Section 2c: Switching and Fluorescence Signal Amplification with Metal Ions in Allosteric Systems Based on 1,3-alternate Thiacalix[4]crown

In section 2b, a fluorogenic chemosensor based on thiacalix[4]arene of 1,3-alternate conformation possessing pyrene moieties was discussed which exhibits excimer emission indicating that the two pyrene units are stacked parallel. The excimer emission gets quenched on addition of Pb$^{2+}$, Hg$^{2+}$ and Cu$^{2+}$ ions. However, chemosensors showing fluorescence enhancement on addition of analytes are preferred over chemosensors showing fluorescence quenching. Fluorescence quenching is unfavourable for a high signal output upon recognition and hampers temporal separation of spectrally similar complexes with time resolved fluorometry. Chemosensor showing fluorescence enhancement permits a lower detection limit and high speed spatial resolution.\(^{179}\)

Therefore, in continuation of this work on fluorogenic molecular switches based on (thia)calix[4]arenes, we have now designed and synthesized new receptors 15 and 18 based on thiacalix[4]arene of 1,3-alternate conformation possessing anthracene and quinoline moieties linked through imine units. The incorporation of imino group is expected to make PET operational which quenches the fluorescence. The binding of a guest ion will make PET un-operational and thus causing fluorescence enhancement. The anthracene is known to combine efficient dual (monomer/excimer) fluorescence emission properties and the ability to generate several types of photodimers whose geometry depends on electronic and conformational factors.\(^{180}\) On the other hand quinoline unit as a fluorophore is introduced to increase the number of soft metal binding sites.

The receptors 15 and 18 exhibit switching behaviour between Fe$^{3+}$/K$^+$ and Hg$^{2+}$/K$^+$ ions respectively (\textit{vide infra}).\(^{181}\) The Fe$^{3+}$ binds to imino nitrogen and K$^+$ binds to the crown ether ring in chemosensor 15. The complexation of 15 with Fe$^{3+}$ "switch off" the recognition of crown ether ring and act as a gateway, which regulates the binding of K$^+$.


ion to crown moiety. On the other hand, receptor 18 showed fluorescence enhancement with switching between (Hg\(^{2+}/K^+\)) ions. The metal ions Fe\(^{3+}\), Hg\(^{2+}\) and K\(^+\) ions are significant in living systems and environment. Iron plays a crucial role in the growth and development of living systems.\(^{182}\) The trivalent form of iron (Fe\(^{3+}\)) is an essential element in human beings. It provides oxygen carrying capacity of heme and acts as a co-factor in many enzymatic reactions involved in mitochondrial respiratory chain.\(^{183}\) Mercury is also one of the most significant cations as compounds of mercury have been used in medicine for example mercury (I) chloride is used as diuretic, disinfectant, and as laxative. The toxic effects\(^{184}\) of mercury in the environment have been well documented and its contamination is wide spread which arises from a variety of natural and anthropogenic sources including oceanic and volcanic emission, gold mining, solid waste incineration and combustion of fossil fuels. The exposure to mercury even at very low concentration, leads to digestive, kidney and especially neurological diseases.\(^{185}\) Similarly K\(^+\) is also an important cation in human body. It is an important blood electrolyte and its plasma concentration must be maintained at defined levels for normal physiological functioning.\(^{186}\) Fluctuation in plasma levels of K\(^+\) can lead to hypokalemia and hyperkalemia.\(^{187}\)

Thus, condensation of thiacalix[4]crown diamine 3 with 9-anthracene carbaldehyde 14 in CHCl\(_3_/MeOH furnished compound 15 (79 %), mp. 240 °C, FAB-MS m/z 1341 (M+1)\(^+\) (Scheme 2.5). We also synthesized a model compound 16 (81%), mp. 235 °C, FAB-MS m/z 1267 (M+1)\(^+\) (Scheme 2.6) from a known precursor 12\(^{172}\) without incorporating the crown ether moiety. The \(^1\)H NMR spectra of compounds 15 and 16 showed two singlets (18H each) at 1.29, 1.29 and 1.36, 1.32 ppm corresponding to the tert-butyl protons, two

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triplets (4H each) at 3.08, 3.66 ppm for NCH$_2$ and 3.51, 3.90 ppm corresponding to OCH$_2$ protons, two singlets (4H each) at 7.43, 7.39 and 7.55, 7.58 ppm corresponding to aromatic protons of thiacalix[4]arene, a multiplet (8H) from 7.26-7.41, 7.30-7.50 ppm, two doublets (4H each) at 7.96, 7.99 and 8.37, 8.43 ppm, a singlet (2H) at 8.47, 8.49 ppm corresponding to aromatic protons of anthracene moiety and a singlet for imino protons (2H each) at 9.41, 9.45 ppm respectively. In addition two broad signals and two triplets (4H each) 3.42, 3.64, 4.01, and 4.40 ppm corresponding to OCH$_2$ protons of crown ring were observed in case of compound 15. A triplet (6H) at 0.67 ppm corresponding to CH$_3$ protons, multiplet (4H) from 1.07-1.19 ppm corresponding to CH$_2$ protons and a triplet (4H) at 4.45 ppm corresponding to OCH$_2$ protons were observed in the case of compound 16. The IR spectra of receptors 15 and 16 showed >C=N stretching bands at 1650 and 1635 cm$^{-1}$ respectively. These spectroscopic data corroborate the structures 15 and 16 for these compounds.

The binding behaviour of compounds 15 and 16 toward different cations (Cu$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Fe$^{3+}$, Fe$^{2+}$, Ba$^{2+}$, Co$^{2+}$, Ag$^{+}$, K$^+$, Na$^+$ and Li$^+$) was investigated by UV-vis and fluorescence spectroscopy. The absorption spectrum of 15 (1 x 10$^{-5}$ M) is characterized by typical absorption bands of anthracene at $\lambda = 340$, 357 and 377 nm in
THF. Of the various metal ions tested (Cu²⁺, Hg²⁺, Pb²⁺, Zn²⁺, Ni²⁺, Cd²⁺, Fe³⁺, Fe²⁺, Ba²⁺, Co²⁺, Ag⁺ K⁺, Na⁺ and Li⁺), a new band is formed at 455 nm only on addition of Fe³⁺ (15.0 equiv.) ions with an isosbestic point at 399 nm (figure 2.39). The formation of a new band at 455 nm is attributed to the interaction of Fe³⁺ ions with the imino nitrogen atoms, leading to intramolecular charge transfer (ICT) from the anthracene moiety to the

**Figure 2.39** UV-vis spectra of 15 (1 x 10⁻⁴ M) in response to the presence of Fe³⁺ ions (15.0 equiv.) in THF.

**Figure 2.40** Fluorescence intensity changes [(I - I₀)/I₀ × 100] of receptor 15 in THF upon addition of various metal perchlorates. The excitation wavelength was 340 nm for 15. I₀ is the fluorescence intensity of each free host, and I is the fluorescence intensity after adding metal ions.

**Figure 2.41** Fluorescence spectra of 15 (1 x 10⁻⁴ M) in response to the presence of Fe⁴⁺ ions (20.0 equiv.) in THF; λₑₓ = 340 nm.

**Figure 2.42** Job’s plot of 15 with Fe⁵⁺ representing stoichiometry 1:1 (host: guest).
The fluorescence spectrum of compound 15 (1 X 10^{-5} M) exhibited weak characteristic anthracene monomer emission at 418 nm with a weak excimer emission band centred at 504 nm in dry THF when excited at 340 nm. The weak monomer emission intensity is attributed to photo induced electron transfer (PET) from imino nitrogen to the photo excited anthracene moiety. The weak excimer band formation indicates that compound 15 exists in partially stacked conformation with two anthracene units close to each other. Upon addition of various metal ions (Cu^{2+}, Hg^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, Ba^{2+}, Co^{2+}, Ag^{+} K^{+}, Na^{+} and Li^{+} ) as their perchlorate salts to solution of compound 15, a change in the fluorescence behaviour was observed. The results are shown in figure 2.40. It was observed that the monomer emission of receptor 15 showed enhancement of 473 % and 200 % on addition of Fe^{3+} (20.0 equiv) and K^{+} (200.0 equiv) ions respectively.

The fluorescence spectra of 15 (1 X 10^{-5} M) at various concentrations of Fe^{3+} ions is shown in figure 2.41. Such fluorescence enhancement with blurring of vibronic bands observed for receptor 15 in the presence of Fe^{3+} ions is attributed to the co-ordination of imino nitrogens of 15 with Fe^{3+} leading to the formation of supramolecular complex, as a result of which the PET from imino nitrogen to anthracene moiety is suppressed. The excimer emission on addition of Fe^{3+} ions initially shows enhancement which slowly budge towards the monomer emission on consequent addition of Fe^{3+} ions and ultimately disappears. This disappearance of excimer emission is a result of conformational change that occurs during the binding of Fe^{3+} ions to the imino nitrogen atoms. In this changed conformation, it is not possible for the anthracene groups to stack in parallel. The binding constant (log β_{1}) was found to be 4.88 between compound 15 and Fe^{3+} ions. Job’s plot^{174} proved 1:1 stoichiometry between 15 and Fe^{3+} ions (figure 2.42). To test the practical applicability of compound 15 as a Fe^{3+} selective fluorescence sensor, competitive experiments were carried out in the presence of Fe^{3+} ions at 20.0 equiv. mixed with other cations (Cu^{2+}, Hg^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, Ba^{2+}, Co^{2+}, Ag^{+} K^{+}, Na^{+} and Li^{+} ) at 100.0 equiv., no significant variation was found by comparison with and without the other metal ions (figure 2.43). This means that compound 15 has a high affinity for Fe^{3+} ions. The fluorescence quantum yield$^{178}$ ($\phi$) of compound 15 in the free and 15.Fe^{3+}
bound state was found to be 0.013 and 0.094, respectively. This substantial increase in the quantum yield\textsuperscript{178} of compound 15 in the presence of Fe\textsuperscript{3+} ions showed its credibility as a good Fe\textsuperscript{3+} sensor. We also carried out a reversibility experiment that proved that Fe\textsuperscript{3+} binding to compound 15 is reversible. The addition of 12.0 equiv of tetrabutylammonium chloride (TBACl) to 15.Fe\textsuperscript{3+} complex restored the fluorescence signal of 15 which is ascribed to strong affinity of chloride ions for Fe\textsuperscript{3+} ions. Thus, chloride ions forms a complex with Fe\textsuperscript{3+} ions which results in decomplexation of the 15.Fe\textsuperscript{3+} complex (figure 2.44). However, the fluorescence was revived again on addition of 30.0 equiv. of Fe\textsuperscript{3+} ions to the solution. The addition of 200.0 equiv. of K\textsuperscript{+} ions to the solution of 15 in THF results in fluorescence enhancement of both monomer and excimer emission (figure 2.45). The reason for the increase in the emission intensity is due to the fact that K\textsuperscript{+} ions bind to the oxygen atoms of the polyether chain as a result of which PET frompolyether unit to the photoexcited anthracene moiety is suppressed resulting in fluorescence enhancement. The binding constant (log \(\beta_1\)) between compound 15 and K\textsuperscript{+} ions was found to be 2.23 and demonstrated 1:1 stoichiometry (host: guest) in solution.
To observe the switching behaviour between Fe$^{3+}$ and K$^+$ ions in chemosensor 15, we carried out experiments wherein we added Fe$^{3+}$ (25.0 equiv.) ions to the solution of 15.K$^+$ complex and K$^+$ ions to 15.Fe$^{3+}$ complex. It was observed that on addition of Fe$^{3+}$ (25.0 equiv.) ions to the solution of 15.K$^+$ complex, the pattern of fluorescence emission was same as that of 15.Fe$^{3+}$ complex (figure 2.46) which indicates that Fe$^{3+}$ moves in and K$^+$ moves out from 15. In the reverse of this metal ion exchange process, when K$^+$ ions were added to solution of 15.Fe$^{3+}$ complex, no change in emission spectrum was observed. Thus, from these experiments we may conclude that
formation of \textbf{15}. Fe\textsuperscript{3+} complex triggers the decomplexation of K\textsuperscript{+} ion and ‘\textit{switch off}’ the recognition ability of crown ether ring.

The binding behaviour of model compound \textbf{16} was evaluated with the help of fluorescence spectroscopy. It was observed that among all the metal ions tested the change in the fluorescence was observed only on addition of Fe\textsuperscript{3+} ions (figure 2.47). No change in fluorescence was observed on addition of K\textsuperscript{+} ions to the receptor \textbf{16} confirming that the fluorescence enhancement observed in the case of \textbf{15} with K\textsuperscript{+} ions is due to interaction of the crown ring with K\textsuperscript{+} ions.

In the next part of investigation anthracene moiety of receptors \textbf{15} and \textbf{16} was replaced with quinoline moiety and receptors \textbf{18} and \textbf{19} were synthesized. Thus, condensation of diamine \textbf{3} with 2-quinoline carboxaldehyde \textbf{17} furnished receptor \textbf{18} (79\%), mp. 238 °C, FAB–MS m/z 1244 (M+1)$^+$ (Scheme 2.7). Using the same procedure we synthesized model compound \textbf{19} (83\%), mp. 242 °C, FAB-MS m/z 1169 (M+1)$^+$ from known precursor \textbf{12}$^{172}$ without a crown motif. The $^1$H NMR spectra of compounds \textbf{18} and \textbf{19} showed two singlets (18H each) at 1.30, 1.26 and 1.35, 1.31 ppm corresponding to the tert-butyl protons, two triplets (4H each) at 3.05, 3.40 ppm for NCH$_2$ and 3.20, 3.87 ppm corresponding to OCH$_2$ protons, two singlets (4H each) at 7.37, 7.27 and 7.41, 7.33 ppm corresponding to aromatic protons of thiacalix[4]arene, and a singlet for imino protons.
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(2H each) at 8.43, 8.48 ppm. In addition two triplets (4H each) at 3.96 and 4.17 ppm and two broad signals (4H each) at 3.39 and 3.60 corresponding to OCH$_2$ protons of crown moiety, two triplets (2H each) 7.55 and 7.70 ppm, one doublet (2H) at 7.81 ppm, one multiplet (6H) from 8.06-8.16 ppm corresponding to aromatic protons of quinoline moiety were observed in case of receptor 18. A triplet (6H) at 0.65 ppm corresponding to CH$_3$ protons, a multiplet (4H) from 1.08-1.27 ppm corresponding to CH$_2$ protons, a triplet (4H) 4.25 ppm corresponding to OCH$_2$ protons, three multiplets (2H, 2H, 4H) from 7.50-7.70, 7.71-7.74, 8.03-8.20 ppm, two doublets (2H each) at 7.82 and 8.32 ppm, corresponding to aromatic protons of quinoline moiety were observed in case of receptor 19. The IR spectra of receptors 18 and 19 showed $>$C=N stretching bands at 1640 and 1635 cm$^{-1}$ respectively. These spectroscopic data corroborate the structures 18 and 9 for these compounds.

The binding behaviour of compound 18 toward different cations was investigated by UV-vis, fluorescence and NMR spectroscopy. The absorption spectrum of 18 (1 x 10$^{-5}$ M) is characterized by typical absorption band of quinoline at $\lambda = 245$ nm in THF. A new absorption band is formed at 335 nm on addition of only Hg$^{2+}$ ions (13.0 equiv.) (figure 2.48) with an isosbestic point at 306 nm, which is ascribed to the interaction of Hg$^{2+}$

![Figure 2.48](image)

**Figure 2.48** UV-vis spectra of 18 (1 X 10$^{-5}$ M) in response to the presence of Hg$^{2+}$ ions (13.0 equiv.) in THF. Inset shows the magnified view of newly formed band on addition of Hg$^{2+}$ ions.

![Figure 2.49](image)

**Figure 2.49** Fluorescence spectra of 18 (1 X 10$^{-5}$ M) in response to the presence of Hg$^{2+}$ ions (16.0 equiv.) in THF; $\lambda_{ex} = 245$ nm.
ions with the imino nitrogen atoms leading to intramolecular charge transfer (ICT) from the quinoline moiety to the imino group. Fluorescence spectroscopy was used to evaluate the binding behaviour of 18 toward different metal ions. The emission spectrum of 18 (1 X 10^{-5} M) in THF showed a characteristic emission band at 315 nm when excited at 245 nm. The addition of only Hg^{2+} (16.0 equiv.) (figure 2.49) and K^{+} (410.0 equiv.) ions (figure 2.50) to the solution of 18 shows enhancement of 903 % and 371 % in the emission intensity (figure 2.51). The fluorescence enhancement with Hg^{2+} ions is attributed to the binding of imino nitrogen of 18 with Hg^{2+} ions which inhibits the PET from imino nitrogen atoms to the quinoline moiety. Under the same conditions as used above for the Hg^{2+} ions, we also tested the fluorescence behaviour of receptor 18 toward various metal ions (Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, Ba^{2+}, Co^{2+}, Ag^{+}, Na^{+} and Li^{+}), no significant variation was observed with any other metal ion (figure 2.51). To test the practical applicability of compound 18 as a Hg^{2+} selective sensor, competitive experiments were carried out using fluorescence spectroscopy in the presence of Hg^{2+} ions at 16.0 equiv mixed with other cations (Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, Fe^{2+}, Ba^{2+}, Co^{2+}, Ag^{+}, Na^{+} and Li^{+}) at 100.0 equiv, no significant variation in the emission was found by comparison with and without the other
metal ions (figure 2.52). Binding constants (log $\beta_i$) of 18 with Hg$^{2+}$ and K$^+$ ions were found to be 5.21 and 2.41 respectively. Job’s plot$^{174}$ proved 1:1 stoichiometry between 18 and Hg$^{2+}$ ions (host/guest) (figure 2.53). The fluorescence quantum yield$^{178}$ ($\phi$) of compound 18 in the free and Hg$^{2+}$ bound state was found to be 0.023 and 0.188, respectively which proved its reliability as good Hg$^{2+}$ sensor. To test if the proposed complex could be reversed, we added tetra butyl ammonium iodide (TBAI) (15.0 equiv.)
to solution of \textbf{18.Hg}^{2+} complex which restored the fluorescence signal of \textbf{18} to its original level (figure 2.54). The fluorescence signal was again revived on addition of Hg\textsuperscript{2+} ions (20.0 equiv.) to the same solution. Fluorescence and NMR spectroscopy was used to observe switching behaviour of receptor \textbf{18} between Hg\textsuperscript{2+} and K\textsuperscript{+} ions. When Hg\textsuperscript{2+} ions (25.0 equiv.) were gradually added to the solution of \textbf{18.K}\textsuperscript{+} complex, the fluorescence intensity shows further enhancement and finally reaches an intensity equivalent to \textbf{18.Hg}^{2+} complex (figure 2.55). This indicates that the addition of Hg\textsuperscript{2+} leads to the decomplexation of \textbf{18.K}\textsuperscript{+} complex with formation of \textbf{18.Hg}^{2+} complex. In the reverse of this metal ion exchange process, when K\textsuperscript{+} ions were added to solution of \textbf{18.Hg}^{2+} complex, no change in emission spectrum was observed. Thus, the formation of \textbf{18.Hg}^{2+} complex triggers the decomplexation of K\textsuperscript{+} ion and ‘switches off’ the recognition ability of crown ether ring. This ‘switch on-switch off’ of the recognition behaviour of \textbf{18} was also studied by a set of \textsuperscript{1}H NMR experiments (figure 2.56). On addition of 1.0 equiv. of

![Figure 2.56 \textsuperscript{1}H NMR spectra of \textbf{18} in CDCl\textsubscript{3}/CD\textsubscript{3}CN (8:2). (A) Free ligand (B) in presence of 1.0 equiv of mercury perchlorate; (C) in presence of 1.0 equiv of potassium perchlorate; (D) addition of 1.0 equiv of mercury perchlorate to ligand/potassium complex. NMR frequency is 300 MHz.](image-url)
Hg\textsuperscript{2+} ions to 18, the signal of imino protons were shifted downfield (Δ\(\delta\) = 1.79 ppm) and signals of protons of quinoline moiety showed an overall downfield shift (\(\delta\) = 7.58-8.59) in comparison to free ligand (\(\delta\) = 7.55-8.41) with further splitting of protons, which indicates that imino nitrogen and nitrogen of quinoline moiety were interacting with Hg\textsuperscript{2+} ions (figure 2.56A and 2.56B). Similarly, on addition of 1.0 equiv of K\textsuperscript{+} ions to 18 there was broadening, splitting and downfield shift of protons of crown moiety (\(\delta\) = 2.8-4.35 ppm) in comparison to free ligand 18 (\(\delta\) = 3.40-4.19 ppm) and there was no shift observed in case of imine protons which indicates that K\textsuperscript{+} ions were interacting with oxygen of crown ring of 18 (figure 2.56C). On adding 1.0 equiv of Hg\textsuperscript{2+} ions to 18.K\textsuperscript{+} complex, \textsuperscript{1}H NMR spectrum of compound completely changed to that of 18.Hg\textsuperscript{2+} complex exhibiting sharp signals of crown moiety and downfield shift of imino protons (Δ\(\delta\) = 1.79 ppm) (figure 2.56D). When K\textsuperscript{+} ions were added to 18.Hg\textsuperscript{2+} complex no spectral changes were observed, which indicates that the complexation of 18 with Hg\textsuperscript{2+} ions suppresses the recognition of K\textsuperscript{+} ion in crown moiety.

The binding behaviour of reference compound 19 was also studied under the similar conditions as used for compound 18. The receptor 19 showed similar fluorescence behaviour with Hg\textsuperscript{2+} ions (figure 2.57) as shown by compound 18 but no change in

![Fluorescence spectra of 19 (1 X 10^{-5} M) in response to the presence of Hg^{2+} ions (20.0 equiv.) in THF; \(\lambda_{ex} = 245\) nm.](image-url)

*Figure 2.57* Fluorescence spectra of 19 (1 X 10^{-5} M) in response to the presence of Hg\textsuperscript{2+} ions (20.0 equiv.) in THF; \(\lambda_{ex} = 245\) nm.
emission was observed on addition of K\(^+\) ions confirming the binding of K\(^+\) in crown ring. To conclude, we designed and synthesised two new ditopic receptors 15 and 18 based on thiacalix[4]arene of 1,3-\textit{alternate} conformation possessing two different complexation sites. A negative allosteric behaviour between cations Fe\(^{3+}/K^+\) and Hg\(^{2+}/K^+\) ions was observed with high selectivity and fluorescence amplification. The cause of negative allosteric behaviour between Fe\(^{3+}/Hg^{2+}\) and K\(^+\) ions in chemosensor 15 and 18 respectively is due to strong binding of Fe\(^{3+}/Hg^{2+}\) with imino nitrogen atoms in comparison to the binding of K\(^+\) ion with the oxygen atoms of crown ether moiety. This is well ascribed from the binding constant values of Fe\(^{3+}/Hg^{2+}\) and K\(^+\) ions with chemosensor 15 and 18 respectively (\textit{vide supra}). The overall switching behaviour in chemosensor 15 and 18 between Fe\(^{3+}/K^+\) and Hg\(^{2+}/K^+\) is shown in figure 2.58.

\[ = \text{15} \quad \text{18} \]

**Figure 2.58** Synthetic allosteric systems 15 and 18 showing negative allosteric behaviour between transition metal ion and K\(^+\) ion.