http://dx.doi.org/10.5012/bkcs.2013.34.5.1378

Fluorescence Enhancement of 7-Diethylamino-4-methylcoumarin by Noncovalent Dipolar Interactions with Cucurbiturils

Mee Ock Park, Myung Gu Moon, and T. J. Kang*

*Department of Chemistry and Applied Chemistry, Daegu University, Gyoungsan 712-714, Korea. *E-mail: tjkang@daegu.ac.kr*
Received December 26, 2012, Accepted February 7, 2013

We have investigated the complex forming behavior of cucurbit[6]urils(CB6) and cucurbit[7]urils(CB7) with 7-diethylamino-4-methylcoumarin(C460) in water. The electronic absorption maximum of C460 shows bathochromic shift with the addition of CB7 and fluorescence intensity is greatly increased, while CB6 has no noticeable effects on the spectroscopic properties of C460. It is noted that CB7 interacts more strongly with C460 than CB6 does. Fluorescence lifetime also significantly increased for the CB7 complex, which is attributed to reduced polarity surrounding C460 and/or C460 being in a restricted environment. The stoichiometry for the complex formation determined from the fluorescence titration measurement indicates that 2:1 complex in which two CB7 molecules bind to C460 is formed. Thus, two step equilibrium processes are suggested for the complex formation and the binding constants are estimated. The semi-empirical electronic structures calculations indicate that C460 is not included in the CB7 cavity but interacts noncovalently with the portal carbonyls of CB7.

Key Words : Cucurbiturils, Complex, Fluorescence, *n*-Diethylamino-4-methylcoumarin

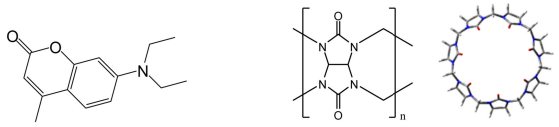
Introduction

Understanding the intermolecular interactions is important in the study of host-guest chemistry. Most of biological processes such as ion transport, enzymatic catalysis, and drug delivery are based on intermolecular recognition and interactions. Over the past decade, cucurbiturils have emerged as a new class of macrocyclic hosts and found various applications in fluorescence sensors and switches, drug binding and delivery, control over chemical reactivity, *etc*.[1-3] Cucurbiturils have highly symmetrical cage structures with two identical portal ends flanked with carbonyl groups, and several homologues of cucurbit[*n*]urils (n = 5,6,7,8) with varying cavity sizes have been synthesized.[4-7] The structural characteristics of cucurbituril host capable of encapsulating guest molecules in a hydrophobic cavity can lead to the formation of inclusion complexes. The cucurbituril cavity imposes structural confinement and the embedded guest molecules can be isolated chemically from the surroundings. Guest molecules embedded in cucurbituril host experiences a modified microscopic environment which may be different from the bulk solution. Consequently, the guest molecules exhibit somewhat different spectroscopic features. Examples of inclusion complexes resulting from the encapsulating interaction of cucurbituril for various fluorescent molecules have been reported and their spectral properties were investigated.[8-11] Fluorophores such as xanthene, coumarin, oxazine dyes have many useful applications for fluorescent labeling, single molecule detection and fluorescent-based assays, *etc*.[12-14] Fluorescent dyes for these purposes require high quantum yield and good chemical stability. Recently, the strategy of host-guest complex formation employing cucurbituril hosts shows a range of desirable effects for fluorescence behavior and chemical stability of dye molecules.[15] Binding of fluorescent dyes with host in some cases involves fluorescence quenching rather than enhancement.[16-19] Some cationic dyes are reported to bind strongly with cucurbiturils while anionic dyes do not form complexes.[15] When charged species are involved in complex formation, coulomb interactions are important. For molecular dyes, host-guest interactions are more complicated with a multitude of interactions including hydrogen boding, π-π interaction and dipolar attractions.

Homologues of different cucurbit[*n*]urils (n = 5-8) with cavity size varying between *ca.* 4 Å and 9 Å show marked difference in guest affinity, which results in different binding stoichiometry and configuration. In particular, cucurbit[7]uril is highly water soluble in contrast to the other homologues, making it possible to study host-guest chemistry in biologically benign aqueous media. In this work, the host-guest complex formation between cucurbituril homologues and 7-diethylamino-4-methylcoumarin in aqueous solvent was investigated using electronic absorption and fluorescence measurements. Although many host-guest systems of cucurbiturils suggest the encapsulation scheme for complex formation, it seems the association complexes rather than the inclusion ones are formed in our study. In order to have some insight into how host and guest molecules are bound to each other, the electronic structure calculations using Gaussian09 was carried out in a semi-empirical level and the results are interpreted in accord with the experimental results.

Experimental

Molecular structures of 7-diethylamino-4-methylcoumarin and macrocyclic host cucurbit[*n*]uril are as following,



7-diethylamino-4-methylcoumarin (C460) Cucurbit[*n*]urils (n=6,7)

C460 was purchased from Exiton and used as received. Cucurbit[6]uril was purchased from Aldrich, and cucurbit[7] uril was synthesized according to an improved method which exploited mechanistic insights into the oligomerization of glycoluril.[20,21] The starting materials for the CB7 synthesis such as formaldehyde, sulfuric acid, and glycoluril were from Aldrich. Some of organic solvents for washing and dissolving purpose are from Duksan Chemicals. The product yield of CB7 with respect to glycoluril was about 3%, which is much lower than the reported value in the literature.[22] The relative ratio of CB7 to the other cucurbituril homologues was reported to vary depending on the reaction temperature that would be responsible for the discrepancy in our work.[20] The synthesized CB7 was characterized with proton NMR and fab mass spectrometry. Stock solutions of 6.88×10^{-5} M CB7 were prepared. Aqueous solution 0.1 M Na_2SO_4 was used in the preparation of CB6 solution to ensure sufficient solubility. The UV/Vis absorption spectra were recorded with photodiode array spectrophotometer by Agilent (HP8453) and the fluorescence measurements were carried out using Jasco spectrofluorometer (FP-6300) with a wavelength resolution of ~1 nm. Fluorescence lifetimes were measured with PicoQuant FluoTime 200 that takes advantage of time-correlated single photon counting method. A pulsed diode laser operated at 20 MHz repetition rate was used as the excitation source. The FWHM of a laser pulse was typically 45 ps and the instrument response function was ~190 ps when the Hamamatzu photomultiplier tube (H5783-01) was used.

Results and Discussions

Effects of Cucurbiturils on the Electronic Absorption and Fluorescence Characteristics of C460. Figure 1 shows the electronic absorption profile changes of C460 upon introducing the macrocyclic hosts, CB6 and CB7 to the dye solution. The cucurbituril homologues are transparent to the ultraviolet as well as the visible region of interest. The possibility of C460 in protonated diethylammonium form in water is ruled out and measured absorption spectra are attributed entirely to the neutral form of C460.[23] With the addition of CB6 (Figure 1(a)), the spectral changes were only nominal with slight increase of absorbance, and no spectral shift was observed. On the other hand, incremental addition of CB7 (Figure 1(b)) resulted in pronounced change in absorption spectrum. At lower concentration of CB7, the gradual bathochromic shift followed by small increase in the absorbance was observed. This trend continues until CB7 concentration is reached at about 10 μM. This number is close to a point where equimolar concentrations of the dye and the macrocyclic host are achieved. The change in the

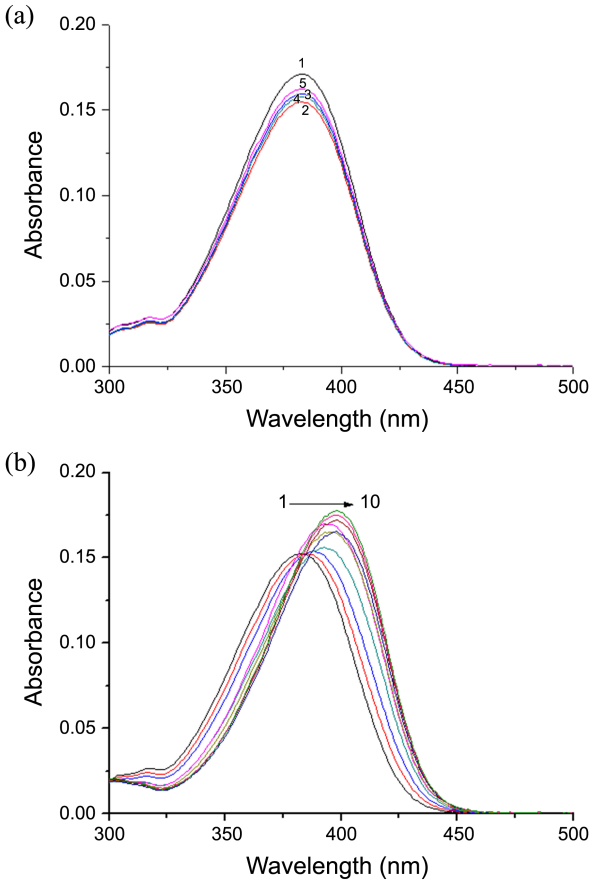


Figure 1. (a) UV/Vis absorption spectra of C460 with various concentrations of CB6. (1) no CB6, (2) 6.25 mM, (3) 1.25 mM, (4) 4.38 mM, (5) 7.50 mM, (b) UV/Vis absorption spectra of C460 with various concentrations of CB7. (1) no CB7, (2) 4.13 mM, (3) 8.26 mM, (4) 12.4 mM, (5) 20.7 mM, (6) 24.8 mM, (7) 41.3 mM, (8) 45.4 mM, (9) 49.6 mM, (10) 54.7 mM.

spectral profile indicates an isosbestic point around 385 nm suggesting an equilibrium process. Further increase of CB7 concentration beyond 20 μM, appreciable increase in the absorbance is observed with the maximum of absorption peak converging at 398 nm. This observation indicates that an additional equilibrium process may exist in the solution and the 2:1 complex formation is occurring in the equilibrium process. Overall bathochromic peak shift of about 16 nm was measured in the absorption spectrum. The bathochromic shift can be explained by asserting that the environment surrounding dye molecules becomes less polar in the presence of CB7. The degree of energy stabilization for the ground electronic state in less polar environment is reduced.

Fluorescence measurements resulted in even more drastic change from CB6 to CB7. There was no change in the peak position of the fluorescence spectra for both macrocyclic hosts. However, the fluorescence intensity of C460 increased significantly upon addition of CB7, as shown in Figure 2. Large increase of fluorescence intensity with a fluorescence maximum at 471 nm was accompanied by a slight decrease of fluorescence bandwidth of 154 cm^{-1}. Almost an order of magnitude increase in the fluorescence intensity was observed

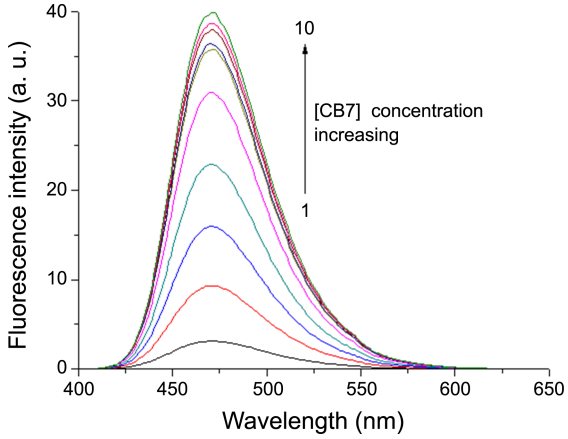


Figure 2. Fluorescence intensity change of C460 with the incremental addition of CB7. (1) no CB7, (2) 4.13 mM, (3) 8.26 mM, (4) 12.4 mM, (5) 20.7 mM, (6) 24.8 mM, (7) 41.3 mM, (8) 45.4 mM, (9) 49.6 mM, (10) 54.7 mM.

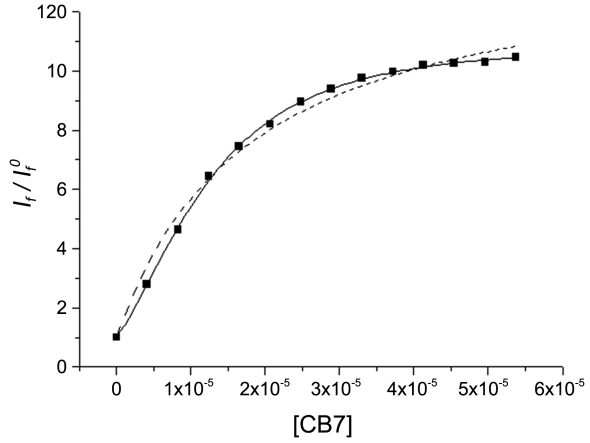


Figure 3. Fluorescence enhancement of C460 as a function of CB7 concentration. The solid line represents the best fit for the 2:1 stoichiometry using Eq. (2) and the dashed line represents 1:1 stoichiometry using Eq. (1).

with CB7, meanwhile changes in the fluorescence spectra with CB6 was insignificant. These notable differences in the fluorescence behavior of C460 by CB6 and CB7 suggest that the degree of molecular interactions is not the same between the two cucurbiturils. Since the molecular interactions between C460 and CB6 are relatively weak and spectral changes are less significant, more emphasis is given to the interactions of C460 with CB7. The dye molecule experiences different physical and chemical environment in the neighborhood when the two different macrocyclic hosts are present. A large increase of fluorescence intensity with the addition of CB7 is further indicative of strong interaction between the dye and CB7. The details of binding interactions can be analyzed by following the fluorescence enhancement of the dye with respect to the CB7 concentration.

Fluorescence Enhancement and the Binding Scheme. To quantify the fluorescence enhancement, the ratio of the integrated fluorescence spectra in the presence of CB7 to the fluorescence spectrum of dye with no CB7 present was obtained. The dependence of the fluorescence enhancement with the increase of CB7 concentration is shown in Figure 2.

The fluorescence enhancement can be described by assuming the complex formation between C460 and CB7. Assuming that only 1:1 binding equilibrium exists such as,

$$\text{CB7} + \text{C460} \overset{K_1}{\rightleftharpoons} \text{CB7·C460}$$

The following equation for the fluorescence intensity change with CB7 concentration can be derived.[23,24]

$$I_f = \frac{I_f^0 + I_1 K_1 [CB7]_0}{1 + K_1 [CB7]_0} \quad (1)$$

where I_f^0 is the fluorescence intensity of C460 without CB7, I_1 is the fluorescence intensity from the 1:1 complex when all C460 molecules form complexes with CB7, $[CB7]_0$ is the initial concentration of CB7, and K_1 is the equilibrium constant for the complex formation. Using a nonlinear regression method, the fluorescence enhancement data are directly fitted with the above equation and the equilibrium constant is estimated.

Figure 3 shows the fluorescence intensity change for C460 as CB7 concentration varies. It is evident that the experimental data show an unsatisfactory fit to the Eq. (1) for usual 1:1 binding stoichiometry. The deviation of experimental data from the presumed binding curve may suggest that a successive 2:1 complex formation may take place. In this case, an additional stepwise equilibrium is considered

$$\text{CB7·C460} + \text{CB7} \overset{K_2}{\rightleftharpoons} \text{CB7·C460·CB7}$$

If one takes into account the additional equilibrium process, the following equation is obtained.[24-26]

$$I_f = \frac{I_f^0 + I_1 K_1 [CB7]_0 + I_2 K_2 [CB7]_0^2}{1 + K_1 [CB7]_0 + K_1 K_2 [CB7]_0^2} \quad (2)$$

where I_2 and K_2 is the fluorescence intensity and the equilibrium constant for 2:1 complex. The fluorescence enhancement data are more accurately described by the above equation. The fit converged well with the correlation coefficient of 0.999. Using the Eq. (2), K_1 and K_2 values were estimated to be $(24 \pm 4) \times 10^3$ and $(139 \pm 14) \times 10^3$ respectively for the binding interaction between CB7 and C460. The large values of binding constant suggest strong binding for dipole-dipole interactions. Comparatively larger value of K_2 indicates the binding of second CB7 molecule to the existing 1:1 complex is energetically more favorable.

The details of binding stoichiometry were investigated with fluorescence titration measurements. The simple method of continuous variation, so called Job's method was applied to investigate the stoichiometry for complex formation. In this method, fluorescence intensity measurements were carried out for a series of solutions where mole fractions of dye and CB7 are varied while the total concentration remains constant. A typical Job's plot obtained for the C460-CB7 complexes is shown in Figure 4. The plot clearly

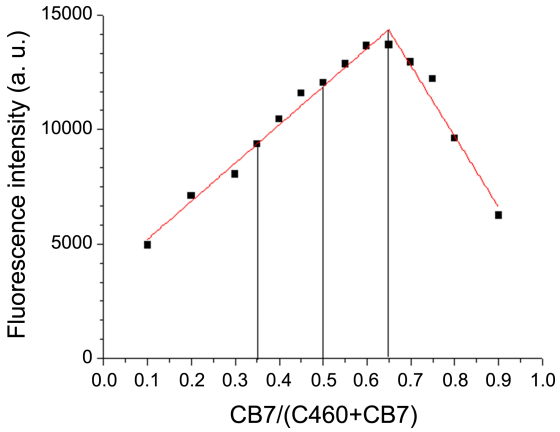


Figure 4. Job's plots obtained for C460-CB7 complexes. The maximum fluorescence intensity is obtained at about 0.65 mole fraction of CB7.

indicates that C460-CB7 complexes are formed mainly with stoichiometric ratio of 1:2. That is, two CB7 molecules associate with a single C460 molecule. From the study of binding stoichiometry, it is quite difficult to conceive that one dye molecule would be included into the cavity of two macrocyclic hosts.

Geometry Optimization for the Complexes: To get some insight into the binding geometry and the nature of interaction between the dye and macrocyclic host, the semi-empirical electronic structure calculations using the Gaussian09 were performed. The ground state geometries for C460, CB7, 1:1 complex, and 2:1 complex were obtained at AM1 level. Geometry optimization was carried out for various conceivable input geometries not to be misguided by any local minima during calculations. The calculations suggest that formation of inclusion complexes is not energetically favored. The embedded structure always resulted in higher energy configuration. The most stable structure for the 1:1 complex was the one in which the alky groups of C460 interacts with the carbonyl group at the portal of CB7. The optimized structure for the 1:1 complex is the one in which the ethyl unit of the diethylamino group of C460 associates with the carbonyl groups of the CB7 portal as shown in Figure 5(a). It is noted that the molecular dimension of C460 along the short molecular axis is about 5.6 Å. This number is comparable to the diameter of CB7 portal which is about 5.4 Å, and inclusion of C460 into the cavity of macrocyclic host may be difficult to take place.[4] In the optimized structure for 2:1 complexes, the dye molecule associates one CB7 with the diethylamino end, and the other CB7 with the methyl end as shown in Figure 5(b). This geometry is consistent with the fact that the fluorescence intensity of the dye is greatly enhanced upon addition of CB7. It is well investigated that the fluorescence quantum yield of C460 decreases in the polar environment since the twisted intramolecular charge transfer state is stabilized.[27] Binding of CB7 with diethylamino end restricts the rotation of the amino group, thus preventing it from forming the twisted intramolecular charge transfer state, which would affect the nonradiative

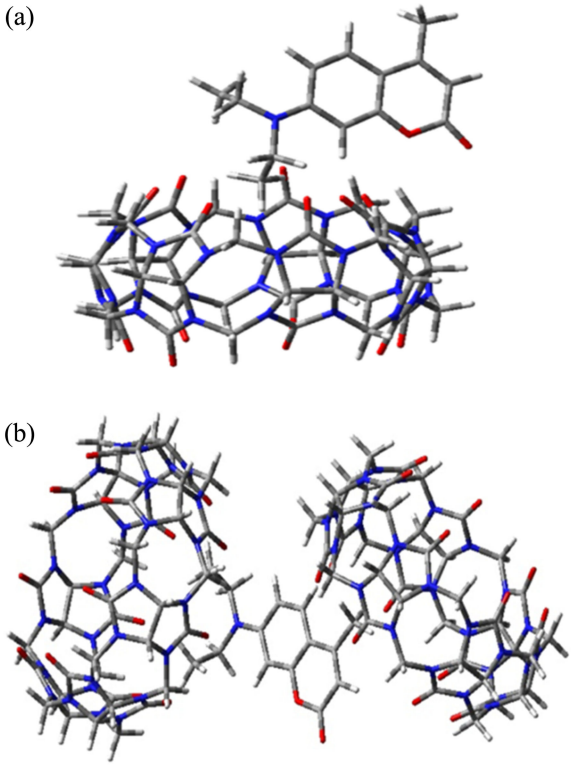


Figure 5. Optimized structures for (a) 1:1 complex and, (b) 2:1 complex calculated with AM1 semi-empirical method.

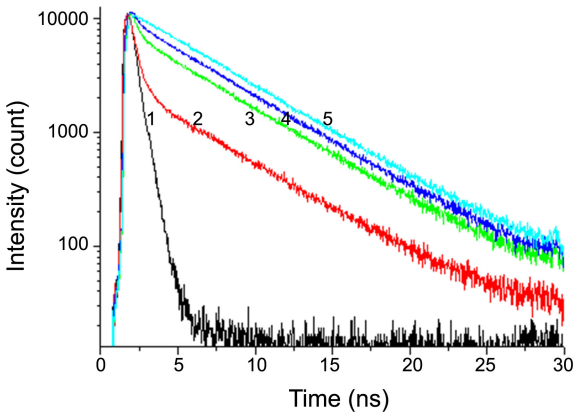


Figure 6. Fluorescence lifetime measurements for C460 with the addition of CB7. The average fluorescence lifetime increases with CB7 concentration. (1) no CB7, (2) 4.13 mM, (3) 8.26 mM, (4) 12.4 mM, (5) 54.7 mM.

decay rate constant.

Effect of CB7 on the Fluorescence Lifetime of C460: Figure 6 shows the fluorescence decay measurements for C460 in the absence and the presence of CB7. It is noted that the decay processes exhibit multiexponential behavior regardless of CB7. These multiexponential decay characteristics of fluorescence in all the measurements are attributed to the different conformational structures for the complexes. In the absence of CB7, the average fluorescence decay time was found to be about 350 ps. As the CB7 concentration increased, the amplitude of fast decaying component is reduced and the fluorescence decay rate becomes slower. The overall average

fluorescence lifetime corresponds to 5.3 ns for 54.7 mM CB7 solution. Besides the restriction of nonradiative decay processes by complex formation, the reduced decay rate may be the consequence of low polarizability of microscopic environment surrounding the dye molecules. According to the conventional theory, the radiative decay rate constant is proportional to the square of the refractive index.[28] The reduced refractive indices of the surrounding medium lead to slower radiative decay rates, and accordingly longer fluorescence lifetimes are measured. The polarizability inside CB7 cavity has been studied[29] and the longer fluorescence lifetimes when the fluorophores were included in the CB7 cavity have been reported.[15] Our results of fluorescence lifetime measurements as well cannot exclude the possibility of low polarizability provided by CB7 in the vicinity of dye molecules when the complexes are formed.

Conclusion

Molecular interactions of macrocyclic cucurbiturils with 7-diethylamino-4-methylcoumarin have been studied using steady state and time-resolved fluorescence measurements along with electronic absorption. It is noted cucurbit[6]uril shows nominal interaction with C460, meanwhile CB7 shows strong affinity toward C460. On the other hand, molecular interactions between CB7 and C460 resulted in significant change in the electronic absorption and fluorescence spectrum of C460. Bathochromic shift in the absorption spectrum with the increased absorption coefficient as well as large enhancement in the fluorescence intensity of C460 are observed. The strong enhancement of fluorescence intensity of C460 by CB7 is attributed to the restriction of the torsional motion of diethyl amino group imposed by the formation of complexes. Job's plot indicates the binding scheme should include both 1:1 and 2:1 stoichiometry complexes. Although tight binding complexes between CB7 and C460 are likely formed with the binding constants of the order of 10^4-10^5, the inclusion of C460 into CB7 cavity is unlikely. Rather some type of cooperative binding is effectively taking place between two CB7 molecules and one C460 molecule. The semi-empirical electronic structure calculations using the Gaussian09 also suggest that inclusion complexes do not conform to the most stable structure and they are not energetically favored. It is suggested that strongly associated complexes are formed in which alkyl side of C460 binds with the carbonyl groups of CB7 portal. Time-resolved fluorescence measurements show that fluorescence lifetime greatly increases as the complexes form, which is consistent with the enhancement of fluorescence intensity. This can be explained by the argument that nonradiative decay rate decreases because of the inhibition of twisted intramolecular charge transfer process due to the restricted movement of the amino group.

Acknowledgments. This work was supported by the Daegu University research grant.

References

1. Parvari, G.; Reany, O.; Keinan, E. *Isr. J. Chem.* **2011**, *51*, 646.
2. Masson, E.; Ling, X.; Joseph, R.; Mensah, L. K.; Lu, X. *RSC Advances* **2012**, *2*, 1213.
3. Walker, S.; Oun, R.; McInnes, F. J.; Wheate, N. J. *Isr. J. Chem.* **2011**, *51*, 616.
4. Lee, J. W.; Samal, S.; Selvapalam, N.; Kim, H. J.; Kim, K. *Acc. Chem. Res.* **2003**, *36*, 621.
5. Isaacs, L. *Isr. J. Chem.* **2011**, *51*, 578.
6. Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem. Int. Ed.* **2005**, *44*, 4844.
7. Kim, J.; Jung, I. S.; Kim, S. Y.; Lee, E.; Kang, J. K.; Sakamoto, S.; Yamaguchi, K.; Kim, K. *J. Am. Chem. Soc.* **2000**, *122*, 540.
8. Wagner, B. D.; Stojanovic, N.; Day, A. I.; Blanch, R. J. *J. Phys. Chem. B* **2003**, *107*, 10741.
9. Wagner, B. D.; Boland, P. G.; Lagona, J.; Isaacs, L. *J. Phys. Chem. B* **2005**, *109*, 7686.
10. Zhou, Y.; Yu, H.; Zhang L.; Sun. J; Wu, L.; Lu, Q.; Wang, L. *J. Incl. Phenom. Macrocycl. Chem.* **2008**, *61*, 259.
11. Choudhury, S. D.; Mohanty, J.; Upadhyaya, H. P.; Bhasikuttan, A. C.; Pal, H. *J. Phys. Chem. B* **2009**, *113*, 1891.
12. Valeur, B. *Molecular Fluorescence*, 3rd Ed, Wiley-VCH: 2006.
13. Slavik, J. *Fluorescent Probes in Cellular and Molecular Biology*, CRC Press: 1994.
14. Wagner, B. D. *Molecules* **2009**, *14*, 210.
15. Nau, W. M.; Mohanty, J. *Int. J. Photoenergy* **2005**, *7*, 133.
16. Limei, Z.; Jiannan, Z.; Yunqian, Z.; Qianjiang, Z.; Saifen, X.; Tao, Z.; Jianxin, Z.; Xin, Z.; Zhanbin, W.; Lasheng, L.; Day, A. I. *Supramol. Chem.* **2008**, 1.
17. Zhau, Y.; Xue, S.; Zhu, Q.; Tao, Z.; Zhang, J.; Wei, Z.; Long, L.; Hu, M.; Xiao, H.; Day, A. I. *Chi. Sci. Bull.* **2004**, *49*, 1111.
18. Montes-Navajas, P.; Garcia, H. *J. Phys. Chem. C* **2010**, *114*, 2034.
19. Wagner, B. D.; Fitzpatrick, S. J.; McManus, G. J. *J. Inc. Phenom. Macrocycl. Chem.* **2003**, *47*, 187.
20. Day, A.; Arnold, A. P.; Blanch, R. J.; Sunshall, B. *J. Org. Chem.* **2001**, *66*, 8094.
21. Oh, K. S.; Yoon, J.; Kim, K. S. *J. Phys. Chem. B* **2001**, *105*, 9726.
22. Marquez, C.; Huang, F.; Nau, W. M. *IEEE trans. Nanobiosci.* **2004**, *3*, 39.
23. Deshpande, A. V.; Jathar, L. V.; Rane, J. R. *J. Fluoresc.* **2009**, *19*, 607.
24. Muñoz de la Peña, A.; Salinas, F.; Gómez, M. J.; Acedo, M. I.; Sánchez Peña, M. *J. Incl. Phenom. Mol. Rec. Chem.* **1993**, *15*, 131.
25. Nigam, S.; Durocher, G. *J. Phys. Chem.* **1996**, *100*, 7135.
26. Singh, M. K.; Pal, H.; Koti, A. S. R.; Sapre, A. V. *J. Phys. Chem. A* **2004**, *108*, 1465.
27. Jones II, G.; Jackson, W. R.; Choi, C.; Bergmark, W. R. *J. Phys. Chem.* **1985**, *89*, 294.
28. Strickler, S. J.; Berg, R. A. *J. Chem. Phys.* **1962**, *37*, 814.
29. Marquez, C.; Nau, W. M. *Angew. Chem. Int. Ed.* **2001**, *40*(23), 4387.