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# Synthesis and Fluorescence Properties of Functionalised Long-wavelength Fluorophores

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## Introduction

In recent years, biophysical and biochemical applications of fluorescence spectroscopy have grown markedly, particularly as a result of advances in instrumentation along with the development of new fluorescent compounds. Despite the large number of fluorescent chromophores some of them have absorption and emission wavelengths in the UV-visible region where the biological matrix exhibits high absorption and auto-fluorescence background. Development of long-wavelength fluorescent dyes has been suggested to circumvent this limitation.[1,2] However, few fluorescent probes exist which absorb in the red or near-infrared region and even fewer are available with a suitable functional group for covalent labelling of the analyte.[3]

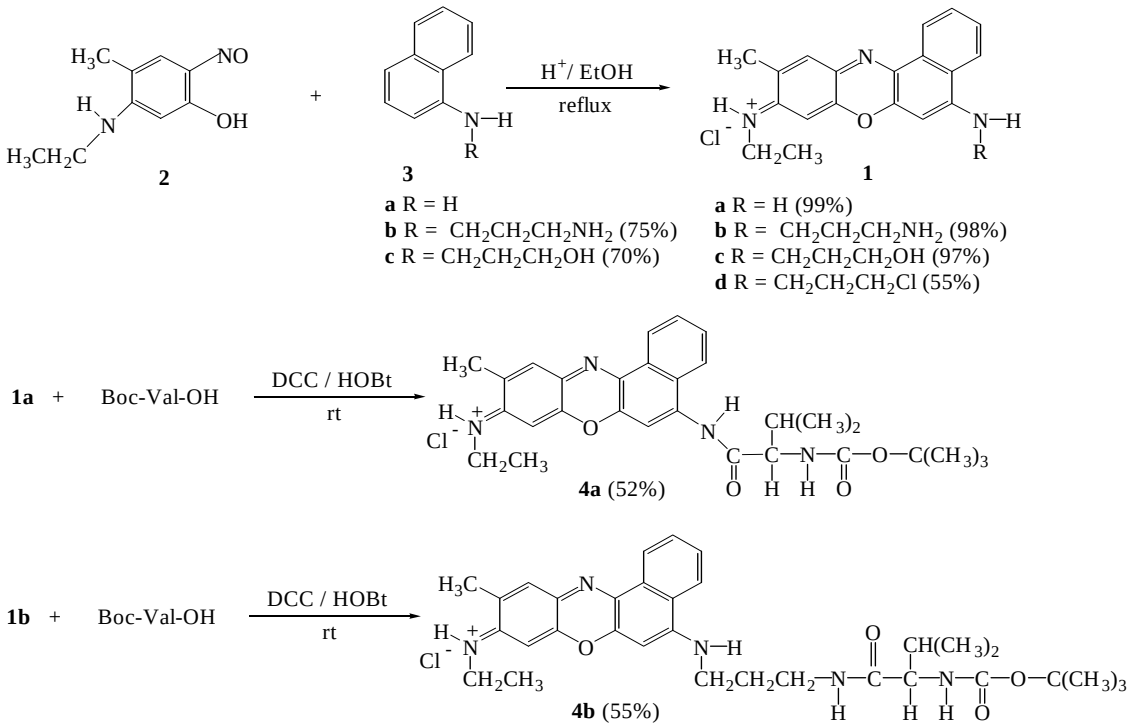
Bearing this in mind and in connection with our preliminary results,[4,5] we present the efficient synthesis of new side-chain functionalised 5,9-benzo[*a*]phenoxazinium salts. The possibility of linking these fluorogenic moities to the carboxylic function of α-amino acid residues was also studied. These compounds exhibit visible absorption in the 565-630 nm region and fluorescence with emission wavelength higher than 600 nm.

## Results and Discussion

Benzo[*a*]phenoxazinium chlorides **1** were prepared by the reaction of 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **2**, with *N*-alkylated-naphthylamine **3** in acidic medium (Scheme 1). The required 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **2** was synthesised by an usual procedure[4] involving treatment of the 3-ethyl-4-aminophenol with sodium nitrite in acid solution. *N*-Alkyl-naphthylamines **3b-c** were prepared by acylation of 1-naphthylamine with 3-bromo-1-propilamine

hydrobromide and 3-bromo-1-propanol, respectively. After purification by dry chromatography, compounds **3b-c** were obtained in 75% (**3b**) and 70% (**3c**) and characterised by high resolution mass spectrometry, IR and NMR ($^{1}H$ and $^{13}C$) spectroscopy.

When compound **2** was refluxed with **3a-c** in ethanol, benzo[*a*]phenoxazinium salts **1a-c** were isolated, after dry chromatography purification, as solid materials in excelent yields. Treatment of compound **1c**, with thionyl chloride, at room temperature gave the corresponding chloride derivative **1d** (55%) (Scheme 1, Table1).




**Scheme 1**

The amino derivatives **1a** and **1b** were used for studying the linkage of these fluorogenic moities to the carboxylic function of α-amino acid residues. Thus, reaction between **1a-b** with Boc-Val-OH was carried out with *N,N'*-dicyclohexylcarbodiimide (DCC), assisted by 1-hydroxybenzotriazole (HOBt) under standard conditions.[6]

After purification by chromatography on silica gel, the corresponding valine derivatives **4a** and **4b** were obtained as blue solids in 52% (**4a**) and 55% (**4b**) yields (Scheme 1).

Fluorescent compounds **1** and **4** were fully characterised by high resolution mass spectrometry, IR, NMR ($^{1}H$ and $^{13}C$) and visible spectroscopy.

The visible spectra of compounds **1a-d** in degassed absolute ethanol showed absorption peaks at 620 nm (**1a**, **1b** and **1c**) and 625 nm (**1d**) with molar extinction coefficient values (ε) ranging from 20282 (**1b**) to 53968 **(1a**) (Table 1). Absorption maxima of compounds **4** was 565 nm (**4a**) and 630 nm (**4b**) with ε values 20741 and 45000, respectively.

**Table 1.** UV/visible and fluorescent data for compounds **1a-d** in ethanol.

| | UV/vis | Fluorescence | | | |
|---|---|---|---|---|---|
| Compound | $\lambda_{max}$ [nm] (ε) | $\lambda_{exc}$ [nm] | $\lambda_{em}$ [nm] | $\phi$ | Stokes' shift [nm] |
| **1a** | 620 (53968) | 580 | 637 | 0.31 | 57 |
| **1b** | 620 (20282) | 580 | 644 | 0.33 | 64 |
| **1c**[5] | 620 (34649) | 590 | 634 | 0.24 | 44 |
| **1d** | 625 (21978) | 580 | 645 | 0.55 | 65 |

The fluorescence properties of the functionalysed benzo[*a*]phenoxazinium salts **1a-d** were measured in degassed absolute ethanol, using Oxazine 1 as standard and are summarised in Table 1. All moieties exhibit fluorescence between 634 nm (**1c**) and 645 nm (**1d**), compound **1d** being apparently the best fluorophore ($\phi = 0.55$). Studies of the fluorescent properties of the labelled valine (**4a**-**b**) are on the way and preliminary results showed that these derivatives exhibit fluorescence with emission wavelength higher than 600 nm.

The efficient synthesis of compounds **1a-d**, their good absorption and emission properties, in connection with the presence of a functional group make these water-soluble cationic dyes potencial long-wavelength fluorochromophores for covalent labelling of analytes.

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