Self-assembled copper(ii) metallacycles derived from asymmetric Schiff base ligands: efficient hosts for ADP/ATP in phosphate buffer†

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Novel asymmetric Schiff base ligands 2: ([3-(3-hydroxy-1-methyl-but-2-enylideneamino)-2,4,6-trimethylphenylimino]-methyl)-phenol (H2L1) and 1: ([3-(3-hydroxy-1-methyl-but-2-enylideneamino)-2,4,6-trimethylphenylimino]-methyl)-naphthalen-2-ol (H2L2) possessing dissimilar N,O-chelating sites and copper(ii) metallacycles (Cul4)1 and (Cul3)2 based on these ligands have been described. The ligands and complexes have been thoroughly characterized by satisfactory elemental analyses, and spectral (IR, 1H, 13C NMR, ESI-MS, UV/vis) and electrochemical studies. Structures of H2L2 and 1 have been unambiguously determined by X-ray single crystal analyses. The crystal structure of H2L2 revealed the presence of two distinct N,O-chelating sites on dissimilar cores (naphthalene and β-ketoaminato groups) offering a diverse coordination environment. Metallacycles 1 and 2 having a cavity created by four Cu(II) centres coordinated in a homo- and heteroleptic fashion with respective ligands act as efficient hosts for adenosine-5′-diphosphate (ADP) and adenosine-5′-triphosphate (ATP) respectively, over other nucleoside polyphosphates (NPPs). The disparate sensitivity of these metallacycles toward ADP and ATP has been attributed to the size of the ligands assuming diverse dimensions and spatial orientations. These are attuned for π–π stacking and electrostatic interactions suitable for different guest molecules under analogous conditions, metallacyle 1 offers better orientation for ADP, while 2 for ATP. The mechanism of the host–guest interaction has been investigated by spectral and electrochemical studies and supported by molecular docking studies.

Introduction

Nucleoside polyphosphates (NPPs) are the most intriguing and important species present in living cells and are vital for numerous biological activities.1,2 Amongst these, adenosine-5′-triphosphate (ATP) and adenosine-5′-diphosphate (ADP) generate energy in the cells via cleavage of the phosphate bond. ATP is known as ‘energy currency’ of the cells and plays a crucial role in protein transport, ion channel regulation, intra-/extracellular signaling and synthesis of diverse macromolecules.1,3 During necrosis/apoptosis the ATP level appreciably decreases and may impose serious health problems like ischemia, Parkinson’s disease, and hypoglycemia.4 On the contrary, ADP is produced by hydrolysis of ATP and also in many metabolic reactions catalyzed by ATPases and kinases.5 Therefore, detection of ADP/ATP is extremely helpful in monitoring various metabolic processes and determination of the catalytic activity of ATPases/kinasases. Furthermore, supramolecular metal complexes (SMCs) are advantageous over organic receptors viz. calixarenes, cyclodextrins, crown ethers and cyclodextrins as these can have supramolecular cavities capable of interacting with guest molecules via modulation of the ligands.6 In addition, coordination complexes, triangles, and tetranuclear squares/rectangles have been recognized as the major receptors for anions, organic molecules and biologically important species.7 Due to the presence of the supramolecular cavities of diverse shape and size, SMCs may find potential applications in molecular recognition, gas storage etc.8,9 The recognition of NPPs by supramolecular receptors is based on hydrogen bonding and/or non-covalent interactions leading to supramolecular assemblies.2 Therefore, designing
and synthesis of the receptors capable of recognizing NPPs have attracted a great deal of attention. In this context, many systems have been developed and utilized in the detection of ADP/ATP and other biomolecules in aqueous media.\textsuperscript{10,11}

Further, \textit{m}-substituted symmetrical bis-salen type Schiff base ligands capable of forming stable binuclear metallacycles with transition metals have drawn special attention.\textsuperscript{12} Such systems find potential applications in diverse areas like asymmetric catalysis, metal ion sensing, DNA cleavage \textit{etc}.\textsuperscript{12,13}

Recently, we have reported some symmetrical salen (H\textsubscript{2}L\textsubscript{3}), pyridyl (L\textsubscript{4}) and mono/di-\(\beta\)-ketoaminato (HL\textsubscript{5}/H\textsubscript{2}L\textsubscript{6}) ligands and their diverse complexation behaviour towards copper(II) salts (Scheme 1, part A).\textsuperscript{12} Amongst these, bis-chelating salen (H\textsubscript{2}L\textsubscript{3}) and pyridyl (L\textsubscript{4}) ligands reacted with Cu(NO\textsubscript{3})\textsubscript{2}-2.5H\textsubscript{2}O to afford symmetrical binuclear metallacycles, while mono/di-\(\beta\)-ketoaminato (HL\textsubscript{5}/H\textsubscript{2}L\textsubscript{6}) ligands gave Cu\textsubscript{4}O\textsubscript{4} cubanes involving transformation of the ligands via oxidation and dearylamidation of the mesitylene ring.\textsuperscript{12d} The divergent results from the salen and \(\beta\)-ketoaminato ligands prompted us to design asymmetric ligands containing both of these moieties and examine their reactivity with Cu(NO\textsubscript{3})\textsubscript{2}-2.5H\textsubscript{2}O. Through this work we describe the synthesis and characterization of two asymmetric Schiff base ligands H\textsubscript{2}L\textsubscript{1} and H\textsubscript{2}L\textsubscript{2} having different N,O-chelating sites (one \(\beta\)-ketoaminato and the other salen/\(\beta\)-hydroxy naphthalene) and tetranuclear copper(II) complexes 1 and 2 based on these ligands (Scheme 1, part B). Also, we describe herein the applicability of 1 and 2 as efficient size selective hosts for ADP/ATP in phosphate buffer saline (PBS).

**Experimental section**

**General information and materials**

Common reagents and solvents were acquired from S.D. Fine Chem. Pvt. Ltd Mumbai. Solvents were dried and distilled following the standard procedures.\textsuperscript{14} Salicylaldehyde, 2-hydroxy-1-naphthaldehyde, 2,4,6-trimethylbenzene-1,3-diamine (mesitylene diamine), adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP), adenosine-5'-mono-phosphate (AMP), guanosine-5'-triphosphate (GTP), guanosine-5'-diphosphate

![Scheme 1](image-url)  
_Scheme 1_ Schematic representation of diverse complexation behaviour of the symmetric and asymmetric Schiff base ligands with Cu(s) salts (the orange sphere shows the cavity suitable for ADP in 1, and yellow for ATP in 2) in the previous (part A) and present (part B) work.
(GDP), cytidine-5′-triphosphate (CTP), uridine-5′-triphosphate (UTP) and uridine-5′-diphosphate (UDP) were procured from Sigma Aldrich Pvt. Ltd. The starting amine HL3 [4-(3-amino-2,4,6-trimethylphenylimino)-pent-2-ene-2-ol] was synthesized by following our earlier procedure.2f

Elemental analyses for C, H, N were performed on a Euro-E 3000 Elemental Analyzer at Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute, (CDRI) Lucknow. Infrared and electronic absorption spectra were obtained on a Perkin Elmer Spectrophotometer. 1H (300 MHz), 13C (75.45 MHz) and 31P (121.50 MHz) NMR spectra were acquired on a JEOL AL300 FT-NMR spectrometer using tetramethylsilane (TMS) as an internal reference for 1H/13C NMR and H3PO4 (85%) as an external reference for 31P NMR. Electrospray ionization mass spectrometric (ESI-MS) data were obtained on a JEOL Accu TOF JMS-T100 LC mass spectrometer. Electrochemical measurements were made using a CHI 620c electrochemical analyzer. All the measurements were made using DMSO under a nitrogen atmosphere in a single compartment cell equipped with a glassy carbon working, platinum wire counter, and Ag/AgCl as the reference electrode.

Preparation of 2-[[3-(3-hydroxy-1-methyl-but-2-enylideneamino)-2,4,6-trimethylphenylimino]-methyl]-phenol (HL1)

Salicylaldehyde (0.244 g, 2.0 mmol) dissolved in methanol (10 mL) was added to a solution of HL3 (0.464 g, 2.0 mmol) in the same solvent (20 mL) along with catalytic amounts of acetic acid and the contents of the flask were heated under reflux for 12 h. After cooling to room temperature the solvent was removed under reduced pressure to afford an oily yellow product. It was purified by extracting with boiling hexane and was removed under reduced pressure to a reflux for 12 h. After cooling to room temperature the solvent acetic acid and the contents of the flask were heated under the same solvent (20 mL), Cu(NO3)2·2.5H2O (0.232 g, 1.0 mmol) dissolved in methanol (10 mL) was added with stirring over half an hour at room temperature. The resulting reaction mixture was stirred for an additional 2 h. A brown coloured precipitate thus obtained was separated by filtration, washed thrice with methanol, diethyl ether and dried under vacuum. Slow diffusion of methanol over a dichloromethane solution of the complex gave dark red facefracture quality crystals within a few days. Yield (0.242 g, 61%). Anal. Calc. for C25H26N2O2 (386.48): C, 77.69; H, 6.25; N, 6.17%. ESI-MS (Calcd Found, m/z): 387.2072, 387.2072 [(M + H)+, 100%]. IR (KBr pellets, cm−1): 3391, 3270 (br), 3067 (m), 2917 (w), 2852 (m), 1668 (s), 1593 (s), 1513 (s), 1468 (s), 1447 (s), 1408 (s), 1377 (m), 1341 (s), 1337 (m), 1327 (s), 1267 (s), 1190 (m), 1147 (m), 1099 (w), 1011 (w), 922 (w), 755 (m). UV/vis (DMSO/PBS; ν/λ, 1:99, λmax nm, ε M−1 cm−1): 336 (2.26 × 104) and 349 (2.41 × 104) nm.

Preparation of (CuL1)4 (1)

To a solution of deprotonated HL1 [obtained by treatment of CuL1 (0.336 g, 1.0 mmol) with KOH (0.112 g, 2.0 mmol) in methanol (20 mL)], Cu(NO3)2·2.5H2O (0.232 g, 1.0 mmol) dissolved in methanol (10 mL) was added with stirring over half an hour at room temperature. The resulting reaction mixture was stirred for an additional 2 h. A brown coloured precipitate thus obtained was separated by filtration, washed thrice with methanol, diethyl ether and dried under vacuum. Slow diffusion of methanol over a dichloromethane solution of the complex gave dark red facefracture quality crystals within a few days. Yield (0.242 g, 61%). Anal. Calc. for C25H26N2O2 (386.48): C, 77.69; H, 6.25; N, 6.17%. ESI-MS (Calcd Found, m/z): 1591.3969, 1591.3943 [(M + H)+, 100%]; 1613.3789, 1613.3768 [(M + Na)+, 25%]. IR (KBr pellets, cm−1): 2918 (w), 1613 (s), 1579 (m), 1532 (s), 1511 (s), 1447 (s), 1403 (s), 1377 (m), 1327 (m), 1290 (m), 1190 (m), 1147 (m), 1099 (w), 1011 (w), 922 (w), 755 (m). UV/vis (DMSO/PBS; ν/λ, 1:99, λmax nm, ε M−1 cm−1): 336 (2.26 × 104) and 349 (2.41 × 104) nm.

Preparation of (CuL2)4 (2)

It was prepared following the above procedure for 1 using HL2 (0.386 g, 1.0 mmol) in place of HL1. Yield (0.284 g, 63%). Anal. Calc. for C25H26N2O2 (386.48): C, 77.69; H, 6.25; N, 6.17%. ESI-MS (Calcd Found, m/z): 1792.4629, 1792.4596 [(M + H)+, 8%]; 1814.4488, 1814.4493 [(M + Na)+, 2%]. IR (KBr pellets, cm−1): 2918 (w), 1618 (s), 1604 (s), 1580 (s), 1538 (s), 1455 (s), 1430 (s), 1399 (s), 1365 (m), 1252 (w), 1186 (m), 1107 (m), 1012 (w), 830 (m), 746 (m). UV/vis (DMSO/PBS; ν/λ, 1:99, λmax nm, ε M−1 cm−1): 410 (2.14 × 104), 319 (3.94 × 104) and 660 nm (ε, 361 M−1 cm−1), d-d transition band appeared at c, 1 × 10−3 M; DMSO/PBS, ν/λ, 1:9.

X-ray structure determinations

Single crystal X-ray data for HL2 and 1 were acquired on a CCD Agilent Technologies (Oxford Diffraction) SUPER NOVA diffractometer. Data were collected using graphite-monochro-
Results and discussion

Synthesis and characterization

The ligands 2-[[3-[3-hydroxy-1-methyl-but-2-enylideneamino]-2,4,6-trimethylphenylimino]-methyl]-phenol \( \text{H}_2\text{L}_1 \) and 1-[[3-[3-hydroxy-1-methyl-but-2-enylideneamino]-2,4,6-trimethylphenylimino]-methyl]-napthalen-2-ol \( \text{H}_2\text{L}_2 \) were synthesized by condensation of 4-(3-amino-2,4,6-trimethylphenylimino)-pent-2-ene-2-ol \( \text{H}_2\text{L}_3 \) with respective aldehydes (salicylaldehyde and 2-hydroxy-1-naphthaldehyde) in methanol in a 1:1 molar ratio under refluxing conditions (Scheme 2). These were purified by extraction with hot hexane and isolated in reasonably good yield (76%, \( \text{H}_2\text{L}_1 \)) and 81%, \( \text{H}_2\text{L}_2 \)). Further, treatment of the deprotonated methanolic solution of \( \text{H}_2\text{L}_1/\text{H}_2\text{L}_2 \) with Cu(NO$_3$)$_2$·2.5H$_2$O afforded tetracarbon(n) complexes \( \text{CuL}_1 \) (1) and \( \text{CuL}_2 \) (2) (Scheme 2). The ligands and complexes have been thoroughly characterized by satisfactory elemental analyses, spectral (IR, \(^{1} \text{H}, ^{13} \text{C} \) NMR, UV/vis, ESI-MS) and electrochemical studies and structures of \( \text{H}_2\text{L}_1 \) and 1 have been authenticated by X-ray single crystal analyses.

The \(^{1} \text{H} \) NMR spectrum of \( \text{H}_2\text{L}_1 \) in CDCl$_3$ displayed three sharp singlets in the downfield region at \( \delta \) 12.95 (Ha, phenolic –OH), 11.93 (Hb, –OH) and 8.29 ppm (Hc, –HC≡N–) (Fig. S1, ESiF). In an analogous manner \( \text{H}_2\text{L}_2 \) also displayed sharp singlets at \( \delta \) 15.05 (Ha, phenolic –OH), 11.96 (Hb, –OH) and 9.08 ppm (Hc, –HC≡N–) (Fig. S2, ESiF). In addition, allylic protons for \( \text{H}_2\text{L}_1 \) and \( \text{H}_2\text{L}_2 \) resonated as a sharp singlet at \( \delta \) 5.22 (Hf) and 5.24 ppm (Hg), respectively. Distinct resonances due to aliphatic and aromatic protons for these ligands were displayed at their usual positions. These observations strongly suggested the formation of \( \text{H}_2\text{L}_1 \) and \( \text{H}_2\text{L}_2 \). \(^{13} \text{C} \) NMR spectral studies corroborated well with the conclusions drawn from \(^{1} \text{H} \) NMR studies (Fig. S1 and S2, ESiF). The resulting data are summarized in the Experimental section.

ESI-mass spectral studies supported the formation of ligands \( \text{H}_2\text{L}_1 \), \( \text{H}_2\text{L}_2 \) and tetracarbon complexes 1 and 2. ESI-MS of \( \text{H}_2\text{L}_1 \) and \( \text{H}_2\text{L}_2 \) displayed molecular ion peaks at \( m/z \) 337.1912 [27%, \( \text{[M + H]} \) and 387.2072 [100%, \( \text{[M + H]} \)] (Fig. S3, ESiF). Complexes 1 and 2 showed molecular ion peaks at \( m/z \) 1591.3943 [7%, \( \text{[M + H]} \) and 1613.3768 [22%, \( \text{[M + Na]} \)] and 1792.4596 [8%, \( \text{[M + H]} \) and 1814.4493 %, \( \text{[M + Na]} \)] (Fig. S4 and S5, ESiF). In addition, the most intense peaks appearing in the ESI-MS at \( m/z \) 817.1899 (for 1) and 448.1226 (for 2) have been assigned to \( \text{[L}^\text{1Cu} \text{Na}^+ \)] and \( \text{[L}^\text{2Cu} \text{Na}^+ \) respectively. These designate the half and one-fourth units of 1 and 2, respectively. The molecular ion peaks for 1 and 2 nicely substantiated with their simulated isopropic patterns and strongly supported the formulation of complexes (insets, Fig. S4 and S5, ESiF).

Crystal structures

Structures of \( \text{H}_2\text{L}_2 \) and 1 have been authenticated by single crystal X-ray diffraction analyses. Details about the data collection, solution and refinement parameters are summarized in the Experimental section and Table 1, while a pertinent view with partial atomic numbering scheme is depicted in Fig. 1.
H$_2$L$_2$ crystallizes in the triclinic system with the P$_1$ space group and its structure clearly showed that one amine group from mesitylenediamine is involved in condensation with 2-hydroxy-1-naphthaldehyde while the other one with acetyl acetone creating two asymmetric N,O-chelating sites [N$_1$O$_1$, $\beta$-ketoaminato; N$_2$O$_2$, 2-hydroxy-1-naphthalene] (Fig. 1). These asymmetric units are disposed in gauche conformation with the bite angle of 42.91° between each arm. The donor sites C$_1$–N$_1$ and C$_{15}$–N$_2$ in H$_2$L$_2$ interact with the nearby oxygen atoms via intramolecular hydrogen bonding (N$_1$⋯H$_1$–O$_1$; 2.663, N$_2$⋯H$_2$–O$_2$; 2.544 Å, Fig. S6, ESI†).

Complex 1 crystallizes in the monoclinic system with the I$_2$/a space group with eight molecules in each unit cell. Moreover, it has been observed that four units of the deprotonated ligands coordinated with four Cu(II) centres in a homo- (Cu$_1$/Cu$_4$) and heteroleptic (Cu$_2$/Cu$_3$) fashion (Fig. 2). The homoleptic copper centres Cu$_1$ and Cu$_4$ are coordinated with two units of each of
the salen (N1O1, N2O2) and β-ketoaminato moieties (N7O7, N8O8). On the other hand the heteroleptic copper centres Cu2 and Cu3 are bonded to two N,O-chelating sites, one from salen (N3O3, N4O4) and the other from the β-ketoaminato (N5O5, N6O6) moiety. The immediate coordination geometry about each copper centre is distorted square planar. The extent of distortion from an ideal square planar geometry has been estimated from inter planar angles ranging from 34.48–49.45° for cis–cis and 88.63–89.88° for the trans–trans N–Cu–O planes. It is noteworthy to mention here that arrangement of the copper centres created a rectangular supramolecular cavity with a separation of ∼7.0 Å between each two adjacent Cu atoms and a diagonal distance of ∼10 Å (Fig. 3). The space filling model too, revealed the presence of a large cavity and signifies supramolecular aspects of 1 (Fig. 2b). Further, the distance for each Cu(i) centre from the centroid lies in the range of 4.800–5.124 Å (Fig. S7, ESI†). The observed bond lengths and bond angles around each copper centre lie in the range for normal square planar systems (Tables 2 and 3).

The crystal structure of H2L2 strongly suggested the occurrence of asymmetrical N,O-chelating sites which may cause dissimilar reactivity. In H2L1 and H2L2, the salen-naphthalene...
core is more reactive relative to a \( \beta \)-ketoaminato site thus, might have acquired asymmetric coordination behaviour with the metal centres.\(^{19}\) The difference in the reactivity for both chelating sites in each ligand leads to tetranuclear Cu(II) complexes unlike symmetrical binuclear metallacycles.\(^{12}\) It has been unequivocally verified by single crystal X-ray analyses on 1 which clearly show a tetrameric structure. Noticeably, the gauche-conformation of the ligand acquires trans-orientation during complexation with copper and forms a tetranuclear complex in contrast to a symmetrical binuclear metallacycle reported in our previous work.\(^{12}\) It is quite significant and may be considered as a major criterion toward formation of the desired tetrameric species using asymmetric ligands.

**Electronic absorption studies**

UV/vis spectra of \( \text{H}_2\text{L}^1, \text{H}_2\text{L}^2, 1 \) and 2 have been acquired in DMSO/PBS solution (\( c, 10 \, \mu\text{M}; \text{v/v}, 1:99; \text{pH}, 7.4, \text{Fig. 4, Table S1, ESI}\)†). The spectra of \( \text{H}_2\text{L}^1 \) and \( \text{H}_2\text{L}^2 \) displayed low energy (LE) bands at 310 (\( \epsilon, 4.57 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1} \)) and 364 nm (\( \epsilon, 2.26 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1} \)) and high energy (HE) bands at 262 (\( \epsilon, 4.41 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1} \)) and 312 nm (\( \epsilon, 4.94 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1} \)), respectively. The complexes 1 and 2 displayed strong absorption bands in the LE region at 386 (\( \epsilon, 2.69 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1} \)) and 410 nm (\( \epsilon, 2.14 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1} \)), respectively assignable to the ligand to metal charge transfer (LMCT) transitions. In addition, the HE bands observed at 323 (\( \epsilon, 4.21 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1}, 1 \)) and 319 nm (\( \epsilon, 3.94 \times 10^4 \, \text{M}^{-1} \, \text{cm}^{-1}, 2 \)) may be associated with intra-ligand charge transfer transitions. Notably, the UV/vis spectra of 1 and 2 do not show bands in the range of d–d transitions at such a low concentration (\( c, 1 \times 10^{-3} \, \text{M} \)), however when verified at a higher concentration (\( c, 1 \times 10^{-3} \, \text{M}; \text{DMSO/PBS, v/v, 1:9} \)) very weak and broad absorption bands appeared at 649 (\( \epsilon, 378 \, \text{M}^{-1} \, \text{cm}^{-1}, 1 \)) and 660 nm (\( \epsilon, 361 \, \text{M}^{-1} \, \text{cm}^{-1}, 2 \)) which may be attributed to d–d transitions (Laporte forbidden, Fig. 4c). A high DMSO/PBS ratio (1:9) has been used to prepare a \( c, 1 \times 10^{-3} \, \text{M} \) solution just to avoid precipitation.

### Table 3

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![Fig. 4](image_url)  
**Fig. 4** UV/vis spectra of \( \text{H}_2\text{L}^1, \text{H}_2\text{L}^2 \) (a), complexes 1–2 (b) and d–d transition bands showing at the low energy region (c).
Interactions of 1 and 2 with ADP/ATP

Large supramolecular cavities present in 1 and 2 contain functional sites/metal centres. These are suitable for non-covalent interactions like π–π stacking, electrostatic interactions and may host appropriate guest molecules. Considering this and to explore host–guest interaction behaviour absorption spectra of 1 and 2 have been acquired in the presence of NPPs (ATP, ADP, AMP, GTP, GDP, CTP, UTP and UDP) and various anions. The absorption spectra exhibited significant changes for both LE and HE bands in the presence of ADP/ATP and remained unperturbed with other NPPs and anions (Fig. S8 and S9, LE and HE bands in the presence of ADP/ATP and remained unperturbed with other NPPs and anions [Fig. S8 and S9, ESI†]). This indicated rather strong interactions of 1 and 2 with ADP/ATP over other NPPs. Afterwards, the effect on the so-called d-d transition bands of 1 and 2 in the presence of ADP/ATP has also been monitored. This experiment has been carried out by using the 1 × 10⁻⁴ M concentration of complexes (vide supra) and demonstrated that addition of ADP/ATP (5.0 equiv.) into the solutions of 1 and 2 do not exhibit significant changes in the d-d transition bands (Fig. S10, ESI†).

To gain deep insights into the interaction between complexes (1 and 2) and ADP/ATP, UV/vis titration studies have been undertaken (Fig. 5 and 6). Addition of ADP (0.5 equiv.) to a solution of 1 (c, 10 μM, DMSO/PBS; v/v, 1 : 99) displayed a significant hypochromic shift for both HE [323 nm (Δε, 1.9 × 10³ M⁻¹ cm⁻¹)] and LE bands [386 nm (Δε, 0.8 × 10³ M⁻¹ cm⁻¹)]. Notably, the HE band also displayed a small bathochromic shift (Δλ = 1 nm) with the appearance of a new band at 259 nm (ε, 4.55 × 10⁴ M⁻¹ cm⁻¹; Fig. 5a). Further additions of ADP (1.0–6.0 equiv.) led to saturation for both HE (Δε, 1.02 × 10⁵ M⁻¹ cm⁻¹; Δλ, 4 nm) and LE bands (Δε, 4.9 × 10⁴ M⁻¹ cm⁻¹) and at this stage it exhibited a large hyperchromic shift for the band at 259 nm (ε, 1.24 × 10⁵ M⁻¹ cm⁻¹) with a clear isosbestic point at 279 nm. Similarly, aliquot additions of ATP (0.5–10.0 equiv.) to a solution of 1 resulted in a continual decrease in the optical density for the HE band (λ, 323 nm) with a small bathochromic shift (Δε, 0.79 × 10⁴ M⁻¹ cm⁻¹; Δλ = 3 nm). In addition, the LE band (λ, 386 nm) also exhibited a significant hypochromic shift (Δε, 2.96 × 10⁴ M⁻¹ cm⁻¹) without alteration in the peak position. Simultaneously, a new strong band emerged at 258 nm (ε, 1.15 × 10⁵ M⁻¹ cm⁻¹) with a distinct isosbestic point at 280 nm (Fig. 5b). The newly generated bands at 259 (1 + ADP) and 258 nm (1 + ATP) are most probably associated with the self-absorbance of ADP and ATP in solution. Significant alterations in both the LE and HE bands (inset, Fig. 5) clearly indicated strong interactions between 1 and ADP/ATP in the given media. Therefore, for precise calculation of the association constant (Kₐ) and limit of detection (LOD), changes for the band at 323 nm have been considered. The Kₐ value estimated by the Benesi–Hildebrand (B–H) method for 1 came out to be 2.12 ± 0.02 × 10⁵ M⁻¹ and 6.31 ± 0.05 × 10⁴ M⁻¹, respectively for ADP and ATP (Fig. S11, ESI†). The LOD for 1 has been calculated to be 1.92 μM (ADP) and 27.12 μM (ATP). Based on these results it has been concluded that 1 exhibits strong binding affinity for ADP relative to ATP.

In an analogous manner the interaction between 2 (c, 10 μM, DMSO/PBS; v/v, 1 : 99) and ADP/ATP has been investigated by UV/vis titration studies under analogous conditions. Addition of ADP (0.5–10.0 equiv.) to a solution of 2 displayed hypochromic as well as bathochromic shifts for the HE band at 319 nm (Δε, 0.73 × 10⁴ M⁻¹ cm⁻¹; Δλ, 4 nm) with a concomitant hypochromic shift for the LE band at 410 nm (Δε, 0.28 × 10⁴ M⁻¹ cm⁻¹). A new band emerged at 257 nm (ε, 1.12 × 10⁵ M⁻¹ cm⁻¹) along with a clear isosbestic point at 281 nm (Fig. 6a). Conversely, addition of ATP (0.5–5.0 equiv.) to a solution of 2 exhibited significant hypochromic as well as bathochromic shifts for the HE band at 319 nm (Δε, 1.04 × 10⁵ M⁻¹ cm⁻¹; Δλ, 4 nm) along with a noticeable hypochromic shift (Δε, 0.38 × 10⁴ M⁻¹ cm⁻¹) for the LE band at 410 nm. It also

![Fig. 5](image-url)

UV/vis titration spectra for 1 with the increasing amount of ADP (0.0–6.0 equiv.) (a) and ATP (0.0–10.0 equiv.) (b), [insets show clear changes in HE and LE bands].
displayed a new band at 257 nm ($\epsilon, 1.62 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) with a clear isosbestic point at 282 nm (Fig. 6b). The association constants ($K_a$) for 2 in the presence of ADP and ATP have been calculated as $1.04 \pm 0.03 \times 10^{5} \text{ M}^{-1}$ and $2.91 \pm 0.01 \times 10^{5} \text{ M}^{-1}$ (Fig. S12, ESI†) and LOD came out to be 13.21 μM and 0.54 μM for ADP and ATP, respectively. High $K_a$ and low LOD value irrefutably suggested strong binding affinity and greater sensitivity of 2 toward ATP relative to ADP. It is noteworthy to mention that sensitivity order for 2 reversed relative to 1. It may be attributed to a large cavity size for 2 relative to 1 which is achieved most likely due to greater steric requirements of the naphthalene than the salen core to stabilize the cavity.

The job’s plot analysis revealed a 1:1 stoichiometry between complexes (1 and 2) and ADP/ATP (Fig. S13 and S14, ESI†) which signifies that the cavity of the hosts may accommodate one unit of the guest molecules via various types of weak/supramolecular interactions. The worth of the supramolecular cavity toward host guest interactions has been substantiated by UV/vis spectral studies using $H_2L_1$ and $H_2L_2$ in the presence of ADP/ATP which displayed insignificant perturbations in the spectral features (Fig. S15, ESI†). This study proved a non-negotiable role of the supramolecular architectures of 1 and 2 to interact with ADP/ATP. It also supported the origin of supramolecular interactions holding the guest moieties only in the complexed form of the ligands $H_2L_1$ and $H_2L_2$ but not in the uncoordinated state.²

**$^1$H and $^{31}$P NMR spectral studies**

To gain deep insights into interactions between complexes and ADP/ATP, NMR titration studies have been performed. $^1$H and $^{31}$P NMR titration studies have been carried out using fixed concentrations of ADP/ATP (c, 1.0 mM) in D$_2$O and varying the amounts of 1 and 2 (c, 5.0 mM, DMSO-$d_6$). The $^1$H NMR spectra of ADP/ATP displayed two distinct singlets in the aromatic region [$\delta$ 8.42 and 8.22 ppm, ADP; 8.41 and 8.24 ppm, ATP] attributable to adenine $\beta$H and $\alpha$H protons. In the presence of 0.5 equiv. of 1, the signals due to the adenine protons exhibited a significant downfield shift ($\Delta\delta, 0.14$ ppm, $H_\beta$; $0.05$ ppm, $H_2$, Fig. 7). Upon further addition of 1 (1.0 equiv.) these resonances broadened and displayed a continual downfield shift ($\Delta\delta, 0.26$, $H_\beta$; $0.08$ ppm, $H_2$). Similarly, addition of 2 (0.5–1.0 equiv.) to a solution of ATP led to a significant downfield shift ($\Delta\delta, 0.20$ ppm, $H_\beta$ and 0.06 ppm, $H_2$) and broadening of the signals due to adenine protons which finally resonated at $\delta$ 8.62 and 8.30 ppm (Fig. S16, ESI†). It clearly indicated that the signal associated with the adenine moiety from ADP/ATP undergoes a significant alteration on gradual addition of 1 and 2 while other resonances hardly show any change. These results affirmed effective $\pi-\pi$ stacking between the adenine moiety of the ADP/ATP and the salen/naphthalene core of 1 and 2. Besides, the observed broadening for signals may also partially be accredited to the presence of paramagnetic Cu(i), but the associated shift in resonances holds the validity of the results.

Further, to deduce information about involvement of the phosphate group in weak interactions, $^{31}$P NMR spectral studies have been carried out under analogous conditions. The $^{31}$P NMR spectra of ADP and ATP displayed two ($\delta$, $-5.07$ ppm, $\alpha$; $-15.84$ ppm, $\beta$) and three ($\delta$, $-6.87$ ppm, $\gamma$; $-7.38$ ppm, $\alpha$; and $-18.89$ ppm, $\beta$) distinct signals due to $^{31}$P nuclei (Fig. 8). Addition of 1 (0.5 equiv.) to a solution of ADP exhibited a significant upfield shift ($\Delta\delta, -0.08$ ppm, $\alpha$; $-0.17$ ppm, $\beta$) and broadening of the signals associated with $^{31}$P nuclei of the phosphate group.²¹ Upon further addition of 1 (1.0 equiv.), the resonances due to the phosphate group assumed an upfield shift [$-0.12$ ($\alpha$) and $-0.29$ ppm ($\beta$)]. Likewise, addition of 2 (0.5 equiv.) to a solution of ATP displayed an upfield shift ($\Delta\delta$) for signals due to $^{31}$P nuclei [$-0.17$ ($\gamma$); $-0.12$ ($\alpha$); $-0.36$ ppm ($\beta$)] (Fig. S17, ESI†). Further addition of 2 (1.0 equiv.) to a solution of ATP showed significant broadening for signals associated with $\alpha$ and $\gamma$ $^{31}$P nuclei which ultimately merged together. Noticeable shifts and broadening of $^{31}$P NMR signals clearly suggested the involvement of the phos-
phosphate group in interaction with the metal centres. On the basis of comparative studies with other related systems the observed changes in $^1$H and $^{31}$P NMR spectra may be taken as quite significant to support strong interactions of ADP/ATP with 1 and 2. Ramaiah et al. reported a Cu(n) metalloccylophane exhibiting strong interactions ($K_a = 1.2 \pm 0.1 \times 10^4$ M$^{-1}$) with GMP. It shows only a decrease in intensity and broadening in $^1$H and $^{31}$P NMR signals without alteration in the peak position. Similarly, other systems also showed comparative changes in their spectral patterns. $^{5a,21}$

$^1$H and $^{31}$P NMR spectral results substantiated the occurrence of strong interactions between 1 and 2 with ADP/ATP. Here, we conclude that the adenine moiety of ADP/ATP interacts with the salen/naphthalene core via $\pi-\pi$ stacking, while phosphate groups are involved in the electrostatic interaction with Cu(n) centres. These interactions most probably orient the nucleotide bases in such a way that they get involved in effective interactions of the Cu(n) via phosphates and also facilitate $\pi-\pi$ interactions between the adenine and the salen/naphthalene core leading to the formation of adducts 1-ADP.

**Fig. 7** $^1$H NMR titration spectra for ADP (D$_2$O) with varying concentrations of 1 (blue dotted lines show downfield shifting in H8 and H2 adenine protons).

**Fig. 8** $^{31}$P NMR titration spectra for ADP (D$_2$O) with varying concentrations of 1 (blue dotted lines show upfield shifting in $^{31}$P signals).
and 2-ATP. Moreover, they supported the selectivity of 1 and 2 toward ADP/ATP over other NPPs owing to an appropriate spatial orientation of the host guest molecules. Due to the significant shift in the $^1$H NMR spectra of ADP/ATP in the presence of 1 and 2, one may presume the binding of adenine with the Cu(u) centre as well. However, this should also be reflected as a significant change in the d-d transition bands in the UV/vis spectra. But, as discussed earlier by the UV/vis experiments, which are complexed supramolecular inorganic moieties and relatively lower intensity of certain peaks related to adducts shifts in the resonance signal may be significantly attributed to the π-π stacking and electrostatic interactions.

ESI-MS studies

The composition of the species resulting from the interaction of 1 and 2 with ADP/ATP has been authenticated by ESI-MS studies in a positive mode (Fig. S18–S21, ESI†). ESI-MS of the ultimate products arising from the interaction of 1 with ADP and ATP showed feeble molecular ion peaks at $m/z$ 2099.2877 [$[1 + (ADP)^{2+} + 3H]^+$; 8%] and 2099.2877 [$[1 + (ATP)^{2+} + 3H]^+$; 7%] which indicated the formation of the adducts in a 1:1 molar ratio (1-ADP and 1-ATP). Likewise, ESI-MS of 2 + ADP/ATP exhibited low intensity peaks at $m/z$ 2219.7501 [$[2 + (ADP)^{2+} + 3H]^+$; 4%] and 2299.5135 [$[2 + (ATP)^{2+} + 3H]^+$; 5%] assignable to 1:1 adducts (2-ADP and 2-ATP). The ensuing spectra for the adducts occurred due to the monocationic species ($2/[2 + (ADP)^{2+} + 3H]^+$) which most probably acquire protons from the solution. Moreover, the adducts are mostly likely anionic and thus negative ion ESI-MS has also been acquired which displayed no clear signals associated with the molecular ions. Further, the isotopic patterns based on natural abundance for the resulting adducts corroborated well with their simulated one (inset, Fig. S18–S21, ESI†). The relatively lower intensity of certain peaks related to adducts which are complexed supramolecular inorganic moieties and can easily undergo extensive fragmentations due to high voltage, particularly used in ESI-MS experiments. Thus, the presence of molecular ion peaks at relevant positions and matching isotopic patterns with the simulated one significantly attested the proposed formulations.

In addition, stability of 1 and 2 in solution has been monitored by UV/vis spectral studies. In this direction the absorption spectra of a fresh solution of 1 and 2 (c, 10 μM) has been compared with the one acquired under analogous conditions after keeping the solution for 10 days. We found that it displayed similar spectral features and concluded that their solutions are quite stable for longer durations (Fig. S22, ESI†). Thus, we conclude that indeed the complexes 1 and 2 undergo strong supramolecular interactions in the presence of ADP/ATP as tetranuclear complexes.

Electrochemical studies

The redox behaviour of $H_2L^1$, $H_2L^2$ and 1–2 (c, 100 μM, DMSO) has been investigated by cyclic voltammetric studies (Fig. 9) and the resulting data are presented in Table S2 in the ESL†. The cyclic voltammograms (CV) of 1 and 2 illustrated irreversible oxidation waves at $E_{pa}$ 0.564 (I, −4.15 μA) and 0.554 V (I, −3.66 μA) associated with ligand moieties (Fig. 9b). In addition, they displayed irreversible reduction double waves [$E_{pc}$, −0.306 (I, 1.671 μA) and −0.719 V (I, 1.620 μA); 1 and −0.314 (I, 1.509 μA) and −0.717 V (I, 1.465 μA); 2] due to the Cu(u) → Cu(i) redox couple in different environments. The first sharp reduction wave (−0.306 V, 1 and −0.314 V, 2) has been tentatively assigned to the Cu(u) → Cu(i) redox couple for the homoleptic copper centre coordinated with salen/naphthalene moieties, while the second broad wave (−0.719 V, 1 and −0.717 V, 2) may be associated with the reduction of other three copper centres coordinated either only with β-ketoamidino or both salen/naphthalene and β-ketoamidino groups.

To have a clear cut idea about the particular Cu(u) centre which is significantly involved in the interaction of the complexes with ADP/ATP, cyclic voltammetric titration studies have been performed on 1 and 2 by varying the concentration of ADP and ATP at room temperature. Addition of ADP (0.5–10.0 equiv.)
and ATP (0.5–12.0 equiv.) to a solution of 1 showed a negative potential shift with an increase in the current intensity ($\Delta E_{pc}$, 20 mV; $\Delta I$, 0.266 $\mu$A; ADP, and $\Delta E_{pc}$, 15 mV; $\Delta I$, 0.221 $\mu$A ATP) for the wave at $-0.306$ V [associated with the salen coordinated homoleptic Cu(II) centre] (Fig. 10 and S23, ESI†). In addition, another reduction wave at $-0.719$ V displayed an increase in the current intensity ($\Delta I$, 0.541 $\mu$A; ADP and $\Delta I$, 0.365 $\mu$A; ATP) without an appreciable potential shift (Table S3, ESI†). Consequently, addition of ADP (0.5–15.0 equiv.) and ATP (0.5–7.0 equiv.) to a solution of 2 displayed changes analogous to 1 (data summarized in Table S3 and Fig. S24 and S25, ESI†). A small potential shift observed for the reduction wave ($-0.306/-0.314$ V; 1/2) effectively suggested that only one Cu(II) centre, homoleptically coordinated to the salen (1) or naphthalene (2) core is involved in the interaction with the phosphate group of the ADP/ATP. This study provided significant information regarding the mechanism of host guest interactions and is consistent with the above spectral observations and conclusions drawn from the job’s plot analysis.

Optimization of the proposed structures

Based on the above spectral results a model for adducts of 1–2 with ADP/ATP has been proposed (Fig. 11a) wherein salen/naphthalene moieties interacted with adenine through $\pi-\pi$
stacking and phosphate groups with Cu(n) centres coordinated with salen/naphthalene through electrostatic interactions by placing the phosphate chain into the cavity. To support the proposed structures of the adducts for 1 and 2 with ADP/ATP, a molecular docking study has been performed which provided ten most preferable orientations for the host and guest molecules. Among these, some orientations display π–π stacking between the salen/naphthalene and the adenine cores while others revealed insertion of the phosphate chain into the cavity of complexes. The docked structure for 2 + ATP suggested that most of the resulting conjugates exhibit π–π stacking between the adenine core and the naphthalene ring. At the same time, the phosphate chain is partly inserted into the cavity (Fig. 11b). Besides, the other orientations illustrated the interaction of phosphates with Cu(n) centres but direct binding of the adenine core to the metal centre has not been evidenced by any of the preferred orientations. Further, the space filling model for one of the ten most stable orientations clearly indicated partial insertion of the phosphate chain into the cavity with π–π stacking and validated the proposed interactions (Fig. 11b). Likewise, docked structures for other adducts also followed the analogous results and thereby supported the presumed models involving the said interactions and remarkably suggest the preferable interaction sites for ADP and ATP in 1 and 2 (Fig. S26–S28, ESI†).

The above studies revealed competitive interactions of 1 and 2 with ADP and ATP which strongly depend on the size of the ligand core, ensuing cavity in the complexes, and an adenine moiety of the nucleotides. The complexes 1 and 2 exhibited the dissimilar sensitivity for ADP/ATP, as the cavity with the salen core in 1 is appropriate for accommodating ADP while 2 offers an apt site for ATP due to the relatively larger size of the naphthalene. UV/vis spectral studies for ligands vs. ADP/ATP displayed insignificant changes and suggested the foremost importance of the supramolecular cavity in host–guest interactions. The results obtained from 1H and 31P NMR spectral studies advocated π–π stacking between the adenine and salen/naphthalene cores and electrostatic interaction between the phosphate and Cu(n) centres. Further, 1:1 binding between each pair of the complex and nucleotide evidenced by job’s plot analysis supported by ESI-MS studies and cyclic voltammetric studies on 1/2 with ADP/ATP signified that only one Cu(n) centre interacts with the phosphate group through weak electrostatic interactions.

Conclusion

In summary, through this work we have described the synthesis and characterization of two novel asymmetric Schiff base ligands H2L1 and H2L2 possessing different N,O-donor sites and tetracnuclear supramolecular Cu(n) complexes 1 and 2. The only difference in ligands in having the salen and the naphthalene cores leads to a dissimilar supramolecular architecture in 1 and 2. Due to this disparity 1 and 2 acquire diverse sensitivity toward ADP and ATP. Based on the spectral investigations it has been shown that ADP can be accommodated with greater sensitivity by 1 while ATP by 2. The mode of binding in host–guest systems has been established by UV/vis, 1H, 31P NMR and ESI-MS studies followed by cyclic voltammetry. The proposed structures of adducts have been supported by molecular docking studies. Thus, the present study proposed a new system rendering a new approach in host–guest chemistry where selectivity and sensitivity toward the guest species are dependent on minute differences in the ligand moieties that may be utilized in future for designing the host guest systems with desired occupancy.

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