DOI: 10.1002/ejic.200500579

Kinetic Inertness and Electrochemical Behavior of Copper(II) Tetraazamacrocyclic Complexes: Possible Implications for in Vivo Stability

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Keywords: Macrocyclic ligands / N ligands / Copper

The kinetic inertness of copper(II) complexes of several carboxymethyl-armed cyclams and cyclens in 5 M HCl have been determined confirming that the complex derived from cross-bridged cyclam (Cu-CB-TE2A) is by far the most resistant to acid decomplexation. FT-IR studies in D_2O solution revealed its unique resistance to full carboxylate protonation and its retention of coordination by both pendant arms even in 1 M DCl. The X-ray structure of its monoprotonated form, [Cu-CB-TE2AH]$^+$, also established full coordination by both COO^- and COOH pendant arms in the solid state. Cyclic voltammograms of four carboxymethyl pendant-armed cyclam and cyclen complexes in aqueous solution were obtained with only Cu-CB-TE2A displaying a quasi-reversible Cu^{II}/Cu^{I} reduction wave. These indicators correlate with the superior in vivo behavior of this complex and its bifunctional conjugate.

Introduction

Carboxymethyl pendant-armed derivatives of tetraazamacrocycles are promising chelators for copper(II)-based radiopharmaceuticals.[1,2] The 14-membered macrocycle (cyclam) derivative TETA (Figure 1) has been evaluated as a ^{64}Cu-carrier by in vivo studies.[1] Despite its high in vitro thermodynamic stability, significant in vivo loss of radio-metal from both ^{64}Cu-TETA and its bifunctional conjugates was observed.[2] In addition to Cu^{II} dissociation from the chelator, its reduction and subsequent complex destabilization prior to Cu^{I} loss may also be a pathway for loss of radio-copper. The latter demetallation mode has been implicated in the selective trapping of ^{64}Cu from its bis(thiosemicarbazone) complexes in hypoxic cells.[3,4] We therefore hypothesize that *both* kinetic inertness to Cu^{II} dissociation in aqueous solution as well as resistance towards Cu^{II}/Cu^{I} reduction and subsequent Cu^{I} loss are useful indicators of desirable in vivo performance of a potential copper-based radiopharmaceutical.

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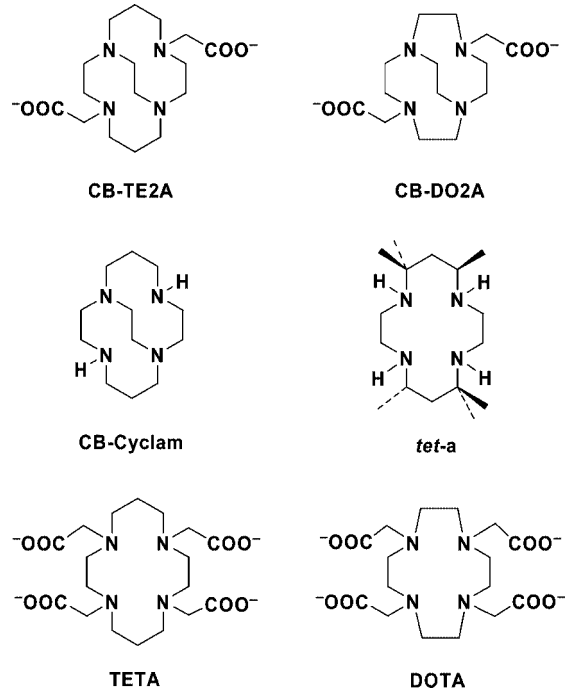

Figure 1. The cross-bridged ligands CB-TE2A, CB-DO2A, and CB-Cyclam with TETA, DOTA, and *tet*-a.

Recently we reported the superior in vivo behavior of a ^{64}Cu-labeled chelator CB-TE2A (Figure 1) and a bioconjugate compared to TETA, DOTA, as well as CB-DO2A analogues.[5–7] Briefly, the respective ligands were labeled

with ^{64}Cu and injected into rats, the animals were sacrificed at appropriate time points, and the biodistribution and metabolism of the labeled complexes assayed out to 20 h post-injection. It was found that ^{64}Cu-CB-TE2A and its bioconjugate showed a marked improvement in both clearance and resistance to demetallation. Presence in the chelator of both a cross-bridged cyclam backbone and at least one carboxymethyl pendant arm appears to be essential for this enhanced performance. To gain insight into these observations and to develop convenient indicators for bio-stability, we have initiated kinetic inertness as well as electrochemical studies of this and related copper complexes in aqueous media. We report herein the extraordinary kinetic inertness of Cu-CB-TE2A to acid decomplexation as well as its unique electrochemical behavior compared to Cu-TETA and their related cyclen analogues, Cu-CB-DO2A and Cu-DOTA (Figure 1).

Table 1. Half-lives of copper(II) complexes.

Complex	Half-life
5 M HCl, 90 °C	
Cu-CB-TE2A	154(6) h
Cu-TETA	4.5(5) min
Cu-DOTA	< 1 min
Cu-CB-cyclam	11.8(2) min
Cu-*tet*-a	1.1(1) h
5 M HCl, 50 °C	
Cu-TETA	3.2(1) h
Cu-*tet*-a	83.7(6) h
5 M HCl, 30 °C	
Cu-TETA	3.5(2) days
Cu-CB-cyclam	18.5(7) days
Cu-CB-DO2A	< 2 min
Cu-cyclam	2.7(1) days
1 M HCl, 30 °C	
Cu-CB-DO2A	4.0(1) h

Results and Discussion

The primary aim of this work is to develop a convenient and practical, albeit qualitative, assay of in vitro inertness for predicting the suitability of each new copper chelator in follow-up biological studies. Since aqueous acid-assisted decomplexation of polyazamacrocyclic copper complexes is a convenient and useful indicator of their kinetic inertness,[8,9] we chose to compare the decomplexation half-lives of Cu-CB-TE2A and analogues under pseudo first-order conditions in 5 M HCl (Table 1). As the detailed decomplexation mechanisms have not yet been elucidated, the data must be interpreted with caution. Nonetheless, we can readily conclude that Cu-CB-TE2A is by far the most kinetically inert amongst the carboxymethyl pendant-armed tetraazamacrocyclic complexes studied, with the relative order: Cu-CB-TE2A >> Cu-DOTA ≈ Cu-TETA > Cu-CB-DO2A. It is noteworthy that even the extremely robust red isomer of Cu-*tet*-a (Figure 1) decomposed more rapidly under these strongly acidic conditions.[8,10] Neither Cu-CB-Cyclam nor Cu-CB-DO2A showed comparable inertness to Cu-CB-TE2A, suggesting that both the carboxymethyl pendant arms and the bicyclo[6.6.2] cross-bridged cyclam backbone are essential for Cu-CB-TE2A's exceptional robustness. Sargeson and co-workers have reported encapsulated Cu^{II} complexes of hexaaza cage ligands which are also extremely inert to acid decomplexation,[11,12] although direct comparisons with Cu-CB-TE2A have not yet been made.

To gain insight into the speciation of these complexes in acidic aqueous media, FT-IR spectra of Cu-CB-TE2A, Cu-CB-DO2A, Cu-TETA, and Cu-DOTA in DCl/D_2O solutions were examined in the 1550–1800 cm^{-1} region. In 0.1 M DCl, all complexes revealed prominent ν_{asym}(COO) bands due to coordinated carboxylate (1598–1620 cm^{-1}) as well as protonated carboxylic acid (1688–1724 cm^{-1}) bands (Figure 2, a and Figure 3). While Cu-CB-DO2A decomposed in 1.0 M DCl within hours, both the Cu-TETA and Cu-DOTA spectra showed carboxylic acid bands (1685–1727 cm^{-1}) as the only major features (Figure 3). The splitting of these carboxylic acid bands is due to the presence of both coordinated as well as uncoordinated pendant arms. By contrast, the Cu-CB-TE2A complex exhibited both carboxylate (1611 cm^{-1}) and carboxylic acid (1702 cm^{-1}) bands (Figure 2, b). Since the free ligand CB-TE2A displayed distinct carboxylate (at pD 12, not shown) and carboxylic acid (1.0 M DCl, Figure 2, b) bands at 1574 and 1718 cm^{-1}, respectively, we can infer retention of *both* carboxylate and carboxylic acid pendant arm coordination at the copper center of Cu-CB-TE2A.

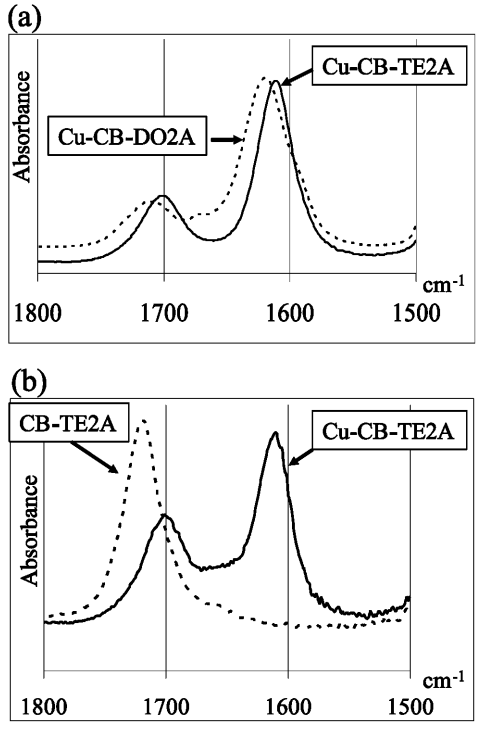

Figure 2. FT-IR spectra of: (a) Cu-CB-TE2A and Cu-CB-DO2A in 0.1 M DCl /D_2O, (b) Cu-CB-TE2A and CB-TE2A in 1.0 M DCl/D_2O.

Slow evaporation of a 0.1 M perchloric acid solution of Cu-CB-TE2A yielded dark-blue crystals of the monopro-

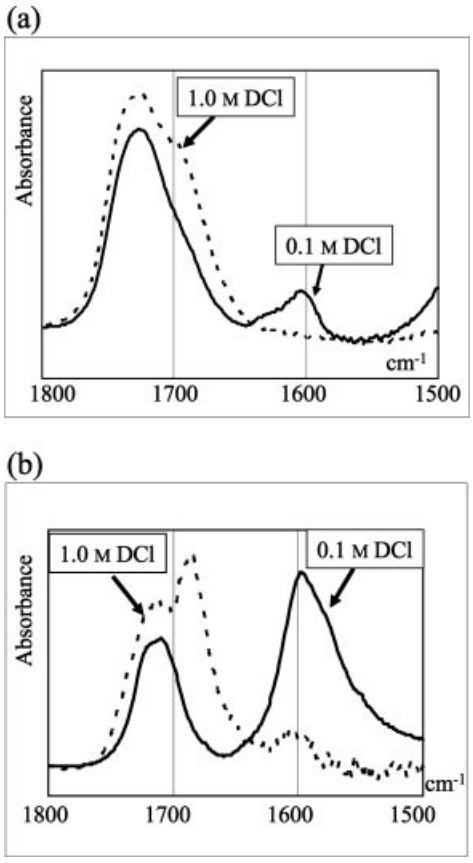

Figure 3. FT-IR spectra in DCl/D_2O solutions of: (a) Cu-DOTA, and (b) Cu-TETA.

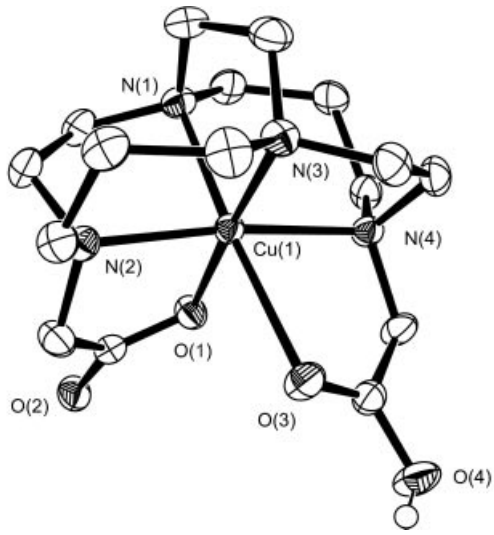

Figure 4. X-ray structure of $[Cu\text{-}CB\text{-}TE2AH]ClO_4$. All non-carboxylic acid protons and perchlorate anion omitted for clarity.

Table 2. Relevant bond lengths [Å] and angles [°] for $[Cu\text{-}CB\text{-}TE2AH]ClO_4$.

Cu(1)–O(1)	1.966(2)	Cu(1)–N(2)	2.057(2)
Cu(1)–O(3)	2.525(2)	Cu(1)–N(3)	2.016(2)
Cu(1)–N(1)	2.216(2)	Cu(1)–N(4)	2.046(2)
O(1)–Cu(1)–N(3)	177.03(8)	O(1)–Cu(1)–N(4)	90.07(4)
O(1)–Cu(1)–N(1)	95.32(7)	O(1)–Cu(1)–N(2)	84.03(7)
N(1)–Cu(1)–N(3)	86.36(8)	N(1)–Cu(1)–N(2)	85.02(8)
N(1)–Cu(1)–N(4)	99.43(7)	N(2)–Cu(1)–N(3)	98.58(8)
N(2)–Cu(1)–N(4)	172.92(8)	N(3)–Cu(1)–N(4)	87.26(8)

tonated complex $[Cu\text{-}CB\text{-}TE2AH]ClO_4$. Its X-ray structure (Figure 4) confirmed the weak coordination of the carboxylic acid pendant arm with a Jahn–Teller elongated Cu(1)–O(3) bond length of 2.525(2) Å as well as strong coordination of the remaining carboxylate arm with a much shorter Cu(1)–O(1) bond at 1.966(2) Å. The C(16)–O(3) distance of 1.219(3) Å relative to C(16)–O(4) of 1.306(3) Å confirmed carbonyl coordination of the COOH group. For comparison, the parent Cu-CB-TE2A complex has less pronounced Jahn–Teller elongated copper-carboxylate bond lengths of 2.30–2.33 Å and undistorted bonds of 2.00–2.01 Å.[13] In both structures, the metal cation fits snugly inside the cross-bridged cyclam ligand's cleft as indicated by nearly linear *trans*-N–Cu–N angles (173–182°). Additional structural data for the $[Cu\text{-}CB\text{-}TE2AH]^+$ cationic complex are listed in Table 2.

The solid-state IR spectrum of the monoprotonated complex displayed both coordinated carboxylate and carboxylic acid COO stretches at 1616 and 1694 cm^{-1}, respectively, reasonably close to the 1.0 M DCl solution values of 1611 and 1702 cm^{-1}. This further supports retention of both carboxylate and carboxylic acid pendant arm coordination of Cu-CB-TE2A in DCl solution. As acid-assisted decomplexation of carboxylate and phosphonate pendant-armed cyclam and cyclen metal complexes typically involves *O*-protonation pre-equilibria,[8,9,14–18] dissociation of protonated arms likely precedes rupture of Cu–N bonds. The 10-membered rings of the ligand adopt [2233] conformations rather than the distorted diamond-lattice [2323] conformation found in the unprotonated analogue. We propose that for protonated Cu-CB-TE2A, at least one coordinated carboxylic acid arm must dissociate from the metal before any Cu–N bond cleavage can occur. Even then, such bond breaking is discouraged by the cross-bridged cyclam's low-energy [2233] or [2323] solution conformation. Indeed, such a ruptured Cu–N bond is predisposed to reform the original complex. Consistent with this rationale, Cu-CB-Cyclam is almost an order of magnitude more kinetically inert than Cu-Cyclam (Table 1).

Since Cu^I has significantly different coordination preferences than Cu^{II}, in vivo reduction of stable Cu^{II} complexes to form Cu^I products can lead to demetallation or disproportionation in an alternative decomplexation pathway. We carried out cyclic voltammetry experiments on Cu-CB-TE2A, Cu-TETA, Cu-DOTA, and Cu-CB-DO2A in 0.1 M aqueous sodium acetate solution adjusted to pH 7 (Figure 5).

Both Cu-TETA and Cu-CB-DO2A yielded irreversible reduction voltammograms. Cu-DOTA gave both a broad reduction peak at –1.00 V (vs. Ag/AgCl) and an attenuated oxidation wave at –0.39 V in the return scan. By contrast, a quasi-reversible reduction was observed for Cu-CB-TE2A at –1.08 V with a peak separation of 120 mV. This voltammogram was reproducible upon repeated cycling between

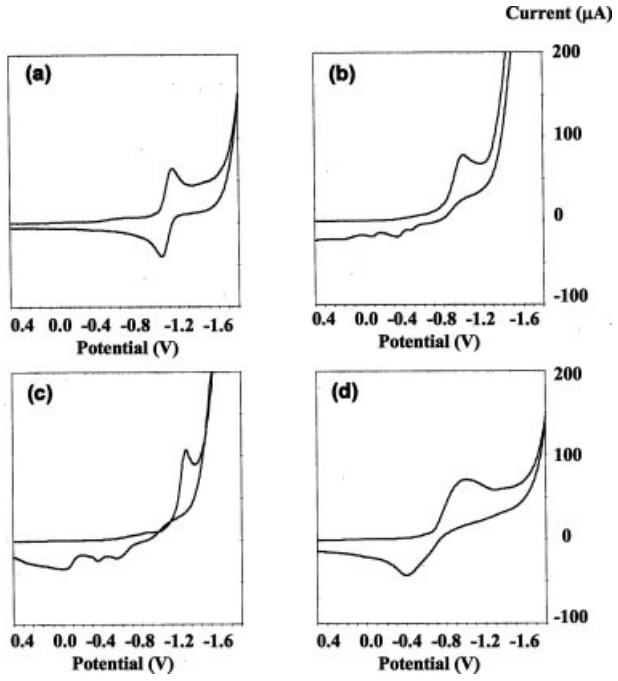

Figure 5. Cyclic voltammograms of (a) Cu-CB-TE2A, (b) Cu-CB-DO2A, (c) Cu-TETA, and (d) Cu-DOTA in 0.1 M sodium acetate, pH 7.0.

+0.8 V and –1.4 V. An increase of the scan rate (ν) resulted in an increased peak separation. A differential pulse voltammogram (0.0 V to –1.4 V at v = 20 mV/s) further confirmed a well-defined reduction peak at –1.06 V. We were also able to observe a similar quasi-reversible reduction of this complex in DMSO ($E_{1/2}$ = –1.23 V, ΔE = 162 mV). The higher chemical reversibility ($i_{pa}/i_{pc} \approx 0.8$) of this redox couple suggests that the reduction product, Cu^{I}-CB-TE2A, must be longer-lived compared to its analogues. Thus none of the related ligands DOTA, TETA, or CB-DO2A appear to be able to adapt to the coordination requirements of Cu^{I} and stabilize it. In contrast, the ability of a dibenzyl derivative of cross-bridged cyclam to accommodate the disparate coordination preferences of both a cuprous as well as a cupric cation has already been demonstrated.[19] Efforts to isolate and characterize the Cu^{I} complex of CB-TE2A are in progress.

Conclusions

Taken together, the relative kinetic inertness, FT-IR and electrochemical data single out the unique properties of Cu-CB-TE2A compared to its closely-related Cu-TETA, Cu-DOTA, and Cu-CB-DO2A analogues. This particular combination of a cross-bridged cyclam backbone together with carboxymethyl pendant arms stabilizes the complex towards *both* acid-assisted and reductive decomplexation and correlate well with the improved in vivo performance of ^{64}Cu-CB-TE2A. Further investigations to confirm these correlations in parallel with biological studies to gain insight into the in vivo mechanism of metal dissociation from ^{64}Cu-labeled tetraazamacrocyclic complexes are underway.

Experimental Section

Caution! Although we encountered no problems, perchlorate salts of organic ligands are potentially explosive and should be handled with care and only in small quantities. All copper(II) complexes of carboxymethyl pendant-armed tetraazamacrocyclic ligands were prepared according to published literature procedures: Cu-TETA,[20] Cu-DOTA,[20] Cu-CB-TE2A,[13] and Cu-CB-DO2A.[6]

Acid-Decomplexation Studies: Sample concentrations of copper complexes studied were between 1–3 mM. Each complex's visible electronic spectrum in 5.0 M HCl at 90 °C was recorded at specific time points using a Cary 50 Bio UV/Vis spectrophotometer. In each case, isosbestic spectra were obtained indicative of a single decomplexation product. The decreasing absorbance at the λ_{max} of each spectrum (Cu-CB-TE2A 529 nm, Cu-CB-DO2A 606 nm, Cu-TETA 615 nm, Cu-DOTA 725 nm, Cu-*tet-a* 516 nm, Cu-cyclam 510 nm, and Cu-CB-cyclam 587 nm) was used to monitor the progress of the decomplexation reaction. Half-lives were calculated from the slopes of the linear *ln*(absorbance) vs. time plots.

Solution FT-IR Spectra: FT-IR absorbance spectra in D_2O solutions were obtained in a barium fluoride cell (0.01 cm pathlength) with a Nicolet 520 FT-IR spectrophotometer. Absorbance spectra were produced by ratioing the single beam spectra of the samples to the corresponding solvents. All spectra were acquired at a nominal 2 cm^{-1} resolution (using a liquid nitrogen-cooled MCT detector).

Synthesis of [Cu-CB-TE2AH]ClO_4: A sample of Cu-CB-TE2A was dissolved in 0.1 M $HClO_4$ and the solvent was evaporated from a beaker to yield dark-blue crystals suitable for X-ray studies. Analytical data for [Cu-CB-HTE2A]ClO_4 $C_{16}H_{29}ClCuN_4O_8(H_2O)_{0.5}$ (513.44): calcd. C 37.43, H 5.89, N 10.91, Cl 6.91; found C 37.47, H 5.78, N 10.82, Cl 6.69. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 3500, 3000–2800,

Table 3. Crystal and refinement data for [Cu-CB-TE2AH]ClO_4.

Formula	$C_{16}H_{29}ClCuN_4O_8$
Mol. mass	504.42
T (K)	213(2)
Crystal system	monoclinic
Space group	$P2_1/n$
a [Å]	7.9631(4)
b [Å]	25.4584(11)
c [Å]	10.0100(5)
α [°]	90.00
β [°]	93.0010(10)
γ [°]	90.00
V [Å^3]	202.52(17)
Z	4
F(000)	1052
$D_{calcd.}$ [g/cm^3]	1.653
Scan type	phi and omega scans
λ [Å]	0.71073
μ [mm^{-1}]	1.263
Crystal size [mm^3]	0.35×0.26×0.21
Index ranges	h: –10; 10
	k: –32; 32
	l: –12; 13
Absorption correction	SADABS
RC = reflections collected	14593
IRC = independent RC	4749
IRCGT = IRC and $I > 2\sigma(I)$	4386
Refinement method	full-matrix least-squares on F^2
Data/parameters	4749/387
R for IRCGT	R_1 = 0.0459, wR_2 = 0.1062
R for IRC	R_1 = 0.0419, wR_2 = 0.1038
Goodness-of-fit	1.096

1694 (ν_{COOH}), 1616 (ν_{COO}), 1123, 1099, 1088, 623. UV/Vis (H_2O): λ_{max} = 621 nm (44 $M^{-1} cm^{-1}$).

X-ray Structural Determination of [Cu-CB-TE2AH]ClO_4: A blue block crystal was found to produce the crystal and refinement data listed in Table 3. All non-hydrogen atoms were refined anisotropically and all hydrogen atoms were treated as idealized contributions. All software used is contained in libraries maintained by Bruker-AXS (Madison, WI).

CCDC-262407 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Electrochemical Studies: Cyclic voltammograms were obtained with an EG&G PAR Model 263A scanning potentiostat with PowerCV data collection and analysis software. A three-electrode cell under argon was used with a glassy carbon disk working electrode, platinum auxiliary electrode, and silver/silver chloride reference electrode. Samples (1 mM) were run in 0.1 M aqueous sodium acetate adjusted to pH 7.0 with glacial acetic acid at scan rates of v = 100 mV/s. Samples in DMSO solution were run using 0.1 M tetra-*n*-butylammonium hexafluorophosphate electrolyte. Differential pulse voltammograms were run with a BAS Model 100B electrochemical system equipped with a PC utilizing BAS software. A similar three-electrode system was used and the scan rate was 20 mV/s.

Acknowledgments

We acknowledge financial support for this research from NIH CA93375 (E. H. W., G. R. W., and C. J. A.), UNH-UROP (K. S. W. and K. J. H.), an NIH-sponsored Chemistry-Biology Interface Training Grant (GM08785-01; C. A. B.), and a U.S. DOE Nuclear Medicine Education Training Grant (DE F0101 NE23051; C. A. B.). We also acknowledge Mr. Evan Southwick's contributions to part of the acid-decomplexation data.

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Received: June 29, 2005
Published Online: October 12, 2005

