Synthesis of a new class of benzo[*a*]phenoxazinium chlorides with ((3-chloropropyl)disulfanyl)propoxy and propoxylamino terminal groups

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Abstract: Fluorescent probes for labeling of biomolecules are widely used for various purposes. In this context we have efficiently synthesized a new benzo[*a*]phenoxazinium chloride with the ((3-chloropropyl)disulfanyl) propoxylamino group at position 5. In this molecule the disulfide bond can be cleaved under mild basic conditions to afford the free thiol group. These heterocycles having the thiol group could act as a capping layer for the passivation of semiconductor QDs and so its location in AOT/cyclohexane was studied by fluorescence spectroscopy as 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 files of science. Especially, long wavelength emission probes are preferred in protein labeling,[1] biological stains[2] and many other purposes.[3] In this connection, benzo[*a*]phenoxazines are also used as biomarkers in the field of medicine.[4,5] Owing to the importance and in continuation of our research works,[6-8] we were interested to synthesize a benzophen[*a*]oxazine compound with a disulfide bond which upon cleavage can be attached to quantum dots to serve many purposes. The water pool entrapped in reversed micelles/microemulsions has been extensively used as a medium to study chemical and biological reactions.[9] Reverse micelles are intensively used in drug delivery systems,[10] and templates for the synthesis of semiconductor.[11] 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.[12] This current work describes the synthesis of benzophenoxazinium chloride with a disulfide bond and the variation in the photophysical behavior in homogeneous media and in AOT reverse micelles.

Experimental

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, 2.7×10^{-4} mol), in ethanol (2.0 mL), *N*-(3-((3-chloropropyl)disulfanyl)propyl)naphthalen-1-amine **2** (0.079 g, 1.37×10^{-4} mol), and concentrated hydrochloride acid (7.0×10^{-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 **1a** was obtained as a blue solid (0.078 g, 64%). ^{1}H NMR (CD_3OD, 400 MHz): δ_H = 1.39 (t, J = 7.6 Hz, 3H, $NHCH_2\underline{CH_3}$), 2.16 (m, 2H, $NHCH_2\underline{CH_2}CH_2SSCH_2CH_2CH_2Cl$), 2.28 (m, 2H, $NHCH_2CH_2CH_2SSCH_2\underline{CH_2}CH_2Cl$), 2.33 (s, 3H, $\underline{CH_3}$), 2.91 (m, 4H, $NHCH_2CH_2\underline{CH_2}SSCH_2CH_2CH_2Cl$ and $NHCH_2CH_2CH_2SS\underline{CH_2}CH_2CH_2Cl$), 3.53 (q, J = 7.2 Hz, 2H, $N\underline{CH_2}CH_3$), 3.67 (t, J = 6.4 Hz, 2H, $NH\underline{CH_2}CH_2CH_2SSCH_2CH_2CH_2Cl$), 3.82 (t, J = 6.8 Hz, 2H, $NHCH_2CH_2CH_2SSCH_2CH_2\underline{CH_2}Cl$), 6.79 (s, 1H, 8-H), 6.92 (s, 1H, 11-H), 7.63 (s, 1H, 6-H), 7.79 (t, J = 6.8 Hz, 1H, 3-H), 7.88 (t, J = 7.2 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}C NMR (CD_3OD, 100.6 MHz): δc = 14.20 ($NHCH_2\underline{CH_3}$), 17.64 ($\underline{CH_3}$), 29.10 ($NHCH_2\underline{CH_2}CH_2SSCH_2CH_2CH_2Cl$), 32.93 ($NHCH_2CH_2CH_2SSCH_2\underline{CH_2}CH_2Cl$), 36.13 ($NHCH_2\underline{CH_3}$), 36.45 ($NHCH_2CH_2CH_2SS\underline{CH_2}CH_2CH_2Cl$), 43.96 ($NHCH_2CH_2CH_2SSCH_2CH_2\underline{CH_2}Cl$), 44.70 ($NH\underline{CH_2}CH_2CH_2SSCH_2CH_2CH_2Cl$), 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 $C_{25}H_{29}ClN_3OS_2$ $[M]^+$ 486.1435; found486.1428.

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

To a solution of naphthalen-1-amine (0.286 g, 2.0×10^{-3} mol) in ethanol (2 mL), 3-chloropropane-1-thiol (0.242 g, 2.20×10^{-3} mol) was added, and the resulting mixture was refluxed for 26 hours. The

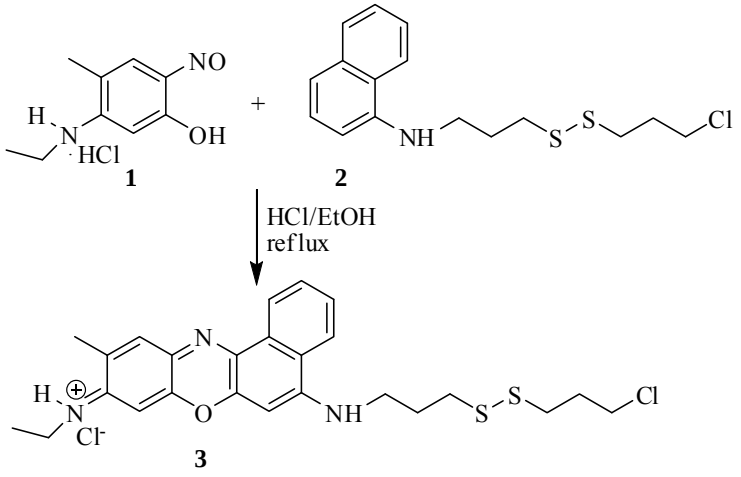
progress of reaction was monitored by TLC (dichloromethane/methanol, 9.5:0.5). After completion of the reaction, the solvent was evaporated and the mixture was purified by column chromatography on silica using dichloromethane and dichloromethane/methanol (99:1), as the eluent. *N*-(3-((3-chloropropyl)disulfanyl)propyl)naphthalen-1-amine **2** was obtained as violet oil (0.401 g, 54%). TLC (dichloromethane/methanol, 9.9:0.1): R_f = 0.71. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 2.18 (m, 4H, $NHCH_2\underline{CH_2}CH_2SSCH_2CH_2CH_2Cl$ and $NHCH_2CH_2CH_2SSCH_2\underline{CH_2}CH_2Cl$), 2.88 (m, 4H, $NHCH_2CH_2\underline{CH_2}SSCH_2CH_2CH_2Cl$ and $NHCH_2CH_2CH_2SS\underline{CH_2}CH_2CH_2Cl$), 3.44 (t, J = 6.8 Hz, $NH\underline{CH_2}CH_2CH_2SSCH_2CH_2CH_2Cl$), 3.66 (m, 2H, $NHCH_2CH_2CH_2SSCH_2CH_2\underline{CH_2}Cl$), 6.68 (d, J = 7.6 Hz, 1H, 2-H), 7.29 (m, 1H, 3-H), 7.40 (t, J = 8.0 Hz, 1H, 6-H), 7.50 (m, 2H, 7-H and 4-H), 7.83 (m, 2H, 5-H and 8-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 28.40 ($NHCH_2\underline{CH_2}CH_2SSCH_2CH_2CH_2Cl$), 31.53 ($NHCH_2CH_2CH_2SSCH_2\underline{CH_2}CH_2Cl$), 35.25 ($NHCH_2CH_2CH_2SS\underline{CH_2}CH_2CH_2Cl$), 36.32 ($NHCH_2CH_2\underline{CH_2}SSCH_2CH_2CH_2Cl$), 42.74 ($NHCH_2CH_2CH_2SSCH_2CH_2\underline{CH_2}Cl$), 43.07 ($NH\underline{CH_2}CH_2CH_2SSCH_2CH_2CH_2Cl$), 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^{-1}): ν = 3422, 3054, 2954, 2926, 2853, 1581, 1527, 1480, 1435, 1408, 1343, 1277, 1265, 1252, 1216, 1118, 1036, 950, 853, 785, 770. HRMS: m/z (EI): calcd. for $C_{16}H_{20}NS_2Cl$ [M^+] 325.0726; found 325.0729.

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 con. 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, in moderate yield. The required 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **1** was obtained 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. The obtained compounds structure confirmed by the usual analytical techniques.[13]

The ^{1}H NMR spectra showed a multiplet at δ 2.91 ppm for two methylenic groups adjacent to the disulfide bond, (-$\underline{CH_2}$-S-S-$\underline{CH_2}$-), the methylene group directly linked to the nitrogen atom ($NH\underline{CH_2}$) exhibited a triplet at δ 3.67 ppm with J = 6.4 Hz, similarly another triplet was observed for the methylene group (-$\underline{CH_2}Cl$) at δ 3.82 ppm with coupling constant 6.8 Hz. The presence of protons of the

methyl group directly linked to the aromatic ring at position 10, which appeared as singlet (δ 2.33 ppm). In addition, spectra showed the expected aromatic protons of the polycyclic system, in particular H-8 (δ 6.79 ppm), H-6 (δ 7.63 ppm), and H-11 (δ 6.92 ppm), which appeared in the form of singlets. The ^{13}C NMR spectra showed the signals of the methylenic groups (-$\underline{CH_2}$-S-S-$\underline{CH_2}$-), at 36.45 ppm, the methylene group directly linked to the nitrogen atom (NH$\underline{CH_2}$) at 44.70 ppm, and methylene group (-$\underline{CH_2}$Cl) at 43.96 ppm. The methyl group linked to the aromatic ring at position 10 showed a signal at (δ 17.64 ppm). Spectra showed the expected aromatic carbons, in particular C-8 (δ 94.25 ppm); C-6 (δ 94.63 ppm) and C-11 (δ 149.26 ppm).



Scheme 1. Synthesis of benzo[*a*]phenoxazinium **3**.

Previous studies on this type of compounds showed that its photophysics in proton-accepting solvents is influenced by acid-base equilibria mainly located at the 5-amino position.[6-8] In ethanol media the absorption spectra are dominated by an acidic form (AH^+) and a ~100nm blue shifted neutral form (A).[6] The fluorescence of the basic form is broad and centered at around 600 nm whilst the acid form (AH^+) shows a band centered above 660 nm with a much higher quantum yield (~0.4).[14] These fluorescence bands are seen to red shift when the medium changes from ethanol to water (data not shown). This is typicall of π-π* 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 normal ethanol media or when acidified with fluoroacetic acid or made basic with tetraethylammonium

hydroxide (TEAH) is shown together with results in water solutions. Additionally, a small band with maximum near 550 nm is observed with 470 nm excitation in water an in acidified ethanol. This could be due to a structure with a partially reduced π-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.[15] 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 ω_o parameter that is defined by the ratio of water to surfactant concentrations.[16]

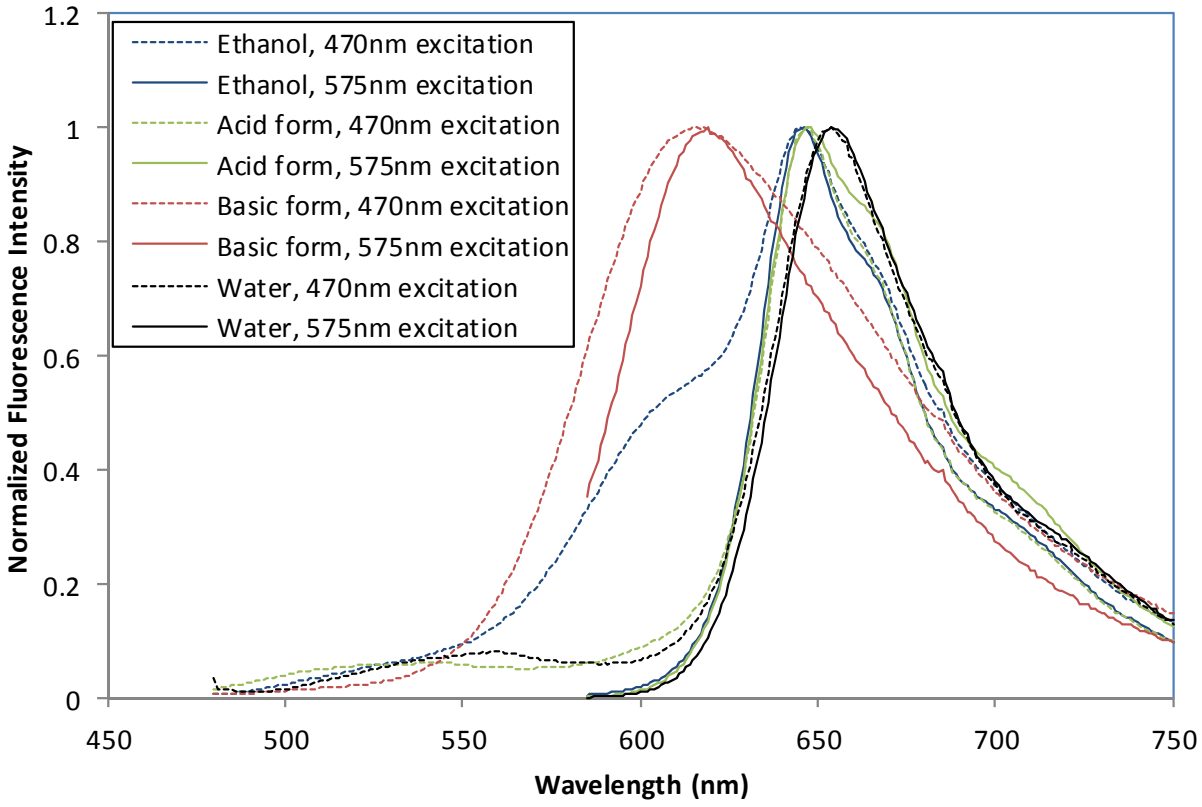


Figure 1: Normalized fluorescence spectra of compound **3** in ethanol, acidified ethanol, basified ethanol and water either at 470 nm excitation wavelength (dashed lines) or at 575 nm excitation wavelength (full lines).

It is expected that benzo[*a*]phenoxazinium chlorides have the potential to probe the water environment in the reverse micelles as already discussed earlier about the dependence of its photophysics on an acid-base equilibria and of a slight solvatochromism through a π-π* electronic transition. The specific case of the synthesized compound is of interest as the disulfide bond can easily be cleaved and the resulting thiol group which can used in coupling with cadmium based quantum dots or with metallic nanoparticles. As these 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.

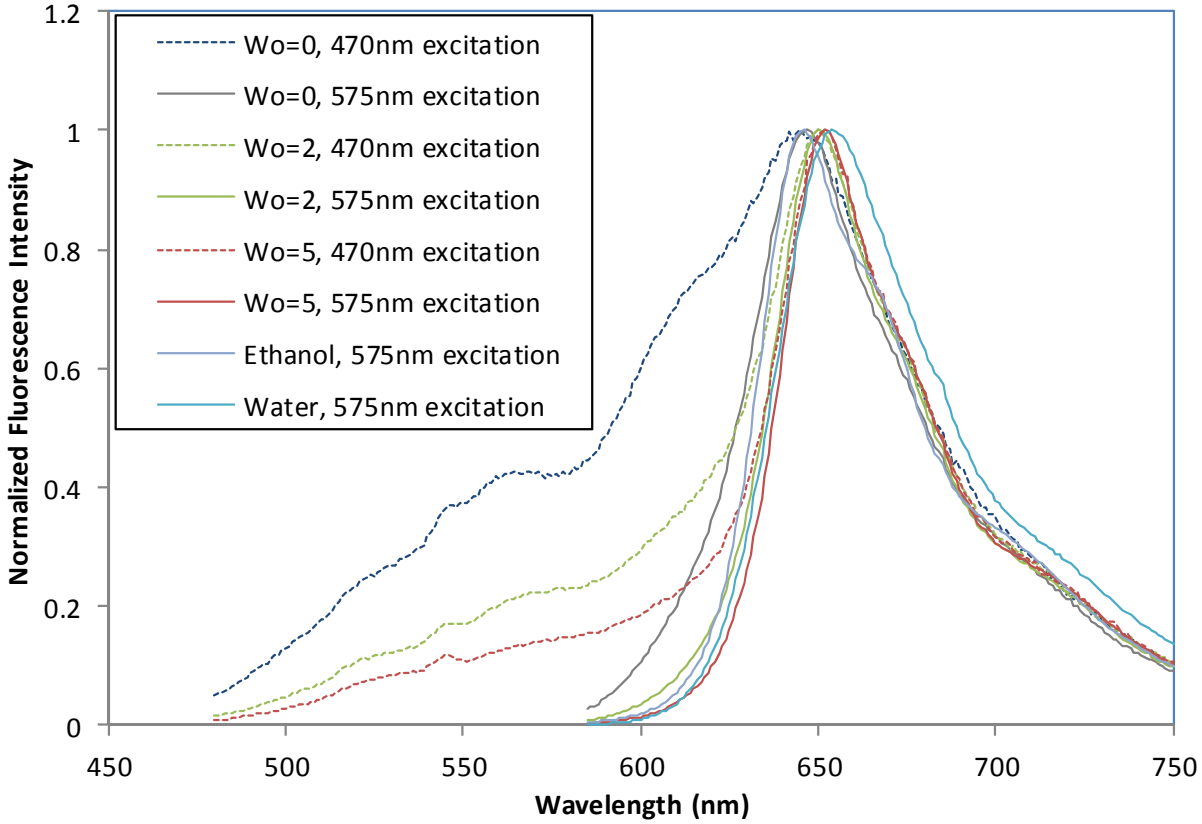


Figure 2: Normalized fluorescence spectra of compound **3** in AOT/ciclohexane reverse micelles either at 470 nm excitation wavelength (dashed lines) or at 575 nm excitation wavelength (full lines). For comparison fluorescence spectra in ethanol and water are also included.

As an initial study (figure 2), fluorescence spectra were obtained in AOT/cyclohexane reverse micelles with varying amounts of added water (ω_o = 0, 2 and 5). From the acid form band it can be seen that the environment changes from ethano-like at ω_o = 0 to a more polar one but still different from bulk water.

With added water the reverse micelle interface gets more hydrated. So, it seems compound **3** resides in the interface and does not go to water even at $\omega_o = 5$ where bulk-like water already exists.[17]
The basic neutral form appears at $\omega_o = 0$ but it gradually disappers as the water content is increased. The band in the 550nm region also is seen to decrease with the increase of ω_o.

Conclusions

We have efficiently synthesized *N*-(5-((3-((3-chloropropyl)disulfanyl)propyl)amino)-10-methyl-9*H*-benzo[*a*]phen oxazin-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 behavior 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 trough the ω_o 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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