Electronic Supplementary Material (ESI) for Chemical Communications

CuproCleav-1, a first generation photocage for Cu^{+}

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General Procedures

All the materials listed below were of a research grade or a spectro-grade in the highest purity commercially available from Acros Organics or TCI America. Dichloromethane (CH_2Cl_2), toluene ($C_6H_5CH_3$) and tetrahydrofuran (THF) were purged with argon and dried by passage through a Seca Solvent Purification System. All chromatography and TLC were performed on silica (230-400 mesh) from Silicycle unless otherwise specified. Activated basic alumina (~150 mesh) was obtained from Acros Organics. TLCs were developed with mixtures of ethyl acetate (EtOAc)/hexanes or CH_2Cl_2/methanol (MeOH) unless otherwise specified and were visualized with 254 and 365 nm light and I_2 or Br_2 vapor. ^1H and ^{13}C NMR spectra were recorded using a Brüker 400 MHz NMR instrument and chemical shifts are reported in ppm on the δ scale relative to tetramethylsilane. IR spectra were recorded on a Nicolet 205 FT-IR instrument and the samples were prepared neat. High resolution mass spectra were recorded at the University of Connecticut mass spectrometry facility using a micromass Q-Tof-2^{TM} mass spectrometer operating in positive ion mode. The instrument was calibrated with Glu-fibrinopeptide B 10 pmol/μL using a 50:50 solution of CH_3CN and H_2O with 0.1% acetic acid (AcOH). Compound **4** **a**nd **8** were synthesized according to the literature procedures.

Methyl-3-amino-3-(3,4-dimethoxyphenyl)propanoate (12). The amino acid **4** **(**10.0 g, 44.4 mmol) and *p*-toluenesulfonic acid monohydrate (16.9 g, 88.8 mmol) were dissolved in methanol (200 mL) and refluxed for 24 h. Solvent was removed, and the resulting solid mixture was dissolved in 50 % NH_4OH (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). Organic layer was dried over $MgSO_4$, and solvent removed under vacuum to yield methyl 3-amino-3-(3,4-dimethoxyphenyl)propanoate as a white solid (9.5 g, 89.0 %). TLC R_f = 0.38 (alumina, EtOAc). ^1H-NMR (400 MHz, $CDCl_3$) δ 6.84-6.93 (m, 3 H), 4.38 (s, 1 H), 3.86 (s, 3 H), 3.81 (s, 3 H), 3.67

(s, 3 H), 2.69-2.65 (m, 2 H). ^{13}C-NMR (100 MHz) δ 172.7, 149.3, 148.5, 137.6, 118.4, 111.4, 109.6, 56.1, 56.0, 52.6, 51.9, 44.4. IR (Neat), 3546.8, 2951.2, 2834.6, 1729.2, 1529.1, 1437.6, 1257.2, 1137.4, 1024.3 cm^{-1}. HRMS (+ES1), calculated for MH^+ 240.1236, observed 240.1255.

Methyl-3-((*tert*-butoxycarbonyl)amino)-3-(3,4-dimethoxyphenyl)propanoate (13). In 200 mL of CH_2Cl_2, the methyl-3-amino-3-(3,4-dimethoxyphenyl)propanoate (9.0 g, 37.6 mmol) and triethylamine (10.5 mL, 75.3 mmol) were added followed by *di-tert*-butyldicarbonate (9.0 g, 41.4 mmol).The reaction was left to stir for at 23 °C for 24 h. The reaction mixture was thereafter washed sequentially with 1 M HCl (100 mL), sat.$NaHCO_3$ (100 mL) and water (100 mL). The organic portion was dried over $MgSO_4$ and concentrated *in vacuo* to yield the product as white solid (12.2 g, 87.2 %). Mp = 106–108 ºC, TLC R_f = 0.5 (silica, 1:1 EtOAc/CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.85 (m, 3 H), 5.40 (s, 1 H), 5.06 (s, 3 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.65 (s, 3 H), 2.85 (m, 2 H), 1.55 (s, 9 H). ^{13}C-NMR (100 MHz) δ 171.6, 155.2, 149.2, 148.6, 134.1, 118.3, 111.4, 109.9, 68.1, 56.1, 56.0, 51.9, 41.0, 28.5, 25.8. IR (Neat), 3397.6, 2971.9, 2838.5, 1732.8, 1688.8, 1513.4, 1280.5, 1165.81, 1145.2, 1023.8, 984.8 cm^{-1}**.** HRMS (+ES1), calculated for MH^+ 340.1760, observed 340.1774.

***tert*-Butyl (1-(3,4-dimethoxyphenyl)-3-hydroxypropyl)carbamate (5).** In a 500 mL round bottom flask methyl 3-((*tert*-butoxycarbonyl)amino)-3-(3,4-dimethoxyphenyl)propanoate (8.50 g, 25.1 mmol) was dissolved in 50 mL of dry THF. The reaction mixture was chilled to 0 °C and borane in THF (10.8 g, 125 mmol) was added slowly. Reaction mixture was left to warm up to room temperature and stirred for 20 h. Reaction mixture was added on to 200 mL of ice cold water cautiously. Extraction was done using CH_2Cl_2 (3 × 100 mL) and the organic extracts were dried over $MgSO_4$. Solvent removal gave a white solid which was washed with ether (50 mL × 3) to furnish the product as a white solid (6.21 g, 79.5 %). TLC R_f = 0.32 (silica, 1:1

EtOAc/CH_2Cl_2). Mp = 87−89 ºC. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.79 (m, 3 H), 5.25 (s, 1 H), 4.77(s, 1 H), 3.90 (d, *J* = 4 Hz, 6 H), 3.62 (s, 2 H), 3.44 (s, 1 H), 1.98 (m, 1 H), 1.82 (m, 1 H), 1.39 (s, 9 H). ^{13}C-NMR (100 MHz) δ 156.6, 149.3, 148.6, 134.9, 118.4, 118.4, 111.5, 110.3, 80.2, 59.3, 56.1, 51.6, 39.7, 28.5. IR (Neat) 3479.5, 3361.4, 2933.7, 2835.5, 1679.9, 1513.9, 1258.4, 1230.4, 1137.2, 1023.16, 966.0 cm^{-1}. HRMS (+ES1), calculated for MH^+ 312.1811, observed 312.1814.

***tert*-Butyl(1-(4,5-dimethoxy-2-nitrophenyl)-3-hydroxypropyl)carbamate (6)**.

Trifluoromethanesulfonic acid (TfOH, 2.28 mL, 25.7 mmol) was dissolved in CH_2Cl_2 (50 mL) followed by anhydrous HNO_3 acid (0.6 mL, 12.9 mmol) which resulted to a white crystalline solid formation and was allowed to stir for 1 h at 23 °C. The reaction temperature was reduced to -43 °C, **5** (2.00 g, 64.3 mmol) dissolved in CH_2Cl_2 (5 mL) was added as one portion. The reaction mixture was maintained at - 43 °C for 6 h with minimal stirring and then quickly poured onto a chilled saturated solution of $NaHCO_3$ (50 mL). Extraction was done using CH_2Cl_2 (3 × 100 mL) and the organic extracts dried over $MgSO_4$. Gravity chromatography on silica (3:7 EtOAc/CH_2Cl_2) furnish the product as a yellow solid (0.91 g, 40.1 %) Mp = 139−143 ºC, TLC R_f = 0.48 (silica, 3:7 EtOAC/CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1 H), 7.02 (s, 1 H), 5.98(m, 1 H), 5.42 (s, 1 H), 3.92 (d, *J* = 12 Hz, 6 H), 3.78 (s, 2 H), 2.11(m, 1 H), 1.98 (m, 1 H), 1.43 (s, 9 H). ^{13}C-NMR (100 MHz) δ, 171.4, 155.9, 153.6, 147.9, 140.8, 110.9, 108.9, 60.6, 56.7, 51.1, 37.4, 28.5, 21.22, 14.37. IR (Neat) 3362.3, 2976.1, 2835.5, 1678.2, 1151.7, 1467.5, 1322.4, 1266.5, 1157.7, 1055.7, 1024.3, 972.5 cm^{-1}. HRMS (+ES1), calculated for MH^+ 357.1662, observed 357.1657.

3-((*tert*-Butoxycarbonyl)amino)-3-(4,5-dimethoxy-2-nitrophenyl)propyl methanesulfonate (7). Methanesulfonyl chloride (0.13 mL, 1.68 mmol) was added

dropwise into a mixture of compound **6** (0.6 g, 1.68 mmol) and triethylamine (0.704 mL, 5.06 mmol) in CH_2Cl_2 (20 mL) at 0 °C. Reaction was left to stir for 1 h resulting to a yellow reaction mixture. 2 M HCl (10 mL) was added followed by extraction using CH_2Cl_2 (3 × 25 mL).The organic fractions were pooled, dried under vacuum to give the product as a white solid (0.62 g, 84.5 %). Mp =140 ºC (decomposes). TLC R_f = 0.64 (silica, 3:7 EtOAC/CH_2Cl_2). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1 H), 6.98 (s, 1 H), 5.55(m, 1 H), 5.39 (m, 1 H), 4.37 (d, J = 12 Hz, 2 H), 3.97 (s, 6 H), 3.08 (s, 3 H), 2.35 (m, 2 H), 1.28 (s, 9 H). ^{13}C-NMR (100 MHz) δ 155.3, 153.9, 149.5, 148.8, 148.3, 118.4, 111.6, 67.1, 56.8, 56.6, 56.2, 37.7, 37.6, 36.1, 28.5. IR (Neat) 3372.7, 2978.5, 2848.9, 1676.3, 1514.4, 1360.6, 1278.9, 1171.9, 1057.1, 971.14, 795.7 cm^{-1}. HRMS (+ES1) , calculated for MH^+ 435.1437, observed 435.1464.

***tert*-Butyl-(3-(bis(2-(ethylthio)ethyl)amino)-1-(4,5-dimethoxy-2-nitrophenyl)propyl)carbamate (14).** A mixture of **8** (0.25 g, 1.31 mmol), K_2CO_3, (0.48 g, 3.58 mmol), 18-C-6 (0.31 g, 1.19 mmol) in CH_3CN (10 mL) was refluxed for 30 min. Compound **7** (0.52 g, 1.19 mmol) in CH_3CN (1 mL) was added dropwise to the refluxing reaction mixture and reflux continued for 1 h resulting to an intense yellow reaction mixture. The mixture was allowed to cool to ambient temperature, water (20 mL) was added, to dilute the reaction and extraction was effected by CH_2Cl_2. The organic portion was washed several times with copious amount of saturated aqueous KCl, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow solid. Gravity chromatography on silica (1:1 EtOAc/hexanes) provided the product as a white solid (0.37 g, 57.9 %). Mp = 143−145 ºC, R_f = 0.51 (silica, 1:1, EtOAc/hexanes). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1 H), 6.95 (s, 1 H), 5.49 (s, 1 H), 5.37 (m, 1 H), 4.40 (m, 2 H), 3.95 (d, J = 8 Hz, 6 H), 3.46 (m, 4 H), 2.70 (m, 4 H), 2.59 (m, 4 H), 2.22 (m, 1 H), 2.04 (m, 1 H) 1.39 (t, 6 H) 1.25 (s, 9 H). ^{13}C-NMR (100 MHz) δ 155.9, 155.2, 153.7, 148.0, 140.6, 110.2, 108.9, 62.8,

56.67, 56.6, 48.6, 48.1, 30.5, 29.7, 28.5, 26.3, 26.2, 15.0. IR (film) 3359.4, 2960.4, 2841.7, 1676.4, 1509.8, 1277.9, 1224.5, 1167.9, 1057.8, 866.5, 794.9 cm^{-1}. HRMS (+ES1) , calculated for MH^+ 532.2515, observed 532.2491.

1-(4,5-Dimethoxy-2-nitrophenyl)-*N3,N3*-bis(2-(ethylthio)ethyl)propane-1,3-diamine (9). Trifluoroacetic acid (CF_3CO_2H, 2.5 mL) was added to a solution of **14** (0.3 g, 0.56 mmol) in CH_2Cl_2 (20 mL) and stirred at 23 °C for 3 h. Reaction mixture was concentrated *in vacuo* and saturated $NaHCO_3$ was added. Extraction was done with CH_2Cl_2 (3 × 30 mL).The combined organic extracts were dried over $MgSO_4$, filtered, and solvent removed *in vacuo* to yield the product (0.21 g, 87.2 %) as a yellow oil. The crude was taken to the next step without further purification. TLC R_f= 0.38 (alumina, 9 : 1, EtOAc/MeOH). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.49 (s, 1 H), 7.29 (s, 1 H), 4.79 (m, 1 H), 4.36 (m, 1 H), 4.20 (m, 1 H), 4.00 (s, 3 H), 3.94 (s, 3 H), 3.47 (m, 4 H), 2.73 (m, 4 H), 2.58(m, 4 H), 2.17 (m, 1 H) 1.93 (m, 1 H) 1.26 (s, 6 H). ^{13}C-NMR (100 MHz) δ 156.2, 153.6, 147.8, 135.9, 109.3, 107.9, 62.9, 56.6, 56.5, 48.6, 47.7, 38.3, 29.7, 26.2, 15.0. IR (Neat) 3468.5, 2927.5, 2831.9, 1690.2, 1516.0, 1421.6, 1268.6, 1197.4, 1055.3, 867.8, 727.4 cm^{-1}. HRMS (+ES1) , calculated for MH^+ 432.1919, observed 432.1940.

Ethyl-2-(bis(2-(ethylthio)ethyl)amino)acetate (15). A solution of **8** (15.0 g, 78.1 mmol), K_2CO_3, (31.6 g, 234 mmol), 18-crown-6 (20.7 g, 781 mmol) in CH_3CN (200 mL) was brought to reflux for 30 min. Ethyl bromoacetate (10.4 mL, 85.9 mmol) was added dropwise and reflux continued for 20 h resulting to a dark reaction mixture. Reaction mixture was concentrated under reduced pressure. Water (100 mL) was added and extraction was effected by CH_2Cl_2. The organic portion was washed several time with copious amount of saturated aqueous KCl and dried over $MgSO_4$. Solvent was removed under reduced pressure resulting to a thick yellow oil (19.6 g, 90.2 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.13 - 4.06 (q, J = 21 Hz, 2 H), 3.37 (s, 2 H),

2.82 (m, 4 H), 2.59 - 2.47 (m, 8 H), 1.23-1.16 (m, 9 H). ^{13}C-NMR (100 MHz) δ 171.4, 60.6, 55.2, 54.5, 30.1, 26.3, 30.1, 26.3, 15.1, 14.4.

2-(Bis(2-(ethylthio)ethyl)amino)acetic acid (16). The ester **15** (19.6 g, 70.5 mmol) was dissolved in 1:1 MeOH: dioxane (100 mL) followed by KOH (19.8 g, 325 mmol) dissolved in 1:1 MeOH: dioxane (40 mL). Reaction mixture was left to stir at 23° C for 20 h. Water (100 mL) was added and the pH was adjusted to 6.8 by 2M HCl. Extraction was done by CH_2Cl_2 (3 × 100 mL), dried over $MgSO_4$ and concentrated under reduced pressure to yield **16** as a white solid (7.8 g, 44.2 %) . ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.37 (s, 2 H), 2.92-2.88 (m, 4 H), 2.70 - 2.67 (m, 4 H), 2.60 - 2.55 (m, 4 H), 1.30 - 1.27 (t, J = 9 Hz, 6 H).^{13}C-NMR (100 MHz) δ 171.8, 57.2, 54.3, 29.5, 26.4, 14.9.

2,5-Dioxopyrrolidin-1-yl-2-(bis(2-(ethylthio)ethyl)amino)acetate (10). 2-(Bis(2-(ethylthio)ethyl)amino)acetic acid (4 g, 16.0 mmol) and *N*-hydroxysuccinimide (1.84 g, 16.0 mmol) were dissolved in CH_2Cl (50 mL). At 0 °C, dicyclohexylcarbodiimide (DCC) (3.63 g, 17.6 mmol) dissolved in CH_2Cl_2 (10 mL) was added drop wise and reaction was left to warm up to 23 °C for a period of 2 h. The white precipitate was filtered off and on concentrating the reaction mixture, a yellow thick oil was obtained. Crystallization was effected by 1:1 ether:hexanes mixture to give a white crystalline solid (3.68 g, 66 %). Mp = 38 ºC, TLC R_f= 0.71 (alumina, 9:1 CH_2Cl_2/EtOAc) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 2 H), 2.98 - 2.94 (m, 4 H), 2.96 (s, 4 H), 2.69-2.65 (m, 4 H), 2.61 - 2.55 (q, J = 24 Hz, 4 H), 1.29 - 1.26 (t, J = 12 Hz, 6 H). ^{13}C-NMR (100 MHz) δ 169.1, 166.4, 54.1, 52.5, 30.1, 26.4, 25.8, 15.1. IR (Neat) 2959.1, 2924.6, 2850.1, 1814.3, 1722.3, 1428.0, 1365.7, 1198.3, 1042.6, 956.9, 851.2 cm^{-1}. HRMS (+ES1), calculated for MH^+ 349.1256, observed 349.1280.

2-(Bis(2-(ethylthio)ethyl)amino)-N-(3-(bis(2-(ethylthio)ethyl)amino)-1-(4,5-dimethoxy-2-nitrophenyl)propyl)acetamide (CuproCleav-1, 1). Compound **10** (0.11 g, 0.25 mmol), K_2CO_3, (0.06 g, 5.06 mmol), 18-crown-6 (0.07 g, 0.25 mmol) were dissolved in CH_3CN (6 mL). Compound **9** (0.09 g, 0.25 mmol) dissolved in CH_3CN (2 mL) was added drop wise to the reaction mixture and left to stir at 23 °C for 2 h. Water (20 mL) was added and extraction was effected by CH_2Cl_2. The organic portion was washed several time with copious amount of saturated aqueous KCl and dried over $MgSO_4$. Solvent was removed under reduced pressure resulting to a yellowish oil. Purification by gravity chromatography on silica using EtOAc as elutant gave 0.07 g, 45.5 %) of the product as a white solid TLC R_f = 0.34 (silica, EtOAc). Mp = 69−71 ºC ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1 H), 7.56 (s, 1 H), 7.06 (s, 1 H), 5.74 (m, 1 H), 4.43 (m, 2 H), 4.00 (s, 3 H), 3.94 (s, 3 H), 3.48 (m, 4 H), 3.15 (d, J = 16 Hz, 2 H), 2.80 - 2.53 (m, 20 H), 2.33(m, 1 H) 2.22 (m, 1 H) 1.40 (m, 12 H). ^{13}C-NMR (100 MHz) δ 170.7, 155.8, 147.9, 141.0, 132.4, 110.6, 108.4, 62.2, 58.4, 56.8, 56.6, 54.4, 48.1, 47.2, 35.1, 29.6, 26.5, 14.9. IR (film) 3298.1, 2962.0, 2825.6, 2246.9, 1693.9, 1518.5, 1422.5, 1270.8, 1195.9, 1058.1, 866.1, 728.7 cm^{-1}. HRMS (+ES1), calculated for MH^+ 665.2899, observed 665.2847.

***N*-Benzyl-2-(bis(2-(ethylthio)ethyl)amino)acetamide (11).** Benzyl amine (0.07 g, 0.65 mmol), K_2CO_3, (0.17 g, 1.3 mmol), 18-crown-6 (0.18 g, 0.65 mmol) were dissolved in CH_3CN (10 mL). Compound **10** (0.23 g, 0.65 mmol) dissolved in CH_3CN (2 mL) was added drop wise to the reaction mixture and left to stir at 23 °C for 2 h. Water (20 mL) was added to dilute the reaction mixture and extraction was effected by CH_2Cl_2. The organic portion was washed several time with copious amount of saturated aqueous KCl, dried over $MgSO_4$, and filtered. Solvent was removed under reduced pressure to give the product as a yellow oil. (0.17 g, 76 %). TLC R_f = 0.48 (silica, EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1 H), 7.32 (m, 5 H), 4.49 (s, 2 H),

3.21 (s, 2 H), 2.75 (m, 4 H), 2.58 (m, 4 H), 2.46 (m, 4 H), 1.19 (t, 6 H). ^{13}C-NMR (100 MHz) δ 171.3, 138.7, 128.8, 128.0, 127.5, 58.8, 54.8, 43.3, 30.0, 26.4, 14.9. IR (film) 3029.6, 2960.2, 1617.9, 1519.6, 1454.1, 1265.1, 1112.7, 700.0 cm^{-1}. HRMS (+ES1), calculated for MH^+ 341.1721, observed 341.1682.

General Spectroscopic Methods.

All solutions were prepared with spectrophotometric grade solvents. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and KCl (99.5 %) were purchased and used as received. Cu^+ stock solutions were prepared from 99.9 % pure $(CH_3CN)_4CuPF_6$ while Zn^{2+}, Hg^{2+}, Cd^{2+} and Cu^{2+} were prepared from their perchlorate salts. Cu^+ solutions was introduced stabilized in thiourea. CuproCleav-1 was introduced to aqueous solution by addition of stock solution in DMSO (5.87 mM). Graphs were manipulated and equations calculated by using Kaleidagraph 4.0. The pH values of solutions were recorded with an Omega PHB 212 glass electrode that was calibrated prior to each use. Absorption spectra were recorded on a Cary 50 UV-visible spectrophotometer under the control of a Pentium IV-based PC running the manufacturer supplied software package. Spectra were routinely acquired at 25 °C, in 1-cm path length quartz cuvettes with a total volume of 3.0 mL. Fluorescence spectra were recorded on a Hitachi F-4500 spectrophotometer under the control of a Pentium-IV PC running the FL Solutions 2.0 software package. Excitation was provided by a 150 W Xe lamp (Ushio Inc.) operating at a current of 5 A. Spectra were routinely acquired at 25 °C, in 1 cm quartz cuvette with a total volume of 3.0 mL using, unless otherwise stated, 5 nm slit widths and a photomultiplier tube power of 700 V. Photolysis experiments were performed at 25 ^{o}C, in 1-cm path length quartz cuvettes illuminated by a 8 W lamps photoreactor source at 350 nm. High performance liquid chromatography (HPLC) was performed on a Perkin Elmer 250 binary pump

equipped with a diode array detector under the control of a Pentium-III PC running the PeakSimple software package. An Apollo silica 5µ column (250 × 4.6 mm) was used for the separation of photoproducts and the peaks were detected at 250 nm. A linear gradient from A in B to 2 % A was run at 0.1 mL/min for 30 min, where A is i-PrOH and B is EtOAc.

Determination of Cu^+ binding constant

Calibration curve. A 2 µM solution of the fluorescent Cu^+ sensor (CS1) (20 mM HEPES, 100 mM KCl, 30 % EtOH, pH 7.0) was prepared from a 2.4 mM CS1 stock solution. A 3.0 mL aliquot of this solution was placed in a quartz cuvette and fluorescence was measured. Five 0.3 µL aliquots of a 2.0 mM $(CH_3CN)_4CuPF_6$ stock solution was added to the above solution and fluorescence was measured after each addition. Fluorescence was corrected for dilution, normalized and plotted against total Cu^+ concentration to generate a calibration curve.

CS1 titration procedure. A 3.0 mL aliquot of a solution containing copper sensor 1 (CS1, 2 μM) at pH 7 (20 mM HEPES, 100 mM KCl, 30% EtOH) was placed in a cuvette and a flourescence spectrum was recorded. A 1.5 μL aliquot of a Cu^+ solution (1.0 µM) was added and the spectrum was recorded. The increase in flourescence at 560 nm results from the formation of $[Cu(CS1)]^+$ complex. Aliquots of the CuproCleav-1 stock solution (5.87 mM) were added in 5.0 μL increments and the flourescence spectra were recorded after each addition. The above procedure was repeated at 1 and 3 μM initial CS1 concentrations to assure reproducibility of results. Since CS1 forms a 1:1 complex with Cu^+, an excess (2 equiv.) of CS1 that is sufficient to put > 99 % of the Cu^+ in the $[Cu(CS1)]^+$ form must be used. Under the experimental conditions 99.99 % of Cu^+ is bound to the sensor at the beginning of the titration. The binding equilibrium

for this system may be expressed by equation 1. The binding constant of the [Cu(CuproCleav-1)]$^+$ complex ($K'_{CuproCleav\text{-}1}$) is obtained by solving equation 2, where K'_{CS1} = 3.6 × 10^{12} M^{-1}.

$$CuproCleav-1 + Cu(CS1) \rightleftharpoons Cu(CuproCleav-1) + CS1 \quad (1)$$

$$\frac{[Cu(CuproCleav-1)][CS1]}{[CuproCleav-1][Cu(CS1)]} = \frac{K'_{CuproCleav-1}}{K'_{CS1}} \quad (2)$$

Equations 3-5 were used to calculate the individual components of equation 2. [Cu(CS1)] was determined from the calibration curve.

$$[CS1] = [CS1]_{total} - [Cu(CS1)] \quad (3)$$

$$[Cu(CuproCleav-1)] = [Cu(CS1)]_{initial} - [Cu(CS1)] \quad (4)$$

$$[CuproCleav-1] = [CuprCleav-1]_{total} - [Cu(Cleav-1)] \quad (5)$$

Quantum Efficiency of Photolysis.

Calibration curve. A 5.87 mM of CuproCleav-1 stock solution was prepared. The stock solution was diluted to make 300, 200 and 100 µM CuproCleav-1 solutions. Similarly, a 300, 200 and 100 µM solutions of [Cu(CuproCleav-1)]$^+$ were prepared. HPLC traces of each solution were recorded and the area under the curve was determined by integration. Solvent of elution was 2 % i-PrOH in 98 % EtOAc and 100 % EtOAc respectively.

Photolysis of [Cu(CuproCleav-1)]$^+$. A 3.0 mL aliquot of a 0.5 mM solution of each CuproCleav-1 and [Cu(CuproCleav-1)]$^+$ was irradiated using an applified photophysics reactor (8 W lamps, λ_{ex} = 350 nm) . HPLC traces of the above solution were recorded after irradiating for 2, 4 and 6 min maintaining the overall concentration at 300 µM. The area of [Cu(CuproCleav-1)]$^+$ peak was obtained by integration and the concentration of remaining

$[Cu(CuproCleav\text{-}1)]^+$ was determined using the calibration curve. The quantum efficiency of photolysis of CuproCleav-1 was obtained by solving equation 6 where N_A is the Avogadro's number.

$$Quantum\,Efficiency = \frac{Change\,in[CuproCleav-1]/Irradiation\,time}{Intensity\,of\,the\,source}.N_A \qquad (6)$$

The intensity of the source was determined as described previously.[1-5]

Release of Caged Cu^+

A 3.0 mL aliquot of a 1.0 μM CS1 solution (20 mM HEPES, 100 mM KCl, 30 % EtOH, pH 7.4) was placed in a quartz cuvette and the fluorescence spectrum was recorded. A 7.5 μL aliquot of a $(CH_3CN)_4CuPF_6$ stock solution (20.6 mM prepared in 0.2 mM thiourea) was added to give a final concentration of 0.5 μM and the fluorescence spectrum was recorded. A 27.5 μL aliquot of the CuproCleav-1 stock solution (5.87 mM) was added to give a final concentration of 50 μM and the fluorescence spectrum was recorded. The cuvette was irradiated in a 4 W lamps photoreactor source at 350 nm continuously and the fluorescence spectra were recorded at 2 min intervals.

Selectivity study

A 3.0 mL aliquot of a solution containing Zinc sensor 1 (ZinPyr-1, 1 μM) at pH 7 (20 mM HEPES, 100 mM KCl, 30 % EtOH) was placed in a cuvette and the fluorescence spectrum recorded. A 3.6 μL aliquot of a Zn^{2+} solution (1.64 mM) was added and another fluorescence spectrum was recorded. The increase in absorbance at ~ 490 nm results from the formation of $[Zn(ZinPyr\text{-}1)]^{2+}$ complex. Aliquots of the CuproCleav-1 stock solution (5.87 mM) were added to a maximum of 300 equivalences and absorption spectra changes were recorded after each addition. This procedure was repeated replacing Zn^{2+} with Cd^{2+}, Hg^{2+} and Cu^{2+} ions.

References

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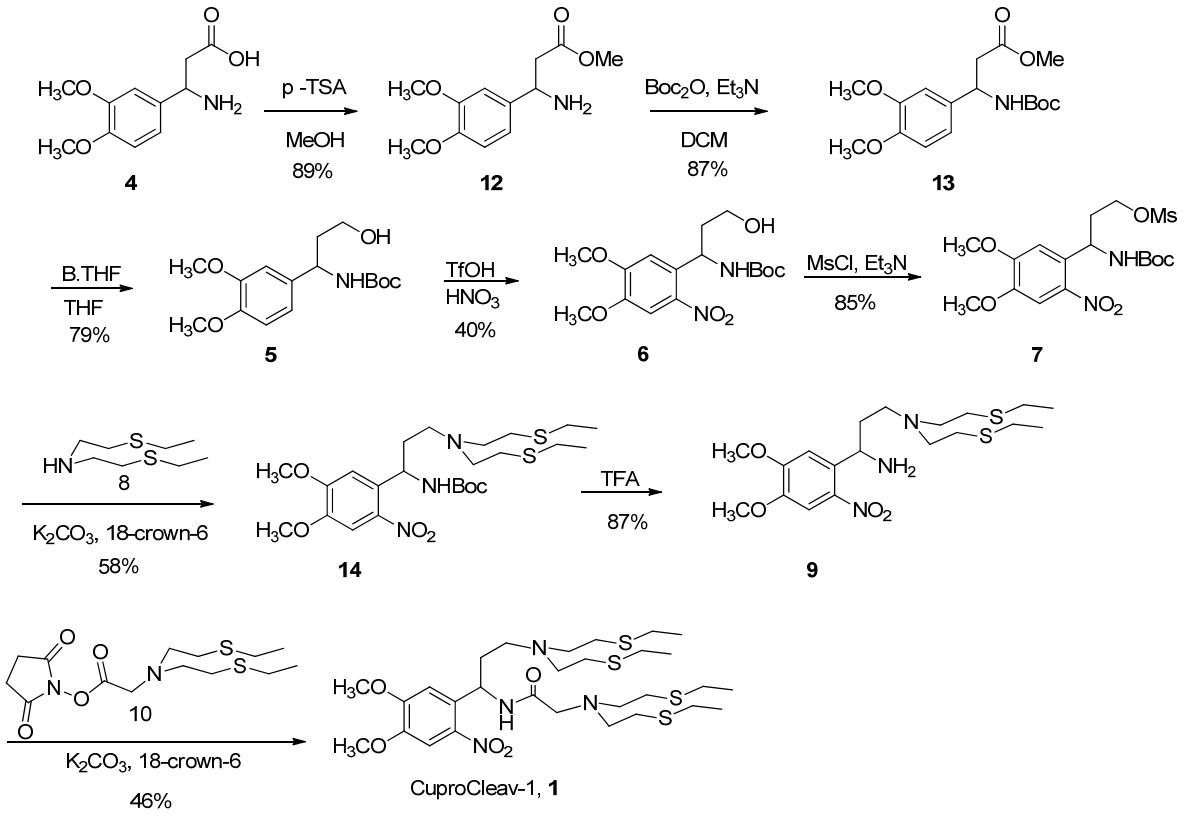
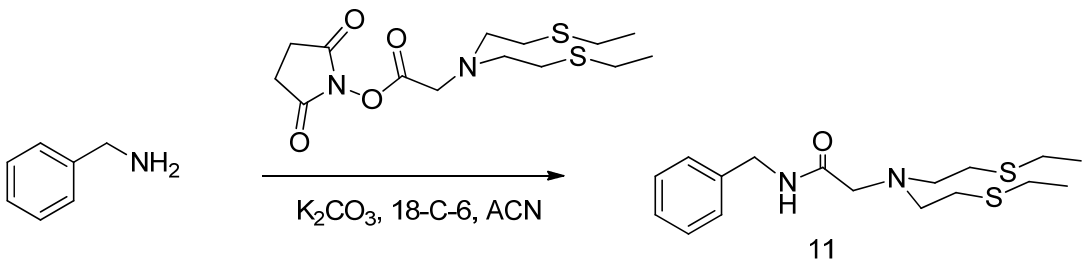


Scheme S1. Expanded synthetic scheme for CuproCleav-1



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Scheme S2. Synthesis of photoproduct model

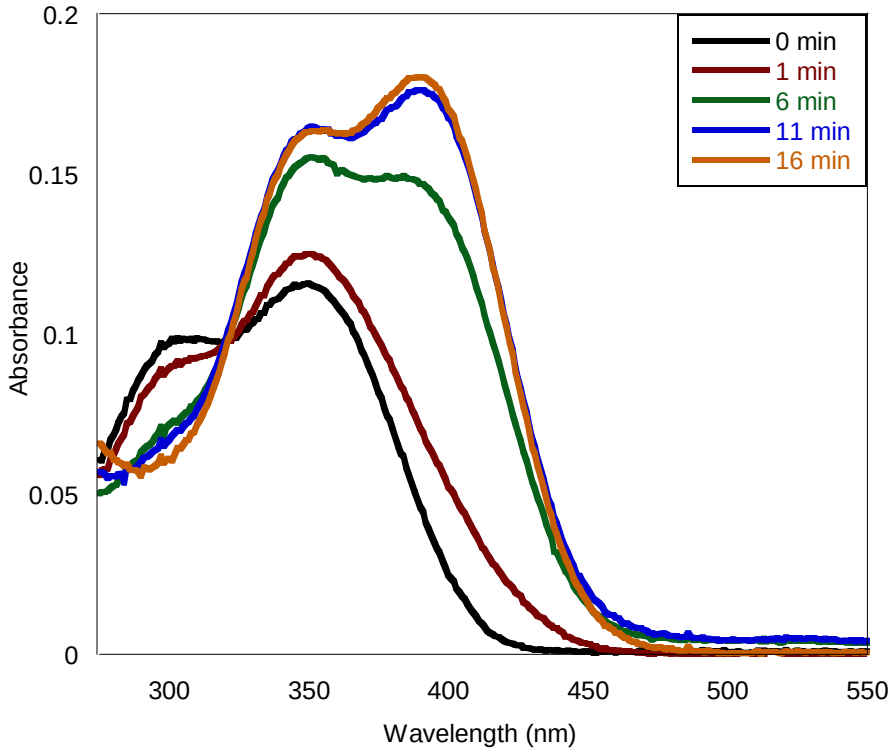

Figure S1. Photolysis of 25 μM CuproCleav-1 in 20 mM HEPES, 100 mM KCl, 30% EtOH, pH 7.0 using an 8 W lamps photoreactor source at 350 nm.