Supplementary Material (ESI) for Chemical Communications

A new bifunctional chelator for copper radiopharmaceuticals: A cage amine ligand with a carboxylate functional group for conjugation to peptides

Supporting Information

Michelle T. Ma[a,b], John A. Karas[b], Jonathan M. White[a,b], Denis Scanlon[b] and Paul S. Donnelly*[a,b]

[a] *School of Chemistry, University of Melbourne, Parkville, Melbourne, Victoria, 3010, Australia, Tel: 61 3 8344 2399; E-mail: pauld@unimelb.edu.au*

[b] *Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Melbourne, Victoria, 3010, Australia*

Experimental

Abbreviations
$(NH_2)_2$sar =1,8-diamino-3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane
t-Boc = N-*tert*-butoxycarbonyl ester
HATU = 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCTU = 2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate

Instrumentation:

Mass spectra were recorded in the positive ion mode on an Agilent 6510 Q-TOF LC/MS Mass Spectrometer coupled to an Agilent 1100 LC system (Agilent, Palo Alto, CA). Data were acquired and reference mass corrected via a dual-spray electrospray ionisation source, using the factory-defined calibration procedure. Each scan or data point on the Total Ion Chromatogram is an average of 9652 transients, producing 1.02 scans s^{-1}. Spectra were created by averaging the scans across each peak. Mass spectrometer conditions: fragmentor: 200 – 300 V; drying gas flow: 7 L/min; nebuliser: 30 psi; drying gas temp: 325°C; V_{cap}: 4000 V; skimmer: 65 V; OCT R_fV: 750 V; scan range acquired: 150 – 3000 *m/z*.

HPLC-MS traces were recorded using an Agilent Eclipse Plus C18 column (5 μm, 2.1 x 150 mm) coupled to the Agilent 6510 Q-TOF LC/MS Mass Spectrometer described above. 1 μL aliquots of each sample were injected onto the column using the Agilent 1100 LC system, with a flow rate of 0.5 mL/min. Data acquisition parameters are the same as those described above for mass spectra, with the exception of the fragmentor (fragmentor voltage: 100 V).

NMR spectra were acquired in D_2O on a Varian FT-NMR 500 spectrometer. ^{1}H NMR spectra were acquired at 500 MHz and ^{13}C NMR spectra were acquired at 125.7 MHz. ^{1}H NMR and ^{13}C NMR spectral shifts were referenced to acetone (δ = 2.22 ppm, δ = 30.89 ppm respectively).

Semi-preparative HPLC purifications were performed using an Agilent 1200 Series HPLC system with a 5 mL/min flow rate. Solvent gradients and column specifications are described below (see *Synthesis*). An automated Agilent 1200 fraction collector collected 1 – 3 mL fractions and fraction collection was based on UV-Vis detection at 214 or 220 nm, with a lower threshold limit between 100 – 400 mAU. Each fraction was analysed using MS and analytical HPLC.

Analytical HPLC traces were acquired using an Agilent 1200 Series HPLC system and an Agilent Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 μm) with a 1 mL/ min flow rate and UV spectroscopic detection at 214 nm, 220 nm and 270 nm.

UV-Vis spectra were acquired on a Cary 300 Bio UV-Vis spectrophotometer, from 800 – 200 nm at 0.500 nm data intervals with a 300.00 nm/min scan rate.

Electrochemistry experiments were performed with an Autolab (Eco Chemie, Utrecht, Netherlands) computer-controlled electrochemical workstation. A standard three-electrode arrangement was used with a glassy carbon disk (d, 3 mm) as working electrode, a Pt wire as auxiliary electrode and a Ag/AgCl reference electrode (silver wire in H_2O (KCl (0.1 M) $AgNO_3$ (0.01 M)). Scan rate: 100 mV/s, sample interval: 1.06 mV, sensitivity: 1 x 10^{-4} A. Potassium ferricyanide was used as a reference ($E^{1/2}$ ($[Fe(CN)_6]^{3-/4-}$) = 0.36 V).

HPLC traces of radiolabelled peptides were acquired using a Waters Comosil C18 column (4.6 x 150 mm) coupled to a Shimadzu LC-20AT with a sodium iodide scintillation detector and a UV-Vis detector. 100 μL aliquots of each radiolabelled sample were injected onto the column, using a flow rate of 1 mL/min.

Reagents:

SP Sephadex and DOWEX cation exchange resin was purchased from Aldrich. Fmoc-L-amino acids, HATU, HCTU and 2-chlorotrityl resin were purchased from GL Biochem Ltd (Shanghai, China). Fmoc-Lys(iv-Dde)-OH and Fmoc-D-amino acids were purchased from Bachem AG (Switzerland). Fmoc-Pal-PEG-PS resin was purchased from Applied Biosystems (Foster City, Califoria).

Synthesis and characterisation:

$[CuL^1](NO_3)_3$ and $[CuL^2](CF_3SO_3)Cl$: A solution of $[Cu(NH_3)_2Sar](CF_3SO_3)_4$ (1.5 g, 1.53 mmol) in anhydrous *N*,*N*-dimethylacetamide (12 mL) was heated under an atmosphere of nitrogen to 70°C. Glutaric anhydride (0.19 g, 1.64 mmol) and diisopropylethylamine (600 μL) were added and the solution was heated at 70°C for two hours. The solution was cooled and water (20 mL) was added. The solution was applied to a column of SP Sephadex C-25 cation exchange (Na^+ form, 30 x 5 cm). The column was eluted with 0.05 M sodium citrate solution to separate three components. (Chromatographic yield: Fraction 1 ~ 30%, fraction 2 ~ 30%, fraction 3 ~ 30%.) Each fraction was applied separately to a DOWEX 50W x 2 cation exhange column (H^+ form, 10 x 5 cm). The column was washed with water (500 mL) and 1 M HCl solution (500 mL) and then eluted with 4 M HCl (350 mL) and the eluent was evaporated to dryness under reduced pressure at 40°C. Fraction 1: **$[CuL^2]Cl_2$.xHCl** (1.00 g) MS: $[CuC_{24}H_{45}N_8O_6]^+$ m/z = 604.27 (experimental), 604.28 (calculated). Fraction 2: **$[CuL^2]Cl_3$.xHCl** (0.82 g) MS: $[CuC_{19}H_{39}N_8O_3]^+$ m/z = 490.24 (experimental), 490.24 (calculated). The dark blue residue from fraction 2 was dissolved in distilled water (30 mL). Concentrated nitric acid (2 mL) was added and the solution was concentrated by rotary evaporation until crystallisation commenced. The mixture

was cooled at 5 °C for 30 mins before the light blue crystals were collected by filtration. **$[CuL^1](NO_3)_3$**: 0.13 g, 13% isolated yield.

Crystals suitable for X-ray diffraction were grown from evaporation of a solution of **$[CuL^1]Cl_3.xHCl$** (20 mg) in ~ 1 M HNO_3 (2 mL) at ambient temperature.

Crystals of **$[CuL^2](CF_3SO_3)Cl$** were suitable for X-ray diffraction studies and were formed as follows: **$[CuL^2]Cl_2.xHCl$** (0.45 g) was dissolved in water (2 mL) and a solution of silver triflate (0.33 g in 2 mL water) was added. This solution was filtered twice (MilliQ syringe filter (0.45 μm)) and evaporated to dryness under reduced pressure to give a dark blue-purple residue. The residue was redissolved in water (8 mL) and over the course of 10 min, blue crystals precipitated from this solution. These were collected and dried by filtration. **$[CuL^2](CF_3SO_3)Cl$**: 0.13 g. Crystals suitable for X-ray diffraction were grown from evaporation of a solution of **$[CuL^2](CF_3SO_3)Cl$** (30 mg) in water (6 mL) at ambient temperature.

Microanalysis: **$[CuL^1](NO_3)_3$**: $CuC_{19}H_{41}N_{11}O_{12}$ – C 34.86%, H 6.40%, N 24.76% (experimental); - C 33.60%, H 6.08%, N 22.69% (calculated); **$[CuL^2](CF_3SO_3)Cl$**: $CuC_{25}H_{46}N_8O_9SClF_3$ - C 38.06%, H 5.92%, N 14.20%, S 3.88% (experimental); - C 37.97%, H 5.86%, N 14.17%, S 4.06% (calculated).

UV-vis: **$[CuL^1](NO_3)_3$** in water, pH 4, λ_{max} = 658 nm, ε = 140 M^{-1} cm^{-1}; **$[CuL^2](CF_3SO_3)Cl$** in water, pH = 4, λ_{max} = 655 nm, ε = 146 M^{-1} cm^{-1}

Electrochemistry: **$[CuL^1](NO_3)_3$** (1mM) in an aqueous solution of $NaBF_4$ (100mM), pH 3.5, E_{red} = -0.722 V, $E°$ = 0.36). **$[CuL^2](CF_3SO_3)Cl$** (1mM) in an aqueous solution of $NaBF_4$ (100mM), pH 3.5, E_{red} = -0.874 V (vs Ag/AgCl, $[Fe(CN)_6]^{3-/4-}$, $E°$ = 0.36).

$[CuL^1](NO_3)_3$ (1mM) in an aqueous solution of tris buffer (100mM), pH 7.4, E_{red} = -1.04 V (vs $[Fe(CN)_6]^{3-/4-}$, $E°$ = 0.36). **$[CuL^2](CF_3SO_3)Cl$** (1mM) in an aqueous solution of tris buffer (100mM), pH 7.4, E_{red} = -1.0 V (vs Ag/AgCl, $[Fe(CN)_6]^{3-/4-}$, $E°$ = 0.36).

L^1: A solution of **$[CuL^1]Cl_3.xHCl$** (0.44 g, ~ 0.73 mmol based on x = 0) in water (4 mL) was deoxygenated by purging with N_2 gas for 20 mins. Sodium sulfide (0.6 g) was added and the solution was stirred overnight at room temperature (under an atomosphere of nitrogen gas). After addition of sodium sulfide, the solution turned a dark green. After ~ 16 hours, a black-brown precipitate was present and the solution appeared a light yellow-green. This mixture was filtered (Whatman Filter Paper 1) and the filtrate diluted with 1 M HCl (250 mL) resulting in the formation of a cloudy, white precipitate. The mixture was filtered (MilliQ syringe filters (0.45 μm)) and applied to a DOWEX 50W x 2 cation exhange column (H^+ form, 10 x 5 cm). The column was washed with 1 M HCl solution (750 mL) (to remove Na_2S) and then eluted with 4 M HCl solution (400 mL). The eluent was evaporated to dryness under reduced pressure to give a clear residue with a slight blue tinge. Because this residue was still slightly blue, the above process was repeated. The final solution was evaporated to dryness to give a clear, colourless residue. **L^1.xHCl**: 0.30 g, 89 % MS: $[C_{19}H_{40}N_8O_3]^+$ 429.34 (experimental), 429.33 (calculated). ^{1}H NMR: δ 1.852, m, 2H, βCH_2 (with respect to COOH); 2.358, t, ^3J = 7.54, 2H, glutarate CH_2; 2.417, t, ^3J =7.18, 2H glutarate CH_2; 3.181, broad s, 6H, cage CH_2; 3.315, broad s, 12H, cage CH_2; 3.694, broad s, 6H, cage CH_2. ^{13}C NMR: δ 20.36, 33.46, 35.54 (glutarate CH_2); 46.46, 48.39, 50.66, 51.18, 55.23, 56.54 (cage); 177.937, 178.516 (CO).

L^1-(*t*-BOC)$_{3\text{-}5}$: **L^1.xHCl** was converted to a trifluoromethanesulfonate salt to increase its solubility in *N*,*N*-dimethylacetamide. In brief, **L^1.xHCl** (0.20 g, 0.43 mmol) was dissolved in water (5 mL) and silver triflate (0.11 g, 0.43 mmol) was added, precipitating silver chloride. The solution was filtered (MilliQ 0.45 μm syringe filter) and evaporated to dryness under reduced pressure to give a colourless, clear hydroscopic residue. $L^1.HCF_3SO_3$: 0.31 g.

$L^1.HCF_3SO_3$ (80 mg, 0.138 mmol) was dissolved in a solution of *N*,*N*-dimethylacetamide:water (4:1) (10 mL). Di-*tert*-butyldicarbonate (0.25 g, 1.15 mmol) and diisopropylethylamine (100 μL) were added and the solution was stirred under an atmosphere of nitrogen gas for 30 min. After this time, the solvent was removed under high vacuum at ~ 40 °C. The residue was dissolved in acetonitrile (15 mL), filtered (MilliQ 0.45 μm syringe filter) and lyophilised to remove traces of *N*,*N*-dimethylacetamide. Once the crude compound was dry, it was dissolved in a solution of A:B (70:30) (A = milliQ water containing 0.1 % trifluoroacetic acid, B = acetonitrile containing 0.1 % trifluoroacetic acid) (5 mL), filtered (MilliQ 0.45 μm syringe filter), and applied to a C18 cartridge (Alltech Maxi-Clean C18 900 mg). The cartridge was washed sequentially with 5 mL A, 5 mL 10% B in A and 5 mL 20% B in A. It was then eluted with 5 mL 80 % B in A, and 1 mL fractions were collected. Most of the desired compound (> 95 %) was collected in the first two fractions. These were lyophilised to yield a mixture of *t*-BOC protected isomers, and the degree of protection ranged from 3-5 *t*-BOC groups per molecule of cage compound. **L^1-(*t*-BOC)$_{3\text{-}5}$**: 30 mg, 25 % yield. MS: $[C_{19}H_{41}N_8O_3(C_5H_8O_2)_3]^+$ 729.49 (experimental), 729.49 (calculated); $[C_{19}H_{41}N_8O_3(C_5H_8O_2)_4]^+$ 829.54 (experimental), 829.54 (calculated); $[C_{19}H_{41}N_8O_3(C_5H_8O_2)_5]^+$ 929.59 (experimental), 929.59 (calculated).

L^1-OCT: Linear [Tyr^3]-octreotate (linear OCT) peptide (dPhe-Cys-Tyr-dTrp-Lys-Thr-Cys-Thr-OH) was synthesised on 2-chlorotrityl chloride resin using standard Fmoc solid phase peptide synthesis procedures. An excess of resin (0.06 g, ~0.8 mmol/g) was swelled in dimethylformamide. L^1-(*t*-BOC)$_{3\text{-}5}$ (25 mg, 0.03 mmol), HCTU (20 mg, 0.05 mmol) and diisopropylethylamine (20 μL) in DMF (1 mL) were added to the resin and the mixture was stirred and left to react overnight. The reaction supernatant was then drained and the resin washed with dimethylformamide (3 x 5 mL) and dichloromethane (3 x 5mL). The resin was transferred to a falcon tube and trifluoroacetic acid (5mL), distilled

water (0.15 mL) and triisopropylsilane (0.15 mL) were added. The falcon tube was placed on a shaker for 40 min. The peptide material was precipitated from the solution using diethyl ether (15 mL) and the mixture was centrifuged (3 mins, 3000 rpm). The supernatant was discarded and the precipitate dissolved in A:B (70:30). This solution was filtered (MilliQ 0.45 μm syringe filter) and lyophilised.

The crude peptide material was purified by semi-preparative reverse phase HPLC (Eclipse XDB-C18 5 μm 9.5 x 250 mm column) using a linear 1% A → B/min gradient. Linear **L^1-OCT** eluted at 30 min (determined by ESI-MS) and fractions containing linear **L^1-OCT** were lyophilised. The dried fractions were then redissolved in 25 mM ammonium acetate (8 mL) and an excess of 2,2-dithiodipyridine (12 mg) was added. The solution was then applied to a semi-preparative reverse phase HPLC column, and purified using a linear 1% A → B/min gradient. Cyclic **L^1-OCT** eluted at 30 min and fractions containing cyclic **L^1-OCT** were lyophilised. **L^1-OCT**: 1-2 mg; HPLC retention time: 12.989 min (linear gradient, 0 → 60% B in A over 25 min) MS: $[C_{68}H_{105}N_{18}O_{14}S_2]^{3+}$ 487.25 (experimental), 487.25 (calculated); $[C_{68}H_{104}N_{18}O_{14}S_2]^{2+}$ 730.37 (calculated), 730.37 (experimental).

L^1-Lys$_3$-BBN: Bombesin peptide (BBN) (1-14) (Pyr-Gln-Lys-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2) was synthesised on Fmoc-PAL-PEG-PS resin using standard Fmoc solid phase peptide synthesis procedures. The side chain of the Lys_3 residue was protected with a iv-DDE (*N*ε-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl) group. This was selectively deprotected using 5 % hydrazine in DMF (3 x 5mL) over one hour. Resin (0.05 g, ~0.2 mmol/g) was swelled in dimethylformamide. L^1-(*t*-BOC)$_{3\text{-}5}$ (10 mg, 0.012 mmol), HATU (10 mg, 0.026 mmol) and diisopropylethylamine (20 μL) in DMF (1 mL) were added to the resin and the mixture was stirred and left to react for 3 hrs. The reaction supernatant was then drained and the resin washed with dimethylformamide (3 x 5 mL) and dichloromethane (3 x 5mL). The resin was transferred to a falcon tube and trifluoroacetic acid (2 mL), distilled water (70 μL) and triisopropylsilane (70 μL) were added. This solution was placed on a shaker for 40 min. The solvent was evaporated under a stream of N_2 gas and the residue dissolved in A:B (70:30). This solution was filtered (MilliQ 0.45 μm syringe filter) and lyophilised.

The crude peptide material was purified by semi-preparative reverse phase hydro HPLC (Phenomenex Synergi 4u Hydro-RP 80A 50 x21.20 mm), using a "slow" linear gradient (0.5 % A → B/min). **L^1-Lys$_3$-BBN** eluted with 25 % B in A and fractions containing **L^1-Lys$_3$-BBN** were lyophilised. The peptide was not pure, so the dried fractions were redissolved in milliQ water and applied to the same semi-preparative reverse phase HPLC column, and purified using a "very slow" linear 0.25 % A → B/min gradient. **L^1-Lys$_3$-BBN** eluted with ~ 26.5 % B in A. An impurity with a lower molecular mass (~ 780) still persisted in these fractions, so the peptide was purified by semi-preparative reverse HPLC (Eclipse XDB-C18 5 μm 9.5 x 250 mm column) using a using a "slow" linear gradient (0.5 % A → B/min). **L^1-Lys$_3$-BBN** eluted with 26 % B in A and fractions containing **L^1-Lys$_3$-BBN** were lyophilised. Analytical reverse phase HPLC indicated that the final fractions contained 95% **L^1-Lys$_3$-BBN**. **L^1-Lys$_3$-BBN**: 1 – 50 μg; HPLC retention time: 13.593 min (linear gradient, 0 → 60% B in A over 25 min); MS: $[C_{90}H_{152}N_{30}O_{20}S_2]^{4+}$: 501.54 (experimental), 501.54 (calculated); $[C_{90}H_{151}N_{30}O_{20}S_2]^{3+}$: 668.39 (experimental), 668.38 (calculated); $[C_{90}H_{150}N_{30}O_{20}S_2]^{2+}$: 1002.07 (experimental), 1002.07 (calculated).

HPLC-ESI-MS of L$_1$-peptides: HPLC-ESI-MS traces were acquired for **L^1-peptides** synthesised. HPLC-ESI-MS traces were also acquired for solutions containing **L^1-peptide** and $CuCl_2$, to determine whether **L^1-peptide** binds "free" Cu^{2+} ion. We used a reverse phase C18 analytical HPLC column with a linear gradient (0 → 60% B in A over 25 min) to determine retention times and molecular masses of peptide species. (Here, A = milliQ water with 0.1% formic acid; B = acetonitrile with 0.1% formic acid.)

L^1-OCT: L^1-OCT (~ 10 μg) was dissolved in milliQ water (50 μL). This solution was further diluted (5 μL of peptide solution in 20 μL of milliQ water) to provide a solution of suitable concentration for HPLC-ESI-MS. A solution containing **L^1-OCT** (5 μL of the original stock peptide solution) and $CuCl_2$ (5 μL of 1 mM $CuCl_2$ solution) in milliQ water (15 μL) was also made up. HPLC-ESI-MS: **L^1-OCT**, R.T.: 12.984 min; $[C_{68}H_{106}N_{18}O_{14}S_2]^{4+}$ 365.69 (experimental), 365.69 (calculated); $[C_{68}H_{105}N_{18}O_{14}S_2]^{3+}$ 487.25 (experimental), 487.25 (calculated); $[C_{68}H_{104}N_{18}O_{14}S_2]^{2+}$ 730.37 (calculated), 730.37 (experimental); **[CuL^1-OCT]$^{2+}$**, R.T.: 13.199 min; $[CuC_{68}H_{104}N_{18}O_{14}S_2]^{4+}$ 380.92 (experimental), 380.92 (calculated); $[CuC_{68}H_{103}N_{18}O_{14}S_2]^{3+}$ 507.56 (experimental), 507.55 (calculated); $[CuC_{68}H_{102}N_{18}O_{14}S_2]^{2+}$ 760.83 (calculated), 760.83 (experimental).

L^1-Lys$_3$-BBN: **L^1-Lys$_3$-BBN** ((~ 1-5 μg) was dissolved in milliQ water (50 μL). The concentration of this solution was suitable for HPLC-ESI-MS. 1 mM $CuCl_2$ solution (2.5 μL) was added to a portion of this solution (20 μL) to determine whether **L^1-Lys$_3$-BBN** binds "free" Cu^{2+} ion. HPLC-ESI-MS: **L^1-Lys$_3$-BBN**, R.T.: 13.520 min; $[C_{90}H_{152}N_{30}O_{20}S]^{4+}$: 501.54 (experimental), 501.54 (calculated); $[C_{90}H_{151}N_{30}O_{20}S]^{3+}$: 668.39 (experimental), 668.38 (calculated); $[C_{90}H_{150}N_{30}O_{20}S_2]^{2+}$: 1002.07 (experimental), 1002.07 (calculated); **[CuL^1-Lys$_3$-BBN]$^{2+}$**, R.T.: 13.769 min; $[CuC_{90}H_{150}N_{30}O_{20}S]^{4+}$: 517.02 (experimental), 517.02 (calculated); $[CuC_{90}H_{149}N_{30}O_{20}S]^{3+}$: 689.03 (experimental), 689.02 (calculated); $[CuC_{90}H_{148}N_{30}O_{20}S]^{2+}$: 1033.03 (experimental), 1033.03 (calculated).

^{64}Cu radiolabelling of L^{1}-peptides: HPLC with a radioactivity counter was used to monitor **L^{1}-peptide** ligation to $^{64}Cu^{2+}$. We employed a reverse phase C18 analytical HPLC column with a linear gradient (0 → 60% B in A over 15 min) to determine retention times of **[^{64}CuL^{1}-peptides]$^{2+}$**. These retention times were compared with retention times of the "cold" **[CuL^{1}-peptides]$^{2+}$** under the same HPLC conditions with UV spectroscopic detection at 275 nm.

[^{64}CuL^{1}-OCT]$^{2+}$: An aliquot of 0.1 M HCl solution containing $^{64}CuCl_2$ (20 μL) was added to an aqueous solution (490 μL) containing **L^{1}-OCT** (0.02 mg/mL) and sodium acetate (0.02 M). The pH was ≥ 5.5. The solution was left at ambient temperature for 20 min before an aliquot (100 μL) was injected onto a reverse phase C18 analytical HPLC column with a linear gradient (0 → 60% B in A over 15 min). Retention time: 12.730 min; Activity: 412150 s^{-1} counts. An aqueous sample of "cold" **[CuL^{1}-OCT]$^{2+}$** (~ 1 mg/mL) was injected onto the HPLC column (injection volume ~ 30 μL) and was eluted using the same linear gradient (0 → 60% B in A over 15 min). Retention time: 12.937 min.

Human male AB serum (200 μL) was added to a solution of **[^{64}CuL^{1}-OCT]$^{2+}$** (200 μL). This solution was incubated in a water bath at 37 °C. At time points of 1 hr, 4 hr and 20 hr an aliquot of this serum solution (100 μL) was removed for radio-HPLC analysis: acetonitrile (200 μL) was added to the serum aliquot to precipitate serum proteins. This mixture was filtered and the acetonitrile evaporated under a stream of argon gas. The final volume was < 100 μL. The solution was frozen at - 70 °C until injection onto the HPLC column. 1hr: Retention time 12.716 min, Activity 134894 counts s^{-1}; 4 hr: Retention time 12.725 min, Activity 54694 counts s^{-1}; 20 hr: Retention time 12.800 min, Activity 31030 counts s^{-1}.

[^{64}CuL^{1}-Lys$_3$-BBN]$^{2+}$: An aliquot of 0.1 M HCl solution containing $^{64}CuCl_2$ (20 μL) was added to an aqueous solution (290 μL) containing **L^{1}-Lys$_3$-BBN** (~ 0.03 mg/mL) and sodium acetate (0.03 M). The solution was left at ambient temperature for 10 min before an aliquot (100 μL) was injected onto a reverse phase C18 analytical HPLC column with a linear gradient (0 → 60% B in A over 15 min). Retention time: 12.642; Activity: 170869 counts s^{-1}. An aqueous sample of "cold" **[CuL^{1}- Lys$_3$-BBN]$^{2+}$** (~ 0.5 mg/mL) was injected onto the HPLC column (injection volume ~ 60 μL) and was eluted using the same linear gradient (0 → 60% B in A over 15 min). Retention time: 13.073 min.

A single time point serum stability study for **[^{64}CuL^{1}-Lys$_3$-BBN]$^{2+}$** was conducted in the same way as studies for **[^{64}CuL^{1}-OCT]$^{2+}$**. An aliquot of **[^{64}CuL^{1}-Lys$_3$-BBN]$^{2+}$** (200 μL) was added to human male AB serum and incubated at 37 °C. After 2 hr, the sample was subjected to the same treatment as described above and injected onto the HPLC column. 2 hr: Retention time 12.828 min, Activity 60066 counts s^{-1}.

Figure S1: An ORTEP representation of the cation present in $[CuL^2](CF_3SO_3)Cl$. Ellipsoids are at the 20% probability level. Hydrogen atoms bound to carbon are omitted for clarity.

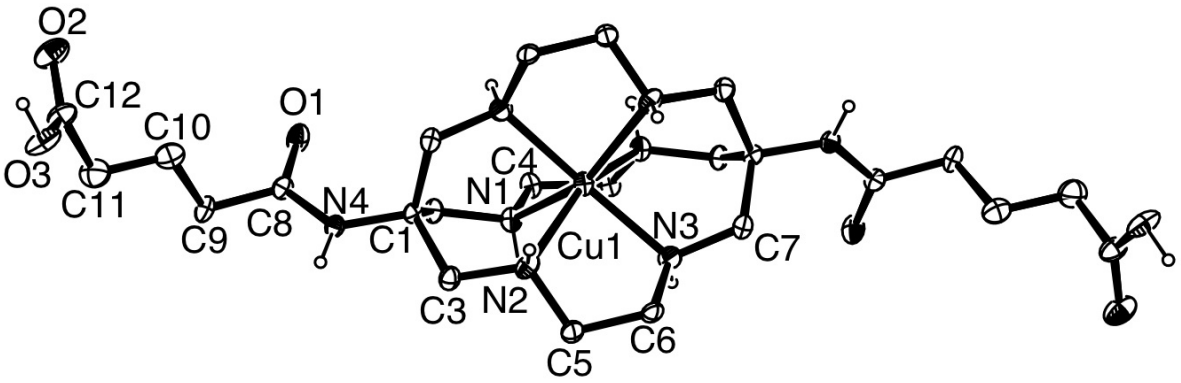


Table S1: Selected crystallographic parameters for $[CuL^1](NO_3)_3$ and $[CuL^2](CF_3SO_3)Cl$

	$[CuL^1](NO_3)_3$	$[CuL^2](CF_3SO_3)Cl$
Formula	$CuC_{19}H_{41}N_{11}O_{12}$	$CuC_{25}H_{44}N_8O_9SF_3Cl$
Formula weight	679.17	788.73
Crystal system	Monoclinic	Orthorhombic
Space Group	$P2_1/c$	$C222_1$
a, b, c (Å)	8.345 (5), 12.231 (5), 26.941 (5)	12.4608 (13), 20.445 (2), 13.2263 (14)
α, β, γ (deg)	90.00, 93.658 (5), 90.00	90.00, 90.00, 90.00
Cell volume ($Å^3$)	2744 (2)	3369.6 (6)
Z	4	4
Temperature (K)	130 (2)	130 (2)
λ (Å)	0.71073	0.71073
Reflections Collected	17656	8844
Independent Reflections	4834	2973
R(int)	0.18	0.03
Flack parameter		0.37(2)
R-factor (%)	6.71	4.76

Figure S2: Radio-HPLC of $[^{64}Cu(L^1\text{-OCT})]^{2+}$ after incubation in human serum for 0 hr (red), 1 hr (blue), 4 hr (yellow) and 20 hr (green)

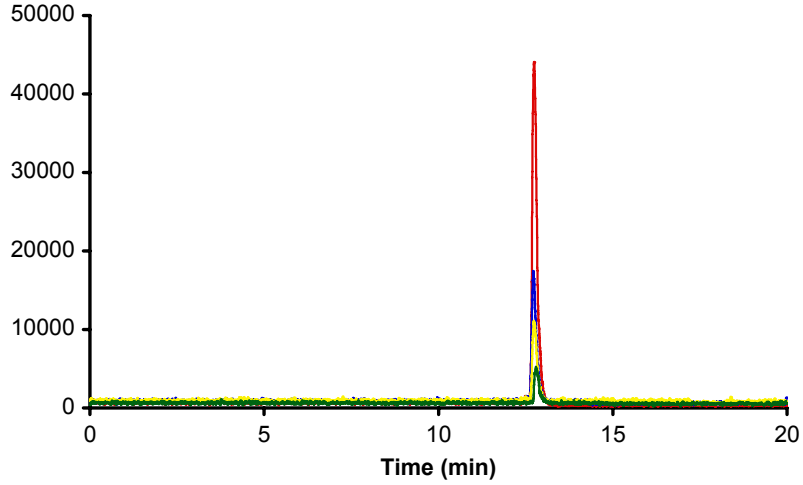
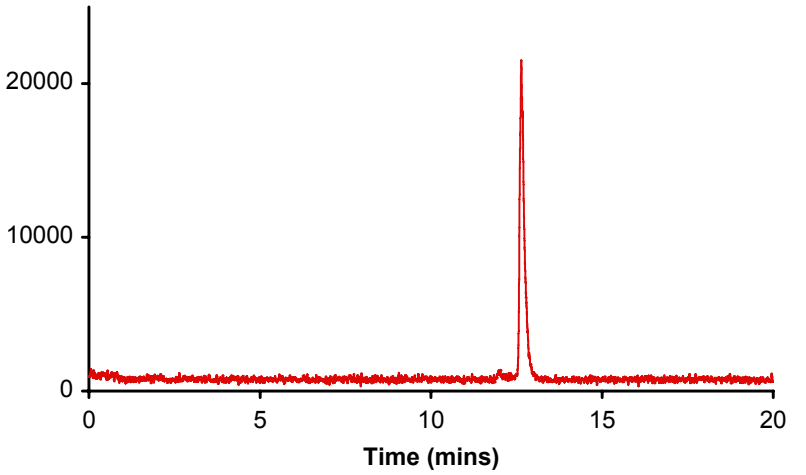
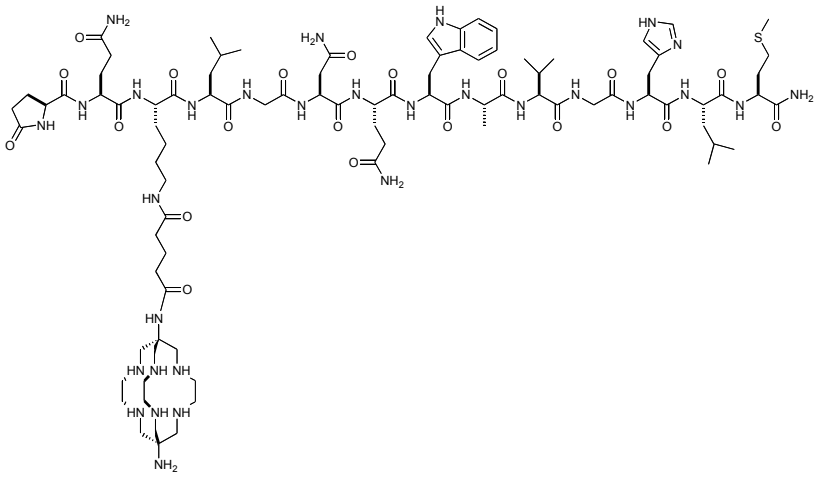
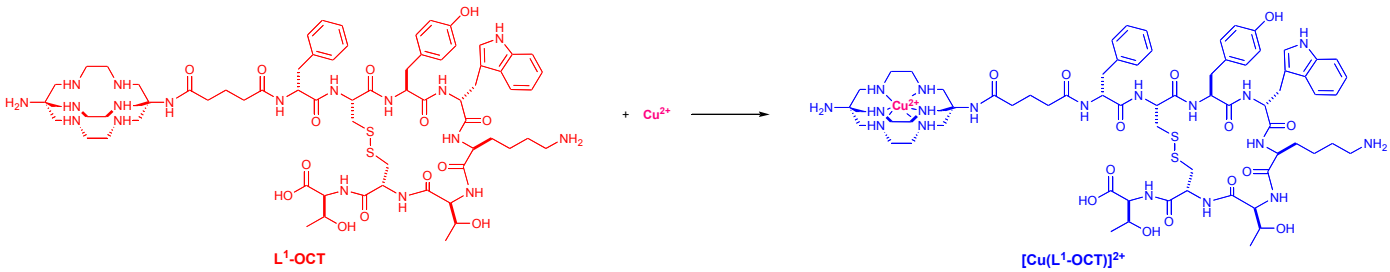


Figure S3: Radio-HPLC of $[^{64}Cu(L^1\text{-Lys}_3\text{-BBN})]^{2+}$

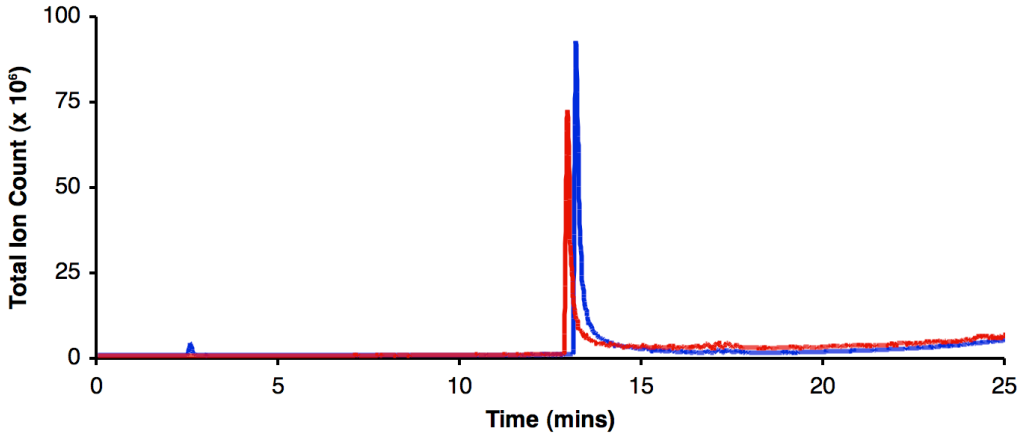




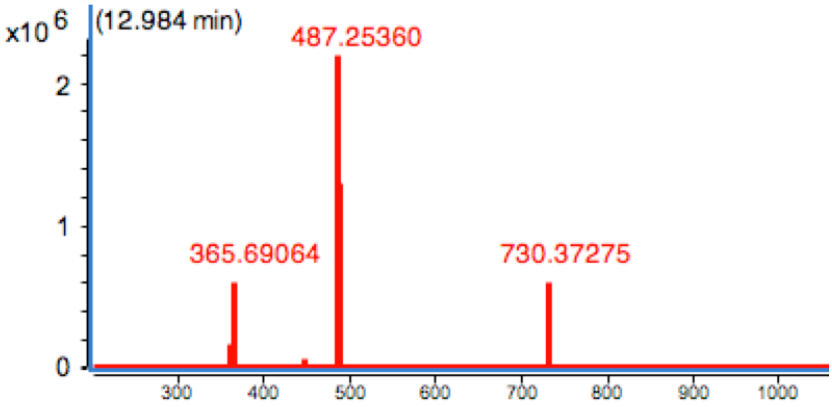
L^1-Lys_3-BBN



(a)



(b)



(c)

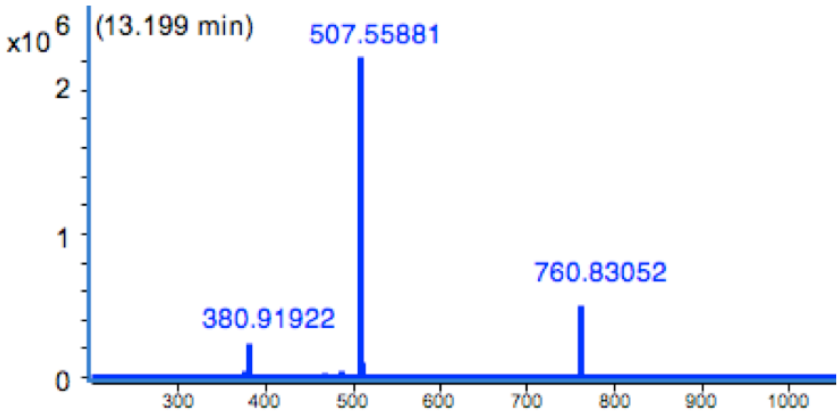


Figure S4: (a) LC-MS trace of **L^1-OCT** (red) and **L^1-OCT** complexed with Cu^{2+} (blue) (b) MS of **L^1-OCT** at 12.98 min (TIC vs m/z) (c) MS of **L^1-OCT** with Cu^{2+} at 13.20 min

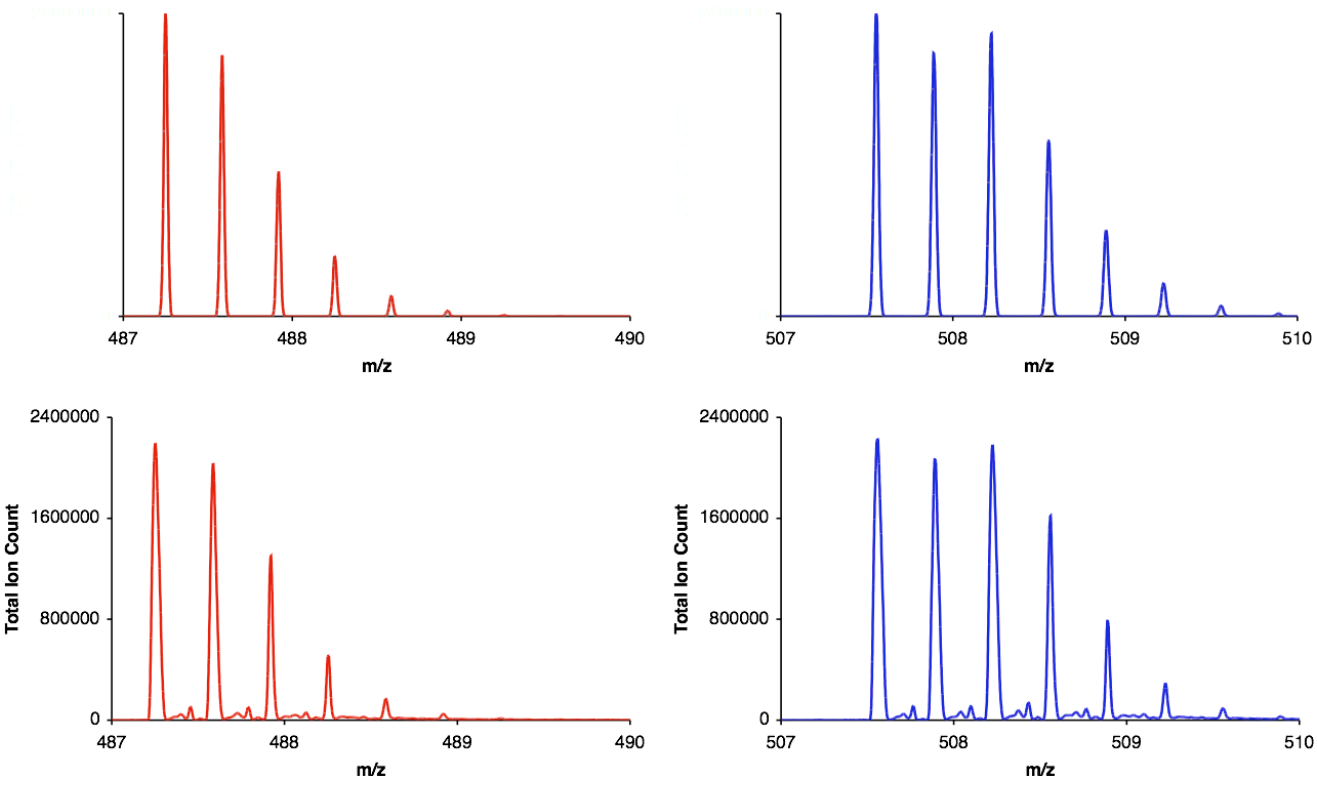
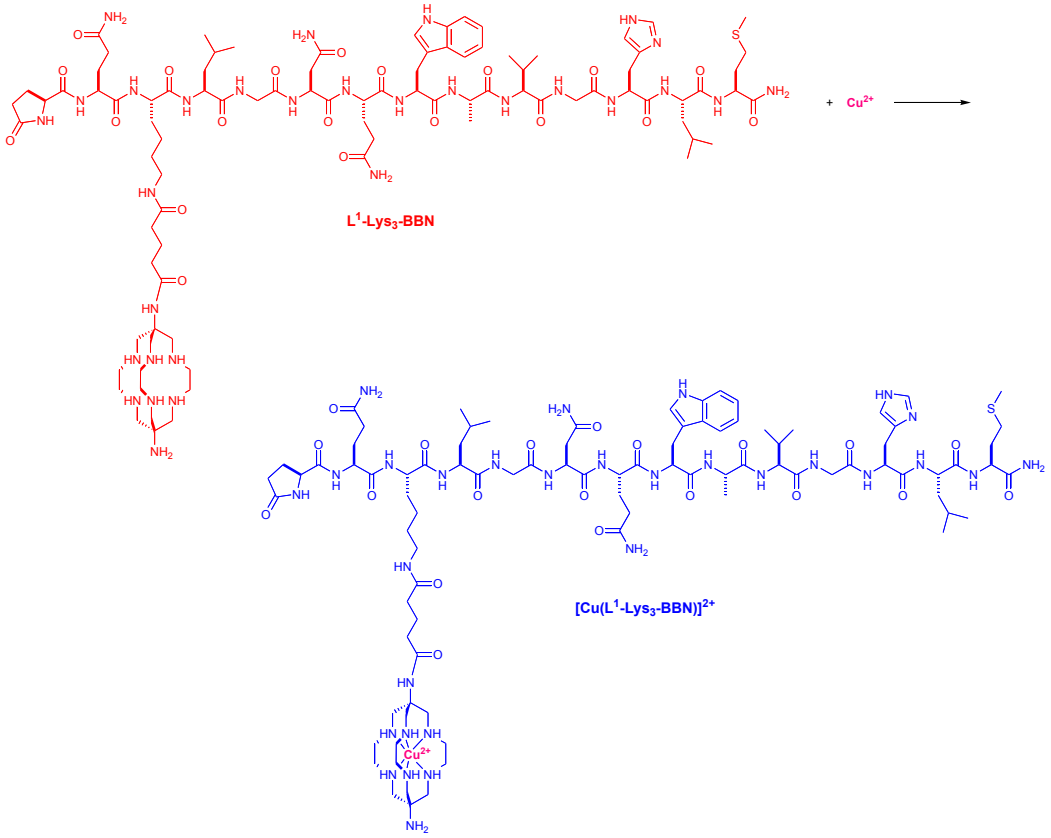


Figure S5: MS signals for gas phase ion of **L^1-OCT**, $[C_{68}H_{105}N_{18}O_{14}S_2]^{3+}$: (red) 487.25 (experimental, bottom), 487.25 (calculated, top); **L^1-OCT** with Cu^{2+}, $[CuC_{68}H_{103}N_{18}O_{14}S_2]^{3+}$: (blue) 507.56 (experimental, bottom), 507.55 (calculated, top);



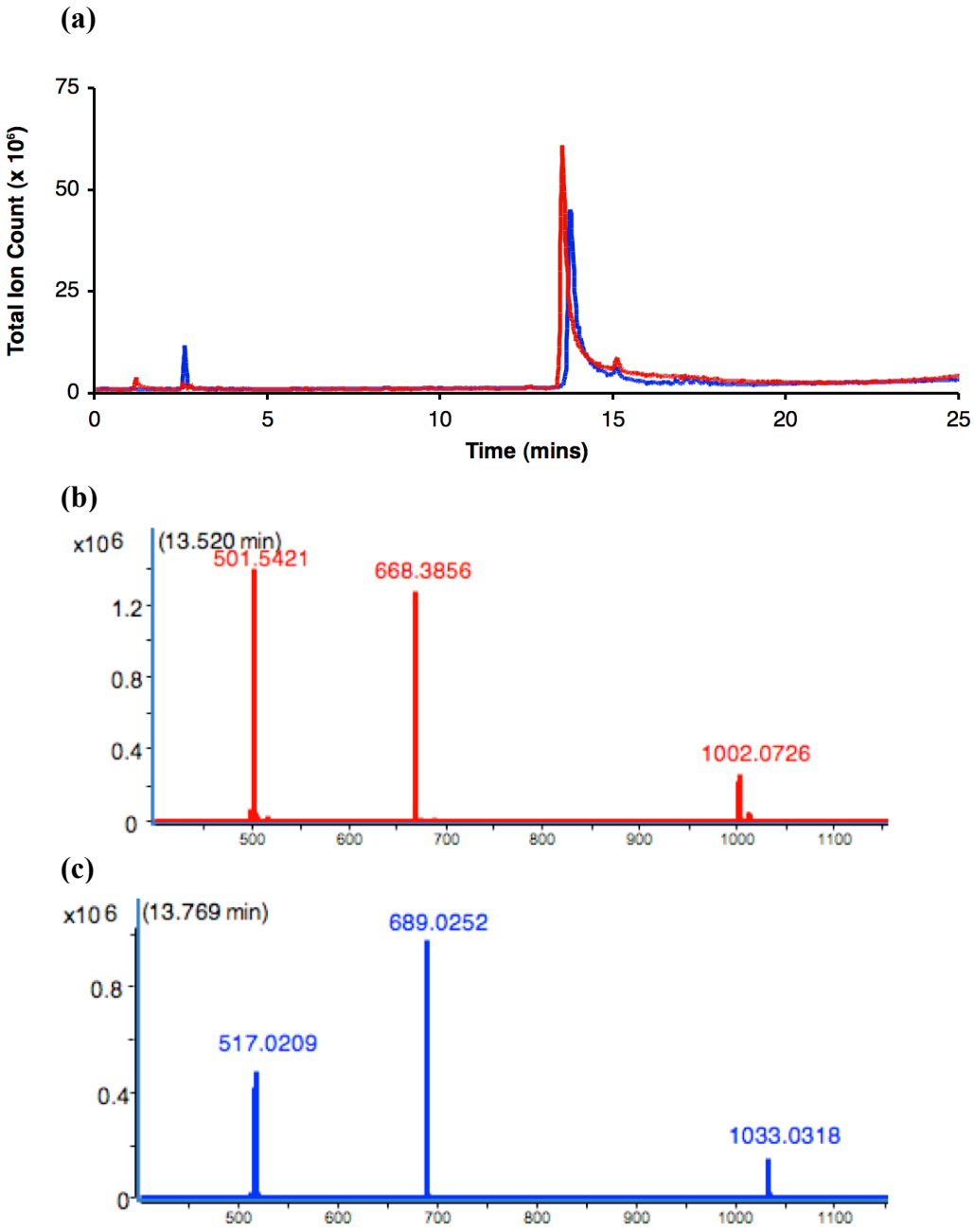


Figure S6: (a) LC-MS trace of **L^1-Lys_3-BBN** (red) and **L^1-Lys_3-BBN** complexed with Cu^{2+} (blue) (b) MS of **L^1-Lys_3-BBN** at 13.52 min (TIC vs m/z) (c) MS of **L^1-Lys_3-BBN** with Cu^{2+} at 13.77 min

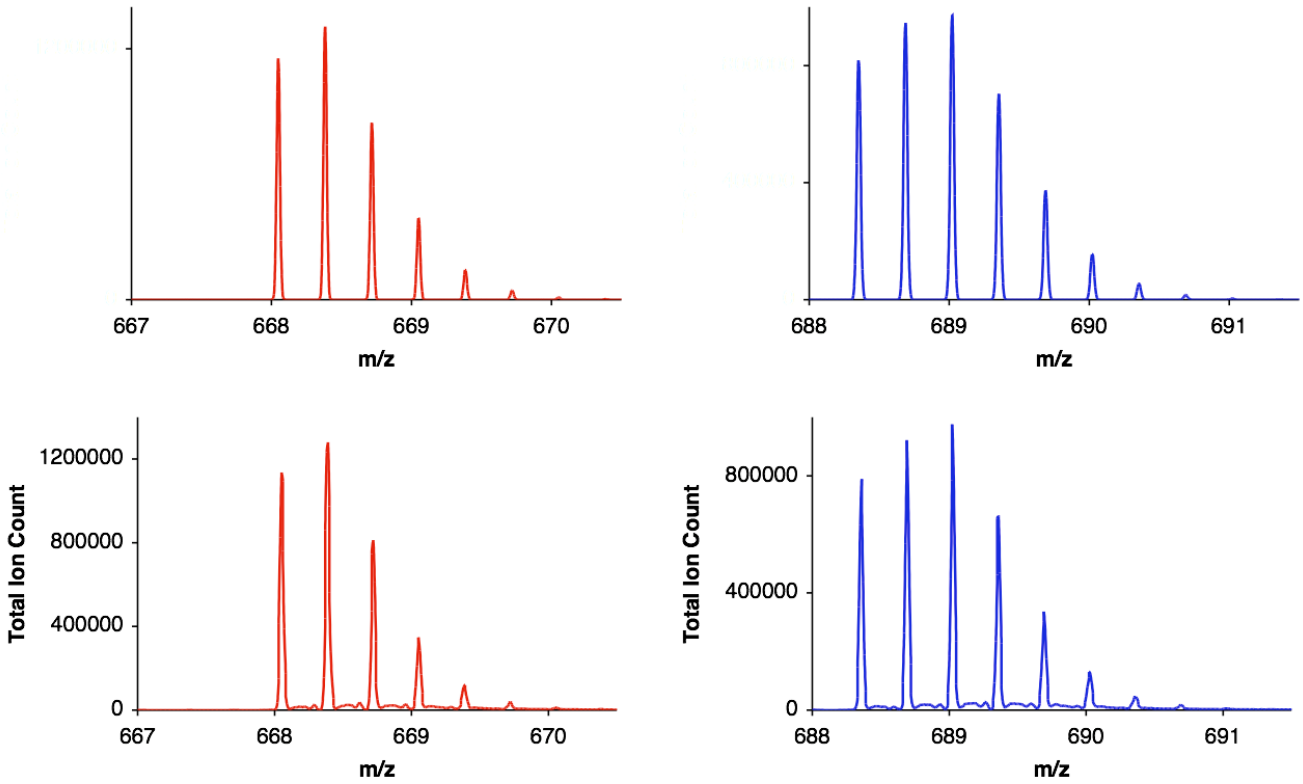


Figure S7: MS signals for **L^1-Lys_3-BBN**, $[C_{90}H_{151}N_{30}O_{20}S]^{3+}$: (red) 668.39 (experimental, bottom), 668.38 (calculated, top); and **L^1-Lys_3-BBN** with Cu^{2+}, $[CuC_{90}H_{149}N_{30}O_{20}S]^{3+}$: (blue) 689.02 (experimental, bottom), 689.02 (calculated, top);