Electronic supplementary information

A supramolecular approach to metal ion sensing: Cystine-based designed systems for Cu$^{2+}$, Hg$^{2+}$, Cd$^{2+}$ and Pb$^{2+}$ sensing

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Experimental Section

Materials and General Instrumentation: All reagents were used without further purification. All solvents employed in the reactions were distilled or dried from appropriate drying agents prior to use. Progress of reactions was monitored by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and silica gel with 60-120 mesh used for column chromatography. Melting points were recorded on a Fisher-Scientific melting point apparatus and were uncorrected. IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets. $^1$H NMR spectra were recorded on Brucker-DPX-300 spectrometer using tetramethylsilane ($^1$H) as an internal standard. Coupling constants are reported in Hz and the $^1$H NMR data are reported as s (singlet), d (doublet), br (broad), t (triplet) and m (multiplet), dd (double doublet). High resolution mass spectra (HRMS) were recorded in Bruker MicrO-TOF- QII model using ESI technique and AB Sciex, 1011273/A model using ESI-technique. UV-Visible spectra were recorded in Shimadzu double beam spectrophotometer, UV-2400. The emission spectra were recorded in HORIBA JOBIN YVON Scientific, fluoromax-4 spectrofluorometer with a slit-width of 5 nm. Lifetime measurements were conducted in IBH-Time correlated Single Photon Counting System with slit-width of 5 nm. Circular dichroism (CD) spectra were recorded on AVIV Model 410 spectrometer using a quartz cell of 1 mm path length. The samples were prepared in acetonitrile with a concentration of 500 µM. The CD spectra were recorded with a scan speed of 1 nm/min and averaged over 3-6 scans. The thermodynamic parameters were estimated by MicroCal iTC$_{200}$ system at 298 K in acetonitrile. Sample solution (S1) of 200µM was taken and 50µM titrant (S2) was taken and delivered over 25 s with an interval of 40 minutes. Roughly 4 equiuvalents of titrant was added to the sample solution. The nonlinear least
square algorithm and the concentration of the sample solution was used to determine the best fit values of change in enthalpy ($\Delta H$), change in entropy ($\Delta S$) and binding constant ($K$).

**Preparation of S1:** To an ice cooled solution of L-Bis Boc-cystine (450 mg, 1.02 mmol) in dry CH$_2$Cl$_2$; N-hydroxysuccinimide (NHS) (242 mg, 2.1 mmol); dicyclohexycarbodiimide (DCC) (432 mg, 2.1 mmol) were added. After 5 minutes, 1-Pyrenemethylamine (560 mg, 2.1 mmol) and triethylamine (0.3 mL, 2.1 mmol) were added and reaction was stirred for 24 h. Reaction mixture was filtered and the organic phase was washed sequentialy with 0.2 N H$_2$SO$_4$, NaHCO$_3$ solution and finally with water. The filtrate was dried over Na$_2$SO$_4$, evaporated and purified by column chromatography using ethyl acetate and hexane as eluents to yield 450 mg (50%) of the compound.

m.p : 176-178 °C

$^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$: 1.27 (s, 18H), 2.82 (dd, $J = 15$Hz, $J = 3$Hz, 2H), 3.05 (dd, $J = 15$ Hz, $J = 3$ Hz, 2H), 4.24 (br m, 2H), 4.88 (br m, 4H), 7.11 (d, $J = 9$ Hz, 2H), 7.91 - 8.30 (m, 18H), 8.65 (t , $J = 6$ Hz, 2H).

$^{13}$C NMR(CDCl$_3$,75 MHz) $\delta$ : 29.7, 42.0, 47.4, 54.3, 79.7, 122.8, 124.3, 124.7, 125.0, 125.3, 126.0, 127.2, 127.4, 127.5, 128.0, 129.1, 130.7, 131.1, 155.6, 170.1.

IR (KBr) : 3426, 2926, 2851, 1689, 1628, 1574, 1529, 1440 cm$^{-1}$.

HRMS: Calculated for C$_{50}$H$_{50}$N$_4$O$_6$S$_2$Na 889.3069, obtained 889.3057.

**Preparation of S2:** To an ice cooled solution of L-Bis Boc-cystine (500 mg, 1.1 mmol) in dry CH$_2$Cl$_2$; NHS (287 mg, 2.4 mmol); DCC (515 mg, 2.4 mmol) were added. After 5 minutes, L-Tryptophan methyl ester (636 mg, 2.4 mmol) and triethylamine (0.34 mL, 2.4 mmol) were added and the reaction was stirred for 24 h. Reaction mixture was filtered, and the organic phase was washed sequentialy with 0.2 N H$_2$SO$_4$, NaHCO$_3$ solution and finally with water. The filtrate
was dried over Na$_2$SO$_4$, evaporated and purified by column chromatography using ethyl acetate and hexane as eluents to yield 550 mg (59%) of the compound.

m.p : 133-135 °C

$^1$H NMR (CDCl$_3$, 300 MHz) δ: 1.41 (s, 18H), 2.82 (dd, $J = 15$ Hz, $J = 3$ Hz, 2H), 2.98 (dd, $J = 15$ Hz, $J = 3$ Hz, 2H), 3.20 (br m, 2H), 3.35 (br m, 2H), 3.67 (s, 6H), 4.61 (m, 2H), 4.86 (m, 2H), 5.41 (d, $J = 9.6$ Hz, 2H), 6.94 (s, 2H), 7.12 (m, 4H), 7.23 (m, 2H), 7.54 (m, 4H), 8.25 (brs, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz) δ: 27.4, 28.2, 44.2, 52.3, 52.9, 54.1, 80.3, 109.8, 111.3, 118.3, 119.4, 122.0, 123.0, 127.3, 136.1, 155.6, 170.3, 172.0.

IR (KBr) : 3338 (br), 3055, 2975, 2927, 2360, 1740, 1665, 1517, 1447, 1366, 1248, 1166, 1105 cm$^{-1}$.

HRMS: Calculated for C$_{40}$H$_{52}$N$_6$O$_{10}$S$_2$Na 863.3084, obtained 863.3074.

**Preparation of S3:** To a solution of S$_2$ (100 mg, 0.11 mmol) in EtOH, NaBH$_4$ (11 mg, 0.35 mmol) was added and the reaction mixture was kept stirred in an inert atmosphere (Argon gas). The course of the reaction was monitored by TLC. After complete reduction, S$_1$ (95 mg, 0.11 mmol) in EtOH was added and kept stirred in oxygen atmosphere at room temperature for 6 h. The reaction mixture was evaporated, residue was re-dissolved in dichloromethane, washed with water and purified by column chromatography using MeOH/CHCl$_3$ as the eluents to obtain 30 mg (31%) of the product.

m.p : 220-222 °C

$^1$H NMR (CDCl$_3$, 300 MHz) δ: 1.46 (s, 18H), 2.80-3.10 (m, 4H), 3.17-3.55 (m, 4H), 3.71 (s, 3H), 4.70 (m, 1H), 4.89 (br m , 2H), 5.38 (br m, 2H), 6.96 (s, 2H), 7.10 (m, 4H), 7.22 (s, 1H), 7.54 (d, $J = 6$ Hz, 2H), 7.90-8.35 (m, 4H).
\[^{13}\text{C}\] NMR (DMSO-\textit{d}_6, 75 MHz) \(\delta\): 24.3, 25.2, 26.9, 28.0, 33.2, 47.4, 51.8, 53.0, 53.7, 78.2, 108.9, 111.3, 117.8, 118.2, 120.8, 122.9, 123.5, 123.8, 124.4, 125.0, 126.0, 126.8, 126.9, 127.2, 127.3, 127.8, 129.9, 130.1, 130.6, 132.3, 136.0, 155.1, 170.2, 171.8.

IR (KBr): 3414 (br), 2966, 2928, 2858, 1756, 1662 (br), 1527, 1458, 1369, 1229, 1168 cm\(^{-1}\).

HRMS: Calculated for C\(_{45}\)H\(_{51}\)N\(_5\)O\(_8\)S\(_2\)Na 876.3077, obtained 876.3068.
**Steady-state fluorescence experiments on S1 and S3**

Solutions of S1 with varying concentrations were made in CH₃CN and fluorescence spectra were recorded using a steady-state fluorescence spectrophotometer with a slitwidth of 5 nm. The ratio of the intensity for the excimer band (I₄₈₀) and monomer band (I₃₈₀) were calculated and plotted against concentration of S1.

S3 is titrated with Cd²⁺ ion solution and the ratio of the intensity for the excimer band (I₄₈₀) and monomer band (I₃₉₀) were calculated and plotted against concentration of S3.

![Figure S1: (a) Concentration vs I₄₈₀/I₃₈₀ of S1 (b) Concentration vs I₄₈₀/I₃₈₀ of S3 upon addition of Cd(II).](image)

**Excitation spectra of S1 and S3**

The ground state dimer formation in S1 was proved by the excitation spectra intensities of excimer (I₄₈₀ nm) and monomer intensity (I₃₈₀ nm). Excitation spectra was recorded on a steady-state fluorescence spectrophotometer with a slitwidth of 5 nm. The emission intensities were plotted against the wavelength. The redshift in emission intensity indicates the ground state dimer formation in S1.

The complex formation for S3 with Cd²⁺ was confirmed by the excitation spectra. Excitation spectra was recorded on a steady-state fluorescence spectrophotometer with a slitwidth of 5 nm.
The emission intensities were plotted against the wavelength. The redshift in emission intensity indicates the complex formation in S3 with Cd\(^{2+}\) and which is the static type complex.

**Figure S2:** (a) Excitation spectra of S1 (b) Excitation spectra of S3 upon Cd(II) complexation

**Metal ion binding studies using fluorescence spectroscopy**

Stock solutions of compounds S1, S2 and S3 with a concentration of \(10^{-5}\) M were made in acetonitrile. Stock solutions of metal perchlorates (\(10^{-5}\) M) were made in acetonitrile. The compounds were titrated against the metal ions and the emission spectra were recorded using HORIBA JOBIN YVON Scientific, fluoromax-4 spectrofluorometer with slitwidth of 5 nm. The titration was continued till a saturation point was observed.
Figure S3: (a) Fluorescence spectral studies of S1 with various metal ions in CH$_3$CN, the results obtained are for 4 equivalents of Cu$^{2+}$ and Hg$^{2+}$ and for 20 equivalents of other metal ions, $\lambda_{ex} = 340$ nm (b) Fluorescence spectral studies of S2 in CH$_3$CN, 14 equivalents other metal ions and 3 equivalents of Cu$^{2+}$ and Hg$^{2+}$, $\lambda_{ex} = 290$ nm (c) Fluorescence spectra of S3 in CH$_3$CN, S3 + 10 equivalents of Cd$^{2+}$, S3 + other metal ions 30-50 equivalents, $\lambda_{ex} = 290$ nm (d) Fluorescence spectra of S1:S2 in CH$_3$CN, S1:S2 + 2 equivalents of Pb$^{2+}$, S1:S2 + 20 equivalents of other metal ions, $\lambda_{ex} = 290$ nm.

Metal ion binding studies using fluorescence spectroscopy

The interactions of S1 and S2 with Cu$^{2+}$ and Hg$^{2+}$ was studied with the help of Stern-Volmer plot.

$$I_0/I = 1 + KQ$$

Where $I$ and $I_0$ are the fluorescence intensity with and without the metal ions; $Q$ is the metal ion concentration; $K$ is the quenching/binding constant.

The binding constant ($K_{21}$) for 2:1 binding of receptor S3 with Cd$^{2+}$ was determined by equation

$$2H + G \rightleftharpoons GH_2$$

$$[G]_{tot} = \frac{a}{2K_{21}(1-a)^2[H]_{tot}} + \frac{a[H]_{tot}}{2}$$

(1)

$[H_{tot}]$ and $[G_{tot}]$ denote the total concentrations of S3 and Cd$^{2+}$, and the term $a$ is the ratio of change in fluorescent intensity ratio ie. $(I - I_0)/(I_i - I_0)$, where $I$ is the fluorescence intensity at added metal ion, and $I_0$ and $I_i$ are the intensity at zero and infinite cation concentrations.

The association constant for the binding of S1:S2 with Pb$^{2+}$ was determined by fluorescence spectroscopy using Benesi-Hildebrand method.
\[
\frac{1}{I_0 - I} = \frac{1}{(I_0 - I_\infty)K[G]} + \frac{1}{(I_0 - I_\infty)}
\]

Where \( I \) and \( I_0 \) are the fluorescence intensities of \( S1:S2 \) with and without \( \text{Pb}^{2+} \) and \( I_\infty \) is the fluorescence intensity for infinite dilution of \( S1:S2 \) with \( \text{Pb}^{2+} \).

**Figure S4:** (a) Stern-Volmer plot for \( S1 \) with \( \text{Cu}^{2+} \) (b) Stern-Volmer plot for \( S2 \) with \( \text{Hg}^{2+} \) (c) Benesi-Hildebrand plot for \( S1:S2 \) with \( \text{Pb}^{2+} \) (d) Benesi-Hildebrand plot for \( S1 \) with \( \text{Cu}^{2+} \) (e) Benesi-Hildebrand plot for \( S2 \) with \( \text{Hg}^{2+} \).

**Comparison of metal ion binding by fluorescence spectroscopy**

An equimolar solutions of \( S1, S2, S3 \) and \( S1:S2 \) as well as metal ions (\( 10^{-5} \) M) were prepared in \( \text{CH}_3\text{CN} \) and the emission intensity of the receptors with \( (I) \) and without \( (I_0) \) metal ions were recorded with steadystate fluorescence. The change in emission intensity \( (I-I_0) \) and \( (I_0-I) \) were calculated and the metal response was found by plotting change in intensity with metal ions.
Figure S5: Selectivity study of (a) S1, (b) S2, (c) S3 and (d) S1:S2 towards various metal ions. The binding was studied by steady-state fluorescence spectroscopic analysis.

**Time resolved fluorescence studies on S1-Cu^{2+} complex**

The dynamic nature of complex formation between S1 and Cu^{2+} was confirmed from the time-resolved fluorescence experiment. The ratio of lifetimes with (\(\tau_0\)) and without (\(\tau\)) Cu^{2+} ion was calculated and plotted against concentration of Cu^{2+}. The ratio of \(\tau_0/\tau\) vs concentration of Cu^{2+} was found to be concentration dependent indicating the dynamic nature. Similarly the ratio of fluorescence intensity without and with Cu^{2+} (\(F_0/F\)) was also found to be concentration dependent of Cu^{2+}.

![Figure S5](image)

Figure S6: The plot of \(\tau_0/\tau\) and \(F_0/F\) against Cu(II) concentration.

**Circular dichorism studies of S1, S2, S3 and S1:S2 with and without metal ions**

Circular dichroism (CD) spectra were recorded on AVIV Model 410 spectrometer using 1 mm length cell. The samples were prepared in acetonitrile with a concentration of 500 \(\mu\)M. The spectra were recorded with a scan speed of 1 nm/min and averaged over 3-6 scans. Metal ion solutions were made in the respective compound solution.
**Figure S7:** CD Spectra of (a) S1 alone and with 3 equivalents of Cu$^{2+}$ (b) S2 alone and with 3 equivalents of Hg$^{2+}$ (c) S3 alone and with 3 equivalents of Cd$^{2+}$ (d) S1:S2 alone and with 3 equivalents of Pb$^{2+}$.

**Determination of stoichiometry of complex**

A series of solutions containing compounds (S1, S2, S3) (1x10$^{-5}$ M) and metal ions (1x10$^{-5}$ M) were prepared in CH$_3$CN. The molefraction of the receptors were varied from 0.1 to 1 and the changes in absorbance/emission intensity after correction factor were plotted against the molefraction.
Figure S8: Job’s plot of (a) for S1 with Cu$^{2+}$ (b) S2 with Hg$^{2+}$ (c) S1 and S2 (d) S1:S2 with Pb$^{2+}$ (e) S3 with Cd$^{2+}$.

Monitoring the color changes upon complex formation of S2 and S3 with various metal ions.

Solutions of compounds and metal salts in CH$_3$CN were prepared in concentration $\sim 10^{-5}$ M concentration. Five equivalents of metal salts was added to each compound and monitored the changes in colour.

Figure S9: Colorimetric response of S2 and S3 with various metal ions (a) for S2 (b) for S3.
NMR titration

The involvement of indole moieties in the metal ion binding was confirmed by $^{13}$C NMR in CD$_3$CN. A solution of S2 (500 μM) in CD$_3$CN was prepared and $^{13}$C NMR was recorded. S2 was added to a solution of Hg (ClO$_4$)$_2$ in CD$_3$CN (500 μM) and $^{13}$C NMR was recorded.

**Figure S10:** $^{13}$C NMR (75 MHz) in CD$_3$CN (i) S2 alone (ii) S2 with 5 equivalents of Hg$^{2+}$. The red dotted lines represent the carbons that show maximum shift upon Hg$^{2+}$ binding.

**Table S1. Comparison of $^{13}$C NMR Chemical shift values of S2 with and without Hg$^{2+}$.**

Highlighted carbon atoms show change in chemical shift values upon Hg$^{2+}$ complexation.

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<th>System</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
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<tr>
<td>S2</td>
<td>28.53</td>
<td>80.59</td>
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<td>54.91</td>
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<td>171.82</td>
<td>54.22</td>
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**Time-resolved fluorescence data for S1, S2 and S1:S2 with metal ions**

Time-resolved fluorescence experiments were performed by using an IBH picosecond single photon-counting system employing an adjustable nano-LED excitation source and a Hamamatsu C4878-02 micro channel plate (MCP) detector with a slitwidth of 5 nm. Data were collected at room temperature with a 4 mm optical path fluorescence cell filled with the compounds. The decay time was calculated by the linear least square fit method and single exponential fit was observed for Trp excitation at 290 nm and double exponential fit for Pyrene excitation at 340 nm. Both compounds and metal ions were prepared in CH$_3$CN with a concentration of 10$^{-5}$M.

**Figure S11:** Time-resolved fluorescence spectra (a) S1 upon adding various equivalents of Cu$^{2+}$, $\lambda_{ex} = 340$ nm, collection at 474 nm (b) S2 with various equivalents of Hg$^{2+}$, $\lambda_{ex} = 290$ nm, collection at 330 nm (c) S1:S2 with various equivalents of Pb$^{2+}$, $\lambda_{ex} = 290$ nm, collection at 330 nm.

**Table S2:** Parameters calculated from time-resolved fluorescence spectra of S1 alone and with Cu$^{2+}$

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<th>Compounds</th>
<th>$\lambda_{ex}$</th>
<th>$\lambda_{collection}$</th>
<th>$T_1$ (ns)</th>
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<td>474 nm</td>
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<td>S1+1eq. Cu(II)</td>
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<td>86.77</td>
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<td>13.34</td>
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Table S3: Parameters calculated from time-resolved fluorescence spectra of S2 alone and with Hg$^{2+}$.

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<td>290 nm</td>
<td>330 nm</td>
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<td>100</td>
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<td>S2 + 1eq. Hg(II)</td>
<td>290 nm</td>
<td>330 nm</td>
<td>0.99</td>
<td>100</td>
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<td>S2 + 2eq. Hg(II)</td>
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<td>330 nm</td>
<td>1.0</td>
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<td>S2 + 3eq. Hg(II)</td>
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<td>330 nm</td>
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Table S4: Parameters calculated from time-resolved fluorescence spectra of S3 alone and with Cd$^{2+}$.

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<tr>
<td>S3</td>
<td>290 nm</td>
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<td>474 nm</td>
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<td>S3 + 1eq. Cd(II)</td>
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<td>474 nm</td>
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<td>S3 + 1eq. Pb(II)</td>
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**Table S5:** Parameters calculated from time-resolved fluorescence spectra of S1:S2 alone and with Pb^{2+}.

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<tr>
<th>Compounds</th>
<th>( \lambda_{\text{ex}} ) (nm)</th>
<th>( \lambda_{\text{collection}} ) (nm)</th>
<th>( \tau_1 ) (ns)</th>
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<th>A2</th>
<th>( \chi^2 )</th>
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<td>100</td>
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<td>0.98</td>
</tr>
<tr>
<td>S1:S2</td>
<td>290</td>
<td>474</td>
<td>3.2</td>
<td>15.32</td>
<td>7.94</td>
<td>92.06</td>
<td>1.01</td>
</tr>
<tr>
<td>S1:S2 + 1eq. Pb(II)</td>
<td>290</td>
<td>330</td>
<td>0.41</td>
<td></td>
<td>100</td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>S1:S2 + 3eq. Pb(II)</td>
<td>290</td>
<td>330</td>
<td>0.38</td>
<td></td>
<td>100</td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>S1:S2 + 1eq. Pb(II)</td>
<td>290</td>
<td>474</td>
<td>3.2</td>
<td>15.32</td>
<td>6.14</td>
<td>93.86</td>
<td>1.01</td>
</tr>
<tr>
<td>S1:S2 + 3eq. Pb(II)</td>
<td>290</td>
<td>474</td>
<td>3.2</td>
<td>15.30</td>
<td>3.23</td>
<td>96.77</td>
<td>1.1</td>
</tr>
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</table>

**ESI-HRMS** showing the 2:1 complex of S3 with Cd\(^{2+}\) and the heterodimeric complex of S1 and S2.

Compound S3 (500 \( \mu \text{M} \)) and Cd(ClO\(_4\))\(_2\) (500 \( \mu \text{M} \)) solutions were made in CH\(_3\)CN and 5 equivalents of Cd(ClO\(_4\))\(_2\) solution was added to S3 and the resulting solution was analysed by ESI technique.

![Figure S12: ESI-HRMS of (a) S3-Cd(II) complex (b) S1:S2 (c) S1:S2-Pb(II) complex](image)
DFT calculations

The DFT calculations were done using the Guassian 09 suite of programs. Theoretical supports to the experimental findings have been obtained from density functional theory (DFT) studies using B3LYP/6-31G* method on S1, S2, S3 and S1:S2.

![Optimized structures](image)

**Figure S13:** (a) Optimized structures of (a) S1 (b) S2 (c) S3 (d) S3-Cd$^{2+}$ complex

**UV-Visible spectral studies of S1 and S2 in CH$_3$CN**

Receptor solutions S1 and S2 were made in CH$_3$CN of the concentration range of 10$^{-5}$ M. S1 is taken as the host solution and S2 was taken as guest. Titration was continued inorder to get 1 eq. of S2 in S1 means (1:1) solution.
Figure S14: The UV spectra of S1, S2, S1:S2 and S1+S2. Here S1+S2 represents the mathematically added UV spectra of S1 and S2 (a) UV-Visible spectra of 1:1 mixture of S1 and S2 in CH$_3$CN (b) UV-Visible spectra showing the interaction of S1 and S2 and the addition spectra of S1 and S2 in CH$_3$CN.

NMR titration data

The heterodimer formation between S1 and S2 was confirmed by the changes in chemical shift by $^1$H NMR in CD$_3$CN. An equimolar solution of S1 and S2 (500 μM) in CD$_3$CN was prepared and the $^1$H NMR was recorded for a 1:1 solution.

Figure S15: (a) Partial $^1$H NMR (300 MHz) spectra of S1, S2 and S1:S2 in CD$_3$CN (b) $^1$H NMR (300 MHz, CD$_3$CN) titration of S1:S2 with Pb$^{2+}$. 

S19
FRET efficiency of S1:S2 upon binding with Pb$^{2+}$

Lifetime of the 1:1 solution of S1 and S2 with and without Pb$^{2+}$ was recorded. The FRET efficiency was calculated with the help of the lifetime data.

\[ E = (1 - \frac{\tau_{AD}}{\tau_D}) \]

Where \( \tau_{AD} \) is the lifetime of the donor acceptor complex and \( \tau_D \) is the lifetime of the donor.

Table S6. FRET efficiency of S1:S2 (\( \lambda_{ex} = 290 \) nm) with Pb(II).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Lifetime</th>
<th>FRET efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \tau_D )</td>
<td>( E = (1 - \frac{\tau_{AD}}{\tau_D}) )</td>
</tr>
<tr>
<td>S2</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>S1:S2</td>
<td>0.48</td>
<td>52 %</td>
</tr>
<tr>
<td>S1:S2 + 1eq. Pb(II)</td>
<td>0.41</td>
<td>59 %</td>
</tr>
<tr>
<td>S1:S2 + 3eq. Pb(II)</td>
<td>0.38</td>
<td>62 %</td>
</tr>
</tbody>
</table>

ITC experiments on complexation

The thermodynamic parameters were determined by using MicroCal iTC$_{200}$ system at 298K in acetonitrile. Sample solution (S1) of 200 \( \mu \)M was taken in the cell and 50 \( \mu \)M of S2 was slowly added. Roughly 4eq. of titrant was added to sample solution. The nonlinear least square algorithm was used to determine the best fit values for change in enthalpy (\( \Delta H \)) and binding constant (K).

Similarly, another experiment was conducted by interchanging the titrant and the sample solutions. Sample solution (S2) of 200 \( \mu \)M was taken and titrated against 50 \( \mu \)M (S1) (Figure...
S18a). Roughly, 4eq. of titrant was added to sample solution. The least square fitting gave the same result as with the previous experiment. As a control, solvent CH$_3$CN was titrated against S2 (Figure S16b).

**Figure 16.** ITC titration plots (a) S1 with S2 as titrant (b) control experiment in which CH$_3$CN titrated against S2.

**Differential Scanning calorimetric (DSC) experiments on S1, S2, S1:S2**

The DSC studies were carried out using a Perkin Elmer Diamond DSC with pan a of volume 30 µL. The samples were heated from 50 °C to 250 °C with a scanning rate of 10 °C.

**Figure S17.** DSC thermograms of (a) S1 (b) S2 (c) S1:S2.
Visual fluorescence color change of S1:S2 with and without Pb$^{2+}$ under UV light

Figure S18: Images of acetonitrile solutions of S1 (1x10^-5 M), S2 (1x10^-5 M) and S1:S2 (1x10^-5 M) with and without addition of 5 equivalents of Pb$^{+2}$. The images were taken under UV light.

Reference:


$^1$H NMR spectrum of S1 in DMSO-$(d_6)$ (300 MHz)

$^{13}$C NMR spectrum of S1 in CDCl$_3$ (75 MHz)
ESI-Mass spectrum of S1

$^1$H NMR spectrum of S2 in CDCl$_3$ (300 MHz)
$^{13}$C NMR spectrum of S2 in CDCl$_3$ (75 MHz)

ESI-Mass spectrum of S2
$^1$H NMR spectrum of S3 in CDCl$_3$ (300 MHz)

$^{13}$C NMR spectrum of S3 in CDCl$_3$ (75 MHz)
ESI-Mass spectrum of S3