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Ultrasound promoted synthesis of Nile Blue derivatives

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ABSTRACT

Ultrasound irradiation was used for the first time towards the synthesis of new Nile Blue related benzo[*a*]phenoxazinium chlorides possessing isopentylamino, (2-cyclohexylethyl)amino and phenethylamino groups at 5-position of the heterocyclic system. The efficacy of sonochemistry was investigated with some of our earlier reported synthesis of benzo[*a*]phenoxazinium chlorides. This newer protocol proved competent in terms of reaction times and enhanced yields. Photophysical studies carried out in ethanol, water and simulated physiological conditions, revealed that emission maxima occurred in the range 644–656 nm, with high fluorescent quantum yields. Other attractive feature exhibited by these materials includes good thermal stability. These properties might be useful in the development of fluorescent probes for biotechnology.

1. Introduction

Fluorescence probes serve as an indispensable tool for cellular imaging, determination of amino acids, proteins, DNA sequencing, and fragment analysis in bioanalytical sciences [1–3]. Among these, oxazine derivatives have been employed as standards in fluorescence measurements of biological stains [4,5]. Phenoxazines and its derivatives are also useful as tranquillisers [6], multidrug resistance modulators in cancer cells [7], drugs for antitumor effects, disregulation of cell cycle, and apoptosis induction in HTLV-I-positive leukaemia cells [8]. Benzo[*a*]phenoxazinium chlorides belonging to the family of phenoxazines are quite attractive due to their high fluorescent quantum yields at long wavelengths (above 600 nm), low triplet yields and good photostabilities [9]. Moreover, the benzo[*a*]phenoxazinium chlorides can also function as covalent probes for organic and biological molecules, namely amino acids [10], proteins [11], peptides and DNA [12], as well as in the non-covalent labelling of nucleic acids in monitoring protein conformations and alterations for therapeutic purposes [13,14]. In addition, these compounds function as potential photosensitizers with cancer and broad antimicrobial photodynamic activities [15,16], also act as antimicrobial and antimalarial agents [17–19].

Ultrasound irradiation is a well established tool which provides specific activation based on a physical phenomenon: acoustic cavitation, and can be use as an alternative source for organic reactions carried out under ordinary heating conditions [20–25]. Moreover, numerous organic transformations can be performed in improved yields, shorter reaction times, increased selectivities or milder conditions with ultrasonic irradiation [26–35].

In continuation of our research interests in the synthesis and applications of oxazine based heterocycles, as well as in alternative green synthetic protocols [10,17,36–42], the present work describes for the first time an efficient synthesis of benzo[*a*]phenoxazinium chlorides by ultrasound-mediated condensation, in comparison with conventional heating conditions. The new benzo[*a*]phenoxazinium chlorides synthesized possesses isopentylamino, (2-cyclohexylethyl)amino and phenethylamino groups at 5-position of the heterocyclic system. Fundamental photophysical studies of the synthesised cationic fluorophores were carried out in ethanol, water and physiological pH. In addition, thermal stability by differential scanning calorimetry was measured for all compounds.

2. Experimental

2.1. General

All melting points were measured on a Stuart SMP3 melting point apparatus. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60F_{254}) and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer. UV–Vis–NIR absorption spectra (200–700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. NMR spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as

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internal reference at 25 °C. All chemical shifts are given in ppm and *J* values are given in Hz. Assignments were carried out through the comparison of chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. Low and high resolution mass spectrometry analyses were performed at the "C.A.C.T.I. – Unidad de Espectrometria de Masas", at the University of Vigo, Spain. Fluorescence spectra were collected using a Spex Fluorolog spectrofluorometer. Ultrassonic assisted reactions were carried out in an ultrasonic instrument set-up (horn type), "Misonix S-4000 Sonicator", USA, on an operating frequency of 20 kHz, operating amplitude: 80; maximum power output: 600 W; bath temperature: 62–64 °C; horn of titanium alloy with dimensions: 12.7 cm (length) × 3.8 cm (diameter); ultrasound irradiating face diameter: 5.1 cm. Differential scanning calorimetric (DSC) analyses were performed in an 821e Mettler Differential Scanning Calorimeter. Commercially available reagents were used as received.

5-(Ethylamino)-4-methyl-2-nitrosophenol hydrochloride (**1**) was obtained by reaction of 3-(ethylamino)-4-methylphenol (1.5 mmol) in ethanol (2 mL) and 12 M hydrochloric acid (0.4 mL) with sodium nitrite (1.2 equiv.), under low temperature (ice-bath), with stirring for 3 h, followed by evaporation of the reaction mixture. This compound was used in the synthesis of benzo[*a*]phenoxazinium chlorides **3a**–**g** without any purification, as previously reported [10,17,36–41,43].

Ethyl 4-(naphthalen-1-ylamino)butanoate (**2d**) and 3-(naphthalen-1-ylamino)propan-1-ol (**2f**) were prepared by reaction of naphthalen-1-amine (**2g**) with ethyl-4-bromobutyrate (1.05 equiv.) or 3-bromo-1-propanol (1.05 equiv.) in ethanol, under reflux conditions during 15 h, followed by silica gel dry chromatography purification as previously reported [10]. 4-(Naphthalen-1-ylamino)butanoic acid (**2e**) resulted from the reaction of compound **2d** with aqueous 1 M sodium hydroxide (1.8 equiv.) in 1,4-dioxane, at room temperature with stirring for 8 h, followed by acidification to pH 2–3 and extraction of the reaction mixture as previously described [10].

2.2. Preparation of precursors ***2a–c***

2.2.1. *N-isopentyl-naphthalen-1-amine (**2a**)*

To a solution of naphthalen-1-amine (0.500 g, 3.50 mmol) in ethanol (2 mL), 1-bromo-3-methylbutane (0.580 g, 3.84 mmol) was added, and the resulting mixture was refluxed for 14 h. 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 gel using dichloromethane and dichloromethane/methanol 99:1, as the eluent. Compound **2a** 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, $NHCH_2CH_2CH(CH_3)_2$), 1.70–1.86 (m, 3H, $NHCH_2CH_2CH(CH_3)_2$ and $NHCH_2CH_2CH(CH_3)_2$), 3.39 (t, *J* = 7.2 Hz, 2H, $NHCH_2CH_2CH(CH_3)_2$), 3.50 (br s, 1H, NH), 7.05 (dd, *J* = 6.2 and 2.4 Hz, 1H, 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, *J* = 7.2 and 2.0 Hz, 1H, 8-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 22.3 ($NHCH_2CH_2CH(CH_3)_2$), 25.9 ($NHCH_2CH_2CH(CH_3)_2$), 36.9 ($NHCH_2CH_2CH(CH_3)_2$), 44.5 ($NHCH_2CH_2CH(CH_3)_2$), 108.8 (C-2), 120.1 (C-3), 120.3 (C-8), 123.8 (C-4a), 125.2 (C-7), 125.8 (C-6), 126.0 (C-4), 128.4 (C-5), 134.1 (C-8a), 140.0 (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, along with **2a**, *N,N-bis(isopentyl)naphthalen-1-amine* was also isolated as brown oil (0.162 g, 17%). TLC (dichloromethane/methanol, 9.9:0.1): R_f = 0.71. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 0.85 (d, *J* = 6.6 Hz, 12H, $N(CH_2CH_2CH(CH_3)_2)_2$), 1.35–1.49 (m, 4H, $N(CH_2CH_2CH(CH_3)_2)_2$), 1.51–1.62 (m, 2H, $N(CH_2CH_2CH(CH_3)_2)_2$), 3.10–3.20 (m, 4H, $N(CH_2CH_2CH(CH_3)_2)_2$), 7.16 (d, *J* = 7.2 Hz, 2-H), 7.41 (t, *J* = 7.8 Hz, 1H, 3-H), 7.43–7.52 (m, 2H, 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 = 22.7 ($N(CH_2CH_2CH(CH_3)_2)_2$), 26.3 $N(CH_2CH_2CH(CH_3)_2)_2$), 36.0 ($N(CH_2CH_2CH(CH_3)_2)_2$), 52.5 ($N(CH_2CH_2CH(CH_3)_2)_2$), 117.7 (C-2), 123.0 (C-4), 124.2 (C-8), 125.0 (C-3), 125.5 (C-7), 125.6 (C-6), 128.1 (C-5), 131.0 (C-4a), 134.8 (C-8a), 148.6 (C-1). IR (KBr 1%, cm^{-1}): *ν* = 3056, 2956, 2926, 2868, 1624, 1583, 1527, 1477, 1409, 1379, 1344, 1286, 1225, 1144, 1108, 767, 570. HRMS: m/z (EI): calcd. for $C_{20}H_{29}N$ [M^+] 283.2300; found 283.2310.

2.2.2. *Synthesis of N-(2-cyclohexylethyl)naphthalen-1-amine (**2b**)*

Starting from naphthalen-1-amine (0.500 g, 3.50 mmol) in ethanol (2 mL), using (2-bromoethyl)cyclohexane (0.735 g, 3.84 mmol), and following the same procedure described before for the preparation of **2a** (reflux time 17 h), compound **2b** was obtained as brown solid (0.260 g, 28%), m.p. 84.7–86.7 °C. TLC (chloroform/methanol, 9.5:0.5): R_f = 0.60. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 1.04–1.17 (m, 2H, 2 × CH Cy), 1.23–1.46 (m, 3H, 3 × CH Cy), 1.50–1.63 (m, 1H, $NHCH_2CH_2CH$), 1.75 (q, *J* = 6.8 Hz, 2H, $NHCH_2CH_2$), 1.80–1.95 (m, 5H, 5 × CH Cy), 3.36 (t, *J* = 7.6 Hz, 2H, $NHCH_2CH_2$), 4.33 (broad s, 1H, NH), 6.71 (d, *J* = 7.6 Hz, 2-H), 7.33 (d, *J* = 8.4 Hz, 1H, 4-H), 7.46 (d, *J* = 7.8 Hz, 1H, 3-H), 7.49–7.57 (m, 2H, 6-H and 7-H), 7.86–7.93 (m, 2H, 8-H and 5-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 26.3 (2 × CH_2 Cy), 26.5 (CH_2 Cy), 33.4 ($NHCH_2CH_2CH$), 35.7 ($NHCH_2CH_2CH$), 37.0 (2 × CH_2 Cy), 41.9 (($NHCH_2CH_2$), 104.1 (C-2), 117.0 (C-4), 119.8 (C-8), 123.3 (C-8a), 124.5 (C-7), 125.6 (C-6), 126.6 (C-3), 128.6 (C-5), 134.3 (C-4a), 143.6 (C-1). IR (KBr 1%, cm^{-1}): *ν* = 3385, 3054, 3010, 2956, 2927, 2869, 2717, 2454, 1623, 1604, 1582, 1528, 1478, 1468, 1410, 1378, 1368, 1345, 1286, 1253, 1224, 1170, 1143, 1108, 1081, 1021, 951, 863, 800, 784, 768, 665. HRMS: m/z (EI): calcd. for $C_{18}H_{23}N$ [M^+] 253.1830; found 253.1838.

In the above reaction, along with **2b**, *N,N-bis(2-cyclohexylethyl)naphthalen-1-amine* was also isolated as colourless oil (0.089 g, 7%). TLC (dichloromethane/methanol, 9.5:0.5): R_f = 0.74. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 0.83 (m, 4H, 4 × CH Cy), 1.17–1.24 (m, 6H, 6 × CH Cy), 1.25–1.34 (m, 2H, $N(CH_2CH_2CH)_2$), 1.38–1.48 (m, 4H, $N(CH_2CH_2CH)_2$), 1.60–1.74 (m, 10H, 10 × CH Cy), 3.14–3.23 (m, 4H, $N(CH_2CH_2CH)_2$), 7.17 (d, *J* = 8.4 Hz, 1H, 2-H), 7.42 (t, *J* = 8.0 Hz, 1H, 3-H), 7.45–7.52 (m, 2H, 6-H and 7-H), 7.56 (d, *J* = 8.4 Hz, 1H, 4-H), 7.81–7.87 (m, 1H, 5-H), 8.29–8.36 (m, 1H, 8-H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 26.3 (4 × CH_2 Cy), 26.6 (2 × CH_2 Cy), 33.4 (4 × CH_2 Cy), 34.5 ($N(CH_2CH_2CH)_2$), 36.0 ($N(CH_2CH_2CH)_2$), 52.0 ($N(CH_2CH_2CH)_2$), 117.7 (C-2), 123.0 (C-4), 124.3 (C-8), 124.9 (C-7), 125.5 (C-6), 125.6 (C-3), 128.1 (C-5), 131.1 (C-8a), 134.9 (C-4a), 148.8 (C-1). IR (KBr 1%, cm^{-1}): *ν* = 3436, 3046, 2922, 2849, 1576, 1526, 1506, 1448, 1401, 1381, 1344, 1263, 1147, 1119, 1082, 1052, 1016, 962, 886, 843, 774, 614. HRMS: m/z (EI): calcd. for $C_{26}H_{37}N$ [M^+] 363.2926; found 363.2931.

2.2.3. *Synthesis of N-(phenethyl)naphthalen-1-amine (**2c**)*

Starting from naphthalen-1-amine (0.500 g, 3.50 mmol) in ethanol (2 mL), using (2-bromoethyl)benzene (0.710 g, 3.84 mmol), and following the same procedure described before for the preparation of **2a** (reflux time 15 h), compound **2c** was obtained as violet solid (0.268 g, 31%). TLC (chloroform/methanol, 9.5:0.5): R_f = 0.69. m.p. 80.8–82.8 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 3.13 (t, *J* = 7.2 Hz, 2H, $NHCH_2CH_2Ph$), 3.62 (t, *J* = 6.9 Hz, 2H, $NHCH_2CH_2Ph$), 4.20 (br s, 1H, NH), 6.77 (d, *J* = 7.5 Hz, 1H, 2-H), 7.32–7.52 (m, 9H,