and measured for radioactivity. The activity eluted corresponds to serum-bound ^{64}Cu; stability versus serum (2 h/24 h; in % complex intact): 97/77.

[^{64}Cu(RGD1)]. R_t of ^{64}Cu radiolabeled product on HPLC (gradient: A: H_2O, 0.1% TFA, B: CH_3CN. 5−100% B linear gradient 30 min): 8.4 min. Stability versus serum (15 min/2 h/24 h; in % complex intact): 94/90/72.

[^{64}Cu(RGD2)]. R_t of ^{64}Cu radiolabeled product on HPLC (gradient: A: H_2O, 0.1% TFA, B: CH_3CN. 5−100% B linear gradient 30 min): 9.2 min. Stability versus serum (15 min/2 h/24 h; in % complex intact): 98/94/88.

RESULTS AND DISCUSSION

Formation of the copper complex with H_2**dedpa** can be followed by the strong, rapid color change of the reaction mixture to a dark turquoise upon mixing of the ligand and metal solution (which exhibits light blue coloring prior to addition). The resulting complex is only soluble in protic solvents despite its neutral charge; hence, the product cannot be precipitated or extracted in order to separate out other inorganic salts present in the mixture. Separation from salts was

dx.doi.org/10.1021/ic300482x | Inorg. Chem. 2012, 51, 6279−6284

done with purification using a C18 reversed phase cartridge. The paramagnetic nature of the Cu(II) metal center hinders NMR spectroscopic investigations, hence the product was characterized through elemental analysis, IR spectroscopy, and mass spectrometry. IR spectral data show a characteristic decrease of carboxylate stretching frequencies upon metal coordination (from 1761[21] to 1621 cm^{-1}). In the mass spectrum, diagnostic $[M + Na]^+$ peaks were easily identified and displayed the characteristic isotope distribution of $^{63}Cu/^{65}Cu$ (414.0374 corresponds to the ^{63}Cu isotope with higher abundance; the M+2 peak is also found, corresponding to the ^{65}Cu isotope).

The product crystallizes forming small blue prism crystals suitable for X-ray diffraction. The complex cocrystallizes with three water molecules in the lattice (Figure 3). Bond length

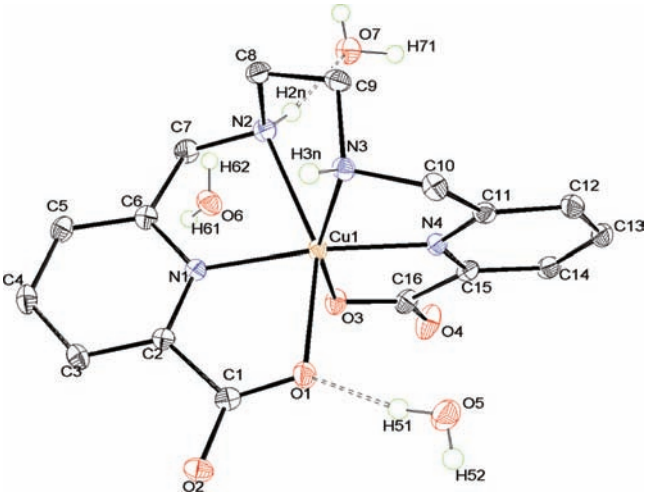


Figure 3. ORTEP drawing of [Cu(**dedpa**)]. Shown is the atom numbering scheme (50% thermal ellipsoids), and hydrogen atoms within the complex structure are omitted for clarity.

comparisons between the metal complex and the ligand provide insight into the coordination environment (Table 2). Most bond lengths in [Cu(**dedpa**)] are between 1.93 and 2.04 Å, except N2−Cu and O1−Cu, which at 2.30 Å show a Jahn−Teller type distortion, typical for octahedral Cu(II) complexes and also observed for [Cu(CB-TE2A)].[7] In order to illustrate the elongation of the axial bonds, a side-on view in Figure 4 is provided, with comparison to $[Ga(\mathbf{dedpa})]^+$. The Cu(II) complex appears to be closer to octahedral geometry; however, both complexes are strongly distorted. The H_2**dedpa** ligand system shows great flexibility in terms of metal ion tolerance (Figure 4).

In order to evaluate the capability of the complex to withstand bioreductive conditions without reduction of Cu(II)

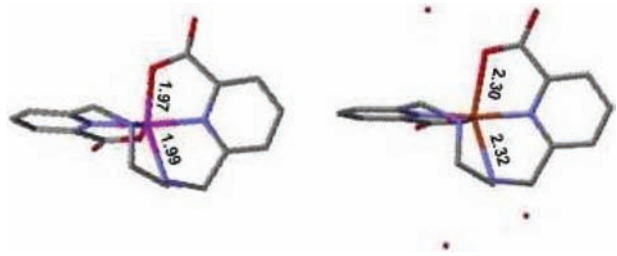


Figure 4. Selected bond lengths in side-on view of $[Ga(\mathbf{dedpa})]^+$ (left) and $[Cu(\mathbf{dedpa})]\cdot 3H_2O$ (right), H atoms, and $[ClO_4]^-$ counterion of Ga complex omitted for clarity. M−O1 and M−N2 distances are given in angstroms. This graphic was generated using Mercury 2.2.[27]

to Cu(I) and subsequent decomplexation, a cyclic voltammogram was measured. A metal based reduction process was observed at −1.12 V (−0.92 V vs NHE, Supporting Information Figure S1), and an oxidation process was observed at 0.02 V (0.22 V vs NHE, Figure S1). The cyclic voltammogram, while irreversible, was found to be reproducible over multiple cycles without decrease of intensities of either peak. In order to show that the two distinct, dissimilar peaks observed are related, a smaller electrochemical potential window was chosen, which excludes the reduction process found at −1.12 V. Subsequently, the oxidation peak at 0.02 was not observed (Supporting Information Figure S2); hence, the two peaks are shown to be related. Due to dissimilar peak shape, it is likely that the coordination mode of $\mathbf{dedpa}^{2-}$ changes upon reduction. Previously reported CV data on Cu(II) complexes with ligands DOTA, TETA, CB-DO2A, and CB-TE2A indicate that complexes that dissociate in vivo are also found to produce irreversible voltammograms, where cycling is not possible, indicating loss of the metal upon reduction (e.g., TETA, CB-DO2A).[12] This was not observed for [Cu(**dedpa**)]; hence, no copper is lost in the course of multiple reduction and reoxidation cycles. The reduction potential lies well below the −0.4 V (NHE) threshold for bioreductants, suggesting that [Cu(**dedpa**)] could have high stability against redox-induced decomplexation in vivo.[3]

The tendency for acid-catalyzed dissociation was demonstrated using HPLC methods. [Cu(**dedpa**)] was found to dissociate rapidly in acid solution ($t_{1/2} \leq 5$ min), indicating decreased kinetic stability similar to ligand systems such as DOTA, NOTA, or cyclam. The thermodynamic instability of a monoprotonated species of [Cu(**dedpa**)] is indicated by the corresponding low thermodynamic stability constant log K_{MHL} (3.10).

The term log K_{ML} was determined to be 19.16(5), corresponding to pM ($-\log[M^{n+}_{free}]$) = 18.5 at $[M^{n+}]$ = 1 μM, [L] = 10 μM. These values are significantly lower than values

Table 2. Selected Bond Lengths (Å) and Angles (deg) in [Cu(dedpa)], Compared to Selected Bond Lengths in $[Ga(\mathbf{dedpa})][ClO_4]$[21]

bond	length [Å] (M = Cu)	length [Å] (M = Ga)	angle (M = Cu)	degree [deg]
N1−M	2.0008(12)	1.992(5)	N(4)−Cu(1)−O(3)	81.17(5)
N2−M	2.3171(13)	1.981(5)	N(4)−Cu(1)−N(3)	78.65(5)
N3−M	2.1364(13)	2.188(5)	N(1)−Cu(1)−N(3)	104.83(5)
N4−M	1.9386(13)	2.159(5)	N(4)−Cu(1)−O(1)	97.86(5)
O1−M	2.3014(11)	1.967(4)	N(1)−Cu(1)−O(1)	76.23(4)
O3−M	2.0430(10)	1.976(4)	N(1)−Cu(1)−N(2)	76.82(5)

dx.doi.org/10.1021/ic300482x | Inorg. Chem. 2012, 51, 6279−6284

found for highly thermodynamically stable Cu(II) complexes such as cyclam (log K_{ML} = 27) or TETA (log K_{ML} = 21).[3] The low stability constant measured for the monoprotonated complex species also suggests facile decomplexation once protonation has taken effect. The **dedpa**$^{2-}$ chelate is acyclic and is therefore expected to have a faster k_{off} rate, which can be highly detrimental to successful in vivo application as a bifunctional chelator. Indications for kinetic lability can be seen from serum stability measurements of corresponding ^{64}Cu complexes at different time points which are more representative of complex inertness in vivo.

Further insight into the instability of [Cu(**dedpa**)] compared to [Ga(**dedpa**)]$^{+}$ can be obtained from modeling of the corresponding electrostatic potentials. While [Ga(**dedpa**)]$^{+}$ displays a mainly positive electrostatic potential throughout the molecular surface (Figure 5, indicated by blue areas),

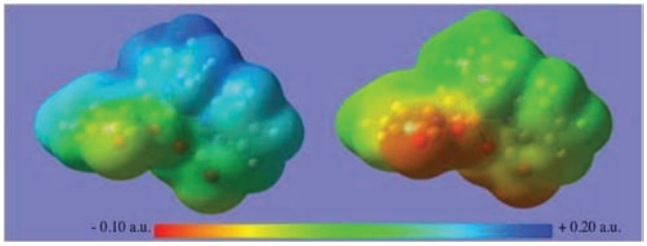


Figure 5. Electrostatic potentials of the complexes of **dedpa**$^{2-}$ with Ga(III) (left) and with Cu(II) (right) mapped onto the electron density. The MEPs represent a maximum potential of 0.20 au, and a minimum of −0.10 au, mapped onto electron density isosurfaces of 0.002 e Å^{-3}.

[Cu(**dedpa**)] is found to have greater areas of electronegative potential (Figure 5, indicated by red areas). This could point toward a greater potential for destabilization through protonation of the Cu complex, opposed to the high stability of the Ga complex.

The labeling of H_2**dedpa** with ^{64}Cu proceeds quantitatively with ligand concentrations as low as 10^{-5} M in 10 min at room temperature. The stability of the radiochemical complex was measured by a competition experiment in mouse serum. While the complex remains relatively stable over the first 2 h (only 3% transchelation), over the course of 24 h, 23% of the ^{64}Cu previously coordinated to **dedpa**$^{2-}$ is associated with serum. It cannot be determined if actual transchelation has happened or if the complex is still intact but associated with serum proteins as a whole entity. Coordination of H_2**RGD1** to ^{64}Cu affords the complex within 10 min at room temperature in 97% radiochemical yield (Figure 6). The subsequent serum challenge experiment revealed that 72% of the radiolabeled complex remained intact after 24 h in the presence of excess serum. PD-10 columns were used to filter off any serum-bound ^{64}Cu. In the case of H_2**RGD2**, the ^{64}Cu complexes are formed within 10 min at room temperature in 96% radiochemical yield (Figure 6). The subsequent serum challenge experiment yields over 88% of the complex remaining intact after 24 h.

Both radiochemical coordination experiments confirm observations with H_2**dedpa**: efficient labeling with radiochemical yields >92% can be observed with ligand concentrations as low as 10^{-5} M. A decrease of radiochemical yield at lower concentrations of ligand was observed due to lower specific activity (compared to what can be observed with ^{67}Ga)[21] of the ^{64}Cu stock solution. ^{64}Cu labeled bioconjugates

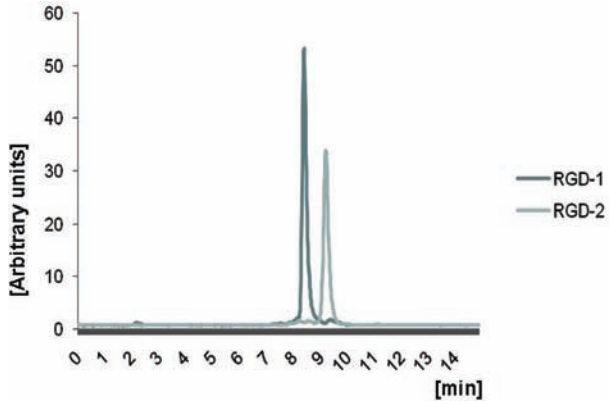


Figure 6. Radioabeling traces of the crude reaction mixtures of [^{64}Cu(**RGD1**)] (left) and [^{64}Cu(**RGD2**)] (right).

show a non-negligible percentage of ^{64}Cu transchelated with serum proteins after only 2 h (comparable to the [Cu(DOTA)]$^{2-}$ complex),[28] which will impose limitations likely too great for the successful use of the H_2**dedpa** system as a bifunctional chelate for ^{64}Cu.

CONCLUSION

The H_2**dedpa** scaffold was investigated for its capability to coordinate Cu(II). It has many promising properties for the coordination of Cu(II) and its radionuclide of interest, ^{64}Cu. The solid state structure shows similarities to the CB-TE2A coordination sphere. Studies of the redox properties of [Cu(**dedpa**)] yielded a reproducible, irreversible cyclic voltammogram with a reduction event at −0.92 V and an oxidation at 0.22 V (vs NHE). Because the two events are related but show dissimilar peak shape, it is likely that the ligand system changes its coordination sphere according to the predominant redox species. Labeling of H_2**dedpa** with ^{64}Cu proceeds within 10 min at room temperature, whereas some of the more established ligand systems require heat and prolonged reaction times. The thermodynamic stability constant was found to be log K_{ML} = 19.16(5), which is a value found for moderately stable Cu(II) complexes. Two bioconjugates of H_2**dedpa**, derivatized with either one or two cRGDyK peptides, were also evaluated for their coordination chemistry with ^{64}Cu. H_2**RGD1** and H_2**RGD2** coordinate the two PET isotopes ^{68}Ga and ^{64}Cu, both of interest to the radiopharmaceutical community, in a fast and efficient manner. Due to low kinetic and thermodynamic stability, as well as decreased serum stability of the conjugates with ^{64}Cu in vitro within 24 h the complex is not suitable for in vivo applications. The search for a universal chelate suitable for all radiometals of great interest to the radiopharmaceutical community continues.

ASSOCIATED CONTENT

Supporting Information

Cyclic voltammetry data, potentiometric titration curves, crystallographic data, and the crystallographic information file (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

dx.doi.org/10.1021/ic300482x | Inorg. Chem. 2012, 51, 6279−6284

AUTHOR INFORMATION

Corresponding Author

*Telephone: 604-822-4449. E-mail: orvig@chem.ubc.ca (C.O.). Telephone: 604-222-7527. E-mail: adam@triumf.ca (M.J.A.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge the University of British Columbia for a Four Year Fellowship (E.B.), NORDION (Canada) and the Natural Sciences and Engineering Research Council (NSERC) of Canada for grant support, and Glen Bremner and Prof. Michael Wolf for their help with CV measurements. C.O. acknowledges the Canada Council for the Arts for a Killam Research Fellowship (2011−2013) and Caterina Ramogida for experimental assistance.

REFERENCES

(1) Anderson, C. J.; Welch, M. J. *Chem. Rev.* **1999**, *99*, 2219−2234.
(2) Bryan, J. N.; Jia, F.; Mohsin, H.; Sivaguru, G.; Miller, W. H.; Anderson, C. J. *Nucl. Med. Biol.* **2005**, *32*, 1473−1480.
(3) Wadas, T. J.; Wong, E. H.; Weisman, G. R.; Anderson, C. J. *Chem. Rev.* **2010**, *110*, 2858−2902.
(4) Wadas, T. J.; Wong, E. H.; Weisman, G. R.; Anderson, C. J. *Curr. Pharm. Des.* **2007**, *13*, 3−16.
(5) Sephton, R. G.; Hodgson, G. S.; De Abrew, S.; Harris, A. W. *J. Nucl. Med.* **1978**, *19*, 930−935.
(6) Heroux, K. J.; Woodin, K. S.; Tranchemontagne, D. J.; Widger, P. C. B.; Southwick, E.; Wong, E. H.; Weisman, G. R.; Tomellini, S. A.; Wadas, T. J.; Anderson, C. J.; Kassel, S.; Golen, J. A.; Rheingold, A. L. *Dalton Trans.* **2007**, *21*, 2150−2162.
(7) Wong, E. H.; Weisman, G. R.; Hill, D. C.; Reed, D. P.; Rogers, M. E.; Condon, J. P.; Fagan, M. A.; Calabrese, J. C.; Lam, K.-C.; Guzei, I. A.; Rheingold, A. L. *J. Am. Chem. Soc.* **2000**, *112*, 10561−10572.
(8) Bevilacqua, A.; Gelb, R. I.; Hebard, W. B.; Zompa, L. J. *Inorg. Chem.* **1987**, *26*, 2699−2706.
(9) Martell, A. E.; Motekaitis, R. J.; Clarke, E. T.; Delgado, R.; Sun, Y.; Ma, R. *Supramol. Chem.* **1996**, *6*, 353−363.
(10) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum Press: New York, 1989; Vol. *1*−*6*.
(11) Bernhardt, P. V.; Bramley, R.; Engelhardt, L. M.; Harrowfield, J. M.; Hockless, D. C.; Korybut-Daszkiewiecz, B. R.; Krausz, E. R.; Morgan, T.; Sargeson, A. M. *Inorg. Chem.* **1995**, *34*, 3589−3599.
(12) Woodin, K. S.; Heroux, K. J.; Boswell, C. A.; Wong, E. H.; Weisman, G. R.; Niu, W.; Tomellini, S. A.; Anderson, C. J.; Zakharov, L. N.; Rheingold, A. L. *Eur. J. Inorg. Chem.* **2005**, *25*, 4829−4833.
(13) DeNardo, S. J.; Liu, R.; Albrecht, H.; Natarajan, A.; Sutcliffe, J. L.; Anderson, C.; Peng, Li; Ferdani, R.; Cherry, S. R.; Lam, K. S. *J. Nucl. Med.* **2009**, *50*, 625−634.
(14) Wei, L.; Ye, Y.; Wadas, T. J.; Lewis, J. S.; Welch, M. J.; Achilefu, S.; Anderson, C. J. *Nucl. Med. Biol.* **2009**, *36*, 277−285.
(15) Jeong, J. M.; Hong, M. K.; Chang, Y. S.; Lee, Y.-S.; Kim, Y. J.; Cheon, G. J.; Lee, D. S.; Chung, J.-K.; Lee, M. C. *J. Nucl. Med.* **2008**, *49*, 830−836.
(16) Liu, S. *Bioconjugate Chem.* **2009**, *20*, 2199−2213.
(17) Boros, E.; Ferreira, C. L.; Cawthray, J. F.; Price, E. W.; Patrick, B. O.; Wester, D. W.; Adam, M. J.; Orvig, C. *Nucl. Med. Biol.* **2012**, DOI: 10.1016/j.nucmedbio.2012.01.003.
(18) Ferreiros-Martinez, R.; Esteban-Gomez, D.; Platas-Igesias, C.; de Blas, A.; Rodriguez-Blas, T. *Dalton Trans.* **2008**, 5754−5758.
(19) *SAINT*; Bruker AXS Inc.: Madison, WI, 1999.
(20) *SADABS*; Bruker AXS Inc.: Madison, WI, 1999.
(21) Boros, E.; Ferreira, C. L.; Cawthray, J. F.; Price, E. W.; Patrick, B. O.; Wester, D. W.; Adam, M. J.; Orvig, C. *J. Am. Chem. Soc.* **2010**, *132*, 15726−15733.
(22) Gran, G. *Analyst* **1952**, *77*, 661−671.
(23) Duckworth, P. private communication.
(24) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739−1753.
(25) Baes, C. F., Jr.; Mesmer, R. E. The Hydrolysis of Cations; Wiley-Interscience: New York, 1976.
(26) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; et al. *Gaussian 09*, Gaussian, Inc.: Wallingford, CT, 2009.
(27) Macrae, C. F.; Edgington, P. R.; McCabe, P.; Pidcock, E.; Shields, G. P.; Taylor, R.; Towler, M.; Streek, J. v. d. *J. Appl. Crystallogr.* **2006**, *39*, 453−457.
(28) Ait-Mohand, S.; Fournier, P.; Dumulon-Perreault, V.; Kiefer, G. E.; Jurek, P.; Ferreira, C. L.; Bénard, F.; Guérin, B. *Bioconjugate Chem.* **2011**, *22*, 1729−1735.