Microwave synthesis of novel water-soluble 2-, 5- and 9-substituted benzo[a]phenoxazinium chlorides in comparison with conventional heating


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Abstract: Microwave irradiation was used for the first time in an efficient synthesis of benzo[a]phenoxazinium chlorides. The main advantage of this protocol is the notable reduction in reaction times and good to excellent yields of the products were achieved in comparison with classical heating conditions as described. These new series of compounds possess 5-amine and/or 2-hydroxyl substituents in the polycyclic system to improve their solubility in aqueous media, in addition to the functional groups as terminals in the side chains, allowing their further use in covalent labeling. Fundamental photophysical studies carried out in ethanol, physiological pH and water revealed that all cationic fluorophores absorbed and emitted in the range of 610-628 nm and 630-652 nm, respectively, with relative fluorescent quantum yields ranging from 0.16 to 0.96.

Introduction

Synthetic fluorescent probes still remain dynamic and growing tools in various fields of modern medicine and science, with applications from spectroscopy to microscopy and imaging, to single molecule detection, to proteomics and genomics. In this context, heterocycles containing oxazine skeleton are of considerable interest due to their impressive use as biological stains,[1,2] laser dyes,[3] indicators in protein labelling,[4] in environmental analysis[5] and as chromophoric compounds in host-guest chemistry.[6] Among the family of oxazine dyes, benzo[a]phenoxazinium salts are attractive fluorophores due to their photostable nature, and favourable photophysical properties in the near infrared spectral region (emission above 600 nm). These compounds can function as covalent probes for organic and biological molecules, namely amino acids,[7] proteins,[8] peptides and DNA,[9] as well as in the non-covalent labelling of nucleic acids in monitoring protein conformation alterations or for therapeutic purposes.[10,11] Moreover, several compounds are
potential photosensitizers with cancer and broad antimicrobial photodynamic activities,\textsuperscript{[12,13]} as well as antimicrobial and antimalarial agents.\textsuperscript{[14-16]}

The synthesis of benzo[a]phenoxazinium salts usually involves the condensation reaction of nitrosoanilines or nitrosonaphthylamines with 1-naphthylamines, 3-aminophenols or 2-naphthol. In the last case, monoaminosubstituted benzo[a]phenoxazinium salts need to be converted into the diaminosubstituted compounds by reaction with amines in the presence of an oxidation agent. Furthermore, para-phenylenediamines can also be used in condensation with 4-amino-substituted 1,2-naphthoquinones. The cyclization occurred in the presence of a strong mineral acid, such as perchloric or hydrochloric acids, using \(N,N\)-dimethylformamide, methanol or ethanol as a solvent, under reflux conditions. The use of acetic acid is also reported, including in the original synthesis of Nile Blue, the most well-known benzo[a]phenoxazinium salt.\textsuperscript{[17-20]} However, these condensations can be performed in the absence of acid, in \(N,N\)-dimethylformamide at 90 °C.\textsuperscript{[21]} Reaction times varied from a few minutes to several hours, resulting in the desired polycyclic heterocycles ranging from low to very good yields.

Microwave energy has rapidly become important for heating and driving organic chemical reactions, since it can be associated to a number of advantages over conventional heating techniques, such as the dramatic reduction of processing times, the improvement of reaction yields and the enhancement of product purities.\textsuperscript{[22-26]}

In conjunction with our previous interests in the synthesis and application of oxygen and nitrogen polycyclic heterocycles, namely benzo[a]phenoxazinium salts, as well as the research in alternative green procedures,\textsuperscript{[27-32]} the present work describes for the first time the efficient synthesis of benzo[a]phenoxazinium chlorides by condensation reactions under microwave irradiation, in comparison to conventional heating conditions. These new compounds possess a combination of substituents at the tetracyclic ring that includes the hydroxyl, aminopropoxyl, as well as amine groups, and monoalkylated amines. Fundamental photophysical characterisation of the synthesised cationic fluorophores in ethanol, water and simulated physiological conditions were carried out.

**Results and Discussion**

**Synthesis:** In view of the potential application of new benzo[a]phenoxazinium chlorides as probes of biological relevant molecules, 5-amine and/or 2-hydroxyl substituents were included in the polycyclic system to improve solubility in aqueous media, namely in physiological conditions. Furthermore, the presence of functional groups as terminals in the side chains allows for their use in
covalent labeling, in addition to their intrinsic ability to act as non-covalent labels due to the cationic character of these fluorochromophores.

Considering these facts, the synthetic approach begins with precursors 2 and 4a,b, which were obtained by the alkylation of 5-aminonaphthalen-2-ol 1 in the amine or hydroxyl functions with the appropriate bromoderivative under heating in the absence or presence of a base, respectively. 5-Aminonaphthalen-2-ol 1 reacted with 1-bromopropane in methanol at reflux to give the N-monosubstituted, 5-(propylamino)naphthalen-2-ol 2, and simultaneously the N-disubstituted derivative, 5-(dipropylamino) naphthalen-2-ol 3 (Scheme 1). The reaction of 5-aminonaphthalen-2-ol 1 with 1-bromopropane in DMF by heating at 75 °C, using potassium carbonate as a base resulted in the alkylation of the hydroxyl group, giving 6-propoxynaphthalen-1-amine 4a. On the other hand, the use of ethyl 4-bromobutanoate, in acetonitrile with heating at 60 °C in the presence of cesium carbonate originated ethyl 4-((5-aminonaphthalen-2-yl)oxy)butanoate 4b, in a better yield and a reaction time lower than that required in the synthesis of the 4a (2.5 h, 4b, 15.3 h, 4a). Thus, the latter conditions were chosen in the subsequent derivatisation of the hydroxyl group. The 4-((5-aminonaphthalen-2-yl)oxy)butanoic acid 4c was obtained by the hydrolysis of the ethyl ester group of intermediate 4b with 1 M sodium hydroxide in 1,4-dioxane, at a low temperature (0 °C). Starting from 5-(propylamino) naphthalen-2-ol 2 and using 3-bromopropan-1-amine in acetonitrile, heated at 60 °C with cesium carbonate, 6-(3-aminopropoxy)-N-propynaphthalen-1-amine 5 was obtained. After purification by silica gel column chromatography (except for 4c, isolated by solvent extraction from the reaction mixture), intermediates 2-5 were obtained as solids (2, 4a-c), an oily solid (5) or an oil (3), in moderate to high yields, and were fully characterised by high resolution mass spectrometry, IR and NMR (1H and 13C) spectroscopy.

The IR spectra of these compounds showed the expected bands due to the stretching vibrations of the amine and hydroxyl functions (3457-3100 cm\(^{-1}\)), the carbonyl group of the ester (4b) (1733 cm\(^{-1}\)) and carboxylic acid (4c) (1711 cm\(^{-1}\)). The 1H NMR spectra measured in deuterated dimethyl sulfoxide (DMSO-d\(_6\)) showed, in addition to aromatic and aliphatic protons, signals of the hydroxyl group directly attached to the aromatic ring as singlets (2, δ 9.49 ppm; 3, δ 9.64 ppm), as well as from the carboxylic acid (4c) as a broad singlet (δ 12.13 ppm). In intermediates monoalkylated in the amine group (2, 5), the NH proton was visible in the form of a broad singlet (2, δ 5.92 ppm) or a triplet (5, δ 6.06 ppm). Compounds 4a-c with a free amine group directly linked to the aromatic ring showed the corresponding protons in the form of singlets or a broad singlet (4c) (δ ~5.63 ppm); in 5 with NH\(_2\) as the terminal of the substituent linked to the naphthalene ring by the oxygen atom, the corresponding signal appeared in the form of a broad singlet (δ 7.80 ppm). In the 13C NMR spectra we highlighted the signals of the carbonyl group from the ester (4b, δ 172.58 ppm), and the carboxylic acid (4c, δ 174.15 ppm).

Next, the other required precursor, nitrosophenol 6, was obtained by the usual procedure involving treatment of the 3-(ethylamino)-4-methylphenol with sodium nitrite in the presence of hydrochloric acid.\[7,14,19,20,27-31,33]\n
The target compounds, benzo[a]phenoxazinium chlorides 7a-f were synthesized by refluxing of the precursor 6 with compound 1, 2, 4a-c or 5, in ethanol or heating in dimethylformamide (4c), in the presence and absence of acid, using an oil bath or under microwave irradiation, respectively (Scheme 2). The benzo[a]phenoxazinium chlorides 7a-f were obtained in low to excellent yields (34-96%) using the conventional heating method in 9 to 280 minutes (4.7 h) (Table 1).
Scheme 2. Synthesis of benzo[a]phenoxazinium chlorides 7a-f.

Table 1. Synthesis of benzo[a]phenoxazinium chlorides 7a-f by conventional heating method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>Temp. (ºC)</th>
<th>Time (min)</th>
<th>Yield (%)[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>Ethanol</td>
<td>65</td>
<td>9</td>
<td>96</td>
</tr>
<tr>
<td>7b</td>
<td>Ethanol</td>
<td>65</td>
<td>280</td>
<td>89</td>
</tr>
<tr>
<td>7c</td>
<td>Ethanol</td>
<td>65</td>
<td>50</td>
<td>77</td>
</tr>
<tr>
<td>7d</td>
<td>Ethanol</td>
<td>65</td>
<td>155</td>
<td>34</td>
</tr>
<tr>
<td>7e</td>
<td>DMF</td>
<td>70</td>
<td>60</td>
<td>66</td>
</tr>
<tr>
<td>7f</td>
<td>Ethanol</td>
<td>65</td>
<td>120</td>
<td>60</td>
</tr>
</tbody>
</table>

[a] Isolated yields

As a preliminary approach to study the effect of microwave irradiation conditions, the reaction of compounds 6 and 1 was carried out in silica gel as the solid support. The choice of silica was related to the fact that its acidic nature surface could act as a catalyst for the reaction. In addition, the crude reaction mixture obtained could be used directly for the subsequent column chromatography purification. However, the reaction did not occur and instead the starting materials were decomposed in the microwave conditions. Then the condensation reaction was tested with different solvents such as acetonitrile, dimethylformamide, ethanol and water, in the absence of acid. It was observed that in ACN, reaction did not occur at 90 ºC, and further upon increasing the temperature to 120 ºC during a long period (40 min) did not result in the completion of the reaction.

In DMF, the reaction was sluggish without any significant improvement in the yield of the required product. Under the same reaction conditions, ethanol was used as the solvent, the resulting
benzo[a]phenoxazine 7a was obtained in excellent yield with reduction in the reaction time (3 min). In water, it was found that condensation reaction did not occur in the tested conditions. Hence, from the above studies we concluded that ethanol could be an appropriate solvent for the synthesis of benzo[a]phenoxazines under the microwave reaction conditions (Scheme 3, Table 2).

Scheme 3. Optimization reaction studies of nitrosophenol 6 with 5-aminonaphthalen-2-ol 1 under microwave conditions.

**Table 2. Optimization reaction studies of nitrosophenol 6 with 5-aminonaphthalen-2-ol 1 under microwave conditions.**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>Yield (%)^[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent free (silica support)</td>
<td>90</td>
<td>10</td>
<td>NR</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>90/120</td>
<td>8/40</td>
<td>59</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>90</td>
<td>5</td>
<td>41</td>
</tr>
<tr>
<td>Ethanol</td>
<td>90</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>Ethanol</td>
<td>90</td>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>water</td>
<td>90</td>
<td>40</td>
<td>NR</td>
</tr>
</tbody>
</table>

^[a] Isolated yields; NR refers to no reaction

The condensation reaction of nitrosophenol 6 with compounds 2, 4 and 5 under microwave irradiation in ethanol or DMF (4c) afforded the corresponding benzo[a]phenoazinium chlorides (except 7e) in good to excellent yields (75-97%, Table 3). It was found that this synthetic methodology resulted in an increase of the yield for compounds 7a-f (excluding 7e), with a time reduction of 9 to 140 times when compared to conventional heating in the same solvent, but in the presence concentrated hydrochloride acid.
**Table 3.** Synthesis of benzo[a]phenoxazinium chlorides 7a-f by microwave irradiations at 90 °C.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>Time (min)</th>
<th>Yield (%)[^a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>Ethanol</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>7b</td>
<td>Ethanol</td>
<td>1.3</td>
<td>93</td>
</tr>
<tr>
<td>7c</td>
<td>Ethanol</td>
<td>1.3</td>
<td>90</td>
</tr>
<tr>
<td>7d</td>
<td>Ethanol</td>
<td>2</td>
<td>86</td>
</tr>
<tr>
<td>7e</td>
<td>DMF</td>
<td>--</td>
<td>NR</td>
</tr>
<tr>
<td>7f</td>
<td>Ethanol</td>
<td>2.5</td>
<td>75</td>
</tr>
</tbody>
</table>

[^a] Isolated yields; NR refers to no reaction

After silica gel column chromatography purification, compounds 7a-f were obtained as blue solids and were fully characterised by the usual analytical techniques.

The IR spectra of these benzo[a]phenoxazinium chlorides showed the expected bands due to the stretching vibrations of the amine and hydroxyl functions (3413-3214 cm⁻¹), the carbonyl group of the ester (1731 cm⁻¹, 7d) and the carboxylic acid (1723 cm⁻¹, 7e).

The ¹H NMR spectra measured in deuterated methanol showed the signals of aliphatic protons from the methylenic groups of substituents of positions 5 and 9, directly linked to the nitrogen atom NHCH₂ that appeared as a broad singlet (7b), triplet (7f) or multiplet (7e) (δ 3.50 to 3.70 ppm), as well as groups closed to the same atom, NHCH₂CH₂ (7b, 7f), which appeared as multiplets (δ 1.80 to 2.0 ppm). This also occurred in the case of the methylenic protons linked to the oxygen atom (7c-f), namely OCH₂ that appeared as broad singlets (7c-e), a triplet (7f) or multiplet (7d of the ester group) (δ 3.65-4.44 ppm), and groups closed to the same atom, OCH₂CH₂, which appeared as broad singlets (7c-e) or multiplets (7f) (δ 1.82 to 2.34 ppm) and OCH₂CH₂CH₂ (7d-f) in the form of multiplets (7d), a broad singlet (7e) or triplet (7f) (δ 2.0 to 3.27 ppm). The methyl protons of oxypropyl (7e), propylamine (7b, 7f) and ethylamine groups in positions 2, 5 and 9 of the heterocycle and ethoxyl of the terminal ester of the substituent in position 2 (7d), appeared as broad singlets, triplets and multiplets (δ 1.09 to 1.50 ppm). There was also the presence of protons of the methyl group directly linked to the aromatic ring at position 10, which appeared as a broad singlet (7b) or singlets (δ 2.21 to 2.38 ppm). In addition, spectra showed the expected aromatic protons, in particular H-8 as singlets, a broad singlet (7b), or multiplet (7e) (δ 6.57 to 6.91 ppm); H-6 as singlets, a broad singlet (7b) or a multiplet (7e) (δ 6.62 to 6.95 ppm); and protons H-11 which appeared in the form of singlets and a broad singlet (7c, 7e) (δ 7.40 to 7.74 ppm).

The ¹³C NMR spectra of benzo[a]phenoxazinium chlorides 7a-f measured in deuterated methanol, showed the signals of the methylenic groups of the substituents of positions 5 and 9, directly linked to the nitrogen atom NHCH₂ (δ ~47.20 or 36.61 ppm, 7e), and groups closed to the
same atom, NHCH$_2$CH$_2$ (7b, 7f) ($\delta \sim 23.2$ ppm). In addition, this also occurred in the methylenic carbons linked to the oxygen atom (7e-f), namely OCH$_2$ ($\delta$ 44.80-71.35 ppm), groups closed to the same atom, OCH$_2$CH$_2$ ($\delta$ 23.18-28.34 ppm), and OCH$_2$CH$_2$CH$_2$ (7d-f) ($\delta$ 25.68-38.47 ppm). The methyl carbons of oxypropyl (7c), propylamine (7b, 7f), ethylamine, and ethoxyl (7d) groups in positions 2, 5 and 9 of the heterocycle and terminal ester of substituent in position 2, were visible ($\delta$ 10.81-14.59 ppm). Furthermore, carbons of the methyl groups directly linked to the aromatic ring at position 10 ($\delta$ 17.47-17.90 ppm) were observed. In addition, spectra showed the expected aromatic protons, in particular C-8 ($\delta$ 93.13-96.33 ppm), C-6 ($\delta$ 92.94-95.33 ppm) and C-11 ($\delta$ 130.86-132.68 ppm). In the case of compounds 7d and 7e we highlighted signals from the carbonyl group of the ester ($\delta$ 174.87 ppm, 7d) and carboxylic acid ($\delta$ 164.0 ppm, 7e).

**Photophysical studies:** In view of the potential application of benzo[a]phenoxazinium chlorides 7a-f as long-wavelength fluorescent probes, namely for biological purposes, fundamental photophysical studies were carried out in ethanol, and aqueous solutions at pH 7.4 (adjusted with a solution of 0.1 M sodium phosphate and a mixed solution of 0.2 M boric acid and 0.05 M citric acid) to simulate physiological conditions, as well as in water.

The longest wavelength of maximum absorption ($\lambda_{abs}$) of all the compounds in the three solvents was located in the region 610-628 nm (Table 4). Despite the similarity in these values, by comparison the $\lambda_{abs}$ of each compound individually in ethanol and both aqueous solutions, a hypsochromic shift (2 to 13 nm; except in 7c at pH 7.4, 7a and 7c in water) was observed in the latter solutions.

In ethanol, by comparing the wavelength of maximum absorption of compounds 7a-d and 7f, a bathochromic shift of 13 nm in 7a and 7c ($\lambda_{abs}$ 615 nm) for 7d ($\lambda_{abs}$ 628 nm) was highlighted. For compounds with propylamine at position 5 (7b, 7f), there was a bathochromic shift of 5 nm from 7b ($\lambda_{abs}$ 623 nm) to 7f ($\lambda_{abs}$ 628 nm). Comparing data of 7a-f, at physiological pH, we highlighted the red shift of 13 nm registered in the $\lambda_{abs}$ by 7f (623 nm) with respect to 7b (610 nm). The remaining compounds (7a, 7c-e) showed equal or very similar values (Figure 1).

In water, a bathochromic shift of 7 nm was observed from 7a, 7c ($\lambda_{abs}$ 617 nm) to 7d ($\lambda_{abs}$ 622 nm), and a hypsochromic shift of 3 nm to 7e. Compounds 7b and 7f displayed closer $\lambda_{abs}$ values (619 nm, 7b; $\lambda_{abs}$ 622 nm, 7f).

The oxazine dyes are known to form H-aggregates in water. These type of dimers possess an absorption band to the blue of the monomer and have a very low fluorescence quantum yield. Given the absorption spectra of compounds 7a-f in ethanol, there was found to be a slight tendency for
aggregation in the concentration studied, which is probably due to a small percentage of water in the solvent (Figure 1).

Table 4. Absorption data of benzo[a]phenoxazinium chlorides 7a-f in ethanol, at pH 7.4 and in water.

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Ethanol</th>
<th>pH 7.4</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{\text{abs}}$ (nm)</td>
<td>$\varepsilon$ (M$^{-1}$cm$^{-1}$)</td>
<td>$\lambda_{\text{abs}}$ (nm)</td>
</tr>
<tr>
<td>7a</td>
<td>615</td>
<td>32000</td>
<td>613</td>
</tr>
<tr>
<td>7b</td>
<td>623</td>
<td>38132</td>
<td>610</td>
</tr>
<tr>
<td>7c</td>
<td>615</td>
<td>52222</td>
<td>615</td>
</tr>
<tr>
<td>7d</td>
<td>628</td>
<td>34000</td>
<td>615</td>
</tr>
<tr>
<td>7e</td>
<td>619</td>
<td>19333</td>
<td>617</td>
</tr>
<tr>
<td>7f</td>
<td>628</td>
<td>24667</td>
<td>623</td>
</tr>
</tbody>
</table>

Figure 1. Normalised absorbance spectra of benzo[a]phenoxazinium chlorides 7a, 7d in ethanol, and 7b, 7f at pH 7.4 (7a, 7d, in ethanol, $C = 3.0 \times 10^{-6}$ M; 7b, 7f at pH 7.4, $C = 6.0 \times 10^{-6}$ M).

Data at pH 7.4 and water showed that all compounds (with the exception of 7f in water) formed dimers (Figures 1 and 2). Considering the behavior at pH 7.4 it was found that in compounds 7a, 7c, 7e and 7f the maximum absorption at the longest wavelength was higher than at the shorter wavelength. This fact suggests that the monomeric form predominates over the dimeric form in aqueous solutions. The aggregation in compound 7f is less pronounced than in 7a, 7c and 7e, which behaved in a similar way. In the case of compounds 7b and 7d the dimeric form dominates.
In water, compound 7f was the one less prone to aggregation, and compounds 7a, 7c, 7e showed a more reduced tendency than 7b and 7d (Figure 2).

Figure 2. Normalized absorbance spectra of benzo[a]phenoxazinium chlorides 7a, 7c and 7f in water (7a, C = 6.0×10^{-6} M; 7c, 7f, C = 4.5×10^{-6} M).

Table 5. Fluorescence data of benzo[a]phenoxazinium chlorides 7a-f in ethanol, at pH 7.4 and in water.

<table>
<thead>
<tr>
<th>Compd</th>
<th>Ethanol</th>
<th>pH 7.4</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ_{em}^{[a]}</td>
<td>Φ_{F}</td>
<td>Δλ^{[a]}</td>
</tr>
<tr>
<td>7a</td>
<td>636</td>
<td>0.72</td>
<td>21</td>
</tr>
<tr>
<td>7b</td>
<td>642</td>
<td>0.81</td>
<td>19</td>
</tr>
<tr>
<td>7c</td>
<td>637</td>
<td>0.96</td>
<td>22</td>
</tr>
<tr>
<td>7d</td>
<td>643</td>
<td>0.66</td>
<td>15</td>
</tr>
<tr>
<td>7e</td>
<td>635</td>
<td>0.95</td>
<td>16</td>
</tr>
<tr>
<td>7f</td>
<td>642</td>
<td>0.79</td>
<td>14</td>
</tr>
</tbody>
</table>

[a] In nm

Studies of the fluorescent properties of benzo[a]phenoxazinium chlorides 7a-f were also carried out in ethanol, aqueous solutions in physiological conditions (pH 7.4) and water. Maximum emission wavelengths (λ_{em}) and relative fluorescence quantum yields (Φ_{F}) were obtained and are summarised in Table 5. For the determination of quantum yields, Oxazine 1, used as a standard (Φ_{F} = 0.11 in ethanol), was excited at 590 nm for all compounds. In the three solvents, benzo[a]phenoxazinium salts 7a-f displayed maximum emission wavelengths in the region of 630-652 nm, with low to moderate Stokes’ shifts (14-37 nm), and relative fluorescence quantum yields between 0.16 and 0.96.
The comparison of the values of $\lambda_{\text{em}}$ in water and ethanol revealed a bathochromic shift in water (except for 7c) between 6 and 9 nm. On the other hand, when comparing $\lambda_{\text{em}}$ in aqueous solutions, it was found that 7a-c displayed a bathochromic shift (1-6 nm) in water, while the remaining compounds practically did not change. In ethanol, and when comparing the $\lambda_{\text{em}}$ of compound 7a (636 nm) with 7b, 7d and 7f (642 nm), a bathochromic shift of 6 nm was observed in all cases. At physiological pH, there was a bathochromic shift from 7a ($\lambda_{\text{em}}$ 641 nm) to 7b-f (except in 7c, which displays a hypsochromic shift of 11 nm, $\lambda_{\text{em}}$ 630 nm), the highest value being that of 11 nm recorded for 7d ($\lambda_{\text{em}}$ 652 nm).

In water, the behavior was similar to the other solvents, especially the bathochromic shift of 10 nm in compound 7d ($\lambda_{\text{em}}$ 652 nm) when compared to 7a ($\lambda_{\text{em}}$ 642 nm); compound 7c displayed a hypsochromic shift of 6 nm ($\lambda_{\text{em}}$ 636 nm) (Figure 3).

![Figure 3. Normalised fluorescence spectra of benzo[a]phenoxazinium chlorides 7c in ethanol and pH 7.4, and 7d in ethanol and water (7c in ethanol, C = 1.8×10^{-6} M; 7c at pH 7.4, C = 6.0×10^{-6} M; 7d in ethanol C = 3.0×10^{-6} M; 7d in water C = 6.0×10^{-6} M).](image)

**Conclusions**

For the first time, it was demonstrated that microwave-assisted procedure was a more convenient method (good to excellent yields in short reaction times) for the preparation of benzo[a]phenoxazinium chlorides by the condensation of nitrosophenol with 5-aminonaphthalen-2-ol, its N- or/and O-alkylated derivatives using ethanol as solvent in the absence of acid.

These new water-soluble compounds emitted between 630 and 652 nm with moderate to excellent relative fluorescence quantum yields in ethanol, at simulated physiological conditions and in water. The presence of functional groups as terminals in the side chains allows their further use in covalent labeling, in addition to their intrinsic ability to act as non-covalent probes due to the cationic
character of these fluorochromophores. Overall, the new series of efficiently synthesised benzo[a]phenoxazinium chlorides possesses the potential of constituting useful probes for organic and biologically relevant molecules.

**Experimental Section**

**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 60F254) and spots were visualised 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. UV-Vis-NIR absorption spectra (200 – 700 nm) were obtained using a Shimadzu UV/2501PC 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 ºC. All chemical shifts are given in ppm and $J$ values are given in Hz. Assignments were carried out through the 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 the University of Vigo, Spain. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. Microwave experiments were carried out in a CEM Focused Microwave Synthesis System Discover S-Class equipment with an infrared temperature sensor positioned below the reaction vessel, using IntelliVent microwave reactor tubes of 10 mL. Commercially available reagents were used as received.

**Preparation of intermediates 2-5**

**5-(Propylamino)naphthalen-2-ol (2):** To a solution of 5-aminonaphthalen-2-ol 1 (0.30 g, 1.89 mmol) in methanol (2 mL), 1-bromopropane (0.182 mL, 1.98 mmol) was added and the resulting mixture was refluxed for 9 h. Then, additional 1-bromopropane (0.182 mL, 1.98 mmol) was added and the reaction mixture was further refluxed for 25 h and monitored by TLC (ethyl acetate/light petroleum 1:3). After evaporation of the solvent and column chromatography purification on silica gel, using ethyl acetate/light petroleum 1:3 as the eluent, compound 2 was obtained as a grey solid (0.124 g, 33 %); $R_f = 0.47$ (ethyl acetate/light petroleum 1:3); m.p. 88.9-91.3°C; $^1$H NMR (300 MHz, [D$_6$]DMSO): $\delta = 0.96$ (t, $J = 7.5$ Hz, 3H; NHCH$_2$CH$_2$CH$_3$), 1.60-1.73 (m, 2H; NHCH$_2$CH$_2$CH$_3$), 3.12-3.15 (m, 2H; NHCH$_2$CH$_2$CH$_3$), 5.92 (broad s, 1H; NH), 6.25 (d, $J = 7.8$ Hz, 1H; H-6), 6.83 (d, $J = 8.1$ Hz, 1H; H-8), 6.89 (dd, $J = 9.2$ and 2.7 Hz, 1H; H-3), 6.94 (d, $J = 2.4$ Hz,
1H; H-1), 7.13 (t, J = 8.1 Hz, 1H; H-7), 7.99 (d, J = 9.0 Hz, 1H; H-4), 9.49 ppm (s, 1H; OH); $^{13}$C NMR (75.4 MHz, [D$_6$]DMSO): $\delta$ = 11.83 (NHCH$_2$CH$_2$CH$_3$), 21.49 (NHCH$_2$CH$_2$CH$_3$), 45.00 (NHCH$_2$CH$_2$CH$_3$), 100.28 (C-6), 109.18 (C-1), 113.79 (C-8), 115.79 (C-3), 117.51 (C-4a), 123.34 (C-4), 127.23 (C-7), 135.83 (C-8a), 144.38 (C-5), 154.97 ppm (C-2); IR (KBr): $\nu$ = 3308, 3100, 2963, 2936, 2875, 1636, 1584, 1513, 1454, 1424, 1384, 1350, 1277, 1224, 1152, 1125, 1078, 1004, 956, 907, 878, 855, 814, 783, 765, 750, 711, 677, 635, 609 cm$^{-1}$. HRMS (TOF EI): $m/z$ calcd for C$_{13}$H$_{15}$NO: 201.1154 [M$^+$]; found: 201.1160.

5-(Dipropylamino)naphthalen-2-ol (3): In the above reaction, along with 2, compound 3 was also isolated as brown oil (0.012 g, 3%); $R_f = 0.67$ ethyl acetate/light petroleum 1:3; $^1$H NMR (400 MHz, [D$_6$]DMSO): $\delta$ = 0.79 (t, J = 7.6 Hz, 6H; N(CH$_2$CH$_2$CH$_3$)$_2$), 1.30-1.50 (m, 4H; N(CH$_2$CH$_2$CH$_3$)$_2$), 3.00 (broad s, 4H; N(CH$_2$CH$_2$CH$_3$)$_2$), 6.90-7.10 (m, 3H; H-6, H-3 and H-1), 7.20-7.40 (m, 2H; H-7 and H-8), 8.07 (d, J = 8.0 Hz, 1H; H-4), 9.64 ppm (s, 1H; OH); $^{13}$C NMR (75.4 MHz, [D$_6$]DMSO): $\delta$ = 11.61 (N(CH$_2$CH$_2$CH$_3$)$_2$), 19.84 (N(CH$_2$CH$_2$CH$_3$)$_2$), 55.63 (N(CH$_2$CH$_2$CH$_3$)$_2$), 109.05 (C-1), 114.96 (C-6), 117.67 (C-3), 121.51 (C-8), 125.28 (C-4), 126.03 (C-7), 130.07 (C-4a), 136.12 (C-8a), 148.06 (C-5), 155.19 ppm (C-2). IR (KBr): $\nu$ = 3354, 3049, 2960, 2930, 2872, 1625, 1580, 1512, 1465, 1433, 1398, 1380, 1344, 1260, 1213, 1151, 1081, 1051, 961, 936, 854, 830, 789, 750, 664 cm$^{-1}$. HRMS (TOF EI): $m/z$ calcd for C$_{16}$H$_{21}$NO: 243.1623 [M$^+$]; found: 243.1627.

6-Propoxynaphthalen-1-amine (4a): To a solution of 5-aminonaphthalen-2-ol 1 (0.06 g, 0.37 mmol) in DMF (2 mL), potassium carbonate (0.246 g, 1.78 mmol) and 1-bromopropane (0.036 mL, 0.39 mmol) were added. The resulting mixture was heated at 75 °C for 15 h and 20 min and monitored by TLC (CH$_2$Cl$_2$/MeOH 9:1). After evaporation of the solvent and column chromatography purification on silica gel, using CH$_2$Cl$_2$/MeOH 9:1 as the eluent, compound 4a was obtained as a grey solid (0.049 g, 65%); $R_f = 0.47$ ethyl acetate/light petroleum 1:3; m.p. 85.0-87.2 °C; $^1$H NMR (300 MHz, [D$_6$]DMSO): $\delta$ = 1.00 (t, J = 7.2 Hz, 3H; OCH$_2$CH$_2$CH$_3$), 1.68-1.84 (m, 2H; OCH$_2$CH$_2$CH$_3$), 3.99 (t, J = 6.9 Hz, 2H; OCH$_2$CH$_2$CH$_3$), 5.63 (s, 2H; NH$_2$), 6.49 (d, J = 7.5 Hz, 1H; H-2), 6.90-7.00 (m, 2H; H-4 and H-7), 7.03-7.07 (m, 2H; H-5 and H-3), 7.94 ppm (d, J = 9.3 Hz, 1H; H-8); $^{13}$C NMR (75.4 MHz, [D$_6$]DMSO): $\delta$ = 10.49 (OCH$_2$CH$_2$CH$_3$), 22.09 (OCH$_2$CH$_2$CH$_3$), 68.80 (OCH$_2$CH$_2$CH$_3$), 105.74 (C-2), 106.80 (C-5), 114.59 (C-4), 115.94 (C-7), 117.94 (C-8a), 124.02 (C-8), 127.31 (C-3), 135.67 (C-4a), 144.70 (C-1), 156.33 ppm (C-6); IR (KBr): $\nu$ = 3413, 3332, 3228, 3050, 2965, 2935, 2872, 1625, 1586, 1514, 1466, 1450, 1380, 1288, 1270, 1227, 1183, 1145, 1128, 1085, 1066, 1048, 1013, 987, 956, 848, 840, 817, 779, 722, 665 cm$^{-1}$. HRMS (EI): $m/z$ calcd for C$_{13}$H$_{15}$NO: 201.1154 [M$^+$]; found: 201.1161.
**Ethyl 4-((5-aminonaphthalen-2-yl)oxy)butanoate (4b):** To a solution of 5-aminonaphthalen-2-ol 1 (0.060 g, 0.377 mmol) in acetonitrile (2 mL), cesium carbonate (0.601 g, 1.84 mmol) and ethyl 4-bromobutanoate (0.063 mL, 0.415 mmol) were added. The resulting mixture was heated at 60 °C for 2.5 h and monitored by TLC (ethyl acetate/light petroleum 1:3). The excess of base was filtered, the solvent was evaporated and after purification by column chromatography on silica gel, using ethyl acetate/light petroleum 1:3 as the eluent, compound 4b was obtained as a pink solid (0.082 g, 80%); Rf = 0.55 ethyl acetate/light petroleum 1:3; m.p. 61.5-63.0 °C; 1H NMR (300 MHz, [D$_6$]DMSO): δ = 1.17 (t, J = 7.2 Hz, 3H; CO$_2$CH$_2$CH$_3$), 1.95-2.08 (m, 2H; OCH$_2$CH$_2$CO$_2$CH$_2$CH$_3$), 2.42-2.58 (m, 2H; OCH$_2$CH$_2$CH$_2$CO$_2$CH$_3$), 4.00-4.18 (m, 4H; OCH$_2$CH$_2$CO$_2$CH$_3$ and CO$_2$CH$_2$CH$_3$), 5.65 (s, 2H; NH$_2$), 6.49 (d, J = 6.9 Hz, 1H; H-6), 6.90-7.02 (m, 2H; H-8 and H-3), 7.06-7.18 (m, 2H; H-1 and H-7), 7.95 ppm (d, J = 9.3 Hz, 1H; H-4); 13C NMR (75.4 MHz, [D$_6$]DMSO): δ = 14.11 (CO$_2$CH$_2$CH$_3$), 24.25 (OCH$_2$CH$_2$CO$_2$CH$_2$CH$_3$), 30.20 (OCH$_2$CH$_2$CH$_2$CO$_2$CH$_3$), 59.87 (CO$_2$CH$_3$), 66.33 (OCH$_2$CH$_2$CO$_2$CH$_2$CH$_3$), 105.82 (C-6), 106.85 (C-1), 114.58 (C-8), 115.85 (C-3), 117.98 (C-4a), 124.05 (C-4), 127.34 (C-7), 135.60 (C-8a), 144.66 (C-5), 156.09 (C-2), 172.58 ppm (CO$_2$CH$_3$); IR (neat): □□□ = 3456, 3376, 2963, 2937, 2870, 1733, 1625, 1586, 1517, 1467, 1451, 1385, 1377, 1318, 1293, 1274, 1249, 1213, 1050, 1029, 975, 921, 863, 842, 816, 780, 750, 666 cm$^{-1}$; HRMS (EI): m/z calcd for C$_{16}$H$_{19}$NO$_3$: 273.1365 [M$^+$]; found: 273.1371.

**4-((5-Aminonaphthalen-2-yl)oxy)butanoic acid (4c):** To a solution of ethyl 4-((5-aminonaphthalen-2-yl)oxy)butanoate 4b (0.050 g, 0.183 mmol) in 1,4-dioxane (2.0 mL), 1 M sodium hydroxide (0.37 mL, 0.366 mmol) was added. The reaction mixture was stirred at room temperature for 9 hours and monitored by TLC (CH$_2$Cl$_2$/MeOH 9.5:0.5). After acidification to pH 2–3 with 1 M potassium hydrogen sulfate, the mixture was extracted with ethyl acetate (5×20 mL), and the organic extracts were dried (magnesium sulfate) and evaporated to dryness giving compound 4c as a light brown solid (0.041 g, 90%); Rf = 0.37 (CH$_2$Cl$_2$/MeOH 8:2); m.p. 148.5-150.1 °C; 1H NMR (400 MHz, [D$_6$]DMSO): δ = 1.93-2.12 (m, 2H; OCH$_2$CH$_2$CH$_2$CO$_2$H), 2.41 (t, J = 7.2 Hz, 2H; OCH$_2$CH$_2$CH$_2$CO$_2$H), 4.06 (t, J = 6.4 Hz, 2H; OCH$_2$CH$_2$CH$_2$CO$_2$H), 5.62 (broad s, 2H; NH$_2$), 6.49 (dd, J = 7.6 and 0.8 Hz, 1H; H-6), 6.92 (m, 2H; H-8 and H-3), 7.07-7.15 (m, 2H; H-1 and H-7), 7.95 (d, J = 9.2 Hz, 1H; H-4), 12.13 ppm (broad s, 1H; OH); 13C NMR (100.6 MHz, [D$_6$]DMSO): δ = 24.28 (OCH$_2$CH$_2$CH$_2$CO$_2$H), 30.19 (OCH$_2$CH$_2$CH$_2$CO$_2$H), 66.45 (OCH$_2$CH$_2$CH$_2$CO$_2$H), 105.82 (C-6), 106.90 (C-1), 114.59 (C-8), 115.89 (C-3), 117.99 (C-4a), 124.05 (C-4), 127.34 (C-7), 135.64 (C-8a), 144.72 (C-5), 156.16 (C-2), 174.15 ppm (CO$_2$H); IR (neat): □□□ = 3457, 3368, 2939, 1711, 1625, 1587, 1517, 1451, 1414, 1386, 1320, 1294, 1271,
Starting from a solution of 5-(propylamino)naphthalen-2-ol 2 (0.02 g, 0.994 mmol), in acetonitrile (4 mL), using cesium carbonate (1.583 g, 4.86 mmol), 3-bromopropan-1-amine (0.478 g, 2.18 mmol), and following the same procedure as described for 4b (heating for 29 h; TLC (CH₂Cl₂/MeOH 9.5:0.5); column chromatography using CH₂Cl₂/MeOH 6:4) gave compound 5 as a brown oily solid (0.113 g, 44%); Rₛ = 0.59 (CH₂Cl₂/MeOH 8:2); ¹H NMR (300 MHz, [D₆]DMSO): δ = 0.95 (t, J = 7.2 Hz, 3H; CH₃), 1.60-1.75 (m, 2H; NHCH₂CH₂CH₃), 2.00-2.15 (m, 2H; OCH₂CH₂CH₂NH₂), 2.97 (t, J = 7.2 Hz, 2H; OCH₂CH₂CH₂NH₂), 3.05-3.15 (m, 2H; NHCH₂CH₂CH₃), 4.14 (t, J = 6.0 Hz, 2H; OCH₂CH₂CH₂NH₂), 6.06 (t, J = 5.4 Hz, 1H; NH), 6.31 (d, J = 7.2 Hz, 1H; H-2), 6.94 (d, J = 8.1 Hz, 1H; H-4), 7.00 (dd, J = 9.3 and 2.3 Hz, 1H; H-7), 7.13 (d, J = 2.4 Hz, 1H; H-5), 7.20 (t, J = 7.8 Hz, 1H; H-3), 7.80 (broad s, 2H; NH₂), 8.09 ppm (d, J = 9.6 Hz, 1H; H-8); ¹³C NMR (75.4 MHz, [D₆]DMSO): δ = 11.83 (NHCH₂CH₂CH₃), 21.49 (NHCH₂CH₂CH₃), 26.91 (OCH₂CH₂CH₂NH₂), 36.32 (OCH₂CH₂CH₂NH₂), 45.00 (NHCH₂CH₂CH₃), 64.56 (OCH₂CH₂CH₂NH₂), 101.11 (C-2), 107.33 (C-5), 114.25 (C-4), 115.68 (C-7), 118.18 (C-8a), 123.59 (C-8), 127.55 (C-3), 135.52 (C-4a), 144.42 (C-1), 155.99 ppm (C-6); IR (KBr): νmax = 3418, 2960, 2929, 2874, 1626, 1586, 1530, 1469, 1432, 1384, 1342, 1282, 1226, 1182, 1149, 1064, 969, 773, 740, 664 cm⁻¹; HRMS (EI): m/z calcld for C₁₆H₂₂N₂O₂: 258.1732 [M⁺]; found: 258.1738.

General method for the preparation of compounds 7a-f

Conventional methodology: ⁷,¹⁴,¹⁹,²⁰,²⁷-⁳¹ To a cold solution (ice bath) of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride 6 (1.26 mmol) in ethanol or dimethylformamide (7e) (2 mL), 5-aminonaphthalen-2-ol 1 or its derivatives (2, 4a-c or 5) (0.63 mmol) and concentrated hydrochloric acid (1.29×10⁻² mL) were added. The mixture was refluxed or heated at 65 °C (7e) during the time as indicated in Table 1, and monitored by TLC (CH₂Cl₂/MeOH). After evaporation of the solvent and column chromatography purification on silica gel, the required dye 7a-f was obtained.

Microwave irradiation using a solid support: After the pre-activation (pre-activation of the silica gel was achieved by microwave irradiation for 5 min, in 60 s intervals, each followed by a 5 s cooling interval) of silica gel (100–200 mesh, 5 times the weight of the starting substrate), an ethanol solution of 1 (0.140 mmol) and 6 (0.280 mmol) was added and then the solvent was evaporated. The resulting homogeneous mixture of the substrates and silica gel was transferred to
the reaction vial of the microwave reactor. Initially the microwave irradiation was set at 150 W, the pressure to 100 psi, the temperature was ramped from room temperature to 90 °C. Once this was reached, the reaction mixture was held at this temperature with stirring for 10 min (Table 2). During this time the power was modulated automatically to keep the reaction mixture at 90 °C. After the reaction was completed (monitored by TLC using CH₂Cl₂/MeOH 95:5 as eluent), the mixture was cooled to room temperature, and purified by column chromatography.

**Microwave irradiation using solvent:** A solution of compound 6 (0.280 mmol) and 1, 2, 4a-c or 5 (0.140 mmol) in ethanol (0.5 mL) was placed in the glass vial of the microwave reactor. Initially the microwave irradiation was set at 150 W, the pressure to 100 psi, the temperature was ramped from room temperature to 90 °C. Once this was reached, the reaction mixture was held at this temperature with stirring for the time mentioned in Tables 2 and 3. During this time the power was modulated automatically to keep the reaction mixture at 90 °C. After the reaction was completed (monitored by TLC using CH₂Cl₂/MeOH 95:5 as eluent), the mixture was cooled to room temperature, the solvent was evaporated, and column chromatography purification on silica gel yielded the required dye 7a-f (except 7e). In the case of acetonitrile, dimethylformamide and water as solvent the procedure was similar.

*N-(5-Amino-2-hydroxy-10-methyl-9H-benzo[a]phenoxazin-9-ylidene)ethanaminium chloride* (7a): Chromatography purification on silica gel, using CH₂Cl₂/MeOH 9.3:0.7, gave a blue solid; Rᵣ = 0.15 (CH₂Cl₂/MeOH 9:1); m.p. > 350 °C; ¹H NMR (300 MHz, [D₆]DMSO): δ = 1.22 (broad s, 3H; NHCH₂CH₃), 2.21 (s, 3H; CH₃), 3.39 (broad s, 2H; NHCH₂CH₃), 6.70 (s, 1H; H-8), 6.82 (s, 1H; H-6), 7.24 (d, J = 7.5 Hz, 1H; H-3), 7.47 (s, 1H; H-11), 8.01 (s, 1H; H-1), 8.40 (d, J = 9.0 Hz, 1H; H-4), 9.82 and 10.07 (2 × broad s, 2H; NH₂), 11.14 ppm (broad s, 1H; OH). ¹³C NMR (75.4 MHz, [D₆]DMSO): δ = 13.87 (NHCH₂CH₃), 17.47 (CH₃), 37.99 (NHCH₂CH₃), 93.45 (C-8), 95.33 (C-6), 108.34 (C-1), 115.04 (C-Ar), 118.74 (C-3), 125.32 (C-Ar), 126.98 (C-4), 127.96 (C-10), 130.86 (C-11), 133.71 (C-Ar), 134.03 (C-Ar), 146.87 (C-Ar), 150.92 (C-Ar), 153.81 (C-9), 160.52 (C-5), 161.61 ppm (C-2). IR (KBr): ν = 3419, 3064, 1644, 1619, 1595, 1549, 1483, 1435, 1359, 1319, 1299, 1265, 1216, 1156, 1131, 1086, 1057, 1028, 1010, 935, 883, 849, 830, 812, 736, 712, 666 cm⁻¹. HRMS (ESI): m/z calcd for C₁₉H₁₈N₃O₂+H⁺: 320.13935 [M⁺+ H⁺]; found: 320.13927.

*N-(2-Hydroxy-10-methyl-5-(propylamino)-9H-benzo[a]phenoxazin-9-ylidene)ethanaminium chloride* (7b): Chromatography purification on silica gel, using CH₂Cl₂/MeOH 9.4:0.6, gave a blue solid; Rᵣ = 0.46 CH₂Cl₂/MeOH 9:1); m.p. 290.3-292.4 °C; ¹H NMR (400 MHz, CD₃OD): δ = 1.12 (broad s, 3H; NHCH₂CH₂CH₃), 1.39 (broad s, 3H; NHCH₂CH₃), 1.80-2.00 (m, 2H;
NH₂CH₂CH₂CH₃), 2.29 (broad s, 3H; CH₃), 3.50 (broad s, 2H; NH₂CH₂CH₃), 3.62 (broad s, 2H; NH₂CH₂CH₂CH₃), 4.60 (broad s, 2H; 2×NH), 6.70 (broad s, 2H; H-6 and H-8), 7.20 (broad s, 1H; H-3), 7.50 (s, 1H; H-11), 8.05 (broad s, 1H; H-4), 8.13 ppm (s, 1H; H-1); ¹³C NMR (100.6 MHz, CD₃OD): δ = 11.75 (NH₂CH₂CH₂CH₃), 14.19 (NH₂CH₂CH₃), 17.65 (CH₃), 23.20 (NH₂CH₂CH₂CH₃), 39.60 (NH₂CH₂CH₃), 47.23 (NH₂CH₂CH₂CH₃), 92.94 (C-6), 94.46 (C-8), 109.86 (C-4), 117.22 (C-Ar), 119.98 (C-3), 126.12 (C-1), 127.58 (C-10), 130.74 (C-Ar), 132.55 (C-11), 134.93 (C-Ar), 135.06 (C-Ar), 148.95 (C-Ar), 153.21 (C-Ar), 156.01 (C-9), 159.03 (C-5), 162.48 ppm (C-2); IR (KBr): δ = 3387, 2965, 2927, 1642, 1596, 1546, 1488, 1455, 1437, 1384, 1294, 1268, 1231, 1158, 1136, 1035, 938, 881, 851, 827, 810, 666 cm⁻¹; HRMS (ESI): m/z calcd for C₂₂H₂₄N₃O₂+H⁺: 362.18630 [M⁺+H⁺]; found: 362.18617.

N-(5-Amino-10-methyl-2-propoxy-9H-benzo[a]phenoxazin-9-ylidene)ethanaminium chloride (7c): Chromatography purification on silica gel, using CH₂Cl₂/MeOH 9:0.5, gave a blue solid; Rᵣ = 0.25 (CH₂Cl₂/MeOH 9:1); m.p. 296.1-297.8 °C; ¹H NMR (400 MHz, CD₃OD): δ = 1.10-1.22 (m, 3H; OCH₂CH₂CH₃), 1.39 (broad s, 3H; NHCH₂CH₃), 1.93 (broad s, 2H; OCH₂CH₂CH₃), 2.27 (s, 3H; CH₃), 3.48 (broad s, 2H; NHCH₂CH₃), 4.11 (broad s, 2H; OCH₂CH₂CH₃), 6.57 (s, 1H; H-8), 6.62 (s, 1H; H-6), 7.23 (broad s; 1H; H-3), 7.45 (broad s; 1H; H-11), 7.98 (broad s; 1H; H-4), 8.07 ppm (s, 1H; H-1); ¹³C NMR (100.6 MHz, CD₃OD): δ = 10.81 (OCH₂CH₂CH₃), 14.21 (NH₂CH₂CH₃), 17.68 (CH₃), 23.58 (OCH₂CH₂CH₃), 39.69 (NHCH₂CH₃), 71.35 (OCH₂CH₂CH₃), 94.50 (C-6), 96.33 (C-8), 107.25 (C-4), 117.35 (C-Ar), 119.94 (C-3), 126.76 (C-1), 127.65 (C-10), 130.63 (C-Ar), 132.68 (C-11), 134.48 (C-Ar), 135.40 (C-Ar), 148.81 (C-Ar), 152.60 (C-Ar), 156.26 (C-9), 161.76 (C-5), 163.69 ppm (C-2). IR (KBr): δ = 3325, 2970, 2927, 1644, 1595, 1548, 1484, 1474, 1440, 1420, 1384, 1308, 1266, 1226, 1160, 1133, 1087, 1064, 1031, 1005, 978, 881, 867, 850, 811, 715, 665 cm⁻¹. HRMS (ESI): m/z calcd for C₂₂H₂₄N₃O₂+H⁺: 362.18630 [M⁺+H⁺]; found: 362.18616.

N-(5-Amino-2-(4-ethoxy-4-oxobutoxy)-10-methyl-9H-benzo[a]phenoxazin-9-ylidene)ethanaminium chloride (7d): Chromatography purification on silica gel, using CH₂Cl₂/MeOH 9:5:0.5, gave a blue solid; Rᵣ = 0.42 (CH₂Cl₂/MeOH 9:1); m.p. 110.6-112.9 °C; ¹H NMR (400 MHz, CD₃OD): δ = 1.24-1.32 (m, 3H; CO₂CH₂CH₃), 1.36-1.45 (m, 3H; NHCH₂CH₃), 2.12 (broad s, 2H; OCH₂CH₂CH₂CO₂CH₂CH₃), 2.15-2.24 (m, 2H; OCH₂CH₂CH₂CO₂CH₂CH₃), 2.22 (s, 3H; CH₃), 3.42-3.54 (m, 2H; NHCH₂CH₃), 3.65 (broad s, 2H; OCH₂CH₂CH₂CO₂CH₂CH₃), 4.14-4.26 (m, 2H; CO₂CH₂CH₃), 6.63 (s, 1H; H-8), 6.71 (s, 1H; H-6), 7.18 (d, J = 7.2 Hz, 1H; H-3), 7.4 (s, 1H; H-11), 7.91 (broad s; 1H; H-1), 8.01 ppm (d, J = 7.6 Hz, 1H; H-4); ¹³C NMR (100.6 MHz, CD₃OD): δ = 14.22 (NH₂CH₂CH₃), 14.59 (CO₂CH₂CH₃), 17.77 (CH₃), 24.85
N-(5-Amino-2-(3-carboxypropoxy)-10-methyl-9H-benzo[a]phenoxazin-9-ylidene)ethanaminium chloride (7e): Chromatography purification on silica gel, using CH₂Cl₂/MeOH 8:2, gave a blue solid; Rᵣ = 0.19 (CH₂Cl₂/MeOH 8:2); m.p. > 350 °C; ¹H NMR (400 MHz, CD₂OD): δ = 1.40–1.50 (m, 3H; NHCH₂CH₃), 2.22 (broad s, 2H; OCH₂CH₂CH₂CO₂H), 2.33 (broad s, 3H; CH₃), 2.60 (broad s, 2H; OCH₂CH₂CH₂CO₂H), 3.50–3.60 (m, 2H; NHCH₂CH₃), 4.30 (broad s, 2H; OCH₂CH₂CH₂CO₂H), 6.65–6.90 (m, 2H; H-6 and H-8), 7.36 (broad s, 1H; H-3), 7.65 (broad s, 1H; H-11), 8.24 ppm (broad s, 2H; H-1 and H-4); ¹³C NMR (100.6 MHz, CD₂OD): δ = 14.16 (NHCH₂CH₃), 17.60 (CH₃), 25.80 (OCH₂CH₂CH₂CO₂H), 31.62 (OCH₂CH₂CH₂CO₂H), 36.61 (NHCH₂CH₃), 68.92 (OCH₂CH₂CH₂CO₂H), 94.61 (C-6), 96.45 (C-8), 107.57 (C-4), 107.70 (C-5), 126.87 (C-1), 127.72 (C-10), 130.90 (C-Ar), 132.86 (C-11), 134.76 (C-Ar), 153.05 (C-Ar), 156.39 (C-9), 162.03 (C-5), 163.59 (C-2), 164.0 ppm (CO₂H); IR (KBr): ν = 3390, 2920, 2851, 1723, 1643, 1594, 1563, 1548, 1524, 1486, 1452, 1384, 1304, 1264, 1228, 1131, 1012, 666 cm⁻¹. HRMS (ESI): m/z calcd for C₂₅H₂₉N₄O₄H⁺: 435.21597 [M⁺+ H⁺]; found: 435.21590.

N-(2-(3-Aminopropoxy)-10-methyl-5-(propylamino)-9H-benzo[a]phenoxazin-9-ylidene)ethanaminium chloride (7f): Chromatography purification on silica gel, using CH₂Cl₂/MeOH 8:2, gave a blue solid; Rᵣ = 0.36 (CH₂Cl₂/MeOH 8:2); m.p. 110.6–112.9 °C; ¹H NMR (400 MHz, CD₂OD): δ = 1.13 (t, J = 7.6 Hz, 3H; NHCH₂CH₂CH₃), 1.40 (t, J = 7.2 Hz, 3H; NHCH₂CH₃), 1.85–1.94 (m, 2H; NHCH₂CH₃), 2.24–2.34 (m, 2H; OCH₂CH₂CH₂NH₂), 2.38 (s, 3H; CH₃), 3.27 (t, J = 7.6 Hz, 2H; OCH₂CH₂CH₂NH₂), 3.57 (q, J = 7.2 Hz, 2H; NHCH₂CH₃), 3.70 (t, J = 7.2 Hz, 2H; NHCH₂CH₂CH₃), 4.44 (t, J = 6.0 Hz, 2H; OCH₂CH₂CH₂NH₂), 6.91 (s, 1H; H-8), 6.95 (s, 1H; H-6), 7.47 (dd, J = 9.2 and 2.4 Hz, 1H; H-3), 7.74 (s, 1H; H-11), 8.37 (d, J = 9.2 Hz, 1H; H-4), 8.43 ppm (d, J = 2.8 Hz, 1H; H-1); ¹³C NMR (100.6 MHz, CD₂OD): δ = 11.81 (NHCH₂CH₂CH₃), 14.25 (NHCH₂CH₃), 17.90 (CH₃), 23.20 (NHCH₂CH₂CH₃), 28.34 (OCH₂CH₂CH₂NH₂), 38.47 (OCH₂CH₂CH₂NH₂), 39.73 (NHCH₂CH₃), 47.23 (NHCH₂CH₂CH₃), 66.96 (OCH₂CH₂CH₂NH₂), 93.24 (C-6), 94.58 (C-8), 107.58 (C-4), 118.50 (C-Ar), 119.30 (C-3),
126.29 (C-1), 128.10 (C-10), 131.14 (C-Ar), 132.58 (C-11), 134.27 (C-Ar), 134.73 (C-Ar), 149.11 (C-Ar), 153.06 (C-10), 158.47 ppm (C-2); IR (KBr): \(\nu = 3413, 2923, 1640, 1596, 1559, 1490, 1454, 1384, 1312, 1266, 1230, 1167, 1136, 1058, 1012, 948, 813, 666\) cm\(^{-1}\). HRMS (ESI): \(m/z\) calcd for \(C_{25}H_{31}N_4O_2+H^+\): 419.24415 \([M^+ + H^+]\); found: 419.24409.

**Supporting Information** Supporting information for this article is available on the WWW under http://www.eurjoc.org/ or from the author.

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