

# Synthesis and photophysics behaviour in AOT/cyclohexane w/o microemulsions of a new benzo[*a*]phenoxazinium chloride with 3-((3-chloropropyl)disulfanyl)propyl)amino group

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**Abstract:** Fluorescent probes for labelling of biomolecules are widely used for various purposes. In this context we have efficiently synthesized a new benzo[*a*]phenoxazinium chloride possessing the 3-((3-chloropropyl)disulfanyl)propyl)amino group at position 5. The disulfide bond can be cleaved under mild basic conditions to afford the free thiol group, and consequently this heterocycle could act as a capping layer for the passivation of semiconductor QDs. The location of the new benzo[*a*]phenoxazinium chloride in AOT/cyclohexane was studied by fluorescence spectroscopy, since this microheterogeneous structures are frequently used for nanoparticle synthesis.

**Keywords:** benzo[*a*]phenoxazines, NIR fluorescent probes, AOT reverse micelles.

## Introduction

The development of fluorescent probes is an area of interest in the present days due to their wide use for the analytical purposes in various fields of science. Specially, long wavelength emission probes are preferred in protein labelling,<sup>1</sup> biological stains<sup>2</sup> and many other purposes.<sup>3</sup> In this connection, benzo[*a*]phenoxazines are also used as biomarkers in the field of medicine.<sup>4,5</sup> Owing to the importance and in continuation of our research works,<sup>6-8</sup> we are now interested in the synthesis of benzo[*a*]phenoxazine derivatives with a disulfide bond, which upon cleavage can be attached to quantum dots to allow advanced probing applications.

The water pool entrapped in reversed micelles/microemulsions has been extensively used as a medium to study chemical and biological reactions.<sup>9</sup> Reverse micelles are intensively used in drug delivery

systems,<sup>10</sup> templates for the synthesis of semiconductor,<sup>11</sup> among others. Previous studies of a commercial benzo[*a*]phenoxazinium perchlorate, Nile Blue, in reverse micelles mainly showed varying amount of normal and deprotonated forms with water content.<sup>12</sup> The current work describes the synthesis of a benzo[*a*]phenoxazinium chloride with a disulfide bond and the variation in the photophysical behaviour in homogeneous media and AOT reverse micelles.

## Experimental

### Synthesis of *N*-(3-((3-chloropropyl)disulfanyl)propyl)naphthalen-1-amine **2**.

To a solution of naphthalen-1-amine (0.286 g,  $2.0 \times 10^{-3}$  mol) in ethanol (2 mL), 3-chloropropane-1-thiol (0.242 g,  $2.20 \times 10^{-3}$  mol) was added, and the resulting mixture was refluxed for 26 hours. The progress of reaction was monitored by TLC (dichloromethane/methanol, 9.5:0.5). After completion of the reaction, the solvent was evaporated and the mixture was purified by column chromatography on silica using dichloromethane and dichloromethane/methanol (99:1), as the eluent. Compound **2** was obtained as violet oil (0.401 g, 54%). TLC (dichloromethane/methanol, 9.9:0.1):  $R_f = 0.71$ . <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_H = 2.14$ -2.23 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl and NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 2.82-2.92 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl and NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 3.44 (t,  $J = 6.8$  Hz, NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 3.65 (t,  $J = 6.4$  Hz, 2H, NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 6.68 (d,  $J = 7.6$  Hz, 1H, 2-H), 7.26-7.31 (m, 1H, 3-H), 7.40 (t,  $J = 8.0$  Hz, 1H, 6-H), 7.44-7.53 (m, 2H, 7-H and 4-H), 7.80-7.86 (m, 2H, 5-H and 8-H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100.6 MHz):  $\delta_C = 28.40$  (NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 31.53 (NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 35.25 (NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 36.32 (NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 42.74 (NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 43.07 (NHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Cl), 104.64 (C-2), 117.65 (C-4), 119.76 (C-8), 123.40 (Ar-C), 124.74 (C-7), 125.71 (C-6), 126.51 (C-3), 128.64 (C-5), 134.27 (Ar-C), 142.87 (Ar-C). IR (KBr 1%, cm<sup>-1</sup>):  $\nu = 3422, 3054, 2954, 2926, 2853, 1581, 1527, 1480, 1435, 1409, 1343, 1277, 1265, 1252, 1216, 1118, 1036, 950, 853, 785, 770$ . HRMS:  $m/z$  (EI): calcd. for C<sub>16</sub>H<sub>20</sub>NS<sub>2</sub><sup>35</sup>Cl [M<sup>+</sup>] 325.0726; found 325.0729. Calcd. for C<sub>16</sub>H<sub>20</sub>NS<sub>2</sub><sup>37</sup>Cl [M<sup>+</sup>] 327.0696; found 327.0693.

**Synthesis of *N*-(5-((3-((3-chloropropyl)disulfanyl)propyl)amino)-10-methyl-9*H*-benzo[*a*]phenoxazin-9-ylidene)ethanaminium chloride **3**.** To a cold solution (ice bath) of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **2** (0.050 g,  $2.7 \times 10^{-4}$  mol), in ethanol (2.0 mL), *N*-(3-((3-

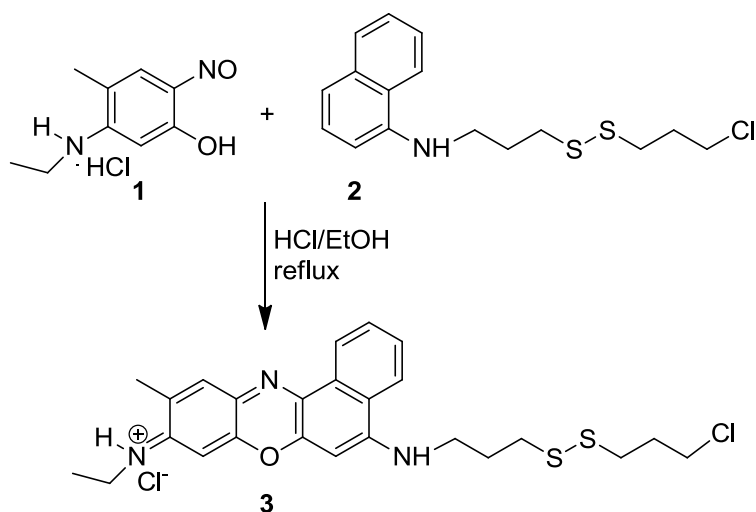
chloropropyl)disulfanyl)propyl)naphthalen-1-amine **2** (0.079 g,  $1.37 \times 10^{-4}$  mol), and concentrated hydrochloride acid ( $7.0 \times 10^{-3}$  mL) were added. The mixture was refluxed for a period of 7 hours, and monitored by TLC (dichloromethane/methanol, 9.5:0.5). After evaporation of the solvent and column chromatography purification on silica gel with dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, compound **3** was obtained as a blue solid (0.078 g, 64%).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta_{\text{H}} = 1.39$  (t,  $J = 7.6$  Hz, 3H,  $\text{NHCH}_2\text{CH}_3$ ), 2.11-2.21 (m, 2H,  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 2.23-2.34 (m, 2H,  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 2.33 (s, 3H,  $\text{CH}_3$ ), 2.85-2.97 (m, 4H,  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$  and  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 3.53 (q,  $J = 7.2$  Hz, 2H,  $\text{NHCH}_2\text{CH}_3$ ), 3.67 (t,  $J = 6.4$  Hz, 2H,  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 3.82 (t,  $J = 6.8$  Hz, 2H,  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 6.79 (s, 1H, 8-H), 6.92 (s, 1H, 11-H), 7.64 (s, 1H, 6-H), 7.79 (t,  $J = 7.2$  Hz, 1H, 3-H), 7.89 (t,  $J = 7.6$  Hz, 1H, 2-H), 8.33 (d,  $J = 8.4$  Hz, 1H, 4-H), 8.87 (d,  $J = 8.0$  Hz, 1H, 1-H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100.6 MHz):  $\delta_{\text{C}} = 14.20$  ( $\text{NHCH}_2\text{CH}_3$ ), 17.64 ( $\text{CH}_3$ ), 29.10 ( $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 32.93 ( $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 36.13 ( $\text{NHCH}_2\text{CH}_3$ ), 36.45 ( $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$  and  $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 43.96 ( $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 44.70 ( $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{CH}_2\text{Cl}$ ), 94.25 (C-8), 94.63 (C-6), 123.87 (Ar-C), 125.47 (C-3), 130.76 (C-4), 131.74 (Ar-C), 132.46 (C-2), 132.54 (C-1), 132.70 (Ar-C), 133.85 (Ar-C), 135.30 (C-10), 149.26 (C-11), 152.62 (Ar-C), 154.07 (C-9), 156.34 (Ar-C), 158.61 (C-5). HRMS:  $m/z$  (ESI): calcd. for  $\text{C}_{25}\text{H}_{29}^{35}\text{ClN}_3\text{OS}_2$   $[\text{M}+\text{H}]^+$  486.14351; found 486.14281. Calcd. for  $\text{C}_{25}\text{H}_{29}^{37}\text{ClN}_3\text{OS}_2$   $[\text{M}+\text{H}]^+$  488.14069; found 488.13387.

## Results and discussion

Benzo[*a*]phenoxazinium chloride **3** was synthesized by condensation of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **1** with *N*-(3-((3-chloropropyl)disulfanyl)propyl)naphthalen-1-amine **2** in presence of concentrated hydrochloric acid in moderate yield (Scheme 1). The intermediate **2** was obtained by alkylation of naphthalen-1-amine with 3-chloropropane-1-thiol in ethanol as the solvent, also in moderate yield. The required 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **1** was synthesised by nitrosation of the corresponding 3-(ethylamino)-4-methylphenol with sodium nitrite in the presence of hydrochloric acid, in a mixture of ethanol-water as the solvent.<sup>13</sup> The structures of the compounds obtained were confirmed by the usual analytical techniques.

The  $^1\text{H}$  NMR spectra of the target molecule **3** showed a multiplet at  $\delta$  2.85-2.97 ppm for two methylenic groups adjacent to the disulfide bond ( $-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-$ ); the methylene group directly linked

to the nitrogen atom ( $\text{NHCH}_2$ ) exhibited a triplet at  $\delta$  3.67 ppm and similarly another triplet was observed for the methylene group adjacent to the chlorine atom ( $-\text{CH}_2\text{Cl}$ ) at  $\delta$  3.82 ppm. The presence of protons of the methyl group directly linked to the aromatic ring at position 10, appeared as a singlet ( $\delta$  2.33 ppm). In addition, spectra showed the expected aromatic protons of the polycyclic system, in particular H-8 ( $\delta$  6.79 ppm), H-6 ( $\delta$  7.64 ppm), and H-11 ( $\delta$  6.92 ppm), which appeared in the form of singlets. The  $^{13}\text{C}$  NMR spectra exhibited the signals of the methylenic groups adjacent to the S-S bond ( $-\text{CH}_2\text{-S-S-CH}_2-$ ) at 36.45 ppm, the methylene group directly linked to the nitrogen atom ( $\text{NHCH}_2$ ) at 44.70 ppm, and methylene group linked to the chlorine atom ( $-\text{CH}_2\text{Cl}$ ) at 43.96 ppm. The methyl group linked to the aromatic ring at position 10 showed a signal at  $\delta$  17.64 ppm. Spectra showed the expected aromatic carbons, in particular C-8 ( $\delta$  94.25 ppm); C-6 ( $\delta$  94.63 ppm) and C-11 ( $\delta$  149.26 ppm).

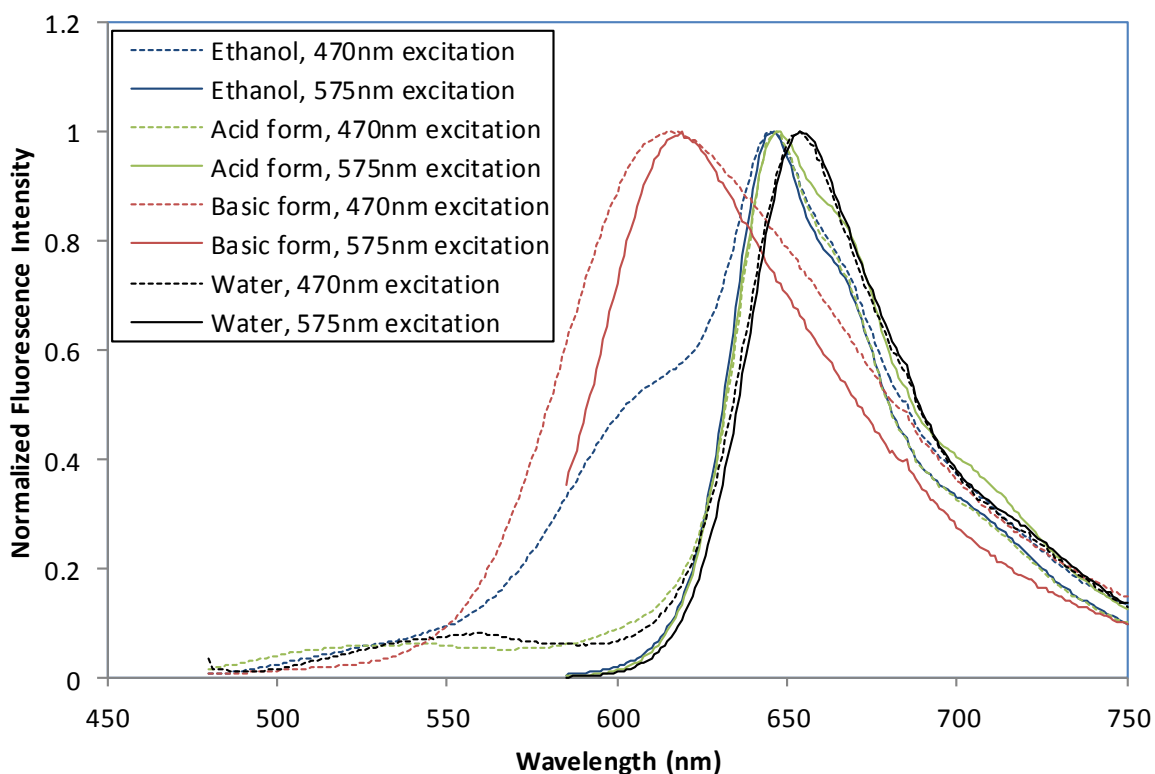


**Scheme 1.** Synthesis of benzo[*a*]phenoxazininium chloride 3.

Previous studies on benzo[*a*]phenoxazininium chlorides showed that their photophysics in proton-accepting solvents is influenced by acid-base equilibria mainly located at the 5-amino position.<sup>6-8</sup> In ethanol media the absorption spectra are dominated by an acidic form ( $\text{AH}^+$ ) and a  $\sim 100$  nm blue shifted neutral form (A).<sup>6</sup> The fluorescence of the basic form is broad and centred at around 600 nm whilst the acid form ( $\text{AH}^+$ ) shows a band centred above 660 nm with a much higher quantum yield ( $\sim 0.4$ ).<sup>14</sup> These fluorescence bands are seen to red shift when the medium changes from ethanol to water (data not shown), which is typical of  $\pi$ - $\pi^*$  electronic transitions.

At 470 nm the basic form is mostly excited with a small fraction of acidic form. At 575 nm the situation is reversed. The above characteristics are confirmed in Figure 1 where fluorescence data in plain ethanol media or when acidified with trifluoroacetic acid or basified with tetraethylammonium hydroxide are shown together with results in water solutions. Additionally, a small emission band with maximum near 550 nm is observed with 470 nm excitation in water and acidified ethanol. This could be due to a structure with a partially reduced  $\pi$ -electron system resulting in a blue shifted emission.

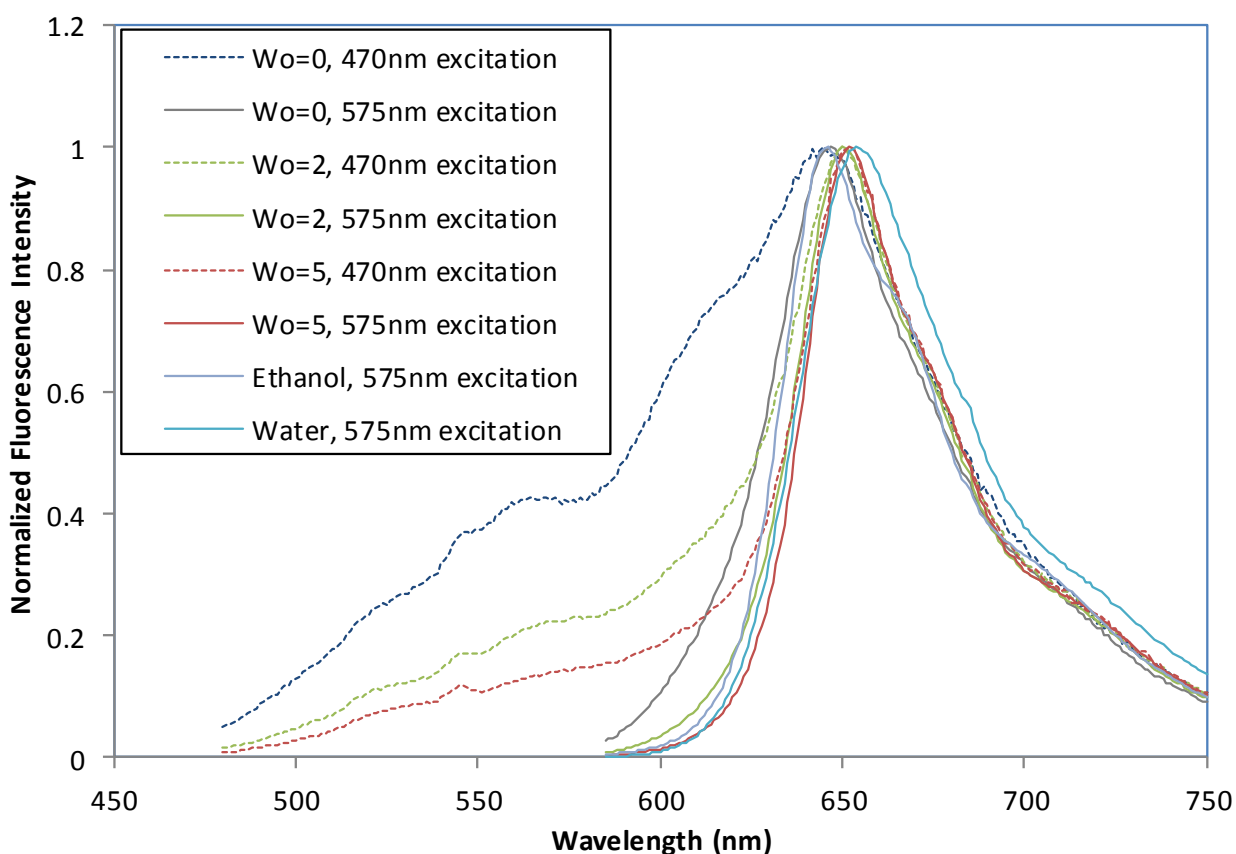
In a reverse micellar system small nanosized water pools are surrounded by a layer of surfactant (AOT) molecules. This organization allows the water phase to be homogeneously dispersed in the organic (cyclohexane) solvent. These nano-pools have been used as chemical reactors allowing, by confinement effects, the synthesis of nanoparticles.<sup>15</sup> Depending on the pool size of the reverse micelles, the water within them has different properties than “bulk” normal water. The size of the reverse micelles is determined by the  $\omega_o$  parameter that is defined by the ratio of water to surfactant concentrations.<sup>16</sup>



**Figure 1:** Normalized fluorescence spectra of compound **3** in ethanol, acidified ethanol, basified ethanol and water either at 470 nm (dashed lines) and 575 nm excitation wavelengths (full lines).

It is expected that benzo[*a*]phenoxazinium chlorides have the potential to probe the water environment in the reverse micelles because of the already discussed dependence of its photophysics on an acid-base equilibria and of a slight solvatechromism through a  $\pi$ - $\pi^*$  electronic transition. The specific case of the synthesized compound **3** is of interest as the disulfide bond can easily be cleaved and the resulting thiol group used in coupling with cadmium based quantum dots or with metallic nanoparticles. As this can be produced within the water pools of reverse micelles it is interesting to know where this type of benzo[*a*]phenoxazinium chlorides reside in surfactant water in oil microemulsions.

As an initial study (Figure 2), fluorescence spectra were obtained in AOT/cyclohexane reverse micelles with varying amounts of added water ( $\omega_o = 0, 2$  and 5). From the acid form band it can be seen that the environment changes from ethanol-like at  $\omega_o = 0$  to a more polar one but still different from bulk water. With added water the reverse micelle interface gets more hydrated. As a result, it seems that compound **3** resides in the interface and does not go to water even at  $\omega_o = 5$  where bulk-like water already exists.<sup>17</sup>



**Figure 2:** Normalized fluorescence spectra of compound **3** in AOT/cyclohexane reverse micelles either at 470 nm (dashed lines) and 575 nm excitation wavelengths (full lines). For comparison fluorescence spectra in ethanol and water are also included.

The basic neutral form appears at  $\omega_0 = 0$  but it gradually disappears as the water content is increased. The band in the 550 nm region also is seen to decrease with the increase of  $\omega_0$ .

## Conclusions

We have efficiently synthesized *N*-(5-((3-((3-chloropropyl)disulfanyl)propyl)amino)-10-methyl-9*H*-benzo[*a*]phenoxazin-9-ylidene)ethanaminium chloride in moderate yield. The photophysics of the acid and basic forms were studied in ethanolic media by adding either a strong acid or a strong base. The behaviour of this compound in AOT/cyclohexane w/o microemulsion allows the study of reverse micelles as they locate in its interface and their fluorescence spectra depends on the water content through the  $\omega_0$  parameter. The localization of the synthesized compound in the reverse micelle interface can be exploited in order to functionalize quantum dots with a benzo[*a*]phenoxazine moiety.

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