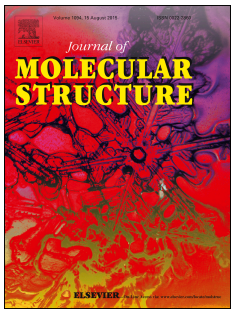

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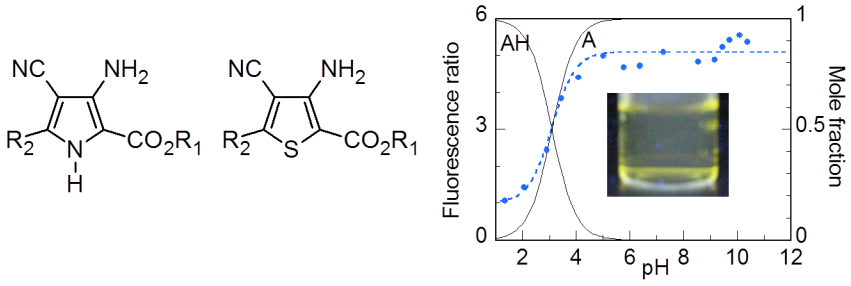

Synthesis and fluorescence properties of aminocyanopyrrole and aminocyanothiophene esthers for biomedical and bioimaging applications

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Abstract
We prepared a series of substituted aminocyanopyrroles and another of aminocynaothiophenes. We describe an efficient new one-step synthetic strategy via the condensation of an alkyl sarcosinate and ethoxymethylenemalononitrile, through a Gewald-like reaction. The UV-visible absorption and steady-state and time resolved fluorescence properties of some representative compounds, as well as their acid-base behavior, is also presented. The compounds might be useful for medicinal applications and as bioimaging probes.

Keywords: Aminocyanothiophene · Aminocyanopyrrole · Sarcosinate · Gewald-like reaction · One-pot synthesis · Fluorescent probes

1. Introduction

Pyrrole and thiophene derivatives are frequently found in naturally occurring compounds, many of which exhibit useful biological activity [1,2]. Polyfunctional derivatives of 3-aminopyrroles are an important family of compounds [1], with many applications as antibacterial, antiviral, anticonvulsant, anti-inflammatory, analgesic and antipyretic agents [3-6]. Pyrroles are also building blocks for porphyrins [7], and polymers of pyrroles have found use as conducting polymers and materials for non-linear optics [8]. Different methods for the preparation of this heterocyclic system have been proposed [9-13]. They can also further undergo a wide variety of chemical reactions including electrophilic substitution reactions, acylation and alkylation reactions at the nitrogen [14].

In the other hand, multi-substituted thiophene compounds have demonstrated a broad spectrum of uses, including in agrochemical applications [15], as potential antioxidant and anti-inflammatory agents [16,17], anti-HIV PR inhibitors [18], anti-breast cancer [19], anti-avian influenza virus (H5N1) [20], multitarget kinase inhibitors [21], AMPK activators [22], antitubercular agents [23], and as fluorescent bioimaging dyes [24]. For this purpose there is also the potential of enhancing the fluorescence properties through aggregation-induced emission by immobilization of the dyes in nanoparticles [25-43].

Hence, the development of substituted aminopyrroles and aminothiophenes is of great interest in organic synthesis as well as in photochemistry.

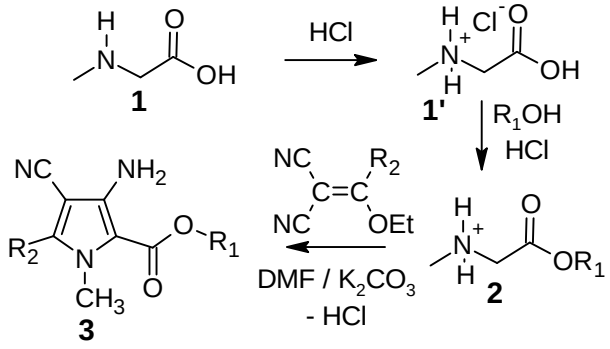
Here we aim to synthesize heterocyclic compounds with pyrrole and thiophene groups functionalized with amine, nitrile and esther groups at specific positions through simple methods. These compounds are potentially biologically active and might serve as building blocks for other useful molecules. We also expect them to have interesting photophysical properties that might make them useful for bioimaging, especially with fluorescence-related microscopies including confocal microscopy.

2. Results and discussion

2.1. Synthesis

Synthetic methods for preparing a series of substituted pyrrole by amines at position 3 have rarely been reported [44-46]. The synthesis of aminocyanopyrroles **3** was carried out, using a multi-component reaction, through two steps. The first step was an esterification reaction of an acetylchloride with an alcohol which generated hydrochloric acid in the medium. Sarcosine **1** was then added, and transformed into the corresponding salt **1'**, which in the presence of HCl and of an excess of the alcohol underwent esterification to give the corresponding hydrochloride of alkyl sarcosinate **2**. In the following steps, we have added to the mixture DMF and K_2CO_3 as alkaline reagent (releasing one molecule of hydrochloric acid and turning **2** into the corresponding alkyl sarcosinate) and ethoxymethylenemalononitrile. The reaction was heated under reflux for 6 hours at 60°C, yielding the corresponding aminocyanopyrrole **3** (Scheme 1).

Several methods for the preparation of thiophenes have been reported as well as their applications in the synthesis of useful compounds [15-24,47,48]. Most thiophenes are formed by reaction of a 3-thionate salt acrylic ester or acrylonitrile



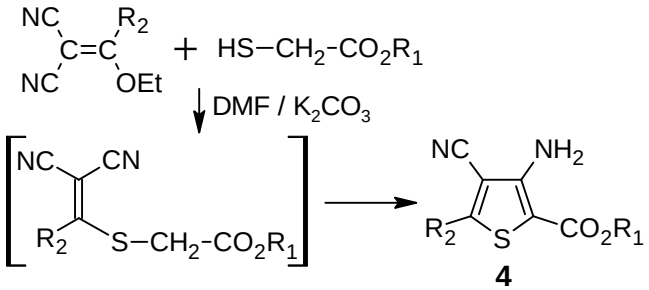
1 **1'** **2** **3**

3a R_1: CH_3, R_2: H; **3b** R_1: CH_3, R_2: CH_3; **3c** R_1: CH_3, R_2: C_2H_5

3d R_1: CH_3, R_2: Ph; **3e** R_1: C_2H_5, R_2: CH_3; **3f** R_1: C_2H_5, R_2: H

Scheme 1. One-pot strategy used for pyrroles synthesis.

with a halogen compound. In this work, the 3-amino-4-cyanothiophene-2-carboxylates **4** (Scheme 2) were synthesized via a single step process of a 3-alkyl-3-alkoxyacrylonitrile malononitrile and a mercaptoacetic ester in DMF and K_2CO_3 by a modified reaction of the Gewald method [48-50]. The structures of the synthesized compounds were confirmed by NMR and IR spectroscopies and by mass spectrometry.



4

4a R_1: CH_3, R_2: CH_3; **4b** R_1: CH_3, R_2: H; **4c** R_1: C_2H_5, R_2: CH_3

Scheme 2. Synthetic strategy for thiophenes **4**.

2.2. Fluorescence, UV-Vis Absorption and Acid-base Properties

The structure of the different compounds is very similar in terms of ionizable groups, so we have studied one representative compound of each of the pyrrole and thiophene families, **3a** and **4a**, as well as the pyrrole derivative with a conjugated phenyl group **3d**.

In Figure 1 we present UV-Vis absorption (*a*), fluorescence emission (*b*) and fluorescence excitation (*c*) spectra of **4a**. Other fluorescence emission spectra of **4a** can be found in Supplementary Material (fig. S1). The molar absorptivity coefficients of the thiophene **4a** (see Figures 1*a*, S2*a* and S3*a* and table S1) and its respective fluorescence are more intense than those of the pyrrole compounds **3a** and **3d**, and those of **3d** (with the phenyl group) are more intense than those of **3a**.

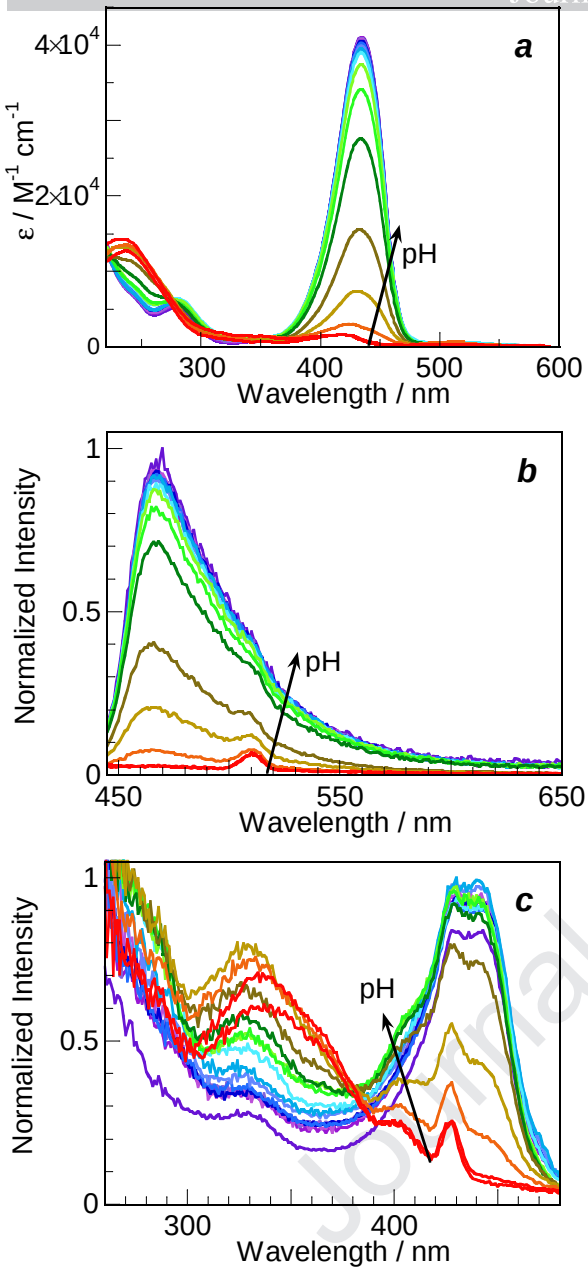


Figure 1. UV-Visible absorption (*a*), fluorescence emission (λ_{exc} = 435 nm) (*b*) and excitation (λ_{em} = 500 nm) (*c*) spectra of **4a** (from red to violet: pH 1.2; 1.8; 2.56; 3.13; 3.60; 4.20; 4.70; 5.23; 5.85; 6.47; 7.06; 7.73; 8.73; 9.53; 10.32; 1.02; 11.60).

In order to have a better view the behavior of the absorption and fluorescence of the compounds depending on the pH and to determine the ionization constants, we show titration curves of the lone compounds at representative wavelengths in Fig. 2. Bearing in mind the possibility of ratiometric imaging [51,52], we show the fluorescence curves as a ratio of fluorescence intensities at two wavelengths. We also present in the plots the speciation curves which were simulated with the HySS2009 software package [53]. By fitting the experimental data with simulated curves of absorption or fluorescence intensity, we obtain the ionization constants (Table 1). In Table S1 we also present the estimations for molar absorption coefficients resulting from the fittings of the absorption spectra with the estimated pK_a values.

The titration of compound **4a** (Fig. 2*a*) shows the existence of one acid-base equilibrium, which provokes a great change in the absorption around 400-450 nm, and the corresponding change in the fluorescence intensity when exciting at this wavelength. The compound can still be excited at 460 nm, making it usable in confocal microscopy. The titration curve shown in Fig. 2*a* was obtained using this excitation wavelength.

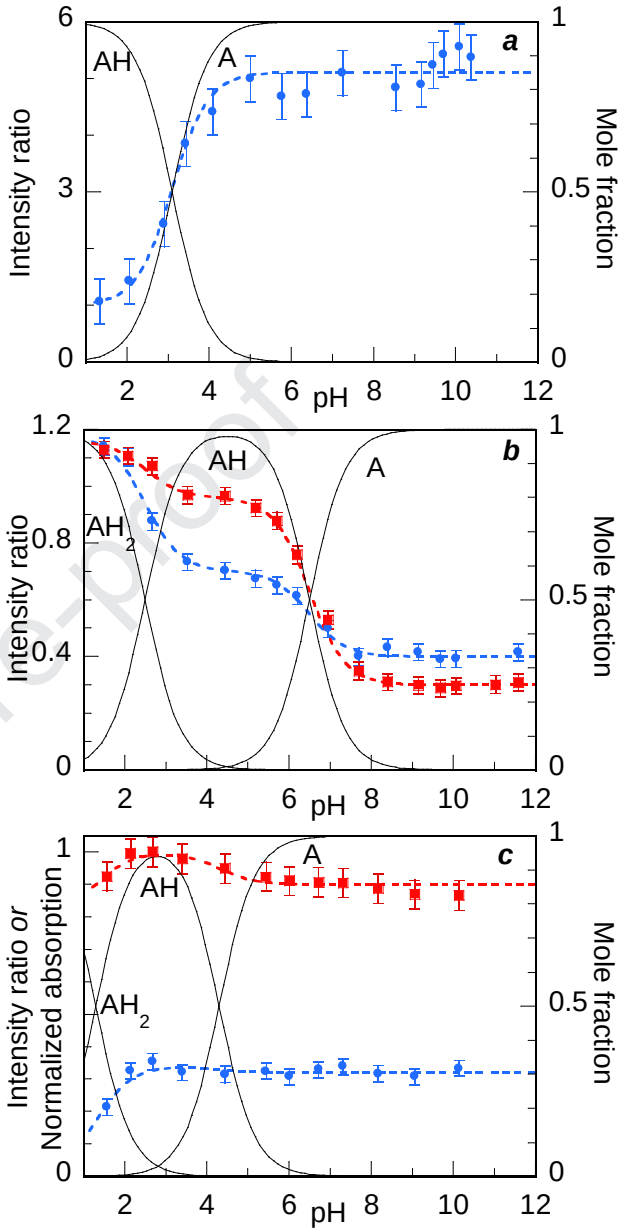


Figure 2. Titration curves overlapped with the calculated speciation curves (thin solid lines) and titration fitting curves (thick dashed lines): (*a*) **4a** (Emission, λ_{exc} = 460 nm. Blue: $I(\lambda_{em}$ = 480 nm)/$I(\lambda_{em}$ = 600 nm); (*b*) **3a** (Emission, $I(\lambda_{em}$ = 370 nm)/$I(\lambda_{em}$ = 430 nm). Blue: λ_{exc} = 270 nm; red: λ_{exc} = 340 nm; (*c*) **3d** (Red: Normalized UV-vis absorption, λ = 285 nm; blue: emission, λ_{exc} = 345 nm, $I(\lambda_{em}$ = 550 nm)/$I(\lambda_{em}$ = 450 nm). *I*: Intensity

In Figure 2*b* and 2*c* we also present the titration curves for the pyrrole compounds **3a** and **3d** at representative wavelengths. Their UV-visible, fluorescence emission and excitation spectra can be seen at Supplementary Material figures S1 and S2. We propose possible acid-base equilibria in Scheme S1.

The pyrrole compounds **3a**, **3d** have an extra ionization constant when compared to the thiophene **4a**, as expected due

to the nitrogen in the pyrrole ring. The presence of the hydrophobic phenyl group makes compound **3d** more acidic than **3a**, possibly in part due to the extra gain in stability in water if the compound is protonated.

We have also measured the fluorescence lifetimes of **4a**, **3a** and **3d** at representative pH values. The decays were analyzed with a sum of three exponential functions, yielding good fitting results (see Supplementary Material Tables S3, S4 and S5). An example of a decay is shown in Figure 3. The remaining decays can be seen in Supplementary Material figures S4 to S22.

Table 1. Ionization constants obtained by fitting of the spectrofluorimetric and spectrophotometric titration curves with the calculated speciation curves. Proposed acid-base equilibria are depicted at Scheme S1 (Supplementary Material)

Compound	pK_{a1}	pK_{a2}
4a	3.1±0.2	-
3a	2.5±0.2	6.5±0.2
3d	1.3±0.2	4.3±0.2

As can be seen in Table S3 (Supplementary Material), in the case of the thiophene compound **4a** we observe that the decays are relatively unchanged by the acid-base equilibria, but are faster for the emission band appearing at 410 nm than when collected at 350 nm. At pH ≈ 2, when exciting at 335 nm and collecting at 420 nm, the decay lifetime values of the different components remain relatively unchanged from when exciting at 285 nm, although the average lifetime is shorter due to a greater weight of the shortest component.

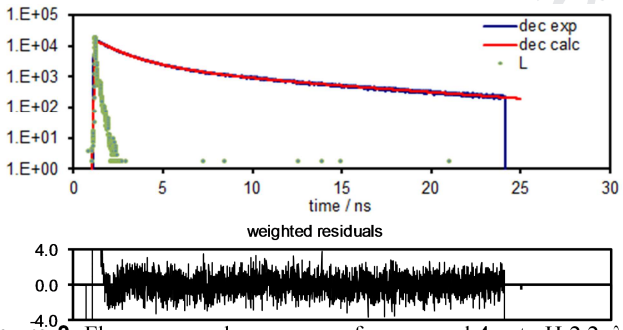


Figure 3. Fluorescence decay curve of compound **4a** at pH 2.2, λ_{exc} = 285 nm, λ_{emi} = 350 nm. Weighted residuals are plotted below the decay curve. The fluorescence decay lifetimes were obtained by fitting the decay curve with a sum of three exponential functions (with weights of each decay component τ_1, τ_2 and τ_3 being w_1, w_2 and w_3). The average decay lifetime is calculated as $\langle\tau\rangle = w_1 \times \tau_1 + w_2 \times \tau_2 + w_3 \times \tau_3$. For **4a** at pH 2.2, τ_1 = 1.01 ns (w_1 = 24%), τ_2 = 2.49 ns (w_2 = 28%), τ_3 = 10.82 ns (w_3 = 48%), $\langle\tau\rangle$ = 6.1 ns and χ^2 = 1.09. "Dec exp": experimental decay; "Dec calc": calculated decay; "L": Light source.

In the case of the pyrrole compound **3a**, the longer components τ_2 and τ_3 are also not too much affected by pH, but there is a diminishing of the value of the shortest component τ_1 with pH, especially when passing from pH ≈ 3 to pH ≈ 9. There is also a decrease in the relative weight w_1 with the corresponding increase in w_2 and w_3 .

In the pyrrole compound **3d**, the decays also are not very much affected by pH. There is, however, a diminishing in the value of τ_2 when exciting at 335 nm and passing from pH ≈ 3 to 6. The average lifetime $\langle\tau\rangle$ is also shorter when exciting at 290 nm and collecting at 450 nm than when exciting at 335 nm due to a greater weight in the shortest component. The average lifetime $\langle\tau\rangle$ is also generally shorter for **3d** than for **3a**.

We conclude that **3d** has a greater molar absorptivity and fluorescence intensity when comparing with **3a** due to the presence of the phenyl group. This group also seems to lower slightly the pK_a values of **3d** relatively to **3a**, and its decays become faster.

Conclusions

In summary, we have developed an efficient new strategy of preparation of 3-amino-4-cyano-1H-pyrrole-2-carboxylates, in two steps from commercially available inexpensive starting materials. We also describe an easy protocol for the preparation of 2-aminothiophenes. These products constitute building blocks useful in the access to many nitrogen and sulphur heterocycles with potential therapeutic interest or which could serve as precursors for many useful biologically active molecules. They could also be potentially useful fluorescent dyes for bioimaging applications, especially in the case of thiophene derivatives, which can be excited at 460 nm, making them usable in confocal microscopy applications.

Some representative compounds described herein were tested for their UV-visible absorption and fluorescence properties, as well as their acid-base behavior. We have seen that the molar absorptivity and fluorescent intensity of the thiophene compound **4a** are significantly higher than those of the pyrrole compounds **3a** and **3d**, and that the presence of the phenyl group of **3d** also affects the absorption and fluorescence properties. The fluorescence and UV-visible absorption capabilities of these compounds might enable further studies of their biological activities by these techniques.

Experimental

IR Spectra were determined for KBr on a Jasco Fourier transform FT–IR 420 spectrometer with precision of 2 cm^{-1} covering the field of 400–4000 cm^{-1}.

The ^1H NMR and ^{13}C NMR spectra were recorded in solution in dimethyl sulfoxide (DMSO-d_6) on a Bruker spectrometer (^1H at 400 MHz, ^{13}C at 100 MHz). The chemical shifts are expressed in parts per million (ppm). The multiplicities of the signals are indicated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quadruplet; and m, multiplet, and coupling constants are expressed in hertz.

Mass spectra were acquired in a Bruker Daltonics micrOTOF-Q spectrometer with Electrospray ionization (ESI).

The melting points were determined in an Electrotherma l9100 apparatus and are not corrected.

The reactions were monitored by thin-layer chromatography (TLC) using aluminium sheets with silica gel 60F_{254} from Merck.

General method for the synthesis of methyl-3-amino-4-cyano-1,5-dimetyhl-1H-pyrrole-2-carboxylate

2.25 g of sarcosine were added to a cold solution of 5 mL of acetyl chloride and 22.5 mL of alcohol. The mixture was then heated under reflux for 3 hours, leading to the formation of sarcosine chlorohydrate. In the second step, sodium carbonate (K_2CO_3) (10 mmol) was added to the mixture with ethoxymethylenemalononitrile (10 mmol) and dimethylformamide (DMF) (30 mL). The reaction was heated for 6 hours at 60°C. The mixture was then poured into 100 ml of water; the compound in the resulting precipitate was then filtered and recrystallized from ethanol.

Compound **3a**: methyl 3-amino-4-cyano-1-methyl-1*H*-pyrrole-2-carboxylate: 62%; mp: 209 ºC. **FTIR**: $\nu_{C=N}$: 2150 cm^{-1}, ν_{NH2}: 3322-3413 cm^{-1}, $\nu_{C=O}$: 1668 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 7.54 (s, 1H, H_9), 5.79 (s, 2H, NH_2); 3.74 (s, 3H, H_7); 3.70 (s, 3H, H_8). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 160.9, C_3 146.0, C_5 133.9, C_2 120.6, C_{10} 115.0, C_4 79.6, C_7 50.5, C_8 37.7. **+MS** m/z 180.07 (M+1)

Compound **3b**: methyl 3-amino-4-cyano-1,5-dimethyl-1*H*-pyrrole-2-carboxylate: 92%; mp: 187 ºC. **FTIR**: $\nu_{C=N}$: 2212 cm^{-1}, ν_{NH2}: 3332-3423 cm^{-1}, $\nu_{C=O}$: 1661 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 5.77 (s, 2H, NH_2), 3.72 (s, 3H, H_7); 3.63 (s, 3H, H_8); 2.24 (s, 3H, H_9). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 160.9, C_3 145.3, C_5 142.4, C_2 115.3, C_{10} 104.2, C_4 80.8, C_7 50.4, C_8 33.1, C_9 11.2. **+MS** m/z 194.09 (M+1).

Compound **3c**: methyl 3-amino-4-cyano-5-ethyl-1-methyl-1*H*-pyrrole-2-carboxylate: 85%; mp: 165 ºC. **FTIR**: $\nu_{C=N}$: 2215 cm^{-1}, ν_{NH2}: 3346-3446 cm^{-1}, $\nu_{C=O}$: 1668 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 5.75 (s, 2H, NH_2), 3.73 (s, 3H, H_7); 3.66 (s, 3H, H_8); 2.66 (q, J=7.6 Hz, 2H, H_9); 1.13 (t, J=7.6Hz, 3H, H_{10}). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 161.0, C_3 147.2, C_5 145.5, C_2 115.1, C_4 104.3, C_7 79.8, C_8 50.4, C_{11} 32.9, C_{10} 18.5, C_9 12.7. **+MS** m/z 208.10 (M+1)

Compound **3d**: methyl 3-amino-4-cyano-1-methyl-5-phenyl-1*H*-pyrrole-2-carboxylate: 82%; mp: 145 ºC. **FTIR:** $\nu_{C=N}$: 2211 cm^{-1}, ν_{NH2}: 3342-3435 cm^{-1}, $\nu_{C=O}$: 1671 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 7.5 (m, 5H, phenyl group); 5.88 (s, 2H, NH_2), 3.78 (s, 3H, H_7); 3.62 (s, 3H, H_8). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 161.1, C_3 145.7, C_5 145.7, C_{10} 143.8, C_9 129.7, C_{12} 128.8, C_{11} 127.9, C_2 115.2, C_{13} 105.6, C_4 81.3, C_7 50.71, C_8 35.1. **+MS** m/z 256.10 (M+1)

Compound **3e**: methyl 3-amino-4-cyano-1-ethyl-5-methyl-1*H*-pyrrole-2-carboxylate: 87%; mp: 164 ºC. **FTIR**: $\nu_{C=N}$: 2193 cm^{-1}, ν_{NH2}: 3328-3425 cm^{-1}, $\nu_{C=O}$: 1668 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 5.73 (s, 2H, NH_2), 4.21 (q, J= 7.2 Hz, 2H, H_7); 3.63 (s, 3H, H_9); 2.25 (s, 3H, H_{10}), 1.28 (t, J = 7.2 Hz, 3H, H_8). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 160.5, C_3 145.3, C_5 142.3, C_2 118.9, C_{11} 104.3, C_4 80.8, C_7 58.9, C_8 33.2, C_9 14.4, C_{10} 11.3. **+MS** m/z 208.10 (M+1)

Compound **3f**: methyl 3-amino-4-cyano-1-ethyl-1*H*-pyrrole-2-carboxylate: 60%; mp : 192ºC. **FTIR**: $\nu_{C=N}$: 2150 cm^{-1}, ν_{NH2}: 3322-3402 cm^{-1}, $\nu_{C=O}$: 1669 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 7.83 (s, 1H, H_{10}), 5.07 (s, 2H, NH_2); 3.51 (s, 3H, H_9); 2.89 (q, J = 7.2 Hz, 2H, H_7), 2.22 (t, J = 7.2 Hz, 3H, H_8). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 163.5, C_3 144.3, C_5 138.4, C_2 120.43, C_{10} 114.2, C_4 80.8, C_7 50.4, C_8 44.9, C_9 28.8. **+MS** m/z 194.09 (M+1)

General method for the synthesis of 3-amino-4-cyano-1,5-dimetyhl-1H-thiophene-2-carboxylate

A mixture of the corresponding 3-alkyl-3-alkoxyacrylonitrile (10 mmol) and mercaptoacetic ester (10 mmol) in DMF (15 ml) with sodium carbonate (K_2CO_3) was heated under reflux for 1h. The resulting precipitate was then washed with water.

Compound **4a**: methyl 3-amino-4-cyano-5-methylthiophene-2-carboxylate: 66%; mp: 240 ºC. **FTIR**: $\nu_{C=N}$: 2208 cm^{-1}, ν_{NH2}: 3388-3421 cm^{-1}, $\nu_{C=O}$: 1665 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 6.81 (s, 2H, NH_2), 3.74 (s, 3H) 2.51 (s, 3H). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 162.9, C_5 157.1, C_3 153.4, C_2 113.0, C_4 101.3, C_9 96.1, C_7 51.3, C_8 15.4. **+MS** m/z 197.03 (M+1)

Compound **4b**: methyl 3-amino-4-cyanothiophene-2-carboxylate: 68%; mp: 250 ºC. **FTIR**: $\nu_{C=N}$: 2215 cm^{-1}, ν_{NH2}: 3345-3446 cm^{-1}, $\nu_{C=O}$: 1669 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 7.7 (s, H_5) 6.80 (s, 2H, NH_2), 3.8 (s, 3H). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 163.1, C_5 158.3, C_3 153.6, C_2 113.1, C_4 101.9, C_8 95.9, C_7 51.8. **+MS** m/z 183.01 (M+1)

Compound **4c**: ethyl 3-amino-4-cyano-5-methylthiophene-2-carboxylate: 78%; mp: 205 ºC. **FTIR**: $\nu_{C=N}$: 2192 cm^{-1}, ν_{NH2}: 3329-3425 cm^{-1}, $\nu_{C=O}$: 1680 cm^{-1}. **NMR ^{1}H** (DMSO-d_6, 400 MHz): 6.79 (s, 2H, NH_2), 4.22 (q, J=7.2 Hz, 2H), 2.51 (s, 3H), 1.24 (q, J=7.2 Hz, 3H). **NMR ^{13}C** (DMSO-d_6, 100 MHz): C_6 162.6, C_5 157.0, C_3 153.4, C_2 113.0, C_4 101.2, C_9 96.4, C_7 60.0, C_8 15.3, C_{10} 14.2. **+MS** m/z 211.05 (M+1)

UV-visible Absorption and Fluorescence Measurements

Absorption, steady-state and time-resolved fluorescence spectra of the different pyrrole and thiophene derivatives were measured in aqueous solutions with sodium pyrophosphate buffer 0.01 M. The pyrrole samples were prepared at the concentration of 1 mM and the thiophene sample at 10 μM, corresponding to an optical density of A ≈ 0.1 at 350 nm (**3a**, **3d**) or 435 nm (**4a**). UV-visible absorption spectra were obtained in a JASCO V-660 spectrometer, using quartz cells (l = 1 cm), unless indicated otherwise. The fluorescence spectra were recorded on a Horiba Jobin Yvon Fluorolog 3-22 spectrofluorimeter using quartz cells (l = 1 cm), excitation slits with 3 nm bandwidth, emission slits with 1 nm bandwidth, integration time of 0.1 s/point, maximum sensitivity, and right angle mode.

The fluorescence quantum yields were determined for **3a**, **3d** and **4a** relative to standards using the procedure recommended by IUPAC [54,55]. The compounds were dissolved in phosphate buffer 0.01 M at neutral, acidic and basic pH. For **3a** and **3d** quinine sulphate in H_2SO_4 0.5 M (ϕ = 0.546, λd_{em} = 350−665 nm, λ_{ex}=340 nm) was used as standard and for **4a** we used coumarin 153 in H_2O (ϕ = 0.12, λd_{em} = 432−650 nm, λ_{ex}=420 nm) as standard [54]. The absorption and emission spectra of each compound and of the corresponding standard (with excitation at the same wavelength) were measured for at least 5 different concentrations.

Fluorescence intensity decay curves with picosecond resolution were obtained by the single-photon timing technique using a laser excitation system that consists of a de-locked Coherent Inova 440-10 argon ion laser synchronously pumping a cavity dumped Coherent 701-2 dye laser (Rhodamine 6G or DCM), which delivers 5-6 ps pulses at a repetition rate of 460 kHz. Intensity decay measurements

were made by alternate collection of excitation and decay curves, using an emission polarizer set at the magic angle. The excitation profile was recorded at the excitation wavelength with a scattering suspension. For the decays, a cut-off filter was used to remove all excitation light. The emission signal passed through a depolarizer, a Jobin-Yvon HR-320 monochromator with a grating of 100 lines/mm and was detected with a Hamamatsu 2809U-01 microchannel plate photomultiplier (MCP-PT). The instrument response had as effective FWHM of 35 ps.

Fluorescence intensity decay curves were obtained by excitation light at 285 or 290 nm using the rhodamine dye laser and at 435 nm using the titanium-sapphire laser. The decay curves were analyzed using home-made non-linear least-square reconvolution software based on the Marquard algorithm [56], and the quality of the fit was evaluated by the reduced χ^2, the weight residuals and the autocorrelation of the residuals.

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Supplementary Material

Supplementary Material related to this article can be found at http://...

Table 1. Ionization constants obtained by fitting of the spectrofluorimetric and spectrophotometric titration curves with the calculated speciation curves. Proposed acid-base equilibria are depicted at Scheme S1 (Supplementary Material)

Compound	pK_{a1}	pK_{a2}
4a	3.1±0.2	-
3a	2.5±0.2	6.5±0.2
3d	1.3±0.2	4.3±0.2

Synthesis and fluorescence properties of aminocyanopyrrole and aminocyanothiophene esthers for biomedical and bioimaging applications

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Highlights:

- A series of substituted aminocyanopyrrole esthers has been synthesized;
- A series of substituted aminocyanotiophene esthers has also been synthesized;
- Efficient one-pot synthetic strategies are described;
- The fluorescence, UV-vis absorbance and acid-base behaviour of the compounds is presented;
- These compounds might be useful for medicinal applications and as bioimaging probes.

Oussama Cherif: Investigation, Methodology, Resources, Writing

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: