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Synthesis of the first thieno-δ-carboline Fluorescence studies in solution and in lipid vesicles

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Abstract

The first thieno-δ-carboline (6,8,9-trimethyl-5*H*-pyrido[3,2-*b*]thieno[3,2-*f*]indole) was prepared in good yield (70%) by intramolecular palladium-assisted cyclization of an *ortho*-chlorodiarylamine. The latter was in turn selectively synthesized in high yield (90%) by C–N palladium-catalyzed cross-coupling of 3-bromo-2-chloropyridine with, the also prepared, 6-amino-2,3,7-trimethylbenzo[*b*]thiophene. Fluorescence studies in solution show that thieno-δ-carboline has a solvatochromic behaviour. Despite the low fluorescence quantum yields in solution, studies of its incorporation in lipid vesicles of DPPC, DOPE and DODAB indicate that thienocarboline is located mainly inside the lipid bilayer, exhibiting different behaviours in gel or liquid-crystalline phases. Our studies are useful for the incorporation of thienocarboline in liposomes and for controlled drug release assays, due to its biological activity.

Keywords: Palladium-assisted reactions; Thieno-δ-carboline; Fluorescence; Solvatochromic probes; Lipid vesicles

1. Introduction

δ-Carbolines are very rare in nature and the best representatives of this family are the benzo-δ-carbolines, quindoline and cryptolepine (Fig. 1), two indoloquinoline alkaloids isolated in 1977 and 1929, respectively, from a west African plant, *Cryptolepis sanguinolenta*, but they are also found in other plants. Considerable interest in this family has been shown by several research groups due to their various and important biological activities [1].

The anti-tumor activity of cryptolepine was also reported by DNA intercalation and interaction with Topoisomerase II [2].

The electronic spectra and photophysics of δ-carboline were recently reported and discussed [3].

In recent years we have been interested in the synthesis of tetracyclic heteroaromatic compounds derivatives of benzo[*b*]thiophenes, analogues of natural anti-tumoral pyridocarbazoles (ellipticine and olivacine), namely thienocarbazoles [4,5]. The photochemistry and photophysics of some of the thienocarbazoles prepared, were already studied by some of us [6].

Here we present the synthesis by palladium-assisted reactions, of the first thieno-δ-carboline and its fluorescence properties in different solvents and in lipid vesicles. Fluorescence spectra of this compound exhibit a strong dependence with solvent polarity, so that it may be considered as a solvatochromic probe. This type of probes has found many applications in areas of biology [7], namely as probes for proteins [8–10], micelles and microemulsions [11–15], and lipid membranes [16–18]. Our results of thienocarboline incorporation in lipid vesicles show that this compound is located mainly inside the bilayer.

Recent studies on the antiproliferative activity of our thieno-δ-carboline in a panel of human tumor cell lines showed a remarkable cytotoxicity upon UVA irradiation [19]. Like its natural analogues (Fig. 1) our compound may exhibit various biological activities and it may be carried as drug in the hydrophobic region of liposomes.

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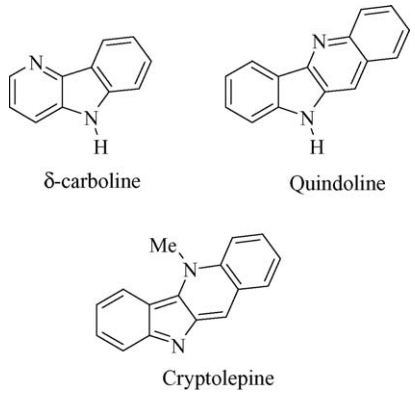


Fig. 1. Structures of some δ-carboline derivatives.

2. Experimental

2.1. Synthesis

2.1.1. General remarks

Melting points were determined on a Gallenkamp apparatus and are uncorrected. The ^{1}H NMR spectra were measured on a Varian Unity Plus at 300 MHz. TMS was used as internal reference. Spin–spin decoupling techniques were used to assign the signals. The ^{13}C NMR spectra were measured in the same instrument at 75.4 MHz (using DEPT θ 45°). Elemental analyses were determined on a LECO CHNS 932 elemental analyser. Mass spectra (EI) and HRMS were made by the mass spectrometry service of University of Vigo-Spain.

Column chromatography was performed on Macherey–Nagel silica gel 230–400 mesh. Petroleum ether refers to the boiling range 40–60 °C. Ether refers to diethyl ether. Chloroform was used in the hotte. P(*t*-Bu)$_3$ was purchased from Strem as an hexane solution. *rac*.BINAP refers to racemic-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene.

2.1.2. N-(2,3,7-trimethylbenzo[b]thien-6-yl)benzophenone imine and 6-amino-2,3,7-trimethylbenzo[b]thiophene

A dry *Schlenk* tube was charged, under Ar, with dry toluene (6 mL), 6-bromo-2,3,7-trimethylbenzo[*b*]thiophene (1.00 g, 3.92 mmol), Pd(OAc)$_2$ (3 mol%), *rac*.BINAP (4 mol%), Cs_2CO_3 (1.4 equiv.), benzophenone imine (1.2 equiv.) and the mixture was heated at 100 °C for 22 h. Water and ether were added after cooling and the phases were separated. The aqueous phase was extracted with more ether and the organic extracts were collected, dried ($MgSO_4$) and filtered. Removal of the solvent gave *N-(2,3,7-trimethylbenzo*[*b*]*thien-6-yl)benzophenone imine* as a yellow solid after several washes with MeOH (1.36 g, quantitative yield), mp 169–171 °C. ^{1}H NMR ($CDCl_3$) 2.21 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.53 (d, J = 8.4 Hz, 1H, ArH), 7.10–7.51 (m, 9H, ArH), 7.80–7.84 (m, 2H, ArH) ppm. ^{13}C NMR ($CDCl_3$) 11.39 (CH_3), 13.74 (CH_3), 16.12 (CH_3), 117.74 (CH), 118.52 (CH), 121.13 (C), 127.65 (C), 127.92 (CH), 128.14 (CH), 128.57 (CH), 129.04 (CH), 129.32 (CH), 130.60 (CH), 131.42 (C), 136.56 (C), 136.97 (C), 139.13 (C), 139.63 (C), 145.51 (C), 167.93 (C) ppm. Anal. Calcd. for $C_{24}H_{21}NS$: C 81.09, H 5.95, N 3.94, S 9.02; found: C 81.05, H 5.85, N 3.92, S 8.97%. To this compound (3.84 mmol) THF (15 mL) and HCl 2 M (3 mL) were added and the mixture was stirred at room temperature for 15 min. HCl 0.5 M (10 mL) and hexane/ethyl acetate 2:1 (6 mL) were added. A precipitate came off and filtration gave a white solid which was then stirred with 20 mL of NaOH 30% for 2 h. Chloroform was added (50 mL) and the phases were separated, the aqueous phase was extracted with more $CHCl_3$ and the organic phases gave, after drying ($MgSO_4$), filtration and removal of the solvent, *6-amino-2,3,7-trimethylbenzo*[*b*]*thiophene* as a colourless solid (0.442 g, overall yield 60%), mp 90–92 °C. ^{1}H NMR ($CDCl_3$) 2.26 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.63 (s, 2H, NH_2), 6.79 (d, J = 8.4 Hz, 1H, H-5), 7.28 (d, J = 8.4 Hz, 1H, H-4) ppm. ^{13}C NMR ($CDCl_3$) 11.37 (CH_3), 13.58 (CH_3), 15.15 (CH_3), 114.03 (CH), 114.52 (C), 119.23 (CH), 127.47 (C), 129.27 (C), 133.82 (C), 140.03 (C), 140.27 (C) ppm. Anal. Calcd. for $C_{11}H_{13}NS$: C 69.07, H 6.85, N 7.32, S 16.76; found: C 68.81, H 6.94, N 7.30, S 16.43%.

2.1.3. 2-Chloro-N-(2,3,7-trimethylbenzo[b]thien-6-yl)pyridin-3-amine (**1**)

In a dry *Schlenk* tube it was poured under Ar with stirring, dry toluene (3 mL), 3-bromo-2-chloropyridine (162 mg, 0.840 mmol), Pd(OAc)$_2$ (5 mol%), P(*t*-Bu)$_3$ (7 mol%), NaO*t*-Bu (5 equiv.) and 6-amino-2,3,7-trimethylbenzo[*b*]thiophene (160 mg, 0.840 mmol). The mixture was heated under Ar at 105 °C for 3 h (following by TLC). After cooling, water and ether were added and the phases were separated. The organic phase was dried ($MgSO_4$), filtered and removal of the solvent under reduced pressure gave an oil. This was submitted to column chromatography using 10% ether/petroleum ether as eluent, to give the *ortho*-chlorodiarylamine **1** as a colourless solid (228 mg, 90%). Crystallization from ether/petroleum ether gave colourless crystals, mp 158–160 °C. ^{1}H NMR ($CDCl_3$) 2.32 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 6.01 (s, 1H, N–H), 6.84 (dd, J = 8.1 and 1.7 Hz, 1H, H-4), 7.00 (dd, J = 8.1 and 4.6 Hz, 1H, H-5), 7.23 (d, J = 8.4 Hz, 1H, ArH), 7.47 (d, J = 8.4 Hz, 1H, ArH), 7.80 (dd, J = 4.6 and 1.7 Hz, 1H, H-6) ppm. ^{13}C NMR ($CDCl_3$) 11.45 (CH_3), 13.87 (CH_3), 15.92 (CH_3), 119.66 (CH), 119.80 (CH), 122.84 (CH), 123.21 (CH), 127.47 (C), 127.77 (C), 132.78 (C), 133.98 (C), 137.15 (C), 137.93 (CH), 139.09 (C), 139.77 (C), 139.89 (C) ppm. Anal. Calcd. for $C_{16}H_{15}ClN_2S$: C 63.46, H 4.99, N 9.25, S 10.59; found: C 63.44, H 5.17, N 9.24, S 10.49%.

2.1.4. 6,8,9-Trimethyl-5H-pyrido[3,2-b]thieno[3,2-f]indole (**2**)

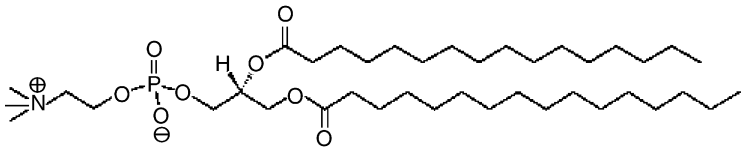
In a dry *Schlenk* tube it was poured under Ar with stirring, dry dioxane (7 mL), Pd(OAc)$_2$ (40 mol%), P(*t*-Bu)$_3$ (40 mol%), the *ortho*-chlorodiarylamine **1** (100 mg, 0.330 mmol) and finely grinded K_3PO_4 (10 equiv.). The mixture was heated under Ar for 20 h at 120 °C. After cooling ethyl acetate was added and the mixture was filtered. Removal of solvents gave a solid which was washed with ether to give the thieno-δ-carboline

2 as a brownish solid (62.0 mg, 70%), mp 287–289 °C. ^{1}H NMR ($CDCl_3$ + DMSO) 2.31 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 7.11 (dd, J = 8.1 and 4.7 Hz, 1H, H-3), 7.58 (dd, J = 8.1 and 1.3 Hz, 1H, H-4), 8.18 (s, 1H, H-10), 8.29 (dd, J = 4.7 and 1.3 Hz, 1H, H-2), 10.19 (1H, broad s, NH). ^{13}C NMR ($CDCl_3$ + DMSO) 11.20 (CH_3), 13.50 (CH_3), 14.61 (CH_3), 109.33 (CH), 111.97 (C), 117.06 (CH), 119.36 (CH), 120.52 (C), 127.09 (C), 129.91 (C), 133.96 (C), 134.77 (C), 137.15 (C), 137.98 (C), 140.35 (CH), 142.29 (C). MS *m/z* (%): 266 (100, M^+), 265 (11, $M^+ - 1$), 251 (17). HRMS $C_{16}H_{14}N_2S$: Calcd. M^+ 266.0878; found 266.0882.

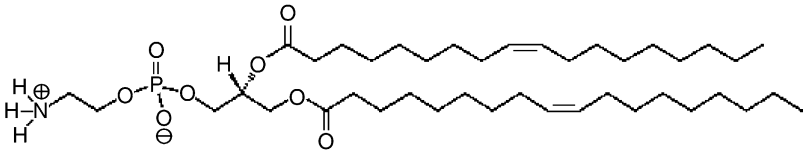
2.2. Vesicles preparation

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), both from Sigma, and dioctadecyldimethylammonium bromide (DODAB), from Tokyo Kasei, were used as received (lipid structures are shown below). Lipid/probe films were prepared from stock solutions in chloroform, by evaporation of solvent under a nitrogen stream. Vesicles were formed by hydration of lipids with an aqueous buffer solution (20 mM HEPES, 10 mM NaCl), at room temperature for DOPE and at 60 °C for DODAB and DPPC (above transition temperature of both lipids), followed by bath-sonication to obtain optically clear solutions [17,20]. The final lipid concentration was 1 mM, with a probe/lipid molar ratio of 1:500.

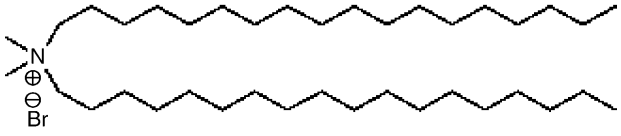
DPPC



DOPE



DODAB



2.3. Spectroscopic measurements

All solutions were prepared using spectroscopic grade solvents. The fluorescence quantum yields (Φ_s) were determined using the standard method (Eq. (1)) [21]. 9,10-Diphenylanthracene in ethanol was used as reference, $\Phi_r = 0.95$ [22].

$$\Phi_s = \left[\frac{A_r F_s n_s^2}{A_s F_r n_r^2}\right] \Phi_r \quad (1)$$

where A is the absorbance at the excitation wavelength, F the integrated emission area and n the refraction index of the solvents used. Subscripts refer to the reference (r) or sample (s) compound.

Absorption spectra were recorded in a Shimadzu UV-3101PC UV–Vis–NIR spectrophotometer. Fluorescence measurements were performed using a Spex Fluorolog 212 spectrofluorimeter, with a temperature controlled cuvette holder. For fluorescence quantum yield determination, the solutions were previously bubbled for 20 min with ultrapure nitrogen. Fluorescence spectra were corrected for the instrumental response of the system.

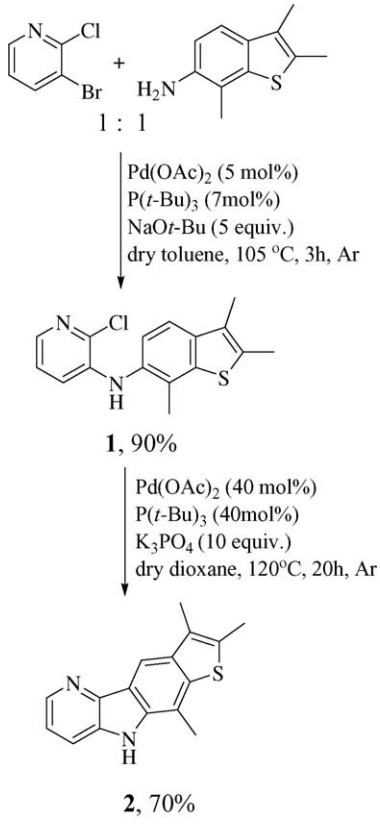
3. Results and discussion

3.1. Synthesis

Here we present the synthesis in good yield (70%) of the first thieno-δ-carboline by palladium-assisted intramolecular cyclization of *ortho*-chlorodiarylamine **1**. The latter was in turn synthesized in high yield (90%) by selective palladium-catalyzed C–N Buchwald–Hartwig cross-coupling [23] of 3-bromo-2-chloropyridine with the also prepared, by a method already described [24], 6-amino-2,3,7-trimethylbenzo[*b*]thiophene, using P(*t*-Bu)$_3$ as ligand and NaO*t*-Bu as base (Scheme 1). These conditions were used by Bedford and Cazin for the preparation in high yield of *ortho*-chlorodiphenylamines from halobenzenes and *ortho*-chloroanilines [25].

Our selective amination observed in the synthesis of compound **1** is in agreement with the results of Maes et al. obtained for the palladium-catalyzed arylaminations of 2-chloro-3-iodo or 2-chloro-5-iodopyridines, despite the use of different reaction conditions [26].

For the cyclization reaction we followed the conditions used by Maes et al. for the cyclization of 3-chloro-2-(4-pyridinylamino)pyridine to the corresponding tricyclic compound [27] but in our case higher amounts of the palladium catalyst were required to obtain compound **2** in good yield. This



Scheme 1. Synthesis of *ortho*-chlorodiarylamine **1** and of thieno-δ-carboline **2**.

reaction can be seen as an intramolecular C–H activation by a Pd(II) complex, resulting of oxydative addition of compound **1** to Pd(0), presumably by an electrophilic displacement mechanism, to give a six membered pallacycle which subsequently yields the thienocarboline **2** by reductive elimination, as described for the synthesis of carbazoles from *ortho*-chlorodiphenylamines [25].

3.2. Fluorescence

Fluorescence spectra of thienocarboline **2** in different solvents are shown in Fig. 2. Absorption spectra are displayed as inset. For the lowest energy absorption band, a small red shift is observed in polar solvents while the spectral shape is maintained. Fluorescence spectra display much larger red shifts with increasing solvent polarity. The absorption and emission maxima and the fluorescence quantum yields (Φ_F) are shown in Table 1. Φ_F values in methanol and water are very low ($\leq$0.001).

Due to the strong dependence of emission spectrum with environment polarity, thienocarboline can be considered as a solvatochromic probe, despite the low Φ_F exhibited (Table 1).

Significant spectral changes are observed for emission in polar solvents (Fig. 2), with a loss of the vibrational structure and increase of the bandwidth which may be attributed to specific solvent effects and/or to a dipolar intramolecular charge transfer (ICT). These processes may be competitive and it is difficult to determine which one is predominant.

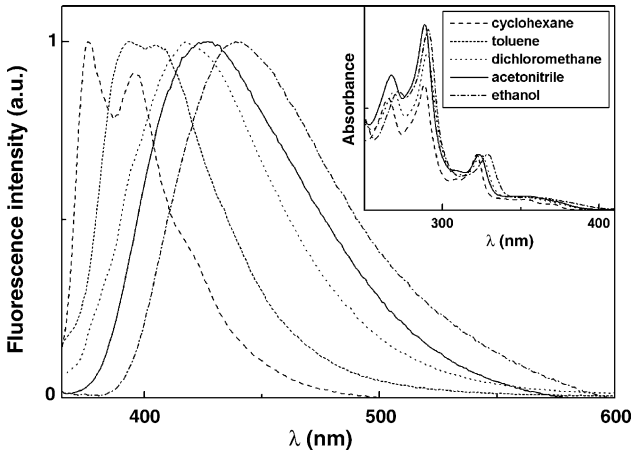


Fig. 2. Normalized fluorescence spectra of thienocarboline (2×10^{-6} M) in different solvents (λ_{exc} = 325 nm). Inset: absorption spectra (normalized in the lowest energy maximum).

Table 1
Fluorescence quantum yields (Φ_F), maximum absorption and emission wavelengths (λ_{abs} and λ_{em}) for thienocarboline in different solvents

Solvent	λ_{abs} (nm)	λ_{em} (nm)	Φ_F[a]
n-Hexane	321	372	0.015
Cyclohexane	322	376	0.02
Toluene	325	392	0.02
Dichloromethane	325	418	0.03
N,*N*-Dimethylformamide	326	424	0.04
Acetonitrile	324	427	0.04
Dimethylsulfoxide	328	429	0.05
1-Butanol	329	435	0.07
Ethanol	329	440	0.04

[a] Relative to 9,10-diphenylanthracene in ethanol (Φ_r = 0.95 [22]).

To investigate the occurrence of specific solvent effects, small amounts of ethanol were added to a thienocarboline solution in cyclohexane, and the emission spectra are shown in Fig. 3. It can be observed that a small amount of ethanol (2.6%) is enough for the complete loss of vibrational structure and a strong red shift

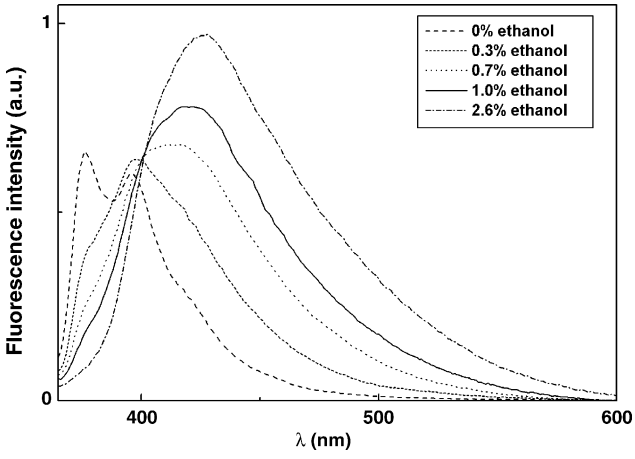


Fig. 3. Fluorescence spectra of thienocarboline **2** (2×10^{-6} M) in mixtures of cyclohexane/ethanol, increasing ethanol percentage (λ_{exc} = 325 nm).

of the emission maximum. This behaviour is very similar to the reported for 2-anilinonaphthalene [28] and 4-aminophthalimide [29] attributed to specific solvent effects [30,31].

Solvatochromic shifts are often described by the Lippert–Mataga equation (2), which relates the energy difference between absorption and emission maxima to the orientation polarizability [30,32],

$$\bar{\nu}_{abs} - \bar{\nu}_{fl} = \frac{1}{4\pi\varepsilon_0}\frac{2\Delta\mu^2}{hcR^3}\Delta f \quad (2)$$

where $\bar{\nu}_{abs}$ is the wavenumber of maximum absorption, $\bar{\nu}_{fl}$ the wavenumber of maximum emission, $\Delta\mu = \mu_e - \mu_g$ the difference in the dipole moment of solute molecule between excited (μ_e) and ground (μ_g) states, R the cavity radius (considering the fluorophore a point dipole at the center of a spherical cavity immersed in the homogeneous solvent), and Δf is the orientation polarizability given by Eq. (3):

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \quad (3)$$

where ε is the static dielectric constant and n is the refractive index of the solvent.

The Lippert–Mataga plot for thieno-δ-carboline **2**, shown in Fig. 4, is reasonably linear, toluene and alcohols exhibiting positive deviations.

From theoretical calculations obtained with a geometrically optimized structure (using a PM3 quantum mechanical model provided by ArgusLab software), a cavity radius of 5.2 Å was estimated. For the dipole moment change, a value of $\Delta\mu = 11.9$ D was calculated from the slope of the Lippert–Mataga plot, this high value indicating the presence of an intramolecular charge transfer mechanism (ICT). In thieno-δ-carboline **2** a significant electron density flow to the pyridinic ring is expected, not only due to the pyrrolic ring as in carbolines [3,34], but also due to the electron donating properties of the thiophene ring (mesomeric effect, +M) and of the methyl groups (inductive effect, +I). These effects may increase the CT character of the emitting state which is consistent with the high dipole moment change obtained [3].

The fluorescence quantum yield (Φ_F butanol > ethanol ≫ methanol, water) seems to decrease with the increase of hydrogen-bonding ability of protic solvents. This type of behaviour can be explained by an increase of singlet → triplet intersystem crossing efficiency through H-bond interaction, as reported for 4-aminophthalimide [29,31]. Fluorescence studies on δ-carboline (structure shown in Fig. 1) evidenced hydrogen bonding formation through the pyrrolic NH (donor) and through the pyridinic N atom (acceptor) [3]. For our thieno-δ-carboline the presence of the S atom may increase the H-bond acceptor character and may also promote the intersystem crossing process by enhancement of spin–orbit coupling interaction [35].

In order to study the behaviour of thienocarboline **2** in lipid membranes, it was incorporated in vesicles of DPPC, DOPE and DODAB. The neutral phospholipids DPPC and DOPE are major components of biological membranes, while cationic liposomes based in DODAB have been used as vehicles for DNA transfection and drug delivery [36].

As the lipid bilayer has an inner and outer aqueous environment and compound **2** is not fluorescent in pure water, its fluorescence was measured in a water/ethanol mixture (1:10). A red shift and enlargement of the emission band is observed together with a significant fluorescence quenching (Fig. 5). Because of the asymmetric nature of the thienocarboline emission band in polar media, the spectra were fitted with lognormal functions, an approach previously used for hydroxypyridine [37] and Nile Red [15,18,38].

In pure ethanol, thienocarboline emission band can be well fitted with one lognormal function, with a maximum at 440 nm, while the spectrum in a 1:10 water/ethanol mixture can only be fitted with a sum of two lognormal functions (inset of Fig. 5), the major component with a maximum at 444 nm (corresponding to the compound in ethanol) and the minor component with a maximum at 520 nm, attributed to a water-rich environment.

In lipid vesicles thienocarboline **2** exhibits significant fluorescence (Fig. 6) which is an indication that it is incorporated

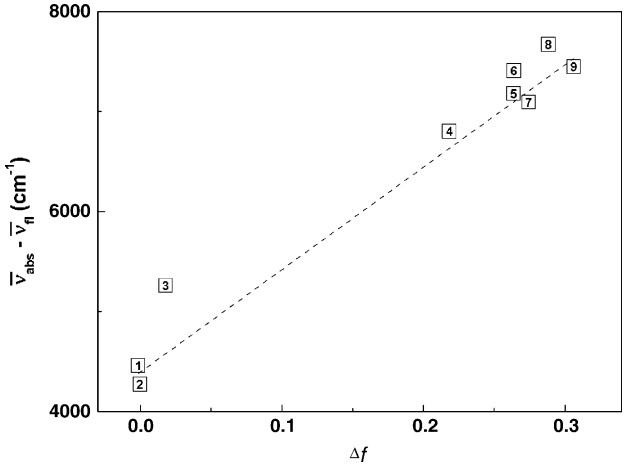


Fig. 4. Lippert–Mataga plot for thieno-δ-carboline **2**. 1: Cyclohexane; 2: *n*-hexane; 3: toluene; 4: dichloromethane; 5: dimethylsulfoxide; 6: 1-butanol; 7: dimethylformamide; 8: ethanol; 9: acetonitrile (values of ε and n are obtained from Ref. [33]).

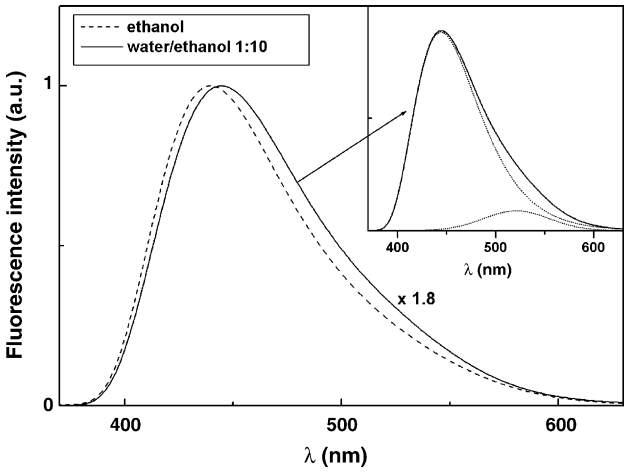


Fig. 5. Normalized fluorescence spectra of thienocarboline **2** in ethanol and in a 1:10 water/ethanol mixture. Inset: the latter spectrum and its two lognormal components.

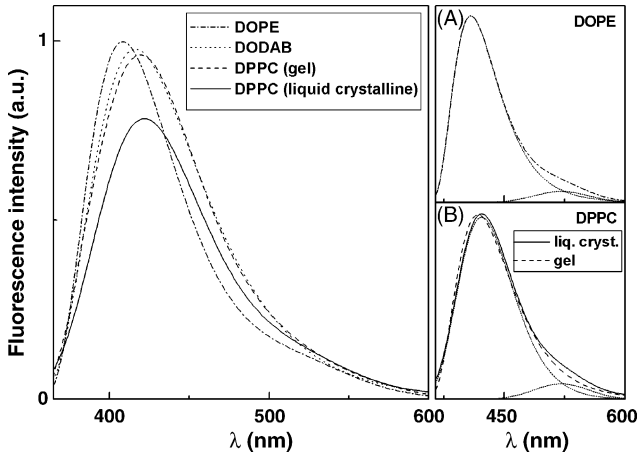


Fig. 6. Fluorescence spectra of compound **2** in lipid vesicles of DOPE, DODAB and DPPC in both gel (25 °C) and liquid-crystalline phases (55 °C). On the right side: spectra of compound **2** in vesicles of DOPE (A) and of DPPC at the liquid-crystalline phase (B) and the corresponding two lognormal components. A normalized one lognormal function in DPPC gel phase is shown for comparison (B).

in the lipid bilayer. The fluorescence intensities are similar for DOPE, DODAB and DPPC gel, although slightly higher for DOPE (Fig. 6). The emission maxima, 410 nm in DOPE, 418 nm in DODAB and 419 nm in DPPC, together with the structureless shape of emission, indicate that compound **2** is located inside the lipid membrane but in interaction with water molecules that diffuse across the bilayer [31].

In DODAB and DPPC vesicles, spectra fit well to one lognormal function, but emission in DOPE is a sum of two lognormal components (Fig. 6A). The major component has a maximum at 408 nm, while the minor one has a maximum at 520 nm, the latter corresponding to the compound in a water-rich environment as observed in the water/ethanol mixture (inset of Fig. 5). The different behaviour of thienocarboline in DOPE vesicles may be due to that at room temperature DOPE is in the liquid-crystalline phase, while the other lipids are both in the gel phase (phase transition temperatures are ca. 41 °C for DPPC [39] and 45 °C for DODAB [20]). To clarify this behaviour, thienocarboline emission was measured in DPPC vesicles at 55 °C (Fig. 6).

Like in DOPE, emission in DPPC liquid-crystalline phase is a sum of two lognormal components, exhibiting the component of $\lambda_{max} \approx 520$ nm (Fig. 6B). This provides evidence that when the lipid is in the more fluid phase (liquid-crystalline) thienocarboline **2** probes two different environments, a water-rich and another deeper in membrane, feeling the penetration of water molecules. Therefore, this compound can be incorporated in the hydrophobic region of liposomes for controlled drug release assays, in order to develop its biological application.

4. Conclusions

We were able to synthesize the first thieno-δ-carboline by palladium-assisted intramolecular cyclization of an *ortho*-chlorodiarylamine prepared selectively by a C–N palladium-catalyzed cross-coupling of 3-bromo-2-chloropyridine with 6-amino-2,3,7-trimethylbenzo[*b*]thiophene.

Fluorescence studies show that thienocarboline has a solvatochromic behaviour. Despite the low fluorescence quantum yields in solution, studies of its incorporation in lipid vesicles show that the compound locates mainly inside the bilayer, exhibiting a different behaviour in the gel and liquid-crystalline phases. Due to the thieno-δ-carboline potential biological activities, including the already proven anti-tumoral activity after photoactivation, our studies may be useful for its incorporation in the hydrophobic region of liposomes and controlled drug release.

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