Ultrasonic mediated synthesis of new benzo[*a*]phenoxazinium chlorides and their photophysical studies

B. Rama Raju[1], Diogo M. F. Sampaio[2], Paulo J. G. Coutinho[1], M. Sameiro T. Gonçalves[2*]

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

*e-mail:*msameiro@quimica.uminho.pt

Abstract: Ultrasonic irradiation was demonstrated to be an efficient technique for activating various organic transformations, allowing short reaction times, high yields and sometimes good selectivity. Three new benzo[*a*]phenoxazinium chlorides possessing isopentylamino, (2-cyclohexylethyl)amino and phenethylamino groups at 5-position of the heterocyclic systems were efficiently synthesized under ultrasound irradiation. Photophysical characterization in ethanol and water, as well as acid-base equilibrium studies of the probes were carried out.

Keywords: benzo[*a*]phenoxazines, ultrasonic irradiation, NIR fluorescent probes, Nile Blue derivatives.

Introduction

Fluorescent chromophores have immense applications in biological sciences, particularly in labeling for diagnosis and analysis.[1,2] Numerous reports reveal that a variety of commercially available dyes fluoresce in the visible region of the spectrum. However, long-wavelength fluorophores (600-1000 nm) are preferred for biological applications which can account their minimum interference from absorption scattering and the natural auto-fluorescence of biological molecules.[3,4] In this context, oxazine derivatives, such as phenoxazines and benzo[*a*]phenoxazines have been reported for various spectroscopic research studies in the near-infrared region.[5]

Ultrasonic irradiation has been considered as a useful and clean protocol in organic synthesis as

compared with the traditional methods due to its convenience. Moreover, a large number of organic transformations can be carried out in short reaction times, high yield or milder conditions under ultrasonic irradiation.[6] Considering these facts and in continuation of our research interests in the synthesis of organic fluorophores,[7-9] we wish to herein report for the first time the use of ultrasound irradiation in the efficient preparation of a new set of benzo[*a*]phenoxazines. Fundamental photophysical characterization of these compounds was carried out.

Experimental

Typical procedure for the synthesis of 3a-c (described for **3a**).

To a solution of naphthalen-1-amine **1** (0.500 g, 3.50 $\times 10^{-3}$ mol) in ethanol (2 mL), 1-bromo-3-methylbutane (0.580 g, 3.84×10^{-3} mol) was added, and the resulting mixture was refluxed for 14 hours. The progress of reaction was monitored by TLC (dichloromethane/methanol, 9.5:0.5). After completion of the reaction, the solvent was evaporated and the mixture was purified by column chromatography on silica using dichloromethane and dichloromethane/methanol (99:1), as the eluent. *N*-isopentylnaphthalen-1-amine **3a** was obtained as violet oil (0.401 g, 54%). TLC (dichloromethane/methanol, 9.9:0.1): R_f = 0.54. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 1.00 (d, J = 6.4 Hz, 6H, 2×$NHCH_2CH_2CH(\underline{CH_3}_2)$), 1.70-1.86 (m, 3H, $NHCH_2CH_2\underline{CH}(CH_3)_2$ and $NHCH_2\underline{CH_2}CH(CH_3)_2$), 3.39 (t, J = 7.2 Hz, 2H, $NH\underline{CH_2}CH_2CH(CH_3)_2$), 3.50 (br s, 1H, NH), 7.05 (dd, 1H, J = 6.2 and 2.4 Hz, 2-H), 7.40-7.46 (m, 2H, 3-H and 4-H), 7.49-7.56 (m, 2H, 7-H and 6-H), 7.85 (dd, J = 7.0 and 2.0 Hz, 1H, 5-H), 8.02-8.08 (dd, 1H, J = 7.2 and 2.0 Hz, 8-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 22.25 ($NHCH_2CH_2CH(\underline{CH_3}_2)$), 25.90 ($NHCH_2CH_2\underline{CH}(CH_3)_2$), 36.90 ($NHCH_2\underline{CH_2}CH(CH_3)_2$), 44.54 ($NH\underline{CH_2}CH_2CH(CH_3)_2$), 108.82 (C-2), 120.11 (C-3), 120.27 (C-8), 123.80 (C-4a), 125.23 (C-7), 125.78 (C-6), 125.98 (C-4), 128.44 (C-5), 134.10 (C-8a), 139.99 (C-1). IR (KBr 1%, cm^{-1}): ν = 3424, 3049, 2926, 2848, 2823, 1623, 1581, 1527, 1471, 1445, 1410, 1383, 1345, 1324, 1289, 1264, 1174, 1143, 1121, 1099, 1033, 784, 765. HRMS: m/z (EI): calcd. for $C_{15}H_{19}N$ [M^+] 213.1517; found 213.1519.

In the above reaction, *N*,*N*-diisopentylnaphthalen-1-amine **3a** was also isolated as brown oil (0.162 g, 17%). TLC (dichloromethane/methanol, 9.5:0.5): R_f = 0.71. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 0.85 (d,

J = 6.6 Hz, 12H, N($CH_2CH_2CH(\underline{CH_3})_2)_2$), 1.35-1.49 (m, 4H, N($CH_2\underline{CH_2}CH(CH_3)_2)_2$), 1.51-1.62 (m, 2H, N($CH_2CH_2\underline{CH}(CH_3)_2)_2$), 3.10-3.20 (m, 4H, N($\underline{CH_2}CH_2CH(CH_3)_2)_2$), 7.16 (d, J = 7.2 Hz, 2-H), 7.41 (t, J = 7.8 Hz, 1H, 3-H), 7.43-7.52 (2H, m, 7-H and 6-H), 7.55 (d, J = 7.8 Hz, 1H, 4-H), 7.78-7.88 (m, 1H, 8-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 22.69 N($CH_2CH_2CH(\underline{CH_3})_2)_2$), 26.29 N($CH_2CH_2\underline{C}H(CH_3)_2)_2$), 36.0 (N($CH_2\underline{CH_2}CH(CH_3)_2)_2$), 52.52 (N($CH_2\underline{CH_2}CH(CH_3)_2)_2$), 117.74 (C-2), 123.03 (C-4), 124.23 (C-8), 124.99 (C-3), 125.47 (C-7), 125.57 (C-6), 128.08 (C-5), 131.04 (C-4a), 134.83 (C-8a), 148.64 (C-1). HRMS: m/z (EI): calcd. for $C_{20}H_{29}N$ [M^+] 283.2300; found 283.2310.

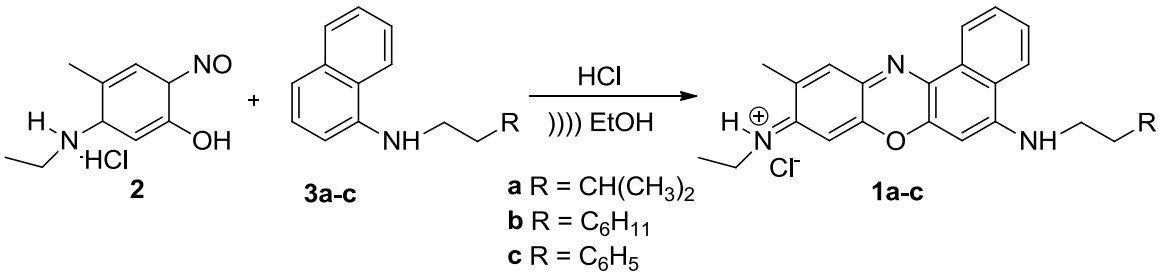
Typical procedure for the preparation of 1a-c (described for **1a**).

To a cold solution (ice bath) of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **2** (0.050 g, 2.7×10^{-4} mol), in ethanol (1.5 mL), *N*-isopentylnaphthalen-1-amine **3a** (0.030 g, 1.37×10^{-4} mol), and concentrated hydrochloride acid (7.0×10^{-3} mL) were added. The mixture was sonicated for a period of 1.5 hours, and monitored by TLC (dichloromethane/methanol, 9.5:0.5). After evaporation of the solvent and column chromatography purification on silica gel with dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, *N*-(5-(isopentylamino)-10-methyl-9*H*-benzo[*a*]phenoxazin-9-ylidene)ethanaminium chloride **1a** was obtained as a blue solid (0.046 g, 90%). ^{1}H NMR (CD_3OD, 400 MHz): δ_H = 1.09 (d, J = 6.4 Hz, 6H, NH($CH_2CH_2CH(\underline{CH_3})_2$), 1.40 (t, J = 7.2 Hz, 3H, $NHCH_2\underline{CH_3}$), 1.73-1.90 (m, 3H, $NCH_2\underline{CH_2}CH(CH_3)_2$ and N($CH_2CH_2\underline{CH}(CH_3)_2)_2$), 2.35 (s, 3H, CH_3), 3.54 (q, J = 7.2 Hz, 2H, $NH\underline{CH_2}CH_3$), 3.72 (t, J = 8.0 Hz, 2H, $NH\underline{CH_2}CH_2CH(CH_3)_2$), 6.84 (s, 1H, 8-H), 6.90 (s, 1H, 6-H), 7.66 (s, 1H, 11-H), 7.81 (dt, J = 7.8 and 1.8 Hz, 1H, 3-H), 7.91 (dt, J = 8.4 and 1.2 Hz, 1H, 2-H), 8.34 (d, J = 8.4 Hz, 1H, 1-H), 8.89 (dd, J = 8.0 and 0.8 Hz, 1H, 4-H). ^{13}C NMR (CD_3OD, 100.6 MHz): δc = 14.15 ($NCH_2\underline{CH_3}$), 17.68 ($\underline{CH_3}$), 22.85 ($NHCH_2CH_2CH(\underline{CH_3})_2$), 27.44 $NHCH_2CH_2\underline{CH}(CH_3)_2$), 38.33 ($NHCH_2\underline{CH_2}CH(CH_3)_2$), 39.72 ($NH\underline{CH_2}CH_3$), 44.14 ($NH\underline{CH_2}CH_2CH(CH_3)_2$), 93.88 (C-6), 94.54 (C-8), 123.70 (C-1), 124.78 (Ar-C), 125.55 (C-4), 128.69 (Ar-C), 130.80 (C-3), 132.07 (Ar-C), 132.54 (Ar-C), 132.71 (C-2), 132.82. (C-11), 134.48 (Ar-C), 149.39 (Ar-C), 152.97 (Ar-C), 156.70 (C-9), 158.60 (C-5). IR (KBr 1%, cm^{-1}): ν =3239, 2926, 1644, 1592, 1565, 1546, 1520, 1450, 1435, 1318, 1296, 1261, 1187, 1164, 1125, 1000, 876. HRMS: m/z (ESI): calcd. for $C_{24}H_{28}N_3O$ $[M+H]^+$ 374.2227; found 374.2222.

Results and discussion

Benzo[*a*]phenoxazinium chlorides **1a-c** were synthesised by condensation of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **2** with *N*-alkylated naphthalen-1-amines **3a-c** in acid media. Intermediates **3a-c** were obtained by alkylation of naphthalen-1-amine with their corresponding alkyl and aryl bromo derivatives in ethanol as the solvent, in low to moderate yields. The required 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **2** was obtained by nitrosation of the corresponding 3-(ethylamino)-4-methylphenol with sodium nitrite in the presence of hydrochloric acid, in a mixture of ethanol-water as the solvent.[10]

The cyclisation reaction of *N*-isopentylnaphthalen-1-amine **3a**, *N*-(2-cyclohexylethyl)naphthalen-1-amine **3b** and *N*-phenethylnaphthalen-1-amine **3c** with 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **2** occurred in the presence of concentrated hydrochloric acid under ultrasonic irradiation in ethanol as the solvent. Purification by silica gel column chromatography gave *N*-(5-(isopentylamino)-10-methyl-, *N*-(5-((2-cyclohexylethyl)amino)-10-methyl-, *N*-(10-methyl-5-(phenethylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)ethanaminium chlorides **1a-c** as blue solids in excellent yields (90-93%, Scheme 1). By using conventional oil bath heating instead of ultrasound irradiation, compound **1a** was also synthesized in excellent yield, but the reaction time increased more than five times (from 1.5 to 8 hours). All compounds obtained were fully characterized by the usual analytical techniques.



Scheme 1. Synthesis of benzo[*a*]phenoxazinium chlorides **1a-c**

The ^{1}H NMR spectra showed the signals of aliphatic protons from the methylenic groups of substituents of positions 5 and 9, directly linked to the nitrogen atom NH$\underline{CH_2}$ that appeared as triplets or a broad singlet (**1b**) (δ 3.72 to 3.91 ppm), as well as groups closed to the same atom, NHCH$_2\underline{CH_2}$, showed as multiplets (δ 1.70 to 1.90 ppm) or a triplet (**1c**, δ 3.16 ppm). The CH proton occurred as a

multiplet (**1a**) or broad singlet (**1b**) (δ 1.53 to 1.90 ppm). The methyl protons of ethyl and isopentyl (**1a**) groups in amines of positions 5 and 9 of the heterocycle appeared as triplets (δ ~ 1.40 ppm) and doublets (isopentyl group of **1a,** δ 1.09 ppm). There was also the presence of protons of the methyl group directly linked to the aromatic ring at position 10, which appeared as singlets (δ 2.30 to 2.36 ppm). In addition, spectra showed the expected aromatic protons of the polycyclic system, in particular H-8 (δ 6.73 to 6.86 ppm), H-6 (δ 6.76 to 6.90 ppm), and H-11 (δ 7.66 to 7.74 ppm), which appeared in the form of singlets.

The ^{13}C NMR spectra showed the signals of the methylenic groups of substituents of positions 5 and 9, directly linked to the nitrogen atom NH$\underline{CH_2}$ (δ 39.72 to 47.0 ppm), as well as groups closed to the same atom, NHCH$_2\underline{CH_2}$ (δ 43.70 to 47.00 ppm) and the CH carbon (δ 27.44 or 36.98 ppm). The methyl carbons of ethyl and isopentyl (**1a**) groups in amines of positions 5 and 9 of the heterocycle also appeared (δ 14.15 to 22.85 ppm). In addition, there was the presence of carbons of the methyl group directly linked to the aromatic ring at position 10 (δ 17.66 to 17.72 ppm). Spectra showed the expected aromatic carbons, in particular C-8 (δ 94.48 to 94.55 ppm); C-6 (δ 93.88 to 93.96 ppm) and C-11 (δ 132.76 to 132.85 ppm).

Fundamental photophysical studies of the fluorescent properties of benzo[*a*]phenoxazinium chlorides **1a** and **1b** were carried out in ethanol, and distilled water. The longest wavelength of maximum absorption (λ_{abs}) of both compounds in the two solvents was located in the region 621-629 nm. The molar absorptivities of these compounds displayed excellent values in ethanol, and decreased in water. Maximum emission wavelengths (λ_{em}) and relative fluorescence quantum yields (Φ_F) were obtained and are summarised in Table 1. For the determination of quantum yields, Oxazine 1, used as a standard (Φ_F = 0.11 in ethanol),[11] was excited at the wavelength of excitation of each compound. In both solvents, benzo[*a*]phenoxazinium salts displayed λ_{em} in the region of 642-651 nm, and fluorescence quantum yields that are superior in ethanol.

Previous studies of benzo[*a*]phenoxazinium salts showed that its photophysics in proton-accepting solvents is influenced by acid-base equilibrium mainly located at the 5-amino position.[7-9] In the case of compounds **1a** and **1b**, only the acid form was observed in ethanol and also in water. However, the sensitivity to acid and basic media was studied in both solvents by using trifluoroacetic acid (TFA) and tetraethylammonium hydroxide (TEAH), respectively. The presence of the acid did not affect significantly λ_{abs} and λ_{em} values, and fluorescence quantum yields are similar or superior (except for **1b** in water). In basic medium a hypsochromic shift in the absorption and emission in ethanol and water

was observed. The Stokes' shifts were superior ($\Delta\lambda$> 100 nm) to those obtained in the absence of TEAH, and as it was expected, the basic form display low fluorescence, being the best Φ_F value 0.08 (compound **1b**, in ethanol).

Table 1. Preliminary photophysical studies of compounds **1a** and **1b** in ethanol, water and after the addition of TFA and TEAH.

Solvent	λ_{abs} (nm)		λ_{em} (nm)		Φ_F	
	1a	**1b**	**1a**	**1b**	**1a**	**1b**
Ethanol	629	628	644	642	0.56	0.67
Water	621	625	651	649	0.14	0.18
Ethanol + TFA	629	628	643	642	0.55	0.71
Ethanol + TEAH	496	502	608	603	0.04	0.08
Water + TFA	623	568	651	649	0.16	0.05
Water + TEAH	456	439	590	568	0.002	0.004

Conclusions

Ultrasonic irradiation was used for the first time in the efficient synthesis of benzo[*a*]phenoxazinium chlorides. The main advantage of this protocol is the reduction of reaction time along with excellent yields of the expected products. Considering the water-solubility and the photophysical characteristics, namely absorption and fluorescent emission in the range 621-651 nm, with good relative fluorescent quantum yields (acid form), the benzo[*a*]phenoxazinium chlorides synthesized can be considered good candidates as long-wavelength fluorescent probes of biomolecules.

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