ZinCast-1: a photochemically active chelator for Zn²⁺

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General Procedures

All materials were purchased from Acros Organic or TCI America. Solvents were dried in Seca Solvent Purification System. All chemistry was performed in a lab with yellow UV filters on the room lights. *N*-phenyl-di(2-picolyl)amine (**3**) was prepared according to known procedures.¹ ¹H and ¹³C NMR spectra were recorded with Bruker 400 MHz spectrometer and referenced to CDCl₃. IR spectra were recorded on a Nicolet 205 FT-IR instrument and samples were analyzed as KBr pellets. High resolution mass spectra were obtained on micromass Q-Tof-2TM operating in positive ion mode.

[4-(Bis-pyridin-2-ylmethyl-amino)-phenyl]-(3,4-dimethoxy-phenyl)-methanone (4). A 10 g potion of 84% polyphosphoric acid (PPA) was added to a suspension of 2,3-dimethoxyobenzoic acid (1.09 g, 5.99 mmol) in 5 mL of CH₂Cl₂, and the resulting mixture was heated to 80 °C. Compound 3 (1.65 g, 5.99 mmol) was added to the red reaction mixture, which was stirred for an additional 2 h at 80 °C. The dark red reaction mixture was cooled to 0 °C and the PPA was neutralized by adding 6 M NaOH dropwise until the solution reached a pH of 9-10. T The product was extracted into CH_2Cl_2 (3 × 50 mL) and the combined organic extracts were dried over Mg₂SO₄. Flash chromatography on basic alumina with diethyl ether afforded **4** as a white solid (2.24 g, 84.9%). Mp = 135-136 °C. ¹H NMR (CDCl₃, 400 MHz) δ 8.60 (2 H, d, J = 4.0, H₆. P_{V}), 7.70 (2 H, d, J = 8.0 Hz, ArH), 7.64 (2 H, t, J = 8.0 Hz, H_{4-Pv}), 7.37 (1 H, s, ArH), 7.32 (1 H, d, J = 8.0 Hz, ArH), 7.24 (2 H, d, J = 8.0 Hz, H_{3-Py}), 7.20 (2 H, t, J = 8.0 Hz, H_{5-5Py}), 6.85 (1 H, d, J = 8.0 Hz, ArH); 6.75 (2 H, d, J = 8.0 Hz, ÅrH); 4.90 (4 H, s, N-CH₂-Py), 3.92 (3 H, s, OCH₃); 3.90 (3 H, s, OCH₃). ¹³C NMR (CDCl₃, 100 MHz) δ 194.1, 157.9, 152.3, 151.8, 150.1, 149.0, 137.1, 132.7, 131.6, 127.0, 124.5, 122.5, 120.9, 112.5, 111.7, 109.9, 57.4, 56.2 ppm. IR (KBr pellet) 3076, 3007, 2963, 2834, 1625, 1461, 1437, 1414, 1390, 1365, 1340, 1321, 1038, 992, 958, 941, 877, 841, 818, 702, 627, 510, 406. HRMS (⁺ESI): Calcd for MH⁺, 440.1974; Found, 440.2010.

[4-(Bis-pyridin-2-ylmethyl-amino)-phenyl]-(4,5-dimethoxy-2-nitro-phenyl)-methanone (5).

Cu(NO₃)₂ × 2.5 H₂O (1.27 g, 5.46 mmol) was added to a solution of **4** (1.60 g, 3.64 mmol) in acetic anhydride (10 mL) at 0 °C and the reaction mixture was stirred for 12 h. Ice and 25 mL of 0.4 M EDTA were added to the solution and the pH was adjusted to 9 with 6M NaOH. The product was extracted into CH₂Cl₂ (3 × 50 mL) and the combined organic extracts were dried over Mg₂SO₄. Flash chromatography on alumina (17:3 CH₂Cl₂/EtOAc) followed by recrystallization from toluene yielded **5** as a white solid (1.00 g, 56.7%). Mp = 200-201 °C. ¹H NMR (CDCl₃, 400 MHz) δ 8.59 (2 H, d, *J* = 4.0 Hz, H₆-Py), 7.69 (1 H, s ArH), 7.64 (2 H, t, *J* = 8.0 Hz, H₄-Py), 7.58 (2 H, d, *J* = 12.0 Hz, ArH), 7.19 (4 H, m), 6.78 (1 H, s, ArH), 6.69 (2 H, d, *J* = 12.0 Hz, ArH), 4.87 (4 H, s, N-CH₂-Py), 3.99 (3 H, s, OCH₃), 3.92 (3 H, s, OCH₃). ¹³C NMR (CDCl₃, 100 MHz) δ 191.7, 157.5, 154.0, 152.8, 150.2, 149.4, 137.2, 131.9, 131.6, 125.5, 122.6, 120.9, 112.0, 110.2, 107.1, 57.3, 56.8, 56.7 ppm. IR (KBr pellet) 3076, 3007, 2963, 2834, 1647, 1592-1570, 1548, 1522, 1453-1440, 1408-1390, 1340, 1284, 1265, 1221, 1196, 1190, 1182, 1145, 1065, 933, 871, 824, 753, 614. HRMS (⁺ESI): Calcd for MH⁺, 485.1825; Found, 485.1780.

[4-(Bis-pyridin-2-ylmethyl-amino)-phenyl]-(4,5-dimethoxy-2-nitro-phenyl)-methanol

(ZinCast-1, 1). Compound 5 (0.875 g, 1.99 mmol) was dissolved in CH₃CN, chilled to 0 °C and NaBH₄ (753 mg, 1.99 mmol) was added slowly over a period of 1-2 minutes. After the addition was complete, the reaction mixture was slowly heated to 70 °C over a period of 2 h and stirred for an additional 12 h. The reaction mixture was diluted into 10 mL of ice water and 1 mL of conc. HCl, and the pH was adjusted to 9 with 6M NaOH. The product was extracted into CH₂Cl₂ (3 × 30 mL) and the combined organic extracts were dried over Mg₂SO₄. Flash chromatography on alumina (4:6 CH₂Cl₂/EtOAc) followed by recrystallization from toluene/hexanes yielded **ZinCast-1** as fine yellow crystals (0.324 g, 33.5%). Mp = 113-114 °C. ¹H-NMR (CDCl₃, 400 MHz) δ 8.55 (2 H, d, *J* = 4.0 Hz, H_{6-Py}), 7.60 (3 H, m), 7.41 (1 H, s, ArH), 7.21 (2 H, d, *J* = 8.0 Hz, H_{3-Py}), 7.15 (2 H, t, *J* = 8.0 Hz, H_{5-Py}), 7.09 (2 H, d, *J* = 12.0 Hz, ArH), 6.61 (2 H, d, *J* = 8.0 Hz, ArH), 6.47 (1 H, s, C<u>H</u>-OH), 4.76 (4 H, s, N-CH₂-Py), 3.95 (3 H, s, OCH₃), 3.92 (3H, s,

OCH₃), 2.93 (1 H, s, OH). ¹³C NMR (CDCl₃, 100 MHz) δ 158.6, 153.4, 149.7, 147.9, 147.7, 139.9, 136.9, 134.8, 130.8, 128.4, 122.1, 120.8, 112.4, 110.1, 108.1, 77.3, 71.3, 57.3, 56.4, 56.3 ppm. IR (KBr pellet) 3285, 2939, 2848, 1610-1570, 1517, 1473-1463, 1439, 1380, 1353, 1326, 1266, 1219, 1181, 1154, 1061 cm⁻¹. HRMS (⁺ESI): Calcd for MH⁺, 487.1981; Found, 487.1946.

(4-Iodo-phenyl)-bis-pyridin-2-ylmethyl-amine (6). Compound 3 (0.507 g, 1.84 mmol) was dissolved in CH₃CN (10 mL) and chilled to 0 °C. [K(18-crown-6)]ICl₂ (0.970 g, 1.94 mmol) was added and the reaction mixture was stirred at room temperature for 30 minutes. After removing the solvent, the reaction residue was diluted with 25 mL of water and the pH was adjusted to 9 with 6 M NaOH. The product was extracted into EtOAc (3×25 mL), washed with saturated NaHSO₃ (25 mL) and brine (3×25 mL) the combined organic extracts were dried over Mg₂SO₄. Flash chromatography on alumina (1:1 hexanes/EtOAc) followed by recrystallization from hexanes yielded **6** as pale yellow needles (0.687 g, 93.0%). Mp = 104-105 °C. ¹H NMR (CDCl₃, 400 MHz) δ 8.59 (2 H, d, J = 8.0 Hz, H₆-Py), 7.62 (2 H, t, J = 8.0 Hz, H₄-Py), 7.39 (2 H, d, J = 8.0 Hz, ArH), 7.22 (2 H, d, J = 4.0 Hz, H₃-Py), 7.18 (2 H, t, J = 8.0 Hz, H₅-Py), 6.49 (2 H, d, J = 8.0 Hz, ArH), 4.79 (4 H, s, N-CH₂-Py). ¹³C-NMR (CDCl₃, 100 MHz) δ 158.5, 150.1, 148.0, 138.1, 137.1, 122.4, 121.0, 115.1, 78.6, 57.6 ppm. IR (KBr pellet) 3048, 3006, 2927, 1862, 1585, 1494, 1469, 1435, 1383, 1357, 1278, 1254, 1235, 1195, 1177, 1151, 1145, 1090, 1049, 1043, 665, 545, 458. HRMS (⁺ESI) : Calcd for MH⁺, 402.0467; Found, 402.0458.

Bis-pyridin-2-ylmethyl-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl]-amine (7). Compound **6** (0.307 g, 0.766 mmol), KOAc (0.226 g, 2.30 mmol), PdCl₂ (4.1 mg, 3 mol%), PPh₃ (12.1 mg, 6 mol%), bis(pinacolato) diboron (0.214 g, 0.843 mmol) and DMSO (10 mL) were combined in a Schlenk tube. After performing three cycles of freeze-pump-thaw, the tube was filled with N₂, sealed and heated at 80 °C ± 5 °C for 12 h. The mixture was cooled, diluted with H₂O (25 mL) and extracted with EtOAc (3 × 25 mL) and dried with Na₂SO₄. Flash chromatography on alumina (1:1 petroleum ether/EtOAc) yielded **7** as a (0.162 g, 52.8%). Mp = 201-202 °C. ¹H-NMR (CDCl₃, 400 MHz) δ 8.59 (2H, d, J=4.0 Hz, H_{6-Py}); 7.63-7.58 (4H, m); 7.20 (2H, d, J=8.0 Hz, H_{3-Py}); 7.17 (2H, t, J=8.0 Hz, H_{5-Py}); 6.69 (2H, d, J=8.0 Hz, ArH); 4.85 (4H, s, N-CH₂-Py); 1.29 (12H, s, -CH₃). ¹³C-NMR (CDCl₃, 100 MHz) δ 158.6, 150.7, 150.0, 137.1, 136.6, 122.3, 120.9, 111.9, 83.5, 77.6, 57.2 ppm. IR (KBr pellet) 616, 652, 750, 810, 964, 991, 1141, 1372, 1470, 1606, 2928, 2973, 3046, 3082. HRMS (⁺ESI) : Calcd for MH⁺, 402.2353; Found, 402.2337.

[4-(Bis-pyridin-2-ylmethyl-amino)-phenyl]-(4,5-dimethoxy-2-nitro-phenyl)-methanol

(ZinCast-1, 1). Compound 7 (0.150 g, 0.374 mmol), 6-nitroveratraldehyde (79.0 mg, 0.374 mmol), tris-1-naphthylphosphine (15.4 mg, 10 mol%), PdCl₂ (6.6 mg, 10 mol%), K₂CO₃ (0.155 g, 1.12 mmol) and THF (10 mL) were combined in a Schlenk tube. The tube was sealed and mixture was stirred at 60 °C \pm 5°C for 60 h. Solvent was removed under reduced pressure and to the resulting dark brown residue was added 50 ml of water, the mixture was extracted with CH₂Cl₂ and dried with Mg₂SO₄. Flash chromatography on alumina with EtOAc provided **ZinCast-1** as fine yellow crystals (95.2 mg, 52.3%).

[4-(Bis-pyridin-2-ylmethyl-amino)-phenyl]-(4,5-dimethoxy-2-nitroso-phenyl)-mathanone

(ZinUnc-1, 2). ZinCast-1 (42.1 mg, mmol) was dissolved in 4 mL of MeCN and loaded into a quartz cuvette. Sealed cuvette was irradiated with a 100 W Xe arc lamp for 4 hr. Solvent was removed under vacuum and the crude residue was flash chromatographed on basic alumina with EtOAc yielding (30 mg, 69%) yellow ZinUnc-1. Mp = 163-165 °C. ¹H-NMR (CDCl₃, 400 MHz) δ 8.59 (2H, d, J=4.0 Hz, H_{6-Py}); 7.77 (4H, m, Ar-H); 7.20 (6H, m, ArH); 6.69 (2H, d, J=8.0 Hz, ArH); 6.33 (1H, s, ArH); 4.89 (4H, s, N-CH₂-Py); 4.03 (3H, s, OCH₃); 3.91 (3H, s, OCH₃). -NMR (CDCl₃, 100 MHz) δ 194.1, 160.0, 157.5, 156.0, 152.8, 150.2, 140.0, 137.2, 132.7, 129.2, 128.2, 122.6, 120.9, 111.8, 109.8, 91.9, 57.3, 57.0, 56.4 ppm. IR (KBr pellet) 755, 1080, 1185, 1225, 1258, 1286, 1525, 1593, 1643, 2924, 3001. HRMS (⁺ESI) : Calcd for MH⁺, 469.1876; Found, 469.1869.

General Spectroscopic Methods. All solutions were prepared with spectrophotometric grade solvents. 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. Analytical photolysis were performed using 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. Photolysis reactions were conducted at 25 °C, in 1 cm quartz cuvette with a total volume of 3.0 mL using, unless otherwise stated, 10 nm slit widths and a photomultiplier tube power of 700 V. Preparative scale photolysis were carried out using a 1000 W Xe small arc lamp. ZinCast-1 was delivered to buffered solutions from a 6.37 mM stock solution in DMSO.

Determination of M²⁺ Binding Constants

Solutions were prepared by adding 11.8 μ L of ZinCast-1 stock solution to 3000 μ L of 20% DMSO/HEPES buffer (50 mM HEPES, 100 mM KCl, pH 7.0). Titration experiments were performed in triplicate with the following aqueous solutions: 5.00 mM Cu(ClO₄)₂, 40.0 mM Zn(ClO₄)₂ and 56.5 mM Cd(ClO₄)₂. After 1 min of equilibration time absorbance spectra were recorded. Spectra were corrected for dilution and analyzed as previously described.² Spectra from the Cu²⁺ titration were also fit using HyperSpec.^{3,4}

Quantum Yields of Photolysis

A 25 μ M solution of ZinCast-1 in 20 % DMSO/HEPES buffer was irradiated at a wavelength of 350 nm and a 10 nm slit width for a total of 3 hours. Absorption spectra were obtained at the following total times of exposure: 5, 15, 30, 60, 120, 180 minutes. The concentration of the photoproduct (ZinCast-UNC) was calculated as follows:

$$[ZinUNC-1] = \frac{Abs_{349} - (\varepsilon_{ZinCast-1} \times C_{ZinCast-1})}{(\varepsilon_{ZinUNC-1} - \varepsilon_{ZinCast-1})} \times \frac{1L}{1000cm^3}$$

Where $\varepsilon_{ZinCast-1}$ is extinction coefficient of ZinCast-1 (5623 M^{-1}), $\varepsilon_{ZinUNC-1}$ is extinction coefficient of ZinUNC-1 (33352 M^{-1}) and $C_{ZinCast-1}$ is total concentration of ZinCast-1 (25 μM). The quantum yield of the photoreaction was calculated as follows:

$$\Phi_{photolysis} = \frac{\Delta [ZinUNC - 1] \times N_A}{\Delta time \times I}$$

Where N_A is Avogadro's number (6.022x10²³), $\Delta time$ is total time elapsed in seconds and I is the intensity of the source as measured by the K₃[Fe(oxalate)₃] actinometer.⁵ The quantum yield of [Zn(ZinCast-1)]²⁺ was measured in the presence of 40 equivalents of Zn(ClO₄)₂. All photolysis experiments were duplicated.

Photolysis in presence of Zn sensor

A 3.0 mL aliquot of a 5 μ M ZP1B solution was placed in a quartz cuvette and the fluorescence spectrum was recorded. An 0.636 μ L aliquot of Zn(ClO₄)₂ stock solution (0.118 M) was added to give a final concentration of 25 μ M and the fluorescence spectrum was recorded. A 58.9 μ L aliquot of the ZinCast-1 stock solution (6.37 mM) was added to give a final concentration of 125 μ M and the fluorescence spectrum was irradiated in Applied

Photophysics Semi-Macro Photochemical Reactor with 350 nm wavelength radiation and the fluorescence spectra were recorded at 5 min intervals.





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Figure S-2. Titration of ZinCast-1 with Zn(ClO₄)₂.



Figure S-3. Titration of ZinCast-1 with Cu(ClO₄)₂.



Figure S-4. Titration of ZinCast-UNC with Cd(ClO₄)₂.



Figure S-5. Titration of ZinCast-UNC with Zn(ClO₄)₂.



Figure S-6. Titration of ZinCast-UNC with Cu(ClO₄)₂.



Figure S-7. Normalized change in absorbance at λ_{max} and λ_{min} for ZinCast-UNC while titrated with Cd(ClO₄)₂, Zn(ClO₄)₂, and Cu(ClO₄)₂.



Figure S-8. Photolysis of 25 µM ZinCast-1 at 350 nm.



Figure S-9. Photolysis of ZinCast-1 in presence of 40 equivalences of Zn(ClO₄)₂ at 350 nm.



Figure S-10. ¹H NMR of compound 4.



Figure S-11. ¹³C NMR of compound 4.



Figure S-12. ¹H NMR of compound 5.



Figure S-13. ¹³C NMR of compound 5.



Figure S-14. ¹H NMR of compound ZinCast-1.



Figure S-15. ¹H NMR of compound ZinCast-1.



Figure S-16. ¹H NMR of compound **6.**



Figure S-17. ¹³C NMR of compound 6.



Figure S-18. ¹H NMR of compound 7.



Figure S-19. ¹³C NMR of compound **7.**



Figure S-20. ¹H NMR of ZinUnc-1.



Figure S-21. ¹³C NMR of ZinUnc-1.

References

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