2,2'-Bipyridine and hydrazide containing peptides for cyclization and complex quaternary structural control

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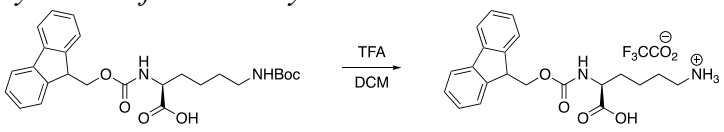
Protocol for preparing Fmoc-L-Lysine(Me,Boc)-OH

Synthesis of Fmoc-L-Lysine-OH

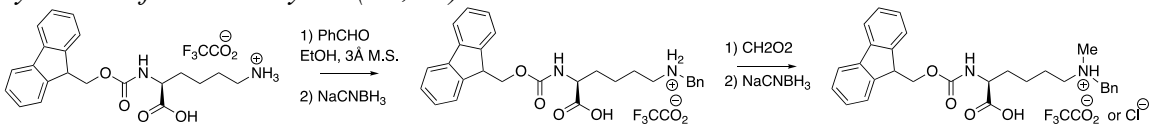


38g Fmoc-L-Lys(Boc)-OH (468.55 mmol) were dissolved in 25mL TFA and 40mL DCM and stirred overnight at room temperature. The solvents were removed by rotary evaporator at 40 °C, then under vacuum. The resulting gum was triturated with ether until it solidified, providing 37g of the trifluoroacetate salt of Fmoc-L-Lys-OH (95% yield).

Purification of benzaldehyde

100mL benzaldehyde was washed several times with saturated sodium bicarbonate to remove any benzoic acid impurities. It was stored over sodium sulfate in an amber bottle, minimizing light exposure.

Synthesis of Fmoc-L-Lysine(Bn,Me)-OH

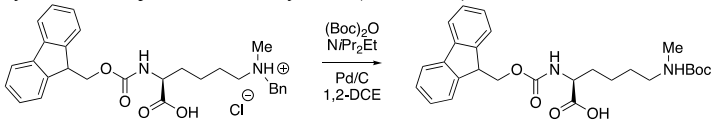


400mL EtOH were placed over 3Å molecular sieves overnight at room temperature. 40g (83 mmol) of the trifluoroacetate salt of Fmoc-Lys-OH were subsequently dissolved in the ethanol, and 10mL (98 mmol) benzaldehyde were added. The solution was stirred overnight at room temperature under argon balloon; the sieves had pulverized by the next morning. Next, 6.3g (99 mmol) $NaCNBH_3$ were added in three portions. LC-MS showed 70% conversion to the mono-benzylated product, with 13% di-benzylated and 17%

uncoverted. 12mL (161 mmol) of 37% aqueous formaldehyde were subsequently added, once again followed by the addition of 7.3g (62.84 mmol) $NaCNBH_3$ in four portions. After one hour stirring at room temperature, LC-MS showed 73% conversion to the mono-methyl/mono-benzyl product, with 10% di-benzylated and 16% di-methylated. The reaction was poured over ice, and 6M HCl was added (in a well-ventilated hood!) to a pH of 2 by litmus paper. Solids were filtered off and rinsed with 0.5 M HCl. 200cc silica were added to the filtrate, and solvent was removed by rotary evaporation and vacuum.

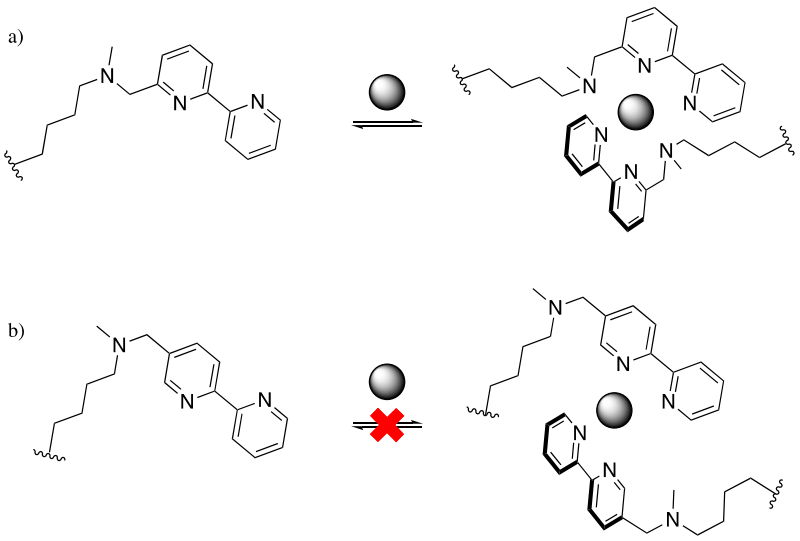
3000cc of silica were packed in 7.5% MeOH/DCM. The crude mixture that had been adsorbed on silica was added to a plug of 7.5% MeOH/DCM at the top of the column. After further packing, sodium sulfate was added on top, and the column was eluted with 3L 7.5% MeOH/DCM and then 10% MeOH/DCM until all the di-benzylated impurity had eluted. The MeOH percentage was then increased from 10 to 35% until all product had eluted, with minimal di-methyl impurity. Solvent was removed from product fractions. However, this product was contaminated with silica that had dissolved in the high-percentage-methanol eluent. The silica was removed by reverse-phase chromatography on Combi-Flash, with a $MeCN/H_2O$ eluent. MeCN was removed from the pure fractions, and the water was lyophilized off to give a white, fluffy solid. LC-MS and ^1H NMR corresponded to literature reports.

Synthesis of Fmoc-L-Lysine(Me,Boc)-OH

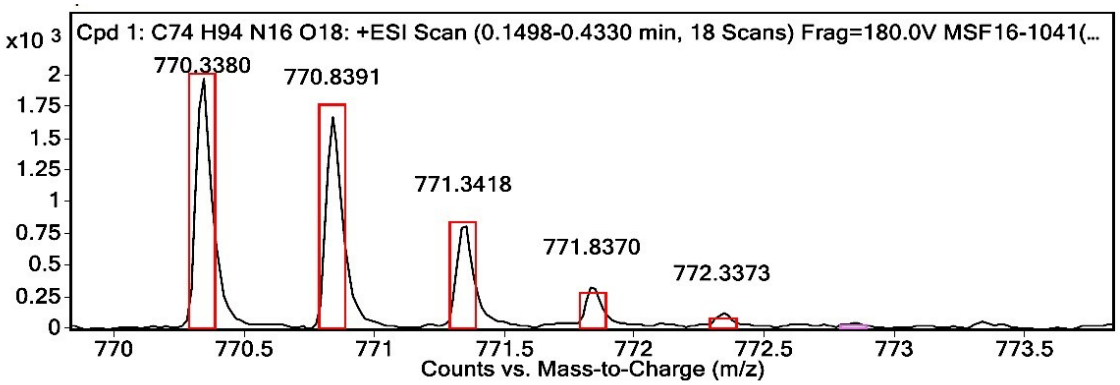


0.75 g (1.47 mmol) of the hydrochloride salt of Fmoc-Lys(Bn, Me)-OH, 0.43 g (2.0 mmol) di-tert-butyl dicarbonate, and 75 mg (0.070 mmol) 10% Pd/C were added to 5 mL

1,2-dichloroethane. The reaction mixture was sparged twice with a H_2 balloon. 620 uL N,N-diisopropylethylamine (3.56 mmol) were then added, and the reaction mixture was stirred under H_2 balloon at room temperature overnight. LC-MS showed ~85% conversion to Fmoc-Lys(Me, BOC)-OH, with ~7% Fmoc deprotected. The Pd/C was filtered off, and the organic filtrate was washed twice with 1 M HCl, once with saturated sodium bicarbonate solution, and once with brine. The washed organics were dried over sodium sulfate and filtered. Solvents were removed by rotary evaporation and vacuum. The product was purified by reverse-phase chromatography on Combi-Flash, using a MeCN/H_2O gradient. MeCN was removed from the pure fractions, and the water was lyophilized to give a white, fluffy solid. LC-MS and ^{1}H NMR corresponded to literature reports.



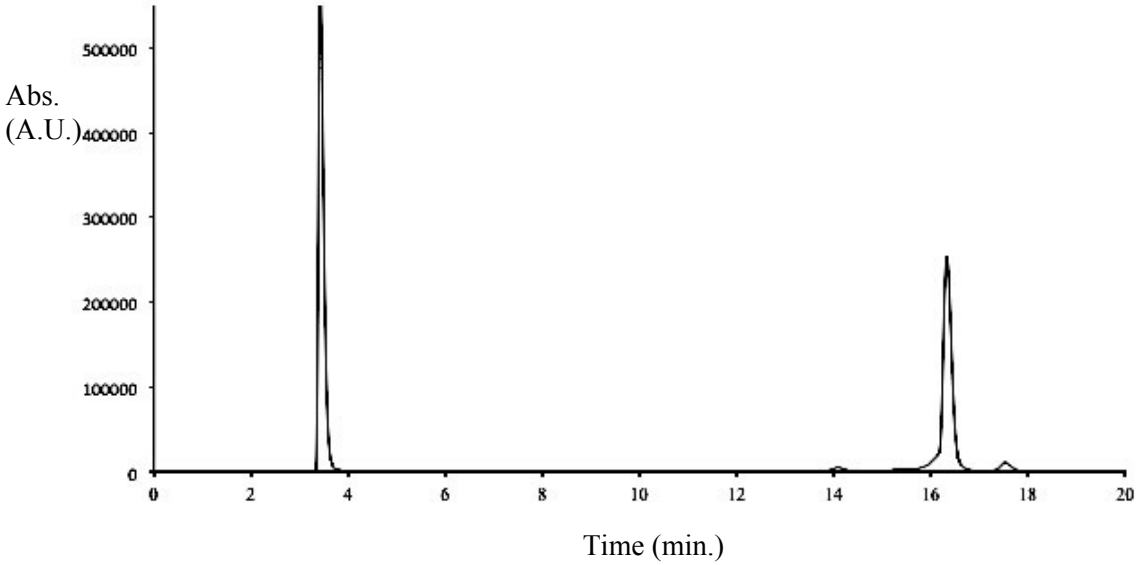
SI Figure-1. Initially proposed binding arrangements when using N_ε-methyl Lysine. a) Proposed binding of bipy when modified at the 6-postion (found not to occur, vida infra). b) Binding of bipy when modified at the 5-position will not bind in a 1:1 stoichiometry, but requires a third ligand (vida infra).



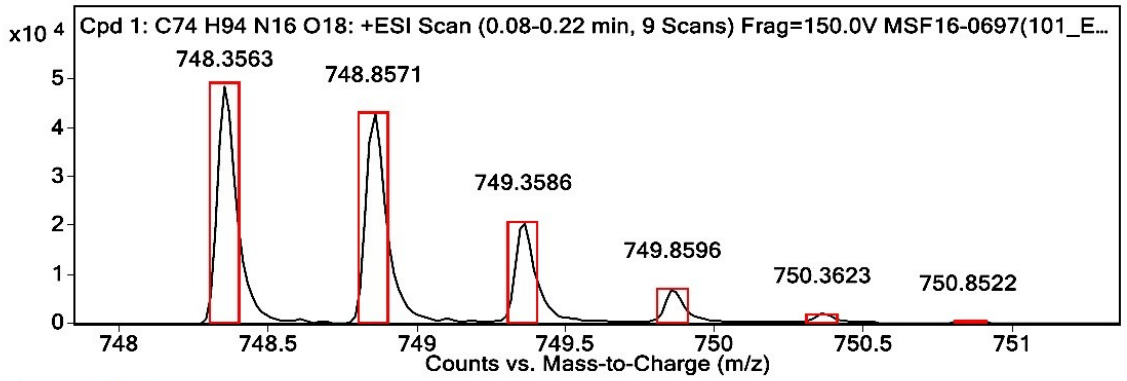
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
499.24200			22563.5			
770.33800	770.33580	2	1978.04	C74H94N16O18	(M+2Na)+2	-2.81
770.83910	770.83730	2	1684.36	C74H94N16O18	(M+2Na)+2	-2.29
771.34180	771.33870	2	837.47	C74H94N16O18	(M+2Na)+2	-3.95
771.83700	771.84010	2	342.13	C74H94N16O18	(M+2Na)+2	4
772.33730	772.34140	2	134.56	C74H94N16O18	(M+2Na)+2	5.31

SI-Figure 2: HRMS for MP-5.



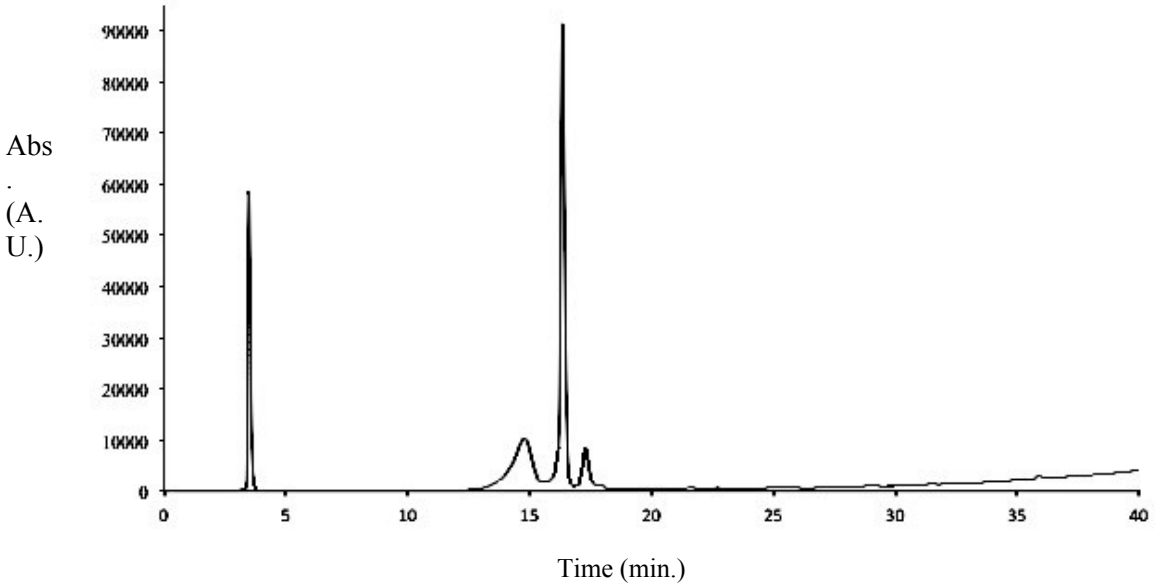
SI-Figure 3: Analytical HPLC trace for MP-5 at 254 nm.



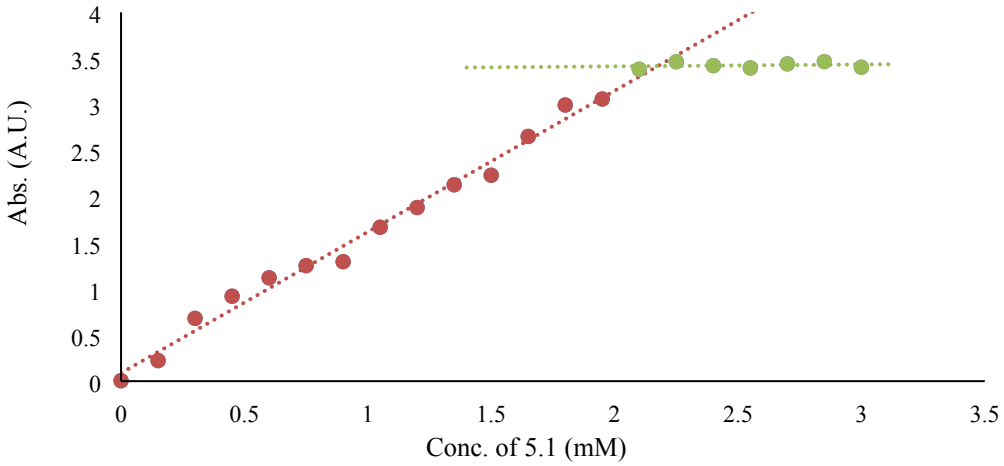
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
499.24110			153640.8			
748.35630	748.35390	2	48860.6	C74H94N16O18	(M+2H)+2	-3.26
748.85710	748.85540	2	43099.37	C74H94N16O18	(M+2H)+2	-2.37
749.35860	749.35680	2	20948.22	C74H94N16O18	(M+2H)+2	-2.44
749.85960	749.85810	2	7048.33	C74H94N16O18	(M+2H)+2	-1.99
750.36230	750.35950	2	1867.83	C74H94N16O18	(M+2H)+2	-3.76
750.85220	750.86080	2	316.37	C74H94N16O18	(M+2H)+2	11.44

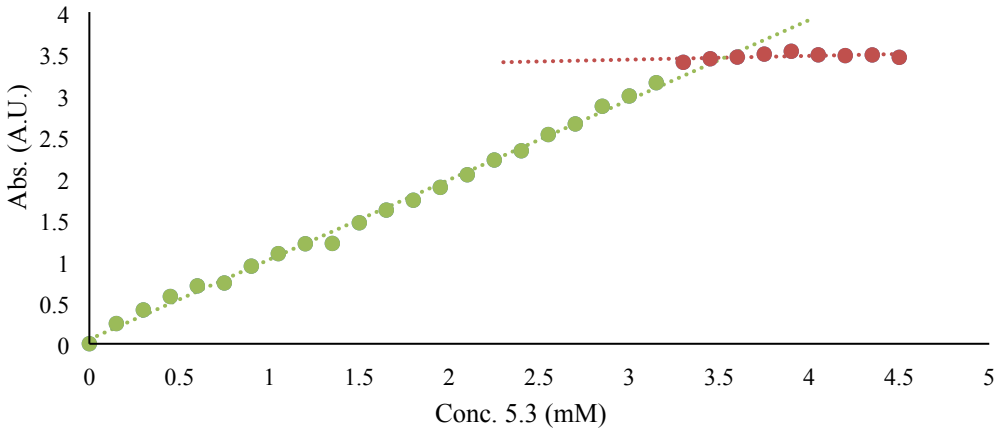
SI-Figure 4: HRMS for MP-6.



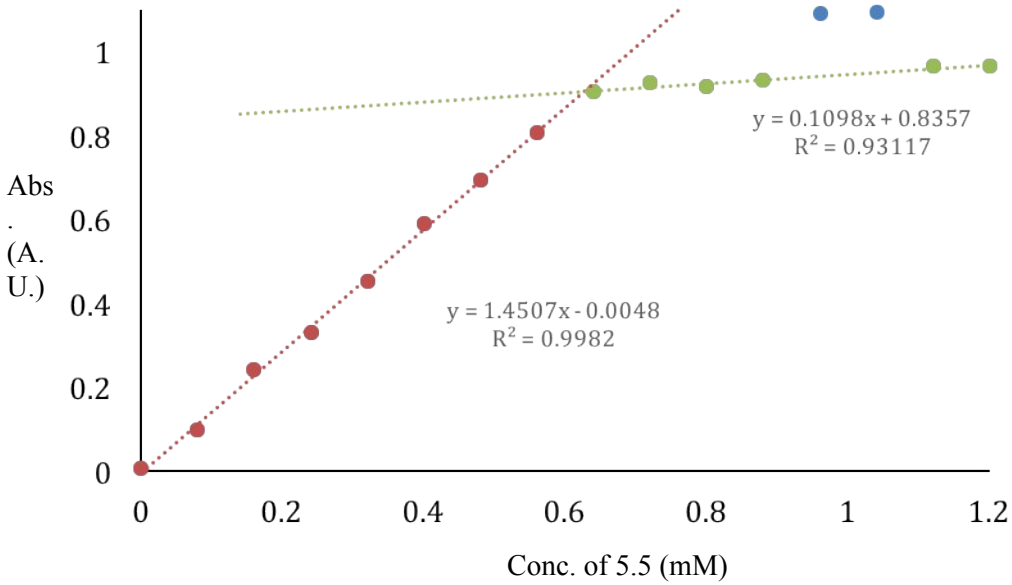
SI-Figure 5: Analytical HPLC trace for MP-6 at 254 nm.



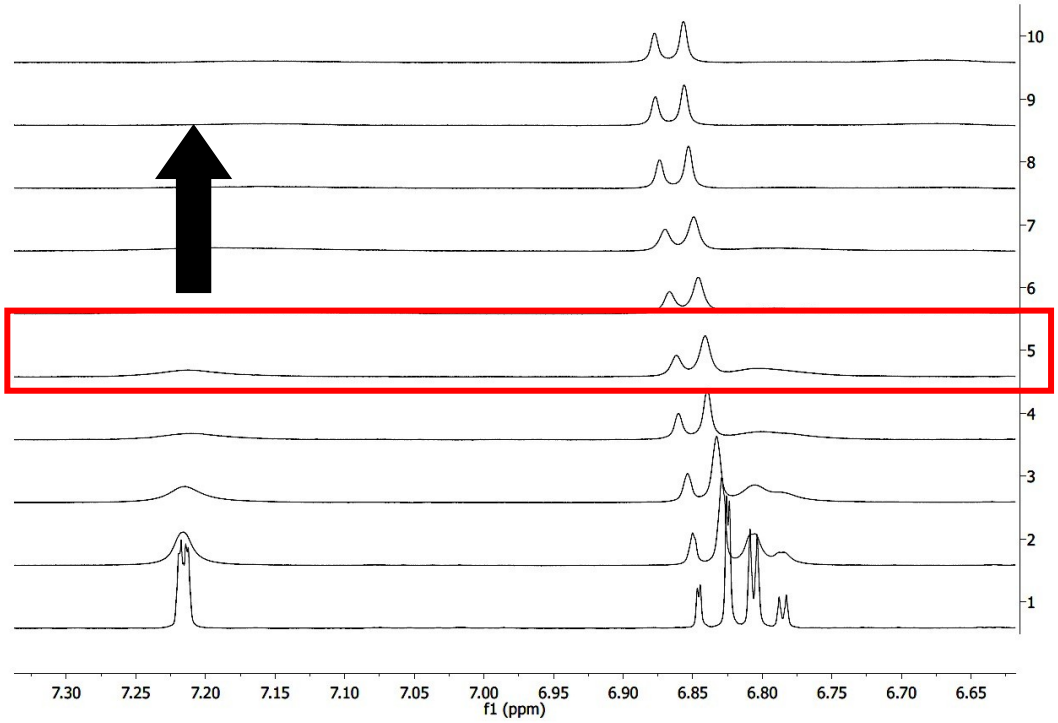
SI-Figure 6: Binding isotherm for 1.



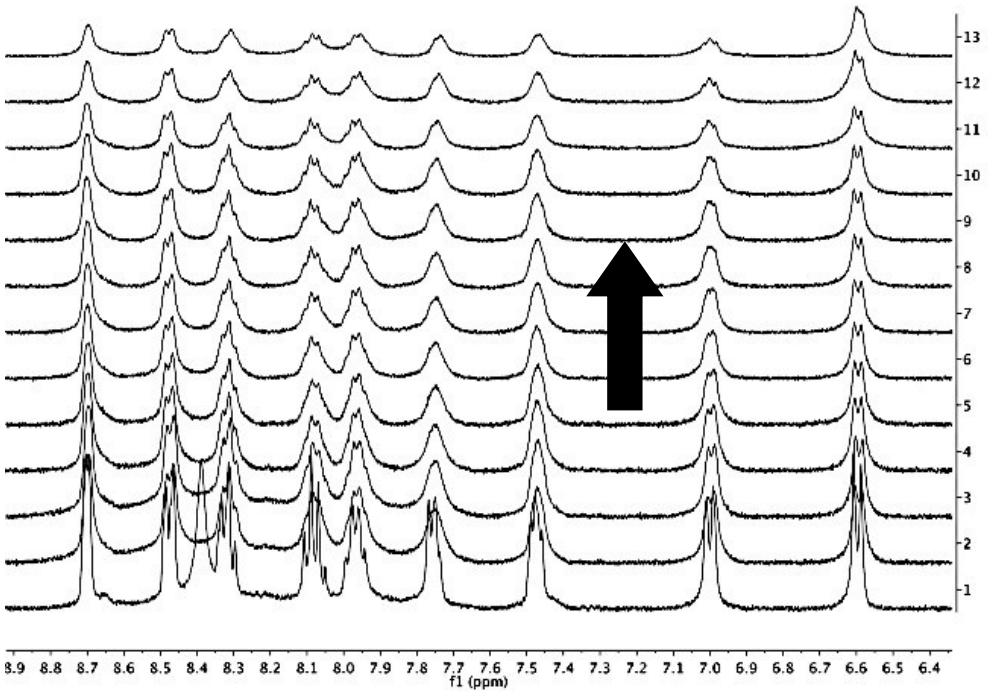
SI-Figure 7: Binding isotherm for 2.



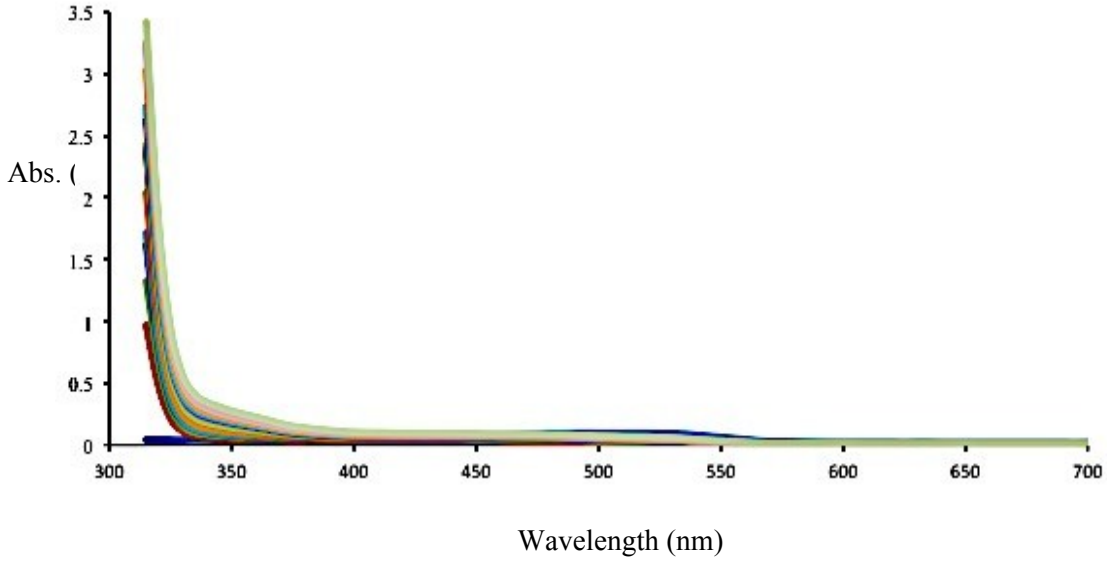
SI-Figure 8: Binding isotherm for 5 at 530 nm.



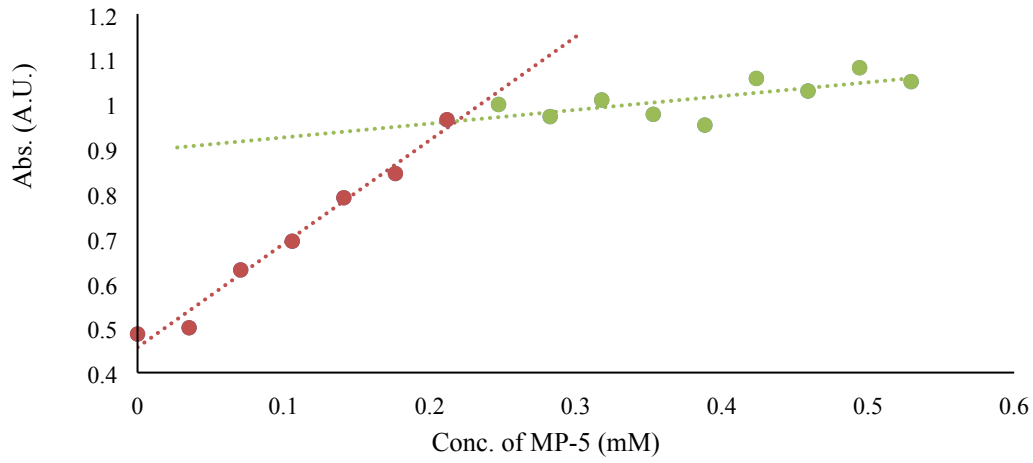
SI-Figure 9: Proton NMR for Zn^{2+} titrated to a solution of 6. Red box indicates saturation reached at 0.33 eqs of Zn^{2+} added. Arrow indicates increase in equivalents: 0, 0.066, 0.13, 0.20, 0.26, 0.33, 0.40, 0.46, 0.53, 0.59, 0.66.



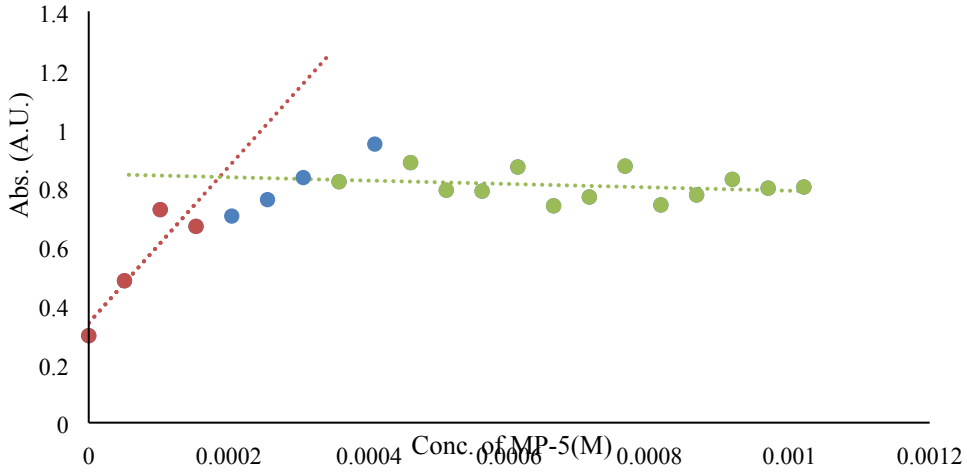
SI-Figure 10: Proton NMR titration of Fe^{2+} to MP-6. Saturation was not reached. Arrow indicates increase in equivalents: 0, 0.13, 0.26, 0.39, 0.52, 0.65, 0.78, 0.91, 1.0, 1.2, 1.3, 1.4, 1.6, 1.7.



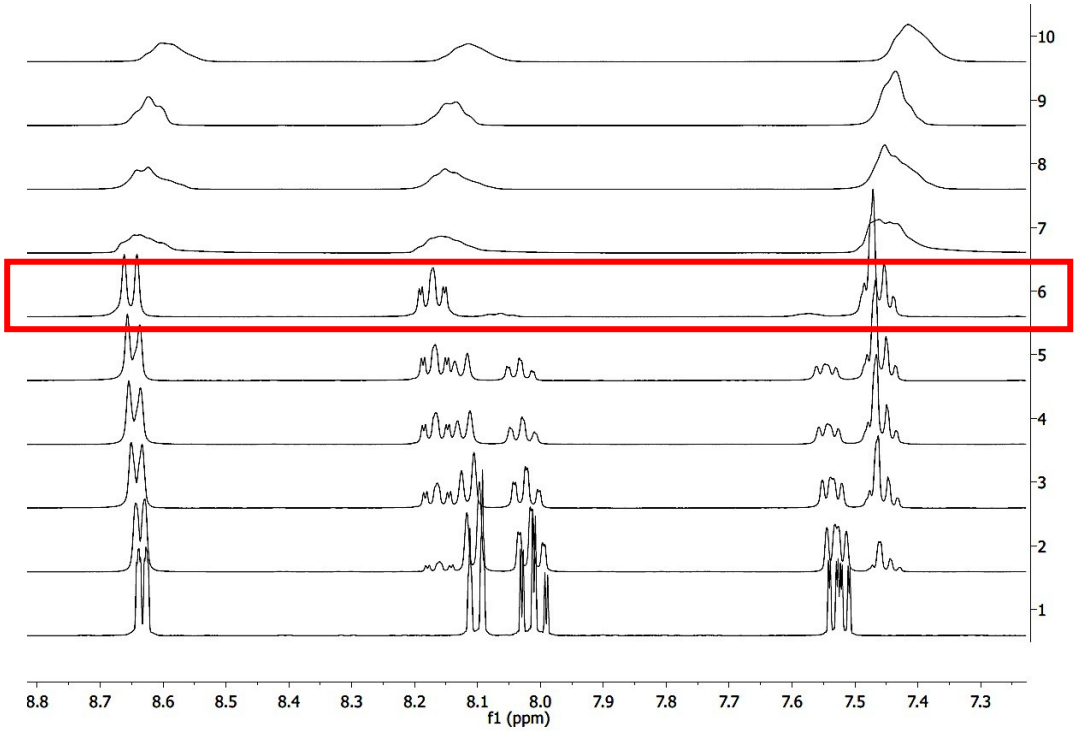
SI-Figure 11: UV-Vis well-plate titration of MP-6 to Fe^{2+}. No colorimetric response was observed.



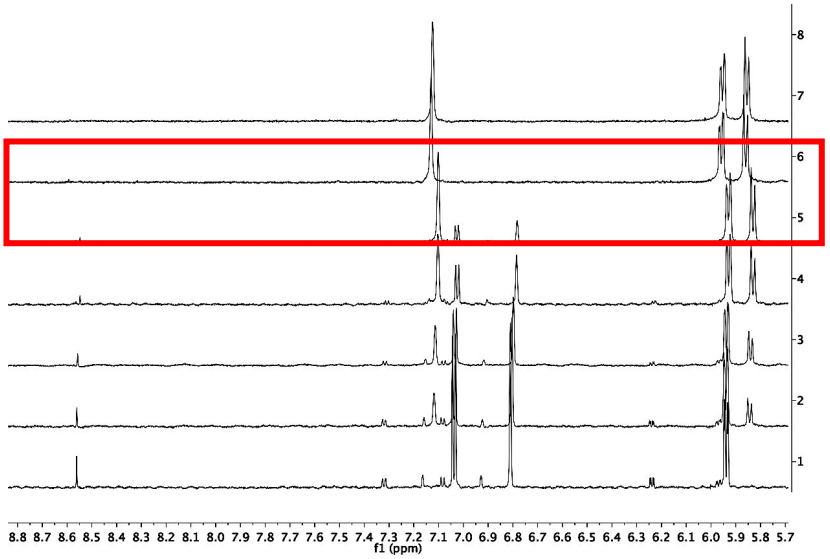
SI-Figure 12: Binding isotherm for Fe^{2+} and compound 3 titrated with MP-5 at 530 nm.



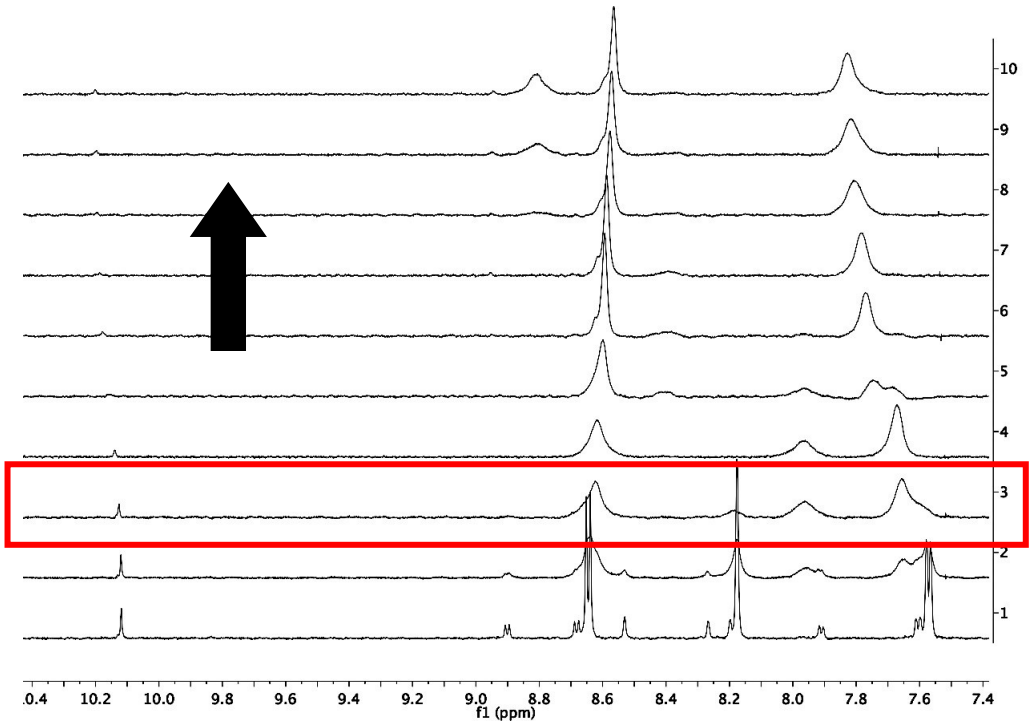
SI-Figure 13: Binding isotherm for Fe^{2+} and compound 5 titrated with MP-5 at 530 nm.



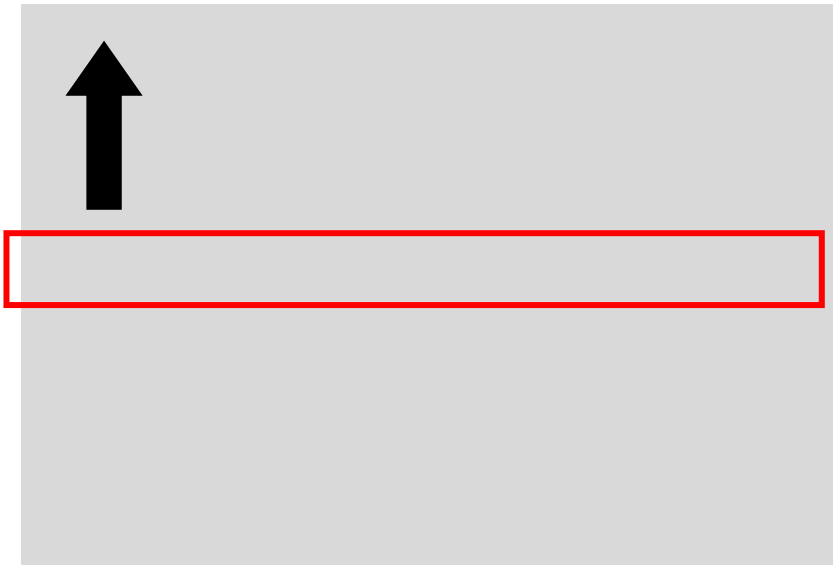
SI-Figure 14: Proton NMR titration of 2-2' bipyridine with Fe^{2+}. Red box indicated 0.33 eq. required of metal required for 3:1 stoichiometry. Arrow indicates increase in equivalents: 0, 0.066, 0.13, 0.20, 0.26, 0.33, 0.40, 0.46, 0.53, 0.59, 0.66.



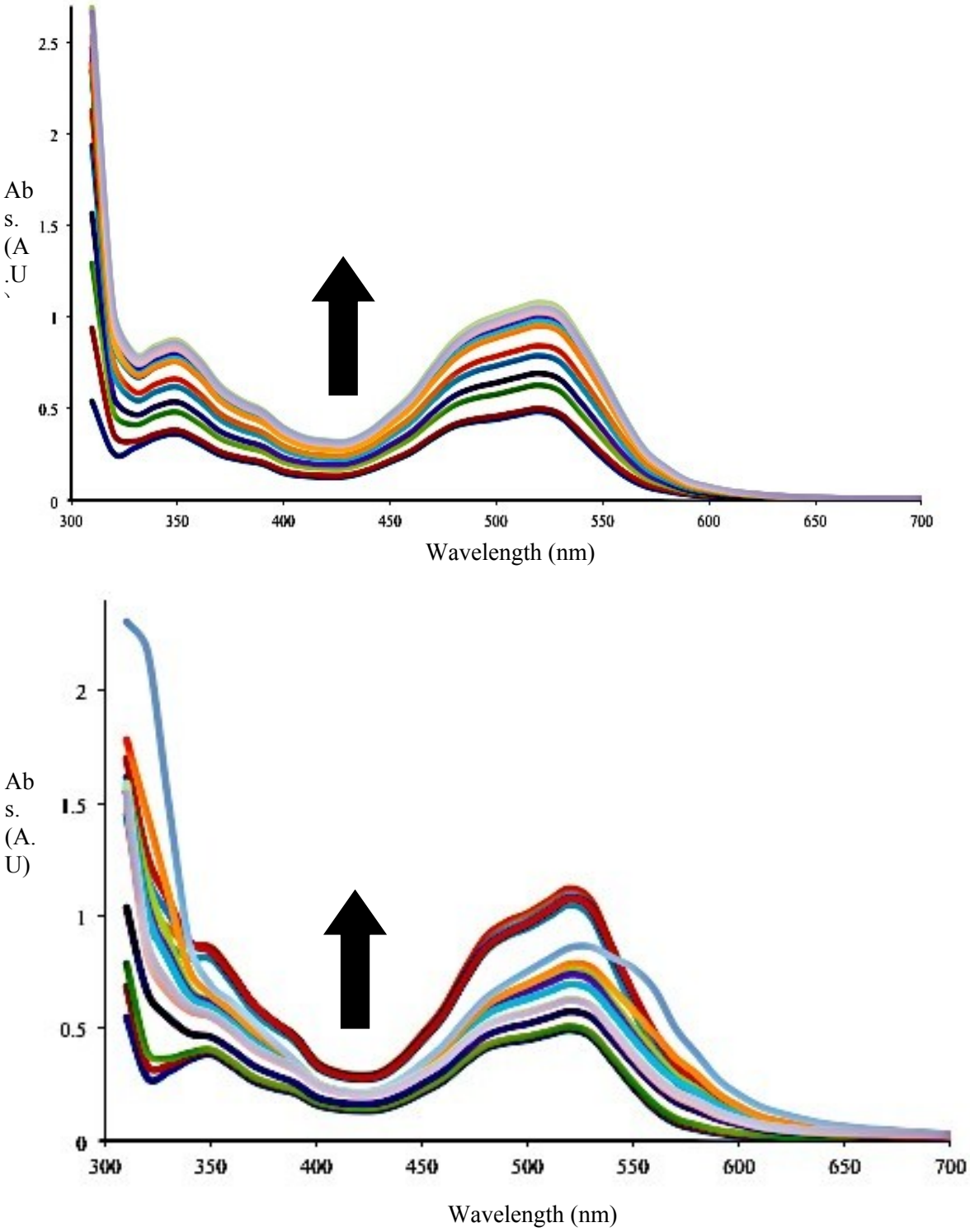
SI-Figure 15: Proton NMR titration of [2,2'-bipyridine]-5,5'-dicarbaldehyde with Fe^{2+}. Red box indicated 0.32-0.42 eq. required of metal for 3:1 stoichiometry. Arrow indicates increase in equivalents: 0, 0.066, 0.13, 0.20, 0.26, 0.33, 0.40, 0.46.



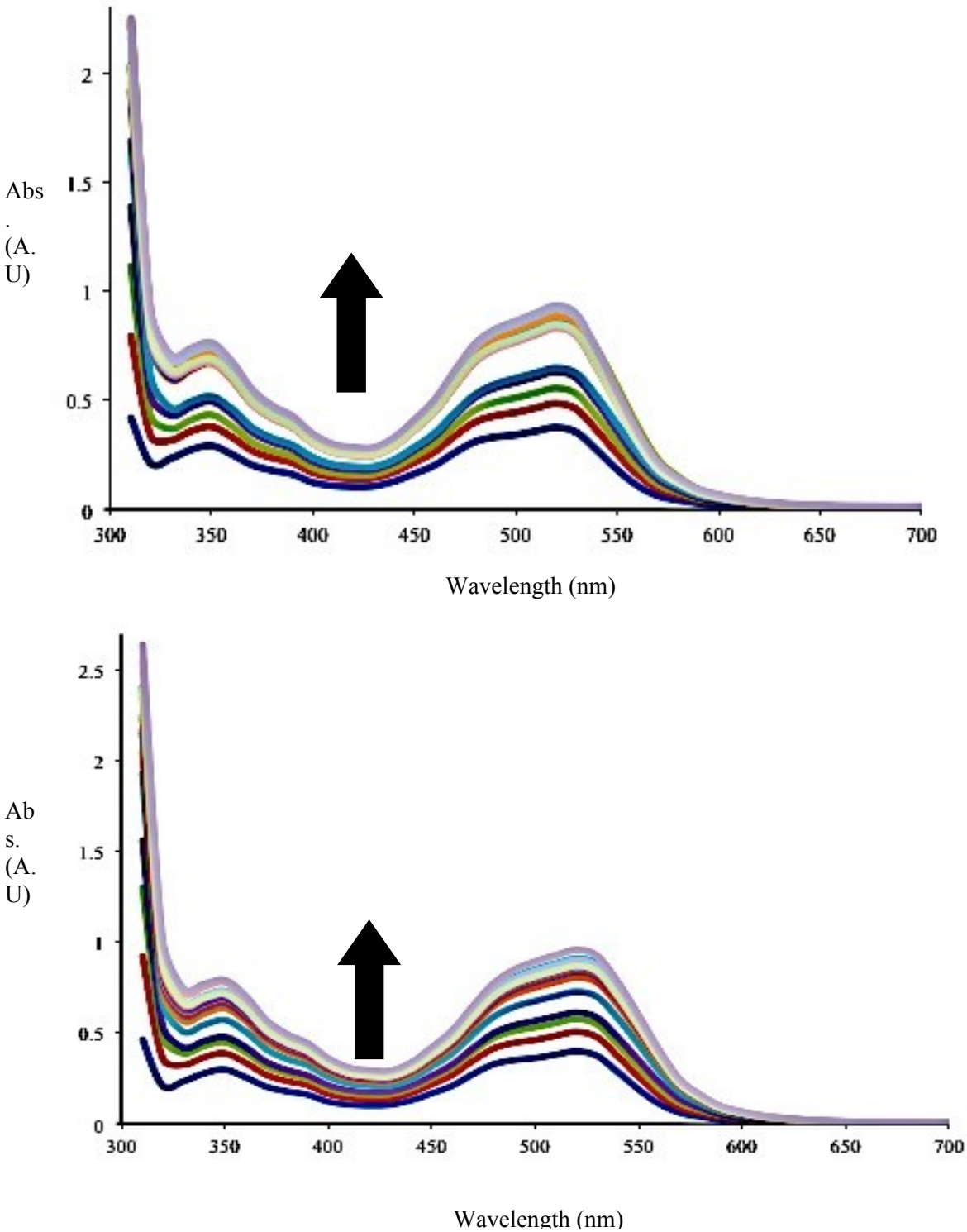
SI-Figure 16: Proton NMR titration of [2,2'-bipyridine]-5,5'-dicarbaldehyde with Zn^{2+}. Red box indicated 0.33 eq. required of metal for 3:1 stoichiometry. Arrow indicates increase in equivalents: 0, 0.11, 0.22, 0.33, 0.44, 0.55, 0.66, 0.77, 0.88, 0.99, 1.1.



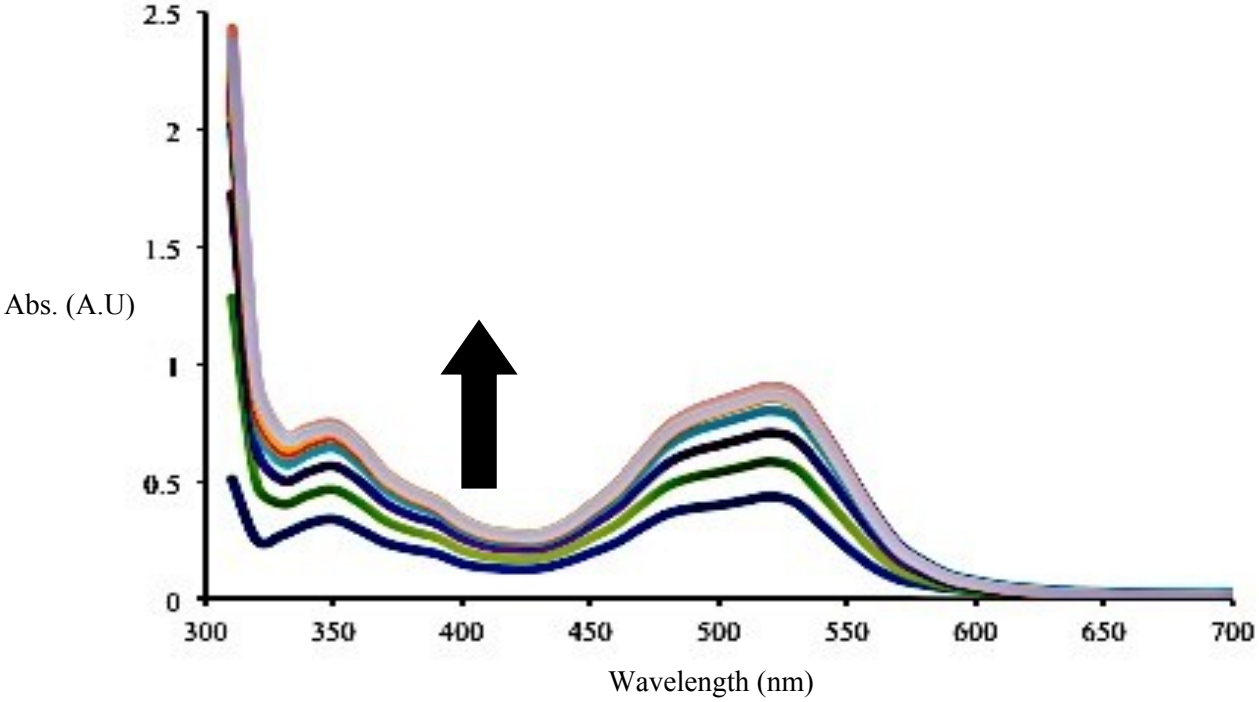
SI-Figure 17: Proton NMR titration of [2,2'-bipyridine]-5,5'-diyldimethanethiol with Zn^{2+}. Red box indicated 0.33 eq. required of metal for 3:1 stoichiometry. Arrow indicates increase in equivalents: 0, 0.066, 0.13, 0.20, 0.26, 0.33, 0.40, 0.46, 0.53, 0.59, 0.66.

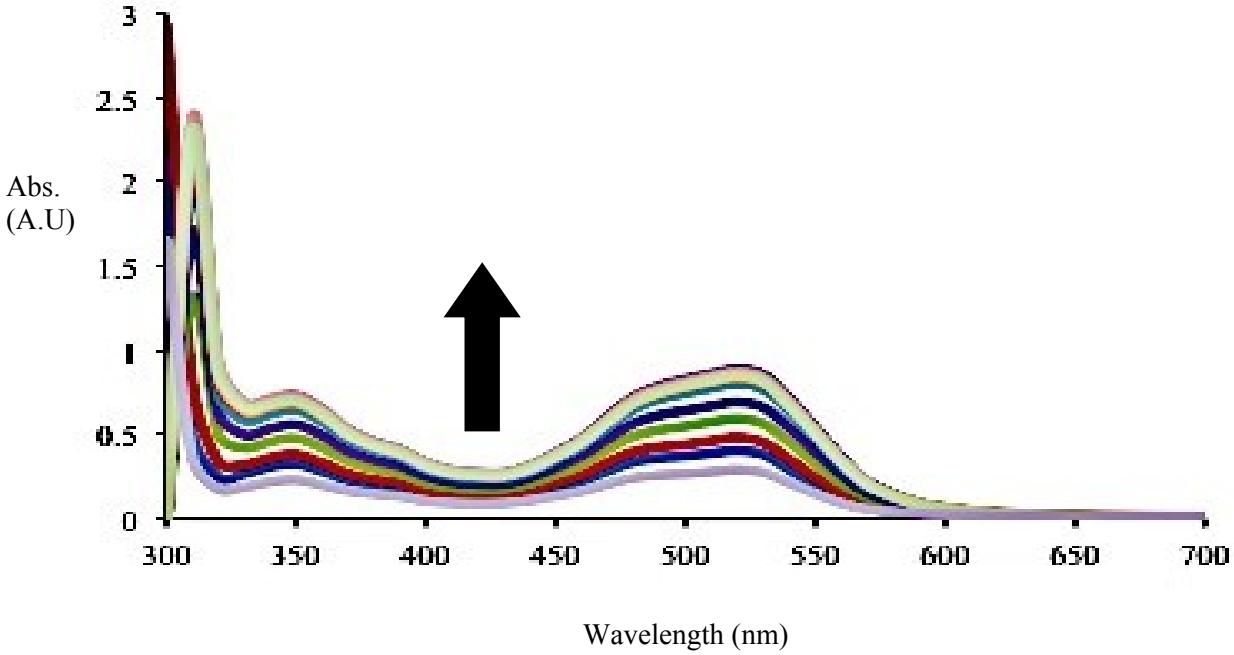


SI-Figure 18: (top) Well-plate titration for MP-5, 2,2'-bipyridine and Fe^{2+} (trial 1). (bottom) Trial 2.

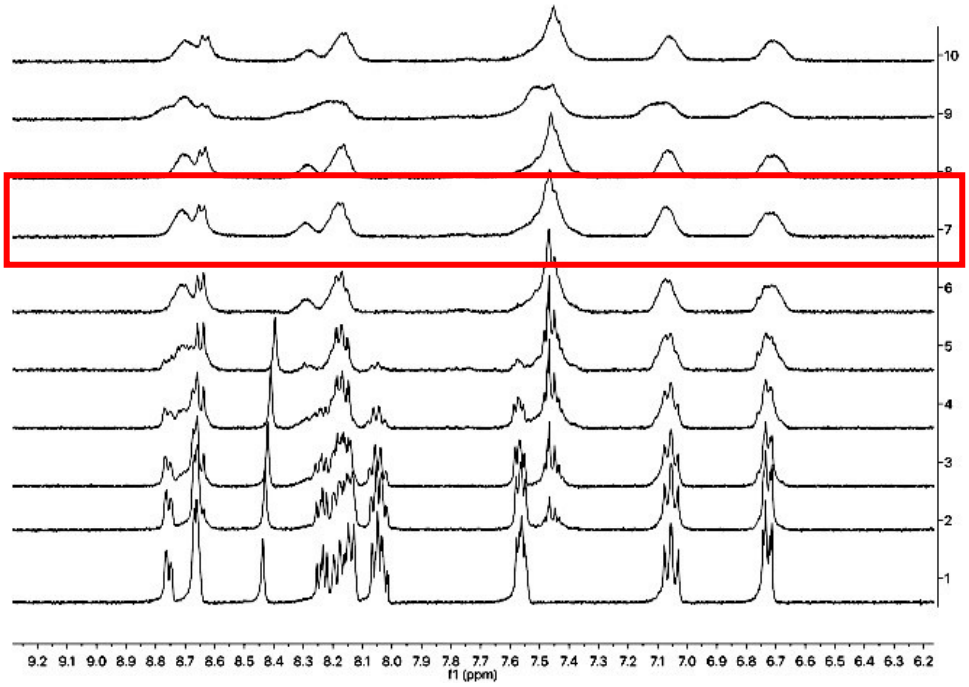


SI-Figure 19: (top) Well-plate titration for MP-5, 2,2'-bipyridine, and Fe^{2+} (trial 3). (bottom) Trial 4.

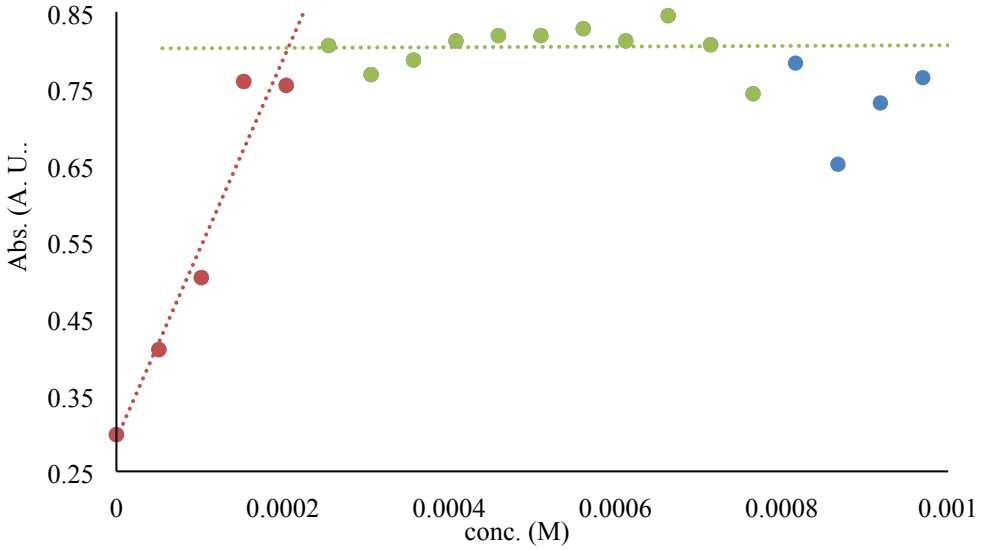




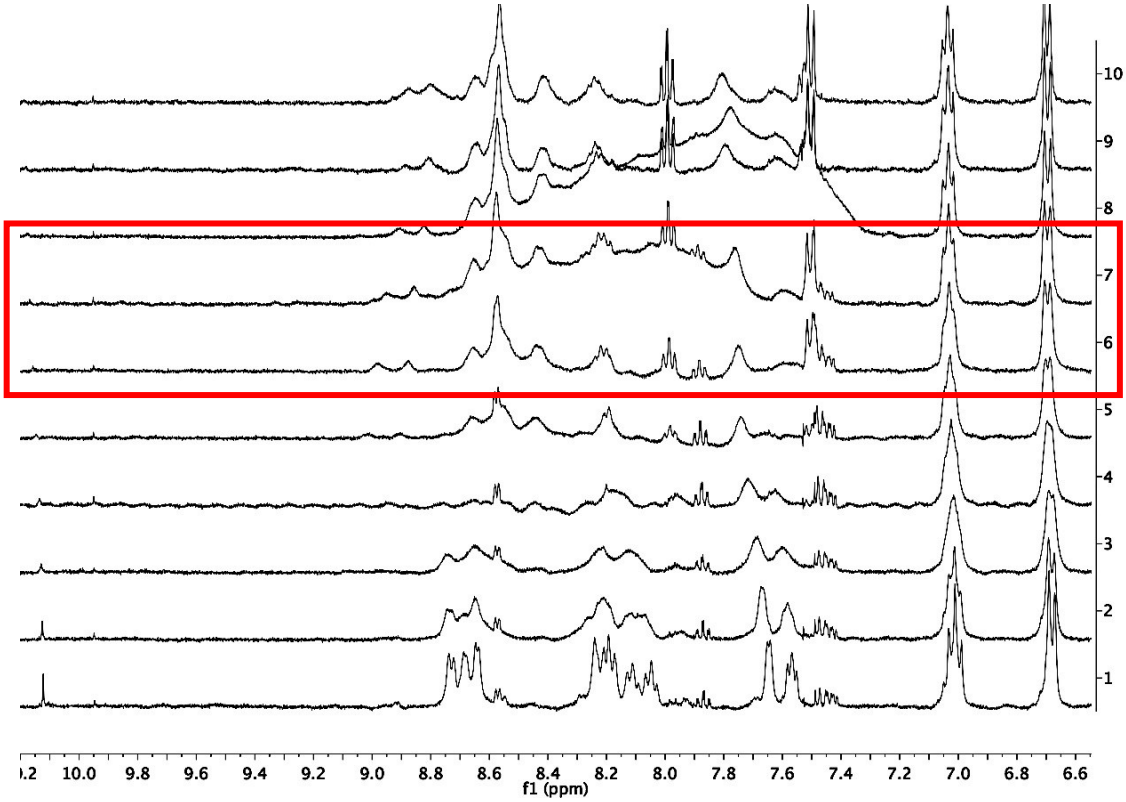
SI-Figure 20: (top) Well-plate titration for MP-5, 2,2'-bipyridine, and Fe^{2+} (trial 5). (bottom) Trial 6.



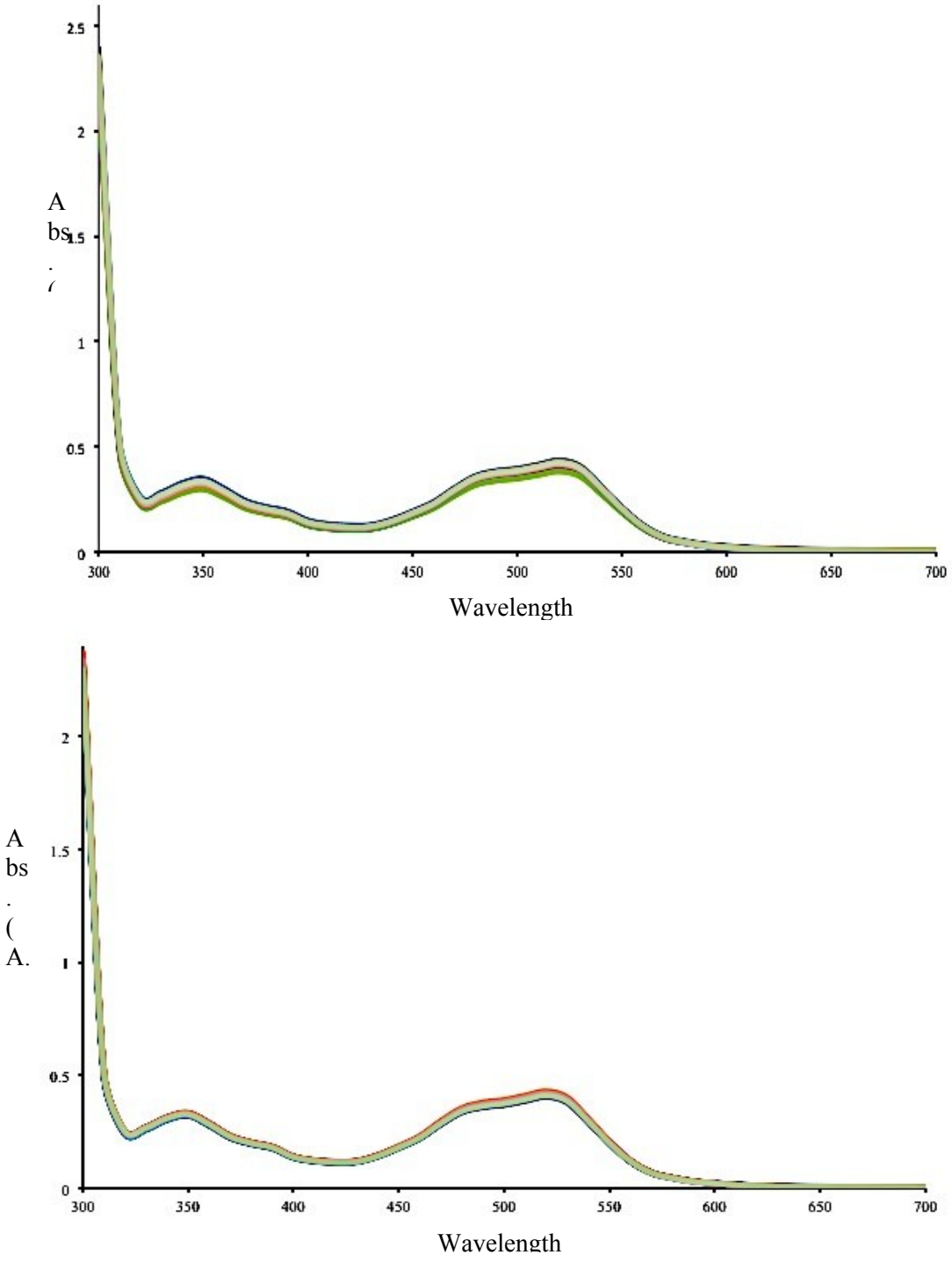
SI-Figure 21: Proton NMR titration for MP-5, 2,2'-bipyridine, and Fe^{2+}. Red box indicates 1.3 eqs of metal were required for cyclization. Arrow indicates increase in equivalents: 0, 0.18, 0.36, 0.54, 0.71, 0.89, 1.1, 1.3, 1.4, 1.6, 1.8.



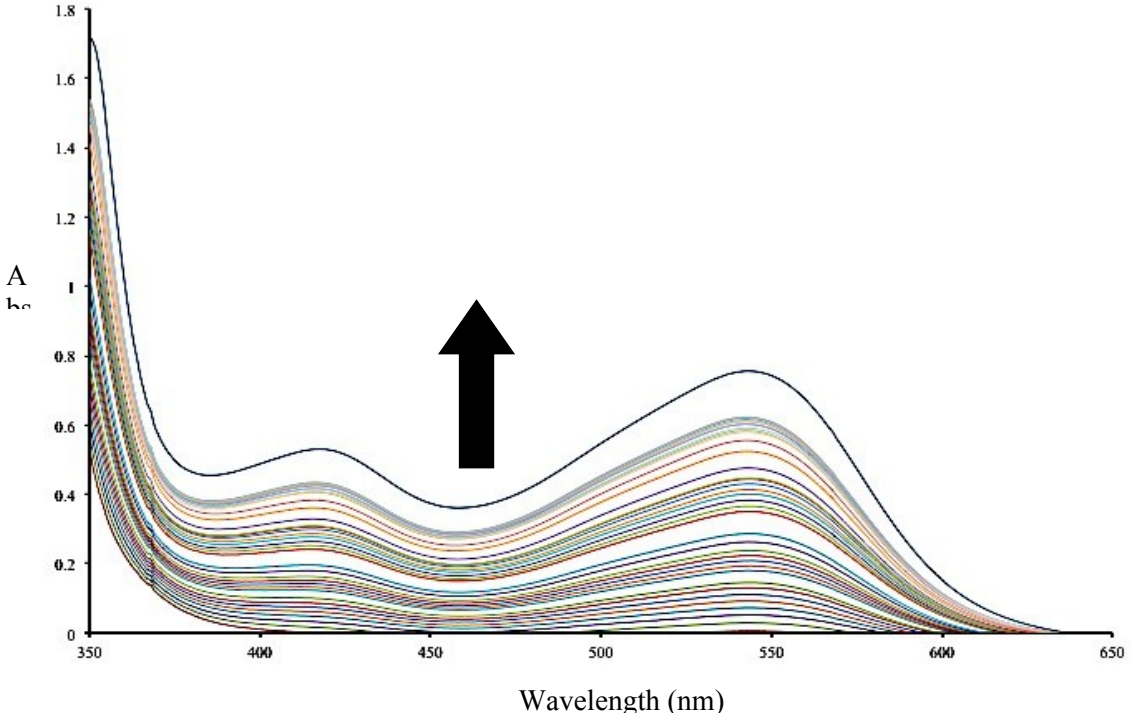
SI-Figure 22: Binding plot at 530 nm for MP-5 (0.206 mM), [2,2'-bipyridine]-5,5'-dicarbaldehyde (0.195 mM), and Fe^{2+} (0,214 mM). Cyclization occurred with a 1.1:1:1.1 stoichiometry.



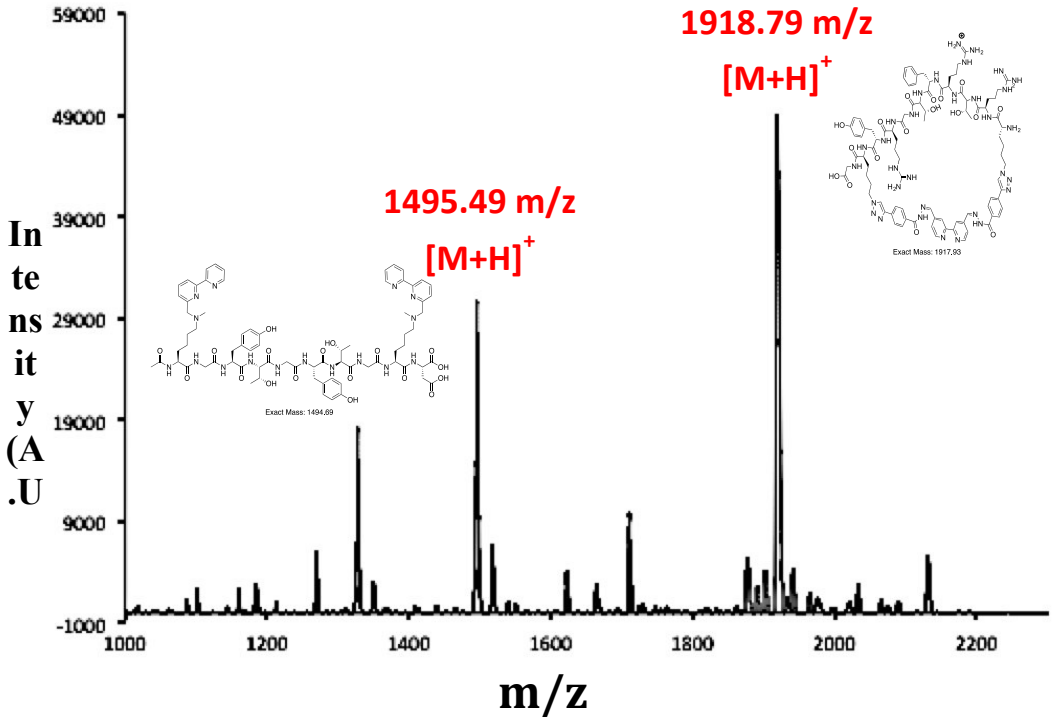
SI-Figure 23: Proton NMR titration for MP-5, 2,2'-bipyridine, and Zn^{2+}. Red box indicates 0.84-1 eq. of metal were required for cyclization. Arrow indicates increase in the equivalents of metal added**:** 0, 0.14, 0.28, 0.42, 0.56, 0.7, 0.84, 0.98, 1.12, 1.26, 1.4.



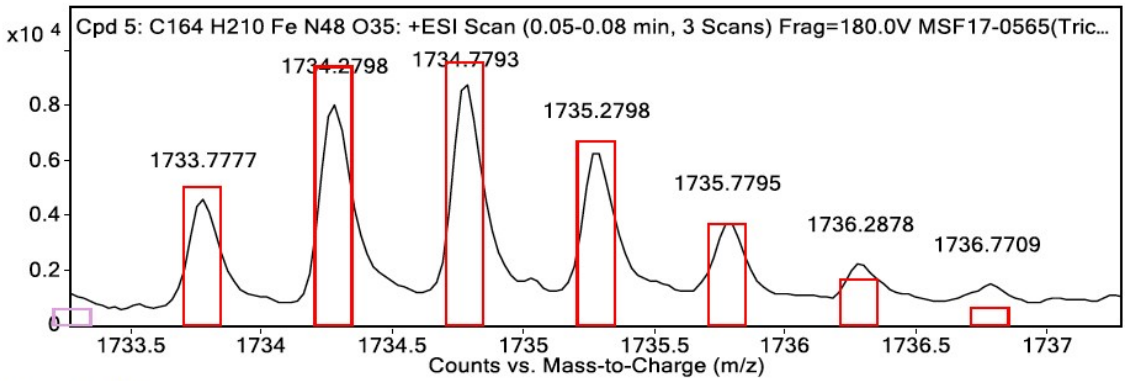
SI-Figure 24: (top) Well-plate titration for MP-6, 2,2'-bipyridine, and Fe^{2+} (trial 1). (bottom) Trial 2. Increasing concentration of peptide led to no change in absorbance.



SI-Figure 25: UV-Vis titration of MP-5, PepHyd-1 Cyclic Bipyridine, and Fe^{2+}.



SI-Figure 26: MALDI-TOF mass specrtrum for formation of cyclic of HydPep in the presence of MP-5 before addition of metal.



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
640.31740			1004976.47			
1733.77770	1733.77390	2	4648.76	C164H210FeN48O35	M+2	-2.16
1734.27980	1734.27500	2	8080.64	C164H210FeN48O35	M+2	-2.76
1734.77930	1734.77620	2	8876.3	C164H210FeN48O35	M+2	-1.75
1735.27980	1735.27750	2	6457.2	C164H210FeN48O35	M+2	-1.31
1735.77950	1735.77880	2	3809.72	C164H210FeN48O35	M+2	-0.35
1736.28780	1736.28010	2	2309.05	C164H210FeN48O35	M+2	-4.41
1736.77090	1736.78140	2	1572.4	C164H210FeN48O35	M+2	6.05
1737.20710	1737.28270	2	1132.19	C164H210FeN48O35	M+2	43.53

SI-Figure 27: High-res mass spectrum for formation of cyclic of HydPep in the presence of MP-5 after addition of metal.