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Article

Design and Synthesis of Disulfide-based Macrocycles and their Heavy Metal Ion Complexation and Self-assembly Studies

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ABSTRACT

A set of disulfide-based macrocycles **C4-C8** with different spacer groups were synthesized. They possess complexing abilities toward heavy metal ions such as Hg^{2+} and Cd^{2+}. **C4** showed specific binding toward Hg^{2+}, while **C6** exhibited binding toward Cd^{2+}. Self-assembly analysis depicted their varied morphologies dependent on spacer moiety and internal molecular arrangement.

Key words: Macrocycle, Click reaction, Heavy metal sensors, Self-assembly, Vesicles.

1. INTRODUCTION

Macrocycles have always owned a special place due to their chemical diversity and usefulness in biochemical research. Cyclic molecules with a variety of backbones have been synthesized and utilized for several synthetic and biological purposes [1-6]. Macrocycles generally possess more rigidity as compared to their acyclic analogs. Macrocyclization has been recognized as an efficient way to restrict conformation and impart the unique self-assembling ability to generate diverse supramolecular assemblies [7-12]. The self-assembly of rigid macrocycles into well-defined nanostructures has been the subject of intense study in recent years, both for basic and applied research.

In general, rigid aromatic macrocycles can self-assemble into cylindrical micelles or spherical structures [13-15]. These supramolecular self-assemblies can be controlled or altered by varying the building blocks. The introduction of amino acids in the macrocycles can impart distinct self-assembling features since amide bonds can facilitate inter and intra-molecular hydrogen bonding [16-20]. They are helpful in introducing in-depth structural control, as they form primary, secondary, tertiary, and quaternary structures.

Macrocycles designed with non-metallic centers can act as receptors for metals. Soft non-metals like sulfur can induce affinity toward heavy metals such as mercury, cadmium, lead, and silver. Heavy metal ions are considered dangerous as they can accumulate in the body through pollution and thus cause serious diseases like prenatal brain damage, cognitive disorders and Minamata diseases, even at low concentrations. Thus, designing molecules that can act as receptors for these ions and are helpful in eliminating them is an important area of concern [21-27].

2. EXPERIMENTAL SECTION

2.1. UV-visible Titration Experiments

Stock solutions of compounds C4-C8 were prepared in spectroscopy grade chloroform/acetonitrile with concentration (10^{-4}–100^{-5} M). Solutions of metal salts were prepared in chloroform/acetonitrile with concentration (10^{-1}–10^{-2} M). The absorbance of blank compound solution and on gradual addition of cation salt was recorded using Shimadzu double beam UV-visible spectrophotometer model UV-2450.

2.2. Calculation of Binding Constants and Stoichiometry

The binding constants were calculated by UV-visible titration experiments using the Benesi-Hildebrand plot as standardized for 1:1 stoichiometry. The stoichiometry of binding was calculated by Job's plot from UV-vis. absorbance data.

2.3. Optical Microscopy

Around 3 mM solution of the sample in HPLC grade methanol/chloroform (1:1) was made and mounted on a glass slide. Samples were viewed using Nikon Eclipse TS100 optical microscope system.

2.4. Transmission Electron Microscopy (TEM)

Around 3 mM solution of the sample in HPLC grade methanol/chloroform (1:1) was used for TEM. All the sample solutions were filtered through a nylon syringe filter (0.2 μm). About 2 μL aliquot of the sample solution was placed on a 200 mesh copper grid and stained with 0.2% wt. Phosphotungstate in water for 2 min. and dried in air. Samples were viewed using a Philips CM 12 TEM.

2.5. Scanning Electron Microscopy (SEM)

Around 3–4 mM solution in 1:1 methanol/chloroform was prepared, and one drop of compound solution was put on glass coverslip, which was pasted on carbon tape mounted on a stub, dried at room temperature, and coated with ~ 10 nm of gold. Samples were analyzed using SEM ZEISS EVO 50 SEM.

2.6. Atomic Force Microscopy (AFM)

Around 3.0–4.0 mM solution in 1:1 methanol/chloroform was prepared, and one drop of compound solution was put on the silicon

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wafer. Samples were analyzed using Dimension Icon AFM operating at tapping mode in air. Images were recorded in air at room temperature and data analysis was performed using nanoscope 5.31r software.

3. RESULTS AND DISCUSSION

3.1. Design and Synthesis

We report here disulfide-based macrocycles **C4-C8** decked with varied spacer groups (Figure 1). The macrocycles were synthesized by Cu-catalyzed azide-alkyne dipolar cyclo-addition reaction. The design of the macrocycles is carried out such that a rigid macrocycle is built around aromatic units using disulfide and triazole units. The S-S linkage not only provided flexibility and preorganization properties to the molecules but also soft centers. Consequently, these scaffolds possess dual characteristics; tuned morphology and heavy metal binders.

3, 3'- Dithiobisbenzoic acid (**C1**) is converted into acid chloride derivative (**C2**) by reacting it with $SOCl_2$. **C2** is further reacted with propargyl alcohol to give di (prop-2-yn- 1-yl) 3, 3'-disulfanediyldibenzoate, which acts as the dialkyne system **C3** (Scheme 1).

C3 is further tethered to different aromatic and aliphatic spacers based on various diazides by click reaction to give macrocycles **C4-C8**. **C3** was reacted with 1, 1'-Biphediazide in the presence of Cu (I) under argon atmosphere to give **C4** (Scheme 2). Similarly, **C3** was reacted with m-xylylene diazide and 1, 6- Diazohexane to give **C5** and **C6**; **C7,** respectively (Scheme 2). The reaction of **C3** with diazidoacetic ester derivative of L-Cystine amino acid diester yielded **C8** (Scheme 2). Synthesis of **C8** was carried out using $CuSO_4.5H_2O$ in the presence of sodium ascorbate in the EtOH/Toluene/water (6:3:1) mixture as the procedure followed for the synthesis of other macrocycles gave very poor yield. **C4-C6** contains only one disulfide linkage, whereas **C7** and **C8** possess two disulfide linkages opposite to each other, which help to provide additional flexibility to the macrocycles. During the synthesis of **C4-C8**, we obtained residues along with the shown structures, which were found to be insoluble in most of the available solvents employed for spectral characterization. Therefore, they were not characterized. The residue **C7** obtained along with **C6** was soluble in dimethylsulphoxide and turned out to be a dimer of it.

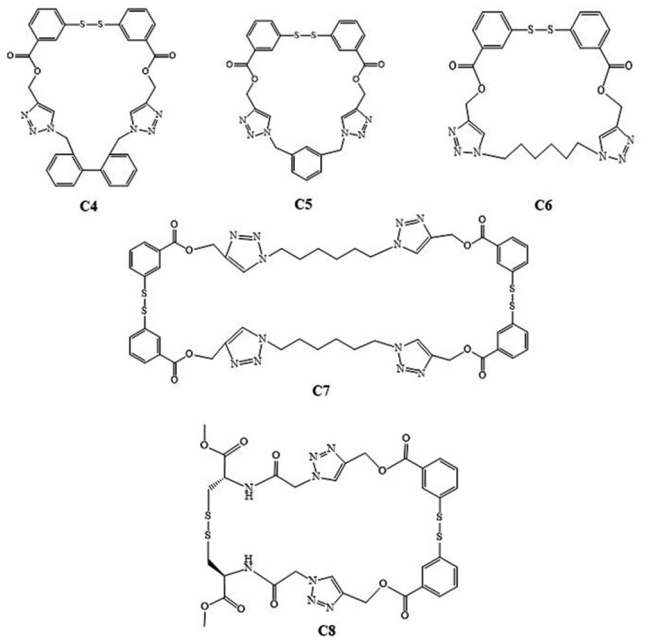
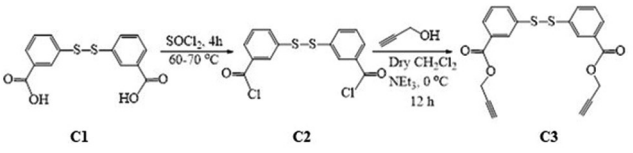


Figure 1: Structural representation of macrocycles **C4-C8**.

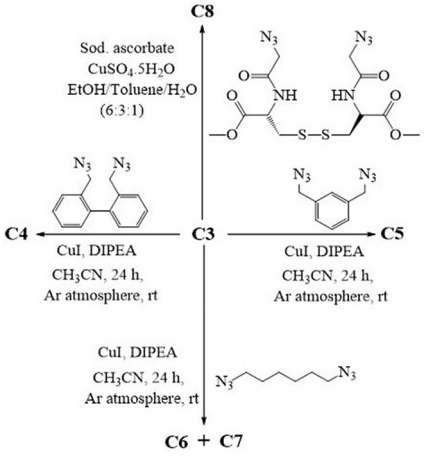
3.2. Metal Ion Complexation Studies

As the macrocycles **C4-C8** contain non-metallic centers such as sulfur and oxygen, which can coordinate to metals and can form complex, we attempted to analyze the metal binding affinity of **C4-C8** toward metal ions such as Hg^{2+}, Pb^{2+}, Ag^{+}, Fe^{2+}, Fe^{3+}, Cd^{2+}, Cu^{+}, Mg^{2+}, Ni^{2+} and Zn^{2+} in 1:1 $CH_3CN/CHCl_3$. The binding studies were carried out with perchlorate salts of metal ions. Binding affinity was evaluated by using UV-vis. spectroscopy titration experiments. UV-vis. titration profile of **C4** with Hg^{2+} showed a red shift in the absorbance peak at 240 nm upon slot-wise addition of metal salt (Figure 2a). The stoichiometry of binding as evaluated by Job's plot is 1:1 with Hg^{2+} (SI, Figure S1a). The binding constant as calculated by the Benesi-Hildebrand plot is 2.7×10^3 M^{-1} (Inset Figure 2a). The specificity of binding of **C4** toward Hg^{2+} is also demonstrated by a UV-Vis. experiment, in which excess (117.0 equiv) of other metal perchlorate salts in the presence of 46.0 equiv of Hg $(ClO_4)_2$ were added. The results showed negligible change in the absorbance of **C4** in the presence of other metal ions while it increases to ~ 3.8 folds in the case of Hg $(ClO_4)_2$, which is a noteworthy observation (Figure 2b).

To investigate the possibility of involvement of pi-bonds of the aromatic/triazole ring in binding, ^{1}HNMR titration experiment was performed by gradual addition of 0.0–40.0 equiv Hg $(ClO_4)_2$ to **C4** in 1:1 $CDCl_3/CD_3CN$. The studies revealed the negligible change in the chemical shift values of ring protons, thereby eliminating the existence of cation-π interaction. Therefore, the mode of binding can be proposed as encapsulated mercuric ion inside **C4** with electrostatic interactions between sulphur/oxygen atoms and Hg^{2+} (Figure 3a). This binding model is supported by the observance of complex **C4** + Hg^{2+} + ClO_4^- in the form of white precipitates upon the addition of 40.0 equiv of Hg $(ClO_4)_2$ salt to the 2 mM solution of **C4** (Figure 3b). It was confirmed by the presence of peak at m/z 947.0896 in ESI-MS (SI, Figure S1b). The dried precipitate showed a melting point of 232–234°C, which is different from that of **C4**; 97–100°C. This kind of precipitation was not observed in the case of other cations. All these observations support the specific affinity of **C4** toward Hg^{2+}.



Scheme 1: Synthesis of dialkyne **C3**.



Scheme 2: Synthesis of macrocycles **C4-C8**.

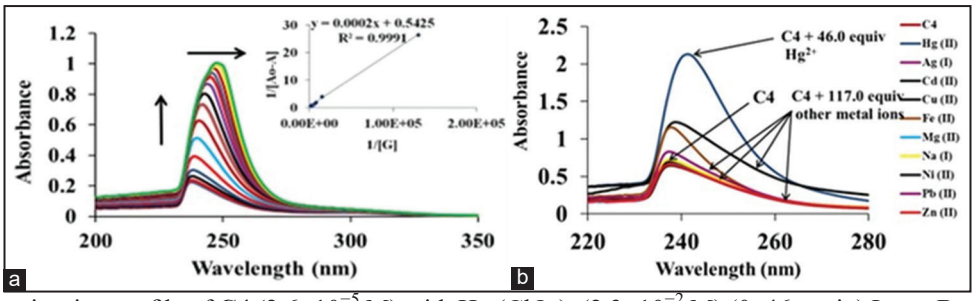


Figure 2: (a) UV-vis. titration profile of **C4** (2.6×10^{-5} M) with Hg $(ClO_4)_2$ (2.3×10^{-2} M) (0–46 equiv) Inset: Benesi-Hildebrand plot of **C4** with Hg^{2+} (b) UV-vis. response of addition of all metal ions to **C4** in 1:1 chloroform/acetonitrile (Other metal ions are added in excess {117.0 equiv} as compared to Hg^{2+} ion).

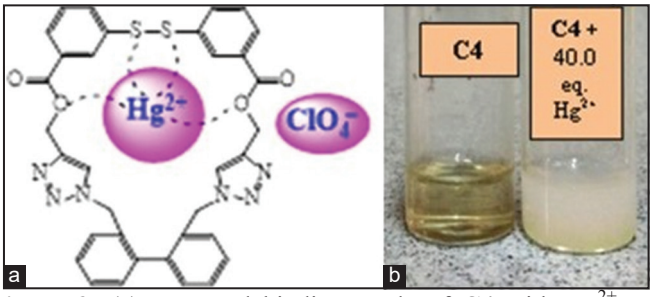


Figure 3: (a) Proposed binding mode of **C4** with Hg^{2+} and structure of complex (**C4** + Hg^{2+} + ClO_4^-) (b) White precipitate observed upon addition of 40.0 equiv. of Hg $(ClO_4)_2$ (2.3×10^{-3} M) to **C4** (2.0×10^{-3}M) in 1:1 chloroform/acetonitrile.

UV-vis. titration profile of **C6** with Cd^{2+} showed decrease in the absorbance at 240 nm upon slot-wise addition of metal salt (SI, Figure S2a). The Job's plot showed a 1:1 stoichiometry with Cd^{2+} (SI, Figure S2b), and the Benesi-Hildebrand plot showed a binding constant of 1.3×10^3 M^{-1} (SI, Figure S2c). The other macrocycles showed no significant binding toward Hg^{2+}, Pb^{2+}, Ag^+, Fe^{2+}, Fe^{3+}, Cd^{2+}, Cu^+, Mg^{2+}, Ni^{2+} and Zn^{2+} metal ions.

3.3. Self-assembly and Encapsulation Studies

As the designed macrocycles contain aromatic units, disulfide bridging and amino acid residue, this can impart unique morphological features to the molecules. Therefore, the self-assembling features of **C4-C8** were analyzed in 1:1 $CH_3OH/CHCl_3$ by using various microscopic techniques such as SEM, TEM, and AFM. The studies revealed the vesicular morphology of **C4-C6**, while **C8** possessed flat bar-like morphology (Figure 4, SI, Figure S3a-e). These studies show the role of spacer groups in controlling morphology. Attaching amino acid spacer introduces additional hydrogen bonding ability and more flexibility to the molecule. The presence of two disulfide bridges in **C8** might have changed the inter-spatial arrangement within the molecule and positioned it for linear self-assembly as compared to other macrocycles. Self-assembly analysis of **C7** in dimethylsulphoxide and **C3** in 1:1 $CH_3OH/CHCl_3$ didn't show any specific morphology.

A closer look at the SEM images showed that the average size of vesicles formed from **C4** and **C5** is nearly same (200–400 nm), while the average size of vesicles of **C6** increases to 3.0 µm (SI, Figure S4). The length of bars in the SEM image of **C8** ranges from 9.2 µm-23 µm while the average width and height are 3.06 µm and 757 nm, respectively (SI, Figure S4).

After discovering the vesicular self-assembly of **C4-C6**, we moved our attention to evaluating the encapsulation potency of

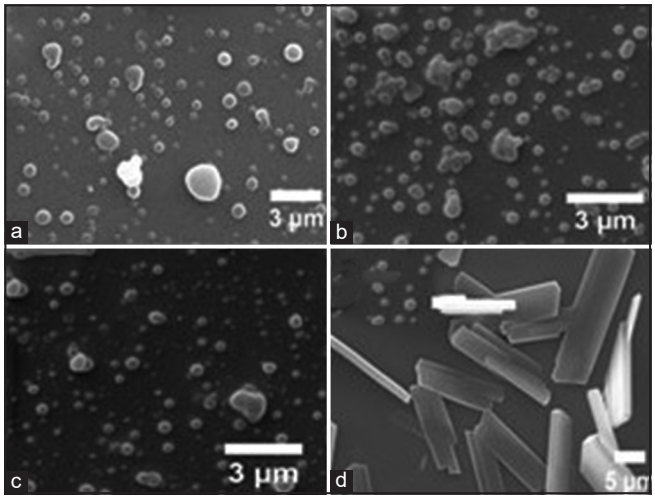


Figure 4: SEM images of macrocycle (a) **C4** (3.1 mM) (b) **C5** (2.8 mM) (c) **C6** (2.96 mM) (d) **C8** (2.8 mM) in 1:1 $CHCl_3$/MeOH respectively.

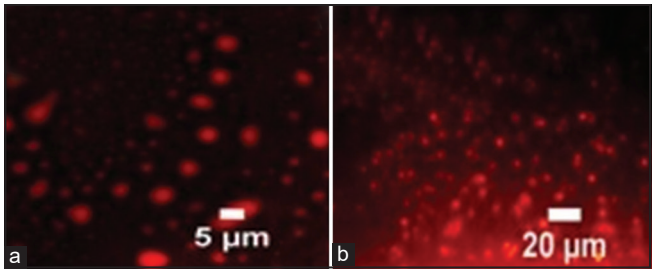


Figure 5: Fluorescence microscopic images (λ_{ex} = 510–560 nm) of rhodamine B encapsulated (a) **C4** (b) **C5** 1:1 $CH_3OH/CHCl_3$.

these vesicles. 0.02 equivalent of rhodamine B loaded vesicles appeared red (λmax = 510–550 nm) in case of **C4** and **C5**, clearly indicating the encapsulation, while **C6** doesn't showed encapsulation (Figure 5a and b). The most plausible reason for this is the presence of lesser number of aromatic units in **C6** to interact with aromatic units in rhodamine B, yielding comparatively less stable interactions resulting in non-encapsulation.

4. CONCLUSION

In summary, we have synthesized disulfide-based macrocycles **C4-C8** containing 1, 2, 3-triazole units. The complexing ability of **C4-C8** toward metal ions was evaluated by UV-vis. spectroscopy. **C4** showed binding toward Hg^{2+} ion while **C6** exhibited binding toward Cd^{2+}. These macrocycles were demonstrated to show distinct

self-assembling features dependent upon the backbone moieties. **C4-C6** showed vesicular self-assembly, while **C8** showed flat bar-like morphology. This study shows that the positioning of aromatic moieties plays a distinct role in controlling self-assembly.

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6. SUPPLEMENTARY MATERIAL

Supplementary data (method of preparation, spectral data, and microscopic images) associated with this article can be found in the attached Word document.

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***Bibliographical Sketch**

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