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Synthesis and photophysical characterisation of long alkyl side-chain derivatives of benzo[*a*]phenoxazinium salts

Carla M. A. Alves[1], Paulo J. G. Coutinho,[2] and M. Sameiro T. Gonçalves[1]

[1]*Centro de Química and* [2]*Centro de Física, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

E-mail: msameiro@quimica.uminho.pt

Abstract – Several fluorescent benzo[*a*]phenoxazinium chlorides possessing a long hydrocarbon chain terminated with methyl, hydroxyl, carboxylic acid or ester groups as substituent at the 5-amino function of the heterocycle were efficiently synthesised. Studies of their photophysical properties revealed that all compounds absorbed and emitted at longer wavelengths with moderate to good fluorescent quantum yields.

Keywords: Benzo[*a*]phenoxazines; Fluorescent labels; Functionalised fluorophores; Long-wavelength fluorophores

1. Introduction

In the last few years, the development of fluorescent dyes for labelling purposes of molecules with biological interest has attracted the interest of researchers.[1-2] A number of biological macromolecules has hydrophobic and hydrophilic zones. The presence of a long hydrocarbon chain in the fluorescence probe allow it easily bind to the hydrophobic parts of biomolecules enabling the fluorophore moiety to probe its environment.[3] Fluorophores with absorption and emission in longer wavelengths (600-1000 nm), including the benzophenoxazine class of dyes, showed minimal background interference from biological material as well as high sensitivity. [4,5]

Bearing this in mind and in connection with our research interest,[6,7] we report the synthesis and characterisation of several fluorescent benzo[*a*]phenoxazinium chlorides bearing long alkyl side-chains with apolar or functionalised ending-groups. These compounds absorbed in the range 616 to 638 nm and fluoresced at 655 nm or at about 677 nm, in ethanol.

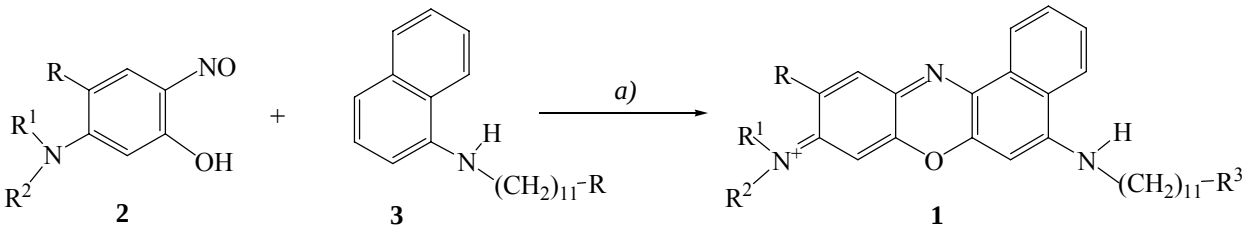
2. Results and Discussion

Benzo[*a*]phenoxazinium chlorides **1a-f** were synthesised by condensation of 5-alkylamino-2-nitrosophenol hydrochlorides **2a-c** with *N*-substituted-naphthylamines **3a-d** in an acidic medium

(Scheme 1). The required 5-alkylamino-2-nitrosophenol hydrochloride **2a-c** was synthesised using the usual procedure[8] involving treatment of the corresponding 3-alkylaminophenol with sodium nitrite in an acid solution. Intermediates **3a-c** were prepared by alkylation, in ethanol, of 1-naphthylamine with the appropriate bromo derivative, 1-bromododecane, 11-bromoundecan-1-ol and 12-bromododecanoic acid, respectively. Hydrolysis of the ester group of the intermediate **3c** (1M NaOH/1,4-dioxane), yielded the corresponding 12-(naphthalen-1-ylamino)dodecanoic acid **3d**. After dry chromatography purification or isolation by extraction (**3d**), these compounds were obtained as oils (**3a**, 44%; **3b**, 73%; **3c**, 70%, together with compound **3d** in 17%) or an oily solid (**3d** , 90%) and were characterised by high resolution mass spectrometry, IR and NMR (^{1}H and ^{13}C) spectroscopy.

Condensation of 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **1a**, 5-diethylamino-2-nitrosophenol hydrochloride **1b** and 5-dimethylamino-2-nitrosophenol hydrochloride **1c** with *N*-dodecylnaphthalen-1-amine **2a**, in the presence of hydrochloric acid, produced the benzo[*a*]phenoxazinium chlorides **3a-c**. The reaction of nitrosofenol **2a** with functionalised precursors **3b** and **3c**, also in the presence of hydrochloric acid, refluxed in ethanol, gave the corresponding benzo[*a*]phenoxazinium chlorides **1d** and **1e**. In the preparation of compound **1f**, the nitroso intermediate **2a** reacted with compound **3d**, in an acidic medium, using DMF as a solvent and heating at 70 ºC.

After purification by dry chromatography, cationic dyes **1a-f** were isolated as solid materials in moderate to high yields (Table 1) and were fully characterised by the usual analytical techniques.



2

a R = Me, R^1 = H, R^2 = Et
b R = H, R^1 = R^2 = Et
c R = H, R^1 = R^2 = Me

3

a R = Me
b R = OH
c R = CO_2Et
d R = CO_2H

1

a R = Me, R^1 = H, R^2 = Et, R^3 = Me
b R = H, R^1 = R^2 = Et, R^3 = Me
c R = H, R^1 = R^2 = R^3 = Me
d R = Me, R^1 = H, R^2 = Et, R^3 = OH
e R = Me, R^1 = H, R^2 = Et, R^3 = CO_2Et
f R = Me, R^1 = H, R^2 = Et, R^3 = CO_2H

Scheme 1. Synthesis of compounds **1a-f**. *Reagents and conditions: a)* H^+, ethanol, reflux or DMF, 70 ºC (**1f**).

Table 1. Synthesis, UV/ visible and fluorescence data for compounds **1a-f** in ethanol.

Compd	Yield [%]	λ_{max} [nm] (ε, $M^{-1}cm^{-1}$)	λ_{em} [nm]	Φ_F	Stokes' shift [nm]
1a	83	627 (63 128)	655	0.27	28
1b	69	638 (46 339)	677	0.09	39
1c	43	629 (55 551)	676	0.12	47
1d	64	616 (110 079)	654	0.29	38
1e	49	629 (57 595)	655	0.28	26
1f	33	625 (51 885)	655	0.24	30

Electronic absorption and emission spectra of 10^{-6} M solutions of benzo[*a*]phenoxazinium chlorides **1a-f** in degassed absolute ethanol were measured and the summarised data are presented in Table 1.

The longest wavelength of maximum absorption (λ_{max}) of all compounds was located between 616 and 638 nm, with molar absorptivities ranging from 46 339 to 110 079 $M^{-1}cm^{-1}$. Regarding fluorescence properties, the quantum yields (Φ_F) were calculated using Oxazine 1 as a standard (Φ_F = 0.11 in ethanol).[9] For the determination of relative quantum yields, Oxazine 1 was excited at 590 nm, the wavelength of maximum excitation found for each one of the compounds to be tested. Emission maxima (λ_{em}) for all compounds in ethanol was at about 655 nm (**1a**, **1d**, **1e** and **1f**) or at about 677 nm (**1b** and **1c**), the Stokes' shifts were from 26 to 47 nm. All compounds exhibited high levels of fluorescence, with Φ_F between 0.09 (**1b**) and 0.29 (**1d**); the highest values being associated with the monoethylamino substituent on the 9-position of the benzo[*a*]phenoxazine.

Non-functionalised and functionalised benzo[*a*]phenoxazinium chlorides were prepared through the usual procedure in moderate to high yields. Regarding their photophysical properties, namely longer wavelength of absorption and emission maxima in connection with the high fluorescence and moderate to good fluorescent quantum yields of all compounds, these cationic dyes are potential candidates to fluorescent non-covalent and/ or covalent labels of biomolecules.

3. Experimental

Typical procedure for the synthesis of **1a-e** (described for **1a**): To a cold solution (ice bath) of 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **2a** (0.087 g; 4.82×10^{-4} mol) in ethanol (2 mL), **3a** (0.151 g; 4.86×10^{-4} mol) and concentrated hydrochloride acid (5.0×10^{-2} mL) were added. The mixture was refluxed for 4 hours and monitored by TLC (silica: chloroform). The

solvent was removed under reduced pressure and the crude mixture was purified by dry chromatography on silica gel using dichloromethane/ *n*-hexane and dichloromethane/ methanol, mixtures of increasing polarity as the eluent. *N*-(5-(Dodecylamino)-10-methyl-9*H*-benzo[*a*]phenoxazin-9-ylidene)ethanaminium chloride (**1a**) was obtained as a blue solid (0.19 g, 83%). mp 93.1-96.0 ºC. R_f = 0.50 (silica: dichloromethane/ methanol, 9:1). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.88 (3 H, t *J* 6.9 Hz, $NH(CH_2)_{11}\underline{CH_3}$), 1.20-1.50 (21 H, 2×m, 9×CH_2 and $NHCH_2\underline{CH_3}$), 1.84 (2 H, broad s, $NHCH_2\underline{CH_2}$), 2.44 (3 H, s, CH_3), 3.10-3.30 (2 H, m, $NH\underline{CH_2}CH_2$), 3.52 (2 H, broad s, $NH\underline{CH_2}CH_3$), 6.19 (1 H, s, 8-H), 6.27 (1 H, s, 6-H), 7.47 (1 H, s, 11-H), 7.80-7.90 (2 H, m, 2-H and 3-H), 8.76-8.90 (1 H, m, 1-H), 9.10-9.24 (1 H, m, 4-H), 11.14 (1 H, broad s, NH) ppm. ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 13.82 ($NHCH_2\underline{CH_3}$), 14.04 ($NH(CH_2)_{11}\underline{CH_3}$), 18.27 ($CH_3$), 22.60 ($CH_2$), 27.12 ($CH_2$), 28.62 ($NHCH_2\underline{CH_2}$), 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 ($NH\underline{CH_2}CH_2$), 44.51 ($NH\underline{CH_2}CH_3$), 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) ppm. IR (KBr, 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 cm^{-1}. HRMS (FAB): calcd for $C_{31}H_{42}N_3O$ [M^+], 472.3328; found 472.3335.

Typical procedure for the synthesis of **3a-c** (described for **3a**): To a solution of 1-naphthylamine (2.0 g, 1.40 × 10^{-2} mol) in ethanol (3 mL), 1-bromododecane (3.55 mL; 1,47×10^{-3} mol) was added and the resulting mixture was refluxed for 11 hours, and monitored by TLC (silica: chloroform). The solvent was removed under reduced pressure and the crude mixture was purified by dry chromatography on silica gel using dichloromethane/ *n*-hexane 1:6, as the eluent. *N*-Octylnaphthalen-1-amine **3a** was obtained as a white oily solid (1.92 g, 44%). R_f = 0.63 (silica: dichloromethane/ *n*-hexane, 3:4). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.92 (3 H, t *J* 7.2 Hz, CH_3), 1.20-1.55 (18 H, 2×m, 9×CH_2), 1.70-1.90 (2 H, m, $NHCH_2\underline{CH_2}$), 3.30 (2 H, t *J* 7.5 Hz, $NH\underline{CH_2}CH_2$), 6.69 (1 H, d *J* 7.2 Hz, 4-H), 7.28 (1 H, d *J* 7.8 Hz, 2-H), 7.38 (1 H, t *J* 7.5 Hz, 3-H), 7.42-7.50 (2 H, m, 6-H and 7-H), 7.78-7.88 (2 H, m, 8-H and 5-H) ppm. ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 14.11 (CH_3), 22.68 (CH_2), 27.33 ($NHCH_2\underline{CH_2}$), 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 ($NH\underline{CH_2}CH_2$), 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) ppm. IR (nujol) 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 cm^{-1}. HRMS (EI): calcd for $C_{22}H_{33}N$ [M^+], 311.2613; found 311.2611.

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