1. INTRODUCTION

In 1987, the award of Noble prize to Charles J. Pedersen, Cram and Lehn stimulated research activity in the field of supramolecular chemistry. Supramolecular chemistry has been defined by Lehn as “chemistry beyond the covalent bond or the chemistry of associates with well defined structure”. The gross structure of the associates is governed by relatively weak forces such as hydrogen bonds, ion-dipole and dipole-dipole interactions and van der Waals interactions. Although supramolecular chemistry has rapidly expanded into a burgeoning research area encompassing a multitude of disciplines, in this introduction the attention is specifically on crown ethers.

Most of the work on crown ethers was concentrated on the design of selective receptors for both alkali and alkaline earth cations, and very little is known even today about receptors (host molecules) which can simultaneously and selectively complex with several different types of alkali, alkaline earth and heavy metal ions. Such selective complexation of cations by crown ethers for example benzo-18-crown-6 (Fig. 1) derivatives find applications as chemical sensors, selective removal of poisonous or radioactive metal cations from waste streams, membrane transport, immobilisation of radioisotopes and as phase-transfer catalysts.

1.1: Crown ether based complexing systems:

Various crown ether containing sensory systems complexing with alkali, alkaline earth, transition metals and radioactive metal ions are examplied below.

1.1.1: Crown ether sensors for alkali and alkaline earth metals:

Potassium (K⁺) is an essential element of biological fluids of the human body. The daily monitoring of its concentration may be one of the most important indices in the early diagnosis of heart disorder, myocardial infarction, insult, and other diseases. The dibenzo-crown ethers bearing hydroxyl and chloromethyl groups (Fig 2) were representative examples of K⁺
complexing systems. These are highly selective ion-sensitive electrodes for determination of K\(^+\) concentration in biological fluids\(^{10}\).

Similarly, Magnesium ion (Mg\(^{2+}\)) is also plays vital roles in many cellular processes. For example, as an enzyme cofactor, stabilization of DNA conformation, ion transport through the membrane, maintenance of cell shape, and signal transduction. As a result, a great deal of effort has been devoted to the design and synthesis of sensitive and selective sensors for magnesium. The synthesized and developed crown ether based sensors\(^{11}\) 5,6 (Fig 3) were effectively play important roles as intracellular messengers in the regulation of cell function.

**Fig 2: Examples of K\(^+\) selective crown ethers**

**Fig 3: Examples of Mg\(^{2+}\) selective crown ethers**

1.1.2: **Crown ether sensors for transition metals and heavy metal cations:**

Out of various transition and heavy metal ions the concentration of Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\) were need to be monitored in biological systems due to their toxic effects. Hence the development of crown ether based sensory systems for above metal ions is of interest several bioorganic chemists. In this view
Copper ($\text{Cu}^{2+}$) is the third-most abundant transition metal in the body and in the brain, but alterations in its cellular homeostasis are connected to serious neurodegenerative diseases, including Menkes and Wilson diseases, familial amyotrophic lateral sclerosis, Alzheimer’s diseases, and prion diseases. Because of its essential yet toxic nature, cells exert strict control over intracellular copper distributions, and the thermodynamically estimated level of free copper in the cytosol of bacterial model systems is less than one ion per cell. A novel fluorene-based copolymer with aza-15-crown-5 as pendant group was synthesized by C, Yang et al$^{12}$ (Fig 4, 7). It is specific detection for CuSO$_4$ by the synergistic effect of counterion. This system may find use in the sensory of some pesticides containing CuSO$_4$.

Zinc ($\text{Zn}^{2+}$) is one of the transition metal, which is used in paint, electroplating, pharmaceutical and chemical industries and thus occurs widely in the environment. It is a vital component in many cellular processes. Although traditionally the study of Zn$^{2+}$ bioinorganic chemistry has focused on its structural role and catalytic functions in proteins, the neurobiology of Zn$^{2+}$ has been a subject of increasing attention. The Zn$^{2+}$ ion has the ability to modulate a variety of ion channels, may play a role in neuronal death during seizures, is pertinent to neurodegenerative disorders and may be vital to neurotransmission. The synthesized compounds$^{13}$ 8,9 (Fig 5) are targeted as Zn$^{2+}$ selective sensors over the other metal cations.
Silver (Ag⁺) ions and silver compounds show a toxic effect on some bacteria, viruses, algae and fungi, typical for heavy metals like lead or mercury, but without the high toxicity to humans that are normally associated with them. Its germicidal effects kill many microbial organisms in vitro. Below dibenzo-16-crown-4 was synthesized¹⁴ (10) by M, Ouchi et al., and is selectively for Ag⁺ and Th⁺.

Fig 6: Examples of Ag⁺ sensitive sensor

Fig 7: Example of Cd²⁺ crown ether
Cadmium (Cd\(^{2+}\)) is an extremely toxic metal commonly found in industrial workplaces, particularly where any ore is being processed or smelted. The effect of its acute poisoning is manifested in a variety of symptoms, including high blood pressure, kidney damage, anemia, hypertension, bone marrow disorders, cancer and toxicity to aquatic biota. 11, 12 (Fig 7) were used as selective chromogenic system for Cd\(^{2+}\) in water\(^{15}\).

Mercury (Hg\(^{2+}\)) is a highly toxic and hazardous environmental contaminant, even at low levels. Excessive exposure of the human body to mercury leads to DNA damage, brain damage, and nervous system defects, including Minamata disease. Considering the extreme toxicity of mercury, the United States Environmental Protection Agency (EPA) provides the standard for the maximum allowed level of mercury in dietary and environmental sources, such as edible fishes, to be 0.55 ppm. The developed fluorescent sensors\(^{16}\) 13, 14 (Fig 8) were utilized effectively in the determination of Hg\(^{2+}\) concentrations in waste effluent streams and drinking water.

![Fluorescent sensor for Hg\(^{2+}\) detection](image1)

Out of several heavy metals and its toxicity effects, lead (Pb\(^{2+}\)) poisoning remains the world’s most common environmentally caused disease. Once introduced into the body, lead is a potent neurotoxin that can interfere with brain development, slow nerve conduction velocity, and trigger behavioral problems. Synthesized fluorescent sensors\(^{17}\) 15, 16, 17 (Fig 9) for detecting Pb\(^{2+}\) concentration in living cells and in water. Leadfluor-l (15) was particularly used as it has attractive optical properties and biological compatibility.
Fig 9: Some of the examples of Pb$^{2+}$ Selective sensors

It is well known that, because of the relatively high mobility of uranium in surface and near surface environments, its measurement in trace amounts in natural waters could be used as a basis for geochemical exploration. Uranium dioxide powder is a starting material for the preparation of fuel pellets, which are widely used in nuclear power reactors. In this process, several steps are necessary including leaching from ores, purification by ion-exchange and solvent extraction, precipitation, reduction, etc. The uranium monitoring in all these process streams as well as in radioactive wastes is of increasing interest. The complexing systems\textsuperscript{18} 18, 19 (Fig 10) used as uranium selective extractants from radioactive waste. It is therefore essential to have uranium ion selective sensor for monitoring environments.

Fig 10: Examples of Uranyl detected crown ethers
1.2: EGTA based sensory systems:

Among the chelating compounds used either for chemical or medicinal purposes, polyaminopolycarboxylic acids stand out as archetypical metal binding agents. Some of the linear polyaminopolycarboxylic acid chelating agents (Fig 11) are ethylenediaminetetraacetic acid (EDTA, 20), 1,2-diaminocyclohexanetetraacetic acid (DCTA, 21), tris-(2-aminoethyl)aminehexaacetic acid (TAAHA, 22) and diethylenetriaminepentaacetic acid (DTPA, 23). EDTA forms stable complexes with a number of physiologically important cations such as Ca\(^{2+}\), Mn\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Fe\(^{2+}\) and Fe\(^{3+}\) and is used to protect against toxic elements in cationic forms.\(^{19}\) DTPA is superior to EDTA for polyvalent cations such as lanthanides and actinides because of its octadentivity.\(^{20}\)

![Chemical structures of EDTA, DCTA, and TAAHA](Fig11)

Among the prominent applications in medical sciences is Gd-complexed aza-crown ethers. Gd\(^{3+}\) ion being most useful paramagnetic,\(^{21}\) species because of its high magnetic moment and efficient relaxation. However, this ion is too toxic to be used \textit{in vivo} and it must be reacted with a chelating agent before being injected into the blood stream in order to facilitate its rapid excretion through the kidneys. Safe and effective contrast agents containing gadolinium should not dissociate in the body, and thus should be highly stable and kinetically inert towards metal ion release. High stability is achieved with the Gd\(^{3+}\) complex of DTPA, and Gd(DTPA)\(^{2-}\) is the most commonly used MRI contrast agent today.

Chelating agents based on azacrown ethers bearing acetic acid moieties such as 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA, 24), 1,4,7-10-tetraazacyclododecane-
N,N’,N”’,N”’-tetraacetic acid (DOTA, 25) and 1,4,8,11-tetraazacyclotetradecane- N,N’,N”’,N”’-tetraacetic acid (TETA, 26) have attracted considerable interest in the design of NMR contrast agents because of their multidentate ligating sites (Fig 12).\textsuperscript{22} Stetter and Frank,\textsuperscript{23} reported that DOTA, 25 forms the most stable Ca\textsuperscript{2+} complex known, with a log K value of 16.5. Kinetic investigation of the lanthanide DOTA chelates by Desreux,\textsuperscript{24} has shown that Gd(DOTA)\textsuperscript{-} dissociates more slowly than Gd(DTPA)\textsuperscript{2-} even in acidic media. Gd(DOTA)\textsuperscript{-} is kinetically remarkably inert and also the complexes of lanthanides with DOTA have formation constants that are several orders of magnitude higher than both the EDTA and TETA. The stability constant for the formation of Gd(DOTA)\textsuperscript{-}, for example, was found to be 10\textsuperscript{27}.

Derivatives of EDTA and DTPA are also used as bifunctional chelating agents with applications in tumour targeting via monoclonal antibody conjugates. In 1974 Mears\textsuperscript{25} synthesized an EDTA derivative bearing a p-amino phenyl substituent 27a (Fig 13) that may be coupled to proteins under mild conditions. Benzyl (27b, 27c), phenethyl 27d and 2-carboxyethyl 28 analogues have subsequently been described. Recently, Gansow\textsuperscript{26} reported DTPA analogue p-SCN-Bz-DTPA 29. A common feature in all these structures is the attachment of the protein reactive function at a methylene carbon away from the polyamine backbone.
Synthesis of polyaminopolycarboxylic acid crown ethers and a study of their complexing abilities, especially towards rare earth metal ions and transition metal ions. The potential utility of such metal complexes of polyaminopolycarboxylic acids as contrast reagents for MRI, is in radio-immunotherapy, in tumour targeting, as models for calcium binding proteins and as antiviral agents against the viruses HIV-1 and 2.

Polyaminopolycarboxylate type chelators exert interesting patterns in selectivity for binding a series of alkaline earth metal ions from Mg$^{2+}$ to Ba$^{2+}$. Interest has been focused on Ca$^{2+}$ because of its importance in physiologically activities such as muscle contraction, neurotransmitter release, hormonal response and blood clotting. Calcium binding proteins, such as troponin C$^{27}$ and calmodulin, $^{28}$ has high affinity for Ca$^{2+}$ over Mg$^{2+}$ and other alkaline earth metal ions because of their polycarboxylate binding sites. In 1980, Schauer and Anderson $^{29}$ synthesized 3,6-dioxaoctane-1,8-diamine-N,N,N’$^,$N’$^{-}$tetraacetic acid (H$_4$ EGTA, 30) and showed it to be an useful model for the Ca$^{2+}$ binding site of proteins (Fig 14).
1.3: BAPTA based complexing systems:

Tsien\textsuperscript{30} synthesized 1,2-bis(o-aminophenoxy)ethane-N,N',N'-tetraacetic acid (H\textsubscript{4} BAPTA, 31) an H\textsubscript{4} EGTA like cheater in which the 1,2-disubstituted benzene rings hold the amino nitrogens in the vicinity of the ethereal oxygens (Scheme 1). In addition, H\textsubscript{4} BAPTA has some useful properties. The existence of two phenyl groups in the molecule endows H\textsubscript{4} BAPTA with an appropriate absorption maximum in its UV spectrum. Thus the chelated and free states of H\textsubscript{4} BAPTA can be followed quite conveniently by UV spectroscopy. In addition an H\textsubscript{4} BAPTA complex faster with Ca\textsuperscript{2+} than does H\textsubscript{4} EGTA, with about the same selectivity for Ca\textsuperscript{2+} over Mg\textsuperscript{2+} as H\textsubscript{4} EGTA. Another BAPTA based example of Ca\textsuperscript{2+} sensor is azid-1\textsuperscript{31}, 35 (Fig 15)

Later developments on applicability of BAPTA based systems to the complexation trasition metal yielded. Zinc\textsuperscript{32} and Cadmium\textsuperscript{33} selective probes 36 and 37 respectively (Fig 15). The chelator 36 was used as Zinc detecting system and as marker for monitoring biological process. Similarly 37 was also gained attention in utilizing as Cd\textsuperscript{2+} marker.
**Fig 15**: BAPTA backbone of different metal selective sensors

\[ \text{Ca}^{2+} \text{ complex of azid-1} \]

\[ \text{Zn}^{2+} \text{ complex of Fluo Zin-3, tetrapotassium salt} \]

\[ \text{Cd}^{2+} \text{ complex of BTC-5N, tetrapotassium salt} \]
2. PRESENT WORK:

In present work, we visined to combine the BAPTA backbone with complexing power crown ether to attain more efficient selective complexing agent. Appending a readout system could become a sensory for detecting the complexing events.

The proposed sensory structure and its retro synthetic analysis is depicted in **Scheme 2**. The azo crown compound 38 could be obtained from commercially available starting material o-nitro phenol 40.

![Scheme 2: Reterosynthetic route to sensor](image)

2.1: Synthesis of Chromogenic Azacrown ether 38:

![Scheme 3](image)
The BAPTA frame of chemosensor 38 starts from 2-nitrophenol 40. Sodium salt of 40 was treated with 1,2-dibromoethane in dimethylformamide under reflux conditions at 125 °C for 3 h to give 1,2-bis(2-nitrophenoxy)ethane 41 in 69% yield (Scheme 3). Compound 41 was characterized from 1H NMR spectrum by appearance of methylene protons at δ 4.55 (4H) as a singlet, aromatic protons at δ 7.80 (dd, J= 8.1, 1.7 Hz, 2H), 7.58 (dt, J= 7.5, 1.5Hz, 2H), 7.33 (d, J= 8.49Hz, 2H), 7.08 (dt, J= 8.3, 0.9Hz, 2H). The structure of the compound 41 was also confirmed by its ESI mass spectrum at m/z 305 (M+H). The nitro functionality of compound 41 was reduced using 10% Pd/C in methanol at 60 °C under H₂ for 3 h to result the 1,2-bis(aminophenoxy)ethane 33 in 96.5% as a brown color solid. From 1H NMR spectra, Shift of methylene protons from δ 4.55 to δ 4.33, broad singlet at δ 3.74 (brs, NH₂, 4H), and aromatic protons appeared as multiplets at 6.80-6.72 (4H), 6.65-6.61 (4H) confirms the reduction of nitro group, IR spectrum showed absorption at 3362 cm⁻¹ for amine functionality and a peak at m/z 245 (M+H) in ESI mass spectrum confirmed the structure of the compound 33.

![Scheme 4](image.png)

The crown ether frame was introduced using 1,2-bis(2-iodoethoxy)ethane 42 as a reagent. The reaction of 42 with compound 33 in acetonitrile, Na₂CO₃ at 95 °C- 100 °C for 6 h afforded the crown compound 43 in 82% yield (Scheme 4). The formation of compound 43 was revealed from 1H NMR spectrum, crown ether protons at δ 4.34 (s, 4H), 3.65 (t, J= 4.93Hz, 4H), 3.58 (s, 4H), 3.24 (m, 4H), the NH group displayed a broad singlet at 4.65 (2H). This structure was further confirmed through its HRMS spectrum, a peak at m/z 359.1944 (M+H).

![Scheme 5](image.png)
The ethyl ester functionality on aza crown frame was introduced through the reaction of 43 in acetonitrile with ethyl bromoacetate, Na$_2$CO$_3$, NaI under reflux conditions using N$_2$ atm. for 8 h to afford compound 39 in 86% yield (Scheme 5). The $^1$H NMR spectrum of the compound 39 showed ethyl proton peaks, quartet at $\delta$ 4.06 (q, $J$ = 14.35, 4H) and triplet at $\delta$ 1.19 (t, $J$ = 7.17, 6H), carbonyl attached -CH$_2$- protons at $\delta$ 4.21 (4H) as singlet and remaining crown protons at $\delta$ 3.64-3.62 (4H), 3.54-3.51 (8H) as multiplets. The structure of the compound 39 was unambiguously confirmed by its ESI mass spectrum which showed a peak at m/z 531 (M$^+$+H).

On chromogenic readout system azo benzene was introduce diazotization followed by coupling reaction. By first carrying out diazoniation on p-nitroaniline in aqueous THF solution (1:1), followed by the addition of compound 39 in aqueous THF. The resulting red residue was purified by flash column chromatography on neutral alumina (EtOAc: hexane 40:60) giving target molecule 38 as a red color semi solid in 58% yield (Scheme 6). The formation of azo product 38 is established from its spectral data. The $^1$H NMR showed aromatic signals at $\delta$ 8.37 (4H), $\delta$ 7.98 (4H), $\delta$ 7.52 (2H), $\delta$ 7.03 (2H) as doublets and $\delta$ 7.69-7.66 (2H) as doublet of doublet, and remaining protons at $\delta$ 4.50 (d, 8H), $\delta$ 4.09 (q, 4H), $\delta$ 3.78 (t, 4H), $\delta$ 3.68 (t, 4H), 3.58 (s, 4H), 1.17 (t, 6H). Molecular ion peak at m/z 851 (M$^+$ + Na), in the ESI mass spectrum confirms the formation of azo product.

2.2: Analysis of solvatochromism in compound 38 by UV-visible spectroscopy:

In order to study binding ability of chromogenic compound 32, various solvent systems, their absorption spectra were examined for suitability using UV-visible spectroscopy.
visible spectra of compound 38, dissolved separately in acetonitrile, chloroform and methanol as solvents at approximately $1.0 \times 10^{-4}$ M concentration revealed that CHCl$_3$ showed maximum absorbivity at $\lambda_{\text{max}}$ at 466 nm (Fig 16). Methanol showed two folds of lower absorbivity at same $\lambda_{\text{max}}$. We have not observed any distinctive shift of $\lambda_{\text{max}}$ with respect to solvent. CH$_3$CN being optimum solvent for absorbivity at $\lambda_{\text{max}}$ as well as a better solvent for dissolving metal perchlorates is therefore chosen as solvent system for further study.

![Absorption of Azacrown compound 38 in various solvents](image)

**Fig. 16**: Absorption of Azacrown compound 38 in various solvents

2.3: Binding characteristics of Chromogenic Azacrown ether compound 38:

2.3.1: UV-visible studies:

The synthesized compound 38 was examined for their interaction with metal perchlorate salts Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Sr$^{3+}$ at same concentration ($10^{-4}$ M) in acetonitrile using UV-visible spectroscopy. When $10^{-4}$ M concentrated solution of 38 was treated with metal cations, a significant bathochromic shift $\lambda_{\text{max}}$ was observed with the addition of Copper perchlorate. Incidentally, the other metal ions (Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Sr$^{3+}$) showed hypsochromic shift. The visible color change was observed from yellow to orange in the solution upon addition of Cu$^{2+}$ metal perchlorate. Such distinctive color was not noticed with other metal perchlorates.
Fig. 17: UV-visible absorption spectra of sensor 38 and shifts in its $\lambda_{\text{max}}$ upon the addition of metal cations.

Fig. 18: changes in the UV-visible spectra of 38 (10$^{-4}$ M) upon titration by Copper salt in acetonitrile as a solvent where the concentration (M) of Copper salt (10$^{-5}$ to 3.5 x 10$^{-4}$)

It was observed that addition of Cu$^{2+}$ ions to a 10$^{-4}$ M solution of 38 in a gradual manner led to a decrease in the intensity of the absorption band centered at 472 nm while the intensity of 38/Cu$^{2+}$ complex absorption band at 520 nm increased with addition of Cu$^{2+}$ ions (10$^{-5}$ to 3.5 x 10$^{-4}$ M) solutions (Fig 17). At concentration rations of 1.5:1 (Cu$^{2+}$: 38) the original absorption band at 472 nm max. disappeared with the appearance of absorption at 520 nm with an isobestic point at
480 nm. When added two moles of Cu$^{2+}$ ions observed max. Absorbivity and max. Wave length $\lambda_{\text{max}}$. The visible color change from yellow to orange was noticed at 2:1 Cu$^{2+}$ complexation ratio. The bathochromic shift observed in the absorption of 38 on addition of Cu$^{2+}$ ion could possibly be ascribed to a change in diploement of the aza group through possible charge transfer induced by Cu$^{2+}$ binding though aza group and oxygen and nitrogen atoms.

3. CONCLUSION:

We have synthesized new chromogenic azo sensor 38 and have examined the interaction with metal cations Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Sr$^{3+}$ as their perchlorate salts. It has been observed that 38 significantly and selectively interacts with Copper ion in preference to other metal cations and exhibits a bathochromic shift in the absorption maxima of 38 in the UV-visible spectroscopic experiments. Quantitative studies on the binding characteristics of 38 with Cu$^{2+}$ ions revealed that two moles of Copper ions is needed to complete binding pocket.
4. EXPERIMENTAL SECTION:

1,2-bis (2-nitrophenoxy)ethane (41):

Sodium salt of 2-nitrophenoxide 40 (23 g, 142.85 mmol), (13.41 g, 71.42 mmol) of 1,2-dibromoethane and 30mL of dimethylformamide were stirred and heated to 125 °C under reflux. After 3hr the reaction mixture was cooled and the product filtered off and washed with more DMF, several portions of cold H_2O and ethanol. After dried yielded 1,2-bis (2-nitrophenoxy)ethane 41 (29.96 g, 69%) as light brown color solid.

M. P : 166-168 °C (Lit. 168-169 °C).

IR (neat) : \( \nu_{\text{max}} \) 3108, 2932, 2873, 1607, 1525, 1486, 1448, 1364, 1282, 1250, 1157, 1057, 935, 852, 742 cm\(^{-1}\).

\(^{1}\)H NMR (300 MHz, CDCl\(_3\) + DMSO-d\(_6\)) : \( \delta \) 7.80 (dd, \( J = 8.1, 1.7 \) Hz, 2H), 7.58 (dt, \( J = 7.5, 1.5 \) Hz, 2H), 7.33 (d, \( J = 8.49 \) Hz, 2H), 7.08 (dt, \( J = 8.3, 0.9 \) Hz, 2H), 4.55 (s, 4H).

\(^{13}\)C NMR (100MHz, CDCl\(_3\)+DMSO-d\(_6\)) : \( \delta \) 150.3, 138.7, 133.0, 123.8, 119.8, 114.3, 67.1.

MS (ESI) : m/z 305 (M\(^+\) + H).

HRMS (ESI) (M\(^+\) + Na) : Calcd for C\(_{14}\)H\(_{12}\)N\(_2\)O\(_6\)Na: 327.0593; found: 327.0577.

1,2-bis(2-aminophenoxy)ethane (33):

1,2-Bis(2-nitrophenexy)ethane 41 (10.2 g, 33.55 mmol), 220mg of 10% palladium-on-charcoal catalyst, and 100 mL of MeOH under H\(_2\) in 250 mL par-shaker at up scaling division. After 3hr reaction mixture filtered through celitepad concentrated that filterate yielded 1,2-bis(2-aminophenoxy)ethane 33 (7.72 g, 96.5%) as a brown colour solid.

M. P : 66-68 °C

IR (neat) : \( \nu_{\text{max}} \) 3446, 3362, 2947, 3060, 1609, 1503, 1459, 1275, 1246, 1211, 1142, 1082, 945, 741 cm\(^{-1}\).
1H NMR (400 MHz, CDCl3) : δ 6.80-6.72 (m, 4H), 6.65-6.61 (m, 4H), 4.33 (s, 4H), 3.74 (brs, 4H).

13C NMR (75MHz, CDCl3) : δ 146.1, 136.7, 121.8, 118.2, 115.2, 112.4, 67.3.

MS (ESI) : m/z 245 (M+ + H).

HRMS (ESI) (M+ + H) : Calcd for C14H17N2O2: 245.1290; found: 245.1286.

1,2-bis(2-iodoethoxy)ethane (42):

To a stirred solution of Triethylene glycol (10.0 g, 66.6 mmol) in dichloromethane (150 mL), imidazole (9.60 g, 133.3 mmol) followed by Triphenylphosphene (39.6 g, 133.3 mmol) were added at 0 °C. After stirring the reaction mixture for 20 min. then added I2 (33.8 g, 133.3 mmol) slowly. Stirring the reaction mixture for 8 h at 0 °C- room temperature, it was quenched with a saturated aqueous solution of Hypo at 0 °C, then separated two layers and organic layer was washed with brine, water and dried over anhydrous Na2SO4 and concentrated in vacuum. Crude residue was chromatographed over silica gel (ethyl acetate/hexane, 1:99) to afforded 1,2-bis(2-iodoethoxy)ethane 42 (19.9 g, 81%) as a liquid.

M. P : liquid

IR (neat) : νmax 2868, 1458, 1354, 1265, 1111, 1037, 981 cm⁻¹.

1H NMR (400 MHz, CDCl3) : δ 3.74 (t, J= 6.59Hz, 4H), 3.64 (s, 4H), 3.23 (t, J= 6.59 Hz, 4H).

13C NMR (75MHz, CDCl3) : δ 71.8, 70.0, 2.9.

MS (ESI) : m/z 388 (M+ + NH4).

HRMS (ESI) (M+ + Na) : Calcd for C6H12O2Na2I2: 392.8824; found: 392.8818.

5,6,7,9,10, 12,13,14, 20,21-decahydrodibenzo[e,q][1,4,10,13,7,16]tetraoxadiazacyclooctadecine (43):

Solution of bis amine 33 (5.0 g, 20.49 mmol) and Na2CO3 (10.8 g, 102.4 mmol) in acetonitrile (250 mL) was stirred at room temperature for 15 min. Then cooled to 10 °C then added 1,2-bis(2-iodoethoxy)ethane 42 (9.85 g, 26.63 mmol) using syringe under Nitrogen atmosphere. After 0.5 h, reaction mixture was placed at reflux temperature for 6 h. After completion of the reaction, concentrated removed all acetonitrile. In that crude added H2O (60
mL), then extracted into EtOAc (2x 60 mL). Organic layer washed with H₂O the concentrated in vacuum to obtained crude product, it was chromatographed over silica gel (ethyl acetate/hexane: 5:95) to afforded 43 (6.01 g, 82%) as a brown color solid.

M. P : 150-152 °C

IR (neat) : \( \nu_{\text{max}} \) 3421, 2920, 2863, 1597, 1522, 1448, 1351, 1279, 1220, 1116, 1095, 938, 714 cm⁻¹.

\(^1\)H NMR (400 MHz, DMSO-

\( \delta \) 6.80-6.76 (m, 4H), 6.57-6.52 (m, 4H), 4.65 (brs, NH, 2H), 4.34 (s, 4H), 3.65 (t, \( J = 4.39 \)Hz, 4H), 3.58 (s, 4H), 3.24 (m, 4H).

\(^13\)C NMR (75MHz, DMSO-

\( \delta \) 145.1, 137.6, 121.2, 1115.9, 110.2, 109.5, 69.6, 68.1, 65.9, 42.1.

MS (ESI) : m/z 359 (M⁺ + H).


Diethyl 2,2’-(6,7,9,10,12,13,20,21-octahydrodibenzo[e,q][1,4,10,13,7,16]tetraoxadiazaacyclo octadecine-5,14-diyl)diacetate (39):

To a solution of 43 (1.0 g, 2.79 mmol) in CH₃CN (15 mL) was added Na₂CO₃ (1.77 g, 16. 75 mmol) NaI (200 mg) at 50 °C and stirred for 20 min. Then added ethylbromo acetate (1.41 g, 8.379 mmol) to the reaction mixture at 50 °C then temperature increased to 95 °C- 100 °C for 8 h.. After completion of the reaction, removal of acetonitrile and added H₂O (20 mL) and dichloromethane (30 mL). The phases were separated, aqueous phase was extracted with dichloromethane (2x 20 mL), and combined organic phases were dried with Na₂SO₄ and concentrated in vaccum. After flash column chromatography to afforded 39 (1.7 g, 86%) as a semi solid.

M. P : Semi solid.

IR (neat) : \( \nu_{\text{max}} \) 3402, 2878, 1735, 1591, 1498, 1452, 1373, 1199, 1092, 1036, 935, 750 cm⁻¹.

\(^1\)H NMR (300 MHz, CDCl₃) : \( \delta \) 7.04-7.01 (m, 2H), 6.90-6.86 (m, 6H), 4.35 (s, 4H), 4.21 (s, 4H), 4.06 (q, \( J = 14.3, 7.1 \)Hz, 4H),
\[\text{Diethyl 2,2'}-(2,17-\text{bis}((E)-(4-nitrophenyl)diazenyl)-6,7,9,10,12,13,20,21-\text{octahydrodibenzo}[e,q][1,4,10,13,7,16]tetraoxadiazacyclooctadecine-5,14-diyl)diacetate (38):}\]

NaNO\(_2\) (0.09 g, 1.14 mmol) was added slowly to a mixture of conc. HCl (2mL) and p-aniline (0.16 g, 1.14 mmol) in water: THF (1:1 v/v, 5 mL) at 0 °C. This suspension was stirred for a further 20 min. at 0 °C. The resulting solution was then added drop wise at 0 °C to a solution of 39 (0.3 g, 0.57 mmol) in water: THF (1:1 v/v, 5 mL). The mixture was allowed to warm to room temperature and stirred for further 12 h at room temperature. The THF solvent was removed under reduced pressure and the solution redissolved in CHCl₃ and washed with H₂O (20 mL). The crude mixture was purified by column chromatography on neutral aluminium oxide with ethyl acete/hexan: 30:70 to give azo sensor 38 as red color semi solid in 58% (0.27 g) yield.

**M. P** : Semi solid.

**\(^{1}\text{H NMR (300 MHz, CDCl\textsubscript{3})}\)**

\[\delta\ 8.37 (d, J= 9.06 \text{ Hz}, 4H), 7.98 (d, J= 9.06 \text{ Hz}, 4H), 7.69 (dd, J= 9.06, 2.26\text{Hz}, 2H), 7.52 (d, J= 1.51\text{Hz}, 2H), 7.03 (d, J= 8.30\text{Hz}, 2H), 4.50 (d, J= 1.51\text{Hz}, 8H), 4.09 (q, J= 14.35, 7.5\text{Hz}, 4H), 3.78 (t, J= 4.5\text{Hz}, 4H), 3.68 (t, J= 4.5\text{Hz}, 4H), 3.58 (s, 4H), 1.17 (t, J= 7.55\text{Hz}, 6H).\]

**\(^{13}\text{C NMR (75MHz, CDCl\textsubscript{3})}\)**

\[\delta\ 171.3, 160.2, 150.0, 144.1, 124.7, 123.0, 122.9, 122.2, 117.7, 103.8, 70.4, 69.4, 66.7, 60.6, 54.1, 52.5, 40.7, 29.6, 14.2.\]

**MS (ESI)**

\[m/z 829 (M^+ + H).\]
5. REFERENCES:


