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Synthesis and photophysical properties of side-chain chlorinated benzo[*a*]phenoxazinium chlorides

B. Rama Raju [a,b], A. Daniela G. Firmino [b], A. Luísa S. Costa [b], Paulo J. G. Coutinho [a], M. Sameiro T. Gonçalves [b,*]

[a] Centre of Physics, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal
[b] Centre of Chemistry, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal

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ABSTRACT

Novel water-soluble benzo[*a*]phenoxazinium chlorides mono- or disubstituted with 3-chloropropyl groups at the amine of position 9, and also at the hydroxyl function at position 2, were synthesized. These compounds possessing one, two or three chlorinated terminals displayed fluorescence emission in ethanol and water in the range 644–666 nm and 649–676 nm, respectively, with fluorescence quantum yields from 0.26 to 0.38.

It was found that this type of molecules is involved in acid–base equilibrium and tautomerization with localized positive charge. Ab-initio ground and excited state DFT calculations were made for the various possible tautomers of a model compound. Preliminary studies of the photophysical behaviour of these compounds in AOT/cyclohexane reverse micelles with low water content (ω_o=[Water]/[AOT]≤5) showed that they interact strongly with the head groups in the interface feeling the presence of an increased amount of water. At very low ω_o values, AOT molecules promote the tautomerization process allowing it to follow both in absorption and emission spectra.

1. Introduction

The synthesis of novel fluorochromophores based on the oxazine core, such as benzo[*a*]phenoxazines possessing substituents, that originate interesting photophysical properties could be important for labelling purposes. This type of probes through its oxazine core can report on local changes of physico–chemical properties namely micropolarity, hydration level, local charge and microviscosity—very important properties to understand the structure, dynamics and functions of self-organized media, such as micelles, microemulsions, liposomes, etc. The cationic nature of these fluorophores is expected to locate themselves at interfaces constituted by anionic surfactants allowing the study of their structure and physico–chemical properties.

When small amounts of water are added to solutions of appropriate surfactants in water immiscible solvents nanometer-sized water pools surrounded by the polar end of the surfactants are formed. These are called reversed micelles and the ratio of water to surfactant concentration (ω_0) is an important parameter as its value determines the average size of the resulting water pools in the surfactant/organic solvent water in oil (w/o) microemulsion. The water pool entrapped in reversed micelles/microemulsions has been extensively used as a medium to study chemical and biological reactions.[1] The entrapped water is heterogeneous in nature and its properties gradually change as a function of ω_0 and depend on the distance from the polar head layer.[2]

The behaviour of water molecules in the nanopool has been the subject of extensive investigation.[3–7] The use of techniques, such as ultrafast infrared vibrational echo spectroscopy,[3] infrared pump-probe spectroscopy,[4] NMR,[5] calorimetry,[6] neutron scattering[7] and molecular dynamics simulation,[8] allowed the identification of various types of water in reverse micelles. The water physically trapped between surfactant head groups, i.e., water directly bound (by H-bonding) to the head groups and relatively unperturbed bulk like water at the centre of the nanopools. There are still some controversy on the size (ω_0 values) of the reverse micelles at which water behaviour in the core becomes similar to that of bulk water[9,10] with the limiting value in dispute being $\omega_0 > 10$.[9]

Due to various applications of reverse micelles, such as drug delivery systems[11] and as templates for the synthesis of semiconductor[12] or metallic nanoparticles,[13] they have been intensively used in recent years. Previous studies of commercial benzo[*a*]phenoxazinium perchlorate, Nile Blue, in reverse micelles mainly showed varying amount of normal and deprotonated forms with water content.[14–16]

Considering these facts and as part of our research interests in the synthesis and characterization of fluorescence probes,[17] this

* Corresponding author. Fax: +351 253604382; e-mail address: msameiro@quimica.uminho.pt (M.S.T. Gonçalves).

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work describes the preparation of a new set of benzo[*a*]phenoxazinium chlorides possessing chlorinated terminals at substituents of the side-chain hydroxyl and/or the amino groups of positions 2- and 9- of the polycyclic system. These terminals can offer the possibility of covalent labelling of the analytes through nucleophilic substitution, in addition to the inherent non-covalent capability of benzo[*a*]phenoxazinium chlorides synthesized. The study of the variation in photophysical behaviour, in homogeneous media and in AOT reverse micelles was performed.

2. Results and discussion

2.1. Synthesis

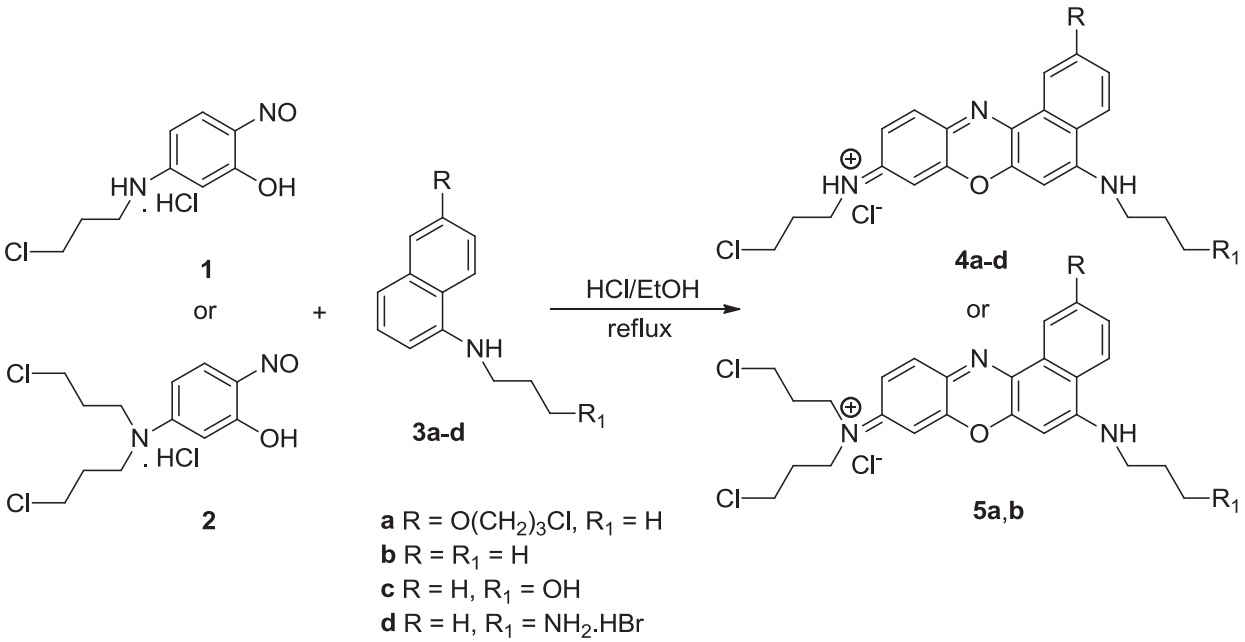
The synthesis of benzo[*a*]phenoxazinium chlorides **4a**–**d** and **5a**,**b** was initiated by the preparation of *N*- or *N*- and *O*-alkylated derivatives of 3-aminophenol, naphthalen-1-amine and 5-aminonaphthalen-2-ol. The choice of these precursors is essential, since they determined the substituents present at 2-, 5- and 9-positions of the tetracyclic aromatic system of the target compounds.

The reaction of 3-aminophenol with 1-bromo-3-chloropropane followed by silica gel column chromatography purification afforded the mono- and di-*N*-alkylated derivatives, namely 3-(3-chloropropylamino)phenol and 3-(bis(3-chloropropyl)amino) phenol, respectively. By nitrosation of these compounds with sodium nitrite in hydrochloric acid solution the required 5-(3-chloropropylamino)-2-nitrosophenol hydrochloride **1** and 5-(bis(3-chloropropyl)amino)-2-nitrosophenol hydrochloride **2** were obtained.[18]

The synthesis of 6-(3-chloropropoxy)-*N*-propylnaphthalen-1-amine **3a** was carried out by the *O*-alkylation of 5-(propylamino) naphthalen-2-ol with 1-bromo-3-chloropropane in the presence of cesium carbonate by heating at 60 °C in acetonitrile. The starting reagent was obtained from the reaction of 5-aminonaphthalen-2-ol with 1-bromopropane by refluxing in methanol, as previously reported.[17f]

Similarly, the synthesis of *N*-propylnaphthalen-1-amine **3b**, 3-(naphthalen-1-ylamino)propan-1-ol **3c** and N^1-(naphthalen-1-yl) propane-1,3-diamine hydrobromide **3d**, having the methyl, hydroxyl and amine groups as terminals of the aliphatic chains was achieved by alkylation of naphthlen-1-amine with 1-bromopropane, 3-bromopropan-1-ol and 3-bromopropan-1-amine hydrobromide, respectively, in ethanol under reflux conditions.[17b,c] After purification by silica gel column chromatography, intermediates **3a**–**d** were isolated as solid and oils (**3c**) in moderate to good yields, and spectroscopic data were in accordance with the expected structures.

The reaction of 3-(3-chloropropylamino)phenol hydrochloride **1** or 3-(bis(3-chloropropyl)amino)phenol hydrochloride **2** with *N*- or *N*- and *O*-alkylated 5-aminonaphthalen-2-ol and naphthalen-1-amine derivatives **3a**–**d** in an acidic medium afforded the corresponding benzo[*a*]phenoxazinium chloride **4a**–**d** or **5a**,**b**, respectively (Scheme 1). Thus, reaction between nitrosophenol **1** with intermediates **3a**–**d** in ethanol, in the presence of concentrated hydrochloric acid, and after silica gel column chromatography purification gave the benzo[*a*]phenoxazinium chlorides **4a**–**d**, possessing the amine of 9-position monosubstituted with the 3-chloropropyl group. Similarly, the nitrosophenol **2** reacted with precursors **3a** and **3b**, producing the cationic dyes **5a**,**b**, but with the 9-position of the polycyclic system disubstituted with 3-chloropropyl groups. All these compounds were obtained as blue solids in moderate yields and were fully characterized by high resolution mass spectrometry, IR and NMR (^{1}H and ^{13}C) spectroscopy.



Scheme 1. Synthesis of benzo[*a*]phenoxazinium chlorides **4a**–**d** and **5a**,**b**.

In the IR spectra of benzo[*a*]phenoxazines **4** and **5** we highlighted the bands of the amine and hydroxyl groups (3427–3184 cm^{-1}), as well as a strong band of the C=N bond (1640–1588 cm^{-1}) due to the fused oxazine ring.

The ^{1}H NMR spectra showed the methyl groups for the terminal of the aliphatic chains as a triplet (δ 1.12–1.14 ppm), the methylene protons directly linked to the nitrogen atom at 5- and 9-positions (NHCH_2 or NCH_2) as multiplets or triplets (in case of NHCH_2CH$_2$CH$_2$Cl or NCH_2CH$_2$CH$_2$Cl) (δ 3.54–4.26 ppm), as well as the adjacent methylene groups, NHCH$_2$$CH_2$ or NCH$_2$$CH_2$ as multiplets (δ 1.80–2.30 ppm), and NHCH$_2$CH$_2$$CH_2$ or NCH$_2$CH$_2$$CH_2$, which appeared as multiplets or triplets (**4c**, **5a**) (δ 3.54–3.85 ppm). The remaining methylene protons attached to oxygen atom (compounds **4a** and **5a**), namely OCH_2 appeared as a triplet (**4a**) or a multiplet (**5a**) (δ 4.38–4.43 ppm), groups close to the same atom,

OCH_2CH_2, which appeared as multiplets (δ 2.30–2.41 ppm) and $OCH_2CH_2CH_2$, in the form of a triplet (**4a**) or a multiplets (**5a**) (δ 3.72–3.87 ppm). In addition to the signals corresponding to the aliphatic *N*- and *O*-substituents in the heterocycle, the ^{13}C NMR spectra displayed the expected aromatic carbons, that we highlighted C-8 (δ 94.13–97.56 ppm), C-6 (δ 94.18–97.47 ppm) and C-11 (δ 133.57–135.0 ppm).

2.2. Photophysical studies

Electronic absorption and fluorescence spectra of solutions of fluorophores **4a**–**d** and **5a**,**b** in degassed absolute ethanol and distilled water were measured. Summarized data of this study is presented in Table 1. The fluorescence quantum yields, Φ_F, were evaluated using oxazine **1** in ethanol as standard (Φ_F=0.11[19]) at 575 nm or 470 nm excitation. Figs. 1 and 2 show absorption and fluorescence spectra, at 470 nm excitation, of the compounds in water (panel A), ethanol (panel B) and basic ethanol (addition of a small amount of tetraethylammonium hydroxide, panel C).

authors in similar compounds[17g,h] (Table 1). Substitution at 2-position seems to have the same effect even without di-alkylation at 9-position (compound **5a**). Also, noteworthy is the much lower molar absorptivity of compound **4d**. In ethanol media, this is explainable by a predominance of the basic form, which always shows lower absorptivity (Table 1) especially for this compound. However, in water where neither basic form nor H-aggregate formation is prominent, other specific interaction arising from the $NH_2 \cdot HBr$ group must be involved. Substitution at the 2-position shows a slight decrease in the molar absorptivity in water and ethanol media.

Fluorescence data from Table 1 exhibit that the maximum emission wavelength of the basic form is between 605 and 626 nm, whereas for the acid form the maximum lies in the region 644–666 nm in ethanol and 649–676 nm in aqueous media. This red shift is consistent with a π–π^* type transition and was observed previously.[17g,j] With the exception of compound **4d** all fluorescence quantum yields in water are between two and seven times lower than in ethanol. Results from earlier studies[17g,h] show

Table 1
Photophysical data of compounds **4a**–**d** and **5a**,**b** in ethanol, basic ethanol and water (C=2×10^{-6} M)

Cpd	Ethanol			Basic ethanol			Water		
	λ_{abs} (nm)	λ_{em} (nm)	Δ (nm)	λ_{abs} (nm)	λ_{em} (nm)	Δ (nm)	λ_{abs} (nm)	λ_{em} (nm)	Δ (nm)
	ε (10^4 M^{-1} cm^{-1})	Φ_F[a]		ε (10^4 M^{-1} cm^{-1})	Φ_F[b]		ε (10^4 M^{-1} cm^{-1})	Φ_F[a]	
		$\Phi_{F,\ Ac}$[a]							
4a	627	663	36	505	614	109	606[c]	663	57
	12.6	0.34		6.5	0.011		4.6	0.048	
		0.40							
4b	620	646	26	498	613	115	613	652	49
	19.2	0.35		10	0.049		11.9	0.16	
		0.43							
4c	620	647	37	498	616	118	615	652	37
	23.1	0.37		13.1	0.051		16	0.17	
		0.41							
4d	613/493	644	31	485	605	120	608	649	41
	1.1/1.6	0.26		1.6	0.017		2.2	0.27	
		0.29							
5a	630	665	35	504	624	120	627	672	45
	12.8	0.27		6.4	0.027		5.4	0.062	
		0.33							
5b	627	666	39	500	626	126	628	676	48
	15.1	0.38		12.3	0.055		14.2	0.075	
		0.44							

[a] Excitation at 575 nm.
[b] Excitation at 470 nm.
[c] 1% Variation between 598 and 624 nm.

As in previous studies of other family of benzo[*a*]phenoxazinium chlorides,[17g,h] the absorption spectra in ethanol media is dominated by an acidic form (BzH^+) and a ~100 nm blue shifted neutral form (Bz), which is clearly observed in ethanol medium in presence of base tetraethylammonium hydroxide (TEAH) (Fig. 1B and C). Among the compounds without substitution at heterocycle position 2, compound **4c** has the higher fraction of acidic form. This could be due to an intramolecular interaction between OH and the N atom at position 5 as this is the main site of deprotonation as shown by ab-initio calculations in a previous study.[17i] On the contrary compound **4d** displayed a huge fraction of basic form even in normal ethanol. This can be explained by the positive charge in NH_3^+, which facilitates the exit of the proton in the 5-amino position.

In aqueous media (Fig. 1A) the basic form is almost absent and the spectra reveals a blue shoulder/enlargement especially for the compounds substituted at the 2-position (**4a** and **5a**). This has been shown to be due to the formation of H-aggregates.[17g] It can also be seen that the di-alkylation at 9-position (compounds **5a** and **5b**) originates a red shift of absorbance as already reported by the that changing mono to di-alkylation at the 9-amino position results in a red shifted emission and also a decrease in fluorescence quantum yield. In the case of compounds reported in this work, it is possible to conclude that the existence of oxy-substituents at position 2 of the heterocycle originates the same red shift but without a decrease in the fluorescence efficiency (compound **4a**). However, it seems that di-alkylation with chlorine terminated side chains in the absence of substitution in 2-position also maintains the high quantum yield (compound **5b**). It is noteworthy that the reported fluorescence quantum yields at 575 nm excitation are less than the corresponding true cationic form quantum yield due to the residual absorbance of light by the basic neutral form. An attempt to exclude the basic form by acidifying ethanol with tetrafluoroacetic acid (data not shown) was not successful as additional fluorescence bands appeared near 540 nm. Assuming that in ethanol in presence of the base (TEAH), only the deprotonated Bz form exists it is possible to estimate the contribution of BzH^+ form to the absorption spectra in neat ethanol. This fraction depends on the compound and lies between 0.81 (compound **4b**) and 0.91 (compound **4d**). In Table 1 the corresponding estimation of the true

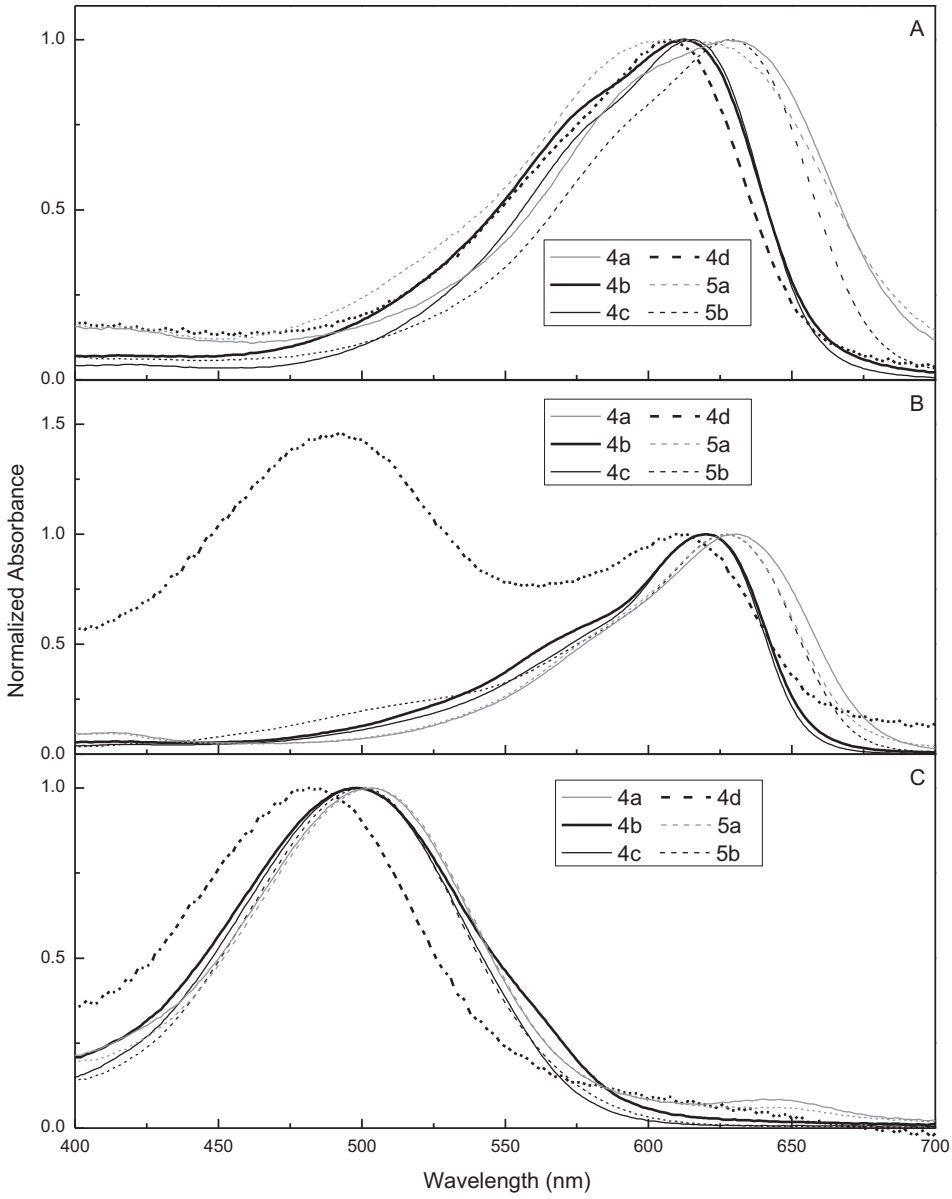


Fig. 1. Normalized absorption spectra of compounds **4a**–**d** and **5a**,**b** in water (A), ethanol (B) and basic ethanol (C) (C=2×10^{-6} M).

fluorescence quantum yield of the cationic BzH^+ form in ethanol is also shown ($\Phi_{F,Ac}$). Compound **4d** shows a lower fluorescence quantum yield and this must again be an effect of the positive charge in NH_3^+ that arises from the NH_2·HBr substituent.

Fluorescence spectra at 470 nm excitation (Fig. 2) reveals the emission of both acid and basic forms, but the latter is predominantly excited (Fig. 1). Although the basic form has far greater absorption at 470 nm, the emission spectra is dominated by the acidic form. This is accountable by the fact that the quantum yield is 10 times greater for the acidic form than the basic form (Table 1). Again an additional band near 540 nm is clearly observed for some of the compounds (**4b**, **4c** and **4d**) both in water and ethanol. This band could arise from tautomerization equilibrium with localized positive charge and a slight loss of resonance among the π-electron system (Scheme 2).

Tautomer A allows full positive charge delocalization. Tautomer B has a positive charge localized in the 9-amino position, whereas tautomer C has the positive charge localized in the 5-amino position.

To confirm this hypothesis ab-initio calculations were performed using Gaussian 09 Rev A.02 software package.[20] In Fig. 3 optimized geometries at the DFT-MPW1PW91 level of theory with a 3-21G+* basis are shown for the tautomers of a model benzo[*a*]phenoxazine without substitution at position 2 of the heterocycle and with single methyl groups at both 5- and 9-amino positions. The calculations were done both in the presence and absence of the chlorine counter-anion and were followed by a frequency calculation at the same level of theory.

In a final step, excited state information was obtained by a TD-DFT calculation with a MPW1PW91 functional and a 6-31G++(d,p) basis. The results of the various ab-initio calculations are shown in Table 2. From the optimized geometries in the absence of counter-anion, it is seen that there is full positive charge delocalization as the structure is completely planar (all N atoms in sp^2 hybridization). The transfer of a proton from one N position to the other (from structure in the left to that in the right in Scheme 2) originates a decrease in the planarity of the N atoms in relation to the heterocycle as one of those changes hybridization from nearly sp^2 to sp^3. Interestingly, the chloride anion seems to allow a partial recovery of the sp^2 hybridization through the formation of Cl—H—N hydrogen-bridge. This interaction is not observed when there is full

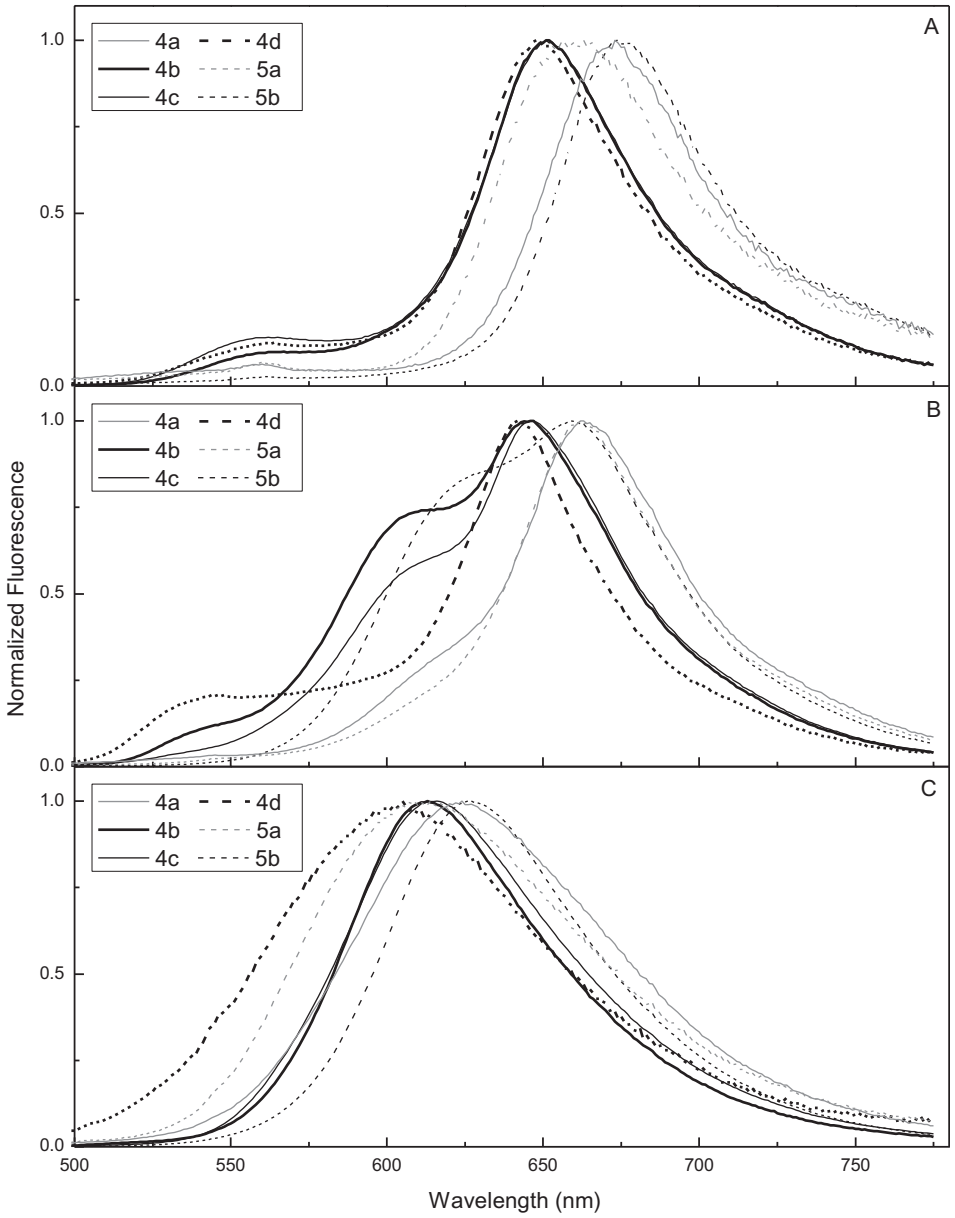


Fig. 2. Normalized fluorescence spectra of compounds **4a**–**d** and **5a**,**b** in water (A), ethanol (B) and basic ethanol (C) at 470 nm excitation (C=2×10^{-6} M).

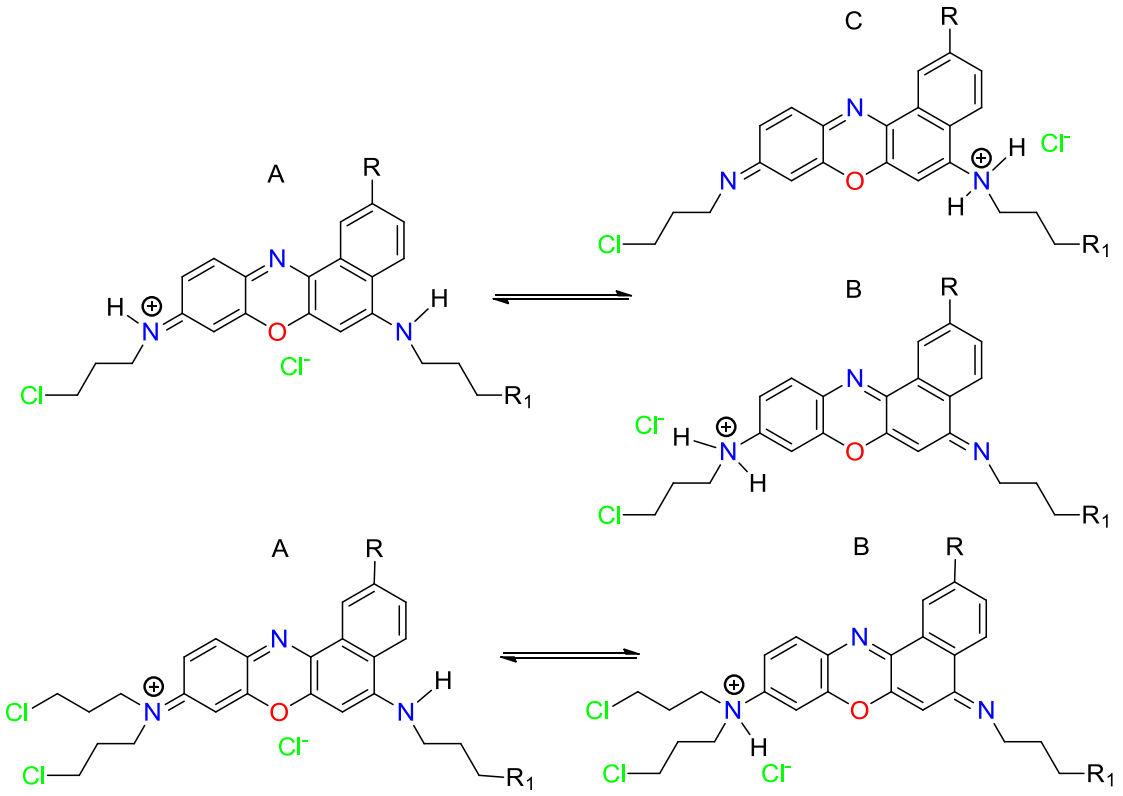
positive charge delocalization (tautomer A) and the chlorine anion locates at 2.48 Å from the heterocycle and in closer distance to one of the methyl groups that slightly bends itself towards chlorine. From Table 2, it is possible to conclude that tautomers B and C are higher in energy than tautomer A when chloride anion is absent. However, this change when the counter-anion is close to the Bz heterocycle with tautomers B having less energy than tautomer A. Tautomer C is slightly more energetic but the excess energy is lower than the thermal energy at 25 °C (3.72 kcal mol^{-1}).

Thus, in situations where ion-pairing or, presumably, association with proton accepting molecules is possible, the tautomerization process can occur. The latter possibility certainly can happen in the studied ethanolic or aqueous media.

The excited state calculations were done in vacuum and for the ground state geometry. As result, the tabulated HOMO–LUMO gaps should correspond to absorption maximum. Assuming a Stokes's shift halfway between the acidic and basic form (~75 nm), it is possible to estimate that tautomers B emit at ~511 nm and tautomer C at ~538 nm. Thus, it is possible to get some more confidence on the assignment of the 540 nm emission band to a tautomer form of the BzH$^+$Cl$^-$ molecule.

Fig. 4 shows the absorption spectra of compounds **5b** and **4d** in AOT/cyclohexane reverse micelles. An absorption band is clearly seen at ~460 nm, which decreases as the water content (ω_o parameter) increases.

This variation is in correspondence with a decrease in a fluorescence band near 550 nm (Fig. 5). It is then possible to conclude that the tautomerization process is occurring in this microemulsion media and its importance decreases with an increase in the water content. Further, the absorption and emission maxima of the band corresponding to the tautomer A (BzH$^+$ with fully delocalized positive charge) is seen shifted to lower energies with the increase in ω_o but does not reach the values observed in aqueous media (Table 1). The fluorescence quantum yield at 575 nm excitation is much higher when ω_o=0 and decreases as the water content increases. All the studied quantities (see the insets of Figs. 4 and 5)



Scheme 2. Tautomerization equilibrium of benzo[*a*]phenoxazinium chlorides.

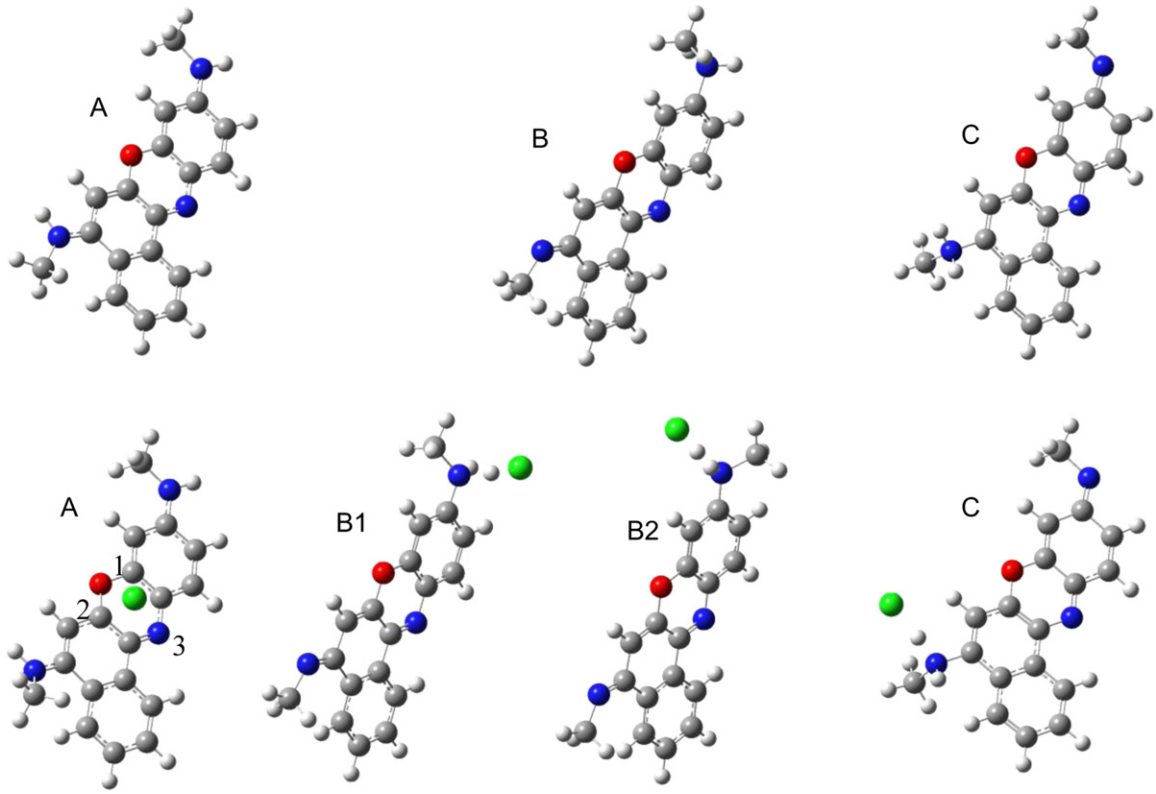


Fig. 3. Optimized ground-state geometries of tautomers A, B and C of a model benzo[*a*]phenoxazine in the absence or presence of chloride counter-anion. In B1 and B2 the chloride anion was initially placed in different sides of the heterocycle.

Table 2
Ab-initio calculation with structures in Fig. 3

DFT calculations (MPW1PW91 functional)	Without chlorine counter-anion			With chlorine counter-anion			
	A	B	C	A	B1	B2	C
Cl distance to nearest N or C atom (Å)	—	—	—	2.48	2.90	2.90	2.83
Cl distance to H atom (Å)	—	—	—	—	1.41	1.41	1.61
Dihedral angle Cl–1–2–3 (°)	—	—	—	−89.1	−112.1	111.5	7.04
Zero point Vibrational energy (Hartree)	0.309904	0.309226	0.308607	0.312896	0.310559	0.311528	0.310021
Ground state energy (Hartree)	−934.663	−934.594	−934.590	−1395.052	−1395.052	−1395.051	−1395.048
Molecular energy relative to tautomer A (kcal mol^{-1})	—	179	188	—	−1.102	−0.456	0.929
HOMO–LUMO gap (nm)	494.2	453.4	471.7	593.8	438.2	434.67	463.12

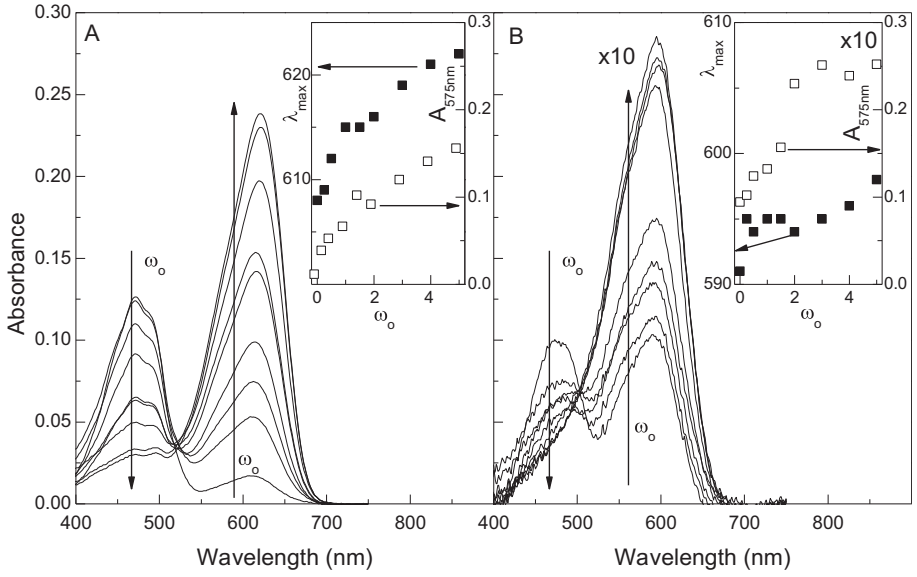


Fig. 4. Absorbance spectra of compound **5b** (A) and **4d** (B), $C=4\times10^{-6}$ M, in AOT/cyclohexane microemulsions as a function of water content (ω_o) with $P_{EtOH}=0.34$. The inset shows the absorbance at 575 nm (open squares, right axis) and the maximum absorption wavelength of the red side band (filled squares, left axis).

practically stabilizes at $\omega_o=3$. Previous studies placed the ω_o limit above which water in reverse micelles behaves as bulk water between 7 and 10.[9,21]

This indicates that the studied compounds are seeing differences in the hydration shell of the AOT layer in the reverse micelles. The maximum fluorescence quantum yield reaches 0.67 for both compounds and it should correspond to a close association between BzH^+ and the negative charged group of the AOT surfactant with diminishing water content. For compound **5b**, the quantum yield stabilizes at a value lower than that observed in ethanol

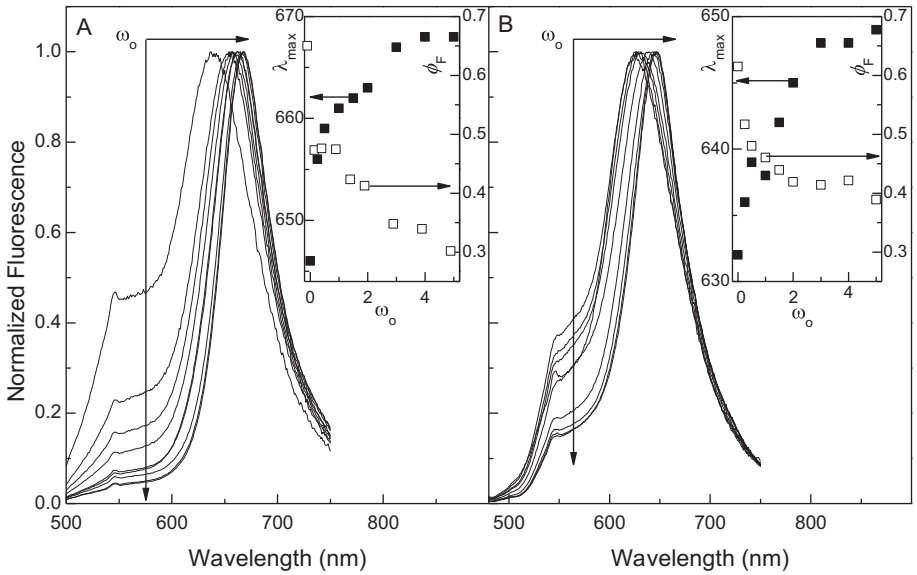


Fig. 5. Normalized fluorescence spectra of compound **5b** (A) and **4d** (B), $C=4\times10^{-6}$ M, in AOT/cyclohexane microemulsions as a function of water content (ω_o) with $P_{EtOH}=0.34$. The inset shows the fluorescence quantum yield at 575 nm excitation (open squares, right axis) and the maximum emission wavelength (filled squares, left axis).

whereas the reverse situation occurs for compound **4d**. This must be due to the $NH_2 \cdot HBr$ substituent that favours the location of the compound close to the AOT head groups even in high water content.

Solutions of compounds **4d** and **5b** in AOT/cyclohexane microemulsions contained small amounts of ethanol. This small polar molecule can either localize on the micelle interface working as a co-surfactant, or be on the water pool. In order to better follow the effect of the presence of ethanol molecules on the AOT w/o microemulsion an equivalent parameter to ω_o is defined: P_{EtOH}=[EtOH]/[AOT], and varied maintaining ω_o fixed at 0 or 2 (Fig. 6). It can be seen that up to P_{EtOH}=0.34 a slight increase of tautomerization is observed. However, when ethanol content is 10 times higher the predominant phenomena is the formation of the basic Bz neutral form (broad emission band near 600 nm), and the fluorescence quantum yield of the remaining acid form markedly decreases for both compounds when ω_o=0, but only for compound **4d** when ω_o=2. This appears to indicate that the stronger association with AOT head groups prompted by the $NH_2 \cdot HBr$ side-chain substituent is perturbed by the presence of ethanol molecules in the reverse micelle interface.

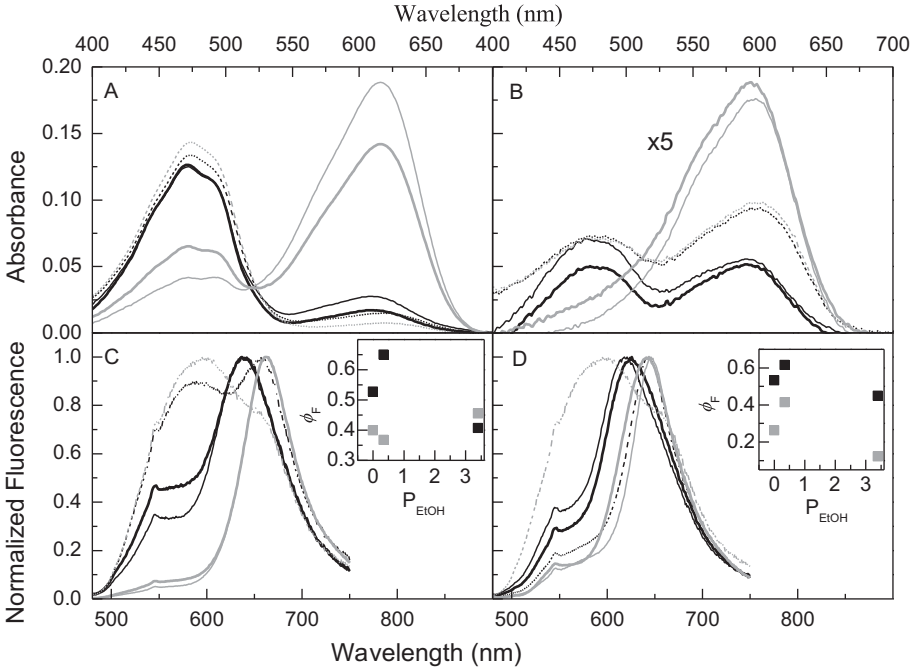


Fig. 6. Absorbance and normalized fluorescence spectra at 470 nm excitation of compound **5b** (A, C) and **4d** (B, D), $C=4\times10^{-6}$ M in AOT/cyclohexane microemulsions at ω_o=0 (black lines) and ω_o=2 (grey lines) with various ethanol content: P_{EtOH}=0 (thin line), P_{EtOH}=0.34 (thick line) and P_{EtOH}=3.4 (dashed line). The inset shows the fluorescence quantum yields at 575 nm both for ω_o=0 (black squares) and ω_o=2 (grey squares).

3. Conclusion

Water-soluble fluorescent benzo[*a*]phenoxazinium chlorides with one, two or three chlorine atoms as terminals of their substituents at 2- and 9-positions were obtained. In addition, two of these cationic fluorophores possess the hydroxyl or amine functions as terminals in the aliphatic chains of position 5, providing them extra potential for covalent labelling beyond their inherent capabilities for non-covalent labelling of (bio)molecules. These molecules were shown to be involved in tautomerization equilibrium leading to localization of the positive charge at one of the N atoms in 5- or 9-positions with chloride counter-anion (or other proton accepting molecule) making an H-bond that allows partial recovery of the molecule's resonance possibilities. The use of these molecules as fluorescence probes in AOT reverse micelles allowed following changes in the hydration shell of the surfactant layer with water content. AOT molecules promote the tautomerization process with the presence of water molecules having the reverse effect. Association with AOT molecules originates a higher fluorescence quantum yield of the molecular form with delocalized cationic charge. It was also concluded that small traces of ethanol affect the properties of the reverse micelle interface to a point that instead of tautomerization the usual deprotonation process occurred.

4. Experimental

4.1. General

All melting points were measured on a Stuart SMP3 melting point apparatus. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualized under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer. NMR spectra were obtained on a Varian Unity Plus Spectrometer at an operating frequency of 300 MHz for ^{1}H and 75.4 MHz for ^{13}C or a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 °C. All chemical shifts are given in parts per million using δ_H Me_4Si=0 ppm as reference, and *J* values are given in hertz. Assignments were made by comparison of chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. Low and high resolution mass spectrometry analyses were performed at the 'C.A.C.T.I. — Unidad de Espectrometria de Masas', at University of Vigo, Spain.

UV–vis absorption spectra (200–800 nm) were obtained using Shimadzu UV/3101PC Spectrophotometers and fluorescence spectra with Spex Fluorolog spectrofluorometer. Commercially available reagents were used as received. Reverse micelles in cyclohexane were prepared by injection of a needed amount of water into a cyclohexane 0.2 M solution of AOT (dioctyl sodium sulfosuccinate) into which a given volume of either 1×10^{-4} M or 1×10^{-3} M

ethanolic solution of the studied compounds was added in order to obtain a final concentration of 4×10^{-6} M. For preparations without ethanol, the required volume of ethanolic mother solution was evaporated with a stream of nitrogen prior to the addition of AOT solution in cyclohexane.

4.2. Synthesis of precursors 1–3

*4.2.1. 5-((3-Chloropropyl)amino)-2-nitrosophenol hydrochloride **1**.* To an ice-cold solution of 3-(3-chloropropylamino)phenol (0.155 g, 8.37×10^{-4} mol) in ethanol (1 mL), concentrated hydrochloric acid (0.28 mL) was added and stirred for 5 min. The solution of sodium nitrite (0.058 g, 9.20×10^{-4} mol) in water (0.1 mL) was then added drop-wise within an interval of 20–25 min. The resulting mixture was stirred for 3 h and monitored by TLC (dichloromethane/methanol, 1:9 and 2:8). After evaporation of the reaction, compound **1** was obtained as an orange oily solid (0.180 g) and was used in the following step without any purification.

*4.2.2. 5-(Bis(3-chloropropyl)amino)-2-nitrosophenol hydrochloride **2**.* Starting from 3-(bis(3-chloropropyl)amino)phenol (0.100 g, 3.81×10^{-4} mol) in ethanol (1 mL) and concentrated hydrochloric acid (0.105 mL), using sodium nitrite (0.03 g, 4.19×10^{-4} mol) in water (0.1 mL), and following the same procedure as described before for the preparation of **1**, compound **2** was obtained as an orange oily solid (0.113 g) and was used in the following step without any purification.

4.2.2.1. 3-(3-Chloropropylamino)phenol. To a solution of 3-aminophenol (1.4 g, 1.28×10^{-2} mol) in ethanol (2 mL), 1-bromo-3-chloropropane (1.27 mL, 1.28×10^{-2} mol) was added, and the resulting mixture was refluxed for 5 h. 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/methanol, mixtures of increasing polarity, as the eluent. The expected product was obtained as brown oil (0.688 g, 29%). TLC (dichloromethane/methanol, 9.5:0.5): R_f=0.54. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H=2.00–2.10 (m, 2H, $NHCH_2CH_2CH_2Cl$), 3.31 (t, J=6.4 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 3.64 (t, J=6.3 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 4.25 (broad s, 2H, NH and OH), 6.14 (t, J=2.1 Hz, 1H, 2-H), 6.18–6.26 (m, 2H, 4-H and 6-H), 7.03 (t, J=8.0 Hz, 1H, 5-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=31.77 ($NHCH_2CH_2CH_2Cl$), 41.02 ($NHCH_2CH_2CH_2Cl$), 42.57 ($NHCH_2CH_2CH_2Cl$), 99.86 (C-2), 104.81 (C-6), 106.06 (C-4), 130.29 (C-5), 149.25 (C-3), 156.73 (C-1). IR (KBr 1%, cm^{-1}): ν=3198, 2947, 2587, 1615, 1505, 1488, 1470, 1372, 1316, 1284, 1233, 1172, 1084, 1020, 1000, 859, 828, 780, 735, 693, 655. HRMS: m/z (EI): calcd for $C_9H_{12}{}^{35}ClNO$ [M^+] 185.0607; found 185.0602. Calcd for $C_9H_{12}{}^{37}ClNO$ [M^+] 187.0578; found 187.0585.

4.2.2.2. 3-(Bis(3-chloropropyl)amino)phenol. In the above reaction, this compound was also isolated as brown oil (0.222 g, 7%). TLC (dichloromethane/methanol, 9.5:0.5): R_f=0.71. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H=1.93–1.99 (m, 4H, $N(CH_2CH_2CH_2Cl)_2$), 3.38 (t, J=7.2 Hz, 4H, $N(CH_2CH_2CH_2Cl)_2$), 3.49 (t, J=6.0 Hz, 4H, $N(CH_2CH_2CH_2Cl)_2$), 5.40 (s, 1H, OH), 6.14–6.26 (m, 3H, 2-H, 6-H and 4-H), 7.00 (t, J=8.4 Hz, 1H, 5-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=29.79 ($N(CH_2CH_2CH_2Cl)_2$), 42.94 ($N(CH_2CH_2CH_2Cl)_2$), 49.59 ($N(CH_2CH_2CH_2Cl)_2$), 100.01 (C-2), 102.58 (C-6), 105.53 (C-4), 130.34 (C-5), 148.68 (C-3), 156.81 (C-1). IR (KBr 1%, cm^{-1}): ν=3189, 2956, 2653, 2552, 1615, 1578, 1505, 1488, 1464, 1451, 1360, 1316, 1288, 1235, 1169, 1117, 1082, 1055, 1011, 998, 945, 928, 879, 828, 785, 733, 698. HRMS: m/z (EI): calcd for $C_{12}H_{17}{}^{35}Cl_2NO$ [M^+] 261.0687; found 261.0689. $C_{12}H_{17}{}^{35}Cl^{37}ClNO$ [M^+] 263.0658; found 263.0659. Calcd for $C_{12}H_{17}{}^{37}Cl_2NO$ [M^+] 265.0628; found 265.0617.

*4.2.2.3. 6-(3-Chloropropoxy)-N-propylnaphthalen-1-amine **3a**.* To a solution of 5-(propylamino) naphthalen-2-ol[17f] (0.070 g, 3.48×10^{-4} mol) in acetonitrile (2 mL), 1-bromo-3-chloropropane (0.038 mL, 3.83×10^{-4} mol) and cesium carbonate (0.554 g, 1.70×10^{-3} mol) were added, and the resulting mixture was heated at 60 °C for 1.5 h. The progress of the reaction was monitored by TLC (ethyl acetate/light petroleum 1:5). The excess of base was filtered out, the solvent was evaporated and the crude mixture was purified by column chromatography on silica gel using ethyl acetate/light petroleum 1:5, as the eluent. Compound **3a** was obtained as a light brown solid (0.09 g, 93%). Mp=97.1–100.2 °C. TLC (ethyl acetate/light petroleum 1:5): R_f=0.67. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H=0.96 (t, J=7.6 Hz, 3H, $NHCH_2CH_2CH_3$), 1.64–1.76 (m, 2H, $NHCH_2CH_2CH_3$), 2.17–2.28 (m, 2H, $OCH_2CH_2CH_2Cl$), 3.15 (t, J=7.6 Hz, 2H, $NHCH_2CH_2CH_3$), 3.82 (t, J=6.4 Hz, 2H, $OCH_2CH_2CH_2Cl$), 4.18 (t, J=6.0 Hz, 2H, $OCH_2CH_2CH_2Cl$), 6.48 (broad s, 1H, H-2), 7.00–7.13 (m, 2H, H-4 and H-7), 7.20 (d, J=2.4 Hz, 1H, H-5), 7.24 (t, J=8.0 Hz, 1H, H-3), 8.09 (d, J=9.2 Hz, 1H, H-8). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ_C=11.67 ($NHCH_2CH_2CH_3$), 21.20 ($NHCH_2CH_2CH_3$), 31.73 ($OCH_2CH_2CH_2Cl$), 42.04 ($OCH_2CH_2CH_2Cl$), 45.92 ($NHCH_2CH_2CH_3$), 64.19 ($OCH_2CH_2CH_2Cl$), 103.00 (C-2), 107.36 (C-5), 116.00 (C-4), 116.21 (C-7), 118.52 (C-8a), 123.48 (C-8), 127.34 (C-3), 135.57 (C-4a), 143.06 (C-1), 156.14 (C-6). IR (KBr 1%, cm^{-1}): ν=3405, 2959, 2926, 2872, 1623, 1587, 1529, 1476, 1460, 1432, 1378, 1341, 1284, 1267, 1224, 1181, 1151, 1070, 1047, 974, 912, 865, 837, 818, 779, 743. HRMS: m/z (EI): calcd for $C_{16}H_{20}NO^{35}Cl$ [M^+] 277.1233; found 277.1235; calcd for $C_{16}H_{20}NO^{37}Cl$ [M^+] 279.1204; found 279.1208.

4.3. General procedure for preparation of compounds 4 and 5

To a cold solution (ice bath) of 5-((3-chloropropyl)amino)-2-nitrosophenol hydrochloride **1** (2 equiv) or 5-(bis(3-chloropropyl) amino)-2-nitrosophenol hydrochloride **2** (2 equiv), in ethanol (2–3 mL), precursors **3a**–**d** (1 equiv) and concentrated hydrochloride acid (0.25 equiv) were added. The mixture was refluxed during the time mentioned below, and monitored by TLC. After evaporation of the solvent and column chromatography purification on silica gel with dichloromethane and dichloromethane/methanol, mixtures of different polarity, as the eluent, the required dye **4** or **5** was obtained as a blue solid.

*4.3.1. 3-Chloro-N-(2-(3-chloropropoxy)-5-(propylamino)-9H-benzo[a]phenoxazin-9-ylidene)propan-1-aminium chloride **4a**.* The product of the reaction of **1** (0.055 g, 2.2×10^{-4} mol) in ethanol (3 mL) and concentrated hydrochloric acid (2.26×10^{-3} mL) with 6-(3-chloropropoxy)-*N*-propylnaphthalen-1-amine **3a** (0.031 g, 1.1×10^{-4} mol) (reflux time 1 h), was chromatographed with dichloromethane and dichloromethane/methanol 8.5:1.5, to give compound **4a** (0.039 g, 37%). Mp=128.3–130.8 °C. R_f=0.33 (dichloromethane/methanol, 9:1). ^{1}H NMR (CD_3OD, 400 MHz): δ_H=1.14 (t, J=7.2 Hz, 3H, $NHCH_2CH_2CH_3$), 1.85–1.95 (m, 2H, $NHCH_2CH_2CH_3$), 2.10–2.24 (m, 2H, $NHCH_2CH_2CH_2Cl$), 2.30–2.41 (m, 2H, $OCH_2CH_2CH_2Cl$), 3.64–3.80 (m, 4H, $NHCH_2CH_2CH_2Cl$ and $NHCH_2CH_2CH_3$), 3.87 (t, J=6.4 Hz, 2H, $OCH_2CH_2CH_2Cl$), 4.26 (t, J=6.8 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 4.38 (t, J=6.0 Hz, 2H, $OCH_2CH_2CH_2Cl$), 6.70 (d, J=2.0 Hz, 1H, H-8), 6.90 (s, 1H, H-6), 7.11 (dd, J=7.8 Hz and 1.6 Hz, 1H, H-3), 7.32–7.38 (m, 1H, H-10), 7.75 (d, J=9.2 Hz, 1H, H-11), 8.18 (d, J=2.4 Hz, 1H, H-1), 8.24 (d, J=9.2 Hz, 1H, H-4). ^{13}C NMR (CD_3OD, 100.6 MHz): δ_C=11.73 ($NHCH_2CH_2CH_3$), 23.31 ($NHCH_2CH_2CH_3$), 31.21 ($NHCH_2CH_2CH_2Cl$), 33.19 ($OCH_2CH_2CH_2Cl$), 41.59 ($NHCH_2CH_2CH_2Cl$), 42.21 ($OCH_2CH_2CH_2Cl$), 43.17 ($NHCH_2CH_2CH_2Cl$), 47.53 ($NHCH_2CH_2CH_3$), 66.47 ($OCH_2CH_2CH_2Cl$), 94.18 (C-6), 97.39 (C-8), 108.00 (C-1), 115.60 (C-3), 118.50 (C–Ar), 120.31 (C-10), 126.33 (C-4), 130.31 (C–Ar), 133.79 (C–Ar), 134.96 (C-11), 136.42 (C–Ar), 149.16 (C–Ar), 153.63 (C–Ar), 155.16 (C-9), 159.93 (C-5), 163.33 (C-2). IR (KBr 1%, cm^{-1}): ν=3389, 2924, 2853,

1640, 1590, 1548, 1461, 1419, 1331, 1281, 1222, 1151, 1127, 1036, 909, 815, 781, 717, 666. HRMS: *m*/*z* (ESI): calcd for $C_{25}H_{28}{}^{35}Cl_2N_3O_2$ [M^++1] 472.15531; found 472.15354. $C_{25}H_{28}{}^{35}Cl^{37}ClN_3O_2$ [M^++1] 474.15252; found 474.15016. Calcd for $C_{25}H_{28}{}^{37}Cl_2N_3O_2$ [M^++1] 476.14951; found 476.15235.

4.3.2. 3-Chloro-N-(5-(propylamino)-9H-benzo[a]phenoxazin-9-ylidene)propan-1-aminium chloride ***4b***. The product of the reaction of **1** (0.139 g, 6.49×10^{-4} mol) in ethanol (2 mL) and concentrated hydrochloric acid (0.170 mL), with *N*-propylnaphthalen-1-amine **3b** (0.060 g, 3.24×10^{-4} mol) (reflux time 7 h) was chromatographed with dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, to give compound **4b** (0.083 g, 31%). Mp=184.9–185.3 °C. TLC (dichloromethane/methanol, 9.5:0.5): R_f=0.20. ^{1}H NMR (CD_3OD, 400 MHz): δ_H=1.13 (t, *J*=7.6 Hz, 3H, $NHCH_2CH_2CH_3$), 1.89–1.99 (m, 2 H, $NHCH_2CH_2CH_3$), 2.16–2.25 (m, 2H, $NHCH_2CH_2CH_2Cl$), 3.59 (t, *J*=6.8 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 3.70–3.82 (m, 4H, $NHCH_2CH_2CH_3$ and $NHCH_2CH_2CH_2Cl$), 6.86 (d, *J*=2.4 Hz, 1H, 8-H), 7.04 (s, 1H, 6-H), 7.12 (d, *J*=9.2 Hz, 1H, 10-H), 7.82–7.87 (m, 2H, 11-H, 3-H), 7.96 (t, *J*=8.0 Hz, 1H, 2-H), 8.38 (d, *J*=8.4 Hz, 1H, 4-H), 8.95 (d, *J*=8.0 Hz, 1H, 1-H). ^{13}C NMR (CD_3OD, 100.6 MHz): δ_C=11.70 ($NHCH_2CH_2CH_3$), 23.02 ($NHCH_2CH_2CH_3$), 32.63 ($NHCH_2CH_2CH_2Cl$), 41.63 ($NHCH_2CH_2CH_2Cl$), 42.96 ($NHCH_2CH_2CH_2Cl$), 47.44 ($NHCH_2CH_2CH_3$), 94.50 (C-6), 96.21 (C-8), 123.81 (C-10), 124.28 (C-4), 125.0 (Ar–C), 125.76 (C-1), 131.10 (C-3), 131.96 (Ar–C), 132.79 (Ar–C), 132.08 (C-2), 134.05 (C-11), 135.39 (Ar–C), 150.34 (Ar–C), 153.52 (Ar–C), 157.97 (C-9), 159.76 (C-5). IR (KBr 1%, cm^{-1}): ν=3206, 2962, 2930, 2873, 1640, 1588, 1547, 1494, 1455, 1433, 1384, 1322, 1278, 1237, 1185, 1159, 1123, 1012, 1000, 976, 855, 827, 774. HRMS: *m*/*z* (ESI): calcd for $C_{22}H_{23}{}^{35}ClN_3O$ [M^++1] 380.15242; found 380.15218. Calcd for $C_{22}H_{23}{}^{37}ClN_3O$ [M^++1] 382.14945; found 382.14947.

4.3.3. 3-Chloro-N-(5-(3-hydroxypropylamino)-9H-benzo[a]phenoxazin-9-ylidene)propan-1-aminium chloride ***4c***. The product of the reaction of **1** (0.180 g, 8.4×10^{-5} mol) in ethanol (2 mL) and concentrated hydrochloric acid (0.236 mL), with 3-(naphthalen-1-ylamino) propan-1-ol **3c** (0.084 g; 4.2×10^{-5} mol) (reflux time 3.5 h) was chromatographed with dichloromethane, dichloromethane/methanol, mixtures of increasing polarity as the eluent, to give compound **4c** (0.151 g, 42%). Mp=228.0–228.9 °C. TLC (dichloromethane/methanol, 9:1): R_f=0.33. ^{1}H NMR (CD_3OD, 400 MHz): δ_H=2.0–2.14 (m, 2H, $NHCH_2CH_2CH_2OH$), 2.15–2.25 (m, 2H, $NHCH_2CH_2CH_2Cl$), 3.54 (t, *J*=6.8 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 3.75–3.85 (m, 6H, $NHCH_2CH_2CH_2OH$, $NHCH_2CH_2CH_2OH$ and $NHCH_2CH_2CH_2Cl$), 6.68 (s, 1H, 8-H), 6.87 (s, 1H, 6-H), 7.05 (d, *J*=9.2 Hz, 1H, 10-H), 7.66 (d, *J*=9.2 Hz, 1H, 11-H), 7.77 (t, *J*=7.6 Hz, 1H, 3-H), 7.86 (t, *J*=8.0 Hz, 1H, 2-H), 8.22 (d, *J*=8.4 Hz, 1H, 4-H), 8.72 (d, *J*=8.0 Hz, 1H, 1-H). ^{13}C NMR (CD_3OD, 100.6 MHz): δ_C=32.14 ($NHCH_2CH_2CH_2OH$), 32.61 ($NCH_2CH_2CH_2Cl$), 41.72 ($NHCH_2CH_2CH_2Cl$), 43.06 ($NHCH_2CH_2CH_2OH$), 43.40 ($NHCH_2CH_2CH_2Cl$), 60.40 ($NHCH_2CH_2CH_2OH$), 94.29 (C-6), 95.95 (C-8), 123.76 (C-4 and C-10), 124.67 (Ar–C), 125.52 (C-1), 131.0 (C-3), 131.79 (Ar–C), 132.33 (C-2), 132.95 (Ar–C), 133.87 (C-11), 134.81 (Ar–C), 149.95 (Ar–C), 152.95 (Ar–C), 157.88 (C-9), 159.26 (C-5). IR (KBr 1%, cm^{-1}): ν=3207, 3084, 2940, 1641, 1592, 1549, 1524, 1498, 1455, 1435, 1372, 1328, 1999, 1279, 1256, 1279, 1237, 1219, 1198, 1188, 1162, 1129, 1089, 1129, 1055, 1005, 951, 923, 871, 838, 779, 744, 706, 665. HRMS: *m*/*z* (ESI): calcd for $C_{22}H_{23}{}^{35}ClN_3O_2$ [M^++1] 396.14733; found 396.14756. Calcd for $C_{22}H_{23}{}^{37}ClN_3O_2$ [M^++1] 398.14438; found 398.14473.

4.3.4. N-(5-((3-Aminopropyl)amino)-9H-benzo[a]phenoxazin-9-ylidene)-3-chloropropan-1-aminium chloride ***4d***. The product of the reaction of **1** (0.232 g, 1.07×10^{-3} mol) in ethanol (2 mL) and concentrated hydrochloric acid (0.287 mL), with N^1-(naphthalen-1-yl) propane-1,3-diamine hydrobromide **3d** (0.151 g, 5.39×10^{-4} mol) (reflux time 9 h) was chromatographed with dichloromethane, dichloromethane/methanol, as the eluent, to give compound **4d** (0.202 g, 37%). TLC (dichloromethane/methanol, 9:1): R_f=0.15. ^{1}H NMR (CD_3OD, 400 MHz): δ_H=2.11–2.20 (m, 2H, $NHCH_2CH_2CH_2NH_2\cdot HBr$), 2.21–2.30 (m, 2H, $NHCH_2CH_2CH_2Cl$), 3.13 (t, *J*=7.6 Hz, 2H, $NHCH_2CH_2CH_2NH_2\cdot HBr$), 3.60–6.69 (m, 2H, $NHCH_2CH_2CH_2NH_2\cdot HBr$), 3.72 (t, *J*=6.4 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 3.77 (t, *J*=6.0 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 6.96 (s, 1H, 8-H), 7.15 (s, 1H, 6-H), 7.85–7.93 (m, 4H, 10-H, 11-H, 3-H and 2-H), 8.51 (d, *J*=7.2 Hz, 1H, 4-H), 8.80 (d, *J*=6.4 Hz, 1H, 1-H). ^{13}C NMR (CD_3OD, 100.6 MHz): δ_C=27.67 ($NHCH_2CH_2CH_2NH_2\cdot HBr$), 29.86 ($NHCH_2CH_2CH_2Cl$), 38.39 ($NHCH_2CH_2CH_2NH_2\cdot HBr$), 42.20 ($NHCH_2CH_2CH_2NH_2\cdot HBr$), 42.97 ($NHCH_2CH_2CH_2Cl$), 43.28 ($NHCH_2CH_2CH_2Cl$), 95.53 (C-6), 96.17 (C-8), 122.46 (C-4), 124.69 (C-10), 125.91 (C-1), 130.16 (C-3), 131.76 (Ar–C), 131.88 (C-2), 132.15 (Ar–C), 133.57 (C-11), 137.36 (Ar–C), 148.34 (Ar–C), 152.04 (Ar–C), 153.60 (C-9), 160.54 (C-5). IR (KBr 1%, cm^{-1}): ν=3396, 3210, 2924, 2853, 1638, 1587, 1545, 1493, 1459, 1436, 1324, 1277, 1260, 1152, 1123, 1008, 826, 770, 704, 665. HRMS: *m*/*z* (ESI): calcd for $C_{22}H_{24}{}^{35}ClN_4O$ [M^++1] 395.16332; found 395.16332. Calcd for $C_{22}H_{24}{}^{37}ClN_4O$ [M^++1] 397.16037; found 397.16082.

4.3.5. 3-Chloro-N-(2-(3-chloropropoxy)-5-(propylamino)-9H-benzo[a]phenoxazin-9-ylidene)-N-(3-chloropropyl)propan-1-aminium chloride ***5a***. The product of the reaction of **2** (0.14 g, 4.32×10^{-4} mol) in ethanol (3 mL) and concentrated hydrochloric acid (4.44×10^{-3} mL), with 6-(3-chloropropoxy)-*N*-propylnaphthalen-1-amine **3a** (0.060 g, 2.16×10^{-4} mol) (reflux time 7 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.5:0.5, to give compound **5a** (0.071 g, 30%). Mp=97.8–99.1 °C. TLC (dichloromethane/methanol, 9:1): R_f=0.52. ^{1}H NMR (CD_3OD, 400 MHz): δ_H=1.12 (t, *J*=7.6 Hz, 3H, $NHCH_2CH_2CH_3$), 1.80–1.93 (m, 2H, $NHCH_2CH_2CH_3$), 2.10–2.30 (m, 4H, $N(CH_2CH_2CH_2Cl)_2$), 2.31–2.40 (m, 2H, $OCH_2CH_2CH_2Cl$), 3.60–3.70 (m, 2H, $NHCH_2CH_2CH_3$), 3.71 (t, *J*=6.0 Hz, 6H, $N(CH_2CH_2CH_2Cl)_2$), 3.72–3.82 (2H, m, $OCH_2CH_2CH_2Cl$), 3.87 (t, *J*=6.0 Hz, 4H, $N(CH_2CH_2CH_2Cl)_2$), 4.38–4.43 (m, 2H, $OCH_2CH_2CH_2Cl$), 6.82 (s, 1H, H-8), 6.86 (d, *J*=2.4 Hz, 1H, H-6), 7.15–7.28 (m, 1H, H-3), 7.29 (dd, *J*=9.0 and 2.4 Hz, 1H, H-10), 7.75 (d, *J*=9.6 Hz, 1H, H-11), 8.11 (d, *J*=2.8 Hz, 1H, H-1), 8.22 (d, *J*=9.2 Hz, 1H, H-4). ^{13}C NMR (CD_3OD, 100.6 MHz): δ_C=11.74 ($NHCH_2CH_2CH_3$), 23.33 ($NHCH_2CH_2CH_3$), 30.20 ($NCH_2CH_2CH_2Cl$), 31.18 ($NCH_2CH_2CH_2Cl$), 33.18 ($OCH_2CH_2CH_2Cl$), 42.18 ($N(CH_2CH_2CH_2Cl)_2$), 42.27 ($OCH_2CH_2CH_2Cl$), 43.28 ($N(CH_2CH_2CH_2Cl)_2$), 47.50 ($NHCH_2CH_2CH_3$), 66.45 ($OCH_2CH_2CH_2Cl$), 94.13 (C-8), 97.47 (C-6), 107.83 (C-1), 115.71 (C-3), 118.43 (C–Ar), 120.22 (C-10), 126.39 (C-4), 130.27 (C–Ar), 133.79 (C-11), 134.81 (C–Ar), 136.22 (C–Ar), 149.13 (C–Ar), 153.50 (C–Ar), 155.39 (C-9), 159.78 (C-5), 163.22 (C-2). IR (KBr 1%, cm^{-1}): ν=3427, 2958, 2926, 1640, 1591, 1548, 1514, 1485, 1461, 1420, 1384, 1332, 1300, 1278, 1222, 1155, 1130, 1039, 909, 820, 665 cm^{-1}. HRMS: *m*/*z* (ESI): calcd for $C_{28}H_{33}{}^{35}Cl_3N_3O_2$ [M^++1] 548.16329; found 548.16515. Calcd for $C_{28}H_{33}{}^{35}Cl_2{}^{37}ClN_3O_2$ [M^++1] 550.16049; found 550.16361. Calcd for $C_{28}H_{33}{}^{35}Cl^{37}Cl_2N_3O$ [M^++1] 552.15753; found 552.16035. Calcd for $C_{28}H_{33}{}^{37}Cl_3N_3O_2$ [M^++1] 554.15448; found 554.16155.

4.3.6. 3-Chloro-N-(3-chloropropyl)-N-(5-(propylamino)-9H-benzo[a]phenoxazin-9-ylidene) propan-1-aminium chloride ***5b***. The product of the reaction of **2** (0.113 g, 3.88×10^{-4} mol) in ethanol (1.5 mL) and concentrated hydrochloric acid (0.105 mL), with **3b** (0.036 g, 1.94×10^{-4} mol) (reflux time 9 h) was chromatographed with dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, to give compound **5b** (0.074 g, 39%). Mp=244.7–246.1 °C. TLC (dichloromethane/methanol, 9.5:0.5): R_f=0.32. ^{1}H NMR (CD_3OD, 400 MHz): δ_H=1.14 (t, *J*=7.2 Hz, 3H, $NHCH_2CH_2CH_3$), 1.86–1.98 (m, 2H, $NHCH_2CH_2CH_3$), 2.22–2.30 (m, 4H, $N(CH_2CH_2CH_2Cl)_2$), 3.74–3.80 (m, 6H, $N(CH_2CH_2CH_2Cl)_2$ and $NCH_2CH_2CH_3$), 3.85 (t, *J*=7.2 Hz, 4H, $N(CH_2CH_2CH_2Cl)_2$), 7.02 (s, 1H, 8-H), 7.08 (s, 1H, 6-H), 7.33 (d, *J*=8.8 Hz, 1H, 10-H), 7.87 (t, *J*=7.6 Hz, 1H, 3-H), 7.90–8.0 (m, 2H, 11-H and 2-H), 8.40 (d,

J=8.0 Hz, 1H, 4-H), 8.96 (d, J=8.0 Hz, 1H, 1-H). ^{13}C NMR (CD_3OD, 100.6 MHz): δ_C=11.70 ($NHCH_2CH_2\mathit{CH}_3$), 23.10 ($NHCH_2\mathit{CH}_2CH_3$), 31.15 ($N(CH_2\mathit{CH}_2CH_2Cl)_2$), 43.14 ($N(CH_2CH_2\mathit{CH}_2Cl)_2$), 47.64 ($NH\mathit{CH}_2CH_2CH_3$), 50.04 ($N(\mathit{CH}_2CH_2CH_2Cl)_2$), 95.01 (C-6), 97.56 (C-8), 116.11 (C-10), 123.98 (C-4), 125.20 (Ar–C), 125.93 (C-1), 131.11 (Ar–C), 131.39 (C-3), 133.31 (C-2), 133.97 (Ar–C), 135.00 (C-11), 136.70 (Ar–C), 149.42 (Ar–C), 153.74 (Ar–C), 155.58 (C-9), 160.32 (C-5). IR (KBr 1%, cm^{-1}): ν=3185, 2963, 1640, 1589, 1546, 1495, 1460, 1433, 1401, 1384, 1333, 1275, 1262, 1226, 1173, 1158, 1125, 1083, 1056, 1014, 1001, 972, 844, 824, 786, 757, 709, 646. HRMS: m/z (ESI): calcd for $C_{25}H_{28}{}^{35}ClN_3O$ [M^++1] 456.16039; found 456.16005. Calcd for $C_{25}H_{28}{}^{37}ClN_3O$ [M^++1] 458.15745; found 458.15728.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2013.01.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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