Tetrahedron 65 (2009) 10441–10452

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Novel long alkyl side chain benzo[*a*]phenoxazinium chlorides: synthesis, photophysical behaviour and DNA interaction

Carla M.A. Alves [a], Sarala Naik [a], Paulo J.G. Coutinho [b], M. Sameiro T. Gonçalves [a,*]

[a] *Centro de Química, Universidade do Minho, Gualtar, 4710-057 Braga, Portugal*
[b] *Centro de Física, Universidade do Minho, Gualtar, 4710-057 Braga, Portugal*

ARTICLE INFO

Article history:
Received 4 August 2009
Received in revised form
30 September 2009
Accepted 6 October 2009
Available online 12 October 2009

Keywords:
Long-wavelength fluorescent dyes
Benzo[*a*]phenoxazinium dyes
Fluorescent labelling
DNA probes
Acid–base probes

ABSTRACT

Several fluorescent benzo[*a*]phenoxazinium chlorides possessing a propyl-, octyl-, decyl-, dodecyl- or tetradecylamino at the 5-position of the heterocyclic moiety were efficiently synthesised. The absorption and emission maxima of all compounds lie in the range 627–638 nm and 654–678 nm, respectively, with good fluorescence quantum yields. Studies of their photophysical properties in ethanol allowed for the estimation of the acid–base dissociation constant, K_a, revealing an enhancement with the increase in the alkyl side-chain length. It is in the aqueous medium only that the acid form is observed as coexisting with H-aggregates. The solubility markedly decreased when the chain length increased. The residual ethanol (0.2% v/v) used to facilitate the solubilisation of the benzo[*a*]phenoxazinium dyes allow for the existence of the basic form in an aqueous solution, possibly through preferential solvation. Photophysical studies in the presence of DNA revealed that the compounds with an alkyl side chain of up to eight carbon atoms could intercalate between DNA nucleotides. Moreover, other forms of DNA binding were found to be operative, involving also the basic form of benzo[*a*]phenoxazinium dyes.

1. Introduction

Several molecules known as ligands interact with DNA through covalent and electrostatic binding or intercalation.[1] Intercalation occurs when ligands of a suitable size and chemical nature fit themselves in between base pairs of DNA. These ligands are usually polycyclic, aromatic and planar structures, and consequently often make good nucleic acid stains. Intensively studied DNA intercalators include ethidium bromide, proflavine, daunomycin, doxorubicin and thalidomide. These compounds are used in chemotherapeutic treatment to inhibit DNA replication in rapidly growing cancer cells. Examples include doxorubicin (adriamycin) and daunorubicin, both of which are used in the treatment of Hodgkin's lymphoma, as well as dactinomycin, which is used in Wilm's tumour, Ewing's sarcoma and rhabdomyosarcoma.

Studies on the interaction between DNA and ligands are extremely important for therapeutic[2] and scientific reasons.[3,4] Nile Blue (NB), a well-known fluorescent cationic dye from the benzo[*a*]phenoxazinium family, possessing a planar and rigid structure, has been used as a DNA probe.[5] The interactions of Nile Blue and DNA proved that this dye is a good intercalator of the DNA double helix.[6] Electrochemical studies on the interaction of NB with DNA on gold electrodes showed that the binding of NB with DNA in solution contains both electrostatic and intercalative interactions, and also suggested that NB can be used as an electrochemical indicator in the preparation of DNA sensors.[7] Furthermore, recently electrochemical studies reported the use of Nile Blue as a DNA redox active intercalator.[8–10] Based on fluorescent spectroscopy techniques, Mitra et al. clearly identified non-specific electrostatic and intercalative modes of interaction of the probe with DNA at lower and higher DNA concentrations, respectively.[11] Comparison of NB to the other conventional intercalators revealed that the use of NB has the advantages of possessing low toxicity and comparable sensitivity for DNA quantification.[5]

Another possibility of interaction of small organic molecules with nucleic acids is groove binding, in which the molecules locate themselves in the minor or major groove of the DNA.[12] In this case, binding occurs through hydrogen bonding, van der Waals and hydrophobic interactions while, in intercalation, the main associative interaction is π–stacking.[13]

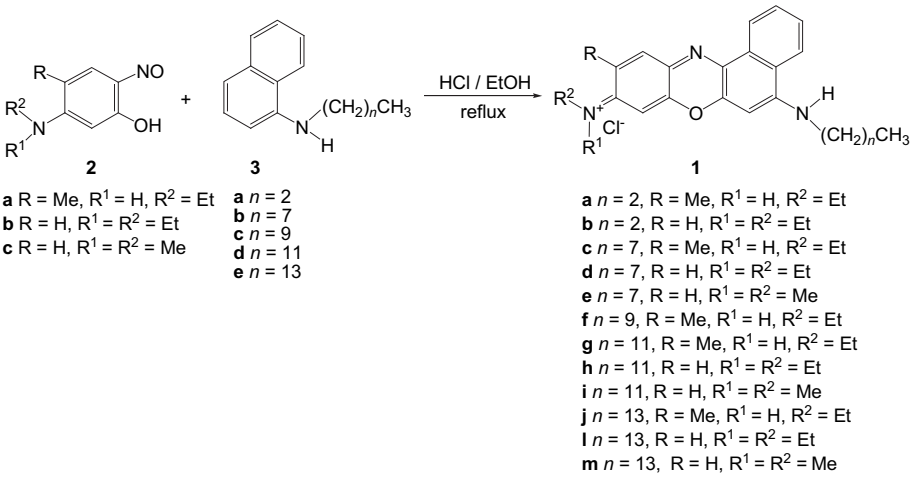
To the best of our knowledge, only limited reports are available on the use of NB despite its importance as a DNA probe, whereas reports concerning the use of other members of the benzo[*a*]phenoxazinium dyes family are almost non-existent.

Our current research interests include the synthesis and characterisation of fluorescent long-wavelength probes, namely 5,9-diaminobenzo[*a*]phenoxazinium dyes, which can be considered as Nile Blue analogues.[14–20]

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

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.10.017

Considering all these facts, we report herein the synthesis and photophysics of several benzo[*a*]phenoxazinium dyes, possessing long alkyl side chains, namely octyl-, decyl-, dodecyl- or tetradecylamino at the 5-position of the tetracyclic system. Fundamental photophysical characterisation of the synthesised compounds, in ethanol and aqueous solutions with 0.2% v/v of ethanol, was carried out with emphasis on an acid/base equilibrium, aggregation and preferential solvation. Studies on the interaction of these fluorochromophores with a self-organised system–DNA were also carried out, as an extension of the previously preliminary research work using Triton® X-100 and cetyltrimethylammonium bromide micelles as biomimicking self-assembled systems.[21]



Scheme 1. Synthesis of benzo[*a*]phenoxazinium chlorides **1a**–**m**.

2. Results and discussion

2.1. Synthesis

The synthesis of benzo[*a*]phenoxazinium chlorides **1a**–**m** was initiated by the preparation of the *N*-alkylnaphthalen-1-amines **3a**–**e**. The choice of these intermediates is crucial, since they are responsible for the size and characteristics of the side chains of the 5-amino position of benzo[*a*]phenoxazinium dyes to be synthesised and studied. Therefore, it was decided to start with the synthesis of *N*-propylnaphthalen-1-amine **3a**, with two methylenic groups (n=2) and then to vary the size of the alkyl chain of 1-naphthylamine to seven (n=7, **3b**), nine (n=9, **3c**), eleven (n=11, **3d**) and thirteen (n=13, **3e**) methylenic groups. Compounds **3a**–**e** were prepared by alkylation of 1-naphthylamine with the appropriate bromo-derivative, by refluxing in ethanol (except for compound **3a**, where methanol was used).

After purification through silica gel dry chromatography, intermediates **3a**–**e** were isolated as oils, solids or as an oily solid (**3d**), in yields ranging from 44 (**3d**) to 88% (**3c**), and were fully characterised by high resolution mass spectrometry, IR and NMR (^{1}H and ^{13}C) spectroscopy.

The IR spectra of these compounds showed the expected bands, due to stretching vibrations of the amine function (3428–3389 cm^{-1}), the C–H linkage of the methylenic and methyl groups (2958–2852 cm^{-1}) and the C–C linkage of the aliphatic chains (1173–800 cm^{-1}).

In addition to the signals of naphthalene protons, the ^{1}H NMR spectra showed the signals corresponding to aliphatic protons, in particular those of methyl groups as triplets (δ 0.91–1.09 ppm) and methylenic groups close to nitrogen atom as multiplets ($NHCH_2CH_2$, δ 1.70–2.10 ppm) and as triplets ($NHCH_2CH_2$, δ 3.29–3.39 ppm). In the ^{13}C NMR spectra of compounds **3a**–**e**, relevant signals were assigned to the aliphatic carbons, namely the methyl groups (δ 11.75–14.11 ppm) and the methylenic groups next to the nitrogen atom: $NHCH_2CH_2$ (δ 22.33–29.37 ppm) and $NHCH_2CH_2$ (δ 44.19–49.08 ppm).

By condensation of the *N*-alkylated-naphthylamines **3a**–**e** with 5-alkylamino-2-nitrosophenol hydrochlorides **2a**–**c** in an acidic medium, benzo[*a*]phenoxazinium chlorides **1a**–**m** were synthesised (Scheme 1). The required 5-alkylamino-2-nitrosophenol hydrochloride **2a**–**c** was synthesised using the usual procedure,[22] involving treatment of the corresponding 3-alkylaminophenol with sodium nitrite in an acid solution.

Reaction between nitrosophenol **2a** and **2b** with intermediate **3a**, in ethanol, in the presence of concentrated hydrochloric acid, gave compounds **1a** and **1b**. Similarly, by reacting nitroso compounds **2a**, **2b** and **2c** with **3b**, benzo[*a*]phenoxazinium dyes **1c**, **1d** and **1e**, were, respectively, obtained. Intermediate **3c** reacted with compound **2a**, resulting in benzo[*a*]phenoxazinium chloride **1f**, while the *N*-alkylated-naphthylamine derivatives **3d** and **3e** reacted with nitrosophenols **2a**, **2b** or **2c**, producing fluorophores **1g**–**m**.

After silica gel dry chromatography purification, compounds **1a**–**m** were obtained as blue solid materials in low to excellent yields (Table 1) and were fully characterised by the usual analytical techniques.

The IR spectra of these benzo[*a*]phenoxazinium dyes showed, as in the case of intermediates **3a**–**e**, the expected bands, due to stretching vibrations of the amine function (3450–3195 cm^{-1}), the C–H linkage of the methyl and methylenic groups (2968–2853 cm^{-1}), the C–C linkage of the aliphatic chains (1201–816 cm^{-1}), as well as a strong band of the C=N bond (1643–1588 cm^{-1}) as result of the oxazine ring.

The ^{1}H NMR spectra showed signals of the methyl group of the aliphatic chains in the form of triplets (δ 0.78–1.15 ppm) and the methyl groups directly linked to the aromatic ring as singlets (δ 2.38–2.45 ppm; **1a**, **1c**, **1f**, **1g**, **1j**). In compounds **1e**, **1i** and **1m** with dimethylamine at 9-position of the benzo[*a*]phenoxazinium ring, the methyl protons occurred as singlets ($\delta \sim 3.25$ or 3.20 ppm). The methyl protons of the ethyl groups (R^1 and/or R^2) appeared as multiplets (δ 1.10–1.50 ppm; **1c**, **1f**, **1g**, **1h**, **1j**, **1l**) or as triplets (δ 1.30–1.42 ppm; **1a**, **1b**, **1d**). For all compounds, the methylenic groups closed to the nitrogen atom ($NHCH_2CH_2$) in 5-position of heterocycles appeared as broad singlets (δ 1.76–1.90 ppm; **1c**, **1f**, **1g**,

Table 1
Synthesis, UV–visible and fluorescence data in ethanol of benzo[*a*]phenoxazinium chlorides **1a–m**

Compound (no.)	*n*	R	R^1	R^2	Yield [%]	λ_{max} [nm] (ε)	λ_{em} [nm]	Φ_F	DS[a] [nm]
1a	2	Me	H	Et	92	628 (46,465)	654	0.46	26
1b	2	H	Et	Et	49	637 (43,471)	670	0.27	33
1c	7	Me	H	Et	98	627 (67,486)	655	0.49	28
1d	7	H	Et	Et	88	636 (68,015)	678	0.21	42
1e	7	H	Me	Me	45	628 (45,378)	676	0.21	48
1f	9	Me	H	Et	96	627 (44,978)	655	0.44	28
1g	11	Me	H	Et	83	627 (63,128)	655	0.48	28
1h	11	H	Et	Et	69	638 (46,339)	677	0.20	39
1i	11	H	Me	Me	43	629 (55,551)	676	0.21	47
1j	13	Me	H	Et	86	626 (90,934)	655	0.47	29
1l	13	H	Et	Et	53	633 (20,860)	677	0.20	44
1m	13	H	Me	Me	17	631 (38,760)	676	0.21	45

[a] Stokes' shift.

1h, **1j**, **1l**) or as multiplets (δ 1.60–2.0 ppm; **1a**, **1d**, **1e**, **1i**, **1l**), and the protons directly linked to the nitrogen atom (NH*CH*$_2$CH$_2$) appeared as multiplets (δ 3.10–3.88 ppm; **1a–e**, **1g**, **1i**, **1m**) or as broad singlets (δ 3.18 or 3.71 ppm; **1f**, **1h**, **1j**, **1m**). Spectra also showed the expected aromatic protons, in particular, H-10 as double doublets (δ 6.91–7.26 ppm; **1b**, **1d**, **1e**, **1l**) or as doublets (δ 6.86 to ~6.93 ppm; **1h**, **1i**, **1m**), H-8 as singlets (δ 6.08–6.50 ppm), *meta* doublets (*J* 2.4 Hz) (δ 6.35 ppm; **1e**, 6.87 ppm; **1b**) and H-11, which appeared as singlets (δ 7.36–7.49 ppm; **1a**, **1c**, **1f**, **1g**, **1i**), or as doublets (δ 7.20–7.65 ppm; **1d**, **1e**, **1h**, **1i**, **1l**, **1m**).

The ^{13}C NMR spectra showed the signals corresponding to the aliphatic *N*-substituents in the heterocycle, namely the methyl groups (δ 11.59–14.09 ppm) and for the R group attached directly to aromatic ring (δ 17.72–18.60 ppm). In compounds **1e**, **1i** and **1m** with dimethylamine at 9-position of the benzo[*a*]phenoxazinium ring, the methyl carbons appeared at δ from 40.81 to 40.93 ppm. For all compounds, carbons of methylenic groups close to the nitrogen atom (NHCH$_2$*CH*$_2$) in 5-position of heterocycles appeared between 22.19 and 29.18 ppm and the carbon atom directly linked to nitrogen (NH*CH*$_2$CH$_2$) occurred between 38.66 and 47.44 ppm. Spectra also showed the expected aromatic signals such as, C-6 (δ 92.23–95.71 ppm), C-8 (δ 93.04–96.99 ppm) and C-11 (δ 130.92–134.10 ppm).

2.2. Photophysical studies

Electronic absorption spectra of 10^{-5} or 10^{-6} M solutions of benzo[*a*]phenoxazinium chlorides **1a–m** in degassed absolute ethanol were measured and data is summarised in Table 1. The longest wavelength of maximum absorption (λ_{max}) of all compounds was located between 626 nm (**1j**) and 638 nm (**1h**). Considering benzo[*a*]phenoxazinium dyes **1c–e**, with the same number of methylenic groups (*n*=7) and different substituents R, R^1 and R^2, compound **1d** (R=H, R^1=R^2=Et) showed the higher wavelength of maximum absorption (636 nm).

Similarly, comparison of other fluorophores with an equal alkyl chain in the amine function of the 5-position, such as benzo[*a*]-phenoxazinium chlorides **1g–i** (*n*=11) and **1j–m** (*n*=13), follows the same trend, i.e., there is a bathochromic shift for the compounds with the *N*-dialkylated amine at the 9-position of the heterocycle R=H, R^1=R^2=Me (2 or 5 nm) and R=H, R^1=R^2=Et (7 or 11 nm) compared to compounds with the substituents R=Me, R^1=H, R^2=Et. Considering this behaviour, it was predicted that increasing the size of the alkyl chain (a change in the values of *n*) of the amine function at position 5 of the benzo[*a*]phenoxazinium ring does not influence the values of λ_{max}, but are dependent on the amine substituent present in the opposite position.

From our previously reported results it was found that, in benzo[*a*]phenoxazinium dyes, the acid and basic forms coexist in organic solvents possessing the ability to donate/accept a proton.[16] In order to study the effect of the alkyl side chain on the acid–base equilibrium, absorption spectra of compounds **1a**, **1b**, **1c**, **1d**, **1g**, **1h**, **1j**, **1l** and **1m** at concentrations between 2 μM and 50 μM were measured. Figure 1 shows the molar absorptivity for compounds **1a** and **1b**. A clear isosbestic point can be observed, which confirms that only two molecular species are at equilibrium. The band at around 500 nm corresponds to the basic neutral form and the band around 630 nm corresponds to the cationic acid form. It was observed that a higher fraction of basic form occurs when R^1 and R^2 are ethyl groups (R=H), and thus in the acid form only one labile proton is present in the amine function at 5-position. The absorption spectra of the acid and basic forms were obtained by adding 10 μL of trifluoroacetic acid (TFA) and 20% methanolic solution of tetraethylammonium hydroxide (TEAH), respectively, to a 2×10^{-5} benzo[*a*]phenoxazinium dye solution (Fig. 2A). This allowed for the determination of the amount of acid and basic forms as a function of total compound concentration by fitting the absorption spectra with a weighed sum of spectra of the pure acid and basic forms. Figure 3 illustrates the result of this procedure for compounds **1a** and **1b**.

In order to better compare the acid–base equilibrium of the synthesised compounds in ethanol, an acid/base dissociation constant, K_a, was estimated using a procedure similar to that used in aqueous solutions (Eqs. 1–4):

$$K_{Et} = \frac{[EtO^-][H^+]}{[EtOH]} \quad (1)$$

$$K_a = \frac{[A][H^+]}{[AH^+]} \quad (2)$$

$$[H^+] + [AH^+] = [EtO^-] + [Cl^-] \quad (3)$$

$$[AH^+] + [A] = C_{AHCl} \quad (4)$$

where K_{Et} is the ethanol dissociation constant, K_a is the benzo[*a*]-phenoxazinium dye dissociation constant, A is the benzo[*a*]phenoxazinium dye in the basic form, AH^+ is the benzo[*a*]phenoxazinium dye in the acid form and C_{AHCl} is the total

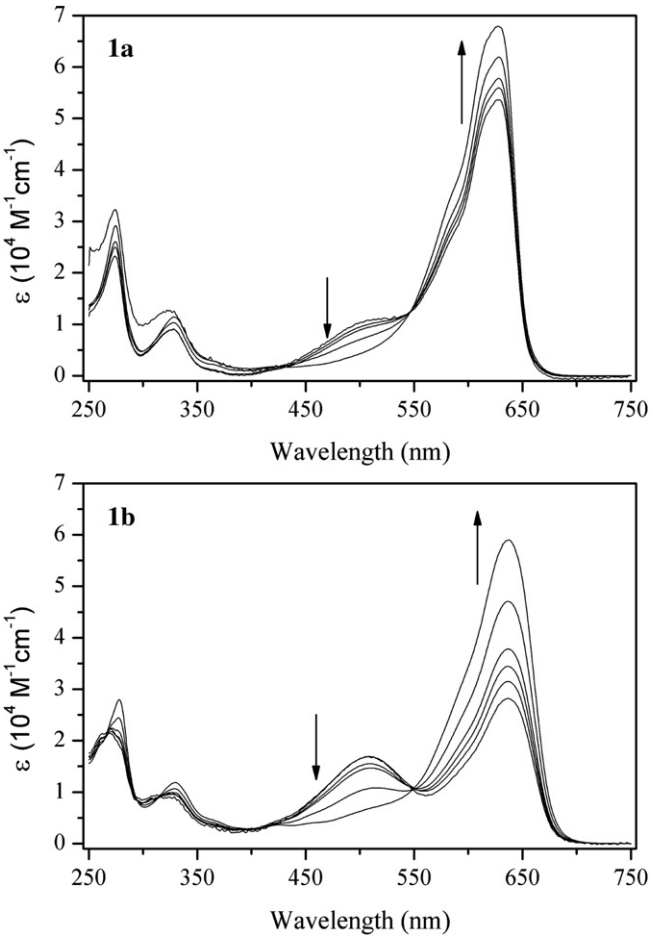


Figure 1. Molar absorptivity of compounds **1a** and **1b** in ethanol at concentrations 2, 5, 7.5, 10, 20 and 50 μM. The arrows point the direction of concentration increase.

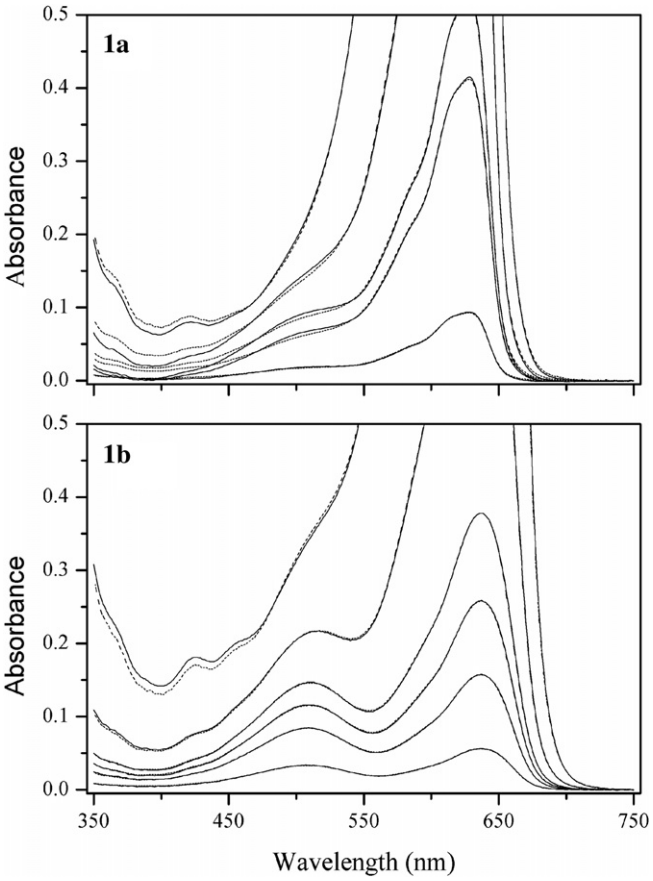


Figure 3. Decomposition of the absorption spectra of compounds **1a** and **1b** (solid line) into a weighted sum of spectra of the corresponding pure acid and basic forms (dotted line).

concentration of benzo[*a*]phenoxazinium dye. The value of K_{Et} is 1.25×10^{-16} M.[23] In Figure 4, the results of fitting the values of the basic form fraction to the solution of Eqs. 1–4 are plotted for fluorophores **1a** and **1b**. In some cases the amount of basic form should decrease with compound concentration, much more than that which was experimentally determined. Nevertheless, the obtained values for K_a give an indication of the extension of acid–base equilibrium in ethanolic solutions.

Figure 5 shows the obtained pK_a values plotted against the number of carbon atoms in the alkyl side chain of the 5-amino position of benzo[*a*]phenoxazinium dye. It was observed that pK_a decreases with the size of the alkyl chain, with an inversion from C_{12} to C_{14}. The fact that the same type of variation was observed for

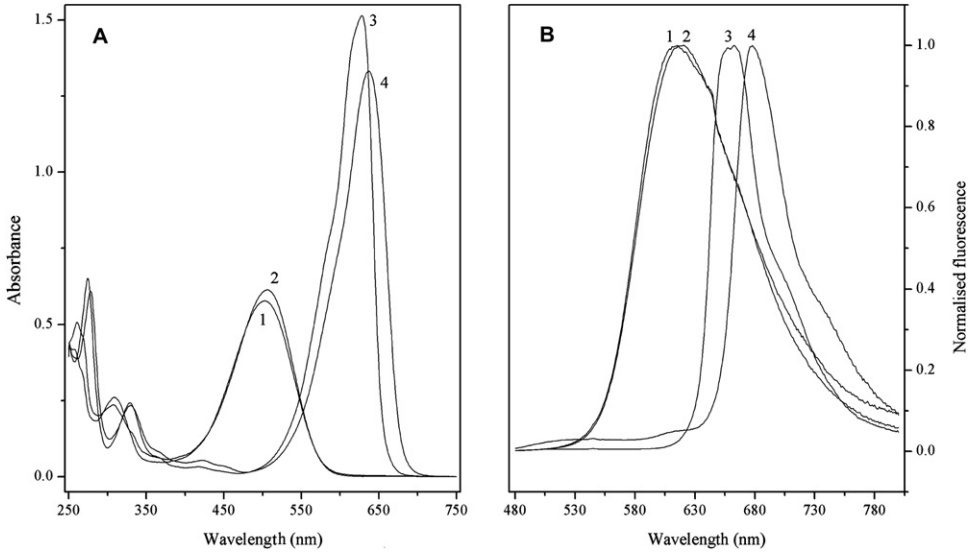


Figure 2. Absorption (A) and fluorescence (B) spectra of compounds **1a**/**1b** in its acid (3/4) or basic forms (1/2) of 2×10^{-5} M solutions (λ_{ex}=470 nm).

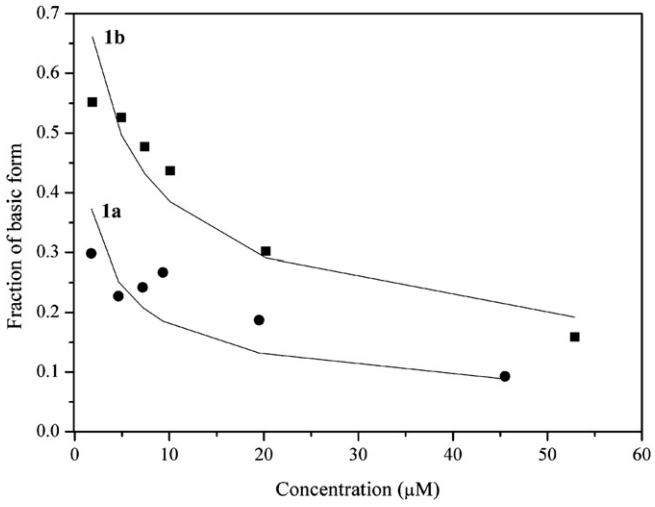


Figure 4. Fraction of basic form as a function of total compound concentration. The line represents the fitting to Eqs. 1–4.

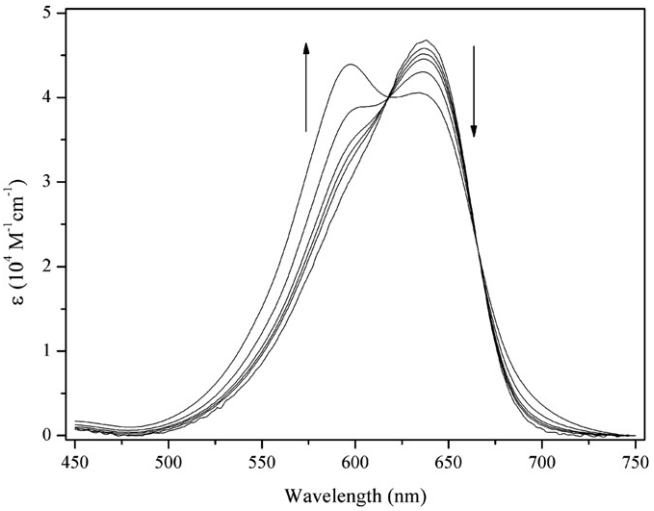


Figure 6. Molar absorptivity of compound **1b** in water at concentrations 2, 5, 7.5, 10, 20 and 50 μM. The arrows point the direction of concentration increase.

compounds with R=H and R=Me is an indication that the main site of deprotonation is always the amine function in the 5-position of benzo[*a*]phenoxazinium dye.

For compounds with high water solubility (**1a** and **1b**) the behaviour in aqueous solutions is completely different owing to non-fluorescent H-aggregate formation.[16] In Figure 6 the absorption spectra of compound **1a** in water at various concentrations is plotted. An isosbestic point was again observed here, which indicates that only one type of aggregates was formed and no basic form is present. It is worth mentioning that in contrast to ethanolic solutions, the main band now decreases with concentration. The absence of the basic form can be understood by hydrogen bonding of water molecules with the deprotonated form of benzo[*a*]phenoxazinium dyes. This interaction removes the lone electron pair from the deprotonated amine and should essentially give the same photophysical behaviour as the acid form.

Studies of the fluorescent properties of these compounds were also carried out in ethanol; the maximum emission wavelengths (λ_{em}) and relative fluorescence quantum yields (Φ_F) were obtained and are summarised in Table 1. For the determination of quantum yields, Oxazine 1, used as a standard (Φ_F=0.11 in ethanol),[24] was excited at 590 nm for all compounds tested.

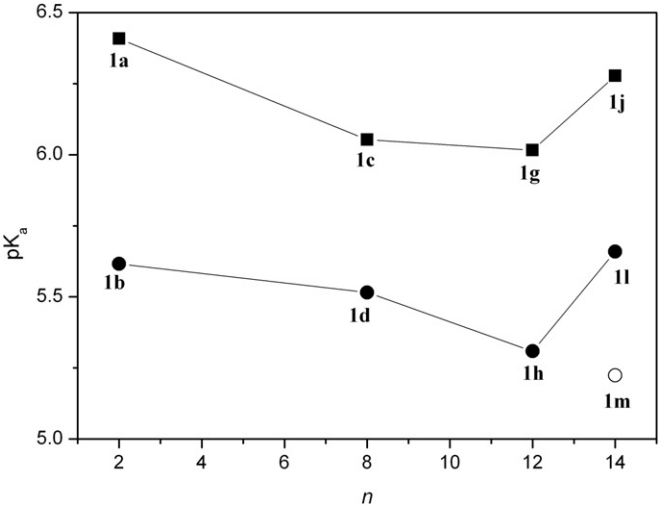


Figure 5. Recovered pK_a values as a function of total compound concentration. ●—Compounds with R=H, ■—compounds with R=Me, ○—compound with R=H and R^1, R^2=Me.

Compounds **1a–m** have a wavelength of maximum emission superior to 654 nm (**1a**) with the highest value of 678 nm (**1d**). Fluorophores with the same combination of substituents R, R^1 and R^2 and a different chain length displayed similar λ_{em}, which suggested that these values are independent from the size of the alkyl chain at the amine of the 5-position of the heterocycle. Thus, when R=Me, R^1=H, R^2=Et, λ_{em} is 655 nm (**1c**, **1f**, **1g**, **1j**) or 654 nm (**1a**). The higher values of λ_{em} arise with the combination R=H, R^1=R^2=Me, where λ_{em} was 676 nm (**1e**, **1i**, **1m**) and R=H, R^1=R^2=Et, where λ_{em} was 678 nm (**1d**) or 677 nm (**1h**, **1l**).

Regarding Stokes' shift, benzo[*a*]phenoxazinium chlorides with the diethyl- or dimethylamine at the 9-position (R=H, R^1=R^2=Et or R=H, R^1=R^2=Me; **1b**, **1d**, **1e**, **1h**, **1i**, **1l**, **1m**) showed higher values (33–48 nm) than fluorophores possessing the monoalkylated amine (R^1=H, R^2=Et) and the methyl group at the 10-position of the heterocycle (26–29 nm; **1a**, **1c**, **1f**, **1g**, **1j**). These results showed that benzo[*a*]phenoxazinium dyes with superior wavelengths of maximum emission displayed the highest Stokes' shifts.

The acid–base equilibrium also influences the fluorescence properties since the acid and basic forms of these compounds have very different fluorescence spectra and relative fluorescence quantum yields.[16] Figure 2 shows the normalised spectra of fluorescence of ethanolic solutions of compounds **1a** and **1b** in its acid and basic forms. It can be observed that the basic form almost does not show the red shift observed in the acid form when R changes from Me to H. Although the fraction of the basic form can be as high as 60%, the absorption at the excitation wavelength (590 nm) is >99% due to the acid form (see Fig. 2), and the results of the fluorescence quantum yields presented in Table 1 correspond to the acid form.

Considering the fluorescence quantum yields of all compounds (**1a–m**), it appears that values are located in two intervals, related to the benzene ring substituents (R, R^1 and R^2) and independent from the size of the alkyl chain of the amine in the naphthalene ring. The values of Φ_F with the combination R=Me, R^1=H, R^2=Et (**1a**, **1c**, **1f**, **1g**, **1j**) were between 0.44 and 0.49 and for other fluorophores (**1b**, **1d**, **1e**, **1h**, **1i**, **1l**, **1m**), these occurred between 0.20 and 0.27.

From our previous study with long alkyl side-chain derivatives of benzo[*a*]phenoxazinium chlorides[21] it was found that they have the ability to associate with positive and non-ionic micelles where

the alkyl chain functions as an anchor. Following the evaluation of these types of molecules as biological fluorescent probes, Figures 7 and 8 show the normalised absorption and fluorescence spectra of compounds **1a**, **1b**, **1c**, **1j**, **1l** and **1m** at a fixed concentration of 2×10^{-6} M and various amounts of DNA corresponding to DNA–phosphate/dye (p/d) molar ratios of 0, 1, 2, 5, 10, 50 and 100. The corresponding normalisation factors (values of maximum absorption and maximum emission intensity) are shown in the insets.

Compounds with higher water solubility (**1a** and **1b**) show simpler absorption features with DNA. Compound **1b** is the most similar to Nile Blue with only an extra three carbon alkyl chain at 5-position of the amine function of benzo[*a*]phenoxazinium ring. For this compound, an enlargement of the blue side of the spectrum is observed up to p/d=10 and, after that point, the spectra regain its original form but are shifted to the red. A slight enlargement in the red side is already observed for p/d=5, 10. The variations in the blue side of the absorption spectra can be interpreted as variations in the

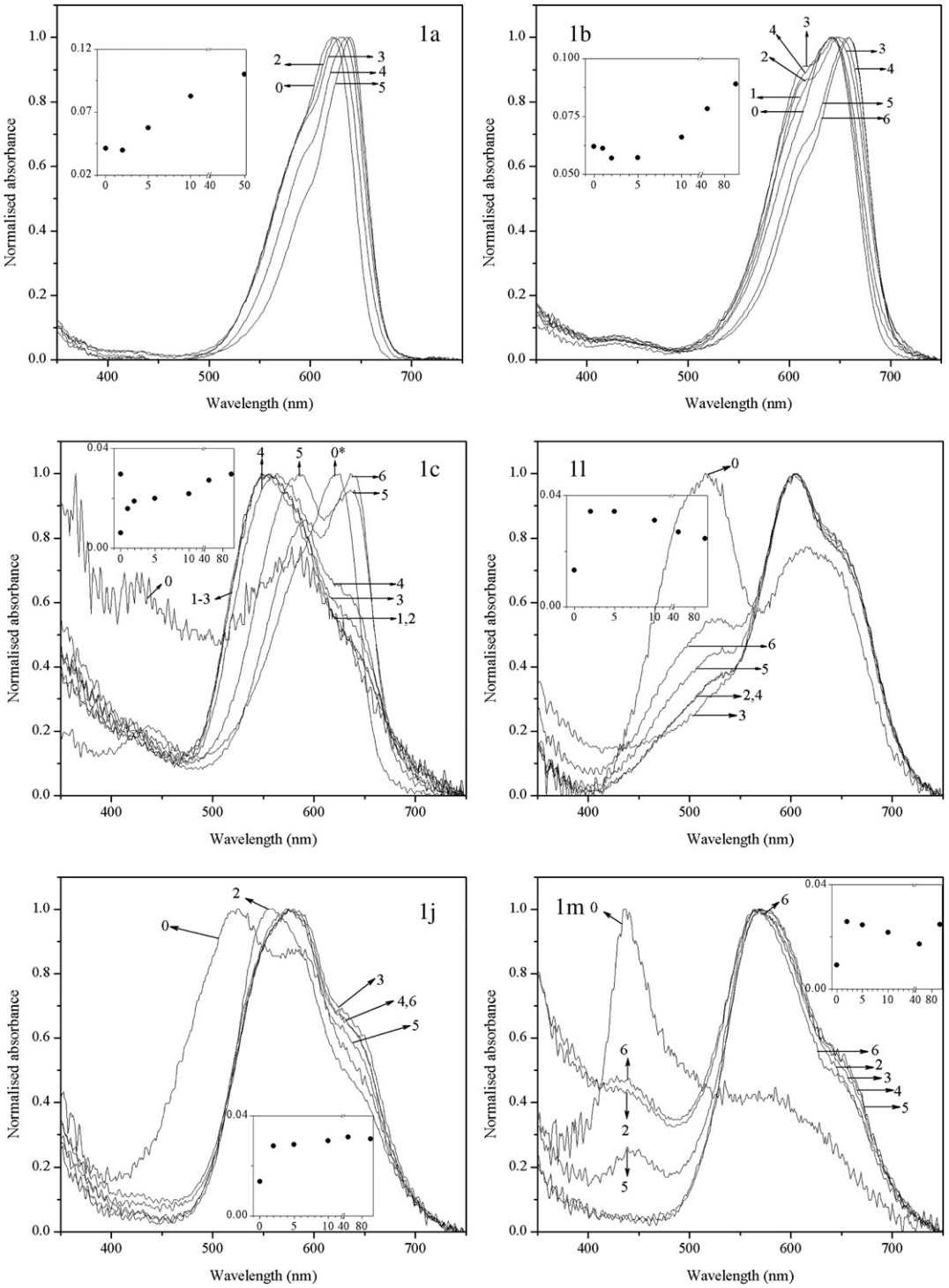


Figure 7. Normalised absorption spectra of compounds **1a**, **1b**, **1c**, **1j**, **1l** and **1m** in buffered DNA aqueous solutions after one day of equilibration. Curves 0–6 correspond to p/d=0, 1, 2, 5, 10, 50, 100. The inset plots the normalisation factors, i.e., maximum absorption. Curve 0* corresponds to absorption spectrum immediately after preparation of the solution.

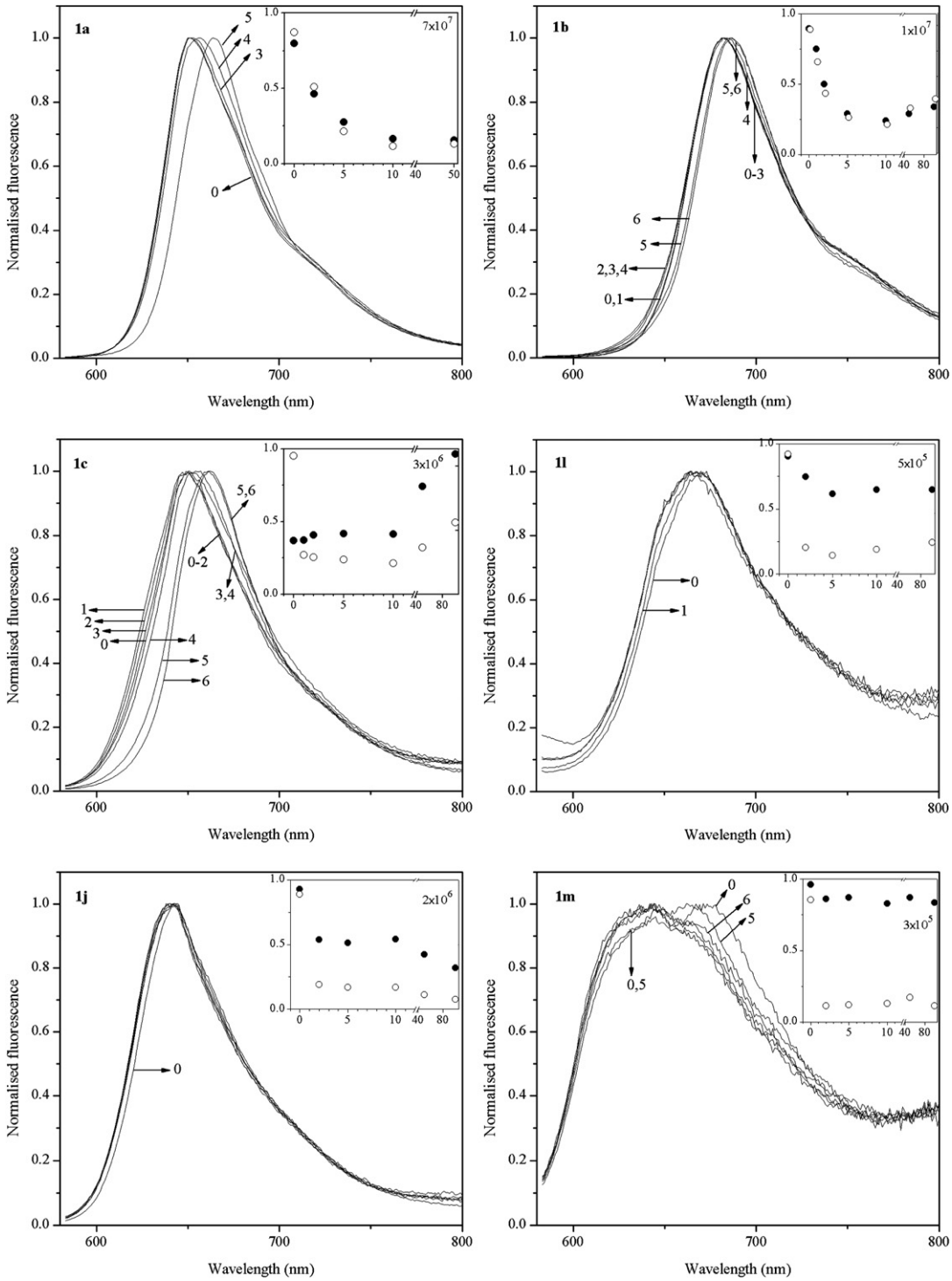


Figure 8. Normalised fluorescence spectra of compounds **1a**, **1b**, **1c**, **1j**, **1l** and **1m** in buffered DNA aqueous solutions after one day of equilibration. Curves 0–6 correspond to p/d=0, 1, 2, 5, 10, 50, 100. The inset plots the normalisation factors, i.e., maximum fluorescence intensity (•) and this quantity divided by the absorbance at the excitation wavelength, λ_{ex}=570 nm (○).The scale factor for maximum fluorescence intensity is indicated in the insets.

fraction of H-aggregates and corresponds to a decrease in fluorescence quantum yield, observed in the corresponding inset in Figure 8, while maintaining the fluorescence spectral shape. This can result from electrostatic binding to DNA with neighbouring dye molecules presenting a greater tendency of aggregating. The amount of aggregates seems to be maximal for p/d=5. The absorption maximum first decreases with p/d, reaches a plateau and then increases from p/d=5 onwards. The decrease is again explainable by an aggregation process (see Fig. 6). If the subsequent increase in absorption is caused by a decrease in aggregate fraction, then fluorescence intensity should increase with no change in the spectral shape. As a significant red shift is observed for both fluorescence (Fig. 8) and absorption (Fig. 7), it seems that a different kind of dye binding to DNA is operative for high p/d values, which can be groove binding or intercalation. However, using single strand DNA, Mitra et al.[11] interpreted similar results for Nile Blue in DNA in terms of electrostatic and intercalative binding. The red shift in absorption points to an intercalation process, also observed

in the case of ethidium bromide, is a typical intercalative binder (the maximum absorbance of 480 nm in water shifts to 525 nm in DNA[25]).

In the case of compounds **1a** and **1c** (easily soluble in water at least up to 10 μM concentration), the same intercalation process seems to occur at high p/d values as the same red shifted absorption and emission are observed. For these compounds there is an additional methyl group at the R position (10-position) and the amine at the 9-position is monoalkylated. Additionally, for compound **1c** there is an eight carbon side chain in the amine at position 5. The binding of compound **1c** to DNA can be further confirmed by the fact that the presence of DNA reduces its adsorption in glass. The adsorption effect can be seen in the corresponding inset of Figure 7, where after one day of storage, the absorption of a 2 μM aqueous solution decreases from 0.03 to 0.006. The DNA samples were measured one day after their preparation; absorbance from 0.016 to 0.03 was observed in the case of compound **1c**. The water solubility of benzo[*a*]phenoxazinium chlorides is expected to decrease with the increase of the alkyl side chain. As a result, for compounds **1c**, **1j**, **1l** and **1m**, the fraction of aggregates is much higher (absorption band at 580–600 nm) and another absorption band appears near 520 nm. This can stem from preferential solvation by residual ethanol (ethanol exists in a concentration of 0.2% v/v, i.e., 34 mM, as a result of the preparation procedure). The preferentially ethanol solvated molecules can be in their neutral basic form, which can justify the absorbance around 520 nm. An additional absorption band at 450 nm appears for compound **1m** in water and can result from H-aggregates of the neutral basic form. In Figure 7 one can see that these forms appear in different relative amounts depending on the R^1, R^2, R (compounds **1j**, **1l** and **1m**) and especially on the size of the alkyl side chain (compounds **1a**, **1c**, and **1j**).

For compound **1c**, the fluorescence intensity is approximately constant up to p/d=10 (Fig. 8). This indicates that electrostatic binding, which enhances H-aggregate formation (also not evident in absorption spectra of Fig. 7 up to p/d=10), has not occurred and the most probable main form of DNA interaction is groove binding with the C8 chain laying along the DNA strand. The additional band that appears for compounds **1c** and **1j** around 550 nm can thus stem from compound molecules in the basic form that bind to DNA grooves, possibly interacting via H-bond. The contribution of this form to the emission spectra is seen for compound **1c** as an enlargement of the blue side of the emission band. For compound **1c** a sudden change is observed in absorption and fluorescence spectra when p/d is above 10. This can be interpreted as binding of the acid form resulting in H-aggregates (absorption at 580 nm) and intercalation (red shifted absorption at ~650 nm). The C8 alkyl chain is also expected to be laying along the DNA strain.

When the side chain is longer (C14 for compounds **1j**, **1l** and **1m**), there is no evidence of intercalative binding. However, these compounds clearly associate with DNA, as is seen in the variations of both absorption maximum and absorption spectral shapes. For compounds **1j** and **1l**, the absorption spectra in the presence of DNA are dominated by H-aggregates and the acid form. In the case of compound **1l**, a small contribution from basic form at around 520 nm is observed increasing with p/d. This indicates that the basic form binds to DNA (probably in the grooves) but differently from the case of compounds **1c** and **1j**.

Compound **1m** is similar to compound **1l** with R^1=R^2=Me instead of Et. Yet, its behaviour is different. The residual acid form appears in the same spectral position (~650 nm) but the 600 nm band, attributed to H-aggregates, shifts to 560 nm; the 520 nm band, attributed to the basic form, goes to 450 nm. Thus, this compound seems highly aggregated in aqueous solutions and reveals little interaction with DNA as is confirmed by a much lower fluorescence emission, with almost no rational p/d variations in spectral shape or intensity.

3. Conclusion

A series of long alkyl side chain benzo[*a*]phenoxazinium chlorides were synthesised in good to excellent yields by the reaction with corresponding 5-alkylamino-2-nitrosophenol hydrochloride and *N*-alkylated-naphthylamines. The photophysical behaviour of these molecules was confirmed to be dominated by acid–base equilibria and H-aggregate formation. The acid/base dissociation constant of the compounds in ethanol was estimated and a tendency to increase with side-chain length was found.

The positive charge of these compounds, together with its structure with four condensed aromatic rings, opens the possibility of DNA binding. As some of the compounds have very little water solubility, it was necessary to use a residual amount of ethanol to help the solubilisation process and allow for the interaction with DNA. The residual ethanol was found to favour the presence of the basic form of the compound, probably through preferential solvation. The photophysical behaviour in a DNA buffered solution showed that these types of compounds intercalate in a DNA but only if the side chain is up to eight carbon atoms long. Other forms of DNA binding (electrostatic and groove) were also found to occur. Overall, the new benzo[*a*]phenoxazinium chlorides synthesised with maxima fluorescence emission in the near-infrared region are promising labels for biomolecules.

4. Experimental

4.1. General

All melting points were measured on a Stuart SMP3 melting point apparatus and are uncorrected. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualised under UV light or with the naked eye. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer, using KBr discs or neat samples. NMR spectra were obtained on a Varian Unity Plus Spectrometer, at an operating frequency of 300 MHz for ^{1}H NMR and 75.4 MHz for ^{13}C NMR, or a Bruker Avance III 400, at an operating frequency of 400 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR using the solvent peak as an internal reference at 25 °C. All chemical shifts are given in parts per million using δ_H Me_4Si=0 ppm as a 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 HMBC and HMQC correlation techniques. Mass spectrometry analyses were performed at the 'C.A.C.T.I.-Unidad de Espectrometria de Masas', at the University of Vigo, Spain. UV–visible absorption spectra (200–800 nm) were obtained using either a Shimadzu UV/2501PC or Shimadzu UV/3101PC spectrophotometers. Fluorescence spectra were collected using either FluoroMax-4 or FluoroMax-3 spectrofluorometers. All chemical reagents were used as received.

Natural double-stranded salmon sperm DNA was obtained from Invitrogen. Mother solutions of salmon sperm DNA were made in 10 mM Tris–HCl buffer (pH=7.4), with 1 mM EDTA. The purity of DNA was checked by monitoring the absorption spectrum and the ratio of the absorbance at 260 and 280 nm, A_{260}/A_{280}=1.95 (good-quality DNA has an A_{260}/A_{280} ratio higher than 1.8).[25] The DNA concentrations in the number of bases (or phosphate groups) were determined from the molar extinction coefficient, ε=6600 M^{-1} cm^{-1} at 260 nm.[26,27] Appropriate amounts of 10^{-3} M ethanolic solutions of the compounds were added to DNA solutions at desired concentrations. The solutions were left at least 24 h to stabilize.

4.2. General method for preparation of compounds 1a–m

To a cold solution (ice bath) of 5-(alkylamino)-2-nitrosophenol hydrochloride **2a–c** in ethanol (2 mL), *N*-alkylated-naphthylamine (**3a–e**) and concentrated hydrochloride acid (5.0×10^{-2} mL) were added. The mixture was refluxed during 2–25 h, and monitored by TLC (dichloromethane/methanol or chloroform/methanol). After evaporation of the solvent and dry chromatography purification on silica gel, the required dye (**1a–m**) was obtained as a blue material.

*4.2.1. N-[10-Methyl-5-(propylamino)-9H-benzo[a]phenoxazin-ylidene]ethanaminium chloride (**1a**).* The product of the reaction of **2a** (0.153 g, 8.51×10^{-4} mol) with **3a** (0.150 g, 8.11×10^{-4} mol) (reflux time 2 h) was chromatographed with dichloromethane/*n*-hexane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, to give compound **1a** (0.28 g, 92%). Mp=125.0–126.2 °C. TLC (dichloromethane/methanol, 6.5:0.5): R_f=0.47. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=1.07 (t, J=5.4 Hz, 3H, $NHCH_2CH_2CH_3$), 1.42 (t, J=5.1 Hz, 3H, $NHCH_2CH_3$), 1.80–2.00 (m, 2H, $NHCH_2CH_2$), 2.41 (s, 3H, CH_3), 3.20–3.35 (m, 2H, $NHCH_2CH_2$), 3.58 (br s, 2H, $NHCH_2CH_3$), 6.30 (s, 1H, 8-H), 6.37 (s, 1H, 6-H), 7.49 (s, 1H, 11-H), 7.80–8.00 (m, 2H, 2-H and 3-H), 8.81 (d, J=6.9 Hz, 1H, 1-H), 9.19 (br s, 1H, 4-H), 11.23 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=11.59 ($NHCH_2CH_3$), 14.03 ($NHCH_2CH_3$), 17.72 (CH_3), 22.19 ($NHCH_2CH_2$), 38.83 ($NHCH_2CH_2$), 46.43 ($NHCH_2CH_3$), 93.04 (C-8), 93.80 (C-6), 124.11 (Ar-C), 124.21 (C-1), 125.70 (C-10), 126.12 (C-4), 129.09 (Ar-C), 129.87 (Ar-C), 130.67 (C-3), 131.29 (C-11), 133.52 (C-2), 135.35 (Ar-C), 146.58 (Ar-C), 151.14 (Ar-C), 153.17 (C-9), 156.79 (C-5). IR (KBr 1%, cm^{-1}): ν=3201, 2968, 2931, 2874, 1642, 1592, 1561, 1544, 1520, 1498, 1451, 1435, 1384, 1343, 1318, 1258, 1234, 1186, 1165, 1144, 1129, 1086, 1056, 1014, 1004, 977, 882, 856, 818, 774. HRMS (FAB): calcd for $C_{22}H_{24}N_3O$ [M^+]: 346.1919; found: 346.1914.

*4.2.2. N-Ethyl-N-[5-(propylamino)-9H-benzo[a]phenoxazin-9-ylidene]ethanaminium chloride (**1b**).* The product of the reaction of **2b** (0.291 g, 1.5×10^{-3} mol) with **3a** (0.185 g, 1×10^{-3} mol) (reflux time 5 h) was chromatographed with chloroform and chloroform/methanol, mixtures of increasing polarity, as the eluent, to give compound **1b** (0.288 g, 49%). Mp=235–237 °C. TLC (chloroform/methanol, 9.4:0.6): R_f=0.19. ^{1}H NMR (CD_3OD, 400 MHz): δ=1.15 (t, J=7.6 Hz, 3H, $NHCH_2CH_2CH_3$), 1.37 (t, J=6.8 Hz, 6H, $2\times NCH_2CH_3$), 1.85–1.98 (m, 2H, $NHCH_2CH_2CH_3$), 3.65–3.79 (m, 6H, $2\times NCH_2CH_3$ and $NHCH_2CH_2CH_3$), 6.87 (d, J=2.4 Hz, 1H, 8-H), 6.90 (s, 1H, 6-H), 7.26 (dd, J=9.4 Hz and 2.4 Hz, 1H, 10-H), 7.76–7.83 (m, 2H, 11-H and 3-H), 7.85–7.94 (m, 1H, 2-H), 8.32 (d, J=8.0 Hz, 1H, 4-H), 8.79 (d, J=8.0 Hz, 1H, 1-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=11.77 ($NHCH_2CH_2CH_3$), 12.98 ($2\times NCH_2CH_3$), 23.06 ($NHCH_2CH_2CH_3$), 47.05 ($2\times NCH_2CH_3$), 47.44 ($NHCH_2CH_2CH_3$), 94.46 (C-6), 96.99 (C-8), 116.64 (C-10), 123.85 (C-4), 124.71 (Ar-C), 125.54 (C-1), 130.90 (C-3), 131.53 (Ar-C), 132.51(Ar-C), 132.95(C-2), 134.10 (C-11), 134.91 (Ar-C), 149.64 (Ar-C), 153.14 (Ar-C), 155.61 (C-9), 159.36 (C-5). IR (KBr 1%, cm^{-1}): ν=3378, 3169, 2966, 2929, 1639, 1586, 1546, 1452, 1434, 1384, 1327, 1276, 1257, 1196, 1166, 1148, 1122, 1073, 1015, 1002, 944, 884, 855, 821, 809, 766. HRMS (FAB): calcd for $C_{23}H_{26}N_3O$ [M^+] 360.20657; found: 360.20704.

*4.2.3. N-[10-Methyl-5-(octylamino)-9H-benzo[a]phenoxazin-9-ylidene]ethanaminium chloride (**1c**).* The product of the reaction of **2a** (0.216 g, 1.20×10^{-3} mol) with **3b** (0.307 g, 1.20×10^{-3} mol) (reflux time 4 h) was chromatographed with dichloromethane/methanol, 59:1, as the eluent, to give compound **1c** (0.49 g, 98%). Mp=147.5–149.0 °C. TLC (dichloromethane/methanol, 5:1): R_f=0.45. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.86 (t, J=6.6 Hz, 3H, $NH(CH_2)_7CH_3$), 1.20–1.50 ($2\times$m, 13H, $5\times CH_2$ and $NHCH_2CH_3$), 1.85 (br s, 2H, $NHCH_2CH_2$), 2.45 (s, 3H, CH_3), 3.20–3.30 (m, 2H, $NHCH_2CH_2$), 3.57 (br s, 2H, $NHCH_2CH_3$), 6.30 (br s, 2H, 8-H and 6-H), 7.49 (s, 1H, 11-H), 7.87 (br s, 2H, 2-H and 3-H), 8.80–8.90 (m, 1H, 1-H), 9.18 (br s, 1H, 4-H), 10.81 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=13.95 ($NHCH_2CH_3$), 14.03 ($NH(CH_2)_7CH_3$), 18.60 (CH_3), 22.55 (CH_2), 27.12 (CH_2), 28.65 ($NHCH_2CH_2$), 29.14 (CH_2), 29.24 (CH_2), 31.71 (CH_2), 38.72 ($NHCH_2CH_2$), 44.40 ($NHCH_2CH_3$), 92.44 (C-6), 93.22 (C-8), 123.46 (Ar-C), 123.99 (C-1), 125.54 (C-4), 127.06 (C-10), 129.66 (Ar-C), 129.94 (C-3), 130.57 (Ar-C), 130.98 (C-11), 131.61 (C-2), 133.48 (Ar-C), 146.72 (Ar-C), 150.57 (Ar-C), 154.03 (C-9), 156.71 (C-5) ppm. IR (KBr 1%, cm^{-1}): ν=3195, 2925, 2853, 1642, 1592, 1561, 1544, 1519, 1498, 1451, 1435, 1315, 1295, 1259, 1232, 1185, 1163, 1129, 1085, 1054, 1010, 881, 817, 773, 733, 708, 666. HRMS (FAB): calcd for $C_{27}H_{34}N_3O$ [M^+]: 416.2702; found: 416.2708.

*4.2.4. N-Ethyl-N-[5-(octylamino)-9H-benzo[a]phenoxazin-9-ylidene]ethanaminium chloride (**1d**).* The product of the reaction of **2b** (0.235 g; 1.21×10^{-3} mol) with **3b** (0.309 g, 1.21×10^{-3} mol) (2 mL) (reflux time 8 h) was chromatographed with chloroform/*n*-hexane and chloroform/methanol, mixtures of increasing polarity, as the eluent, to give compound **1d** (0.46 g, 88%). Mp=113.0–115.8 °C. TLC (chloroform/methanol, 9.5:0.5): R_f=0.33. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.84 (t, J=7.2 Hz, 3H, CH_3), 1.30–1.38 (m, 10H, $5\times CH_2$), 1.32 (t, J=7.2 Hz, 6H, $N(CH_2CH_3)_2$), 1.40–1.50 (m, 2H, CH_2), 1.84–1.92 (m, 2H, $NHCH_2CH_2$), 3.56 and 3.60 ($2\times$d, J=7.2 and 6.8 Hz, 2H, $N(CH_2CH_3)_2$), 3.72–3.82 (m, 2H, $NHCH_2CH_2$), 6.50 (s, 1H, 8-H), 6.51 (s, 1H, 6-H), 6.95 (dd, J=9.4 and 2.8 Hz, 1H, 10-H), 7.65 (d, J=9.2 Hz, 1-H, 11-H), 7.67–7.76 (m, 2H, 2-H and 3-H), 8.65 (d, J=9.6 Hz, 1H, 1-H), 9.26 (d, J=8.8 Hz, 1H, 4-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=12.60 ($N(CH_2CH_3)_2$), 13.99 (CH_3), 22.53 (CH_2), 27.12 ($NHCH_2CH_2$), 28.98 (CH_2), 29.13 (CH_2), 29.26 (CH_2), 31.70 (CH_2), 44.96 ($NHCH_2CH_2$), 45.84 ($N(CH_2CH_3)_2$), 93.21 (C-6), 95.58 (C-8), 113.71 (C-10), 123.91 (C-1), 124.16 (Ar-C), 126.21 (C-4), 128.35 (Ar-C), 130.44 (C-3), 130.58 (Ar-C), 131.78 (C-2), 132.38 (C-11), 135.26 (Ar-C), 147.26 (Ar-C), 151.33 (Ar-C), 152.84 (C-9), 158.46 (C-5). IR (KBr 1%, cm^{-1}): ν=3424, 2956, 2925, 2853, 1640, 1588, 1547, 1498, 1454, 1435, 1383, 1330, 1273, 1255, 1164, 1123, 1073, 1012, 751, 707, 666. HRMS (EI): calcd for $C_{28}H_{36}N_3O$ [M^+]: 430.28541; found: 430.28529.

*4.2.5. N-Methyl-N-[5-(octylamino)-9H-benzo[a]phenoxazin-9-ylidene]methanaminium chloride (**1e**).* The product of the reaction of **2c** (0.20 g; 1.31×10^{-3} mol) with **3b** (0.34 g, 1.31×10^{-3} mol) (reflux time 12 h) was chromatographed with chloroform/*n*-hexane and chloroform/methanol, mixtures of increasing polarity, as the eluent, to give compound **1e** (0.24 g, 45%). Mp=174.9–176.2 °C. TLC (chloroform/methanol, 9:1): R_f=0.43. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.86 (t, J=5.1 Hz, 3H, CH_3), 1.20–1.50 (m, 10H, $5\times CH_2$), 1.80–1.90 (m, 2H, $NHCH_2CH_2$), 3.24 (s, 6H, $N(CH_3)_2$), 3.70–3.85 (m, 2H, $NHCH_2CH_2$), 6.35 (d, J=2.4 Hz, 1H, 8-H), 6.46 (s, 1H, 6-H), 6.91 (dd, J=9.2 and 2.4 Hz, 1H, 10-H), 7.56 (d, J=9.2 Hz, 1H, 11-H), 7.68–7.77 (m, 2H, 2-H and 3-H), 8.58–8.65 (m, 1H, 1-H), 9.20–9.30 (m, 1H, 4-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=14.03 ($NH(CH_2)_7CH_3$), 22.57 (CH_2), 27.16 (CH_2), 29.03 (CH_2), 29.18 ($NHCH_2CH_2$), 29.30 (CH_2), 31.74 (CH_2), 40.89 ($N(CH_3)_2$), 45.06 ($NHCH_2CH_2$), 93.28 (C-6), 95.71 (C-8), 113.78 (C-10), 123.99 (C-1), 124.15 (Ar-C), 126.28 (C-4), 128.22 (Ar-C), 130.47 (C-3), 130.51 (Ar-C), 131.81 (C-11), 131.88 (C-2), 135.39 (Ar-C), 146.66 (Ar-C), 151.25 (Ar-C), 154.44 (C-9), 158.46 (C-5). IR (KBr 1%, cm^{-1}): ν=3428, 2956, 2925, 2854, 1641, 1592, 1553, 1538, 1500, 1456, 1427, 1380, 1334, 1293, 1202, 1180, 1148, 1127, 1009, 906, 816, 784, 741, 716, 666. HRMS (TOF EI): calcd for $C_{26}H_{32}N_3O$ [M^+]: 402.25403; found: 402.25399.

*4.2.6. N-[5-(Decylamino)-10-methyl-9H-benzo[a]phenoxazin-9-ylidene]ethanaminium chloride (**1f**).* The product of the reaction of **2a** (0.18 g, 1.05×10^{-3} mol) with **3c** (0.21 g, 7.3×10^{-3} mol) (reflux

time 8 h) was chromatographed with chloroform/*n*-hexane and chloroform/methanol, mixtures of increasing polarity, as the eluent, to give compound **1f** (0.32 g, 96%). Mp=168.8–171.8 °C. TLC (chloroform/methanol, 5:1): R_f=0.40. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.86 (t, J=6.3 Hz, 3H, $NH(CH_2)_9CH_3$), 1.18–1.46 (2×m, 17H, 7×CH_2 and $NHCH_2CH_3$), 1.80 (br s, 2H, $NHCH_2CH_2$), 2.39 (s, 3H, CH_3), 3.18 (br s, 2H, $NHCH_2CH_2$), 3.42 (br s, 2H, $NHCH_2CH_3$), 6.08 (s, 1H, 8-H), 6.16 (s, 1H, 6-H), 6.78 (br s, 1H, NH), 7.36 (s, 1H, 11-H), 7.70–7.90 (m, 2H, 2-H and 3-H), 8.74 (d, J=8.7 Hz, 1H, 1-H), 8.91 (br s, 1H, 4-H), 10.59 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=13.74 ($NHCH_2CH_3$), 14.06 ($NH(CH_2)_9CH_3$), 17.95 (CH_3), 22.61 (CH_2), 27.11 (CH_2), 28.55 ($NHCH_2CH_2$), 29.24 (2×CH_2), 29.29 (CH_2), 29.49 (CH_2), 31.81 (CH_2), 38.67 ($NHCH_2CH_2$), 44.69 ($NHCH_2CH_3$), 92.36 (C-6), 93.10 (C-8), 123.56 (C-1), 123.75 (Ar-C), 125.25 (C-4), 126.83 (C-10), 129.48 (Ar-C), 129.97 (C-3), 130.61 (Ar-C), 130.92 (C-11), 131.50 (C-2), 133.65 (Ar-C), 146.68 (Ar-C), 150.50 (Ar-C), 154.00 (C-9), 156.93 (C-5). IR (KBr 1%, cm^{-1}): ν=3440, 2956, 2925, 2853, 1643, 1592, 1424, 1384, 1316, 1185, 1126, 1012, 666. HRMS (FAB): calcd for $C_{29}H_{38}N_3O$ [M$^+$]: 444.3015; found: 444.3021.

*4.2.7. N-[5-(Dodecylamino)-10-methyl-9H-benzo[a]phenoxazin-9-ylidene]ethanaminium chloride (**1g**).* The product of the reaction of **2a** (0.087 g, 4.82×10^{-4} mol) with **3d** (0.151 g, 4.86×10^{-4} mol) (reflux time 4 h) was chromatographed with dichloromethane/*n*-hexane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, to give compound **1g** (0.19 g, 83%). Mp=93.1–96.0 °C. TLC (dichloromethane/methanol, 9:1): R_f=0.50. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.88 (t, J=6.9 Hz, 3H, $NH(CH_2)_{11}CH_3$), 1.20–1.50 (2×m, 21H, 9×CH_2 and $NHCH_2CH_3$), 1.84 (br s, 2H, $NHCH_2CH_2$), 2.44 (s, 3H, CH_3), 3.10–3.30 (m, 2H, $NHCH_2CH_2$), 3.52 (br s, 2H, $NHCH_2CH_3$), 6.19 (s, 1H, 8-H), 6.27 (s, 1H, 6-H), 7.47 (s, 1H, 11-H), 7.80–7.90 (m, 2H, 2-H and 3-H), 8.76–8.90 (m, 1H, 1-H), 9.10–9.24 (m, 1H, 4-H), 11.14 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=13.82 ($NHCH_2CH_3$), 14.04 ($NH(CH_2)_{11}CH_3$), 18.27 (CH_3), 22.60 (CH_2), 27.12 (CH_2), 28.62 ($NHCH_2CH_2$), 29.27 (CH_2), 29.30 (CH_2), 29.50 (CH_2), 29.53 (CH_2), 29.58 (CH_2), 30.87 (CH_2), 31.83 (CH_2), 38.66 ($NHCH_2CH_2$), 44.51 ($NHCH_2CH_3$), 92.33 (C-6), 93.08 (C-8), 123.62 (Ar-C), 123.94 (C-1), 125.51 (C-4), 127.0 (C-10), 129.55 (Ar-C), 129.99 (C-3), 130.59 (Ar-C), 130.92 (C-11), 131.54 (C-2), 133.53 (Ar-C), 146.71 (Ar-C), 150.58 (Ar-C), 154.06 (C-9), 156.92 (C-5). IR (KBr 1%, cm^{-1}): ν=3450, 3210, 2956, 2923, 2852, 1643, 1592, 1561, 1544, 1520, 1451, 1436, 1384, 1317, 1295, 1262, 1233, 1185, 1163, 1129, 1085, 1010, 878, 816, 773, 733, 666. HRMS (FAB): calcd for $C_{31}H_{42}N_3O$ [M$^+$]: 472.3328; found: 472.3335.

*4.2.8. N-[5-(Dodecylamino)-9H-benzo[a]phenoxazin-9-ylidene]-N-ethylethanaminium chloride (**1h**).* The product of the reaction of **2b** (0.121 g, 6.25×10^{-4} mol) with **3d** (0.194 g, 6.25×10^{-4} mol) (reflux time 7 h) was chromatographed with dichloromethane/*n*-hexane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, to give compound **1h** (0.26 g, 69%). Mp=130.3–131.4 °C. TLC (dichloromethane/methanol, 9.5:0.5): R_f=0.42. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.78 (t, J=6.6 Hz, 3H, $NH(CH_2)_{11}CH_3$), 1.10–1.40 (3×m, 24H, $N(CH_2CH_3)_2$ and 9×CH_2), 1.82 (br s, 2H, $NHCH_2CH_2$), 3.40–3.60 (m, 4H, $N(CH_2CH_3)_2$), 3.71 (br s, 2H, $NHCH_2CH_2$), 6.43 (s, 2H, 8-H and 6-H), 6.92 (d, J=9.3 Hz, 1H, 10-H), 7.56 (d, J=9.0 Hz, 1H, 11-H), 7.58–7.70 (m, 2H, 2-H and 3-H), 8.48–8.58 (m, 1H, 1-H), 9.13 (br s, 1H, 4-H), 11.47 (br s, 1H, N–H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=12.51 ($N(CH_2CH_3)_2$), 13.86 ($NH(CH_2)_{11}CH_3$), 22.40 (CH_2), 26.99 (CH_2), 28.83 ($NHCH_2CH_2$), 29.07 (CH_2), 29.19 (CH_2), 29.35 (CH_2), 29.38 (CH_2), 30.70 (CH_2), 31.63 (CH_2), 44.74 ($NHCH_2CH_2$), 45.81 ($N(CH_2CH_3)_2$), 92.90 (C-6), 95.41 (C-8), 113.93 (C-10), 123.68 (C-1), 123.79 (Ar-C), 125.74 (C-4), 128.38 (Ar-C), 129.91 (C-3), 130.33 (Ar-C), 131.43 (C-2), 132.23 (C-11), 134.50 (Ar-C), 147.06 (Ar-C), 150.95 (Ar-C), 152.85 (C-9), 157.92 (C-5). HRMS (EI): calcd for $C_{32}H_{44}N_3O$ [M$^+$]: 486.34800; found: 486.34789.

*4.2.9. N-[5-(Dodecylamino)-9H-benzo[a]phenoxazin-9-ylidene]-N-methylmethanaminium chloride (**1i**).* The product of the reaction of **2c** (0.22 g, 1.45×10^{-3} mol) with **3d** (0.301 g, 9.67×10^{-4} mol) (reflux time 25 h) was chromatographed with chloroform/*n*-hexane and chloroform/methanol, mixtures of increasing polarity, as the eluent, to give compound **1i** (0.19 g, 43%). Mp=163.0–163.8 °C. TLC (chloroform/methanol, 9.5:0.5): R_f=0.57. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.87 (t, J=6.9 Hz, 3H, $NH(CH_2)_{11}CH_3$), 1.12–1.60 (2×m, 18H, 9×CH_2), 1.62–2.0 (m, 2H, $NHCH_2CH_2$), 3.25 (s, 6H, $N(CH_3)_2$), 3.70–3.88 (m, 2H, $NHCH_2CH_2$), 6.41 (s, 1H, 8-H), 6.49 (s, 1H, 6-H), 6.93 (d, J=7.8 Hz, 1H, 10-H), 7.20 (d, J=9.0 Hz, 1H, 11-H), 7.68–7.80 (m, 2H, 2-H and 3-H), 8.60–8.72 (m, 1H, 1-H), 9.21 (br s, 1H, 4-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=14.08 ($NH(CH_2)_{11}CH_3$), 22.64 (CH_2), 27.22 (CH_2), 29.17 (CH_2), 29.31 (CH_2), 29.39 (CH_2), 29.56 (CH_2), 29.59 (2×CH_2), 29.62 (CH_2), 31.87 (CH_2), 40.93 ($N(CH_3)_2$), 45.24 ($NHCH_2CH_2$), 93.48 (C-6), 95.85 (C-8), 113.62 (C-10), 124.05 (C-1), 124.47 (Ar-C), 126.20 (C-4), 128.21 (Ar-C), 130.52 (C-3), 130.56 (Ar-C), 131.83 (C-11), 131.90 (C-2), 135.84 (Ar-C), 146.74 (Ar-C), 151.19 (Ar-C), 154.38 (C-9), 158.35 (C-5). IR (KBr 1%, cm^{-1}): ν=3448, 2955, 2924, 2853, 1641, 1590, 1552, 1513, 1500, 1459, 1427, 1378, 1332, 1292, 1201, 1177, 1149, 1125, 1009, 905, 864, 817, 783, 715, 666. HRMS (EI): calcd for $C_{30}H_{40}N_3O$ [M$^+$]: 458.31711; found: 458.31659.

*4.2.10. N-[10-Methyl-5-(tetradecylamino)-9H-benzo[a]phenoxazin-9-ylidene]ethanaminium chloride (**1j**).* The product of the reaction of **2a** (0.110 g, 6.13×10^{-4} mol) with **3e** (0.200 g, 6.13×10^{-4} mol) (reflux time 6 h) was chromatographed with chloroform/methanol, mixtures of increasing polarity, as the eluent, to give compound **1j** (0.27 g, 86%). Mp=113.9–115.5 °C. TLC (chloroform/methanol, 9:1): R_f=0.30. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.87 (t, J=6.9 Hz, 3H, $NH(CH_2)_{13}CH_3$), 1.20–1.50 (2×m, 25H, 11×CH_2 and $NHCH_2CH_3$), 1.80 (br s, 2H, $NHCH_2CH_2$), 2.38 (s, 3H, CH_3), 3.18 (br s, 2H, $NHCH_2CH_2$), 3.43 (br s, 2H, $NHCH_2CH_3$), 6.10 (s, 1H, 8-H), 6.18 (s, 1H, 6-H), 7.39 (s, 1H, 11-H), 7.81 (br s, 2H, 2-H and 3-H), 8.77 (d, J=7.5 Hz, 1H, 1-H), 8.92 (br s, 1H, 4-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=13.77 ($NHCH_2CH_3$), 14.09 ($NH(CH_2)_{13}CH_3$), 17.84 (CH_3), 22.65 (CH_2), 27.14 (CH_2), 28.57 ($NHCH_2CH_2$), 29.32 (2×CH_2), 29.53 (CH_2), 29.57 (CH_2), 29.62 (2×CH_2), 29.66 (2×CH_2), 31.88 (CH_2), 38.71 ($NHCH_2CH_2$), 44.74 ($NHCH_2CH_3$), 92.45 (C-6), 93.18 (C-8), 123.79 (Ar-C), 124.03 (C-1), 125.36 (C-4), 126.69 (C-10), 129.46 (Ar-C), 130.15 (C-3), 130.66 (Ar-C), 131.00 (C-11), 131.62 (C-2), 133.92 (Ar-C), 146.69 (Ar-C), 150.64 (Ar-C), 153.96 (C-9), 157.16 (C-5). IR (KBr 1%, cm^{-1}): ν=3448, 3230, 2954, 2919, 2852, 1644, 1592, 1562, 1546, 1520, 1453, 1436, 1384, 1316, 1261, 1185, 1164, 1129, 1087, 1010, 880, 774, 666. HRMS (FAB): calcd for $C_{33}H_{46}N_3O$ [M$^+$]: 500.3641; found: 500.3637.

*4.2.11. N-Ethyl-N-[5-(tetradecylamino)-9H-benzo[a]phenoxazin-9-ylidene]ethanaminium chloride (**1l**).* The product of the reaction of **2b** (0.195 g, 1.00×10^{-3} mol) with **3e** (0.341 g, 1.00×10^{-3} mol) (reflux time 10 h) was chromatographed with dichloromethane/methanol 99:1, as the eluent, to give compound **1l** (0.27 g, 53%). Mp=71.1–73.4 °C. TLC (dichloromethane/methanol, 9:1): R_f=0.68. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.81 (t, J=7.2 Hz, 3H, $NH(CH_2)_{13}CH_3$), 1.10–1.50 (3×m, 28H, 11×CH_2 and $N(CH_2CH_3)_2$), 1.80–2.0 (m, 2H, $NHCH_2CH_2$), 3.54 and 3.58 (2×d, J=7.2 Hz, 4H, $N(CH_2CH_3)_2$), 3.70–3.80 (m, 2H, $NHCH_2CH_2$), 6.45 (s, 2H, 8-H and 6-H), 6.92 (dd, J=9.2 and 2.4 Hz, 1H, 10-H), 7.58 (d, J=9.2 Hz, 1H, 11-H), 7.60–7.70 (m, 2H, 2-H and 3-H), 8.50–8.60 (m, 1H, 1-H), 9.16 (br s, 1H, 4-H), 11.67 (br s, 1H, N–H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=12.57 ($N(CH_2CH_3)_2$), 13.95 ($NH(CH_2)_{13}CH_3$), 22.50 (CH_2), 27.08 (CH_2), 28.92 ($NHCH_2CH_2$), 29.17 (CH_2), 29.27 (CH_2), 29.43 (CH_2), 29.54 (2×CH_2), 29.47 (CH_2), 29.49 (CH_2), 29.51 (CH_2), 31.73 (CH_2), 44.93 ($NHCH_2CH_2$), 45.82 ($N(CH_2CH_3)_2$), 93.05 (C-6), 95.51 (C-8), 113.80 (C-10), 123.79 (Ar-C),

124.02 (C-1), 125.94 (C-4), 128.35 (Ar-C), 130.15 (C-3), 130.45 (Ar-C), 131.56 (C-2), 132.29 (C-11), 134.85 (Ar-C), 147.15 (Ar-C), 151.11 (Ar-C), 152.85 (C-9), 158.20 (C-5). IR (KBr 1%, cm^{-1}): ν=3436, 2956, 2923, 2852, 1641, 1590, 1548, 1514, 1438, 1385, 1329, 1277, 1255, 1168, 1151, 1125, 1073, 1013, 705, 666. HRMS (EI): calcd for $C_{34}H_{48}N_3O$ [M^+]: 514.37906; found: 514.37919.

*4.2.12. N-Methyl-N-[5-(tetradecylamino)-9H-benzo[a]phenoxazin-9-ylidene]methanaminium chloride (**1m**).* The product of the reaction of **2c** (0.15 g, 9.56×10^{-4} mol) with **3e** (0.324 g, 9.56×10^{-4} mol) (reflux time 14 h) was chromatographed with dichloromethane/*n*-hexane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, to give compound **1m** (0.079 g, 17%). Mp=154.0–156.7 °C. TLC (dichloromethane/methanol, 9:1): R_f=0.55. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=0.84 (t, J=5.2 Hz, 3H, NH$(CH_2)_{13}CH_3$), 1.10–1.50 (3×m, 22H, 11×CH_2), 1.76–1.90 (br s, 2H, NHCH$_2CH_2$), 3.20 (s, 6H, N$(CH_3)_2$), 3.71 (br s, 2H, NHCH_2CH_2), 6.0 (s, 1H, 8-H), 6.40 (s, 1H, 6-H), 6.86 (d, J=8.0 Hz, 1H, 10-H), 7.48 (d, J=8.8 Hz, 1H, 11-H), 7.60–7.70 (m, 2H, 2-H and 3-H), 8.52 (d, J=7.6 Hz, 1H, 1-H), 8.95 (br s, 1H, 4-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C=13.99 (NH$(CH_2)_{13}CH_3$), 22.54 (CH_2), 27.11 (CH_2), 28.89 (NHCH$_2CH_2$), 29.01 (CH_2), 29.11 (CH_2), 29.21 (CH_2), 29.31 (CH_2), 29.36 (CH_2), 29.51 (CH_2), 29.54 (CH_2), 29.56 (CH_2), 31.77 (CH_2), 40.81 N$(CH_3)_2$), 45.10 (NHCH_2CH_2), 93.09 (C-6), 95.65 (C-8), 113.94 (C-10), 123.87 (Ar-C), 123.92 (C-1), 125.44 (C-4), 128.36 (Ar-C), 130.15 (C-3), 130.40 (Ar-C), 131.60 (C-2), 131.83 (C-11), 139.13 (Ar-C), 146.59 (Ar-C), 150.99 (Ar-C), 154.51 (C-9), 158.17 (C-5). IR (KBr 1%, cm^{-1}): ν=3449, 2956, 2923, 2853, 1640, 1590, 1552, 1459, 1428, 1384, 1332, 1292, 1178, 1125, 1009, 905, 818, 774, 715, 666. HRMS (EI): calcd for $C_{32}H_{44}N_3O$ [M^+]: 486.34727; found: 486.34789.

4.3. General method for preparation of compounds 3a–e

To a solution of 1-naphthylamine in methanol or ethanol, 1-bromoalkane was added and the resulting mixture was refluxed for 11–33.5 h, and monitored by TLC (chloroform/*n*-hexane). After evaporation of the solvent and dry chromatography purification on silica gel, the required intermediate (**1a–e**) was obtained.

*4.3.1. N-Propylnaphthalen-1-amine (**3a**).* The product of the reaction of 1-naphthylamine (2.0 g, 1.4×10^{-2} mol) with 1-bromopropane (1.81 mL, 0.020 mol) in ethanol (2 mL) (reflux time 18 h) was chromatographed with chloroform/*n*-hexane, mixtures of increasing polarity, as the eluent, to give compound **3a** as a dark brown oil (1.55 g, 60%). TLC (chloroform/*n*-hexane, 5:2): R_f=0.55. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.09 (t, J=7.8 Hz, 3H, CH_3), 1.80–1.90 (m, 2H, NHCH$_2CH_2$), 3.29 (t, J=7.2 Hz, 2H, NHCH_2CH_2), 6.75 (d, J=7.2 Hz, 1H, 4-H), 7.29 (d, J=8.1 Hz, 1H, 2-H), 7.38 (t, J=7.8 Hz, 1H, 3-H), 7.42–7.50 (m, 2H, 6-H and 7-H), 7.78–7.82 (m, 1H, 8-H), 7.84–7.90 (m, 1H, 5-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=11.75 (CH_3), 22.33 (NHCH$_2CH_2$), 46.65 (NHCH_2CH_2), 105.62 (C-4), 118.03 (C-2), 119.89 (C-5), 123.51 (C-4a), 124.83 (C-7), 125.73 (C-6), 126.52 (C-3), 128.66 (C-8), 134.30 (C-8a), 142.65 (C-1). IR (neat, cm^{-1}): ν=3427, 2958, 2923, 2852, 1624, 1583, 1475, 1463, 1455, 1409, 1379, 1345, 1280, 1142, 1080, 1016, 975, 858, 798, 768, 666. HRMS (EI): calcd for $C_{13}H_{16}N$ [M^+]: 186.12823; found: 186.12773.

*4.3.2. N-Octylnaphthalen-1-amine (**3b**).* The product of the reaction of 1-naphthylamine (1.0 g, 6.98×10^{-3} mol) with 1-bromooctane (1.97 mL, 9.81×10^{-3} mol) in ethanol (2 mL) (reflux time 33 h 30 min) was chromatographed with chloroform/*n*-hexane, 1:1, as the eluent, to give compound **3b** (0.46 g, 51%) as a brown oil. TLC (chloroform/*n*-hexane, 1:2): R_f=0.61. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.97 (t, J=7.2 Hz, 3H, CH_3), 1.30–1.60 (m, 10H, 5×CH_2), 1.78–1.90 (m, 2H, NHCH$_2CH_2$), 3.33 (t, J=7.5 Hz, 2H, NHCH_2CH_2), 6.85 (d, J=7.2 Hz, 1H, 4-H), 7.35 (d, J=8.1 Hz, 1H, 2-H), 7.45 (t, J=8.1 Hz, 1H, 3-H), 7.38–7.56 (m, 2H, 6-H and 7-H), 7.80–7.88 (m, 1H, 8-H), 7.90–7.98 (m, 1H, 5-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=13.99 (CH_3), 22.50 (2×CH_2), 26.86 (CH_2), 27.13 (CH_2), 29.03 (CH_2), 31.62 (CH_2), 49.08 (NHCH_2CH_2), 114.57 (C-4), 120.81 (C-5), 124.75 (C-7), 125.65 (C-2), 126.17 (C-4a), 126.47 (C-3), 126.57 (C-6), 128.73 (C-8), 134.33 (C-8a), 136.21 (C-1). IR (neat, cm^{-1}): ν=3389, 3058, 2956, 2925, 2855, 2469, 1625, 1605, 1582, 1531, 1465, 1410, 1377, 1344, 1282, 1254, 1216, 1173, 1142, 1081, 1016, 953, 861, 800, 770, 724, 666. HRMS (EI): calcd for $C_{18}H_{25}N$ [M^+]: 255.1987; found: 255.1985.

*4.3.3. N-Decylnaphthalen-1-amine (**3c**).* The product of the reaction of 1-naphthylamine (0.50 g, 3.5×10^{-3} mol) with 1-bromodecane (1.11 mL, 5.35×10^{-3} mol), in ethanol (2 mL) (reflux time 22 h 30 min) was chromatographed with chloroform/*n*-hexane, mixtures of increasing polarity, as the eluent, to give compound **3c** as a brown oil (0.87 g, 88%). TLC (chloroform/*n*-hexane, 2:3): R_f=0.46. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.91 (t, J=6.9 Hz, 3H, CH_3), 1.20–1.40 (2×m, 14H, 7×CH_2), 1.80–2.0 (m, 2H, NHCH$_2CH_2$), 3.39 (t, J=7.8 Hz, 2H, NHCH_2CH_2), 7.22 (d, J=7.5 Hz, 1H, 4-H), 7.41 (t, J=8.4 Hz, 1H, 3-H), 7.48–7.60 (m, 3H, 2-H, 6-H and 7-H), 7.85 (dd, J=7.5 and 1.8 Hz, 1H, 8-H), 8.09 (d, J=8.1 Hz, 1H, 5-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=14.06 (CH_3), 22.61 (CH_2), 27.00 (NHCH$_2CH_2$), 27.80 (CH_2), 29.19 (CH_2), 29.21 (2×CH_2), 29.44 (CH_2), 31.81 (CH_2), 47.65 (NHCH_2CH_2), 111.61 (C-4), 120.50 (C-5), 122.29 (C-2), 124.33 (C-4a), 125.94 (C-3), 126.0 (C-7), 126.21 (C-6), 128.69 (C-8), 134.31 (C-8a), 138.33 (C-1). IR (neat, cm^{-1}): ν=3428, 3055, 3010, 2954, 2924, 2853, 1624, 1583, 1527, 1478, 1466, 1409, 1376, 1344, 1281, 1253, 1170, 1141, 1114, 1088, 1018, 861, 843, 800, 783, 767, 722, 699, 666. HRMS (EI): calcd for $C_{20}H_{29}N$ [M^+]: 283.2300; found: 283.2302.

*4.3.4. N-Dodecylnaphthalen-1-amine (**3d**).* The product of the reaction of 1-naphthylamine (2.0 g, 1.40×10^{-4} mol) with 1-bromododecane (3.55 mL, 1.469×10^{-3} mol), in ethanol (3 mL) (reflux time 11 h) was chromatographed with dichloromethane/*n*-hexane, 1:6, as the eluent, to give compound **3d** as a white oily solid (1.92 g, 44%). TLC (dichloromethane/*n*-hexane, 3:4): R_f=0.63. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.92 (t, J=7.2 Hz, 3H, CH_3), 1.20–1.55 (2×m, 18H, 9×CH_2), 1.70–1.90 (m, 2H, NHCH$_2CH_2$), 3.30 (t, J=7.5 Hz, 2H, NHCH_2CH_2), 6.69 (d, J=7.2 Hz, 1H, 4-H), 7.28 (d, J=7.8 Hz, 1H, 2-H), 7.38 (t, J=7.5 Hz, 1H, 3-H), 7.42–7.50 (m, 2H, 6-H and 7-H), 7.78–7.88 (m, 2H, 8-H and 5-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=14.11 (CH_3), 22.68 (CH_2), 27.33 (NHCH$_2CH_2$), 29.28 (CH_2), 29.35 (CH_2), 29.47 (CH_2), 29.61 (2×CH_2), 29.63 (CH_2), 29.66 (CH_2), 31.91 (CH_2), 44.59 (NHCH_2CH_2), 104.89 (C-4), 117.56 (C-2), 119.83 (C-5), 123.41 (C-4a), 124.69 (C-7), 125.69 (C-6) 126.59 (C-3) 128.65 (C-8), 134.30 (C-8a), 143.09 (C-1). IR (Nujol, cm^{-1}): ν=3395, 3054, 3009, 2925, 2853, 1623, 1583, 1523, 1464, 1409, 1378, 1346, 1292, 1280, 1253, 1231, 1214, 1170, 1132, 1119, 1095, 1078, 1038, 1029, 1015, 956, 945, 768, 742, 723, 666. HRMS (EI): calcd for $C_{22}H_{33}N$ [M^+]: 311.2613; found: 311.2611.

*4.3.5. N-Tetradecylnaphthalen-1-amine (**3e**).* The product of the reaction of 1-naphthylamine (1.0 g, 6.98×10^{-3} mol) with 1-bromotetradecane (2.2 mL, 7.33×10^{-3} mol), in ethanol (3 mL) (reflux time 12 h.) was chromatographed with chloroform/methanol, mixtures of increasing polarity, as the eluent, to give compound **3e** as a brown oil (0.790 g, 67%). TLC (chloroform/*n*-hexane, 6:4): R_f=0.68. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.99 (t, J=6.6 Hz, 3H, CH_3), 1.26–1.60 (m, 22H, 11×CH_2), 1.76–1.86 (m, 2H, NHCH$_2CH_2$), 3.32 (t, J=6.9 Hz, 2H, NHCH_2CH_2), 6.68 (d, J=7.2 Hz, 1H, 4-H), 7.31 (d, J=7.8 Hz, 1H, 2-H), 7.43 (t, J=7.8 Hz, 1H, 3-H), 7.46–7.56 (m, 2H, 6-H and 7-H), 7.87 (d, J=8.6 Hz, 2H, 5-H and 8-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C=14.11 (CH_3), 22.69 (CH_2), 27.35 (CH_2), 29.37 (NHCH$_2CH_2$), 29.41 (CH_2), 29.50 (CH_2), 29.62 (CH_2), 29.64 (CH_2), 29.68 (CH_2), 29.69 (2×CH_2), 29.71 (CH_2), 31.93 (CH_2), 44.19

(NH*CH*$_2$CH$_2$), 104.15 (C-4), 117.01 (C-5), 119.74 (C-2), 123.30 (C-4a), 124.48 (C-7), 125.55 (C-6), 126.62 (C-3) 128.60 (C-8), 134.28 (C-8a), 143.56 (C-1). IR (neat, cm^{-1}): ν=3395, 3050, 3009, 2923, 2852, 2730, 1623, 1590, 1583, 1524, 1463, 1408, 1378, 1344, 1283, 1269, 1254, 1222, 1131, 1120, 1098, 1082, 1054, 1039, 1027, 1015, 955, 946, 925, 890, 876, 850, 768. HRMS (EI): calcd for $C_{24}H_{37}N$ [M$^+$]: 339.2926; found: 339.2923.

Acknowledgements

Thanks are due to *Fundação para a Ciência e Tecnologia* (Portugal) for its financial support of a BPD to Sarala Naik (SFRH/BPD/37840/2007) and *Centro de Química* and *Centro de Física* (*Universidade do Minho*). The NMR spectrometer Bruker Avance II 400 is part of the National NMR Network and was acquired with funds from FCT and FEDER.

References and notes

1. Richards, A. D.; Rodgers, A. *Chem. Soc. Rev.* **2007**, *36*, 471–483.
2. Lippard, S. J. *Acc. Chem. Rev.* **1978**, *11*, 211–217.
3. Johnston, D. H.; Glasgow, K. C.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 8933–8938.
4. Blackburn, G. M.; Gait, M. J. *Nucleic Acids in Chemistry and Biology*; IRL: New York, NY, 1990.
5. Chen, Q.; Li, D.; Yang, H.; Zhu, Q.; Xu, J.; Zhao, Y. *Analyst* **1999**, *124*, 901–907.
6. Lakowicz, J. R.; Piszczek, G.; Kang, J. S. *Anal. Biochem.* **2001**, *288*, 62–75.
7. Ju, H.; Ye, Y.; Zhu, Y. *Electrochim. Acta* **2005**, *50*, 1361–1367.
8. Gorodetsky, A. A.; Ebrahim, A.; Barton, J. K. *J. Am. Chem. Soc.* **2008**, *130*, 2924–2925.
9. Gorodetsky, A. A.; Hammond, W. J.; Hill, M. G.; Slowinski, K.; Barton, J. K. *Langmuir* **2008**, *24*, 14282–14288.
10. Chen, Z.-W.; Balamurugan, A.; Chen, S.-M. *Bioelectrochemistry* **2009**, *75*, 13–18.
11. Mitra, R. K.; Sinha, S. S.; Pal, S. K. *J. Fluoresc.* **2008**, *18*, 423–432.
12. Yang, X.-L.; Wang, A. H.-J. *Pharmacol. Ther.* **1999**, *83*, 181–215.
13. Mahadevan, S.; Palaniandavar, M. *Inorg. Chim. Acta* **1997**, *254*, 291–302.
14. Frade, V. H. J.; Gonçalves, M. S. T.; Moura, J. C. V. P. *Tetrahedron Lett.* **2005**, *46*, 4949–4952.
15. Frade, V. H. J.; Gonçalves, M. S. T.; Moura, J. C. V. P. *Tetrahedron Lett.* **2006**, *47*, 8567–8570.
16. Frade, V. H. J.; Gonçalves, M. S. T.; Coutinho, P. J. G.; Moura, J. C. V. P. *J. Photochem. Photobiol., A* **2007**, *185*, 220–230.
17. Frade, V. H. J.; Coutinho, P. J. G.; Moura, J. C. V. P.; Gonçalves, M. S. T. *Tetrahedron* **2007**, *63*, 1654–1663.
18. Frade, V. H. J.; Barros, S. A.; Moura, J. C. V. P.; Gonçalves, M. S. T. *Tetrahedron Lett.* **2007**, *48*, 3403–3407.
19. Frade, V. H. J.; Barros, S. A.; Moura, J. C. V. P.; Coutinho, P. J. G.; Gonçalves, M. S. T. *Tetrahedron* **2007**, *63*, 12405–12418.
20. Frade, V. H. J.; Sousa, M. J.; Moura, J. C. V. P.; Gonçalves, M. S. T. *Tetrahedron Lett.* **2007**, *48*, 8347–8352.
21. Alves, C. M. A.; Naik, S.; Coutinho, P. J. G.; Gonçalves, M. S. T. *Tetrahedron Lett.* **2009**, *50*, 4470–4474.
22. Crossley, M. L.; Turner, R. J.; Hofmann, C. M.; Dreisbach, P. F.; Parker, R. P. *J. Am. Chem. Soc.* **1952**, *74*, 578–584.
23. Streitwieser, A., Jr.; Heathcook, C. H. *Introduction to Organic Chemistry*, 2nd ed.; Macmillan: New York, NY, 1981; p 238.
24. Sens, R.; Drexhage, K. H. *J. Lumin.* **1981**, *24*, 709–712.
25. Wolfbeis, O. S. *Fluorescence Spectroscopy in Biology—Advanced Methods and their Applications to Membranes, Proteins, DNA and Cells*, 1st ed.; Springer: Berlin, 2005; p 305.
26. Cao, Y.; He, X.-W. *Spectrochim. Acta A* **1998**, *54*, 883–892.
27. Renault, E.; Fontaine-Aupart, M. P.; Tfibel, F.; Gardes-Albert, M.; Bisagni, E. *J. Photochem. Photobiol., B: Biol.* **1997**, *40*, 218–227.