Electronic Supplementary Material (ESI) for Chemical Communications

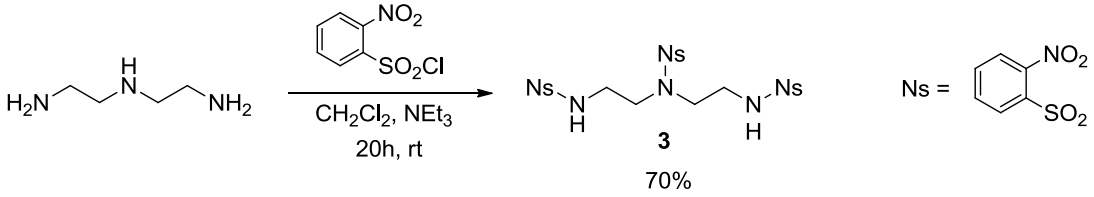
A potent antioxidant small molecule aimed at targeting metal-based oxidative stress in neurodegenerative disorders.

Kimberly M. Lincoln, Paulina Gonzalez, Timothy E. Richardson, David A. Julovich, Ryker Saunders, James W. Simpkins and Kayla N. Green*

Supporting Information.

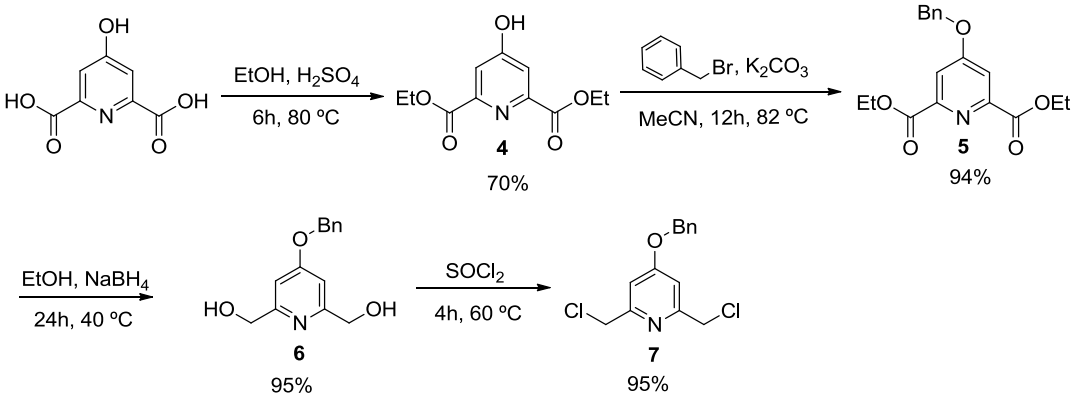
Experimental Methods. All reagents were purchased from commercial sources and used as received unless noted. Ligand and $CuSO_4$ or $Zn(OAc)_2$ solutions were prepared in MilliQ water and diluted to the desired concentration using buffer (15 mM) phosphate buffer with NaCl (15 mM). Each experiment was performed in triplicate with a peptide to copper to ligand ratio of 1:2:4 respectively.

Physical Methods. A Molecular Devices Spectra Max MS microplate reader was employed to obtain the turbidity and UV-Vis results. Fluorescence measurements were recorded on a Varian Cary Eclipse with voltage set to high, and the instrument was set to record the average of three scans. HR-MS was performed using the Agilent 6224 Accurate-Mass Time-Of-Flight (TOF) MS. A Varian Mercury 300 was utilized to obtain the NMR spectra in deuterated solvents as specified in the sections below.



Scheme S1. Nosyl amine protection of diethylenetriamine.[1]

1,4,7-Tris(2-nitrobenzenesulfonyl)-1,4,7-triazaheptane (3). Tri-nosylate protection of diethylenetriamine was performed using a modified procedure based on the work of Nicak *et al*.[1] A solution of diethylenetriamine (14.1 mmol, 1.52 mL) and triethylamine (23.7 mmol, 3.30 mL) in CH_2Cl_2 (100 mL) were added, under N_2, to a stirring solution of 2-nitrobenzenesulfonyl chloride (10.00 g, 45.1 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred at room temperature for 20 h and the solvent removed under reduced pressure. Next, the orange residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. After washing, the organic layer was extracted with H_2O (3 × 75 mL), dried over sodium sulfate, and solvent removed under reduced pressure to yield an orange foam. Finally, the crude product was recrystallized from $CH_2Cl_2/CHCl_3$, 9:1 to give **3** as an off white solid in 70% yield (6.53 g)**.** ^{1}H NMR (DMSO) δ: 3.04 (t, C*H*$_2$NH, 4H), 3.36 (t, C*H*$_2$NH-Nos, 4H), 7.77 (m, Ar H, 12 H). ^{13}C NMR (DMSO) δ: 42.0, 48.6, 125.2, 125.3, 130.1, 130.3, 131.7, 133.0, 133.3, 133.4, 134.8, 135.4, 148.2. HR-MS (m/z): Found: 659.0513 [**3**+H]$^+$ (100%) Theoretical: 659.0536 [**3**+H]$^+$ (100%).



Scheme S2. Synthetic route to 4-Benzyloxy-2,6-bis(chloromethyl)pyridine (**7**).

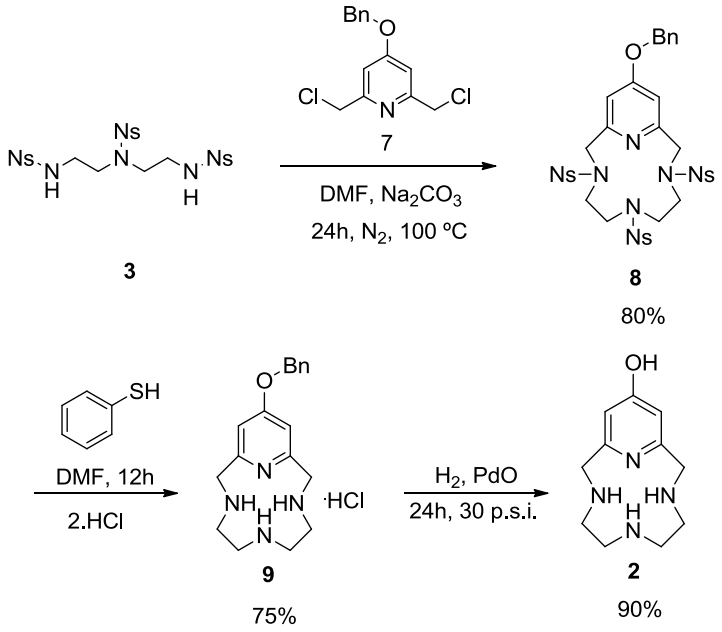
Diethyl-4Hydroxypyridine-2,6-dicarboxylate (4). Protection of the Chelidamic acid was accomplished according to Kazufumi *et al*.[2] Chelidamic acid (5.0 g, 27.3 mmol) was dissolved in anhydrous ethanol (200 mL), and 5 mL of $SOCl_2$. The solution was heated at reflux (80 °C) for 6 h and evaporated to dryness. Toluene was added to the resulting residue, 50 mL × 2. Diethyl ether and water, 100 mL each, was added to the flask containing the residue. The flask was capped and shaken three times; upon which a white crystalline solid became suspended between these layers. The white crystalline solid was filtered and dried over vacuum with no further purification necessary. Yield: 5.56 g, (70%). ^{1}H NMR ($CDCl_3$), δ: 1.38 (t, $CH_2C\underline{H}_3$, 6H), 4.38 (q, $C\underline{H}_2CH_3$, 4H), 7.43 (s, py H, 2H), 9.2 (s, O*H*, 1H). ^{13}C NMR ($CDCl_3$) δ: 14.2, 18.3, 58.3, 63.0, 118.0, 163.5.

Diethyl 4-(Benzyloxy)pyridine-2,6-dicarboxylate (5). Compound **5** was also produced using the methods of Froidevaux.[3] Compound **4** (5.0 g, 20.9 mmol) was dissolved in dry acetonitrile (150 mL) followed by sequential addition of K_2CO_3 (4.5 g, 32.8 mmol) and benzyl bromide (20.9 mmol, 2.4 mL). The reaction mixture was heated at reflux (82 °C) under N_2, for 12 h. The reaction mixture was cooled to room temperature, filtered to remove the inorganic salts, and evaporated to dryness. The oily residue was crystallized from hot hexane to yield **5** as a white solid. Yield 7.55 g (94%). ^{1}H NMR ($CDCl_3$), δ: 1.43 (t, $CH_2C\underline{H}_3$, 6H), 4.43 (q, $C\underline{H}_2CH_3$, 4H), 5.22 (s, $C\underline{H}_2$Bz, 2H), 7.39 (m, Ar H, 5H), 7.87 (s, py H, 2H) ^{13}C NMR ($CDCl_3$) δ: 14.1, 62.4, 70.7, 114.6, 127.7, 128.7, 128.8, 135.0, 150.0, 164.8, and 166.6.

4-(Benzyloxy)-2,6-bis(hydroxymethyl)pyridine (6). Reduction of the esters was performed according to Busto[3], with a slight modification of the procedure, as CH_2Cl_2 was used in place of ethyl acetate to extract the product. The previous product **5** (5.0 g, 20.9 mmol) was dissolved in absolute EtOH (320 mL) and then $NaBH_4$ (3.80 g, 100 mmol) was added in one portion. The mixture was heated to 40 °C and stirred for 24 h. The resulting mixture was quenched with H_2O (50 mL) and filtered to remove the boric acid precipitate. Next, the bulk of the ethanol was removed under reduced pressure and the resulting aqueous extracted with CH_2Cl_2 (3 × 100 mL). It should be noted if there is difficulty in visible separation of the two layers, the addition of more water is necessary for ideal separation. The organic phases were combined and dried over sodium sulfate to yield **6** as a white, crystalline solid with no further purification necessary. Yield 3.56 g (95%). ^{1}H NMR (DMSO), δ: 4.42 (s, $C\underline{H}_2$OH, 4H), 5.14 (s, $CH_2O\underline{H}$, 2H), 5.36 (s, $C\underline{H}_2C_6H_5$, 2H), 6.90 (s, py H, 2H), 7.30 (m, Ar H, 5H). ^{13}C NMR ($CDCl_3$) δ: 62.6, 67.6, 103.3, 126.3, 126.7, 127.1, 135.0, 161.7, 164.4.

4-Benzyloxy-2,6-bis(chloromethyl)pyridine (7). The reported method by Chessa[4] was used for synthesis of the alkyl halide with MeOH used instead of EtOH for recrystallization resulting in higher yields in our hands. Thionyl chloride (22 mL, 234 mmol) was slowly added to **6** (2.37 g, 9.6 mmol), the clear solution was heated under reflux at 60°C for 4 h, cooled to room temperature and excess thionyl chloride was removed under reduced pressure. The residue was neutralized with a cold solution of 10% Na_2CO_3. The beige solid was filtered and washed thoroughly with cold water, and recrystallized from hot methanol to give **7** as white, crystalline needles. Yield, 2.35

g (90%). ^{1}H NMR ($CDCl_3$) δ: 6.61 (s, C$\underline{H_2}$Cl, 4H), 5.14 (s, C$\underline{H_2}C_6H_5$, 2H), 7.03 (s, py H, 2H), 7.41 (m, Ar H, 5H). ^{13}C NMR ($CDCl_3$) δ: 46.7, 70.4, 109.0, 127.9, 128.8, 129.0, 135.8, 158.2, 168.3.



Scheme S3. Macrocyclization and deprotection reactions used to produce **2**.

12-Benzyloxy-3,6,9-tris(2-nitrobenzenesulfonyl)-3,6,9,15-tetraazabicyclo[9.3.1]penta-deca-1(15),11,13-triene (8). Macrocyclization was done according to Siaugue and co-workers[5], also with a modification of the procedure due the observation that the fully protected macrocyclic product is not soluble in CH_2Cl_2. A solution of **7** (2.22, 7.8 mmol) in anhydrous DMF (125 mL) was added drop wise over 4 h, under N_2, to a stirred solution of **3** (5.19 g, 7.8 mmol) and Na_2CO_3 (3.8 g, 31.2 mmol) in anhydrous DMF (125 mL) at 100 °C. The bright yellow solution was heated at reflux overnight, and the solvent was removed under reduced pressure to give an orange solid. The orange solid was taken up in CH_2Cl_2, filtered, washed with 0.1 M NaOH (200 mL), washed with water (2 × 100 mL) followed by diethyl ether (2 × 100 mL) to give **8** as a fine, powdery and bright yellow solid. Yield 5.53 g (80%). ^{1}H NMR (DMSO) δ: 3.47 (t, 4H), 3.73 (t, 4H), 4.53 (s, 4H), 5.16 (s, 2H), 6.98 (s, 2H), 7.35 (m, 5H), 7.83 (m, 12 H). ^{13}C NMR (DMSO) δ: 31.2, 46.3, 54.9, 70.6, 111.2, 124.4, 124.6, 128.0, 128.7, 129.0, 131.1, 131.5, 132.3, 134.2, 156.6. HR-MS (m/z): Found: 868.1517 [**8**+H]$^{+}$ (100%), Theoretical: 868.1377 [**8**+H]$^{+}$.

12-Benzyloxy-3,6,9,15-tetraazabicyclo[9.3.1]penta-deca-1(15),11,13-triene (9). Removal of the nosylates was done according to reported methods of Favre-Reguillon[6], with no modification. Thiophenol (4 mL, 38.2 mmol) was added to a stirred solution of **8** (5.53 g, 6.3 mmol) and Na_2CO_3 (8.0 g, 75 mmol) in anhydrous DMF (80 mL). The dark orange solution was stirred overnight at room temperature. The solvent was evaporated to give an oily orange residue which was triturated with aqueous HCl (1M). The yellow aqueous phase was extracted with diethyl ether (2 × 100 mL), and the organic layer was discarded. The yellow aqueous solution was adjusted to pH 12 with NaOH, and concentrated under reduced pressure. The yellow solid was taken up in CH_2Cl_2 (200 mL), dried over sodium sulfate, and solvent removed under reduced pressure to give a yellow oil. Diethyl ether was added to the yellow oil, followed by drop wise addition of concentrated HCl until precipitation of a yellowish solid occurred. The solution containing the precipitate was filtered to give **9** as a fine and powdery light yellow solid. Yield 3.30 g, (75%). ^{1}H NMR (D_2O) δ: 3.39 (m, 8H), 5.13 (s, 2H), 7.02 (s, 2H), 7.28 (m, 5H). HR-MS (m/z): Found: 313.2569 [**9**+H]$^{+}$ (100%), Theoretical: 313.2028 [**9**+H]$^{+}$.

3,6,9,15-tetraazabicyclo[9.3.1]penta-deca-1(15),11,13-trien-13-ol (2). Debenzylation was performed as stated by Mochizuki[7], with no change in procedure as the authors note that the HCl salt is necessary for

debenzylation to occur. A suspension of **9** (1.5 g, 4.8 mmol) in H_2O (25 mL) containing PdO (0.55 g, 4.5 mmol) was exposed to H_2(g) (30 psi) using a PARR Hydrogenator System for 24 h. The clear solution was filtered and lyophilized to give **2**. ^{1}H NMR (D_2O) δ: 3.2 (m, 8H), 4.40 (s, 4H), 6.85 (s, 2H). ^{13}C NMR (D_2O) δ: 43.3, 49.2, 111.7, 151.7, 166.3. White, fluffy solid. Yield 0.97 g (90%). HR-MS (m/z): Found: 223.1834 [**9**+H]$^+$ (100%), Theoretical: 223.1559 [**9**+H]$^+$. HPLC analysis revealed 96 % purity, H_2O/MeCN (80:20), 0.1 mL/min, Retention time = 1.26 min.

General procedure for metal complexation with (2). Metal complexes were prepared by dissolving 50 mg of **2** in 2 mL of water, and the pH of the solution was adjusted to 7.0 with concentrated NaOH. Perchlorate salts of zinc or copper were added to the ligand in a ratio of 1:1 and stirred for 2 days at room temperature. Next, the solvent was removed and the complexes were taken up in a minimum amount of methanol. The resulting solutions were filtered through a syringe filter. ESI-MS and UV-Vis were used to indicate complex formation of all solutions.

[Cu2●Cl]ClO_4. Blue crystals suitable for x-ray crystallography were obtained by slow evaporation of MeOH. ESI-MS (m/z): Found 284.1143 (60%) [M-H]$^+$, 320.0950 (100%) [M+Cl$^-$]$^+$, Theoretical 285.0777 [M]$^+$.

[Zn2●Cl]ClO_4. Faint yellow crystals suitable for x-ray crystallography were obtained by slow evaporation of MeOH. ESI-MS (m/z): Found 287.1288 [M+H$^+$]$^+$, 321.0942 [M+Cl$^-$]$^+$, 385.0786 [M+ClO_4^-]$^+$, Theoretical 286.0772 [M]$^+$.

DPPH Assay. DPPH stock solution was prepared by dissolving 25 mg in 100 mL of absolute EtOH. The working radical solution was prepared by dilution with absolute EtOH to an absorbance of 1.3 ± 0.002 units at 515 nm.[8] Stock solutions of BHT (positive control), **1** and **2** were dissolved in 95% EtOH to a concentration of 5mM, and serial dilutions were done in 95% EtOH to reach the desired concentrations. For the analysis, 2 mL of 95 % EtOH (control) BHT, or ligands (625 μM-37.5 μM) were combined with 2 mL of the DPPH working solution and incubated in the dark for 24 hours. Analysis of the solutions was performed as follows: 1 mL of the sample solution was diluted with 1 mL of absolute EtOH in a cuvette, shaken for 30 s and absorbance measured at 515 nm. Analysis of each sample was performed in triplicate. EtOH (95 %) was used as a control in this experiment, and all data were normalized to the average (n = 3) absorbance of this sample. In addition, 1 mL of DPPH working solution (incubated for 24 hours) was diluted with 1 mL of absolute EtOH, repeated in triplicate, and the absorbance was normalized to the average of the control (95% EtOH) absorbance value.[1]

Preparation of $A\beta_{1\text{-}40}$ stock. Synthetic Beta Amyloid 1-40 peptide was purchased from Twenty-First Century Biochemicals. A peptide stock solution was prepared by dissolving 1.9 mg of $A\beta_{1\text{-}40}$ in 1 mL of buffer, followed by NaOH (20 mM, 300 μL). The solution was sonicated for one minute then adjusted to pH 7.4 using HCl (0.5 M, ~10.5 μL). The solution was sonicated for another minute and diluted to 200 μM using MQ water.

Turbidity Studies. Two separate turbidity studies (absorbance at 405 nm) were performed using amyloid, copper or zinc, and ligands one to determine disaggregation capability and another to determine preventive capability of copper induced beta amyloid formation. Turbidity studies were repeated with HEPES Buffer (0.02 HEPES, 0.154 M NaCl, pH=7.4) giving identical results.

(a) **Disaggregation**. Zinc (II) acetate or copper (II) sulfate solution (400 μM, 40 μL) was added to a solution of $A\beta_{40}$ (200 μM, 40 μL) and incubated at 37 °C for one day. After incubation the ligand stock solutions were added (800 μM, 40 μL) to aggregated peptide solution and incubated for a further 12 hours at 37 °C. For analysis of turbidity, 20 μL sample aliquots were diluted with buffer (180 μL) and absorbance recorded using a Microplate reader (Softmax Pro M5). The value of the blank absorbance was subtracted from each sample value.

(b) **Preventative**. Ligand stock solutions (800 μM, 40 μL) were added to a solution of $A\beta_{40}$ (200 μM, 40 μL) and incubated for five minutes at room temperature followed by addition of $Zn(OAc)_2$ or $CuSO_4$ (400 μM, 40 μL). All samples were incubated at 37 °C for one day. Absorbance measurements were carried out in the same manner as described above.

Tyrosine Fluorescence. Tyrosine fluorescence studies were carried out on samples used for the turbidity experiments. For sample preparation, 50 μL of sample was diluted with 270 μL of buffer and agitated for 30 s. Excitation and emission values utilized were 278 and 305 nm, respectively.[9]

DCFH-DA Assay. Fibroblasts obtained from a 30 year old FRDA patient from The Coriell Institute (Camden, NJ, USA) were kept in Dulbecco's Modified Eagle Medium (DMEM; ThermoScientific, Waltham, MA, USA) with 10% charcoal-stripped fetal bovine serum (FBS; ThermoScientific), 1% GlutaMAX (ThermoScientific) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C, 5% CO_2 and 90% humidity. HT-22 cells were prepared in a similar manner. The FRDA or HT-22 cells were plated at 5,000 cells per well on a 96-well plate, then treated for 12 hours with either ligands (various concentrations) and/or BSO (1mM). After 12 hours of treatment the media was removed from each well of the 96-well plate, and 100 μL of a 1 μM 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA; AnaSpec Inc., Fremont, CA, USA) in phosphate buffer (PBS) was added to each well. The plates were returned to a 37°C incubator for 20 minutes, then each well was washed three times with PBS and the plate was read on a Tecan Infinite M200 plate reader with an absorbance of 495 nm and an emission of 529 nm, respectively.

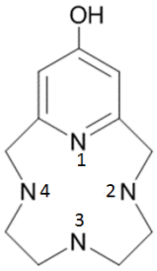
Calcein AM Cell Viability Assay. FRDA cells were plated on a 96-well plate at a density of 3,000 cells per well, then treated with ligands (various concentrations) and/or BSO (1mM). After 48 hours of BSO and ligand treatment, the media was removed, and 1 μg/mL Calcein AM (CalBiochem, San Diego, CA, USA) in phosphate buffer pH 7.2 (PBS; Fisher Scientific, Pittsburg, PA, USA) was added to each well and the plate was incubated for 10 minutes at 37°C. Cell viability was determined with a Tecan Infinite M200 plate reader with an excitation of 490 nm and emission of 520 nm.

Table S1. Bond distances of interest for the Cu^{II} and Zn^{II} derivatives of **1** and **2.** [10]

M =	Cu		Zn	
	$M(\mathbf{1})^{2+}$	$M(\mathbf{2})^{2+}$	$M(\mathbf{1})^{2+}$	$M(\mathbf{2})^{2+}$
M-N(1)	1.965	1.9392	2.055	2.0413
M-N(2)	2.054	2.0796	2.212	2.2092
M-N(3)	2.147	2.2264	2.048	2.0876
M-N(4)	2.076	2.0821	2.245	2.2078
M-Cl	2.384	2.2264	2.245	2.2141
C-OH	-	1.340	-	1.340

Table S2. Bond angles of interest for the Cu^{II} and Zn^{II} derivatives of **1** and **2.** [10]

M =	Cu		Zn	
	$M(\mathbf{1})^{2+}$	$M(\mathbf{2})^{2+}$	$M(\mathbf{1})^{2+}$	$M(\mathbf{2})^{2+}$
N(1)-M-N(2)	82.537	81.79	76.871	78.62
N(2)-M-N(3)	86.219	84.49	84.05	84.37
N(1)-M-N(3)	102.24	98.89	113.32	97.47
N(1)-M-N(4)	80.863	81.87	77.769	79.22
N(3)-M-N(4)	84.57	84.44	108.95	83.96
N(2)-M-N(4)	158.83	158.57	145.15	153.31



Legend for Tables S1 and S2.

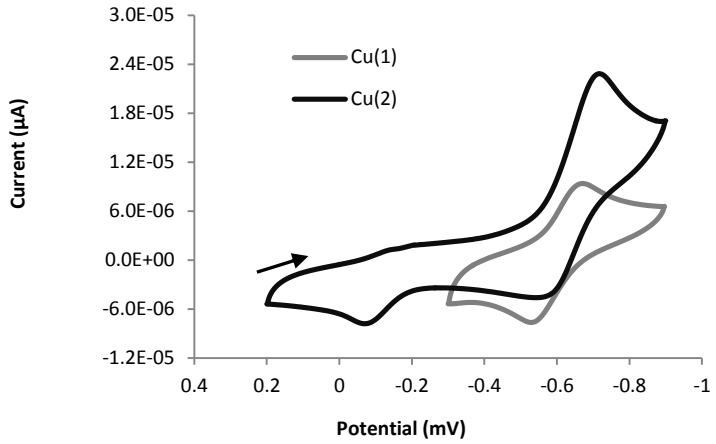


Figure S1. Cyclic voltammograms of Cu(**1**) and Cu(**2**), [3 mM], in phosphate buffer at pH = 7.4, referenced to a Ag/AgCl electrode. Scan rate = 100 mV/s. *The oxidation peak of Cu(**2**) around -100 mV is ligand based.

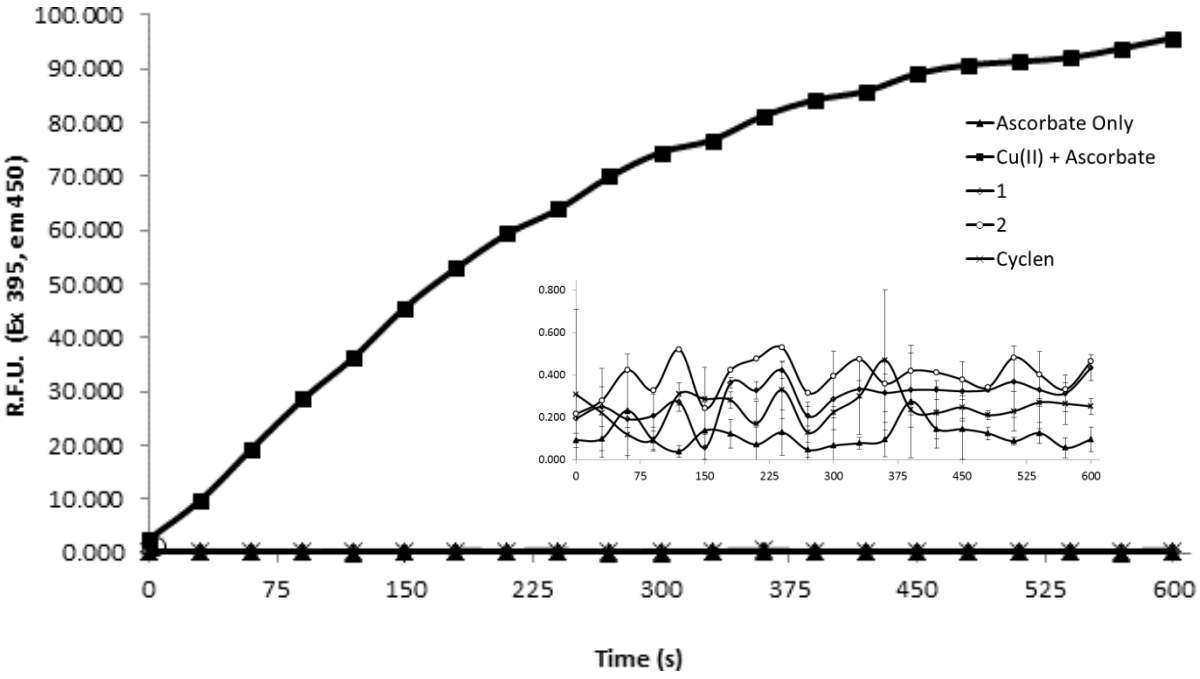


Figure S2. Fluorescence intensity of 7-hydroxy-CCA after incubation of CCA [100µM] and ascorbate [300µM] with Cu^{II}(■)[40µM]. Compound **1**(◊), **2**(○), and cyclen(X) [40µM] were added at t = 0 s prior to Cu^{II}. Asc(▲) is a negative control with buffer and ascorbate only. All solutions, except $Cu(NO_3)_2$ (milli-Q water only) were dissolved and diluted in phosphate buffer [15 mM] containing desferryl [2 µM]. Final volume = 4 mL, n = 3 for each sample.

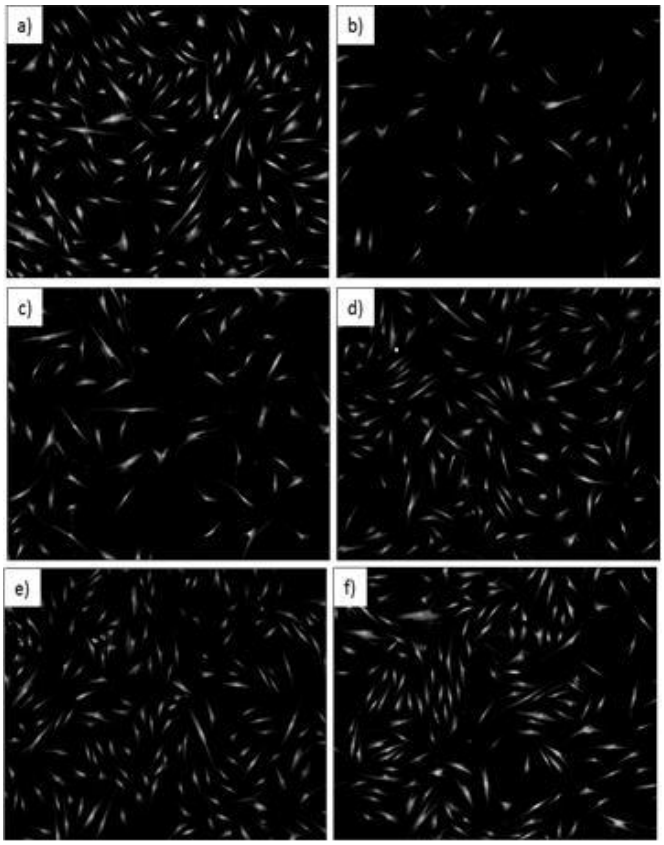


Figure S3. Fluorescence response of FRDA cells incubated with Calcein AM viability indicator (a) FRDA cells in media only (b) + BSO [1 mM] (c) b + 12.5 nM 2 (d) b + 125 nM 2 (e) b + 1.25 uM 2 (f) b + 12.5 uM **2**.

Turbidity Studies: Copper (II) or zinc (II) ion addition to a solution of amyloid$_{1\text{-}40}$ results in a turbid solution which scatters light with a consequential increased absorbance signal using absorption spectrophotometry.[25, 27] Turbidity results show that incubation of **1** or **2** with A$\beta_{1\text{-}40}$ prior to addition of Cu^{II} or Zn^{II} prevents formation of aggregations compared to amyloid incubated with metal-ions alone. These studies were also carried out with other N-heterocylic amines and open chain chelators which have been studied by others and show that our ligands are equally effective in terms of anti-aggregate activity.[25] Moreover, amyloid aggregates formed by metal ion co-incubation can be disaggregated by the addition of **1** or **2**.

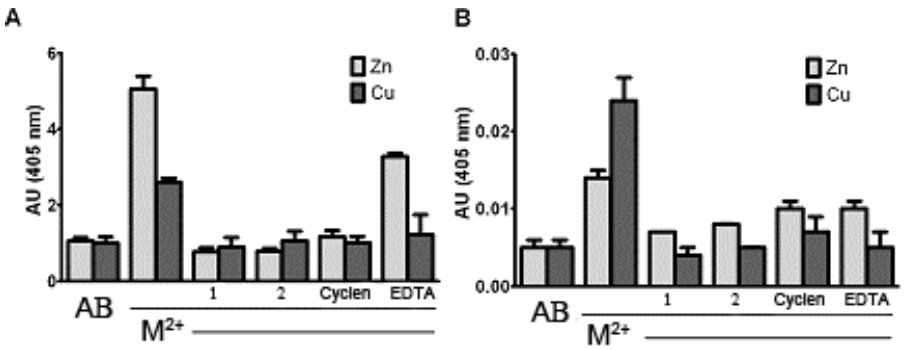


Figure S4. Turbidity Assay showing (a) preventative and (b) disaggregative capability of **2** compared to **1**, cyclen and EDTA to prevent amyloid plaques. [A$\beta_{1\text{-}40}$] = 200 nM, [$CuSO_4$] or $Zn(OAc)_2$ = 400 nM, [chelator] = 800 nM. n=3 for each sample.

Tyr-10 Studies: Copper(II) binding to Aβ quenches the signal via paramagnetic effects while Zinc(II) changes reflect a change in aggregate state.[33] Chelators 1 and 2 are equally effective in preventing signal decrease and re-constituting the Tyr-10 signal for both types of metal induced quenching. These results are compared to macrocyclic and open-chain chelators cyclam and EDTA, which show an equivalent capacity. These results are consistent with the turbidity studies suggesting that the Tyr-10 signal can be used to follow the aggregates formation as well.

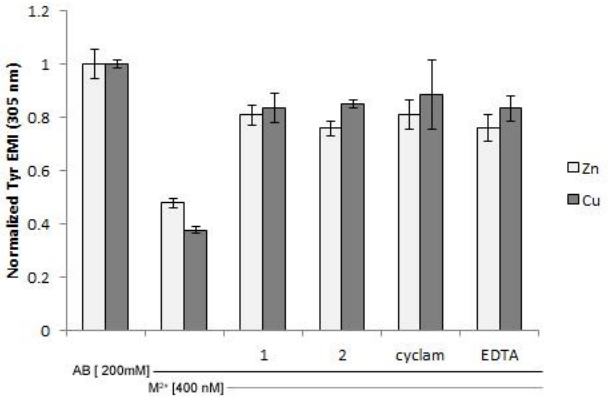


Figure S5. Tyr fluorescence results for protective capability study. All ligands display protective effects against copper induced aggregation, with EDTA showing to be least effective. Solutions were prepared in phosphate buffer. [$A\beta_{1-40}$] = 200 nM, [$CuSO_4$] or [$Zn(OAc)_2$] = 400 nM, [chelator] = 800 nM. n=3 for each sample.

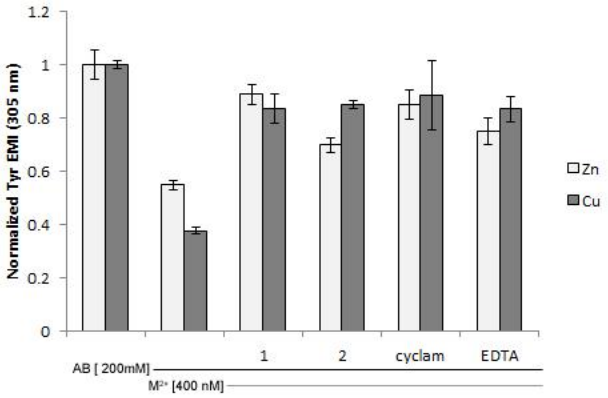


Figure S6. Tyr fluorescence results for the disaggregative capability study. All ligands display protective effects against copper induced aggregation, with EDTA showing to be least effective. Solutions were prepared in phosphate buffer. [$A\beta_{1-40}$] = 200 nM, [$CuSO_4$] or [$Zn(OAc)_2$] = 400 nM, [chelator] = 800 nM. n=3 for each sample.

Part II. Crystallographic Details.

Table 1. Sample and crystal data for [Cu(2)●Cl][ClO_4].

Identification code	082412	
Chemical formula	$C_{11}H_{21}Cl_2CuN_4O_5$	
Formula weight	423.76	
Temperature	220(2) K	
Wavelength	0.71073 Å	
Crystal size	0.300 x 0.400 x 0.500 mm	
Crystal habit	blue block	
Crystal system	monoclinic	
Space group	P 1 21/c 1	
Unit cell dimensions	a = 11.454(5) Å	α = 90°
	b = 8.985(4) Å	β = 122.113(19)°
	c = 18.166(6) Å	γ = 90°
Volume	1583.5(11) $Å^3$	
Z	4	
Density (calculated)	1.777 g/cm^3	
Absorption coefficient	1.747 mm^{-1}	
F(000)	872	

Table 2. Data collection and structure refinement for [Cu(2)●Cl][ClO_4].

Theta range for data collection	2.10 to 27.62°
Index ranges	-14<=h<=14, -11<=k<=11, -23<=l<=22
Reflections collected	13671
Independent reflections	3617 [R(int) = 0.0255]
Coverage of independent reflections	98.5%
Absorption correction	multi-scan
Max. and min. transmission	0.7456 and 0.6196
Structure solution technique	direct methods
Structure solution program	SHELXS-97 (Sheldrick, 2008)
Refinement method	Full-matrix least-squares on F^2
Refinement program	SHELXL-97 (Sheldrick, 2008)
Function minimized	$\Sigma\, w(F_o^2 - F_c^2)^2$
Data / restraints / parameters	3617 / 292 / 283
Goodness-of-fit on F^2	1.024
Δ/σ_{max}	0.001
Final R indices	3283 data; I>2σ(I) R1 = 0.0245, wR2 = 0.0638
	all data R1 = 0.0277, wR2 = 0.0660
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0325P)^2+1.0702P]$ where $P=(F_o^2+2F_c^2)/3$
Largest diff. peak and hole	0.433 and -0.432 eÅ^{-3}
R.M.S. deviation from mean	0.062 eÅ^{-3}

Table 3. Bond lengths (Å) for [Cu(2)●Cl][ClO_4].

Cu1-N3	1.9392(16)	Cl3-O4	1.425(7)	C11-H11	0.94
Cu1-N2	2.0821(16)	Cl3-O2'	1.434(12)	Cl3-O1	1.376(5)
Cu1-Cl1	2.2264(9)	Cl3-O2"	1.448(3)	Cl3-O3"	1.398(5)
O9-H9	0.8193	O1'-O3'	1.65(3)	Cl3-O2	1.416(6)
N1-C8	1.473(3)	Cu1-N4	2.0796(16)	Cl3-O3	1.432(6)
N2-C3	1.483(2)	Cu1-N1	2.1730(19)	Cl3-O1"	1.441(4)
N2-H2	0.9001	O9-C10	1.340(2)	Cl3-O3'	1.464(12)
N3-C5	1.347(2)	N1-C1	1.466(3)		
N4-C6	1.484(2)	N1-H1	0.9		
C1-C2	1.522(3)	N2-C2	1.487(3)		
C1-H1B	0.98	N3-C4	1.341(2)		
C2-H2B	0.98	N4-C7	1.482(2)		
C3-H3A	0.98	N4-H4	0.9001		
C4-C11	1.369(3)	C1-H1A	0.98		
C5-C6	1.503(2)	C2-H2A	0.98		
C6-H6B	0.98	C3-C4	1.516(2)		
C7-H7A	0.98	C3-H3B	0.98		
C8-H8A	0.98	C5-C9	1.375(2)		
C9-C10	1.396(3)	C6-H6A	0.98		
C10-C11	1.401(3)	C7-C8	1.520(3)		
Cl3-O4"	1.376(4)	C7-H7B	0.98		
Cl3-O4'	1.390(11)	C8-H8B	0.98		
Cl3-O1'	1.401(14)	C9-H9A	0.94		

Table 4. Bond angles (°) for [Cu(2)●Cl][ClO_4].

N3-Cu1-N4	81.79(6)	N3-Cu1-N2	81.87(6)
N4-Cu1-N2	158.57(6)	N3-Cu1-N1	98.89(6)
N4-Cu1-N1	84.49(7)	N2-Cu1-N1	84.44(7)
N3-Cu1-Cl1	154.20(5)	N4-Cu1-Cl1	100.07(5)
N2-Cu1-Cl1	100.69(5)	N1-Cu1-Cl1	106.91(5)
C10-O9-H9	109.3	C1-N1-C8	117.78(16)
C1-N1-Cu1	103.52(12)	C8-N1-Cu1	104.04(12)
C1-N1-H1	107.8	C8-N1-H1	107.8
Cu1-N1-H1	116.3	C3-N2-C2	113.64(15)
C3-N2-Cu1	109.77(11)	C2-N2-Cu1	106.09(12)
C3-N2-H2	108.7	C2-N2-H2	108.7
Cu1-N2-H2	109.9	C4-N3-C5	120.88(15)
C4-N3-Cu1	119.42(12)	C5-N3-Cu1	119.34(12)
C7-N4-C6	113.33(15)	C7-N4-Cu1	105.64(12)
C6-N4-Cu1	110.53(11)	C7-N4-H4	108.9
C6-N4-H4	108.7	Cu1-N4-H4	109.7
N1-C1-C2	107.86(16)	N1-C1-H1A	110.1
C2-C1-H1A	110.1	N1-C1-H1B	110.1
C2-C1-H1B	110.1	H1A-C1-H1B	108.4
N2-C2-C1	111.02(16)	N2-C2-H2A	109.4
C1-C2-H2A	109.4	N2-C2-H2B	109.4
C1-C2-H2B	109.4	H2A-C2-H2B	108.0
N2-C3-C4	111.19(14)	N2-C3-H3A	109.4
C4-C3-H3A	109.4	N2-C3-H3B	109.4

C4-C3-H3B	109.4	H3A-C3-H3B	108.0
N3-C4-C11	121.22(16)	N3-C4-C3	114.28(15)
C11-C4-C3	124.49(16)	N3-C5-C9	121.42(16)
N3-C5-C6	114.65(15)	C9-C5-C6	123.88(15)
N4-C6-C5	111.30(14)	N4-C6-H6A	109.4
C5-C6-H6A	109.4	N4-C6-H6B	109.4
C5-C6-H6B	109.4	H6A-C6-H6B	108.0
N4-C7-C8	110.86(16)	N4-C7-H7A	109.5
C8-C7-H7A	109.5	N4-C7-H7B	109.5
C8-C7-H7B	109.5	H7A-C7-H7B	108.1
N1-C8-C7	107.81(16)	N1-C8-H8A	110.1
C7-C8-H8A	110.1	N1-C8-H8B	110.1
C7-C8-H8B	110.1	H8A-C8-H8B	108.5
C5-C9-C10	117.88(16)	C5-C9-H9A	121.1
C10-C9-H9A	121.1	O9-C10-C9	122.53(17)
O9-C10-C11	117.35(18)	C9-C10-C11	120.11(17)
C4-C11-C10	118.35(17)	C4-C11-H11	120.8
C10-C11-H11	120.8	O4"-Cl3-O1	140.2(4)
O4"-Cl3-O4'	33.4(11)	O1-Cl3-O4'	115.9(10)
O4"-Cl3-O3"	111.8(4)	O1-Cl3-O3"	97.6(5)
O4'-Cl3-O3"	144.5(11)	O4"-Cl3-O1'	132.8(14)
O1-Cl3-O1'	37.3(14)	O4'-Cl3-O1'	134.3(19)
O3"-Cl3-O1'	68.4(15)	O4"-Cl3-O2	102.2(6)
O1-Cl3-O2	108.9(5)	O4'-Cl3-O2	98.3(16)
O3"-Cl3-O2	80.4(7)	O1'-Cl3-O2	123.2(15)

O4"-Cl3-O4	32.3(6)	O1-Cl3-O4	111.2(7)
O4'-Cl3-O4	11.8(17)	O3"-Cl3-O4	142.9(6)
O1'-Cl3-O4	123.7(16)	O2-Cl3-O4	110.0(7)
O4"-Cl3-O3	78.1(6)	O1-Cl3-O3	113.1(7)
O4'-Cl3-O3	110.5(16)	O3"-Cl3-O3	40.4(5)
O1'-Cl3-O3	75.7(17)	O2-Cl3-O3	109.1(6)
O4-Cl3-O3	104.5(6)	O4"-Cl3-O2'	106.2(10)
O1-Cl3-O2'	110.4(10)	O4'-Cl3-O2'	110.1(11)
O3"-Cl3-O2'	64.9(16)	O1'-Cl3-O2'	114.5(19)
O2-Cl3-O2'	15.6(18)	O4-Cl3-O2'	121.4(15)
O3-Cl3-O2'	94.9(17)	O4"-Cl3-O1"	111.6(4)
O1-Cl3-O1"	29.7(3)	O4'-Cl3-O1"	95.0(11)
O3"-Cl3-O1"	110.7(4)	O1'-Cl3-O1"	42.5(14)
O2-Cl3-O1"	135.8(6)	O4-Cl3-O1"	86.9(7)
O3-Cl3-O1"	105.2(6)	O2'-Cl3-O1"	139.9(10)
O4"-Cl3-O2"	111.5(3)	O1-Cl3-O2"	83.2(4)
O4'-Cl3-O2"	89.3(14)	O3"-Cl3-O2"	106.8(4)
O1'-Cl3-O2"	113.2(15)	O2-Cl3-O2"	34.7(6)
O4-Cl3-O2"	99.6(7)	O3-Cl3-O2"	142.7(5)
O2'-Cl3-O2"	47.9(15)	O1"-Cl3-O2"	104.1(3)
O4"-Cl3-O3'	77.1(11)	O1-Cl3-O3'	107.1(10)
O4'-Cl3-O3'	107.6(10)	O3"-Cl3-O3'	47.6(14)
O1'-Cl3-O3'	70.3(17)	O2-Cl3-O3'	119.4(15)
O4-Cl3-O3'	100.0(15)	O3-Cl3-O3'	10.3(15)
O2'-Cl3-O3'	105.1(11)	O1"-Cl3-O3'	95.8(12)

O2"-Cl3-O3'	152.7(14)	Cl3-O1'-O3'	56.7(11)
Cl3-O3'-O1'	53.1(9)		

Table 5. Sample and crystal data for [Zn(2)●Cl][ClO_4].

Identification code	111512	
Chemical formula	$C_{11}H_{18}Cl_2N_4O_5Zn$	
Formula weight	422.57	
Temperature	89(2) K	
Wavelength	0.71073 Å	
Crystal habit	clear light yellow block	
Crystal system	monoclinic	
Space group	P 1 21/n 1	
Unit cell dimensions	a = 11.5092(5) Å	α = 90°
	b = 9.0075(4) Å	β = 97.410(2)°
	c = 15.4711(7) Å	γ = 90°
Volume	1590.48(12) $Å^3$	
Z	4	
Density (calculated)	1.777 g/cm^3	
Absorption coefficient	1.910 mm^{-1}	
F(000)	876	

Table 6. Data collection and structure refinement for [Zn(2)●Cl][ClO_4].

Theta range for data collection	2.08 to 38.45°
Index ranges	-20<=h<=20, -15<=k<=15, -27<=l<=26
Reflections collected	91677
Independent reflections	8871 [R(int) = 0.0392]
Coverage of independent reflections	99.3%
Absorption correction	multi-scan
Structure solution technique	direct methods
Structure solution program	SHELXS-97 (Sheldrick, 2008)
Refinement method	Full-matrix least-squares on F^2
Refinement program	SHELXL-97 (Sheldrick, 2008)
Function minimized	$\Sigma\, w(F_o^2 - F_c^2)^2$
Data / restraints / parameters	8871 / 47 / 239
Goodness-of-fit on F^2	1.019
Δ/σ_{max}	0.001
Final R indices	6731 data; I>2σ(I) R1 = 0.0453, wR2 = 0.1106
	all data R1 = 0.0684, wR2 = 0.1251
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0586P)^2+2.2207P]$ where $P=(F_o^2+2F_c^2)/3$
Largest diff. peak and hole	1.525 and -1.152 eÅ^{-3}
R.M.S. deviation from mean	0.126 eÅ^{-3}

Table 7. Bond lengths (Å) for [Zn(2)●Cl][ClO_4].

Zn1-N1	2.0413(17)	Cl2-O3	1.468(7)	N4-H3A	0.88
Zn1-N4	2.2078(17)	Cl2'-O5'	1.441(9)	Cl2-O2	1.377(6)
Zn1-Cl1	2.2141(6)	Cl2'-O2'	1.486(6)	Cl2-O4	1.444(7)
Zn1-H2A	1.7826	Zn1-N3	2.0876(19)	Cl2'-O4'	1.375(8)
C1-N1	1.339(2)	Zn1-N2	2.2092(19)	Cl2'-O3'	1.470(9)
C1-C11	1.509(3)	Zn1-H1A	1.8991	Cl2'-O3'	1.470(9)
C2-H2	0.95	Zn1-H3A	1.9028		
C3-C4	1.400(3)	C1-C2	1.381(3)		
C4-H4	0.95	C2-C3	1.394(3)		
C5-C6	1.512(3)	C3-O1	1.340(3)		
C6-H6A	0.99	C4-C5	1.380(3)		
C7-N2	1.478(3)	C5-N1	1.341(3)		
C7-H7A	0.99	C6-N2	1.479(3)		
C8-N3	1.478(3)	C6-H6B	0.99		
C8-H8B	0.99	C7-C8	1.520(3)		
C9-C10	1.520(3)	C7-H7B	0.99		
C9-H9B	0.99	C8-H8A	0.99		
C10-H10A	0.99	C9-N3	1.476(3)		
C11-N4	1.470(3)	C9-H9A	0.99		
C11-H11B	0.99	C10-N4	1.476(3)		
N3-H2A	0.88	C10-H10B	0.99		
O1-H1	0.84	C11-H11A	0.99		
Cl2-O5	1.385(7)	N2-H1A	0.88		

Table 8. Bond angles (°) for 111512.

N1-Zn1-N3	97.47(7)	N1-Zn1-N4	79.22(6)
N3-Zn1-N4	83.96(7)	N1-Zn1-N2	78.62(7)
N3-Zn1-N2	84.37(7)	N4-Zn1-N2	153.31(7)
N1-Zn1-Cl1	143.46(5)	N3-Zn1-Cl1	119.04(5)
N4-Zn1-Cl1	101.96(5)	N2-Zn1-Cl1	104.69(5)
N1-Zn1-H1A	94.0	N3-Zn1-H1A	99.5
N4-Zn1-H1A	172.7	N2-Zn1-H1A	23.2
Cl1-Zn1-H1A	82.0	N1-Zn1-H2A	122.2
N3-Zn1-H2A	24.7	N4-Zn1-H2A	90.1
N2-Zn1-H2A	89.2	Cl1-Zn1-H2A	94.4
H1A-Zn1-H2A	95.7	N1-Zn1-H3A	93.2
N3-Zn1-H3A	100.5	N4-Zn1-H3A	23.2
N2-Zn1-H3A	171.0	Cl1-Zn1-H3A	79.7
H1A-Zn1-H3A	157.7	H2A-Zn1-H3A	98.3
N1-C1-C2	121.84(17)	N1-C1-C11	116.39(16)
C2-C1-C11	121.76(16)	C1-C2-C3	118.33(18)
C1-C2-H2	120.8	C3-C2-H2	120.8
O1-C3-C2	122.9(2)	O1-C3-C4	117.5(2)
C2-C3-C4	119.5(2)	C5-C4-C3	118.27(19)
C5-C4-H4	120.9	C3-C4-H4	120.9
N1-C5-C4	121.71(17)	N1-C5-C6	115.71(18)
C4-C5-C6	122.57(18)	N2-C6-C5	111.99(17)
N2-C6-H6A	109.2	C5-C6-H6A	109.2
N2-C6-H6B	109.2	C5-C6-H6B	109.2

H6A-C6-H6B	107.9	N2-C7-C8	111.35(16)
N2-C7-H7A	109.4	C8-C7-H7A	109.4
N2-C7-H7B	109.4	C8-C7-H7B	109.4
H7A-C7-H7B	108.0	N3-C8-C7	109.55(18)
N3-C8-H8A	109.8	C7-C8-H8A	109.8
N3-C8-H8B	109.8	C7-C8-H8B	109.8
H8A-C8-H8B	108.2	N3-C9-C10	109.55(18)
N3-C9-H9A	109.8	C10-C9-H9A	109.8
N3-C9-H9B	109.8	C10-C9-H9B	109.8
H9A-C9-H9B	108.2	N4-C10-C9	110.80(17)
N4-C10-H10A	109.5	C9-C10-H10A	109.5
N4-C10-H10B	109.5	C9-C10-H10B	109.5
H10A-C10-H10B	108.1	N4-C11-C1	112.82(15)
N4-C11-H11A	109.0	C1-C11-H11A	109.0
N4-C11-H11B	109.0	C1-C11-H11B	109.0
H11A-C11-H11B	107.8	C1-N1-C5	120.13(17)
C1-N1-Zn1	118.81(13)	C5-N1-Zn1	119.63(13)
C7-N2-C6	114.97(19)	C7-N2-Zn1	103.24(14)
C6-N2-Zn1	109.71(12)	C7-N2-H1A	122.5
C6-N2-H1A	122.5	Zn1-N2-H1A	58.2
C9-N3-C8	114.61(17)	C9-N3-Zn1	107.29(13)
C8-N3-Zn1	106.11(13)	C9-N3-H2A	122.7
C8-N3-H2A	122.7	Zn1-N3-H2A	57.9
C11-N4-C10	114.21(16)	C11-N4-Zn1	109.91(12)
C10-N4-Zn1	103.09(12)	C11-N4-H3A	122.9

C10-N4-H3A	122.9	Zn1-N4-H3A	58.5
O2-Cl2-O5	106.0(4)	O2-Cl2-O4	136.3(5)
O5-Cl2-O4	112.2(6)	O2-Cl2-O3	111.8(5)
O5-Cl2-O3	80.5(4)	O4-Cl2-O3	95.0(5)
O4'-Cl2'-O5'	111.0(6)	O4'-Cl2'-O3'	102.0(5)
O5'-Cl2'-O3'	144.4(6)	O4'-Cl2'-O2'	81.1(4)
O5'-Cl2'-O2'	93.1(5)	O3'-Cl2'-O2'	105.1(5)

References:

1. Nikac, M.; Brimble, A. M.; Crumbie, L. R. *Tetrahedron*. **2007**, *63*, 5220-5226.

2. Froidevaux, P.; Harrowfield, J. M.; Sobolev, N. A. *Inorg. Chem.* **2000**, *39*, 4678-4687.

3. Busto, E.; Gonzalez-Alvarez, A.; Gotor-Fernandez, V.; Alfonso, I. *Tetrahedron*. **2010**, *66*, 6070-6077.

4. Chessa, G.; Scrivanti, A. *Perkin Transactions 1*. **1996**, 307-311.

5. Siaugue, M. J.; Dioury-Segat, F.; Sylvestre, I.; Favre-Reguillon.; Foos, J.; Madic, C.; Guy, A. *Tetrahedron*. **2001**, *57*, 4713-4718.

6. Favre-Reguillon, A.; Segat-Dioury, F.; Nait-Bouda, L.; Cosma, C.; Siaugue, M. J.; Foos, J.; Guy, A. *Synlett*. **2000**, *6*, 868-870.

7. Mochizuki, K.; Sugita, T.; Kuriowa, S.; Yamada, F.; Hayano, K.; Ohgami, Y.; Suzui, M.; Kato, S. *Inorganica Chimica Acta*. **2010**, *363*, 3151-3157.

8. (a) Mensor, L. L.; Menezes, F. S.; Leitão, G. G.; Reis, A. S.; dos Santos, T. C.; Coube, C. S.; Leitão, S. G. *Phytother. Res.* **2001**, *15*, 127-130. (b) Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D. H. *J. Food Composition and Analysis* **2006**, *19*, 669-675.

9. (a) Rolinski, O. J.; Amaro, M.; Birch, D. J. S. *Biosensors and Bioelectronics* **2010**, *25*, 2249-2252. (b) Amaro, M.; Birch, D. J. S.; Rolinski, O. J. Phys. Chem. Chem. Phys. **2011**, *13*, 6434-6441. (c) Maji, S. K.; Amsden, J. J.; Rothschild, K. J.; Condron, M. M.; Teplow, D. B. *Biochemistry* **2005**, *44*, 13365-13376. (d)Yang, C. –A.; Chen, Y. –H.; Shyue-Chu, K.; Chen, Y.-R.; Huang, H.-B. Lin, Chen, Y.-C. *Int. J. Alzheimer's Disease.* **2011**, 1-7.

10. V. Félix, J. Costa, R. Delgado, M. G. B. Drew, M. T. Duarte and C. Resende, *Dalton Transactions*, 2001, 1462–1471.