Electronic Supplementary Information (ESI)

Revival of TE2A; a better chelate for Cu(II) ions than TETA?

Darpan N. Pandya,[a] Jung Young Kim,[b] Jeong Chan Park,[a] Hochun Lee,[c] Prasad B. Phapale,[d] Wonjung Kwak,[a] Tae Hyun Choi,[b] Gi Jeong Cheon,[b] Young-Ran Yoon[d] and Jeongsoo Yoo*[a]

[a] Department of Molecular Medicine and Nuclear Medicine, Kyungpook National University, Daegu 700-422, Republic of Korea, Tel: +82-53-420-4947, Fax: +82-53-426-4944, E-mail: yooj@knu.ac.kr

[b] Molecular Imaging Research Center, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Republic of Korea

[c] Department of Applied Chemistry, Kumoh National Institute of Technology, Yangho-dong 1, Gumi, Kyeongbuk 730-701, Republic of Korea

[d] Department of Molecular Medicine, Kyungpook National University School of Medicine & Clinical Trial Center, Kyungpook National University Hospital, Daegu 700-422, Republic of Korea

Materials and methods: Cyclam, TETA and DOTA were purchased from CheMatech, France. All other reagents and solvents were purchased from Sigma-Aldrich and were used as received. Copper-64 was produced at KIRAMS, Korea by the $^{64}Ni(p,n)^{64}Cu$ nuclear reaction. CB-TE2A was synthesized by the modified method of reported procedure.

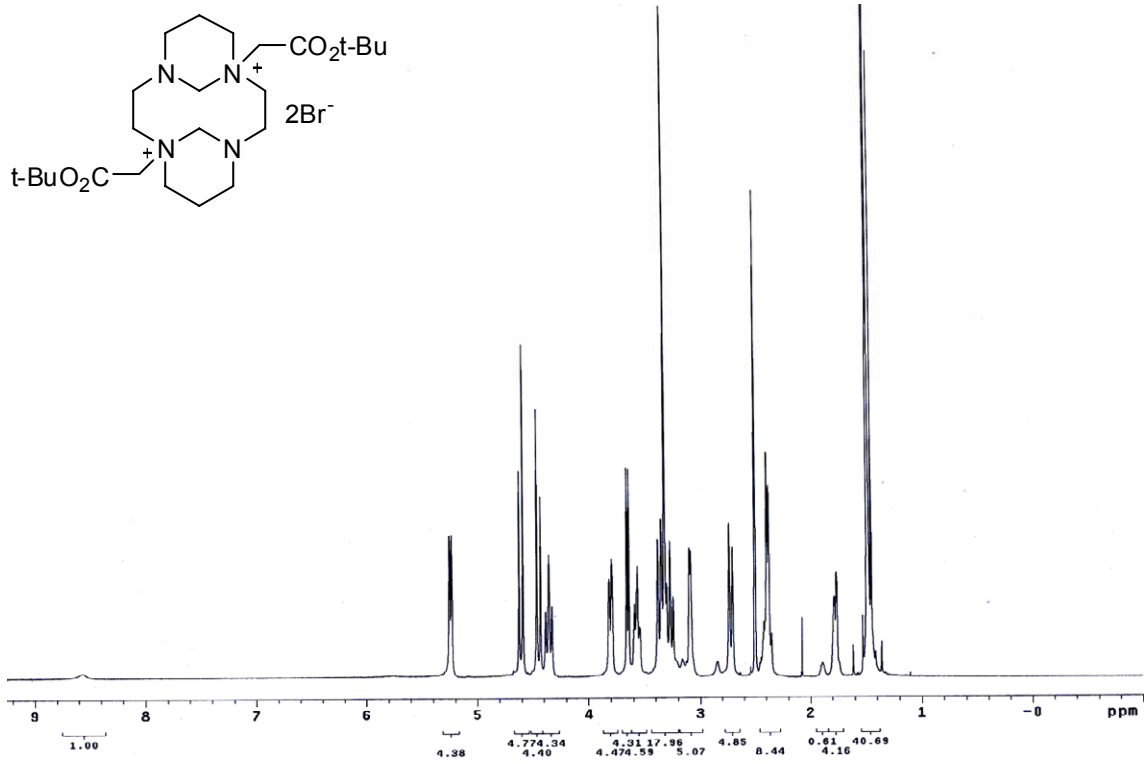
Instrumention: All ^{1}H NMR and ^{13}C NMR spectra were measured on Varian Unity Inova 500 MHz instrument. High–resolution mass spectra (HRMS) were recorded on JEOL JMS700 or Quattro Premier XE mass spectrometer. Elemental analyses were carried out at KAIST and Kyungpook National University, Korea. UV-Vis spectra were acquired on a Shimadzu UV-Vis spectrophotometer (UV-1650PC). Analytical HPLC traces were acquired using Waters 600 series HPLC system and Waters Xbridge C18 column (4.6 X 150 mm, 5 ìm) with an isocratic method (30 mM citric acid, 1 mL/min flow rate).

Synthesis of 1,4,8,11-Tetraazatricyclo[9.3.1.1^{4,8}]-hexadecane (2). The bisaminal compound **2** was prepared by modification and scale-up of the previously reported method by R. Guilard, C. Lecomte *et al*. Briefly, two equivalent of formaldehyde (15.1 mL, 37% in water) was added rapidly to an aqueous solution of cyclam, **1** (20.3 g, 0.10 mol in 200 mL) at 0-5°C. Then the reaction mixture was allowed to warm up to room temperature and stirred for 2 h. The reaction mixture was again cooled to 0-5°C, then the white precipitate formed was filtered and washed with chilled water (2 X 10 mL). The resulting white solid was taken up in $CHCl_3$ (200 mL) and dried over $MgSO_4$. Complete evaporation of $CHCl_3$ under reduced pressure gave a white solid **2** (20.95 g, 92% yield). Spectroscopic data of Compound **2** was exactly matched with the earlier reported one.

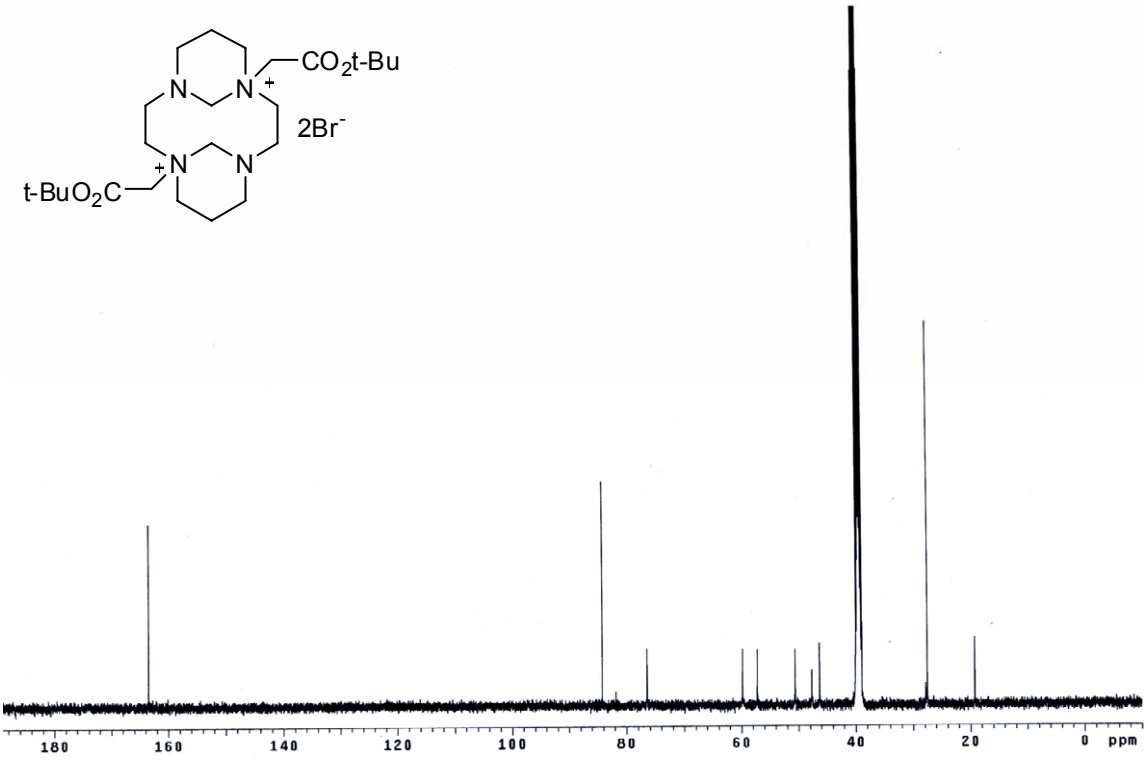
Synthesis of 1,8-N,N'-bis-(carbo-*tert*-butoxymethyl)-4,11-diazoniatricyclo[9.3.1.1^{4,8}] hexadecane dibromide (3). Four equivalent of *t*-butylbromoacetate (1.70 g, 8.74 mmol) was added in one portion to a stirred solution of **2** (0.49g, 2.18 mmol) in MeCN (20 mL). The reaction mixture was stirred at room temperature for 24h. The yellowish white precipitate formed was then filtered, washed with MeCN (2 X 10 mL) and dried under vacuum. The crude product was recrystallized in ethanol to give white solid **3** (1.27 g, 95% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.48(s, 18H), 1.76-1.78(d, 2H, J = 8.5 Hz), 2.35-2.45(m, 4H), 2.70-2.73(d, 2H, J = 15Hz), 3.08-3.09(d, 2H, J = 5Hz), 3.24-3.38(m, 4H), 3.53-3.58(m, 2H),

3.64-3.66(d, 2H, *J* = 10Hz), 3.79-3.81(d, 2H, *J* = 11.5Hz), 4.33-4.38(t, 2H, *J* = 14Hz), 4.43-4.46(d, 2H, *J* = 16.5 Hz), 4.59-4.62(d, 2H, *J* = 16.5 Hz), 5.23-5.25(d, 2H, *J* = 9.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.5, 84.2, 76.5, 59.8, 57.2, 50.6, 47.7, 46.3, 27.5, 19.2**;** HRMS (ESI) calculated for $C_{24}H_{47}N_4O_4$, 455.3597 [$(M+H)^+$] , found: 455.3594 [$(M+H)^+$]; Anal. Calcd. For $C_{24}H_{46}Br_2N_4O_4$.: C 46.91, H 7.55, N 9.12; Found C 46.63, H 7.74, N 9.21.

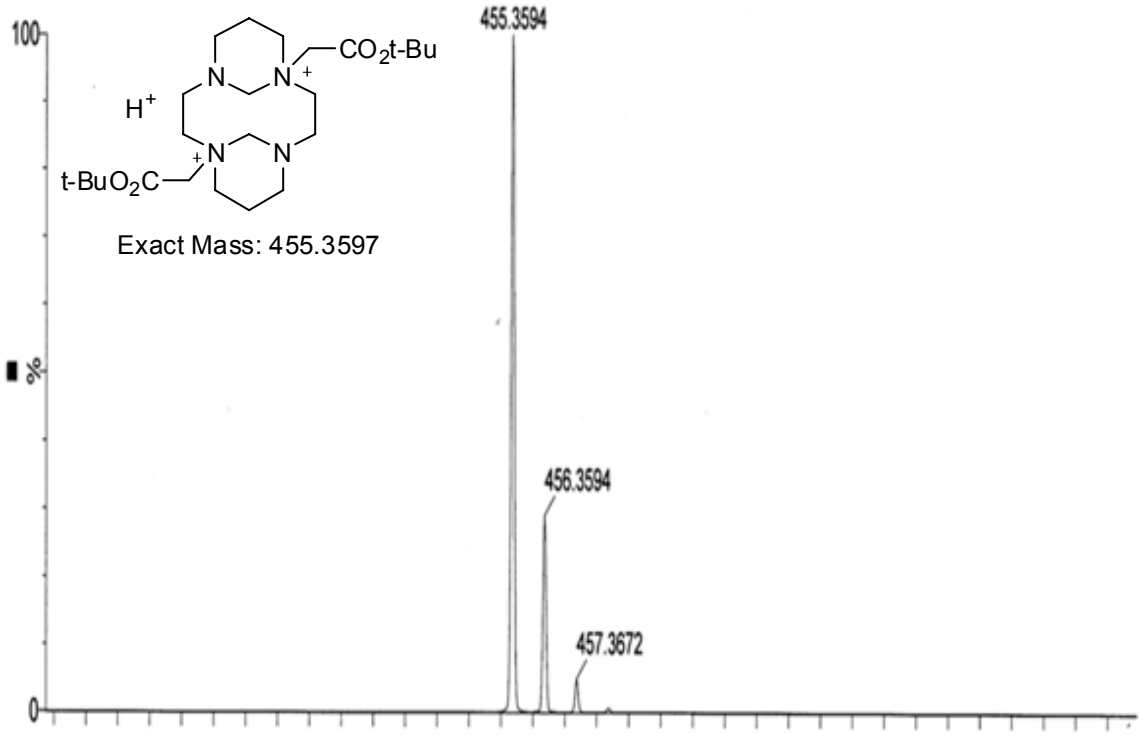
^{1}H-N.M.R. (DMSO-d_6 -500 MHz)



^{13}C-N.M.R. (DMSO-d_6 -500 MHz)

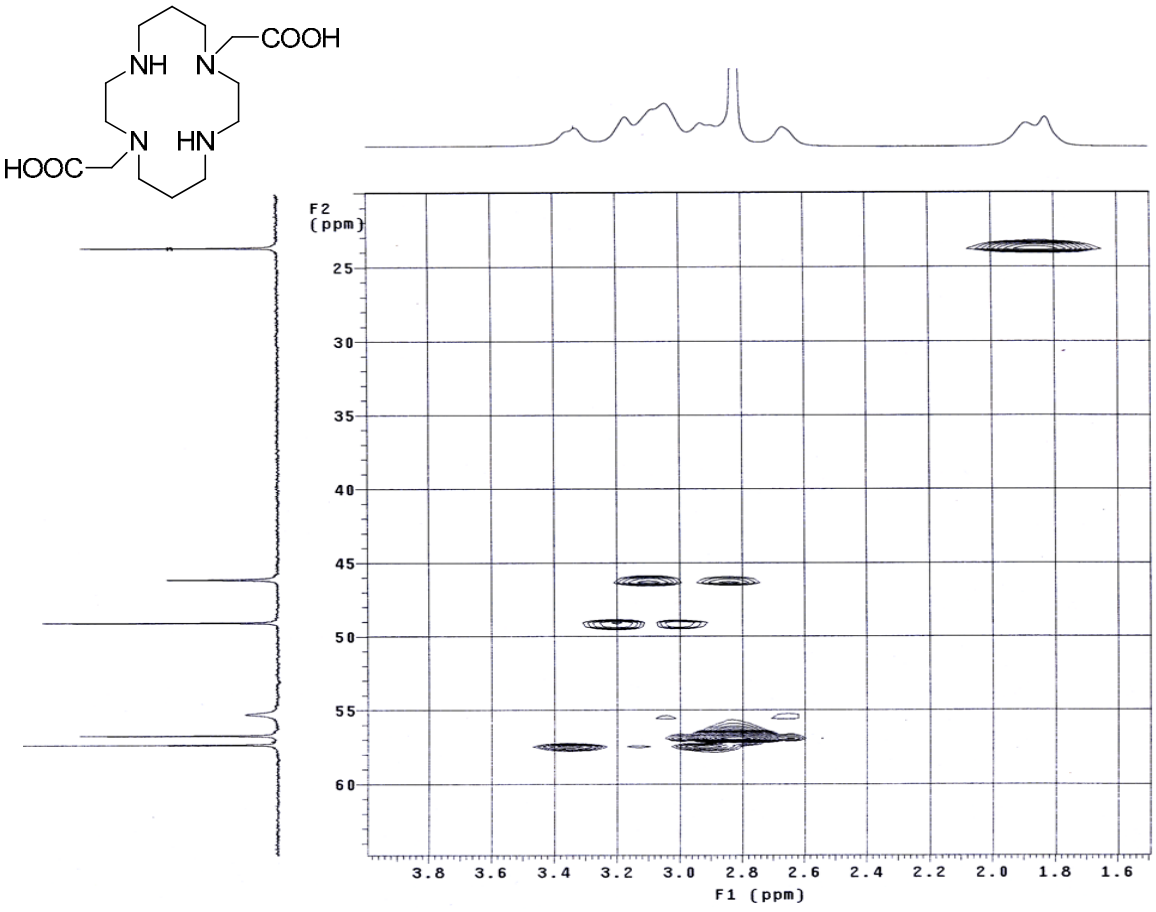


HRMS

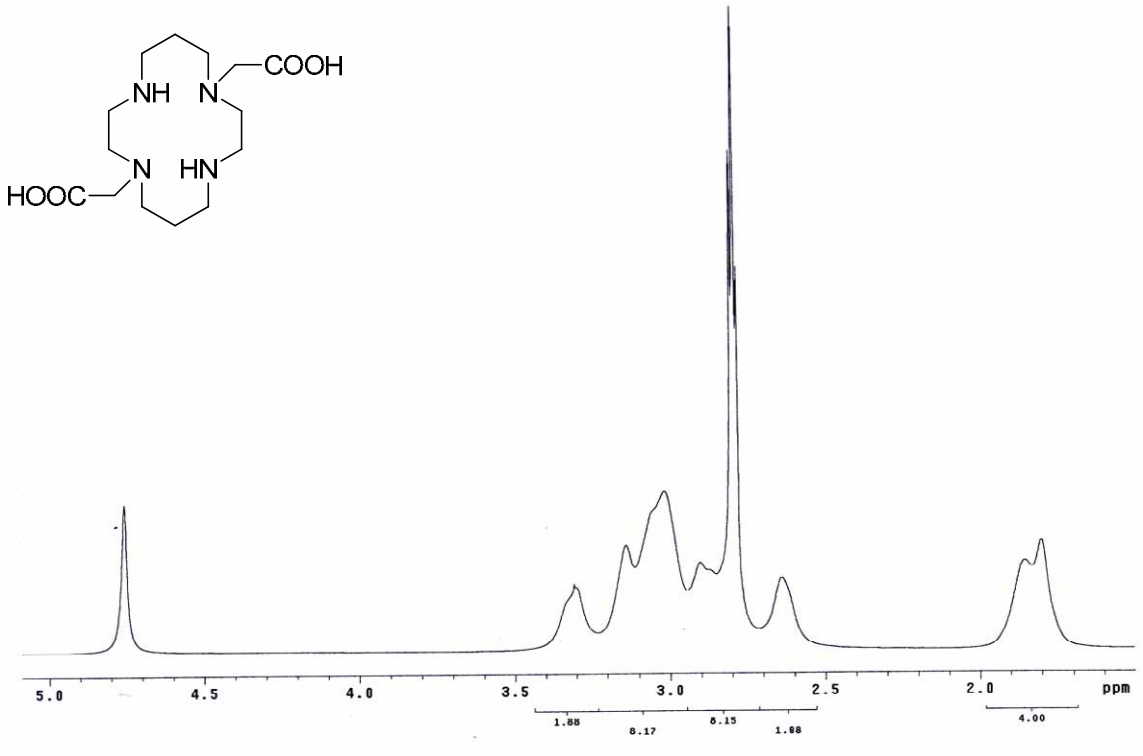


Synthesis of 1,8-*N*,*N*'-bis-(carboxymethyl)-1,4,8,11-tetraazacyclotetradecane, TE2A (4). KOH (0.55 g, 9.76 mmol) was added to a stirred solution of **3** (1.2 g, 1.95 mmol) in EtOH/H_2O (1:1, 20 mL) mixture. The reaction was stirred at 80°C for 8 h. After complete evaporation of all solvents, the resulting yellowish white solid was dissolved in the minimum amount of water. Then the resulting solution was loaded on a DOEWX 1X8 (OH^- form) anion exchange resin and washed with water first, then the product was eluted by 0.5 M HCl. The complete dryness of water from acidic eluates afforded the hydrochloride salt of TE2A as a white solid (0.89 g, 98% yield) ^{1}H NMR (500 MHz, D_2O): δ 3.31(brs, 2H), 3.22-2.95(m, 8H), 2.94-2.72(m, 8H), 2.64(brs, 2H) 1.83(brs, 4H); ^{13}C NMR (125 MHz, D_2O): δ 180.9, 57.5, 56.9, 55.4, 49.2, 46.2, 23.7; HRMS (FAB): Calculated for $C_{14}H_{29}N_4O_4$, 317.2189[$(M+H)^+$] Found: 317.2185 [$(M+H)^+$]; Anal Calcd For $C_{14}H_{28}N_4O_4.3.7HCl.H_2O$: C 35.83, H, 7.24, N 11.94, Found: C 35.81, H 7.42, N 12.06.

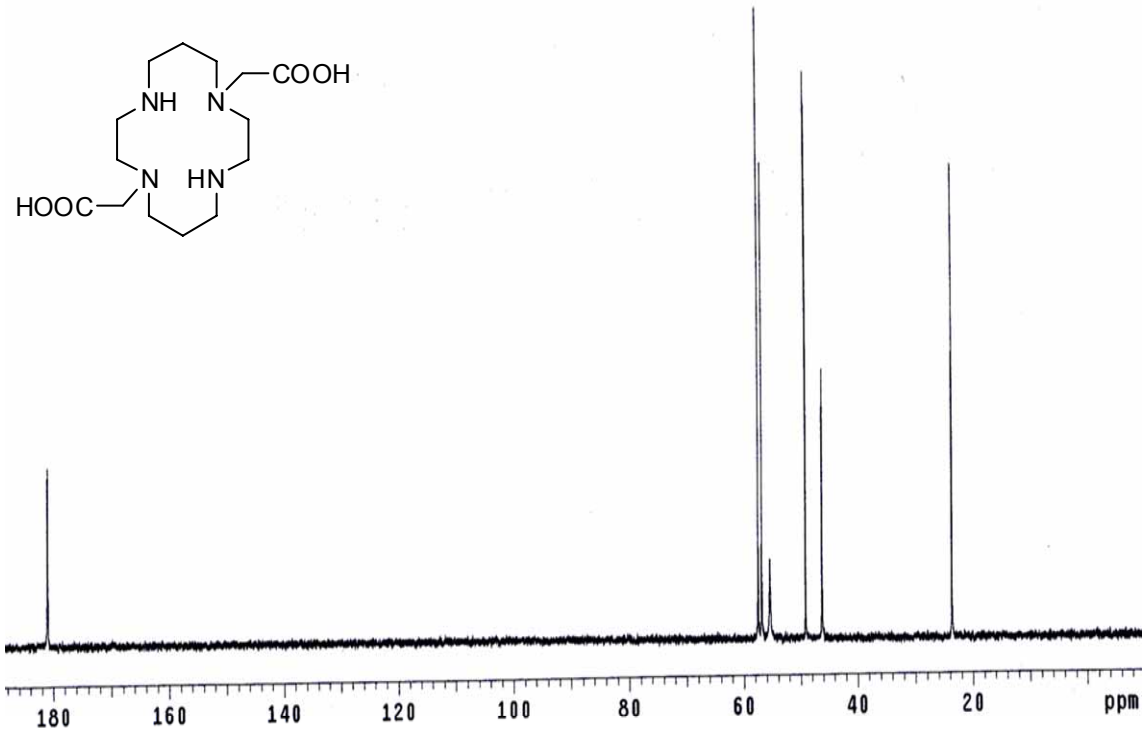
2D-N.M.R. (D_2O -500 MHz)



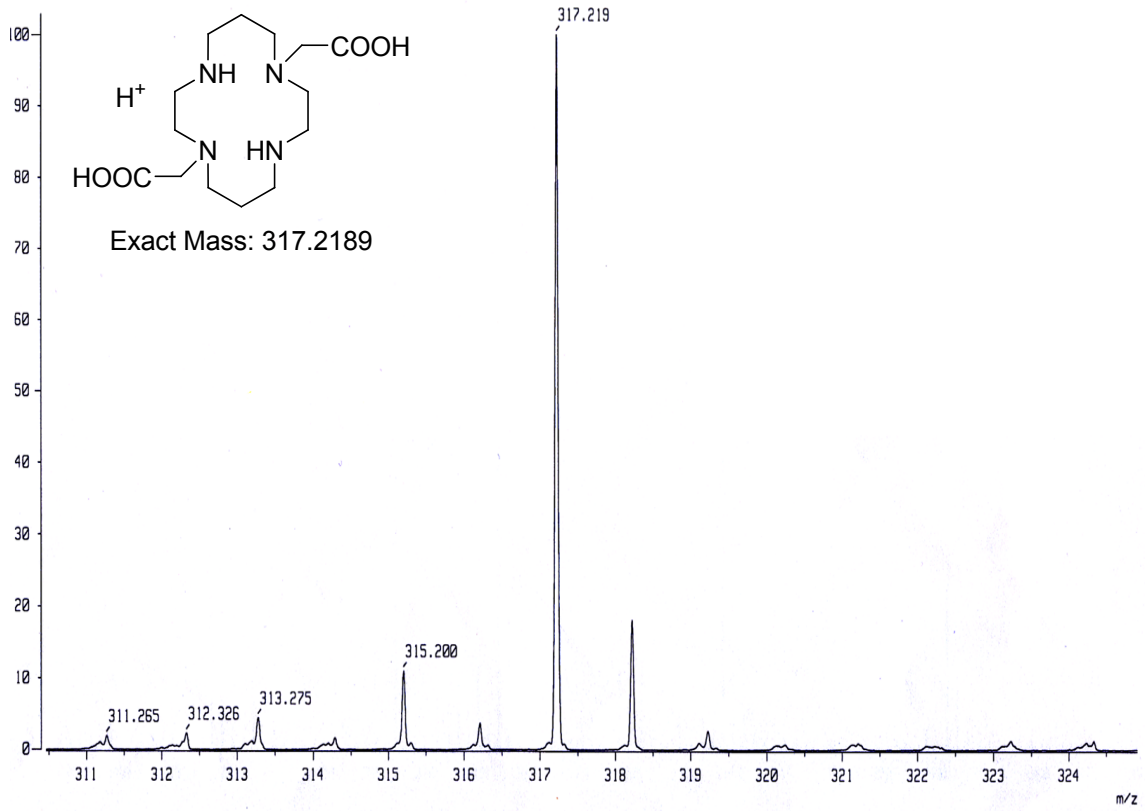
^{1}H-N.M.R. (D_2O -500 MHz)



^{13}C-N.M.R. (D_2O -125 MHz)



HRMS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N
H+
N
HN
HOOC
Exact Mass: 317.2189
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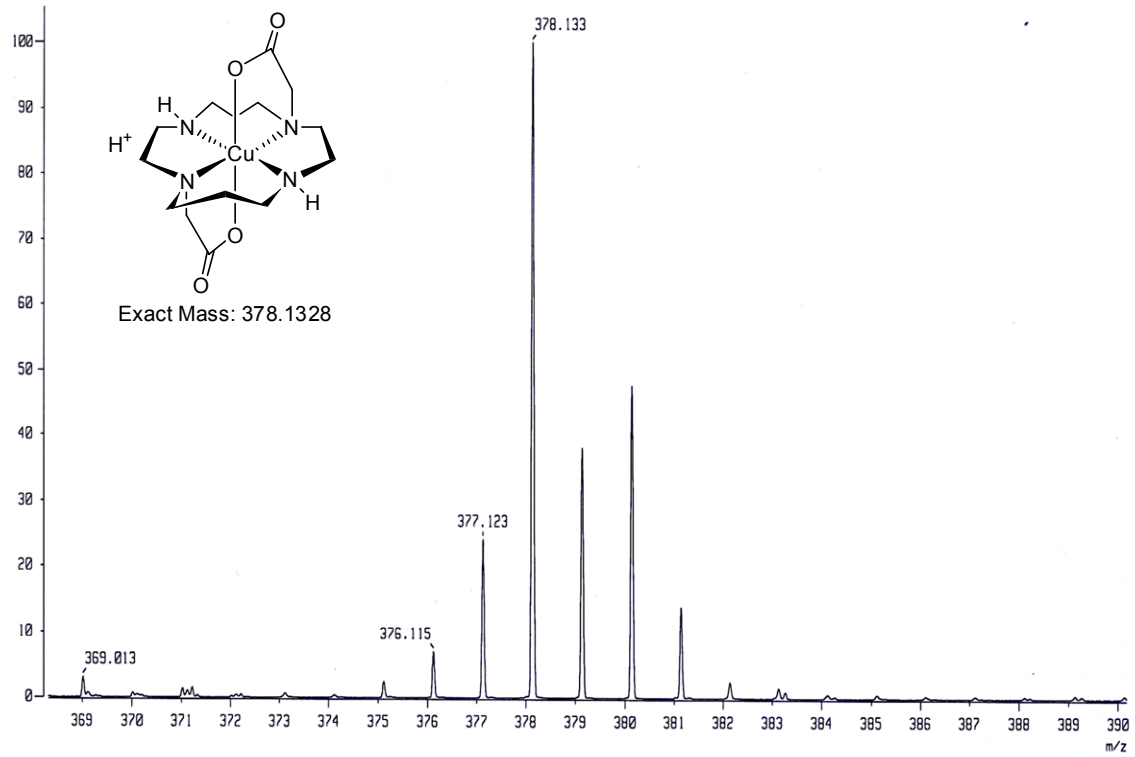
311.265
312.326
313.275
100
90
80
70
60
50
40
30
20
10
0
311
312
313
314
315
316
317
318
319
320
321
322
323
324
m/z

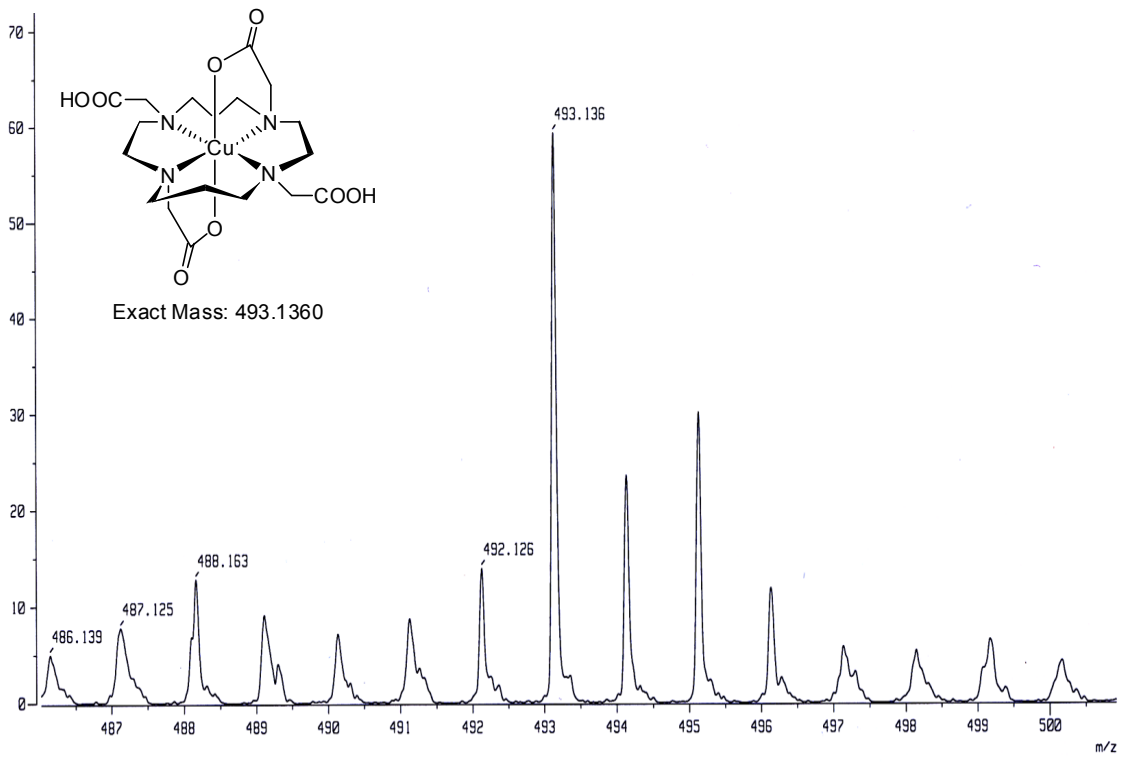
Synthesis of Cu-TE2A. To a solution of TE2A (**4**) (227 mg, 0.48 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (178 mg, 0.48 mmol) in 20 mL of methanol was added 1M aqueous solution of NaOH (2.88 mmol). The resulting clear blue solution was refluxed for 2h, cooled, and filtered through celite bed. The filtrate was subjected to diethyl ether diffusion. The deposited blue crystals were collected and dried. (223 mg, 89% yield). HRMS (FAB): Calculated for $C_{14}H_{27}CuN_4O_4$, 378.1328 $[(M+H)^+]$ Found: 378.1332 $[(M+H)^+]$; Anal Calcd For $C_{14}H_{26}CuN_4O_4 \cdot NaClO_4 \cdot H_2O$: C 32.44, H, 5.44, N 10.81, Found: C 32.74, H 5.48, N 10.26. Visible electronic spectrum: λ_{max} (H_2O)/568 nm (ε = 71 $M^{-1}cm^{-1}$); λ_{max} (5 M HCl)/549 nm (ε = 80 $M^{-1}cm^{-1}$).

HRMS



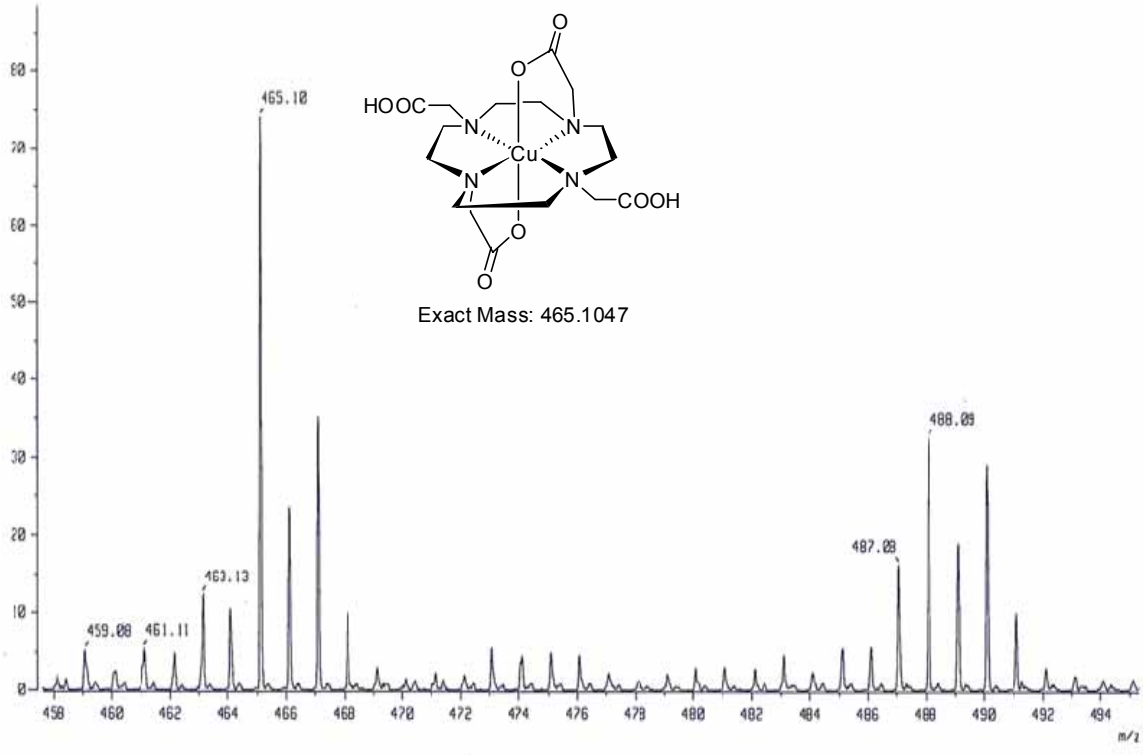
Synthesis of Cu-TETA. To a solution of TETA (235 mg, 0.54 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (201 mg, 0.54 mmol) in 18 mL of methanol was added 1M aqueous solution of NaOH (3.24 mmol). The resulting clear blue solution was refluxed for 2h, cooled, and filtered through celite bed. The filtrate was subjected to diethyl ether diffusion. The deposited blue crystals were collected and dried. (344 mg, 90% yield). HRMS (FAB): Calculated for $C_{18}H_{30}CuN_4O_8$, 493.1360[$(M)^+$] Found: 493.1356 [$(M)^+$]; Anal Calcd For $C_{18}H_{30}CuN_4O_8 \cdot 1.45NaClO_4 \cdot 1.8H_2O$: C 30.71, H, 4.81, N 7.96, Found: C 30.73, H 4.93, N 7.73. . Visible electronic spectrum: λ_{max} (H_2O)/640 nm (ε = 102 $M^{-1}cm^{-1}$); λ_{max} (5 M HCl)/615nm (ε = 97 $M^{-1}cm^{-1}$).

HRMS



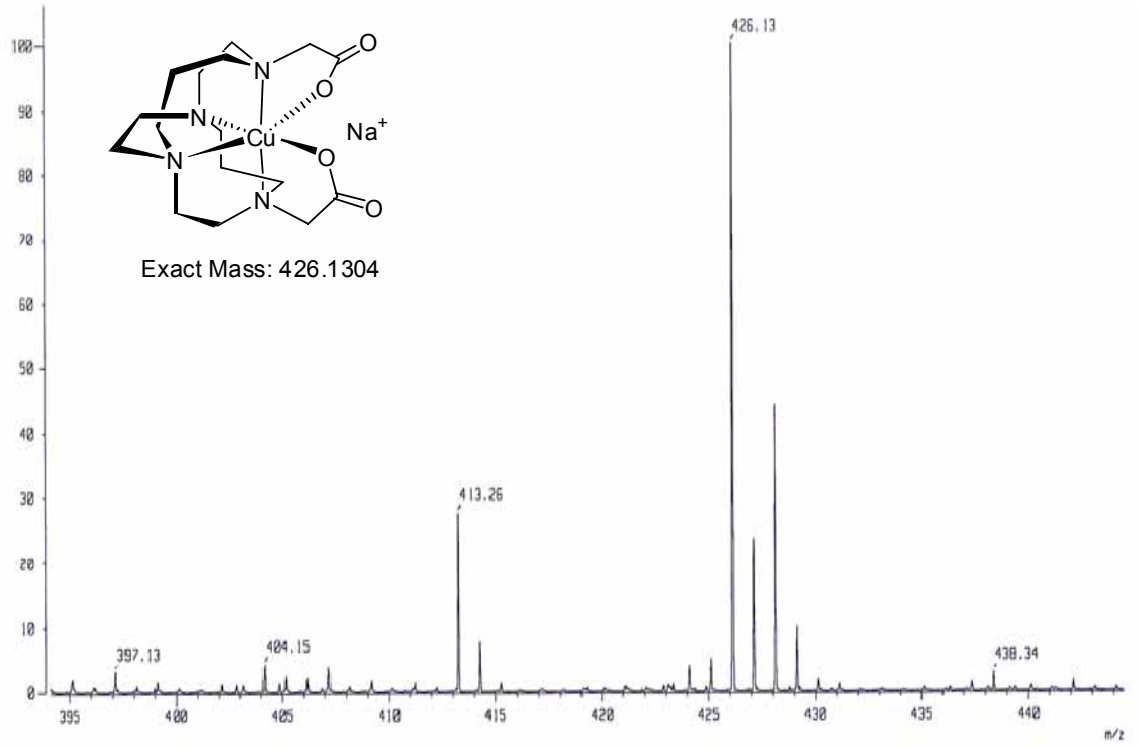
Synthesis of Cu-DOTA. To a solution of DOTA (215 mg, 0.53 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (197 mg, 0.53 mmol) in 20 mL of methanol was added 1M aqueous solution of NaOH (3.18 mmol). The resulting clear blue solution was refluxed for 2h, cooled, and filtered through celite bed. The filtrate was subjected to diethyl ether diffusion. The deposited blue crystals were collected and dried. (344 mg, 90% yield). HRMS (FAB): Calculated for $C_{16}H_{26}CuN_4O_8$, 465.1047$(M)^+$] Found: 465.1045 [$(M)^+$]; Anal Calcd For $C_{18}H_{30}CuN_4O_8 \cdot 1.45NaClO_4 \cdot 1.8H_2O$: C 30.71, H, 4.81, N 7.96, Found: C 30.73, H 4.93, N 7.73. Visible electronic spectrum: λ_{max} (5 M HCl)/689nm (ε = 571 $M^{-1}cm^{-1}$).

HRMS

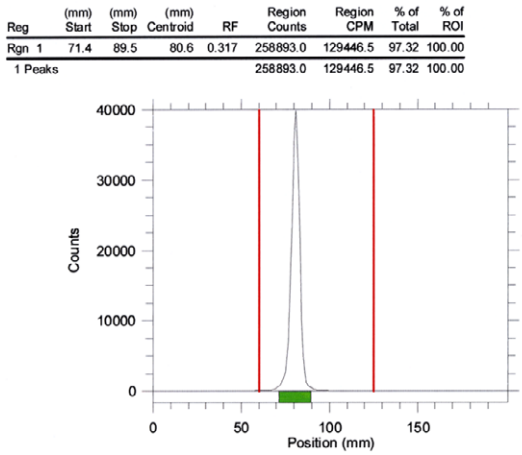


Synthesis of Cu-CB-TE2A. To a solution of CB-TE2A (208 mg, 0.61 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (226 mg, 0.61 mmol) in 20 mL of methanol was added 1M aqueous solution of NaOH (3.66 mmol). The resulting clear blue solution was refluxed for 2h, cooled, and filtered through celite bed. The filtrate was subjected to diethyl ether diffusion. The deposited blue crystals were collected and dried. (297 mg, 86% yield). HRMS (FAB): Calculated for $C_{16}H_{28}CuN_4NaO_4$, 426.1304 $[(M+Na)^+]$ Found: 426.1308 $[(M+Na)^+]$; Anal Calcd For $C_{16}H_{28}CuN_4O_4 \cdot 1.2NaClO_4 \cdot H_2O$: C 33.78, H 5.32, N 9.85, Found: C 33.89, H 5.42, N 9.88. Visible electronic spectrum: λ_{max} (5 M HCl)/624nm ($\varepsilon = 91\ M^{-1}cm^{-1}$).

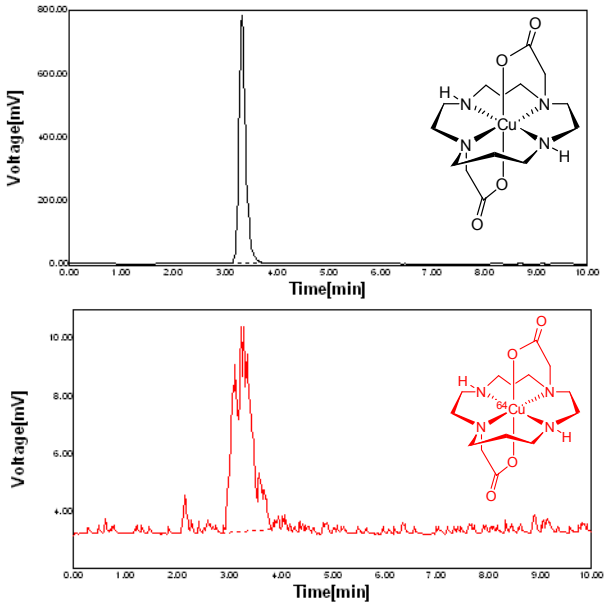
HRMS



Radio labeling of TE2A with ^{64}Cu. Complexation of the ^{64}Cu with TE2A was achieved by the addition of no-carrier added $^{64}CuCl_2$ in 0.01 N HCl (5 ìL, 0.1-0.5 mCi) to a 100 ìL of 5 mM solution of the ligand TE2A in 0.1M ammonium acetate (pH = 6.8) followed by 20 min incubation at 30°C. Formation of the complex was verified by radio TLC using a mobile phase 1:1 Methanol: 10% ammonium acetate on a silica plate. Radio-HPLC analysis of ^{64}Cu-TE2A was accomplished using Xbridge C18 column (4.6 X 150 mm, 5 ìm) with an isocratic method (30 mM citric acid, 1 mL/min flow rate)

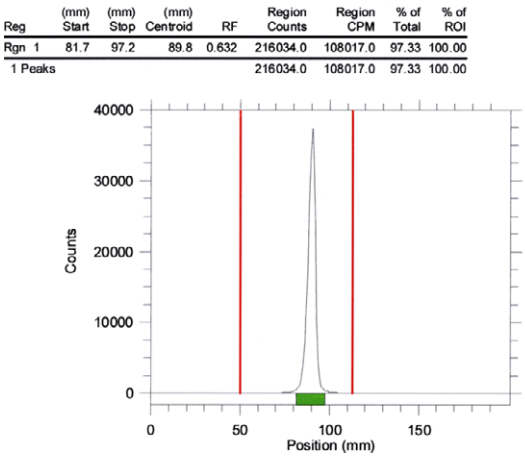


Radio-TLC of ^{64}Cu-TE2A

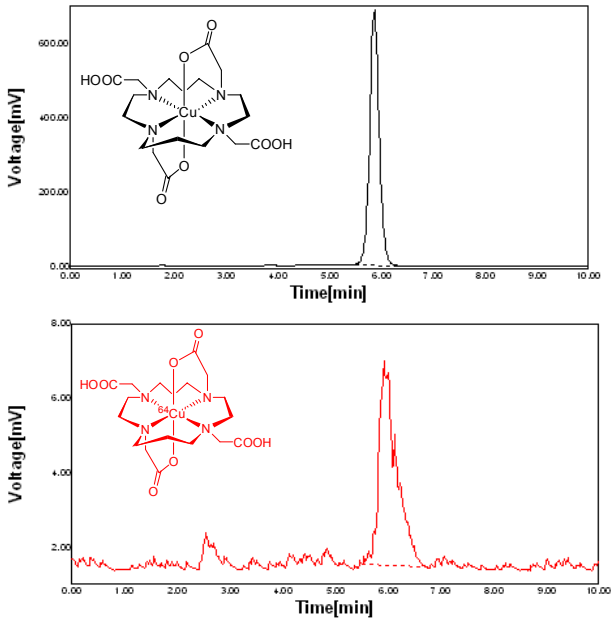


UV chromatogram (280 nm) of non-radioactive Cu-TE2A (top) and radio-chromatogram of ^{64}Cu-TE2A (bottom) in HPLC.

Radio labeling of TETA with ^{64}Cu. Complexation of the ^{64}Cu with TETA was achieved by the addition of no-carrier added $^{64}CuCl_2$ in 0.01 N HCl (5 ìL, 0.1-0.5 mCi) to a 100 ìL of 5 mM solution of the ligand TETA in 0.1M ammonium acetate (pH = 6.8) followed by 30 min incubation at 50°C. Formation of the complex was verified by radio TLC using a mobile phase 1:1 Methanol: 10% ammonium acetate on a silica plate. Radio-HPLC analysis of ^{64}Cu-TETA was accomplished using Xbridge C18 column (4.6 X 150 mm, 5 ìm) with an isocratic method (30 mM citric acid, 1 mL/min flow rate)



Radio-TLC of ^{64}Cu-TETA



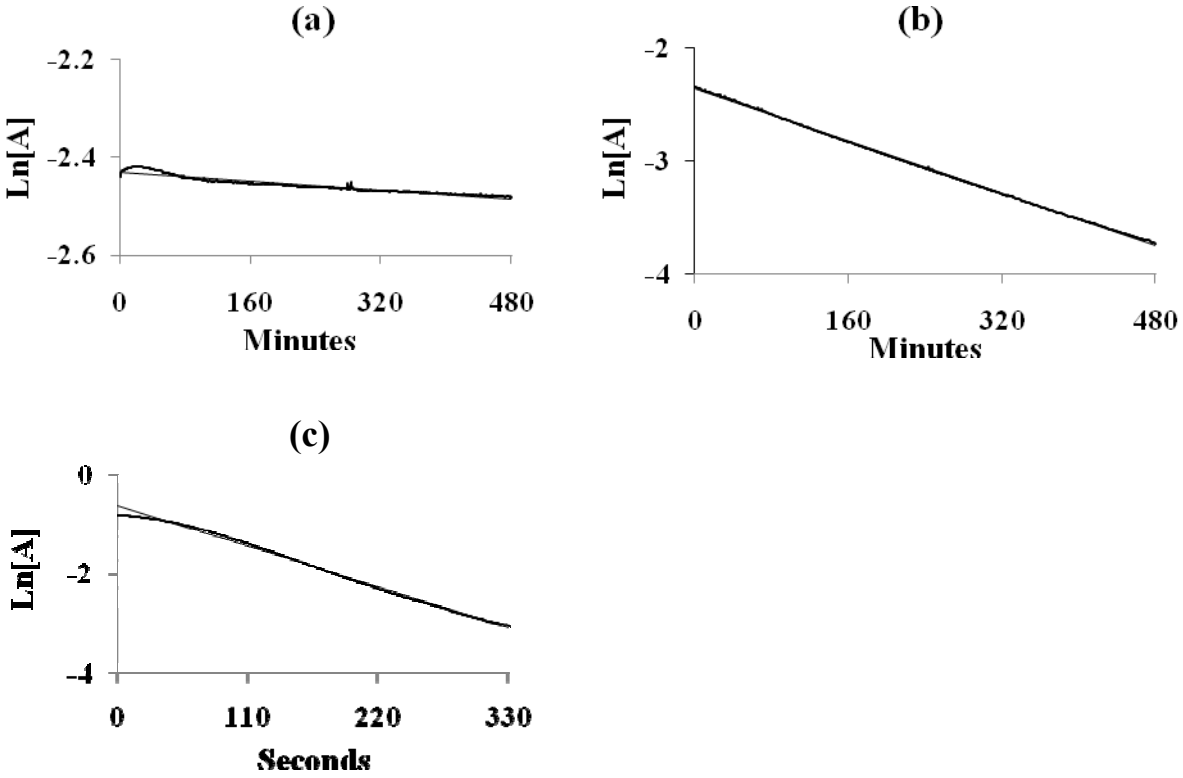
UV chromatogram (280 nm) of non-radioactive Cu-TETA (top) and radio-chromatogram of ^{64}Cu-TETA (bottom) in HPLC.

In Vitro Serum Stability: In vitro serum stability of ^{64}Cu-TE2A and ^{64}Cu-TETA were carried out by adding 50 ìL of ^{64}Cu-TE2A and ^{64}Cu-TETA (2 mM, 50ìCi) to 500 ìL of FBS (Fetal Bovine Serum). The solution was incubated at 37°C, and samples were analyzed by radio-TLC at 10, 30, and 60 min and at 2, 4, 10 and 24 h postadministration to FBS.

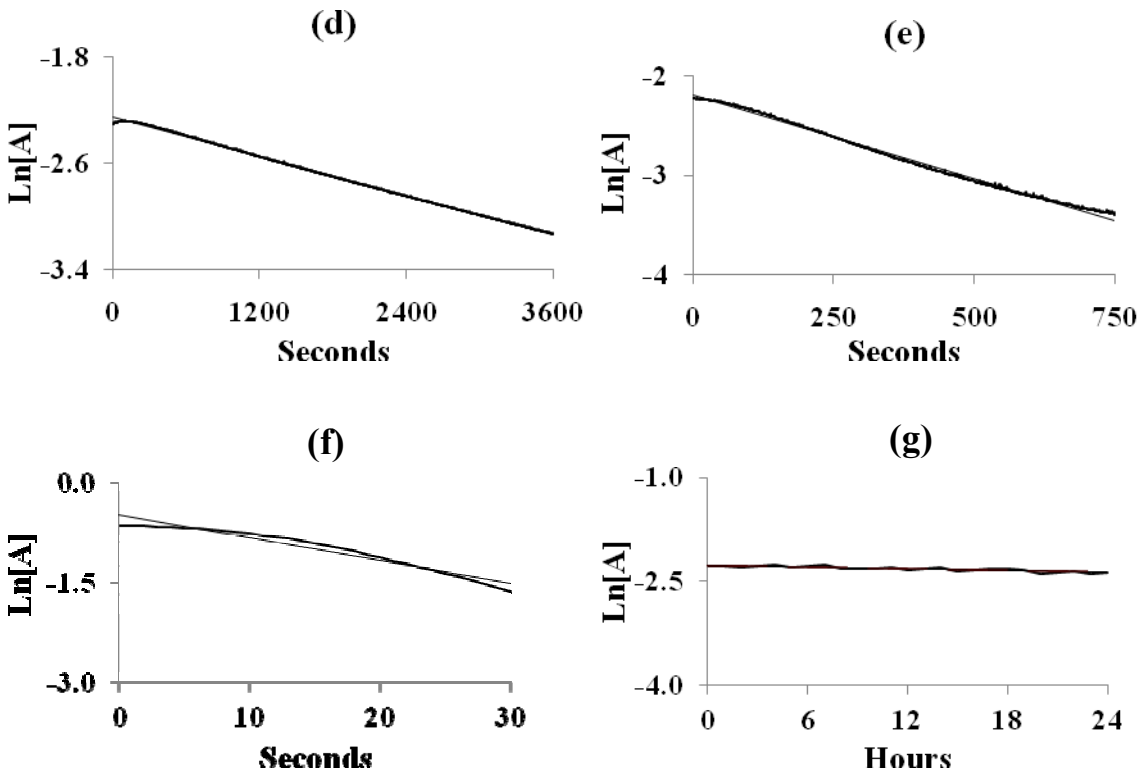
Comparative biodistribution of ^{64}Cu-TE2A and ^{64}Cu-TETA: Mature, female, Sprague-Dawely rats (180-190 g, n = 5) were injected via tail-vein with ^{64}Cu-TE2A and ^{64}Cu-TETA (ca. 20 ìCi in 200 ìL per rat). Animals were sacrificed at 24h post-injection. Organs and tissues of interest (Blood, Heart, Lung, Muscle, Fat, Bone, Spleen, Kidney and Liver) were removed, weighted, and counted using gamma-counter. The %ID per gram (%ID/g) was calculated by comparison to a weighted, counted standard.

Tissue/ Organ	%ID/g	
	^{64}Cu-TETA	^{64}Cu-TE2A
Blood	0.041 ± 0.006	0.038 ± 0.010
Heart	0.028 ± 0.001	0.020 ± 0.002
Lung	0.046 ± 0.005	0.022 ± 0.002
Muscle	0.024 ± 0.004	0.022 ± 0.003
Fat	0.053 ± 0.016	0.040 ± 0.016
Bone	0.055 ± 0.011	0.041 ± 0.009
Spleen	0.052 ± 0.006	0.031 ± 0.003
Kidney	0.240 ± 0.038	0.139 ± 0.030
Liver	0.154 ± 0.012	0.073 ± 0.013

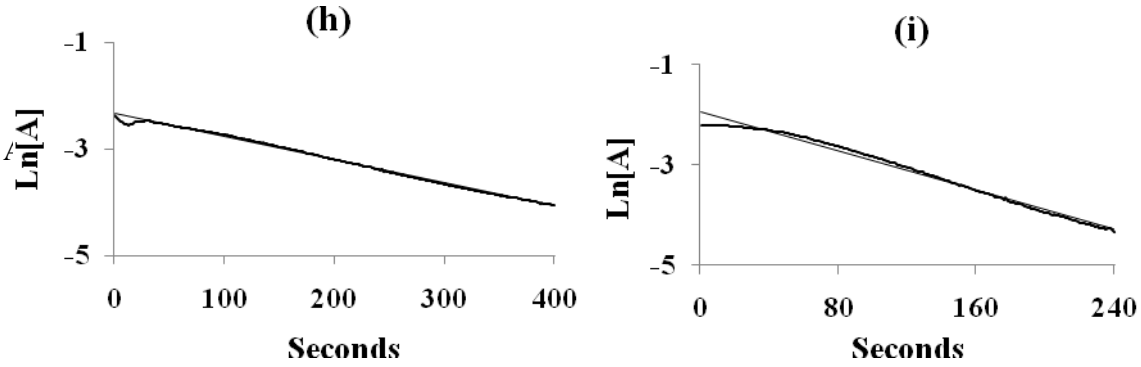
Acid decomplexation studies by spectrophotometer: Acid-decomplexation studies were performed under *pseudo* first-order conditions using sample concentrations of 2.2 mmol in 5 M or 12 M HCl at 50°C or 90°C. Changes in the absorption maxima with time were monitored using a Shimadzu UV-Vis spectrophotometer (UV-1650PC) in thermostated cells. The decreasing absorbance at the λ_{max} of each spectrum (Cu-TE2A 549 nm, Cu-TETA 615 nm, Cu-DOTA 689 nm, Cu-CB-TE2A 624 nm) was used to monitor the progress of the decomplexation reaction. Half-lives were calculated from the slopes of linear ln(absorbance) *vs.* time plots. Each experiment was repeated two to three times and mean values of half-lives are reported. Typical absorbance to time plots for each case are depicted below.



Natural log(absorbance) *vs.* time plots for (a) Cu-TE2A, (b) Cu-TETA, and (c) Cu-DOTA at 50°C in 5M HCl



Natural log(absorbance) *vs.* time plots for (d) Cu-TE2A, (e) Cu-TETA, (f) Cu-DOTA, and (g) Cu-CB-TE2A at 90°C in 5M HCl



Natural log(absorbance) *vs.* time plots for (h)Cu-TE2A, and (i)Cu-TETA at 90°C in 12M HCl

Acid decomplexation studies by HPLC: Sample concentrations of copper complexes (Cu-TE2A and Cu-TETA) studied were 2.2 mmol in 5 M HCl. Each complex's visible HPLC spectrum in 5 M HCl at 90°C was recorded at specific time points by injecting an aliquot (20 ìL) onto a reverse phase Xbridge C18 column (4.6 X 150 mm, 5 ìm) with an isocratic method (30 mM citric acid, 1 mL/min flow rate). The decreasing absorbance at the λ_{max} of each HPLC spectrum (Cu -TE2A 549 nm, Cu-TETA 615 nm) was used to monitor the progress of the decomplexation reaction.

Electrochemical studies: Cyclic voltammetry was conducted with a Biologic model SP-150 with three-electrode configuration. The working electrode was a glassy carbon (diameter = 3 mm), Ag/AgCl (sat. KCl) reference electrode, and Pt wire counter electrode. Samples (2 mM) were run in 0.1 M aqueous acetic acid adjusted to pH 7.0 with glacial acetic acid at scan rate 100 mV/s. The solutions were deoxygenated for 30 min with Ar prior to use and kept under Ar atmosphere during measurement.

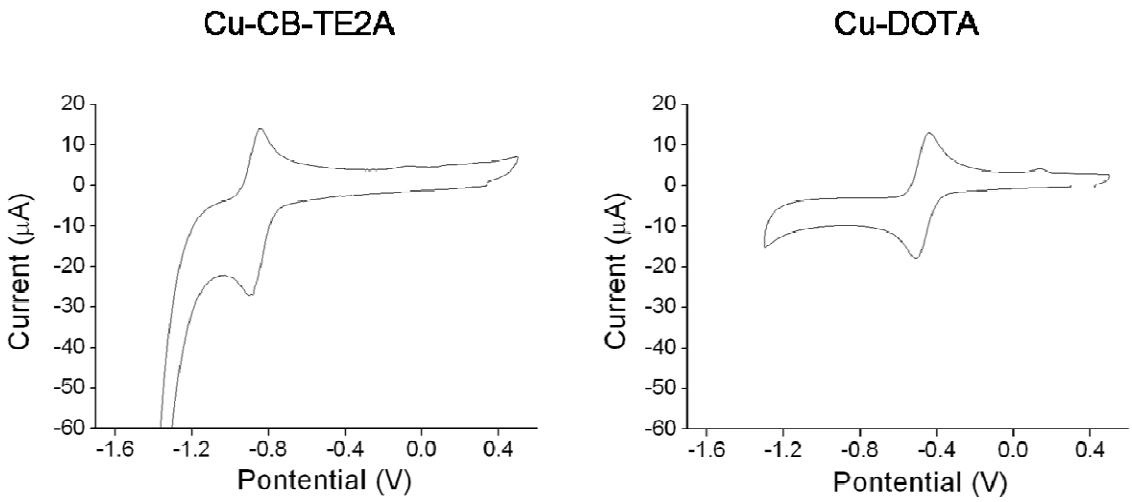


Table S1. Half-lives for acid decomplexation[a] and reduction potentials of copper(II) complexes

Complex	5M HCl, 50°C	5M HCl, 90°C	12M HCl, 90°C	E_{red}(V) vs Ag/AgCl
Cu-TETA	4.1(3) h	4.7(4) min	1.1(3) min	-0.88 (irrev)
Cu-TE2A	92.6(2) h	46.2(8) min	2.6(5) min	-1.10 (irrev)
Cu-DOTA	1.5(5) min	< 1 min		-0.51 (quasi-rev)
Cu-CB-TE2A		158(7) h		-0.90 (quasi-rev)

[a] Half-lives are mean values of 2-3 experiments.