Selective detection of pyrophosphate by new tripodal amine calix[4]arene-based Cu(II) complexes using indicator displacement strategy

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ABSTRACT

Mono- and dinuclear Cu(II) complexes of *p-tert*-butylcalix[4]arene (**CuL1** and **CuL2**, respectively) were synthesized, and their anion recognition abilities were explored. Recognition is efficiently signaled through the displacement of pyrocatechol violet bound to the receptor. For **CuL2**, recognition selectivity is ascribed to the tuning of the distance between donor atoms of anion guests and their ability to encompass the Cu^{2+}–Cu^{2+} distance within the cleft of **CuL2**. In addition, the preorganization of calix[4]arene in the cone conformation and steric hindrance of two bulky tripodal amine moieties are important factors in controlling the Cu^{2+}–Cu^{2+} distance. These factors caused **CuL2** to recognize pyrophosphate selectively with respect to other inorganic anions in 80/20 (v/v%) $MeCN/H_2O$ solution buffered with 10 mM HEPES at pH 6.4.

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Significant attention has been given to the development of anion sensing by indicator displacement assays (IDAs).[1] This method is a simple, convenient, and increasingly popular approach to naked-eye anion sensors because an indicator is bound to a receptor by non-covalent interactions. IDAs rely upon competition between the indicator and the analyte in the host cavity. Consequently, a receptor is designed to bind a target analyte with a desired affinity, and an indicator must have a weaker affinity with the receptor than the analyte. Importantly, the indicator must absorb or emit light differently upon binding to the host and being in free form in solution.

Generally, anion recognition in the aqueous system is very challenging due to the strong hydration effects of anions. The utilization of a metal complex as a binding site for anions has been found to be the most successful strategy.[2] Therefore, metal complexes are often used as IDA receptors.[3] Normally, metal-bound ligands can bind anions more efficiently than water, allowing the detection of anions in aqueous solution. The metal center must have an unsaturated coordination sphere to accommodate the incoming anion guest.

IDA receptors for pyrophosphate ($P_2O_7^{4-}$, PPi), the product of ATP hydrolysis and involved in DNA polymerization in biological reactions,[4] have been developed by many research groups utilizing dinuclear zinc complexes of phosphotriesterase enzyme as the receptor module.[5] Recently, Hong and Fabbrizzi reported that dinuclear Cu^{2+}–DPA complexes can be employed as PPi fluorescence sensors using the IDA concept.[6] The metal–metal distance is found to play a key role in analyte preference. In order to obtain the selectivity toward PPi over other anions, especially phosphate anion (PO_4^{3-}), the metal–metal distance should not be less than 3.4 Å which is the Zn–Zn distance in phosphotriesterase.[7]

Our group is currently working on the synthesis of calix[4]arenes containing tripodal amine as the recognition unit for use as ionophores for ion selective electrodes (ISEs). The rigidity of calix[4]arenes in the cone conformation plays an important role by providing a specific cavity to recognize specific guest molecules, due to preorganization of its skeleton.[8] The transannular distances in the lower rim of original calix[4]arene and its derivatives are in the range of 3.74–4.20 Å,[9] which are longer than the Zn–Zn distance in the dinuclear zinc enzyme. Therefore, calix[4]arenes may be a suitable building block for PPi using IDA strategies. In addition, the side arms attached to the calix[4]arene can control the size and shape of the recognition cavity of calix[4]arene derivatives.[10]

In this work, calix[4]arenes containing a tripodal amine have been chosen as IDA receptors for PPi. Furthermore, we expected that steric interactions between the tripodal amine and the rigidity of the calix[4]arene framework would play crucial roles in optimizing the metal–metal distance and provide more selectivity toward PPi. Herein, we report the synthesis and characterization of ionophores based on calix[4]arenes **L1** and **L2** and their mononuclear and dinuclear complexes with $CuCl_2$ (**CuL1** and **CuL2**). We also

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demonstrate that the dinuclear complex, **CuL2**, is a suitable receptor for indicator displacement assay of PPi.

Ionophores **L1** and **L2** were synthesized in two steps according to Scheme 1. The mono-calixaldehyde[11] and bis-calixaldehyde[12] (for **L1** and **L2**, respectively) were reacted with 2-[bis(2-pyridylmethyl)aminomethyl]aniline[13] in dichloromethane, followed by in situ reduction with $NaBH_4$ in methanol to yield **L1** in 12% and **L2** in 20%, respectively. The HRMS-ESI spectra of **L1** and **L2** show parent peaks at m/z 1085.6671 and 1521.8715 assigned to the molecular ions [L+H]+ and provide evidence that the calix[4]arene derivatives are the 1:1[14] and 1:2[15] condensation products. The two calix[4]arene derivatives are in the cone conformation, as supported by their NMR spectra (Figs. S1–S4, Supplementary data). The ^1H NMR spectrum of **L1** was consistent with an asymmetric calix[4]arene structure. In particular, two pairs of doublets for the protons of the methylene bridges were observed.

Two OH singlets were observed for **L1** at low field, 9.47 and 10.18 ppm (ratio 2:1). These strong downfield shifts for the OH protons are indicative of a circular hydrogen bond at the lower rim of these derivatives, in agreement with the results reported by Frkanec et al.[16] The ^1H NMR spectrum of **L2** features a pair of doublets at 3.32 ppm and 4.43 ppm corresponding to the equatorial and the axial protons of the methylene bridging groups, respectively. We deduce that the cone structure is the major conformation of **L1** and **L2** in solution.

Addition of $CuCl_2$ to methanolic solutions of **L1** and **L2** gave green complexes of **CuL1** and **CuL2** in 72%[17] and 42%[18] yields,

respectively. The mass spectrum of **CuL1** shows the parent peak at m/z 1182.5424 which is assigned to the molecular ion of the mononuclear complex [CuL1Cl]+. For **CuL2**, the parent peak at m/z 1751.6365 corresponds to the molecular ion of the dinuclear complex [Cu2**L2**Cl3]+. A crystal of **CuL1** was obtained upon slow evaporation of a methanolic solution and the structure was determined by X-ray crystallography, Figure 1.[19] It is clearly seen that the calixarene skeleton adopts a cone conformation. It should be noted that the phenolic hydrogen atoms are involved in strong intramolecular O–H···O hydrogen bonding with the neighboring oxygen atoms to stabilize the cone conformation, in agreement with the solution structure deduced from the ^1H NMR spectrum.

The crystal structure of **CuL1** also shows that two Cu(II) centers coordinate with four nitrogen donors from the tripodal amine unit and two chloride bridging ligands to give a distorted octahedral geometry. The substantial elongation of the axial Cu1–Cl1_2 and Cu1–N1 bonds [2.984 and 2.553(4) Å, respectively] compared to the equatorial Cu1–Cl1, Cu1–N2, Cu1–N3, and Cu1–N4 [2.278(12), 2.066(3), 1.977(4), and 1.994(4) Å, respectively] is caused by the active Jahn–Teller distortion of the Cu^{2+} ion. Interestingly, the mass spectrum of the **CuL1** complex suggests that it is a mononuclear complex in solution. This implies that the mononuclear complex of **CuL1** is the most stable species in solution while the dinuclear complex is the most stable species in the solid state.

In light of the crystal structure of **CuL1**, we expect that the dye and anions might occupy the bimetallic cleft of dinuclear complex **CuL2**. In this work, we chose pyrocatechol violet (PV) as a

L1 (12%)   **CuL1** (72%)

L2 (20%)   **CuL2** (42%)
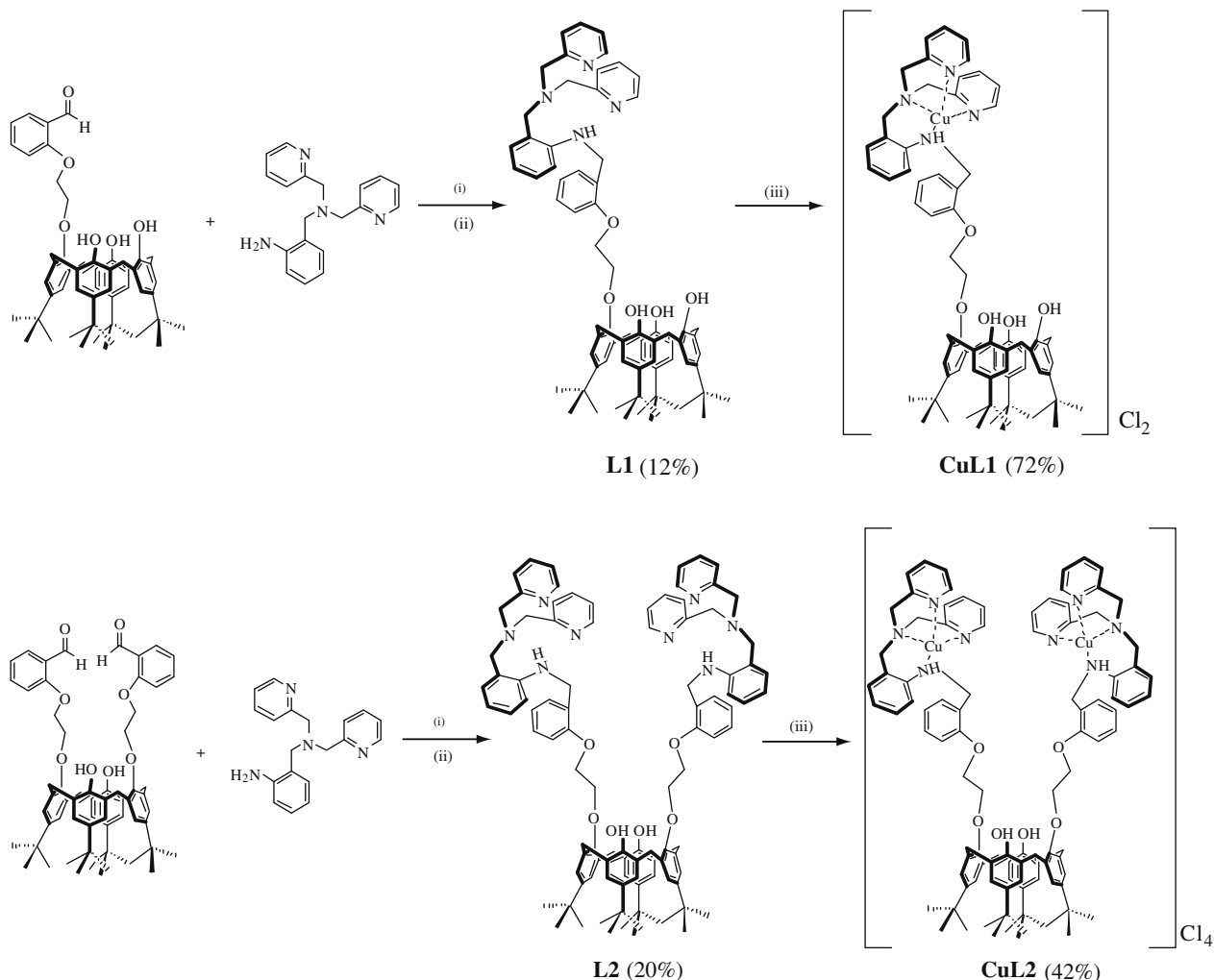
Scheme 1. Synthetic procedures for **L1**, **L2**, **CuL1**, and **CuL2**. Reagents and conditions: (i) anhyd CH_2Cl_2, anhyd $MgSO_4$, rt; (ii) $NaBH_4$, CH_3OH, reflux; (iii) $CuCl_2$, MeOH, rt.
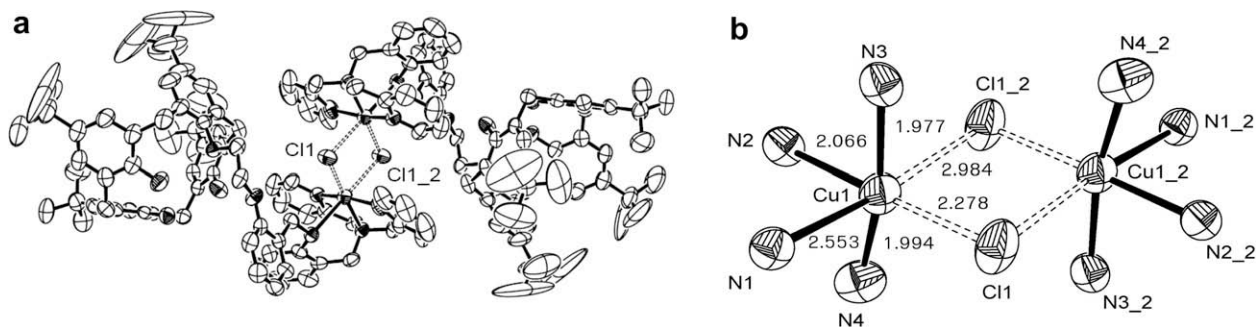
Figure 1. (a) ORTEP representation of the solid state structure of the dinuclear complex of **CuL1** with two bridging chloride ligands and (b) the coordination environment of Cu^{2+} in the complex. Thermal ellipsoids are drawn at 50% probability level (CCDC 767461).

competitive indicator.[20] The yellow solution of PV was prepared in 80% acetonitrile aqueous solution buffered with 10 mM HEPES pH 6.4, which was then titrated with increasing amounts of **CuL2** using the same solvent at 25 °C. It was found that addition of **CuL2** led to the disappearance of the absorption band of PV at 430 nm, with the simultaneous appearance of a new band at 670 nm and a color change from yellow to green (Fig. 2a). In addition, an isosbestic point was found at 488 nm, suggesting the presence of two equilibrium species. A Job plot (at 670 nm) was also obtained and suggested that the complex between **CuL2** and PV was formed with a 1:1 stoichiometry (inset of Fig. 2a). Using the Benesi–Hildebrand method, the association constant (K_a) between PV and **CuL2** was found to be $1.30 \times 10^4 \, M^{-1}$.[21]

Upon addition of various anions (as tetrabutylammonium salts, 3 equiv) to the ensemble [**CuL2**·PV] solutions, only PPi was able to turn the color from green to yellow of the unbound dye, while other anions did not give rise to UV–vis spectral changes (Fig. 2b) or any color changes (Fig. 2c). Moreover, we also carried out displacement of PV from the **CuL2** cavity by phosphate containing biomolecules (AMP, ADP, and ATP). Results showed that both ADP and ATP were able to displace PV from the cleft of **CuL2**, whereas AMP was not (Fig. S5, Supplementary data). Therefore, **CuL2** possessed high selectivity toward PPi over other anions. We tried to change the dye from PV to fluorescein. However,

results of this ensemble did not show specific selectivity to any anions (Fig. S6, Supplementary data).

Titrating PPi with an ensemble solution [**CuL2**·PV] caused an absorbance increase around 430 nm and an absorbance decrease around 670 nm (hypsochromic shift), with a color change to yellow, revealing that the indicator was displaced from the cleft of **CuL2** by the analyte (Fig. 3). The UV–vis spectrum at 670 nm was completely saturated at 1.5 equiv of PPi. The binding constant between **CuL2** and PPi was estimated by the competitive spectrophotometric method[22] and found to be $5.2 \times 10^5 \, M^{-1}$. The electrospray ionization mass spectrum (positive mode) of **CuL2** complex with PPi showed a molecular ion peak at $m/z = 1824.57$ (Fig. S7, Supplementary data). The result thus confirmed the 1:1 complex species of **CuL2**:PPi.

Xu et al. have characterized crystallographically a ternary system complex of PPi with a mononuclear Cu^{2+} ion and a 2,2′-dipyridylamine (hdpa) ligand, $[Cu(dhpa)]^{2+}$.[23] In this case, one PPi anion acted as the bridging ligand to bring two units of $[Cu(dhpa)]$ together, forming a dinuclear complex. Simultaneously, two PPi ions coordinating to the oxygen atoms of a discrete dinuclear complex also acted as the bridging atoms to hold those two discrete dimeric species together to form a tetranuclear complex. Compared to our system, we assume that PPi is bound within the bimetallic cleft of **CuL2**. Two oxygen atoms on each phosphorus of PPi coordinated
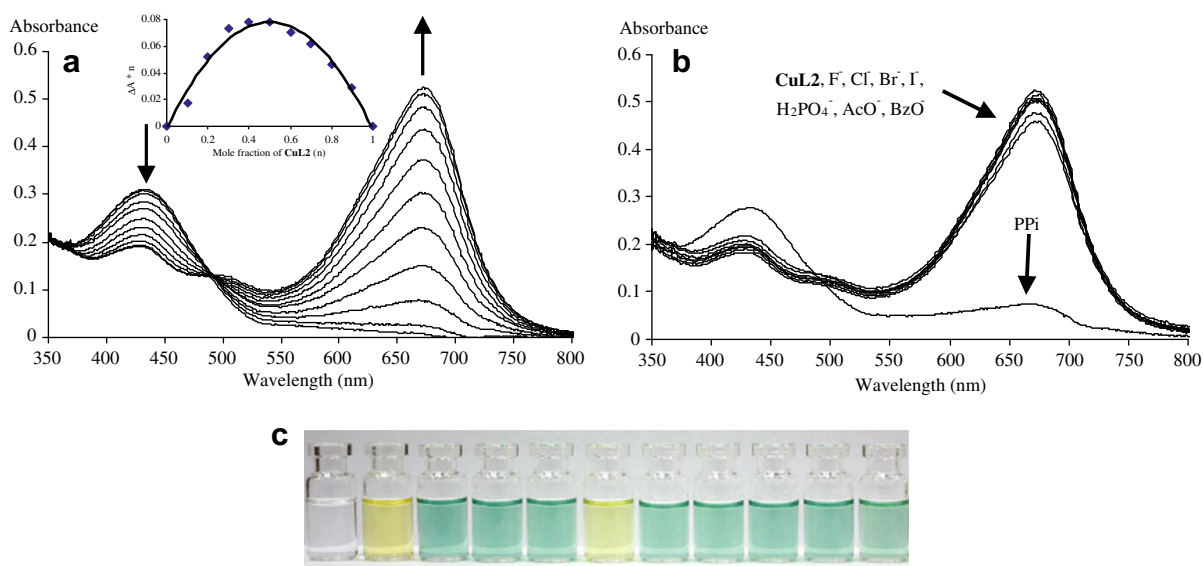

Figure 2. (a) UV–vis spectra obtained by addition of **CuL2** (400 μM) to PV (20 μM) solution; (b) UV–vis spectra obtained by addition of various anions (3 equiv of tetrabutylammonium salts) to an ensemble solution [**CuL2**·PV] (20 μM); and (c) color changes of the ensemble [**CuL2**·PV] 20 μM after addition of various anions (3 equiv of tetrabutylammonium salts). From left to right: **CuL2**, PV, [**CuL2**·PV], $H_2PO_4^-$, AcO^-, PPi, BzO^-, I^-, Br^-, Cl^-, and F^-. All experiments were carried out in 80/20 (v/v) MeCN/H_2O solution buffered with 10 mM HEPES at pH 6.4.
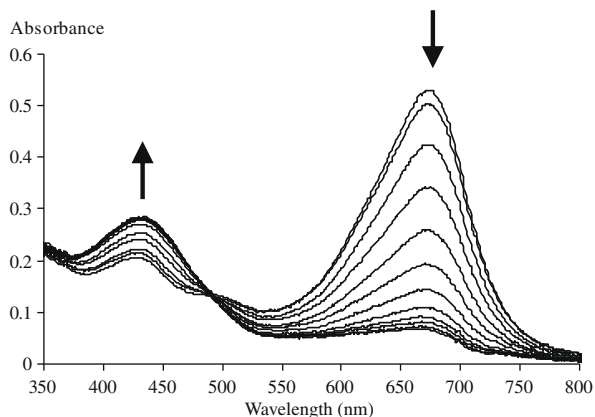
Figure 3. UV–vis spectra obtained by addition of PPi (400 μM) to an ensemble solution of [**CuL2**·PV] (20 μM) in 80/20 (v/v) MeCN/H$_2$O solution buffered with 10 mM HEPES at pH 6.4.

through one Cu^{2+} ion, which was similar to the binding mode of PPi with the dinuclear DPA–2Zn^{2+} derivatives reported by Yoon and co-workers[24] and Hong and co-workers.[5b]

Similar experiments have been run with the mononuclear **CuL1** complex. The results showed that in the presence of any anions in an ensemble solution of [**CuL1**·PV] the yellow solution of the unbound dye was not observed (Fig. S8, Supplementary data). This result strongly supports the fact that the cooperative action of two Cu^{2+} ions in solution is required for selective sensing of PPi.

In conclusion, we have successfully synthesized mono- and dinuclear Cu(II) complexes of calix[4]arene containing a tripodal amine, **CuL1** and **CuL2**. **CuL2** was demonstrated to be a remarkable IDA receptor for PPi. A rationale to account for the selectivity of **CuL2** toward PPi requires matching of the distance between the donor atoms of PPi with the Cu^{2+}–Cu^{2+} distance in the **CuL2** cavity. In addition, the preorganization of calix[4]arene in the cone conformation and steric hindrance between the two bulky tripodal amine parts are the most important factors controlling the Cu^{2+}–Cu^{2+} distance. This resulted in selective recognition of **CuL2** toward PPi over other anions. Further studies are underway in our laboratory to prepare anion selective electrodes from **CuL2**.

Acknowledgments

Supplementary data

Supplementary data (additional ^{1}H and ^{13}C NMR spectra of **L1** and **L2**, and displacement results of **CuL1** with various anions are available) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.095.

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14. Ionophore **L1**, ^{1}H NMR (400 MHz, CDCl$_3$, ppm): δ 10.20 (s, 1H, –OH), 9.49 (s, 2H, –OH), 8.44 (d, 2H, J = 4.0 Hz, ArH), 7.49 (t, 2H, J = 6.0 Hz, ArH), 7.37 (d, 2H, J = 4.0 Hz, ArH), 7.34 (d, 2H, J = 8.0 Hz, ArH), 7.13 (s, 2H, ArH), 7.12 (s, 1H, ArH), 7.07 (d, 2H, J = 2.0 Hz, ArH), 7.05 (s, 2H, ArH), 7.03 (s, 2H, ArH), 7.02 (s, 2H, ArH), 6.94 (m, 2H, ArH), 8.82 (s, 1H, –NH–), 6.54 (t, 1H, J = 7.6 Hz, ArH), 6.40 (d, 1H, J = 8.0 Hz, ArH), 4.70 (d, 2H, J = 3.2 Hz, –CH$_2$–NH–), 4.64 (s, 4H, –O–CH$_2$–O–), 4.53 (d, 2H, J = 12.8 Hz, Ar–CH$_2$–Ar), 4.24 (d, 2H, J = 13.6 Hz, Ar–CH$_2$–Ar), 3.83 (s, 4H, –CH$_2$–N), 3.69 (s, 2H, –CH$_2$–N), 3.41 (dd, 4H, J = 7.6 Hz, J = 12.8, 13.6 Hz, Ar–CH$_2$–Ar), 1.23 (s, 36H, p-tert-butyl); ^{13}C NMR (100 MHz, CDCl$_3$, ppm): δ 159.24, 156.11, 149.12, 149.04, 148.39, 148.29, 148.09, 147.85, 143.50, 143.12, 136.30, 133.61, 130.94, 128.74, 128.53, 128.12, 128.07, 127.75, 127.69, 126.56, 125.82, 125.71, 125.66, 123.18, 121.90, 121.51, 121.10, 115.32, 110.80, 110.20, 74.78, 60.13, 58.47, 41.73, 34.26, 33.99, 33.93, 33.00, 32.18, 31.50, 31.25; HRMS-ESI: [M+H]$^+$ calcd for C$_{72}$H$_{84}$N$_4$O$_5$, 1085.6670: found 1085.6671.
15. Ionophore **L2**, ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.42 (d, 4H, J = 4.0 Hz, ArH), 7.65 (d, 2H, J = 2.4 Hz, ArH), 7.46 (m, 4H, ArH), 7.35 (d, J = 7.6 Hz, 2H, ArH), 7.30 (s, 4H, ArH), 7.19 (m, 2H, ArH), 7.06 (m, 8H, ArH), 7.05 (m, 2H, ArH), 6.95 (d, 4H, J = 8.4 Hz, ArH), 6.89 (s, 4H, ArH) 6.86 (d, 2H, J = 7.2 Hz, ArH), 6.72 (s, 2H, –NH–), 6.54 (m, 2H, ArH), 6.44 (d, 2H, J = 8.0 Hz, ArH), 4.53 (d, 4H, J = 4.8 Hz, –CH$_2$–NH–), 4.44 (d, 4H, J = 12.8 Hz, Ar–CH$_2$–Ar), 4.35 (s, 8H, –CH$_2$–O–), 3.80 (s, 8H, –CH$_2$–N), 3.68 (s, 4H, –CH$_2$–N), 3.33 (d, 4H, J = 13.2 Hz, Ar–CH$_2$–Ar), 1.26 (s, 18H, p-tert-butyl), 1.03 (s, 18H, p-tert-butyl); ^{13}C NMR (100 MHz, CDCl$_3$, ppm): δ 159.23, 156.24, 150.35, 149.73, 149.07, 148.21, 147.34, 141.69, 136.29, 133.12, 130.97, 128.77, 128.56, 128.52, 127.93, 127.63, 125.72, 125.20, 123.20, 121.91, 121.55, 120.71, 115.35, 110.78, 110.32, 74.16, 66.65, 60.16, 58.47, 41.76, 34.04, 33.84, 31.83, 31.66, 31.10; HRMS-ESI: [M+H]$^+$ calcd for C$_{100}$H$_{112}$N$_8$O$_6$, 1521.8705: found 1521.8715.
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17. **CuL1**; A methanolic solution of CuCl$_2$·2H$_2$O (24 mg, 0.14 mmol) in 5 mL CH$_3$OH was added to a suspension of **L1** (0.108 g, 0.09 mmol) in methanol giving a deep-green solution. The solution was allowed to stand at room temperature. After 1 week, green block-shaped X-ray diffraction quality single crystals of **CuL1** were obtained (80 mg, 73%). HRMS-ESI: [M+Cl]$^+$ calcd for C$_{72}$H$_{84}$ClCuN$_4$O$_5$, 1182.5426: found 1182.5424.
18. **CuL2**; A methanolic solution of CuCl$_2$·2H$_2$O (26 mg, 0.15 mmol) was added to a methanolic suspension of ionophore **L2** (110.9 mg, 0.06 mmol); the color of the solution changed to deep-green immediately. After standing the green solution at room temperature for 1 week, the deep-green solid appeared. This was filtered and washed with MeOH to give **CuL2** in 42% yield (111 mg). HRMS-ESI: [M+Cl]$^+$ calcd for C$_{100}$H$_{112}$Cl$_3$Cu$_2$N$_8$O$_6$, 1751.6362: found 1751.6365.

19. Crystal data for **CuL1**: $C_{146}H_{180}Cl_2Cu_2N_8O_{14}$, Mr = 2539.99, T = 293(2) K, triclinic, space group $P\bar{1}$ a = 13.3699(10), b = 15.5074(12), c = 17.9970(13) Å, α = 94.853° (2), β = 90.013° (2), γ = 99.694° (2), V = 3664.5(5) Å^3, ρ_{calcd} = 1.151 g cm^{-3}, μ = 0.422 mm^{-1}, Z = 1, reflections collected: 50619, independent reflections: 17717 (R_{int} = 0.0556), final R indices [I >2σI]: $R1$ = 0.0974, w$R2$ = 0.2936, R indices (all data): $R1$ = 0.1511, w$R2$ = 0.3344. Crystallographic data for **CuL1** are available upon request from the Cambridge Crystallographic Data Base (CCDC 767461).
20. All spectrophotometric titrations were performed in 80/20 (v/v) MeCN/H$_2$O solution buffered with 10 mM HEPES at pH 6.4, thermostated at 25 °C. Receptor/indicator affinity constants were determined by adding aliquots of a 400 μM **CuL2** complex solution to a 20 μM solution of PV. After each addition, the UV–vis absorption spectra of the indicator solution were recorded in a quartz cuvette. Similar titration experiments were performed with PPi. In typical titrations, aliquots of PPi (400 μM) were added to an ensemble solution (20 μM) of [**CuL2**·PV].
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