Chapter 5

Zinc Ensemble of Hexaphenylbenzene Derivative for Detection of Anions, Biomolecules and Explosives

Quinoline appended hexaphenylbenzene derivative 36 namely hexakis(N-quinolin-2-ylmethylene)-4-amine-[1,1′-biphenyl]benzene has been synthesized. The compound 36 behaves as a highly selective and sensitive chemosensor for Zn$^{2+}$ ions among various metal ions tested. Derivative 36 can penetrate the cellular membrane of living PC-3 cells and exhibits high emission after binding with the intracellular zinc. Further, the zinc ensemble of hexaphenylbenzene derivative 36 exhibits sensitive response toward adenosine monophosphate (AMP) and H$_2$PO$_4^-$ ions. In addition, the derivative 36 mimics the functions of multichannel molecular keypad in the presence of chemical inputs of Zn$^{2+}$ ions, H$_2$PO$_4^-$ ions and AMP.
5.1 Introduction

Zinc is second most abundant transition metal present in human body after iron and is required as a key component of numerous enzymes and transcription factors. Loose bound \( \text{Zn}^{2+} \) ions have gained a lot of interest due to their strong binding with protein macromolecules. Loosely bound or free zinc occurs in certain regions of the body, including the hippocampus and olfactory bulb in the brain, the mitochondria of the prostate and the pancreas where release of \( \text{Zn}^{2+} \) plays an important role in physiological signaling and other functions. Zinc levels are highly regulated by transporters and disruption of zinc homeostasis leads to several pathologies including ischemia, Alzheimer’s disease and prostate cancer. In this context, design of new zinc imaging tools suitable for studies in living cells is of great interest and significant advances in biological zinc sensor design have been made. Further, the development of chemosensors that can discriminate \( \text{Zn}^{2+} \) from \( \text{Cd}^{2+} \) is still a challenge as both cadmium and zinc have similar properties due to their presence in the same group of the modern periodic table and cause similar spectral changes upon interactions with chemosensors. Thus, designing fluorescent sensors for zinc ions has drawn worldwide attention. On the other hand among anions, phosphate ions are of particular interest as they are key substrates for many biochemical reactions and are main components of biomolecules. Recently, metal based receptors particularly \( \text{Zn}^{2+} \) complexes have been reported for fluorescent sensing of biphosphylated, triphosphylated and polyphosphylated species. However, a few reports are available on metal based receptors for fluorescent sensing of monophosphylated species. Hexaarylbenezene based molecules have been of continuing interest to many researchers for metal inclusion chemistry. Rathore et al., Shionoya et al. and Wang et al. have used this scaffold for sensing of metal ions and to generate metal ions assisted supramolecular architectures like molecular cages and organic capsules. However, the potential of hexaphenylbenzene derivatives is not much explored for fluorogenic chemosensing of transition metal ions, anions, biomolecules and explosives. These conjugated non-planar systems are highly favourable for making thin amorphous layers which can be helpful to fabricate chemosensing devices with practical applications. Keeping this in view, we have designed and synthesized a symmetrically substituted star shaped hexaphenylbenzene derivative appended with quinoline moieties which shows remarkable fluorescence enhancement in the presence of \( \text{Zn}^{2+} \) ions and upholds exceptional selectivity over other physiologically relevant divalent cations. Derivative responds to \( \text{Zn}^{2+} \) ions even in blood
serum milieu, is cell permeable and exhibits dramatic fluorescence enhancement on binding with zinc ions present in the PC-3 cells without the need for additional extracellular zinc, unlike many reported sensors,⁹ thus, is a promising candidate for biological use. Further, we have used the Zn²⁺ ensemble of compound 36 as potential fluorescent probe for H₂PO₄⁻ and AMP and picric acid. Interestingly, this fluorescence Zn²⁺ ensemble is also capable of detecting vapors of PA. In addition, fluorescent TLC strips made of zinc ensemble of derivative 36 have been used as portable, convenient and low cost method for trace detection of PA.

5.2 Results and Discussion

Hexaphenylbenzene, 32 was treated with bromine to give hexakis(4-bromophenyl)benzene, 33¹⁰ as white powder in 92% yield (Scheme 5.1). The synthesis of derivative 26¹¹ has been discussed in chapter 4, page 97. Further, six-fold Suzuki-Miyuara cross coupling of hexakis(4-bromophenyl)benzene, 33¹⁰ with 4-(4,4,5,5-tetramethyl-1,3,2dioxaborolane-2-yl)aniline, 26 furnished hexakis(4-amino(1,1'-biphenyl)-4'-yl)benzene, 34 in 70% yield (Scheme 5.2) which on condensation with 2-quinolinecarboxaldehyde, 35 in N,N-dimethylformamide at room temperature for 3h furnished compound 36 in 71% yield (Scheme 5.3). The structures of compound 33, 34 and 36 were confirmed from their spectroscopic and analytical data. The ¹H NMR spectrum of compound 33 shows two doublets at 6.61 and 7.06 ppm corresponding to the aromatic protons. The ¹H NMR spectrum of compound 34 exhibits four doublets at 6.40, 6.82, 7.01 and 7.07 ppm corresponding to aromatic protons. The ESI-MS mass spectrum of derivative 34 exhibits molecular ion peak of (M+H)⁺ at m/z 1082.718. The ¹H NMR spectrum of derivative 36 exhibits seven doublets at 6.98, 7.26, 7.36, 7.85, 8.14 and 8.22 ppm, one triplet at 7.75 ppm and one multiplet at 7.55-7.57 ppm corresponding to the aromatic protons. In addition to this, it shows a singlet at 8.80 ppm corresponding to the protons of imino groups. A parent ion peak for M⁺ was observed at m/z 1916. 79 in ESI-MS spectrum of compound 36. These spectroscopic data corroborate with the structures 33, 34 and 36 for these compound.

Scheme 5.1
5.2a Binding Studies

To evaluate binding ability of compound 36 toward different metal ions, we carried out UV-vis and fluorescence experiments in ethanol:THF (3:1) by adding aliquots of different metal ions (Cu$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Hg$^{2+}$, Co$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Ag$^{+}$, Ba$^{2+}$, Mg$^{2+}$, K$^{+}$, Na$^{+}$, and Li$^{+}$) as their perchlorate salts. The absorption spectrum of compound 36 (10µM) in a solvent mixture of ethanol:THF (3:1) exhibits two absorption bands at $\lambda_{\text{abs}}$ 287 and 355 nm corresponding to hexaphenylbenzene and quinoline, respectively. Upon addition of Zn$^{2+}$ ions (0-20 equiv.), the band at 355 nm disappeared and the band at 287 nm is red shifted to 305 nm (Figure 5.1). Two isosbestic points are observed at 296 and 325 nm indicating formation of 36-Zn$^{2+}$ complex.

In the fluorescence spectrum, compound 36 (5µM) does not exhibit any fluorescence emission in ethanol:THF (3:1) (Figure 5.2) when excited at $\lambda_{\text{exc}}$ = 287 nm or at $\lambda_{\text{exc}}$ = 355 nm. This is due to photoinduced electron transfer (PET) from imino nitrogen to photoexcited hexaphenylbenzene moiety. Upon addition of increasing amounts of Zn$^{2+}$ ions (20 eq) to the solution of 36 in ethanol:THF (3:1), a fluorescence emission band appeared at 438 nm.
This fluorescence emission band is attributed to the formation of the $36\text{-Zn}^{2+}$ complex due to interaction between Zn$^{2+}$ ions and imino nitrogens and the nitrogen atoms of the quinoline moieties as a result of which the PET from the imino nitrogens to the hexaphenylbenzene moiety is suppressed resulting into fluorescence enhancement. The detection limit of compound 36 as a fluorescent sensor for the analysis of Zn$^{2+}$ ions was found to be 4.5 µM (Figure 5.3). The fluorogenic and colorimetric change in solution of derivative 36 on addition of Zn$^{2+}$ ions can be seen by naked eyes (Figure 5.4A and B).

Under the same conditions as used above for zinc ions, we also tested the fluorescence response of compound 36 to other metal ions such as Cu$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Hg$^{2+}$, Co$^{2+}$, Pb$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Ag$^{+}$, Ba$^{2+}$, Mg$^{2+}$, K$^+$, Na$^+$, and Li$^+$ but no change in emission was observed in the presence of these metal ions (Figure 5.4 and 5.5A). Further, to check the practical applicability of compound 36 as Zn$^{2+}$ sensor, we carried out competitive experiments in the presence of 20 eq of Zn$^{2+}$ mixed with Pb$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Hg$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Mg$^{2+}$,
Ba$^{2+}$, Ag$^+$, K$^+$, Na$^+$, and Li$^+$ of at 2000 equivalent (Figure 5.5B), and no significant variation in the fluorescence intensity change was found by comparison with or without the other metal ions.

Fitting the changes in the fluorescence spectra of compound 36 with Zn$^{2+}$ ions using the nonlinear regression analysis program SPECFIT gave a good fit and demonstrated that 3:1 stoichiometry (metal:receptor) was the most stable species in the solution with binding constant (log $\beta$) =11.0215 with 79% complexation. To elucidate the binding mode of receptor 36 with zinc perchlorate, the IR spectrum of 36-Zn$^{2+}$ complex was compared with IR spectrum of compound 36. The important change was in absorption band corresponding to imino group which shifts from 1625.51 to 1595.02 cm$^{-1}$.
Biological applicability of 36 to sense Zn\textsuperscript{2+} ions was checked by carrying out fluorescence titrations of \textit{in situ} prepared Zn\textsuperscript{2+} complex and titrating it by varying the concentrations of bovine serum albumin (BSA) (Figure 5.6) as well as human blood serum\textsuperscript{13} (Figure 5.7), capable of capturing Zn\textsuperscript{2+} ions. As is evident from the histogram (Figure 5.8), no significant change in the fluorescence intensity of the emission band of ensemble 36-Zn\textsuperscript{2+} at 438 nm was observed either in presence of BSA (Figure 5.6) or blood serum milieu (Figure 5.7).

![Figure 5.8 Histograms showing the fluorescence response of 36 (5 \textmu M) with Zn\textsuperscript{2+} (100 mM) in the presence of varying amounts (\mu l) of either BSA or serum. (a) 10 \mu l; (b) 20 \mu l; (c) 30 \mu l; (d) 40 \mu l; (e) 50 \mu l at pH=7.0.]

Figure 5.9 Fluorescence and brightfield images of PC3 cells lines (on right). (a) Brightfield image of cells (b) Blue fluorescence images of cells treated with probe 36 (1.0 \mu M) only for 20 min at 37 \textdegree C. (c) Overlay of (a) and (b).

We also evaluated the potential biological application of the receptor 36 for detection of Zn\textsuperscript{2+} ions present in prostate cancer (PC-3) cells since the malignant prostate cells possess the ability to accumulate high zinc levels. The prostate cancer (PC3) cell lines were incubated with receptor 36 (5.0 \mu M in ethanol:THF, 3:1), in RPMI-1640 medium for 20 min at 37 \textdegree C and washed with phosphate buffered saline (PBS) buffer (pH 7.4) to remove excess of receptor 36. Microscopic images showed a strong blue fluorescence which indicates that the compound 36 complexes with labile Zn\textsuperscript{2+} ions present in cells (Figure 5.9).
5.2b Zinc Ensemble as Chemosensor for Biomolecules and Anions

In present scenario, the use of metal centers for coordination of analytes has offered an alternative strategy for the design of chemosensors as stereochemical preferences of transition metal ions can impart selective binding tendencies toward various analytes.\textsuperscript{14,15,16} Keeping this in view, the binding ability of the \textit{36-Zn}\textsuperscript{2+} ensemble\textsuperscript{17} in ethanol:THF (3:1) was studied toward different anions and biomolecules (F\textsuperscript{-}, Cl\textsuperscript{-}, Br\textsuperscript{-}, I\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, OH\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-}, OAc\textsuperscript{-}, CN\textsuperscript{-}, H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}, AMP, ADP and ATP). The binding studies exhibited the fast, sensitive and distinct response toward monophosphate ions and AMP in comparison to the other anions, ADP and ATP. In the fluorescence spectrum, upon addition of 6 eq of H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-} ions to \textit{36-Zn}\textsuperscript{2+} ensemble, the emission band at 438 nm was quenched (Figure 5.10). The quenching in emission band at 438 nm can be attributed to decomplexation of the existing \textit{36-Zn}\textsuperscript{2+} ensemble due to interaction of phosphate ions with Zn\textsuperscript{2+} ions so as to revive PET phenomenon (Scheme 5.4). On further addition of H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-} ions upto 12 eq, a new blue shifted band at 366 nm was observed (Figure 5.10) which may be attributed to the formation of stable hydrogen bonded complex between nitrogen atoms of quinoline moieties of derivative \textit{36} and OH group of excess phosphate ions in solution (Scheme 5.4).

On the other hand, addition of AMP leads to enhancement of emission intensity along with slight blue shifting of signal from 438 nm to 431 nm (Figure 5.11). This result suggests that AMP is binding to Zn\textsuperscript{2+} ions without breaking the existing \textit{36-Zn}\textsuperscript{2+} bond. The fluorescence enhancement on addition of AMP to \textit{36-Zn}\textsuperscript{2+} can be attributed to the additional hydrogen bonding between hydrogen of phosphate group of AMP and nitrogen of the quinoline moiety (Scheme 5.4). Such type of emission behaviour in the presence of AMP has been previously reported.\textsuperscript{18} In the case of other anions such as F\textsuperscript{-}, Cl\textsuperscript{-}, Br\textsuperscript{-}, I\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, CH\textsubscript{3}COO\textsuperscript{-}, CN\textsuperscript{-}, ClO\textsubscript{4}\textsuperscript{-},
OH\(^{-}\) and ADP and ATP, no significant change in emission intensity is observed (Figure 5.12). The stronger binding affinity of 36-Zn\(^{2+}\) ensemble towards phosphate ions and AMP over other anions including acetate and halide ions may be attributed to strong coordination of Zn\(^{2+}\) to the monophosphate unit.\(^{19}\) It was found that 36-Zn\(^{2+}\) ensemble has detection limit of \(9 \times 10^{-8}\) and \(9 \times 10^{-7}\) M for \(\text{H}_2\text{PO}_4^{-}\) ions and AMP, respectively (Figure 5.13 and 5.14).

![Scheme 5.4](image)

**Figure 5.12** Fluorescence multispectra of 36-Zn\(^{2+}\) ensemble (ethanol:THF, 3:1) on addition (12 equivalents) of various anions, ATP, ADP and AMP.

**Figure 5.13** The plot of % change in fluorescence of 36-Zn\(^{2+}\) ensemble vs concentration of \(\text{H}_2\text{PO}_4^{-}\) ions.
5.2c Construction of Multichannel Keypad

Recently, there has been a lot of interest in development of molecular logic systems for construction of molecular devices\(^2^{0}\) that have memory elements. Among these devices, the molecular keypad locks have drawn more attention as these systems are based on the sequence of the correct combination of chemical inputs that signifies more secure and classified password. Shanzer et al. has reported a molecular keypad system which unlocks when exposed to the correct password, a sequence of chemicals (base and EDTA) and UV light.\(^2^{1}\) Recent developments in the field of biocomputing have led to biomolecular systems that use chemical information to mimic digital electronics. Katz et al. has constructed a keypad which powers itself in a biofuel cell.\(^2^{2}\) Although there are many reports on customary ON-OFF keypad locks that oscillate between ON state and OFF state,\(^2^{3}\) yet there is no report of such molecular device that generates two different ON states depending upon sequence of chemical inputs. Such circuits have shown a great interest in multiuser gadgets where the different personal user accounts can be operated by applying different passwords without the interference of other users. For instance, multichannel decoder, cellular phones, multichannel signaling, multiuser computers, ATM and security alarms are generally based on these multichannel keypad locks.

Keeping this in view, we have constructed a multichannel keypad system based on chemical inputs that switches between two different fluorescent outputs. To construct this multichannel molecular keypad, we carried out fluorescence titrations of receptor 36 with \(\text{Zn}^{2+}\), \(\text{H}_2\text{PO}_4^-\) and AMP in different sequences in ethanol:THF, 3:1. The emission band was observed at 366 nm when \(\text{Zn}^{2+}\) ions were added to compound 36 followed by the addition of \(\text{H}_2\text{PO}_4^-\).
In another sequence, the fluorescence emission band at 431 nm was observed when AMP was added after the addition of Zn\(^{2+}\) ions in chemosensor 36 (Figure 5.12). Thus depending upon the three different inputs (Zn\(^{2+}\), H\(_2\)PO\(_4\)- and AMP), we have constructed a multichannel ‘ON-ON’ keypad system in which the chemosensor 36 can switch between two different outputs. The three inputs Zn\(^{2+}\), H\(_2\)PO\(_4\)- and AMP are designated as ‘A’, ‘R’ and ‘S’, respectively and two outputs at 431 nm and 366 nm are designated by ‘K’ and ‘T’, respectively. In the first sequence, ‘A’ is added to 36 followed by the addition of ‘R’ to generate the output ‘T’ whereas in the reverse order, the output ‘N’ is generated which represents the off state of emission at 366 nm. In second sequence, ‘A’ is added to 36, followed by the addition of S to generate the output ‘K’ whereas when input sequence is reversed, it leads to the output ‘W’ which represents the off state of fluorescence emission at 431 nm. Thus, we can switch the chemosensor 36 in two different fluorescence emission wavelengths by changing the sequence of the three different inputs to generate a multichannel keypad (Figure 5.15) based on chemical inputs where every sequence characterizes a different password.

5.2d Zinc Ensemble as Chemosensor for Nitro Derivatives

Recently, identification and discrimination of closely related nitroaromatics is a challenge and is a significant need for national security and law enforcement. Nitroaromatics have a great tendency to bind with oxidized fluorophores and quench their fluorescence due to an electron transfer process. The chemosensing ensemble approach involving use of metal centers for coordination of analytes has attracted a lot of interest. In this connection, we planned to explore the possibility of utilizing 36-Zn\(^{2+}\) ensemble in ethanol:THF (3:1) as fluorescent sensor for nitro-aromatic/aliphatic compounds. For efficient fluorescence sensing of nitro-aromatic/aliphatic compounds, strong affinity between analytes and emissive component and adequate energy level matching are key factors.

The binding ability of 36-Zn\(^{2+}\) ensemble in ethanol:THF (3:1) as fluorescent sensor for nitro-aromatic/aliphatic compounds such as picric acid (PA), 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT), 1,4-dinitrobenzene (DNB) and 2,3-dimethyl-2,3-dinitrobutane (DMNB) was studied. Upon addition of increasing amount of picric acid in 36-Zn\(^{2+}\) ensemble, the absorption band at 305 nm is red shifted to 321 nm along with the increase in absorption (Figure 5.16) which shows the interaction between picric acid and 36-Zn\(^{2+}\) ensemble. The fluorescence emission changes of chemosensing ensemble 36-Zn\(^{2+}\) (5 μM) shows quenching upon addition of increasing amounts of picric acid (35 μM) as shown in Figure 5.17. A linear Stern-Volmer plot of quenching with PA was observed (Figure 5.18)
with high Stern-Volmer quenching constant of $1.6 \times 10^5 \text{ M}^{-1}$. The detection limit of $36-\text{Zn}^{2+}$ ensemble was found to be 50 nM for PA (Figure 5.19).

Interestingly, under similar conditions, no significant change in fluorescence emission of $36-\text{Zn}^{2+}$ ensemble was observed in the presence of other nitroderivatives (Figure 5.20). The fluorescence quenching is probably due to energy transfer from the excited state of the $36-\text{Zn}^{2+}$ ensemble to the ground state of nitro-derivatives indicated by spectral overlap of picric acid with the emission spectra of ensemble in the wavelength region of 370-470 nm (Figure 5.21). It is evident from the fluorescence studies that the most electron-deficient aromatic substrates engendered the greatest quenching i.e. the greater the number of electron-withdrawing nitro (-NO$_2$) groups present on benzene/toluene core, the more extensive the degree of fluorescence quenching. The better quenching efficiency of picric acid may be attributed to high polarizability of PA in comparison to other nitro derivatives. The histogram of fluorescence titrations of $36-\text{Zn}^{2+}$ ensemble with various nitroderivatives shows that the $36-\text{Zn}^{2+}$ ensemble can be used for selective detection of PA over other explosives (Figure 5.20).
To enhance the practical application of this ensemble based chemosensor, we studied the vapour phase detection of PA by 36-Zn\(^{2+}\) ensemble. We exposed solution of 36-Zn\(^{2+}\) ensemble in EtOH:THF (3:1) to vapors of PA by inserting the vial containing solution into a sealed vial containing solid picric acid at room temperature. The 11% quenching of fluorescence of 36-Zn\(^{2+}\) ensemble was observed within five minutes which went up to 64% quenching of emission intensity after 45 min at room temperature (Figure 5.22). This result reveals the sensitivity of 36-Zn\(^{2+}\) ensemble towards picric acid in solution as well as in vapor phase.

To turn the detection of PA easier and sensitive, we prepared fluorescent TLC strips by running them in solution of 36-Zn\(^{2+}\) ensemble and drying under the vacuum (Figure 5.23B). These test strips were placed in the sealed vial carrying PA powder for 30 minutes and change in fluorescence was observed. Interestingly, a significant quenching in fluorescence of TLC strip was observed with naked eye (Figure 5.23A). To check the detection of traces PA in contact mode, the fluorescent TLC strip was run in solution of PA (10\(^{-10}\) M) resulted in
fluorescence quenching observable to the naked eye (Figure 5.23C). This provides an easy and economical way for trace detection of PA in vapour as well as contact modes.

5.3 Conclusion
In conclusion, we designed and synthesized a novel hexaphenylbenzene derivative which showed fluorescence enhancement in the presence of Zn$^{2+}$ ions. Derivative 36 can penetrate the cellular membrane of living PC-3 cells and exhibits high emission after binding the intracellular zinc. Further, zinc ensemble serves as an efficient chemosensor for adenosine monophosphate and H$_2$PO$_4^-$ ions. It works as a multichannel keypad system by using Zn$^{2+}$, phosphate and AMP as chemical inputs and emission wavelengths as outputs. Besides, the Zinc ensemble of derivative 36 has been used for selective and sensitive detection of PA in solution phase as well as in vapour phase.

5.4 Experimental Section

5.4a General Experimental Methods:

Physical Measurements: Same as given in chapter 2, page 70.

5.4b UV-vis and Fluorescence Titrations:

UV-vis titrations were performed at 10 µM solution of compound 36 in EtOH:THF (3:1). Fluorescence titrations were performed using 5 µM solution of compound 36 in EtOH:THF (3:1). In titration experiments, each time a 3 ml solution of derivative 36 (5 µM in EtOH:THF, 3:1) were filled in a quartz cuvette (path length, 1 cm) and solutions of metal ions were added into the quartz cuvette by using a micro-pipet.

5.4c Fluorescence Quantum Yield
Same as given in chapter 3, page 88.

5.4d Experimental Details of Determining Detection Limit
Same as given in chapter 2, page 71.

5.4e Experimental Details of Preparation of TLC Test Strips
The TLC strip was run in solution of 36-Zn$^{2+}$ ensemble and dried in air and used for detection of PA in vapor mode and in contact mode.

5.4f Experimental Detail of Vapour Phase Detection of PA
Same as given in chapter 2, page 71.
5.4g Syntheses

**Synthesis of Hexakis(4-bromophenyl)benzene (33)** A slurry of compound 32 (0.20 g, 0.37 mmol) and bromine (0.41 ml, 2.6 mmol) was stirred at room temperature for 1h. After 1h, a saturated solution of NaHSO₃ was added to the reaction mixture. The resulting precipitates were filtered and washed with acetone to give compound 33. Yield (0.350, 92 %). ¹H NMR (300 MHz, CDCl₃) δ (ppm) 6.61 (d, J = 9 Hz, 12H, ArH), 7.06 (d, J = 9 Hz, 12H, ArH).

**Synthesis of Hexakis(4-amino(1,1'-biphenyl)-4'-yl)benzene (34)** To a solution of hexakis(4-bromophenyl)benzene 33 (0.30 g, 0.30 mmol) and boronic ester 26 (0.46 g, 2.10 mmol) in 14ml of THF:Toluene (1:1) were added powdered NaOH (0.30 g, 7.50 mmol) and PdCl₂(PPh₃)₂ (0.11 g, 0.15 mmol) under inert atmosphere and reaction mixture was refluxed at 110 °C for overnight. After the completion of the reaction, the THF and toluene was removed under vacuum and the residue so obtained was treated with methanol. The resulting solid was filtered and recrystallized from THF and methanol to give compound 34 (0.23 g, 70 %); ¹H NMR (300 MHz, CDCl₃:DMSO-d₆, 4:6): δ (ppm) = 6.40 (d, J = 6.0 Hz, 12H), 6.82 (d, J = 6.0 Hz, 12H), 7.01 (d, J = 6.0 Hz, 12H), 7.07 (d, J = 6.0 Hz, 12H). ¹³C NMR (100 MHz, DMSO-d₆): δ (ppm) 79.12, 114.10, 123.00, 126.51, 131.43, 136.66, 137.83, 139.99, 148.01. ESI-MS: m/z 1082 (M+H⁺). Elemental analysis Calcd for C₁₂H₈N₂: C, 86.64 %; H, 5.59 %; N, 7.77%; Found: C, 86.58%; H, 5.46 %; N, 7.66 %.

**Synthesis of Hexakis(4-(quinolin-2-ylmethylene)amino(1,1'-biphenyl)-4'-yl)benzene (36)** A clear solution of compound 34 (0.05 g, 0.05 mmol) and quinoline-2-carboxaldehyde 35 (0.06 g, 0.38 mmol) in dry DMF (4ml) was stirred at room temperature. After 3h, the reaction mixture turned turbid. The chloroform (10-15 ml) was added to make this turbid mixture clear. The solution was concentrated under vacuum and treated with methanol resulting in formation of precipitates. The precipitates were filtered and recrystallized from chloroform:methanol (1:9) to afford the yellow coloured compound 36 (0.06 g, 71 %); ¹H NMR (300 MHz, CDCl₃) δ 6.98 (d, J = 9Hz, 12H, ArH), 7.26 (d, J = 9Hz, 12H, ArH ), 7.36
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(d, J = 9Hz, 12H, ArH), 7.53 (m, 24H, ArH), 7.75 (t, J = 3Hz, 6H, ArH), 7.85 (d, J = 6Hz, 6H, ArH), 8.14 (d, J = 6Hz, 6H, ArH), 8.22 (d, J = 6Hz, 6H, ArH), 8.35 (d, J = 6Hz, 6H, ArH), 8.80 (s, 6H, (N=CH)). 13H NMR (75MHz, CDCl₃) δ 27.82, 115.26, 118.71, 121.63, 125.26, 127.26, 127.71, 128.86, 129.69, 129.85, 131.12, 131.86, 132.05, 134.12, 136.54, 147.97, 154.93. ESI-MS: m/z 1916.79. IR (KBr): νmax = 1625 cm⁻¹. Elemental analysis Calcd for C₁₂H₈N₂: C, 86.49 %; H, 4.73 %; N, 8.77%; Found: C, 86.40 %; H, 4.62 %; N, 8.65 %.

5.5 References


The blood serum was isolated by centrifugation of the fresh blood sample of a healthy volunteer after fasting at 4000 rpm for 20 minutes at 4°C. The stock solution of blood serum was prepared by dissolving 100 µl of serum in 1 ml solution of HEPES buffer at pH = 7.0


