

[a039] **Synthesis and spectral characterisation of naphtho[2,3-*a*]phenoxazinium salts**

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Abstract - A series of new functionalised naphtho[2,3-*a*]phenoxazinium chlorides was synthesised by condensation of 5-ethylamino-2-nitrosophenol hydrochloride with 1-aminoanthracene and its *N*-substituted derivatives. These cationic dyes showed maximum absorption in the range between 590 and 647 nm and strong fluorescence with maximum emission wavelengths between 630 and 674 nm, as well as high fluorescent quantum yields in ethanol and water at physiological pH.

1. Introduction

Long-wavelength fluorescent dyes have been synthesised and used in different important applications such as laser dyes, sequencing studies and drug displacement.¹⁻³

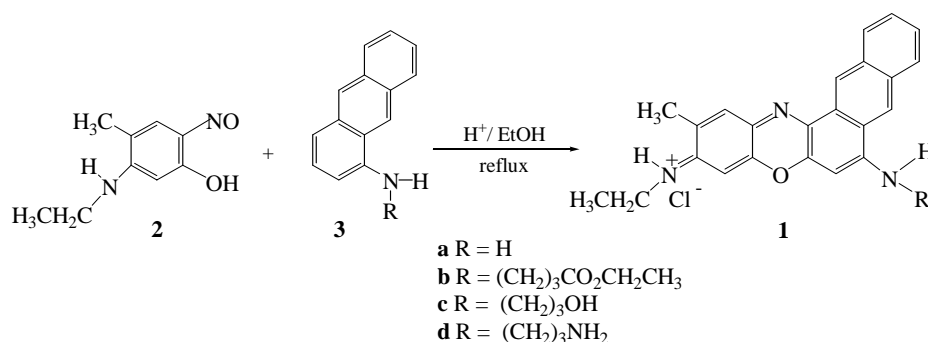
Oxazine derivatives are included in this category of dyes and they have been used in various biomedical applications,^{4,5} as biomarkers for nucleic acid detection⁶ and protein labelling.⁷

Although the large amount of fluorescent reagents has been reported, development of new long-wavelength fluorophores still continues to be a subject of great interest.

Bearing this in mind, and following our previous research in the synthesis and application of benzo[*a*]phenoxazine dyes,⁸⁻¹⁰ we decided to extend our previous work to the synthesis of new fluorochromophores, such as naphtho[2,3-*a*]phenoxazinium salts. These cationic compounds were obtained in low to excellent yields and evaluation of their absorption and emission properties was carried out in ethanol and water (pH 7.4).

2. Results and Discussion

Naphtho[2,3-*a*]phenoxazinium chlorides **1a-d** were prepared by the reaction of 5-ethylamino-2-nitrosophenol hydrochloride **2**, with 1-aminoanthracene or its *N*-substituted-derivatives **3a-d** in an acidic medium (Scheme 1). The required nitrosophenol **2** was synthesised using the usual procedure involving treatment of the corresponding 3-ethylamino-4-methylphenol with sodium nitrite in an acid solution. Compounds **3a-d** were prepared by alkylation of 1-aminoanthracene with the appropriate bromo reagent in yields of about 53% (**3c** and **3d**; **3b** was not isolated).



Scheme 1.

The cationic dyes **1a-d** were obtained as blue solids in yields ranging from 16 to 98% (Table 1) and were fully characterised by high resolution mass spectrometry, IR and NMR (¹H and ¹³C) and visible spectroscopy.

Table 1. Synthesis and visible data of naphtho[2,3-*a*]phenoxazininium salts **1a-d**.

| Compound | Yield / % | Vis ^a | Vis ^b |
|-----------|-----------------|--|--|
| | | λ_{max} [nm] (ϵ) | λ_{max} [nm] (ϵ) |
| 1a | 98 | 615 (62112) | 615 (46296) |
| 1b | 16 ^c | 647 (19595) | -- ^d |
| 1c | 61 | 640 (35000) | 590 (20538) |
| 1d | 98 | 645 (4781) | -- ^d |

^a Spectra measured in ethanol. ^b Spectra measured in water (pH 7.4).

^c Yield calculated from 1-aminoanthracene. ^d Compound insoluble in water (pH 7.4).

The visible absorption spectra of 10⁻⁶ M solutions of compounds **1a-d** in degassed absolute ethanol showed absorption peaks between 615 nm (**1a**) and 647 nm (**1b**) with ϵ values ranging from 4781 (**1d**) to 62112 (**1a**) (Table 1).

The fluorescent properties of these polycyclic heterocycles measured in the same solvent, using Oxazine 1 as a standard,¹¹ are summarised in Table 2. All compounds exhibited high levels of fluorescence, with quantum yields (Φ_{F}) between 0.20 (**1b**) and 0.37 (**1c**) and showed moderate to good Stokes' shifts (44 - 99 nm).

The photophysical properties of compounds **1a** and **1c** were also studied in water at physiological pH (Tables 1 and 2). Their maximum absorption (λ_{max}) and emission (λ_{em}) wavelengths in water and ethanol were equal for compound **1a** (615 nm), whereas for compound **1c** a hypsochromic shift of 50 nm (λ_{max}) or 42 nm (λ_{em}) occurred from ethanol to water. When compared to ethanol, Φ_{F} in

water was superior for compound **1a** (0.47 in water, 0.34 in ethanol) and inferior for compound **1c** (0.37 in ethanol < 0.1 in water).

Table 2. Fluorescence data for compounds **1a-d** in ethanol and water (pH 7.4).

| Compd | Fluorescence ^a | | | Stokes' shift | Fluorescence ^b | | | Stokes' shift |
|-----------|---------------------------|----------------|----------|---------------|---------------------------|----------------|----------|---------------|
| | λ_{exc} | λ_{em} | Φ_F | | λ_{exc} | λ_{em} | Φ_F | |
| 1a | 590 | 634 | 0.34 | 44 | 590 | 632 | 0.47 | 42 |
| 1b | 585 | 674 | 0.20 | 89 | -- ^c | -- | -- | -- |
| 1c | 600 | 672 | 0.37 | 72 | 600 | 630 | 0.072 | 30 |
| 1d | 575 | 674 | 0.24 | 99 | -- ^c | -- | -- | -- |

Units of λ_{exc} , λ_{em} and Stokes' shift: nm. ^a Spectra measured in ethanol. ^b Spectra measured in water (pH 7.4). ^c Compound insoluble in water (pH 7.4).

In this work, naphtho[2,3-*a*]phenoxazinium salts **1a-d**, containing different functional groups, were synthesised in low to excellent yields. The photophysical properties of these long-wavelength polycyclic heterocycles in ethanol and water at physiological pH, strongly justifies further studies with them as fluorescent probes in biological applications.

3. Experimental

Typical procedure for the synthesis of **1a-d** (described for **1c**): To a cold solution (ice bath) of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **2** (82 mg; 4.54×10^{-4} mol) in ethanol (2 mL), 3-(anthracen-1-ylamino) propanol **3c** (114 mg; 4.54×10^{-4} mol) and concentrated hydrochloride acid (5.0×10^{-2} mL) were added. The mixture was refluxed for 4 hours and 30 minutes and monitored by TLC (silica: dichloromethane/ methanol, 6:1). The solvent was removed under reduced pressure and the crude mixture was purified by dry chromatography (silica: dichloromethane/ methanol, 5.5:0.5). *N*-(7-(3-hydroxypropylamino)-2-methyl-3*H*-naphtho[2,3-*a*]phenoxazin-3-ylidene) ethanaminium **1c** was obtained as a blue solid (113.5 mg, 61%). Mp above 300 °C. R_f 0.68 (silica: dichloromethane/ methanol, 6:1). FTIR (Nujol): ν_{max} 3406, 2954, 2924, 2854, 1638, 1549, 1463, 1455, 1377, 1278, 1124, 1016 cm^{-1} . ¹H NMR (CD₃OD, 300 MHz): δ 1.20-1.40 (3 H, m, NHCH₂CH₃), 2.10-2.20 (2 H, m, NHCH₂CH₂CH₂), 2.31 (2 H, broad s, NHCH₂CH₂CH₂), 2.36 (3 H, s, CH₃), 3.50-3.60 (2 H, m, NHCH₂CH₃), 3.85 (2 H, broad s, NHCH₂CH₂CH₂), 6.71 (1 H, broad s, 4-H), 6.91 (1 H, broad s, 6-H), 7.63 (1 H, s, 1-H), 7.73 (2 H, broad s, 6-H and 7-H naphthyl), 8.16 (2 H, broad s, 5-H and 8-H naphthyl), 8.80 (1 H, broad s, 1-H naphthyl), 9.28 (1 H, broad s, 4-H) ppm. ¹³C NMR (CD₃OD, 75.4 MHz): δ 14.66 (NHCH₂CH₃),

18.33 (CH₃), 30.21 (NHCH₂CH₂CH₂), 32.75 (NHCH₂CH₂CH₂), 40.06 (NHCH₂CH₃), 43.95 (NHCH₂CH₂CH₂), 94.27 (4-C), 94.67 (6-C), 122.00 (Ar-C), 125.28 (Ar-C), 125.45 (Ar-C), 127.83 (4-C naphthyl), 127.94 (1-C naphthyl), 129.45 (7-C or 6-C naphthyl), 130.08 (5-C and 8-C naphthyl), 130.61 (7-C or 6-C naphthyl), 132.19 (1-C), 133.87 (Ar-C), 134.95 (Ar-C), 135.06 (Ar-C), 135.49 (Ar-C), 148.08 (Ar-C), 154.0 (3-C), 155.50 (2-C), 159.42 (Ar-C) ppm. The assignments were supported by HMQC technique. HRMS (FAB): calcd for C₂₆H₂₆N₃O₂ [M⁺]: 412.2025; found 412.2021.

Acknowledgments

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