

N-(4-amino-7-nitrobenzaoxa-1,3-diazole)-substituted aza crown ethers: complexation with alkali, alkaline earth metal ions and ammonium

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Received: 11 November 2009 / Accepted: 15 April 2010 / Published online: 7 May 2010
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Abstract Three novel aza-crown ether derivatives incorporating 4-amino-7-nitrobenzaoxa-1,3-diazole (NBD) chromophore were synthesized and their structure confirmed by $^1\text{H-NMR}$, IR and elemental analysis. The influence of the solvent polarity and protonation on the photophysical properties of NBD-15-crown-5 was studied by UV/Vis and fluorescence methods. The influence of the investigated cations on the absorption spectra of the ligands was negligible, however emission was strongly affected. Complexation and binding stability of NBD-aza-15-crown-5 and NBD-aza-18-crown-6 were studied using fluorescence spectroscopy. NBD-aza-18-crown-6 exhibits strong selectivity toward Ca^{2+} and Sr^{2+} ions with formation constants about 10^3 times higher than the formation constants with the other ions included in the study.

Keywords Aza-crown ethers · Complexation · Metal ions · Fluorescence · Absorption

Introduction

NBD derivatives find extensive use in biological applications [1]. NBD chloride [2] has often been used for labeling proteins with a fluorescent group and in studies of protein

structure and conformational changes [3, 4]. A large number of phospholipids and cholesterol analogues with an NBD group as fluorophore find application in the study of biological and model membranes [5–7]. The fluorescence quenching of the NBD moiety by transition metal ions has also been used in determining the location of the fluorophore in complex media [8]. Several studies reported excited-state charge transfer and conformational change in some NBD derivatives as well as the fluorescence response of a few NBD derivatives toward different guests [9–13].

NBD derivatives are most often synthesized by reacting 4-chloro- or 4-fluoro-7-nitrobenzofurazan with primary or secondary amines. The compounds have been utilized for theoretical investigations or chemosensors. Samantha et al. [14] described the reactions of 4-chloro-7-nitrobenzofurazan with sodium azide in DMSO and *N,N* dimethylethylenediamine in toluene to obtain NBD derivatives for purposes of studying their photophysical properties.

Balaban et al. [15] obtained numerous NBD derivatives for theoretical and experimental studies of their absorption and emission properties. Buldt and Karst [16] reported the synthesis and utilization of *N*-methyl-4-hydrazino-7-nitrobenzofurazan, for monitoring of aldehydes and ketones in air.

We reported previously the synthesis and complexation properties of aza-crown ether containing chromo- and fluoroionophores where the process depends strongly on the properties of the heterocycle conjugated with the crown ether [17–20].

The current study describes novel NBD-crown ethers and investigates their binding abilities.

NBD serves as excellent chromophore and its absorption and emission properties can be utilized for detection of species that are able to change the electron distribution in ground and excited state and exhibit concentration

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dependant response. From another side, crown ethers offer a convenient and selective recognition site able to accommodate ionic and molecular species by non-covalent interactions. Electrostatic bonds, hydrogen bonds and π - π interactions play an important role in this process. The combination of both results in effective, sensitive and selective sensor system for detection of metal ions and amino acids. Since both the absorption and emission properties are affected by the complex formation we obtained a concentration dependent response for both while the fluorescence response is more sensitive due to the intrinsic low-concentration performance of the method.

Experimental

Materials and methods

All chemicals were purchased from Sigma-Aldrich and were ACS grade. They were used without further purification. Melting points were determined on Mel Temp 3.0 instrument and were uncorrected. ^1H NMR spectra were recorded on a Varian Gemini HX 300 MHz spectrometer using CDCl_3 as a solvent and the chemical shifts are expressed in parts per million (δ , ppm) downfield from TMS as an internal standard. Infrared spectra (IR) were recorded, for KBr discs, on a Perkin-Elmer FTIR 1430 spectrometer. Elemental analyses were performed for C, H, and N (Galbraith Laboratories, Inc., Knoxville, TN, USA) and were within $\pm 0.4\%$ of the theoretical values. The UV/Vis absorption of the solutions was measured using the Shimadzu UV-2401 PC spectrophotometer instrument. Fluorescence measurements were done on the Perkin Elmer LS55 Fluorescence spectrofluorimeter.

Synthesis

Scheme 1 represents the synthesis of the aza-crown ether derivatives.

A typical synthetic procedure utilized in this study is described below:

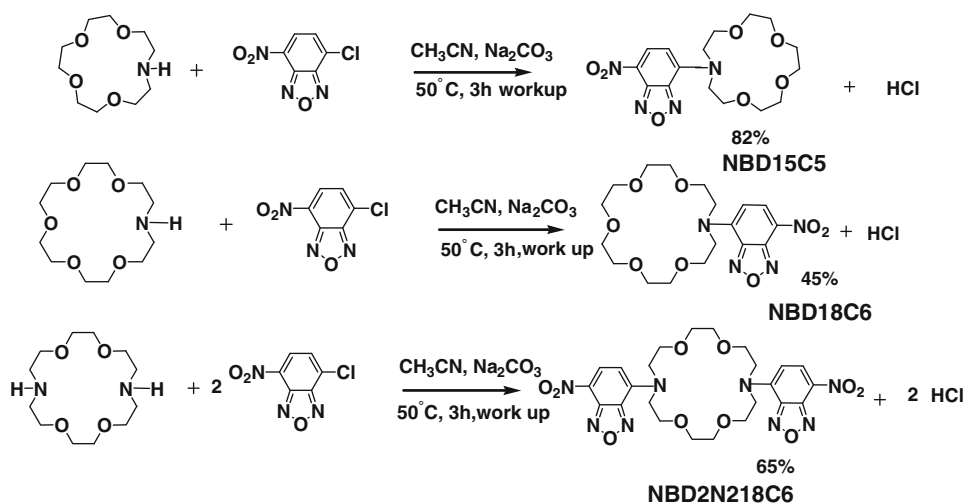
Synthesis of *N*-(4-amino-7-nitrobenzoxa-1, 3-diazole)-1, 4, 7, 10-tetraoxa-13-azacyclopentadecane (NBD15C5)

A total of 0.264 g (1.2 mmol) aza-15-crown-5 (1, 4, 7, 10-tetraoxa-13-azacyclopentadecane, N15C5, and 0.225 g (1.13 mmol) 4-chloro-7-nitrobenzofurazan (NBD-Cl, Sigma-Aldrich) were dissolved in 15 ml of acetonitrile. Then 0.212 g (2.0 mmol) of sodium carbonate was added to the solution along with a stirring bar. The mixture was heated at 50 °C for 3 h. After completion of the reaction, the solution was purified by filtration, the residue washed with methylene chloride and the combined solutions were concentrated. The resulting dark orange oil was re-dissolved in methylene chloride, the solution washed with 3×100 ml of 0.1 M tetrabutylammonium hydroxide (Sigma-Aldrich) and dried over magnesium sulfate (Sigma-Aldrich). The resulting solution was concentrated and a brown solid resulted. The brown solid was purified by column chromatography with silica gel (Sigma) and ethyl acetate as eluent, resulting in final product weight of .315 g (82%) [16].

NBD15C5: ^1H NMR (CDCl_3): 3.64–3.68 (m, 20H, crown); 6.29 (d, 1H, $J = 9.0$ Hz, aromatic H^2); 8.4 (d, 1H, $J = 8.7$ Hz, aromatic H^2).

IR: (ν cm^{-1}): (solid state): 2820–2910, 1615, 1544, 1255.

Scheme 1 Synthesis of NBD-aza-crown ether derivatives



Elemental analysis ($C_{16}H_{22}N_4O_7$): (%calculated/found): C:50.26/49.90; H: 5.80/5.95; N: 14.65/14.80; m.p. 120–124 °C.

NBD18C6: 1H NMR ($CDCl_3$): 3.63–3.9 (m, 24H, crown); 6.35 (d, 1H, $J = 9.3$ Hz, aromatic H^2); 8.4 (d, 1H, $J = 9.0$ Hz, aromatic H^2).

IR (ν cm^{-1}): (solid state): 2800–2900, 1610, 1540, 1250. Elemental analysis ($C_{18}H_{26}N_4O_8$): (%calculated/found): C: 50.70/50.20; H: 6.15/5.95; N: 13.14/12.65. m.p. 58–60 °C.

NBD2N218C6: 1H NMR ($DMSO-d_6$): 3.60–3.85 (m, 24H, crown); 6.35 (d, 2H, $J = 9.2$ Hz, aromatic H^2); 8.4 (d, 2H, $J = 9.1$ Hz, aromatic H^2).

IR: (ν cm^{-1}) : (solid state) : 2850–2920, 1618, 1545, 1245.

Elemental analysis ($C_{24}H_{28}N_8O_{10}$): (%calculated/found): C:48.98/49.25; H: 4.80/4.65; N:19.04/20.06 m.p. 244–246 °C decomp.

Spectroscopic studies

All crown ether solutions ($1-3 \times 10^{-5}$ M) were prepared from a stock solution in acetonitrile (spectroscopic grade, Sigma-Aldrich). The solutions of the guest were also prepared in acetonitrile and different volumes were added to the host solutions. The concentration of the NBD crown ethers was kept constant while the concentration of the metal salts was changing. The corresponding volumes of the guest and the host were mixed in 25 mL volumetric

flasks and the volume adjusted to the mark with acetonitrile. All measurements were performed at 25 °C.

Results and discussion

Properties of the NBD chromophore. Solvatochromic effect

Figure 1 represents the absorption spectra and Fig. 2 the emission spectra of NBD15C5 in solvents of different polarities. Table 1 summarizes the data from the above mentioned experiments.

The broad structureless absorption band with maximum at 480 nm in acetonitrile can be assigned to an intramolecular charge-transfer transition. The fluorescence spectra of the compound ($\lambda_{ex} = 480$ nm) are also structureless typical for charge-transfer transition. The origin of the other absorption maxima is not completely clear. PPT calculations performed on NBD derivatives [21] used optimized geometries obtained from the ab initio STO-3G calculations considered only the π -system of conjugated molecules. This method predicted very well the absorption maxima of the compounds.

Because the lowest energy transition is of intramolecular charge transfer type, the first excited state of each molecule is expected to have a larger dipole moment than the ground state. Consequently, on moving any of these derivatives from a non-polar to a polar environment we would expect the first excited state to be more stabilized than the ground state, the result being a decrease in energy gap between the

Fig. 1 Absorption spectra of NBD15C5 (1.0×10^{-5} M) in presence of solvents of different polarity: 1-hexane, 2-ethyl acetate, 3-chloroform, 4-methanol, 5-acetonitrile

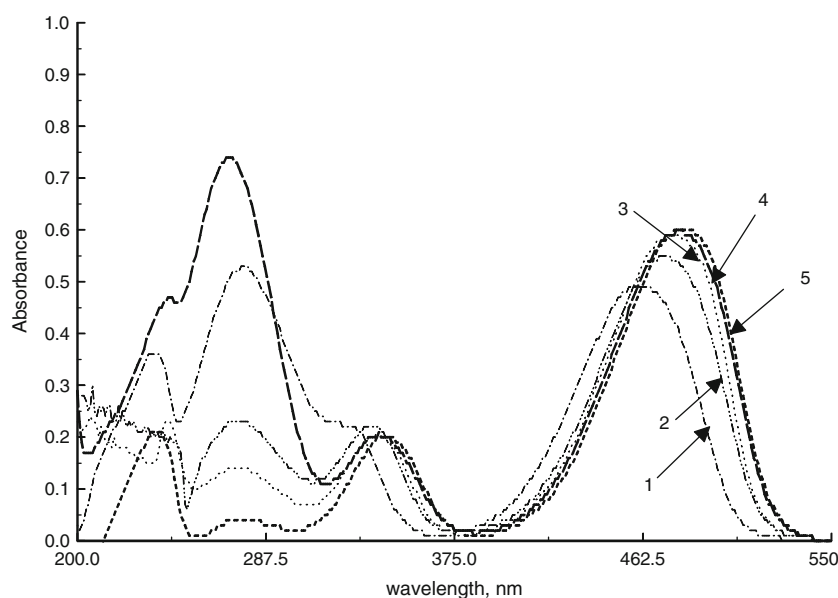


Fig. 2 Emission spectra of NBD15C5 (1.0×10^{-5} M) in presence of solvents of different polarity: 1-acetonitrile, 2-methanol, 3-ethyl acetate, 4-chloroform, 5-hexane

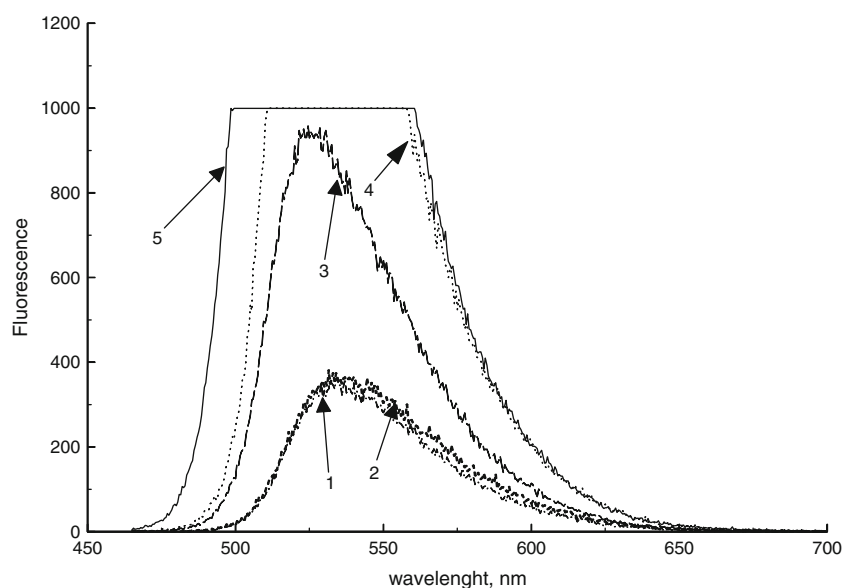


Table 1 Absorption and emission data for NBD15C5 in different solvents

Solvent	Absorption Maximum (nm)	Absorption intensity	Emission maximum (nm)	Emission intensity	Dielectric constant
Chloroform	478	0.59	N/A	N/A	4.8
Hexane	460	0.49	N/A	N/A	2.0
Acetonitrile	480	0.60	533	358	37.5
Methanol	482	0.60	530	380	32.6
Ethyl acetate	472	0.55	525	957	6.0

two states and a corresponding increase in the wavelength of light required for excitation (red shift) [21].

Protonation of the NBD chromophore. Absorption and fluorescence in presence of HCl and H₂O

Increasing amounts of deionized water cause bathochromic shift ($\Delta\lambda_{\text{max}} = 10$ nm) combined with slight increase in the absorption intensity (Fig. 3). This effect is consistent with the increasing of the solvent polarity and stabilizing the excited state of the molecule. Figure 4 represents the effect of the solvent polarity on the absorption maxima of NBD15C5. There is an excellent correlation between the absorption maximum wavelength and the percent water in acetonitrile.

At the same time, addition of same volume of 6 M HCl results in much smaller bathochromic shift and absorption increase (Fig. 5). The effect is a result of two processes taking place which have opposite effects on the absorption and emission of the chromophore: the first is the increased solvent polarity which was discussed above, and the second is a process of complex formation between the proton and the nitrogen atom of the crown ether. The latter engages the

electron pair of the nitrogen and decreases its participation in a charge transfer process. The fluorescence spectra also confirm this conclusion (Fig. 6).

Effect of cations on the absorption properties of the NBD chromophore

The complexation properties of the ligands were investigated in acetonitrile. The amount of host was kept constant and the absorption and emission spectra were recorded in presence of increasing amounts of the guest. Metal ions cause only a slight decrease in the absorption intensity ($\Delta A = 0.05$ – 0.1) of the charge transfer band combined with a slight hypsochromic shift ($\Delta\lambda = 1$ – 5 nm) and an isosbestic point was observed as well (Fig. 7).

While the position of the emission maximum changes very slightly upon addition of the guest species, the emission intensity increases significantly and the changes are concentration dependent thus allowing to determine the stability constants and the free energy changes of the process (Fig. 8, Tables 2, 3). We did not observe direct correlation between the sizes of the cations and the

Fig. 3 Absorption spectra of NBD15C5 (1.0×10^{-5} M) acetonitrile in presence of increasing amounts of DI water (increasing 1 → 5)

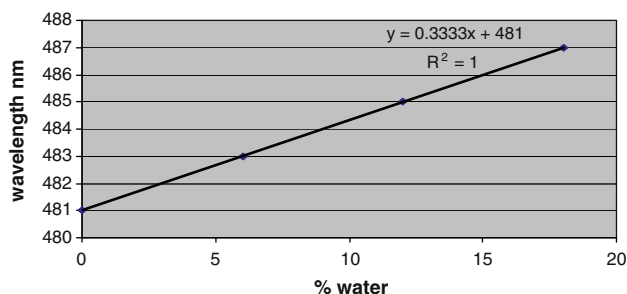
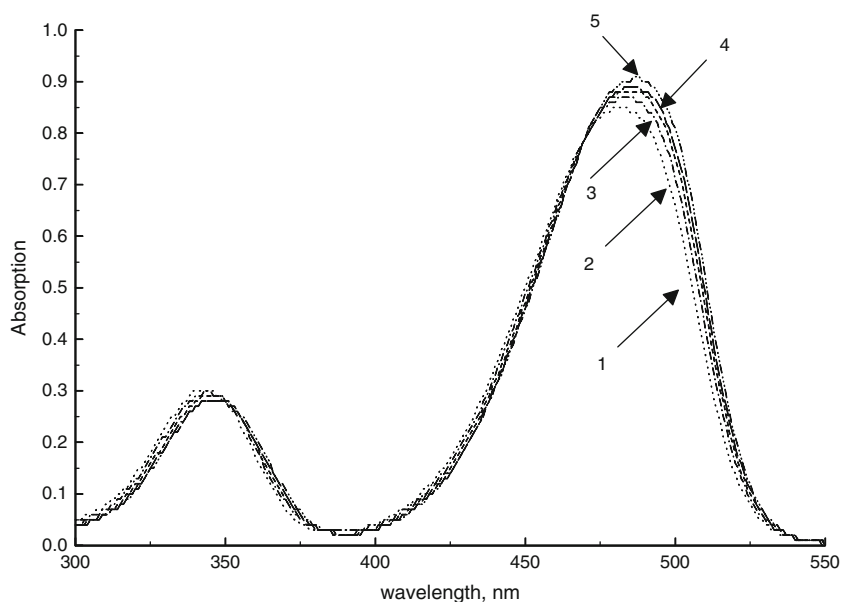
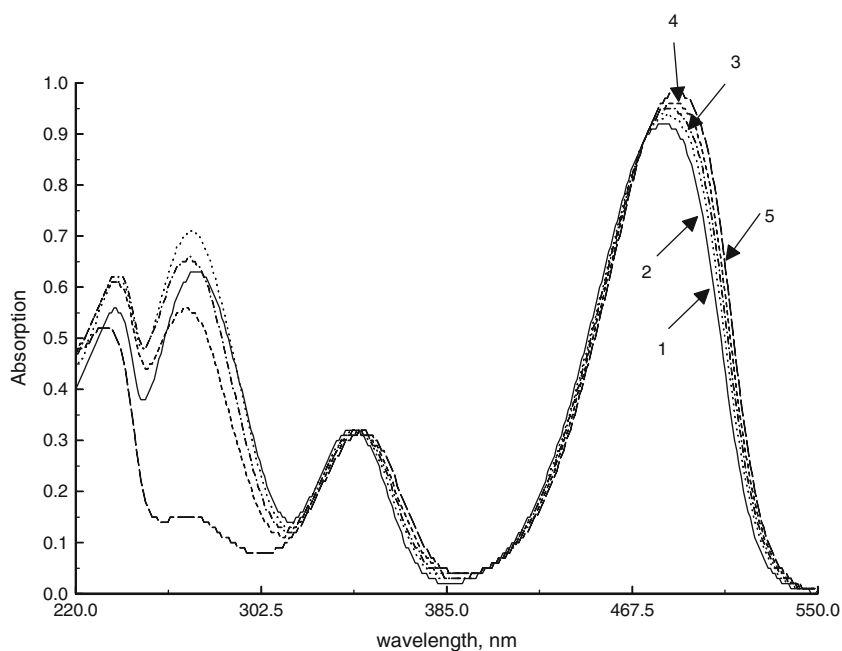


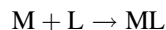
Fig. 4 Dependence of absorption maxima on the solvent polarity (%water in acetonitrile) at 25 °C. The concentration of NBD15C5 was 3.0×10^{-5} M

Fig. 5 Absorption spectra of NBD15C5 (1.0×10^{-5} M) in acetonitrile in presence of increasing amounts of hydrochloric acid (from 1 to 5—0 to 2.4 M HCl)



emission and absorption changes assuming that conformational changes take place during the complexation process to accommodate the metal ion (Tables 2, 3, 4).

The complexation process can be described by the following:



The apparent formation constant K_f was calculated according to Valeur et al. [22] using the equation

$$\frac{I_0}{I_0 - I} = \frac{a}{K_f M} + a$$

Fig. 6 Emission spectra of NBD15C5 (1.0×10^{-5} M) in acetonitrile in presence of increasing amounts of hydrochloric acid (from 1 to 5—0 to 2.4 M HCl)

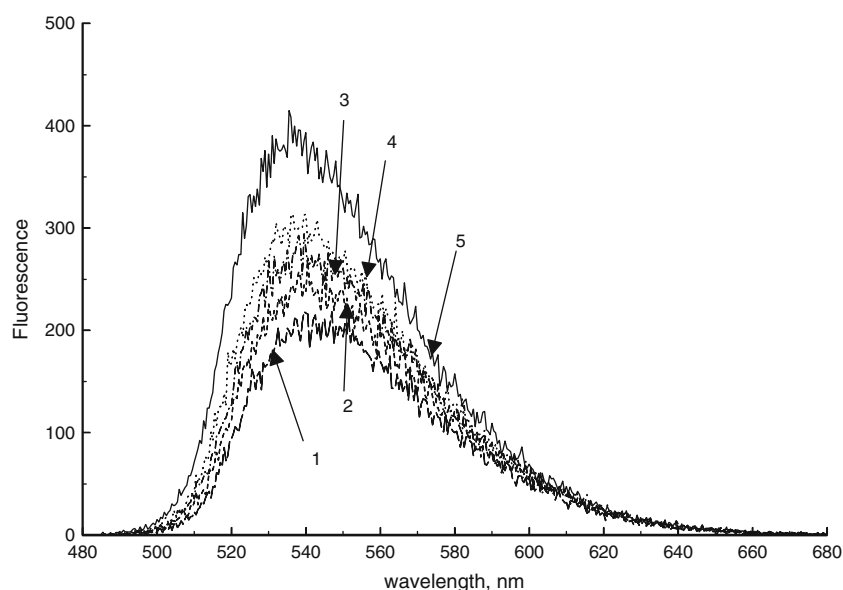
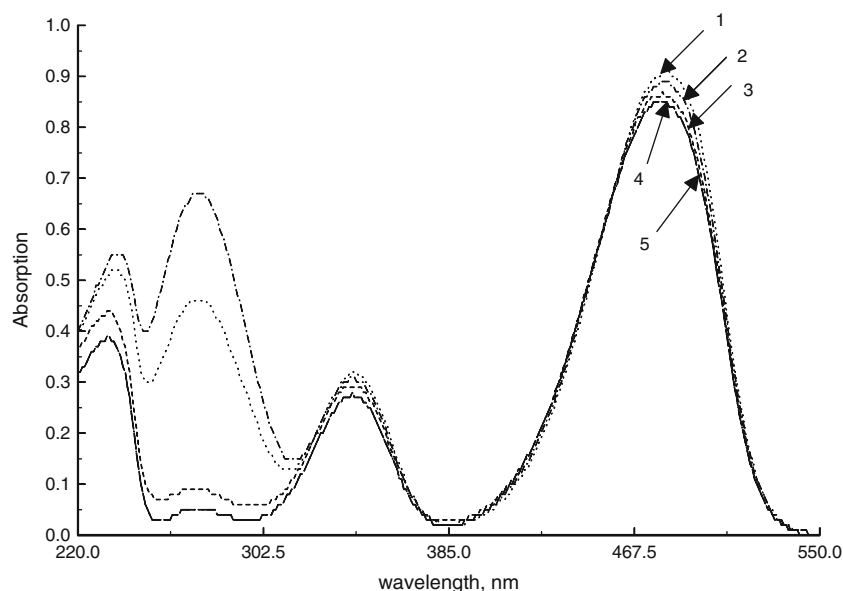


Fig. 7 Absorption spectra of NBD15C5 (1.0×10^{-5} M) in acetonitrile in presence of increasing amounts of 1.0 M sodium perchlorate



where a is a constant incorporating the factors of molar absorptivities and fluorescence quantum yields for the free ligand and complex. Under the conditions of $C_M \gg C_1$ and hence, $[M] \sim C_M$, a plot of $I_0/(I - I_0)$ versus $1/C_M$ is linear, and K_f is found as a ratio of the intercept at the OY axis and the slope.

Tables 5 and 6 summarize the data for the apparent formation constants for the studied complexation processes. Since the changes in the absorption spectra were much smaller and eventual calculations would lead to bigger error all calculations were performed on the basis of the fluorescence changes. The spectral changes induced by the Mg^{2+} ion were inconsistent and no isosbestic point was observed indicating the presence of several equilibria in the

mixture. The formation constant data show that NBD18C6 exhibits strong affinity toward Ca^{2+} and Sr^{2+} ions with the formation constants about 1000 times higher than those with the other metal ions included in the study.

Conclusions

NBD modified crown ethers were synthesized, purified and their structure confirmed by proton NMR, IR and elemental analysis. UV/vis and fluorescence studies demonstrated the strong influence of the solvent polarity on the photophysical properties of the compounds. Metal ions cause fluorescence enhancement which is concentration dependant.

Fig. 8 Emission spectra of NBD15C5 (1.0×10^{-5} M) in acetonitrile presence of increasing amounts of 1.0 M sodium perchlorate

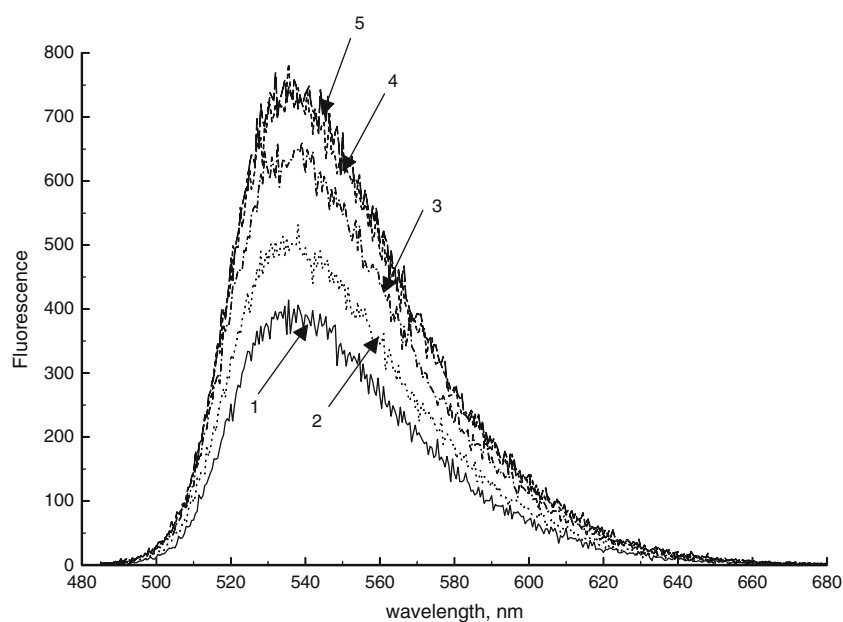


Table 2 Effect of metal cations on the emission maxima and emission intensity of NBD15C5 and NBD18C6

Ion	NH_4^+		Li^+		Na^+		K^+	
	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI
NBD15C5	-1 to 2	+38	0	+445	0	+332	+4	-120
NBD18C6	-8	+225	0	+212	0	+631	0	+114

Table 3 Effect of metal cations on the emission maxima and emission intensity of NBD15C5 and NBD18C6

Ion	Mg^{2+}		Ca^{2+}		Sr^{2+}		Ba^{2+}	
	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI	$\Delta\lambda_{\text{max}}$ (nm)	ΔFI
NBD15C5	0	0	0	0	0	+198	0	+46
NBD18C6	0	Inc.	-5	+658	-5	+296	-8	+304

Table 4 Ionic radii and cavity sizes of the crown ethers included in this study

Ion	Ionic radius A
Li^+	0.6
Mg^{2+}	0.82
Na^+	0.95
Ca^{2+}	1.18
K^+	1.33
Sr^{2+}	1.32
Ba^{2+}	1.53
Crown ether	Cavity size
Aza-18-crown-6	1.38
Aza-15-crown-5	0.85

Table 5 Apparent formation constants for the complexation between NBD crown ethers, alkali metal ions and ammonium

Ion:	Li^+	Na^+	K^+	NH_4^+
Ligand:	K_f	K_f	K_f	K_f
NBD15C5	55	9.7	16	33
NBD18C6	46	30	2.59	309

Table 6 Apparent formation constants, K_f for the complexation between NBD crown ethers and alkaline earth metal ions

Ion	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
Ligand	K_f	K_f	K_f	K_f
NBD15C5	N/A	55	40	82
NBD18C6	N/A	4.75×10^5	1.27×10^4	935

Double-charged ions exhibit stronger binding to both NBD15C5 and NBD18C6. NBD18C6 is Ca^{2+} and Sr^{2+} selective with binding constants several orders of magnitude higher than the other ions included in this study.

Acknowledgements This research was supported by Title III program at Florida A&M University. The authors also would like to thank Dr. Maurice Edington for his constant support and encouragement as well as Dr. Wang Zang from the College of Pharmacy and Pharmaceutical Sciences for recording the NMR spectra.

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