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Synthesis and spectral properties of long-wavelength fluorescent dyes

Vânia H.J. Frade [a], M. Sameiro T. Gonçalves [a,*], Paulo J.G. Coutinho [b], João C.V.P. Moura [a]

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

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Abstract

Benzo[*a*]phenoxazinium salts were synthesised by reacting 5-alkylamino-2-nitrosophenol hydrochloride with *N*-alkylated-naphthylamine in good to excellent yields. Photophysical properties of these fluorophores with emphasis in solvent effects were studied. Remarkable shifts in the absorption and emission maximum have been observed as a function of polarity and proton accepting capability of the solvents. The influence on the fluorescence quantum yield was also studied.
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1. Introduction

The fluorometric detection method has been widely used in medical area and diagnostics as well as in environmental analysis and material sciences. To make this method more useful, advances in instrumentation along with the synthesis of new fluorescent reagents are of extreme importance. In recent years, many fluorescence compounds have been reported to label biomolecules. However, only a few are long-wavelength absorbing and fluorescent dyes. The development and use of these type of fluorophores are valuable, because the background autofluorescence from biological samples, typically in the blue or green region of the spectrum, could interfere in the measurement of the label emission. Therefore, it is desirable to enhance the sensitivity of detection by using fluorophores with absorption and emission in the red or near-infrared spectral region.

Long-wavelength excitation and emission probes have been synthesised [1–3] and used in protein labelling [4], chromatography studies [5], measurements in blood [6] and DNA analysis [7]. Among these reagents, oxazine derivatives have been reported in several spectroscopic investigations because of their wide use as a dye laser in the range 600–900 nm. They are also used as standards for fluorescence measurements, and in biological stains [8,9]. These studies showed that the absorption and emission properties of this family of compounds are affected by the solvent physical and chemical characteristics. Benzophenoxazines and benzo[*a*]phenoxazines have been used in various biomedical applications [10–13], and also as biomarkers for nucleic acid detection [14,15], and protein labelling [16]. Despite their importance for bioassays purposes, fewer have reactive groups, such as carboxylic function.

With this in mind and the fact that only dyes having a suitable functional group, which will react with the analyte, appropriate for covalent labelling, we prepared new functionalised benzo[*a*]phenoxazininium salts. Thus, a carboxylic acid or ester (which can be hydrolysed) was chosen as the reactive functional group, and this was achieved by using the corresponding naphthyl carboxylic acid or ethyl ester precursor. To extend our previous work [17], we now report the efficient synthesis and characterisation of several side-chain substituted 5,9-diaminobenzo[*a*]phenoxazinium dyes **1**. Slight variations in the side-chain substituents can have marked effects on the photophysic behaviour of these chromophores. The effects were studied using the set of new benzo[*a*]phenoxazininium

* Corresponding author. Tel.: +351 253 604386; fax: +351 253 678983.
E-mail address: msameiro@quimica.uminho.pt (M.S.T. Gonçalves).

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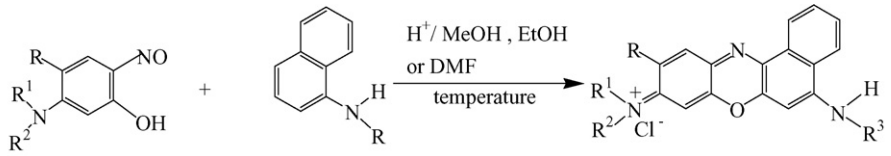

2a R = H, $R^1 = R^2 = CH_3$
2b R = H, $R^1 = R^2 = CH_2CH_3$
2c R = H, R^1 = H , $R^2 = (CH_2)_5CH_3$
2d R = CH_3, R^1 = H , $R^2 = CH_2CH_3$

3a R = $(CH_2)_2CO_2H$
3b R = $(CH_2)_2CO_2CH_2CH_3$
3c R = $(CH_2)_2CO_2CH_3$
3d R = Ph
3e R = H

1a R = H, $R^1 = R^2 = CH_3$, $R^3 = (CH_2)_2CO_2CH_3$
1b R = H, $R^1 = R^2 = CH_3$, $R^3 = (CH_2)_2CO_2H$
1c R = H, $R^1 = R^2 = CH_3$, $R^3 = (CH_2)_2CO_2CH_2CH_3$
1d R = H, $R^1 = R^2 = CH_3$, R^3 = Ph
1e R = H, $R^1 = R^2 = CH_3$, R^3 = H
1f R = H, $R^1 = R^2 = CH_2CH_3$, $R^3 = (CH_2)_2CO_2CH_3$
1g R = H, $R^1 = R^2 = CH_2CH_3$, $R^3 = (CH_2)_2CO_2H$
1h R = H, R^1 = H, $R^2 = (CH_2)_5CH_3$, R^3 = Ph
1i R = CH_3, R^1 = H, $R^2 = CH_2CH_3$, $R^3 = (CH_2)_2CO_2H$
1j R = CH_3, R^1 = H, $R^2 = CH_2CH_3$, $R^3 = (CH_2)_2CO_2CH_2CH_3$
1l R = CH_3, R^1 = H, $R^2 = CH_2CH_3$, $R^3 = (CH_2)_2CO_2CH_3$
1m R = CH_3, R^1 = H, $R^2 = CH_2CH_3$, R^3 = Ph

Scheme 1.

derivatives synthesised. Thus, spectral properties, i.e. absorption and fluorescence, emphasising on the solvent influence were also studied.

2. Results and discussion

2.1. Synthesis

Among the few synthetic methodologies for the synthesis of benzo[*a*]phenoxazinium salts that have been reported in the literature [18], we decided to prepare benzo[*a*]phenoxazinium chlorides **1** by the reaction of 5-alkylamino-2-nitrosophenol hydrochloride **2a–d**, with *N*-alkylated-naphthylamine **3a–e** in acidic medium (Scheme 1). The required 5-alkylamino-2-nitrosophenol hydrochloride **2a–d** was synthesised by usual procedure involving treatment of the corresponding 3-alkylaminophenol with sodium nitrite in acid solution. 3-Alkylaminophenols used were commercial reagents, except in case of 3-hexylaminophenol which was obtained by refluxing 3-aminophenol with 1-bromohexane in ethanol by the usual procedure.

N-alkyl-naphthylamines **3a–c** were prepared by alkylation of 1-naphthylamine with chloropropionic acid, 3-ethyl-3-bromopropionate and 3-methyl-3-bromopropionate, respectively. After purification by dry chromatography, compounds **3a–c** were obtained as solid (**3a**) and oils (**3b** and **3c**) in yields ranging from 45 to 55%, and were characterised by high resolution mass spectrometry, IR and NMR (^{1}H and ^{13}C) spectroscopy. The presence of the carbonyl group was confirmed by IR, which showed a strong band at 1717 (**3a**), 1727 (**3b**) and 1731 cm^{-1} (**3c**). In the ^{13}C NMR, signals at δ 164.4 (**3a**) and about 173.0 ppm (**3b** and **3c**) were assigned to the functional group. Compounds **3d** and **3e** used were commercial reagents.

When 5-alkylamino-2-nitrosophenol hydrochloride (**2a**) reacted with 3-(naphthalen-1-ylamino) propanoic acid (**3a**) in the presence of hydrochloric acid, reflux in methanol, benzo[*a*]phenoxazinium chloride **1a** was isolated by dry chromatography purification, as the major product (77%). Although esterification occurred, the carboxylic acid derivative (**1b**) was also obtained in 22%. Reaction of **2a** with the ethyl 3-(naphthalen-1-ylamino) propanoate (**3b**), in ethanol, gave **1c** in a quantitative yield (99%). In the preparation of dyes **1d** (76%) and **1e** (66%) the nitrosophenol component used was also **2a** which reacted with *N*-phenyl-1-naphthylamine (**3d**) and 1-naphthylamine (**3e**), respectively.

Starting with 5-(diethylamino)-2-nitrosophenol hydrochloride (**2b**) and **3a**, reaction in methanol, both dyes **1f** (64%) and **1g** (7%) were obtained. The reaction between 5-(hexylamino)-2-nitrosophenol (**2c**) and **3d** yielded compound **1h** in 46%.

To avoid the possibility of esterification, compound **1i** (45%) was prepared by reaction of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride (**2d**) with propanoic acid derivative **3a**, using DMF as solvent and heating at 70 °C. Reaction of the same nitroso precursor (**2d**) with the alkylated naphthylamines **3b** and **3c** under reflux in ethanol gave benzo[*a*]phenoxazinium salts **1j** and **1l** in high yields (90%, **1j**; 75%, **1l**) (Table 1). Dye **1m** was prepared in 54% yield by reaction of compounds **2d** and **3d** using the same conditions reported above.

Although compounds **1d** and **1e** were reported before [19–23], we decided to prepare them, for comparison with the new fluorophores, at the same conditions, in the absorption and fluorescence studies. In addition their full characterisation was also presented, which from the knowledge of the authors, had never been reported.

Thus, all dyes were obtained as blue materials and were fully characterised by elemental analysis or high resolution mass spectrometry, IR, NMR (^{1}H and ^{13}C) and visible spectroscopy.

The IR shows bands due to the ester group for compounds **1a**, **1c**, **1f**, **1j** and **1l** between 1723 (**1c** and **1l**) and 1738 cm^{-1} (**1a**). In ^{13}C NMR, signals from δ 172.0 (**1c**) to δ 173.3 ppm (**1l**) and from δ 164.3 (**1i**) to δ 169.3 ppm (**1g**) also confirmed the presence of the carbonyl function of the ester (**1a**, **1c**, **1f**, **1j** and **1l**) and the acid (**1b**, **1g** and **1i**). The ^{1}H and ^{13}C NMR spectra of derivatives **1d**, **1h** and **1m** showed the expected signals due

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