Supporting Information

A Supramolecular Cu(II) Metallocyclophane Probe for Guanosine 5'–Monophosphate

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1. Experimental Techniques

General Methods. The equipment and procedures for melting point determination and spectral recordings have been described elsewhere.[1] ^{1}H and ^{13}C NMR spectra were measured on a 300 or 500 MHz Bruker advanced DPX spectrometer. ^{31}P NMR spectra were measured on a 500 MHz Bruker advanced DPX spectrometer using orthophosphoric acid as external standard. The electronic absorption spectra were recorded on a Shimadzu UV-VIS-NIR spectrophotometer. Fluorescence spectra were recorded on a SPEX-Fluorolog F112X spectrofluorimeter. MALDI-TOF MS analysis was performed with a Shimadzu Biotech Axima CFRplus instrument equipped with a nitrogen laser in the linear mode using 2,5-dihydroxybenzoicacid (DHB) as the matrix. Electrochemical measurements were carried out in a Bioanalytical Systems Inc., BAS-CV50W cyclic voltammeter. Isothermal titration calorimetry was carried out using a Micorcal VP-ITC instrument. Doubly distilled water was used in all the studies. All experiments were carried out at room temperature (25 ± 1 °C), unless otherwise mentioned.

Starting Materials. Guanosine 5'-monophosphate (5'-GMP), adenosine 5'-monophosphate (5'-AMP), adenosine 5'-triphosphate (5'-ATP), adenosine 5'-diphosphate (5'-ADP), guanosine 5'-diphosphate (5'-GDP), guanosine 5'-triphosphate (5'-GTP), guanosine 3',5'-cyclic monophosphate (3',5'-cGMP), guanosine and adenosine were purchased from Sigma Aldrich and used as received with out any further purification. Anthracene, imidazole, NaH, HBr in glacial acetic acid and $CuCl_2$ was purchased locally and used without further purification. All the solvents used were purified and distilled before use.

Calculation of association constants (K_{ass}). Nucleotide, nucleoside (Sigma Aldrich) and metallocyclophane $[\mathbf{1}.CuCl_2]_2$ solutions were prepared in distilled water and the ligand **1** in 20% DMSO-water. The binding affinities were calculated using Benesi-Hildebrand equation 1,

$$\frac{1}{(I_f - I_{ob})} = \frac{1}{(I_f - I_{fc})} + \frac{1}{K(I_f - I_{fc})[\text{Ligand}]} \quad \text{eq 1}$$

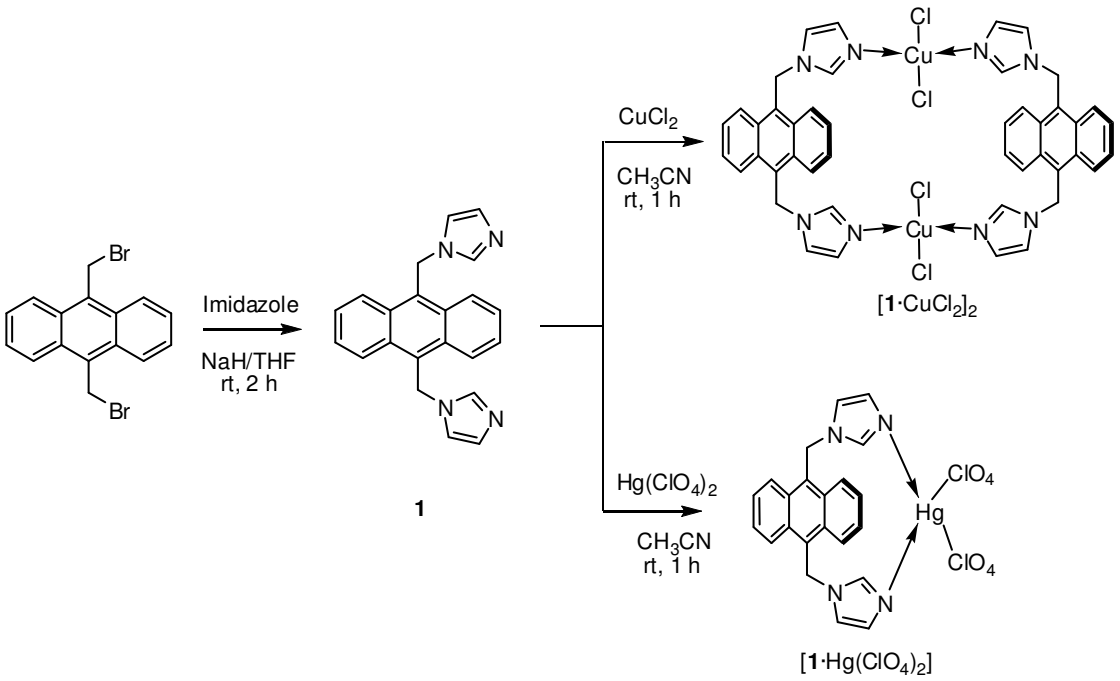
where, K is the equilibrium constant, I_f is the fluorescence of ligand **1**, I_{ob} is the observed fluorescence in the presence of various ligands and I_{fc} is the fluorescence at saturation. The linear dependence of $1/(I_f - I_{ob})$ on the reciprocal of the ligand concentration indicates the formation of a 1:1 molecular complex between ligand and the host.

2. Synthesis of ligand 1 and complexes $[\mathbf{1}.CuCl_2]_2$ and $[\mathbf{1}.Hg(ClO_4)_2]$

Synthesis of the ligand **1** and the complexes $[\mathbf{1}.CuCl_2]_2$ and $[\mathbf{1}.Hg(ClO_4)_2]$ were achieved as outlined in Scheme 1.

Synthesis of 9,10-bis(imidazolylmethyl)anthracene (**1**). To a reaction solution of imidazole (500 mg, 7.4 mmol) in dry THF (100 mL) was added NaH (330mg, 13.8 mmol) at 0 ºC. After the reaction mixture was stirred for 30 min at 0 ºC, 9,10-bis(bromomethyl)-anthracene (500 mg, 1.4 mmol) was added. The reaction mixture was then additionally stirred for 2 h at room temperature, it was poured into 100 mL of water and extracted with dichloromethane. The organic layer was then separated, dried over anhydrous sodium sulfate and evaporation of the solvent under vacuum yielded a residue which was purified by column chromatography over silica gel. Elution of the column with ethyl acetate yielded 370 mg (78%) of the ligand **1**, which was then re-crystallized from a mixture of acetonitrile and ethyl acetate (4:1); mp 246-247 ºC; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 6.30 (s, 4H), 6.78 (s,

2H), 6.94 (s, 2H), 7.68 – 7.74 (m, 6H), 8.62 – 8.64 (m, 4H); ^{13}C NMR (125.75 MHz, DMSO-d_6, TMS) δ 41.98, 119.00, 124.76, 126.79, 128.23, 129.01, 130.09, 136.9; HRMS (FAB): *m/z* Calcd for $C_{22}H_{19}N_4$: 339.4132; found 339.4208 $(M + H)^+$.



Scheme 1

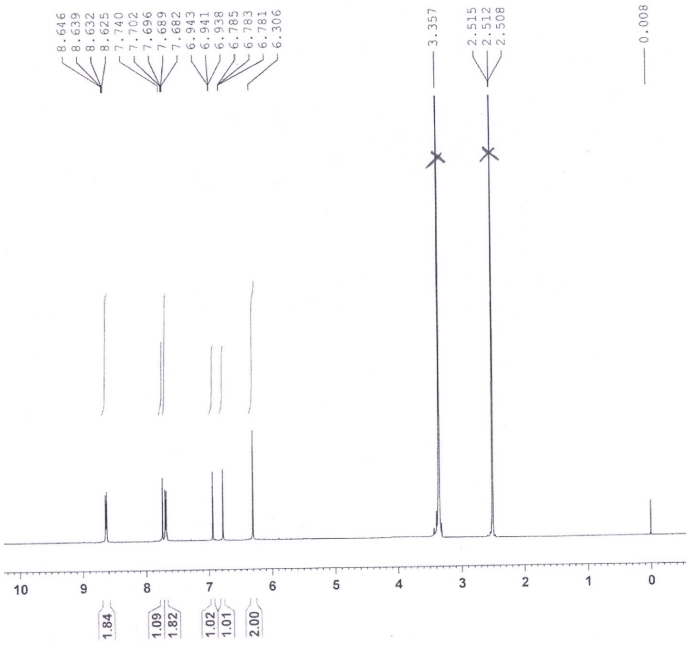
*Synthesis of metallocyclophane [**1**.$CuCl_2$]$_2$.* To a solution of 9,10-bis(imidazolyl-methyl) anthracene (**1**, 250 mg, 0.74 mmol) in a mixture of acetonitrile (50 mL) and methanol (5 mL) was added $CuCl_2$ (100 mg, 0.74 mmol). As shown above, the reaction of the anthracene-imidazole conjugate **1** with one equivalent of $CuCl_2$ in methanol-acetonitrile mixture at room temperature readily gave the metallocyclophanes in moderate yields. It was further purified by re-crystallization from acetonitrile to yield 124 mg (17%) of the metallocyclophane [**1**.$CuCl_2$]$_2$; MALDI-TOF MS: *m/z* calcd for $[C_{44}H_{36}Cu_2Cl_4N_8]$: 945.7; found 944.67 $(M - H)^+$.

*Synthesis of metallocyclophane [**1**.$Hg(ClO_4)_2$].* To a solution of 9,10-bis(imidazolyl-methyl)anthracene (**1**, 250 mg, 0.74 mmol) in a mixture of acetonitrile (50 mL) and methanol (5 mL) was added $Hg(ClO_4)_2$ (120 mg, 0.74 mmol). As shown above, the reaction of the anthracene-imidazole conjugate **1** with one equivalent of $Hg(ClO_4)_2$ in methanol-acetonitrile mixture at room temperature readily gave the metallocyclophanes in moderate yields. It was further purified by re-crystallization from acetonitrile to yield 112 mg (15%) of the metallocyclophane [**1**.$Hg(ClO_4)_2$]; MALDI-TOF MS: *m/z* calcd for $[C_{22}H_{18}Cl_2HgN_4O_8]$: 738.02; found 737.4 $(M - H)^+$.

References

1 R. R. Avirah, K. Jyothish, D. Ramaiah, *J. Org. Chem.* 2008, **73**, 274; K. Jyothish, M. Hariharan, D. Ramaiah, *Chem. Eur. J.* 2007, **13**, 5944.

A)



B)

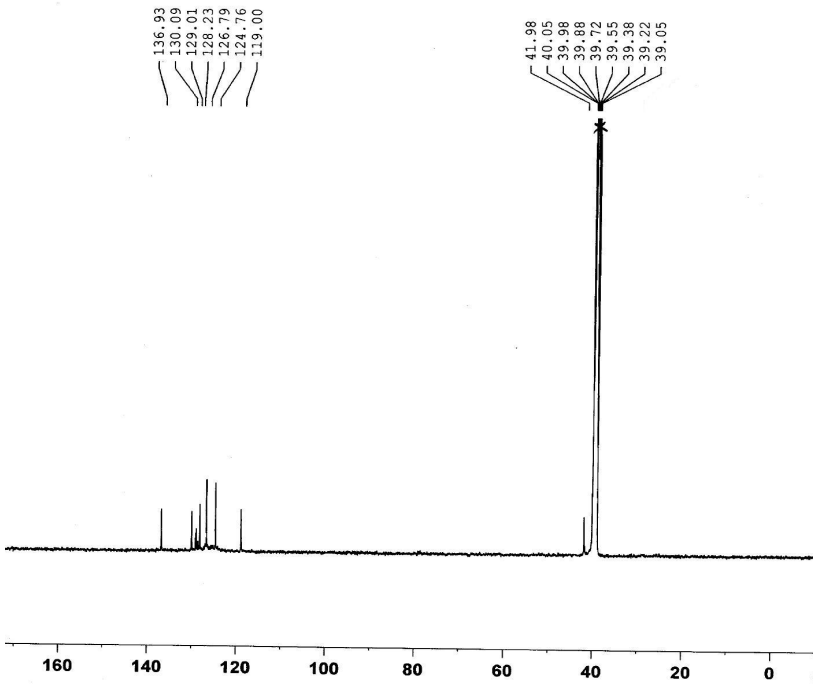


Fig. S1 A) ^{1}H and B) ^{13}C NMR spectra of the ligand **1** in DMSO–d_6.

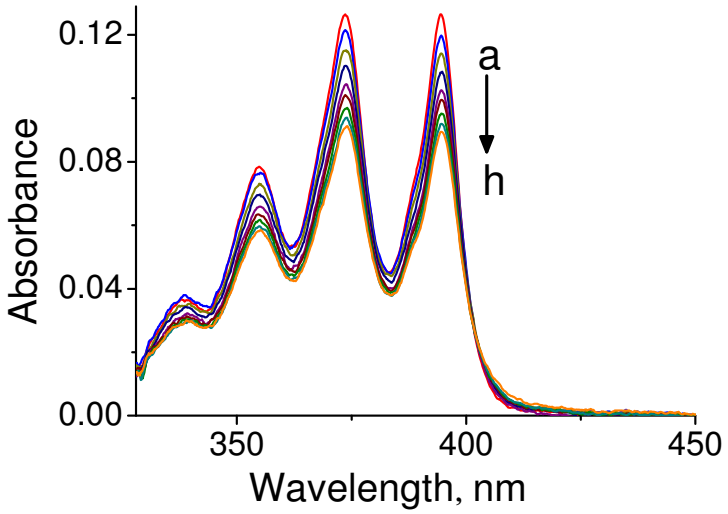


Fig. S2 Changes in the absorption spectrum of the ligand **1** (20 μM) with the addition of $CuCl_2$ in 20% DMSO–water. [$CuCl_2$], a) 0, and h) 156 μM.

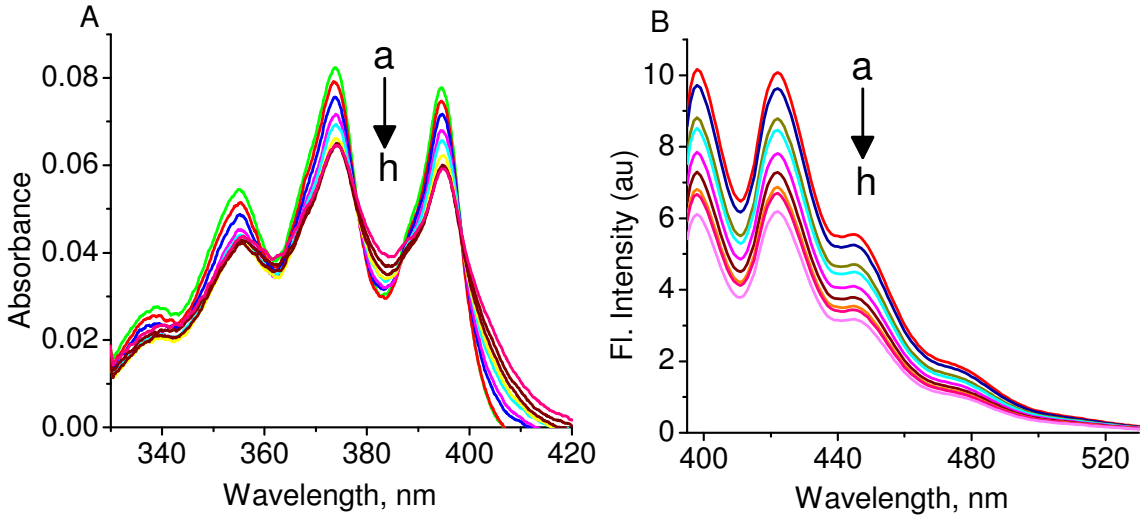


Fig. S3 Changes in the A) absorption and B) emission spectra of ligand **1** with the addition of $Hg(ClO_4)_2$. [$Hg(ClO_4)_2$] (a) 0, and (h) 156 μM. λ_{ex}, 379 nm.

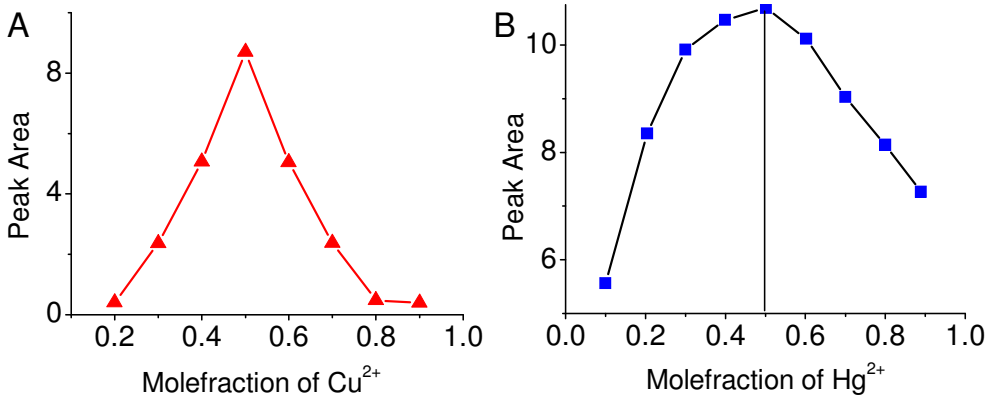


Fig. S4 Job's plot for the binding of A) $CuCl_2$ and B) $Hg(ClO_4)_2$ with the ligand **1** in 20% DMSO-water.

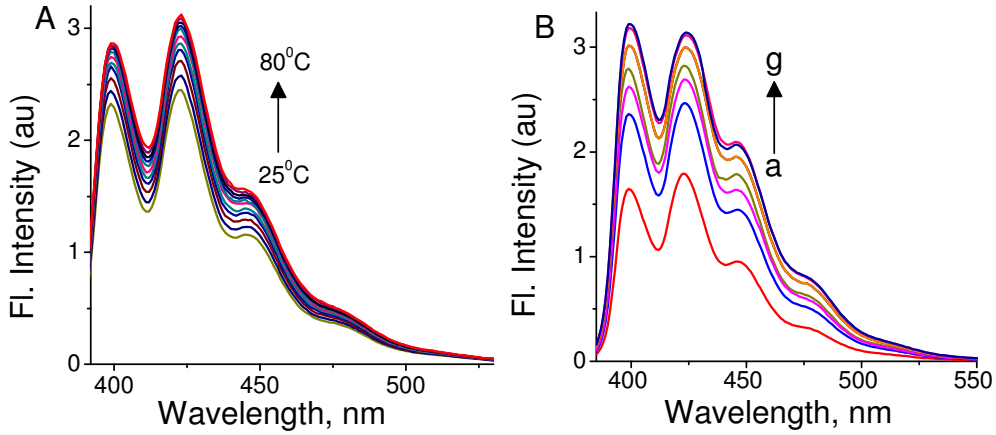
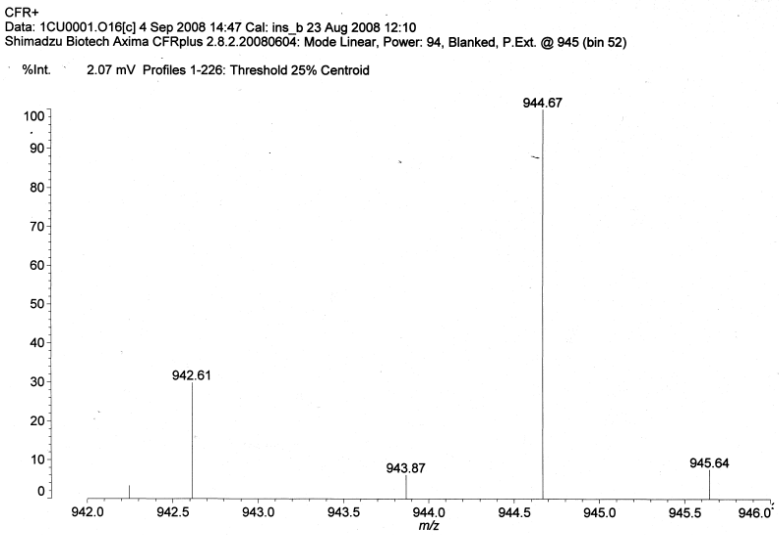


Fig. S5 Changes in the emission spectrum of $[\mathbf{1}.CuCl_2]_2$ with A) increasing temperature and B) addition of EDTA. [EDTA] (a) 0, and (g) 625 μM. λ_{ex}, 379 nm.

A)



B)

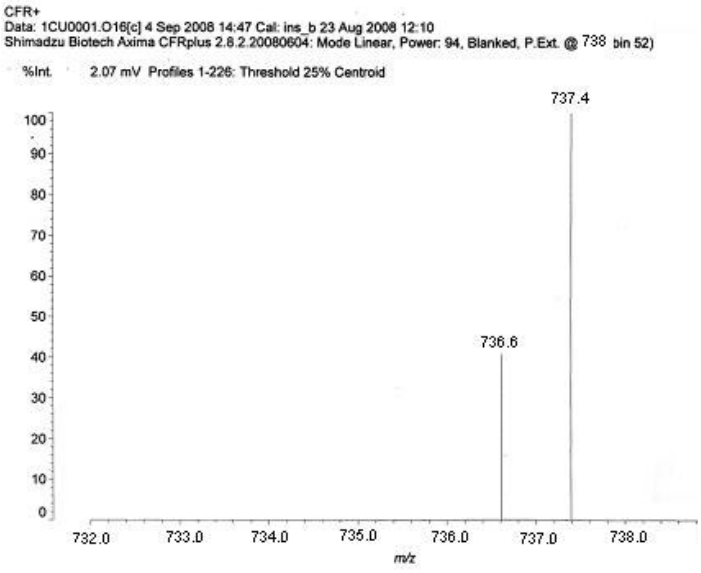


Fig. S6 MALDI-TOF mass spectra of the metallocyclophane A) [**1**.$CuCl_2$]$_2$ and B) the complex [**1**.$Hg(ClO_4)_2$].

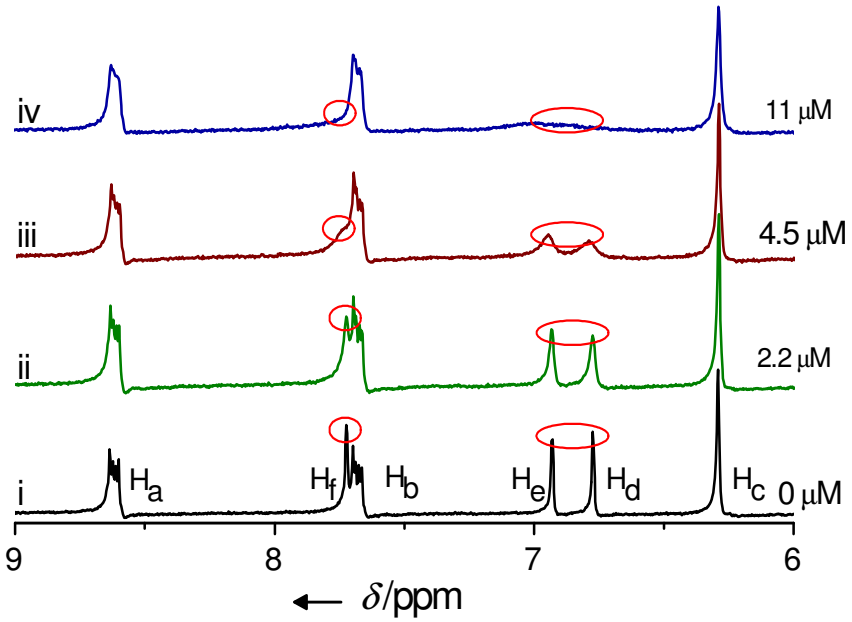


Fig. S7 Changes in the ^{1}H NMR spectrum of the ligand **1** (4.9 mM) with the addition of $CuCl_2$ in DMSO-d_6.

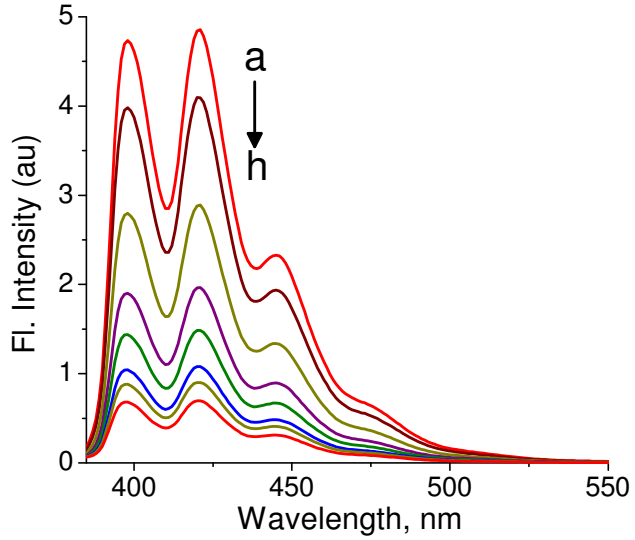


Fig. S8 Changes in the fluorescence intensity of [**1**.$CuCl_2$]$_2$ with the addition of 5'-GMP in the aqueous medium. [5'-GMP], a) 0 and h) 625 μM. λ_{ex}, 379 nm.

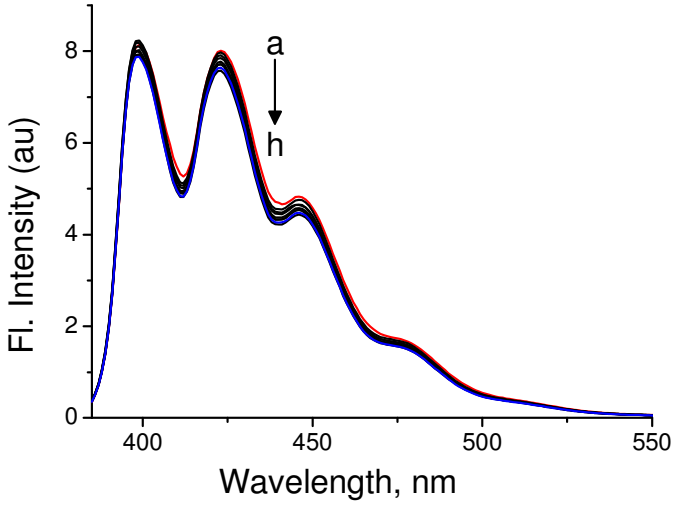


Fig. S9 Changes in the fluorescence intensity of [**1**.Hg(ClO_4)$_2$] with the addition of 5'-GMP. [5'-GMP], a) 0 and h) 625 μM. λ_{ex}, 379 nm.

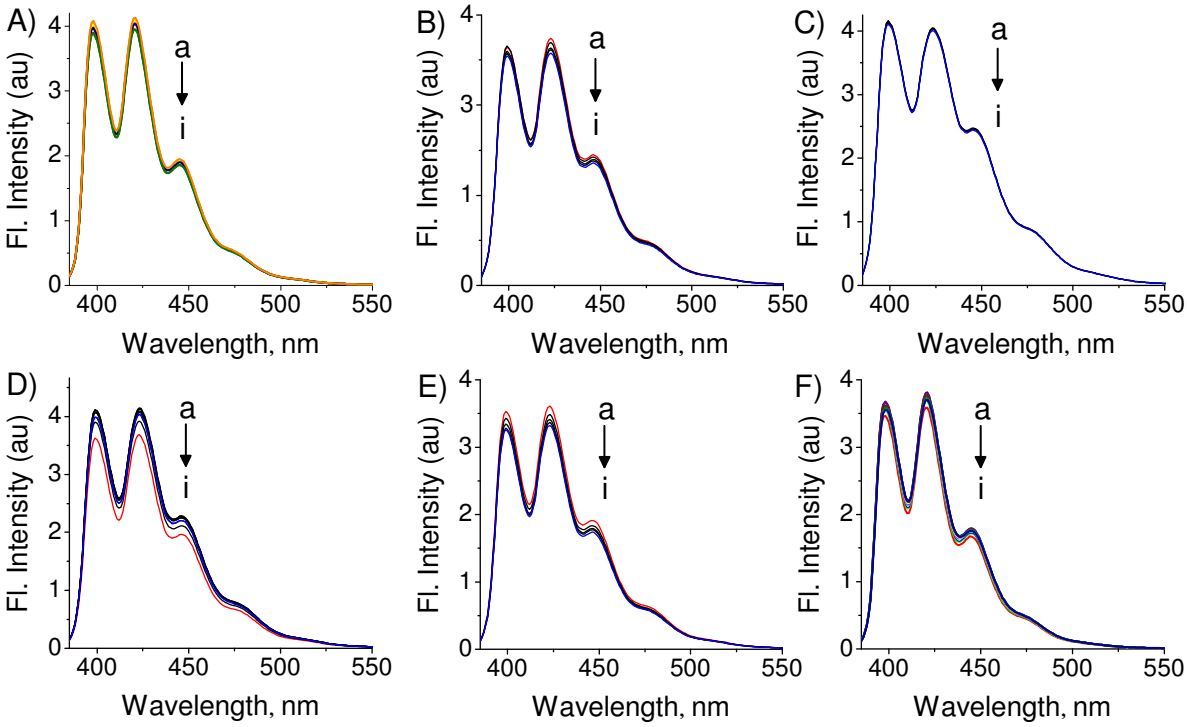


Fig. S10 Changes in the fluorescence spectrum of the metallocyclophane [**1**.$CuCl_2$]$_2$ with the addition of A) 5'-AMP, B) adenosine, C) 5'-ATP, D) 5'-GDP, E) guanosine and F) 5'-GTP in the aqueous medium. [Ligand], (a) 0, and (i) 625 μM. λ_{ex}, 379 nm.

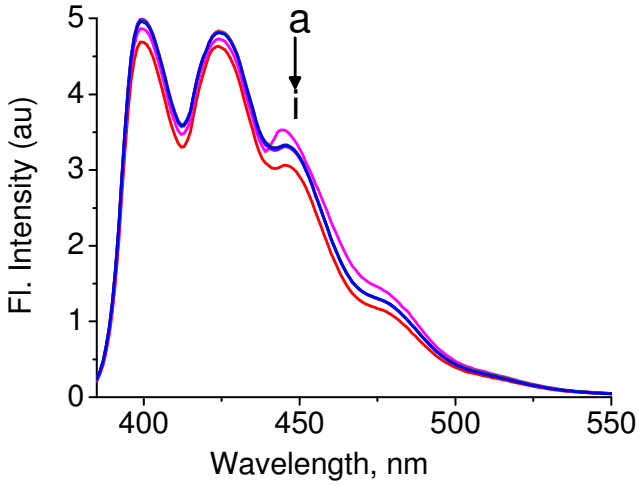


Fig. S11 Changes in the fluorescence spectrum of the metallocyclophane [**1**.$CuCl_2$]$_2$ with the addition of 3',5'-cGMP. [Ligand], (a) 0, and (i) 625 μM. λ_{ex}, 379 nm.

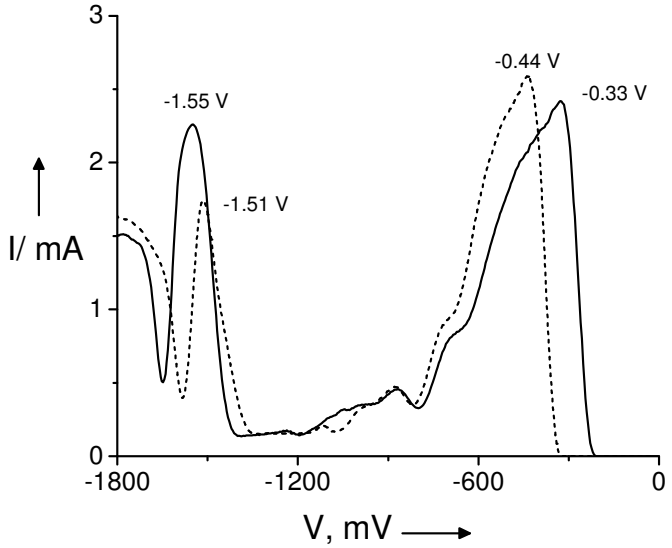


Fig. S12. Differential pulse voltammogram of the metallocyclophane [**1**.$CuCl_2$]$_2$ (0.98 mM) in the absence (——) and presence (- - -) of 5'-GMP (20 μM) in the aqueous medium.

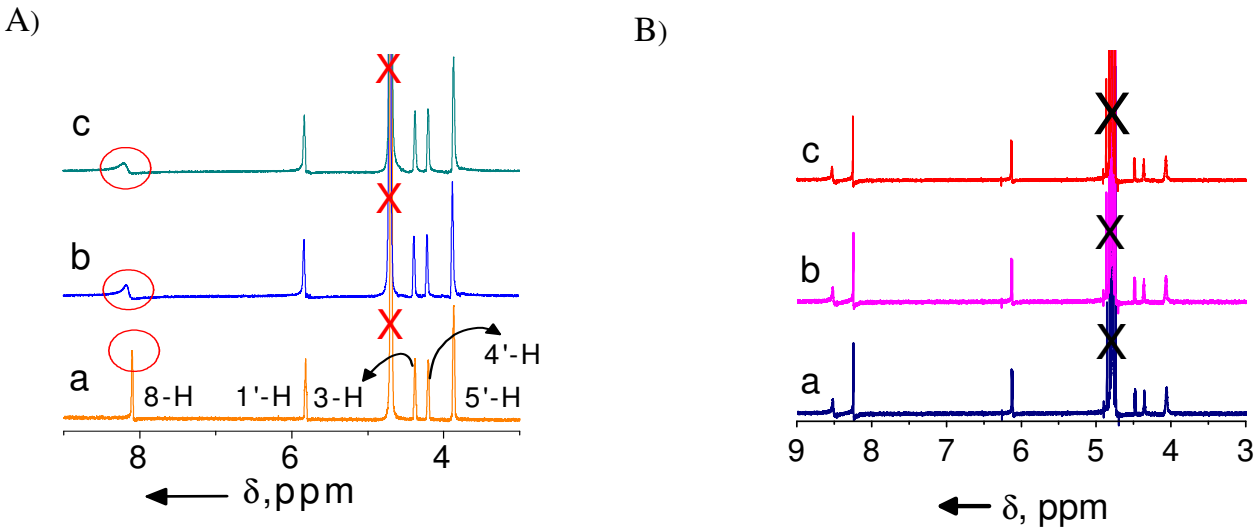


Fig. S13 Changes in the ^{1}H NMR spectra of A) 5'-GMP (10 mM) and B) 5'-AMP (10 mM) with the addition of the metallocyclophane [**1**.$CuCl_2$]$_2$ in D_2O. [**1**.$CuCl_2$]$_2$, a) 0, b) 3.2, and c) 15.8 μM.

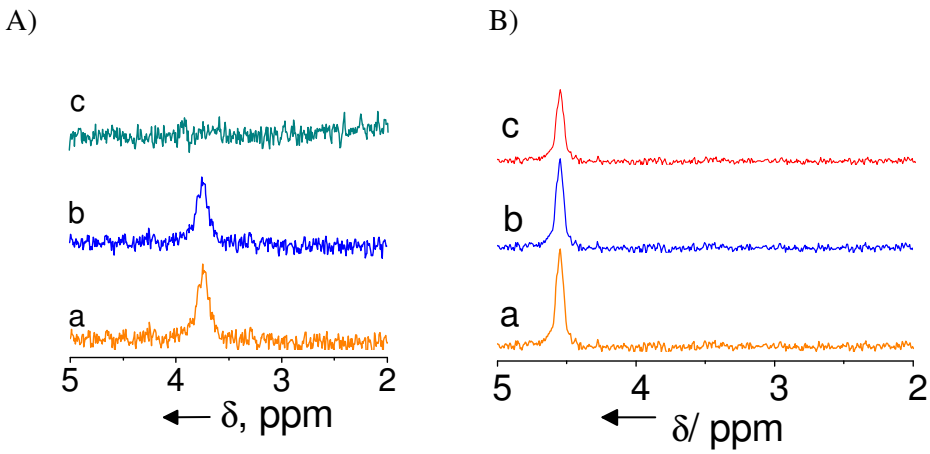


Fig. S14 Changes in the ^{31}P NMR spectra of the A) 5'-GMP (10 mM) and B) 5'-AMP (10 mM) with the addition of [**1**.$CuCl_2$]$_2$in D_2O. [**1**.$CuCl_2$]$_2$, a) 0, b) 7.4 and c) 15.8 μM.